VIP *Very Important Paper*

Substrate Profiling of Anion Methyltransferases for Promiscuous Synthesis of *S*-Adenosylmethionine Analogs from Haloalkanes

Kai H. Schülke[+],[a] Felipe Ospina[+],[a] Kathrin Hörnschemeyer,[a] Sebastian Gergel,[a] and Stephan C. Hammer*[a]

Biocatalytic alkylation reactions can be performed with high chemo-, regio- and stereoselectivity using *S*-adenosyl-L-methionine (SAM)-dependent methyltransferases (MTs) and SAM analogs. Currently, however, this methodology is limited in application due to the rather laborious protocols to access SAM analogs. It has recently been shown that halide methyltransferases (HMTs) enable synthesis and recycling of SAM analogs with readily available haloalkanes as starting material. Here we expand this work by using substrate profiling of the anion MT enzyme family to explore promiscuous SAM analog synthesis. Our study shows that anion MTs are in general very promiscuous with respect to the alkyl chain as well as the halide leaving group. Substrate profiling further suggests that promiscuous anion MTs cluster in sequence space. Next to iodoalkanes, cheaper, less toxic, and more available bromoalkanes have been converted and several haloalkanes bearing short alkyl groups, alkyl rings, and functional groups such as alkene, alkyne and aromatic moieties are accepted as substrates. Further, we applied the SAM analogs as electrophiles in enzyme-catalyzed regioselective pyrazole allylation with 3-bromopropene as starting material.

Introduction

Biocatalytic alkylation chemistry is a flourishing research field.[1] Several enzymes have recently been developed that catalyze highly selective sp^3 C–C/N/O/S bond formations. Within this field, *S*-adenosyl-L-methionine (SAM)-dependent enzymes are attracting significant attention due to their ability to perform highly selective alkylation reactions. In nature, methyltransferases (MTs) utilize SAM as co-substrate in methyltransfer reactions and produce *S*-adenosyl-L-homocysteine (SAH) as byproduct that is recycled in a multistep reaction sequence.[2] While SAM chemistry is mostly limited to methyltransfer, several enzymatic methods have lately been developed to synthesize SAM analogs.[2–10] In a subsequent reaction, this SAM-related sulfonium species can be used as co-substrates by natural and engineered MTs in highly selective sp^3 C–C/N/O/S bond forming reactions that are otherwise very difficult to achieve.[1,11]

A current limitation in biocatalytic alkylation with electrophilic sulfonium species originates from the rather laborious synthesis of SAM analogs.[12] Traditionally, SAM analogs have been synthesized by chemical alkylation of SAH, yielding diastereomeric mixtures of SAM-related sulfonium species that are commonly separated by reverse-phase chromatography (Figure S1).[11] An alternative chemoenzymatic approach depends on stoichiometric amounts of L-methionine derivatives and ATP[2,10,13,14] or 5′-halo-5′-deoxyadenosine[6,15] (Figure 1A and S1). As L-methionine derivatives are not commercially available, their chemical synthesis from haloalkanes and L-homocystine further hampers straightforward biocatalytic alkylation chemistry. In this respect, the enzyme system developed by Liao and Seebeck is an attractive alternative, as it enables synthesis and recycling of SAM directly from simple iodomethane and SAH.[7] This reaction is catalyzed by halide methyltransferases (HMTs) that in nature perform the reverse reaction; the SAM-dependent methylation of halides to produce halomethanes.[16–18] The Seebeck system was originally established for SAM synthesis and recycling but has recently been expanded towards SAM analogs by replacing iodomethane with other readily available haloalkanes (Figure 1B). This was achieved through enzyme engineering[8] as well as by the identification of promiscuous HMTs.[9] It has been proposed that HMT-catalyzed synthesis of SAM analogs might soon become the method of choice to perform biocatalytic alkylation chemistry.[12] Currently, however, this approach has been proven itself only in SAM analog synthesis with 5–6 different haloalkanes, namely isotope-labelled iodomethane, iodoethane, -1-iodopropane, 3-iodopropene and fluoro(iodo)methane.[8,9,19,20]

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

[+] *These authors contributed equally to this work.*

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbic.202100632

This article is part of the Special Collection ChemBioTalents2021. Please see our homepage for more articles in the collection.

