Electronic Supplementary Information

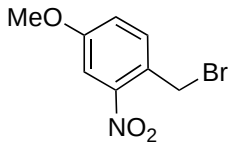
Content

General Methods

All chemicals and solvents were obtained from commercial suppliers (Sigma-Aldrich; VWR) and used without further purification. HPLC analysis and purifications were carried out using a High Performance Liquid Chromatograph/Mass Spectrometer LCMS-2020 (ESI, operating both in positive and negative mode) equipped with a SPD-M20A Prominence Photo Diode Array Detector in preparative mode and a SPD-20A Prominence Dual Wavelength UV Detector in analytical mode, all from Shimadzu. For analytical analyses the solvent delivery module LC-20AD was used; for preparative purifications two LC-20AP units were used. Analytical separations were performed on A Waters XBridge BEH300 C_{18} column (10 µm, 4.6 x 250 mm). Preparative separations were performed on a Waters XBridge BEH300 Prep C_{18} column (5 µm, 19 x 150 mm) at a flow rate of 20 mL/min. The solvents used were water + 0.1% formic acid (solvent A) and HPLC-grade acetonitrile + 0.1% formic acid (solvent B). NMR spectra were recorded at the University of Heidelberg on a Varian 500 MHz NMR spectrometer. High-resolution mass spectra were obtained on a Bruker Maxis EDT II ultra-high resolution time-of-flight (TOF) mass spectrometer. UHPLC-MS measurements were performed on a Shimadzu Nexera X2 UHPLC LCMS 8050 (triple quadrupole ESI, operating both in positive and negative mode) using an Acquity UHPLC peptide CSH C_{18} column (130 Å, 1.7 µm, 2.1 x 100 mm). Irradiation experiments were performed at λ = 365 nm using a UV hand lamp (VILBER VL-6.LC lamp, 6 W) or a LED (Thorlabs, LED M365L2-C1, 700 mA, 4,4V).

Synthesis of co-substrates 2 5

1-(Bromomethyl)-4-methoxy-2-nitrobenzene (8)

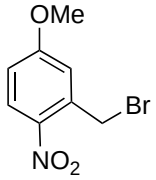


4-Methoxy-1-methyl-2-nitrobenzene (1) (2.50 g, 15.0 mmol, 1.00 equiv.) was suspended in tetrachloromethane (22.5 mL). *N*-Bromosuccinimide (3.99 g, 22.4 mmol, 1.5 equiv.) and benzoyl peroxide (36.2 mg, 150 µmol, 0.01 equiv.) were added to the solution. The reaction mixture was heated to 90 °C and refluxed for 2.5 h. Next, the suspension was filtered and the filtrate was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The obtained crude product was purified via FCC (cyclohexane / acetone, 9:1) to yield 2 (2.00 g, 8.13 mmol, 54%) as a yellowish solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.56 (d, J = 2.7 Hz, 1H, H3), 7.46 (d, J = 8.6 Hz, 1H, H6), 7.13 (dd, J = 8.6, 2.7 Hz, 1H, H5), 4.80 (s, 2H, CH_2), 3.89 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ =160.23, 133.74, 124.91, 120.09, 110.50, 56.13, 29.27.

2-(Bromomethyl)-4-methoxy-1-nitrobenzene (9)[1]

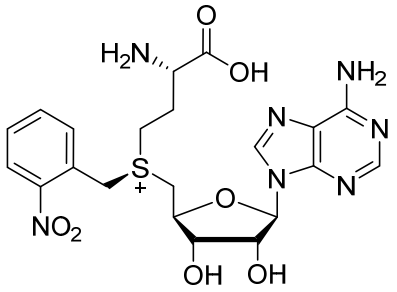


4-methoxy-2-methyl-1-nitrobenzene (3) (6.69 g, 40.0 mmol, 1.00 equiv.) was suspended in tetrachloromethane (60.0 mL). *N*-bromosuccinimide (10.68 g, 60.0 mmol, 1.5 equiv.) and benzoyl peroxide (96.9 mg, 400 µmol, 0.01 equiv.) were added to the solution. The reaction mixture was heated to 90 °C and refluxed for 6 h. Subsequently, the suspension was filtered and the filtrate was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The obtained crude product was purified via FCC (cyclohexane / acetone, 9:1) to yield 4 (1.39 g, 5.64 mmol, 14%) as a yellowish solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 8.14 (d, J = 9.1 Hz, 1H, H6), 7.02 (d, J = 2.8 Hz, 1H, H3), 6.92 (dd, J = 9.1, 2.8 Hz, 1H, H5), 4.86 (s, 2H, CH_2), 3.91 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): (ppm) = 163.57, 140.84, 135.82, 128.53, 117.73, 114.15, 56.17, 30.00.

