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Norcoclaurine Synthase-Mediated Stereoselective Synthesis of 1,1′-Disubstituted, Spiro- and Bis-Tetrahydroisoquinoline Alkaloids

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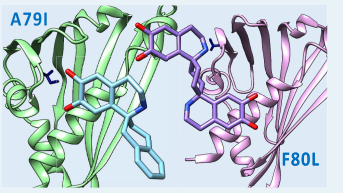
ABSTRACT: The Pictet−Spenglerase norcoclaurine synthase (NCS) catalyzes the formation of (*S*)-norcoclaurine, an important intermediate in the biosynthetic pathway of benzylisoquinoline alkaloids. NCS has been used as a biocatalyst with *meta*-hydroxy phenethylamines and aldehydes for the preparation of single-isomer tetrahydroisoquinoline alkaloids (THIAs). Recently, it was also reported that some ketones can be accepted as substrates, including 4-substituted cyclohexanones and phenyl acetones. Here, we report the use of wild-type NCS and selected variants with aliphatic, cyclic, α-substituted cyclic, heterocyclic, and bicyclic ketones to access challenging non-natural THIAs. Remarkably, fused bicyclic ketones as well as diketones could also be accepted by some of the NCS variants, and in silico modeling was used to provide insights into the rationale for this.



KEYWORDS: *biocatalysis, norcoclaurine synthase, Pictet−Spengler, tetrahydroisoquinoline, in silico modeling*

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INTRODUCTION

Alkaloids are found primarily in plants and are particularly prevalent in certain families of flowering plants. Tetrahydroisoquinoline alkaloids (THIAs) are a structurally diverse class of compounds, with a history of human use dating back thousands of years.[1] In addition to their prominent role in traditional medicine, THIAs have a wide variety of pharmacological applications, for example, as antitussives, antimicrobials, and antispasmodics.[1−3] Recently, preliminary studies have also uncovered new potential applications in treating cancer, malaria, and many other diseases.[2−9] Among all THIAs, a particularly interesting group are the 1,1′-spiro-tetrahydroisoquinolines, such as the natural products ochotensine **1**[10] and the related *N*-oxide **2**,[6] as well as the antiplasmodial compound **3** (Figure 1).[8] Indeed, when **3** was

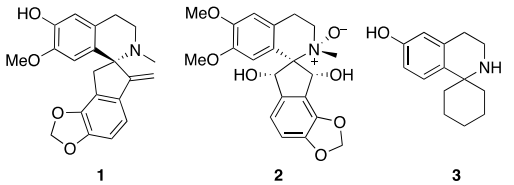

Figure 1. Examples 1,1′-spiro tetrahydroisoquinoline alkaloids **1**−**3**.

used in in vitro assays against *P. falciparum*, compared to a range of other THIAs, the antimalarial activity significantly increased, and it was suggested that this was due to the rigidity in this spiro analog.[8] The structurally related *Erythrina* class of alkaloids, also spiro-THIAs, has furthermore been used for a plethora of, for example, ethnomedicine applications.

Traditionally, many alkaloids such as morphine have been extracted from plant materials. However, typically, they are present as part of a mixture of components, and low extraction yields are obtained by the end of the process.[11] To overcome these limitations, different synthetic strategies have been developed. To date, there are many reported approaches to access THIAs, although the Bischler−Napieralski cyclization/reduction sequence[12] or Pictet−Spengler reactions (PSRs)[13,14] have been most frequently utilized. For asymmetric synthetic strategies, transfer hydrogenation with transition metals and chiral ligands have been successfully employed with a range of substrates for the synthesis of a variety of THIAs.[15,16] Although many of these methods are very useful, protection of phenolic groups is typically required; reaction efficacy varies, as does the stereoselectivities achieved; and the approach cannot be used to generate 1,1′-disubstituted THIAs. In order to access these, Brønsted acid or Lewis acid catalysts have been utilized (Scheme 1a); however, harsh reaction conditions are normally required.[17−20] More recently, the use of aqueous phosphate media[21] (Scheme 1b) has enabled access to a range of 1,1′-disubstituted THIAs under mild conditions.[22] Here, the main limitation is that racemic products are formed, unless single-isomer chiral substrates are used, which could also lead to the formation of diastereoselective products.

