VIP Biocatalysis Very Important Paper

How to cite: *Angew. Chem. Int. Ed.* **2022**, *61*, e202213056
International Edition: doi.org/10.1002/anie.202213056
German Edition: doi.org/10.1002/ange.202213056

Selective Biocatalytic *N*-Methylation of Unsaturated Heterocycles

Felipe Ospina[+], *Kai H. Schülke*[+], *Jordi Soler, Alina Klein, Benjamin Prosenc, Marc Garcia-Borràs,* and Stephan C. Hammer**

Abstract: Methods for regioselective *N*-methylation and -alkylation of unsaturated heterocycles with "off the shelf" reagents are highly sought-after. This reaction could drastically simplify synthesis of privileged bioactive molecules. Here we report engineered and natural methyltransferases for challenging *N*-(m)ethylation of heterocycles, including benzimidazoles, benzotriazoles, imidazoles and indazoles. The reactions are performed through a cyclic enzyme cascade that consists of two methyltransferases using only iodoalkanes or methyl tosylate as simple reagents. This method enables the selective synthesis of important molecules that are otherwise difficult to access, proceeds with high regioselectivity (r.r. up to >99 %), yield (up to 99 %), on a preparative scale, and with nearly equimolar concentrations of simple starting materials.

Unsaturated *N*-heterocycles such as benzimidazoles, benzotriazoles, imidazoles and indazoles are privileged scaffolds in pharmaceuticals and bioactive compounds.[1] These aromatic rings are frequently *N*-methylated or -alkylated, with the *N*-substitution pattern controlling their biological function (Figure 1A). Due their usefulness, many methods for the preparation of *N*-substituted heterocycles exist, but their synthesis is typically tedious and often proceeds through multistep *de novo* ring construction (see Figure 1B).[2,3] Synthetic access to *N*-substituted heterocycles is particular laborious when multiple derivatives need to be synthesized, since the *N*-substitution pattern is set early in the synthetic sequences.

As countless unsaturated *N*-heterocycles are commercially available at low cost, selective alkylation with "off the shelf" reagents could directly generate *N*-substituted heterocycles. However, at present there is no general reagent, protecting group strategy, or catalyst that can selectively *N*-alkylate such molecules to access different regioisomers.[4–6] Regioselective *N*-alkylations are challenging because tautomerization generates nitrogen atoms of very similar reactivity (Figure S1). Consequently, conventional chemistry with alkylation reagents results in product mixtures which are challenging to separate (Figure S2). Not surprisingly, the regioselective *N*-methylation and -alkylation of readily available heterocycles is on the wish list of many chemists (Figure 1C),[7,8] as it would drastically shorten synthesis, especially in the direct conversion of lead structures into potentially more active derivatives.

We have recently started to develop a biocatalytic platform to control regioselectivity in such challenging C–N bond forming alkylation reactions.[9–11] This approach utilizes two *S*-adenosyl-L-methionine (SAM)—dependent methyltransferases (MTs) for selective alkylation reactions in a cyclic two-enzyme cascade (Figure 2A).[12] The cascade is driven by a promiscuous anion MT that uses iodo- and bromoalkanes as well as methylsulfates and -sulfonates as simple precursors to generate SAM or analogs thereof through enzymatic alkylation of *S*-adenosyl-L-homocysteine.[9,11,13,14] A second MT utilizes SAM (or its analogue) as electrophile in highly selective methylation/alkylation reactions. In a first study, we reported that this biocatalytic system can be engineered to methylate pyrazoles with high regioselectivity using iodomethane as reagent.[9] While our recently reported system describes a first catalytic regioselective pyrazole alkylation, the method is currently limited to low conversion (typically <10 %), high excess of the iodoalkane (10 equiv) and to pyrazoles as substrates.[9–11]

Here we report the enzymatic methylation or ethylation of various *N*-heterocycles, including benzimidazoles, benzotriazoles, imidazoles and indazoles with high regioselectivity (r.r. >99:1), regiodivergence, high activity (up to 99 % yield) and on preparative scale. We further describe that this enzyme cascade can be performed with almost stoichiometric amounts of methyl tosylate as simple methylation reagent.

A significant drawback in synthesis with SAM-dependent MTs is their limited substrate scope as well as the regeneration of SAM and analogs thereof.[15–21] While the

[*] F. Ospina,[+] K. H. Schülke,[+] A. Klein, B. Prosenc, Prof. Dr. S. C. Hammer
Faculty of Chemistry, Organic Chemistry and Biocatalysis
Bielefeld University
Universitätsstraße 25, 33615 Bielefeld (Germany)
E-mail: stephan.hammer@uni-bielefeld.de

J. Soler, Dr. M. Garcia-Borràs
Institut de Química Computacional i Catàlisi (IQCC) and Departament de Química, Universitat de Girona
Carrer Maria Aurèlia Capmany 69, Girona 17003, Catalonia (Spain)
E-mail: marc.garcia@udg.edu

[+] These authors contributed equally to this work.

