Supporting Information

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

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I. Materials and methods

(A) Chemicals and enzymes: All chemicals and solvents, as not described otherwise, were purchased from commercial suppliers (Sigma Aldrich, Abcr, Alfa Aesar, TCI, BLDpharm, Chiramer Inc, Chemsolute) and used without additional purification. S-Adenosyl-L-methione (SAM) disulfate tosylate was purchased from Abcr (cat-#: AB436584), S-adenosyl-L-homocystein (SAH) was purchased from Chiramer, Inc (cat-#: CR-1273-CV) and lysozyme was purchased from Carl Roth® (cat-#: 8259.2). T5 exonuclease, DpnI and Phusion® HF DNA polymerase and Taq DNA ligase were purchased from New England Biolabs (NEB), DNasel from Gold Biotechnology® (cat-#: D-300-5)

(B) NMR spectroscopy: 1H and 13C NMR spectra were recorded on a Bruker Avance III 500 HD spectrometer working at a frequency of 500 MHz (protons) using CDCl3 as solvent. Chemical shifts (δ) are given in ppm and referenced to the residual solvent peak. Coupling constants (Hz) and signal multiplicity (s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, ddt = doublet of doublets of triplets, t = triplet, m = multiplet) are noted in the conventional form.

(C) HPLC analysis of SAM analogs: High pressure liquid chromatography (HPLC) based detections and quantifications of SAH, SAM and SAM analogs were conducted with an Agilent 1290 Infinity II series instrument equipped with a diode-array detector (DAD). The substrate profile screen was performed at a wavelength of 260 nm using a SUPELCOSIL™ LC-18-T HPLC column (15 cm × 4.6 mm, 3 µM). To separate the charged SAM and SAM analogs, an ion-pairing reversed-phase (IP RP) HPLC method was used.[1] Therefore, as mobile phase (A) 10 mM sodium phosphate containing 5 mM 1-heptansulfonate as ion-pairing reagent and (B) pure acetonitrile was used with a flow rate of 1 mL/min with 1 µL injection volume and column temperature of 40°C. Gradient: begin with 95% A, 5 min to 80% A, hold for 2 min, 1 min to 20% A, hold for 1 min, 1 min to 95% A, hold for 2 min. For the reaction with iodoalkane 15, the gradient was elongated: begin with 95% A, 20 min to 80% A, hold for 2 min, 1 min to 20% A, hold for 1 min, 1 min to 95% A, uphold for 2 min.

High resolution mass spectrometry (HRMS) was performed using electrospray ionization (ESI) in positive mode on an Agilent 6200 Accurate-Mass TOF, LC- coupled with an Agilent 1200 series and equipped with a SeQuant® ZIC®-pHILIC 5 µm polymer LC column (50 × 4.6 mm). Mass data were collected in the range from 100 m/z to 1500 m/z. Capillary was set at 3500 V, nebulizer at 2 bar, dry heater at 325 °C and dry gas flow at 5.0 L/min. The mobile phase consists of buffer A (90% ACN, 20 mM ammonium formate, pH 2.8) and buffer B (10% ACN, 20 mM ammonium formate, pH 2.8) with a flow rate of 0.5 mL/min with 1 µL injection
volume and a column temperature of 40°C. Gradient: begin with 80% A, hold for 1 min, 12 min to 50%, hold for 3 min, 4 min to 80%, hold for 10 min.

(D) Gas chromatography-mass spectrometry (GC-MS) analysis: GC-MS analysis for the cascade reactions with 3-cyclopropyl-1H-pyrazole (20) and 3-bromo-1-propene (13) were performed with an Agilent 8860 GC instrument equipped with an Agilent 5977B mass spectrometer. An Agilent HP-5 MS UI column (30 m × 250 µm × 0.25 µm) was operated with helium as carrier gas. 1 µL sample injections were made with a split ratio of 1:15 and with an inlet temperature of 300°C. The column flow was set to 1.2 mL/min. Electron ionization of the analyte was carried out with 70 eV acceleration voltage. Temperature profile: Start at 110°C, hold for 1 min, 30°C/min to 165°C, hold for 0.7 min. SIM mode with 148.1 m/z (allylic products 21 and 22), 109.1 m/z (substrate 20), 148.9 m/z (Internal standard 2,6-dichloropyridine).
II. General procedures

(A) Cloning of HMTs and NNMTs variants: HMT genes derived from *Kordia algicida* (*kal*, UniProtKB: A9DNE6), *Methanosarcina acetivorans* (*mac*, UniProtKB: Q8TNZ0), *Ustilago maydis* (*uma*, UniProtKB: A0A0D1DT00), *Vibrio parahaemolyticus* (*vpa*, UniProtKB: Q87H19) and *Brassica rapa chinensis* (*bra*, UniProtKB: A1YSK2) were codon optimized for *E. coli* using the codon optimization tool from IDT (https://eu.idtdna.com/CodonOpt), ordered as synthetic gene fragments from Twist Bioscience (San Francisco, US) and inserted into pET 28a(+), containing a C-terminal His\(_6\)-tag with a LE-Linker, by Gibson Assembly.[2] After a final purification the derived plasmid was transformed through electroporation into a 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase (mtn) deficient *E. coli* BL21(DE3) strain described and kindly donated by the group of Prof. Bornscheuer.[1]

The HMT variant pET28a(+)-*ath*-V140T derived from *Arabidopsis thaliana* (*ath*, UniProtKB: Q0WP12) was provided by the group of Prof. Bornscheuer[1] and mutated to the wildtype using overlap extension PCR. The obtained PCR products were digested with DpnI, purified and ligated using the Gibson Assembly method.[2] After a final purification, the derived plasmid was transformed by electroporation into the 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase (mtn) deficient *E. coli* BL21(DE3) strain.

Genes of the HMTs derived from *Aspergillus clavatus* (*acl*, UniProtKB: A1CIS5), *Batis maritima* (*bma*, UniProtKB: Q7ZSZ7), *Burkholderia xenovorans* (*bxe*, UniProtKB: Q145N6), *Synechococcus elongatus* (*sel*, UniProtKB: Q31S13), *Chloracidobacterium thermophilum* (*cth*, UniProtKB: G2LF24) were part of a previous report of our group[3] and were already cloned in the pBAD33 vector containing a C-terminal His\(_6\)-tag with LE-linker. The plasmids were transformed into the SAHN-knockout *E. coli* strain JW0155 (Keio-collection) via electroporation.

The pET28a(+) vector was used for the nicotinamide N-methyltransferase derived from *Homo sapiens* (*wtNNMT*, UniProtKB: P40261) as well as for the previously constructed FuncLib derived variants[3]. All plasmids were transformed through electroporation into the 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase (mtn) deficient *E. coli* BL21(DE3) strain.