In order to overcome these issues, the use of biocatalysis has emerged as a powerful tool to generate high-value single isomer products. For instance, imine reductases (IREDs) have been used to produce enantiopure THIAs from the

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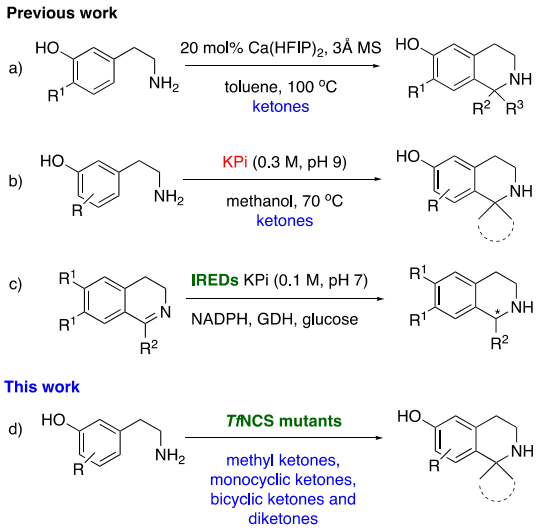
Scheme 1. Nonenzymatic and Enzymatic Routes to 1-Monosubstituted and 1,1′-Disubstituted PSRs[a]



[a]Parts (a) and (b) previous routes to 1,1′-disubstituted THIAs, (c) use of imine reductases to enantiopure THIAs, and (d) this work using norcoclaurine synthase (NCS) to 1,1′-disubstituted THIAs.

corresponding dihydroisoquinoline (Scheme 1c).[23] Pictet–Spenglerases are lyases (EC 4) that have been used as biocatalysts for the stereoselective synthesis of a range of alkaloids.[24−26] The main advantage of this group of enzymes is that no cofactor such as NADPH is required, which reduces the overall cost of processes compared to other biocatalysts that require, for example, glucose dehydrogenase redox cofactor recycling systems. Synthesis of functionalized starting materials for enzymes such as IRED is also required.

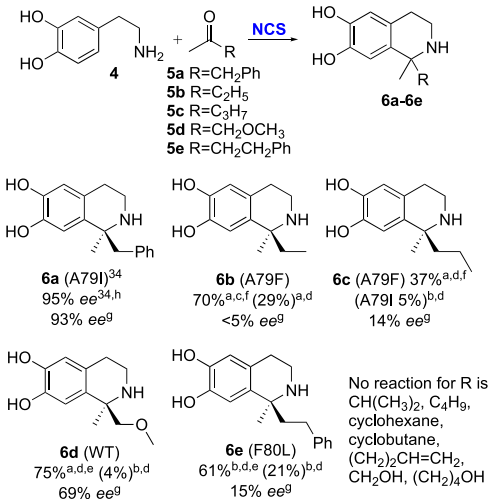
The Pictet–Spenglerase NCS catalyzes the PSR between dopamine and 4-hydroxylphenylacetaldehyde (4-HPAA) to form the key compound (*S*)-norcoclaurine, which is then biosynthetically converted into more than 2500 other alkaloids such as morphine. Despite the acceptance of a range of aldehydes by NCS,[25−32] very few ketones have been tolerated. Previously, some α-ketoacid acceptance was described,[33] and recently, it was reported that several unactivated ketones have been accepted in high yields.[34] For this, wild-type (WT) *Thalictrum flavum* NCS (*Tf*NCS) was used, together with the selected single-point active-site mutants, with some variants leading to higher product yields with cyclic ketones (e.g., A79F), while others were more productive toward the methyl ketones (e.g., A79I), and computational substrate docking experiments highlighted putative orientations of the imine intermediates in the active site.[34] In silico docking experiments and protein X-ray crystallographic studies have also been performed to understand better the NCS mechanism and role of key active site residues.[32,35,36]

Here, our aim was to fully explore the ketone substrate promiscuity of NCSs to enhance the applications of using this PSR more widely synthetically. As part of this, a more effective method for determining reaction stereoselectivities was required. Remarkably, using a wide range of cyclic, acyclic, fused bicyclic ketones and diketones, THIAs were synthesized and the reaction conditions were optimized and successfully applied to several phenethylamines (Scheme 1d). Importantly, some of the THIAs synthesized also have important bioactivities.

