Supporting Information

Enhanced chemical stability of AdoMet analogs for improved methyltransferase-directed labeling of DNA

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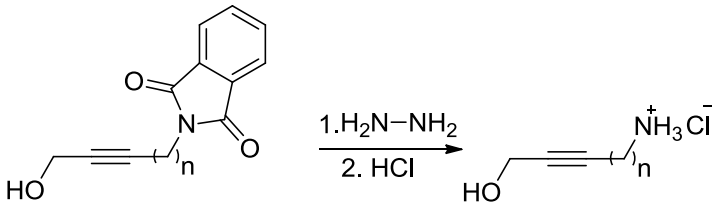


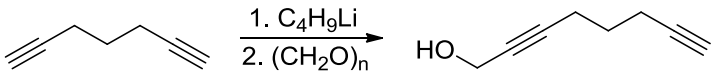
n = 1, 4-Aminobut-2-yn-1-ol hydrochloride, **14**
n = 3, 6-Aminohex-2-yn-1-ol hydrochloride, **15**

A solution (150 ml) of 4-phthalimidobut-2-yn-1-ol (7.6 g, 35 mmol, 1 equiv, prepared from 2-butyn-1,4-diol according to Thomson methodology)[1] or 6-phthalimidohex-2-yn-1-ol (8.5 g, 35 mmol, 1 equiv, prepared from 5-chloro-1-pentyne according to adapted methodologies)[2] in methanol was treated with hydrazine hydrate (3.46 ml, 70 mmol, 2 equiv). The reaction mixture was heated with reflux for 2 h and after cooling to room temperature the solvent was removed under reduced pressure (100 mmHg, 40°C). Water and ethanol (100 ml, 1:1 mixture) and conc. hydrochloric acid (100 ml) were added to the residue. The mixture was heated with reflux for 20 min and the precipitate removed by filtration. The filtrate was concentrated under reduced pressure (10 mmHg, 60 °C). The resulting 4-aminobut-2-yn-1-ol hydrochloride residue was crystallized from methanol as a white solid. The 6-aminohex-2-yn-1-ol was used in further reactions without crystallization.

4-Aminobut-2-yn-1-ol hydrochloride (14), yield 82%. ^{1}H NMR (300 MHz, D_2O, δ): 3.77 (t, J = 2.0 Hz, -CH_2-, 2H), 4.18 (t, J = 2.0, -CH_2-, 2H); ^{13}C NMR (75 MHz, D_2O, δ): 32.09, 52.23, 79.15, 87.96.

6-Aminohex-2-yn-1-ol hydrochloride (15), yield 70%. ^{1}H NMR (300 MHz, D_2O, δ): 1.80-1.90 (m, -CH_2-, 2H), 2.39 (tt, J = 7.0, 2.2Hz, -CH_2-, 2H), 3.13 (t, J = 7.0 Hz, -CH_2-, 2H), 4.22 (t, J = 2.2Hz, -CH_2-, 2H); ^{13}C NMR (75 MHz, D_2O, δ): 15.49, 25.78, 38.80, 49.91, 79.58, 84.62. HRMS: m/z $[M+H]^+$ calcd for $C_6H_{12}NO$: 114.0913; found: 114.0914

Preparation of octa-2,7-diyn-1-ol (**16**)



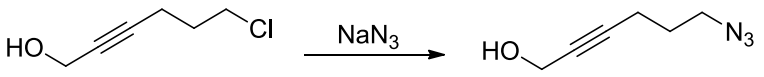
Octa-2,7-diyn-1-ol, **16**

To a cooled solution (–80 °C) of the hepta-1,6-diyne (2 g, 25.6 mmol, 1 equiv) in 30 ml THF butyllithium solution in hexanes (3 ml, 30 mmol, 1.2 equiv) was added (modified method described in [3]). Mixture was stirred for 1 h and paraformaldehyde powder (0.85 g, 25.6 mmol, 1 equiv) was added in one portion. The stirring mixture was allowed to warm to room temperature over ~4 hr. The suspension of paraformaldehyde gradually dissolved during this period. The reaction was quenched by addition of 50 ml of ice-cold water. The aqueous layer was separated and extracted with three 50 ml portions of diethyl ether. Combined organic layers were dried over anhydrous magnesium sulfate and evaporated. Product was purified *via* flash chromatography (silica gel flushed with chloroforme, target product eluted with ethyl acetate step gradient). Two separately eluting fraction were collected which

gave clear oil. NMR spectra confirmed that one fraction is octa-2,7-diyn-1-ol (0.8 g, 7.4 mmol, 29% yield) and the other - nona-2,7-diyne-1,9-diol (0.67 g, 4.9 mmol, 19% yield).

Octa-2,7-diyn-1-ol (16), 29% yield.[1]H NMR (300 MHz, $CDCl_3$, δ): 1.63-1.75 (m, -CH_2-, 2H), 1.94 (t, J = 2.6 Hz, ≡C*H*, 1H), 2.09 (br s, -O*H*, 1H), 2.30 (m, -CH_2-, 4H), 4.21 (t, J = 2.2 Hz, -CH_2-, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 17.99, 24.80, 26.61, 51.24, 51.46, 79.34, 83.73, 85.35.

Preparation of 6-azidohex-2-yn-1-ol (**17**)

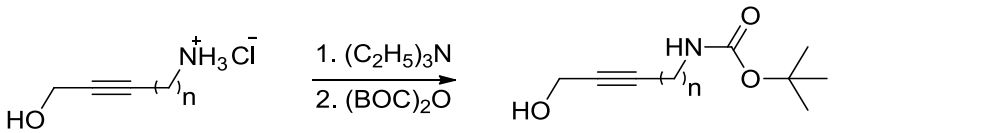


6-Azidohex-2-yn-1-ol, **17**

To a solution of the 6-chlorohex-2-yn-1-ol (0.1 g, 0.74 mmol, 1 equiv) in 5 ml DMF sodium azide (0.147 g, 2.26 mmol, 3 equiv) and tetrabutylammonium bromide (0.024 g, 0.074 mmol, 0.1 equiv) were added. The mixture was stirred for 24 h at 80 °C (sand bath) temperature, then 5 ml of water was added to reaction vessel. Target product was extracted with diethyl ether (3 x 10 ml), combined organic layers were dried over magnesium sulphate and evaporated. Purification of product *via* flash chromatography (silica gel 5–40 μm, flushed with benzene, target product eluted with dichloromethane) afforded clear oil after removal of eluent under reduced pressure.

6-Azidohex-2-yn-1-ol (17), 70% yield. [1]H NMR (300 MHz, $CDCl_3$, δ): 1.81 (m, -CH_2- and –O*H*, 3 H), 2.37 (tt, J = 6.9, 2.4 Hz, -C≡CCH_2-, 2H), 3.44 (t, J = 6.9Hz, -CH_2-, 2H), 4.28 (t, J = 2.4 Hz, -CH_2-, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 16.3, 27.9, 50.0, 51.5, 79.6, 84.7; IR: ν (cm^{-1}) = 3350 (OH), 2223 (C≡C), 2100 (N_3).

Protection of reactive amino group



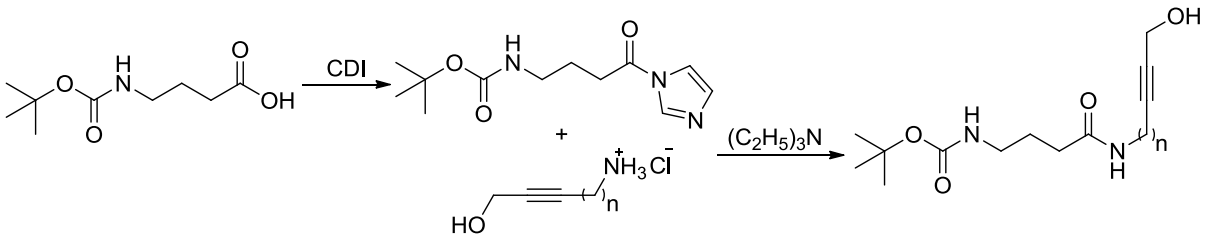
n = 1, 4-(*N*-Boc-amino)but-2-yn-1-ol, **18**
n = 3, 6-(*N*-Boc-amino)hex-2-yn-1-ol, **19**

Protection of a primary amino group in aminoalcohols was performed according to published procedures.[4]

4-(*N*-Boc-amino)but-2-yn-1-ol (18), yield 90%. [1]H NMR (300 MHz, $CDCl_3$, δ): 1.45 (s, -C(CH_3)$_3$, 9H), 3.95 (s, -CH_2-, 2H), 4.26 (s, -CH_2-, 2H); 5.08 (br s, -NH-, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 28.25, 30.50, 50.62, 80.08, 81.39, 81.55, 155.58.

6-(*N*-Boc-amino)hex-2-yn-1-ol (19), yield 80%. [1]H NMR (300 MHz, $CDCl_3$, δ): 1.35 (s, -C(CH_3)$_3$, 9H); 1.56-1.65 (m, -CH_2-, 2H), 2.18 (tt, J = 6.9, 2.0 Hz, -CH_2-, 2H), 3.08-3.18 (m, -CH_2-, 2H), 3.48 (br s, -OH, 1H), 4.14 (br s , -CH_2-, 2H), 4.90 (br s, -NH-, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 16.39, 28.65, 28.86, 39.76, 51.05, 79.56, 79.82, 84.89, 156.46. HRMS: m/z $[M+Na]^+$ calcd for $C_{11}H_{19}NO_3$: 236.1257; found: 236.1276.

Extending aminoalcohols by CDI coupling



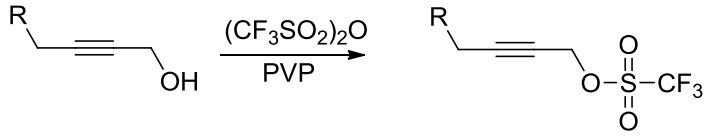
n = 1, 4-[4-(*N*-Boc-amino)butanamido]but-2-yn-1-ol, **20**
n = 3, 6-[4-(*N*-Boc-amino)butanamido]hex-2-yn-1-ol, **21**

4-(***N***-Boc-amino)butanoic acid (2 g, 10 mmol, 1 equiv, prepared in analogy to [4]) was dissolved in anhydrous tetrahydrofuran (20 ml), carbonyldiimidazole (CDI) (1.8 g, 11 mmol, 1.1 equiv) was added, and the resulting clear solution was stirred at room temperature for 2H. Then, the aminoalcohol (1.2 g 4-aminobut-2-yn-1-ol hydrochloride or 1.5 g 6-aminohex-2-yn-1-ol hydrochloride, 10 mmol, 1 equiv) and triethylamine (2.8 ml, 20 mmol, 2 equiv) were added and stirring was continued at room temperature for 2 h. The solvent was removed under reduced pressure (50 mmHg, 40 °C) and the crude product was purified by column chromatography (silica gel, 40 g, chloroform/ethylacetate 1:1). Product containing fractions were pooled and solvent was removed under reduced pressure.

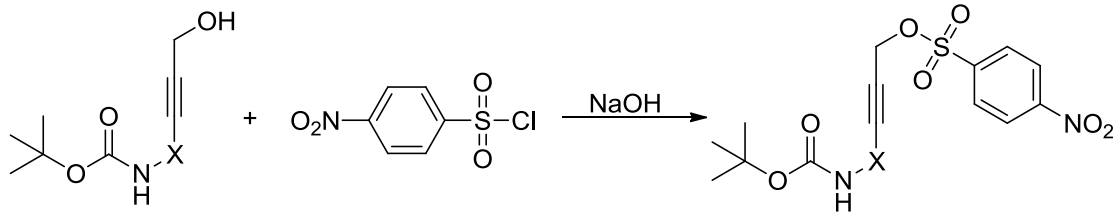
4-[(4-*N*-Boc-amino)butanamido]but-2-yn-1-ol (20), yield 52%. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.41 (s, -C(C*H*$_3$)$_3$, 9H), 1.74-1.83 (m, -C*H*$_2$-, 2H), 2.22 (t, *J* = 7.1Hz, -C*H*$_2$-, 2H,), 3.09-3.18 (m, -C*H*$_2$-, 2H), 4.03-4.09 (m, -C*H*$_2$-, 2H), 4.09-4.14 (m, -C*H*$_2$-, 2H), 4.84 (br s, 1H, -N*H*-), 6.67 (br s, 1H, -N*H*-); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 26.38, 28.64, 29.62, 33.52, 40.05, 50.74, 79.63, 81.22, 81.86, 153.87, 171.53.

6-[(4-*N*-Boc-amino)butanamido]hex-2-yn-1-ol (21), yield 60%. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.45 (s, -C(C*H*$_3$)$_3$, 9H), 1.69–1.87 (m, -C*H*$_2$-,4 H), 3.16 (t, *J* = 6.5 Hz, -C*H*$_2$-, 2H), 3.39 (q, *J* = 6.5, -C*H*$_2$-, 2H), 4.24 (t, *J* = 2.2Hz, -C*H*$_2$-, 2H), 5.06 (br s, 1H), 6.81 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 16.74, 26.65, 28.21, 28.66, 33.89, 39.01, 40.14, 51.12, 79.73, 80.08, 84.99, 159.93, 173.41.

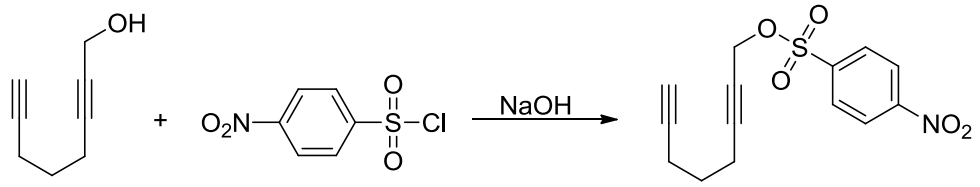
Activation of alcohols by sulfonylation



R = -H, But-2-yn-1-ol triflate, **22**
R = $-CH_3$, Pent-2-yn-1-ol triflate, **23**



X = $-CH_2-$, 4-(*N*-Boc-amino)but-2-ynyl 4-nitrobenzenesulfonate , **24**
X = $-(CH_2)_3-$, 6-(*N*-Boc-amino)hex-2-ynyl 4-nitrobenzenesulfonate, **25**
X = $-CH_2-NH-C(O)-(CH_2)_3-$, 4-[(4-*N*-Boc-amino)butanamido]but-2-yn-1-yl 4-nitrobenzenesulfonate, **26**
X = $-(CH_2)3-NH-C(O)-(CH_2)_3-$, 6-[(4-*N*-Boc-amino)butanamido]hex-2-yn-1-yl 4-nitrobenzenesulfonate, **27**



Octa-2,7-diynyl 4-nitrobenzenesulfonate, **28**

An but-2-yn-1-ol (**22**) and pent-2-yn-1-ol triflates (**23**) were prepared according to [5, 6] and used for the AdoHcy alkylation without purification.

