Electronic Supplementary Material (ESI) for Chemical Communications.

SUPPORTING INFORMATION

Reversible Modification of DNA by Methyltransferase-catalyzed Transfer and Light-triggered Removal of Photo-caging Groups

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Supporting Information

General Information

All chemicals were purchased from *Sigma Aldrich*, *Alfa Aesar* or *TC*I unless otherwise noted. The plasmid pBR322 was purchased from *NEB*. The HPLC grade acetonitrile was purchased from *VWR*. All components were used without further purification.

^{1}H and ^{13}C NMR spectra were measured at 299 K on a Bruker AV-300, AV-400 MHz or on a Varian VNMR 500 MHz spectrometer. The chemical shifts (δ) were reported in ppm relative to deuterated solvents as internal standard (δ_H: $CDCl_3$ 7.26 ppm and δ_C: 77.13 ppm). Multiplicities are indicated as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet) and m (multiplet).

High-resolution mass spectra were recorded on a *Thermo Scientific* Orbitrap LTQ XL or on a Bruker MicroTof instrument. GC-MS spectra were recorded on a *Shimadzu* QP5050/GC17A. LC-MS measurements were performed on a *Bruker* maXis II ultra-high resolution QTOF coupled to a *Thermo Scientific* UltiMate 3000® UHPLC.

Absorption measurements were recorded using an Eppendorf BioPhotometer and a TECAN Infinite M1000 PRO®.

HPLC analysis was performed on an Agilent 1260 Infinity HPLC equipped with a diode array detector (DAD) (190-640 nm) using a Nucleodur® C18 Pyramid reversed-phase column (5 µm, 125 x 10 mm, 4 mm ID) from Macherey-Nagel. Preparative HPLC purification was carried out on the same HPLC using a Nucleodur® C18 Pyramid reversed-phase column (5 µm, 125 x 10 mm, 10 mm ID).

UV irradiation experiments were conducted using a 365 nm UV LED LZ1-00UV00 (700 mA, 3.5 V) from *LED Engin*.

TaqI Methyltransferase (M. TaqI) and restriction enzyme Taq$^{\alpha}$I (R. TaqI) were purchased from *NEB*, FastDigest BamHI and FastAP from *Thermo Scientific*, Proteinase K from *Applichem* and nuclease P1 from *Sigma Aldrich*. MTAN and LuxS were recombinantly expressed and purified as previously described.[1]

Purification of AdoMet analogs

Purification of all AdoMet analogs was performed using a semi-preparative reversed-phase HPLC on a NUCLEODUR© C18 Pyramid (5 µm, 125×10 mm) column from *Macherey-Nagel*. Elution was performed at a flow rate of 5 mL/min applying a linear gradient of H_2O supplemented with 0.01% trifluoroacetic acid and acetonitrile supplemented with 0.01% trifluoroacetic acid. Product peaks were concentrated by lyophilization and the products were redissolved in water (40 µL). The respective aliquots were stored at -20 °C. Concentrations were determined by UV absorption analysis with ε_{260} = 15.400 $L \cdot mol^{-1} \cdot cm^{-1}$. For HPLC analysis, a NUCLEODUR® C18 Pyramid (5 µm, 125×4 mm) column from *Macherey-Nagel* was used. Elution was performed at a flow rate of 1 mL/min applying a linear gradient of buffer A (50 mM ammonium acetate, pH = 6.0) and buffer B (50% buffer A, 50% acetonitrile).

Enzymatic plasmid DNA modification and subsequent UV irradiation

The plasmid pBR322 (0.5 µg) was incubated with AdoMet (0.8 mM) or the respective AdoMet analog (1.0 – 3.0 mM), M. TaqI (20 U), MTAN (4 µM), LuxS (4 µM) in 1x CutSmart buffer (NEB) in a total volume of 10 µL for 2 h at 60 °C. For photo-cleavage, the respective samples were irradiated with UV light (365 nm) for 10 min or 30 min. Afterwards, 15 µL of 1x CutSmart buffer and R. TaqI (5 U) were added to the reactions and the mixture was incubated for 45 min at 65 °C. Plasmid DNA was linearized by incubation with BamHI for 45 min at 37 °C and deactivated for 5 min at 80 °C. Samples were directly loaded onto a 1% agarose gel (100 V, 1 h). The gel was stained with ethidium bromide and scanned using a Typhoon FLA9500 laser scanner (GE healthcare).

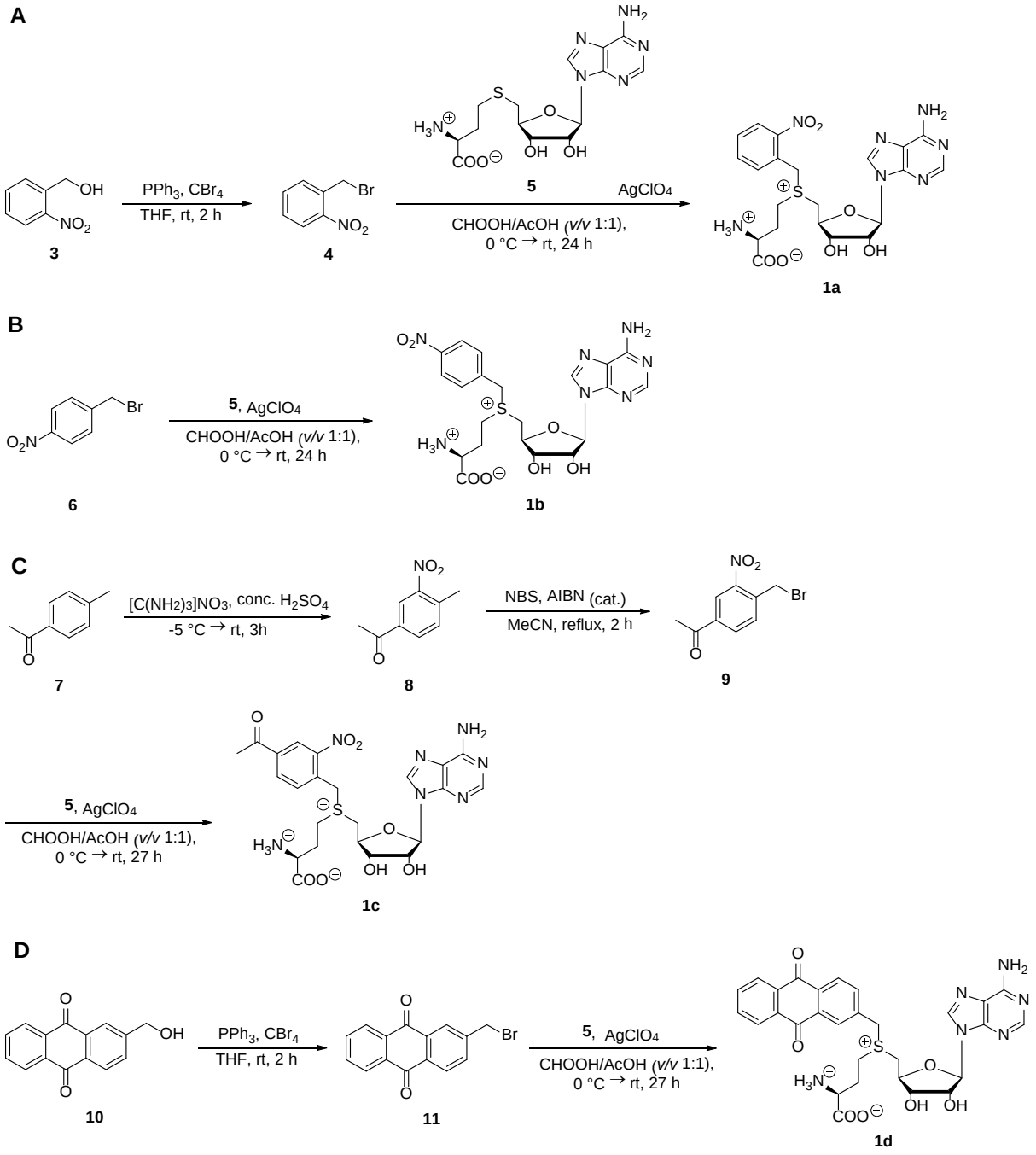
Enzymatic modification of short DNA and subsequent digestion for mass analysis