(B) Expression of HMTs and MTs for enzyme purification: Precultures of 5 mL LB media containing antibiotics (pET28a(+): 50 µg/mL kanamycin, pBAD33: 34 µg/mL chloramphenicol) were inoculated from glycerol stocks (stored at -80°C) and incubated at 37°C at 180 rpm overnight. For the main culture, 500 mL TB-media (containing a final concentration of 50 µg/mL kanamycin for pET28a(+)) and 34 µg/mL chloramphenicol for pBAD33) was inoculated to a concentration of 1% (v/v) from the overnight culture and incubated at 37°C for 3 h at 100 rpm.
Before induction, the cells were cooled for 15 min in an ice bath and induced by adding either IPTG (pET28a(+) expression system) to a final concentration of 0.5 mM or L-arabinose (pBAD33 expression system) to a final concentration of 0.02% (w/v). Protein expression was performed for 20 h at 20°C and 100 rpm. The cells were harvested at 4.331 × g at 4°C for 15 min, frozen and stored at -20°C.

(C) Protein purification and storage: Frozen cell pellets were thawed at room temperature and resuspended in 3 mL lysis buffer (0.05 M KPi, 0.5 M NaCl, 0.01 M imidazole, 5% glycerol, pH 7.5, 1 mg/mL lysozyme, 0.2 mg/mL DNaseI) per 1 g of cell pellet and incubated at room temperature for 2 h. The insoluble fraction of the lysate was separated by centrifugation (13,000 × g, 4°C for 10 min) and the soluble fraction was filtered through 0.2 µM sterile filters. Enzyme purification was conducted at room temperature using a HisTrap™ HP 1 mL column loaded with Ni²⁺. The column was equilibrated with buffer A (0.05 M KPi, 0.5 M NaCl, 0.01 M imidazole, 5% glycerol, pH 7.5) for 10 column volumes (cv) at a flow rate of 2 mL/min before the lysate was loaded and washed for 10 cv with buffer A. The enzymes were eluted with an imidazole gradient starting with 20 mM imidazole for 4 cv, 40 mM imidazole for 4 cv, 70 mM imidazole for 4 cv, 100 mM imidazole for 4 cv, and 500 mM imidazole for 10 cv. The enzyme-containing fractions were identified by SDS PAGE analysis, combined, and dialyzed with a dialysis bag (12-14 kDa cutoff) against a 50 mM KPi buffer with a pH of 7.5 containing 5% glycerol overnight and once for 3 h the next day. The protein concentration was determined via absorption at 280 nm as well as by the Pierce™ BCA Protein Assay Kit (Thermo Scientific, US) and aliquots were stored at -20°C until further use.

(D) Protein stability analysis: The thermal stabilities of the purified anion methyltransferases were determined using a thermal shift assay.[4] Therefore, duplicates of each purified enzyme were diluted to 0.2 mg/mL and mixed with a Sypro® Orange (Thermo Fisher Scientific) solution to a final concentration of 1×. The interaction between the dye and the hydrophobic amino acids during a temperature gradient starting at 25°C and increasing with 1°C/min to 95°C was monitored using the StepOnePlus™ Real-Time PCR system (Applied Biosystems) under control of the StepOne™ software v2.3. The melting point (Tm) of each enzyme was determined according to the highest slope (inflection point) of fluorescence increase.

(E) Screening and rescreening of SAM analogs formation mediated by anion MTs: For the substrate profile screening, single 750 µL reactions carried out in 1.5 mL glass vials for each anion methyltransferase and haloalkane substrate combination was performed. Therefore, KP buffer (50 mM, pH 7.5) was mixed with SAH (as 100× DMSO stock) to a final
concentration of 1 mM as well as 10 µM (1 mol%) of purified anion methyltransferase and the reaction was started by adding haloalkane substrate (as 100× DMSO stock) to a final concentration of 10 mM for all haloalkanes except of 3-iodo-1-propene and 3-bromo-1-propene (2 mM final conc.) and 3-bromoprop-1-yne (1 mM final conc.). The reactions were performed at room temperature, 450 rpm for either 20 h or 5 h (in case of the 3-iodo-1-propene and 3-bromo-1-propene reaction). The negative controls were treated the same way and consist of the same concentration of SAH and haloalkanes but without the anion methyltransferase. For the sample preparation, the reactions were mixed with 750 µL acetonitrile, vortexed, incubated for 30 min at RT and centrifuged for 10 min at 15,000 × g before analysis via HPLC as described in section (C) of Materials and methods. In the rescreening, the same reactions and negative controls were carried out in triplicates for the best enzyme/substrate combinations.

(F) Chemical synthesis of SAM analogs: For the confirmation of the SAM analog formation, the retention times of biotransformation reactions were compared to chemically synthesized standards. Analytical standards of SAM analogs were synthesized according to the protocol described by the group of Gruber-Khadjawi. Therefore, 250 µL reactions were performed in glass vials. For each reaction, 20 mM SAH (1 eq.) and 60 mM of silver trifluoromethanesulfonate (3 eq.) were solved in pure formic acid before 1 M of haloalkanes (50 eq.) was added to start the reaction. The reactions were incubated at room temperature and shaken at 450 rpm on a bench plate shaker for 5 h. For sample preparation, 50 µL reaction mixture was mixed with 450 µL water and extracted 3× with 1 mL diethyl ether. The water phase was diluted with 500 µL acetonitrile and centrifuged for 15 min at 15,000 × g. The HPLC analysis was carried out as described in section (C) of Materials and methods.

(G) Screening of the FuncLib NNMT-variants for allylation of pyrazole 20: Frozen cells (NNMT variants) in 96 DWP were thawed and lysed with 200 µL/well lysis buffer (50 mM KPi, pH 7.0, 1 mg/mL lysozyme and 0.2 mg/mL DNaseI) for 2 h at 37°C and 480 rpm. Simultaneously, acl containing cells were lysed using 3 mL lysis buffer (50 mM KPi, pH 7.0, 1 mg/mL lysozyme and 0.2 mg/mL DNaseI) per g of cell paste. The 96 DWP lysates (NNMT variants) were centrifuged at 4°C and 4347 × g for 10 min to receive the soluble supernatant. The acl containing lysate was distributed over 2.0 mL Eppendorf tubes and centrifuged at 4°C and 21130 × g for 10 min to receive the soluble supernatant.

