Chemoenzymatic cascades towards methylated tetrahydroprotoberberine and protoberberine alkaloids

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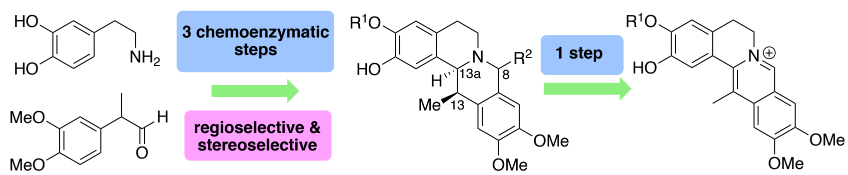
Supporting Information Placeholder



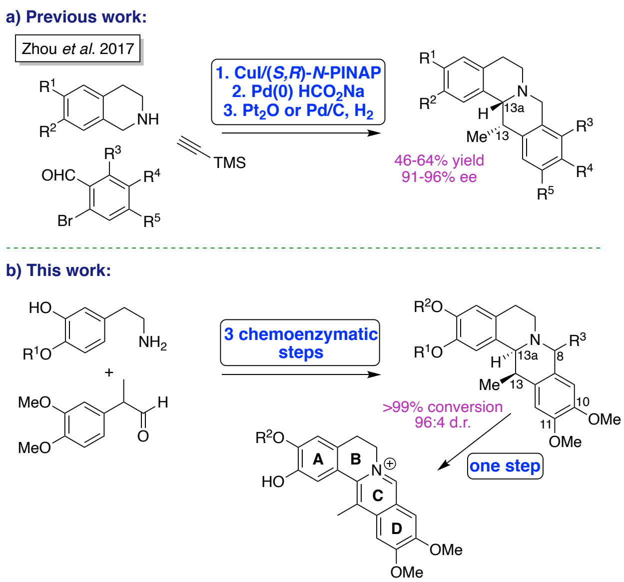
ABSTRACT: Tetrahydroprotoberberine and protoberberine alkaloids are a group of biologically active natural products with complex molecular scaffolds. Isolation from plants is challenging and stereoselective synthetic routes, particularly of methylated compounds are limited, reducing the potential use of these compounds. In this work, we describe chemoenzymatic cascades towards various 13-methyl-tetrahydroprotoberberbine scaffolds using a stereoselective Pictet-Spenglerase, regioselective catechol *O*-methyltransferases and selective chemical Pictet-Spengler reactions. All reactions could be performed sequentially, without the workup or purification of any synthetic intermediates. Moreover, the naturally occurring alkaloids have the (+)-configuration and importantly here, a strategy to the (-)-isomers was developed. A methyl group at C-8 was also introduced with some stereocontrol, influenced by the stereochemistry at C-13. Furthermore, a single step reaction was found to convert tetrahydroprotoberberine alkaloids into the analogous protoberberine scaffold, avoiding the use of harsh oxidizing conditions or a selective oxidase. This work provides facile, selective routes towards novel analogues of bioactive alkaloids.

Natural products and related analogues are a major source of therapeutics, making up 42% of FDA-approved drugs from 1981-2019.[1] Many have a diverse range of molecular scaffolds, often with multiple, defined stereocenters. However, accessing natural products is challenging and different approaches are used with varying successes. For example, isolation from plant sources is hindered by low production and issues with separation from other structurally similar metabolites.[2] Total synthesis is commercially viable in some cases, although can be unattainable or not cost effective for more complex products.[3] *In vivo* fermentation processes involve significant bioengineering efforts and achieving high enantiopurities can be challenging.[4,5] High regio- and stereoselective control under benign conditions, with minimal side-reactions can be achieved however by mimicking the biosynthesis *in vitro*, using recombinantly-expressed enzymes.[6] Nevertheless, this approach can be limited by challenges such as enzyme stability issues. Cascade processes, using a combination of traditional synthetic methods and biocatalytic enzymes, can alternatively lead to the generation of complex products, in fewer steps with easier purification processes than solely *in vivo* or organic synthetic routes.[7–9]