A short double stranded DNA (500 pmol) was incubated with the respective AdoMet analog (2 - 4 mM), M. TaqI (30 U), MTAN (4 µM) and LuxS (4 µM) in 1x CutSmart buffer (NEB) in a total volume of 20 µL for 3 h at 37 °C. Proteinase K (0.6 µU) was added to the reactions followed by incubation for 1 h at 55 °C. Then, nuclease P1 (0.33 U) and FastAP (1 U) were added to the reaction followed by incubation over night at 37 °C. Samples were directly used for LC-MS measurements.

Sequence of double stranded DNA (recognition sequence of M. TaqI is underlined):

5'…CTTGGAGCCACTATCGACTACGCGATCATGG…3'
3'…GAACCTCGGTGATAGCTGATGCGCTAGTACC…5'

Supplementary Figures



Scheme S1: Synthesis of AdoMet analogs **1a-d** modified with photo-cleavable groups. **4** and **11** were synthesized as described by Su *et al*. and **9** was synthesized as described by Kammari *et al*.[2, 3]

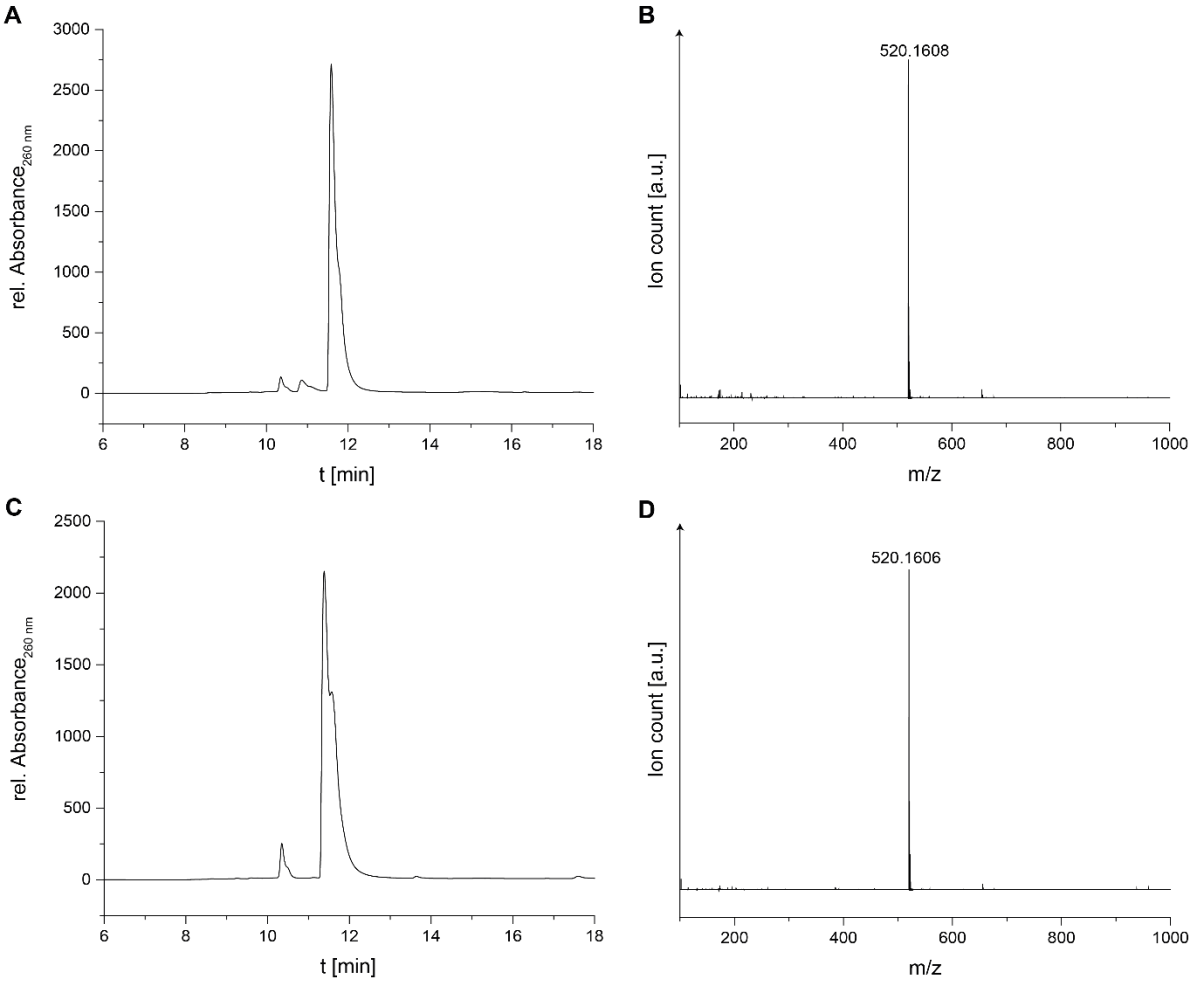


Figure S1: HPLC traces and mass spectrometric analysis of purified AdoMet analogs **1a** (**A**, **B**) and **1b** (**C**, **D**). (**B**) **1a**: Calculated mass of $[C_{21}H_{26}N_7O_7S]^+$ = 520.1609 $[M]^+$, found: 520.1608. (**D**) **1b**: Calculated mass of $[C_{21}H_{26}N_7O_7S]^+$ = 520.1609 $[M]^+$, found: 520.1606.

