SYNTHESIS AND STRUCTURE - ACTIVITY STUDIES
OF NOVEL POTASSIUM ION CHANNEL BLOCKERS

A thesis presented in partial fulfilment
of the requirements for the
Doctor of Philosophy Degree
of the University of London

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March 1997

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Abstract

Small conductance Ca^{2+} activated potassium channels (SK_{Ca}) occur in many cells but, until recently, have been relatively little studied. Interest in this field has been generated by the discovery of agents which selectively block the channel, notably several insect and anthropod toxins with high potency, and a variety of smaller molecules, the most active of which is dequalinium. This thesis describes the synthesis of novel compounds as potential blocking agents of the SK_{Ca} channel, in order to examine the nature of the interaction with the channel protein, and in particular to identify any structural features additional to the two arginine residues in the peptide toxins which have been proposed to participate in binding. Each compound was assayed in vitro for its ability to block the after-hyperpolarisation (mediated by the opening of the SK_{Ca} channels) that follows the action potential in rat sympathetic neurones.

An initial series of compounds was prepared comprising various elaborations on a fumaric acid-based bis-guanidinium compound, which had possessed weak activity. Incorporation of an additional aminoalkyl substituent resulted in a significant increase in potency. Replacement of the guanidine groups in this compound by 4-aminoquinoline increased the activity 100 fold to a level greater than dequalinium. A variety of derivatives where three and four aminoquinolinyl groups are attached by alkyl chains to a meta-substituted aromatic central unit was also prepared, based on a 1,6-disubstituted indane as a mimic for an \( \alpha \)-helix. All were found to be more potent than dequalinium.

The relative potencies are discussed in terms of the energy changes of the binding process, and it is concluded that only two of the electropositive groups interact with the channel protein. A proposal for their relative spatial relationship is discussed, and is consistent with current hypotheses concerning the dependence on charge delocalisation, and the involvement of a hydrophobic contribution, probably operating at short range, in addition to the primary electrostatic interaction of two charged groups separated by approximately 11 A. Quaternisation of the basic residues is unnecessary, provided that the groups are protonated at physiological pH; the linkage position of the chains through the exocyclic or endocyclic nitrogen of each aminoquinoline is also not critical.
Acknowledgements

I would like to thank Professor C.R Ganellin for giving me the opportunity to work on this project, and for his advice, support and encouragement. Special thanks are due to colleagues at Wyeth Research, U.K, in particular Mr George Weston for giving me the impetus to begin the work, and to Dr Ken Heatherington for his assistance in obtaining and interpreting analytical and spectroscopic data.

I am grateful to Dr Phil Dunn of the Department of Pharmacology for performing the electrophysiological testing of the compounds and to Professor D.H Jenkinson for helpful discussions.

I would like to thank Wyeth Research for providing financial support during the majority of the study period.

Finally, but by no means least, I would like to thank all members of the Ganellin group for their support and comraderie during my visits.
To my parents, my wife and 'the tribe'.

'Patience achieves more than force'
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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>abs EtOH</td>
<td>absolute ethanol</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>ACh</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine 5'-diphosphate</td>
</tr>
<tr>
<td>AHP</td>
<td>after-hyperpolarisation</td>
</tr>
<tr>
<td>Ala</td>
<td>alanine</td>
</tr>
<tr>
<td>4-AP</td>
<td>4-aminopyridine</td>
</tr>
<tr>
<td>Ar</td>
<td>aryl</td>
</tr>
<tr>
<td>Arg</td>
<td>arginine</td>
</tr>
<tr>
<td>Asn</td>
<td>asparagine</td>
</tr>
<tr>
<td>Asp</td>
<td>aspartic acid</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine 5'-triphosphate</td>
</tr>
<tr>
<td>Boc</td>
<td>t-butoxycarbonyl</td>
</tr>
<tr>
<td>br</td>
<td>broad</td>
</tr>
<tr>
<td>BKCa</td>
<td>high conductance Ca(^{2+})-activated K(^+) channel</td>
</tr>
<tr>
<td>Bu</td>
<td>t-butyl</td>
</tr>
<tr>
<td>[Ca(^{2+})](_i)</td>
<td>intracellular concentration of Ca(^{2+})</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine -3',5'-monophosphate</td>
</tr>
<tr>
<td>CBZ</td>
<td>benzyloxy carbonyl</td>
</tr>
<tr>
<td>CDCl(_3)</td>
<td>deuterated chloroform</td>
</tr>
<tr>
<td>ChTX</td>
<td>charybdotoxin</td>
</tr>
<tr>
<td>CMA 100</td>
<td>chloroform/methanol/0.91 NH(_3), 100/10/1</td>
</tr>
<tr>
<td>CMA 200</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>Cys</td>
<td>cysteine</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton (molecular mass unit)</td>
</tr>
<tr>
<td>DCCU</td>
<td>1,3-Dicyclohexylurea</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethylformamide</td>
</tr>
<tr>
<td>DMSO-d(_6)</td>
<td>deuterated dimethylsulphoxide</td>
</tr>
<tr>
<td>ED(_{50})</td>
<td>dose (of drug) that causes 50% of maximum response</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact</td>
</tr>
<tr>
<td>EMR</td>
<td>equiffective molar ratio</td>
</tr>
<tr>
<td>Et</td>
<td>ethyl</td>
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<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
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</table>
fmol femtomole \((10^{-15})\)
g gramme
G guanidino
Gln glutamine
Glu glutamic acid
Gly glycine
h hour
H5 putative pore forming region of K\(^+\) channel proteins
HOBT 1-hydroxybenzotriazole
Har homoarginine
His histidine
HPLC high performance liquid chromatography
5-HT 5-hydroxytryptamine
Hz hertz
IbTX iberiotoxin
icv intracerebroventricularly
IK\(_{\text{Ca}}\) intermediate conductance Ca\(^{2+}\)-activated K\(^+\) channel
IR infra red
IRK1 cloned K\(_{\text{IR}}\) channel
J coupling constant (NMR)
K\(_{\text{A}}\) A-channel (transient outward, K\(^+\) channel subtype)
K\(_{\text{ATP}}\) ATP-sensitive K\(^+\) channel
K\(_{\text{D}}\) dissociation constant
kDa kilodalton \((10^3\) daltons\)
Leu leucine
Lys lysine
M1, M2 segments of internally rectifying/ ATP activated K\(^+\) channel
channel proteins
MIBK methyl isobutyl ketone
nM nanomolar
NMR nuclear magnetic resonance
NTX noxiustoxin
P\(_1\), P\(_2\) protecting groups (general)
Ph phenyl
Phe phenylalanine
pK\(_{\text{a}}\) \(-\log K\(_{\text{a}}\)\)
pM picomolar \((10^{-12}\) molar\)
PO5 peptide toxin isolated from the venom of the scorpion
*Androctonus mauretanecus mauretanicus*

Pro proline

pS picosiemens ($10^{-12}$ siemens)

q quartet

ROMK1 cloned ATP-regulated K$^+$ channel

RP reverse phase

RT room temperature

s singlet (NMR), strong (IR)

S serotonin

S1-S6 segments of voltage-dependent K$^+$ channel proteins

SCyTX leiurotoxin I

SKCa small conductance Ca$^{2+}$-activated K$^+$ channel

t triplet

TEA triethylammonium; toluene/abs ethanol/0.91 NH$_3$, 80/20/1

TFA trifluoroacetic acid

THF tetrahydrofuran

Thr threonine

TLC thin layer chromatography

TMS trimethylsilane

Trp tryptophan

TS thermospray

TsOH p-toluene sulphonic acid

Tyr tyrosine

UCL identification tag (University College London)

UV ultraviolet

V.I.P vasoactive intestinal peptide

Val valine

w weak
## Thesis compounds

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<th>Page of experimental</th>
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## Thesis tables

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### Table 1 - Structure and biological results for the compounds in this study

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<th>EMR*</th>
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<td>1597</td>
<td>100800</td>
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<td>1796/105</td>
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<td>1796/119</td>
<td><img src="image" alt="Structure 5" /></td>
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<td>Structure</td>
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<td>1796/281</td>
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(* E.M.R ref dequalinium)
Over 40 years ago Hodgkin and Huxley described the current generated in the excitation of a nerve\(^1\). Since that time there has been considerable scientific interest and development of pharmaceutical agents acting on calcium and sodium channels, but, until recently, comparatively little exploitation of the therapeutic potential of potassium channels\(^2,3\). This situation has now changed, with the discovery of toxins which selectively block specific types of potassium channels, and improvement in electrophysiological techniques for isolating the potassium current allowing better interpretation of results. Leading the way was the discovery in 1979 of apamin as a blocker of the calcium activated potassium channel\(^4,5\). Since then a variety of other agents active on K\(^+\) channels have been discovered, including components from scorpion venoms.

The interest in identifying the pharmacophore of apamin and its related blocking agents, and consequent implications for the elucidation of the channel structure has prompted this study, via the synthesis of a number of small potential blocking agents.

### 1.1. Ion Channels

The role of potassium channels in controlling the excitability of a cell during an action potential is well understood:

#### 1.1.1. Membranes and cell resting potential

All electrical potentials in biological systems are generated across cell membranes, which are constructed from a lipid bilayer impermeable to ions and other water soluble substances. These materials only traverse the membrane through proteins embedded in the bilayer - the ion channels\(^6\). Nerve cells at rest maintain a voltage across the membrane of -60 to -70 mVolts, arising from the unequal distribution of charge on each side.
of the cell. Inside cells the major cation is potassium, outside it is sodium. The concentration of Ca\textsuperscript{2+} in the resting cell is very low. The major anions inside are organic molecules (eg aspartate, pyruvate), outside chloride. Individual resting potentials, ignoring the effects of other ions, can be calculated. The values are summarised below:

<table>
<thead>
<tr>
<th>Ion</th>
<th>Conc inside cell</th>
<th>Conc outside cell</th>
<th>Resting potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na\textsuperscript{+}</td>
<td>50mmol</td>
<td>460mmol</td>
<td>+55mV</td>
</tr>
<tr>
<td>K\textsuperscript{+}</td>
<td>400mmol</td>
<td>10mmol</td>
<td>-92mV</td>
</tr>
<tr>
<td>Cl\textsuperscript{-}</td>
<td>40mmol</td>
<td>540mmol</td>
<td>-67mV</td>
</tr>
<tr>
<td>A\textsuperscript{-}</td>
<td>400mmol</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

The permeability of the resting cell is different for different ions, being most permeable to K\textsuperscript{+}, least to Na\textsuperscript{+}, intermediate to Cl\textsuperscript{-}, impermeable to organic anions. Thus more K\textsuperscript{+} ions pass through the membrane to the outside, and Cl\textsuperscript{-} to the inside, than Na\textsuperscript{+} ions passing to the inside, causing a build up of negative charge inside the cell. The equilibrium is reached when the electrostatic attraction in the cell balances the diffusion force, at -65mV.\textsuperscript{7-9}

1.1.2. Receptor and action potentials

Sensory stimulae from the environment impinge on receptors producing receptor potentials, which lead to generation of action potentials through chains of cells. This can carry information great distances in the brain and other organs.

There are important differences between these two types of cell excitation, summarised below:
1). Amplitudes of receptor potentials are proportional to intensity of the stimulus, whereas all action potentials are of the same size, approx 0.1 mVolt.
2). Receptor potentials are maintained for the duration of the stimulus, action potentials are constant duration, 1.5msec.
3. A tiny stimulus may produce a receptor potential, whereas action potentials require a potential change of >15mV for generation.

The effect of passing a small amount of current into a giant squid axon was first examined by Hodgkin & Huxley. If the membrane potential is changed by > +15mV, in the depolarising direction, an additional change is observed across the membrane. The physical effect of this depolarisation is to make the membrane much more permeable to Na⁺ (>20 times the K⁺ conductance). Na⁺ ions diffuse into the cell, causing more depolarisation. If the change is below 15mV, this is offset by effusion of K⁺, and the cell remains passive. >15mV 'positive feedback' occurs and the potential rises rapidly over approx 1msec to +55mV, close to the Na⁺ potential.

After this time the membrane becomes much more permeable to K⁺ while the permeability to Na⁺ rapidly subsides, and the voltage now falls to approx -90mV, close to that for K⁺, before it gradually returns to the resting potential. Thus, the potassium conductance increases after a latency of 1msec, to a maximum after 2-3msec. This component of the action potential is called after-hyperpolarisation.
1.1.3. Gating

The process of opening and closing of the channel, called gating, is an important aspect of ion channel function. In essence, this can be viewed in terms of the operational sequence below:

\[ \text{Resting} \leftrightarrow \text{Open} \leftrightarrow \text{Inactivated} \]

Insight into the operation of a voltage gated K\(^+\) channel was made possible recently by the cloning of the \textit{Shaker} gene from \textit{Drosophila}\textsuperscript{11-13}, which codes for the K\(^+\) channel protein\textsuperscript{14}. This enabled the amino acid sequence of the transmembrane protein to be determined, and subsequent hydrophobicity analysis led to prediction of the membrane spanning section\textsuperscript{15,16}. Peptide cleavage analysis demonstrated that both amino and carboxy ends of the peptide were in the cytoplasmic side of the membrane. Earlier conclusions that the protein consisted of six transmembrane \(\alpha\)-helical sections (S1-S6) each of 22 amino acid residues appeared to be confirmed\textsuperscript{17,18}. Additionally, the 20 residues at the amino end were found to adopt a ball structure, which was attached to the S1 region by a chain of 20 residues\textsuperscript{19,20}.

![Diagram of a voltage gated channel](image)

\textbf{Fig 2}

Operation of the gate begins on depolarisation. The initial delay, mentioned above, presumably arises as the protein passes through several closed conformations before it can open. The rate of opening is strongly dependent
on membrane voltage, varying from several msecs at 0V to <1msec at +50mV. The physical sensor which initiates opening is believed to lie on the S4 membrane spanning region, which consists of positively charged residues, lysine/arginine repeat sequences, every fourth residue\textsuperscript{21}. It is believed that when the voltage across the membrane changes, the entire S4 helix moves across the membrane in a spiral path exchanging ion pair partners between its positively charged residues and fixed negatively charges in surrounding membrane segments, in a 'sliding screw' model\textsuperscript{18,22}, resulting in channel opening. Many regions of the protein are highly conserved between a wide variety of species and cell types, which is unprecedented in other channels. In particular the ball and chain fragment and the S4 segment, indicating the importance of these regions, and implying very strong evolutionary pressures to conserve the K\textsuperscript{+} channel\textsuperscript{23}. This homology has also been observed in sodium and calcium channels\textsuperscript{24}. The currently favoured theory describing the mechanism for opening is as follows, although several theories have been proposed\textsuperscript{25-27}.

On opening to allow passage of ions, a receptor on the cytoplasmic side of the protein becomes exposed, to which the ball binds, blocking the channel. The length of time for which the channel stays open before inactivating is independent of applied voltage, being related to the electrostatic attraction and diffusion to the receptor. Evidence for the ball/receptor binding theory is provided by observing the effect of modification of that region; point deletions from the chain speed up inactivation, whereas removal of the ball and chain section causes the channel to fail to inactivate. Inactivation is then restored by addition of synthetic forty unit sequence peptide to the internal solution—furthermore, the rate of restored inactivation is linearly dependent on peptide concentration\textsuperscript{28}.

![Fig 3](image-url)
The inactivated channel slowly returns to the resting state. The rate of return is somewhat variable between cell types, the ball and chain model providing a physical rationale for the wide variety in inactivation rates, from non-inactivating to fully inactivating, reflecting efficiency of binding. The various rates of return are important in different cell functions. For example, the non-inactivating type are important for rapid transmission of electrical responses such as those used in escape behaviour, whereas the faster inactivating channels may be particularly applicable to rhythmic (neuronal) firing, where, in a constant depolarising potential, on returning to the resting state after a constant time the channel again senses a depolarising potential and repeats the cycle, for example in feeding, and respiration.

1.1.4. Ion selectivity and the Pore.

In order to operate successfully a potassium channel must be selective in only allowing the passage of potassium ions, in sufficient quantity to provide an adequate mechanism for restoring the cell potential. This selectivity is achieved so that generally the rate of diffusion for Na⁺ compared to K⁺ is 1 to 1,000. In order to pass through the channel, the ions must lose some of their surrounding water molecules. This loss of hydration energy must be recovered by solvation with liganding groups of the amino acid residues of the channel. K⁺ achieves this by virtue of its tight fit in the channel. In the case of Na⁺, however, the smaller diameter (0.15nm) results in a space between the ion and channel walls, generating additional unfavourable electrostatic energy, so that the energy lost in dehydration is not sufficiently recovered.

In the case of the sodium channel, the narrowest place in the channel pore has dimensions ca 0.3 x 0.5nm, slightly larger than the K⁺ channel, just wide enough for a Na⁺ ion and one water molecule to fit through, but too narrow for a K⁺ ion and a water molecule. Thus, although K⁺ slowly diffuses through the channel, it must shed more of its water than sodium, and is accordingly
less energetically favourable. In conclusion, the ion must make a tight fit to the walls of the channel in order to pass a selectivity constriction\textsuperscript{30,31}.

![Fig 4](image)

Note that only a small part of the pore is the selectivity filter- if the channel were narrow for its entire length the conductance would be too low. Most channels (fig 5) consist of a wide antechamber (A), narrowing to the mouth (M), before the short tunnel (T) is reached\textsuperscript{32}.

![Fig 5](image)

It is the mouth of the channel which is plugged by the toxic agents, which are too wide to enter the pore. Analysis of the amino acid residues in the \textit{Shaker} channel which specifically influence the binding of TEA and the scorpion venom charybdotoxin, has provided evidence of the part of the protein molecule which comprises the pore\textsuperscript{33}. Glutamate and aspartate residues in the section between the trans membrane spanning helices S5 and S6.
been found to have a major influence on toxin binding\textsuperscript{34}, which occurs via an electrostatic interaction\textsuperscript{35}. Several other residues have been shown to play lesser roles in influencing binding through local effects across a distance of 5-15 Å, indicating that the K\textsuperscript{+} conduction pore lies solely in a 39 residue section between the S5 and S6 units\textsuperscript{36}. Pore probing studies indicate that residues 14 (glutamate) and 32 (threonine) lie just on the outside of the channel, 24 (threonine) on the inside. This section between 14 and 32 in \(\alpha\)-helical form would be much too short to bridge the membrane, which has been estimated to be 30 Å wide. In fully extended form the length is 60 Å, strongly indicating a \(\beta\)-hairpin type conformation for this section (H5), below (fig 6).

A contribution from a hydrophobic interaction cannot be ruled out. A study with a series of quaternary ammonium compounds showed that the concentration required to block the pore increased with increasing size/nature of the hydrophilic groups, indicating that the agent blocks by attaching to a hydrophobic region within the fairly large tunnel mouth\textsuperscript{37,38}. In common with Na\textsuperscript{+} and Ca\textsuperscript{2+} channels, the channel is believed to be a tetrameric structure consisting of four identical subunits, so that the pore is at the centre of an eight-stranded antiparallel \(\beta\)-barrel\textsuperscript{39}. The S1, S2 and S3 segments provide an electrostatic shield between the hydrophobic membrane interior and the highly charged S4 sequence of the voltage sensor\textsuperscript{40-42} (fig 7).
This structure appears to be common to the majority of $K^+$ channels. Recently, however, the inward rectifier and ATP regulated $K^+$ channels have been shown to consist of only two transmembrane spanning hydrophobic segments (M1 and M2), corresponding to S5 and S6 of the voltage-gated channels, as well as a sequence which exhibits extensive similarity to the H5 region. The sequence homology of these two channel types suggests that they may belong to a new family of $K^+$ channels which are related to, but distinct from, voltage gated $K^+$ channels. Thus the region relating to ion specificity and conduction is retained, but the superfluous voltage sensing (S4) and surrounding (S1-S3) regions are absent. In the ATP regulated channel, a single putative ATP-binding site associated with a cluster of phosphorylation sites and basic amino acids may constitute the regulatory domain.

The most widely accepted theory describing the transfer of the $K^+$ ion across the membrane involves the existence of an aromatic $K^+$ binding site part way down the pore. In this context the gly-tyr-gly sequence, which is conserved in a wide range of $K^+$ channels may act as the selectivity filter. A computer
modelled structure of the *Shaker* and ROMK1 K⁺ channel indicates the tyrosines from the four loops of the barrel are oriented so that a square planar hole is obtained, this hole being the ideal size to let the K⁺ ion through by forming an oxygen cage.

It has been widely proposed that the mechanism of ion transport across membranes involves the presence of rings of negatively charged amino acids in the channel pores, in cases as diverse as the acetylcholine receptor. The binding site for the ball in the inactivation of the channel has not yet been established.

1.1.5. Modulation and second messengers.

Several mechanisms are possible for preventing the flow of ions through a channel:

1). Binding to the open ion channels inhibits ion flow as long as the drug is bound, but also prevents channel closing.

2). Binding to the channel in its closed conformation prevents opening.

3). Binding to an allosteric site changes the channel gating, leading to a decrease of the channel open time or increase in the probability of channel opening.

Mechanisms (1) and (2) arise from channel blockers, (3) gate modifiers. The mechanism of channel opening is complicated by modulation by various ligands, such as neurotransmitters or hormones, and ions. As an illustration,
in the case of a calcium activated K⁺ channel, a single calcium ion must bind before the channel can open, after which a second calcium ion binds, followed possibly by a third and fourth. Thus, unlike the solely voltage dependent channels, in which the protein senses the applied voltage and undergoes a conformational change, in this case it is the binding of calcium to the channel protein that senses the applied voltage, the channel itself maybe voltage insensitive but is modulated by calcium as a second messenger⁴⁷,⁴⁸.

![Fig 10](image)

The high conductance calcium activated potassium channel has been shown to contain at least six Ca²⁺ binding sites involved in the activation process⁴⁹. This phenomenon also occurs by binding of a neurotransmitter or other ligand binding at a site remote from the channel, which releases a second messenger which blocks or modulates the channel conduction.

Gating, which essentially describes the way in which the particular channel can modify its ion flux, occurs in an all or none manner, so that variations in the macroscopic K⁺ current generally reflects variations in the number of open channels.

A common modulation mechanism is from phosphorylation of key residues of the channel/ enzyme protein. This changes the stabilising electrostatic interactions such as hydrogen bonding between amino acid residues (eg serine) which become exposed on opening of the channel. It can also affect the concentration of Ca²⁺ ions near the phosphorylated site by virtue of the increase in electronegativity which phosphorylation creates, leading to facilitation of channel activation⁵⁰.
1.2. Potassium channels

Potassium channels are the most widespread and diverse of all ion channels, being found in all cell types and tissues of the body\(^{51}\). Principal differences occur in their gating and regulation; other properties, for example the almost universal block by quaternary ammonium ions, may be shared or incidental to function, suggesting a common origin.

Some potassium channels regulate the pace of the heartbeat, while others may mediate learning and rhythmic motor output, such as digestion, muscle function, secretion and respiration. The mode of regulation is determined largely by the characteristics of the action potentials generated by the individual cell type. For example, some cells show no spontaneous activity, whereas others fire action potentials at fixed regular intervals. Others produce irregular patterns of discharge, or produce repetitive bursts separated by profound hyperpolarisations of the membrane. The mode of action of some drugs already in use clinically, discovered years ago, as acting on K\(^+\) channels, has only recently become understood, eg sulphonylureas, tolbutamide (hypoglycaemics) acting as channel blockers, and pinacidil, chromokalin, nicorandil (vasodilators, antiarrhythmics) as channel openers\(^2\).

Unfortunately the study of potassium channels has been difficult since they are present in the cell only at comparatively low concentration, forming only a minor part of the cell.

Potassium channels can be broadly classified according to their mode of regulation, kinetics of activation and inactivation, and sensitivity to pharmacological agents, the main types being voltage-dependent, calcium activated, ATP regulated and agonist modulated. By far the majority or work has been carried out on the first two types, since no toxins have yet been discovered that act specifically on ATP regulated channels. The main types are subdivided into many subtypes. It is not yet understood why cells should have so many conductance pathways for one ion, but presumably in this way the electrical activity can be modulated in different ways over a wide range of voltages.
1.2.1. Voltage regulated channels

1.2.1a. Delayed rectifier

This is the classical type studied by Hodgkin & Huxley in the Giant Squid Axon, described above. It is found in most but not all neurones, the conductance varying widely between 2 and 200pS. It slowly activates after an initial brief delay on depolarisation, persists while the stimulus is maintained, then inactivates slowly. Recovery times can be as great as one minute in some cells. Accordingly, the pharmacological function is believed to be to prevent repetitive firing, which can lead to cumulative inactivation of this channel, altering the efficacy of release of neurosecretary products via Ca\(^{2+}\) influx.

Binding sites for TEA have been detected on both intra and extracellular sides of the channel. This current is blocked by a number of polypeptide scorpion venoms. High affinity selective probes for this channel have been identified in noxiustoxin and margatoxin, which have been characterised, but no studies have yet been reported on the toxin-binding protein.

1.2.1b. Transient (A) channel (KA)

This is the type examined in the Shaker gene of drosophyila. It is a small current (ca 10pS), often largely inactivated at voltages more positive than -40mV, close to the resting potential for many cells. In order to elicit this current by depolarisation, the membrane potential must be set to -90mV for several hundred msecs to remove the steady state voltage dependent inactivation. On depolarisation to greater than -45mV, the channel rapidly activates, then unlike the delayed rectifier it closes spontaneously even if the stimulus is removed, thus termed a fast channel. This enables activation to occur at fairly negative potentials, often below that needed for action potential generation.

Because the A current is in the sub-threshold region of membrane potentials, it is thought to play a role in the frequency of firing in neurons that are spontaneously active or fire repetitively in response to tonic depolarisation-
the transient activation of an outward current at low negative voltage will slow
the return of the membrane potential and help to prolong the interspike
interval. TEA blocks this channel only weakly. Dendrotoxins from the
African mamba snake block this channel selectively and with high affinity, as does MCD peptide from the same honey bee venom as apamin, and
charybdotoxin.

1.2.1c. Anomalous rectifying channel

This channel type can pass larger currents in the inward than the outward
direction, being activated by hyperpolarisation and able to pass sustained
currents (conductance ca 5-25pS) thus mirroring the characteristics of
delayed rectifiers. The current can be regulated by external K+. It is usually
found in cells that are subject to long lasting depolarisations, and appears to
be the potassium conductance that determines the resting potential of
skeletal muscle.

1.2.2 Receptor regulated potassium channels

1.2.2a. M channel

This current begins to activate after an initial delay at voltages more positive
than -50mV, and activation is usually complete by -20mV. It does not
spontaneously inactivate, so that it can be part of the resting potassium
current in the cell. Although it is a very small current, its activation in the
critical region between the resting and threshold for firing allows it to play an
important role in limiting repetitive activity. It is turned off by a variety of
putative neurotransmitters, eg acetylcholine. Inhibition should increase the
excitability of the cell by allowing repetitive firing to a maintained depolarising
current.

1.2.2b. S channel

This channel exhibits similar behaviour to the M current, but is turned off by
serotonin. The channel, which is only weakly dependent on membrane
potential, contributes to the background resting potential, being open at this potential.

1.2.3. Miscellaneous potassium channels

1.2.3a. ATP regulated channel

This type of channel is found in many cell types, but the physiological role has been established only in pancreatic β-cells, where they are involved in insulin secretion\(^64\), and in some heart\(^65-67\) and skeletal cells\(^68,69\), where they have been suggested to provide an energy-saving mechanism during conditions of metabolic insufficiency. \(K_{ATP}\) channels show strong inward rectification\(^70\), regarding which their structural resemblance and sequence identities to \(K_{IR}\) is relevant.

The mode of action of sulphonylureas for the treatment of hypoglycaemia, for a long time unknown, is thought to be via blockage of this channel. Tolbutamide has been shown to block the \(K_{ATP}\) channel, as its primary site of action, with an IC\(_{50}\) of 7 \(\mu\)M\(^71,72\).

1.2.3b. \(Na^+\) activated and cell volume sensitive \(K^+\) channels

These two channel types have not been extensively studied, and their physiological importance remains unknown. The former have been found in heart cells\(^73\) and neurons\(^74\), the latter in hepatocytes\(^75\). The cell volume channels are blocked by cetiedil, bepridil and quinine.

1.2.4. \(Ca^{2+}\) activated channels

This type of channel, probably the most extensively studied\(^76\), is activated by \(Ca^{2+}\) which enters the cell through the voltage dependent calcium channels following a depolarisation pulse. The phenomenon was first observed forty years ago during experiments on red blood cells, in which when the cells are depleted of ATP there is a large \(K^+\) efflux, but only if \(Ca^{2+}\) is present in the external solution\(^77\). The voltage dependence of this \(K^+\) current is very similar to that of the \(Ca^{2+}\) current, showing a steep increase with depolarisation and
a decline at very positive potentials. Pharmaceutical agents which block the
Ca\(^{2+}\) current thus also block this K\(^{+}\) current, but it can be reactivated by
injection of intracellular Ca\(^{2+}\) \(^{78,79}\).

This channel type is divided into three subtypes, distinguished by the channel
conductance: The channel structures are undoubtably related, but the ability
of high affinity toxins to discriminate among the subtypes indicates that slight
modification of the channel proteins can result in large changes in binding
interactions and hence affinity for a particular toxin.

1.2.4a. BK\(_{Ca}\) channel

This subtype is characterised by a relatively large conductance, 150-200pS.
This has a number of consequences, imposing a structural requirement for
a short selectivity filter and large channel mouth. Only a few of the typical
50-100 per cell are needed to be open to have a large influence on
conductance. Both membrane potential and intracellular Ca\(^{2+}\) ions
concentration control the gating, although the sensitivity to Ca\(^{2+}\) varies
markedly between cells, from 0.2 to 10\(\mu\)molar\(^{-}\)\(^{80,81}\). The involvement of as
many as six Ca\(^{2+}\) ions in the initiation step of the channel opening is
discussed above.

