ChemCatChem

Research Article
doi.org/10.1002/cctc.202400492

Chemistry Europe
European Chemical Societies Publishing

www.chemcatchem.org

# An Alternative Cascade for the Selective Methylation of Catechols and Tetrahydroisoquinolines by *O*-Methyltransferases

Matthew T. Salinger,[a] Daniel Castellano Garrido,[a] Eleanor D. Lamming,[a] John M. Ward,[b] Thomas S. Moody,[c] Jack W. E. Jeffries,[b] and Helen C. Hailes*[a]

Methyltransferases are gaining traction as a method to achieve greener and regioselective methylations of a wide array of substrates. In this work, a halide methyltransferase biomethylation cascade was successfully adapted with the incorporation of a methionine adenosyltransferase to generate the *S*-adenosyl-L-methionine (SAM) methylation cofactor *in situ* from less costly ATP and L-methionine, instead of directly adding SAM to start the cascade. Furthermore, this cascade was applied to achieve novel catechol and tetrahydroisoquinoline methylations with the *O*-methyltransferases *Rn*COMT, *Mx*SafC and *Np*N4OMT with high conversions and regioselectivities. In addition, the cascade was successfully trialled with the less toxic methyl group donor, methyl tosylate.

## Introduction

Methyltransferases (MTs) are a class of enzymes that catalyse the transfer of methyl groups to substrates ranging from proteins and nucleic acids, to regulate their activities, to small molecule metabolites where they are a key step in the diversification of natural products.[1–4] Most MTs require the cofactor *S*-adenosyl-L-methionine (SAM) as the electrophilic methyl donor and target specific moieties in their substrates, typically by an $S_N2$ mechanism, with the most significant being *O*-MTs, *C*-MTs and *N*-MTs.[4–7] However, there are also SAM-dependent MTs that operate via a free radical mechanism.[8,9]

Methylation reactions are of pharmaceutical interest, as they can modulate drug solubilities, conformations and metabolic profiles.[4,10,11] Using MTs for methylations presents several advantages over chemical methods, the most notable being the stereoselectivity and regioselectivity offered by enzyme catalysis, whereas chemical methylations often add methyl groups to substrates non-selectively.[4] Additionally, MTs provide a sustainable synthetic route and can facilitate the late-stage modification of drug molecules under mild conditions.[4–7,12]

Various *O*-MTs with different substrate specificities have been reported, particularly catechol-*O*-methyltransferases (COMTs) which have relaxed substrate specificities. Native mammalian COMTs such as the enzyme from *Rattus norvegicus* (*Rn*COMT) typically exhibit *meta*-selectivities, against substrates such as dopamine, and 3,4-dihydroxybenzoic acid (3,4-DHBA), where the polar substituents are more likely to be positioned pointing out of the active site.[4–7] *Para*-selectivities have been noted for more hydrophobic compounds, as these moieties may point towards the so called hydrophobic wall rather than the solvent.[4,13] Bacterial *O*-MTs such as that from the saframycin biosynthetic pathway in *Myxococcus xanthus* (*Mx*SafC) selectively methylate L–DOPA at the *para*-position, but *meta*-products predominated with 3,4-DHBA and 3,4-dihydroxybenzaldehyde, suggesting that catechol side-chain active site interactions were important.[14,15] When *Rn*COMT and *Mx*SafC were used with tetrahydroisoquinolines (THIQs), *Rn*COMT methylated predominantly at the *meta*-hydroxyl (giving 6-OMe THIQs), while *Mx*SafC gave a mixture of isomers (6-OMe and 7-OMe products). However, C-1 spiro-THIQs altered this selectivity, and a C-8 fluoro-analogue gave 6-OMe-selectivities with both enzymes,[16–18] highlighting subtle changes to the position or conformation of the substrates in the active site that can influence the regioselectivities. *Mx*SafC was also able to generate some dimethylated catechols.[16]

SAM is an expensive cofactor, and combined with poor stability and a stochiometric requirement can impact on the costs of MT catalysed reactions. To overcome this issue, methods have been found to generate SAM *in situ* via biocatalytic cascades. Previously a linear cascade was developed with *in situ* SAM generation from ATP and L-methionine using a

[a] M. T. Salinger, D. Castellano Garrido, Dr. E. D. Lamming, Prof. H. C. Hailes
Department of Chemistry
University College London
20 Gordon Street
London, WC1H 0AJ (UK)
E-mail: h.c.hailes@ucl.ac.uk

[b] Prof. J. M. Ward, Dr. J. W. E. Jeffries
Department of Biochemical Engineering
University College London
Gower Street, Bernard Katz Building
London WC1E 6BT (UK)

[c] Prof. T. S. Moody
Almac Sciences
Department of Biocatalysis and Isotope Chemistry
Almac House, 20 Seagoe Industrial Estate
Craigavon BT63 5QD (UK)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cctc.202400492

