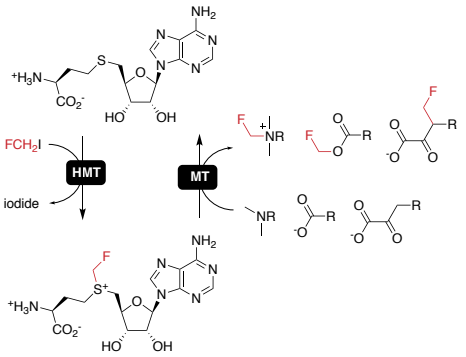
Fluorinated S-adenosylmethionine as a reagent for enzyme-catalyzed fluoromethylation

Jiaming Peng[1,2], Cangsong Liao,[1,2] Carsten Bauer[1] and Florian P. Seebeck[1]*

[1] Department of Chemistry, University of Basel, Mattenstrasse 24a, Basel 4002, Switzerland

[2] These authors contributed equally

Abstract. Strategic replacement of protons with fluorine atoms or functional groups with fluorine-containing fragments has proven a powerful strategy to optimize the activity of therapeutic compounds. For this reason, the synthetic chemistry of organofluorides has been the subject of intense development and innovation for many years. By comparison, the literature on fluorine biocatalysis still makes for a slim chapter. In this report we introduce *S*-adenosyl-methionine (SAM) dependent methyltransferases as a new tool for the production of fluorinated compounds. We demonstrate the ability of halide methyltransferases to form fluorinated SAM (*S*-adenosyl-(fluoromethyl)-L-homocysteine) from *S*-adenosylhomocysteine and fluoromethyliodide. Fluorinated SAM (F-SAM) is too unstable for isolation, but is accepted as a substrate by C-, N- and O-specific methyltransferases for enzyme-catalyzed fluoromethylation of small molecules.



Keyword: fluorine biocatalysis, methyltransferase, monofluoromethlyation,

Introduction. Fluorine is a popular substituent in the development and optimization of pharmaceutical and agrochemical compounds. Although fluorine is almost isosteric with a proton, strategic hydrogen-to-fluorine replacement can significantly change conformational preferences, metabolic stability, reactivity and solubility of a given compound.[1] In addition, fluorine atoms attached to metabolites and drugs are valuable NMR probes (^{19}F) and radiotracers (^{18}F) for theranostic applications.[2] The growing need for such compounds inspired the development of a broad range of techniques in organofluorine chemistry, including direct fluorination by C-H activation,[3] by substitution of functional groups, or through the transfer of fluorinated fragments such as mono-, di- or trifluoromethyl or trifluoromethoxy groups.[4] The monofluoromethyl group (hereafter referred to as fluoromethyl group) is of particular interest because its steric similarity to methyl groups, primary alcohols, thiols and terminal amines.[4a] Fluoromethylated drugs include the muscle-relaxant *afloqualone*, the anesthetic *sevoflurane* or the anti-inflammatory drug *fluticasone* or the potential anticonvulsant fluoromethylglutamic acid (Figure 1).[4a] The search for more efficient and selective protocols for fluoromethylation has unearth a broad variety of reagents for electrophilic, nucleophilic and radical fluoromethylation.[4a, 5]