RESULTS AND DISCUSSION

In a previous work, it was reported that NCS enzymes could accept phenyl acetones.[34] Initial studies here focussed on NCS reactivities toward dopamine **4** with phenylketone **5a** and aliphatic methyl ketones **5b-e** to give THIAs **6a-6e** to understand the structure–activity trends, and also methods were developed to help determine the stereoselectivities of the reactions. While **5a** readily formed **6a** as previously described (87% isolated yield),[34] for methyl ketones **5b-e**, they were screened against WT-*Tf*NCS and variants A79I, A79F, L76A, L76V, F80L, M79F, and Y108F as cell lysates. From this, the highest performing variant was identified (Supporting Information Figure S1.1), reaction conditions were optimized, including the addition of the antioxidant sodium ascorbate to avoid side reactions due to the oxidation of catechols,[37] and THIAs were generated (Scheme 2). Reactions were monitored

Scheme 2. Use of *Tf*NCSs with Methyl Ketones 5a-5e to Synthesize 6a-6e



Reaction conditions: Small-scale reactions were performed using **4** (10–15 mM), ascorbate (10–15 mM), methyl ketone (10–150 mM), and dimethyl sulfoxide (DMSO, 10%) at 37 °C in HEPES buffer (100 mM pH 7.5) using the NCS and time indicated. *NCS*: [a]purified NCS (1.6 mg/mL), [b]NCS lysate (1.7 mg/mL NCS). *Reaction time*: [c]1 day; [d]7 days; or more. *Reaction conversions* by: [e]dopamine depletion by HPLC analysis, [f]HPLC analysis against product standards. *Determination of ees*: [g]Marfey's reagent and ^{1}H NMR spectroscopy, [h]chiral HPLC. Isolated yields are given in parenthesis. For further details and larger scale reactions for characterization purposes, see the Supporting Information.

to determine conversion yields by high-performance liquid chromatography (HPLC) analysis (dopamine depletion or product formation) and/or ^{1}H NMR spectroscopy using an internal standard as indicated because of the reported challenges of product isolation which frequently lowers the isolated yield. Larger scale reactions were also performed for product characterization purposes.[18,22] It was clear that regardless of the NCS variant used, increasing the length of the alkyl chain decreased the THIA yield. For example with **5b**, **6b** was formed in 70% conversions, while with **5c**, the conversion decreased to 37% for **6c**, with the best variants A79F and A79I, respectively (Scheme 2). Indeed, no reaction was observed with 3-methylbutan-2-one, longer aliphatic chain methyl ketones, and 1-cyclic-methylketones, presumably

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ACS Catal. 2021, 11, 131−138

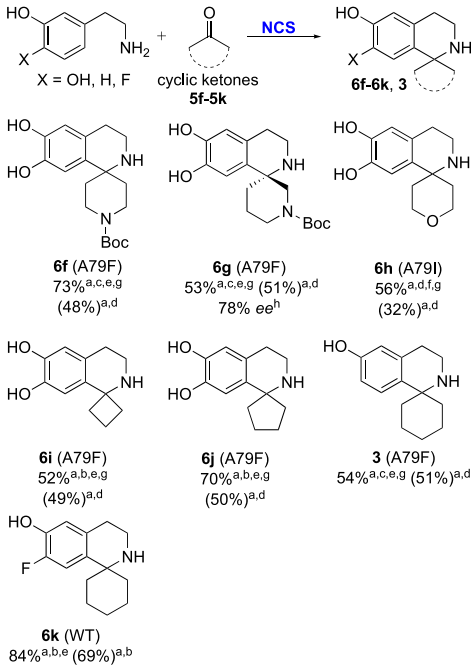
because of unfavorable steric interactions (Scheme 2, Supporting Information Figure S1.2). Interestingly, a 75% conversion was observed with methoxyacetone **5d** to give **6d**, although problems were encountered during its purification, while hydroxylated methyl ketones were not accepted. Benzylacetone **5e** gave **6e** in conversions of 61%, perhaps reflecting its structural similarity to the natural substrate 4-HPAA. Overall, isolated yields were in the range of 4−29%.

Previously, the stereoselectivity of the 1,1′-disubstituted THIAs produced by NCS was determined by chiral HPLC, compared to racemic standards. The absolute stereoselectivity of the major isomer was also assigned based upon the known selectivity with aldehydes.[34] However, for **6b**-**6e** chiral separations could not be achieved using a range of chiral HPLC columns and methods. Alternative methodologies were therefore investigated using **6a** as the *ee*s, which had previously been established,[34] including the preparation of Moshers's amide which has been reported with THIAs formed using NCS and aldehydes.[25] However, with the more sterically congested C-1 center, the ^{1}H NMR spectrum of the Mosher's products could not readily be assigned because of the amide rotamers formed. An alternative method to determine *ee*s is the use of Marfey's reagent,[38,39] which avoids the amide rotamer problems and is attached via S_NAr reaction. Using (rac)-**6a**, formed via the recently reported basic potassium phosphate (KPi) reaction conditions,[22] and (1*S*)-**6a** generated using *Tf*NCS (95% *ee* via chiral HPLC analysis[34]), protocols were developed using Marfey's reagent which confirmed an *ee* of 93% (Supporting Information Figure S1.3).