400 µL biotransformations were performed in 96 DWP with a KPi buffer (50 mM, pH 7.0), 150 µL of the NNMT variant lysates (from the 96 DWP), 2 mM of 3-cyclopropyl-1H-pyrazole (20), 0.1 mM SAH, 50 µL of the acl lysate, 3-bromo-1-propene (13) (10 mM, 5 eq.), and 3% (v/v) DMSO. The screening reactions were run for 5 h at room temperature and 450 rpm on a bench plate shaker. Extraction was performed using 900 µL/well of an extracting
solution (ethyl acetate/cyclohexane 1:1) and 2,6-dichloropyridine was added as internal standard for GC-MS analysis (see section (D) of Materials and methods).

(H) Cascade reactions: 500 µL biotransformations were performed in 1.5 mL screw vials using a KPi buffer (50 mM, pH 7.0 with a final 1.2% (v/v) DMSO). In each vial, 3-cyclopropyl-1H-pyrazole (20) (1 mM) was mixed with SAH (10 µM, 1 mol%), the purified enzymes NNMT (V-28 or V-36) and acl (10 µM, 1 mol% each), and finally with 3-bromo-1-propene (13) (1 mM, 1 eq.). The reactions were incubated over 18 h (covered from light) at room temperature and 480 rpm on a bench plate shaker. The reactions were stopped by addition of 1000 µL dichloromethane and further extraction of the organic layer. 2,6-Dichloropyridine was added as the internal standard for GC-MS measurement (see section (D) of Materials and methods).
III. Supporting figures

Fig. S1: Chemo, chemoenzymatic and pure enzymatic methods for SAM analog synthesis

(a) Chemical synthesis of SAM analogs

\[
\begin{align*}
    R-X & \quad + \quad \overline{\text{OOC-}} \quad \text{S-S} \quad \text{NH}_3 \\
    X = I, Br
\end{align*}
\]

(b) Chemoenzymatic synthesis of SAM analogs using L-methionine derivatives

(c) One step enzymatic synthesis of SAM analogs

Chemical, chemoenzymatic and pure enzymatic methods for the synthesis of SAM analogs. (a) The synthesis of SAM analogs by chemical alkylation of SAH with different haloalkanes typically requires separation of diastereomeric mixtures by preparative HPLC.\(^5\) (b) Two chemoenzymatic approaches are described to generate SAM analogs.\(^6,7\) However, both approaches depend on the initial chemical preparation of L-methionine derivatives from L-homocystine and haloalkanes. (c) Enzymatic SAM analog synthesis as studied in this manuscript.
**Fig. S2**: SDS-PAGE analysis of the purified anion-MTs.

A total amount of 0.5 µg of each purified enzyme and 1 µL of the cth-lysate was used for SDS-PAGE analysis. The marker Color Prestained Protein Standard, Broad Range (10-250 kDa) was used as the size standard and protein separation was performed using a pre-cast gel (Mini-Protean TGX gel, 4-20%, 12-well comb) run at 240 V for 24 min. Protein sizes can be found in **Table S1** and the expected specific protein size of the cth anion-Mt is highlighted with a red triangle.
The thermal stability was determined by measuring the fluorescence increase as result of the interaction of the hydrophobic amino acids with the Sypro® orange dye during the thermally induced protein unfolding (for details see (D) in general procedures).
Fig. S4: Substrate profiling of anion MTs for the promiscuous synthesis of SAM analogs.

HPLC yields as raw data (top) are compared to the heatmap data (bottom) of Fig. 2b. SAM analogs were quantified with a SAM calibration curve. HPLC yields (in %) were calculated by dividing the quantified SAM analog conc. by the SAH substrate conc. (1 mM). The limit of quantification corresponds to 0.1% yield. For the HPLC conversion shown in the heatmap (Fig. 2b), the background + twice the standard deviation (Background SD) of the background reaction (quintuplicate) was subtracted from the raw HPLC yields. Please note that non-enzymatic background SAH alkylation was not generally observed, yet, in some cases (iodomethane, and haloalkanes bearing cyclopropyl, allyl or propargyl moieties) we could determine a low level of highly reproducible background activity. For the construction of the heatmap, we defined an HPLC yield of 0.5% as the threshold to report promiscuous activities. Experiments were performed and analyzed as described in section (E) of General procedures and section (C) of Materials and methods.
IV. Supporting tables

**Table S1**: Overview of the anion-MTs chosen from the SSN. Given are the three letter abbreviations based on the genus and species of the organism, the corresponding kingdom of the organism, the UniProt protein identifier, the molecular weight and the experimentally determined melting temperatures.

| Anion-MT | Organism | Kingdom     | UniProt Identifier | MW [kDa] | Tm [°C] |
|----------|----------|-------------|--------------------|----------|---------|
| ath      | *Arabidopsis thaliana*¹⁶, ³⁰, ³¹ | Plantae     | Q0WP12             | 27.5     | 60.0    |
| bra      | *Brassica rapa chinensis* ³⁰  | Plantae     | A1YSK2             | 26.2     | 59.5    |
| cth      | *Chloracidobacterium thermophilum*¹⁶, ²⁶, ³¹  | Bacteria    | G2LF24             | 23.7     | 55.3    |
| kal      | *Kordia algicida* ³⁰ | Bacteria    | A9DNE6             | 24.2     | 55.3    |
| mac      | *Methanosarcina Acetivorans* ³⁰  | Archaea    | Q8TNZ0             | 23.9     | 60.0    |
| uma      | *Ustilago maydis* ³⁰ | Fungi       | A0A0D1DT00         | 29.5     | 44.6    |
| vpa      | *Vibrio parahaemolyticus* ³⁰  | Bacteria    | Q87H19             | 24.1     | 37.6    |
| acl      | *Aspergillus clavatus*¹⁶, ³⁰ | Fungi       | A1CIS5             | 33.4     | 48.6    |
| bma      | *Batis maritima*¹⁶, ³⁰ | Plantae     | Q7ZSZ7             | 26.9     | 55.0    |
| bxe      | *Burkholderia xenovorans*¹⁶, ³⁰  | Bacteria    | Q145N6             | 23.9     | 51.1    |
| sel      | *Synechococcus elongates*¹⁶, ³⁰  | Bacteria    | Q31S13             | 23.4     | 51.7    |
Table S2: Rescreening of the best substrate/anion-MT combination. The reactions were performed in triplicates with the reaction conditions described in II General procedures (E). Conversions are given as a mean of three measurements ± the standard deviation. The recovery rate of the quantified compounds is generally between 85 and 100%, which confirms high quality of the quantification. Our analysis did not quantify potential decomposition products of SAM and SAM analogs.[8]