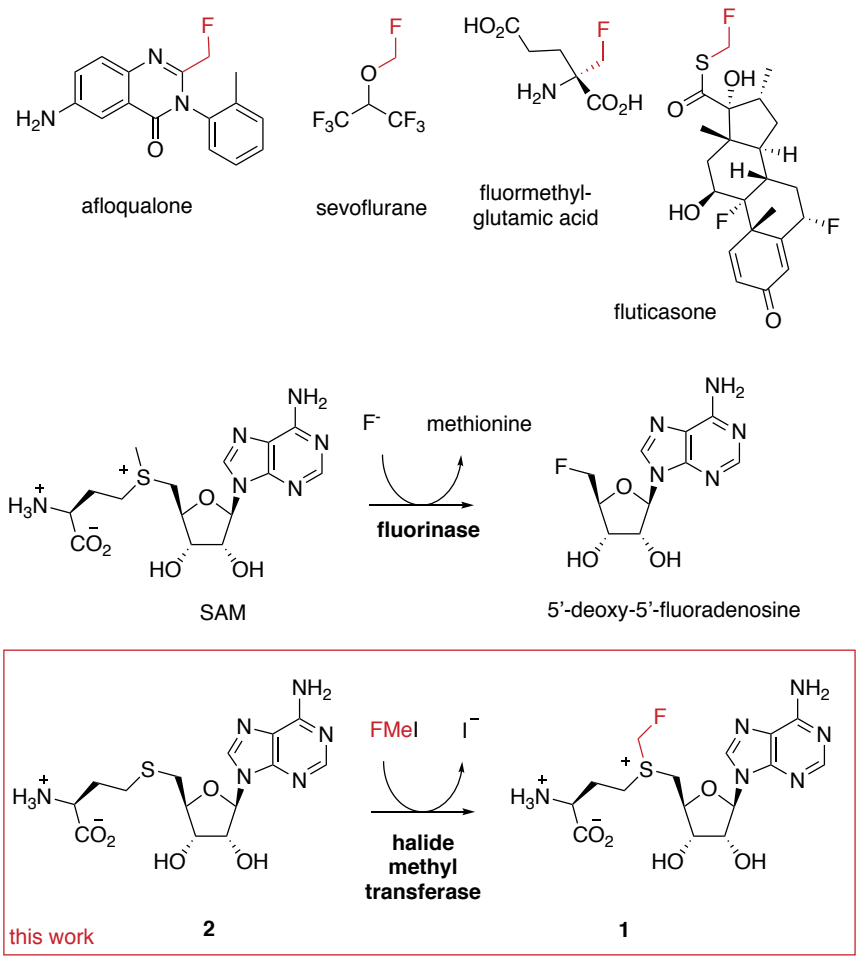


Figure 1: Top: Fluoromethyl-containing drugs. **Middle:** Fluorinase-catalyzed conversion of S-adenosylmethionine (SAM) to 5'-fluoradenosine. **Bottom:** Halide methyltransferase (HMT) catalyzed formation of *S*-adenosyl-(fluoromethyl)-L-homocysteine (F-SAM, **1**) from *S*-adenosylhomocysteine (SAH, **3**) and fluoromethyliodide (FMeI).

By comparison, fluorine biocatalysis is an underdeveloped field.[6] Fluorine containing natural products are rare,[7] and the only enzyme known to produce an organofluoride in a biosynthetic pathway is fluorinase (EC 2.5.1.63).[6a, 8] This enzyme produces 5'-deoxy-5'-fluoradenosine from *S*-adenosylmethionine (SAM) and fluoride via nucleophilic substitution (Figure 1). On the other hand, recent reports on organofluoride production by engineered fluorinases, aldolases or cytochromes indicate that fluorine biocatalysis may indeed be a target-rich area.[9]

In this report we introduce SAM-dependent methyltransferases as a new tool for fluoromethylation. We found that halide methyltransferases (HMT, EC 2.1.1.165) can produce *S*-adenosyl-(fluoromethyl)-L-homocysteine (F-SAM, **1**, Figure 1) from fluoromethyliodide (FMeI) and *S*-adenosylhomocysteine (SAH, **2**). Under physiological conditions, F-SAM hydrolyzes rapidly to homoserine (**3**), 5'-deoxy-5'-thioadenosine (**4**), formaldehyde and fluoride. Despite this instability F-SAM can be accepted by secondary methyltransferases that transfer the fluoromethyl group to N-, C- or O-nucleophiles.

Results and Discussion. In a previous study we have shown that HMTs can produce SAM from SAH and methyl iodide (MeI).[10] Combinations of an HMT with other SAM-dependent methyltransferases were shown to transfer methyl groups from MeI to a variety of accceptor substrates using SAH as a catalyst that transfers activate methyl groups between the two methyl transferases.[10b, 11] Other labs have shown that this methodology can also be applied for the transfer of ethyl, propyl or even allyl groups, suggesting that methyltransferases may provide a general platform of alkyl transfer biocatalysis.[12] Indeed, engineered methyltransferases have been shown to accept SAM derivatives that deliver an even broader range of fragments such as carboxylated alkyl groups,[13] tags for biorthogonal modification or photo-cleavable fragments.[14] Encouraged by these results, we explored as to whether methyltransferase cascades could also transfer fluoromethyl groups.

To this end, we produced HMT from *Burkholderia xenovorans* (protein family 05724) in a strain of *Escherichia coli* that is SAH-nucleosidase-deficient (*E. coli* Δmtn, DE3, Figure S1).[10a] A reaction containing 30 μM of HMT, 6 mM FMeI, 0.5 mM SAH in a 100 mM phosphate buffer at pH 8.0 was incubated at 25°C and analyzed by cation-exchange HPLC. This reaction consumed 80 % of SAH within the first 30 min (Figure 2B). In this early phase two new adenosine species emerged (**1** and **7**) and vanished in the later phase as a more stable species (**4**) accumulated. This stable compound was identified as 5'-deoxy-5'-thioadenosine (**4**) by ^{1}H NMR (Figure S3) and high-resolution electrospray mass spectrometry (HR-ESI-MS, $[C_{10}H_{13}N_5O_3S]^+$ calculated 284.0812, found 284.0812). Homoserine was identified as the second accumulating product (**3**, Figure S2). These are rather unusual degradation products of SAM or SAH.[15] The only known enzyme that produces **4** is the type C radical SAM methyltransferase NosN.[16] In the absence of FMeI, degradation of SAH proceeds orders of magnitudes more slowly either by formation of adenine and *S*-ribosylmethionine, or by formation of 5'-deoxy-5'-methylthioadenosine and homoserine lactone.[10a, 15, 17] The unusual degradation

products and the observation that degradation depends on FMeI provide strong evidence that HMT does fluoromethylate SAH,[10a] and that the produced F-SAM is activated for hydrolysis.

