Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Comparative *S*-adenosyl-L-methionine analogue generation for selective biocatalytic Friedel-Crafts alkylation

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Author Contributions

S.C.H., A.R and N.C. conceived the project. A.H., K.H.S. and N.C. designed and performed experiments. All authors discussed the results. S.C.H., A.R and N.C. wrote the paper.

This PDF file includes:

I. Materials and Methods

Chemicals and enzymes

All chemicals and solvents were purchased from commercial suppliers (Sigma-Aldrich, Merck KGaA, TCI, abcr, Fluorochem, Honeywell Riedel-de-Haën, VWR, Carl Roth, Acros Organics B.V.B.A., Chiramer, Inc., and CHEMSOLUTE) and used without further purification. Pierce™ Universal Nuclease for Cell Lysis was purchased from Thermo Fisher Scientific Inc., and lysozyme was purchased from Carl Roth® (cat-#: 8259.2).

High-pressure liquid chromatography (HPLC)

HPLC Method A

High-pressure liquid chromatography (HPLC)-based analysis and quantifications of SAH, SAM, and SAM analogues were conducted with an Agilent 1290 Infinity II series instrument equipped with a diode-array detector (DAD). The SAM analogue analysis was performed at a wavelength of 260 nm using a SUPELCOSIL™ LC-18-T HPLC column (150 × 4.6 mm, 3 µM). To separate the charged SAM and SAM analogues, an ion-pairing reversed-phase (IP RP) HPLC method was used.[1] Therefore, as mobile phase (A) 5 mM sodium phosphate containing 1 mM 1-heptansulfonate as ion-pairing reagent and (B) pure acetonitrile was used with a flow rate of 1 mL/min (1 µL injection volume, 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.

HPLC Method B

HPLC analysis was performed on an Agilent 1260 Infinity HPLC equipped with a diode array detector (DAD) using Nucleodur® C_{18} Pyramid reversed-phase column (5 µm, 125 x 4 mm) from Macherey-Nagel with a flow rate of 1 mL/min at 20 °C. Buffer A: 100 mM K_2HPO_4/KH_2PO_4 pH = 6.5; buffer B: 1:1 of buffer A and MeCN. Gradient: 0 min: 100 % A, 0 % B; 5 min: 100 % A, 0 % B; 18 min: 40 % A, 60 % B; 20 min: 0 % A, 100 % B; 25 min: 0 % A, 100 % B; 28 min: 100 % A, 0 % B; 33 min: 100 % A, 0 % B.

HPLC Method C

HPLC analysis was performed on an Agilent 1260 Infinity HPLC equipped with a diode array detector (DAD) using Nucleodur® C_{18} Pyramid reversed-phase column (5 µm, 125 x 4 mm) from Macherey-Nagel with a flow rate of 1 mL/min at 20 °C. Buffer A: 100 mM K_2HPO_4/KH_2PO_4 pH = 6.5; buffer B: 1:1 of buffer A and MeCN. Gradient: 0 min: 100 % A, 0 % B; 5 min: 100 %

A, 0 % B; 20 min: 0 % A, 100 % B; 25 min: 0 % A, 100 % B; 28 min: 100 % A, 0 % B; 33 min: 100 % A, 0 % B.

LC-TOF-MS

LC-TOF-MS Method A

High-resolution mass spectrometry (HRMS) was performed using an Agilent 6220 Accurate-Mass TOF system consisting of a dual ESI source and coupled with an Agilent 1200 series HPLC instrument equipped with a SeQuant® ZIC®-pHILIC 5 µm polymer column (50 × 4.6 mm). Mass data were collected in the range from 100 m/z to 1500 m/z, and the dual-ESI source was operated at a spray voltage of 3.5 kV in positive mode. 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 (1 µL injection volume, 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.

LC-TOF-MS Method B

LC-TOF-MS analysis was performed on a Bruker maXis II ultra-high resolution QTOF coupled to a Thermo Scientific UltiMate 3000® UHPLC. Runs are referenced to high mass standard m/z = 1222.0000 (Agilent, part: G1982-85001). Nucleodur® C_{18} Pyramid reversed-phase column (5 µm, 125 x 4 mm) from Macherey-Nagel was used with a flow rate of 0.6 mL/min at 20 °C. Buffer A: 7 mM NH_4HCO_2 and 12 mM HCOOH, pH = 3.5; buffer B: MeOH. Gradient: 0 min: 100 % A, 0 % B; 5 min: 100 % A, 0 % B; 65 min: 0 % A, 100 % B; 70 min: 100 %A, 0 % B; 75 min: 100 % A, 0 % B; 80 min: 0 % A, 100 % B; 85 min: 100 % A, 0 % B; 90 min: 100 % A, 0 % B.

II. General Procedures

Cloning

Aspergillus clavatus Halide methyltransferase (AclHMT) was already cloned and reported in our previous works.[1-2] The gene encoding the AclHMT was inserted into the pBAD33 vector containing chloramphenicol resistance, transformed by electroporation, and expressed in a SAHN knockout *E. coli* strain JW0155 (Keio collection).

Methanocaldococcus jannaschii wildtype methionine adenosyltransferase WT-MjMAT was obtained from Prof. Jennifer Andexer (University of Freiburg). The variant MjMAT L147A/I351 (PC-MjMAT) was generated by side directed mutagenesis as previously reported.[3]

Expression of AclHMT

The AclHMT was expressed in batches of 500 mL terrific broth (TB) media (12 g/L tryptone, 24 g/L yeast extract, 5 g/L glycerol, 89 mM KPi pH 7.4) containing 34 µg/mL chloramphenicol. Therefore, 5 mL lysogenic broth (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl, 34 µg/mL chloramphenicol) precultures were inoculated from glycerol stocks and allowed to grow over night at 37°C and 180 rpm. The whole preculture was used to inoculate the 500 mL TB media and allowed to grow to an OD_{600} between 0.6-0.8 at 37°C at 100 rpm before the cultures were cooled down in an ice bath for 10 min. Protein expression was induced through the addition of arabinose (0.02 w/v%) and was performed over 20 h at 25°C. The cells were harvested through centrifugation (15 min, 4°C, 4,300 rcf), and the residual cell paste was stored at -20°C.

Expression of PCMjMAT

For PC-MjMAT (MjMAT L147A/I351A) production, transformed *E. coli* BL21 (DE3) cells were cultivated in LB medium with kanamycin at 37 °C to an OD_{600} of 0.6. Expression was induced with 0.5 mM IPTG at 18 °C for 16 h. The cells were harvested through centrifugation, and the pellet was stored at -80 °C until purification.

Expression of NovO

For NovO production, transformed *E. coli* BL21 (DE3) cells were cultivated in LB medium with kanamycin at 37 °C to an OD_{600} of 0.6. Expression was induced with 0.5 mM IPTG at 18 °C for 16 h. The cells were harvested through centrifugation, and the pellet was stored at -80 °C until purification.

Expression of MTAN

For MTAN production, transformed *E. coli* BL21 (DE3) cells were cultivated in LB medium with ampicillin at 37 °C to an OD_{600} of 0.6. Expression was induced with 0.2 mM IPTG at 18 °C for 16 h. The cells were harvested through centrifugation, and the pellet was stored at -80 °C until purification.

AclHMT purification and storage

Frozen cell paste pellets were thawed and resuspended on ice with pre-cooled 3 mL lysis buffer (50 mM KPi, 500 mM NaCl, 10 mM imidazole, 5 v/v% glycerol, pH 7.5) per 1 g of cell paste. The resuspended cell suspension was sonicated (Bandelin Sonoplus HD 2070.2, probe: MS 73) three times on ice, each time at 50% amplitude with 5 sec on / 5 sec off for 5 min. Insoluble cell debris was separated from the soluble biocatalyst-containing fraction through centrifugation. The cleared soluble fraction was filtered (0.2 µM sterile filters) and applied to a preconditioned HisTrap™ HP 5 mL column loaded with Ni^{2+} and eluted through an imidazole gradient (20 mM, 40 mM, 70 mM and 100 mM). SDS Page analysis identified protein-containing fractions and verified the purity of the protein. Fractions containing pure protein were pooled, dialysed 2 times 1:100 against 50 mM KPi buffer (5 v/v% glycerol, pH 7.5), and concentrated through ultrafiltration (Amicon® Ultra, 10 kDa Cut-off). The resulting protein concentration was determined through UV absorbance at 280 nm and verified through PierceTM BCA Protein Assay Kit (Thermo Scientific, US) before being aliquoted and stored at -80°C until further usage.

PCMjMAT purification and storage

PC-MjMAT (MjMAT L147A/I351A) was purified by heat purification (incubation at 80 °C for 20 min) and immobilized metal affinity chromatography (IMAC) using the ÄKTApurifier system. The protein was concentrated, and aliquots were flash-frozen in liquid nitrogen and stored at -80 °C.

IMAC binding buffer	50 mM Tris-HCl pH = 8, 300 mM NaCl
IMAC elution buffer	50 mM Tris-HCl pH = 8, 300 mM NaCl, 500 mM imidazole
Storage buffer	25 mM Tris-HCl pH = 8, 80 mM KCl, 10 % glycerol

NovO purification and storage

NovO was purified by immobilized metal affinity chromatography (IMAC) using the ÄKTApurifier system. The protein was concentrated, and aliquots were flash-frozen in liquid nitrogen and stored at -80 °C.

IMAC binding buffer	50 mM Tris-HCl pH = 8, 300 mM NaCl
IMAC elution buffer	50 mM Tris-HCl pH = 8, 300 mM NaCl, 500 mM imidazole
Storage buffer	50 mM Tris-HCl pH = 8, 200 mM NaCl, 10% glycerol

MTAN purification and storage

MTAN was purified by immobilized metal affinity chromatography (IMAC) using the ÄKTApurifier system. The protein was concentrated, and aliquots were flash-frozen in liquid nitrogen and stored at -80 °C.

IMAC binding buffer	50 mM sodium phosphate buffer pH = 7.5
IMAC elution buffer	50 mM sodium phosphate buffer pH = 7.5, 250 mM imidazole
Storage buffer	50 mM sodium phosphate buffer pH = 7.5, 50 mM Hepes and 10% glycerol

Biotransformations for the SAM analogue synthesis with AclHMT

AclHMT-catalysed reactions (500 µL) for the SAM analogue synthesis from SAH and haloalkanes were performed as technical triplicates. Therefore, 1 mM SAH (12.5 µL, 40 mM DMSO stock) was mixed with 10 mol% purified AclHMT (100 µM) in 50 mM HEPES buffer (pH 7.4) before 2.5 eq of the corresponding haloalkane was added, resulting in overall 5 v/v% DMSO. Reactions were mixed by gently shaking until homogeneity and incubated for 2 h at 37°C with 450 rpm on a bench top shaker. Reactions were stopped through the addition of 500 µL acetonitrile and vortexing for 10 sec before incubating at RT for 30 min to precipitate the protein. The final samples were obtained after separating the denatured protein through centrifugation for 10 min with >21.000 rcf. HPLC analysis (HPLC Method A) was carried out, and obtained areas were quantified using standard curves of SAH and SAM to calculate the

conversion of 1 mM SAH and the recovery rate. BSA control experiments and buffer control experiments were performed in the same way to monitor the background reactivity.

Biotransformations for the THPC alkylation cascade with NovO and AclHMT

Cascade reaction with NovO and AclHMT were carried out as triplicates of 50 µL reactions. For these reactions, HEPES Buffer, AclHMT, NovO and 4,5,7-trihydroxy-3-phenylcoumarin (THPC) were mixed together and the reaction was started by the addition of alkyl halide. The resulting reaction mixture consists of 1 mM THPC (25 mM DMSO stock), 10 mol% AclHMT (100 µM), 5 mol% NovO and 2.5 mM alkyl halide (2.5 eq., 250 mM DMSO stock) leading to a final DMSO concentration of 5 v/v% DMSO. Buffer control reaction without enzymes were carried out and treated the same way. Cascade and control reactions were incubated for 2 h at 37°C and stopped by the addition of 150 µl acetonitrile (3 x reaction volume) and mixing with a vortex mixer. Precipitated protein was separated by centrifugation (10 min, 4°C, 20,000 rcf). For HPLC analysis, 30 µL of the cleared supernatant was transferred to a glass vial with inlet and HPLC analysis according to HPLC Method C was performed. The obtained areas were compared as area%.

PC-MjMAT reactions

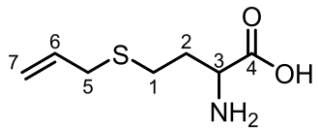
For the MAT assay, methionine analogues **3a**-**e** (5 mM) were incubated with 5 mM ATP and 100 µM PC-MjMAT for 1 h at 37 °C or 65 °C. The reaction buffer consisted of 50 mM HEPES, 10 mM MgCl2, 5 mM KCl (pH = 7.4). The reaction was stopped by adding 10 % (v/v) of 1 M $HClO_4$. The 0 h samples were stopped by the immediate addition of $HClO_4$. After centrifugation (10 min at 21,000 x g), the supernatant was injected into the HPLC (HPLC method B).

Biotransformations for the THPC alkylation cascade with NovO and PC-MjMAT

The PC-MjMAT/NovO cascade reactions were performed with 100 µM PC-MjMAT, 10 µM MTAN, 50 µM of NovO and 1 mM **6** (THPC). Together with the respective Methionine analogues **3a**-**e** (5 mM) the reaction mixture were incubated for 2 h at 37 °C in a total volume of 20 µL. The reaction buffer consisted of 50 mM HEPES, 10 mM $MgCl_2$, 5 mM KCl (pH = 7.4). The reactions were stopped by the addition of three volumes of acetonitrile (MeCN). All samples were centrifuged at 21,000 x g for 10 min and injected directly into the HPLC. (HPLC method C).

III. Synthetic procedures

Synthesis of allyl-D,L-homocysteine (3c)



D,L-homocysteine thiolactone hydrochloride (691 mg, 4.5 mmol, 1.5 eq.) was dissolved under argon in 10 mL of freshly prepared NaOH (5 M, ddH_2O, degassed). The obtained mixture was stirred for 10 min, and the cleavage of thiolactone was monitored by thin layer chromatography (TLC). Sodium bicarbonate (420 mg, 5 mmol, 1.7 eq.) was added to the solution and acidified to a pH = 9 using conc. HCl.

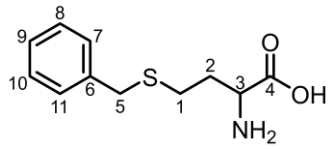
After the addition of 1,4-dioxane (3 mL), allyl bromide (260 µL, 3 mmol, 1.0 eq) was slowly added under argon to the amino acid solution. The reaction mixture was stirred at room temperature for 4h and then acidified to a pH = 6 with 1 M HCl. The crude product was dissolved in 10 mL DMSO and separated via flash chromatography (column: HP-C18-Aq. 120 g, gradient: 100 % A: NH4Ac 50 mM to 20 % B: ACN). The product was obtained as a white powder (218 mg, 1.24 mmol, 42 %).

HRMS: Calculated for $C_6H_{11}NO_2SNa^+$ $[M+Na]^+$ = 198.05592 Da, found 198.05592 m/z.