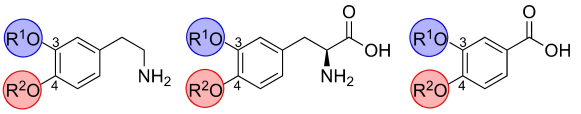
methionine adenosyltransferase (MAT), together with an MT and then methylthioadenosine nucleosidase (MTAN), to hydrolyse the inhibitory *S*-adenosyl-L-homocysteine (SAH) by-product.[19] This system is straightforward to adopt, but SAH is not recycled. Later reports substituted the MTAN for SAH hydrolase (SAHH), to hydrolyse SAH to adenosine and L-homocysteine, with adenosine then being recycled to ATP using polyphosphate and three kinases.[20] Systems describing the use of L-homocysteine *S*-methyltransferase from yeast (*Sc*HSMT) to re-methylate L-homocysteine to L-methionine have been reported.[21] Other SAM supply approaches described include the use of a halide methyltransferase (HMT) biomethylation cascade with SAM regeneration from SAH after the MT step using methyl iodide (MeI) (Scheme 1a).[22] This MT-HMT system has some advantages, such as the need for only two enzymes and catalytic SAM (typically 10 mol%), but MeI is very toxic, and the HMT/MT require purification or expression in SAHH/MTAN knockdown strains before use to remove endogenous MTANs and SAHHs which can hydrolyse SAH and terminate the cascade. The cascade also requires the addition of some SAM/SAH. To tackle this issue thiopurine methyltransferases from *Ustilago maydis* and *Kordia algicida* have enabled MeI to be replaced with methyl tosylate with similar activities.[23]

Here we report the development of a biomethylation cascade combining aspects of the MAT-MT-MTAN and MT-HMT cascades (Scheme 1b). Notably, the addition of MAT (with ATP and L-methionine) – and referred to as a 'primed cascade' to enable the initial *in situ* generation of SAM for use with the MT and then HMT and avoid the requirement for SAM/SAH. Furthermore, we report the application of this cascade with *Rn*COMT, *Mx*SafC, and the norbelladine 4'-*O*-methyltransferase from *Narcissus pseudonarcissus* (*Np*N4OMT),[24] to give products with high regioselectivities.

## Results and Discussion

### MAT-MT-HMT Cascade Functionality

To demonstrate the viability of the MAT-MT-HMT cascade, *Escherichia coli* MAT (*Ec*MAT),[20] HMTs derived from *Paraburkholderia xenovorans* and *Arabidopsis thaliana* (*Px*HMT and *At*HMT, respectively),[23] and *Rn*COMT and *Mx*SafC were prepared as purified enzymes. Since there is a literature precedent for dopamine **1**, L–DOPA **2**, and 3,4-dihydroxybenzoic acid (3,4-DHBA) **3** methylation by *Rn*COMT[7,15,25] and *Mx*SafC,[14,15] these substrates were selected to assess the cascade functionality. The results revealed that the MAT-MT-HMT cascade readily formed the corresponding methylated products in >93% yield (determined by HPLC analysis against product standards) without the addition of any SAM or SAH to initiate the cascade. Product standards were prepared throughout via established procedures, or via scaled-up biocatalytic reactions, and regioisomers separated by preparative HPLC (SI, Section 1.2).



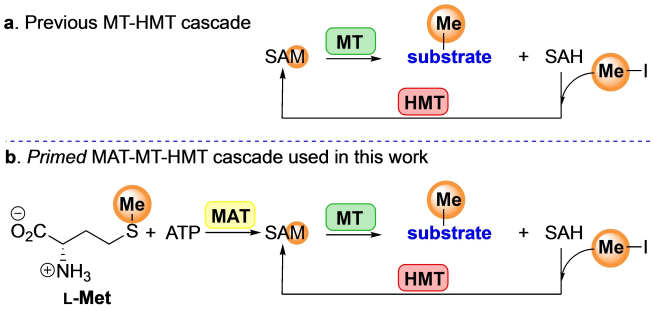

**1** $R^1$=$R^2$=H, dopamine
3-OMe-**1** $R^1$=Me, $R^2$=H
4-OMe-**1** $R^1$=H, $R^2$=Me

**2** $R^1$=$R^2$=H, L-DOPA
3-OMe-**2** $R^1$=Me, $R^2$=H
4-OMe-**2** $R^1$=H, $R^2$=Me

**3** $R^1$=$R^2$=H, 3,4-DHBA
3-OMe-**3** $R^1$=Me, $R^2$=H
4-OMe-**3** $R^1$=H, $R^2$=Me

Regioselectivities were consistent with the literature, with *Rn*COMT giving the *meta*-methylation products 3-OMe-**1**, 3-OMe-**2** and 3-OMe-**3** (3-OMe:4-OMe, ~88:12, 95:5, 73:27, respectively) as the major isomers and *Mx*SafC the *para*-products 4-OMe-**1** and 4-OMe-**2** (3-OMe:4-OMe, ~13:87, 1:99, respectively) for **1** and **2**, and *meta*-isomer 3-OMe-**3** for **3** (77:23) (Figure 1).[15] This different methylation pattern for *Mx*SafC has been noted previously.[15] Additionally, *Px*HMT and *At*HMT gave comparable yields in the cascade.

