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Enzymatic Allylation of Catechols

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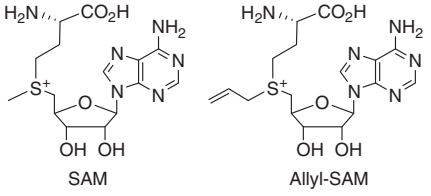
Enzymatic allylation of catechols was realized via catechol *O*-methyltransferase (COMT) using an allylated *S*-adenosyl-L-methionine (allyl-SAM) analog, with relatively good chemo- and regioselectivities. This new reaction offered an alternative procedure for allylation of catechols, which can be expanded as a biocatalytic allylation method in organic synthesis.

Allylation is a valuable reaction in organic synthesis, as it introduces an allyl group that can be further modified by various transformation protocols. Although several synthetic methods have been developed for allylation on *C*-, *O*-, *N*-, or *S*-centers,[1–3] no natural counterpart has been found in the biological system. Recently, the cofactor specificity of methyltransferases (MTases) have been relaxed to include *S*-adenosyl-L-methionine (SAM) analogs, leading to alkylation of biomacromolecules including protein, DNA, and RNA.[4–7] In particular, an allylated SAM (allyl-SAM, Scheme 1) analog has been used as a SAM surrogate by some wild-type protein MTases for protein labeling.[5] As allyl-SAM and SAM share high structural similarity, it is intriguing to explore allyl-SAM as an allyl donor by a wider spectrum of MTases for enzymatic allylation of small molecules. However, only a few examples have been reported on this subject. Two *C*-MTases, NovO and CouO, found in the biosynthesis pathway of the antibiotics coumermycin A1 and novobiocin, respectively, mediate Friedel–Crafts allylation on coumarin intermediate using allyl-SAM as the cofactor.[8] In another example, a sugar *O*-MTase, RebM, catalyzes the *O*-allylation of a rebeccamycin congener in an enzyme-coupled system while allyl-SAM was produced in situ from ATP and *S*-allylhomocysteine.[9] In these examples, the scope of the allyl acceptor, i.e., the substrate of the corresponding MTase, was not explored, most probably because it was difficult to access structurally diversified acceptors.

Regioselectively alkylated catechols are important entities for the preparation of bioactive natural products and drug intermediates.[10,11] Chemical synthesis of allylated catechols are associated with several disadvantages such as low selectivity and tedious protection–deprotection procedures.[12–14] Therefore, enzymatic allylation of catechols would be of particular interest.



Scheme 1. Chemical structures of SAM and allyl-SAM.

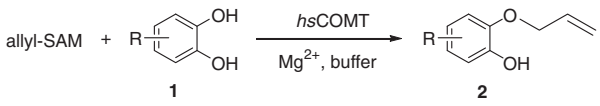
Catechol *O*-MTases (COMTs) are primarily responsible for the methylation of one hydroxy group of a catechol structure. COMTs especially human-soluble COMT (*hs*COMT) are widely studied due to their importance as drug targets.[15] Large-scale production of active *hs*COMT was once reported on fermentation conditions for pharmaceutical trials.[16] Microorganisms have been engineered to overproduce COMT for the production of highly valuable metabolites.[17,18] The enormous biocatalytic potential of COMTs, such as their accessible source, good chemo- and regioselectivities, and wide substrate scope,[19,20] encourage us to expand their catalytic capacities to allylation of different catechol derivatives. In this paper, we report the allylation of catechols by *hs*COMT using allyl-SAM as the allyl donor.

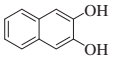
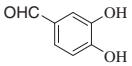
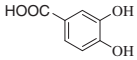
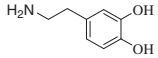
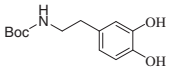
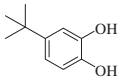
We overexpressed *hs*COMT in engineered *Escherichia coli* cells and purified the recombinant protein.[21] Allyl-SAM was synthesized in gram-scale from readily available chemicals according to a reported procedure.[5] Our initial experiment was performed at 37 °C for 15 h in a 250-μL reaction mixture of *hs*COMT (1.92 mg mL^{-1}, 78.7 μM), allyl-SAM (400 μM), and catechol **1a** (800 μM), and the reaction was followed by TLC analysis.[22] The corresponding allylated product was formed over time. When the reaction was performed on a larger scale with crude lysate-expressed *hs*COMT, the product was isolated in 48.3% yield (Table 1). Under the standard reaction conditions, allylation was also observed for compounds **1b**–**1i** and the products were successfully isolated regardless the substituents on the aromatic nucleus. The yield can be improved as an increased conversion of **1a** was detected by adding more enzymes. Although *hs*COMT showed a wide substrate scope, a catechol-containing structure remained essential. Thus, no allylation products were detected in case of salicylic acid, phenylenediamine, and phenol.

