

**The Synthesis And Biological Evaluation Of Several Series Of
Melatonin Agonists and Antagonists**

A Thesis Submitted by

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In partial fulfilment of the requirements for the degree of

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of the
University of London**

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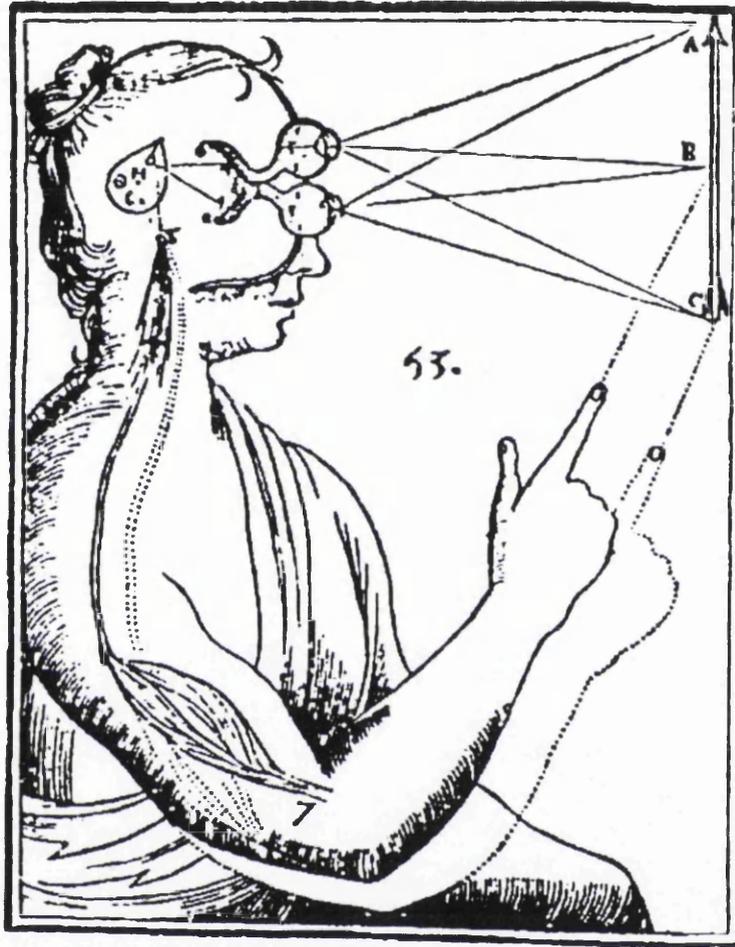
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"The changing of bodies into light, and light into bodies, is very comfortable to the course of nature, which seems delighted with transmutations."

- Isaac Newton

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Abstract

The work contained in this thesis forms part of an ongoing programme to examine the structure of the melatonin receptor by synthesis of indole based melatonin analogues.

The thesis is divided into five chapters. In Chapter One, an overview of the biosynthesis and physiological activity of the pineal hormone melatonin is presented. Receptor structure and distribution are also discussed, followed by a review of analogue studies reported in the literature up to the end of 1998.

Chapter Two describes the results of studies aimed at the introduction of an element of conformational restriction to the C3 side chain of melatonin. A series of 1-methylcycloalkan[b]indoles was synthesised which had high affinity in the 2-[¹²⁵I]-iodomelatonin radioligand binding assay and excellent biological potency in the *Xenopus Laevis* assay. Several racemic compounds from this series were resolved into enantiomers by chromatography and the melatonin receptor shown, for the first time, to be capable of chiral discrimination. The (R)-(-) enantiomers were demonstrated to be considerably better (up to 130 fold) at binding to the melatonin receptor and up to 400 times more potent than the (S)-(+) enantiomer. Several routes towards 1-H-cycloalkan[b]indoles were investigated.

Chapter Three reports the synthesis and results of an exploratory investigation into the potential of *N*_b-acyltetrahydro-β-carbolines as ligands for the melatonin receptor. No significant new agonists were obtained and many of the compounds showed antagonist behaviour.

Chapter Four includes the synthesis of several individual analogues or small sets of compounds aimed at fulfilling a specific requirement. Attempts were made to synthesise some novel analogues by direct substitution reactions on melatonin itself, and some attempts were made to alter postulated hydrogen bonding sites between ligand and receptor by replacing oxygen atoms in the pharmacophores with sulphur.

Chapter Five contains the experimental details for Chapters Two to Four.

Abbreviations

Ag	Agonist
Ant	Antagonist
Aq.	Aqueous
Bz	Benzyl
CDCl ₃	Deuteriochloroform
DCM	Dichloromethane
DMF	N,N-dimethylformamide
DMSO	Dimethylsulphoxide
EC ₅₀	Effective concentration required to achieve 50% of the maximal response.
E.I.M.S.	Electron impact mass spectrometry
ETOAc	Ethyl acetate
Eq.	Equivalent
FAB	Fast atom bombardment
HOAt	Hydroxyazabenzotriazole
HPLC-MS	High pressure liquid chromatography – Mass spectrometry
h.	Hour
IC ₅₀	The concentration of test compound which blocks the maximal effect of melatonin by 50%
IR	Infra red
K _d	Dissociation constant
K _i	Inhibition constant
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
ppm	Parts per million
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography

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Chapter 1

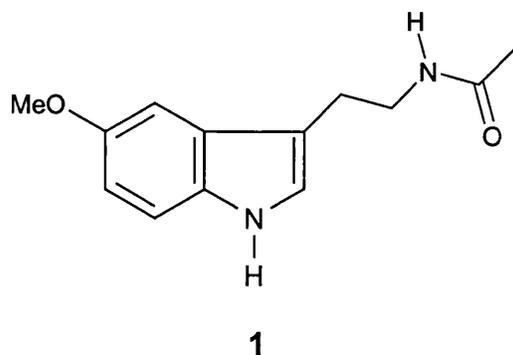
Physiological Activity of the Pineal Hormone Melatonin

Aim

This review constitutes a limited overview of aspects of melatonin research up to the end of 1998. It will attempt to focus mainly on data obtained in mammals, and in particular humans, which outline the mechanism of melatonin production and its effects on biological and endocrine function. The significance of melatonin in the establishment of circadian rhythms and sleep has been the main thrust of research to date, although in recent years a proliferation of reports have emerged suggesting effects in a wide variety of other areas. Publications can be arbitrarily divided into two types, those investigating the broad effects of melatonin on every living cell and those investigating the effects specifically mediated by high affinity cell membrane receptors. This chapter will concentrate largely on the latter. Some of the potential and actual clinical applications of melatonin will also be discussed.

1.1 Introduction

The 17th century mathematician and philosopher Rene Descartes sparked great interest in the pineal gland by identifying it as the seat of the human soul. The advent of empirical science, however, relegated the pineal gland to the role of a non-functioning vestigial organ, a view that was widely held as recently as the 1950's. Since then the wheel has turned full circle and a burgeoning research community has grown around the role of the pineal gland as an important neuroendocrine transducer. As early as 1917 McCord and Allan had demonstrated the ability of pineal extract to blanch tadpole skin¹ but it was not until 1958 that melatonin (1) was first isolated from bovine pineal glands by Lerner *et al.*² and the active constituent was subsequently identified as 5-methoxy-*N*-acetyltryptamine.³ Research has concentrated on the pineal gland as the primary site of melatonin biosynthesis, although the contribution of extrapineal sites of melatonin synthesis such as the retina and the gut to circulating levels of the hormone has become a matter of debate.⁴



The pineal is a pea sized, cone shaped gland contained within the connective tissue extensions of the dorsal surface of the thalamus (Figure 1). It weighs approximately 1 mg in the rat and 100 mg in humans and is heavily innervated by fibres from the superior cervical ganglia.⁵ The pineal gland is found in all vertebrates and the principal cell type is the pinealocyte.

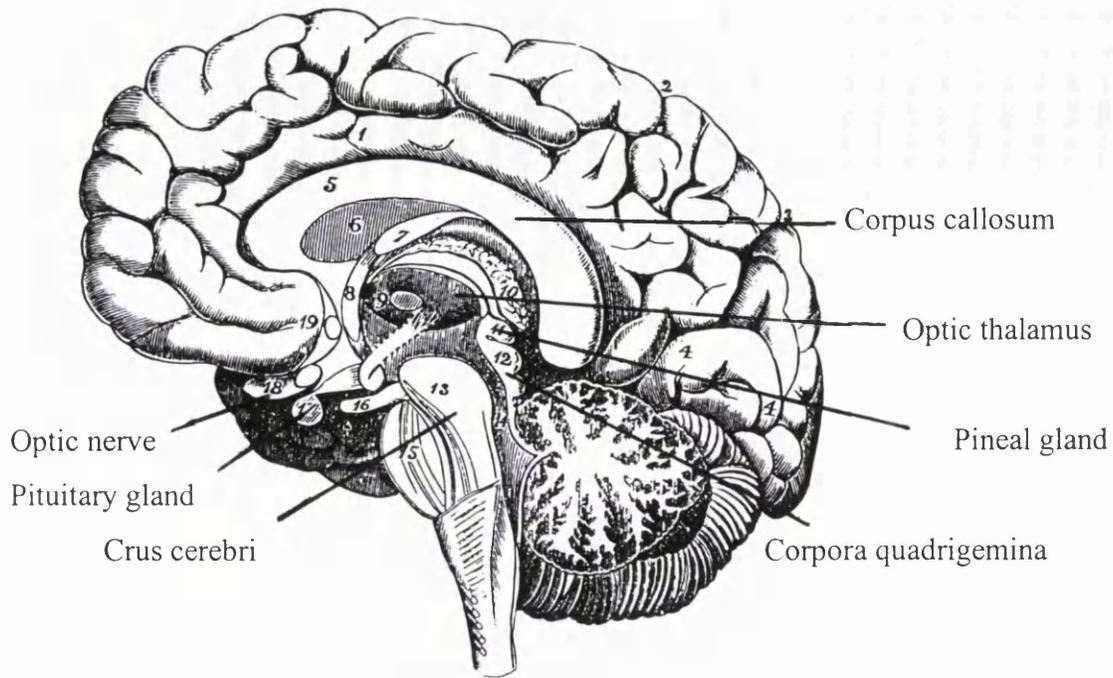


Figure 1a Vertical median section of the encephalon.⁶

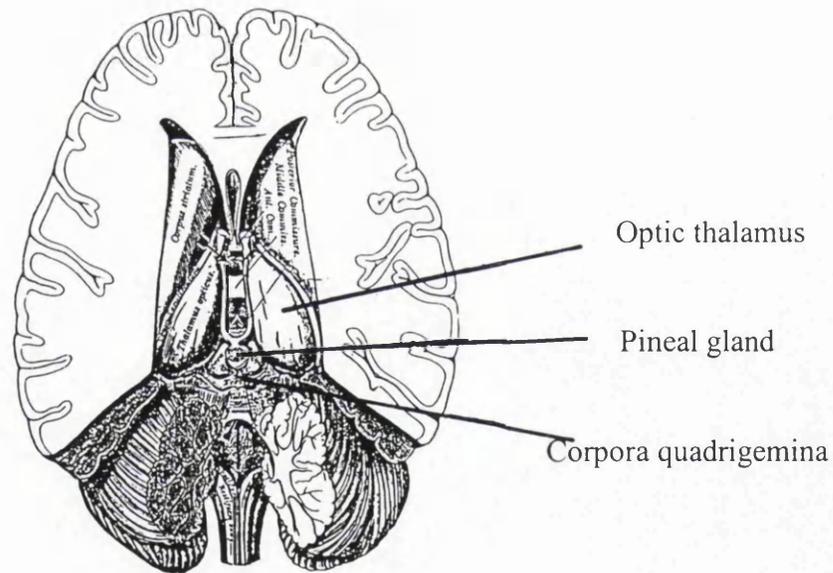


Figure 1b Third and fourth ventricles.⁷

Whilst being physically connected to the brain, the pineal gland is one of a series of structures known as the circumventricular organs which are on the peripheral

side of the blood-brain barrier. These organs are recognised as important sites for communication between both the brain and the cerebrospinal fluid, and the brain and peripheral organs, *via* blood borne chemicals. While the pineal has become particularly associated with melatonin production, several other peptides and hormones have also been isolated from the gland. Some of these appear to modulate gonadotropin secretion and it has been suggested that melatonin might serve as a local hormone, regulating the release of these compounds into the systemic circulation.⁸

Quay⁹ and Wurtman^{10,11} first reported the cyclic secretion of melatonin by the pineal gland during the 1960's and, to date, all of the vertebrate organisms studied show this pattern of secretion. The importance of pineal melatonin as the primary source of nocturnal melatonin was re-inforced by the finding that either pinealectomy or sympathetic denervation of the gland resulted in loss of the nightly increase in circulating hormone levels,¹²⁻¹⁴ although daytime melatonin levels were almost unaffected.¹³ The increase in melatonin level occurs at night in all species regardless of whether they are nocturnal, such as the hamster, or diurnal, such as humans, hence it is a true physiological indicator of night, the 'hormone of darkness'.

In 1965 Hoffmann and Reiter published results directly linking melatonin levels to the length of the photoperiod.¹⁵ Today the role of melatonin as a chemical mediator of environmental cues to animal physiological and behavioural systems is widely accepted. The most thoroughly examined function of melatonin in mammals is the mediation of physiological responses to seasonal changes, particularly *via* the process of phototransduction and synchronisation of circadian cycles. For animals in a temperate climate there are many environmental challenges associated with the change of season and a clear benefit to an individual organism in being able to predict an imminent change in the environment and to initiate the appropriate physiological responses. Seasonal changes in night length induce parallel changes in the duration of elevated melatonin secretion which in turn, trigger seasonal changes in behaviour. When

the duration of nocturnal melatonin secretion increases, indicating that days are becoming shorter, winter responses are triggered, and when the duration of secretion decreases spring and summer responses are initiated. These changes may affect a wide range of functions such as regulation of reproductive cycles,¹⁶ body weight,¹⁷ temperature,¹⁸ and metabolic rate.¹⁹ Some researchers have proposed that melatonin secretion is also sensitive to non photic stimuli, such as changes in temperature²⁰ or strength of magnetic fields.²¹ The fact that this hormone is involved in the modulation of such a wide range of physiological and behavioural processes has made it difficult to ascribe clear cut actions for the molecule.

While many species use changes in nocturnal melatonin secretion to convey information to cells about change of season, they may differ in how they use this information. A shortening of the melatonin signal in spring triggers gonadal recrudescence and initiates breeding in hamsters but, in contrast, the same shortening will trigger gonadal regression and cessation of breeding in sheep.²²

The role of the pineal gland and melatonin in seasonally breeding animals is well documented. However, in nonseasonal species such as humans the physiological significance of the pineal gland remains obscure. While the circadian pattern of secretion in humans is established, the amplitude of the pattern may vary considerably between individuals. Approximately 1-5% of the population have extremely low melatonin levels with no evidence of circadian secretion but both the reason for this and its physiological consequences remain unclear. Like other endocrine glands, the pineal gland is subject to an age dependent calcification process and in mammals an age related lowering of absolute levels of melatonin secretion is observed throughout the life of an individual.²³ This has been confirmed in several studies by direct measurement of blood serum levels as well as those of the major urinary metabolite 6-hydroxymelatonin.²⁴

It would appear that unlike many hormones, melatonin is not essential for the process of bare existence. Animals which have been subjected to pinealectomy can survive without the major source of circulating melatonin and several species

of laboratory mice are unable to synthesise melatonin at all, yet live and breed normally. These environments are relatively protected, however, and it is not certain whether these animals would flourish or even survive in the 'real world'.

In evolutionary terms melatonin is a highly ubiquitous molecule, being found in some of the most ancient of organisms, such as blue-green algae. It would be reasonable to suggest that melatonin could have taken on various roles during this time, although its role in controlling activity-rest cycles has remained conserved. Menaker has used lampreys and sparrows, two species separated by 400 million years of evolution, to illustrate this. In both species, melatonin is synthesised at night in the pineal gland and retina under the control of circadian oscillators, and in both species, pinealectomy has the effect of abolishing circadian locomotor activity.²⁵

Foetal/neonatal entrainment is a recent mammalian role of melatonin for which no nonmammalian equivalent has been demonstrated. It has been suggested by Davis²⁶ to result from the fact that the earliest mammals were nocturnal and nested in dark burrows which restricted access to the environmental light cycle. Since melatonin readily crosses the placenta and is present in maternal milk, its foetal and prenatal effects are probably mediated by the maternal pineal rhythm, which also ensures that siblings are synchronised.²⁷ Postnatally, these effects are mediated by the maturing organisms own melatonin rhythm, which in humans is observed to develop from the age of approximately three months. Melatonin production then develops and becomes circadian, reaching peak levels between 1-3 years of age. During the remainder of childhood, melatonin levels decline by 80% but it is likely that this is largely accounted for by an increasing body mass in the face of a constant hormone production. In adulthood the levels drop by another 10%, the significance of which is unclear.²⁴ It has been suggested that the specific physiological responses caused by melatonin may be qualitatively or quantitatively different at different stages in an organism's life history and not all necessarily related to environmental cycles.²⁸ Indeed, the greater abundance and broader distribution of melatonin receptors in embryos and fetuses relative to

mature animals suggests that the developmental effects of melatonin may be greater and more diverse.²⁹

1.2. Regulation of rhythmic melatonin production

Melatonin has been shown to be synthesised in several tissues but rhythmic synthesis is primarily localised in the pinealocyte cells of the pineal gland and retinae. Retinal perception of environmental light levels are transmitted to the suprachiasmatic nucleus (SCN) via the retinohypothalamic tract. Axons from ganglion cells in the retina synapse directly onto the dendrites of the SCN neurons, which respond to the luminance of light stimuli rather than orientation or motion. The SCN are a pair of tiny neuron clusters in the hypothalamus comprising roughly 8000 cells each with a volume of approximately 0.3 mm² in humans. The SCN is known as the biological clock since each cell has a spontaneous circadian firing rhythm which is maintained even when dissected and cultured *in vitro*.³⁰ This has made the tissue popular as an experimental model. The endogenous firing rhythm expressed by the cells of the SCN has a periodicity that differs from 24 hr and environmental light is the main mechanism by which the rhythm is reset on a daily basis.

The neuronal firing of the SCN is also inhibited by melatonin in a dose dependent manner,¹⁷ although the response to drug is dependent on time of administration. During late subjective day approximately 90% of the neurons are sensitive to melatonin whereas at early subjective day or subjective night only 20%-40% of the neurons are sensitive to melatonin administration.³¹ The cellular mechanism by which this is facilitated is currently unknown.

Output neurons from the SCN innervate nearby regions of the hypothalamus, particularly the paraventricular nuclei (PVN) which have been shown to act as a relay station in the SCN-pineal neural pathway by viral tracing studies.⁵ SCN neurons use γ -aminobutyric acid (GABA) as the primary neurotransmitter and so

are inhibitory in action. Impulses from the PVN are transmitted to the intermediolateral nucleus of the spinal chord via the medial forebrain bundle and conducted through sympathetic nerve fibres to the superior cervical ganglia.³² Axons from this area synapse directly to pinealocyte cells of the pineal gland³³ whose activity is enhanced by adrenaline release and interaction with both α and β adrenergic receptors in the pinealocyte membrane. The exact pathway by which environmental information is transmitted to the physiological clock probably varies between species and recent evidence suggests that even within the same animal more than one pathway may operate.³⁴

β_1 -Adrenergic stimulation results in a large increase in cyclic adenosine monophosphate (cAMP) and subsequent induction of enzymes required for melatonin synthesis.³⁵ The rapidity with which cAMP stimulation induces melatonin synthesis varies greatly between species. Thus in some animals, such as sheep, the night-time rise in melatonin production essentially accompanies the increase in cAMP levels, whereas for others, such as hamsters, the rise in melatonin level may be delayed by several hours.³⁶ The human melatonin pattern falls somewhere between these two extremes, showing a gradual rise in melatonin production from the onset of darkness. While the functional significance of these different patterns remains unknown the reason for the differences presumably lies in the timing of the post cAMP enzyme induction mechanisms. Melatonin itself has been shown to provide a negative feedback input in several tissues by inhibiting cAMP accumulation but the mechanism by which this is achieved is not known.³⁷

Stimulation of the α_1 adrenergic receptors triggers the inositol lipid signalling pathway, activating protein kinase C and inositol triphosphate (InsP₃). InsP₃ binds to the InsP₃ receptor channel on the endoplasmic reticulum and releases calcium from the intracellular stores.³⁸ Calcium sensitive fluorescent dyes allow the monitoring of Ca²⁺ changes in individual cells, and the mobilisation and increase in cellular calcium is followed by an increase in cAMP. Thus, the α_1

pathway can potentiate the effects of $\beta 1$ stimulation. An increase in intracellular calcium induces cyclic changes in membrane potential. Hyperpolarisation of the cell membrane results in an action potential and this is, in turn, followed by a depolarisation phase when calcium influx occurs through voltage sensitive channels in the plasma membrane. There is evidence that melatonin itself blocks the influx of Ca^{2+} from the extracellular medium by blocking the voltage sensitive channels, possibly by inducing hyperpolarisation.³⁹ Melatonin has also been demonstrated to inhibit release of Ca^{2+} from the intracellular stores.⁴⁰

Several other hormones and neurotransmitters may play a role in fine tuning the melatonin signal. The characterisation of pineal binding sites for GABA, dopamine D2,⁴¹ benzodiazepines, vasoactive intestinal polypeptide (VIP) and neuropeptide Y (NPY) has been established,⁴² although their effects are not fully understood. A decrease in nocturnal melatonin level has been reported to be caused by GABA⁴³ and benzodiazepines⁴⁴ while physiological concentrations of certain circulating hormones, such as luteinising hormone or growth hormone, produced elevated melatonin levels.⁴⁵ NPY⁴⁶ and VIP⁴⁷ in particular, cause potent stimulation of cAMP and increase melatonin synthesis significantly. Thus, in the absence of light, the SCN signals the pineal gland by the route described above, resulting in melatonin production. During the day, light, *via* the retinohypothalamic tract, inhibits the SCN from stimulating the pineal gland and melatonin production is suppressed (Figure 2).

1.3. Biosynthesis of melatonin

The initial substrate in the biosynthesis of melatonin is the amino acid L-tryptophan (2). This molecule is hydroxylated at the C5 position by the enzyme tryptophan hydroxylase (TH) to form 5-hydroxytryptophan (3). The factors regulating tryptophan uptake in the pineal gland have not been investigated, although the level of intracellular tryptophan does not follow the diurnal rhythm

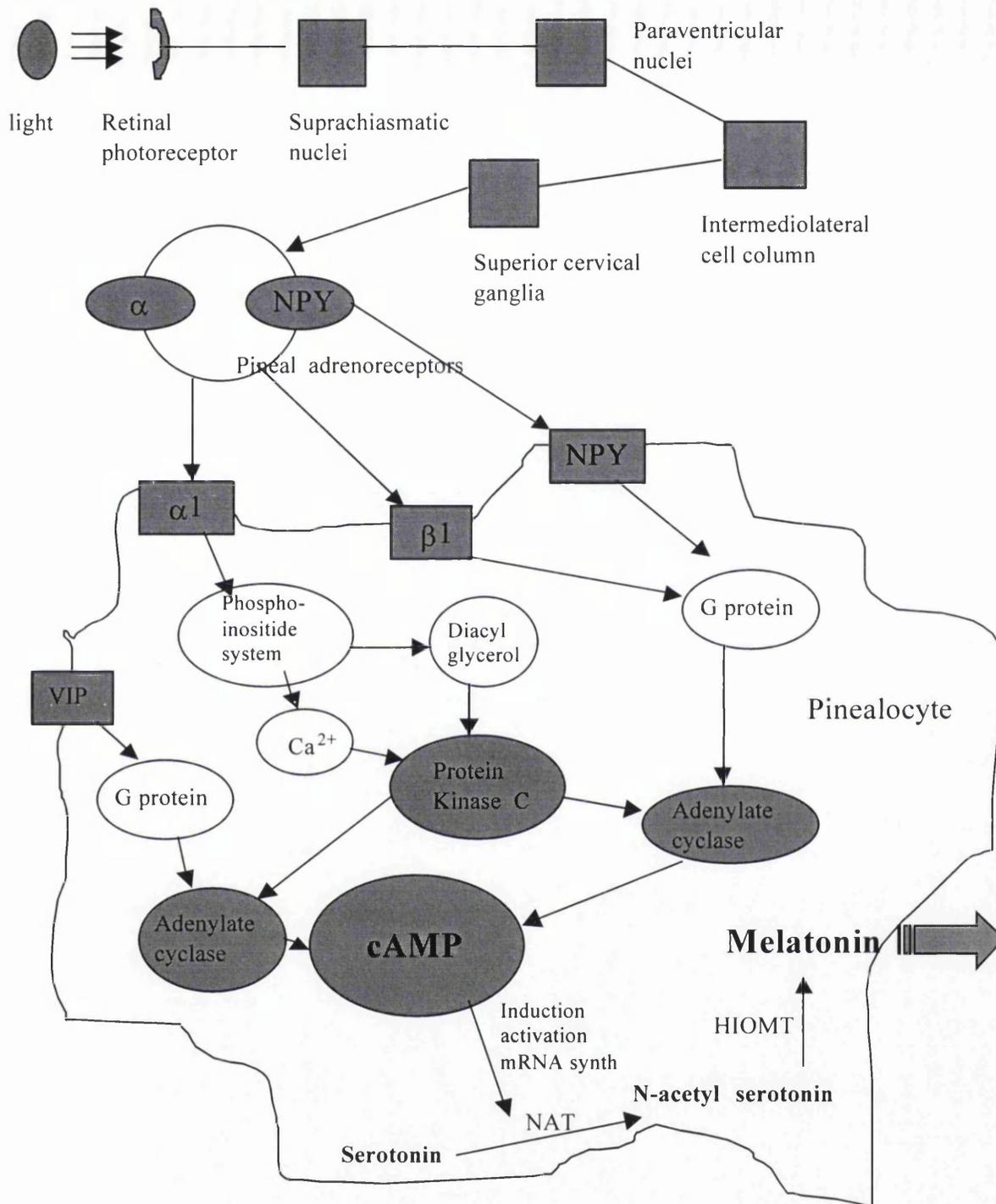
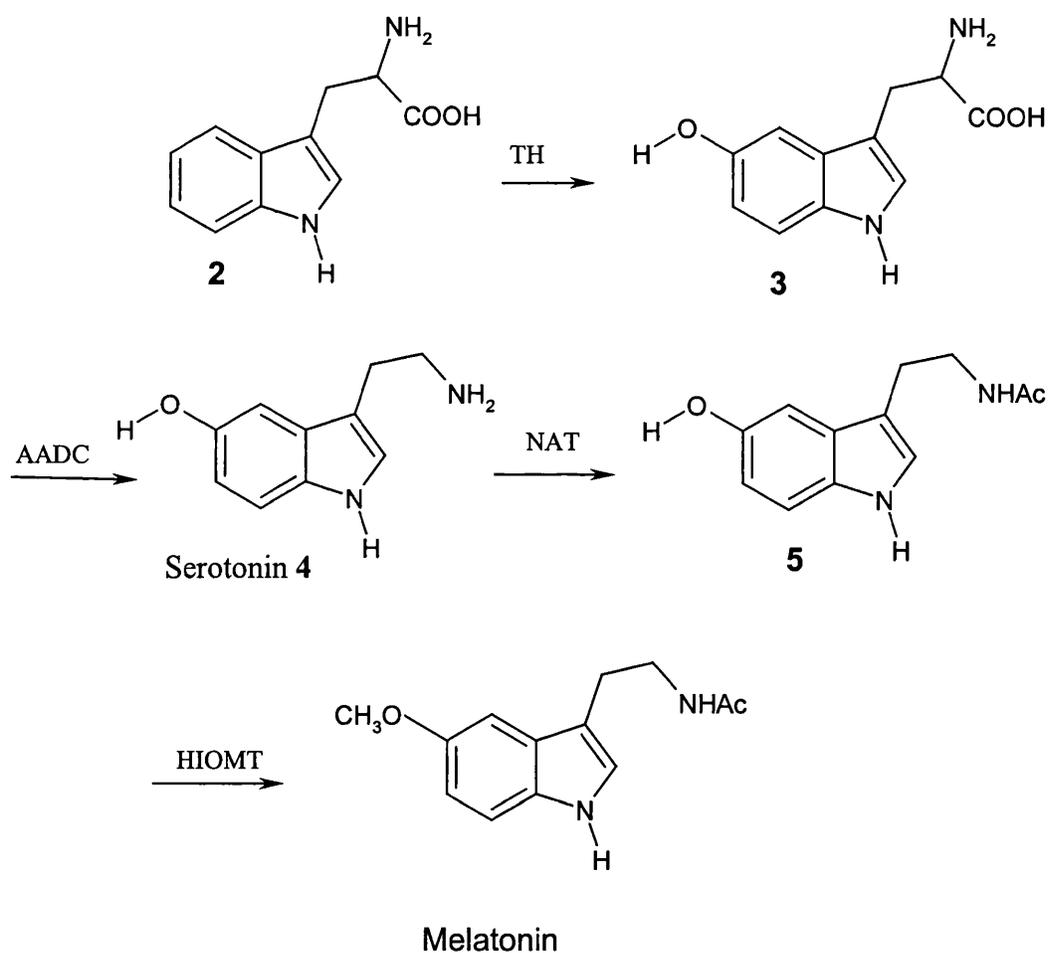


Figure 2 Illustration of some possible pathways for the transduction of environmental light cues.

of the blood indicating that a storage mechanism is operating.⁴⁸ The enzyme itself, a 30 Kda protein, is present in high concentration in the pineal gland and has been reported to also catalyse the hydroxylation of phenylalanine. Night-time activity of TH has been reported to be twice that of its daytime activity,⁴⁹ but whether this rhythm actually contributes to the generation of the melatonin signal is not known. Aromatic amino acid decarboxylase (AADC) then converts this substrate (**3**) to 5-hydroxytryptamine (serotonin, **4**). At night, serotonin is converted by *N*-acetyltransferase (NAT) to *N*-acetylserotonin (**5**) and subsequently to melatonin by the enzyme hydroxyindole-O-methyltransferase (HIOMT).

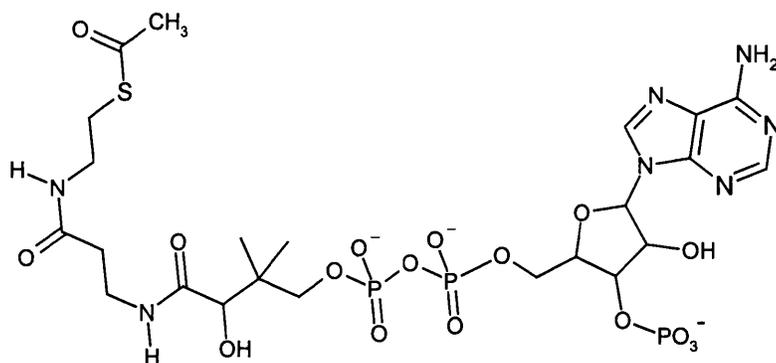


Scheme 1

Serotonin exists in the pineal gland at concentrations 100 fold greater than levels in the brain where it is an important neurotransmitter with numerous functions. In tryptophan deficient animals the brain levels of serotonin drop well in advance of any drop in pineal levels indicating that a storage mechanism is in operation and suggesting the importance of maintaining a stable melatonin production capacity.⁵⁰

Acylation by NAT and methylation by HIOMT are rate limiting steps in the production of melatonin with the former considered to be the main determinant of melatonin production.⁵¹

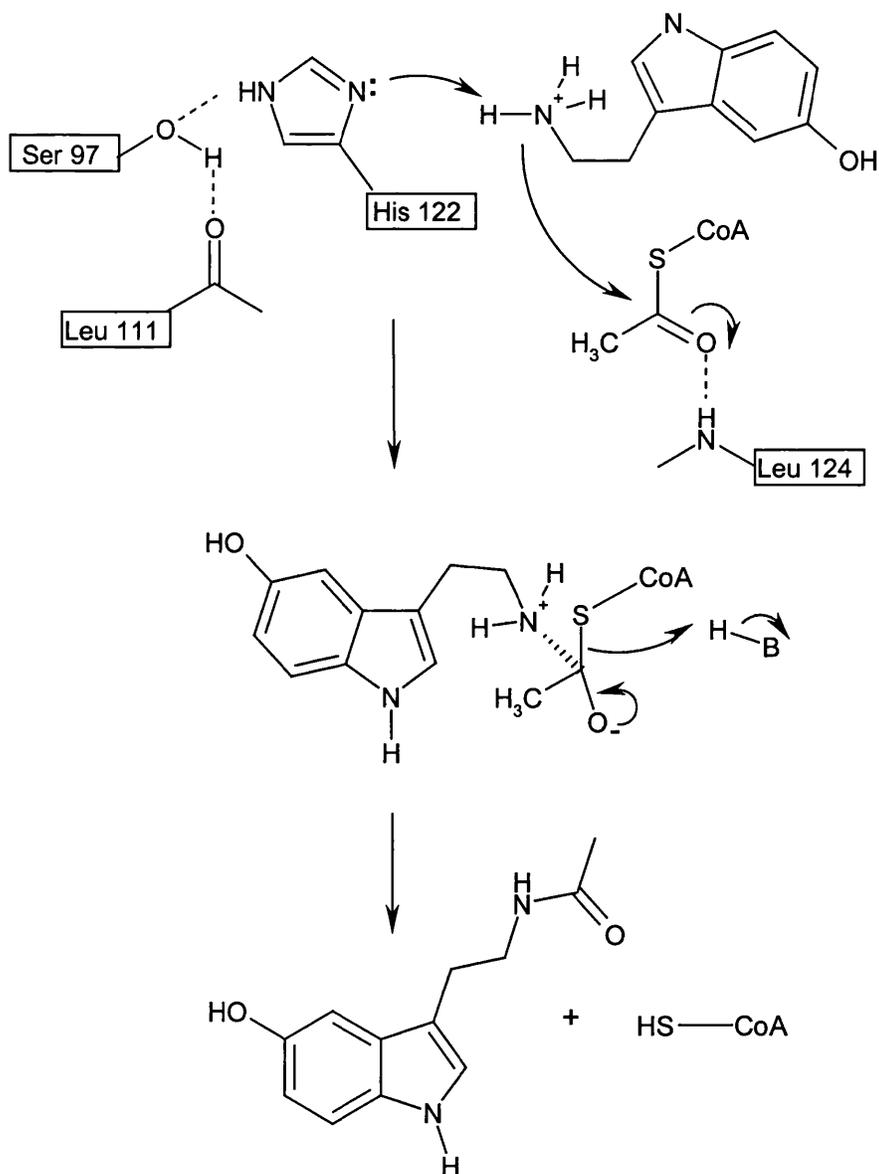
The structure of serotonin *N*-acetyltransferase has recently been published by Dyda, Klein and Burgess Hickman.⁵² They have proposed a catalytic mechanism for acetylation, which requires acetyl coenzyme A (6) as a co-factor, and involves two conserved histidine residues in close proximity in the active site.



6

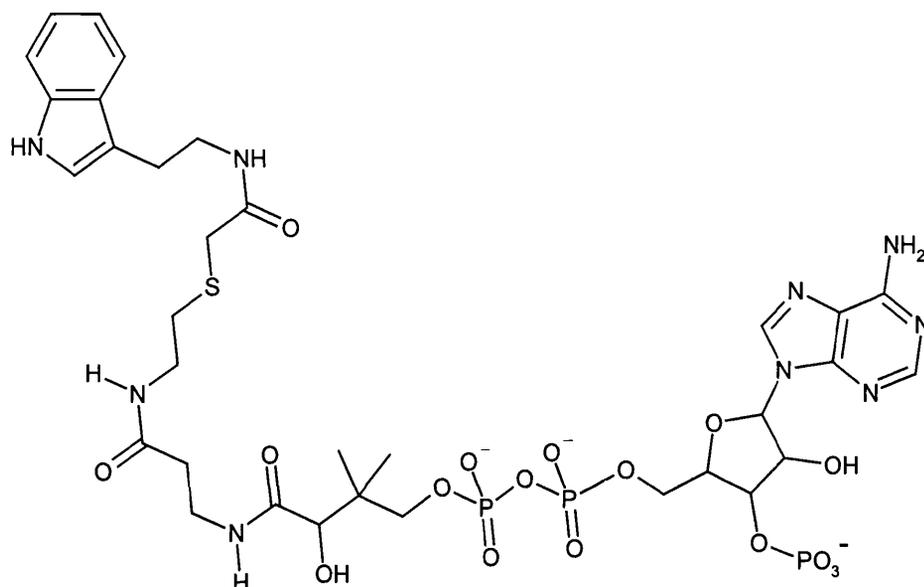
The enzyme is a globular protein consisting of an eight stranded β sheet flanked by five α helices, and is unusual in having a deeply buried binding site for the acyl CoA and arylalkylamine substrate. The aromatic amine is funnelled towards the interior of the protein by a ring of hydrophobic residues, and the transformation is thought to proceed by the initial binding of acetyl CoA, followed by serotonin. One of the two conserved histidines in the active site is

held by a network of hydrogen bonds that maintains the imidazole ring in a deprotonated form. The deprotonated imidazole can act as a general base in an initial deprotonation step and, subsequently, as a general acid catalyst to protonate the incipient thiolate anion of CoA formed in the critical transfer step. After acetyl transfer, *N*-acetylserotonin is released, and CoA subsequently diffuses out of the active site (scheme 2).



Scheme 2

This information was rapidly put to use by Khalil and Cole, who synthesised the biosubstrate analogue (7) by joining the CoA fragment with tryptamine.⁵³



7

They hypothesised that this molecule would be recognised by the active site of the enzyme but, since the acetylation could not be carried out, it should act as an enzyme inhibitor. This proved to be the case *in vitro* ($IC_{50} = 159$ nM) and they are currently attempting the synthesis of less polar analogues for *in vivo* analysis. Nocturnal NAT activity in the rat is thirty times that of the daytime activity and in short photoperiods (winter) NAT is high for a long time and in long photoperiods (summer) NAT levels are high for a short time. In this way the length of the night is measured and translated into a chemical signal via melatonin secretion (Figure 3). Nocturnal NAT activity is extremely sensitive, as exposure to light at night causes a decrease in NAT activity with a half-life of approximately 3 minutes.⁵⁴ Light, by influencing SCN neuronal output, suppresses NAT activity and melatonin secretion in a dose dependent fashion.⁵⁵

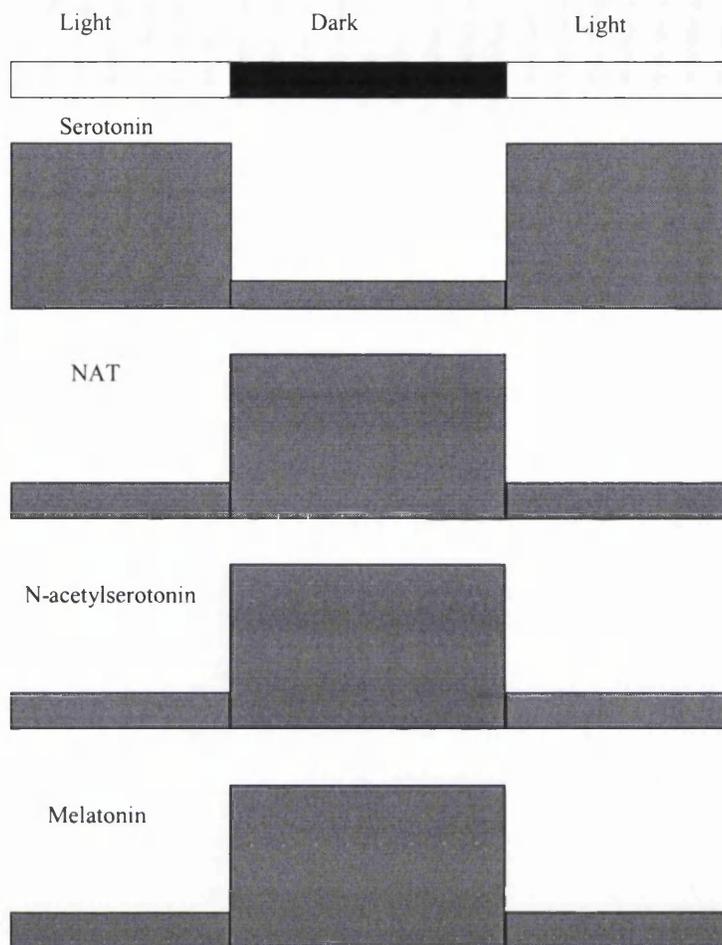


Figure 3. Correlation of melatonin and its biosynthetic precursors with photoperiod.

The final enzyme in the cascade is HIOMT which has been isolated from the pineal gland of several species and shown to be composed of two identical monomer units of approximately 38 kDa.⁵⁶ HIOMT in the pineal gland does not appear to possess an intrinsic diurnal rhythm, and can O-methylate a range of hydroxyindoles. However, *N*-acetylserotonin has a 10-20 fold greater affinity for the enzyme than the other substrates, and is therefore presumed to be the natural substrate *in vivo*.⁵⁷

1.4 Regulation of melatonin biosynthesis

Many receptors types continue to be identified in pinealocyte membranes, and the means by which melatonin synthesis is regulated are not known. Oxenkrug and co-workers⁵⁸ have reported a circadian rhythm of β_1 adrenergic receptor expression on human pinealocytes whose peak values are reached between 16.00 hr and 20.00 hr. The general result of stimulation of these receptors is an increase in cAMP and induction of enzyme synthesis. This is followed by an increase in serotonin and *N*-acetylserotonin which reach maximal values between 20.00 hr and 24.00 hr, and a rise in measured plasma melatonin. This level peaks between 24.00 hr. and 04.00 hr. with measured serum concentrations of 30-150 pg mL^{-1} compared to daytime levels of less than 5 pg mL^{-1} .⁵⁹

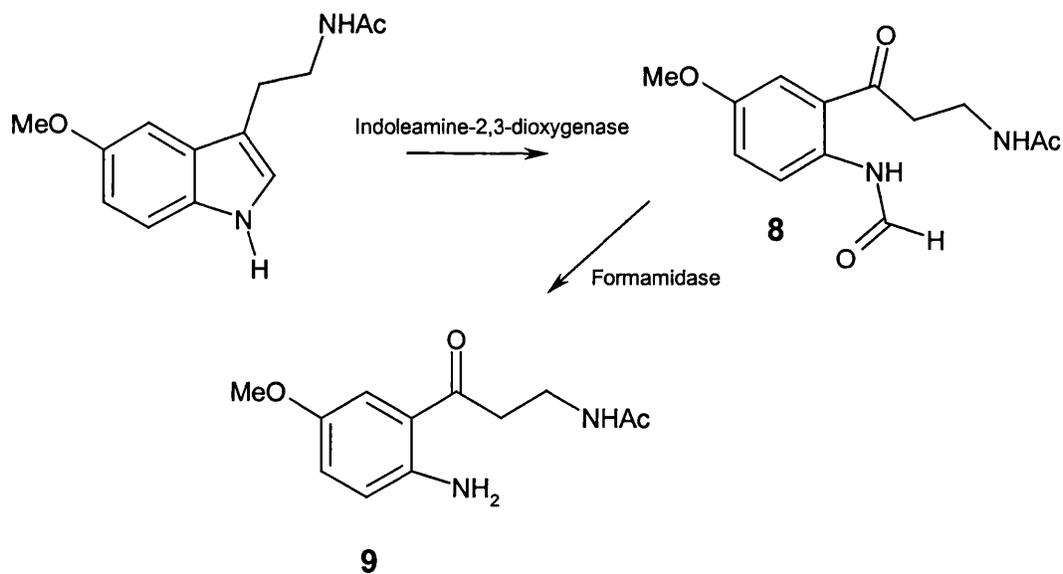
Melatonin secretion is rapidly suppressed if the subject is exposed to light during the dark period. Minimal suppressive effects of light are observed with full spectrum intensities of 200-300 lux, whereas complete suppression of melatonin synthesis is obtained with light intensities above 2000-2500 lux. The response to light is rapid, and only 15 min of bright light exposure is necessary to shut down melatonin production completely. Removal of the light source is however, sufficient to quickly restore pre-exposure levels. The SCN contains a high density of melatonin receptors which may provide feedback on circulating levels of the hormone. The potent cAMP-induced gene transcription repressor ICER (Inducible cAMP early repressor) is also activated in conjunction with NAT, and represents a mechanism that limits the nocturnal production of melatonin by effectively switching off enzyme synthesis.⁵¹

1.5 Melatonin metabolism

Once produced, melatonin is rapidly released into the bloodstream *via* the capillaries serving the pineal gland. The molecule is not stored, and its small size and lipophilicity aid passive diffusion from the gland to the bloodstream, so that no specialised transport mechanism seems to be required. Some evidence indicates that the levels of melatonin in the cerebrospinal fluid (CSF) are higher than those in the blood.⁶⁰ In the CSF melatonin is not bound to proteins, whereas in blood 70% of it is bound to albumin.⁶¹ In human blood the half life of melatonin is 30-40 minutes,⁶² largely due to its massive metabolism in the liver where 90% is C6 hydroxylated on the first pass. The hydroxylated metabolite is then excreted as the water soluble sulphate or glucuronide conjugates.⁶³

A second metabolic pathway, which occurs largely in brain tissue, is *via* oxidative cleavage of the indole C2-C3 bond by indoleamine-2,3-dioxygenase (Scheme 3).⁶⁴

This enzyme pathway is described in an excellent review by Botting.⁶⁵



Scheme 3.

The enzyme has a fairly broad specificity and will accept as substrates D- and L-tryptophan, melatonin, tryptamine, 5-hydroxytryptamine and 5-hydroxytryptophan, together with a number of others.⁶⁶ The immediate product from the oxidation of melatonin is the *N*-formylkynurenine analogue (8), which is rapidly hydrolysed to the methoxy-kynurenine (9) by kynurenine formamidase. Kynurenine is at a branch point in the metabolic pathway and can be metabolised by several different routes. Kynurenines have themselves been reported as having potent physiological activity and it is possible that some of the effects of melatonin could in fact be due to its metabolites.⁶⁷

The main site of melatonin synthesis outside of the pineal gland is in the retina, and its synthesis in this tissue also shows a daily rhythm.⁶⁸ In the retina it appears that melatonin functions only locally, as the circulating blood plasma rhythm is abolished by pinealectomy.⁶⁹ A probable reason for this is the high rate of catabolism by the enzyme aryl-acylamidase which converts melatonin to 5-methoxytryptamine.⁷⁰

1.6. Melatonin Receptor Distribution

A number of tissues display high affinity melatonin binding sites and in vertebrates the species specific distribution pattern is a notable feature. In most mammals high concentrations of receptors can be found in the SCN where they probably mediate circadian activities. Several other areas of the brain are enriched with melatonin receptors, such as the hypothalamus of photoperiodic rodents where they may mediate the regulation of reproductive functions.⁷¹ A major receptor concentration is found in the pars tuberalis of the pituitary gland which has a role in seasonal behaviour. Receptors in the retinae may mediate the effects of melatonin on retinal physiology.⁷² A wide range of peripheral melatonin binding sites have been reported, including the thymus gland, where it may have a modulatory role on aspects of the immune system.⁷³ The

identification of receptors in several arterial locations such as the basilar, caudal, carotid and cerebral arteries⁷⁴ has led to suggestions of a cardiovascular role for melatonin. In rodents, melatonin appears to influence thermoregulation by an effect on vascular tone of the caudal arteries.⁷⁵

Kopin *et al.* reported in 1961⁷⁶ that exogenous melatonin was sequestered by brain tissue from the general circulation and the sites of uptake were narrowed down to tissues of the hypothalamus and midbrain by Anton-Tay and Wurtman in 1969, using tritiated (³H) melatonin.⁷⁷ Unfortunately, as a pharmacological tool (³H) melatonin left much to be desired, due in large part to its relatively low specific activity and instability. The studies were of limited value, often due to problems with reproducibility, although several tissues were shown to contain low affinity (³H) melatonin binding sites.⁷⁸

The development of 2-(¹²⁵I)-melatonin, first reported by Vakkuri and co-workers in 1984, has great advantages over its tritiated counterpart.⁷⁹ While retaining the biological activity of melatonin, its much greater specific activity improves the sensitivity of assays to the degree that picomolar 2-(¹²⁵I)-melatonin binding sites are now regularly characterised as opposed to those in the 10-700 nanomolar range assayed by (³H) melatonin. 2-(¹²⁵I)-melatonin is a strong emitter of both β and γ radiation and the power of this ligand for identifying receptor binding sites is greatest when combined with the technique of autoradiography.

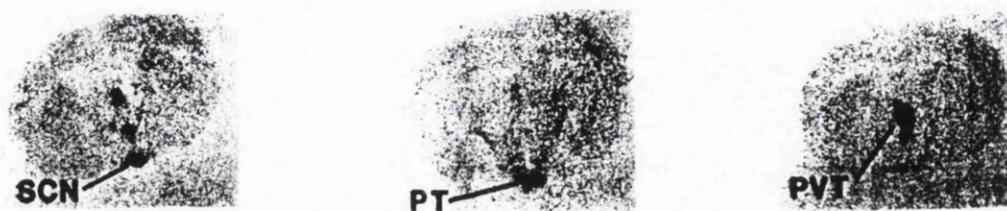
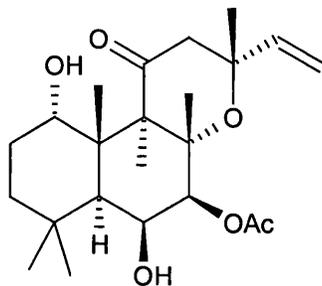


Figure 4 Autoradiographs showing melatonin receptor distribution in Hamster brain.⁸⁰

Historically, three main tissues have proved popular as models for mechanistic studies on melatonin and its analogues. The pars tuberalis (PT) of the ovine pituitary has been a widely used model for quantitative comparison of melatonin analogue potency. The measured response in this tissue is usually the inhibition of forskolin induced cAMP synthesis. Forskolin (**10**) is a diterpenoid isolated from *Coleus forskohlii* which has been shown to stimulate levels of cAMP and protein kinase A.⁸¹



10

Whilst this has proved a good model for comparing analogue potency there is debate about the mechanism by which this occurs. Evidence is needed to demonstrate that the cAMP reduction is a direct consequence of G protein receptor mediated adenylate cyclase inhibition. Some researchers have suggested an indirect mechanism, possibly via a modulation of intracellular K^+ levels.⁸²

A second popular tissue amongst melatonin researchers has been the *pars distalis* (PD) of the neonatal rat. This is a distinct area of tissue adjoining the PT in the pituitary body, which is largely involved in the release of leutenising hormone (LH). LH is a glycoprotein comprised of an 89 amino acid residue α chain and an 115 amino acid β chain cross-linked by hydrogen bonds and disulphide bridges, with 3 carbohydrate chains attached at specific sites. It is an important gonadotroph whose release is stimulated by leutenising hormone releasing hormone (LHRH), itself a decapeptide (**11**). Melatonin and its analogues have

however,⁸⁸ and this serves to emphasise the tissue-specific nature of melatonin receptor signaling.

1.7 Receptor classification

Initial attempts to characterise the receptors identified in the growing number of tissues focussed on demonstrating the presence of a high affinity binding site for 2-(¹²⁵I)-iodomelatonin. Original studies suggested the existence of two subtypes based on different binding affinity for 2-(¹²⁵I)-iodomelatonin and on different rank order of affinity for a conventional set of ligands.⁸⁹ One receptor population, originally referred to as ML₁, had binding affinity in the 10-300 pM range and a rank order 2-iodomelatonin (IMel) > melatonin (Mel) >> *N*-acetylserotonin (NAS) >> Serotonin (5-HT). A second population had lower affinity for 2-(¹²⁵I)-iodomelatonin (1-10 nM), and a rank order of Imel > NAS > MLT >> 5-HT.

It is important to have a clear correlation between binding affinity and biological activity for comparative structure activity relationship studies (SAR). A pharmacological potency ranking for inhibition of 2-(¹²⁵I)-melatonin binding in various tissues has been established in which certain assays appear to correlate well. For example, there appears to be good correlation between binding affinity in chick brain and biological potency in *Xenopus* pigment aggregation studies. The correlation is particularly good for retinal sites as illustrated by comparison of affinity and activity data in table 1. Competitive binding activity with 2-(¹²⁵I)-melatonin was measured in the retina and compared with the ability to inhibit the [³H] dopamine response in the same tissue.⁹⁰

In the last few years several melatonin receptor clones have been isolated from various vertebrate tissues by Reppert's group and others. The first receptor was cloned from *Xenopus* dermal melanophores (Figure 5) by Reppert *et al.* in 1994⁹¹ and was soon followed by cloned receptors from several mammals, including humans.⁹²

Binding Assay (retina)

Functional Assay

Compound	Potency ranking	Affinity (K _i nM)	Potency ranking	Affinity (IC ₅₀ nM)
2-iodomelatonin	1	2.5	1	0.1
6-chloromelatonin	2	4	2	0.5
melatonin	3	6.3	3	1
6-hydroxymelatonin	4	74	4	30
6-methoxymelatonin	5	460	5	100
5-methoxytryptamine	8	4600	6	200
<i>N</i> -acetylserotonin	7	3000	7	300
<i>N</i> -acetyltryptamine	6	1600	8	> 1000
Serotonin	9	> 10000	9	> 10000
5-methoxy tryptophol	10	46400	10	> 10000

Table 1. Correlation of binding and inhibition of [³H] dopamine release for a series of melatonin analogues.⁹⁰

The identified receptors seem to belong to the guanine nucleotide binding protein (G protein) coupled receptor superfamily. As is usual for this family of receptors, GTP and its analogues can convert the high affinity state (~ 40 pM) to a low affinity state (~ 400 pM) in which the melatonin binding site and the G protein have uncoupled. In addition, the effects of melatonin can be inhibited by pre-treatment of the tissue with pertussis toxin, indicating the intermediacy of a G protein.⁹³

All G protein coupled receptors are thought to have a fundamental structural similarity, mainly because they have a significant degree of sequence similarity. The structure is generally accepted to consist of seven transmembrane helices interposed by intracellular and extracellular loops, with the N terminus occupying the extracellular space, and the C-terminus, the cell cytoplasm (figure 5).

The melatonin receptors so far cloned, appear to be distinct from the other known

classes of receptors, but have some structural features in common with certain amine and peptide receptors. These include cysteine residues in the extracellular loops which are thought to form a sulfur bridge, proline residues in the transmembrane domains which induce turns in the α helices and influence conformation, and an NAXXY motif in transmembrane 7 that is present in most other known 7TM receptors as NPXXY (see appendix, table 17 for nomenclature). Despite the close chemical similarities between melatonin and serotonin, the amino acid sequence analysis of the melatonin receptor bears little homology with those of serotonin.

The cloning of these melatonin receptors led to a new sequence based classification system which was divided into the designations Mel_{1a}, Mel_{1b}, and Mel_{1c}. The first clone was produced from a *Xenopus* cDNA library and a mammalian cell expression strategy.⁹² Based on this sequence and using a polymerase chain reaction (PCR) approach, a high affinity melatonin receptor was subsequently cloned from several mammals including humans. These mammalian receptors were designated Mel_{1a} and showed more than 80% sequence homology with each other and were greater than 60% homologous with the frog receptor at the amino acid level.

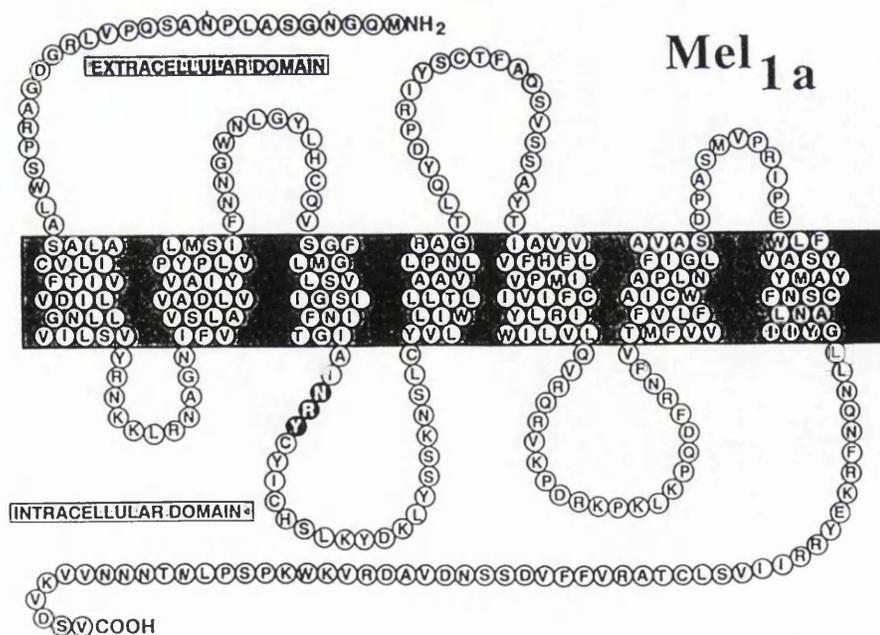


Figure 5 Membrane topology of the human Mel_{1a} receptor.⁹²

A third melatonin receptor subtype exists and has been cloned from chickens but has not yet been identified from mammalian tissues.⁹⁵ Designated Mel_{1c}, it has similar pharmacological and functional properties to mammalian Mel_{1a} and Mel_{1b} receptors and is 80% identical to the frog receptor at the amino acid level.

The pharmacological profile of these three cloned receptor subtypes in terms of ranked binding affinity for a set of ligands appears to be the very similar.⁹⁶ 6-Chloromelatonin, however, has been reported to have equal ranking with melatonin for the Mel_{1b} receptor as illustrated in Figure 7, and slightly lower affinity for the others.

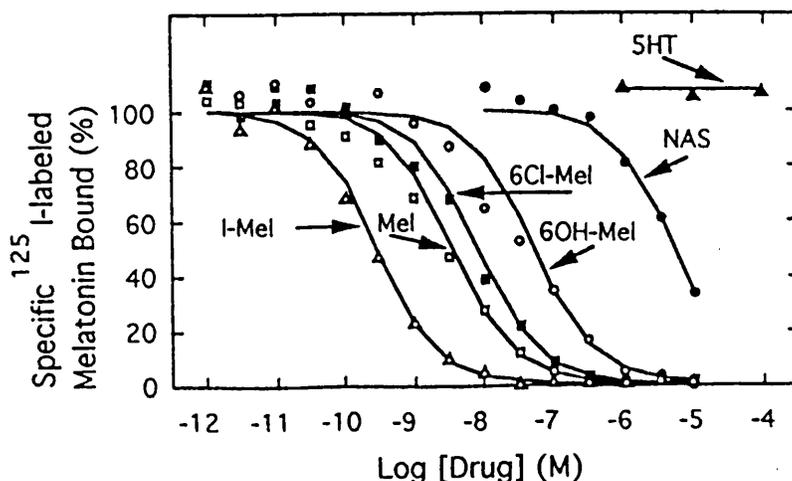


Figure 7. Competition by various ligands for 2-(¹²⁵I)-melatonin binding in chick brain homogenates. Cells were incubated with 100 pM 2-(¹²⁵I)-melatonin and various concentrations of the ligands.⁹⁶

Recently, melatonin was shown to bind with nanomolar affinity to a cloned nuclear orphan receptor known as the retinoid Z receptor β (RZR β).^{97,98} Nuclear orphan receptors are members of a family of structurally related receptors for which no ligand has yet been identified. This melatonin related receptor is G protein coupled and unusual in having a carboxyl tail more than 300 amino acids long. While the overall sequence homology in the transmembrane domains is

55%, it does not bind to either 2-(¹²⁵I)-melatonin or (³H) melatonin. Previous studies have shown a nuclear localisation of melatonin in a number of different mammalian tissues and a nuclear function has been proposed by several researchers.⁹⁹ The possibility that another structurally related compound is the true physiological ligand cannot be excluded, however. The distribution of this receptor is largely confined to the CNS with particularly high concentrations in the pineal gland. More evidence, however, is required before it can be accepted as a genuine melatonin receptor (Figure 8).

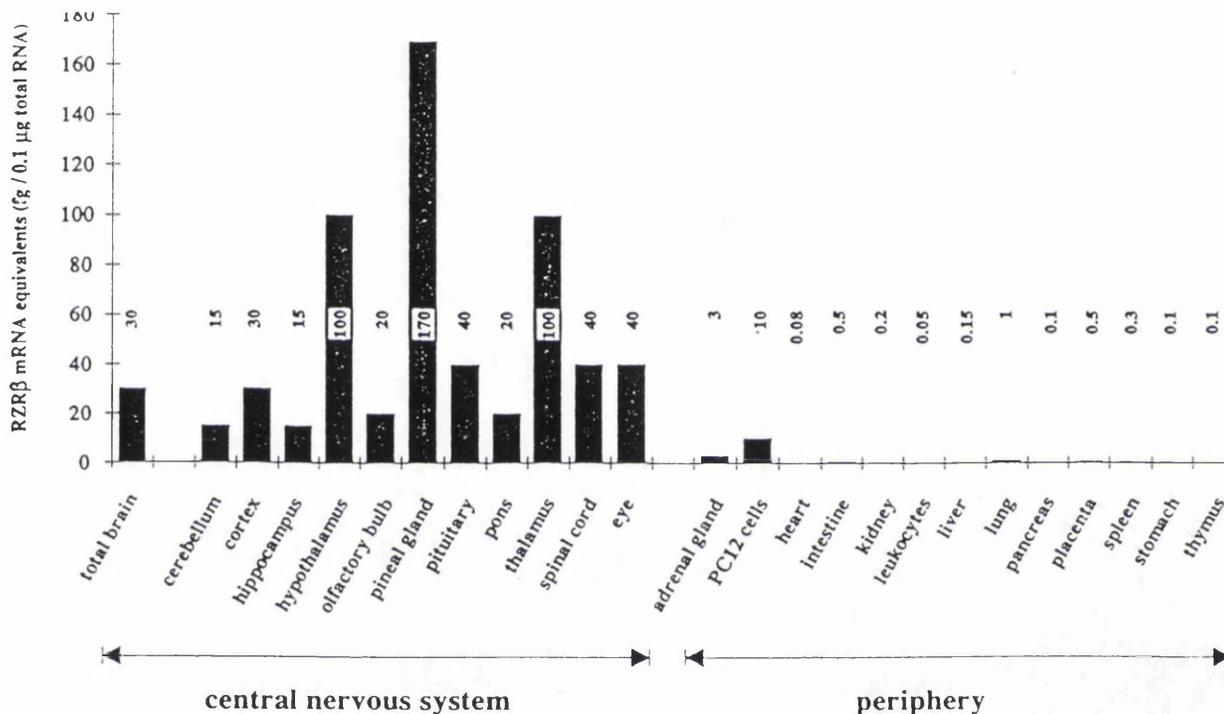


Figure 8 Tissue distribution of the orphan retinoid receptor β .⁹⁹

The cloning and molecular characterisation of melatonin receptors has begun to open new avenues for the exploration all aspects of melatonin action. While the receptor proteins are now beginning to be produced in laboratory expression systems, the data regarding precise localisation of these receptors and how this distribution varies with species is limited. With the increasing number of receptor clones being reported there is a need for a classification nomenclature system. A

system of nomenclature and classification of melatonin receptors was recently approved by the International Union of Pharmacology (IUP). Three classifications have been designated at present:

MT receptor subtype with known molecular structure and function

mt receptor subtype with known molecular structure and but undefined function.

MT receptor subtype with known functional pharmacology but unknown molecular structure.

Thus, the previously named Mel_{1b} receptor has a defined function (inhibition of dopamine release) and has been characterised in a native tissue (retina) so is designated **MT₂**. The old Mel_{1a} receptor subtype cloned by Reppert *et al.* has a known structure but functional characteristics in native tissue still need to be proven and is therefore at present designated as **mt₁**.

With a recognised classification system now in place, the need to overcome the general lack of selectivity observed with the currently available ligand set poses a major challenge for the future.

It is a general assumption that the biological action of any hormone is dependent on the existence of highly specific receptor proteins in the target tissue where the hormone exerts its effect. The presence of melatonin receptors certainly indicates a target but does not preclude other more basic effects of the hormone in cells devoid of membrane based receptors. This is particularly true for melatonin whose small size and lipophilic character enable it to readily penetrate the cell and thus bind to both extracellular and intracellular targets. One effect of melatonin which has received attention is the intracellular binding to calmodulin which may modulate tubulin association and thereby influence cytoskeletal and mitotic cellular functions.¹⁰⁰ Melatonin has also been shown to be a potent hydroxyl radical scavenger which in the cell nucleus may help to protect DNA from the destructive effect of these cytotoxic agents.¹⁰¹

1.8 Circadian Rhythms

Rhythms are ubiquitous in the mammalian CNS. They can range from brain wave signals spanning a fraction of a second through to events such as the return of migrating swallows to Capistrano in California which has happened on the 19th March every year, with only two exceptions, in 200 years of records. The pineal gland has a major influence on the control of rhythmic adaptations to daily and seasonal cycles (circadian rhythms). Through the melatonin signal it can provide both time of day information (amplitude of serum levels) and time of year information (duration of elevated serum levels).

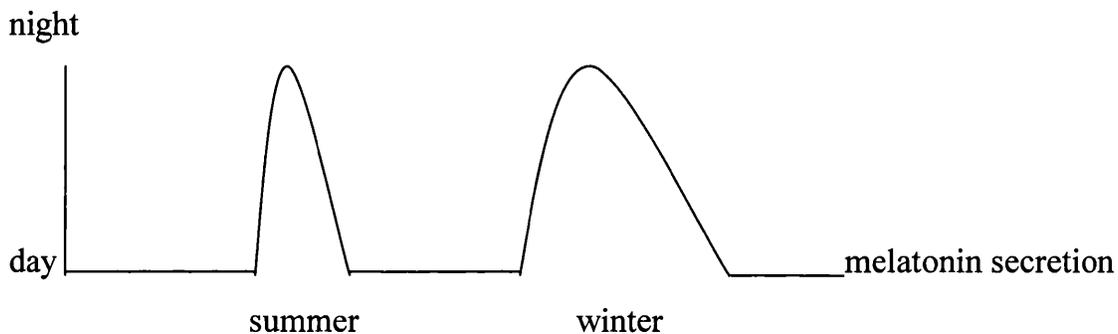


Figure 9. Effect of photoperiod on amplitude (y axis) and length of melatonin secretion (x axis).

The circadian system has many elements, of which the main components are the SCN, the pineal gland, and the retinal photoreceptors. The major oscillator component of the biological clock, from which the other rhythms derive, is probably located in the cells of the SCN and it is usually assumed that melatonin exerts its zeitgeber (time giving) effect by acting on these cells. In reptiles and birds the pineal gland itself contains sufficient oscillators to generate its own circadian rhythm of melatonin production but this ability has been lost by mammals who require external signals from the SCN.¹⁰² In these species, disruption of any element in the neural pathway between SCN and pineal gland

results in loss of the rhythmic melatonin signal. Importantly, the intrinsic rhythm of the SCN, known as the tau period, is not 24 hours in duration but in humans, for example, is closer to 25 hours.¹⁰³ As has been noted previously there is a high density of melatonin receptors in the SCN and the ability of melatonin to reset, or entrain, the biological clock by synchronising SCN output has been demonstrated in vitro.¹⁰⁴ Application of melatonin to rodent SCN slices has been shown to phase advance the peak in neuronal firing rate.¹⁰⁵ The mechanism by which this occurs is unknown.

As a consequence of the endogenous tau period, the absence of a mechanism for re-setting this clock on a daily basis *via* signals from the photoreceptors would result in melatonin production peaking approximately 1 hour later on consecutive days, a situation known as 'free running'. In multicellular animals circadian rhythms are often measured in behaviours such as eating, drinking or locomotor activity. These behaviours are usually measured digitally and displayed in the form of an actogram as illustrated below (Figure 10).

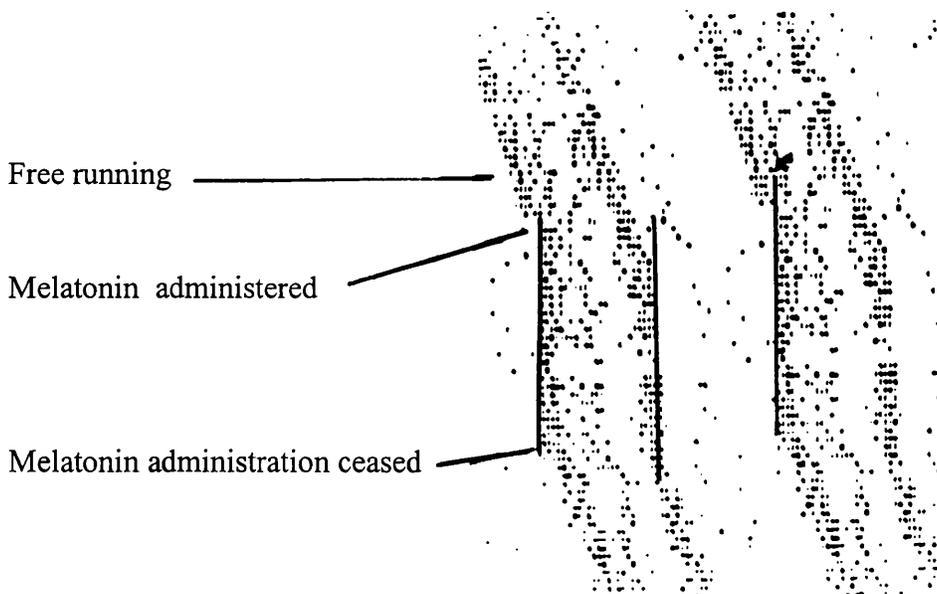


Figure 10. Effects of daily melatonin injections on circadian rhythm of wheel running in rats kept in total darkness.¹⁰⁶

Daily injections of melatonin were shown to entrain wheel running rhythms in rodents and it was after this observation that efforts began in earnest to elucidate the role of melatonin in the modulation of human circadian rhythms. In humans it is not easy to determine the circadian phase position by activity levels. A currently used protocol for assessing the circadian phase position is to assay blood drawn under dim light and determine the point of melatonin onset when levels begin to rise. In most individuals this occurs at circadian time (CT) 14, that is, 14 hours after the 'lights on' point.¹⁰⁷

It is generally accepted that melatonin is the main mechanism by which the biological clock is set each day. Melatonin, or a melatonin agonist, may therefore provide a treatment for disorders associated with disruptions in circadian rhythms such as those resulting from jet lag, blindness, shift work, or delayed/advanced sleep-phase syndromes.¹⁰⁸ Given the involvement of melatonin in the modulation of circadian rhythms, a role in the depressive seasonal affective disorder (SAD) has been postulated. Administration of melatonin has been found to exacerbate the symptoms associated with SAD, while mimicking longer photoperiods by exposure to bright light reverses these symptoms.¹⁰⁹

The following section briefly looks at some of the more active areas of current melatonin research as extracted from literature reports. Because of the ubiquity of melatonin in biological tissue and the impact of circadian rhythms on many aspects of physiology much of the data presented is open to alternative interpretation and is, in some cases, vigorously debated. Some of the relationships which sparked off an area of investigation are correlative and this does not necessarily mean that a causal relationship will eventually be proved. In addition, evidence from studies which have used pharmacological concentrations of drug, never likely to be experienced by cells under normal physiological conditions, must be treated with caution.

1.9 Melatonin and Reproduction

There is extensive literature on the seasonality of reproductive functions and it is generally accepted that melatonin is the hormone responsible for synchronising these seasonal adaptations with the environment. Seasonal mammals are often divided into those who slowly shut down reproductive function in response to a shortening photoperiod (long day breeders) and those who use the same signal to initiate reproductive capability or (short day breeders). For most vertebrates the timing of birth is critical to the young animals chance of survival with spring obviously being the most favourable time. Long day breeders are typically birds or small mammals that mate in spring, produce offspring in the summer and shut down reproductive function in the autumn. Short day breeders are usually larger mammals who use a long gestation strategy and mate in the autumn. In both cases the young are produced at the most favourable time of year.

It is thought that the melatonin receptors which mediate the changes in reproductive physiology are likely to be those situated on the *pars tuberalis* of the pituitary gland. This gland has a major influence on the secretion of many hormones associated with reproductive physiology. By controlling the release of gonadotropins melatonin can regulate the functional status of the gonads and influence reproductive capability in a seasonal manner.¹¹⁰ Studies on several species have shown that administering melatonin to pinealectomised animals duplicates the effect of the photoperiod on the reproductive system.¹¹¹ This has led to the commercial use of melatonin in short day breeders such as sheep, mink and deer to encourage them to breed out of season.¹¹² To mimic the effect of summer by the administration of a melatonin antagonist should in theory enable the same result to be achieved in long day breeders although this has not been reported. An interesting adjunct to the use of melatonin in this way, is the fact that it has not been possible to maintain reproductive status indefinitely through the use of melatonin. Thus in hamsters administration of melatonin induces

gonadal regression for 3-4 months but then spontaneous recrudescence of the gonads occurs even in the presence of continued melatonin treatment.¹¹³ This emphasises the role of melatonin in the specific timing of reproductive transitions. The mechanisms that regulate reproductive changes may be classified under two categories. The first category is that of 'activational' mechanisms because they effect a direct gonadal change as a result of activation e.g. the gonadotropins. The second category of mechanisms are responsible for timing and synchronising of reproductive changes and melatonin clearly has a major effect in this area. However, the relationship between the pineal gland and the reproductive system is complex and a direct action of melatonin has not been ruled out. A direct 'activational' effect of melatonin on the gonads is less likely as, despite the widespread presence of receptors on these tissues, the same signal can produce opposing effects in different species and under different physiological conditions. Humans have been reported to show modest seasonal variations in reproductive function based on physiological measures¹¹⁴ but there is no real evidence that endogenous melatonin regulates reproduction. Correlation of melatonin with the serum levels of several hormones have been noted but the physiological significance is unclear. There is fairly strong evidence, however, that exogenous melatonin can influence reproductive functions. An example is the night-time amplification of LH during the follicular phase of the menstrual cycle which can be reproduced during the day by administration of melatonin.¹¹⁵ Cagnacci has suggested that amplification of LH levels beyond a critical time in the 24 hour period can have deleterious effects on ovulation.¹¹⁶ This offers the possibility that the LH surge associated with long nights may influence reproduction and suggest an endogenous effect. What is certain though is that high doses of melatonin given on the day of prestrus blocks ovulation in many species.¹¹⁷ Several patents for melatonin analogues have mentioned ovulatory inhibition as a pharmaceutical indication.

1.10 Melatonin and Sleep

Melatonin became linked with sleep almost immediately after its identification in the 1960's, and the realisation that melatonin secretion in humans is concurrent with nocturnal sleep. Research on the link between the two has been encouraged by the hypothesis that many psychiatric and sleep disorders might be examples of disturbed circadian rhythms¹¹⁸ and several studies have shown decreased melatonin levels in individuals presenting with insomnia.¹¹⁹ Sleep disturbance is a problem that everyone will experience at some point, if only transiently, and a drug which addresses this effectively has a massive potential market.

The age related insomnia associated with the elderly population has been correlated with a parallel reduction in melatonin levels and it has been reported that insomniacs in this age group tend to have lower absolute serum levels of the pineal hormone than good sleepers of the same age.¹²⁰ A high incidence of sleep disturbance amongst the blind population is thought to be a consequence of the absence of a mechanism for photic entrainment. This results in a 'free running' situation in which the individual gradually loses synchronisation with the environment. The use of melatonin to achieve the daily re-setting of the biological clock has potential in this clinical application.

Many studies have shown that an increase in serum melatonin level as a result of exogenous administration is associated with sedation as well as increased sleep efficiency.^{121,122} Many of these early studies used doses in the pharmacological range but, more recently, doses which produce physiological peak serum levels have been shown to decrease sleep onset latency, temperature, and vigilance in human volunteers.¹²³ The mechanism behind the hypnotic properties of melatonin are not known but the sedative effect is not blocked by flumazenil, which suggests that benzodiazepine receptors are not involved.¹²⁴ This offers the prospect of a therapy which may be free from the typical side effects associated with the classical sedative hypnotics such as barbiturates or benzodiazepines.

In humans, the sleep-wake cycle is closely associated with the circadian rhythm of melatonin secretion. Sleep architecture is complex and has been divided into several different stages. Melatonin may have influence at all of these stages but most evidence to date suggests that it has greatest impact during the initiation and consolidation steps. Under normal conditions the rise in serum melatonin level precedes the onset of sleep, is high during the sleep period and declines on waking. Because of the strong correlation between melatonin and core body temperature (CBT) many studies have used this parameter as a marker for the underlying circadian cycle of melatonin. In normal subjects the high point for the CBT occurs between 20.00 and 22.00 hours. After this there is a sudden increase in sleep propensity which co-incides with the emergence of the melatonin surge and an accompanying drop in CBT. This has been referred to as the opening of the sleep gate or the 'dissipation of the circadian drive for wakefulness'.¹²⁵ Given that the opening of this sleep gate is preceded by a period of increased arousal which is abruptly terminated by the opening of the sleep gate, Shochat *et al.* have suggested that the role of melatonin in the induction of sleep is to inhibit the wakefulness producing mechanisms.¹²⁶ Interestingly, Edgar and co-workers have investigated sleep wake cycles in SCN lesioned monkeys and have come to the conclusion that a primary site for the wakefulness regulating mechanism might be the SCN.¹²⁷ The SCN is densely populated with melatonin receptors and if the output of the SCN is responsible for maintaining wakefulness then a possible mechanism for the action of melatonin is suggested.¹²⁸

There are much fewer studies published on the relationship between melatonin and the later stages of sleep. Some of the evidence is contradictory but most studies do not suggest any strong measurable and reproducible effects of the type seen at the sleep induction stage. Exogenous melatonin does appear to have a strong effect on the EEG measurements of slow waves monitored during non REM sleep but the significance and mechanism for this is not known.¹²⁹ More studies using physiological concentrations of melatonin are necessary to establish the role of melatonin in the endogenous circadian sleep rhythm.

1.11 Melatonin and Thermoregulation

The peak of melatonin secretion in humans is roughly simultaneous with the nadir in core body temperature¹³⁰ (Figure 11). Studies in humans have shown that exogenous administration of melatonin during the day causes core body temperature to drop by 0.3-0.4 °C, and that suppression of melatonin at night enhances it to a similar degree.¹³¹ This relationship has also been confirmed in other species and melatonin is thought likely to be exerting this effect in the hypothalamus where thermoregulation is controlled.¹³² Once again, the amounts used in studies were in the pharmacological range, and one study using 0.1-0.3 mg doses which have been shown to give rise to physiological blood levels of 48 and 121 pg mL⁻¹ respectively, failed to detect a reduction of core body temperature.¹²³ Menaker has demonstrated an underlying circadian rhythm of body temperature in lizards comparable with that in mammals (1 ° to 2 °C) which is abolished by pinealectomy.¹³³ The adaptive significance of this in a reptile whose body temperature normally varies by up to 20 °C in a 24 hour period is unknown and may represent the initiation of a 'set point' for some alternative system such as the general metabolic rate.

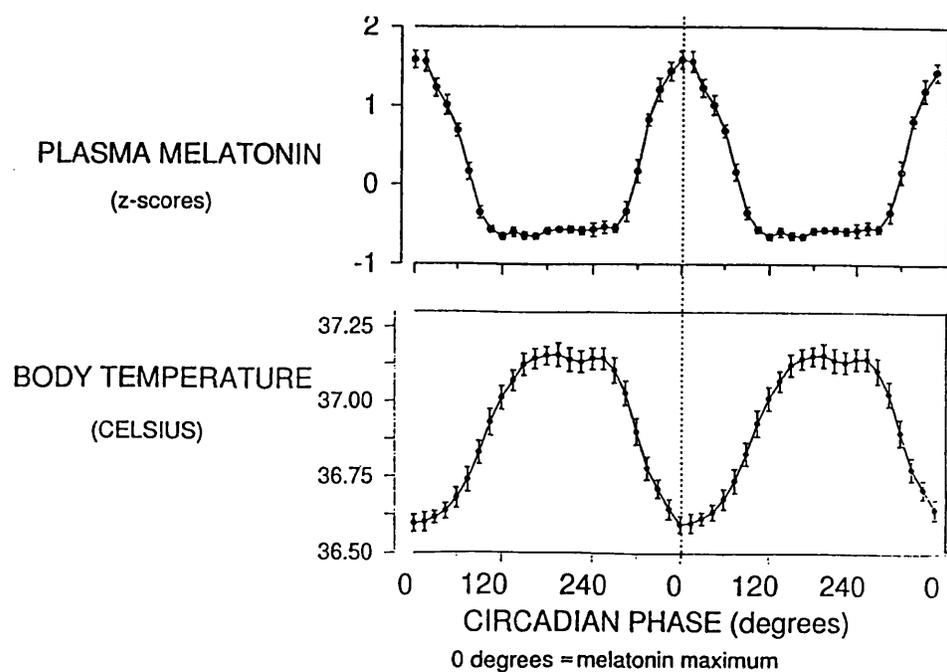


Figure 11. Endogenous circadian rhythm of core body temperature and melatonin.¹³¹

Mechanistically, the action of melatonin may be either directly on the thermoregulatory centres or by influencing the mechanisms of heat production and loss. It has been shown in rats that melatonin influences the tone of both cerebral and peripheral arteries¹³⁴ and this may directly influence the thermosensitive neurons in the hypothalamus by altering local blood flow. In the rat, melatonin administration has been shown to cause vasoconstriction in the caudal artery with a consequent reduction in heat dissipation.¹³⁵ However, the precision and reproducibility of the effect when melatonin is administered makes it unlikely that it operates by purely modifying vascular heat loss. A central mode of action is supported by detection of high affinity melatonin binding sites in the neurons of the preoptic anterior hypothalamus, the known site of thermoregulation.

1.12 Melatonin and the immune system

Day length appears to affect immune systems in many species and melatonin secretion is thought to play a part in mediating this. Nelson and Demas have published an excellent review on seasonal changes in immune function, hypothesising that a mechanism has evolved for allowing individuals to anticipate recurrent periods of immunologically challenging conditions, for example winter.¹³⁶ Their review of studies carried out in the laboratory concludes that exposure to reduced photoperiod results in an enhancement of immune function in every species so far studied, as measured by variables such as lymph tissue size and antibody levels. At present, little is known about the mechanism by which melatonin interacts with the immune system apart from in its capacity as a photoperiod transducer. Interestingly, male sex hormones whose levels show a photodependance are known to generally suppress immune function¹³⁷ so enhancement of immune function could be a result of melatonin transduced steroid hormone suppression. Effects on the female immune system are less clear

since an increase in estrogens can result in immunological stimulation or suppression under different conditions.¹³⁷ As in other areas, most of the studies in animals have used high concentrations of hormone. Spleen cells from antigen primed mice have been incubated with physiological concentrations (0.1-50 nM) of melatonin and supernatants from the cultures tested for immunological activity.¹³⁸ At 0.2-0.5 nM concentrations a marked stimulation of response was reported and the effect ascribed to melatonin induced opioid release. In general it is thought that melatonin plays a critical role, both directly and indirectly through its effects on other hormones, in mediating photoperiodic modulation of the immune system.

1.13 Melatonin as an anti-oxidant and neuroprotective agent

A potential role for melatonin in the treatment of epilepsy has been reported, although earlier studies in rodents used rather high doses.¹³⁹ A later study showed a significant reduction in frequency and severity of pentylenetetrazole induced seizures in rodents and used lower doses.¹⁴⁰ The results of this study were promising enough to initiate trials in humans.

Melatonin was recently shown to be a potent antioxidant and free radical scavenger with a greater efficiency than that of vitamin E^{141,142} and military research establishments are examining melatonin analogues for protection against the effects of radiation.¹⁴³ Once inside the cell, the hormone appears to be widely distributed, unlike most of the known antioxidants such as vitamin E (restricted to lipid cell membranes), or vitamin C (aqueous cytosol). Melatonin has been reported to be associated within the nucleus with DNA and a specific role in protecting the genetic material from free radical attack has been postulated.¹⁴⁴ These reports have sparked much hypothesising about possible roles as an anti-ageing drug. It must be emphasised, however, that there is a considerable difference between demonstrating the capacity for melatonin to sequester radicals

and demonstrating that this is a major component of its physiological role, particularly at the picomolar concentrations experienced intracellularly.

Oxidative stress as a result of free radical generation is known to be a major contributing factor to a variety of neurodegenerative pathologies. This process may be particularly important in neural tissue where oxygen consumption, and presumably free radical generation, is relatively high and low serum melatonin levels have been reported in populations with dementia.¹⁴⁵ Of particular interest was a clinical case involving the treatment of a patient with Alzheimer's disease¹⁴⁶ and a recent study on inhibition of β -fibrillogenesis by melatonin in the physiological range.¹⁴⁷ The researchers initially ascribed the results to general antioxidant properties but subsequently proposed a specific interaction between melatonin and a peptide residue which inhibits β sheet formation and amyloid fibrils. Some of the proposed interactions with this peptide involve the disruption of His-Asp salt bridges which are critical to the amyloid structure and the same residues have been suggested to determine binding with the membrane bound melatonin receptor.

1.14 Melatonin as an oncostatic agent

Blask,¹⁴⁸ and Panzer and Viljoen¹⁴⁹ have published excellent reviews on the evidence for an oncostatic action of melatonin. It is not surprising that a compound reported to have sleep promoting, anti-depressive, immunostimulatory and oxidative effects should attract attention as an oncostatic agent. Bartsch *et al.* have reviewed the studies reporting melatonin levels in unoperated cancer patients and reported a depression of melatonin secretion which is tumour size dependent.¹⁵⁰ In animals, pinealectomy was shown to enhance tumour development and this growth was arrested when melatonin was administered to the cells.¹⁴⁸ It is still a controversial area, however, as at least one study has found melatonin to stimulate tumour growth.¹⁵¹ Interpretation of clinical data on the

association between malignancies and melatonin is fraught with difficulty due to the different possible modes of action, different diseases and stage of disease, as well as individual variations in normal melatonin levels. In addition, most studies to date have involved late stage patients who have failed to respond to other therapies, which might suggest a lower chance of a positive outcome in any case.

In vitro studies using exogenous melatonin have shown an inhibition of tumour growth in several different cell types.¹⁴⁸ The greatest body of research has been carried out on estrogen receptor positive human breast cancer cells (MCF-7) where melatonin exerts its greatest anti-proliferative effect. In a study which used a range of concentrations from 10^{-5} M (pharmacological) to 10^{-15} M (sub-physiological) Hill and Blask found the greatest effect with sequential 12 hr 10^{-9} – 10^{-11} M melatonin concentration, that is, conditions which mimic the physiological rhythm of serum concentration. When these same cells were treated with fresh melatonin deficient medium the proliferation returned to previous levels.¹⁵² In contradiction to this, Bartsch has reported a melatonin free pineal extract to inhibit cancer growth,¹⁵³ while Shellard reports that the metabolite 6-hydroxymelatonin has greater cytotoxicity than melatonin itself.¹⁵⁴

Despite the often conflicting results there is a growing opinion that a dynamic interaction between the pineal gland and tumour growth exists and that it merits further research.

1.15 Toxicity

There is no evidence to suggest that melatonin has any major toxicity in the immediate term although no scientific research on the long term effects of its use have been published. There is much anecdotal evidence testifying to its safety from various sources, although some of the many effects described in the previous sections would advise caution, particularly in groups such as pregnant or lactating women. Lerner, the leader of the team who originally isolated the hormone in

1958, began trials on humans almost immediately and a patient dosed intravenously with 200 mg per day for 5 days was reported to show no signs of delayed toxicity 18 years later. It has been pointed out that this equated to the melatonin content of a million pineal glands daily.¹⁵⁵ Arendt reports the administration of 0.05 mg-10 mg doses of melatonin to over 500 individuals during the course of 16 years without noting any significant side effects apart from drowsiness.

In a study in rats it proved impossible to find an oral LD₅₀ even at doses as high as 3.2 g/kg¹⁵⁶ and in humans 250 mg melatonin taken orally 4 times daily for 25-30 days showed no discernible signs of toxicity in terms of blood pressure, pulse rate, ECG, blood count, electrolytes or liver enzymes.¹⁵⁷ The only side effect reported was that of drowsiness. If melatonin is to be used in the clinic, clearly more studies on long term toxicity need to be undertaken, and the consequences of activating receptors outside the target area would have to be addressed.

1.16 Structure-Activity Relationships of melatonin analogues

Four main tissue types have been used in the majority of structure activity relationship (SAR) studies on binding affinity, namely, whole chicken brain, *Xenopus* melanophores, ovine *pars tuberalis*, and chicken retina. Different researchers have used different assays as well as different tissues in their studies and the exact nature of the receptor subtype(s) present was not determined. This may make interpretation of trends less certain when comparing results from different groups. Against this caveat is the fact that, in general, it has not proved possible to differentiate between the native receptors in most of these tissues on the basis of the currently available ligand set and so reasonable comparisons across tissues and assays can be made.

A short time after the discovery that melatonin was the active constituent of pineal extract in the amphibian skin lightening test, Heward and Hadley published the

first examination of some melatonin analogue SAR's.¹⁵⁸ All of the early studies on melatonin and its analogues were carried out in frog or fish dermal pigment aggregation assays, and measure biological activity only, since a binding affinity assay was not available.

The demonstration by Heward and Hadley that *N*-acetyltryptamine could block the effects of melatonin in the frog dermal melanophore pigment aggregation assay, but had no intrinsic activity of its own, led to the conclusion that the *N*-acetyltryptamine moiety was the principal binding motif, whilst the 5-methoxy group was necessary to trigger a biological response. In this pharmacophore model the indole nucleus was generally viewed as 'spacer'.

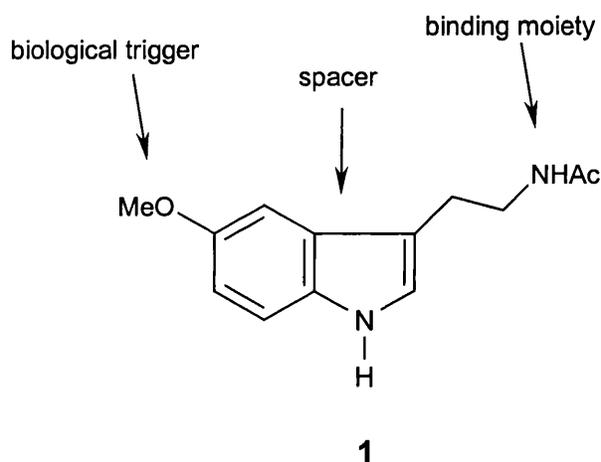
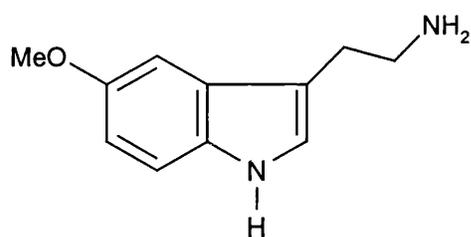


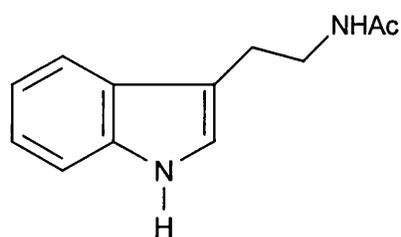
Figure 12. Pharmacophore model proposed by Heward and Hadley.

This concept has been used as the basis for explaining agonist and antagonist properties of melatonin analogues, but more recent studies, having the advantage of being able to measure binding affinity, have shown the model to be rather simplistic. Sugden and Chong have demonstrated that both 5-methoxytryptamine (**12**), an analogue lacking the *N*-acetyl group, and *N*-acetyltryptamine (**13**), an analogue lacking the 5-methoxy group, had no significant agonist potency in the

melanophore pigment aggregation assay.⁹² The binding of both of these compounds in 2-(¹²⁵I)-iodomelatonin binding assays was also considerably reduced, the K_i for **13** being 530 nM or ca. 1000 fold less than melatonin ($K_i = 508$ pM), and the K_i of **12** being 1623 nM, some 3000 fold less. This illustrates the importance of having both pharmacophores present to achieve very high affinity binding. Similar binding results have been reported by Morgan in ovine *pars tuberalis* tissue.¹⁵⁹



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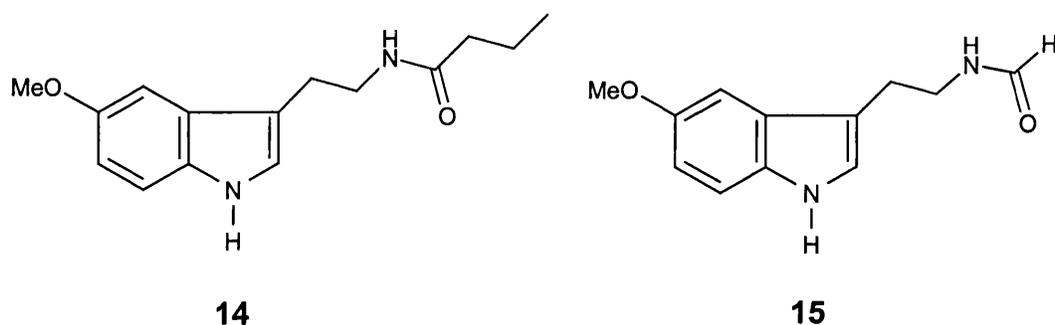
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Whilst the significance of the contribution of the 5-methoxyl group to binding has gained recognition, recent results have suggested that it is not absolutely essential for biological activity. Garratt *et al.* have synthesised various 2-substituted *N*-acyltryptamines lacking a 5-methoxyl group which showed good but slightly reduced binding ($K_i \sim 100$ nM) in chicken brain, but importantly, were melatonin agonists in the *Xenopus* melanophore assay.¹⁶⁰

1.17 C 3 substituents

One or two carbon homologation of the acetyl substituent of melatonin results in improved binding affinity in the 2-(¹²⁵I)-iodomelatonin binding assay in both chicken brain, and sheep *pars tuberalis*.⁹² The optimal binding affinity was obtained with 5-methoxy-*N*-butanoyltryptamine (**14**, $K_i = 45$ pM, *N*-propanoyl =

110 pM and *N*-acetyl = 508 pM) representing an 11 fold increase over melatonin. This pattern was repeated in the *N*-acyltryptamine series which lack the 5-methoxyl group and have correspondingly lower binding affinities (*N*-butanoyltryptamine $K_i = 68$ nM, *N*-propanoyl = 228 nM and *N*-acetyl = 730 nM) and an 8 fold improvement for the optimal substituent. Any further extension of this side chain, or the introduction of branching, as in the cyclopropyl or cyclobutyl derivatives, results in a rapid, progressive, reduction in affinity. A reduction in the size of the acyl substituent is also deleterious (5-methoxy-*N*-formyltryptamine, **15**, $K_i = 12050$ nM, *N*-formyl tryptamine = 157820 nM).¹⁶¹



Other modifications in this part of the molecule including the substitution of the amide bond for that of a urea, thiourea, or carbamate also resulted in a loss of binding affinity.¹⁶² Put together, this evidence points to the presence of a small hydrophobic pocket in the receptor with which the acetyl group of melatonin interacts.

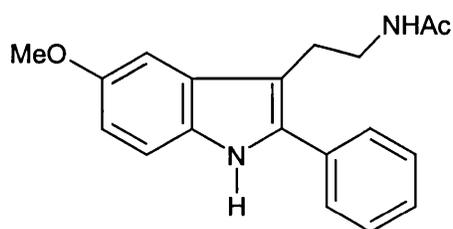
1.18 C 2 substituents

One of the first modifications at the C2 position of melatonin was the addition of a methyl group in an attempt to block *in vivo* metabolism by the indole 2,3-dioxygenase family of enzymes.¹⁶³ Alkyl substituents at this position have

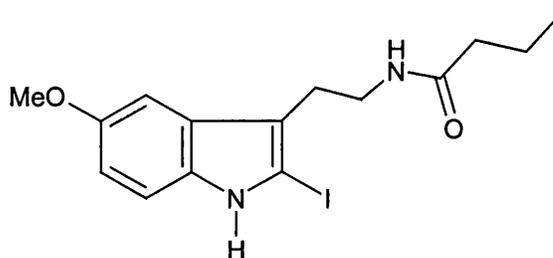
proved to be well tolerated, with 2-phenylmelatonin (**16**, $K_i = 24$ pM in quail brain), showing the highest binding affinity in a series of alkyl substituted melatonin analogues (melatonin $K_i = 1.1$ nM).¹⁶⁴ The increased binding in this case is great enough to significantly offset the loss associated with the removal of the 5-methoxy group (*N*-acetyl-2-phenyltryptamine $K_i = 100$ nM, in chicken brain).

Using an alternative approach, Kennaway, in a series of experiments on ewes, has reported that saturation of the indole 2,3 double bond results in some blocking of metabolism without compromising biological activity.¹⁶⁵

Sugden and Chong have demonstrated that halogenation at the C2 position confers enhanced binding potency as well as an increase in agonist activity in the *Xenopus* melanophore assay.¹⁶¹ This is in agreement with studies by Stankov *et al.* in the rabbit parietal cortex and quail brain, that ranked substituents in terms of competitive binding against 2-(¹²⁵I)-melatonin in the order 2-Cl (K_i 24 pM) > 2-Br (K_i 45 pM) > 2-I (K_i 58 pM).¹⁶⁴ Combining C2 iodination with an *N*-butanoyl moiety in the C3 side chain (**17**) has resulted in further large gains in both binding affinity ($K_i = 15$ pM) and biological activity in the melanophore assay ($EC_{50} = 6$ pM).¹⁶¹



16



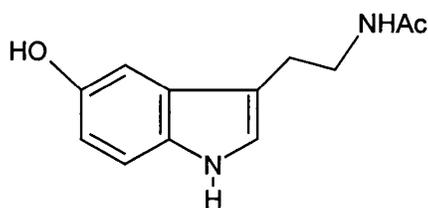
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Several hypotheses have been advanced to explain the enhanced binding and activity associated with C2 substitution by halogen or alkyl groups. The enhancement could be the result of a steric influence on the conformation of the

C3 ethylamido side chain or it could indicate the presence of a lipophilic pocket covering this part of the molecule as suggested by Stankov and co-workers.¹⁶⁴ Stankov has furthermore suggested that the increase in lipophilicity of these molecules compared to melatonin might result in improved distribution, or that the metabolic half lives could be longer.

1.19 C 5 substituents

Modification of the C5 position has attracted little interest in terms of publication of SAR data since changes do not appear to be well tolerated. Replacing the 5-methoxyl group with methyl results in a 250 fold decrease in binding (chick brain), while *N*-acetylserotonin (**5**) has a K_i of 1100 nM in the same assay, a 560 fold reduction (melatonin $K_i = 0.508$ nM).¹⁶¹

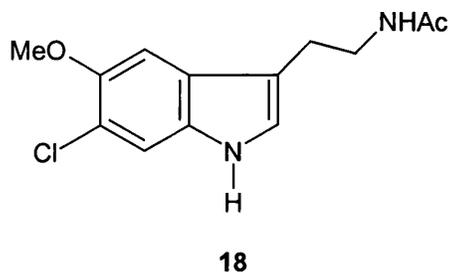


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1.20 C 6 substituents

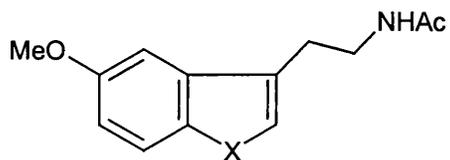
Substitution at the C6 position of the indole ring does appear to be reasonably well tolerated. This position has attracted considerable synthetic attention in view of the fact that rapid C6 hydroxylation in the liver appears to be the metabolic fate of the majority of the circulating hormone. A range of C6 substituted analogues have therefore been prepared in an attempt to gain some resistance to metabolic degradation. 6-Chloromelatonin, **18** ($K_i = 0.58$ nM), was found to have a comparable binding affinity with melatonin, but a serum half-life

of 27 minutes following intra-venous administration, which was an improvement on the 12-15 minutes reported for melatonin.¹⁶⁶ In studying the binding affinity of a range of melatonin analogues, Sugden and Chong reported that the halogenated analogues 6-F and 6-chloromelatonin showed a slight increase in potency ($K_i = 0.36$ nM, and 0.58 nM respectively), whereas the introduction of 6-OH ($K_i = 6.3$ nM), or 6-OMe ($K_i = 31.7$ nM), resulted in a slight decrease relative to melatonin.¹⁶¹

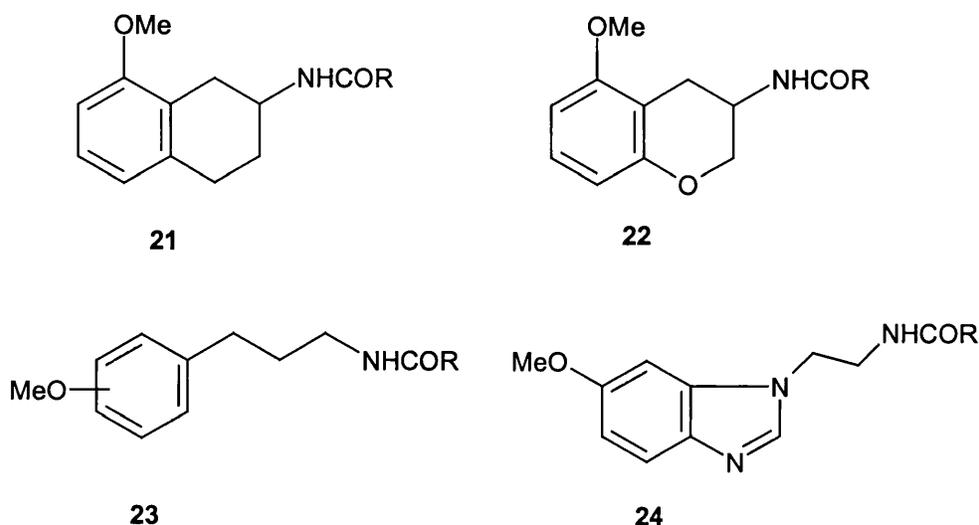


1.21 Indole Bioisosteres

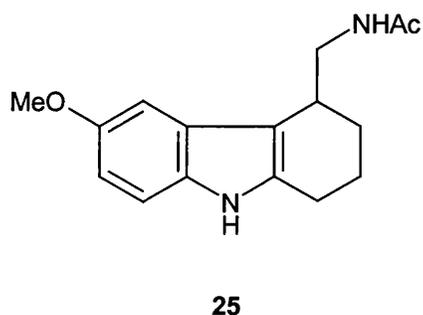
The pharmacophore model of Heward and Hadley suggested that the indole ring serves only to act as a scaffold which presents the methoxyl and acetyl groups in a suitable position for interaction at the receptor. Some of the earliest attempts to replace the indole template involved simply replacing the nitrogen of the indole ring with sulfur (benzo-[b]-thiophenes, **19**), or oxygen (benzo-[b]-furans, **20**).¹⁶⁷ These replacements result in a slight loss of affinity and activity but support the idea that the indole core itself is not crucial for melatonin receptor recognition.



