# Synthesis and Characterization of Compounds 1–13

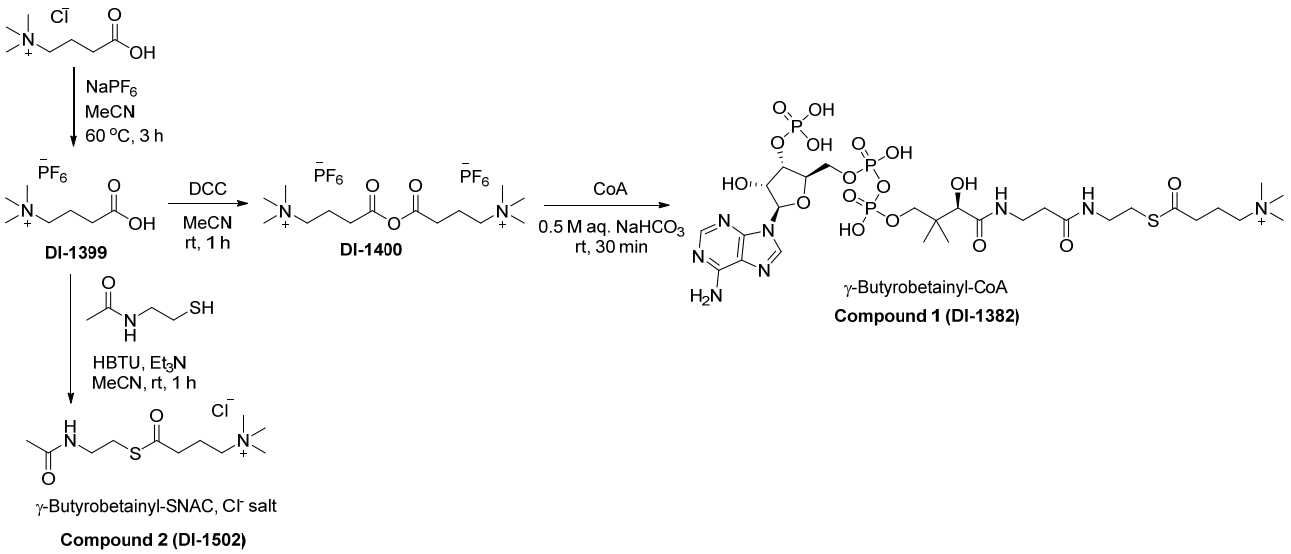
Reagents and starting materials were obtained from commercial sources or synthesized according to literature procedures. TLC was performed on Merck 60 F254 pre-coated silica gel plates and was visualized with UV light at 254 nm. Flash column chromatography was carried out using Kieselgel (0.035–0.070 mm) silica gel. NMR spectra were recorded at 298 K (25 °C) on Bruker 400 AVANCE NEO NMR spectrometer equipped with 5 mm Z149000_0043 (CPP BBO 400S1 BB-H&F-D-05 Z) probe and at 298 K (25 °C) on 300 MHz Bruker Fourier with probe 5-mm 1H / 13C Dual EasyProbe with Z-Grad spectrometers with chemical shift values (δ) in parts per million using the residual solvent signal as an internal standard ($D_2O$: $^1H$ 4.79; DMSO-$d_6$: $^1H$ 2.50, $^{13}C$ 39.52; $CDCl_3$: $^1H$ 7.26, $^{13}C$ 77.16; MeOD: $^1H$ 3.31, $^{13}C$ 49.00). Reversed-phase chromatography was done on a Biotage Isolera One system using SNAP KP-C18-HS cartridges. GC/MS analyses were performed on an Agilent 7890A gas chromatograph combined with a 5975C mass spectrometer using a J&W Scientific HP-5MS silica column (30 m × 0.25 mm, 0.25 µm). Exact molecular masses (HRMS) were determined on a Waters SYNAPT G2-Si hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer equipped with an electrospray ionization (ESI) source.

The substrate and analogues 1–13 were synthesized and characterized as described in Schemes S1–S10 and the corresponding experimental procedures in the Supporting Information; internal DI identifiers are listed in Table S1.

# Abbreviation list

CDI 1,1'-Carbonyldiimidazole
COMU (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate
DCC N,N'-Dicyclohexylcarbodiimide
HBTU 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
PAN Pantetheine
PyBOP Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
SNAC N-Acetylcysteamine

*Compounds **1 (DI-1382)** and **2 (DI-1502)***




Scheme S1. Synthesis of compounds 1 (DI-1382) and 2 (DI-1502).

**3-Carboxy-*N*,*N*,*N*-trimethylpropan-1-aminium hexafluorophosphate (DI-1399)**

γ-Butyrobetaine hydrochloride (0.20 g, 1.10 mmol) was suspended in MeCN (3.0 mL), $NaPF_6$ (0.19 g, 1.10 mmol) was added and mixture was stirred at 60 °C for 3 h. The mixture was cooled down to rt, precipitate was filtered off. The filtrate was concentrated under reduced pressure to provide **DI-1399** (0.28 g, 87%), white solid. The crude residue containing product was used in the next step without any purification. $^1$H NMR (400 MHz, $D_2O$) δ 3.41 – 3.33 (m, 2H), 3.15 (s, 9H), 2.53 (t, *J* = 7.1 Hz, 2H), 2.16 – 2.05 (m, 2H). $^{13}$C NMR (101 MHz, $D_2O$) δ 176.4, 65.3 [t, $^1J_{(N,C)}$ = 3.1 Hz], 52.8 [t, $^1J_{(N,C)}$ = 4.0 Hz], 29.9 [unresolved t, $^1J$ = 1.7 Hz], 17.9. HRMS calculated for $C_7H_{16}NO_2$: 146.1181, found: 146.1185 $[M]^+$.

**4,4'-Oxybis(*N*,*N*,*N*-trimethyl-4-oxobutan-1-aminium) hexafluorophosphate (DI-1400)**

To a stirred solution of **DI-1399** (100 mg, 0.34 mmol) in MeCN (3.0 mL), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (35 mg, 0.17 mmol) was added and the mixture was stirred at rt for 1 h. Precipitate was filtered off, filtrate concentrated under reduced pressure to the volume of 0.4 mL and used in the next step without purification.

**γ-Butyrobetainyl-CoA (compound 1; DI-1382)**

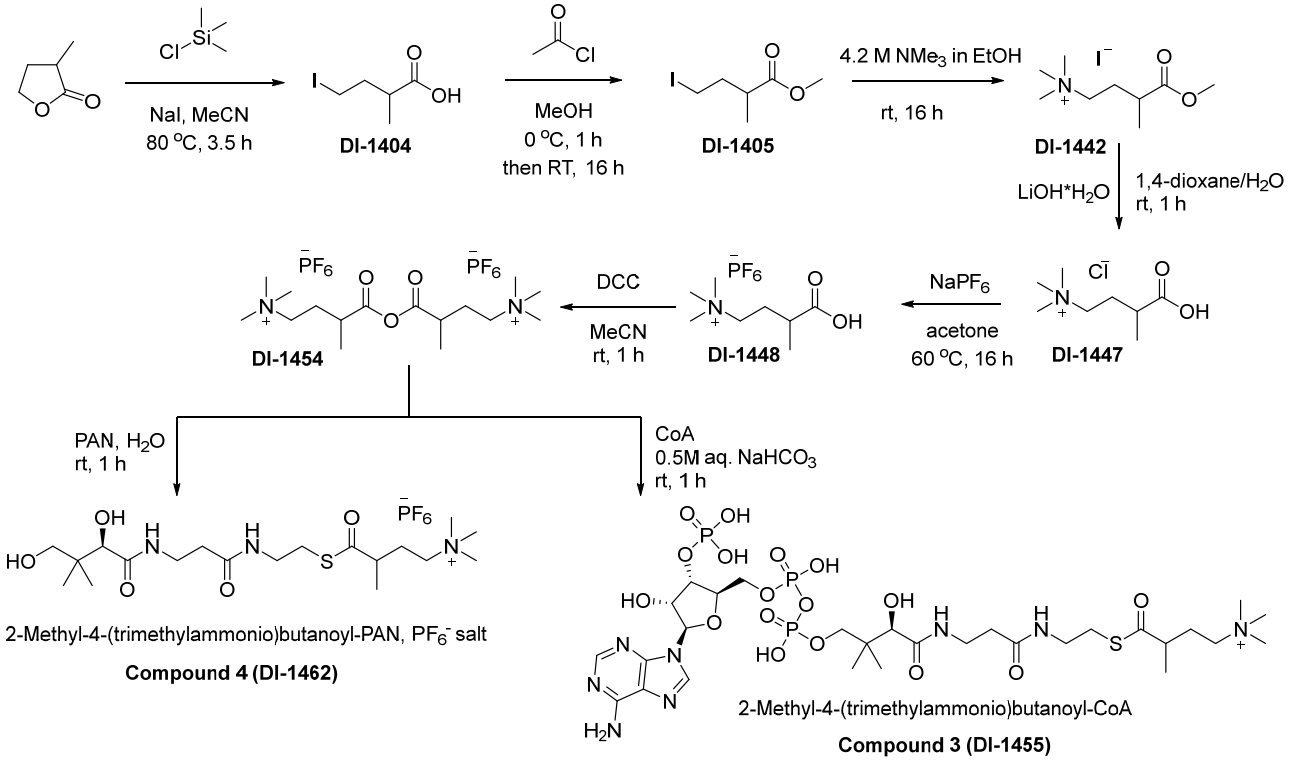
To the solution of coenzyme A as a sodium salt (40 mg, 52 μmol dissolved in 0.73 mL of 0.5 M aq. $NaHCO_3$) solution of anhydride **DI-1400** (94 mg, 0.17 mmol) was added, and the mixture was stirred at rt for 30 min. Next, the mixture was quenched with dropwise addition of 1 M aq. HCl to adjust the pH 3. Precipitate was filtered off, MeCN was evaporated from the filtrate under reduced pressure and the residue was purified by flash chromatography on RP-C18, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 1** (17 mg, 37%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 8.70 (s, 1H, Ade-8), 8.46 (s, 1H, Ade-2), 6.24 (d, J = 5.2 Hz, 1H, Rib-1'), 4.95 – 4.86 (m, 2H, Rib-2',3'), 4.67 – 4.60 (m, 1H, Rib-4'), 4.36 – 4.24 (m, 2H, Pan-1), 4.07 (s, 1H, Pan-3), 3.90 (dd, *J* = 9.8, 4.6 Hz, 1H, Rib-5'$_a$), 3.65 (dd, *J* = 9.8, 4.5 Hz, 1H, Rib-5'$_b$), 3.49 (t, *J* = 6.6 Hz, 2H, βA-1), 3.42 – 3.28 (m, 4H, CA-1, Ac-4), 3.14 (s, 9H, $NMe_3$), 3.05 (t, *J* = 6.3 Hz, 2H, CA-2), 2.79 (t, *J* = 7.1 Hz, 2H, Ac-2), 2.47 (t, *J* = 6.5 Hz, 2H, βA-2), 2.20 – 2.09 (m, 2H, Ac-3), 0.98 (s, 3H, Pan-Me$_a$), 0.86 (s, 3H, Pan-Me$_b$). $^{13}$C NMR (101 MHz, $D_2O$) δ 201.5, 174.7, 174.0, 149.9, 148.5, 144.7, 142.5, 118.5, 87.5, 83.5, 74.2, 74.1, 74.0, 73.9, 71.9, 65.1, 65.0, 52.8 [t, $^1J_{(N,C)}$ = 4.1 Hz], 39.3, 38.4, 38.3, 35.4, 35.3, 28.3, 20.9, 18.4, 18.3. HRMS calculated for $C_{28}H_{50}N_8O_{17}P_3S$: 895.2227, found: 895.2232 $[M]^+$.

**γ-Butyrobetainyl-SNAC, $Cl^-$ salt (compound 2; DI-1502)**

To a solution of **DI-1399** (50 mg, 0.17 mmol) and $Et_3N$ (59 μL, 0.43 mmol) in MeCN (1.0 mL) HBTU (65 mg, 0.17 mmol) was added. The mixture was stirred at rt for 30 min. Next, *N*-acetylcysteamine (SNAC, 23 mg, 0.19 mmol) was added and reaction mixture was stirred at rt for 1 h. Solvent was evaporated from the

reaction mixture under reduced pressure. The residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 2** (13 mg, 27%), as a colorless foam. $^{1}$H NMR (400 MHz, $D_2O$) δ 3.43 – 3.32 (m, 4H, Ac-4, CA-1), 3.15 (s, 9H, $NMe_3$), 3.11 – 3.05 (m, 2H, CA-2), 2.80 (t, *J* = 7.1 Hz, 2H, Ac-2), 2.22 – 2.08 (m, 2H, Ac-3), 1.98 (s, 3H, Ac). $^{13}$C NMR (101 MHz, $D_2O$) δ 201.5, 174.3, 65.1 [t, $^{1}J_{(N,C)}$ = 3.1 Hz], 52.8 [t, $^{1}J_{(N,C)}$ = 4.0 Hz], 39.2 [unresolved t, $^{1}J$ = 1.7 Hz], 38.5, 28.2, 21.8, 18.4. HRMS calculated for $C_{11}H_{23}N_2O_2S$: 247.1480, found: 247.1487 $[M]^+$.

*Compounds **3 (DI-1455)** and **4 (DI-1462)***




Scheme S2. Synthesis of compounds 3 (DI-1455) and 4 (DI-1462).

**4-Iodo-2-methylbutanoic acid (DI-1404)** J. Med. Chem. 2014, 57, 2213−2236.

γ-Valerolactone (5.5 g, 54.9 mmol) was added to a solution of NaI (11.1 g, 74.2 mmol) in MeCN (50 mL), followed by trimethylsilyl chloride (TMSCl) (9.4 mL, 74.2 mmol). The mixture was stirred at 80 °C for 3.5 h. The mixture was cooled down to room temperature, poured into ice/water (120 mL) and stirred for 20 min. The mixture was extracted with $Et_2O$ (4 × 50 mL), organic layer was washed with $H_2O$ (2 × 40 mL), 5% aq. $Na_2S_2O_3$ solution (20 mL) and aq. saturated NaCl (2 × 20 mL). The organic layer was evaporated at 20 °C/130 mbar to afford **DI-1404** (12.0 g, 96%), as a slightly brown oil.

**Methyl 4-iodo-2-methylbutanoate (DI-1405)** J. Med. Chem. 2014, 57, 2213−2236

Acid **DI-1404** (12.00 g, 52.6 mmol) was added to a solution of cold MeOH (100 mL) and acetyl chloride (5.60 mL, 78.90 mmol). Resulting mixture was stirred at 0 °C during 1 h, then overnight at room temperature. Reaction mixture was poured into ice/water (120 mL), extracted with $Et_2O$ (2 × 50 mL), organic layer was washed successively with brine (20 mL), aq. saturated $NaHCO_3$ solution (20 mL), 5% aq. $Na_2S_2O_3$ solution (20 mL), and brine (2 × 20 mL). Organic layer was evaporated at 30 °C/95 mbar. The residue was purified by column chromatography on silica, eluting with $CHCl_3$ in *n*-hexane (gradient from 20% to 65%) to afford **DI-1405** (8.70 g, 68%), as a slightly brown oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 3H), 3.18 (t, *J* = 7.2 Hz, 2H), 2.68 – 2.55 (m, 1H), 2.29 – 2.15 (m, 1H), 1.96 – 1.83 (m, 1H), 1.17 (d, *J* = 7.0 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 176.0, 51.9, 40.2, 37.2, 16.7, 3.5. GC−MS (EI, 70 eV) *m/z* ($I_{rel}$, %) 115.1 $[M - I]^+$ (100), 183.0 $[M - COOCH_3]^+$ (13), 211.0 $[M - CH_3O]^+$ (18).

**4-Methoxy-*N*,*N*,*N*,3-tetramethyl-4-oxobutan-1-aminium iodide (DI-1442)**

To a solution of 4.2 M $Me_3N$ in EtOH (2.95 mL, 12.39 mmol) was added **DI-1405** (2.00 g, 8.26 mmol) solution in EtOH (1 mL) dropwise at 0 °C. Reaction mixture was stirred at rt for 16 h. Obtained precipitate was collected by filtration, washed successively with $Et_2O$ (3 × 5 mL), EtOAc (2 × 5 mL), dried in vacuo to afford **DI-1442** (1.85 g, 74%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 3.76 (s, 3H), 3.40 (pd, *J* = 12.7, 4.9 Hz, 2H), 3.16 (s, 9H), 2.75 – 2.63 (m, 1H), 2.25 – 2.10 (m, 1H), 2.00 (tt, *J* = 12.0, 5.5 Hz, 1H), 1.26 (d, *J* = 7.1 Hz,

3H). $^{13}$C NMR (101 MHz, $D_2O$) δ 178.1, 64.4 [t, $^1J_{(N,C)}$ = 3.1 Hz], 52.9 [t, $^1J_{(N,C)}$ = 4.0 Hz], 52.6, 36.6, 36.6, 36.6, 25.8, 16.3. HRMS calculated for $C_9H_{20}NO_2$: 174.1494, found: 174.1502 $[M]^+$.

**3-Carboxy-*N*,*N*,*N*-trimethylbutan-1-aminium chloride (DI-1447)**

To a solution **of DI-1442** (1.82 g, 6.00 mmol) in 1,4-dioxane/$H_2O$ (12 mL, 1:1) solid LiOH*$H_2O$ (0.38 g, 9.00 mmol) was added. The reaction mixture was stirred at rt for 1 h. Then it was acidified with 1M aq. HCl to pH 3 and concentrated under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0.01% HCl in water to afford **DI-1447** (0.71 g, 60%), as a yellowish oil. $^1$H NMR (400 MHz, $D_2O$) δ 3.45 – 3.28 (m, 2H), 3.13 (s, 9H), 2.68 – 2.53 (m, 1H), 2.19 – 2.08 (m, 1H), 2.01 – 1.89 (m, 1H), 1.24 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $D_2O$) δ 180.2, 178.1, 64.4 [t, $^1J_{(N,C)}$ = 3.2 Hz], 52.8 [t, $^1J_{(N,C)}$ = 4.1 Hz], 52.5, 37.0, 25.9, 25.7, 16.6, 16.2. HRMS calculated for $C_8H_{18}NO_2$: 160.1338, found: 160.1347 $[M]^+$.

**3-Carboxy-*N*,*N*,*N*-trimethylbutan-1-aminium hexafluorophosphate (DI-1448)**

Acid **DI-1447** (0.71 g, 3.63 mmol) was suspended in acetone (10 mL), $NaPF_6$ (0.73 g, 4.35 mmol) was added and mixture was stirred at 60 °C for 16 h. The mixture was cooled down to rt, concentrated under reduced pressure. After addition of water (10 mL) to the oily residue, precipitate formed. Obtained precipitate was collected by filtration and dried in vacuo to afford **DI-1448** (0.32 g, 29%), slightly yellow solid. $^1$H NMR (400 MHz, $D_2O$) δ 3.46 – 3.29 (m, 2H), 3.13 (s, 9H), 2.66 – 2.54 (m, 1H), 2.20 – 2.06 (m, 1H), 2.03 – 1.90 (m, 1H), 1.25 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $D_2O$) δ 179.8, 64.4 [t, $^1J_{(N,C)}$ = 3.2 Hz], 52.8 [t, $^1J_{(N,C)}$ = 4.1 Hz], 36.7, 25.8, 16.4. HRMS calculated for $C_8H_{18}NO_2$: 160.1338, found: 160.1347 $[M]^+$.

**4,4'-Oxybis(*N*,*N*,*N*,3-tetramethyl-4-oxobutan-1-aminium) hexafluorophosphate (DI-1454)**

To a stirred solution of **DI-1448** (100 mg, 0.33 mmol) in MeCN (1.5 mL), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (34 mg, 0.16 mmol) was added and mixture was stirred at rt for 1 h. Reaction mixture was concentrated under reduced pressure to the volume of 0.4 mL and used in the next step without purification.

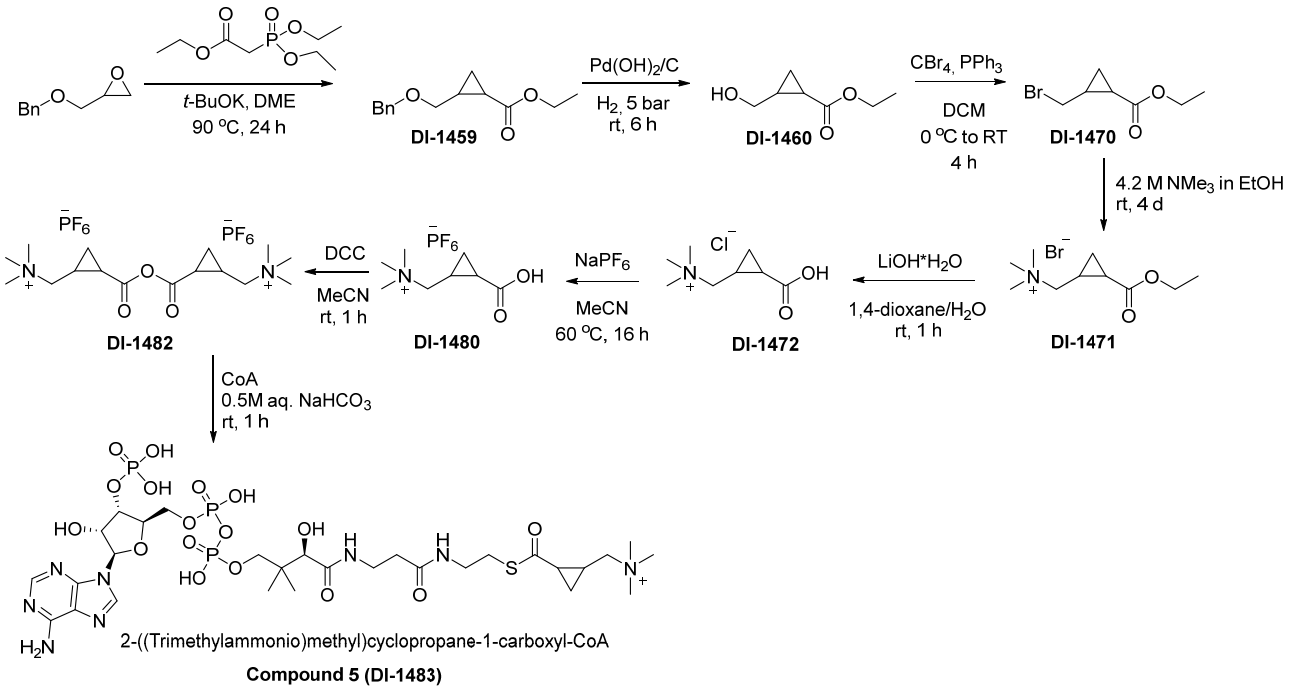
**2-Methyl-4-(trimethylammonio)butanoyl-CoA (compound 3; DI-1455)**

To the solution of coenzyme A as a sodium salt (20 mg, 26 μmol dissolved in 0.36 mL of 0.5 M aq. NaHCO3) solution of anhydride **DI-1454** (95 mg, 0.16 mmol) in MeCN (0.4 mL) was added, and the mixture was stirred at rt for 1 h. Next, the mixture was quenched with dropwise addition of 1 M aq. HCl, to adjust the pH 3. MeCN was evaporated from the reaction mixture under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 3** (16.2 mg, 67%), as a white foam. $^1$H NMR (400 MHz, $D_2O$) δ 8.58 (s, 1H, Ade-8), 8.28 (s, 1H, Ade-2), 6.18 (d, J = 7.1 Hz, 1H, Rib-1'), 4.84 – 4.80 (m, 1H, Rib-2'), 4.78 – 4.74 (m, 1H, Rib-3'), 4.61 – 4.55 (m, 1H, Rib-4'), 4.29 – 4.18 (m, 2H, Pan-1), 4.03 (s, 1H, Pan-3), 3.83 (dd, *J* = 9.9, 5.0 Hz, 1H, Rib-5'$_b$), 3.56 (dd, *J* = 9.9, 4.9 Hz, 1H, Rib-5'$_a$), 3.46 (t, *J* = 6.6 Hz, 2H, βA-1), 3.41 – 3.20 (m, 4H, Ac-4, CA-1), 3.11 (s, 9H, $NMe_3$), 3.07 – 2.93 (m, 2H, CA-2), 2.84 – 2.76 (m, 1H, Ac-2), 2.44 (t, *J* = 6.5 Hz, 2H, βA-2), 2.18 – 2.07 (m, 1H, Ac-3), 2.00 – 1.88 (m, 1H, Ac-3), 1.22 (d, J = 7.1 Hz, 3H, Ac-5), 0.89 (d, *J* = 1.1 Hz, 3H, Pan-$Me_a$), 0.76 (s, 3H, Pan-$Me_b$). $^{13}$C NMR (101 MHz, $D_2O$) δ 205.9, 174.7 [d, $^1J_{(N,C)}$ = 2.3 Hz], 174.0, 155.7, 152.9, 149.4, 139.9, 118.6, 86.5, 84.1, 74.4, 74.1, 73.6, 71.8, 65.8, 64.0, 52.8 [t, $^1J_{(N,C)}$ = 4.1 Hz], 38.4, 38.3, 35.4, 28.2, 26.0, 20.8, 18.2 [d, 1J = 2.2 Hz], 17.3 [d, 1J = 2.2 Hz]. HRMS calculated for $C_{29}H_{52}N_8O_{17}P_3S$: 909.2384, found: 909.2421 $[M]^+$.

**2-Methyl-4-(trimethylammonio)butanoyl-PAN, $PF_6^-$ salt (compound 4; DI-1462)**

Obtained from the anhydride **DI-1454** (68 mg, 0.12 mmol) and pantetheine (PAN) (16 mg, 0.06 mmol) analogous to **Compound 3**. The residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in $H_2O$. Product containing fractions were lyophilized to afford **Compound 4** (9.0 mg, 28%), as a colorless foam. $^1$H NMR (400 MHz, $D_2O$) δ 4.00 (s, 1H, Pan-3), 3.57 – 3.46 (m, 3H, Pan-1, Ac-4), 3.45 – 3.35 (m, 4H, βA-1, CA-1), 3.29 (td, *J* = 12.7, 4.6 Hz, 1H, Ac-4), 3.13 (s, 9H, $NMe_3$), 3.13 – 3.00 (m, 2H, CA-2), 2.96 – 2.84 (m, 1H, Ac-2), 2.49 (t, *J* = 6.6 Hz, 2H, βA-2), 2.18 (tdd, *J* = 12.5, 8.0, 4.6 Hz, 1H, Ac-3), 2.06 – 1.93 (m, 1H, Ac-3), 1.27 (d, *J* = 7.0 Hz, 3H, Ac-5), 0.93 (s, 3H, Pan-$Me_a$), 0.90 (s, 3H, Pan-$Me_b$). $^{13}$C NMR (101 MHz, $D_2O$) δ 206.1, 175.1, 174.0, 75.7, 68.3, 64.0 [t, $^1J_{(N,C)}$ = 3.2 Hz], 52.8 [t, $^1J_{(N,C)}$ = 4.0 Hz], 45.2, 38.6, 38.4, 35.4, 35.3, 28.1, 26.0, 20.5, 19.1, 17.4. HRMS calculated for $C_{19}H_{38}N_3O_5S$: 420.2532, found: 420.2542 $[M]^+$.

*Compound **5 (DI-1483)***




Scheme S3. Synthesis of compound 5 (DI-1483).

**Ethyl 2-((benzyloxy)methyl)cyclopropane-1-carboxylate (DI-1459)**

To a solution of triethyl phosphonoacetate (6.14 g, 27.4 mmol) in DME (45 mL) was added *t*-BuOK (3.08 g, 27.4 mmol) at 0 °C under Ar atmosphere. After addition, the mixture was stirred at 20 °C for 1 h, and then (*R*,*S*)-2-(benzyloxymethyl)oxirane (3.0 g, 18.3 mmol) in DME (4 mL) was added dropwise at 20 °C. The resulting mixture was stirred at 90 °C for 24 h. The reaction mixture was quenched by aq. satd. $NH_4Cl$ (30 mL) at 0 °C, and then diluted with EtOAc (30 mL) and extracted with EtOAc (3 × 40 mL). Combined organic layers were washed with brine and evaporated. The residue was purified by column chromatography on silica, eluting with EtOAc in *n*-hexane (gradient from 0% to 90%) to afford **DI-1459** (2.84 g, 66%), as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.24 (m, 5H), 4.52 (s, 2H), 4.12 (qd, *J* = 7.1, 1.8 Hz, 2H), 3.44 (dd, *J* = 10.4, 6.1 Hz, 1H), 3.37 (dd, *J* = 10.4, 6.5 Hz, 1H), 1.80 – 1.67 (m, 1H), 1.63 – 1.51 (m, 1H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.21 (dt, *J* = 9.0, 4.6 Hz, 1H), 0.86 (ddd, *J* = 8.4, 6.3, 4.3 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 173.9, 138.3, 128.5, 127.8, 72.8, 71.7, 60.6, 21.7, 18.7, 14.4, 13.1. HRMS calculated for $C_{14}H_{18}O_3Na$: 257.1154, found: 257.1154 $[M + Na]^+$.