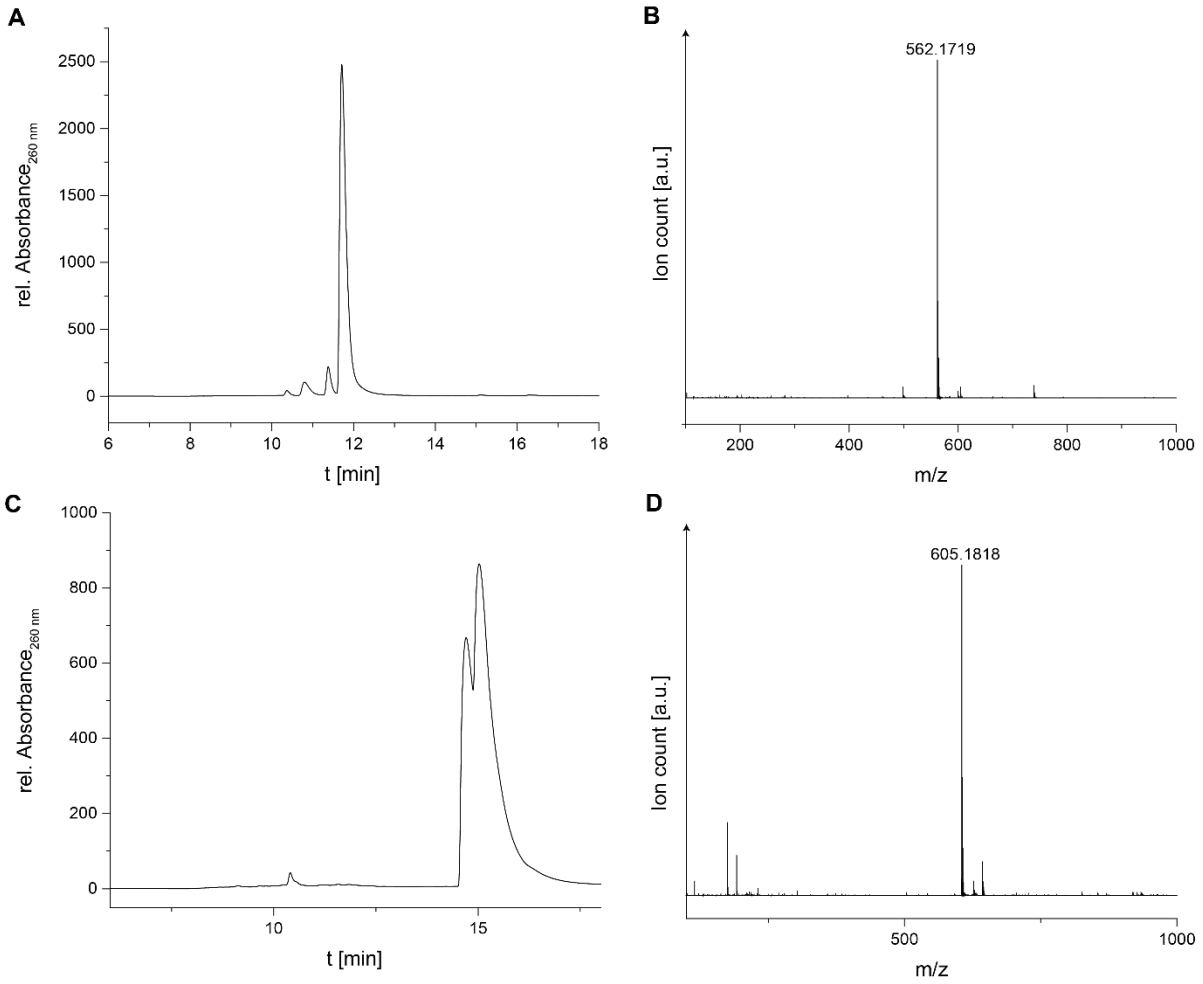


Figure S2: HPLC traces of and mass spectrometric analysis of purified AdoMet analogs **1c** (**A**, **B**) and **1d** (**C**, **D**). (**B**) **1c**: Calculated mass of $[C_{23}H_{28}N_7O_8S]^+$ = 562.1715 $[M]^+$, found: 562.1719. (**D**) **1d**: Calculated mass of $[C_{29}H_{29}N_6O_7S]^+$ = 605.1813 $[M]^+$, found: 605.1818.

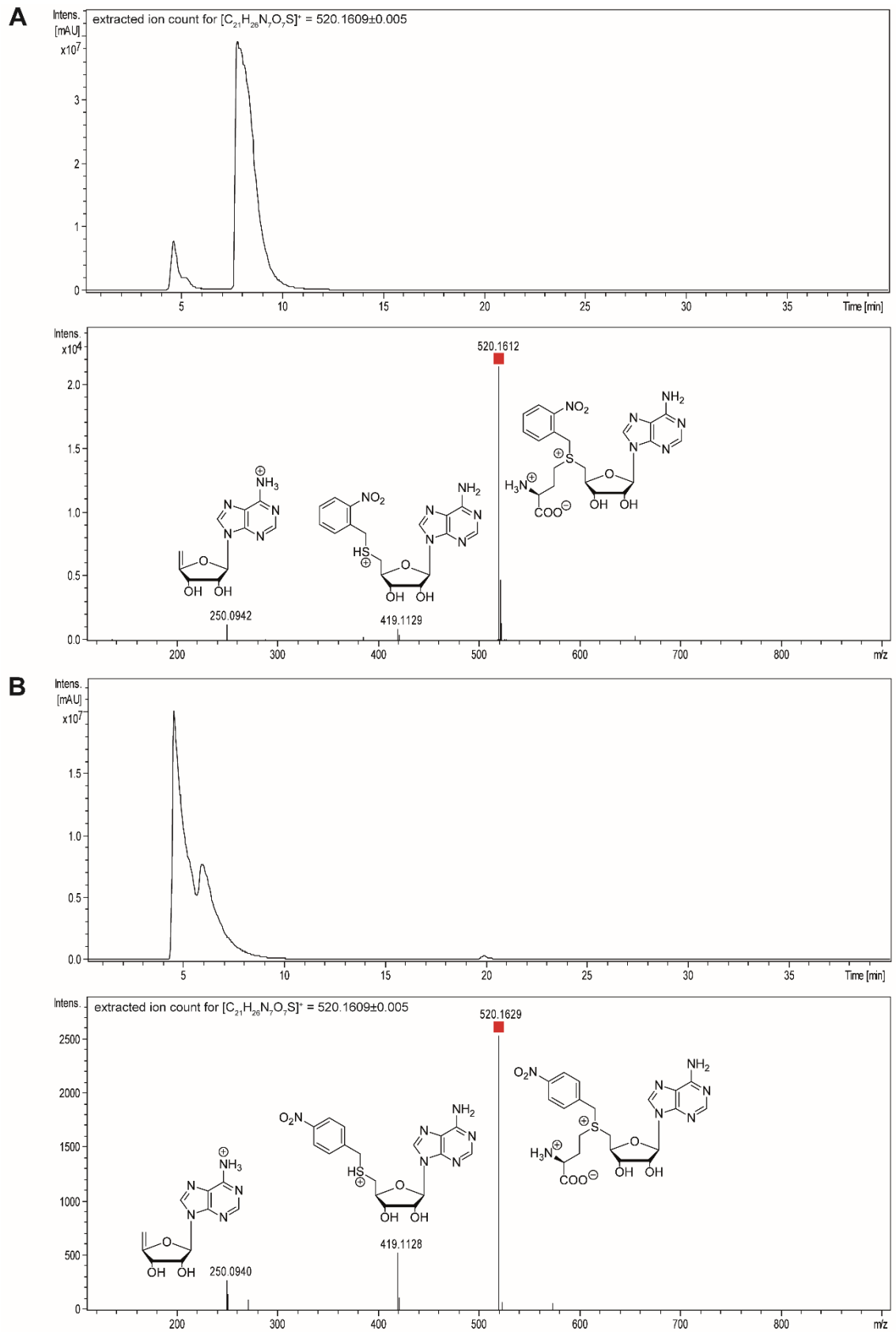


Figure S3: CID-MS/MS analysis of purified AdoMet analogs **1a** (**A**) and **1b** (**B**). Fragmentation patterns were obtained by low energy CID at 25 eV. Masses for the precursor ions are marked with a red square.