Decomposition of F-SAM to **3** and **4** necessarily occurs via the intermediate 5'-deoxy-5'-fluoromethylthioadenosine (F-MTA, **7**, Figure 2B). Indeed, a transiently accumulating species (maximal concentration of 10 μM) could be identified a **7** by HR-ESI-MS ($[C_{11}H_{15}FN_5O_3S]^+$ calculated m/z 316.0874, found 316.0871, Figure S42), but isolation was precluded by the inherent instability of fluoromethylthioethers.[18] The second transient species, which accumulated to a maximal concentration of 0.5 μM, elutes from the cation exchange column with a similar retention time as SAM and was therefore assigned as F-SAM (**1**, Figure 2B). Again, this species was too unstable for further characterization. We also observed the transient accumulation of 5 μM SAM, owing to a small (> 0.1 %) contamination of MeI in FMeI. This signal was identified by co-injection of authentic SAM (data not shown). After four hours when most SAH was consumed, the signal for SAM vanished, presumably due to HMT-catalyzed demethylation of SAM. To compare the rates of HMT-catalyzed methylation and fluoromethylation, we monitored the time-dependent consumption of SAH (initial concentration 50 μM) in reactions containing 5 mM MeI or FMeI. Under these conditions 1 μM HMT was found to methylate or fluoromethylate SAH with a frequency of $(0.24 \pm 0.01)\ s^{-1}$ or $(0.013 \pm 0.001)\ s^{-1}$ respectively, and to consume more than 90 % of SAH after about 2.5 or 50 min respectively (Figure S4 and S5). The 20-fold difference in alkylation rate is consistent with the higher intrinsic electrophilicity of monohalomethanes compared to that of dihalogenmethanes.

A second experiment was designed to test whether *in situ* generated F-SAM could be used as a reagent for enzyme-catalyzed fluoromethlytion. We combined HMT with the histidine-specific methyltransferase EgtD from *Mycobacterium smegmatis* (EC 2.1.1.44, protein family 10017).[19] This enzyme catalyzes three successive methylation steps that convert histidine to *N*-α-trimethylhistidine (TMH). A reaction containing 1 mM *N*-α-dimethylhistidine (DMH), 10 mM FMeI, 50 μM SAH, 50 μM EgtD and 50 μM HMT in 100 mM phosphate buffer, pH 8.0 converted 97 % of DMH to the fluorinated version of TMH (F-TMH, **6**) within 8 h (Figure 2C and D). In the same time period the concentration of SAH diminished only by 25 %, suggesting that dealkylation of F-SAM by EgtD successfully competes with hydrolytic degradation (Figure 2D, inset). No F-TMH was formed in reactions without HMT (Figure 2C, a). A 2 ml scale reaction was used to produce 0.8 mg F-TMH for characterization by ^1H and ^{13}C NMR (Figure S6 and S7). The analytical data was consistent with that collected from authentic F-TMH made by chemical synthesis (Figure S31 – S34).