Interestingly, control reactions using MT-HMT cascades (in the absence of *Ec*MAT and SAM) still demonstrated the nearly full conversion of **1** to *O*-methylated products (Figure 2A). This was only apparent for cascades with *Rn*COMT (not *Mx*SafC), with *Px*HMT enhancing this effect compared to *At*HMT (Figure 2A–C). Furthermore, high conversions persisted when ATP and L-methionine were also removed from the cascade. This




**Scheme 1. a**. Previous reported MT-HMT methylation cascade.[22] **b**. Primed MAT-MT-HMT cascade investigated in this work with novel substrates and *Rn*COMT, *Mx*SafC and *Np*N4OMT. L–Methionine, L–Met; MAT, L-methionine adenosyltransferase; MT, methyltransferase; HMT, halide methyltransferase.

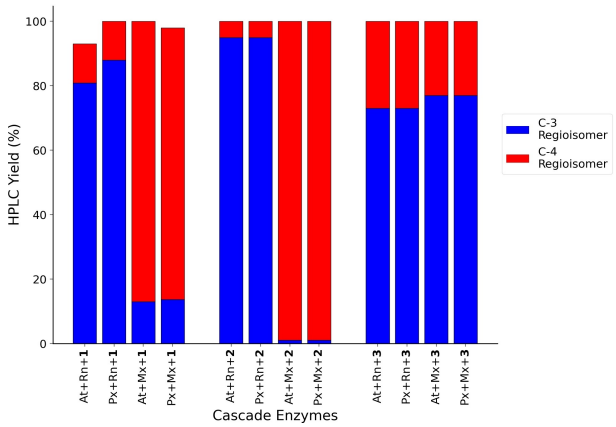



**Figure 1.** Use of the MAT-MT-HMT cascade with different MTs and HMTs for the methylation of **1**–**3** to give 3/4-OMe **1** –**3**. Px is *Px*HMT; At is *At*HMT; Rn is *Rn*COMT; Mx is *Mx*SafC. *Reaction conditions*: 24 h at 25 °C, 700 rpm, 5 mM **1**–**3**, 5 mM sodium ascorbate, 50 mM MeI, 0.5 mM ATP, 0.5 mM L-methionine, 20 mM $MgCl_2$, 200 mM KCl, 50 mM HEPES (pH 7.5), 5% *v*/*v* $CH_3CN$, 0.63 mg/mL *Ec*MAT, 0.96 mg/mL *Px*HMT/0.74 mg/mL *At*HMT, 1.4 mg/mL *Rn*COMT/*Mx*SafC. Reactions were performed in triplicate and HPLC yields were determined by HPLC analysis against product standards.

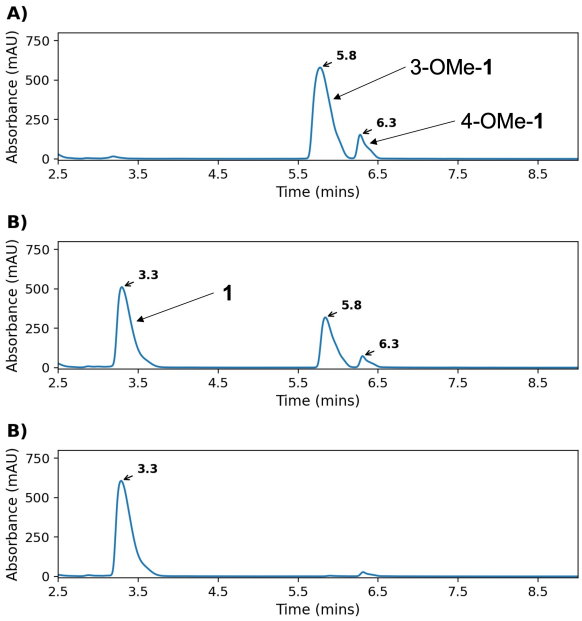



**Figure 2.** (**A**) An analytical HPLC trace of the *Px*HMT-*Rn*COMT assay without *Ec*MAT, showing a quantitative conversion of **1** to products 3-OMe-**1** and 4-OMe-**1**, with no addition of SAM. (**B**) An analytical HPLC trace of the *At*HMT-*Rn*COMT assay without *Ec*MAT, showing significant, but comparatively lower conversion to methylated products of **1**. (**C**) An analytical HPLC trace of the *At*HMT-*Mx*SafC assay without *Ec*MAT, showing essentially a trace conversion to methylated products of **1** as expected when there is no SAM supply system.

methylation in the presence of MT-HMT but absence of SAM or SAM supply system to start the cascades is currently unreported to the best of our knowledge. This is possibly due to endogenous SAM and SAH from *Rn*COMT expression being retained in the enzyme active site after enzyme purification, which has been previously described ($K_M$ for SAM binding is reported to be 30 μM).[26] Residual SAM/SAH is then sufficient to start the cascade due to the requirements for only catalytic amounts of SAM/SAH, as the cofactor SAM is regenerated by the HMT. This SAM supply system independence opens up the possibility for MT-HMT cascades to operate without the need for the costly input of SAM/SAH or precursors such as ATP and L-methionine if MTs (or HMTs) with a high SAM/SAH affinity are used.