| Substrate         | Anion MT | HPLC yield [%] | Recovery [%] |
|-------------------|----------|----------------|--------------|
| Iodomethane 1     | acl      | 83 ± 0.2       | 83 ± 0.2     |
|                   | uma      | 83 ± 0.7       | 83 ± 0.7     |
|                   | mac      | 13 ± 0.5       | 87 ± 7.9     |
|                   | Control  | 3.0 ± 0.1      | 94 ± 4.0     |
|                   | BSA Control | 2.9 ± 0.1 | 93 ± 0.2     |
| Iodoethane 2      | acl      | 75 ± 2.3       | 75 ± 2.3     |
|                   | uma      | 78 ± 1.6       | 78 ± 1.6     |
|                   | Control  | 0.0 ± 0.0      | 94 ± 1.1     |
| Bromoethane 3     | acl      | 98 ± 0.7       | 98 ± 0.7     |
|                   | uma      | 22 ± 0.4       | 102 ± 1.0    |
|                   | bma      | 18 ± 0.7       | 104 ± 0.7    |
|                   | Control  | 0.0 ± 0.0      | 102 ± 6.6    |
| 1-Iodopropane 4   | acl      | 8.8 ± 0.1      | 105 ± 0.8    |
|                   | uma      | 2.2 ± 0.1      | 103 ± 3.0    |
|                   | Control  | 0.0 ± 0.0      | 104 ± 3.8    |
| 1-Bromopropane 5  | acl      | 4.6 ± 0.1      | 103 ± 2.3    |
|                   | sel      | 0.8 ± 0.0      | 105 ± 0.9    |
|                   | Control  | 0.0 ± 0.0      | 94 ± 8.2     |
| Compound                        | acl     | uma    | Control | (Bromomethyl)cyclobutane 11 | acl     | uma    | vpa     | Control | (Iodomethyl)cyclopropane 9 | acl     | uma    | Control |
|--------------------------------|---------|--------|---------|-----------------------------|---------|--------|---------|---------|-----------------------------|---------|--------|---------|
| 1-Iodobutane 7                 | 4.7 ± 0.0 | 99 ± 3.8 | 0.0 ± 0.0 | 8.4 ± 0.2 | 107 ± 1.9 | 2.6 ± 0.1 | 111 ± 0.9 | 1.5 ± 0.0 | 107 ± 1.6 |
| I-Me                            |         |        |         | (Bromomethyl)cyclobutane 11 |         |        |         |         | 0.6 ± 0.0 | 102 ± 0.6 |         |        |         |
| Control                         |         |        |         | (Bromomethyl)cyclobutane 11 |         |        |         |         | 0.0 ± 0.0 | 102 ± 0.1 |         |        |         |
| 3-Iodoprop-1-ene 12             | 76 ± 0.7 | 86 ± 0.7 | 4.6 ± 0.0 | 76 ± 0.7 | 86 ± 0.7 | 18 ± 0.2 | 100 ± 0.5 | 18 ± 0.1 | 99 ± 0.6 |
| 3-Bromoprop-1-ene 13            | 33 ± 0.5 | 88 ± 1.4 | 3.4 ± 0.3 | 33 ± 0.5 | 88 ± 1.4 | 7.5 ± 0.2 | 101 ± 2.0 | 11 ± 0.0 | 94 ± 2.8 |
| (2-Iodoethyl)benzene 15         | 1.0 ± 0.0 | 100 ± 0.6 | 0.0 ± 0.0 | 1.0 ± 0.0 | 100 ± 0.6 |         |        |         |         |
| 3-Bromoprop-1-yne 18            | 25 ± 0.2 | 99 ± 0.8 | 1.4 ± 0.0 | 25 ± 0.2 | 99 ± 0.8 | 12 ± 0.1 | 101 ± 0.5 |         |        |         |
| BSA Control                     | 4.3 ± 0.0 | 103 ± 0.1 |         | 4.3 ± 0.0 | 103 ± 0.1 |         |        |         |         |
V. DNA and amino acid sequences

For the determination of the protein concentration, the absorbance at 280 nm was measured and the used molecular weight and molecular extinction coefficient were calculated using the ProtParam tool provided on expasy (https://web.expasy.org/protparam/). These results were confirmed with the Pierce™ BCA Protein Assay Kit (Thermo Scientific, US).

**Brassica rapa chinensis** anion MT (**bra**)

**DNA sequence**

ATGGCCGCGAATGTCACAAAATGCCCCAATCTGACCCGAGGAAAATATATATTTATCTCCTGAGGATGATAGCTAATGTTTACCGGAA
GACTGTCGAGGCGGGGGCTGGGAGAGGGTGTTGGGAAGACGGTGTTACGCCGTGGGATCAGGGGCGTGCTACGCCACTTGTGG
TTCACCTCTCGGAAATCTACAGCTTTCCTCCTCCGCGGCCTGGCTCTGTACCAGGTGTTGCTGCTGTTGCTGCGATG
GGCTCCCGCGAGCTATCTGTGGGGGCAGTATTTTACCAATCAGCCGTCCTGGGAAACAGCAGGAGTTGCTGATCTGATTACG
ATTGTTGTTGTTGCTCCATTACGTCTGACGAGCCCGCCCGCTGGGCTAAGGCGGAGTTGAGTTATTAAACCAAGGATGGCGATG
GCGTCCCCGGAGCGCTATGTTGTGGGGCTTGATATTTCCGAATCCGCCTTGGAAAAGGCGGCGGAGACTTACGGGTCTTCTCC
TAAAGCCAAGTACTTTACGTTTGTAAAGGAAGACTTTTTCACTTGGCGTCCAAACGAGTTGTTCGATCTGATTTTCGATTACG
TGGTGTTCTGCTGCCATCGACTGCTGACGAGCCCGCCCGCTGGGCTAAGGCGGAGTTGAGTTATTAAACCAAGGATGGCGATG
GCGTCCCCGGAGCGCTATGTTGTGGGGCTTGATATTTCCGAATCCGCCTTGGAAAAGGCGGCGGAGACTTACGGGTCTTCTCC

**Amino acid sequence** (MW: 26.221 kDa, Molecular extinction coefficient: 46535 M⁻¹·cm⁻¹)

MAEVQQNSAHINGENIIPPEDVAKFLPKTVEEGGWEKCWEDGVTPWDQGRATPLVVHLVESSSLPLGLRALVGCGGHDVYM
ASPERSVYVGDISEALEKAAETYSSPKKAYFTFTFDFEDTFWPNELFDLIFYVDVVFCAIEPTFRPAKAMYELKPDGEL
ITLMPYITDHDGGPPYKFVASTYEDVLVPFGKAVISIBNPYESIATRGKKEKLARWKKINLHHHHHH*