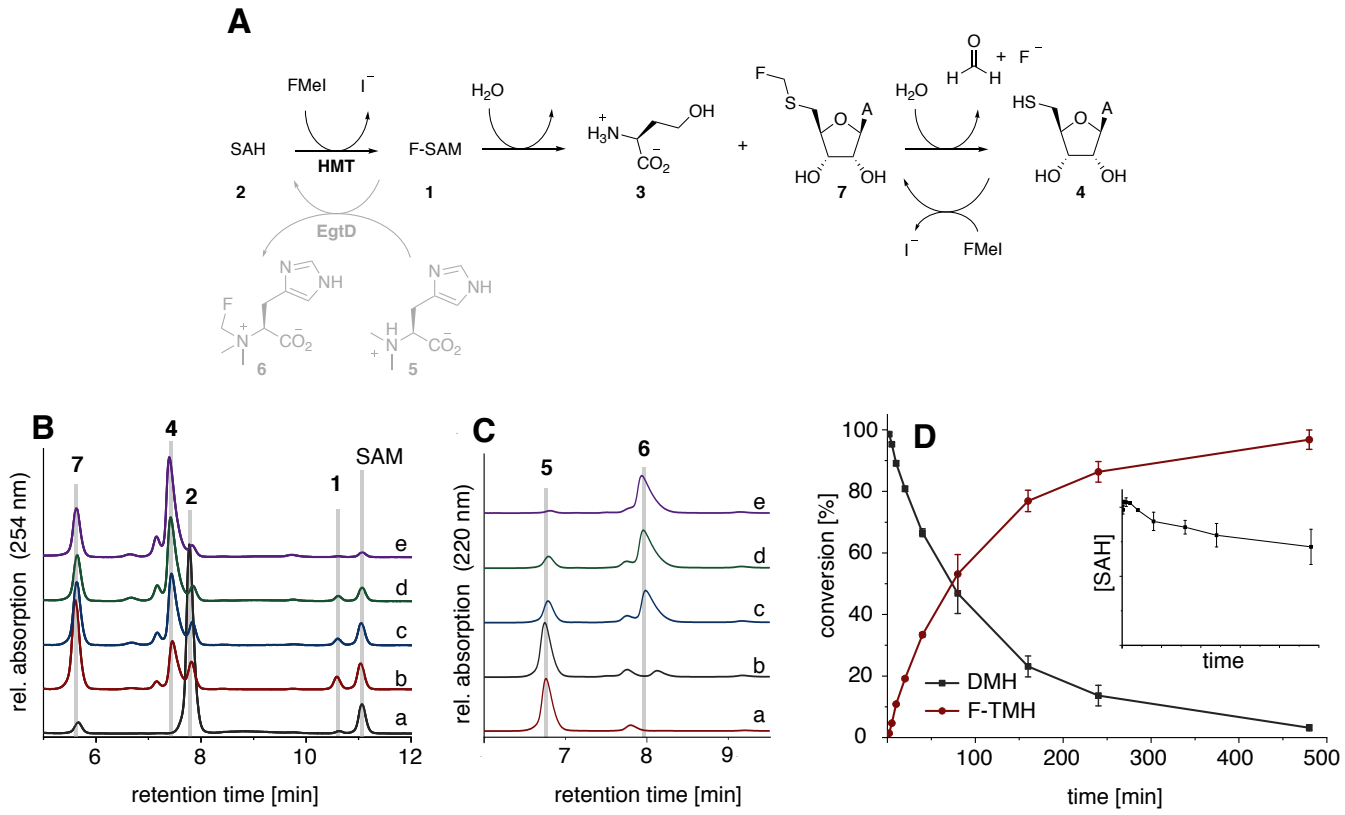


Figure 2. A: HMT-catalyzed generation F-SAM (**1**). F-SAM is unstable and degrades by hydrolysis to homoserine (**3**), 5'-deoxy-5'-thioadenosine (**4**), formaldehyde and fluoride with 5'-deoxy-5'-fluoromethylthioadenosine (F-MTA, **7**) as intermediate. In gray: EgtD can accept F-SAM as substrate to alkylate *N*-α-dimethylhistidine, (DMH, **5**) to produce fluoro-*N*-α-trimethylhistidine (F-TMH, **6**). **B:** Cation-exchange HPLC analysis of time-dependent consumption of SAH by HMT in a FMeI-dependent reaction (a: 1 min; b: 30 min; c: 60 min; d: 120 min; e: 240 min). The reaction products homoserine (**3**) and 5'-deoxy-5'-thioadenosine (**4**) were identified by ^{1}H NMR (Figure S3). This reaction also produced a small amount of SAM as a result of MeI contamination (< 0.1 %) in commercially available FMeI. **C**: Cation-exchange HPLC analysis of a cascade reaction containing 1 mM DMH, 10 mM FMeI, 50 µM SAH, 50 µM EgtD and 50 µM HMT (a: reaction after 480 min without HMT; b: 40 min; c: 160 min; d: 240 min; e: 480 min). **D**: Time-dependent production of F-TMH from DMH. Inset: [SAH] during the same time period.

The HMT-EgtD cascade with histidine as acceptor substrate produced no stable *N*-methylated products. Interestingly, this reaction did not degrade SAH either. These observations suggest that HMT-produced F-SAM could not accumulate and degrade because it was efficiently dealkylated by the EgtD:histidine complex (Figure S8). The product of this reaction, *N*-α-fluoromethyl histidine, did not accumulate, because it decayed to histidine, formaldehyde and fluoride. The instability of *N*-α-fluoromethyl histidine illustrates that stable fluoromethylation is only possible for nucleophiles with poorly donating lone pairs, such as C-nucleophiles, tertiary amines, electron-deficient alcohols or thioacids.

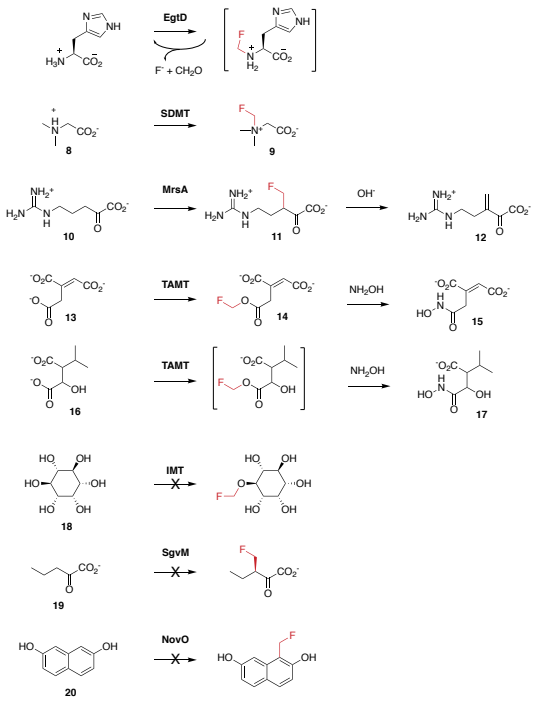


Figure 3: Methyltransferases that were tested for their ability to transfer fluoromethyl groups from F-SAM to their native acceptor substrate.

