Supporting information

General information

All chemicals were purchased from Sigma-Aldrich, Alfa Aesar or TCI unless otherwise noted. The cap analog and the vaccinia capping system were purchased from NEB. DBCO-SRB 568/585 was purchased from Click Chemistry Tools, TAMRA-tetrazine and biotin-tetrazine were obtained from Jena Bioscience. All components were used without further purification.

Electroporation was performed on an Eppendorf Eporator® (Eppendorf, Hamburg). Sonication was carried out using a Sonoplus GM3100 (Bandelin, Berlin). Absorption measurements were performed with the Infinite M1000 PRO® (TECAN, Salzburg, Austria).

Protein purification was performed by affinity chromatography (Ni-NTA, GE Healthcare) on an ÄKTA Prime purifier system (GE Healthcare). For RNase-free preparation of proteins additional gel filtration was performed using a Superdex 200 Increase 10/300 GL (GE Healthcare).

NMR spectra were recorded at ambient temperature on a Bruker AV300 spectrometer or on a Varian VNRMS 600 spectrometer. Multiplicities are indicated as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet) and m (multiplet). Spectra were annotated using gCOSY, gHSQC and gHMBC.

High-resolution mass spectra were obtained on a Thermo Scientific Orbitrap LTQ XL or on a Bruker MicroTof. MALDI spectra were obtained on a Bruker MALDI Autoflex speed. LC-MS measurements were performed on a maXis II ultra-high resolution QTOF (Bruker, Bremen) coupled to an UltiMate 3000® UHPLC (Thermo Scientific, Waltham, USA).

HPLC was performed on an Agilent 1260 Infinity HPLC equipped with a diode array detector (DAD) (190-640 nm).

Ecm1, MTAN and LuxS were recombinantly expressed and purified as previously described.[1] M. TaqI and R. TaqI were purchased from NEB.

Enzymatic transfer reactions to the mRNA cap

Enzymatic transfer reactions were typically performed using GpppA (0.3 mM), 20 mol% Ecm1, the respective AdoMet analog (2 mM), 4 µM MTAN and 4 µM LuxS in a final volume of 20-25 µL. Reactions were incubated for up to 3 h at 37 °C (typically near-quantitative conversions were already obtained after 1 h). Afterwards, proteins were precipitated by addition of 1/10th volume of acetonitrile and directly analysed by reversed-phase HPLC (analysis at an absorption wavelength of 260 nm). For analysis a NUCLEODUR® C18 Pyramid (125×4 mm) column from Macherey-Nagel was used. Elution was performed at a flow rate of 1 mL/min in a linear gradient of buffer A (100 mM K_2HPO_4/KH_2PO_4, pH = 6.5) and buffer B (50 % buffer A, 50 % MeCN).

Enzymatic plasmid DNA modification

The plasmid *pBR322* (0.5 µg) was mixed with AdoMet or the respective AdoMet analog (0.4 mM), M. TaqI (1:1 dilutions starting from 5 U/reaction), 4 µM MTAN, 4 µM LuxS and 1 µL of 10×reaction buffer (CutSmart, NEB) in a total volume of 10 µL. The reactions were incubated at 60 °C for 2 h. Afterwards, 15 µL of 1×reaction buffer (CutSmart, NEB) and 5 U of R. TaqI were added to the reactions and incubated for 45 min at 65 °C. Plasmid DNA was linearized using BamHI and directly loaded onto a 1 % agarose gel (100 V, 50 min). Staining was performed using ethidium bromide and scanning on a Typhoon FLA9500 laser scanner (GE healthcare).

Kinetic analysis

Enzymatic modification of GpppA (0.6 mM) with Ecm1 and the respective AdoMet analogs (50 – 750 µM) were performed in the presence of 4 µM MTAN and LuxS in 1×PBS buffer at 37 °C. 4 mol% Ecm1 were used for all AdoMet analogs except for AdoViBe and AdoNB where 2 mol% were used. In order to determine initial velocities, reactions were stopped at defined time-points (0 – 5 min) by adding 1/10th volume 1 M $HClO_4$. Conversions were analyzed by reversed-phase HPLC as mentioned above. Afterwards, the enzymatic activity (µM min^{-1}) was calculated by linear regression and plotted against the concentration of the respective AdoMet analog. Michaelis-Menten equation in Origin 2016 was used for fitting in a range from 50 µM-750 µM.

Tetrazine ligations

Tetrazine ligations were performed using the respectively-modified cap analog (vinyl-benzyl, norbornene; 300 µM) with a 3-15-fold excess of the respective tetrazine conjugate and incubated for 30 min at 37 °C. Analysis was directly performed by HPLC, LC-MS or in-gel analysis.

Purification of AdoMet analogs

Purification of all AdoMet analogs was conducted by semi-preparative reversed-phase HPLC on a NUCLEODUR© C18 Pyramid (5 µm, 125×10 mm) from Macherey-Nagel. Elution was performed at a flow rate of 5 mL/min in a linear gradient of H_2O supplemented with 0.01 % TFA and acetonitrile supplemented with 0.01 % TFA. Product peaks were lyophilized and redissolved in ddH_2O to give a final concentration of 10 mM.

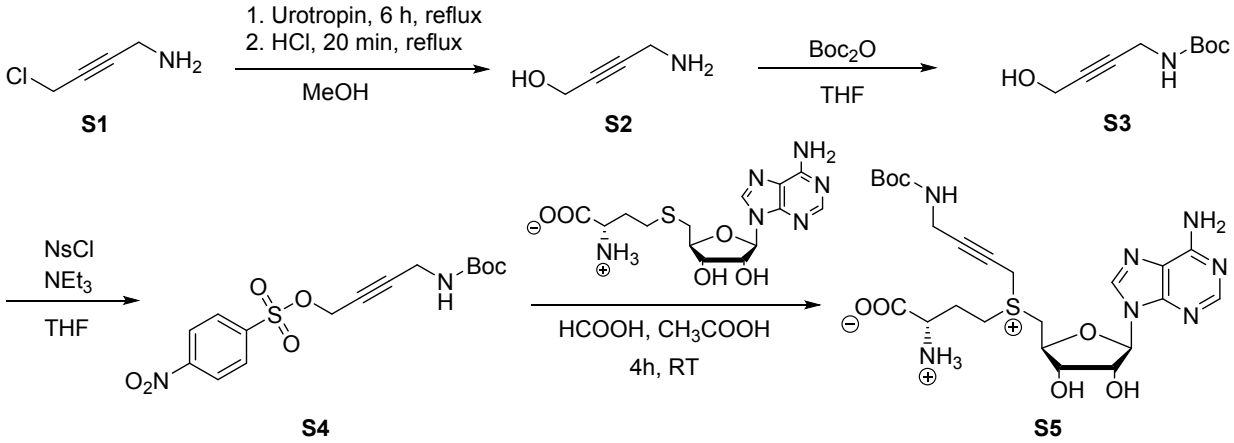


Fig. S1: Scheme for the synthesis of alkyne-linker containing AdoMet analog **S5**.[2]

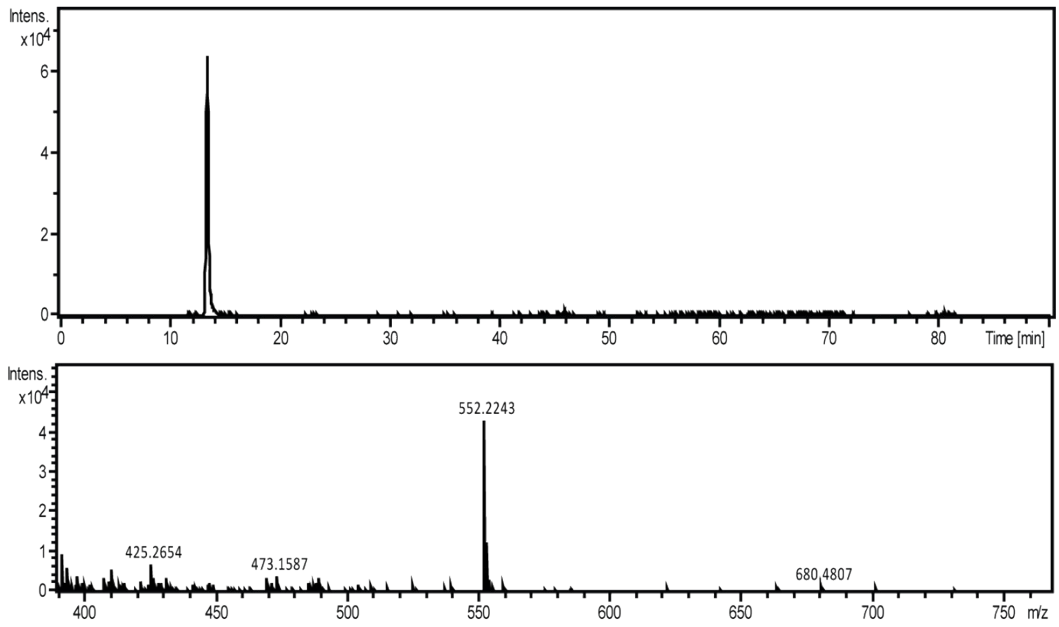


Fig. S2: LC-MS analysis of the crude product of alkyne-modified AdoMet analog **S5**. Expected mass for $C_{23}H_{34}N_7O_7S^+$ = 552.2235 $[M]^+$, found: 552.2243.

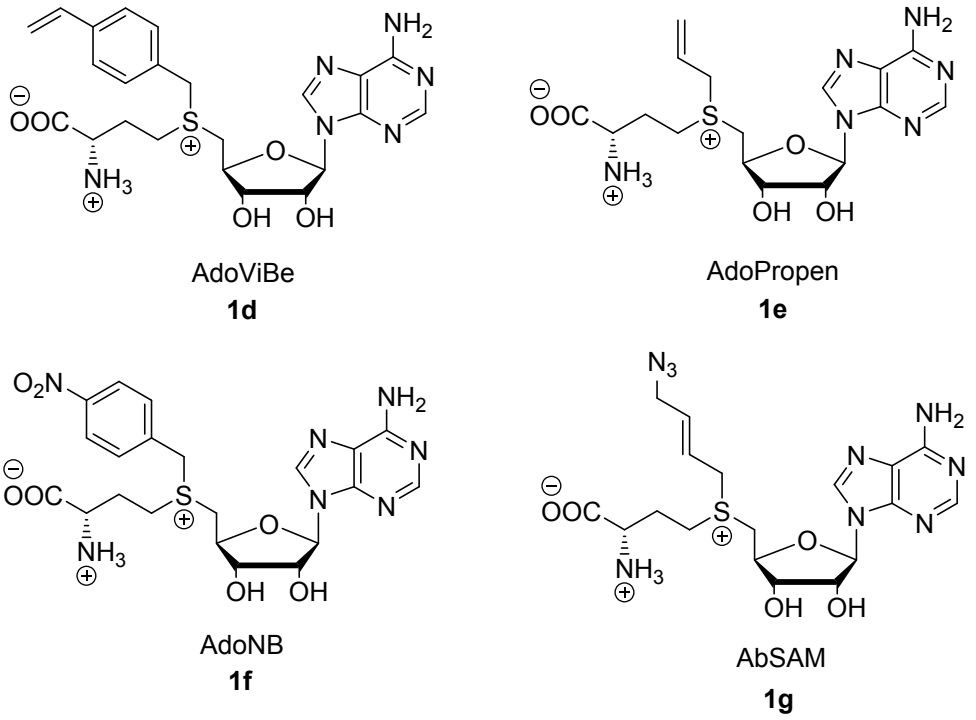


Fig. S3: Structures of used AdoMet analogs.

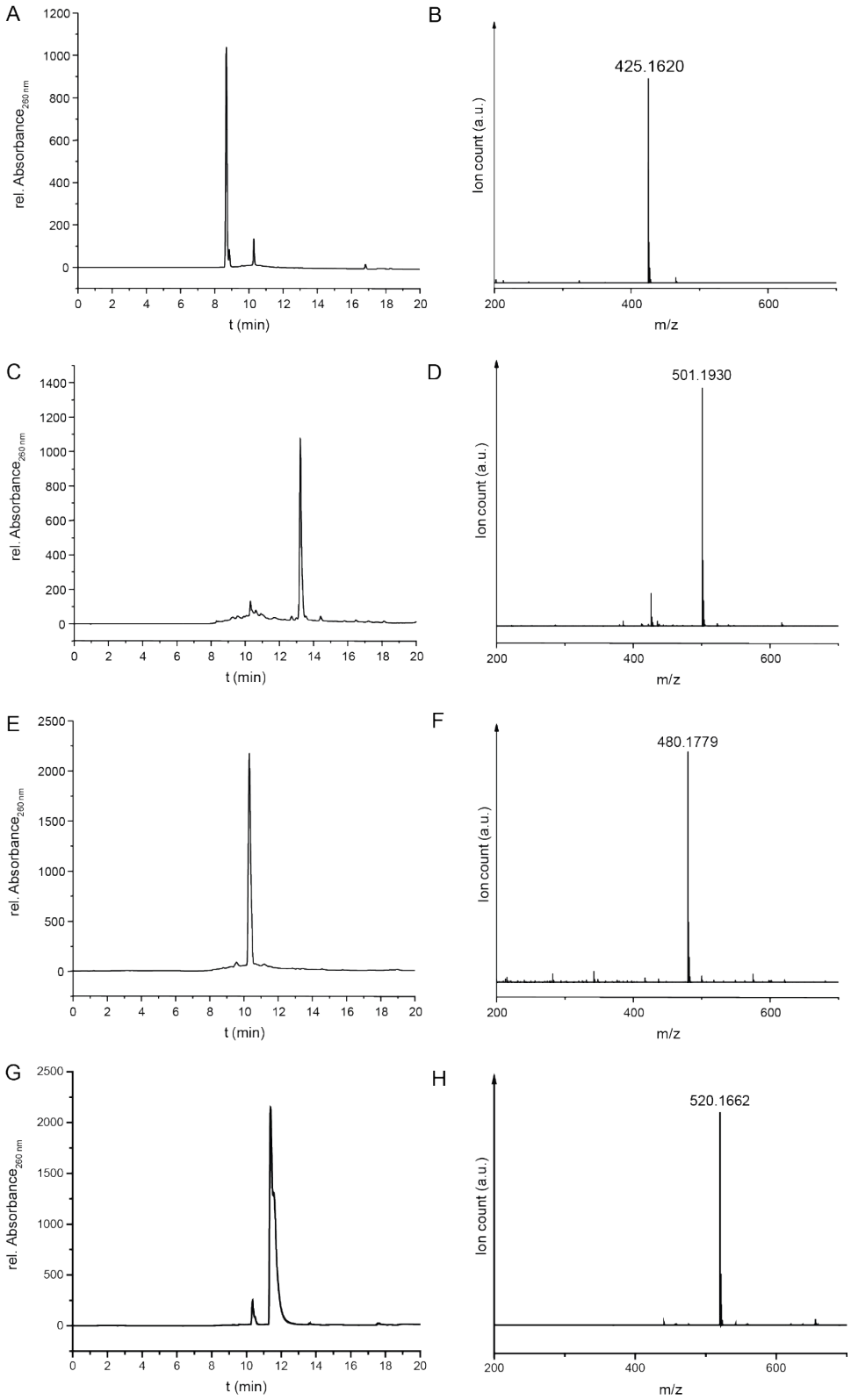


Fig. S4: HPLC and mass spectrometric analysis of AdoPropen (A and B, expected mass for $C_{17}H_{25}N_6O_5S^+$ = 425.1602 $[M]^+$, found: 425.1620), AdoViBe (C and D, expected mass for $C_{23}H_{29}N_6O_5S^+$ = 501.1915 $[M]^+$, found: 501.1930), AbSAM (E and F, expected mass for $C_{18}H_{26}N_9O_5S^+$ = 480.1772 $[M]^+$; found: 480.1779) and AdoNB (G and H, expected mass for $C_{21}H_{26}N_7O_7S^+$ = 520.1609 $[M]^+$; found: 520.1662).

