Supplementary information for the manuscript

"***S*-adenosylhomocysteine as a methyl transfer catalyst in biocatalytic methylation reactions**"

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Supplementary Methods

General information

All methyltransferase-encoding pET28a expression plasmids were purchased from BioCat GmbH. Proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions. The gels were stained with Coomassie brilliant blue.

Analytical high-performance liquid chromatography (HPLC) was performed using a Shimadzu LC-20AT HPLC with a Shimadzu SPD-M20A diode array detector. Phenomenex Luna® 5 µm SCX 100 Å, LC Column 100 x 4.6 mm was used for cation exchange. Size exclusion chromatography was performed on AKTA Explorer 100 FPLC System with a HiLoad® 16/600 Superdex® 200 pg column. High-resolution mass spectra were obtained on a Bruker maXis 4G UHR-TOF Mass Spectrometer. Chiral HPLC-UV analysis was performed on Agilent 1100 system with CHIRAL-AGP column from Chrom Tech, Inc. ^{1}H NMR spectra were recorded on a a Bruker Avance Neo NMR spectrometer operating at 500 MHz proton frequency and chemical shifts were internally referenced to residual proton signals of solvents. Chemical shifts (δ) were reported in parts per million (ppm). Standard abbreviations indicating multiplicity were used as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), and m (multiplet). Coupling constants (J) were reported in Hertz (Hz).
Unless otherwise noted, all chemicals and reagents were purchased from Sigma Aldrich and used as recieved. Antibiotics were purchased from PanReac AppliChem. Ingredients for buffers were purchased from Acros Organics. L-Histidine was purchased Fluka™. *Myo*-inositol was purchased from Apollo Scientific ltd. 2,7-dihydroxynaphthalene was purchased from FluoroChem Co. Deuterated solvents were purchased from Cambridge Isotope Laboratories.

Protein expression

E. coli BL21(DE3) pLysE cell: pET-28a plasmids containing genes were transformed using standard heat-shock protocols for chemically competent *E. coli* BL21(DE3) cells. *E. coli* cells containing the plasmid were collected from LB-AGAR plates with Chloramphenicol (35 µg/ml) & Kanamycin (50 µg/ml) and used to inoculate LB medium with Chloramphenicol (35 µg/ml) & Kanamycin (50 µg/ml) (20 ml). After incubation at 37°C overnight, 15 ml of pre-culture was used to inoculate fresh LB Medium (1L) with Chloramphenicol (35 µg/ml) & Kanamycin (50 µg/ml).[1] The cells were grown in 3L shaking flask at 37 °C (170 rpm) for about 4 h until OD600 reached 0.6~0.7 and then the culture was cool to 18 °C and Isopropyl β-D-thiogalactoside (IPTG) was added to final concentration of 100 µM. Expression was performed for 20 hours. Cells were harvested by centrifugation at 10,000 x g for 30 minutes and stored at -20 °C.
E. coli Δmtn (DE3): pET28a expression plasmids were transformed by electroporation. Cells containing the plasmid were collected from LB-AGAR plates with Kanamycin (50 µg/ml) and used to

inoculate LB medium. After inoculation, expression was done by following the same protocol as that of *E. coli* BL21(DE3) pLysE cell.

For purification, 10 g of cell pellet was suspended in 40 ml lysis buffer (50 mM phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0). Cells were disrupted by sonication for 3 x 60 s with Branson sonifier 450 (output control 5, 50% duty cycle). Lysates were centrifuged at 10,000 x g for 1.0 h at 4 °C. The cleared lysate was mixed with 1 ml of Ni^{II} NTA agarose at 4 °C for 20 min and loaded onto a column. The agarose beads were washed with 10 ml lysis buffer containing sodium phosphate buffer containing 10 mM and 20 mM imidazole respectively. The protein was eluted in a lysis buffer solution containing 250 mM imidazole. Protein containing fractions were collected and dialyzed against dialysis buffer (100 mM phosphate, pH 8), aliquoted and stored at -80 °C.

Construction of MTA/SAH nucleosidase knockout *E. coli* strains that carry inducible T7 RNA Polymerase

During our *S*-adenosyl methionine regeneration assay, co-factor was converted to adenine as a side reaction. This reaction was catalyzed by MTA/SAH nucleosidase, which is an impurity from protein purification. We still observed the activity of this side reaction after we purified our proteins by size exclusion chromatography. Therefore, an MTA/SAH nucleosidase knockout *E. coli* strains carrying inducible T7 RNA Polymerase was constructed.

Plasmid pKD46 was transformed to an *E. coli* cell strain from the KEIO collection (CGSC #: 8422,[2] MTA/SAH nucleosidase knockout) by electroporation to delete the chromosomal kanamycin resistant cassette.[3] The cells were inoculated onto plate with ampicillin and incubated at 30 °C. Single colony was picked and suspended in 50 μL LB-medium and then spreaded onto plate without any antibiotic and incubated at 42 °C overnight. The incubation at 42 °C was repeated for three times. Three single colonies were picked and suspended in 50 μL LB-medium and inoculated onto plate without any antibiotic and plates with kanamycin or ampicillin respectively to verify removal of plasmid pKD46 and deletion of kanamycin resistant cassette. One clone that only grew on plates without any antibiotic was chosen for λDE3 Lysogenization.

Lysogenization was done by following the protocol of the Novagen λDE3 Lysogenization Kit provided by the producer. Firstly, cells from single colonies were grown in LB medium supplement with 0.2% maltose, 10 mM $MgSO_4$ at 37 °C to an OD_{600} of 0.5. Secondly, 10 μL cultures were mixed with 5 μL of mixture containing 10^8 pfu λDE3, 10^8 pfu Helper Phage, and 10^8 pfu Selection Phage and incubated at 37 °C for 20 min. Then the mixtures were spread onto LB plates and plates were incubated overnight at 37 °C. Colonies were picked from plates and used to prepare cell cultures for further verification, storage and protein production. This cell strain was named as *E. coli* Δmtn (DE3).

Protein sequences

HMT (accession number: GenBank: AEP12557.1)
MGHHHHHHAENLYFQGSGLGMDADTASFWEEKYRADLTAWDRGGVSPALEHWLAEGALKPGRILIPGCG
YGHEVLALARRGFEVWGLDIALTPVRRLQEKLAQAGLTAHVVEGDVRTWQPEQPFDAVYEQTCLCALSP
EDWPRYEAQLCRWLRPGGRLFALWMQTDRPGGPPYHCGLEAMRVLFALERWRWVEPPQRTVPHPTGFFE
YAAILERLV
(Ext. coefficient: 65680, Molecular weight: 24617.03)

EgtD (GenBank: CKI49089.1)
MGSSHHHHHHSSGLVPRGSHMALSLANYLAADSAAEALRRDVRAGLTATQKSLPPKWFYDAVGSDLFDQ
ITRLPEYYPTRTEAQILRTRSAEIISAAGADTLVELGSGTSEKTRMLLDAMRDAELLRRFIPFDVDAGV
LRSAGAAIGAEYPGIEIDAVCGDFEEHLGKIPHVGRRLVVFLGSTIGNLTPAPRAEFLSTLADTLQPGD
SLLLGTDLVKDTGRLVRAYDDAAGVTAAFNRNVLAVVNRELSADFDLDAFEHVAKWNSDEERIEMWLRA
RTAQHVRVAALDLEVDFAAGEEMLTEVSAKFRPENVVAELAEAGLRQTHWWTDPAGDFGLSLAVR
(Ext. Coefficient: 36440, Molecular weight: 37180.86)

Inositol 4-methyltransferase (IMT) (GenBank: AAA33032.1)
MGHHHHHHAENLYFQGSGMTTYTNGNYTQPKTLDKDEQLAGLAVTLANAAAFPMILKSAFELKILDIFS
KAGEGVFVSTSEIASQIGAKNPNAPVLLDRMLRLLASHSVLTCKLQKGEGGSQRVYGPAPLCNYLASND
GQGSLGPLLVLHHDKVMMESWFHLNDYILEGGVPFKRAHGMIQFDYTGTDERFNHVFNQGMAHHTILVM
KKLLDNYNGFNDVKVLVDVGGNIGVNVSMIVAKHTHIKGINYDLPHVIADAPSYPGVEHVGGNMFESIP
QADAIFMKWVLHDWSDEHCVKILNKCYESLAKGGKIILVESLIPVIPEDNLESHMVFSLDCHTLVHNQG
GKERSKEDFEALASKTGFSTVDVICCAYDTWVMELYKK
(Ext. coefficient: 41745, Molecular weight: 42374.51)

Putrescine N-methyltransferase (PMT) (GenBank: AAT99576.1)
MGHHHHHHAENLYFQGSGMEVISNHNNGSTTKIILKNGSICNGNVNGNSHSHEKIENKLVECTNSIKPG
WFSEFSALWPGEAFSLKIEKLLFQGKSDYQDVMLFESATYGKVLTLDGAIQHTENGGFPYTEMIVHLPL
GSIPSPKKVLIIGGGIGFTLFEVSRYPTIETIDIVEIDDVVVDVSRKFFPYLAAGFDDPRVTLIIGDGA
AFVKAAQPGYYDAIIVDSSDPIGPAKDLFERPFFEALAKALRPGGVVCTQAESIWLHMHLIKQIIANCR
QVFKGSVNYAWTTVPTYPTGVIGYMLCSTEGPEVNFKNPVNSIDKDTSHVKSKGPLKFYNSDIHKAAFI
LPSFARDLVEF
(Ext. coefficient: 40130, Molecular weight: 39252.78)

C3-methyltransferase (SgvM) (GenBank: AGN74875.1)
MGHHHHHHAENLYFQGSGMATHDIAAQHLADGIAASGPAPDLAAAAAFLEMGDRLGVVAHLDPDRTLET
AEVAAALDLPEPALVRYLDAVESAGLVIREGEGRYRACPDFDTIRHQAGYISWTMNANRPFIENARDFF
TDWDKAARTHVRDYREVAVSSQWMGSHAFYPTALATIIDAAPRKVVDLGAGTCRLLIEVLGAVPGSTGV
GLDFAADACRAAEQAVAQAGMTDRLTVVERTIQSVATDPGVLEGADVIHAGFVFHDMLPEEEDVCDQVL
ANCRESLAPGGFLAITDAVPYLRNDRERRFSAAVSYYHGEFMRRRLQSEEEWVERLRGAGFSDVRALTL
AFPTGRLFLAHR
(Ext. coefficient: 35660, Molecular weight: 38947.74)

C-Methylation of Coumarin (NovO) (GenBank: AAF67508.2)
MKIEAITGSEAEAFHRMGSQASHRYDEFVDLLVGAGIADGQTVVDLCCGSGELEVILSSRFPSLNLVGV
DLSEDMVRIAREYAAEQGKALEFRHGDAQLLAGMEDLAGKADLVVSRNAFHRLTRLPAAFDTMLRLAKP
GGAVLNCSFIHPSDFDESGFRAWVTFLNQRPWDSEMQIVWALAHHYAPRLDDYREALAQAARETPVSEQ
RVWIDDQGYGVPTVKCFARRAAAHHHHHH
(Ext. coefficient: 29700, Molecular weight: 26221.55)

Product characterization of cascade reactions

L-*Nα*-trimethyl histidine (5), HR-ESI-MS (*m/z* 220.1057 $[M+Na]^+$, calcd for $C_9H_{15}N_3NaO_2$, 220.1056). ^1H NMR (D_2O, 500 MHz) δ_H 3.19-3.28 (m, 2H), 3.27 (s, 9H), 3.90 (dd, *J* =4.3, 11.1 Hz, 1H), 7.00 (s, 1H), 7.73 (s, 1H).

Preparative–scale reaction of TMH-d9 (*d9*-5). The same conditions were used as for the preparative enzymatic synthesis of TMH (see main text). CD_3I was used instead of methyl iodide. Deuteration was confirmed by both HR-ESI-MS and 2D NMR. *d9*-**5**, HR-ESI-MS (*m/z* 229.1617 $[M+Na]^+$, calcd for $C_9H_6D_9N_3NaO_2$, 229.1621). ^1H NMR (D_2O, *p*D 5.0, 500 MHz) δ_H 3.37 (dd, *J* =12.1, 14.1 Hz, 1H), 3.49 (dd, *J* = 3.8, 14.1 Hz, 1H), 3.98 (dd, *J* =3.8, 12.0 Hz, 1H), 7.34 (s, 1H), 8.60 (s, 1H). ^{13}C NMR (125 MHz). δ_c 169.1, 133.5, 126.2, 116.9, 76.0, 50.6, 22.0.

Ononitol (9).