### Novel Catechol and Tetrahydroisoquinoline Methylations

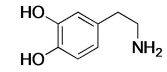
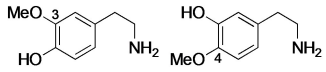
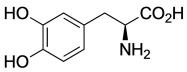
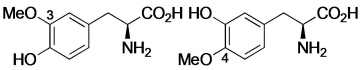
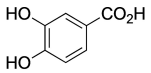
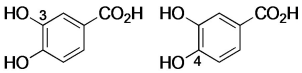
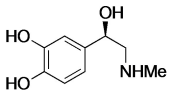
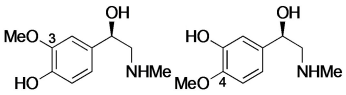
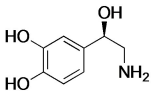
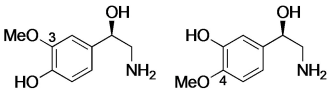
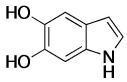
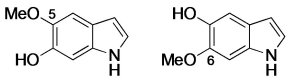
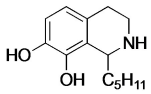
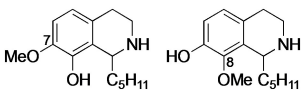
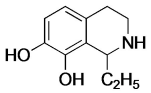
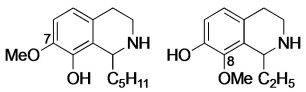
The class I *O*-MT enzyme *Np*N4OMT is involved in the biosynthesis of galanthamine, where the natural substrate is norbelladine.[24] Previous work using the recombinant enzyme in *E. coli* demonstrated *para*-catechol regioselectivity with norbelladine and dopamine, although caffeic acid and 3,4-dihydroxybenzaldehyde were not accepted as substrates.[24] With our interest in using productive MTs for biocatalytic applications, recombinant *Np*N4OMT in *E. coli* was generated as reported.[24] The relatively lower solubility of *Np*N4OMT limited the concentration it could be used in the assays, so all *O*-MTs were used at this concentration (0.25 mg/mL enzyme) for comparison purposes. Using the MAT-MT-HMT cascade for substrates **1**–**3** (Table 1), similar selectivities and good to excellent yields were observed as above (Figure 1) for *Rn*COMT and *Mx*SafC. With *Np*N4OMT and **1**, very high *para*-regioselectivity was observed giving 3-OMe-**1**:4-OMe-**1** in a ratio of 2:98, indicating greater selectivity than for *Mx*SafC. However, for **2** and **3** possessing carboxylate groups, trace amounts of products were formed. To expand the substrates used, the dopamine analogues epinephrine **4** and norepinephrine **5** were used,[25] where again *Rn*COMT produced the *meta*-products as the major isomers (97:3 and 96:4, respectively), and *Mx*SafC the *para*-products (12:88 and 6:94) (Table 1). Notably, with *Np*N4OMT high *para*-regioselectivity was observed with the new substrates **4** and **5**, giving 3-OMe:4-OMe products in 6:94 and 3:97 ratios (respectively), and good HPLC yields (determined by HPLC analysis against product standards) were also observed of up to 78%. The substrate selectivity with *Np*N4OMT is interesting and future structural studies will provide insights into substrate binding modes.

Encouraged by these results, the cascade was used with *Rn*COMT, *Mx*SafC and *Np*N4OMT and 5,6-dihydroxyindole **6**, an eumelanin precursor.[27] Substrate **6** was accepted as a substrate by all three *O*-MTs, with good yields for *Rn*COMT and *Mx*SafC at higher enzyme concentrations, whilst lower yields were observed in all cases at the lower enzyme concentrations of 0.25 mg/mL. Interestingly, *Rn*COMT displayed a small preference for C-5 *O*-methylation,[28] as did *Mx*SafC, whilst *Np*N4OMT had a notable regioselectivity (ratio 6:94) towards the C-6 hydroxyl group, despite *Mx*SafC and *Np*N4OMT previously exhibiting similar regioselectivities (Table 1). Furthermore, *Mx*SafC performed a double *O*-methylation of **6**. *In silico* docking analysis of **6** with *Rn*COMT (PDB code: 2ZTH) and *Mx*SafC (PDB code: 5LOG) (Figure 3) revealed that for *Rn*COMT the 5-OH methylation is likely to occur due to the C-5 hydroxyl position hydrogen bonding to Tyr147 in the active site, which positions it adjacent to the catalytic $Mg^{2+}$ cofactor and the SAM methyl group for $S_N2$ nucleophilic attack.This pose is further favoured by Lys144 electrostatically interacting with the indole nitrogen of **6**. Rotating **6** in the active site to reverse the regioselectivity was also an acceptable pose, with the Tyr147 and $Mg^{2+}$ interaction maintained and the indole N–H facing towards the polar aqueous exterior of the active site. This could explain the poor regioselectivity for *Rn*COMT (Figure 3A).