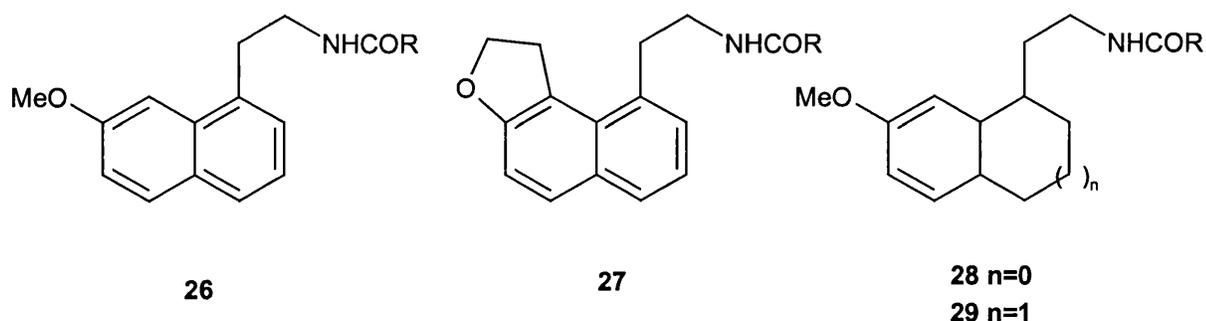
Alternative templates have been reported, including tetralins **21**, and chromans **22**, the best examples of both showing rather modest affinity (**21** R=Me $K_i = 46$ nM, and **22** R= nPr $K_i = 94$ nM).^{168,169} Both phenethylamides **23**, and benzimidazoles **24** have also been investigated as potential templates.



The phenethylamide series exemplified by **23** probably represents the minimum of structural features necessary for receptor recognition and has been examined by Garratt *et al.*¹⁷⁰ They report an excellent binding affinity for the butanamide (R = nPr, $K_i = 5.5$ nM), despite its simplicity. Garratt and co-workers have also taken the alternative approach of restricting the acyl side chain in order to gain insight into the side chain conformational requirements. Binding affinities comparable with melatonin are reported for the racemic 1,2,3,4-tetrahydrocarbazole **25**, and the (-) enantiomer was shown to have considerably higher binding affinity and activity than the other.¹⁷¹



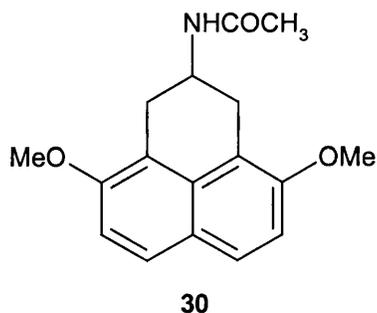
Amongst the non indole bioisosteres, the naphthalene ring system has perhaps undergone the most intensive investigation. The naphthalene compound **26** has equivalent affinity to melatonin in ovine *pars tuberalis*¹⁷² and efficacy in *pars tuberalis* of the sheep.¹⁷³ *In vitro* studies show **26** to be equipotent with melatonin in phase advancing the neuronal activity of isolated cells of rat SCN and this activity is paralleled in free running rats *in vivo*. The compound is now in clinical trials for potential sleep dysfunction and depression/anxiety applications.¹⁷⁴



Analogue studies in the naphthalene series indicate that the addition of a second methoxy group to compound **26** in the position ortho to the acetamido side chain is well tolerated (binding affinity $K_i = 0.1$ nM in ovine *pars tuberalis*).¹⁷⁵ This result is mirrored in the phenalkylamide series, **23**, and gives credence to the suggestion that a binding pocket exists in this region, similar to that which would be occupied by the 2-substituent of the indole ring.

Tricyclic compounds represented by **27**, where the methoxyl group is constrained within a carbocyclic ring also demonstrate binding affinities which are generally equal to those of the corresponding methoxynaphthalene. Similar results were observed with the saturated tetrahydronaphthalenes, **28** and **29**.¹⁷⁶ Incorporation of the acetamido side chain into a rigid carbocycle, however, appears to result in the loss of some of this potency. The phenylene compound **30** shows a binding affinity ($K_i = 0.7$ nM in chicken brain) which is an order of magnitude poorer than

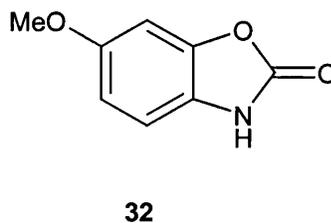
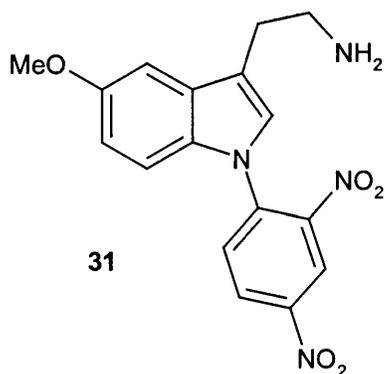
the corresponding naphthalene suggesting that this is not attaining the optimal conformation for binding at the receptor.¹⁷⁷



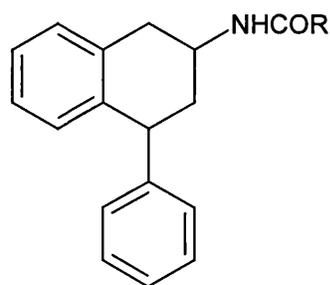
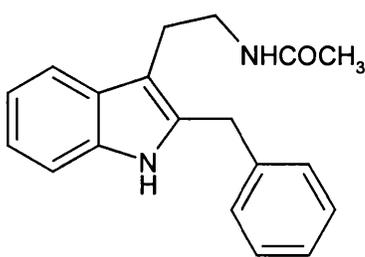
1.22 Antagonists

Understanding of the factors governing antagonist activity is complicated by the fact that in some cases the same ligand can be classified as an agonist, partial agonist or antagonist depending on the biological test employed. 2-Phenylmelatonin for instance, is a full agonist in the *Xenopus* dermal melanophore assay,¹⁷⁸ a partial agonist in rabbit parietal cortex tissue, and an antagonist in the Syrian hamster gonadal regression model.¹⁶⁴

Early reports of melatonin antagonists based on *in vivo* activity have mostly proved to be unfounded. The first of these, in keeping with the SAR theory of Heward and Hadley, was *N*-acetyl-5-hydroxytryptamine (**5**). Subsequent experiments, however, have shown this compound to be a rather weak partial agonist.⁹² Other compounds reported to show antagonist activity based on observed biological effects but subsequently found to be ineffective at the melatonin receptor were the substituted tryptamine **31**,^{179,180} and the cyclic carbamate **32**.^{181,182}

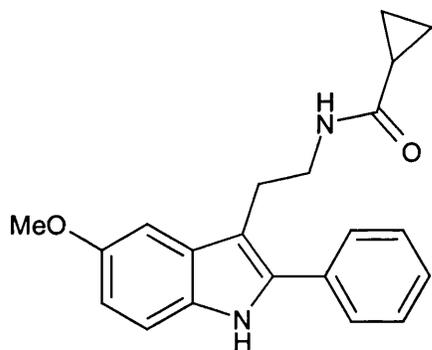


The first genuine competitive melatonin antagonist, Luzindole **33**, was reported in 1988 by Dubocovich.¹⁸³ Luzindole has been shown *in vitro*, to inhibit the Ca^{2+} dependent release of [^3H] dopamine in the rabbit retina, and also to reverse the melatonin induced aggregation of pigment in the *Xenopus* assay.¹⁸⁰ Luzindole has a relatively low intrinsic binding affinity presumably due largely to the lack of the 5-methoxyl group (K_i 1.0, 1.6 and 1.7 μM in ovine *pars tuberalis*, chick brain and chick retina respectively).¹⁸⁰ Despite this low affinity, compound **33** has been used extensively in pharmacological studies. Copinga and co-workers have used a similar idea, to produce a series of 4-phenyl-2-amidotetralins, represented by **34**, as putative melatonin antagonists.¹⁸⁴ These compounds have reasonable binding affinities (ca. 120-500 nM in chick retina) and have been shown to antagonise melatonin in the rabbit retina assay.

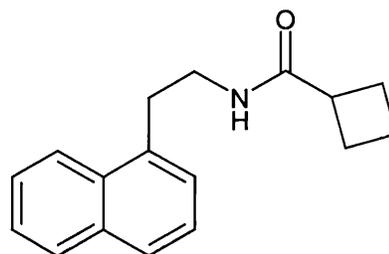


Several groups have tried to overcome the inherent low affinities implied by the absence of a 5-methoxyl group by re-introducing this substituent, particularly in

combination with a cyclic acylating substituent. Compound **35** has a binding affinity $K_i = 240$ pM in quail brain and antagonises melatonin in the syrian hamster gonadal regression model.¹⁶⁴ The naphthalene derivative **36** has been shown to block the action of melatonin in both the *Xenopus* melanophore assay and the forskolin stimulated cAMP accumulation assay in the *pars tuberalis* of sheep.¹⁸⁵ It has been selected for pre-clinical development as a melatonin antagonist.¹⁸⁶



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A recent report from Dubocovich suggests that the lack of selectivity observed for current agonists may not be reflected for antagonists. This study correlates binding data in a range of cloned receptors with potency, measured by [³H] dopamine release from rabbit retina. While agonists did not differentiate, and showed a correlation across all of the substrates, antagonist binding and activity only correlated for the MT_2 receptors and not for mt_1 . Affinity was also higher at the MT_2 receptor with selectivity ratios of greater than 300 for some analogues. This opens the possibility of using melatonin antagonists for pharmacological characterisation of this receptor subtype.⁸⁹

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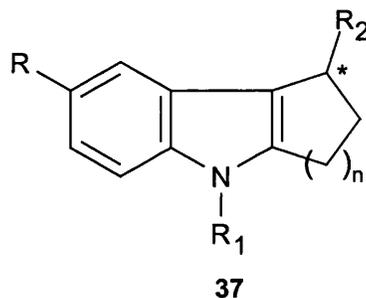
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Chapter 2

Conformationally constrained melatonin analogues

Aim

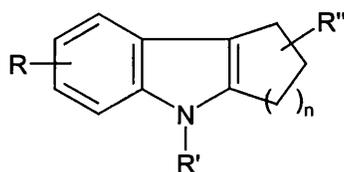
The work described in this chapter forms part of an ongoing programme to map the melatonin receptor by the synthesis of melatonin analogues in which the acyl side chain is conformationally restricted by incorporation into a carbocyclic ring. It has previously been shown that a number of *N*-acyl-aminomethyl-cycloalkan[b]indoles (**37**) have high affinity for the melatonin receptor in chick brain and are agonists in the pigment aggregation test involving isolated melanophores from the neural crest of *Xenopus Laevis* embryos.¹



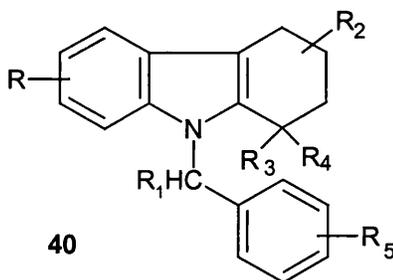
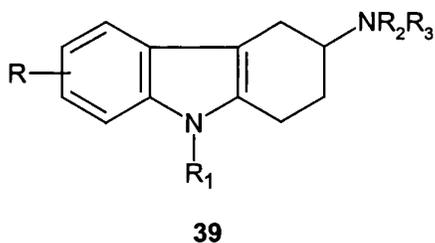
N-acyl-aminomethyl-cycloalkan[b]indoles such as **37** are chiral, having one asymmetric carbon centre (*) at the substituent bearing position of the cycloalkyl ring. The first objective was to vary the size of the annelating ring ($n = 1, 2$ and 3) and obtain compounds in sufficient quantity for preparative chiral separation by HPLC. Biological evaluation of the individual enantiomers would allow us to establish whether the receptor is capable of chiral discrimination and to use the absolute stereochemistry of the compounds to probe its spatial requirements. We also wished to extend the series of compounds by varying both the substituents R and R₂, and to attempt to prepare some compounds which would allow us to compare the two series where R₁ = H or CH₃.

2.1 Introduction

Cycloalkan[b]indoles of general structure **38** (see appendix, Fig. 49 for ring nomenclature) have been reported as antibacterial agents.² Compounds of this type have also been synthesised for examination as antihypertensives,³ and tumour inhibitors.^{4,5}

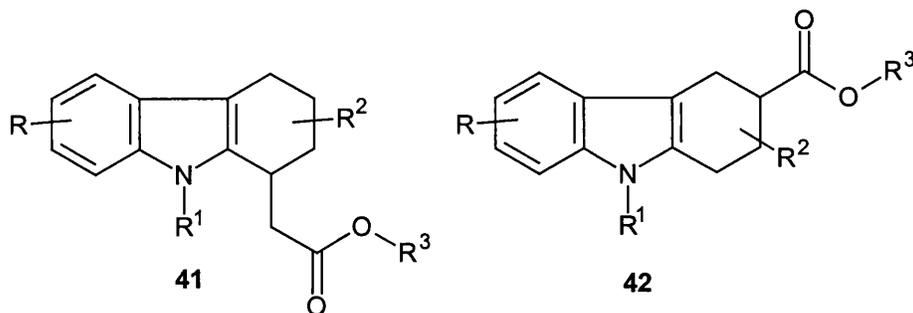


Tetrahydrocarbazoles ($n = 2$) have been shown to have effect in the central nervous system, notably anti parkinson activity⁶ and sedation effects.⁷ Compounds of type **39** have found use in research as serotonin receptor agonists⁸ and have potential for use in conditions such as migraine, while compounds of type **40** have been patented as inhibitors of leukotriene synthesis and as prostaglandin antagonists.⁹



Several 1,2,3,4-tetrahydrocarbazoles have been reported to show antimicrobial and pesticidal activity but were found to be of limited use.¹⁰ This was also the case for a series of tetrahydrocarbazoles (**41** and **42**), patented in the 1970's as

anti-inflammatory compounds. These were particularly aimed at rheumatoid arthritis¹¹ but were rapidly superseded by more effective compounds.



SAR studies on melatonin receptor agonists confirm the importance of having both the 5-methoxyl and the *N*-acetyl pharmacophores present (see chapter 1). Previous work within our group by Vonhoff had suggested that the relative spatial arrangement of these two pharmacophores is important for conferring good binding and activity.¹² In melatonin itself, there are two rotatable C-C bonds between the indole ring and the acetamide group and Vonhoff has synthesised a series of tricyclic indole derivatives in which these bonds were constrained to different degrees (Fig 13). In compound **43** for instance, one of the bonds (**a**) was fixed by incorporation into the ring of a tetrahydrocarbazole as a way of introducing conformational constraint to the *N*-acetyl side chain whilst still allowing a degree of flexibility about the second bond. In a second set of compounds, illustrated by compound **44**, both bonds (**a** and **b**) were fixed within the same rigid tricycle.

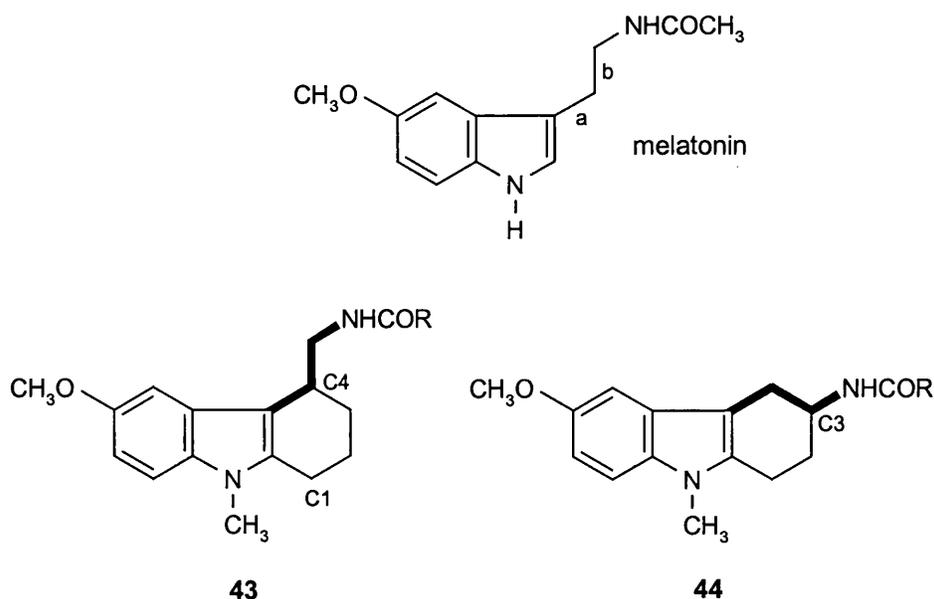


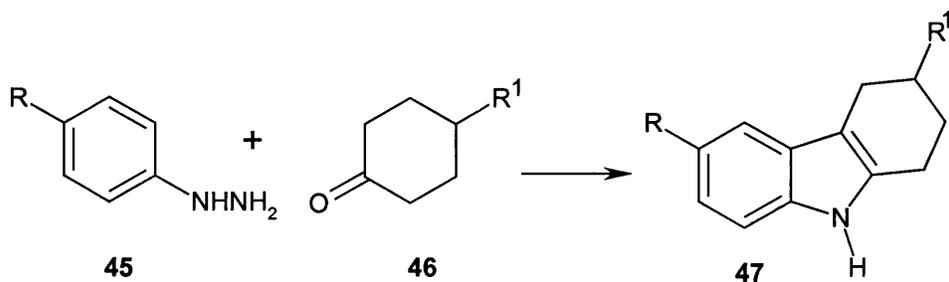
Figure 13 Conformational restriction of the flexible side chain of melatonin.

The results obtained from this work demonstrated that the 4-substituted 1,2,3,4-tetrahydrocarbazole **43** was comparable with melatonin in terms of biological potency. Restriction of both bonds by the positioning of the acetyl side chain at C3 however, as in compound **44**, resulted in almost complete loss of both binding and activity.¹³ This is probably because, in **44**, the pharmacophores have been forced to adopt an unfavourable relative orientation and cannot both bind successfully at the receptor. Compounds such as **43**, however, have a greater degree of freedom and may therefore adopt the correct conformation.

We therefore decided to investigate further the 4-substituted 1,2,3,4-tetrahydrocarbazoles in order to gain more information on SAR in this series.

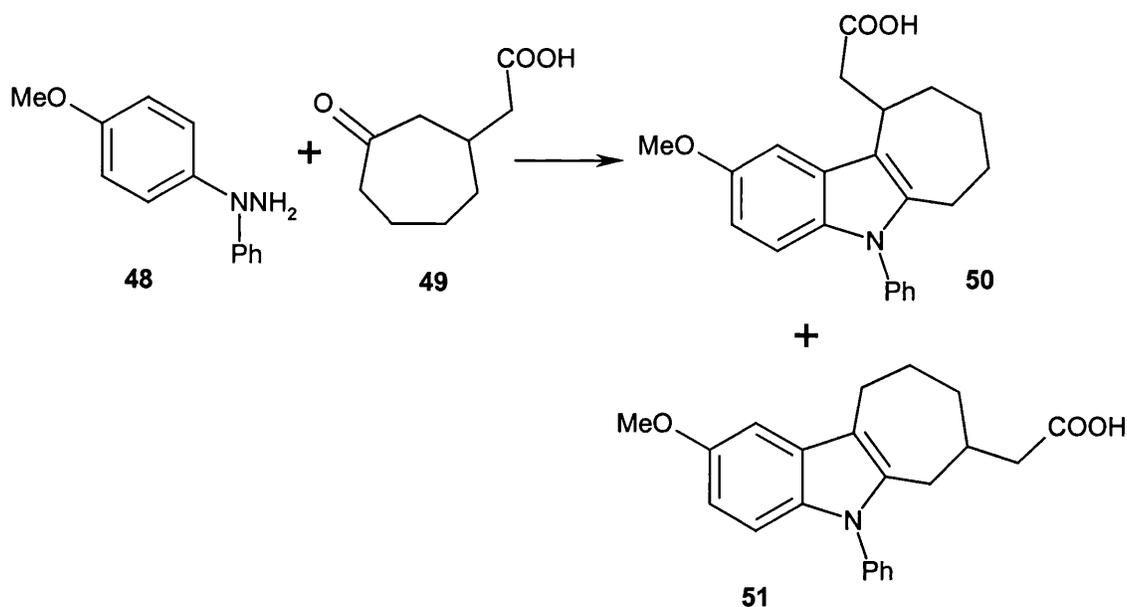
2.2. Synthetic approach

Cycloalkan[b]indoles have been synthesised using the Fischer indole reaction between a substituted phenylhydrazine **45**, and cycloalkanone **9** (scheme 4).¹⁴



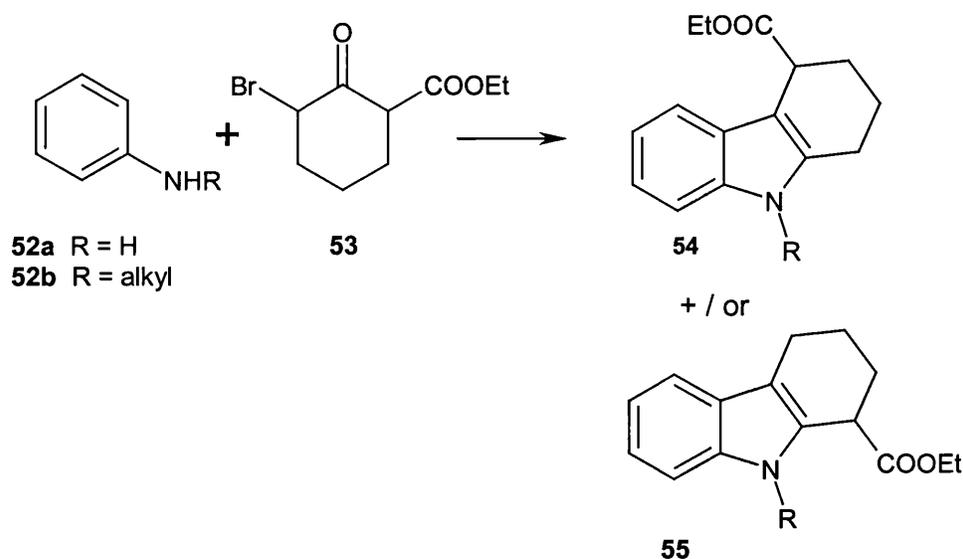
Scheme 4

This route did not seem attractive to us since the reported yields of these Fischer reactions are usually low and the functionalised phenylhydrazines and cycloalkanones may themselves require the investment of considerable synthetic effort. In addition, the problem of low yield is exacerbated by the issue of regioselectivity. The reaction between *N*-phenyl-4-methoxyphenylhydrazine **48** and cycloheptanone-3-acetic acid **49**, for example, gives a 1:1 mixture of two regioisomers **50** and **51** (scheme 5).¹⁵



Scheme 5.

A more attractive route from our point of view was the modified Bischler reaction reported by Julia and co-workers.¹⁶⁻²⁰ This reaction, between α -halogenoketones and anilines (scheme 6), has been extensively studied by Julia's group. They observed that the reaction of *N*-alkylanilines (**52b**) with 2-bromo-6-carbethoxycyclohexanone (**53**) gave the 4-substituted product (**54**) exclusively, while anilines (**52a**) gave exclusively the opposite regioisomer (**55**).



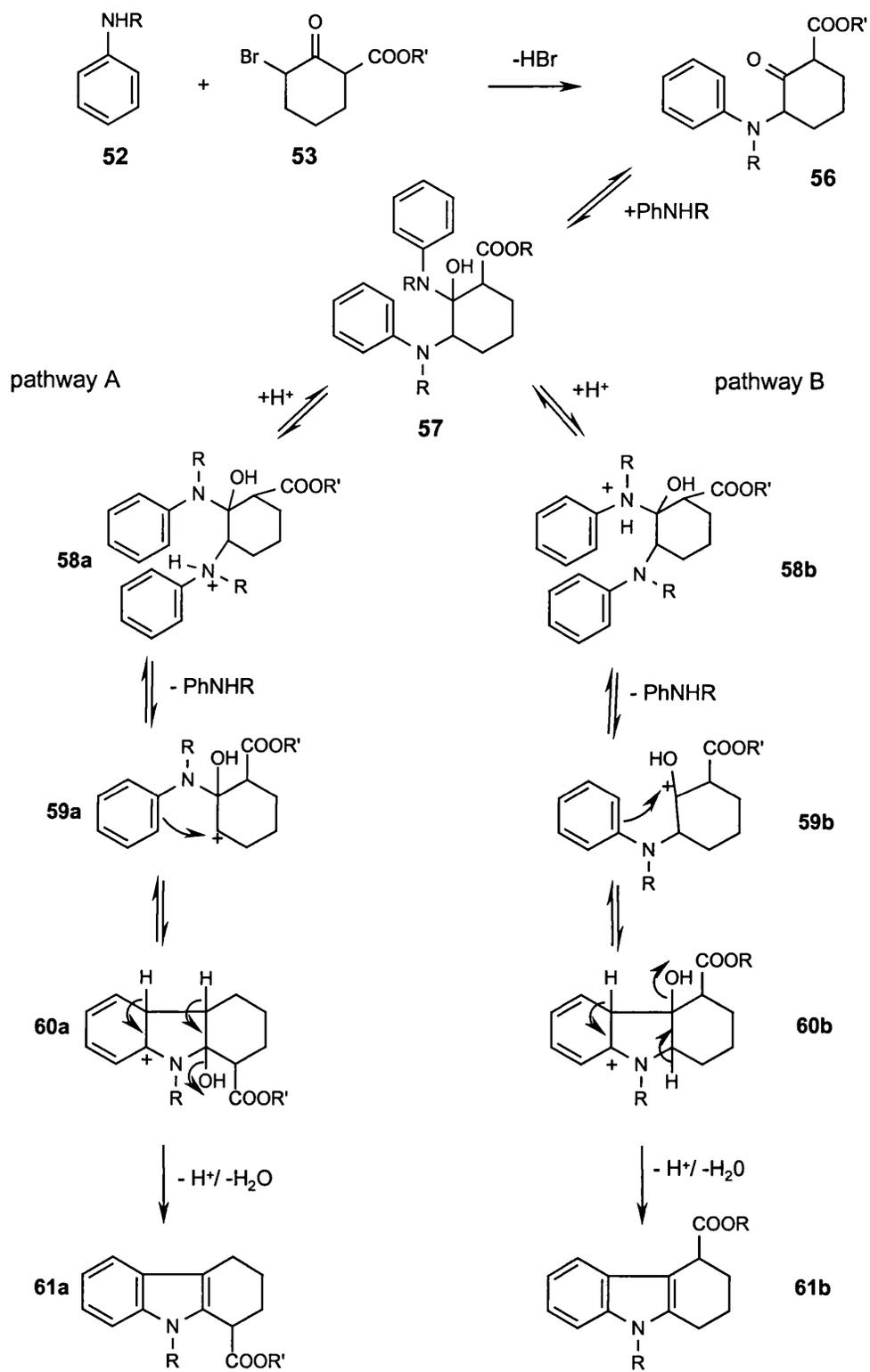
Scheme 6

This modification of the Bischler synthesis has been carried out within our group in order to produce several 1,2,3,4-tetrahydrocarbazoles in small quantity as racemates and Julia's observation on regiochemistry was confirmed by NMR and X-ray studies.¹² The route also has the merit of allowing relatively easy access to a range of aryl-substituted analogues *via* commercially available anisidines. Having established that some of these compounds exhibited nanomolar binding affinity at the melatonin receptor we decided to prepare sufficient quantity of some of them for separation into enantiomers. A consequence of using *N*-methylanilines to effect regioselective cyclisation was that the corresponding N-H compounds (**37**, R = H) were not directly available. In the *Xenopus* dermal

melanophore assay *N*-methyl melatonin has been shown to be approximately 50 times less active than melatonin itself ²¹ and this prompted us to attempt the synthesis of some analogues which were unsubstituted on the indole nitrogen.

2.3 Mechanism of the Bischler Reaction

The mechanism of this reaction has been investigated by several groups and the evidence summarised in scheme 7.²²⁻²⁵ The initially formed anilinoketone **56** reacts with a second molecule of the aniline **52** to give the dianiline **57**. Protonation of either nitrogen and subsequent loss of an aniline component, gives a carbocation **59a/59b** via pathways A or B. Evidence for this intermediate has been obtained from crossover reactions involving different anilines as well as radiolabelling experiments.¹² The evidence also fits the observation of Julia *et al.* regarding the reaction of *N*-alkylanilines (**52b**), with 2-bromo-6-carbomethoxycyclohexanone (**53**) which gave the 4-substituted products, apparently resulting from pathway B exclusively. Anilines (**52a**) however, gave exclusively the 1-substituted product, apparently resulting from pathway A. A possible explanation for this observation may be the steric interaction that occurs between the *N*-alkyl group and the carboxyl substituent on the cycloalkane ring. For R = alkyl these interactions are minimised in intermediates **59b** and **60b**, favouring the cyclised product **60b**. When R = H the steric interaction is minimal and the reaction can proceed via pathway A to give the cyclised product **60a**. This is possibly aided by hydrogen bond formation between N-H and the carboxyl substituent. The intermediates **60a** and **60b** are then cyclised to the indoles by electrophilic attack of the carbocation at the ortho position of the aniline ring followed by dehydration and subsequent aromatisation.

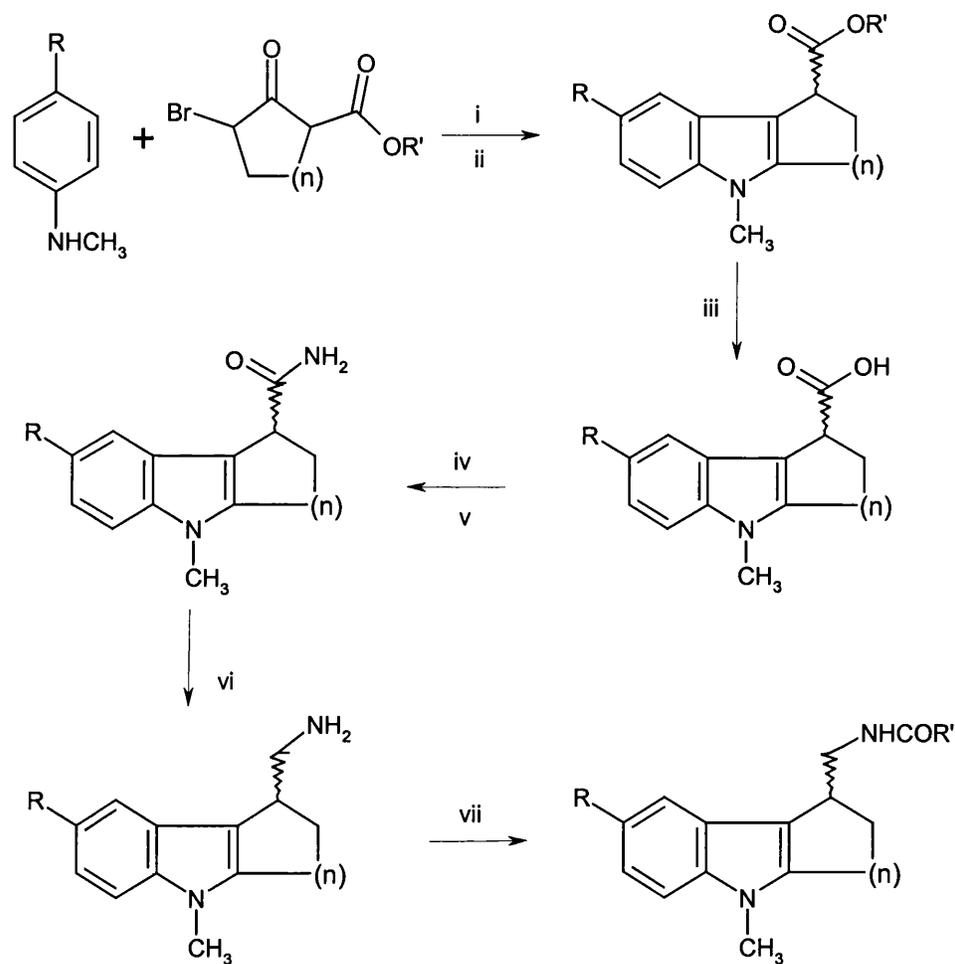


Scheme 7

2.4 Synthesis

The synthesis of a series of cycloalkan[b]indoles is outlined below (scheme 8). The *N*-methylanilines used were commercially available, and the α -halogenoketones were prepared by bromination of the appropriate ethyl or methyl 2-oxocycloalkanecarboxylate. These materials were heat labile oils and received only limited purification. The modified Bischler reaction was carried out by treating the α -bromoketone with 2 equivalents of *N*-methylaniline, in the absence of solvent, at 50 °C for 3 hours. When the starting materials were both solid, the reagents were mixed in diethylether or toluene and evaporated to give an intimate mixture prior to heating. After 3 hours, the resulting cake, containing the anilinoketone, was dissolved in 2-propanol and refluxed in the presence of anhydrous zinc chloride to effect cyclisation. The esters obtained as a result were purified by column chromatography and then saponified to give the carboxylic acids, which were crystalline solids. In the case of the tetrahydrocarbazoles and cyclohept[b]indole derivatives, only one regioisomer was obtained in reasonable yield at this stage, but the cyclopent[b]indoles gave a mixture of regioisomers, as deduced from the ¹H NMR spectra. The five membered ring compound was also unstable and required extensive purification. However, we eventually obtained a sufficient amount of the required acid to continue. The NMR spectrum of this compound was compared to that of the compound previously synthesised and characterised in a series of X-ray and NMR studies by Vonhoff.¹² In the next step, the deprotonated carboxylic acid was reacted with ethyl chloroformate to generate the mixed anhydride and subsequently quenched with gaseous ammonia to give the primary carboxamide. These compounds were all crystalline solids and the I.R spectra showed a characteristic shift of the carbonyl absorption from ca. 1700 cm⁻¹ for the acids to ca. 1650 cm⁻¹ for the carboxamides. Characteristic of the NMR spectra of these compounds were the two broad NH signals occurring at between $\delta = 5$ and 6.5 ppm in CDCl₃ or between $\delta = 6.5$ and 7.5 ppm in d₆-DMSO. The carboxamides were subsequently reduced by lithium aluminium

hydride in THF to give the amines. The crude amines were subjected to column chromatography but showed signs of instability on standing at room temperature, by a noticeable and rapid darkening. Therefore, in the final step the amines were immediately acylated with an alkanolic anhydride, or an acid chloride if the former was not commercially available. The reaction using the milder anhydride conditions were preferable and generally gave higher yields than the corresponding acid chloride.



Reagents: i) 3hr, 50 °C. ii) ZnCl_2 , propan-2-ol, reflux o/n. iii) NaOH , $\text{H}_2\text{O}/\text{EtOH}$, reflux 6 hr. iv) Et_3N , DCM , EtCOOCl , 0 °C. v) NH_3 , 0 °C. vi) LiAlH_4 . vii) Et_3N , DCM , $(\text{RCO})_2\text{O}$ or RCOCl .

Scheme 8

In addition to varying the size of the cycloalkane ring, several compounds were prepared in the tetrahydrocarbazole series by variation of both the aromatic substituent (R) and the acyl moiety (R'). These are illustrated in table 2.

62 (+/-) R = OMe R' = Et	25 (+/-) R = OMe R' = Me	63 (+/-) R = OMe R' = Et
62 (+) R = OMe R' = Et	25 (-) R = OMe R' = Me	63 (+) R = OMe R' = Et
62 (-) R = OMe R' = Et	25 (+) R = OMe R' = Me	63 (-) R = OMe R' = Et
	64a (+/-) R = H R' = Me	
	64 (+) R = H R' = Me	
	64 (-) R = H R' = Me	
	65 R = Cl R' = Me	
	66 R = Cl R' = cC ₃ H ₅	
	67 R = Cl R' = cC ₄ H ₇	
	68 R = OCF ₃ R' = Me	
	69 R = OCF ₃ R' = Et	
	70 R = OCF ₃ R' = cC ₄ H ₇	
	71 R = Me R' = Me	
	72 R = Me R' = Et	
	73 R = Me R' = cC ₄ H ₇	
	74 R = Et R' = Et	

Table 2 The synthesised cycloalkan[b]indoles

The series of compounds produced, and included in table 2, were all purified by column chromatography and/or HPLC, and characterised by NMR, IR and mass spectrometry. In general, the alicyclic protons displayed a complex overlapping pattern in the NMR spectrum with the C4 proton occurring at ca. $\delta = 3.1-3.3$ ppm. The methylene protons of the side chain were well separated from those of the ring and were found in the $\delta = 3.5-3.6$ ppm region of the spectrum. *N*-Methyl protons of the pyrrole ring were readily observed as a singlet at 3.6 ppm, while the acyl N-H is visible as a poorly resolved triplet ($J \sim 5.6$ Hz) in the $\delta = 5.4-5.7$ ppm region (CDCl_3), or between $\delta = 7.7$ and 8.0 ppm in d_6 -DMSO. Aromatic protons of the 6-substituted compounds show three distinct aromatic signals: a doublet with $J \sim 8.8$ Hz assigned to the C5 proton, a doublet with $J \sim 2.2$ Hz assigned to the C8 proton, and a doublet of doublets with both the above coupling constants assigned to the proton at C7. Examples of a typical ^1H and ^{13}C spectrum of these compounds are included in the appendix (figures 35 and 36).

2.5 Chiral chromatographic separation

An important aspect of this work was to carry out a chiral resolution on some of the compounds in order to determine whether the receptor itself displayed any degree of chiral discrimination. The compounds illustrated below are chiral, with one asymmetric carbon atom at C4. (Figure 14)

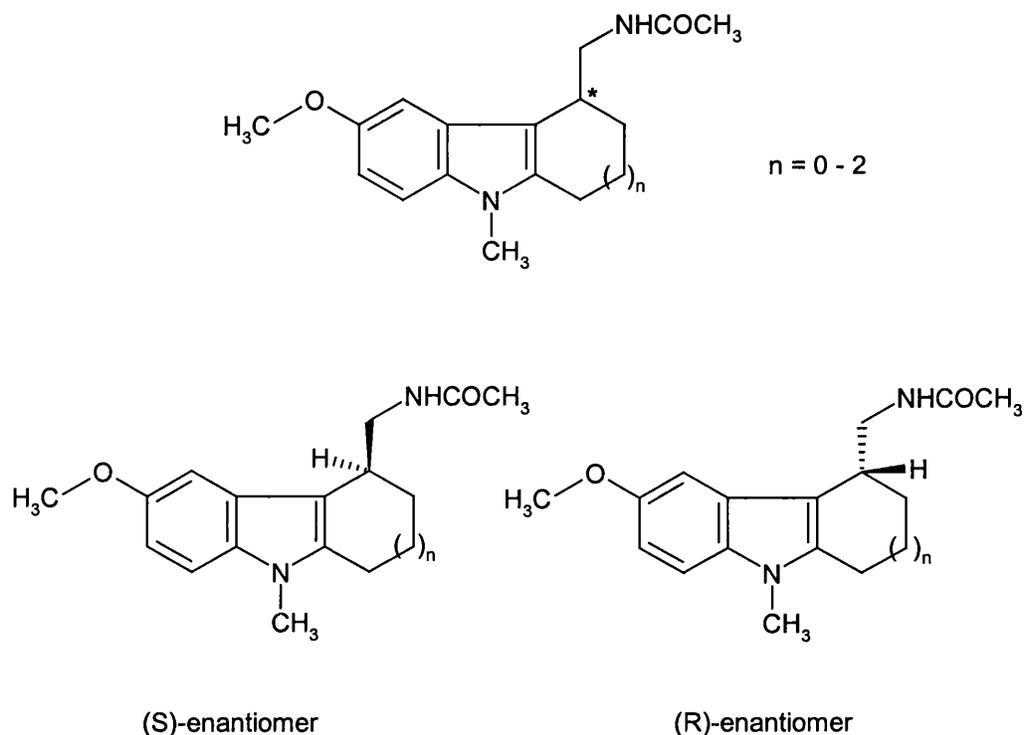


Figure 14 Enantiomers of tetrahydrocarbazole 25

Each racemate was examined analytically on three column types; 'Chiralcel OJ', 'Chiralcel OD' and Chiralcel AD. One column, (OJ), is composed of a cellulose ester derivative coated on silica gel. The second, (OD), is a cellulose carbamate derivative and the third, (AD), is an amylose carbamate derivative (Figure 15). Trial separations showed the carbamate 'AD' and 'OD' columns to be considerably more effective at resolving the enantiomers than the 'OJ' column.

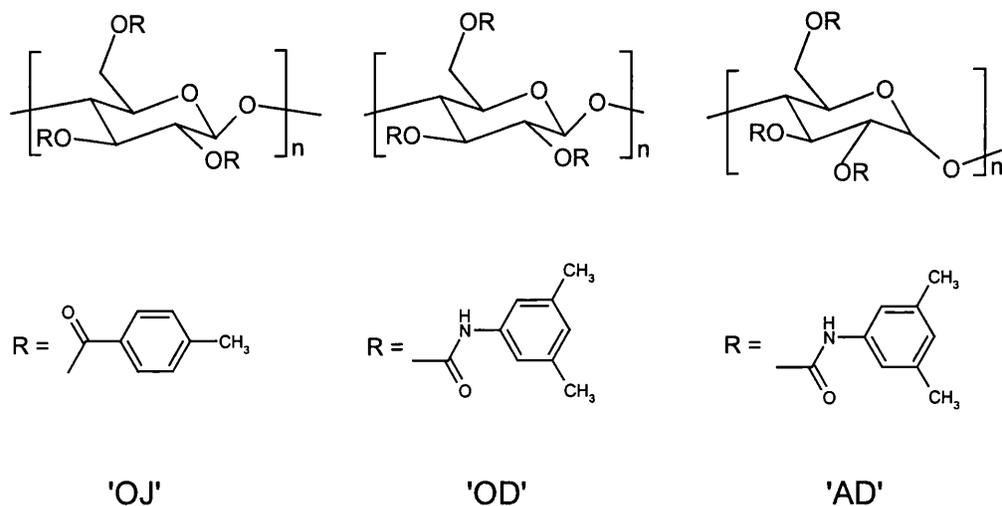


Figure 15. Types of chiral column adsorbant.

The compounds, dissolved in the minimum volume of solvent, were applied to the column and eluted isocratically with ethanol/hexane, the percentage of each being optimised beforehand. In some cases a baseline separation allowed direct collection of resolved material but, when this was not achieved, re-cycling of impure fractions was carried out in order to obtain the necessary purity.

The purity of each enantiomer was confirmed by analytical chiral HPLC. Optical rotation and circular dichroism studies (CD) gave supporting evidence that the relationship of each member of a pair to the other was enantiomeric in nature.

X-ray studies on *N*-acetyl-4-aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole, **64**, have shown the (-) enantiomer to have the S absolute configuration and the (+) enantiomer the R absolute configuration (Figure 16).

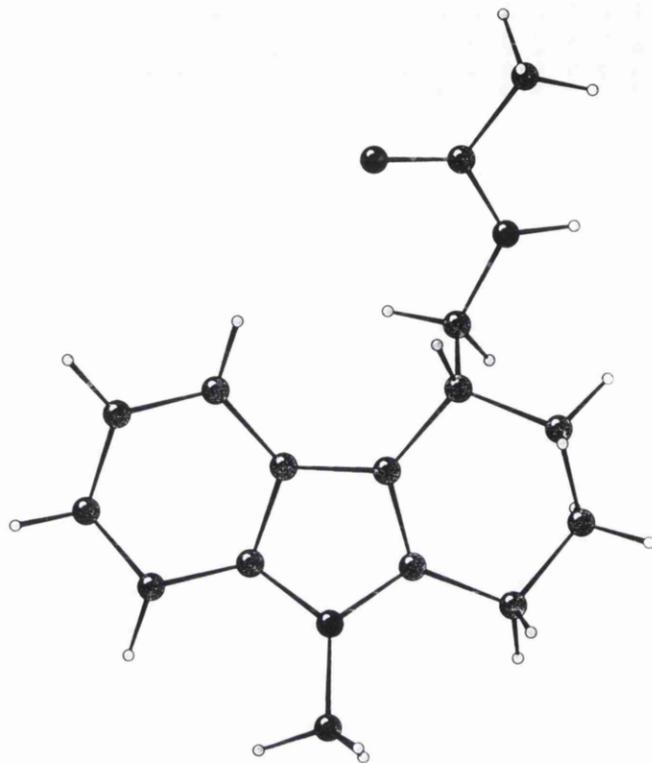


Figure 16. X-ray diagram of R-(+)-*N*-Acetyl-4-aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole.

In the crystal, the core of the molecule is virtually planar with C-2 and C-3 atoms of the saturated ring forming a half chair. The *N*-acetyl side chain at C-4 is at an angle of 112.7° to the ring plane and is hydrogen bonded through the carbonyl and N-H groups to adjacent molecules. The side chain extends away from the core but slightly deviates away from the aromatic ring, probably as a consequence of hydrogen bonding. Two views of (-) – (S)-**64** are shown in Figure 17.

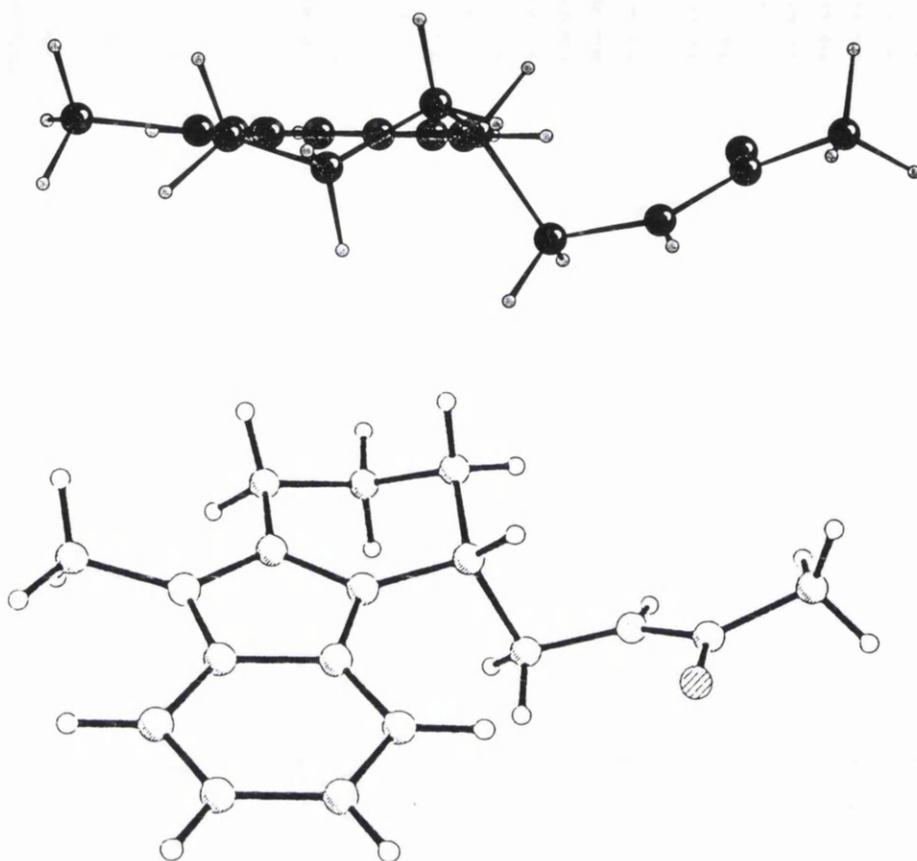


Figure 17. Two views of *S*-(-)-*N*-Acetyl-4-aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole.

Circular dichroism measures both rotation and absorbance simultaneously and the spectrum represents the differential absorption of left and right circularly polarised light. For a pair of enantiomers the CD spectra of each should be of equal magnitude but opposite sign to the other.

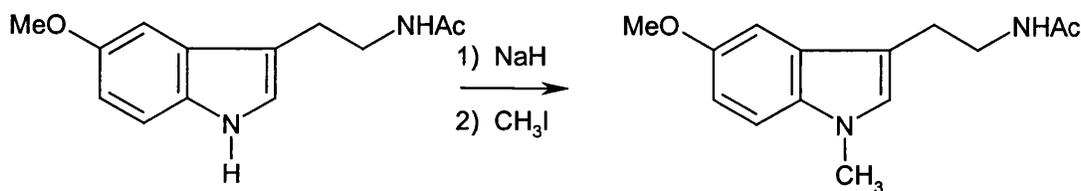
In every separation we performed, the (+) enantiomer eluted first from the column and the signs of the Cotton curves, determined from CD studies, were the same for all of the (+) enantiomers. We therefore made the assumption that the stereochemistry at each of these centres was identical and subsequently assigned absolute configuration to the other pairs of enantiomers in this series on the basis of their CD spectra. It has been previously demonstrated that compounds of a

given family with the same configuration exhibit CD spectra of similar pattern and magnitude.²⁶ An example of the CD spectra of the resolved compounds are shown in the appendix (figure 37) and display the expected mirror image attributes expected for pairs of enantiomers.

These cycloalkan[b]indoles are currently included in SAR studies and this information on structure and conformation will be of use in the construction and refinement of receptor models.²⁷⁻³⁰ Melatonin receptor models of this type are in the early stages of development, as are the complementary site directed mutagenesis studies necessary to define the crucial residues involved in receptor binding.³¹ Any credible model will have to take into account and explain the binding affinities of a large range of active ligands and also any stereoselectivity observed within a given series of compounds. The behaviour of these enantiomers in various assays and models may therefore contribute to the clarification of the issue of pharmacophore chirality and be of use in testing the predictive power of new models.

2.6 Synthesis of 9-H-tetrahydrocarbazoles

N-Methyl melatonin was synthesised by the treatment of melatonin with sodium hydride, and alkylation of the generated anion with methyl iodide (scheme 9).

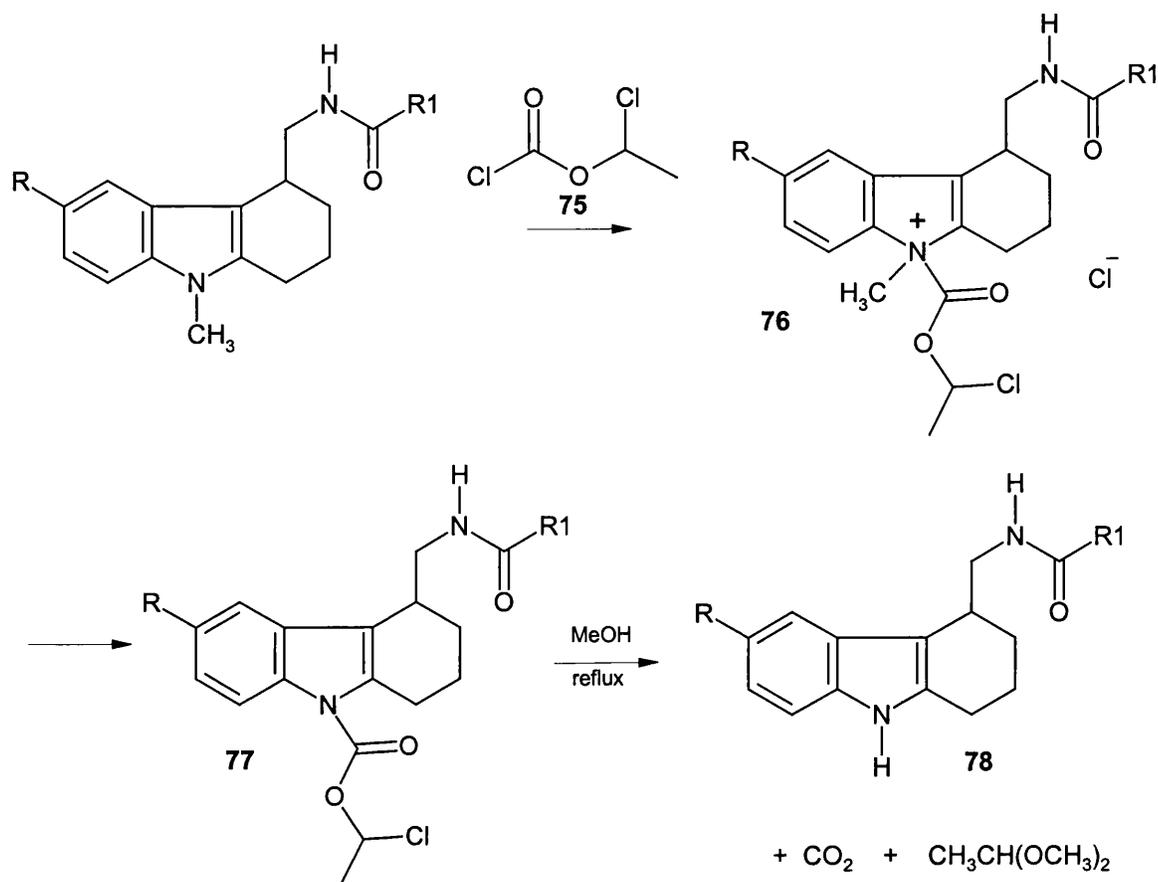


Scheme 9

The *N*-methyl compound was found to be have approximately 20 times poorer affinity than melatonin itself in the 2-[¹²⁵I] iodomelatonin binding assay (K_i *N*-

methylmelatonin = 5.5 nM, while K_i melatonin = 0.28 nM). The *N*-benzyl melatonin analogue was considerably worse ($K_i = 897 \pm 199$ nM)¹² indicating that substitution on this nitrogen is undesirable. This prompted us to attempt the synthesis of cycloalkan[b]indoles in which the indole nitrogen is unsubstituted.

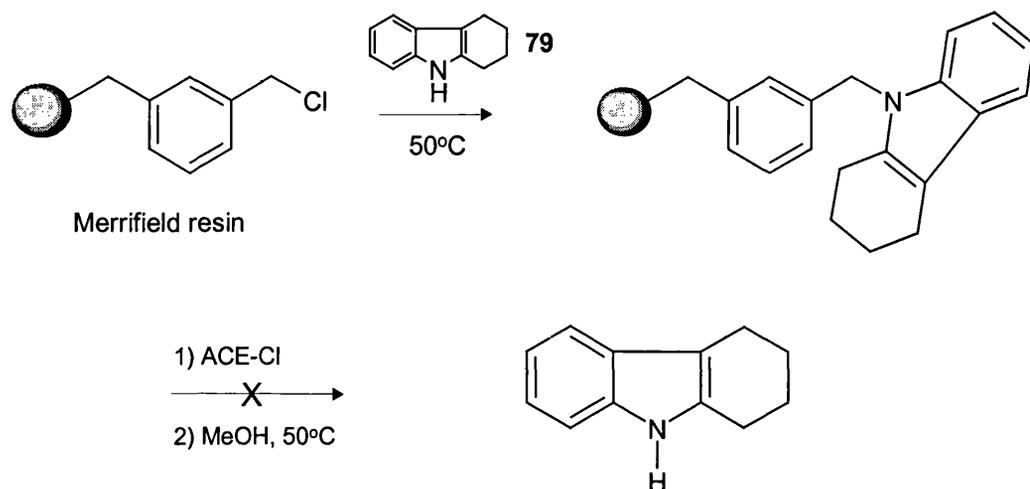
Our initial approach was to attempt dealkylation of the compounds we had already obtained by the method of Julia and co-workers. Preliminary attempts at debenylation of *N*-benzyl tetrahydrocarbazoles within our group, using sodium in ammonia or catalytic hydrogenation had been unsuccessful.¹² De-protection was not achieved in either case and as *N*-benzyl removal can sometimes be problematic we elected to look at other approaches. The reagent α -chloroethyl chloroformate (ACE-Cl, **75**) has been proven as an effective reagent for the selective debenylation of tertiary amines and has also been applied to demethylation procedures. Yang *et al.* treated a range of tertiary *N*-benzyl compounds with ACE-Cl in dichloromethane and isolated a carbamate intermediate which was obtained *via* quaternisation of the tertiary nitrogen.³² This carbamate decomposes on heating in methanol to give the secondary amine hydrochloride. Olofson rationalised this by suggesting that the CHClCH_3 unit of **76** is too hindered to undergo competitive $\text{S}_{\text{N}}2$ attack by chloride ion, and the related cation too unstable to permit $\text{S}_{\text{N}}1$ substitution, thus forcing the ejection of the methyl fragment as chloromethane.³³ We envisaged using this strategy as illustrated in scheme 10.



Scheme 10.

The use of ACE-Cl, as outlined in the literature, did not effect demethylation of compounds **25**, **65**, or **71** in our hands. Prolonged treatment resulted in the formation of a complex reaction mixture and HPLC analysis indicated that no single component was dominant. As the starting materials themselves required considerable synthetic effort we did not feel that this route merited pursuing. However, recent reports of the use of ACE-Cl as a reagent for cleavage of amines from Merrifield resin was of interest since this is effectively the use of a solid support as an *N*-benzyl protecting group.³⁴ In this case, the intermediate carbamate formed by quaternisation is subject to nucleophilic attack by chloride at the benzylic carbon atom. This regenerates the starting resin and releases the secondary amine as a hydrochloride. A model reaction was carried out by

attaching the commercially available tetrahydrocarbazole (**79**) to Merrifield resin *via* the anion generated by sodium hydride in DMF. Treatment of the resulting polymer bound material with ACE-Cl yielded none of the required product (Scheme 11).



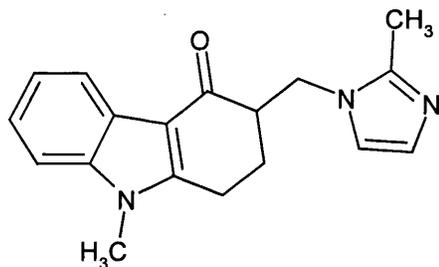
Scheme 11

These preliminary investigations suggested that the N-H compounds would not be readily accessible from *N*-alkyl analogues so we briefly looked at other protecting groups for use with the same chemistry. The acidic conditions of the cyclisation step precluded the use of acid labile protecting groups, so we initially chose to look at base labile protecting groups such as fluorenylmethoxycarbonyl (Fmoc), and trifluoroacetyl. The protected anilines were prepared according to literature methods^{35,36} but no cyclisation to the tetrahydrocarbazole was observed, either under standard conditions or on prolonged reaction. In both cases the protected anilines were recovered in good yield. A possible reason for the failure to react could be that the electron withdrawing effect of the acyl or carbamate group renders the aniline too unreactive. At this point we decided to try a completely new approach towards the target molecules.

Tetrahydrocarbazolones

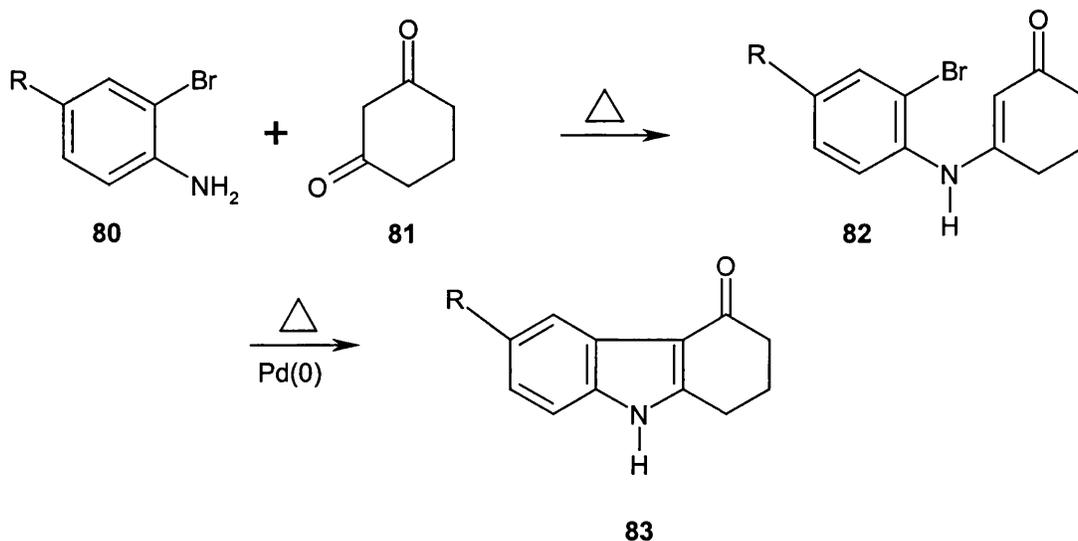
2.7 Introduction

A major area of research during the past two decades has been in the field of 5-HT receptors and many tetrahydrocarbazolones have activity in assays aimed at these targets. The raised serotonin levels usually associated with clinical efficacy against many disease conditions were often associated with side effects, such as nausea and vomiting, and serotonin receptor blockade with 5HT-3 antagonists was found to alleviate these side effects in many instances. The most efficacious compound in relieving these symptoms at the time was the carbazolone Ondansetron.



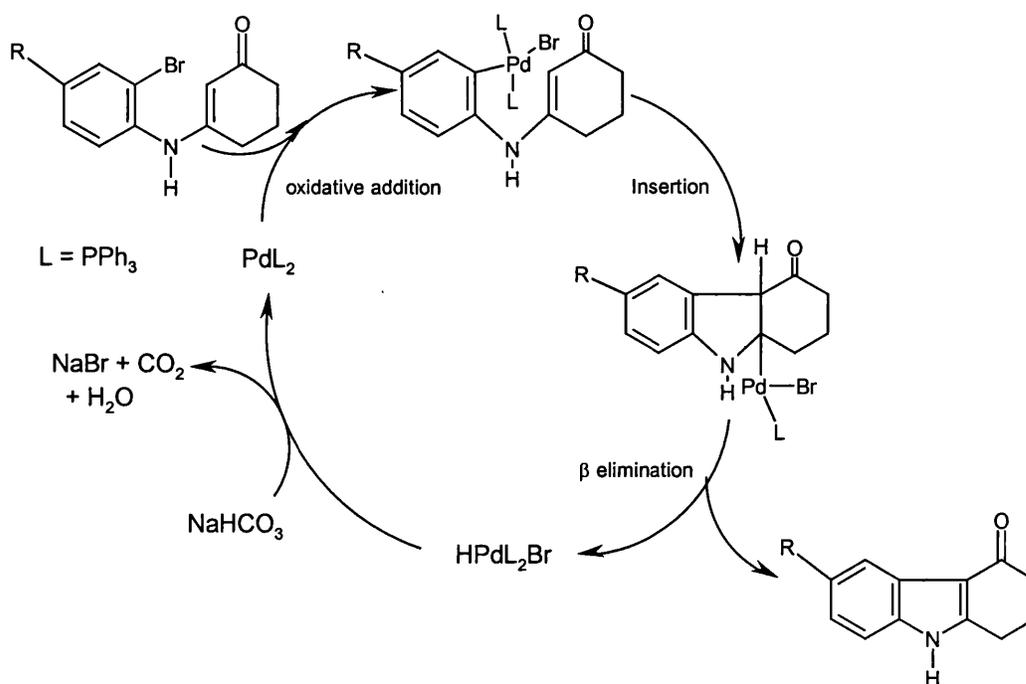
Ondansetron

Several groups were prompted to investigate the synthesis of this type of compound, including the group of Kibayashi, who published a convenient two step route to tetrahydrocarbazolones.³⁷ This was an extension of earlier work by Akermark *et al.* in 1975³⁸ and involves the condensation of a 2-bromoaniline with 1,3-cyclohexanedione followed by palladium catalysed cyclisation (scheme 12).³⁹ The initially formed bromo enaminone (**82**) is treated with a catalytic amount of triphenylphosphine, palladium acetate and sodium bicarbonate to effect a Heck cyclisation which yields the required tetrahydrocarbazole (**83**).



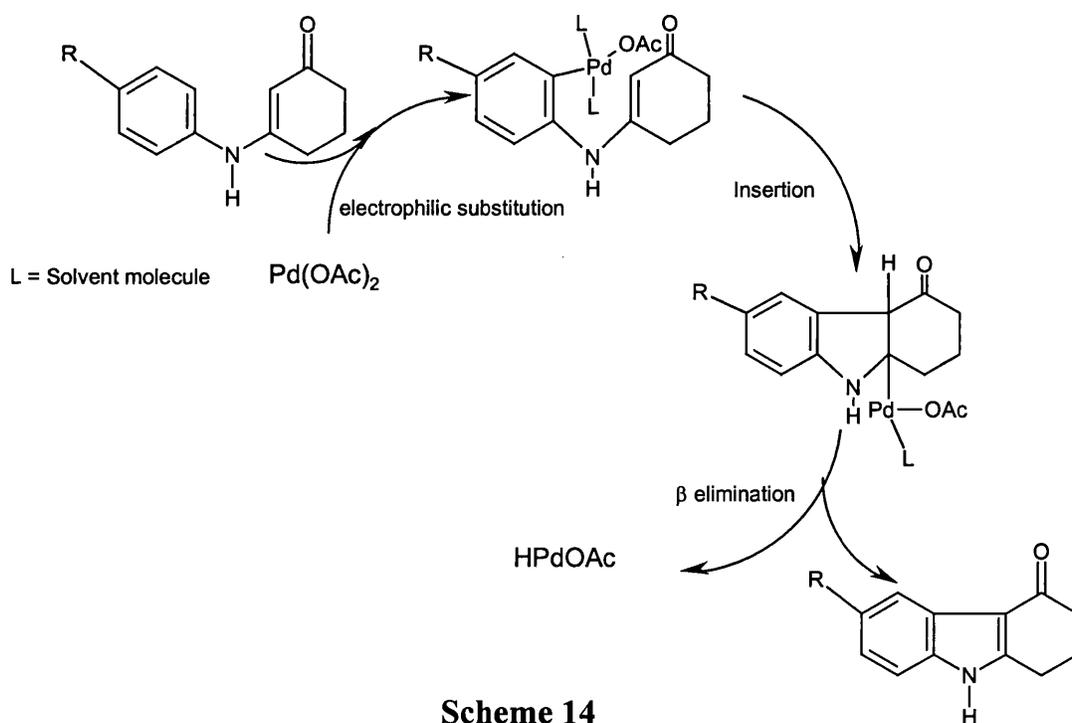
Scheme 12

Kibayashi has postulated that the reaction is likely to proceed by oxidative addition of the aryl halide to the Pd(0) species followed by insertion of the double bond of the enaminone system into the aryl-Pd bond. This is probably followed by β -elimination of a palladium hydride species that subsequently collapses. Palladium(0) may then be regenerated by sodium bicarbonate (**scheme 13**).

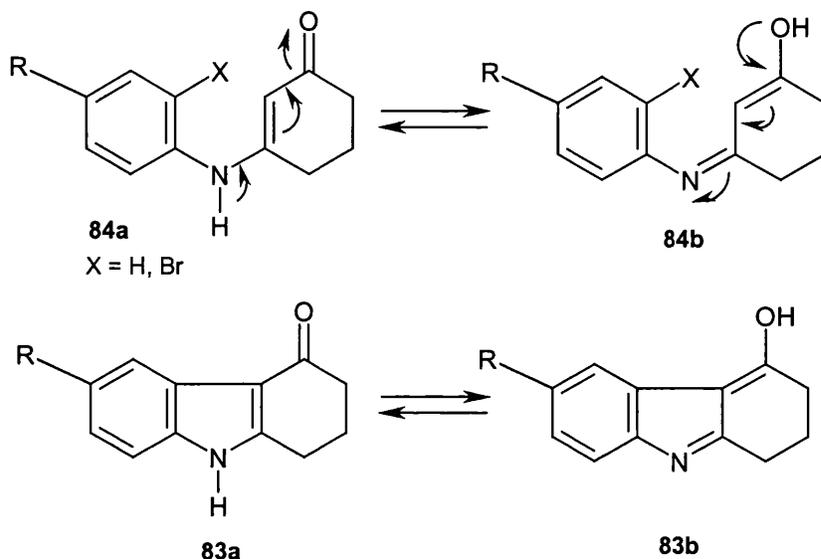


Scheme 13

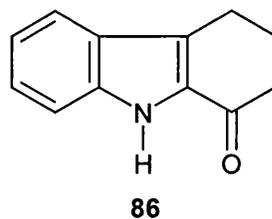
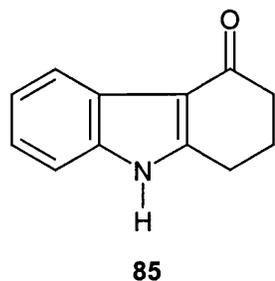
Cyclisation can also be effected in the absence of an *ortho* bromine atom with the use of stoichiometric palladium acetate, as reported by Akermark, or catalytically by the addition of cupric acetate and oxygen to the palladium acetate. The large number of commercially available anilines made this stoichiometric route extremely attractive in terms of potential analogue generation. Both Kibayashi and Akermark have suggested that mechanistically this route involves direct electrophilic substitution of the aromatic ring by palladium(II) to generate the aryl palladium acetate (scheme 14). Some evidence to support this is obtained from observations on the effect of varying certain reaction parameters. Electron donating aromatic substituents result in an acceleration of the reaction, whilst electron withdrawing substituents may both slow the rate considerably and require the use of greater than stoichiometric amounts of palladium acetate. The reaction is also catalysed by organic acids, such as acetic or methanesulphonic acid. Once formed, the palladium substituted intermediate may co-ordinate intramolecularly with the double bond of the enaminone system to give an intermediate which can be incorporated into a catalytic cycle as described above.



Because most of the reported work in this area has been directed towards 5-HT targets, synthetic effort has tended to concentrate on functionalisation of the C3 position. The enaminone and tetrahydrocarbazolone can exist in two forms via a classic 'keto-enol' tautomerism as illustrated below. Greenhill has shown from pKa studies on a series of 3-amino-cyclohex-2-enones and their derivatives that the carbonyl form **84a** is generally favoured over the enol form **84b**.⁴⁰



Mann and Willcox⁴¹ compared the IR spectra of 1,2,3,4-tetrahydrocarbazol-1-one (**86**) and 1,2,3,4-tetrahydrocarbazol-4-one (**85**). They found that while the spectrum of the former showed a strong sharp band at 3275 cm^{-1} due to the NH group and a strong C=O band at 1642 cm^{-1} , the spectrum of **85** displayed a strong band at 1610 cm^{-1} and a rather broad band at 3060 cm^{-1} . It was suggested that this may either represent the existence of a resonance hybrid structure with some hydrogen bonding, or that the bands at 1610 and 3060 cm^{-1} represent the conjugated N=C-C=C system and OH bond stretch, respectively, indicating that **85** is in the enol form. Masager *et al.*⁴² and Patir also published spectroscopic data consistent with an enol form.⁴³



Rodriguez *et al.* noted a general lack of reactivity of the tetrahydrocarbazolone carbonyl group with nucleophiles or Wittig ylids when the substituent was at the 4-position.⁴⁴ This was in marked contrast to the normal reactivity observed when the carbonyl was at the C3 position. A thorough investigation of the crystal structure of 1,2,3,4-tetrahydrocarbazol-4-one by X-ray diffraction was carried out and it was shown to exist as the ketone tautomer. The occurrence of strongly associated molecular aggregates, with intermolecular nitrogen-hydrogen-oxygen bridges between the N-H of the indole and the carbonyl group, were suggested to be a cause of the lack of reactivity (Figure 18).

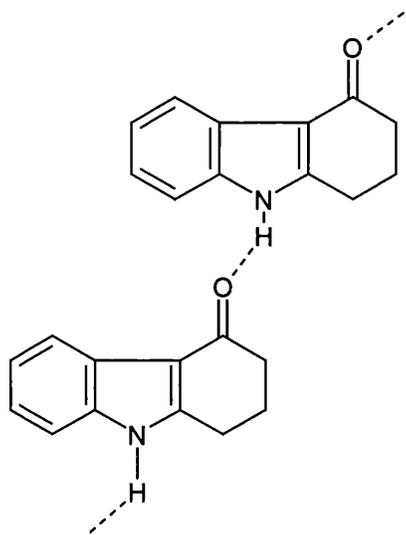


Figure 18. Extended Hydrogen bonding interactions.⁴⁴

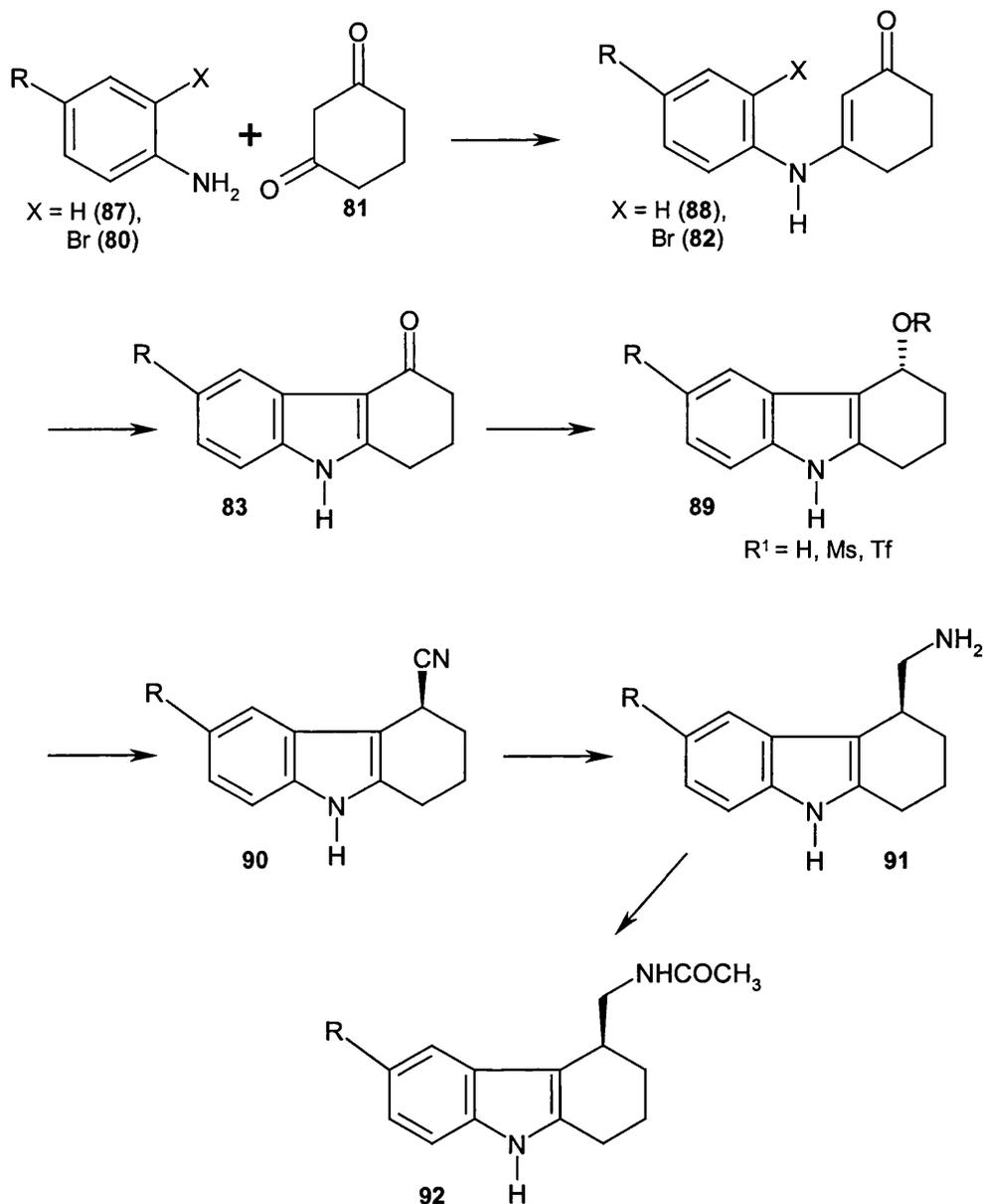
Previous conformational analysis has indicated that the cycloalkane ring adopts an envelope conformation with the C2 atom at the 'flap'.⁴⁵ Rodriguez suggested

that this rigid and stable conformation, along with the intermolecular bridging, was responsible for the apparent inert character of the carbonyl group.

Caubere and co-workers prepared a series of 1,2,3,4-tetrahydrocarbazol-4-ones which had spectra consistent with an enol form.⁴⁶ They prepared an *N*-methanesulphonate derivative which possessed all the spectral properties expected for the ketone, supporting the suggestion that the unsubstituted compound was in the enol form. However, X-ray examination of the crystal structures gave data corresponding to a hydrogen bonded ketone structure similar to that postulated by Rodriguez and suggested that the compounds were not in the enolic form. Caubere's group reached the same conclusion as Rodriguez, suggesting that strong bridging interactions between the N-H and the carbonyl group were maintained even in solution, and that this could account for the unusual IR and ¹H NMR data.

2.8 Synthesis

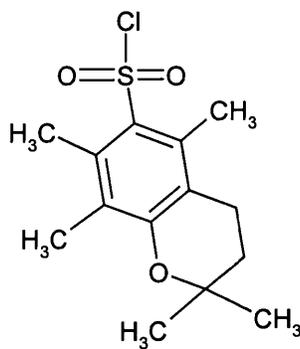
Our aim was to introduce a carbon chain at C4 and we envisaged a possible route starting from chiral reduction of the tetrahydrocarbazolone to give an alcohol. This might subsequently be converted to a nitrile, with inversion of configuration, using one of a range of literature procedures. Reduction by lithium aluminium hydride and subsequent acetylation of the amine might then give a chiral synthesis of the required compounds (scheme 15).



Scheme 15

A series of 6-substituted carbazalones were prepared as described in scheme 12. The aromatic substituent, R, was varied to investigate the possible influence on the reactivity of the carbonyl, *via* an effect on the electron donating or withdrawing ability of the nitrogen atom. In addition, this set of compounds was treated with pentamethylchromansulphonylchloride (Pmc-Cl, **93**) according to literature procedure⁴⁷ in order to protect the indole nitrogen. This protecting

group was chosen as it is readily and rapidly removed by trifluoroacetic acid in 1-3 hours.



93

A set of compounds were prepared with the substitution shown below in table 3.

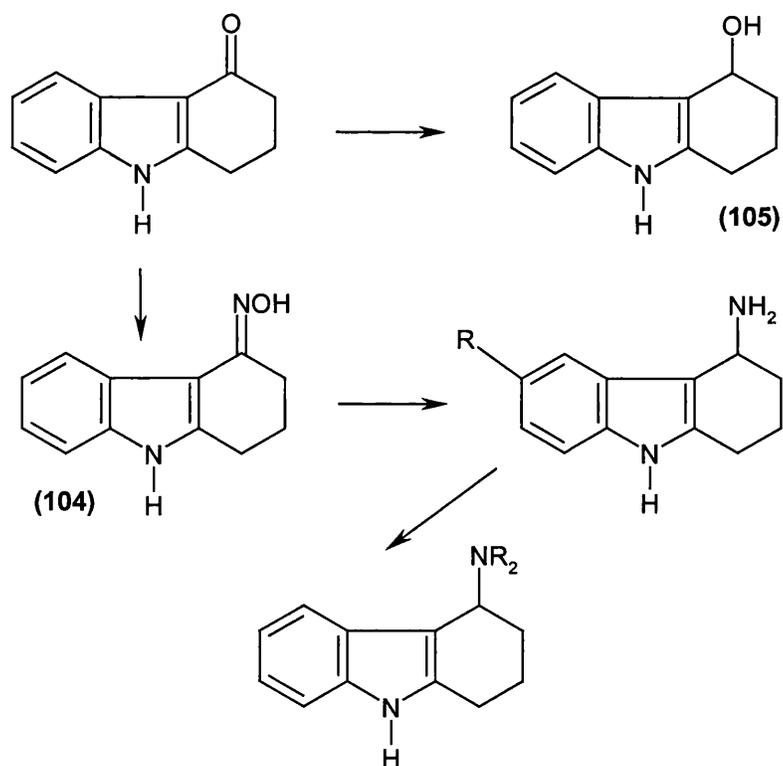
	R'	R	Compound
	H	H	94
	H	CH ₃	95
	H	OCH ₃	96
	H	Cl	97
	H	F	98
	H	(6,7)-O-CH ₂ -O-	99
	Pmc	H	100
	Pmc	CH ₃	101
	Pmc	OCH ₃	102
	Pmc	Cl	103

Table 3 Tetrahydrocarbazolones synthesised.

The IR spectra of both the tetrahydrocarbazolones and the enaminone intermediates were in agreement with the literature spectra described previously. The spectra displayed the characteristic strong, broad bands at ca. 3250 cm⁻¹ and a sharp band at ca. 1600 cm⁻¹, indicative of an enol structure. When these compounds were *N*-sulfonylated with the acid labile Pmc group, the resulting

compounds had IR spectra consistent with the ketonic form, having a strong band in the 1660cm^{-1} region and a somewhat weaker, but sharp, band in the 3000cm^{-1} region of the spectrum.

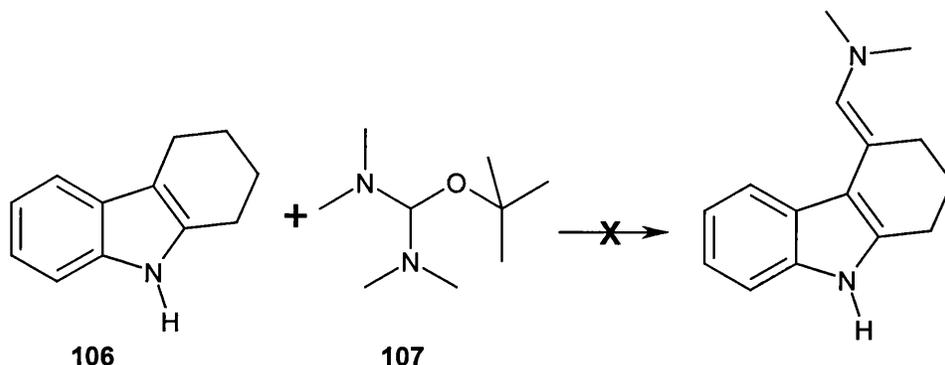
A search of the literature revealed a single paper which reported the successful reduction of **94** using sodium borohydride as the reducing agent.⁴⁸ The bulk of the paper deals with formation of an oxime (**104**) from the tetrahydrocarbazolone and its subsequent manipulation (scheme 16). The borohydride reduced product was characterised by melting point and CHN analysis only and was not subjected to further transformation. We attempted this reduction many times, on all of the prepared tetrahydro-carbazol-4-ones, **94-103**, as well as with various batches of borohydride and polymer supported borohydride, but could only recover starting material. In monitoring the reaction by HPLC and mass spectrometry, we were unable to detect the required product (**105**).



Scheme 16

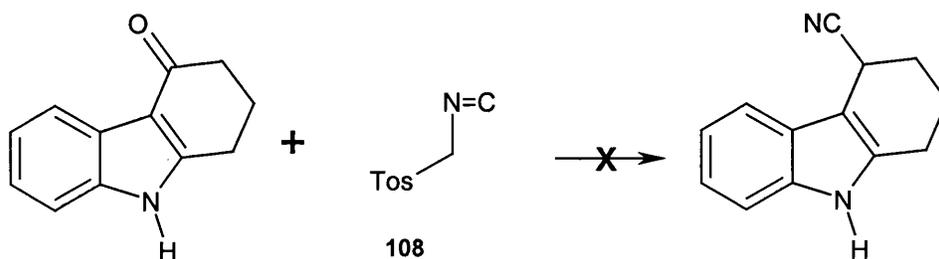
Treatment of **95** with lithium aluminium hydride however, resulted in complete reduction of the carbonyl function to the known cycloalkane **106**.⁴⁸ With this

compound in hand we attempted the direct functionalisation of this CH₂ position with tert-butoxybis(dimethylamino)methane (**107**, Brederick's reagent). Brederick's reagent has been used to functionalise compounds via enamination of active methylene groups⁴⁹ prior to nucleophilic displacement (scheme 17). However, no reaction occurred in our hands and starting material was recovered.



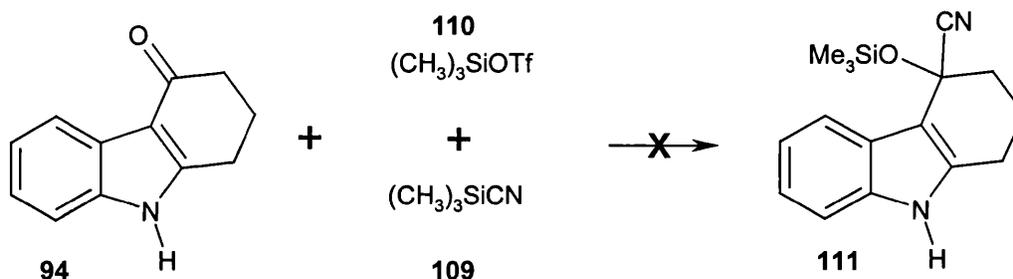
Scheme 17

The main route by which we hoped to achieve the synthesis of the N-H compounds required the formation of a nitrile (scheme 15). We first tried to introduce the nitrile functionality directly to a range of 1,2,3,4,9-tetrahydrocarbazol-4-ones (**94**, **97**, **99**, **100**, and **102**) using tosylmethyl isocyanide (**108**, TosMIC),⁵⁰ a proven reagent for the conversion of ketones to nitriles (scheme 18). Experiments using a range of reaction conditions (1-3 eq. of TosMIC, 3-6 eq. t-BuOK, 0 °C or 50 °C, and DME or DMSO as solvent) failed to yield any of the required material and the products were either starting material or complex mixtures.



Scheme 18.

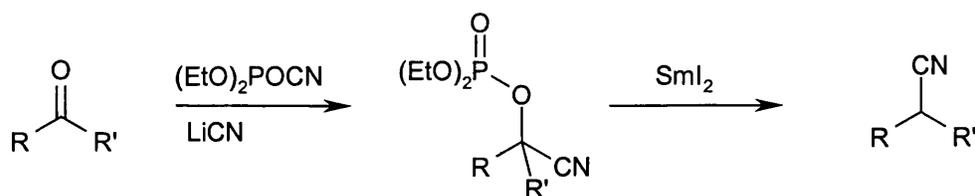
Noyori and co-workers⁵¹ have used a combination of TMS-cyanide **109** and TMS-triflate **110** to effect the formation of nitriles from ketones *via* a cyanohydrin trimethylsilyl ether **111** (scheme 19). This reaction was completely unsuccessful in all of our attempts to apply it to **94**, **95** or **97**.



Scheme 19.

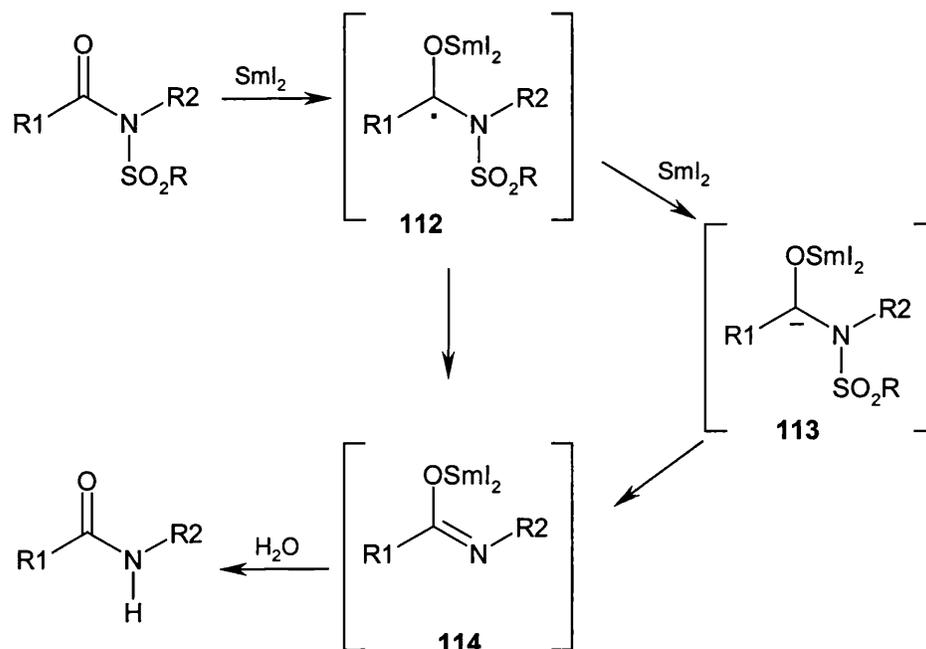
The use of TMS-CN/ZnI₂/POCl₃⁵² was also unsuccessful using **96** and a complex mixture containing ca. 40% starting material was obtained as the major product. Treatment of **95**, **96** and **98** with methanesulphonyl chloride in pyridine only gave starting material but in the case of **46** a small amount of material of lower molecular mass was also obtained which we were unable to identify. Similarly, attempts to prepare the TMS ether of compounds **95** and **100**, prior to reaction with sodium cyanide⁵³ were unsuccessful. Compound **94** was treated with nitromethane and sodium ethoxide in an attempt to prepare the 4-nitromethylcyclohexanol, but only starting material was recovered.

The cyanophosphorylation of carbonyl compounds has been successfully carried out by Kurihara⁵⁴ using diethyl phosphorocyanidate and lithium cyanide. The same group had converted the crude cyanophosphonates to nitriles by reaction with samarium iodide (scheme 20).



Scheme 20

No reaction occurred upon treatment of **94** and **95** under these conditions. However, we did observe smooth deprotection of the indole nitrogen when the pmc protected compounds **101**, **102**, or **103** were treated in the same way. Unfortunately, this deprotection was not accompanied by formation of the required nitrile, but a search of the literature revealed desulfonylation by samarium iodide to be a known reaction.⁵⁷⁻⁵⁹ Parsons has suggested two possible mechanisms for this deprotection.⁵⁹ The first involves electron transfer from samarium iodide to the sulphonyl group in line with literature precedent. An alternative possibility might involve electron transfer to the amide carbonyl to give a samarium (III) species **112**, which can either undergo subsequent β -elimination of a sulfonyl radical to give **114**, or, as a result of further reduction by samarium iodide, loss of a sulphonyl anion *via* **113** (scheme 21).

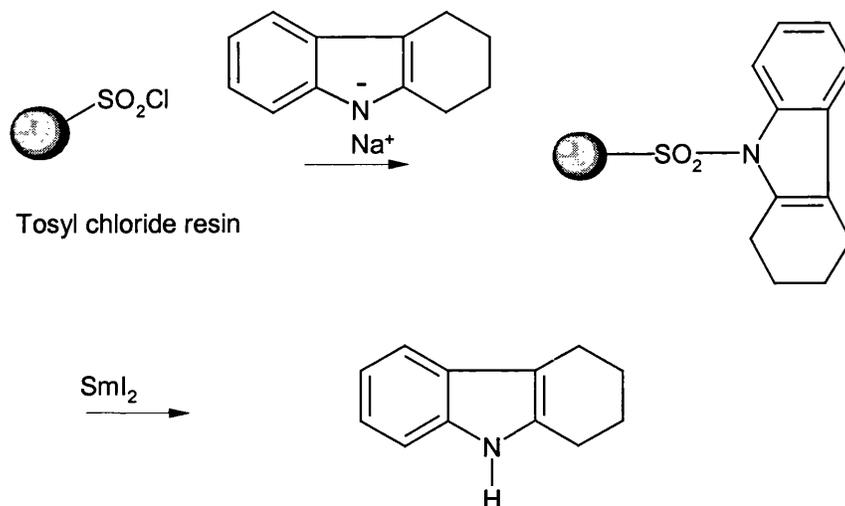


Scheme 21

2.9 Solid Phase Attempts

The effectiveness of the samarium iodide deprotection led us to consider the possibility of a solid phase approach to the target compounds. A major advantage of solid phase chemistry is the ability to drive reactions to completion by the use of excess reagents. These can then be washed away at the end of each step, thus avoiding the need for extensive purification at each stage of a synthesis. We envisaged the possibility of using a polymer supported sulphonyl chloride to tether an aniline, indole or tetrahydrocarbazole and at the end of the synthesis releasing the final products by a samarium iodide cleavage. As a solid phase reagent samarium iodide is particularly attractive as it is supplied (Aldrich) as a standardised solution in THF. This solvent is highly compatible with the use of polystyrene type resins, such as the commercially available tosyl chloride resin (Argonaut technologies). In our initial experiments, the anion of 1,2,3,4-tetrahydrocarbazole was generated in DMF by sodium hydride and then reacted

with polymer supported tosyl chloride. The resin bound material was subsequently treated with an 0.1 M solution of samarium iodide in THF and a pale yellow solid recovered on work-up which was identical by mass and nmr spectra to the 1,2,3,4-tetrahydrocarbazole starting material (scheme 22).

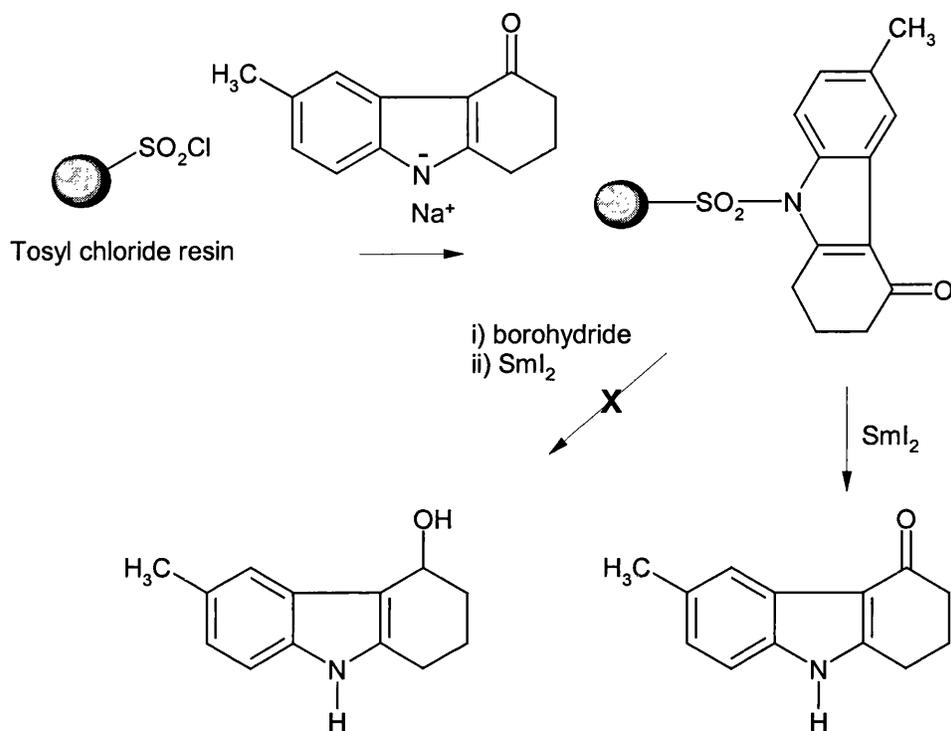


Scheme 22

Compound **95** was attached to tosyl chloride resin *via* the anion, using the above protocol, and cleavage of a small sample of resin by samarium iodide returned the starting material in good yield and high purity. Several attempts to reduce this polymer bound ketone with sodium borohydride, sodium triacetoxyborohydride or lithium borohydride failed, although the starting ketone was recovered each time, on cleavage with samarium iodide (scheme 23).

These attempts were made using either dry THF or THF containing 1% water as the solvent since the resin swelling properties of the solvent are an important consideration. THF is clearly not a particularly good choice of solvent for the borohydrides and the more commonly used aqueous or alcohol based solvents are not well tolerated by polystyrene type resins. It is possible that an alternative solvent/resin combination might give a different result, however, limited time did

not allowed us to pursue this further.



Scheme 23

While the attempts outlined here, both in solution and on solid phase were not exhaustive, the carbonyl group of these tetrahydrocarbazolones did appear to be relatively inert to a range of chemistry. This was probably either due to enol formation, or to the intermolecular hydrogen bond formation suggested by Rodriguez and Caubere. Time did not permit a more thorough and methodical investigation into the chemistry of these compounds.

Biological results

2.10 Binding Affinity Studies

In-vitro screening of compounds at the high affinity melatonin binding site in chicken brain membranes was determined in a competition radioligand binding

assay using 2-[¹²⁵I] iodomelatonin and was carried out by Dr. D. Sugden and co-workers, Kings College, London. White Leghorn chicks (*Gallus domesticus*, Orchard Farms, Buckinghamshire, U.K.) were purchased at 1 day of age and housed for two weeks under controlled lighting conditions (12 L : 12 D, lights on at 06.00 h) in a temperature controlled room (28 +/- 2 °C). Chicks were decapitated between 14.00 and 15.00 h and the whole brain rapidly removed and frozen in liquid nitrogen. Whole brain membranes were prepared as previously described,⁵⁸ suspended in Tris -HCl (50 mM, pH 7.4) containing phenylmethylsulphonyl chloride (1mM), leupeptin (50 µg/ml) and EGTA (1 mM) and aliquots stored in liquid nitrogen at -70 °C at a concentration of approximately 1-2 mg of protein /ml. In competition experiments, duplicate membrane aliquots were incubated (25 °C, 60 min) with competing drugs and 70-80 pM of 2-[¹²⁵I] iodomelatonin (2200 Ci/mmol, DuPont U.K. Ltd. Stevenage, UK). The assays were terminated by the addition of ice cold Tris-HCl buffer and filtered immediately through glass fibre filters. After twice washing the filter with buffer the filtrate was counted on a LKB 1282 Compugamma CS counter. Non specific binding was defined using cold melatonin (1mM; Sigma, Poole Dorset, UK).

2.11 Biological Activity

The biological activity of the melatonin analogues *in vitro* was assessed using the pigment aggregation response in *Xenopus laevis* melanophores. The black pigment in these cells is contained in small granules called melanosomes which when dispersed, cause the cells to appear black, and upon aggregation, white. Addition of melatonin agonists to these cells triggers a rapid aggregation of pigment granules towards the centre of the cell, an effect which can be reversed by the application of melatonin antagonists. This change in pigment granule distribution in primary cultures of melanophores has been used previously^{59,60} to

construct concentration–response curves of various melatonin analogues, in order to define agonist and antagonist activity. The present study used a clonal *Xenopus laevis* dermal melanophore cell line provided by Dr. Michael Lerner (Department of Dermatology, University of Texas, USA). The melanophores were grown as previously described⁶¹ in 96-well tissue culture plates and changes in pigment granule distribution were quantitated by measuring absorbance (630 nm) before and after the addition of a methanolic solution of the test compounds. Drugs were prepared as stock solutions (10 mM) in methanol and stored at -20°C in the dark. Immediately prior to use, the drug solutions were diluted with de-ionised water and added to cultures from the diluted stock solutions. The maximum concentration of methanol (1%) did not alter specific binding or pigment granule distribution in the melanophores. Concentration response curves were determined using a range of concentrations of analogues in 2-4 wells of melanophores. EC_{50} values, representing the concentration of the analogue necessary to produce 50% of the maximal pigment aggregation, were determined after 10 minutes incubation. Antagonist potency was measured by adding test compounds to melanophores 1 hour before challenging with melatonin (10^{-9} M), a concentration which consistently produces a maximal aggregation of pigment. Each concentration of antagonist was tested on 2-4 wells of melanophores. IC_{50} values, representing the concentration of the test compound which blocked melatonin induced pigment aggregation by 50%, were calculated.

2.12 Data analysis

Binding affinity is reported as an inhibition constant (K_i), indicating the ability of melatonin analogues to displace the radioligand 2-[^{125}I] iodomelatonin from receptors in chick brain membranes. IC_{50} values were determined in competition assays using the ALLFIT programme⁶² using the equation:

$$Y = \frac{A - D}{1 + (X/C)^B} + D$$

Where X and Y are the concentration of competing compound and percentage inhibition of 2-[¹²⁵I] iodomelatonin binding respectively. A is the maximal binding (in the absence of competitor), B is the slope factor, C is the IC₅₀, and D is the nonspecific binding.

The inhibition constants were calculated using the Cheng-Prussoff equation:⁶³

$$K_i = \frac{IC_{50}}{1 + (Ligand / K_d)}$$

Where IC₅₀ is the concentration of competing drug which reduces specific binding by 50% and K_d is the dissociation constant obtained from kinetic experiments.

Agonist efficacy is reported as an EC₅₀ value, which represents the concentration of the analogue needed to produce 50% of the maximal pigment aggregation.

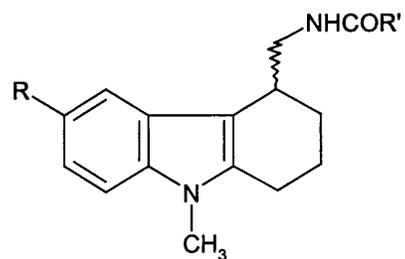
Results and discussion

The results of the radioligand binding and melanophore assays for the *N*-acyl-4-aminomethyl-9-methyltetrahydrocarbazole compounds are shown in table 4. Any interpretation of the receptor binding data reported here must take account of the fact that the results were obtained on chicken brain membrane which may contain multiple receptor sub-types. It is possible that differential binding of the various analogues to different receptor subtypes may have occurred and that a different profile may be observed when we are able to test the compounds on specific sub-types.

2.13 Binding Affinity

All of the 6-substituted derivatives have a higher binding affinity than the parent 6-H compound **64** pointing to the existence of a binding interaction in this region. We, and others, have proposed that the oxygen of the methoxyl group may be involved in a hydrogen bonding interaction with a residue within the receptor.¹³ Replacing the methyl of the 6-methoxyl group with the trifluoromethyl group results in a dramatic loss of binding affinity which may indicate that the methyl is occupying a neutral hydrophobic pocket not easily accessed by the more polar OCF₃ group. Replacing the methoxyl group with a small alkyl group such as methyl (**71**) or ethyl (**74**) results in compounds having lower binding affinities, as we might predict on the basis of the absent oxygen atom. Interestingly, the 6-chloro derivative **65** has a high binding affinity, almost comparable with that of the 6-methoxy compound **25**.

Previous studies within the group¹ have shown that the size of the acylating group on the aminomethyl side chain is important and this observation is re-inforced by the results for the current series. For a given substituent in the 6 position, variation of the C-3 acylating group follows a pattern in which binding increases slightly from acetyl to propanoyl and then decreases rapidly with increasing size, as in the cyclopropyl and cyclobutyl compounds. The binding affinity and biological activity of this series of compounds is reported in tables 4 and 5.