The 4-nitrobenzenesulfonyl addition was performed following amodified previously described procedure.[7] 4-Nitrobenzenesulfonyl chloride (0.90 g, 4 mmol, 1.1 equiv) and sodium hydroxide (0.74 g, 18.5mmol, 5 equiv) were added to a solution of protected aminoalcohol (3.6 mmol, 1 equiv) in methylene chloride (15 ml) at 0°C. After stirring the reaction mixture for 3 h at room temperature sodium hydroxide was filtered, the reaction was quenched with 20 ml of cold water, extracted with methylene chloride (3 x 10 ml) and the combined organic layers dried over sodium sulfate. The sample was passed through a glass filter and concentrated under reduced pressure (200 mmHg, 30 °C) as a slightly yellow solid.

But-2-yn-1-ol triflate (22). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.93 (t, *J*=2.5 Hz, $-CH_3$, 3 H), 5.07 (t, *J*=2.5 Hz, , $-CH_2O-$, 2H), ^{13}C NMR (75 MHz, $CDCl_3$, δ): 3.7, 65.2, 69.8, 89.6, 118.7 (q, *J*(CF) =318 Hz; $-CF_3$);.

4-(*N*-Boc-amino)but-2-ynyl 4-nitrobenzenesulfonate (24), yield 45%. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.47 (s, $-C(CH_3)_3$, 9H), 3.84 (s, $-CH_2-$, 2H), 4.61 (br s, -NH-, 1H), 4.87 (s, $-CH_2-$, 2H); 8.09-8.26 (m, Ar H, 2H), 8.38-8.53 (m, Ar H, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 28.55, 30.57, 59.34, 74.28, 80.71, 87.32, 124.67, 129.72, 142.36, 151.18, 171.87.

6-(*N*-Boc-amino)hex-2-ynyl 4-nitrobenzenesulfonate (25), yield 27%. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.41 (s, $-C(CH_3)_3$, 9H); 1.49-1.58 (m, $-CH_2-$, 2H), 2.09 (tt, *J* = 7.0, 2.2Hz, $-CH_2-$, 2H), 3.03-3.10 (m,

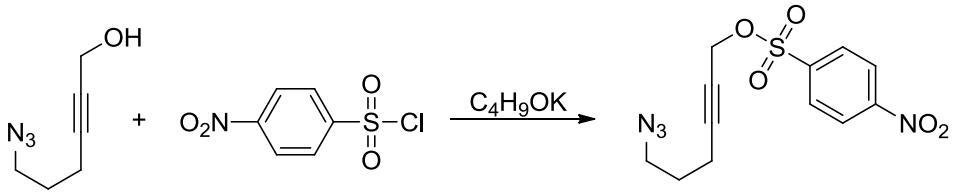
-CH_2-, 2H), 4.57 (br s, -NH-, 1H), 4.80 (t, J = 2.2Hz, -CH_2-, 2H), 8.10-8.14 (m, Ar H, 2H), 8.36-8.41 (m, Ar H, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 16.35, 28.56, 28.63, 39.72, 60.03, 72.23, 79.65, 90.76, 124.61, 129.74, 142.55, 151.05, 156.14. HRMS: m/z $[M+Na]^+$ calcd for $C_{17}H_{22}N_2O_7$: 421.1040; found: 421.1046.

4-[(4-*N*-Boc-amino)butanamido]but-2-yn-1-yl 4-nitrobenzenesulfonate (26), yield 55%. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.43 (s, -C(CH_3)$_3$, 9H), 1.71-1.81 (m, -CH_2-, 2H), 2.19 (t, J = 7.1Hz, -CH_2-, 2H), 3.10-3.16 (m, -CH_2-, 2H), 3.92 (dt, J = 1.8, 5.3 Hz, -CH_2-, 2H), 4.74 (br s, -NH-, 1H), 4.83 (t, J = 1.8 Hz, -CH_2-, 2H), 6.61 (br s, -NH-, 1H), 8.09-8.17 (m, Ar H, 2H), 8.38-8.45 (m, Ar H, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 26.96, 28.64, 29.26, 33.37, 39.66, 59.33, 74.19, 79.89, 87.08, 124.69, 129.74, 142.36, 151.20, 162.76, 172.64.

6-[(4-*N*-Boc-amino)butanamido]hex-2-yn-1-yl 4-nitrobenzenesulfonate (27), yield 50%. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.37 (s, -C(CH_3)$_3$, 9H); 1.50-1.59 (m, -CH_2-, 2H), 1.69-1.79 (m, -CH_2-, 2H), 2.09 (tt, J = 7.1, 2.2Hz, -CH_2-, 2H), 2.19 (t, J = 7.1Hz, -CH_2-, 2H), 3.03-3.21 (m, -CH_2-, 4H), 4.77 (t, J = 2.2Hz, -CH_2-, 2H), 5.13 (br s, -NH-, 1H), 6.87 (br s, -NH-, 1H), 8.07-8.13 (m, Ar H, 2H), 8.33-8.40 (m, Ar H, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 16.48, 26.59, 27.95, 28.59, 33.57, 38.75, 39.98, 60.11, 72.23, 79.48, 90.72, 124.65, 129.69, 142.36, 151.04, 156.87, 173.45.

Octa-2,7-diynyl 4-nitrobenzenesulfonate (28), yield 51 %. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.54 (m, -CH_2-, 2H), 1.95 (t, J = 2.6 Hz, ≡CH, 1H), 2.15 (m, -CH_2-, 4H), 4.83 (t, J = 2.2 Hz, -CH_2-, 2H), 8.10-8.15 (m, Ar H, 2H), 8.36-8.42 (m, Ar H, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 17.78, 27.04, 34.65, 60.13, 69.59, 72.34, 83.06, 90.68, 124.61, 129.71, 142.71, 151.04.

Preparation of 6-azidohex-2-ynyl-4-nitrobenzenesulfonate

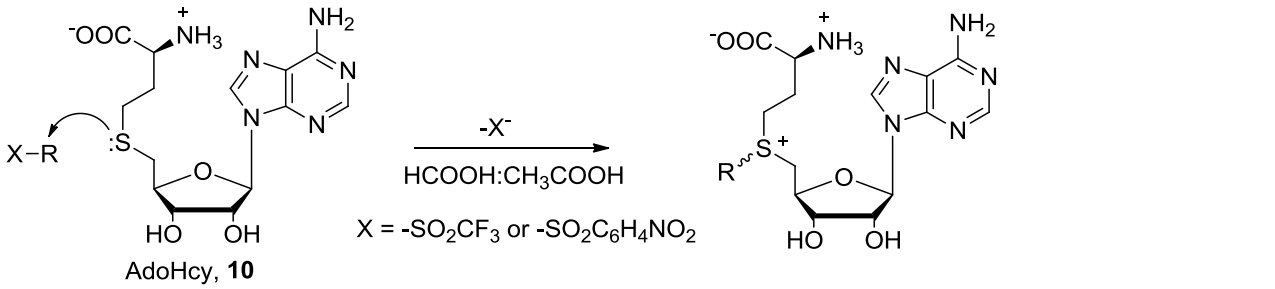


6-Azidohex-2-ynyl 4-nitrobenzenesulfonate, **29**

To cooled (0°C) solution of 6-azidohex-2-yn-1-ol (**14**) (0.073 g, 0.52 mmol, 1 equiv) in anhydrous THF (5 ml) potassium *tert*-butoxide (0.055 g, 0.49 mmol, 0.9 equiv) was added, after several minutes to clear solution a 4-nitrobenzenesulfonyl chloride (0.110 g, 0.49 mmol, 0.9 equiv) was added. Reaction mixture was stirred for 2 h, then solvent was evaporated under reduced pressure, and residue purified *via* flash chromatography (silica gel 5–40 μm, flushed with benzene, target product eluted with dichloromethane). Removal of eluent afforded target product as yellowish powder (m.p. 37–38 °C).

6-azidohex-2-yn-1-yl 4-nitrobenzenesulfonate (29), 42% yield. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.67 (tt, J = 6.9, 6.6 Hz, -CH_2-, 2H), 2.23 (tt, J = 6.9, 2.4 Hz, -CH_2-, 2H), 3.33 (t, J = 6.6 Hz, -CH_2-, 2H), 4.86 (t, J = 2.4Hz, -CH_2-, 2H,), 8.19 (d, J = 9 Hz, Ar H, 2H), 8.46 (d, J = 9 Hz, Ar H, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 16.1, 27.4, 50.1, 59.8, 72.7, 89.9, 124.5, 129.6, 142.5, 151.0. IR: ν (cm^{-1}) = 2146 (C≡C), 2100 (N_3), 1532, 1348 (NO_2). HRMS: m/z $[M+H]^+$ calcd for $C_{12}H_{12}N_4O_5S$: 325.0601; found: 325.0595.

S-Alkylation of S-adenosyl-L-homocysteine (**10**)



AdoHcy, **10**

Cofactor **2**, R = $-CH_2C{\equiv}CCH_3$
Cofactor **3**, R = $-CH_2C{\equiv}CCH_2CH_3$
Cofactor **4**, R = $-CH_2C{\equiv}CCH_2NH_2$
Cofactor **5**, R = $-CH_2C{\equiv}CCH_2NHC(O)(CH_2)_3NH_2$
Cofactor **6**, R = $-CH_2C{\equiv}C(CH_2)_3NH_2$
Cofactor **7**, R = $-CH_2C{\equiv}C(CH_2)_3NHC(O)(CH_2)_3NH_2$
Cofactor **8**, R = $-CH_2C{\equiv}C(CH_2)_3C{\equiv}CH$
Cofactor **9**, R = $-CH_2C{\equiv}C(CH_2)_3N_3$

An activated alcohol (100–200 equivalents of triflate, prepared according to (Ross et al., 2000), or 10-30 equivalents of 4-nitrobenzenesulfonyl ester, or 6 equivalents 6-Azidohex-2-ynyl-4-nitrobenzene-sulfonate (see above)) was slowly added to *S*-adenosyl-L-homocysteine (AdoHcy, **10**, 10–30 mg, 1 equiv) in a 1:1 mixture of formic acid and acetic acid (0.5–1.0 ml) at 0 °C. The solutions were allowed to warm up to room temperature and incubated with shaking. After specified times (2-24 h) the reactions were quenched by adding water (5–10 ml). The aqueous phase was extracted three times with equal volume of diethyl ether and water was removed in rotary evaporator (10 mmHg, 30 °C). Residue was dissolved in 10 ml of HPLC buffer. Purification of cofactors **2** and **3** was performed by preparative reversed-phase HPLC. The 4-nitrobenzenesulfonate was removed by passing solution through Dowex 1 anion exchanger column prior the HPLC purification.
Deprotection of amino group was performed by adding two volumes of CF_3COOH to the water solution of AdoMet analogue and incubating for 1h at room temperature. This procedure completely removes Boc protecting group since no protected cofactor peak appeared after such treatment.

Purification of AdoMet analogs

Purification of AdoMet analogs was performed by preparative reversed-phase HPLC (Supelco Discovery C18 10 x 250, 5 µm or Supelco Discovery HS C18 10 x 150, 5 µm) eluting with a linear gradient of solvents A (20 mM $HCOONH_4$, pH 3.5) and B (80% methanol solution in water) at a flow rate of 4.5 ml/min. The diastereomers of cofactors **8** and **9** were separated using preparative reversed-phase HPLC (column - Agilent Prep-C18, dimensions: 30×150, 10 µm, PN 413910-302) eluting at a flow rate of 33 ml/min using 20 mmol/L ammonium formate buffer (pH = 3.5) as eluent A and 60% methanol in water as eluent B. Compounds were detected by their absorption at 260 and 280 nm. Enriched Fractions were pooled, lyophilized under reduced pressure in a rotary evaporator (10 mmHg, 30 °C) and desalted by passing through reverse phase C-18 silica gel. Structure of novel compound was confirmed by NMR measurements and yields determined by UV absorption of the adenine chromophore (ε_{260} = 15 400 l mol^{-1} cm^{-1}).
Cofactor **2**, yield 12 + 21%. ^1H NMR of *S*-and *R*-isomers mix (300 MHz, D_2O, δ): 1.70–1.73 (m, $H4''_R$, 0.9H), 1.89 (t, *J* = 2.1 Hz, $H4''_S$, 2.1H), 2.27-2.37 (m, $H\beta_{S/R}$, 2H), 3.42–3.85 (m, $H\gamma_{S/R}$, $H\alpha_{S/R}$, $H5'_R$,

3.6H), 3.93–4.00 (m, H5'$_S$, 1.4H), 4.18–4.23 (m, H1"$_R$, 0.6H), 4.27–4.32 (m, H1"$_S$, 1.4H), 4.49–4.57 (m, H4'$_{S/R}$, 1H), 4.63 (t, J = 5.7 Hz, H3'$_{S/R}$, 1H), 4.92–4.97 (m, H2'$_{S/R}$, 1H), 6.07 (d, J = 4.2 Hz, H1'$_S$, 0.7H), 6.11 (d, J = 2.9 Hz, H1'$_R$, 0.3H), 8.25 (s, Ar H$_{S/R}$, 1H), 8.27 (s, Ar H$_S$, 0.7H), 8.28 (s, Ar H$_R$, 0.3H). ESI-MS: m/z (relative intensity): 437 (100) $[M]^+$, 336 (33) [5'-(but-2-ynyl)thio-5'-deoxyadenosine + H]$^+$, 250 (75) [5'-deoxyadenosine]$^+$

Cofactor **3**, yield 7 + 8%. ESI-MS: m/z (relative intensity): 451 (100) $[M]^+$, 350 (30) [5'-(pent-2-ynyl)thio-5'-deoxyadenosine + H]$^+$, 250 (70) [5'-deoxyadenosine]$^+$

Cofactor **4**, yield 17 + 14 %. ^{1}H NMR of *S*-isomer (300 MHz, D_2O, δ): 2.11-2.29 (m, Hβ, 2H), 3.48-3.68 (m, Hα, Hγ, 3H), 3.69-3.92 (m, H5', H4'', 4H), 4.29-4.40 (m, H1'', 0.9H*), 4.48-4.55 (m, H4', 1H), 4.57-4.61 (m, H3', 1H), 4.86 (dd, J = 3.5, 3.1 Hz, H2', 1H), 6.05 (d, J = 3.5 Hz, H1', 1H), 8.21 (br s, Ar H, 2H). * time-dependent loss of resonance in D_2O due to exchange of H for D. ESI-MS: m/z (relative intensity): 452 (100) $[M]^+$, 351 (25) [5'-(aminobut-2-ynyl)thio-5'-deoxyadenosine + H]$^+$, 250 (65) [5'-deoxyadenosine]$^+$