The current is blocked selectively by a number of pharmacological agents,
notably charybdotoxin\(^{82}\) with high affinity, at \(K_D=10\)nM, noxiustoxin, at
450nM\(^{83}\), and iberiotoxin\(^{84}\). Internal Na\(^{+}\), and particularly Ba\(^{2+}\) can
reversibly block the channel.

In common with agents which block other channels, blockage appears to
occur by binding to specific residues in the mouth of the channel. The nature
of the interactions is discussed below.

1.2.4b. IK\(_{Ca}\) channel

This channel subtype has an intermediate conductance, between 35-60 pS,
and is the one found in red blood cells, described above. There is large
variation in the number of channels of this type in the cell, from 10 to as many
as 200.
Agents which block this channel include cetydil, quinine and charybdotoxin. However, the absence of a specific blocking agent means that little structural work has been done.

1.2.4c. \(SK_\text{Ca}\) channel

This subtype has a conductance of 6-15pS\(^{85-88}\), which is a slow current appearing to act as a brake to limit repetitive firing, and in termination of bursting, being best suited to generate long-lasting (several hundred msecs) after-hyperpolarisation that follows the action potential. Although only a small current, it can still play a critical role in determining the characteristics of the rhythmic activity of the cell, as it can be active under circumstances where the larger currents are not. Indeed, the interplay of this and other small currents may determine whether, and with what time course, the membrane potential reaches the threshold for activation of the larger currents.

The existence of the BK\(_\text{Ca}\) and \(SK_\text{Ca}\) channels as separate entities was first hypothesised in 1984 to explain the mutual exclusivity in the blockage of two types of channels found in muscle cells with apamin and TEA\(^{89}\). This interpretation was proven correct shortly afterwards when the apamin sensitive/ TEA insensitive channel current was measured in these cells\(^{90}\).

It has since been identified in many cell types, including neuroblastoma cells\(^{91}\), skeletal muscle\(^{92}\) and hepatocytes\(^{93,94}\). In the brain this channel has been implicated in the function of learning and long term memory.

Activation of \(SK_\text{Ca}\) channels in intestinal smooth muscle mediates the inhibitory action of \(\alpha_1\)-adrenoceptors and of receptors for neurotensin and ATP\(^{95-96}\).

In most cells the conductance shows little voltage dependence and high sensitivity to \(\text{Ca}^{2+}\), having ten times higher \(\text{Ca}^{2+}\) sensitivity at negative membrane potentials compared to the BK\(_\text{Ca}\) channel\(^{97}\).

There has been much interest in the \(SK_\text{Ca}\) channel, since a number of agents have been discovered which block this channel with very high
selectivity. The first of these was apamin, whose discovery as a selective blocking agent of the SK\textsubscript{Ca} channel really initiated the recent proliferation of investigations into potassium channels\textsuperscript{4,5,98}

1.3. Apamin

1.3.1. Isolation and characteristics

Apamin is a small polypeptide isolated from the venom of the honey bee, comprising 2\% of the dry weight. It is unusual in its ability to cross the blood brain barrier and in its extreme potency, being active at picomolar concentrations. The first description of symptoms that can ascribed to apamin were reported sixty years ago\textsuperscript{99,100}. However, little work of significance was done until 1965 when the active component was isolated\textsuperscript{101-103} and purified\textsuperscript{104,105}, and was thus free from the other venom constituents which masked its activity. The disulphide bridging was established shortly after this\textsuperscript{106} and the residue sequence was later confirmed by synthesis using the Merrifield technique\textsuperscript{107,108}. This created the opportunity for the synthesis of various derivatives, enabling structural activity relationships to be performed.

1.3.2. Location of binding sites

Apamin binding sites have been found in a wide range of tissues from the central nervous system\textsuperscript{109,110} to peripheral organs\textsuperscript{111,112}, using the technique of iodination of the terminal histidine residue, which produces a very sensitive detection method\textsuperscript{113}. The number of binding sites often changes during development, and under different pathological conditions. The target site for its toxicity was found to be blockage of the K\textsuperscript{+} efflux induced by either ATP or adrenalin in smooth muscle, by blocking specifically the permeability which results from receptor activation and ensures inhibition. Blockage occurs at very low concentrations, and dissociation of apamin from the receptor is very slow, indicating very strong binding. In the brain, the location of receptor sites appears to be quite heterogeneous, acting
preferentially on limbic and motor regions while other regions have little affinity. An endogenous apamin-like substance has been detected in mammalian brain, suggesting this channel plays an important physiological role in modulating neuronal excitability.

The role of the apamin sensitive channel is in some cases known, generally underlying the maintained hyperpolarisation that follows action potentials in neurons, eg in neuroblastoma, sympathetic ganglion and motor neurons, appearing to act to control repetitive firing.

Although not present in adult skeletal muscle, a receptor for apamin has been found in foetal cells, and in patients suffering from Myotonic Muscular Dystrophy, a genetic condition in which muscle fibres fire repetitive action potentials. A method of generation is a lower resting potential close to that for Na\(^+\), the Ca\(^{2+}\) activated K\(^+\) channel creating an AHP of the channel which reactivates the Na\(^+\) channel, creating repetitive bursts of activity. This also may be a mechanism in apamin's central toxicity.

The variation in ability of apamin to displace V.I.P. from its receptor in certain organs provides an indication of considerable variation in receptor structure between various organs. This receptor multiplicity in mammalian organisms has also been observed for other neurotransmitters, such as dopamine, 5-HT, and enkephalins.

1.3.3. Structure

Although apamin has not yet been isolated as a crystalline material, the primary and secondary structure has been well documented. Apamin is smaller than most other toxins acting on potassium channels, being an octadecapeptide bridged by two disulphide bonds, between cysteine residues 1-11 and 3-15. The molecule is unusually rigid and is highly stabilised by intramolecular hydrogen bonds.
The secondary structure has been determined by nmr, CD, a combination of two-dimensional nmr and distance geometry, and optimised by energy minimisation techniques. Residues 2-5 are folded into a β-sheet. A type II β-turn begins at pro-6, ending at ala-9. The section 9-18 forms an α-helix, with increasing disorder from residue 15 onwards, so that the his-18 residue is comparatively mobile in solution. Hydrogen bonds between the helix and residues near the N terminal further stabilise the structure.
The arrangement of an α-helix linked to a β-sheet by disulphide bonds has significant similarities to those found in other toxins, see below, suggesting a common ancestry. However, the comparatively small, compact size of apamin may be important in its potency as well as selectivity, being able to get further into the channel mouth.

1.3.4. Structure of the apamin binding site

Although apamin has a very high affinity for the putative channel acceptor protein, the density of binding sites found on all tissues is low, approx. 100 times less than for the Na⁺ channel, being approx. 1,500 /neuroblastoma cell⁹¹. This, together with the apparent instability after solubilisation from the membrane¹²⁴, has made purification and identification of the binding protein a difficult task.

The molecular weight of the protein was first estimated to be 250 kDa¹²⁵. More recent studies using iodinated apamin cross-linked to the receptor have indicated three groups of binding proteins mol wt 86 kDa, 58 kDa and 23 kDa¹²⁶, although it is suggested that the 58 kDa unit is a fragment of the 86 kDa¹²⁷. Cross-linking experiments with disuccimidyl suberate has led to the identification of a 28 kDa protein¹²⁸, and later a 33 kDa unit¹²⁹. It has been concluded that the SKCa channel is generally composed of two main subunits, these being 86 and ca 30 kDa proteins, although different channel subtypes may exist.

Incorporation of the smaller subunit is much weaker in some cells, perhaps indicating a change in conformation in some tissue types, implying the existence of several structurally different receptor populations¹³⁰,¹³¹. It is still as yet uncertain whether the channel is constructed from oligomeric subunits, but it seems that the 86kDa subunit contains the apamin binding site, since the intensity of the other peptide components fluctuates between cell types.

Recently the apamin binding protein of smooth muscle was cloned, representing an important breakthrough in the elucidation of the precise nature of the SKCa channel. The protein appeared to consist of 438 amino
acids with four potential transmembrane domains, one putative Ca\(^{2+}\) binding site and a protein kinase C phosphorylation site\(^{132}\).

The physical rationale for the difference in conductance from the BK\(_{Ca}\) channel presumably lies in the length of the selectivity pore. In order to produce a large conductance in the latter, this region of the channel must be quite short, whereas for the SK\(_{Ca}\) channel, it must be comparatively longer. Intuitively, this may also have implications for the relatively slow nature of the SK\(_{Ca}\) channel in restoring the voltage during the action potential, since transport of the ions through this long section must be slow.

Binding affinity is extremely sensitive to external K\(^+\) concentration, suggesting a modulatory role for K\(^+\). Both K\(^+\) and Rb\(^+\) are able to increase binding when applied to the extracellular side, while other ions such as Li\(^+\), Na\(^+\) and guanidinium do not inhibit binding\(^{110}\). This has been interpreted to indicate the existence of two different ion binding sites, one for K\(^+\) (or Rb\(^+\)) and the other for the electropositive region of apamin. Possibly the ion binding site is close and functionally associated with the channel itself.

Increasing K\(^+\) concentration from 0.1 and 10 mmolar led to an eight-fold increase in specific binding. Further observations indicate that occupation of this K\(^+\) binding site that saturates near the physiological concentration for K\(^+\) is necessary for formation of the receptor complex.

It is thought highly likely that apamin binds directly to the channel and not to an associated regulatory component, thereby altering the gating properties of the channel (i.e. by reduction in opening time/ increase in probability of opening). This is supported by the displacement of apamin by TEA in high concentrations, in a manner consistent with competitive inhibition at a single class of site- it is generally believed that TEA blocks channels because it has the same diameter as the K\(^+\) ion, allowing it to interact with the mouth of the pore but not to penetrate the selectivity filter. This binding cannot be reversed by increasing K\(^+\) ion concentration, indicating that TEA does not compete with K\(^+\) ions for the regulatory cationic site\(^{133}\).
1.3.5. Structure-activity relationships

Early structure-activity studies were carried out by examining the degree of toxic effect of apamin analogues. The approach to this was that commonly used in which selected residues are modified and the change in toxic effect determined. It was quickly established that the two arginine residues 13 and 14 must both be present for a lethal potency for apamin to occur. Treatment of the arginine groups with cyclohexanedione abolished the activity. In the same study, acetylation of either cys-1 or lys-4 NH$_2$, or treatment of his-18 with diethylpyrocarbonate, only reduced the activity by a factor of 2. However, on modification of cys-1, lys-4 and his-18 the effect was synergistic, completely abolishing activity.

Later studies were facilitated by the ability to prepare synthetic apamin analogues, and confirmed that replacement of both arginines by non basic groups (13,14 Orn) resulted in loss of neurotoxicity, whereas replacement of one (either) arginyl by lysine only slightly reduced the toxicity, both by lysine this was reduced to 7%, by har, ca 10% (LD$_{50}$). On replacement by a single phenylalanine, which places the amine at the same distance from the peptide backbone as lysine, the toxicity was reduced by 1000.

In the most recent study, synthetic analogues missing sequential C-terminal residues were examined for both binding affinity and toxicity. The results are summarised below, and suggest that gln-17, not gln-16, plays a significant role in the binding:

<table>
<thead>
<tr>
<th>relative toxicity</th>
<th>rel. binding affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>apamin</td>
<td>100</td>
</tr>
<tr>
<td>apamin (1-17)</td>
<td>16</td>
</tr>
<tr>
<td>apamin (1-16)</td>
<td>0.35</td>
</tr>
<tr>
<td>apamin (1-15)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

1.3.6. Mode of action

The location of both arginines on the same side of the helix implies a concentration of two points of negative charge on the receptor protein. Although evidence from replacement by non basic groups, or amines having
a much lower pK illustrates the importance of these residues, from the above
evidence the high affinity of apamin for its binding site cannot be explained by
the presence of the two arginine and phenylalanine groups alone. In view of
the much reduced toxicity on replacement with har, the length of the
sidechains also appears important, indicating some hydrophobic contribution
to the binding.
The gln-17 residue, and to lesser extent his-18, appear to play important
roles in the interaction with the receptor, in conjunction with the two arginines,
presumably through hydrogen bonding of the gln-17 side chain amide and
terminal his-18 amide group, although a contribution from the peptide
backbone cannot be excluded. Leu-10, the only hydrophobic residue on the
same side was shown to make little contribution.
The spatial proximity of arg 13, arg-14 and gln-17 suggests a rather limited
interaction predominantly through these residues with specific residues in the
mouth of the SKCa channel, and not a long range electrostatic attraction with
a general area of negative charge on the channel. The retention of toxic
effect on replacing arg-13 and arg-14 indicates that although these two
residues form part of the pharmacophore, other amino acids must contribute.
The parallelism between binding affinity and toxicity is sometimes
inconsistent, particularly for synthetic analogues. For example, apamin
substituted at 4 and 14 with har had only 2% of the potency in binding but still
retained 19% of the toxicity. This may be due to poorer/ better penetration of
the blood/brain barrier than apamin, but this may also indicate an effect on
the expression of the toxin, as differentiated from channel blockage (see
below). In this respect the terminal his-NH2 may also play a role.

1.4. Leiurotoxin I

Leiurotoxin I is a 31 residue polypeptide comprising 0.02% of the venom
of the scorpion Leirus quinquestriatus hebraeus, which was recently
found to compete with apamin binding at nanomolar concentrations in
hepatocytes 139,140, albeit with slightly lower affinity than apamin.
Remarkably, although the biological activities are very similar, there is no
structural homology between apamin and leiurotoxin I. Accordingly, it has
been concluded that it is the spatial arrangement of certain key residues that provides the similarity in activity.

\[
\text{H}_2\text{N-Ala-Phe-Cys-Asn-Leu-Arg-Met-Cys-Gln-Leu-Ser-Cys-Arg-Ser-}
\]

\[
\text{S} \quad \text{S} \quad \text{S} \quad \text{S} \quad \text{S} \\
\]

\[
\text{Val-Lys-His-CO.NH}_2
\]

Fig 13 - primary structure of leiurotoxin

Iodination of tyr-2 permitted elucidation of the binding sites, which appeared to be colocalised with apamin. Other similarities were found; the dependence of binding affinity on K\(^+\) and Rb\(^+\) ion concentration, inhibition by Na\(^+\), Ca\(^{2+}\) and guanidinium ions, also the existence of two polypeptides linking leiurotoxin I to its receptor protein (27kDa and 57kDa), previously identified with apamin\(^{142}\). An endogenous equivalent of leiurotoxin I has been characterized in phaeochromocytoma cells\(^{141}\).

In common with other scorpion venom toxins, leiurotoxin I consists of an antiparallel β-sheet, residues 18-30, linked by three disulphide bonds (12-28, 3-21 & 8-26) to an α-helix, residues 6-16 (fig 14). The region 1-5 is a short extended region. The structure is also quite rigid, being stabilised by intramolecular hydrogen bonds between the sheet and helix as well as hydrogen bonds between compatible residues within these sections. Notably, leiurotoxin I has two arginine residues (arg-6 and arg-13) situated on the helix, and although not sequential they are on the same side of the helix two turns apart. The flexibility of the two side chains allows sufficient opportunity to adopt a similar spatial position to apamin. A glutamine residue is also found one turn of the helix below arg-6.
In common with other toxins the construction of the peptide backbone is the reverse of that found in apamin, the amino end being at the a-helix end of the molecule, suggesting that precise definition of the peptidic backbone is not essential for activity. The carboxyl end is again a his-NH$_2$ residue. A small number of structure activity studies have been carried out. The importance of the two arginines for activity was confirmed by modification with 1,2-cyclohexanedione, the resulting derivative being unable to inhibit apamin binding. Substitution of arg-6 by leu-6 drastically weakened, but did not abolish, binding to the receptor. Substitution of met-7 by arg-7 increased biological activity to a similar potency to apamin. Another important residue appeared to be his-31, iodination of which caused a forty-fold decrease in binding affinity (hence the reason for tyr-2 iodination in receptor site study—modification of this residue does not alter binding), whereas amidation resulted in a four-fold increase.

Modification of the residues on the b-sheet region of the molecule also yielded interesting results. Conversion of lys-20, 25 and 30 to homoarginine did not affect the binding interaction of the peptide with brain receptor, but reduced the contraction inducing property by a factor of 70. A similar phenomenon was observed when glu-27 was modified, the binding again being unaffected while the contraction induction was reduced by a factor of 30. This suggests that although the region around the two arginines is essential for binding, the b-sheet region may be involved in a step following binding that affects the functional expression of the toxin, ie in channel blockade.
1.5. PO5

Five components from the venom of the scorpion *Androctonus mauretanicus mauretanicus* have been shown to compete with apamin binding to rat synaptosome membranes. The most active and first to be isolated was PO5. It exhibits 87% sequence homology with leiurotoxin I, being more active (IC50 $2 \times 10^{-11}$μM, LD50 6 pM) than the latter and almost as potent as apamin.147.

PO5 has a similar secondary structure to leiurotoxin I (fig 14), being also a 31 residue polypeptide linked by three disulphide bridges (8-26, 12-28, 3-21) between a β-sheet (residues 17-29), an α-helix (5-14) and an extended section (1-4). It has three arginine residues, all found on the same, solvent exposed side of the helix, 6, 7 and 13. The only sequence similarity to apamin is the arg-arg-cys-gln sequence located on the helix, the region thought to be vital for biological activity.

It has been suggested that arg-6, arg-7 and gln-9 of PO5-NH2 occupy similar spatial positions to arg-13, arg-14 and gln-16 in apamin, respectively. However, this correlation is apparently inconsistent with the finding above that it is gln-17 which is the important residue for the activity of apamin, whereas gln-16 is not a critical feature. The calculated electrostatic potential is highly assymmetric, as for apamin and leiurotoxin, with the greatest positive potential located on gln-9148.

The limited number of structure activity studies have confirmed the need for arg-6 & arg-7 for high potency. Replacement of both arg-6 and arg-7 by lys
reduced the IC₅₀ to 2 nM (i.e. by a factor of 100), but the LD₅₀ was only reduced by a factor of 10 (60 pM). Replacement by leu reduced the IC₅₀ to 0.1 μM, while the derivative was not toxic below 10 nM. Amidination of the C-terminal his-31 residue caused binding to be irreversible, a strengthening also observed in leiurotoxin, suggesting a multipoint interaction with the receptor since this residue is quite remote from the charged region.¹⁴⁹

1.6. Binding and toxicity

The relationship between binding affinity and pharmacological effect is complex. It is evident that there is no tight correlation between the capacity of the molecules to bind to the apamin receptor in vitro and their neurotoxicity/contraction inducing ability in vivo. The proposition that a part of the molecule distinct from the region which binds to the receptor protein is involved in functional expression appears reasonable since this mechanism is also found in many drug-receptor interactions.

It has been widely proposed that upon interaction with a binding agent, the receptor protein may undergo a conformational change, which is ultimately observed as a pharmacological response.¹⁵⁰

Molecules that can adopt the conformation required for binding may act as agonists or antagonists. An agonist is able to elicit a response by virtue of the presence of a key functional group (C), whereas an antagonist may be bound to the receptor site (e.g. through groups A and B below) but not trigger the pharmacological response because of the absence or conformational restriction of the key functional group.

![Diagram](image.png)

*Fig 16*
1.7. Other scorpion toxins

In view of the many common structural features of potassium channels, useful insight into the interaction of apamin, leiurotoxin I and PO5 with the SKCa channel can be gained from consideration of the mode of action of charybdotoxin and related toxins on the BKCa channel, which has been extensively studied.

The first scorpion venom to have a direct effect on potassium channels was noxiustoxin isolated from the Mexican scorpion Centruroides noxius, which was found to block the delayed rectifier current in the giant squid axon. This discovery led to investigation of other venom components as K+ current blockers. Two related toxins, charybdotoxin and iberiotoxin, were discovered which are both blockers of the BKCa channel, and have proved very useful for probing the activity of this channel and providing indications of the structural basis for interactions with the receptor protein and for differentiation of the various toxins for BKCa and SKCa channels. Charybdotoxin has been a particularly important tool in the study of K+ channels. With its use, it has been possible to purify the channel proteins, and hence carry out site directed mutagenesis studies on the receptor and toxin to elucidate the exact nature of the binding interaction.

1.7.1. A common structure

The secondary structures are very similar to that of leiurotoxin I, being much shorter than the Na+ current toxins derived from snake venom such as dendrotoxin and bungarotoxin (which contain 60-70 residues). The structure consists of a β-sheet (residues 25-36) joined to an α-helix (11-19) and an extended fragment (1-9) by three disulphide bonds (7-28, 13-33, 17-35). The major structural difference from leiurotoxin, arising from the smaller number of residues, is the possession a much shorter N-terminal extended fragment.

This structural motif of an α-helix linked to an antiparallel β-sheet by disulphide bonds is common to all known scorpion toxins and insect
defensins\textsuperscript{154,155}, for example the other scorpion toxins discussed below. Sequences of hydrophobic residues (e.g. thr-3, val-5, val-16, leu-20, cys-33) are conserved in these toxins, which is thought to contribute greatly towards the structural stability. The location of charged residues is, however, quite different, and accounts for the selectivity in both binding and functional expression.

![Fig 17-structure of charybdotoxin](image)

1.7.2. Charybdotoxin.

Charybdotoxin accounts for 0.2% of the total venom protein of the scorpion \textit{Leiurus quinquestriatus}. It blocks both the BK\textsubscript{Ca} and voltage activated (KV\textsubscript{1.3}) channels with high affinity. Addition of extracellular charybdotoxin results in single silent periods (ca. 10 secs) interspersed with normal channel activity, indicating that it acts by reversibly blocking channel currents, without affecting channel gating\textsuperscript{151}. The binding appears to involve interaction of one molecule of toxin with the channel mouth near the pore\textsuperscript{156}, supported both by the competition with TEA and by the observation that internal K\textsuperscript{+} destablises the interaction in a manner consistent with binding of K\textsuperscript{+} to a site within the channel pore\textsuperscript{157}. The ionic composition of the external medium exerts a huge influence on the interaction, in a way expected for a non specific through-space electrostatic effect involving a region of fixed negative charge density near the binding site, the affinity being considerably reduced with increasing ionic strength.
Increasing ionic strength screens the local surface charge and reduces the toxin affinity\cite{158}. The affinity of charybdotoxin for the A-type $K^+$ channel was lowered by 12 on replacing glu-422, near the pore mouth, by lys, whereas replacement by asp had no effect, indicating that the positively charged toxin is electrostatically focussed towards its binding site by the negative potential set up by this glu-422\cite{159}. The secondary structure consists of an $\alpha$-helix linked to a $\beta$-sheet, in common with the structural motif shown in figure 17. An important feature of the residue distribution in charybdotoxin is the location of six of its eight charged residues (arg-25, lys-27, lys-31, lys-32, arg-34, his-37 CO$_2$H) in the $\beta$-sheet section\cite{160}. These residues are thought to constitute the functionally important part of the molecule, in particular the side chains of arg-25, lys-27 and arg-34 which are at the surface of the sheet, and close to tyr-36\cite{152}.

Other crucial residues are located on the same side of the molecule, some of which have strong hydrophobicity (trp-14, met-29 & tyr-36) and others hydrogen bonding capacity (ser-10 & asn-30). As a consequence the receptor site must be reciprocally endowed with varied, complementary residues and that specific block of the toxin relies upon hydrophobic as well as polar interactions. Mutation of these residues gives rise to large changes in dissociation rate and significantly weakens the binding\cite{161}. The association rate is little affected, suggesting that these residues in particular form close range interactions with the channel protein.

Mutation of lys-27 to gln removed the toxin's voltage dependence in dissociation rate. Since voltage dependence reflects an interaction between $K^+$ ions in the pore and the bound toxin, it has been proposed that this residue lies close to where the wide mouth begins to narrow to the pore-neutralisation would remove the electrostatic repulsion between pore associated $K^+$ and charybdotoxin bound at the receptor site and would thus abolish the voltage dependence of the off rate\cite{162}.

Subsequent studies on the BK$\text{Ca}$ channel have led to the conclusion that the receptor site consists of a flat outer vestibule of approx $30 \times 20$ A to which a small area of the surface of the toxin makes close contact, blocking the channel. A shallow 7 A depression is also incorporated, which is the contact
site for tetraethylammonium ion, before further narrowing to the 3 A diameter pore. 

The Shaker channel vestibule appears to be very similar. Additionally in this case a close interaction of a steric nature has been demonstrated between thr-8 or thr-9 on the toxin surface and the Shaker phe-425 residue, from which it was concluded that this residue is located 10-15 A above the floor of the ChTX receptor. Replacement of either phe-425 or thr-8/9 with larger residues substantially weakened binding.

In a recent study, deletion of the first six residues considerably reduced the toxicity, although leaving the structure unchanged, perhaps indicating a role for this section in tuning the specificity and potency of the toxin for the target.
1.7.3. Structure of the charybdotoxin-binding protein.

The BKCa channel protein has been shown to consist of two subunits, α and β, so that a functional channel should be formed by association of four α and four β subunits.

The α-subunit forms the pore (MW 62 kDA), whereas the β-subunit (MW 31 kDA) appears to assist largely in modulation of the biophysical and pharmacological properties of the channel complex166, principally by the presence of a 'ball' of aminoacid residues which moderates the channel inactivation process in a similar manner to the mechanism for the α-subunit, described above167-169.

This may be the site of interaction of an indole diterpene series of compounds which has been found to either assist or hinder ChTX binding to the BKCa channel by an allosteric mechanism170. Cross-linkage to the lys-32 residue of charybdotoxin indicates that this subunit is separated from the main subunit by approx 11 Å171.

Recent evidence has indicated that Na⁺ and Ca²⁺ channels can exist as heteromeric complexes in which the accessory subunit appears to modify the gating or pharmacology of the pore-forming subunit172. In view of this, and the common ancestry of many K⁺ channels, the role and structure of the putative two main subunits in the apamin receptor132 may be very similar to the models for the ChTX-binding channels above.
1.7.4. Iberiotoxin.

The amino acid sequences of charybdoxin and iberiotoxin, isolated from the scorpion *Buthus tamulus*, are 68% identical. Iberiotoxin displays similar binding characteristics to charybdotoxin. Despite their structural similarities, IbTX and ChTX differ in their selectivities for the $B{K}_{Ca}$ channel and $Kv^3.1$ voltage activated channel. Whereas ChTX blocks both with high affinity, IbTX blocks only the $B{K}_{Ca}$ channel.

There are other more subtle differences; the blocking periods for IbTX are longer and the association rate five times slower than charybdotoxin. These differences are thought to be due to the much lower nett positive charge of iberiotoxin, +1, compared to charybdotoxin, +5, primarily due to the substitution of four residues in the $\alpha$-helix region of charybdoxin for negatively charged, aspartate, residues. These cause a lower 'on-rate' to the channel due to less electrostatic attraction, as well as affecting the global dipole orientation of the molecule, which may alter the manner in which the toxin is steered towards the channel mouth.

If the lack of affinity of IbTX for the $Kv^1.3$ channel is due to the additional negatively charged residues on the $\alpha$-helix side of the molecule, this may indicate that this side of the molecule is involved in binding to the $Kv^1.3$ channel, whereas interaction with the $B{K}_{Ca}$ channel is dominated by the positively charged residues on the $\beta$-sheet side. An alternative reason may be that the negatively charged residues may form specific contacts with the channel after binding of the $\beta$-sheet side, stabilising the toxin/receptor interaction, and leading to a lower dissociation rate.

As a result of structure activity studies on several chimeric IbTX / ChTX toxins$^{173}$, the following factors have been found to determine selectivity:

1) Toxin selectivity for the particular channel type resides in the $\beta$-sheet part of the molecule, it being likely that any or all of three residues, gly-22, asp-24 and gly-30 in IbTX, are determinant. Thus attraction to the $B{K}_{Ca}$ channel is dominated by the electrostatic interactions from the conserved residues in this part of the molecule, principally from lys-27. The lack of affinity of IbTX
for the Kv1.3 may in part be due to the additional negatively charged asp residues.

2). The four N-terminal residues of ChTX, in particular phe-2, appear to impart high affinity for the BKCa channel but have little effect on the affinity for the Kv1.3. It may be particularly relevant that the side chain of phe-2 normally interacts with the functionally important residues gly-27 and arg-34. Several other toxins in this family have been discovered more recently, notably kaliotoxin\textsuperscript{174} and margatoxin\textsuperscript{175}. Further study of their respective selectivities for each type of potassium channel will no doubt provide additional information about which residues are determinant for each channel type\textsuperscript{176}.

1.7.5. Channel selectivity

In summary, the above studies suggest the role of both the helix and sheet regions of the toxins as determinant factors in binding and achievement of selectivity, the major contributions to the binding depending generally on the specific charges and location of key residues of the particular toxin. It is evident that minor variations in peptide residues can markedly alter the specificity. A recent study\textsuperscript{177} has shown that a single phenylalanine to glycine mutation in the presumed mouth of the channel pore of Kv1.3 abolishes the toxin block that distinguishes this member of the Shaker family from its close relatives.

Charybdotoxin and iberiotoxin interaction with the BKCa channel is dominated by the charged region on the \(\beta\)-sheet side of the molecule, whereas apamin, leiurotoxin and PO\textsubscript{5} interact with the SKCa channel through the highly electropositive, \(\alpha\)-helical side.

Leiurotoxin I and PO\textsubscript{5} have a very similar structure to ChTX, but present a very different \(\beta\)-sheet surface; arg-25, lys-31, arg-34 and tyr-36 being replaced by much more hydrophobic residues; leu, asn, glu and val respectively. Apamin also has no region corresponding to the charged
β-sheet region. Furthermore, one of the important arginine residues in leiurotoxin I is replaced in charbdotoxin, albeit with another charged residue (lys), lowering the electropositive nature of the helical side of the molecule. In the cases of charbdotoxin and iberiotoxin, the helical side of the molecule appears to play a modulatory role in the functional expression of the toxin. Whether the opposite is also true of those agents active on the $SK_{Ca}$ channel is at present inconclusive, but in this respect residues like his-31 in PO$_5$ and leiurotoxin and his-18 in apamin may contribute.