AdoNB (2)[2,3]



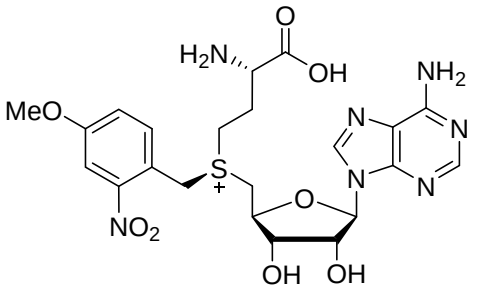
1-(Bromomethyl)-2-nitrobenzene (561 mg, 2.60 mmol, 50.0 equiv.) was added to S-(5'-deoxyadenos-5'-yl)-L-homocysteine (SAH) dissolved in a mixture of acetic acid and formic acid (1:1, 1.00mL). Next, silver trifluoromethanesulfonate (26.7 mg, 104 µmol, 2 equiv.) dissolved in a mixture of acetic acid and formic acid (1:1, 400 µL) were added to the solution. The mixture was shaken under exclusion of light for 24 h. Another batch of silver trifluoromethanesulfonate (26.7 mg, 104 µmol, 2 equiv.) dissolved in a mixture of acetic acid and formic acid (1:1, 400 µL) was added and the mixture was shaken for additional 24 h. Hydrochloric acid (1.80 mL, 10%) was added to quench the reaction. After 1 h, H_2O containing 0.1% formic acid (900 µL) was added and the suspension was filtered over Celite. H_2O containing 0.1% formic acid was added (4.50 mL) and the solution was washed with diethyl ether (3 x 9.00mL). The aqueous layer was concentrated by lyophylization. The crude product was purified by preparative HPLC to yield two diastereomers of 10 separated (4.74 mg, 9.11 µmol, 17%). Aliquots of 11 µL (10mM in H_2O, pH 2.0) were stored at −80 °C.

^{1}H NMR (500 MHz, ^{1}H-^{1}H-COSY, DMSO-d_6): δ = 8.32 (s, 1H, H8), 8.13 (s, 1H, H2), 812 (s, 1H, NH_2), 7.98 (dd, *J* = 8.1, 1.4 Hz, 1H, arom. H), 7.58 (td, *J* = 7.5, 1.4 Hz, 1H, arom. H), 7.51 (td, *J* = 7.8, 1.5 Hz, 1H, arom. H), 7.44 (dd, *J* = 7.6, 1.5 Hz, 1H, arom. H), 7.28 (s, 2H, NH_2), 5.87 (d, *J* =

5.4 Hz, 1H, H1'), 5.49 (d, *J* = 5.9 Hz, 1H, 2'-OH), 5.28 (d, *J* = 5.3 Hz, 1H, 3'-OH), 4.71 (q, *J* = 5.5 Hz, 1H, H2'), 4.43 – 4.32 (m, 1H, Hγ), 4.25 – 4.16 (m, 1H, Hγ), 4.13 (q, *J* = 5.0 Hz, 1H, H3'), 4.10 – 3.99 (m, 3H, benz. H, Hα), 4.01 – 3.92 (m, 1H, H4'), 2.83 (dd, *J* = 13.9, 5.5 Hz, 1H), 2.71 (dd, *J* = 14.0, 7.2 Hz, 1H), 2.46 (m, under DMSO signal, 1H, Hβ), 2.15 – 1.98 (m, 1H, Hβ).

HRMS (ESI): m/z: $[M]^+$ calculated for $[C_{21}H_{26}N_7O_7S]^+$: 520.1609; found: 520.1612.

Ado4MNB (3)[2,3]



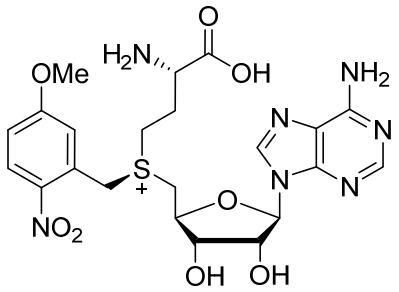
1-(Bromomethyl)-4-methoxy-2-nitrobenzene (2) (640 mg, 2.60 mmol, 50.0 equiv.) was added to SAH dissolved in a mixture of acetic acid and formic acid (1:1, 1.40mL). Silver trifluoromethanesulfonate (26.7 mg, 104 µmol, 2 equiv.) was dissolved in a mixture of acetic acid and formic acid (1:1, 400 µL) and added to the solution. The mixture was shaken under exclusion of light for 6 h. Hydrochloric acid (1.80 mL, 10%) was added to quench the reaction. After 1 h, H_2O containing 0.1% formic acid (900 µL) was added and the suspension was filtered over Celite. H_2O containing 0.1% formic acid was added (4.50 mL) and the solution was washed with diethyl ether (3 x 9.00 mL). The aqueous layer was concentrated by lyophylization. The crude product was purified by preparative HPLC to yield epimeric pure Ado4MNB (4.42 mg, 8.03 µmol, 15%). Aliquots of 11 µL (10mM in H_2O, pH 2.0) were stored at −80 °C.