^{1}H NMR (600 MHz, D2O + 1 % NaOD) δ (ppm) = 5.79 (ddt, *J* = 17.2, 10.0, 7.3 Hz, 1H, H-C(6)); 5.14 (dq, *J* = 17.0, 1.4 Hz, 1H, H-C(7)); 5.11 (dd, *J* = 10.1, 1.7 Hz, 1H, H-C(7)); 3.26 (dd, *J* = 7.4, 5.5 Hz, 1H, C-H(3)); 3.16 (dt, *J* = 7.3, 1.1 Hz, 2H, C-H(5)); 2.55 – 2.44 (m, 2H, H-C(1)); 1.85 (m, *J* = 14.4, 9.1, 7.1, 5.5 Hz, 1H, H-(C2)), 1.78 – 1.69 (m, 1H, H-(C2)).

^{13}C-{^{1}H}-NMR (151 MHz, D2O + 1 % NaOD) δ (ppm) = 85.29 (C(4)); 136.58 (C(6)); 120.12 (C(7)); 57.87 (C(3)); 36.04 (C(5)); 30.78 (C(2)); 28.99 (C(1))

Synthesis of benzyl-D,L-homocysteine (3d)



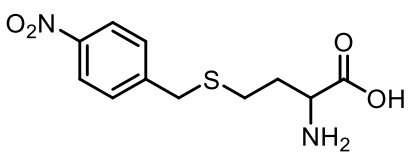
D,L-homocysteine thiolactone hydrochloride (691 mg, 4.5 mmol, 1.5 eq.) was dissolved under argon in 10 mL of freshly prepared NaOH (5 M, ddH_2O, degassed). The obtained mixture was stirred for 10 min, and the cleavage of thiolactone was monitored by thin layer chromatography (TLC). Sodium bicarbonate (420 mg, 5 mmol, 1.7 eq.) was added to the solution and acidified to a pH = 9 using conc. HCl. After the addition of 1,4-dioxane (3 mL), benzyl bromide (357 µL, 3 mmol, 1.0 eq) was slowly added under argon to the amino acid solution. The reaction mixture was stirred at room temperature for 2h and then acidified to a pH = 6 with 1 M HCl. The crude product was dissolved in 10 mL DMSO and separated via flash chromatography (column: HP-C18-Aq. 120 g, gradient: 100 % A: NH4Ac 50 mM to 100 % B: ACN). The product was obtained as a white powder (270.3 mg, 1.2 mmol, 40 %).

HRMS: Calculated for $C_{11}H_{15}NO_2SNa^+$ $[M+Na]^+$ = 248.071518Da, found 248.07159 m/z.

^{1}H NMR (600 MHz, D2O + 1 % NaOD) δ (ppm) = 7.40 – 7.32 (m, 4H, H-C(ar)); 7.32 – 7.26 (m, 1H, H-C(ar)); 3.74 (s, 2H, H-C(5); 3.23 (dd, *J* = 7.5, 5.4 Hz, 1H, H-(C3)); 2.51 – 2.39 (m, 2H, H-C(1)); 1.86 (m, *J* = 14.2, 9.1, 6.9, 5.3 Hz, 1H, H-C(2)); 1.72 (m, *J* = 13.5, 9.0, 7.5, 6.1 Hz, 1H, H-C(2)).

^{13}C-{^{1}H}-NMR (151 MHz, D2O + 1 % NaOD) δ (ppm) = 185.16 (C(4)); 141.09, (C(6)); 131.41 (C(7,8,10,11)); 129.77 (C(9)); 57.84 (C(3)); 37.54 (C(5)); 36.92 (C(2)); 29.72 (C(1)).

Synthesis of benzyl-D,L-homocysteine (3e)



Under argon D,L-homocysteine thiolactone hydrochloride (691 mg, 4.5 mmol, 1.5 eq.) was dissolved in 10 mL freshly prepared NaOH (ddH_2O, degassed, 5 M). The mixture was stirred for 10 min and the thiolactone cleavage was monitored by TLC. Sodium bicarbonate (420 mg, 5 mmol, 1,7 eq.) was added and the mixture was acidified to pH = 9 using conc. HCl.

A solution of 4-nitrobenzyl bromide in 1,4-dioxane (6 mL) was prepared under argon and slowly added to the amino acid solution. The reaction was stirred at room temperature over 4 h and the progress was monitored *via* HPLC. The crude mixture was acidified to pH = 6 using 1 M HCl. The residual solvent was evaporated under reduced pressure. The crude product was dissolved in 10 mL DMSO and separated via flash chromatography (column: HP-C18-Aq. 100 g, gradient: 100 % A: NH_4Ac 50 mM to 60 % B: ACN). The product was obtained as white powder (533.9 mg, 1.98 mmol, 66 %).

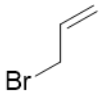
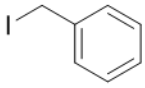
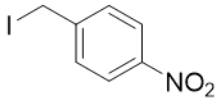
HRMS: Calculated for $C_{11}H_{13}N_2O_4S^-$ $[M-H]^-$ = 269.060124 Da, found 269.05998 m/z.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) = 8.17 (d, *J* = 8.7 Hz, 2H, H-C(7,11)); 7.60 (d, *J* = 8.7 Hz, 2H, H-C(8,10)); 3.85 (s, 2H,H-C(5)); 3.32 – 3.22 (m, 1H, H-C(3)); 2.57 – 2.41 (m, 2H, H-C(1)); 2.10 – 1.84 (m, 2H,H-C(2)).

^{13}C-{^{1}H}-NMR (101 MHz, DMSO-d_6) δ (ppm) = 172.90 (C(4); 170.36; 147.35 (C(9)); 146.30 (C(6)); 130.10 (C(7,11)); 123.57 (C(8,10)); 53.15 (C(3)); 34.02 (C(5)); 31.21 (C(2)); 27.19 (C(1)); 22.52 ppm.

IV. Supporting figures

Table S 1: Synthesis of SAM and SAM analogues by enzymatic alkylation of SAH with AclHMT. HPLC yields are calculated by HPLC quantification of the SAM analogues using 1 mM SAH as the basis. The limit of quantification corresponds to a yield of 0.1%. Conversions are given as the average of triplicates ± standard deviation of the three measurements. n.d. = not detectable

		Conversion as mean of triplicates ± SD [%]	Recovery as mean of triplicates ± SD [%]
Methyl iodide 	acl	99.5 ± 0.4	100.7 ± 0.4
	BSA	0.26 ± 0.1	98.5 ± 0.4
	Buffer	0.23 ± 0.02	98.3 ± 1.6
Ethyl iodide 	acl	98.9 ± 1.4	101.7 ± 2.1
	BSA	n. d.	100.4 ± 1.7
	Buffer	n. d.	99.7 ± 0.8
Ethyl bromide 	acl	63.3 ± 12.0	101.9 ± 0.6
	BSA	n. d.	98.6 ± 1.3
	Buffer	n. d.	98.6 ± 1.4
Allyl iodide 	acl	59.4 ± 0.7	80.2 ± 0.7
	BSA	3.3 ± 0.2	97.2 ± 1.7
	Buffer	3.6 ± 0.1	95.6 ± 0.5
Allyl bromide 	acl	78.5 ± 0.9	83.8 ± 0.9
	BSA	2.4 ± 0.1	98.0 ± 0.3
	Buffer	2.5 ± 0.1	97.6 ± 0.8
Benzyl iodide 	acl	28.6 ± 1.2	73.3 ± 0.3
	BSA	8.7 ± 0.3	88.5 ± 0.5
	Buffer	10.3 ± 0.4	88.1 ± 0.5
p-Nitrobenzyl iodide 	acl	5.7 ± 0.3	77.1 ± 1.6
	BSA	n. d.	97.8 ± 0.3
	Buffer	n. d.	98.0 ± 0.5

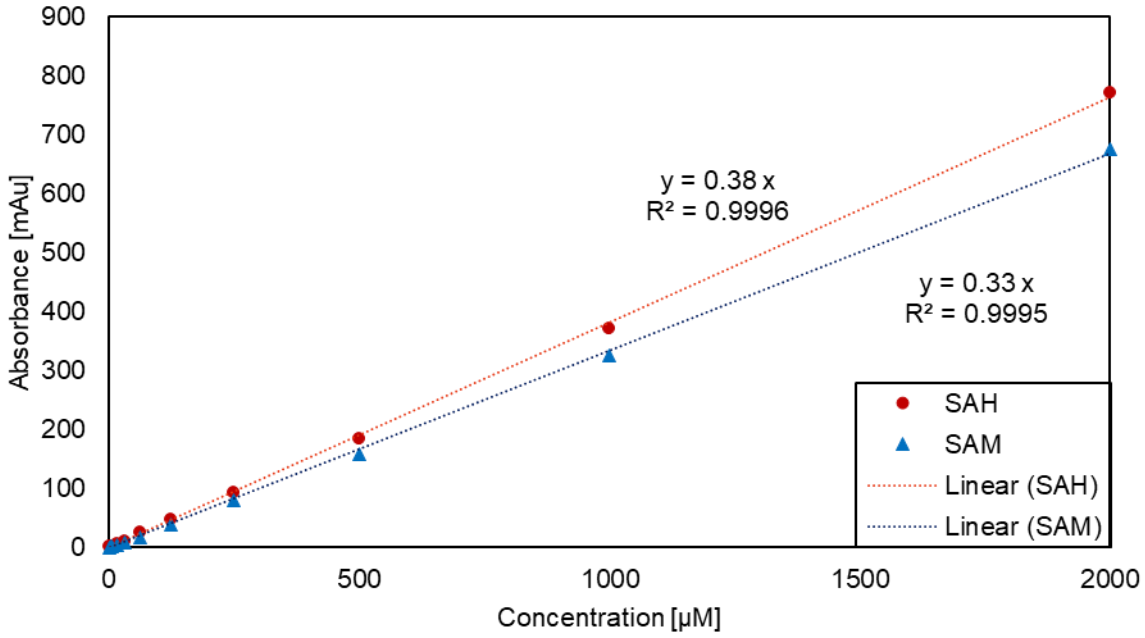


Figure S 1: The calibration curve of SAM and SAH were measured using external standards *via* HPLC-DAD analysis.

Formation of SAM analogues formed by the AclHMT catalysed alkylation of SAH with alkyl halides was confirmed by LC-MS measurements using ESI ionization (LC-TOF-MS Method A). The DAD traces were determined at 260 nm, and the mass spectra for the specific time interval is given with the structure and the theoretical calculated mass. In a previous study, we reported the LC-MS pattern for the formation of *S*-adenosyl-L-methionine, *S*-adenosyl-L-ethionine and allyl SAM analogue.[1a] Here, we add the characterization of the benzyl SAM analogue and para-nitrobenzyl SAM analogue formation.

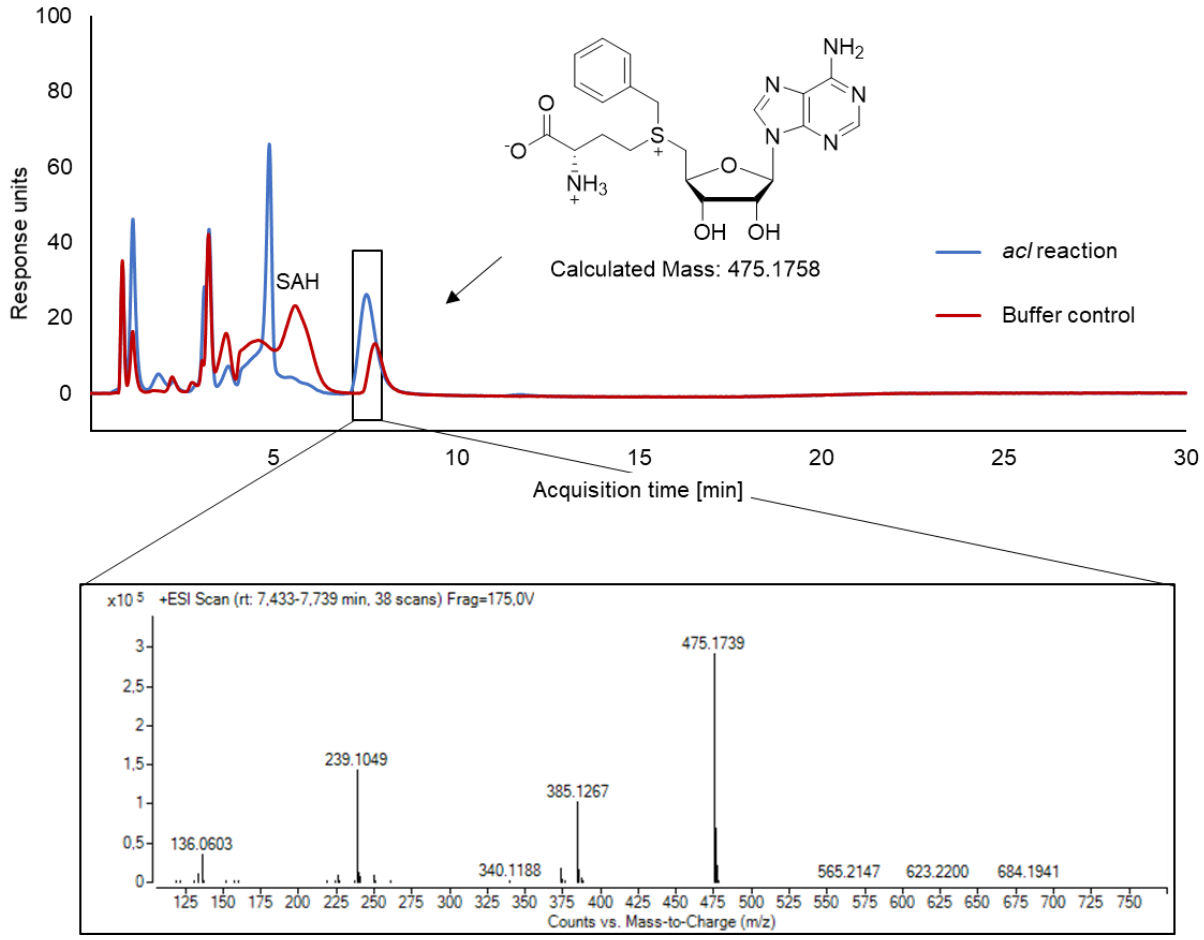


Figure S 2: Biotransformation with SAH, benzyl iodide and AclHMT were shaken for 2 h at 37°C. The given DAD traces show an overlay of the enzymatic reaction (blue) and buffer control w/o enzyme (red).