**Kortia algicida** anion MT (**kal**)

**DNA sequence**

ATGAAACGCGATGCGACTAAGGGAATATTGAGGCACCAACGTTTACAGGAACAAATTTCAACTTGTTGGAACATTGGCTCCCATCAC
ACCACTTAAAACATCCTAGCCGTTCAAGCCGAAATCTCCAAAATTATCTCATCAGCAGGCGGAAACGGCGATAGGCGAAG
AAATCTCTTCAACAGGAGGTTCAAGGATAATATTATCTCTGTTGATCTCGGAGTACCCCGTGGGACGCCAACCGTCCTCAGCCAGCC
CGGTTTGCCGCTGCTGGAACCAAGATTGACTCTTGGAGACGACACCCGAGCATCCATCATATGATTTGATTATGGAACAGACATT
CTTCTGCTCTTCCTCCTTATCGACCCGAAACCGCCAGGAAACCCCGAGGAGCTGCTGCTGTTGCTGCTGCTGCTGCTGCTGCTGCTG
TGGAGCCCTTTTGGTTGATTTCCTCCGTGAGGCACTGAGGTAGGCTCCGGGAACCGGCCTGGGAGTACCATCAGCTGAGG
TACCTCAAGCCTTTACCTCAGATTTAAGACCTTTGGAAGCGCTCATAATTCTTATATCTGCTCTCGCGCGGCAACCGAGCTTTGC
GATCTTTATCAAATCTTCGACCGACCCACCCACCCAGCACTGAGA

**Amino acid sequence** (MW: 24.228 kDa, Molecular extinction coefficient: 34505 M⁻¹·cm⁻¹)

MNSDATKEYMQSRYKDSTQGIDSPSTPPLKTDYQIQQLKRIKLIILPGAGNAYEAYEYLLQGFTN1Y1LDSEIEPLQFQKN
PEF5SDRLCLFDFTTHKNYDLI1FIIQFFCSPFFPEPRAQYAKHMADLLNPNGKLVGLWDFPLTDDELKRRPGSSKEEYEL
YFKPYFDVKTPEFYKANSIPRAGNELGFIFIKSEHHHHHH*

**Ustilago maydis** anion MT (**uma**)

**DNA sequence**

ATGACATCTTCTCTTAAGGAAATATTGAGGCACCAACGTTTACAGGAACAAATTTCAACTTGTTGGAACATTGGCTCCCATCAC
ACCACTTAAAACATCCTAGCCGTTCAAGCCGAAATCTCCAAAATTATCTCATCAGCAGGCGGAAACGGCGATAGGCGAAG
AAATCTCTTCAACAGGAGGTTCAAGGATAATATTATCTCTGTTGATCTCGGAGTACCCCGTGGGACGCCAACCGTCCTCAGCCAGCC
CGGTTTGCCGCTGCTGGAACCAAGATTGACTCTTGGAGACGACACCCGAGCATCCATCATATGATTTGATTATGGAACAGACATT
CTTCTGCTCTTCCTCCTTATCGACCCGAAACCGCCAGGAAACCCCGAGGAGCTGCTGCTGTTGCTGCTGCTGCTGCTGCTGCTGCTG
TGGAGCCCTTTTGGTTGATTTCCTCCGTGAGGCACTGAGGTAGGCTCCGGGAACCGGCCTGGGAGTACCATCAGCTGAGG
TACCTCAAGCCTTTACCTCAGATTTAAGACCTTTGGAAGCGCTCATAATTCTTATATCTGCTCTCGCGCGGCAACCGAGCTTTGC
GATCTTTATCAAATCTTCGACCGACCCACCCACCCAGCACTGAGA

**Amino acid sequence** (MW: 24.228 kDa, Molecular extinction coefficient: 46535 M⁻¹·cm⁻¹)

UWWNQCSSSHQRTQKDSTOGIDSPSTPPLKTDYQIQQLKRIKLIILPGAGNAYEAYEYLLQGFTN1Y1LDSEIEPLQFQKN
PEF5SDRLCLFDFTTHKNYDLI1FIIQFFCSPFFPEPRAQYAKHMADLLNPNGKLVGLWDFPLTDDELKRRPGSSKEEYEL
YFKPYFDVKTPEFYKANSIPRAGNELGFIFIKSEHHHHHH*
**Amino acid sequence** (MW: 29.521 kDa, Molecular extinction coefficient: 46075 M⁻¹·cm⁻¹)

MTSSLSKKDLQKNLRLFDAVPFDNDPIKADQWIDSTTDWNRPQAPALVELLEGDAHADAKVQPDGVNGLIEQAPIFKDGD
TAVVPVCDGCCGDYOARVGAERGLTSYGVISNANAYNNKIDQDLPLTELDKVNFAEADFFTGLGSKSLVLESLKPGQATLAMY
DFTFLCAIIPPSLRTTWEAYTTLRAKKGCLVLALVFPIHGDPRPGPPSFISPQLVRELLGSOKNAAMAATWELKPKGEPTR
RPDVERMVWRRSLEH****

**Vibrio parahaemolyticus** anion MT (*vpa*)

**DNA sequence**

GATTATACGTTTCTTTGGCGATCCCGCAAGCTCGGCAACATGCGACAGATCTACAGCTACACGTTTATTAGCAAAACATGGTGTTTTAATCGCTTTAGTGTTTCCTATCCACGGCGATCGCCCAGGGGGTCCACCGTTCTCTATCTCCCCTCAGTTGGTACGGGA

**Amino acid sequence** (MW: 24.122 kDa, Molecular extinction coefficient: 38055 M⁻¹·cm⁻¹)

MKSKDSPIINEQRFWDALFFNGTMPWDRSQTPNELKHYLRKIAKTHSVFIPCGAAYEVSHVFVDCGHDV1AMDYSAEAVNLAK
SQLGQHQKVMULGDDVNAFDSREFDVIYERAFPLAALPREWGDVFAMIERLLLPSNGLLVGYVFISDDYRFRPPFCLRSGEIE
QKLEANFHIL1ESTPVTDSDVFVKEWQWQM3****

**Methanosarcina acetivorans** anion MT (*mac*)

**DNA sequence**

MFWDEVYKGTFFWDIDHQFPAFQALESSEIRIGPRALGGCGRGENAIMLKNGCDVTGIDLAKAIS
DAAKAIERHKVKNFIVGVLVDMDQLFTEDDFIVMIDSLFLHVITDEERLLFTRRHVKVLKGGKYM
LCFSDKEPGEYELPRRAKAEIESTFSPLFNIYIKDVIFDSLLLNPGRQAYLSSAKTSLEH****
Arabidopsis thaliana anion MT (ath)

DNA sequence

ATGGGCCAGTAGTCTATCATCATATCACCACATATAGCTGTTCTGCTGTCCGCGCGCGACGCTATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAGATGTCGTACGATATGCTGGTCTGGTGCCGCGCGGCAGTCATATGGCCGAAGAACAGCAGAATAG