^{1}H NMR (500 MHz, ^{1}H-^{1}H -COSY, DMSO-d_6) δ = 8.71 (s, 1H, arom. H), 8.49 (s, 1H, arom. H), 8.38 (d, *J* = 5.4 Hz, 2H, NH_2), 7.68 (d, *J* = 8.6 Hz, 1H, arom. H), 7.59 (d, *J* = 2.7 Hz, 1H, arom. H), 7.34 (dd, *J* = 8.6, 2.7 Hz, 1H, arom. H), 5.95 (d, *J* = 5.7 Hz, 1H, H1'), 4.97 (s, 2H, benz. H), 4.68 (t, *J* = 5.3 Hz, 1H, H2'), 4.15 – 4.13 (m, 1H, H3'), 4.09 – 4.02 (m, 1H, H4'), 3.95 (q, *J* = 5.8

Hz, 1H, Hα), 3.87 (s, 3H, OMe), 2.95 (dd, *J* = 13.9, 6.0 Hz, 1H, H5'), 2.86 (dd, *J* = 13.9, 7.0 Hz, 1H, H5'), 2.73 – 2.64 (m, 2H, Hγ), 2.11 – 1.97 (m, 2H, Hβ).

HRMS (ESI) m/z: [M]+ calculated for $[C_{22}H_{28}N_7O_8S]^+$: 550.1715; found: 550.1717 .

Ado5MNB (4)[2,3]

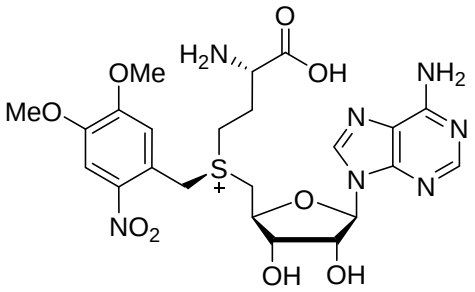


2-(Bromomethyl)-4-methoxy-1-nitrobenzene (4) (640 mg, 2.60 mmol, 50.0 equiv.) was added to SAH dissolved in a mixture of acetic acid and formic acid (1:1, 1.40mL). Silver trifluoromethanesulfonate (26.7 mg, 104 µmol, 2 equiv.) was dissolved in a mixture of acetic acid and formic acid (1:1, 400 µL) and added to the solution. The mixture was shaken under exclusion of light for 24 h. Hydrochloric acid (1.80 mL, 10%) was added to quench the reaction. After 1 h, H_2O containing 0.1% formic acid (900 µL) was added and the suspension was filtered over Celite. H_2O containing 0.1% formic acid was added (4.50 mL) and the solution was washed with diethyl ether (3 x 9.00mL). The aqueous layer was concentrated by lyophylization. The crude product was purified by preparative HPLC to yield epimeric pure Ad5MNB (1.61 mg, 2.92 µmol, 6%). Aliquots of 11 µL (10mM in H_2O, pH 2.0) were stored at −80 °C.

^{1}H NMR (500 MHz, ^{1}H-^{1}H -COSY, DMSO-d_6): δ = 8.30 (s, 1H, arom. H), 8.14 (s, 1H, arom. H), 8.11 (s, 1H, arom. H), 8.07 (d, *J* = 8.8 Hz, 1H, arom. H), 7.27 (s, 2H, NH_2), 7.03 (s, 1H, arom. H), 5.86 (d, *J* = 5.4 Hz, 1H, H1'), 5.51 (m, 1H, 2'-OH), 5.29 (m, 1H, 3'-OH), 4.70 (t, *J* = 5.3 Hz, 1H, H2'), 4.17 – 4.10 (m, 2H, H3', Hγ), 4.07 (s, 2H, benz. H), 4.00 – 3.94 (m, 1H, H4'), 3.82 (s, 3H, OMe), 3.76 (m, 1H, Hα), 2.86 (dd, *J* = 14.0, 5.6 Hz, 1H, H5'), 2.74 (dd, *J* = 14.0, 7.1 Hz, 1H, H5'), 2.44 – 2.38 (m, 1H, Hβ), 2.02 – 1.94 (m, 1H, Hβ).

HRMS (ESI) m/z: [M]+ calculated for $[C_{22}H_{28}N_7O_8S]^+$: 550.1715; found: 550.1720.