Compound	R	R ¹	Binding [Ki], nM	Agonist EC ₅₀ (nM)	Antagonist IC ₅₀ (μM)
Melatonin			0.59 +/- 0.1	0.69	N/A
64 (+/-)	H	CH ₃	227 +/- 39	599	N/A
64 (+)	H	CH ₃	708 +/- 60	N/A	6.4
64 (-)	H	CH ₃	40 +/- 3.1	189	N/A
25 (+/-)	OCH ₃	CH ₃	0.97 +/- 0.2	0.70	N/A
25 (+)	OCH ₃	CH ₃	48 +/- 8	52.3	N/A
25 (-)	OCH ₃	CH ₃	0.372 +/- 0.01	0.23	N/A
65	Cl	CH ₃	2.12 +/- 0.01	5.2	N/A
66	Cl	c-C ₃ H ₅	131 +/- 39	1511	33
67	Cl	c-C ₄ H ₇	187 +/- 58	N/A	14
68	CF ₃ O	CH ₃	141 +/- 58	2030	42
69	CF ₃ O	C ₂ H ₅	40 +/- 6	733	6
70	CF ₃ O	c-C ₄ H ₇	1240 +/- 240	N/A	41
71	CH ₃	CH ₃	16.1 +/- 2.7	108	N/A
72	CH ₃	C ₂ H ₅	7.7 +/- 1.2	128	N/A
73	CH ₃	c-C ₄ H ₇	555 +/- 92	1450	N/A
74	C ₂ H ₅	C ₂ H ₅	27.5 +/- 4.8	305	62

Table 4 Binding affinity and biological activity of various cycloalkan[b]indoles.

The difference in binding between the pairs of enantiomers was of particular interest and is illustrated for one pair in Figure 19. The affinity of the (S) (-) enantiomer of **25** at the 2-[¹²⁵I] iodomelatonin binding site was 130 fold higher than the affinity of the (R) (+) enantiomer of **25** and comparable with that of melatonin itself. The racemate, **25**, had an affinity which is close to what would be expected for a 50:50 mixture of the two enantiomers.

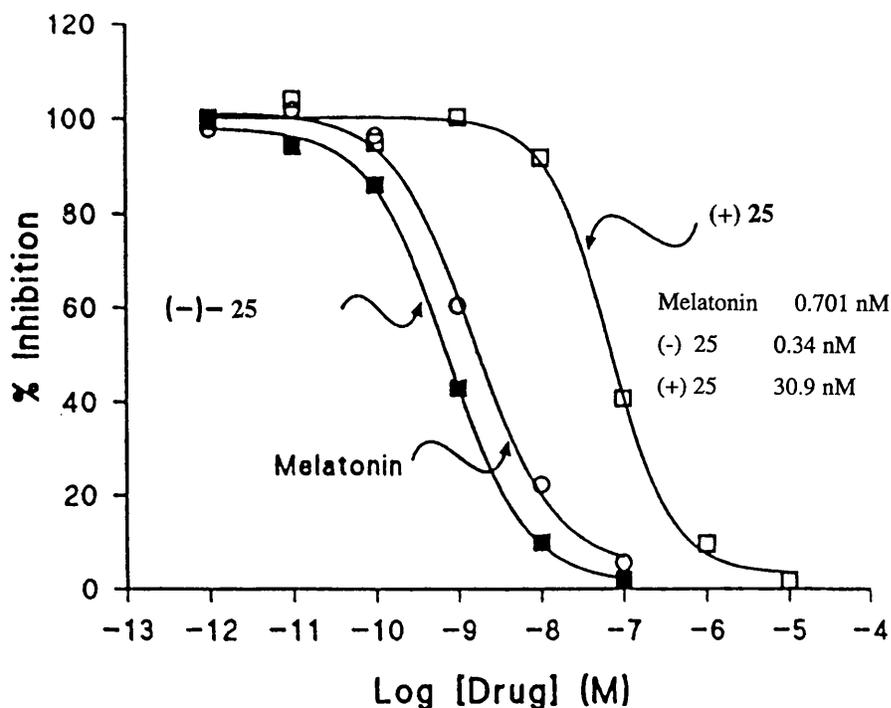
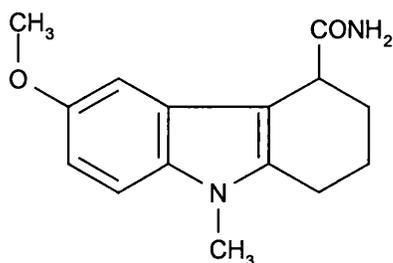


Figure 19. Inhibition of 2-(¹²⁵I) iodomelatonin binding in chicken brain membranes by melatonin and the enantiomers **25** (-) and **25** (+).

An 18 fold difference in binding affinity between enantiomers was also observed between the unsubstituted compounds (+) **64** and (-) **64** (R = H) despite the intrinsically lower affinity of the racemate **64** compared to **25**. Interestingly, chiral HPLC separation of the synthetic intermediate **115** gave two enantiomers

which also demonstrated a 5 fold difference in binding affinity in favour of the (S)-(-) enantiomer.

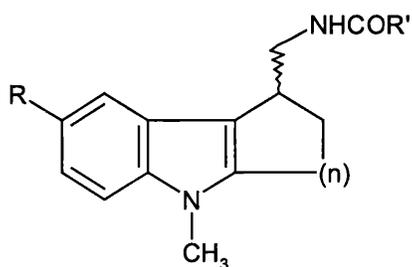


115 (+) Ki 2170 +/- 21 nM

115 (-) Ki 448 +/- 45 nM

The large difference in binding affinity between the two enantiomers (+) **25** and (-) **25** suggests that the orientation as well as the distance between the methoxyl and the ethanamide side chain is of great importance. The observation that there is still a substantial difference between the enantiomers, (+) **64** and (-) **64**, of the 6-H compound indicates that the binding site also has a geometrical requirement for the relative orientation of the ethanamide side chain and the tetrahydrocarbazole core.

The effect of increasing or decreasing the size of the annelating ring is shown in table 5.



Compound	n	R	R ¹	Receptor binding Ki (nM)	Agonist EC ₅₀ (nM)	Antagonist IC ₅₀ (μM)
62 (+/-)	1	OCH ₃	C ₂ H ₅	16 +/- 2.2	408	N/A
62 (+)	1	OCH ₃	C ₂ H ₅	243 +/- 24	789	N/A
62 (-)	1	OCH ₃	C ₂ H ₅	1.7 +/- 0.14	3.4	N/A
116	1	OCH ₃	CH ₃	161 +/- 20	423	N/A
25 (+/-)	2	OCH ₃	CH ₃	0.97 +/- 0.2	0.7	N/A
25 (+)	2	OCH ₃	CH ₃	48 +/- 8	52.3	N/A
25 (-)	2	OCH ₃	CH ₃	0.372	0.23	N/A
117	3	OCH ₃	CH ₃	24 +/- 3.5	258	10
63 (+/-)	3	OCH ₃	C ₂ H ₅	7 +/- 0.8	91 ^a	Ant
63 (+)	3	OCH ₃	C ₂ H ₅	201.5 +/- 20	N/A	Ant
63 (-)	3	OCH ₃	C ₂ H ₅	6.5 +/- 0.7	118 ^a	Ant

Table 5 Binding affinity and activity data for cycloalkan[b]indoles with different annelating ring size.

The highest binding affinity value of the three racemic compounds was obtained with the tetrahydrocarbazole **25** and the observed difference in binding between the (S) and (R) enantiomers of **25** is paralleled in the different binding affinities of

the hexahydrocyclohept[b]indole and tetrahydrocyclopent[b]indole enantiomers. The binding affinity data for the 6-methoxytetrahydrocarbazoles shows that the chick brain melatonin binding site displays a 130 fold preference for (S)-(-)-**25** compared to the (R)-(+)-**25** enantiomer. The 6-methoxyhexahydrocyclohept[b]indoles show a smaller difference between (S)-(-)-**63** and (R)-(+)-**63** of approximately 30 fold and for the 6-methoxytetrahydrocyclopent[b]indole the difference between (S)-(-)-**62** and (R)-(+)-**62** is 140 fold (Figure 20).

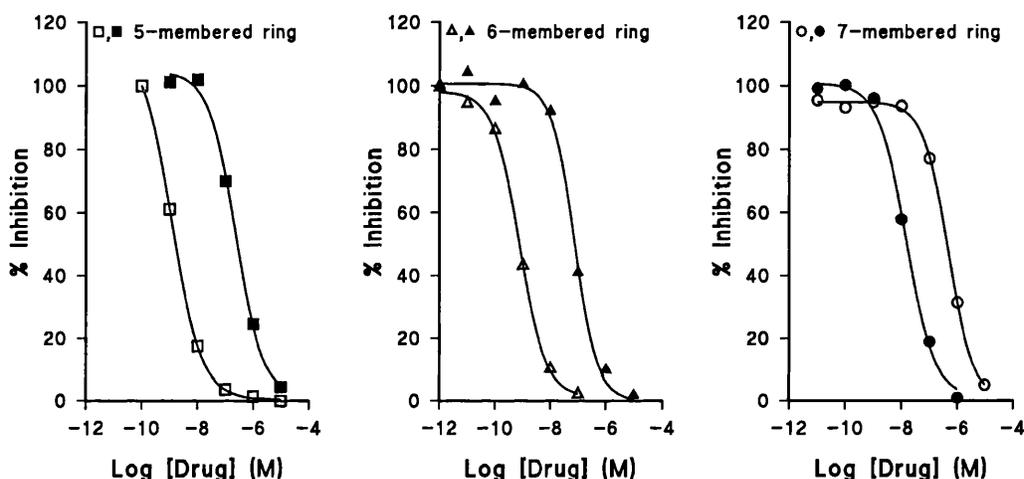


Figure 20. Differences in binding affinity between enantiomers. S-enantiomers shown by open symbols, R-enantiomers by filled symbols.

The observation that the (S)-(-)-enantiomers of the cyclopent[b]indole and cyclohept[b]indole derivatives also have higher binding affinities supports the view that the relative orientation of the acyl side chain to the substituted indole ring is of great importance.

The energy minimised structures for the racemic compounds **25**, **116** and **117** are shown in figure 21. Superimposing the models of the tetrahydrocarbazole and cyclohept[b]indole derivatives demonstrates a near coincidence of the

aminomethyl side chains, with that of the cyclopent[b]indole derivative being somewhat displaced. This re-inforces our view,⁶⁴ and that of others from modelling of non-indole derivatives,^{65,27} that the spatial relationship of the methoxy group and the acetamido side chain is of major importance in determining the binding affinity.

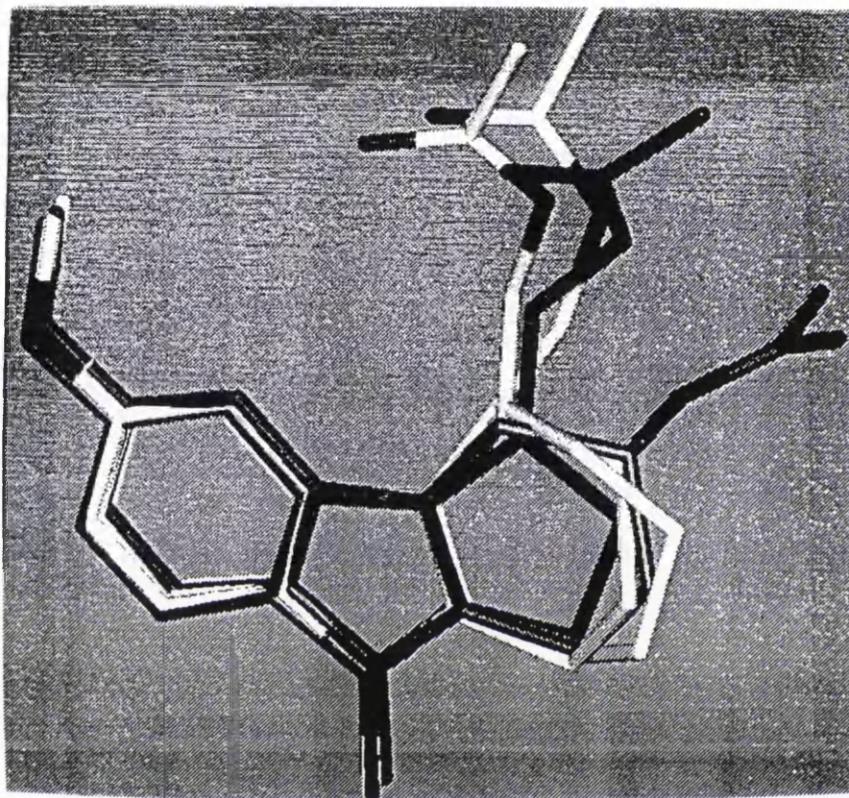


Figure 21. Overlay of the energy minimised structures of *N*-acetyl-aminomethyl-cycloalkan[b]indoles **25**, **117**, **116** and the inactive compound **44**.¹

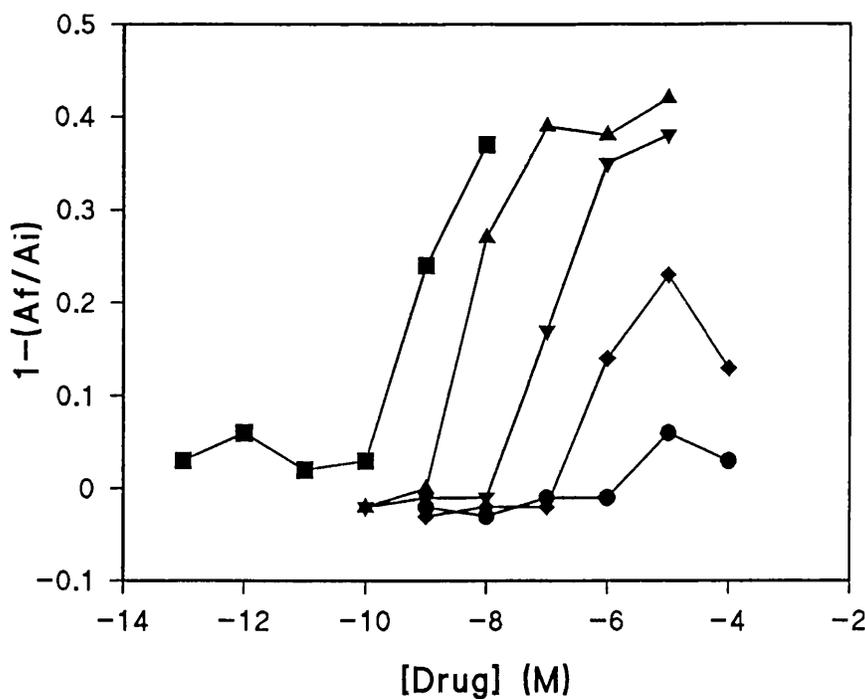
N-Methylmelatonin was also prepared and assayed, in order to determine the likely effect of having this substituent on the pyrrole nitrogen (see chapter 4). The results of the assay show an approximately 20 times lowering of binding affinity as a result of having this substituent in place. It does appear to be well tolerated

however, and we would anticipate that a similar increase in binding would be observed for the unsubstituted cycloalkan[b]indoles.

2.14 Biological activity

Tables 4 and 5 also contain the biological activity data obtained from this set of compounds. The melatonin antagonist luzindole, **33** was also assayed and found to have a K_i of 1606 +/- 143 nM, and an IC_{50} of 2.1 μ M. Luzindole is an antagonist which shows a modest selectivity for MT_2 and the relatively low K_i values obtained in these experiments suggests that the observed aggregation response may be a mt_1 receptor mediated event.

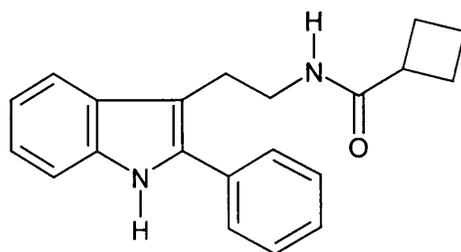
All of the compounds having a 6-methoxyl substituent evoked a full biological response as agonists at nanomolar concentrations and elicited no antagonist effect at concentrations of up to 100 μ M. Substitution of the 6-methoxyl by a methyl group causes a reduction in activity, but the compounds still behave as full agonists with no antagonist effect at concentrations of up to 100 μ M. Replacing the methoxyl with a trifluoromethoxy group, however, not only reduces binding affinity and agonist potency, but produces compounds with antagonist activity. Compounds with a 6-chloro substituent have binding affinities comparable those with a 6-methoxyl group as previously noted, but the compounds show marked differences in terms of biological activity. The acetyl compound **65** has a high binding affinity, and is a full agonist but on changing the acylating agent to an alicyclic group, as in **66** or **67**, the resulting compounds have little or no agonist activity but clear, if relatively weak, antagonist activity. To date, all known antagonists at the melatonin receptor are relatively weak (μ M compared to nM) compared to the agonists. The effect of the 6 substituent on agonist potency is shown in figure 22.



Legend: ■ 25, ▲ 65, ▼ 71, ◆ 64, ● 68.

Figure 22. Concentration-response curve showing the effect of changing the R group on agonist potency. Melanophore cells were grown in 96 well plates and initial absorbance (A_i , 630 nm) of the cells was measured. The cells were then treated with the concentrations of the analogues indicated. The final absorbance (A_f) was measured after 60 minutes and the fractional change $[1 - (A_f/A_i)]$ calculated.

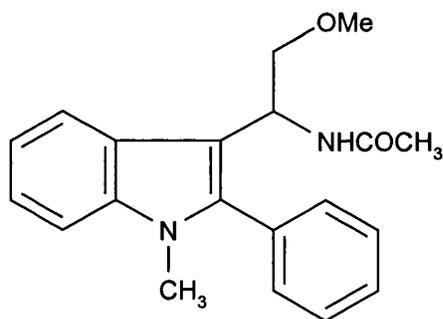
The observation that derivatives lacking a methoxyl group but having an alicyclic acylating group, such as *N*-(cyclobutylcarbonyl)-2-phenyltryptamine, **119**, can behave as antagonists was reported previously.⁶⁵



119

In the present series of analogues those having a larger aliphatic acyl group, such as cyclobutyl, also show antagonist activity, as does the trifluoromethoxyl compound 70. The cyclohept[b]indole compounds 63, (+) 63 and (-) 63 are unusual in that they show antagonistic activity while still retaining the methoxyl group that is so important in conferring a high receptor binding affinity. It is possible that this antagonism arises from competitive binding of these derivatives to the active site without evoking a corresponding biological response.

A racemic 2-phenyl melatonin analogue prepared by Jones,⁶⁶ had been shown to exert an antagonist effect in the *Xenopus* melanophore assay. We separated a small sample of this compound, 120, by chiral HPLC and observed a small but distinct difference in binding between the two enantiomers (ca. 3 fold). This lower degree of discrimination between the enantiomers can perhaps be explained by the greater degree of freedom in the branched acyl side chain compared to the more constrained cyclic compounds. Alternatively, it may reflect a general lowering of sensitivity associated with the properties of an antagonist.



120	(+)	Ki 303 +/- 12 nM
120	(-)	Ki 110 +/- 5 nM

The difference in binding affinity observed between enantiomers is reflected in the large differences in biological potency. The enantiomers (+) 64 and (-) 64 show weak biological responses generally, with the (S)-(-) enantiomer binding more strongly and showing weak agonism. The (R)-(+ enantiomer has no detectable agonism but shows some antagonistic activity. Introduction of the 6-methoxyl group increases both binding and biological activity, both enantiomers

behaving as full agonists. The compound (S)-(-) **25** shows a 230 fold increase in potency over the (R)-(+)-enantiomer (Figure 23).

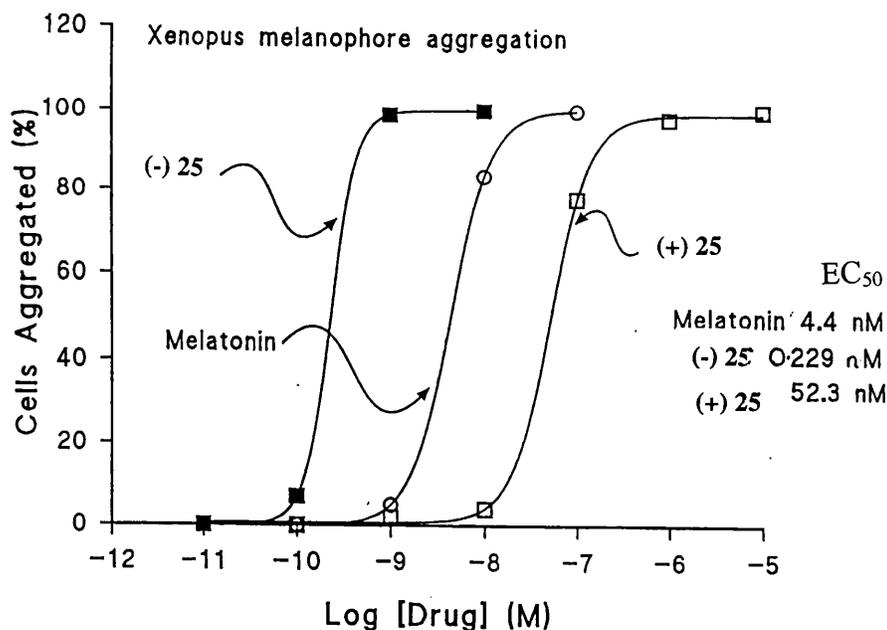


Figure 23 Pigment granule aggregation concentration-response curves for melatonin, (-) **25**, and (+) **25**. The percentage of melanophores showing clear pigment aggregation in response to concentration of compound is plotted.

The tetrahydrocarbazole enantiomers (S)-(-) **25** and (R)-(+)-**25** have begun to be used in studies on melatonin action and the pharmacological identification of receptor subtype. Wilson *et al.* have examined the vasoconstrictor action of melatonin in rat caudal arteries and used various indole based analogues to characterise the receptor.⁶⁷ The published results demonstrate a 400 fold difference in activity between the two enantiomers of compound **25** in this model (figure 24).

This behaviour of the enantiomers was cited as supporting evidence for characterising the rat caudal receptors as belonging to the mt₁ subtype and

underlining the essential similarity between vascular and non vascular mt_1 receptors. The two enantiomers were also used in a study on fish retinae where they were found to block light induced dopamine release at the horizontal cell D1 receptor sites specifically.⁶⁸ Interactions of melatonin receptors with dopamine D1 receptors which resulted in the blockade of dopamine induced cAMP accumulation had previously been shown in chick retinal neurones and the authors therefore deduce the presence of melatonin receptors on teleost cone horizontal cells.

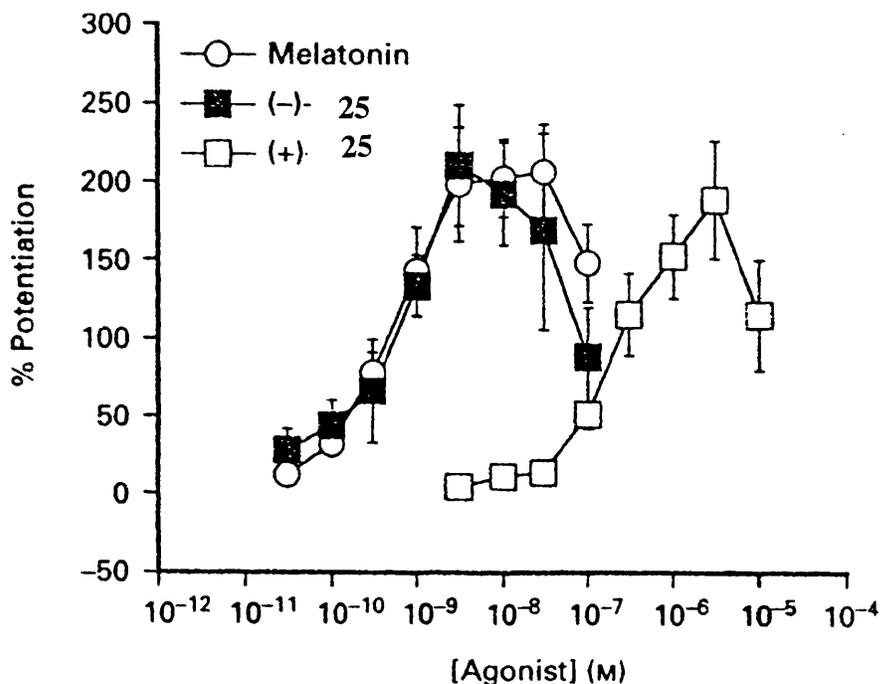


Figure 24 The effect of melatonin, (+) 25 and (-) 25 on electrically evoked contractions of the isolated tail arteries from juvenile male rats.⁶⁷

Agonism is a complex biological response and is system dependant. A common experiment to confirm true agonist behaviour is to investigate the effect of the addition of a competitive antagonist on cells treated with the putative agonist.

This should result in reversal of the observed biological effect and indicates that both compounds are likely to be competing for the same receptor.

The aggregation of melanophores induced by compounds (+) **25** and (-) **64** could be reversed by melatonin antagonists such as luzindole and *N*-cyclobutanecarbonyl 2-phenyltryptamine (Figure 25). This data was obtained by measuring the area occupied by pigment in individual cells before treatment (clear bars), 15 min after agonist treatment (melatonin 10^{-8} M, hatched bars) and 30 min after subsequent addition of *N*-cyclobutanecarbonyl 2-phenyltryptamine (10^{-8} M; double hatched bars).

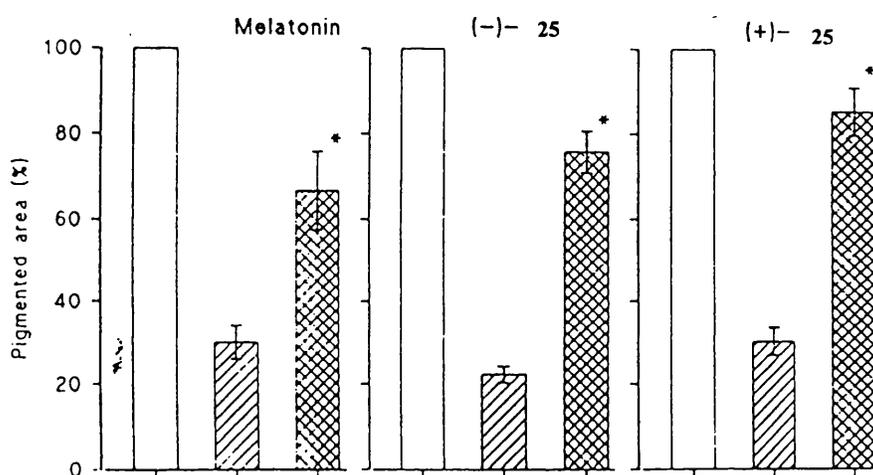
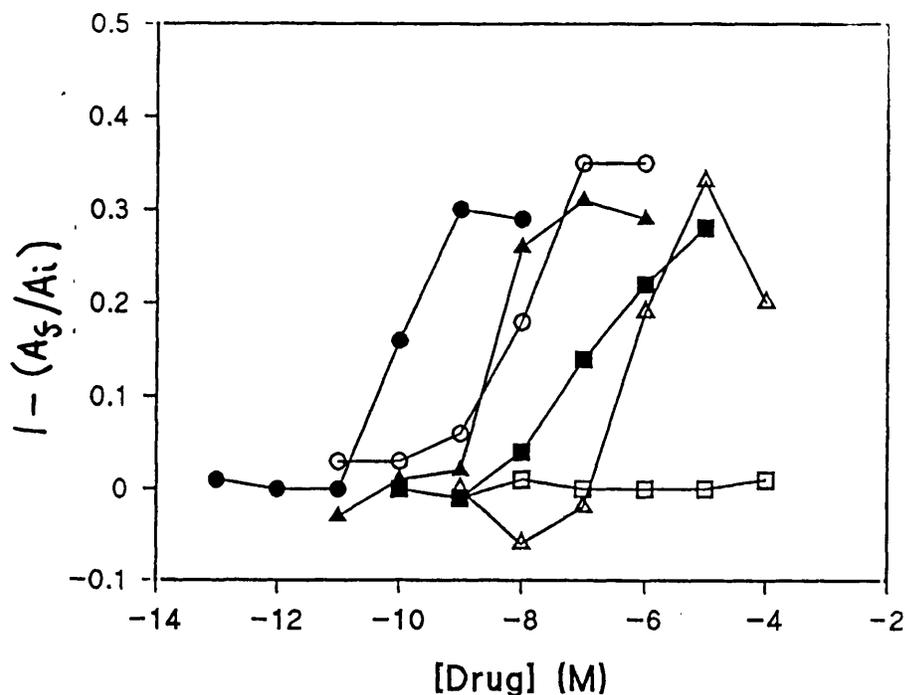


Figure 25. Reversal of the agonist effect of (+)-**25** and (-)-**25** by melatonin antagonists.

The biological responses of the 6-methoxy-tetrahydrocyclopent[b]indole enantiomers are similar to those of the tetrahydrocarbazoles. Both are full agonists with the (S)-(-) **63** enantiomer having a 230 fold greater potency than the (R)-(+)**63** enantiomer. In contrast to this, the hexahydro cyclohept[b]indole compounds (+) **62** and (-) **62** exert only weak effects and show either partial or no agonist behaviour while both display a degree of antagonist activity. Despite this,

the enantiomers still display a difference in potency, albeit less than for the other series. The (S)-(-) **63** enantiomer is 30 fold more active than the (R)-(+) **63** enantiomer. The differences in potency of the pairs of enantiomers are illustrated in figure 26.



Legend: ● (-) **25**, ○ (+) **25** ▲ (-) **62** △ (+) **62**, ■ (-) **63** □ (+) **63**.

Figure 26. Graphical comparison of the potencies of enantiomers of some cycloalkan[b]indoles with different annelating ring sizes.

The observed differences in binding and biological activity between (S) and (R) enantiomers re-enforces the view that orientation as well as distance between the methoxy and acetamido groups is important. The annelating ring, is clearly important in presenting the C4 side chain in a favourable orientation, with the S(-) enantiomer of **25** most closely resembling that adopted by melatonin itself, in its active conformation. It is possible that for the (R) enantiomer to achieve the

correct orientation relative to the methoxy group it is necessary for the cycloalkan[b]indole core to tilt, thus introducing a destabilising effect.

Before discussing a possible model for the way in which melatonin might bind to the receptor it is worth summarising the main points that have arisen from the study of the cycloalkan[b]indole series.

- 1) The presence of a 6-methoxyl group confers high binding affinity at the melatonin receptor but is not an essential requirement for biological activity.
- 2) There is a distance requirement for the binding motifs present at the positions corresponding to C3 and C5 of melatonin
- 3) The size and nature of the substituent on the ethanamine side chain is important in conferring agonist or antagonist behaviour.
- 4) The orientation of the binding motifs present at the positions corresponding to C3 and C5 of melatonin are critical.
- 5) The greater affinity and efficacy of the (S) (-) enantiomers suggests that this orientation most closely approximates the conformation adopted by melatonin itself when interacting with the receptor
- 6) There may be a geometrical requirement for the relative orientation of the ethanamine side chain and the tetrahydrocarbazole core independent of the relative orientation of the ethanamine side chain and the methoxyl group.

The binding site for small molecules in G-protein coupled receptors is thought to involve the residues in the hydrophobic transmembrane domains,⁶⁹ and it is generally believed that a three point interaction is at least necessary to achieve the high affinity, selectivity and efficacy observed in most natural ligands. The low water solubility of melatonin and its analogues suggests that the receptor pocket is largely lipophilic in nature.

Structure activity investigations, site directed mutagenesis studies, and the clear homology with the transmembrane domains of bacteriorhodopsin have led to proposed binding site models for catecholamine and 5-HT receptors.^{70,71} These models postulate hydrogen bonding between hydroxyl groups and specific amino acid residues in the fifth transmembrane helix and π - π stacking in the sixth. A similar putative binding motif (Asp in helix III and Ser in helix V) is observed for dopaminergic, adrenergic and serotonergic receptors. In the 5-HT_{2c} receptor site model⁷² an alignment of helices II-VI in an approximately parallel symmetric arrangement gave rise to an interhelical channel of appropriate dimensions for binding to 5-HT. The putative site lies near the top of this channel and contains an aspartate residue in helix III which pairs to the amino group and a serine residue in the fifth transmembrane helix which acts as a hydrogen bond donor/acceptor with the indole OH group. The recent cloning of distinct melatonin receptor subtypes from various species has allowed the comparison of the melatonin receptor with that of the catecholamine and 5-HT receptors at the level of amino acid sequence.

The similarity of melatonin to 5-HT is strong, although the former is obviously much less polar and less basic, with the amine functionality of 5-HT being replaced by an amide and the hydroxyl group being replaced by a methoxyl. Modelling of the putative transmembrane regions of the cloned melatonin receptor of *Xenopus melanophores*⁷³ allows us to speculate on a possible binding model for melatonin which is consistent with this data and the structure of *N*-acetyl-4-(aminomethyl)-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole **25**.

We have suggested⁷⁴ that the absence of a conserved serine residue in the fifth transmembrane helix and the occurrence of a conserved histidine residue in all melatonin receptors so far cloned can be rationalised by the histidine acting as a proton donor to the C5 methoxy group of melatonin or the C6 methoxy group of **25**. Mutagenesis studies have indicated that a conserved aspartate residue in the third transmembrane helix provides a counterion for the basic amine group of the 5HT receptor ligand. This residue is not conserved in the cloned melatonin

receptors and we have suggested⁶⁵ that the two conserved serine residues in the third transmembrane helix might be acting as hydrogen bond donor/acceptors to the *N*-acetyethanamine side chain (N-H and C=O moieties). A phenylalanine in the sixth transmembrane helix of the 5HT receptor is thought to interact with the aromatic ring by a π - π stacking arrangement and is conserved in all receptors which bind aromatic biogenic amine ligands. The sixth transmembrane domain of all the cloned melatonin receptors so far obtained have two conserved phenylalanine residues together with a conserved tryptophan residue, all of which would be suitable for π - π stacking and provide a fourth point of interaction at the receptor site. A schematic model showing the possible molecular interactions of (S)-(-)-*N*-acetyl-4-aminomethyl-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole, in the melatonin receptor pocket is shown in Figure 27.

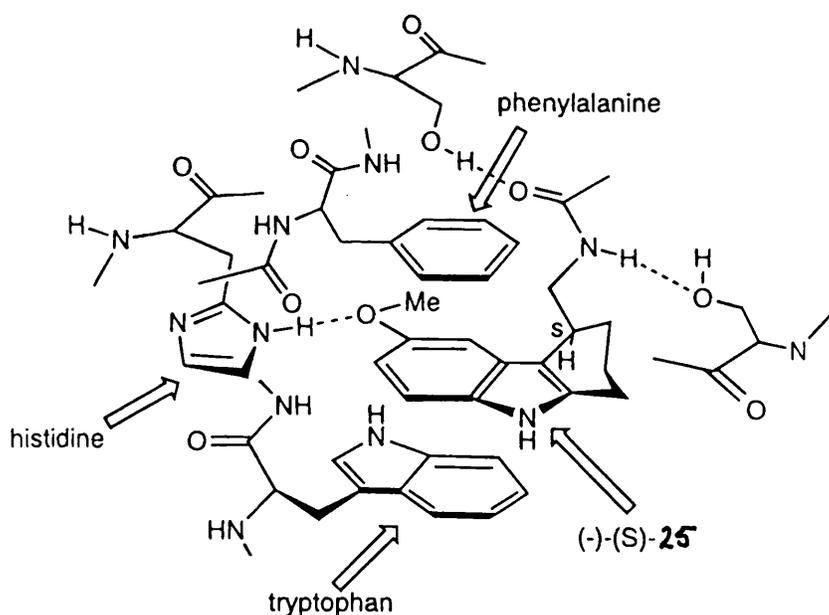


Figure 27. Schematic model showing possible interactions between (S)-(-) 25 and conserved amino acids within transmembrane domains of the melatonin receptor.

2.15 Summary

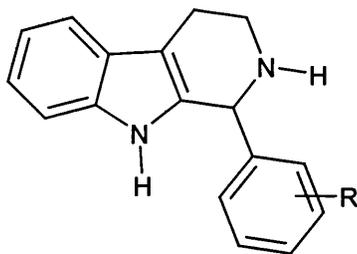
We have prepared a series of conformationally constrained melatonin analogues and resolved several racemic compounds into individual enantiomers by chromatographic means. The ability of the melatonin receptor to display chiral discrimination was demonstrated for the first time and the information gained about conformational preference will be used in future modelling studies. The enantiomeric compounds have begun to find use in receptor distribution studies and may be of some use in distinguishing between receptor subtypes. Variation of the substituent at the C6 position of the cycloalkan[b]indoles has given some information about the likely size and nature of the binding regions which interact with these parts of the molecule.

Chapter 3

Tetrahydro- β -carbolines

Aim

Structure activity relationship studies support the assertion that high potency at the melatonin receptor is dependant on attaining an optimal distance and spatial arrangement between the methoxyl and acetamido moieties. Several research groups have incorporated these two pharmacophores into alternative indole or non indolic templates and demonstrated high binding affinities at the melatonin receptor (see chapter 1). We wished to examine the possibility of using a different indole based template to present the two pharmacophores in a biologically active conformation, and gain more insight into structure activity relationships. A class of compounds which appeared attractive both from this viewpoint and from their ease of synthesis were the N_b -acyltetrahydro- β -carbolines. These compounds are readily accessible by appropriate substitution of tetrahydro- β -carbolines (121).

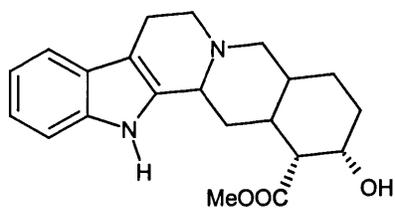


(121)

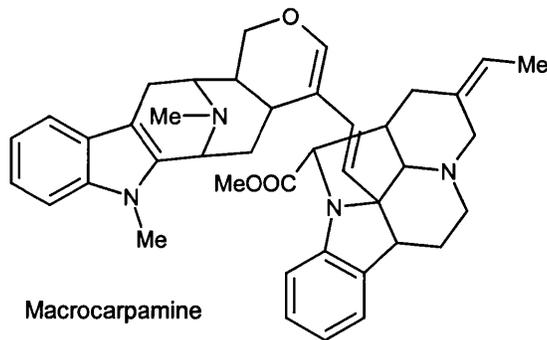
We wished to prepare a series of tetrahydro- β -carbolines in which the substituent R contained a methoxy or acetamido moiety and to introduce the second pharmacophore by acylation of the carboline N-H with an appropriate acid. We also wished to synthesise a number of compounds containing alternative substituents.

3.1. Introduction

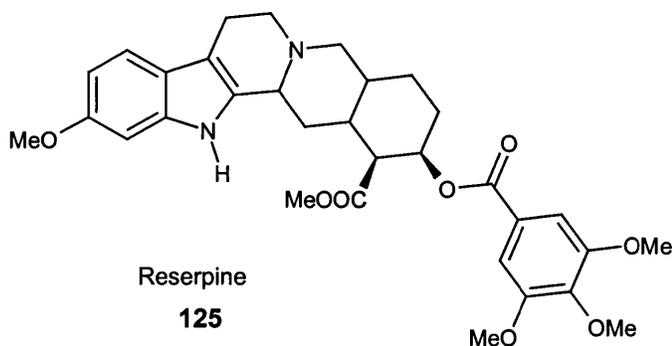
The tetrahydro- β -carboline ring system is an important constituent of numerous naturally occurring indole alkaloids with diverse biological properties. These have been isolated from many plants of South American and African origin and have often been employed for medicinal purposes or as intoxicating snuffs.^{75,76} The α 2-adrenoreceptor agonist yohimbine (**122**) enhances norepinephrine release and increases general sympathetic central nervous system activity.⁷⁷ It has found widespread use as an anxiogen in clinical studies on conditions such as agoraphobia and Panic Disorder.^{78,79} Macrocarpamine (**123**), isolated from the roots of *Alsonia augustifolia*, has antiameobic and antiplasmodial activity and ajmaline (**124**), isolated from *Vinca Rosea*, has marked cardiovascular effects and both are the subject of current research.⁸⁰⁻⁸² Reserpine (**125**), was used for a long time as an anti-hypertensive, but fell out of use as it was overtaken by other compounds with better side-effect profiles.



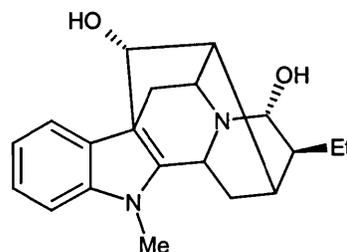
Yohimbine
122



Macrocarpamine
123



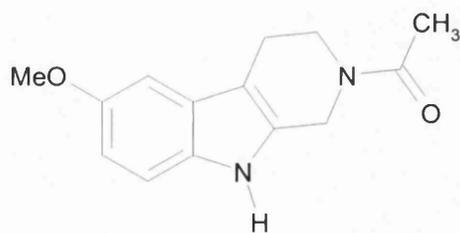
Reserpine
125



Ajmaline
124

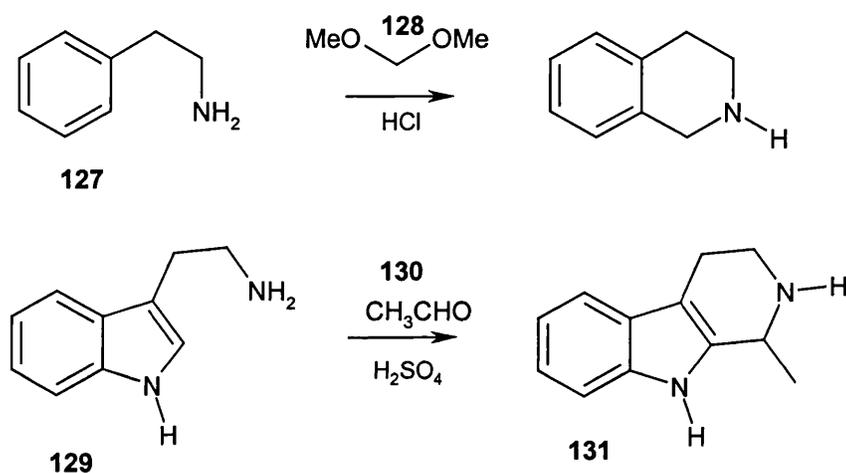
Cook and co-workers have prepared a series of tetrahydro- β -carbolines and used them to study benzodiazepine binding sites in the CNS, where they are effective ligands.⁸³⁻⁸⁵ Several tetrahydro- β -carbolines derived from tryptamine and 5-methoxytryptamine have been shown to inhibit monoamine oxidase A and to bind with nanomolar affinity to serotonin receptors in the CNS.⁸⁶⁻⁸⁸ Investigations on antidepressant drug interactions at the [³H] Imipramine binding site by Langer *et al.* led to the discovery of a high concentration of 6-methoxy-tetrahydro- β -carboline in the human pineal gland.⁸⁹ The [³H] Imipramine binding site is associated with uptake of serotonin and is thought to act as a receptor which modulates serotonin transport mechanisms. Levels of 6-methoxy-tetrahydro- β -carboline were found to be comparable with the concentration of melatonin and Langer has suggested that the pineal gland may synthesise and store this compound as an endogenous ligand for the [³H] Imipramine binding site.

The *N*_b-acetyl-6-methoxy-tetrahydro- β -carboline (**126**) was included in a study on melatonin analogues by the Spadoni group²⁸ who found poor binding at the melatonin receptor. The work described in the previous chapter (chapter 2) could explain this as being due to the incorrect relative orientation of the 6-methoxyl and *N*-acetyl groups.

**126**

3.2 Synthetic approach

The Pictet-Spengler reaction has become established as one of the most powerful methods for the construction of both the tetrahydro- β -carboline and tetrahydroisoquinoline templates. The condensation of phenethylamine (**127**) with dimethoxymethane (**128**) to provide tetrahydroisoquinoline was first reported by Pictet and Spengler in 1911.⁹⁰ Tatsui extended the scope of this reaction in 1928 to provide a synthesis of tetrahydro- β -carbolines (THBC's).⁹¹

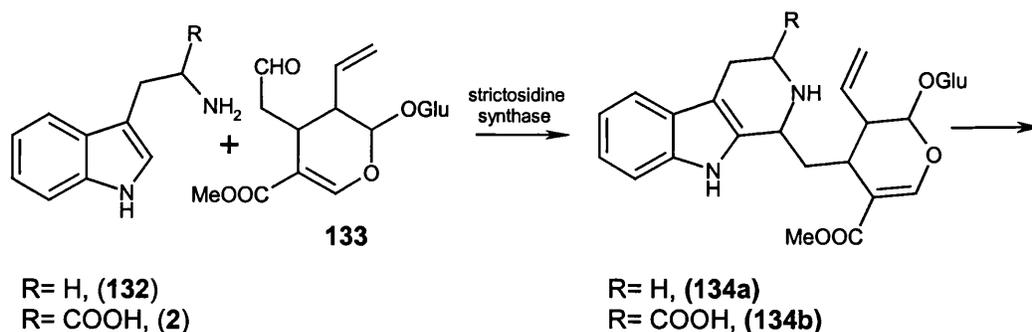


Scheme 24

The reaction is tolerant towards a wide range of aldehyde components and is commonly carried out in protic solvents, in the presence of an acid catalyst. Cook and co-workers considerably extended the scope of this reaction by performing Pictet-Spengler cyclisations with various esters of tryptophan in nonacidic, aprotic media.^{92,93} Further work by this group and others has concentrated on asymmetric aspects of the synthesis, particularly involving the use of tryptophan as the amine substrate.⁹⁴⁻⁹⁷ It had originally been assumed that endogenous THBC's are formed non-enzymatically through a Pictet-Spengler reaction. This *in vivo* formation was

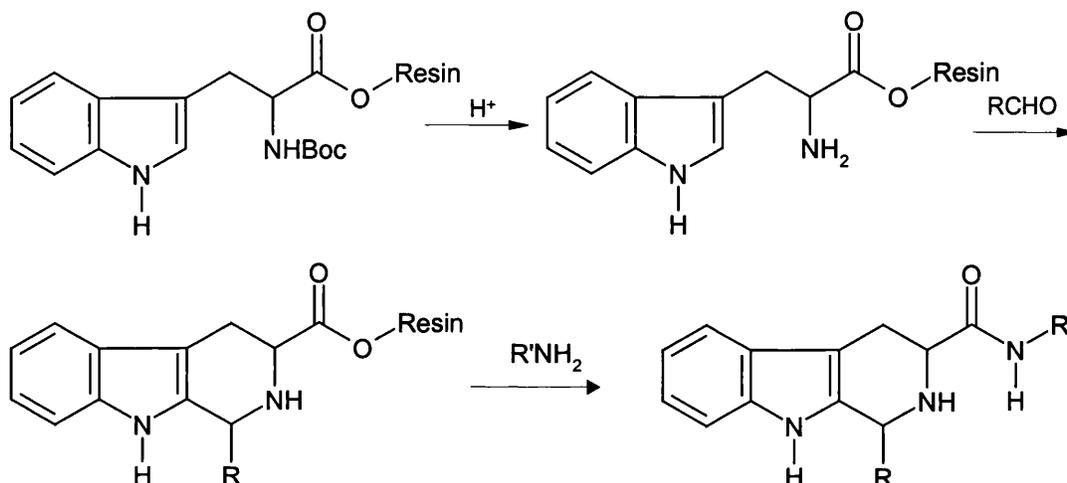
initially doubted, on the grounds that the acidic extraction procedure itself might have been responsible for their production.⁸⁷ Callaway *et al.* have recently studied the reaction between biogenic tryptamines and four potential biological carbonyl substrates in buffered aqueous solution at 37 °C and physiological pH.⁸⁶ The report notes rapid formation of the expected 1-methyl-THBC's, such as **131**, resulting from the use of acetaldehyde, **130**, as the carbonyl substrate, as well as the formation of THBC-1-carboxylic acids from sodium glyoxylate. The reaction failed, however, if formaldehyde or sodium pyruvate were used as the carbonyl component. Apart from showing that the non-enzymatic reaction was possible for some substrates, these results are of interest in the study of certain clinical conditions, such as alcoholism and depression. Excessive consumption of alcohol has been shown to cause a sharp rise in the serum levels of acetaldehyde (0.9 g produced from every gram of ethanol consumed), followed by a corresponding elevation in 1-methyl-THBC's.⁸⁶ As mentioned previously, THBC's are capable of modifying serotonergic activity in the CNS and abnormalities in this system have been implicated in several neurological disorders including depression⁹⁸ and alcoholism.⁹⁹

Enzymatically catalysed Pictet-Spengler reactions have also been reported.¹⁰⁰ The condensation of tryptamine (**132**) or tryptophan (**2**) with secologanin (**133**) is a key step in the biogenic pathway to a series of monoterpene indole alkaloids (scheme 25).



Scheme 25

The Pictet-Spengler reaction has recently been applied to solid phase combinatorial synthesis.¹⁰¹⁻³ In one example, the initial point of attachment to a hydroxymethyl functionalised resin was *via* the acid side chain of *N*-boc-tryptophan. Removal of the Boc group, by TFA, preceded a Pictet-Spengler cyclisation, with subsequent nucleophilic cleavage to give the required products in good yield and purity (scheme 26).

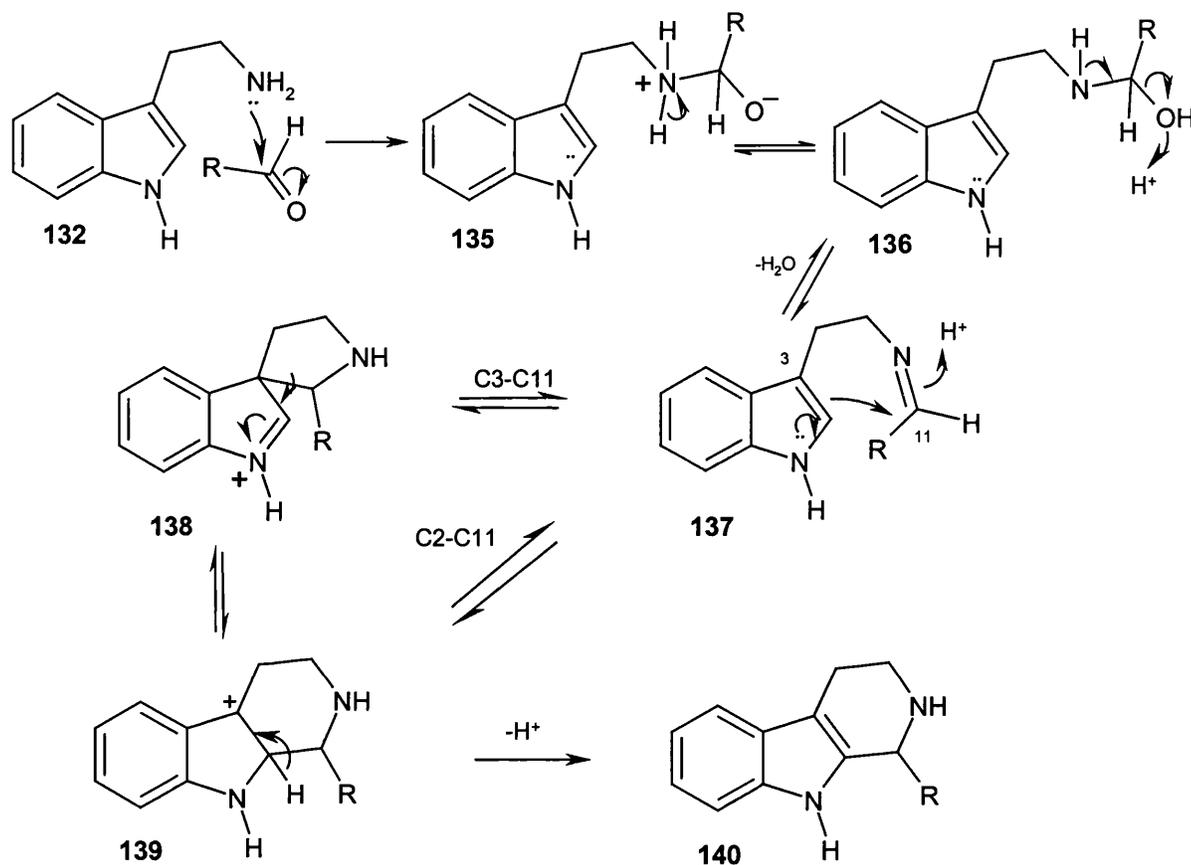


Scheme 26

3.3 Mechanism

The mechanism of the Pictet-Spengler cyclisation has been the subject of much discussion (scheme 27).¹⁰⁴ The first step is the generation of an imine intermediate **137**, and the subsequent cyclisation has generally been thought to proceed *via* a spiroindolenine intermediate **138**.^{24,105,106} Casnati has demonstrated that it can be the result of direct attack at position 2 of the indole (path C2-C11) when very reactive electrophiles are employed.¹⁰⁷ Regardless of which path the reaction takes, the main driving force for the cyclisation is the electrophilic nature of the imine double bond.

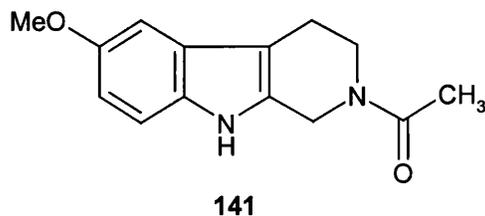
Once formed, the cationic intermediate **139** rapidly loses a proton to restore aromaticity to the pyrrole ring and produce the tetrahydrocarboline product **140**.



Scheme 27

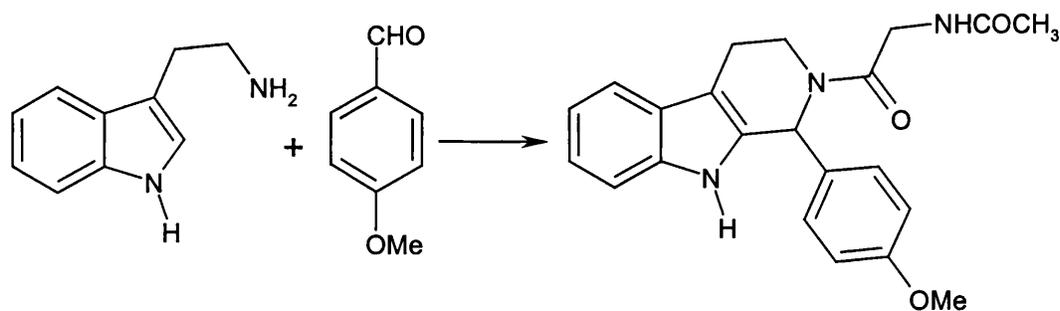
3.4 Synthesis of β -carbolines

A literature search revealed no precedent for investigation into the effect of β -carboline derivatives on melatonin receptors, although subsequent to this work being undertaken Spadoni *et al.* have reported the inactivity of a single compound, **141**.²⁸



The aim of this exercise was, therefore, to identify a new way of displaying the two pharmacophores and, if successful, to generate some SAR data around the best compounds. The synthetic targets were chosen to include the two pharmacophores in a variety of substitution patterns whilst retaining the methoxyl on an aromatic ring. In the tetrahydrocarbazole series we had noted that replacement of methoxyl by chlorine appeared to be well tolerated, so we included this substituent in the compound set.

A variety of conditions for the Pictet-Spengler synthesis of tetrahydro- β -carbolines have been reported in the literature. We initially carried out some trial reactions which varied the solvent (DCM, H₂O, AcOH) and acid (HCl, H₂SO₄, AcOH, TFA) components using 4-methoxybenzaldehyde and tryptamine as substrates.



Scheme 28

As a result of these trials we decided to use glacial acetic acid as both solvent and catalyst, in part due to its excellent solvating ability but also because it could be easily removed by lyophilisation at the end of the reaction. We subsequently found that the use of glacial acetic acid meant that almost all of the cyclisations could be

readily effected at ambient temperature. The crude products were liberated from their acetate salts by aqueous sodium bicarbonate and purified by column chromatography and recrystallisation. This synthetic protocol only failed on one occasion, when 4-dimethylaminophenylbenzaldehyde was used. In this case the intermediate imine appeared to be particularly stable and attempts to use forcing conditions to effect the cyclisation, such as high temperature or prolonged reaction times, resulted in the recovery of complex mixtures. This particular compound was not of high priority and time constraints did not allow further investigation.

In the NMR spectra of the β -carbolines, the proton attached to the carboline nitrogen (N2, Figure 28) was not always visible, being broad and variable in position. In d_6 -DMSO the signal often coincided with the solvent signal but it was usually more clearly visible in $CDCl_3$.

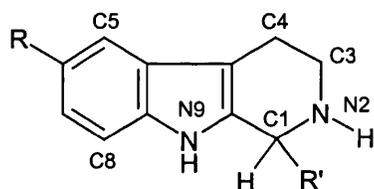
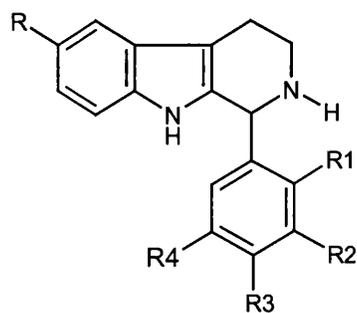


Figure 28 Carboline nomenclature.

A characteristic of the 1H NMR spectra of these β -carbolines was the singlet signal at $\delta = 4.5-5.5$ ppm, assigned to the proton attached to C1. The cycloalkane signals of the protons at C3 and C4 were observed in the $\delta = 2.5-3.5$ ppm range. The N2 atom has more of an effect on the C3 protons, which resonate slightly downfield of the C4 protons, and this difference is also observed in the ^{13}C NMR spectra. Aromatic signals from R' and the indole ring were often overlaid in both proton and carbon NMR spectra, and were not easily assigned. Examples of both a proton and carbon NMR spectrum are appended in Figures 38 and 39. Table 6 shows the compounds made in this way.



Compound	R	R1	R2	R3	R4
142	H	H	H	OCH ₃	H
143	H	OCH ₃	H	H	OCH ₃
144	H	OCH ₃	OCH ₃	H	H
145	H	H	H	OCF ₃	H
146	H	H	H	NHCOCH ₃	H
147	H	H	CF ₃	H	H
148	H	Cl	H	Cl	H
149	OCH ₃	H	H	OCF ₃	H
150	OCH ₃	H	OCH ₃	OCH ₃	OCH ₃
151	OCH ₃	H	H	OCH ₃	OCH ₃
152	H	H	OCH ₃	OCH ₃	OCH ₃
153	H	H	H	OCH ₃	OCH ₃
154	OCH ₃	H	H	OCH ₃	H
155	OCH ₃	OCH ₃	H	H	H
156	H	OCF ₃	H	H	H
157	OCH ₃	OCF ₃	H	H	H
158	H	H	OCH ₃	H	H
159	OCH ₃	H	OCH ₃	H	H
160	H	H	OCF ₃	H	H
161	H	Cl	H	H	H
162	H	H	H	Cl	H
163	H	H	Cl	H	H

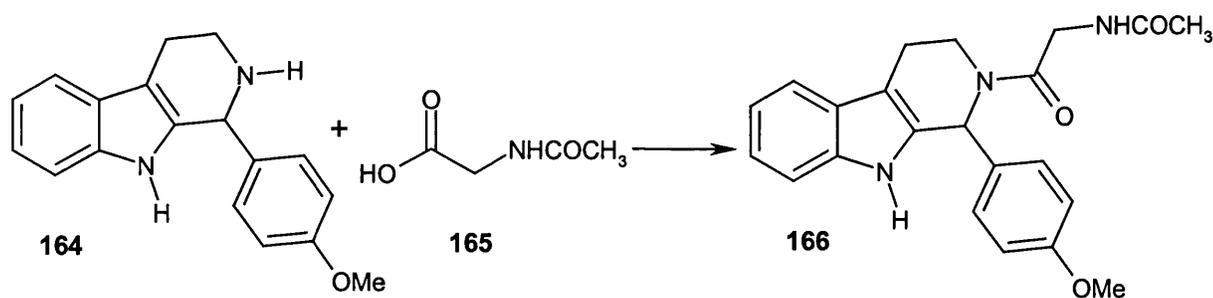
Table 6 C1 substituted β -carbolines.

Acylation of Tetrahydro- β -carbolines

An appropriately substituted two, three or four carbon chain pendant to the β nitrogen of the carboline appeared to be a reasonable starting point for the construction of analogues with potential for binding at the melatonin receptor. This was simply based on a bond counting exercise between pharmacophores and no attempts to model the compounds were made. A small set of commercially available acids was chosen to allow ready access to the desired target molecules, but the method of coupling required some consideration.

3.5 Coupling conditions

Peptide chemists have developed a range of reagents and protocols for efficient formation of the amide bond.^{108,109} Four of these were examined in the model acylation of 1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (**164**) with *N*-acetyl glycine (**165**).



Scheme 29

The coupling reagents chosen for examination are shown in Figure 2. These were:

- 1) O-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (**167**, HATU).
- 2) Benzotriazol-1-yloxy-tris(pyrrolidino)phosphonium hexafluoro phosphate (**168**, PyBOP).
- 3) 1-Ethyl-3-(3'-dimethyl aminopropyl) carbodiimide (**169**, WSCDI), and
- 4) Polymer supported WSCDI.

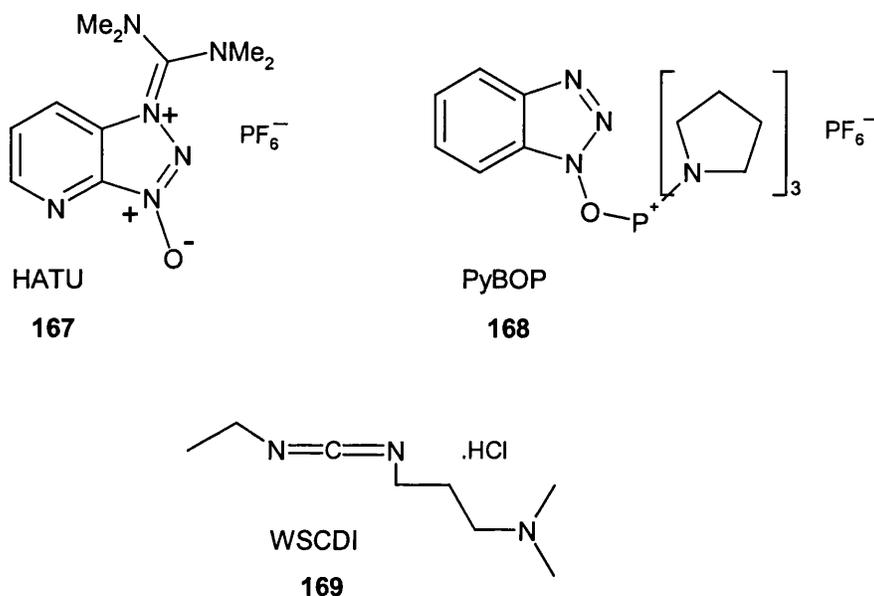
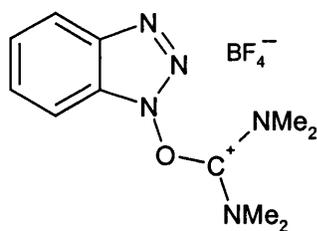


Figure 29. Coupling reagents

Trial reactions were carried out at room temperature, using DMF as solvent and diisopropylethylamine as base, the reaction being worked up after 18 hours and examined by HPLC-MS. WSCDI and the polymer supported WSCDI were not effective, yielding mainly starting material with approximately 15% and 5% of the required product respectively. PyBOP gave mainly the required product but still contained a significant amount of starting material (approx 20%), whereas the reaction using HATU was observed to proceed in almost quantitative yield. The results of these trial reactions are in agreement with the generally accepted ranking of

the reagents based on studies involving difficult peptide sequences.¹¹⁰ HATU is one of the most potent of the currently available coupling reagents and is generally used in the more difficult couplings.¹¹¹ This reagent was therefore used in all subsequent couplings. We also examined the effect of carrying out the acylation using an excess of *N*-acetylglycine, or an excess of both *N*-acetylglycine and coupling agent. The excess reactant can be removed by the technique of resin scavenging¹¹² which involved the addition of polymer supported carbonate as a sequestering agent. Excess *N*-acetylglycine was effectively sequestered by this method but a significant overall improvement in yield was not achieved. The use of excess (1.5 eq.) coupling reagent did not result in significantly improved yield and purification of the crude product was made more difficult.

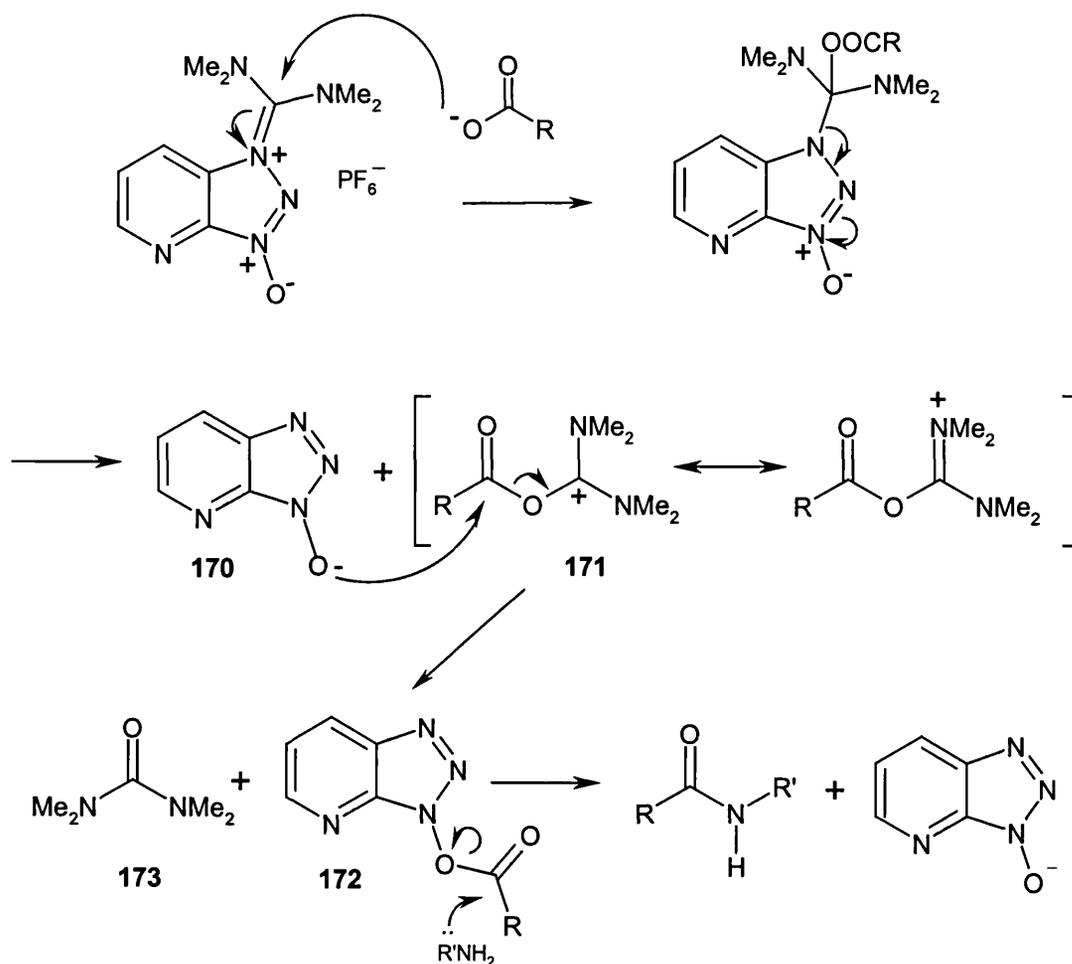
The HATU reagent was first developed and reported by Carpino¹¹³ as an aza analogue of the uronium coupling reagent 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU).



TBTU

Although initially assigned a uronium type structure analogous to that of TBTU,¹¹⁴ X-ray analysis has recently shown HATU to crystallise as the guanidinium *N*-oxide.^{115,116} The mechanism of coupling is not known for certain but it has been postulated that the key coupling step involves the intermediacy of an active ester **172**. A possible mechanism is described below (scheme 30) involving initial guanidination of the acid, followed by loss of the stabilised cation **171** and generation of the HOAt

anion **170**. This anion may then attack the carbonyl group of the carboxylic acid, resulting in the formation of an HOAt ester **172** and the stable urea **173**. This active ester could subsequently react with an amine to yield the amide product and regenerate the anion of HOAt.



Scheme 30

A mechanistic consequence of incorporating a nitrogen atom at position 7 is the possibility of invoking a neighbouring group participation effect in the active ester intermediate **172** (figure 30).

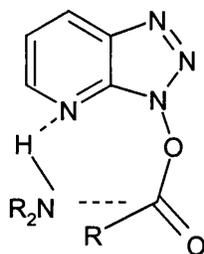
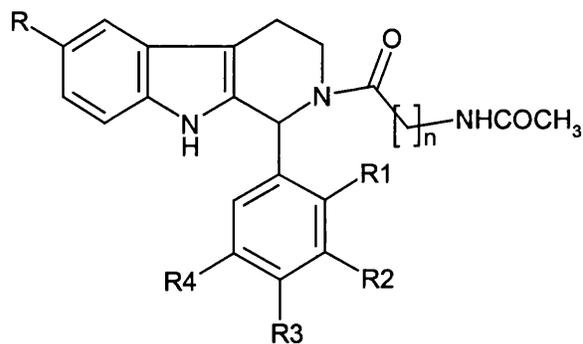


Figure 30. Possible acceleration of HATU couplings by a neighbouring group effect.

Another contributory factor, which may provide a possible explanation for the increased efficiency of HATU compared with PyBop, for example, is found in the electronic nature of the ring and its influence on the stability of reaction intermediates. The incorporation of an electron withdrawing nitrogen at position 7 of the aromatic ring in HATU would be expected to stabilise the anion **170**, making it a better leaving group than the corresponding HOBT anion generated from PyBOP or TBTU. This effect is reflected in the pKa values of the hydroxy-azabenzotriazole and hydroxy-benzotriazole which are 3.5 and 4.6 respectively.

3.6 Synthesis

The fact that HATU was more efficient than the other tested reagents for these coupling reactions suggests that the acylation of the tetrahydro- β -carbolines is not trivial and this may possibly be due to steric hinderance. The HATU coupling method did, however, prove effective for all the reactions carried out in this series and table 7 lists all the compounds made in this way.

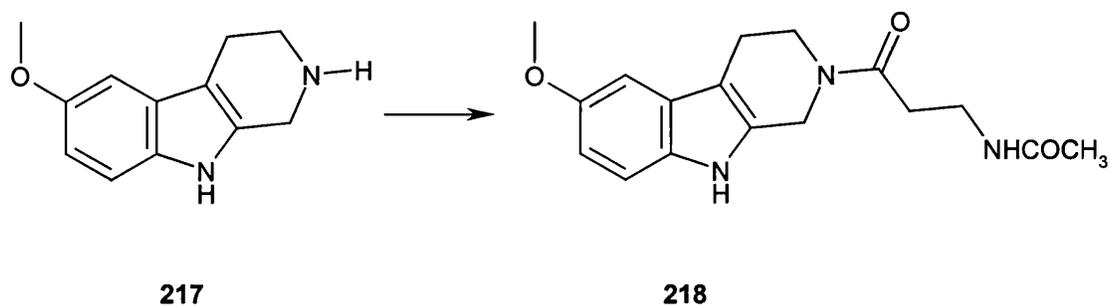
Table 7. β -carbolines acylated at N2

Compd	n	R	R1	R2	R3	R4
174	2	H	H	H	OCH ₃	H
175	2	H	OCH ₃	H	H	OCH ₃
176	2	H	OCH ₃	OCH ₃	H	H
177	2	H	H	H	OCF ₃	H
178	1	H	H	H	OCH ₃	H
179	1	H	OCH ₃	H	H	OCH ₃
180	1	H	H	H	OCF ₃	H
181	3	H	H	H	OCF ₃	H
182	3	H	H	H	OCH ₃	H
183	1	OCH ₃	H	OCH ₃	OCH ₃	OCH ₃
184	2	OCH ₃	H	OCH ₃	OCH ₃	OCH ₃
185	3	OCH ₃	H	OCH ₃	OCH ₃	OCH ₃
186	2	OCH ₃	H	H	OCF ₃	H
187	3	OCH ₃	H	H	OCF ₃	H
188	1	OCH ₃	H	H	OCF ₃	H
189	2	OCH ₃	OCH ₃	H	H	H
190	3	OCH ₃	OCH ₃	H	H	H
191	1	H	OCF ₃	H	H	H
192	2	H	OCF ₃	H	H	H

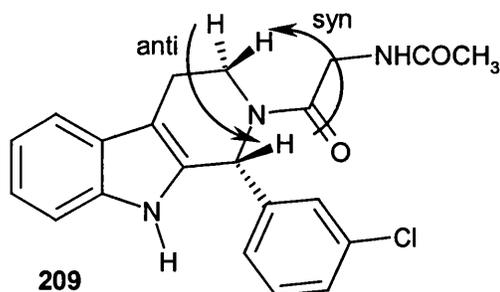
193	3	H	OCF ₃	H	H	H
194	2	H	H	OCH ₃	H	H
195	3	H	H	OCF ₃	H	H
196	1	H	H	OCH ₃	H	H
197	3	H	H	OCH ₃	H	H
198	3	OCH ₃	H	OCH ₃	H	H
199	2	OCH ₃	H	OCH ₃	H	H
200	1	OCH ₃	H	OCH ₃	H	H
201	2	H	H	OCF ₃	H	H
202	1	H	H	OCF ₃	H	H
203	3	OCH ₃	H	OCH ₃	OCH ₃	H
204	2	H	H	CF ₃	H	H
205	1	H	Cl	H	Cl	H
206	2	H	Cl	H	Cl	H
207	3	H	Cl	H	Cl	H
208	1	H	H	H	Cl	H
209	1	H	H	Cl	H	H
210	3	H	H	H	Cl	H
211	2	H	Cl	H	H	H
212	3	H	Cl	H	H	H
213	2	H	H	Cl	H	H
214	2	H	H	H	Cl	H
215	3	H	H	Cl	H	H
216	1	H	Cl	H	H	H

Table 7 (contd) β -carbolines acylated at N2.

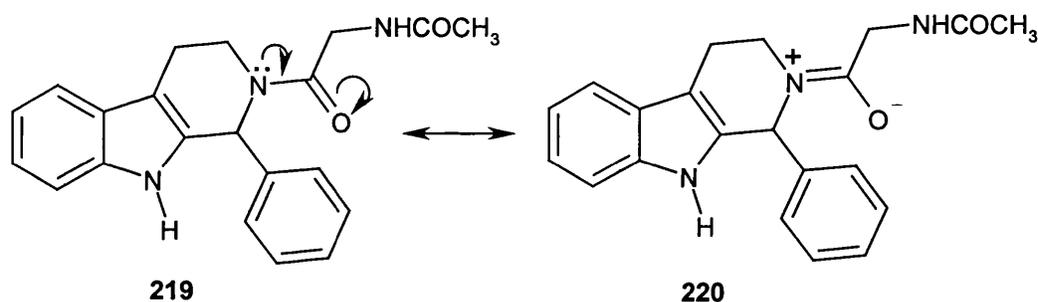
In addition to these compounds we also synthesised two compounds (217 and 218) which carried the 6-methoxyl substituent, but not the phenyl group at C1.

**Scheme 31**

The proton NMR spectra of the acylated compounds **174-216** are generally quite complex, as illustrated by the spectra of compound **209** (appendix, figures 40 and 41). In both acylated and nonacylated β -carbolines, the C3 protons are observed at a lower resonance than those of C4, as would be expected from the greater negative inductive effect exerted by an adjacent nitrogen. The two protons at C3 (and those at C4) are distinct due to their relative relationship - syn or anti - with the C1 proton (figure 31). This diastereotopic relationship gives rise to the unequal coupling which is observed for these protons.

**Figure 31.** Diastereotopic relationship between protons at C1 and C3

After acylation, the protons at C4 are relatively unaffected, but the anisotropic effect of the amide bond has a strong effect on the adjacent C3 protons of the tetrahydro- β -carboline ring. Where the signals assigned to the two C3 protons were only separated by *ca.* 0.15 ppm in the nonacylated tetrahydro- β -carbolines, on acylation the separation is increased to *ca.* 0.8 ppm. This is possibly explained by the nature of the amide bond, the stability of which is partly due to resonance stabilisation resulting from conjugation, as illustrated in scheme 32. Efficient conjugation requires that the 3 atoms must be in a planar configuration in order to ensure good overlap between the orbitals of the nitrogen lone pair and the sp^2 hybridised carbonyl π bond. This requirement is responsible for the introduction of a degree of rigidity to this part of the molecule.

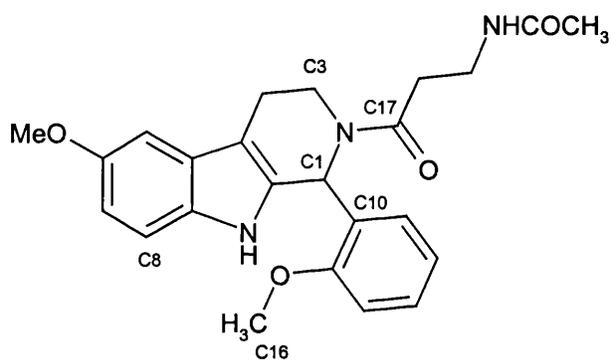


Scheme 32

A possible explanation for the observed increase in the difference in chemical shift between the C3 proton signals in the NMR spectra is that one of the protons is situated in approximately the same plane as the amide group. This proton would thus experience a greater degree of deshielding due to contribution from resonance structure **220** and might result in a downfield shift of the signal in the NMR spectrum. The other proton of C3 may lie out of the plane of the amide group and, consequently, might not experience such a significant shift.

The signal assigned to the C1 proton also experiences a considerable downfield shift on acylation, for example from $\delta = 5.10$ ppm in compound **163** to $\delta = 6.8$ ppm in compound **209**.

A doubling of signals in the ^{13}C and ^1H spectra was also observed in some compounds, resulting in complex spectra (appendix, figures 42 and 43). This was particularly evident when the pendant aromatic ring contained a substituent in the *ortho* position as in **189**. A possible explanation for this is that free rotation of bonds in this highly substituted part of the molecule is restricted by steric crowding.



189

The rotation of the substituted phenyl ring may be restricted by interaction of the *ortho* substituent with the amide group and the indole N-H. This could result in there being two favorable conformations, one in which the aromatic substituent is situated above the plane of the core and one in which it is situated below the plane. As a result of this rotational barrier, several of the signals in the ^1H and ^{13}C spectra are doubled. Two signals assigned to the indole N-H proton of **189** are observed at ($\delta = 10.60$ ppm, and $\delta = 10.50$ ppm) in a ratio of approximately 4:3. Two signals, also split in the same ratio ($\delta = 3.91$ ppm, and $\delta = 3.82$ ppm), are assigned to the methoxyl protons from C16. Some of the compounds with *meta* substitution in the phenyl ring show a small degree of rotational restriction (appendix, Figure 40) with the ratio of

major to minor signals being of the order of *ca.* 15:1. None of the parent unsubstituted tetrahydro- β -carboline spectra showed any sign of this behaviour, probably reflecting the lower degree of steric crowding.

Variable temperature proton NMR experiments confirmed that this observed splitting of signals was a rotameric effect. A marked simplification of the spectrum was observed as the result of an averaging out of all of the signals at higher temperatures. For example, the shape of the indole N-H signal changed when the sample was heated, the two signals coalescing to a single peak at *ca.* 120 °C. This behaviour was also seen for the protons assigned to the C16 methoxyl, which are observed as a singlet ($\delta = 3.80$ ppm) at 120 °C. Signals assigned to the aromatic protons also become more distinct at this temperature, whereas at 30 °C they were duplicated because of the presence of two rotamers. The aromatic signals sharpened further at 140 °C.

The NMR spectra of compound **189** at 30 °C, 120 °C and 140 °C are shown in the appendix (figures 42 and 43). It is possible to obtain an estimate for the free energy barrier for interconversion (ΔG^\ddagger) of the rotameric forms using equation 1¹¹⁷ derived from the analysis carried out by Gutowski and Holm.¹¹⁸

$$\Delta G^\ddagger = 2.303RT_c (10.319 - \log_{10}k_1 + \log_{10}T_c) \quad \text{Equation 1}$$

where R = Gas constant (8.31441 J K⁻¹ mol⁻¹)

T_c = temperature of coalescence as calculated by from equation 2:

$$k_1 = \frac{\pi \Delta\nu}{\sqrt{2}} \quad \text{Equation 2}$$

$\Delta\nu$ = frequency separation of the resolved signals at low temperature.

For compound **189**, the temperature of coalescence was not determined but the methoxyl proton signals for the two rotamers were distinct at 80 °C and had coalesced to a sharp singlet by 120 °C. The separation between the signals at the lower temperature ($\Delta\nu$) was measured as 15 Hz. Substituting these figures into equations 2 and 1, a lower limit for ΔG^\ddagger of 77 kJ mol⁻¹ and an upper limit of 86 kJ mol⁻¹ can be derived. These values, though approximate, indicate that there is severe restriction to rotation in compound **189**. This suggests that **189** and similar compounds are sterically crowded, leading to their existence as distinct rotameric forms.

Clayden *et al.* have carried out studies on conformationally restricted amides of binaphthyls¹¹⁹ (figure 32), and have made the observation that when the substituent X is heteroatomic, such as methoxyl or N-dimethylamino, a much greater degree of rotational restriction results than might be expected purely on the basis of size.

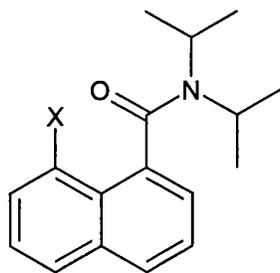


Figure 32

Clayden suggests that a bonding effect between the lone pair of the heteroatom and the amide carbonyl may be in operation. It is possible that similar interactions could also contribute to the restriction observed in the 2-methoxy substituted carboline system *via* H bonding with the indole N-H.

Biological results

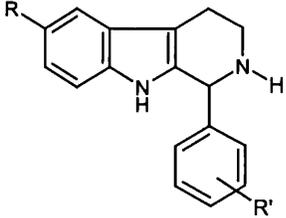
All compounds were screened for agonist and antagonist activity by Dr. David Sugden and co-workers, Kings College, London. The assay determined the pigment aggregation response of *Xenopus laevis* dermal melanophores after administration of drug and is described in section 2.9.2. Data is given in the form of EC₅₀ for full agonists which represents the concentration of drug required to produce 50% of the maximum aggregation of melanophores. For partial agonists the dose producing a given percentage of maximum pigment aggregation is reported. Antagonism is reported as an IC₅₀ value representing the dose required to reverse 50% of the maximum aggregation resulting from addition of a single dose of melatonin at 1 nM. For partial antagonists the dose producing a given percentage of the maximum inhibition of 1 nM [-9] melatonin stimulated aggregation is reported. Binding data have not been obtained for these compounds.

3.7 Results and discussion

The results of the melanophore assay for the tetrahydro- β -carbolines are shown in table 8. Several of these compounds display weak agonism at the melatonin receptor and a few are partial agonists. All of the compounds having a 6-methoxyl group (analogous to the 5-methoxyl of melatonin) show some form of agonism whereas only one, **142**, of the 6-H compounds does so. This is displayed most clearly for those pairs of compounds which are identical, except at the 6-position, such as **150** and **152**, or **156** and **157**. Assuming that these compounds are acting at the same site as melatonin, it is likely that the receptor is simply recognising the methoxy-indole moiety and it is this interaction which confers the weak activity

observed. Interestingly, the only two compounds lacking a 6-methoxyl which did show any agonism were **142** (weak agonist) and **153** (partial agonist), both of which contained a para-methoxyl in the phenyl ring pendant to C1. This gave us some encouragement that we may be able to observe some activity once the *N*-acetyl pharmacophore was in place. Two compounds, **143** and **145**, also exhibited some weak antagonist activity, and some evidence of toxicity was observed for some of the chloro-substituted compounds at higher concentration.

Table 8. Biological activity of tetrahydro- β -carbolines substituted at C1. (for structures see Table 6, p138)

	Compound	Agonist EC ₅₀ (μ M)	Antagonist IC ₅₀ (μ M)
		melatonin	0.00059
	142	54.9	-
	143	-	181.9
	144	-	-
	145	-	204.1
	146	-	-
	147	-	-
	148	[-5] 51%	-
	149	[-5] 36%	-
	150	0.3	-
	151	0.4	-
	152	-	-
	153	[-4] 33%	-
	154	0.4	-
	155	0.09	-
	156	-	-
	157	0.05	-

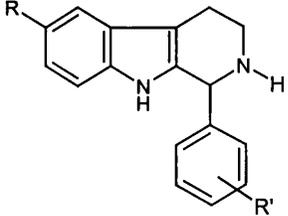
	Compound	Agonist EC ₅₀ (x 10 ⁻⁶)	Antagonist IC ₅₀ (x 10 ⁻⁶)
		158	-
	159	1.1	[-4] 43%
	160	[-4] Toxic	-
	161	-	-
	162	[-4] Toxic	-
	163	[-4] Toxic	-

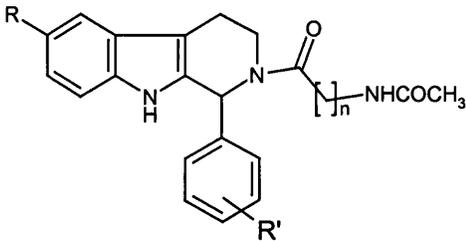
Table 8 (contd) Biological activity of tetrahydro- β -carbolines substituted at C1.

The results of the melanophore assay for the *N*₆-acyl-tetrahydro- β -carbolines are shown in tables 9 and 10. Disappointingly, only four compounds amongst this entire set showed full agonist activity at a concentration of 10⁻⁹ M. The most active of these, **174** (EC₅₀ = 0.52 μ M), was approximately 1000 times less potent than melatonin itself. Three of these four compounds, **183**, **184** and **188**, (EC₅₀ = 0.52 μ M, 117.4 μ M and 2.0 μ M respectively) had a 6-methoxyl substituent, which was probably largely responsible for the activity, since the parent carboline of two of these compounds, **150**, had greater potency (EC₅₀ = 0.3 μ M) than either acylated derivative. The only remaining compound which showed any agonism, **174**, was derived from carboline **142**, which was itself a full agonist. This compound was unusual in that acylation with *N*-acetyl- β -alanine gave rise to a *ca.* 100 fold increase in potency. However, increasing or decreasing the *N*-acetyl chain length by one carbon unit, resulted in compounds which behaved as full antagonists. The lack of any other similar results in this set makes it hard to draw any conclusions.

Whilst some of the 6-methoxyl substituted compounds displayed partial antagonism at higher concentrations, full antagonist behaviour was observed in only one compound with this substitution (**186**, EC₅₀ = 11.5 μ M). This compound was unusual in that it also appeared to act as an agonist at higher concentrations ($\geq 10^{-4}$

M). Full antagonism was common, however, in those compounds where the 6-position was unsubstituted.

Table 9 Biological activity of *N*-acyl-tetrahydro- β -carbolines. (Structures in Table 7, p144).

	Compound	Agonist	Antagonist
		EC ₅₀ (μ M)	IC ₅₀ (μ M)
	174	0.54	-
	175	-	38.9
	176	-	36.3
	177	-	21.9
	178	[-5] 68%	93.3
	179	-	34.7
	180	-	36.3
	181	-	72.4
	182	-	26.9
	183	0.52	-
	184	117.4	-
	185	-	-
	186	[-4] 21%	11.7
	188	2.0	[-4] 55%
	189	-	[-4] 32%
	190	-	[-4] 55%
	191	-	[-4] 75%
	192	-	25.1
	193	-	[-4] 69%
	194	-	12.5
195	-	17.3	

	Compound	Agonist	Antagonist
		EC ₅₀ (μ M)	IC ₅₀ (μ M)
	196	-	41.6
	197	-	23.9
	198	-	21.3
	199	-	[-4] 75%
	200	-	27.5
	201	-	28.1
	202	-	38.0
	203	-	[-4] 49%
	204	-	[-4] 52%
	205	-	7.5
	206	-	19.9
	207	-	24.5
	208	-	[-4] 79%
	209	-	35.4
	210	-	14.1
	211	-	28.8
	212	-	[-4] 69%
	213	-	[-4] 60%
	214	-	[-4] 81%
	215	-	19.0
	216	-	[-4] 56%

Table 9 (contd) Biological activity of *N_b*-acyl-tetrahydro- β -carbolines. (For structures see Table 7, p 144-145).

In addition to the compounds listed above, a further set of four compounds was synthesised from **146**. In these compounds the *N*-acetyl moiety is at position 4 of the phenyl ring pendant to the C1 position and a methoxyl group is introduced by appropriate acylation of the carboline nitrogen. These compounds are shown below and had no biological activity at concentrations below 10^{-4} M.

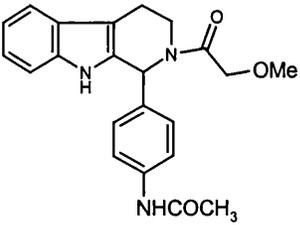
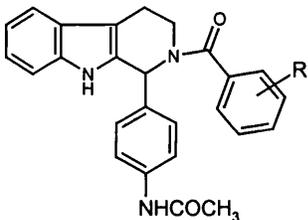
	R	Compound	Agonist	Antag.
	-	221	-	-
	2-OMe	222	-	-
	3-OMe	223	-	-
	4-OMe	224	-	-

Table 10 Biological activity of some *N_b*-acyl-tetrahydro- β -carbolines.

3.8 Summary

It appears that this set of *N_b*-acyl-tetrahydro- β -carbolines does not constitute a class of compounds which are able to evoke a good agonist response at the melatonin receptor in the *Xenopus laevis* melanophore assay. We did not succeed in presenting

the two pharmacophores in an active conformation, using the β -carboline template, and this series yielded no agonists of any significance.

In general, melatonin agonists are considerably more potent (low nanomolar range) than currently known antagonists (micromolar to nanomolar range, see chapter 1), and it is possible that further evaluation of this class of compound might yield antagonists of interest. The low intrinsic biological activity of these compounds, however, has resulted in our not obtaining any binding data for these compounds. It would have been useful to know whether the inactivity was due to poor binding, for example because of their large size, or whether it is because they are unable to adopt an active conformation at the receptor site. The high degree of steric crowding and apparent stability of certain N_b -acyl-tetrahydro- β -carboline rotamers suggests that these compounds may be less flexible than we would have wished in an exploratory approach. The phenyl ring pendant to C1 might be expected to prefer an orientation which puts the plane of the ring at an angle of approximately 90° to the central carboline core in order to minimise steric interactions. In addition, any hydrogen bonding analogous to that observed by Clayden (figure 32) would also tend to restrict the degree of conformational freedom. This would have major implications for the amount of space occupied by the molecule as a whole, as well as determining those regions of space accessible to the methoxyl and N -acetyl pharmacophores. It is also possible that the putative π -stacking of the indole core with aromatic residues in the receptor, which is postulated for melatonin, could be disrupted by the shape of these molecules.

X-ray and modeling studies would be useful to addressing these issues but were not carried out due to time constraints.

Chapter 4

Miscellaneous Melatonin Analogues

Aim

The main aim of the work described in this chapter was to investigate the effect of various point changes on the molecule of melatonin. We wished to investigate the nature and size of certain indole substituents in order to probe the spatial requirements of the receptor pocket and to obtain information about the mode of binding of ligands to the melatonin receptor. Melatonin analogues bearing halogen substituents at the C2 position were required for receptor studies and for supply to an external research group for ^{11}C positron emission studies. We also wished to attempt the preparation of the novel halogenated analogue, 2-fluoromelatonin.

A series of compounds was required, in which the alkyl substituent on the 5-methoxyl pharmacophore was varied in order to examine the steric requirements of the receptor at this position.

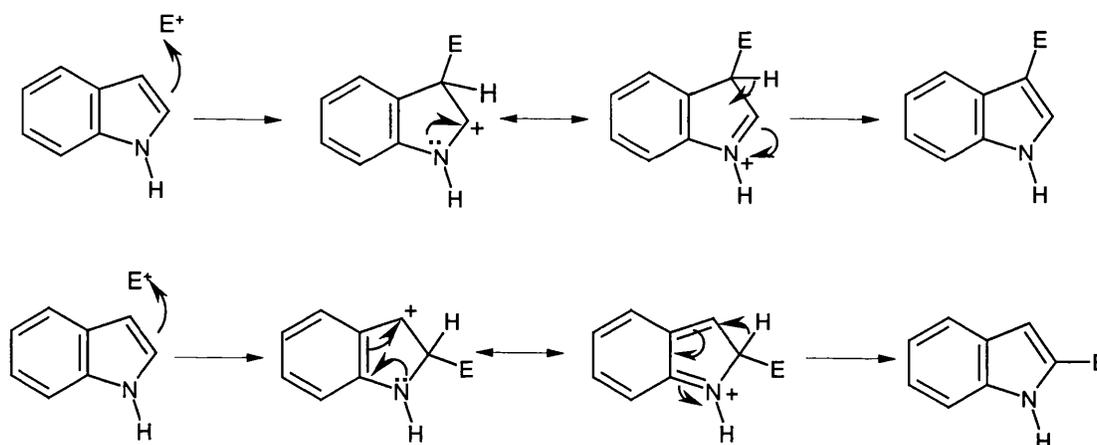
The possible biological activity of a major metabolic product of melatonin was also of interest and we wished to synthesise this compound for biological evaluation.

Finally, we wished to carry out the systematic replacement of the oxygen atoms of melatonin by sulfur, in order to examine the effect on binding and activity.

2-Halomelatonin analogues

4.1 Introduction

2-Iodomelatonin, 2-bromomelatonin and 2-chloromelatonin are melatonin agonists with greater potency than melatonin itself in a range of assays.¹²⁰⁻¹²² The electron rich character of the five membered ring of indole makes electrophilic substitution relatively easy, with a strong preference for attack at C3. Substitution by electrophiles at C2 is also favoured, although to a lesser degree, and a comparison of the reaction intermediates for the two potential pathways is usually used to explain the preference for substitution at C3. (scheme 33).

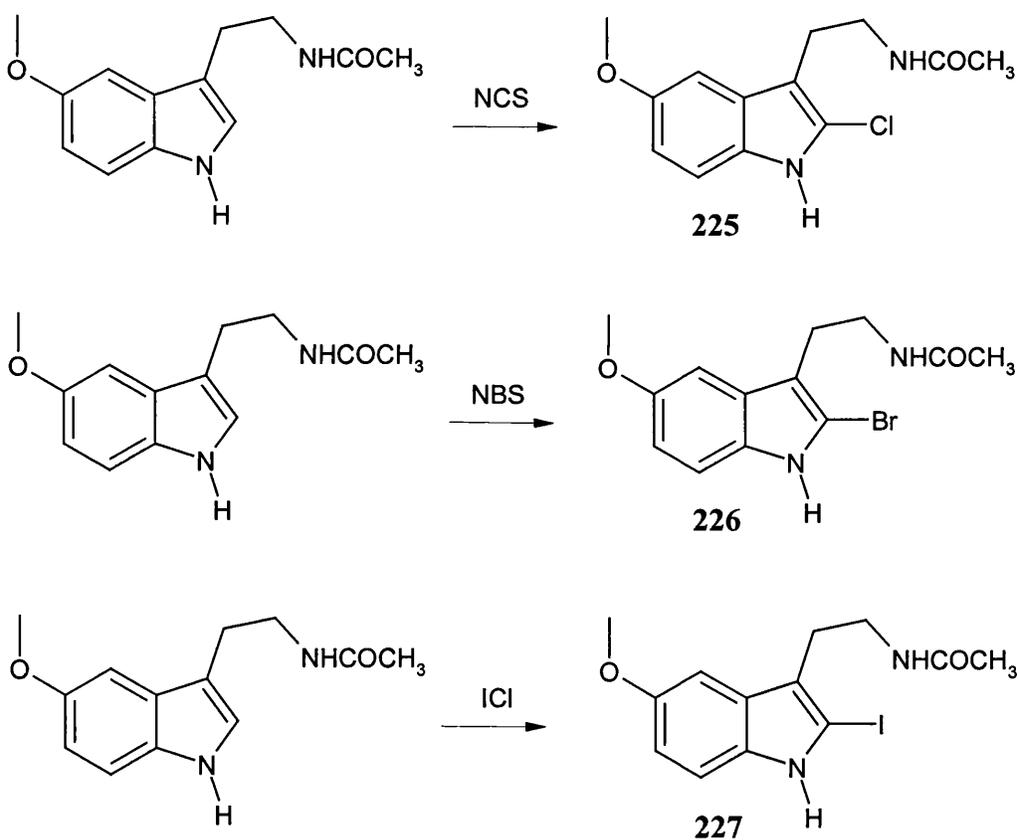


Scheme 33.

The intermediate resulting from electrophilic attack at C3 is stabilised by having the positive charge located adjacent to the electronegative nitrogen. Electrophilic attack at C2 is less favoured since it involves disruption of aromaticity in the phenyl ring and results in a higher energy intermediate than that resulting from

attack at C3. C2 substitution is most commonly encountered when the C3 position is blocked, when an initial attack at C3 is followed by a 3-2 migration, or when *N*-blocked indoles are *ortho* lithiated.

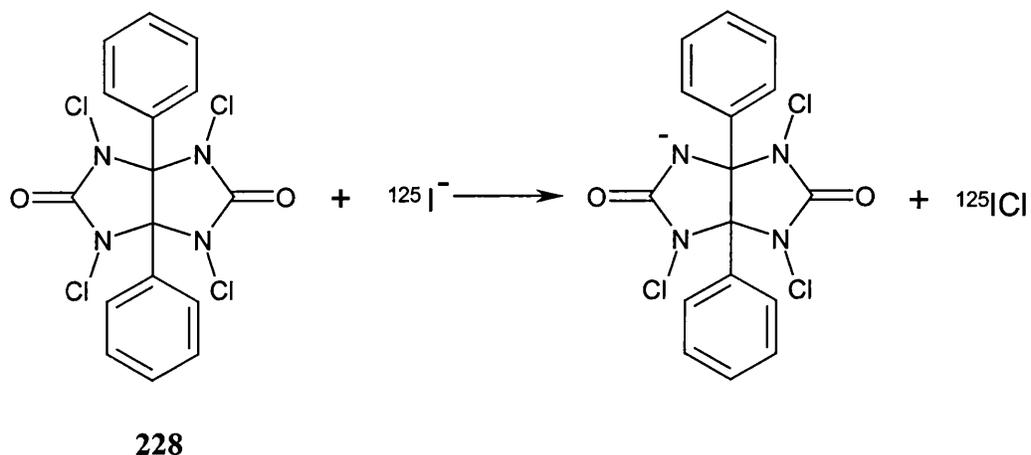
The known 2-halomelatonin analogues have all been prepared directly from melatonin, and their syntheses are included in an excellent review on the chemistry of melatonin by Kennaway and Hugel.¹²³ 2-Chloro and 2-bromo melatonin (**225** and **226**),^{123,124} have been prepared by reaction with *N*-chlorosuccinimide (NCS) and *N*-bromosuccinimide (NBS) respectively, while 2-iodomelatonin (**227**) is prepared *via* iodine monochloride (scheme 34).^{125,126}



Scheme 34

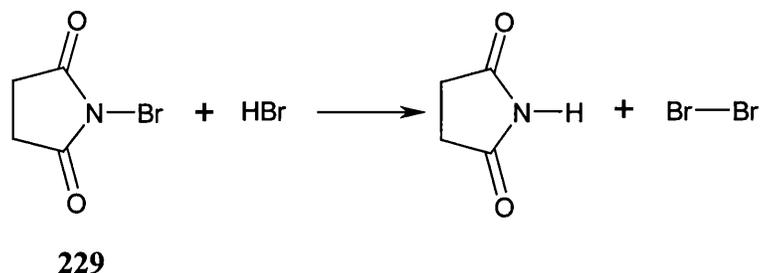
The preparation of radiolabelled 2-iodomelatonin by Vakkuri and co-workers was a milestone in the search for melatonin receptors, as was previously discussed in

section 1.6. The radiolabel is incorporated at the C2 position by treating melatonin with $K^{125}I$ in the presence of Iodogen (1,2,4,6-tetrachloro-3a-6a-diphenylglycouril, **228**) in chloroform at room temperature, which generates electrophilic ICl (scheme 35).¹²⁶



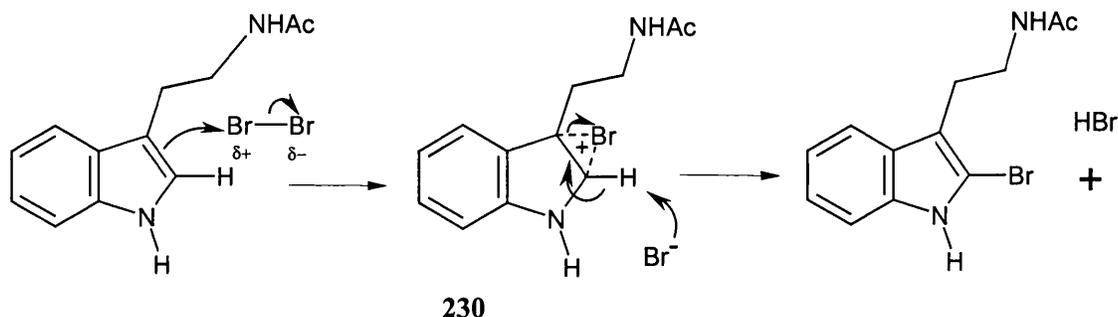
Scheme 35

Melatonin can potentially be brominated at several positions, but selective halogenation at C2 is possible using NBS (**229**) in chloroform, according to the literature method of Duranti *et al.*¹²⁴ This reagent supplies a constant low concentration of bromine which favours the electrophilic substitution route over that of the less discriminating free radical addition. At temperatures below $80^{\circ}C$, a trace amount of HBr is required to initiate the reaction and form molecular bromine (scheme 36).



Scheme 36

The conventional mechanism for electrophilic substitution by molecular bromine involves the formation of a cyclic bromonium ion (**230**), with the proton abstracting species being the concomitantly formed bromide ion.¹²⁷ The HBr which is formed can then react with a molecule of NBS, as in scheme 2, to regenerate molecular bromine (scheme 37).



Scheme 37

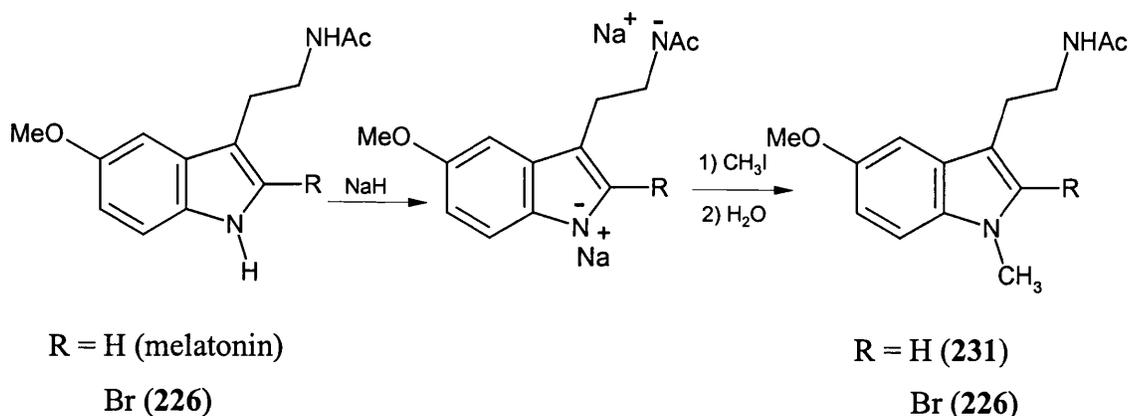
4.11 Synthesis

We wished to prepare samples of 2-bromomelatonin, 1-methyl-2-bromomelatonin and 1-methylmelatonin as research tools for supply to external collaborators, as well as for our own use in analogue SAR studies and as assay references. 1-Methylmelatonin was of particular interest, since the cycloalkan[b]indoles synthesised in chapter 2 all bear this *N*-substituent and we wished to estimate the degree to which the binding of these compounds might be affected as a consequence.