Cofactor **5**, yield 50%. ^{1}H NMR of *S*- and *R*-isomer (300 MHz, D_2O, δ): 1.88-1.97 (m, H7", 2H), 2.29-2.44 (m, H6", Hβ, 4H), 3.01 (t, J = 7.7 Hz, H8", 2H), 3.45-3.75 (m, Hγ, 2H), 3.83 (t, J = 6.3 Hz, Hα, 1H), 4.00 (d, J = 5.9Hz, H5', 2H), 4.05 (br s, H4", 2H), 4.41 (br s, H1", 2H), 4.50-4.56 (m, H4', 1H), 4.62-4.67 (m, H3', 1H), 4.93 (dd, J = 3.8, 5.3 Hz, H2', 1H), 5.09 (t, J = 1.7 Hz, NH, 1H), 6.11 (d, J = 4.2 Hz, H1', 1H), 8.30-8.35 (m, Ar H, 2H). HRMS: m/z $[M]^+$ calcd for HRMS: m/z $[M]^+$ calcd for $C_{22}H_{33}N_8O_6S$: 537.2238; found: 537.2214

Cofactor **6**, yield 3 + 5%. ^{1}H NMR of *S*- and *R*-isomer (300 MHz, D_2O, δ): 1.57–1.80 (m, H5"$_{R/S}$, 2H), 1.96-2.34 (m, H4"$_{R/S}$, Hβ$_{S/R}$, 4H), 2.83 (t, J = 7.9 Hz, H6"$_R$, 1H), 2.92 (t, J = 7.7 Hz, H6"$_S$, 1H), 3.27–3.44 (m, Hγ$_{S/R}$, 2H), 3.45- 3.76 (m, Hα$_{S/R}$, H5'$_R$, 3H), 3.80–3.86 (m, H5'$_S$, 1H), 4.12–4.25 (m, H1"$_{R/S}$, 1.2H*), 4.37–4.47 (m, H4'$_{S/R}$, 1H), 4.59-4.68 (m, H3'$_{S/R}$, 1H), 4.78–4.84 (m, H2'$_{S/R}$, 1H), 5.96 (d, J = 3.8 Hz, H1'$_S$, 0.5H), 5.99 (d, J = 2.8 Hz, H1'$_R$, 0.5H), 8.12-8.16 (m, Ar H$_{S/R}$, 2H). * time-dependent loss of resonance in D_2O due to exchange of H for D. HRMS: m/z $[M]^+$ calcd for $C_{20}H_{30}N_7O_5S$: 480.2024; found: 480.2020

Cofactor **7***, yield 30 %. ^{1}H NMR* of *S*- and *R*-isomer (300 MHz, D_2O, δ): 1.44-1.55 (m, X_{10}, 1H), 1.50-1.59 (m, H5'', 3H), 1.82-1.92 (m, H9'', X_5, 6H), 2.08 (q, X_9, 1.2H), 2.20-2.35 (m, Hβ, H9'', H4'', X_4, 10H), 2.50 (t, X_6, 1.5H), 2.93-3.00 (m, H10'', 5.6H), 3.06 (t, X_{11}, 1H), 3.14 (t, H6''$_R$, 1H), 3.22 (t, H6''$_S$, 1H), 3.42-3.64 (m, H5'$_R$, Hγ, 2.5 H), 3.75-3.80 (m, Hα$_{R/S}$, 1H), 3.93-3.94 (m, H5'$_S$, 0.5H), 4.29 (br s, H1''$_R$, 1H), 4.32 (br s, H1''$_S$, 1H), 4.48-4.55 (m, H4'$_{R/S}$, 1H), 4.59-4.65 (m, H3'$_{R/S}$, 1H), 4.68 (t, X_1, 1.8H,), 4.87-4.92 (m, H2'$_{R/S}$, 1H), 6.03-6.06 (m, H1'$_{R/S}$, 1H) 8.20-8.23 (m, Ar H$_{R/S}$, 2H). * X indicated signals from 6-(4-aminobutanamido)hex-2-yn-1-ol in Cofactor **7** NMR spectrum. HRMS: m/z $[M]^+$ calcd for $C_{24}H_{37}N_8O_6S$: 565.2551; found: 565.2537.

Cofactor **8**, yield 26 %.^{1}H NMR of *S*- and *R*-isomer (300 MHz, D_2O, δ): 1.39-1.48 (m, H5"$_R$, 0.4H), 1.54-1.63 (m, H5"$_S$, 1.6H) 2.03-2.34 (m, H6"$_{R/S}$, H4"$_{R/S}$, H8"$_{R/S}$, Hβ$_{R/S}$, 7H), 3.36–3.90 (m, Hγ$_{R/S}$, Hα$_{R/S}$, H5'$_{R/S}$, 5H), 4.20 (br s, H1"$_R$, 0.4H), 4.27 (br s, H1"$_S$, 1.6H), 4.43–4.51 (m, H4'$_{R/S}$, 1H), 4.55-4.60 (m, H3', 1H), 4.84–4.89 (m, H2', 1H), 6.00 (d, J = 2.8 Hz, H1'$_S$, 0.75H), 6.02 (d, J = 4.0 Hz, H1'$_R$, 0.25H), 8.17-20 (s, Ar H$_{R/S}$, 2H). HRMS: m/z $[M]^+$ calcd for $C_{22}H_{29}N_6O_5S$: 489.1915; found 489.1915.

Cofactor **9,** yield 12.1 % ^{1}H NMR of *S*-isomer (300 MHz, D_2O, δ): 1.42-1.56 (m, H5", 2H), 2.0-2.12 (m, H4", 2H), 2.12-2.24 (m, Hβ, 2H), 3.15 (t, J = 6.3 Hz, H6", 2H), 3.3-3.48 (m, Hγ, 2H), 3.5-3.8 (m, Hα, H5', 3H), 4.18-4.21 (m, H1", 2H), 4.41-4.49 (m, H4', 1H), 4.86 (dd, J = 5.7, 5.1 Hz, H2', 1H), 6.01 (d, J = 5.7 Hz, H1', 1H), 8.17 (s, Ar H, 1H), 8.18 (s, ArH, 1H), signal of C3'-H in the furane ring overlaps with residual water signal in D_2O. HRMS: m/z $[M]^+$ calcd for $C_{20}H_{28}N_9O_5S$: 506.1934; found: 506.1929

Supplementary Tables

Supplementary Table S1. Proton chemical shifts of -CH_2- groups around the sulfonium center and activated transferrable moiety in AdoMet and its synthetic analogs.

Cofactor	Chemical shift (ppm)				Reference
	1" -C*H*$_2$-*/-C*H*$_3$	5' -C*H*$_2$-	γ -C*H*$_2$-	4" -C*H*$_2$-	
1	3.0	3.9	3.5	none	[8]
2	4.2–4.3	3.9–4.0	3.4–3.9	1.7–1.9	[6]
4	4.3–4.4	3.7–3.9	3.5–3.7	3.7–3.9	This work
5	4.4	4.0	3.5–3.8	4.1	This work
6	4.1–4.3	3.5–3.9	3.3–3.5	2.0–2.3	This work
7	4.3	3.4–3.6	3.3–3.8	2.2–2.4	This work
8	4.2–4.3	3.4–3.9	3.4–3.9	2.0–2.3	This work
9	4.2	3.5–3.8	3.3–3.5	2.0–2.1	This work

* time-dependent loss of resonance in D_2O due to exchange of H with D.

Supplementary Table S2.

Apparent catalytic turnover rates (h^{-1}) of M.HhaI variants in reactions with AdoMet and its analogs as determined in DNA protection assays (see Figure S5).

Cofactor	M.HhaI		Reference
	Q82A/N304A	Q82A/Y254S/N304A	
1	16	16	[9]
2	512	512	[9]
3	128	64	[9]
4	0.25/ 2	n.d.	Figure S5
5	1	n.d.	[10]
6	4	64	Figure S5
7	8	16	Figure S5 and [9]
8	n.d.	64	Figure S5

Supplementary Figures

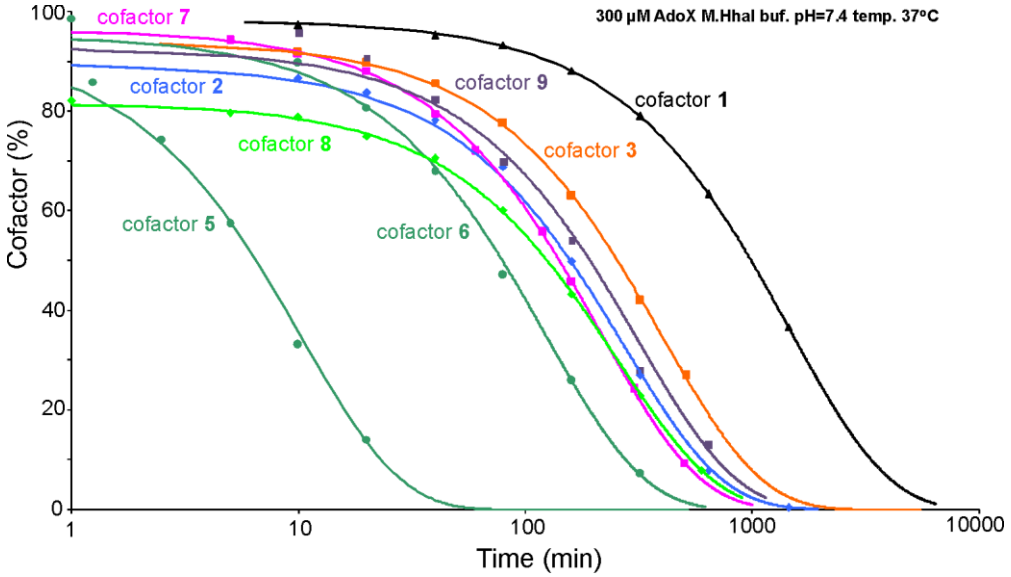


Figure S1. Decomposition kinetics of cofactors **1**–**9** in M.HhaI buffer (50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 2 mM 2-mercaptoethanol, 10 mM NaCl, 0.2 mg/ml BSA) at 37 °C.

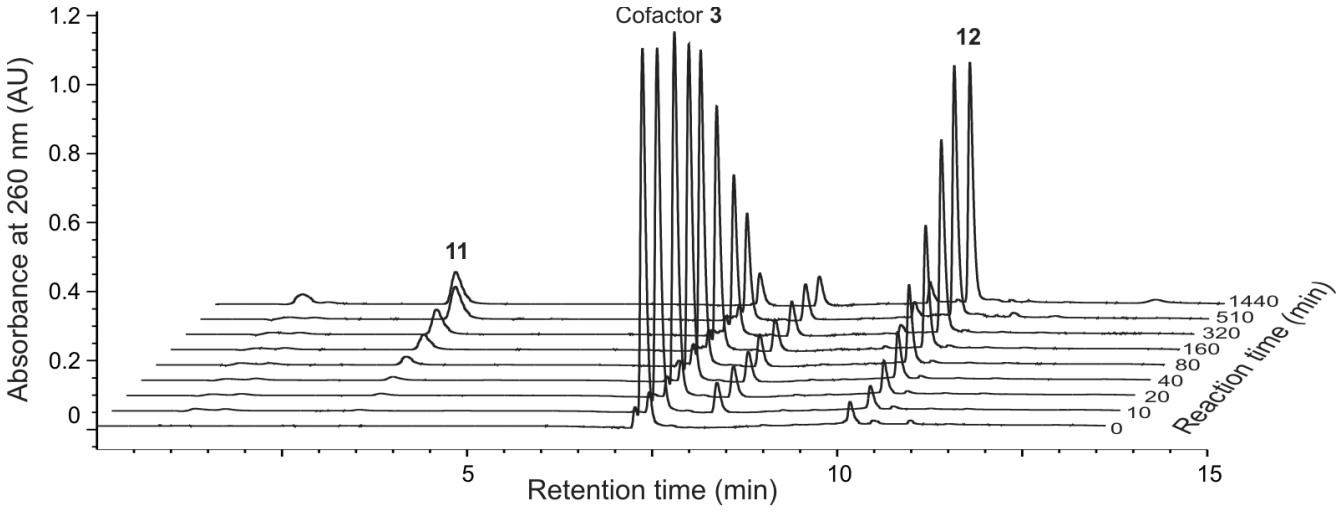


Figure S2. Decomposition timecourse of cofactor **3** in M.HhaI buffer (50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 2 mM 2-mercaptoethanol, 10 mM NaCl, 0.2 mg/ml BSA) at 37 °C as monitored by HPLC. Reaction products were identified by UV spectra and mass spectrometry.

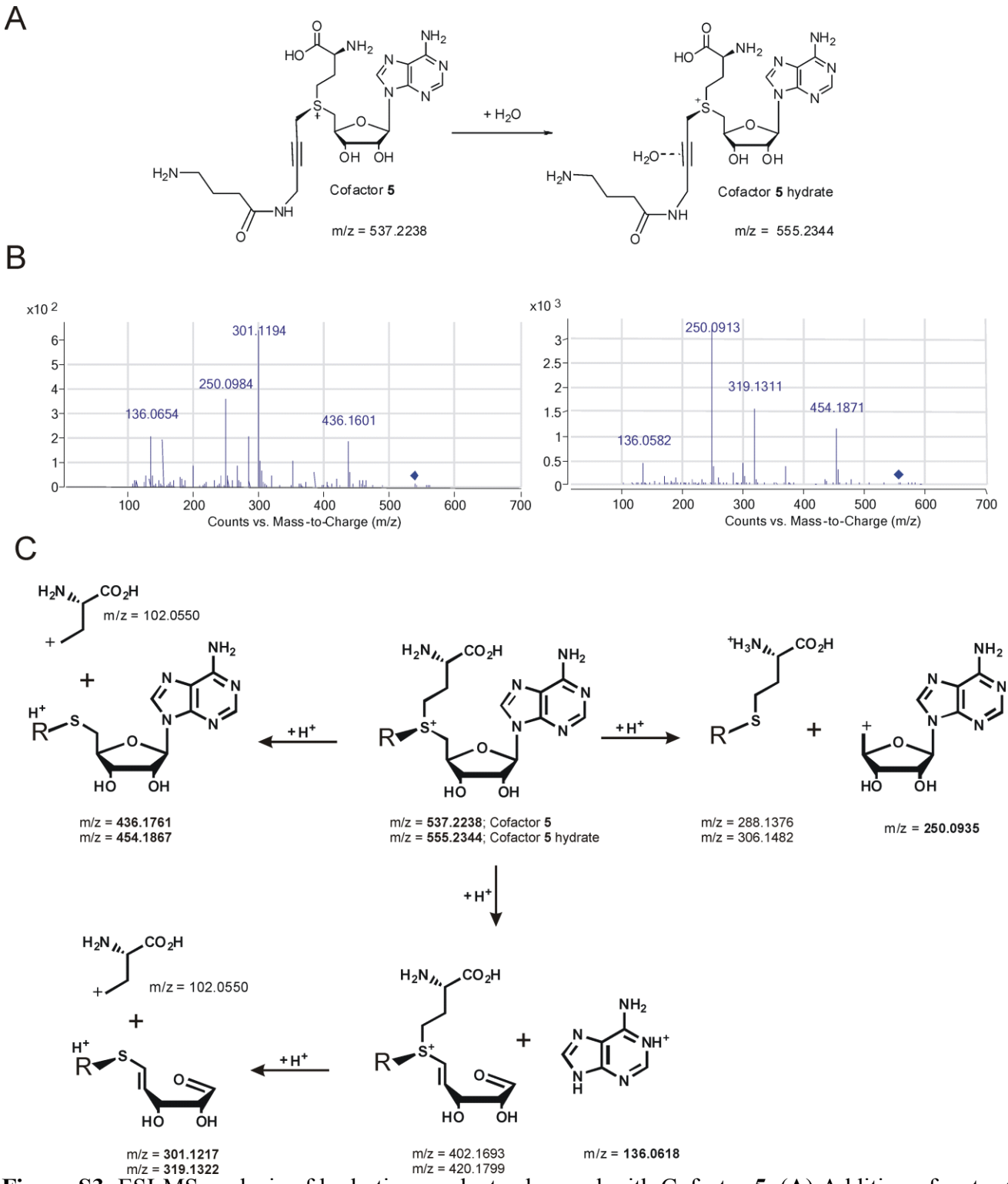


Figure S3. ESI-MS analysis of hydration products observed with Cofactor **5**. (**A**) Addition of water to the cofactor **5** (theoretical mass 537.2238) leads to a product of (555.2344); (**B**) MS/MS fragmentation spectra of precursor ions (marked with diamonds) for cofactor **5** (left panel) and its hydration product (right panel); (**C**) Fragmentation pathways and experimentally observed products (theoretical masses shown in boldface) indicate that the hydration occurs to the side chain R of the cofactor.