**Ethyl 2-(hydroxymethyl)cyclopropane-1-carboxylate (DI-1460)**

A solution of **DI-1459** (2.84 g, 12.12 mmol) in EtOH (10 mL) was hydrogenated over $Pd(OH)_2$/C (0.31 g, 11 wt%) under hydrogen pressure (5 bar) for 6 h at rt. The reaction mixture was filtered through celite, filter cake was washed with MeOH (3 × 10 mL), filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica, eluting with EtOAc in *n*-hexane (gradient from 0% to 80%) to afford **DI-1460** (1.29 g, 74%), as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) 4.12 (q, J = 7.1, 2H), 3.62 (dd, J = 11.4, 6.0 Hz, 1H), 3.47 (dd, J = 11.4, 6.9 Hz, 1H), 1.72 (ddtd, J = 8.8, 6.9, 6.1, 4.1 Hz, 2H), 1.56 (ddd, J = 8.7, 4.8, 4.1 Hz, 1H), 1.25 (t, J = 7.1 Hz, 3H), 1.21 (dt, J = 9.0, 4.7 Hz, 1H), 0.85 (ddd, J = 8.4, 6.3, 4.3 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 173.9, 64.8, 60.7, 24.3, 18.5, 14.3, 12.8. GC−MS (EI, 70 eV) ***m/z*** ($I_{rel}$, %) 55.0 (100), 73.0 (87), 88.0 (49), 99.1 $[M – C_2H_5O]^+$ (58), 117.1 (7).

**Ethyl 2-(bromomethyl)cyclopropane-1-carboxylate (DI-1470)**

To a solution of **DI-1460** (0.5 g, 3.47 mmol) in abs. DCM (20 mL) were added $CBr_4$ (1.73 g, 5.20 mmol) and $PPh_3$ (1.36 g, 5.20 mmol) in portions at 0 °C under Ar. The reaction mixture was stirred at rt for 4 h. The organic solvent was removed under reduced pressure. The residue was purified by column chromatography on silica, eluting with 10% EtOAc in *n*-hexane to afford **DI-1470** (0.36 g, 50%), as a slightly yellow oil. $^1$H

NMR (400 MHz, $CDCl_3$) δ 4.12 (qd, J = 7.1, 1.3 Hz, 2H), 3.37 (dd, *J* = 10.5, 7.3 Hz, 1H), 3.31 (dd, *J* = 10.5, 7.5 Hz, 1H), 1.96 – 1.84 (m, 1H), 1.64 (ddd, *J* = 8.9, 5.1, 4.0 Hz, 1H), 1.44 – 1.34 (m, 1H), 1.26 (t, *J* = 7.2 Hz, 3H), 0.95 (ddd, *J* = 8.6, 6.1, 4.7 Hz, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.9, 60.9, 35.4, 24.3, 22.8, 17.04, 14.3. GC−MS (EI, 70 eV) *m/z* ($I_{rel}$, %) 99.1 (100), 127.1 [M – Br]$^+$ (32), 161.0 (44), 178.0 (62).

**1-(2-(Ethoxycarbonyl)cyclopropyl)-*N*,*N*,*N*-trimethylmethanaminium bromide (DI-1471)**

To a solution of **DI-1470** (0.36 g, 1.74 mmol) in EtOH (2 mL), 4.2 M $NMe_3$ solution in EtOH (1.24 mL, 5.22 mmol) was added under Argon. The reaction mixture was stirred at rt for 4 d. The organic solvent was removed under reduced pressure. Residue was dissolved in DCM (10 mL) and washed with $H_2O$ (10 mL). Water layer containing product was evaporated under reduced pressure to give crude **DI-1471** (0.35 g, 76%), as a white solid. Product was used in the next step without further purification. $^1$H NMR (400 MHz, $CDCl_3$) δ 4.16 – 4.02 (m, 2H), 3.88 (dd, *J* = 13.3, 6.8 Hz, 1H), 3.81 (dd, *J* = 13.4, 7.6 Hz, 1H), 3.43 (s, 9H), 1.95 (ddd, *J* = 8.9, 5.1, 4.1 Hz, 1H), 1.63 – 1.50 (m, 1H), 1.35 (dt, *J* = 8.5, 4.8 Hz, 1H), 1.27 (ddd, *J* = 8.7, 6.2, 4.7 Hz, 1H), 1.21 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.3, 67.8, 61.5, 53.6, 19.5, 15.1, 14.3, 14.1. HRMS calculated for $C_{10}H_{20}NO_2$: 186.1494, found: 186.1501 [M]$^+$.

**1-(2-Carboxycyclopropyl)-*N*,*N*,*N*-trimethylmethanaminium chloride (DI-1472)**

To a solution of **DI-1471** (0.34 g, 1.28 mmol) in 1,4-dioxane/$H_2O$ (4 mL, 1:1) solid LiOH*$H_2O$ (0.08 g, 1.92 mmol) was added. The reaction mixture was stirred at rt for 1 h. Reaction mixture was acidified with 1 M aq. HCl to pH 3 and concentrated under reduced pressure. The residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0.01% aq. HCl to afford **DI-1472** (0.22 g, 89%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 3.49 – 3.41 (m, 1H), 3.41 – 3.31 (m, 1H), 3.21 (s, 9H), 1.89 – 1.80 (m, 2H), 1.53 – 1.43 (m, 1H), 1.22 – 1.13 (m, 1H). $^{13}$C NMR (101 MHz, $D_2O$) δ 176.9, 68.3 [t, $^1J_{(N,C)}$ = 3.1 Hz], 52.9 [t, $^1J_{(N,C)}$ = 4.0 Hz], 19.3, 15.2, 13.6. HRMS calculated for $C_8H_{16}NO_2$: 158.1181, found: 158.1190 [M]$^+$.

**1-(2-Carboxycyclopropyl)-*N*,*N*,*N*-trimethylmethanaminium hexafluorophosphate (DI-1480)**

**DI-1472** (0.22 g, 1.14 mmol) was suspended in MeCN (5 mL), $NaPF_6$ (0.23 g, 1.34 mmol) was added and mixture was stirred at 60 °C for 16 h (white precipitate formed). The mixture was cooled down to rt, precipitate was filtered off. Filtrate was concentrated under reduced pressure to afford **DI-1480** (0.26 g, 75%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 3.49 – 3.39 (m, 1H), 3.38 – 3.30 (m, 1H), 3.20 (s, 9H), 1.88 – 1.78 (m, 2H), 1.52 – 1.41 (m, 1H), 1.22 – 1.11 (m, 1H). $^{13}$C NMR (101 MHz, $D_2O$) δ 177.0, 68.4 [t, $^1J_{(N,C)}$ = 3.1 Hz], 52.2 [t, $^1J_{(N,C)}$ = 4.0 Hz], 19.3, 15.1, 13.5. HRMS calculated for C8H16NO2: 158.1181, found: 158.1177 [M]$^+$.

**1,1'-((Oxybis(carbonyl))bis(cyclopropane-2,1-diyl))bis(*N*,*N*,*N*-trimethylmethanaminium) hexafluorophosphate (DI-1482)**

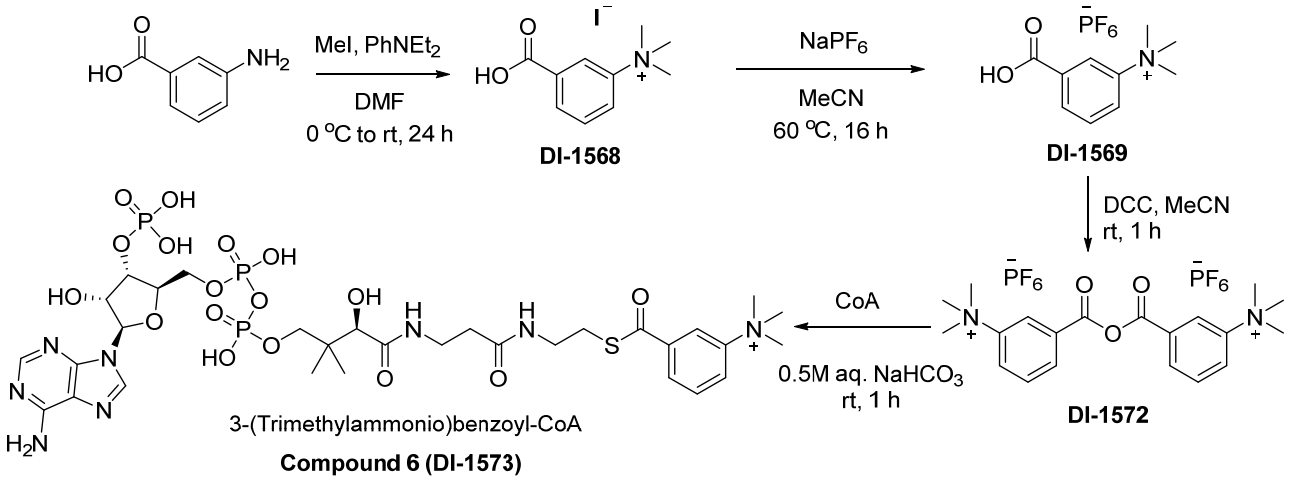
To a stirred solution of **DI-1480** (50 mg, 0.17 mmol) in MeCN (2.0 mL), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (17 mg, 0.08 mmol) was added and the mixture was stirred at rt for 1 h. Reaction mixture was concentrated under reduced pressure to the volume of 0.4 mL and used in the next step without purification.

**2-((Trimethylammonio)methyl)cyclopropane-1-carboxyl-CoA (compound 5; DI-1483)**

To the solution of coenzyme A as a sodium salt (20 mg, 26 μmol dissolved in 0.36 mL of 0.5 M aq. $NaHCO_3$) solution of anhydride **DI-1482** (47 mg, 0.08 mmol) in MeCN (0.2 mL) was added, and the mixture was stirred at rt for 1 h. Next, the mixture was quenched with dropwise addition of 1 M aq. HCl, to adjust the pH 3. MeCN was evaporated from the reaction mixture under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Product containing fractions were lyophilized to afford **Compound 5** (7.0 mg, 30%), as a white foam. $^1$H NMR (400 MHz, $D_2O$) δ 8.57 (s, 1H, Ade-8), 8.27 (s, 1H, Ade-2), 6.18 (d, *J* = 7.1 Hz, 1H, Rib-1'), 4.94 – 4.87 (m, 2H, Rib-2',3'), 4.63 – 4.54 (m, 1H, Rib-4'), 4.29 – 4.18 (m, 2H, Pan-1), 4.04 (s, 1H, Pan-3), 3.84 (dd, *J* = 9.8, 4.9 Hz, 1H, Rib-5'$_b$), 3.56 (dd, *J* = 9.9, 4.8 Hz, 1H, Rib-5'$_a$), 3.46 (t, *J* = 6.5 Hz, 2H, βA-1), 3.41 – 3.33 (m, 4H, Ac-4, CA-1), 3.17 (s, 9H, $NMe_3$), 3.05 – 2.98 (m, 2H, CA-2), 2.44 (t, *J* = 6.5 Hz, 2H, βA-2), 2.35 – 2.27 (m, 1H, Ac-2), 1.96 – 1.84 (m, 1H, Ac-3), 1.54 (dt, *J* = 9.4, 5.0 Hz, 1H, Ac-5), 1.25 – 1.16 (m, 1H, Ac-5), 0.89 (d, *J* = 1.6 Hz, 3H,

Pan-$Me_a$), 0.76 (s, 3H, Pan-$Me_b$). $^{13}C$ NMR (101 MHz, $D_2O$) δ 200.2, 174.7, 174.0, 155.7, 152.9, 149.4, 139.9, 118.6, 86.5, 84.1, 74.4, 74.1, 73.7, 71.8, 68.1, 65.8, 53.0 [t, $^1J_{(N,C)}$ = 3.9 Hz], 48.8, 38.4, 38.3, 35.4, 28.5, 28.2, 20.9, 20.8, 18.2, 17.3, 15.3. HRMS calculated for $C_{29}H_{50}N_8O_{17}P_3S$: 907.2227, found: 907.2241 $[M]^+$.

*Compound **6 (DI-1573)***




Scheme S4. Synthesis of compound 6 (DI-1573).

**3-Carboxy-*N*,*N*,*N*-trimethylbenzenaminium iodide (DI-1568)** [SI1]

Methyl iodide (2.27 mL, 36.50 mmol) was added slowly to the solution of 3-aminobenzoic acid (0.50 g, 3.65 mmol) and *N*,*N*-diethylphenylamine (1.19 mL, 7.48 mmol) in DMF (1.0 mL) at 0 °C in an ice bath. The mixture was stirred at rt for 24 h. Formed precipitate was collected by filtration and washed with DCM (5 mL), and MeCN (5 mL) to afford **DI-1568** (0.84 g, 76%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 9.55 – 9.48 (m, 1H), 9.27 (d, *J* = 7.8 Hz, 1H), 9.23 – 9.14 (m, 1H), 8.86 (t, *J* = 8.2 Hz, 1H), 4.79 (s, 9H). $^{13}$C NMR (101 MHz, $D_2O$) δ 168.6, 146.6, 132.4, 131.5, 130.9, 124.4, 121.0, 57.0. HRMS calculated for $C_{10}H_{14}NO_2$: 180.1025, found: 180.1031 $[M]^+$.

**3-Carboxy-*N*,*N*,*N*-trimethylbenzenaminium hexafluorophosphate (DI-1569)**

Acid **DI-1568** (0.20 g, 0.65 mmol) was suspended in MeCN (3.0 mL), $NaPF_6$ (0.13 g, 0.78 mmol) was added and mixture was stirred at 60 °C for 16 h (white precipitate formed). The mixture was cooled down to rt, precipitate was filtered off. Filtrate was concentrated under reduced pressure and residue was purified by flash chromatography on RP-$C_{18}$, eluted with $H_2O$ to afford **DI-1569** (70 mg, 33%), as a slightly yellow solid. $^1$H NMR (400 MHz, MeOD) δ 8.52 – 8.46 (m, 1H), 8.27 – 8.19 (m, 2H), 7.79 (t, *J* = 8.1 Hz, 1H), 3.77 (s, 9H). $^{13}$C NMR (101 MHz, MeOD) δ 167.6, 148.7, 134.8, 132.5, 132.1, 125.6, 122.1, 57.9. HRMS calculated for $C_{10}H_{14}NO_2$: 180.1025, found: 180.1034 $[M]^+$.

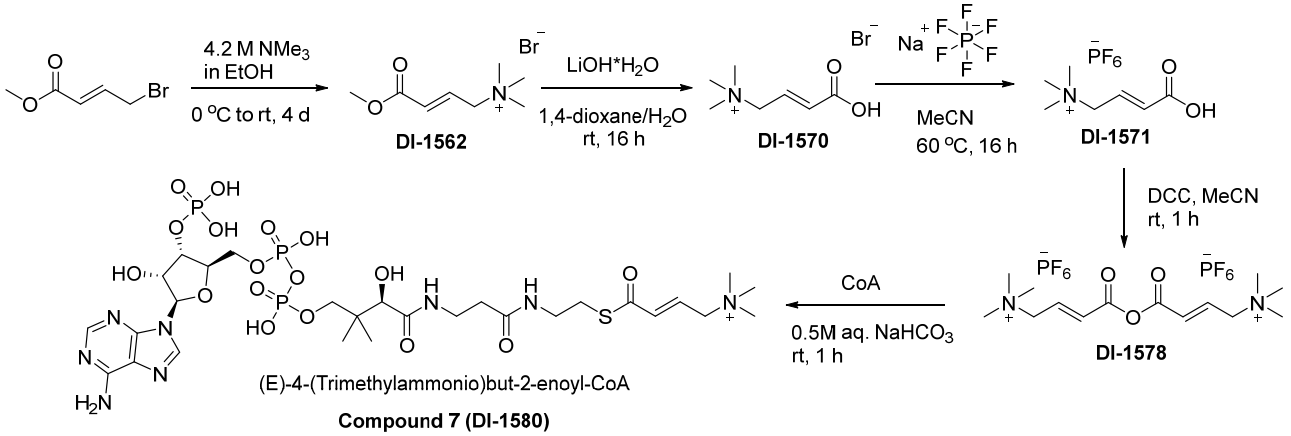
**3,3'-(Oxybis(carbonyl))bis(*N*,*N*,*N*-trimethylbenzenaminium) hexafluorophosphate (DI-1572)**

To a stirred solution of **DI-1569** (60 mg, 0.18 mmol) in MeCN (1.0 mL), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (19 mg, 0.09 mmol) was added and the mixture was stirred at rt for 30 min. Reaction mixture was concentrated under reduced pressure to the volume of 0.4 mL and used in the next step without purification.

**3-(Trimethylammonio)benzoyl-CoA (compound 6; DI-1573)**

To the solution of Coenzyme A as a sodium salt (20 mg, 26 μmol dissolved in 0.36 mL of 0.5 M aq. $NaHCO_3$) solution of **DI-1572** (57 mg, 0.09 mmol) in MeCN (0.4 mL) was added and the mixture was stirred at rt for 1 h. Next, the mixture was quenched with dropwise addition of 1 M aq. HCl, to adjust the pH 3. MeCN was evaporated from the reaction mixture under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 6** (6.0 mg, 25%), as a white foam. $^1$H NMR (400 MHz, $D_2O$) δ 8.69 (br s, 1H, Ar-2), 8.43 (s, 1H, Ade-8), 8.29 (dd, *J* = 2.9, 1.4 Hz, 1H, Ade-2), 8.20 – 8.10 (m, 2H, Ar-3,4), 7.80 (t, *J* = 8.2 Hz, 1H, Ar-5), 6.18 (d, *J* = 4.8 Hz, 1H, Rib-1'), 4.96 – 4.83 (m, 2H, Rib-2',3'), 4.64 – 4.52 (m, 1H, Rib-4'), 4.38 – 4.22 (m, 2H, Pan-1), 4.01 (s, 1H, Pan-3), 3.93 – 3.81 (m, 1H, Rib-5'$_b$), 3.71 (s, 9H, $NMe_3$), 3.67 – 3.57 (m, 1H, Rib-5'$_a$), 3.55 – 3.41 (m, 4H, βA-1, CA-1), 3.28 (t, *J* = 6.2 Hz, 2H, CA-2), 2.47 (t, *J* = 6.4 Hz, 2H, βA-2), 0.93 (s, 3H, Pan-Me$_a$), 0.80 (s, 3H, Pan-Me$_b$). $^{13}$C NMR (101 MHz, $D_2O$) δ 193.1, 174.7, 174.1, 149.8, 146.9, 144.7, 142.5, 138.1, 131.4, 129.3, 124.9, 118.1, 87.5, 83.4, 74.2, 73.9, 72.0, 65.1, 57.0, 38.4, 35.4, 35.3, 28.7, 20.8, 18.3. HRMS calculated for $C_{31}H_{48}N_8O_{17}P_3S$: 929.2071, found: 929.2081 $[M]^+$.

*Compound **7 (DI-1580)***




Scheme S5. Synthesis of compound 7 (DI-1580).

**(*E*)-4-Methoxy-*N*,*N*,*N*-trimethyl-4-oxobut-2-en-1-aminium bromide (DI-1562)**

To a solution of 4.2 M $Me_3N$ in EtOH (4.95 mL, 20.80 mmol) was added methyl-4-bromocrotonate (0.71 mL, 5.20 mmol) solution in EtOH (1 mL) dropwise at 0 °C. Reaction was stirred at rt for 4 d. Formed precipitate was collected by filtration, washed successively with $Et_2O$ (3 × 5 mL), EtOAc (2 × 5 mL), dried in vacuo to afford **DI-1562** (0.85 g, 69%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 7.11 – 6.97 (m, 1H), 6.48 – 6.38 (m, 1H), 4.18 (d, *J* = 7.5 Hz, 2H), 3.84 (s, 3H), 3.20 (s, 9H). $^{13}$C NMR (101 MHz, $D_2O$) δ 167.1, 133.0, 131.7, 65.9 [t, $^1J_{(N,C)}$ = 3.4 Hz], 53.0 [t, $^1J_{(N,C)}$ = 3.9 Hz], 52.6. HRMS calculated for $C_8H_{16}NO_2$: 158.1181, found: 158.1183 $[M]^+$.

**(*E*)-3-Carboxy-*N*,*N*,*N*-trimethylprop-2-en-1-aminium bromide (DI-1570)**

To a solution of **DI-1562** (0.85 g, 3.55 mmol) in 1,4-dioxane/$H_2O$ (12 mL, 1:1) LiOH*$H_2O$ (0.22 g, 5.32 mmol) was added. The reaction mixture was stirred at rt for 16 h. Reaction mixture was acidified with 1 M aq. HCl to pH 1 and concentrated under reduced pressure. The residue was purified by flash chromatography on RP-$C_{18}$, eluted with $H_2O$ to afford **DI-1570** (0.61 g, 77%), as a slightly brown oil. $^1$H NMR (400 MHz, $D_2O$) δ 7.12 – 6.93 (m, 1H), 6.45 – 6.34 (m, 1H), 4.18 (d, *J* = 7.6 Hz, 2H), 3.20 (s, 9H). $^{13}$C NMR (101 MHz, $D_2O$) δ 168.4, 133.0, 132.4, 65.9 [t, $^1J_{(N,C)}$ = 3.4 Hz], 53.02 [t, $^1J_{(N,C)}$ = 3.9 Hz]. HRMS calculated for $C_7H_{14}NO_2$: 144.1025, found: 144.1026 $[M]^+$.

**(*E*)-3-carboxy-*N*,*N*,*N*-trimethylprop-2-en-1-aminium hexafluorophosphate (DI-1571)**

Acid **DI-1570** (0.61 g, 2.72 mmol) was suspended in MeCN (8.0 mL), $NaPF_6$ (0.55 g, 3.27 mmol) was added and mixture was stirred at 60 °C for 16 h (white precipitate formed). The mixture was cooled down to rt, precipitate was filtered off. Filtrate was concentrated under reduced pressure (a slightly brown oil) and triturated with acetone (10 mL). Solidified material was collected by filtration to afford product **DI-1571** (0.35 g, 45%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 7.09 – 6.94 (m, 1H), 6.46 – 6.32 (m, 1H), 4.17 (d, *J* = 7.6 Hz, 2H), 3.19 (s, 9H). $^{13}$C NMR (101 MHz, $D_2O$) δ 168.4, 133.0, 132.5, 65.9 [t, $^1J_{(N,C)}$ = 3.3 Hz], 53.0 [t, $^1J_{(N,C)}$ = 3.8 Hz]. HRMS calculated for $C_7H_{14}NO_2$: 144.1025, found: 144.1032 $[M]^+$.

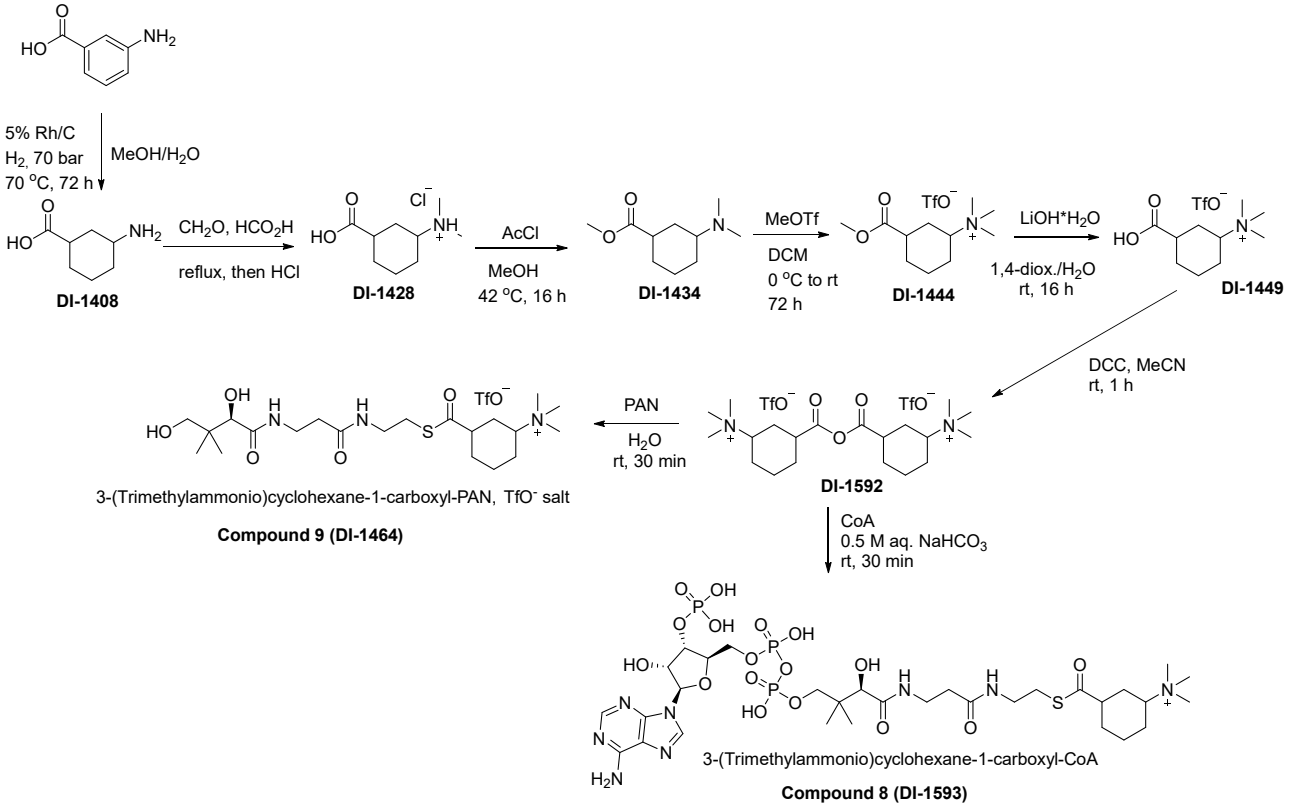
**(*E*)-*N*,*N*,*N*-trimethyl-4-oxo-4-(((*E*)-4-(trimethylammonio)but-2-enoyl)oxy)but-1-en-1-aminium hexafluorophosphate (DI-1578)**

To a stirred solution of **DI-1571** (30 mg, 0.11 mmol) in MeCN (1.0 mL), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (11 mg, 0.05 mmol) was added and the mixture was stirred at rt for 1 h. Reaction mixture was concentrated under reduced pressure to the volume of 0.4 mL and used in the next step without purification.