A 1 ml reaction containing 100 mM sodium phosphate buffer, 5 mM inositol (**8**), 50 μM SAH, 10 mM methyl iodide 10 μM HMT and 40 μM IMT was incubated at 25 °C for 24 hours. Reaction mixture was centrifuged at 14,000 x g for 10 minutes and precipitated protein was removed. Supernatant was centrifuged and lyophilized. Reaction mixture was dissolved in 600 µL D_2O for ^1H NMR recording. Conversion was determined based on integral of protons from both substrate and product. After 24 hours the reaction mixture (see main text) was centrifuged at 14,000 x g for 10 minutes and precipitated protein was removed. Additional HMT and IMT were added to the same final concentration and incubated for 24 hours. Centrifugation and addition of new enzymes were repeated once more. After another 24 hours, the reaction was centrifuged and lyophilized. After lyophilization, product was dissolved in 600 µL D_2O for ^1H NMR recording. ^1H NMR (D_2O, *p*D 8.4, 500Hz). δ_H 3.35 (m, 1H), 3.38 (m, 1H), 3.49 (dd, *J* = 3.0, 10.0 Hz, 1H), 3.59 (s, 3H), 3.61 (m, 1H), 3.63 (m, 1H), 4.04 (dd, *J* = 2.8, 3.0 Hz, 1H). The ^1H NMR spectrum is consistent with that reported in literature.[4]

***N*,*N*′-dimethylputrescine (11)**.

2 ml reaction containing 100 mM sodium phosphate buffer, 2 mM putrescine, 20 μM SAH, 8 mM methyl iodide, 10 μM HMT and 20 μM PMT were incubated at 25 °C for 72 hours and lyophilized. Reaction mixture was dissolved in 600 µL D_2O for ^1H NMR recording. Conversion was determined based on integral of protons from both substrate and product.

HR-ESI-MS (*m/z* 117.1386 $[M+H]^+$, calcd for $C_6H_{17}N_2$, 117.1386). ^1H NMR (D_2O, *p*D 8.4, 500 MHz) δ_H 1.80 (m, 4H), 2.71 (s, 6H), 3.06 (m, 4H). The ^1H NMR spectrum is consistent with that reported in literature.[5]

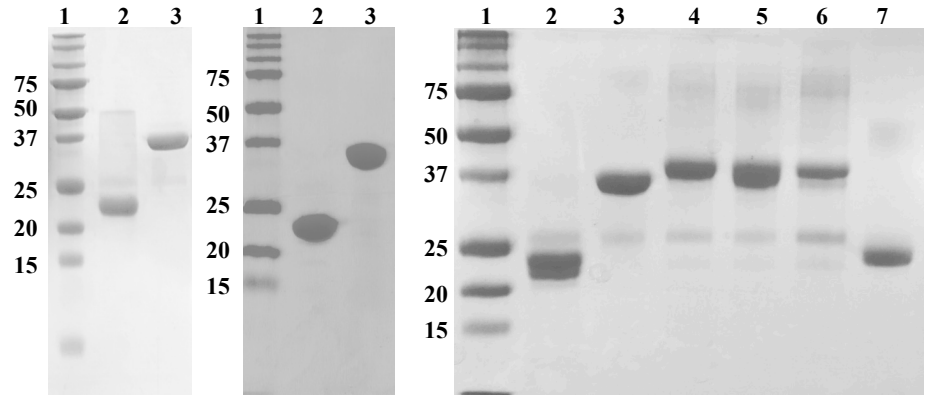
***R*-3-methyl-2-oxovalerate (13)**.

A 2 ml reaction containing 100 mM sodium phosphate buffer, 2 mM 2-oxovaleric acid (**12**), 20 μM SAH, 4 mM methyl iodide, 10 μM HMT and 20 μM SgvM were incubated at 25 °C for 72 hours and lyophilized. The reaction mixture was dissolved in 600 µL D_2O for ^1H NMR recording. Conversion was determined based on integral of protons from both substrate and product. HR-ESI-MS (*m/z* 129.0559 $[M-H]^-$, calcd for $C_6H_9O_3$, 129.0557) ^1H NMR (D_2O, *p*D 8.4, 500Hz). δ_H 0.89 (t, *J* = 7.5 Hz, 3H), 1.09 (d, *J* = 7.0 Hz, 3H), 1.43-1.50 (m, 1H), 1.66-1.74 (m, 1H), 2.89-2.96 (m, 1H). The ^1H NMR spectrum is consistent with that reported in literature.[6]

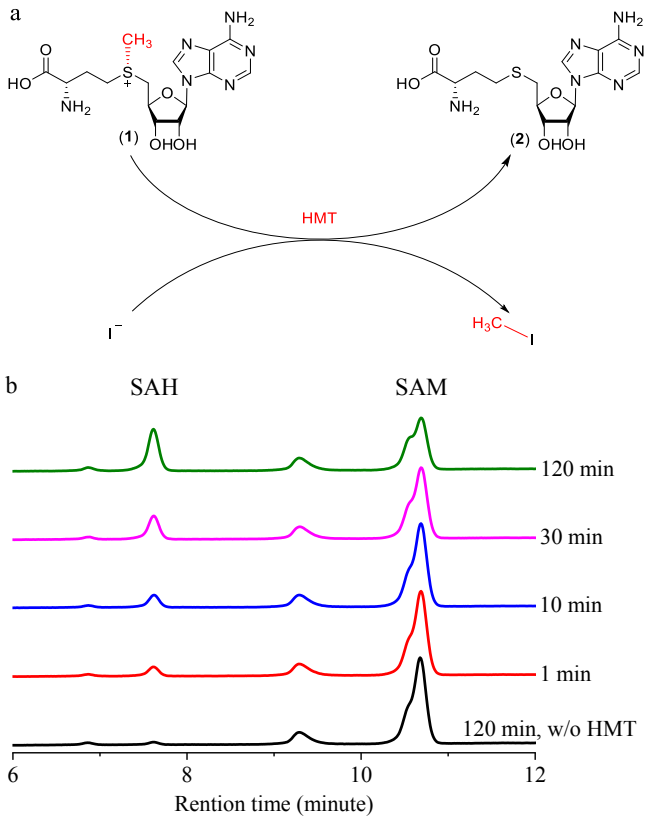
2,7-dihydroxy-1-methylnaphthalene (15).

A 1 ml reaction containing 100 mM sodium phosphate buffer, 5 mM 2,7-dihydroxynaphthalene (**14**), 50 μM SAH, 10 mM methyl iodide 10 μM HMT and 40 μM NovO were incubated at 25 °C for 24 hours. Reaction mixture was centrifuged at 14,000 x g for 10 minutes and precipitated protein was removed. Supernatant was centrifuged and lyophilized. Reaction mixture was dissolved in 600 µL D_2O for ^1H NMR recording. Conversion was determined based on integral of protons from both substrate and product.For product characterization: Firstly, 1 ml reaction was run as before. After 24 hours, reaction mixture was centrifuged at 14,000 x g for 10 minutes and precipitated protein was removed. Additional HMT and NovO were added to the same final concentration. After another 24 hours, the reaction was centrifuged. The solution was extracted 3x with 2.5 ml dichloromethane. The combined organic solutions were evaporated to dryness. Product was dissolved in 600 µL DMSO-*d*6 for ^1H NMR recording. ^1H NMR (DMSO-*d*6, 500Hz). δ_H 2.28 (s, 3H), 6.82 (dd, *J* = 2.3, 8.7 Hz, 1H), 6.90 (d, *J* = 8.8 Hz, 1H), 7.03 (d, *J* = 2.3 Hz, 1H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 9.29 (s, 1H), 9.52 (s, 1H). The ^1H NMR spectrum is consistent with that reported in literature.[7]

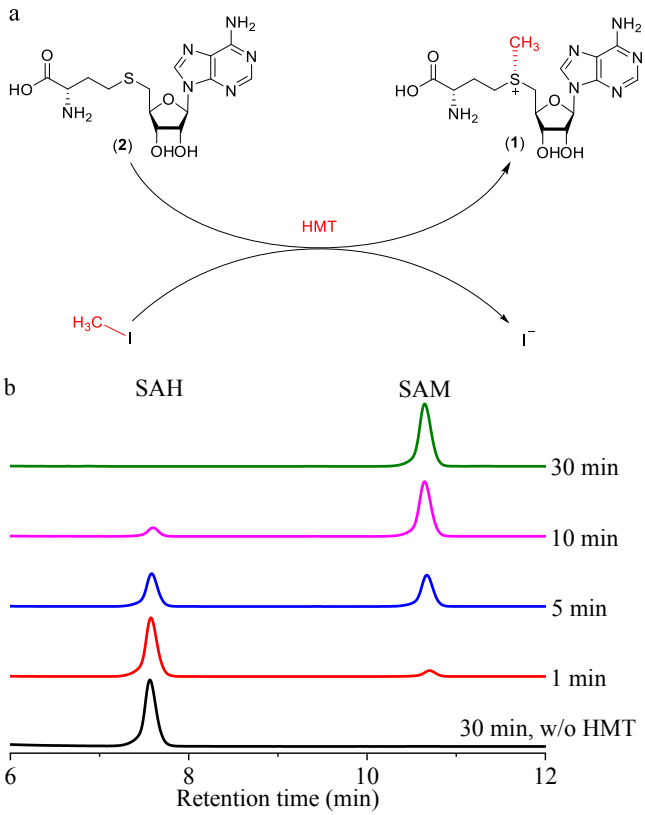
Supplementary Figures and Tables



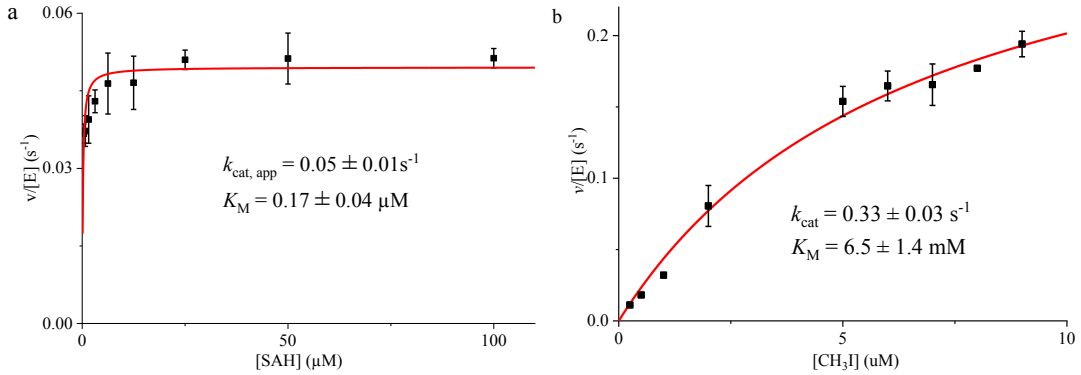
Supplementary Figure 1. SDS-PAGE of recombinant enzymes. Left: HMT and EgtD expression in *E. Coli* BL21 (DE3) pLysE and purified by Ni^{II}-affinity chromatography, lane 1. Marker with annotated MS (kD). lane 2. HMT, lane 3, EgtD. Middle: HMT and EgtD expression in *E. Coli* BL21(DE3) pLysE and purified by Ni^{II}-affinity chromatography and size exclusion chromatography, lane 1. Marker with annotated MS. lane 2. HMT, lane 3, EgtD. Right: Enzymes expressed in new *E. coli* Δmtn (DE3) strain and purified by Ni^{II}-affinity chromatography, lane 1. Marker with annotated MS. lane 2. HMT, lane 3. EgtD, lane 4. IMT, lane 5. SgvM, lane 6. PMT, lane 7, NovO.



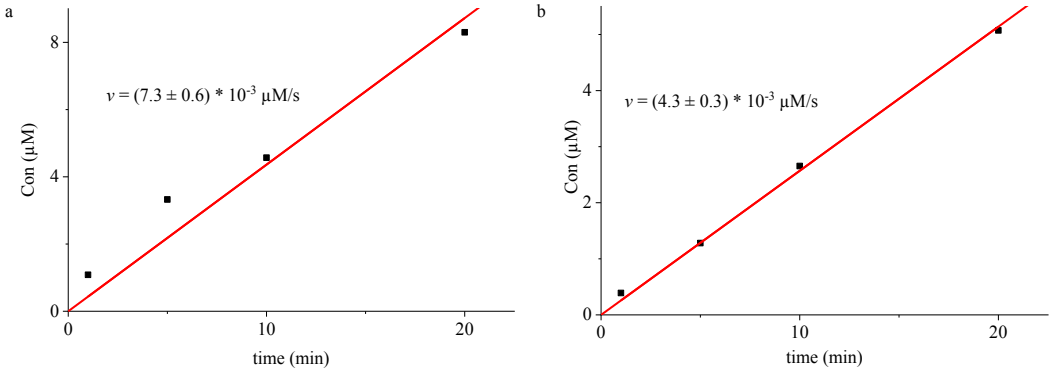
Supplementary Figure 2. Reactivity of HMT catalyzing methyl group transfer from SAM to iodide. a: Reaction scheme. b: HPLC trace of native reaction monitoring SAH and SAM. A 250 µL reaction containing 100 mM sodium phosphate buffer (pH 8.0), 10 mM of iodide, 1 μM HMT and 1 mM SAM was incubated at 25 °C. Close inspection of the SAM-related peak shows a slight shoulder that grows more significant as SAM is consumed. This shoulder is due to the inactive (*R*,*S*)-isomers of SAM.[8] Importantly, HMT-produced SAM does not show this shoulder (see Supplementary Figure 3), suggesting that freshly produced SAM contains a significantly larger (*S*,*S*) to (*R*,*S*) ratio.