© 2021 The Authors. ChemBioChem published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. ChemBioChem published by Wiley-VCH GmbH

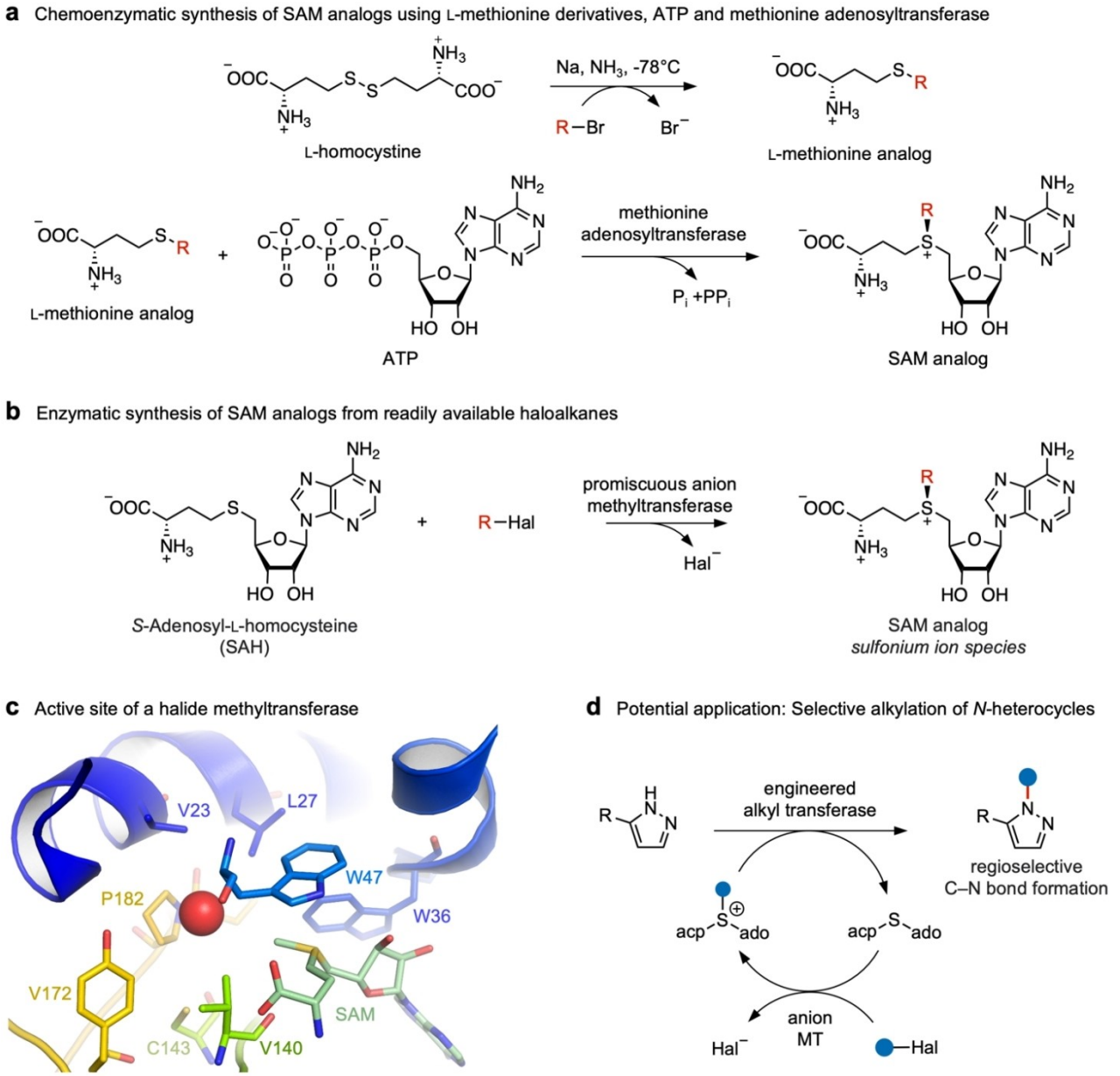

Figure 1. Synthesis and application of SAM-related sulfonium species. (a) Chemoenzymatic synthesis of SAM analogs depends on the synthesis of L-methionine derivatives and stoichiometric amounts of ATP or related adenosines.[11] R = alkyl group. (b) SAM-related sulfonium species can also be directly accessed by enzymatic SAH alkylation. This methodology benefits from readily available haloalkanes that can be used as substrates. R = alkyl group. (c) SAH alkylation with haloalkanes is catalyzed by HMTs, which naturally catalyze the reverse reaction, the SAM-dependent methylation of halides to produce halomethanes. The structure (PDB 3LCC) shows a halide-binding pocket. SAM is modelled into the active site of *ath* (PDB 3LCC) by structural alignment with PDB 1NW3 that contains a co-crystallized SAM molecule. SAM is shown as pale green sticks. The potential halide-binding site is visualized by a chloride (shown as red sphere). (d) Enzymatic alkylation of SAH might enable highly efficient and selective alkylation chemistry, given that the SAM analog synthesis can be combined in a two-enzyme cascade with selective alkyl-transfer reactions. In such cyclic enzyme cascades non-natural analogs of SAM are synthesized and recycled using readily available haloalkanes as starting material; acp = (*S*)-3-amino-3-carboxypropyl. ado = adenosyl.

Here we report substrate profiling of eleven HMT homologs with 19 different haloalkanes. The studied enzymes belong to a bigger enzyme family that we propose to classify as anion methyltransferase enzyme family. Substrate profiling reveals that anion MTs are highly promiscuous biocatalysts in the synthesis of SAM-related sulfonium species from SAH and haloalkanes. Our study further supports that this chemistry is not limited to iodoalkanes as various bromoalkanes are accepted as substrates. This is proven by the first regioselective allylation of pyrazoles.

Results and Discussion

To shed light on the promiscuous activities of HMT homologs, we started with a bioinformatic analysis to identify the scope of

© 2021 The Authors. ChemBioChem published by Wiley-VCH GmbH

the natural enzyme pool. We used several experimentally verified HMTs as seed sequence to search for homologs with a Basic Local Alignment Search Tool (BLAST) using default parameters.[21]