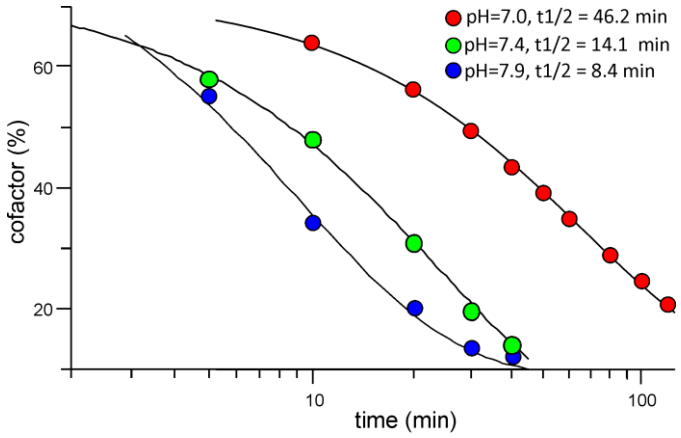


Figure S4. Kinetics of cofactor **5** decomposition at different pH.

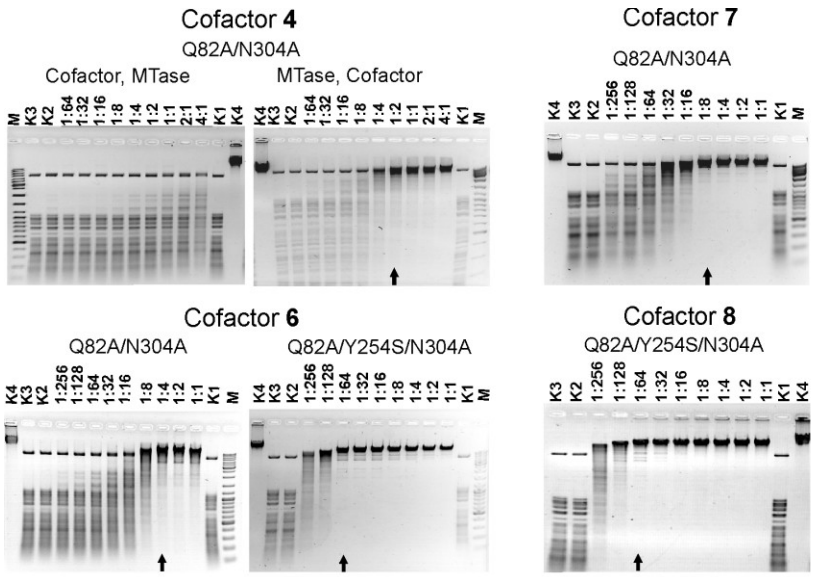


Figure S5. Evaluation of the utility of AdoMet analogs in transalkylation reactions using DNA protection assays. An engineered variant of M.HhaI as indicated was incubated with lambda DNA and cofactor (300 μM) at 37 °C for 1 h at a series of dilutions (molar ratios of MTase to its target sites are as indicated). Modified DNA was treated with R.Hin6I endonuclease and analyzed by agarose gel electrophoresis. Two upper left panels indicate experiments in which either the cofactor **4** or the MTase was added first in the reaction mixture indicating poor reproducibility of the reaction due to an extremely fast decay of the cofactor. Control reactions: K1 - no MTase, K2 - no cofactor, K3 - no MTase, no cofactor, K4 - untreated DNA. Lanes representing highest MTase dilutions that render full protection of target sites in DNA are marked with arrows. Full protection of substrate DNA from endonuclease cleavage in reaction in which a MTase is present in an *N*-fold dilution relative to its target sites indicates that the enzyme carried out at least *N* turnovers; the turnover rate was calculated by dividing the number of turnovers by reaction time *t* ($k_{obs} \geq N/t$)

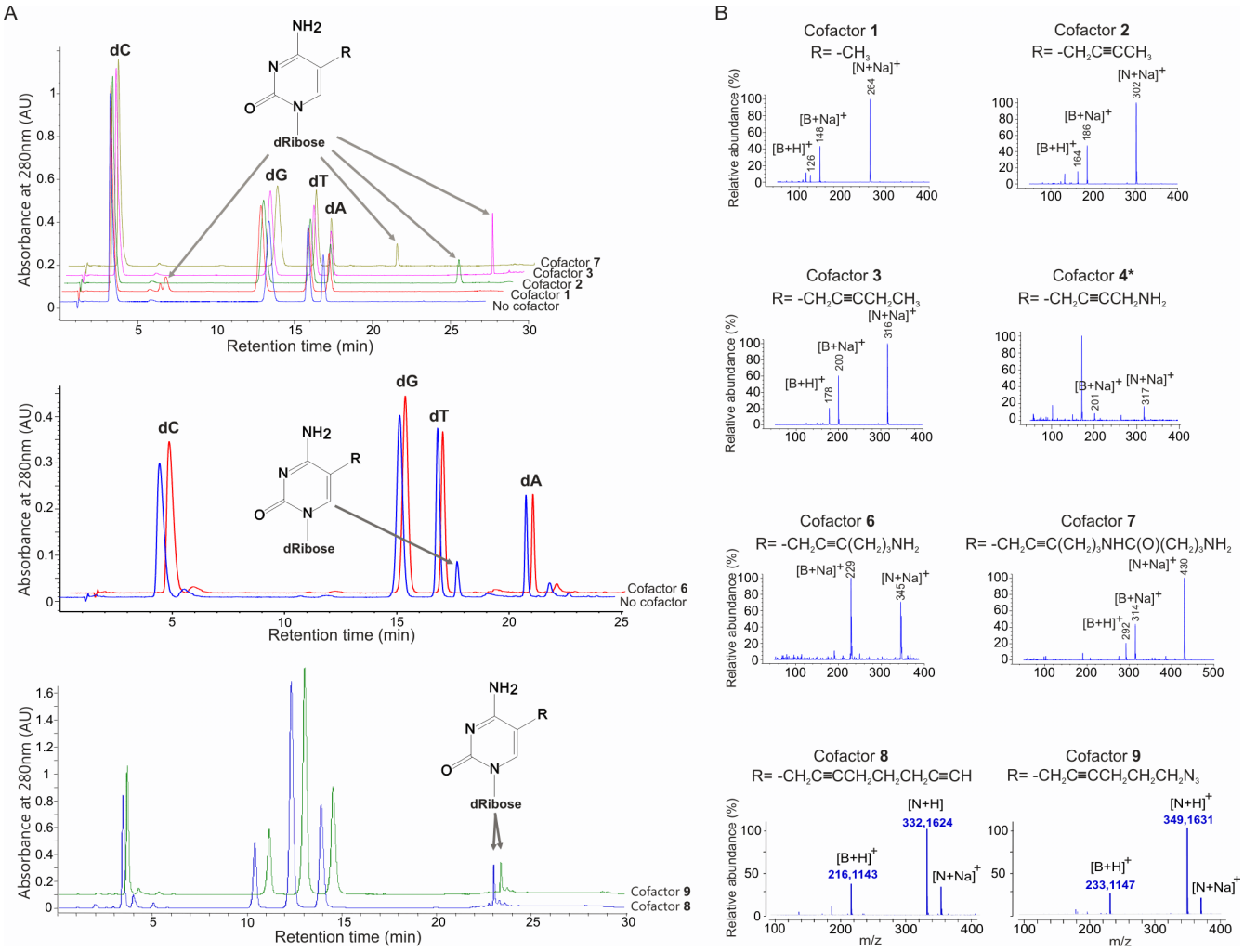


Figure S6. HPLC-MS analysis of mTAG transalkylation products formed in duplex oligodeoxynucleotides with cofactors **1–4** and **6–9** and eM.HhaI. (**A**) Nucleoside HPLC UV traces of enzymatically fragmented duplex oligodeoxynucleotide obtained after modification with eM.HhaI in the presence of AdoMet analogs. (**B**) ESI-MS analysis of HPLC fractions corresponding to modified nucleosides. N denotes 2'-deoxynucleoside; B – nucleobase.

*Reaction product of cofactor **4** co-elutes with and its UV signal is fully obscured by dC (trace not shown), but is identified by MS.

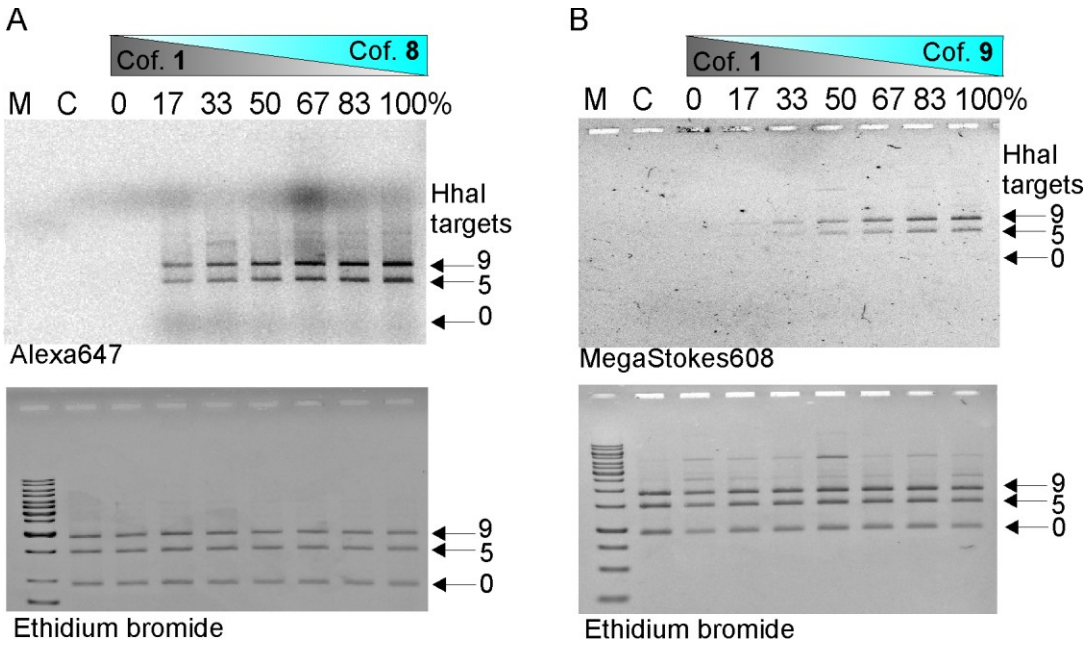


Figure S7. Sequence-specific click-labeling of DNA using eM.HhaI and cofactors **8** and **9** in mixtures with **1** (AdoMet) *in vitro*. Plasmid pΔGH$_6$E119H DNA (0.25 μM targets) was modified with eM.HhaI (0.125 μM) in the presence of cofactor **1** and synthetic cofactor **8** or **9** combined in different ratios as indicated (total cofactor concentration 50 μM). Control sample (lanes C) contained no cofactor. Modified DNA was labeled with a suitable dye and then treated with R.HincII and R.PscI endonucleases to give three DNA fragments containing 9, 5 or 0 HhaI target sites. The resulting fragments were separated on an agarose gel and scanned for labeling dye and EtBr fluorescence. M – 1 kb DNA Ladder, Fermentas.

(**A**) mTAG labeling with cofactor **8** and Alexa647 azide. Modified DNA was analyzed by agarose gel electrophoresis then stained with ethidium bromide and scanned for Alexa647 (635 nm laser) and EtBr (473 nm laser) fluorescence; (**B**) mTAG labeling with cofactor **9** and Alkyne MegaStokes608 dye. The gel was first scanned for MegaStokes fluorescence (473 nm laser), then stained with ethidium bromide and scanned again to visualize bulk DNA.

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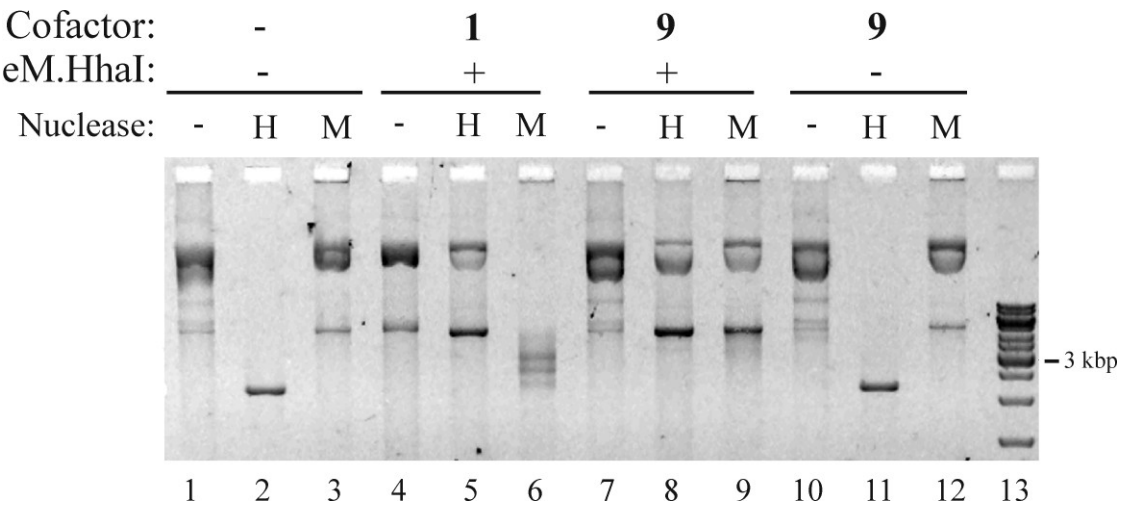

Figure S8. Methylation and modification in crude cell lysate. *E.coli* ER2267 cells carrying the pΔGH$_6$E119H plasmid were lysed with lysozyme and the lyzate was complemented with eM.HhaI (2 μM) and cofactor **1** (control) or **9** (50 μM). After modification the plasmids were purified from the lysate and aliquots digested with the R.Hin6I (lanes H), or McrBC (lanes M) nuclease. R.Hin6I cleaves DNA at unmodified HhaI target sites (lanes 2 and 11), but does not cleave at modified sites (lanes 5 and 8); McrBC fragments DNA in the vicinity of methylated HhaI targets (lane 6); both nucleases are inactive on GCGC sites modified with extended alkyl groups.[9] Lane 13 – GeneRuler DNA Ladder mix, Fermentas. Resistance of DNA modified with eM.HhaI and cofactor **9** to the action of both nucleases (lanes 8 and 9) indicates its complete alkylation of the GCGC sites.