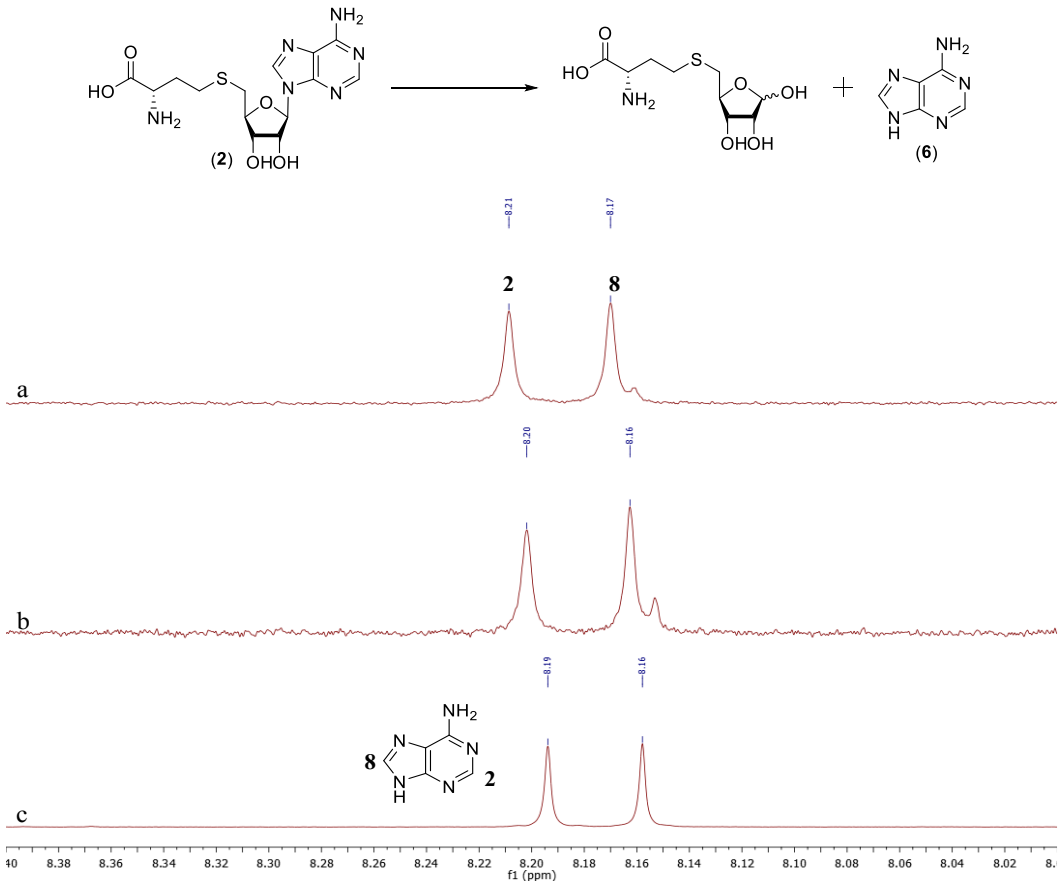
Supplementary Figure 3. Reactivity of HMT catalyzing methyl group transfer from methyl iodide to SAH. a: Reaction scheme. b: HPLC trace of reaction monitoring SAH consumption and SAM formation. A 250 µL reaction containing 100 mM sodium phosphate buffer (pH 8.0), 10 mM of methyl iodide, 1 μM HMT and 1 mM SAH was incubated at 25 °C.



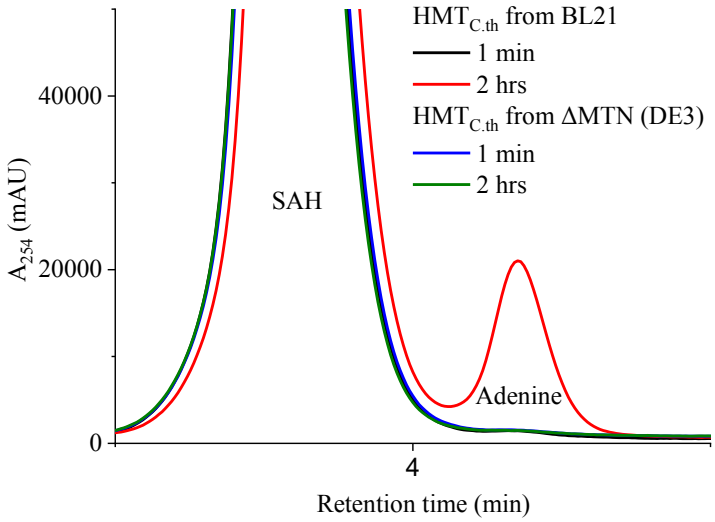
Supplementary Figure 4. Michaelis-Menten analysis of SAM formation catalyzed by HMT. 250 µL reactions containing 100 mM sodium phosphate buffer (pH 8.0), 0.5 μM HMT and different concentration of SAH were incubated at 25 °C. 30 µL of reaction mixture was quenched by adding to 15 µL of 1M phosphoric acid at 1, 2, 3, and 4 minutes. Concentrations of both SAH and SAM were monitored by Cation-exchange HPLC according to the standard curve obtained from solution of commercial SAH. The velocities were fitted to equation of $v = v_{max} * [s] / (K_M + [s])$. a) Concentration of SAH was varied from 100 μM to 0.39 μM while concentration of methyl iodide was 1 mM. The maximal rate determined with variable [SAH] is an apparent k_{cat} ($k_{cat, app}$) because of a limiting [methyl iodide]. Due to a lack of data points at [SAH] below K_M the determined value for $K_{M,SAH}$ should be viewed as an upper estimate of the true value. b) Concentration of methyl iodide was varied from 10 mM to 0.25 mM while concentration of SAH was 50 μM. Error bars represent standard deviations from three independent measurements.



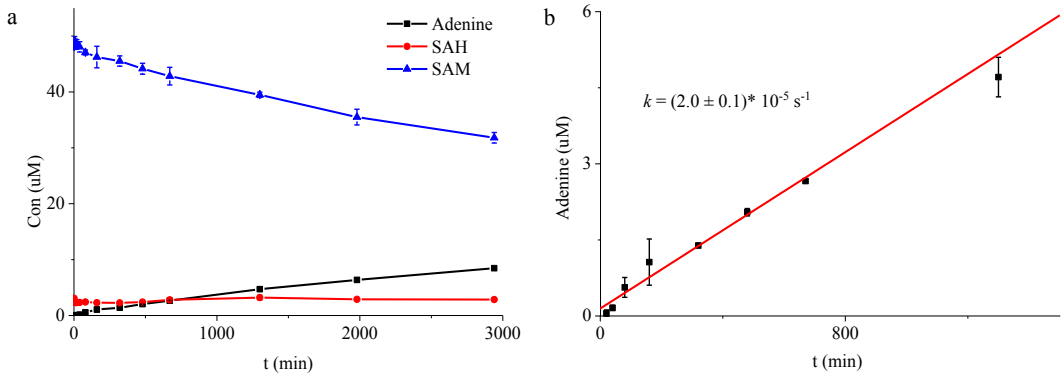
Supplementary Figure 5. Initial SAH degradation rate during HMT_EgtD cascade reaction. Product was identified to be adenine (Supplementary Figure 6). Therefore, concentration of degradation product was calculated based on the calibration curve of SAH. Concentrations of first four time points were fit versus time as linear curve. Slope of each curve were shown in figure as velocity. a: Proteins were purified from BL21 by Ni(II)-NTA agarose affinity chromatography. b: Proteins were re-purified by size-exclusion chromatography.



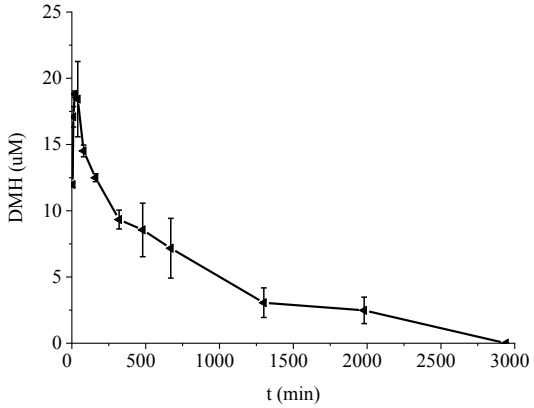
Supplementary Figure 6. ^{1}H NMR of SAH degradation product and authentic adenine. Degradation product: A 500 µL of 1 mM SAH and 40 μM EgtD in 100 mM sodium phosphate buffer was incubated overnight and lyophilized. The mixture was dissolved in 500 µL D_2O. a: Mixture of product and authentic adenine. b: Product of SAH degradation. c: Authentic adenine.



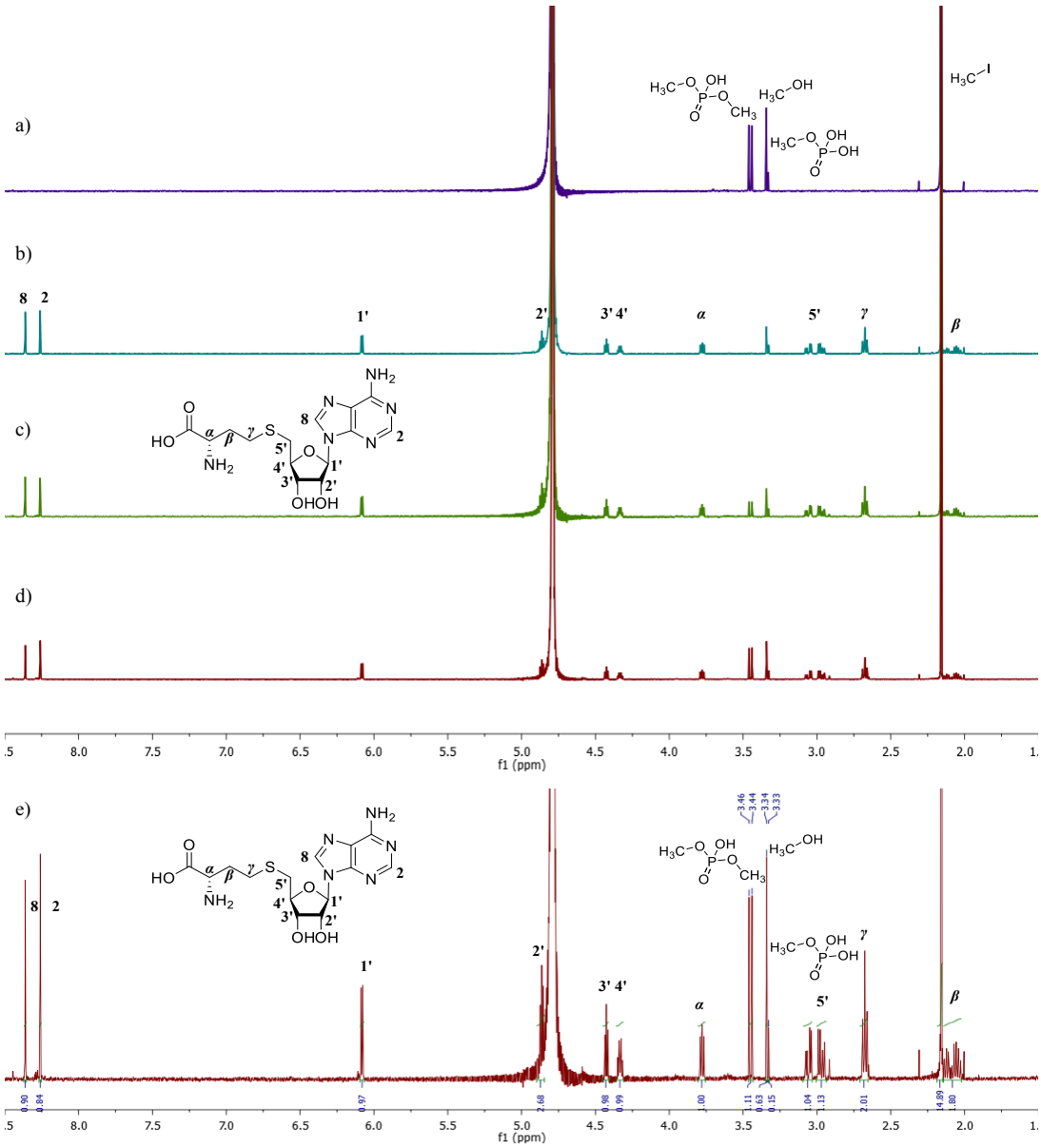
Supplementary Figure 7. SAH nucleosidase activity of impurity within HMT from new strain. Reactions containing 1 mM of SAH and 10 μM of HMT purified from two strains were incubated at room temperature for 2 hours. No significant adenine was observed from the reaction of HMT purified from *E. coli* Δmtn (DE3) strain after 2 hours.