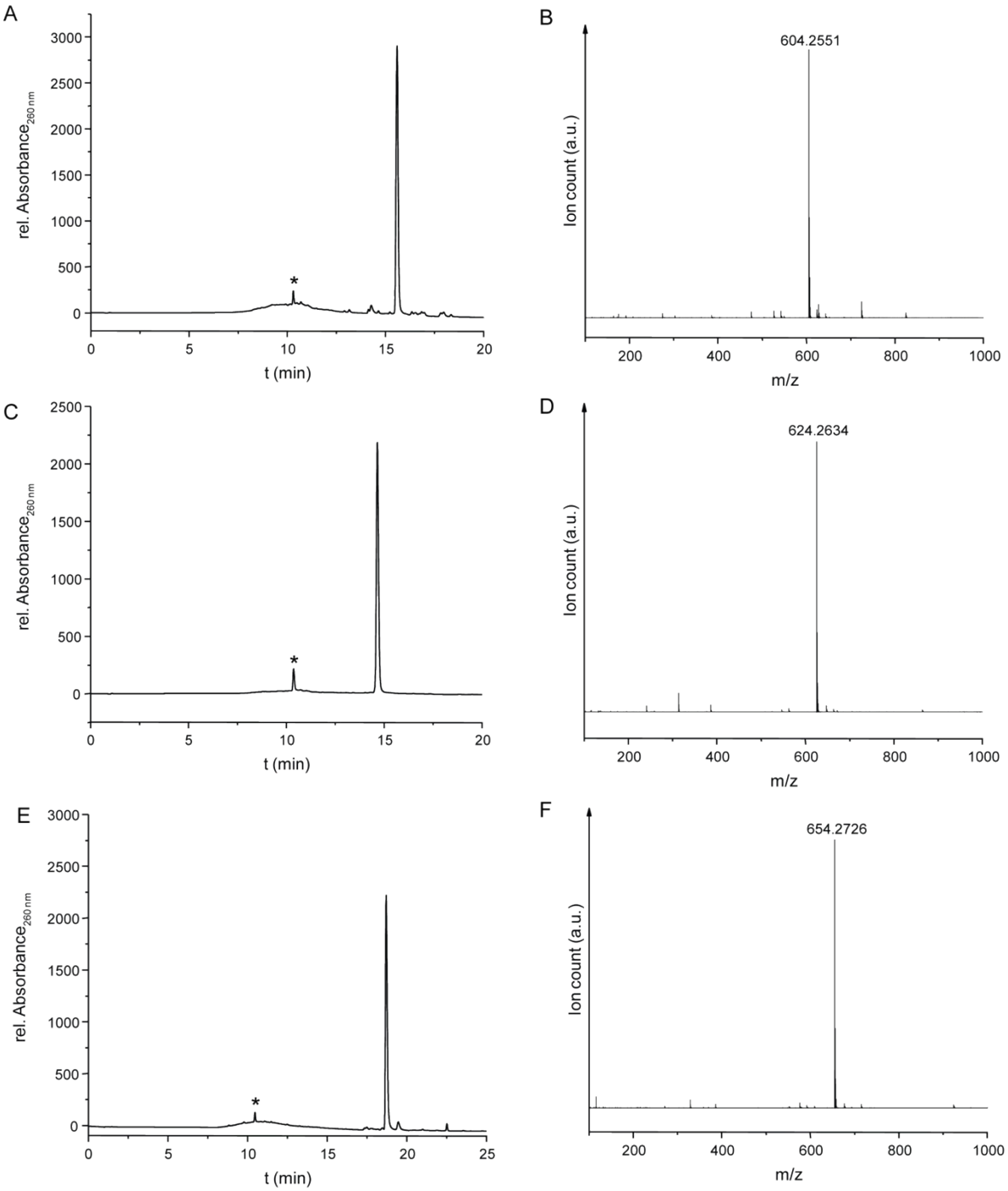


Fig. S5: HPLC (A) **1a** (C) **1b** (E) **1c** and mass respective spectrometric analyses of purified AdoMet analogs. (B) **1a**. Expected mass for $C_{27}H_{38}N_7O_7S^+$ = 604.2548 $[M]^+$, found: 604.2551. (D) **1b**. Expected mass for $C_{30}H_{38}N_7O_6S^+$ = 624.2599 $[M]^+$, found: 624.2634. (F) **1c**. Expected mass for $C_{31}H_{40}N_7O_7S^+$ = 654.2704 $[M]^+$, found: 654.2726. Peaks marked with an asterisk are due to slow degradation of the respective AdoMet analog and formation of *S*-adenosyl-L-homocysteine.

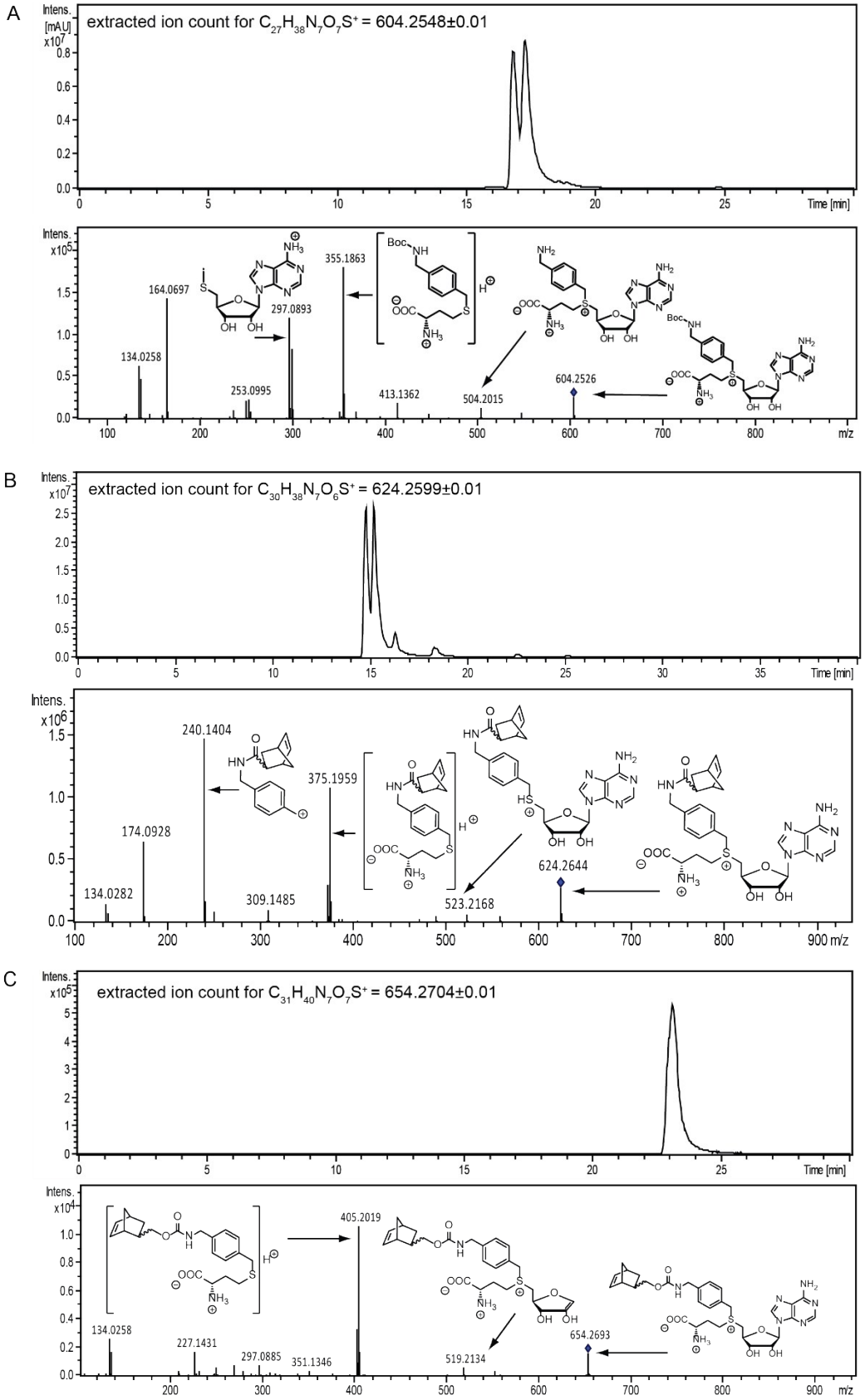


Fig. S6: CID-MS/MS analysis of purified AdoMet analogs. Precursor masses are denoted with a blue diamond. Possible structures for fragments are depicted. (A) **1a** (B) **1b** (C) **1c**.

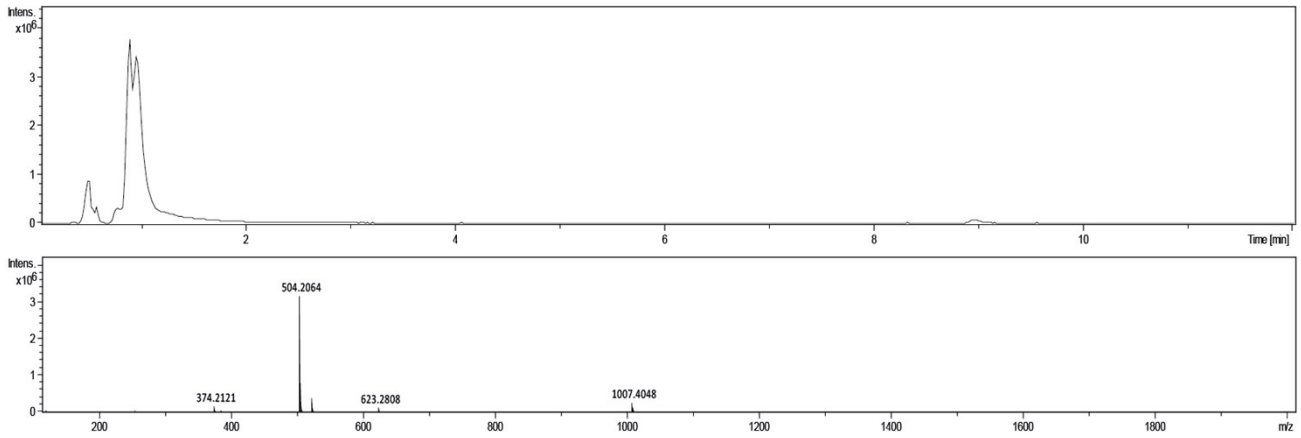


Fig. S7: LC-MS analysis of AdoMet analog **1a** after deprotection of the BOC-moiety, yielding the free amine. Expected mass for $C_{22}H_{30}N_7O_5S^+$ = 504.2024; found: 504.2064.

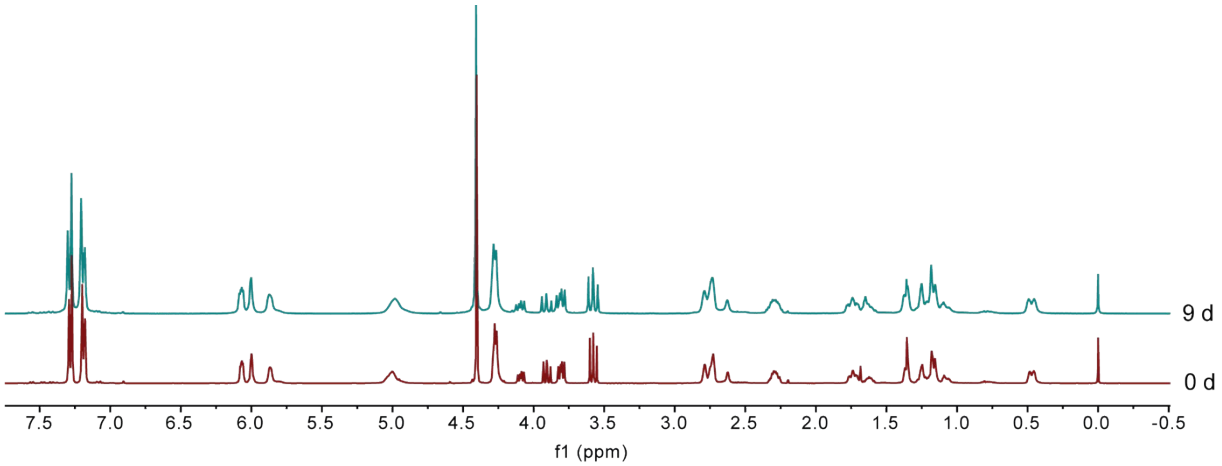


Fig. S8: ^{1}H-NMR analysis of norbornene-linker conjugate **6c** directly after synthesis (0 d) and after incubation for 9 d at room temperature in $CDCl_3$.

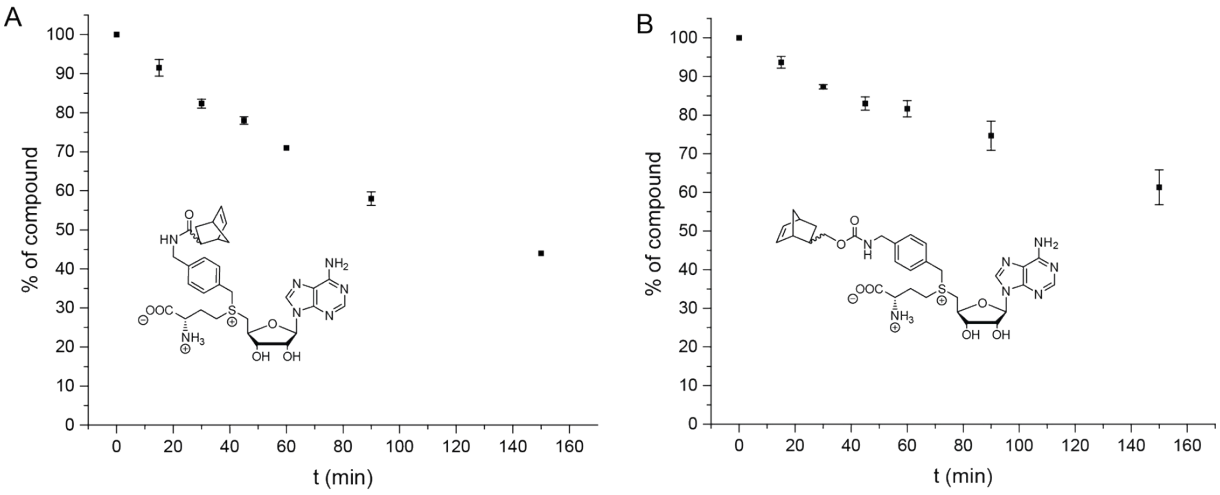


Fig. S9: Decay of the AdoMet analogs **1b** (A) and **1c** (B) in Ecm1 dialysis buffer (pH = 8). The AdoMet analog was incubated in Ecm1 dialysis buffer (pH = 8) at 37 °C, samples were taken after indicated time-points, acidified using $HClO_4$ and directly injected into the HPLC for analysis. The cofactor half-lives ($\tau_{1/2}$) were determined to be approx. 2 h for **1b** and 4 h for **1c**.