The seed panel included enzymes from *Arabidopsis thaliana*, *Aspergillus clavatus*, *Batis maritima*, *Burkholderia xenovorans*, *Chloracidobacterium thermophilum* and *Synechococcus elongatus*.[9,18] All the seed HMTs are proven to produce halomethanes from halides in a SAM-dependent manner. Initial BLAST searches revealed that these enzymes belong to a bigger superfamily that is classified as thiopurine methyltransferase (TPMT) superfamily in the Pfam database (No. PF05724). This superfamily harbors more than 12.000 sequences and can be further divided into two subfamilies: MTs that methylate small organic molecules such as thiopurine as well as MTs that methylate small anions including halides,[17] hydrogen sulfide anions[22,23] and thiocyanate.[17,24] To study promiscuity for SAM-analog synthesis from SAH and different haloalkanes, we focused on MTs that methylate small anions and neglected MTs that methylate organic molecules. This is based on our assumption that, in analogy to HMTs (Figure 1C), related anion MTs provide anion-binding pockets that might be important to activate the leaving group in the reverse reaction.

We identified a representative panel of anion MTs by generating a sequence similarity network (SSN)[25] (Figure 2A). All sequences that clustered with well-known thiopurine MTs were neglected and individual sequences of potential anion MTs were chosen from the remaining main clusters. We selected a total of eleven anion MTs, which were produced by overexpression in *E. coli* and purified via immobilized metal affinity chromatography using a hexa histidine-tag (Figure S2). The proteins were readily purified, stored at −20 °C and showed typical melting points in the range of 50–60 °C (Figure S3 and Table S1). Please note that in our hands the recently studied HMT from *Chloracidobacterium thermophilum* (*cth*)[7] did not express particularly well in a soluble form (Figure S2). As the *E. coli* cell lysate harboring low concentrations of *cth* produced SAM from SAH and iodomethane, we decided to study the promiscuity of *cth* as cell lysate preparation and not as purified protein.

We have chosen a broad panel of haloalkanes as substrates, including short-chain (**1**–**7**) and branched haloalkanes (**8**), haloalkanes bearing cyclic groups (**9**–**11**) as well as functionalized haloalkanes bearing alkene, alkyne, aromatic and trifluoromethyl moieties (**12**–**19**). Besides promiscuity with respect to the alkyl chain, we also started to explore leaving group promiscuity (iodide, bromide and chloride). The substrate profiling was performed with purified enzymes and the reactions were analyzed by HPLC analysis.