Reaction stereoselectivities were then established using this method for the NCS reactions with the most productive variants toward **5b**-**5e** (Scheme 2) and for this, racemic compounds were also synthesized.[22] With 2-butanone **5b**, **6b** was formed in a negligible *ee*, while **5c** with a slightly longer aliphatic chain gave **6c** in a 14% *ee*. Clearly, where similar sized groups are present either side of the carbonyl moiety, there is little stereodifferentiation in the active site. The incorporation of the methyl ether in **5d** improved the *ee* in **6d** to 69%, reflecting the impact of possible hydrogen bonding in the side chain on the stereochemistry. Surprisingly, benzyl acetone **5e** only gave **6e** in 15% *ee*. Following the stereopreference for the WT and NCS variants with aldehydes, the major isomers were assigned as having the (1*S*)-stereochemistry, other than **6d**, which because of the Cahn−Ingold−Prelog priority rules was assigned as (1*R*)-**6d**.

Previously, it was reported that NCS enzymes could readily accept several 4-substituted cyclohexanones, but we were curious as to whether the presence of different heteroatoms on the cyclohexane ring or different ring sizes could affect the reaction. For the cyclic ketones leading to **11**−**15**, as before, they were screened against WT *Tf*NCS and available variants as lysates to identify the highest performing variant for use in reactions (Supporting Information Figure S1.4). In previous works using the 4-substituted cyclohexanones, A79F was noted as a productive variant, as was the case here using **4** and 1-*N*-Boc-4-piperidone **5f**: with A79F, **6f** was formed, with a large Boc group at the 4-position relative to the carbonyl group, in a conversion yield of 73% after 72 h (48% isolated yield) (Scheme 3). When 1-*N*-Boc-3-piperidone **5g** was reacted with **4**, again with A79F, the conversion to give **6g** after 72 h was 53% (51% isolated yield) and an *ee* of 78% was observed. Following the general stereochemical preference for aldehydes, the major isomer in **6g** due to the Cahn−Ingold−Prelog priority rules was assigned as (1*R*)-**6g**. Despite the good reactivities observed with **5f** and **5g**, the corresponding analogues with *N*-benzyl groups did not seem to be accepted, which may have been due to substrate solubility issues (Supporting Information Figure S1.2). Next, tetrahydro-4*H*-pyran-4-one **5h** was investigated with **4** and several variants readily gave **6h**, including A79I in 56% conversion yield (32% isolated yield). Alternative ring sizes using **4** with cyclobutanone **5i** and cyclopentanone **5j** gave **6i** and **6j** after 24 h in 52 and 70% conversions, respectively, using A79F, and both were isolated in ∼50% yield. Interestingly, the five-membered ring analogue of **5g**, 1-*N*-Boc-3-pyrrolidinone, was not accepted, nor was the analogue 1-*N*-benzyl-3-pyrrolidinone (Supporting Information Figure S1.2). In addition to using alternative cyclic ketones, selected substituted phenethylamines were investigated. 2-(3-Hydroxyphenyl)ethylamine and a fluorinated analogue were prepared following reported procedures[40] and reacted with cyclohexanone **5k** and A79F to give **3** and WT to give **6k**, in 54 and 84% conversion yields (51 and 69% isolated yields), respectively. Compound **3** is particularly interesting, as it has been reported to possess good antimalarial properties compared to other THIAs screened.[8] Overall, it was clear that a range of cyclic ketones were accepted by the NCSs and cell-free lysates could readily be used for these reactions; however, rings possessing bulkier

Scheme 3. Use of *Tf*NCSs with Cyclic Ketones 5f-5k to Synthesize 6f-6k and 3



Reaction conditions: Small-scale reactions were performed using **4**, 2-(3-hydroxyphenyl)ethylamine or 5-(2-aminoethyl)-2-fluorophenol (10−15 mM), ascorbate (10−15 mM), ketone (10−150 mM), and DMSO (10%) at 37 °C in HEPES buffer (100 mM pH 7.5) using the NCS and time indicated. *NCS*: [a]NCS lysate (1.7 mg/mL NCS; for **3**, **6k** 8.5 mg/mL). *Reaction time*: [b]1 day, [c]3 days, [d]7 days. *Reaction conversions* by: [e]amine depletion by HPLC analysis, [f]HPLC analysis against product standards, [g]^{1}H NMR spectroscopy against an internal standard. *Determination of ees*: [h]Marfey's reagent and ^{1}H NMR spectroscopy. Isolated yields are given in parenthesis. For larger-scale reactions for characterization purposes, see the Supporting Information.