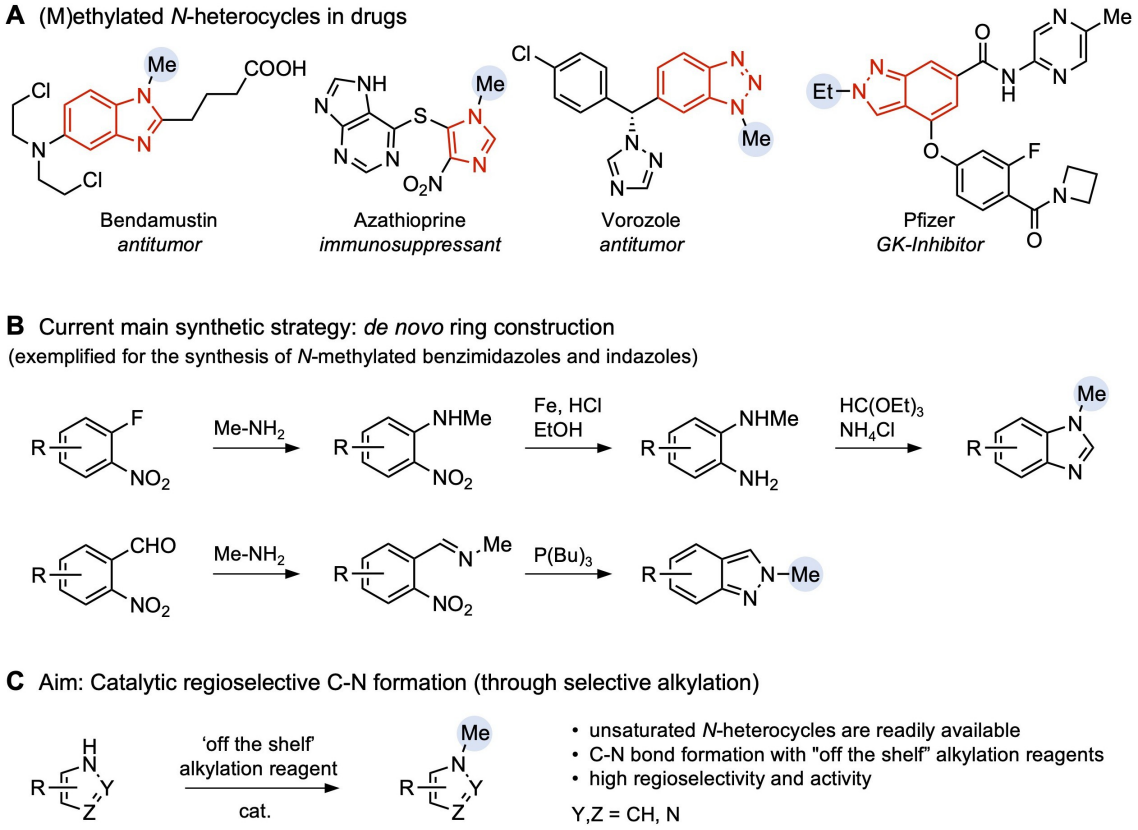

Figure 1. *N*-(m)ethylated heterocyclic compounds. A) Examples of pharmaceuticals containing *N*-(m)ethylated heterocycles. B) Synthetic strategies to access *N*-substituted heterocycles typically involve *de novo* ring construction, here shown in the selective synthesis of *N*-methylated benzimidazoles and indazoles as an example. Such syntheses proceed via multistep reaction sequences, with the *N*-methylation/-alkylation pattern being set right in the beginning. This can be laborious, especially in the direct conversion of lead structures into several derivatives. C) Catalytic regioselective alkylation: As countless *N*-heterocycles are commercially available, a direct regioselective methylation/alkylation with synthetic reagents would significantly shorten synthesis.

latter is currently being addressed through the development of cyclic enzyme cascades that use simple haloalkanes to synthesize and recycle artificial cosubstrates (SAM analogues, Figure 2A),[9,11,12,15,22] the whole technology is largely limited to natural products as substrates. Typical starting materials used by MTs in synthetic applications are a small set of coumarins, phenols, amino acids, alkaloids, or sugars.[12,13,23–28] To investigate whether the substrate scope of MTs can be easily extended towards the *N*-methylation/alkylation of various heterocycles, we aimed to identify an enzyme panel that shows high activity and selectivity for a broad spectrum of molecules. Our MT panel consists of 59 enzymes (Table S2): i) 50 variants from a computationally designed enzyme library (v1–v50) of the human nicotinamide *N*-methyltransferase (NNMT),[9,29,30] ii) five single point variants of NNMT (v51–55) and iii) three NNMT homologs, namely a histamine *N*-methyltransferase (HNMT),[31] a phenylethanolamine *N*-methyltranferase (PNMT)[32] as well as an indolethylamine *N*-methyltranferase (INMT).[30,33] The computationally designed enzyme library (v1–v50) was built using the FuncLib algorithm[9,34] and each variant contains 4–5 active site mutations compared to NNMT wildtype, generated to explore a larger section of the sequence space (Table S2).

We have chosen benzimidazoles, benzotriazoles, imidazoles and indazoles as substrates for profiling because they bear multiple competing *N*-nucleophiles of comparable reactivity (Figure 2C). Although benzimidazoles, indazoles and benzotriazoles are privileged scaffolds in manmade pharmaceuticals and bioactive compounds, they are largely unknown moieties in natural products. The selected substrates mainly contain substitutions remote to the competing nitrogen nucleophiles. Therefore, these are substrates that depend on a very high level of catalyst-control for selective C–N bond formation. Please note that non-enzymatic methylations/alkylations of these molecules generated almost equal mixtures of regioisomers (Figure S2).