Amino acid sequence (MW: 27.452 kD, Molecular extinction coefficient: 42065 M⁻¹·cm⁻¹)

MGSSHHHHHHSSGLVPRGSHMAEEQQNSDQSNGGNVIPTPEEVATFLHKTVEEGWKEWEEIEIPWDQGRATPLTVLVHDTS

Aspergillus clavatus anion MT (acl)

DNA sequence

ATGTCAACGCCCAGTTTAATTCCATCCGGTGTGCACGAAGTATTAGCAAAATACAAAGACGGTAACTATGTCGACGGGTGGGC

Amino acid sequence (MW: 33.396 kDa, Molecular extinction coefficient: 62255 M⁻¹·cm⁻¹)

MSTPSLIPSGVHEVLAKYKDGNYVDGWAELWDKSKGDRLPWDRGFPNPALEDTLLQKRAIGGIPQDDQADGKTVYKALLVPVGCGRGVDVLLASFGYDAYGLEYASTATAVDVCQEEAOORKIIPQDQFDFFDPWEDATLKLDRNQYDFYDYTF

Batis maritima anion MT (bma)

DNA sequence

ATGTCTACTGTAGCTAATATCGCTCCCGTATTCACGGGCGACTGCAAAACTATCCCAACGCCGAGATGTCGCTACCTTCTT

Amino acid sequence (MW: 26.851 kDa, Molecular extinction coefficient: 43805 M⁻¹·cm⁻¹)

MSTVANIAPVFTGDCKTIPTPEECATFLYKVVNSDQQAGKGTVYKALYVPGCGRGVDVLLASFGYDAYGLEYASTATAVDVCQEEAOORKIIPQDQFDFFDPWEDATLKLDRNQYDFYDYTF
**Burkholderia xenovorans** anion MT (bxe)

**DNA sequence**

ATGTCGGCACAAGCGCAGCCGGGTCCGGAATCTCGAATCTGATCCAAATTCTCCGGCATTGGATGAGGTAGTGTTCGGACATGTCGAAGGACGAGCTTGGGACCCCGAAGCTCAATTTTGGGAGCAGCGTTATCAAGAAGGGTCCGATCGTTGGGACCTTGGGCAGGCGGCACCTGTCTGGCGTTCACTTTTGGCGGGGACTAATGCCCCGGCACCTGGTCGCATTGCAGTATTAGGCTGTGGACGCGGTCACGATGCTCGCTTATTTGCCGAGCAAGGCTTCGAGGTTGTCGGCTTTGACTTTGCGCCATCCGCCATTGCCGCAGCACAAGCGTGCGGCCAGGGAACTACAGCGCAGTTTCTTCAGCGTGATATTTTTGCGTTGCCTCAAGAATTCGCGGGTCAGTTCGACACTGTTCTGGAGCATACATGCTTTTGCGCCATTGATCCTGACCGCCGTGCCGAGTATGTTGAGGTTGTGCGCCAAATTTTGAAACCTAAGGGTGCCTTCTTGGCTTGTTTTGGTGTCATGATCGTCCGTCCGGCCCTCCCTATGGATGTTCTTTAACAGAACTGCGCGATCGTTTCGCTCAGGGCTGGCAAGAAGAGCAGTTAGAATCTGTCACAGAATCCGTGGAGGGTCGCCGTGGTGAGGAATACTTGGGACGGTTGGCGTCGTCTGGATCTGGAACATCATCACCACCATCATTAA

**Amino acid sequence** (MW 23.914 kDa, Molecular extinction coefficient: 40575 M⁻¹ ‧ cm⁻¹)

MSDPTQPAVPDFETDRPNSFAVLFVRLVQEEQDFVFPPAWVFAIRPLALLARDYARHMDLLLPGGALLAGFFFLGATPKGPPFGEERAELDLATPYFDSLIDEAVHDSIAVFGERWLTWRRRALEHHHHH*

**Synechococcus elongatus** anion MT (sel)

**DNA sequence**

ATGACTAATGCCGTTAACCAAGCTCAATTTTGGGAGCAGCGTTATCAAGAAGGGTCCGATCGTTGGGACCTTGGGCAGGCGGCACCTGTCTGGCGTTCACTTTTGGCGGGGACTAATGCCCCGGCACCTGGTCGCATTGCAGTATTAGGCTGTGGACGCGGTCACGATGCTCGCTTATTTGCCGAGCAAGGCTTCGAGGTTGTCGGCTTTGACTTTGCGCCATCCGCCATTGCCGCAGCACAAGCGTGCGGCCAGGGAACTACAGCGCAGTTTCTTCAGCGTGATATTTTTGCGTTGCCTCAAGAATTCGCGGGTCAGTTCGACACTGTTCTGGAGCATACATGCTTTTGCGCCATTGATCCTGACCGCCGTGCCGAGTATGTTGAGGTTGTGCGCCAAATTTTGAAACCTAAGGGTGCCTTCTTGGCTTGTTTTGGTGTCATGATCGTCCGTCCGGCCCTCCCTATGGATGTTCTTTAACAGAACTGCGCGATCGTTTCGCTCAGGGCTGGCAAGAAGAGCAGTTAGAATCTGTCACAGAATCCGTGGAGGGTCGCCGTGGTGAGGAATACTTGGGACGGTTGGCGTCGTCTGGATCTGGAACATCATCACCACCATCATTAA

**Amino acid sequence** (MW 23.374 kDa, Molecular extinction coefficient: 39335 M⁻¹ ‧ cm⁻¹)

MTNAVNQAQFWEQRYEQSGRDFWLGQAAPQVFPRWLSLANTNAPGRAVLGCGGRHDLRAEQGFEVGFDFPSAIAAAALAGQTAAQFQRDIFALPQEFAGQFDTVLEHTCFACIDFRDALEYEVVRQILKPKGLGLFWKCHDRGSFPYGCSTELRDRFAQGWQEEQLESVSTESVEGRGEYLGRWRRLDEHHHHH*