2-Bromomelatonin (**226**) was prepared from melatonin in 32% yield, using NBS, as described in section 4.1.1. (scheme 37) The proton NMR spectrum was in agreement with that previously published by Duranti *et al.*¹²⁴

1-Methylmelatonin (**231**, 79% yield) and 1-methyl-2-bromomelatonin (**232**, 46% yield) were readily prepared from melatonin and **226** respectively, by initial

deprotonation with two equivalents of sodium hydride in THF. The anion produced was then alkylated by the addition of one equivalent of iodomethane (scheme 38).¹²⁸



Scheme 38

Monitoring of the reaction by thin layer chromatography was difficult in both cases, due to the similarity of starting material and product. However, reverse phase HPLC, using ACN/H₂O buffered with 0.1% TFA as the mobile phase, was found to be a good means of distinguishing the starting material from product. The reaction was worked up when HPLC indicated complete consumption of starting material.

4.3 Results and Discussion

The binding data for the three compounds, in the chick brain homogenate assay were obtained by Dr David Sugden and co-workers, Kings College, London, as detailed in section 2.9.1. The results are reported in table 11.

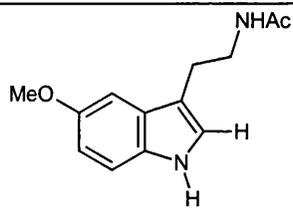
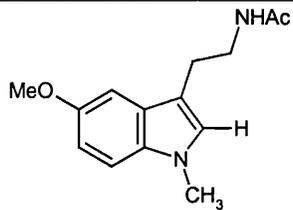
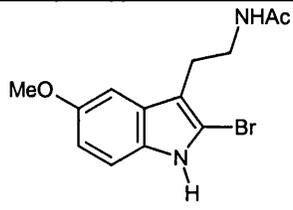
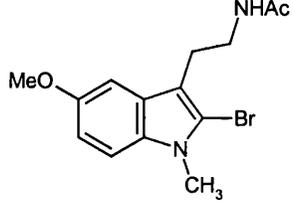
	Compound	Number	Binding (K_i , nM)
	melatonin	1	0.28
	1-methylmelatonin	231	5.5
	2-bromomelatonin	226	0.046
	1-methyl-2-bromomelatonin	232	0.22

Table 11. Binding affinity of some melatonin analogues.

In this assay, 1-methylmelatonin ($K_i = 5.5$ nM), was observed to bind with approximately 20 times lower affinity than melatonin itself ($K_i = 0.28$ nM). On methylation, a corresponding reduction in affinity was also observed for the 2-bromo compound, although it was a smaller reduction of approximately 5 fold. Figure 33 shows some typical binding curves to illustrate this. It appears that the

substitution of hydrogen by bromine at C2 is more than able to compensate for the drop in binding affinity associated with methylation of the indole nitrogen.

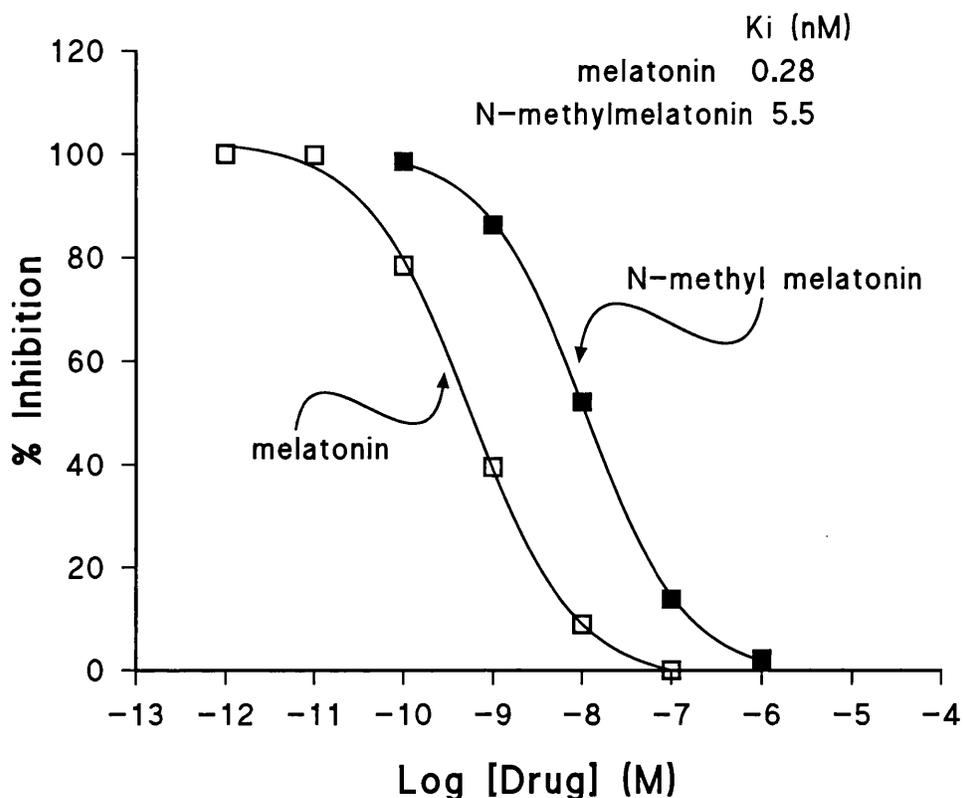


Figure 33. Binding affinity data for melatonin and 1-methyl melatonin (chick brain homogenate).

It is noteworthy that 2-bromomelatonin has a six fold greater binding affinity than melatonin itself in this assay, and 1-methyl-2-bromomelatonin a 25 fold greater affinity than 1-methylmelatonin. Initially, we had postulated that the steric effect of a bulky group at C2 would be to force the side chain at C3 into a conformation that was closer to that required for optimal binding. This would agree with the results in chapter 2 on conformational restriction of the C3 side chain and with the results published by Jones and co-workers on the highly active 2-phenylmelatonin

compounds (2-phenylmelatonin $K_i = 0.059$ nM).⁶⁴ Recently however, Stankov *et al.* have suggested that this might indicate the presence of a lipophilic pocket in this region of the molecule.¹²⁹ It is possible that the halogen atoms, and perhaps the C1 or C2 methylene groups of the cycloalkan[b] indoles described in chapter 2, are able to access this pocket, if it exists. Further investigations into the interaction of this region of the molecule with the receptor would be of great interest. One consequence, for example, may be that the 1-H cycloalkan[b] indoles that we unsuccessfully attempted to prepare in chapter 2 might not demonstrate such a great rise in activity as we had originally thought, based on a direct comparison between melatonin and *N*-methyl melatonin.

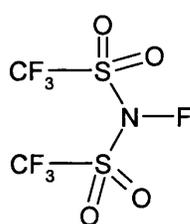
Attempted Synthesis of 2-Fluoromelatonin

4.4 Introduction

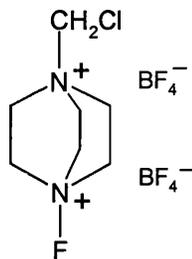
Several research groups have used 2-halomelatonin analogues as pharmacological tools, but there have been no reports of the preparation of 2-fluoromelatonin in the literature. We decided to investigate the possibility of preparing this compound by making use of some recent developments in commercially available fluorinating reagents.

Agents for the fluorination of organic molecules can be broadly divided into three categories; sources of fluoride ion (F^-), fluorine radicals (F^\cdot), and compounds that can deliver electrophilic fluorine (F^+). The direct fluorination of an electron rich centre such as the 2 position of indoles by nucleophilic F^- reagents is not synthetically feasible. A radical approach to the direct fluorination of organic compounds is well documented, usually via elemental fluorine, but the radicals can sometimes display indiscriminate reactivity and the yields are often poor.¹³⁰

Recent years have seen the emergence of a variety of safe and selective sources for electrophilic fluorine. Compounds such as xenon difluoride XeF_2 and trifluoromethyl hypofluorite CF_3OF were among the first reagents reported¹³¹ but more recently a whole class of NF fluorinating agents with a wide range of fluorinating power have found use as sources of F^+ .¹³² These are either neutral R_2NF compounds such as ‘DesMarteau’s compound’ (233) or quaternary $\text{R}_3\text{N}^+\text{F}^-$ salts, for example ‘selectfluor’ (234), where A is a non nucleophilic anion,.

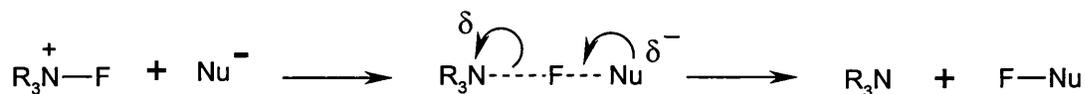


233

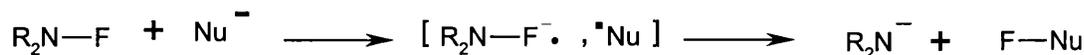


234

The R_2N and R_3N^+ fragments are chosen for their effectiveness as leaving groups and, generally speaking, the quaternary compounds are more powerful fluorinating agents than the R_2NF type in the reaction with nucleophiles. While there is little doubt that reagents such as ‘selectfluor’ react with nucleophiles in a manner which results in the transfer of F^+ , there is currently no single widely accepted mechanism for this.¹³² The main debate centres on whether the process occurs by discreet single electron transfer steps or by direct attack of the nucleophile at fluorine, as shown in scheme 39.

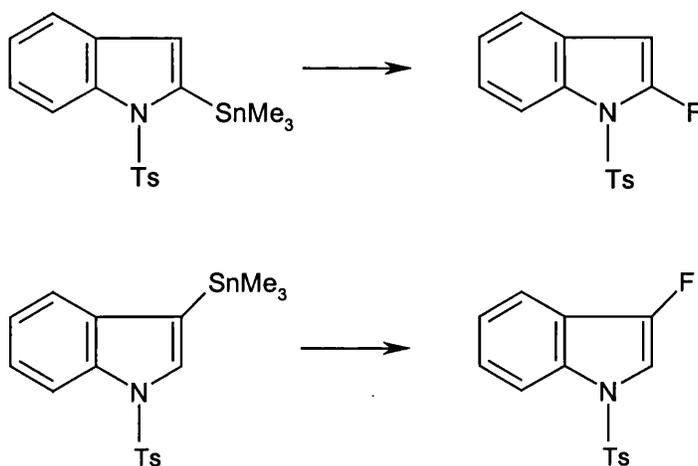


SET pathway



Scheme 39.

The favoured site of attack for an electrophile such as F^+ will tend to be at the most nucleophilic centre of the substrate molecule. By pre-forming an organometallic derivative at the required site of substitution in the nucleophilic species it should be possible to achieve selective fluorination. The electrophilic fluorination of alkenyl heteroarylstannanes with xenon difluoride is documented,¹³³ and the preparation of 2-fluoroindoles and 3-fluoroindoles from the corresponding stannylated indole has been reported by Madge and co-workers using selectfluor (scheme 40).¹³⁴



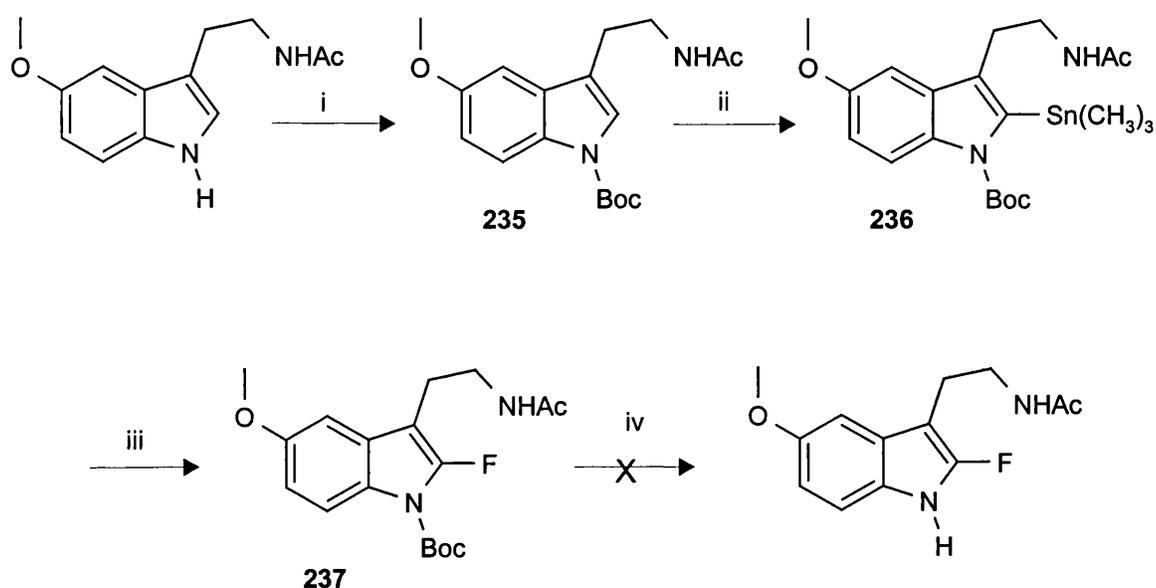
Scheme 40

The most viable method for incorporating the trimethylstannyl moiety into the 2-position of melatonin appeared to be *via* lithiation at C2 and subsequent

quenching with trimethyltin chloride. This had the advantage of synthetic brevity and avoided construction of the indole ring itself. For the fluorination reaction, 'selectfluor' was chosen on the basis of its commercial availability, ease of use and synthetic precedent.

Synthesis

Application of the chemistry described above in scheme 40, to the synthesis of 2-fluoromelatonin was attempted as outlined below (scheme 41).

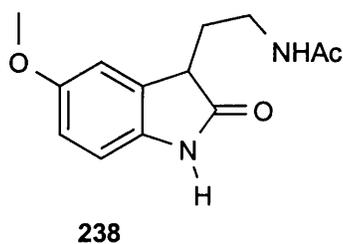


Reagents: i) NaH, THF, (CH₃)₃COCO₂N=C(C₆H₅)CN ii) LDA, THF, ClSn(CH₃)₃, -70°C iii) 'selectfluor', ACN, -30°C iv) H⁺

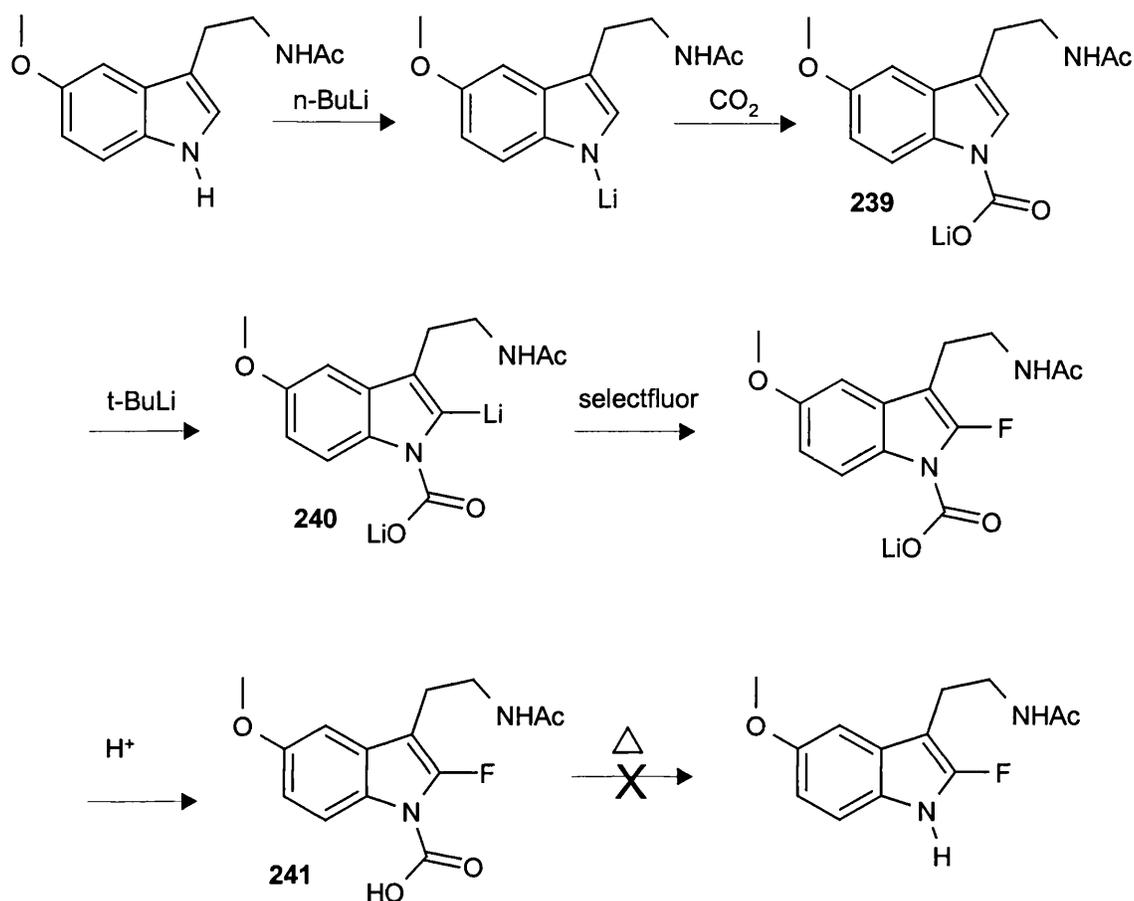
Scheme 41

The initial stages of the synthesis proceeded as expected and in reasonable yield. Compound **235** was obtained, in quantitative fashion, by reaction of 2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-On), with the anion derived from the treatment of melatonin with sodium hydride. Direct lithiation at the C2

position by freshly prepared LDA and treatment with trimethyl tin chloride in THF gave the stannylated indole (**236**) in 90% yield. The procedure required treatment with potassium fluoride to complex volatile and toxic tin residues. The NMR spectra of this compound showed the disappearance of the signal assigned to the proton at C2, accompanied by the appearance of a very distinctive signal at approximately 0.3 ppm, with two satellite signals (doublets), which are assigned to the trimethylstannyl protons. The main singlet signal arises from the trimethylstannyl protons which are attached to the major tin isotopes, ^{116}Sn and ^{118}Sn , while the satellite signals arise from coupling to the ^{119}Sn and ^{117}Sn isotopes and reflect their natural relative abundance of ~8% and ~7% respectively (appendix, figure 44a). The mass spectrum of compound **236** sometimes failed to give a molecular ion, but showed a fragmentation consistent with the required product and displayed a diagnostic isotope pattern for tin (appendix, figure 44b). The stannylated product could be subjected to chromatography, but in order to alleviate the problem of silica induced cleavage of the carbon – tin bond, the silica was pre-washed with eluent containing 5% triethylamine. Fluorine was successfully introduced by selectfluor, in 40% yield, using standard procedures and acetonitrile as solvent. Protecting group removal from compound **237** was achieved using several different methods (TFA, HCl in dioxan, HCl in glacial acetic acid, and HF in pyridine) but the required product was never isolated. The reaction usually gave several spots on tlc and the only product isolated had a mass and NMR spectrum consistent with that of 2-oxomelatonin (**238**). Hugel and Kennaway have reported the synthesis of this oxindole compound by treatment of melatonin with hydrochloric acid in DMSO.¹²³



An alternative *N*-protection strategy, developed by Katritzky and Akutagawa, was also employed (scheme 42).¹³⁵



Scheme 42.

In a one-pot procedure, melatonin was treated with *n*-BuLi at $-78\text{ }^{\circ}\text{C}$ followed by solid carbon dioxide to generate the lithium salt of the indole-1-carboxylic acid (**239**). This intermediate was then treated *in situ* with 1 *eq.* of *t*-BuLi, in order to lithiate the indole C2 (**240**). Selectfluor was then added to fluorinate the 2-position and the reaction mixture acidified at $0\text{ }^{\circ}\text{C}$ to give hopefully, the indole-1-carboxylic acid (**241**). This product was gently warmed to $130\text{ }^{\circ}\text{C}$ in order to effect decarboxylation. Instead of the desired 2-fluoromelatonin, however, the only product isolated was the oxindole **238**, identical to that previously obtained

by the Boc protection strategy. As none of the intermediates were isolated we are unable to determine at which point this sequence failed, but we intend to re-visit the route at a later date.

A possible explanation for the failure of these reactions to give the required product is that under acidic conditions C3 is readily protonated and the fluorine at C2 is then sufficiently labile to be readily replaced by nucleophiles including water, present in the work up procedure. This was reinforced by our observation of replacement of fluorine by methoxyl if methanol was added during the work up procedure. We still believe that it should be possible to achieve the synthesis given the right protection strategy. One interesting possibility might be the samarium iodide mediated deprotection of a tosyl group using anhydrous THF, as discussed in chapter 3. Unfortunately time constraints did not allow any further work on this target, although the binding affinities of the intermediates were evaluated in the chick brain assay (described in section 2.9.1).

Biological Results

The assay results for all synthetic intermediates are shown in table 12.

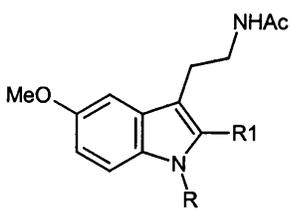
	R	R1	Compound	Binding (K_i, nM)
	H	H	melatonin	0.28
	Boc	H	235	156.5 +/- 23.1
	Boc	Sn(CH ₃) ₃	236	6200 +/- 800
	Boc	F	237	9.3 +/- 2.4

Table 12 Binding affinities of some 2-substituted melatonin analogues.

The large, electron withdrawing Boc protecting group clearly has a negative effect on binding affinity. The Boc substituent is considerably worse (*ca.* 28 fold) than the smaller 1-methyl group reported in section 4.1.4 above, which itself has a 20 fold reduction in affinity compared to melatonin. This negative effect on binding is largely reversed however, on the introduction of fluorine to the 2 position, indicating that 2-fluoromelatonin would be a very potent compound indeed. This effect is similar to that observed with bromine and iodine (see previous section), however, since fluorine is a relatively small molecule, it might not be expected to promote the occupancy of the preferred conformation of the ligand at the melanophore for purely steric reasons. This result might therefore support the proposal of a lipophilic binding pocket with which this part of the molecule can interact. Unfortunately, the apparent lability of this substituent at the 2 position suggests that it may find little use as a pharmacological tool. 2-Fluoro-1-methylmelatonin might, however, be a significant target molecule for a highly potent melatonin agonist. Time precluded the completion of the synthesis of this compound although it should be prepared in the near future.

***O*-Alkyl melatonin analogues**

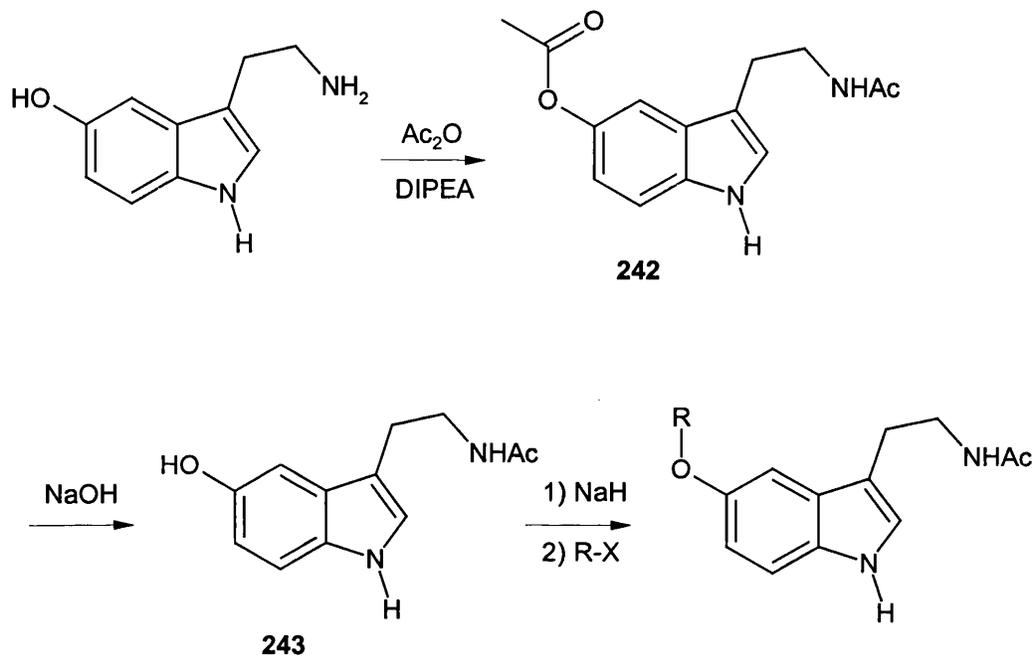
4.7 Introduction

Early studies by Heward and Hadley demonstrated the importance of the 5-methoxy group of melatonin for imparting the property of agonism at the melatonin receptor.¹³⁶ Replacing the methyl group with hydrogen (*N*-acetyl-5-hydroxytryptamine) results in loss of activity and we wished to examine the effect of replacing the methyl with a set of larger alkyl chain substituents on receptor binding and biological activity. Whilst many of these compounds have been tested in melatonin assays, this had not been done in the *Xenopus* assay system. In addition, the advent of literature reports of cloned melatonin receptors

suggested that it would be worthwhile to have these compounds in hand for later studies.

4.11 Synthesis

Using the procedure of Flaugh *et al.*¹³⁷ we have prepared a set of 5-alkoxyl analogues from commercially available 5-hydroxytryptamine (scheme 43).



Scheme 43

5-Hydroxytryptamine was *O,N*-diacetylated (**242**) with acetic anhydride in chloroform and a subsequent selective hydrolysis of the *O*-acyl group with sodium hydroxide gave *N*-acetyl-5-hydroxytryptamine (**243**) in 73% overall yield. This product was spectrally identical with that reported by Flaugh and co-workers. Deprotonation of the 5-hydroxyl group by sodium hydride, allowed subsequent alkylation of the resulting anion with a range of alkyl halides. The compounds prepared by this method are shown in table 13.

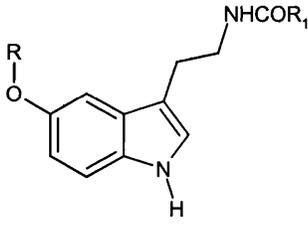
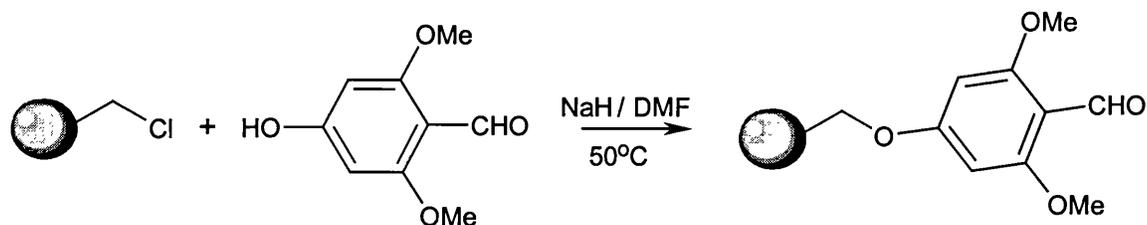
	Compound	R	R ₁
	239	COCH ₃	CH ₃
	243	H	CH ₃
	Melatonin	CH ₃	CH ₃
	244	CH ₂ CH ₃	CH ₃
	245	CH ₂ CH ₂ CH ₃	CH ₃
	246	cBu	CH ₃
	247	Bz	CH ₃
	248	4-MeO-Bz	CH ₃

Table 13. 5-alkoxyl melatonin analogues.

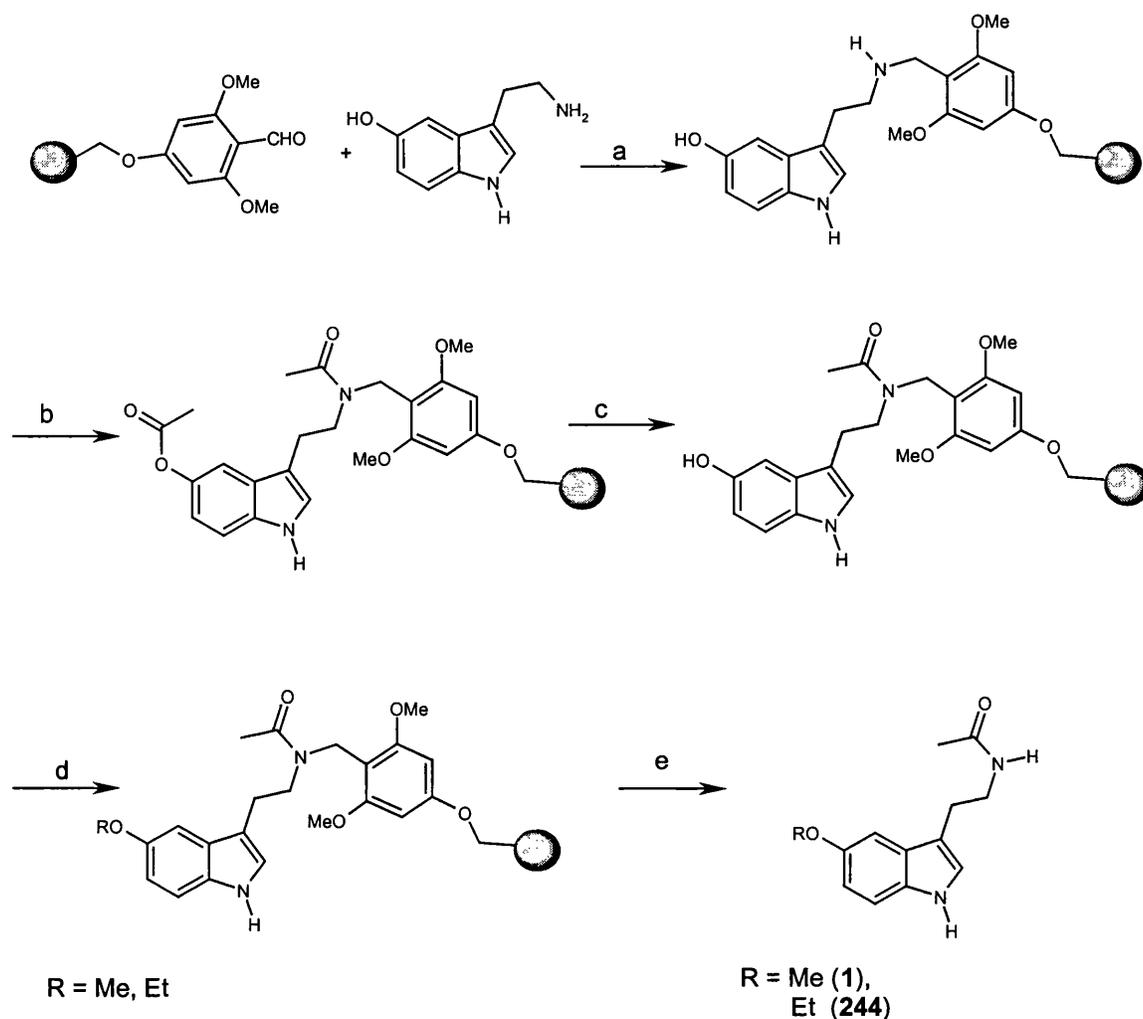
We have also investigated the possibility of preparing these compounds on solid phase, since this route might allow the rapid generation of a large number of analogues from a common polymer supported intermediate. For this, we required a resin bound aldehyde, which had been used previously by Ellman to attach a variety of amino esters on to resin prior to elaboration into benzodiazepine-2,5-diones.¹³⁸ Following Ellmans protocol, commercially available Merrifield resin was derivatised with the 2,6-dimethoxyaldehyde linker as shown in scheme 44. The presence of electron donating methoxyl groups is necessary to facilitate the final acid promoted cleavage step.



Scheme 44

It is difficult to monitor the progress of reactions on solid phase without actually cleaving a sample of resin. In this case, quantification of the resin loading was not attempted, but a small sample of the resin was treated with a solution of 2,4-dinitrophenylhydrazine (2,4-DNP) in DMF and a red colouration of the beads was noted, indicating the presence of an aldehyde group. In addition, the IR spectrum showed a strong aldehyde carbonyl stretch at 1690 cm^{-1} . 5-Hydroxytryptamine was attached to this resin by reductive amination using sodium triacetoxyborohydride and 1% glacial acetic acid in DMF as solvent. After 18 hrs, a small amount of the resin was removed and treated with a solution of chloranil in toluene, as a functional group test which confirmed the presence of a secondary amine.¹³⁹ In addition, the 2,4-DNP test was negative and the IR spectrum obtained from the resin showed the disappearance of the aldehyde peak at 1690 cm^{-1} . We then treated this resin with acetic anhydride (after which a negative chloranil test was obtained), and subsequently hydrolysed the *O*-acetyl group with 50% 2M sodium hydroxide in THF. The alkylation step was carried out using cesium carbonate and methyl or ethyl iodide in DMF. Cleavage of compound from the resin was then effected with 50% TFA in DCM (scheme 45). The required compounds ($R = \text{CH}_3$ and C_2H_5) were obtained in good purity although a small but significant amount of material was observed in the MS-HPLC which we could not characterise. This impurity was observed in both samples, as well as in the products from other experiments carried out on solid phase. We suspect that it is derived from the leaching of material from the tubes used to contain the reaction, since the impurities were not observed when the reactions were carried out in glass.

The presence of these impurities might be tolerated in a synthesis aimed at a lead discovery programme where characterisation need not initially be so rigorous. For the purposes of this piece of work, however, the need to purify enough material for characterisation meant that there was no real advantage in using a solid phase route.

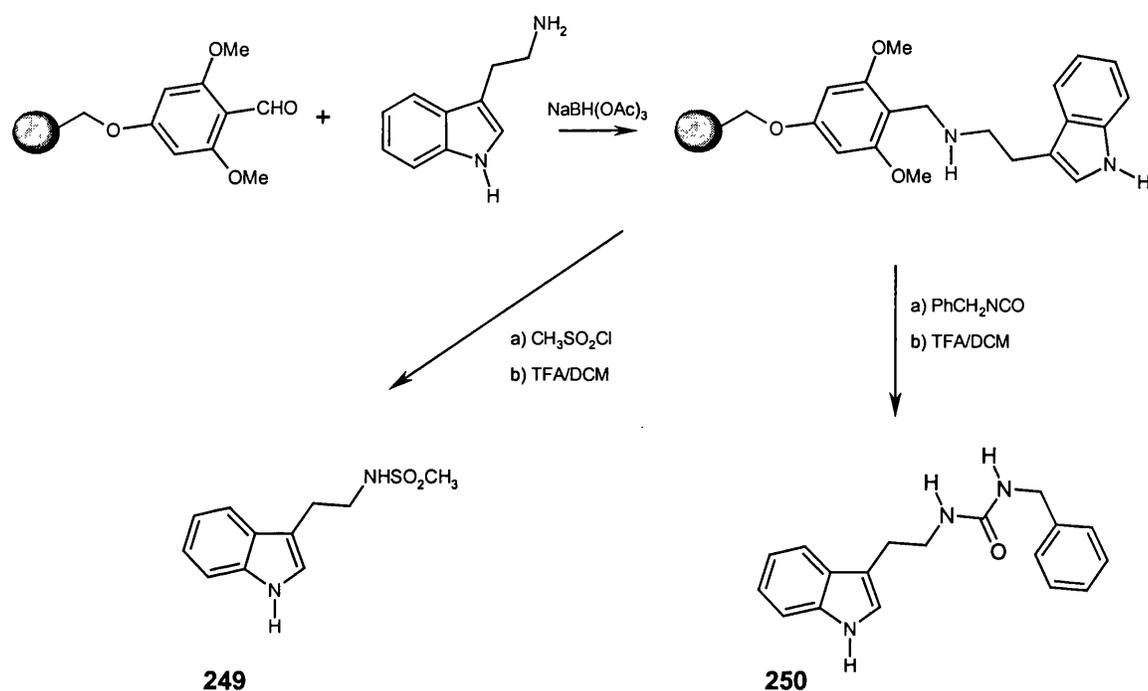


Reagents: a) $\text{NaBH}(\text{OAc})_3$, 1% Acetic acid in DMF. b) Ac_2O , DCM, DIPEA.
 c) 50% 2M NaOH in THF. d) CsCO_3 , R-I, DMF. e) 50% TFA in DCM.

Scheme 45

One of the attractions of this route is the possibility of varying the *N*-substituent by using a range of reagents such as acid chlorides, sulfonyl chlorides, isocyanates etc. and two trial reactions were performed in order to test the feasibility of this approach. Reductive amination of the aldehyde resin with tryptamine and treatment of the polymer bound amine with methanesulfonyl chloride or benzyl isocyanate gave the required sulphonamide (**249**) and urea (**250**) products in good

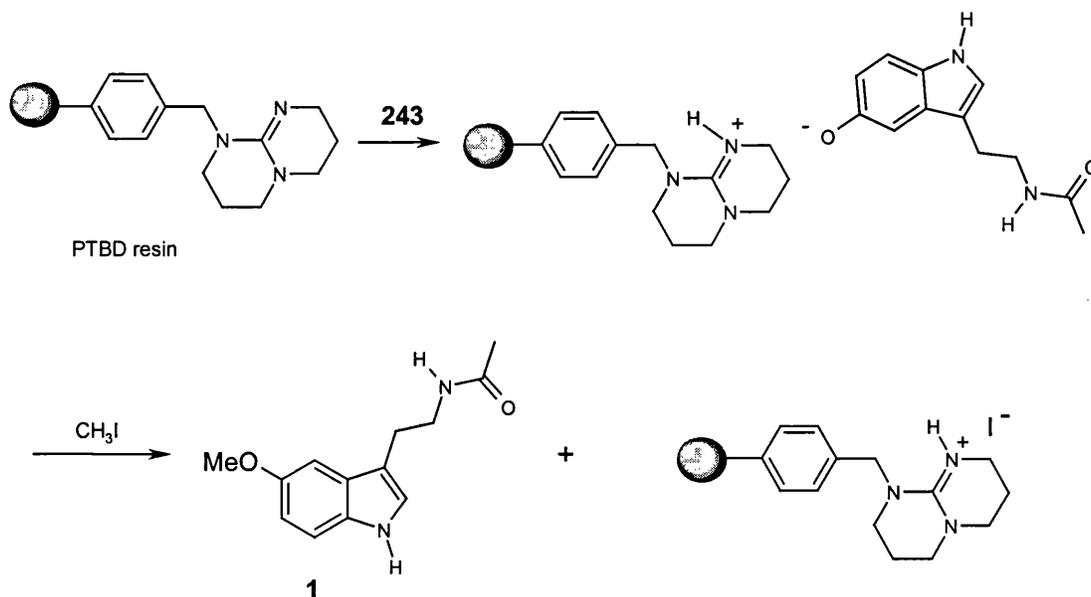
purity (> 90% based on HPLC-MS evidence, scheme 46). We did not characterise these products by any other means, but have used the approach successfully in other chemistry not reported in this thesis.



Scheme 46

Since this investigation several new aldehyde functionalised resins have become commercially available, including the one used here.

We have also attempted an alternative approach suggested by a publication from Xu and co-workers, who report *O*-alkylation of phenols with a polymer supported guanidine base (PTBD).¹⁴⁰ Our attempts to apply their methodology to the alkylation of *N*-acetyl-5-hydroxytryptamine (scheme 47) were disappointing as, despite obtaining evidence for the required products by HPLC-MS, the recovery was extremely low (< 25% with a reaction time of 1 week).



Scheme 47

We therefore decided not to pursue either of these routes and prepared the compounds by the established procedure of Flaugh *et al.*

4.9 Results and Discussion

All compounds were screened for agonist and antagonist activity by Dr. David Sugden and co-workers, Kings College, London. The assay determined the pigment aggregation response of *Xenopus Laevis* dermal melanophores after administration of drug and is described in section 2.9.2. Data is given in the form of EC_{50} for full agonists which represents the concentration of drug required to produce 50% of the maximum aggregation of melanophores. Antagonism is reported as an IC_{50} value representing the dose required to reverse 50% of the maximum aggregation resulting from addition of a single dose of melatonin at 1 nM. The results are shown in table 14 below. Binding data has not been obtained for these compounds at present.

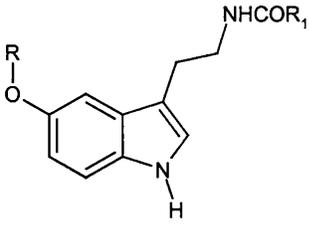
	Compound	Agonist EC ₅₀ (nM)	Antagonist IC ₅₀ (nM)
	239	-	21380
243	1100	-	
Melatonin	0.08	-	
244	3.72	-	
245	60.3	-	
246	-	22387	
247	-	218776	
248	-	234423	

Table 14 Biological activity of some 5-alkoxy melatonin analogues (for structures see Table 13, p175).

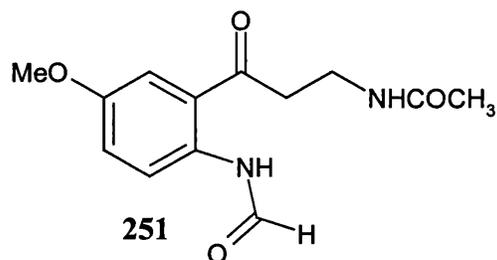
Our results support the widely held belief that the 5-methoxyl group occupies a fairly small lipophilic pocket in the receptor and that there is little scope for chemical modification at this position without significant loss of activity. The poor biological activity of *N*-acetylserotonin (**243**) in this assay can be explained by the polar nature of the hydroxyl group, by its potential as a hydrogen bonding proton donor, or by a combination of these with its smaller size. This lack of activity is not surprising, since 5-HT is present at serum levels far in excess of melatonin and a structural basis for achieving the necessary selectivity must be crucial. There is clearly a diminution of activity with increasing chain length of the *O*-alkylating group (Me > Et > Pr), with the EC₅₀ values for these compounds being *ca.* 0.1 nM, 4 nM and 60 nM respectively. Introduction of both the cyclobutyl (**246**) or benzyl substituent (compounds, **247** and **248**) resulted in the observation of antagonist activity. The cyclobutyl derivative **246**, however, is considerably more potent than the benzyl (**247**) or 4-methoxybenzyl (**248**) compounds which are both rather weak antagonists.

The results confirm that the receptor pocket in this region has quite specific size requirements with the methyl group found in the natural ligand being optimum. The receptor does not tolerate large substituents at this position, with the antagonist properties of the bulkier compounds probably being the result of a steric exclusion effect. Compounds **239** and **246** are reasonably potent in terms of antagonist activity and may be of some interest on this account.

Synthesis of a formylkyneurenine metabolite of melatonin

4.10 Introduction

Two major pathways are involved in the primary metabolism of melatonin, both of which are common to many members of the tryptophan class of molecule. One major pathway involves hydroxylation of melatonin at the C6 position, followed by excretion as the water soluble sulphate or glucuronate. These compounds have been, and continue to be, useful markers of melatonin levels in many studies. The second pathway is *via* oxidative cleavage of the 2,3-indole bond, which results, initially, in the formation of *N*-acetyl-4-methoxy-formylkyneurenine (**251**).



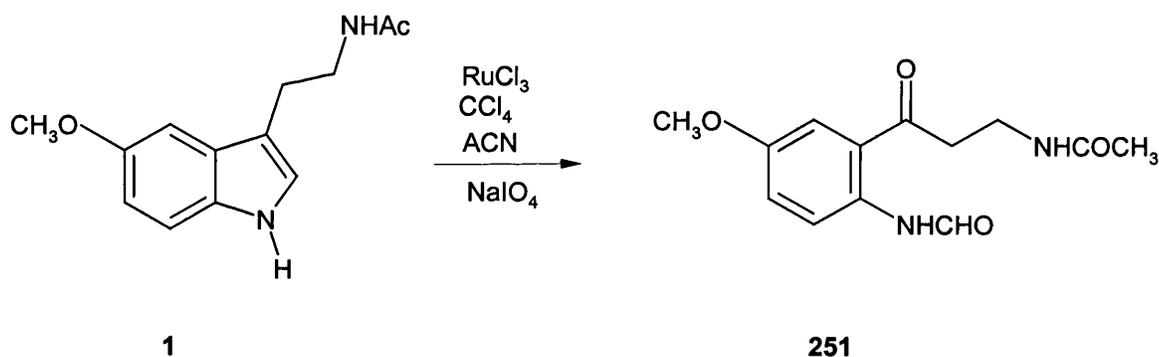
Botting has recently compiled an excellent review of the chemistry of the kyneurenine pathway of tryptophan metabolism.¹⁴¹ Kyneurenine is a key metabolic intermediate which is at a branch point for several important biosynthetic pathways, such as the aromatic amino acid pathway and the quinolinic pathway. The importance of the kyneurenines is emphasised by the

fact that in peripheral tissue, more than 95% of dietary tryptophan is converted to kynurenine compared to less than 1% which is converted to 5-HT.

This has given rise to speculation about a possible physiological role for the indole metabolite and it has been suggested that the methoxy-kynurenamine **251** might be the actual mediator of many of the biological effects ascribed to melatonin. We were therefore interested in synthesising this compound for biological evaluation.

4.11 Synthesis

Compound **251** was prepared from melatonin using a ruthenium chloride catalysed periodate oxidation (scheme 48). The product was obtained in only 19% yield after chromatography but as this was sufficient for our purposes no attempts were made to optimise this procedure.



Scheme 48

4.12 Results

Compound **251** was assayed for biological activity by Dr David Sugden and co-workers as described in section 2.9.2. The compound had an EC₅₀ of 6.34 nM which while being 100 fold lower than melatonin itself, still represents significant biological potency. Much investigation is necessary to discover the actual role and subsequent fate of this metabolite and a systematic study of the compound and some analogues has been initiated within the Garratt group.

4.13 Sulfur containing analogues of melatonin

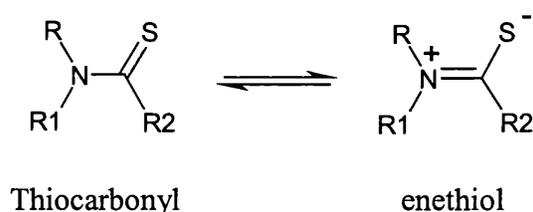
Introduction

Depreux *et al.* have examined the effect on ovine *pars tuberalis* membrane homogenates, of replacing the indole nitrogen of melatonin with sulfur and oxygen.¹⁴² These changes are well tolerated (*ca.* 1 nanomolar activity for both compounds), indicating that the heteroatom itself is playing little part in the binding or biological activity of melatonin agonists. This led to the suggestion that the indole ring was simply acting as a 'spacer' between the methoxyl and *N*-acetyl pharmacophores. It is likely, however, (see section 2.14) that there may be an important π -stacking interaction between the indole ring and a tryptophan or phenylalanine residue in the receptor, which contributes to the overall binding affinity observed for melatonin.

Most current 7TM receptor models are designed on the basis of hydrogen bonding interactions between the ligand and specific amino acid residues in the receptor. For melatonin, the most obvious sites for interactions such as these are the methoxyl and acetyl oxygens, as hydrogen bond acceptors, and the acetyl N-H as a putative hydrogen bond donor. By examination of melatonin receptor sequences and their difference to 5HT receptors (see section 2.14), we have postulated that the oxygen of the 5-methoxyl group may be acting as a hydrogen bond acceptor from a conserved histidine residue in the fifth transmembrane helix. In addition, the presence of two conserved serine residues in the third transmembrane helix might indicate a role as hydrogen bond donor/acceptors to the N-H and C=O moieties of the *N*-acetyl side chain. In order to test this idea, we decided to prepare and examine some compounds in which the oxygen atoms of the melatonin pharmacophores were replaced by sulfur.

Much of the chemistry of thiocarbonyl compounds is influenced by the relatively weak C=S bond compared to that of C=O. Calculated bond dissociation energies show that the strength of the C=O bond exceeds that of the C=S bond by

approximately 40 kJ mol^{-1} .¹⁴³ Whilst this relative weakness results in the instability of many simple aliphatic thiocarbonyl compounds, this is offset in thioamides by an increased resonance stabilisation through electron release from nitrogen and a more pronounced tendency towards tautomeric change (scheme 49). X-ray studies indicate that a significant contribution towards stability is made by the enethiol form, with the key atoms situated in a plane similar to that in normal olefinic double bonds.¹⁴⁴ This contribution appears to be greater in thioamides than the corresponding amides and results in rotational barriers for the former being $12\text{-}20 \text{ kJ mol}^{-1}$ higher than the latter.¹⁴⁵

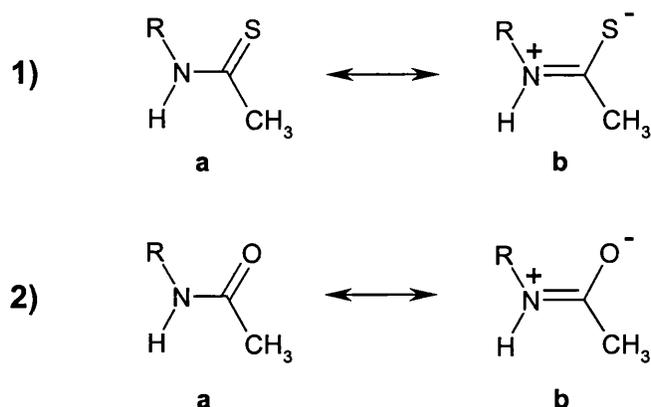


Scheme 49

We have based most of our suggestions about receptor binding on possible hydrogen bonding interactions between the ligand and the receptor. Several groups have studied this type of interaction, and their work has been recently reviewed by Abraham *et al.*¹⁴⁶ Dudek and Dudek¹⁴⁷ (from NMR studies) and Gramstad and Sandstrom¹⁴⁸ (from I R studies) have used experimental evidence to suggest that the N-H of a thioamide is a stronger proton donor and the C=S moiety a weaker acceptor than the corresponding amide analogues.

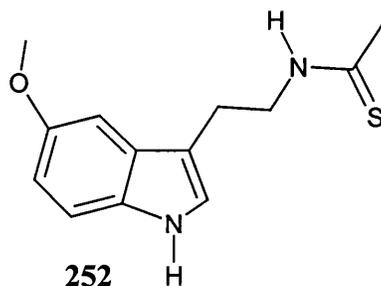
Hydrogen bond donor ability seems to be largely restricted to N-H or O-H, while H bond acceptors are more widely distributed and include N, O, S, and F. The ability to function as proton donor is a consequence of the fact that hydrogen atoms bound to electronegative elements, such as N or O, acquire a partial positive charge because of the high polarity of the corresponding bond. Generally for a donor R-A-H, as R becomes more electronegative then A (and consequently

H) becomes more electron deficient and better H bond donation is observed. This can be applied to rationalise the relative proton donor ability of thioamides (scheme 50, **1a/b**) and amides (scheme 50, **2a/b**).



Scheme 50

The lower electronegativity of sulfur relative to oxygen might be expected to have the effect of decreasing the hydrogen bond donating ability of the adjacent nitrogen in thioamides relative to the analogous amide. This σ inductive effect on nitrogen however, is diminished by the intervening carbon and overcome by the greater contribution of the enethiol form **1b**. This results in nitrogen having a greater electron deficiency in thioamides relative to amides and makes the former much better proton donors than the latter. A deshielding effect is observed experimentally in the ^1H NMR spectra of compound **252**, in the lower resonance (relative to the amide), of the signal assigned to the thioamide N-H. A corresponding downfield shift of *ca.* 30 ppm is also observed for the signal assigned to the carbon attached directly to the sulfur in the ^{13}C NMR spectrum.



The intrinsic electronegativity of the heteroatom plays a larger role in determining the proton acceptor characteristics of a chemical group. The outer shell electrons in the smaller oxygen atom are held more tightly by the nucleus with the result that the negative charge is relatively concentrated around the nucleus. For sulfur, the increased size and correspondingly increased diffusion of the electronic charge results in the thioamide compounds being substantially weaker hydrogen bond acceptors than the corresponding amides.¹⁴⁹

A feature of hydrogen bond interactions is their directional character. The usual representation of O-H donation to oxygen, for example, is depicted in figure 34a. The donor O-H bond is derived from overlap between the hydrogen s orbital with an sp^3 orbital of the oxygen to which it is bonded. The δ^+ hydrogen is then aligned in a purely 'electrostatic' attraction with the lone pair sp^3 orbital on the acceptor oxygen atom. Recent experiments, however, indicate that the lone pair electrons of the acceptor atom spend a 'non negligible' amount of time in the vicinity of the hydrogen, resulting in a continuous wavefunction between the two oxygens as depicted in fig. 34b.¹⁵⁰

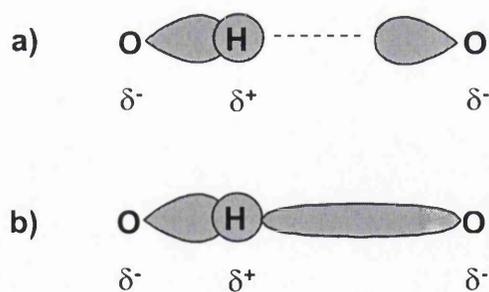
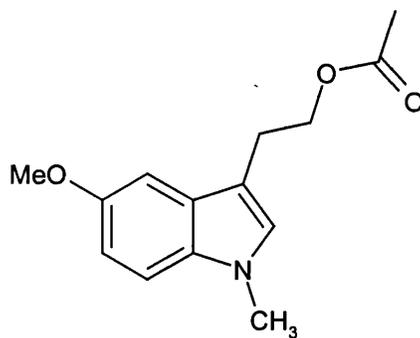


Figure 34

This covalent element to hydrogen bonding may represent a significant contribution to the interaction but can only occur when the orbitals are correctly aligned. When the donor group is essentially fixed in space, as a residue in a receptor might be, the importance of geometry for the lone pair of the ligand molecule is emphasised.

We should note that most of our postulations regarding activity and binding are based on the idea of a 'rigid receptor', that is to say, we assume that the residues on the receptor are fixed in space and we envisage trying to fit the ligand to these fixed points. This is an assumption however, and might not be true. The act of binding, for example, may induce conformational changes in the receptor and / or ligand, which in turn determines the range of interactions available to the receptor-ligand complex. This may be one of the physical bases for the property of antagonism, in that a conformational change induced on the binding of one pharmacophore may preclude the necessary interaction in another part of the molecule, which is fundamental to biological activity.

If the primary function of the acetamide pharmacophore in the melatonin model is to act as a hydrogen bond acceptor, then replacement by the bioisosteric ester or ketone groups would be expected to result in small changes in potency. If it is primarily a hydrogen bond donor then these changes should reduce potency, and if it is acting as a non-interactive 'spacer' then little change should be observed. Vonhoff has synthesised the methyl ester analogue (**253**) of *N*-methylmelatonin, and found no binding affinity at less than 10000 nM concentrations.¹² This tends to imply that hydrogen bond donation by the amide N-H is very important in binding of the ligand to the receptor. It is necessary to qualify this however, since the geometry of the ester carbonyl is different to that of the amide and this may disrupt any hydrogen bond acceptor interactions involving this oxygen.



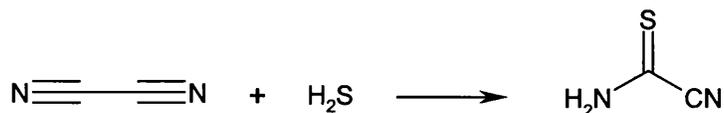
253

Replacing the 5-methoxyl group with the thiomethyl moiety might also be expected to have consequences for biological activity, particularly if hydrogen bonding to the receptor is important in this position (see also sect. 4.2.2). Ethers are capable of acting as hydrogen bond acceptors whereas this is generally not thought to be true for arylalkylthioethers.¹⁴⁶ Allen and co-workers, using data retrieved from the Cambridge structural database, report that, of 1811 sulfur containing compounds that co-occur with N-H or O-H donors less than 5% form S...H-N(O) bonds.¹⁵¹ This implies that if hydrogen bonding between the receptor and the 5-methoxyl oxygen is very important we might expect to see a decrease in activity for the 5-thiomethyl melatonin analogue.

Thus, replacement of oxygen atoms by sulfur may yield some information about putative hydrogen bonding interactions at these sites of exchange. Melatonin, and the 5-ethoxyl melatonin analogue **244** (described in section 4.2) were selected as substrates for the exchange procedures.

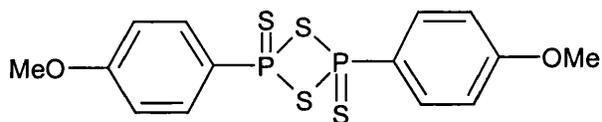
4.14 Synthesis

The thioamide or thioether melatonin analogues we chose to prepare were not reported in the literature, however, several approaches to the synthesis of these compounds recommended themselves. The chemical transformation of the carbonyl group to a thiocarbonyl derivative has been the subject of synthetic effort for over a century.¹⁵² The first thioamide was reported in 1815 by Gay-Lussac from the reaction between hydrogen sulfide and cyanogen (scheme 51).¹⁵³



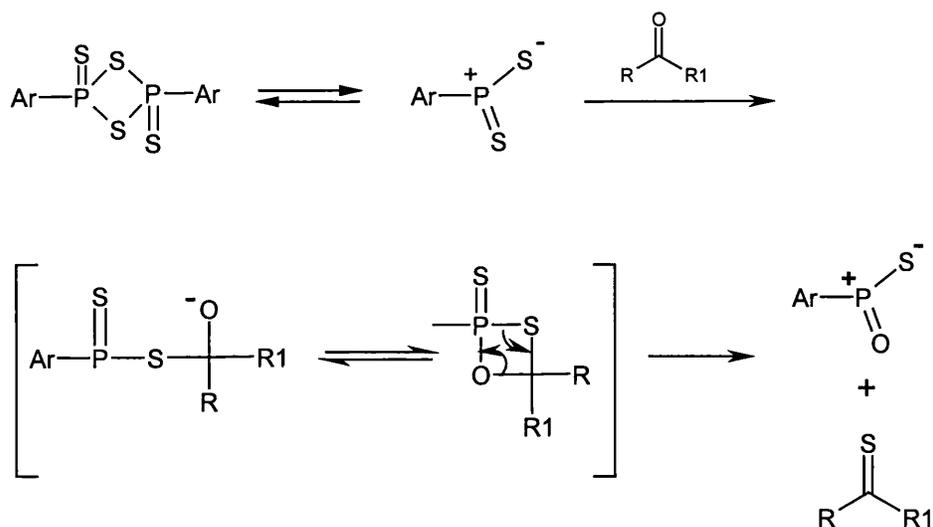
Scheme 51

In 1869 Henry¹⁵⁴ and Wislicenus¹⁵⁵ independently reported the successful thionation of carbonyl compounds by heating with phosphorous(V) pentasulfide and for much of the last century most of the reported syntheses have relied on one of these two methods. In recent years the most commonly used reagent has been 2,4-bis(4-methoxyphenyl)-1,3-dithiadiphosphetaine-2,4-disulfide (**254**), now commonly referred to as Lawesson's reagent.¹⁵⁶ This compound has been successfully applied in the thionation of a wide range of carbonyl groups and the transformations are covered in an excellent review by Cava and Levinson.¹⁵⁷



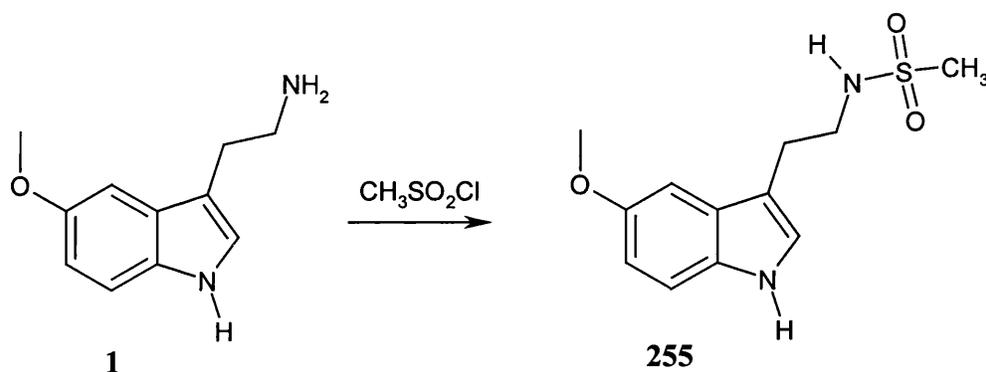
254

The mechanism of the reaction is thought to involve the intermediacy of a highly reactive dithiophosphine ylid and probably involves a four membered ring akin to the betaine structure postulated for the Wittig reaction (scheme 52).^{158,159}



Scheme 52

In addition to these series of compounds, we also would like to have varied the oxidation state of the introduced sulfur, by synthesis of sulfoxide and sulphone analogues. The geometries of these groups are markedly different to that found in the same position of melatonin and we wished to determine what effect this alteration might have on receptor binding. Time did not allow the completion of this part of the exercise, with the exception of the simple sulphonylation of 5-methoxytryptamine, using methanesulphonyl chloride (scheme 53).



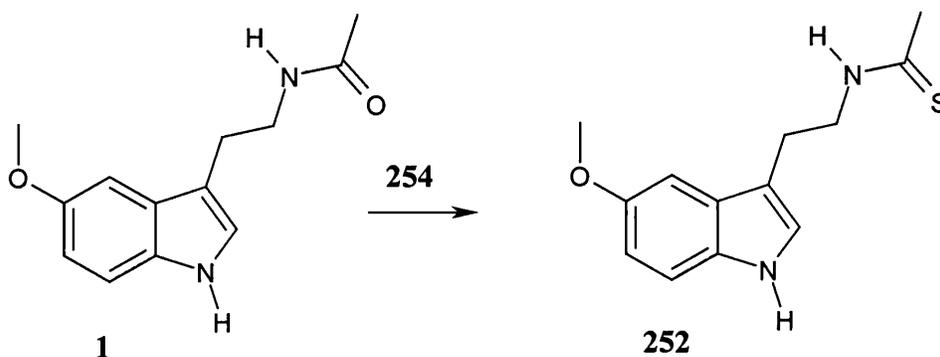
Scheme 53

Table 15 lists the series of compounds that were prepared for this investigation.

	Compound	R	X	R1
	melatonin	CH ₃	O	COCH ₃
	252	CH ₃	O	CSCH ₃
	256	CH ₃	S	COCH ₃
	244	CH ₂ CH ₃	O	COCH ₃
	257	CH ₂ CH ₃	S	COCH ₃
	258	CH ₂ CH ₃	O	CSCH ₃
	259	CH ₂ CH ₃	S	CSCH ₃
	255	CH ₃	O	SO ₂ CH ₃

Table 15. Sulfur containing melatonin analogues.

We chose to begin the preparation of this series of atom replacements by focussing on the conversion of the acetyl group to a thioacetamide using Lawesson's reagent. Literature precedent (for simple thionations)¹⁵⁷ recommended the use of 0.5 molar equivalents of the Lawesson's reagent and initial attempts using this stoichiometry yielded the required product, **252**, from melatonin, in 29% yield after chromatography (scheme 54). The experiment was repeated with the addition of a second 0.5 molar equivalent of Lawesson's reagent after 2 hr and the yield was increased to 69%.



Scheme 54

A possible explanation for the increased yield is that the oxygen of the 5-methoxyl group may be competing for the electrophilic phosphorus of the thionating reagent. This effect has been proposed by Baxter and Bradshaw to explain the lack of reactivity towards thionation by esters which also contain an ether functionality.¹⁶⁰

Subsequent attempts to improve the yield further, by increasing the molar ratio of thionating agent, were not successful, possibly because purification of the resulting crude reaction mixture became more difficult. The possibility of preparing a polymer supported Lawessons reagent, perhaps tethered *via* one of the methoxyl groups (as illustrated in figure 35), appealed to us since it might considerably simplify the purification procedure and possibly result in improved recovery of product. We did not have time to pursue this however.

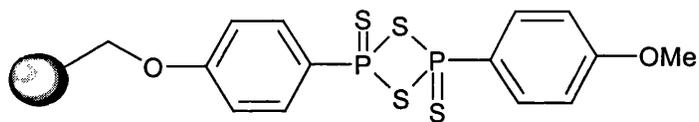
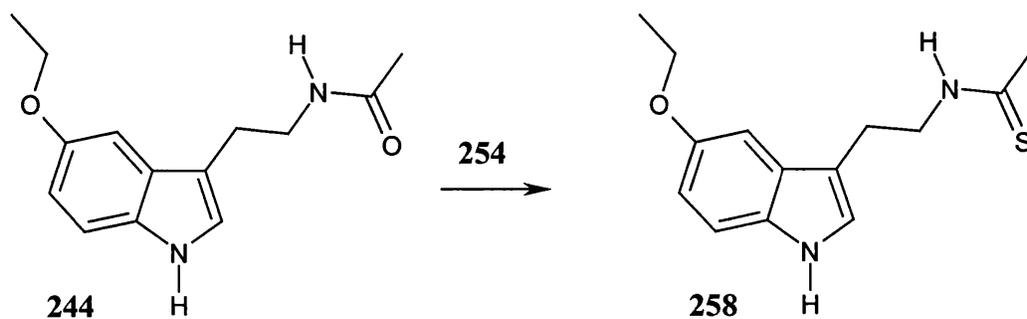


Figure 35

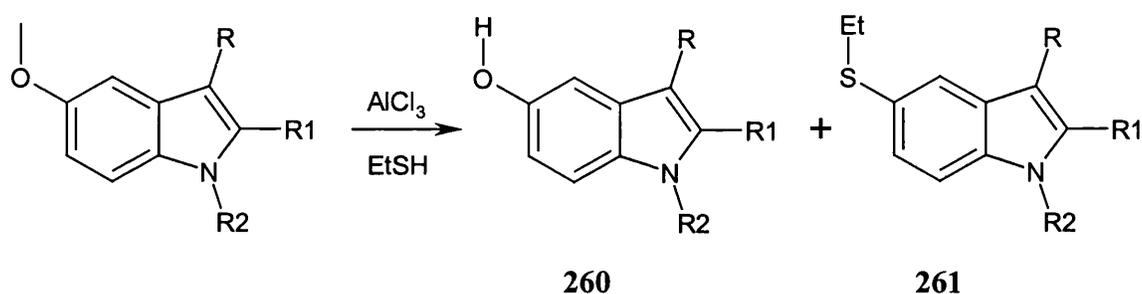
The ^1H NMR spectrum of **252** shows several notable shifts as a result of thionation. The signal assigned to the methyl group of the *N*-acetyl side chain of melatonin ($\delta = 1.93$ ppm) is shifted downfield to *ca.* $\delta = 2.5$ ppm, whilst the signal assigned to the methylene group adjacent to the thioamide experiences a similar shift, from $\delta = 3.58$ ppm in melatonin, to $\delta = 3.98$ ppm in **252**. Signals assigned to the aromatic protons and the 5-methoxyl group are essentially unchanged. The proton assigned to the acetamide NH is, however, shifted from *ca.* $\delta = 5.6$ ppm in melatonin to *ca.* $\delta = 7.4$ ppm in **252**. The ^{13}C NMR spectrum reflects the changes in the ^1H NMR spectrum, with the signal assigned to C=S being particularly diagnostic at *ca.* $\delta = 200$ ppm. The NMR spectra of melatonin and **252** are included in appendix (figures 45-48). In the IR spectrum, the absence of an amide C=O stretch at *ca.* 1650 cm^{-1} is observed.

We then applied the same reaction to the ethoxyl compound **244**, and obtained the thioamide **258**, in 23% yield (scheme 55).



Scheme 55

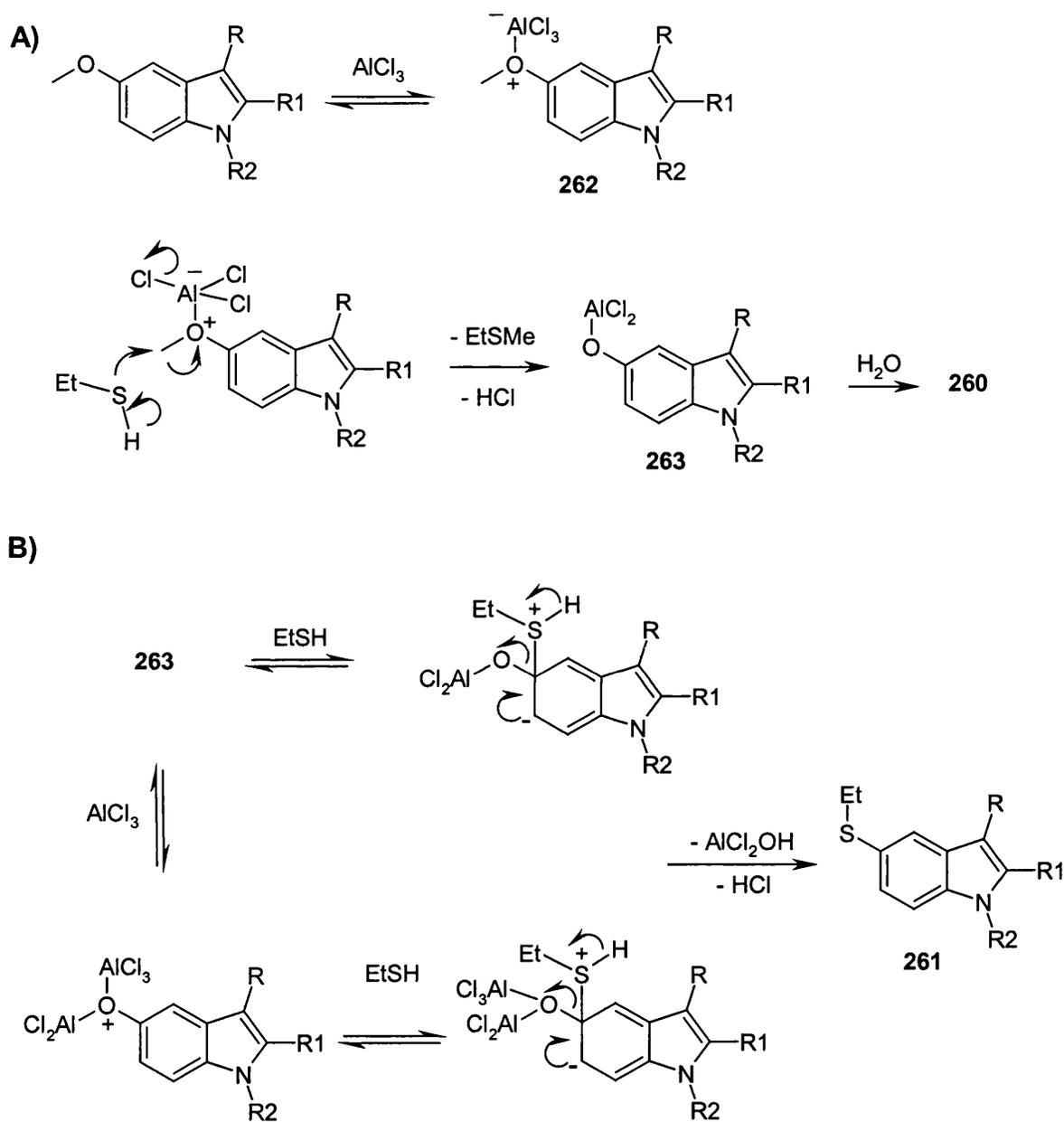
Having established a method for thionation of the acyl oxygen, we wished to effect a similar replacement of sulfur for oxygen in the 5-methoxyl and 5-ethoxyl groups. Fortunately, the direct substitution of 5-methoxyl for an SEt moiety had recently been reported by Caubere and co-workers.¹⁶¹ Whilst trying to demethylate some aryl methyl ethers Caubere *et al.* serendipitously observed alkylthiolation, when using aluminium halides associated with thiols. The group subsequently applied this protocol to 5-methoxyindoles and found that a combination of aluminium chloride and ethanethiol, both in large excess, efficiently thioalkylated the substrates (scheme 56).



Scheme 56

Aluminium chloride was shown to be superior to aluminium bromide in the formation of **261**, whilst the latter reagent was better at producing compound **260**. The success of the substitution relies on a large excess of reagents and is dependant on the nucleophilicity of the thiol used. The best results were obtained with PhCH₂SH. Caubere *et al.* support a mechanism for the formation of **261** proposed by Fujita and collaborators (scheme 57).¹⁶² The proposed mechanism postulates an initial complexation of the aluminium trihalide (path A), with the ether oxygen of the indole to give a charged intermediate **262**. Subsequent attack by the nucleophilic thiol leads to the formation of a thioether and the charge neutral intermediate **263**. Path A terminates with hydrolysis of **263** and the

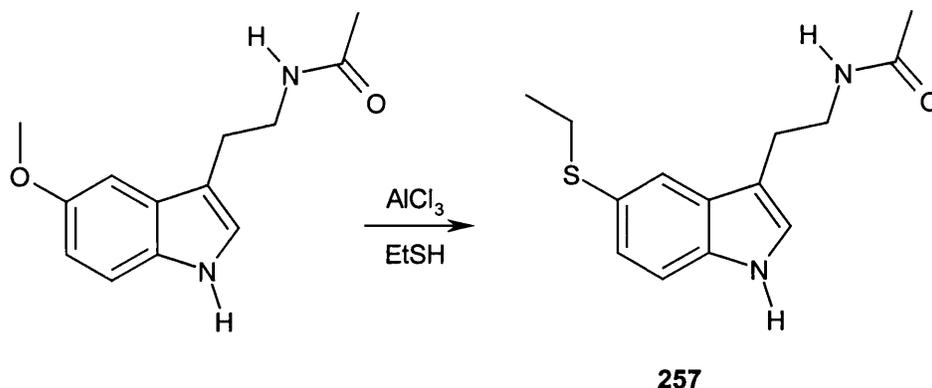
formation of compound **260**, or alternatively, it may react further (path **B**) in the presence of a large excess of reagents, leading to the substituted product **261**.



Scheme 57

We applied this reaction to melatonin and obtained the required product (**257**) in a yield of 15%, after chromatography (scheme 58). This was repeated and the yield

increased to 29%, however, as in the previous synthesis of **252**, we felt that the isolation and purification of the compound may have been partly responsible for the poor yield.

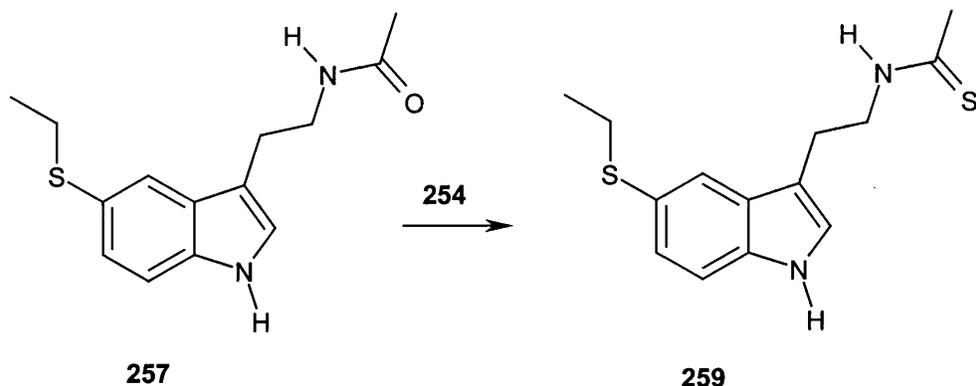


Scheme 58

The ^1H NMR of **257** is almost identical with that of **244**, with the exception that the signals assigned to the ethyl protons are observed to resonate slightly further upfield in **257** ($\delta = 1.24$ ppm and $\delta = 2.9$ ppm) compared to those of **244** ($\delta = 1.43$ ppm and $\delta = 4.0$ ppm respectively). The signals assigned to aromatic protons are themselves shifted very slightly downfield (by *ca.* 0.2-0.4 ppm). In the ^{13}C NMR spectrum, the signal assigned to the *N*-acetyl C=O is clearly visible at *ca.* $\delta = 170$ ppm, showing that this oxygen has not been affected. This is supported by the IR spectrum which has a clear C=O stretch at *ca.* 1650 cm^{-1} .

With compound **257** in hand, we used Lawesson's reagent to thionate the remaining oxygen atom and obtain the fully thionated compound **259** (scheme 59). The reaction between **257** and Lawesson's reagent gave the required product in only 28% yield, after chromatography, despite the fact that the reaction itself was monitored by t.l.c throughout and appeared quite clean in terms of the number of identifiable components. We strongly suspected that in both reactions, involving Lawesson's reagent or ethanethiol/aluminium chloride, the purification process was responsible for a significant drop in recovered material and that there

was potentially much scope for improvement in the yield. However, since we had obtained sufficient material for spectral and assay purposes we did not pursue this further.

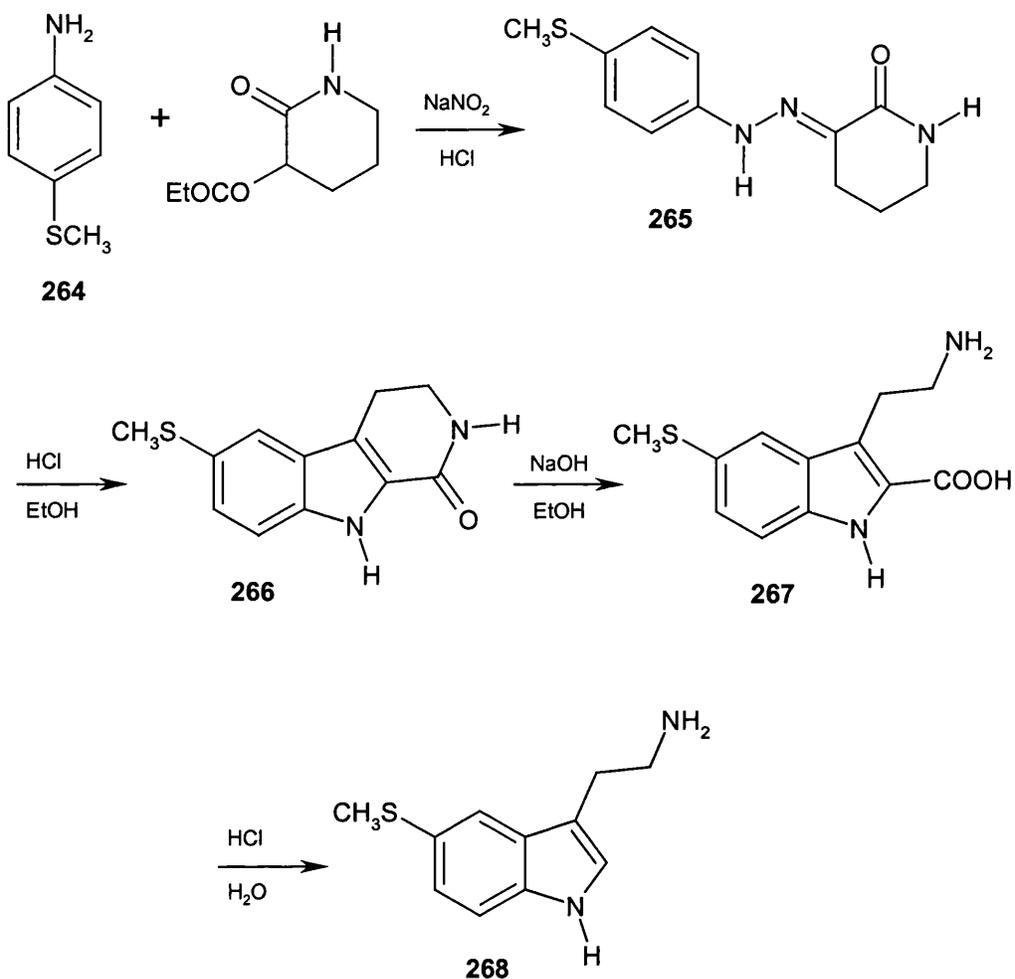


Scheme 59

Compound **259** has a similar ^1H NMR spectrum to **257** except that the signal assigned to the methyl protons of the *N*-acetyl group is shifted downfield from $\delta = 1.95$ to $\delta = 2.5$ ppm. The signals assigned to the methylene protons adjacent to the $\text{NHC}=\text{S}$ group are also shifted downfield from *ca.* $\delta = 3$ ppm in **257** to *ca.* $\delta = 4$ ppm in **259**.

Since methanethiol was not suitable as a nucleophile in the procedure previously used to introduce the thioethyl moiety, we required an alternative synthesis for the direct melatonin analogue. There were a limited number of reports in the literature for the preparation of 2-(5-methylthio-indol-3-yl) ethylamine (**268**) or its derivatives. The most commonly reported procedure for the preparation of **268** was that employed by Adlerova *et al.*¹⁶³ and subsequently by Kline *et al.*¹⁶⁴ and Guengoer and co-workers.¹⁶⁵ This route uses commercially available 4-thiomethylaniline (**264**) and proceeds via diazonium ion formation utilising the Japp-Klingmann variation of the Fischer indole synthesis with piperidine-2,3-dione-4-methylthio-phenylhydrazone (**263**). The resulting tetrahydro- β -carboline

(266) is then treated with base to yield 3-(2-aminomethyl)-5-methylthio-1H-indole-2-carboxylic acid (267), which was decarboxylated in refluxing HCl to give the required tryptamine (scheme 60).

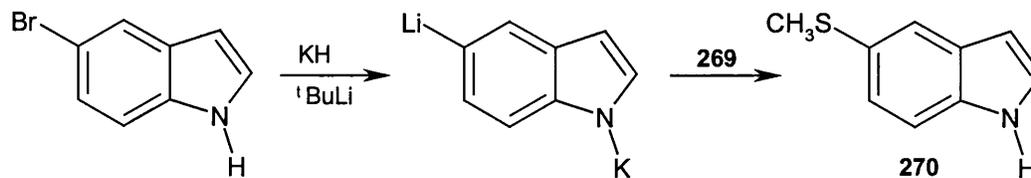


Scheme 60

The overall yield reported for this synthesis was 6% and the potential for achieving significantly less than this did not encourage us.

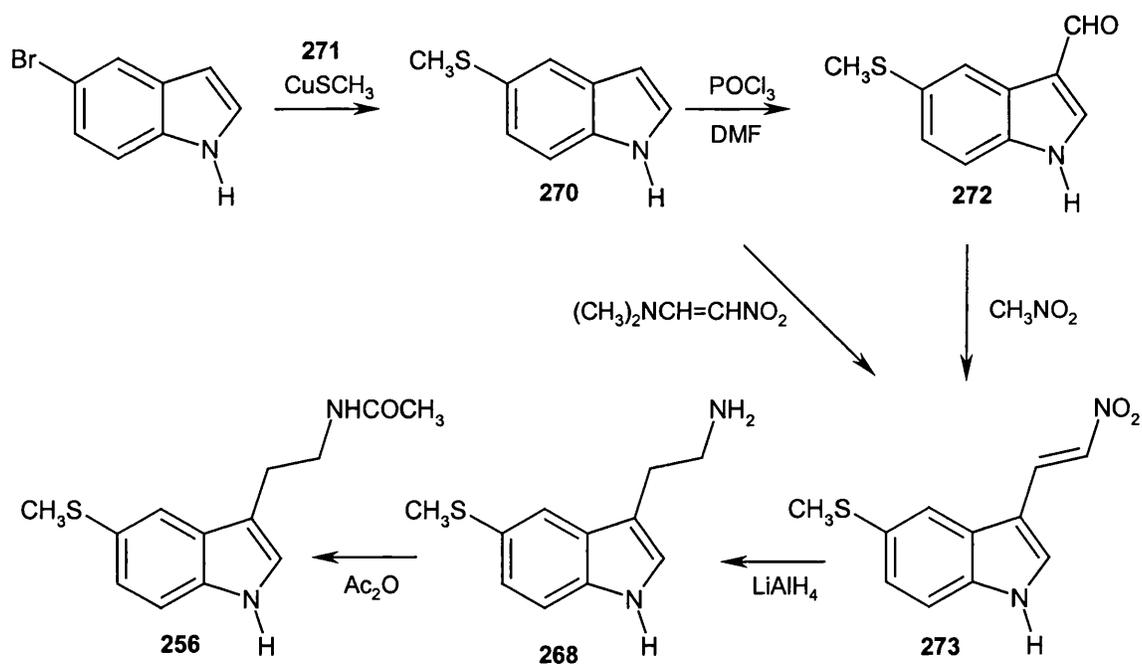
More recently Yang *et al.* published a procedure for the preparation of 5-substituted indoles, including the thiomethylindole, by employing a halogen metal exchange strategy.¹⁶⁶ In this synthesis, commercially available 5-bromoindole

was converted to the 1-potassio derivative in order to prevent C2 metalation, and then subjected to halogen-metal exchange using tertiary butyl lithium. The lithiated species was then treated with dimethyl disulfide (CH_3SSCH_3 , **269**) and was reported to give the required indole (**270**) in > 90% yield (scheme 61).



Scheme 61

We decided to prepare 5-methylthioindole from 5-bromoindole, using the safer and more convenient alternative employed by Guillaume *et al.*¹⁶⁷ This route employs methylthiocopper (**271**) in order to effect the interconversion of bromine to the thiomethyl group. This organocopper reagent is readily prepared, following literature procedure,¹⁶⁸ is used immediately, and does not require even transient protection of the indole nitrogen. With the indole (**270**) in hand, the remaining synthetic steps to the target would involve nitro-olefination of the indole, either directly, or *via* the 3-formyl compound (**272**). The resulting nitro-olefin (**273**) could then be reduced by lithium aluminium hydride and the tryptamine (**268**) acylated to give the required product **256** (scheme 62).



Scheme 62

Our first attempt at preparing 5-methylthioindole using this methodology, resulted in a 1:1 mixture of the required product and 5-bromoindole, according to NMR and G.C. analysis. The two compounds co-eluted under most conditions and proved impossible to separate at this stage. The problem was solved by repeating the reaction, using freshly prepared methylthiocopper and we attributed the initial failure to poor quality of this reagent. The 1H NMR spectrum ($CDCl_3$) was in agreement with published data and showed a singlet at $\delta = 2.5$ ppm which was assigned to the thiomethyl protons. The yield of this reaction was 55% and gave enough material to continue with the synthesis. Some of this material was converted to the 3-formyl intermediate (272) using phosphorous oxychloride and DMF in a Vilsmeier-Haack procedure.¹⁶⁹ The signal in the 1H NMR (d_6 -DMSO) spectrum assigned to the aldehyde group was clear at $\delta = 9.90$ ppm and the IR spectrum showed a large C=O stretch at *ca.* 1650 cm^{-1} . This aldehyde was then reacted with nitromethane in a Henry reaction¹⁷⁰ to form the nitrovinylindole (273) in a combined yield of 19% for the two steps. Alternatively, the 5-

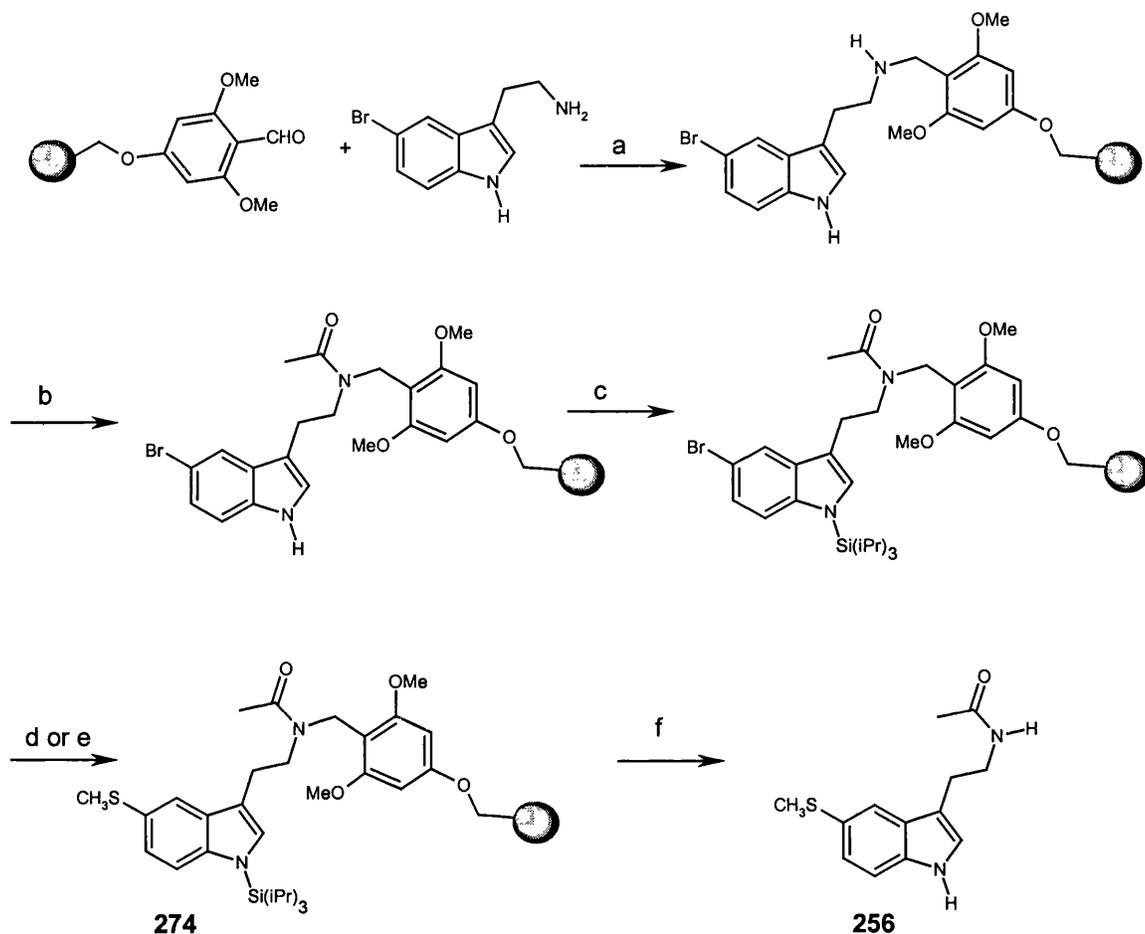
methylthioindole was nitro-olefinated according to the one step protocol of Buchi and Mak.¹⁷¹ In this procedure, treatment of the indole with 1-dimethylamino-2-nitroethylene in TFA at 0 °C gave the required product, as an intense orange crystalline solid, in 35% yield, after chromatography. Whilst not being greatly superior in terms of yield, the ease and speed of this procedure made it preferable to the two step protocol in this case. The ¹H NMR spectrum of compound **273** showed a diagnostic AB quartet between $\delta = 7.90$ ppm and $\delta = 8.4$ ppm which was assigned to the vinyl protons of the nitro side chain.

Compound **273** was subsequently reduced with lithium aluminium hydride in THF to give the required tryptamine (**268**) in 27% yield. Once again, tlc sampling of aliquots of the reaction mixture indicated efficient conversion and we ascribe the poor yield to incomplete recovery of product. The acylation of **268** by acetic anhydride was carried out in 37% yield after chromatography to give the required melatonin analogue **256**. The overall yield for this synthesis was an extremely poor 1.9%.

With the material we had in hand (*ca.* 50 mg), we attempted to thionate the *N*-acetyl group of **256** using Lawessons reagent. Disappointingly, while observing mass spectral evidence for the required material in sampled aliquots of the reaction mixture, we did not manage to isolate any pure material. Time constraints did not allow an attempt at re-synthesis of **256** in greater quantity.

The purification and efficient recovery of some of these compounds has been a major obstacle throughout this investigation and we plan to address this in future by the use of the solid phase chemistry. A particular advantage of this approach would be the ability to use a large excess of reagent to drive reactions towards completion, coupled with a trivial purification process (washing the resin), for the supported intermediates. We are currently attempting the synthesis of compound **256** on solid phase by attaching 5-bromotryptamine to the 2,6-dimethoxyaldehyde resin used in section 4.8. Acylation of the secondary amine, and protection of the indole nitrogen should allow facile lithiation of the aryl bromide. This intermediate could then be treated with dimethyl disulfide, or the

thiomethylcopper reagent, or possibly with a palladium catalyst, to give compound **256** after cleavage from the resin (scheme 63). The released compounds should require relatively simple purification at this stage.



Reagents: a) $\text{NaBH}(\text{OAc})_3$, 1% Acetic acid in DMF, b) Acetic anhydride, DCM, DIPEA. c) NaH , TIPS-Cl, DMF d) CuSMe e) BuLi , CH_3SSCH_3 f) TFA.

Scheme 63

This chemistry is being pursued on a multi-gram scale since it offers the possibility of generating a series of analogues with the thiomethyl substituent at the 5-position using the chemistry illustrated in scheme 46. If the aromatic substitution proves successful we also plan to attempt thionation of the polymer

supported *N*-acyl compound (**274**) with Lawessons reagent and to apply the chemistry to obtaining compound **252** via reductive amination with 5-methoxytryptamine

4.15 Results and Discussion

Biological activity was obtained as described in section 4.2.2. The results are recorded in table 16.

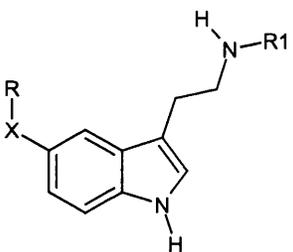
	Compound	Agonist EC ₅₀ (nM)	Antagonist IC ₅₀ (nM)
		melatonin	0.08*
	252	0.57	-
	256	169	-
	244	3.72	-
	257	1.55	-
	258	A/R	A/R
	259	74.1	-
	255	21.4	-

Table 16. Biological activity of some sulfur containing melatonin analogues

(A/R – awaiting result, * Averaged figure). For structures see Table 15, page 186.

Melatonin, the endogenous ligand, was undoubtedly the most potent compound in this set. Replacing the oxygen of the *N*-acetyl pharmacophore with sulfur (compound **252**) resulted in a 7 fold decrease in biological activity. Clearly, this is not very large and may be explained in several ways. It might reflect the fact that hydrogen bond donation by the N-H group is not very important, since we had rationalised that the donor ability should increase as a result of the atom exchange (NH-C=S cf. NH-C=O). However, since the hydrogen bond acceptor

properties of this pharmacophore are detrimentally affected by the change of atoms (C=S cf. C=O), the result might equally well represent an averaged effect of the two influences. Another possibility is that the maximum effect is already achieved by the amide bond N-H of melatonin, so that increasing the donor ability, in **252**, is effectively superfluous. It is not possible to distinguish between these explanations with the limited data obtained here.

Replacing the acetyl group completely with a methanesulphonyl moiety, as in compound **255**, results in a drop in potency of *ca.* 270 fold. This group can obviously still provide hydrogen bond donor/acceptor properties but they will be orientated differently in space and one or the other functions may not be available to the residues in the receptor normally involved in such interactions.

Simply replacing the 5-methoxyl oxygen with sulfur (compound **256**) results in an astonishing 2000 fold reduction in potency. This suggests that hydrogen bond donation to this oxygen is crucial for activity at the melatonin receptor.

The results for the 5-ethoxyl series, however, are not in agreement with those obtained for the 5-methoxyl series. The ethoxyl analogue **244**, has an intrinsic potency which is *ca.* 45 fold lower than melatonin. Surprisingly, replacing oxygen of the 5-ethoxyl group with sulfur, as in compound **257**, actually improves biological activity slightly (2 fold). We currently await results for compound **258** in order to determine the effect of replacing the *N*-acetyl oxygen with sulfur, although the effect of making both replacements simultaneously is deleterious, since a drop in potency of *ca.* 20 fold is observed. The changes observed, however, in this series are in no way as dramatic as those observed for the 5-methoxyl compounds.

It is not possible to explain the differences in the results for these two series at present and a definitive experiment comparing all of the compounds in the same assay is required. It would be very interesting to compare the binding affinities alongside the potency and we currently await this data. The results so far, are interesting enough to warrant further investigation and future work will be carried out in this area to try to clarify the issues. Introduction of conformational

restriction to the oxygen at the 5-position, by incorporation into a ring for example, would be of interest.

4.16 Summary

It would seem from the results of this chapter that the 5-methoxyl group of melatonin interacts with a small lipophilic pocket in the receptor and that there is some indication of a binding region in the C2 region of the melatonin molecule. We did not succeed in synthesising the novel analogue 2-fluoromelatonin, apparently due to the lability of the halogen under our reaction conditions, but have indications from testing the intermediates that it would be very potent. Compounds containing larger substituents at either C5 or at the *N*-acetyl pharmacophore may have antagonist properties and we hope to find a use for some of these compounds in subtype differentiation studies in the future. We have also tried to gain some insight into possible hydrogen bonding interactions between ligand and receptor and their impact on biological activity, by chemical modification of putative donor/acceptor sites within the pharmacophores and future work is planned in this area.

Our current models for binding at the melatonin receptor are largely based on other similar receptor models, as discussed in chapter 2. These suggest the involvement of conserved amino acids, in particular histidine and aspartate residues in the fifth and third helices respectively, which are known to be important for binding in other systems. Site directed mutagenesis studies targeting these residues have started to be reported¹⁷² and will be crucial in refining these models and allowing a better evaluation of the results of analogue assays so far obtained.

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Chapter 5

Experimental

General Information

Chemical reagents were purchased from Aldrich Chemical company, Lancaster and BDH and usually used without purification. Column chromatography was carried out using silica gel 9385 supplied by Merck chemicals. Melting points were measured on a Gallenkamp MFB apparatus and are uncorrected.

Proton nuclear magnetic resonance (^1H nmr) spectra were recorded using a Bruker 360 MHz, Bruker 400 MHz, or Bruker AC 200 MHz spectrometer. The spectra were measured in deuteriochloroform (CDCl_3) or dimethylsulphoxide- d_6 (d_6 -DMSO) solution. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane. Apparent J values are given in Hertz and multiplicities are recorded: s, singlet; d, doublet; t, triplet; q, quartet; qi, quintet. Unresolved aromatic protons are recorded Ar. Carbon nuclear magnetic resonance (^{13}C nmr) spectra were recorded at 90 MHz on a Bruker 360 MHz spectrometer, or at 100 MHz on a Bruker 400 MHz instrument. Signals are reported as δ values using the resonances of CDCl_3 (δ_{C} 77.0 ppm, t) or d_6 -DMSO (δ_{C} 39.7 ppm, heptet) as reference.

E.I. Mass spectra are recorded on a Concept 32 Kratos system or a Fisons V.G. Platform LC.M.S system and FAB mass spectra were recorded on a MS50 Kratos system. High resolution accurate mass spectra were obtained on a Kratos Concept 32 spectrometer or Fisons V.G. Autospec instrument.

Infra red spectra were recorded on a Bruker IFS66 spectrometer or Bio-Rad FTS155 spectrometer and obtained by the diffuse reflectance technique. Circular Dichroism spectra were acquired on a Jasco J600 spectrometer and U.V. spectra recorded on a Perkin Elmer 330 spectrometer by the Department of Physical Sciences, Wellcome Research Laboratories. Rotations were measured using an optical activity polar 2001 polarimeter. $[\alpha]_D$ values are given in units of $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$

The X-ray structure determination was performed by Oxford University X-ray Services. Microanalyses samples were dried *in vacuo* over phosphorous pentoxide

and the microanalyses were carried out in the Department of Physical Sciences, Wellcome Research Laboratories, Beckenham, Kent, or by the Butterworth Laboratories Ltd, Teddington, Middlesex.

General Procedures

Preparation Of α -Bromoketones

Bromine (1.1 eq.) was added dropwise at 0 °C over 2 h to a solution of the ketoester (1 eq.) in ether (300 mL for 1 mole) and the reaction mixture was stirred overnight at room temperature. The reaction was then washed with water (2 x 150 mL), saturated *aq.* sodium bicarbonate solution (2 x 150 mL) and dried over anhydrous magnesium sulphate. The solution was filtered, and the filtrate evaporated *in vacuo* to yield the crude bromoketone as a viscous oil. These oils appeared to be heat labile and so were used without further purification.

Bischler Indole Synthesis

A mixture of the *N*-methylaniline (2 eq) and the bromoketone (1 eq) were stirred at 50 °C for 3 h. The resulting mixture (often a caked solid) was dissolved in anhydrous propan-2-ol (100 mL for 0.1 mole of aniline) and treated with anhydrous zinc chloride (3 eq.). The reaction mixture was then refluxed under nitrogen for 18 h. After evaporation of the crude reaction mixture, the product was extracted by partitioning between 2M hydrochloric acid and ethyl acetate. The dark organic layer was washed with water (2 x 75 mL) and saturated *aq.* sodium bicarbonate solution (2 x 75 mL) and dried over magnesium sulphate. The solution was filtered and the filtrate evaporated *in vacuo* to give a dark oil

which was purified by column chromatography. Elution with ethyl acetate/hexane 1:1 gave the required indole.

Saponification Of Bischler Indoles

The ester obtained from the cyclisation stage was dissolved in hot 90% aq. ethanol (100 mL for 0.01 mole). Sodium hydroxide pellets (10 eq.) were added and the reaction mixture refluxed for 6 h. The alcohol was removed by evaporation *in vacuo* and the alkaline residue washed with dichloromethane (2 x 75 mL). The product was precipitated by pouring into an excess of ice-cold 10% hydrochloric acid. After filtration and washing on the filter with cold water, the product was dried in a vacuum dessicator at room temperature.

Synthesis Of Primary Amides

The carboxylic acid (1 eq.) was dissolved in dichloromethane (10 mL for 1 g) and triethylamine (1 eq.) added. The solution was cooled to 0 °C and after 10 min ethyl chloroformate (1.1 eq.) was added dropwise. The reaction was stirred for 30 min at room temperature and then ammonia was bubbled through the solution for two minutes. A white solid precipitated and stirring was continued for a further hour. The reaction was then poured into a separating funnel and washed with water (1x 10 mL), 2M hydrochloric acid (2 x 10 mL), 2M sodium hydroxide (2 x 10 mL) and finally water (1 x 10 mL). The organic layer was dried over magnesium sulphate, filtered, and the filtrate evaporated *in vacuo* to give the crude amide which was purified by recrystallisation from methanol or ethanol.