To identify promiscuous enzyme activity, we tested all substrates with the enzyme panel as cell lysate using stoichiometric amounts of SAM as methyl source. Remarkably, the enzyme panel revealed broad activity-selectivity profiles for all *N*-heterocycles (Figures 2B and S3), high regioselectivity (r.r. >99 %), and regiodivergent solutions. The regiodivergence is visible in the activity-selectivity profile for 5-bromobenzimidazole (Figure 2B) through the

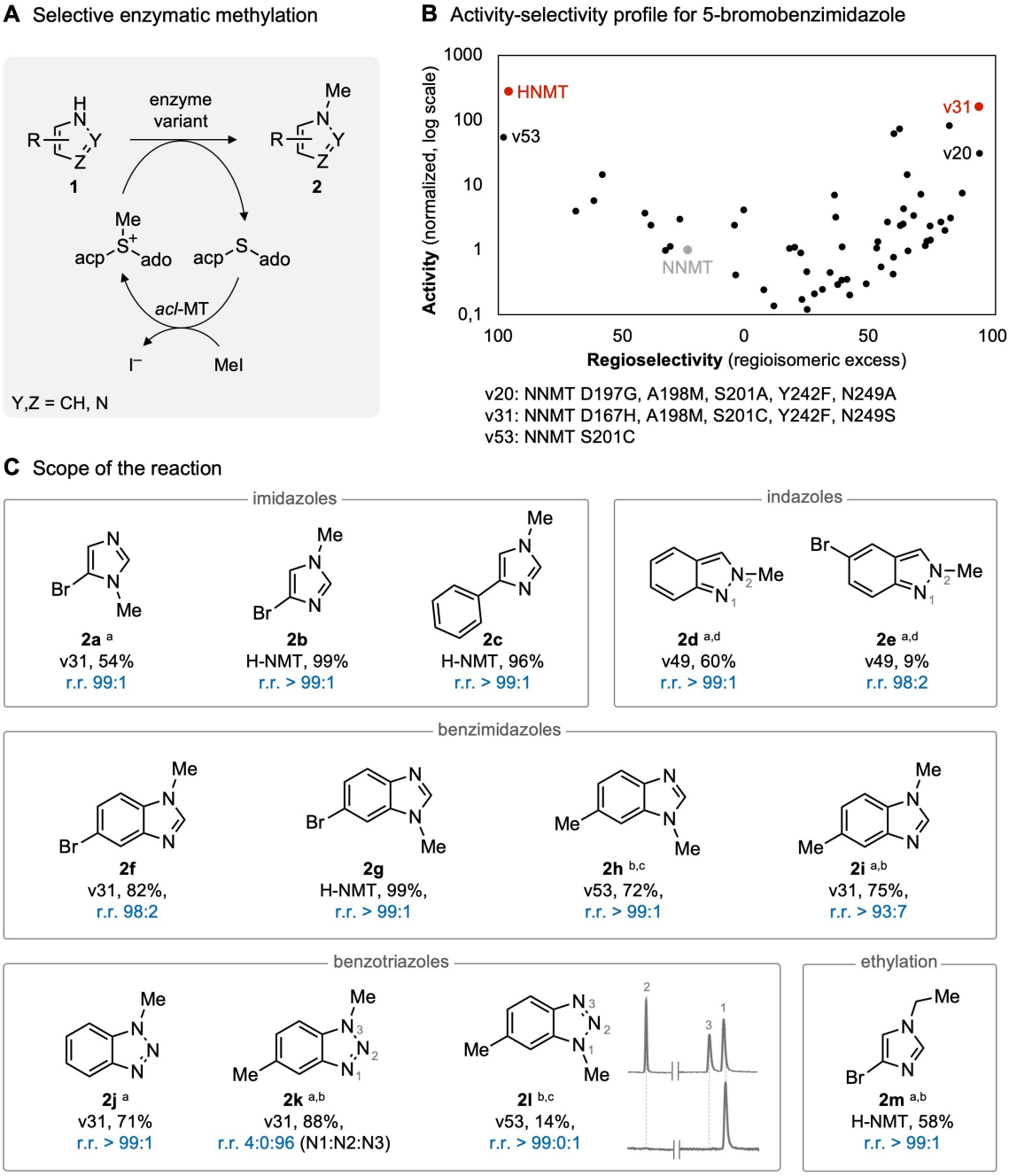

Figure 2. Regioselective biocatalytic methylation. A) Cyclic enzyme cascade applied in this study. SAM is used as cosubstrate by methyltransferases in selective alkylation reactions. The produced SAH byproduct can be methylated by an anion MT using simple methyl iodide. B) We identified a panel of MTs (engineered variants and wildtype enzymes) that show broad activity-selectivity profiles for different *N*-heterocycles. The figure shows such a profile for 5-bromobenzimidazole as substrate. While the wildtype NNMT (grey) shows low activity and selectivity in the methylation reaction using SAM as cosubstrate, various variants of NMMT (v20, v31, v53) and the wildtype HNMT revealed high activity and selectivity in the promiscuous C–N bond formation. C) Regioselective (m)ethylation of various *N*-heterocycles using the cascade of Figure 2A. Each product is shown with the corresponding enzyme. The yield is given as mean value from triplicate experiments. The regioselectivity is reported as regioisomeric ratios (r.r., in blue). Standard reaction conditions: 2 mM substrate, 1 mol% of both enzymes and SAH, 5 equiv haloalkane, 2% DMSO, 20 h, RT. [a] 2 mol% enzyme variant, 48 h. [b] 1 mM substrate. [c] 5 mol% enzyme variant, 48 h. [d] 3.5% DMSO. acp = (*S*)-3-amino-3-carboxypropyl. ado = adenosyl.