Alkaloids are important nitrogen-containing natural products, many of which are biologically active.[10] Protoberberine (PB) and tetrahydroprotoberberine (THPB) alkaloids isolated from plants of the *Corydalis* genus are unique amongst other isolated alkaloids of this type as they possess a methyl group at C-13.[11,12] Many 13-Me-PB and 13-Me-THPB alkaloids have been shown to have promising bioactivities,[13] including as inhibitors of reverse transcriptase activity[14] and enterovirus 71.[15] Others have been shown to be dopamine D-1 receptor agonists and to have anti-hepatitis B activities.[16,17]

Synthetic routes towards racemic 13-Me-PB and 13-Me-THPB alkaloids have been reported,[18–20] and between them only a few are stereoselective. The asymmetric synthesis of natural 13-Me-THPBs has been described by Zhou *et al*. (Scheme 1a).[21,22] After the four-step synthesis of an enantiopure PINAP ligand, the (13*R*,13a*S*)-13-Me-THPBs were synthesized in three-steps in yields of 47-65% and high selectivities (91-96% ee). Previous work in our group has shown that the Pictet-Spenglerase[23] norcoclaurine synthase (NCS) can accept α-methyl-substituted aldehydes as substrates.[24] Notably, an active site variant of *Thalictrum flavum* NCS (*Tf*NCS) M97V gave (1*S*,1'*R*)-tetrahydroisoquinolines (THIQs) in high yields (up to 96%) and diastereomeric ratios (d.r. = 98:2) in a single-step. Herein, the application of this methodology in combination with other biocatalytic or chemical steps is described to generate a range of 13-Me-THPBs isolated from *Corydalis* plants. Importantly, the products generated have opposing stereochemistry to those isolated from plants, thus providing routes to useful natural product analogues (Scheme 1b).

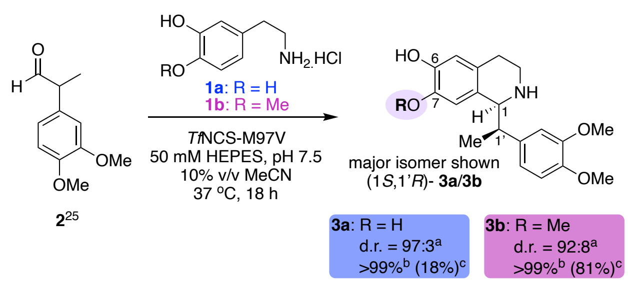
Scheme 1: Routes towards 13-Me-THPB alkaloids (a) Previous route using a chiral catalyst;[19] (b) This work using a chemoenzymatic approach.



To generate the desired THIQ scaffold (Scheme 2), NCS-mediated reactions between dopamine **1a** and the aldehyde **2**[24,25] were investigated. Variants of *Tf*NCS, M97V, L76V and M97F, that previously gave improved selectivities compared with the wild type for the acceptance of α-methyl phenylacetaldehyde, were explored (SI Figure S6): when this racemic aldehyde had been used in NCS catalyzed reactions, the *R*-enantiomer was accepted preferentially over the *S*-isomer.[24] This had been determined by performing reactions with single enantiomer α-methyl aldehydes. Notably, it resulted in the generation of two defined chiral centers in the resulting THIQs, with the major product assigned as (1*S*,1'*R*).[24] Aldehyde **2** was prepared in three steps[25] and after the final step taken through without purification due to its oxidative sensitivity. *Tf*NCS-M97V gave the THIQ products with **2** in quantitative yield by HPLC analysis, with the major diastereomer generated assigned as (1*S*,1'*R*)-**3a** (d.r. = 96:4).[24] The minor diastereomer observed was (1*S*,1'*S*)-**3a** as no racemic NCS background reaction was observed and NCS has been shown to generate the *S*-stereochemistry at C-1.[26,27] Compound **3a** was isolated by preparative HPLC (with the reaction performed on a 0.10 mmol scale, with 20 mg **1a**) for characterization purposes but otherwise was taken through directly for the cascade process to telescope the synthetic approach. While these initial experiments used 2 equiv. of **2**, similar results and slightly higher stereoselectivities were observed when using just one equiv. (>99% conversion, 18% isolated, d.r. = 97:3) as **2** can racemize *in situ*.[28] The challenges of THIQ product isolation have previously been reported[29] which can lower isolated yields, highlighting the advantages of directly taking material through to the next step using cascaded reaction sequences which is described below. This reaction was also amenable to scale up with conversions and stereoselectivities retained on a 50 mL, 10 mM scale.