Reduction Of Primary Amides

A solution of the amide (1 eq.) in anhydrous THF (10 mL for 1 g) was added dropwise to a suspension of lithium aluminium hydride (10 eq.) in anhydrous THF (20 mL for 1 g). On completion of addition the reaction was refluxed for 2 h. Excess lithium aluminium hydride is decomposed by the careful addition of water (1 mL for 1 g of LiAlH_4), 2M sodium hydroxide (3 mL for 1 g of LiAlH_4), and finally water (1 mL for 1 g of LiAlH_4). The reaction mixture was then filtered and washed through with ethyl acetate. The filtrate was washed with water and the product was then extracted into dilute hydrochloric acid (2 x 20 mL). After washing the *aq.* layer with ethyl acetate, 2 M sodium hydroxide was added to liberate the amine and the product was extracted with ethyl acetate (3 x 25 mL). The organic layer was dried over magnesium sulphate, filtered, and the filtrate evaporated *in vacuo* to give the crude amine, which was purified by column chromatography. The colourless amines darkened on standing and were therefore acylated immediately.

Acylation Of Primary Amines

The amine was dissolved in dichloromethane (25 mL for 1 g of amine). After cooling to 0 °C, triethylamine (5 mL for 1 g of amine) was added followed by acetic or propionic anhydride (1.1 eq.). The reaction was allowed to reach room temperature and stirred further for 1 h. Ether was then added and the organic layer washed with water (1 x 20 mL), 2M hydrochloric acid (2 x 20 mL), saturated *aq.* sodium bicarbonate solution (2 x 20 mL), and finally saturated sodium chloride solution (1 x 20 mL). The organic layer was dried over magnesium sulphate, filtered, and the filtrate evaporated *in vacuo* to give a crude solid which was purified by column chromatography. Elution with ethyl acetate gave the required product.

Condensation Of Anilines And Cyclohexane-1,3-diol

The aniline and cyclohexane-1,3-diol (1 eq.) were heated together at 125 °C in the absence of solvent for 3 h. The contents of the flask were cooled and triturated with ether to give a solid of sufficient purity for use in the next step.

Synthesis Of Tetrahydrocarbazolones - Catalytic Palladium Acetate

The cyclohex-3-enone was added to a suspension of palladium acetate (0.055 equivalent), triphenylphosphine (0.11 equivalent) and sodium bicarbonate (2.2 equivalent) in DMF (11 mL per mmole of cyclohex-3-enone). The reaction mixture was stirred at 125 °C for 36 h, and was then filtered whilst hot. The filtrate was evaporated *in vacuo* and then partitioned between water and warm ethyl acetate. The organic layer was cooled to 0 °C and the precipitate collected.

Synthesis Of Tetrahydrocarbazolones - Stoichiometric Palladium Acetate

The cyclohex-3-enone was added to a suspension of palladium acetate (1.1 eq.), triphenylphosphine (0.11 eq.) and sodium bicarbonate (2.2 eq.) in DMF (11 mL for each mmole). The reaction mixture was stirred at 125 °C for 36 h, and was then filtered whilst hot. The filtrate was evaporated *in vacuo* and then partitioned between water and warm ethyl acetate. The organic layer was cooled to 0 °C and the precipitate collected.

PMC Protection Of Tetrahydrocarbazolones

Tetrahydro-carbazol-4-one was dissolved in acetone (4 mL for 1 mmole) and 4 N sodium hydroxide (1.25 mL for 1 mmole). PMC-Cl (1.5 eq.) in acetone (1.5 mL

for 1 mmole) was added dropwise and the reaction stirred for 3 h under nitrogen. The acetone was removed by evaporation *in vacuo* and the residual solution was partitioned between ethyl acetate and 5% citric acid. The organic layer was separated, washed with water, dried over magnesium sulphate and evaporated *in vacuo*. The crude material was purified by chromatography.

Attachment Of Tetrahydrocarbazoles To Tosyl Chloride Resin

The tetrahydrocarbazole (3 eq), was dissolved in DMF (5 mL for 1 mmol) at 0 °C. Sodium hydride (3 eq) was added portionwise over 15 minutes and the reaction stirred for a further 2 h under nitrogen. Tosyl chloride resin was then added in one portion, followed by a further aliquot of DMF (20 mL) and the reaction agitated by swirling for 18 h at room temperature. The resin was then removed by filtration and washed sequentially with DMF (5 x 20 mL), methanol (5 x 20 mL), DCM (5 x 20 mL) and finally diethylether (3 x 10 mL).

Cleavage Of Polymer Bound Tetrahydrocarbazoles

Resin bound tetrahydrocarbazole was suspended in THF (50 mL for 1g resin), and cooled to 0 °C. A solution of samarium iodide (1 eq. from an 0.1 M solution in THF) was added dropwise over 10 min and the reaction stirred at 0 °C for a further 30 min, followed by 15 min at room temperature. Saturated sodium hydrogen carbonate (30 mL) was added and the reaction diluted with ethyl acetate (100 mL). The resin was removed by filtration and the filtrate separated. The organic layer was washed with water, dried over anhydrous magnesium sulphate and evaporated *in vacuo*.

Preparation Of Tetrahydro- β -carbolines

The required tryptamine was dissolved in glacial acetic acid (3 ml for 1 mmole) and the aldehyde (1 eq.) was added. The reaction was stirred under nitrogen for 18 hours at ambient temperature. The solvent was then removed by lyophilisation and the residue dissolved in ethyl acetate (15 ml for 1 mmole). The organic solution was washed with saturated *aq.* sodium hydrogen carbonate (4x 10 ml), followed by water (10 ml) and dried over magnesium sulphate. The solution was then filtered and the filtrate evaporated *in vacuo*. The crude product was usually purified by trituration with diethylether and recrystallisation otherwise, it was subjected to column chromatography, eluting with ethyl acetate. The purified fractions were combined and evaporated *in vacuo*.

Acylation Of Tetrahydro- β -carbolines

The acid was dissolved in anhydrous dimethylformamide (5 ml for 1 mmole) and *N,N*-diisopropylethylamine (2 eq.) was added followed by HATU (1 eq.). The mixture was allowed to stand for 5 min with occasional agitation, and then a solution of the tetrahydro- β -carboline (1 eq.) in anhydrous dimethylformamide (5 ml for 1 mmole) was added. The solution was allowed to stand for 18 h and was then evaporated *in vacuo*. The residue was dissolved in ethyl acetate (20 ml for 1 mmole) and washed with 5% *aq.* citric acid (10 ml) followed by saturated *aq.* sodium hydrogen carbonate (3x 10 ml) and finally brine (10 ml). The organic layer was dried over magnesium sulphate, filtered and the filtrate evaporated *in vacuo*. The crude product was purified by column chromatography followed by recrystallisation.

Reductive Amination Of Polymer Bound Aldehyde

The amine (3 eq.) was added to a suspension of polymer bound aldehyde in 1% acetic acid in DMF (30 mL for 1 g of resin). The mixture was agitated for 1 hr and then sodium triacetoxyborohydride (4 eq.) added. The reaction mixture was shaken for 12 hr and then filtered and washed sequentially with DMF (8 x 30 mL), methanol (4 x 30 mL), DCM (5 x 30 mL) and finally ether (2 x 30 mL).

Acylation Of Polymer Bound Secondary Amines

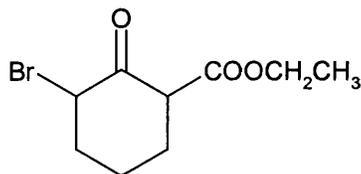
The resin bound amine was suspended in anhydrous DCM (3 mL for 100 mg resin) and diisopropylethylamine added (20 eq.), followed by acetic anhydride (10 eq.). The mixture was agitated for 18 hr and then filtered and washed sequentially with DMF (8 x 5 mL), methanol (4 x 5 mL), DCM (5 x 5 mL) and finally ether (2 x 5 mL).

Cleavage Of Amides From Aldehyde Resin

The resin bound amide was suspended in 50% TFA in DCM (3 mL for 100 mg resin) which had been cooled to 0 °C. The mixture was agitated for 30 minutes and then filtered. The resin was washed with DCM (3 mL) and the filtrates combined and evaporated *in vacuo*.

Experimental for Chapter 2

2-Bromo-6-ethoxycarbonylcyclohexanone (53)¹



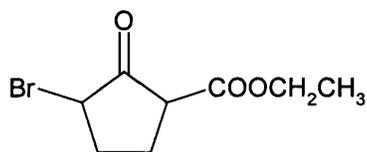
Ethyl-2-oxocyclohexanecarboxylate (50 g, 0.3 M) was dissolved in 400 mL of diethyl ether and cooled to 0 °C. Bromine (52.0 g, 0.33 M) was added dropwise over 4 h with a gentle stream of dry nitrogen being passed into the solution. At the end of addition the cooling bath was removed and the reaction allowed to stir for a further 2 h by which time t.l.c. indicated that reaction was complete.

The solution was then de-gassed for 30 min to remove excess hydrogen bromide and then washed with saturated *aq* sodium hydrogen carbonate (2 x 300 mL) followed by water (300 mL). The organic layer was dried over magnesium sulphate, filtered, and the filtrate evaporated *in vacuo* to give a pale yellow oil. This was distilled under vacuum to give a colourless oil, 70.9 g, 0.28 M, 95% yield, b.p. 112-114°C / 0.6 mm Hg.

IR ν_{\max} cm^{-1} 2945, 1740, 1655, 1613, 1402, 1391, 1294, 1257, 1221, 1190, 1099, 906. ^1H NMR (200 MHz; CDCl_3) δ_{H} 1.30-2.30 (t, $J = 7.0$ Hz, 3H, CH_3), 1.70-2.55 (m, 6H, CH_2), 4.22 (q, $J = 7.0$ Hz, 2H, CH_2), 4.68 (t, $J = 3.0$ Hz, 1H, CH), 12.06 (s, 1H, OH enol). ^{13}C NMR (90 MHz; D_6 -DMSO) δ_{C} 14.0 (CH_3), 17.7 (CH_2), 22.2 (CH_2), 32.1 (CH_2), 45.8 (CH), 60.8 (CH_2), 99.7 (CH), 166.4 (C=O), 172.1 (C=O). E.I.M.S. m/e 248/250 (M^+ 40%), 202/204 (30%), 169 (75%), 141 (40%), 123 (90%), 95 (80%), 67 (70%), 55 (100%), 39 (60%). Found: C, 41.98%, H, 4.90%. $\text{C}_9\text{H}_{13}\text{BrO}_3$ requires C, 43.39% H, 5.26%.

The following compounds were prepared in the same way:

2-Bromo-5-ethoxycarbonylcyclopentanone¹

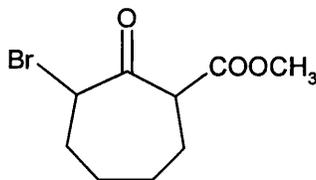


Ethyl-2-oxocyclopentanecarboxylate (25 g, 0.16 M) and bromine (28.25 g, 0.17 M) gave a brown oil. Attempts at distillation were curtailed due to extensive decomposition and the remaining material was dissolved in hot ethanol and treated with charcoal. The solution was then filtered and the filtrate evaporated *in vacuo* before being re-dissolved in ethyl acetate and passed through a short silica column. Elution with ethyl acetate and evaporation of the major fraction gave a colourless oil, 7.59 g, 0.03M, 19% yield.

IR ν_{\max} cm^{-1} 2991, 1759, 1729, 1666, 1626, 1417, 1369, 1355, 1255, 1227, 1180, 1153, 800. E.I.M.S. m/e 234/236 (M^+ 15%), 188/190 (20%), 155 (50%), 127 (20%), 109 (100%), 55 (85%).

Material used immediately without further characterisation.

2-Bromo-7-methoxycarbonylcycloheptanone¹

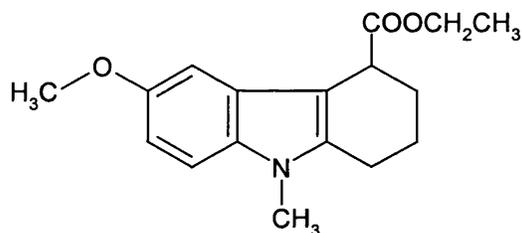


Methyl-2-oxocycloheptanecarboxylate (10 g, 0.058 M) and bromine (10.39 g, 0.065 M) gave a brown oil. Attempts at distillation were curtailed due to extensive decomposition and the remaining material was dissolved in hot ethanol and treated with charcoal. The solution was then filtered and the filtrate evaporated *in vacuo* before being re-dissolved in ethyl acetate and passed through a short silica

column. Elution with ethyl acetate and evaporation of the major fraction gave a pale yellow oil, 9.98 g, 0.04 M, 69% yield.

IR ν_{\max} cm^{-1} 2935, 2959, 1747, 1714, 1645, 1441, 1360, 1302, 1239, 1205, 1180, 1149, 1005. E.I.M.S. m/e 248/250 (M^+ 15%), 217/219 (15%), 169 (40%), 109 (60%), 55 (100%). Material used immediately without further characterisation

Ethyl 6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate¹



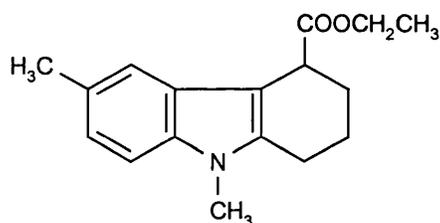
4-Methoxy-*N*-methylaniline (10.0 g, 0.072 mol) and 2-bromo-6-ethoxycarbonylcyclohexanone (9.55 g, 0.036 mole) were heated in the absence of solvent at 50 °C under N_2 for 3 hr. The resulting deep red solution was then diluted with 70 mL of dry propan-2-ol and anhydrous zinc chloride (14.09 g, 0.103 mol) added. After refluxing for 18 hrs the reaction mixture was evaporated *in vacuo* and the residue partitioned between 2M hydrochloric acid (150 mL) and ethyl acetate (100 mL). The aqueous layer was re-extracted with ethyl acetate (100 mL) and the organic layers combined and washed sequentially with water (100 mL), saturated *aq.* sodium bicarbonate (2 x 100 mL), and water (100 mL). The solution was then dried over magnesium sulphate, filtered, and the filtrate evaporated *in vacuo* to give a dark brown oil which yielded a white solid on cooling and trituration with ether, 7.15 g, brown oil which yielded a white solid on cooling and trituration with ether, 7.15 g, 0.025 M, 69% yield, m.p. 85-86 °C.

IR ν_{\max} cm^{-1} 2945, 1726, 1600, 1580, 1495, 1456, 1327, 1254, 1221, 1184, 1173, 1155, 1072, 1043, 1026, 939, 908. ^1H NMR (200 MHz; CDCl_3) δ_{H} 1.26 (t, J = 7.1 Hz, 3H, CH_3), 1.80-2.00 (m, 2H, CH_2), 2.10-2.30 (m, 2H, CH_2), 2.60-2.80 (m,

2H, CH₂), 3.56 (s, 3H, CH₃), 3.83 (s, 3H, CH₃), 3.85 (t, 1H, CH), 4.08-4.24 (m, 2H, m, CH₂), 6.79 (dd, J = 8.8 Hz, J = 2.5 Hz, 1H, ArH), 7.02 (d, J = 2.3 Hz, 1H, ArH), 7.12 (d, J = 8.8 Hz, 1H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_C 14.3 (CH₃), 20.4 (CH₂), 21.8 (CH₂), 26.4 (CH₂), 29.0 (CH₃), 38.5 (CH), 55.9 (CH₃), 60.4 (CH₂), 101.2 (ArCH), 104.9 (C4a), 109.1 (ArCH), 110.5 (ArCH), 128.1 (ArC), 132.0 (ArC), 137.3 (ArC), 153.8 (ArC), 175.0 (C=O). E.I.M.S. m/e 287 (M⁺ 60%), 214 (100%), 199 (30%), 183 (20%), 170 (30%), 141 (40%). Found: C, 70.77%, H, 7.37%, N, 4.87%. C₁₇H₂₁NO₃ requires C, 71.05% H, 7.37%, N, 4.72%.

The following compounds were prepared in the same way:

Ethyl 6-methyl-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate

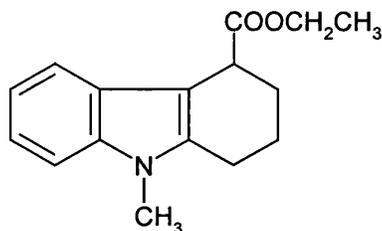


Ethyl 3-bromo-2-oxocyclohexanecarboxylate (9.07 g, 0.0345 mol) and *N*-methyl-toluidine (8.36 g, 0.069 mol) gave the title compound as a pale yellow solid which was triturated with ether to give a white solid, 8.53 g, 0.031 M, yield 92%, m.p. 107-108 °C.

IR ν_{\max} cm⁻¹ 2935, 1722, 1497, 1450, 1375, 1333, 1300, 1175, 1159, 1072, 1041, 1029, 814. ¹H NMR (360 MHz; CDCl₃) δ_H 1.30 (t, J = 7.1 Hz, 3H, CH₃), 1.90-2.05 (m, 2H, CH₂), 2.15-2.30 (m, 2H, CH₂), 2.40 (s, 3H, CH₃), 2.60-2.80 (m, 2H, CH₂), 3.56 (s, 3H, CH₃), 3.85 (t, 1H, CH), 4.15 (q, 2H, CH₂), 6.89 (dd, J = 8.8 Hz, J = 2.5 Hz 1H, ArH), 7.10 (d, J = 2.3 Hz, 1H, ArH), 7.30 (d, J = 8.8 Hz, 1H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_C 14.6 (CH₃), 21.0 (CH₂), 21.8 (CH₃), 22.2

(CH₂), 27.0 (CH₂), 29.3 (CH₃), 39.1 (CH), 60.8 (CH₂), 106.2 (C4a), 108.6 (ArCH), 118.9 (ArCH), 122.7 (ArCH), 127.4 (ArC), 128.5 (ArC), 135.8 (ArC), 137.1 (ArC), 175.4 (C=O). E.I.M.S. m/e 271 (M⁺ 40%), 198 (100%), 182 (30%), 168 (20%). Found: C, 75.00%, H, 7.86%, N, 5.21%. C₁₇H₂₁NO₂ requires C, 75.24% H, 7.80% N, 5.16%.

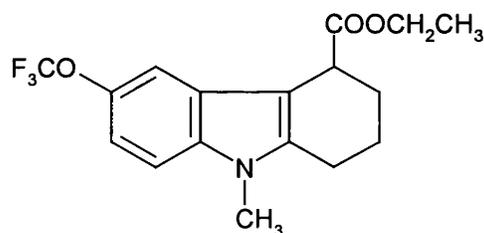
Ethyl 9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate



Ethyl 3-bromo-2-oxocyclohexanecarboxylate (9.55 g, 0.036 mol) and *N*-methyl aniline (7.85 g, 0.072 mol), gave the title compound as a yellow solid, 8.97 g, 0.035 mol, yield 97%, m.p. 81-82 °C.

IR ν_{\max} cm⁻¹ 2945, 1730, 1475, 1395, 1313, 1252, 1178, 1155, 1079, 1061, 744. ¹H NMR (360 MHz; CDCl₃) δ_{H} 1.28 (t, J = 7.1 Hz, 3H, CH₃), 1.90-2.00 (m, 2H, CH₂), 2.10-2.25 (m, 2H, CH₂), 2.55-2.80 (m, 2H, CH₂), 3.56 (s, 3H, CH₃), 3.85 (t, 1H, CH), 4.20 (q, J = 7.1 Hz, 2H, CH₂), 7.05 (m, 1H, ArH), 7.15 (m, 1H, ArH), 7.25 (m, 1H, ArH), 7.50 (d, 1H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_{C} 14.7 (CH₃), 21.0 (CH₂), 22.2 (CH₂), 27.0 (CH₂), 29.3 (CH₃), 39.1 (CH), 60.8 (CH₂), 106.8, (C4a) 108.9 (ArCH), 119.1 (ArCH), 119.4 (ArCH), 121.2 (ArCH), 127.3 (ArC), 137.1 (ArC), 137.5 (ArC), 175.3 (C=O). E.I.M.S. m/e 257 (M⁺ 35%), 184 (100%), 167 (60%), 157 (25%), 128 (25%) 110 (30%), 92 (30%). Found: C, 73.60%, H, 7.55%, N, 5.21%. C₁₆H₁₉NO₂ requires C, 74.68%, H, 7.44%, N, 5.44%.

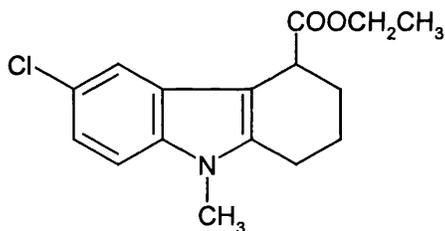
Ethyl 6-trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate



Ethyl 3-bromo-2-oxocyclohexanecarboxylate (6.89 g, 0.026 mol) and *N*-methyl-4-trifluoromethoxy aniline (10.0 g, 0.052 mol) gave the title compound as a pale yellow solid which was triturated with ether to give a white solid, 7.39 g, 0.021 mol, yield 83%. The compound was saponified immediately.

E.I.M.S. m/e 341 (M^+ 50%), 268 (100%), 252 (20%), 199 (10%), 191(25%), 182 (15%).

Ethyl 6-chloro-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate

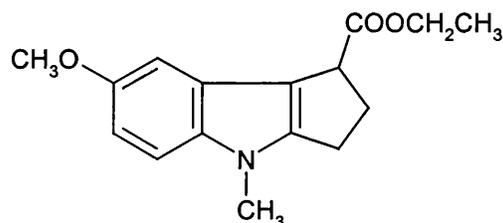


Ethyl 3-bromo-2-oxocyclohexanecarboxylate (9.55 g, 0.036 mol) and *N*-methyl-4-chloroaniline (10.20 g, 0.072 mol) gave the title compound as a pale yellow solid, 5.53 g, 0.019 mol, yield 53%, m.p. 115-116 °C.

IR ν_{\max} cm^{-1} 2928, 1720, 1477, 1369, 1333, 1294, 1254, 1188, 1161, 1070, 1041, 1026, 812. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.26 (t, $J = 7.2$ Hz, 3H, CH_3), 1.85-2.00 (m, 2H, CH_2), 2.15-2.30 (m, 2H, CH_2), 2.55-2.80 (m, 2H, CH_2), 3.54 (s, 3H, CH_3), 3.82 (t, 1H, CH), 4.20 (m, 2H, CH_2), 7.00-7.20 (m, 2H, ArH), 7.50 (d, $J = 0.8$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 14.6 (CH_3), 20.8 (CH_2), 22.2 (CH_2), 26.7 (CH_2), 29.5 (CH_3), 38.8 (CH), 61.0 (CH_2), 106.5 (C4a), 109.9

(ArCH), 118.7 (ArCH), 121.3 (ArCH), 125.2 (ArC), 128.2 (ArC), 135.8 (ArC), 138.6 (ArC), 174.9 C=O). E.I.M.S. m/e 291 (M^+ 65%), 218 (100%), 183 (70%), 167 (45%), 91 (20%). Found: C, 65.87%, H, 6.30%, N, 4.80%. $C_{16}H_{18}NClO_2$ requires C, 65.86%, H, 6.22%, N, 4.80%.

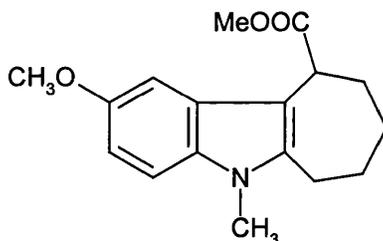
Ethyl 5-methoxy-8-methyl-1,2,3-trihydrocyclopent[b]indole-3-carboxylate¹



4-Methoxy-*N*-methylaniline (6.94 g, 0.05 mol) and 2-bromo-5-ethoxycarbonyl-cyclopentanone (5.87 g, 0.025 mol) gave the title compound as a brown oil which failed to crystallise on trituration or after passing through a short silica column, 3.63 g, 0.013 mol, yield 53%. The crude product was saponified directly without further characterisation.

FAB Mass.Spec 274 (M^+ 100%), 246 (50%), 199 (50%), 157 (30%), 111 (30%).

Methyl 7-methoxy-10-methyl-1,2,3,4,5-pentahydrocyclohept[b]indole-5-carboxylate¹

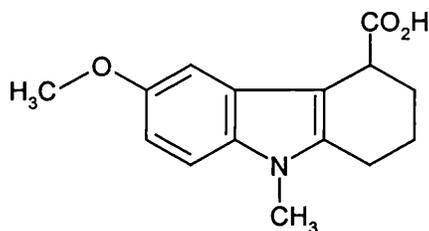


4-Methoxy-*N*-methylaniline (6.94 g, 0.05 mol) and 2-bromo-7-methoxycarbonyl-cycloheptanone (5.87 g, 0.025 mol) gave the title compound as a cream coloured

solid on trituration with ether, 4.27 g, 0.014 mol, 29% yield. The crude product was saponified directly without further purification.

^1H NMR (360 MHz; CDCl_3) δ_{H} 1.52-1.65 (m, 1H, CH_2), 1.80-2.08 (m, 4H, CH_2), 2.40-2.47 (m, 1H, CH_2), 2.90-2.95 (m, 2H, CH_2), 3.62 (s, 3H, CH_3), 3.65 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 4.15-4.18 (m, 3H, $\text{CH}+\text{CH}_2$), 6.79 (dd, $J = 8.7$ Hz, $J = 2.5$ Hz, 1H, ArH), 6.90 (d, $J = 2.3$ Hz, 1H, ArH), 7.10 (d, $J = 8.7$ Hz, 1H, Ar-H).
 ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 26.1 (CH_2), 27.3 (CH_2), 27.4 ($\text{CH}_2 \times 2$), 30.0 (CH_3), 31.2 (CH_2), 41.4 (CH_3), 52.0 (CH), 56.5 (CH_3), 100.4 (ArCH), 109.9 (C5a), 110.0 (ArCH), 110.9 (ArCH), 128.4 (ArC), 131.8 (ArC), 141.3 (ArC), 154.5 (ArC), 175.2 (C=O). E.I.M.S. m/e 302 (M^+ 80%), 228 (100%), 213 (25%), 200 (35%), 187 (35%), 172 (25%), 156 (25%).

6-Methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid¹



Ethyl 6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate (6.5 g, 0.022 mol) was saponified with 200 mL of hot 90% *aq.* ethanol and 9.10 g of sodium hydroxide pellets by heating on a steam bath for 5 hr. The solution was evaporated *in vacuo* and the residue dissolved in water and washed with dichloromethane (2 x 100 mL). The volume of the *aq.* layer was reduced by evaporation *in vacuo* and the residue poured into cold excess 10% hydrochloric acid to yield a white solid which was dried in a vacuum dessicator, 4.05 g, 0.0157 mol, 71% yield, m.p. 176-177 °C.

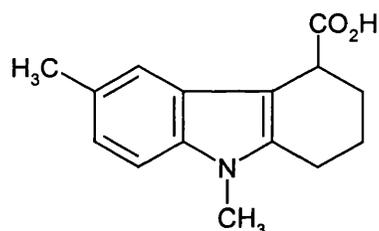
IR ν_{max} cm^{-1} . 2850, 1701, 1581, 1497, 1458, 1419, 1302, 1252, 1221, 1149, 799.

^1H NMR (200 MHz; CDCl_3) δ_{H} 1.80-2.30 (m, 4H, CH_2), 2.50-2.80 (m, 2H, CH_2),

3.54 (s, 3H, CH₃), 3.77 (s, 3H, CH₃), 3.89 (t, 1H, CH), 6.79 (dd, J = 8.8 Hz, J = 2.5 Hz, 1H, ArH), 6.99 (d, J = 2.4 Hz, 1H, ArH), 7.10 (d, J = 8.7 Hz, 1H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_C 20.3 (CH₂), 21.8 (CH₂), 26.4 (CH₂), 29.1 (CH₃), 38.2 (CH), 55.9 (CH₃) 101.0 (ArCH), 105.0 (C4a), 109.3 (ArCH), 110.7 (ArCH), 126.6 (ArC), 132.2 (ArC), 137.5 (ArC), 154.9 (ArC), 161.0 (C=O). E.I.M.S. m/e 259 (M⁺ 20%), 214 (100%), 197 (30%), 182 (20%), 170 (25%), 97 (20%), 81 (30%), 69 (60%), 57 (70%), 43 (80%). Found: C, 68.94%, H, 6.56%, N, 5.22%. C₁₅H₁₇NO₃ requires C, 69.48%, H, 6.61%, N, 5.40%.

The following compounds were prepared in a similar manner:

6-Methyl-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid

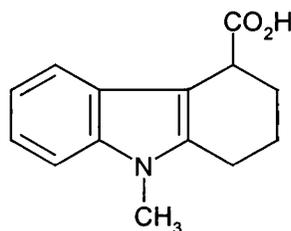


Ethyl 6-methyl-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate (8.45 g, 0.031 mol) dissolved in 200 mL of 90% *aq.* ethanol and 13.20 g (0.33 mol) of sodium hydroxide pellets gave the title compound as a white solid, 7.47 g, 0.03 mol, 99% yield, m.p. 201-202 °C.

IR ν_{\max} cm⁻¹. 3050-2500, 1701, 1485, 1416, 1375, 1302, 1227, 789. ¹H NMR (360 MHz; CDCl₃) δ_H 1.85-2.00 (m, 2H, CH₂), 2.05-2.30 (m, 2H, CH₂), 2.40 (s, 3H, CH₃), 2.55-2.80 (m, 2H, CH₂), 3.56 (s, 3H, CH₃), 3.89 (m, 1H, CH), 6.79 (dd, J = 7.9 Hz, J = 0.9 Hz, 1H, ArH), 7.10 (d, J = 8.0 Hz, 1H, ArH), 7.30 (d, J = 0.9 Hz, 1H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_C 20.8 (CH₂), 21.8 (CH₃), 22.2 (CH₂), 26.9 (CH₂), 29.4 (CH₃), 38.60 (CH), 105.4 (C4a), 108.7 (ArCH), 118.6 (ArCH), 122.9 (ArCH), 127.2 (ArC), 128.9 (ArC), 135.8 (ArC), 137.3 (ArC),

180.7 (C=O). E.I.M.S. m/e 243 (M^+ 75%), 198 (100%), 181 (55%), 168 (35%), 99 (30%), 91 (40%), 69 (20%). Found: C, 70.63%, H, 6.73%, N, 5.48%. $C_{15}H_{17}NO_2$ requires C, 74.07% H, 5.76% N, 5.76%.

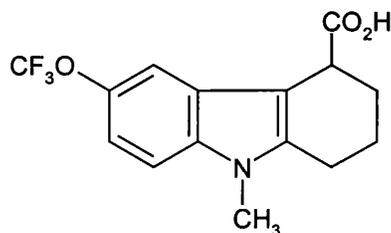
9-Methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid



Ethyl 9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate (8.50 g, 0.033 mol) dissolved in 250 mL of 90% aq. ethanol and 25 g of sodium hydroxide pellets gave the title compound as a white solid, 5.01 g, 0.22 mol, 66% yield, m.p. 207-208 °C.

IR ν_{\max} cm^{-1} . 3050-2500, 1699, 1475, 1412, 1315, 1250, 1225, 744. 1H NMR (360 MHz; d_6 -DMSO) δ_H 1.80-2.10 (m, 4H, CH_2), 2.60-2.80 (m, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.75 (t, 1H, CH), 6.95 (dd, $J = 8.0$ Hz 1H, ArH), 7.05 (dd, $J = 8.0$ Hz, 1H, ArH), 7.35 (d, $J = 8.0$ Hz, 1H, ArH), 7.40 (d, $J = 8.0$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_C 21.1 (CH_2), 22.0 (CH_2), 27.2 (CH_2), 29.6 (CH_3), 39.1 (CH), 107.0 (C4a), 109.7 (ArCH), 119.0 (ArCH), 119.3 (ArCH), 121.1 (ArCH), 127.4 (ArC), 137.5 (ArC), 137.6 (ArC), 176.6 (C=O). F.A.B Mass Spec. 230 (M^+ 90%), 184 (100%), 167 (20%), 91 (20%). Found: C, 72.99%, H, 6.73% N, 5.95% $C_{14}H_{15}NO_2$ requires C, 73.34% H, 6.59% N, 6.11%.

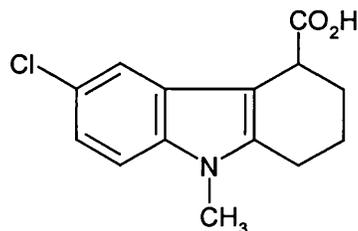
6-Trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid



Ethyl 6-trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate (7.0 g, 0.02 mol) in 200 mL of 90% *aq.* ethanol and 9.20 g (0.23 mol) of sodium hydroxide pellets gave the title compound as a white solid, 6.08 g, 0.195 mol, 97% yield, m.p. 191-192 °C.

IR ν_{\max} cm^{-1} 3100-2750 (br.), 1705, 1485, 1418, 1265, 1217, 1155, 1142, 956, 802. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.80-2.15 (m, 4H, CH_2), 2.60-2.80 (m, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.75 (t, 1H, CH), 7.02 (dd, $J = 8.8$ Hz, $J = 1.6$ Hz, 1H, ArH), 7.35 (d, $J = 0.9$ Hz, 1H, ArH), 7.44 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 20.9 (CH_2), 22.1 (CH_2), 26.9 (CH_2), 29.9 (CH_3), 38.8 (CH), 107.6 (C4a), 110.7 (ArCH), 111.3 (ArCH), 114.3 (ArCH), 121.4 (q, $J = 259$ Hz, CF_3), 127.4 (ArC), 135.9 (ArC), 140.2 (ArC), 142.5 (ArC), 176.6 (C=O). E.I.M.S. m/e 313 (M^+ 25%), 268 (100%), 252 (10%) 182 (10%). Found: C, 57.24%, H, 4.46% N, 4.33% $\text{C}_{15}\text{H}_{14}\text{NF}_3\text{O}_3$ requires C, 57.51%. H, 4.47%, N, 4.47%.

6-Chloro-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid



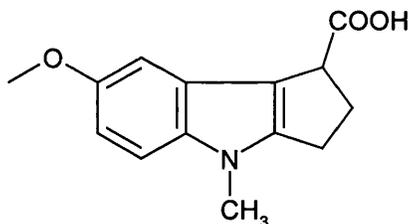
Ethyl 6-chloro-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylate (5.00 g, 0.017 M) dissolved in 175 mL of 90% *aq.* ethanol and 6.80 g (0.17 mol) of sodium hydroxide pellets, gave the title compound as a white solid, 4.42 g, 0.016 mol, 99% yield, m.p. 186-187 °C.

IR ν_{\max} cm^{-1} . 3000-2500, 1699, 1472, 1416, 1369, 1288, 1256, 1219, 941, 797.

^1H

NMR (360 MHz; d_6 -DMSO) δ_{H} 1.70-2.15 (m, 4H, CH_2), 2.65-2.75 (m, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.55-3.65 (m, 1H, CH), 7.02 (dd, $J = 8.8$ Hz, $J = 2.3$ Hz, 1H, ArH), 7.35-7.40 (m, 2H, ArH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 21.3 (CH_2), 22.2 (CH_2), 28.1 (CH_2), 29.9 (CH_3), 39.8 (CH), 108.0 (C4a), 111.3 (ArCH), 118.0 (ArCH), 120.7 (ArCH) 123.9 (ArC), 128.3 (ArC), 136.0 (ArC), 139.8 (ArC), 176.9 (C=O). E.I.M.S. m/e 263 (35%), 218 (100%), 181 (30%), 167 (20%). Found: C, 63.53%, H, 5.40%, N, 5.23%. $\text{C}_{14}\text{H}_{14}\text{NClO}_2$ requires C, 63.81% H, 5.86% N, 5.23%.

5-Methoxy-8-methyl-1,2,3-trihydrocyclopent[b]indole-3-carboxylic acid

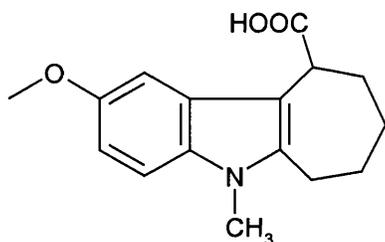


Ethyl 5-methoxy-8-methyl-1,2,3-trihydrocyclopent[b]indole-3-carboxylate (3.50 g, 0.012 mol) dissolved in 175 mL of 90% *aq.* ethanol and 4.80 g (0.12 mol) of sodium hydroxide pellets gave an off-white solid (1.06 g). This was subjected to column chromatography and eluted with dichloromethane to give a white solid, 0.43 g, 0.0018 mole, 15% yield, which was used without further purification.

^1H NMR (360 MHz; CDCl_3) δ_{H} 2.70-3.10 (m, 4H, CH_2), 3.65 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 4.10 (m, 1H, CH), 6.80 (dd, $J = 8.8$ Hz, $J = 1.6$ Hz, 1H, ArH), 7.05

(d, $J = 1.6$ Hz, 1H, ArH), 7.14 (d, $J = 8.8$ Hz, 1H, ArH). E.I.M.S. m/e 245 (M^+ 25%), 200 (100%), 184 (15%), 156 (40%), 40 (25%).

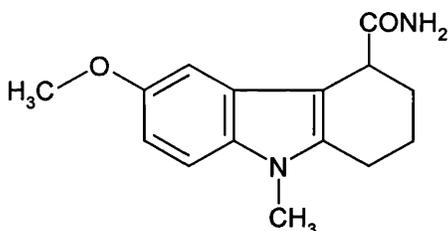
7-Methoxy-N-methyl-1,2,3,4,5-pentahydrocyclohept[b]indole-5-carboxylic acid¹



Methyl 7-methoxy-10-methyl-1,2,3,4,5-pentahydrocyclohept[b]indole-5-carboxylate (4.0 g, 0.015 mol) dissolved in 150 mL of 90% *aq.* ethanol and 6.0 g (0.15 mol) of sodium hydroxide pellets gave an off-white solid, 2.94 g, 0.01 mol, 72% yield, m.p. 191-193 °C.

E.I.M.S. m/e 273 (M^+ 85%), 228 (100%), 200 (35%), 187 (25%), 168 (25%), 156 (20%), 128(10%), 115 (10%), 43 (20%).

6-Methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide (115)



6-Methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid (3.75 g, 14.7 mmol) was dissolved in 25 mL of dichloromethane and triethylamine (1.48 g, 14.7 mmol) was added at 0 °C. After stirring at this temperature for ten minutes, ethyl chloroformate (1.56 g, 14 mmol) was added dropwise and the reaction stirred at 0 °C for 90 mins followed by 30 mins at room temperature. Gaseous

ammonia was then bubbled through the solution for two minutes and stirring continued for one hour. The mixture was poured into a separating funnel and washed sequentially with water (1 x 10 mL), 2M hydrochloric acid (2 x 10 mL), 2M sodium hydroxide (2 x 10 mL) and finally water (1 x 10 mL). The organic layer was dried over magnesium sulphate, filtered and the filtrate evaporated *in vacuo* to give an off-white product which was recrystallised from methanol to give a white solid, 3.24 g, 12.6 mmol, 86% yield, m.p. 165-166 °C.

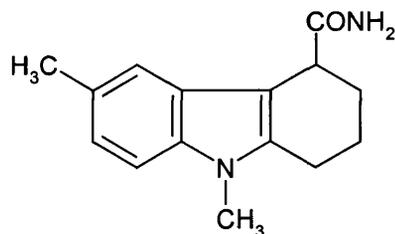
IR ν_{\max} cm^{-1} 3464, 3203, 2939, 1680, 1653, 1593, 1578, 1487, 1454, 1416, 1369, 1221, 1173, 1147, 796. ^1H NMR (200 MHz; CDCl_3) δ_{H} 1.85-2.10 (m, 3H, CH_2), 2.25-2.40 (m, 1H, CH_2), 2.60-2.80 (m, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.70 (t, 1H, CH), 3.82 (s, 3H, CH_3), 5.65 (br s, 1H NH_2), 5.77 (br.s, 1H NH_2), 6.82 (dd, $J = 8.8$ Hz, $J = 2.4$ Hz, 1H, ArH), 6.92 (d, $J = 2.3$ Hz, 1H, ArH), 7.17 (d, $J = 8.7$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 20.4 (CH_2), 22.1 (CH_2), 27.4 (CH_2), 29.1 (CH_3), 40.0 (CH), 55.9 (CH_3), 100.1 (ArCH), 106.2 (C4a), 109.5 (ArCH), 110.9 (ArCH), 126.6 (ArC), 132.2 (ArC), 138.0 (ArC), 154.1 (ArC), 177.6 (C=O). E.I.M.S. m/e 258 (M^+ 80%), 240 (50%), 214 (100%), 199 (70%), 183 (50%), 170 (60%), 156 (40%), 128 (30%), 115 (30%), 107 (40%), 77 (20%), 44 (45%). Found: C, 69.25%, H, 7.16%, N, 10.62%. $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_2$ requires C, 69.74% H, 7.02% N, 10.84%.

Chromatographic separation of racemic amide 115

The racemic amide (**115**, 100 mg) was dissolved in ethanol (10 mg/mL) and injected in 1 mL aliquots onto a 25 cm x 2 cm Chiralcel AD preparative HPLC column. The eluting solvent was 85% Hexane/15% Ethanol and the eluent monitored at $\lambda = 280$ nm. These conditions afforded baseline separation and allowed each enantiomer to be collected directly. Evaporation of the eluted material *in vacuo*, yielded two white solids **115 (+)** (41 mg) and **115 (-)** (43 mg). The enantiomeric purity of each was confirmed by analytical HPLC and circular dichroism spectra obtained.

The following compounds were prepared in a similar way:

6-Methyl-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide

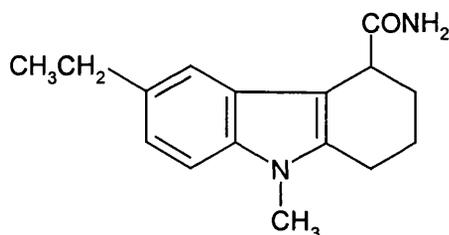


6-Methyl-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid (7.00 g, 0.0288 mol) gave an off-white solid which was recrystallised from methanol to give a white solid, 5.87 g, 0.0243 mol, 84% yield, m.p. 187-188 °C.

IR ν_{\max} cm^{-1} 3402, 3192, 2941, 2914, 1655, 1485, 1406, 1373, 1288, 1248, 781.

^1H NMR (360 MHz; d_6 -DMSO) δ_{H} 1.70-2.15 (m, 4H, CH_2), 2.39 (s, 3H, CH_3) 2.65-2.75 (m, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.55-3.65 (m, 1H, CH), 6.80 (br.s, 1H NH_2), 6.88 (dd, $J = 8.8$ Hz, $J = 2.4$ Hz, 1H, ArH), 7.10 (br.s, 1H, NH_2), 7.20-7.30 (m, 2H, ArH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 21.4 (CH_2), 22.1 (CH_3), 22.2 (CH_2), 28.4 (CH_2), 29.6 (CH_3), 40.3 (CH), 107.5 (C4a), 109.4 (ArCH), 118.5 (ArCH), 122.5 (ArCH), 127.4 (ArC), 127.5 (ArC), 136.0 (ArC), 137.9 (ArC), 177.3, (C=O). E.I.M.S. m/e 242 (M^+ 15%), 198 (75%), 170 (25%), 157 (30%), 141 (30%), 112 (30%), 101 (100%), 73 (60%), 55 (60%), 43 (70%). Found: C, 73.74%, H, 7.28%, N, 11.50%. $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}$ requires C, 74.22% H, 7.49% N, 11.57%.

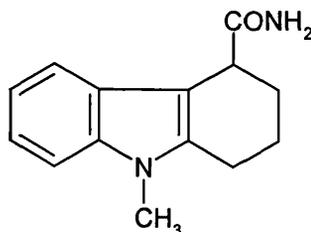
6-Ethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide



6-Ethyl-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid (1.70 g, 6.6 mmol) gave an off-white solid which was recrystallised from methanol to give a white solid, 1.14 g, 4.45 mmol, 67% yield, m.p. 180-181 °C.

IR ν_{\max} cm^{-1} 3450, 3129, 2964, 1674, 1481, 1456, 1375, 806. ^1H NMR (360 MHz; d_6 -DMSO) δ_{H} 1.25 (t, $J = 7.8$ Hz, 3H, CH_3), 1.85-2.10 (m, 3H, CH_2), 2.30-2.40 (m, 1H, CH_2), 2.60-2.80 (m, 4H, CH_2), 3.60 (s, 3H, CH_3), 3.65-3.75 (m, 1H, CH), 5.75 (br.s, 1H NH_2), 5.85 (br.s, 1H NH_2), 7.02 (dd, $J = 8.8$ Hz, $J = 2.4$ Hz, 1H, ArH), 7.15 (d, $J = 8.8$ Hz, 1H, ArH), 7.30 (d, $J = 2.4$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 17.0 (CH_3), 21.1 (CH_2), 22.6 (CH_2), 28.0 (CH_2), 29.5 (CH_2), 29.6 (CH_3), 40.6 (CH), 107.0 (C4a), 109.2 (ArCH), 117.1 (ArCH), 122.2 (ArCH), 127.2 (ArC), 136.2 (ArC), 136.3 (ArC), 138.0 (ArC), 178.3 (C=O). E.I.M.S. m/e 256 (M^+ 30%), 212 (100%), 196 (15%), 182 (30%), 167 (15%), 99 (20%). Found: C, 74.77%, H, 7.80%, N, 10.89%. $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}$ requires C, 74.93% H, 7.86% N, 10.97%.

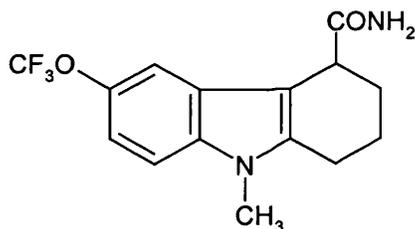
9-Methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide



9-Methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid (4.50 g, 0.0197 mol) gave an off-white solid which was recrystallised from methanol to give a white solid, 4.35 g, 0.0191 mol, 97% yield, m.p. 212-213 °C.

IR ν_{\max} cm^{-1} 3346, 2941, 1692, 1370, 1276, 1244, 741. ^1H NMR (400MHz; CDCl_3) δ_{H} 1.85-2.05 (m, 3H, CH_2), 2.35-2.40 (m, 1H, CH_2), 2.65-2.75 (m, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.55-3.65 (m, 1H, CH), 5.60 (br.s, 1H NH_2), 5.70 (br.s, 1H, NH_2), 7.02 (1H, m, ArH), 7.10 (m, 1H, ArH), 7.25 (d, 1H, ArH), 7.40 (d, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 20.8 (CH_2), 22.4 (CH_2), 27.7 (CH_2), 29.6 (CH_3), 39.8 (CH), 106.9 (C4a), 109.4 (ArCH), 118.5 (ArCH), 120.0 (ArCH), 121.8 (ArCH), 127.0 (ArC), 137.6 (ArC), 138.4 (ArC), 177.6 (C=O). E.I.M.S. m/e 228 (M^+ 35%), 184 (100%), 156 (30%), 49 (61%). Found: C, 73.26%, H, 6.99%, N, 12.31%. $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}$ requires C, 73.66% H, 7.07% N, 12.27%.

6-Trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide

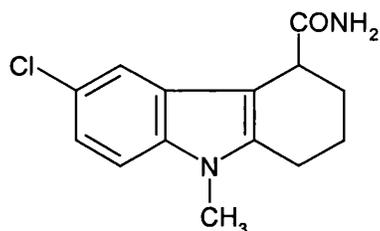


6-Trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid (6.00 g, 0.019 mol) gave an off-white solid which was recrystallised from methanol to give a white solid 4.21 g, 0.0135 mol, 71% yield, m.p. 188-189 °C.

IR ν_{\max} cm^{-1} 3321, 3298, 3163, 1655, 1495, 1410, 1290, 1271, 1254, 1213, 1157, 1139. ^1H NMR (360 MHz; d_6 -DMSO) δ_{H} 1.70-2.15 (m, 4H, CH_2), 2.70 (t, 2H, CH_2), 3.60 (s, 3H, CH_3), 3.55-3.65 (m, 1H, CH), 6.84 (br.s, 1H, NH_2), 6.99 (dd, $J = 8.8$ Hz, $J = 1.2$ Hz, 1H, ArH), 7.35 (br.s, 1H, NH_2), 7.29 (d, $J = 1.2$ Hz, 1H, ArH), 7.42 (d, $J = 8.7$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 20.3 (CH_2), 21.2 (CH_2), 27.1 (CH_2), 28.9 (CH_3), 38.8 (CH), 107.6 (C4a), 109.7

(ArCH), 109.9 (ArCH), 113.1 (ArCH), 121.4 (q, $J = 259$ Hz, CF_3), 126.2 (ArC), 134.9 (ArC), 139.4 (ArC), 141.4 (ArC), 175.8 (C=O). E.I.M.S. m/e 312 (M^+ 15%), 268 (100%), 252 (10%), 182 (10%), 168 (10%). Found: C, 57.30%, H, 4.77%, N, 8.84%. $\text{C}_{15}\text{H}_{15}\text{N}_2\text{F}_3\text{O}_2$ requires C, 57.69% H, 4.84% N, 8.97%.

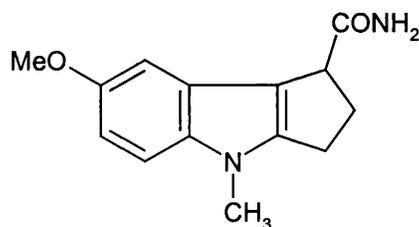
6-Chloro-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide



6-Chloro-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxylic acid (4.00 g, 0.015 mol) gave an off-white solid which was recrystallised from methanol to give a white solid, 3.78g, 0.014 mol, 96% yield, m.p.177-178 °C.

IR ν_{\max} cm^{-1} 3385, 3198, 2945, 1655, 1473, 1412, 1265, 1242, 1063, 959, 791. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.75-2.10 (m, 4H, CH_2), 2.60-2.75 (m, 2H, CH_2), 3.57 (s, 3H, CH_3), 3.71 (m, 1H, CH), 7.03 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.34 (d, 1H, ArH), 7.40 (d, $J = 8.8$ Hz, 1H, ArH). 12.15 (br.s. 2H, NH_2). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 20.9 (CH_2), 22.1 (CH_2), 26.9 (CH_2), 29.9 (CH_3), 38.8 (CH), 106.9 (C4a), 111.3 (ArCH), 118.3 (ArCH), 120.8 (ArCH), 124.1 (ArC), 128.4 (ArC), 136.0 (ArC), 139.4 (ArC), 176.3 (C=O). E.I.M.S. m/e 262 (M^+ 40%), 218 (100%), 183 (70%), 167 (40%), 107 (40%), 91 (70%). Found: C, 63.74%, H, 5.71%, N, 10.45%. $\text{C}_{14}\text{H}_{15}\text{N}_2\text{ClO}$ requires C, 64.12% H, 5.77% N, 10.72%.

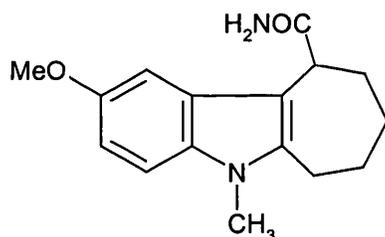
5-Methoxy-8-methyl-1,2,3-trihydrocyclopent[b]indole-3-carboxamide¹



5-Methoxy-8-methyl-1,2,3-trihydrocyclopent[b]indole-3-carboxylic acid (0.4 g, 1.6 mmol) gave an off-white solid which was recrystallised from methanol/ether to give a white solid, 220mg, 0.89 mmol, 56% yield, m.p. 207-208 °C. This solid was used immediately.

E.I.M.S. m/e 244 (M⁺ 30%), 200 (100%).

7-Methoxy-10-methyl-1,2,3,4,5-pentahydrocyclohept[b]indole-3-carboxamide¹



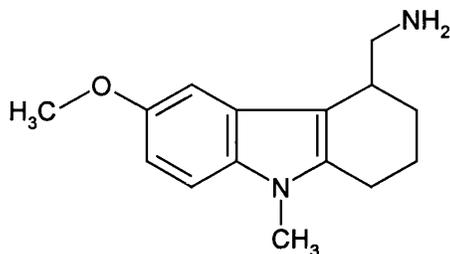
7-Methoxy-N-methyl-1,2,3,4,5-pentahydrocyclohept[b]indole-5-carboxylic acid (2.8 g, 10 mmol) gave an off-white solid which was recrystallised from methanol to give a white solid, 2.10g, 7.72 mmol, 77% yield, m.p.189-191 °C.

IR ν_{\max} cm⁻¹ 3419, 2929, 1676, 1616, 1499, 1456, 1367, 1227, 1155, 1050, 823.

¹H NMR (360 MHz; CDCl₃) δ_{H} 1.54-2.10 (m, 5H, CH₂), 2.60-2.80 (m, 2H, CH₂), 2.9-3.0 (m, 1H, CH₂) 3.62 (s, 3H, CH₃), 3.81 (s, 3H, CH₃), 3.97 (m, 1H, CH), 5.68 (br.s., 1H, NH₂), 5.87 (br.s., 1H, NH₂), 6.81 (dd, J = 8.7 Hz, J = 2.5 Hz, 1H, ArH), 6.90 (d, J = 2.5 Hz, 1H, ArH), 7.12 (d, J = 8.80 Hz, 1H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_{C} 26.5 (CH₂), 27.0 (CH₂), 27.2 (CH₂), 30.1 (CH₃), 30.6

(CH₂), 42.3 (CH), 56.4 (CH₃), 100.0 (C5a), 110.2 (ArCH), 110.8 (ArCH), 111.6 (ArCH), 128.4 (ArC), 132.0 (ArC), 140.7 (ArC), 154.8 (ArC), 177.0 (C=O). E.I.M.S. m/e 272 (M⁺ 40%), 254 (15%), 228 (100%), 213 (15%), 200 (10%), 187 (10%), 174 (10%), 44 (15%). Found: C, 69.92%, H, 7.58%, N, 10.08%. C₁₆H₂₀N₂O₂ requires C, 70.56% H, 7.40% N, 10.08%.

4-Aminomethyl-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole¹



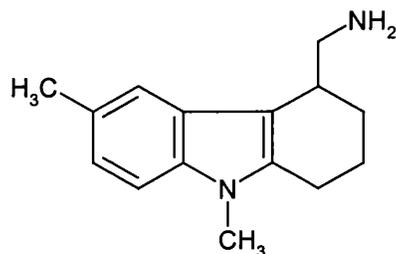
6-Methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide (3.0 g, 11.4 mmol) was added portionwise to a stirred suspension of lithium aluminium hydride (5.20 g, 13 mmol) in 40 mL of dry tetrahydrofuran at 0 °C. The reaction was stirred at reflux for 3 h and then cooled in ice. To the reaction was added water (6 mL), followed by 2N sodium hydroxide (18 mL), and finally water (6 mL). The mixture was filtered and the filtrate diluted with ethyl acetate (50 mL), separated, and the aqueous layer re-extracted with ethyl acetate (50 mL). The organic layers were combined and the product extracted into 0.5M HCl (2 x 25 mL). The aqueous layers were combined and washed with ethyl acetate (25 mL) and then basified with 2N NaOH. The crude amine was extracted into ethyl acetate (4 x 50 mL), dried over magnesium sulphate, filtered and the filtrate evaporated *in vacuo* to give an orange oil. This was subjected to column chromatography using ethyl acetate as eluent. The ninhydrin positive fractions were combined to give a beige solid, 0.88 g, 3.61 mmol, 31% yield.

IR ν_{\max} cm⁻¹ 3500-2900, 1620, 1578, 1495, 1460, 1416, 1302, 1225, 1153, 1032, 799. E.I.M.S. m/e 244 (M⁺ 50%), 228 (50%), 214 (100%), 199 (70%), 184

(40%), 170 (60%), 156 (30%), 128 (20%), 115 (20%). This product was acylated without further characterisation.

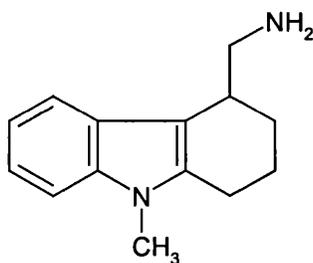
The following compounds were similarly prepared:

4-Aminomethyl-6-methyl-9-methyl-1,2,3,4-tetrahydrocarbazole



6-Methyl-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide (5.75 g, 23.7 mmol) gave the title compound as a pale yellow solid on evaporation, 1.56 g, 29% yield. E.I.M.S. m/e 228 (M^+ 25%), 212 (80%), 198 (100%). This product was acylated immediately.

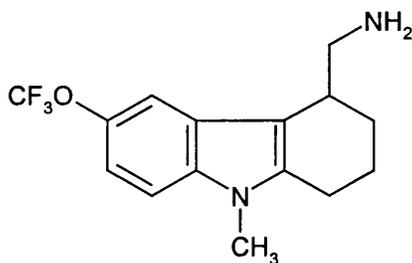
4-Aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole



9-Methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide (4.0 g, 0.0176 M) gave the title compound as a pale yellow viscous oil, 2.17 g, 58% yield.

E.I.M.S. m/e 214 (M^+ 30%), 198 (100%), 184 (100%). This product was acylated immediately.

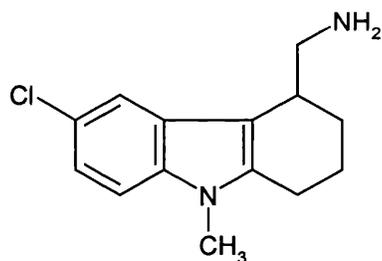
4-Aminomethyl-6-trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole



6-Trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide (4.0 g, 12.8 mmol) gave the title compound as a pale yellow solid on evaporation, 1.15g, 30% yield.

E.I.M.S. m/e 298 (M^+ 35%), 282 (90%), 168 (100%). This product was acylated without further characterisation.

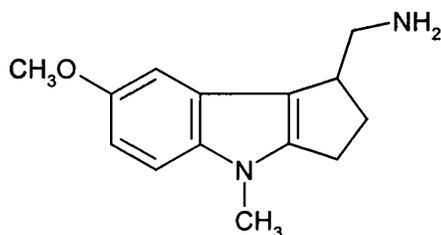
4-Aminomethyl-6-chloro-9-methyl-1,2,3,4-tetrahydrocarbazole



6-Chloro-9-methyl-1,2,3,4-tetrahydrocarbazole-4-carboxamide (3.40 g, 13 mmol) gave the title compound as a pale yellow viscous oil, 1.55 g, 48% yield.

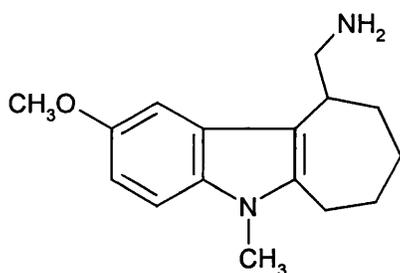
E.I.M.S. m/e 248 (M^+ 25%), 232 (80%), 218 (100%), This product was acylated without further characterisation.

3-Aminomethyl-5-methoxy-8-methyl-1,2,3-trihydrocyclopent[b]indole



5-Methoxy-8-methyl-1,2,3-trihydrocyclopent[b]indole-3-carboxamide (0.20 g, 0.8 mmol) gave the title compound as a pale yellow viscous oil, 65mg, 35% yield. This was acylated immediately.

5-Aminomethyl-7-methoxy-10-methyl-1,2,3,4,5-pentahydrocyclohept[b]indole

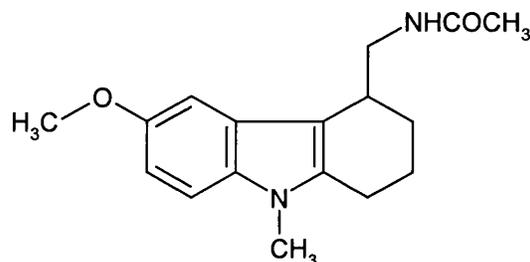


7-Methoxy-10-methyl-1,2,3,4,5-pentahydrocyclohept[b]indole-3-carboxamide (2.0 g, 7.6 mmol) gave the title compound as a pale yellow solid, 1.27 g, 65% yield.

IR ν_{\max} cm^{-1} 2995, 2924, 2854, 1618, 1579, 1485, 1458, 1288, 1228, 1217, 1157, 1034, 793 752. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.50-2.15 (m, 8H, CH_2+NH_2), 2.68-2.98 (m, 4H, CH_2), 3.14-3.23 (m, 1H, CH), 3.60 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 6.79 (dd, $J = 8.8$ Hz, $J = 2.5$ Hz, 1H, ArH), 6.98 (d, $J = 2.4$ Hz, 1H, ArH), 7.07-7.12 (d, $J = 8.7$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 26.1 (CH_2), 26.2 (CH_2), 27.9 (CH_2), 30.4 (CH_2), 38.5 (CH_3), 45.6 (CH_2), 50.6 (CH), 56.5 (CH_3), 100.7 (ArCH), 109.9 (ArCH), 110.7 (ArCH), 114.3 (C5a), 129.1 (ArC),

131.9 (ArC), 139.6 (ArC), 154.4 (ArC). E.I.M.S. m/e 258 (M^+ 20%), 228 (100%), 213 (20%), 200 (20%), 187 (20%), 174 (20%), 157 (15%), 115 (10%).

N-Acetyl-4-aminomethyl-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole (25)¹



4-Aminomethyl-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole (0.80 g, 3.27 mmol) was dissolved in 10 mL of dry DCM and acetic anhydride (510 mg, 5.0 mmol) in 3 mL dichloromethane added dropwise over 15 minutes. The reaction was stirred at room temperature for two hours and then 10 mL of water were added and the solution separated. The aqueous layer was washed with dichloromethane (2 x 10 mL) and the organic layers combined, washed with water (10 mL), 2M hydrochloric acid (10 mL), saturated aqueous sodium bicarbonate (2 x 10 mL), and finally with saturated aqueous sodium chloride (10 mL). The organic layer was dried over magnesium sulphate, filtered, and the filtrate evaporated *in vacuo* to give an off-white solid. The crude product was subjected to column chromatography using ethyl acetate as eluent. The pure fractions were combined and evaporated *in vacuo* then triturated with ether to give a white solid which was recrystallised from ethyl acetate, 285 mg, 1 mmol, 31% yield, m.p. 162-163 °C.

IR ν_{\max} cm^{-1} 3290, 2931, 1649, 1579, 1554, 1487, 1450, 1417, 1371, 1300, 1259, 1225, 1151, 1032, 800. ^1H NMR (200 MHz; CDCl_3) δ_{H} 1.76-2.06 (m, 4H, CH_2), 1.95 (s, 3H, CH_3), 2.60-2.70 (m, 2H, CH_2), 3.14-3.24 (m, 1H, CH), 3.55-3.65 (m, 2H, CH_2), 3.58 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 5.61 (br.s, 1H, NH), 6.81 (dd, $J = 8.8$ Hz, $J = 2.4$ Hz, 1H, ArH), 7.08 (d, $J = 2.4$ Hz, 1H, ArH), 7.15 (d, $J = 8.8$ Hz,

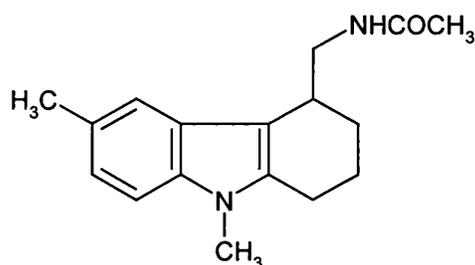
¹H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_C 19.6 (CH₂), 22.1 (CH₂), 23.5 (CH₃), 26.7 (CH₂), 29.1 (CH₃) 32.6 (CH), 43.4 (CH₂), 56.1 (CH₃), 100.9 (ArCH), 109.1 (C4a), 109.3 (ArCH), 110.4 (ArCH), 127.0 (ArC), 132.2 (ArC), 137.6 (ArC), 153.9 (ArC), 170.2 (C=O). E.I.M.S. m/e 286 (M⁺ 45%), 227 (80%), 214 (100%), 199 (50%), 184 (30%), 170 (40%), 156 (20%) 128 (10%), 43 (45%).

Enantiomeric resolution. The racemic tetrahydrocarbazole (**25**, 280 mg) was dissolved in ethanol (10 mg mL⁻¹) and injected in 0.5 ml aliquots onto a 25 cm x 2 cm Chiralcel AD preparative HPLC column. The eluting solvent was 85% hexane/15% ethanol and the eluent monitored at λ = 280 nm. These conditions afforded baseline separation and allowed each enantiomer to be collected directly. Evaporation of the eluent yielded two white solids, **25**(-) (147 mg) and **25**(+) (117 mg). Optical rotation measurements were obtained at a concentration of 10 mg mL⁻¹ in EtOH, **25**(-) [α]_D = -29° **25**(+) [α]_D = +28.5°

The enantiomeric purity of each sample was confirmed by analytical HPLC and by Circular Dichroism studies. The CD spectrum of the two enantiomers are included in the appendix (figure 37).

The following compounds were similarly prepared;

N-Acetyl-4-aminomethyl-6-methyl-9-methyl-1,2,3,4-tetrahydrocarbazole (71)

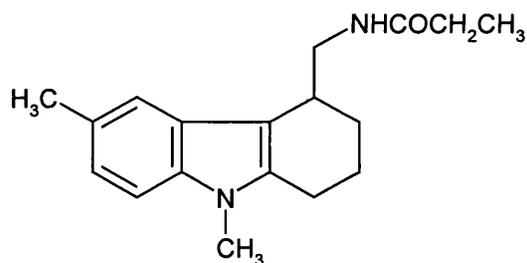


4-Aminomethyl-6-methyl-9-methyl-1,2,3,4-tetrahydrocarbazole (0.50 g, 2.28 mmol) and acetic anhydride (275 mg, 2.7 mmol) gave the title compound on

trituration with ether and recrystallisation from ethyl acetate, as a white solid, 390 mg, 1.45 mmol, 64% yield, m.p. 171-172 °C.

IR ν_{\max} cm^{-1} 3351, 2933, 2852, 1651, 1545, 1487, 1437, 1373, 1292, 1167, 1094, 795. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.76-2.05 (m, 4H, CH_2), 1.92 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.60-2.70 (m, 2H, CH_2), 3.16-3.24 (m, 1H, CH), 3.57 (s, 3H, CH_3), 3.55-3.65 (m, 2H, CH_2), 5.54 (br.s, 1H, NH), 6.97 (dd, $J = 8.3$ Hz, $J = 1.3$ Hz, 1H, ArH), 7.13 (d, $J = 8.3$ Hz, 1H, ArH), 7.15 (d, $J = 0.6$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 20.2 (CH_2), 21.8 (CH_3), 22.5 (CH_2), 23.8 (CH_3), 27.2 (CH_2), 29.4 (CH_3), 33.0 (CH), 44.0 (CH_2), 108.8 (ArCH), 109.3 (C4a), 118.6 (ArCH), 122.6 (ArCH), 126.6 (ArC), 128.6 (ArC), 135.7 (ArC), 137.2 (ArC), 170.5 (C=O). E.I.M.S. m/e 270 (M^+ 35%), 211 (80%), 198 (100%), 182 (40%), 168 (25%). Found: C, 75.30%, H, 8.05%, N, 10.42%. $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}$ requires C, 75.52% H, 8.20% N, 10.36%.

N-Propionyl-4-aminomethyl-6-methyl-9-methyl-1,2,3,4-tetrahydrocarbazole (72)



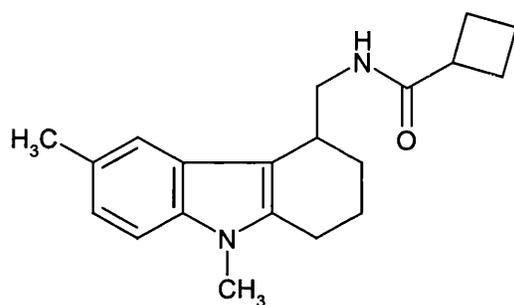
4-Aminomethyl-6-methyl-9-methyl-1,2,3,4-tetrahydrocarbazole (0.40 g, 1.75 mmol) and propionyl chloride (200 mg, 1.75 mmol) gave the title compound on trituration with ether and recrystallisation from ethyl acetate, as a white solid, 260 mg, 0.92 mmol, 52% yield, m.p. 153-154 °C.

IR ν_{\max} cm^{-1} 3288, 2931, 2868, 1659, 1556, 1487, 1462, 1379, 1299, 1238, 789. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.11 (t, $J = 7.6$ Hz 3H, CH_3), 1.75-2.05 (m, 4H, CH_2), 2.14 (q, $J = 7.6$ Hz, 2H, CH_2), 2.43 (s, 3H, CH_3), 2.58-2.74 (m, 2H, CH_2), 3.16-3.24 (m, 1H, CH), 3.57 (s, 3H, CH_3), 3.55-3.65 (m, 2H, CH_2), 5.51 (br.s, 1H,

NH), 6.97 (dd, $J = 8.3$ Hz, $J = 1.2$ Hz, 1H, ArH), 7.13 (d, $J = 8.3$ Hz, 1H, ArH), 7.35 (d, $J = 0.5$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 10.1 (CH_3), 20.1 (CH_2), 21.8 (CH_3), 22.5 (CH_2), 27.2 (CH_2), 29.3 (CH_3), 30.2 (CH_2), 33.0 (CH), 43.9 (CH_2), 108.8 (ArCH), 109.3 (C4a), 118.6 (ArCH), 122.6 (ArCH), 127.4 (ArC), 128.5 (ArC), 135.5 (ArC), 137.3 (ArC), 173.9 (C=O). E.I.M.S. m/e 284 (M^+ 20%), 211 (80%), 198 (100%), 182 (30%), 168 (15%). Found: C, 75.22%, H, 8.36%, N, 9.77%. $\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}$ requires C, 75.52% H, 8.48% N, 9.86%.

N-Cyclobutanoyl-4-aminomethyl-6-methyl-9-methyl-1,2,3,4-tetrahydrocarbazole

(73)



4-Aminomethyl-6-methyl-9-methyl-1,2,3,4-tetrahydrocarbazole (0.40 g, 1.75 mmol) and cyclobutane carbonylchloride (300 mg, 1.75 mmol) gave the title compound on trituration with ether and recrystallisation from ethyl acetate, as a white solid, 240 mg, 0.77 mmol, 44%, m.p.169-170 °C.

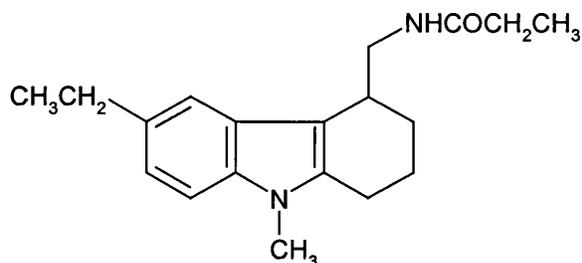
IR ν_{max} cm^{-1} 3244, 2937, 2872, 1659, 1654, 1560, 1485, 1466, 1379, 1261, 787.

^1H NMR (360 MHz; CDCl_3) δ_{H} 1.57-2.35 (m, 10H, m, CH_2), 2.43 (s, 3H, CH_3), 2.58-2.74 (m, 2H, CH_2), 2.85-2.95 (m, 1H, m, CH), 3.16-3.24 (m, 1H, CH), 3.57 (s, 3H, CH_3), 3.55-3.65 (m, 2H, CH_2), 5.45 (br.s, 1H, NH), 6.90 (dd, $J = 8.3$ Hz, $J = 1.4$ Hz, 1H, ArH), 7.13 (d, $J = 8.1$ Hz, 1H, ArH), 7.34 (d, $J = 0.4$ Hz, 1H, ArH).

^{13}C NMR (90 MHz; CDCl_3) δ_{C} 18.6 (CH_2), 20.0 (CH_2 x2), 21.9 (CH_3), 22.5 (CH_2), 25.7 (CH_2), 27.2 (CH_2), 30.5 (CH_3), 33.0 (CH), 40.6 (CH), 43.9 (CH_2), 108.8 (ArCH), 109.3 (C4a), 118.6 (ArCH), 122.6 (ArCH), 127.0 (ArC), 128.6 (ArC), 135.5 (ArC), 138.0 (ArC), 170.5 (C=O). E.I.M.S. m/e 310 (M^+ 10%), 211

(65%), 198 (100%), 182 (15%), 168 (10%). Found: C, 77.18%, H, 8.20%, N, 8.97%. $C_{20}H_{26}N_2O$ requires C, 77.38% H, 8.44% N, 9.02%.

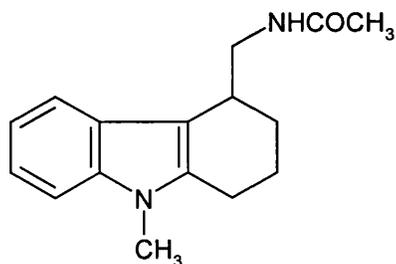
N-Propanoyl-4-aminomethyl-6-ethyl-9-methyl-1,2,3,4-tetrahydrocarbazole (74)



4-Aminomethyl-6-ethyl-9-methyl-1,2,3,4-tetrahydrocarbazole (0.50 g, 2.06 mmol), propanoyl chloride (203 mg, 2.2 mmol) and triethylamine (505 mg, 5 mmole) gave the title compound on trituration with ether and recrystallisation from ethyl acetate, as a white solid, 290 mg, 0.97 mmol, 47% yield, m.p. 158-159 °C.

IR ν_{\max} cm^{-1} 3351, 2933, 2852, 1651, 1545, 1487, 1437, 1373, 1292, 1167, 1094, 795. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.15 (t, $J = 8.8$ Hz, 3H, CH_3), 1.25 (t, $J = 8.8$ Hz, 3H, CH_3), 1.76-2.05 (m, 4H, CH_2), 2.15 (q, $J = 8.8$ Hz, 2H, CH_2), 2.60-2.80 (m, 4H, CH_2), 3.20-3.30 (m, 1H, CH), 3.60 (s, 3H, CH_3), 3.55-3.70 (m, 2H, CH_2), 5.60 (br.s, 1H, NH), 7.00 (dd, $J = 8.3$ Hz, $J = 1.0$ Hz, 1H, ArH), 7.18 (d, $J = 8.3$ Hz, 1H, ArH), 7.38 (d, $J = 1.0$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 10.0 (CH_3), 16.8 (CH_3), 19.9 (CH_2), 22.3 (CH_2), 27.0 (CH_2), 29.2 (CH_3), 29.3 (CH_2), 30.1 (CH_2), 32.8 (CH), 43.8 (CH_2), 108.8 (ArCH), 109.4 (C4a), 117.2 (ArCH), 121.3 (ArCH), 127.2 (ArC), 135.3 (ArC), 135.7 (ArC), 137.2 (ArC), 170.5 (C=O). E.I.M.S. m/e 298 (M^+ 30%), 225 (80%), 212 (100%), 196 (15%), 182 (35%). Found: C, 76.34%, H, 8.65%, N, 9.23%. $C_{19}H_{26}N_2O$ requires C, 76 46% H, 8.78% N, 9.39%.

N-Acetyl-4-aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole (64)

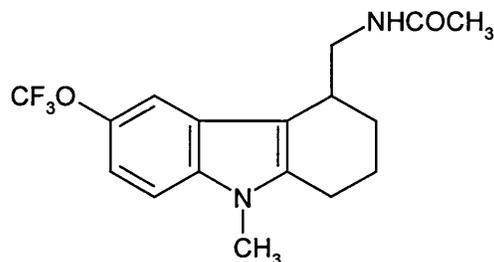


4-Aminomethyl-9-methyl-1,2,3,4-tetrahydrocarbazole (1.95 g, 9.15 mmol) and acetic anhydride (1.42 g, 14 mmol) gave a white solid, on recrystallisation from ethyl acetate, 1.42 g, 5.57 mmol, 61% yield m.p. 173-174 °C.

IR ν_{\max} cm^{-1} 3306, 2943, 2926 1649, 1558, 1470, 1375, 1294, 1163, 737, 729. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.76-2.06 (m, 4H, CH_2), 1.95 (s, 3H, CH_3), 2.60-2.75 (m, 2H, CH_2), 3.19-3.26 (m, 1H, CH), 3.60 (s, 3H, CH_3), 3.50-3.70 (m, 2H, CH_2), 5.66 (br.s, 1H, NH), 7.06 (t, $J = 7.0$ Hz, $J = 0.9$ Hz, 1H, ArH), 7.15 (t, $J = 7.0$ Hz, $J = 0.9$ Hz, 1H, ArH), 7.25 (d, $J = 7.9$ Hz, 1H, ArH), 7.58 (d, $J = 7.9$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 20.2 (CH_2), 22.5 (CH_2), 23.7 (CH_3), 27.1 (CH_2), 29.4 (CH_3), 33.1 (CH), 43.4 (CH_2), 109.1 (ArCH), 109.9 (C4a), 118.7 (ArCH), 119.5 (ArCH), 121.2 (ArCH), 127.2 (ArC), 137.3 (ArC), 137.5 (ArC), 170.5 (C=O). E.I.M.S. m/e 256 (M^+ 35%), 197 (85%), 184 (100%), 167 (60%), 154 (20%), 128 (20%), 115 (20%).

Chromatographic Separation: The racemic tetrahydrocarbazole (64, 1.20 g) was dissolved in ethanol (10 mg/mL) and injected in 0.5 mL aliquots onto a 25 cm x 2 cm cm 'Chiralcel OD' preparative HPLC column. The eluting solvent was 90% hexane/10% ethanol and the eluent monitored at $\lambda = 280$ nm. These conditions afforded baseline separation and allowed each enantiomer to be collected directly. Evaporation of the eluent yielded two white solids 64(+) (0.41 g) and 64(-) (0.47 g). Optical rotation measurements were recorded at a concentration of 10 mg mL^{-1} in DCM, 64(+) $[\alpha]_{\text{Hg}} = +15.9^\circ$, 64(-) $[\alpha]_{\text{Hg}} = -16.5^\circ$

N-Acetyl-4-aminomethyl-6-trifluoromethoxy-9-methyl-1,2,3,4-tetrahydro-
carbazole (68)

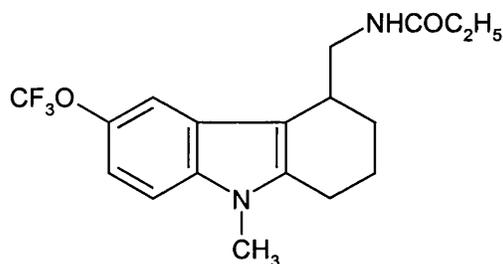


4-Aminomethyl-6-trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole (0.40 g, 1.34 mmol) and acetic anhydride (212 mg, 2 mmol) gave the title compound on trituration with ether and recrystallisation from ethyl acetate as a white solid, 290 mg, 0.62 mmol, 46% yield, m.p. 183-184 °C.

IR ν_{\max} cm^{-1} 3261, 2935, 1634, 1564, 1485, 1435, 1375, 1277, 1257, 1219, 1163.

^1H NMR (360 MHz; CDCl_3) δ_{H} 1.75-2.04 (m, 4H, CH_2), 1.93 (s, 3H, CH_3), 2.58-2.75 (m, 2H, CH_2), 3.14-3.20 (m, 1H, CH), 3.60 (s, 3H, CH_3), 3.52-3.60 (m, 2H, m, CH_2), 5.55 (br.s, 1H, NH), 7.01 (dd, $J = 8.7$ Hz, $J = 0.9$ Hz, 1H, ArH), 7.19 (d, $J = 8.7$ Hz, 1H, ArH), 7.40 (d, $J = 0.9$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 19.7 (CH_2), 22.5 (CH_2), 23.6 (CH_3), 26.8 (CH_2), 29.5 (CH_3), 32.9 (CH), 44.0 (CH_2), 109.5 (ArCH), 110.7 (C4a), 111.2 (ArCH), 114.8 (ArCH), 127.3 (ArC), 135.7 (ArC), 139.2 (ArC), 143.2 (ArC), 170.5 (C=O), (CF_3 not observed). E.I.M.S. m/e 340 (M^+ 35%), 281 (90%), 268 (100%), 252 (50%), 196 (15%), 182 (35%), 168 (30%), 154 (10%), 43 (25%). Found: C, 59.55%, H, 5.58%, N, 8.08%. $\text{C}_{17}\text{H}_{19}\text{N}_2\text{F}_3\text{O}_2$ requires C, 59.99%, H, 5.63% N, 8.23%.

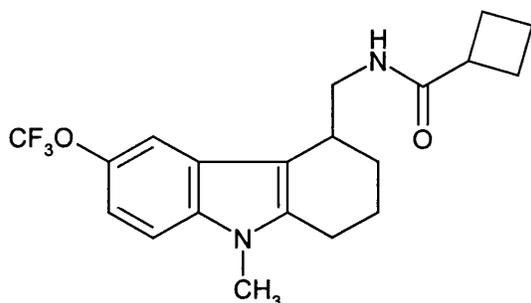
N-Propionyl-4-aminomethyl-6-trifluoromethoxy-9-methyl-1,2,3,4-tetrahydro-
carbazole (69)



4-Aminomethyl-6-trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole (0.37 g, 1.24 mmol) and propionyl chloride (138 mg, 1.24 mmol) gave the title compound on trituration with ether and recrystallisation from ethyl acetate as a white solid, 240 mg, 0.67 mmol, 55%, m.p. 170-171 °C.

IR ν_{\max} cm^{-1} 3439, 2978, 2935, 1637, 1559, 1493, 1256, 1217, 1151, 1047, 789, 694. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.12 (t, $J = 7.6$ Hz, 3H, CH_3), 1.75-2.05 (m, 4H, CH_2), 2.14 (q, $J = 7.6$ Hz, 2H, CH_2), 2.58-2.74 (m, 2H, CH_2), 3.16-3.24 (m, 1H, CH), 3.60 (s, 3H, CH_3), 3.50-3.62 (m, 2H, CH_2), 5.51 (br.s, 1H, NH), 7.01 (dd, $J = 7.6$ Hz, $J = 0.9$ Hz, 1H, ArH), 7.19 (d, $J = 7.6$ Hz, 1H, ArH), 7.40 (d, $J = 0.9$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 10.1 (CH_3), 19.7 (CH_2), 22.6 (CH_2), 26.8 (CH_2), 29.5 (CH_3), 30.2 (CH_2), 33.0 (CH), 43.8 (CH_2), 109.5 (ArCH), 110.7 (C4a), 111.3 (ArCH), 114.7 (ArCH), 127.3 (ArC), 135.7 (ArC), 139.2 (ArC), 143.2 (ArC), 174.2 (C=O), (CF_3 not observed). E.I.M.S. m/e 354 (M^+ 20%), 281 (95%), 268 (100%), 252 (30%), 182 (20%). Found: C, 60.54%, H, 5.96%, N, 7.80%. $\text{C}_{18}\text{H}_{21}\text{N}_2\text{F}_3\text{O}_2$ requires C, 61.01% H, 5.96% N, 7.91%.

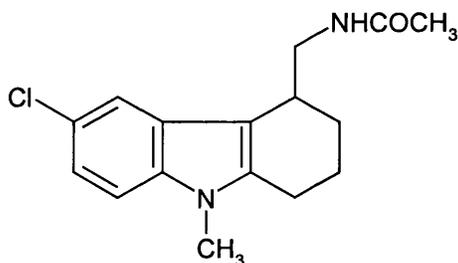
N-Cyclobutanoyl-4-aminomethyl-6-trifluoromethoxy-9-methyl-1,2,3,4-tetrahydro-
carbazole (70)



4-Aminomethyl-6-trifluoromethoxy-9-methyl-1,2,3,4-tetrahydrocarbazole (350 g, 1.17 mmol) and cyclobutanecarbonylchloride (205 mg, 1.20 mmol) gave the title compound on trituration with ether and recrystallisation from ethyl acetate as a white solid, 90 mg, 0.24 mmol, 20%, m.p. 152-153 °C.

IR ν_{\max} cm^{-1} 3439, 2978, 2935, 1637, 1559, 1493, 1256, 1217, 1151, 1047, 789, 694. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.75-2.30 (m, 10H, m, CH_2), 2.58-2.74 (m, 2H, CH_2), 2.88-2.98 (m, 1H, CH), 3.14-3.22 (m, 1H, CH), 3.60 (s, 3H, CH_3), 3.50-3.60 (m, 2H, CH_2), 5.42 (br.s, 1H, NH), 7.05 (dd, $J = 8.7$ Hz, $J = 1.1$ Hz, 1H, ArH), 7.19 (d, $J = 8.8$ Hz, 1H, ArH), 7.38 (d, $J = 0.9$ Hz, 1H, NH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 18.5 (CH_2), 19.7 (2x CH_2), 22.6 (CH_2), 25.7 (CH_2), 25.8 (CH_2), 29.5 (CH), 33.0 (CH), 40.5 (CH_3), 43.7 (CH_2), 109.4 (ArCH), 110.7 (C4a), 111.3 (ArCH), 114.7 (ArCH), 127.3 (ArC), 135.7 (ArC), 139.2 (ArC), 143.9 (ArC), 175.4 (C=O), (CF_3 not observed). E.I.M.S. m/e 380 (M^+ 10%), 281 (95%), 268 (100%), 252 (30%), 182 (40%), 168 (30%), 55 (65%). Found: C, 62.70%, H, 5.98%, N, 7.20%. $\text{C}_{20}\text{H}_{23}\text{N}_2\text{F}_3\text{O}_2$ requires C, 63.15% H, 6.05% N, 7.36%.

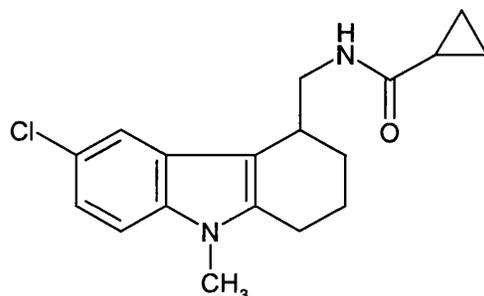
N-Acetyl-4-aminomethyl-6-chloro-9-methyl-1,2,3,4-tetrahydrocarbazole (65)



4-Aminomethyl-6-chloro-9-methyl-1,2,3,4-tetrahydrocarbazole (0.50 g, 2 mmol) and acetic anhydride (345 mg, 0.34 mmol) gave the title compound on trituration with ether and recrystallisation from ethyl acetate as a pale yellow solid, 320 mg, 1.1 mmol, 55% yield m.p. 163-164 °C.

IR ν_{\max} cm^{-1} 3275, 2931, 1738, 1647, 1637, 1556, 1473, 1433, 1371, 1296, 1242, 1075, 1005, 739. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.75-2.05 (m, 4H, CH_2), 1.95 (s, 3H, CH_3), 2.58-2.74 (m, 2H, CH_2), 3.13-3.20 (m, 1H, CH), 3.60 (s, 3H, CH_3), 3.50-3.60 (m, 2H, CH_2), 5.50 (br.s, 1H, NH), 7.06-7.18 (m, 2H, ArH), 7.51 (d, $J = 1.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 19.8 (CH_2), 22.5 (CH_2), 23.9 (CH_3), 26.7 (CH_2), 29.6 (CH_3), 32.8 (CH), 43.8 (CH_2), 110.1 (ArCH), 110.2 (C4a), 118.2 (ArCH), 121.1 (ArCH), 125.0 (ArC), 128.1 (ArC), 135.5 (ArC), 138.6 (ArC), 171.6 (C=O). E.I.M.S. m/e 290 (M^+ 20%), 231 (90%), 218 (100%), 183 (50%), 167 (30%), 154 (15%), 43(55%). Found: C, 64.17%, H, 6.70%, N, 8.64%. $\text{C}_{16}\text{H}_{19}\text{N}_2\text{ClO}$ requires C, 66.08% H, 6.58% N, 9.63%.

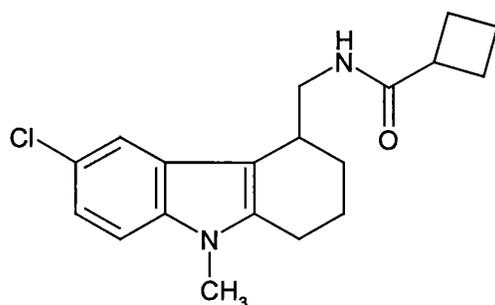
(66)



4-Aminomethyl-6-chloro-9-methyl-1,2,3,4-tetrahydrocarbazole (0.50 g, 2 mmol) and cyclopropane carbonylchloride (345 mg, 0.34 mmol) gave the title compound on trituration with ether and recrystallisation from ethyl acetate as a white solid, 130 mg, 0.41 mmol, 21% yield, m.p.173-174 °C.

IR ν_{\max} cm^{-1} 3305, 2931, 2858, 1637, 1553, 1473, 1437, 1254, 1240, 795, 700, 677. ^1H NMR (360 MHz; CDCl_3) δ_{H} 0.64-0.80 (m, 2H, CH_2), 0.92-1.04 (m, 2H, CH_2), 1.22-1.30 (m, 1H, CH), 1.70-2.05 (m, 4H, CH_2), 2.58-2.74 (m, 2H, CH_2), 3.14-3.22 (m, 1H, CH), 3.58 (s, 3H, CH_3), 3.52-3.60 (m, 2H, CH_2), 5.69 (br.s, 1H, NH), 7.05-7.15 (m, 2H, ArH), 7.54 (d, $J = 1.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 7.4 (CH_2), 7.6 (CH_2), 15.3 (CH), 19.7 (CH_2), 22.5 (CH_2), 26.7 (CH_2), 29.6 (CH_3), 32.9 (CH), 44.1 (CH_2), 110.0 (ArCH), 110.1 (C4a), 118.3 (ArCH), 121.1 (ArCH), 125.1 (ArC), 128.2 (ArC), 135.6 (ArC), 138.7 (ArC), 174.1 (C=O). E.I.M.S. m/e 316 (M^+ 10%), 231 (60%), 218 (100%), 183 (25%), 167 (20%). Found: C, 67.72%, H, 6.56%, N, 8.71%. $\text{C}_{18}\text{H}_{21}\text{N}_2\text{ClO}$ requires C, 68.23% H, 6.68% N, 8.84%.

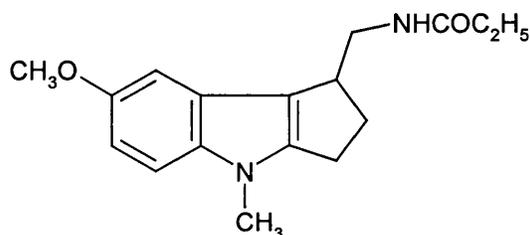
(67)



4-Aminomethyl-6-chloro-9-methyl-1,2,3,4-tetrahydrocarbazole (0.50 g, 2 mmol) and cyclobutane carbonylchloride (403 mg, 0.34 mmol) gave the title compound on trituration with ether and recrystallisation from ethyl acetate as a white solid, 240 mg, 0.73 mmol, 36% yield, m.p. 185-186 °C.

IR ν_{\max} cm^{-1} 3269, 2935, 1635, 1549, 1473, 1431, 1379, 1292, 1256, 791. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.75-2.35 (m, 10H, CH_2), 2.60-2.74 (m, 2H, CH_2), 2.90-3.00 (m, 1H, CH), 3.14-3.22 (m, 1H, CH), 3.60 (s, 3H, CH_3), 3.54-3.62 (m, 2H, CH_2), 5.40 (br.s, 1H, NH), 7.07-7.18 (m, 2H, ArH), 7.51 (d, $J = 1.8$ Hz, 1H, ArH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 18.6 (CH_2), 19.6 (CH_2), 22.5 (CH_2), 25.8 (2 x CH_2), 26.6 (CH_2), 29.6 (CH_3), 32.8 (CH), 40.5 (CH), 43.8 (CH_2), 110.0 (ArCH), 110.1 (C4a), 118.2 (ArCH), 121.1 (ArCH), 125.1 (ArC), 128.1 (ArC), 135.6 (ArC), 138.6 (ArC), 175.5 (C=O). E.I.M.S. m/e 330 (M^+ 10%), 231 (70%), 218 (100%), 182 (25%), 167 (10%), 55 (30%). Found: C, 68.36%, H, 6.82%, N, 8.25%. $\text{C}_{19}\text{H}_{23}\text{N}_2\text{ClO}$ requires C, 68.97% H, 7.12% N, 8.46%.

N-Propanoyl-3-aminomethyl-5-methoxy-8-methyl-1,2,3,-trihydrocyclopent[b]indole (62)

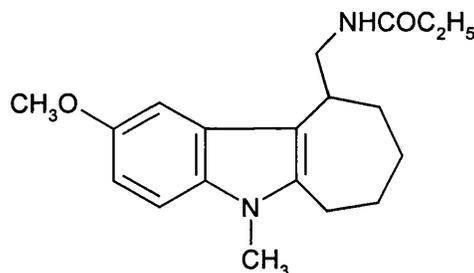


3-Aminomethyl-5-methoxy-8-methyl-1,2,3-trihydrocyclopent[b]indole (60 mg, 0.26 mmol) and propionic anhydride (65 mg, 0.5 mmol) gave the title compound on trituration with ether, as a white solid, 43 mg, 0.15 mmol, 58% yield, m.p. 118-120 °C.

^1H NMR (360 MHz; CDCl_3) δ_{H} 1.13 (t, $J = 7.6$ Hz, 3H, CH_3), 2.04-2.15 (m, 3H, CH_2), 2.50-2.80 (m, 3H, CH_2), 3.40-3.50 (m, 3H, $\text{CH} + \text{CH}_2$), 3.53 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 5.43 (br.s, 1H, NH), 6.70 (dd, $J = 8.8$ Hz, $J = 2.4$ Hz, 1H, ArH), 6.81 (d, $J = 2.4$ Hz, 1H, ArH), 7.02 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 10.2 (CH_3), 24.3 (CH_2), 30.3 (CH_2), 31.2 (CH_3), 33.2 (CH_2), 39.4 (CH), 44.3 (CH_2), 56.4 (CH_3), 101.5 (ArCH), 110.2 (ArCH), 110.5 (ArCH), 117.7 (C3a), 124.8 (ArC), 137.4 (ArC), 148.2 (ArC), 154.6 (ArC), 174.2 (C=O). E.I.M.S. m/e 286 (M^+ 15%), 213 (65%), 200 (100%), 185 (20%), 168 (10%), 157 (20%). Found: MH^+ 286.16713. Formula requires 286.16813.

Chromatographic Separation The racemic cyclopent[b]indole (**62**, 43 mg) was dissolved in ethanol (5 mg mL^{-1}) and injected in 0.5 mL aliquots onto a 25 cm x 2 cm 'Chiralcel OD' preparative HPLC column. The eluting solvent was 93% hexane, 7% ethanol and the eluent was monitored at $\lambda = 280$ nm. Even though these conditions gave relatively poor resolution, recycling of impure fractions enabled enough material to be obtained for biological evaluation. Evaporation of the eluent yielded two white solids **62**(-) (9.7 mg) and **62**(+) (8.3 mg). The purity of each was confirmed by chiral HPLC and CD.

N-Propanoyl-5-aminomethyl-7-methoxy-10-methyl-1,2,3,4,5-pentahydrocyclohept[b]indole (63)



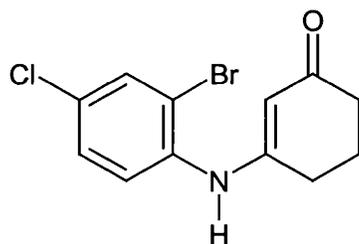
5-Aminomethyl-7-methoxy-10-methyl-1,2,3,4,5-pentahydrocyclopent[b]indole (1.20 g, 4.65 mmol) and propionic anhydride (0.90 g, 7 mmol) gave the title compound on trituration with ether, as a white solid, 1.01 g, 3.23 mmol, 69% yield, m.p. 125-127 °C.

IR ν_{\max} cm^{-1} 3340, 2916, 1641, 1524, 1499, 1448 1230, 1155, 1036, 831, 793. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.02 (t, $J = 7.6$ Hz, 3H, CH_3), 1.45-1.55 (m, 1H, CH_2), 1.70-1.80 (m, 1H, CH_2), 1.80-2.20 (m, 4H, CH_2), 2.04 (q, $J = 7.6$ Hz, 2H, CH_2), 2.65-2.75 (m, 1H, CH_2), 2.92-3.02 (m, 1H, CH_2), 3.22-3.42 (m, 2H, $\text{CH}+\text{CH}_2$), 3.63 (s, 3H, CH_3), 3.70 (m, 1H, CH_2), 3.83 (m, 3H, CH_3), 5.40 (br.s, 1H, NH), 6.80 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.96 (d, $J = 2.2$ Hz, 1H, ArH), 7.12 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 10.2 (CH_3), 26.2 (CH_2), 26.5 (CH_2), 27.8 (CH_2), 30.1 (CH_3), 30.2 (CH_2), 31.1 (CH_2), 34.8 (CH), 43.4 (CH_2), 56.5 (CH_3), 100.0 (ArCH), 110.2 (ArCH), 111.1 (ArCH), 113.4 (C5a), 128.8 (ArC), 131.6 (ArC), 140.0 (ArC), 154.5 (ArC), 174.1 ($\text{C}=\text{O}$). E.I.M.S. m/e 314 (M^+ 85%), 241 (75%), 228 (100%), 213 (35%), 200 (20%), 187 (20%), 157 (15%) 57 (15%), 43 (15%). Found: C, 72.73%, H, 8.55%, N, 8.79%. $\text{C}_{19}\text{H}_{26}\text{N}_2\text{O}$ requires C, 72.58%, H, 8.33%, N, 8.91%.

Chromatographic Separation: The racemic cyclohept[b]indole (63, 400 mg) was dissolved in ethanol (10 mg mL^{-1}) and injected in 0.5 mL aliquots onto a 'Chiralcel OD' preparative HPLC column. The eluting solvent was 95% hexane, 5% ethanol and the eluent was monitored at $\lambda = 280$ nm. These conditions did not

give baseline separation and it was therefore necessary to take fractions and recycle. Evaporation of the eluent yielded two white solids, **63(+)** (142 mg) and **63(-)** (127 mg). The enantiomeric purity was determined by analytical HPLC and by Circular Dichroism studies. Optical rotations were measured at a concentration of 10 mg mL⁻¹ in DCM: **63(+)** [α]_D = + 31.5°, **63(-)** [α]_D = - 30.0°.

3-(2-Bromo-4-chloro-phenylamino)-cyclohex-2-enone

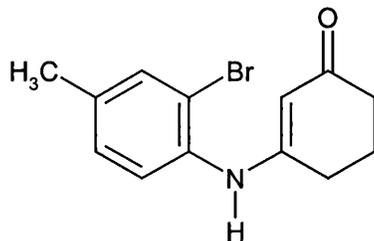


2-Bromo-4-chloroaniline (4.5 g, 0.021 mol) and cyclohexane-1,3-diol (2.16 g, 0.021 mol) were heated together at 125 °C for 3 h. The contents of the flask were cooled and triturated with ether to give the title compound as a yellow solid, 4.97 g, 0.016 M, 84% yield, m.p. 184-185 °C.

IR ν_{\max} cm⁻¹. 3230, 1605, 1591, 1560, 1514, 1360, 1300, 1242, 1194, 1134, 925. ¹H NMR (360 MHz; CDCl₃) δ_{H} 2.05 (qi, J = 6.4 Hz, 2H, CH₂), 2.35 (t, J = 6.4 Hz, 2H, CH₂), 2.52 (t, J = 6.4 Hz, 2H, CH₂), 5.40 (s, 1H, CH), 6.19 (br.s, 1H, NH), 7.24-7.29 (m, 2H, ArH), 7.59 (d, J = 2.2 Hz, 1H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_{C} 22.0 (CH₂), 30.0 (CH₂), 36.9 (CH₂), 101.8 (CH), 119.3 (C), 126.8 (ArCH), 128.8 (ArCH), 131.8 (ArC), 133.2 (ArCH), 135.8 (ArC), 161.0 (ArC), 198.4 (C=O). E.I.M.S. m/e 301/299 (M⁺ 100%), 273/271 (70%), 220 (70%), 192 (100%), 164 (70%), 157 (40%), 68 (60%). Found: C, 47.73%, H, 3.48%, N, 4.64%. C₁₂H₁₁NBrClO requires C, 47.90%, H, 3.66%, N, 4.66%.

The following compounds were similarly prepared;

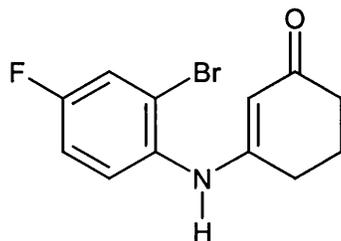
3-(2-Bromo-4-methyl-phenylamino)-cyclohex-2-enone²



2-Bromo-4-methylaniline (5.55 g, 0.03 mol) and cyclohexane-1,3-diol (3.36 g, 0.03 mol) gave the title compound as a yellow solid, 7.87 g, 0.028 M, 94% yield, m.p. 157-158 °C. (lit 160-162 °C).

IR ν_{\max} cm^{-1} . 3250, 1600, 1526, 1521, 1233, 1178, 1144, 1039, 833, 678. ^1H NMR (360 MHz; CDCl_3) δ_{H} 2.07 (qi, $J = 6.4$ Hz, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.38 (t, $J = 6.4$ Hz, 2H, CH_2), 2.55 (t, $J = 6.4$ Hz, 2H, CH_2), 5.43 (s, 1H, CH), 6.15 (br.s, 1H, NH), 7.11 (dd, $J = 8.0$ Hz, $J = 1.2$ Hz, 1H, ArH), 7.26 (d, $J = 8.0$ Hz, 1H, ArH), 7.44 (d, $J = 1.2$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 20.9 (CH_3), 22.2 (CH_2), 30.0 (CH_2), 36.9 (CH_2), 101.1 (CH), 119.1 (C), 126.5 (ArCH), 129.3 (ArCH), 133.9 (ArCH), 134.2 (ArC), 137.8 (ArC), 161.8 (ArC), 198.5 (C=O). E.I.M.S. m/e 281/279 (M^+ 50%), 253/251 (50%), 200 (60%), 172 (70%), 144 (100%). Found: C, 55.82%, H, 5.13%, N, 4.83%. $\text{C}_{13}\text{H}_{14}\text{NBrO}$ requires C, 55.73%, H, 5.04%, N, 4.99%.

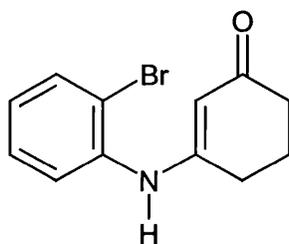
3-(2-Bromo-4-fluoro-phenylamino)-cyclohex-2-enone



2-Bromo-4-Fluoro-aniline (1.00 g, 5.3 mmol) and cyclohexane-1,3-diol (0.59 g, 5.3 mmol) gave the title compound as a yellow solid, 1.27 g, 4.49 mmole, 85% yield, m.p. 190-191 °C.