**(*E*)-4-(Trimethylammonio)but-2-enoyl-CoA (compound 7; DI-1580)**

To the solution of coenzyme A as a sodium salt (20 mg, 26 μmol dissolved in 0.36 mL of 0.5 M aq. $NaHCO_3$) solution of anhydride **DI-1578** (29 mg, 0.05 mmol) in MeCN (0.4 mL) was added and the mixture was stirred at rt for 1 h. Next, the mixture was quenched with dropwise addition of 1 M aq. HCl, to adjust the pH 3. MeCN was evaporated from the reaction mixture under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 7** (7.0 mg, 30%), as a white foam. $^1$H NMR (400 MHz, $D_2O$) δ 8.68 (s, 1H, Ade-8), 8.46 (s, 1H, Ade-2), 6.92 (dt, *J* = 15.1, 7.5 Hz, 1H, Ac-3), 6.71 (d, *J* = 15.4 Hz, 1H, Ac-2), 6.28 – 6.17 (m, 1H, Rib-1'), 4.97 – 4.87 (m, 2H, Rib-2',3'), 4.66 – 4.58 (m, 1H, Rib-4'), 4.35 – 4.24 (m, 2H, Pan-1), 4.20 – 4.10 (m, 2H, Ac-4), 4.05 (s, 1H, Pan-3), 3.90 (dd, *J* = 9.9, 4.1 Hz, 1H, Rib-5'$_b$), 3.64 (dd, *J* = 9.8, 4.0 Hz, 1H, Rib-5'$_a$), 3.48 (t, *J* = 6.6 Hz, 2H, βA-1), 3.43 (t, *J* = 6.2 Hz, 2H, CA-1), 3.28 – 3.05 (m, 11H, CA-2, $NMe_3$), 2.47 (t, *J* = 6.5 Hz, 2H, βA-2), 0.97 (s, 3H, Pan-Me$_a$), 0.85 (s, 3H, Pan-Me$_b$). $^{13}$C NMR (101 MHz, $D_2O$) δ 191.8, 174.7, 174.1, 149.9, 148.5, 144.7, 142.5, 137.7, 128.6, 118.5, 87.5, 83.5, 74.2, 73.9, 72.0, 65.2, 65.1, 53.0, 38.3, 35.4, 35.3, 28.4, 20.8, 18.3. HRMS calculated for $C_{28}H_{48}N_8O_{17}P_3S$: 893.2071, found: 893.2079 $[M]^+$.

*Compounds **8 (DI-1593)** and **9 (DI-1464)***




Scheme S6. Synthesis of compounds 8 (DI-1593) and 9 (DI-1464).

**3-Aminocyclohexane-1-carboxylic acid (DI-1408)** [SI2]

Solution of 3-aminobenzoic acid (1.00 g, 7.29 mmol) in MeOH (38 mL) and $H_2O$ (2 mL) was hydrogenated on 5% Rh/C (20 wt%, 0.20 g) at 70 bar and 70 °C for 72 h. Catalyst was filtered off on a celite pad and washed with MeOH (3 × 15 mL). The filtrate was concentrated to 5 mL to effect crystallization. Precipitate was collected by filtration to provide **DI-1408** (0.77 g, 74%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 3.29 – 3.16 (m, 1H), 2.28 (tt, *J* = 12.1, 3.4 Hz, 1H), 2.23 – 2.13 (m, 1H), 2.06 – 1.97 (m, 1H), 1.97 – 1.87 (m, 2H), 1.52 – 1.19 (m, 4H). $^{13}$C NMR (101 MHz, $D_2O$) δ 183.8, 49.8, 44.8, 33.4, 29.8, 28.4, 23.2. HRMS calculated for $C_7H_{14}NO_2$: 144.1025, found: 144.1033 $[M + H]^+$.

**3-Carboxy-*N*,*N*-dimethylcyclohexan-1-aminium chloride (DI-1428)** [SI3]

A mixture of **DI-1408** (1.31 g, 9.15 mmol), 37% aq. formaldehyde (3.60 mL, 46.65 mmol) and formic acid (3.69 mL, 97.80 mmol) was stirred at reflux for 16 h. The mixture was cooled down to rt and concd. aq. HCl (2.44 mL, 29.28 mmol) was added. Solvent was removed under reduced pressure, the residue was dissolved in 2-propanol (5 mL) and sedimented by addition of $Et_2O$ (17 mL). Precipitate was collected by filtration, washed with $Et_2O$ and dried in vacuo to afford **DI-1428** (1.30 g, 68%), as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.37 (br s, 1H), 10.95 (br s, 1H), 3.23 – 3.08 (m, 1H), 2.65 (d, *J* = 3.8 Hz, 6H), 2.33 (tt, *J* = 12.1, 3.4 Hz, 1H), 2.28 – 2.22 (m, 1H), 2.08 – 1.95 (m, 1H), 1.90 – 1.79 (m, 2H), 1.45 (q, *J* = 12.2 Hz, 1H), 1.39 – 1.11 (m, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 175.3, 62.7, 41.10, 38.7, 38.7, 28.1, 27.6, 25.1, 23.2. HRMS calculated for $C_9H_{18}NO_2$: 172.1338, found: 172.1345 $[M + H]^+$.

**Methyl 3-(dimethylamino)cyclohexane-1-carboxylate (DI-1434)**

**DI-1428** (1.30 g, 6.25 mmol) was added to a solution of cold MeOH (40 mL) and AcCl (0.89 mL, 12.50 mmol). Resulting mixture was stirred at 42 °C during 16 h. Reaction mixture was concentrated under reduced pressure, DCM (45 mL) and $K_2CO_3$ (0.95 g, 6.88 mmol) were added, and the mixture was stirred at rt for 16 h. Precipitate was filtered off, filtrate was evaporated to afford **DI-1434** (0.95 g, 82%), as a slightly

brown oil. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 3.58 (s, 3H), 2.31 (tt, $J$ = 12.0, 3.5 Hz, 1H), 2.23 (tt, $J$ = 11.5, 3.4 Hz, 1H), 2.16 (s, 6H), 1.99 – 1.91 (m, 1H), 2.00 – 1.90 (m, 3H), 1.32 – 1.02 (m, 4H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 175.1, 61.7, 51.3, 41.9, 40.9, 30.3, 28.4, 27.5, 24.0. HRMS calculated for $C_{10}H_{20}NO_2$: 186.1494, found: 186.1501 $[M + H]^+$.

**3-(Methoxycarbonyl)-*N*,*N*,*N*-trimethylcyclohexan-1-aminium trifluoromethanesulfonate (DI-1444)**

To the **DI-1434** (0.95 g, 5.13 mmol) dissolved in DCM (5 mL), MeOTf (0.62 mL, 5.64 mmol) was added at 0 °C and resulting mixture was stirred at rt for 72 h. The reaction mixture was concentrated under reduced pressure, solid residue granulated in $Et_2O$ (25 mL). Obtained precipitate was collected by filtration to afford **DI-1444** (1.45 g, 81%), as a white solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 3.63 (s, 3H), 3.43 – 3.35 (m, 1H), 3.01 (s, 9H), 2.48 – 2.40 (m, 1H), 2.40 – 2.31 (m, 1H), 2.17 – 2.07 (m, 1H), 1.98 – 1.80 (m, 2H), 1.54 (q, $J$ = 12.1 Hz, 1H), 1.47 – 1.18 (m, 3H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 174.0, 71.85 [t, $^1J_{(N,C)}$ = 2.4 Hz], 51.7, 50.5 [t, $^1J_{(N,C)}$ = 4.0 Hz], 41.5, 27.8, 27.2, 24.7, 23.5. HRMS calculated for $C_{11}H_{22}NO_2$: 200.1651, found: 200.1659 $[M]^+$.

**3-Carboxy-*N*,*N*,*N*-trimethylcyclohexan-1-aminium trifluoromethanesulfonate (DI-1449)**

To a solution of **DI-1444** (1.42 g, 4.06 mmol) in 1,4-dioxane/$H_2O$ (10 mL, 1:1) solid LiOH*$H_2O$ (0.26 g, 6.10 mmol) was added. The reaction mixture was stirred at rt for 16 h. Reaction mixture was acidified with 1 M aq. HCl to pH 1 and concentrated under reduced pressure. The residue was purified by flash chromatography on RP-$C_{18}$, eluted with $H_2O$ to afford **DI-1449** (1.20 g, 88%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 3.42 (tt, J = 12.0, 3.2 Hz, 1H), 3.10 (s, 9H), 2.62 – 2.46 (m, 2H), 2.31 – 2.21 (m, 1H), 2.14 – 1.99 (m, 2H), 1.67 (q, J = 12.0 Hz, 1H), 1.60 – 1.26 (m, 3H). $^{13}$C NMR (101 MHz, $D_2O$) δ 178.8, 73.1 [t, $^1J_{(N,C)}$ = 2.2 Hz], 50.8 [t, $^1J_{(N,C)}$ = 4.2 Hz], 42.0, 27.8, 27.3, 25.0, 23.5. HRMS calculated for $C_{10}H_{20}NO_2$: 186.1494, found: 186.1501 $[M]^+$.

**3,3'-(Oxybis(carbonyl))bis(*N*,*N*,*N*-trimethylcyclohexan-1-aminium) trifluoromethanesulfonate (DI-1592)**

To a stirred solution of **DI-1449** (0.10 g, 0.30 mmol) in MeCN (2.0 mL), *N*,*N*'-dicyclohexylcarbodiimide (DCC) (31 mg, 0.15 mmol) was added and the mixture was stirred at rt for 1 h. Reaction mixture was concentrated under reduced pressure to the volume of 0.4 mL and used in the next step without purification.

**3-(Trimethylammonio)cyclohexane-1-carboxyl-CoA (compound 8; DI-1593)**

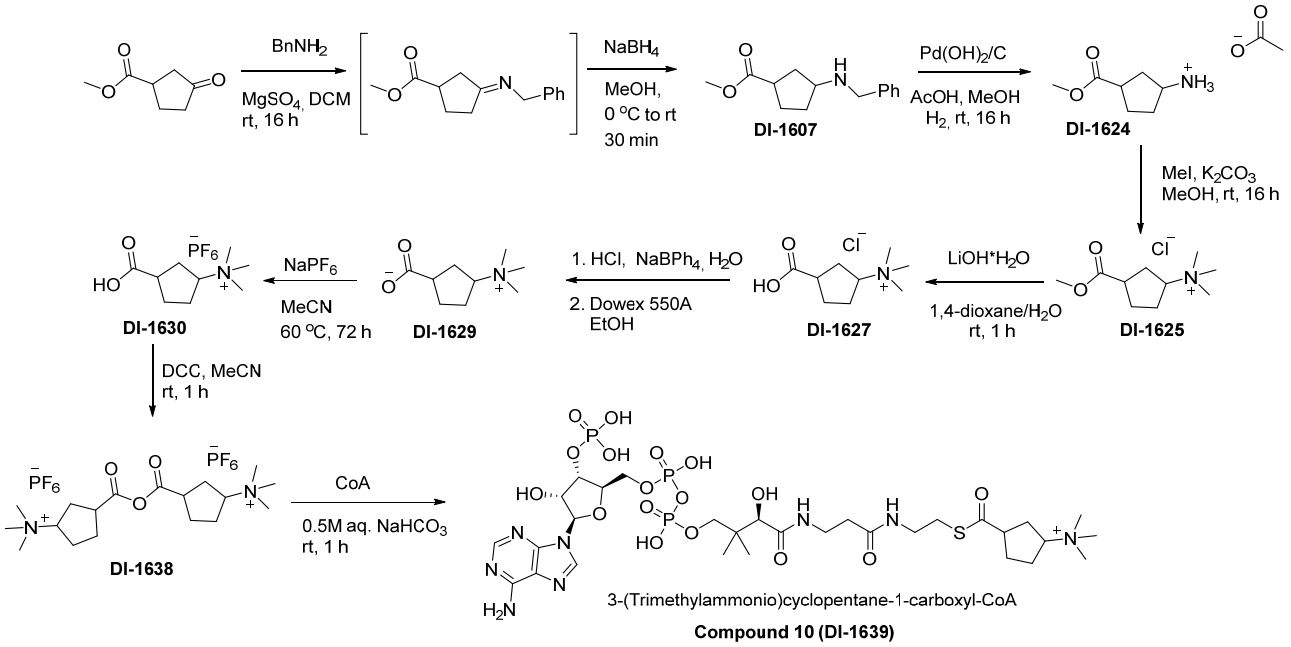
To the solution of coenzyme A as a sodium salt (20 mg, 26 μmol dissolved in 0.36 mL of 0.5 M aq. $NaHCO_3$) solution of anhydride **DI-1592** (51 mg, 0.08 mmol) in MeCN (0.2 mL) was added and the mixture was stirred at rt for 30 min. Next, the mixture was quenched with dropwise addition of 1 M aq. HCl to adjust the pH 3. MeCN was evaporated from the reaction mixture under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 8** (9.0 mg, 38%), as a white foam. $^1$H NMR (400 MHz, $D_2O$) δ 8.67 (s, 1H, Ade-8), 8.45 (s, 1H, Ade-2), 6.22 (d, J = 5.5 Hz, 1H, Rib-1'), 4.96 – 4.86 (m, 2H, Rib-2',3'), 4.65 – 4.58 (m, 1H, Rib-4'), 4.37 – 4.23 (m, 2H, Pan-1), 4.04 (s, 1H, Pan-3), 3.94 – 3.84 (m, 1H, Rib-5'$_b$), 3.71 – 3.60 (m, 1H, Rib-5'$_a$), 3.48 (t, J = 6.6 Hz, 2H, βA-1), 3.45 – 3.39 (m, 1H, Ac-4), 3.37 (t, J = 6.2 Hz, 2H, CA-1), 3.08 (s, 9H, $NMe_3$), 3.07 – 2.99 (m, 2H, CA-2), 2.87 – 2.75 (m, 1H, Ac-2), 2.50 – 2.39 (m, 3H, βA-2, Ac-3$_a$), 2.32 – 2.19 (m, 1H, Cy), 2.12 – 2.02 (m, 1H, Cy), 2.02 – 1.92 (m, 1H, Cy), 1.68 (q, J = 11.9 Hz, 1H, Cy), 1.58 – 1.27 (m, 3H, Cy), 0.97 (s, 3H, Pan-$Me_a$), 0.85 (s, 3H, Pan-$Me_b$). $^{13}$C NMR (101 MHz, $D_2O$) δ 204.6, 174.7, 174.0, 149.8, 148.5, 144.7, 142.5, 118.5, 87.5, 83.4, 74.3, 74.2, 73.9, 72.9 [unresolved t, $^1J_{(N,C)}$ = 1.9 Hz], 72.1, 65.1, 50.8 [t, $^1J_{(N,C)}$ = 4.0 Hz], 50.5, 38.4, 35.4, 35.3, 28.1, 28.0, 24.9, 23.4, 20.8, 18.3. HRMS calculated for $C_{31}H_{54}N_8O_{17}P_3S$: 935.2540, found: 935.2535 $[M]^+$.

**3-(Trimethylammonio)cyclohexane-1-carboxyl-PAN, $TfO^-$ salt (compound 9; DI-1464)**

Compound 9 analogously was obtained from the solution of pantetheine (PAN) (11 mg, 38 μmol dissolved in 55 μL of $H_2O$) and anhydride **DI-1592** (50 mg, 0.08 mmol) in MeCN (0.2 mL). Evaporated reaction mixture was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in $H_2O$. Target fractions were lyophilized to afford **Compound 9** (7.5 mg, 33%), as a white foam. $^1$H NMR (400 MHz, $D_2O$) δ 4.00 (s, 1H, Pan-3), 3.57 – 3.47 (m, 3H, Ac-4, Pan-1), 3.47 – 3.37 (m, 4H, CA-1, βA-1), 3.17 – 3.03 (m, 11H, CA-2, NMe3), 2.89 – 2.78 (m, 1H, Ac-2), 2.52 – 2.42 (m, 3H, Ac-3$_a$, βA-2), 2.32 – 2.21 (m, 1H, Cy), 2.13 – 2.05 (m, 1H, Cy), 2.06 – 1.95 (m, 1H, Ac-3$_b$), 1.71 (q, $J$ = 11.9 Hz, 1H, Cy), 1.61 – 1.29 (m, 3H, Cy), 0.93 (s, 3H, Pan-

$Me_a$), 0.90 (s, 3H, Pan-$Me_b$). $^{13}C$ NMR (101 MHz, $D_2O$) δ 204.7, 175.1, 174.0, 75.7, 73.0, 68.3, 50.8 [t, $^1J_{(N,C)}$ = 4.1 Hz], 50.6, 38.6, 38.5, 35.4, 35.3, 28.1, 28.0, 27.9, 24.9, 23.4, 20.5, 19.1. HRMS calculated for $C_{21}H_{40}N_3O_5S$: 446.2691, found: 446.2689 $[M]^+$.

*Compound **10 (DI-1639)***




Scheme S7. Synthesis of compound 10 (DI-1639).

**Methyl 3-(benzylamino)cyclopentane-1-carboxylate (DI-1607)**

A mixture of ketone (0.50 g, 3.52 mmol), benzylamine (0.38 g, 3.52 mmol), and powdered anhydrous $MgSO_4$ (1.27 g, 10.56 mmol) in DCM (15 mL) was stirred at rt for 16 h. Inorganics were filtered off, filtrate was concentrated under reduced pressure, and the residue was dried under vacuum (7 mmHg) for 1 h. The crude imine was dissolved in MeOH (5 mL), and the solution was cooled to 0 °C. Solid $NaBH_4$ (0.13 g, 3.46 mmol) was added at 0 °C. The reaction mixture was stirred at rt for 30 min. The organic solvent was removed under reduced pressure. The residue was purified by column chromatography on silica, eluting with EtOAc in *n*-hexane (gradient from 20% to 65%) to afford **DI-1607** (0.48 g, 59%), as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.21 (m, 5H), 3.83 – 3.72 (m, 2H), 3.73 – 3.62 (m, 3H), 3.36 – 3.11 (m, 1H), 2.88 (m, 1H), 2.24 – 2.04 (m, 1H), 1.94 – 1.63 (m, 5H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 176.8, 128.6, 128.6, 128.4, 128.3, 127.2, 127.1, 58.9, 58.6, 52.6, 52.5, 51.9, 51.8, 42.4, 42.2, 36.8, 36.5, 33.1, 32.5, 28.4, 27.7. HRMS calculated for $C_{14}H_{20}NO_2$: 234.1494, found: 234.1493 $[M + H]^+$.

**3-(Methoxycarbonyl)cyclopentan-1-aminium acetate (DI-1624)**

A solution of **DI-1607** (0.14 g, 0.60 mmol) and AcOH (40 mg, 0.66 mmol) in MeOH (5 mL) was hydrogenated over $Pd(OH)_2/C$ (20 wt%, 30 mg) under $H_2$ atmosphere (1 bar) for 72 h. The reaction mixture was filtered through a pad of celite and the filter cake was washed with MeOH (3 × 5 mL). The filtrate was concentrated under reduced pressure to afford **DI-1624** (0.11 g, 90%), as a white solid. Product was used in the next step without any purification. $^1$H NMR (400 MHz, $CDCl_3$) δ 3.70 – 3.65 (m, 3H), 3.64 – 3.48 (m, 1H), 3.13 – 2.78 (m, 1H), 2.29 – 2.17 (m, 1H), 2.17 – 1.59 (m, 9H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.9, 176.7, 176.2, 52.2, 52.0, 51.7, 42.4, 41.9, 36.3, 35.9, 32.4, 28.3, 28.1, 23.6. HRMS calculated for $C_7H_{14}NO_2$: 144.1025, found: 144.1026 $[M + H]^+$.

**3-(Methoxycarbonyl)-*N*,*N*,*N*-trimethylcyclopentan-1-aminium chloride (DI-1625)**

To a mixture of **DI-1624** (0.10 g, 0.49 mmol) in MeOH (5 mL), MeI (0.70 g, 4.90 mmol) and $K_2CO_3$ (0.34 g, 2.45 mmol, anhydrous powder) were added and the mixture was stirred vigorously for 16 h. Precipitate was filtered off and washed with MeOH (2 × 5 mL). Obtained filtrate was concentrated under reduced pressure, acidified with 1 M aq. HCl to pH 3, concentrated under reduced pressure. Obtained residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-5% of MeCN in 0.01% aq. HCl to afford **DI-1625** (75 mg, 69%), as a slightly yellow solid. $^1$H NMR (400 MHz, $D_2O$) δ 4.11 – 3.93 (m, 1H), 3.79 – 3.71 (m, 3H), 3.22 –

3.00 (m, 10H), 2.52 – 2.34 (m, 1H), 2.30 – 1.81 (m, 5H). $^{13}$C NMR (101 MHz, $D_2O$) δ 177.4, 177.3, 75.0 [t, $^1J_{(N,C)}$ = 2.5 Hz], 74.8 [t, $^1J_{(N,C)}$ = 2.5 Hz], 52.6, 52.6, 51.4, 51.3, 51.3, 51.2, 51.2, 51.1, 41.9, 41.5, 29.1, 28.5, 27.7, 26.9, 26.2, 25.2. HRMS calculated for $C_{10}H_{20}NO_2$: 186.1494, found: 186.1498 $[M]^+$.

**3-Carboxy-*N*,*N*,*N*-trimethylcyclopentan-1-aminium chloride (DI-1627)**

To a solution of **DI-1625** (0.11 g, 0.52 mmol) in dioxane/$H_2O$ (2 mL, 1:1) solid LiOH*$H_2O$ (44 mg, 1.04 mmol) was added. The reaction mixture was stirred at rt for 1 h. Reaction mixture was acidified with 1 M aq. HCl to pH 3 and concentrated under reduced pressure to afford **DI-1627** (150 mg, contains inorganic material), as a white solid. Product was used in the next step without additional purification. $^1$H NMR (400 MHz, $D_2O$) δ 4.04 – 3.84 (m, 1H), 3.08 – 2.89 (m, 10H), 2.43 – 2.23 (m, 1H), 2.15 – 1.73 (m, 5H). $^{13}$C NMR (101 MHz, $D_2O$) δ 178.9, 75.0, 74.8, 51.3 [t, $^1J_{(N,C)}$ = 4.0 Hz], 51.2 [t, $^1J_{(N,C)}$ = 4.0 Hz], 41.9, 41.5, 29.1, 28.5, 27.7, 26.9, 26.2, 25.0. HRMS calculated for $C_9H_{18}NO_2$: 172.1338, found: 172.1334 $[M]^+$.

**3-(Trimethylammonio)cyclopentane-1-carboxylate (DI-1629)**

Acid **DI-1627** (0.15 g, 0.505 mmol, 70% purity) was dissolved in $H_2O$ (3 mL) and acidified with 1 M aq. HCl to pH 3. A solution of $NaBPh_4$ (0.25 g, 0.72 mmol, 20% in $H_2O$) was added to ensure a complete precipitation of the ammonium salt (white precipitate formed). The precipitate was collected by filtration and triturated with EtOH (40 mL, partially soluble). Insolubles were filtered off and the filtrate was concentrated under reduced pressure. Residue was dissolved in EtOH (20 mL) and passed through Dowex Monosphere 550A (OH) ion-exchange resin column, followed by a slow elution with EtOH (40 mL). The eluate containing expected product was concentrated under reduced pressure and the residue was dried in vacuo to afford **DI-1629** (70 mg, 81%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 4.09 – 3.90 (m, 1H), 3.16 – 2.97 (m, 10H), 2.49 – 2.30 (m, 1H), 2.28 – 1.81 (m, 5H). $^{13}$C NMR (101 MHz, $D_2O$) δ 178.9, 178.8, 75.0 [t, $^1J_{(N,C)}$ = 2.5 Hz], 74.8 [t, $^1J_{(N,C)}$ = 2.5 Hz], 51.3 [t, $^1J_{(N,C)}$ = 4.0 Hz], 51.1 [t, $^1J_{(N,C)}$ = 4.2 Hz], 41.8, 41.5, 29.0, 28.4, 27.7, 27.0, 26.2, 25.0. HRMS calculated for $C_9H_{18}NO_2$: 172.1338, found: 172.1341 $[M + H]^+$.

**3-Carboxy-*N*,*N*,*N*-trimethylcyclopentan-1-aminium hexafluorophosphate (DI-1630)**

Betaine **DI-1629** (30 mg, 0.17 mmol) was heated at 60 °C with 1 M aq. HCl (2 mL) for 16 h. The reaction mixture was evaporated and the residue was mixed with water (5 mL) and again evaporated. The residue was suspended in MeCN (3 mL, turbid solution), $NaPF_6$ (31 mg, 0.19 mmol) was added and mixture was stirred at 60 °C for 72 h (precipitate formed). The mixture was cooled down to rt, precipitate was filtered off. Filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with $H_2O$ to afford **DI-1630** (35 mg, 65%), as a slightly yellow solid. $^1$H NMR (400 MHz, $D_2O$) δ 4.09 – 3.90 (m, 1H), 3.17 – 2.97 (m, 10H), 2.50 – 2.33 (m, 1H), 2.30 – 1.80 (m, 5H). $^{13}$C NMR (101 MHz, $D_2O$) δ 181.4, 181.3, 77.6 [t, $^1J_{(N,C)}$ = 2.5 Hz], 77.3 [t, $^1J_{(N,C)}$ = 2.5 Hz], 53.8 [t, $^1J_{(N,C)}$ = 4.0 Hz], 53.6 [t, $^1J_{(N,C)}$ = 4.0 Hz], 44.4, 44.0, 31.6, 31.0, 30.3, 29.5, 28.7, 27.5. HRMS calculated for $C_9H_{18}NO_2$: 172.1338, found: 172.1345 $[M]^+$.

**3,3'-(Oxybis(carbonyl))bis(*N*,*N*,*N*-trimethylcyclopentan-1-aminium) hexafluorophosphate (DI-1638)**

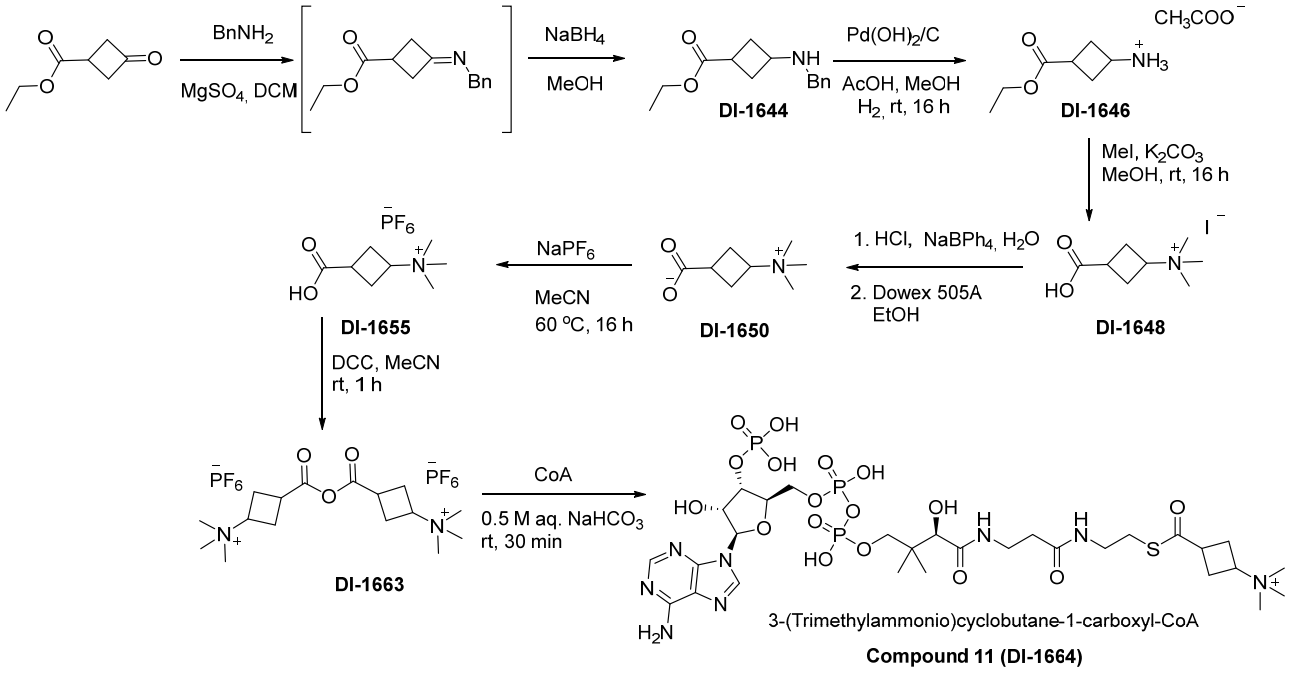
To a stirred solution of **DI-1630** (35 mg, 0.11 mmol) in MeCN (1 mL), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (12 mg, 0.06 mmol) was added and the mixture was stirred at rt for 30 min. Reaction mixture was concentrated under reduced pressure to the volume of 0.4 mL and used in the next step without purification.