AdoDMNB (5)



1-(Bromomethyl)-4,5-dimethoxy-2-nitrobenzene (500 mg, 1.81 mmol, 34.8 equiv.) suspended in toluene (800 µL) was added to SAH (20 mg, 52 µmol, 1 eq) dissolved in a mixture of acetic acid and formic acid (1:1, 2.00mL). Silver trifluoromethanesulfonate (26.7 mg, 104 µmol, 2 equiv.) dissolved in a mixture of acetic acid and formic acid (1:1, 400 µL) was added to the suspension. The mixture was shaken under exclusion of light for 24 h. Another batch of silver trifluoromethanesulfonate (26.7 mg, 104 µmol, 2 equiv.) dissolved in a mixture of acetic acid and formic acid (1:1, 400 µL) was added and the mixture was shaken for additional 24 h. Hydrochloric acid (3.60 mL, 10%) was added to quench the reaction. After 1 h, H_2O containing 0.1% formic acid (1.80 mL) was added and the suspension was filtered over Celite. H_2O containing 0.1% formic acid was added (9 mL) and the solution was washed with diethyl ether (3 x 18.00mL). The crude product was purified by preparative HPLC to yield epimeric pure AdoDMNB (2.87 mg, 4.94 µmol, 10%). Aliquots of 11 µL (10mM in H_2O, pH 2.0) were stored at −80 °C.

^{1}H NMR (500 MHz, ^{1}H-^{1}H -COSY, DMSO-d_6): δ = 8.44 (s, 1H, arom. H), 8.42 (s, 1H, arom. H), 8.36 (s, 2H, NH_2), 7.65 (s, 1H, arom. H), 5.50 (s, 1H, arom. H), 7.27 (s, 2H, NH_2), 7.03 (s, 1H, arom. H), 5.93 (d, *J* = 5.1 Hz, 1H, H1'), 4.69 (t, *J* = 5.4 Hz, 1H, H2'), 4.31 (bs, 2H, benz. H), 4.15 – 4.11 (m, 1H, H3'), 4.07 -4.03 (m, 2H, Hγ, H4'), 3.91, 3.88 (2 x s, 2 x 3H, OMe), 3.82 (m, 1H, Hα), 2.95 (dd, *J* = 14.0, 6.1 Hz, 1H, H5'), 2.86 (dd, *J* = 14.0, 6.8 Hz, 1H, H5'), 2.72 – 2.62 (m, 1H, Hβ), 2.10 – 1.98 (m, 1H, Hβ).

HRMS (ESI) m/z: $[M]^+$ calculated for $[C_{23}H_{30}N_7O_9S]^+$: 580.1820; found: 580.1825.