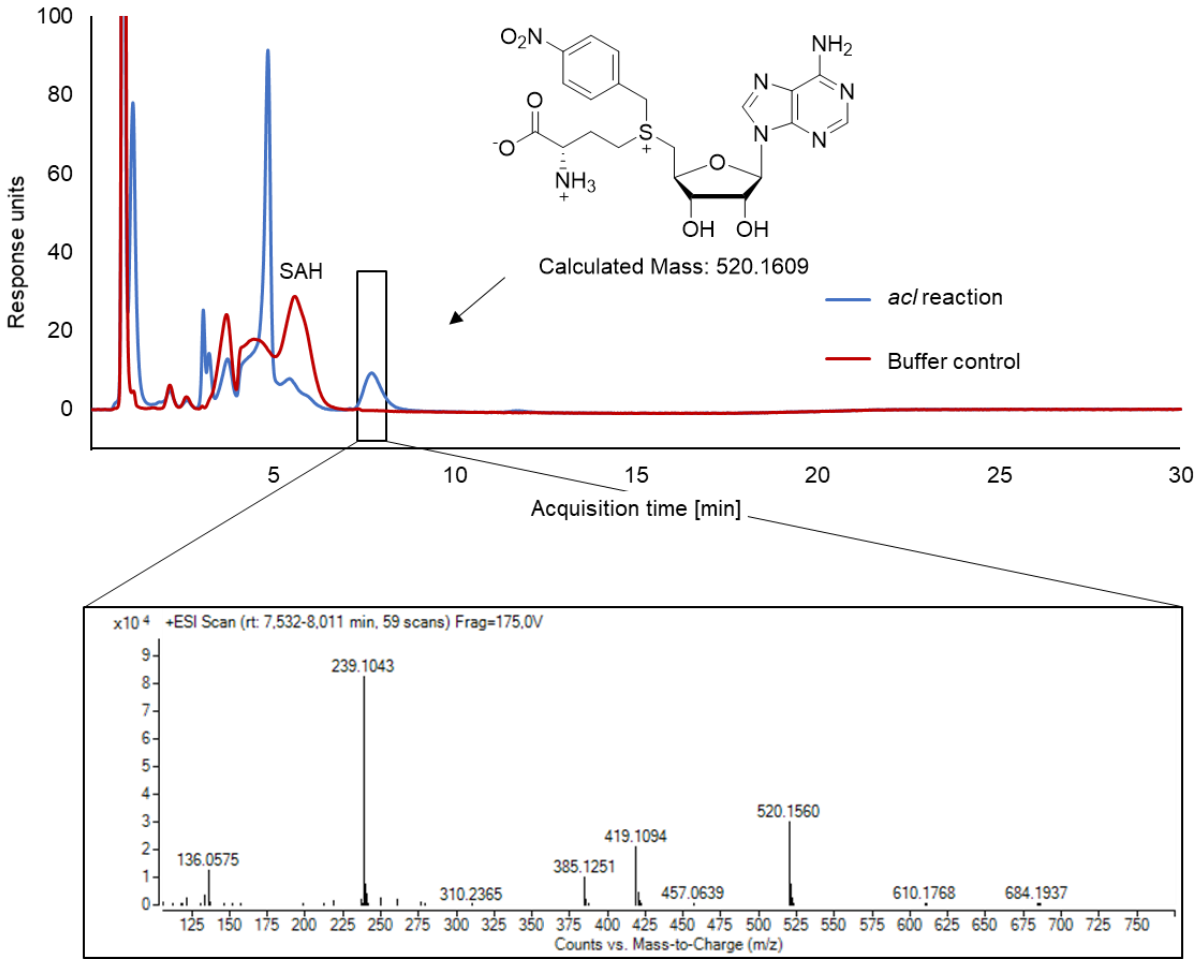


Figure S 3: Biotransformation with SAH, para-nitrobenzyl iodide and AclHMT were shaken for 2 h at 37 °C. The given DAD traces show an overlay of the enzymatic reaction (blue) and buffer control w/o enzyme (red).

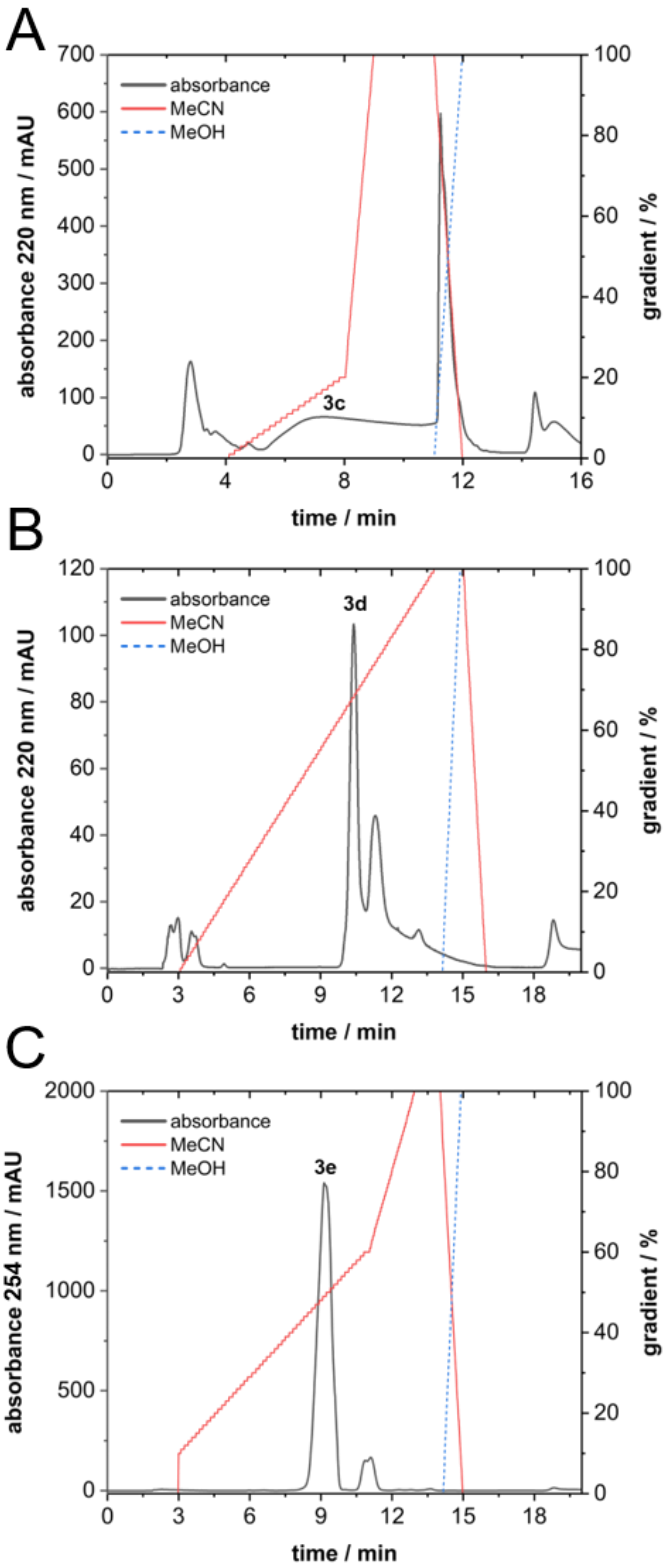


Figure S 4: Prep-HPLC-Purification of methionine analogues **3c**-**e** using BÜCHI C-700. A: allyl-D,L-homocysteine (**3c**), B: benzyl-D,L-homocysteine (**3d**), C: para-nitrobenzyl-D,L-homocysteine (**3e**).

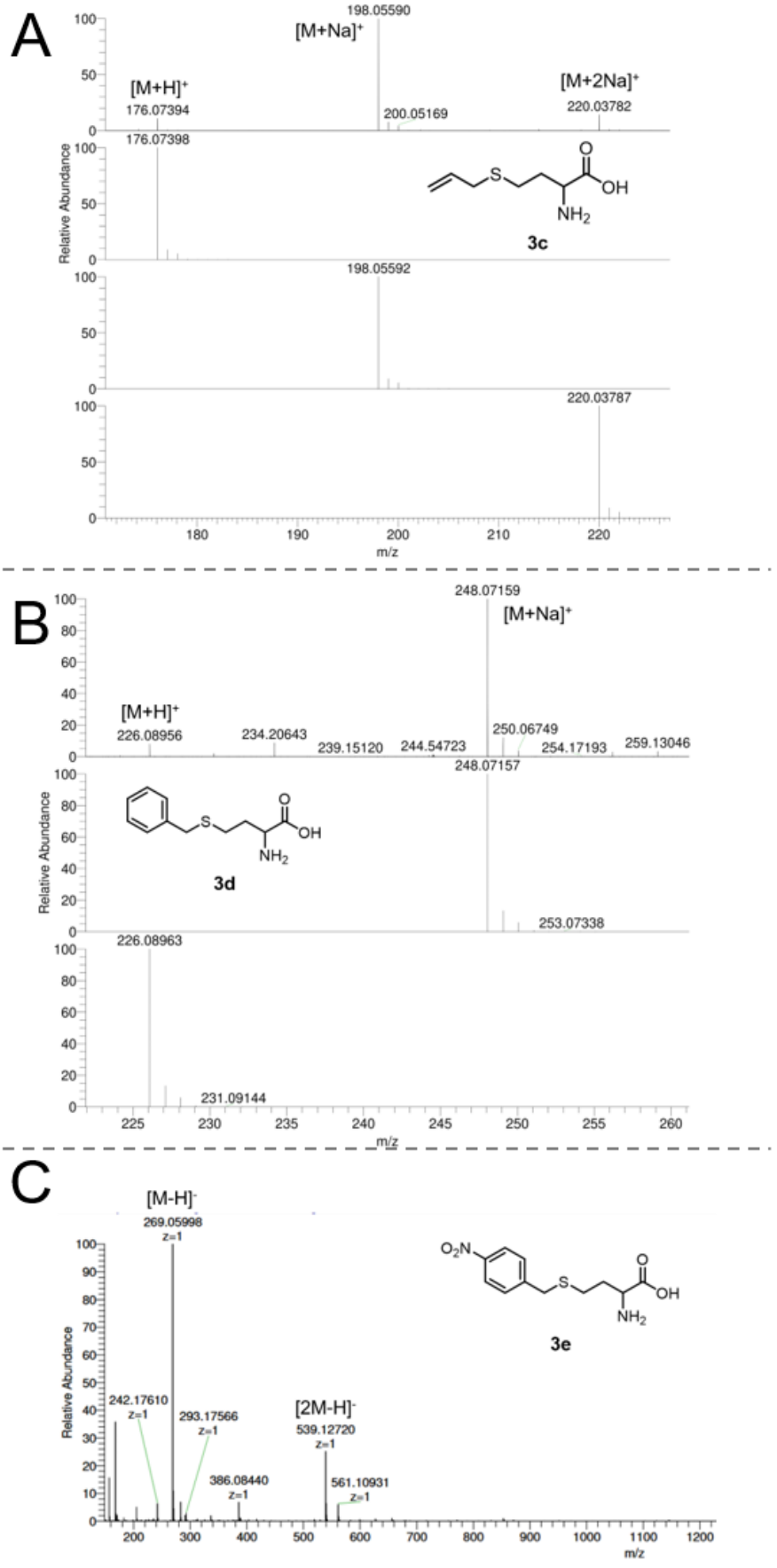


Figure S 5: High-Resolution MS spectra of methionine analogues (**3a**-**f**). ESI-MS with LTQ Orbitrap XL (Thermo-Fisher Scientific) in positive mode (A and B) and negative Mode (C). Spectrum A and B show the obtained masses (above) and the calculated masses (below) for the methionine analogues **3c** and **3d**. Spectrum C shows the obtained masses for the methionine analogue **3e**.

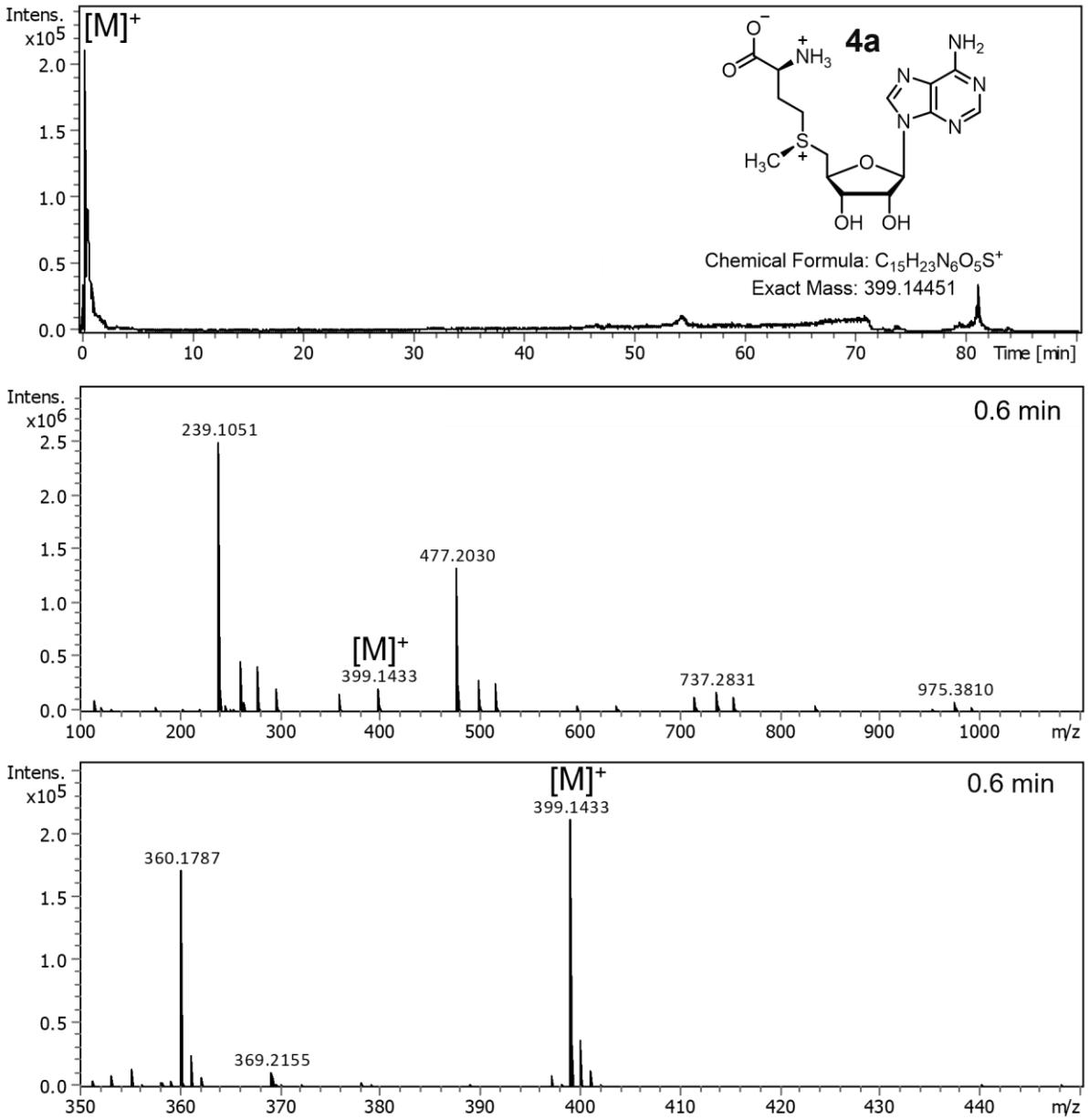


Figure S 6: HPLC/MS analysis of MAT assay with **3a** show the formation of **4a**. Calculated mass for $[M]^+$ = 399.144476 Da, found m/z = 399.1433 Da.