For *Mx*SafC, *in silico* modelling highlighted that the Asn169 residue can coordinate to the C-6 hydroxyl, with the C-5 hydroxyl adjacent to $Mg^{2+}$ and the SAM methyl group and coordinating to Lys145. Residues Trp172 can also form a π-stacking interaction with the indole ring to hold the substrate in place (Figure 3B). C-5 regioselectivity would then be guided by the Thr40 sidechain weakly hydrogen bonding to the indole N–H to hold **6** in the correct orientation for C-5 hydroxyl methylation (Figure 3B). Interestingly, these residue interactions closely align with those reported for *Mx*SafC using **1**, with Lys145, Asn169 and Thr40 displaying equivalent roles in the active site.[15]

Further expansion of the range of substrates accepted by these *O*-MTs, and application of the MAT-MT-HMT cascade was

**Table 1.** Use of the MAT-MT-HMT cascade with compounds **1**–**8**, together with yields (by HPLC analysis) and regioisomeric ratios.[a]

| Substrate | Products | *Rn*COMT HMT | *Rn*COMT HPLC Yield Products & isomeric ratio | *Mx*SafC HMT | *Mx*SafC HPLC Yield Products & isomeric ratio | *Np*N4OMT HMT | *Np*N4OMT HPLC Yield Products & isomeric ratio |
|---|---|---|---|---|---|---|---|
|  **1** |  3-OMe-**1** 4-OMe-**1** | *Px* | Quant. 3-OMe-**1**:4-OMe-**1** 87:13 | *At* | 73% 3-OMe-**1**:4-OMe-**1** 15:85 | *At* | 37% 3-OMe-**1**:4-OMe-**1** 2:98 |
|  **2** |  3-OMe-**2** 4-OMe-**2** | *Px* | Quant. 3-OMe-**2**:4-OMe-**2** 93:7 | *Px* | 89% 3-OMe-**2**:4-OMe-**2** 1:99 | *Px* | 6% 3-OMe-**2**:4-OMe-**2** n.d. |
|  **3** |  3-OMe-**3** 4-OMe-**3** | *Px* | 97% 3-OMe-**3**:4-OMe-**3** 70:30 | *At* | 61% 3-OMe-**3**:4-OMe-**3** 75:25 | *Px* | 2%, 3-OMe-**3**:4-OMe-**3** n.d. |
|  **4** |  3-OMe-**4** 4-OMe-**4** | *Px* | Quant. 3-OMe-**4**:4-OMe-**4** 97:3 | *Px* | 73% 3-OMe-**4**:4-OMe-**4** 12:88 | *Px* | 74% 3-OMe-**4**:4-OMe-**4** 6:94 |
|  **5** |  3-OMe-**5** 4-OMe-**5** | *Px* | Quant. 3-OMe-**5**:4-OMe-**5** 96:4 | *Px* | 76% 3-OMe-**5**:4-OMe-**5** 6:94 | *Px* | 78% 3-OMe-**5**:4-OMe-**5** 3:97 |
|  **6** |  5-OMe-**6** 6-OMe-**6** | *At* | 32% [b]Quant. 5-OMe-**6**:6-OMe-**6** [b]55:45 | *Px* | 23% [b]Quant. 5-OMe-**6**:6-OMe-**6** [b]68:26:6* [*Di-O–Me-**6** product] | *At* | 35% 5-OMe-**6**:6-OMe-**6** 6:94 |
|  **7** |  7-OMe-**7** 8-OMe-**7** | *At* | 5% [b]18% 7-OMe-**7**:8-OMe-**7** 100:0 | *Px* | 20% [b]48% 7-OMe-**7**:8-OMe-**7** 100:0 | *At* | 2% 7-OMe-**7**:8-OMe-**7** 100:0 |
|  **8** |  7-OMe-**8** 8-OMe-**8** | *At* | 9% [b]30% 7-OMe-**8**:8-OMe-**8** 100:0 | *Px* | 55% [b]75% 7-OMe-**8**:8-OMe-**8** 100:0 | *At* | 3% 7-OMe-**8**:8-OMe-**8** 100:0 |

[a]*Reaction conditions*: 24 h at 25 °C and 700 rpm, 5 mM of catechol/tetrahydroisoquinoline/indole substrate, 5 mM sodium ascorbate, 50 mM MeI, 0.5 mM ATP, 0.5 mM L-methionine, 20 mM $MgCl_2$, 200 mM KCl, 50 mM HEPES (pH 7.5), 5% *v/v* $CH_3CN$ (5% *v/v* $CH_3CN$ + 5% *v/v* DMSO for **6**; 7.5% *v/v* $CH_3CN$ for **7** and **8**), 0.63 mg/mL *Ec*MAT, 0.8-0.9 mg/mL *Px*HMT or *At*HMT, 0.25 mg/mL *Rn*COMT/MxSafC/*Np*N4OMT. Reactions were performed in triplicate and yields were determined by HPLC analysis against product standards; Not determined (n.d.) due to low yields. [b]1.4 mg/mL enzyme for *Rn*COMT/MxSafC.