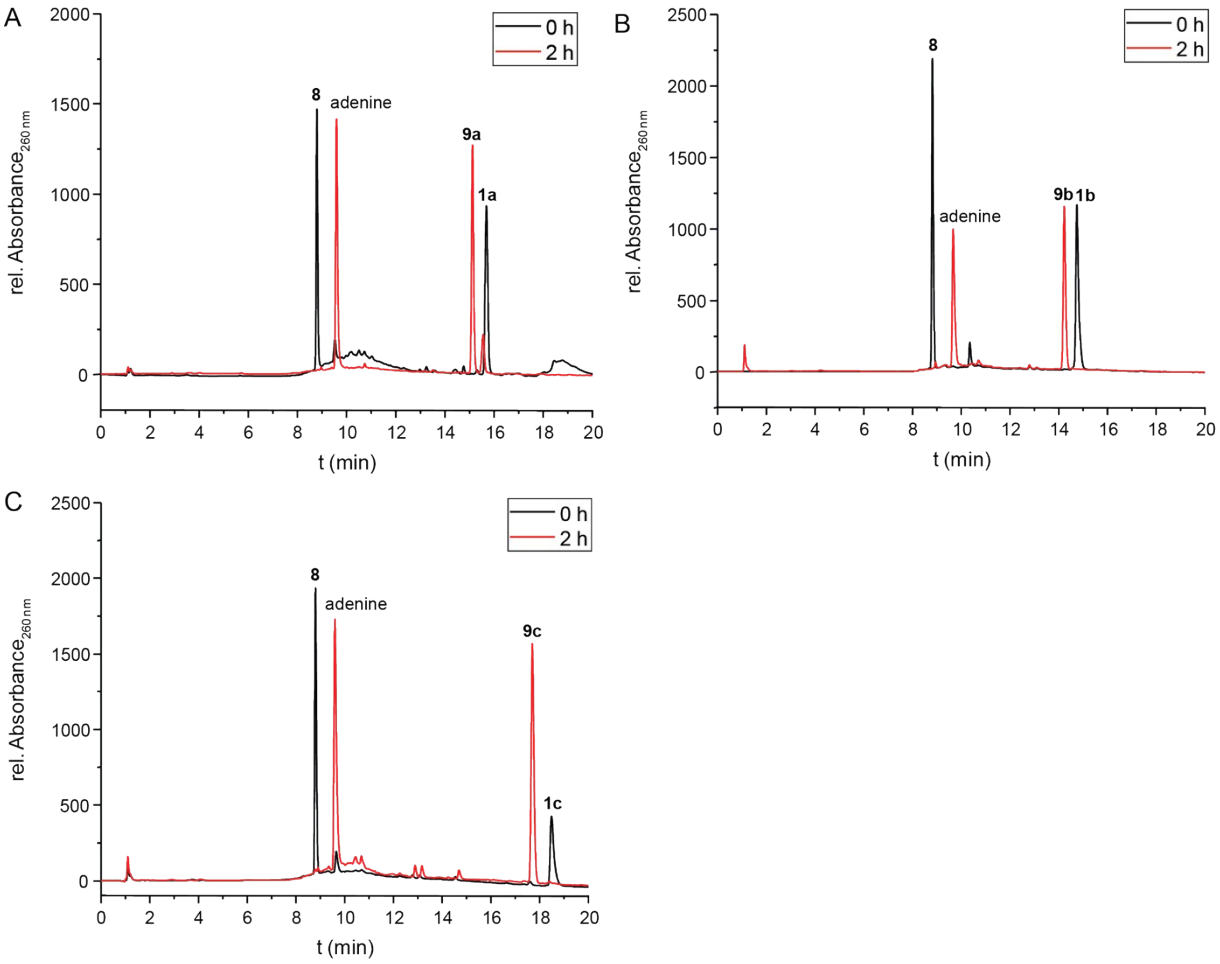


Fig. S10: HPLC analyses of enzymatic transfer reactions, giving modified cap analogs (A) **9a** (B) **9b** (C) **9c**.

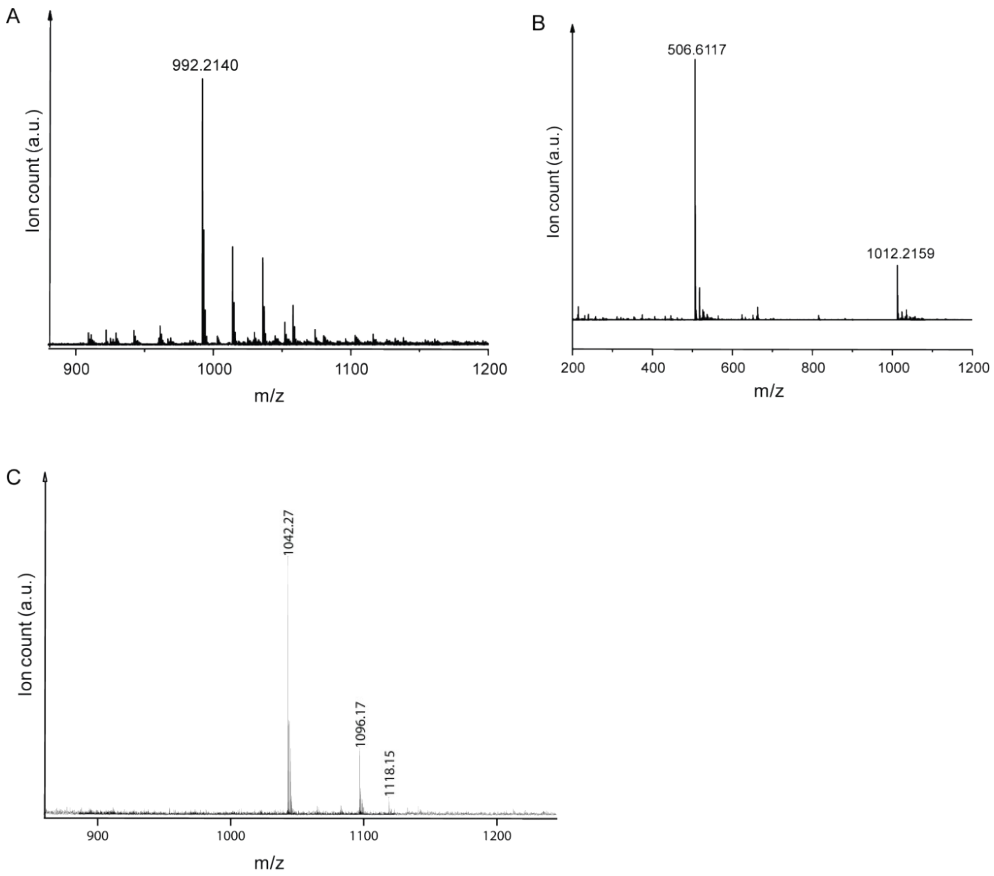


Fig. S11: Mass spectrometric analyses of enzymatic GpppA modification using Ecm1 (A) **9a**. Expected mass for $C_{33}H_{45}N_{11}O_{19}P_3^+$ = 992.2101 $[M]^+$, found: 992.2140 (B) **9b**. Expected mass for $C_{36}H_{45}N_{11}O_{18}P_3^+$ = 1012.2151 $[M]^+$, found: 1012.2159; expected mass for $C_{36}H_{46}N_{11}O_{18}P_3^{2+}$ = 506.6112 $[M+H]^+$, found: 506.6117 (C) **9c**. Expected mass for $C_{37}H_{47}N_{11}O_{19}P_3^+$ = 1042.2257 $[M]^+$, found: 1042.27 (MALDI-TOF measurement).

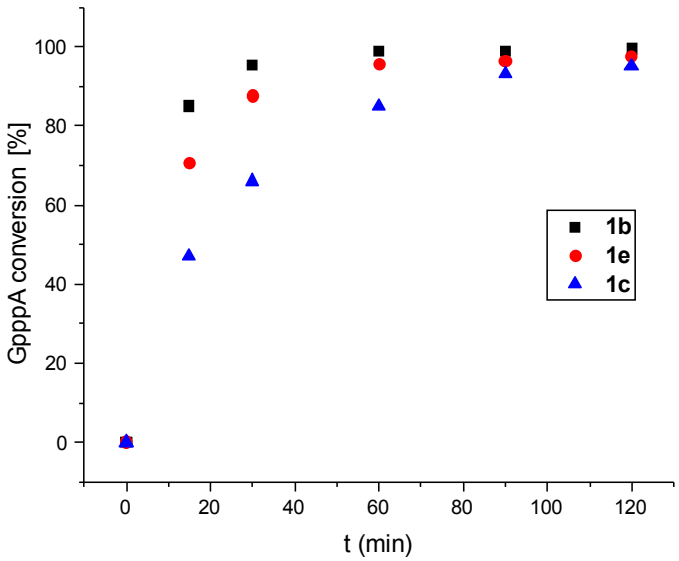


Fig. S12: Time-course of enzymatic transfer using GpppA (300 µM), 20 mol% Ecm1 and different AdoMet analogs (2 mM). The reaction mixture was incubated at 37 °C and aliquots were taken after indicated time points. Reactions were stopped by addition of a final concentration of 0.1 M $HClO_4$, samples were centrifuged and the supernatant was directly injected into the HPLC for analysis.

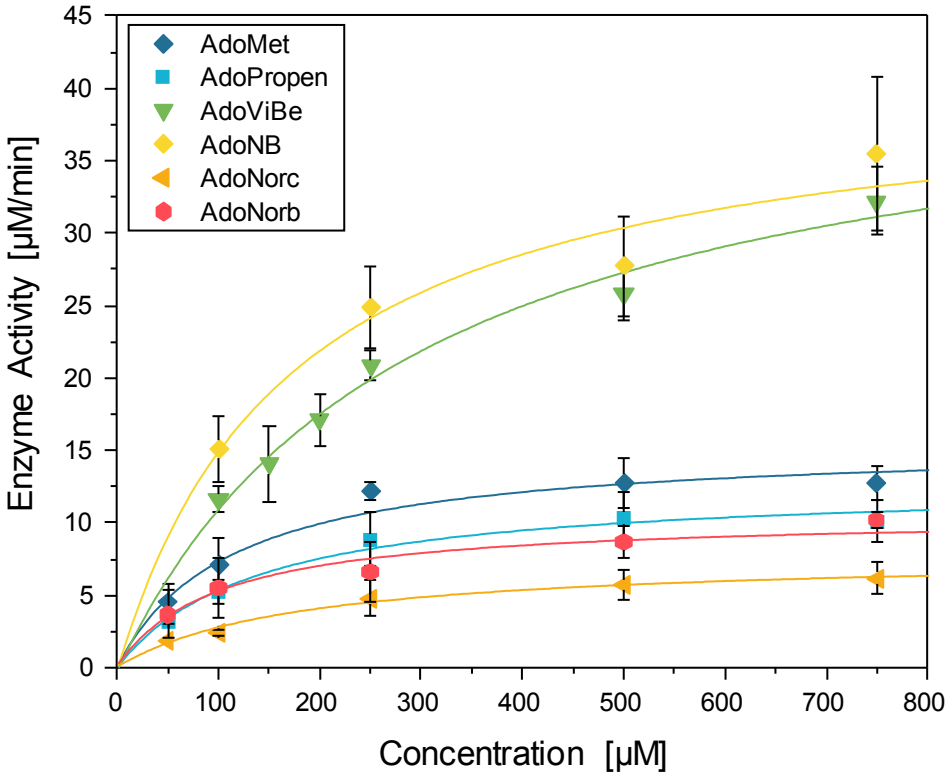


Fig. S13: Enzymatic activity of Ecm1 using the respective AdoMet analog and the model substrate GpppA.

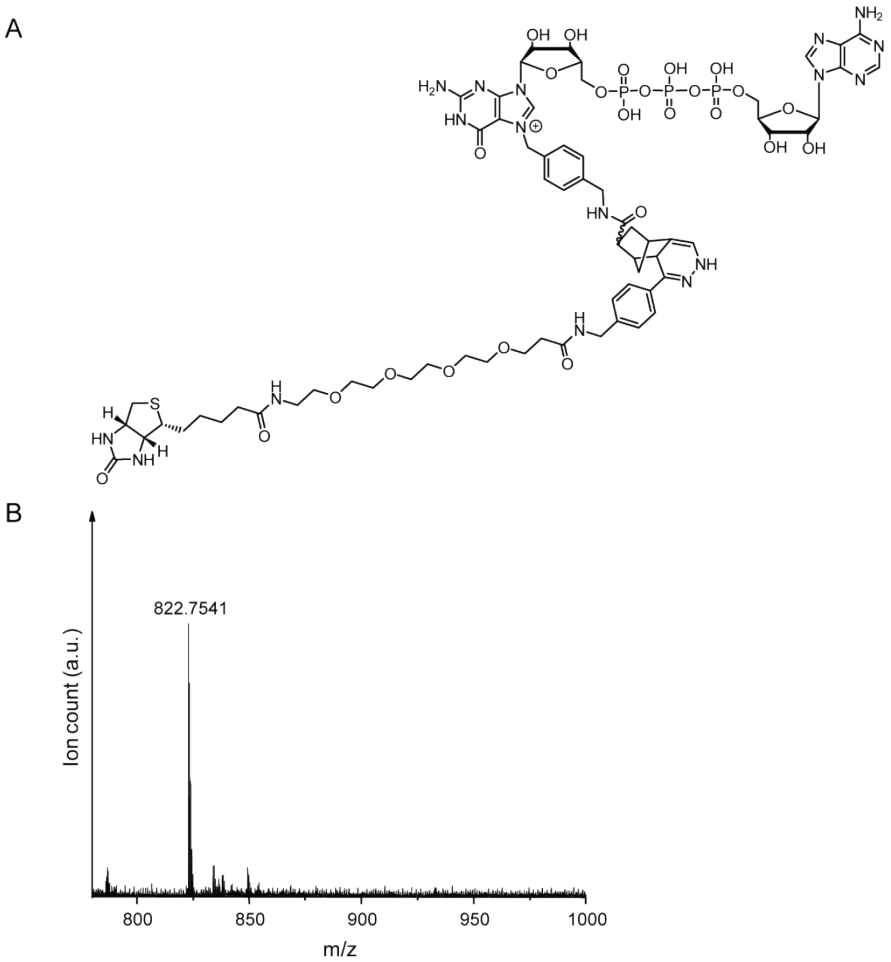


Fig. S14: LC-MS analysis of the tetrazine ligation between norbornene-modified GpppA and biotin-tetrazine (A) Structure of biotin-modified GpppA (**13b**). (B) LC-MS analysis of biotin-modified GpppA. Expected mass for $C_{66}H_{90}N_{17}O_{25}P_3S^{2+}$ = 822.7608 $[M+H]^{2+}$; found: 822.7541.

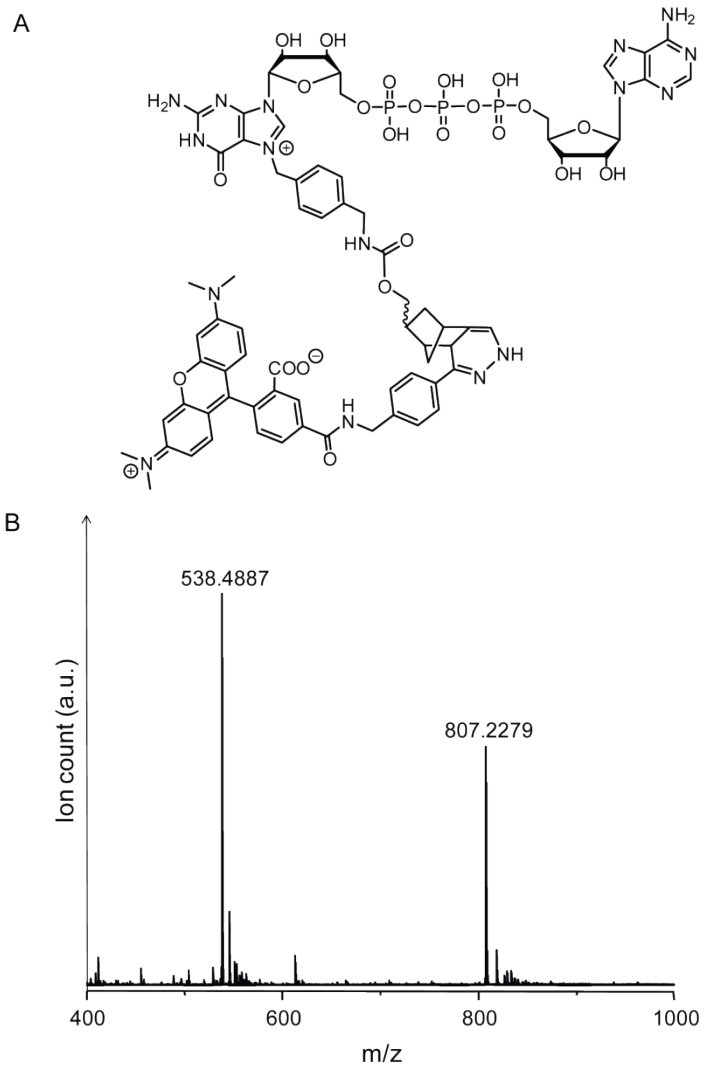


Fig. S15: LC-MS analysis of the tetrazine ligation between norbornene-modified GpppA and tetrazine-TAMRA **11**, giving conjugate **14c**. (A) Structure of TAMRA-modified GpppA. (B) LC-MS analysis of TAMRA-modified GpppA. Expected mass for $C_{71}H_{77}N_{16}O_{23}P_3^{2+}$ = 807.2275 $[M+H]^{2+}$, found: 807.2279; expected mass for $C_{71}H_{78}N_{16}O_{23}P_3^{3+}$ = 538.4874 $[M+2H]^{3+}$, found: 538.4887.