**Chloracidobacterium thermophilum** anion MT (cth)

**DNA sequence**

ATGCCTTGGTATGGACGCGCTATCGGCAAGTTTTTTGGGAGAGAAGGATATTATCGTGCAATGGCTGGCCCGCTGGCGTCTCCGACCTGGGACCCCGAAGCTCAATTTTGGGAGCAGCGTTATCAAGAAGGGTCCGATCGTTGGGACCTTGGGCAGGCGGCACCTGTCTGGCGTTCACTTTTGGCGGGGACTAATGCCCCGGCACCTGGTCGCATTGCAGTATTAGGCTGTGGACGCGGTCACGATGCTCGCTTATTTGCCGAGCAAGGCTTCGAGGTTGTCGGCTTTGACTTTGCGCCATCCGCCATTGCCGCAGCACAAGCGTGCGGCCAGGGAACTACAGCGCAGTTTCTTCAGCGTGATATTTTTGCGTTGCCTCAAGAATTCGCGGGTCAGTTCGACACTGTTCTGGAGCATACATGCTTTTGCGCCATTGATCCTGACCGCCGTGCCGAGTATGTTGAGGTTGTGCGCCAAATTTTGAAACCTAAGGGTGCCTTCTTGGCTTGTTTTGGTGTCATGATCGTCCGTCCGGCCCTCCCTATGGATGTTCTTTAACAGAACTGCGCGATCGTTTCGCTCAGGGCTGGCAAGAAGAGCAGTTAGAATCTGTCACAGAATCCGTGGAGGGTCGCCGTGGTGAGGAATACTTGGGACGGTTGGCGTCGTCTGGATCTGGAACATCATCACCACCATCATTAA

**Amino acid sequence** (MW 23.735 kDa, Molecular extinction coefficient 64190 M⁻¹ ‧ cm⁻¹)

MLGMDADTASFWEEKYRADLTAWDRGGVSPALEHWLAEGALKPGRILIPGCGYGHEVLALARRGFEVWGLDIALTPRRLQEKLAQAGLTAHVVEGDRLTWWPRFQPEAQFDVTFVLHEHCTFCADFRRRLEYEVVRQILKPKLGLGLFWKCHDRGSGFPYGCSLTELDRRAFAQGWQEEQLESVSTESVEGRGEYLGRWRRLDEHHHHH*

**Amino acid sequence** (MW 23.739 kDa, Molecular extinction coefficient 33958 M⁻¹ ‧ cm⁻¹)

MLGMDADTASFWEEKYRADLTAWDRGGVSPALEHWLAEGALKPGRILIPGCGYGHEVLALARRGFEVWGLDIALTPRRLQEKLAQAGLTAHVVEGDRLTWWPRFQPEAQFDVTFVLHEHCTFCADFRRRLEYEVVRQILKPKLGLGLFWKCHDRGSGFPYGCSLTELDRRAFAQGWQEEQLESVSTESVEGRGEYLGRWRRLDEHHHHH*
Variant derived from *Homo sapiens* nicotinamide N-MT (NNMT): NNMT-v28[3]

**DNA sequence**

ATGGAGAGTGGATTTACGAGTAAGGACACTTTATTTTAAACCCGGCCGACTACTTTAGAAAATCTACAAAGTGGG
AAAGCCGCAATTCAGGAGGAAATCCAAATCTCTGAAACACTTTTTAAAAAGAACTGAGGCGGTTTACAGGAAGC
GAGATTTGTTGATTGACATCGGATCAGGTCCCACAATTTACCAACTTCTGTCCGCTTGTGAGAGTTTTAAGGAGATCGTCGTG
ACGGACTATTTGCTTCAGCTCTGACATATCTGAGTGCATATGCTGACATACGCGGATCTGCAGCAGTCTGGGTGCTGC
Amino acid sequence (MW: 30.678kDa, Molecular extinction coefficient 34880 M⁻¹·cm⁻¹)

MESGFTSKDYTLYSHNPRTYELKYYKFGSRHSAESQILKHLLKNLFKIFCCLDGKVKGDLILDIGSPTIYQLLSACESEFKEIVV
TDSQDIQHELWLKKEFEDWSVPVTYVCDLEGNKPGPEEKLRLQAVKVLKCDVTSQPLGVPLFPAVDCVSLSTLCL
HAACPDLPYCRALRNLSLLKPGFGLVIMDALKCSYMYIEQKFSMLPLGREAVEAAVKAEGYTIWEFEVISPYSSTMANAA
EGLFSLVARKLSRPLLEHHHHH* 

Variant derived from *Homo sapiens* nicotinamide N-MT (NNMT): NNMT-v36[3]

**DNA sequence**

ATGGAGAGTGGATTTACGAGTAAGGACACTTTATTTTAAACCCGGCCGACTACTTTAGAAAATCTACAAAGTGGG
AAAGCCGCAATTCAGGAGGAAATCCAAATCTCTGAAACACTTTTTAAAAAGAACTGAGGCGGTTTACAGGAAGC
GAGATTTGTTGATTGACATCGGATCAGGTCCCACAATTTACCAACTTCTGTCCGCTTGTGAGAGTTTTAAGGAGATCGTCGTG
ACGGACTATTTGCTTCAGCTCTGACATATCTGAGTGCATATGCTGACATACGCGGATCTGCAGCAGTCTGGGTGCTGC
Amino acid sequence (MW: 30.678kDa, Molecular extinction coefficient 33515 M⁻¹·cm⁻¹)

MESGFTSKDYTLYSHNPRTYELKYYKFGSRHSAESQILKHLLKNLFKIFCCLDGKVKGDLILDIGSPTIYQLLSACESEFKEIVV
TDSQDIQHELWLKKEFEDWSVPVTYVCDLEGNKPGPEEKLRLQAVKVLKCDVTSQPLGVPLFPAVDCVSLSTLCL
HAACPDLPYCRALRNLSLLKPGFGLVIMDALKCSYMYIEQKFSMLPLGREAVEAAVKAEGYTIWEFEVISPYSSTMANAA
EGLFSLVARKLSRPLLEHHHHH* 

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**Notes:**

1. Variant derived from *Homo sapiens* nicotinamide N-MT (NNMT): NNMT-v28
2. DNA sequence
3. Amino acid sequence (MW: 30.678kDa, Molecular extinction coefficient 34880 M⁻¹·cm⁻¹)
4. Variant derived from *Homo sapiens* nicotinamide N-MT (NNMT): NNMT-v36
5. DNA sequence
6. Amino acid sequence (MW: 30.678kDa, Molecular extinction coefficient 33515 M⁻¹·cm⁻¹)
VI. Calibration curves

The calibration curve of SAM and SAH were measured using external standards via HPLC-DAD analysis. For the enzymatic cascade, the calibration curves of compounds 20, 21 and 22 were calculated using internal standard via GC-MS.
VII. HPLC-DAD traces and MS characterization of SAM-analog formation

Mass spectrometric detection of enzymatically formed SAM-analogs. Enzymatic synthesis of SAM analogs were carried out as described in section E of General procedures and confirmed by LC-MS measurements using ESI ionization as described in section C of Materials and methods. The HPLC-DAD traces were determined at 260 nm and the calculated theoretical mass of each analog is shown beneath the structure.