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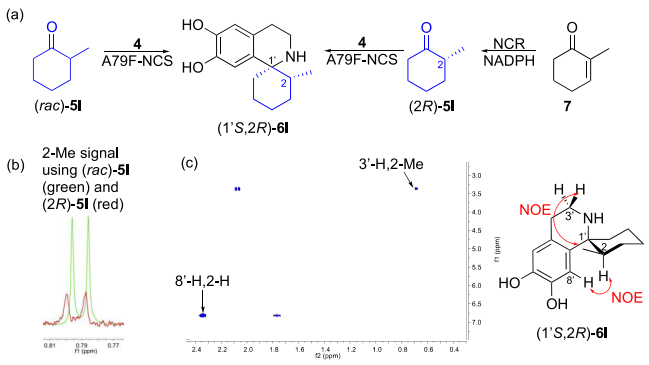
ACS Catal. 2021, 11, 131−138

side groups were not tolerated, most likely because of steric factors, and so this was explored in more detail with α-substituted cyclohexanones.

In a previous work, it was reported that α-substituted cyclic ketones yielded no products most likely because of steric reasons.[34] However, as α-methyl-substituted aldehydes can be accepted by NCS,[32] and the acceptance of α-substituted cyclohexanones by NCS could be useful in developing routes to the erythrina alkaloids, this was explored further. Initial attempts using 2-chlorocyclohexanone led to substrate degradation, and no THIA product was generated. However, with 2-methylcyclohexanone **5l** and WT-NCS, THIA formation was noted by LC−mass spectrometry (MS). Reactions were optimized, particularly using higher equivalents of ketone, higher enzyme concentrations, longer reaction times, different cosolvents (DMSO gave the highest yields), and enzyme variants. Variants A79I, A79F, Y108F, and WT that gave rise to some of the higher conversions after 24 h were used in reactions for longer periods of time, leading to improved conversions: a preparative scale reaction, for example, with *rac*-**5l** (A79F, 7 d) gave **6l** in 15% isolated yield; longer reaction times reported higher conversions of >70%, but they were evaluated as unsatisfactory for synthetic applications (Supporting information Figure S1.5). In theory, up to four stereoisomers could be generated, but chiral HPLC analysis suggested that there was predominantly one diastereoisomer present. To confirm whether one isomer of **5l** was accepted, a two-step one-pot reaction cascade was developed. Compound (2*R*)-**5l** was prepared from 2-methyl-2-cyclohexen-1-one **7** using the ene-reductase NCR from *Zymomonas mobilis* expressed in *E. coli*[41] (and NADPH and G6PDH cofactor recycling system), and then A79F-*Tf*NCS was added to give **6l** (Scheme 4a). Both products formed using (rac)-**5l** and (2*R*)-**5l**

Scheme 4. Use of 5l and Key NMR Analyses to Establish the Stereochemistry of 6l



(a) A79F-*Tf*NCS-catalyzed PSR between **4** and (rac)- **5l** or (2*R*)-**5l** generated from 2-methyl-2-cyclohexen-1-one and the ene-reductase NCR; (b) C-2 Me signals in the ^{1}H NMR spectra of **6l** starting from (rac)-**5l** (green) and (2*R*)-**5l** (red); and (c) Key ^{1}H-^{1}H NOESY correlations in **6l** produced from (rac)-**5l**.[42]

gave the same single product by ^{1}H NMR spectroscopy (see the methyl signal region in Scheme 4b). ^{1}H NMR spectroscopic analysis revealed key Nuclear Overhauser effects (NOEs) between 2-H and 8'-H and 3'-H and 2-CH_3, and because (2*R*)-**5l** was readily accepted by A79F-*Tf*NCS and the equatorial C-2-methyl substituent is more likely, the stereochemistry of **6l** was assigned as (1'*S*,2*R*), consistent with the NOE data (Scheme 4c) and the *S*-selectivity normally observed with aldehydes and NCS. Interestingly, in a previous work, (3*R*)-methylcyclohexanone was accepted by A79F-*Tf*NCS to give the (1*R*,3'*R*)-product, but with the methyl group at the 3-position, different steric restraints will be acting within the active site.[34]

A computational docking study also suggested that the imine intermediate formed between dopamine and (2*R*)-**5l** was preferred in the active site, compared to (2*S*)-**5l**, with the methyl group adopting an equatorial conformation (Supporting Information Figure S1.6). Because A79 is located at the entrance of the active site, the improved reactivity of A79F toward **5l** could be explained by the bulkier amino acid residue helping to orientate the imine intermediate into a reactive conformation, promoting the cyclization to give **6l**. The acceptance of **5l** by NCS highlights its ability to achieve both enantioselective and stereoselective PSRs, which is chemically challenging to achieve by other methods. In addition to **5l**, 2-ethylcyclohexanone was also used and a small amount of the corresponding THIA was detected by LC−MS but could not be isolated.