The allylation reaction showed good chemoselectivity. In protocatechuic acid (**1d**) and dopamine (**1e**) (Table 1, Entries 4 and 5), only one of the two hydroxys was allylated, and no allylation was found on the carboxyl group or the amino group. Thus, only mono-allylated products were obtained regardless the presence of excessive allyl-SAM. This provided an attractive route for the preparation of mono-allylated catechol derivatives for other applications. These results indicated that enzymatic allylation was advantageous over regular chemical synthesis as tedious and costly protection–deprotection procedures may be omitted.

It is known that *meta*-methylation is preferred by COMTs.[19] For protocatechuic aldehyde **1c** and **1d**, our results indicated that *hs*COMT afforded the *meta*-methylation product and the *para*-product in the ratios of 2.1:1 and 3.0:1, respectively, which were consistent with literature results.[23,24] To determine whether *hs*COMT followed a similar regioselectivity for allylation, we

Table 1. Enzymatic allylation of catechols[a]



Entry	1		Isolated yield/%	*meta*/*para* allylation ratio[b]
1		**1a**	48	—
2		**1b**	36	—
3		**1c**	34	0.8:1
4		**1d**	41	50:1
5		**1e**	—[c]	3.5:1
6		**1f**	21	1.1:1
7		**1g**	15	1:0
8		**1h**	30	7.4:1[d]
9		**1i**	14	2.1:1[d]

[a]Reactions conditions: *hs*COMT crude lysate (6–10 mg mL^{-1}, containing 60% *hs*COMT), allyl-SAM (4 mM) and **1** (4.4 mM), Tris-HCl buffer, 37 °C, 14 h. [b]Ratio determined by ^{1}H NMR or HPLC analysis. Reactions conditions: purified *hs*COMT (1.0 mg mL^{-1}, 40 μM), catechols (2 mM), allyl-SAM (3 mM). [c]Yield was not determined. [d]*meta*/*ortho* allylation ratio.

measured the distribution of different allylated products. The allylation of **1d**, **1e**, and 4-*tert*-butylbenzene-1,2-diol (**1g**) (Table 1, Entries 4, 5, and 7) as well as 3-fluorobenzene-1,2-diol (**1h**) and 3-methoxybenzene-1,2-diol (**1i**) (Table 1, Entries 8 and 9) were all confirmed to proceed with *meta*-site preference regardless the choice between *meta* and *para*-site or *meta* and *ortho*-site. It was exciting that almost only *meta*-allylated products were obtained for **1d** and **1g**. However, there was no remarkable regioselectivity for **1c** and *tert*-butyl 3,4-dihydroxyphenethylcarbamate **1f** (Table 1, Entries 3 and 6) while a *para*-allylated product was obtained by chemical synthesis in case of

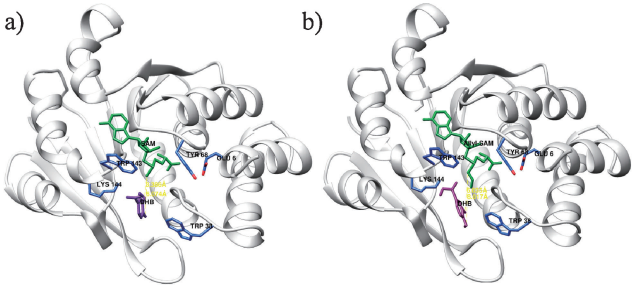


Figure 1. Docking results of *hs*COMT (PDB: 3A7E) complexed with **1d** and an alkyl donor. Residues involved in close contact with substrates were shown in stick representation. a) The distances between the carbon atom of the methyl group of SAM and the oxygen atom of OH group at the *meta*- and the *para*-position of **1d** were 3.38 and 6.07 Å, respectively. b) The distances between the methylene carbon atom of the allyl group of allyl-SAM and the OH group at the *meta*- and the *para*-position of **1d** were 6.03 and 8.01 Å, respectively.