**3-(Trimethylammonio)cyclopentane-1-carboxyl-CoA (compound 10; DI-1639)**

To the solution of coenzyme A as a sodium salt (20 mg, 26 μmol dissolved in 0.36 mL of 0.5 M aq. $NaHCO_3$) solution of anhydride **DI-1638** (35 mg, 0.06 mmol) in MeCN (0.4 mL) was added, and the mixture was stirred at rt for 0.5 h. Next, the mixture was quenched with dropwise addition of 1 M aq. HCl, to adjust the pH 3. MeCN was evaporated from the reaction mixture under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 10** (11 mg, 46%), as a white foam. $^1$H NMR (400 MHz, $D_2O$) δ 8.73 (br s, 1H, Ade-8), 8.47 (s, 1H, Ade-2), 6.24 (d, *J* = 4.2 Hz, 1H, Rib-1'), 5.00 – 4.86 (m, 2H, Rib-2',3'), 4.69 – 4.56 (m, 1H, Rib-4'), 4.44 – 4.19 (m, 2H, Pan-1), 4.06 (s, 1H, Pan-3), 4.04 – 3.96 (m, 1H, Ac-4), 3.96 – 3.84 (m, 1H, Rib-5'$_b$), 3.73 – 3.60 (m, 1H, Rib-5'$_a$), 3.49 (t, *J* = 6.5 Hz, 2H, βA-1), 3.44 – 3.24 (m, 3H, Ac-2, CA-1), 3.13 – 3.02 (m, 11H, CA-2, $NMe_3$), 2.46 (t, *J* = 6.6 Hz, 2H, βA-2), 2.42 – 1.77 (m, 6H, Ac-3,5,6), 0.98 (s, 3H,

Pan-$Me_a$), 0.86 (s, 3H, Pan-$Me_b$). $^{13}C$ NMR (101 MHz, $D_2O$) δ 204.6, 204.5, 174.7, 174.0, 149.9, 144.7, 142.6, 87.5, 83.4, 74.9, 74.7, 74.2, 73.9, 72.1, 65.1, 51.3 [t, $^1J_{(N,C)}$ = 4.0 Hz], 51.1 [t, $^1J_{(N,C)}$ = 4.0 Hz], 50.5, 49.9, 38.4, 35.4, 35.3, 29.2, 28.8, 28.7, 28.3, 28.2, 27.9, 26.7, 25.0, 20.8, 18.3. HRMS calculated for $C_{30}H_{52}N_8O_{17}P_3S$: 921.2384, found: 921.2381 $[M]^+$.

*Compound **11 (DI-1664)***




Scheme S8. Synthesis of compound 11 (DI-1664).

**Ethyl 3-(benzylamino)cyclobutane-1-carboxylate (DI-1644)**

A mixture of ketone (0.50 g, 3.52 mmol), benzylamine (0.38 g, 3.52 mmol), and powdered anhydrous $MgSO_4$ (1.27 g, 10.56 mmol) in DCM (15 mL) was stirred at rt for 72 h. Precipitate was filtered off, the filtrate was concentrated under reduced pressure, and the residue was dried under vacuum (7 mmHg) for 1 h. Obtained residue was dissolved in MeOH (5 mL), and the solution was cooled to 0°C. Solid $NaBH_4$ (0.13 g, 3.33 mmol) was added at 0°C. The reaction mixture was stirred at rt for 30 min. The organic solvent was removed under reduced pressure. The residue was purified by column chromatography on silica, eluting with EtOAc in *n*-hexane (gradient from 20% to 65%) to afford **DI-1644** (0.23 g, 31%), as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 5H), 4.18 – 3.98 (m, 2H), 3.69 – 3.44 (m, 2H), 3.22 – 2.91 (m, 1H), 2.71 – 2.36 (m, 3H), 2.15 – 1.80 (m, 2H), 1.22 – 1.10 (m, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 175.1, 140.1, 128.4, 128.2, 127.0, 60.7, 60.5, 51.2, 51.0, 49.4, 37.0, 34.7, 33.3, 33.0, 31.3, 14.2. HRMS calculated for $C_{14}H_{20}NO_2$: 234.1494, found: 234.1498 $[M + H]^+$.

**3-(Ethoxycarbonyl)cyclobutan-1-aminium acetate (DI-1646)**

A solution of **DI-1644** (0.23 g, 0.99 mmol) and AcOH (65 mg, 1.08 mmol) in MeOH (10 mL) was hydrogenated over $Pd(OH)_2/C$ (20 wt%, 30 mg) under $H_2$ atmosphere for 16 h. The reaction mixture was filtered through a pad of celite and the filter cake was washed with MeOH (3 × 5 mL). The filtrate was concentrated under reduced pressure to afford **DI-1646** (0.17 g, 87%), as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 4.22 – 4.06 (m, 2H), 3.85 – 3.32 (m, 1H), 2.81 – 2.46 (m, 3H), 2.24 – 2.00 (m, 2H), 1.99 (s, 2H), 1.32 – 1.17 (m, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 176.8, 175.1, 174.9, 63.5, 60.8, 45.2, 43.7, 37.1, 35.6, 33.8, 32.6, 31.1, 29.2, 22.4, 14.3. HRMS calculated for $C_7H_{14}NO_2$: 144.1025, found: 144.1021 $[M]^+$.

**3-Carboxy-*N,N,N*-trimethylcyclobutan-1-aminium iodide (DI-1648)**

To a solution of **DI-1646** (0.17 g, 0.80 mmol) in MeOH (7 mL), MeI (1.2 g, 8.10 mmol) and $K_2CO_3$ (0.56 g, 4.05 mmol, anhydrous powder) were added and the mixture was stirred vigorously for 16 h at rt. Inorganics were filtered off and washed on filter with MeOH (2 × 5 mL). Obtained filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with water to afford **DI-1648** (0.20 g, 86%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 4.49 – 4.14 (m, 1H), 3.36 – 2.96 (m, 10H), 2.93 – 2.61 (m, 4H). $^{13}$C NMR (101 MHz, $D_2O$) δ 178.2, 177.2, 66.1, 63.6, 50.3 [t, $^1J_{(N,C)}$ = 3.7 Hz],

50.1 [t, $^{1}J_{(N,C)}$ = 4.0 Hz], 30.1, 28.2, 27.2, 26.4. HRMS calculated for $C_8H_{16}NO_2$: 158.1181, found: 158.1181 $[M]^+$.

**3-(Trimethylammonio)cyclobutane-1-carboxylate (DI-1650)**

Acid **DI-1648** (0.20 g, 0.70 mmol) was dissolved in $H_2O$ (2 mL) and acidified with 1 M aq. HCl to pH 3. A solution of $NaBPh_4$ (20% in $H_2O$, 0.24 g, 0.70 mmol) was added to ensure a complete precipitation of the ammonium salt (white precipitate formed). The precipitate was collected by filtration and triturated with EtOH (40 mL, not completely soluble). Insolubles were filtered off and the filtrate was concentrated under reduced pressure. Residue was dissolved in EtOH (20 mL) and passed through Dowex Monosphere 550A (OH) ion-exchange resin column, followed by a slow elution with EtOH (40 mL). The eluate, containing expected product, was concentrated under reduced pressure and the residue was dried in vacuo to afford **DI-1650** (50 mg, 45%), as a white solid. $^{1}$H NMR (400 MHz, $D_2O$) δ 4.26 – 3.91 (m, 1H), 3.10 – 2.98 (m, 9H), 2.80 – 2.32 (m, 5H). $^{13}$C NMR (101 MHz, $D_2O$) δ 182.4, 181.5, 66.7, 63.6, 49.8 [t, $^{1}J_{(N,C)}$ = 3.7 Hz], 49.6 [t, $^{1}J_{(N,C)}$ = 4.0 Hz], 32.1, 30.2, 27.7, 26.8. HRMS calculated for $C_8H_{16}NO_2$: 158.1181, found: 158.1182 $[M + H]^+$.

**3-Carboxy-*N*,*N*,*N*-trimethylcyclobutan-1-aminium hexafluorophosphate (DI-1655)**

Betaine **DI-1650** (50 mg, 0.32 mmol) was dissolved in 1M aq. HCl (3 mL) heated at 60 °C for 72 h. The reaction mixture was concentrated under reduced pressure, the residue was mixed with water (5 mL) and evaporated again. Obtained residue was suspended in MeCN (4 mL, turbid solution), $NaPF_6$ (59 mg, 0.35 mmol) was added and mixture was stirred at 60 °C for 16 h (precipitate formed). The mixture was cooled down to rt, precipitate was filtered off. Filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with water to afford **DI-1655** (65 mg, 67%), as a white solid. $^{1}$H NMR (400 MHz, $D_2O$) δ 4.38 – 4.00 (m, 1H), 3.24 – 2.92 (m, 10H), 2.85 – 2.52 (m, 4H). $^{13}$C NMR (101 MHz, $D_2O$) δ 177.2, 63.5, 49.8 [t, $^{1}J_{(N,C)}$ = 3.8 Hz], 49.6, 28.0, 26.9, 26.1. HRMS calculated for $C_8H_{16}NO_2$: 158.1181, found: 158.1185 $[M]^+$

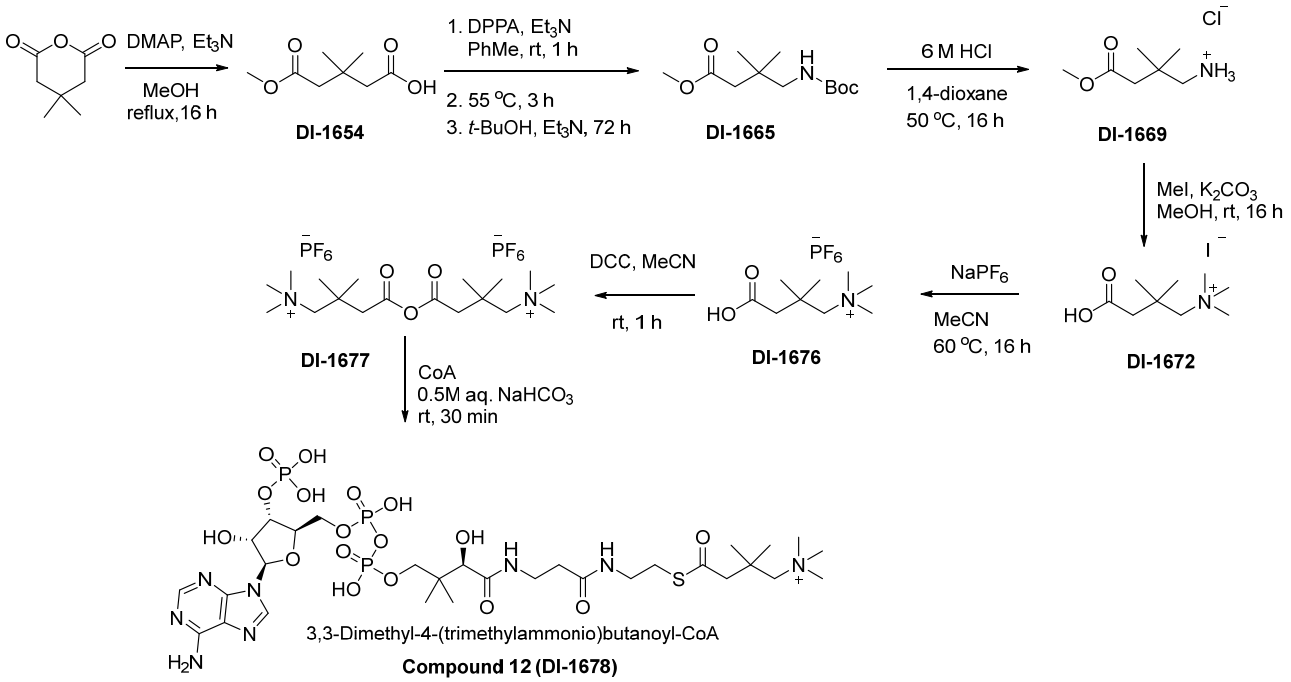
**3,3'-(Oxybis(carbonyl))bis(*N*,*N*,*N*-trimethylcyclobutan-1-aminium) hexafluorophosphate (DI-1663)**

To a stirred solution of **DI-1655** (43 mg, 0.14 mmol) in MeCN (1 mL), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (14 mg, 0.07 mmol) was added and the mixture was stirred at rt for 30 min. Reaction mixture was concentrated under reduced pressure to the volume 0.4 mL and used in the next step without purification.

**3-(Trimethylammonio)cyclobutane-1-carboxyl-CoA (compound 11; DI-1664)**

To the solution of coenzyme A as a sodium salt (20 mg, 26 μmol dissolved in 0.36 mL of 0.5 M aq. $NaHCO_3$) solution of anhydride **DI-1663** (41 mg, 0.07 mmol) in MeCN (0.4 mL) was added, and the mixture was stirred at rt for 30 min. Next, the mixture was quenched with dropwise addition of 1 M aq. HCl, to adjust the pH 3. MeCN was evaporated from the reaction mixture under reduced pressure, precipitate was filtered off and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 11** (8 mg, 34%), as a white foam. $^{1}$H NMR (400 MHz, $D_2O$) δ 8.72 (br s, 1H, Ade-8), 8.46 (s, 1H, Ade-2), 6.23 (d, *J* = 4.4 Hz, 1H, Rib-1'), 4.98 – 4.86 (m, 2H, Rib-2',3'), 4.66 – 4.57 (m, 1H, Rib-4'), 4.40 – 4.26 (m, 2H, Pan-1), 4.26 – 4.06 (m, 1H, Ac-4), 4.06 (s, 1H, Pan-3), 3.96 – 3.85 (m, 1H, Rib-5'$_b$), 3.72 – 3.60 (m, 1H, Rib-5'$_a$), 3.53 – 3.47 (m, 2H, βA-1), 3.47 – 3.15 (m, 3H, Ac-2, CA-1), 3.11 – 3.01 (m, 11H, CA-2, $NMe_3$), 2.84 – 2.54 (m, 4H, Ac-3,5), 2.52 – 2.41 (m, 2H, βA-2), 0.97 (s, 3H, Pan-Me$_a$), 0.86 (s, 3H, Pan-Me$_b$). $^{13}$C NMR (101 MHz, $D_2O$) δ 203.6, 202.4, 174.7, 174.0, 149.9, 144.7, 142.6, 87.51, 83.4, 74.2, 73.9, 72.0, 65.6, 65.1, 63.2, 49.8, 49.7, 38.4, 38.0, 35.8, 35.4, 35.3, 28.5, 28.3, 27.6, 26.8, 20.8, 18.3. HRMS calculated for $C_{29}H_{50}N_8O_{17}P_3S$: 907.2227, found: 907.2247 $[M]^+$.

*Compound **12 (DI-1678)***




Scheme S9. Synthesis of compound 12 (DI-1678).

**5-Methoxy-3,3-dimethyl-5-oxopentanoic acid (DI-1654)**

A mixture of 3,3-dimethylglutaric anhydride (0.68 g, 4.80 mmol), $Et_3N$ (0.68 mL, 4.80 mmol) and DMAP (0.059 g, 0.48 mmol) in MeOH (15 mL) was stirred at reflux for 16 h. The mixture was cooled down to rt and concentrated under reduced pressure. The residue was purified by column chromatography on silica, eluting with MeOH in DCM (gradient from 3% to 10%) to afford **DI-1654** (0.73 g, 87%), as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 3H), 2.47 (s, 2H), 2.45 (s, 2H), 1.14 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.5, 172.8, 51.5, 45.0, 44.9, 32.6, 27.9. HRMS calculated for $C_8H_{13}O_4$: 173.0814, found: 173.0819 [M - H]$^-$.

**Methyl 4-((tert-butoxycarbonyl)amino)-3,3-dimethylbutanoate (DI-1665)**

To a solution of **DI-1654** (0.73 g, 4.19 mmol) and $Et_3N$ (0.64 mL, 4.61 mmol) in PhMe (10 mL) diphenylphosphoryl azide (DPPA) (1.27 g, 4.61 mmol) was added under Ar atmosphere. The resulting mixture was stirred at ambient temperature for 1 h. Then the temperature was increased and the reaction mixture was stirred at 60 °C for 3 h. Next, the reaction mixture was concentrated to the half of volume and *t*-BuOH (5 mL) was added, followed by $Et_3N$ (0.64 mL, 4.61 mmol), and the resulting solution was stirred at 60 °C for 72 h. The mixture was cooled down to rt and concentrated under reduced pressure. The residue was purified by column chromatography on silica, eluting with MTBE in *n*-hexane (10%) to afford **DI-1665** (0.45 g, 44%), as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$) δ 4.86 (br s, 1H), 3.66 (s, 3H), 3.03 (d, *J* = 6.8 Hz, 2H), 2.22 (s, 2H), 1.44 (s, 9H), 0.98 (s, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 172.8, 156.4, 79.2, 51.5, 50.1, 44.0, 35.3, 28.5, 25.5. HRMS calculated for $C_{12}H_{23}NO_4Na$: 268.1525, found: 268.1538 [M + Na]$^+$.

**4-Methoxy-2,2-dimethyl-4-oxobutan-1-aminium chloride (DI-1669)**

A 6 M solution of HCl in 1,4-dioxane (1.5 mL, 9.17 mmol) was added to a solution of **DI-1665** (0.45 g, 1.83 mmol) in 1,4-dioxane (2.0 mL). The reaction mixture was stirred at 50 °C for 16 h. The mixture was cooled down to rt and concentrated under reduced pressure. The residue was purified by flash chromatography on RP-$C_{18}$, eluted with water to afford **DI-1669** (0.33 g, 99%), as a white solid (mixture of ester and corresponding acid 3:1). $^1$H NMR (400 MHz, $D_2O$) δ ester 3.73 (s, 3H), 3.03 (s, 2H), 2.48 (s, 2H), 1.11 (s, 6H); acid 3.05 (s, 2H), 2.46 (s, 2H), 1.14 (s, 6H). $^{13}$C NMR (101 MHz, $D_2O$) ester δ 174.3, 52.2, 48.5, 43.4, 32.1, 24.1; acid 175.8, 48.5, 43.8, 31.9, 24.2. HRMS calculated for ester $C_7H_{16}NO_2$: 146.1181, found: 146.1181 [M + H]$^+$; calculated for acid $C_6H_{14}NO_2$: 132.1025, found: 132.1024 [M + H]$^+$.

**3-Carboxy-*N*,*N*,*N*,2,2-pentamethylpropan-1-aminium iodide (DI-1672)**

To a mixture of **DI-1669** (0.33 g, 1.82 mmol) in MeOH (10 mL), MeI (2.58 g, 18.20 mmol) and $K_2CO_3$ (1.26 g, 9.10 mmol) were added and the mixture was stirred vigorously for 16 h at rt. Inorganics were filtered off and washed on filter with MeOH (2 × 5 mL). Obtained filtrate was concentrated under reduced pressure and purified by flash chromatography on RP-$C_{18}$, eluted with water to afford **DI-1672** (0.30 g, 55%), as a white solid. $^1$H NMR (400 MHz, $D_2O$) δ 3.54 (s, 2H), 3.25 (s, 9H), 2.34 (s, 2H), 1.29 (s, 6H). 13C NMR (101 MHz, $D_2O$) δ 177.1, 72.8 [t, $^1J_{(N,C)}$ = 2.1 Hz], 53.2 [t, $^1J_{(N,C)}$ = 4.0 Hz], 47.4, 32.6, 24.8. HRMS calculated for $C_9H_{20}NO_2$: 174.1494, found: 174.1499 $[M]^+$.

**3-Carboxy-*N*,*N*,*N*,2,2-pentamethylpropan-1-aminium hexafluorophosphate (DI-1676)**

To a mixture of **DI-1672** (0.30 g, 1.00 mmol) in MeCN (7.0 mL) $NaPF_6$ (0.19 g, 1.10 mmol) was added. The mixture was stirred at 60 °C for 16 h. The mixture was cooled down to rt and concentrated under reduced pressure. Residue solidified upon addition of $H_2O$ (3.0 mL). Precipitate was collected by filtration to afford the first crop of the product as $PF_6^-$ salt. Aqueous filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with water, to afford the second crop. Combined yield of **DI-1676**: 0.17 g (53%), as a colorless solid. $^1$H NMR (400 MHz, MeOD) δ 3.59 (s, 2H), 3.25 (s, 9H), 2.54 (s, 2H), 1.31 (s, 6H). $^{13}$C NMR (101 MHz, MeOD) δ 174.3, 75.2, 56.2 [t, $^1J_{(N,C)}$ = 3.8 Hz], 45.6, 36.1, 28.1. HRMS calculated for $C_9H_{20}NO_2$: 174.1494, found: 174.1502 $[M]^+$.

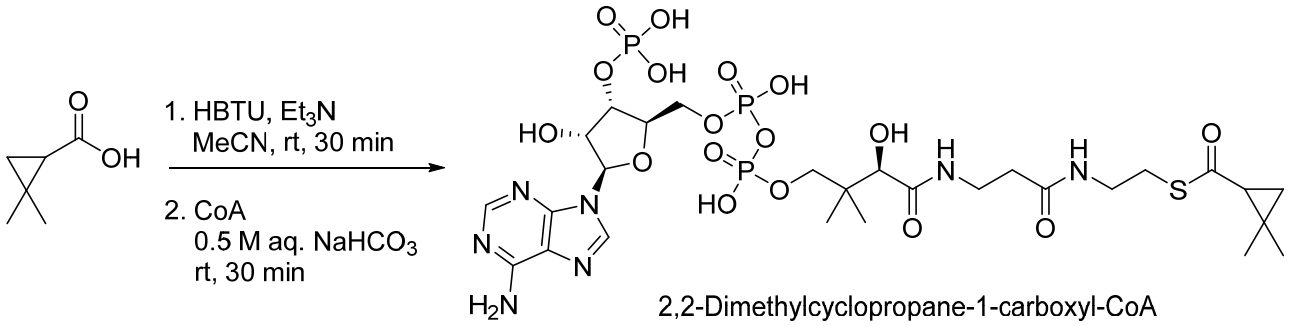
**4,4'-Oxybis(*N*,*N*,*N*,2,2-pentamethyl-4-oxobutan-1-aminium) hexafluorophosphate (DI-1677)**

To a stirred solution of **DI-1676** (43 mg, 0.13 mmol) in MeCN (1.0 mL), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (14 mg, 0.07 mmol) was added and the mixture was stirred at rt for 30 min. Reaction mixture was concentrated under reduced pressure to the volume of 0.4 mL and used in the next step without purification.

**3,3-Dimethyl-4-(trimethylammonio)butanoyl-CoA (compound 12; DI-1678)**

To the solution of coenzyme A as a sodium salt (20 mg, 26 μmol dissolved in 0.36 mL of 0.5 M aq. $NaHCO_3$) solution of **DI-1677** (41 mg, 0.07 mmol) in MeCN (0.4 mL) was added and the mixture was stirred at rt for 30 min. Next, the mixture was quenched with dropwise addition of 1 M aq. HCl, to adjust the pH 3. MeCN was evaporated from the reaction mixture under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 12** (9.0 mg, 38%), as a white foam. $^1$H NMR (400 MHz, $D_2O$) δ 8.70 (br s, 1H, Ade-8), 8.45 (s, 1H, Ade-2), 6.22 (d, *J* = 5.0 Hz, 1H, Rib-1'), 4.98 – 4.87 (m, 2H, Rib-2',3'), 4.67 – 4.57 (m, 1H, Rib-4'), 4.40 – 4.22 (m, 2H, Pan-1), 4.05 (s, 1H, Pan-3), 3.98 – 3.85 (m, 1H, Rib-5'$_b$), 3.73 – 3.61 (m, 1H, Rib-5'$_a$), 3.54 – 3.45 (m, 4H, Ac-4, βA-1), 3.37 (t, *J* = 6.3 Hz, 2H, CA-1), 3.23 (s, 9H, $NMe_3$), 3.03 (t, *J* = 6.3 Hz, 2H, CA-2), 2.85 (s, 2H, Ac-2), 2.46 (t, *J* = 6.5 Hz, 2H, βA-2), 1.28 (s, 6H, Ac-Me), 0.97 (s, 3H, Pan-Me$_a$), 0.86 (s, 3H, Pan-Me$_b$). $^{13}$C NMR (101 MHz, $D_2O$) δ 200.4, 174.7, 174.0, 149.9, 144.7, 142.6, 87.5, 83.3, 74.2, 73.9, 72.1, 65.1, 55.5 [t, $^1J_{(N,C)}$ = 4.2 Hz], 53.3, 45.1, 38.4, 35.8, 35.4, 35.3, 28.5, 26.7, 26.7, 20.8, 18.4. HRMS calculated for $C_{30}H_{54}N_8O_{17}P_3S$: 923.2540, found: 923.2538 $[M]^+$.

*Compound **13 (DI-1271)***




**Compound 13 (DI-1271)**

Scheme S10. Synthesis of compound 13 (DI-1271).

**2,2-Dimethylcyclopropane-1-carboxyl-CoA (compound 13; DI-1271)**

To a solution of 2,2-dimethylcyclopropane-1-carboxylic acid (6 mg, 0.05 mmol) and $Et_3N$ (18 µL, 0.13 mmol) in MeCN (0.5 mL) HBTU (22 mg, 0.06 mmol) was added. The mixture was stirred at rt for 30 min. Next, the solution of coenzyme A as a sodium salt (20 mg, 26 µmol dissolved in 0.36 mL of 0.5 M aq. NaHCO3) was added and the mixture was stirred at rt for 1 h. The mixture was quenched with dropwise addition of 1 M aq. HCl, to adjust the pH 3. MeCN was evaporated from the reaction mixture under reduced pressure and the residue was purified by flash chromatography on RP-$C_{18}$, eluted with 0%-20% of MeCN in 0.01% aq. HCl. Target fractions were lyophilized to afford **Compound 13** (6 mg, 27%), as a white foam. $^1$H NMR (400 MHz, $D_2O$) δ 8.57 (s, 1H, Ade-8), 8.28 (s, 1H, Ade-2), 6.19 (d, J = 6.4 Hz, 1H, Rib-1'), 4.90 – 4.82 (m, 2H, Rib-2',3'), 4.64 – 4.56 (m, 1H, Rib-4'), 4.32 – 4.20 (m, 2H, Pan-1), 4.04 (s, 1H, Pan-3), 3.85 (dd, J = 9.9, 4.2 Hz, 1H, Rib-5'$_b$), 3.57 (dd, J = 9.7, 4.1 Hz, 1H, Rib-5'$_a$), 3.52 – 3.39 (m, 2H, βA-1), 3.38 – 3.24 (m, 2H, CA-1), 3.07 – 2.91 (m, 2H, CA-2), 2.43 (t, J = 6.6 Hz, 2H, βA-2), 2.08 – 1.98 (m, 1H, Ac-2), 1.24 (t, J = 4.9 Hz, 1H, Ac-4), 1.16 (s, 3H, Ac-Me), 1.07 (s, 3H, Ac-Me), 1.03 (dd, J = 7.7, 4.4 Hz, 1H, Ac-4), 0.91 (s, 3H, Pan-Me$_a$), 0.77 (s, 3H, Pan-Me$_b$). 13C NMR (101 MHz, $D_2O$) δ 201.0, 174.7, 173.9, 155.6, 152.8, 139.9, 86.3, 83.6, 74.2, 74.2, 74.1, 73.8, 71.9, 65.3, 38.8, 38.4, 38.3, 36.4, 35.4, 35.3, 28.2, 27.4, 25.7, 23.5, 20.9, 18.1, 17.7. HRMS calculated for $C_{27}H_{45}N_7O_{17}P_3S$: 864.1805, found: 864.1808 $[M + H]^+$.