Next, more challenging bicyclic ketone substrates were explored affording a family of otchosentine **1**[10] natural product analogues. Again, the use of a range of NCS variants was explored, and the highest yielding variants used are given in Scheme 5 (also see Supporting Information Figure S1.7). Remarkably, when using β-tetralone **5m**, **6m** was formed in 79% conversion yield and 86% *ee* using A79I. Using other β-tetralones **5n**-**5p**, good conversions and isolated yields were achieved, together with similar *ee*s. Indeed, 6-substituted-β-tetralones with electron-withdrawing chloro- or bromo-groups gave **6n** with WT-NCS and **6o** with A79I in 50 and 54% isolated yields, respectively. The use of **5p** with an electron-donating group at the 7-methoxy-position was also readily accepted, giving **6p** in 55% isolated yield with M97F. The purification of THIAs **6m**-**6p** was readily achieved by acid−base extraction procedures without the requirement for chromatographic purifications, enabling the facile scalability of such approaches. All NCS variants used, however, failed to accept β-tetralone-bearing methoxy groups at C-5 or C-8 (Supporting Information Figure S1.2), presumably because steric reasons when entering the active site. Assignment of the major enantiomer shown (Scheme 5) was tentatively made based upon preference for formation of the (*S*)-isomer with aldehydes.[25,26] 2-Indanone **5q** was readily accepted to give **6q** in 76% conversion yield and 59% isolated yield.

To understand better the results with these challenging bicyclic ketones, some docking experiments were performed. Here, both the affinity energies and key distances of the five NCS mutants used (A79I, A79F, M97V, F80L, and L76V) and the WT-NCS (Supporting information Figures S1.8 and S1.9) with imine intermediates toward **6m** and **6o** were determined. Previous works have described the 'dopamine-first mechanism' where the Pictet−Spengler cyclization is triggered by the deprotonation of the (dopamine-derived) *meta*-OH by Lys122, and the crystal structure containing a mimic highlighted this key interaction with bond distances of ∼3 Å.[35,36] Notably, with both intermediates modeled (Figure 2), they fitted into the active site of all the structures, but A79I gave either the shortest distance (∼3 Å) from *meta*-OH to the Lys122 residue combined with a folded conformation (**6o**) or one of the best binding affinities and a folded conformation (**6m**) with *meta*-OH to Lys122 approaching ∼4.0 Å when a weak interaction occurs. Indeed, the modeling with L76V to give **6m** gave a less

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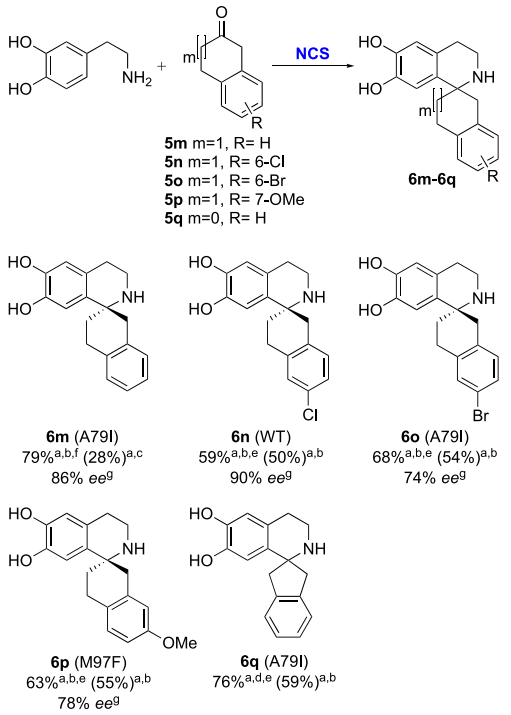
Scheme 5. Use of *Tf*NCSs with Dopamine and Tetralones 5m-5p and Indanone 5q to Synthesize Spiro-THIA Alkaloids



Reaction conditions: Small-scale reactions were performed using **4**, ascorbate (10−15 mM), ketone **5m-5q** (10−150 mM), and DMSO (10%) at 37 °C in HEPES buffer (100 mM pH 7.5) using the NCS and time indicated. *NCS*: [a]NCS lysate (1.7 mg/mL: for **6n-q** 8.5 mg/mL). *Reaction time*: [b]1 day, [c]3 days, [d]7 days. *Reaction conversions* by: [e]dopamine depletion by HPLC analysis, [f]HPLC analysis against product standards. *Determination of ees*: [g]Marfey's reagent and ^{1}H NMR spectroscopy (compared to racemic standards). Isolated yields are given in parenthesis. For further details and larger-scale reactions for characterization purposes, see the Supporting Information.