Supporting References

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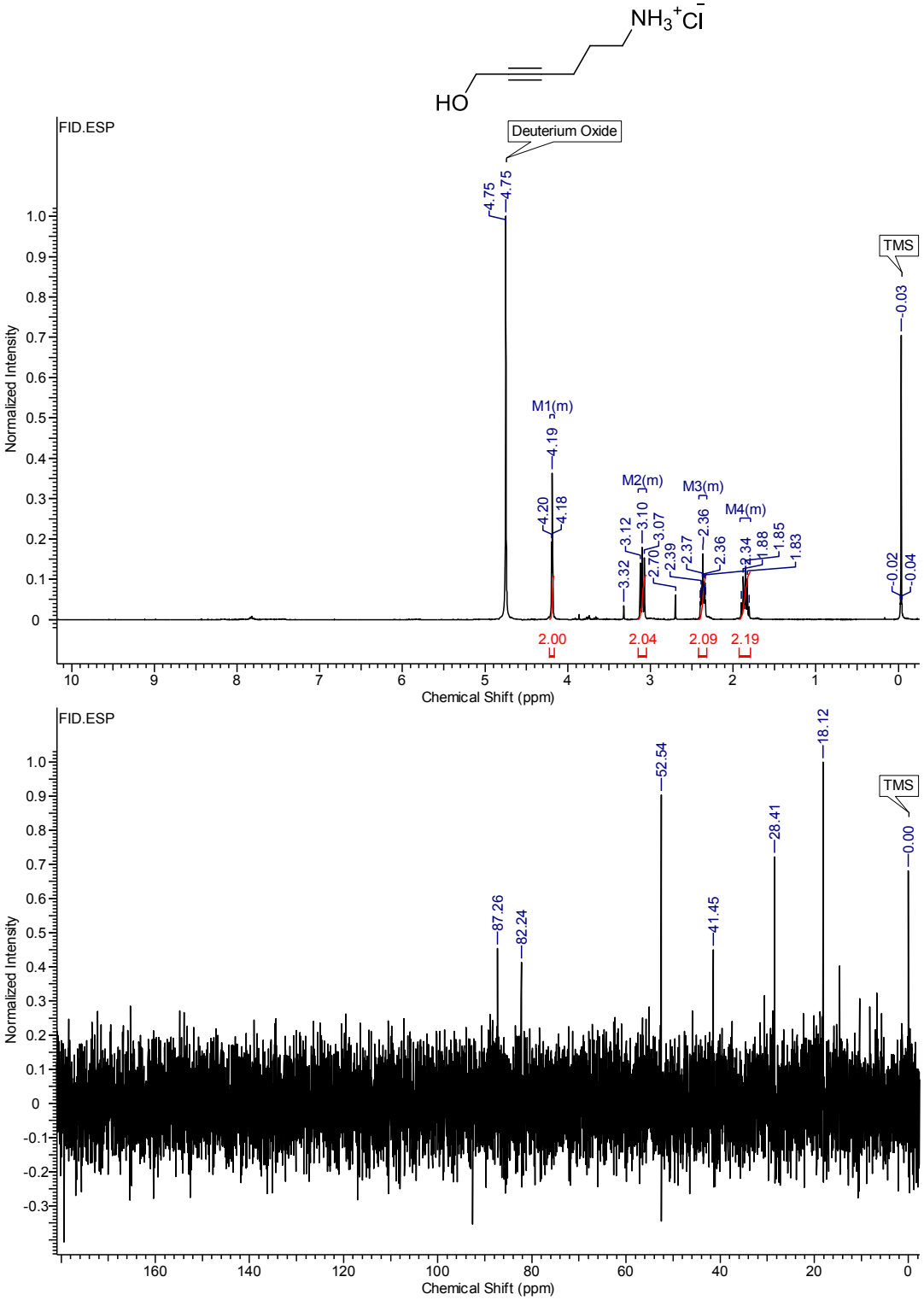
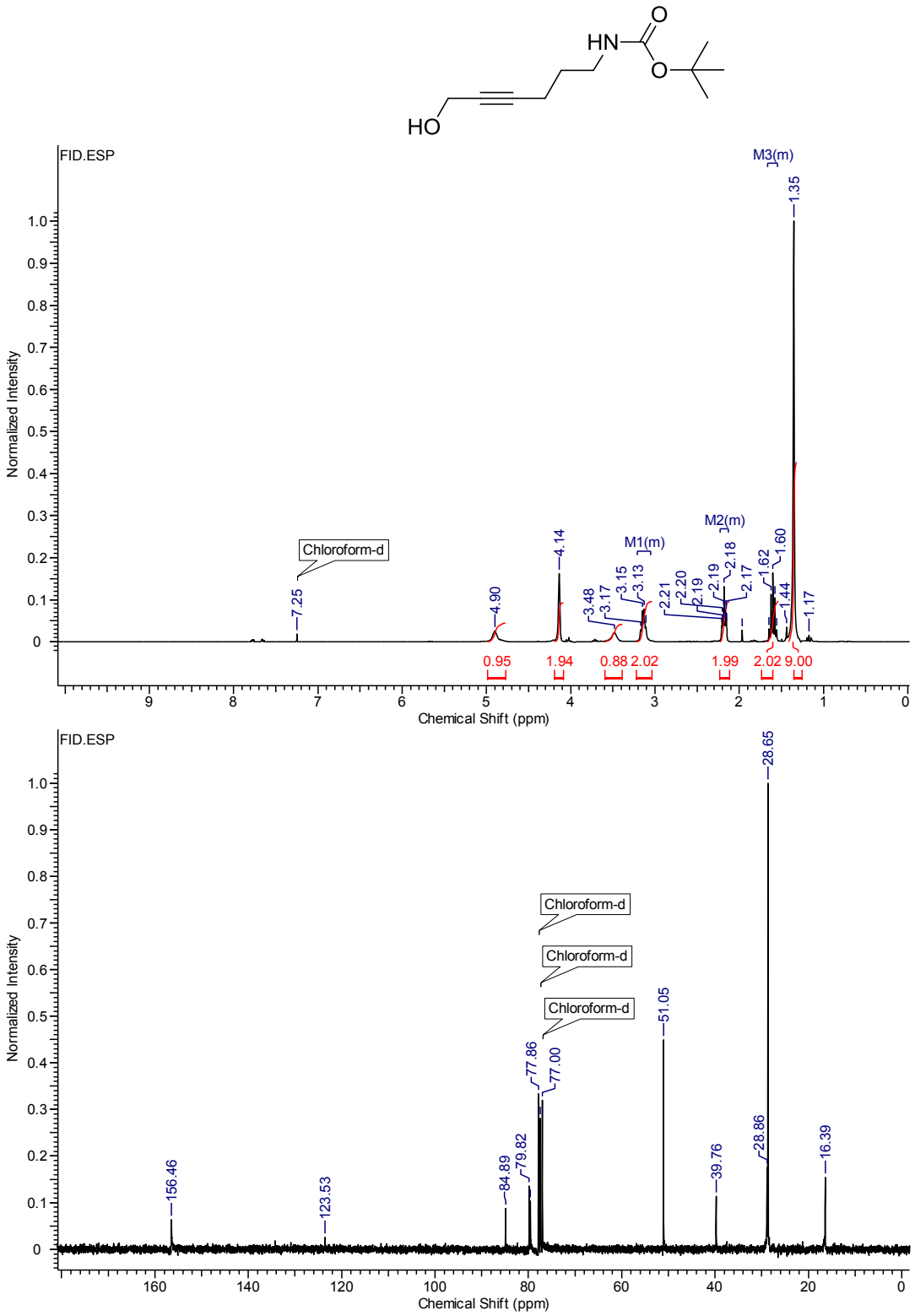
NMR spectra of synthetic compounds

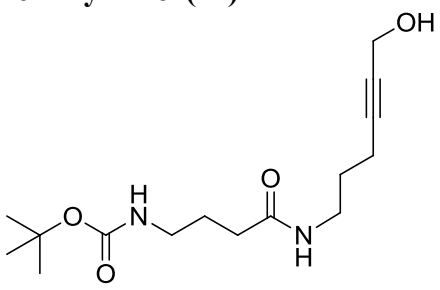
6-Aminohex-2-yn-1-ol hydrochloride (15)

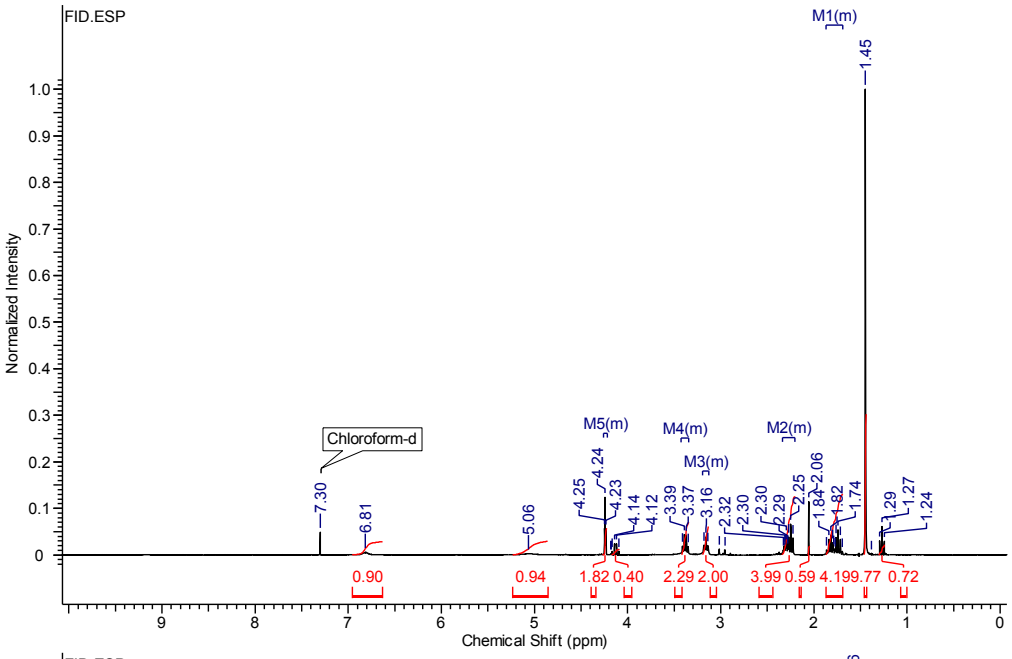


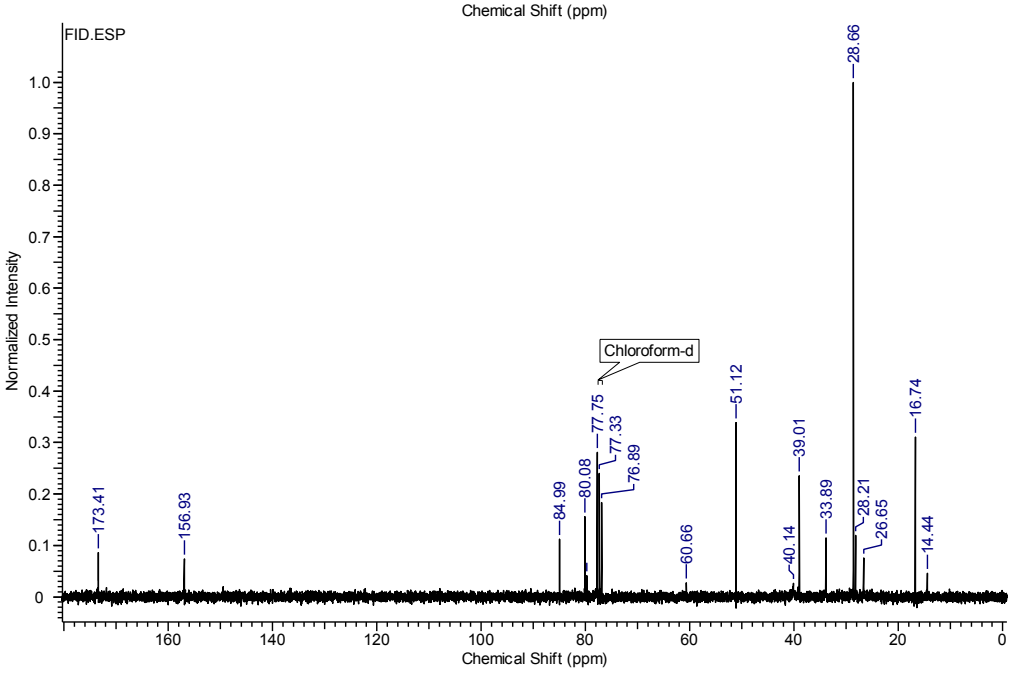
6-(*N*-Boc-amino)hex-2-yn-1-ol (19)



