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Biocatalytic production of tetrahydroisoquinolines

Bettina M. Ruff [a,b], S. Bräse [b], Sarah E. O'Connor [c,d,*]

[a] Massachusetts Institute of Technology, Department of Chemistry, 77 Massachusetts Avenue, Cambridge, MA 02139, USA
[b] Karlsruhe Institute of Technology, Institute of Organic Chemistry, Fritz-Haber-Weg 6, 76137 Karlsruhe, Germany
[c] The John Innes Centre, Department of Biological Chemistry, Norwich NR4 7UH, UK
[d] The University of East Anglia, Norwich NR4 7TJ, UK

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ABSTRACT

The promiscuity of the enzyme norcoclaurine synthase is described. This biocatalyst yielded a diverse array of substituted tetrahydroisoquinolines by cyclizing dopamine with various acetaldehydes in a Pictet–Spengler reaction. This enzymatic reaction may provide a biocatalytic route to a range of tetrahydroisoquinoline alkaloids.

The tetrahydroisoquinoline moiety is found in many natural products and synthetic pharmaceuticals. In particular, this chiral N-heterocyclic scaffold is an integral part of all tetrahydroisoquinoline alkaloid natural products. The benzylisoquinoline biosynthetic pathways in plants lead to a large group of alkaloids including the well-known analgesics morphine and codeine, which are isolated from the opium poppy. Additional examples of industrially important tetrahydroisoquinolines include noscapine (**1**, Fig. 1), which has been used as an antitussive since the 19th century, and the anticancer properties of this compound were recently reported.[1] The (*S*)-configured antimuscarinic drug Michellamine B (**2**),[2] isolated from Ancistrocladus plants, is one of the many tetrahydroisoquinolines with anti-human immunodeficiency virus (HIV) activity.[3] The synthetic compound Solifenacin (**3**) has a urinary antispasmodic effect.[4]

Norcoclaurine synthase (NCS)—one of the three known Pictet–Spenglerases[5]—catalyzes the C–C bond forming reaction between 4-hydroxyphenylacetaldehyde (4-HPAA, **4**) and dopamine (**5**) to afford (*S*)-norcoclaurine (**6**) (Table 1), a compound with antiallergic[6] and β-adrenergic[7] properties, and the biosynthetic precursor to all known naturally occurring tetrahydroisoquinoline alkaloids. Uncatalyzed Pictet–Spengler reactions with phenylethylamines and aldehydes yield racemic mixtures of tetrahydroisoquinolines.[8] Several catalysts have been developed for Pictet–Spengler reactions utilizing substituted tryptamines to yield tetrahydro-β-carbolines.[9]

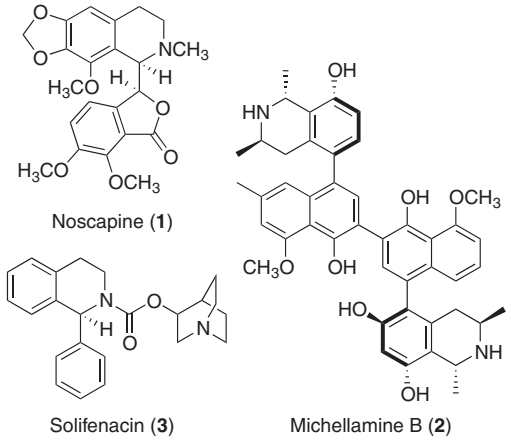

Figure 1. Examples of naturally occurring and synthetic pharmaceutically active tetrahydroisoquinoline alkaloids.

For the production of tetrahydroisoquinolines, the use of asymmetric catalysts for the enantioselective hydrogenation of dihydroisoquinoline derivatives[10] can be numbered among the few stereoselective chemical methods reported to produce this class of compounds.

The Pictet–Spenglerase enzymes could, in principle, be used to produce enzymatically tetrahydroisoquinolines in an enantioselective fashion. The stereoselectivity of the NCS catalyzed reaction to yield (*S*)-configured products has already been shown in various

* Corresponding author.
E-mail address: sarah.o'connor@jic.ac.uk (S.E. O'Connor).