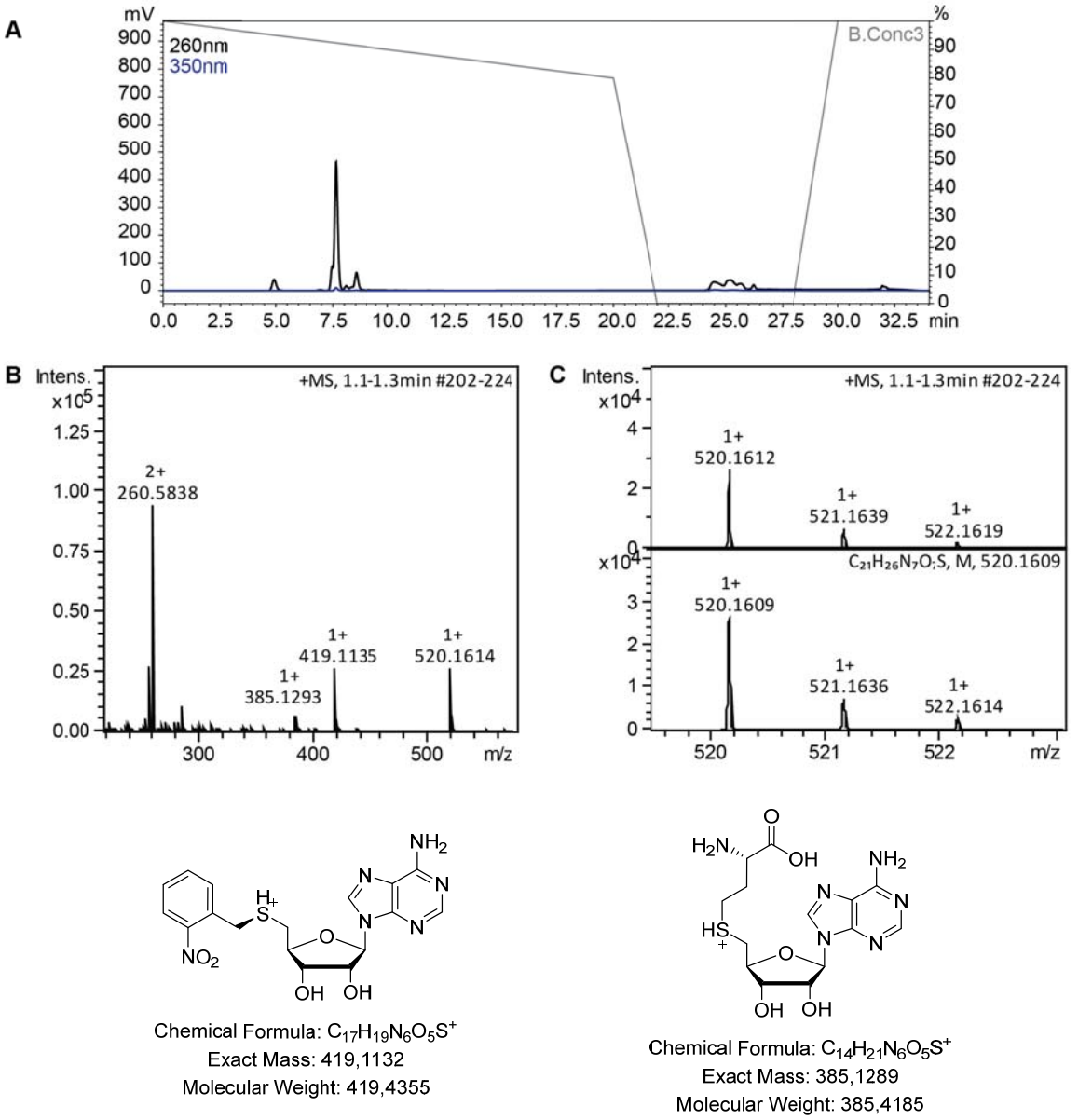


Fig. S1 Reversed phase HPLC chromatogram **(**A) monitored at λ = 260 nm and 350 nm as well as ESI-TOF analysis in positive mode (B & C) of purified AdoNB (**2**). Due to fragmentation of the cosubstrate during MS analysis additional peaks resulting from S-adenosylhomocysteine (SAH) and the respective thioadenosine can be detected (structures shown below). C) Measured MS profile (top) in comparison with the calculated MS profile (bottom) is shown. Measured and calculated spectra were obtained using Bruker Compass MS Software DataAnalysis 4.1.

