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Bioalkylation Strategies to Synthesize Allylated Tetrahydroisoquinolines by Using Norcoclaurine Synthase and *O*-Methyltransferases

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O-Methyltransferase (*O*-MT)-mediated alkylations are of growing interest for the regioselective modification of bioactive motifs, although there are still few examples of applications with more structurally complex compounds. In this work, we have used *O*-MTs for the allylation of various catechol and tetrahydroisoquinoline substrates via a biocatalytic cascade involving additionally methionine adenosyltransferases (MATs) and a methylthioadenosine nucleosidase (MTAN). Furthermore, we have integrated norcoclaurine synthase into this cascade to stereoselectively generate (*S*)-THIQs in situ as both intermediates and products in the allylation cascade. Notably, a variation in the order in which NCS and MAT-MT-MTAN are added can significantly affect the regioselective outcome, enabling exquisite control of both stereochemistry and regiochemistry in the products. We also identified *Ureaplasma urealyticum* MAT as an effective enzyme for the formation of the *S*-adenosyl-*S*-allyl-L-homocysteine required as the cofactor for the *O*-MTs and established that the racemate rather than the single isomer of *S*-allyl-homocysteine can effectively be used in the cascades with MATs.

1. Introduction

Stereoselective alkylations, such as allylations, are important for the tailored modification of structural motifs to alter compound bioactivities or introduce click handles for use in chemical biology applications.[1] Enzymatic alkylations typically use methyltransferase (MT) enzymes, which in native reactions catalyze the transfer of methyl groups to target molecules and are rapidly gaining favor as a method for green and regioselective chemical synthesis.[2–4] Most MTs require the methyl group donor cofactor *S*-adenosyl-L-methionine (SAM) as the electrophile, with the reaction operating via an S_N2 mechanism. Using MTs in alkylation reactions presents several advantages over traditional chemical methods, such as enhanced regioselectivities, mild reaction conditions, and the avoidance of toxic alkylating reagents such as methyl iodide and dimethyl sulfate. However, the key requirement for stoichiometric quantities of SAM in biomethylations can be a problem in synthetic applications, as it is expensive and relatively unstable.[5] To overcome this issue, SAM can be generated in situ from ATP and L-methionine using methionine adenosyltransferase (MAT).[6] Methylthioadenosine nucleosidase (MTAN) can also be added to hydrolyze the *S*-adenosyl-L-homocysteine (SAH) by-product and prevent MT feedback inhibition.[6] This three-enzyme MAT-MT-MTAN methylation cascade has been used with several substrates,[6] including tetrahydroisoquinolines (THIQs).[7] Other studies have produced cyclic regeneration systems using SAH hydrolase instead of MTAN, with polyphosphate and kinases to convert the adenosine produced into ATP, and L-homocysteine *S*-methyltransferase to transform the L-homocysteine into L-methionine.[8,9] Also, halide methyltransferases (HMTs) have been described to regenerate SAM from SAH, using methyl iodide or less hazardous methyl tosylate as methyl donors.[10,11] HMTs have additionally been combined into MAT-MT-HMT cascades to avoid priming the methylations with SAM.[12]

There has been a recent expansion in the range of MT-catalyzed alkylations. For instance, ATP and L-ethionine or *S*-allyl-L-homocysteine (*S*)-**1** were used with a human MAT mutant I322 V hMAT2A to give *S*-adenosyl-L-ethionine (SAE) and *S*-adenosyl-*S*-allyl-L-homocysteine (SAA), respectively, then used in *N*-MT reactions with THIQs, although conversions were not indicated.[13] Similarly, SAE and SAA were prepared from L-ethionine and *S*-allyl-L-homocysteine using *Thermococcus kodakarensis* (Tk) MAT and used to *O*- or *N*-alkylate amino nitrophenols, with up to 40% conversions noted.[14] In addition,

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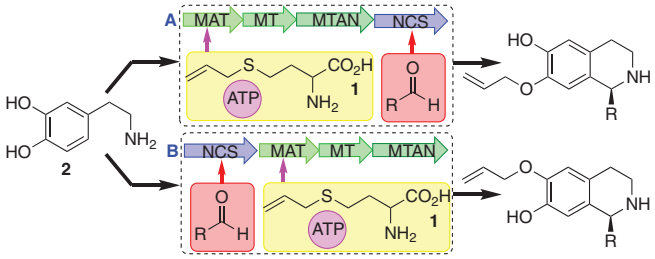


Scheme 1. The allylation cascade, via routes A or B to THIQs (major regioisomers shown).

engineered enzymes were used for *O*-carboxymethylations of catechols and *N*-carboxylmethylations of THIQs.[15] HMTs have also been mutated for the in situ generation of SAE and SAA from SAH and then applied in the ethylation of luteolin and allylation of 3,4-dihydroxybenzaldehyde in up to 48% conversions.[16]

THIQs are important pharmacophores due to the various antibiotic, hypotensive, and antitumor properties associated with this scaffold.[17–20] The synthesis of single-isomer THIQs by traditional chemical methods is challenging due to the high density of functional groups present.[21] However, biocatalytic approaches have recently proven to be particularly effective in generating a range of THIQs in high enantiomeric purities using the Pictet–Spenglerase enzyme norcoclaurine synthase (NCS), a key enzyme in the biosynthetic pathway to such alkaloids.[22] NCS catalyzes the stereoselective Pictet-Spengler condensation between dopamine and analogues and a range of aldehydes to form (*S*)-THIQs.[22,23] Indeed, mutagenesis of *Thalictrum flavum* NCS (*Tf*NCS) has led to a wider range of aromatic and aliphatic aldehydes, as well as ketones, being accepted as substrates.[24–26] Recently, studies have successfully combined NCS into in vitro enzyme cascades or in vivo biosynthetically inspired pathways to produce a range of alkaloids.[27–30] Moreover, MTs have been used to perform a range of biocatalytic *N*- and *O*-methylations with (*S*)-THIQs, achieving impressive regioselectivities,[13] some together with NCS in cascades.[7,28]

While MTs show much promise for the selective methylation of molecular scaffolds, allylations have been less explored. In this work, we report biocatalytic cascades for the *O*-allylation of (*S*)-THIQs by *Rattus norvegicus* catechol *O*-MT (*Rn*COMT) and *Myxococcus xanthus* SafC-*O*-MT (*Mx*SafC) from the saframycin biosynthetic pathway, demonstrating successful allylations on these complex motifs. A key consideration was at what stage in the biocatalytic pathway the allylation should be performed, either before the NCS step to produce (*S*)-THIQs (Scheme 1: route **A**) or after (Scheme 1: route **B**), and the impact of these two routes on the cascade's stereoselectivity is presented. Additionally, we established that *S*-allyl-DL-homocysteine (*rac*-**1**), which is readily synthesized in one step, can be used as an allyl donor in a MAT-MT-MTAN allylation cascade. Furthermore, we identified that *Ureaplasma urealyticum* MAT (*Uu*MAT) is highly effective in forming the allyl analogue of SAM,[31] negating the need for higher temperatures, which has been reported with other MATs.[32,33]

2. Results and Discussion

2.1. Initial *O*-Allylations Using the MAT-MT-MTAN Cascade

With few allylations reported to date, initially MAT-MT-MTAN allylation cascades with dopamine **2**, L-DOPA **3**, and 3,4-dihydroxybenzoic acid **4** as representative catechols were explored. This would establish assay conditions and identify the best MAT for the formation of SAA from *S*-allyl-L-homocysteine (*S*)-**1**. *Rn*COMT, which preferentially methylates catechol 3-OH groups, and *Mx*SafC, which typically methylates 4-OH groups, were also selected for use in this new *O*-allylation cascade.[6,7,34,35]

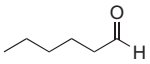
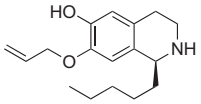
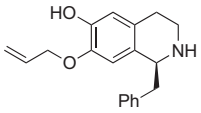
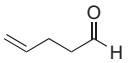
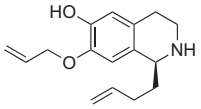
MATs from *Escherichia coli* (*EcMAT*),[8] *Methanocaldococcus jannaschii* (*Mj*MAT),[36] *T. kodakarensis* (*Tk*MAT)[14,19,32] and *Uu*MAT,[31] were screened with *Rn*COMT and *Mx*SafC, with the MTAN derived from *E. coli* (*Ec*MTAN),[6] (*S*)-**1** prepared as previously reported,[37] ATP and **2**–**4**. All recombinant enzymes (in *E. coli*) were added as clarified lysates for these initial assays. HPLC analysis revealed that all assay combinations with *Uu*MAT achieved the highest HPLC yields (28%), with assay combinations containing *Ec*MAT also giving moderate amounts of allylated products (Figure 1).

Conversely, *Mj*MAT and *Tk*MAT both performed poorly, which was contrary to previous allylations with *Tk*MAT and nitrophenols.[14] All the methylation positive controls using L-methionine instead of **1** showed the complete consumption of dopamine irrespective of the MAT/MT combinations used, indicating all MATs were active, and it is unclear why *Uu*MAT is more productive here. Furthermore, *Rn*COMT retained its 3-OH regioselectivity for **2**–**4** to give **2a**–**4a**, while *Mx*SafC retained the typical 4-OH regioselectivity for **2** and **3** but interestingly displayed C-3 regioselectivity for **4** as has been reported for methylations of this substrate by *Mx*SafC.[34] The reaction with **4** (61 mg) and *Rn*COMT was also performed at a preparative enzymatic scale to give **4a**.