enzymes HNMT or v53 producing isomer **2g** (see Figure 2C) and the enzymes v31 or v20 generating isomer **2f**. After profiling all enzymes with the substrate panel in a 96-well plate format, we continued to analyze the most active and selective biocatalysts as purified enzyme. Next to high regioselectivity in the methylation of benzimidazoles (**2f**, **2g**,

2h and **2i**) and imidazoles (**2a**, **2b** and **2c**), we observed highly selective indazole methylation. The generated 2-methyl indazole products (**2d** and **2e**) are notable, as they are the thermodynamically less-stable regioisomers.[35] We have also studied benzotriazoles which contain three competing *N*-nucleophiles, leading to an almost equal mixture of three regioisomers in non-enzymatic methylation (Figure S2). To our delight, highly regioselective C–N bond formations (r.r. >99:0:1) could be identified with benzotriazoles as substrates, as highlighted by the formation of product **2l** using v53 (Figure 2C). This high level of regiocontrol is outstanding, as the catalysts must discriminate between the orientation of a small methyl group distal to the three competing *N*-nucleophiles. This highlights the advantage that enzymes have in synthesis due to their high degree of confinement (molecular recognition).[36]

The enzymes did not only show high regiocontrol with various heterocycles as substrates, but also high activity. While in some cases the wildtype HNMT could be identified as a selective and active biocatalyst, in many other cases variants from the computational enzyme library showed astonishing performance. For example, v31, which contains five active site mutations (D167H, A198M, S201C, Y242F, N249S), is not only regioselective (r.r. >99:1 compared to 38:62 for NNMT wildtype) but also two orders of magnitude more active than the NNMT wildtype (Figure 2B). Such a high increase in activity and selectivity is rare in enzyme engineering and has been observed for different substrates in this study (Figure S3). This high increase in activity and selectivity underlines the efficiency of computational enzyme library design using Funclib.[34] As SAM is not a cheap methyl donor in synthesis, we applied the identified enzymes in the cyclic enzyme cascade (Figure 2A). Here, an anion MT from *Aspergillus clavatus* (*acl*-MT) regenerates SAM through methylation of SAH using simple iodomethane (MeI). We have found that most of the regioisomers can be synthesized with high regioselectivity and high yield (up to 99%, see Figure 2C). Please note that this catalytic approach (Figure 2A) can transfer groups other than methyl by using different reagents.[9,11,13,22] In this context, we utilized iodoethane as reagent to ethylate 5-bromoimidazole to **2m** with outstanding regioselectivity (r.r. >99:1, Figure 2C). The HNMT performed the regioselective ethylation at the same nitrogen atom as the methylation reaction (please compare **2b** and **2m**), but with lower yield (58% instead of 99%). Overall, this strongly supports that engineered MTs offer a generalizable catalytic approach for regioselective *N*-alkylation of heterocycles which could significantly shorten synthesis and might be very powerful to efficiently diversify heterocyclic lead structures through selective biocatalytic alkylation.

We next aimed to perform some of the reactions on a preparative scale. As methylation reagents differ in their reactivity, we were interested to investigate various reagents as methyl/alkyl source.[6] We have already reported that anion MTs are not limited to iodoalkanes but also readily accept cheaper and more abundant bromoalkanes to synthesize SAM analogs.[9,11] Very recently the Seebeck group has also reported that methylsulfates and -sulfonates can be used as methyl source.[14] Since anion MTs and related enzymes do not only bind halides,[37] but also hydrogen sulfide anion,[38,39] thiocyanate[37,40] and phosphonates,[41] we postulated that such enzymes could also activate a broader range of leaving groups for SAH methylation (and SAM regeneration). We studied the promiscuous activity of our in-house anion MT library to activate phosphates, mesylates and tosylates as leaving groups (Figure S14). We found that methyl tosylate (MeOTs) is well accepted to synthesize SAM from SAH (Figure 3A), which further confirms the original work from the Seebeck group.[14] To us, this is of interest as MeOTs is a "hard" reagent that may show different non-enzymatic background methylation behavior in biotransformations than the "soft" MeI, therefore expanding this methodology towards a broader range of potential nucleophiles. In addition, tosylates are not only commercially available but also directly accessible from alcohols, thus, generating a link between abundant and cheap alcohols and selective biocatalytic alkylation chemistry using MTs. We further investigated MeOTs-driven SAM formation with *acl*-MT, as this enzyme revealed high conversion and was also the most promiscuous enzyme with respect to different haloalkanes.[11] The time course of the *acl*-MT-catalyzed reaction showed that MeOTs is almost as efficient as MeI in SAM formation (Figure 3B), making MeOTs an interesting reagent for preparative scale applications.