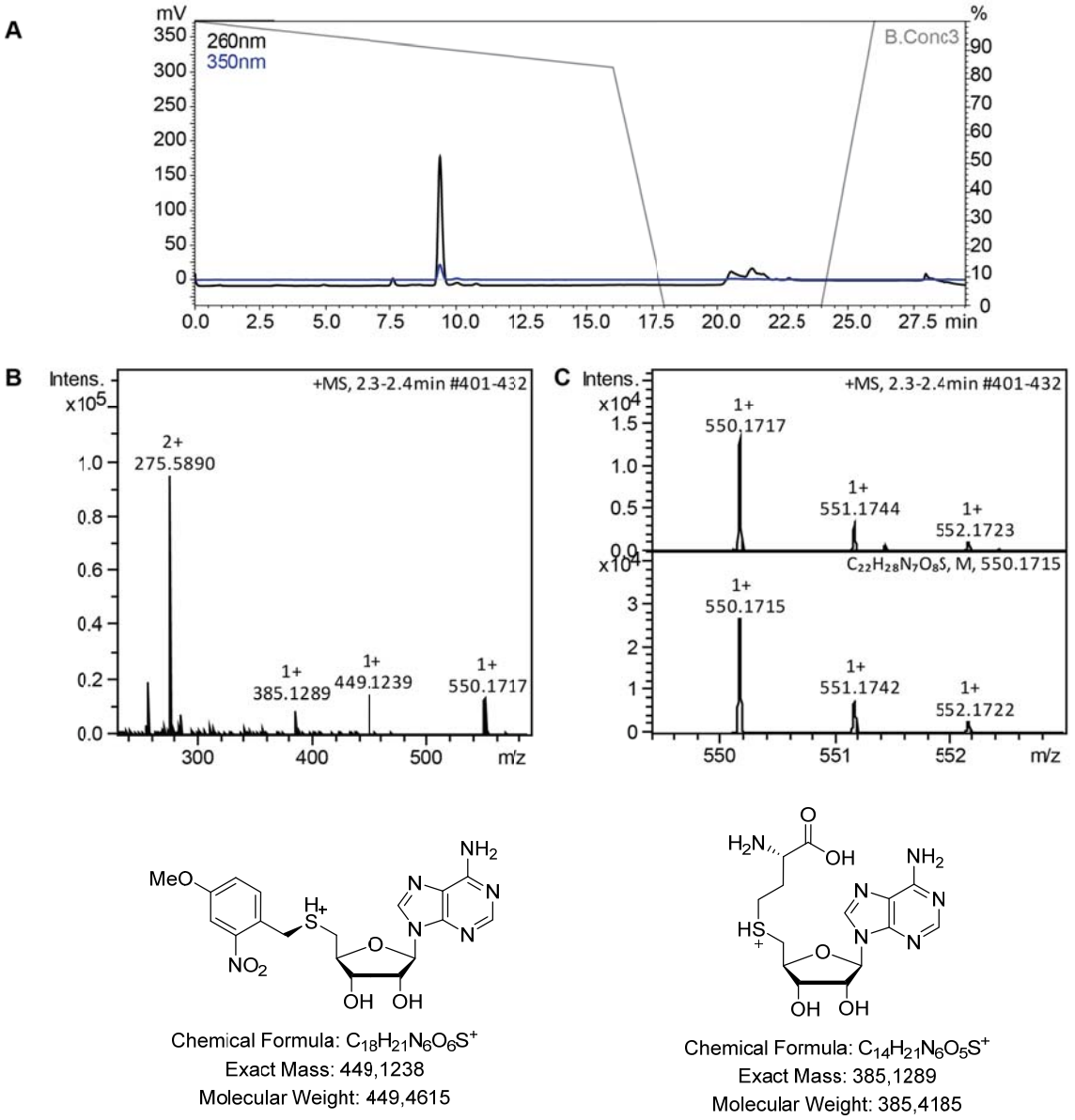


Fig. S2 Reversed phase HPLC chromatogram **(**A) monitored at λ = 260 nm and 350 nm as well as ESI-TOF analysis in positive mode (B & C) of purified Ado4MNB (**3**). Due to fragmentation of the cosubstrate during MS analysis additional peaks resulting from S-adenosylhomocysteine (SAH) and the respective thioadenosine can be detected (structures shown below). C) Measured MS profile (top) in comparison with the calculated MS profile (bottom) is shown. Measured and calculated spectra were obtained using Bruker Compass MS Software DataAnalysis 4.1.

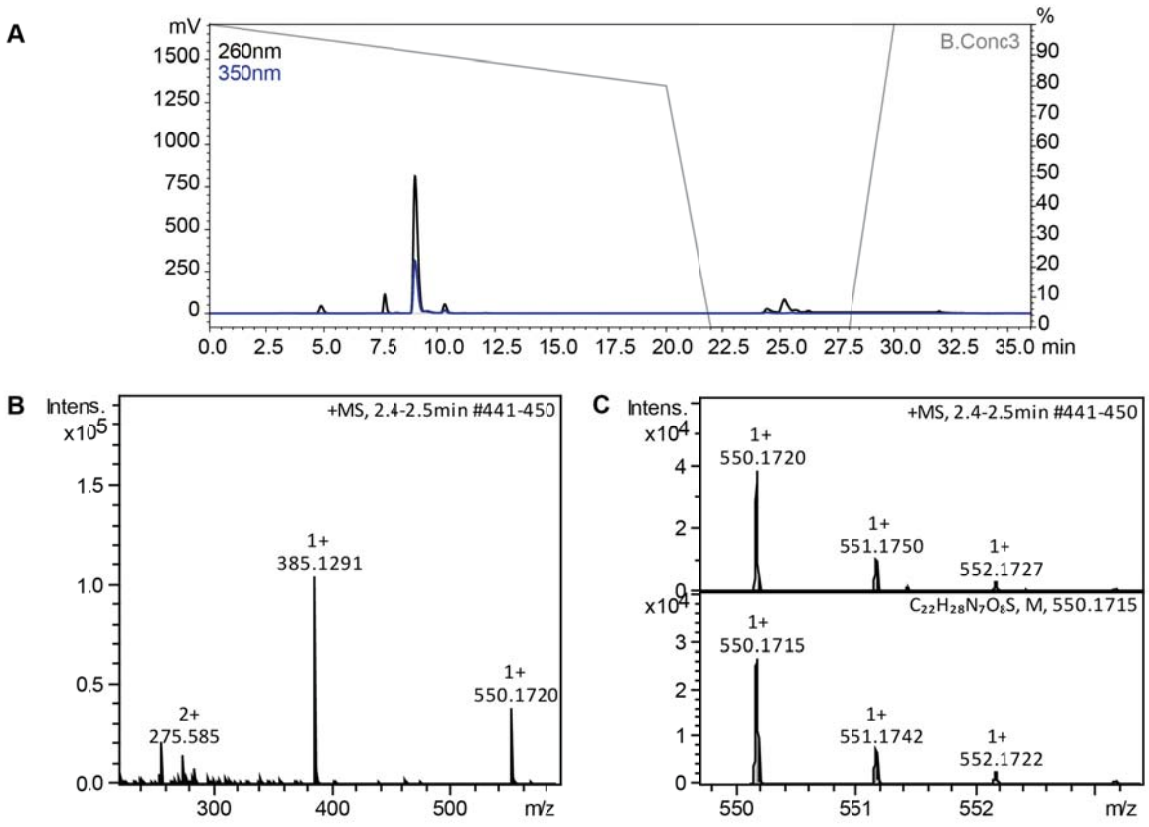


Fig. S3 Reversed phase HPLC chromatogram **(**A) monitored at λ = 260 nm and 350 nm as well as ESI-TOF analysis in positive mode (B & C) of purified Ado5MNB (**4**). Due to fragmentation of the cosubstrate during MS analysis an additional peak resulting from S-adenosylhomocysteine (SAH) can be detected. C) Measured MS profile (top) in comparison with the calculated MS profile (bottom) is shown. Measured and calculated spectra were obtained using Bruker Compass MS Software DataAnalysis 4.1.