The *para*-hydroxyl group of dopamine **1** is non-essential for a productive NCS reaction,[30] so the 7-OMe THIQ **3b** was also generated using a *Tf*NCS-M97V reaction between **2** and the 7-OMe dopamine analogue, **1b**[26] on a 0.10 mmol scale (with 20 mg of **1b**). This reduced the oxidative sensitivity of the THIQ scaffold. The product, (1*S*,1'*R*)-**3b** (Scheme 2) was generated in high yield (>99% HPLC conversion, 81% isolated) and in a reasonable d.r. (92:8).

Scheme 2: Stereoselective synthesis of the THIQ scaffold using norcoclaurine synthase

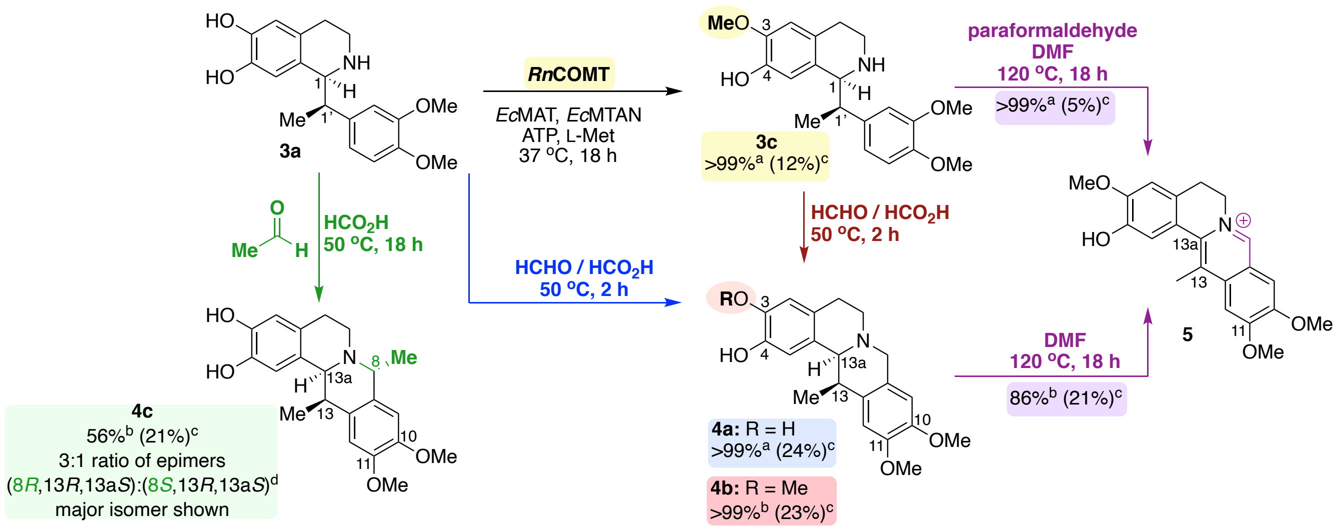


[a]d.r.s correspond to the ratio of the two diastereomers formed, (1*S*,1'*R*):(1*S*,1'*S*) and were determined by ^{1}H NMR spectroscopy and HPLC (method 2). [b]Conversions were determined by HPLC analysis, based upon calibration curves of the purified products (See SI). [c]Isolated yields after preparative HPLC purification.

To generate the analogous C6-OMe-THIQ (**3c**), use of regioselective catechol *O*-methyltransferases (*O*-MTs) were explored. Previous work has shown that two promising *O*-MTs, *Rn*COMT (isolated from *Rattus norvegicus*) and *Mx*SafC (isolated from *Myxococcus xanthus*) are capable of regioselectively methylating the C6-OH or C7-OH of various 1-benzylic and 1-aryl-THIQs.[26,31–34] Both are *S*-adenosyl-L-methionine (SAM) dependent enzymes and although SAM is expensive and has issues of instability, recent developments in SAM supply systems have meant that such biocatalytic methylations are viable on preparative scales.[35,36] Here, we used a previously described *in situ* SAM generation system which utilizes ATP, L-methionine, and a methionine adenosyl transferase from *Escherichia coli* (*Ec*MAT E.C.2.5.1.6). After the methylation reaction, *S*-adenosyl-L-homocysteine (SAH) is generated which can inhibit the *O*-MT, so another enzyme methylthioadenosine/SAH nucleosidase (*Ec*MTAN E.C.3.2.2.9) is used to breakdown SAH.[35,37] The use of other SAM generation systems was not attempted, but other in situ SAM supply methods could be applied.[38,39]