1.8. Other blockers of the $SK_{Ca}$ channel

At the beginning of this study, a number of smaller molecules had been found to inhibit the $K^+$ current in the $SK_{Ca}$ channel, albeit with affinity several orders of magnitude lower than the natural toxins$^{178-180}$. These molecules had one common feature, thought to account for their activity- the possession of two positively charged nitrogen moieties separated by approximately 11Å.
Table 2- SKca blocking agents

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
<th>Compound</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>150</td>
<td>Decamethonium</td>
<td>450</td>
</tr>
<tr>
<td>Cinchonine</td>
<td>320</td>
<td>Hexamethonium</td>
<td>2000</td>
</tr>
<tr>
<td>Quinacrine</td>
<td>73</td>
<td>Dibucaine</td>
<td>470</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>200</td>
<td>Tetrathylammonium</td>
<td>7900</td>
</tr>
<tr>
<td>Quinidine</td>
<td>240</td>
<td>Neurotensin</td>
<td>0.4</td>
</tr>
<tr>
<td>Cinchonidine</td>
<td>370</td>
<td>Dequalinum</td>
<td>1.1</td>
</tr>
<tr>
<td>Strychnine</td>
<td>190</td>
<td>Amiloride</td>
<td>0.25</td>
</tr>
<tr>
<td>Atracurium</td>
<td>3.0</td>
<td>Apamin</td>
<td>0.000006</td>
</tr>
<tr>
<td>Tubocurarine</td>
<td>3.0</td>
<td>Leiurotoxin I</td>
<td>0.0004</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>3.5</td>
<td>PO5</td>
<td>0.00002</td>
</tr>
</tbody>
</table>

The activity of neurotensin can be explained by the two adjacent arginine residues. The conformation is much less well defined than that found in apamin. However, the presence of the two adjacent proline residues is thought to generate a preference for an α-helix structure as a low energy conformation, explaining the relatively higher activity of neurotensin when compared to smaller peptides having two adjacent arginines.

1.9. Drug design

Motivated by the biological significance of a wide range of oligomeric polypeptides, interest in the design of small molecules which mimic the pharmacophore of the natural product has accelerated greatly in recent years. This has been primarily driven by the increasing sophistication of molecular modelling computer software. While previously attempts to identify a biological lead relied only on random screening and examination of known (natural or synthetic) small active molecules, today the power of the
modelling programmes enables the modeller to not only perform energy minimisation and measurement of interatomic relationships, but also to perform structural overlay to obtain a better fit of the smaller molecule to the predetermined global structure of the polypeptide. Electrostatic potential energy maps can be calculated, enabling the various interatomic interactions to be assessed. There have been surprisingly few structure activity relationships carried out on sections of peptide chains from the toxins described above. This could yield useful results, as was the case with a recent study on noxiustoxin; a synthetic nine amino acid peptide corresponding to the N-terminal sequence was shown to be almost as active as the natural toxin, indicating that the pharmacophore lies within this region of the molecule. However, when viewed as a challenge for synthetic chemistry, this approach was not entirely suitable, nor especially applicable in this study. Furthermore, the conformation of the small peptide unit cannot be guaranteed to match exactly that of the same region in the global polypeptide, so that if the small unit proves insufficiently active, this may be due to misalignment of the key residues and not to simple omission of these residues. An alternative approach, which has gained much in popularity and success over recent years, is to design small molecules in which the spatial arrangement of the key groups of atoms suspected as comprising the pharmacophore are reproduced, creating a structure termed a peptidomimetic.

1.9.1. Peptidomimetics

The design of peptidomimetics has been surveyed by Farmer, who established a set of guidelines based on known examples, electrostatic interactions, and steric considerations, below. There are three main types of interaction; ionic, hydrogen-bonded and hydrophobic. As seen above, hydrophobic residues are important for receptor binding whereas polar residues are essential for activity. Ideally, it is usually advisable to include both types.
i) It is generally unnecessary to reproduce extensively the topology of the peptide; a distance through non-critical space can be spanned by an alkyl chain, or a cyclic unit if greater rigidity/interatomic specification is required.

ii) In non-critical areas, the spatial volume can be exceeded, although not advised, but if the spatial boundaries are exceeded in critical areas the chance of interaction with the receptor is greatly reduced.

iii) The design should not rely heavily on mimicking the peptide backbone exactly, since in most instances the side chains of key residues are likely to play the most important part in binding; thus residues such as gly, ala, cys, pro usually only serve to dictate the secondary structure, and do not contribute to binding. It may not be necessary to reproduce the whole side chain, only the spatial relationship of the polar groups (but note (ii) above).

iv) Conformational flexibility should be maximised until a lead compound is discovered, for even a fairly rigid bioactive peptide may undergo local conformational changes on binding. Central to this is the molecular components strategy, in which the non-peptidic analogue is assembled on a 'silent' entity, which may be a single carbon atom, and the putative active component parts fixed to this point. This central entity is usually a hydrophobic unit. A single carbon is ideal for small distances between active groups; where the distance is large and long hydrophobic chains required, a larger central unit may be preferred.

Progress in the development of peptide mimetics has been reviewed. Pre-determined conformational restraints can be imposed with a close match of alignment of key residues and occupation of the same spatial volume as the original peptide. In this way redundant regions of the molecule can be replaced by a single, large central group. This manner of design has to date chiefly concentrated on mimicking β-turns or Ω-loops, as in the examples shown in fig 21.
Assessment of replacement of the peptide bond has been reviewed by Weinstein\textsuperscript{186}. Since the main function of the peptide backbone is often to provide framework for the allignment of the essential sidechain residues, straightforward surrogates of one of the four elements which constitute the peptide bond are obvious alternatives, i.e., -CO\textsuperscript{2}-, -CO.NR-, -CO.CH\textsubscript{2}-, -CS.NH-, or reversal -HN.OC-. These have met with some success. The trans C=\text{C} bond has also been shown to be an excellent surrogate peptide bond in terms of bond lengths and bond angles. However, as with replacement by other units, a major consideration is the effect of altering the hydrophilic nature of the bond. The peptide bond itself interacts through hydrogen bonding either from carbonyl or N-H. For example, replacement by C=\text{C} exchanges this interaction for a hydrophobic one, CH\textsubscript{2}N creates a new ionic centre. Either may alter the binding to the receptor, and may also affect ultimately in vivo physiological metabolism and transport through the body to the active site.

1.9.2. General considerations

The technique of designing the binding agent to fit a known substrate morphology is complicated by the conformational changes which occur on
binding, so that the two substrates do not have to be complementary. Even if the secondary structure of the peptide is known, the exact conformation adopted on binding to the receptor site may be quite different, in accordance with the widely accepted theory of induced conformational changes.\textsuperscript{187} The strength of the binding interaction is thought to be determined by the following major factors:

i). The main attractive forces are are hydrophobic, electrostatic and hydrogen bonds.

ii). The main repulsive forces are electrostatic repulsions and steric bulk interactions.

iii). Only a small fraction of the amino acid residues are directly involved in the specificity of the interaction, but some that are far from the active site may play a role in determining the necessary geometry of the essential amino acids.

Conformational changes may occur in either or both the binding agent\textsuperscript{188} and the substrate\textsuperscript{189,190}. This hypothesis was the origin of the induced fit theory\textsuperscript{191-193}, which has been used to explain interactions as diverse as the complexation of a substrate with an enzyme, to the mode of action of some drugs. Thus, when a drug comes into contact with a receptor, which usually is part of a protein molecule, it causes a reversible perturbation or change in the protein's tertiary structure. This change, which in certain instances is an allosteric transition\textsuperscript{194}, causes the observed biological effect.

For example, it has been suggested that acetylcholine may interact with the muscarinic receptor of postganglionic parasympathetic nerves and with acetylcholinesterase in the full extended conformation and, in a different, more folded structure, with the nicotinic receptors at ganglia and neuromuscular junctions\textsuperscript{195-197}.

Evidently, the rational design of nonpeptide compounds is only feasible if sufficient information is known about structure-activity relationships and conformational properties of the peptide structures. In spite of the uncertainties, this approach is often successful\textsuperscript{198}. 
CHAPTER 2

SELECTION OF COMPOUNDS

As mentioned in the introduction, relatively simple molecules such as gallamine, dequalinium and tubocurarine are potent and selective blockers of the $SK_{Ca}$ channel. This information supports the assertion that it should be possible to design small molecules which mimic the pharmacophore of apamin and related toxins.

The objective of this work has been to prepare a range of potential blockers of the $SK_{Ca}$ channel, in support of the project at U.C.L., and in parallel with other members of the research group, to explore the nature of the interaction with the channel and examine what structural features are important for blocking activity. Several aspects of the blocking mechanism were investigated:

1. Exploration of a possible role for gln-17 in the binding of apamin by elaboration of fumaric and maleic acid derived bis guanidinium compounds, in which the two guanidines were intended to mimic the corresponding groups in arg-13 and arg-14 of apamin.

2. Comparison of the relative binding affinity of guanidinium versus 4-amino quinolinium substituents.

3. Examination of the nature of the interaction of aminoquinolininium groups linked through the exocyclic nitrogen, in which the spatial orientation of two of these groups was so designed as to mimic the $\alpha$-helical arrangement found between the two adjacent arginines in apamin. A third aminoquinolininium substituent was also incorporated to investigate the possible participation of gln-17 in the binding of apamin.

4. Investigation of the effect of introducing a fourth aminoquinolinyl substituent.

It is an important point to note that although the selection and synthesis of each group of compounds (1-4) was chronological and based on the
potencies of the previous groups, together with other compounds made by fellow researchers, all compounds within each group were tested simultaneously and accordingly the choice of each individual compound in a particular group was not made with the prior knowledge of the potency of others in the same group.

The spatial conformation of apamin has been well studied, and the structure optimised by computer molecular modelling. Accordingly, the molecular coordinates were transferred from the energy-minimised structure of Freeman\textsuperscript{122} into the Chemex modelling system, protein module, for determination of spatial relationships between the various residues of interest. Examination of the position of the two arginine residues, 13 & 14, showed the guanidine groups indeed to be separated by a distance of approx 11 Å. The side chains, which are very mobile in solution, protruded noticeably from the fairly globular molecule on the same side of the helix. Gln-17 was also located on this side, being one turn below the two arginines and almost directly below arg-14.

2.1. Fumaric/ maleic acid-based compounds

At the beginning of this study, initial attempts to design small molecules which may mimic arg-13 and arg-14 had been made, comprising short peptides which contained the arg-arg combination, and various fumaric acid-derived diamides having two guanidine residues separated by approx 11 Å\textsuperscript{199}. The following group was found to be inactive at concentrations < 10\textsuperscript{-5} molar.

\begin{equation}
\text{Gly-Phe-Arg-Arg-Ile-Leu-Arg-Arg-Ala-Ser-Leu-Gly-NH}_2
\end{equation}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig22}
\caption{}
\end{figure}
In the same study, two fumaric-acid based bisguanidinium derivatives, (1) and (2), modelled on the arg-arg pharmacophore were also examined. Both exhibited only weak activity (30% inhibition at 300μmolar).

Thus, although the arg-arg unit is necessary for biological activity, the above structures did not adequately define the spatial arrangement of the two guanidine groups for recognition by the SKCa channel. In addition, it was considered that the lower activity than compounds such as tubocurarine and pancuronium may be due to the lack of sufficient hydrophobic character in the above molecules. The strong evidence for both participation of gln-17 and a hydrophobic contribution suggested that the design of further candidates should take into consideration both arg-13, arg-14 and gln-17. The negative charges of the binding site were proposed to lie at the bottom of pockets or wells, so that it was thought desirable to aim for structures with three arms (fig 24).
The principal contributing factor to the binding of gln-17 was thought to be from a hydrogen bonded interaction of the glutamine side chain amide since the primary attraction to the channel mouth is electrostatic, although there may also be contribution from the ethyl side chain, as discussed in Chapter 1. The hydrogen bonding is probably between the N-H of the amide and a negatively charged group on the receptor, in view of the electronegative nature of residues near the channel mouths of all K+ channels cloned to date.

At the time of consideration of initial compounds for examination, it was decided to elaborate on the fumaric acid based structure (2) in order to provide a link with the previous compounds. Retention of the trans double bond as the central unit was considered to be worthwhile since it has been successfully used as an isostere in other instances, and introduces a degree of hydrophobicity.

The most obvious point for this elaboration was to introduce a substituent by transforming one of the secondary amides into a tertiary amide. Molecular modelling calculations (Chemex) carried out on potential candidates, in comparison with that of apamin, showed that the desired spatial arrangement could be achieved by incorporation of an octylamino group on one of the amide nitrogens, in UCL 1190. The primary amine group was selected in view of the suspected mode of binding of gln-17 being due to its hydrogen donor ability, while the alkyl chain provided a degree of hydrophobicity.

![Chemical Structure](UCL 1190)
The relatively planar central unit is rigid, but sufficient conformational flexibility was theoretically present in the alkyl chains to accommodate the desired spatial arrangement, through twisting of the octyl chain, as fig 26:

![Fig 26](image)

A disadvantage of the above conformation is its relatively high energy due to steric interactions. In addition there are several minimum energy conformers corresponding to the *trans* and *gauche* amide rotamers, which create a wide variation in relative spatial positions of the three basic groups.

In order to test the theory that any resulting increase in activity might be due to the twisting of the octyl chain, a second candidate was selected having the n-octyl chain replaced by a biphenyl, this unit being of similar length (15 A) to the alkyl analogue in its linear conformation, and is unable to twist to adopt the conformation in fig 26. In the resulting molecule, UCL1641, the spatial relationships are much more closely defined since the biphenyl moiety is essentially linear.

![UCL1641](image)

Fig 27
The biphenyl unit is becoming increasingly popular as a spacer in drug design\textsuperscript{201,202}, introducing characteristics of hydrophobicity and specificity of orientation which make it an ideal 'silent' residue. For example, 4-(4-benzylamino)-benzoic acid has been used to replace two turns of an $\alpha$-helix in V.I.P\textsuperscript{185}. It was thought that the greater lipophilicity of the biphenyl group compared to the alkyl linkage might also be advantageous if a hydrophobic interaction is important in the binding.

Several analogues having a cis double bond central unit were found to possess affinity for the SK$_{Ca}$ channel. Accordingly the cis isomer of UCL1190, UCL1597, based on the maleic acid central unit, was selected for synthesis.

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\includegraphics[width=0.5\textwidth]{fig28.png}};
\node at (0,0) {UCL1597};
\end{tikzpicture}
\end{center}

2.2. Replacement of the guanidinium groups by aminoquinolinium

At the same time as the above candidates were being prepared, structure activity studies were being performed on a series of derivatives of dequalinium (fig 29) by other members of the research group\textsuperscript{203,204}.

\begin{center}
\begin{tikzpicture}
\node at (0,0) {\includegraphics[width=0.5\textwidth]{fig29.png}};
\node at (0,0) {Fig 29};
\end{tikzpicture}
\end{center}
One of the conclusions from the observed activities was that replacement of the two guanidines corresponding to arg-13 and arg-14 in apamin with aminoquinolium groups significantly increased the binding affinity. For example, the 70-fold increase in potency between the two derivatives in fig 30.

![Chemical structures](image)

UCL 1609, IC$_{50}$ = 25μM  
UCL 1488, IC$_{50}$ = 0.17μM

Fig 30

This may seem surprising in view of Farmer's Rules, in view of the much greater steric bulk of the aminoquinoline substituent inhibiting a fit between the blocking agent and residues in the channel pore. However, from these studies it was apparent that there was a degree of steric tolerance in the interaction. The increased activity was concluded to arise from an improved hydrophobic interaction, discussed in Chapter 4, which had overcome any steric constraint.

Further examples of inhibition of potassium channels by aminoquinoline-based agents was reported recently, the compound (3) below being particularly active against the voltage activated, n-type channel $^{205}$. 
Accordingly, in order to bridge the gap between the fumaric acid diamide compounds and the dequalinium-type derivatives, it was decided to prepare an analogue of UCL1190 with the guanidines replaced by aminoquinolinyl-type substituents.

Whilst it would be preferrable to replace the two guanidine residues directly with quaternary aminoquinolinium groups (i.e (5)) in order to provide a direct comparison with the majority of the compounds of the dequalinium series made by other group members203,204, after assessment of the various synthetic options this was not attempted; potential routes involving alkylation of 4-aminoquinoline on the ring nitrogen with dihalopropyl substrates (e.g (4), fig 32) were rejected due poor synthetic accessibility arising from the predictable instability of the halide precursor and its earlier intermediates.

A more synthetically accessable alternative which appeared worth exploration was thought to be UCL1716, in which the aminoquinolininyl groups were linked through the exocyclic nitrogen.
Although 4-aminoquinoline is less basic ($\text{pK}_a 9.2^{206}$) than guanidine ($\text{pK}_a 12.5^{207}$), and non-quaternized, the above derivative was considered to be a suitable target compound for examination, since earlier results had shown that quaternization was not an essential feature for channel blockade, provided that the heterocycle is sufficiently basic to be protonated at physiological pH$^{204}$ (although aminoquinolines alkylated on the ring nitrogen are not true quaternary salts, the term is used for convenience in this thesis).

2.3. Trisubstituted aryl centred compounds

2.3.1. 1,2,4 and 1,3,5 Trisubstituted benzenes

Molecular modelling (using Chemex) of the fumaric acid-based group of compounds prepared above (UCL 1190, 1641, 1716), shows their most energetically favourable conformation to be essentially planar. Although there is reasonable flexibility in the alkylamino chains to adopt a three-legged-type structure, this is a relatively high energy conformation. In order to design target compounds in which the desired conformation would be more energetically favourable, it was thought necessary to depart from the planar fumaric acid-based template. An excellent way of accomplishing this was considered to be to use an aromatic ring as a spacer between one turn of the helix. On this basis molecular modelling studies were carried out on a variety of potential templates. As a result it was concluded that a 1,6-disubstituted indane should act as an excellent template for two adjacent residues of an $\alpha$-helix. This conclusion was supported independently by other molecular modelling studies, as in the example (phe,leu) below$^{208}$. 

![Diagram of compound UCL1716]
Omission of amide linking groups was thought reasonable on considering the evidence that the peptide backbone in apamin and related toxins apparently serves only to provide a rigid framework for assembly of the various aminoacid side chains in the correct structural orientation to provide activity. Accordingly, it was decided to select a range of candidates for examination based on this template.

Thus, the above model proposes location of the two arginine side chains at the 1 and 6 positions of indane in order to simulate arg-13 and arg-14 in apamin. The gln-17 residue could then be incorporated in this type of structure as in fig 35:
Retrosynthetic analysis of the above target compounds revealed no straightforward synthetic route. No indanes with the desired substitution patterns have been prepared to date. As an alternative, use of indoline as a template was considered, but this also appeared to be synthetically very difficult.

An additional feature of the above molecules is their optical activity, (7) having two chiral centres (C\textsubscript{1} and C\textsubscript{3}). Only one of the enantiomers of (6) and (7) represents the correct direction of turn of the helix, and so resolution or a chirally selective synthesis would ideally be required.

A simpler version of the 1,6-indane template was considered to be a meta-substituted benzene derivative. Although possessing more rotational freedom than the bicyclic unit, a very similar spatial conformation of arg-13 (G\textsubscript{1} below) and arg-14 (G\textsubscript{2}) is achievable in this more synthetically accessible monocyclic analogue (fig 36). An additional advantage is that the issue of the multiplicity of enantiomers is overcome since the proposed molecule has a plane of symmetry in the plane of the aromatic ring, and so is not optically active. This type of central unit would enable the gln-17 residue (G\textsubscript{3}) to be incorporated by a third substituent in the 4 or 5 position.

![Fig 36](image)

While sp\textsuperscript{3} hybridisation is required for the correct orientation of the substituents in 1 and 3 positions (X), the length of the spacer could be varied by having either sp\textsuperscript{2} or sp\textsuperscript{3} hybridisation at the third ring position (Y).
These two series were examined using a (Chemex) molecular modelling programme, and after energy minimisation the interatomic distances were determined and compared with the respective key distances in apamin (fig 36).

### Table 2 - Interatomic distances in apamin & prospective model compounds

<table>
<thead>
<tr>
<th></th>
<th>Apamin 1,3,5-</th>
<th>1,3,5-</th>
<th>1,2,4-</th>
<th>1,2,4-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$sp_3$</td>
<td>$sp_2$</td>
<td>$sp_3$</td>
<td>$sp_2$</td>
</tr>
<tr>
<td>$a_1-b$</td>
<td>5.9A</td>
<td>X$_1$-Y=5.1A</td>
<td>X$_1$-C$_1$=6.0A</td>
<td>X$_1$-Y=5.6A</td>
</tr>
<tr>
<td>$a_2-b$</td>
<td>5.5A</td>
<td>X$_2$-Y=5.1A</td>
<td>X$_2$-C$_1$=5.2A</td>
<td>X$_2$-Y=3.0A</td>
</tr>
<tr>
<td>$g_1-g_2$</td>
<td>10.8A</td>
<td>G$_1$-G$_2$=10.6A</td>
<td>G$_1$-G$_2$=11.2A</td>
<td>G$_1$-G$_2$=11.1A</td>
</tr>
<tr>
<td>$g_1-g_3$</td>
<td>12.8A</td>
<td>G$_1$-G$_3$=10.7A</td>
<td>G$_1$-G$_3$=12.9A</td>
<td>G$_1$-G$_3$=12.9A</td>
</tr>
<tr>
<td>$g_2-g_3$</td>
<td>8.0A</td>
<td>G$_2$-G$_3$=10.7A</td>
<td>G$_2$-G$_3$=10.7A</td>
<td>G$_2$-G$_3$=10.7A</td>
</tr>
</tbody>
</table>

(1 helix turn = 5.38 A)

$sp_2$ hybridisation can be achieved by using anilines; it is a feature of the compounds having N joined to the aromatic ring that the C$_1$ atom also lies in the plane of the ring, since delocalisation of the lone pair of the nitrogen with the $\pi$-electrons of the ring causes it to be trigonal. The energy barrier to rotation between the two resulting isomers is, however, small.

![Fig 37](image)

The following candidates were selected from amongst the various potential alternatives by examining not only fulfillment of the structural requirements described above, but also in terms of availability of raw materials and ease of synthesis.
In these compounds it was decided to mimic the gln-17 by using a third aminoquinolinium substituent, principally for synthetic accessibility. This proposition was considered to be acceptable as an assessment of the binding of the 'gln-17' through its H-donor properties, from interaction of the N-H protons of the primary amide with a negatively charged residue in the channel mouth rather than as a proton acceptor through the carbonyl group.

Accordingly, following the previously defined criteria, two groups of target compounds were selected. In the first group, having 1,3,5-trisubstitution, in order to examine the effect of varying the nature and length of the 5-substituent on potency, X was kept as O, and Y chosen to be O, N or C (-N), as below. A similar variation was explored in the second group, having 1,2,4-substitution.

**Group 1 (1,3,5-trisubstituted benzenes)**
**Group 2 (1,2,4 trisubstituted benzenes)**

Preparation of structural types containing nitrogen linkages, which fulfilled the principal requirements outlined above, would be additionally informative as a result of the presence of the amine group, since this might aid in the binding to the receptor by providing an additional polar centre. An important feature in these derivatives was the scope for production of a range of variations at the 4/5 position, not only through alteration of the N-alkyl group, but also by incorporation of a fourth substituent (see section 2.4).

It had been intended to prepare several analogues with ethoxy linkages to provide an indication of the effect of altering the maximum distance between the aminquinolinyls. However, because of difficulties discussed in the synthesis section, this was not carried out.
2.3.2. 1,2,3 Trisubstituted benzenes

The affinity of gallamine for the SK$_{Ca}$ channel was discussed above, having an IC$_{50}$ of 12 µM. In view of the greater activity of compounds possessing the aminoquinolinyl-type substituent in place of an aliphatic, electropositive (e.g. guanidine) group, it was decided to prepare the analogue of gallamine having the triethylammonium groups replaced by 4-aminoquinolinyl, (55) below, as a further probe of this interaction, and for comparison with the series above. In the event, because of difficulty with the synthesis the propoxy analogue, UCL 1822, was prepared instead.

Unlike the compounds prepared above it was thought that it should be easier to form a quaternary salt derivative in the case of UCL1822, since the alkylation should be less susceptible to the side reactions of other analogues, such as alkylation of other amines in the molecule, or ring alkylation, since only two of the substituents (1 & 3) reinforce each other towards electrophilic substitution (in the 4 position). The triethyl analogue, UCL 1836 (fig 40), was selected. The relative potency of this compound compared to UCL 1822 would provide valuable information for investigation of the important issue of the need for quaternisation of the aminoquinoline.
2.4. Tetrasubstituted aryl centred compounds

A deliberate feature of the selection of derivatives containing nitrogen linkages was the potential for introduction of a fourth substituent. In this way as many features as possible could be retained with minimum disruption to the rest of the molecule, so that the effect of the elaboration could be accurately assessed. In particular, incorporation of a fourth aminoquinoline was considered to be worthwhile, predominantly as a measure of a statistical contribution to the binding. For this purpose it was desirable to keep a similar separation between each pair of aminoquinolines. With regard to the exact length of the linking chain, this was chosen to correspond with the placement of his-18 in apamin, a possibly contributary residue in the binding interaction. From measurement of the relevant interatomic distances in the energy minimized molecular model of apamin, the linking chain was chosen to be n-pentyl, resulting in selection of UCL1821 and 1823.
CHAPTER 3

SYNTHESIS OF COMPOUNDS

3.1. UCL1190

3.1.1. Synthetic strategy

On assessing the various possible approaches to UCL1190, the principal difficulty identified was to devise a strategy for the selective introduction of the two guanidine substituents and the primary amine. There are only a limited number of preparative methods for guanidines, the most frequently used being from the respective primary amines. Accordingly there were two alternative options, involving protection of either the two guanidines or the primary amine, with removal of the particular protecting group being the final stage in the synthesis.

A variety of protecting groups has been used for guanidines, the most popular being nitro, arylsulphonyl and acyl. However, selective removal is often a problem as forcing conditions are required, usually hydrogenation or strong acid hydrolysis, both of which would affect other parts of the UCL1190 molecule.

It was therefore preferred to protect the primary amine, since protecting groups are available for this group which can be removed under much milder conditions. Since chemical manipulation of guanidines is difficult due to their high basicity and water solubility, formation would desirably be the penultimate stage.

Various reagents have been used for the guanidination of primary amines. O-methyl isourea was thought unsuitable in view of the high basicity and instability. An alternative more recently favoured, 3,5-dimethyl pyrazole-1-carboxamidine nitrate (fig 42), which requires an optimum pH of 9.5 and milder conditions, was selected. This was preferred to S-methyl isothiourea, which generates methane thiol and formamidine sulphinic acid, after which it would be difficult to isolate the product.
Retrosynthetic analysis of the remainder of the UCL1190 synthesis is summarised in fig 43.

Other prominent disconnection points are the two amide bonds, leaving an unsymmetrically substituted fumaric acid, and two amines, 1,3-diamino propane and 1,8-diamino-1-(3-aminopropyl)-octane. In order to achieve selective coupling, a strategy was required to enable these amines to be joined sequentially to the fumaric acid component. A suitable substrate was provided by fumaric acid monoethyl ester, for after coupling the first amine and the acid, the ester group could be hydrolysed and then joined to the second amine.

A further consideration was that, in each coupling reaction, the amines in the diamine components which are not required to react must be appropriately protected, the selected group being different to that used for the octylamine. Disconnection points for 1,8-diamino-(3-aminopropyl)-octane are located at the secondary amine. The synthetic strategy for this intermediate was largely dictated by availability of 1,8-disubstituted octane raw materials, 8-bromo-1-octanol being the only relatively cheap alternative available in sufficiently large quantities.

After consideration of the stability of a range of protecting groups to the proposed conditions in the synthesis, the strategy chosen was to protect the octylamine as the trifluoroacetamide and the other two amines as the t-boc carbamate derivatives. These two types of protecting group were selected for their markedly different behaviour under various pH's, t-boc being stable to pH >7 and being increasingly labile with decreasing pH$^{215}$, whereas
HN

HN(CH₂)₃HN.OC

(Ch₂)₃NH + HN(CH₂)₃NHP₂

H₂N(CH₂)₃NHP₂

X(CH₂)₃NHP₂

X(CH₂)₃NHP₁

HN(CH₂)₃NHP₁

CO₂H

H₂N(CH₂)₃NHP₂

RO₂C

RO₂C

HN(CH₂)₃NHP₁

Fig 43, Retrosynthetic analysis of UCL1190
trifluoroacetamide is stable to acidic conditions and readily cleaved under mild base. This would enable the t-boc protecting groups to be removed (acidic conditions) and guanidinated, before liberating the octylamine with aqueous base.

The amides could be theoretically formed in either order. However, if the tertiary amide were prepared first, the trifluoroacetyl protecting group would be labile under the basic conditions used in the ester hydrolysis. Accordingly, the secondary amide was prepared before the tertiary amide. This has the additional advantage of being a more convergent synthesis, thereby making best use of the secondary amine (14), since its preparation requires five steps. Thus the route outlined in fig 44 was adopted.

3.1.2. Synthesis of UCL1190

For the synthesis of t-boc-1,3-diaminopropane, di-t-butyl dicarbonate was added slowly to a large excess of the diamine in order to minimise formation of the di-t-boc derivative.

The unreacted diamine remained in the aqueous phase on work up; the pure product being extracted after filtering off diprotected material. Extraction into ether gave product which could be stored without significant deterioration, whereas use of methylene chloride, which extracted more product, resulted in decomposition on storage.

Reaction with monoethyl fumarate was carried out using 1,3-dicyclohexyl carbodiimide as coupling agent to form the activated ester in the presence of 1-hydroxybenzotriazole. This reagent is commonly used in peptide chemistry to suppress racemisation and minimise side reactions, which usually arise from decomposition of the initially formed O-acyl urea.