IR ν_{\max} cm^{-1} . 3245, 2950, 1650, 1590, 1556, 1504, 1255, 1194, 1037, 880, 829, 716. ^1H NMR (360 MHz; d_6 DMSO) δ_{H} 1.85 (qi, $J = 7.9$ Hz, 2H, CH_2), 2.10 (t, $J = 7.0$ Hz, 2H, CH_2), 2.48 (t, $J = 7.0$ Hz, 2H, CH_2), 4.58 (s, 1H, CH), 7.20-7.40 (m, 2H, ArH), 7.68 (dd, $J = 7.2$ Hz, $J = 1.8$ Hz, 1H, ArH), 8.70 (br.s, 1H, NH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 21.6 (CH_2), 27.8 (CH_2), 36.4 (CH_2), 98.1 (CH), 115.7 (ArCH), 122.0 (C), 130.6 (ArCH), 133.8 (ArCH), 158.1 (ArC), 162.0 (ArC), 163.9 (ArC), 195.4 (C=O). E.I.M.S. m/e 284/286 (MH^+ 100%).

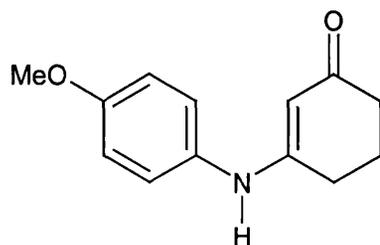
3-(2-Bromo-phenylamino)-cyclohex-2-enone²



2-Bromo-aniline (1.72 g, 0.01 mol) and cyclohexane-1,3-diol (1.12 g, 0.01 mol) gave the title compound as a yellow solid, 2.59 g, 0.009 M, 90% yield, m.p. 165-166 °C. (Lit. 167-168 °C).

IR ν_{\max} cm^{-1} . 3217, 3018, 1599, 1591, 1572, 1520, 1472, 1365, 1246, 1184, 1146, 754, 723. ^1H NMR (360 MHz; d_6 DMSO) δ_{H} 1.95 (q, $J = 6.4$ Hz, 2H, CH_2), 2.14 (t, $J = 6.4$ Hz, 2H, CH_2), 2.50 (t, $J = 6.4$ Hz, 2H, CH_2), 4.70 (s, 1H, CH), 7.15-7.25 (m, 1H, ArH), 7.30-7.38 (m, 1H, ArH), 7.40-7.48 (m, 1H, ArH), 7.72 (d, $J = 7.8$ Hz, 1H, ArH), 8.65 (br.s, 1H, NH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 22.5 (CH_2), 28.8 (CH_2), 37.3 (CH_2), 101.1 (CH), 99.4 (CH), 122.0 (C), 129.2 (CH), 129.5 (CH), 130.0 (CH), 134.2 (CH), 138.2 (ArC), 164.2 (ArC), 196.2 (C=O). E.I.M.S. m/e 268/266 (MH^+ 100%).

3-(4-methoxy-phenylamino)-cyclohex-2-enone²

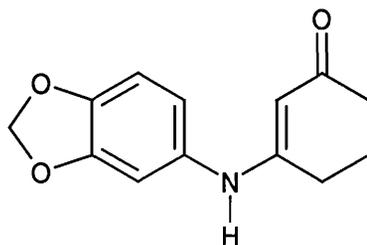


p-Anisidine (4.92 g, 0.04 mol) and cyclohexane-1,3-diol (4.12 g, 0.04 mol) gave the title compound as a yellow solid, 4.02 g, 0.019 M, 46% yield, m.p. 170-171 °C. (Lit. 164-166 °C)

IR ν_{\max} cm^{-1} . 3217, 3043, 1572, 1518, 1414, 1365, 1277, 1242, 1182, 1139, 1050, 870. ^1H NMR (360 MHz; d_6 -DMSO) δ_{H} 1.87 (q, $J = 6.4$ Hz, 2H, CH_2), 2.12 (t, $J = 6.4$ Hz, 2H, CH_2), 2.46 (t, $J = 6.4$ Hz, 2H, CH_2), 3.74 (s, 3H, OCH_3), 5.09 (s, 1H, CH), 6.91-6.94 (m, 2H, ArH), 7.06-7.10 (m, 2H, ArH), 8.58 (br.s, 1H, NH).

^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 22.5 (CH_2), 29.3 (CH_2), 40.1 (CH_2), 56.2 (CH_3), 98.1 (CH), 115.3 (ArCH x2), 126.2 (ArCH x2), 132.6 (C), 157.4 (ArC), 163.9 (ArC), 196.1 (C=O). E.I.M.S. m/e 217 (M^+ 100%), 200 (25%), 189 (80%), 174 (80%), 160 (30%), 146 (30%). Found: C, 71.26%, H, 7.10%, N, 6.36%. $\text{C}_{13}\text{H}_{15}\text{NO}_2$ requires C, 71.87%, H, 6.96%, N, 6.45%.

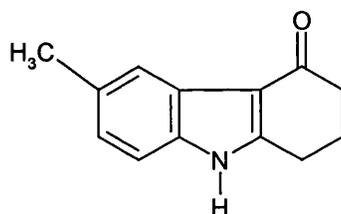
3-(4,5-methylenedioxy-phenylamino)-cyclohex-2-enone



3,4-Methylenedioxy-aniline (5.48 g, 0.04 mol) and cyclohexane-1,3-diol (4.12 g, 0.04 mol) gave the title compound as a tan solid, 2.32 g, 0.01 M, 25% yield, m.p.159-160 °C.

IR ν_{\max} cm^{-1} . 3236, 3057, 1583, 1539, 1499, 1452, 1367, 1256, 1242, 1186, 1036, 925, 855. ^1H NMR (360 MHz; d_6 -DMSO) δ_{H} 1.84 (q, $J = 6.4$ Hz, 2H, CH_2), 2.11 (t, $J = 6.4$ Hz, 2H, CH_2), 2.43 (t, $J = 6.4$ Hz, 2H, CH_2), 5.09 (s, 1H, CH), 5.99 (s, 2H, CH_2), 6.59 (dd, $J = 8.0$, $J = 2.0$, 1H, ArH), 6.71 (d, $J = 2.0$ Hz, 1H, ArH), 6.86 (d, $J = 8.0$ Hz, 1H, ArH), 8.55 (br.s, 1H, NH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 22.4 (CH_2), 29.2 (CH_2), 40.1 (CH_2), 98.6 (CH), 102.2 (CH_2), 106.4 (CH), 109.1 (ArCH), 118.0 (ArCH), 133.9 (C), 145.3 (ArC), 148.5 (ArC), 163.8 (ArC), 196.2 (C=O). E.I.M.S. m/e 231 (M^+ 100%), 214 (40%), 203 (70%), 174 (90%), 136 (50%). Found: C, 67.01%, H, 5.83%, N, 5.57%. $\text{C}_{13}\text{H}_{13}\text{NO}_3$ requires C, 67.52%, H, 5.67%, N, 6.06%.

6-Methyl-1,2,3,4,9-tetrahydro-carbazol-4-one (95)³



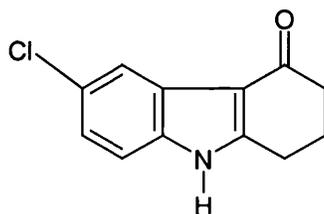
3-(2-Bromo-4-methyl-phenylamino)-cyclohex-2-enone (5.0 g, 18 mmol) was added to a suspension of palladium acetate (225 mg, 1 mmol), triphenylphosphine (524 mg, 2 mmol) and sodium bicarbonate (3.40 g, 40 mmole) in DMF (200 mL). The reaction mixture was stirred at 125 °C for 36 h, then filtered whilst hot through 'Hyflo'. The filtrate was evaporated *in vacuo* and then partitioned between water and warm ethyl acetate. The required product, as a brown insoluble material, was removed by filtration (1.85 g) and the organic layer separated, cooled in ice and a second crop of precipitate collected (1.30 g). The

brown solids were identical by tlc and were combined and recrystallised from ethanol to give a grey solid, 3.15 g, 15.8 mmole, 88%, 281-282 °C. (Lit. 283-284 °C).

IR ν_{\max} cm^{-1} . 3188, 3155, 3120, 2035, 1616, 1472, 1215, 1134, 1122, 799. ^1H NMR (360 MHz; d_6 -DMSO) δ_{H} 2.10 (q, $J = 6.4$ Hz, 2H, CH_2), 2.37 (s, 3H, CH_3), 2.41 (t, $J = 6.4$ Hz, 2H, CH_2), 2.93 (t, $J = 6.4$ Hz, 2H, CH_2), 6.98 (d, $J = 8.3$ Hz, 1H, ArH), 7.28 (d, $J = 8.3$ Hz, 1H, ArH), 7.77 (d, $J = 8.3$ Hz, 1H, ArH), 11.63 (br.s, 1H, NH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_3), 23.7 (CH_2), 24.3 (CH_2), 38.7 (CH_2), 112.0 (ArCH), 112.4 (C4a), 121.0 (ArCH), 124.6 (ArCH), 125.7 (ArC), 131.1 (ArC), 135.0 (ArC), 153.0 (ArC), 193.5 (C=O). E.I.M.S. m/e 199 (M^+ 90%), 171 (70%), 143 (40%), 91 (100%). Found: C, 77.58%, H, 6.57%, N, 7.03%. $\text{C}_{13}\text{H}_{13}\text{NO}$ requires C, 47.90%, H, 6.53%, N, 7.03%.

The following compounds were prepared in a similar manner:

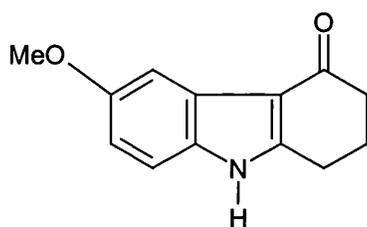
6-Chloro-1,2,3,4,9-tetrahydro-carbazol-4-one (97)³



3-(2-Bromo-4-chloro-phenylamino)-cyclohex-2-enone (2.0 g, 6.6 mmol), palladium acetate (100 mg, 0.44 mmol), triphenylphosphine (234 mg, 0.88 mmol) and sodium bicarbonate (1.11 g, 13.2 mmol) in DMF (60 mL) gave the title compound as a white solid, on precipitation from ethyl acetate and recrystallisation from ethanol, 760 mg, 3.5 mmole, 53% yield, m.p. 285-287 °C. (Lit. 285 °C).

IR ν_{\max} cm^{-1} . 3140, 1630, 1555, 1464, 1258, 1131, 774. ^1H NMR (360 MHz; CDCl_3) δ_{H} 2.00 (q, $J = 6.4$ Hz, 2H, CH_2), 2.31 (m, 2H, CH_2), 2.84 (t, $J = 6.4$ Hz, 2H, CH_2), 7.04 (dd, $J = 8.5$ Hz, $J = 2.1$ Hz, 1H, ArH), 7.28 (d, $J = 8.5$ Hz, 1H, ArH), 7.79 (d, $J = 2.1$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 22.1 (CH_2), 22.6 (CH_2), 37.0 (CH_2), 110.8 (C4a), 112.5 (ArCH), 118.6 (ArC), 121.7 (ArCH), 125.1 (ArCH), 125.5 (ArC), 133.8 (ArC), 153.0 (ArC), 192.2 (C=O). E.I.M.S. m/e 221/219 (M^+ 90%), 193/191 (100%), 165/163 (50%), 96 (30%).

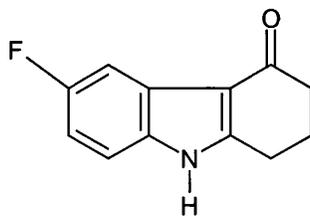
6-Methoxy-1,2,3,4,9-tetrahydro-carbazol-4-one (96)²



3-(4-Methoxy-phenylamino)-cyclohex-2-enone (2.0 g, 9.2 mmol), palladium acetate (2.06 g, 9.2 mmol), triphenylphosphine (234 mg, 0.88 mmol) and sodium bicarbonate (1.11 g, 13.2 mmol) in DMF (60 mL) gave the title compound as a tan solid on precipitation from ethyl acetate and recrystallisation from ethanol-diethylether, 940 mg, 4.4 mmole, 48% yield, m.p. 223-224 °C. (Lit. 245-247 °C).

IR ν_{\max} cm^{-1} . 3140, 1625, 1584, 1468, 1251, 868. ^1H NMR (360 MHz; CDCl_3) δ_{H} 2.00 (q, $J = 6.0$ Hz, 2H, CH_2), 2.32 (t, $J = 6.0$ Hz, 2H, CH_2), 2.83 (t, $J = 5.4$ Hz, 2H, CH_2), 3.66 (s, 3H, CH_3), 6.68 (dd, $J = 8.5$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.18 (d, $J = 8.5$ Hz, 1H, ArH), 7.39 (d, $J = 2.2$ Hz, 1H, ArH), 11.55 (br.s, 1H, NH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 25.7 (CH_2), 26.2 (CH_2), 40.6 (CH_2), 58.2 (CH_3), 105.7 (ArCH), 114.4 (ArCH), 114.6 (C4a), 115.0 (ArCH), 128.2 (ArC), 133.5 (ArC), 155.2 (ArC), 158.1 (ArC), 195.5 (C=O). E.I.M.S. m/e 215 (M^+ 100%), 187 (75%), 159 (35%).

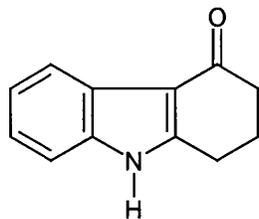
6-Fluoro-1,2,3,4,9-tetrahydro-carbazol-4-one (98)



3-(2-Bromo-4-fluoro-phenylamino)-cyclohex-2-enone (1.0 g, 3.5 mmol), palladium acetate (10 mg, 0.044 mmol), triphenylphosphine (80 mg, 0.3 mmol) and sodium bicarbonate (336 mg, 4 mmol) in DMF (20 mL) gave the title compound as a white solid, on precipitation from ethyl acetate and recrystallisation from ethanol, 365 mg, 1.8 mmole, 51% yield, m.p. 279-280 °C.

IR ν_{\max} cm^{-1} . 3140, 1630, 1555, 1464, 1258, 1131, 774. ^1H NMR (250 MHz; d_6 -DMSO) δ_{H} 2.20 (qi, $J = 6.0$ Hz, 2H, CH_2), 2.50 (t, $J = 6.0$ Hz, 2H, CH_2), 3.03 (t, $J = 6.0$ Hz, 2H, CH_2), 7.10 (dd, $J = 8.0$ Hz, $J = 1.8$ Hz, 1H, ArH), 7.50 (d, $J = 8.0$ Hz, 1H, ArH), 7.71 (d, $J = 1.8$ Hz, 1H, ArH). ^{13}C NMR (63 MHz; d_6 -DMSO) δ_{C} 22.9 (CH_2), 23.4 (CH_2), 37.8 (CH_2), 105.2 (ArCH), 110.2 (ArCH), 112.0 (C4a), 121.7 (ArCH), 125.1 (ArC), 132.3 (ArC), 153.9 (ArC), 158.5 (d, $J = 216$ Hz, ArC), 193.0 (C=O). E.I.M.S. m/e 407 (2MH^+ 30%), 204 (MH^+ 100%), 176 (20%).

1,2,3,4,9-Tetrahydro-carbazol-4-one (94)²

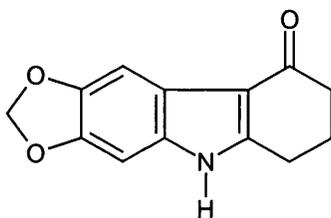


3-(2-Bromophenylamino)-cyclohex-2-enone (2.0 g, 7.5 mmol), palladium acetate (1.68 g, 7.5 mmol), triphenylphosphine (190 mg, 0.72 mmol) and sodium

bicarbonate (0.90 g, 10.8 mmol) in DMF (50 mL) gave the title compound as a light brown solid on precipitation from ethyl acetate and recrystallisation from ethanol, 970 mg, 5.2 mmole, 70% yield, m.p. 220-221 °C. (Lit 217-219 °C).

IR ν_{\max} cm^{-1} . 3148, 3055, 2953, 1612, 1589, 1473, 1462, 1260, 1173, 1030, 831, 756, 561. ^1H NMR (360 MHz; d_6 -DMSO) δ_{H} 2.10 (q, $J = 6.0$ Hz, 2H, CH_2), 2.40 (t, $J = 6.0$ Hz, 2H, CH_2), 2.94 (t, $J = 5.4$ Hz, 2H, CH_2), 7.10-7.22 (m, 2H, ArH), 7.38-7.45 (m, 1H, ArH), 7.90-8.00 (m, 1H, ArH), 11.88 (br.s, 1H, NH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 22.8 (CH_2), 23.5 (CH_2), 37.9 (CH_2), 111.6 (ArCH), 111.9 (C4a), 120.3 (ArCH), 121.6 (ArCH), 122.5 (ArCH), 124.7 (ArC), 136.0 (ArC), 152.4 (ArC), 193.0 (C=O). E.I.M.S. m/e 186 (MH^+ 100%).

6,7-Methylenedioxy-1,2,3,4,9-tetrahydro-carbazol-4-one (99)

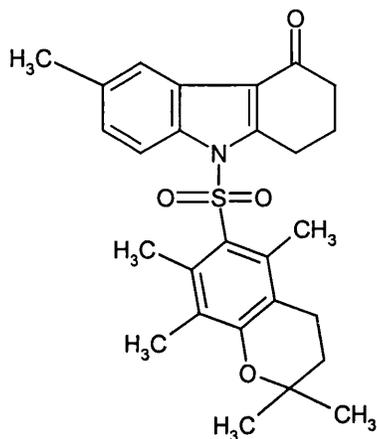


3-(4,5-Methylenedioxy-phenylamino)-cyclohex-2-enone (2.0 g, 8.6 mmol), palladium acetate (1.92 g, 9.2 mmol), triphenylphosphine (234 mg, 0.88 mmol) and sodium bicarbonate (1.11 g, 13.2 mmol) in DMF (60 mL) gave the title compound as a brown solid on precipitation from ethyl acetate, 940 mg, 4.12 mmole, 48% yield, m.p. 193-194 °C.

IR ν_{\max} cm^{-1} . 3168, 1625, 1590, 1468, 1226, 1134, 781. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.99 (q, $J = 6.0$ Hz, 2H, CH_2), 2.29 (t, $J = 6.0$ Hz, 2H, CH_2), 2.79 (t, $J = 6.0$ Hz, 2H, CH_2), 5.87 (s, 2H, CH_2), 6.85 (s, 1H, ArH), 7.28 (s, 1H, ArH), 11.48 (br.s, 1H, OH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 24.4 (CH_2), 25.2 (CH_2), 40.6 (CH_2), 94.5 (ArCH), 100.9 (ArCH), 102.2 (CH_2), 113.8 (C4a), 120.0 (ArC), 132.2 (ArC), 145.3 (ArC), 146.0 (ArC), 152.1 (ArC), 194.4 (C=O). E.I.M.S. m/e

229 (M^+ 100%), 201 (40%), 173 (60%). Found: C, 67.96%, H, 4.71%, N, 6.06%. $C_{13}H_{11}NO_3$ requires C, 68.11%, H, 4.84%, N, 6.11%.

9-(N^G -2,2,5,7,8-Pentamethylchroman-6-benzenesulphonyl-amino)-1,2,3,9-tetrahydro-carbazol-4-one (101)



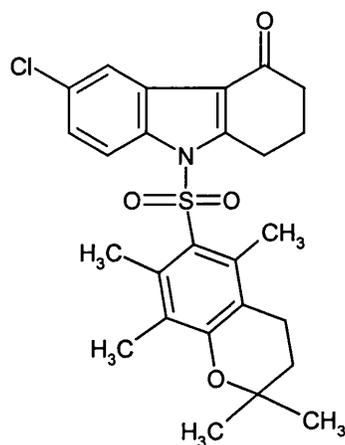
6-Methyl-1,2,3,4,9-tetrahydro-carbazol-4-one (200 mg, 1 mmol) was stirred at room temperature in acetone (4 mL) and 4 M sodium hydroxide (1.25 mL). PMC-Cl (450 mg, 1.5 mmol) in 2 mL of acetone was added dropwise and the dark solution stirred for 3 hr. The reaction mixture was partitioned between ethyl acetate (25 mL) and 5% citric acid (20 mL) and the organic layer was separated and washed with water (4 x 20 mL). The organic layers were combined, dried over magnesium sulphate and evaporated *in vacuo*. The crude material was then subjected to column chromatography and eluted with ethyl acetate to give a white solid, 210 mg, 45% yield, m.p. 155-156 °C.

IR ν_{\max} cm^{-1} . 1666, 1533, 1456, 1396, 1360, 1350, 1217, 1163, 1151, 1121, 1105, 1070, 1028, 621, 546. $^1\text{H NMR}$ (360 MHz; CDCl_3) δ_{H} 1.32 (s, 6H, CH_3), 1.82 (t, $J = 6.8$ Hz, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.10 (t, $J = 6.2$ Hz, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.50 (q, $J = 6.3$ Hz, 2H, CH_2), 2.59 (t, $J = 6.3$ Hz, 2H, CH_2), 2.83 (t, $J = 6.0$ Hz, 2H, CH_2), 7.04 (dd, $J = 8.5$ Hz, J

= 0.9 Hz, 1H, ArH), 7.60 (d, J = 8.5 Hz, 1H, ArH), 8.07 (d, J = 0.9 Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 13.1 (CH_3), 17.5 (CH_3), 18.4 (CH_3), 21.8 (CH_2), 22.1 (CH_3), 24.1 (CH_2), 24.3 (CH_2), 27.5 (2 x CH_3), 33.0 (CH_2), 38.6 (CH_2), 76.2 (ArC), 114.2 (ArCH), 116.5 (ArC), 120.8 (ArC), 122.2 (ArCH), 125.8 (ArC), 125.9 (ArC), 127.3 (ArCH), 129.5 (ArC), 134.6 (ArC), 135.4 (ArC), 137.7 (ArC), 138.2 (ArC), 152.7 (ArC), 156.9 (ArC), 195.6 (C=O). E.I.M.S. m/e 465 (M^+ 45%), 267 (65%), 219 (40%), 203 (95%), 147 (100%). Found: C, 69.24%, H, 6.64%, N 3.00%. $\text{C}_{27}\text{H}_{31}\text{NSO}_4$ requires C, 69.65%, H, 6.71%, N, 3.01%.

The following compounds were similarly prepared:

9-(N^G -2,2,5,7,8-Pentamethylchroman-6-benzenesulphonyl-amino)-6-chloro-1,2,3,9-tetrahydro-carbazol-4-one (103)

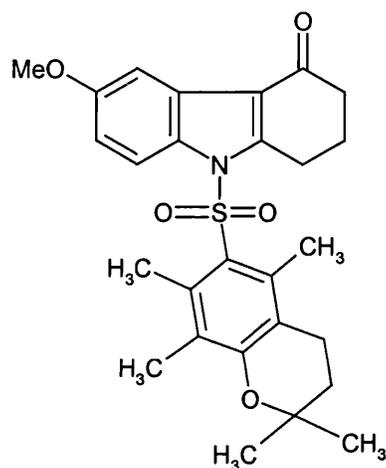


6-Chloro-1,2,3,4,9-tetrahydro-carbazol-4-one (400 mg, 1.7 mmol) and PMC-Cl (830 mg, 2.75 mmol) gave the title compound after chromatographic purification, with ethyl acetate as eluent, to give a grey solid, 170 mg, 0.35 mmole, 21% yield, m.p. 189-190 °C.

IR ν_{max} cm^{-1} . 1670, 1551, 1441, 1394, 1157, 1070, 780, 608. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.31 (s, 6H, CH_3), 1.82 (t, J = 6.8 Hz, 2H, CH_2), 2.07 (s, 3H, CH_3),

2.08 (q, $J = 6.8$ Hz, 2H, CH₂), 2.29 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 2.48 (t, $J = 6.8$ Hz, 2H, CH₂), 2.58 (t, $J = 6.8$ Hz, 2H, CH₂), 2.80 (t, $J = 6.1$ Hz, 2H, CH₂), 7.18 (dd, $J = 9.0$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.64 (d, $J = 9.0$ Hz, 1H, ArH), 8.24 (d, $J = 2.2$ Hz, 1H, ArH). E.I.M.S. m/e 485 (M^+ 30%), 267 (65%), 219 (30%), 203 (100%), 147 (95%). Found: C, 64.27%, H, 5.78%, N, 2.90%. C₂₆H₂₈NClO₄S requires C, 64.25%, H, 5.81%, N, 2.88%.

9-(*N*^G-2,2,5,7,8-Pentamethylchroman-6-benzenesulphonyl-amino)-6-methoxy-1,2,3,9-tetrahydro-carbazol-4-one (102)



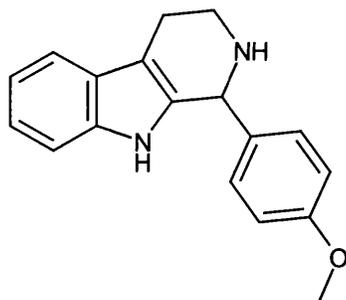
6-Methoxy-1,2,3,4,9-tetrahydro-carbazol-4-one (750 mg, 3.67 mmol) and PMC-Cl (1.65 g, 5.5 mmol) gave the title compound after chromatographic purification, with ethyl acetate as eluent, to give a grey solid, 705 mg, 1.47 mmole, 40% yield, m.p. 160-161 °C .

IR ν_{\max} cm⁻¹. 3000, 1662, 1458, 1402, 1356, 1155, 1030, 617, 588. ¹H NMR (360 MHz; CDCl₃) δ_H 1.30 (s, 6H, CH₃), 1.81 (t, $J = 6.7$ Hz, 2H, CH₂), 2.07 (s, 3H, CH₃), 2.08 (q, $J = 6.8$ Hz, 2H, CH₂), 2.29 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 2.48 (t, $J = 6.7$ Hz, 2H, CH₂), 2.58 (t, $J = 6.8$ Hz, 2H, CH₂), 2.82 (t, $J = 6.1$ Hz, 2H, CH₂), 3.84 (s, 3H, OCH₃), 6.82 (dd, $J = 8.9$ Hz, $J = 2.7$ Hz, 1H, ArH), 7.57 (d, $J = 9.0$ Hz, 1H, ArH), 7.74 (d, $J = 2.5$ Hz, 1H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_C

14.2 (CH₃), 18.8 (CH₃), 19.8 (CH₃), 23.4 (CH₂), 25.5 (CH₂), 25.8 (CH₂), 28.8 (2 x CH₃), 34.6 (CH₂), 40.0 (CH₂), 57.9 (CH₃), 76.8 (ArC), 105.9 (ArCH), 116.4 (ArCH), 116.6 (ArCH), 118.3 (ArC), 121.0 (ArC), 127.5 (ArC), 127.9 (ArC), 131.3 (ArC), 133.5 (ArC), 139.3 (ArC), 139.4 (ArC), 153.6 (ArC), 158.2 (ArC), 159.2 (ArC), 197.1 (C=O). E.I.M.S. m/e 481 (M⁺ 100%), 267 (25%), 219 (15%), 203 (40%), 147 (45%). Found: C, 65.48%, H, 6.42%, N, 2.80%. C₂₇H₃₁NO₅S requires C, 67.34%, H, 6.49%, N, 2.91%.

Experimental for Chapter 3

1-(4-Methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline (142)⁴



Tryptamine (1.20 g, 7.5 mmol) and 4-methoxybenzaldehyde (1.02 g, 7.5 mmol) were dissolved in 15 mL glacial acetic and the reaction mixture stirred under nitrogen for 18 h. The solvent was then removed by lyophilisation and the residue dissolved in ethyl acetate (50 mL). The organic solution was washed with saturated *aq.* sodium bicarbonate (4 x 30 mL), followed by water (30 mL), dried over magnesium sulphate,

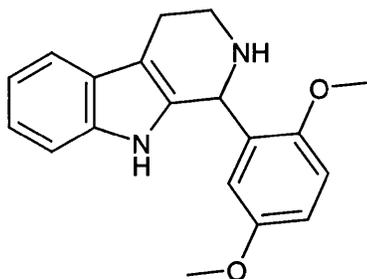
filtered and the filtrate evaporated *in vacuo*. The crude product was subjected to column chromatography, eluting with ethyl acetate, and the purified fractions were

combined and evaporated *in vacuo*. Trituration with ether, followed by recrystallisation from ethyl acetate, gave the title compound as a white solid, 1.16 g, 4.17 mmol, 56%, m.p. 143-144 °C. (Lit. reported as hydrochloride).

I.R. ν_{\max} cm^{-1} 3142, 2846, 1609, 1514, 1504, 1454, 1285, 1255, 1178, 873, 832, 747. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.6-2.77 (m, 2H, CH_2), 2.87-2.96 (m, 1H, CH_2), 3.02-3.05 (m, 1H, CH_2), 3.73 (s, 3H, CH_3), 5.02 (s, 1H, CH), 6.89 (dd, $J = 8.8$ Hz, 2H, ArH), 6.91-7.01 (m, 2H, ArH), 7.18-7.24 (m, 3H, ArH), 7.40 (d, $J = 8.8$ Hz, 1H, ArH), 10.35 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.6 (CH_2), 41.7 (CH_2), 55.4 (CH_3), 56.4 (CH), 108.0 (ArC), 111.4 (ArCH), 113.8 (ArCH x2), 117.8 (ArCH) 118.5 (ArCH), 120.8 (ArCH), 127.2 (ArC), 129.8 (ArCH x2), 135.6 (ArC), 136.5 (ArC), 136.6 (ArC), 159.0 (ArC). E.I.M.S. m/e 279 (100%). Found: MH^+ 279.148795. Formula requires 279.149738. Found: C, 77.25%, H, 6.48%, N, 9.80%. $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}$ requires C, 77.67%, H, 6.52%, N, 10.06%.

The following compounds were prepared in a similar manner:

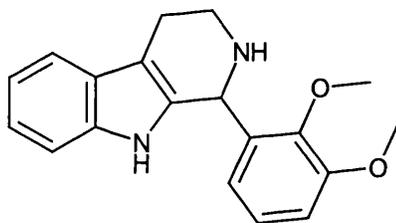
1-(2,5-Dimethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (143)⁵



Tryptamine (1.20 g, 7.5 mmol) and 2,5-methoxybenzaldehyde (1.25 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 0.99 g, 3.2 mmol, 43%, m.p. 149-150 °C. (Lit. m.p. of free amine not reported).

I.R. ν_{\max} cm^{-1} . 3294, 2937, 2831, 1498, 1470, 1448, 1352, 1298, 1225, 1213, 1177, 1041, 862, 812, 737, 706. ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.76-2.91 (m, 2H, CH_2), 2.99-3.16 (m, 1H, CH_2), 3.28-3.34 (m, 1H, CH_2), 3.67 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 5.58 (s, 1H, CH), 6.71 (d, $J = 3.0$ Hz 1H, ArCH), 6.77-6.81 (m, 1H, ArCH), 6.89 (d, $J = 7.8$ Hz 1H, ArCH), 7.07-7.14 (m, 2H, ArCH), 7.19-7.23 (m, 1H, ArCH), 7.51 (dd, $J = 7.8$ Hz, $J = 1.6$ Hz, 1H, ArCH), 7.83 (br.s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.5 (CH_2), 42.3 (CH_2), 51.2 (CH), 55.7 (CH_3), 56.2, (CH_3), 110.1 (ArC), 110.8 (ArCH), 111.8 (ArCH), 113.0 (ArCH), 115.2 (ArCH), 118.0 (ArCH), 119.1 (ArCH), 121.4 (ArCH), 127.4 (ArC), 131.3 (ArC), 134.3 (ArC), 135.7 (ArC), 151.3 (ArC), 153.7 (ArC). E.I.M.S. m/e 309 (100%), 280 (80%). Found: MH^+ 309.160313. $\text{C}_{19}\text{H}_{21}\text{N}_2\text{O}_2$ requires 309.160303.

*1-(2,3-Dimethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (144)*⁵

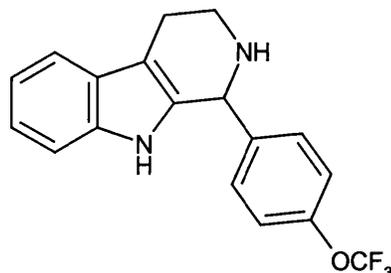


Tryptamine (1.20 g, 7.5 mmol) and 2,3-methoxybenzaldehyde (1.25 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 1.44 g, 4.67 mmol, 62%, m.p.166-167 °C. (Lit. reported as hydrochloride).

I.R. ν_{\max} cm^{-1} . 3345, 2938, 2836, 1587, 1481, 1466, 1315, 1295, 1277, 1213, 1170, 1066, 1003, 799, 748. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.60-2.77 (m, 2H, CH_2), 2.88-2.96 (m, 1H, CH_2), 3.03-3.10 (m, 1H, CH_2), 3.82 (s, 6H, OCH_3), 5.42 (s, 1H, CH), 6.48 (dd, $J = 8.8$ Hz, 1H, ArH), 6.9-7.0 (m, 4H, ArH), 7.21 (d, $J = 8.8$ Hz, 1H, ArH), 7.40 (d, $J = 8.8$ Hz, 1H, ArH), 10.35 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.6 (CH_2), 40.4 (CH_2), 49.6 (CH_3), 55.0 (CH), 60.0 (CH_3), 107.8 (ArC), 110.3 (ArCH), 111.1 (ArCH), 116.7 (ArCH), 117.4 (ArCH), 119.7 (ArCH), 120.3 (ArCH), 122.7 (ArCH), 126.2 (ArC), 134.6 (ArC), 135.2

(ArC), 135.7 (ArC), 146.0 (ArC), 151.8 (ArC). E.I.M.S. m/e 309 (100%). Found: MH^+ 309.159282. Formula requires 309.160303. Found: C, 73.72%, H, 6.51%, N, 8.87%. $C_{19}H_{20}N_2O_2$ requires C, 74.00%, H, 6.54%, N, 9.08%.

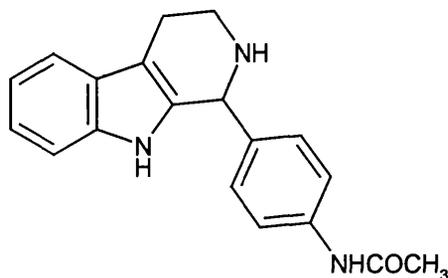
1-(4-Trifluoromethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (145)



Tryptamine (1.20 g, 7.5 mmol) and 4-trifluoromethoxybenzaldehyde (1.42 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 1.60 g, 4.82 mmol, 64%, m.p. 169-170 °C.

I.R. ν_{max} cm^{-1} . 3401, 3058, 2843, 1502, 1449, 1285, 1234, 849, 748. 1H NMR (400 MHz; d_6 -DMSO) δ_H 2.62-2.74 (m, 2H, CH_2), 2.88-3.05 (m, 3H, CH_2 + NH), 5.02 (s, 1H, CH), 6.93-7.03 (m, 2H, ArH), 7.22 (d, $J = 8.8$ Hz, 1H, ArH), 7.30-7.36 (m, 2H, ArH), 7.38-7.42 (m, 3H, ArH), 10.50 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_C 22.5 (CH_2), 41.4 (CH_2), 56.1 (CH), 108.0 (ArC), 111.4 (ArCH), 117.9 (ArCH), 118.6 (ArCH), 120.9 (ArCH), 121.0 (ArCH x2), 126.4 (ArC), 130.6 (ArCH x2), 135.5 (ArC), 136.6 (ArC), 143.1 (ArC), 148.0 (ArC) OCF_3 not observed. E.I.M.S. m/e 333 (100%). Found: MH^+ 333.120429. Formula requires 333.121473. Found: C, 64.81%, H, 4.54%, N, 8.02%. $C_{18}H_{15}N_2F_3O$ requires C, 65.06%, H, 4.55%, N, 8.43%.

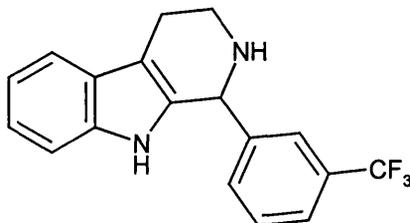
1-(4-Acetamidophenyl)-2,3,4,9-tetrahydro-1H- β -carboline (146)



Tryptamine (1.20 g, 7.5 mmol) and 4-acetamidobenzaldehyde (1.42 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 1.27 g, 4.16 mmol, 56%, m.p. 246-247 °C.

I.R. ν_{\max} cm^{-1} 3246, 1670, 1595, 1541, 1416, 1315, 1308, 1285, 834, 799, 739. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.03 (s, 3H, CH_3), 2.60-2.76 (m, 2H, CH_2), 2.88-3.11 (m, 2H, CH_2), 5.02 (s, 1H, CH), 6.92-7.01 (m, 2H, ArH), 7.16-7.23 (m, 3H, ArH), 7.40 (d, $J = 8.8$ Hz, 1H, ArH), 7.52 (m, 2H, ArH), 9.90 (s, 1H, NH), 10.50 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 20.5 (CH_2), 22.2 (CH_3), 39.6 (CH_2), 54.5 (CH), 106.4 (ArC), 109.3 (ArCH), 115.7 (ArCH), 116.4 (ArCH), 117.0 (ArCH x2), 118.7 (ArCH), 124.6 (ArC), 126.9 (ArCH x2), 133.9 (ArC), 134.2 (ArC), 136.0 (ArC), 136.6 (ArC), 167.0, (ArC). E.I.M.S. m/e 306 (30%), 277 (100%), 263 (20%). Found: MH^+ 306.161357. Formula requires 306.16063. Found: C, 74.56%, H, 6.23%, N, 13.80%. $\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}$ requires C, 74.73%, H, 6.27%, N, 13.81%

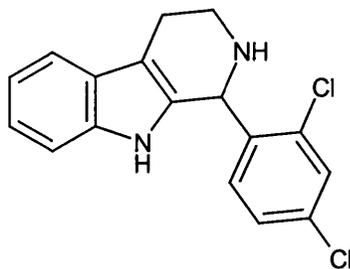
1-(3-Trifluoromethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (147)



Tryptamine (1.20 g, 7.5 mmol) and 3-trifluoromethoxybenzaldehyde (1.30 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 1.02 g, 3.23 mmol, 43%, m.p. 207-208 °C.

I.R. ν_{\max} cm^{-1} . 3192, 2832, 1426, 1341, 1323, 1165, 1118, 1068, 862, 808, 735, 707. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.63-2.80 (m, 2H, CH_2), 2.90-3.07 (m, 2H, CH_2), 5.20 (s, 1H, CH), 6.92-7.05 (m, 2H, ArH), 7.23 (d, $J = 8.8$ Hz, 1H, ArH), 7.42 (d, $J = 8.8$ Hz, 1H, ArH), 7.58 (m, 2H, ArH), 7.64 (m, 2H, ArH), 10.50 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 23.1 (CH_2), 42.3 (CH_2), 57.1 (CH), 109.6 (ArC), 112.1 (ArCH), 118.7 (ArCH), 119.3 (ArCH), 121.8 (ArCH), 125.0 (ArCH), 125.5 (q, $J = 265$ Hz, CF_3), 125.9 (ArCH), 127.8 (ArC), 130.0 (ArC), 130.2 (ArCH), 133.7 (ArCH), 135.6 (ArC), 137.0 (ArC), 145.7 (ArC). E.I.M.S. m/e 317 (10%), 288 (100%). Found: MH^+ 317.127351. Formula requires 317.126558. Found: C, 68.05%, H, 4.66%, N, 8.80%. $\text{C}_{18}\text{H}_{15}\text{N}_2\text{F}_3$ requires C, 68.35%, H, 4.78%, N, 8.86%.

1-(2,4-Dichloromethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (148)



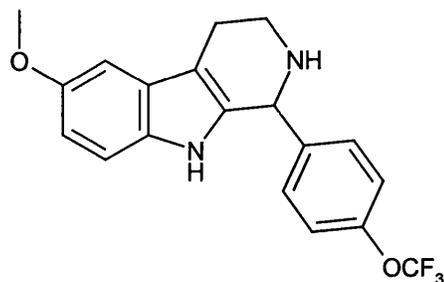
Tryptamine (1.20 g, 7.5 mmol) and 2,4-dichloromethoxybenzaldehyde (1.30 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 1.73 g, 5.45 mmol, 73%, m.p. 178-179 °C.

I.R. ν_{\max} cm^{-1} . 3066, 2842, 1464, 1455, 1428, 1092, 865, 853, 825, 747, 735, 709. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.60-2.78 (m, 2H, CH_2), 3.30-3.42 (m, 2H, CH_2), 5.47 (s, 1H, CH), 6.90-7.05 (m, 3H, ArH), 7.22 (d, $J = 8.8$ Hz, 1H, ArH), 7.30 (d, $J = 8.8$ Hz, 1H, ArH), 7.43 (d, $J = 8.8$ Hz, 1H, ArH), 7.68 (s, 1H, ArH),

10.50 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.4 (CH_2), 40.6 (CH_2), 52.7 (CH), 109.7 (ArC), 111.5 (ArCH), 118.0 (ArCH), 118.7 (ArCH), 121.2 (ArCH), 127.1 (ArC), 127.3 (ArCH), 129.2 (ArCH), 131.9 (ArCH), 132.8 (ArC), 133.9 (ArC), 134.6 (ArC), 136.3 (ArC), 139.5 (ArC). E.I.M.S. m/e 318 (100%). Found: MH^+ 317.061230. Formula requires 317.061229. Found: C, 63.95%, H, 4.44%, N, 8.80%. $\text{C}_{17}\text{H}_{14}\text{N}_2\text{Cl}_2$ requires C, 64.37%, H, 4.45%, N, 8.83%.

6-Methoxy-1-(4-trifluoromethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline

(149)

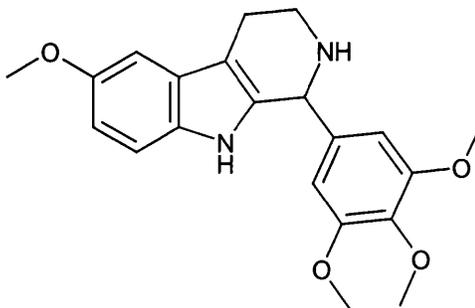


5-Methoxytryptamine (1.43 g, 7.5 mmol) and 4-trifluoromethoxybenzaldehyde (1.42 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 0.95 g, 2.62 mmol, 35%, m.p. 177-178 °C.

I.R. ν_{max} cm^{-1} . 1624, 1490, 1510, 1490, 1440, 1269, 1216, 1200, 1166. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.60-2.78 (m, 2H, CH_2), 2.90-3.08 (m, 3H, CH_2 + NH), 3.75 (s, 3H, OCH_3), 5.15 (s, 1H, CH), 6.67 (dd, $J = 8.8$ Hz, $J = 2$ Hz, 1H, ArH), 6.92 (d, $J = 2.2$ Hz, 1H, ArH), 7.11 (d, $J = 8.8$ Hz, 1H, ArH), 7.29-7.42 (m, 4H, ArH), 10.40 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.3 (CH_2), 41.2 (CH_2), 55.3 (CH), 55.6 (CH_3), 99.8 (ArCH), 108.2 (ArC), 110.4 (ArCH), 111.7 (ArCH), 120.5 (q, $J = 259$ Hz, CF_3), 120.8 (ArCH x2), 127.1 (ArC), 130.3 (ArCH x2), 131.0 (ArC), 135.1 (ArC), 142.2 (ArC), 147.5 (ArC), 153.1 (ArC). E.I.M.S. m/e 363 (100%), 334 (25%). Found: C, 62.77%, H, 4.71%, N, 7.68%. $\text{C}_{19}\text{H}_{17}\text{N}_2\text{F}_3\text{O}_2$ requires C, 62.98%, H, 4.73%, N, 7.73%.

6-Methoxy-1-(3,4,5-trifluoromethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline

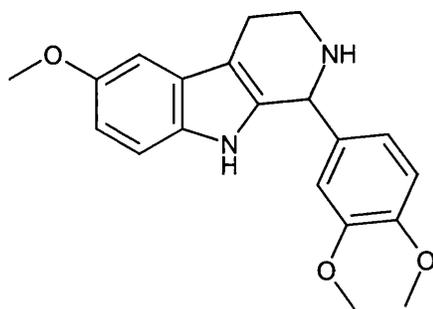
(150)⁶



5-Methoxytryptamine (1.43 g, 7.5 mmol) and 3,4,5-trimethoxybenzaldehyde (1.47 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 2.09 g, 5.68 mmole, 76%, m.p. 114-115 °C. (Reported in lit. as hydrochloride).

I.R. ν_{\max} cm^{-1} . 3356, 1628, 1587, 1560, 1454, 1423, 1370, 1336, 1261, 1220, 1127, 1005, 837, 663, 617. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.58-2.77 (m, 2H, CH_2), 2.89-2.96 (m, 1H, CH_2), 3.14-3.21 (m, 1H, CH_2), 3.65 (s, 3H, CH_3), 3.72 (s, 6H, CH_3), 3.74 (s, 3H, CH_3), 5.01 (s, 1H, CH), 6.63-6.67 (m, 3H, ArH), 6.90 (d, $J = 2.2$ Hz, 1H, ArH), 7.12 (d, $J = 8.8$ Hz, 1H, ArH), 10.15 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 42.1 (CH_2), 55.2 (OCH_3), 55.6 (OCH_3 x2), 57.3 (OCH_3), 59.7 (CH), 99.7 (ArCH), 105.3 (ArCH x2), 107.6 (ArC), 110.0 (ArCH), 111.5 (ArCH), 126.9 (ArC), 130.8 (ArC), 136.1 (ArC), 136.5 (ArC) 138.4 (ArC), 152.5 (ArC x2), 152.8 (ArC). E.I.M.S. m/e 369 (100%), 737 (10%), Found: C, 68.16%, H, 6.39%, N, 7.58%. $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_4$ requires C, 68.46%, H, 6.57%, N, 7.60%.

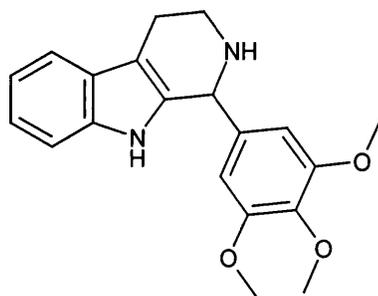
6-Methoxy-1-(3,4,-dimethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline (151)⁵



5-Methoxytryptamine (1.43 g, 7.5 mmol) and 3,4-dimethoxybenzaldehyde (1.25 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 1.79 g, 5.30 mmole, 71%, m.p.164-165 °C. (Lit. reported as hydrochloride).

I.R. ν_{\max} cm^{-1} . 2947, 1614, 1549, 1523, 1484, 1463, 1435, 1401, 1263, 1240, 1220, 1136, 1023, 812, 654. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.58-2.75 (m, 2H, CH_2), 2.88-2.95 (m, 1H, CH_2), 3.07-3.13 (m, 1H, CH_2), 3.71 (s, 3H, CH_3), 3.73 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 5.00 (s, 1H, CH), 6.63 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.73 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.88-6.91 (m, 2H, ArH), 6.95 (d, $J = 2.2$ Hz, 1H, ArH), 7.10 (d, $J = 8.8$ Hz, 1H, ArH), 10.15 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.0 (CH_2), 40.5 (CH_2), 54.1 (CH_3), 54.2 (CH_3), 54.3 (CH_3), 55.5 (CH), 98.5 (ArCH), 106.6 (ArC), 108.9 (ArCH), 110.2 (ArCH), 110.4 (ArCH), 110.9 (ArCH), 119.1 (ArCH), 125.9 (ArC), 129.7 (ArC), 134.3 (ArC), 135.3 (ArC), 146.8 (ArC), 147.3 (ArC), 151.7 (ArC). E.I.M.S. m/e 339 (100%), 677 (10%), Found: C, 69.67%, H, 6.48%, N, 8.10%. $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_3$ requires C, 71.00%, H, 6.53%, N, 8.28%.

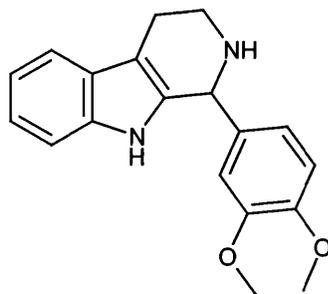
1-(3,4,5-Trimethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (152)⁷



Tryptamine (1.20 g, 7.5 mmol) and 3,4,5-trimethoxybenzaldehyde (1.47 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate 1.31 g, 3.88 mmole, 52%, m.p. 157–158 °C. (Lit. reported as hydrochloride)

I.R. ν_{\max} cm^{-1} . 3335, 1597, 1551, 1510, 1458, 1427, 1333, 1257, 1126, 1001, 744.
 ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.63-2.70 (m, 1H, CH_2), 2.74-2.82 (m, 1H, CH_2), 2.92-3.00 (m, 1H, CH_2), 3.16-3.23 (m, 1H, CH_2), 3.64 (s, 3H, CH_3), 3.72 (s, 6H, CH_3), 5.05 (s, 1H, CH), 6.66 (s, 2H, ArH), 6.92-7.02 (m, 2H, ArH), 7.24 (d, $J = 8$ Hz, 1H, ArH), 7.40 (d, $J = 8$ Hz, 1H, ArH), 10.30 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.3 (CH_2), 42.5 (CH_2), 56.1 (CH), 57.7 ($\text{OCH}_3 \times 2$), 60.2 (OCH_3), 105.9 (ArCH $\times 2$), 108.4 (ArC), 111.4 (ArCH), 117.9 (ArCH), 118.5 (ArCH), 120.9 (ArCH), 127.3 (ArC), 135.7 (ArC), 136.3 (ArC), 138.5 (ArC), 153.2 (ArC $\times 2$), 160.7 (ArC). E.I.M.S. m/e 339 (100%), 677 (10%), Found: C, 70.86%, H, 6.46%, N, 8.20%. $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_3$ requires C, 71.00%, H, 6.53%, N, 8.28%.

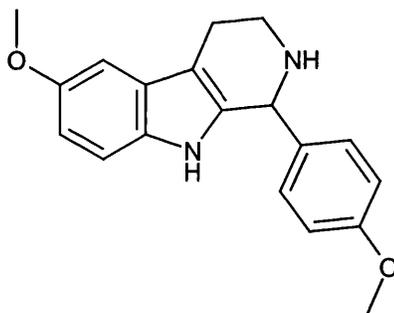
1-(3,4-Dimethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (153)⁵



Tryptamine (1.20 g, 7.5 mmol) and 3,4-dimethoxybenzaldehyde (1.25 g, 7.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 670 mg, 2.18 mmole, 29%, m.p. 109-111 °C. (Lit. reported as hydrochloride).

I.R. ν_{\max} cm^{-1} . 1514, 1505, 1462, 1454, 1416, 1258, 1231, 1141, 1024, 749. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.60-2.77 (m, 2H, CH_2), 2.88-2.96 (m, 1H, CH_2), 2.98-3.14 (m, 1H, CH_2), 3.35 (br.s, 1H, NH), 3.70 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 5.02 (s, 1H, CH), 6.74 (dd, $J = 8.0$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.88-7.01 (m, 4H, ArH), 7.22 (d, $J = 8.0$ Hz, 1H, ArH), 7.39 (d, $J = 8.0$ Hz, 1H, ArH), 10.30 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.3 (CH_2), 40.7 (CH_2), 54.5 (OCH_3), 54.6 (OCH_3), 55.7 (CH), 107.0 (ArC), 110.1 (ArCH), 110.4 (ArCH), 111.2 (ArCH), 116.5 (ArCH), 117.2 (ArCH), 119.4 (ArCH x2), 126.9 (ArC), 137.2 (ArC), 137.4 (ArC), 137.5 (ArC), 147.1 (ArC), 147.7 (ArC). E.I.M.S. m/e 309 (100%), 617 (10%). Found: C, 73.62%, H, 6.38%, N, 9.14%. $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_2$ requires C, 74.00%, H, 6.54%, N, 9.08%.

6-Methoxy-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H β -carboline (154)

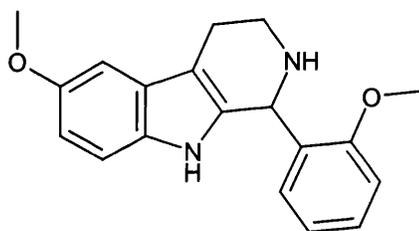


5-Methoxytryptamine (0.95 g, 5.0 mmol) and 4-methoxybenzaldehyde (0.68 g, 5.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 0.81 g, 2.62 mmole, 53%, m.p. 200-201 °C.

I.R. ν_{\max} cm^{-1} . 2953, 2836, 1609, 1552, 1513, 1479, 1454, 1433, 1399, 1300, 1247, 1221, 1203, 1175, 1031, 843, 648. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H}

2.60-2.74 (m, 2H, CH₂), 2.88-2.96 (m, 1H, CH₂), 3.03-3.11 (m, 1H, CH₂), 3.73 (s, 3H, CH₃), 3.76 (s, 3H, CH₃), 5.04 (s, 1H, CH), 6.63 (dd, J = 8.8 Hz, J = 2.2 Hz, 1H, ArH), 6.88 (d, J = 8.8 Hz, 2H, ArH), 6.90 (s, 1H, ArH), 7.10 (d, J = 8.8 Hz, 1H, ArH), 7.18 (d, J = 8.8 Hz, 2H, ArH), 10.20 (s, 1H, NH). ¹³C NMR (100 MHz; d₆-DMSO) δ_C 22.4 (CH₂), 41.6 (CH₂), 55.4 (CH₃), 55.7 (CH₃), 56.4 (CH), 100.1 (ArCH), 108.1 (ArC), 110.6 (ArCH), 112.0 (ArCH), 113.8 (ArCH x2), 127.4 (ArC), 129.9 (ArCH x2), 131.2 (ArC), 135.1 (ArC), 136.5 (ArC), 153.4 (ArC), 159.0 (ArC). E.I.M.S. m/e 309 (100%), 280 (80%), 266 (30%). Found: C, 73.88%, H, 6.50%, N, 8.98%. C₁₉H₂₀N₂O₂ requires C, 74.00%, H, 6.54%, N, 9.08%.

6-Methoxy-1-(2-methoxyphenyl)-6-methoxy-2,3,4,9-tetrahydro-1H-β-carboline
(155)

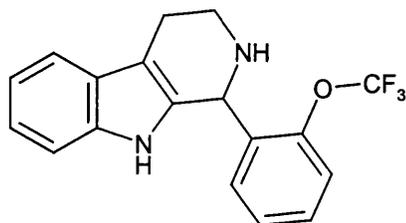


5-Methoxytryptamine (0.76 g, 4.0 mmol) and 2-methoxybenzaldehyde (0.54 g, 4.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 0.77 g, 2.50 mmole, 63%, m.p. 139-140 °C.

I.R. ν_{max} cm⁻¹. 2939, 2832, 1590, 1486, 1461, 1455, 1436, 1288, 1242, 1212, 1104, 1093, 1028, 800, 756. ¹H NMR (400 MHz; CDCl₃) δ_H 2.10 (br.s, 1H, NH), 2.70-2.80 (m, 2H, CH₂), 2.93-3.01 (m, 1H, CH₂), 3.10-3.18 (m, 1H, CH₂), 3.79 (s, 3H, CH₃), 3.81 (s, 3H, CH₃), 5.51 (s, 1H, CH), 6.71 (dd, J = 8.8 Hz, J = 2.2 Hz, 1H, ArH), 6.80 (t, J = 7.8 Hz, 1H, ArH), 6.87 (d, J = 8.8 Hz, 1H, ArH), 6.90-6.98 (m, 3H, ArH), 7.22 (t, J = 8.8 Hz, 1H, ArH), 8.22 (s, 1H, NH). ¹³C NMR (100 MHz; CDCl₃) δ_C 23.0 (CH₂), 42.1 (CH₂), 51.3 (CH), 55.9 (CH₃), 56.4 (CH₃), 100.7 (ArCH), 110.3 (ArC), 111.0 (ArCH), 111.5 (ArCH), 111.9

(ArCH), 120.9 (ArCH), 128.1 (ArC), 129.3 (ArCH), 129.7 (ArCH), 130.4 (ArC), 131.4 (ArC), 135.7 (ArC), 154.2 (ArC), 157.6 (ArC). E.I.M.S. m/e 309 (100%), 280 (90%), 266 (10%). Found: C, 73.91%, H, 6.50%, N, 9.04%. $C_{19}H_{20}N_2O_2$ requires C, 74.00%, H, 6.54%, N, 9.08%.

1-(2-Trifluoromethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (156)

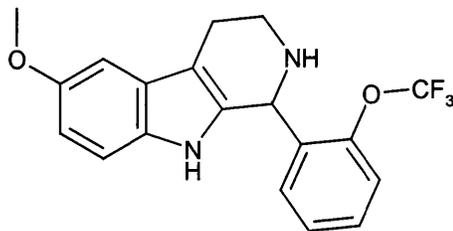


Tryptamine (0.80 g, 5.0 mmol) and 2-trifluoromethoxybenzaldehyde (0.95 g, 5.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 0.56 g, 1.69 mmole, 34%, m.p. 106-107 °C.

I.R. ν_{\max} cm^{-1} . 3060, 2944, 1450, 1287, 1270, 1248, 1222, 1208, 1166, 1147, 1086, 899, 765, 743, 737. ^1H NMR (400 MHz; CDCl_3) δ_{H} 1.93 (br.s, 1H, NH), 2.78-2.96 (m, 2H, CH_2), 3.11-3.19 (m, 1H, CH_2), 3.25-3.32 (m, 1H, CH_2), 5.60 (s, 1H, CH), 7.10-7.15 (m, 2H, ArH), 7.18-7.24 (m, 3H, ArH), 7.30-7.36 (m, 2H, ArH), 7.52-7.60 (m, 2H, ArH + NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 22.4 (CH_2), 42.4 (CH_2), 50.6 (CH), 110.9 (ArCH), 111.0 (ArC), 118.3 (ArCH), 119.5 (ArCH), 120.7 (q, $J = 259$ Hz, OCF_3), 120.8 (ArCH), 121.9 (ArCH), 127.2 (ArCH), 127.2 (ArC), 129.3 (ArCH), 130.5 (ArCH), 133.1 (ArC), 134.4 (ArC), 135.9 (ArC), 147.4 (ArC). E.I.M.S. m/e 333 (90%), 304 (100%), 266 (10%). Found: MH^+ 333.121329. Formula requires 333.121473. Found: C, 64.86%, H, 4.49%, N, 8.22%. $C_{18}H_{15}N_2F_3O$ requires C, 65.06%, H, 4.55%, N, 8.43%.

6-Methoxy-1-(2-trifluoromethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline

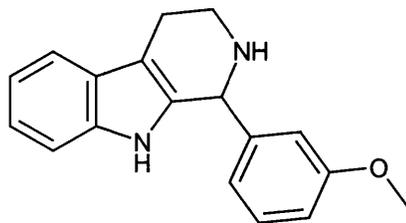
(157)



5-Methoxytryptamine (0.76 g, 4.0 mmol) and 2-trifluoromethoxybenzaldehyde (0.76 g, 4.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 0.6 g, 1.66 mmole, 41%, m.p. 123-124 °C.

I.R. ν_{\max} cm^{-1} . 3152, 2947, 2841, 1489, 1454, 1433, 1293, 1256, 1214, 1177, 1137, 1089, 1041, 929, 874, 865, 807, 767. ^1H NMR (400 MHz; CDCl_3) δ_{H} 1.95, (br.s, 1H, NH), 2.74-2.92 (m, 2H, CH_2), 3.10-3.18 (m, 1H, CH_2), 3.24-3.32 (m, 1H, CH_2), 3.87 (s, 3H, OCH_3), 5.59 (s, 1H, CH), 6.79 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.99 (d, $J = 1.8$ Hz, 1H, ArH), 7.09 (d, $J = 8.8$ Hz, 1H, ArH), 7.19 (m, 2H, ArH), 7.32 (m, 2H, ArH), 7.49 (br.s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 22.4 (CH_2), 42.4 (CH_2), 50.7 (CH), 56.0 (CH_3), 110.5 (ArCH), 110.8 (ArC), 111.5 (ArCH), 111.7 (ArCH), 120.6 (q, $J = 259$ Hz, CF_3), 120.7 (ArCH), 127.2 (ArCH), 127.6 (ArC), 129.3 (ArCH), 130.5 (ArCH), 131.0 (ArC), 134.0 (ArC), 134.4 (ArC), 147.4 (ArC), 154.1 (ArC). E.I.M.S. m/e 363 (100%), 334 (90%). Found: C, 62.80%, H, 4.66%, N, 7.65%. $\text{C}_{19}\text{H}_{17}\text{N}_2\text{F}_3\text{O}_2$ requires C, 62.98%, H, 4.73%, N, 7.73%.

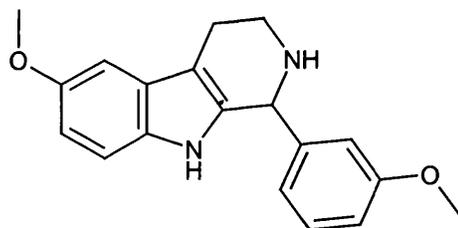
6-Methoxy-1-(3-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline (158)⁸



Tryptamine (0.80 g, 5.0 mmol) and 3-methoxybenzaldehyde (0.68 g, 5.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate 0.87 g, 3.13 mmole, 63%, m.p. 156-157 °C. (Lit. 140-141 °C)

I.R. ν_{\max} cm^{-1} . 3299, 3050, 2893, 2841, 1596, 1492, 1454, 1427, 1296, 1271, 1252, 1040, 856, 794, 747. ^1H NMR (400 MHz; CDCl_3) δ_{H} 1.88 (br.s, 1H, NH), 2.76-2.96 (m, 2H, CH_2), 3.10-3.17 (m, 1H, CH_2), 3.34-3.41 (m, 1H, CH_2), 3.76 (s, 3H, CH_3), 5.12 (s, 1H, CH), 6.85-6.90 (m, 3H, ArH), 7.09-7.15 (m, 2H, ArH), 7.18-7.22 (m, 1H, ArH), 7.23-7.29 (m, 1H, ArH), 7.53 (dd, $J = 7.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.62 (br.s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 22.5 (CH_2), 43.0 (CH_2), 55.3 (CH_3), 58.6 (CH), 108.0 (ArC), 110.1 (ArCH), 113.8 (ArCH x2), 118.2 (ArCH) 119.4 (ArCH), 120.7 (ArCH), 121.7 (ArCH), 127.4 (ArC), 129.8 (ArCH), 134.4 (ArC), 135.9 (ArC), 143.4 (ArC), 160.0 (ArC). E.I.M.S. m/e 279 (70%), 250 (100%). Found: MH^+ 279.149586 Formula requires 279.149738.

6-Methoxy-1-(3-methoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (159)

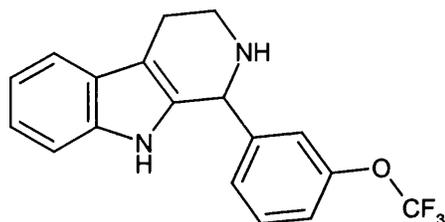


5-Methoxytryptamine (0.76 g, 4.0 mmol) and 3-methoxybenzaldehyde (0.54 g, 4.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate 0.68 g, 2.21 mmole, 55%, m.p. 136-137 °C.

I.R. ν_{\max} cm^{-1} . 3304, 2953, 2832, 1594, 1486, 1463, 1453, 1429, 1272, 1238, 1210, 1166, 1039, 856, 792, 704. ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.19 (br.s, 1H, NH), 2.72-2.92 (m, 2H, CH_2), 2.97-3.15 (m, 1H, CH_2), 3.33-3.40 (m, 1H, CH_2), 3.73 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 5.09 (s, 1H, CH), 6.78 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.83-6.90 (m, 3H, ArH), 6.99 (d, $J = 2.2$ Hz, 1H, ArH), 7.07 (d,

$J = 8.8$ Hz, 1H, ArH), 7.22-7.27 (m, 1H, ArH), 7.58 (s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 22.5 (CH_2), 43.0 (CH_2), 55.3 (CH_3), 56.0 (CH_3), 58.2 (CH), 100.5 (ArCH), 109.9 (ArC), 111.5 (ArCH x2), 113.7 (ArCH), 113.8 (ArCH), 120.7 (ArCH), 127.8 (ArC), 129.8 (ArCH), 131.0 (ArC), 135.3 (ArC), 143.3 (ArC), 154.0 (ArC), 160.0 (ArC). E.I.M.S. m/e 309 (100%), 280 (100%), 266 (10%). Found: C, 73.88%, H, 6.44%, N, 8.96%. $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_2$ requires C, 74.00%, H, 6.54%, N, 9.08%.

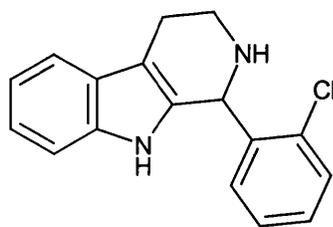
1-(3-Trifluoromethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (160)



Tryptamine (0.80 g, 5.0 mmol) and 3-trifluoromethoxybenzaldehyde (0.95 g, 5.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 0.67 g, 2.02 mmole, 40%, m.p. 147-148 °C.

I.R. ν_{max} cm^{-1} . 3037, 2935, 2871, 1587, 1449, 1247, 1214, 1177, 806, 777, 747, 704. ^1H NMR (400 MHz; CDCl_3) δ_{H} 1.85 (br.s, 1H, NH), 2.79-2.98 (m, 2H, CH_2), 3.09-3.17 (m, 1H, CH_2), 3.30-3.38 (m, 1H, CH_2), 5.17 (s, 1H, CH), 7.10-7.27 (m, 6H, ArH), 7.34-7.39 (m, 1H, ArH), 7.53-7.58 (m, 2H, ArH + NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 22.8 (CH_2), 43.0 (CH_2), 57.9 (CH), 111.0 (ArC), 111.3 (ArCH), 118.8 (ArCH), 120.0 (ArCH), 120.8 (q, $J = 259$ Hz, OCF_3), 120.9 (ArCH), 121.5 (ArCH), 122.4 (ArCH), 127.2 (ArCH), 127.7 (ArC), 130.6 (ArCH), 133.8 (ArC), 136.3 (ArC), 144.7 (ArC), 150.0 (ArC). E.I.M.S. m/e 333 (100%), 304 (100%), Found: MH^+ 333.121403. Formula requires 333.121473.

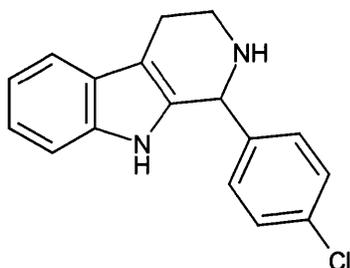
1-(2-Chlorophenyl)-2,3,4,9-tetrahydro-1H- β -carboline (161)⁷



Tryptamine (0.80 g, 5.0 mmol) and 2-chloromethoxybenzaldehyde (0.70 g, 5.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 0.61 g, 2.16 mmole, 43%, m.p. 130-131 °C. (Lit. reported as hydrochloride).

I.R. ν_{\max} cm^{-1} . 3394, 3056, 2913, 2841, 1699, 1467, 1449, 1298, 1267, 1242, 1048, 1035, 742. ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.64-2.78 (m, 3H, CH_2 +NH), 2.90-2.98 (m, 2H, CH_2), 5.51 (s, 1H, CH), 6.90-7.03 (m, 3H, ArH), 7.17-7.31 (m, 3H, ArH), 7.44 (d, $J = 7.6$ Hz, 1H, ArH), 7.50 (d, $J = 7.8$ Hz, 1H, ArH), 10.55 (s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 21.9 (CH_2), 40.6 (CH_2), 52.4 (CH), 108.9 (ArC), 110.8 (ArCH), 117.3 (ArCH), 117.9 (ArCH), 120.4 (ArCH), 126.4 (ArCH), 126.4 (ArC), 128.6 (ArCH), 129.1 (ArCH), 129.9 (ArCH), 133.0 (ArC), 133.6 (ArC), 135.7 (ArC), 139.7 (ArC). E.I.M.S. m/e 283 (100%), 285 (50%), 254 (35%).

1-(4-Chloromethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (77)⁷

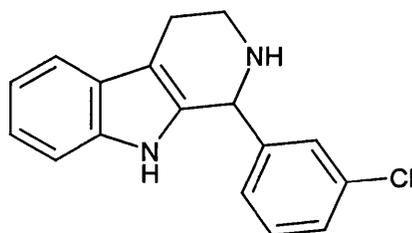


Tryptamine (0.80 g, 5.0 mmol) and 4-chloromethoxybenzaldehyde (0.70 g, 5.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl

acetate, 1.01 g, 3.58 mmole, 72%, m.p. 170-171 °C. (Lit. reported as hydrochloride)

I.R. ν_{\max} cm^{-1} . 3292, 2910, 2841, 1485, 1452, 1437, 1408, 1306, 1101, 1065, 1005, 850, 839, 813, 797, 747. ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.60-2.78 (m, 2H, CH_2), 2.88-3.05 (m, 3H, $\text{CH}_2 + \text{NH}$), 5.09 (s, 1H, CH), 6.91-7.02 (m, 2H, ArH), 7.22 (d, $J = 7.8$ Hz, 1H, ArH), 7.25-7.42 (m, 5H, ArH), 10.50 (s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 21.8 (CH_2), 40.0 (CH_2), 54.7 (CH), 107.3 (ArC), 109.9 (ArCH), 116.5 (ArCH), 117.1 (ArCH), 119.5 (ArCH), 125.7 (ArC), 126.9 (ArCH x 2), 129.2 (ArCH x 2), 130.6 (ArC), 133.8 (ArC), 134.8 (ArC), 141.1 (ArC). E.I.M.S. m/e 283 (100%), 285 (50%), 254 (35%).

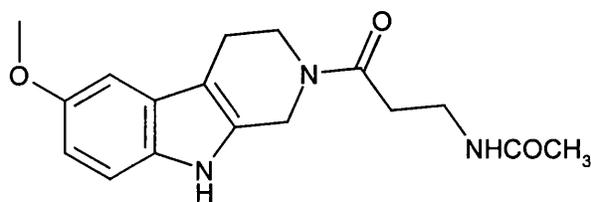
1-(3-Chloromethoxyphenyl)-2,3,4,9-tetrahydro-1H- β -carboline (163)



Tryptamine (0.80 g, 5.0 mmol) and 3-chloromethoxybenzaldehyde (0.70 g, 5.0 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 0.93 g, 3.30 mmole, 66 %, m.p. 166-167 °C.

I.R. ν_{\max} cm^{-1} . 3158, 2895, 2848, 1597, 1474, 1454, 1445, 1342, 1296, 1270, 1098, 873, 864, 795, 749, 688. ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.63-2.78 (m, 2H, CH_2), 2.85-3.08 (m, 3H, $\text{CH}_2 + \text{NH}$), 5.10 (s, 1H, CH), 6.93-7.05 (m, 2H, ArH), 7.22-7.36 (m, 5H, ArH), 7.43 (d, $J = 7.6$ Hz, 1H, ArH), 10.50 (s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 22.5 (CH_2), 41.5 (CH_2), 56.4 (CH), 108.9 (ArC), 111.5 (ArCH), 118.0 (ArCH), 118.7 (ArCH), 121.0 (ArCH), 127.2 (ArC), 127.5 (ArCH x 2), 128.6 (ArCH), 130.3 (ArCH), 133.2 (ArC), 135.1 (ArC), 136.4 (ArC), 146.2 (ArC). E.I.M.S. m/e 283 (100%), 285 (50%), 254 (35%).

1-Phenyl-2-(N-acetyl-β-alanyl)-2,3,4,9-tetrahydro-1H-β-carboline (218)

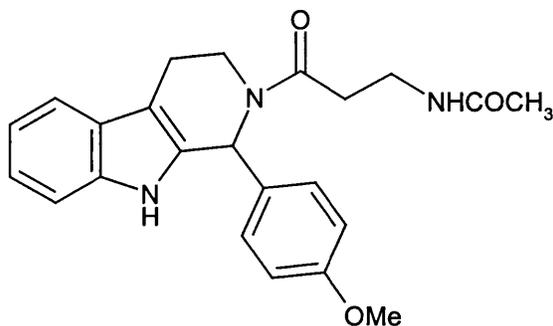


N-acetyl-β-alanine (66 mg, 0.5 mmol) was dissolved in anhydrous DMF (5 mL) and *N,N*-diisopropylethylamine (101 mg, 1 mmol) was added, followed by HATU (115 mg, 0.5 mmol). The reaction mixture was allowed to stand for 5 minutes and then DJD35 (153 mg, 0.5 mmol) was added. The solution was stood at room temp for 18 h and then evaporated *in vacuo*. The residue was dissolved in ethyl acetate (30 mL) and washed with 5% *aq.* citric acid (2 x 15 mL), followed by saturated *aq.* sodium bicarbonate (3 x 15 mL) and finally brine (15 mL). The organic layer was dried over magnesium sulphate, filtered and the filtrate evaporated *in vacuo*. The crude product was subjected to column chromatography and eluted with ethyl acetate. The pure fractions were combined and evaporated *in vacuo*. Trituration with ether gave a pale yellow solid which was recrystallised from ethyl acetate, 105 mg, 0.33 mmol, 67% yield, m.p. 125-127 °C.

I.R. ν_{\max} cm^{-1} 3328, 2902, 1659, 1650, 1632, 1537, 1486, 1454, 1326, 1293, 1223, 1041, 821, 729, 601; ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} . 1.78 (s, 3H, CH_3), 2.57-2.77 (m, 4H, CH_2), 2.79-2.85 (m, 2H, CH_2), 3.25-3.33 (m, 3H, CH_2), 3.73 (s, 3H, CH_3), 3.92-4.01 (m, 1H, CH_2), 6.68 (dd, $J = 7.8$ Hz, $J = 1.6$ Hz, 1H, ArH), 6.90 (d, $J = 1.6$ Hz, 1H, ArH), 7.19 (d, $J = 7.8$ Hz, 1H, ArH), 7.90 (t, $J = 5.4$ Hz, 1H, NH), 10.65 (s, 1H, NH); ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.1 (CH_2), 22.0 (CH_3), 33.0 (CH_2), 35.5 (CH_2), 40.2 (CH_2), 43.6 (CH_2), 55.7 (CH_3), 100.2 (ArCH), 106.9 (ArC), 110.7 (ArCH), 111.9 (ArCH), 127.0 (ArC), 131.6 (ArC), 132.8 (ArC), 153.0 (ArC), 169.4 (C=O), 170.0 (C=O); E.I.M.S. m/e 316 (MH^+ , 60%), 203 (100%), 186 (40%), 160 (25%). Found: C, 63.98%, H, 6.66%, N, 13.29%. $\text{C}_{17}\text{H}_{21}\text{N}_3\text{O}_3$ requires C, 64.74%, H, 6.71%, N, 13.32%.

The following compounds were prepared in a similar manner:

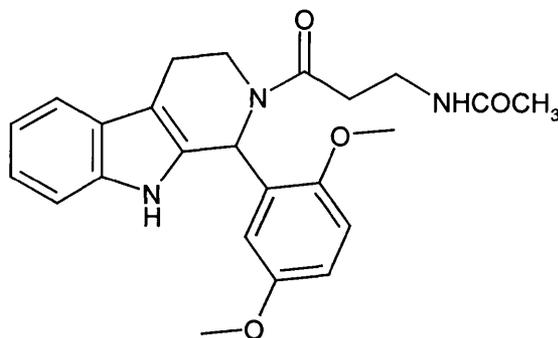
N-{3-[1-(4-methoxy-phenyl)-1,3,4,9-tetrahydro-beta-carbolin-2-yl]-3-oxo-propyl}-acetamide (174)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **142** (139 mg, 0.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 96 mg, 0.24 mmol, 49% yield, m.p. 156-157 °C.

I.R. ν_{\max} cm^{-1} . 3280, 1647, 1614, 1537, 1510, 1454, 1366, 1301, 1247, 1175, 1033, 837, 744. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} . 1.77 (s, 3H, CH_3), 2.55-2.64 (m, 2H, CH_2), 2.78-2.85 (m, 2H, CH_2), 3.15-3.35 (m, 3H, CH_2), 3.80-3.90 (m, 1H, CH_2), 3.72 (s, 3H, OCH_3), 6.81 (s, 1H, CH). 7.00 (q, $J = 83$ Hz, $J = 8.0$ Hz, 4H, ArH), 7.01 (t, $J = 7.8$ Hz, 1H, ArH), 7.12 (d, $J = 7.8$ Hz, 1H, ArH), 7.29 (d, $J = 7.8$ Hz, 1H, ArH), 7.47 (d, $J = 7.8$ Hz, 1H, ArH), 7.90 (br.t, $J = 5.6$ Hz, 1H, AcNH), 10.95 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.4 (CH_2), 23.4 (CH_3), 33.5 (CH_2), 36.0 (CH_2), 39.9 (CH_2), 51.4 (CH_3), 56.0 (CH), 109.3 (ArCH), 112.0 (ArC), 114.6 (ArCH x2), 118.7 (ArCH), 119.4 (ArCH), 121.5 (ArCH), 127.0 (ArC), 130.1 (ArCH x2), 133.0 (ArC), 133.2 (ArC), 136.9 (ArC), 159.5 (ArC), 169.5 (C=O), 170.0 (C=O). E.I.M.S. m/e 392 (MH^+ , 90%), 279 (80%), 262 (80%), 236 (60%), 783 (2MH^+ , 20%). Found: C, 70.44%, H, 6.41%, N, 10.72%. $\text{C}_{23}\text{H}_{25}\text{N}_3\text{O}_3$ requires C, 70.50%, H, 6.44%, N, 10.73%.

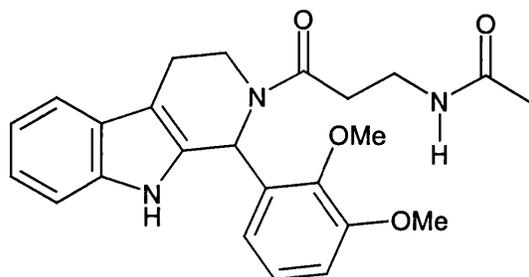
N-{3-[1-(2,5-dimethoxy-phenyl)-1,3,4,9-tetrahydro-beta-carbolin-2-yl]-3-oxo-propyl}-acetamide (175)



N-Acetyl β -alanine (66 mg, 0.5 mmol) and **143** (154 mg, 0.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate 93 mg, 0.22 mmol, 44 % yield, m.p. 118-120 °C.

I.R. ν_{\max} cm^{-1} 3328, 1655, 1638, 1472, 1466, 1459, 1303, 1276, 1247, 1231, 1064, 1004, 747. I.R. ν_{\max} cm^{-1} . ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} . 1.69 + 1.77 (2 x s - rotamers, 3H, CH_3), 2.54-2.90 (m, 4.5H, CH_2), 3.25-3.40 (m, 3H, CH_2), 3.81 (s, 3H, CH_3), 3.86 (2 x s - rotamers, 3H, CH_3), 3.96-4.04 (m, 0.5H, CH_2), 6.38 (m, 1H, CH), 6.90-7.09 (m, 5H, ArH), 7.28 (d, $J = 7.8$ Hz, 1H, ArH), 7.45 (d, $J = 7.8$ Hz, 1H, ArH), 7.89 (br.t, $J = 5.0$ Hz, 1H, AcNH), 10.75 (d, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) single rotamer reported δ_{C} 21.9 (CH_2), 22.7 (CH_3), 33.0 (CH_2), 35.3 (CH_2), 39.8 (CH_2), 51.9 (CH), 56.1 (CH_3), 60.1 (CH_3), 108.5 (ArC), 111.5 (ArCH), 113.1 (ArCH), 116.7 (ArCH), 118.6 (ArCH), 121.6 (ArCH x2), 124.0 (ArCH), 127.2 (ArC), 132.9 (ArC), 134.0 (ArC), 136.2 (ArC), 148.2 (ArC), 153.1 (ArC), 169.5 (C=O), 170.0 (C=O). E.I.M.S. m/e 422 (MH^+ , 100%), 309 (85%), 292 (90%), 266 (40%), 151 (10%), 843 (10%), Found: C, 68.25%, H, 6.39%, N, 9.89%. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4$ requires C, 68.39%, H, 6.46%, N, 9.97%.

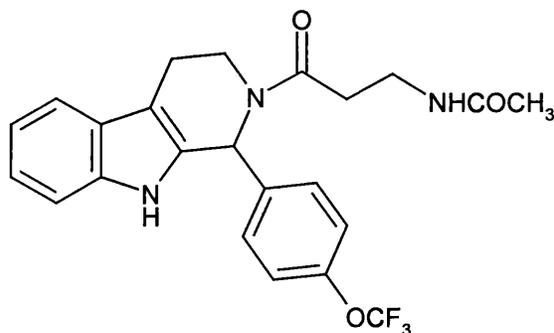
N-{3-[1-(2,3-dimethoxy-phenyl)-1,3,4,9-tetrahydro-beta-carbolin-2-yl]-3-oxo-propyl}-acetamide (176)



N-Acetyl β -alanine (66 mg, 0.5 mmol) and **144** (154 mg, 0.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 90 mg, 0.21 mmol, 58% yield, m.p. 203-204 °C.

I.R. ν_{\max} cm^{-1} . 3341, 3192, 2944, 1740, 1658, 1649 1627, 1503, 1470, 1453, 1426, 1275, 1235, 1052, 793, 742, 607. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.69 + 1.78 (2 x s - rotamers, 3H, CH_3), 2.54-2.90 (m, 4H, CH_2), 2.95-3.04 (m, 0.5H, CH_2), 3.25-3.33 (m, 2.5H, CH_2), 3.56 + 3.58 (2 x s - rotamers, 3H, CH_3), 3.78 + 3.86 (2 x s - rotamers, 3H, CH_3), 3.98-4.05 (m, 0.5H, CH_2), 4.49-4.56 (m, 0.5H, CH_2), 6.34 (s, 1H, CH), 6.83-6.92 (m, 1H, ArH), 6.96-7.10 (m, 4H, ArH), 7.30 (d, $J = 7.8$ Hz, 1H, ArH), 7.47 (t, $J = 7.6$ Hz, 1H, ArH), 7.89 (br.s., 1H, NH), 10.65 (d, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) single rotamer reported δ_{C} 20.7 (CH_2), 22.1 (CH_3), 33.0 (CH_2), 35.6 (CH_2), 40.2 (CH_2), 51.0 (CH), 55.7 (CH_3), 56.3 (CH_3), 109.0 (ArC), 111.6 (ArCH), 112.8 (ArCH), 116.7 (ArCH), 117.3 (ArCH), 118.2 (ArCH), 118.9 (ArCH), 121.7 (ArCH), 126.5 (ArC), 128.9 (ArC), 132.1 (ArC), 136.6 (ArC), 151.5 (ArC), 153.3 (ArC), 170.0 (C=O), 170.8 (C=O). E.I.M.S. m/e 422 (MH^+ , 100%), 309 (100%), 292 (70%), 266 (50%). Found: C, 68.27%, H, 6.40%, N, 9.94%. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4$ requires C, 68.39%, H, 6.46%, N, 9.97%.

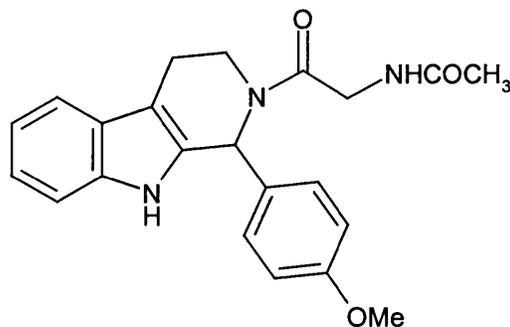
N-{3-[1-(4-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro-beta-carbolin-2-yl]-3-oxo-propyl}-acetamide (177)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **145** (166 mg, 0.5 mmol) gave the title compound as a pale yellow solid on recrystallisation from ethyl acetate, 71 mg, 0.16 mmol, 32% yield, m.p. 115-116 °C.

I.R. ν_{\max} cm^{-1} . 3284, 1647, 1632, 1504, 1448, 1258, 1220, 1162, 743. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} . 1.78 (s, 3H, CH_3), 2.56-2.70 (m, 2H, CH_2), 2.75-2.90 (m, 2H, CH_2), 3.14-3.24 (m, 1H, CH_2) 3.25-3.38 (m, 2H, CH_2), 3.98-4.05 (m, 1H, CH_2), 6.88 (s, 1H, CH), 6.99-7.12 (m, 2H, ArH), 7.29-7.39 (m, 5H, ArH), 7.48 (d, $J = 7.8$ Hz, 1H, ArH), 7.90 (t, $J = 5.6$ Hz, 1H, NH) 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 23.2 (CH_2), 24.2 (CH_3), 34.3 (CH_2), 36.8 (CH_2), 39.6 (CH_2), 52.1 (CH), 110.2 (ArC), 112.9 (ArCH), 119.7 (ArCH), 120.4 (ArCH), 122.8 (ArCH x2), 123.1 (ArCH), 127.9 (ArC), 131.7 (ArCH x2), 133.1 (ArC), 137.9 (ArC), 141.5 (ArC), 149.7 (ArC), 171.4 (C=O), 172.0 (C=O). E.I.M.S. m/e 446 (MH^+ , 100%), 333 (75%), 290 (50%), Found: C, 63.62%, H, 6.22%, N, 13.88%. $\text{C}_{23}\text{H}_{22}\text{N}_3\text{F}_3\text{O}_3$ requires C, 63.78%, H, 6.31%, N, 13.95%.

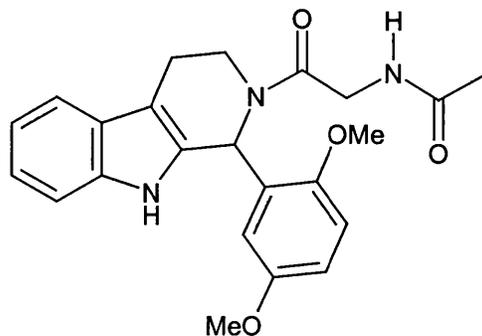
N-{3-[1-(4-methoxy-phenyl)-1,3,4,9-tetrahydro-beta-carbolin-2-yl]-3-oxo-ethyl}-acetamide (178)



N-Acetyl glycine (59 mg, 0.5 mmol) and **142** (139 mg, 0.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 88 mg, 0.23 mmol, 47% yield, m.p. 134-136 °C.

I.R. ν_{\max} cm^{-1} . 3285, 2932, 1647, 1637, 1510, 1454, 1247, 1175, 1031, 744. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.89 (s, 3H, CH_3), 2.72-2.92 (m, 2H, CH_2), 3.19-3.28 (m, 1H, CH_2), 3.72 (s, 3H, OCH_3), 3.92-4.00 (m, 1H, CH_2), 4.07 (d, $J = 5.8$ Hz, 2H, CH_2), 6.76 (s, 1H, CH), 7.02 (q, $J = 83$ Hz, $J = 8.0$ Hz, 4H, ArH), 7.01 (t, $J = 7.6$ Hz, 1H, ArH), 7.08 (t, $J = 7.6$ Hz, 1H, ArH), 7.29 (d, $J = 8.0$ Hz, 1H, ArH), 7.48 (d, $J = 8$ Hz, 1H, ArH), 8.09 (t, $J = 5.6$ Hz, 1H, NH), 10.95 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.9 (CH_2), 22.8 (CH_3), 38.6 (CH_2), 41.0 (CH_2), 51.4 (CH), 55.5 (CH_3), 108.5 (ArC), 111.5 (ArCH), 114.1 (ArCH x2), 118.3 (ArCH), 119.0 (ArCH), 121.6 (ArCH), 126.5 (ArC), 129.7 (ArCH x2), 132.4 (ArC), 132.7 (ArC), 136.5 (ArC), 159.2 (ArC), 167.8 (C=O), 169.8 (C=O). E.I.M.S. m/e 378 (MH^+ , 100%), 279 (50%), 262 (60%), 236 (40%), Found: C, 69.91%, H, 6.08%, N, 11.10%. $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_3$ requires C, 70.01%, H, 6.14%, N, 11.13%,

N-{3-[1-(2,5-dimethoxy-phenyl)-1,3,4,9-tetrahydro-beta-carbolin-2-yl]-3-oxo-ethyl}-acetamide (179)

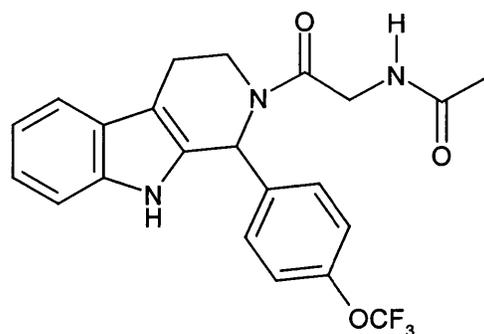


N-Acetyl glycine (59 mg, 0.5 mmol) and **143** (154 mg, 0.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 55 mg 0.13 mmol, 27% yield, m.p. 137-139 °C.

I.R. ν_{\max} cm^{-1} 3310, 1681, 1647, 1631, 1480, 1454, 1271, 1068, 1000, 844, 745.

^1H NMR (400 MHz; d_6 -DMSO) δ_{H} . 1.85 (d, 3H, CH_3), 2.70-2.95 (m, 2H, CH_2), 3.25-3.33 (m, 1H, CH_2), 3.57 + 3.59 (2 x s - rotamers, 3H, CH_3), 3.75 + 3.89 (2 x s - rotamers, 3H, CH_3), 4.45-4.65 (m, 1H, CH_2), 6.32 (s, 1H, CH), 6.80-6.95 (m, 1H, ArH), 6.97-7.15 (m, 4H, ArH), 7.22-7.30 (m, 1H, ArH), 7.40-7.47 (m, 1H, ArH), 8.00 + 8.04 (2 x t - rotamers, $J = 5.0$ Hz, 1H, NH), 10.65 + 10.80 (2 x s - rotamers, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} major rotamer reported 20.6 (CH_2), 21.5 (CH_3), 34.6 (CH_2), 37.2 (CH_2), 38.9 (CH_2), 45.5 (CH_3), 54.0 (CH), 58.8 (CH_3), 99.3 (ArC), 109.5 (ArCH), 110.8 (ArCH), 115.9 (ArCH), 116.8 (ArCH), 119.3 (ArCH x2), 121.7 (ArCH), 124.3 (ArC), 131.0 (ArC), 131.9 (ArC), 134.3 (ArC), 151.0 (ArC), 151.7 (ArC), 165.8 (C=O), 167.6 (C=O). E.I.M.S. m/e 408 (MH^+ , 100%), 309 (40%), 292 (50%), 266 (20%), 151 (10%), 815 (20%), Found: C, 67.61%, H, 6.12%, N, 10.27%. $\text{C}_{23}\text{H}_{25}\text{N}_3\text{O}_4$ requires C, 67.80%, H, 6.18%, N, 10.31%.

N-{3-[1-(4-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro-beta-carbolin-2-yl]-3-oxo-ethyl}-acetamide (180)

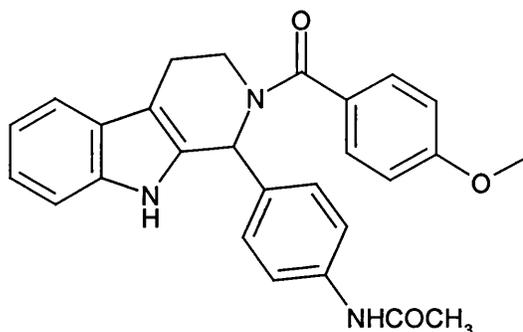


N-Acetyl glycine (59 mg, 0.5 mmol) and **145** (166 mg, 0.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 147 mg, 0.34 mmol, 68% yield, m.p. 130-132 °C.

I.R. ν_{\max} cm^{-1} . 3290, 1659, 1643, 1510, 1448, 1256, 1221, 1163, 853, 744. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.89 (s, 3H, CH_3), 2.80-2.95 (m, 2H, CH_2), 3.17-3.27 (m, 1H, CH_2), 3.98-4.05 (m, 1H, CH_2), 4.10 (t, $J = 1.5$ Hz, 2H, CH_2), 6.81 (s, 1H, CH), 7.00-7.12 (m, 2H, ArH), 7.30-7.40 (m, 5H, ArH), 7.49 (d, $J = 7.8$ Hz, 1H, ArH), 8.11 (t, $J = 5.6$ Hz, 1H, NH), 11.0 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.4 (CH_2), 22.5 (CH_3), 38.7 (CH_2), 40.7 (CH_2), 51.8 (CH), 108.6 (ArC), 111.3 (ArCH), 118.1 (ArCH), 118.8 (ArCH), 121.2 (ArCH x2), 121.5 (ArCH), 126.1 (ArC), 130.1 (ArCH x2), 131.4 (ArC), 136.2 (ArC), 139.7 (ArC), 148.0 (ArC), 167.9 (C=O), 169.7 (C=O). E.I.M.S. m/e 432 (MH^+ , 100%), 333 (50%), 316 (20%), Found: C, 61.23%, H, 4.60%, N, 9.76%. $\text{C}_{22}\text{H}_{20}\text{N}_3\text{F}_3\text{O}_3$ requires C, 61.25%, H, 4.67%, N, 9.74%.

N-{4-[2-(4-methoxybenzoyl)-2,3,4,9-tetrahydro-1*H*- β -carbolin-1-yl]phenyl}

acetamide (224)



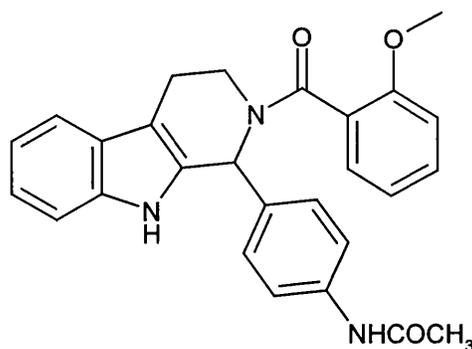
4-Methoxybenzoic acid (148 mg, 0.5 mmol) and **146** (139 mg, 0.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 127 mg, 0.29 mmol, 58%, m.p. 228-229 °C.

I.R. ν_{\max} cm^{-1} . 1680, 1604, 1541, 1509, 1428, 1313, 1243, 1182, 1032, 839, 755.

^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.03 (s, 3H, CH_3), 2.73-2.93 (m, 2H, CH_2), 3.25-3.40 (m, 2H, CH_2), 3.79 (s, 3H, CH_3), 6.88 (s, 1H, CH), 7.01 (m, 3H, ArH), 7.10 (t, $J = 8$ Hz, 1H, ArH), 7.22-7.42 (m, 5H, ArH), 7.48 (d, $J = 8$ Hz, 1H, ArH), 7.58 (d, $J = 8$ Hz, 2H, ArH), 10.0 (s, 1H, AcNH), 11.0 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.6 (CH_2), 23.7 (CH_3), 41.0 (CH_2), 51.1 (CH_3), 55.0 (CH), 107.6 (ArC), 111.0 (ArCH), 113.5 (ArCH x2), 117.7 (ArCH), 118.4 (ArCH), 118.7(ArCH x2), 121.0 (ArCH), 126.0 (ArC), 128.0 (ArC), 128.2 (ArCH x2), 131.6 (ArC), 134.5 (ArC), 136.0 (ArC), 138.5 (ArC), 138.6 (ArC), 160.0 (ArC), 168.0 (C=O), 169.5 (C=O). E.I.M.S. m/e 440 (MH^+ , 100%), 879 (2MH^+ , 10%). Found: MH^+ 440.198565. Formula requires 440.197417. Found: C, 72.63%, H, 5.69%, N, 9.42%. $\text{C}_{27}\text{H}_{25}\text{N}_3\text{O}_3$ requires C, 73.79%, H, 5.73%, N, 9.56%.

N-{4-[2-(2-methoxybenzoyl)-2,3,4,9-tetrahydro-1*H*- β -carbolin-yl-phenyl]}

acetamide (222)

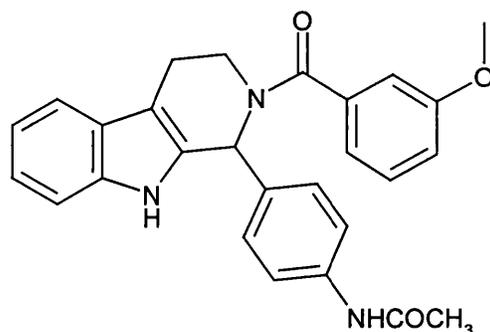


2-Methoxybenzoic acid (148 mg, 0.5 mmol) and **146** (139 mg, 0.5 mmol) gave the title compound as a white solid on recrystallisation from ethyl acetate, 188 mg, 0.43 mmol, 86% yield, m.p. 266-267 °C.

I.R. ν_{\max} cm^{-1} . 3298, 1680, 1600, 1536, 1531, 1514, 1469, 1454, 1434, 1321, 1246, 1026, 738. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.03 (s, 3H, CH_3), 2.61-2.82 (m, 2H, CH_2), 3.15-3.25 (m, 1H, CH_2), 3.35-3.45 (m, 1H, CH_2), 3.71 + 3.77 (2 x s - rotameric, 3H, CH_3), 6.91 (s, 1H, CH), 6.96-7.12 (m, 5H, ArH), 7.20-7.28 (m, 2H, ArH), 7.33 (br.t. $J = 7$ Hz, 1H, ArH), 7.39-7.52 (m, 2H, ArH), 7.60 (d, $J = 8$ Hz, 2H, ArH), 10.05 (s, 1H, AcNH), 11.05 + 11.15 (2 x s - rotameric, 1H, NH); ^{13}C NMR (100 MHz; d_6 -DMSO) major rotamer reported δ_{C} 22.0 (CH_2), 24.3 (CH_3), 40.9 (CH_2), 51.0 (CH_3), 55.6 (CH), 108.3 (ArC), 111.6 (ArCH), 111.7 (ArCH), 118.3 (ArCH), 119.0 (ArCH), 119.2 (ArCH x2), 119.4 (ArCH), 121.1 (ArCH), 121.6 (ArCH), 126.2 (ArC), 128.4 (ArCH), 128.6 (ArCH), 130.6 (ArCH), 132.1 (ArC), 133.1 (ArC), 134.6 (ArC), 136.4 (ArC), 139.3 (ArC), 155.3 (ArC), 168.5 (C=O), 169.0 (C=O). E.I.M.S. m/e 440 (MH^+ , 100%), 879 (2MH^+ , 15%). Found: MH^+ 440.198819. Formula requires 440.197417. Found: C, 72.65%, H, 5.75%, N, 9.57%. $\text{C}_{27}\text{H}_{25}\text{N}_3\text{O}_3$ requires C, 73.79%, H, 5.73%, N, 9.56%

N-{4-[2-(3-methoxybenzoyl)-2,3,4,9-tetrahydro-1*H*- β -carbolin-1-yl]phenyl}

acetamide (223)

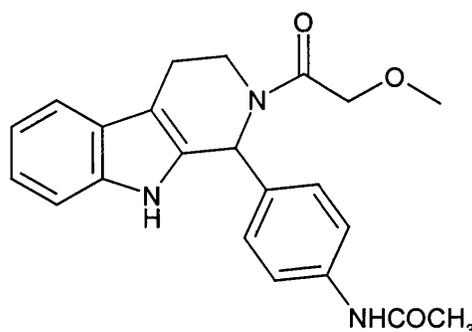


3-Methoxybenzoic acid (148 mg, 0.5 mmol) and **146** (139 mg, 0.5 mmol) gave the title compound as a cream coloured solid, on recrystallisation from ethyl acetate, 145 mg, 0.33 mmol, 66% yield, m.p. 199-200 °C.

I.R. ν_{\max} cm^{-1} . 3284, 1681, 1614, 1604, 1579, 1546, 1511, 1465, 1451 1432, 1416, 1323, 1041, 846, 743. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.01 (s, 3H, CH_3), 2.70-2.90 (m, 2H, CH_2), 3.24-3.33 (m, 1H, CH_2), 3.60-3.70 (m, 1H, CH_2), 3.79 (s, 3H, CH_3), 6.89-6.98 (m, 3H, CH + ArH), 7.00-7.13 (m, 3H, ArH), 7.23-7.40 (m, 4H, ArH), 7.48 (d, $J = 8$ Hz, 1H, ArH), 7.59 (d, $J = 8$ Hz, 2H, ArH), 10.05 (s, 1H, AcNH), 11.10 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.1 (CH_2), 24.3 (CH_3), 41.4 (CH_2), 51.6 (CH_3), 55.6 (CH), 108.3 (ArC) 111.6 (ArCH), 111.9 (ArCH), 115.7 (ArCH), 118.3 (ArCH), 118.6 (ArCH), 119.0 (ArCH), 119.4(ArCH x2), 121.7 (ArCH), 126.6 (ArC), 128.8 (ArCH x2), 130.2 (ArCH), 132.1 (ArC), 135.2 (ArC), 136.6 (ArC), 138.0 (ArC), 139.3 (ArC), 159.6 (ArC), 168.7 (C=O), 169.5, (C=O). E.I.M.S. m/e 440 (MH^+ , 100%), 879 (2MH^+ , 10%). Found: MH^+ 440.198874. Formula requires 440.197417. Found: C, 72.96%, H, 5.69%, N, 9.41%. $\text{C}_{27}\text{H}_{25}\text{N}_3\text{O}_3$ requires C, 73.79%, H, 5.73%, N, 9.56%.

N-{4-[2-(2-methoxyacetyl)-2,3,4,9-tetrahydro-1*H*- β -carbolin-1-yl]phenyl}

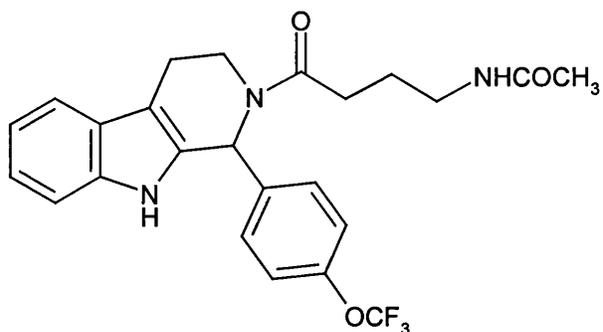
acetamide (221)



Methoxyacetic acid (68 mg, 0.75 mmol) and **146** (152 mg, 0.75 mmol) gave the title compound as a white solid, on recrystallisation from dichloromethane/hexane, 144 mg, 0.38 mmol, 51% yield, m.p. 224-225 °C.