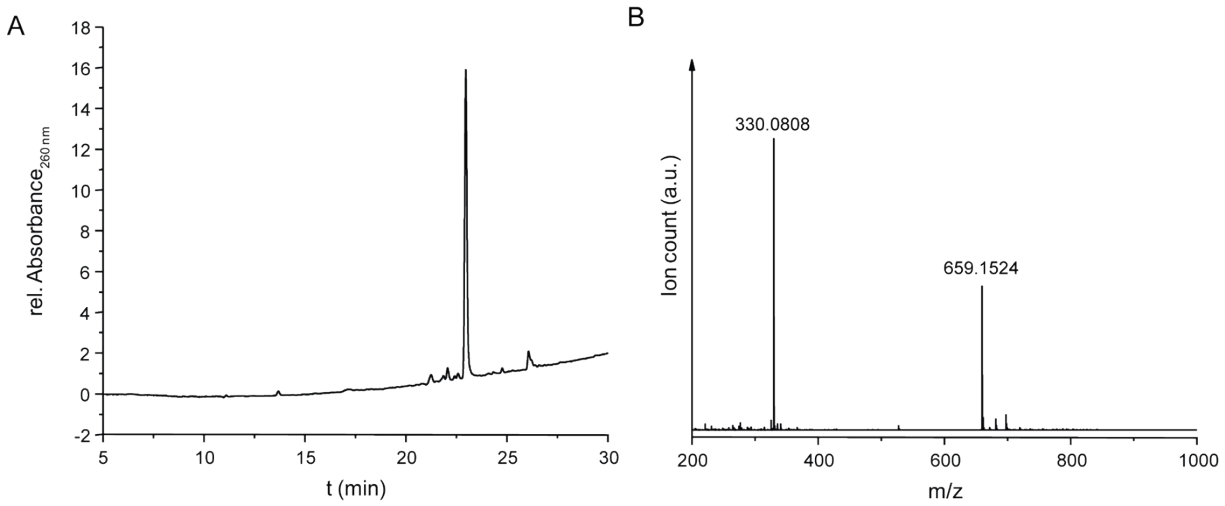


Fig. S16: HPLC (A) and mass spectrometric analysis (B) of tetrazine-Oregon Green 488 (**12**). Expected mass for $C_{35}H_{21}F_2N_6O_6^+$ = 659.1485 $[M+H]^+$, found: 659.1524; expected mass for $C_{35}H_{22}F_2N_6O_6^{2+}$ = 330.0779 $[M+2H]^{2+}$, found: 330.0808.

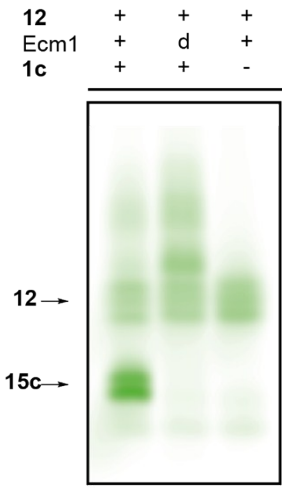


Fig. S17: Reaction of norbornene-GpppA **9c** with the fluorescent dye Oregon Green 488-tetrazine (**12**), giving conjugate **15c** and separation on a 10 % denaturing PAGE. A new fluorescent band was only observed if all components were present. Additional fluorescent bands are derived from AdoMet analog **1c** or its degradation products which are also reactive in the tetrazine ligation.

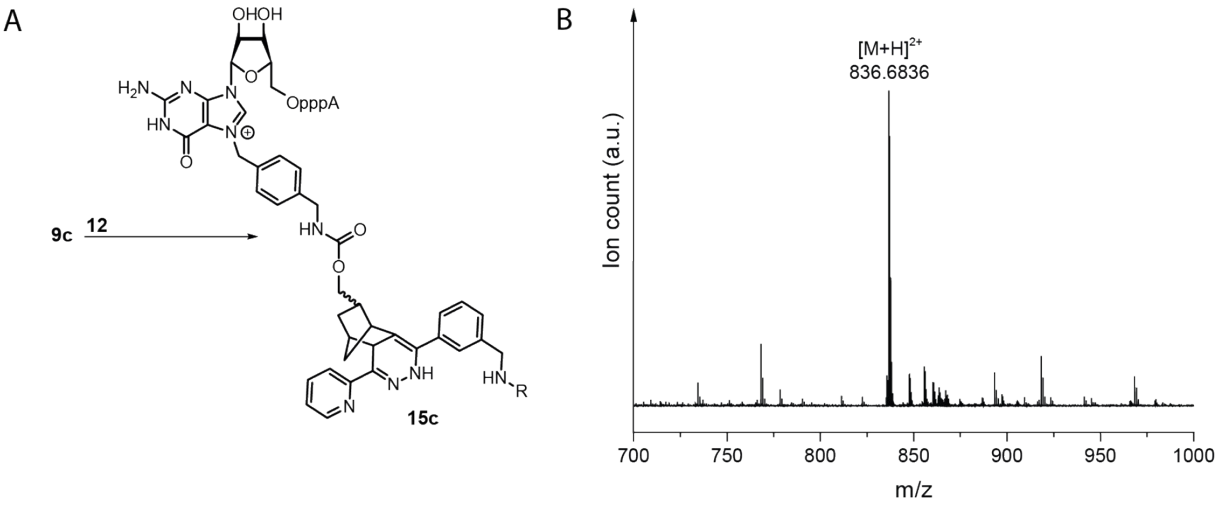


Fig. S18: (A) Scheme for the reaction of norbornene-modified GpppA **9c** with Oregon Green 488-tetrazine **12** to give the fluorescent cap analog **15c**. (B) Identification of the reaction product **15c** by LC-MS analysis. Expected mass for $C_{72}H_{68}F_2N_{15}O_{25}P_3^{2+}$ = 836.6840 $[M+H]^{2+}$, found: 836.6836.

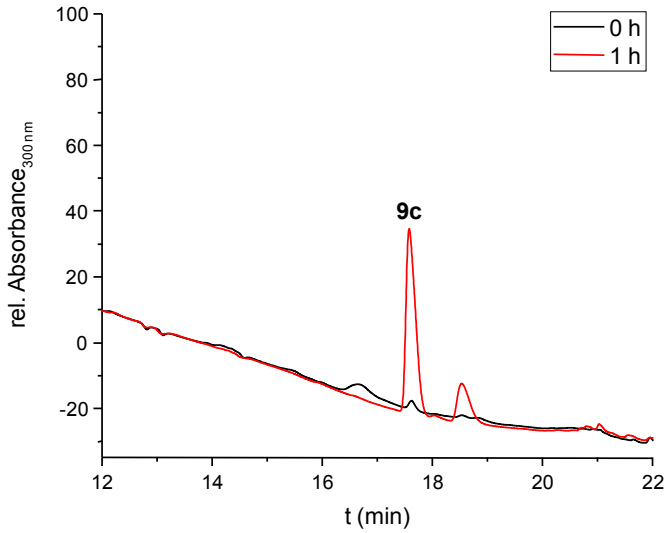


Fig. S19: Enzymatic conversion of GpppA (**8**) using Ecm1 and AdoMet analog **1c** in eukaryotic cell lysate. After incubation for 1 h at 37 °C, 10 % ACN was added for protein precipitation, the reaction mixture was centrifuged and directly analyzed by HPLC. Formation of **9c** was detected at an absorption wavelength of 300 nm (instead of 260 nm) which gives a weaker but specific signal for modified cap analogs.

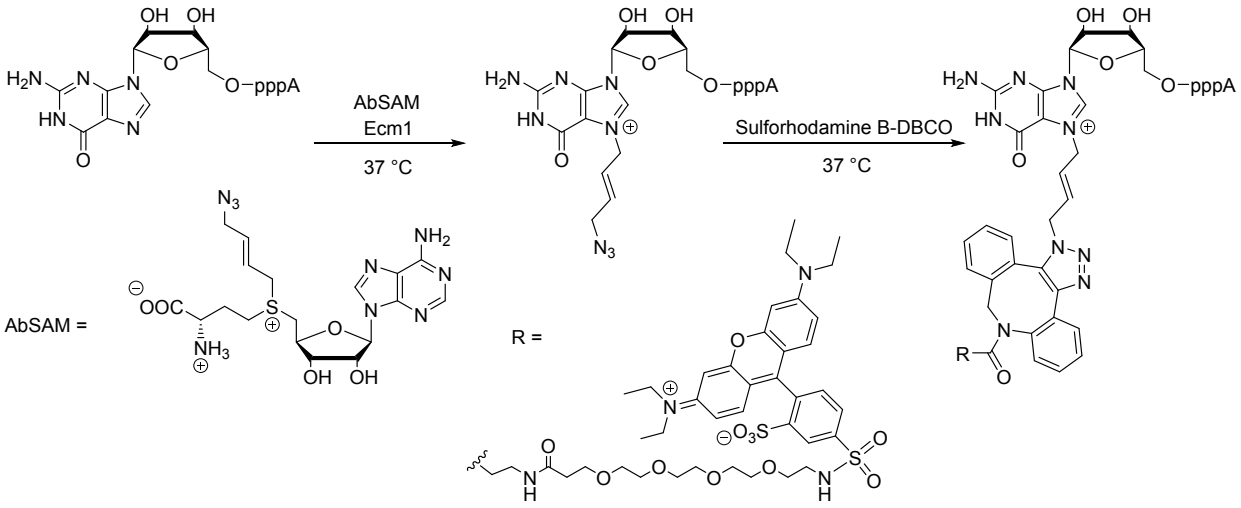


Fig. S20: Reaction scheme for the enzymatic modification of the cap analog GpppA (**8**) using Ecm1 and AbSAM followed by SPAAC reaction of the *N7*-azido-modified GpppA with the fluorescent dye conjugate Sulforhodamine B-DBCO.

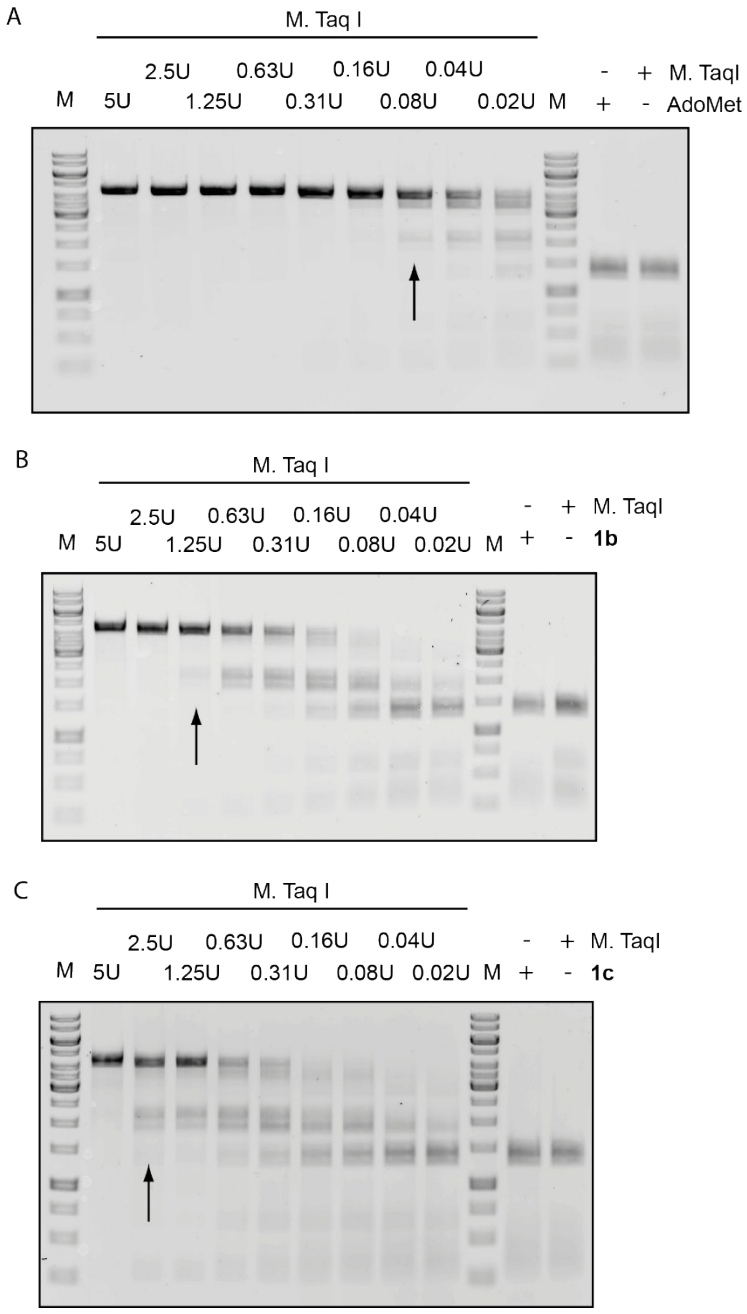


Fig. S21: Comparison of modification efficiencies using M. TaqI and either AdoMet, **1b** or **1c**, respectively. The substrate *pBR322* was incubated together with decreasing amounts of M. TaqI and AdoMet or the respective AdoMet analog. After incubation at 60 °C for 2 h, R. TaqI was added and incubated for 45 min at 65 °C. For linearization of the plasmid, BamHI was added to the reaction mixture and incubated for another 45 min at 37 °C. Analysis was performed by agarose gel electrophoresis (1 % agarose gel, 100 V, 45 min) and staining using ethidium bromide. (A) AdoMet (B) **1b** (C) **1c**. Arrows indicate the highest concentration of M. TaqI which does not give complete protection of the plasmid DNA. Control reactions omitting either the respective AdoMet analog or M. TaqI were performed, proving the specificity of the reaction.