In a next step we explored as to whether methyltransferases from other protein families may also catalyze fluoromethylation using in situ generated F-SAM as a co-substrate. The examined cascade reactions contained 50 µM HMT, 50 µM SAH, 10 mM FMeI, 50 µM of the tested methyltransferase and 1 mM of the respective methylation substrate. First, we examined the sarcosine dimethylglycine methyltransferase from the thermophilic eukaryote *Galdieria sulphuraria* (SDMT, EC 2.1.1.157, protein family 08241, Figure 3).[20] Although the reaction catalyzed by this enzyme – methylation of *N*-dimethylglycine (DMG, **8**) to form glycine betaine – is very similar to that catalyzed by EgtD, the two enzymes belong to different protein families, and the structure of their active sites are quite different (PDB code SDMT: 2O57, EgtD: 4PIN).[19b] Despite these differences, the HMT-SDMT cascade fully converted 1 mM DMG to fluoro-glycine betaine (**9**) as inferred by ^{1}H NMR (Figure S9 and S36).

The 2-ketoarginine methyltransferase from *Pseudomonas syringae* (MrsA, 2.1.1.243, protein family PF13649) catalyzes methylation of carbon 3 of 2-ketoarginine (**10**, Figure 3) as a key step in the production of β-methyl arginine.[21] The recombinant enzyme, in combination with HMT, SAH and FMeI consumed more than 90 % of 2-ketoarginine in the first hour. The primary accumulating species could be isolated under acidic conditions and identified as 5-guanidine-3-fluoromethyl-2-oxopentanoate (**11**) by HR-ESI-MS ($[C_7H_{13}FN_3O_3]^+$ calculated 206.0936, found 206.0932) and ^{1}H and ^{19}F NMR (Figures S10 – S14). In the second phase of the reaction this compound slowly decomposed to HF and 5-guanidine-3-methylene-2-oxopentanoate (**12**, $[C_7H_{12}N_3O_3]^+$ calculated

186.0874, found 186.0862). Despite the instability of **11** at neutral pH, this experiment demonstrates the ability of MrsA to transfer a fluoromethyl group from F-SAM to a C-nucleophile.

A cascade reaction containing the trans-aconitate 3-methyltransferase from *Saccharomyces cerevisiae* (TAMT, EC 2.1.1.145, protein family PF13847)[22] as secondary methyltransferase, and trans-aconitate (**13**) as substrate also gave an unstable fluoromethylated product. HPLC analysis showed that 90 % of **13** was converted to one new species in the first three hours (Figure S15). Analysis of the reaction mixture after 45 min by ^{19}F NMR revealed the accumulation of a fluorine species containing a fluoromethyl ester (Figure S16), suggesting that fluoromethyl-trans-aconitate (**14**) was formed. However, this product proved too unstable for further characterization. To trap this unstable ester we supplemented the cascade reaction with 5 mM hydroxylamine. This reaction produced a more stable compound with a mass consistent with that of the hydroxamic acid of trans-aconitate (**15**, $C_6H_7NO_6^-$ [M-H]$^-$ 188.0200, found [M-H]$^-$ 188.0200, Figure S17). The same cascade with MeI instead of FMeI produced trans-aconitate methyl ester as a stable product (Figure S18 and S19). Addition of hydroxylamine to this reaction did not produce any traces of **15**.

The second physiological substrate of yeast TAMT has been identified as the leucine biosynthesis intermediate 3-isopropylmalate (**16**).[23] We examined the products of a reaction containing initially 1 mM of **16**, 5 mM FMeI together with 50 µM of each SAH, HMT and TAMT. To this reaction we added 5 mM hydroxylamine for in situ conversion of the labile fluoromethylester of **16** to the more stable hydroxamic acid **17**. In 210 min at 25°C this reaction converted more than 90 % of **16** to **17** as inferred by ^1H and ^{13}C NMR and HR-ESI-MS ([$C_7H_{12}NO_5$]$^-$ 190.0718 found, 190.0720 calculated, Figure S20 – S22).

Analogous cascade reactions with the α-keto acid methyltransferase SgvM from *Streptomyces griseoviridis* (protein family PF08242),[24] the coumarin methyltransferase NovO from *Streptomyces niveus* (EC 2.1.1.284, protein family PF13649)[25] or the inositol 4-methyltransferase from *Mesembryanthemum crystallinum* (IMT, EC 2.1.1.129 protein family PF00891)[26] did not produce fluoromethylated versions of their respective substrates (**18**, **19** and **20**), as inferred by ^1H NMR (Figures S24, S26 and S28). Furthermore, HPLC analysis of the HMT-SgvM and the HMT-IMT cascade reactions showed that SAH was degraded to **4** at a similar rate and via the same intermediates (**1** and **7**) as observed in the reaction with HMT alone (Figure S23 and S25). This behavior suggests that SgvM and IMT do not turnover F-SAM efficiently under these conditions.