2.2. Cascade A: MAT-MT-MTAN then NCS

Following the successful allylation of dopamine **2** in preliminary experiments, 4-*O*-allyl dopamine **2b** was considered as a substrate for the Pictet–Spenglerase *Tf*NCS, along with aldehydes, to form 7-*O*-allylated THIQs. Trials were performed using **2b** (synthesized non-enzymatically and regioisomers (~1:1) separated by preparative HPLC (Supporting Information) and wild-type (WT) *Tf*NCS with hexanal, phenylacetaldehyde, or 4-pentenal. NCS mechanistic studies have indicated a "dopamine first" mechanism with Lys122 deprotonating the *meta*-OH group, enabling subsequent cyclization onto the imine.[38] With substrate **2b** containing an *O*-allyl group at C-4, assays were carried out with different concentrations of *Tf*NCS to enhance yields and optical purities with this more sterically challenging substrate. Pleasingly, **2b** was accepted by *Tf*NCS with all three aldehydes to give the corresponding THIQs (*S*)-**5b**, (*S*)-**6b**, and (*S*)-**7b** in high HPLC yields and enantiomeric excesses (ees) (Table 1). Furthermore, only minor enhancements in yields and ees were achieved when the *Tf*NCS concentration was raised from 0.5 to 1.0 mg/mL.

Table 1. WT *Tf*NCS assays using 4-*O*-allylated dopamine 2b and either hexanal, phenylacetaldehyde, or 4-pentenal as the aldehyde substrate.[a)]

Substrates	Product	Yield by HPLC (ee)	
		*Tf*NCS 0.5 mg/mL	*Tf*NCS 1.0 mg/mL
2b + 	 (*S*)-**5b**	77% (88% ee)	82% (91% ee)
2b + 	 (*S*)-**6b**	78% (96% ee)	82% (98% ee)
2b + 	 (*S*)-**7b**	84% (92% ee)	88% (95% ee)

[a)] Reaction conditions: 24 h at 37 °C, 800 rpm, 5 mM 2b, 5 mM sodium ascorbate, 7.5 mM aldehyde, 50 mM HEPES (pH 7.5), and WT *Tf*NCS, 5% v/v MeCN. Assays were performed in triplicate with a volume of 200 µL. HPLC yields and ee values were determined by HPLC analysis against racemic product standards.

Given that *Tf*NCS accepts 4-*O*-allylated dopamine **2b**, it was conceived that adding the *Uu*MAT-*Mx*SafC-*Ec*MTAN cascade as a preceding step to generate **2b** for the *Tf*NCS would enable direct access to novel allylated THIQs. *Uu*MAT was shown above to be the most effective MAT to produce SAA, whilst *Mx*SafC had demonstrated 4-OH regioselectivity towards **2**. This property of *Mx*SafC is particularly useful, as **2a**, with no *meta*-OH group, cannot undergo the Pictet–Spengler reaction.[38,39] The *Mx*SafC-mediated allylation of **2** gave a regioisomeric ratio of ~1:6, **2a**:**2b**, compared to the non-enzymatic synthesis, which gave a ratio of 1:1, highlighting the advantage of the allylation cascade. Moreover, to improve the cascade with respect to substrate cost, *rac*-**1** was used instead of (*S*)-**1** to examine if the presence of (*R*)-**1** elicited any inhibitory effects on the cascade. Notably, *rac*-**1** was synthesized in one step and a reasonable yield (55%) compared to (*S*)-**1**, which requires protection and deprotection steps, as well as the methyl to allyl group switch, in an overall yield of 14%–38%.[37] Interestingly, performing the *step 1* *Uu*MAT-*Mx*SafC-*Ec*MTAN cascade (purified enzymes) worked remarkably well, giving **2b** in a 51% HPLC yield, and suggested that *Uu*MAT enzyme did not experience inhibition due to the presence of (*R*)-**1**.

After centrifugation of the reaction mixture, the supernatant was directly used in the *Tf*NCS *step 2* of the telescoped cascade to give (*S*)-**5b**–**7b** in high HPLC yields and ees (Scheme 2), which corresponded well to the previous single-step *Tf*NCS assay using **2b** (Table 1). Furthermore, 100% 7-*O*-allylated regioselectivity was observed in (*S*)-**5b**–**7b** products, with minor quantities of **1a** produced in *step 1* not participating in the reaction. A slight reduction in ee was noted in the assays forming (*S*)-**5b** and (*S*)-**7b**, which could be due to the release of phosphate and diphosphate in the MAT reaction catalyzing the non-enzymatic Pictet–Spengler condensation with aliphatic aldehydes.[39]

2.3. Cascade B: NCS then MAT-MT-MTAN

To enable access to 6-*O*-allylated regioselectivity, cascade B was investigated with the NCS reaction first to form the (*S*)-THIQ, followed by the allylation cascade (Scheme 3). *Rn*COMT and *Mx*SafC were selected as the *O*-MTs, as previous work established that *Rn*COMT typically methylated the 6-OH position (~90:10) with *Mx*SafC showing variable regioselectivities, including for 7-OH, depending on the functionality at C-1.[7,25]

Using **2**, hexanal, phenylacetaldehyde, and 4-pentenal as previously, the formation of THIQs was readily achieved using *Tf*NCS in yields of 85%–99% and excellent ees (*S*)-**5** (>99% ee), (*S*)-**6** (>99% ee), and (*S*)-**7** (99% ee), in agreement with previous work.[7,22] For the second step of the cascades, allylations proceeded with good conversions, with HPLC yields (*S*)-**5a** > (*S*)-**6a** > (*S*)-**7a**. The ee was confirmed to be the same after the allylation step, as confirmed for substrate **5a**, where no (*R*)-**5a** was detected (see Supporting Information). Additionally, for all (*S*)-THIQ compounds tested, there was clear regioselectivity for the 6-OH, with (*S*)-**5a**-**7a** being formed in preference to (*S*)-**5b**–**7b** (Scheme 3). This was the case for *Rn*COMT, and interestingly *Mx*SafC, contrary to the typical preference for *para*-alkylation with **2** and SAM and other catechols, that this *O*-MT displays.[4,34] However, the 6-OH regioselectivity has been observed with some THIQ substrates in *Mx*SafC methylations with fluorine at C-8 or bulkier functional groups at C-1.[7] The regioselective preferences observed here for *Mx*SafC compared with **2** and SAM are

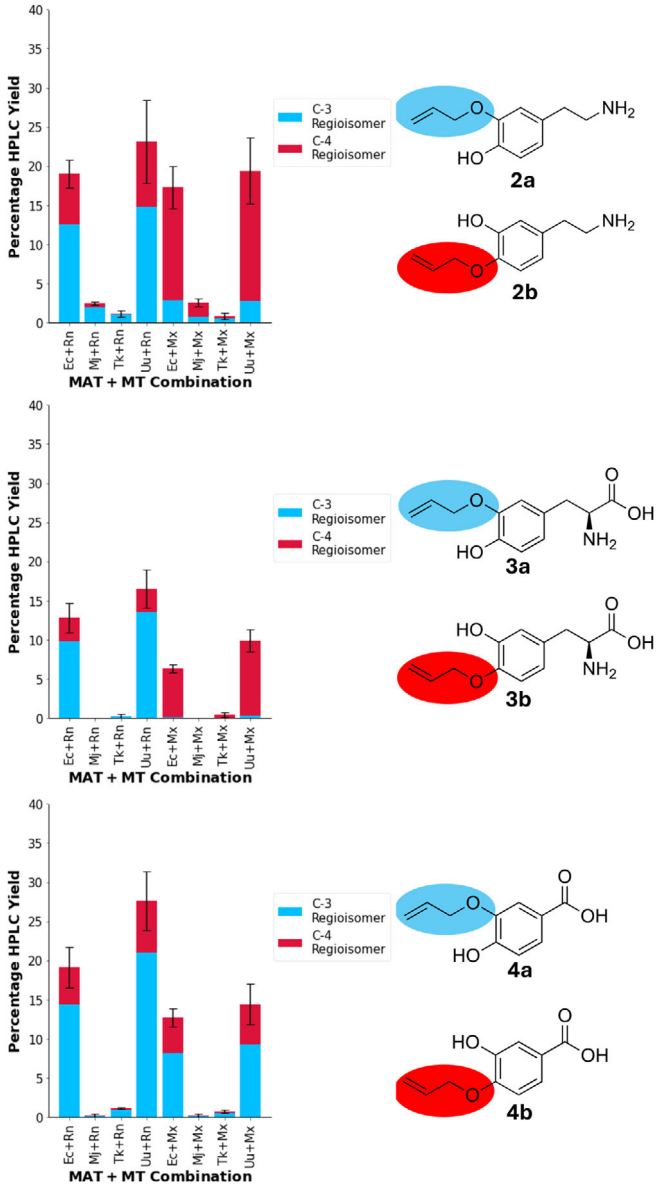
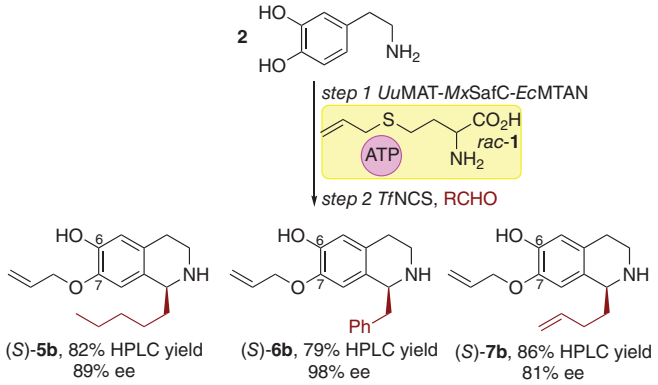
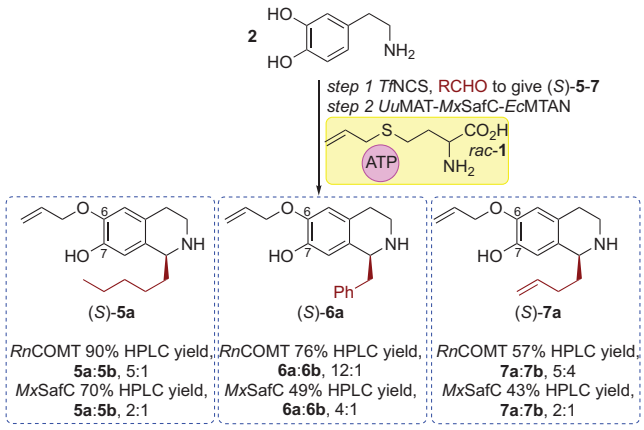


Figure 1. Initial experiments with catechol substrates 2–4 and *S*-allyl-L-homocysteine (*S*)-1 with different MAT and MT combinations to give the 3-OH allylated products (2a–4a) and the 4-OH allylated products (2b–4b). Ec is *Ec*MAT; Mj is *Mj*MAT; Tk is *Tk*MAT; Uu is *Uu*MAT; Rn is *Rn*COMT; and Mx is *Mx*SafC. Reaction conditions: 24 h at 37 °C, 800 rpm, 5 mM 1–3, 5 mM sodium ascorbate, 6 mM ATP, 6 mM (*S*)-1), 20 mM $MgCl_2$, 200 mM KCl, 50 mM HEPES (pH 7.5), 20% v/v clarified lysate for all MATs, 20% v/v clarified lysates for all MTs, 2.5% v/v *Ec*MTAN clarified lysate. Assays were carried out in triplicate and at a volume of 200 µL, with standard deviation bars given. HPLC yields were determined by HPLC analysis against product standards.