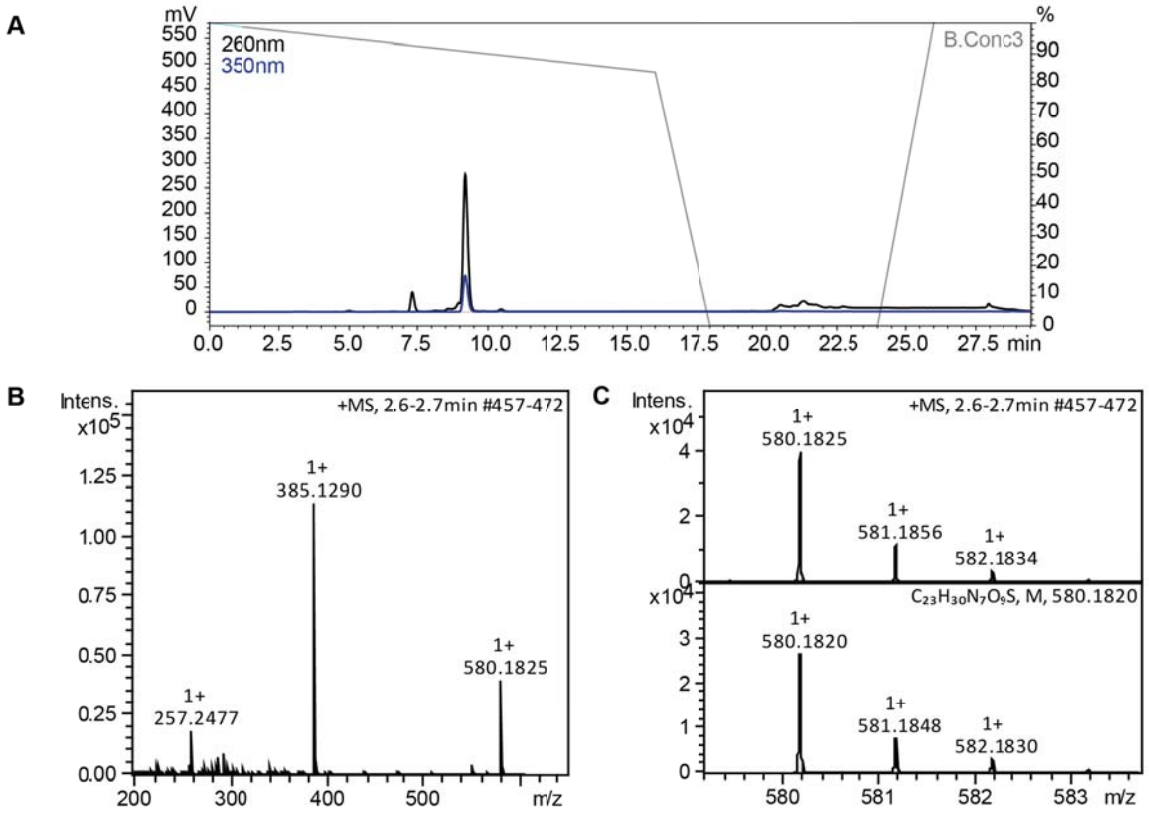


Fig. S4 Reversed phase HPLC chromatogram **(**A) monitored at λ = 260 nm and 350 nm as well as ESI-TOF analysis in positive mode (B & C) of purified AdoDMNB (**5**). Due to fragmentation of the cosubstrate during MS analysis an additional peak resulting from S-adenosylhomocysteine can be detected. C) Measured MS profile (top) in comparison with the calculated MS profile (bottom) is shown. Measured and calculated spectra were obtained using Bruker Compass MS Software DataAnalysis 4.1.

Stability measurements of cosubstrates 2–5

Cosubstrate derivative **2**–**5** (1.00 mM) was incubated in HEPES buffer (50.0 mM, pH 7.0) at 37 °C with tryptophan added as an internal standard. Samples were taken every 30 min and analyzed via HPLC-MS. Quantification of remaining cosubstrate over time revealed half-lives of about 3 h (AdoNB **2**), 3 h (Ado5MNB **4**), 3.5 h (AdoDMNB **5**) and 4 h (Ado4MNB **3**) for the cosubstrate analogues (Fig. S5 C). By means of mass spectrometry, lactonization was confirmed to be the dominant degradation pathway under the described conditions. In addition, the formation of small amounts of SAH as well as slow epimerization could be observed (Fig. S5 B and D).