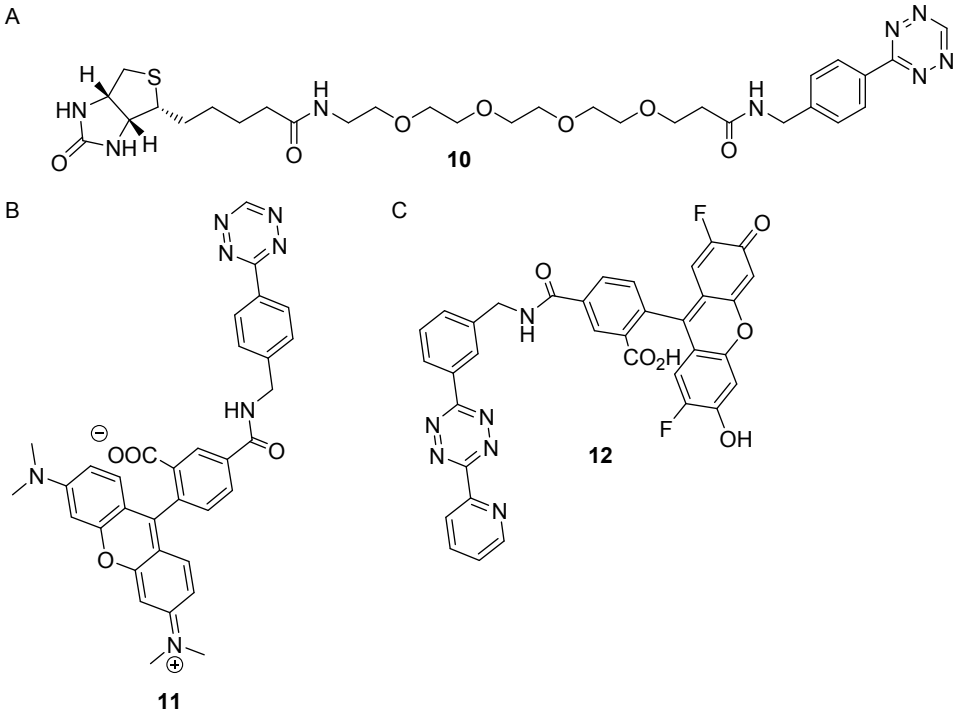
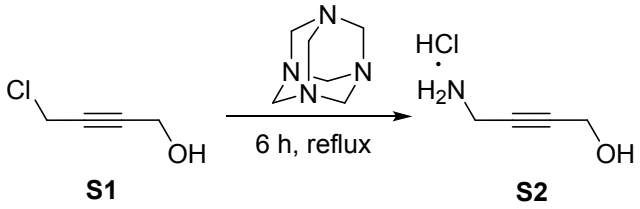


Fig. S22: Complete structures of (A) tetrazine-biotin (**10**) (B) tetrazine-5-TAMRA (**11**) (C) tetrazine-Oregon Green 488 (**12**).

Chemical synthesis

4-Aminobut-2-yn-1-ol (S2)



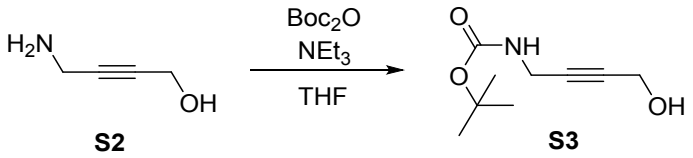
S1 **S2**

4-chlorobut-2-yn-1-ol (1 g, 9.6 mmol, 1.0 equiv.) and hexamethylenetetramine (1.48 g, 10.6 mmol, 1.1 equiv.) were dissolved in $CHCl_3$ (5 mL) and refluxed for 6 h. Solvent was removed under reduced pressure and the crude product was washed with chloroform and diethylether. The solid was then refluxed in a mixture of concentrated HCl and MeOH for 20 min. The solution was filtered and the solid was washed with conc. HCl/MeOH. Solvent was removed from the filtrate under reduced pressure to give a white solid which was recrystallized from ethanol (0.58 g, yield: 70 %). Spectroscopic data were in accordance with reported values.

^{1}H-NMR (MeOD, 300 MHz): δ [ppm] 4.21 (t, J = 2.0 Hz, 2H), 3.81 (t, J = 2.0 Hz, 2H)

MS (ESI-pos): expected mass for $C_4H_8NO^+$ = 86.0600 $[M + H]^+$; found: 86.0595.

***Tert*-butyl (4-hydroxybut-2-yn-1-yl)carbamate (S3)**

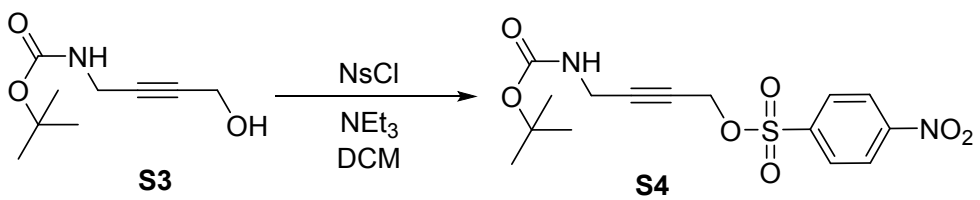


S2 **S3**

4-aminobut-2-yn-1-ol (0.25 g, 2.9 mmol, 1.0 equiv.), di-tert-butyl dicarbonate (0.64 g, 627 µL, 1.0 equiv.) and triethylamine (0.59 g, 814 µL, 2.0 equiv.) were dissolved in THF (20 mL) and stirred for 4 h at room temperature. The solvent was evaporated under reduced pressure and the product dried under high vacuum. The product was used without further purification.

MS (ESI-pos): expected mass for $C_9H_{15}NNaO_3^+$ = 208.0944 $[M+ Na]^+$; found: 208.0948.

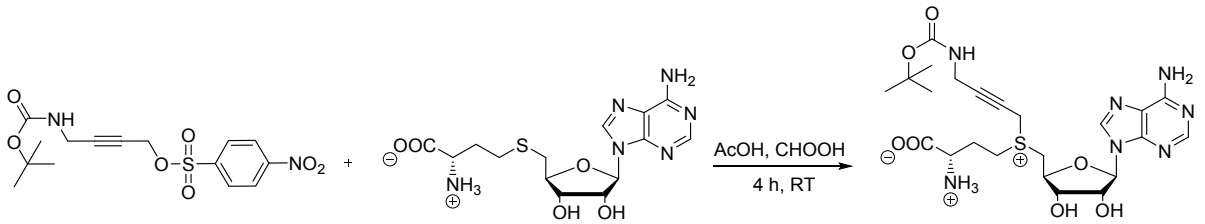
4-((*Tert*-butoxycarbonyl)amino)but-2-yn-1-yl 4-nitrobenzenesulfonate (S4)



S3 **S4**

Tert-butyl (4-hydroxybut-2-yn-1-yl)carbamate **S3** (0.19 g, 1.03 mmol, 1.0 equiv.) was dissolved in DCM (10 mL) and cooled down to 0 °C, 4-nitrobenzenesulfonyl chloride (NsCl, 0.25 g, 1.13 mmol, 1.1 equiv.) and NaOH (0.21 g, 5.15 mmol, 5.0 equiv.) were added and the reaction was stirred for 3 h at room temperature. The reaction was stopped by addition of cold H_2O (5 mL) and extracted with DCM (3×15 mL). The organic phase was dried over Na_2SO_4 and solvent was removed under reduced pressure to give a yellow solid (0.30 g, 78% yield) which was used without further purification.

5'-[(*R/S*)(3*S*)-3-Amino-3-carboxypropyl]-4-((*tert*-butoxycarbonyl)amino)but-2-yn-1-yl-sulfonio]-5'-deoxyadenosine (S5)

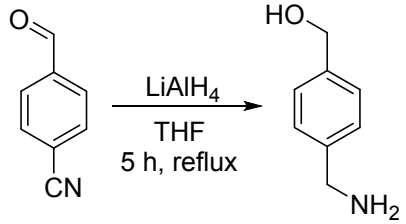


S4 **7** **S5**

To a stirred solution of *S*-adenosyl-L-homocysteine (5 mg, 0.013 mmol, 1.0 equiv.) in 0.75 mL formic acid and 0.75 mL acetic acid, nosylate **S4** was added (100 mg, 0.27 mmol, 20.8 equiv.). The solution was stirred for 3 h at room temperature, diluted with H_2O (3 mL) and extracted twice with diethylether (15 mL). Identity of the AdoMet analog was confirmed by HPLC and LC-MS. The product was obtained as a mixture of *R*- and *S*-epimers, but could not be isolated.

MS(ESI-pos): expected mass for $C_{23}H_{34}N_7O_7S^+$ = 552.2235 $[M]^+$, found: 552.2243.

(4-Aminomethyl)phenylmethanol (3)



2 **3**

(4-Aminomethyl)phenylmethanol **3** was synthesized according to a literature procedure.[3] Briefly, 4-cyanobenzaldehyde **2** (1.0 g, 7.6 mmol, 1.0 equiv.) was dissolved in dry THF, $LiAlH_4$ (1.74 g, 45.8 mmol, 6.0 equiv.) in 30 mL of dry THF was slowly added at 0 °C and the reaction mixture was stirred at reflux for 5 h. Afterwards, the reaction was cooled again to 0 °C, quenched by addition of H_2O (2 mL) and NaOH (6 mL, 2 M) and stirred for 10 min. The white suspension was filtered and the filtrate was washed with ethyl acetate (250 mL). After removal

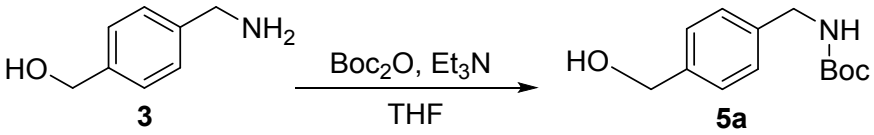
of the solvent under reduced pressure, the product was obtained as a white solid. Spectroscopic data were in accordance with reported values.

^{1}H-NMR (MeOD, 300 MHz): δ [ppm] = 7.30 (s, 4H), 4.57 (s, 2H), 3.75 (s, 2H).

MS (ESI-pos): expected mass for $C_8H_{12}NO^+$ = 138.0913 $[M]^+$, found: 138.0921.

Yield: quant.

***Tert*-butyl(4-(hydroxymethyl)benzyl)carbamate (5a)**



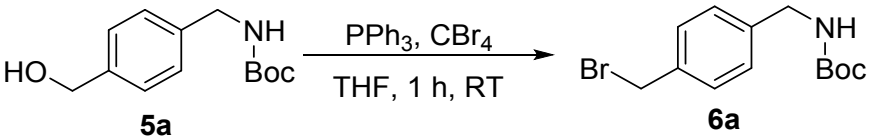
3 **5a**

Tert-butyl(4-(hydroxymethyl)benzyl)carbamate **5a** was synthesized according to a literature procedure.[4] Briefly, (4-aminomethyl)phenyl)methanol (**3**, 0.44 g, 3.2 mmol, 1.0 equiv.) was dissolved in dry THF (20 mL), triethylamine (0.50 ml, 3.6 mmol, 1.1 equiv.) and di-*tert*-butylcarbonate (0.76 ml, 3.2 mmol, 1.0 equiv.) were added under constant stirring and stirred for another 3.5 h. The reaction mixture was washed with saturated $NaHCO_3$, extracted with ethyl acetate (3×30 mL), the organic phases were dried over Na_2SO_4, solvent was removed under reduced pressure and the product was obtained as a yellow solid. The product was used for the next step without further purification.

MS (ESI-pos): expected mass for $C_{13}H_{19}NNaO_3^+$ = 260.1257 $[M + Na]^+$, found: 260.1266; expected mass for $C_{26}H_{38}N_2NaO_6^+$ = 497.2622 $[2M + Na]^+$, found: 497.2627.

Yield: 0.75 g, 3.12 mmol, 98 %.

***Tert*-butyl(4-(bromomethyl)benzyl)carbamate (6a)**

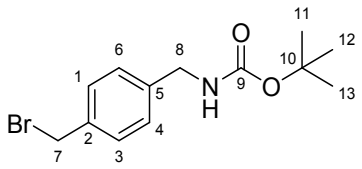


5a **6a**

Tert-butyl(4-(bromomethyl)benzyl)carbamate **6a** (0.50 g, 3.65 mmol, 1.0 equiv.), tetrabromomethane (1.82 g, 5.48 mmol, 1.5 equiv.) and triphenylphosphine (1.44 g, 5.48 mmol, 1.5 equiv.) were dissolved in dry THF under argon atmosphere. The reaction mixture was stirred for 1 h at room temperature, filtered and the solvent of the filtrate was removed under reduced pressure. The product was purified by column chromatography (cyclohexane, ethyl acetate 5:1).

Yield: 0.32 g, 1.1 mmol, 30 %.

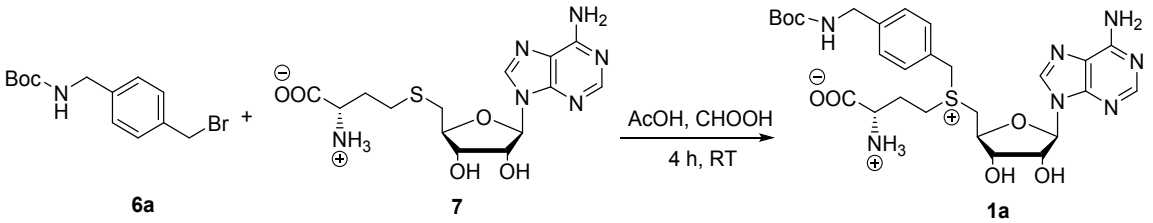
MS (ESI-pos): expected mass for $C_{13}H_{18}BrNNaO_2^+$ = 322.0412 $[M+Na]^+$, found: 322.0413; expected mass for $C_{26}H_{36}Br_2N_2NaO_4^+$ = 621.0934 $[2M + Na]^+$, found: m/z = 621.0905.



^{1}H-NMR (MeOD, 300 MHz): δ [ppm] = 7.29 (m, 4H, **1, 3, 4, 6**), 4.51 (s, 2H, **7**), 4.18 (s, 2H, **8**), 1.43 (s, 9H, **11, 12, 13**).

^{13}C-NMR (MeOD, 75 MHz): δ [ppm] = 158.50 (C-**9**), 141.29 (C-**5**), 138.37 (C-**2**), 130.26 (C-**4, 6**), 128.42 (C-**1, 3**), 80.29 (C-**10**), 64.97 (C-**7**), 44.78 (C-**8**), 28.77 (C-**11, 12, 13**).

5'-[(*R/S*)(3*S*)-3-Amino-3-carboxypropyl]-4-[((*tert*-butoxycarbonyl)amino)-methyl)benzyl -sulfonio]-5'-deoxyadenosine (1a)



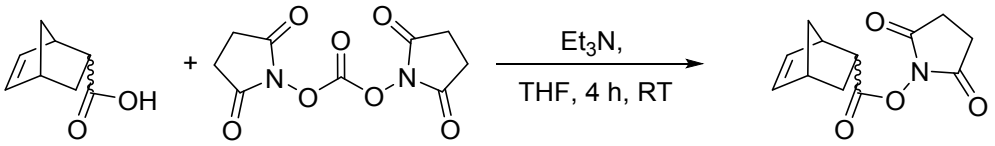
Tert-butyl(4-(bromomethyl)benzyl)carbamate **6a** (0.10 g, 0.33 mmol., 25.0 equiv.) was dissolved in formic acid (0.7 mL) and acetic acid (0.7 mL) and *S*-adenosyl-L-homocysteine (5 mg, 0.013 mmol, 1.0 equiv.) and $AgClO_4$ (2.7 mg, 0.013 mmol, 1.0 equiv.) were added at 0 °C. The reaction mixture was stirred for 4 h at room temperature, diluted with H_2O (3 mL) and extracted twice with diethylether (15 mL). The aqueous phase was lyophilized, redissolved in H_2O and purified by semi-preparative HPLC. Purity and identity of the AdoMet analog were confirmed by HPLC and mass spectrometric analysis. The product was obtained as mixture of *R*- and *S*-epimers and stored at -20 °C for further use.

Yield: 60 % (derived from integration of the HPLC peaks)

MS(ESI-pos): expected mass for $C_{27}H_{38}N_7O_7S^+$ = 604.2548 $[M]^+$; found: 604.2549.

^{1}H-NMR (D_2O, 600 MHz): δ [ppm] = 8.37 (s, 0.5H, CH_{arom}), 8.32 (s, 0.5H, CH_{arom}), 8.31 (s, 0.5H, CH_{arom}), 8.29 (s, 0.5H, CH_{arom}), 7.35 (d, 1H, J = 4 Hz, CH_{arom}), 7.31-7.25 (m, 3H, CH_{arom}), 6.10 (dd, 1H, J = 9.5 Hz, 3.6 Hz, H1'), 4.62 (dt, 2H, J = 11.7 Hz, 6.0 Hz), 4.57-4.53 (m, 1H), 4.50-4.45 (m, 1H), 4.26-4.22 (d, 2H, J = 7.7 Hz), 3.96-3.89 (m, 2H), 3.84-3.79 (m, 2H), 3.68-3.61 (m, 2H), 3.58-3.51 (m, 2H), 2.41-2.32 (m, 2H, Hß), 1.44 (s, 9H).