investigated using the THIQ derivatives **7** and **8**, due to this particular scaffold's association with antimicrobial activity.[29] For **7**, conversion to 7-OMe-**7** only was observed and no 8-OMe-**7** was detected, with *Mx*SafC being the best performing *O*-MT, and *Np*N4OMT forming a trace amount of the same isomer. For **8**, analogous results were observed with only the 7-OMe-**8** regioisomer formed, but in higher yields (Table 1). Higher enzyme concentrations were also used for *Rn*COMT and *Mx*SafC and yields of up to 75% were observed. The generally lower yields with these substrates probably reflects the greater sterically hindered catechol group, and the lack of C-8 *O*-methylation will also be affected by lack of accessibility to this moiety by residues in the active site. Compounds **7** and **8** are racemates so products 7-OMe-**7** and 7-OMe-**8** generated by

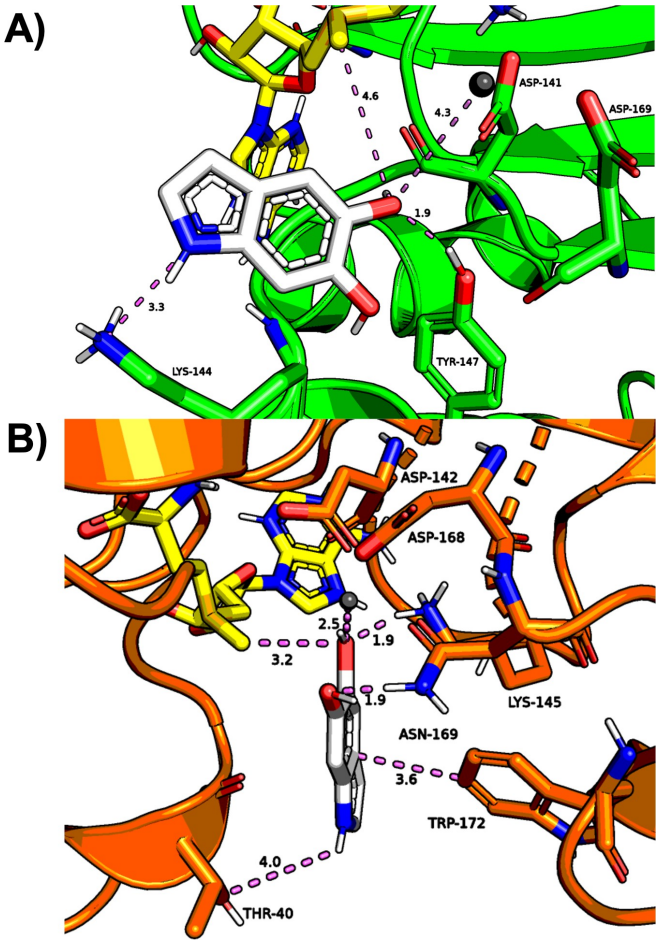



**Figure 3.** (**A**) The *Rn*COMT (green) active site, showing the favoured pose of **6** (white), with the C-5 hydroxyl positioned by the SAM (yellow) methyl group and $Mg^{2+}$ (grey); Asp141 and Asp169 coordinate $Mg^{2+}$ in the active site. (**B**) The *Mx*SafC (orange) active site, showing the favoured pose of **6**, with the C-5 hydroxyl positioned by the SAM methyl group and $Mg^{2+}$; Asp142 and Asp168 coordinate $Mg^{2+}$ in the active site.

*Mx*SafC in the MAT-MT-HMT cascade were analysed by chiral HPLC, but no enantioselectivities were observed (Figure S3). In addition to these substrates, resorcinol analogues (**9**–**12**, SI Figure S4) were also trialled with *Rn*COMT, *Mx*SafC and *Np*N4OMT in the cascades, however, no *O*-methylation was observed with any of these substrates, reflecting the catechol *O*-MT selectivities.

### Alternative Methyl Donors

Due to the toxicity of methyl iodide, the use of less volatile and hazardous methyl donors methyl tosylate (MeOTs) and dimethyl carbonate (DMC) were trialled in MAT-MT-HMT cascade. MeOTs has previously been described with HMTs,[23] while DMC though less electrophilic is used in methylation reactions. They were both screened with *Rn*COMT and **3**, as a representative substrate, together with *Px*HMT/*At*HMT and *Ec*MAT as before. Donor MeOTs was effective in this cascade, albeit only with *Px*HMT where quantitative conversion to the 3/4-OMe-**3** was observed, with similar regioselectivities to those indicated previously (Table 1). The tolerance of *Px*HMT, compared to *At*HMT, towards donors other than methyl iodide has previously been noted.[23] For *At*HMT cascades low product formation was observed, arising from methylation by the SAM formed from initial *Ec*MAT catalysis (Figure 4). For all DMC cascades, no methyl donor activity was observed (SI, Figure S5).