**Table S1. Correspondence between manuscript compound numbers, internal DI codes, synthetic schemes, and analytical figures.**

| Cmpd. | Substance | DI code | Scheme | Figures |
|---|---|---|---|---|
| 1 | γ-Butyrobetainyl-CoA | DI-1382 | S1 | S1–S3 |
| 2 | γ-Butyrobetainyl-SNAC | DI-1502 | S1 | S4–S6 |
| 3 | 2-Methyl-4-(trimethylammonio)butanoyl-CoA | DI-1455 | S2 | S7–S9 |
| 4 | 2-Methyl-4-(trimethylammonio)butanoyl-PAN | DI-1462 | S2 | S10–S12 |
| 5 | 2-((Trimethylammonio)methyl)cyclopropane-1-carboxyl-CoA | DI-1483 | S3 | S13–S15 |
| 6 | 3-(Trimethylammonio)benzoyl-CoA | DI-1573 | S4 | S16–S18 |
| 7 | (E)-4-(Trimethylammonio)but-2-enoyl-CoA | DI-1580 | S5 | S19–S21 |
| 8 | 3-(Trimethylammonio)cyclohexane-1-carboxyl-CoA | DI-1593 | S6 | S22–S24 |
| 9 | 3-(Trimethylammonio)cyclohexane-1-carboxyl-PAN | DI-1464 | S6 | S25–S27 |
| 10 | 3-(Trimethylammonio)cyclopentane-1-carboxyl-CoA | DI-1639 | S7 | S28–S30 |
| 11 | 3-(Trimethylammonio)cyclobutane-1-carboxyl-CoA | DI-1664 | S8 | S31–S33 |
| 12 | 3,3-Dimethyl-4-(trimethylammonio)butanoyl-CoA | DI-1678 | S9 | S34–S36 |
| 13 | 2,2-Dimethylcyclopropane-1-carboxyl-CoA | DI-1271 | S10 | S37–S39 |

**Table S2. NMR assignments abbreviations**

| Module | Labels |
|---|---|
| Adenine | Ade-2 to Ade-8 |
| Ribose | Rib-1′ to Rib-5′ |
| Pantoate | Pan-1 to Pan-4 |
| β-Alanine | βA-1, βA-2 |
| Cysteamine | CA-1, CA-2 |
| Variable acyl group | Ac-1 to Ac-6 |
| Aromatic acyl group 6 | Ar-1 to Ar-6 |
| Trimethylammonium | NMe3 |
| SNAC acetyl group | Ac |

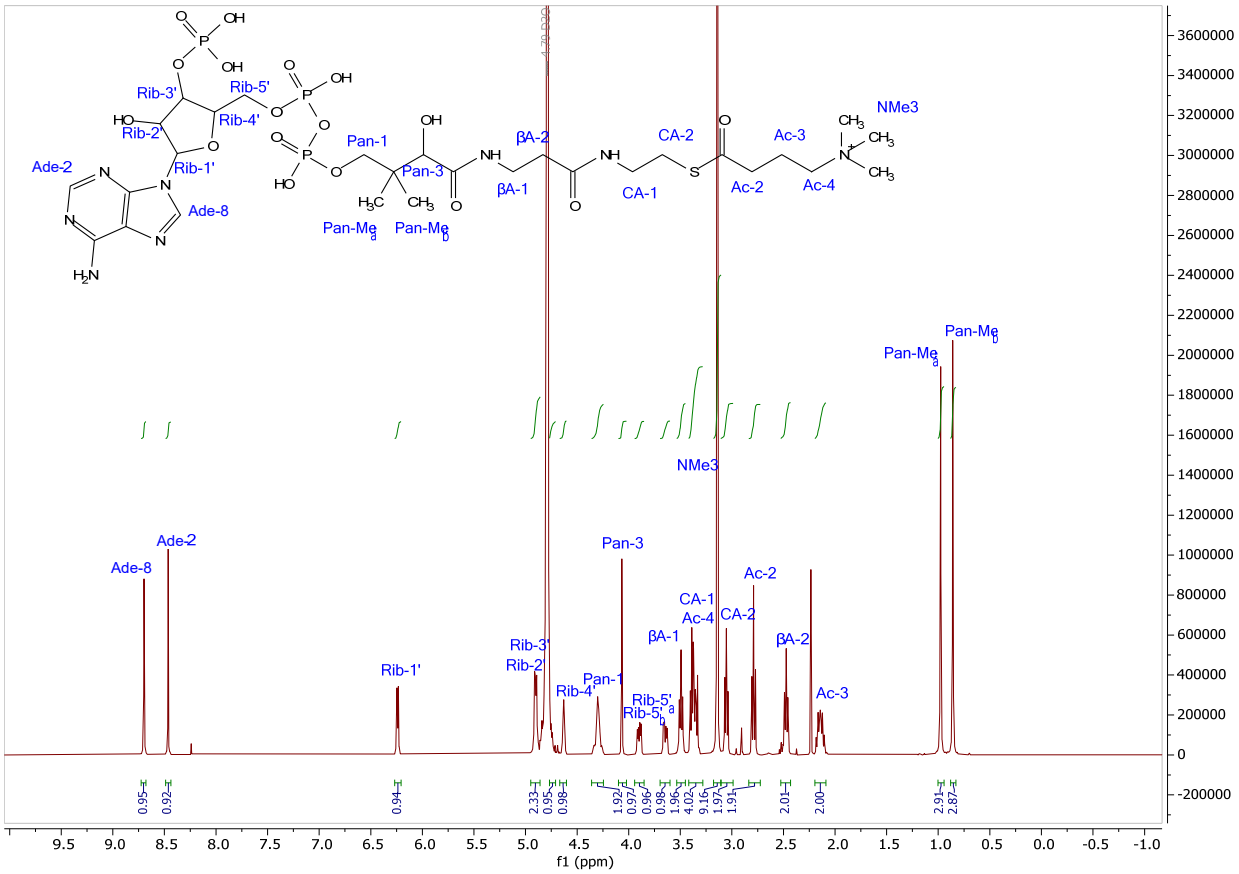



**Figure S1.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 1 (DI-1382)**

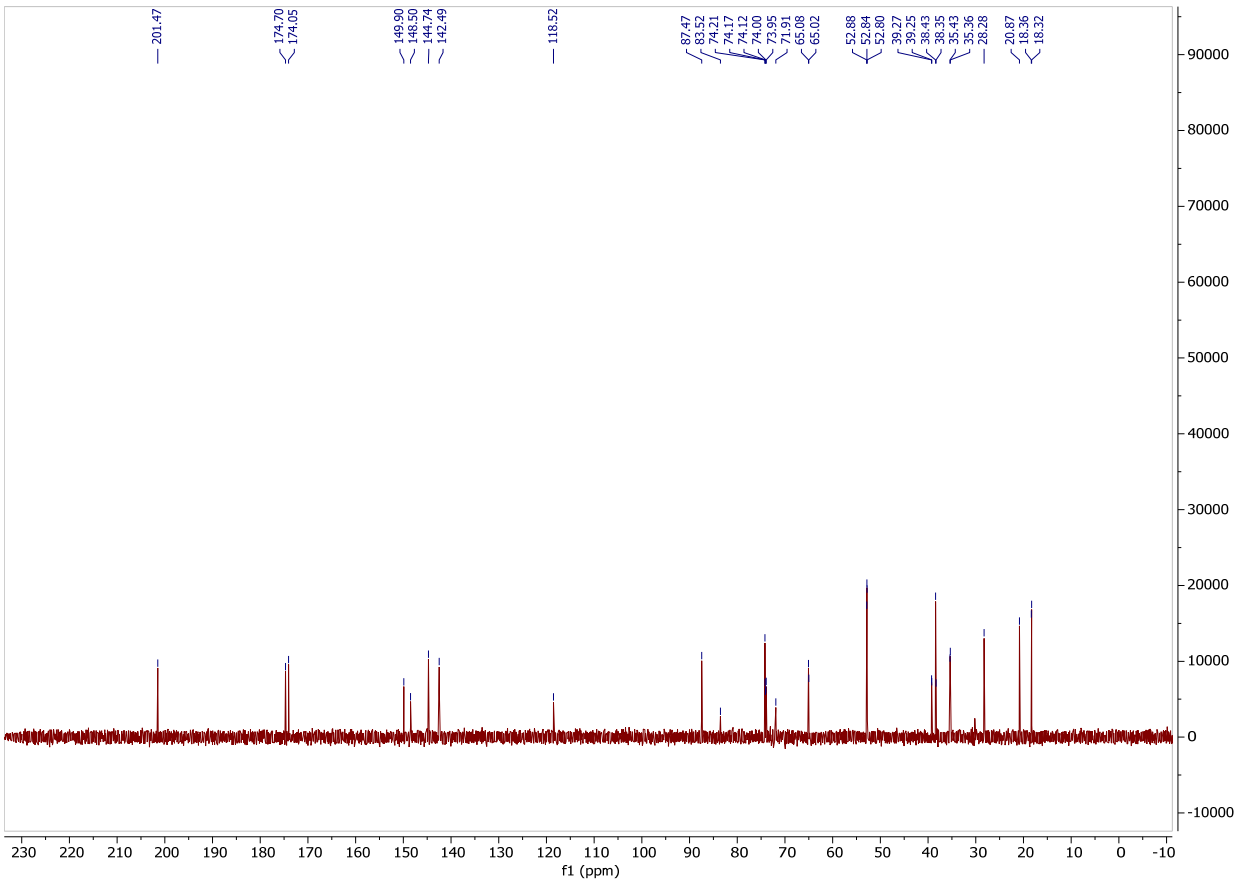



**Figure S2.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 1 (DI-1382)**

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -0.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
3172 formula(e) evaluated with 13 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-150 H: 1-150 N: 0-20 O: 0-20 P: 3-3 S: 1-1

1469 Zaca DI-1382

HRMS_2025_08_492 413 (1.187) Cm (412:415-371:377)

OSI/FOKL-MS
Synapt G2-Si
1: TOF MS ES+
4.95e+006

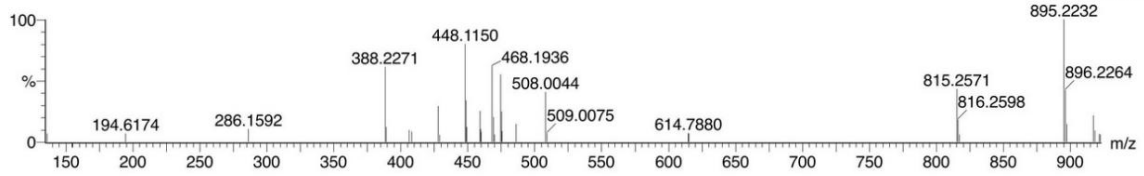



**Figure S3**. HRMS (ESI$^+$, TOF) spectrum of **compound 1 (DI-1382)**. m/z: $[M]^+$ calculated for $C_{28}H_{50}N_8O_{17}P_3S^+$, 895.2227; found, 895.2232; Δ = +0.6 ppm.

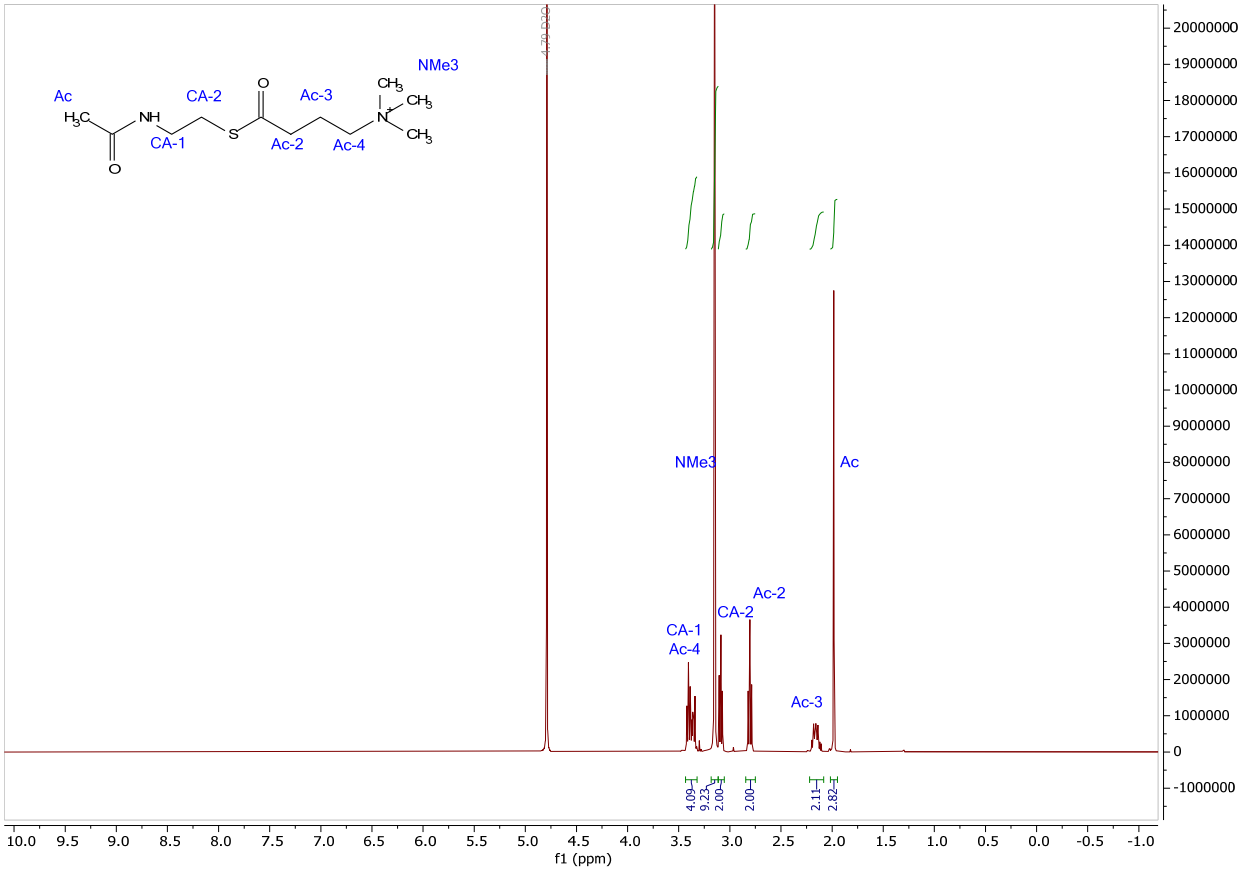



**Figure S4.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 2 (DI-1502)**

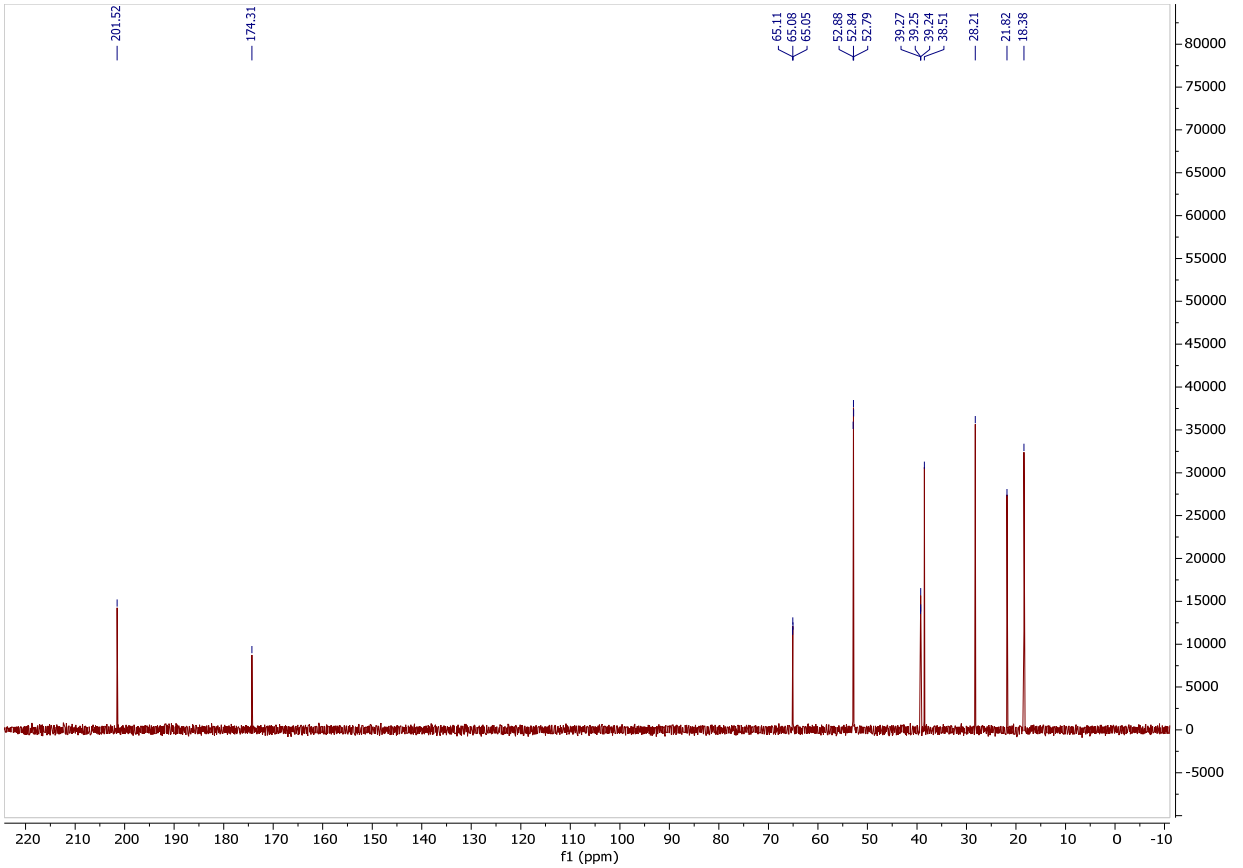



**Figure S5.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 2 (DI-1502)**

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -0.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
219 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-120 H: 1-150 N: 0-15 O: 0-15 S: 1-1

1006 Zaca DI-1502

HRMS_2025_05_465 139 (0.414) Cm (138:141-125:128)

OSI/FOKL-MS
Synapt G2-Si
1: TOF MS ES+
4.39e+006
247.1487

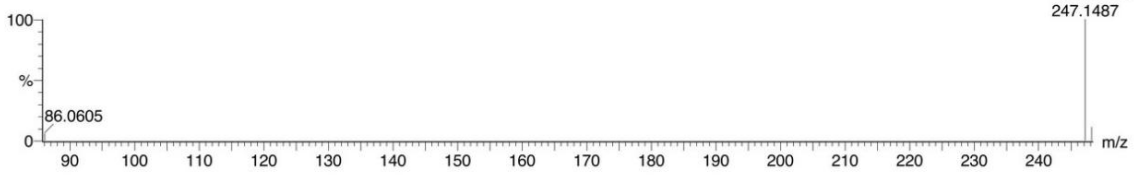



**Figure S6**. HRMS (ESI$^+$, TOF) spectrum of **compound 2 (DI-1502).** m/z: [M]$^+$ calculated for $C_{11}H_{23}N_2O_2S^+$, 247.1480; found, 247.1487; Δ = +2.8 ppm.

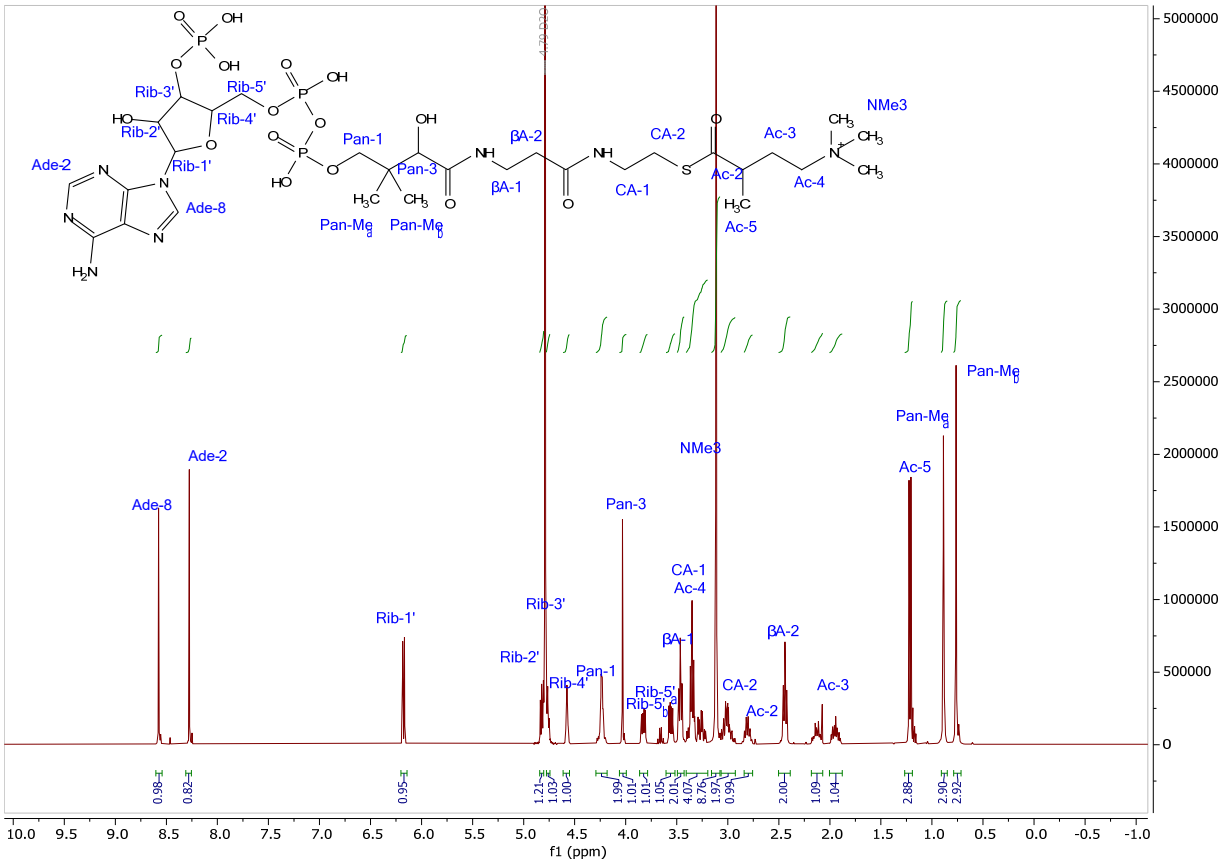



**Figure S7.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 3 (DI-1455)**

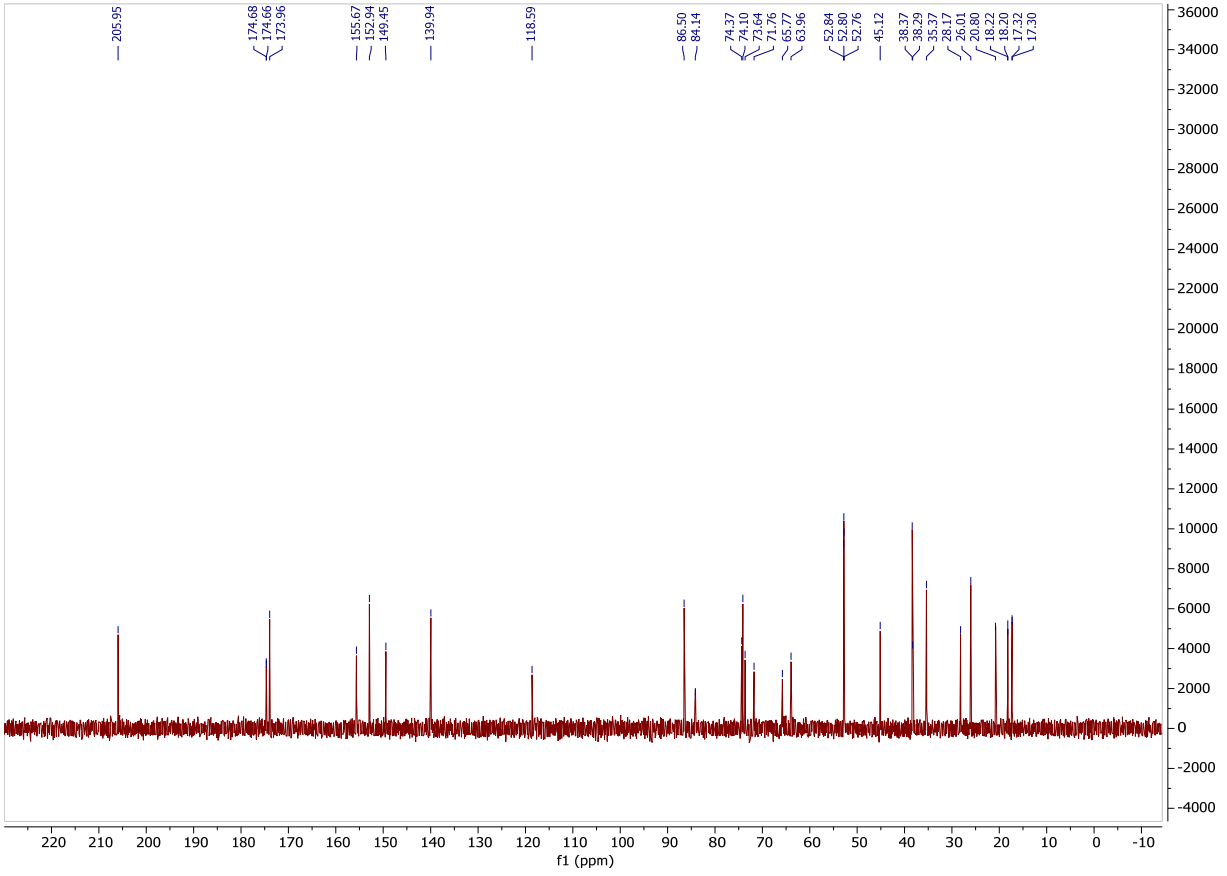



**Figure S8.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 3 (DI-1455)**

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions
4056 formula(e) evaluated with 29 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-150 H: 0-150 N: 0-25 O: 0-20 P: 3-3 S: 1-1
2138 Zaca DI-1455
HRMS_2025_12_079 129 (0.387) Cm (126:129-117:119)

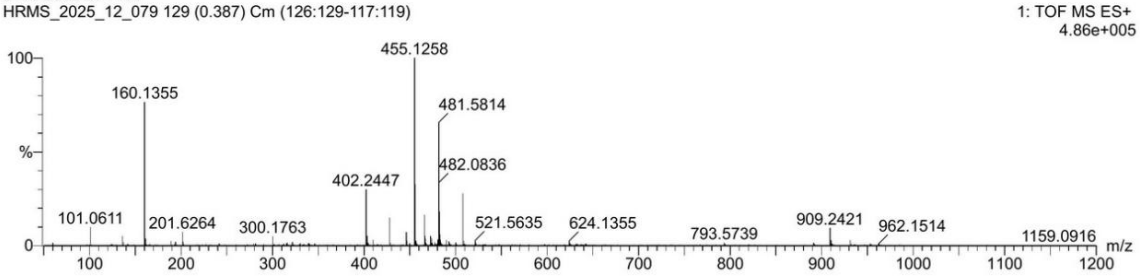



**Figure S9**. HRMS (ESI$^+$, TOF) spectrum of **compound 3 (DI-1455)**. m/z: $[M]^+$ calculated for $C_{29}H_{52}N_8O_{17}P_3S^+$, 909.2384; found, 909.2421; Δ = +4.1 ppm.