Generally, it can be concluded that anion MTs show a high level of substrate promiscuity and accept various haloalkanes to produce SAM analogs (Figure 2B and S4). In more detail, our studies confirmed SAM analog formation with 12 out of 19 tested haloalkanes. Next to short chain haloalkanes **2**–**5** and **7**, substrates bearing cyclopropyl and cyclobutyl moieties (**9** and **11**) were accepted. We could even confirm conversion with bulky (2-iodoethyl)benzene **15** as substrate as well as with functionalized haloalkanes bearing alkene and alkyne groups (**12**, **13** and **18**). Please note that propargyl-analogs of SAM are not particularly stable and hydrolyze under buffer conditions to the corresponding ketone-analogs of SAM.[26] Product formation could not be confirmed with haloalkanes bearing trifluoromethyl moieties (**16** and **17**), chloropropane **6**, the branched haloalkane **8** as well as with (bromomethyl)cyclopropane **10**. Substrate profiling further supported that bromoalkanes (e.g. **3**, **5** and **13**) are well accepted substrates. This is of interest because bromoalkanes are cheaper, less toxic and more readily available than the corresponding iodoalkanes. In addition, acceptance of bromoalkanes can expand the scope of haloalkanes towards substrates that are too reactive as iodides. This include, for example, propargyl-containing haloalkanes, which can be unstable in the iodo-form but are readily available as bromo-compounds (see substrate **18**). We also observed detectable product formation using chloroalkanes **14** and **19** (Figure S4). This agrees with the observation that the forward reaction also allows synthesis of chloromethane from SAM and chloride, but with lower activity compared to iodomethane synthesis.[17,18] The activity with chloroalkanes should be treated with caution as the yield is very low (< 0.5 % product formation) and only 3–4 times higher than the non-enzymatic background activity that was observed for reactive halo-propenes (**12**–**14**) and -propynes (**18**–**19**).

Analysis of the enzyme panel highlights that all eleven biocatalysts can convert SAH and haloalkanes to the corresponding SAM-related sulfonium species (Figure 2B). While some enzymes (e.g. *acl* and *uma*) offer a broad substrate scope accepting various haloalkanes, other enzymes seem to be largely limited to iodomethane as substrate (e.g. *cth* and *bxe*). In an extreme case (*mac*), iodomethane was barely accepted as substrate to produce SAM from SAH, but other haloalkanes were converted with decent promiscuous activity. It is noticeable that the very promiscuous (*acl* and *uma*) as well as the less promiscuous enzymes accumulate in different clusters in the SSN. This suggests that the clusters containing the anion MTs from the fungi *Aspergillus clavatus* (*acl*) and *Ustilago maydis* (*uma*) might provide more promiscuous enzymes for future studies. The anion MT from the methane producing microbe *Methanosarcina acetivorans* (*mac*) is part of an independent cluster in the SSN and might not be an HMT as it does not accept iodomethane to produce SAM from SAH.

We confirmed SAM analog formation by comparison to chemically synthesized standards as well as by LC/MS characterizations (see supporting information). Further, we confirmed the yields from the substrate profiling heat map (Figure 2B) by rescreening the two most active enzymes per substrate (Table S2). The identified promiscuous activities are low to moderate with yields typically ranging from 0.5 to 25 %. As it has been shown for many other promiscuous enzymes,[27] we postulate that these activities can be enhanced by directed evolution and other protein engineering techniques. The breadth of identified promiscuous activities is exciting and suggests that even complex SAM analogs can be synthesized from readily available haloalkanes. This is important as it paves the way to utilize such SAM-related sulfonium species as