Its success lies in the rapidity with which it acylates the O-acyl urea to form a 'peptide ester' which acylates the amine (fig 45).
The resulting amidic ester, (9), was hydrolysed with aq base to give the acid (10).

8-Bromo-1-octanol was converted into aminooctanol (12) following the widely used Gabriel synthesis, involving sequential treatment with potassium phthalimide and hydrazine\textsuperscript{219}. Phthalimidooctanol (11) was obtained in fairly low yield, the major byproduct being the cyclic ether (nmr). Reaction with hydrazine hydrate afforded aminooctanol in quantitative yield, which was trifluoroacetylated to give (13). This was converted to the tosylate, which was successfully processed without purification; t-boc diaminopropane being used as its own base for conversion to (14). The hydrochloride of the product was extractable into chloroform, providing a convenient method of purification.

Coupling of (10) with (14) proceeded in virtually quantitative yield. Removal of the t-boc protecting groups was accomplished with HCl in methanol.
An effervescence was produced due to the liberation of carbon dioxide and formation largely of isobutylene:

\[
\begin{align*}
R & \quad \text{RNH}_2 + \text{CO}_2 + \text{H}_3\text{C} & \quad \text{CH}_3 \\
\text{O} & \quad \text{CH}_3 & \quad \text{CH}_3
\end{align*}
\]

Fig 46

Carefully defined conditions were, however, necessary during the work up to avoid removal of the trifluoroacetyl group. Cleavage of this group is known to occur even with Na\textsubscript{2}CO\textsubscript{3} in aq methanol\textsuperscript{220}. The triamine produced was not easy to separate from (16), and attempted purification resulted in further removal. After the reaction the pH was kept between 8.5-9.0, just sufficient to liberate the free primary amines, and the extraction carried out at low temperature to minimise decomposition. The high water solubility of (16) made extraction difficult. If methanol was removed before work up, (16) remained in the aqueous phase. However, taking these precautions, the product was obtained in good yield and purity.

**UCL1190**

Carefully defined conditions were also required for the guanidination of (16); the pH being particularly important. At the optimum pH of 9.5 (16) rapidly decomposes. At pH<6, no reaction occurs since the amines are fully protonated. Accordingly the pH was maintained at 7.0-8.5, by periodic addition of NaOH, as the pH falls during the reaction due to formation of the weakly basic 3,5-dimethylpyrazole. Hydrolysis of the dimethylpyrazole carboxamidine to dimethylpyrazole and N-cyanocarboxamide also takes place during the reaction, hence the use of a large excess of guanidinating agent. After removal of the trifluoroacetyl group with excess NaOH, excess dimethylpyrazole carboxamidine and dimethylpyrazole were conveniently removed by washing with chloroform. Acidification with nitric acid then
gave crude UCL1190, which was purified and freed from inorganic material by recrystallisation from ethanol.

3.2. UCL1641

The logical approach to this compound involved preparation of (21), the biphenyl analogue of aminoctanol. Unsymmetrical biphenyls are comparatively rare in terms of commercial availability.

Use of the Ullman reaction on two different aryl halides invariably gives the symmetrical byproduct\(^\text{221}\). Thus the most common route to 4-4'- bifunctionality is via electrophilic substitution\(^\text{222,223}\). A route to (21) from 4-bromobiphenyl was devised, shown in fig 48.

\[\text{(19)}\]

4-Bromobiphenyl was acetylated under Friedel Crafts conditions using \(\text{AlCl}_3\) as Lewis acid catalyst to give exclusively the 4'-acetyl derivative (17). The aryl ring is highly activating towards electrophilic substitution, the positive charge of the arenium ion intermediate being delocalised through the two rings (fig 47)- no ortho substitution was observed due to steric hindrance to attack at this position.

![Fig 47](image-url)
Fig 48
Bromoform oxidation to the carboxylic acid, (18), was accomplished in almost quantitative yield; the selectivity and facile nature of this particular transformation probably being promoted by the high percentage of the enolate tautomeric of the deprotonated aryl methyl ketone.

The acid was esterified to (19) by sequential treatment with thionyl chloride and methanol in high yield- attempted esterification in methanol alone proved very slow due to poor solubility of the starting material.

The bromo substituent of (19) was converted to the nitrile (20) with copper (I) cyanide in DMF, using the Rosemund Von Braun reaction. The mechanism is thought to involve an aryl copper intermediate.

(21)

Attempted catalytic or LiAlH₄ reduction of (20) to (21) resulted in hydrogenolysis, giving predominantly 4-(4-toluyl)-benzyl alcohol (nmr). Use of LiBH₄ in THF was more successful, although the yield was variable. A variety of borate complexes were produced incorporating a large amount of the nitrile alcohol intermediate (nmr, IR) which could not be further reduced.

UCL 1641

(21) was processed by application of the same methodology as used for UCL 1190. Reactivity of the respective intermediates varied: Displacement of the benzylic tosyl group with bocdiaminopropane to give (22) proceeded rapidly, in accordance with the much greater susceptibility to nucleophilic substitution as a result of delocalisation of the positive charge from the benzylic carbonium ion through the aromatic rings. The lower solubility of (22) in comparison to the octylamine analogue (14) necessitated addition of DMF in the coupling reaction with (10) to give (23) in order to achieve reaction. The product was converted to UCL 1641 via hydrolytic cleavage of the t-boc protecting groups, followed by treatment with 3,5-dimethylpyrazole carboxamidine nitrate and removal of the trifluoracetyl group, as for UCL 1190.
3.3 UCL1597

Retrosynthetic analysis suggested a similar approach to UCL1190 above, starting from maleic anhydride in place of monoethyl fumarate (fig 50). This was initially explored, treating maleic anhydride with t-boc 1,3-diamino propane to give (24) (fig 51).

A major problem was encountered with the coupling of (14) with (24), the cis analogue of (10), for under a variety of conditions complete isomerisation occurred, giving the trans isomer. This was believed to be due to deprotonation of the secondary amide, followed by rotation to the more thermodynamically stable trans configuration:

If isomerisation had occurred by the above mechanism, it was thought that it should be possible to circumvent this by first forming the tertiary amide (26), which has no available 'acidic' amide proton. Coupling of this acid with t-boc 1,3-diaminopropane would then give the cis diamide, (25). This approach proved successful, in practice giving exclusively the cis isomer. Deprotection was carried out with HCl in methanol, as for the trans isomer. However, attempted extraction of the resulting diamine (27), proved unsuccessful; the product in this case being too water soluble. The work up
Fig 50, Retrosynthetic analysis of UCL1597
Fig 51
procedure was therefore modified, adjusting the pH of the final reaction mixture to 8 with sodium methoxide in methanol and filtering off the liberated NaCl before treatment with dimethylpyrazole carboxamidine nitrate under similar conditions as for the trans isomer. No isomerisation was observed following the coupling stage.

3.4. UCL1716

3.4.1. Synthetic strategy
In view of the similarity to UCL1190, the general synthetic strategy adopted was based on this earlier synthesis. Thus, it was thought preferable to remove the trifluoroacetoxy protecting group in the final stage, and similarly make disconnections at the two amide bonds of fumaric acid. Following retrosynthetic analysis (fig 52), in the case of UCL 1716 it was considered that protection of the two propylamines should be unnecessary, since it should be possible to incorporate the aminoquinoline substituents directly into the synthesis. Alkylation of the two liberated propylamines with 4-chloroquinoline as the penultimate stage would thus be avoided. This would be highly likely to be unsuccessful, resulting in decomposition, predominantly via loss of the CF₃CO protecting group (and consequent alkylation on the liberated octylamine) under the forcing conditions required. The exocyclic nitrogens of the aminoquinoline substituents were not anticipated to significantly create difficulties in the synthesis since the lone pair on the NH is conjugated with the π-system of the aromatic ring, rendering this group much less nucleophilic than the primary and secondary 'aliphatic' amines.

The route shown in fig 53 was selected, in which trifluoroacetamidooctyl tosylate prepared for the synthesis of UCL 1190 and UCL 1597 could conveniently be used to prepare the secondary amine (31), by nucleophilic displacement with the primary amine of 3-aminopropyl-4-aminoquinoline (28). The latter could also be utilised to prepare the mono amide section of the molecule, (29).
Fig 52, Retrosynthetic analysis of UCL1716
The presence of the amine group made the product of the reaction difficult to isolate. The reaction was repeated several times with success, and the isolated material was found to be the desired product. The reaction was performed using diisopropylethylamine as an activating agent and diethyl ether as the solvent. The yield of the isolated compound was high, although the yield was comparatively low (30%).

(28) was alkylated with the reagent 3,4-diethylaminobenzyl bromide at room temperature to give (32). After purification, the isolated material was found to be the desired product. The reaction was performed using diisopropylethylamine as an activating agent and diethyl ether as the solvent. The yield of the isolated compound was high, although the yield was comparatively low (30%).
3.4.2. Synthesis of UCL1716

(28)
Alkylation of t-boc-1,3-diaminopropane with 4-chloroquinoline proceeded in good yield to give (27) with no evidence of deprotection and formation of dialkylated diaminopropane. The presence of the aryl group made the product relatively easily extractable, unlike t-boc diaminopropane, described previously. Deprotection gave the primary amine (28), which required storage in an airtight container to prevent the rapid formation of the carbonate on exposure to the atmosphere.

(30)
Coupling of (28) with monoethyl fumarate to give the amidic ester (29) was performed using dicyclohexyl carbodiimide as activating agent. No advantage was obtained using HOBT in this case. Hydrolysis of (29) was carried out in aqueous methanol with NaOH. Isolation of the zwitterionic product by pH adjustment and extraction proved unsuccessful due to its high water solubility. However, it was found that on acidifying the final reaction mixture with conc. HCl, the hydrochloride salt was slowly precipitated, as the hydrate, effectively being salted out as a result of the large quantity of NaCl generated. The purity of the isolated (30) hydrochloride was high, although the yield was comparatively low (30%), due to both the difficulty in isolation and hydrolysis of the aminquinoline to 4-quinolone (nmr).

(31)
(28) was alkylated with trifluoroacetamidoctyl tosylate in DMF at room temperature to give (31). Alkylation on the ring nitrogen competed strongly with the primary amine. The lone pair of the ring nitrogen is not conjugated with the ring and is free to attack an electrophile, as in fig 54.
It is noteworthy that acylation on the ring nitrogen, for example during the coupling of (28) with monoethylfumarate, is reversible, in contrast to alkylation. 4-Aminoquinoline gives rise to the 4-acyl derivative on treatment with acetic anhydride (fig 55). However, this appears to be less favourable than acylation of the primary amine in (28) due to the lower nucleophilicity of the aniline nitrogen.

The resulting secondary amine (31) was coupled with (30) hydrochloride using dicyclohexylcarbodiimide, DMF being chosen as solvent in order to
solubilise the hydrochloride salt of the acid. Activation of the amino acid hydrochloride proceeded readily, the resulting activated ester precipitating out of solution as the hydrochloride. Subsequent addition of (31) to the reaction mixture resulted in its immediate protonation, and so coupling was then achieved by addition of diisopropylethylamine to effect deprotonation. The trifluoroacetyl protecting group was readily removed from (32) by treatment in methanol with aq NaOH at room temperature. The presence of the aromatic groups facilitated the work up, when compared with the guanidine analogues above, UCL1716 being extracted into chloroform then isolated by precipitation from diethyl ether.

3.4.3. UCL1716 - diquaternary salt (33)

In order to explore the effect of ring alkylation on potency, preparation of (33) was attempted. Alkylation of the trifluoroacetylated precursor (32) was tried, under a range of conditions (e.g. Mel, EtI in DMF, 1-pentanol, acetone, MIBK, no solvent), since direct alkylation of UCL1716 would clearly be unsuccessful due to competing alkylation of the octylamine. However all reactions produced multicomponent mixtures, from which no product could be isolated.
3.5. UCL1714

3.5.1. Synthetic strategy
A readily available 1,2,4-trisubstituted aromatic is dopamine, from which it was considered that UCL1714 could be prepared.

From retrosynthetic analysis (fig 57) the three aminoquinolinium groups could either be incorporated simultaneously, in one final stage, or at least one be introduced earlier in the synthesis, as with UCL1716. Disconnections at either side of each ether linkage strongly favoured their formation by alkylation of the diphenoxide salt with an appropriate alkyl halide, in the Williamson synthesis. Phenols, being more acidic ($pK_a$ 8-11) than aliphatic alcohols ($pK_a$ 16), undergo this type of reaction more readily than aliphatic alcohols, which require much stronger bases to effect deprotonation and hence alkylation.

The route via a disubstituted halopropyl intermediate (i.e (34)) was rejected due to expected instability during synthesis; Preparation of this type of intermediate via alkylation with a bromochloropropane was predicted to be severely disfavoured due to formation of cyclic/intermolecular ethers. Introduction of the ether linkage directly via a halopropylaminoquinolinyl intermediate (e.g (35)) was also discarded, in view of the likely instability of this material to self alkylation, giving quaternary salts.

For a similar reason the route through N-(4-quinolinyl)-dopamine was also rejected; Its formation by treatment of dopamine with 4-chloroquinoline would probably be complicated by alkylation of the phenol groups with the chloroquinoline, and subsequent O-dialkylation of the product would again be difficult due to quaternisation. Accordingly, it was decided to introduce the aminoquinolinyl substituents via a protected bromopropylamine, having first protected the dopamine NH$_2$ to prevent competing alkylation on the phenethylamine nitrogen. t-Boc was chosen as the protecting group in both cases in view of its expected stability to the basic conditions required for
Fig 57, Retrosynthetic analysis of UCL1714
the alkylation and ease of removal (fig 58). This approach generated a triprotected triamine which after deprotection was alkylated with excess 4-chloroquinoline.

\[
\begin{align*}
\text{OH} & \quad \text{OH} \\
\text{NH}_{2}\text{HCl} & \quad \text{OH} \\
& \quad \text{Br(CH}_2\text{)}_3\text{NH.Boc} \\
& \quad \text{Br(CH}_2\text{)}_3\text{NH.HBr} \\
\text{NHBoc} & \quad \text{Boc.HN(CH}_2\text{)}_3\text{O} \\
& \quad \text{O(CH}_2\text{)}_3\text{NH.Boc} \\
& \quad \text{NH.Boc} \\
& \quad \text{HN(CH}_2\text{)}_3\text{O} \\
& \quad \text{O(CH}_2\text{)}_3\text{NH} \\
& \quad \text{UCL1714} \\
\end{align*}
\]
3.5.2. Synthesis of UCL1714

**t-Bocdopamine (36)**

Dopamine possesses considerable zwitterionic character due to the acidity of the two hydroxyl groups\(^2\)\(^2\)\(^7\), which makes handling of this highly water soluble material very difficult:

\[
\begin{align*}
\text{OH} & \quad \text{OH} \\
\text{NH}_2 & \quad \text{NH}_3^+ \\
\end{align*}
\]

![Fig 59](image)

**Fig 59**

`t-Bocdopamine was conveniently prepared by adding sodium methoxide to a mixture of dopamine hydrochloride and di-t-butyl dicarbonate in methanol. In this way, isolation of the zwitterion was avoided, by liberating the free amine in situ for immediate reaction with the protecting agent. Reaction of the protecting agent with the methanol solvent and the phenol groups was negligible due to the much greater nucleophilicity of the amine. The product was also found to be highly water soluble, and so a non-aqueous work up was performed.**

**t-Boc-3-bromopropylamine (37)**

`t-Boc-3-bromopropylamine was prepared by a similar manner to boc-dopamine, adding sodium methoxide to 3-bromopropylamine\(\text{HBr}\) and di-t-butyl dicarbonate in methanol. In this case the competing reaction, involving self alkylation of bromopropylamine base was suppressed by generating the base in situ, which was immediately quenched by protecting agent. The product was found to be unstable on storage, nmr evidence indicating gradual hydrolysis to t-boc-hydroxypropylamine, which caused difficulties in purification at subsequent stages in this and other syntheses.`
Although phenols are sufficiently acidic ($pK_a$ 8-11) to be deprotonated by aq NaOH or even weaker bases\textsuperscript{229}, non protic conditions were chosen to form the dianion from (36). This was both to prevent competing hydrolytic attack on the labile bromide of bromopropylamine, and to promote nucleophilic displacement by non-solvation of the phenoxide anions; polar aprotic solvents such as DMF are favoured for this type of reaction\textsuperscript{230}. Potassium t-butoxide was chosen as the base for convenience. Although t-butanol is formed, it has been shown that polar protic solvent in concentrations slightly above the anion reactant do not sharply lower the reaction rate, being of much lower $pK_a$ (14-17\textsuperscript{227}), and in the case of t-butanol comparatively more hindered.

After washing out acidic material from the crude product, the purity was still poor, containing material from the competing reaction involving elimination of HBr from the alkylating agent (nmr). Purification to 98% was achieved by recrystallisation from diisopropyl ether.

**UCL 1714**

Although the three t-butoxycarbonyl protecting groups could be removed using hydrochloric acid or HCl in methanol, the excess acid was difficult to remove after deprotection since the triamine could not be extracted from water. Accordingly trifluoroacetic acid was used, this acid being anhydrous and low boiling, so that excess reagent could be easily removed under mild conditions, by distillation.

The resulting trifluoroacetate could not be obtained crystalline. However, it was found to be possible to convert the product directly to UCL1714 without further manipulation, for after basifying with NaOMe the resulting crude triamine could be successfully alkylated in pentanol with 4-chloroquinoline.
UCL1714 a stronger base (pK$_a$ 4-aminoquinoline 9.2) than quinoline (pK$_a$ 4.94)$^{206}$, since the conjugate acid is stabilised by delocalisation of the positive charge into the para amino substituent (fig 60).

![Chemical structure](image)

Fig 60

In view of this it was thought that it may be possible to carry out the reaction without additional base, the product being sufficiently basic to prevent protonation of the primary amine (pK$_a$ 10.8) groups of the triamine, rendering them inactive. In the event, however, very little reaction took place.

Since nucleophilic attack on the quinoline ring should be acid catalysed (see earlier), successive additions of acid were made and the rate monitored. These conditions also proved unsuccessful, possibly due to indeterminacy in judgment of the degree of protonation from the prior basification with sodium methoxide.

Use of diisopropylethylamine was successful, this being chosen as base because of its low nucleophilicity and comparatively high base strength (pK$_a$ 11.2)$^{206}$. Progress of the reaction could be followed well by tlc, three spots for mono and three for dialkylated intermediates being observed, in decreasing polarity up to the trialkylated product.

On work up the product was too insoluble to extract into methylene chloride alone, addition a small quantity of methanol being necessary to facilitate this operation. The resulting highly crude product was purified by column chromatography to remove excess 4-chloroquinoline, plus a considerable amount of polar material before crystallisation, affording UCL1714.
3.6. (39)
Preparation of (39), the lower homologue of UCL1714, was attempted following two approaches:

Sequential treatment of t-bocdopamine with KOBu and t-bocbromoethylamine in DMF gave no dialkylation and only ca 10% monoalkylated product (largely on the 4-position-nmr), which would not undergo further alkylation even on addition of extra base and alkylation agent. Thus there is a marked difference in reaction pathways between the ethyl and propyl halides. This was thought to be due principally to the predominance in the ethyl analogue of the competing side reaction involving elimination of HBr, believed to be promoted by stablisation of the developing double bond by conjugation with the lone pair of the nitrogen atom on the β-carbon. Another mechanism which generates HBr, and consequently involves loss of base, is via formation of an aziridine. This highly reactive species would be attacked by any nucleophiles present in the reaction mixture. Since one of these is the phenol, from the absence of any of the expected product either this side reaction does not predominate, or the aziridine is prefentially
attacked by another nucleophile, such as butanol/butoxide. An alternative reaction, involving formation of an oxazoline, was considered less likely even though base promoted cyclisation of β-haloacetamides has been used to prepare oxazolines\textsuperscript{231}, since this would also possible in the propyl analogue, which was not observed. A similar outcome resulted using bromoethylamine protected as the phthalimido derivative. In an alternative approach, dicyanomethylation of t-boc dopamine, to give (40), was accomplished with bromoacetonitrile and potassium carbonate in methyl ethyl ketone, following the procedure used for 2,6-xylenol\textsuperscript{213}. However, after subsequent LiAlH\textsubscript{4} reduction no product was extracted on work up, thought to be due to high water solubility, and hydrogenation gave a complex mixture.

3.7. Other 1,2,4-trisubstituted compounds

3.7.1. Aminocatechols

Treatment of benzene-1,2,4-triol with primary amines is reported in the literature to produce stable 2,4-dihydroxyanilines\textsuperscript{232}. These may also be prepared by oxidative amination of catecho\textsuperscript{233,234}.

Amination of 1,2,4-benzene triol with t-boc diaminopropane was initially explored, with the aim of subsequently preparing the tri-aminoquinolinyl propylbenzene (42) (fig 62).

![Diagram of chemical structures](image-url)
On heating benzene-1,2,4-triol with t-boc diaminopropane in THF/toluene while removing water by azeotropic distillation, one major product was formed, which was isolated by concentration and purified to 95% by column chromatography. The resulting orange gum was confirmed as (41) (nmr,ms). However, on storage the material rapidly darkened, even in the absence of air and light at 0°C, giving polymeric material. An attempt to minimise any decomposition by immediate alkylation was unsuccessful, addition of KOBu to the solution in DMF giving a black oily tar.

The aerial oxidation of some para-hydroxyanilines is reported in the literature. Indeed the parent compound is rapidly oxidised and is used as a photographic agent. The oxidation pathway is usually via coupling of the rings, giving rise to humic acid type polymers. (41) appeared to be similarly unstable, unlike the derivatives reported in the literature.

In view of the product instability, the alternative approach from catechol was not explored.

3.7.2. 1,2,4-Trialkoxybenzenes

The aerial oxidation of some para-hydroxyanilines is reported in the literature. Indeed the parent compound is rapidly oxidised and is used as a photographic agent. The oxidation pathway is usually via coupling of the rings, giving rise to humic acid type polymers. (41) appeared to be similarly unstable, unlike the derivatives reported in the literature.

In view of the product instability, the alternative approach from catechol was not explored.

3.7.2. 1,2,4-Trialkoxybenzenes

Preparation of (43) was attempted initially as an approach to (45). On sequential treatment of 1,2,4-benzene triol with KOBu and boc bromo propylamine in DMF a multicomponent mixture was obtained, containing large amounts of ring alkylated byproducts. It is noteworthy that in this case...
ring alkylation further activates the ring to further substitution, by reinforcing the o/p-directing hydroxyl groups. Purification and further processing of the crude mixture proved ineffective. As an alternative approach, in an attempt to produce a more crystalline intermediate which might allow purification, preparation of the tri-CBZ protected analogue (44) was attempted. CBZ-bromopropylamine was prepared by treatment of 3-bromopropyl amine.HBr and benzyl chloroformate with sodium methoxide in methanol, the product being extracted into diethyl ether after dilution with water, and crystallised from di-isopropyl ether. On using this material in the alkylation of benzene-1,2,4-triol a multi-component mixture was again obtained. However, after work up and initial purification by column chromatography, (44) was successfully isolated in 95% purity by crystallisation from diethyl ether. Unfortunately, on attempted deprotection by catalytic hydrogenation, even under mild conditions (palladium hydroxide at 3 bar), extensive reduction of the aromatic ring occurred. Attempts to prepare this product were therefore also abandoned.

3.8. 1,3,5-Trisubstituted benzenes

Phloroglucinol provides a versatile substrate for access to target compounds having the 1,3,5-aromatic substitution pattern, being widely used on industrial scale and a constituent of many natural products.

3.8.1. 1,3,5-Phloroglucinol triethers
As an approach to (46), trialkylation of phloroglucinol with t-boc-3-bromo propylamine was attempted.
Electrophilic substitution (E, below) in phloroglucinol is particularly easy, since all three hydroxyl groups stabilise the positive charge of the arenium ion intermediate through delocalisation.

This activity is further magnified under basic conditions, invariably resulting in polysubstitution. Friedel Crafts substitution takes place without need for acid catalysis, giving a variety of C- and O- alkylated products, depending on the conditions. Studies on the alkylation of resorcinol and phloroglucinol have shown the following factors affect the mode and position of substitution$^{236}$. 
1). Water or hydroxylic solvents which solvate the negatively charged oxygens promote attack on carbon over oxygen.

2). Use of DMF or DMSO, which are excellent cation-solvating solvents but poor at solvating anions, favours attack on the more electronegative oxygen.

3). Less polar, non-solvating solvents like hexane and toluene favour carbon alkylation.

4). As the alkyl group increases in size, the amount of C alkylation is reduced.

5). Sodium salts give far less C alkylation than lithium salts.

For example, Kaufler obtained exclusively mono-, di- and tri-benzyl ethers of phloroglucinol, in which it acts as a phenol, but initial methylation was always on the ring237. Despite the observation that certain minor variations in temperature, concentration and water content can profoundly affect the composition238, from the above information it was concluded that use of potassium butoxide in DMF should provide suitably favourable conditions for O-alkylation. However, under a variety of conditions C-alkylation overwhelmingly predominated (nmr). Use of NaH in DMF or DMSO gave a similar result. Nevertheless, the former conditions proved successful for other alkylations in this project.

3.8.2 UCL1715

A property of phloroglucinol and other phenols with m-hydroxy groups is the many reactions exhibited which are typical of ketones, even though there is no structural or spectroscopic evidence for the existence of enol-keto equilibria239. Typical is the displacement of a hydroxyl group by a primary amine, although under reportedly forcing conditions, to give an aniline (fig 66), thought to occur via an imine intermediate240.
It was proposed that reaction of phloroglucinol with boc-1,3-diaminopropane would provide a route to UCL1715 via similar methodology to UCL1714, as outlined in figure 69. Alkylation of the 'aniline' nitrogens was expected to be negligible, since the nucleophilicity is very low, as a result of the lone pair being delocalised with the aromatic ring through π-conjugation (fig 67).

It was anticipated that a second hydroxy group of phloroglucinol could be displaced by t-boc diaminopropane, leading to the derivative (50).

Q=4-Quinolinyl
3.8.3 Synthesis of UCL1715

(47) & (49)
Reaction of t-bocdiaminopropane with phloroglucinol proceeded rapidly without acid catalyst, the hydroxy group possessing sufficient acidity to catalyse the desired 'imine' formation. An equilibrium mixture of ca 1:1 (49)/phloroglucinol was formed initially, which was driven to near completion by azeotropic removal of the water formed, giving a mixture of (47) and (49), which was successfully separated. Evidently the second phenolic OH also possesses enough ketonic character to be susceptible to nucleophilic attack.

UCL1715
Dialkylation of (49), to give (51), was accomplished by sequential treatment in DMF with KOBu and t-bocbromopropylamine. Some ring alkylation was observed (nmr), but significantly less than for the attempted trialkylation of phloroglucinol. (51) was deprotected and treated with 4-chloroquinoline under similar conditions to those used for (38). The low nucleophilicity of the aniline nitrogen was expected to prevent its alkylation (by chloroquinoline). This was supported by the successful synthesis of UCL1715 and the observation of few non-polar byproducts.

3.8.4. (50)
Alkylation of (47) with t-bocbromopropylamine proceeded in high yield to give (48). Deprotection under analogous conditions as used for (51) gave the essentially pure triamine (tlc), but on heating with 4-chloroquinoline/diisopropylethylamine in 1-pentanol a large number of products were formed. Aerial oxidation was considered to be a possible source of side reactions. However, on carrying out the reaction with exclusion of air a similar result was obtained. The nucleophilic displacement of aminopropylamine substituents may also be a factor, from solvolysis or attack by the free primary amines present, leading to polymeric products (fig 70).

Indeed phloroglucinol is manufactured by hydrolysis of the 'trianiline' and can be prepared by acid hydrolysis of the trimethyl ether.
Since the rate of this reaction is probably pH dependent, several repeat preparations were carried out varying the proportion of base added. No single significant component could be identified which corresponded to the expected Rf on tlc for (50) (expected to be slightly more polar than UCL1715).

3.9. UCL1735

3.9.1. Synthetic strategy
As discussed above, the viability of a synthetic route to UCL1735 was a major consideration in its selection as a synthetic target. Disconnection points to the central unit are evident at the ether linkages at the 3 and 5 positions, as for UCL1715, and either side of the secondary amine of the 1-chain.

Formation of the key secondary amine below (fig 71) was initially attempted by reductive amination of a 3,5-disubstituted benzaldehyde with t-bocdiamino propane, particularly since it may be possible to apply this method to other analogues.
Formation of the imine was successful (ir), being essentially quantitative without need for azeotropic removal of the water. However, attempted catalytic reduction either produced no reaction (e.g. Raney nickel) or resulted in partial reduction of the aromatic ring (Pt, Pd, Rh on C/Al₂O₃); none of the desired product being isolated. In this context the facile reduction of resorcinol to cyclohexanedione is noteworthy. Chemical reduction proved unsuccessful, due to difficulty in extracting the zwitterionic product. 3,5-Dihydroxybenzyl alcohol subsequently became commercially available, which suggested an alternative approach, by displacement of a suitable leaving group from a benzylic substrate with a bocbromopropylamine. This reaction was predicted to be facile in view of the ease with which benzylic substrates undergo nucleophilic displacement.

From retrosynthetic analysis it was concluded that formation of the two ether linkages must precede preparation of the benzylic leaving group, since the intermediate produced in the reverse scenario would predictably be unstable due to self alkylation, giving aryl benzyl ethers. Thus the synthetic route outlined in fig 73 was adopted.

Selective alkylation of the two phenol groups of 3,5-dihydroxybenzyl alcohol was possible due to the much greater acidity of the phenol groups compared to the alcohol (see above), enabling them to be preferentially deprotonated and thence considerably more nucleophilic than the alcohol. The difference in acidities is a result of the stabilisation possible in the phenoxide ion through delocalisation of the negative charge into the aromatic ring. Although the neutral phenol molecule is similarly stabilised, this occurs to a lesser extent as it involves charge separation, and so formation of the phenoxide is therefore more energetically favourable than an alkoxide (fig 72).