1c.[13,14] Compared to chemical synthesis, opposite regioselectivity was also observed for **1d** and **1h**. The enzymatic allylation is mechanistically different from conventional chemical methods, as the regioselectivity attributed little relationship to the acidity of the hydroxy groups. The decreased regioselectivity after the protection of the amino group in **1e** reconfirmed this predication. This enzymatic allylation method provided a new mode for allylation of catechols.

To get more insights into the regioselectivity of *hs*COMT-catalyzed alkylation reaction, in silico docking experiments were performed using the AutoDock Vina program. Both the alkyl donor (SAM or allyl-SAM), and the substrate **1d**, were docked onto the *hs*COMT active sites (Figure 1). Five parallel molecule simulation assays were carried out, and nine docking conformations were obtained in each docking. Two of the best docking results are shown in Figure 1. According to the literature,[25,26] the cofactor is accommodated inside the active sites in a high-conservation conformation. Tyr 68 is located in the SAM binding site, and the highly conserved residue Trp143 is involved in van der Waals interaction with the adenosine ring of SAM. This residue may also impose a π-hydrogen bond interaction with the carboxylic group of **1d**, leading to a less flexible pose compared with **1c**, and higher alkylation regioselectivity of *hs*COMT in **1d** versus **1c**. The catalytic base Lys144 deprotonates the catechol to form the oxyanion responsible for nucleophilic attack of the alkyl group. The molecular interaction and the distances between the reactive carbon center and two hydroxy groups of **1d** suggested a favorable regioselectivity for alkylation at the *meta*-position. It revealed that both allyl-SAM and SAM bound to the same sites. However, allyl-SAM slightly changed the binding model for **1d**, resulting in a longer distance between the two reactive groups, which also explained the increased regioselectivity for allylation at *meta*-position compared with that of methylation.

The kinetics of *hs*COMT-catalyzed allylation of **1a** was determined in comparison to the corresponding methylation reaction (Table 2). The K_m values for SAM and allyl-SAM were 15.93 and 18.24 μM, respectively, indicating that the two alkyl donors had a similar affinity with *hs*COMT. This was consistent with the docking result where it showed that the SAM-binding

Table 2. Kinetics of *hs*COMT-catalyzed alkylation of **1a**

Alkyl donor	Kinetic parameters		
	K_m/μM	k_{cat}/min^{-1}	V_{max} /μM min^{-1}
Allyl-SAM	18.24 ± 4.46	0.50 ± 0.05	0.35 ± 0.03
SAM	15.93 ± 1.57	22.08 ± 0.78	0.76 ± 0.03

site was tailored to bind allyl-SAM as well. However, the k_{cat} values were widely different. The calculated k_{cat}/K_m value with allyl-SAM as the alkyl donor was approximately 50-fold lower than that with SAM, suggesting that *hs*COMT had significantly lower catalytic efficiency for transferring an allyl group to **1a**. Similar kinetic profiles are also known for human thiopurine *S*-MTase-catalyzed alkylation using other SAM analogs.[27] However, in another report it was demonstrated that the *C*-MTase, NovO, had higher catalytic efficiency in transferring an allyl group than a methyl group.[28] Although the presence of the unsaturated carbon–carbon bond in allyl-SAM stabilized the transition state formed in the MTase-directed S_N2 reactions, thus facilitating the reaction,[6] in case of *hs*COMT, the molecular interaction between the enzyme and the alkyl acceptor might play a more important role. In fact, the docking results showed that the binding of allyl-SAM could push the alkyl acceptor away from the alkyl donor. As a result, while *hs*COMT could bind allyl-SAM almost as well as it could bind SAM, the binding event had a significant consequence on the orientation of the alkyl acceptor at the active site, which led to drastic differences in the catalytic efficiency.

In summary, we have demonstrated that enzymatic allylation of catechols can be readily achieved by recombinant *hs*COMT. The reaction has relatively good chemo- and regioselectivities, and thus provides a "green" strategy for allylation of catechols. It should be pointed out that strategies have been developed for in vivo synthesis of SAM analogs[29] and engineering of MTase-involved pathways.[16,17] Our results may promote further studies on applying MTases as catalysts for biocatalytic alkylation to expand the structural space of natural and synthetic small molecules.

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Supporting Information is available electronically on J-STAGE.