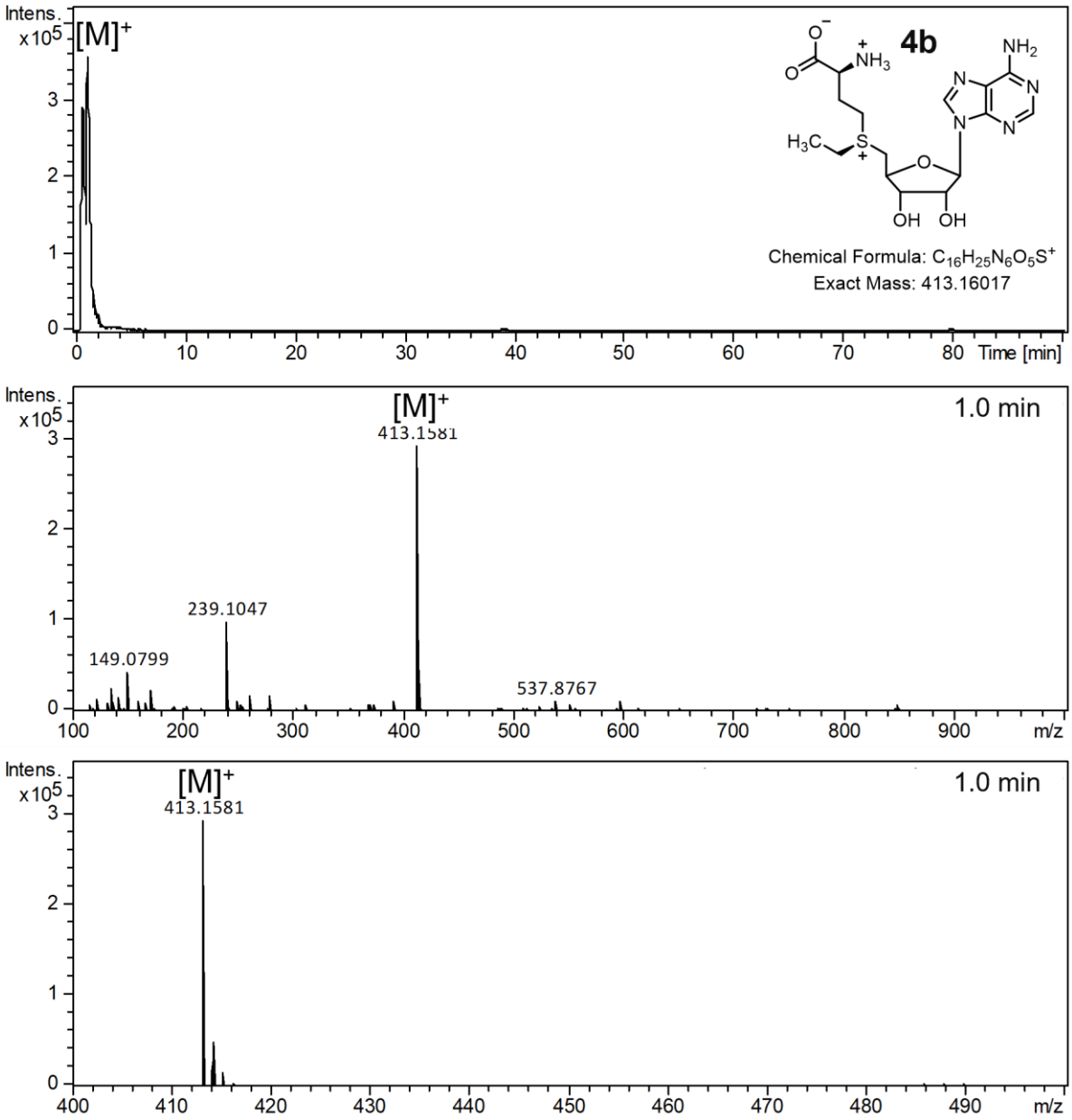


Figure S 7: HPLC/MS analysis of MAT assay with **3b** show the formation of **4b**. Calculated mass for $[M]^+$ = 413.160116 Da, found m/z = 413.1581 Da

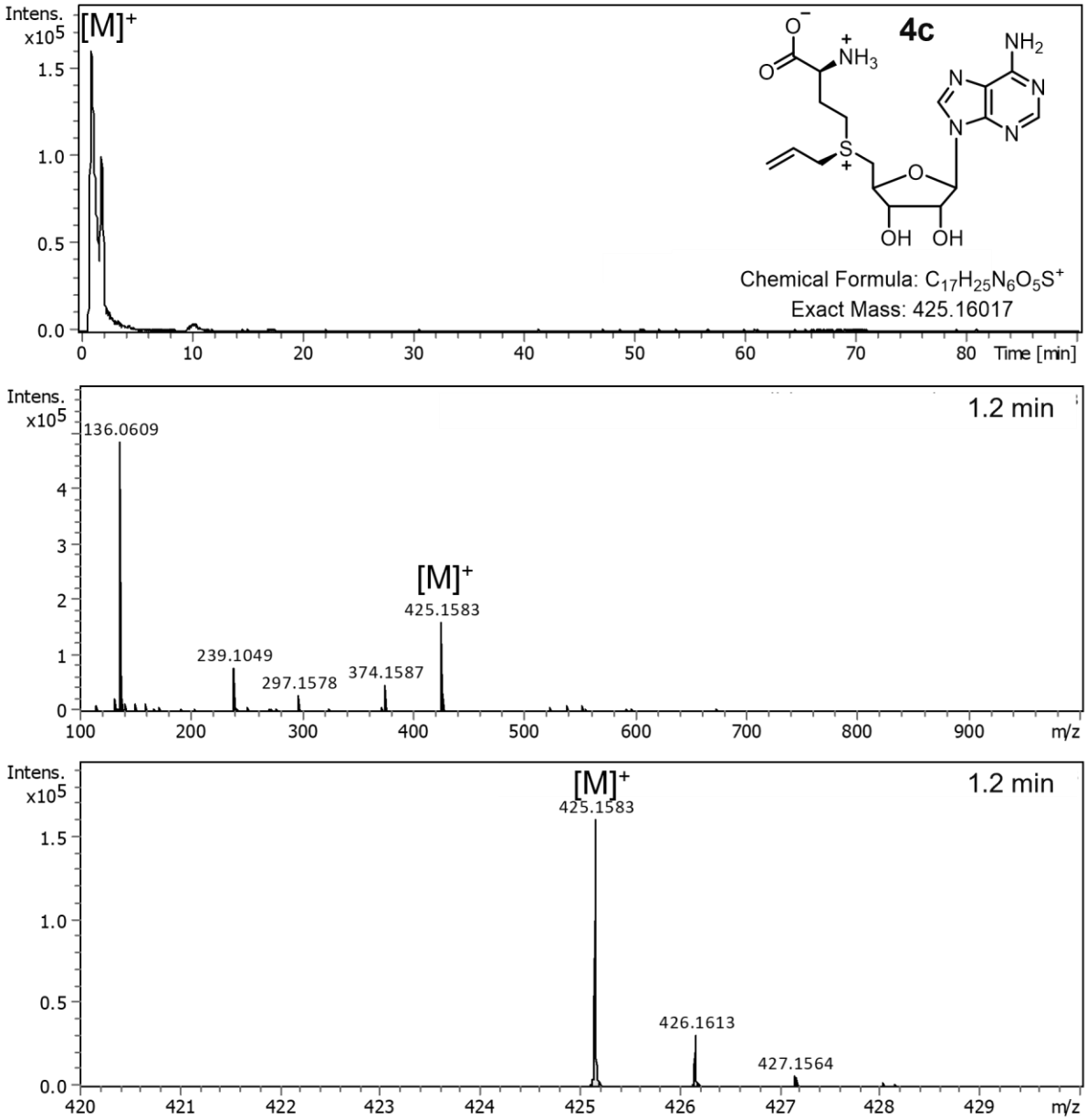


Figure S 8: HPLC/MS analysis of MAT assay with **3c** show the formation of **4c**. Calculated mass for $[M]^+$ = 425.160176 Da, found m/z = 425.1583 Da.

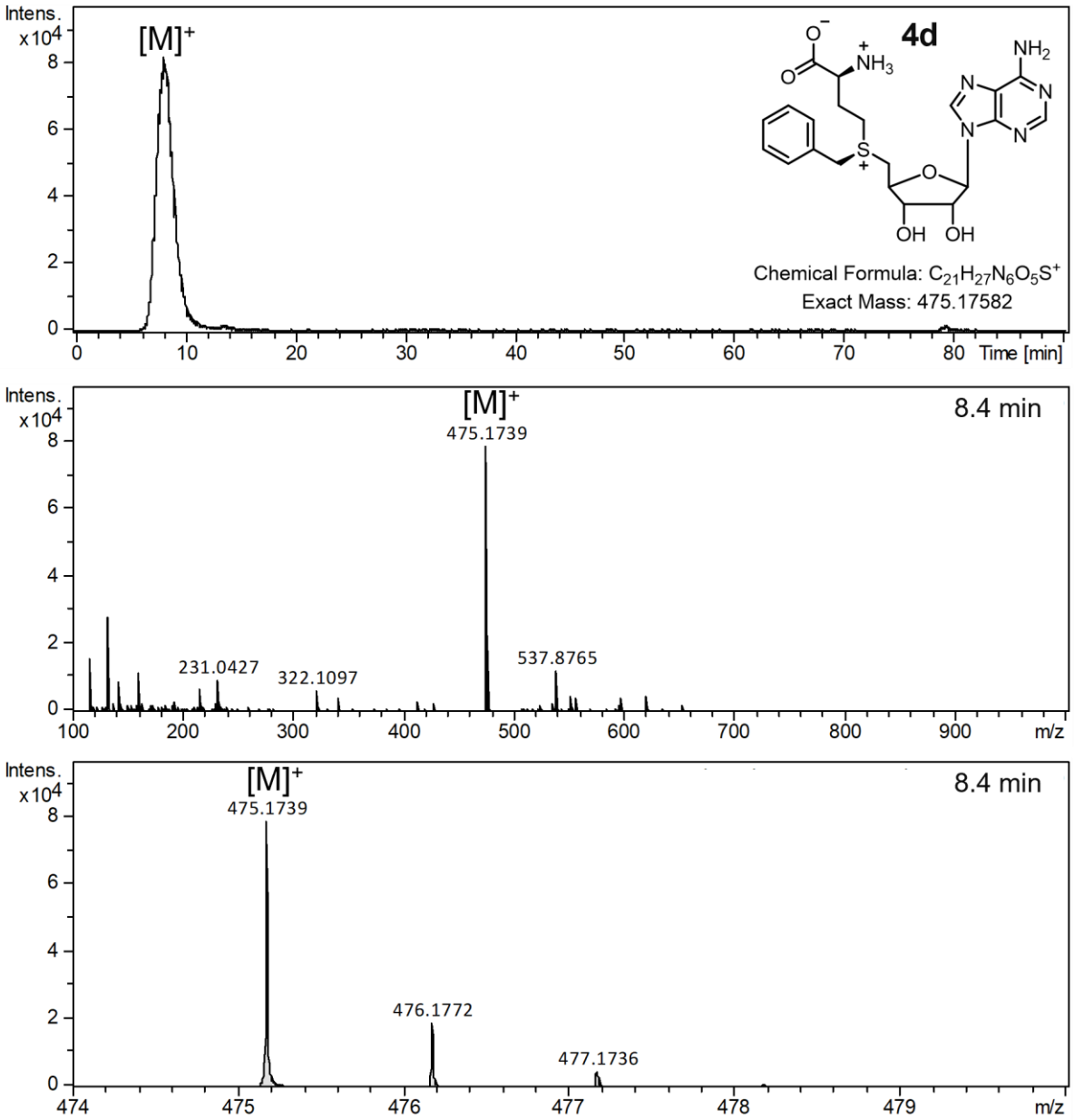


Figure S 9: HPLC/MS analysis of MAT assay with **3d** show the formation of **4d**. Calculated mass for $[M]^+$ = 475.175816 Da, found m/z = 475.1739 Da.

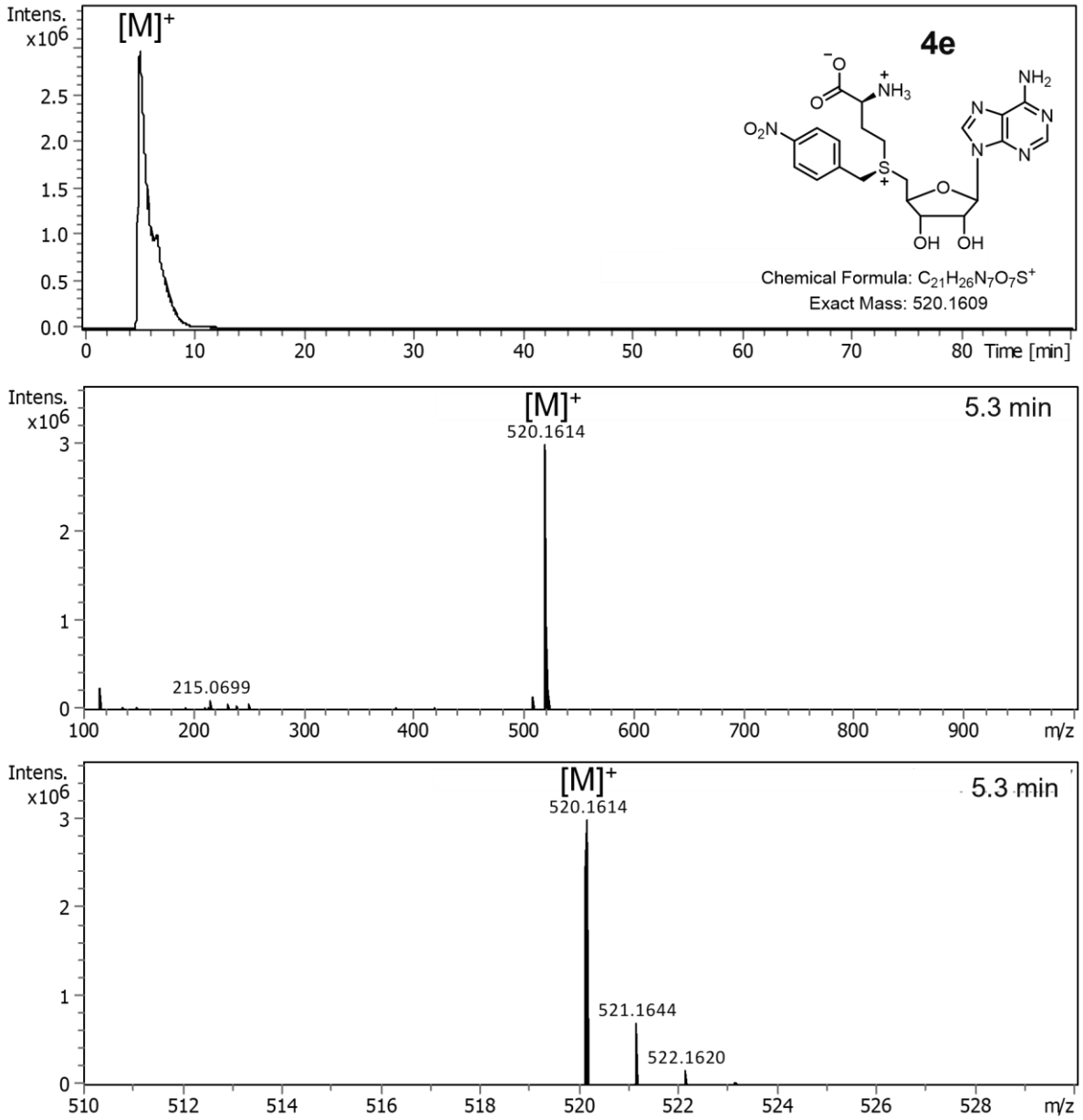


Figure S 10: HPLC/MS analysis of MAT assay with **3e** show the formation of **4e**. Calculated mass for $[M]^+$ = 520.1609 Da, found m/z = 520.1614 Da.