To shed light on the molecular basis for the promiscuous MeOTs activity, we carried out computational modelling using *acl*-MT (see Supporting Information for details). Molecular Dynamics (MD) simulations revealed that the *acl*-MT binding site, in the presence of SAH, is shielded by two rather flexible loops (*N*-terminus loop 1, residues 1–8, and loop 2, residues 35–47, see Figures 3C, S15 and S16). The large conformational flexibility observed for these two loops allows the exploration of alternative loop conformations that generate a large space in the binding pocket, near the SAH sulfur atom (Figures 3C, S15, S16 and S19). This enlarged cavity allows the efficient binding of bulkier MeOTs in a catalytically relevant, near attack conformation which enables an effective methyl transfer for SAM formation (Figures 3C, S15, S20, S21 and S23), as characterized from combined model DFT calculations and MD simulations (see Supporting Information). The conformational flexibility of the *acl*-MT active site loops might generally explain the observed substrate promiscuity of this particular enzyme.[11] The flexible loop structures of *acl*-MT are in contrast to the crystal structure of the homolog anion MT from *Arabidopsis thaliana* (*ath*-MT, PDB: 3LCC), displaying a more ordered *N*-terminus region that forms an α-helix which covers the SAH/halide binding site.[37] These conclusions on the role of the disordered *N*-terminus in more efficient binding of bulkier substrates is in agreement with results from the Seebeck group obtained through X-ray crystallography.[14]

Having selective enzymes and simple reagents in hand, we finally aimed to understand the scope and limitation of this approach in preparative scale reactions. We synthesized the methylated benzimidazoles **2f** and **2g** using either MeI