The THIQ substrate **3a** formed (using *Tf*NCS-M97V), was directly lyophilized and since the *O*-MT enzymes are known to be highly selective towards the catechol moiety and no **1a** remained after the NCS reaction, there was no need for a purification step. Using previously reported conditions,[26,31] *Rn*COMT was used as clarified cell lysate and *Mx*SafC was used as a purified enzyme. Both *O*-MTs interestingly exhibited high regioselectivity towards the 6-OH, generating the product **3c** (Scheme 3) with complete conversions by HPLC analysis and no observable methylation on the 7-OH.

Scheme 3: Regioselective methylations, chemical Pictet-Spengler reactions and generation of the 13-Me-PB scaffold[a-d]



[a]Conversions were determined by HPLC analysis against product standards. [b]Conversions were determined by HPLC analysis, based upon starting material depletion (see calibration curves SI, Figures S7-9). [c]Isolated yields after preparative HPLC purification. [d]Epimeric ratio determined based upon previous studies in which the stereochemistries at C-13 and C-13a were determined, and NOESY ^{1}H NMR analysis of the isolated product (see SI). Isolated yields were lower than HPLC yields/conversions due to issues of oxidative sensitivity of the compounds generated and minor impurities having similar retention times to the desired products by preparative HPLC. To obtain the compounds in high purity for characterization purposes, isolated yields are lower than those typically reported.

Reactions were performed on a 0.10 mmol scale (20 mg **1a**). Since both enzymes exhibited similar reactivities, yet *Rn*COMT could be used as clarified cell lysate, *Rn*COMT was used in subsequent reactions. Regioselectivities were established using Nuclear Overhauser Effect Spectroscopy (NOESY). This result is consistent with the reported regioselectivity of these enzymes towards THIQs with a phenyl or cyclohexane ring at C-1.[26,31] However, with substrates such as dopamine, *Rn*COMT methylates the *meta*-OH and *Mx*SafC the *para*-OH.[36] Such variations in the regioselectivity highlights that bulkier groups attached to C-1 can hinder methylation at the C7-OH position in THIQs for steric reasons. Reactions were performed on a preparative scale (20 mL, 5 mM) using *Rn*COMT to give (1*S*,1'*R*)-**3c** in quantitative conversion by HPLC against product standards and a 12% isolated yield.

Biosynthetic routes to give the THPB scaffold involve the berberine bridge enzyme (BBE).[40] The enantioselective production of various (*S*)-THPBs from racemic, *N*-methylated, 1-benzylic THIQs using recombinant BBE has been achieved in high conversions (98%) and selectivities (>97% ee).[41] Although a highly productive route, there is the requirement to generate the starting material and *in situ* deracemization of the (*R*)-THIQ is needed, involving a selective monoamine oxidase and borane reduction. Here, to generate the tetracyclic THPB scaffold, chemical Pictet-Spengler (PS) reactions were explored (Scheme 3a).[42] Previous work has demonstrated that a phosphate-mediated PS reaction with formaldehyde was capable of converting 1-benzylic-THIQs into THPBs in high yields and good regioselectivities (7:1 10,11-OMe:9,10-OMe THPB).[43] Both reported reaction conditions were attempted here with **3c**, but no conversion was observed, presumably because the aromatic dimethoxy groups reduce the reactivity of the substrates compared to phenolic groups. Instead, formic acid catalyzed PS reactions were explored as described by Qian *et al*.[44] A telescoped synthesis was then performed, with crude, lyophilized **3a** or **3c** used. Complete conversion, by monitoring the consumption of starting material by HPLC to the products (13*S*,13a*R*)-**4a** and (13*S*,13a*R*)-**4b**, was observed with 24% and 23% isolated yields, over two or three steps, respectively. Reactions were performed on a 5 mL, 10 mM scale. Complete regioselectivity was observed on the D-ring (see Scheme 1), with solely 10,11-dimethoxy-substituted products generated. Nuclear Overhauser Spectroscopy (NOESY) analysis of the products formed (**4a** and **4b**) also provided further confirmation of the stereochemistries at C-13 and C-13a i.e., *a syn*-relationship between the two protons at these positions.