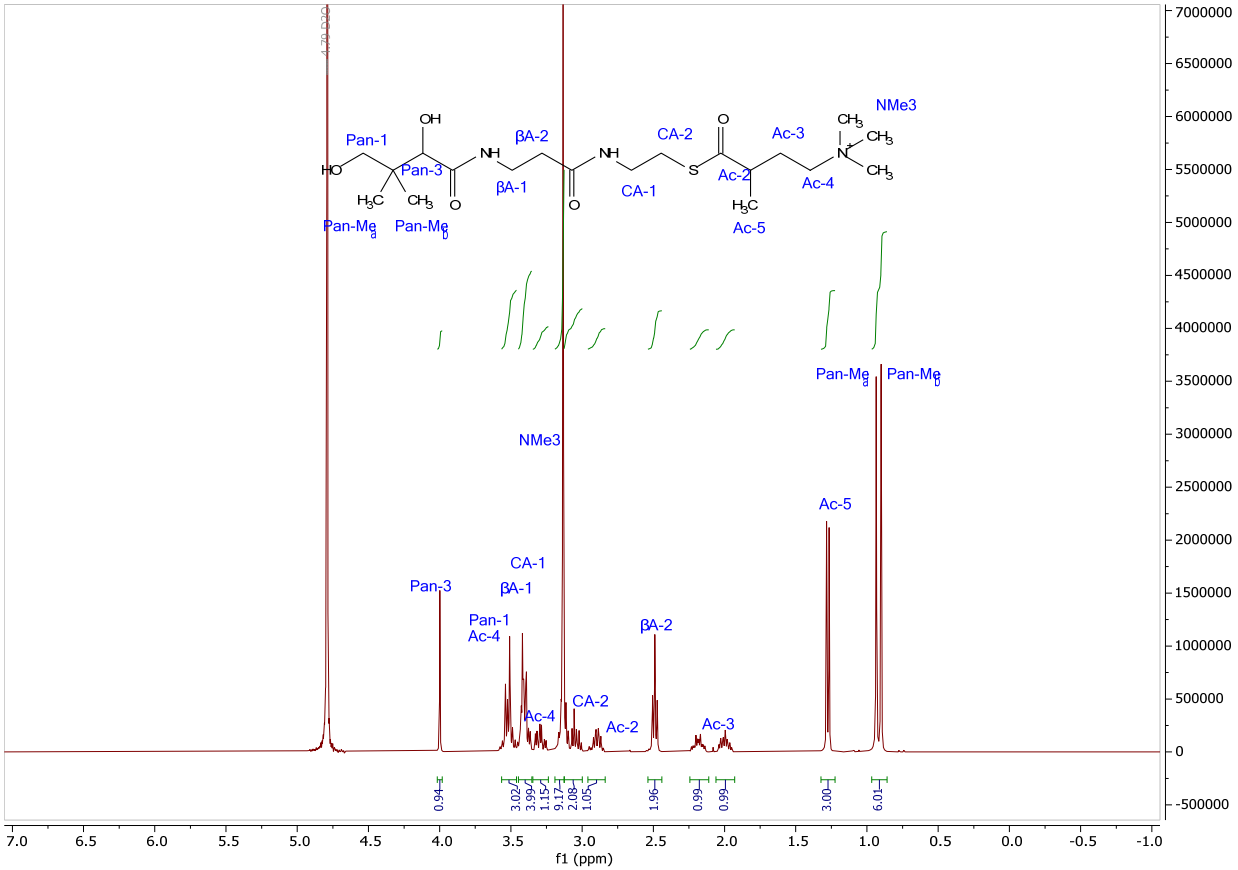



**Figure S10.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 4 (DI-1462)**

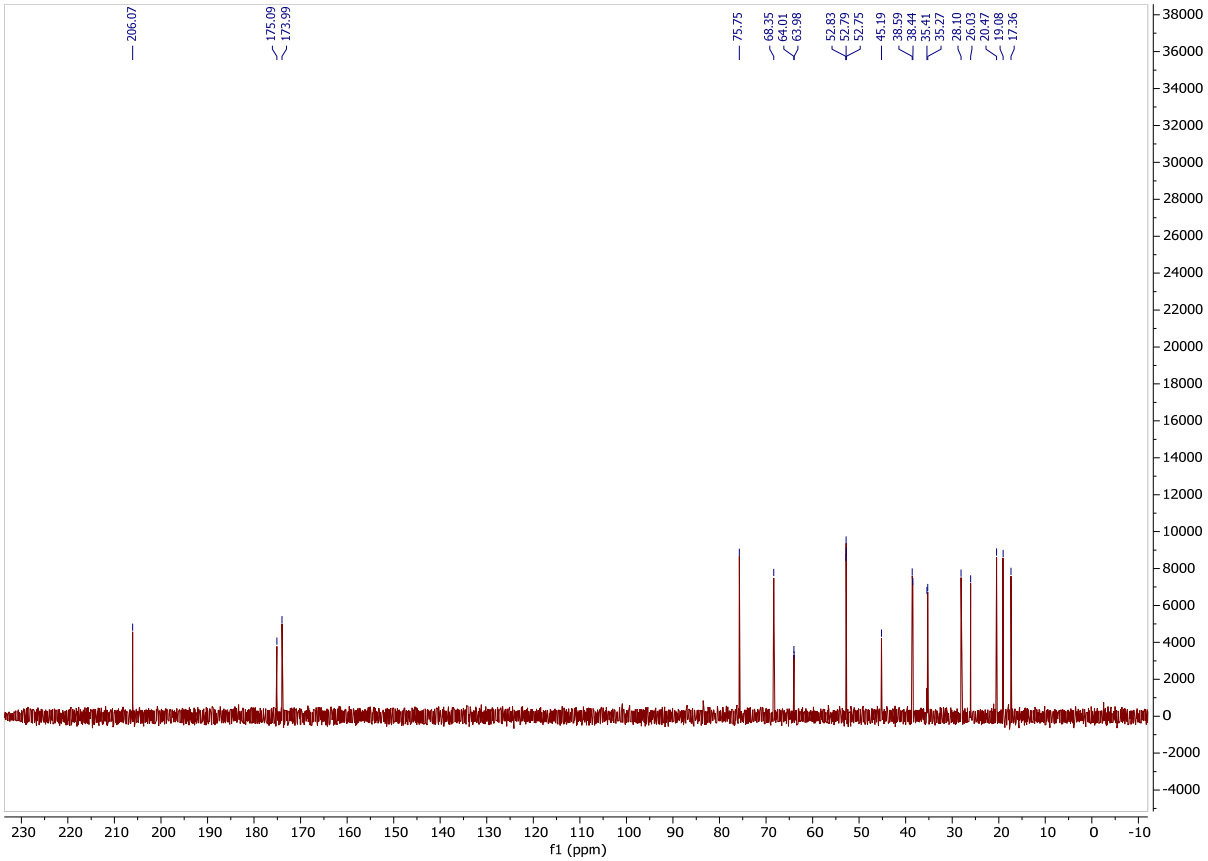



**Figure S11.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 4 (DI-1462)**

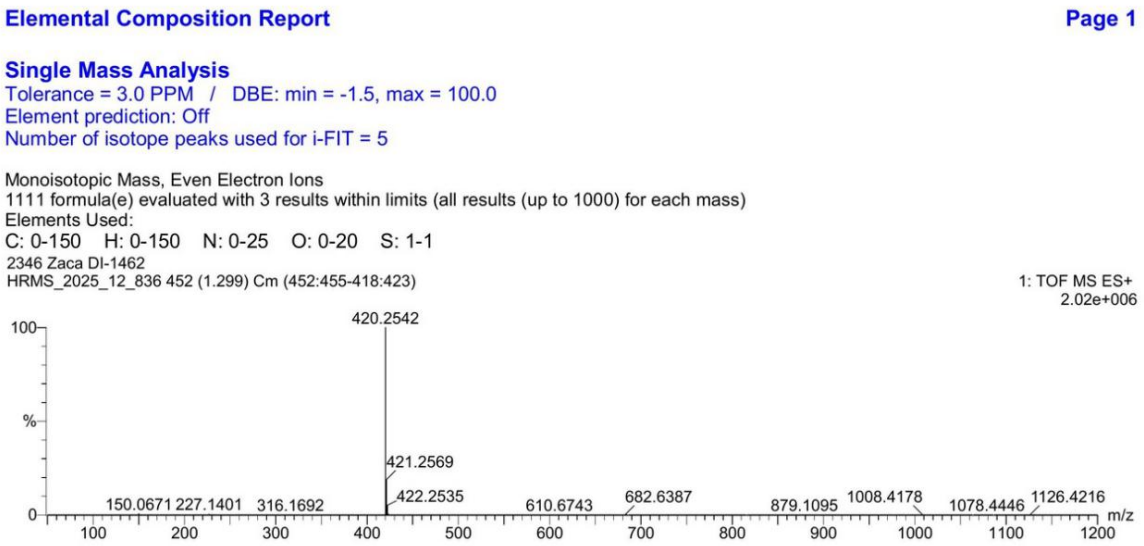



**Figure S12**. HRMS (ESI$^+$, TOF) spectrum of **compound 4 (DI-1462).** m/z: $[M]^+$ calculated for $C_{19}H_{38}N_3O_5S^+$, 420.2532; found, 420.2542; Δ = +2.4 ppm.

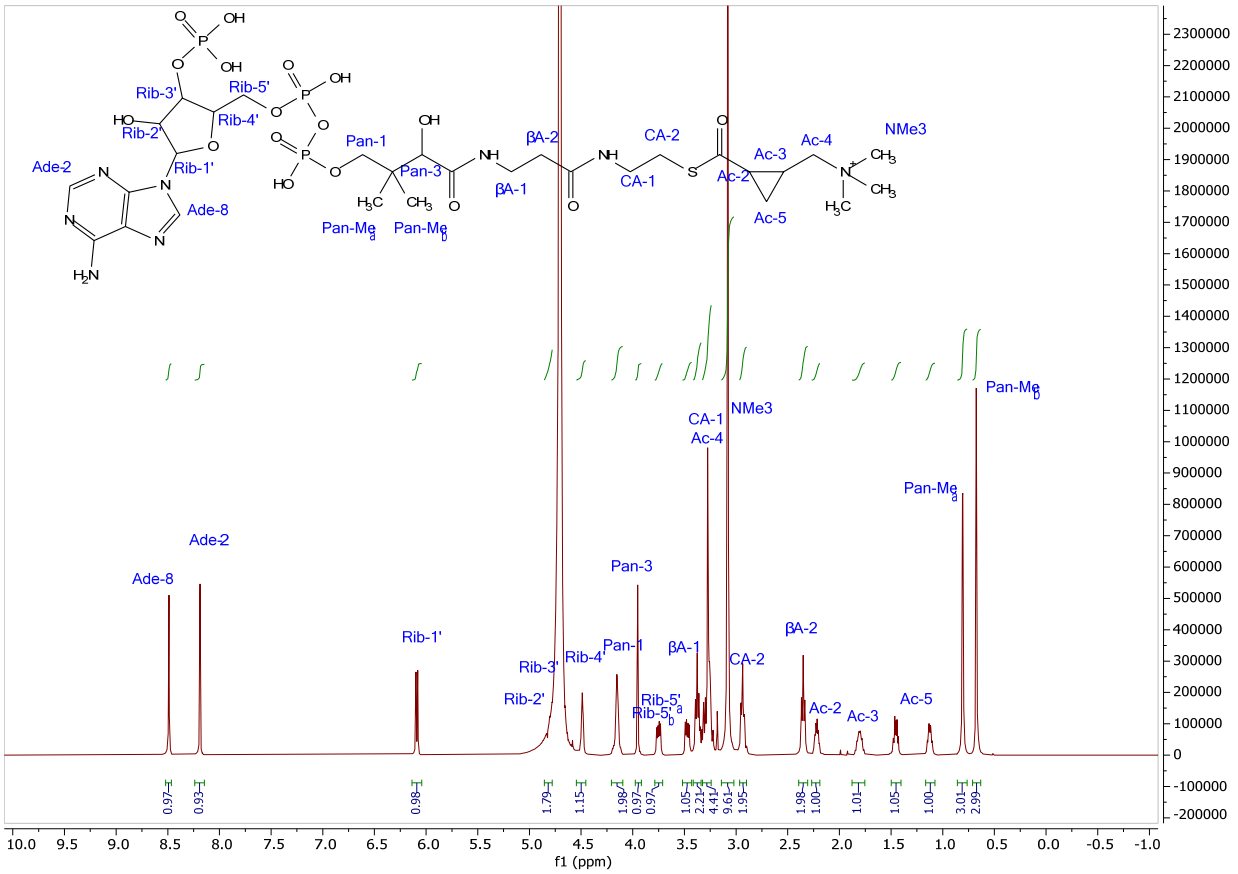



**Figure S13.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 5 (DI-1483)**

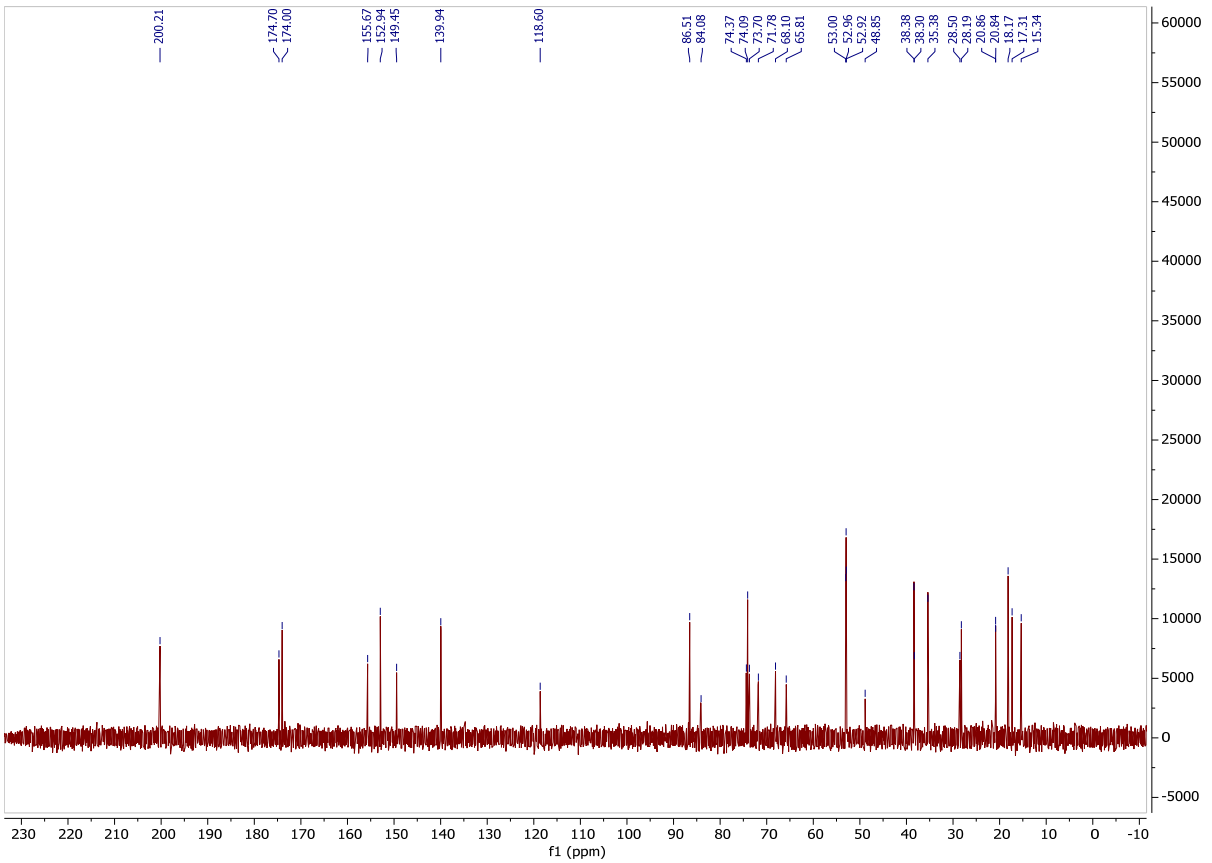



**Figure S14.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 5 (DI-1483)**

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -0.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
3874 formula(e) evaluated with 17 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-150 H: 1-150 N: 0-25 O: 0-20 S: 1-1 P: 3-3

1650 Zaca DI-1606

HRMS_2025_09_534 141 (0.420) Cm (141:145-118:123)

OSI/FOKL-MS
Synapt G2-Si
1: TOF MS ES+
7.39e+006

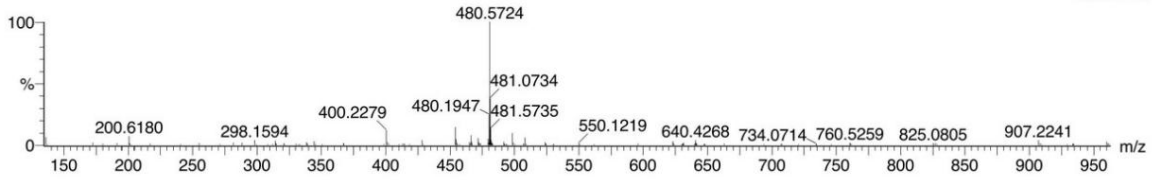



**Figure S15**. HRMS (ESI$^+$, TOF) spectrum of **compound 5 (DI-1483)**. m/z: $[M]^+$ calculated for $C_{29}H_{50}N_8O_{17}P_3S^+$, 907.2227; found, 907.2241; Δ = +1.5 ppm.

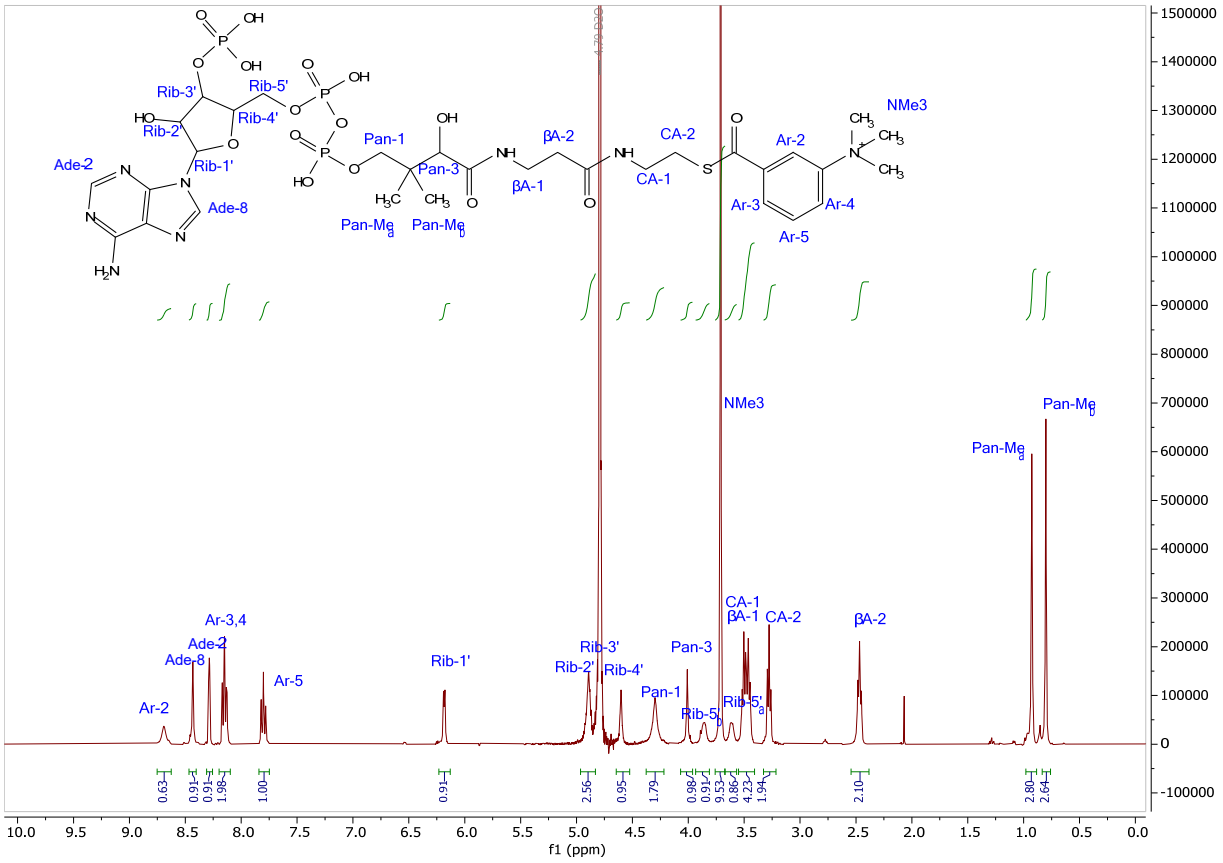



**Figure S16.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 6 (DI-1573)**

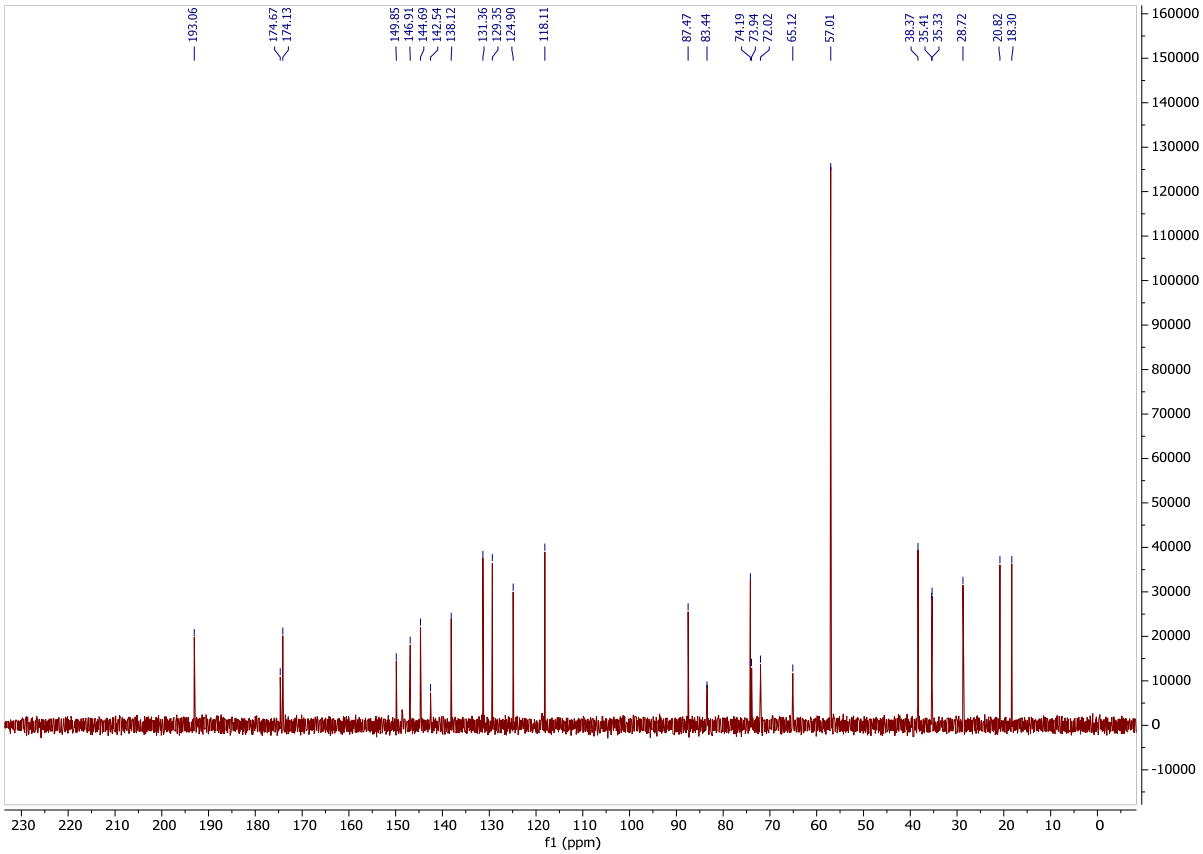



**Figure S17.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 6 (DI-1573)**

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -0.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
3347 formula(e) evaluated with 13 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-150 H: 1-150 N: 0-20 O: 0-20 P: 3-3 S: 1-1

1460 Zaca DI-1573

HRMS_2025_08_494 420 (1.211) Cm (417:420-405:409)

OSI/FOKL-MS
Synapt G2-Si
1: TOF MS ES+
8.01e+006

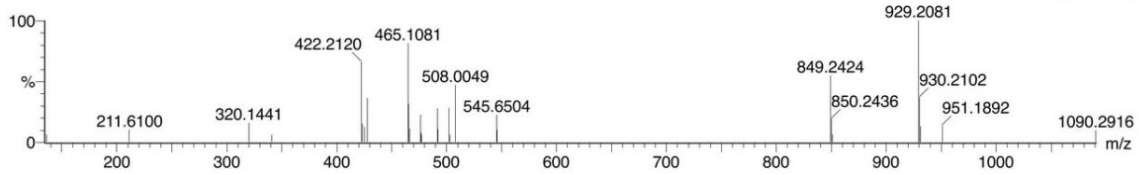



**Figure S18**. HRMS (ESI$^+$, TOF) spectrum of **<u>compound 6 (DI-1573)</u>**. m/z: $[M]^+$ calculated for $C_{31}H_{48}N_8O_{17}P_3S^+$, 929.2071; found, 929.2081; Δ = +1.1 ppm.

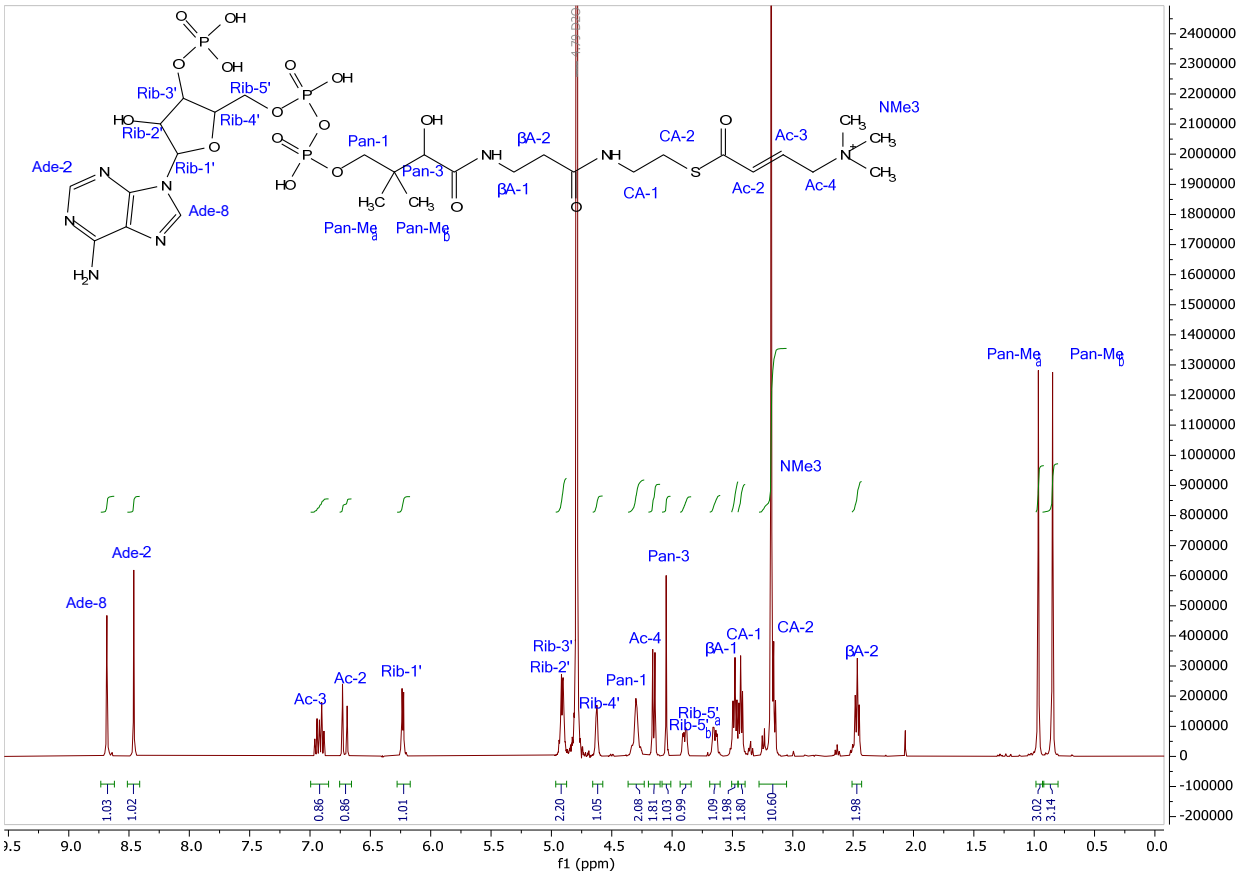



**Figure S19.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 7 (DI-1580)**

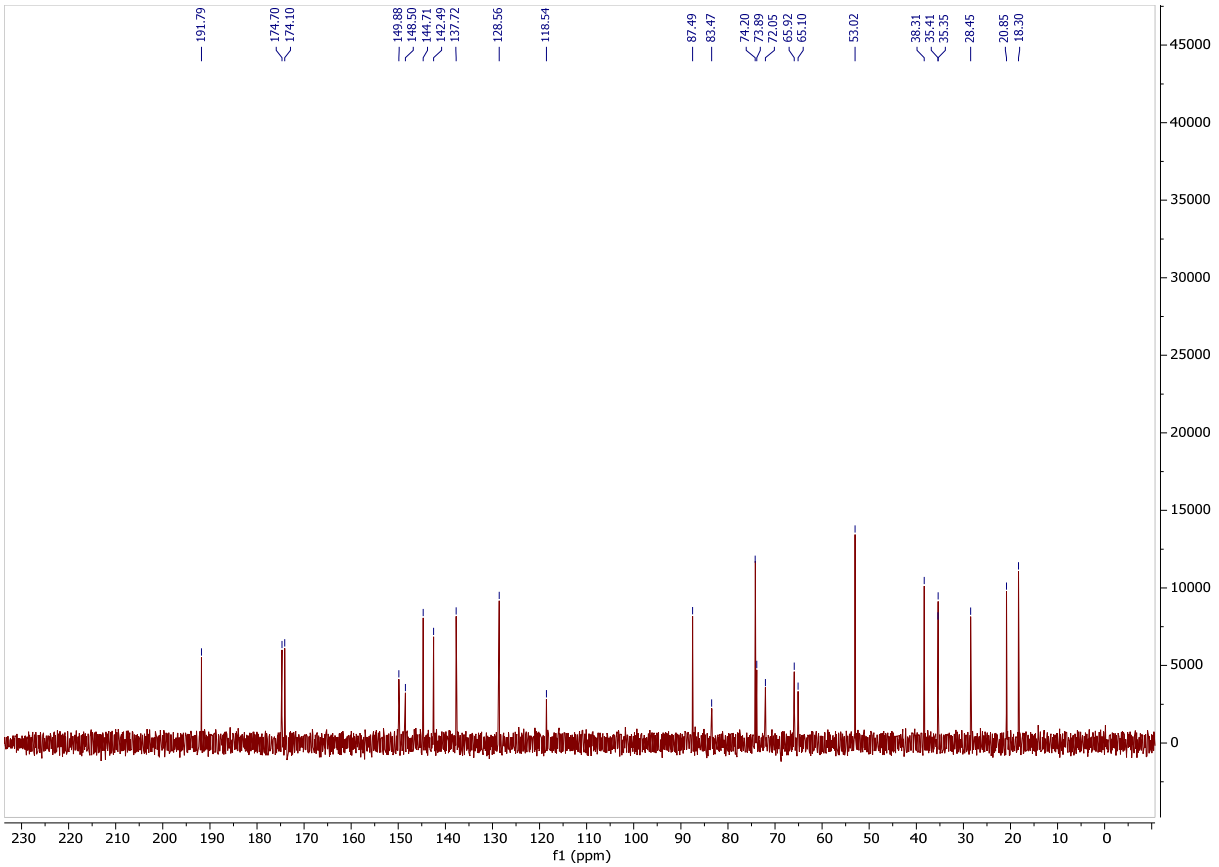



**Figure S20.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 7 (DI-1580)**

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -0.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
3158 formula(e) evaluated with 14 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-150 H: 1-150 N: 0-20 O: 0-20 S: 1-1 P: 3-3

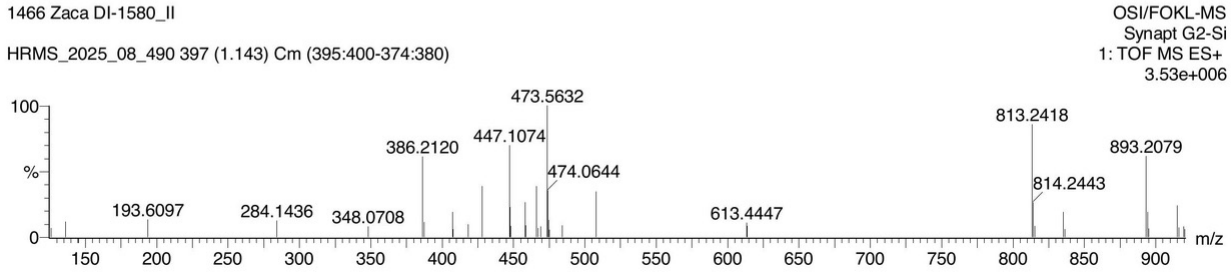



**Figure S21**. HRMS (ESI$^+$, TOF) spectrum of **compound 7 (DI-1580)**. m/z: $[M]^+$ calculated for $C_{28}H_{48}N_8O_{17}P_3S^+$, 893.2071; found, 893.2079; Δ = +0.9 ppm.