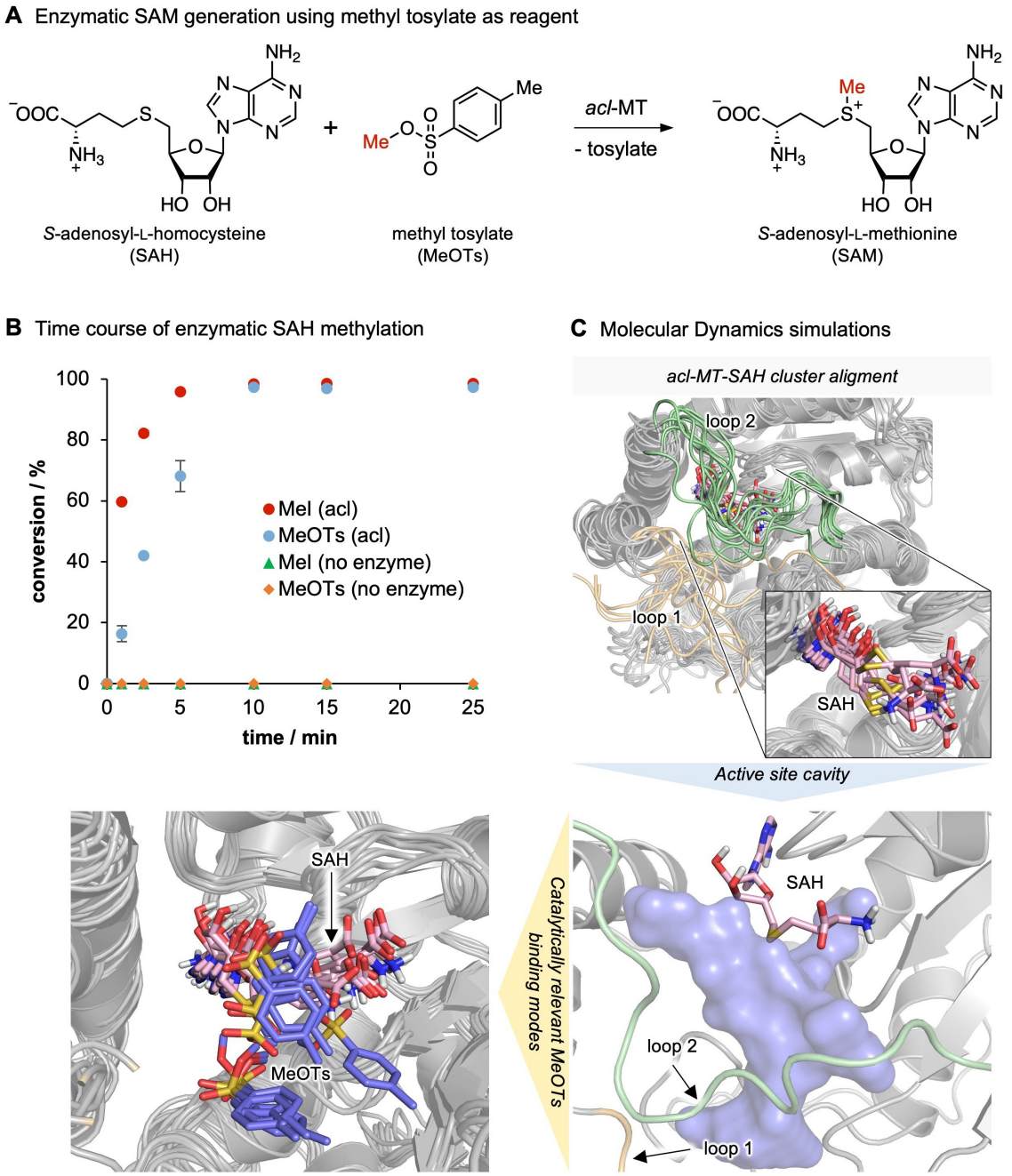

Figure 3. Enzymatic SAH alkylation using methyl tosylate as reagent. A) Reaction scheme of the enzymatic SAM formation by SAH methylation using MeOTs. B) Time course for the *acl*-catalyzed SAM formation using MeI (red) and MeOTs (blue). The reaction leads to 100% conversion within minutes. Conversion is shown as mean value ($n=3$) with standard deviation as error bars. Control experiments without enzyme (*acl*-MT) are shown in green (MeI) and orange (MeOTs). Reaction conditions: 1 mM SAH, 1 mol% *acl*-MT, 1.5 equiv MeI or MeOTs, RT. C) Molecular Dynamics (MD) simulations, a total of 2.5 μs of MD simulation time from 5 independent replicas, were used to characterize the conformational dynamics of *acl*-MT with SAH bound. The flexible *N*-terminus loop 1 (residues 1–8) and loop 2 (residues 35–47) are highlighted in yellow and green, respectively. The accessible empty space in the *acl*-MT with SAH bound active site is shown as a blue surface in a representative snapshot obtained from MD simulations. The catalytically relevant binding modes of MeOTs, bound in a near attack conformation (NAC) for an efficient methyl transfer to SAH, were characterized using restrained-MD simulations. MeOTs can occupy the space generated in the active site by the displacement of the flexible loops. See Supporting Information (Figures S15–S23) for more details.

or MeOTs with excellent isolated yield (96% and 89%, respectively) and high regioselectivity (Figure 4). These preparative scale reactions revealed advantages and disadvantages of both reagents. While MeI as volatile compound (b.p. 42 °C) is typically applied with significant excess with respect to the substrate (3 equiv in our example in Figure 4), MeOTs is barely volatile (b.p. >290 °C) and can thus be applied at almost stoichiometric concentrations (1.5 equiv,

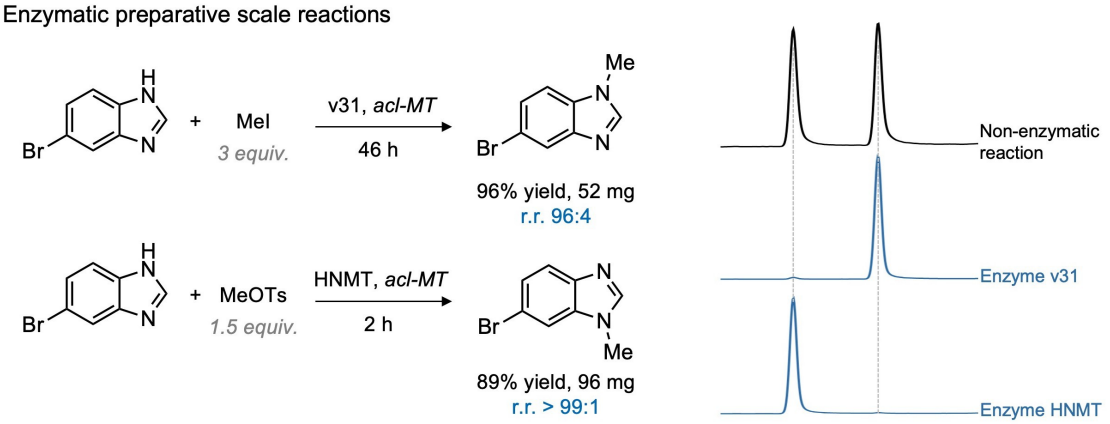

Figure 4. Enzymatic preparative scale methylation. 5-bromobenzimidazole was selectively methylated using either MeI or MeOTs as methyl source. The GC chromatograms on the right highlight the selectivities achieved. Reaction conditions with MeI: 1 mM substrate, 2% *i*-PrOH, 0.5 mol% *acl*-MT, 2 mol% v31, 1 mol% SAH. Reaction conditions with MeOTs: 2 mM substrate, 2% *i*-PrOH, 0.5 mol% *acl*-MT, 1 mol% HNMT and SAH. All enzymes have been used in purified form.

Figure 4). However, MeOTs possesses a higher inherent reactivity than MeI, which can result in a significantly unselective background methylation if the *N*-heterocycles are not efficiently converted by the enzyme. Consequently, upscaling to produce **2f** was performed with MeI and the rather inefficient v31 (46 h reaction time) while **2g** was produced with the more efficient HNMT (2 h reaction time) using only 1.5 equiv of MeOTs.

What stands out is that the starting materials applied (e.g. benzimidazole and MeOTs) are abundant and cheap. In synthetic chemistry there is currently no reagent, catalyst or protecting group strategy that enables regiocontrol using these simple starting materials.[7,8] However, applying these two molecules (5-bromobenzimidazole and MeOTs) in water with the enzymes described here enables the synthesis of important molecules that are otherwise difficult to access, with high regioselectivity, yield, on preparative scale, and with nearly equimolar concentration of starting material (Figure 4).