© 2021 The Authors. ChemBioChem published by Wiley-VCH GmbH

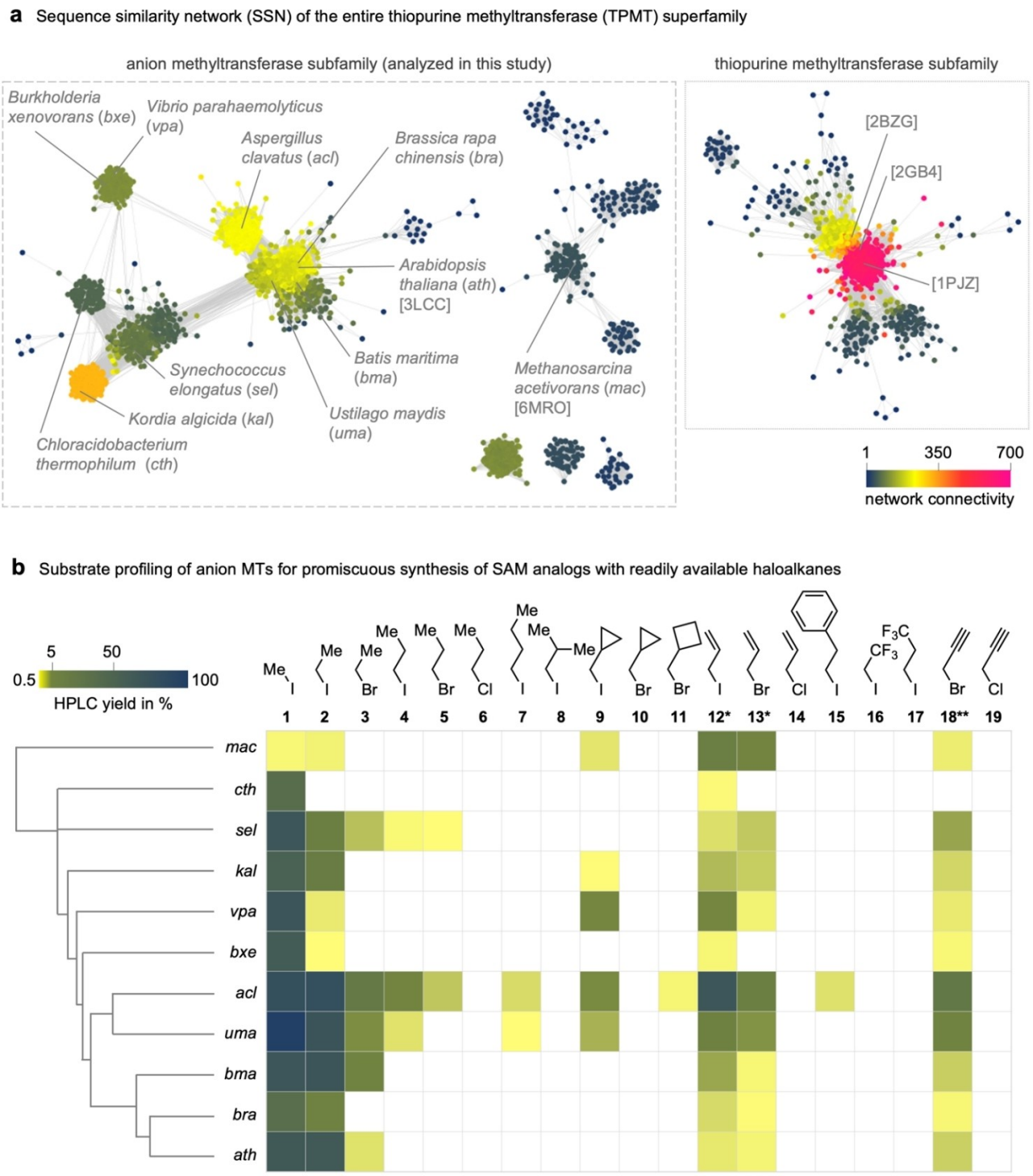

Figure 2. Sequence similarity network and promiscuous activities of anion MTs. (a) Sequence similarity network of the thiopurine methyltransferase (TPMT) superfamily (Pfam accession number: PF05724). This superfamily can be divided into MTs that methylate organic molecules such as thiopurine and MTs that methylate anions such as halides or pseudohalides. To the best of our knowledge, the cluster labelled thiopurine methyltransferases mainly contains enzymes that methylate organic molecules and has thus not been considered in the substrate profiling study. The resulting clusters contain enzymes that methylate anions and build the basis for our study. The chosen enzymes are labeled with the name of the organism and a 3-letter code in round brackets. The PDB codes of solved protein structures are shown in square bracket. Please note that a few sequences (< 100) of the TPMT superfamily did not cluster under the criteria of the generated SSN (filter type: E-value, E-value: 5, minimum AA length 150, maximum AA length 330, alignment score 50) and are not shown in the figure. (b) Heatmap of the substrate profiling experiment. The color code ranges from yellow (0.5% yield) to blue (100% yield). A yield of 0.5% was defined as the cut-off to report promiscuous activities. Values below 0.5% yield are shown colorless. The limit of quantification in our analytical protocol corresponds to 0.1% yield. Standard reaction conditions: 1 mM SAH, 10 equiv. haloalkane, 1 mol% biocatalyst, 20 h at 22 °C. *Standard conditions but with 2 equiv. haloalkane and 5 h reaction time to minimize non-enzymatic background activity. ** Standard conditions but with 1 equiv. haloalkane to minimize non-enzymatic background activity. Enzyme *cth* has been applied as cell lysate.