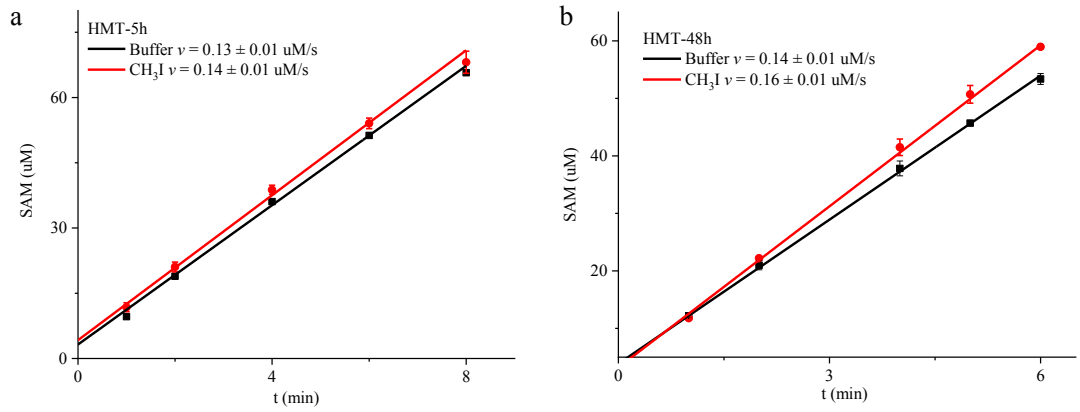
Supplementary Figure 8. Co-factor and its degradation during the cascade reaction using enzymes purified from *E. coli* Δmtn (DE3). A 500 µL reaction containing 1 mM histidine, 6 mM methyl iodide, 50 μM SAH, 10 μM HMT, 10 μM EgtD was run in 100 mM sodium phosphate buffer at 25 °C. a) Time curve of SAH, SAM and adenine. b) Adenine formation by non-enzymatic hydrolysis of SAH. Concentrations of adenine were fitted to linear curve and the slope was divided by concentration of SAH to get the reaction constant. Error bars represent standard deviations from three independent measurements.



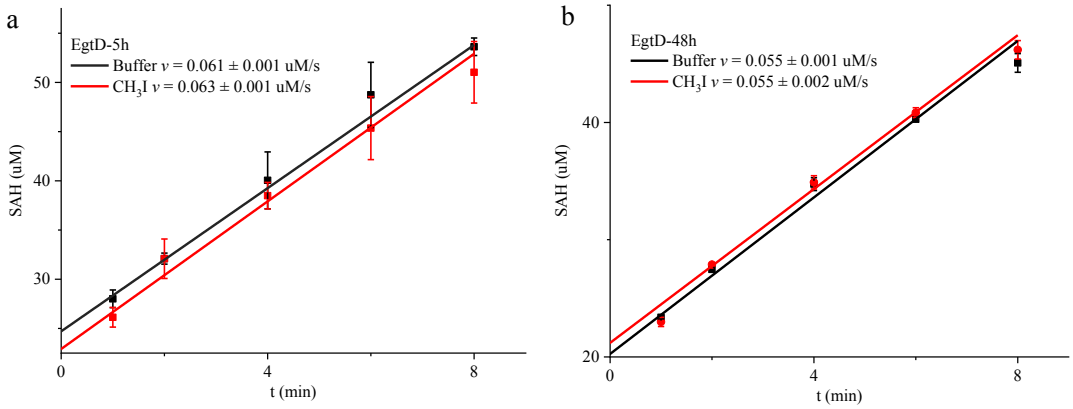
Supplementary Figure 9. Concentration of N_α-dimethyl histidine during the cascade reaction. Reaction condition was same as in Figure 2G and H. Concentration of N_α-dimethyl histidine increased for 20 minutes and reached highest of 18 μM. Subsequently, it gradually decreased and was lower than 1 μM after 48 hours. Error bars represent standard deviations from three independent measurements.



Supplementary Figure 10. Non-enzymatic methylation of S-adenosylhomocysteine and buffer at pD 8. 600 µL of 100 mM phosphate D_2O buffer (pD 8) containing 1 mM S-adenosylhomocysteine and 6 mM methyl iodide was incubated in NMR tube at room temperature (20°C). Another reaction without S-adenosylhomocysteine was run in parallel as control. ^{1}H NMR was recorded at 30 minute, 20 h and 48 h. a). ^{1}H NMR of methyl iodide solution in 100 mM phosphate D_2O buffer after 48 h. b). ^{1}H NMR of S-adenosylhomocysteine and methyl iodide after 30 minutes. c). S-adenosylhomocysteine and methyl iodide after 20 h. d). S-adenosylhomocysteine and methyl iodide after 48 h. e). Quantification of methylphosphate and methanol after 48 hours: Integral of methylphosphate is 0.3 (δ_H 3.33),[9] which indicates the concentration is 100 μM. Integral of dimethylphosphate is 1.11 (δ_H 3.46),[9] which indicates the concentration of dimethylphosphate is 185 μM. Integral of methanol is 0.48 (δ_H 3.34),[10] which indicates the concentration is 160 μM.



Supplementary Figure 11. Methylation effect on activity of HMT. HMT was incubated with 5 mM of methyl iodide or phosphate buffer respectively at 25 °C before it was used for activity assay. Rate of SAM formation was determined in reactions containing 100 mM phosphate buffer, 5 mM methyl iodide, 500 μM SAH and 1 μM HMT. a: SAM formation after incubation of 5 hours. b: SAM formation after incubation of 48 hours. Measured rates were within 10 % of difference in all four conditions. The shown data represents averages of three independent measurements. Error bars represent standard deviations from three independent measurements.



Supplementary Figure 12. **Methylation effect on activity of EgtD**: EgtD was incubated with 5 mM of methyl iodide or phosphate buffer respectively at 25 °C before it was used for activity assay. Rate of SAH formation was determined in reactions containing 100 mM phosphate buffer, 1 mM SAM, 1 mM L-histidine and 1 μM EgtD. a: SAH formation after incubation of 5 hours. b: SAH formation after incubation of 48 hours. Rates of SAH formation are identical in reactions that enzyme was pre-incubated with buffer and methyl iodide. Rates after 48 hours were 10 % lower than that after 5 hours. Error bars represent standard deviations from three independent measurements.

Protein methylation determined by trypsin digestion

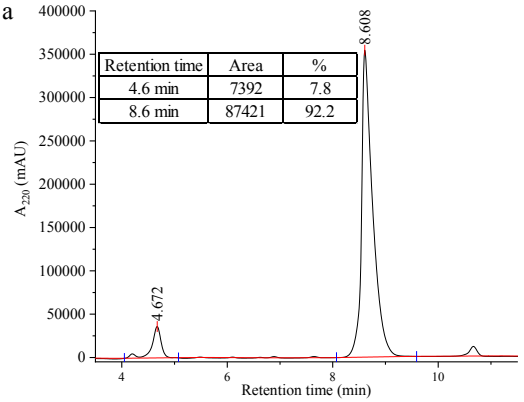
Solution containing 1 mg of protein was incubated with 5 mM methyl iodide overnight at room temperature and protein was precipitated by Chloroform/Methanol. Denatured pellet was harvested by centrifugation and dissolved in 100 µL of 6M urea. Mixture was incubated at room temperature for 60 minutes after addition of 5 µL DTT solution (200 mM). 20 µL of iodoacetamide (200 mM) was added and reaction was incubated for another 60 minutes. Reaction mixture was diluted by 775 µL of MilliQ-H_2O and 20 µg of tryposin or chymotrypsin was added. Digestion was performed overnight at 37 °C and solution was filtered and submitted to LC-HR-ESI-MS analysis. In control experiment, protein was incubated with phosphate buffer instead of methyl iodide before precipitation. All the following steps are the same. Fragments of proteins and their mass were analyzed on ExPASy (https://web.expasy.org/peptide_mass/). Mass analysis was run on a Shimadzu Nexera-x2 HPLC coupled with Bruker maXis II ESI-TOF.

All methylated fragments which were detected by MS contain cysteine residue. It is revealed that cysteine residue rather than any other residue got methylated because the mass of acetamide adduct was only detected in control while methyl adduct was detected in samples treated with methyl iodide.

Supplementary Table 1. Fragments and detected ions of methyltransferases after methylation and trypsin (chymotrypsin) digestion.

Protein	Whole protein MS (Methylated MS)	Methylation degree	sequences of cysteine-containing fragments	Residue number	Calculated MS	Detected mass of ions	
						Acetylamide adduct from control (+57.0215)	CH_3I (+14.0266)
HMT	24483.9 (24540.8)	3	EQTCL	128-132	593.2599	650.2660	607.2621
			CRW	148-150	464.2074	521.2289	478.2118
			IPGCGY	64-69	609.2701	666.2915	623.2718
			QTDRPGGPPYHCGL	163-176	1497.69	n.d.	n.d.
EgtD	37049.0 (37048.0)	0	RSAGAAIGAEYPGIEIDAVC GDF	140-162	2282.07	n.d.	n.d.
IMT	42245.4 (42301.0)	4	LLASHSVLTCK	103-113	1171.65	1228.6718	1185.6449
			CNY	131-133	399.1333	456.1438	413.1390
			NKCY	300-303	527.2282	584.2366	541.2316
			DCHTL	336-340	588.2446	n.d.	n.d.
			SDEHCVKIL	291-299	1043.519	n.d.	n.d.
			STVDVICCAY	364-373	1073.464	n.d.	n.d.
PMT	n.d. (n.d.)	n.d.	QIIANCR	270-276	817.4349	874.4563	831.4336
			VECTNSIKPGW	60-70	1233.593	n.d.	n.d.
			NGSICNGNVNGNSHSHEK	37-54	1867.81	n.d.	n.d.
			RPGGVVCTQAESIW	249-262	1502.742	n.d.	n.d.
SgvM	38817.4 (38817.4)	0	ACPDFDTIR	106-114	1037.472	1094.4935	1051.472
			VVDLGAGTCR	183-192	990.5037	1047.5251	1004.5007
			AADACRAAEQAVAQAGM	211-227	1633.742	n.d.	n.d.
			LPEEEDVCDQVL	264-275	1388.625	n.d.	n.d.
			ANCRESL	276-282	792.3668	n.d.	n.d.
NovO	26277.1 (26306.0)	2	NCSF	144-147	470.1704	527.1798	484.1749
			GVPTVKCF	217-224	850.4491	907.4520	864.4464
			CCGSGEL	668.2378	47-53	n.d.	n.d.

a



Retention time	Area	%
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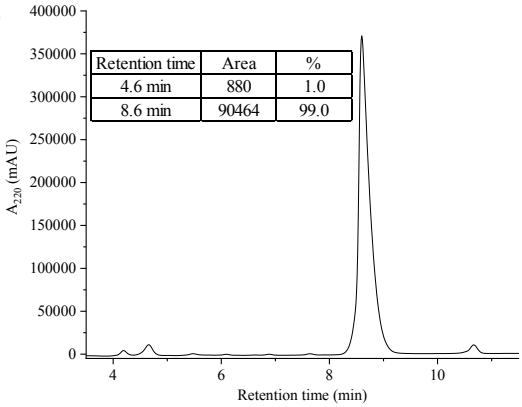
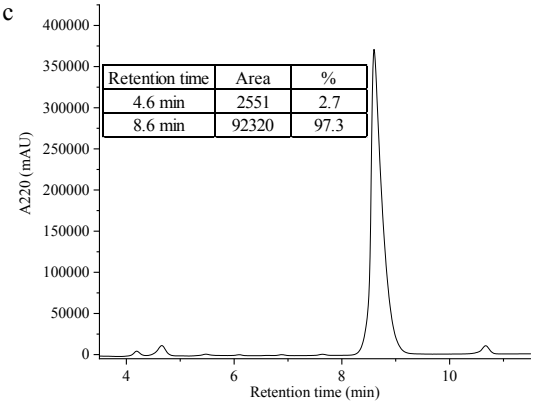
8.6 min	87421	92.2

b



Retention time	Area	%
4.6 min	880	1.0
8.6 min	90464	99.0

c



Retention time	Area	%
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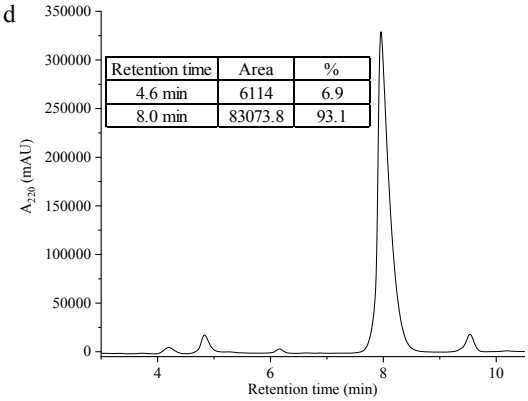
8.6 min	92320	97.3