Scheme 2. The 2-step telescoped cascade to (*S*)-**5b**–**7b**. Reaction conditions—Step 1 to give **2b**: 50 mM HEPES (pH 7.5), 200 mM KCl, 20 mM $MgCl_2$, 5 mM dopamine hydrochloride, 5 mM sodium ascorbate, 12 mM ATP, 12 mM *rac*-1, 0.78 mg/mL *Uu*MAT, 0.90 mg/mL *Mx*SafC, and 0.27 mg/mL *Ec*MTAN (51%, **2a**:**2b** 15:85, where only 2b is converted in Step 2); Step 2: 50% v/v of Step 1 supernatant, 50 mM HEPES (pH 7.5), 3.75 mM aldehyde, 0.56 mg/mL *Tf*NCS, 2.5% v/v MeCN. Step 1 was performed as a single preparative assay at a volume of 5 mL (**2b** 51% yield; **2a**:**2b** 17:83), while step 2 assays were performed in triplicate and at a volume of 200 µL. HPLC yields and ees were determined by HPLC analysis against product standards (racemic where applicable).



Scheme 3. The 2-step telescoped cascade to (*S*)-**5a**-**7a** as the major regioisomers. Reaction conditions—Step 1: 50 mM HEPES (pH 7.5), 5 mM dopamine hydrochloride, 5 mM sodium ascorbate, 7.5 mM aldehyde, and 0.56 mg/mL purified *Tf*NCS, 5% v/v MeCN. Assays were left for 24 h at 37 °C, 800 rpm, and were carried out at a volume of 1 mL. HPLC yields and % ee values were determined by HPLC analysis against racemic product standards. Step 2: 50 mM HEPES (pH 7.5), 200 mM KCl, 20 mM $MgCl_2$, 20% v/v *Tf*NCS assay supernatant, 2.4 mM ATP, 2.4 mM *rac*-1, 0.14 mg/mL purified *Uu*MAT, 0.18 mg/mL purified *O*-MT (either *Mx*SafC or *Rn*COMT), and 0.05 mg/mL purified *Ec*MTAN. Assays were performed in triplicate and at a volume of 200 µL. HPLC yields and regioselectivity ratios were determined by HPLC analysis against synthetic product standards.

most likely due to subtle changes in the substrate orientation in the active site in the presence of the THIQs and SAA. The highest regioselectivities were achieved using (*S*)-**6** and *Rn*COMT, giving (*S*)-**6a** in good yield.

In addition to examining the *Rn*COMT and *Mx*SafC enzymes for allylations with (*S*)-THIQs, these *O*-MTs were also tested with *rac*-THIQs as the substrate; this was performed to determine any possible *Rn*COMT or *Mx*SafC *O*-allylation enantioselectivities. To this end, the *Uu*MAT-MT-*Ec*MTAN cascade was repeated with *rac*-**5**; however, no enantioselectivities were observed with *Rn*COMT or *Mx*SafC by chiral HPLC analysis.

3. Conclusion

In summary, here we report the successful regioselective *O*-allylation of a variety of catechol and THIQ substrates via the

MAT-MT-MTAN cascade in good yields. Firstly, it was observed that *Uu*MAT was the most effective MAT trialed for the formation of allyl-SAM from ATP and *S*-allyl-L-homocysteine, being able to catalyze this reaction at physiological temperatures with good conversions achieved. In addition, it was established that *Tf*NCS can accept analogues of dopamine as substrates, with the *para*-OH group being allylated, to give products in high ees. To the best of our knowledge, this is the first known example of NCSs accepting such analogues, and this opens up other opportunities for the structural diversification of this scaffold.

The design of cascades incorporating TfNCS to generate (*S*)-THIQ products with either the allylation step first (cascade A) or second (cascade B) was able to generate products in excellent to good regioselectivities, respectively. Indeed, adding the MAT-MT-MTAN cascade first, then NCS exploited the requirement of Pictet–Spengler condensations to have an unmodified C-3 hydroxyl group on the catechol substrate, thereby leading to 7-OH allylated (*S*)-THIQ products exclusively. Conversely, adding NCS first, then the MAT-MT-MTAN cascade with *Rn*COMT and *Mx*SafC as the O-MTs, leads to, in most cases, predominantly the 6-OH-allylated (*S*)-THIQs in regioisomer ratios of up to 12:1. In this manner, opposite regioselectivities in the (*S*)-THIQ products can be favored, which is an interesting strategy that can be employed in related enzymatic cascades in the future. In addition to this, it was also found that the use of the racemic allyl donor **1** was also compatible with this cascade. This is advantageous, given the ease of synthesis of *rac*-**1** relative to the multistep and low-yielding synthesis of the (*S*)-enantiomer.

4. Experimental Section

4.1. General Methods (Synthetic)

Reagents and standards were obtained from commercial suppliers, unless stated otherwise. Solvents were removed in vacuo using Büchi rotary evaporators. All thin layer chromatography (TLC) was performed using Merck aluminum-backed Silica Gel 60 F_{254} fluorescent-treated silica, which was visualized using basic potassium permanganate stain solution. Flash chromatography was performed using Geduran Silica Gel 60, with a particle size of 40–63 μm. ^{1}H and ^{13}C NMR spectra were recorded using Bruker Avance Neo 700, Bruker Avance III 600, and Bruker Avance Neo 500 spectrometers. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the respective residual solvent peak. Coupling constants (J) are quoted in Hertz (Hz). The ^{1}H NMR spectra are reported as follows: ppm (multiplicity, coupling constants, number of protons, assignment). Two-dimensional (COSY, HSQC, HMBC, NOESY/ROESY) NMR spectroscopy was used to assist the assignment. Infrared (IR) spectra were recorded on the Perkin Elmer Spectrum 100 FTIR Spectrometer operating in ATR mode. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). Low-resolution mass spectra (LRMS) were recorded on a Waters Acquity UPLC-SQD or Waters Acquity UPLC-SQD2, both operating in ESI mode. High-resolution mass spectra (HRMS) were recorded on a Waters LCT Premier XE Q-TOF mass spectrometer. Melting points (MP) were recorded on a Stuart SMP11 machine in degrees Celsius (°C). Optical rotations ($[\alpha]_D$) were recorded on a Bellingham + Stanley ADP430 polarimeter in the solvent indicated.

4.2. HPLC

Reverse-phase achiral preparative high-performance liquid chromatography was carried out on an Agilent 1260 Infinity II Analytical-Scale LC Purification System using a reverse-phase Discovery BIO Wide Pore C18 column (250 × 21.2 mm). Reverse-phase achiral analytical high-performance liquid chromatography was carried out on an Agilent 1260 Infinity II LC system using a reverse-phase ACE 5 C18-AR column (150 × 4.6 mm).

Normal-phase chiral high-performance liquid chromatography was carried out on an Agilent 1260 Infinity II LC system using a normal-phase ChiralPak AD-H column (250 × 4.6 mm) or a ChiralPak OD-H column (250 × 4.6 mm). Reverse-phase chiral high-performance liquid chromatography was carried out on an Agilent 1260 Infinity II LC system using a reverse-phase Supelco Astec Chirobiotic T Chiral column (25 cm × 4.6 mm) or a Supelco Astec Chirobiotic T2 Chiral column (25 cm × 4.6 mm).

4.3. Synthesis of HPLC Standards

1-Pentyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (**5**) and 1-benzyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol (**6**) were prepared as previously described.[24]

4.3.1. tert-*Butyl (3,4-Dihydroxyphenethyl)carbamate*

The procedure was adapted from the literature, where L-DOPA was the reactant.[40] A solution of dopamine hydrochloride (250 mg, 1.32 mmol) in aqueous $NaHCO_3$ (222 mg, 2.64 mmol in 3 mL water) was degassed with argon. Di-*tert*-butyl-dicarbonate (Boc_2O) (288 mg, 1.32 mmol) was then added alongside tetrahydrofuran (THF) (1.5 mL), and the resulting mixture stirred for 18 h at room temperature. The THF was removed in vacuo and the mixture diluted with water (1 mL), followed by extraction with ethyl acetate (EtOAc) (3 × 15 mL). The organic layer was washed with 1 M HCl (2 × 2 mL), then saturated $NaHCO_3$ (1 × 2 mL) and water (1 × 2 mL). The organic layer was dried with Na_2SO_4 and concentrated in vacuo to yield the product as a brown oil (303 mg, 91%). IR (film) ν_{max}/cm^{-1}: 3330, 2977, 2931, 1681, 1518; ^{1}H NMR (700 MHz; CD_3OD) δ 6.71 (d, J = 7.9 Hz, 1H, 5-H), 6.67 (s, 1H, 2-H), 6.55 (d, J = 7.9 Hz, 1H, 6-H), 3.22 (t, J = 7.4 Hz, 2H, 2′-H), 2.63 (t, J = 7.4 Hz, 2H, 1′-H), 1.46 (s, 9H, $COOC(\underline{CH_3})_3$); ^{13}C NMR (176 MHz; CD_3OD) δ 158.5, 146.2, 144.7, 132.2, 121.0, 116.9, 116.3, 79.9, 43.4, 36.6, 28.8; *m/z* [LRMS ESI−] 252 ($[M - H]^-$); *m/z* [HRMS ESI+] found $[M + H]^+$ 254.1382, $C_{13}H_{20}NO_4$ requires 254.1387.