![Fig 72](image)
Following an approach similar to that used for the synthesis of UCL1190, it was decided to convert (52) to the benzylic tosylate. Although one route to UCL1735 from this intermediate would be to treat it with t-boc diamino propane, giving the triprotected triamine, it was considered that it should be possible to introduce the 4-quinolinylaminopropyl unit at the benzylic position directly by reaction with (28). The aminoquinolinyl group should not be significantly affected during subsequent removal of the t-boc protecting groups.

Dialkylation of (28) by the tosylate (53), X = OTs, and alkylation on the benzylamine nitrogen of (54) by 4-chloroquinoline during the preparation of UCL1735 were predicted to be minimal, being prevented by steric hindrance from the bulky substituents on this secondary amine.

3.9.3 Synthesis of UCL1735

(53)

3,5-Dihydroxybenzyl alcohol was successfully alkylated with boc 3-bromo propylamine, giving the diether (52). Treatment with tosyl chloride/triethylamine in chloroform was intended to give the tosylate, in a method analogous to the preparation of trifluoracetamidotosylate for UCL1190. However, in the event this resulted in almost exclusive formation of the benzyl chloride (53), X=Cl.

It appeared that the tosylate leaving group was sufficiently labile in the benzylic system to be displaced with Cl⁻, a displacement which is irreversible. This was in contrast to the aliphatic case (UCL1190) where the tosylate formed was stable; the α-carbon being less susceptible to nucleophilic substitution.

UCL 1735

Displacement of the chloride was facile in DMF; the amine (28) being used in excess in order to also function as the base for combination with the liberated HCl. Deprotection and alkylation of (54) were performed under similar conditions as for the analogues described above. The crude product contained a large number of predominantly less polar byproducts, but was
successfully purified by column chromatography and 'crystallisation' from ethyl acetate to give UCL 1735.

3.10. Pyrogallol-based compounds

3.10.1. (55)
Synthesis of (55) from pyrogallol was attempted, using methodology based on the literature preparation of gallamine.

![Chemical structure of (55)](image)

1,2,3-Tri-(β-diethylaminoethoxy) benzene, the intermediate in the synthesis of gallamine, is prepared from pyrogallol and diethylaminoethyl chloride with either NaH in toluene⁴⁴ or NaOEt in ethanol⁴⁵. However, no reaction occurred with t-boc bromoethylamine under these conditions.

3.10.2 UCL1822
The difficulty in achieving alkylation with the protected β-haloethylamine was encountered in previous syntheses, but was not found with the propyl analogues. Since exploration of the nature of the drug-ion channel interaction is at an early stage in this study, and there appears to be a degree of
tolerance in the binding, it was considered that examination of the tri-
aminoquinolinylpropyl analogue of gallamine, UCL 1822 (fig 75) would be
equally informative as (55).

Fig 75
Alkylation with boc-3-bromopropylamine under conditions used to prepare the gallamine intermediate produced no reaction. Use of KOBu in DMF, however, proved successful. The crude product consisted of several ring alkylated species (fig 76). In common with analogues above, ring alkylation competes with O-alkylation and activates the ring to further substitution. Any of the various intermediates are susceptible, the major byproducts (57a) and (57b) not just arising from over alkylation of the triether product.

\[
\begin{align*}
\text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{OH} \\
\text{OH} & \quad \text{OH} & \quad \text{OH} & \quad \text{OH} \\
\text{R} = \text{Br(CH}_2)_3\text{NHBOc}
\end{align*}
\]

The target triether (56) was, however, successfully isolated in high purity from this crude mixture.

**UCL1822**

(56) was deprotected (TFA) and alkylated with 4-chloroquinoline (diisopropyl ethylamine/ 1-pentanol). As with other analogues, progress of the reaction was followed by observing the decreasingly polar intermediates formed. Several byproducts of similar polarity to the main component were observed. Although these were not removed by the initial purification by column chromatography, they were almost completely removed by recrystallisation of the product UCL 1822 from ethyl acetate.
3.10.3 UCL1836

A method was developed for preparation of a tris-ring N-alkylated quinolinium salt of UCL1822, overcoming the difficulties encountered in previous experience with poor solubility of mono (and di) alkylated intermediates.

\[
\text{HN(CH}_2\text{)}_2\text{O} \quad \text{UCL1836} \quad \text{HN(CH}_2\text{)}_2\text{O}
\]

Ring N-alkylation of UCL1822 in solvents normally employed for this type of reaction such as acetone, MIBK and pentanol proved unsuccessful due to the extremely poor solubility of the partially alkylated intermediates. More forcing conditions and use of polar solvents in previous experience had invariably resulted in decomposition and multi component mixtures, any product which was formed requiring preparative hplc to obtain pure product.

However, carefully defined conditions were developed in this case which successfully produced a tris-ring N-alkylated salt in high purity without the need for chromatography:

Alkylation was carried out in ethyl iodide as solvent while adding the minimum quantity of DMF to maintain the partially alkylated intermediates in solution. In this way the reaction was almost instantaneous and any solvent-promoted decomposition minimised. This procedure has not been previously reported elsewhere and may be of general synthetic utility for poly-N-alkylated salts where insolubility is a major obstacle to reaction.
3.11. UCL1821

3.11.1 Synthetic strategy
As mentioned earlier, the general synthetic approach would follow that used for UCL1715, employing a protected secondary amine in place of boc-1,3-diaminopropane (figure 79). This could be constructed in several ways; the favoured choice being from reaction of one of two alternative protected haloalkylamines with a monoprotected diaminoalkane. For reasons of availability, t-boc-3-bromopropylamine and t-boc-1,5-diaminopentane were selected, 5-Bromopentylamine was unavailable, whereas 1,5-diaminopentane is widely produced, and t-boc-3-bromopropylamine has been used in previous syntheses.

3.11.2 Synthesis

(59) Separation of amines by selective extraction
t-Boc-1,5-diaminopentane was prepared from 1,5-diaminopentane using conditions developed for the propyl analogue above, involving treatment with di-t-butyl dicarbonate in methanol, removal of the diprotected byproduct by filtration after dilution with water, and extraction of the product. Reaction of bocdiaminopentane with bocbromopropylamine in DMF in the presence of potassium carbonate as base gave a mixture of (59), the secondary amine product, and tertiary amine byproduct.

\[
\begin{align*}
\text{Br(CH}_2)_3\text{NH.Boc} + \text{BocNH(CH}_2)_5\text{NH}_2 & \rightarrow (\text{CH}_2)_3\text{NH.Boc} + \text{BocNH(CH}_2)_5\text{NH(CH}_2)_3\text{NBoc} \\
\text{BocNH(CH}_2)_5\text{NH(CH}_2)_3\text{NBoc} & + \text{BocNH(CH}_2)_5\text{NH}_2 \\
(59) & \\
\text{Fig 78}
\end{align*}
\]
This previously unreported procedure can be used to separate a large variety of amines having very similar base strengths, and has since been demonstrated to be suitably useful on a large scale. The reaction is only of theoretical interest, as byproduct arising from a solution of the 4-chlorobenzylamine is precipitated upon addition of water to a solution of the 4-chlorobenzylamine.

The reaction was carried out using a mixture of water and ethanol, and the byproduct was isolated by column chromatography. Further purification and reduction in the level of the minor byproduct, which has a very similar retention time to UCL1821 (and UCL1821 was achieved by repetitive recrystallisation from any solvent used for UCL1821 in acceptable purity.

Fig 79
A method for separation of the two products using the difference in base strengths was discovered which, although exploiting a small pKₐ variation, was sufficient to enable almost complete separation by partition at the correct acidity:

After dilution of the reaction mixture with water and t-butyl methyl ether, hydrochloric acid was added in 10% molar equivalent until the more basic secondary amine was extracted into the aqueous phase, leaving the tertiary amine in the organic phase. After separation of the two layers, (59) was recovered by basification of the aqueous phase with aq NaOH and reextraction.

This previously unreported procedure can be used to separate a large variety of amines having very similar base strengths, and has since been demonstrated to be particularly useful on a large scale and/or where separation by chromatography or crystallisation is difficult.

**UCL1821**

(59) was condensed with phloroglucinol under similar conditions used for (49), azeotropically removing water from a solution of the two materials in THF/toluene. Very little byproduct arising from displacement of two of the phenol groups was observed, presumably due to steric hindrance.

(60) was dialkylated with bocbromopropylamine following a procedure similar to the analogues above to give (61), which was deprotected (TFA) and alkylated with 4-chloroquinoline. Many byproducts were produced, of higher and lower polarity than the product.

Byproducts of substantially different polarity were removed by column chromatography, giving ca 60% pure product. Further purification and reduction in the level of the major byproduct, which had a very similar rf (0.58) to UCL1821 (rf 0.55) was achieved by repetitive recrystallisation from ethyl acetate, giving the product UCL1821 in acceptable purity.
3.11.3. (62)

Prior to preparation of UCL1823, the derivative below, (62), was prepared.

![Chemical structure of (62)](image)

Fig 80

T-Boc-bromoethylamine was treated with t-boc-1,5-diaminopentane (K$_2$CO$_3$ in DMF), giving the ethyl analogue of (59). A similar product composition was produced to that for the propyl analogue. Interestingly, this is in contrast to the experiences on attempting O-alkylation of phenol groups with boc-bromoethylamine, described above. This was thought to be due to the comparatively low basicity of the amine substituent, generating less elimination.

The secondary amine product was converted to (62) using similar methodology to the synthesis of UCL1821. However, insufficient material was made to obtain a satisfactory measurement of the binding affinity and, in view of the lack of increased potency of UCL1821 relative to the trisubstituted derivatives, the synthesis was not repeated.

3.12. UCL1823

3.12.1. Synthetic strategy

Having already prepared the secondary amine (59), and the 3,5-disubstituted benzyl chloride, (53), the most convenient synthesis of this compound was
to couple these components, then protect and alkylate in accordance with fig 81.

3.12.2. Synthesis of UCL1823

Alkylation of (59) with (53) required considerably more forcing conditions (80°C) than for t-boc-1,3-diaminopropane (25°C), due to steric hindrance of the approach of the more bulky secondary amine. After removal of the four protecting groups (in TFA), alkylation with 4-chloroquinoline in 1-pentanol gave a large number of products. Comparison of the tlc profile with UCL1821 provided an indication of the approximate R_f for the expected product, taking into account the slightly more basic nature of the benzylic amine in comparison to the aniline in UCL1821. This permitted preliminary purification by chromatography. Several recrystallisations from ethyl acetate, incorporating hot filtrations to remove insoluble material, resulted in isolation of UCL1823, in 95% purity. Comparison with the reaction mixture composition showed that this material was not the major tlc-mobile product from the reaction.
Electrophysiological testing on neurones was carried out in the Department of Pharmacology at U.C.L by Dr P.M.Dunn in Professor D.H.Jenkinson's group. The ability of the compounds to block the $\text{SK}_{\text{Ca}}$ channel was determined by measuring the inhibition of the AHP in cultured rat sympathetic neurones. Cultures of neurones were prepared from rat sympathetic ganglia by incubation firstly in a Ca$^{2+}$ and Mg$^{2+}$-free balanced salt solution containing collagenase and bovine serum albumin, then a similar solution containing trypsin and bovine serum albumin. The ganglia were dissociated by pipette, plated and grown in a medium of nutrients for between 6 hours and 10 days. Intracellular recordings were made with conventional microelectrodes filled with 1M KCl solution. An action potential was applied, which was followed by an AHP duration 300-500 ms. Each compound was then applied to the external solution at 2 to 4 concentrations on at least 3 cells and the amplitude of the slow component of the AHP measured. On washing with salt solution the cells returned to the resting potential (-60mV)$^{246,247}$. 

The extent of the blockade is measurable because of the separability of the effect of the action potential on the other K$^+$ channels. Efflux through the $\text{BK}_{\text{Ca}}$ channel and voltage-sensitive channels occurs rapidly after the hyperpolarising
spike, and is complete within 50 ms. The $SK_{Ca}$ channel is much more sensitive to the $Ca^{2+}$ concentration, and since the $Ca^{2+}$ concentration returns only slowly to the resting level, the $SK_{Ca}$ channel is largely responsible for the long duration of the AHP. Accordingly, the effects of this smaller current can be distinguished.

All compounds tested appeared selective for the $SK_{Ca}$ channel, as indicated by the shape of the AHP. However, test concentrations > 30μM were avoided as a precaution against the possible action on $Ca^{2+}$ channels.

Inhibition at the various concentrations of test compound was translated into a 'dose-response' plot, for example fig 83.
CHAPTER 5

RESULTS AND DISCUSSION

For the purposes of this discussion, the results can be conveniently separated into three groups:

1). Fumaric and maleic acid based compounds.
2). Trisubstituted aryl centred compounds.
3). Tetrasubstituted aryl centred compounds.

The binding of a small molecule (e.g., a drug) to a macromolecule has been extensively examined on a quantitative basis using free energy determinations. The calculation involves assessment of the change in both entropy and enthalpy content of the drug for the free and bound states. In qualitative terms, the following factors affect the binding:

i). There is a loss in entropy, in particular, degrees of rotational and translational freedom, as a result of the binding, since the bound molecule has comparatively limited movement. The loss is broadly proportional to the molecular weight; this being dependent on how much of the molecule is tightly bound.

ii). As a result of induced fit (see chapter 1), there may be an amount of unfavourable conformational strain energy in the bound molecule.

iii). There is a change in the intrinsic binding energies of the functional groups in the molecule on binding, through its polar, hydrogen bonded, hydrophobic, and Van der Waals interactions.

iv). There is a change in the energy arising from interactions with the solvent. In the case of hydrophobic residues, this favours binding, whereas for more polar residues, the change may be positive or negative, being influenced by factors such as intermolecular hydrogen bonds.
Table 3 - Biological results for compounds in Series 1

<table>
<thead>
<tr>
<th>UCL No.</th>
<th>Notebook No.</th>
<th>Structure</th>
<th>IC₅₀ (µM)</th>
<th>E.M.R*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1190</td>
<td>1713/83</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>31</td>
<td>27</td>
</tr>
<tr>
<td>1641</td>
<td>1713/209</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>1597</td>
<td>1713/173</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>&gt;300</td>
<td>&gt;300</td>
</tr>
<tr>
<td>1716</td>
<td>1796/143</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>0.58</td>
<td>0.51</td>
</tr>
</tbody>
</table>

(*E.M.R. reference to dequalinium.)

5.1. Fumaric and maleic acid based compounds

The lack of activity of small peptides possessing arg-arg indicated that this dipeptide unit, and other bis guanidinium compounds, do not define the pharmacophore sufficiently well to be recognized by the potassium channel.
The weak activity of the fumaric acid based bis-guanidinylpropyl compound (2) prompted the synthesis of the first group of compounds in this study. The major purpose in elaboration of (2) by attaching the octylamino substituent was to establish an interaction with a possible binding site for gln-17 of apamin on the channel protein, there being a strong indication that this residue plays an important role in the toxin binding (see table 3, page 119).

5.1.1 UCL 1190 & UCL 1641
The potencies of UCL 1190 and UCL 1641 represented a significant improvement on the analogue having no additional substituent, (2), although they were still markedly lower than dequalinium (IC$_{50}$ 1.1µM$^{246}$), used as comparator in the studies. The octylamino substituent was selected since from molecular modelling studies it could theoretically achieve the desired spatial arrangement corresponding to gln-17, one turn of the helix below the arg-arg dipeptide. This would not be a low energy conformer, since several of the hydrogen atoms in the neighbouring methylene groups would be 'skew' or 'eclipsed'. However, it is acceptable that the conformation of a binding agent may alter to that of a higher energy, in accordance with the induced fit theory, if sufficient energy is recovered through the stability attained from the binding interactions. There is a small energy barrier to free rotation about the C-C single bond (ca 3 kcal/mol)$^{258}$, but this is small enough to allow ready interconversion of conformers through the energy of thermal motions at room temperature. As the bond it rotated the energy fluctuates as the conformation changes through trans, skew or eclipsed:

![Staggered Skew Eclipsed Skew](image-url)
In UCL 1190 there is still appreciable mobility in the alkyl chains, particularly the n-octyl linkage. Molecules that are not rigid do not provide as much information as do rigid molecules because the former can exist in a much larger number of conformations. For this reason UCL1641 was prepared, which cannot twist in the manner of UCL1190 to achieve an interaction with any proposed gln-17 binding site. There are a number of possible interpretations for the relative potencies of UCL1190 and UCL1641.

i). The similar increase in affinity of UCL1190 and UCL1641 compared to (2) was due to a weak interaction between the primary amine substituent and a residue of the channel protein. This explanation appears unlikely since the rigidity of the biphenyl linkage places the NH$_2$ group on the opposite side of the molecule from arg-13 and arg-14, in a position corresponding to cys-1 NH$_2$, and there is no evidence to suggest that this residue, or any on this side of the apamin molecule, participate in the binding.

ii). The increase compared to (2) was due to a hydrophobic interaction with the linking chain. This also appears unreasonable since if this were so the aryl linkage in UCL1641 would be expected to give rise to a more potent compound than the alkyl in UCL1190. Furthermore, this hydrophobic interaction does not appear to be the major determinant in the primary attraction to the channel protein.
iii). A more likely cause of the increase is that the presence of the additional substituent places restrictions on the various conformational isomers by virtue of increased electrostatic and steric repulsions, with the result that the two guanidinium groups are in a more advantageous position to interact with corresponding residues in the channel protein. Both the central fumaric acid diamide and the guanidinylpropyl groups are affected. The central portion is planar due to the \( \pi \)-orbital delocalisation. Several discrete conformational isomers are possible, corresponding to rotation about the C-N bonds (fig 85). Evidence of these rotamers was observed in the nmr spectra of UCL1190, UCL1641 and their intermediates.

![Fig 85](image)

Rotational isomers about the vinylogous C-C bond are also possible, giving, for example, the forms in fig 86.
These conformers are more strongly disfavoured due to steric hindrance between the amide substituents and the olefinic hydrogens, also accentuated in UCL1190 and UCL1641 compared to (2). Conformations such as (a) and (c), which minimise the repulsive forces, are therefore favoured. The proportion of these conformations would be increased in UCL1190 and UCL1641 compared to (2) due to the increased electrostatic and steric interactions. In such conformations the two guanidine groups are approximately 11 A apart, the distance between the guanidine groups in apamin and related toxins, thus favouring interaction with corresponding residues in the channel protein.

It is evident from the relative potencies, however, that the interaction is still weak and hence probably non-specific in nature.

5.1.2. UCL1597

The lack of activity of the maleic acid-based isomer UCL1597 can also be explained by analysis of the various conformational isomers. In this case formation of a hydrogen bond between the N-H of the secondary amide and the carbonyl oxygen of the tertiary amide is possible, which stabilises this particular configuration. No such hydrogen bond is possible for the \textit{trans} isomer, in UCL1190 and UCL1641.
Molecular modelling measurements showed that in the above configuration the two guanidines are placed approx 6A apart, considerably different from the optimum (11A). Thus it appears that insufficient energy can be recovered from binding to compensate for the loss of the intrinsic energy from breaking the hydrogen bond.

The above theory is supported by the finding that UCL1609, with an IC\(_{50}\) of 25 \(\mu\)M (see page 57), is equipotent with UCL1190 and UCL1641. Although having cis configuration about the central double bond, no hydrogen bond is possible in UCL1609, the rigidity of the aryl linkage placing the two guanidine groups approx 11 A apart. Accordingly the preferred conformation of UCL1597 is very different to UCL1609, providing a likely explanation for the variation in potencies.

### 5.1.3 UCL1716

Replacement of the two guanidine groups in UCL1190 with aminoquinolines, linked through exocyclic nitrogen, in UCL1716, resulted in a 100 fold increase in potency. A similar magnitude increase had been observed in other compounds where the guanidines were replaced with aminoquinolinium groups linked through the ring nitrogens, such as UCL1488 (see page 57).

The similarity in magnitude of the increase provides further evidence that quaternisation of the aminoquinoline is unnecessary for effective binding, provided that the quinoline is basic enough to be protonated at physiological pH. Furthermore, it supports the assertion that linking of the two aminoquinolinyl
groups through the exocyclic nitrogen atoms rather than the ring nitrogen, as in dequalinium, is tolerated\textsuperscript{204}.

On assessing those differences between the guanidine and quinoline groups which might account for the superiority of the latter, it has been shown previously that delocalisation of the positive charge is the most important factor. In a study of the series of dequalinium analogues below, the more electron releasing the substituent (R), the more potent the compound\textsuperscript{203}.

![Figure 88](image)

There is a build up of positive charge above and below the quinolinium ring, producing a ring shaped field. The ring hydrogen atoms contain partial positive charges (fig 89), each charge being fixed in space by the rotational constraints of the ring.

![Figure 89](image)
The guanidinium ion is also a flat delocalised cation. It is on the basis of this common feature that a correlation can be drawn between the arginines in the various toxins and the aminoquinolium groups in dequalinium, which are thought to interact with the same residues in the channel protein. The delocalisation of charge in the guanidinium group is, however, less extensive than for aminoquinolium.

![Diagram](image)

The much higher water solubility and associated solvation of the guanidinium ion leads to a much greater loss of energy from desolvation during binding, also limiting the strength of its interaction with the receptor. The existence of rings of negatively charged aromatic acids in many ion channels may provide a rationale for the importance of the charge delocalisation, in that the strength of the binding may be as a result of a cation-π interaction between the delocalised positive charge in the binding agent and the hydrophobic regions of these residues in the channel. It is apparent from the activity of UCL1716 that there is a degree of steric tolerance in the requirements for binding in order to accommodate the quinolinium ring, the delocalisation probably reducing the distance demands at the receptor site. This is supported by previous data which showed that variation in the number of methylene groups (6-12) in the linking chain of a series of dequalinium analogues hardly had any effect on the potency.

In view of the similarity in potency to UCL1488, it was concluded that the octylamino substituent does not form a discrete interaction with a corresponding residue in the channel protein. The main influence may be to define more precisely the spatial relationship of the two aminoquinolines through minimisation of steric interactions, in a similar manner to UCL1190 and UCL1641. The need for two aminoquinolinium groups for potent and selective blockade of the SKCa channel has been concluded by other researchers in the group.
5.2. Trisubstituted aryl centred compounds

This series was principally selected to define more precisely the relative positions of the two aminoquinoline groups, as isosteres for the guanidine groups of arg-13 and arg-14 in apamin, by designing a central template which more accurately represented an α helix. Use of an aryl ring for this purpose had enabled a third aminoquinoline to be attached corresponding to gln-17 as a putative third binding residue in apamin.

Similar binding affinities were observed in all compounds prepared in this series (table 4).

The presence of three aminoquinoline groups raises the important question of how many are contributing to the binding. The observed activity may be due to a close interaction between either one, two or three aminoquinolines with up to three receptor sites in the channel. Thus, for example, the affinity may be artificially high due to a statistical effect from having three potential binding groups existing in the molecule approaching one or two binding site(s). These sites may be equivalent, corresponding to identical residues on separate subunits of the channel, or non-equivalent.

All compounds in this series have similar potencies to both UCL1716, UCL1488 and dequalinium, each of which has two aminoquinolinium substituents separated by approximately 11A. Thus it is highly probable that the interaction with the channel involves just two aminoquinolines, as concluded for UCL1716. Furthermore it appears that no statistical advantage had been gained from the presence of the third substituent. The close similarity between these compounds suggests that they all interact with the channel by virtue of their ability to adopt a similar conformation. However, on the evidence of the compounds tested it cannot be conclusively determined which of the substituents in this series interacts most strongly.

Valuable insight can be gained from a computer-generated model of
Table 4 - Biological activities of compounds in series 2

<table>
<thead>
<tr>
<th>UCL No.</th>
<th>Notebook No.</th>
<th>Structure</th>
<th>IC(_{50}) (µM)</th>
<th>EMR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1714</td>
<td>1796/105</td>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td>1715</td>
<td>1796/119</td>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>0.27</td>
<td>0.31</td>
</tr>
<tr>
<td>1735</td>
<td>1796/201</td>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>0.53</td>
<td>0.56</td>
</tr>
<tr>
<td>1822</td>
<td>1796/257</td>
<td><img src="image4.png" alt="Structure 4" /></td>
<td>0.23</td>
<td>0.25</td>
</tr>
<tr>
<td>1836</td>
<td>1796/281</td>
<td><img src="image5.png" alt="Structure 5" /></td>
<td>0.14</td>
<td>0.16</td>
</tr>
</tbody>
</table>

(*ref dequalinium)
UCL1822 which by virtue of the substitution pattern has the most accurately defined conformation of this aryl centred series. The 1,2,3 trisubstitution pattern in UCL1822 places the three substituents in close proximity. Molecular modelling using Chemex indicates that as a result of the electrostatic and steric repulsion, a strongly energetically favourable conformation is produced in which the 1 and 3 substituents are above the ring and the central group below the ring plane (fig 91):

Since the above compound has less intrinsic freedom than the other trisubstituted aryl centred compounds, if significant deviation from the above conformation were required for binding of UCL1822, this would greatly increase the electrostatic and steric interactions. This would result in lower potency, assuming that this loss in energy is not recovered by binding interactions with the channel protein. Thus, in view of the similarity in potencies, it is likely that the conformation adopted is not very different from that indicated in fig 91. However, the relative spatial positions were not defined with sufficient accuracy to produce a very high potency. In most cases the exact conformation needs to be very precise in order to achieve this result, as evidenced by the length of the alkyl chain in (3).
The lack of increased potency of UCL1836 compared to UCL1822 is further support of the assertion that ring alkylation of the aminoquinoline is not a requirement for activity.

5.3 Tetrasubstituted aryl centred compounds
Principally to investigate further the presence of a statistical contribution, two derivatives having four aminoquinolinyl substituents, UCL1821 and UCL1823, were prepared, the additional substituent being chosen so as to represent his-18 in apamin.

Table 5 - Biological activities of compounds in Series 3

<table>
<thead>
<tr>
<th>UCL No.</th>
<th>Notebook No.</th>
<th>Structure</th>
<th>IC50 (µM)</th>
<th>EMR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1821</td>
<td>1796/261</td>
<td></td>
<td>0.15</td>
<td>0.17</td>
</tr>
<tr>
<td>1823</td>
<td>1796/235</td>
<td></td>
<td>0.21</td>
<td>0.23</td>
</tr>
</tbody>
</table>

(*EMR reference to dequalinium)

From the relative potencies it appeared that no advantage, statistical or otherwise, had resulted. Although the absence of such an effect for these compounds and the trisubstituted series may have been due to the additional residues being in an incorrect spatial orientation to interact with corresponding residues in the channel protein, this appears unlikely in view of the flexibility incorporated in each structure and relative equivalence of the four alkylaminoquinolinyl groups. Furthermore it is unlikely that the molecule is too large to approach the channel entrance, since this would probably result
in a relatively lower affinity for UCL1821 and UCI1823 than for the trisubstituted series. In this respect it is noteworthy that the spatial volumes of these two compounds are still much smaller than those of the natural toxins.

5.4. Conclusion
Thus from the above results it is tentatively concluded that there are only two points of close contact where an electrostatic interaction takes place between the binding agent and channel protein. The strength of this interaction is dependent on the degree of delocalisation of the positive charge in the two principal key groups of the binding agent. This is exemplified by the 70-fold increase in potency on replacing the triethylammonium groups in gallamine, which have a spherical charge distribution, with the flat, highly delocalised aminoquinoline groups.

When considering the exact topology of the interaction with the channel, firm conclusions are difficult to draw because little is known of the channel structure. Furthermore, in view of the relatively low affinities compared to the natural toxins, the contact with specific channel residues must still be rather distant.

By analogy with the BK\textsubscript{Ca} channel, and from current structural evidence, the SK\textsubscript{Ca} channel is likely to consist of several identical \(\alpha\)-subunits with associated, smaller \(\beta\)-subunits. The spatial proximity of the residues which are at present considered important in apamin, arg-13, arg-14 and gln-17, suggests a comparatively small area of interaction with the channel protein. However, no support for the participation of gln-17 was established as a result of compounds prepared in this or other studies within the group.

There is strong evidence for the non-involvement of the peptide backbone of the toxins in binding. In this respect, incorporation of amine groups in the linking chains, in UCL1715 and UCL1735, did not appear to confer any advantage (or disadvantage) to the binding of the molecule.
The blocking agent, be it a toxin or a smaller molecule, probably binds through interaction with residues in two of the subunits, in the same way that charybdotoxin blocks the \( \text{BK}_{\text{Ca}} \) channel by forming multiple contacts with residues of several of the subunits up to ca 15\( \text{A} \) from the centre of the pore. In this case the homotetrameric channel displays four energetically equivalent binding sites, only one of which is occupied at any one time. It is likely that apamin and related blockers interact in a similar manner. The primary points of contact are probably with negatively charged residues on different subunits, likely to be aromatic acids, separated by a distance of ca 11\( \text{A} \). Although the principal force of attraction to the channel is electrostatic, there is strong evidence that this is complemented by a hydrophobic interaction, operating at close range. This may be largely a secondary interaction, operating after the particular binding agent has approached the channel as a result of the primary electrostatic attraction, but may be a fundamental requirement for high activity. The activity, albeit considerably reduced, of toxins in which the adjacent arginines have been removed is supportive of this assertion.
CHAPTER 6

EXPERIMENTAL

Commercially available compounds were obtained from either Aldrich Chemical Company or Lancaster Synthesis.

Nuclear Magnetic Resonance (NMR) spectra were recorded on either a Brucker AC200 (200 MHz) or AC400 (400 MHz) spectrometer, and chemical shifts (δ) are reported relative to TMS. Signals are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Infrared (I.R) spectra were run on a Perkin-Elmer 16PC Fourier Transform spectrophotometer. Signals are measured in ν cm⁻¹, and were designated as follows: s, strong; m, medium; w, weak.