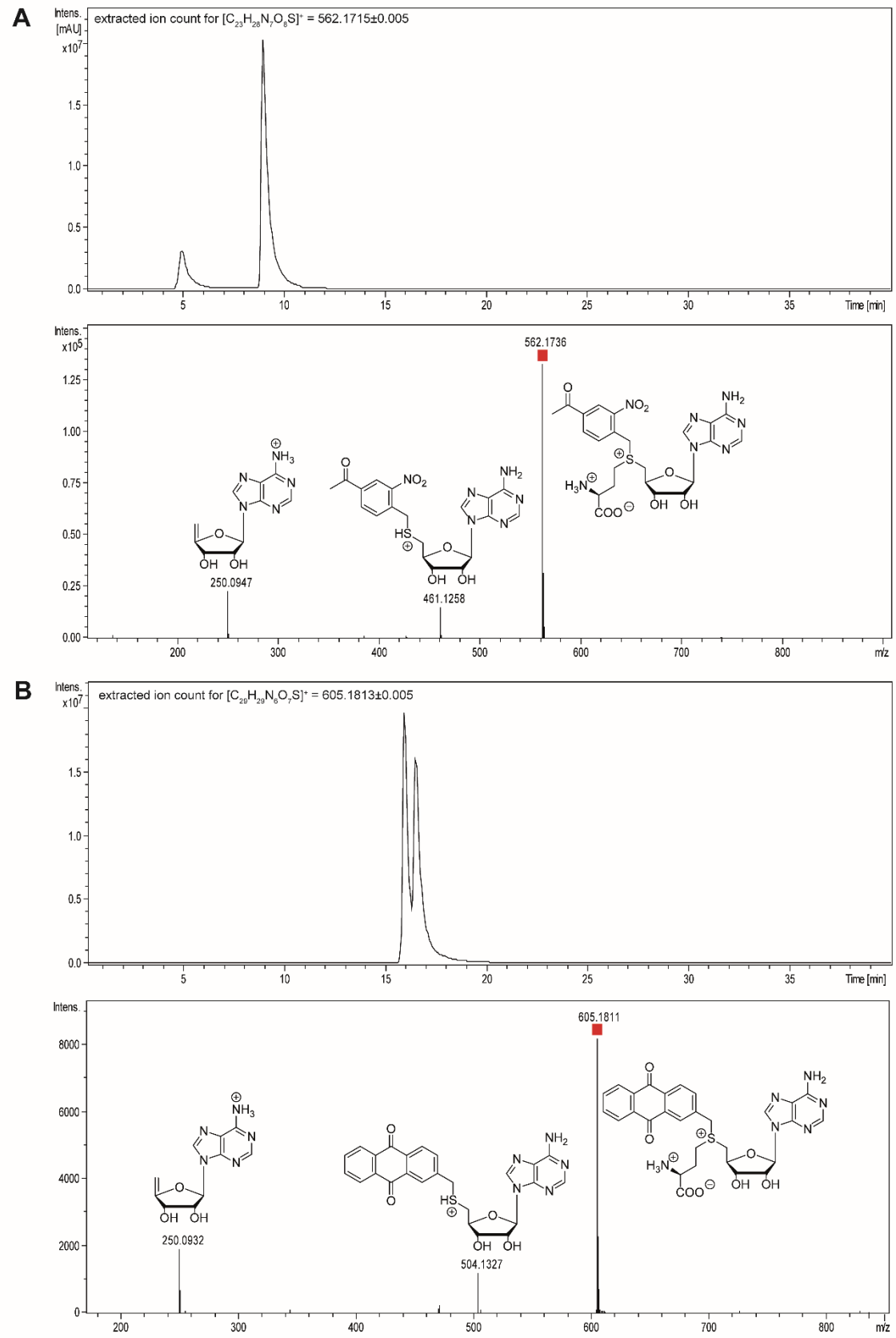


Figure S4: CID-MS/MS analysis of purified AdoMet analogs **1c** (**A**) and **1d** (**B**). Fragmentation patterns were obtained by low energy CID at 25 eV. Masses for the precursor ions are marked with a red square.

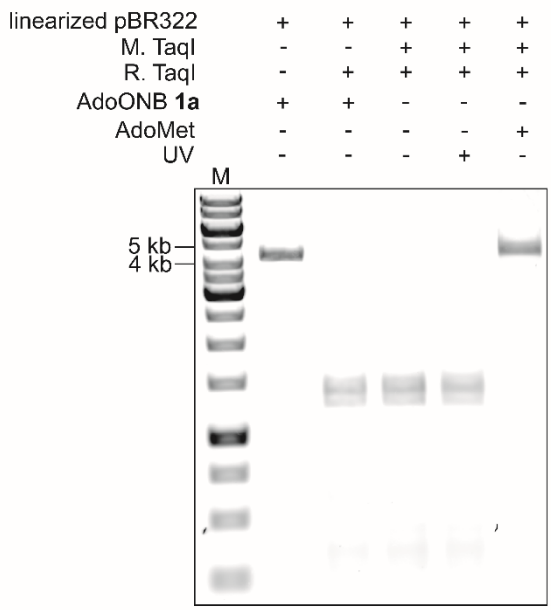


Figure S5: Controls for enzymatic plasmid DNA modification using M.TaqI. Analysis was performed by agarose gel electrophoresis (1% agarose gel, 100 V, 60 min) and staining using ethidium bromide. M: GeneRuler™ 1 kb DNA ladder (*Thermo Fisher Scientific*).