5-Norbornene-2-acetic acid succinimidyl ester (4b)

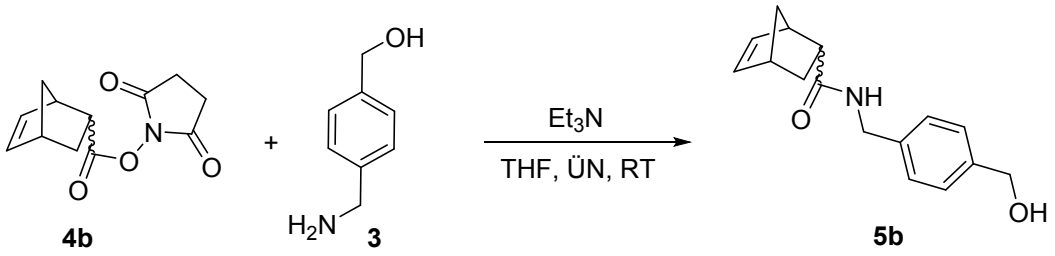


4b

5-Norbornen-2-succinimidylester (**4b**) was synthesized according to a literature procedure.[5] Briefly, 5-norbornene-2-carboxylic acid (0.50 g, 3.62 mmol, 1.0 equiv., mixture of exo and endo, mainly endo) and N, N'-disuccinimidyl carbonate (0.93 g, 3.62 mmol, 1.0 equiv.) were dissolved in dry THF (10 mL), triethylamine (0.55 ml, 3.98 mmol, 1.1 equiv.) was added and the reaction was stirred for 4 h at room temperature. The solvent was removed under reduced pressure, H_2O (10 mL) and ethyl acetate (10 mL) were added and phases were separated. The aqueous phase was extracted twice with ethyl acetate, combined organic phases were dried over Na_2SO_4 and solvent was removed under reduced pressure to give a white solid. The product was used without further purification.

MS (ESI-pos): expected mass for $C_{12}H_{13}NNaO_4^+$ = 258.0742 $[M+Na]^+$, found: 258.0737; $C_{13}H_{17}NNaO_5^+$ = 290.0999 found: 290.1005; expected mass for $C_{24}H_{26}N_2NaO_4^+$ = 493.1581 $[2M+Na]^+$, found: 493.1581.

(1*R*, 4*R*)-*N*-(4-(Hydroxymethyl)benzyl)bicyclo[2.2.1]hept-5-ene-2-carboxamide (5b)

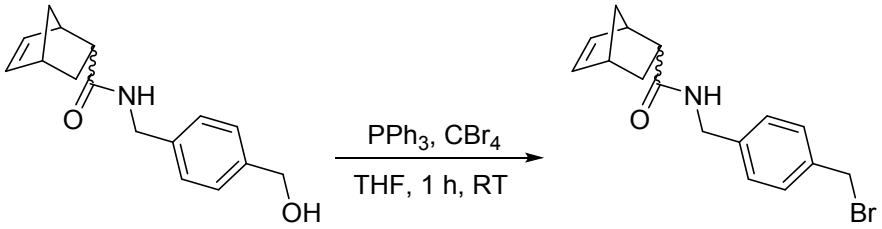


4b **3** **5b**

5-Norbornen-2-succinimidylester **4b** (0.35 g, 1.5 mmol, 1.0 equiv.) and (4-aminomethyl)phenylmethanol **3** (0.20 g, 1.5 mmol, 1.0 equiv.) were dissolved in dry THF (5 mL), triethylamine (0.23 ml, 1.65 mmol, 1.1 equiv.) was added and the reaction was stirred overnight at room temperature. The white suspension was filtered and the solvent was removed from the filtrate under reduced pressure to give a white solid. The product was used for the next step without further purification.

MS (ESI-pos): expected mass for $C_{16}H_{19}NNaO_2^+$ = 280.1308 $[M+Na]^+$, found: 280.1328; expected mass for $C_{32}H_{38}N_2NaO_4^+$ = 537.2724 $[2M+Na]^+$, found: 537.2709.

***N*-(4-(Bromomethyl)benzyl)bicyclo[2.2.1]hept-5-ene-2-carboxamide (6b)**

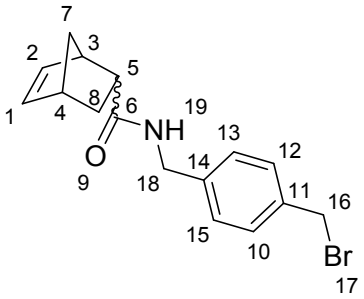


5b **6b**

(1*R*, 2*R*, 4*R*)-*N*-(4-(Hydroxymethyl)benzyl)bicyclo[2.2.1]hept-5-ene-2-carboxamide **5b** (386 mg, 1.50 mmol, 1.0 equiv.), tetrabromomethane (0.75 g, 2.25 mmol, 1.5 equiv.) and triphenylphosphine (0.59 g, 2.25 mmol, 1.5 equiv.) were dissolved in dry THF (8 mL) and stirred for 1 h at room temperature. The resulting orange suspension was filtered, solvent was removed under reduced pressure and the crude product was purified by column chromatography (cyclohexane, ethyl acetate 5:1) to give a white solid. NMR analysis revealed that **6b** consisted mainly of the endo isomer.

Yield: 165 mg, 0.5 mmol, 33 %.

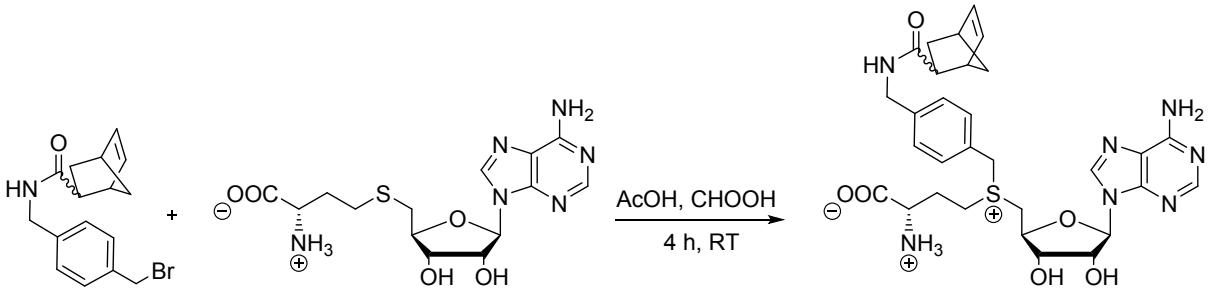
MS (ESI-pos): expected mass for $C_{16}H_{19}BrNO^+$ = 320.0645 $[M+H]^+$, found: 320.0660;
expected mass for $C_{16}H_{18}BrNNaO^+$ = 342.0464 $[M+Na]^+$, found: 342.0483.



^{1}H-NMR ($CDCl_3$, 300 MHz): δ [ppm] = 7.33 (d, 2H, J = 8.1 Hz, **13, 15**), 7.21 (d, 2H, J = 8.1 Hz, **10, 12**), 6.26-6.22 (m, 1H, **2**), 5.99-5.95 (m, 1H, **1**), 5.78-5.71 (bs, 1H, **19**), 4.47 (s, 2H, **16**), 4.44-4.32 (m, 2H, **18**), 3.17-3.12 (m, 1H, **5**), 2.99-2.84 (m, 2H, **3, 4**), 2.00-1.91 (m, 1H, **7**), 1.5-1.22 (m, 3H, **7, 8**).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ [ppm] = 174.6 (C-**6**), 139.5 (C-**14**), 138.3 (C-**2**), 137.4 (C-**1**), 132.7 (C-**11**), 129.8 (C-**10, 12**), 128.5 (C-**13, 15**), 50.5 (C-**7**), 46.7 (C-**5**), 45.3 (C-**3**), 43.6 (C-**18**), 43.2 (C-**4**), 33.6 (C-**16**), 30.3 (C-**8**).

5'-[(*R/S*)(3*S*)-3-Amino-3-carboxypropyl]-4-[((bicyclo[2.2.1]-hept-5-ene-2-carboxamido)-methyl)benzyl-sulfonio]-5'-deoxyadenosine (1b)



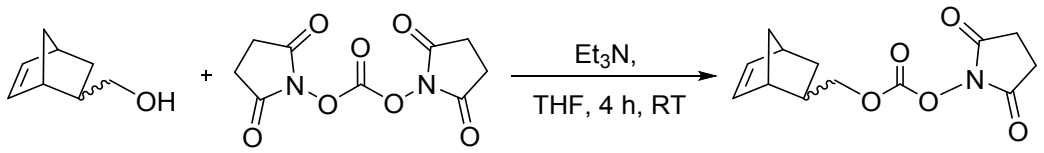
6b **7** **1b**

Compound **1b** was synthesized and purified as described for compound **1a**. Purity and identity of the AdoMet analog were confirmed by HPLC and mass spectrometric analysis. The product was obtained as a mixture of *R*- and *S*-epimers and stored at -20 °C for further use.

MS(ESI-pos): expected mass for $C_{30}H_{38}N_7O_6S^+$ = 624.2599 $[M]^+$; found: 624.2627.

Yield: 49 % (derived from integration of the HPLC peaks).

Bicyclo[2.2.1]hept-5-en-2-yl)methyl (2,5-dioxopyrrolidin-1-yl)carbonate (4c)

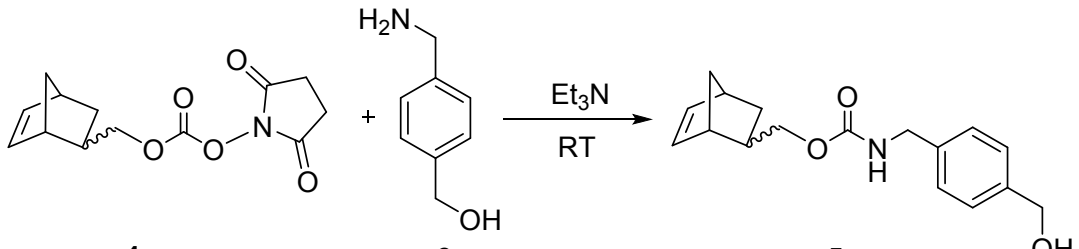


4c

Compound **4c** was synthesized starting from 5-norbornene-2-methanol in a similar procedure as described for compound **4b**. The product was used without further purification.

MS (ESI-pos): expected mass for $C_{13}H_{15}NNaO_5^+$ = 288.0842 $[M + Na]^+$; found: 288.0842.

Bicyclo[2.2.1]hept-5-en-2-yl)methyl (4-(hydroxymethyl)benzyl)carbamate (5c)

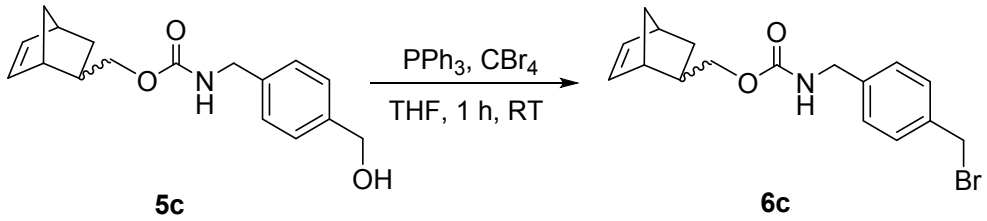


4c **3** **5c**

Compound **5c** was synthesized as described for compound **5b**. The product was used without further purification.

MS (ESI-pos): expected mass for $C_{17}H_{22}NO_3^+$ = 288.1594 $[M + H]^+$; found: 288.0844.

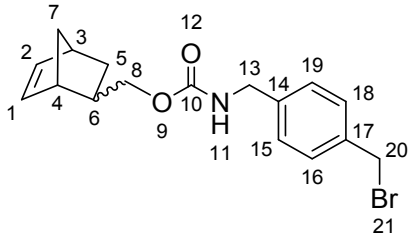
Bicyclo[2.2.1]hept-5-en-2-yl)methyl (4-(bromomethyl)benzyl)carbamate (6c)



5c **6c**

Compound **6c** was synthesized as described for compound **5c**. Purification was performed by column chromatography (cyclohexane, ethyl acetate 5:1) to give a white solid (200 mg, 0.57 mmol, yield: 39 %). NMR revealed that **6c** consisted of a 7:3 mixture of endo/exo isomers.

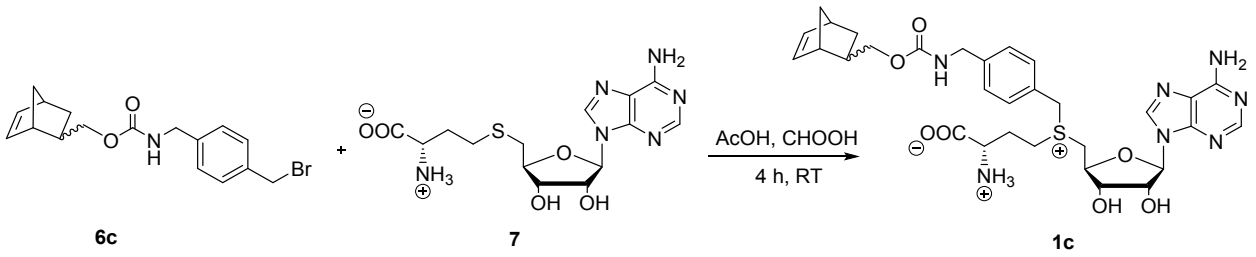
MS (ESI-pos): expected mass for $C_{17}H_{20}BrNNaO_2^+$ = 372.0570 $[M + Na]^+$; found: 372.0562.



^{1}H-NMR ($CDCl_3$, 300 MHz): δ [ppm] = 7.29 (d, J = 8.1 Hz, 2H, **15**, **19**), 7.21 (d, J = 8.1 Hz, 2H, **16**, **18**), 6.2-5.8 (m, 2H, **1, 2**), 5.14-5.03 (bs, 1H, **11**), 4.42 (s, 2H, **20**), 4.33-4.23 (m, 2H, **13**), 4.2-3.6 (m, 2H, **8**), 2.9-2.6 (m, 2H, **3,4**), 2.4-2.3 (m, 0.7H, **6**), 1.8 (m, 0.7H, **5, 7**), 1.7 (m, 0.3H, **6**), 1.5-1.1 (m, 2.6H, **5,7**), 0.6 (m, 0.7H, **7**)

^{13}C-NMR ($CDCl_3$, 75 MHz): δ [ppm] = 156.8 (C-**10**), 139.2 (C-**14**), 137.6 (C-**1, 2**), 137.1 (C-**17**), 137.0 (C-**1, 2**), 136.3 (C-**1, 2**), 132.3 (C-**1, 2**), 129.4 (C-**16**, **18**), 128.0 (C-**15**, **19**), 69.2 (C-**8**), 68.6 (C-**8**), 49.4 (C-**5**, **7**), 45.0 (C-**5,7**), 44.7 (C-**13**), 43.9 (C-**3, 4**), 43.7 (C-**3, 4**), 42.3 (C-**3, 4**), 41.6 (C-**3, 4**), 38.4 (C-**6**), 38.2 (C-**6**), 33.3 (C-**20**), 29.7 (C-**7**), 28.9 (C-**7**).