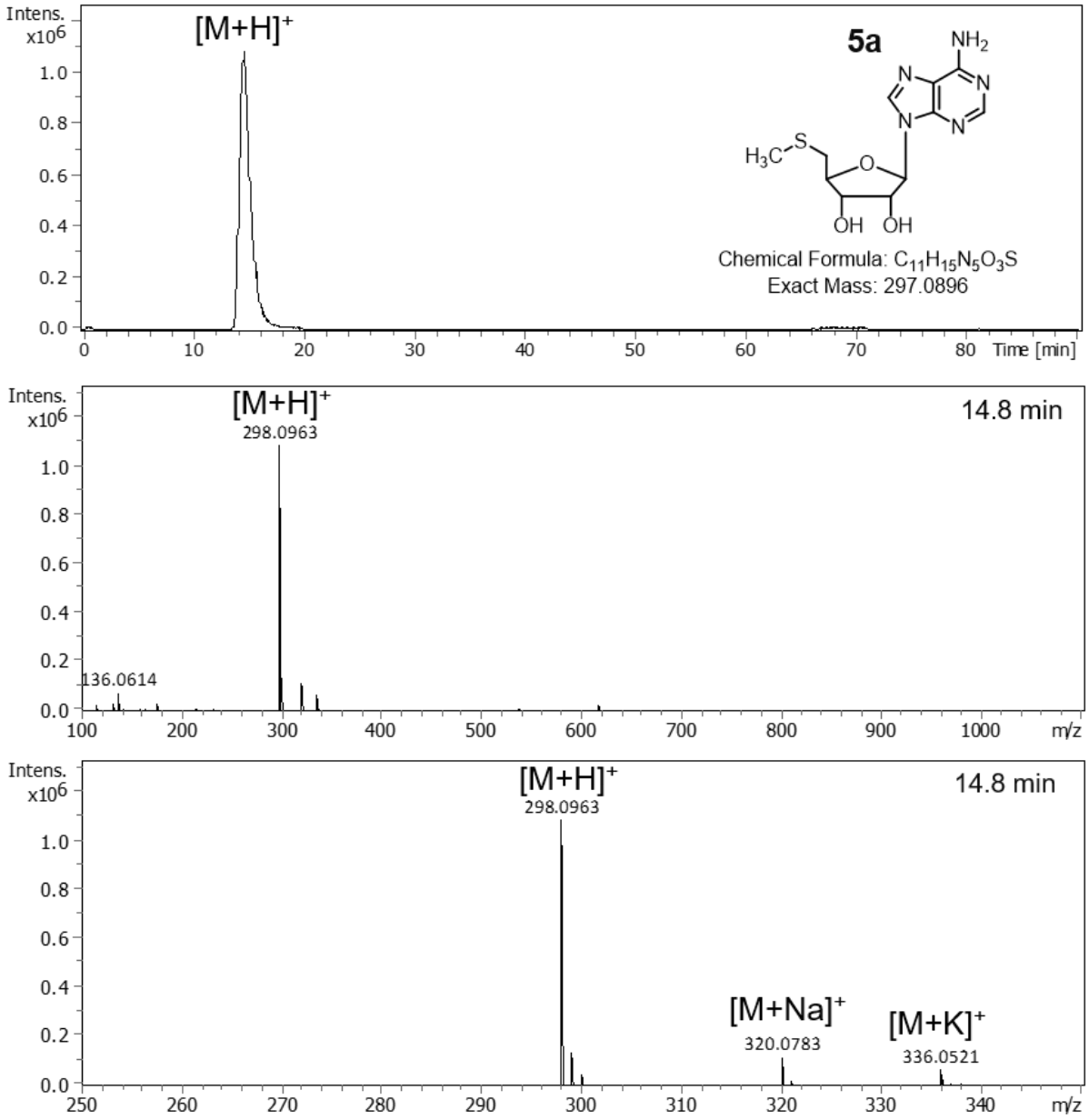


Figure S 11: HPLC/MS analysis of MAT assay with **3a** show the formation of **5a**. Calculated mass for $[M+H]^+$ = 298.096876 Da, found m/z = 298.0963 Da. Calculated mass for $[M+Na]^+$ = 320.078818 Da, found m/z = 320.0783 Da. Calculated mass for $[M+K]^+$ = 336.052758 Da, found m/z = 336.0521 Da.

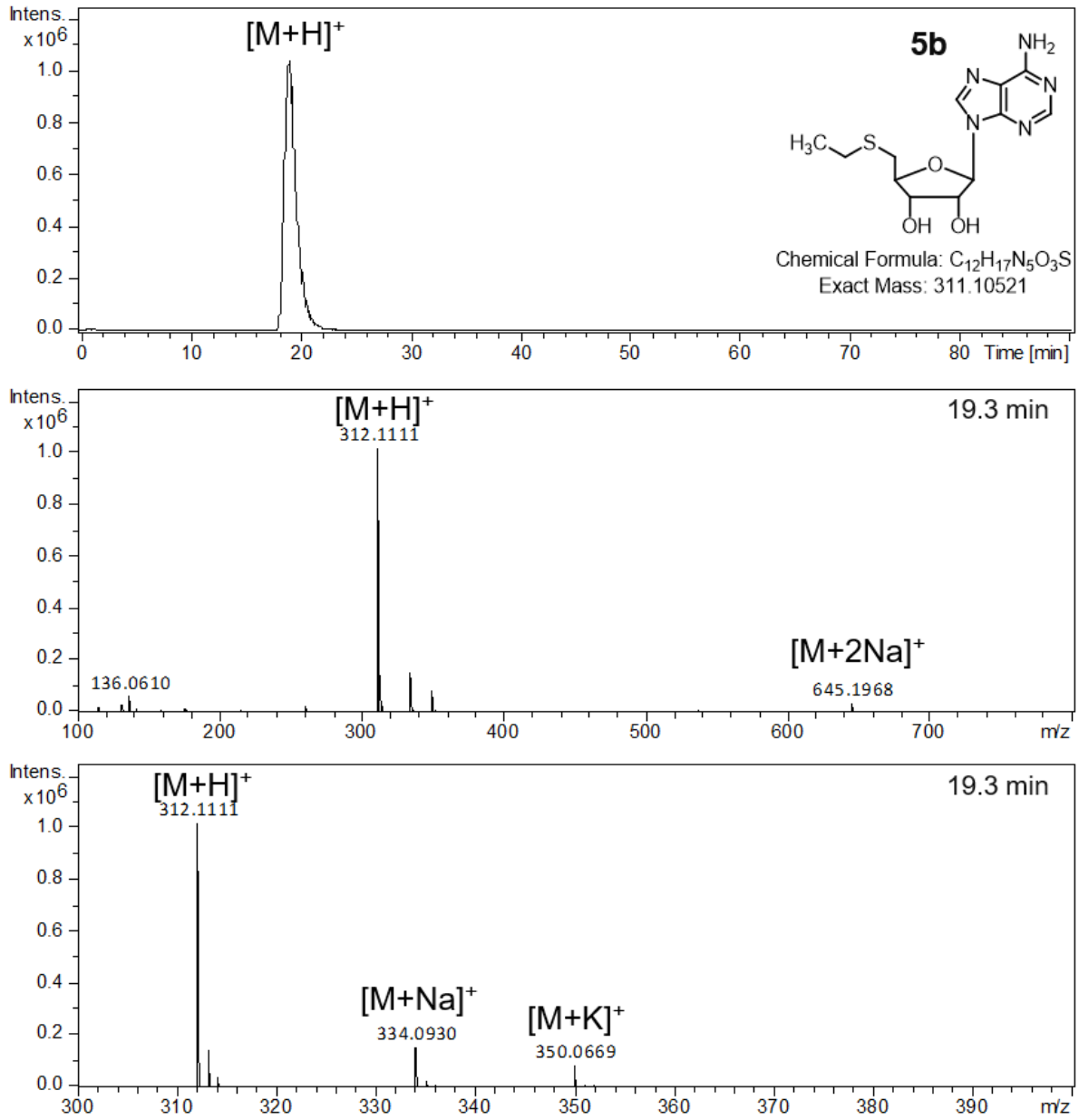


Figure S 12: HPLC/MS analysis of MAT assay with **3b** show the formation of **5b**. Calculated mass for $[M+H]^+$ = 312.112486 Da, found m/z = 312.1111 Da. Calculated mass for $[M+Na]^+$ = 334.094428 Da, found m/z = 334.0930 Da. Calculated mass for $[M+K]^+$ = 350.068368 Da, found m/z = 350.0669 Da. Calculated mass for $[2M+Na]^+$ = 645.199638 Da, found m/z = 645.1968 Da.

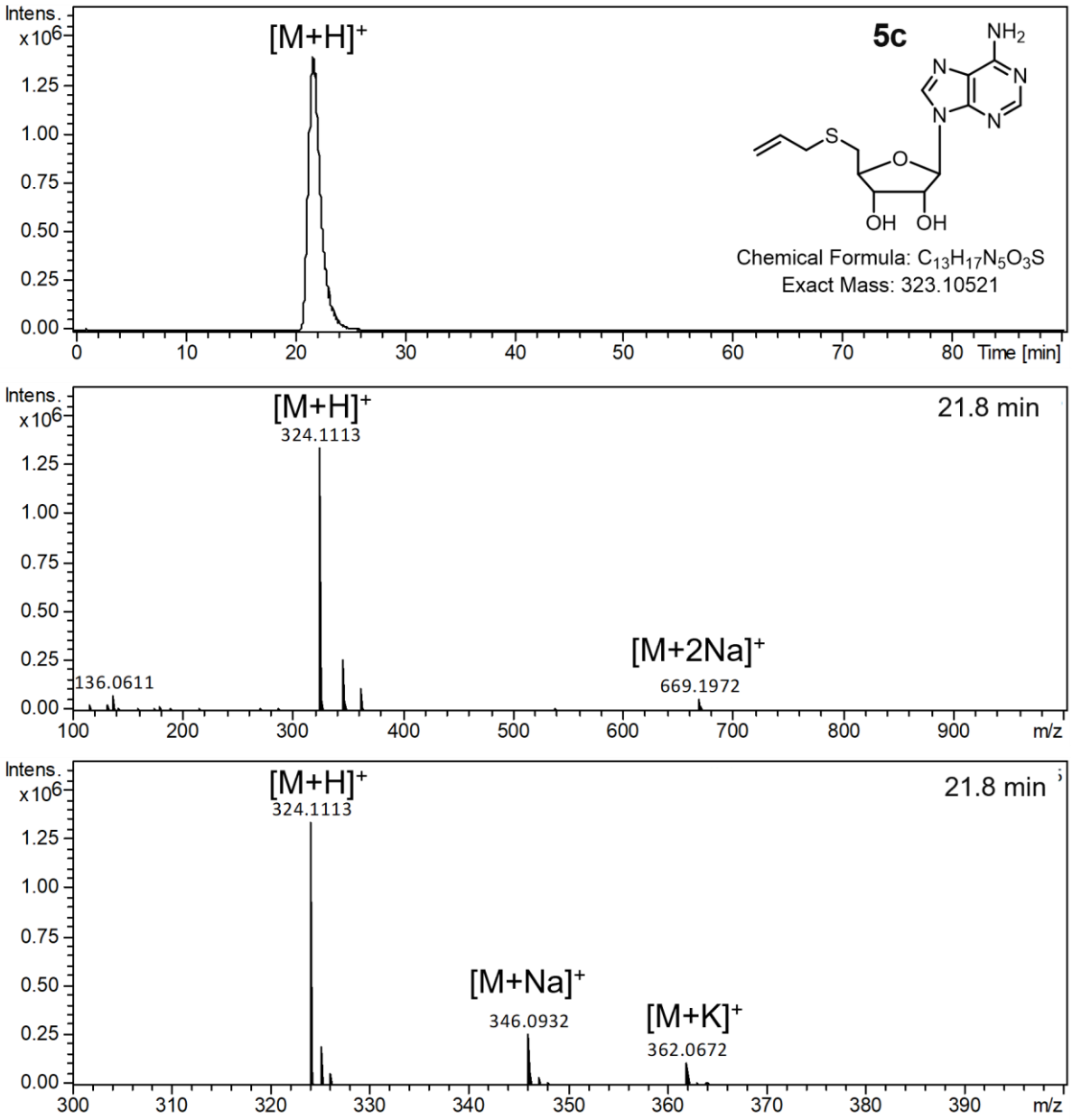


Figure S 13: HPLC/MS analysis of MAT assay with **3c** show the formation of **5c**. Calculated mass for $[M+H]^+$ = 324.112486 Da, found m/z = 324.1113 Da. Calculated mass for $[M+Na]^+$ = 346.094428 Da, found m/z = 346.0932 Da. Calculated mass for $[M+K]^+$ = 362.068368 Da, found m/z = 362.00672 Da. Calculated mass for $[2M+Na]^+$ = 669.199638 Da, found m/z = 669.1972 Da.

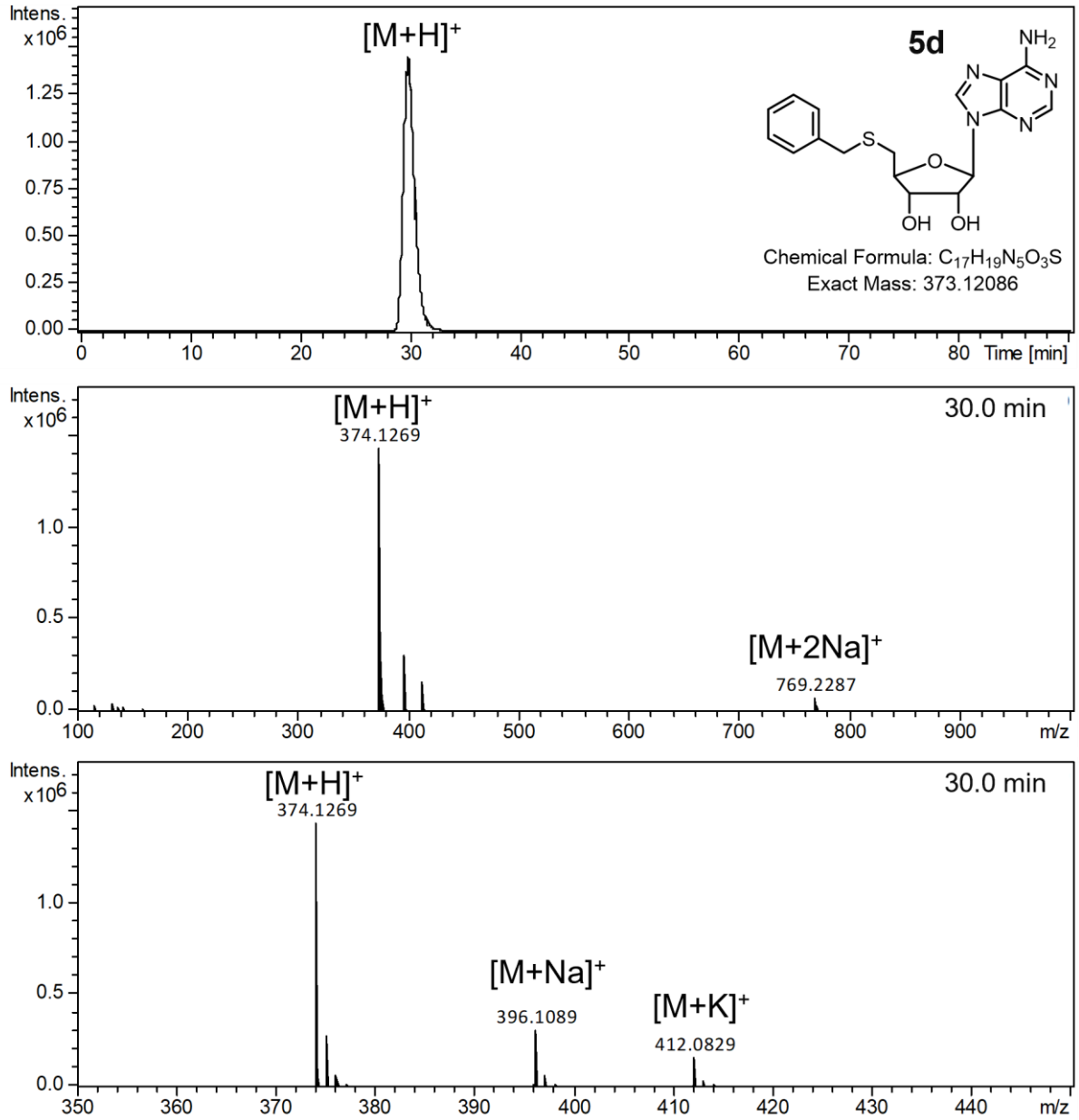


Figure S 14: HPLC/MS analysis of MAT assay with **3d** show the formation of **5d**. Calculated mass for $[M+H]^+$ = 374.128136 Da, found m/z = 374.1269 Da. Calculated mass for $[M+Na]^+$ = 396.110078 Da, found m/z = 396.1089 Da. Calculated mass for $[M+K]^+$ = 412.084018 Da, found m/z = 412.0829 Da. Calculated mass for $[2M+Na]^+$ = 769.230938 Da, found m/z = 769.2287 Da.