d



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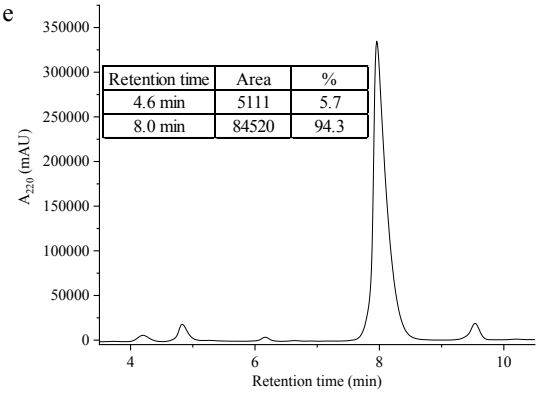
8.0 min	83073.8	93.1

e



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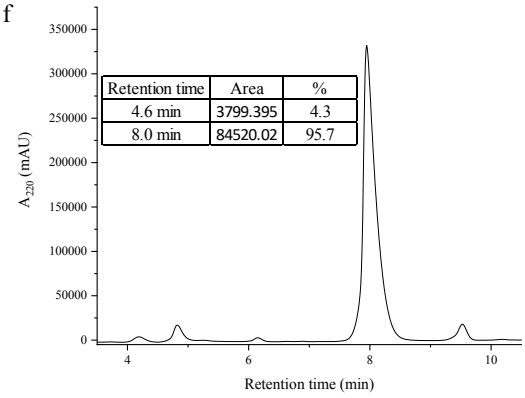
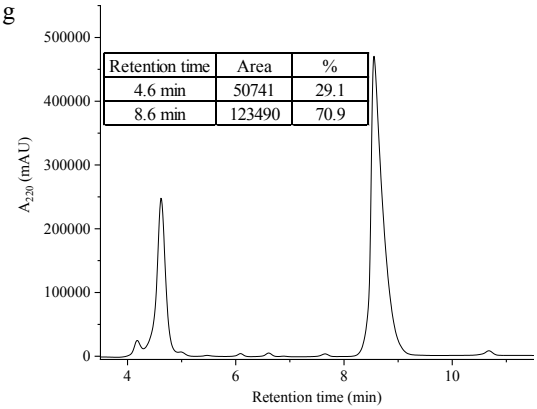
8.0 min	84520	94.3

f



Retention time	Area	%
4.6 min	3799.395	4.3
8.0 min	84520.02	95.7

g



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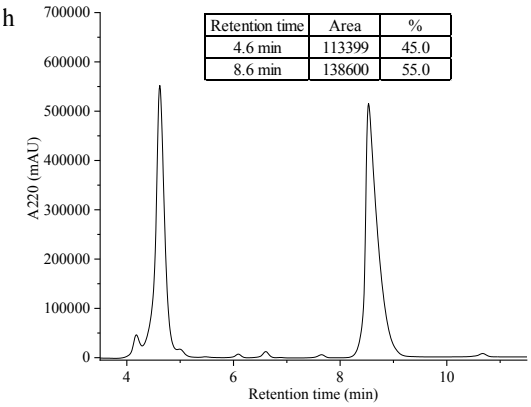
8.6 min	123490	70.9

h



Retention time	Area	%
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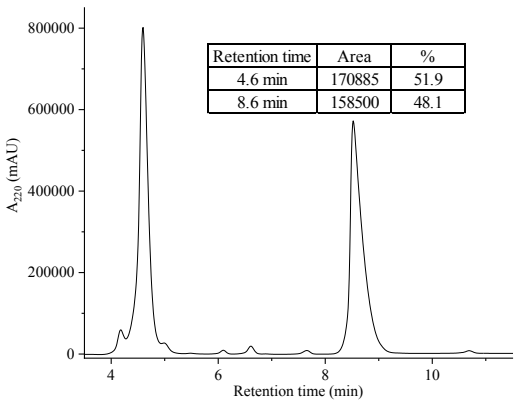
8.6 min	138600	55.0

i



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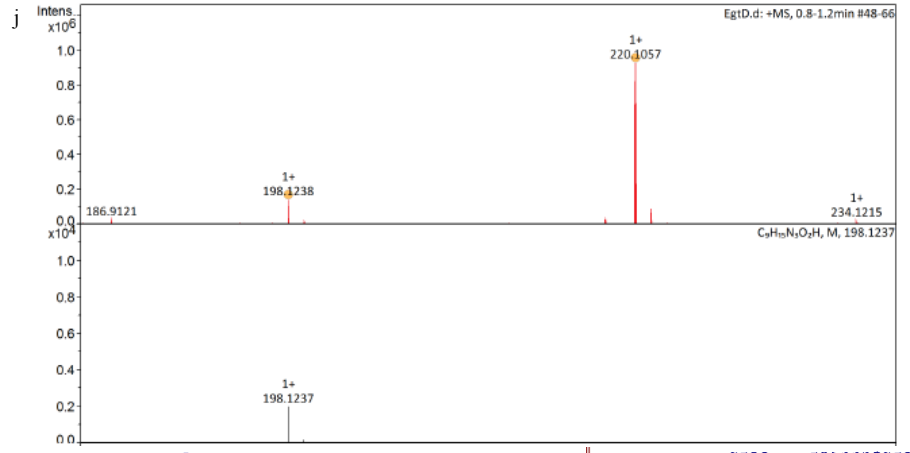

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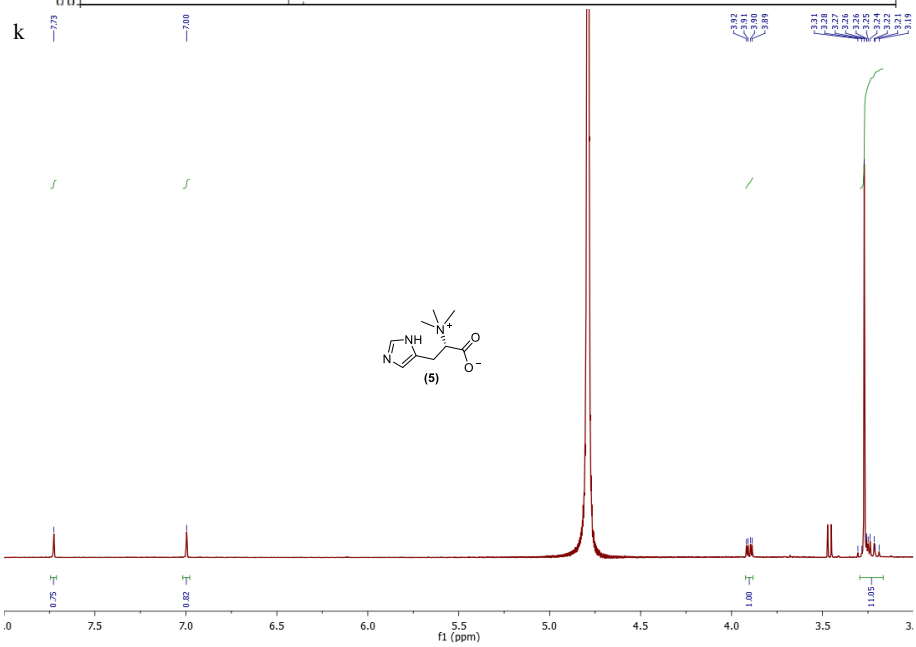
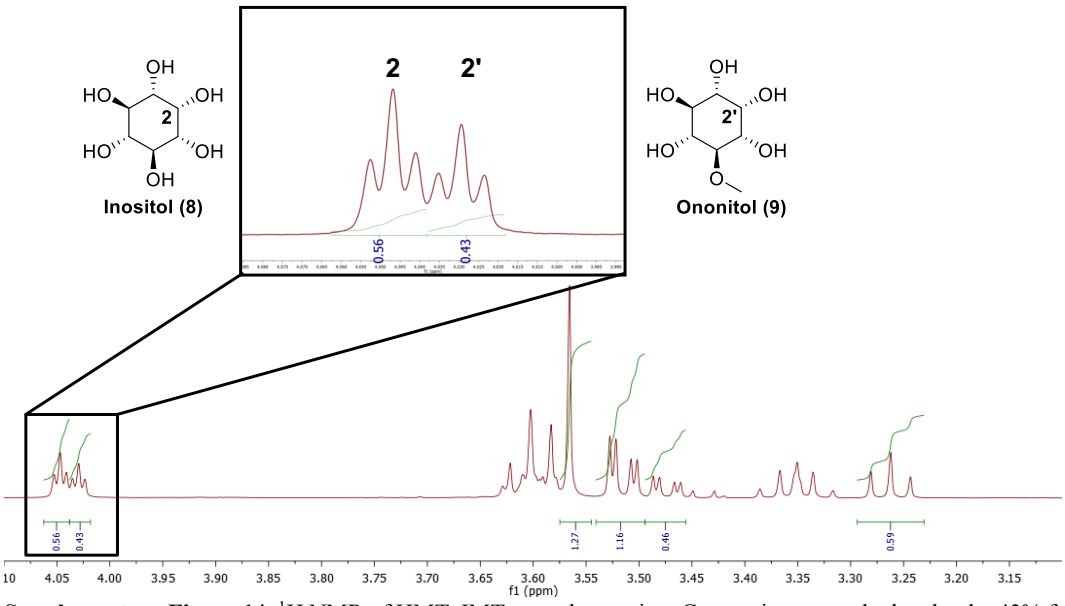
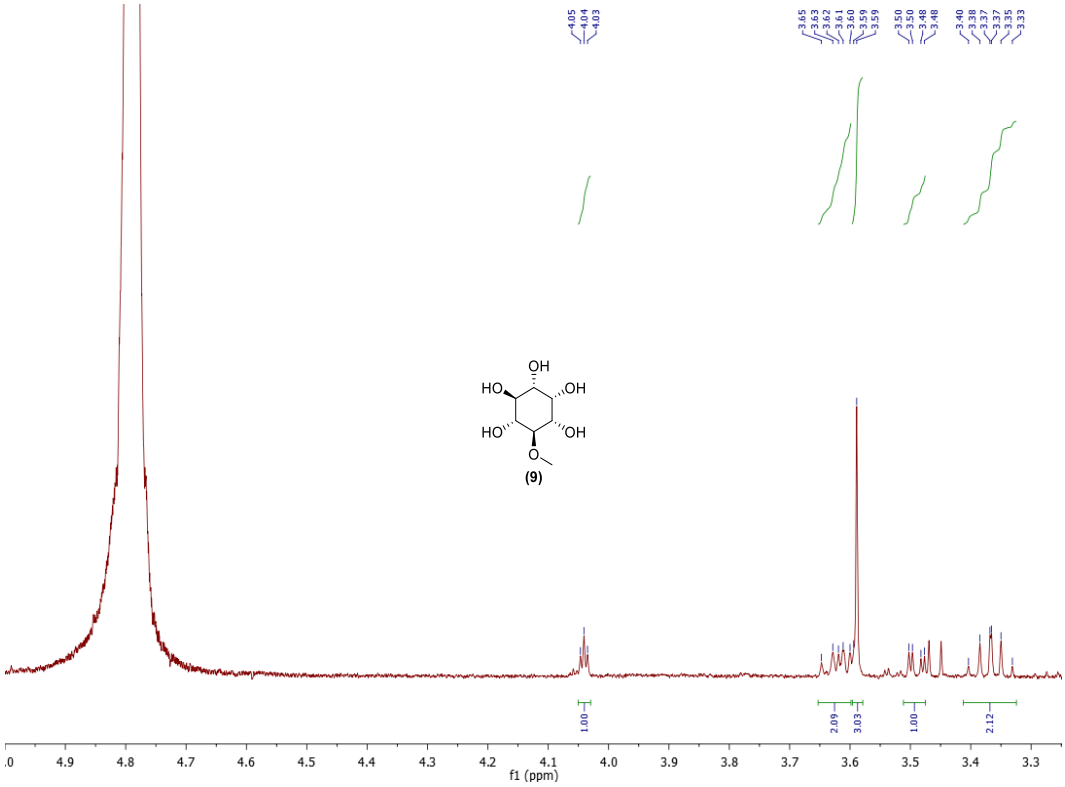


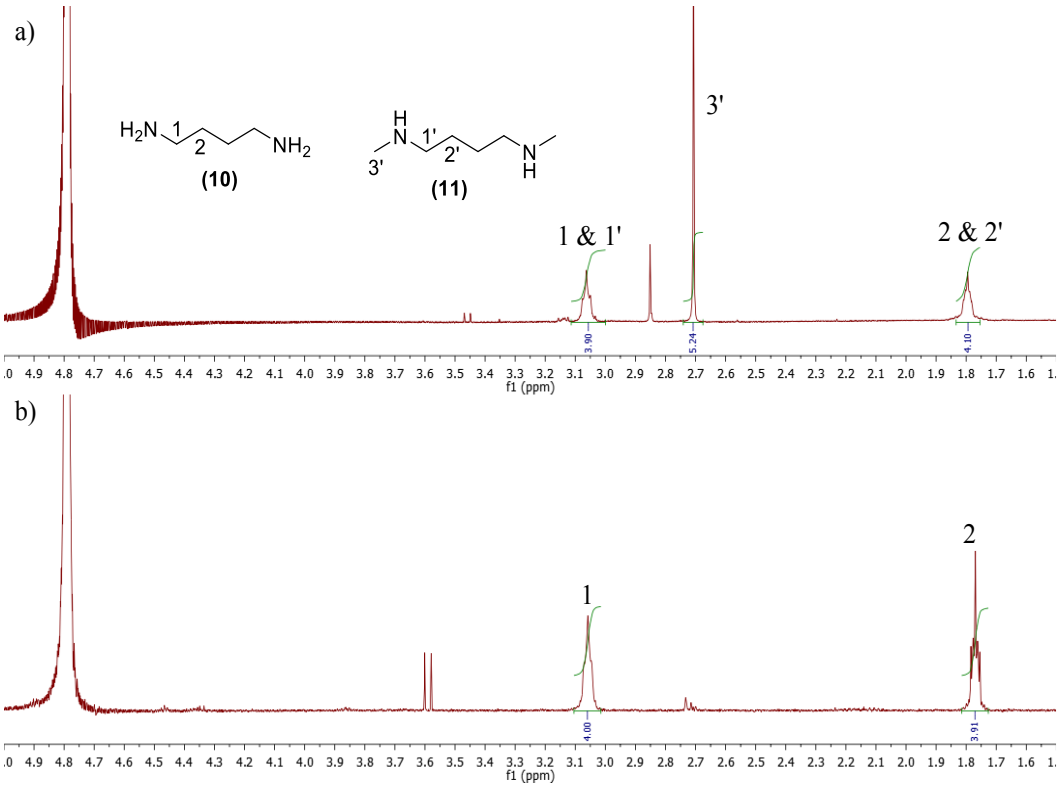
Supplementary Figure 13. Conversion of HMT_EgtD measured by HPLC (Table 1, entry 4-12) and product characterization. Area of both substrate (4.6 min) and product (8.6 min) were shown. a: entry 4; b: entry 5; c: entry 6; d: entry 7; e: entry 8; f: entry 9; g: entry 10; h: entry 11; i: entry 12; j: Measured and calculated HRESIMS of TMH (**5**), k) ^{1}H NMR spectrum of TMH (**5**).