5'-[(*R/S*)(3*S*)-3-Amino-3-carboxypropyl]-4-[(((bicyclo[2.2.1]-hept-5-ene-2-methoxy)-carbonyl)amino)methyl)benzyl -sulfonio]-5'-deoxyadenosine (1c)



6c 7 1c

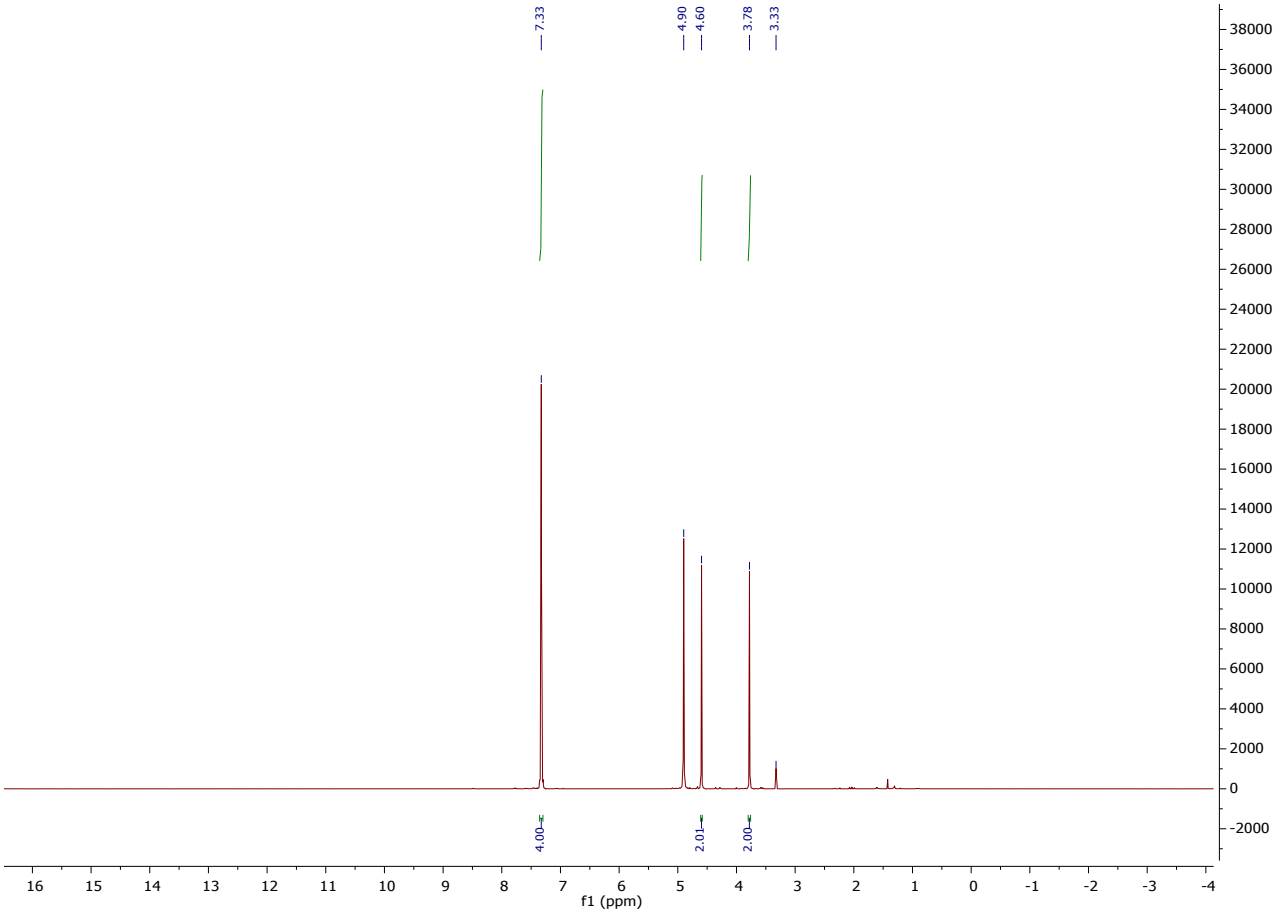
Compound **1c** was synthesized and purified as described for compound **1b**. Purity and identity of the AdoMet analog were confirmed by HPLC and mass spectrometric analysis. The product was obtained as mixture of *R*- and *S*-epimers and stored at -20 °C for further use.

Yield: 41 % (derived from integration of the HPLC peaks)

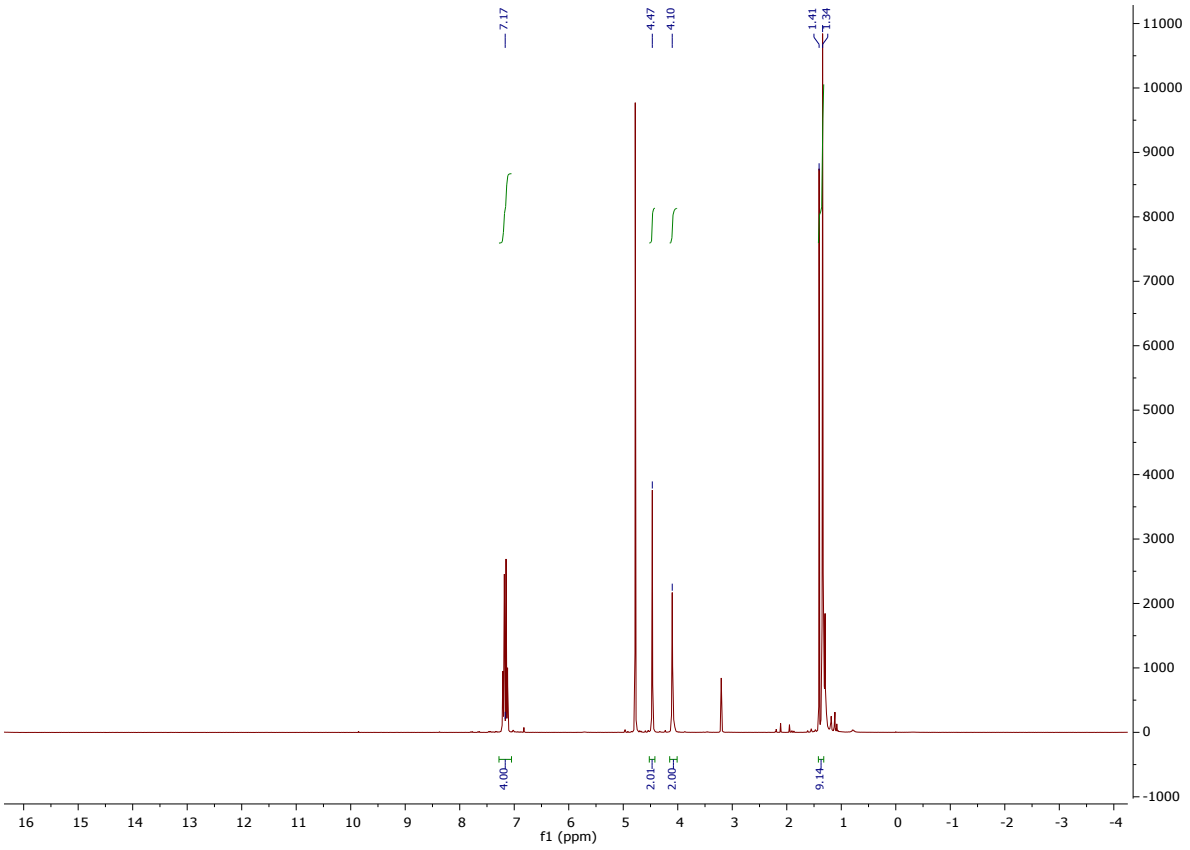
MS(ESI-pos): expected mass for $C_{31}H_{40}N_7O_7S^+$ = 654.2704 $[M]^+$; found: 654.2703.

NMR spectra of novel compounds

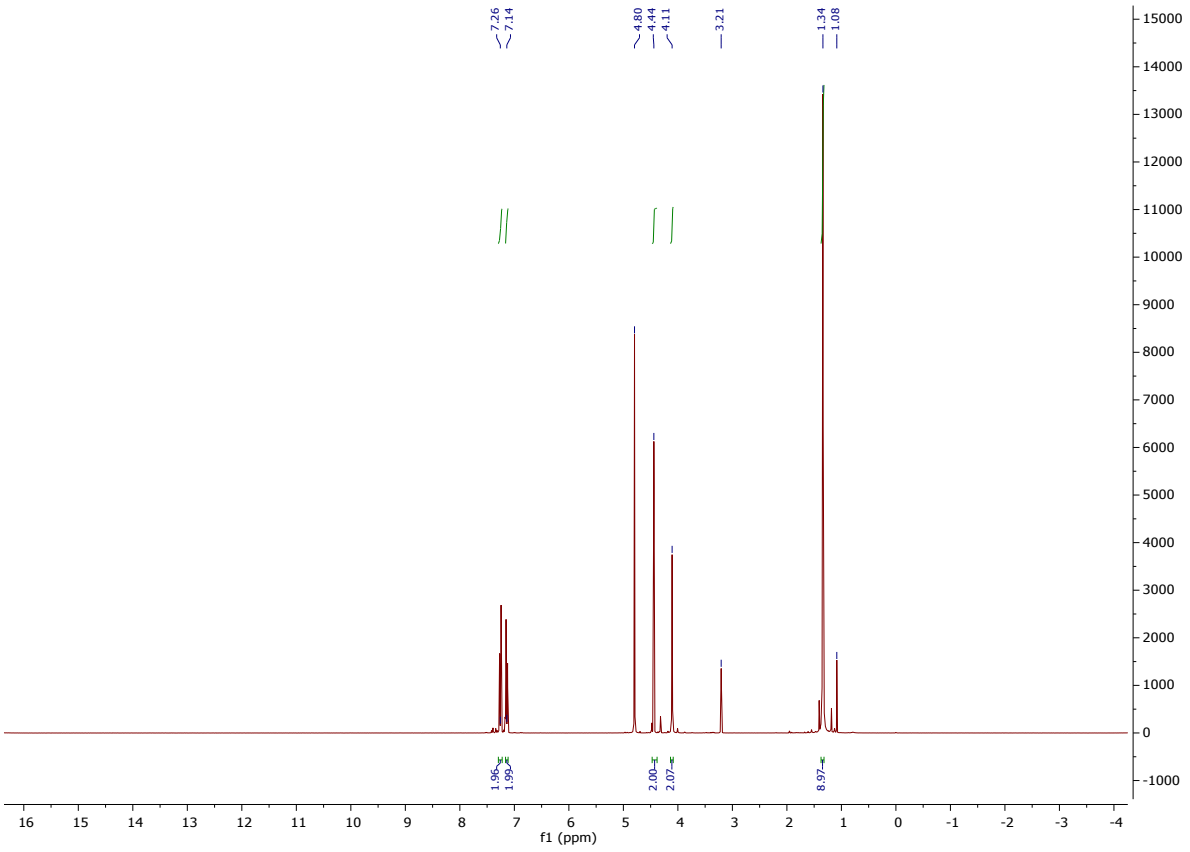
^{1}H-NMR spectrum of (4-aminomethyl)phenylmethanol (3)



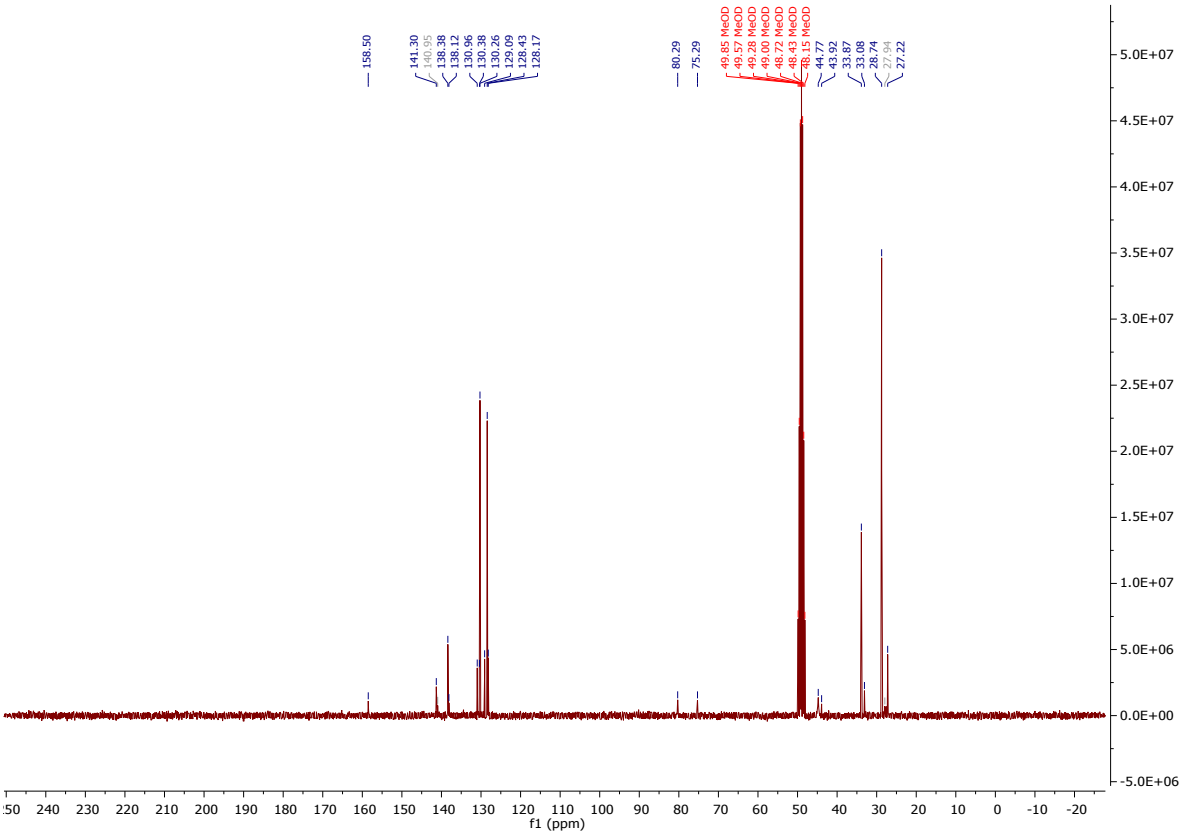
^{1}H-NMR spectrum of *tert*-butyl(4-(hydroxymethyl)benzyl)carbamate (5a)



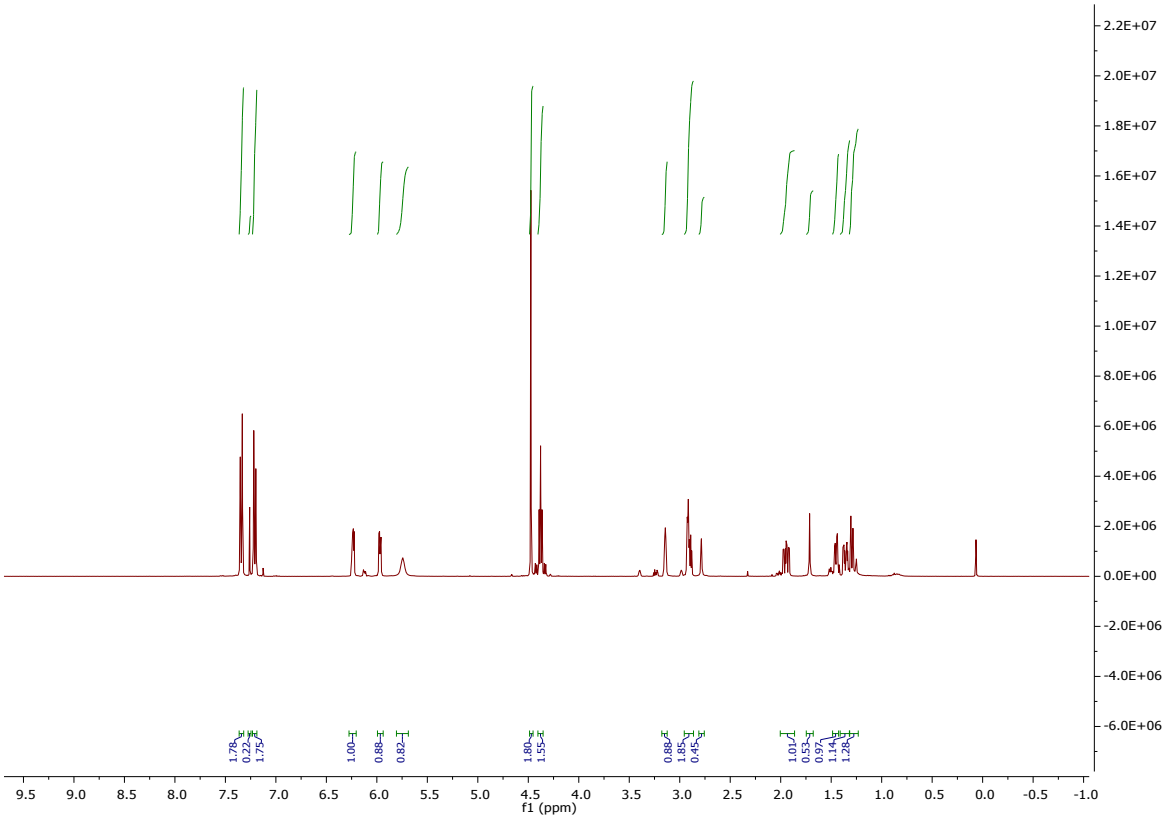
^{1}H-NMR spectrum of *tert*-butyl(4-(bromomethyl)benzyl)carbamate (6a)