4.3.2. 2-(Allyloxy)-4-(2-aminoethyl)phenol (2a)

The procedure was adapted from the literature, where L-DOPA was the reactant and non-allyl groups were added to the catechol hydroxyls.[41] A solution of *tert*-butyl (3,4-dihydroxyphenethyl)carbamate (248 mg, 0.98 mmol) in anhydrous dimethylformamide (DMF) (3 mL) was cooled to 0 °C and degassed with argon. Cesium carbonate (319 mg, 0.98 mmol) was then added, followed by allyl bromide (89 μL, 1.03 mmol) dropwise. The resulting mixture was stirred for 16 h. Formation of the intermediate was observed by TLC analysis (1:4 EtOAc:petroleum ether 40–60, R_f = 0.26), and the mixture was diluted with water (7 mL) and extracted with methyl *tert*-butyl ether (MBTE) (2 × 5 mL). The organic layer was washed with water (5 mL), then brine (2.5 mL), dried with Na_2SO_4 and concentrated in vacuo to yield the Boc-allylated intermediate. This was resuspended in dichloromethane (CH_2Cl_2) (2.8 mL) and degassed with argon, followed by the addition of trifluoroacetic acid (TFA) (2 mL). The mixture was stirred for 2 h,

then the solvent was removed in vacuo to yield the crude product. This was purified by reverse-phase preparative HPLC using Method A1 (see Supporting Information 3.1). The product fractions were combined, and the solvent removed in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a colorless solid (24 mg, 11%). MP 174–176 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3010, 2817, 2753, 1444; ^1H NMR (500 MHz; D_2O) δ 6.99 (d, J = 1.7 Hz, 1H, 2-H), 6.93 (d, J = 8.1 Hz, 1H, 5-H), 6.84 (dd, J = 8.1, 1.7 Hz, 1H, 6-H), 6.10 (ddt, J = 17.4, 10.7, 5.5 Hz, 1H, $OCH_2CH = CH_2$), 5.42 (dd, J = 17.4, 1.3 Hz, 1H, $OCH_2CH = CHH$), 5.33 (dd, J = 10.7, 1.3 Hz, 1H, $OCH_2CH = CHH$), 4.67 (d, J = 5.5 Hz, 2H, $OCH_2CH = CH_2$), 3.24 (t, J = 7.3 Hz, 2H, 2′-H), 2.92 (t, J = 7.3 Hz, 2H, 1′-H); ^{13}C NMR (126 MHz; D_2O) δ 147.0, 145.1, 133.8, 129.8, 123.0, 119.3, 116.9, 115.8, 70.8, 41.5, 33.0; m/z [LRMS ESI+] 194 ($[M + H]^+$); m/z [HRMS ESI+] found $[M + H]^+$ 194.1171, $C_{11}H_{16}NO_2$ requires 194.1176; preparative HPLC retention time = 7.8 min.

4.3.3. 2-(Allyloxy)-5-(2-aminoethyl)phenol (2b)

2-(Allyloxy)-5-(2-aminoethyl)phenol was co-synthesized with **2a** using the same procedure and purified via the same reverse-phase preparative HPLC method on account of the different retention time. The product fractions were combined, and the solvent was removed in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a white solid (10 mg, 5%). MP: 113–114 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3134, 2964, 2933, 2863; ^1H NMR (500 MHz; D_2O) δ 7.05 (d, J = 8.3 Hz, 1H, 5-H), 6.88 (d, J = 1.3 Hz, 1H, 2-H), 6.84 (dd, J = 8.3, 1.3 Hz, 1H, 6-H), 6.09 (ddt, J = 16.5, 10.5, 5.5 Hz, 1H, $OCH_2CH = CH_2$), 5.41 (d, J = 16.5 Hz, 1H, $OCH_2CH = CHH$), 5.33 (d, J = 10.5 Hz, 1H, $OCH_2CH = CHH$), 4.66 (d, J = 5.5 Hz, 2H, $OCH_2CH = CH_2$), 3.24 (t, J = 7.2 Hz, 2H, 2′-H), 2.90 (t, J = 7.2 Hz, 2H, 1′-H); ^{13}C NMR (126 MHz; D_2O) δ 146.3, 145.9, 133.8, 131.2, 121.8, 119.4, 117.0, 115.8, 70.9, 41.4, 32.8; m/z [LRMS ESI+] 194 ($[M + H]^+$); m/z [HRMS ESI+] found $[M + H]^+$ 194.1175, $C_{11}H_{16}NO_2$ requires 194.1176; preparative HPLC retention time: 8.6 min.

4.3.4. Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(3,4-dihydroxyphenyl)propanoate[40]

A solution of thionyl chloride (0.498 mL, 6.86 mmol) in methanol (MeOH) (3 mL) was cooled to 0 °C and degassed with argon. L-DOPA (250 mg, 1.27 mmol) was then slowly added, and the resulting mixture stirred for 24 h. The solvent was removed in vacuo and resuspended in water (3 mL) with $NaHCO_3$ added (213 mg, 2.54 mmol), then tetrahydrofuran (THF) (1.5 mL) along with Boc_2O (272 mg, 1.27 mmol) and the mixture degassed with argon and stirred for 18 h. THF was then in vacuo and the mixture was diluted with water (1 mL), followed by extraction with EtOAc (3 × 15 mL). The organic layer was washed with 1 M HCl (2 × 2 mL), then saturated $NaHCO_3$ (2 mL) and water (2 mL). The organic layer was dried with Na_2SO_4 and concentrated in vacuo to yield the product as a pink oil (315 mg, 80%). R_f (1:4 EtOAc:petroleum ether 40–60) = 0.07; $[\alpha]_D$ + 32 (c 0.5, $CHCl_3$, 24 °C); IR (film) ν_{max}/cm^{-1}: 3358, 2978, 1682, 1517; ^1H NMR (500 MHz; CD_3OD) δ 6.68 (d, J = 8.0 Hz, 1H, 5-H), 6.63 (d, J = 1.8 Hz, 1H, 2-H), 6.51 (dd, J = 8.0, 1.8 Hz, 1H, 6-H), 4.29 (dd, J = 8.4, 5.9 Hz, 1H, 2′-H), 3.69 (s, 3H, $COOCH_3$), 2.93 (dd, J = 13.8, 5.9 Hz, 1H, 1′-HH), 2.79 (dd, J = 13.8, 8.4 Hz, 1H, 1′-HH), 1.41 (s, 9H, $COOC(CH_3)_3$); ^{13}C NMR (126 MHz; CD_3OD) δ 174.4, 157.8, 146.3, 145.3, 129.6, 121.6, 117.3, 116.3, 80.7, 56.8, 52.5, 38.2, 28.7; m/z [LRMS ESI+] 312 ($[M + H]^+$); m/z [HRMS ESI+] found $[M + H]^+$ 312.1441, $C_{15}H_{22}NO_6$ requires 312.1441.

4.3.5. (S)-3-(3-(Allyloxy)-4-hydroxyphenyl)-2-aminopropanoic Acid (3a)

This procedure was adapted from literature, where non-allyl groups were added to the catechol hydroxyls of L-DOPA.[41] A solution of methyl (*S*)-2-((tert-butoxycarbonyl)amino)-3-(3,4-dihydroxyphenyl)propanoate (638 mg, 2.05 mmol) in anhydrous DMF (6 mL) was cooled to 0 °C and degassed with argon. Cesium carbonate (668 mg, 2.05 mmol) was then added, followed by allyl bromide (186 µL, 2.15 mmol) dropwise. The resulting mixture was stirred for 16 h. Formation of the intermediate was observed by TLC analysis (1:4 EtOAc:petroleum ether 40–60, R_f = 0.17). The mixture was then diluted with water (14 mL) and extracted with MBTE (2 × 10 mL). The organic layer was washed with water (10 mL), then brine (5 mL) and dried with Na_2SO_4 and concentrated in vacuo to yield the crude Boc-methylated-allylated intermediate. This was resuspended in dichloromethane (6.75 mL) and degassed with argon, followed by the addition of TFA (4.5 mL). The mixture was stirred for 2 h, the solvent was removed in vacuo, then resuspended in THF (6.75 mL) at 0 °C and degassed with argon, with 5 M aqueous lithium hydroxide (LiOH) then added dropwise (6.75 mL). The mixture was stirred for 2 h at room temperature, after which it was neutralized to pH 7 by the dropwise addition of 6 M HCl and the solvent was removed in vacuo to yield the crude product. This was purified by reverse-phase preparative HPLC using Method A1 (Supporting Information 3.1). The product fractions were combined and dried in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a pale orange solid (94 mg, 17%). $[\alpha]_D$ -11 (c 0.5, H_2O, 25 °C); MP 164–166 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 2925, 1731, 1602, 1513, 1439, 1424; ^1H NMR (500 MHz; CD_3OD) δ 6.85 (d, J = 1.8 Hz, 1H, 2-H), 6.79 (d, J = 8.1 Hz, 1H, 5-H), 6.72 (dd, J = 8.1, 1.8 Hz, 1H, 6-H), 6.09 (ddt, J = 17.4, 10.6, 5.4 Hz, 1H, $OCH_2CH = CH_2$), 5.40 (dd, J = 17.4, 1.3 Hz, 1H, $OCH_2CH = CHH$), 5.25 (dd, J = 10.6, 1.3 Hz, 1H, $OCH_2CH = CHH$), 4.59 (d, J = 5.4 Hz, 2H, $OCH_2CH = CH_2$), 4.18 (dd, J = 7.6, 5.3 Hz, 1H, 2′-H), 3.20 (dd, J = 14.6, 5.3 Hz, 1H, 1′-HH), 3.05 (dd, J = 14.6, 7.6 Hz, 1H, 1′-HH); ^{13}C NMR (126 MHz; CD_3OD) δ 171.3, 148.3, 147.8, 134.9, 126.4, 123.5, 118.0, 117.0, 115.8, 71.0, 55.2, 36.9; m/z [LRMS ESI+] 238 ($[M + H]^+$); m/z [HRMS ESI+] found $[M + H]^+$ 238.1075, $C_{12}H_{16}NO_4$ requires 238.1074; preparative HPLC retention time = 7.6 min.