A) For the biotransformation, SAH and iodomethane 1 and acl-anion-MT was shaken for 20 h. The chromatogram shows the overlay of the biotransformation (green) and the negative control (w/o enzyme, blue). The LC-MS fragmentation pattern for the formed S-adenosyl-L-methionine (theoretical mass: 399.1146 m/z) is shown below the chromatogram.
B) For the biotransformation, SAH and iodoethane 2 and acl-anion-MT was shaken for 20 h. The chromatogram shows the overlay of the biotransformation (green) and the negative control (w/o enzyme, blue). The LC-MS pattern for the formed S-adenosyl-L-ethionine (theoretical mass: 413.1602 m/z) is shown below the chromatogram.
C) For the biotransformation, SAH, 1-iodopropane 4 and acl-anion-MT was shaken for 20 h. The chromatogram shows the overlay of the biotransformation (blue) and the negative control (w/o enzyme, green). The LC-MS pattern for the formed S-adenosyl-L-propionine (theoretical mass: 427.1759 m/z) is shown below the chromatogram.
D) For the biotransformation, SAH, 1-iodobutane 7 and acl-anion-MT was shaken for 20 h. The chromatogram shows the overlay of the biotransformation (blue) and the negative control (w/o enzyme, green). The LC-MS pattern for the formed S-adenosyl-L-buthionine (theoretical mass: 441.1915 m/z m/z) is shown below the chromatogram.
E) For the biotransformation, SAH, 3-iodoprop-1-ene 12 and acl-anion-MT was shaken for 5 h. The chromatogram shows the overlay of the biotransformation (blue) and the negative control (w/o enzyme, green). The LC-MS pattern for the formed S-allyl homocysteine (theoretical mass: 425.1602 m/z) is shown below the chromatogram.
For the biotransformation, SAH, 3-iodoprop-1-yne 18 and acl-anion-MT was shaken for 20 h. The chromatogram shows the overlay of the biotransformation (green) and the negative control (w/o enzyme, blue). The propargylic SAM-analog synthesized from SAH and 3-bromoprop-1-yne is unstable in water with a t½ of 1 min forming the measured S-propanone homocysteine.[9] The LC-MS pattern for the formed S-propanone homocysteine (theoretical mass: 441.1551 m/z) is shown below the chromatogram.
G) For the biotransformation, SAH, (iodomethyl)cyclopropane 9 and acl-anion-MT was shaken for 20 h. The chromatogram shows the overlay of the biotransformation (green) and the negative control (w/o enzyme, blue). The LC-MS pattern for the formed S-cyclopropylmethyl homocysteine (theoretical mass: 439.1759 m/z) is shown below the chromatogram.
H) For the biotransformation, SAH, (bromomethyl)cyclobutane 11 and acl-anion-MT was shaken for 20 h. The chromatogram shows the overlay of the biotransformation (green) and the negative control (w/o enzyme, blue). The LC-MS pattern for the formed S-cyclobutylmethyl homocysteine (theoretical mass: 453.1915 m/z) is shown below the chromatogram.
A) For the biotransformation, SAH, (2-iodoethyl)benzene 15 and acl-anion-MT was shaken for 20 h. The S-phenylethyl homocysteine SAM analogs could not be separated on the HILIC column from the SAH. Therefore, the extracted ion counts (EIC) between 485-495 m/z are shown as overlay of the biotransformation (red) and the negative control (w/o enzyme, black). The LC-MS pattern for the formed S-phenylethyl homocysteine (theoretical mass: 489.1915 m/z) is shown below the chromatogram.

**Extracted Ion Counts 485-495 m/z**
VIII. Chemical synthesis of the regioisomeric allyl-cyclopropylpyrazole standards

The standards were synthesized following a modification of a reported protocol. In a 2 mL round-bottomed flask, 3-cyclopropyl-1H-pyrazole (20) (107.1 mg, 0.99 mmol) was dissolved in acetonitrile (4 mL) and cooled at 0°C under stirring. After 10 min, an aqueous NaOH solution (50% w/v, 400 µL) was added. After additional 10 min, 1.5 equivalents of 3-bromo-1-propene were added to the mixture and the reaction was allowed to warm at room temperature after 5 min. Reaction progress was checked by TLC [cyclohexane/ethyl acetate 2:1, Rf(21, 22) = 0.6 and 0.7 respectively]. The reaction was quenched after 5 h by addition of 20 mL ddH2O and further extracted three times with CH2Cl2 (20 mL). The organic layers were combined and dried with MgSO4. After rotary evaporation under reduced pressure, the resulting crude extract was purified by column chromatography [cyclohexane/ethyl acetate 2:1], leading to 104.8 mg of a colorless oil (76% yield of the combined regioisomeric mixture). For identification purposes, 50 mg of the regioisomeric mixture was purified by another column chromatography using the same conditions. 6 mg and 30 mg of products 21 and 22 respectively were collected as pure fractions (colorless oils).

1-Allyl-3-cyclopropyl-1H-pyrazole (21): "H NMR (500 MHz, CDCl3) δ 7.18 (d~s, 1H), 5.93 (ddt, J = 17.0, 10.2, 5.9 Hz, 1H), 5.81 (d~s, 1H), 5.16 (dd~d, J = 10.2 Hz, 1H), 5.11 (dd~d, J = 17.0 Hz, 1H), 4.59 (dt~d, J = 5.9, 2H), 1.88 (m, 1H), 0.83 (m, 2H), 0.62 (m, 2H) ppm; 13C NMR (126 MHz, CDCl3) δ 155.38, 133.28, 129.58, 118.42, 101.68, 77.41, 77.16, 76.91, 54.51, 9.28, 8.00 ppm.

1-Allyl-5-cyclopropyl-1H-pyrazole (22): "H NMR (500 MHz, CDCl3) δ 7.37 (d, J = 1.4 Hz, 1H), 6.00 (ddt, J = 17.1, 10.3, 5.4 Hz, 1H), 5.84 (d, J = 1.4 Hz, 1H), 5.20 (dd, J = 1.2 Hz, 10.3 Hz, 1H), 5.03 (dd, J = 1.2 Hz, 17.1 Hz, 1H), 4.82 (dt~d, J = 5.4, 2H), 1.74 – 1.67 (m, 1H), 0.94 (m, 2H), 0.65 (m, 2H) ppm; 13C NMR (126 MHz, CDCl3) δ 145.10, 138.50, 133.46, 117.22, 102.64, 51.92, 7.04, 6.14 ppm.

The "H NMR analysis of the mixture showed a regioisomeric ratio (r.r.) 21:22 of 2.6:1
IX. NMR spectra

\$^{1}\text{H-NMR spectrum of 1-allyl-3-cyclopropyl-1H-pyrazole (21): in CDCl}_3.\$
X. References

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