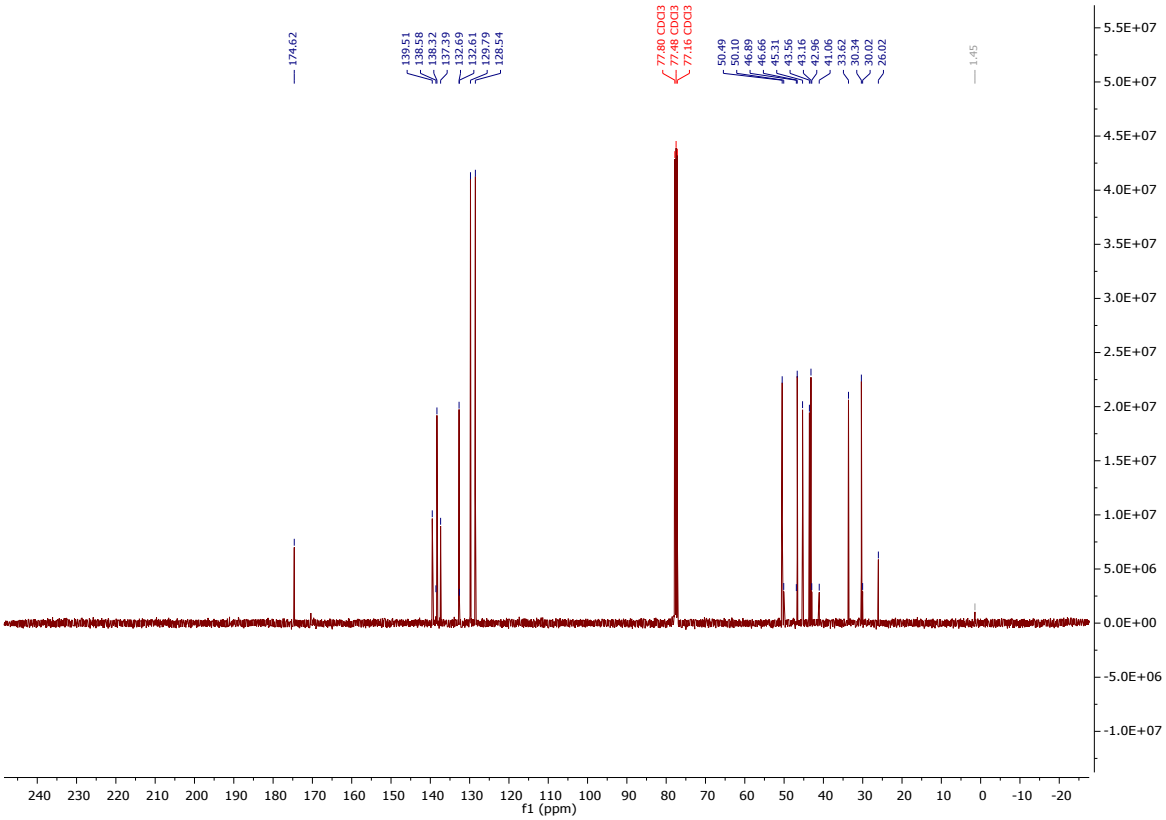
^{13}C-NMR spectrum of *tert*-butyl(4-(bromomethyl)benzyl)carbamate (6a)



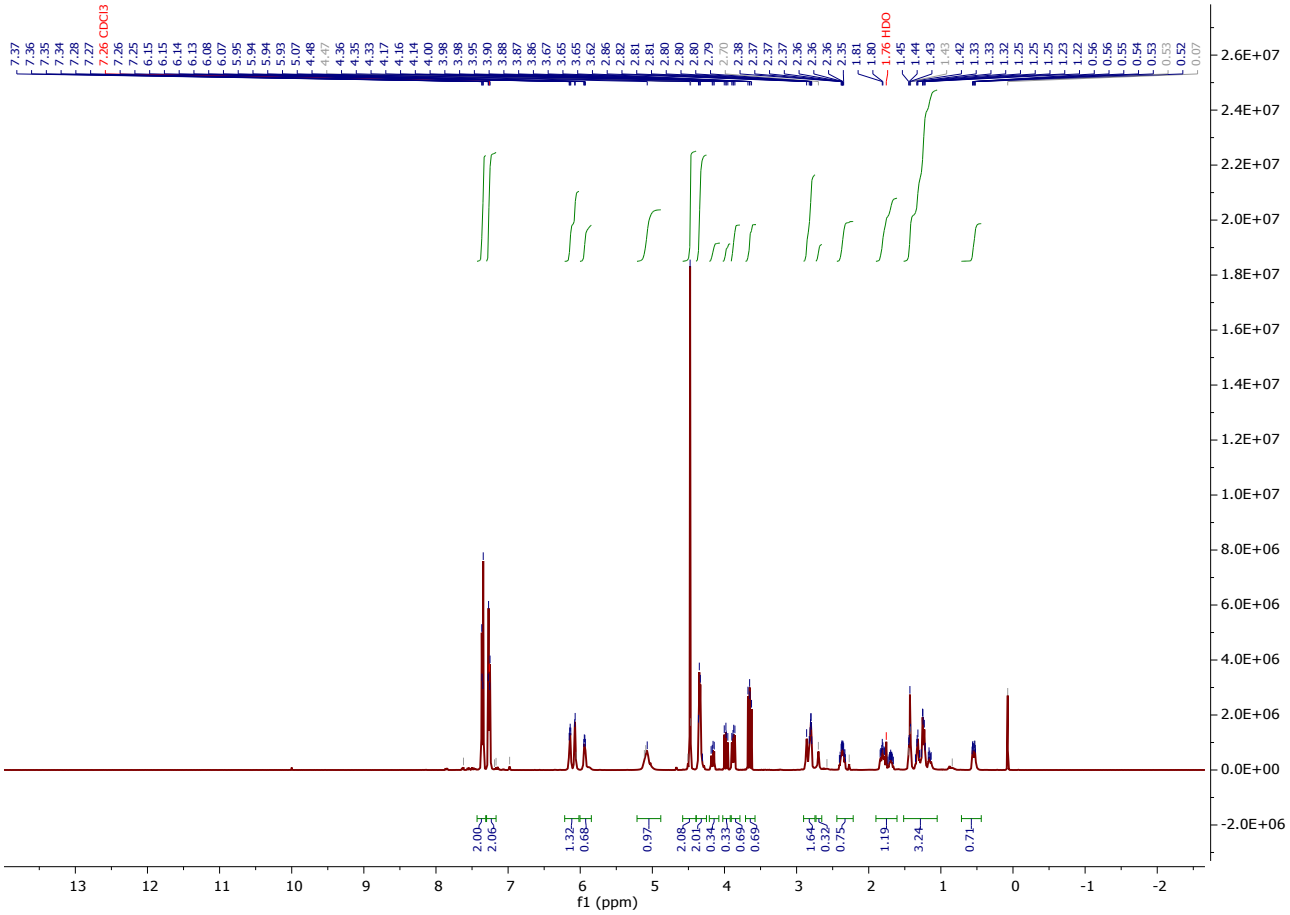
^{1}H-NMR spectrum of *N*-(4-(bromomethyl)benzyl)bicyclo[2.2.1]hept-5-ene-2-carboxamide (6b)



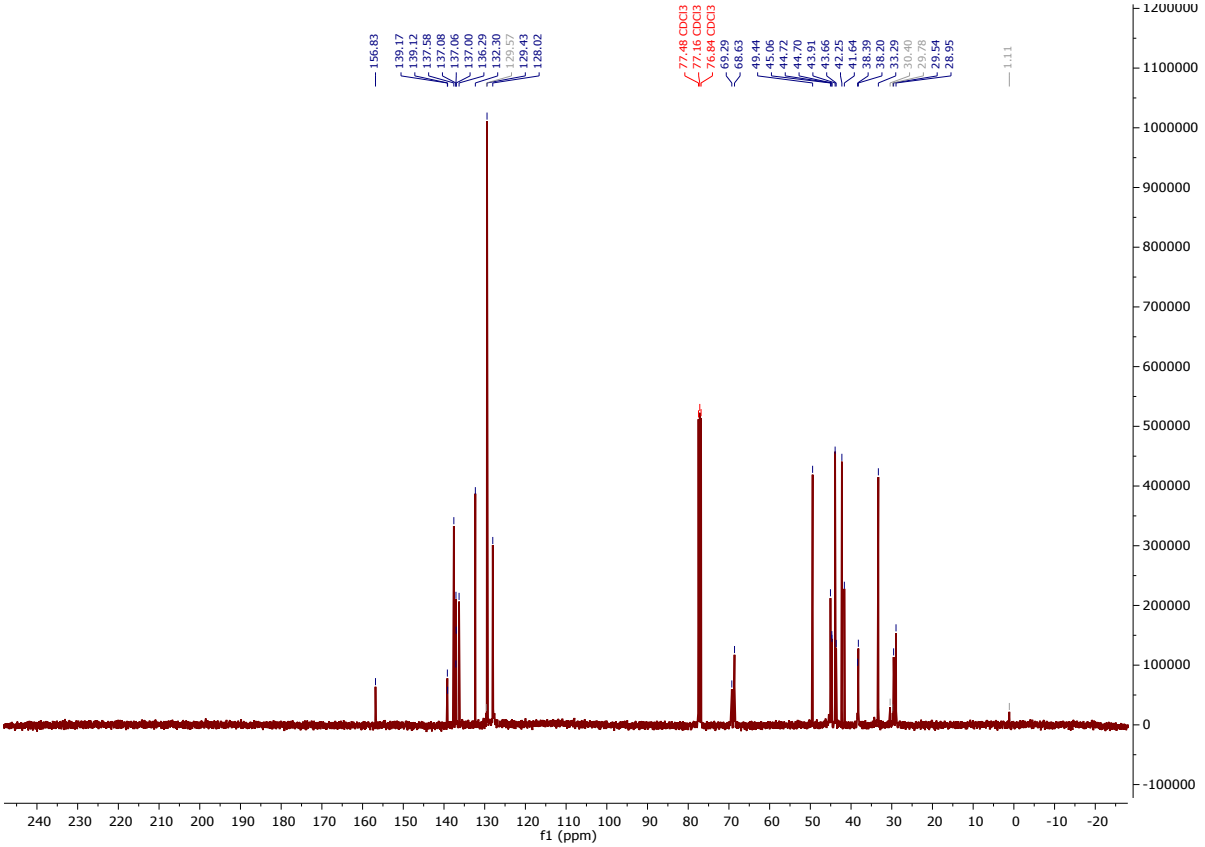
^{13}C-NMR spectrum of *N*-(4-(bromomethyl)benzyl)bicyclo[2.2.1]hept-5-ene-2-carboxamide (6b)



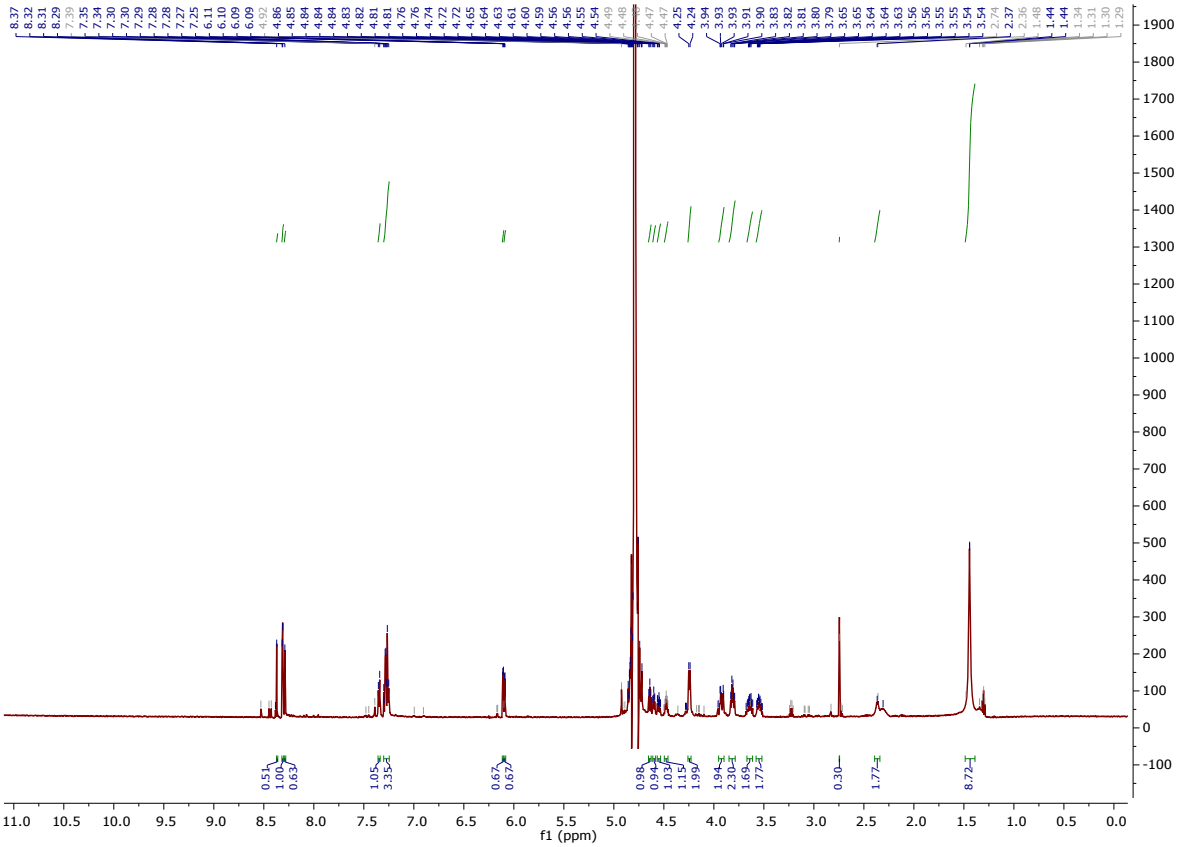
^{1}H-NMR spectrum of bicyclo[2.2.1]hept-5-en-2-yl)methyl (4-(bromomethyl)benzyl)carbamate (6c)



^{13}C-NMR spectrum of bicyclo[2.2.1]hept-5-en-2-yl)methyl (4-(bromomethyl)benzyl)carbamate (6c)



^{1}H-NMR spectrum of 5'-[(*R/S*)(3*S*)-3-amino-3-carboxypropyl]- 4-[((*tert*-butoxycarbonyl)ami-no)-methyl)benzyl-sulfonio]-5'-deoxyadenosine (1a)



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