Mass spectra were run on a V.G.Quattro spectrophotometer.

Analytical high performance liquid chromatography (hplc) was carried out on a Varian Star Workstation, using Varian 9065 Polychrom DAD detector, 9010 Pump and rheodyne 7125 Injector Valve with 20μl loop. Several types of columns were used, which are specified in the relevant experimental sections.

Melting points (m.p) were obtained on an Electrothermal melting point apparatus and are uncorrected.

Thin layer chromatography (tlc) was done on silica gel F-254 plates with a large variety of solvent systems. Components were visualised with either u.v, iodine, or hexaiodoplatinate spray reagent. Quantitative estimates of purity were performed by spotting appropriate volumes of diluted product solution, in order to estimate impurity levels.
3-(N-(t-butoxycarbonyl)amino) propylamine

A solution of di t-butyl dicarbonate (327g, 1.5 mol) in MeOH was added to 1,3-diaminopropane (450ml, 7 mol) in MeOH (1.5l) over 40 minutes at 5-10°C. The resulting suspension was stirred for 18 hours and filtered. The filtrate was concentrated to low volume, then diluted with water (2l), filtered to remove di-(t-boc)-diamino propane and extracted with diethyl ether (6 x 500ml). The extracts were combined, concentrated in vacuo to low volume, and the residue dried by azeotropic distillation with toluene. Further removal of solvent gave 100g product as a toluene concentrate.

Yield 55% (118g), orange-brown oil.
Purity ca 99% (nmr).

$1^H$ NMR (CDCl$_3$): 6.0 (br t, 1H, NH), 3.15 (q, 2H, CH$_2$NH), 2.7 (t, 2H, CH$_2$NH$_2$), 1.6 (m, 2H, CH$_2$), 1.3 (s, 9H, C(CH$_3$)$_3$), 1.2 (s, 2H, NH$_2$).

Ethyl-N-(N'-(3-t-butoxycarbonylaminopropyl)-carbamoyl)-trans-propenamide (9)

A mixture of monoethyl fumarate (43.2g, 0.3 mol) and anhydrous 1-hydroxy benzotriazole (40.5g, 0.3 mol) in THF (300ml) was treated at 20°C with a solution of 1,3-dicyclohexylcarbodiimide (62.0g, 0.3 mol) in THF (200ml) over 10 minutes. A thick suspension was produced soon after the end of the addition, which was stirred for 18 hours at 20°C. A solution of 3-(N-(t-boc) amino) propylamine (toluene concentrate-contained weight 52.2g, 0.3 mol) in THF (100ml) was added over 10 minutes and the reaction mixture stirred for 2 hours before being filtered. The filtrate was concentrated to low volume and the residue dissolved in chloroform (500ml), acidified to pH2 with
2M citric acid, then filtered. The organic phase was washed sequentially with water, sodium bicarbonate, water, then dried (magnesium sulphate) and solvent removed *in vacuo* to give an oil, which solidified on cooling.

Yield 70% (62g), cream powder.
Purity ca 90% (nmr).

mp 95-97°C.

I.R. (Nujol): 3230 (N-H), 1720 (C=O), 1660 (C=C) cm⁻¹

^1^H NMR (CDCl₃): 8.1 (br t, 1H, NH.CO), 6.9 (d, J=15Hz, 1H, HC=CH), 6.5 (d, J=15Hz, 1H, HC=CH), 6.05 (br t, 1H, NHCO), 4.15 (q, 1H, CH₂CH₃), 3.3 (q, 2H, CH₂N), 3.0 (q, 2H, CH₂N), 1.65 (m, 2H, CH₂), 1.35 (s, 9H, C(CH₃)₃).

3-N-(N’-3-(t-butoxycarbonylaminopropyl)-carbamoyl)-trans-propenoic acid (10)

A solution of (9) (50g, 0.17 mol) and potassium hydroxide (11 g, 0.20 mol) in a mixture of ethanol (300ml) and water (25ml) was stirred for 18 hours at 20°C. The reaction mixture was then acidified to pH2 with conc. HCl (ca 25ml), diluted with water (500ml) and extracted with chloroform (4 x 250ml). The extracts were combined, dried (MgSO₄) and concentrated to low volume to give an oil, which was diluted with ethyl acetate (500ml) to precipitate (10).

Yield 71% (32g), white powder.
Purity >90% (nmr).

mp 143-145°C

^1^H NMR (d₆ DMSO): 12.8 (br s, 1H, CO₂H), 8.4 (t, 1H, NH.CO), 6.9 (d, J=15Hz, 1H, HC=CH), 6.5 (d, J=15Hz, 1H, HC=CH), 3.15 (q, 2H, CH₂N), 2.95 (q, 2H, CH₂N), 1.55 (m, 2H, CH₂), 1.35 (s, 9H, C(CH₃)₃).

M.S: (FB⁺): 273 (M⁺ + 1)
8-Phthalimidoctanol (11)

Anhydrous potassium carbonate (140g, 1.0 mol) was added to a solution of 8-bromoctanol (60.5g, 0.29 mol) and phthalimide (42.8g, 0.29 mol) in DMF at 60°C. The reaction mixture was maintained at 60°C for 18 hours, then cooled to 20°C, filtered, and the bulk of the solvent removed in vacuo at 50°C. The resulting oil was diluted with brine (300ml) and extracted with methylene chloride (3 x 250ml). The extracts were combined, dried (MgSO₄), and concentrated to low volume. The resulting oil was diluted with ether, filtered, and the filtrate evaporated to constant weight give the product as an oil, which slowly solidified.

Yield 42% (33g), cream powder.

Purity 90% (nmr).

mp 60-62°C.

¹H NMR (CDCl₃): 7.8 (br s, 4H, phth), 3.6 (m, 4H, 2CH₂N), 1.7-1.3 (m, 13H 6CH₂, OH)

8-Amino-1-octanol (12)

A solution of 8-phthalimidoctanol (33g, 0.12mol) and hydrazine monohydrate (23.5g, 0.47 mol) in ethanol (200ml) was stirred for 18 hours at 20°C, giving a thick precipitate. The reaction mixture was diluted with 4M hydrochloric acid (400ml), filtered and the filtrate washed with methylene chloride (2 x 150ml), before being basified to pH 14 with sodium hydroxide and extracted with methylene chloride (5 x 150ml). The extracts were combined, dried (MgSO₄), and concentrated in vacuo to low volume. The resulting oil was diluted with di-isopropylether to precipitate the product.
Yield 82% (15g), white crystals.
Purity >95% (nmr).
mp 59-61°C.
$^1$H Nmr (CDCl$_3$): 3.6 (t, 2H, CH$_2$O), 2.7 ( , 2H, CH$_2$N), 1.8 (br s, 3H, NH$_2$ & OH), 1.7-1.3 (m, 12H,6CH$_2$).
M.S: (FB+): 146 (M$^+$ + 1)

**8-Trifluoroacetamido-1-octanol (13)**

Methyl trifluoroacetate (7.1g, 0.05 mol) was added to a solution of 8-amino-1-octanol (7.3g, 0.05mol) in methanol (25ml) at 15°C over 10 minutes. The reaction mixture was stirred for 1 hour at 15°C, concentrated to low volume, and the resulting oil diluted with cyclohexane (100ml) to precipitate the product.

Yield 93% (11.2g), cream powder.
Purity (nmr) 95%.
mp 49°C.
$^1$H NMR (CDCl$_3$): 7.5 (br s, 1H, NH.CO), 3.5 (t, 2H, CH$_2$O), 3.2 (q, 2H, CH$_2$N), 2.9 (s, 1H, OH), 1.7-1.3 (br s, 12H, 6CH$_2$).

**N-(trifluoroacetamido)-N'-(3-t-butoxycarbonylaminopropyl)-1,8-diaminooctane (14)**

A solution of 8-trifluoroacetamido-1-octanol (10.6g, 44mmol) and p-toluene sulphonylchloride (13.8g, 73mmol) in chloroform (75ml) was treated with triethylamine (8.6g, 85mmol) over 5 minutes, the temperature rising from 15°C to 26°C. After 2 hours the solvent was removed by distillation *in vacuo* to low volume and the resulting oily solid diluted with ethyl acetate (100ml). The precipitated triethylamine.HCl was filtered off and the filtrate concentrated to
dryness. The crude product was purified by column chromatography on silica gel with ethyl acetate, to give the tosylate as a yellow oil. The tosylate was stirred in DMF (50ml) with N-(t-butoxycarbonyl)amino propylamine (33g, 170mmol) at 20°C for 18 hours. The reaction mixture was then diluted with 2M hydrochloric acid (300ml) and extracted with methylene chloride (2 x 100ml). The extracts were combined, treated with excess triethylamine (15g) and washed with water (2 x 100ml) before concentrating in vacuo. The crude product was absorbed onto silica gel and chromatographed with methanol/ammonia (100/1) to give the product as a pale yellow oil, which slowly solidified.

Yield 68% (12g), cream powder.
Purity >90% (nmr, tlc).
mp 55-57°C.

I.R. (Nujol): 3260 (N-H), 1700 (C = O) cm⁻¹

¹H NMR (CDCl₃): 7.25 (br s, 1H, NH.CO), 5.3 (br s, 1H, NH.CO), 3.4 (q, 2H, CH₂N), 3.2 (q, 2H, CH₂N), 2.75 (t, 2H, CH₂N), 2.55 (t, 2H, CH₂N), 1.65 (m, 6H, 3CH₂), 1.45 (s, 9H, C(CH₃)₃), 1.3 (br s, 8H, 4CH₂).

M.S: (EI⁺): 397 (M⁺), 324 (M - tBu)

N-(3-t-butoxycarbonylaminopropyl)-N-(8-trifluoroacetamido)-3-(N'-t-butoxycarbonylaminopropyl)-carbamoyl-trans-propenamide (15)

(10) (8.6g, 32mmol) and anhydrous 1-hydroxybenzotriazole (4.7g, 35mmol) were stirred in THF (75ml) while adding 1,3-dicyclohexylcarbodiimide (7.2g, 35mmol) in THF (75ml), the solid dissolving to give a yellow solution. The reaction mixture was stirred for 18 hours at 20°C to give a thick suspension, which was treated with (14) (11.0g, 28mmol) in THF (50ml). After 6 hours the dicyclohexylurea was filtered off and the filtrate concentrated to low volume
by distillation in vacuo. The residue was diluted with methylene chloride (100ml) and 1M citric acid (75ml). The organic phase was washed sequentially with water, dilute potassium carbonate, water, before removing the solvent by distillation to give an oily solid. This was absorbed onto silica gel and chromatographed with ethyl acetate/ methanol (20/1) to give (15), crystallised from diethyl ether.

Yield 95% (17.4g), white crystals.
Purity 95% (nmr, tlc).
mp 100-102°C.

$^1$H NMR (CDCl$_3$): 7.3 (d, J=15Hz, 1H, HC=CH) 7.1 (br s, 2H, NH.CO), 6.9 (d, J=15Hz, 1H, HC=CH), 5.4 (br t, 1H, NH.CO), 4.85 (br t, 1H, NH.CO), 3.55-3.3 (m, 8H, 4CH$_2$N), 3.25-3.05 (m, 4H, 2CH$_2$N), 1.75-1.55 (m, 8H, 4CH$_2$), 1.45 (s, 18H, 2C(CH$_3$)$_3$), 1.3 (br s, 8H, 4CH$_2$).

N-(3-Guanidinopropyl)-N-(8-aminoctyl)-3-(N'-(3-guanidinopropyl)-carbamoyl)-trans-propenamide, UCL1190

A solution of (15) (2.0g) in 15% w/w HCl/ methanol (25ml) was stirred for 2 hours at 20°C, then diluted with water, basified to pH9 with sodium hydroxide, keeping the temperature below 0°C, and extracted with chloroform (6 x 50ml). The extracts were combined, dried (MgSO$_4$) and evaporated in vacuo to give (16) (1.6g) as a yellow oil; yield 95%, purity (nmr,tlc,hplc) 95%.

A solution of (16) (1.6g, 3.5mmol) and 3,5 dimethylpyrazole carboxamidine nitrate (2.6g, 13mmol) in water was heated at 60°C for 24 hours, maintaining the pH at 7-8.5 by periodic addition of 1M sodium hydroxide solution. The reaction mixture was then cooled, basified to pH14 with 40% NaOH solution (1ml) and stirred for 2 hours at 20°C before being washed with

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chloroform (2 x 20ml). Nitric acid was then added to the aqueous phase to pH 1 and the solution concentrated in vacuo to dryness. The residue was stirred in ethanol heated under reflux, filtered hot, then cooled to produce an orange oil. 'Recrystallisation' from ethanol gave UCL 1190 trinitrate (300mg) as a yellow glass.

Purity 95% (hplc), >90% (nmr), trihydrate (CHN, IR)

M.S. (LC/MS, TS⁺): 440 (M + 1), 398 (M - HN=C-NH₂), 324 , 244 (base peak)

¹H NMR (dmso): 8.55 (1H, m, NH.CO), 7.7 (3H, br s, NH₃⁺), 7.5 (2H, m, NH), 7.2 (1H, d, HC=CH, J = 15Hz), 7.1 (8H, br s, NH₂⁺), 6.85 (1H, d, J = 15Hz, HC=CH), 3.45 (4H, m, CH₂N), 3.2 (6H, m, CH₂N), 2.75 (2H, m, CH₂N), 1.8 (4H, m, CH₂), 1.65 (4H, m, CH₂), 1.35 (8H, br s, CH₂)

I.R. (film): 3350, 3200 (NH₂⁺,H₂O), 1665 (C=O), 1635 (C=N)

Hplc: Lichrosorb RP Select B (MeOH/ 0.05% aq TFA, 80/20), 224nm

Product rt 1.7 min, impurities 1.85, 2.05 mins (nitrate rt 2.8 mins)

CHN: calculated for C₂₀H₄₁N₉O₂ trinitrate 3H₂O: 35.19, 7.38, 24.62; found, 35.37, 7.31, 24.48
4-Bromo-4'-acetylbiphenyl (17)

A solution of 4-bromobiphenyl (250g, 1.07 mol) in nitrobenzene (900ml) was treated with anhydrous aluminium chloride (190g, 1.42 mol) over 15 minutes, keeping the temperature below 25°C. Acetyl chloride (110g, 1.42 mol) was added over 30 minutes at 20-25°C, with cooling. The reaction mixture was stirred at room temperature for 4 hours, then added to conc hydrochloric acid (500ml) and ice (1kg). The aqueous phase was extracted with methylene chloride (2 x 1l). The organic layers were combined, dried (MgSO₄) and concentrated to give an oil, which was diluted with hexane (3l) to crystallise the product.

Yield 91% (269g), yellow flakes.
Purity >98% (nmr, tlc).

m.p. 124-126°C lit 129-130°C

¹H NMR: 8.05 (d, 2H, CH(Ar)), 7.65 (d, 2H, CH(Ar)), 7.60 (d, 2H, CH(Ar)), 7.50 (d, 2H, CH(Ar)), 2.65 (s, 3H, CH₃CO)

I.R (Nujol): 16800 cm⁻¹ (C=O)

4-(4-Bromophenyl)-benzoic acid, (18)

Bromine (770g) was added to 5M sodium hydroxide (3.2l) over 20 minutes at 0-5°C. The resulting solution was added to a solution of 4-bromo-4'-acetylbiphenyl (268g) in dioxan (2l) at 35-40°C over 1 hour. The reaction mixture was stirred for 2 hours, before adding a solution of sodium metabisulphite (350g) in water (1l) and then concentrating to approx 4l volume. The concentrate was cooled to room temperature, diluted with water (2l) and acidified with conc hydrochloric acid to pH1 to precipitate the product, which was filtered off, washed with water (1l) then acetone (2l).
Yield 96%, white powder.
Purity 95% (nmr).
m p 302-304°C lit 300-302°C

$^1$H NMR: 13.1 (br s, 1H, CO$_2$H), 8.05 (d, 2H, CH(Ar)), 7.80 (d, 2H, CH(Ar)), 7.7 (s, 4H, CH(Ar))

I.R (Nujol): 1685 cm$^{-1}$ (C=O)

M.S. (EI$^+$): 278 (M$^+$ + 1), 152 (M - C$_7$H$_5$O$_2$)

Methy 4-(4-bromophenyl)-benzoate (19)

4-(4-bromophenyl)-benzoic acid (257g) was heated under reflux in thionyl chloride (2l) for 1 hour. The solvent was then replaced by distillation at constant volume with toluene to a head temperature of 110°C (2 hours), before concentrating the solution to dryness. The resulting oil was diluted with methanol (1.5l) to precipitate the product.

Yield: 90% (244g), red-brown crystals.
Purity 98% (nmr).
m p 138-142°C lit 140-142°C

$^1$H NMR: 8.15 (d, 2H, CH(Ar)), 7.65 (d, 2H, CH(Ar)), 7.55 (d, 2H, CH(Ar)), 7.50 (d, 2H, CH(Ar)), 3.95 (s, 3H, CH$_3$O.OC)

I.R (Nujol): 1715 cm$^{-1}$ (C=O)

M.S. (EI$^+$): 292 (M$^+$ + 1), 260 (M - CH$_3$O), 152 (M - C$_8$H$_7$O$_2$)

Methy 4-(4-cyanophenyl)-benzoate (20)

Methyl 4-(4-bromophenyl)-benzoate (240g, 0.8mol) was heated in DMF (1.5l) with copper (I) cyanide (170g, 1.9mol) at 130°C for 16 hours. The resulting suspension was cooled to room temperature then added to water (3l) and methylene chloride (2l), and filtered. The organic phase was washed with water (2x 2l) then dried (MgSO$_4$), before concentrating in vacuo to give an oil, which was diluted with di-isopropyl ether (1l) to precipitate the product.
Yield: 40% (79g), yellow powder.
Purity >98% (nmr,tlc).

m p 142-144°C  lit 140-142°C

$^1$H NMR: 8.15 (d, 2H, CH(Ar)), 7.75 (s, 4H, CH(Ar)), 7.65 (d, 2H, CH(Ar)), 3.95 (s, 3H, CH$_3$O.OC)

I.R (Nujol): 2230 (CN), 1720 (C = O), 1710 (C = O) cm$^{-1}$

4-(4-aminomethylphenyl)-benzyl alcohol (21)

(20) (78g) was treated in THF (800ml) with 2M lithium borohydride in THF (200ml). The resulting solution was heated under reflux for 4 hours to give a thick suspension, which was then cooled and added to water (1.5l) and chloroform (1l). The mixture was filtered and the layers separated. The aqueous phase was extracted with chloroform/ methanol 5/1 (2 x 1l). The organic layers were combined, dried (MgSO$_4$) and solvent removed by distillation in vacuo to give an oily solid. This was absorbed onto silica gel and chromatographed with chloroform/methanol 5/1, the product being crystallised from di-isopropyl ether.

Yield 14% (10g), yellow powder.
Purity 98% (nmr).

m p 164-168°C.

$^1$H NMR: 7.65 (d, 2H, CH(Ar)), 7.60 (d,2H, CH(Ar)), 7.45 (d, 2H, CH(Ar)), 7.40 (d, 2H, CH(Ar)), 4.55 (s, 2H, CH$_2$O), 3.8 (s, 2H, CH$_2$N), 3.2 (br s, 2H, NH$_2$)

4-(4-trifluoroacetamidomethylphenyl)-benzyl alcohol

A suspension of (21) (7.8g) in methanol (100ml) was treated with methyl trifluoroacetate (7.0g, 1.5 mol/mol) until a solution was obtained. The solvent was then removed by distillation in vacuo and the residue purified by column chromatography on silica gel with methylene chloride/ methanol 5/1. The product was crystallised from di-isopropylether.
Yield 53% (5.9g), white powder.
Purity 98% (nmr).
m p 179-181°C.

^1^H NMR: 10.05 (b rt, 1H, NH.CO), 7.65 (d, 2H, CH(Ar)), 7.60 (d, 2H, CH(Ar)), 7.45 (d, 2H, CH(Ar)), 7.40 (d, 2H, CH(Ar)), 5.25 (t, 1H, OH), 4.55 (d, 2H, CH_2O), 4.45 (d, 2H, CH_2N)
I.R. (Nujol): 3290 (N-H), 1700 (O=O), 1560 (C=C) cm^-1

4-(4-trifluoracetamidomethylphenyl)-benzyl 4-toluenesulphonate

DMF (8ml) was added to a mixture of 4-(4-trifluoroacetamidomethylphenyl)-benzyl alcohol (3.7g), toluene sulphonyl chloride (5.1g, 2.25mol/mol) and triethylamine (1.8g, 1.5 mol/mol) in chloroform (30ml) until a solution was obtained. The reaction mixture was heated to reflux and more triethylamine (ca 0.8g) added dropwise until the pH rose to 7, before heating under reflux for a further 1 hour. The solution was cooled, washed with 1M hydrochloric acid (50ml), water (50ml), dried (MgSO_4) and concentrated in vacuo to give an oil, which was triturated with hexane to crystallise the product.

Yield 42% (2.3g), white crystals.
Purity 98% (tlc).
m p 169-171°C.
4-(4-trifluoroacetamidomethylphenyl)-N-(3-t-butoxycarbonylamino propyl) benzylamine (22)

A solution of 4-(4-trifluoroacetamidomethylphenyl)-benzyl 4-toluenesulphonate (2.3g) and t-boc diamino propane (3.4g, 4 mol/ mol) in DMF (12 ml) was stirred for 2 hours at room temperature, then diluted with water and extracted with methylene chloride (2 x 30ml). The extracts were combined, washed with water (15ml), dried (MgSO₄) and concentrated to give an oil, which was absorbed onto silica gel and chromatographed with methylene chloride/ methanol 3/1. The product was isolated by crystallisation from di-isopropyl ether.

Yield 69% (1.6g), white powder.
Purity 98% (nmr).
m.p 195-197°C.

¹H NMR: 7.60 (d, 2H, CH(Ar)), 7.50 (d, 2H, CH(Ar)), 7.40 (d, 2H, CH(Ar)), 7.35 (d, 2H, CH(Ar)), 6.90 (br t, 1H, NH.CO), 5.35 (br t, 1H, NH.CO), 4.45 (d, 2H, CH₂N.CO), 3.85 (s, 2H, CH₂N.CO), 3.35 (t, 2H, CH₂NAr), 2.75 (t, 2H, CH₂N), 1.7 (m, 2H, CH₂), 1.45 (s, 9H, ¹Bu)

M.S. (FB⁺): 466 (M⁺ + 1), 410 (M - C₄H₉), 292 (M - ¹BocO.CO.NH(CH₂)₃NH₂)
N-(3-t-butoxycarbonylamino propyl)-N-(4-(4'-trifluoroacetamidomethyl phenyl)benzyl)-3-N'(3-t-butoxycarbonylamino propyl)-carbamoyl-trans-propenamide (23)

\[
\begin{array}{c}
\text{BocNH} (\text{CH}_2)_3 \text{HNOC}
\end{array}
\]

A solution of (10) (0.7g, 2.57mmol), 1-hydroxybenzotriazole (0.35g, 2.60mmol) and 1,3-dicyclohexylcarbodiimide (0.55g, 2.70mmol) in DMF (7ml) was stirred at room temperature for 2 hours. The resulting thick suspension was treated with (22) (1.1g, 2.35mmol) before being stirred for a further 2 hours. The reaction mixture was filtered, diluted with water (30ml) and extracted with methylene chloride (3 x 20ml). The extracts were combined, washed with water (20ml), dried (MgSO\(_4\)) and concentrated to low volume. The residue was absorbed onto silica gel and chromatographed with ethyl acetate, the product being crystallised from di-isopropyl ether.

Yield 77% (1.3g), white powder.

Purity 98% (nmr, tlc).

\text{m.p 134-136}^\circ\text{C.}

\text{\(^1\text{H NMR: 10.05 (t, 1H, NH.CO), 7.7 (d, 2H, CH(Ar), 7.4 (d, 2H, CH(Ar), 7.15 (d, 2H, J = 15Hz, HC=C), 6.7 (d, 2H, J = 15Hz, C=CH), 4.7 (s, 2H, CH}_2\text{N), 4.45 (d, 2H, CH}_2\text{N), 3.35 (m, 2H, CH}_2\text{N), 3.15 (m, 2H, CH}_2\text{N), 2.95 (m, 4H, CH}_2\text{N), 1.65 (m, 4H, CH}_2\text{), 1.55 (m, 4H, CH}_2\text{), 1.35 (s, 9H, CH}_2\text{-satellite signals due to rotamers.}}

\text{I.R. (Nujol) 3320 (N-H), 1705 (C=O), 1680 (C=O), 1630, 1610 cm}^{-1}

\text{M.S. (LC/MS, TS}^+\text{): 720 (M}^+\text{ + 1), daughter fragments at 292 (as above) & 427}
N-(3-Guanidinopropyl)-N-(4-(4'-aminomethylphenyl))benzyl-3-(N'-(3-guanidinopropyl)-carbamoyl-trans-propenamide: UCL1641

(23) (1.2g) was dissolved in a solution of HCl in methanol (17% w/w, 10ml). The reaction mixture kept at room temperature for 2 hours, before adjusting the pH to 6 with 30% w/w NaOMe in methanol and filtering. The methanol was removed by distillation in vacuo, and the residue diluted with water (20ml). 3,5-Dimethylpyrazole carboxamidine nitrate (1.6g) was then added and the pH adjusted to 8 with 1M aq NaOH. The reaction mixture was then heated at 60°C for 18 hours, maintaining the pH between 7.5-8.5 by periodic addition of 1M aq NaOH. After cooling to room temperature, 40% w/v aq NaOH (0.5ml) was added, to pH14, and the solution stirred for 1 hour before washing with chloroform (2 x 25ml). The aqueous phase was acidified to pH1 with 50% nitric acid and concentrated in vacuo to give a gum, which was stirred in methanol (30ml), filtered, and the solvent removed by distillation. This operation was repeated twice more, the resulting gum was dissolved in hot ethanol (40ml) and the solution cooled to give the product as a yellow glass (120 mg).

Purity (nmr, hplc) 95%.
Contains 13% w/w inorganic material (CHN), 0.3 m/m EtOH (nmr, CHN).
M.S (FAB+): 509 (M+ + 1), 467 (M - HN=C-NH₂), 425 (M - C=C-NH-C=NH-NH₂)
CHN; calculated for C₂₆H₃₇N₉O₂ trinitrate + 13% w/w inorg + 0.3 m/m ethanol), 39.2, 5.12, 20.5; found 39.2, 5.10, 20.3
Hplc: Lichrosorb RP Select B (MeOH/0.05M aq NH$_4$OAc, 95/5), 210nm

Product rt 2.8 min (impurity rt 2.4 min, nitrate rt 2.0 min)

$^1$H NMR: 8.70 (m, CO.NH), 8.35 (br s, 3H, NH$_3^+$), 7.85 (br s, 8H, NH), 7.70-7.55 (m, 6H, CH(Ar)), 7.40 (m, 2H, CH(Ar)), 7.35 (d, J=15Hz, 1H, HC=C), 7.10 (d, J=15Hz, 1H, C=CH), 4.70 (m, 2H, CH$_2$Ar), 4.10 (m, 2H, CH$_2$N), 3.55 (m, 2H, CH$_2$N), 3.35 (m, 2H, CH$_2$N), 3.20 (m, 2H, CH$_2$N), 3.00 (m, 2H, CH$_2$N), 1.90 (m, 4H, CH$_2$)-satellite signals due to rotamers.
**UCL1597, N-(3-Guanidinopropyl)-N-(8-aminoctyl)-3-(N’-(3-guanidinopropyl) carbamoyl)-cis-propenamide**

N-(3-(t-butoxycarbonylaminopropyl)-N-8-trifluoroacetamidoctyl) maleiamide (26)

Maleic anhydride (1.4g, 1.43mmol) was dissolved in methanol (20ml), and a solution of (14) (5.3g, 1.34mmol) in methanol (10ml) added over 10 minutes. The reaction mixture was stirred at room temperature for 2 hours, before removing the solvent by distillation *in vacuo*, then diluting with methylene chloride (100ml) and 1M hydrochloric acid (50ml). The organic phase was dried (MgSO₄), concentrated to low volume and the product crystallised by dilution with di-isopropyl ether.

Yield 98% (6.5g), white crystals

Purity 98% (nmr).

m p: 92-94°C.

1H NMR: 8.55 (br t, 1H, NH.CO), 7.45 (d, 1H, C=CH), 7.30 (d, 1H, HC=C), 5.0 (br t, 1H, NH.CO), 3.45 (q, 2H, CH₂N), 3.25 (q, 2H, CH₂N), 1.75 (quintet, 2H, CH₂), 1.45 (s, 9H, tBu)

IR 3380, 3250, 1680, 1646, 1540 cm⁻¹

N-(3-(t-butoxycarbonylaminopropyl)-N-(8-trifluoroacetamido)-3-(N’-(3-(t-butoxycarbonylaminopropyl) carbamoyl)-cis-propenamide (25)

(26) (6.5g) and 1-hydroxysuccinimide (1.8g, 1.2mol/mol) were dissolved in methylene chloride (50ml). A solution of 1,3-dicyclohexylcarbodiimide (3.0g, 1.1 mol/mol) in methylene chloride (10ml) was added, and the
reaction mixture stirred 16 hours at room temperature before adding t-boc-1,3-diaminopropane (2.4g, 1.1 mol/mol). After a further 3 hours at RT, the reaction mixture was filtered and the filtrate was washed with water (2 x 30ml), dried (MgSO₄) and concentrated in vacuo to low volume.

The product was purified by column chromatography on silica gel with ethyl acetate/ methanol 20/1.