Interestingly, in the HMT-NovO reaction the concentration of SAH remained unchanged (Figure S27). A control reaction without NovO produced the same result, but in a reaction without the substrate 2,7-dihydroxy naphthalene (**20**), SAH was degraded to **4** (Figure S29). Apparently, the substrate, not the enzyme, protects SAH from degradation. Most likely, **20** reacts with F-SAM to form

an unstable O-fluoromethylated product that does not accumulate. This uncatalyzed reaction outcompetes hydrolysis of F-SAM and therefore prevents depletion of the SAH pool. We have not observed a similar uncatalyzed reaction between **20** and SAM.

Five of the eight enzymes in this surveil (HMT, EgtD, SDMT, MrsA and TAMT) were able to transfer fluoromethyl groups to C-, N-, O- and S-nucleophiles, suggesting that many more small molecule methyltransferases and possibly also methyltransferases with macromolecular substrates may accept F-SAM as a co-substrate. However, this ability is not general, as illustrated by the unproductive cascades with SgvM, IMT and NovO. Because fluorine and hydrogen are of similar size, it is unlikely that these enzymes distinguish SAM from F-SAM based on size. SgvM and NovO both accept the more sterically demanding substrate *S*-adenosylethionine (SAE).[24, 27] Alternatively, SAM and F-SAM may adopt different conformations in solution. However, the structures of EgtD from *M. smegmatis* (PDB: 4PIO)[19b], HMT from *Arabidopsis thaliana* (PDB: 3LCC),[28] NovO from *S. niveus* (PDB: 5MGZ)[25] and TAMT from *S. cerevisiae* (PDB: 3G5T) show that these enzymes bind SAM or SAH in essentially the same conformation. Therefore, we conclude that the observed discrimination between SAM and F-SAM must occur based on reactivity rather than on shape. In this regard it is interesting to note that under saturating conditions HMT, EgtD, GsSDMT and MrsA catalyze methyl transfers with frequencies in the range of 1 s^{-1} (Table S1). The turnover frequencies of NovO and SgvM are almost 100-fold smaller. In cascade reactions where HMT-catalyzed alkylation of SAH outpaces dealkylation of F-SAM by the secondary methyltransferase, F-SAM may accumulate and hence degradation to **4** becomes the dominant process. Based on this model we predict that methyltransferases with low turnover frequencies may not be suitable for the construction of fluoromethyl transfer cascades.

Conclusions. In this report we demonstrate the ability of HMT to catalyze fluoromethylation of SAH to produce F-SAM. This labile alkylation reagent has not been described before. The fleeting stabilities of fluoromethylethers as in fluoromethyl-homocysteine or F-MTA (**7**) limit the prospects for preparative access to F-SAM via chemical synthesis,[29] or via biocatalysis using enzymes such as methionine adenosyltransferase (EC 2.5.1.6) or chlorinase (EC 2.5.1.94) which have been used successfully to produce SAM and more stable SAM-derivatives.[27, 30] Our results show that the F-SAM producing activity of HMT can be combined with secondary methyltransferase that transfers fluoromethyl groups to acceptor substrates with C-, N-, and O-nucleophiles. We anticipate that these findings will pave the way to recruit methyltransferase biocatalysis for the production of high value organofluorides for pharmacological and theranostic applications.

Acknowledgment. This project was supported by the "Professur für Molekulare Bionik", the 'Innovationsraum Biokatalyse', Project #C1842 of the BNF "Nationales Qualifizierungsprogramm" and a Proof of Concept grant from the European Research Council (ERC-2018-PoC).

References

[1] aL. Hunter, *Beilstein J Org Chem* **2010**, *6*; bM. Aufiero, R. Gilmour, *Acc. Chem. Res.* **2018**, *51*, 1701 - 1710; cY. Zhou, J. Wang, Z. Gu, S. Wang, W. Zhu, J. L. Aceña, V. A. Soloshonok, K. Izawa, H. Liu, *Chem. Rev.* **2016**, *116*, 422 - 518; dS. K. Holmgren, K. M. Taylor, L. E. Bretscher, R. T. Raines, *Nature* **1998**, *392*, 666 - 667; eL. Merkel, N. Budisa, *Org. Biomol. Chem.* **2012**, *10*, 7241 - 7261; fW. Zhu, P. F. Doubleday, D. S. Catlin, P. M. Weerawarna, A. Butrin, S. Shen, Z. Wawrzak, N. L. Kelleher, D. Liu, R. B. Silverman, *J. Am. Chem. Soc.* **2020**, *142*, 4892 - 4903.