4.3.6. (S)-3-(4-(Allyloxy)-3-hydroxyphenyl)-2-aminopropanoic Acid (3b)

(*S*)-3-(4-(Allyloxy)-3-hydroxyphenyl)-2-aminopropanoic acid was co-synthesized with **3a** in the same procedure and purified via the same reverse-phase preparative HPLC method on account of the different retention time. The product fractions were combined, and the solvent was removed in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a pale orange solid (150 mg, 27%). $[\alpha]_D$ -19 (c 0.5, H_2O, 25 °C); MP: 180–182 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 2866, 1731, 1595, 1508, 1486, 1446, 1423; ^1H NMR (500 MHz; CH_3OD) δ 6.91 (d, J = 8.2 Hz, 1H, 5-H), 6.79 (d, J = 1.8 Hz, 1H, 2-H), 6.71 (dd, J = 8.2, 1.8 Hz, 1H, 6-H), 6.09 (ddt, J = 17.4, 10.6, 5.4 Hz, 1H, $OCH_2CH = CH_2$), 5.40 (dd, J = 17.4, 1.3 Hz, 1H, $OCH_2CH = CHH$), 5.26 (dd, J = 10.6, 1.3 Hz, 1H, $OCH_2CH = CHH$), 4.61 (d, J = 5.4 Hz, 2H, $OCH_2CH = CH_2$), 4.18 (dd, J = 7.7, 5.4 Hz, 1H, 2′-H), 3.21 (dd, J = 14.7, 5.4 Hz, 1H, 1′-HH), 3.03 (dd, J = 14.7, 7.7 Hz, 1H, 1′-HH); ^{13}C NMR (176 MHz; CH_3OD) δ 171.3, 148.5, 147.7, 135.0, 128.4, 121.6, 117.9, 117.6, 115.1, 71.0, 55.2, 36.7; m/z [LRMS ESI+] 238 ($[M + H]^+$); m/z [HRMS ESI+] found $[M + H]^+$ 238.1070, $C_{12}H_{16}NO_4$ requires 238.1074; Preparative HPLC retention time: 8.2 min.

4.3.7. Methyl 3,4-Dihydroxybenzoate

A solution of thionyl chloride (0.636 mL, 8.76 mmol) in MeOH (3 mL) was cooled to 0 °C and degassed with argon. 3,4-Dihydroxybenzoic acid (250 mg, 1.62 mmol) was then slowly added, and the resulting mixture was stirred for 24 h. The solvent was removed in vacuo, and the mixture was resuspended in water (2 mL), followed by extraction with EtOAc (3 × 15 mL). The organic layer was washed with 1 M HCl (2 × 2 mL), saturated $NaHCO_3$ (2 mL), and water (2 mL), then dried with Na_2SO_4, and concentrated in vacuo to yield the product as a white solid (252 mg, 92%). R_f (1:4 EtOAc:petroleum ether 40–60) = 0.08; MP: 124–126 °C (EtOAc); IR (film) ν_{max}/cm^{-1}: 3273, 2955, 1684, 1601, 1523, 1436; ^1H NMR (500 MHz; CD_3OD) δ 7.42 (m, 2H, 6-H and 2-H), 6.81 (d, J = 8.7 Hz, 1H, 5-H), 3.84 (s, 3H, $COOCH_3$); ^{13}C NMR (126 MHz; CD_3OD) δ 168.9, 151.7, 146.2, 123.6, 122.7, 117.5, 115.9, 52.2; *m/z* [LRMS ESI+] 169 ($[M + H]^+$); *m/z* [HRMS ESI−] found $[M - H]^-$ 167.0338, $C_8H_7O_4$ requires 167.0350.

4.3.8. 4-(Allyloxy)-3-hydroxybenzoic Acid (4b)

This procedure was adapted from literature, where non-allyl groups were added to the catechol hydroxyls of L-DOPA.[41] A solution of methyl 3,4-dihydroxybenzoate (255 mg, 1.52 mmol) in anhydrous DMF (3 mL) was cooled to 0 °C and degassed with argon. Cesium carbonate (495 mg, 1.52 mmol) was then added, followed by allyl bromide (138 µL, 1.59 mmol) dropwise. The resulting mixture was stirred for 16 h, after which the intermediate formation was observed by TLC analysis (1:4 EtOAc:petroleum ether 40–60, R_f = 0.40). The mixture was then diluted with water (7 mL) and extracted with MBTE (2 × 5 mL). The organic layer was washed with water (5 mL), then brine (2.5 mL), and dried with Na_2SO_4 and concentrated in vacuo to yield the crude methylated-allylated intermediate. The intermediate was then resuspended in THF (4.5 mL) at 0 °C and degassed with argon, with 5 M aqueous LiOH then being added dropwise (4.5 mL). The mixture was stirred for 2 h at room temperature, after which it was neutralized to pH 7 by the dropwise addition of 6 M HCl and the solvent was removed in vacuo to yield the crude product. This was purified by reverse-phase preparative HPLC using Method A2 (Supporting Information: 3.1). The product fractions were combined and dried in vacuo, then lyophilized to yield the product as a white solid (24 mg, 8%). MP 161–163 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3402, 2872, 2559, 1682, 1614, 1582, 1512; ^1H NMR (500 MHz; CD_3OD) δ 7.54 (dd, J = 8.5, 2.1 Hz, 1H, 6-H), 7.48 (d, J = 2.1 Hz, 1H, 2-H), 7.00 (d, J = 8.5 Hz, 1H, 5-H), 6.13 (ddt, J = 17.3, 10.7, 5.4 Hz, 1H, $OCH_2CH = CH_2$), 5.45 (dd, J = 17.3, 1.5 Hz, 1H, $OCH_2CH = CHH$), 5.31 (dd, J = 10.7, 1.5 Hz, 1H, $OCH_2CH = CHH$), 4.71 (d, J = 5.4 Hz, 2H, $OCH_2CH = CH_2$); ^{13}C NMR (126 MHz; CD_3OD) δ 169.9, 152.1, 147.6, 134.5, 124.7, 123.5, 118.2, 117.7, 113.5, 70.7; *m/z* [LRMS ESI+] 195 ($[M + H]^+$); *m/z* [HRMS ESI+] found $[M + H]^+$ 195.0651, $C_{10}H_{11}O_4$ requires 195.0652; preparative HPLC retention time = 8.7 min.

4.3.9. 3-(Allyloxy)-4-hydroxybenzoic Acid (4a)

An aqueous solution of 3,4-dihydroxybenzoic acid 4 (61.6 mg, 5 mM) mixed with HEPES (50 mM; pH 7.5), KCl (200 mM), $MgCl_2$ (20 mM), sodium ascorbate (5 mM), ATP (12.5 mM), and (*RS*)-S-allylhomocysteine. TFA (289 mg, 12.5 mM), 1.36 mg/mL *Uu*MAT clarified lysate (27% of total protein), 1.61 mg/mL *Rn*COMT clarified lysate (25% of total protein), and 0.46 mg/mL *Ec*MTAN clarified lysate (28% of total protein) and were made up to a total volume of 80 mL with distilled water. The mixture was incubated for 24 h at 37 °C and 200 rpm, then quenched with 8 mL 2 M HCl and centrifuged (3,900 rpm, 15 min, 4 °C). The supernatant volume was then reduced in vacuo and then purified by reverse-phase preparative HPLC using Method A3 (see 3.1.). The product fractions were combined, and the solvent was removed in vacuo, then lyophilized to yield product as a white solid (20 mg, 25%). MP: 135–137 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3512, 3373, 2931, 2874, 2561, 1673, 1595, 1516; ^1H NMR (500 MHz; CD_3OD) δ 7.56 (m, 2H, 2-H and 6-H), 6.86 (d, J = 8.8 Hz, 1H, 5-H), 6.11 (ddt, J = 17.4, 10.6, 5.4 Hz, 1H, $OCH_2CH = CH_2$), 5.43 (dd, J = 17.4, 1.3 Hz, 1H, $OCH_2CH = CHH$), 5.28 (dd, J = 10.6, 1.3 Hz, 1H, $OCH_2CH = CHH$), 4.66 (d, J = 5.4 Hz, 2H, $OCH_2CH = CH_2$); ^{13}C NMR (126 MHz; CD_3OD) δ 170.0, 153.0, 147.4, 134.7, 125.5, 123.0, 118.2, 116.1, 115.9, 71.0; *m/z* [LRMS ESI+] 195 ($[M + H]^+$); *m/z* [HRMS ESI+] found $[M + H]^+$ 195.0651, $C_{10}H_{11}O_4$ requires 195.0652; preparative HPLC retention time: 12.6 min.

4.3.10. 1-(But-3-en-1-yl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol (7)

This procedure was adapted from the literature.[24] An aqueous solution of dopamine hydrochloride (500 mg, 2.64 mmol) in 0.3 M sodium phosphate (pH 6) (5 mL) was mixed with sodium ascorbate (258 mg, 1.32 mmol) and acetonitrile (5 mL). The mixture was degassed with argon, and 4-pentenal (521 µL, 5.27 mmol) was added. The reaction was stirred at 55 °C for 16 h. The resulting mixture was concentrated in vacuo to yield the crude product. This was then purified by reverse-phase preparative HPLC using Method A6 (Supporting Information: 3.1). The product fractions were combined, and the solvent was removed in vacuo, then resuspended in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as an orange solid (440 mg, 65%). MP: 56–58 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3370, 3113, 2936, 2782, 1614, 1523; ^1H NMR (500 MHz; CD_3OD) δ 6.66 (s, 1H, 8-H), 6.62 (s, 1H, 5-H), 5.90 (ddt, J = 17.0, 10.3, 6.6 Hz, 1H, 11-H), 5.17 (dd, J = 17.0, 1.6 Hz, 1H, 12-HH), 5.09 (dd, J = 10.3, 1.6 Hz, 1H, 12-HH), 4.37 (dd, J = 8.3, 5.7 Hz, 1H, 1-H), 3.52 (m, 1H, 3-HH), 3.35 (m, 1H, 3-HH)*, 3.05–2.88 (m, 2H, 4-H), 2.28 (m, 2H, 10-H), 2.12 (m, 1H, 9-HH), 2.00 (m, 1H, 9-HH); ^{13}C NMR (126 MHz; CD_3OD) δ 146.8, 145.9, 137.8, 124.0, 123.7, 116.7, 116.3, 113.9, 56.0, 40.8, 34.5, 30.5, 25.6; *m/z* [LRMS ESI+] 220 ($[M + H]^+$); *m/z* [HRMS ESI+] found $[M + H]^+$ 220.1327, $C_{13}H_{18}NO_2$ requires 220.1332; preparative HPLC retention time: 6.1 min.