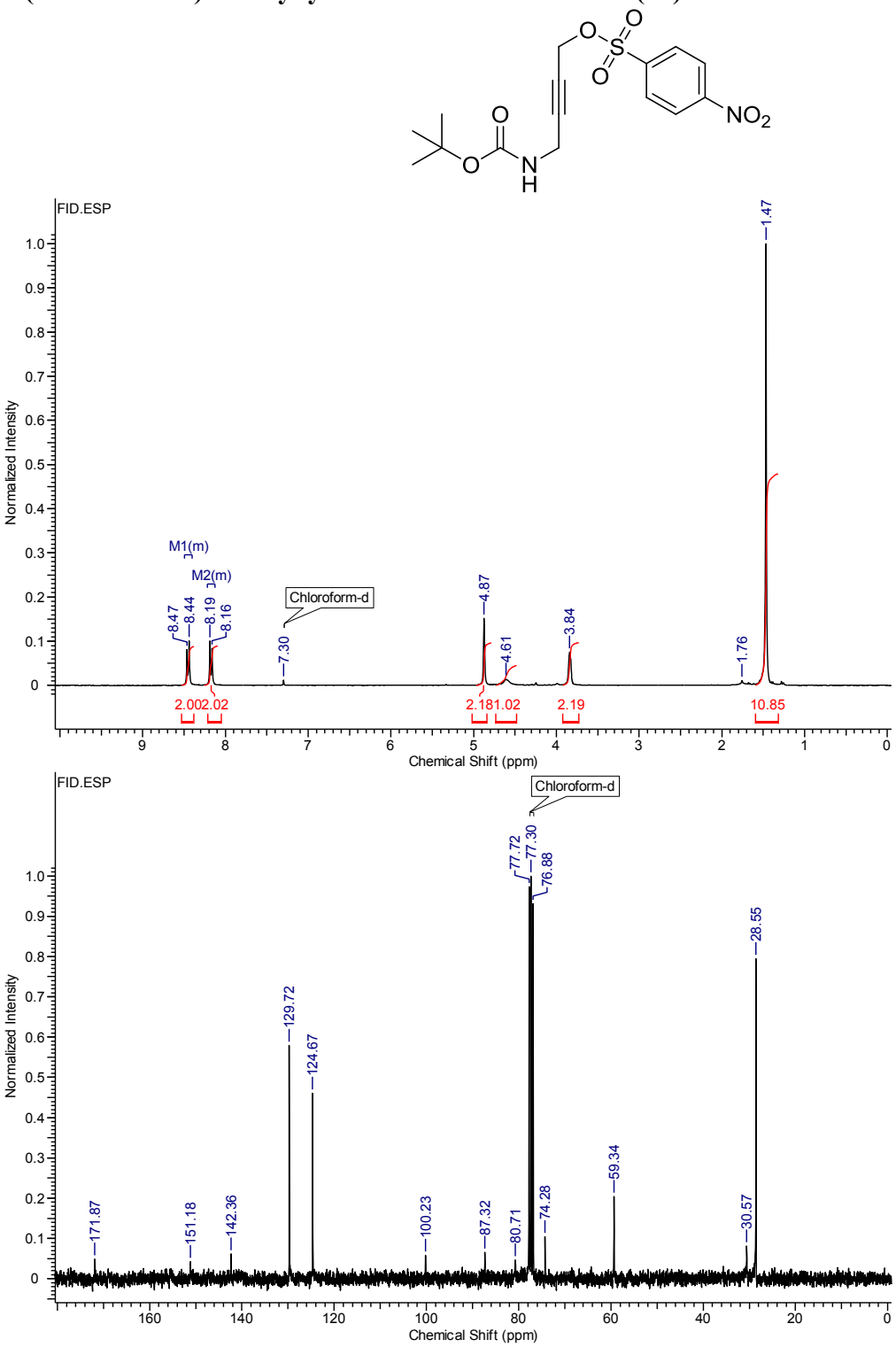
6-[(4-*N*-Boc-amino)butanamido]hex-2-yn-1-ol (21)



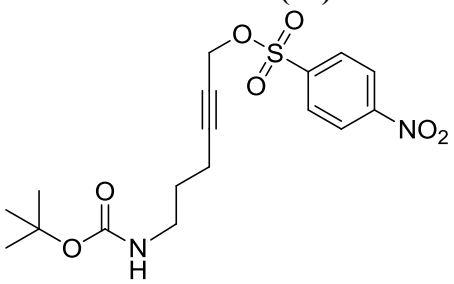


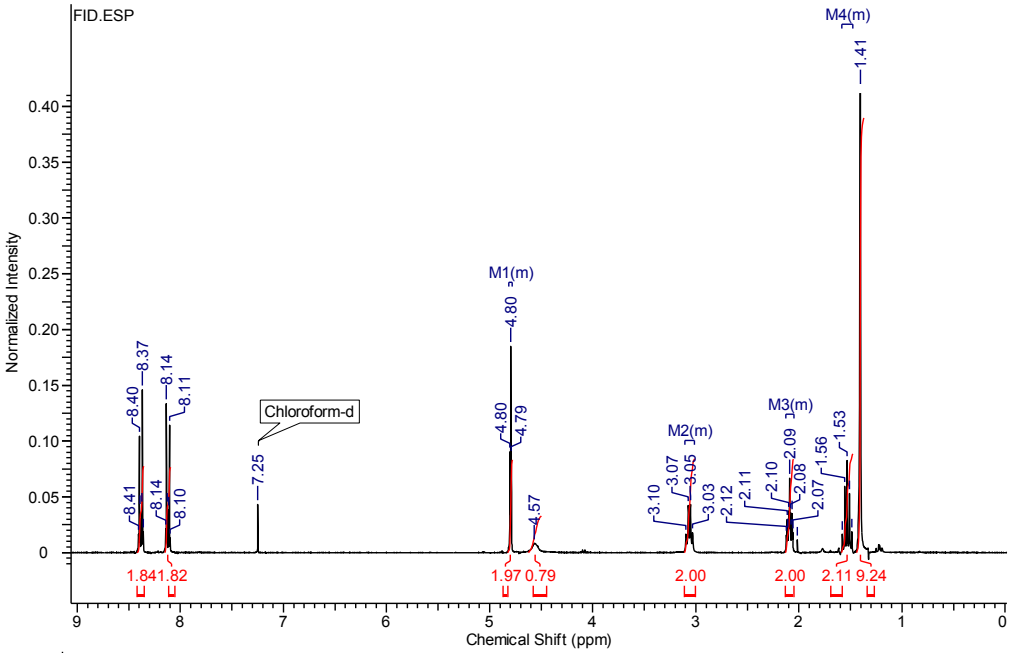


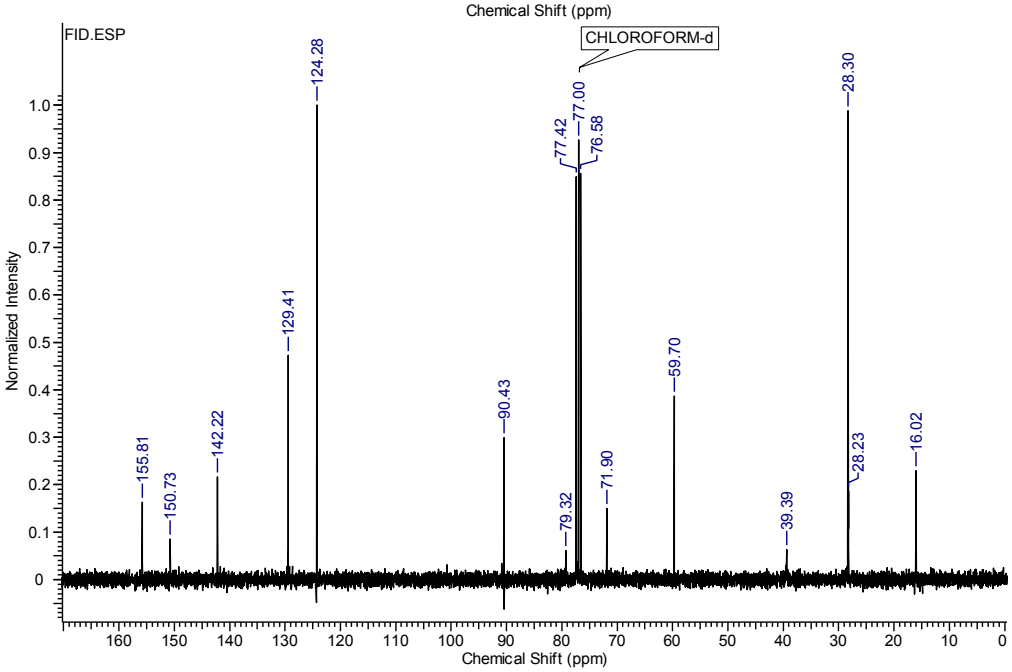
4-(*N*-Boc-amino)but-2-ynyl 4-nitrobenzenesulfonate (24)



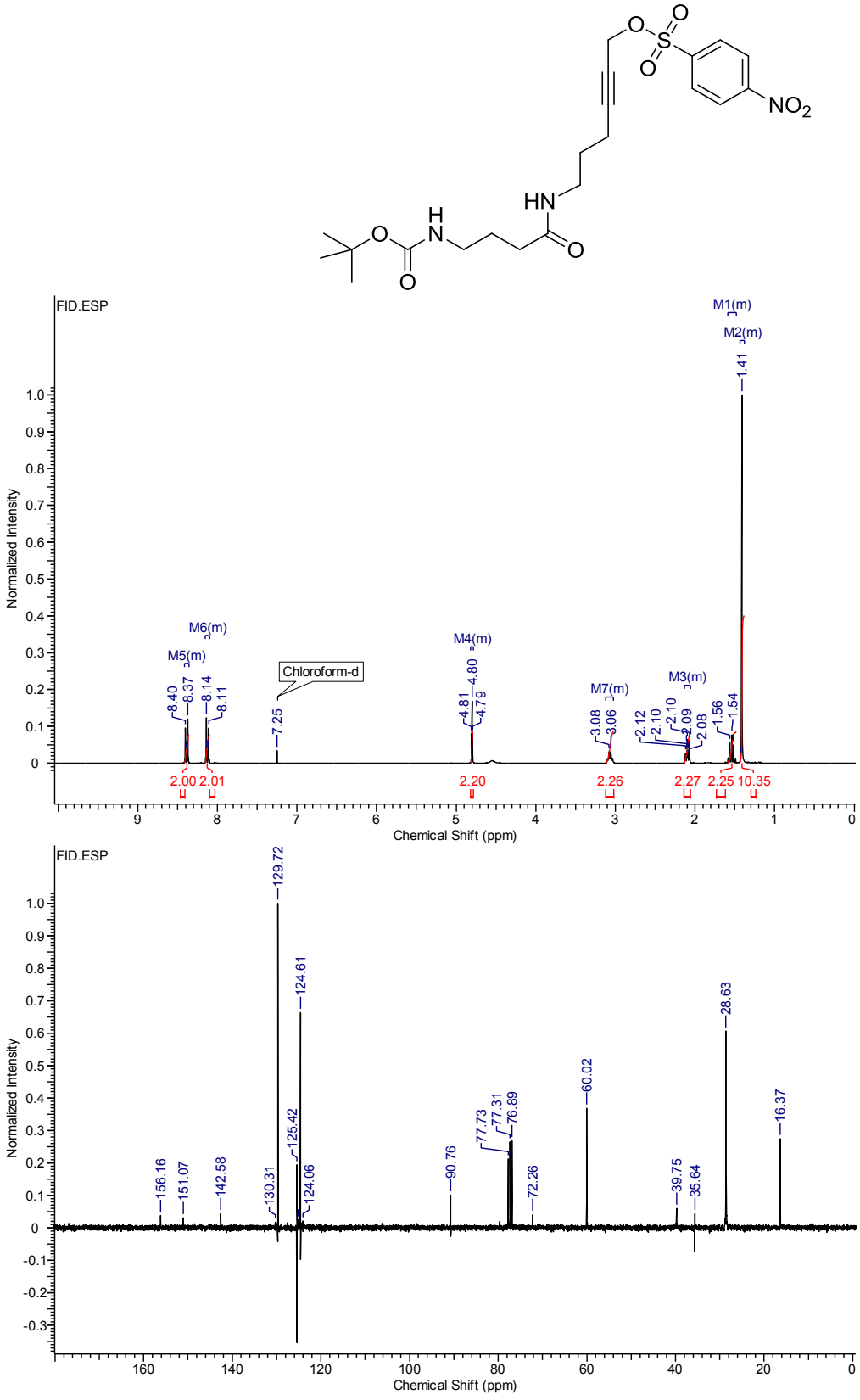
6-(*N*-Boc-amino)hex-2-ynyl 4-nitrobenzenesulfonate (25)