**Yield** 80% (6.9g), yellow gum.

**Purity** 98% (nmr, tlc).

**¹H NMR:**
- 8.0 (br t, 2 rotamers, 1H, NH.CO), 7.1 (br t, 2 rotamers, 1H, NH.CO), 6.4 (d, J=12.7Hz, 1H, HC=C), 6.1 (d, J=12.7Hz, 1H, C=CH), 5.4 (br t, 2 rotamers, 1H, NH.CO), 5.1 (br t, 2 rotamers, 1H, NH.CO), 3.45 (m, 2H, CH₂N), 3.3 (m, 6H, CH₂N), 3.1 (m, 2H, CH₂N), 1.7 (m, 2H, CH₂), 1.6 (m, 2H, CH₂), 1.5 (m, 4H, CH₂), 1.45 (s, 18H, Bu), 1.3 (m, 8H, CH₂).

**M.S:** (LC/MS, TS⁺): 652 (M⁺)

**I.R. (film):** 3350 (N-H), 1705, 1700 (O = O), 1610, 1520cm⁻¹

**N-(3-Guanidinopropyl)-N-(8-aminooctyl)-3-(N'-(3-guanidinopropyl)carbamoyl-propenamide, UCL 1597**

(25) (3.2g) was dissolved in a solution of HCl in methanol (22% w/w, 30ml).

The reaction mixture was kept at room temperature for 2 hours, before adjusting to pH6 with NaOH in methanol and filtering. The methanol was removed by distillation in vacuo and the resulting oil diluted with water (20ml). 3,5-Dimethylpyrazole carboxamidine nitrate (3.7g) was added and the pH adjusted to 8 with 1M aq NaOH. The reaction mixture was heated at 60°C for 26 hours, maintaining the pH between 7.5-8.5 by periodic addition of 1M aq NaOH.
After cooling to room temperature, 40% w/v aq NaOH (1ml) was added (to pH14), and the solution stirred 1 hour before washing with chloroform (2 x 30ml). The aqueous phase was acidified to pH1 with 50% nitric acid and concentrated *in vacuo* to dryness. The residue was stirred in methanol (30ml), filtered, and reconcentrated. This operation was repeated twice more, before recrystallising the product twice from ethanol (2 x 30ml).

Yield 100mg, yellow glass, 3.5 H$_2$O.

Purity 97 % (hplc, nmr) .

$^1$H NMR: 8.40 (1H, NH.CO), 7.7 (br s, 3.5, NH$_3^+$), 7.5 (2H, m, NH), 7.1 (6H, br s, NH$_2^+$), 6.5 (1H, m, J = 12.7Hz, HC=CH), 6.0 (1H, m, J = 12.7Hz, HC=CH), 3.55 (2H, m CH$_2$N), 3.3 (6.5H, m, CH$_2$N), 2.8 (4H, m, CH$_2$N), 1.8 (4H, m, CH$_2$), 1.4 (4H, m, CH$_2$), 1.2 (8H, br m, CH$_2$)

M.S. (LC/MS, TS$^+$): 440 (M + 1), 244 (base peak)

Hplc: Lichrosorb PR Select B (MeOH/ 0.5% aq TFA; 80/20), 224nm

Product rt 1.7 min; Impurity (rt 2.1 min) (nitrate rt 2.5 min)

C.H.N: calculated for C$_{20}$H$_{41}$NgO$_2$ trinitrate 3.5 H$_2$O; 34.73, 7.43, 24.30; found 34.46, 7.13, 24.12
UCL1716. N-(3-(4-quinolinylamino)-propyl)-N-(8-aminooctyl)-3-N'-(3-(4-quinolinylamino)-propyl)-carbamoyl-trans propenamide

N-(4-quinolinyl)-N'-(t-butoxycarbonyl)-1,3-diaminopropane (27)

\[
\begin{array}{c}
\text{NH(CH}_2\text{)}_3\text{NH.Boc}
\end{array}
\]

t-Bocdiaminopropane (32.6g, 0.18 mol) and diisopropylethylamine (25.2g, 0.19 mol) were added to a solution of 4-chloroquinoline (24.5g, 0.15 mol) in 1-pentanol (200ml). The reaction mixture was heated under reflux for six hours, giving a thin suspension, which was concentrated in vacuo. The resulting oil was diluted with methylene chloride (250ml) and washed with 2M NaOH (2 x 120ml). After the second wash a precipitate was produced, which was filtered off and washed with toluene (50ml). The organic phase from the filtrate was concentrated in vacuo and the residue diluted with toluene (150ml) to give a further precipitate, which was also filtered off and washed with toluene. The two crops were combined and recrystallised from toluene (300ml) to provide (27).

Yield 84% (38g), cream powder
Purity: 98% (nmr, tlc)
mp: 165-167°C

\(^1\text{H nmr (CDCl}_3\): 8.50 (d, 1H, CH(Ar)), 8.0 (d, 1H, CH(Ar)), 7.95 (d, 1H, CH(Ar)), 7.60 (m, 1H, CH(Ar)), 7.45 (m, 1H, CH(Ar)), 6.45 (d, 1H, CH(Ar)), 6.0 (br s, 1H, NH.CO), 4.85 (br s, 1H, NH), 3.45 (q, 2H, CH\textsubscript{2}N), 3.30 (q, 2H, CH\textsubscript{2}N), 1.85 (qu, 2H, CH\textsubscript{2}), 1.50 (s, 9H, \textsuperscript{t}Bu).

N-(4-quinolinyl)-1,3-diaminopropane (28)

\[
\begin{array}{c}
\text{NH(CH}_2\text{)}_3\text{NH}_2
\end{array}
\]
(27) (30.1g, 0.10mol) was dissolved in TFA (200ml), producing a moderate
effervescence, which subsided after ca 2-3 minutes. After 1 hour at room
temperature the solution was concentrated in vacuo to low volume, diluted
with toluene (200ml) and reconcentrated to remove the excess TFA. The
resulting oil was diluted with water (200ml), basified to pH14 with 40% w/v
aq. NaOH (50ml), and extracted with chloroform (2 x 150ml). The extracts
were combined, dried (MgSO4) and evaporated to give the product as a pale
oil, which slowly solidified on storage.

Yield 95% (19g), white powder
m p: 75-77°C
Purity: >98% (nmr, tlc)
1H nmr (CDCl3): 8.55 (d, 1H, CH(Ar)), 7.95 (d, 1H, CH(Ar)), 7.80 (d, 1H,
CH(Ar)), 7.65 (m, 1H, CH(Ar)), 7.40 (m, 1H, CH(Ar)), 7.25 (br t, 1H, NH.CO),
6.35 (d, 1H, CH(Ar)), 3.45 (t, 2H, CH2N), 3.05 (t, 2H, CH2N), 1.90 (qu, 2H,
CH2), 1.75 (br s, 9H, tBu).

**Ethyl N-(N'-3-(4-quinolinyaminopropyl)-carbamoyl)-trans propenamide (29)**

![Chemical structure](image)

A solution of monoethylfumarate (8.6g, 0.06 mol) in THF (120ml) was treated
with 1,3-dicyclohexyl carbodiimide (13.6g, 0.06 x 1.10 mol) in one portion, the
temperature rising from 22 to 33°C (no cooling). The reaction mixture was
stirred for 2 hours to give a thick suspension, to which a solution of (28)
(12.0g, 0.06 mol) in THF (50ml) was added in one portion. After a further
2 hours, the mixture was filtered, washing the collected dicyclohexylurea with
THF (50ml). The filtrate was concentrated and the residue triturated with
diethyl ether to crystallise (29).
Yield: 60% (11.7g), cream powder
Purity: 85% (nmr, ca 15% dicyclohexylurea)
m.p: 194-196°C

^1^H nmr: 8.50 (d, 1H, CH(Ar)), 8.00 (m, 2H, CH(Ar)), 7.65 (m, 1H, CH(Ar)), 7.50 (m, 1H, CH(Ar)), 7.00 (d, J=15Hz, 1H, HC=C), 6.90 (d, J=15Hz, 1H, C=CH), 6.50 (t, 1H, NH.CO), 6.30 (d, 1H, CH(Ar)), 4.30 (q, 2H, CH₂O), 3.55 (m, 2H, CH₂N), 3.40 (m, 2H, CH₂N), 1.90 (m, 2H, CH₂), 1.30 (t, 3H, CH₃).

N-(4-quinolinylaminopropyl)-carbamoyl-trans-propenoic acid (30)

A suspension of (29) (9.85g, 0.03 mol) in methanol (30ml) and water (120ml) was stirred while adding 40% w/v NaOH (30ml, 0.3 mol), allowing the temperature to rise to 35°C. The mixture was warmed to 50°C in order to dissolve the remaining solid, then left to cool to room temperature over 3 hours.

The resulting solution was acidified to pH1 with conc hydrochloric acid (60g, 0.7 mol) and stored at 5°C, slowly precipitating (30).HCl, which was filtered off and washed with cold water (10ml).

Yield 30% (3.0g), white powder
Purity: 98% (nmr),
m.p: 179-181°C

NMR (d₆-dmso): 9.50 (t, 1H, CH(Ar)?/NH), 8.85 (t, 1H, NH.CO), 8.60 (d, 1H, CH(Ar)), 8.50 (d, 1H, CH(Ar)), 7.95 (m, 2H, CH(Ar)), 7.70 (m, 1H, CH(Ar)), 6.95 (d, J=15Hz, 1H, HC=C), 6.80 (d, 1H, CH(Ar)), 6.50 (d, J=15Hz, 1H, C=CH), 1.90 (qu, 2H, CH₂), other 4ppm masked by H₂O.

CHN: calculated for C₁₆H₁₇N₃O₃.1.5H₂O:52.97, 5.83, 11.58; found: 52.70, 5.68, 11.36.
N-(trifluoroacetamido)-N’-(3-(4-quinolinvl)-1,3-aminopropvl)-1,8-
diaminoctane (31)

A solution of (28) (5.0g, 50 mmol), trifluoroacetamidooctyl tosylate (10.0g, 26 mmol) and diisopropylethylamine (3.5g, 27.5 mmol) in DMF was stirred for 16 hours at room temperature. The reaction mixture was then diluted with water (150ml) and extracted with methylene chloride (2 x 100ml). The extracts were combined, washed with water (100ml) and dried (MgSO₄), before being concentrated in vacuo to give the crude product, which purified by column chromatography on silica gel with CMA 200.

Yield: 34% (3.5g), yellow oil
Purity: 97% (nmr, tlc)

^H nmr (CDCl₃): 8.50 (d, 1H, CH(Ar)), 7.95 (m, 1H, CH(Ar)), 7.85 (m, 1H, CH(Ar)), 7.75 (br s, 1H, NH?), 7.70 (m, 1H, CH(Ar)), 7.40 (m, 1H, CH(Ar)), 6.70 (br s, 1H, NH?), 6.35 (d, 1H,CH(Ar)), 3.45 (m, 2H, CH₂N), 3.35 (q, 2H, CH₂N), 2.95 (m, 2H, CH₂N), 2.70 (m, 2H, CH₂N), 1.95 (qu, 2H, CH₂), 1.50 (m, 4H, CH₂), 1.25 (br s, 8H, CH₂).

N-(3-(4-quinolinyl)-aminopropyl)-N-(8-trifluoracetamidoctyl)-N’-(3-(4-
quinolinyl)-aminopropyl)-carbamoyl-trans propenamide (32)
A solution of (30)·HCl (3.4g, 10mmol) in DMF (20ml) was treated with 1,3-dicyclohexylcarbodiimide (2.5g, 12 mmol) in one portion, the temperature rising from 25 to 33°C. The mixture was stirred for 3 hours, giving a thick suspension, which was treated sequentially with (31) (4.3g, 10mmol) and diisopropylethylamine (1.6g, 12.5 mmol), most of the precipitate dissolving shortly after the base addition.

After stirring for 2 hours, the mixture was filtered to remove dicyclohexylurea, diluted with water (100ml) and extracted with methylene chloride (2 x 100ml). The extracts were combined, washed with water (50ml) and dried (MgSO₄) before being concentrated in vacuo to give a yellow oil, which was absorbed onto silica gel and eluted with TEA. The product was further purified by crystallisation from diethyl ether.

Yield 22% (1.5g), cream crystals.

m p: 127-129°C
Purity: 80-90% (nmr, contains ca 10-20% DCCU)

^1H nmr (CDCl₃): 8.50 (m, 2H, CH(Ar)), 7.95 (m, 4H, CH(Ar)), 7.60 (m, 2H, CH(Ar)), 7.40 (d, J=15Hz, 1H, HC=C), 7.35 (m, 2H, CH(Ar)), 7.05 (d, J=15Hz, 1H, C=CH), 6.55 (t, 1H, NH.CO), 6.35 (d, 2H, CH(Ar)), 6.10 (t,1H, NH.CO), 3.55 (m, 4H, CH₂N), 3.35 (m, 8H, CH₂N), 1.95 (m, obscured partially by DCCU), 1.55 (m, 4H, CH₂), 1.20 (br s, 8H, CH₂).

I.R (nujol): 3400 (br m, N-H), 1710 (m, C =O), 1630 (m, C =C), 1590 (C-H), 1550 (C-H)

N-(3-(4-quinolinylamino)-propyl)-N-(8-aminoctyl)-3-N’-(3-(4-quinolinylamino)-propyl)-carbamoyl-trans propenamide, UCL1716
40% aq NaOH (ca 0.5 ml) was added to a solution of (32) (0.4g) in a mixture of methanol (5ml) and water (3ml). After 1 hour at room temperature, the reaction mixture was extracted with chloroform (2 x 20ml). The extracts were combined, dried (MgSO₄), and the solvents removed by distillation in vacuo to give a pale yellow gum, which was redissolved in chloroform/ methanol (20/1, 10ml), washed with 0.5M aq NaOH (2 x 5ml), dried (MgSO₄) and reconcentrated to dryness. The residue was dissolved in absolute ethanol and diluted with diethyl ether to 'precipitate' UCL1716 as a cream glass (30mg).

Purity: 98% (hplc), 90% (nmr- partly protonated + 0.3 mol/mol EtOH)

¹H nmr (CDCl₃): 8.55 (m, 2H, CH(Ar)), 7.95 (m, 4H, CH(Ar)), 7.60 (m, 2H,CH(Ar)), 7.45 (d, J=15Hz, 1H, HC=C), 7.40 (m, 2H, CH(Ar)), 7.05 (d, J=15Hz, 1H, C=CH), 6.90 (t, 1H, NH), 6.55 (t, 1H, NH), 6.40 (m, 2H, CH(Ar)), 6.15 (t, 1H, NH), 3.55 (m, 4H, CH₂N), 3.40 (m, 4H, CH₂N), 3.30 (m, 2H, CH₂N), 2.65 (m, 2H, CH₂N), 1.90 (m, 4H, CH₂), 1.65 (m, 2H, CH₂), 1.45 (m, 2H, CH₂), 1.30 (br s, 8H, CH₂).

M.S (TS⁺): 610 (M⁺ + 1)

Hplc (Phenyl BDS, MeOH, 0.05M aq NH₄OAc 90/10, 215nm, 1.5 ml/min): UCL1716 at rt 1.9 min (impurity at rt 1.5 min)

Tlc: CMA 200/ silica; UCL1716 R.f 0.2, (32) R.f 0.3

CHN: calculated for C₂₆H₄₇N₇O₂ 3.5H₂O + 0.3 m/m EtOH: 63.20, 8.00, 14.01; found: 63.24, 7.42, 13.86.

I.R (nujol): 3250 (m, N-H), 1675 (s, C=O), 1550 (C-H(Ar))
UCL1714, N-(4-aminoquinolinvl)-3,4-di-(3-(4-aminoquinolinvl)-propoxv)-phenethvlamine

N-(t-butoxycarbonyl) dopamine (36)

A solution of dopamine.HCl (38.0g, 0.2 mol) and di-t-butyl dicarbonate (48.0g, 0.22 mol) in methanol (200ml) was treated with 25% w/v sodium methoxide in methanol (50ml, 0.23 mol) over 20 minutes at 18-25°C with ice-water cooling. The reaction mixture was stirred at room temperature for a further 20 minutes, then concentrated in vacuo to a third of the volume, before diluting with ethyl acetate (800ml). The precipitated inorganic salts were filtered off and the filtrate concentrated to give a dark orange oil, which was stirred in di-isopropyl ether to precipitate the product.

Yield 65% (32.9g), pale grey powder
Purity: ca 95% (nmr, tlc)
m.p: 132-134°C

H nmr (d6-dmso): 8.80 (br s, 2H, OH), 6.80 (t, 1H, NH.CO), 6.65 (d, 1H, CH(Ar)), 6.60 (m, 1H, CH(Ar)), 6.35 (m, 1H, CH(Ar)), 3.10 (q, 2H, CH2N), 2.45 (t, 2H, CH2Ar), 1.35 (s, 9H, tBu).
M.S. (EI+): 253 (M+ + 1)

I.R. (nujol): 3500 (s, sharp, O-H), 3380 (s, sharp, N-H), 1680 (s, broad, C=O), 1480 (m, C-H).

t-Butoxycarbonyl-bromopropylamine (37)

A solution of 3-bromopropylamine.HBr (217g, 1.0 mol) and di-t-butyl dicarbonate (229g, 1.05 mol) in methanol (400ml) was treated with 25% w/v sodium methoxide in methanol (450 ml, 2.1 mol) over 1 hour at 19-28°C, with ice-water cooling. After being stirred for a further 15 minutes the reaction mixture was diluted with water (3l) and extracted with methylene chloride (2 x 3l). The first extract was washed sequentially with 1M hydrochloric acid (1.5l), water (1.5l), saturated sodium bicarbonate (1.5l)
(1.5l) and finally water (1.5l), back extracting each wash with the second extract. The organic layers were then combined, dried (MgSO₄) and the solvent removed by distillation in vacuo to give the product as a pale yellow oil, which slowly solidified.

Yield 66% (204g), white crystals
Purity: ca 95% (nmr) - decomposes slowly on storage (to give an oil)
m p: 32-37°C

\(^1\)H nmr (CDCl₃): 5.00 (t, 1H, NH.CO), 3.30 (t, 2H, CH₂Br), 3.15 (q, 2H, CH₂N), 1.95 (qu, 2H, CH₂), 1.35 (s, 9H, tBu)
I.R (nujol): 3350 (br, s, N-H), 1680 (br, s, O = O), 1520 (s, C-H)

N-(t-butoxycarbonyl)-3,4-di-(3-(t-butoxycarbonyl)aminopropoxy)-phenethylamine (38)

A solution of boc-dopamine (12.6g, 0.05 mol) in DMF (200ml) was treated with potassium t-butoxide (13.0g, 0.05 x 2.3 mol) portionwise over 15 minutes, the temperature rising to 12-23°C. The resulting green suspension was stirred 10 minutes, then boc-3-bromopropylamine (25.0g, 0.05 x 2.1 mol) added in one portion, the temperature rising to 33°C and a turbid solution being produced. After 30 minutes, the reaction mixture was diluted with water (800ml) and extracted with methylene chloride (2 x 600ml). The first extract was washed sequentially with dilute aq NaOH and water, back extracting each wash with the second extract. The organic layers were then combined, dried (MgSO₄) and concentrated in vacuo. The resulting product was purified by recrystallisation from diisopropyl ether.
Yield 33% (8.5g), cream crystals
Purity: 98% (nmr, tlc)
m.p. 91-93°C

$^1$H nmr (CDCl$_3$): 6.80 (d, 1H, CH(Ar)), 6.70 (m, 2H, CH(Ar)), 5.30 (br s, 2H, NH), 4.60 (br s, 1H, NH), 4.00 (m, 4H, CH$_2$O), 3.35 (m, 6H, CH$_2$N), 2.70 (t, 2H, CH$_2$Ar), 2.00 (m, 4H, CH$_2$), 1.40 (s, 9H, ^t$^1$Bu)

M.S (TS$^+$): 568 (M$^+$ + 1)

I.R (nujol): 3350 (s, N-H), 1680 (s, C=O), 1520 (s, C-H)

UCL1714, N-(4-aminoquinolinyl)-3,4-di-(3-(4-aminoquinolinyl)-propoxy)-phenethylamine

(38) (5.7g, 10 mmol) was dissolved in trifluoroacetic acid (30ml), producing an effervescence. After stirring at room temperature for 1 hour, the excess acid was removed by distillation in vacuo to give a yellow oil. This was dissolved in methanol (50ml) and 25% w/v NaOMe in methanol added until pH 10. The methanol was then replaced with 1-pentanol by distillation at constant volume.

4-Chloroquinoline (3.3g, 40 mmol) and diisopropylethylamine (3.0g, 40mmol) were added and the reaction mixture heated under reflux for 7 hours, before concentrating to low volume. The resulting oil was absorbed onto silica gel and eluted with CMA 200 to give a yellow gum, which was triturated with diethyl ether to provide UCL1714 (30mg) as a yellow glass.
Purity: 98% (nmr), 95% (hplc)

$^1$H nmr (d-MeOH): 8.30 (d, 1H, CH(Ar)), 8.20 (d, 2H, CH(Ar)), 8.00 (m, 3H, CH(Ar)), 7.75 (m, 3H, CH(Ar)), 7.55 (m, 3H, CH(Ar)), 7.35 (m, 3H, CH(Ar)), 6.95 (d, 1H, CH(Ar)), 6.85 (m, 2H, CH(Ar)), 6.45 (m, 3H, CH(Ar)), 4.20 (t, 2H, CH$_2$O), 4.00 (t, 2H, CH$_2$O), 3.55 (m, 4H, CH$_2$N), 3.45 (m, 2H, CH$_2$N), 2.90 (t, 2H, CH$_2$Ar), 2.15 (m, 2H, CH$_2$), 2.05 (m, 2H, CH$_2$)

Hplc (Partisil ODS3, MeOH/0.05M aq NH$_4$OAc (pH4) 90/10, 0.5 ml/min, 215nm): UCL1714 at 5.6 min, impurity (5%) at 4.0 min.

Tlc (CMA 200/ SiO$_2$): UCL1714 R.f 0.6 (impurity R.f 0.65), 'mono alkyls'

R.f 0.1-0.3, 'diamine' R.f 0.05.

M.S (ES$^+$): 649 (M$^+$ + 1)

I.R. (nujol): 3250 (s, N-H), 1700 (C=O), 1540 (C-H)

CHN: calculated for C$_{41}$H$_{40}$N$_6$O$_2$. 2H$_2$O: 71.82, 6.42, 12.26, found: 71.82, 6.17, 11.88.
Phloroglucinol (63g, 0.5 mol) and t-boc diaminopropane (100g, 0.575 mol) were heated under reflux in THF (500ml), giving a clear solution after 30 minutes. Toluene (800ml) was then added and the solvent removed by distillation in vacuo. The residue was redissolved in THF (500ml), refluxed for a further 30 minutes and the toluene addition/concentration operation repeated, giving a dark brown gum. This was absorbed onto silica and chromatographed with ethyl acetate/methylene chloride (1/1). The mobile fraction was concentrated to low volume and the residue stirred in ethyl acetate (500ml) to precipitate (47). The mother liquors were concentrated to constant weight then purified by column chromatography on silica with diethyl ether to give (49) as a gum.

(49):

Yield 56% (80g), brown gum
Purity >95% (nmr, tlc)

$^1$H nmr (d$_6$-dmso): 8.70 (2, 2H, OH), 6.80 (t, 1H, NH.CO), 5.50 (m, 3H, CH(Ar)), 5.30 (t, 1H, NH.CO), 3.10 (m, 2H, CH$_2$N), 3.00 (m, 2H, CH$_2$N), 1.40 (s, 9H, t-Bu)

I.R (film): 3400 (s, br, O-H, N-H), 1690 (s, C=O), 1630 (s, C=N), 1525 (s, C-H)
Yield 13% (28g), dark cream powder
Purity: >95% (nmr, tlc)
m.p: 122-124°C
Nmr (d6-dmso): 8.45 (s, 1H, OH), 6.80 (t, 2H, NH.CO), 5.30 (m, 3H, CH(Ar)), 5.10 (t, 2H, NH), 3.00 (q, 4H, CH2N), 2.85 (q, 4H, CH2N), 1.60 (m, 4H, CH2), 1.40 (s, 9H, tBu)
MS (TS+): 439 (M+ + H)
Tlc: Et2O/silica: phloroglucinol R.f 0.4, (49) R.f 0.45, (47) R.f 0.1
EtOAc/silica: phloroglucinol R.f 0.7, (49) R.f 0.5, (47) R.f 0.45

1-(3-(N-(t-butoxycarbonyl))-aminopropoxy)-3,5-di-(3-(t-butoxycarbonyl)-amino propyl)-amino-benzene (48)

Potassium t-butoxide (1.3g, 115 mmol) was added in one portion to a solution of (47) (4.4g, 100 mmol) in DMF (30ml), the temperature rising from 15 to 22°C. The resulting purple solution was stirred 10 minutes, before adding t-boc-bromopropylamine (2.4g, 100 mmol), after which the temperature rose to 39°C.

The reaction mixture was stirred 1 hour, then diluted with water (120 ml) and extracted with methylene chloride (2 x 100ml). The extracts were combined, washed sequentially with 1M aq NaOH and water, dried (MgSO4) and concentrated in vacuo to give a brown gum, which was absorbed onto silica and chromatographed with diethyl ether. The product was crystallised from diethyl ether.
Yield 58% (3.5g), cream powder
Purity: >95% (nmr, tlc)
m p: 133-135°C

\[
^{1}H \text{ nmr } (\text{CDCl}_3): \text{ 5.60 (s, 2H, CH(Ar)), 5.50 (s, 1H, CH(Ar)), 4.85 (br s, 1H, NH.CO), 4.75 (br s, 1H, NH.CO), 3.95 (t, 2H, CH}_2\text{O), 3.80 (s, 2H, NH), 3.30 (q, 2H, CH}_2\text{N), 3.20 (q, 4H, CH}_2\text{N), 3.10 (t, 6H, CH}_2\text{N), 1.95 (qu, 2H, CH}_2\text{), 1.80 (qu, 4H, CH}_2\text{), 1.45 (s, 27H, iBu).}
\]

M.S. (ES\textsuperscript{+}): 597 (M\textsuperscript{+} + 1), (impurity at 754)
I.R. (nujol): 3450 (w, N-H), 3380 (s, N-H), 1700 (s, C=O), 1620 (m, C=N), 1520 (s, C-H)

1-\{(N-(t-butoxycarbonyl)-aminopropyl)-3,5-di-((t-butoxycarbonyl)-aminopropyl)-amino-benzene (51)

Potassium t-butoxide (5.3g, 46 mmol) was added in one portion to a solution of (49) (5.7g, 20mmol) in DMF (50ml), the temperature rising from 16 to 28°C and a thick precipitate forming. After 10 minutes, t-boc bromopropylamine (9.5g, 40 mmol) was added, producing a further increase in temperature to 35°C. The resulting turbid solution was stirred 30 minutes, before diluting with water (200ml) and extracting with methylene chloride (2 x 150ml). The extracts were combined, washed sequentially with 1M aq NaOH and water, dried (MgSO\textsubscript{4}), and concentrated to give a brown oil (8g). This was absorbed onto silica gel and eluted with diethyl ether to give (51) as a pale yellow oil, which slowly solidified.

Yield 20% (2.3g), cream crystals
Purity: 98% (nmr, tlc)
m p: 125-127°C.
$^1$H nmr (CDCl$_3$): 5.85 (m, 1H, CH(Ar)), 5.80 (m, 2H, CH(Ar)), 4.85 (t, 2H, NH.CO), 4.80 (t, 1H, NH.CO), 3.95 (m, 4H, CH$_2$O), 3.30 (q, 4H, CH$_2$N), 3.20 (m, 2H, CH$_2$N), 3.10 (m, 2H, CH$_2$N), 1.95 (m, 4H, CH$_2$), 1.80 (m, 2H, CH$_2$), 1.40 (s, 27H, $^1$Bu)

M.S (ES$^+$): 597 (M$^+$ + 1), (impurities at 654, 698)

I.R (nujol): 3380 (m, br, N-H), 1700 (s, C=O), 1620 (m, C=N), 1520 (s, C-H)

N-(3-(4-aminoquinoliny1)-propyl)-3,5-di-(3-(4-aminoquinoliny1)-propoxy)-aniline, UCL1715

(51) (2.0g, 3.3mmol) was dissolved in trifluoroacetic acid (30ml), producing a mild effervescence. After 1 hour the excess acid was removed by distillation in vacuo and the resulting green oil diluted with methanol. Sodium methoxide in methanol was added until pH12, before replacing the solvent with 1-pentanol, and treating with 4-chloroquinoline (2.2g, 13mmol) plus diisopropylethylamine (2.2g, 16.5mmol).

The reaction mixture was heated under reflux for 4 hours, then concentrated to low volume, diluted with 1M aq NaOH (100ml) and extracted with methylene chloride/methanol (10/1, 2 x 100ml). The extracts were combined, washed with 1M NaOH (50ml), dried (MgSO$_4$), and concentrated to give a dark brown oil, which was absorbed onto silica gel and eluted with CMA 200. The product was isolated as a pale yellow glass (0.5g) by trituration with diethyl ether.
Purity 95% (nmr), 92% (hplc)
'm p 102°C'

$^1$H nmr (d$_6$-dmso): 8.40 (m, 3H, CH(Ar)), 8.25 (d, 3H, CH(Ar)), 7.75 (m, 3H, CH(Ar)), 7.60 (m, 3H, CH(Ar)), 7.40 (m, 3H, CH(Ar)), 7.20 (m, 3H, NH), 6.45 (m, 3H, CH(Ar)), 5.80 (m, 3H, CH(Ar')), 5.70 (t, 1H, NH), 3.95 (t, 4H, CH$_2$O), 3.50 (t, 4H, CH$_2$N), 3.45 (t, 2H, CH$_2$N), 3.20 (t, 2H, CH$_2$), 2.10 (q, 4H, CH$_2$), 1.90 (q, 2H, CH$_2$).

Hplc (IB-SIL Phenyl-BDS. MeOH/ 0.05M aq NH$_4$OAc, 90/10, 215 nm, 0.5 ml/min): UCL1715 at 2.6 min (impurity at 1.6 min)

M.S (EI$^+$): 678 (M$^+$ + 1)

CHN: calculated for C$_{42}$H$_{43}$N$_7$O$_2$. 2H$_2$O: 70.58, 6.58, 13.72; found: 70.30, 6.50, 13.38.