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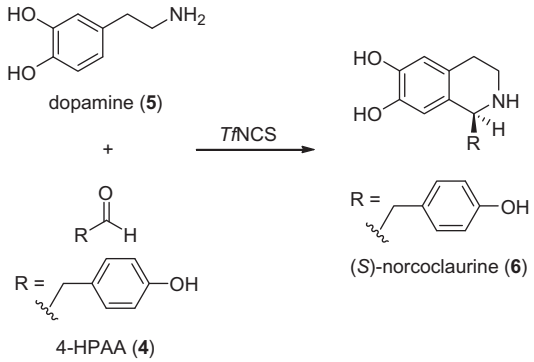
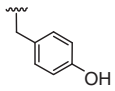
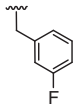
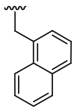
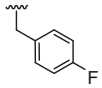
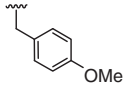
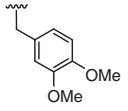
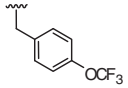
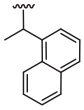
Table 1
Percent conversion of aldehydes **4**, **7**–**25** with *Tf*NCS after 3 h reaction time. Reaction conditions were as described in the text (dopamine (**5**) (1 mM), aldehyde **4**, **7**–**25** (1 mM), *Tf*NCS (300 μM), TRIS buffer (100 mM, pH 7)). All enzymatic reactions were performed alongside a control using boiled enzyme to ensure that no reaction occurs in the absence of enzyme



R=	Product	% Conv. after 3 h	R=	Product	% Conv. after 3 h
 **4**	**6**	60	 **16**	**35**	51
 **7**	**26**	65	 **17**	**36**	68
 **8**	**27**	66	 **18**	**37**	61
 **9**	**28**	71	 **19**	**38**	71
 **10**	**29**	66	 **20**	**39**	42
 **11**	**30**	58	 **21**	**40**	52
 **12**	**31**	57	 **22**	Not detected	–
 **13**	**32**	69	 **23**	Not detected	–
 **14**	**33**	69	 **24**	Not detected	–
 **15**	**34**	65	 **25**	Not detected	–

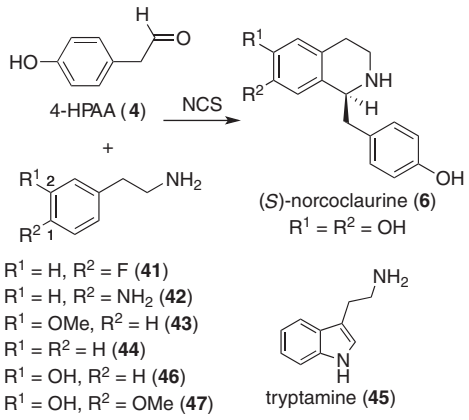
examples in the literature.[5c,d,11,12] However, a key limitation of biocatalytic approaches is the strict substrate specificity of many enzymes. An enzyme must have broad substrate specificity to be practical for wide synthetic application. Here we describe the sub-

strate scope and limitations of the Pictet–Spenglerase NCS, a study that provides a foundation for developing a general biocatalytic strategy for the synthesis of tetrahydroisoquinolines.

Affinity-tagged NCS from *Thalictrum flavum* (*Tf*NCS) was overexpressed in *Escherichia coli* and purified using nickel resin in good yields as previously reported.[12] This readily produced heterologous enzyme was used to probe systematically the enzymatic requirements for the aldehyde substrate using, in addition to the natural substrate 4-HPAA (**4**),[13] 19 aldehyde analogs (**7**–**24**). These aldehydes were synthesized either by oxidation of the corresponding alcohols with Dess–Martin periodinane (**7**–**15**, **17**, **19**, **20**) or by reduction of the corresponding Weinreb amide with LAH (**18**), or were commercially available (**16**, **21**–**24**).[14] For the enzymatic assay, we chose to use one of the reported conditions under which NCS had been assayed in previous studies. Specifically we used TRIS-buffered aqueous media at neutral pH with substrate concentrations at 1 mM. While substantial background reaction can often be observed for the Pictet–Spengler reaction, under these conditions no tetrahydroisoquinoline product was detected after 3 h reaction time with boiled enzyme by HPLC using UV detection at 228 nm. We further controlled for the non-enzymatic background reaction by including a control using boiled (inactive) enzyme with each reaction; in each case, no tetrahydroisoquinoline product was detected by HPLC. Therefore, we could be certain that all tetrahydroisoquinoline products observed were generated enzymatically. Briefly, *Tf*NCS (300 μM) was added to a solution of TRIS buffer (100 mM, pH 7.0) containing dopamine (1 mM) and aldehyde (1 mM). The enzymatic reaction was allowed to proceed at room temperature for 1 h, after which it was quenched with MeOH, and then analyzed by electrospray LC–MS. Authentic standards of each norcoclaurine analogs **26**–**40** were synthesized on a milligram scale, fully characterized by NMR and HRMS analysis and run along with the enzymatic assay (Supplementary data).