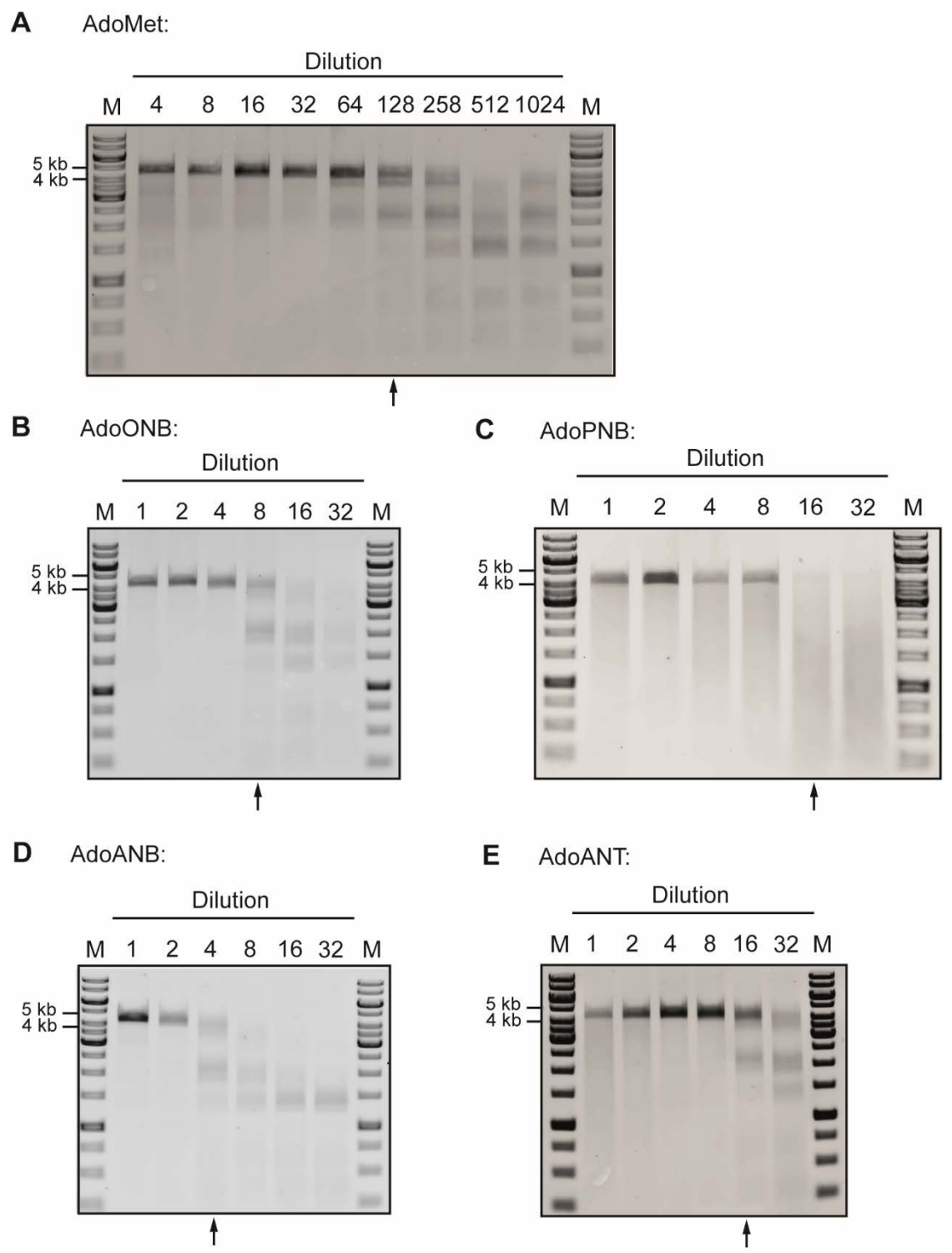


Figure S6: Comparison of modification efficiencies using M. TaqI and either AdoMet (**A**), **1a** (**B**), **1b** (**C**), **1c** (D) or **1d** (**E**) respectively. In dilution 1, the used enzyme concentration of M. TaqI corresponded to 40 U. Analysis was performed by agarose gel electrophoresis (1% agarose gel, 100 V, 60 min) and staining using ethidium bromide. Arrows indicate the highest concentration of M. TaqI which does not give complete protection of the plasmid DNA. M: GeneRuler™ 1 kb DNA ladder (*Thermo Fisher Scientific*).

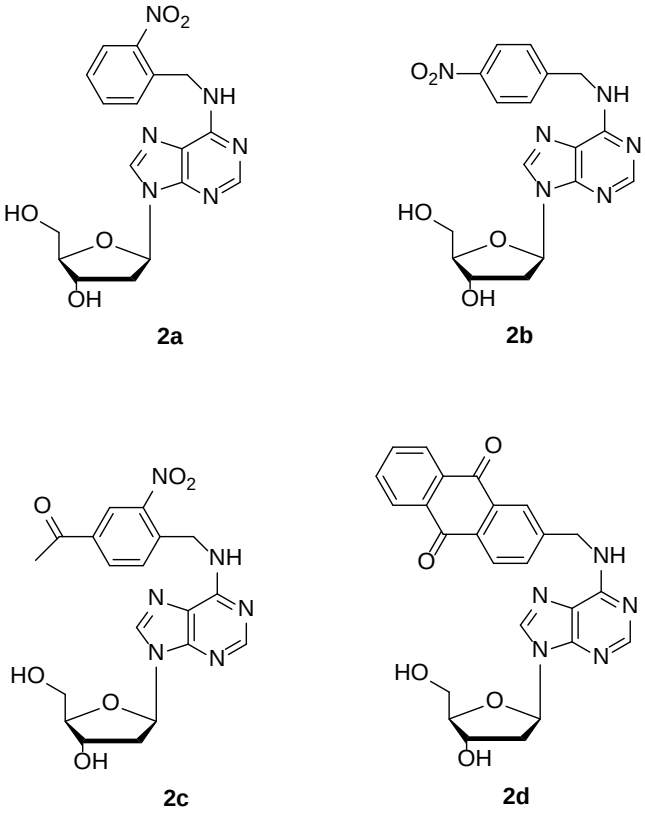


Figure S7: Resulting 2′-deoxyadenosines **2a-d** modified at the N^6 position with photo-cleavable groups.

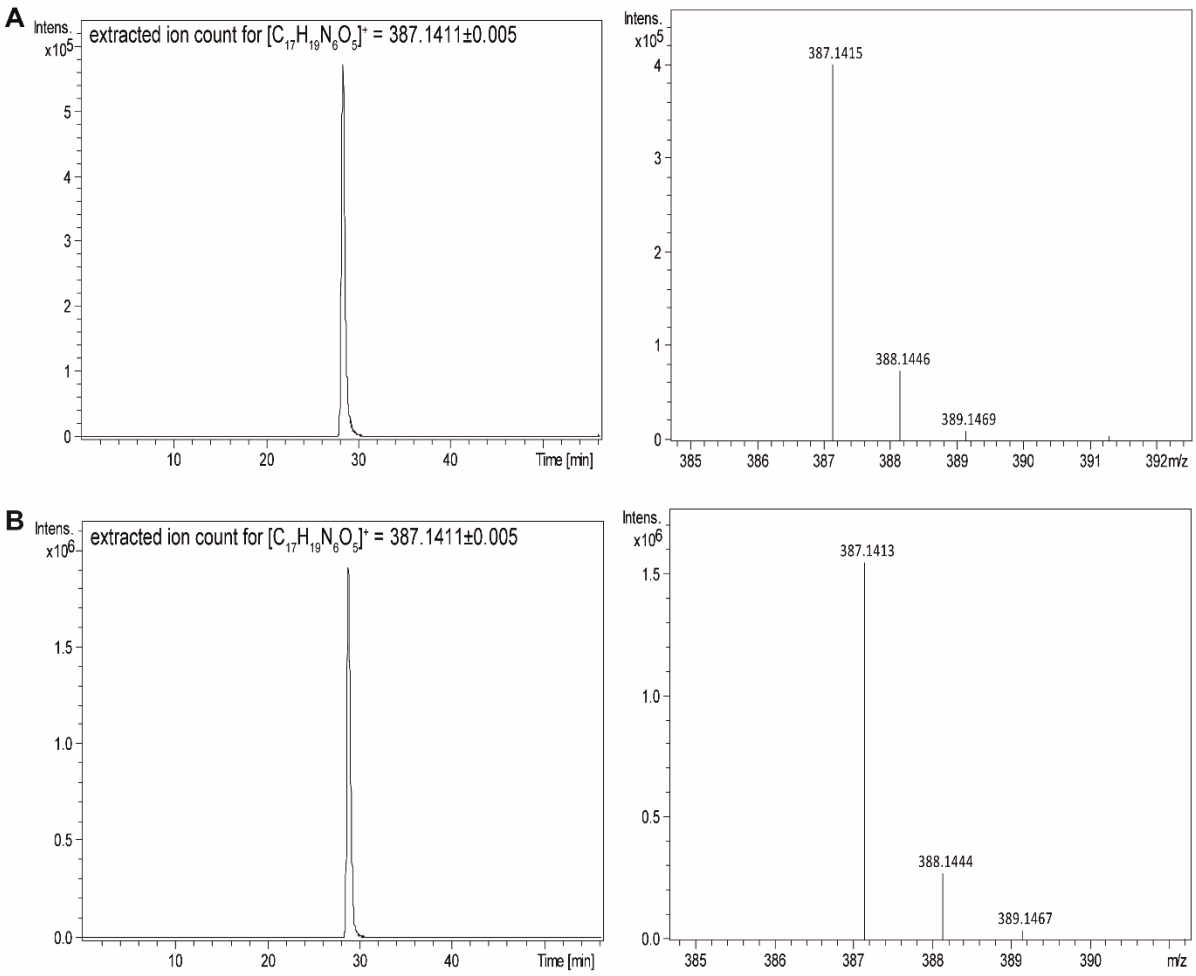


Figure S8: LC-MS analysis of 2′-deoxyadenosines **2a** and **2b** after modification of short double stranded DNA with M.Taq I, digestion, and dephosphorylation. (**A**) **2a**: Calculated mass of $[C_{17}H_{19}N_6O_5]^+$ = 387.1411 $[M+H]^+$, found 387.1415. (**B**) **2b**: Calculated mass of $[C_{17}H_{19}N_6O_5]^+$ = 387.1411 $[M+H]^+$, found 387.1413.