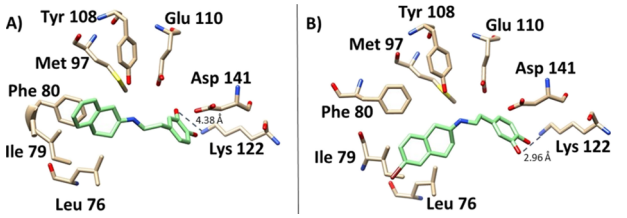
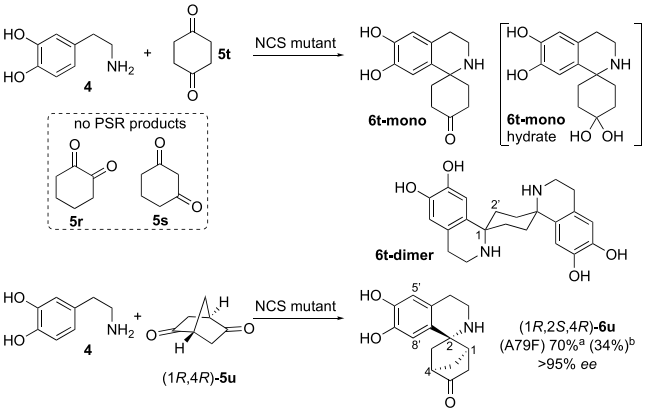


Figure 2. (A) Predicted conformation of the imine-β-tetralone intermediate to give **6m** in the A79I-*Tf*NCS (modeled) active site and key residues. (B) Predicted conformation of the imine-β-bromotetralone intermediate in the A79I-*Tf*NCS active site to give **6o** (subunit A, 5N8Q). Reaction intermediates were docked in the *Tf*NCS active site (subunit A, 5N8Q) using AUTODOCK VINA[43] and UCSF Chimera.[44] For further details, see the Supporting Information.

favorable binding affinity, which was consistent with the lower conversions observed. The modeling overall revealed that productive conformations of the imine intermediates can remarkably fit into the WT-NCS active site and that of variants, with *meta*-OH proximal to Lys122 and the bicyclic system occupying the entrance into the active site, thus highlighting many interesting potential applications of these enzymes.

Finally, it was decided to explore the use of diketones **5r-5t** and 1,2-, 1,3-, and 1,4-cyclohexanedione to establish whether mono-PSR products could be formed with ketone functionalities for further derivatization. With **5r** and **5s**, no PSR products were observed (Supporting Information Figure S1.2). With cyclohexan-1,4-dione, surprisingly, we observed formation of both mono- and a symmetrical di-PSR-product, with the selectivity depending on the NCS variant used (Scheme 6).

Scheme 6. Application of the *Tf*NCS-Catalyzed PSR between Dopamine and Different di-Ketones to Synthesize 1,1′-Disubstituted and Spiro-THIA Alkaloids



Reaction conditions: Reactions were performed using **4** (10−15 mM), ketone **5r-5u** (10−150 mM), purified NCS (1.6 mg/mL), sodium ascorbate (1 eq with respect to **4**), and 10% (v/v) DMSO in HEPES buffer (pH 7.5, 100 mM) at 37 °C for 1 day. [a]Conversion yield, [b]isolated yield.

Moderate conversions were recorded in all cases (20−35%). Notably, WT-*Tf*NCS gave mono-product **6t-mono**: **6t-dimer** in a ratio of 8:1, and **6t-mono** was isolated in 28% yield. Enzyme variants with larger residues at positions toward the entrance to the active site (A79I, A79F) gave similar product ratios of 9:1. However, replacement of the bulky phenylalanine residue with leucine in F80L reversed the selectivity for **6t-mono**: **6t-dimer** to 1:3, enabling **6t-dimer** to be isolated more easily, and the structure shown, which was the same with all mutants, was consistent with the spectroscopic data. Both the formation of di-PSR products and changes in such reaction selectivities are unprecedented, and control reactions gave no PSR products. Compound **6t-mono** was also observed to form some of the hydrate by NMR spectroscopy.