© 2021 The Authors. ChemBioChem published by Wiley-VCH GmbH

electrophiles in natural and engineered transferases to build complex molecules by coupling simple fragments with high selectivity (Figure 1D).

Finally, we proved that the identified promiscuous activities can be used in very challenging bond forming reactions. We focused on regioselective *N*-allylation of pyrazoles as target reaction. This is particular challenging because tautomerization leads to *N*-atoms with comparable reactivity (Figure 3a). There are currently no catalysts or reagents that control regioselective allylation of pyrazoles (and related heterocycles), which partly complicates the synthesis of many pharmaceuticals containing *N*-heteroaromatic compounds.[9,28] We screened our inhouse MT library[9] to identify enzymes that utilize an allyl-analog of SAM as substrate for regioselective allylation of the model pyrazole (**20**). Chemical allylation is a substrate-controlled process and generates a 2.6:1 mixture of both regioisomers (**21** and **22**) with the more sterically demanding **22** as the minor product (Figure 3c). We identified two regiocomplementary MTs and combined them with *acl*-catalyzed SAM-analog synthesis using 3-bromopropene **13**. Pyrazole (**20**) was allylated with high regioselectivity (up to r.r. 98:2, Figure 3b), which describes the first regioselective allylation of pyrazoles. At the current stage, activities are low (<10% product formation), reflecting that these MTs are not optimized for pyrazole allylation. This enzymatic allylation uses readily available bromoalkanes as allyl-source, which is an advantage over purely biological systems for regioselective allylation that depend on allyl-diphosphates.[29] Following this initial results, it is likely that many of the SAM-related sulfonium species shown here (Figure 2B) can be used to form very challenging sp^3 C–C/N/O/S bonds using readily available haloalkanes as starting material.

Conclusion

We have explored a natural pool of enzymes for their ability to synthesize SAM-related sulfonium species from readily available haloalkanes and SAH. Our studies suggest that a large enzyme family of anion MTs promiscuously catalyzes SAM-analog synthesis and that the more promiscuous enzymes accumulate in a bigger cluster of the TPMT superfamily. Surprisingly, we have found a HMT homolog that does not accept iodomethane to produce SAM, but accepts larger haloalkanes to generate SAM analogs.

From a synthetic perspective, this work further establishes a new route to make and recycle SAM-related sulfonium species from simple starting materials. These SAM analogs can be utilized as electrophiles in highly selective enzymatic nucleophilic substitutions and it will be exciting to see what kind of challenging bond formations might be accessible through enzyme-controlled alkylation reactions. We have expanded this chemistry from iodoalkanes to bromoalkanes, which might help to further establish this methodology. Finally, a first proof of concept highlights that SAM-related sulfonium intermediates can be used as electrophiles in regioselective allylation of pyrazoles with simple 3-bromopropene as starting material. This work opens up new avenues for highly selective synthesis, given that all these new enzyme activities can be optimized