References and Notes

1 A. Chaskar, K. Murugan, *Catal. Sci. Technol* **2014**, *4*, 1852.
2 W. Liu, X. Zhao, *Synthesis* **2013**, *45*, 2051.
3 F. Liron, J. Oble, M. M. Lorion, G. Poli, *Eur. J. Org. Chem.* **2014**, 5863.
4 H. Guo, R. Wang, W. Zheng, Y. Chen, G. Blum, H. Deng, M. Luo, *ACS Chem. Biol.* **2014**, *9*, 476.
5 Y. Zhang, Y. Pan, W. Yang, W. Liu, H. Zou, Z. K. Zhao, *ChemBioChem* **2013**, *14*, 1438.
6 C. Dalhoff, G. Lukinavičius, S. Klimašauskas, E. Weinhold, *Nat. Chem. Biol.* **2006**, *2*, 31.
7 D. Schulz, J. M. Holstein, A. Rentmeister, *Angew. Chem., Int. Ed.* **2013**, *52*, 7874.
8 H. Stecher, M. Tengg, B. J. Ueberbacher, P. Remler, H. Schwab, H. Griengl, M. Gruber-Khadjawi, *Angew. Chem., Int. Ed.* **2009**, *48*, 9546.
9 S. Singh, J. Zhang, T. D. Huber, M. Sunkara, K. Hurley, R. D. Goff, G. Wang, W. Zhang, C. Liu, J. Rohr, S. G. Van Lanen, A. J. Morris, J. S. Thorson, *Angew. Chem., Int. Ed.* **2014**, *53*, 3965.
10 T. J. Donohoe, A. Flores, C. J. R. Bataille, F. Churruca, *Angew. Chem., Int. Ed.* **2009**, *48*, 6507.
11 H.-J. Lo, Y.-K. Chang, T.-H. Yan, *Org. Lett.* **2012**, *14*, 5896.
12 M. Hayashida, M. Ishizaki, H. Hara, *Chem. Pharm. Bull.* **2006**, *54*, 1299.
13 X. Wang, T. Ju, X. Li, X. Cao, *Synlett* **2010**, 2947.
14 G. L. Plourde, R. R. Spaetzel, *Molecules* **2002**, *7*, 697.
15 C. Montag, M. Jurkiewicz, M. Reuter, *CNS Neurol. Disord.: Drug Targets* **2012**, *11*, 236.
16 R. Silva, S. Ferreira, M. J. Bonifácio, J. M. L. Dias, J. A. Queiroz, L. A. Passarinha, *J. Biotechnol.* **2012**, *160*, 161.
17 K. Li, J. W. Frost, *J. Am. Chem. Soc.* **1998**, *120*, 10545.
18 E. H. Hansen, B. L. Møller, G. R. Kock, C. M. Bünner, C. Kristensen, O. R. Jensen, F. T. Okkels, C. E. Olsen, M. S. Motawia, J. Hansen, *Appl. Environ. Microbiol.* **2009**, *75*, 2765.
19 D. Tsao, S. Liu, N. V. Dokholyan, *Chem. Phys. Lett.* **2011**, *506*, 135.
20 J. Taskinen, B. T. Ethell, P. Pihlavisto, A. M. Hood, B. Burchell, M. W. H. Coughtrie, *Drug Metab. Dispos.* **2003**, *31*, 1187.
21 J. Zhang, J. P. Klinman, *J. Am. Chem. Soc.* **2011**, *133*, 17134.
22 For experimental details, see Supporting Information.
23 N. J. H. Cotton, B. Stoddard, W. W. Parson, *J. Biol. Chem.* **2004**, *279*, 23710.
24 T. Lotta, J. Vidgren, C. Tilgmann, I. Ulmanen, K. Melén, I. Julkunen, J. Taskinen, *Biochemistry* **1995**, *34*, 4202.
25 K. Rutherford, I. Le Trong, R. E. Stenkamp, W. W. Parson, *J. Mol. Biol.* **2008**, *380*, 120.
26 D. Tsao, L. Diatchenko, N. V. Dokholyan, *PLoS ONE* **2011**, *6*, e24287.
27 B. W. K. Lee, H. G. Sun, T. Zang, B. J. Kim, J. F. Alfaro, Z. S. Zhou, *J. Am. Chem. Soc.* **2010**, *132*, 3642.
28 M. Tengg, H. Stecher, P. Remler, I. Eiteljörg, H. Schwab, M. Gruber-Khadjawi, *J. Mol. Catal. B: Enzym.* **2012**, *84*, 2.
29 R. Wang, K. Islam, Y. Liu, W. Zheng, H. Tang, N. Lailler, G. Blum, H. Deng, M. Luo, *J. Am. Chem. Soc.* **2013**, *135*, 1048.