4.3.11. 6-(Allyloxy)-1-pentyl-1,2,3,4-tetrahydroisoquinolin-7-ol (5a)

A solution of 1-pentyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol hydrochloride (200 mg, 0.74 mmol) in aqueous $NaHCO_3$ (124 mg, 1.47 mmol in 10 mL water) was degassed with argon. Boc_2O (161 mg, 0.74 mmol) was then added alongside THF (5 mL), and the resulting mixture stirred for 18 h at room temperature. The THF was removed in vacuo and the mixture diluted with water (8 mL), followed by extraction with ethyl acetate (EtOAc) (3 × 15 mL). The organic layer was washed with 1 M HCl (2 × 8 mL), then saturated $NaHCO_3$ (2 × 8 mL) and water (8 mL). The organic layer was dried with Na_2SO_4 and concentrated in vacuo to yield the crude Boc-protected intermediate which was confirmed by LCMS [ESI-] (m/z of 334, $[M - H]^-$). This was then resuspended in anhydrous DMF (5 mL), cooled to 0 °C and degassed with argon. Cesium carbonate (208 mg, 0.64 mmol) was then added, followed by allyl bromide (55 µL, 0.64 mmol) dropwise. The resulting mixture was stirred for 16 h at room temperature, after which the formation of the O-allylated intermediate was observed by TLC analysis (1:4 EtOAc:petroleum ether 40–60, R_f = 0.52). The mixture was then diluted with water (6 mL) and extracted with MBTE (2 × 5 mL). The organic layer was washed with water (6 mL), then brine (3 mL), dried with Na_2SO_4 and concentrated in vacuo. This material was resuspended in dichloromethane (4 mL) and degassed with argon, followed by the addition of TFA (2 mL). The mixture was stirred for 2 h, and the solvent was removed in vacuo to yield the crude product, which was purified by reverse-phase preparative HPLC using Method A7 (Supporting Information: 3.1). The product fractions were

combined, and the solvent was removed in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a white solid (23 mg, 10%). MP 134–136 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3182, 3149, 2923, 2852, 2777, 2632, 2552, 1611, 1594, 1527, 1511; ^1H NMR (500 MHz; CD_3OD) δ 6.78 (s, 1H, 5-H), 6.72 (s, 1H, 8-H), 6.09 (ddt, J = 17.3, 10.6, 5.3 Hz, 1H, $OCH_2CH = CH_2$), 5.41 (dq, J = 17.3, 1.6 Hz, 1H, $OCH_2CH = CHH$), 5.27 (dq, J = 10.6, 1.6 Hz, 1H, $OCH_2CH = CHH$), 4.62 (dt, J = 5.3, 1.6 Hz, 2H, $OCH_2CH = CH_2$), 4.38 (dd, J = 8.0, 4.8 Hz, 1H, 1-H), 3.54 (m, 1H, 3-HH), 3.36 (m, 1H, 3-HH)*, 3.02 (m, 2H, 4-H), 2.05 (m, 1H, 9-HH), 1.89 (m, 1H, 9-HH), 1.52 (m, 2H, 10-H), 1.42 (m, 4H, 11-H and 12-H), 0.96 (t, J = 6.9 Hz, 3H, 13-H); ^{13}C NMR (126 MHz; CD_3OD) δ 147.9, 147.3, 134.7, 125.9, 123.5, 118.0, 114.5, 114.1, 70.8, 56.6, 40.9, 35.0, 32.7, 26.1, 25.8, 23.5, 14.3; *m/z* [LRMS ESI+] 276 ($[M + H]^+$); *m/z* [HRMS ESI+] found $[M + H]^+$ 276.1954, $C_{17}H_{26}NO_2$ requires 276.1958; preparative HPLC retention time = 22.2 min.

4.3.12. 7-(Allyloxy)-1-pentyl-1,2,3,4-tetrahydroisoquinolin-6-ol (5b)

7-(Allyloxy)-1-pentyl-1,2,3,4-tetrahydroisoquinolin-6-ol was co-synthesized with **5a** in the same procedure and purified via the same reverse-phase preparative HPLC method on account of the different retention time. The product fractions were combined, and the solvent was removed in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a white solid (25 mg, 11%). Preparative HPLC retention time = 23.1 min. MP: 137–139 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3182, 2951, 2929, 2867, 2806, 1594, 1530; ^1H NMR (500 MHz; CD_3OD) δ 6.78 (s, 1H, 8-H), 6.67 (s, 1H, 5-H), 6.08 (ddt, J = 17.3, 10.6, 5.4 Hz, 1H, $OCH_2CH = CH_2$), 5.39 (dq, J = 17.3, 1.5 Hz, 1H, $OCH_2CH = CHH$), 5.26 (dq, J = 10.6, 1.5 Hz, 1H, $OCH_2CH = CHH$), 4.62 (dt, J = 5.4, 1.5 Hz, 2H, $OCH_2CH = CH_2$), 4.40 (dd, J = 8.1, 5.2 Hz, 1H, 1-H), 3.52 (m, 1H, 3-HH), 3.34 (m, 1H, 3-HH)*, 3.01 (m, 1H, 4-HH), 2.93 (m, 1H, 4-HH), 2.06 (m, 1H, 9-HH), 1.90 (m, 1H, 9-HH), 1.51 (m, 2H, 10-H), 1.42 (m, 4H, 11-H and 12-H), 0.95 (t, J = 6.9 Hz, 3H, 13-H); ^{13}C NMR (126 MHz; CD_3OD) δ 148.2, 147.2, 135.0, 125.5, 124.1, 118.0, 116.5, 112.8, 71.1, 56.8, 40.7, 35.1, 32.8, 26.1, 25.7, 23.5, 14.3; *m/z* [LRMS ESI+] 276 ($[M + H]^+$); *m/z* [HRMS ESI+] found $[M + H]^+$ 276.1956, $C_{17}H_{26}NO_2$ requires 276.1958.

4.3.13. 6-(Allyloxy)-1-benzyl-1,2,3,4-tetrahydroisoquinolin-7-ol (6a)

A solution of 1-benzyl-1,2,3,4-tetrahydroisoquinoline-6,7-diol hydrochloride (200 mg, 0.69 mmol) in aqueous $NaHCO_3$ (115 mg, 1.37 mmol in 10 mL water) was degassed with argon. Boc_2O (150 mg, 0.69 mmol) was then added alongside THF (5 mL), and the resulting mixture stirred for 18 h at room temperature. The THF was removed in vacuo and the mixture diluted with water (8 mL), followed by extraction with ethyl acetate (EtOAc) (3 × 15 mL). The organic layer was washed with 1 M HCl (2 × 8 mL), then saturated $NaHCO_3$ (2 × 8 mL) and water (8 mL). The organic layer was dried with Na_2SO_4 and concentrated in vacuo to yield the Boc-protected intermediate, which was confirmed by LCMS [ESI+] (*m/z* 356, $[M - H]^+$). This was resuspended in anhydrous DMF (5 mL), cooled to 0 °C and degassed with argon. Cesium carbonate (176 mg, 0.54 mmol) was then added, followed by allyl bromide (47 µL, 0.54 mmol) dropwise. The resulting mixture was stirred for 16 h at room temperature, after which the formation of the O-allylated intermediate was observed by TLC analysis (1:4 EtOAc:petroleum ether 40–60, R_f = 0.43). The mixture was concentrated in vacuo, diluted with water (6 mL) and extracted with MBTE (2 × 5 mL). The organic layer was washed with water (6 mL), then brine (3 mL), dried with Na_2SO_4 and concentrated in vacuo. This intermediate was then suspended in dichloromethane (4 mL) and degassed with argon, followed by the addition of TFA (2 mL). The mixture was stirred for 2 h, and the solvent was removed in vacuo to yield the crude product, which was purified by reverse-phase preparative HPLC using Method A7 (Supporting Information: 3.1). The product fractions were combined, and the solvent removed in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a white solid (40 mg, 18%). MP 179–181 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3064, 2920, 2779, 1614, 1528; ^1H NMR (600 MHz; CD_3OD) δ 7.38–7.29 (m, 5H, 11-H and 12-H and 13-H), 6.76 (s, 1H, 5-H), 6.62 (s, 1H, 8-H), 6.06 (ddt, J = 17.3, 10.6, 5.3 Hz, 1H, $OCH_2CH = CH_2$), 5.37 (dq, J = 17.3, 1.7 Hz, 1H, $OCH_2CH = CHH$), 5.23 (dq, J = 10.6, 1.7 Hz, 1H, $OCH_2CH = CHH$), 4.67 (dd, J = 8.7, 5.8 Hz, 1H, 1-H), 4.58 (dt, J = 5.3, 1.7 Hz, 2H, $OCH_2CH = CH_2$), 3.46 (m, 2H, 3-HH and 9-HH), 3.25 (m, 1H, 3-HH)*, 3.04 (m, 2H, 4-HH and 9-HH), 2.94 (dt, J = 17.0, 6.2 Hz, 1H, 4-HH); ^{13}C NMR (151 MHz; CD_3OD) δ 148.1, 147.1, 136.6, 134.7, 130.6, 130.3, 128.8, 125.2, 123.6, 118.0, 114.4, 114.4, 70.8, 57.7, 41.2, 40.9, 25.9; *m/z* [LRMS ESI+] 296 ($[M + H]^+$); *m/z* [HRMS ESI+] found $[M + H]^+$ 296.1643, $C_{19}H_{22}NO_2$ requires 296.1645; preparative HPLC retention time = 18.9 min.