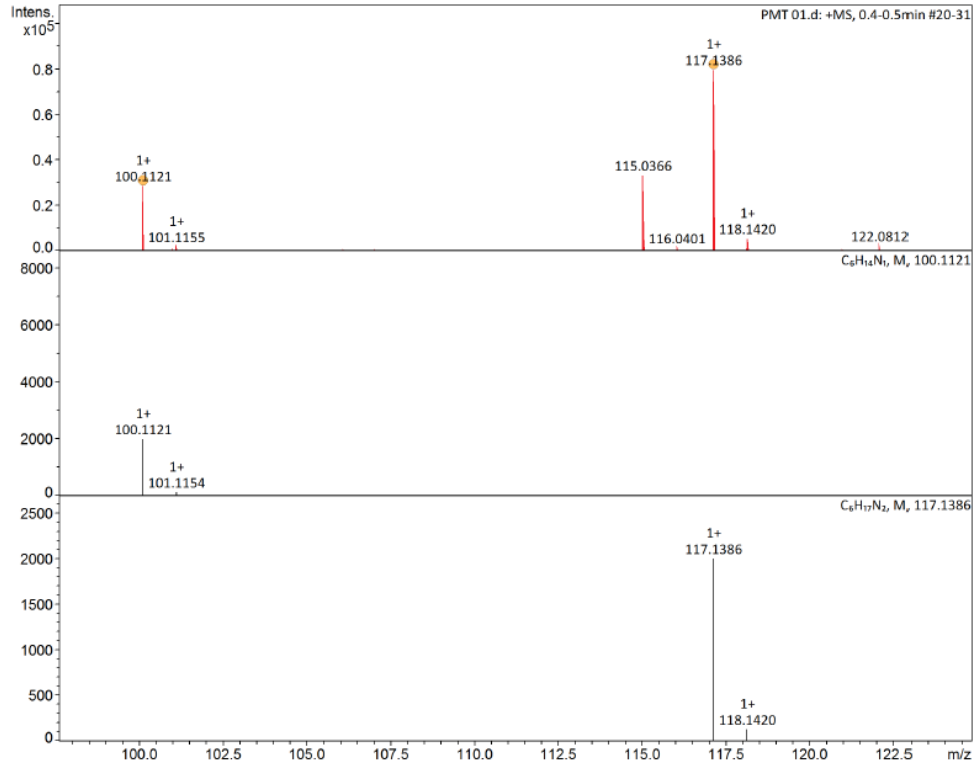
Supplementary Figure 14. ^{1}H NMR of HMT_IMT cascade reaction. Conversion was calculated to be 43% from integrals of reaction mixture by following: C = 2'/(2 + 2').



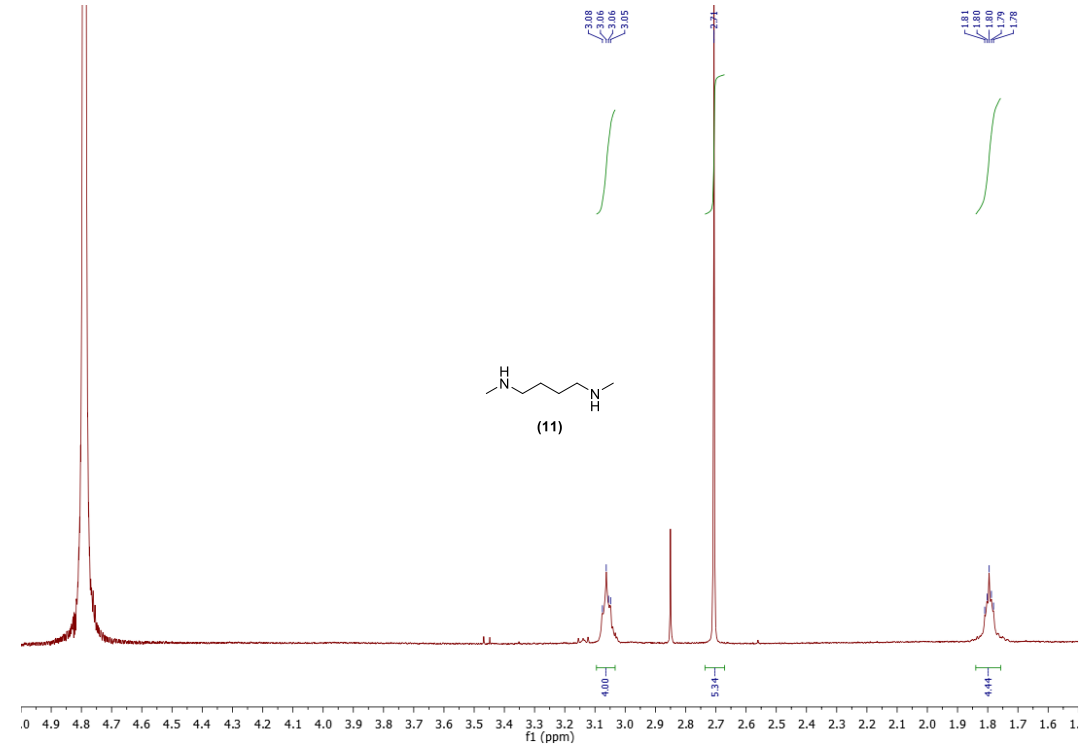
Supplementary Figure 15. ^{1}H NMR spectrum of Ononitol (**9**)



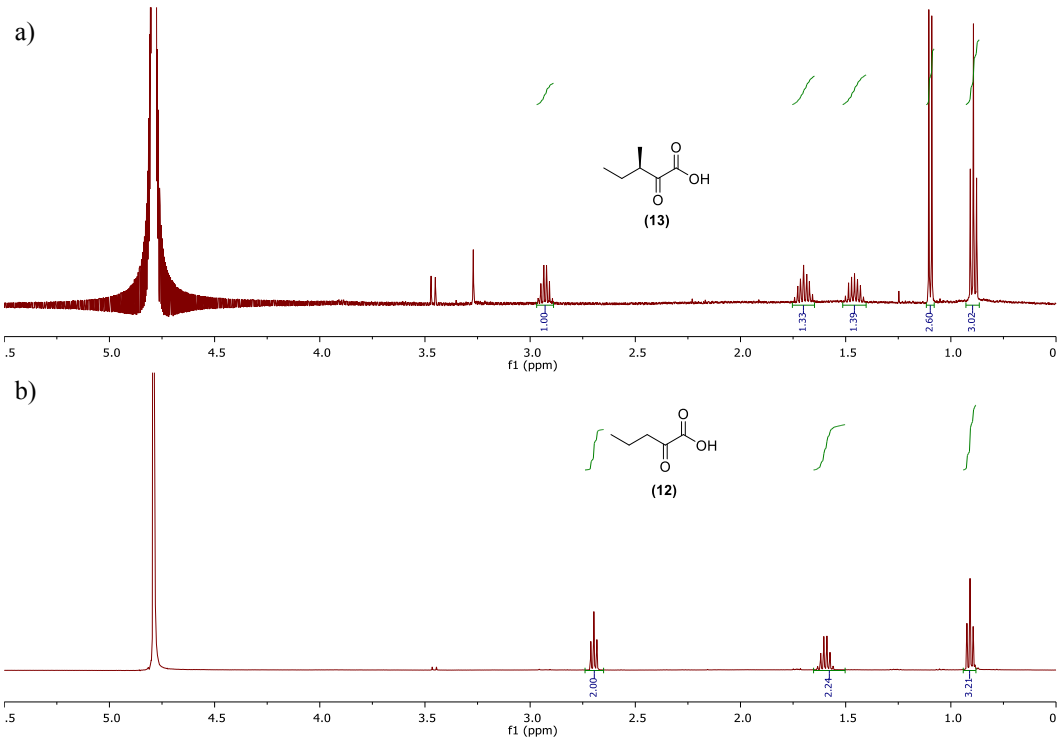
Supplementary Figure 16. ^{1}H NMR of HMT_PMT cascade reaction. Spectrum of reaction mixture was shown in (a). Signals of product and substrate (b) are overlapping. Conversion was calculated to be 87% from integrals of reaction mixture by following: C = 3'/[(1 + 1' + 2 + 2')*1.5].



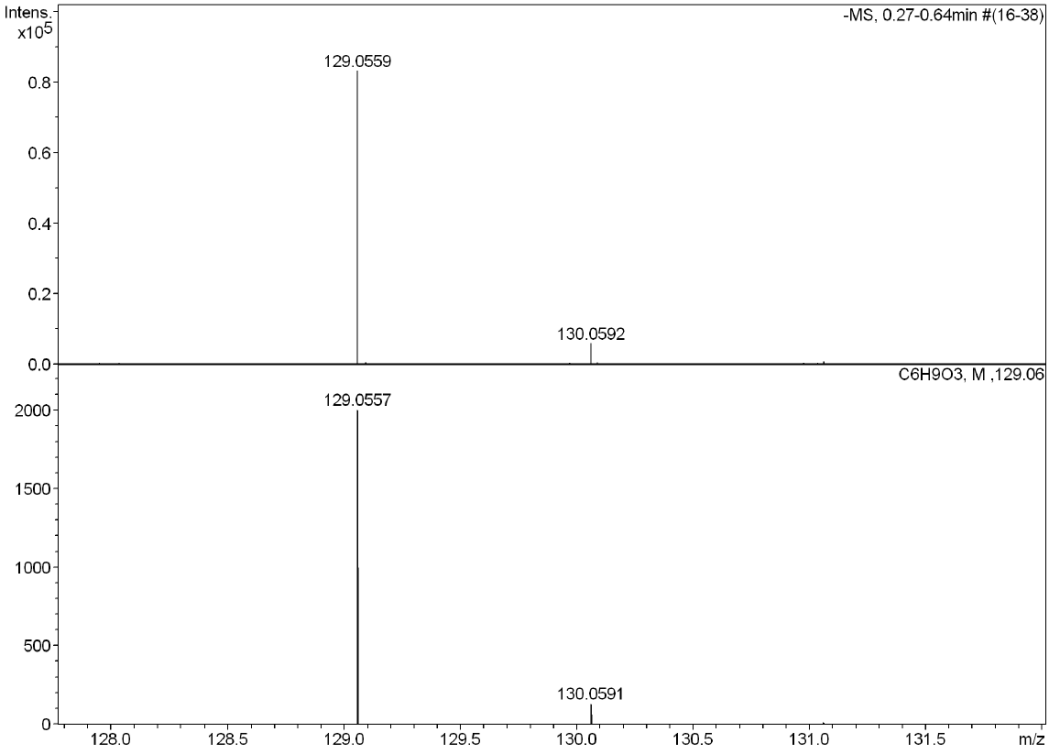
Supplementary Figure 17. Measured and calculated HR-ESI-MS of *N*,*N*′-Dimethyl-1,4-butanediamine (**11**)