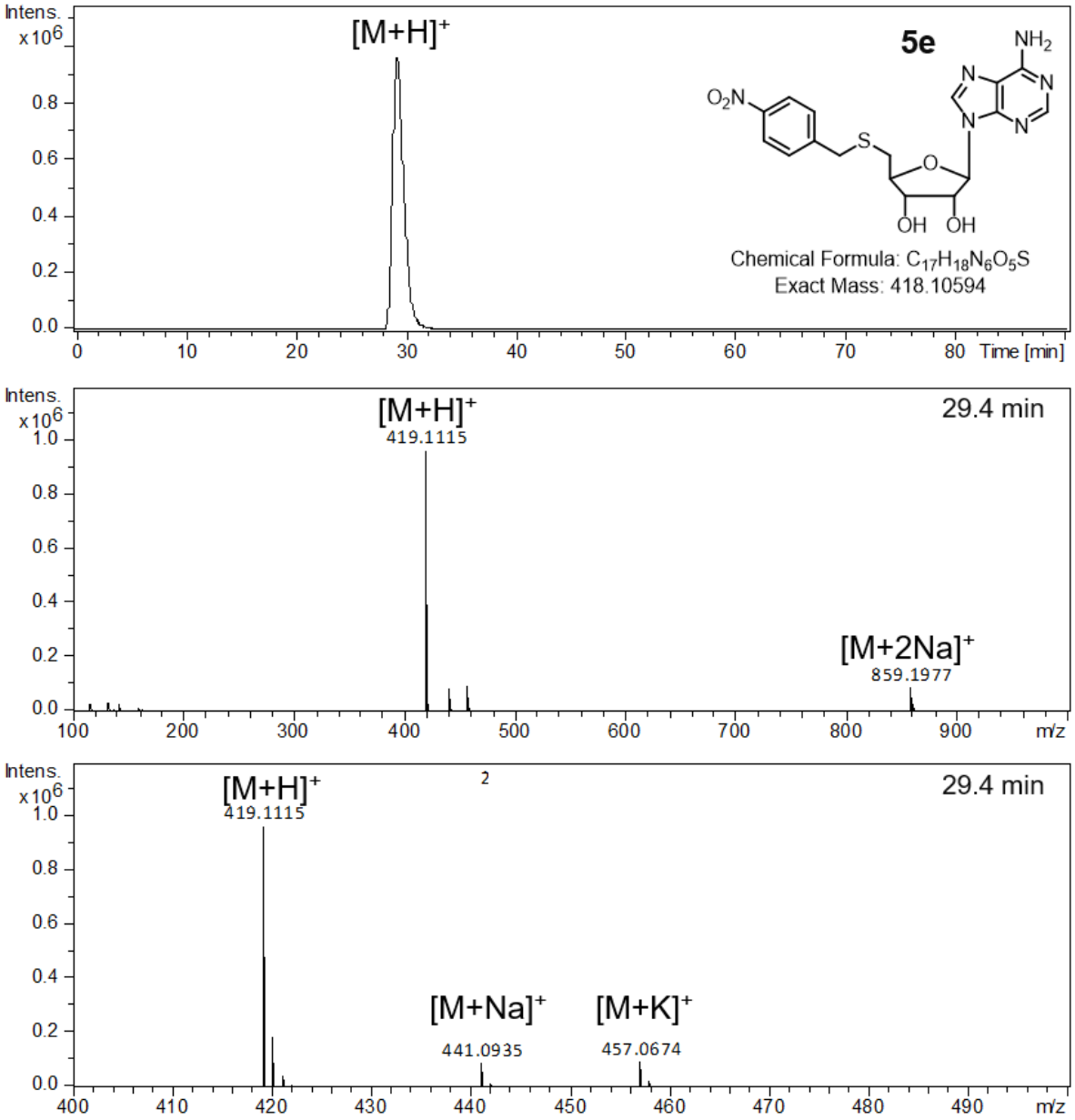


Figure S 15: HPLC/MS analysis of MAT assay with **3e** show the formation of **5e**. Calculated mass for $[M+H]^+$ = 419.113216 Da, found m/z = 419.1115 Da. Calculated mass for $[M+Na]^+$ = 441.095158 Da, found m/z = 441.0935 Da. Calculated mass for $[M+K]^+$ = 457.069098 Da, found m/z = 457.0674 Da. Calculated mass for $[2M+Na]^+$ = 859.201098 Da, found m/z = 859.1977 Da.

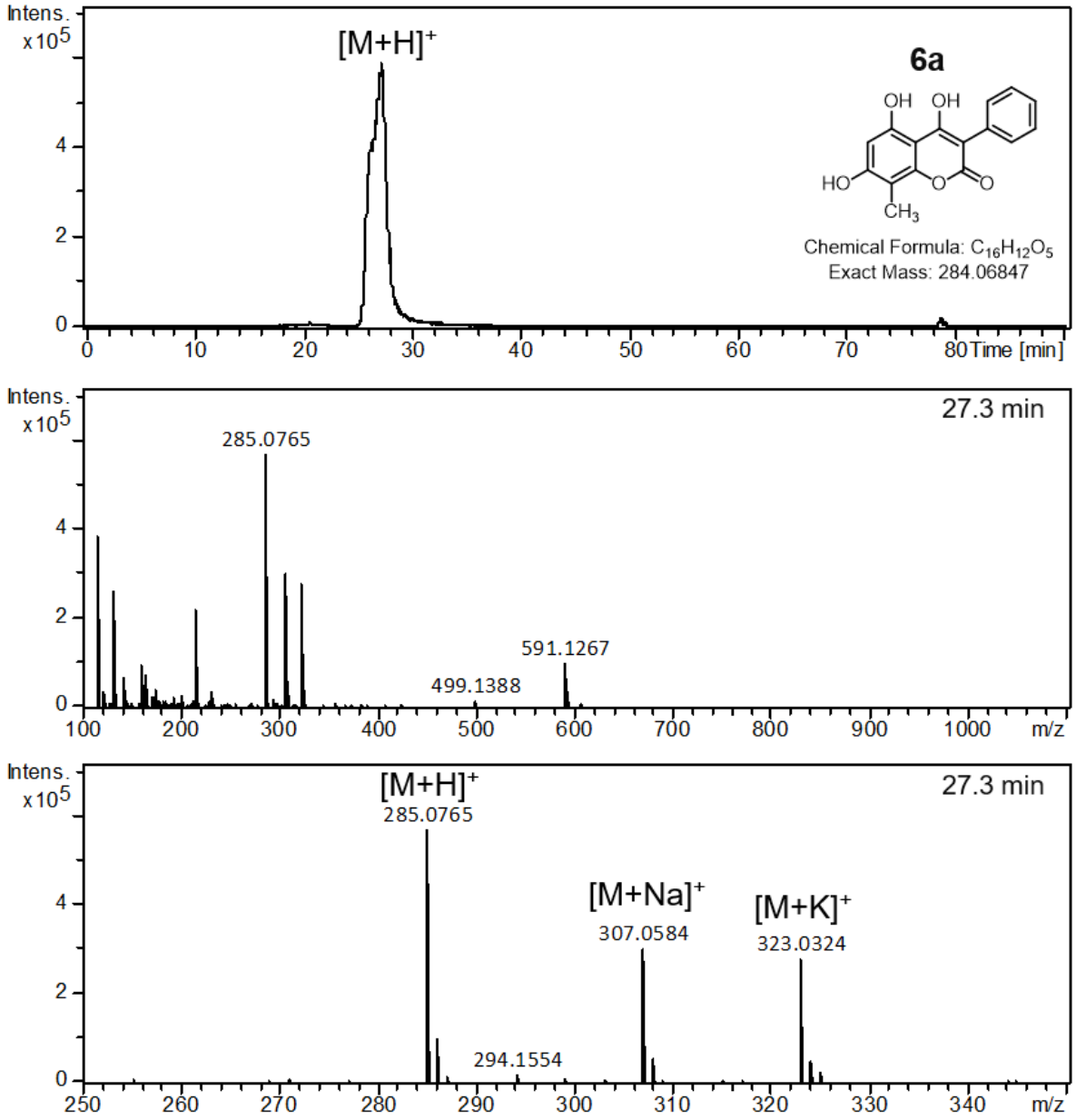


Figure S 16: HPLC/MS analysis of PC-MjMAT/NovO cascade reaction with **3a** show the formation of **6a**. Calculated mass for $[M+H]^+$ = 285.07575 Da, found m/z = 285.0765 Da. Calculated mass for $[M+Na]^+$ = 307.057688 Da, found m/z = 307.0584 Da. Calculated mass for $[M+K]^+$ = 323.031628 Da, found m/z = 323.0324 Da.

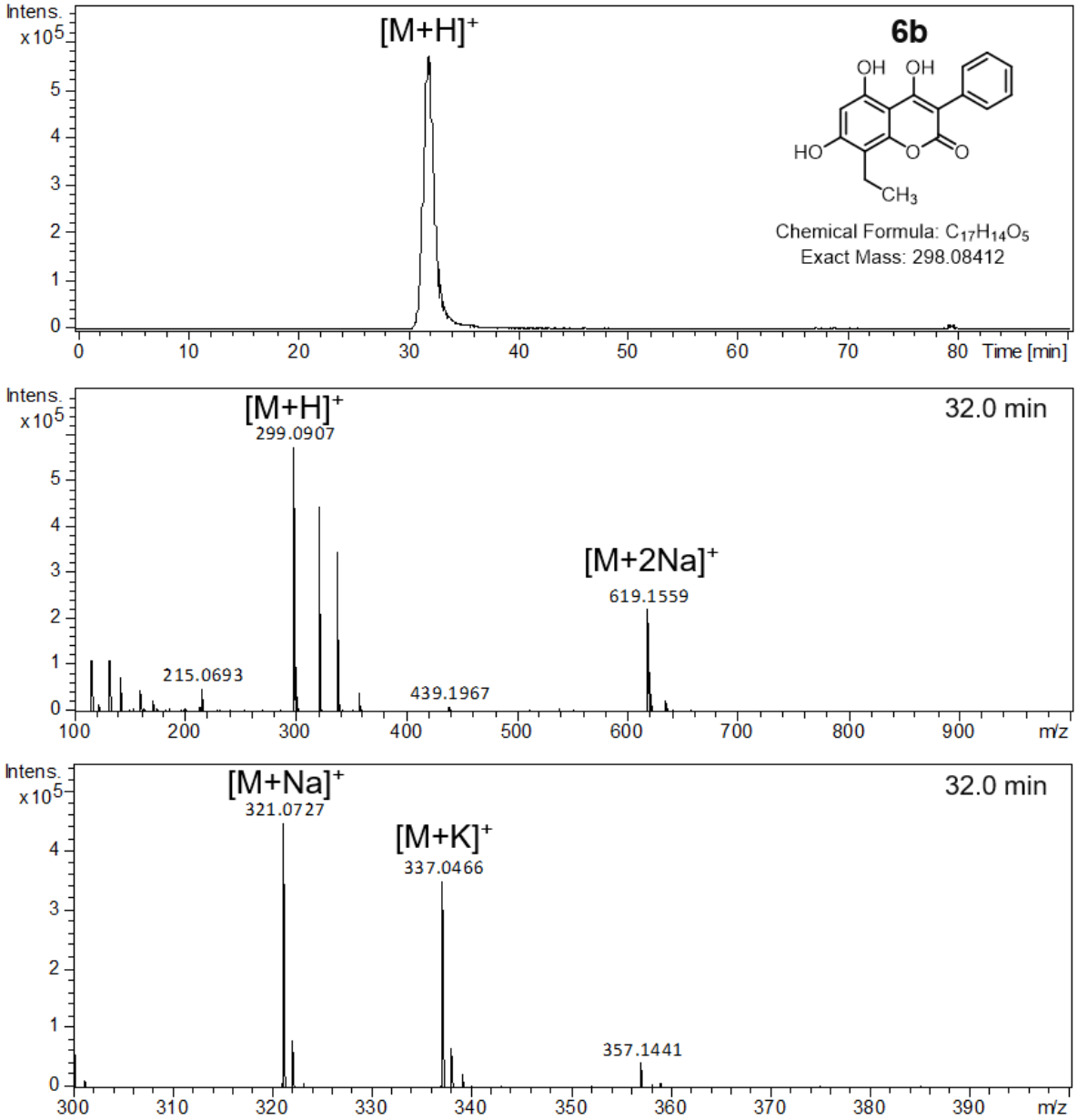


Figure S 17: HPLC/MS analysis of PC-MjMAT/NovO cascade reaction with **3b** show the formation of **6b**. Calculated mass for $[M+H]^+$ = 299.091396 Da, found m/z = 299.0907 Da. Calculated mass for $[M+Na]^+$ = 321.073338 Da, found m/z = 321.0727 Da. Calculated mass for $[M+K]^+$ = 337.047278 Da, found m/z = 337.0466 Da. Calculated mass for $[2M+Na]^+$ = 619.157458 Da, found m/z = 619.1559 Da.