I.R. ν_{\max} cm^{-1} . 3290, 1683, 1647, 1604, 1542, 1512, 1459, 1438, 1418, 1317, 1130, 752. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.02 (s, 3H, CH_3), 2.74-2.92 (m, 2H, CH_2), 3.15-3.25 (m, 1H, CH_2), 3.30 (s, 3H, CH_3), 3.87-3.94 (m, 1H, CH_2), 4.22 (s, 2H, CH_2), 6.75 (s, 1H, CH), 7.00 (t, $J = 8$ Hz, 1H, ArH), 7.08 (t, $J = 8$ Hz, 1H, ArH), 7.14 (d, $J = 8$ Hz, 2H, ArH), 7.30 (d, $J = 8$ Hz, 1H, ArH), 7.48 (d, $J = 8$ Hz, 1H, ArH), 7.53 (d, $J = 8$ Hz, 2H, ArH), 10.00 (s, 1H, AcNH), 10.95 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 24.3 (CH_3), 38.9 (CH_2), 51.2 (CH_3), 58.7 (CH), 71.3 (CH_2), 108.3 (ArC), 111.6 (ArCH), 118.2 (ArCH), 118.9 (ArCH), 119.3 (ArCH x2), 121.6 (ArCH), 126.6 (ArC), 128.8 (ArCH x2), 132.4 (ArC), 135.3 (ArC), 136.7 (ArC), 139.5 (ArC), 168.5 (C=O), 169.2 (C=O). E.I.M.S. m/e 378 (MH^+ , 100%), 755 (2MH^+ , 10%), 243 (10%). Found: MH^+ 378.182014. Formula requires 378.181767. Found: C, 69.46%, H, 6.12%, N, 10.96%. $\text{C}_{22}\text{H}_{23}\text{N}_3\text{O}_3$ requires C, 70.01%, H, 6.14%, N, 11.13%,

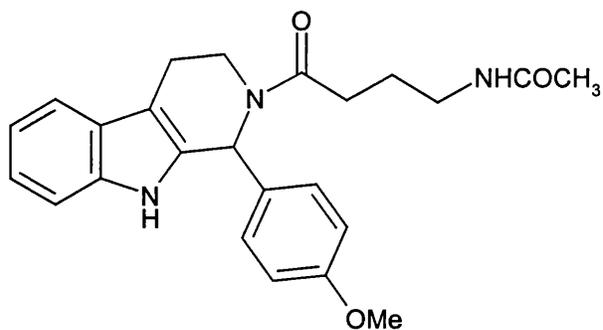
N-{3-[1-(4-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (181)



N-Acetyl butanoic acid (73 mg, 0.5 mmol) and **145** (166 mg, 0.5 mmol) gave the title compound as a pale yellow solid on recrystallisation from ethyl acetate-ether, 110 mg, 0.24 mmol, 48% yield, m.p. 111-113 °C.

I.R. ν_{\max} cm^{-1} . 3434, 2944, 1659, 1632, 1622, 1507, 1466, 1448, 1260, 1221, 1162, 846, 745, 558. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.20-1.30 (m, 2H, CH_2), 1.68 (d, $J = 9$ Hz, 2H, CH_2), 1.79 (s, 3H, CH_3), 2.80-2.95 (m, 2H, CH_2), 3.05-3.15 (q, $J = 6$ Hz, 2H, CH_2), 3.15-3.24 (m, 1H, CH_2), 4.00-4.08 (m, 1H, CH_2), 6.88 (s, 1H, CH), 7.01 (t, $J = 7.5$ Hz, 1H, ArH), 7.09 (t, $J = 7.5$ Hz, 1H, ArH) 7.29-7.40 (m, 5H, ArH), 7.49 (d, $J = 7.5$ Hz, 1H, ArH), 7.87 (t, $J = 5$ Hz, 1H, NH), 10.95 (s, 1H, NH). ^{13}C NMR (100 MHz; D_6 -DMSO) δ_{C} 21.9 (CH_2), 23.1 (CH_3), 25.2 (CH_2), 30.3 (CH_2), 38.5 (CH_2), 39.4 (CH_2), 50.7 (CH), 108.9 (ArC), 111.6 (ArCH), 118.3 (ArCH), 119.1 (ArCH), 121.4 (ArCH x2), 121.6 (ArCH), 126.5 (ArC), 130.3 (ArCH x2), 131.7 (ArC), 136.7 (ArC), 140.5 (ArC), 148.2 (ArC), 169.4 (C=O), 171.3 (C=O), OCF_3 not observed. E.I.M.S. m/e 919 (2MH^+ , 10%), 460 (MH^+ , 100%). Found: C, 62.61%, H, 5.15%, N, 9.10%. $\text{C}_{24}\text{H}_{24}\text{N}_3\text{F}_3\text{O}_3$ requires C, 62.74%, H, 5.27%, N, 9.15%.

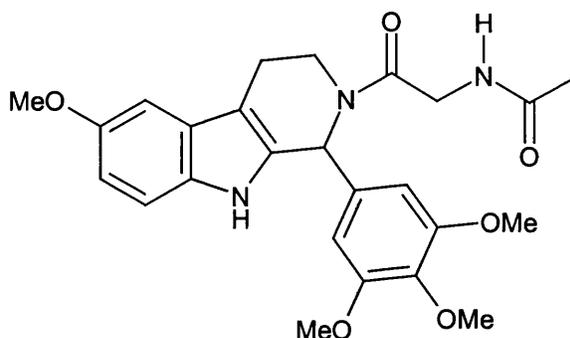
N-{3-[1-(4-methoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-butyl]-acetamide (182)}



N-Acetyl butanoic acid (73 mg, 0.5 mmol) and **142** (139 mg, 0.5 mmol) gave the title compound as a pale yellow solid on trituration with ether and recrystallisation from ethyl acetate, 52 mg, 0.13 mmol, 26% yield, m.p. 114-116 °C.

I.R. ν_{\max} cm^{-1} 3275, 2928, 1659, 1650, 1633, 1614, 1510, 1453, 1249, 1175, 1041, 844, 744. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.20-1.30 (m, 2H, CH_2), 1.60-1.70 (m, 2H, CH_2), 1.78 (s, 3H, CH_3), 2.70-2.90 (m, 2H, CH_2), 3.05 (q, $J = 6$ Hz, 2H, CH_2), 3.15-3.24 (m, 1H, CH_2), 3.72 (s, 3H, OCH_3), 3.95-4.02 (m, 1H, CH_2), 6.81 (s, 1H, CH), 6.99 (q, $J = 7.8$ Hz, $J = 8.0$ Hz, 4H, ArH), 7.01 (t, $J = 7.8$ Hz, 1H, ArH) 7.08 (t, $J = 7.8$ Hz 1H, ArH), 7.29 (d, $J = 7.8$ Hz, 1H, ArH), 7.46 (d, $J = 7.8$ Hz, 1H, ArH), 7.84 (t, $J = 5$ Hz, 1H, NH), 10.95 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 22.8 (CH_3), 25.3 (CH_2), 30.4 (CH_2), 38.6 (CH_2), 39.3 (CH_2), 50.8 (CH), 55.5 (CH_3), 108.5 (ArC), 111.5 (ArCH), 114.1 (ArCH x2), 118.3 (ArCH), 118.9 (ArCH), 121.6 (ArCH), 126.5 (ArCH), 129.6 (ArCH x2), 132.3 (ArC), 132.9 (ArC), 136.5 (ArC), 159.2 (ArC) 167.7 (C=O), 169.8 (C=O). E.I.M.S. m/e 811 (2MH^+ , 10%) 406 (MH^+ , 100%). Found: C, 69.94%, H, 6.67%, N, 10.28%. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_3$ requires C, 71.09%, H, 6.71%, N, 10.36%.

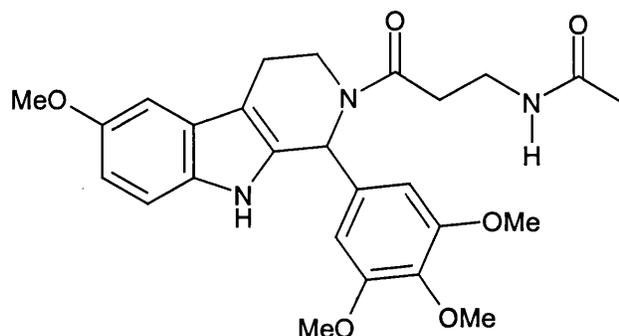
N-{3-[1-(3,4,5-trimethoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-ethyl}-acetamide (183)



N-Acetyl glycine (59 mg, 0.5 mmol) and **150** (169 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 107 mg, 0.23 mmol, 46% yield, m.p. 175-176 °C.

I.R. ν_{\max} cm^{-1} 3284, 1642, 1596, 1507, 1457, 1436, 1241, 1219, 1147, 1132. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.89 (s, 3H, CH_3), 2.78-2.86 (m, 2H, CH_2), 3.30-3.38 (m, 1H, CH_2), 3.64 (s, 3H, OCH_3), 3.67 (s, 6H, OCH_3), 3.78 (s, 3H, OCH_3), 3.99-4.04 (m, 1H, CH_2), 4.09 (d, $J = 5.6$ Hz, 1H, CH_2), 6.51 (s, 2H, ArH+CH), 6.66 (s, 1H, ArH), 6.71 (dd, $J = 8.0$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.99 (d, $J = 2.2$ Hz, 1H, ArH) 7.19 (d, $J = 8.0$ Hz 1H, ArH), 8.10 (t, $J = 5.6$ Hz, 1H, NH), 10.80 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 22.8 (CH_3), 39.4 (CH_2), 41.1 (CH_2), 52.3 (CH_3), 55.1 (CH_3), 55.7 (CH), 56.2 (CH_3), 60.3 (CH_3), 100.5 (ArCH), 105.8 (ArCH x2), 108.4 (ArC), 111.5 (ArCH), 112.2 (ArCH), 126.9 (ArC), 131.6 (ArC), 132.8 (ArC), 136.6 (ArC), 137.9 (ArC), 153.4 (ArC x2), 154.0 (ArC) 168.3 (C=O), 170.1 (C=O). E.I.M.S. m/e 935 (2MH^+ , 10%), 468 (MH^+ , 100%). Found: C, 64.19%, H, 6.23%, N, 9.02%. $\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_6$ requires C, 64.23%, H, 6.25%, N, 8.99%.

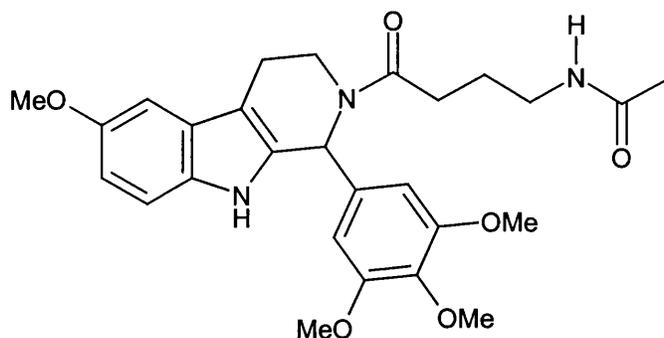
N-{3-[1-(3,4,5-trimethoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-propyl}-acetamide (184)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **150** (169 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 129 mg, 0.27 mmol, 54% yield, m.p. 177-178 °C.

I.R. ν_{\max} cm^{-1} 3336, 1671, 1627, 1616, 1592, 1487, 1464, 1436, 1326, 1238, 1218, 1151, 1119. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.77 (s, 3H, CH_3), 2.55-2.70 (m, 2H, CH_2), 2.78-2.82 (m, 2H, CH_2), 3.28-3.40 (m, 3H, CH_2), 3.60 (s, 3H, OCH_3), 3.64 (s, 6H, OCH_3), 3.78 (s, 3H, OCH_3), 6.51 (s, 2H, ArH+CH), 6.69-6.75 (m, 2H, ArH), 6.98 (d, $J = 2.2$ Hz, 1H, ArH) 7.19 (d, $J = 8.0$ Hz 1H, ArH), 7.89 (t, $J = 5.6$ Hz, 1H, NH), 10.80 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.1 (CH_2), 22.9 (CH_3), 33.1 (CH_2), 35.5 (CH_2), 39.2 (CH_2), 51.8 (CH_3), 55.7 (CH), 56.3 (2 x CH_3), 60.3 (CH_3), 100.5 (ArCH), 105.9 (ArCH x2), 108.4 (ArC), 111.4 (ArCH), 112.2 (ArCH), 127.0 (ArC), 131.7 (ArC), 132.8 (ArC), 136.7 (ArC), 137.7 (ArC), 153.5 (ArC x2), 154.0 (ArC) 170.1 (C=O), 170.3 (C=O). E.I.M.S. m/e 963 (2MH^+ , 10%), 482 (MH^+ , 100%). Found: C, 64.43%, H, 6.52%, N, 8.73%. $\text{C}_{26}\text{H}_{31}\text{N}_3\text{O}_6$ requires C, 64.85%, H, 6.49%, N, 8.73%.

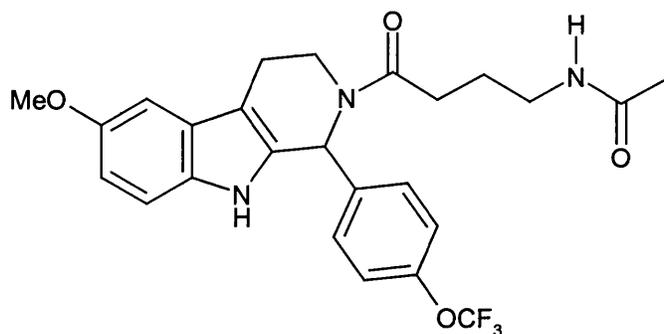
N-{3-[1-(3,4,5-trimethoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (185)



N-Acetyl-butanoic acid (80 mg, 0.5 mmol) and **150** (169 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 98 mg, 0.20 mmol, 40% yield, m.p. 198-199 °C.

I.R. ν_{\max} cm^{-1} 3343, 1671, 1617, 1592, 1464, 1455, 1424, 1325, 1234, 1220, 1149, 1127, 1116. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.62-1.72 (m, 2H, CH_2), 1.79 (s, 3H, CH_3), 2.40-2.54 (m, 2H, CH_2), 2.78-2.82 (m, 2H, CH_2), 3.04-3.10 (m, 2H, CH_2), 3.27-3.34 (m, 1H, CH_2), 3.64 (s, 3H, OCH_3), 3.67 (s, 6H, OCH_3), 3.77 (s, 3H, OCH_3), 3.99-4.07 (m, 1H, CH_2), 6.51 (s, 2H, ArH+CH), 6.69-6.75 (m, 2H, ArH), 6.97-6.99 (m, 1H, ArH), 7.19 (d, $J = 8.0$ Hz 1H, ArH), 7.84 (t, $J = 5.6$ Hz, 1H, NH), 10.80 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.1 (CH_2), 22.9 (CH_3), 25.3 (CH_2), 30.4 (CH_2), 38.6 (CH_2), 39.9 (CH_2), 51.7 (CH_3), 55.7 (CH_3), 55.9 (CH), 56.2 (CH_3), 60.1 (CH_3), 100.5 (ArCH), 105.8 (ArCH x2), 108.6 (ArC), 111.4 (ArCH), 112.2 (ArCH), 126.9 (ArC), 131.5 (ArC), 132.8 (ArC), 136.6 (ArC), 137.8 (ArC), 153.4 (ArC x2), 153.9 (ArC) 169.6 (C=O), 171.4 (C=O). E.I.M.S. m/e 991 (2MH^+ , 5%), 496 (MH^+ , 100%). Found: C, 65.21%, H, 6.68%, N, 8.42%. $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_6$ requires C, 65.44%, H, 6.71%, N, 8.48%.

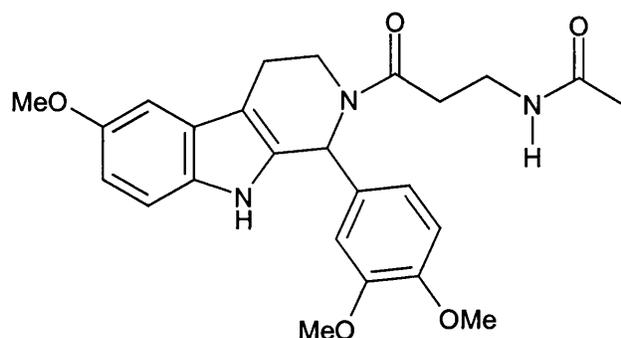
N-{3-[1-(4-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (186)



N-Acetyl-butanoic acid (80 mg, 0.5 mmol) and **149** (181 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 118 mg, 0.24 mmol, 48% yield, m.p. 133-135 °C.

I.R. ν_{\max} cm^{-1} 1652, 1633, 1505, 1485, 1456, 1436, 1259, 1218, 1168, 847. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.65-1.73 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.45-2.53 (m, 2H, CH_2), 2.72-2.90 (m, 2H, CH_2), 3.06-3.12 (m, 2H, CH_2), 3.14-3.22 (m, 1H, CH_2), 3.79 (s, 3H, OCH_3), 4.00-4.07 (m, 1H, CH_2), 6.74 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.89 (s, 1H, CH), 7.00 (d, $J = 2.2$ Hz, 1H, ArH), 7.21 (d, $J = 8.8$ Hz, 1H, ArH), 7.31-7.39 (m, 4H, ArH), 7.89 (t, $J = 5.6$ Hz, 1H, NH), 10.85 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 22.9 (CH_3), 25.2 (CH_2), 30.3 (CH_2), 38.6 (CH_2), 39.6 (CH_2), 50.8 (CH), 55.3 (CH_3), 100.2 (ArCH), 108.8 (ArC), 111.5 (ArCH), 112.2 (ArCH), 120.4 (q, $J = 259$ Hz, CF_3), 121.4 (ArCH x2), 126.9 (ArC), 129.8 (ArCH x2), 131.1 (ArC), 132.0 (ArC), 136.8 (ArC), 147.6 (ArC), 153.3 (ArC), 168.9 (C=O), 170.7 (C=O). E.I.M.S. m/e 490 (MH^+ , 100%). Found: C, 61.23%, H, 5.30%, N, 8.56%. $\text{C}_{25}\text{H}_{26}\text{F}_3\text{N}_3\text{O}_4$ requires C, 61.34%, H, 5.35%, N, 8.58%.

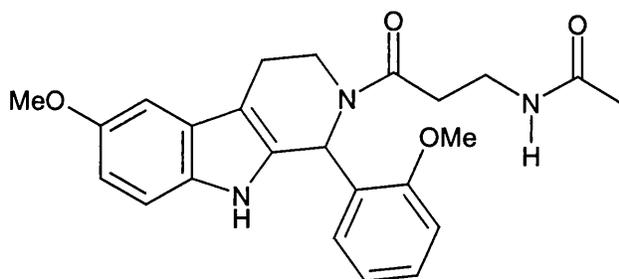
N-{3-[1-(3,4,-dimethoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-propyl}-acetamide (188)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **151** (169 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 87 mg, 0.19 mmol, 39% yield, m.p. 153-154 °C.

I.R. ν_{\max} cm^{-1} 1650, 1643, 1619, 1513, 1465, 1433, 1268, 1253, 1218, 1154, 1135, 845, 557. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.77 (s, 3H, CH_3), 2.55-2.70 (m, 2H, CH_2), 2.74-2.83 (m, 2H, CH_2), 3.21-3.34 (m, 2H, CH_2), 3.55-3.64 (m, 1H, CH_2), 3.69 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.95-4.01 (m, 1H, CH_2), 6.61 (dd, $J = 8.0$ Hz, $J = 1.2$ Hz, 1H, ArH), 6.72 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.79 (s, 1H, CH), 6.86 (m, 2H, ArH), 6.97 (d, $J = 2.2$ Hz, 1H, ArH), 7.19 (d, $J = 8.8$ Hz, 1H, ArH), 7.91 (t, $J = 5.6$ Hz, 1H, NH), 10.80 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.1 (CH_2), 22.9 (CH_3), 33.1 (CH_2), 35.5 (CH_2), 39.5 (CH_2), 51.3 (CH_3), 53.9 (CH), 55.4 (CH_3), 55.7 (CH_3), 55.9 (CH_3), 100.4 (ArCH), 108.2 (ArC), 111.3 (ArCH), 111.7 (ArCH), 112.1 (ArCH), 112.2 (ArCH), 120.7 (ArCH), 126.7 (ArC), 131.4 (ArC), 133.0 (ArC), 133.1 (ArC), 148.5 (ArC), 148.7 (ArC), 153.3 (ArC), 169.4 (C=O), 169.8 (C=O). E.I.M.S. m/e 903 (2MH^+ , 15%), 452 (MH^+ , 100%). Found: C, 66.41%, H, 6.42%, N, 9.25%. $\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_5$ requires C, 66.50%, H, 6.47%, N, 9.31%.

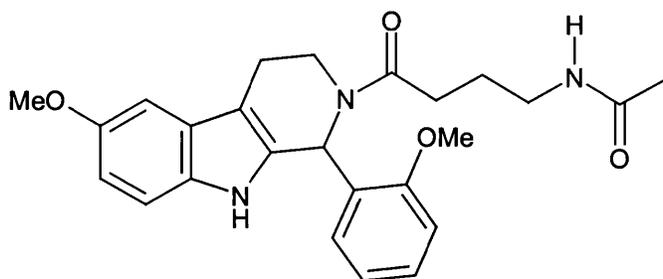
N-{3-[1-(2-methoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-propyl}-acetamide (189)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **155** (154 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 55 mg, 0.13 mmol, 26% yield, m.p. 191-192 °C.

I.R. ν_{\max} cm^{-1} 1653, 1622, 1598, 1545, 1490, 1457, 1436, 1425, 1245, 1232, 1213, 1098, 757. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.71 + 1.79 (2 x s - rotameric, 3H, CH_3), 2.55-2.90 (m, 4H, CH_2), 3.00-3.10 (m, 0.5H, CH_2), 3.25-3.34 (m, 2.5H, CH_2), 3.77 (s, 3H, OCH_3), 3.82 + 3.90 (2 x s - rotameric, 3H, OCH_3), 3.94-4.04 (m, 0.5H, CH_2), 4.48-4.55 (m, 0.5H, CH_2), 6.37 (s, 1H, CH), 6.61 (dd, $J = 8.0$ Hz, $J = 1.2$ Hz, 1H, ArH), 6.69-6.87 (m, 2H, ArH), 6.94-6.98 (m, 1H, ArH), 7.02-7.11 (m, 1H, ArH), 7.19 (d, $J = 8.8$ Hz, 1H, ArH), 7.25-7.35 (m, 1H, ArH), 7.90 (t, $J = 5.6$ Hz, 1H, NH), 10.55 (d, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} major rotamer reported 22.2 (CH_2), 22.8 (CH_3), 35.7 (CH_2), 35.7 (CH_2), 40.2 (CH_2), 51.0 (CH_3), 55.6 (CH), 55.7 (CH_3), 100.3 (ArCH), 109.5 (ArC), 111.1 (ArCH), 111.4 (ArCH), 112.3 (ArCH), 120.3 (ArCH), 127.7 (ArC), 129.4 (ArCH), 129.9 (ArCH), 131.5 (ArC), 133.2 (ArC), 133.8 (ArC), 153.7 (ArC), 157.2 (ArC) 169.8 (C=O), 170.8 (C=O). E.I.M.S. m/e 843 (2MH^+ , 15%), 422 (MH^+ , 100%). Found: C, 68.41%, H, 6.44%, N, 9.97%. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4$ requires C, 68.39%, H, 6.46%, N, 9.97%.

N-{3-[1-(2-methoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (190)

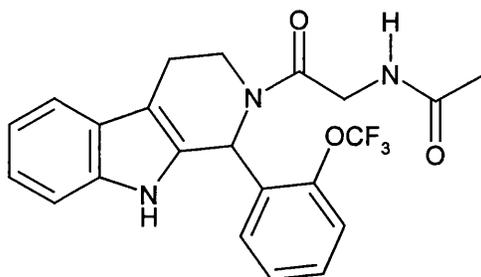


N-Acetyl-butanoic acid (80 mg, 0.5 mmol) and **155** (154 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 87 mg, 0.20 mmol, 40% yield, m.p. 183-184 °C.

I.R. ν_{\max} cm^{-1} 1655, 1611, 1558, 1489, 1457, 1439, 1427, 1247, 1218, 1127, 761.

^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.60-1.75 (m, 2H, CH_2), 1.78 + 1.79 (2 x s - rotamers, 3H, CH_3), 2.30-2.90 (m, 4H, CH_2), 3.02-3.10 (m, 2H, CH_2), 3.25-3.40 (m, 1H, CH_2), 3.76 + 3.80 (2 x s - rotamers, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.05-4.10 (m, 1H, CH_2), 6.40 (s, 1H, CH), 6.65-6.75 (m, 2H, ArH), 6.80-6.87 (q, $J = 7.8$ Hz, 1H, ArH), 6.94-6.97 (m, 1H, ArH) 7.01-7.12 (m, 1H, ArH), 7.18 (d, $J = 7.8$ Hz, 1H, ArH), 7.25-7.34 (m, 1H, ArH), 7.84 (t, $J = 5.4$ Hz, 1H, NH), 10.50 + 10.60 (2 x s - rotamers, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.5 (CH_2), 23.0 (CH_3), 25.4 (CH_2), 30.4 (CH_2), 38.6 (CH_2), 40.1 (CH_2), 47.1 (CH_3), 51.0 (CH), 55.7 (CH_3), 55.9 (CH_3), 100.2 (ArCH), 108.5 (ArC), 111.1 (ArCH), 111.5 (ArCH), 112.3 (ArCH), 120.3 (ArCH), 126.8 (ArC), 128.1 (ArC), 129.4 (ArCH), 129.9 (ArCH), 131.8 (ArC), 133.8 (ArC), 153.7 (ArC), 157.5 (ArC) 169.7 (C=O), 171.6 (C=O). E.I.M.S. m/e 436 (MH^+ , 100%). Found: C, 68.88%, H, 6.66%, N, 9.60%. $\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_4$ requires C, 68.95%, H, 6.71%, N, 9.65%.

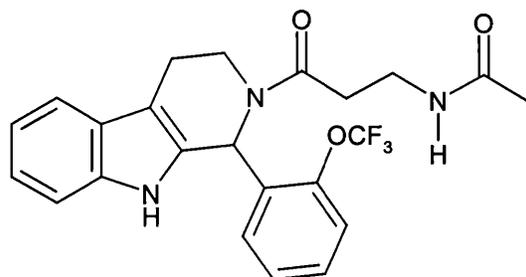
N-{3-[1-(2-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-ethyl}-acetamide (191)



N-Acetyl-glycine (59 mg, 0.5 mmol) and **156** (166 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 89 mg, 0.21 mmol, 41% yield, m.p. 216-217 °C.

I.R. ν_{\max} cm^{-1} 1664, 1641, 1520, 1490, 1464, 1455, 1429, 1260, 1211, 1173, 1162, 752. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.88 (s, 3H, CH_3), 2.76-2.99 (m, 2H, CH_2), 3.19-3.29 (m, 1H, CH_2), 3.92-4.02 (m, 2H, CH_2), 4.12-4.20 (m, 1H, CH_2), 6.90 (d, $J = 7.8\text{Hz}$, 1H, ArH), 7.00-7.12 (m, 2H, ArH), 7.14 (s, 1H, CH), 7.25-7.32 (m, 2H, ArH), 7.40-7.51 (m, 3H, ArH), 8.10 (t, $J = 5.6\text{ Hz}$, 1H, NH), 10.80 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.8 (CH_2), 22.8 (CH_3), 36.7 (CH_2), 40.8 (CH_2), 47.2 (CH), 109.3 (ArC), 111.7 (ArCH), 118.3 (ArCH), 119.1 (ArCH), 120.8 (ArCH), 121.8 (ArCH), 126.7 (ArC), 127.3 (ArCH), 130.3 (ArCH), 131.5 (ArC), 131.6 (ArCH), 132.3 (ArC), 135.6 (ArC), 147.2 (ArC) 168.4 (C=O), 169.7 (C=O), CF_3 not observed. E.I.M.S. m/e 432 (MH^+ , 100%). Found: C, 61.18%, H, 4.60%, N, 9.70%. $\text{C}_{22}\text{H}_{20}\text{N}_3\text{F}_3\text{O}_3$ requires C, 61.25%, H, 4.67%, N, 9.74%.

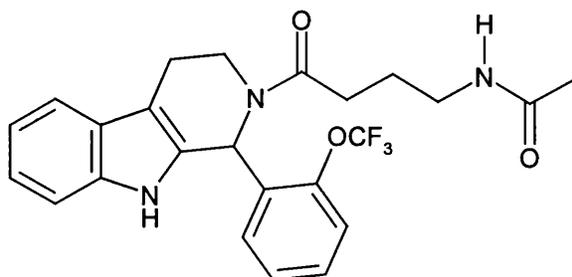
N-{3-[1-(2-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-propyl}-acetamide (192)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **156** (166 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 110 mg, 0.25 mmol, 50% yield, m.p. 149-150 °C.

I.R. ν_{\max} cm^{-1} 1652, 1641, 1621, 1442, 1418, 1254, 1210, 1160, 1050, 764, 742. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.78 (s, 3H, CH_3), 2.52-2.68 (m, 2H, CH_2), 2.78-2.90 (m, 2H, CH_2), 3.13-3.24 (m, 1H, CH_2), 3.28 (q, $J = 5.6$ Hz, 2H, CH_2), 3.93-3.99 (m, 1H, CH_2), 6.85 (d, $J = 7.8$ Hz, 1H, ArH), 7.02 (t, $J = 7.8$ Hz, 1H, ArH), 7.09 (t, $J = 7.8$ Hz, 1H, ArH), 7.21 (s, 1H, CH), 7.24-7.33 (m, 2H, ArH), 7.40-7.50 (m, 3H, ArH), 7.83 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 22.9 (CH_3), 32.9 (CH_2), 35.5 (CH_2), 39.2 (CH_2), 46.6 (CH), 109.4 (ArC), 111.7 (ArCH), 118.3 (ArCH), 119.1 (ArCH), 120.7 (ArCH), 121.8 (ArCH), 126.6 (ArC), 127.2 (ArCH), 130.2 (ArCH), 131.5 (ArC), 131.6 (ArCH), 132.5 (ArCH), 136.7 (ArC), 147.3 (ArC) 169.8 (C=O), 170.4 (C=O). E.I.M.S. m/e 991 (2MH^+ , 5%), 446 (MH^+ , 100%). Found: C, 61.67%, H, 4.90%, N, 9.41%. $\text{C}_{23}\text{H}_{22}\text{N}_3\text{F}_3\text{O}_3$ requires C, 62.02%, H, 4.98%, N, 9.43%.

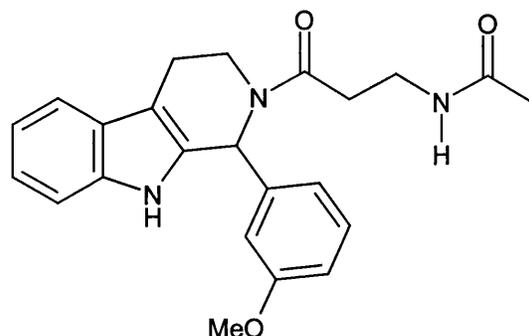
N-{3-[1-(2-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (193)



N-Acetyl butanoic acid (80 mg, 0.5 mmol) and **156** (166 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 90 mg, 0.20 mmol, 39% yield, m.p. 199-200 °C.

I.R. ν_{\max} cm^{-1} . 3331, 1656, 1639, 1548, 1452, 1437, 1257, 1251, 1214, 1168, 1160, 745. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.61-1.71 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.38-2.52 (m, 2H, CH_2), 2.78-2.91 (m, 2H, CH_2), 3.02-3.10 (m, 2H, CH_2), 3.12-3.22 (m, 1H, CH_2), 3.93-4.01 (m, 1H, CH_2), 6.83 (d, $J = 7.8$ Hz, 1H, ArH), 7.01 (t, $J = 7.8$ Hz, 1H, ArH), 7.09 (t, $J = 7.8$ Hz, 1H, ArH), 7.22 (s, 1H, CH), 7.24-7.32 (m, 2H, ArH), 7.40-7.50 (m, 3H, ArH), 7.89 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 22.9 (CH_3), 25.3 (CH_2), 30.2 (CH_2), 36.2 (CH_2), 38.6 (CH_2), 46.6 (CH), 109.4 (ArC), 111.7 (ArCH), 118.2 (ArCH), 119.1 (ArCH), 120.7 (ArCH), 121.7 (ArCH), 126.6 (ArC), 127.2 (ArCH), 130.7 (ArCH), 131.5 (ArC), 131.6 (ArCH), 132.6 (ArCH), 136.5 (ArC), 147.3 (ArC) 169.8 (C=O), 171.7 (C=O). E.I.M.S. m/e 460 (MH^+ , 100%). Found: C, 62.78%, H, 5.23%, N, 9.14%. $\text{C}_{24}\text{H}_{24}\text{N}_3\text{F}_3\text{O}_3$ requires C, 62.74%, H, 5.27%, N, 9.15%.

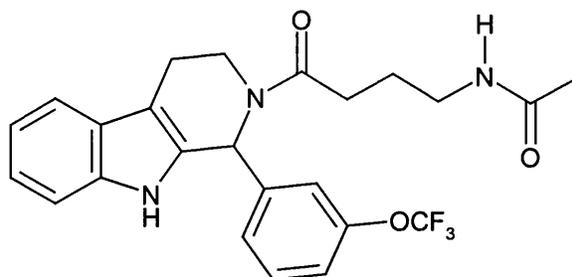
N-{3-[1-(3-methoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-propyl}-
acetamide (194)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **158** (139 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 124 mg, 0.32 mmol, 63% yield, m.p. 176-177 °C.

I.R. ν_{\max} cm^{-1} . 3308, 3212, 1658, 1612, 1581, 1469, 1454, 1356, 1307, 1262, 1048, 742. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.80 (s, 3H, CH_3), 2.60-2.70 (m, 2H, CH_2), 2.74-2.88 (m, 2H, CH_2), 3.20-3.40 (m, 3H, CH_2), 3.70 (s, 3H, CH_3), 3.98-4.05 (m, 1H, CH_2), 6.78-6.90 (m, 4H, ArH+CH), 7.01 (t, $J = 7.8$ Hz, 1H, ArH), 7.10 (t, $J = 7.8$ Hz, 1H, ArH), 7.20-7.38 (m, 2H, ArH), 7.48 (d, $J = 7.8$ Hz, 1H, ArH), 7.94 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.9 (CH_2), 22.9 (CH_3), 33.0 (CH_2), 35.5 (CH_2), 39.7 (CH_2), 51.4 (CH_3), 55.4 (CH), 108.6 (ArC), 111.6 (ArCH), 112.8 (ArCH), 114.6 (ArCH), 118.3 (ArCH), 119.0 (ArCH), 120.5 (ArCH), 121.6 (ArCH), 126.5 (ArC), 129.8 (ArCH), 132.2 (ArC), 136.5 (ArC), 142.4 (ArC), 159.7 (ArC), 169.6 (C=O), 169.9 (C=O). E.I.M.S. m/e 783 (2MH^+ , 100%), 392 (MH^+ , 100%). Found: C, 70.39%, H, 6.39%, N, 10.70%. $\text{C}_{23}\text{H}_{25}\text{N}_3\text{O}_3$ requires C, 70.57%, H, 6.44%, N, 10.73%.

N-{3-[1-(3-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (195)

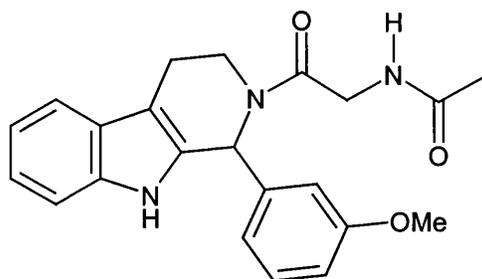


N-Acetyl butanoic acid (80 mg, 0.5 mmol) and **160** (166 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 86 mg, 0.19 mmol, 37% yield, m.p. 188-189 °C.

I.R. ν_{\max} cm^{-1} . 3288, 1650, 1620, 1564, 1462, 1421, 1268, 1230, 1215, 1163, 744.

^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.71 (qn, $J = 7.8$ Hz, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.42-2.59 (m, 2H, CH_2), 2.75-2.92 (m, 2H, CH_2), 3.10 (q, $J = 6.0$ Hz, 2H, CH_2), 3.16-3.24 (m, 1H, CH_2), 4.01-4.10 (m, 1H, CH_2), 6.91 (s, 1H, CH), 7.02 (t, $J = 7.8$ Hz, 1H, ArH), 7.11 (t, $J = 7.8$ Hz, 1H, ArH), 7.20-7.26 (m, 2H, ArH), 7.30-7.37 (m, 2H, ArH), 7.46-7.52 (m, 2H, ArH), 7.89 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.9 (CH_2), 22.9 (CH_3), 25.2 (CH_2), 30.3 (CH_2), 38.6 (CH_2), 39.7 (CH_2), 51.0 (CH), 109.1 (ArC), 111.6 (ArCH), 118.4 (ArCH), 119.1 (ArCH), 120.5 (ArCH), 120.7 (ArCH), 121.8 (ArCH), 126.5 (ArC), 127.3 (ArCH), 130.4 (ArCH), 131.1 (ArC), 136.7 (ArC), 143.7 (ArC), 149.0 (ArC), 169.4 (C=O), 171.4 (C=O), CF_3 not observed. E.I.M.S. m/e 460 (MH^+ , 100%). Found: C, 62.70%, H, 5.24%, N, 9.25%. $\text{C}_{24}\text{H}_{24}\text{N}_3\text{F}_3\text{O}_3$ requires C, 62.74%, H, 5.27%, N, 9.15%.

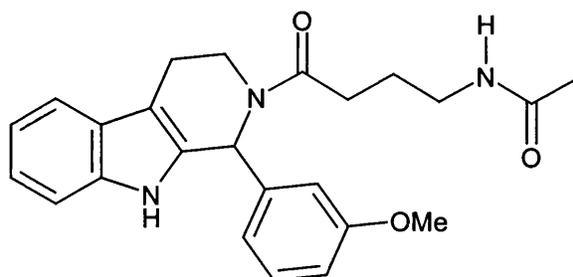
N-{3-[1-(3-methoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-ethyl]-acetamide (196)}



N-Acetyl glycine (59 mg, 0.5 mmol) and **158** (166 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 106 mg, 0.28 mmol, 56% yield, m.p. 194-195 °C.

I.R. ν_{\max} cm^{-1} . 3270, 1666, 1632, 1620, 1595, 1555, 1487, 1469, 1453, 1276, 1244, 1155, 744. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.90 (s, 3H, CH_3), 2.75-2.92 (m, 2H, CH_2), 3.20-3.30 (m, 1H, CH_2), 4.01-4.10 (m, 1H, CH_2), 3.70 (s, 3H, CH_3), 3.97-4.04 (m, 1H, CH_2), 4.10 (d, $J = 6.0$ Hz, 2H, CH_2), 6.76-6.81 (m, 3H, ArH+CH), 6.86-6.91 (m, 1H, ArH), 7.01 (t, $J = 7.8$ Hz, 1H, ArH), 7.09 (t, $J = 7.8$ Hz, 1H, ArH), 7.21-7.33 (m, 2H, ArH), 7.48 (d, $J = 7.8$ Hz, 1H, ArH), 8.11 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.8 (CH_2), 22.8 (CH_3), 37.2 (CH_2), 41.0 (CH_2), 51.9 (CH_3), 55.4 (CH), 108.6 (ArC), 111.6 (ArCH), 112.9 (ArCH), 114.7 (ArCH), 118.3 (ArCH), 119.0 (ArCH), 120.5 (ArCH), 121.7 (ArCH), 126.5 (ArC), 129.9 (ArCH), 132.0 (ArC), 136.5 (ArC), 142.1 (ArC), 159.7 (ArC), 168.0 (C=O), 169.8 (C=O). E.I.M.S. m/e 755 (2MH^+ , 100%), 378 (MH^+ , 100%), 279 (50%), 262 (70%). Found: C, 69.88%, H, 6.11%, N, 11.10%. $\text{C}_{24}\text{H}_{22}\text{N}_3\text{O}_3$ requires C, 70.01%, H, 6.14%, N, 11.13%.

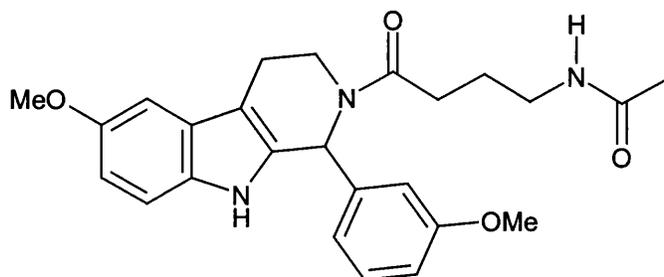
N-{3-[1-(3-methoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (197)



N-Acetyl butanoic acid (80 mg, 0.5 mmol) and **158** (139 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 90 mg, 0.22 mmol, 44% yield, m.p. 192-3 °C.

I.R. ν_{\max} cm^{-1} . 3351, 1651, 1620, 1552, 1485, 1443, 1303, 1277, 1254, 1053, 755, 745. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.65-1.75 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.40-2.52 (m, 2H, CH_2), 2.70-2.90 (m, 2H, CH_2), 2.96-3.12 (m, 2H, CH_2), 3.19-3.38 (m, 1H, CH_2), 3.70 (s, 3H, CH_3), 3.98-4.15 (m, 1H, CH_2), 6.75-6.80 (m, 2H, ArH+CH), 6.83-6.90 (m, 2H, ArH), 7.00 (t, $J = 7.8$ Hz, 1H, ArH), 7.09 (t, $J = 7.8$ Hz, 1H, ArH), 7.25-7.34 (m, 2H, ArH), 7.48 (d, $J = 7.8$ Hz, 1H, ArH), 7.87 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 23.0 (CH_3), 25.3 (CH_2), 30.4 (CH_2), 38.6 (CH_2), 39.7 (CH_2), 51.3 (CH), 55.4 (CH_3), 108.6 (ArC), 111.6 (ArCH), 112.8 (ArCH), 114.6 (ArCH), 118.3 (ArCH), 119.0 (ArCH), 120.5 (ArCH), 121.6 (ArCH), 126.7 (ArC), 129.8 (ArCH), 132.4 (ArC), 136.5 (ArC), 142.3 (ArC), 159.6 (ArC), 169.4 (C=O), 171.1 (C=O). E.I.M.S. m/e 406 (MH^+ , 100%). Found: C, 69.98%, H, 6.64%, N, 10.32%. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_3$ requires C, 71.09%, H, 6.71%, N, 10.36%.

N-{3-[1-(3-methoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (198)

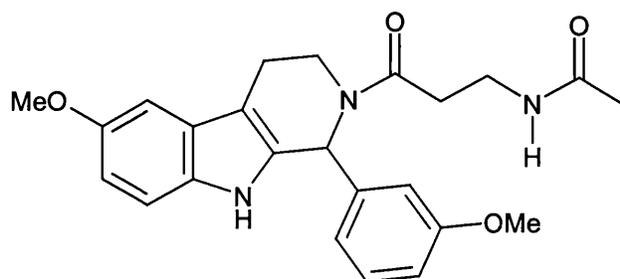


N-Acetyl butanoic acid (80 mg, 0.5 mmol) and **159** (154 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 114 mg, 0.26 mmol, 52% yield, m.p. 163-164 °C.

I.R. ν_{\max} cm^{-1} . 3329, 1654, 1636, 1596, 1544, 1465, 1438, 1265, 1214, 1040, 702.

^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.65-1.75 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.40-2.52 (m, 2H, CH_2), 2.70-2.90 (m, 2H, CH_2), 3.09 (q, $J = 6.0$ Hz, 2H, CH_2), 3.18-3.28 (m, 1H, CH_2), 3.70 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 3.98-4.50 (m, 1H, CH_2), 6.71-6.82 (m, 4H, ArH+CH), 6.85-6.90 (m, 1H, ArH), 6.99 (d, $J = 1.6$ Hz, 1H, ArH), 7.19-7.28 (m, 2H, ArH), 7.88 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 23.0 (CH_3), 25.3 (CH_2), 30.3 (CH_2), 38.6 (CH_2), 39.4 (CH_2), 51.4 (CH), 55.4 (CH_3), 55.7 (CH_3), 100.4 (ArCH), 108.5 (ArC), 111.4 (ArCH), 112.2 (ArCH), 112.8 (ArCH), 114.5 (ArCH), 120.4 (ArCH), 126.9 (ArC), 129.8 (ArCH), 131.6 (ArC), 132.9 (ArC), 142.6 (ArC), 153.6 (ArC), 159.6 (ArC), 169.4 (C=O), 171.1 (C=O). E.I.M.S. m/e 436 (MH^+ , 100%). Found: C, 68.86%, H, 6.64%, N, 9.60%. $\text{C}_{25}\text{H}_{29}\text{N}_3\text{O}_4$ requires C, 68.95%, H, 6.71%, N, 9.65%.

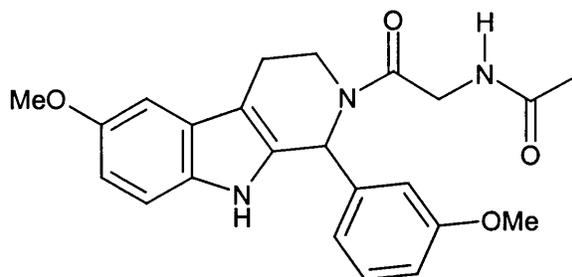
N-{3-[1-(3-methoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-ethyl}-acetamide (199)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **159** (154 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 120 mg, 0.29 mmol, 57% yield, m.p. 175-176 °C.

I.R. ν_{\max} cm^{-1} . 3317, 3162, 1660, 1619, 1595, 1487, 1450, 1434, 1271, 1214, 1037, 804, 702. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.80 (s, 3H, CH_3), 2.58-2.88 (m, 4H, CH_2), 3.18-3.40 (m, 3H, CH_2), 3.70 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 3.97-4.04 (m, 1H, CH_2), 6.71-6.82 (m, 4H, ArH+CH), 6.85-6.90 (m, 1H, ArH), 6.99 (d, $J = 2.2$ Hz, 1H, ArH), 7.19-7.28 (m, 2H, ArH), 7.91 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 22.9 (CH_3), 33.0 (CH_2), 35.5 (CH_2), 39.7 (CH_2), 51.4 (CH_3), 55.4 (CH), 55.7 (CH_3), 100.4 (ArCH), 108.4 (ArC), 111.4 (ArCH), 112.2 (ArCH), 112.8 (ArCH), 114.6 (ArCH), 120.5 (ArCH), 126.8 (ArC), 129.8 (ArCH), 131.5 (ArC), 132.9 (ArC), 142.4 (ArC), 153.6 (ArC), 159.7 (ArC), 169.6 (C=O), 169.9 (C=O). E.I.M.S. m/e 422 (MH^+ , 100%). Found: C, 63.29%, H, 6.43%, N, 9.94%. $\text{C}_{24}\text{H}_{27}\text{N}_3\text{O}_4$ requires C, 68.39%, H, 6.46%, N, 9.97%.

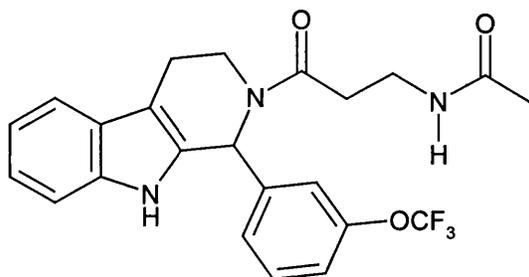
N-{3-[1-(3-methoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-ethyl}-acetamide (200)



N-Acetyl glycine (59 mg, 0.5 mmol) and **159** (154 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 112 mg, 0.28 mmol, 55% yield, m.p. 216-217 °C.

I.R. ν_{\max} cm^{-1} . 3366, 3191, 1655, 1627, 1598, 1521, 1471, 1430, 1267, 1250, 1218, 1040, 856, 700. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.90 (s, 3H, CH_3), 2.73-2.91 (m, 2H, CH_2), 3.20-3.30 (m, 1H, CH_2), 3.70 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 3.96-4.04 (m, 1H, CH_2), 4.10 (d, $J = 6.0$ Hz, 2H, CH_2), 6.71-6.81 (m, 4H, ArH+CH), 6.85-6.90 (m, 1H, ArH), 6.98-7.01 (m, 1H, ArH), 7.20-7.29 (m, 2H, ArH), 8.11 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.9 (CH_2), 22.8 (CH_3), 39.1 (CH_2), 41.0 (CH_2), 52.0 (CH), 55.0 (CH_3), 55.4 (CH_3), 100.5 (ArCH), 108.4 (ArC), 112.2 (ArCH), 112.9 (ArCH), 113.2 (ArCH), 114.7 (ArCH), 120.5 (ArCH), 126.9 (ArC), 129.8 (ArCH), 131.7 (ArC), 132.6 (ArC), 143.0 (ArC), 153.6 (ArC), 159.9 (ArC), 168.3 (C=O), 170.9 (C=O). E.I.M.S. m/e 408 (MH^+ , 100%). Found: C, 67.80%, H, 6.19%, N, 10.30%. $\text{C}_{23}\text{H}_{25}\text{N}_3\text{O}_4$ requires C, 67.80%, H, 6.18%, N, 10.31%.

N-{3-[1-(3-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-propyl}-acetamide (201)

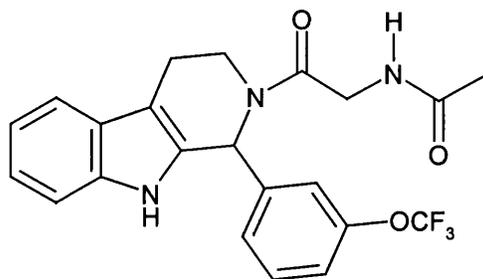


N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **160** (154 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 120 mg, 0.29 mmol, 57% yield, m.p. 194-195 °C.

I.R. ν_{\max} cm^{-1} . 3342, 1658, 1633, 1620, 1556, 1448, 1362, 1273, 1216, 1153, 745.

^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.80 (s, 3H, CH_3), 2.60-2.72 (m, 2H, CH_2), 2.76-2.92 (m, 2H, CH_2), 3.18-3.26 (m, 1H, CH_2), 3.29-3.41 (m, 2H, CH_2), 4.00-4.10 (m, 1H, CH_2), 6.91 (s, 1H, CH), 7.01 (t, $J = 7.8$ Hz, 1H, ArH), 7.12 (t, $J = 7.8$ Hz, 1H, ArH), 7.21-7.28 (m, 2H, ArH), 7.30-7.37 (m, 2H, ArH), 7.46-7.252 (m, 2H, ArH), 7.93 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.8 (CH_2), 22.8 (CH_3), 33.0 (CH_2), 35.4 (CH_2), 39.4 (CH_2), 51.0 (CH), 109.1 (ArC), 111.6 (ArCH), 118.4 (ArCH), 119.1 (ArCH), 120.5 (ArCH), 120.8 (ArCH), 121.8 (ArCH), 126.5 (ArC), 127.3 (ArCH), 128.7 (q, $J = 258$ Hz, OCF_3), 130.8 (ArCH), 131.3 (ArC), 136.6 (ArC), 143.5 (ArC), 148.8 (ArC), 169.6 (C=O), 170.2 (C=O). E.I.M.S. m/e 446 (MH^+ , 100%), 333 (40%). Found: C, 61.94%, H, 4.90%, N, 9.45%. $\text{C}_{23}\text{H}_{22}\text{N}_3\text{F}_3\text{O}_3$ requires C, 62.02%, H, 4.98%, N, 9.43%.

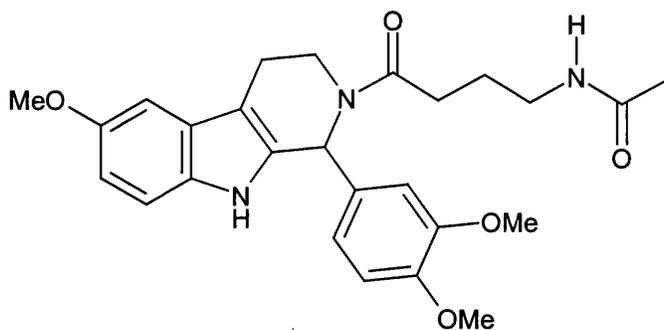
N-{3-[1-(3-trifluoromethoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-ethyl}-acetamide (202)



N-Acetyl glycine (59 mg, 0.5 mmol) and **160** (166 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 102 mg, 0.24 mmol, 48% yield, m.p. 194-195 °C.

I.R. ν_{\max} cm^{-1} . 3352, 1652, 1644, 1632, 1525, 1470, 1431, 1254, 1232, 1220, 1160, 751. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.90 (s, 3H, CH_3), 2.80-2.95 (m, 2H, CH_2), 3.19-3.28 (m, 1H, CH_2), 3.96-4.04 (m, 1H, CH_2), 4.01-4.09 (m, 1H, CH_2), 4.10 (d, $J = 5.4$ Hz, 1H, CH_2), 6.83 (s, 1H, CH), 7.03 (dd $J = 7.8$ Hz, 1H, ArH), 7.11 (dd $J = 7.8$ Hz, 1H, ArH), 7.20-7.26 (m, 2H, ArH), 7.31-7.34 (d, $J = 7.8$ Hz, 2H, ArH), 7.47-7.52 (m, 2H, ArH), 8.11 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.7 (CH_2), 22.8 (CH_3), 39.2 (CH_2), 41.0 (CH_2), 51.6 (CH), 109.0 (ArC), 111.7 (ArCH), 118.4 (ArCH), 119.1 (ArCH), 120.0 (q, $J = 259$ Hz, CF_3), 120.4 (ArCH), 120.8 (ArCH), 121.9 (ArCH), 126.4 (ArC), 127.4 (ArCH), 130.8 (ArCH), 131.1 (ArC), 136.6 (ArC), 143.2 (ArC), 148.8 (ArC), 168.9 (C=O), 170.2 (C=O). E.I.M.S. m/e 863 (2MH^+ , 10%), 432 (MH^+ , 100%), 333 (70%), 316 (10%). Found: C, 61.20%, H, 4.66%, N, 9.72%. $\text{C}_{22}\text{H}_{20}\text{N}_3\text{F}_3\text{O}_3$ requires C, 61.25%, H, 4.67%, N, 9.74%.

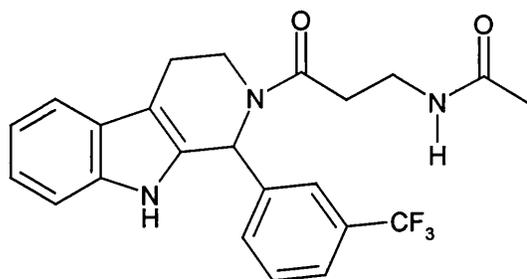
N-{3-[1-(3,4-dimethoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (203)



N-Acetyl butanoic acid (80 mg, 0.5 mmol) and **151** (169 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 108 mg, 0.23 mmol, 46% yield, m.p. 157-158 °C.

I.R. ν_{\max} cm^{-1} . 3348, 1645, 1628, 1607, 1590, 1480, 1466, 1437, 1226, 1214, 847, 840, 557. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.63-1.73 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.40-2.54 (m, 2H, CH_2), 2.74-2.90 (m, 2H, CH_2), 3.06-3.16 (m, 2H, CH_2), 3.19-3.28 (m, 1H, CH_2), 3.68 (s, 3H, CH_3), 3.71 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 3.96-4.03 (m, 1H, CH_2), 6.60 (d, $J = 8.0$ Hz, 1H, ArH), 6.72 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.79 (s, 1H, CH), 6.85-6.90 (m, 2H, ArH), 6.98 (d, $J = 1.8$ Hz, 1H, ArH), 7.19 (d, $J = 8.8$ Hz, 1H, ArH), 7.87 (t, $J = 5.6$ Hz, 1H, NH), 10.80 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.1 (CH_2), 22.9 (CH_3), 25.3 (CH_2), 30.4 (CH_2), 38.6 (CH_2), 39.5 (CH_2), 51.2 (CH), 53.9 (CH_3), 55.7 (CH_3), 55.9 (CH_3), 100.4 (ArCH), 108.4 (ArC), 111.3 (ArCH), 111.7 (ArCH), 112.1 (ArCH), 112.2 (ArCH), 120.6 (ArCH), 128.9 (ArC), 131.5 (ArC), 133.4 (ArC), 134.0 (ArC), 148.9 (ArC), 149.4 (ArC), 153.6 (ArC), 169.7 (C=O), 171.0 (C=O). E.I.M.S. m/e 948, (2MH^+ , 10%), 466 (MH^+ , 100%). Found: C, 66.98%, H, 6.69%, N, 9.00%. $\text{C}_{26}\text{H}_{31}\text{N}_3\text{O}_5$ requires C, 67.07%, H, 6.71%, N, 9.03%.

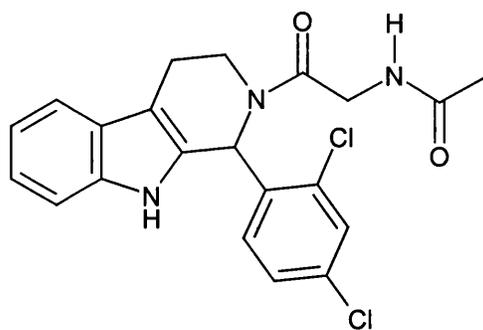
N-{3-[1-(3-trifluoromethyl-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-propyl}-acetamide (204)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **147** (158 mg, 0.5 mmol) gave the title compound as a pale yellow solid on recrystallisation from ethyl acetate, 158mg, 74% yield, m.p. 85-86 °C.

I.R. ν_{\max} cm^{-1} . 3279, 2934, 1640, 1622, 1465, 1442, 1329, 1183, 1164, 1125, 1074, 844, 745, 704, 558. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.79 (s, 3H, CH_3), 2.60-2.72 (m, 2H, CH_2), 2.83-2.92 (m, 2H, CH_2), 3.12-3.40 (m, 3H, CH_2), 4.02-4.10 (m, 1H, CH_2), 6.94 (s, 1H, CH), 7.03 (t, $J = 7.8$ Hz 1H, ArH), 7.11 (t, $J = 7.8$ Hz 1H, ArH), 7.36 (d, $J = 7.8$ Hz, 1H, ArH), 7.50-7.54 (m, 2H, ArH), 7.58-7.78 (m, 3H, ArH), 7.93 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.8 (CH_2), 22.8 (CH_3), 33.0 (CH_2), 35.4 (CH_2), 38.5 (CH_2), 51.2 (CH), 109.2 (ArC), 111.7 (ArCH), 118.4 (ArCH), 119.1 (ArCH), 121.9 (ArCH), 124.7 (ArCH), 124.9 (ArCH), 126.5 (ArC), 127.6 (q, $J = 256$ Hz, CF_3), 129.8 (ArC), 130.0 (ArCH), 131.3 (ArC), 132.4 (ArCH), 136.6 (ArC), 142.2 (ArC), 169.6 (C=O), 170.3 (C=O). E.I.M.S. m/e 430 (MH^+ , 100%). Found: C, 64.27%, H, 5.12%, N, 9.73%. $\text{C}_{23}\text{H}_{22}\text{N}_3\text{F}_3\text{O}_3$ requires C, 64.33%, H, 5.16%, N, 9.78%.

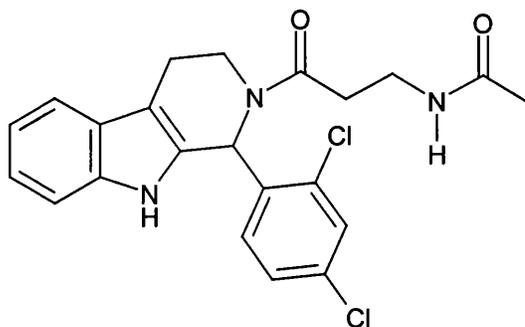
N-{3-[1-(2,4-dichloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-ethyl]-acetamide (205)}



N-Acetyl glycine (59 mg, 0.5 mmol) and **148** (158 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 132 mg, 0.32 mmol, 63% yield, m.p. 223-224 °C.

I.R. ν_{\max} cm^{-1} . 3367, 3295, 1649, 1505, 1472, 1459, 1430, 1215, 1050, 838, 826, 747, 591. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.87 (s, 3H, CH_3), 2.80-2.97 (m, 2H, CH_2), 3.20-3.30 (m, 1H, CH_2), 3.96-4.08 (m, 2H, CH_2), 4.15-4.20 (m, 1H, CH_2), 6.86 (d, $J = 8.0$ Hz 1H, ArH), 7.01 (s, 1H, CH), 7.02 (dd, $J = 7.8$ Hz, 1H, ArH), 7.09 (dd, $J = 7.8$ Hz, 1H, ArH), 7.29-7.35 (m, 2H, ArH), 7.49 (d, $J = 7.8$ Hz, 1H, ArH), 7.69 (d, $J = 1.2$ Hz, 1H, ArH), 8.09 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.8 (CH_2), 22.8 (CH_3), 39.2 (CH_2), 41.0 (CH_2), 49.8 (CH), 108.6 (ArC), 111.7 (ArCH), 118.4 (ArCH), 119.2 (ArCH), 121.9 (ArCH), 127.5 (ArCH), 129.6 (ArCH), 131.6 (ArC), 132.6 (ArCH), 132.8 (ArC), 134.0 (ArC), 134.7 (ArC), 136.7 (ArC), 136.9 (ArC), 168.9 (C=O), 169.8 (C=O). E.I.M.S. m/e 416/418 (MH^+ , 100%). Found: C, 60.55%, H, 4.57%, N, 10.04%. $\text{C}_{21}\text{H}_{19}\text{N}_3\text{Cl}_2\text{O}_2$ requires C, 60.59%, H, 4.60%, N, 10.09%.

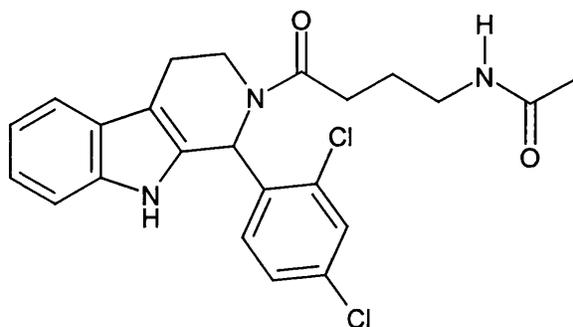
N-{3-[1-(2,4-dichloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-propyl}-acetamide (206)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **148** (158 mg, 0.5 mmol) gave the title compound as a pale yellow solid on recrystallisation from ethyl acetate, 158mg, 0.36 mmol, 74% yield, m.p. 209-210 °C.

I.R. ν_{\max} cm^{-1} . 3285, 1650, 1644, 1537, 1469, 1428, 1368, 1289, 1246, 1207, 1046, 820, 746. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.79 (s, 3H, CH_3), 2.54-2.74 (m, 2H, CH_2), 2.76-2.90 (m, 2H, CH_2), 3.18-3.30 (m, 3H, CH_2), 3.97-4.02 (m, 1H, CH_2), 6.81 (d, $J = 8.0$ Hz, 1H, ArH), 6.98-7.12 (m, 3H, CH + ArH), 7.29-7.35 (m, 2H, ArH), 7.48 (d, $J = 7.8$ Hz, 1H, ArH), 7.68 (s, 1H, ArH), 7.90 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 22.9 (CH_3), 33.1 (CH_2), 35.5 (CH_2), 39.8 (CH_2), 49.2 (CH), 109.2 (ArC), 111.7 (ArCH), 118.3 (ArCH), 119.1 (ArCH), 121.9 (ArCH), 126.6 (ArC), 127.4 (ArCH), 129.6 (ArCH), 131.6 (ArC), 132.6 (ArCH), 133.8 (ArC), 134.5 (ArC), 136.8 (ArC), 137.1 (ArC), 169.9 (C=O), 170.8 (C=O). E.I.M.S. m/e 430/432 (MH^+ , 100%). Found: C, 61.23%, H, 4.87%, N, 9.71%. $\text{C}_{22}\text{H}_{21}\text{N}_3\text{Cl}_2\text{O}_2$ requires C, 61.40%, H, 4.92%, N, 9.76%.

N-{3-[1-(2,4-dichloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-butyl}-acetamide (207)

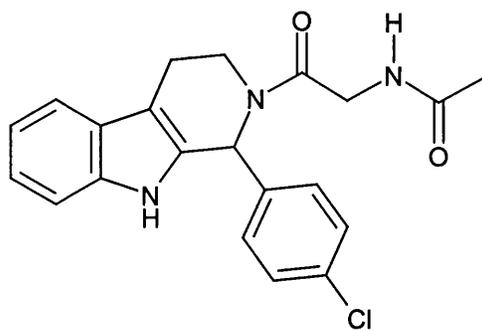


N-Acetyl butanoic acid (80 mg, 0.5 mmol) and **148** (158 mg, 0.5 mmol) gave the title as a white solid compound on trituration with ether and recrystallisation from ethyl acetate-ether, 100 mg, 0.23 mmol, 45% yield, m.p. 174-175 °C.

I.R. ν_{\max} cm^{-1} . 3300, 1650, 1631, 1567, 1557, 1469, 1428, 1373, 1273, 1185, 738.

^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.62-1.71 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.40-2.60 (m, 2H, CH_2), 2.78-2.92 (m, 2H, CH_2), 3.08 (q, $J = 6.0$ Hz 2H, CH_2), 3.17-3.26 (m, 1H, CH_2), 3.96-4.02 (m, 1H, CH_2), 6.80 (d, $J = 8.0$ Hz, 1H, ArH), 7.01 (dd, $J = 7.8$ Hz, 1H, ArH), 7.07-7.11 (m, 2H, CH + ArH), 7.29-7.35 (m, 2H, ArH), 7.49 (d, $J = 7.8$ Hz, 1H, ArH), 7.49 (d, $J = 1.2$ Hz, 1H, ArH), 7.87 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.0 (CH_2), 23.0 (CH_3), 25.3 (CH_2), 30.4 (CH_2), 37.1 (CH_2), 38.6 (CH_2), 49.2 (CH), 109.3 (ArC), 111.7 (ArCH), 118.3 (ArCH), 119.1 (ArCH), 121.8 (ArCH), 126.6 (ArC), 127.4 (ArCH), 129.6 (ArCH), 132.0 (ArC), 132.6 (ArCH), 133.7 (ArC), 134.5 (ArC), 136.8 (ArC), 137.4 (ArC), 169.6 (C=O), 172.1 (C=O). E.I.M.S. m/e 444/446 (MH^+ , 100%). Found: C, 62.13%, H, 5.15%, N, 9.41%. $\text{C}_{23}\text{H}_{23}\text{N}_3\text{Cl}_2\text{O}_2$ requires C, 62.17%, H, 5.22%, N, 9.46%.

N-{3-[1-(4-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-ethyl}-
acetamide (208)

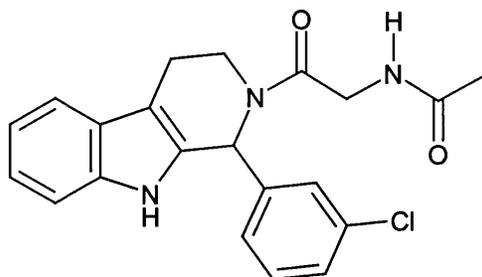


N-Acetyl glycine (59 mg, 0.5 mmol) and **162** (158 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 132 mg, 0.32 mmol, 63% yield, m.p. 189-190 °C.

I.R. ν_{\max} cm^{-1} . 3377, 3282, 1657, 1650, 1644, 1504, 1462, 1430, 1216, 846, 747.

^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.90 (s, 3H, CH_3), 2.78-2.94 (m, 2H, CH_2), 3.15-3.25 (m, 1H, CH_2), 3.96-4.08 (m, 1H, CH_2), 4.09-4.12 (m, 2H, CH_2), 6.80 (s, 1H, CH), 7.01 (dd, $J = 7.8$ Hz 1H, ArH), 7.02 (dd, $J = 7.8$ Hz, 1H, ArH), 7.09 (dd, $J = 7.8$ Hz, 1H, ArH), 7.23 (d, $J = 8.0$ Hz, 2H, ArH), 7.31 (d, $J = 7.8$ Hz, 1H, ArH), 7.41 (d, $J = 8.0$ Hz, 1H, ArH), 7.49 (d, $J = 7.8$ Hz, 1H, ArH), 8.11 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.8 (CH_2), 22.8 (CH_3), 39.0 (CH_2), 41.0 (CH_2), 51.4 (CH), 108.8 (ArC), 111.6 (ArCH), 118.4 (ArCH), 119.1 (ArCH), 121.8 (ArCH), 126.5 (ArC), 128.8 (ArCH x2), 130.3 (ArCH x2), 131.6 (ArC), 132.8 (ArC), 136.6 (ArC), 139.6 (ArC), 169.1 (C=O), 169.8 (C=O). E.I.M.S. m/e 382/384 (MH^+ , 100%). Found: C, 65.87%, H, 5.30%, N, 10.99%. $\text{C}_{21}\text{H}_{20}\text{N}_3\text{ClO}_2$ requires C, 66.05%, H, 5.28%, N, 11.00%.

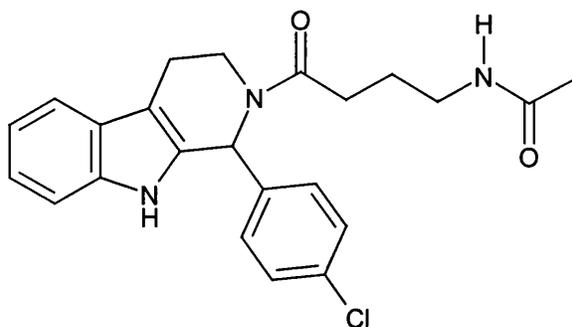
N-{3-[1-(3-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-ethyl}-
acetamide (209)



N-Acetyl glycine (59 mg, 0.5 mmol) and **163** (141 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 106 mg, 0.28 mmol, 56% yield, m.p. 208-209 °C.

I.R. ν_{\max} cm^{-1} . 3288, 1653, 1632, 1527, 1467, 1457, 1434, 1217, 742. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.90 (s, 3H, CH_3), 2.80-2.92 (m, 2H, CH_2), 3.19-3.28 (m, 1H, CH_2), 4.00-4.08 (m, 1H, CH_2), 4.09-4.13 (m, 2H, CH_2), 6.78 (s, 1H, CH), 7.02 (dd, $J = 7.8$ Hz 1H, ArH), 7.10 (dd, $J = 7.8$ Hz, 1H, ArH), 7.15-7.20 (m, 1H, ArH), 7.26 (s, 1H, ArH), 7.31 (d, $J = 7.8$ Hz, 1H, ArH), 7.38-7.42 (m, 2H, ArH), 7.50 (d, $J = 7.8$ Hz, 1H, ArH), 8.12 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.7 (CH_2), 22.8 (CH_3), 39.2 (CH_2), 41.0 (CH_2), 51.6 (CH), 109.1 (ArC), 111.7 (ArCH), 118.4 (ArCH), 119.1 (ArCH), 121.9 (ArCH), 126.5 (ArC), 127.0 (ArCH), 128.2 (ArCH x2), 130.8 (ArCH), 131.4 (ArC), 133.6 (ArC), 136.7 (ArC), 143.1 (ArC), 168.1 (C=O), 170.1 (C=O). E.I.M.S. m/e 763/765 (2MH^+ , 10%), 382/384 (MH^+ , 100%), 283/285 (20%). Found: C, 65.97%, H, 5.20%, N, 10.97%. $\text{C}_{21}\text{H}_{20}\text{N}_3\text{ClO}_2$ requires C, 66.05%, H, 5.28%, N, 11.00%.

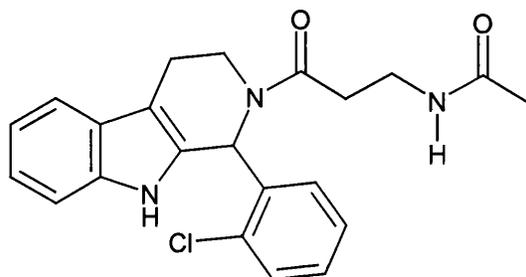
N-{3-[1-(4-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-butyl}-
acetamide (210)



N-Acetyl butanoic acid (80 mg, 0.5 mmol) and **162** (141 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 100 mg, 0.24 mmol, 49% yield, m.p. 156-157 °C.

I.R. ν_{\max} cm^{-1} . 3355, 1665, 1631, 1556, 1441, 1361, 1277, 1216, 1189, 740. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.65-1.72 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.40-2.56 (m, 2H, CH_2), 2.72-2.92 (m, 2H, CH_2), 3.02-3.21 (m, 3H, CH_2), 3.98-4.05 (m, 1H, CH_2), 6.87 (s, 1H, CH), 7.01 (dd, $J = 7.8$ Hz, 1H, ArH), 7.09 (dd, $J = 7.8$ Hz, 1H, ArH), 7.21 (d, $J = 7.8$ Hz, 2H, ArH), 7.30 (d, $J = 8.0$ Hz, 1H, ArH), 7.42 (d, $J = 7.8$ Hz, 2H, ArH), 7.49 (d, $J = 8.0$ Hz, 1H, ArH), 7.87 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.9 (CH_2), 23.0 (CH_3), 25.2 (CH_2), 30.3 (CH_2), 38.6 (CH_2), 39.4 (CH_2), 50.8 (CH), 108.9 (ArC), 111.6 (ArCH), 118.3 (ArCH), 119.0 (ArCH), 121.7 (ArCH), 126.5 (ArC), 128.8 (ArCH x2), 130.2 (ArCH x2), 131.9 (ArC), 132.6 (ArC), 136.6 (ArC), 139.9 (ArC), 169.4 (C=O), 171.2 (C=O). E.I.M.S. m/e 410/412 (MH^+ , 100%). Found: C, 67.28%, H, 5.87%, N, 10.22%. $\text{C}_{23}\text{H}_{24}\text{N}_3\text{ClO}_2$ requires C, 67.39%, H, 5.90%, N, 10.25%.

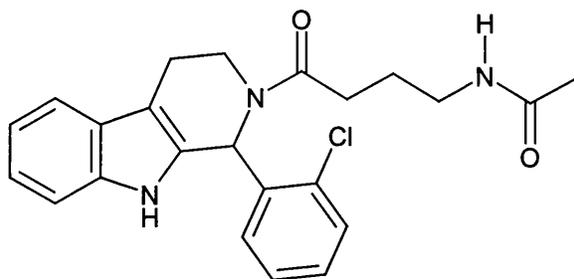
N-{3-[1-(2-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-propyl]-acetamide (211)}



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **161** (141 mg, 0.5 mmol) gave the title compound as a pale yellow solid on recrystallisation from ethyl acetate, 112 mg, 0.28 mmol, 57% yield, m.p. 218-219 °C.

I.R. ν_{\max} cm^{-1} . 3322, 3176, 1650, 1640, 1556, 1437, 1414, 1358, 1047, 759. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.79 (s, 3H, CH_3), 2.52-2.74 (m, 2H, CH_2), 2.76-2.92 (m, 2H, CH_2), 3.19-3.31 (m, 3H, CH_2), 3.92-4.00 (m, 1H, CH_2), 6.80 (d, $J = 8.0$ Hz, 1H, ArH), 7.01 (dd, $J = 7.8$ Hz 1H, ArH), 7.09 (dd, $J = 7.8$ Hz 1H, ArH), 7.12 (s, 1H, CH), 7.20-7.37 (m, 3H, ArH), 7.45-7.53 (m, 2H, ArH), 7.90 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.1 (CH_2), 22.9 (CH_3), 33.0 (CH_2), 35.5 (CH_2), 39.6 (CH_2), 49.6 (CH), 109.1 (ArC), 111.7 (ArCH), 118.3 (ArCH), 119.1 (ArCH), 121.7 (ArCH), 126.5 (ArC), 127.2 (ArCH), 130.0 (ArCH), 130.2 (ArCH), 131.4 (ArCH), 132.0 (ArC), 133.6 (ArC), 136.5 (ArC), 137.8 (ArC), 169.7 (C=O), 170.8 (C=O). E.I.M.S. m/e 396/398 (MH^+ , 100%). Found: C, 66.71%, H, 5.56%, N, 10.59%. $\text{C}_{22}\text{H}_{22}\text{N}_3\text{ClO}_2$ requires C, 66.75%, H, 5.60%, N, 10.61%.

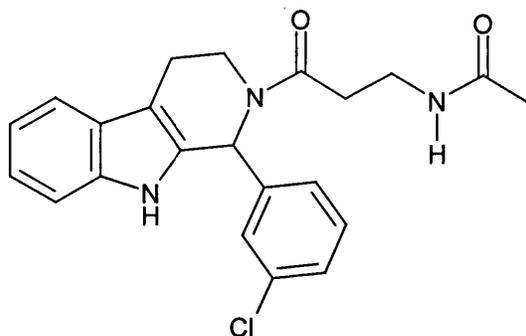
N-{3-[1-(2-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-butyl]-acetamide (212)}



N-Acetyl butanoic acid (80 mg, 0.5 mmol) and **161** (141 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 128 mg, 0.31 mmol, 63% yield, m.p. 216-217 °C.

I.R. ν_{\max} cm^{-1} . 3282, 1640, 1613, 1567, 1449, 1427, 1271, 1209, 741. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.62-1.71 (m, 2H, CH_2), 1.79 (s, 3H, CH_3), 2.37-2.59 (m, 2H, CH_2), 2.78-2.92 (m, 2H, CH_2), 3.00-3.09 (m, 2H, CH_2), 3.10-3.28 (m, 1H, CH_2), 3.94-4.03 (m, 1H, CH_2), 6.70-6.80 (d, $J = 7.8$ Hz, 1H, ArH), 6.98-7.15 (m, 3H, CH + ArH), 7.20-7.40 (m, 3H, ArH), 7.45-7.55 (m, 2H, ArH), 7.84 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 22.1 (CH_2), 23.0 (CH_3), 25.3 (CH_2), 30.4 (CH_2), 38.6 (CH_2), 39.6 (CH_2), 49.6 (CH), 109.1 (ArC), 111.7 (ArCH), 118.2 (ArCH), 119.0 (ArCH), 121.7 (ArCH), 126.6 (ArC), 127.2 (ArCH), 129.9 (ArCH), 130.2 (ArCH), 131.4 (ArCH), 132.3 (ArC), 133.6 (ArC), 136.5 (ArC), 138.1 (ArC), 169.6 (C=O), 172.2 (C=O). E.I.M.S. m/e 410/412 (MH^+ , 100%). Found: C, 67.25%, H, 5.82%, N, 10.19%. $\text{C}_{23}\text{H}_{24}\text{N}_3\text{ClO}_2$ requires C, 67.39%, H, 5.90%, N, 10.25%.

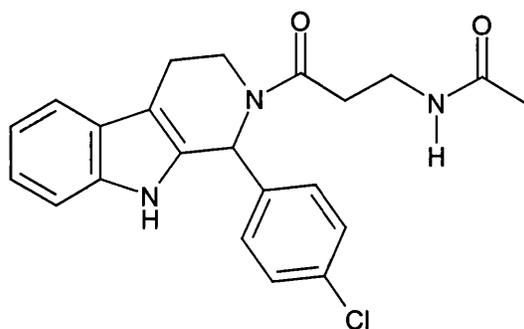
N-{3-[1-(3-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-propyl}-
acetamide (213)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **163** (158 mg, 0.5 mmol) gave the title compound as a pale yellow solid on recrystallisation from ethyl acetate, 147 mg, 0.37 mmol, 74% yield, m.p. 208-209 °C.

I.R. ν_{\max} cm^{-1} . 3309, 3191, 1657, 1614, 1556, 1470, 1454, 1426, 1355, 1305, 739, 710. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.80 (s, 3H, CH_3), 2.60-2.73 (m, 2H, CH_2), 2.76-2.92 (m, 2H, CH_2), 3.19-3.28 (m, 1H, CH_2), 3.30-3.40 (m, 2H, CH_2), 4.00-4.08 (m, 1H, CH_2), 6.89 (s, 1H, CH), 7.02 (dd, $J = 7.8$ Hz, 1H, ArH), 7.11 (dd, $J = 7.8$ Hz 1H, ArH), 7.19-7.23 (m, 1H, ArH), 7.28 (s, 1H, ArH), 7.32-7.42 (m, 3H, ArH), 7.49 (d, $J = 8.0$ Hz, 1H, ArH), 7.93 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.9 (CH_2), 22.9 (CH_3), 33.0 (CH_2), 35.4 (CH_2), 39.4 (CH_2), 51.1 (CH), 109.0 (ArC), 111.7 (ArCH), 118.4 (ArCH), 119.1 (ArCH), 121.8 (ArCH), 126.5 (ArC), 127.0 (ArCH), 128.1 (ArCH x2), 130.7 (ArCH), 131.5 (ArC), 133.5 (ArC), 136.6 (ArC), 143.3 (ArC), 169.6 (C=O), 170.2 (C=O). E.I.M.S. m/e 396/398 (MH^+ , 100%). Found: C, 66.73%, H, 5.59%, N, 10.60%. $\text{C}_{22}\text{H}_{22}\text{N}_3\text{ClO}_2$ requires C, 66.75%, H, 5.60%, N, 10.61%.

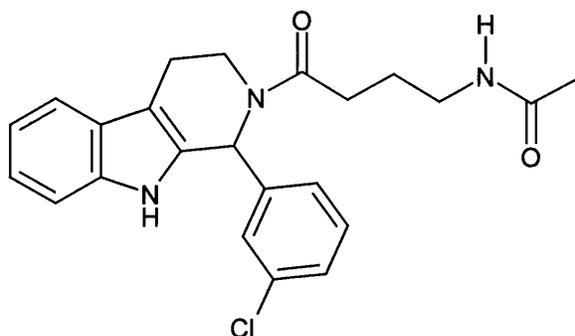
N-{3-[1-(4-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-propyl}-
acetamide (214)



N-Acetyl- β -alanine (66 mg, 0.5 mmol) and **162** (158 mg, 0.5 mmol) gave the title compound as a pale yellow solid on recrystallisation from ethyl acetate, 160 mg, 0.40 mmol, 80% yield, m.p. 211-212 °C.

I.R. ν_{\max} cm^{-1} . 3305, 3189, 1657, 1614, 1470, 1454, 1426, 1354, 739, 710. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.79 (s, 3H, CH_3), 2.60-2.69 (m, 2H, CH_2), 2.74-2.90 (m, 2H, CH_2), 3.13-3.22 (m, 1H, CH_2), 3.29-3.39 (m, 2H, CH_2), 3.97-4.04 (m, 1H, CH_2), 6.88 (s, 1H, CH), 7.01 (dd, $J = 7.8$ Hz, 1H, ArH), 7.09 (dd, $J = 7.8$ Hz 1H, ArH), 7.24 (d, $J = 8.0$ Hz, 2H, ArH), 7.32 (d, $J = 8.0$ Hz, 1H, ArH), 7.40 (d, $J = 8.0$ Hz, 2H, ArH), 7.49 (d, $J = 8.0$ Hz, 1H, ArH), 7.91 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.9 (CH_2), 22.9 (CH_3), 33.0 (CH_2), 35.5 (CH_2), 39.6 (CH_2), 50.8 (CH), 108.9 (ArC), 111.6 (ArCH), 118.3 (ArCH), 119.1 (ArCH), 121.7 (ArCH), 126.5 (ArC), 128.8 (ArCH x2), 130.2 (ArCH x2), 131.8 (ArC), 132.7 (ArC), 136.6 (ArC), 139.8 (ArC), 169.6 (C=O), 170.0 (C=O). E.I.M.S. m/e 396/398 (MH^+ , 100%). Found: C, 66.71%, H, 5.58%, N, 10.58%. $\text{C}_{22}\text{H}_{22}\text{N}_3\text{ClO}_2$ requires C, 66.75%, H, 5.60%, N, 10.61%.

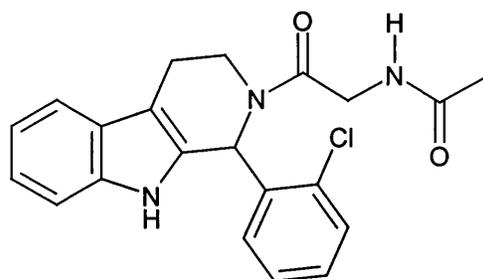
N-{3-[1-(3-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-butyl]-acetamide (215)}



N-Acetyl butanoic acid (80 mg, 0.5 mmol) and **163** (158 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 100 mg, 0.23 mmol, 45% yield, m.p. 205-206 °C.

I.R. ν_{\max} cm^{-1} . 3364, 1651, 1628, 1538, 1470, 1454, 1441, 1355, 1301, 1210, 1191, 743. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.65-1.75 (m, 2H, CH_2), 1.80 (s, 3H, CH_3), 2.45-2.56 (m, 2H, CH_2), 2.78-2.92 (m, 2H, CH_2), 3.04-3.13 (m, 2H, CH_2), 3.16-3.25 (m, 1H, CH_2), 4.00-4.08 (m, 1H, CH_2), 6.86 (s, 1H, CH), 7.01 (dd, $J = 7.8$ Hz, 1H, ArH), 7.11 (dd, $J = 7.8$ Hz, 1H, ArH), 7.15-7.25 (m, 2H, ArH), 7.30-7.40 (m, 3H, ArH), 7.49 (d, $J = 8.0$ Hz, 1H, ArH), 7.89 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.9 (CH_2), 23.0 (CH_3), 25.2 (CH_2), 30.3 (CH_2), 36.7 (CH_2), 38.6 (CH_2), 51.0 (CH), 109.0 (ArC), 111.6 (ArCH), 118.4 (ArCH), 119.1 (ArCH), 121.8 (ArCH), 126.5 (ArC), 127.0 (ArCH), 128.0 (ArCH x2), 130.8 (ArCH), 131.5 (ArC), 133.5 (ArC), 136.6 (ArC), 143.4 (ArC), 169.4 (C=O), 171.4 (C=O). E.I.M.S. m/e 410/412 (MH^+ , 100%). Found: C, 67.30%, H, 5.86%, N, 10.23%. $\text{C}_{23}\text{H}_{24}\text{N}_3\text{ClO}_2$ requires C, 67.39%, H, 5.90%, N, 10.25%.

N-{3-[1-(2-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-ethyl}-
acetamide (216)

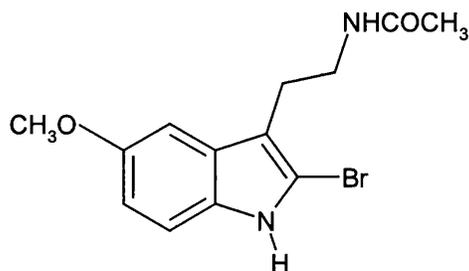


N-Acetyl glycine (59 mg, 0.5 mmol) and **161** (141 mg, 0.5 mmol) gave the title compound as a white solid on trituration with ether and recrystallisation from ethyl acetate-ether, 106 mg, 0.28 mmol, 56% yield, m.p. 229-230 °C.

I.R. ν_{\max} cm^{-1} . 3271, 3206, 1660, 1633, 1622, 1548, 1469, 1441, 1277, 1218, 842, 759, 728. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.90 (s, 3H, CH_3), 2.80-2.98 (m, 2H, CH_2), 3.22-3.32 (m, 1H, CH_2), 3.95-4.08 (m, 2H, CH_2), 4.14-4.22 (m, 1H, CH_2), 6.84 (m, 1H, ArH), 7.00-7.13 (m, 3H, ArH+CH), 7.20-7.40 (m, 3H, ArH), 7.46-7.55 (m, 2H, ArH), 8.09 (t, $J = 5.6$ Hz, 1H, NH), 11.00 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 21.8 (CH_2), 22.8 (CH_3), 39.1 (CH_2), 40.9 (CH_2), 50.1 (CH), 109.1 (ArC), 111.7 (ArCH), 118.3 (ArCH), 119.1 (ArCH), 121.8 (ArCH), 126.5 (ArC), 127.0 (ArC), 127.3 (ArCH), 130.0 (ArCH), 130.2 (ArCH), 131.4 (ArCH), 133.6 (ArC), 136.7 (ArC), 143.1 (ArC), 168.1 (C=O), 170.1 (C=O). E.I.M.S. m/e 763/765 (2MH^+ , 10%), 382/384 (MH^+ , 100%). Found: C, 66.03%, H, 5.25%, N, 10.97%. $\text{C}_{21}\text{H}_{20}\text{N}_3\text{ClO}_2$ requires C, 66.05%, H, 5.28%, N, 11.00%.

Experimental for Chapter 4

2-Bromomelatonin (226)⁹

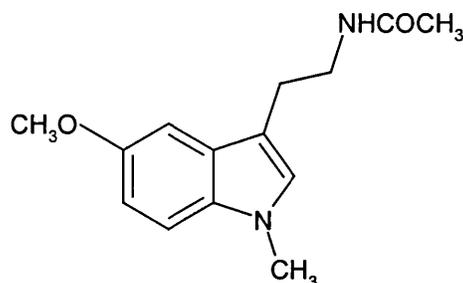


Melatonin (3.48 g, 15 mmol,) was dissolved in glacial acetic acid (30 mL) under nitrogen. *N*-Bromosuccinimide (2.67 g, 15 mmol) in glacial acetic acid (100 mL) was added dropwise over 3 hr and the reaction left to stir at room temperature for a further 3 hr. After neutralisation with 2M sodium hydroxide, the product was extracted with ethyl acetate (3 x 50 mL), washed with saturated *aq.* sodium chloride (2 x 100 mL) and dried over magnesium sulphate. The solution was filtered and the filtrate evaporated *in vacuo* to give a yellow oil which was purified by column chromatography using ethyl acetate as eluent. A white solid was obtained on evaporation *in vacuo* of the pure fractions, 1.47 g, 4.73 mmole, 32% yield, m.p. 144-145 °C.

I.R. ν_{\max} cm^{-1} 221, 3105, 1641, 1616, 1576, 1485, 1435, 1309, 1244, 1219, 1176, 797. ^1H NMR (360 MHz; d_6 -DMSO) δ_{H} 1.78 (s, 3H, CH_3), 2.75 (t, $J = 7.0$ Hz, 2H, CH_2), 3.23 (q, $J = 7.0$ Hz, 2H, CH_2), 3.75 (s, 3H, CH_3), 6.73 (dd, $J = 8.7$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.02 (d, $J = 2.2$ Hz, 1H, ArH), 7.17 (d, $J = 8.8$ Hz, 1H, ArH), 7.85 (s, 1H, NH), 11.37 (br.s, 1H, NH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 23.6 (CH_3), 25.8 (CH_2), 39.5 (CH_2), 56.4 (CH_3), 101.0 (ArCH), 109.7 (ArC), 112.2 (ArCH), 112.3 (ArCH), 112.4 (ArC), 128.6 (ArC), 132.3 (ArC), 154.4 (ArC), 169.9 (C=O). *mz* 310\312(40%), 253/251(100%), 240/238(100%), 231(80%), 225\223(50%), 197\195(35%), 159(50%), 116(40%), 89(40%), 43(75%). Found: MH^+ 310.03031. Formula requires 310.03169. Found: C,

49.89%, H, 4.83%, N, 8.86%. $C_{13}H_{15}N_2BrO_2$ requires C, 50.17%, H, 4.86%, N, 9.00%.

N-methyl-melatonin (231)¹⁰



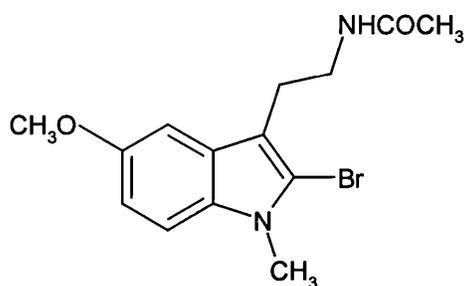
Melatonin (232 mg, 1 mmol) was dissolved in anhydrous THF (15 mL) and cooled to 0 °C. Sodium hydride (80 mg of a 60% suspension in mineral oil, 2 mmol) was then added portionwise over fifteen minutes under a nitrogen blanket. The cooling bath was removed and the reaction stirred at room temperature for 2 hr prior to the dropwise addition of methyl iodide (142 mg, 1 mmole). The reaction was stirred for 5 hr at room temperature (monitored by HPLC) and then partitioned between ethyl acetate (30 mL) and water (30 mL) when all of the starting material had been consumed. The aqueous layer was back-washed with ethyl acetate (30 mL), and the combined organic layers washed with saturated sodium chloride (2 x 30 mL) and dried over magnesium sulphate. The solution was filtered and the filtrate evaporated *in vacuo* to give a pale yellow solid. This product was subjected to column chromatography eluting with ethyl acetate and the pure fractions combined and evaporated *in vacuo*. Recrystallisation from ethyl acetate gave the title compound as a white solid, 195 mg, 0.79 mmole, 79% yield, m.p. 101-102 °C (Lit. m.p. 101-102 °C).

I.R. ν_{\max} cm^{-1} 3310, 2928, 1622, 1567, 1495, 1429, 1230, 1180 1039, 858, 784.

1H NMR (250 MHz; $CDCl_3$) δ_H 1.92 (s, 3H, CH_3), 2.92 (t, $J = 7.0$ Hz, 2H, CH_2), 3.55 (q, $J = 7.0$ Hz, 2H, CH_2), 3.72 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 5.65 (br.s, 1H, NH), 6.85 (s, 1H, ArH), 7.00 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH),

7.02 (d, $J = 2.2$ Hz, 1H, ArH), 7.34 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (63 MHz; CDCl_3) δ_{C} 23.3 (CH_3), 25.2 (CH_2), 32.8 (CH_3), 39.9 (CH_2), 55.9 (CH_3), 100.6 (ArCH), 110.0 (ArCH), 110.1 (ArC), 112.0 (ArCH), 127.3 (ArCH), 128.0 (ArC), 132.5 (ArC), 153.8 (ArC), 170.0 (C=O). mz 2M^{+1} 493(20%), M^{+1} 247(100%). Found: MH^+ 247.144564. Formula requires 247.144563.

1-methyl-2-bromomelatonin (232)

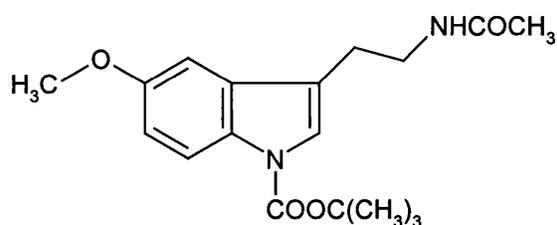


2-Bromomelatonin (**226**, 311 mg, 1 mmol) was dissolved in dry THF (15 mL) at 0 °C and 60% sodium hydride (80 mg, 2 mmol) was added portionwise over 15 min. The cooling bath was removed and the reaction stirred at room temperature for 2 hr and then methyl iodide (142 mg, 1 mmol) was added dropwise. The reaction was stirred for five hours at room temperature and then partitioned between ethyl acetate and water. The aqueous layer was back-washed with ethyl acetate, and the combined organic layers washed with saturated sodium chloride and dried over magnesium sulphate. The solution was filtered and the filtrate evaporated *in vacuo* to give a yellow solid. This product was triturated with ether to give a white solid which was recrystallised from ethyl acetate to give the required product, 150 mg, 0.46 mmole, 46% yield, m.p. 132-133 °C.

I.R. ν_{max} cm^{-1} 3210, 1640, 1612, 1568, 1480, 1248, 1210, 802. ^1H NMR (360 MHz; d_6 -DMSO) δ_{H} 1.77 (s, 3H, CH_3), 2.79 (t, $J = 7.0$ Hz, 2H, CH_2), 3.25 (q, $J = 7.0$ Hz, 2H, CH_2), 3.68 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 6.81 (dd, $J = 8.8$ Hz, $J = 2.4$ Hz, 1H, ArH), 7.06 (d, $J = 2.4$ Hz, 1H, ArH), 7.34 (d, $J = 8.8$ Hz, 1H, ArH), 7.85 (s, 1H, NH). ^{13}C NMR (90 MHz; d_6 -DMSO) δ_{C} 23.1 (CH_3), 25.6

(CH₂), 31.8 (CH₃), 39.1 (CH₂), 55.7 (CH₃), 100.3 (ArCH), 111.1 (ArCH), 111.6 (ArC), 111.8 (ArCH) 113.7 (ArC), 127.4 (ArC), 132.3 (ArC), 154.0 (ArC), 169.5 (C=O). *mz* 326\324 (70%), 267\265 (95%), 254\252 (100%), 239\237 (70%), 211\209 (50%). Found: C, 51.58%, H, 5.17%, N, 8.52%. C₁₄H₁₇N₂BrO₂ requires C, 51.71%, H, 5.27%, N, 8.61%.

N-Butoxycarbonyl-melatonin (235)

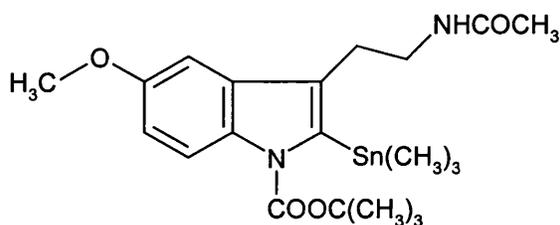


Melatonin (2.80 g 12 mmol) was dissolved in dry THF (35 mL) and added dropwise to a stirred suspension of 2.40 g, 60% sodium hydride (2.40 g, 60 mmole) in anhydrous THF (50 mL) at 0 °C under dry nitrogen. The reaction was stirred for 1.5 hr at room temperature and then cooled to 0 °C in an ice bath. A solution of Boc-ON (14.78 g, 60 mmol) in anhydrous THF (90 mL) was added in a slow trickle and the reaction left to stir at room temperature until all of the starting material had been consumed (approx 4 hr). The reaction was cooled in ice and 15 mL of water carefully added. After stirring at room temperature for 15 min the reaction mixture was separated and the *aq.* layer extracted with ether (4 x 30 mL). The organic layers were combined, dried over magnesium sulphate and filtered. Evaporation of the filtrate *in vacuo* gave a yellow oil which was triturated with 5% ethyl acetate in hexane to give a white solid which was filtered and dried on the pad, 3.59 g, 10.8 mmole, 90% yield, m.p.126-127 °C. A second crop was obtained from the filtrate and recrystallised from ethyl acetate-hexane, 0.31 g, 0.93 mmole m.p.126-127 °C, to give a combined yield of 98%.

I.R. ν_{\max} cm⁻¹ 1726, 1637, 1556, 1479, 1450, 1395, 1294, 1257, 1161, 1086, 1041. ¹H NMR (360 MHz; CDCl₃) δ_{H} 1.65 (s, 9H, CH₃), 1.95 (s, 3H, CH₃),

2.85 (t, $J = 7.0$ Hz, 2H, CH₂), 3.55 (q, $J = 7.0$ Hz, 2H, CH₂), 3.85 (s, 3H, CH₃), 5.95 (br.s, 1H, NH), 6.90 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.00 (d, $J = 2.2$ Hz, 1H, ArH), 7.40 (s, 1H, ArH), 8.00 (d, $J = 8.8$ Hz, 1H, ArH). ¹³C NMR (90 MHz; CDCl₃) δ_C 23.6 (CH₃), 25.5 (CH₂), 28.6 (CH₃ x3), 39.6 (CH₂), 56.1 (CH₃), 83.8 (C), 102.3 (ArCH), 113.5 (ArCH), 116.4 (ArCH), 118.0 (ArC) 124.1 (ArCH), 130.7 (ArC), 131.7 (ArC), 150.0 (C), 156.4 (ArC), 170.5 (C=O). *mz* M⁺ 332 (25%), 232 (25%), 217 (70%), 173 (100%), 160 (95%), 145 (25%), 117 (20%), 57 (85%). Found: C, 65.27%, H, 7.40%, N, 8.44%. C₁₈H₂₄N₂O₄ requires C, 65.06%, H, 7.28%, N, 8.43%.

2-Trimethylstannyl-N-butoxycarbonyl-melatonin (236)

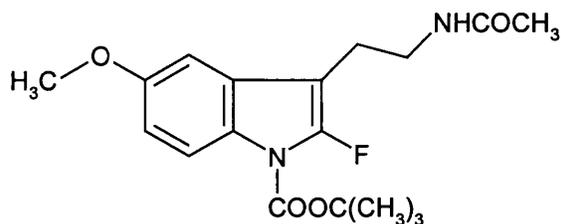


Freshly distilled di-isopropylamine (2.13 mL, 15.2 mmol) was added to anhydrous THF (15 mL) at -15 °C and n-butyl lithium (8.9 mL, 14.2 mmol) was then added *via* syringe. The reaction was stirred at -15 °C for 20 min before being cooled to -75 °C. A cold solution of N-boc melatonin (**235**, 2.25 g, 6.7 mmol) in of anhydrous THF (30 mL) was added *via* syringe over 10 min. The reaction was then stirred at -70 °C for 3 hr before the addition of a solution of a 1M solution of trimethyl tin chloride in THF (7.0 mL, 7.0 mmole). Stirring was continued at -70 °C for 90 min and then at room temperature for 18 hr. The reaction was quenched by the addition of saturated *aq.* potassium fluoride (25 mL) and the reaction stirred for 30 mins. The organic layer was separated, dried over magnesium sulphate and filtered. Evaporation of the filtrate *in vacuo* gave a yellow oil which was subjected to column chromatography using ethyl acetate/cyclohexane/ triethylamine 60/39/1 as eluent. Evaporation of the major

product fractions gave white needle-like crystals, 2.97 g, 5.98 mmole, 89% yield, m.p.118-119 °C.

I.R. ν_{\max} cm^{-1} 2980, 1707, 1665, 1537, 1470, 1446, 1375, 1329, 1286, 1248,1225, 1161, 1099, 758. ^1H NMR (360 MHz; CDCl_3) δ_{H} 0.30 (t, $J = 29$ Hz, 9H, CH_3), 1.65 (s, 9H, CH_3), 1.90 (s, 3H, CH_3), 2.95 (t, $J = 7.0$ Hz, 2H, CH_2), 3.50 (q, $J = 7.0$ Hz, 2H, CH_2), 3.85 (s, 3H, CH_3), 5.55 (br.s, 1H, NH), 6.85 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.05 (d, $J = 2.2$ Hz, 1H, ArH), 7.85 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} -4.38 (C-Sn x3), 23.7 (CH_3), 26.0 (CH_2), 28.6 (CH_3 x3), 40.8 (CH_2), 56.2 (CH_3), 84.4 (C), 101.3 (ArCH), 113.3 (ArCH), 116.5 (ArCH), 129.1(ArC), 132.4 (ArC), 133.5 (ArC), 140.4 (ArC), 152.5 (C), 156.3 (ArC), 170.3 (C=O). FAB mass spec shows a weak M^{+1} 497 (10%) and $\text{M}^{+\text{Na}}$ 519 (85%). E.I. mass.spec shows fragmentation consistent with structure i.e 481 (M^{Me} , 70%), 425 (100%), 381 (80%), 349 (80%), 322 (60%), 173 (50%), 161 (50%). The clustering pattern around each peak agrees with the theoretical isotope pattern for Sn. Found: C, 50.75%, H, 6.37%, N, 5.69%. $\text{C}_{21}\text{H}_{32}\text{N}_2\text{O}_4\text{Sn}$ requires C, 50.93%, H, 6.46%, N, 5.66%.