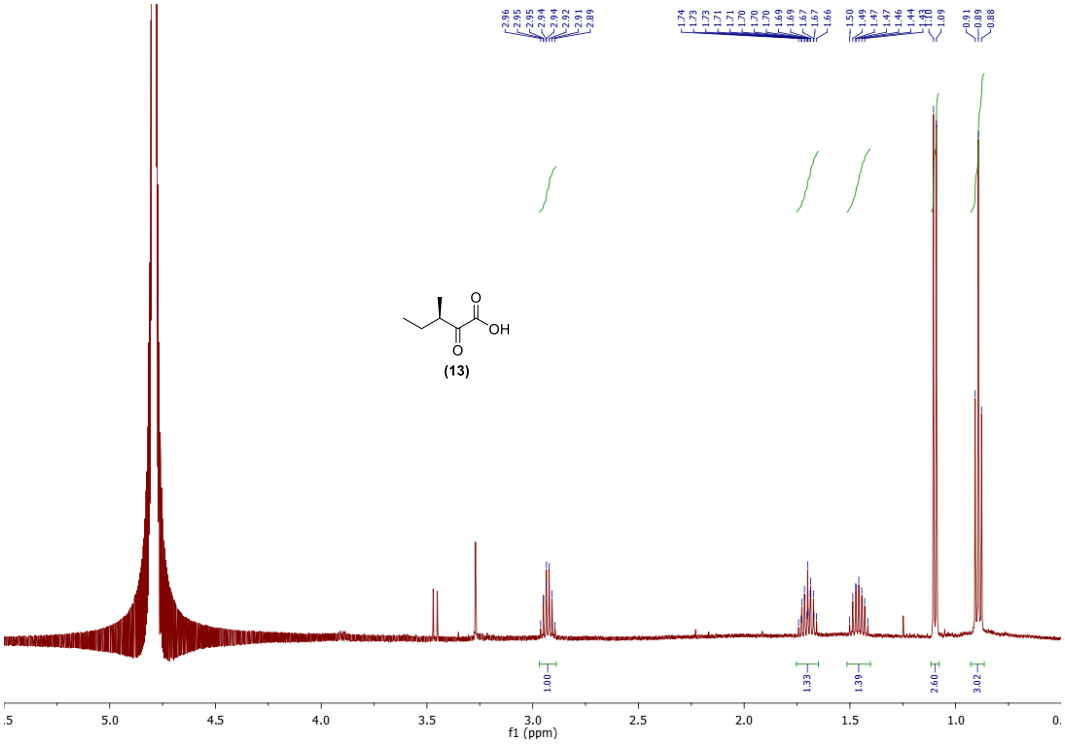
Supplementary Figure 18. ^{1}H NMR spectrum of *N*,*N*′-Dimethyl-1,4-butanediamine (**11**)



Supplementary Figure 19: ^{1}H NMR of HMT_SgvM cascade reaction. Spectrum of reaction mixture was shown in a). No Signal of substrate (b) was observed in reaction mixture. Therefore, the conversion was estimated to be over 95%.



Supplementary Figure 20. Measured and calculated HR-ESI-MS of *R*-3-methyl-2-oxovalerate (**13**)

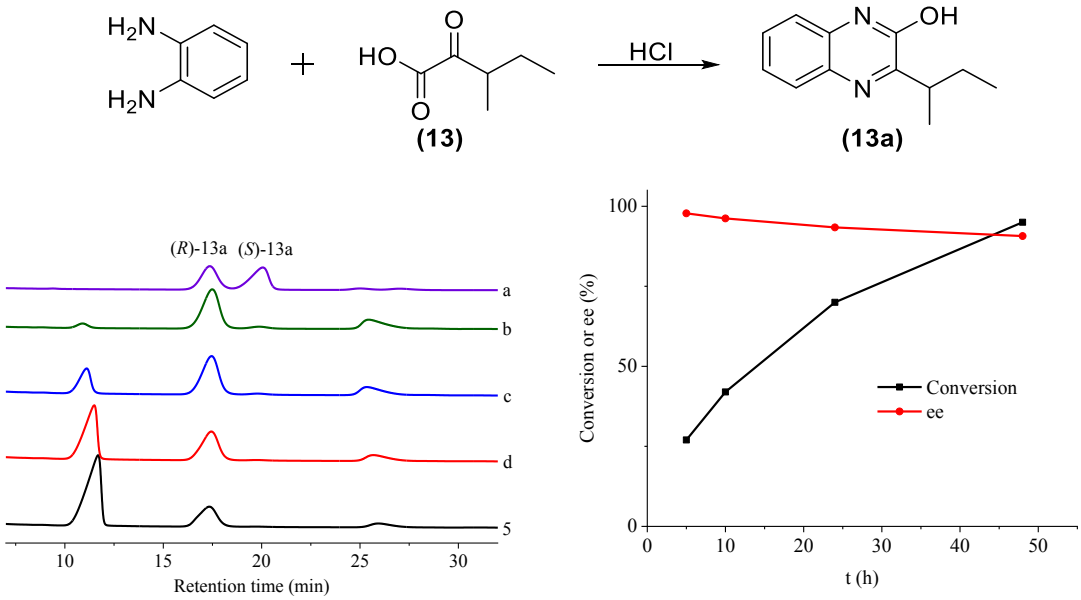


Supplementary Figure 21. ^{1}H NMR spectrum of *R*-3-methyl-2-oxovalerate (**13**)

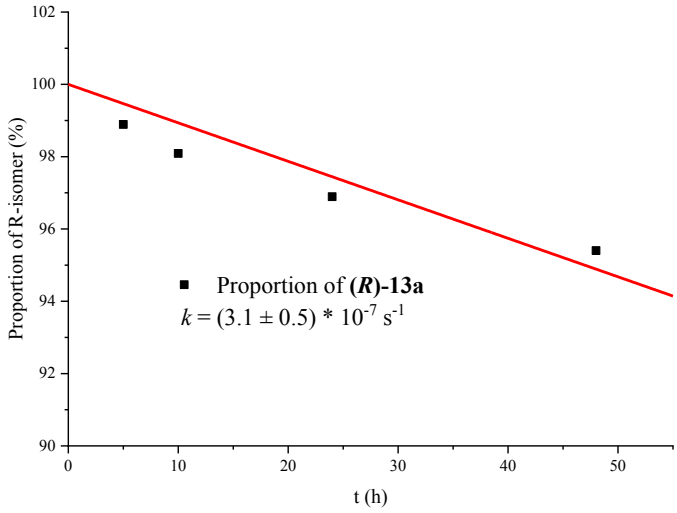
Conversion of HMT_SgvM and enantiomeric excess of product

A 5 ml reaction containing 100 mM sodium phosphate buffer (pH 8.0), 2 mM 2-oxovaleric acid, 20 μM SAH, 4 mM methyl iodide, 10 μM HMT and 20 μM SgvM was incubated at 25 °C. Reaction was analyzed after 5, 10, 24 and 48 hours. Derivatization of acid was following procedure from literature. 500 µL of reaction mixture was mixed with 250 µL o-phenylenediamine in HCl (10 mg/ml in 1 M HCl) and incubated at 35 °C for 30 minutes (Supplementary Figure 17 Top). The sample was lyophilized after neutralized by NaOH. After lyophilization, 300 µL 2-propanol was added and sonicated for 10 minutes. Insoluble solid was filtered before the sample was submitted to Chiral HPLC analysis. HPLC-UV analysis was performed on Agilent 1100 system with CHIRAL-AGP column from Chrom Tech, Inc. Compounds were eluted by a mobile phase of 4% 2-propanol in 10 mM phosphate buffer (pH 7.0) with a flow rate of 0.8 ml/min for 40 minutes.

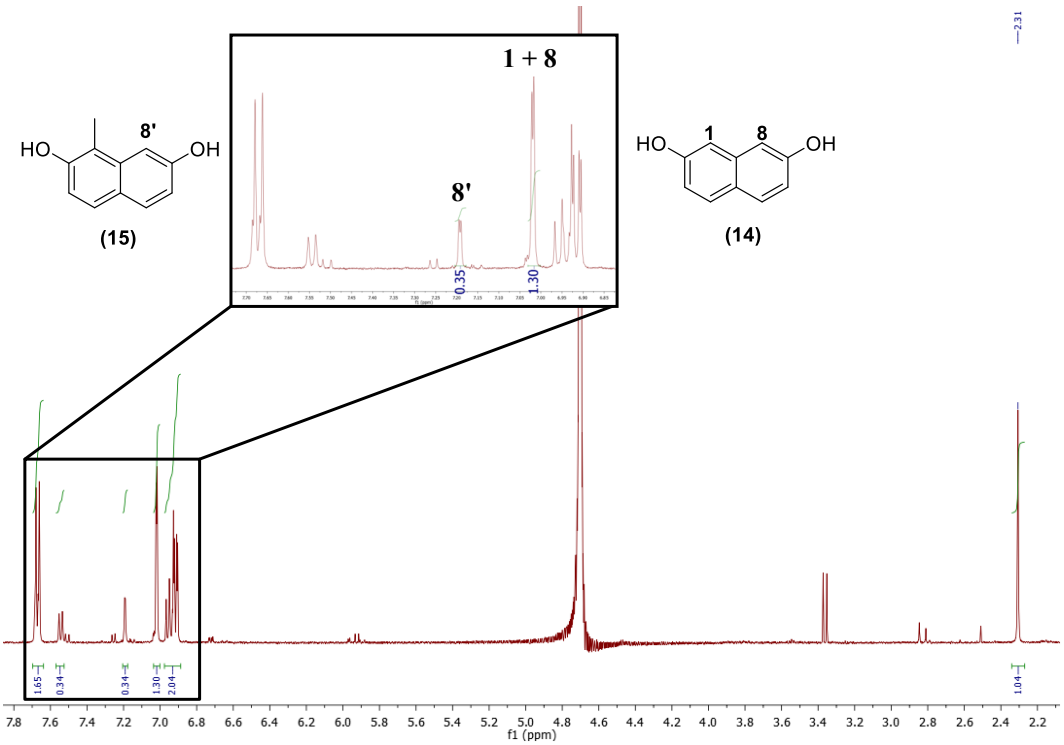
Firstly, authentic samples were separated after optimization of HPLC method and gave two pairs of signals. This might owe to side reaction during derivatization. Reaction samples were analyzed after 5, 10, 24 and 48 hours. One enantiomer of each pair was produced by enzymatic reaction (Supplementary Figure 17, Bottom feft). Conversion and enantiomeric excess (*ee*) values were calculated and shown Supplementary Figure 17 (Bottom right). Conversion of the enzymatic was determined to be 27, 42, 70 and 95%. enantiomeric excess (*ee*) value was calculated based on the ratio of (*R*)-**13a** and (*S*)-**13a** and determined to be 97.8, 96.2, 93.8 and 90.8% after 5, 10, 24 and 48 hours respectively. *ee* value was decreasing because product racemized after it was formed. Structure of the other minor derivatization product was unknown



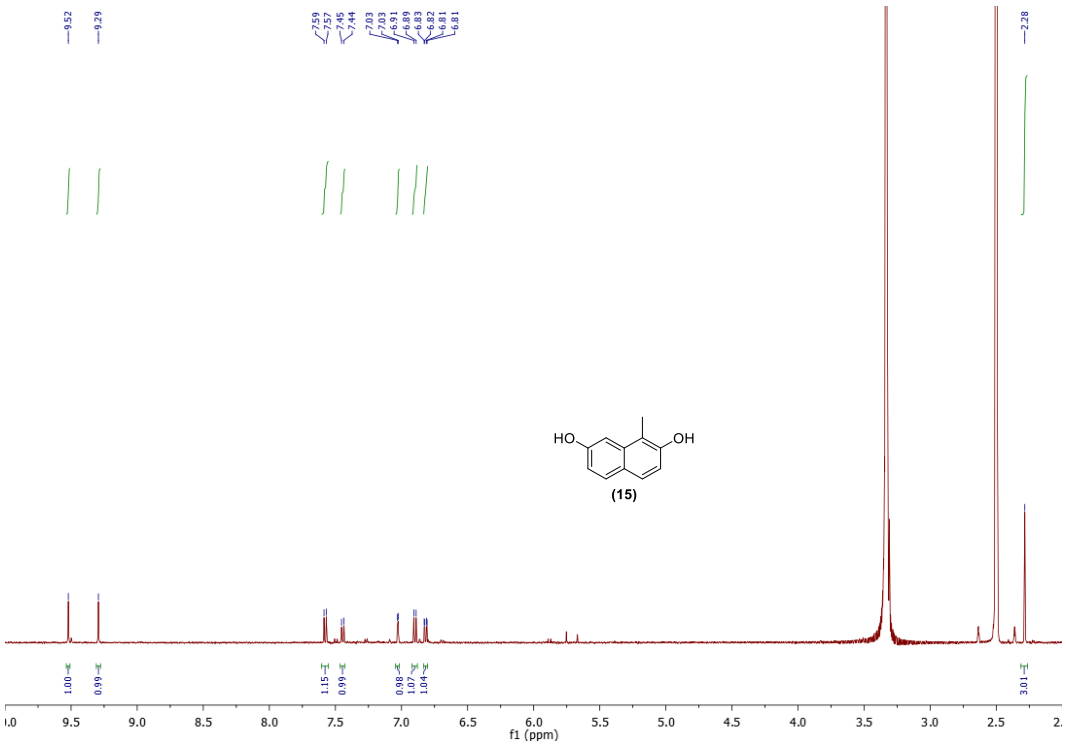
Supplementary Figure 22. Derivatization of keto acid with o-phenylenediamine. Top: reaction scheme. Bottom left: HPLC-UV of HMT-SgvM enzymatic reaction after derivatization. a) derivatized authentic enantiomers, b) reaction after 48 hours, c) reaction after 24 hours, d) reaction after 10 hours, e) reaction after 5 hours. Bottom right: Time dependent curve of conversion and enantiomeric excess of HMT-SgvM enzymatic reaction.