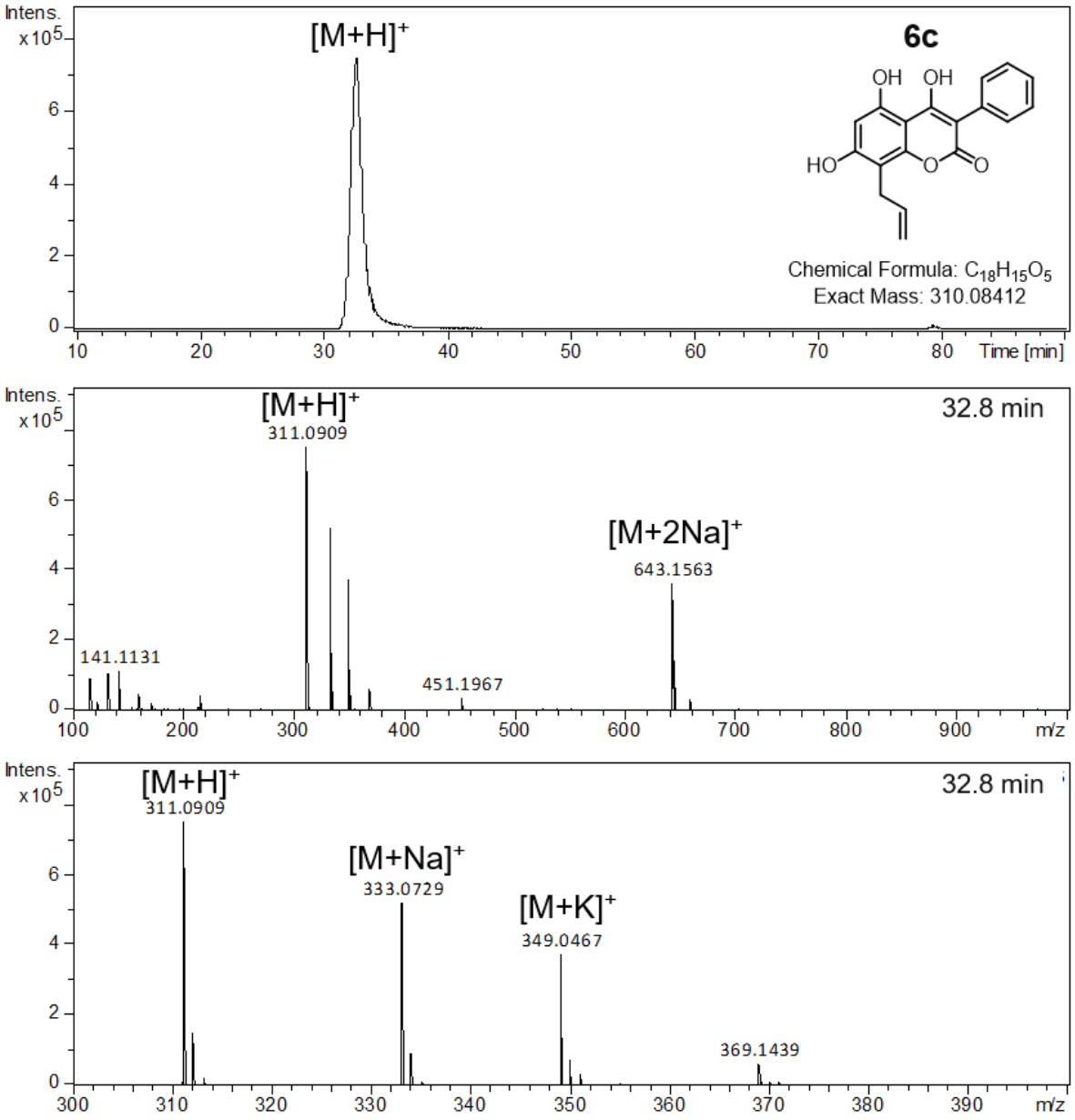


Figure S 18: HPLC/MS analysis of PC-MjMAT/NovO cascade reaction with **3c** show the formation of **6c**. Calculated mass for $[M+H]^+$ = 311.091396 Da, found m/z = 311.0909 Da. Calculated mass for $[M+Na]^+$ = 333.073338 Da, found m/z = 333.0729 Da. Calculated mass for $[M+K]^+$ = 349.047278 Da, found m/z = 349.0467 Da. Calculated mass for $[2M+Na]^+$ = 643.157458 Da, found m/z = 643.1563 Da.

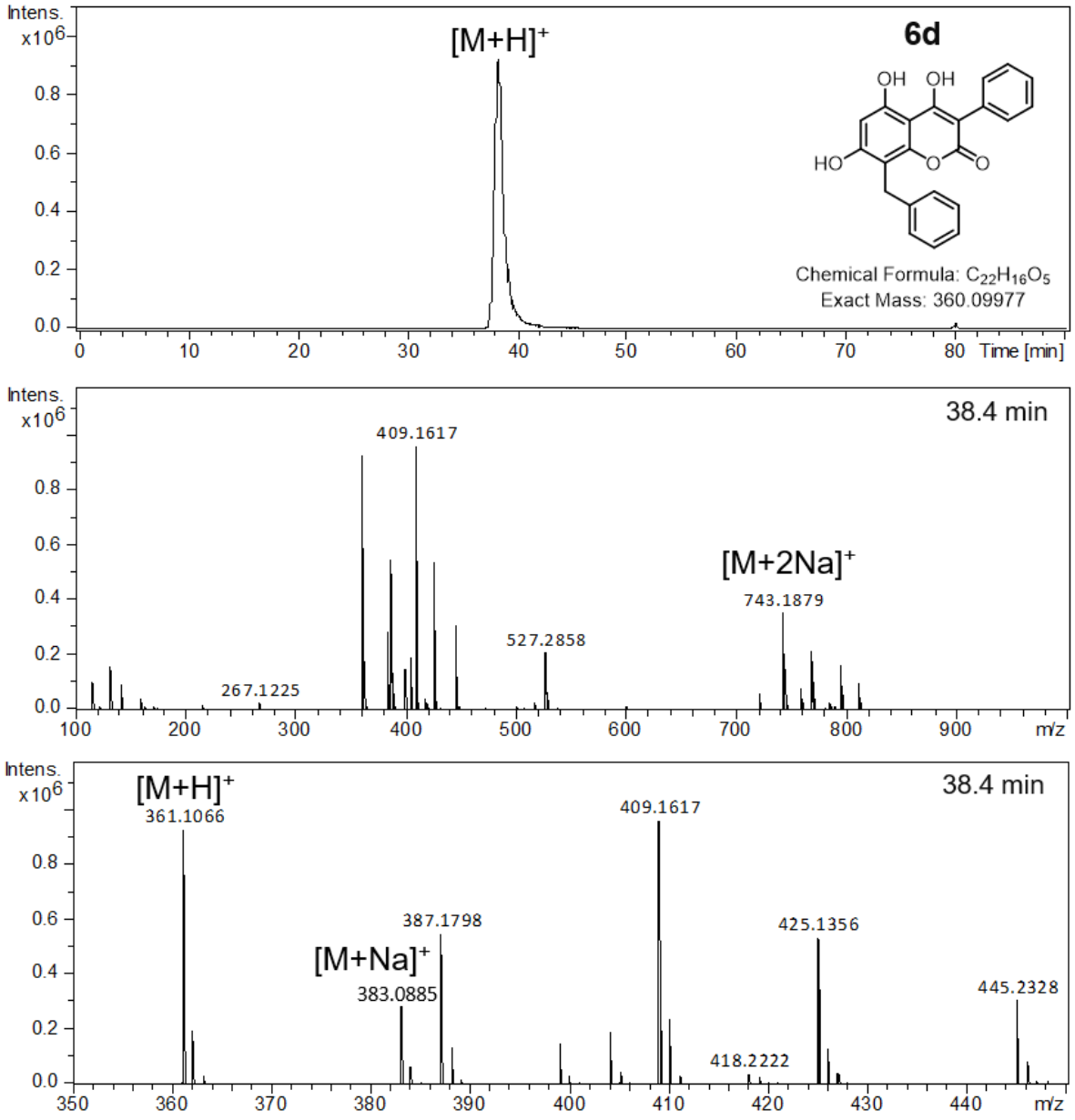


Figure S 19: HPLC/MS analysis of PC-MjMAT/NovO cascade reaction with **3d** show the formation of **6d**. Calculated mass for $[M+H]^+$ = 361.107046 Da, found m/z = 361.1066 Da. Calculated mass for $[M+Na]^+$ = 383.088988 Da, found m/z = 383.0885 Da. Calculated mass for $[2M+Na]^+$ = 743.188758 Da, found m/z = 743.1879 Da.

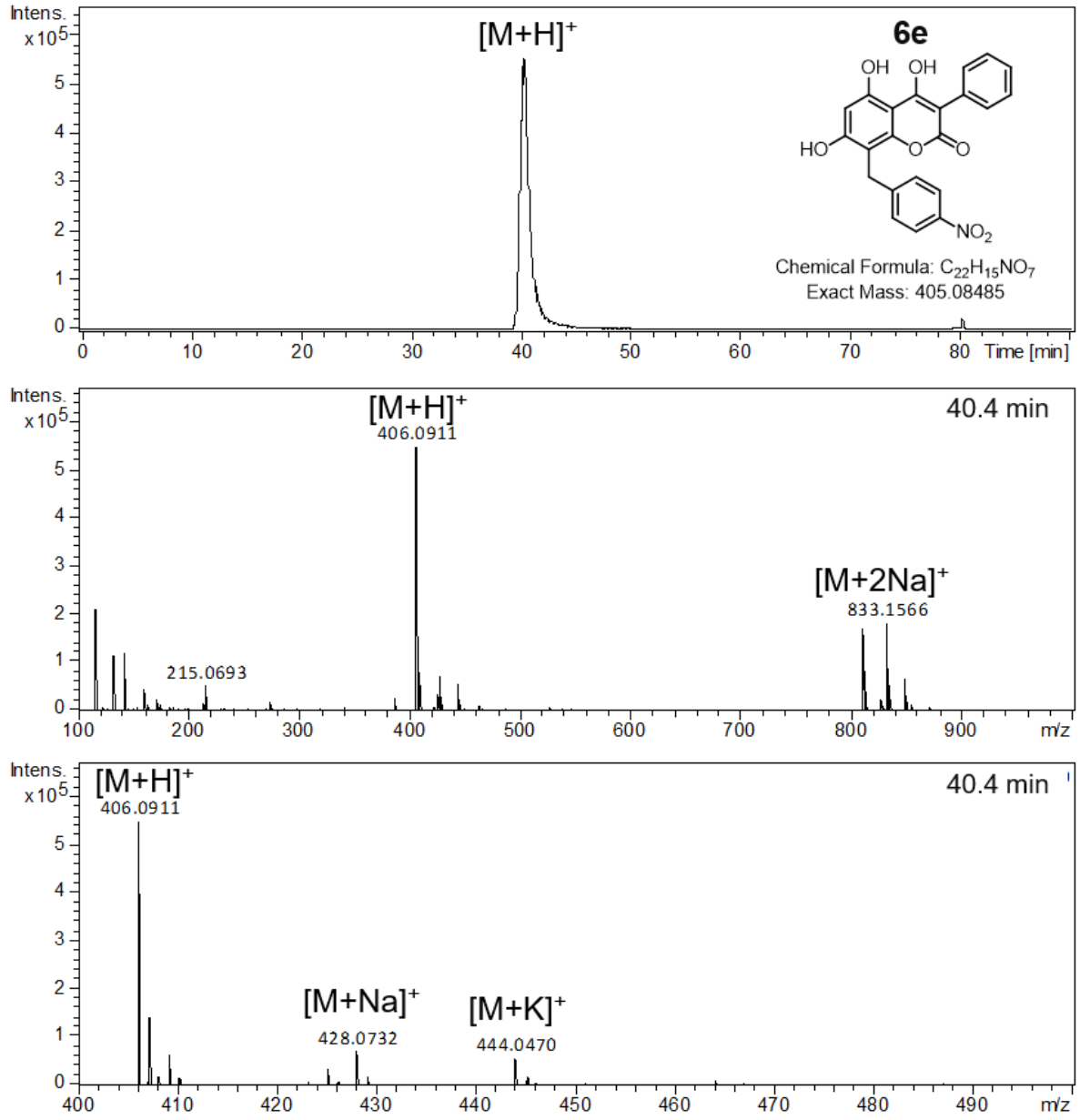


Figure S 20: HPLC/MS analysis of PC-MjMAT/NovO cascade reaction with **3e** show the formation of **6e**. Calculated mass for $[M+H]^+$ = 406.092126 Da, found m/z = 406.0911 Da. Calculated mass for $[M+Na]^+$ = 428.074068 Da, found m/z = 428.0732 Da. Calculated mass for $[M+K]^+$ = 444.048008 Da, found m/z = 444.0470 Da. Calculated mass for $[2M+Na]^+$ = 833.158918 Da, found m/z = 833.1566 Da.

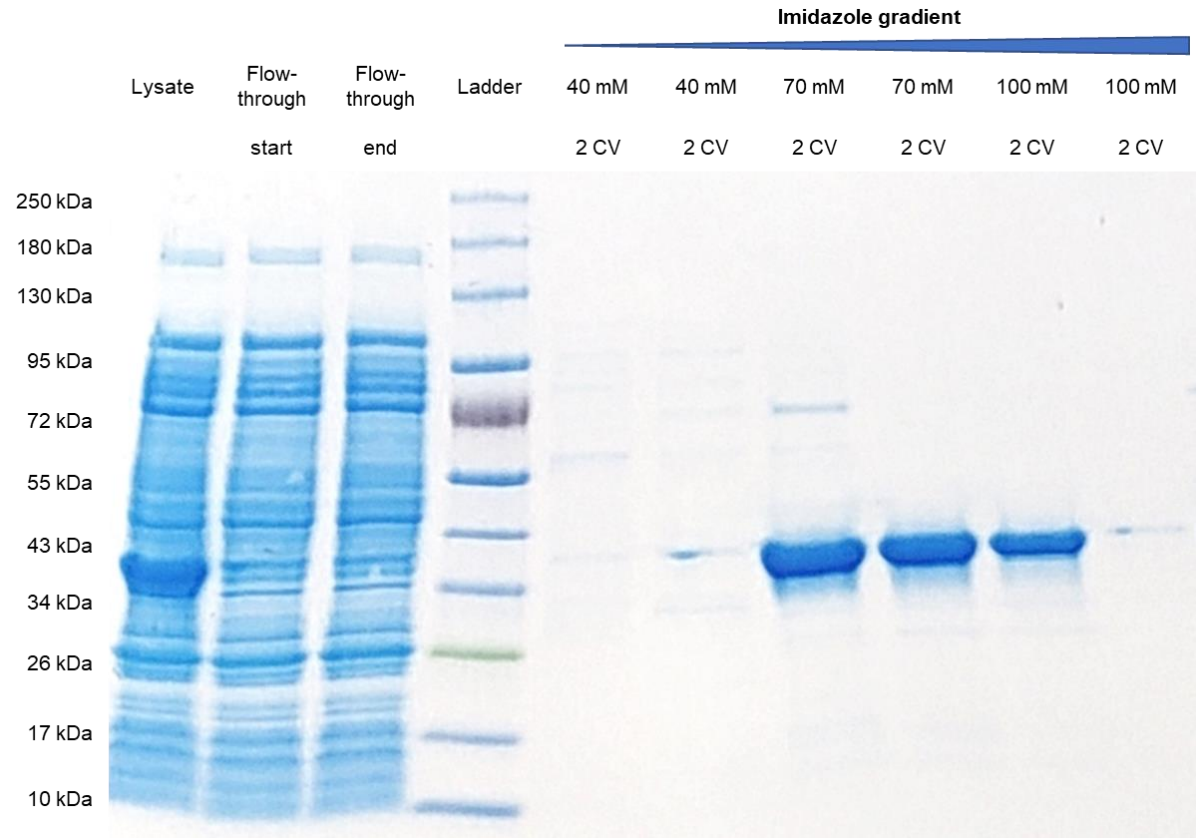


Figure S 21: SDS Page analysis of the AclHMT purification through immobilized metal affinity chromatography (IMAC). Color Prestained Protein Standard, Broad Range (10-250 kDa) from NEB (Catalog#: P7719S) was used as size standard and protein separation was performed using pre-cast gel (Mini-Protean TGX gel, 4-20%) for 24 min at 240 V. Samples: Lysate after cell disruption (sonification), Flow-through of the 5 mL Ni^{2+} IMAC column (start and end) and collected imidazole fractions 40-100 mM (each 2 cv).