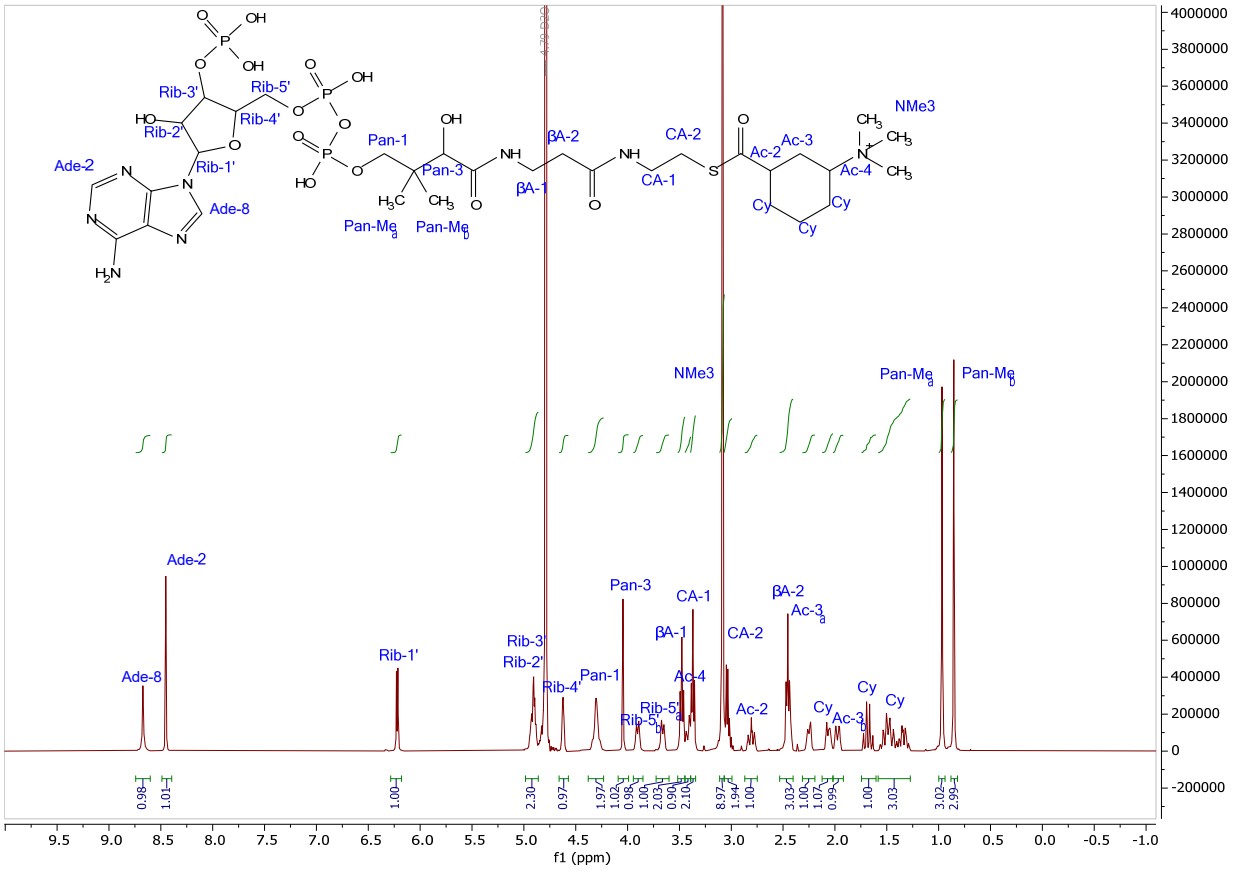



**Figure S22.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 8 (DI-1593)**

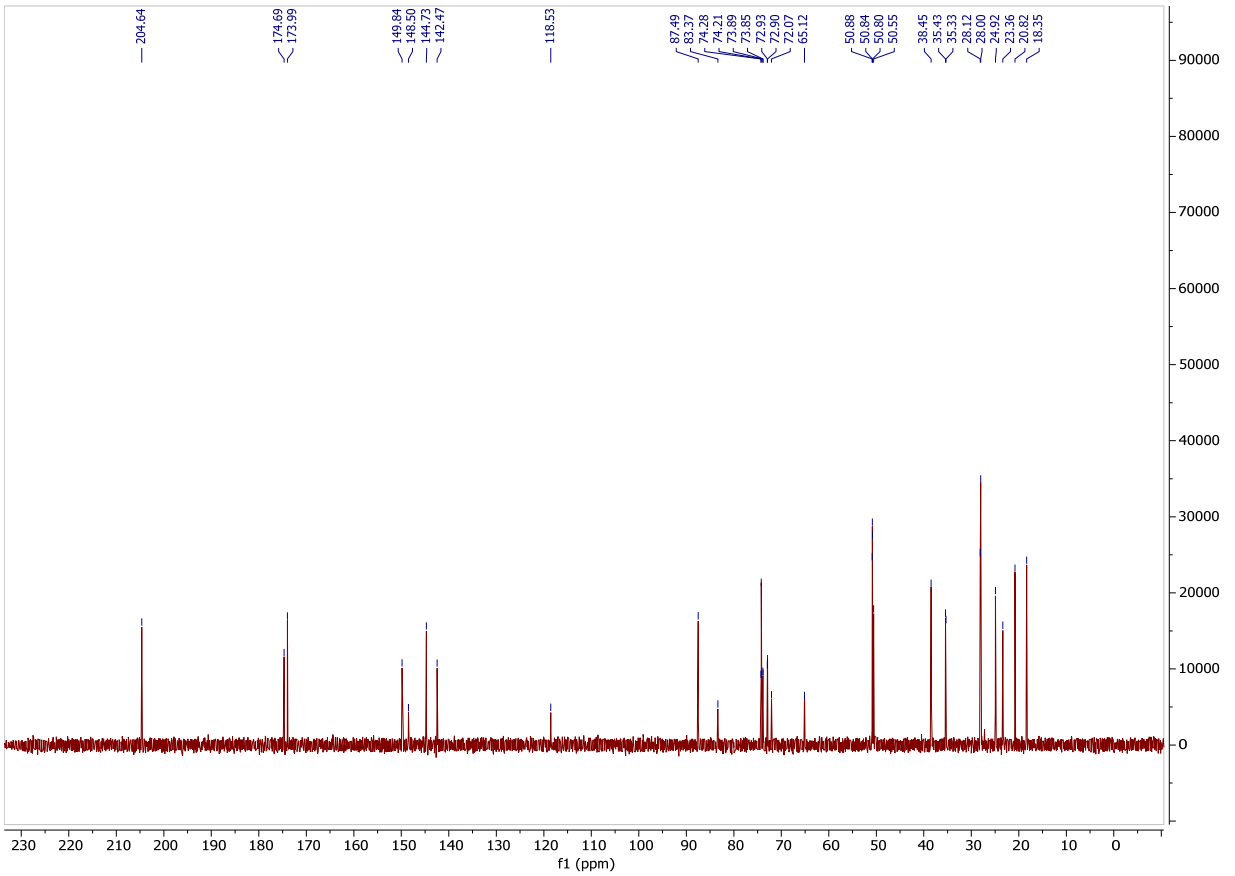



**Figure S23.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 8 (DI-1593)**

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 3.0 PPM / DBE: min = -0.5, max = 200.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
4056 formula(e) evaluated with 16 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 1-150 H: 1-150 N: 0-25 O: 0-20 P: 3-3 S: 1-1

1651 Zaca DI-1593

HRMS_2025_09_542 176 (0.538) Cm (173:181)

OSI/FOKL-MS
Synapt G2-Si
1: TOF MS ES+
3.45e+007

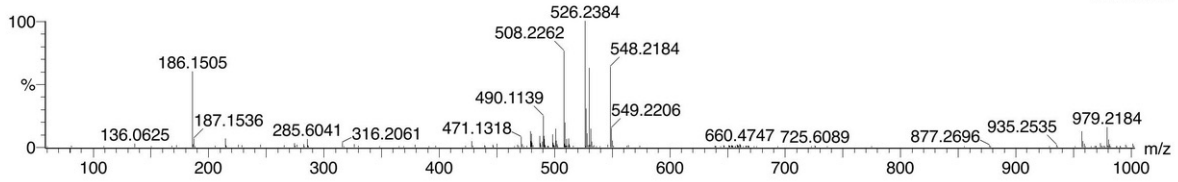



**Figure S24**. HRMS (ESI$^+$, TOF) spectrum of **compound 8 (DI-1593)**. m/z: [M]$^+$ calculated for $C_{31}H_{54}N_8O_{17}P_3S^+$, 935.2540; found, 935.2535; Δ = -0.5 ppm.

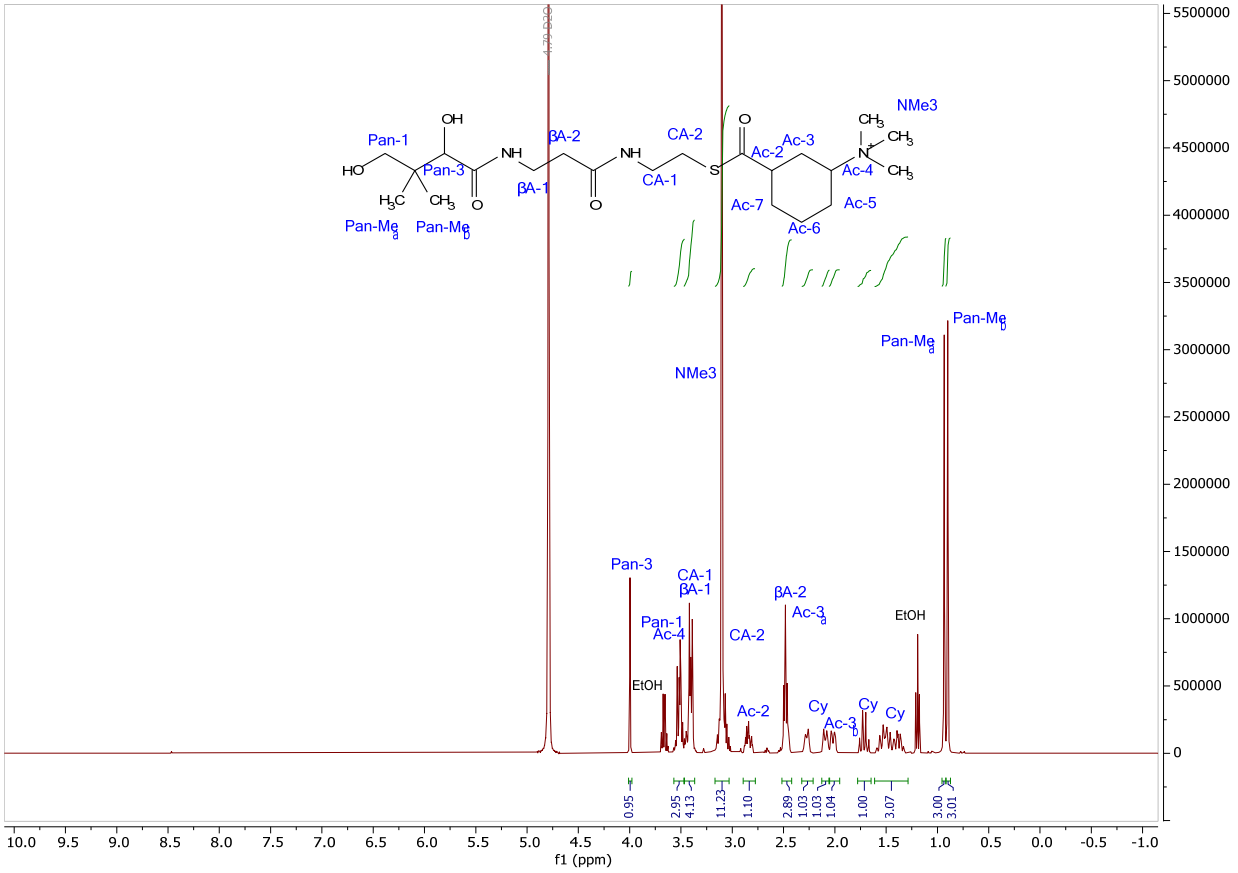



**Figure S25.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 9 (DI-1464)**

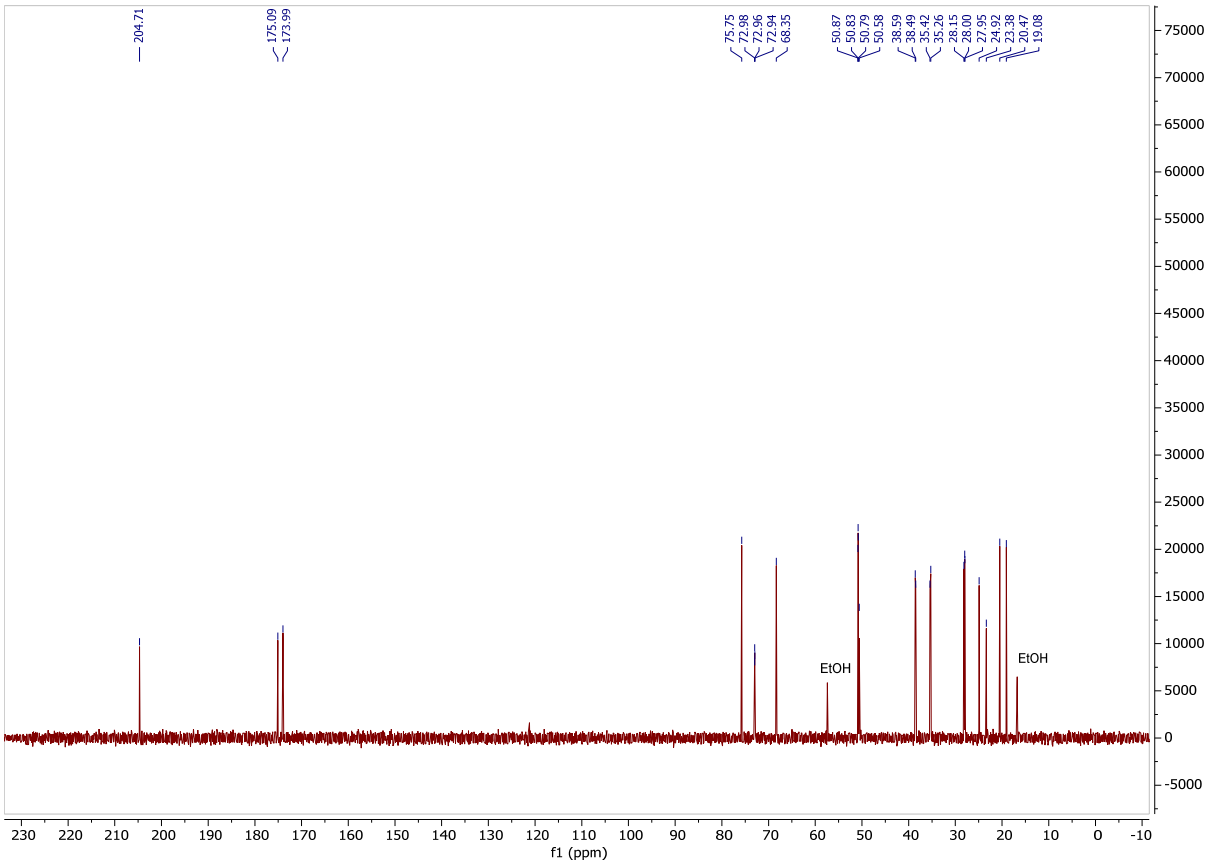



**Figure S26.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 9 (DI-1464)**

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 7

Monoisotopic Mass, Even Electron Ions
1296 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-150 H: 0-150 N: 0-25 O: 0-20 S: 1-1
1004 Zaca DI-1464
HRMS_2025_05_461 467 (1.340) Cm (467:469-444:447)

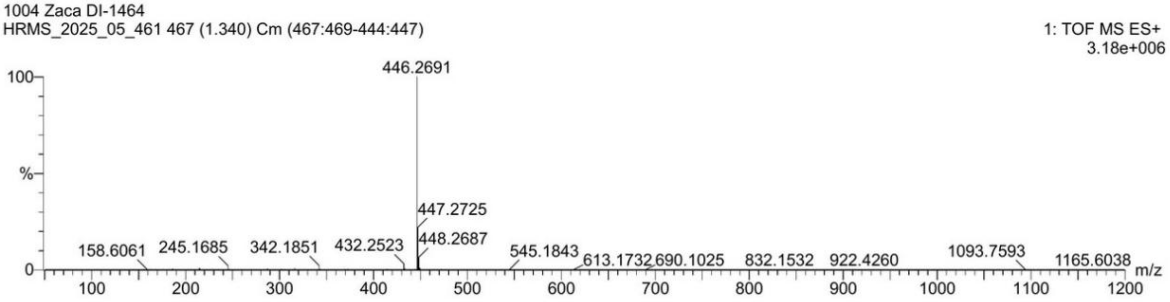



**Figure S27**. HRMS (ESI$^+$, TOF) spectrum of **compound 9 (DI-1464)**. m/z: $[M]^+$ calculated for $C_{21}H_{40}N_3O_5S^+$, 446.2691; found, 446.2689; Δ = -0.4 ppm.

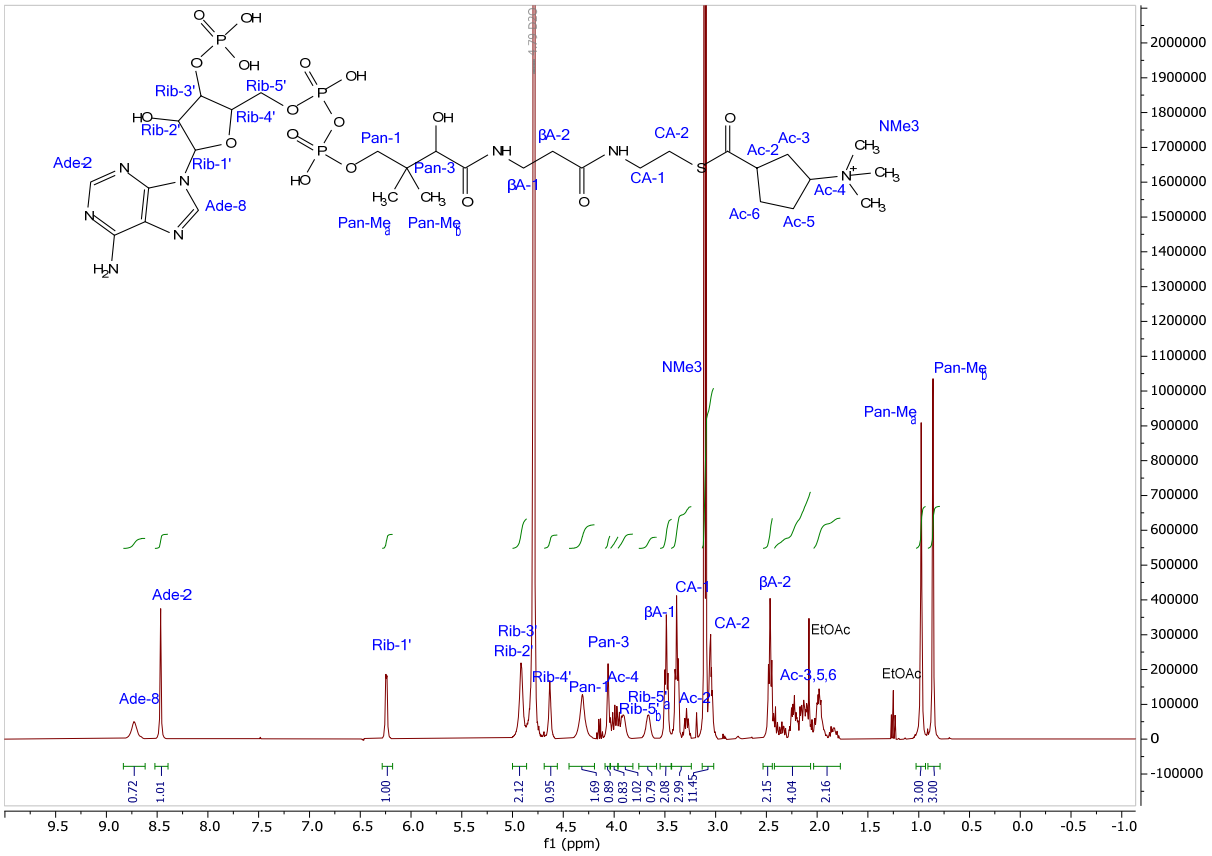



**Figure S28.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 10 (DI-1639)**

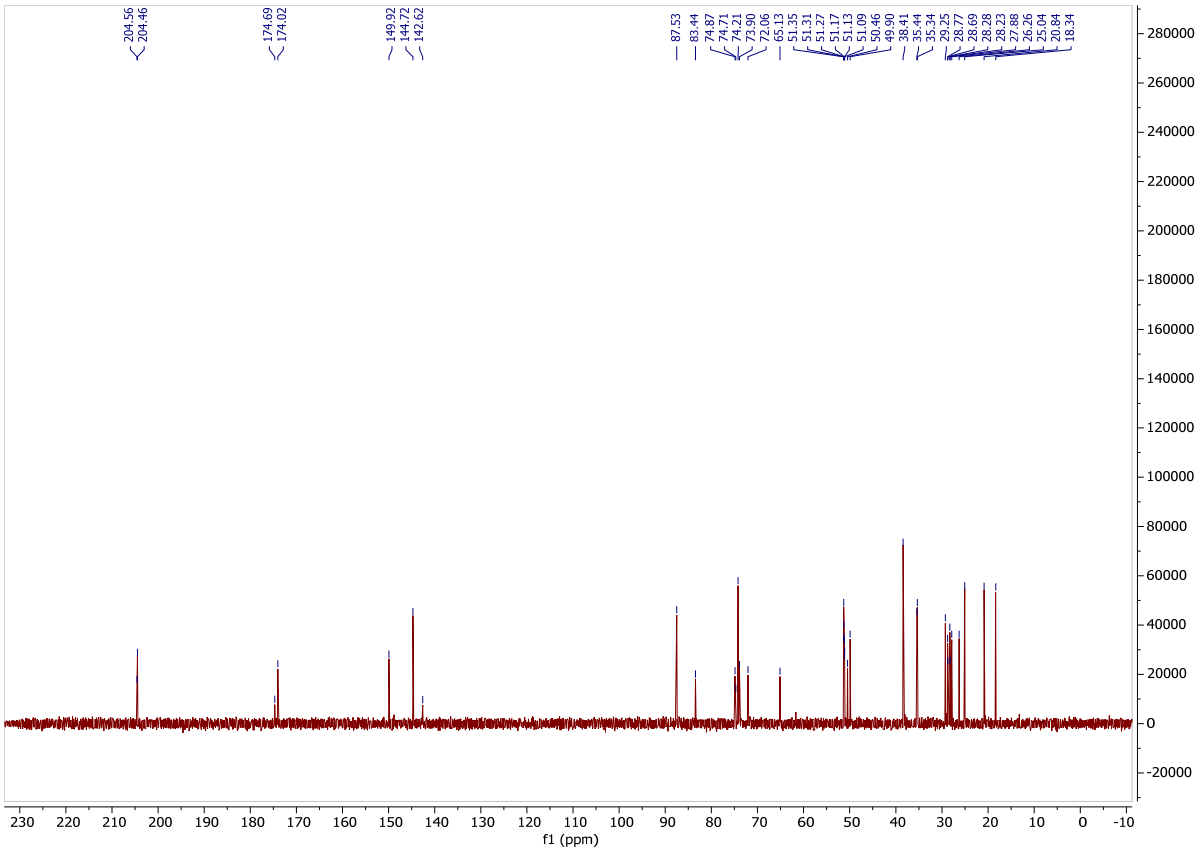



**Figure S29.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 10 (DI-1639)**

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Odd and Even Electron Ions
4134 formula(e) evaluated with 30 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-150 H: 0-150 N: 0-25 O: 0-20 P: 3-3 S: 1-1
1901 Zaca DI-1639
HRMS_2025_11_142 133 (0.398) Cm (132:135-122:124)

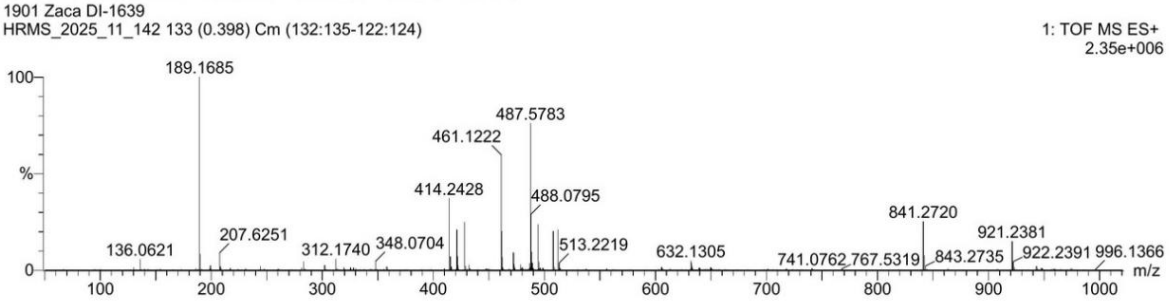



**Figure S30**. HRMS (ESI$^+$, TOF) spectrum of **compound 10 (DI-1639)**. m/z: $[M]^+$ calculated for $C_{30}H_{52}N_8O_{17}P_3S^+$, 921.2384; found, 921.2381; Δ = -0.3 ppm.