[2] aP. W. Miller, N. J. Long, R. Vilar, A. D. Gee, *Angew. Cgem. Int. Ed. Engl.* **2008**, *47*, 8998 - 9033; bD. van der Born, A. Pees, A. J. Poot, R. V. A. Orru, A. D. Windhorst, D. J. Vugts, *Chem. Soc. Rev.* **2017**, *46*, 4709 - 4773; cR. A. Werner, T. Derlin, C. Lapa, S. Sheikbahaei, T. Higuchi, F. L. Giesel, S. Behr, A. Drzezga, H. Kimura, A. K. Buck, F. M. Bengel, M. G. Pomper, M. A. Gorin, S. P. Rowe, *Theranostics* **2020**, *10*, 1 - 16.

[3] R. Szpera, D. F. J. Moseley, L. B. Smith, A. J. Sterling, V. Gouverneur, *Angew Chem Int Ed Engl* **2019**, *58*, 14824 - 14848.

[4] aM. Reichel, K. Karaghiosoff, *Angew Chem Int Ed Engl* **2020**, *59*, 12268 - 12281; bG. Parisi, M. Colella, S. Monticelli, G. Romanazzi, W. Holzer, T. Langer, L. Degennaro, V. Pace, R. Luisi, *J. Am. Chem. Soc.* **2017**, *139*, 13648 - 13651; cT. Ding, L. Jiang, J. Yang, Y. Xu, G. Wang, W. Yi, *Org. Lett.* **2019**, *21*, 6025 - 6028; dG. K. Prakash, S. Chacko, S. Alconcel, T. Stewart, T. Mathew, G. A. Olah, *Angew Chem Int Ed Engl* **2007**, *46*, 4933 - 4936; eT. Scattolin, S. Bouayad-Gervais, F. Schoenebeck, *Nature* **2019**, *573*, 102 - 107; fT. Koike, M. Akita, *Acc. Chem. Res.* **2016**, *49*, 1937 - 1945; gJ. Charpentier, N. Früh, A. Togni, *Chem. Rev.* **2015**, *115*, 650 - 682; hJ. W. Lee, K. N. Lee, M. Y. Ngai, *Angew Chem Int Ed Engl* **2019**, *58*, 11171 - 11181; iS. L. Clarke, G. P. McGlacken, *Chemistry* **2017**, *23*, 1219 - 1230; jV. Fasano, N. Winter, A. Noble, V. K. Aggarwal, *Angew Chem Int Ed Engl* **2020**, *59*, 8502 - 8506; kJ. Moschner, V. Stulberg, R. Fernandes, S. Huhmann, J. Leppkes, B. Koksch, *Chem Rev* **2020**, *119*, 10718 - 10801.

[5] aH. Zheng, Z. Li, J. Jing, X. S. Xue, J. P. Cheng, *Angew Chem Int Ed Engl* **2021**, *60*, 9401 - 9406; bR. Reichel, S. Sile, A. Kornath, K. Karaghiosoff, *J. Org. Chem.* **2021**, *86*, 4423 - 4431; cJ. Sheng, H. Q. Ni, S. X. Ni, Y. He, R. Cui, G. X. Liao, K. J. Bian, B. B. Wu, X. S. Wang, *Angew Chem Int Ed Engl* **2021**, *60*, 15020 - 15027.

[6] aD. O'Hagan, H. Deng, *Chem. Rev.* **2015**, *115*, 634 - 649; bL. Wu, F. Maglangit, H. Deng, *Curr. Opin. Chem. Biol.* **2020**, *55*, 119 - 126.

[7] M. F. Carvalho, R. S. Oliveira, *Crit Rev Biotechnol.* **2017**, *37*, 880 - 897.

[8] K. Markakis, P. T. Lowe, L. Davison-Gates, D. O'Hagan, S. J. Rosser, A. Elfick, *ChemBioChem* **2020**, *21*, 1856 - 1860.

[9] aX. Huang, M. Garcia-Borràs, K. Miao, S. B. J. Kan, A. Zutshi, K. N. Houk, F. H. Arnold, *ACS Cent. Sci.* **2019**, *5*, 270 - 276; bJ. Fang, D. Hait, M. Head-Gordon, M. C. Y. Chang, *Angew Chem Int Ed Engl* **2019**, *58*, 11841 - 11845; cH. Sun, W. L. Yeo, Y. H. Lim, X. Chew, D. J. Smith, B. Xue, K. P. Chan, R. C. Robinson, E. G. Robins, H. Zhao, E. L. Ang, *Angew Chem Int Ed Engl* **2016**, *55*, 14277 - 14280; dD. M. Carminati, J. Decaens, S. Couve-Bonnaire, P. Jubault, R. Fasan, *Angew Chem Int Ed Engl* **2020**, *60*, 7072 - 7076.