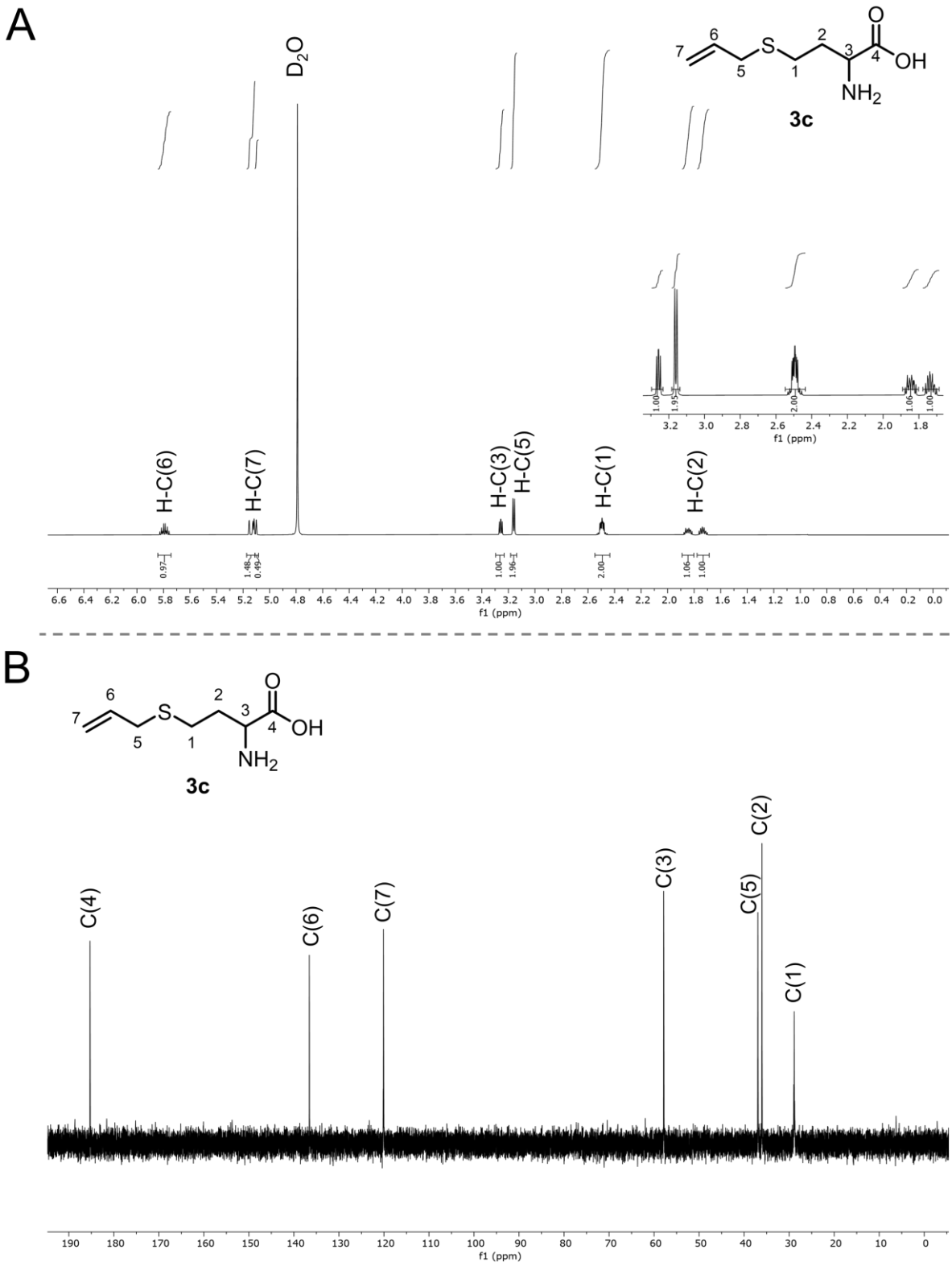


Figure S 22: NMR analysis of allyl-D,L-homocysteine (**3c**). A: ^{1}H NMR analysis at 600 MHz. B: 13C-{^{1}H}-NMR analysis at 151 MHz.

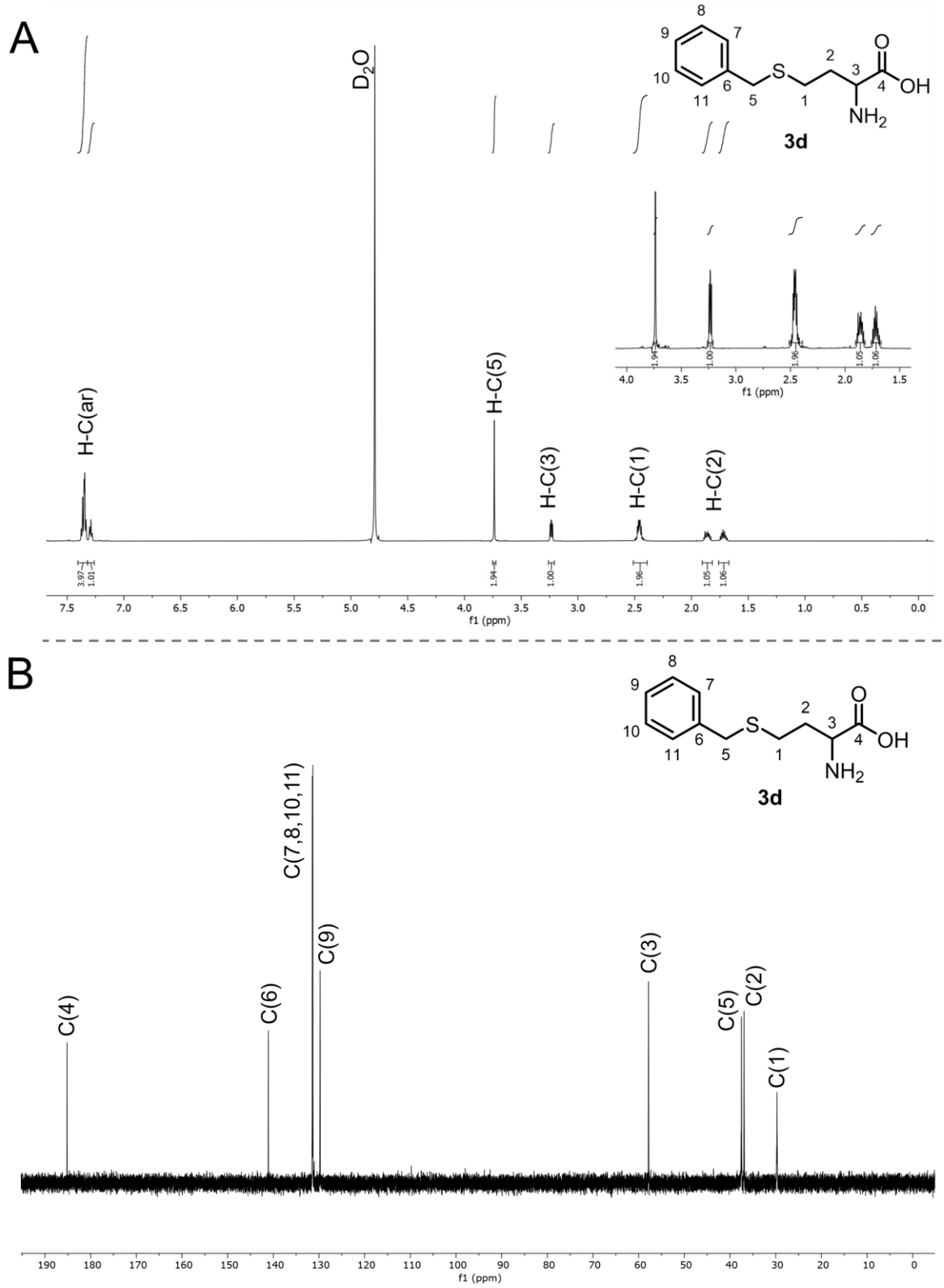


Figure S 23: NMR analysis of benzyl-D,L-homocysteine (**3d**). A: ^{1}H NMR analysis at 600 MHz. B: ^{13}C-{^{1}H}-NMR analysis at 151 MHz.

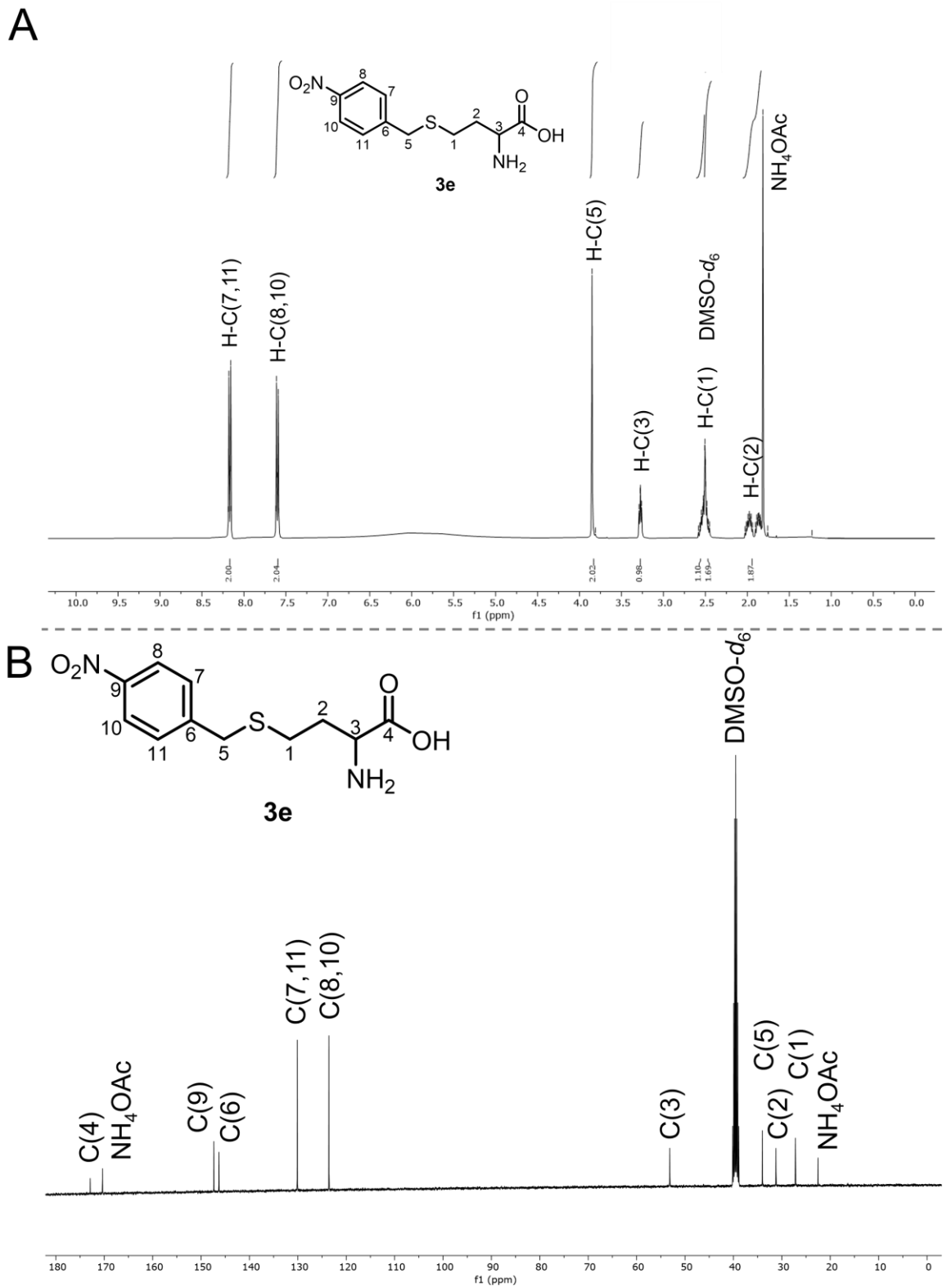


Figure S 24: NMR analysis of para-nitrobenzyl-DL-homocysteine (**3e**). A: ^{1}H NMR analysis at 400 MHz. B: ^{13}C-{^{1}H}-NMR analysis at 101 MHz.

V. DNA / AA Sequences

The sequence of the AclHMT was already published in a previous study.[1a, c]

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

***Aspergillus clavatus* halide methyltransferase (AclHMT)**

DNA sequence

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Amino acid sequence (MW: 33.396 kDa, Molecular extinction coefficient: 62255 $M^{-1}\cdot cm^{-1}$)

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***Methanocaldococcus jannaschii* methionine adenosyltransferase variant L147A/I351A (PC-MjMAT)**

DNA sequence

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Amino acid sequence (MW: 47.331 kDa, Molecular extinction coefficient: 25120 $M^{-1}\cdot cm^{-1}$)

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DNIMEVQKMIVEGKVTTF*

Streptomyces niveus coumarin-C-methyltransferase (NovO)

DNA sequence

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Amino acid sequence (MW: 27.121 kDa, Molecular extinction coefficient: 29700 $M^{-1}\cdot cm^{-1}$)

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PVSEQRVWIDDQGYGVPTVKCFARRAAAKLAAALE**HHHHHH***

***Escherischia coli* SAH nucleosidase (MTAN)**

DNA sequence

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Amino acid sequence (MW: 28.157 kDa, Molecular extinction coefficient: 11920 $M^{-1}\cdot cm^{-1}$)

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EATAIAHVCHNFNVPFVVVRAISDVADQQSHLSFDEFLAVAAKQSSLMVESLVQKLAHG*
```

VI. References

[1] K. H. Schülke, F. Ospina, K. Hörnschemeyer, S. Gergel and S. C. Hammer, *Chembiochem* **2022**, *23*, e202100632.

[2] a) F. Ospina, K. H. Schülke, J. Soler, A. Klein, B. Prosenc, M. Garcia-Borras and S. C. Hammer, *Angew. Chem. Int. Ed.* **2022**; b) L. L. Bengel, B. Aberle, A. N. Egler-Kemmerer, S. Kienzle, B. Hauer and S. C. Hammer, *Angew. Chem. Int. Ed.* **2020**.

[3] a) A. Peters, E. Herrmann, N. V. Cornelissen, N. Klöcker, D. Kümmel and A. Rentmeister, *ChemBioChem* **2022**, *23*, e202100437; b) F. Michailidou, N. Klöcker, N. V. Cornelissen, R. K. Singh, A. Peters, A. Ovcharenko, D. Kümmel and A. Rentmeister, *Angew. Chem. Int. Ed.* **2021**, *60*, 480-485.