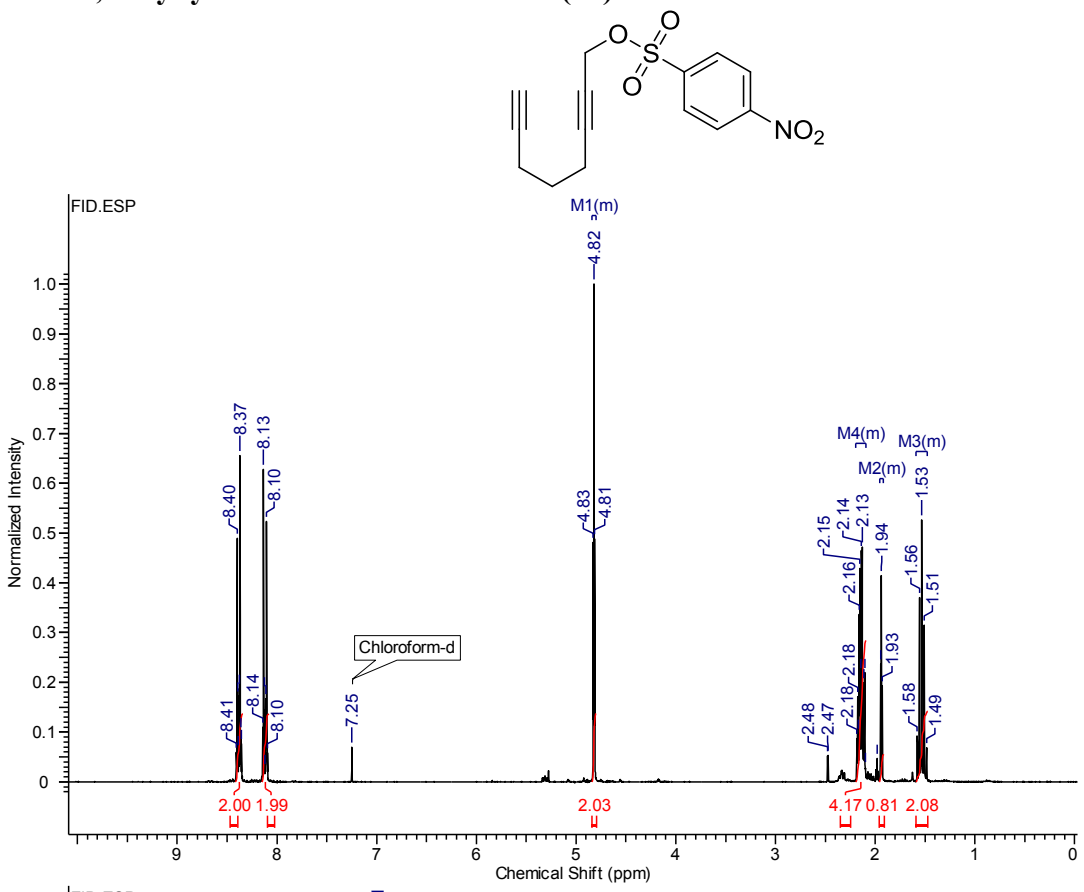


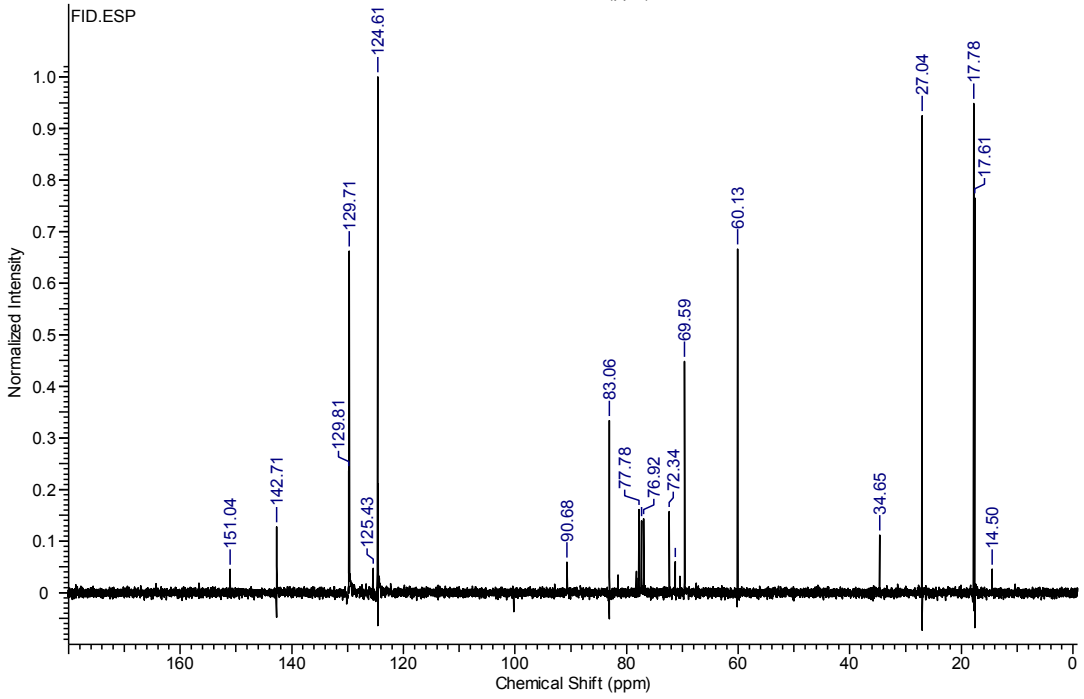


6-[(4-*N*-Boc-amino)butanamido]hex-2-yn-1-yl 4-nitrobenzenesulfonate (27)

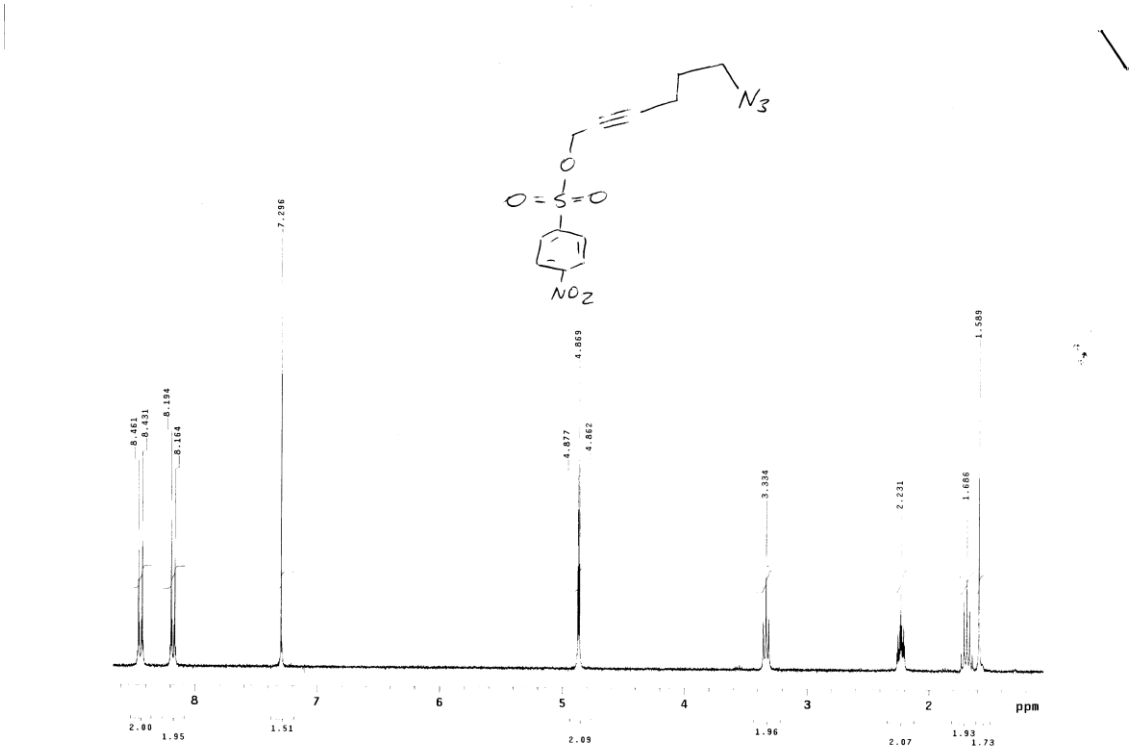


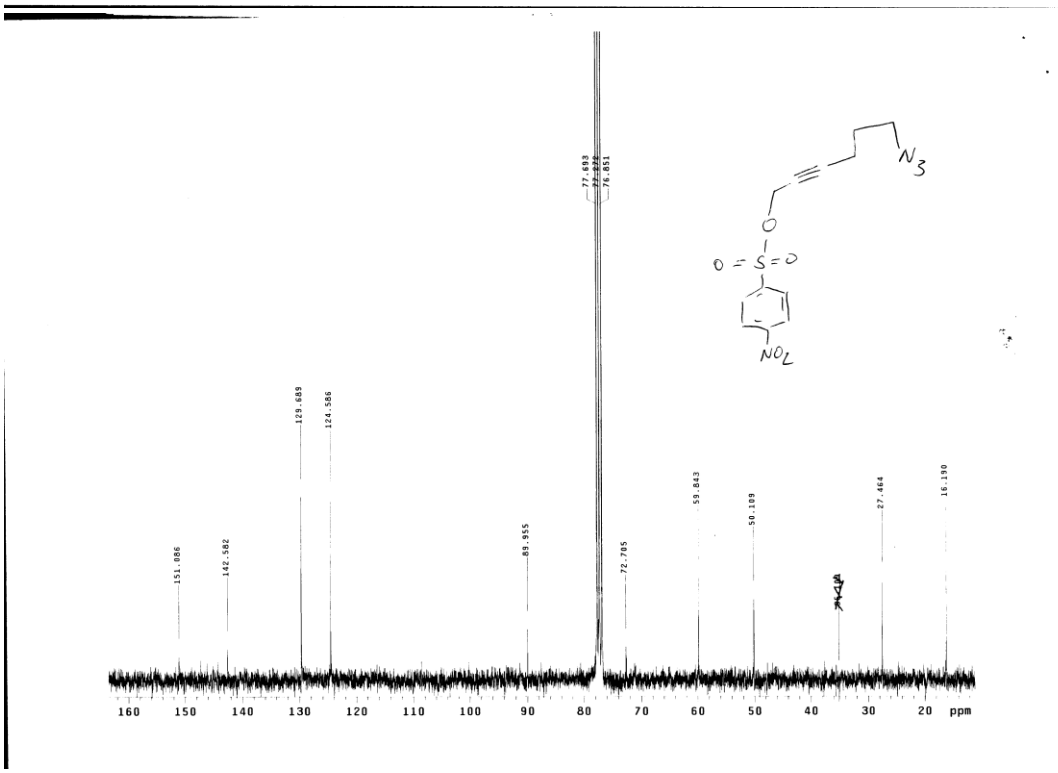
Octa-2,7-diynyl 4-nitrobenzenesulfonate (28)



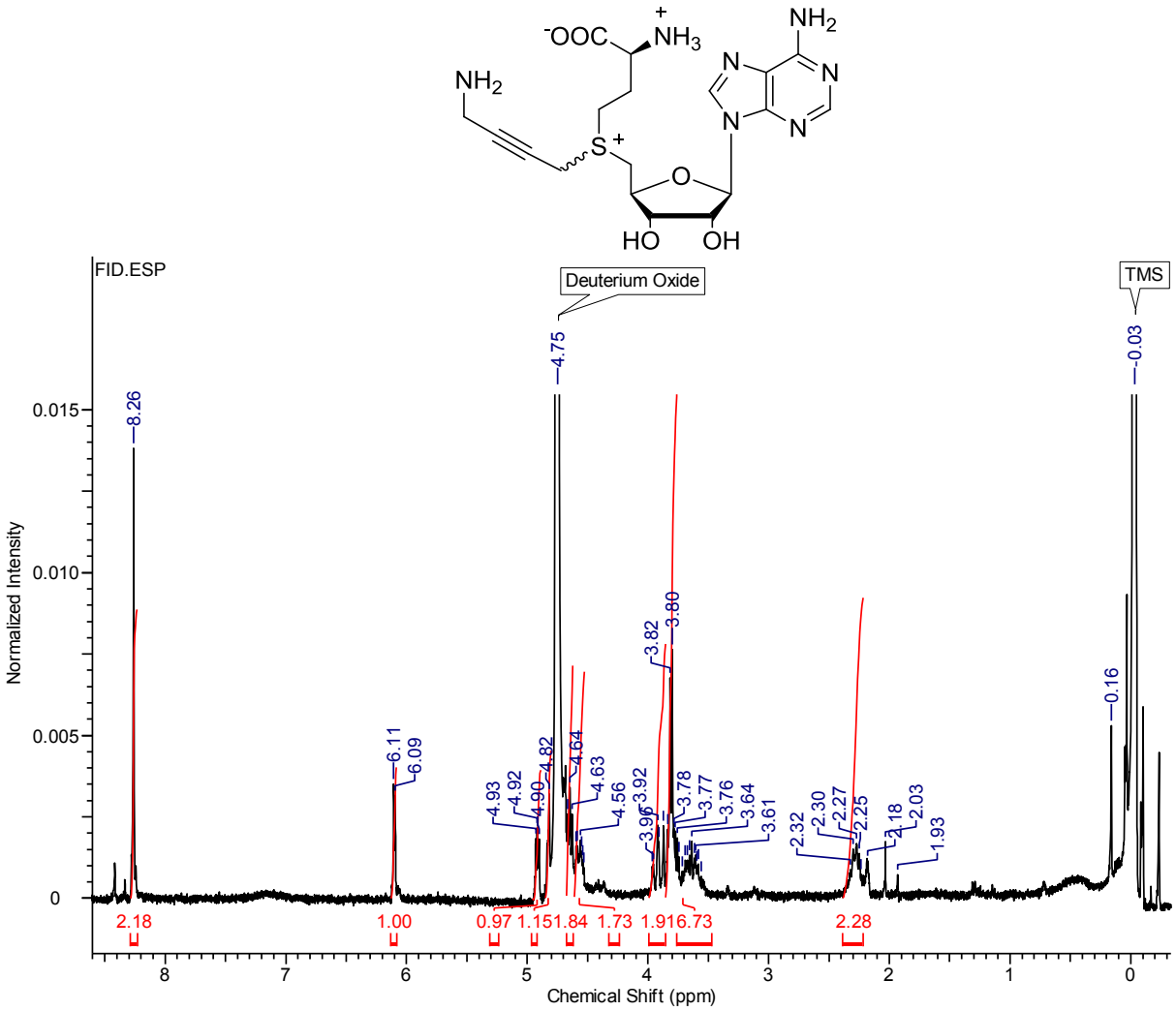


6-azidohex-2-yn-1-yl 4-nitrobenzenesulfonate (29)