2-Fluoro-N-butoxycarbonyl-melatonin (237)

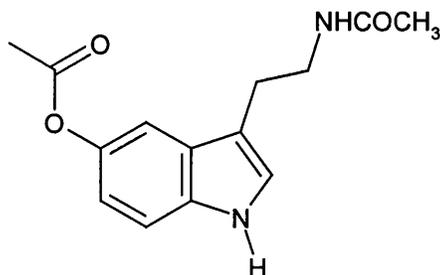


2-Trimethylstannyl-*N*-*boc* melatonin (**236**, 2.50 g, 7.5 mmol) was dissolved in dry acetonitrile (40 mL) and cooled to -30 °C. Selectfluor (3.25 g, 7.5 mmol) was added in one portion and the reaction stirred at -30 °C until tlc indicated that the starting material had been consumed (approx 3 hr). The reaction was allowed to reach room temperature, diluted with 25 mL of saturated *aq.* potassium fluoride and stirred for 30 minutes. The reaction mixture was then evaporated *in vacuo* and the residue partitioned between ether (60 mL) and saturated *aq.* potassium

fluoride (40 mL). The organic layer was washed sequentially with water (25 mL), 1M *aq.* potassium hydrogen sulphate (25 mL), saturated *aq.* sodium hydrogen carbonate (25 mL), and finally, saturated *aq.* sodium chloride (2 x 25 mL). The organic layer was dried over magnesium sulphate, filtered and the filtrate evaporated *in vacuo* to give a viscous yellow oil which was purified by column chromatography on silica gel using ethyl acetate as eluent. The single major component was isolated and evaporated to give a white solid, 1.04 g, 2.97 mmole, 40% yield, m.p.111-112 °C. This solid yellowed slightly on storage at 0 °C.

I.R. ν_{\max} cm^{-1} . 1738, 1655, 1552, 1479, 1456, 1371, 1325, 1240, 1140, 758. ^1H NMR (360 MHz; CDCl_3) δ_{H} 1.65 (s, 9H, CH_3), 1.93 (s, 3H, CH_3), 2.82 (t, $J = 7.0$ Hz, 2H, CH_2), 3.52 (q, $J = 7.0$ Hz, 2H, CH_2), 3.85 (s, 3H, CH_3), 5.65 (br.s, 1H, NH), 6.90 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.98 (d, $J = 2.2$ Hz, 1H, ArH), 7.95 (d, $J = 8.8$ Hz, 1H, ArH). ^{13}C NMR (90 MHz; CDCl_3) δ_{C} 22.5 (CH_2), 23.6 (CH_3), 28.5 (CH_3 x3), 39.1 (CH_2), 56.1 (CH_3), 84.7 (C), 95.1 (ArC), 102.5 (ArCH), 112.3 (ArCH), 116.3 (ArCH), 124.9 (ArC), 127.9 (ArC), 148.6 (C), 150.8 (d, $J = 265$ Hz, C-F), 156.9 (ArC), 170.4 (C=O). *mz.* 350 (M^+ 40%), 250 (70%), 230 (25%), 191 (100%), 178 (95%), 163 (40%), 148 (20%), 135 (50%), 57 (100%), 43 (80%). Accurate Mass Measurement: Calcd: 350.16419 found: 350.16448. Found: C, 61.37%, H, 6.40%, N, 8.14%. $\text{C}_{18}\text{H}_{23}\text{N}_2\text{FO}_4$ requires C, 61.70%, H, 6.61%, N, 8.00%.

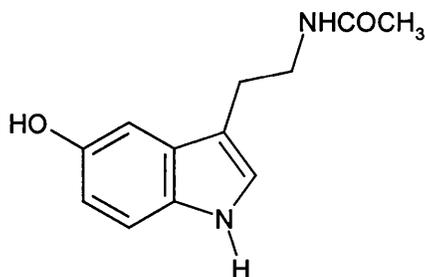
5-Acetoxy-3-(2 acetylamino-ethyl)-indole (242)¹⁰



Serotonin hydrochloride (10 g, 47 mmole) was stirred vigorously with sodium carbonate (10 g, 94 mmole) in 100 mL chloroform and 1 mL water. Acetic anhydride (20 mL, 18.5 g, 180 mmole) was added dropwise over 30 min and the mixture stirred for a further 3 hours. The mixture was then poured carefully into 500 mL of 10% *aq.* sodium carbonate solution and stirred for 1 hour. The chloroform layer was then separated and dried over magnesium sulphate. The solution was filtered and the filtrate evaporated *in vacuo* to give a highly viscous colourless oil, 11.97 g, 46 mmole, 98% yield, m.p. 103-104 °C.

I.R. ν_{\max} cm^{-1} . 3371, 2934, 1746, 1632, 1572, 1485, 1373, 1234, 800, 623, 607. ^1H NMR (400 MHz; CDCl_3) δ_{H} 1.83 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.78 (t, $J = 7.8$ Hz, 2H, CH_2), 3.40 (q, $J = 7.8$ Hz, 2H, CH_2), 5.85 (t, $J = 5.4$ Hz, 1H, NH), 6.80 (d, $J = 2.2$ Hz, 1H, H2), 6.82 (dd, $J = 8.0$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.20 (d, $J = 8.0$ Hz, 1H, ArH), 7.22 (d, $J = 2.2$ Hz, 1H, ArH), 9.04 (br.s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 21.6 (CH_3), 23.6 (CH_3), 25.4 (CH_2), 40.3 (CH_2), 111.0 (ArCH), 112.3 (ArCH), 113.1 (ArC), 116.2 (ArCH), 124.2 (ArCH), 128.0 (ArC), 134.8 (ArC), 144.3 (ArC), 171.0 (C=O), 171.4 (C=O). *mz* 261 (MH^+ 100%), 219 (20%), 202 (10%). Found: MH^+ 261.124282. Formula requires . Found: C, 63.34%, H, 6.13%, N, 10.55%. $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_3$ requires C, 64.60%, H, 6.20%, N, 10.55%.

3-(2-acetylamino-ethyl)-indol-5-ol (243)¹⁰



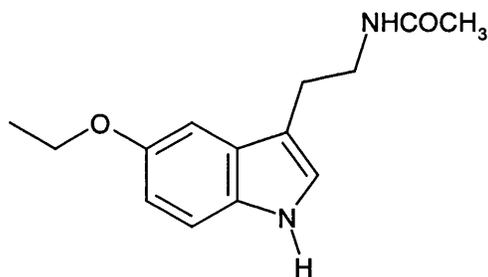
5-Acetoxy-3-(2-acetylamino-ethyl)-indole (242, 9.07 g, 35 mmole) was treated with 1M sodium hydroxide (90 mL) and just enough methanol to give a clear

solution. This was allowed to stand at room temperature overnight and then poured into 1000 mL saturated *aq.* sodium chloride. The solution was extracted with ethyl acetate (6x 100 mL) and the organic layers combined, dried over magnesium sulphate and filtered. The filtrate was evaporated *in vacuo* to give a very pale yellow solid after trituration with ether and recrystallisation from ethyl acetate, 5.68 g, 26 mmole, 74% yield, m.p. 122-123 °C (Lit. m.p. 121-122 °C).

I.R. ν_{\max} cm^{-1} . 3371, 2934, 1746, 1632, 1572, 1485, 1373, 1234, 800, 623, 607.

^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.81 (s, 3H, CH_3), 2.72 (t, $J = 7.8$ Hz, 2H, CH_2), 3.40 (q, $J = 7.8$ Hz, 2H, CH_2), 6.59 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.81(s, 1H, H2), 7.03 (s, 1H, ArH), 7.11 (d, $J = 8.8$ Hz, 1H, ArH), 7.93 (br.s, 1H, NH), 8.60 (s, 1H, OH), 10.50 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 20.9 (CH_3), 23.5 (CH_2), 37.6 (CH_2), 100.4 (ArCH), 109.1 (ArC), 109.4 (ArCH), 109.8 (ArCH), 121.2 (ArCH), 126.1 (ArC), 129.0 (ArC), 148.3 (ArC), 167.2 (C=O). *mz* 2 MH^+ 437 (15%), MH^+ 219 (100%), 160 (70%).

N-2-(5-ethoxy-indol-3-yl)-ethyl acetamide (244)¹¹



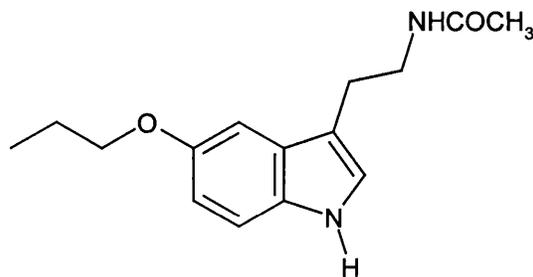
3-(2-acetylamino-ethyl)-indol-5-ol (**243**, 436 mg, 2 mmole) was dissolved in 20 mL anhydrous DMF and cooled to 5°C. Sodium Hydride (2 mmole, 80 mg of a 60% suspension in mineral oil) was added and the reaction stirred under nitrogen for 20 minutes. Ethyl iodide (620 mg, 4 mmole) was added dropwise and the reaction stirred at room temperature for 18 hours. The mixture was then carefully poured into 200 mL water and extracted several times with dichloromethane. The organic fractions were combined and washed sequentially with 5% citric acid,

saturated aq. sodium bicarbonate, and finally with brine. The organic layer was dried over magnesium sulphate, filtered and the filtrate evaporated *in vacuo* to give a pale yellow oil. This was subjected to column chromatography (eluting with EtOAc) and the title compound obtained as an oily solid. Lyophilisation from dioxan gave a white solid, 142 mg, 0.58 mmole, 29% yield, m.p. 84-85 °C (Lit m.p. 82-83 °C).

I.R. ν_{\max} cm^{-1} . 3245, 2934, 1667, 1650, 1585, 1572, 1477, 1297, 1216, 1112, 1048, 872, 799. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.43 (t, $J = 7.8$ Hz, 3H, CH_3), 1.91 (s, 3H, CH_3), 2.87 (t, $J = 7.8$ Hz, 2H, CH_2), 3.40 (q, $J = 7.8$ Hz, 2H, CH_2), 4.11 (q, $J = 7.8$ Hz, 2H, CH_2), 6.81 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.11 (s, 1H, ArH), 7.19 (s, 1H, ArH), 7.32 (d, $J = 8.8$ Hz, 1H, ArH), 8.07 (br.s, 1H, H), 10.60 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 15.3 (CH_3), 23.0 (CH_3), 25.6 (CH_2), 39.8 (CH_2), 63.7 (CH_2), 101.5 (ArCH), 111.8 (ArCH), 112.0 (ArC), 112.3 (ArCH), 123.6 (ArCH), 126.2 (ArC), 131.7 (ArC), 152.6 (ArC), 169.4 (C=O). m/z 2M^{+1} 493 (10%), M^{+1} 247 (100%). Found: MH^{+} 247.145469. Formula requires 247.144653. Found: C, 67.27; H, 7.42; N, 10.84. $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_2$ requires C, 68.27%, H, 7.37%, N, 11.37%

The following compounds were prepared in a similar manner:

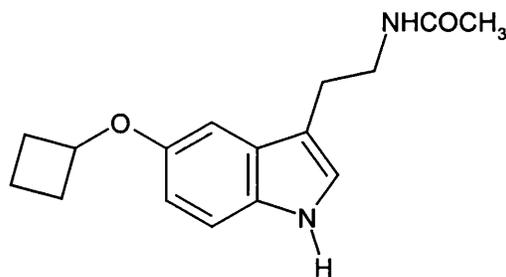
N-2-(5-propyloxy-indol-3-yl)-ethyl acetamide (245)¹²



3-(2-acetylamino-ethyl)-indol-5-ol (**243**, 436 mg, 2 mmole) and 3-bromopropane (488 mg, 4 mmole) gave the title compound as a white solid after crystallisation from ethylacetate/ether, 312 mg, 1.20 mmole, 60% yield, m.p. 96-97 °C (Lit. no m.p. recorded).

I.R. ν_{\max} cm^{-1} . 3251, 2963, 1625, 1550, 1462, 1287, 1216, 1017, 829, 700. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.00 (t, $J = 7.8$ Hz, 3H, CH_3), 1.75 (q, $J = 7.8$ Hz, 2H, CH_2), 1.81 (s, 3H, CH_3), 2.76 (t, $J = 7.8$ Hz, 2H, CH_2), 3.30 (q, $J = 7.8$ Hz, 2H, CH_2), 3.90 (t, $J = 7.8$ Hz, 2H, CH_2), 6.70 (dd, $J = 8.0$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.01 (s, 1H, ArH), 7.09 (s, 1H, ArH), 7.21 (d, $J = 8.0$ Hz, 1H, ArH), 7.94 (br.s, 1H, NH), 10.60 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} . 10.9 (CH_3), 22.7 (CH_2), 23.1 (CH_3), 25.6 (CH_2), 39.8 (CH_2), 69.8 (CH_2), 101.6 (ArCH), 111.8 (ArCH), 112.0 (ArC), 112.3 (ArCH), 123.6 (ArCH), 128.1 (ArC), 131.7 (ArC), 152.6 (ArC), 169.4 (C=O). mz 2M^{+1} 521 (80%), M^{+1} 261 (100%). Found: MH^{+} 261.160053. Formula requires 261.160303. Found: C, 67.98; H, 7.68; N, 10.34. $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_2$ requires C, 69.20%, H, 7.74%, N, 10.76%.

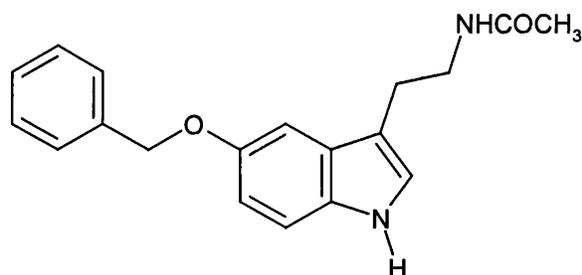
N-2-(5-cyclobutyloxy-indol-3-yl)-ethyl acetamide (**246**)



3-(2-acetylamino-ethyl)-indol-5-ol (**243**, 436 mg, 2 mmole) and cyclobutyl bromide (536 mg, 4 mmole) gave the title compound as a white solid after recrystallisation from ethylacetate/ether, 255 mg, 0.98 mmole, 49% yield, m.p. 106-107 °C.

I.R. ν_{\max} cm^{-1} . 3304, 1632, 1567, 1484, 1293, 1211, 1197, 1090, 829, 806. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.60-1.80 (m, 2H, CH_2), 1.81 (s, 3H, CH_3), 2.00-2.10 (m, 2H, CH_2), 2.38-2.47 (m, 2H, CH_2), 2.74 (t, $J = 7.8$ Hz, 2H, CH_2), 3.28 (q, $J = 7.8$ Hz, 2H, CH_2), 4.65 (qi, $J = 7.8$ Hz, 1H, CH), 6.65 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 6.90 (d, $J = 2.2$ Hz, 1H, ArH), 7.10 (d, 1H, ArH), 7.21 (d, $J = 8.8$ Hz, 1H, ArH), 7.96 (br.s, 1H, NH), 10.60 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} . 13.2 (CH_2), 23.1 (CH_3), 25.7 (CH_2), 30.8 (2x CH_2), 39.8 (CH_2), 71.4 (CH), 102.1 (ArCH), 111.9 (ArCH), 112.0 (ArC), 112.4 (ArCH), 123.6 (ArCH), 127.4 (ArC), 132.3 (ArC), 148.5 (ArC), 167.0 (C=O). m/z 2M^{+1} 521 (80%), M^{+1} 261 (100%). Found: MH^+ 273.159819. Formula requires 273.160303. Found: C, 70.09; H, 7.36; N, 10.26. $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}_2$ requires C, 70.56%, H, 7.40%, N, 10.29%.

N-2-(5-benzyloxy-indol-3-yl)-ethyl acetamide (247) ¹²

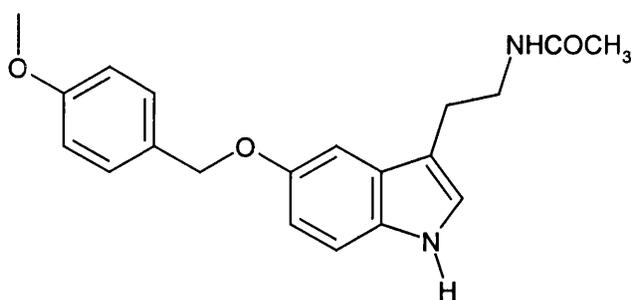


3-(2-acetylamino-ethyl)-indol-5-ol (243, 436 mg, 2 mmole) and benzyl bromide (680 mg, 4 mmole) gave the title compound as a white solid on recrystallisation from ethyl acetate, 296 mg, 0.96 mmole, 48% yield, m.p. 123-124 °C (Lit m.p. 132-133 °C).

I.R. ν_{\max} cm^{-1} . 3364, 3261, 1643, 1551, 1464, 1225, 1010, 796, 750, 704. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.81 (s, 3H, CH_3), 2.77 (t, $J = 7.8$ Hz, 2H, CH_2), 3.30 (q, $J = 7.8$ Hz, 2H, CH_2), 5.09 (s, 2H, CH_2), 6.70 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.12 (dd, $J = 12.0$ Hz, $J = 2.2$ Hz, 2H, ArH), 7.23 (d, $J = 8.8$ Hz, 1H, ArH), 7.33 (m, 1H, ArH), 7.38 (m, 2H, ArH), 7.49 (m, 2H, ArH), 7.97 (br.s, 1H,

NH), 10.60 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} . 23.1 (CH₃), 25.2 (CH₂), 39.9 (CH₂), 70.2 (CH₂), 101.0 (ArCH), 110.8 (ArCH), 110.9 (ArC), 111.2 (ArCH), 122.6 (ArCH), 126.7 (ArC), 126.8 (ArCH), 126.9 (2x ArCH), 127.6 (2x ArCH), 130.8 (ArC), 136.9 (ArC), 151.2 (ArC), 168.2 (C=O). mz 2M^{+1} 617(80%), M^{+1} 309(100%). Found: MH^{+} 309.160303. Formula requires 309.160303. Found: C, 73.78; H, 6.38; N, 8.93. C₁₉H₂₀N₂O₂ requires C, 74.00%, H, 6.54%, N, 9.08%.

N-2-{5-(4-methoxybenzyloxy)-indol-3-yl}-ethyl acetamide (248)

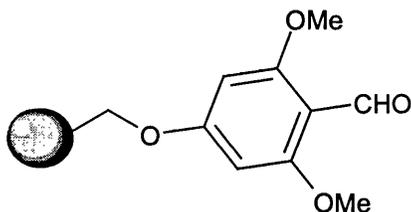


3-(2-acetylamino-ethyl)-indol-5-ol (**243**, 436 mg, 2 mmole) and 4-methoxybenzyl bromide (800 mg, 4 mole) gave the title compound as a white solid after recrystallisation from ethyl acetate, 230 mg, 0.68 mmole, 34% yield, m.p. 145-146 °C.

IR ν_{max} cm^{-1} . 3260, 1628, 1582, 1510, 1485, 1377, 1290, 1250, 1228, 1189, 1033, 820, 798. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 1.81 (s, 3H, CH₃), 2.77 (t, J = 7.8 Hz, 2H, CH₂), 3.29 (q, J = 7.8 Hz, 2H, CH₂), 3.75 (s, 3H, CH₃), 5.00 (s, 2H, CH₂), 6.76 (dd, J = 8.8 Hz, J = 2.2 Hz, 1H, ArH), 6.93 (dd, J = 12 Hz, J = 2.2 Hz, 2H, ArH), 7.11 (m, 2H, ArH), 7.22 (d, J = 8.8 Hz, 1H, ArH), 7.40 (m, 2H, ArH), 7.97 (br.s, 1H, NH), 10.60 (s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} . 23.1 (CH₃), 25.6 (CH₂), 39.8 (CH₂), 55.4 (CH₃), 69.9 (CH₂), 102.2 (ArCH), 112.0 (ArCH), 112.1 (ArC), 112.3 (ArCH), 114.1 (2x ArCH), 123.7 (ArCH), 127.4 (ArC), 129.8 (2x ArCH), 130.0 (ArC), 132.0 (ArC), 152.1 (ArC), 158.6 (ArC),

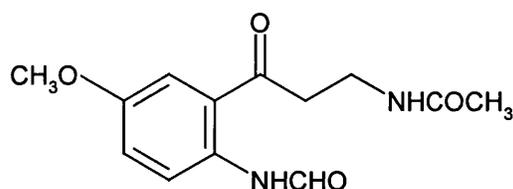
169.2 (C=O). m/z $2M^{+1}$ 677(80%), M^{+1} 339(100%). Found: MH^{+} 339.171611. Formula requires 339.170868. Found: C, 67.77%, H, 6.18%, N, 7.95%. $C_{20}H_{22}N_3O_3$ requires C, 68.17%, H, 6.29%, N, 7.95%.

Polymer supported 2,5-dimethoxybenzaldehyde



Sodium hydride (380 mg of a 60% suspension in mineral oil, 9.5 mmole) was added portionwise, over 20 mins, to a solution of 4-hydroxy-2,6-dimethoxy benzaldehyde (1.82 g, 10 mmole) in anhydrous DMF (75 mL). After 30 minutes, Merrifield resin was added (1.0 g, 0.72 mm/g substitution, 0.72 mMole) and the reaction heated at 50 °C for 36 hrs. The resin was then filtered and washed sequentially with DMF (8 x 50 mL), methanol (4 x 50 mL), DCM (5 x 50 mL) and finally ether (2 x 50 mL). A small sample of resin gave a deep orange colour on treatment with 2,4-dinitrophenylhydrazine indicating the presence of an aldehyde and the IR showed a strong peak at 1690.

N-{3-(2-formylamino-5-methoxyphenyl)-3-oxo-propyl} acetamide (251)¹³

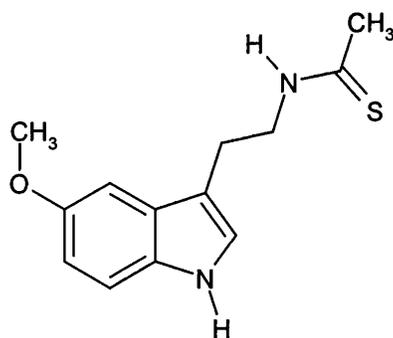


Melatonin (250 mg, 1.07 mmole) was dissolved in acetonitrile (8 mL), carbon tetrachloride (8 mL), and water (16 mL). Anhydrous ruthenium chloride (8 mg,

2.2 mol%) was added and the mixture stirred under nitrogen for twenty minutes. Sodium periodate (220 mg, 0.5 mMol) was added in one portion and stirring maintained for 14 hours. A further portion of sodium periodate (725 mg, 1.65 mmole) was then added and stirring continued for a further 48 hours. The reaction mixture was filtered through 'Hyflo' and evaporated *in vacuo* to give a dark residue which was purified by column chromatography (EtOAc/MeOH 19:1). Evaporation of the major fraction and trituration with ether gave a white solid which rapidly coloured to pale mauve, 55 mgs, 0.21 mmole, 19% yield, m.p. 151-152 °C (lit 153.5-154 °C). This product was pure by HPLC (C8 column, H₂O/ACN/O.02% TFA as eluent).

I.R. ν_{\max} cm^{-1} 3331, 2800 1673, 1651, 1539, 1425, 1395, 1290, 1269, 1194, 1047. ¹H NMR (360 MHz; d₆-DMSO) δ_{H} 1.78 (s, 3H, CH₃), 3.17 (t, J = 6.1 Hz, 2H, CH₂), 3.36 (t, J = 6.1 Hz, 2H, CH₂), 3.81 (s, 3H, CH₃), 7.17 (dd, J = 8.8 Hz, J = 2.9 Hz, 1H, ArH), 7.39 (d, J = 2.9 Hz, 1H, Ar-H), 7.85 (s, 1H, Ar-H), 8.15 (br.s, 1H, NH), 8.33 (br.s, 1H, NH), 10.65 (br.s, 1H, CHO). Found: C, 47.73%, H, 3.48%, N, 4.60%. C₁₃H₁₆N₂O₄ requires C, 47.95%, H, 3.68%, N, 4.64%.

N-{2-(5-Methoxy-indol-3-yl) ethyl} thioacetamide (252)

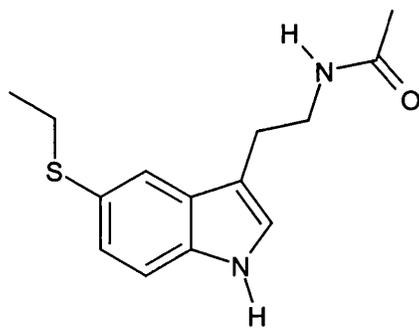


Melatonin (464 mg, 2 mmole) and Lawessons reagent (404 mg, 1 mmole) were refluxed in toluene (10 mL), until HPLC indicated total consumption of starting material (approx 5 hr). The solvent was removed by evaporation *in vacuo* and the

crude material purified by chromatography on silica gel using dichloromethane as eluent. A colourless gum was obtained after trituration with cold ether and this was subjected to lyophilisation from dioxan to give a white solid, 340 mg, 1.37 mmole, 69% yield, m.p. 89-90 °C.

I.R. ν_{\max} cm^{-1} . 3419, 3284, 3199, 2991, 2956, 1547, 1468, 1452, 1441, 1226, 1214, 1137. ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.48 (s, 3H, CH_3), 3.10 (t, $J = 7.0$ Hz, 2H, CH_2), 3.87 (s, 3H, CH_3), 3.98 (q, $J = 7.0$ Hz, 2H, CH_2), 6.88 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.02 (s, 1H, ArH), 7.09 (d, $J = 2.2$ Hz, 1H, ArH), 7.26 (d, $J = 8.8$ Hz, 1H, ArH), 7.38 (br.s, 1H, NH), 8.12 (br.s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 23.1 (CH_2), 33.8(CH_3), 45.8 (CH_2), 55.5 (CH_3), 99.9 (ArCH), 111.6 (ArCH), 112.2 (ArCH), 112.6 (ArC), 122.4 (ArCH), 127.4 (ArC), 131.2 (ArC), 153.8 (ArC), 200.4 (C=S). m/z M^+ 249 (100%) and 174 (20%). Found: C, 62.68%, H, 6.40%, N, 11.14%. $\text{C}_{13}\text{H}_{16}\text{N}_2\text{SO}$ requires C, 62.87%, H, 6.49%, N, 11.28%.

N-{2-(5-Thioethyl-indol-3-yl) ethyl} acetamide (257)

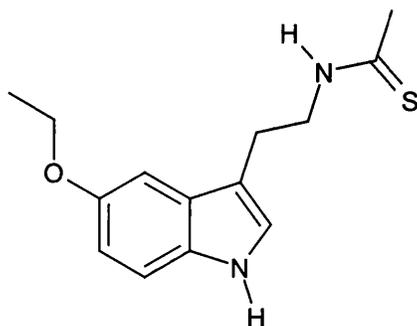


Melatonin (696 mg, 3 mmole) was dissolved in dichloromethane (5 mL) and added dropwise to an ice cold mixture of aluminium chloride (600 mg, 4.5 mmol) and ethanethiol (3.73 g, 4.44 mL, 60 mmole). The reaction was stirred for 2 hr at 0 °C and then a further portion of aluminium chloride (600 mg) and ethanethiol (3.73 g, 4.44 mL) added. Stirring was continued at 0 °C for a further 3 hr before a

third and final portion of aluminium chloride (600 mg) and ethanethiol (3.73 g, 4.44 mL) was added. The reaction was then allowed to warm to room temperature whilst stirring for a further 18 hr after which time HPLC indicated consumption of starting material. After cooling to 0 °C, 1N hydrochloric acid (6 mL) was added and the mixture extracted with dichloromethane (4 x 25 mL). The organic layer was washed with brine (2 x 25 mL) and dried over magnesium sulphate. The solution was filtered and the filtrate evaporated *in vacuo* to give a red viscous oil which was subjected to column chromatography. Elution with ethyl acetate and trituration with ether gave the title compound as a sticky white solid, which was lyophilised from dioxan 114 mg, 0.44 mmole, 15% yield, m.p. 95-96 °C.

I.R. ν_{\max} cm^{-1} . 3403, 3284, 2925, 1653, 1559, 1545, 1457, 1369, 1230, 1103, 801. ^1H NMR (250 MHz; CDCl_3) δ_{H} 1.27 (t, $J = 7.0$ Hz, 3H, CH_3), 1.95 (s, 3H, CH_3), 2.82-3.00 (m, 4H, CH_2), 3.59 (q, $J = 7.0$ Hz, 2H, CH_2), 5.62 (br.s, 1H, NH), 7.01 (d, $J = 1.8$ Hz, 1H, ArH), 7.28-7.32 (m, 2H, ArH), 7.68 (s, 1H, ArH), 8.42 (br.s, 1H, NH). ^{13}C NMR (63 MHz; CDCl_3) δ_{C} 14.6 (CH_3), 23.3 (CH_3), 25.1 (CH_2), 30.4 (CH_2), 39.7 (CH_2), 111.6 (ArCH), 112.6 (ArC), 122.6 (ArCH x2), 125.2 (ArC), 126.5 (ArCH), 128.0 (ArC), 135.5 (ArC), 170.1 (C=O). m/z 2M^{+1} 525(40%), M^{+1} 263(100%). Found 263.121751, $\text{C}_{14}\text{H}_{19}\text{N}_2\text{OS}$ requires 263.121810. Found: C, 63.74%, H, 6.76%, N, 10.48%. $\text{C}_{14}\text{H}_{18}\text{N}_2\text{OS}$ requires C, 64.09%, H, 6.92%, N, 10.68%.

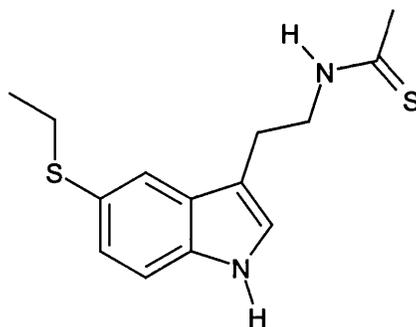
N-{2-(5-Ethoxy-indol-3-yl) ethyl} thioacetamide (258)



N-2-(5-ethoxy-indol-3-yl)-ethyl acetamide (**244**, 100 mg, 0.4 mmole) and Lawessons reagent (101 mg, 0.25 mmole) were refluxed in toluene (10 mL), until HPLC indicated total consumption of starting material (approx 5 hr). The solvent was removed by evaporation *in vacuo* and the crude material purified by chromatography on silica gel using ethyl acetate as eluent. A white solid was obtained after trituration with cold ether, 24 mg, 0.092 mmole, 23% yield, m.p. 103-104 °C.

I.R. ν_{\max} cm^{-1} . 3331, 2974, 1599, 1535, 1481, 1460, 1389, 1298, 1205, 1142, 1034, 953, 827, 800, 542. ^1H NMR (400 MHz, CDCl_3) δ_{H} 1.45 (t, $J = 7.0$ Hz, 3H, CH_3), 2.48 (s, 3H, CH_3), 3.10 (t, $J = 7.0$ Hz, 2H, CH_2), 3.99 (q, $J = 7.0$ Hz, 2H, CH_2), 4.09 (q, $J = 7.0$ Hz, 2H, CH_2), 6.89 (dd, $J = 8.8$ Hz, $J = 1.8$ Hz, 1H, ArH), 7.04 (s, 1H, ArH), 7.09 (d, $J = 1.8$ Hz, 1H, ArH), 7.28 (d, $J = 8.8$ Hz, 1H, ArH), 7.30 (br.s, 1H, NH), 7.99 (br.s, 1H, NH). ^{13}C NMR (63 MHz; CDCl_3) δ_{C} 15.5 (CH_3), 24.0 (CH_2), 34.8 (CH_3), 46.7 (CH_2), 64.7 (CH_2), 101.9 (ArCH), 112.4(ArCH), 113.6 (ArCH), 113.7 (ArC), 123.2 (ArCH), 128.2 (ArC), 132.6 (ArC), 154.1 (ArC), 201.3 (C=S). mz M^{+1} 263 (30%), 188 (100%). Found: C, 63.88%, H, 6.85%, N, 10.53%. $\text{C}_{14}\text{H}_{18}\text{N}_2\text{OS}$ requires C, 64.09%, H, 6.92%, N, 10.68%.

N-{2-(5-Thioethyl-indol-3-yl) ethyl} thioacetamide (**259**)

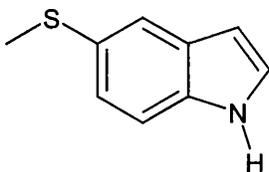


5-Ethylthio-melatonin (**254**, 94 mg, 0.35 mmole) and Lawessons reagent (81 mg, 0.2 mmole) were refluxed in anhydrous toluene (5 mL) for 6 hr. The solvent was removed by evaporation *in vacuo* and the crude material purified by chromatography on silica gel using dichloromethane as eluent. A sticky white solid was obtained after trituration with cold ether and lyophilisation from dioxan, 27 mg, 0.098 mmole, 28% yield.

I.R. ν_{\max} cm^{-1} . 3398, 3175, 2980, 1552, 1460, 1441, 1367, 1220, 1135. ^1H NMR (400 MHz; CDCl_3) δ_{H} 1.22 (t, $J = 7.0$ Hz, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.89 (q, $J = 7.0$ Hz, 2H, CH_2), 3.02 (t, $J = 7.0$ Hz, 2H, CH_2), 3.90 (q, $J = 7.0$ Hz, 2H, CH_2), 6.99 (d, $J = 1.8$ Hz, 1H, ArH), 7.20-7.30 (m, 2H, ArH), 7.69 (s, 1H, ArH), 8.52 (br.s, 1H, NH). ^{13}C NMR (63 MHz; CDCl_3) δ_{C} 14.7 (CH_3), 23.4 (CH_2), 30.2 (CH_2), 34.0 (CH_3), 46.4 (CH_2), 111.8 (ArCH), 111.9 (ArC), 122.4 (ArCH), 122.9 (ArCH), 125.2 (ArC), 126.2 (ArCH), 127.9 (ArC), 135.4 (ArC), 200.6 (C=S). m/z M^{+1} 279 (100%).

Cuprous methylmercaptide - CuSCH_3 (**271**)¹⁴

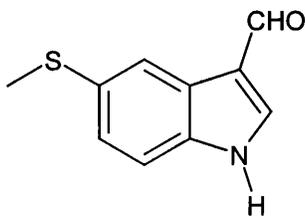
2-Methyl-2-thiopseudourea sulphate (12.0 g, 0.043 mole) and cuprous oxide (7.0 g, 0.049 mole) were stirred in degassed ethanol (400 mL) under nitrogen and 1M sodium hydroxide (150 mL) was added dropwise over 1 hr. The mixture was stirred at reflux for a further 2.5 hr and then cooled to room temperature. The precipitate was filtered and washed on the pad sequentially with water (200 mL), ethanol (100 mL) and finally ether (100 mL). The sand coloured solid was dried under vacuum for 24 hours and used immediately, 9.40 g, 0.085 mole, 98% yield.



Cuprous methyl mercaptide (**271**, 3.54 g, 32 mmol), was stirred at 160 °C under nitrogen with 5-bromoindole (4.90 g, 25 mmole), quinoline (50 mL) and pyridine (9 mL) for 18 hr. The reaction mixture was then cooled and diluted with 2M hydrochloric acid (200 mL) and ethyl acetate (200 mL). The mixture was stirred for 5 minutes, filtered, and the insoluble material washed on the pad with a further 100 mL of ethyl acetate. The filtrates were combined and the phases separated. The organic layer was washed with 2M hydrochloric acid (3x 150 mL), water (2 x 150 mL) and finally brine (100 mL), and then dried over magnesium sulphate. The solution was filtered and the filtrate evaporated *in vacuo* to give a colourless oil. This crude material was subjected to column chromatography and eluted with ethyl acetate/cyclohexane 4:1. Evaporation of the major fraction gave a colourless oil 2.24 g, 13.7 mmole, 55% yield.

¹H NMR (400 MHz; CDCl₃) δ_H 2.47 (s, 3H, CH₃), 6.38 (s, 1H, ArH), 7.08 (dd, J = 8.4 Hz, J = 2.0 Hz, 1H, ArH), 7.32-7.40 (m, 2H, ArH), 7.52 (s, 1H, ArH), 11.10 (br.s, 1H, NH). ¹³C NMR (63 MHz; CDCl₃) δ_C 18.7 (CH₃), 103.0 (ArCH), 113.2 (ArCH), 122.6 (ArCH), 126.2 (ArCH), 127.1 (ArCH), 129.7 (ArC), 130.6 (ArC), 135.9 (ArC). G.C.M.S. *mz* M⁺ 163 (100%), 148 (90%), 104 (25%).

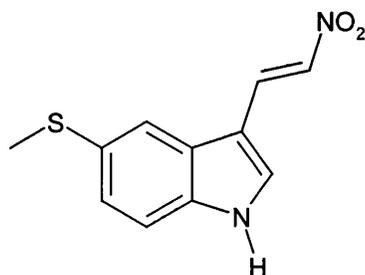
5-Methylthio indole-3-carboxaldehyde (272)



Phosphorous oxychloride (1.00 g, 6.5 mmol) was added at 10 °C under nitrogen to anhydrous DMF (10 mL) and a solution of 5 methylthio-indole (**269**, 1.00 g, 6.13 mmole) in DMF (10 mL) was added dropwise. The reaction was warmed to 45 °C and stirred at this temperature for 2 hours, during which time a precipitate formed. The mixture was then cooled in ice and water (10 mL) added followed by 5M sodium hydroxide (5 mL). The reaction was then heated at 90 °C for 30 min, cooled and then diluted with saturated *aq.* sodium chloride (20 mL). This solution was extracted with ethyl acetate (3x 30 mL) and the organic layers combined and dried over magnesium sulphate. The solution was filtered and the filtrate evaporated *in vacuo* to give an orange oil. A small volume of dichloromethane was added and the title compound crystallised as yellow needles, 0.72 g, 3.75 mmole, 57% yield, m.p. 169-170 °C.

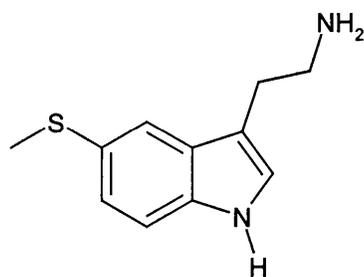
I.R. ν_{\max} cm^{-1} . 3177, 2810, 1654, 1647, 1637, 1522, 1459, 1391, 1286, 1233, 1131, 791, 685, 614, 590. ^1H NMR (400 MHz; d_6 -DMSO) δ_{H} 2.51 (s, 3H, CH_3), 7.22 (d, $J = 8.8$ Hz, 1H, ArH), 7.49 (d, $J = 8.8$ Hz, 1H, ArH), 8.02 (s, 1H, ArH), 8.30 (s, 1H, ArH), 9.90 (br.s, 1H, CHO), 12.20 (br.s, 1H, NH). ^{13}C NMR (100 MHz; d_6 -DMSO) δ_{C} 16.9 (CH_3), 113.4 (ArCH), 118.1 (ArC), 119.3 (ArCH), 123.9 (ArCH), 125.2 (ArC), 131.2 (ArC), 135.5 (ArC), 139.1 (ArCH), 185.4 (C=O). mz M^{+1} 192 (100%). Found: C, 62.57%, H, 4.67%, N, 16.58%. $\text{C}_{10}\text{H}_9\text{NSO}$ requires C, 62.80%, H, 4.74%, N, 16.77%.

3-(2-Nitrovinyl)-5-methylthiindole (273)



1-Dimethylamino-2-nitroethylene (854 mg, 7.36 mmol) was added at 0 °C under nitrogen to trifluoroacetic acid (10 mL) and 5 methylthio-indole (**269**, 1.20 g, 7.36 mmol) added. The reaction was warmed to 45 °C and stirred at this temperature for 10 minutes. The mixture was then cooled in ice and poured into ice cold water (40 mL). The solution was extracted with ethyl acetate (3x 30 mL) and the organic layers combined, washed with saturated *aq.* sodium bicarbonate (2 x 30 mL) followed by brine (2 x 30 mL) and dried over magnesium sulphate. The solution was filtered and the filtrate evaporated *in vacuo* to give an orange oil. A small volume of dichloromethane was added and the title compound crystallised as yellow needles, 0.60 g, 2.58 mmole, 35% yield, m.p. 184-185 °C^d.

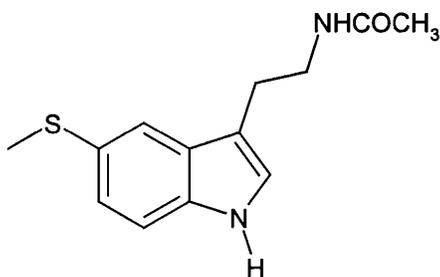
I.R. ν_{\max} cm⁻¹. 3410, 1618, 1559, 1555, 1459, 1310, 1285, 1237, 1110, 884, 798, 593. ¹H NMR (400 MHz; d₆-Acetone) δ_{H} 2.58 (s, 3H, CH₃), 7.29 (dd, J = 8.4 Hz, J = 2.0 Hz, 1H, ArH), 7.53 (d, J = 8.4 Hz, 1H, ArH), 7.88 (d, J = 1.6 Hz, 1H, ArH), 7.92 (dd, J = 13.0 Hz, 1H, CH), 8.14 (s, 1H, ArH), 8.16 (dd, J = 13.0 Hz, 1H, CH), 12.20 (br.s, 1H, NH). ¹³C NMR (100 MHz; d₆-Acetone) δ_{C} 16.7 (CH₃), 107.9 (ArC), 113.4 (ArCH), 118.9 (ArCH), 123.6 (ArCH), 125.8 (ArC), 131.0 (ArCH), 131.5 (ArC), 134.5 (CH) 136.0 (CH), 136.2 (ArC). *mz* M⁺¹ 234 (40%), 218 (100%). Found: C, 56.88%, H, 4.28%, N, 11.92%. C₁₁H₁₀N₂SO₂ requires C, 56.93%, H, 4.30%, N, 11.96%.



3-(2-Nitrovinyl)-5-thiomethylindole (**273**, 800 mg, 3.43 mmol) was added portionwise to a stirred suspension of lithium aluminium hydride (190 mg, 5 mmol) in 20 mL of dry tetrahydrofuran at 0 °C. The reaction was stirred at reflux for 3 h and then cooled in ice. To the reaction was added water (2 mL), followed by 2N sodium hydroxide (6 mL), and finally water (2 mL). The mixture was filtered and the filtrate diluted with ethyl acetate (30 mL), separated, and the aqueous layer re-extracted with ethyl acetate (2 x 20 mL). The organic layers were combined and the product extracted into 0.5M HCl (2 x 25 mL). The aqueous layers were combined and washed with ethyl acetate (25 mL) and then basified with 2M NaOH. The crude amine was extracted into ethyl acetate (4 x 50 mL), dried over magnesium sulphate, filtered and the filtrate evaporated *in vacuo* to give an orange oil. This was subjected to column chromatography using ethyl acetate as eluent. The major fraction was evaporated *in vacuo* to give a colourless gum, 191 mg, 0.93 mmol, 27% yield. This product was acylated immediately.

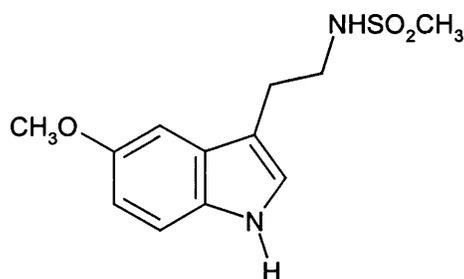
I.R. ν_{\max} cm^{-1} . 3169, 2911, 2851, 1578, 1460, 1448, 1308, 1227, 1103, 877, 796. ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.51 (s, 3H, CH_3), 2.84 (t, $J = 6.4$ Hz, 2H, CH_2), 2.97 (t, $J = 6.4$ Hz, 2H, CH_2), 6.90 (s, 1H, ArH), 7.14-7.26 (m, 2H, ArH), 7.58 (s, 1H, ArH), 8.90 (br.s, 1H, NH), NH_2 protons not observed. ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 17.3 (CH_3), 27.3 (CH_2), 40.3 (CH_2), 110.3 (ArCH), 111.1 (ArC), 118.1 (ArCH), 121.6 (ArCH), 122.3 (ArCH), 125.5 (ArC), 126.5 (ArC), 133.6 (ArC). mz M^{+1} 207 (80%), 190 (100%).

N-{2-(5-thiomethyl-indol-3-yl)-ethyl}-acetamide (256)



5-Thiomethyltryptamine (266, 150 mg, 0.73 mmol) was dissolved in 12 mL of dry dichloromethane and acetic anhydride (86 mg, 0.85 mmol) in 3 mL dichloromethane added dropwise over 15 minutes. The reaction was stirred at room temperature for two hours and then 10 mL of water were added and the solution separated. The aqueous layer was washed with dichloromethane (2 x 10 mL) and the organic layers combined, washed with water (10 mL), 2M hydrochloric acid (10 mL), saturated aqueous sodium bicarbonate (2 x 10 mL), and finally with saturated aqueous sodium chloride (10 mL). The organic layer was dried over magnesium sulphate, filtered, and the filtrate evaporated *in vacuo* to give an off-white solid. The crude product was subjected to column chromatography using ethyl acetate as eluent. The pure fractions were combined and evaporated *in vacuo* then triturated with ether to give a white solid which was recrystallised from ethyl acetate, 67 mg, 0.27 mmol, 37% yield, m.p. 109-111 °C.

I.R. ν_{\max} cm^{-1} . 3273, 2918, 1657, 1643, 1536, 1545, 1434, 1276, 1104, 895, 796, 736, 617. ^1H NMR (400 MHz; CDCl_3) δ_{H} 1.91 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.90 (t, $J = 7.0$ Hz, 2H, CH_2), 3.49-3.57 (t, $J = 6.4$ Hz, 2H, CH_2), 5.94 (br.t, $J = 5.4$ Hz, 1H, NH), 6.96 (s, 1H, ArH), 7.17-7.23 (m, 1H, ArH), 7.25-7.29 (m, 1H, ArH), 7.58 (s, 1H, ArH), 8.90 (br.s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} 18.3 (CH_3), 22.7 (CH_3), 24.6 (CH_2), 39.4 (CH_2), 111.5 (ArCH), 111.7 (ArC), 118.9 (ArCH), 122.5 (ArCH), 123.4 (ArCH), 126.7 (ArC), 127.5 (ArC), 134.7 (ArC), 170.0 (C=O). m/z M^+ 249 (20%), 190 (100%), 143 (20%). Found: C, 62.58%, H, 6.39%, N, 11.16%. $\text{C}_{13}\text{H}_{16}\text{N}_2\text{SO}$ requires C, 62.87%, H, 6.49%, N, 11.28%.



5-methoxy tryptamine (190 mg, 2 mmole) was dissolved in anhydrous dichloromethane (10 mL) under a blanket of nitrogen and triethylamine (0.2 mL) added. Methanesulphonyl chloride (228 mgs, 2 mmole) in dichloromethane (3 mL) was added dropwise over 15 min and stirring continued at room temperature for 1 hr. The reaction was quenched by the addition of water (10 mL) and the solution separated. The aqueous layer was washed with dichloromethane (2 x 30 mL) and all of the organic layers combined and washed sequentially with water (30 mL), 2N HCl (30 mL), saturated sodium bicarbonate (30 mL), and finally with saturated sodium chloride (30 mL). The solution was dried over magnesium sulphate, filtered and the filtrate evaporated *in vacuo* to give an off-white solid which was subjected to column chromatography using ethyl acetate as eluent. The pure fractions were combined, evaporated *in vacuo* and triturated with ether. Recrystallisation from ethyl acetate gave the title compound as a white solid, 202 mg, 0.75 mmole, 38% yield, m.p. 107-108 °C.

I.R. ν_{\max} cm^{-1} . 3431, 3247, 1485, 1420, 1321, 1296, 1236, 1162, 1066, 805, 764, 518. ^1H NMR (400 MHz; CDCl_3) δ_{H} 2.82 (s, 3H, CH_3), 3.01 (t, $J = 7.8$ Hz, 2H, CH_2), 3.43 (q, $J = 7.8$ Hz, 2H, CH_2), 3.87 (s, 3H, CH_3), 4.38 (br t, 1H, NH), 6.88 (dd, $J = 8.8$ Hz, $J = 2.2$ Hz, 1H, ArH), 7.02-7.04 (m, 2H, H2 + ArH), 7.28 (m, 1H, ArH), 8.04(br.s, 1H, NH). ^{13}C NMR (100 MHz; CDCl_3) δ_{C} . 26.1 (CH_2), 40.2 (CH_3), 43.3 (CH_2), 56.0 (CH_3), 100.4 (ArCH), 111.3 (ArC), 112.2 (ArCH), 112.5 (ArCH), 123.4 (ArCH), 127.0 (ArC), 131.8 (ArC), 154.0 (ArC). m/z 2M^{+1} 537(80%), M^{+1} 269(100%). Found: MH^{+} 339.171611. Formula requires

339.170868. Found: C, 53.37%, H, 5.81%, N, 10.20%. C₁₂H₁₆N₂SO₃ requires C, 53.72%, H, 6.01%, N, 10.44%.

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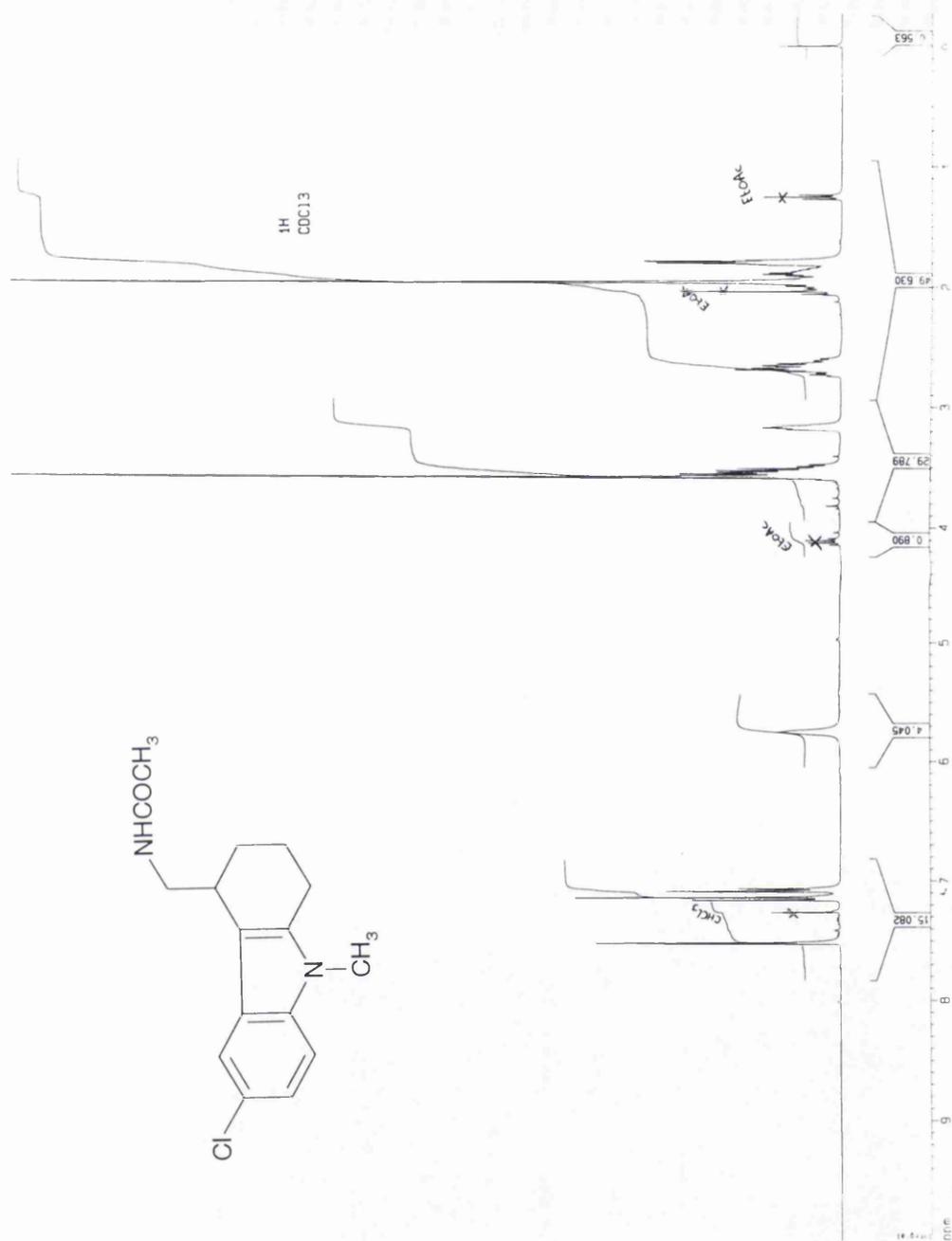


Figure 35 ^1H NMR spectrum of *N*-Acetyl-4-aminomethyl-6-chloro-9-methyl-1,2,3,4-tetrahydrocarbazole (**65**).

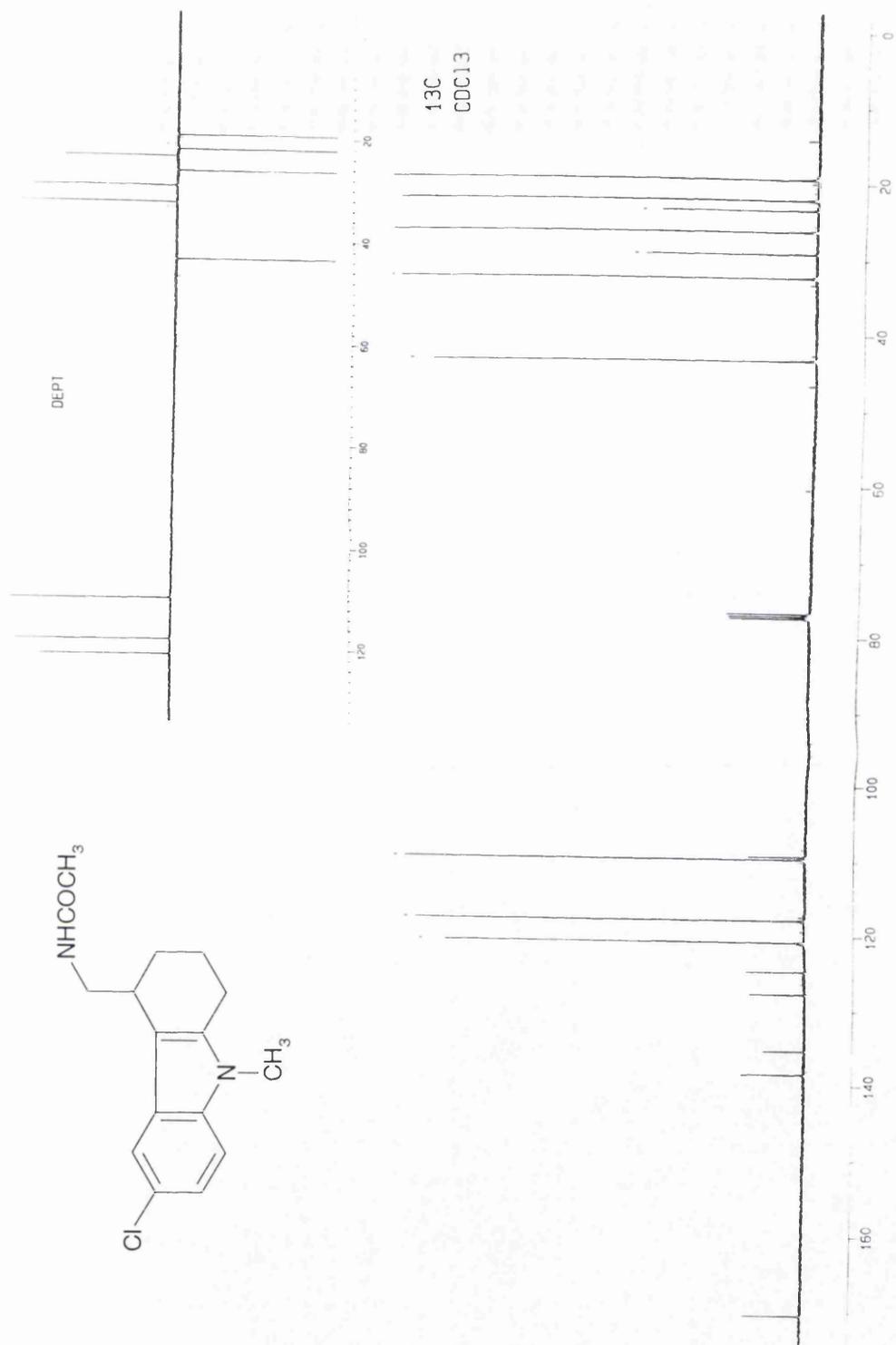


Figure 36 ¹³C NMR spectrum of *N*-Acetyl-4-aminomethyl-6-chloro-9-methyl-1,2,3,4-tetrahydrocarbazole (**65**).

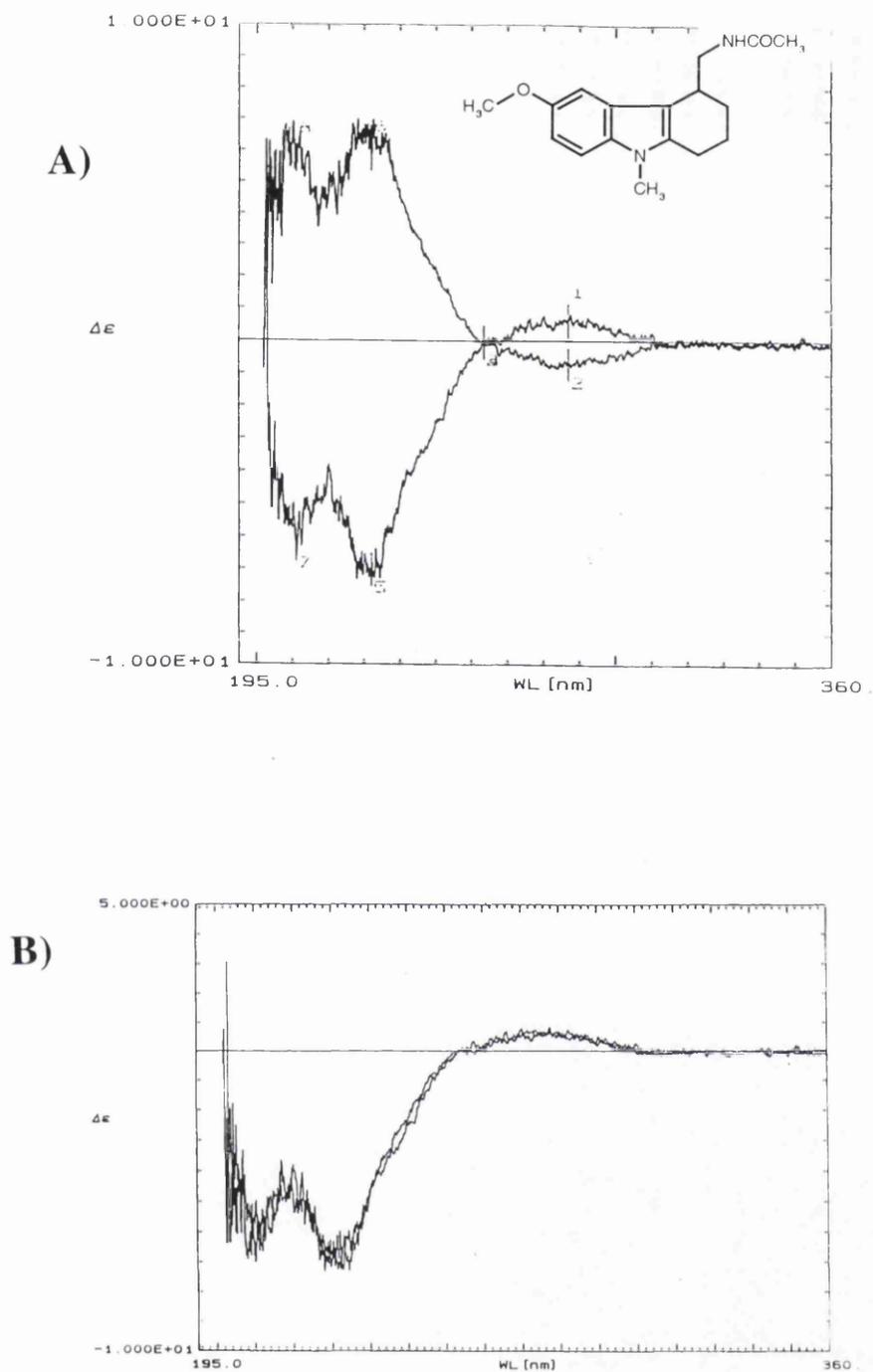


Figure 37 a) CD spectra of the enantiomers of *N*-Acetyl-4-aminomethyl-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole (-)-**(25)**, and (+)-**(25)**. b) CD spectra of (+)-**(25)** inverted and overlaid on that of (-)-**(25)**.

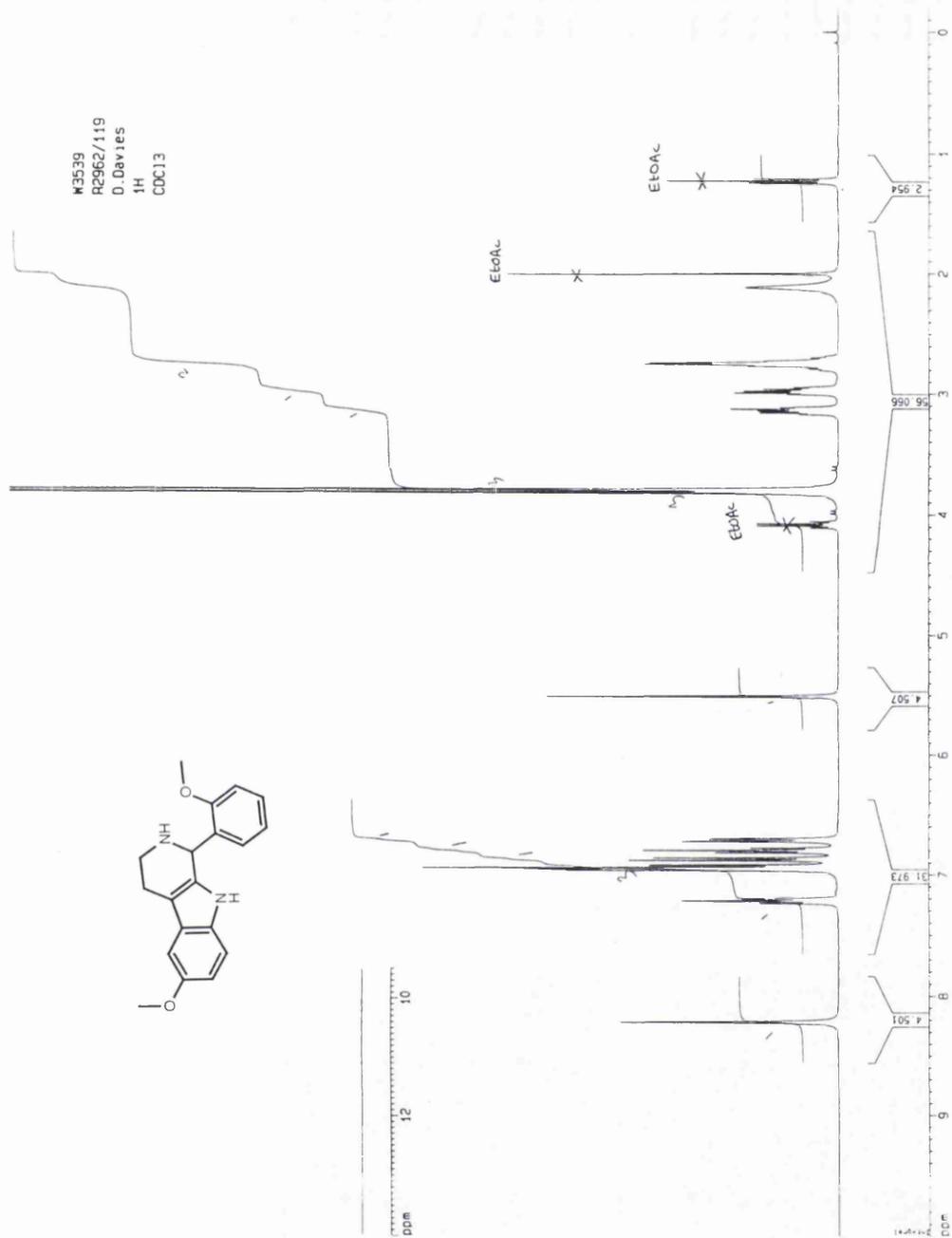


Figure 38 ^1H NMR spectrum of 6-Methoxy-1-(2-methoxyphenyl)-6-methoxy-2,3,4,9-tetrahydro-1H- β -carboline (**155**)

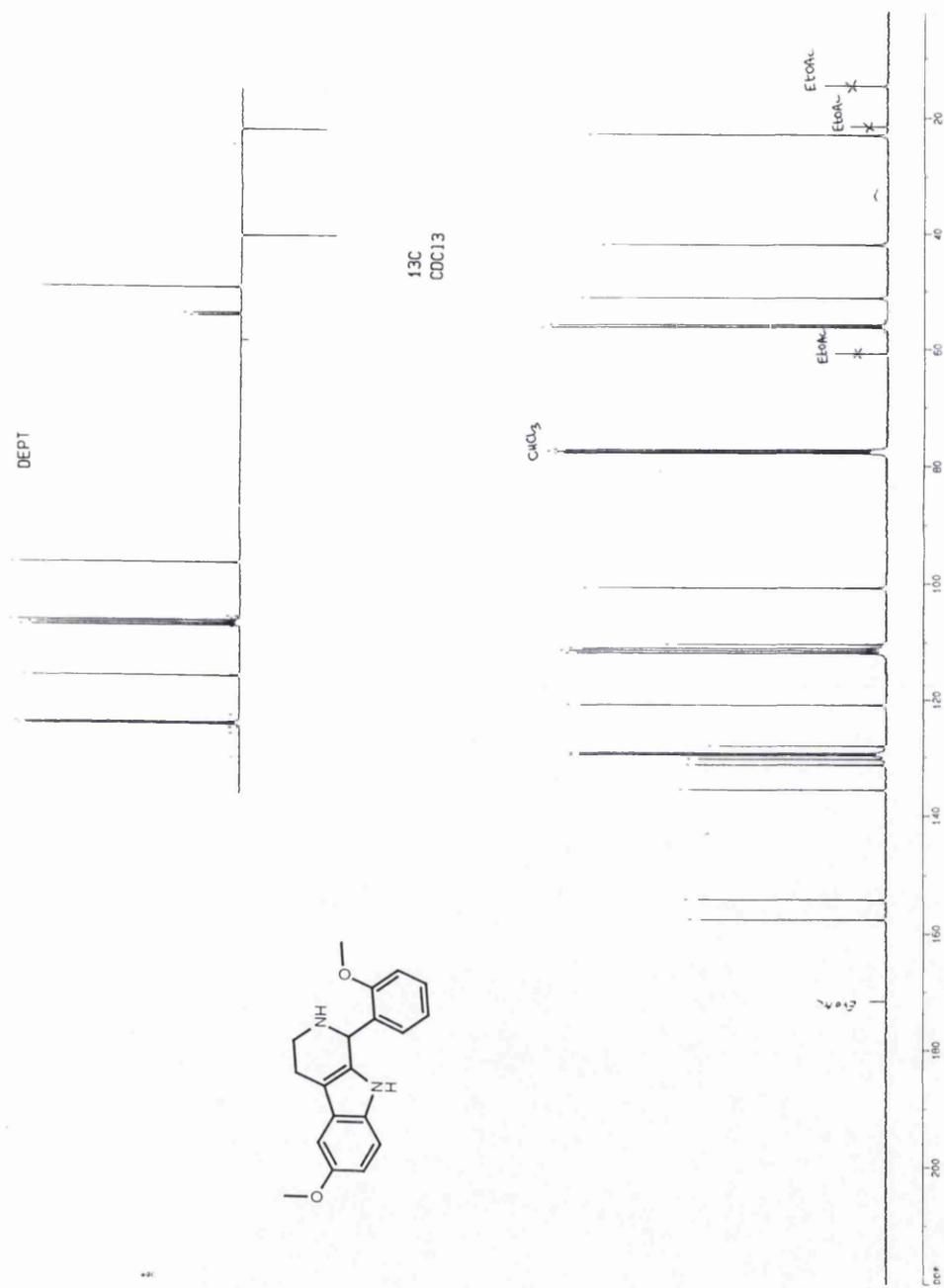


Figure 39 ^{13}C NMR spectrum of 6-Methoxy-1-(2-methoxyphenyl)-6-methoxy-2,3,4,9-tetrahydro-1H- β -carboline (**155**)

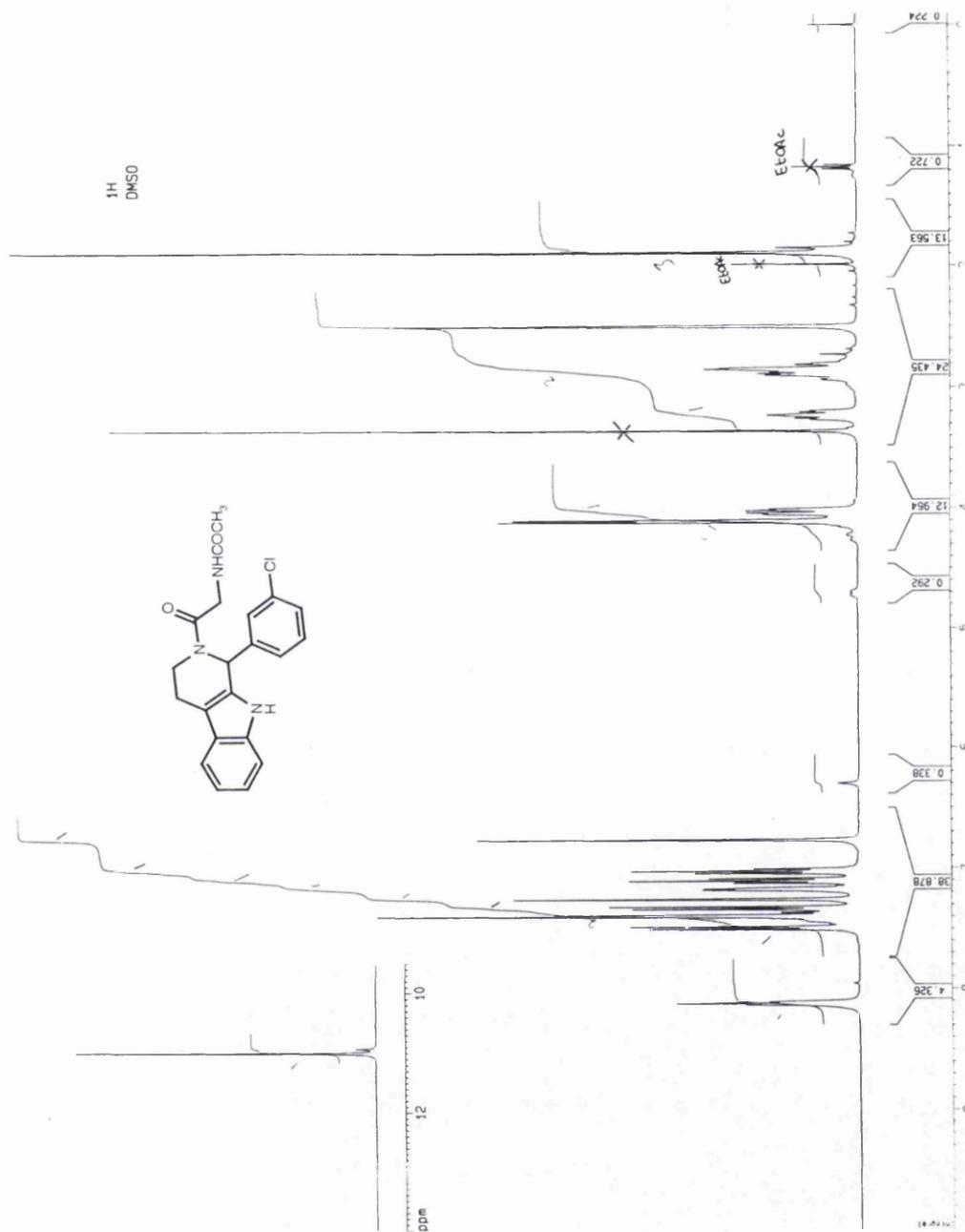


Figure 40 ¹H NMR spectrum of *N*-{3-[1-(3-chloro-phenyl)-1,3,4,9-tetrahydro-β-carboline-2-yl]-3-oxo-ethyl}-acetamide (**209**)

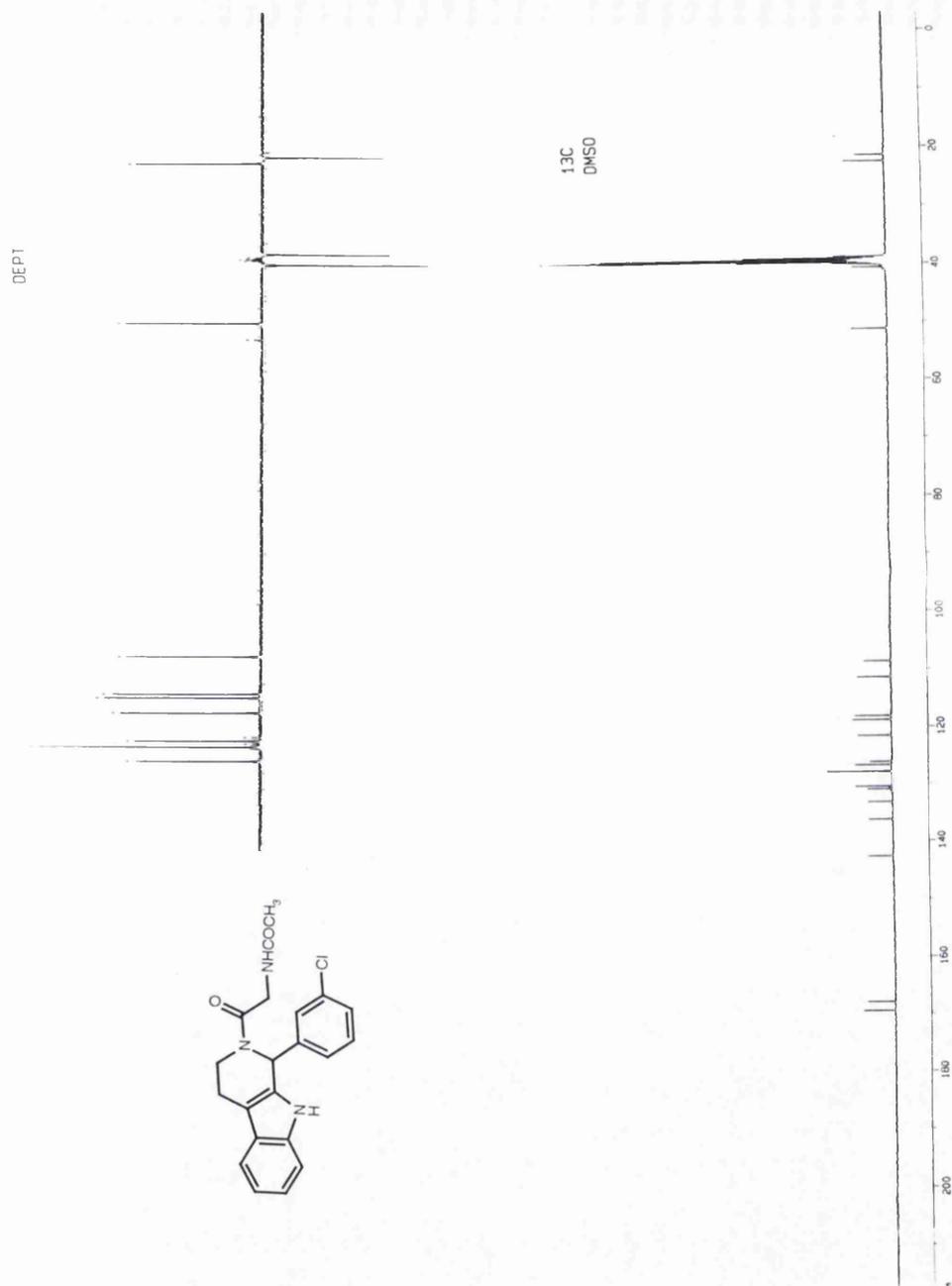


Figure 41 ^{13}C NMR spectrum of *N*-{3-[1-(3-chloro-phenyl)-1,3,4,9-tetrahydro- β -carbolin-2-yl]-3-oxo-ethyl}-acetamide (**209**)

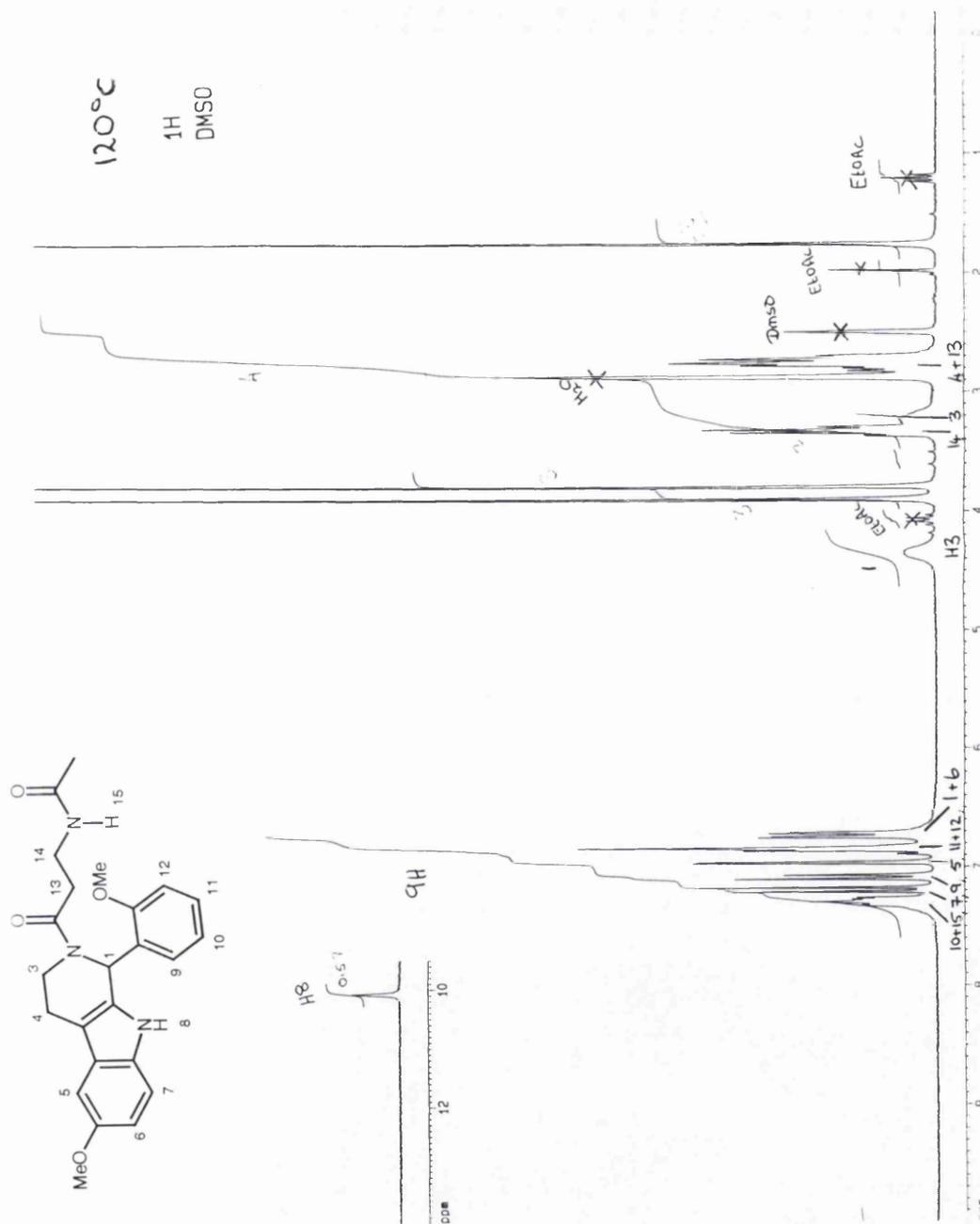


Figure 43 ^1H NMR spectrum of *N*-{3-[1-(2-methoxy-phenyl)-1,3,4,9-tetrahydro-6-methoxy- β -carboline-2-yl]-3-oxo-propyl}-acetamide (**189**) at 120 °C.

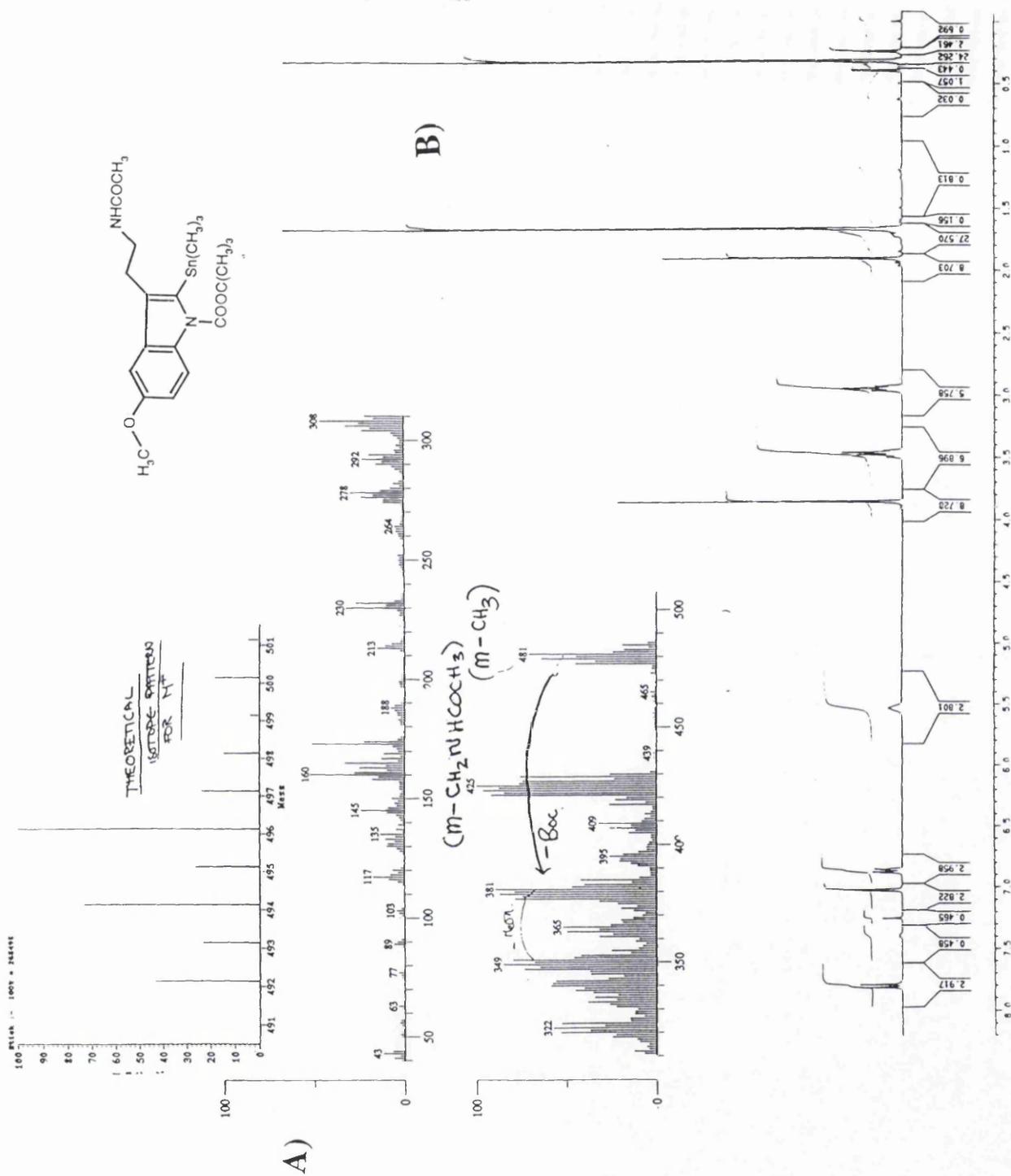


Figure 44 a) Mass spectrum of **236** and its theoretical isotope pattern. b) 1H NMR signal assigned to the trimethylstannyl protons of compound **236**.

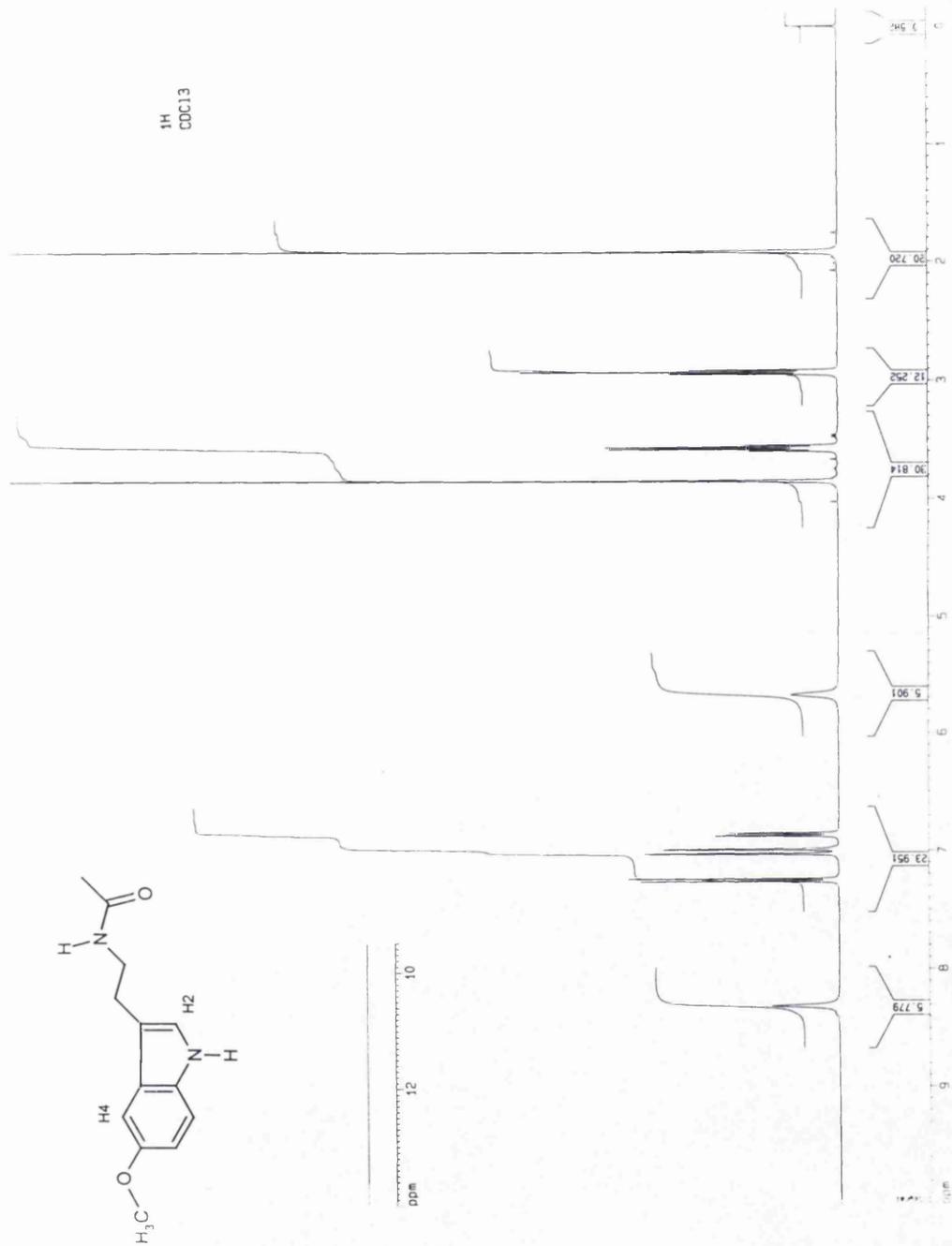


Figure 45 ^1H NMR spectrum of Melatonin (1)

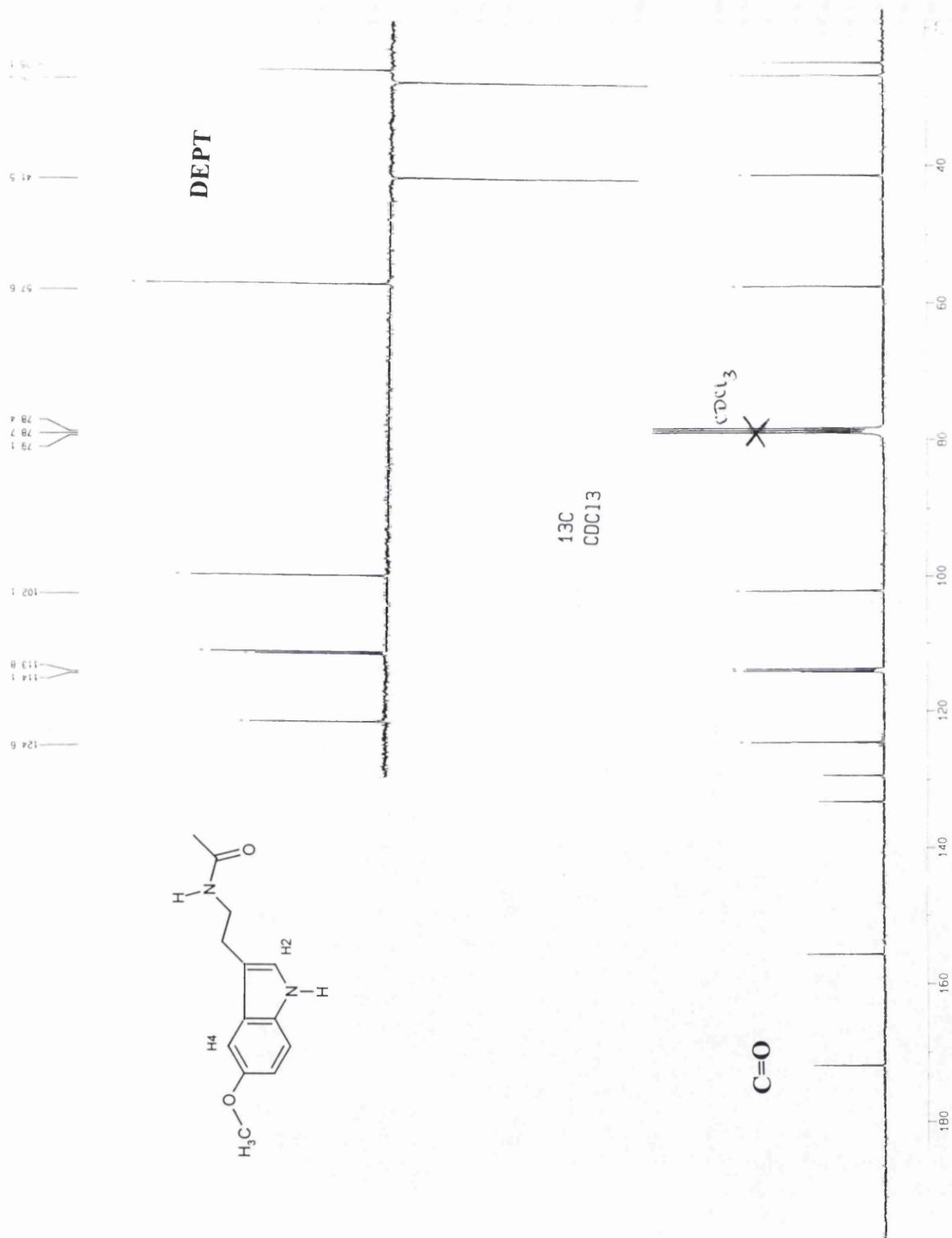


Figure 46 ^{13}C NMR spectrum of Melatonin (1)

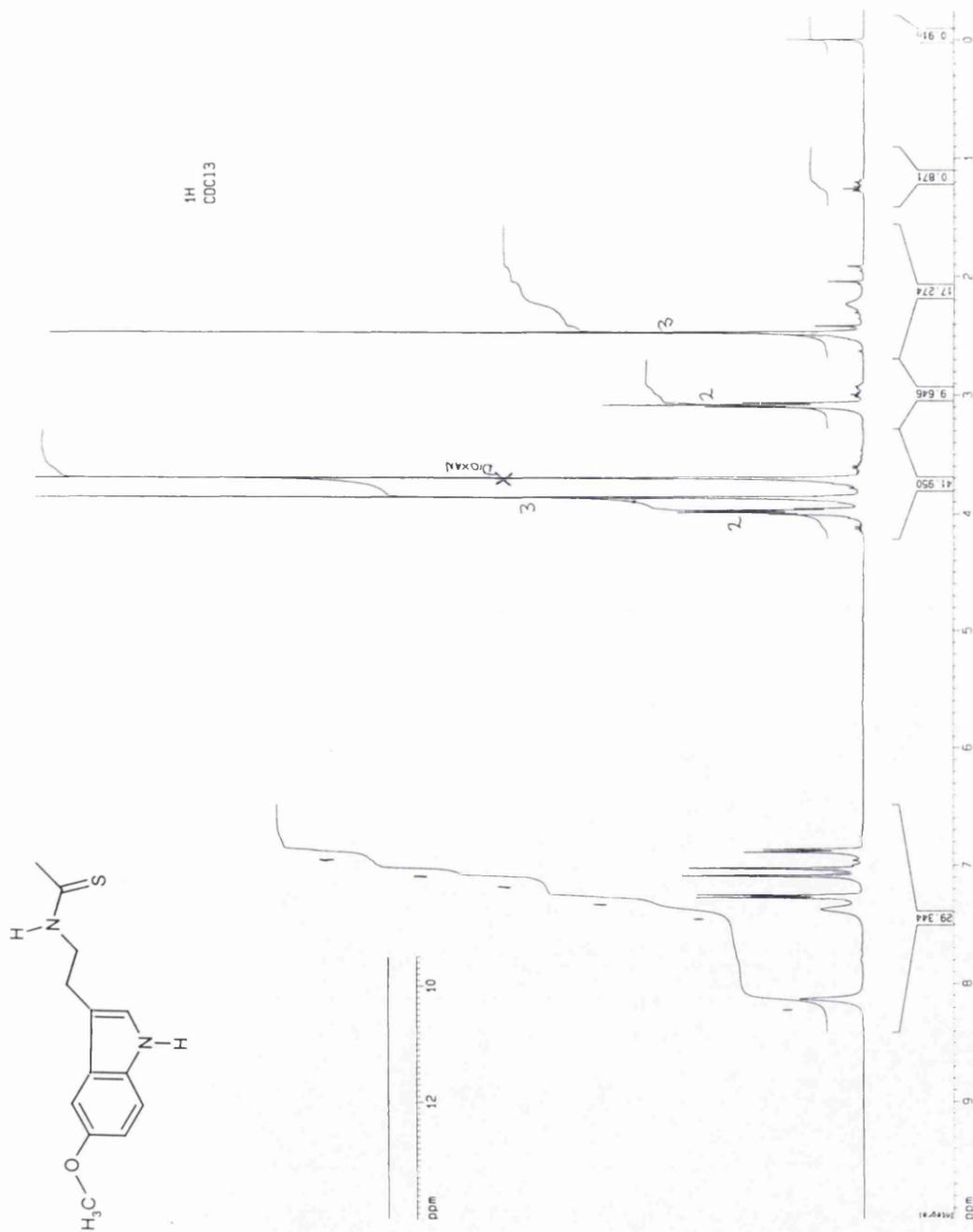


Figure 47 ^1H NMR spectrum of *N*-{2-(5-methoxy-indol-3-yl) ethyl} thioacetamide (252)

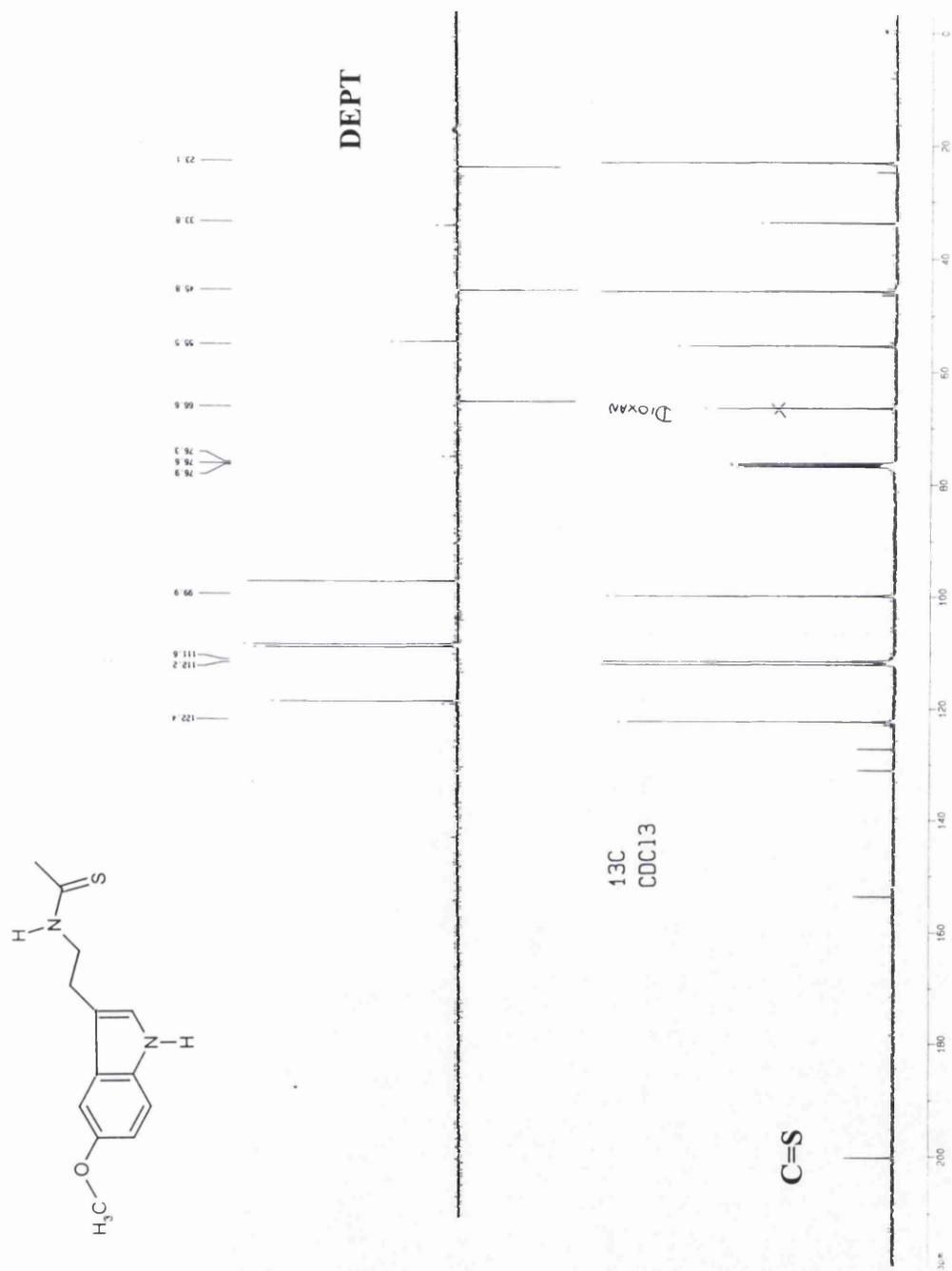
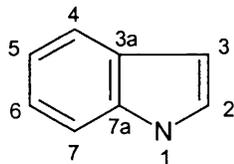


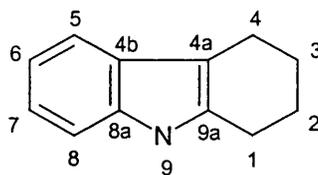
Figure 48 ¹³C NMR spectrum of *N*-{2-(5-methoxy-indol-3-yl) ethyl} thioacetamide (252).

Ring Nomenclature

Indoles



Cycloalkan[b]indoles



Tetrahydro- β -carbolines

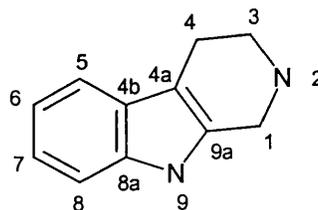


Figure 49. Numbering of the major ring structures discussed in Chapters 1-4.

X ray data for N-acetyl-4-aminomethyl-9-methyl-tetrahydrocarbazole(64)

C₁₆H₂₀N₂O MWt = 256.35, orthorhombic, space group P212121, $a = 9.68$ (1), $b = 11.23$ (1), $c = 12.83$ (1) Angstroms, cell angle $\alpha = 90^\circ$ (3), $\beta = 90^\circ$ (3), $\gamma = 90^\circ$ (3), $v = 1394.73$ Angstrom³. Symmetry space group name H-M 'P 21 21 21, $D_c = 1.22$ gcm⁻³, experimental absorption coefficient $\mu = 5.69$ cm⁻¹, $F(000) 553.41$.

Data for a crystal of dimensions 0.5 x 0.5 x 1.20 mm measured on an Enraf-nonius CAD4 diffractometer with graphite monochromated radiation using a fine focus sealed tube. 4451 independent reflections were measured ($\theta \leq 75$) of which 2567 had $[F_o] > 3\sigma([F_o])$ and were considered to be observed. The data were corrected for absorption (DIFABS)¹ and numerical correction minimum and maximum transmission factors were 0.98 and 1.00 respectively. The structure was solved by direct methods² and refinement was by full matrix least squares³ to $R = 4.3883$, $R_w = 4.7593$. The maximum shift/error in the final refinement was 0.015206. Data was collected using CAD-4 software and computations were carried out using the SHELXS86 PC program system.

References

- [1] Walker, N., Stuart, D. *Acta Cryst.* **1983**, A39, 158.
- [2] Sheldrick, G. M. *Acta Cryst.* **1990**, A46, 467.
- [3] G.M. Sheldrick, SHELXL93, Program for the Refinement of Crystal Structures. University of Gottingen, Germany.

Alanine	Ala	A	Lysine	Lys	K
Arginine	Arg	R	Methionine	Met	M
Asparagine	Asn	N	Phenylalanine	Phe	F
Aspartic acid	Asp	D	Proline	Pro	P
Cysteine	Cys	C	Serine	Ser	S
Glutamic acid	Glu	E	Threonine	Thr	T
Glutamine	Gln	Q	Tryptophan	Trp	W
Glycine	Gly	G	Tyrosine	Tyr	Y
Histidine	His	H	Valine	Val	V
Leucine	Leu	L			

Table 17: Abbreviated nomenclature of the naturally occurring amino acids.