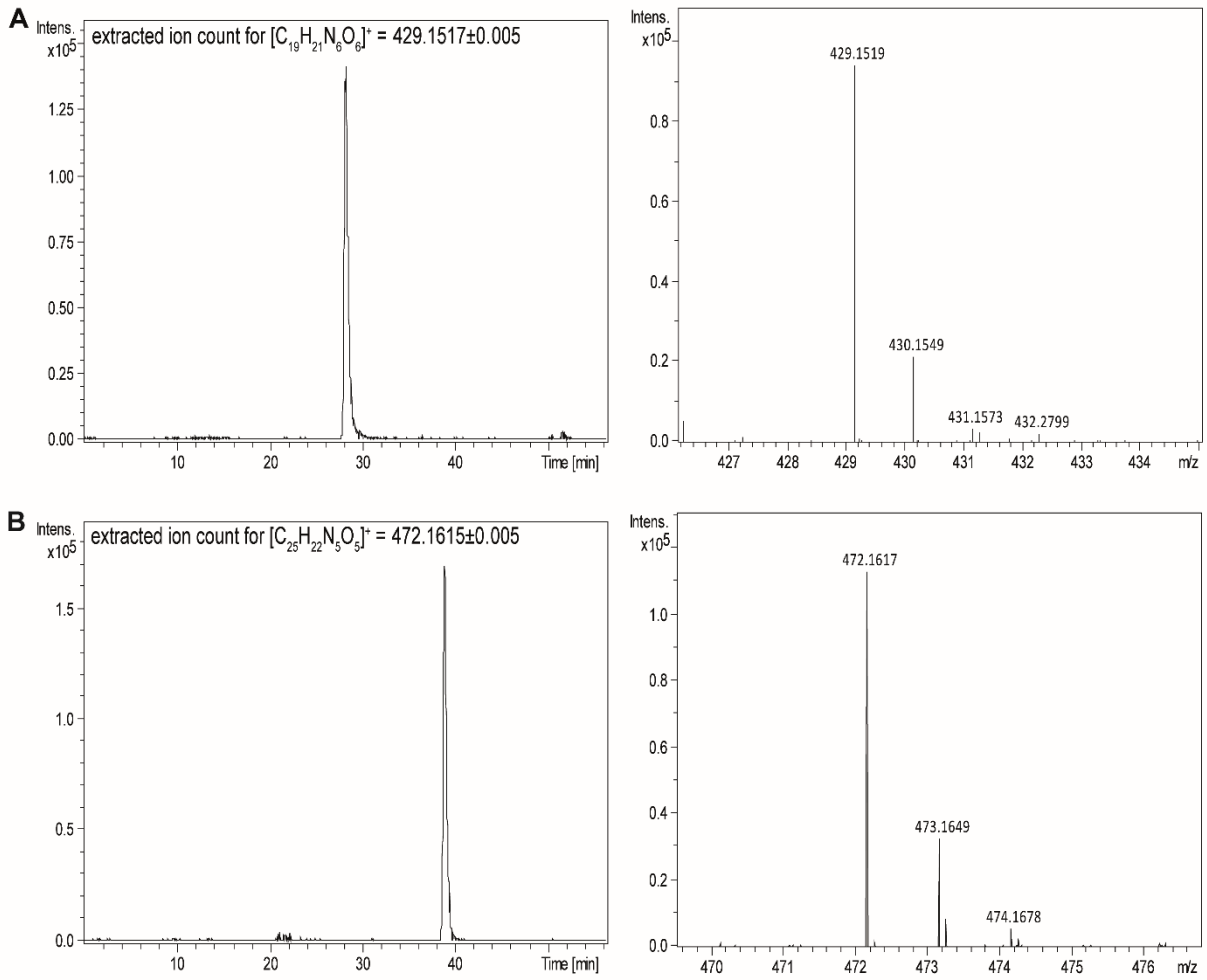
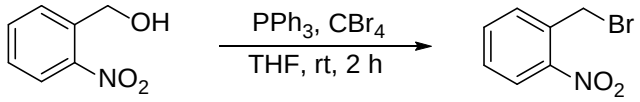


Figure S9: LC-MS analysis of 2′-deoxyadenosines **2c** and **2d** after modification of short double stranded DNA with M.Taq I, digestion, and dephosphorylation. (**A**) **2c**: Calculated mass of $[C_{19}H_{21}N_6O_6]^+$ = 429.1517 $[M+H]^+$, found 429.1519. (**B**) **2d**: Calculated mass of $[C_{25}H_{22}N_5O_5]^+$ = 472.1616 $[M+H]^+$, found 472.1617.

Chemical Synthesis

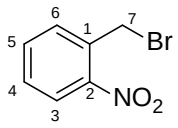
1-(1-Bromomethyl)-2-nitrobenzene (4)



3 **4**

Compound **4** was synthesized according to a procedure by Su *et al.*[2]

Under an atmosphere of argon, 1-(2-nitrophenyl)methanol (**3**) (383 mg, 2.50 mmol, 1.00 equiv), triphenylphosphine (984 mg, 3.75 mmol, 1.50 equiv) and tetrabromomethane (1.25 g, 3.75 mmol, 1.50 equiv) were dissolved in dry tetrahydrofurane (10 mL) and stirred for 2 h at room temperature. The solution was filtered and concentrated. The crude product was purified using flash column chromatography on silica gel (cyclohexane / ethyl acetate 4:1, R_f: 0.5) to give **4** (509 mg, 2.36 mmol, 94% yield) as a pale yellow solid.

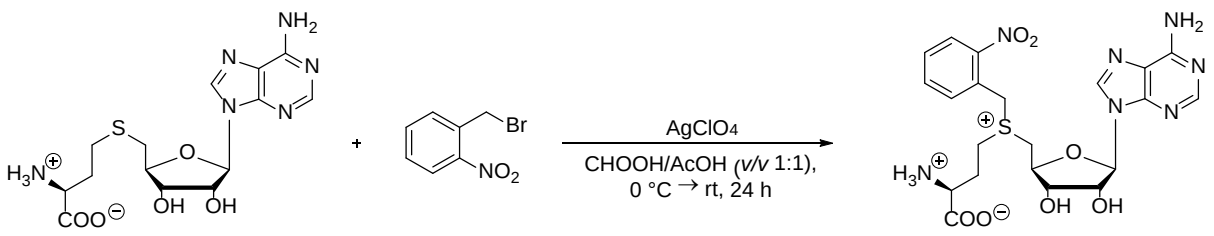


^{1}H-NMR (300 MHz, $CDCl_3$, 299 K): δ [ppm] = 8.05 (dd, 1H, H-3, $^3J_{3,4}$ = 8.0 Hz, $^3J_{3,5}$ = 1.3 Hz), 7.65-7.55 (m, 2H, H-4, H-6), 7.50 (1H, H-5, $^3J_{5,4}$ = 8.1 Hz, $^3J_{5,6}$ = 6.9 Hz, $^4J_{5,3}$ = 1.3 Hz), 4.84 (s, 2H, H 7).

^{13}C-NMR (75 MHz, $CDCl_3$, 299 K): δ [ppm] = 145.7 (C-2), 133.8 (C-5), 133.0 (C-1), 132.7 (C-6), 129.8 (C-4), 125.7 (C-3), 29.0 (C-7).

MS (EI, GC/MS): calculated mass of $[C_7H_6NO_2]^+$ = 136.04 $[M\text{-}Br]^+$; found: 136.1.