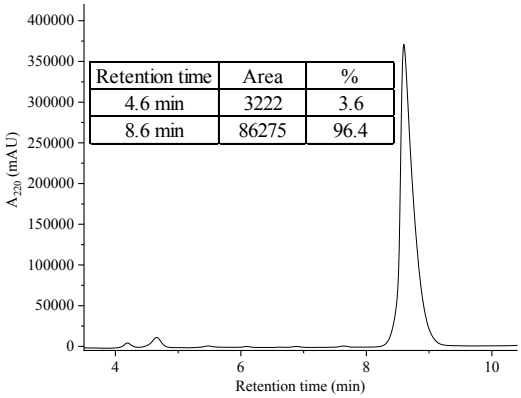
Supplementary Figure 23. Racemization rate of SgvM product. Reaction was run as described above (Supplementary Figure 22). Since only 5% of **(*R*)-13a** was consumed after 48 hours, we considered the racemization as first order reaction. Proportion of the starting isomer was fitted to a linear curve to obtain a first order reaction constant. Reaction constant was estimated to be (3.1 ± 0.5) * 10^{-7} s^{-1}. The given standard deviations represents the uncertainty of the linear fit.



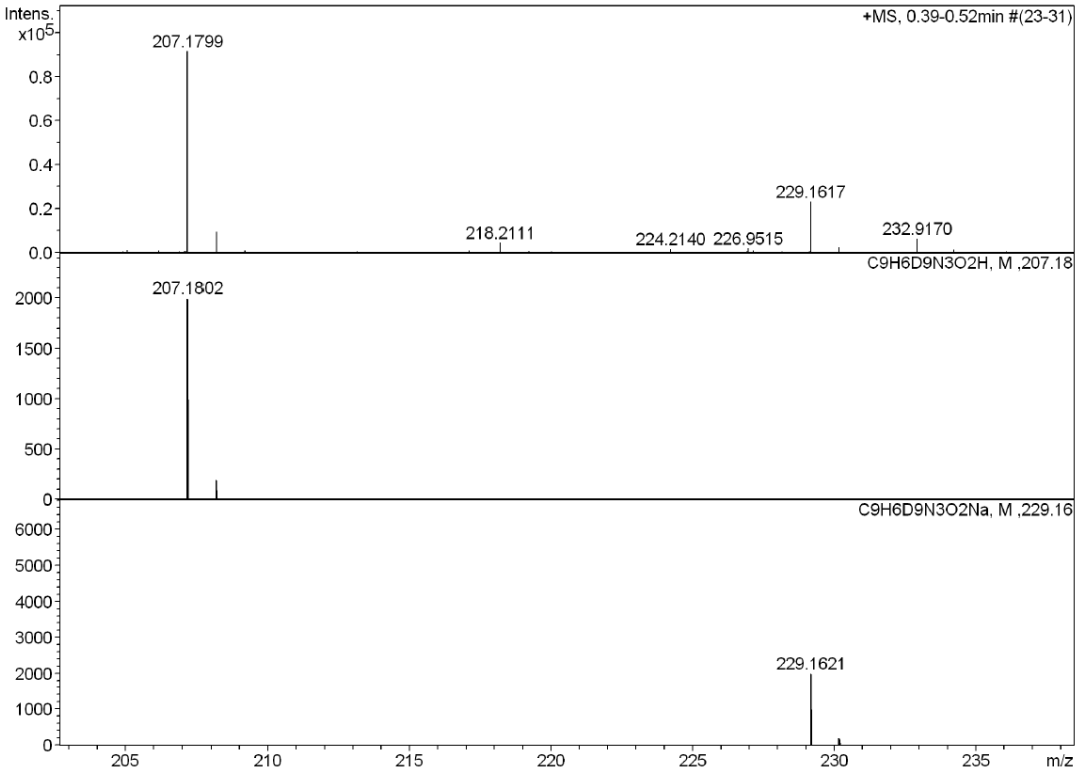
Supplementary Figure 24. ^{1}H NMR of HMT_NovO cascade reaction. Conversion was calculated to be 35% from integrals of reaction mixture by following: C = 8'/[(1 + 8)*0.5 + 8')].



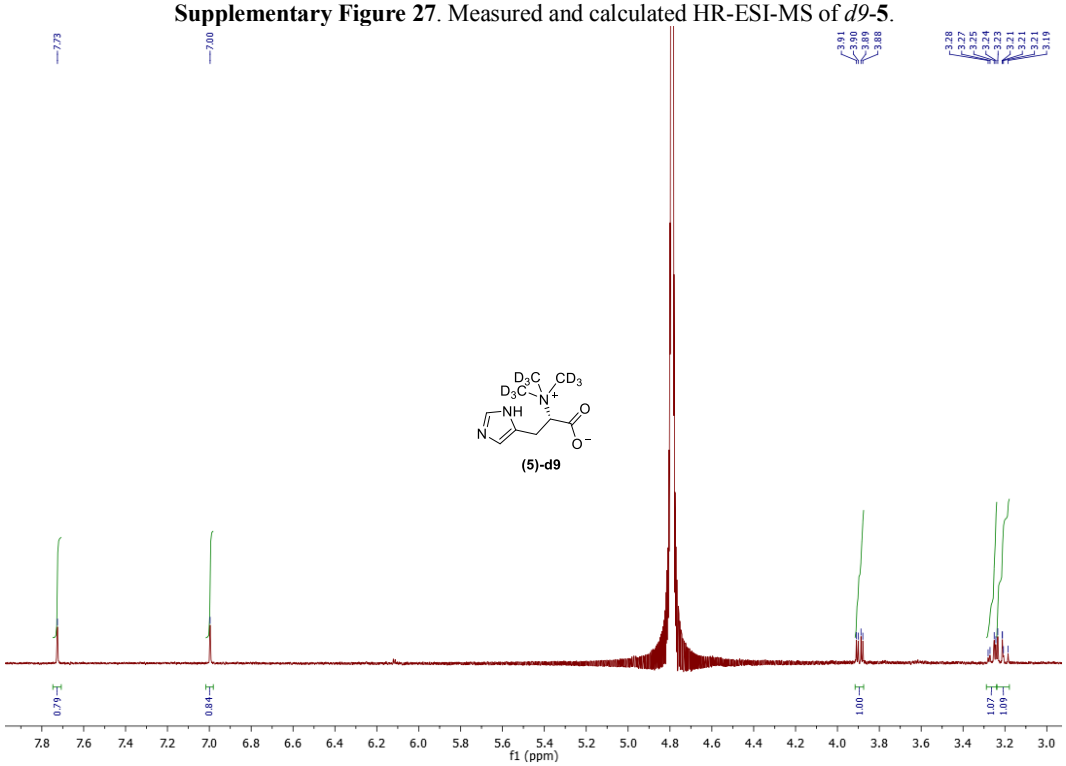
Supplementary Figure 25. ^{1}H NMR spectrum of 2,7-dihydroxy-1-methylnaphthalene (**15**)



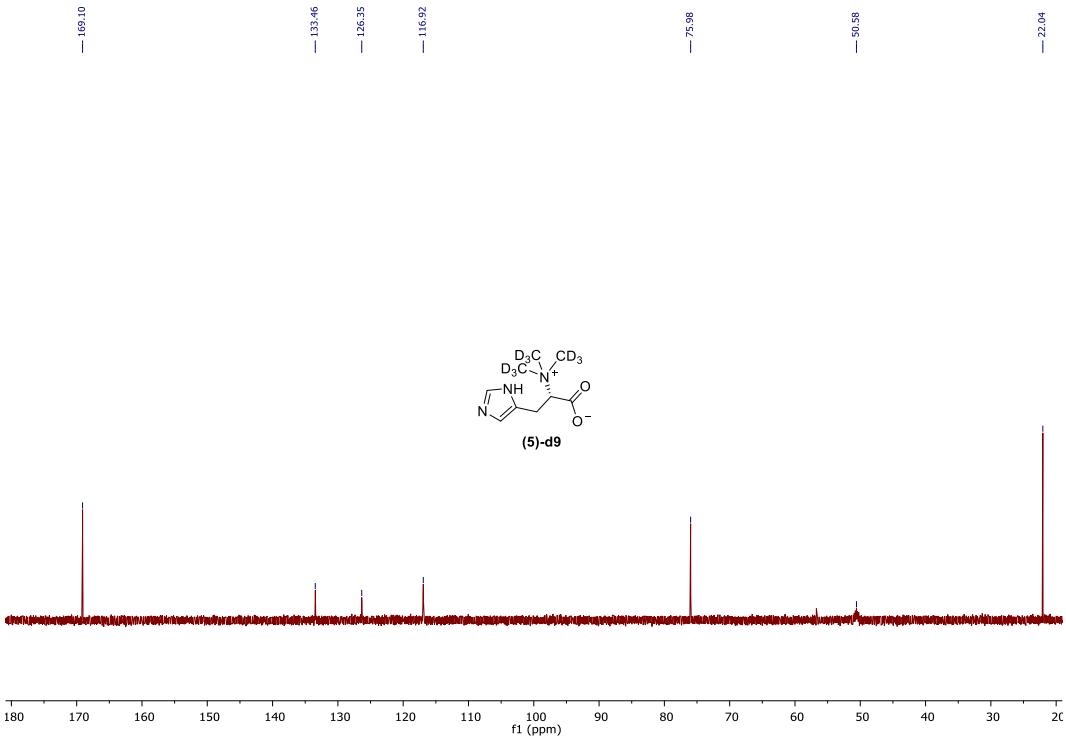
Supplementary Figure 26 Conversion of HMT_EgtD cascade accepting Deuterated methyl iodide measured by HPLC. Area of both substrate (4.6 min) and product (8.6 min) were shown.



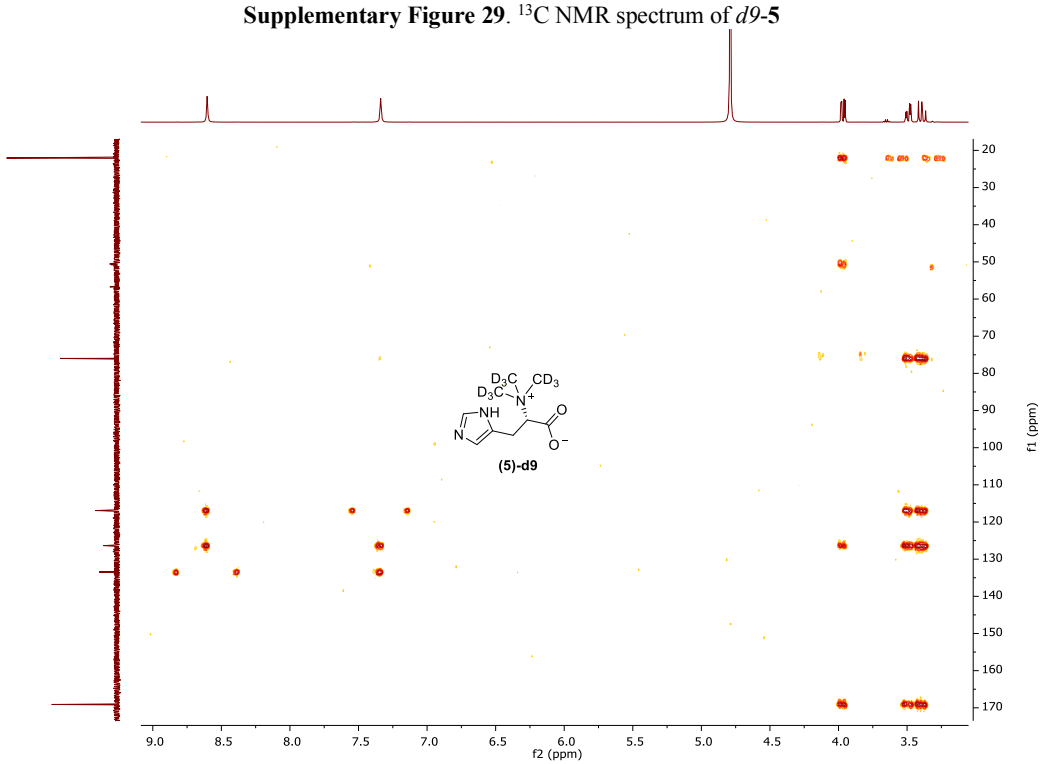
Supplementary Figure 27. Measured and calculated HR-ESI-MS of *d9*-**5**.



Supplementary Figure 28. ^{1}H NMR spectrum of *d9*-**5**



Supplementary Figure 29. ^{13}C NMR spectrum of *d9*-**5**



Supplementary Figure 30. 2D HMBC spectrum of *d9*-**5**

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