## Conclusions

In summary, here we report the full functionality of a new MAT-MT-HMT cascade with a variety of catechol and THIQ substrates. It was found that SAM formed *in situ* by *Ec*MAT from ATP and L-methionine was an appropriate substitute for direct SAM or SAH addition, with complete conversions to methylated products achieved in many cases. Also, an MT-HMT cascade was successfully demonstrated without the addition of SAM/SAH when using an MT (*Rn*COMT) with good SAM binding affinities. This ability to retain SAM in the MT active site post-purification to initiate the cascade, lead to complete conversion to the products.

Furthermore, with this cascade novel *O*-methylations by *Rn*COMT, *Mx*SafC and *Np*N4OMT were achieved with excellent regioselectivities, particularly for **7** and **8**, where complete preference for the C-7 methoxy regioisomer was apparent, most likely due to steric effects. In many cases switching the *O*-MT used between *Rn*COMT and *Mx*SafC/*Np*N4OMT was sufficient to induce a reversal of the regioiselectivity, thereby showing the advantages of enzyme-catalysed reactions as

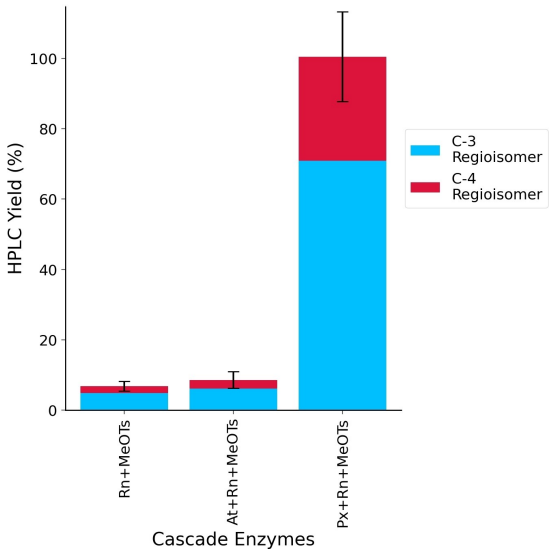



**Figure 4.** The results of the methylation of **3** by *Rn*COMT (Rn) with *At*HMT (At) or *Px*HMT (Px) relative to the no HMT negative control in the MAT-MT-HMT cascade with MeOTs. All assays were performed in triplicate. *Reaction conditions*: 24 h at 25 °C and 700 rpm, 5 mM of **3**, 5 mM sodium ascorbate, 50 mM MeOTs, 0.5 mM ATP, 0.5 mM L-methionine, 20 mM $MgCl_2$, 200 mM KCl, 50 mM HEPES (pH 7.5), 5% *v/v* $CH_3CN$, 0.64 mg/mL *Ec*MAT, 0.8–0.9 mg/mL *Px*HMT or *At*HMT, 0.25 mg/mL *Rn*COMT. Reactions were performed in triplicate and yields were determined by HPLC analysis against product standards.

opposed to non-specific chemical methylations. As demonstrated previously, the interaction of the catechol group and other functional groups attached can influence reaction yields and regioselectivities in the methylation reaction. Additionally, application of the MAT-MT-HMT cascade was achieved using MeOTs as a methyl group donor, thereby avoiding the use of toxic MeI.

## Supporting Information

The authors have cited additional references within the Supporting Information.[30–33]

## *Acknowledgements*

We are grateful for financial support from the BBSRC LIDo PhD programme (BB/T008709/1) and Almac Sciences Limited for funding M.T.S. Also from the EPSRC Centre for Doctoral Training in BioDesign Engineering (EP/S022856/1) for funding D.C.G. We acknowledge 700 MHz NMR equipment support by EPSRC (EP/P020410/1). We thank K. Karu (UCL Mass Spectrometry Facility) and A. E. Aliev (UCL NMR Facility) in the Department of Chemistry at UCL. In addition, we thank Jennifer N. Andexer (Institute of Pharmaceutical Sciences, University of Freiburg) for *Rn*COMT, *Mx*SafC, and *Ec*MAT plasmids.