4.3.14. 7-(Allyloxy)-1-benzyl-1,2,3,4-tetrahydroisoquinolin-6-ol (6b)

7-(Allyloxy)-1-benzyl-1,2,3,4-tetrahydroisoquinolin-6-ol was co-Synthesized with **6a** using the same procedure and purified via the same reverse-phase preparative HPLC method on account of the different retention time. The product fractions were combined, and the solvent was removed in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a white solid (32 mg, 14%). Preparative HPLC retention time = 20.3 min. MP: 172–173 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3174, 3145, 3062, 3027, 2916, 2691, 1613, 1582, 1533, 1496; ^1H NMR (700 MHz; CD_3OD) δ 7.43 (t, J = 7.3 Hz, 2H, 12-H), 7.38 (t, J = 7.3 Hz, 1H, 13-H), 7.35 (d, J = 7.3 Hz, 2H, 11-H), 6.71 (s, 1H, 5-H), 6.49 (s, 1H, 8-H), 6.00 (ddt, J = 17.3, 10.6, 5.4 Hz, 1H, $OCH_2CH = CH_2$), 5.33 (dq, J = 17.3, 1.6 Hz, 1H, $OCH_2CH = CHH$), 5.23 (dq, J = 10.6, 1.6 Hz, 1H, $OCH_2CH = CHH$), 4.73 (t, J = 7.5 Hz, 1H, 1-H), 4.40 (tt, J = 5.4, 1.6 Hz, 2H, $OCH_2CH = CH_2$), 3.56 (m, 1H, 3-HH), 3.43 (dd, J = 14.0, 7.5 Hz, 1H, 9-HH), 3.37 (m, 1H, 3-HH)*, 3.20 (dd, J = 14.0, 7.5 Hz, 1H, 9-HH), 3.03 (m, 2H, 4-H); ^{13}C NMR (176 MHz; CD_3OD) δ 148.3, 146.8, 136.9, 134.7, 130.8, 130.2, 128.8, 125.3, 123.2, 118.0, 116.5, 113.1, 70.9, 57.6, 41.3, 40.3, 25.6; *m/z* [LRMS ESI+] 296 ($[M + H]^+$); *m/z* [HRMS ESI+] found $[M + H]^+$ 296.1642, $C_{19}H_{22}NO_2$ requires 296.1645.

4.3.15. 6-(Allyloxy)-1-(but-3-en-1-yl)-1,2,3,4-tetrahydroisoquinolin-7-ol (7a)

A solution of 1-(but-3-en-1-yl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol hydrochloride (200 mg, 0.78 mmol) in aqueous $NaHCO_3$ (131 mg, 1.56 mmol in 10 mL water) was degassed with argon. Boc_2O (171 mg, 0.78 mmol) was then added alongside THF (5 mL), and the resulting mixture stirred for 18 h at room temperature. The THF was removed in vacuo and the mixture diluted with water (8 mL), followed by extraction with EtOAc (3 × 15 mL). The organic layer was washed with 1 M HCl (2 × 8 mL), then saturated $NaHCO_3$ (2 × 8 mL) and water (8 mL). The organic layer was dried with Na_2SO_4 and concentrated in vacuo to yield the crude Boc-protected intermediate, which was confirmed by LCMS [ESI+] (*m/z* 320, $[M - H]^+$). This intermediate was then resuspended in anhydrous DMF (5 mL), then cooled to 0 °C and degassed with argon. Cesium carbonate (200 mg, 0.61 mmol) was added, followed by allyl bromide (53 µL, 0.61 mmol) dropwise. The resulting mixture was stirred for 16 h at room temperature, and the intermediate was observed by TLC analysis (1:4

EtOAc:petroleum ether 40–60, R_f = 0.54). The mixture was concentrated in vacuo, diluted with water (6 mL) and extracted with MBTE (2 × 5 mL). The organic layer was washed with water (6 mL), and brine (3 mL) and dried with Na_2SO_4 and concentrated in vacuo, then resuspended in dichloromethane (4 mL) and degassed with argon, followed by the addition of TFA (2 mL). The mixture was stirred for 2 h, the solvent was removed in vacuo and the crude was purified by reverse-phase preparative HPLC using Method A7 (Supporting Information: 3.1). The product fractions were combined, and the solvent was removed in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a white solid (36 mg, 16%). MP 187–189 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3488, 2924, 2740, 2632, 2548, 1594, 1516; ^{1}H NMR (600 MHz; CD_3OD) δ 6.74 (s, 1H, 5-H), 6.67 (s, 1H, 8-H), 6.04 (ddt, J = 17.4, 10.6, 5.3 Hz, 1H, OCH_2CH = CH_2), 5.87 (ddt, J = 17.1, 10.3, 6.6 Hz, 1H, 11-H), 5.36 (dq, J = 17.4, 1.6 Hz, 1H, OCH_2CH = CHH), 5.22 (dq, J = 10.6, 1.6 Hz, 1H, OCH_2CH = CHH), 5.13 (dq, J = 17.1, 1.6 Hz, 1H, 12-HH), 5.05 (dq, J = 10.3, 1.6 Hz, 1H, 12-HH), 4.57 (dt, J = 5.3, 1.6 Hz, 2H, OCH_2CH = CH_2), 4.37 (dd, J = 7.9, 5.0 Hz, 1H, 1-H), 3.51 (m, 1H, 3-HH), 3.32 (m, 1H, 3-HH), 3.01 (m, 1H, 4-HH), 2.93 (m, 1H, 4-HH), 2.24 (m, 2H, 10-H), 2.10 (m, 1H, 9-HH), 1.96 (m, 1H, 9-HH); ^{13}C NMR (151 MHz; CD_3OD) δ 148.0, 147.3, 137.8, 134.7, 125.6, 123.5, 118.0, 116.7, 114.5, 114.1, 70.8, 55.9, 40.8, 34.4, 30.5, 25.8; *m/z* [LRMS ESI+] 260 ([M + H]$^+$); *m/z* [HRMS ESI+] found [M + H]$^+$ 260.1644, $C_{16}H_{22}NO_2$ requires 260.1645; preparative HPLC retention time = 17.5 min.

4.3.16. 7-(Allyloxy)-1-(but-3-en-1-yl)-1,2,3,4-tetrahydroisoquinolin-6-ol (7b)

7-(Allyloxy)-1-(but-3-en-1-yl)-1,2,3,4-tetrahydroisoquinolin-6-ol was co-synthesized with **7a** in the same procedure and purified via the same reverse-phase preparative HPLC method on account of its different retention time. The product fractions were combined, and the solvent was removed in vacuo, then redissolved in 0.5 M HCl and lyophilized to yield the hydrochloride salt of the product as a white solid (28 mg, 12%). Preparative HPLC retention time = 18.3 min. MP: 137–138 °C (H_2O); IR (film) ν_{max}/cm^{-1}: 3225, 2944, 2911, 2792, 2708, 2565, 1614, 1592, 1531, 1462, 1428, 1415; ^{1}H NMR (700 MHz; CD_3OD) δ 6.80 (s, 1H, 8-H), 6.69 (s, 1H, 5-H), 6.09 (ddt, J = 17.3, 10.7, 5.5 Hz, 1H, OCH_2CH = CH_2), 5.92 (ddt, J = 17.0, 10.3, 6.5 Hz, 1H, 11-H), 5.41 (dq, J = 17.3, 1.6 Hz, 1H, OCH_2CH = CHH), 5.27 (dq, J = 10.7, 1.6 Hz, 1H, OCH_2CH = CHH), 5.18 (dq, J = 17.0, 1.5 Hz, 1H, 12-HH), 5.10 (dq, J = 10.3, 1.5 Hz, 1H, 12-HH), 4.63 (dt, J = 5.5, 1.6 Hz, 2H, OCH_2CH = CH_2), 4.44 (dd, J = 7.6, 5.2 Hz, 1H, 1-H), 3.55 (m, 1H, 3-HH), 3.36 (m, 1H, 3-HH), 3.03 (m, 1H, 4-HH), 2.96 (m, 1H, 4-HH), 2.29 (m, 2H, 10-H), 2.16 (m, 1H, 9-HH), 2.03 (m, 1H, 9-HH); ^{13}C NMR (176 MHz; CD_3OD) δ 148.3, 147.2, 137.9, 134.9, 125.6, 123.8, 118.0, 116.6, 116.6, 112.9, 71.1, 56.1, 40.6, 34.5, 30.5, 25.6; *m/z* [LRMS ESI+] 260 ([M + H]$^+$); *m/z* [HRMS ESI+] found [M + H]$^+$ 260.1640, $C_{16}H_{22}NO_2$ requires 260.1645.

*These NMR peaks were obscured by the CD_3OD (δ 3.34) solvent peak in the ^{1}H spectra; however, ^{1}H spectra of these compounds in D_2O or DMSO-d_6 were acquired to resolve the integrations and multiplicities of these peaks. CD_3OD was originally used due to greater compound solubility in this solvent.