8-Me-THPB and 8-Me-PB alkaloids have been isolated from *C. ochotensis*, and one 8-Me-PB has been shown to act as an anti-leukemic agent.[45] Stereoselective routes to 8-Me THPBs are limited[46] and routes to analogues are useful for drug discovery purposes. A chemical PS reaction in formic acid with acetaldehyde was therefore explored, with the aim of generating functionalized 8-THPBs. Crude, lyophilized **3c** was used again in a telescoped synthesis (0.050 mmol scale). Product **4c** was formed in a 56% conversion yield (starting material consumption by HPLC) and 21% isolated yield over two steps (Scheme 3). Two epimers at C-8 of the product were observed in a 3:1 ratio by NMR spectroscopy. The stereochemistries at C-13 and C-13a were retained and the major epimer was assigned by NOESY analysis as (8*S*,13*R*,13a*S*)-**4c**. Modelling of both epimers was used to rationalize these results. In both cases, the C-ring is held in a half-chair conformation and for the minor isomer, the two methyl groups are *cis* to each other in pseudo-axial orientations giving an unfavorable steric interaction (SI Figure S10). Therefore, the stereochemistry at C-13 can lead to stereocontrol at C-8 in the cyclisation to give **4c**.

The regioselectivity of D-ring formation has been reported to be solvent-mediated,[47,48] with the other regioisomer of product preferentially formed in apolar, aprotic solvents such as toluene or dichloroethane. As **3c** has poor solubilities in such solvents, DMF was used with reactions performed at 120 ºC on a 0.10 mmol scale (20 mg **1a**). Complete consumption of **3c** was observed after 18 h and interestingly **5** was formed via a cyclisation and oxidation in 5% isolated (yield over three steps from **1a** and **2** with no purification of the intermediates). The oxidation is presumably mediated by trace oxidants in the crude material taken through and may prove useful in generating the PB scaffold from the analogous THPB as the oxidation of the C-ring must occur after the PS reaction. It would seem that the presence of activating methoxy groups on the D-ring and the use of high temperatures also helps to drive the reaction. The unpurified THPB **4b** (formed from **3c**) was also heated in DMF at 120 ºC for 18 h with 86% of starting material converted by HPLC and this gave **5** as the only product in 21% isolated yield. This is a useful route to PBs from the analogous THPB, avoiding the addition of oxidase enzymes[49] or oxidants (I_2/EtOH, reflux).[50] Since the stereochemistry is lost during this oxidation, the racemic THIQ starting material could also be formed by a phosphate-mediated PS reaction which has been shown to be regioselective with α-methyl substituted aldehydes.[24] The PB scaffold is synthetically useful as the iminium ion is susceptible to nucleophilic attack, leading to C-8 functionalization.[51]

In summary, a range of novel (-)-13-Me-THPB alkaloids have been generated with opposing stereochemistries at C-13 and C-13a to the naturally occurring 13-Me-THPB alkaloids from *Corydalis* plants. Single regio- and diastereomer 13-Me-THPBs were formed through two subsequent PS reactions; one enzymatic reaction to generate a THIQ scaffold with two defined stereocenters followed by a chemical PS reaction to generate the tetracyclic scaffold. Regioselective methylation of a single hydroxyl group of the THIQ was also achieved using *O*-MTs. Heating the 13-Me-THPBs in DMF provided a facile route to the analogous 13-Me-PB. All enzymatic reactions were high yielding and there was no requirement for the isolation of material at each reaction step. High stereoselectivity was also obtained without the need for chiral ligands or precursors, and toxic reagents.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications Web site. This includes experimental procedures and $^{1}H/^{13}C$ NMR spectra for the compounds synthesized. The Supporting Information is available free of charge at xxxxx.

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Author Contributions

The manuscript was written through contributions of all authors. R.R. and J.B. performed chemical syntheses. R.R. performed synthesis and characterization, enzyme expression and purification, and the chemoenzymatic cascades. F.S. provided input on enzymatic reactions. The project was supervised by H.C.H, N.H.K. and J.M.W. The manuscript was written by R.R. and H.C.H. All authors have given approval to the final manuscript.

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