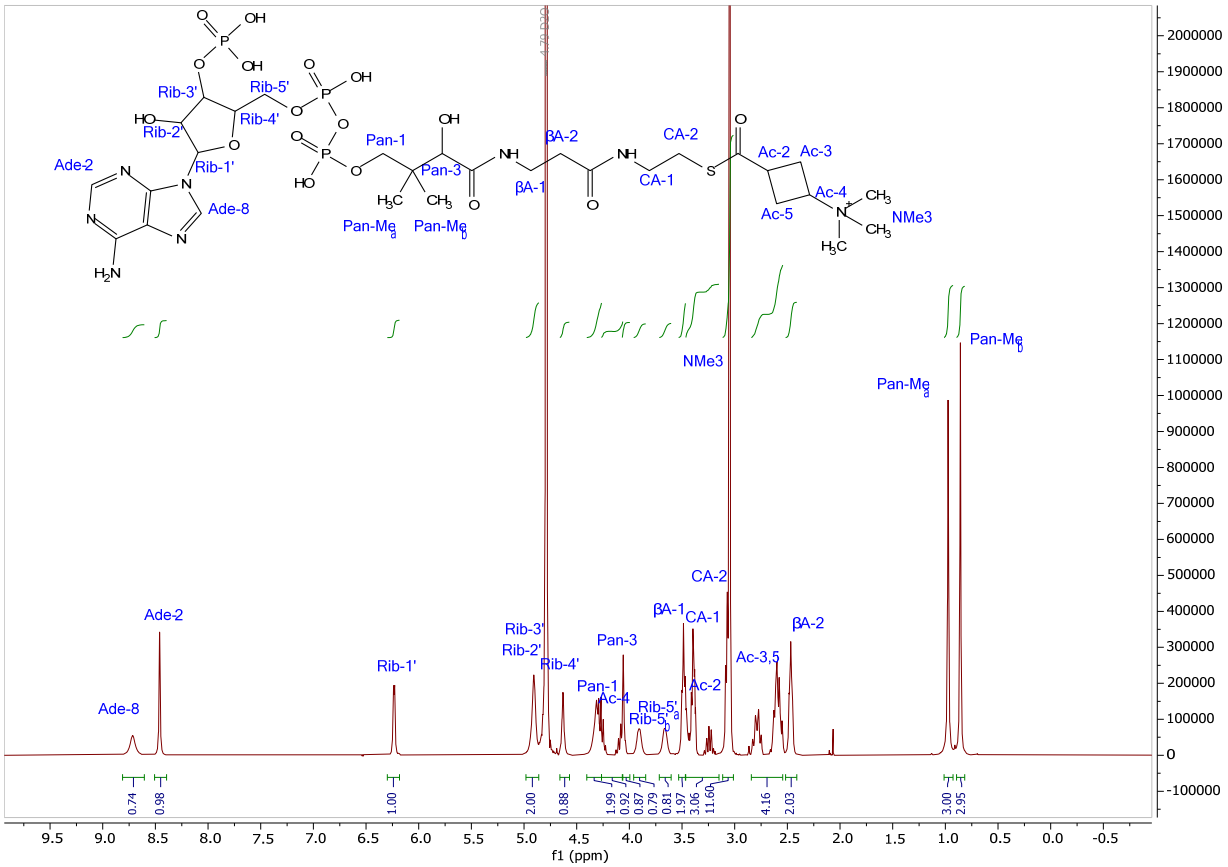



**Figure S31.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 11 (DI-1664)**

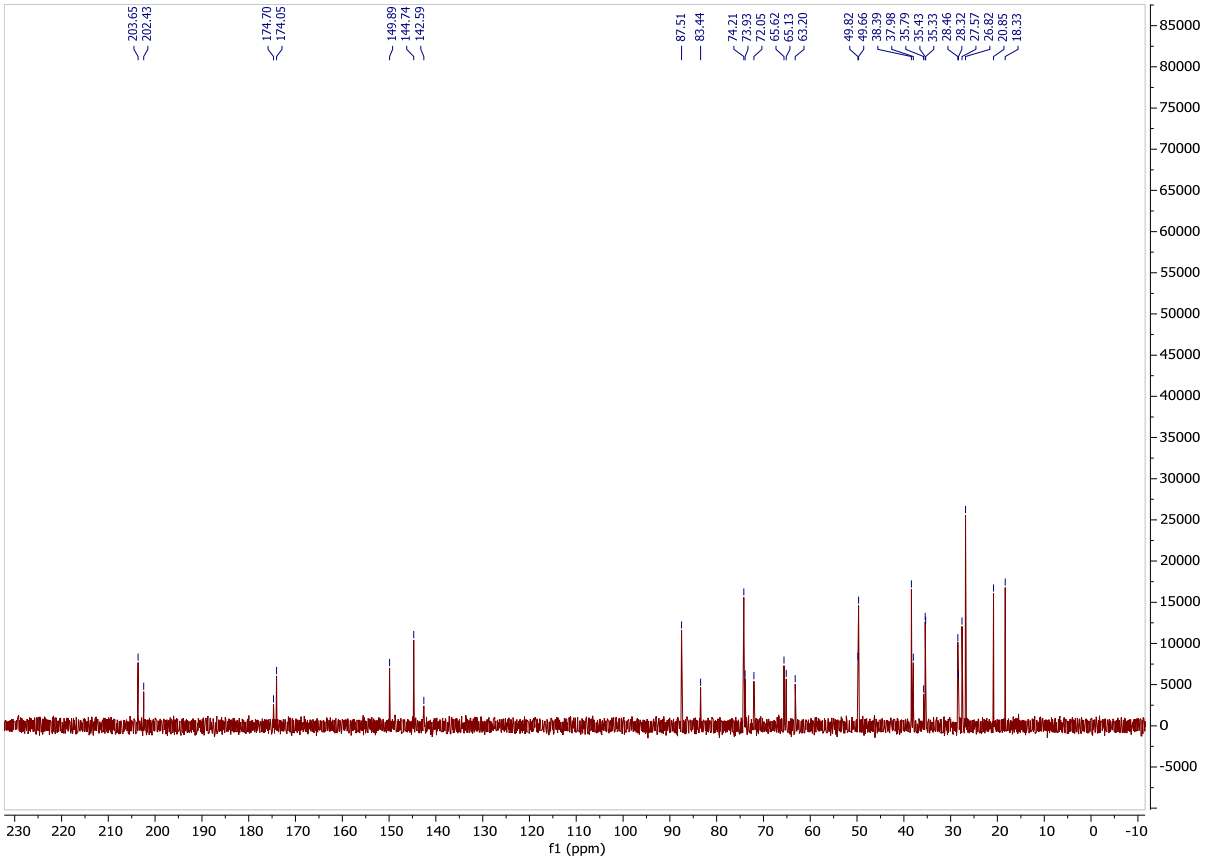



**Figure S32.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 11 (DI-1664)**

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 5

Monoisotopic Mass, Even Electron Ions
4043 formula(e) evaluated with 17 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-150 H: 0-150 N: 0-25 O: 0-20 S: 1-1 P: 3-3

2019 Zaca DI-1664
HRMS_2025_11_587 134 (0.401) Cm (134:138-117:121)

1: TOF MS ES+
3.78e+006

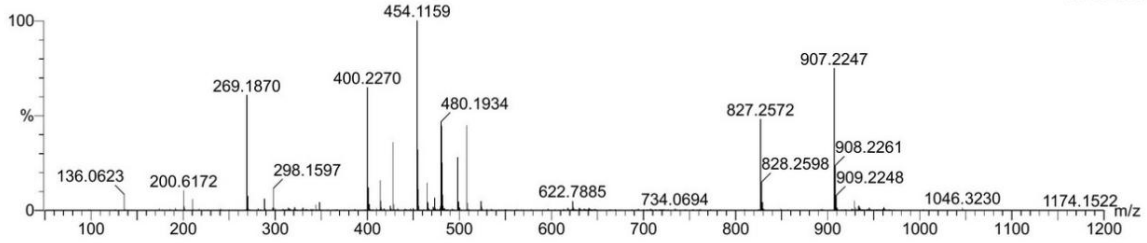



**Figure S33**. HRMS (ESI$^+$, TOF) spectrum of **compound 11 (DI-1664)**. m/z: $[M]^+$ calculated for $C_{29}H_{50}N_8O_{17}P_3S^+$, 907.2227; found, 907.2247; Δ = +2.2 ppm.

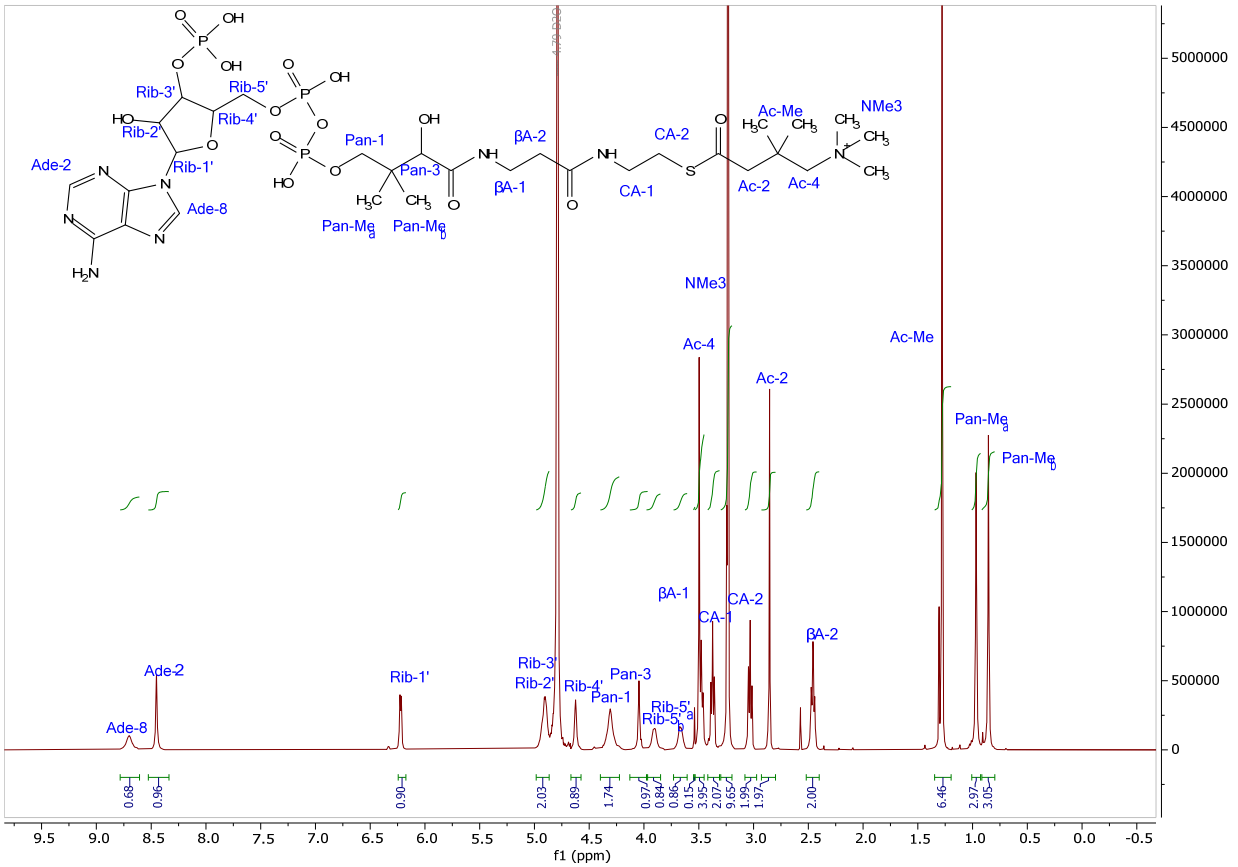



**Figure S34.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 12 (DI-1678)**

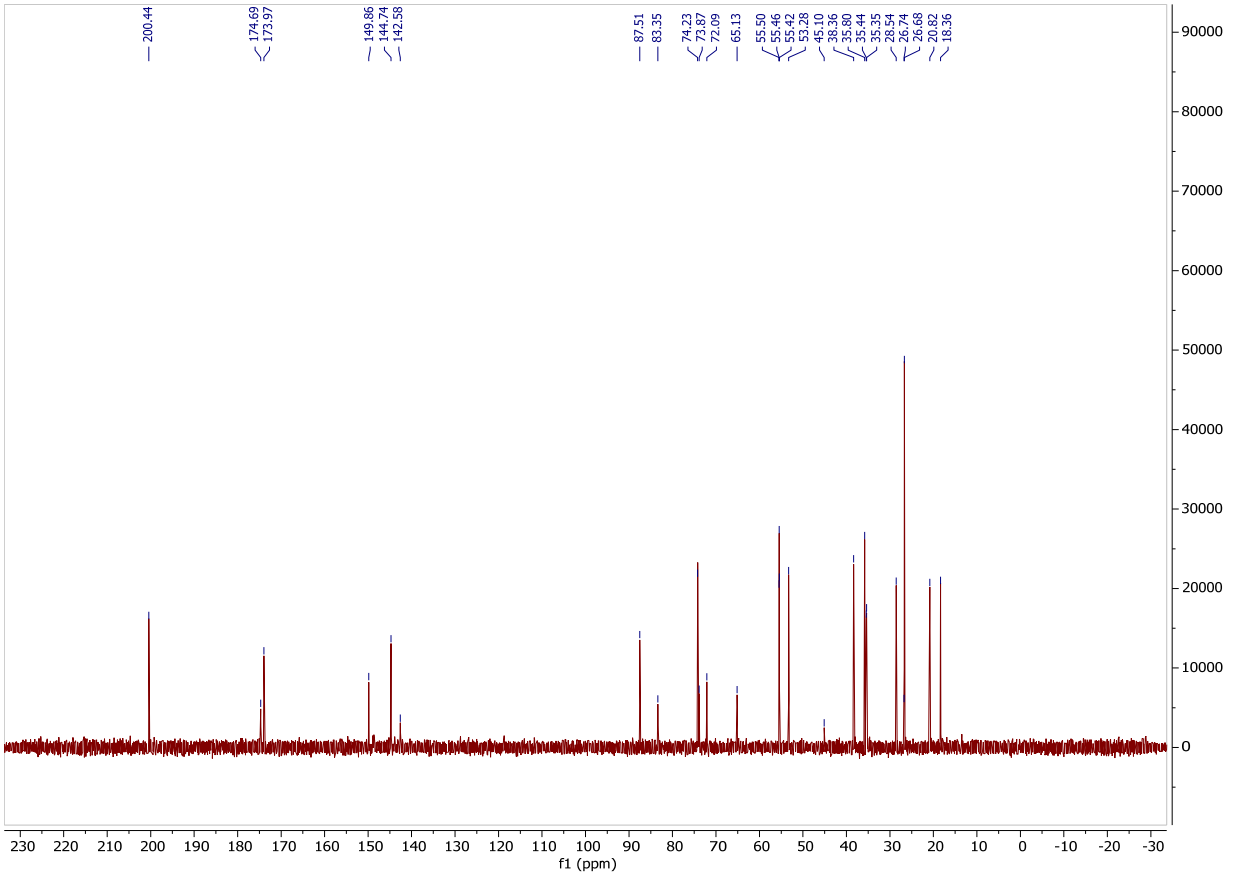



**Figure S35.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 12 (DI-1678)**

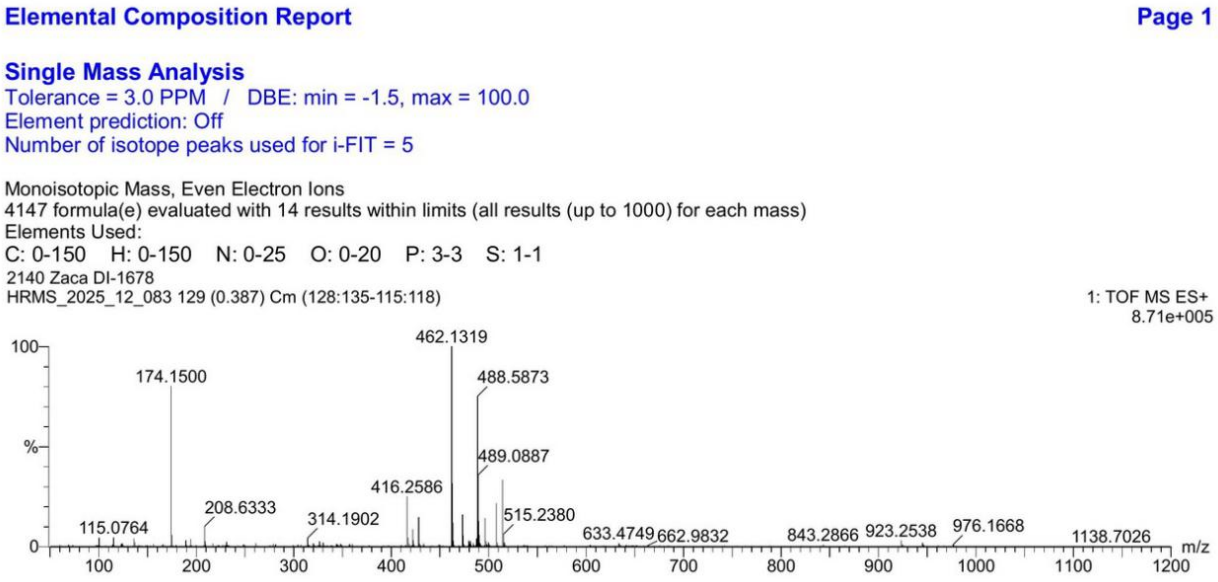



**Figure S36**. HRMS (ESI$^+$, TOF) spectrum of **<u>compound 12 (DI-1678)</u>**. m/z: $[M]^+$ calculated for $C_{30}H_{54}N_8O_{17}P_3S^+$, 923.2540; found, 923.2538; Δ = -0.2 ppm.

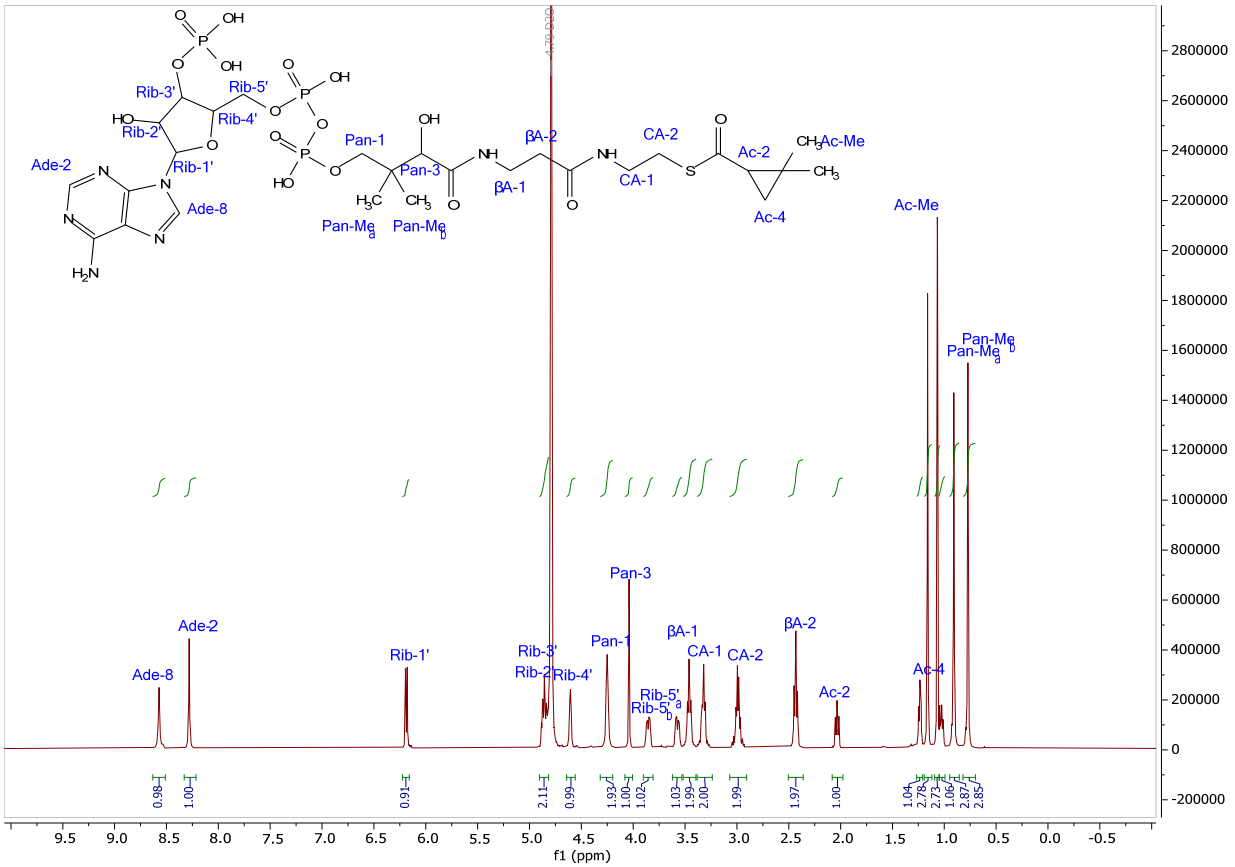



**Figure S37.** $^{1}$H NMR (400 MHz, $D_2O$) spectrum of **compound 13 (DI-1271)**

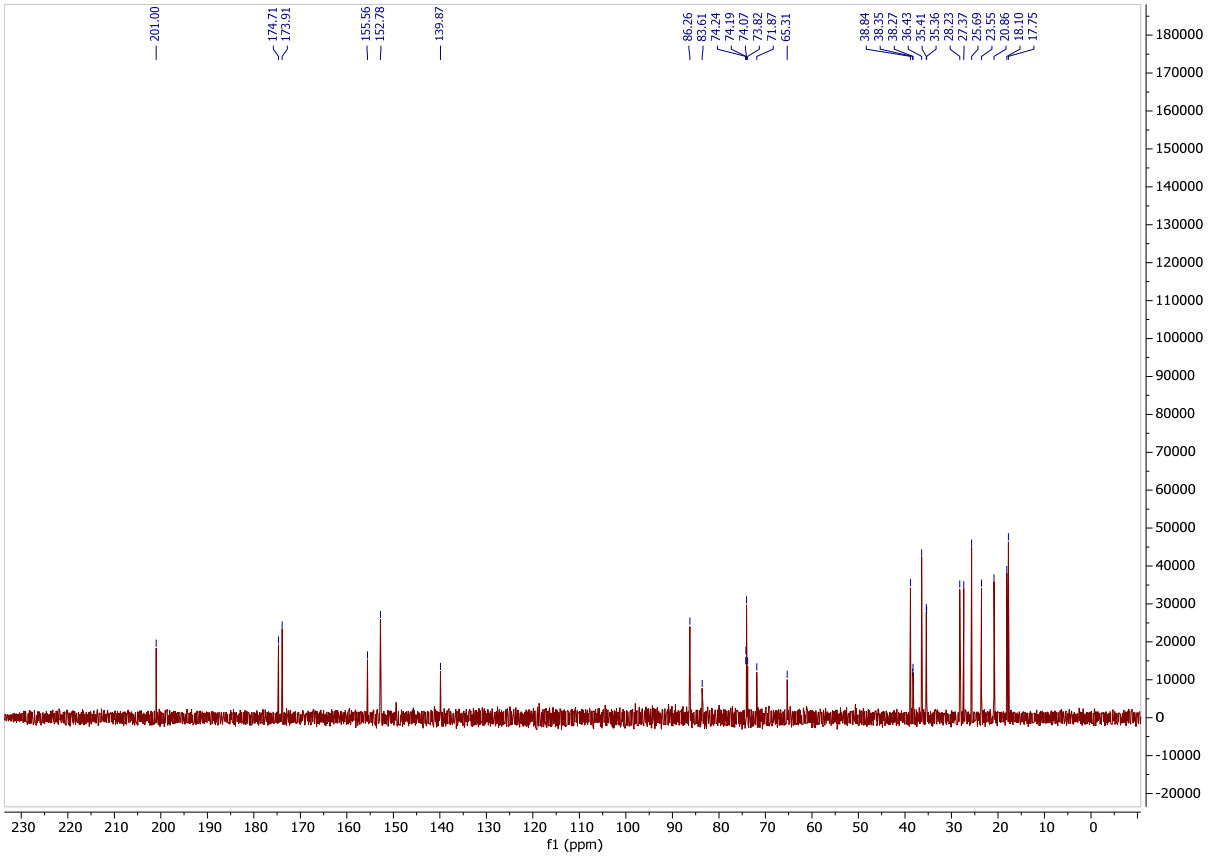



**Figure S38.** $^{13}$C NMR (101 MHz, $D_2O$) spectrum of **compound 13 (DI-1271)**

**Elemental Composition Report** **Page 1**

**Single Mass Analysis**
Tolerance = 3.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 7

Monoisotopic Mass, Even Electron Ions
3809 formula(e) evaluated with 16 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-150 H: 0-150 N: 0-25 O: 0-20 P: 3-3 S: 1-1
350 Zaca DI-1271
HRMS_2026_02_072 549 (1.577) Cm (546:555-511:529)

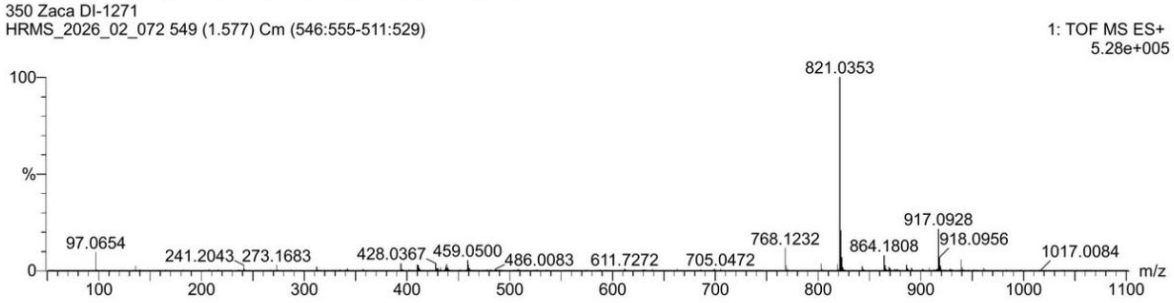



**Figure S39**. HRMS (ESI$^+$, TOF) spectrum of **compound 13 (DI-1271)**. m/z: $[M+H]^+$ calculated for $C_{27}H_{45}N_7O_{17}P_3S^+$, 864.1805; found, 864.1808; Δ = +0.3 ppm.

**Supplementary references:**

[SI1] Cui, L. Q., Dong, Z. L., Liu, K. and Zhang, C. “Design, synthesis, structure, and dehydrogenation reactivity of a water-soluble o-iodoxybenzoic acid derivative bearing a trimethylammonium group.” *Organic letters*, 13 (2011): 6488–6491.

[SI2] Badland, M., Bains, C. A., Howard, R., Laity, D. and Newman, S. D. “An improved synthesis of (1R,3S)-3-[(tert-butoxycarbonyl)amino]cyclohexanecarboxylic acid.” *Tetrahedron: Asymmetry* 21 (2010): 864–866.

[SI3] Tars, K., Leitans, J., Kazaks, A., Zelencova, D., Liepinsh, E., Kuka, J., Makrecka, M., Lola, D., Andrianovs, V., Gustina, D., Grinberga, S., Liepinsh, E., Kalvinsh, I., Dambrova, M., Loza, E. and Pugovics, O. “Targeting carnitine biosynthesis: discovery of new inhibitors against γ-butyrobetaine hydroxylase.” *Journal of medicinal chemistry* 57 (2014): 2213–2236.