4.4. Synthesis of L-Methionine Analogues

Methyl (*tert*-butoxycarbonyl)-L-methioninate and *S*-allyl-L-homocysteine ((*S*)-**1**) were prepared following literature procedures.[37]

*4.4.1. S-Allyl-*dl*-homocysteine (1)*

This procedure was adapted from literature, where non-allyl groups were added to the starting material.[42] DL-Homocysteine thiolactone hydrochloride (1.00 g, 6.51 mmol) was added to 1 M $NaOH_{(aq)}$ solution (23 mL) and degassed with argon. The mixture was then left stirring for 30 mins at 0 °C. After this time, allyl bromide (0.59 mL, 6.83 mmol) was added at 0 °C, and the resulting mixture was stirred for 3 h at 50 °C. The reaction mixture was then neutralized with 6 M HCl and the solvent was removed in vacuo to yield the crude product. This crude was purified by reverse-phase preparative HPLC using Method A9 (Supporting Information: 3.1). The product fractions were combined, and the solvent was removed in vacuo, then lyophilized to yield the trifluoroacetate salt of the product as a white solid (1.04 g, 55%). IR (film) ν_{max}/cm^{-1}: 2919, 2626, 1728, 1664, 1614, 1585, 1562, 1498; MP: 232–234 °C (H_2O); ^{1}H NMR (700 MHz; D_2O) δ 5.87 (ddt, J = 17.2, 9.9, 7.2 Hz, 1H, SCH_2CH = CH_2), 5.25–5.18 (m, 2H, SCH_2CH = CH_2), 4.08 (dd, J = 6.7, 6.0 Hz, 1H, 1-H), 3.26 (dt, J = 7.3, 1.1 Hz, 2H, SCH_2CH = CH_2), 2.68 (t, J = 7.6 Hz, 2H, 3-H), 2.26 (m, 1H, 2-HH), 2.18 (m, 1H, 2-HH); ^{13}C NMR (176 MHz; D_2O) δ 173.3, 134.4, 118.4, 53.3, 34.0, 30.2, 25.6; *m/z* [LRMS ESI−] 174 ([M − H]$^-$); *m/z* [HRMS ESI$^+$] found [M + H]$^+$ 176.0736, $C_7H_{14}NO_2S$ requires 176.0740; preparative HPLC retention time: 9.4 min.

4.5. General Methods (Biological)

All chemicals were obtained from commercial suppliers. Lyophilization was carried out using a VirTis BenchTop Pro with an Omnitronics Freeze Dryer. Thermoshakers used were a BIOER Mixing Block MB-102. Centrifuges used were an Eppendorf Centrifuge 5415R, Eppendorf Centrifuge 5810R, Eppendorf Centrifuge 5430R and Beckman Coulter Allegras x-15R. Autoclaves used were a Priorclave TACTROL 2 and Priorclave TACTROL 3. Where sterilization of waste and media was performed, the temperature was held at 121 °C for 30 min. UV–vis spectrometry was performed on a Thermo Scientific NanoDrop One^C spectrophotometer. The sonicator used was a Soniprep 150. Terrific broth (TB) media contained 47.6 g of TB granules and 4 mL glycerol in 1 L of distilled water, which was then autoclaved. Lysogeny broth (LB) media contained 25 g of LB granules in 1 L of distilled water, which was then autoclaved.

4.6. Enzyme Expression

For every enzyme expressed, starter cultures (10 mL) were prepared in sterile LB broth supplemented with kanamycin (50 μg/mL) and inoculated from glycerol stocks, then incubated and left overnight at 37 °C and 200 rpm. Cells were then subcultured using 1% v/v starter culture in 2 L baffled flasks containing 500 mL of sterile TB broth supplemented with kanamycin (50 μg/mL) and incubated at 37 °C with shaking at 140 rpm until induction. All enzymes (excluding *Tf*NCS) were induced with 0.25 mM IPTG (0.50 mM IPTG for *Tf*NCS) when growing in the early exponential phase (OD_{600} = 0.6 to 0.9), and the temperature was then reduced to 25 °C at 140 rpm overnight. Cells were then harvested by centrifugation (10,000 rpm, 10 mins, 4 °C), with the pellet resuspended in 15 mL 50 mM HEPES buffer (pH 7.5) and sonicated on ice using 10 cycles of 10 s on and 10 s off at 15 W. The sonicated suspension was centrifuged (4,000 rpm, 50 min, 4 °C), with the supernatant being filtered through 0.45 μm syringe filters to yield clarified lysate.

4.7. Enzyme Purification

If the clarified lysates produced required purification, then they were then purified by Ni-NTA HisTrap columns, which were equilibrated by flowing through distilled water and then equilibration buffer (20 mM imidazole, 100 mM NaCl, 50 mM HEPES, pH 7.5). After equilibration, clarified lysate was added to the column, followed by wash buffer (40 mM imidazole, 100 mM NaCl, 50 mM HEPES, pH 7.5) to elute non-specifically bound proteins. Elution buffer (500 mM imidazole, 100 mM NaCl, 50 mM HEPES, pH 7.5) was then passed through the columns to elute the His-tagged protein of interest, which was detected by collecting the flowthroughs into Eppendorf tubes and taking 2 µL aliquots from each tube into 96-well plates and adding 150 µL Bradford reagent. Blue coloration in these wells indicated the His-tagged protein of interest was present, with the tube samples they originated from being combined and concentrated by centrifugation in VIVASPIN TURBO 10 K MWCO concentrator tubes (3000 rpm, 4 °C, variable times) until a volume of 2.5 mL was achieved. To desalt the purified enzyme solution, PD10 columns were prepared by washing with distilled water, then 50 mM HEPES (pH 7.5). The 2.5 mL purified enzyme solution was added to the PD10 columns and allowed to enter the column bed, at which point 3.5 mL of 50 mM HEPES (pH 7.5) was added to the column and the flowthrough of desalted, purified enzyme was collected. Glycerol was then added to the purified enzyme and mixed well to give 10% v/v glycerol solutions for cryoprotection during freezing.

4.8. Cascade A

4.8.1. MAT-MT-MTAN Allylation Cascade with Dopamine then TfNCS

In the first step of this cascade, a single assay was made up to a total volume of 5 mL. This assay contained HEPES (50 mM, pH 7.5), KCl (200 mM), $MgCl_2$ (20 mM), **2**.HCl (5 mM), sodium ascorbate (5 mM), ATP (12 mM), *S*-allyl-DL-homocysteine. TFA (12 mM), 0.78 mg/mL purified *Uu*MAT, 0.90 mg/mL purified *Mx*SafC, and 0.27 mg/mL purified *Ec*MTAN. The rest of the assay volume was made up with distilled water. Negative control assays were run in parallel at a 200 µL volume, whereby the only differences, apart from the volume, were the omission of the *Uu*MAT in the first control, the omission of *Mx*SafC in the second control, the omission of *Ec*MTAN in the third control, and the omission of all enzymes in the fourth control. A positive control assay was also run in parallel, which was identical to the non-control assays apart from *S*-allyl-DL-homocysteine being replaced with L-methionine (the native substrate for MATs) to assess cascade functionality. Assays were then left on a shaker incubator for 24 h at 37 °C and 200 rpm. After this time, each assay was centrifuged (4000 rpm, 20 min, 4 °C) and an aliquot of the resulting supernatant was then analyzed by reverse-phase analytical HPLC using Method B1 (Supporting Information: 3.1.) to assess intermediate conversion. For the second step, 100 µL aliquots of the supernatant of the 5 mL non-control assay were taken and supplemented with HEPES (50 mM, pH 7.5), 3.75 mM aldehyde (either hexanal, phenylacetaldehyde or 4-pentenal), and 0.56 mg/mL purified *Tf*NCS. The assay volume was then made up to 200 µL with distilled water and MeCN, with the final volume containing 2.5% v/v MeCN. These assays were performed in triplicate. Negative control assays were run in parallel under identical conditions to the non-control assays, apart from these assays having *Tf*NCS omitted. Assays were then left on a shaker incubator for 24 h at 37 °C and 800 rpm. After this time, each assay was lyophilized and resuspended in 200 µL MeOH and centrifuged (13,000 rpm, 20 min, 4 °C). The resulting supernatant was then analyzed by reverse-phase analytical HPLC using Method B1 (Supporting Information: 3.1), and by normal-phase chiral HPLC using Method C1 for all hexanal-containing assays, Method C2 for all phenylacetaldehyde-containing assays, and Method C6 for all 4-pentenal-containing assays (Supporting Information: 3.1).

4.9. Cascade B

4.9.1. TfNCS with Dopamine then MAT-MT-MTAN Allylation Cascade

In the first step of this cascade, assays were made up to a total volume of 1 mL. These assays contained HEPES (50 mM, pH 7.5), **2**.HCl (5 mM), sodium ascorbate (5 mM), 7.5 mM aldehyde (either hexanal, phenylacetaldehyde, or 4-pentenal), and 0.56 mg/mL purified *Tf*NCS. The rest of the assay volume was made up with distilled water and MeCN, with the final volume containing 5% v/v MeCN. Negative control assays were run in parallel at a 1 mL volume, whereby the only difference was the omission of the *Tf*NCS in these controls. Assays were then left on a shaker incubator for 24 h at 37 °C and 800 rpm. After this time, each assay was centrifuged (13,200 rpm, 15 min, 4 °C), and aliquots of the resulting supernatant were then analyzed by reverse-phase analytical HPLC using Method B1 (Supporting Information: 3.1) and by reverse phase chiral HPLC using Method C4 (Supporting Information: 3.1), to assess intermediate conversion and enantiomeric excess. For the second step, 40 µL aliquots of the supernatant of the 1 mL first step assays were taken and supplemented with HEPES (50 mM, pH 7.5), KCl (200 mM), $MgCl_2$ (20 mM), ATP (2.4 mM), *S*-allyl-DL-homocysteine. TFA (2.4 mM), 0.14 mg/mL purified *Uu*MAT, 0.18 mg/mL purified MT (either *Rn*COMT or *Mx*SafC), and 0.05 mg/mL purified *Ec*MTAN. The assay volume was then made up to 200 µL with distilled water. These assays were performed in triplicate. Negative control assays were run in parallel at a 200 µL volume, whereby the only difference to the non-control assays was the omission of *Uu*MAT in the first set of controls, the omission of MTs in the second set of controls, the omission of *Ec*MTAN in the third set of controls, and the omission of all enzymes in the fourth set of controls. A set of positive control assays was also run in parallel, which were identical to the non-control assays apart from *S*-allyl-DL-homocysteine being replaced with L-methionine (the native substrate for MATs) to assess cascade functionality. Assays were then left on a shaker incubator for 24 h at 37 °C and 800 rpm. After this time, each assay was quenched with 50 µL MeOH and centrifuged (13,200 rpm, 15 min, 4 °C). The resulting supernatant was then analyzed by reverse-phase analytical HPLC using Method B2 (Supporting Information: 3.1).

Supporting Information

The authors have cited an additional reference within the Supporting Information.[43]

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords: Allylation · Biocatalysis · Methyltransferases · Pictet–Spenglerases · Tetrahydroisoquinolines

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