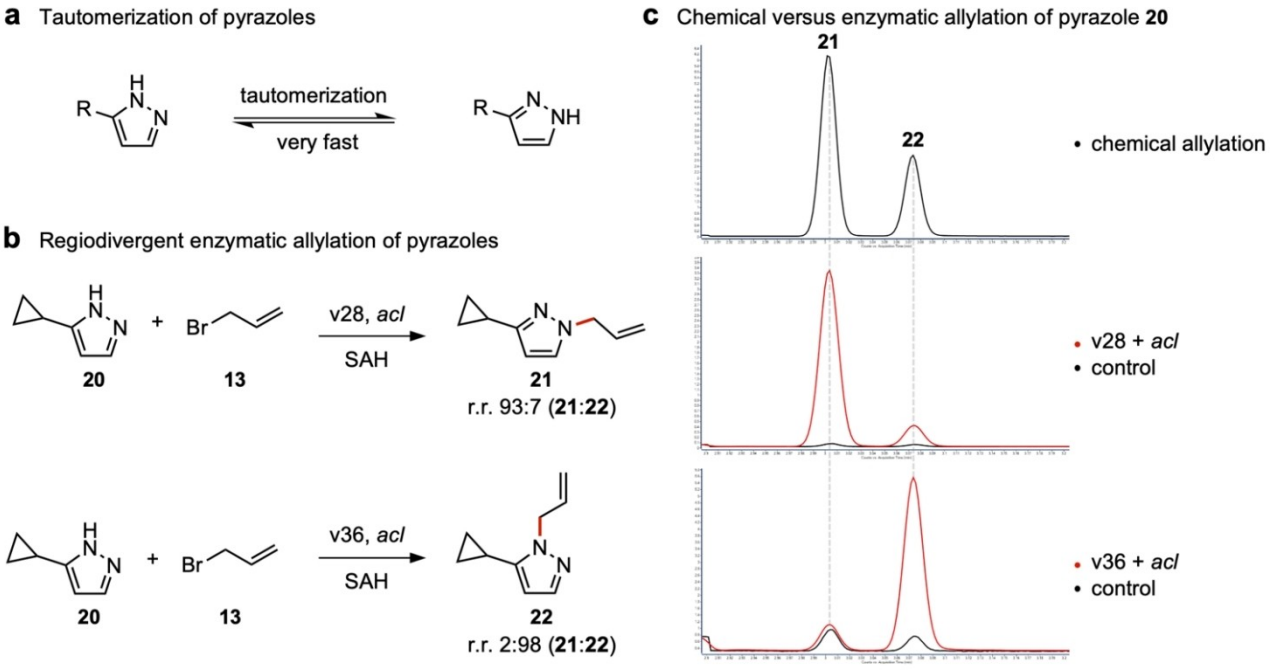

Figure 3. Regiodivergent pyrazole allylation. (a) Tautomerization of pyrazoles generates *N*-atoms with comparable reactivity. (b) The regiodivergent pyrazole allylation was achieved with a cyclic two-enzyme cascade as shown in Figure 1D. The yields of **21** and **22** are 6% and 1%, respectively. Reaction conditions: 1 mM pyrazole **20**, 1 equiv. haloalkane **13**, 1 mol% of each enzyme (purified) and SAH, 18 h at 22 °C. The enzyme variants v28 and v36 are enzyme designs from recent work[9] that originate from the *homo sapiens* nicotinamide *N*-methyltransferase (NMT). v28 = NMT D167H, S201C, S213M, N249A. v36 = NMT Y24F, D167C, A198T, S201C, S213A. (**c**) GC-chromatograms comparing chemical and enzymatic pyrazole allylation using 3-bromopropene as starting material.

© 2021 The Authors. ChemBioChem published by Wiley-VCH GmbH

with directed evolution and other enzyme engineering techniques.

Acknowledgements

The authors thank Alina Klein for support in enzyme purification, Nicole Streser for assistance in LC/MS analysis, Christopher A. Voigt (Massachusetts Institute of Technology, US) for providing plasmid DNA, Gabriele Fischer von Mollard and Simon Koopmeiners for assistance with the thermal shift assay, Uwe Bornscheuer (University of Greifswald, DE) for providing an mtn-*deficient* E. coli *strain, as well as Florian Seebeck (University of Basel, CH), Ludwig Bengel (University of Stuttgart, DE) and Benjamin Aberle (University of Stuttgart, DE) for fruitful discussions. We acknowledge funding from Deutsche Forschungsgemeinschaft via the Emmy Noether program (HA 7668/2-1, to S.C.H.) and Deutsche Bundesstiftung Umwelt via a PhD scholarship (20020/690, to K.H.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: allylation · biocatalysis · methyltransferases · promiscuity · pyrazole