*Tf*NCS proved to have exceptionally broad aldehyde substrate specificity, turning over aldehydes **4**, **7**–**21** (Table 1). The structures of substrates **4**, **7**–**21** vary widely, and include phenylacetaldehydes substituted with various electron-withdrawing or donating groups, heteroaromatic moieties, aromatic bicyclics, aliphatic (hetero) cycles and aliphatic open-chained compounds. Only the products of the smallest, acetaldehyde (**23**) and propionaldehyde (**24**) and the α-substituted aldehydes, benzaldehyde (**22**) and **25** could not be detected.

Reaction rates of aldehydes **4**, **7**–**21** were evaluated by measuring the amount of dopamine consumed over time by HPLC using 0.5, 1, 2 and 3 h time points (Supplementary data). These assays show that **4**, **7**–**21** are turned over at a comparable rate with an average consumption of 65% dopamine (**5**) after 3 h. A slight disfavor (42–52%) for the aliphatic compounds **20** and **21** as well as the unsubstituted phenylacetaldehyde **16** could be observed (Table 1). In summary, we found that *Tf*NCS was able to catalyze the reaction between dopamine and acetaldehydes containing more than 3 carbon atoms and that are unsubstituted at the α-position. Aromatic acetaldehydes appear to be slightly superior substrates compared to bulky aliphatic compounds. Overall, however, all the aldehydes appeared to be converted in qualitatively similar rates. The aldehyde substrate flexibility provides a general method for the enzymatic preparation of 1-(*ortho*-, *meta*- and *para*-substituted benzyl)-1,2,3,4-tetrahydroisoquinolines.



Scheme 1. Phenethylamines **41**–**47** assayed with *Tf*NCS and with co-substrate 4-HPAA (**4**).

In contrast to the relaxed substrate specificity observed for the aldehyde substrate, NCS showed a strict requirement for the amine substrate, dopamine (**5**). We failed to detect any product for enzymatic reactions utilizing the native aldehyde substrate 4-HPAA (**4**) and the commercially available phenethylamines **41**–**44** (Scheme 1). Tryptamine (**45**), the natural substrate of strictosidine synthase, the Pictet–Spenglerase that catalyzes the formation of tetrahydro-β-carbolines,[15] was also not turned over. Overall, this substrate specificity is consistent with earlier mechanistic studies of NCS. Luk et al.[12] proposed the formation of a quinoid at the C-2 position of the aromatic ring, which requires that the phenethylamine substrate contains a hydroxy group at the C-2 position. Therefore, while **46** and **47** (Scheme 1) could be turned over by NCS,[12] substrates **41**–**45** could not.

Figure 2. (A) The norcoclaurine active site (2VQ5) surrounding the aldehyde substrate **4**. The co-substrate **5** is in blue. Lys122 and Glu110 are important for catalysis.[16] All conserved residues that surround **5** are in green; residues that vary among annotated NCS enzymes are in gray. (B) Alignment of annotated NCS enzymes. Residues labeled with * are shown in panel **A** and are conserved; * are in panel **A** but are not conserved among the NCS homologs.

The crystal structure of *Tf*NCS, which was recently published in 2009,[16] revealed that this enzyme harbors a relatively shallow active site (Fig. 2), which is consistent with the range of aldehydes that can be turned over by NCS. With the exception of the residues that directly interact with the aldehyde functional group, the binding pocket for substrate **4** appears to consist largely of hydrophobic interactions that could presumably accommodate substrate analogs **7**–**21**. The aromatic 4-HPAA (**4**) and dopamine (**5**) appear to stack together in the enzyme active site (Fig. 2A), but given the turnover of aliphatic aldehyde substrates, this stacking interaction must not be essential. We note that Ile143 appears to be close to the alpha carbon of **4**, and this residue may be responsible for preventing turnover of α-substituted aldehydes. We hypothesize that the strict amine substrate specificity is not governed by the confines of the enzyme active site, but instead by the reactivity requirements of the substrate.

In summary, we have showed that the enzyme norcoclaurine synthase can be used as a biocatalyst to yield a variety of substituted tetrahydroisoquinolines. We further note that the potential of the enzyme in a multi-gram scale enantioselective preparation of (*S*)-norcoclaurine itself was recently described.[11] The easy overexpression in *E. coli* and purification on large scale, as well as the stability of the protein, suggests potential applications in synthetic chemistry.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedures and spectral characterization) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.12.089.

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