5'-((*R*/*S*)(3*S*)-3-Amino-3-carboxypropyl)-4-(2-nitrobenzylsulfonio)-5'-deoxyadenosine (AdoONB, 1a)



5 **4** **1a**

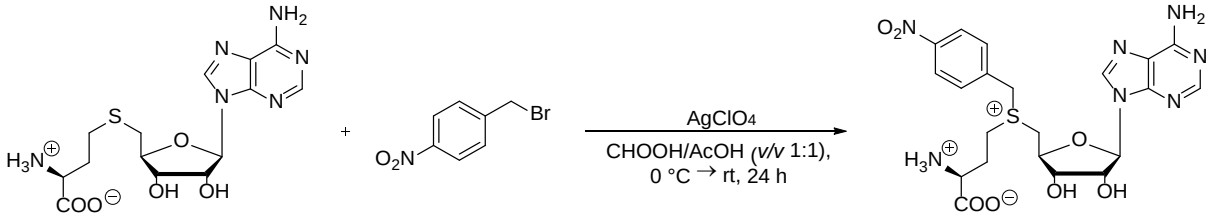
1-(1-Bromomethyl)-2-nitrobenzene (**4**) (170 mg, 0.787 mmol, 30.0 equiv) was dissolved in formic acid (750 µL) and acetic acid (750 µL) and *S*-adenosyl-L-homocysteine (**5**) (10.0 mg,

26.0 µmol, 1.00 equiv) and silver perchlorate (2.70 mg, 13.0 µmol, 0.50 equiv) were added at 0 °C. The reaction mixture was stirred for 24 h at room temperature, diluted with water (3 mL) and extracted with diethyl ether (15 mL). The aqueous phase was lyophilized, redissolved in water (1.5 mL) supplemented with 0.01% trifluoroacetic acid and purified by semi-preparative HPLC. Purity and identity of the AdoMet analog was confirmed by HPLC and mass spectrometric analysis. The product **1a** was obtained as a mixture of *S*- and *R*-epimers and stored at -20 °C for further use.

MS (ESI-pos): calculated mass of $[C_{21}H_{26}N_7O_7S]^+$ = 520.1609 $[M]^+$; found: 520.1608.

Isolated yield: 5 % (determined from concentrations by UV absorption).

5'-((*R*/*S*)(3*S*)-3-Amino-3-carboxypropyl)-4-(4-nitrobenzylsulfonio)-5'-deoxyadenosine (AdoPNB, 1b)



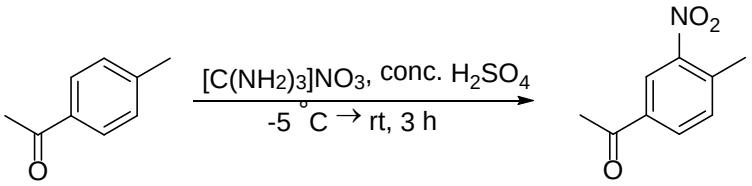
5 **6** **1b**

1-(1-Bromomethyl)-4-nitrobenzene (**6**) (100 mg, 0.463 mmol, 35.0 equiv) was dissolved in formic acid (750 µL) and acetic acid (750 µL) and *S*-adenosyl-L-homocysteine (**5**) (5.00 mg, 13 µmol, 1.00 equiv) and silver perchlorate (2.7 mg, 13 µmol, 1.00 equiv) were added at 0 °C. The reaction mixture was stirred for 24 h at room temperature, diluted with water (3 mL) and extracted with diethyl ether (15 mL). The aqueous phase was lyophilized, redissolved in water (1.5 mL) supplemented with 0.01% trifluoroacetic acid and purified by semi-preparative HPLC. Purity and identity of the AdoMet analog was confirmed by HPLC and mass spectrometric analysis. The product **1b** was obtained as a mixture of *S*- and *R*-epimers and stored at -20 °C for further use.

MS (ESI-pos): calculated mass of $[C_{21}H_{26}N_7O_7S]^+$ = 520.1609 $[M]^+$; found: 520.1606.

Isolated yield: 9 % (determined from concentrations by UV absorption).

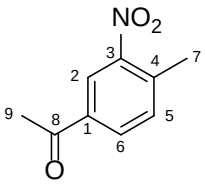
1-(4-Methyl-3-nitrophenyl)ethanone (8)



7 **8**

Compound **8** was synthesized according to a procedure by Kammari *et al*.[3]

Guanidinium nitrate (2.27 g, 18.6 mmol, 1.00 equiv) was slowly added to a mixture of concentrated sulfuric acid (35 mL) and 4-methylacetophenone (**7**) (2.50 g, 18.6 mmol, 1.00 equiv) added at -5 ºC. The mixture was stirred at room temperature for 3 h and subsequently poured into 100 mL of crushed ice. The resulting crystalline product was filtered off, washed thoroughly with water (2 x 75 mL) and concentrated to give **8** (2.82 g, 15.7 mmol, 85% yield) as a pale yellow solid.

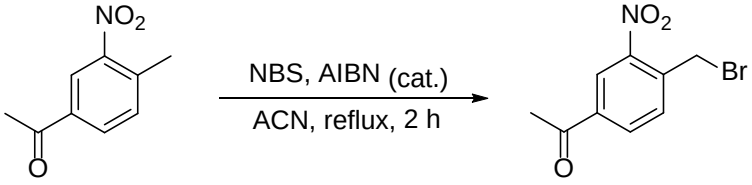


^{1}H-NMR (300 MHz, $CDCl_3$, 299 K): δ [ppm] = 8.51 (dd, 1H, H-2, $^{4}J_{2,6}$ = 1.8 Hz), 8.07 (dd, 1H, H-6, $^{3}J_{6,5}$ = 8.0 Hz, $^{4}J_{6,2}$ = 1.8 Hz), 7.60 (d, 1H, H-5, $^{3}J_{5,6}$ = 8.0 Hz), 2.66 (s, 3H, H-9), 2.64 (s, 3H, H-7).

^{13}C-NMR (125 MHz, $CDCl_3$, 299 K): δ [ppm] = 195.8 (C-8), 149.4 (C-3), 138.8 (C-4), 136.2 (C-1), 133.5 (C-7), 132.1 (C-6), 124.8 (C-2), 26.7 (C-9), 20.8 (C-5).

MS (ESI-pos): calculated mass of $C_9H_9NNaO_3^+$ = 202.0475 $[M+Na]^+$; found: 202.0485.

1-(4-Bromomethyl-3-nitrophenyl)ethanone (9)

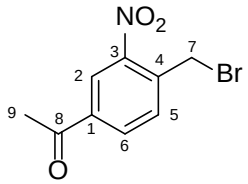


8 **9**

Compound **9** was synthesized according to a procedure by Kammari *et al*.[3]

N-bromosuccinimide (NBS, 1.17 g, 6.58 mmol, 1.18 equiv) and azobisisobutyronitrile (AIBN, 91.6 mg, 0.558 mmol, 0.10 equiv) were added to a solution of **8** (1.00 g, 5.58 mmol, 1.00 equiv)

in acetonitrile (20 mL). The mixture was refluxed for 2 h and then cooled to room temperature and concentrated. The crude product was dissolved in toluene (20 mL) and the resulting precipitate was filtered off. The filtrate was dried over anhydrous magnesium sulfate and concentrated. The crude product was purified by flash column chromatography on silica gel (cyclohexane / ethyl acetate 2:1, R_f: 0.5) to give **9** (601 mg, 3.35 mmol, 60% yield) as a pale yellow solid.