## *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 supplementary material of this article.

**Keywords:** biocatalysis · methyltransferases · methylation · cascade · catechols

[1] M. T. Bedford, S. G. Clarke, *Mol. Cell.* **2009**, *33*, 1–13.
[2] E. L. Greer, Y. Shi, *Nat. Rev. Genet.* **2012**, *13*, 343–357.
[3] K. D. Robertson, *Nat. Rev. Genet.* **2005**, *6*, 597–610.
[4] E. Abdelraheem, B. Thair, R. F. Varela, E. Jockmann, D. Popadić, H. C. Hailes, J. M. Ward, A. M. Iribarren, E. S. Lewkowicz, J. N. Andexer, P.-L. Hagedoorn, U. Hanefeld, *ChemBioChem* **2022**, *23*, e202200212.
[5] M. Richter, *Nat. Prod. Rep.* **2013**, *30*, 1324–1345.
[6] D. K. Liscombe, G. V. Louie, J. P. Noel, *Nat. Prod. Rep.* **2012**, *29*, 1238–1250.
[7] A. W. Struck, M. L. Thompson, L. S. Wong, J. Micklefield, *ChemBioChem* **2012**, *13*, 2642–2655.
[8] M. R. Challand, R. C. Driesener, P. L. Roach, *Nat. Prod. Rep.* **2011**, *28*, 1696–1721.
[9] Y. Nicolet, *Nat. Catal.* **2020**, *3*, 337–350.
[10] H. Schönherr, T. Cernak, *Angew. Chem. Int. Ed.* **2013**, *52*, 12256–12267.
[11] P. d. S. M. Pinheiro, L. S. Franco, C. A. M. Fraga, *Pharmaceuticals* **2023**, *16*, 1157.
[12] E. Romero, B. S. Jones, B. N. Hogg, A. Rué Casamajo, M. A. Hayes, S. L. Flitsch, N. J. Turner, C. Schnepel, *Angew. Chem. Int. Ed.* **2021**, *60*, 16824–16855.
[13] B. J. C. Law, M. R. Bennett, M. L. Thompson, C. Levy, S. A. Shepherd, D. Leys, J. Micklefield, *Angew. Chem. Int. Ed.* **2016**, *55*, 2683–2687.
[14] J. T. Nelson, J. Lee, J. W. Sims, E. W. Schmidt, *Appl. Environ. Microbiol.* **2007**, *73*, 3575–3580.
[15] J. Siegrist, J. Netzer, S. Mordhorst, L. Karst, S. Gerhardt, O. Einsle, M. Richter, J. N. Andexer, *FEBS Lett.* **2017**, *591*, 312–321.
[16] F. Subrizi, Y. Wang, B. Thair, D. Méndez-Sánchez, R. Roddan, M. Cárdenas-Fernández, J. Siegrist, M. Richter, J. N. Andexer, J. M. Ward, H. C. Hailes, *Angew. Chem. Int. Ed.* **2021**, *60*, 18673–18679.
[17] R. Roddan, F. Subrizi, J. Broomfield, J. M. Ward, N. H. Keep, H. C. Hailes, *Org. Lett.* **2021**, *23*, 6342–6347.
[18] Y. Wang, F. Subrizi, E. M. Carter, T. D. Sheppard, J. M. Ward, H. C. Hailes, *Nat. Commun.* **2022**, *13*, 5436.
[19] J. Siegrist, S. Aschwanden, S. Mordhorst, L. Thöny-Meyer, M. Richter, J. N. Andexer, *ChemBioChem* **2015**, *16*, 2576–2579.
[20] S. Mordhorst, J. Siegrist, M. Müller, M. Richter, J. N. Andexer, *Angew. Chem. Int. Ed.* **2017**, *56*, 4037–4041.
[21] D. Popadić, D. Mhaindarkar, M. H. N. Dang Thai, H. C. Hailes, S. Mordhorst, J. N. Andexer, *RSC Chem. Biol.* **2021**, *2*, 883–891.
[22] C. Liao, F. P. Seebeck, *Nat. Catal.* **2019**, *2*, 696–701.
[23] X. Wen, F. Leisinger, V. Leopold, F. P. Seebeck, *Angew. Chem. Int. Ed.* **2022**, *61*, e202208746.
[24] M. B. Kilgore, M. M. Augustin, C. M. Starks, M. O'Neil-Johnson, G. D. May, J. A. Crow, T. M. Kutchan, *PLoS One* **2014**, *9*, e103223.
[25] H. C. Guldberg, C. A. Marsden, *Pharmacol. Rev.* **1975**, *27*, 135–206.
[26] P. N. Palma, M. L. Rodrigues, M. Archer, M. J. Bonifácio, A. I. Loureiro, D. A. Learmonth, M. A. Carrondo, P. Soares-da-Silva, *Mol. Pharmacol.* **2006**, *70*, 143–153.
[27] M. d'Ischia, A. Napolitano, A. Pezzella, *Eur. J. Org. Chem.* **2011**, *2011*, 5501–5516.
[28] J. Axelrod, A. B. Lerner, *Biochim. Biophys. Acta* **1963**, *71*, 650–655.
[29] L. T. Martin, E. D. Lamming, A. Maitra, P. N. Mortazavi, R. Roddan, J. M. Ward, S. Bhakta, H. C. Hailes, *Front. Antibiot.* **2023**, *2*, 1095013.
[30] D. Giuri, K. A. Jacob, P. Ravarino, C. Tomasini, *Eur. J. Org. Chem.* **2020**, *2020*, 7144–7150.
[31] P. R. Kym, E. Voight (AbbVie Inc.), WO2017184871, **2017**.
[32] Z. Huang, O. Kwon, H. Huang, A. Fadli, X. Marat, M. Moreau, J.-P. Lumb, *Angew. Chem. Int. Ed.* **2018**, *57*, 11963–11967.
[33] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248–254.

Manuscript received: March 13, 2024
Revised manuscript received: April 24, 2024
Accepted manuscript online: May 6, 2024
Version of record online: June 10, 2024