Overall, selective biocatalytic alkylation chemistry with SAM-dependent MTs and "off the shelf" reagents is currently flourishing.[9,11,13,15,16,18,20,22,42,43] This approach is rapidly expanding to other alkylation reagents,[9,11,13,14,22] but currently mainly limited to methylate/alkylate a small set of natural products.[12,13,22,44] Here we reported that natural and engineered enzymes can be used in challenging C–N bond formations with *N*-heterocycles, including benzimidazoles, benzotriazoles, imidazoles and indazoles. These reactions are sought-after and so far have no general solution. It is motivating to see that all these enzyme activities could be identified from a single enzyme library and a couple of wildtype MTs. We envision that this platform can be further expanded towards a technology to directly diversify bioactive lead structures to various potentially more active derivatives while dramatically shortcutting synthesis efforts.

Acknowledgements

We thank Florian Seebeck (University of Basel) for fruitful discussion, Bernhard Hauer (Stuttgart University) for providing plasmid DNA of the wildtype MTs, Kathrin Hörnschemeyer for generating the single point mutants (v51–55) and Jan-Hendrik Schröder for support in the preparative scale reactions. This work was supported by the Deutsche Forschungsgemeinschaft via the Emmy Noether program (fellowship HA 7668/2-1) and the Deutsche Bundesstiftung Umwelt via a PhD scholarship (20020/690, to K.H.S.). This work was partially supported by the Spanish MICINN (Ministerio de Ciencia e Innovación) PID2019-111300GA-I00 project and the Ramón y Cajal program via the RYC2020-028628-I fellowship (to M.G.B), and the Spanish MIU (Ministerio de Universidades) predoctoral FPU fellowship FPU18/02380 (to J.S.). Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

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: Alkylation · Biocatalysis · Heterocycles · Methyltransferase · SAM Recycling

[1] R. D. Taylor, M. Maccoss, A. D. G. Lawson, *J. Med. Chem.* **2014**, *57*, 5845–5859.
[2] S. G. Zhang, C. G. Liang, W. H. Zhang, *Molecules* **2018**, *23*, 2783.