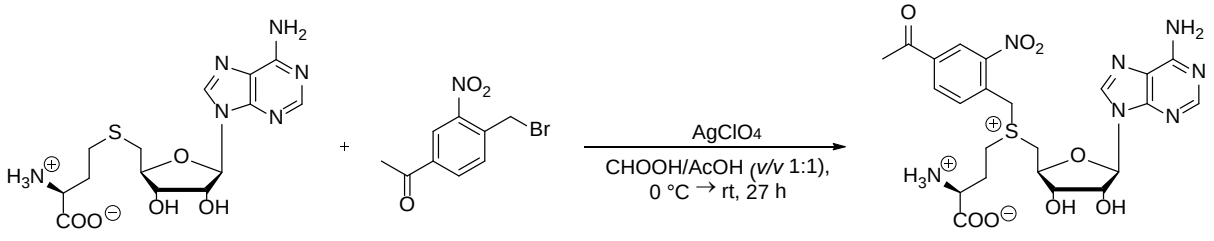
^{1}H-NMR (400 MHz, $CDCl_3$, 299 K): δ [ppm] = 8.56 (dd, 1H, H-2, $^4J_{2,6}$ = 1.8 Hz), 8.16 (dd, 1H, H-6, $^3J_{6,5}$ = 8.0 Hz, $^4J_{6,2}$ = 1.8 Hz), 7.70 (d, 1H, H-5, $^3J_{5,6}$ = 8.0 Hz), 4.84 (s, 3H, H-7), 2.66 (s, 3H, H-9).

^{13}C-NMR (100 MHz, $CDCl_3$, 299 K): δ [ppm] = 195.2 (C-8), 148.3 (C-3), 138.1 (C-4)^{I}, 137.3 (C-1)^{I}, 133.3 (C-6)^{II}, 132.8 (C-5)^{II}, 125.4 (C-2), 28.0 (C-7), 26.8 (C-9).

^{I,II} Specific assignments are given wherever possible.

MS (ESI-pos): calculated mass of $[C_9H_7NBrNaO_3]^+$ = 279.9580 $[M+Na]^+$; found: 279.9588.

5'-((*R*/*S*)(3*S*)-3-Amino-3-carboxypropyl)-4-(4-acteyl-2-nitrobenzylsulfonio)-5'-deoxy-adenosine (AdoANB, 1c)



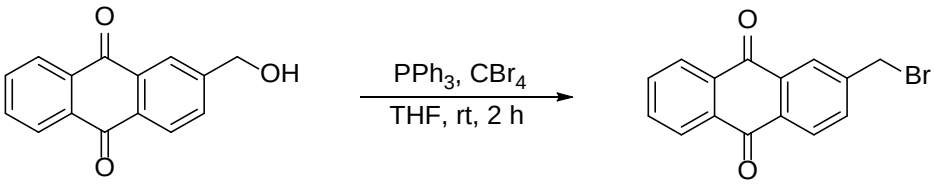
1-(4-Bromomethyl-3-nitrophenyl)ethanone (**9**) (150 mg, 581 µmol, 45.0 equiv) was dissolved in formic acid (750 µL) and acetic acid (750 µL) and *S*-adenosyl-L-homocysteine (**5**) (5.00 mg, 13 µmol, 1.00 equiv) and silver perchlorate (2.70 mg, 13 µmol, 1.00 equiv) were added at 0 °C. The reaction mixture was stirred for 27 h at room temperature, diluted with water (3 mL) and extracted with diethyl ether (15 mL). The aqueous phase was lyophilized, redissolved in water (1.5 mL) supplemented with 0.01% trifluoroacetic acid 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 a mixture of *S*- and *R*-epimers and stored at -20 °C for further use.

MS (ESI-pos): calculated mass of $[C_{23}H_{28}N_7O_8S]^+$ = 562.1715 $[M]^+$; found: 562.1719.

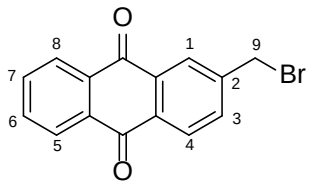
Isolated yield: 7 % (determined from concentrations by UV absorption).

2-(Bromomethyl)anthracene-9,10-dione (11)



10 **11**

Compound **11** was synthesized from **10** as described for compound **4**. Purification was performed by flash column chromatography on silica gel (DCM, R_f = 0.64) to give **11** (158 mg, 0.526 mmol, yield: 79 %) as a pale yellow solid.



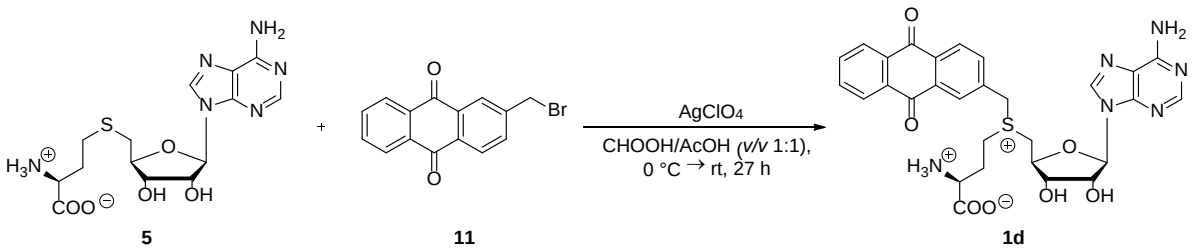
^{1}H-NMR (500 MHz, CDCl3, 299 K): δ [ppm] = 8.29-8.25 (m, 4H, H-5, H-6, H-7, H-8), 7.85-7.79 (m, 3H, H-1, H-3, H-4), 4.59 (s, 2H, H-9).

^{13}C-NMR (125 MHz, $CDCl_3$, 299 K): δ [ppm] = 162.8 (CCO), 162.7 (CCO), 144.3 (C-2), 134.7 (C-3), 134.4 (C-6)^{I}, 134.4 (C-7)^{I}, 134.0 (Cq), 133.6 (Cq), 133.6 (Cq), 133.3 (Cq), 128.2 (C-1)^{II}, 127.7 (C-4)^{II}, 127.5 (C-5)^{III}, 127.5 (C-8)^{III}, 31.6 (C-9).

$^{I, II, III}$ Specific assignments are given wherever possible.

MS (ESI-pos): calculated mass of $[C_{15}H_9BrNaO_2]^+$ = 322.9678 $[M+Na]^+$; found: 322.9683.

5'-((*R*/*S*)(3*S*)-3-Amino-3-carboxypropyl)-4-(9,10-dioxo-9,10-dihydroanthracen-2-yl-sulfonio)-5'-deoxy-adenosine (AdoANT, 1d)



5 **11** **1d**

2-(Bromomethyl)anthracene-9,10-dione (**11**) (100 mg, 0.332 mmol., 25.5 equiv) was dissolved in tetrahydrofurane (5 mL), formic acid (1.25 mL) and acetic acid (1.25 mL) and *S*-adenosyl-L-homocysteine (**5**) (5.00 mg, 0.013 mmol, 1.00 equiv) and silver perchlorate (2.70 mg, 13 µmol, 1.00 equiv) were added at 0 °C. The reaction mixture was stirred for 27 h at room temperature, diluted with water (3 mL) and extracted with diethyl ether (15 mL). The aqueous phase was lyophilized, redissolved in water (1.5 mL) supplemented with 0.01% trifluoroacetic acid 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 a mixture of *S*- and *R*-epimers and stored at -20 °C for further use.

MS (ESI-pos): calculated mass of $[C_{29}H_{29}N_6O_7S]^+$ = 605.1813 $[M]^+$; found: 605.1818.

Isolated yield: 6 % (determined from concentrations by UV absorption).

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