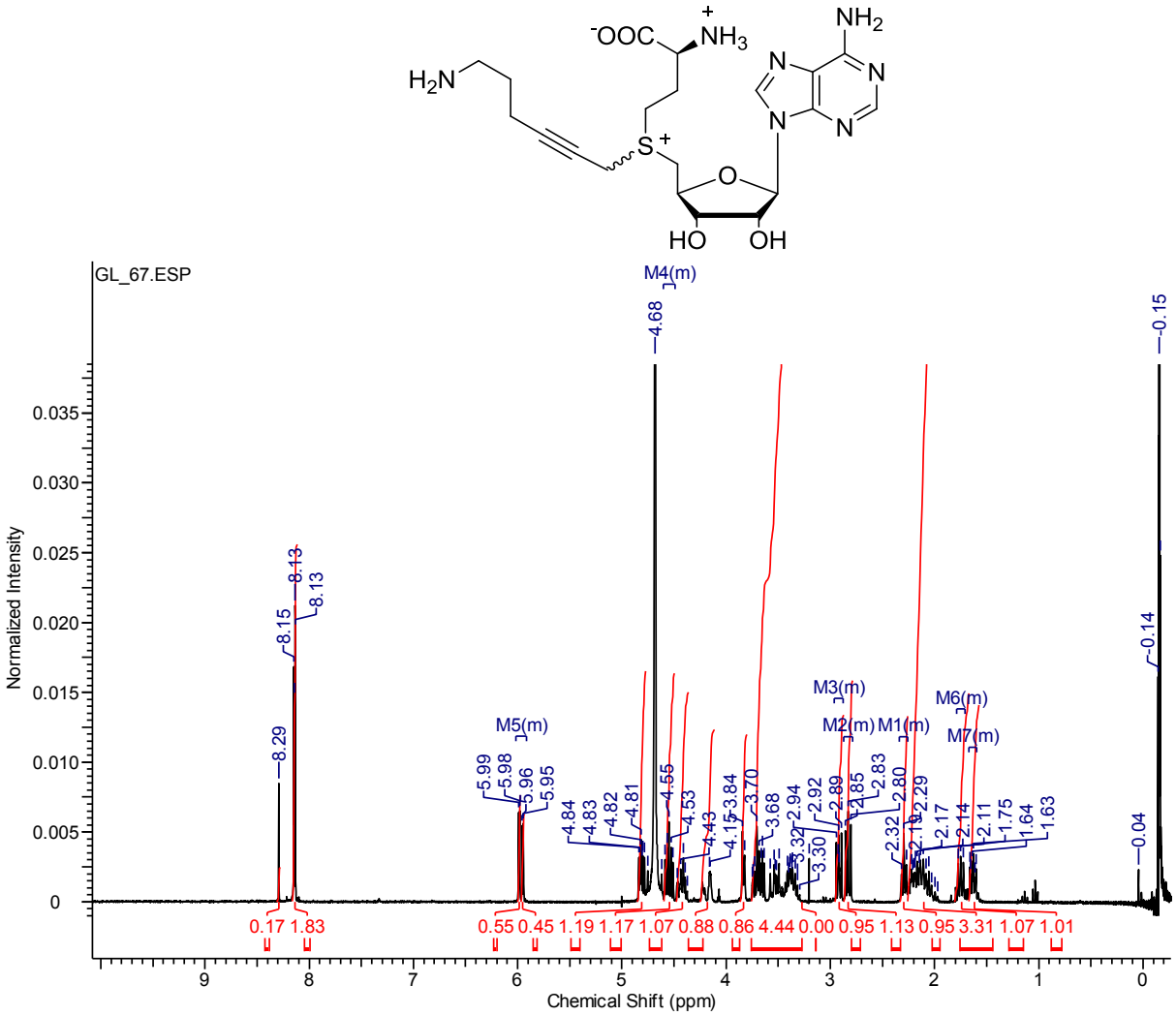




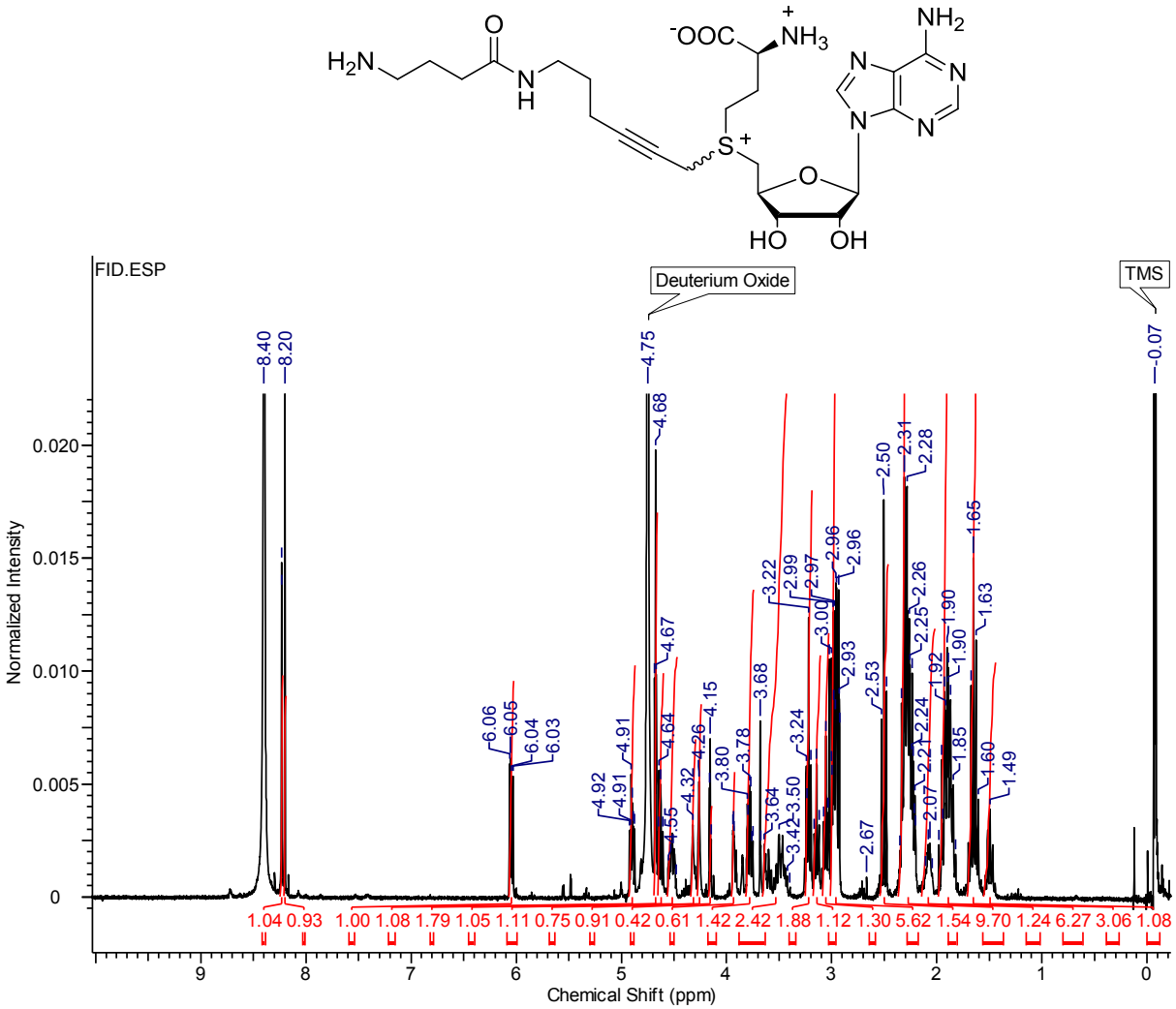
Cofactor **4**


FID.ESP
Deuterium Oxide
TMS
Normalized Intensity
Chemical Shift (ppm)
8.26
6.11
6.09
4.93
4.92
4.90
4.82
4.75
4.64
4.63
4.56
3.96
3.92
3.82
3.80
3.78
3.77
3.76
3.64
3.61
2.32
2.30
2.27
2.25
2.18
2.03
1.93
0.16
0.03
2.18
1.00
0.97
1.15
1.84
1.73
1.91
6.73
2.28

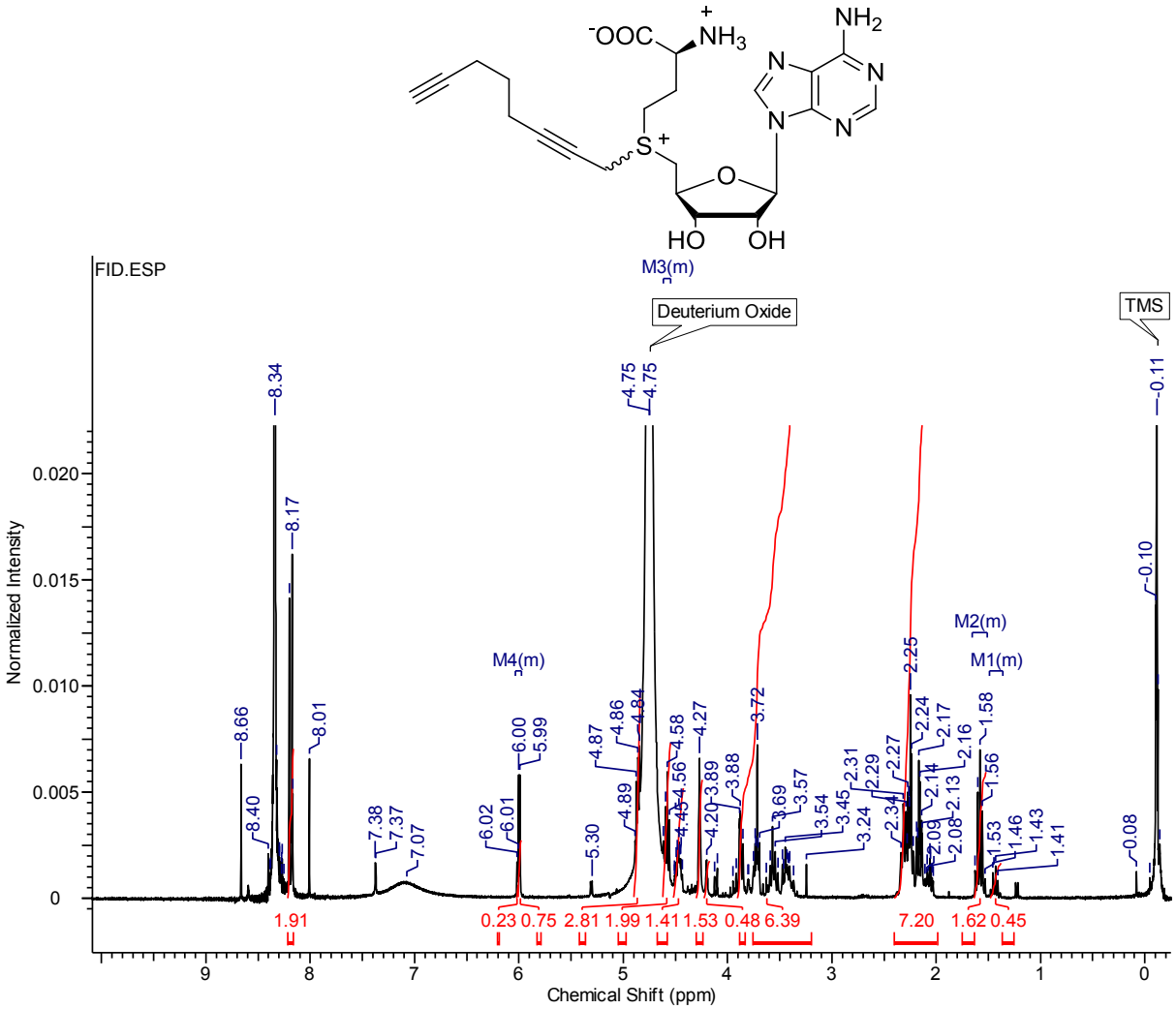
Cofactor **6**


GL_67.ESP
Normalized Intensity
Chemical Shift (ppm)
M4(m)
M5(m)
M3(m)
M2(m)
M1(m)
M6(m)
M7(m)
8.29
8.15
8.13
8.13
4.68
5.99
5.98
5.96
5.95
4.84
4.83
4.82
4.81
4.55
4.53
4.43
4.15
3.84
3.70
3.68
2.94
2.92
2.89
2.85
2.83
2.80
2.32
2.29
2.17
2.14
2.11
1.75
1.64
1.63
0.15
0.14
0.04
0.17 1.83
0.55 0.45 1.19 1.17 1.07 0.88 0.86 4.44 0.00 0.95 1.13 0.95 3.31 1.07 1.01

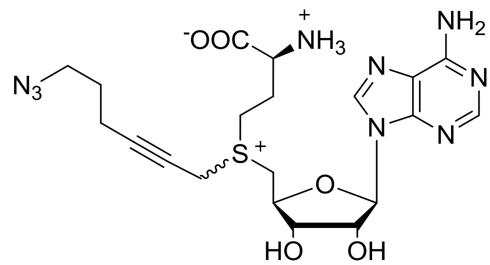
Cofactor **7**


FID.ESP
Deuterium Oxide
TMS
Normalized Intensity
Chemical Shift (ppm)
8.40
8.20
4.75
4.68
4.67
4.64
4.55
4.92
4.91
4.91
4.32
4.26
4.15
3.80
3.78
3.68
3.64
3.50
3.42
3.24
3.22
2.99
2.97
2.96
2.96
3.00
2.93
2.67
2.53
2.50
2.31
2.28
2.26
2.25
2.24
2.21
2.07
1.92
1.90
1.90
1.85
1.65
1.63
1.60
1.49
-0.07
6.06
6.05
6.04
6.03
1.04 0.93 1.00 1.08 1.79 1.05 1.11 0.75 0.91 0.42 0.61 1.42 2.42 1.88 1.12 1.30 5.62 1.54 9.70 1.24 6.27 3.06 1.08

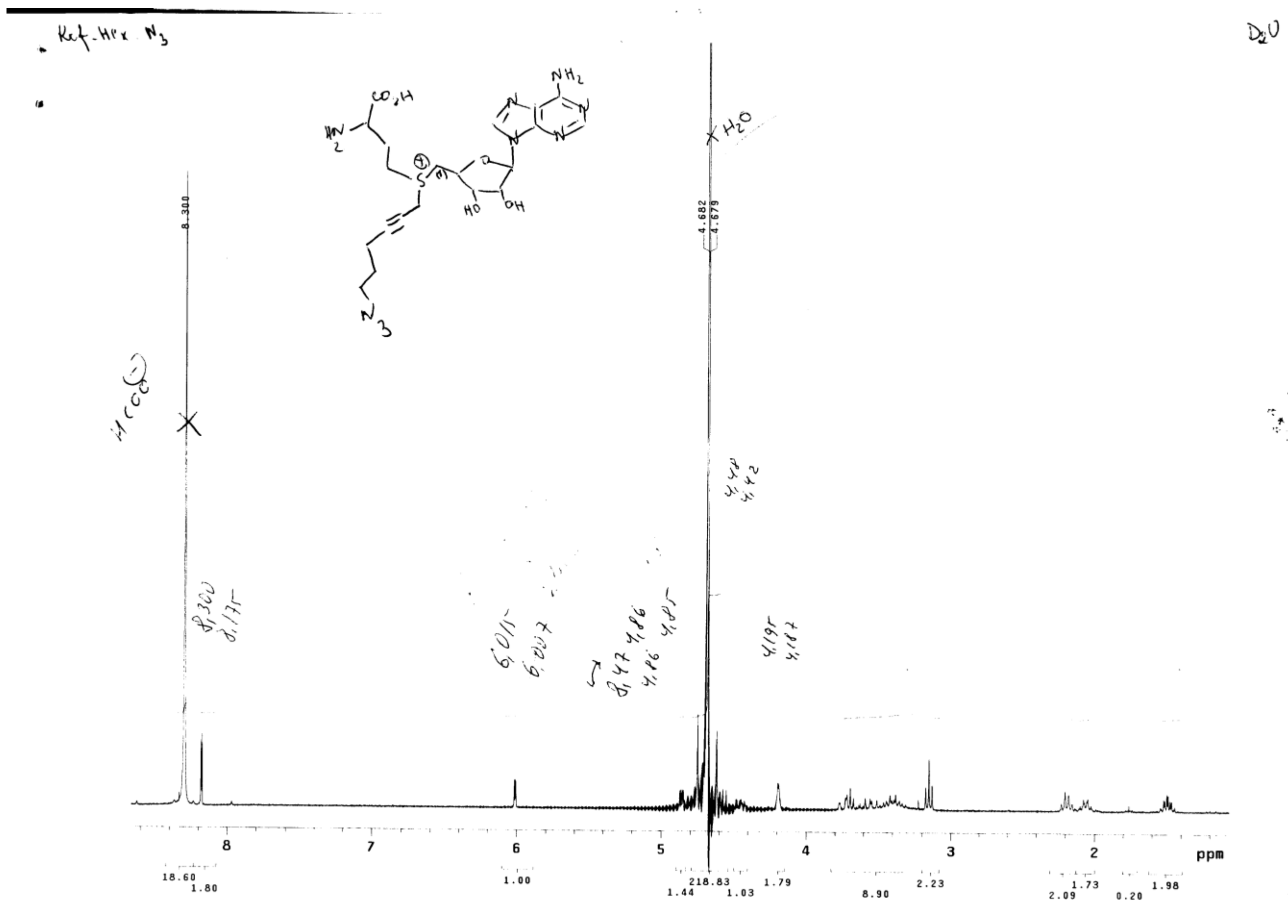
Cofactor **8**


FID.ESP
Deuterium Oxide
TMS
M1(m)
M2(m)
M3(m)
M4(m)
Normalized Intensity
Chemical Shift (ppm)
1.91
0.23 0.75 2.81 1.99 1.41 1.53 0.48 6.39
7.20 1.62 0.45

Cofactor **9**



$^{-}$OOC
$\overset{+}{N}H_3$
NH_2
N
N
N
N
N_3
S+
O
HO
OH



8.300
4.682
4.679
8
7
6
5
4
3
2
ppm
18.60
1.80
1.00
218.83
1.44
1.03
1.79
8.90
2.23
1.73
2.09
0.20
1.98