As an alternative, diketone (1*R*,4*R*)-**5u** was also used. Up to 70% yields of **6u** were observed after 20 h with the most productive variant A79F (Supporting Information Figure S1.10), and the formation of one isomer was observed. The product **6u** was purified by preparative HPLC and isolated in 34% yield. Based on the selectivity observed with aldehydes and the α-methyl cyclohexanone, the isomer formed was assigned as (1*R*,2*S*,4*R*)-**6u** with the new (2*S*)-spiro-sterocenter at C-2/C-1′. No disubstituted PSR products were formed with any of the mutants screened, presumably because of steric effects with **5u**.

In order to probe further the results with these diketones, some molecular docking experiments with the **6t-dimer** were carried out. Using WT-*Tf*NCS (subunit A, 5N8Q), **6t-dimer** did not fit into the active site, presumably indicating the preference for the **6t-mono** that was formed. Furthermore,

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ACS Catal. 2021, 11, 131−138

modeling with F80L (homology model) allowed entrance of the imine diproduct intermediate into the active site, which must occur after the first reaction to afford **6t-dimer** (Figure 3A): similar modeling with WT-*Tf*NCS (Figure 3B) revealed

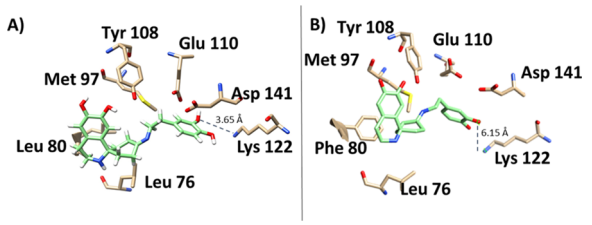

Figure 3. (A) Predicted conformation of the imine diproduct intermediate to give **6t-dimer** in the F80L-*Tf*NCS (modeled) active site and key residues. (B) Predicted conformation of the imine diproduct intermediate to give **6t-dimer** in the WT-*Tf*NCS active site (subunit A, 5N8Q). Reaction intermediates were docked in the *Tf*NCS active site (subunit A, 5N8Q) using AUTODOCK VINA[43] and UCSF Chimera.[44] For further details, see the Supporting Information.

no suitable potential binding modes because long distances between the mechanistically important *meta*-OH and key residue Lys122. This could explain the preference of the WT-*Tf*NCS for **6t-mono** formation. Also, modeled A79I and A79F with the dimer intermediate showed either poorer binding affinities or longer *meta*-OH to Lys122 distances, or both.

In summary, the Pictet−Spenglerase NCS was found to accept a large range of unnatural substrate ketones to synthesize 1,1′-substituted and spiro-THIAs. Several selected NCS variants, in particular A79I/F, F80L, M97F, and Y108F, were identified to effectively catalyze the synthesis of more than 20 diverse THIAs from linear aliphatic, α-substituted, cyclic, and bicyclic ketones and diketones. The key parameter for reactivity with these substrates is, besides a folded productive conformation in the active site and good binding affinity, a short distance between the mechanistically important Lys122 and (dopamine or more generally the phenethylamine) *meta*-OH to initiate the reaction. If steric or other factors prevent this key interaction, then it is not possible for the reaction to proceed. This study also highlights the high degree of enzyme promiscuity displayed by NCSs and good stereoselectivities that can be achieved. NCS is proving to be a very useful, sustainable catalyst to access 1,1′-substituted and spiro-THIAs which are chemically challenging to synthesize.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscatal.0c04704.

Experimental methods, supporting figures and tables and chemical characterization are given (PDF).

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Author Contributions

[‖]J.Z. and D.M.-S. contributed equally. J. Z. and D. M.-S. performed chemical syntheses, chemical characterization, and enzymatic assays and R.R. expressed *Tf*NCS mutants and performed enzymatic assays. The project was supervised by J.M.W and H.C.H. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

NCS,norcoclaurine synthase; PSR,Pictet-Spengler reaction; THIA,tetrahydroisoquinoline alkaloids; *Tf*,*Thalictrum flavum*; 4-HPAA,4-hydroxyphenylacetaldehyde; IRED,imine reductase; NMR,nuclear magnetic resonance; *de*,diastereomeric excess; *ee*,enantiomeric excess; GDH,glucose dehydrogenase; G6PDH,glucose-6-phosphate dehydrogenase; HEPES,4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC,high performance liquid chromatography; DMSO,dimethylsulfoxide; LC−MS,liquid chromatography-mass spectrometry; WT,wild-type.

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