[10] aC. Liao, F. P. Seebeck, *Nat. Catal.* **2019**, *2*, 696 - 701; bC. Liao, F. P. Seebeck, *Angew Chem Int Ed Engl* **2020**, *59*, 7184 - 7187; cI. J. W. McKean, P. A. Hoskisson, G. A. Burley, *Chembiochem* **2020**, *21*, 2890 - 2897.

[11] aP. Schneider, B. Henßen, B. Paschold, B. P. Chapple, M. Schatton, F. Seebeck, T. Classen, J. Pietruszka, *Angew Chem Int Ed Engl* **2021**, *in press*; bM. A. Beliaeva, R. Burn, D. Lim, F. P. Seebeck, *Angew. Chem. Int. Ed. Engl.* **2021**, *60*, 5209 - 5212.

[12] aQ. 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 Engl* **2021**, *60*, 1524 - 1527; bL. L. Bengel, B. Aberle, A. N. Egler-Kemmerer, S. Kienzle, B. Hauer, S. C. Hammer, *Angew Chem Int Ed Engl* **2021**, *60*, 5554 - 55560.

[13] A. J. Herbert, S. A. Shepherd, V. A. Cronin, M. R. Bennett, R. Sung, J. Micklefield, *Angew Chem Int Ed Engl* **2020**, *59*, 14950 - 14956.

[14] F. Michailidou, A. Rentmeister, *Org. Biomol. Chem.* **2021**, *19*, 3756 - 3762.

[15] D. F. Iwig, S. J. Booker, *Biochemistry* **2004**, *43*, 13496 - 13509.

[16] aW. Ding, Y. Li, J. Zhao, X. Ji, T. Mo, H. Qianzhu, T. Tu, Z. Deng, Y. Yu, F. Chen, Q. Zhang, *Angew Chem Int Ed Engl* **2017**, *56*, 3857 - 3861; bX. Ji, D. Mandalapu, J. Cheng, W. Ding, Q. Zhang, *Angew Chem Int Ed Engl* **2018**, *57*, 6601 - 6604.

[17] A. S. Eustáquio, J. Härle, J. P. Noel, B. S. Moore, *ChemBioChem* **2008**, *9*, 2215 - 2219.

[18] M. E. J. Houston, J. F. Honek, *J. Chem. Soc. Chem. Commun.* **1989**, *12*, 761 - 762

[19] aL. Misson, R. Burn, A. Vit, J. Hildesheim, M. Beliaeva, W. Blankenfeldt, F. P. Seebeck, *ACS Chem Biol.* **2018**, *13*, 1333 - 1342; bA. Vit, L. E. Misson, W. Blankenfeldt, F. P. Seebeck, *ChemBioChem* **2015**, *16*, 119 - 125.

[20] J. G. McCoy, L. J. Bailey, Y. H. Ng, C. A. Bingman, R. Wrobel, A. P. M. Weber, B. G. Fox, G. N. Phillips, *Proteins* **2009**, *74*, 368-377.

[21] S. D. Braun, J. Hofmann, A. Wensing, M. S. Ullrich, H. Weingart, B. Volksch, D. Spiteller, *Appl. Environ. Microbiol.* **2010**, *76*, 2500-2508.

[22] H. Cai, D. Dumlao, J. E. Katz, S. Clarke, *Biochemistry* **2001**, *40*, 13699 - 13709.

[23] J. E. Katz, D. S. Dumlao, J. I. Wasserman, M. G. Lansdown, M. E. Jung, K. F. Faull, S. Clarke, *Biochemistry* **2004**, *43*, 5976 - 5986.

[24] C. Sommer-Kamann, A. Fries, S. Mordhorst, J. N. Andexer, M. Muller, *Angew. Chem. Int. Ed. Engl.* **2017**, *56*, 4033-4036.

[25] J. C. Sadler, C. H. Chung, J. E. Mosley, G. A. Burley, L. D. Humphreys, *ACS Chem Biol.* **2017**, *12*, 374 - 379.

[26] D. M. Vernon, H. J. Bohnert, *EMBO J.* **1992**, *11*, 2077 - 2085.

[27] I. J. W. McKean, J. C. Sadler, A. Cuetos, A. Frese, L. D. Humphreys, G. Grogan, P. A. Hoskisson, G. A. Burley, *Angew Chem Int Ed Engl* **2019**, *58*, 17583 - 17588.

[28] J. W. Schmidberger, A. B. James, R. Edwards, J. H. Naismith, D. O'Hagan, *Angew Chem Int Ed Engl* **2010**, *49*, 3646 - 3648.

[29] I. R. Bothwell, M. Luo, *Org. Lett.* **2014**, *16*, 3056 - 3059.

[30] aJ. M. Lipson, M. Thomsen, B. S. Moore, R. P. Clausen, J. J. La Clair, M. D. Burkart, *Chembiochem* **2013**, *14*, 950 - 953; bS. Mordhorst, A. Maurer, D. Popadic, J. Brech, J. N. Andexer, *ChemCatChem* **2017**, *9*, 4164 - 4168.