[1] F. Ospina, K. H. Schülke, S. C. Hammer, *ChemPlusChem* **2021**, https://doi.org/10.1002/cplu.202100454.
[2] 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.
[3] 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.
[4] 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.
[5] F. Michailidou, N. Klöcker, N. V. Cornelissen, S. K. Rohit, A. Peters, A. Ovcharenko, D. Kümmel, A. Rentmeister, *Angew. Chem. Int. Ed.* **2021**, *60*, 480–485; *Angew. Chem.* **2021**, *133*, 484–489.
[6] 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.
[7] C. Liao, F. P. Seebeck, *Nat. Catal.* **2019**, *2*, 696–701.
[8] 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.
[9] L. L. Bengel, B. Aberle, A. Egler-Kemmerer, S. Kienzle, B. Hauer, S. C. Hammer, *Angew. Chem. Int. Ed.* **2021**, *60*, 5554–5560; *Angew. Chem.* **2021**, *133*, 5614–5620.
[10] S. Singh, J. Zhang, T. D. Huber, M. Sunkara, K. Hurley, R. D. Goff, G. Wang, W. Zhang, C. Liu, J. Rohr, et al., *Angew. Chem. Int. Ed.* **2014**, *53*, 3965–3969; *Angew. Chem.* **2014**, *126*, 4046–4050.
[11] T. D. Huber, B. R. Johnson, J. Zhang, J. S. Thorson, *Curr. Opin. Biotechnol.* **2016**, *42*, 189–197.
[12] Q. Tang, I. V. Pavlidis, C. P. S. Badenhorst, U. T. Bornscheuer, *ChemBioChem* **2021**, *22*, 2584–2590.
[13] B. J. C. Law, A.-W. Struck, M. R. Bennett, B. Wilkinson, J. Micklefield, *Chem. Sci.* **2015**, *6*, 2885–2892.
[14] F. Muttach, A. Rentmeister, *Angew. Chem. Int. Ed.* **2016**, *55*, 1917–1920; *Angew. Chem.* **2016**, *128*, 1951–1954.
[15] M. Thomsen, S. B. Vogensen, J. Buchardt, M. D. Burkart, R. P. Clausen, *Org. Biomol. Chem.* **2013**, *11*, 7606–7610.
[16] A. M. Wuosmaa, L. P. Hager, *Science* **1990**, *249*, 160–162.
[17] 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.
[18] T. S. Bayer, D. M. Widmaier, K. Temme, E. A. Mirsky, D. V. Santi, C. A. Voigt, *J. Am. Chem. Soc.* **2009**, *131*, 6508–6515.
[19] M. A. Beliaeva, R. Burn, D. Lim, F. P. Seebeck, *Angew. Chem. Int. Ed.* **2021**, *60*, 5209–5212; *Angew. Chem.* **2021**, *133*, 5269–5272.
[20] J. Peng, C. Liao, C. Bauer, F. P. Seebeck, *Angew. Chem. Int. Ed.* **2021**, *60*, 27178–27183; *Angew. Chem.* **2021**, *133*, 27384–27389.
[21] M. Johnson, I. Zaretskaya, Y. Raytselis, Y. Merezhuk, S. McGinnis, T. L. Madden, *Nucleic Acids Res.* **2008**, *36*, W5-W9.
[22] J. M. Attieh, A. D. Hanson, H. S. Saini, *J. Biol. Chem.* **1995**, *270*, 9250–9257.
[23] N. Itoh, H. Toda, M. Matsuda, T. Negishi, T. Taniguchi, N. Ohsawa, *BMC Plant Biol.* **2009**, *9*, 116.
[24] J. M. Attieh, R. Djiana, P. Koonjul, C. Étienne, S. A. Sparace, H. S. Saini, *Plant Mol. Biol.* **2002**, *50*, 511–521.
[25] J. A. Gerlt, J. T. Bouvier, D. B. Davidson, H. J. Imker, B. Sadkhin, D. R. Slater, K. L. Whalen, *Biochim. Biophys. Acta* **2015**, *1854*, 1019–1037.
[26] I. R. Bothwell, K. Islam, Y. Chen, W. Zheng, G. Blum, H. Deng, M. Luo, *J. Am. Chem. Soc.* **2012**, *134*, 14905–14912.
[27] S. C. Hammer, A. M. Knight, F. H. Arnold, *Curr. Opin. Green Sustain. Chem.* **2017**, *7*, 23–30.
[28] D. C. Blakemore, L. Castro, I. Churcher, D. C. Rees, A. W. Thomas, D. M. Wilson, A. Wood, *Nat. Chem.* **2018**, *10*, 383–394.
[29] M. Liebhold, X. Xie, S. M. Li, *Org. Lett.* **2012**, *14*, 4882–4885.

Manuscript received: November 15, 2021
Revised manuscript received: December 18, 2021
Accepted manuscript online: December 20, 2021
Version of record online: January 5, 2022

© 2021 The Authors. ChemBioChem published by Wiley-VCH GmbH