I.R (nujol): 3250 (s, N-H), 1580 (s, C-H), 1550 (m, C-H)
UCL 1735, N-(3-(4-quinolinvl)-aminopropvn-3.5-di-(3-(4-(quinolinvl)-
aminopropoxv)-benzvlamine

3.5-Di-(t-butoxycarbonylaminoproxy)-benzyl alcohol (52)

A solution of potassium t-butoxide (25g, 0.22 mol) in DMF (100ml) was added in one portion to 3,5-dihydroxybenzyl alcohol (14g, 0.1 mol) in DMF (50ml), the temperature rising over 10 minutes from 20 to 45°C (no cooling). A sticky cream precipitate was initially formed, which became an easily stirrable yellow granular form soon after the addition.

After stirring for 15 minutes, t-bocbromopropylamine (50g, 0.21 mol) was added over 10 minutes, the temperature rising to 64°C and a turbid solution being produced. This was cooled to room temperature, diluted with water (300ml) and extracted with ethyl acetate (3 x 150ml). Each extract was washed with 1M aq NaOH (100ml) then water (100ml) before being combined, dried (MgSO₄) and concentrated in vacuo. Trituration of the residue with di-isopropyl ether (300ml) precipitated (52).

Yield 71% (30.2g), cream powder
Purity; 95% (nmr)
m p: 77-79°C

1H nmr (CDCl₃): 6.50 (s, 2H, CH(Ar)), 6.35 (s, 1H, CH(Ar)), 4.75 (br s, 2H, NH.CO), 4.60 (s, 2H, ArCH₂O), 4.00 (t, 4H, CH₂O), 3.30 (q, 4H, CH₂N), 1.95 (qu, 4H, CH₂), 1.45 (s, 18H, tBu).

I.R (nujol): 3380 (m, N-H), 1690 (s, C=O), 1585 (m, C-H), 1520 (s, C-H)
3.5-di-(t-butoxycarbonylaminopropoxy) benzyl chloride (53)

A solution of (62) (11.3g, 25 mmol) and triethylamine (3.3g, 32 mmol) in chloroform (100ml) was treated with p-toluene sulphonyl chloride (6.0g, 31 mmol). The reaction mixture was stirred at room temperature for 16 hours before replacing the solvent with ethyl acetate by distillation to precipitate triethylamine.HCl. This was filtered off and the filtrate concentrated in vacuo. The resulting oil was absorbed onto silica gel and eluted with ethyl acetate to give the product as a yellow gum, which was crystallised by stirring in diisopropyl ether.

Yield 42% (5.0g), cream powder
Purity: 98% (nmr)
Mp: 129-131°C

$^1$H nmr (CDCl$_3$): 6.50 (s, 2H, CH(Ar)), 6.40 (s, 1H, CH(Ar)), 4.75 (br s, 2H, NH.CO), 4.50 (s, 2H, CH$_2$Cl), 4.00 (t, 4H, CH$_2$O), 3.30 (q, 4H, CH$_2$N), 1.95 (qu, 4H, CH$_2$), 1.45 (s, 18H, Bu)

I.R (nujol): 3380 (m, N-H), 1690 (s, C=O), 1600, 1520 (m, C-H)

N-(3-(4-quinoliny)-aminopropyl) 3.5-di-(3-(t-butoxycarbonyl) aminopropoxy)-benzylamine (54)
(28) (4.0g, 20 mmol) was added to a solution of (53) (2.35g, 5mmol) in DMF (20ml) and the reaction mixture stirred at room temperature for 16 hours, giving a thin precipitate. After dilution with water (80 ml), the product was extracted into ethyl acetate (2 x 80ml). The extracts were combined, washed with water, concentrated in vacuo and the resulting crude (54) purified by column chromatography on silica gel with CMA 200.

Yield 83% (2.6g), yellow oil
Purity: 90% (nmr; rotamers observed)

$^1$H nmr (CDCl$_3$): 8.45 (d, 1H, CH(Ar)), 7.90 (d, 1H, CH(Ar)), 7.60 (m, 1H, CH(Ar)), 7.25 (m, 2H, CH(Ar)), 6.40 (s, 2H, CH(Ar')), 6.30 (s, 1H, CH(Ar')), 6.25 (d, 1H, CH(Ar')), 4.80 (br s, 2H, NH.CO), 3.80 (t, 4H, CH$_2$O), 3.70 (s, 2H, ArCH$_2$N), 3.30 (t, 2H, CH$_2$N), 3.20 (q, 4H, CH$_2$), 2.80 (t, 2H, CH$_2$), 1.85 (qu, 2H, CH$_2$), 1.80 (qu, 4H, CH$_2$), 1.35 (s, 18H, tBu).

M.S (ES$^+$): 638 (M$^+$ + 1), base peak at 356
I.R (film): 3280 (m, N-H), 1700 (s, C=O), 1580 (s, C-H)

N-(3-(4-quinolinvl)aminpropvl) 3,5-di-(3-(4-quinolinvl)aminoproxy) benzyllamine, UCL1735

(54) (1.9, 3.0 mmol) was dissolved in trifluoroacetic acid (20ml), producing a mild effervescence. After 1 hour, the excess acid was removed by distillation in vacuo and the resulting oil dissolved in methanol. Sodium methoxide in
methanol was added to pH10 and the solvent was then removed by distillation in vacuo to dryness, before diluting with 1-pentanol (20ml).
4-Chloroquinoline (2.0g, 12 mmol) and diisopropylethylamine (2.0g, 15 mmol) were then added and the reaction mixture heated under reflux for 4 hours, before concentrating to low volume, diluting with 1M aq NaOH (50ml) and extracting with methylene chloride/methanol 10/1 (2 x 50ml). The extracts were combined, concentrated to low volume and the resulting oil absorbed onto silica gel and eluted with CMA 200. The product fraction was dissolved in methylene chloride, filtered to clarify, then solvent removed by distillation and the residue triturated with ethyl acetate to provide UCL1735 (0.2g) as a yellow foam.

Purity: >95% (nmr), 95% (tlc)

^1\text{H} \text{nmr} (\text{CDCl}_3): 8.55 (m, 3H, CH(Ar)), 7.95 (m, 3H, CH(Ar)), 7.75 (m, 3H, CH(Ar)), 7.50 (m, 3H, CH(Ar)), 7.30 (m, 3H, CH(Ar)), 6.50 (s, 2H, CH(Ar')), 6.40 (m, 3H, CH(Ar')), 4.90 (t, 4H, CH₂O), 3.80 (s, 2H, ArCH₂N), 3.45 (m, 4H, CH₂N), 3.35 (m, 2H, CH₂N), 2.85 (m, 2H, CH₂), 2.40 (br s, 3H, NH), 2.05 (m, 4H, CH₂), 1.90 (m, 2H, CH₂).

M.S (ES\textsuperscript{+}): 692 (M\textsuperscript{+} + 1)

CHN: calculated for C\textsubscript{43}H\textsubscript{45}N\textsubscript{7}O\textsubscript{2}. 2.5H\textsubscript{2}O: 70.00, 6.78, 13.29; found: 70.49, 6.77, 12.88.

I.R (nujol): 3250 (s, N-H, H\textsubscript{2}O), 1710 (w, C=O), 1580 (s, C-H), 1530 (s, C-H)
UCL1822, 1,2,3-tri-(3-(4-quinolinvl)aminopropoxv)-benzene.

1,2,3-tri-(3-(t-butoxvcarbonvl)aminopropoxv)-benzene (56)

\[
\begin{array}{c}
\text{Boc.HN(CH}_2)_3\text{O} \\
\text{O(CH}_2)_3\text{NH.Boc} \\
\text{O(CH}_2)_3\text{NH.Boc}
\end{array}
\]

A solution of potassium t-butoxide (9.3g, 82 mmol) in DMF (50 ml) was added in one portion to pyrogallol (3.2g, 25 mmol) dissolved in DMF (20ml), the temperature rising from 18° to 36°C. A thick yellow suspension was produced which was stirred for 10 minutes before adding t-boc bromo propylamine (18.0g, 75 mmol) in one portion, the temperature rising to 48°C. The resulting dark brown suspension was stirred for 1 hour, then diluted with water (200ml) and extracted with ethyl acetate (3 x 100ml). Each extract was washed sequentially with the same portions of 1M aq NaOH (100ml) and water (100ml). The extracts were then combined and the solvents removed by distillation in vacuo to give a dark brown oil, which was absorbed onto silica gel and eluted with diisopropyl ether/ diethyl ether 1/1. The product was isolated as a pale yellow oil, which was crystallised by trituration with diethyl ether.

Yield 20% (3.0g), white powder
Purity: >98% (nmr)
m p. 84-86°C

\(^1\)H nmr (CDCl\(_3\)): 6.95 (t, 1H, CH(Ar)), 6.55 (d, 2H, CH(Ar)), 5.45 (br s, 1H, NH.CO), 5.15 (br s, 2H, NH.CO), 4.05 (q, 6H, CH\(_2\)O), 3.40 (m, 2H, CH\(_2\)N), 3.30 (m, 4H, CH\(_2\)N), 2.00 (qu, 4H, CH\(_2\)), 1.90 (qu, 2H, CH\(_2\)), 1.40 (s, 27H, \(^t\)Bu)
M.S. (ES\(^+\)): 598 (M\(^+\) + H), 620 (M\(^+\) + Na)
I.R (nujol): 3280 (m, N-H), 1700 (s, C=O), 1580 (w, C-H), 1520 (s, C-H)
UCL1822, 1,2,3-Tri-(3-(4-quinolinyl)aminopropoxy)-benzene

(55) (2.4g, 4 mmol) was dissolved in trifluoroacetic acid (20ml), producing a mild effervescence. After 1 hour, the excess acid was removed by distillation in vacuo and the resulting oil diluted with methanol (50ml) before basifying to pH 10 with sodium methoxide in methanol. The solvent was then replaced with 1-pentanol (80 ml) by distillation at constant volume, before adding 4-chloroquinoline (2.6g, 16 mmol) plus diisopropylethylamine (2.6g, 20 mmol). The reaction mixture was then heated under reflux for 6 hours, concentrated to low volume, diluted with 1M aq NaOH (100ml) and extracted with methylene chloride/ methanol (20/1; 2 x 100ml). The extracts were combined, dried (MgSO₄), and the solvent removed by distillation in vacuo to give an orange oil, which was absorbed onto silica and eluted with CMA 200. The product was isolated as a yellow glass, and was further purified by recrystallisation from ethyl acetate, giving UCL1822 (200mg) as a white powder.

Purity >98% (nmr, tlc)

1H nmr (CDCl₃): 8.50 (m, 3H, CH(Ar)), 8.00 (m, 3H, CH(Ar)), 7.70 (m, 3H, CH(Ar)), 7.55 (m, 3H, CH(Ar)), 7.35 (m, 3H, CH(Ar)), 7.05 (t, 1H, CH(Ar')), 6.65 (d, 2H, CH(Ar')), 6.30 (m, 3H, CH(Ar)), 6.20 (t, NH), 5.25 (t, 2H, NH), 4.25 (t, 2H, CH₂O), 4.15 (t, 4H, CH₂O), 3.45 (m, 6H, CH₂N), 2..15 (m, 6H, CH₂).
Tlc: CMA 200/SiO₂: UCL1822 R.f 0.6 (byproducts R.f 0.65, 0.70)
M.S. (ES⁺): 679 (M⁺ + 1)
CHN: calculated for C₄₂H₄₂N₆O₃·1.25 H₂O + 0.2 EtOAc: 71.50, 6.46, 11.69;
found: 71.49, 6.24, 11.68.
I.R (nujol): 3250 (m, N-H, H₂O), 1690 (C=O, EtOAc), 1580 (s, C-H).
UCL1836, 1,2,3-tri-(3-(4-(N-ethyl)-quinoliumyl)-aminopropoxy) benzene

UCL1822 (0.3g) was dissolved in ethyl iodide (25 ml) and the suspension heated to reflux. DMF (2 ml) was added to the resulting oily suspension to give a clear solution. The reaction mixture was then heated under reflux for a further 10 hours, adding more DMF, in 1 ml portions (total 10ml), to redissolve the liberated oil, throughout the reflux period. After cooling to room temperature, water (100ml) was added to precipitate a gum. The supernatant liquor was decanted away, after which more water (100ml) was added and this wash also removed. The remaining residue was dissolved in hot methanol (80 ml), filtered to remove insoluble material and the filtrate cooled to precipitate a red gum, which was further purified by recrystallisation from ethanol to provide UCL1836.3I⁻ as a yellow powder (80mg).

Purity: ca 95% (nmr)

¹H nmr (d₅-dmso): 9.15 (m, 3H, CH(Ar)), 8.65 (m, 3H, CH(Ar)), 8.50 (m, 3H, CH(Ar)), 8.10 (m, 3H, CH(Ar)), 7.70 (m, 3H, CH(Ar)), 7.05 t, 1H, CH(Ar')), 6.90 (m, 3H, CH(Ar')), 6.70 (d, 2H, CH₂N⁺), 6.60 (q, 6H, CH₂N⁺), 4.60 (q, 6H, CH₂O), 3.75 (q, 6H, CH₂N), 2.10 (qu, 6H, CH₂), 1.40 (t, 9H, CH₃).

M.S (ES⁺): 763 (M⁺ - 2)

CHN: calculated for C₄₈H₅₇N₆O₃.3I⁻. 3.9H₂O: 47.37, 5.37, 6.91; found: 47.12, 5.02, 6.86.

I.R. (nujol): 3400 (m), 3250 (m), 1650 (s), 1575 (s)
UCL1821, N-(3-(4-(aminoquinolinvl))-propyl-N-(5"-(4-(aminoquinolinivl))-
pentyl-3,5-di-(3-(4-(aminoquinolinivl))-propoxv) aniline

**t-Bocdiaminopentane**

A solution of t-butyldicarbonate (60g, 275 mmol) in methanol (200ml) was added to 1,5-diaminopentane (100g, 1.0 mol) in methanol (100ml) over 30 minutes, keeping the temperature below 30°C. The resulting suspension was stirred at room temperature for 15 minutes, before removing half of the solvent by distillation and diluting with water (1300ml) plus 40% w/w aq NaOH (60ml, 1.5 mol). After stirring for 16 hours, the precipitated diprotected diamine was filtered off and the filtrate extracted with t-butyl methyl ether (5 x 600ml). The extracts were combined, diluted with toluene (1l) and the solvent removed by distillation in vacuo to constant weight.

Yield 64% (35g), red oil.

Purity: 98% (nmr, tlc)

**N-(3-(t-butoxycarbonyl)-aminopropyl))-N'-(5-(t-butoxycarbonyl))-diaminopropane (59)**

A solution of t-bocbromopropylamine (35.6g, 0.15 mol) and t-boc-diamino pentane (30.3g, 0.15 mol) in DMF (200ml) was stirred with anhydrous potassium carbonate (27g, 0.45 mol) at room temperature for 16 hours. The reaction mixture was then diluted with water (800ml) and extracted with t-butyl methyl ether (2 x 1l). The extracts were combined and washed with water (600 ml), before stirring with water (600 ml) and adding conc HCl in portions until the product was extracted into the aqueous phase. The aqueous phase was then separated off, basified with aq NaOH, and extracted with t-butyl methyl ether.
(2 x 600ml). These extracts were combined, washed with water (400ml),
dried (MgSO₄), and concentrated in vacuo to give the product as a red oil,
which solidified on cooling.

Yield 39% (20g), orange powder
Purity: >98% (nmr)
mp: 33-35°C
M.S (Cl⁺, NH₃): 346 (M⁺ + 1)
Tlc (CMA 200/silica): (59) at R.f 0.6, tertiary amine R.f 0.8.

N-(3-(t-butoxycarbonyl) aminopropyl)-N-(5-(t-butoxycarbonyl) aminopentyl)
3,5-dihydroxyaniline(60)

Phloroglucinol (4.4g, 35 mmol) and (59) (12.0g, 35 mmol) were dissolved in
THF (30 ml). The solution was diluted with toluene (50 ml) to give a thin
suspension, which was heated under reflux for 2 hours, the solid dissolving to
give a dark brown solution. Solvent was then removed by distillation, before
rediluting with THF (30 ml) and toluene (100ml). The reaction mixture was
heated under reflux for a further 2 hours, then concentrated to low volume.
The resulting product was purified by column chromatography on silica gel
with ethyl acetate.

Yield 61% (10g), orange-brown gum
Purity: 95% (nmr, tlc)
¹H nmr (d₆-dmso): 8.80 (s, 2H, OH), 6.85 (t, 1H, NH.CO), 6.68 (t, 1H,
NH.CO), 5.65 (s, 2H, CH(Ar)), 5.55 (s, 1H, CH(Ar)), 3.15 (m, 4H, CH₂N), 2.90
(m,4H, CH₂N), 1.65 (m, 2H, CH₂), 1.45 (m, 4H, CH₂), 1.40 (s, 18H, ¹Bu)
M.S (El⁺): 468 (M⁺ + 1), impurity at 700.
I.R (nujol): 3380 (s, O-H, N-H), 1680 (s, C=O), 1620 (s, C=N), 1580 (m, C-H), 1520 (m, C-H).

N-(3-(t-butoxycarbonyl)-aminopropyl)-N-(5-(t-butoxycarbonyl)-aminopentyl)-3,5-di-(3-(t-butoxycarbonyl)-aminopropoxy)-aniline (61)

A solution of potassium t-butoxide (4.9g, 44 mmol) in DMF (50ml) was added over 5 minutes to (60) (9.4g, 20 mmol) in DMF (100ml), the temperature rising from 18 ° to 32°C. The resulting suspension was stirred for 15 minutes, before adding boc bromopropylamine (10.0g, 42 mmol) in one portion, the temperature rising to 62°C.

The reaction mixture was then cooled to room temperature, diluted with water (400 ml), and extracted with ethyl acetate (3 x 300ml). Each extract was washed sequentially with 1M aq NaOH (200ml) and water (200 ml). The extracts were then combined, dried (MgSO₄), and concentrated in vacuo to give (61) as a dark red oil, which was purified by column chromatography on silica with diethyl ether.

Yield 45% (7.0g), orange-brown oil.
Purity: ca 80% (nmr, contains t-bocNH(CH₂)₃OH-decomp of bromide starting material)

¹H nmr (CDCl₃): 6.05 (s, 1H, CH(Ar)), 5.85 (s, 2H, CH(Ar)), 4.95 (br s, 1H, NH.CO), 4.85 (br s, 2H, NH.CO), 4.75 (br s, 1H, NH.CO), 4.00 (m, 4H, CH₂O), 3.35 (m, 8H, CH₂N), 3.15 (m, 4H, CH₂N), 1.95 (m, 4H, CH₂), 1.75 (m, 2H, CH₂), 1.65 (m, 4H, CH₂), 1.40 (s, 38H, 4 ¹Bu + CH₂).

M.S (ES⁺): 782.5 (M⁺ + 1)
I.R. (film): 3380 (br, m, N-H), 1700 (br, s, C=O), 1620 (m, C=N), 1520 (s, C-H).
N-(3-(4-aminquinolinyl)-propyl)-N-(5-(4-aminquinolinyl)-pentyl-3,5-di-(3-(4-
aminquinolinyl)-propoxy)-aniline, UCL1821

(61) (5.9g, 7.5 mmol) was dissolved in trifluoroacetic acid (30ml), producing a
vigorous effervescence. After 1 hour at room temperature, excess acid was
removed by distillation in vacuo, and the resulting oil diluted with methanol
(100 ml). Sodium methoxide in methanol was then added until pH 10, before
replacing the solvent with 1-pentanol by distillation at constant volume.
4-Chloroquinoline (7.3g, 45 mmol) and diisopropylethylamine (6.8g, 53 mmol)
were then added and the reaction mixture was heated under reflux for 6
hours, then concentrated to low volume, diluted with water (150 ml), and
extracted with methylene chloride/ methanol (20/1; 2 x 100ml). The extracts
were combined, concentrated, and the resulting brown oil absorbed onto
silica and eluted with CMA 100. The resulting product was ca 70% pure, and
was further purified by recrystallisation twice from ethyl acetate, filtering the
hot supernatant each time from insoluble material, to give UCL1821 as a
fawn foam.

Purity: 90% (tlc; nmr, contains 0.5 m/m ethyl acetate)

$^1$H nmr (CDCl$_3$): 8.55 ((m, 4H, CH(Ar)), 7.95 (m, 4H, CH(Ar)), 7.75 (m, 4H,
CH(Ar)), 7.60 (m, 4H, CH(Ar)), 7.35 (m, 4H, CH(Ar)), 6.40 (m, 4H, CH(Ar)),
5.90 (s, 3H, CH(Ar')), 3.95 (t, 4H, CH$_2$O), 3.5-3.2 (m, 13H, CH$_2$N), 2.10
(m, 6H, CH$_2$), 1.65 (m, 4H, CH$_2$), 1.35 (m, 2H, CH$_2$).

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M.S (El⁺): 890 (M⁺ + 1)
CHN: calculated for C₅₆H₅₉N₉O₂⋅3.5H₂O + 0.5 m/m EtOAc: 68.98, 7.07, 12.63; found 68.99, 6.77, 2.50.
Tlc (CMA 200/SiO₂): UCL 1821 R.f 0.6, triaminoquinolinyl R.f 0.4, impurity R.f 0.65.
I.R. (nujol): 3250 (w, N-H, H₂O), 1690 (w, C=O, EtOAc), 1580 (s, C-H).
UCL1823, N-(3-(4-quinoliny1) aminopropyl)-N-(5-(4-quinoliny1) aminopentyl) 3,5-di-(3-(4-quinoliny1) aminopropoxy) benzylamine

N-(3-(t-butoxycarbonyl) aminopropyl)-N-(5-(t-butoxycarbonyl) aminopentyl) 3,5-di-(3-(t-butoxycarbonyl) aminopropoxy) benzylamine (62)

A solution of (63) (3.3g, 7.0 mmol) and (59) (2.7g, 7.7 mmol) in DMF (30ml) was heated at 80°C with anhydrous K₂CO₃ (1.3g, 9.5 mmol) for 3 hours. The reaction mixture was then cooled, diluted with water (100ml) and extracted with ethyl acetate (2 x 100ml). The extracts were combined and washed with water (50 ml), before being dried (MgSO₄) and concentrated to give an oil, which was purified by column chromatography on silica gel with ethyl acetate to provide (62).

Yield 100% (5.6g, pale yellow oil)
Purity: 90% (nmr, ca 8% DMF)

¹H nmr (CDCl₃): 6.50 (s, 2H, CH(Ar)), 6.30 (s, 1H, CH(Ar)), 5.15 (br s, 1H, NH.CO), 5.00 (br s, 1H, NH.CO), 4.75, 4.00 (t, 4H, CH₂), 3.45 (s, 4H, CH₂Ar), 3.35 (q, 4H, CH₂N), 3.15 (m, 2H, CH₂N), 3.10 (m, 2H, CH₂N), 2.40 (m, 4H, CH₂N), 1.95 (qu, 4H, CH₂), 1.60 (qu, 2H, CH₂), 1.45 (m, 2H, CH₂), 1.40 (s, 36H, tBu)

M.S (ES⁺): 796 (M⁺ + 1)
N-(3-(4-Quinolinyl)-aminopropyl)-N-(5-(4-quinolinyl)-aminopentyl)-3,5-di-(3-(4-quinolinyl)-aminopropoxy)-benzylamine, UCL1823

(62) (4.8g, 6.0 mmol) was dissolved in trifluoroacetic acid (40ml), producing a vigorous effervescence. After 1 hour at room temperature, the excess acid was removed by distillation *in vacuo* and methanol (50ml) added, followed by sodium methoxide in methanol until pH 10. The solvent was then replaced with 1-pentanol by distillation at constant volume.

4-Chloroquinoline (4.9g, 30 mmol) and diisopropylethylamine (4.5g, 35 mmol) were then added and the reaction mixture refluxed for 8 hours, before being concentrated to low volume, diluted with 1M aq NaOH (100ml) and extracted with methylene chloride (2 x 100ml). The extracts were combined, dried (MgSO₄), and the solvent removed by distillation *in vacuo*.

The residue was purified by column chromatography on silica with CMA 200, giving UCL1823 of 70% purity, which was further purified by recrystallisation from ethyl acetate twice, filtering off insoluble material while hot, giving the product as a cream foam (100mg).
Purity: 95% (nmr, tlc)

CHN: calculated for C$_5$H$_6$N$_9$O$_2$. 1.75 H$_2$O + 0.66 m/m EtOAc: 72.08, 7.08, 12.68; found 71.98, 6.91, 12.69.

$^1$H nmr (CDCl$_3$): 8.50 (m, 4H, CH(Ar)), 7.95 (m, 4H, CH(Ar)), 7.75 (m, 4H, CH(Ar)), 7.55 (m, 4H, CH(Ar)), 7.35 (m, 4H, CH(Ar)), 6.85 (t, 1H, NH), 6.45 (s, 2H, CH(Ar')), 6.35 (m, 4H, CH(Ar')), 6.25 (t, 1H, CH(Ar')), 5.65 (t, 2H, NH), 5.10 (t, 1H, NH), 3.80 (t, 4H, CH$_2$O), 3.55 (s, 2H, ArCH$_2$N), 3.35 (m, 4H, CH$_2$N), 3.25 (m, 4H, CH$_2$N), 2.60 (m, 4H, CH$_2$N), 2.00 (m, 4H, CH$_2$), 1.85 (m, 2H, CH$_2$), 1.65 (m, 4H, CH$_2$), 1.45 (m, 2H, CH$_2$)

M.S (ES$^+$): 904.7 (M$^+$ + 1)

Tlc (CMA 200/silica): UCL1823 at R.f 0.5, impurity at R.f 0.55.

byproducts R.f 0.4, 0.45, 0.60-0.65)

I.R (nujol): 3250 (w, N-H), 1690 (C=O, EtOAc), 1580, 1530 (s, C-H)
REFERENCES


29. Latorre R. & Miller C., Conduction and selectivity in $K^+$ channels. 

30. Bezanilla F. & Armstrong C.M., Negative conductance caused by entry of 
   sodium and cesium ions into the $K^+$ channels of squid axons. \textit{J. Gen. 
   Physiol.}, (1972), 60, 588-608.

31. Hille B, Potassium channels in myelinated nerve-selectivity to small 

32. Yellen G., Permeation in potassium channels- Implications for channel 

33. MacKinnon R. & Miller C., Mutant potassium channels with altered 
   binding of charybdotoxin, a pore-blocking peptide inhibitor. \textit{Science}, 

34. Anderson O., MacKinnon R., Smith O. & Miller C., Charybdotoxin block of 
   single $Ca^{2+}$ activated $K^+$ channels- effects of channel gating, voltage and 

35. MacKinnon R., Latorre R. & Miller C., Role of surface electrostatics in the 
   operation of a high-conductance $Ca^{2+}$ activated $K^+$ channel. 

36. Swenson R., Inactivation of potassium current in squid axon by a variety 

37. MacKinnon R., Heginbotham T. & Abramson T., Mapping the receptor site 
   for charybdotoxin, a pore blocking potassium channel inhibitor. \textit{Neuron}, 
   (1990), 5, 767-771.


47. Moczydlowski E. & Latorre R., Gating kinetics of Ca\textsuperscript{2+} activated K\textsuperscript{+} channels from rat muscle incorporated into planar bilayers. *J. Gen. Physiol.*, (1983), 82, 511-542.


49. Golowasch J., Kirkwood A. & Miller C., Allosteric effects of Mg\textsuperscript{2+} on the gating of Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels from mammalian skeletal muscle. *J. Exp. Biol.*, (1986), 124, 5-13.


215. Greene, Protective groups in organic synthesis.


237. Kauffler K., Concerning the tautomers of phloroglucinol. *Montash*, (1900), 21, 993-1006


240. Essenberger E. & Ness R., N-Perisubstitution-3.5-diaminophenol and 


242. McKillop A., Howarth B.D. & Kobylecki P.J., A simple and inexpensive 
   procedure for the preparation of phloroglucinol and phloroglucinol 

243. Fray G.I., Reaction of phloroglucinol with sodium borohydride. 

244. Bovet M.D., Depierre F. & deLestrange Y., U.S. Patent No 2,544,076, 
   (1951).


246. Dunn P.M., Dequalinium, a selective blocker of the slow AHP in rat 
   194.

247. Dunn P.M., Ganglion blocking activity of dequalinium in frog and rat 


249. Page M.L. & Jencks W.P., Entropic contributions to the rate 
   accelerations in enzymic and intramolecular reactions and the chelate 
Lal A.R., Nicholls I.A., Salter C.J. & Mitchell R.C., Towards the semi-
quantitative estimation of binding constants. Guides for peptide-peptide 

251. Williams D.H., Searle M.S., Westwell M.S., Gerhard U. & Holroyd S.E., 
Towards a semi-quantitative description of bimolecular association 
involving weak interactions in aqueous solution. *Phil. Trans. R. Soc. Lond. 

252. Williams D.H., Searle M.S., Mackay J.P., Gerhard U. & Maplestone R.A., 
Towards an estimation of binding constants in aqueous solution: Studies 

253. Searle M.S. & Williams D.H., The cost of conformational disorder: 
114, 10690-10697.

254. Searle M.S., Williams D.H. & Gerhard U., Partitioning of free energy 
contributions in the estimation of binding constants: Residual motions and 

255. Searle M.S. & Williams D.H., On the stability of nucleic acid structure in 
solution: enthalpy-entropy compensations, internal rotations and 

256. Cox J.P.L., Nicholls I.A. & Williams D.H., Molecular recognition in 
aqueous solution: An estimate of the intrinsic binding energy of an amide-
343.

bond in aqueous and non-polar solvents. *J. Am. Chem. Soc.*, (1992), 114, 
338-343.