[3] M. Faheem, A. Rathaur, A. Pandey, V. Kumar Singh, A. K. Tiwari, *ChemistrySelect* **2020**, *5*, 3981–3994.
[4] N. E. Genung, L. Wei, G. E. Aspnes, *Org. Lett.* **2014**, *16*, 3114–3117.
[5] J. M. Joo, B. B. Touré, D. Sames, *J. Org. Chem.* **2010**, *75*, 4911–4920.
[6] Y. Chen, *Chem. Eur. J.* **2019**, *25*, 3405–3439.
[7] D. C. Blakemore, L. Castro, I. Churcher, D. C. Rees, A. W. Thomas, D. M. Wilson, A. Wood, *Nat. Chem.* **2018**, *10*, 383–394.
[8] K. Krämer, "A Wish List for Organic Chemistry," can be found under https://www.chemistryworld.com/news/the-five-reactions-on-every-organic-chemists-wish-list/3010150.article, **2019**.
[9] L. L. Bengel, B. Aberle, A.-N. Egler-Kemmerer, S. Kienzle, B. Hauer, S. C. Hammer, *Angew. Chem. Int. Ed.* **2021**, *60*, 5554–5560; *Angew. Chem.* **2021**, *133*, 5614–5620.
[10] B. List, J. L. Kennemur, *Synfacts* **2021**, *17*, 0322.
[11] K. H. Schülke, F. Ospina, K. Hörnschemeyer, S. Gergel, S. C. Hammer, *ChemBioChem* **2022**, *23*, e202100632.
[12] C. Liao, F. P. Seebeck, *Nat. Catal.* **2019**, *2*, 696–701.
[13] Q. Tang, C. W. Grathwol, A. S. Aslan-Üzel, S. Wu, A. Link, I. V. Pavlidis, C. P. S. Badenhorst, U. T. Bornscheuer, *Angew. Chem. Int. Ed.* **2021**, *60*, 1524–1527; *Angew. Chem.* **2021**, *133*, 1547–1551.
[14] X. Wen, F. Leisinger, V. Leopold, F. P. Seebeck, *Angew. Chem. Int. Ed.* **2022**, *61*, e202208746; *Angew. Chem.* **2022**, *134*, e202208746.
[15] Q. Tang, I. V. Pavlidis, C. P. S. Badenhorst, U. T. Bornscheuer, *ChemBioChem* **2021**, *22*, 2584–2590.
[16] 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.
[17] M. R. Bennett, S. A. Shepherd, V. A. Cronin, J. Micklefield, *Curr. Opin. Chem. Biol.* **2017**, *37*, 97–106.
[18] F. Michailidou, A. Rentmeister, *Org. Biomol. Chem.* **2021**, *19*, 3756–3762.
[19] T. D. Huber, B. R. Johnson, J. Zhang, J. S. Thorson, *Curr. Opin. Biotechnol.* **2016**, *42*, 189–197.
[20] I. J. W. McKean, P. A. Hoskisson, G. A. Burley, *ChemBioChem* **2020**, *21*, 2890–2897.
[21] L. A. Wessjohann, J. Keim, B. Weigel, M. Dippe, *Curr. Opin. Chem. Biol.* **2013**, *17*, 229–235.
[22] J. Peng, C. Liao, C. Bauer, F. P. Seebeck, *Angew. Chem. Int. Ed.* **2021**, *60*, 27178–27183; *Angew. Chem.* **2021**, *133*, 27384–27389.
[23] I. J. W. McKean, J. Sadler, A. Cuetos, A. Frese, L. Humphreys, G. Grogan, P. Hoskisson, G. A. Burley, *Angew. Chem. Int. Ed.* **2019**, *58*, 17583–17588; *Angew. Chem.* **2019**, *131*, 17747–17752.
[24] A. J. Herbert, S. A. Shepherd, V. A. Cronin, M. R. Bennett, R. Sung, J. Micklefield, *Angew. Chem. Int. Ed.* **2020**, *59*, 14950–14956; *Angew. Chem.* **2020**, *132*, 15060–15066.
[25] C. Sommer-Kamann, A. Fries, S. Mordhorst, J. N. Andexer, M. Müller, *Angew. Chem. Int. Ed.* **2017**, *56*, 4033–4036; *Angew. Chem.* **2017**, *129*, 4091–4094.
[26] S. Mordhorst, J. Siegrist, M. Müller, M. Richter, J. N. Andexer, *Angew. Chem. Int. Ed.* **2017**, *56*, 4037–4041; *Angew. Chem.* **2017**, *129*, 4095–4099.
[27] 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*, 3965–3969; *Angew. Chem.* **2014**, *126*, 4046–4050.
[28] H. Stecher, M. Tengg, B. J. Ueberbacher, P. Remler, H. Schwab, H. Griengl, M. Gruber-Khadjawi, *Angew. Chem. Int. Ed.* **2009**, *48*, 9546–9548; *Angew. Chem.* **2009**, *121*, 9710–9712.
[29] H. S. Loring, P. R. Thompson, *Biochemistry* **2018**, *57*, 5524–5532.
[30] L. L. Bengel, *Enzymkatalysierte Regioselektive N-Methylierung und N-Alkylierung von Pyrazolen*, Universität Stuttgart, **2021**.
[31] C. Dent, F. Nilam, I. R. Smith, *Biochem. Pharmacol.* **1982**, *31*, 2297–2300.
[32] N. Drinkwater, C. L. Gee, M. Puri, K. R. Criscione, M. J. McLeish, G. L. Grunewald, J. L. Martin, *Biochem. J.* **2009**, *422*, 463–471.
[33] M. A. Thompson, R. M. Weinshilboum, *J. Biol. Chem.* **1998**, *273*, 34502–34510.
[34] O. Khersonsky, R. Lipsh, Z. Avizemer, Y. Ashani, M. Goldsmith, H. Leader, O. Dym, S. Rogotner, D. L. Trudeau, J. Prilusky, P. Amengual-Rigo, V. Guallar, D. S. Tawfik, S. J. Fleishman, *Mol. Cell* **2018**, *72*, 178–186.
[35] D. D. Gaikwad, A. D. Chapolikar, C. G. Devkate, K. D. Warad, A. P. Tayade, R. P. Pawar, A. J. Domb, *Eur. J. Med. Chem.* **2015**, *90*, 707–731.
[36] B. Mitschke, M. Turberg, B. List, *Chem* **2020**, *6*, 2515–2532.
[37] J. W. Schmidberger, A. B. James, R. Edwards, J. H. Naismith, D. O'Hagan, *Angew. Chem. Int. Ed.* **2010**, *49*, 3646–3648; *Angew. Chem.* **2010**, *122*, 3728–3730.
[38] N. Itoh, H. Toda, M. Matsuda, T. Negishi, T. Taniguchi, N. Ohsawa, *BMC Plant Biol.* **2009**, *9*, 116.
[39] J. M. Attieh, A. D. Hanson, H. S. Saini, *J. Biol. Chem.* **1995**, *270*, 9250–9257.
[40] J. M. Attieh, R. Djiana, P. Koonjul, C. Étienne, S. A. Sparace, H. S. Saini, *Plant Mol. Biol.* **2002**, *50*, 511–521.
[41] J. H. Lee, B. Bae, M. Kuemin, B. T. Circello, W. W. Metcalf, S. K. Nair, W. A. Van Der Donk, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 17557–17562.
[42] F. Ospina, K. H. Schülke, S. C. Hammer, *ChemPlusChem* **2022**, *87*, e202100454.
[43] P. Schneider, B. Henßen, B. Paschold, B. P. Chapple, M. Schatton, F. P. Seebeck, T. Classen, J. Pietruszka, *Angew. Chem. Int. Ed.* **2021**, *60*, 23412–23418; *Angew. Chem.* **2021**, *133*, 23600–23606.
[44] C. Liao, F. P. Seebeck, *Angew. Chem. Int. Ed.* **2020**, *59*, 7184–7187; *Angew. Chem.* **2020**, *132*, 7251–7254.

Manuscript received: September 4, 2022
Accepted manuscript online: October 6, 2022
Version of record online: October 26, 2022