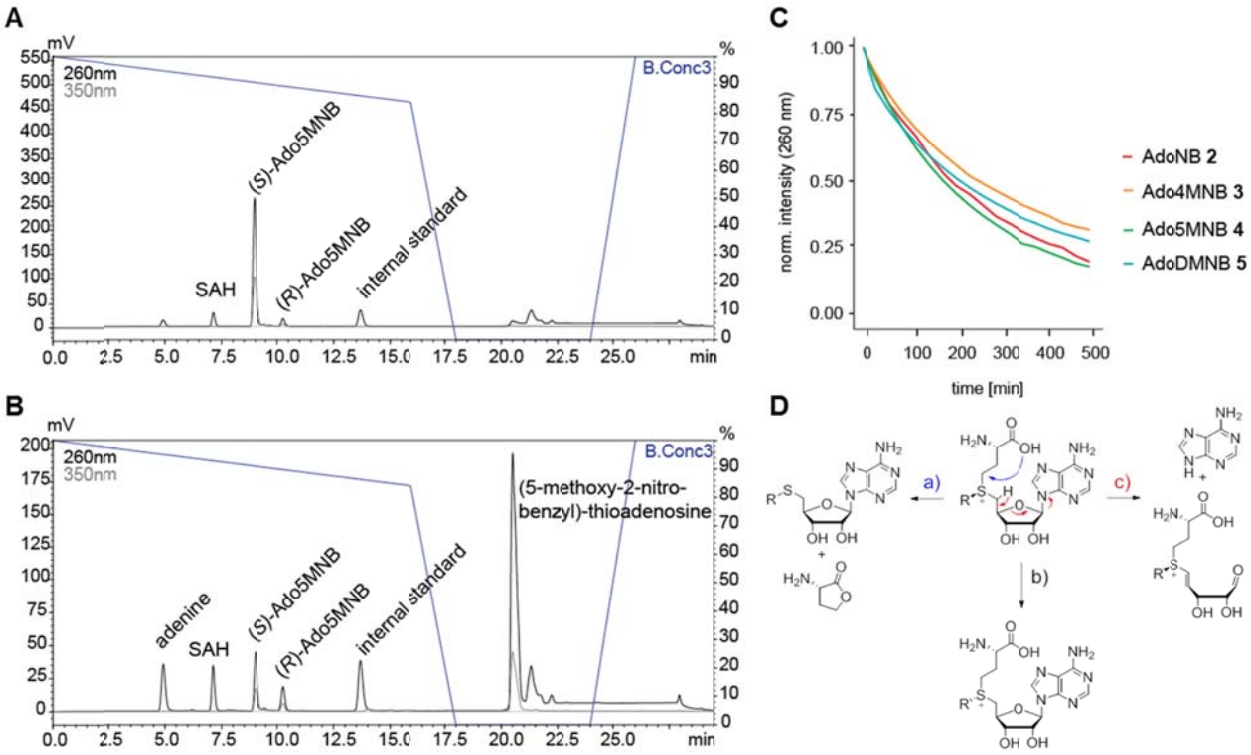


Fig. S5 Stability of the nitrobenzyl derivatives **2**–**5** of AdoMet under physiological conditions: representative HPLC chromatograms of Ado5MNB at t = 0 min (A) and t = 540 min (B). Degradation products were identified via HPLC-MS. C) Decrease of cosubstrate concentration over time followed by HPLC-MS (absorption at λ = 260 nm). D) Common degradation routes of AdoMet drivatives:[4] a) lactonization with the release of an alkyl-thioadenosine derivative; b) epimersation at the sulfur atom; c) base-catalyzed proton abstraction and release of adenine.

Cloning, expression and purification of M.TaqI

M.TaqI methyltransferase was a kind gift from New England Biolabs Inc. and was amplified by PCR from an pBR322 plasmid using primers containing NdeI and XhoI restriction sites (fwd: 5'-ATT-ACATATGGGCCTGCCACCCCTTCTGTCCTTAC-3', rev: 5'-AATTCTCGAGTCAAAAGTTTCGAGCGCT-TTCTGGGCTTG-3'). The amplified DNA construct and the vector pET28a were digested with NdeI and XhoI and ligated using T4 DNA ligase to allow expression of the protein under the control of a T7 promotor as a fusion carrying an N-terminal hexahistidine tag followed by a thrombin cleavage site. Accuracy of the construct was validated by Sanger sequencing using standard T7 promoter and terminator primers.

Single-site mutangenesis of pET28a-M.TaqI was generated with the In-Fusion® HD Cloning Kit (Takara Clontech, France) using following primers: fwd: 5'-GGCCGGGGGGAGACCCCCCC-3'; rvd: 5'-GGTCTCCCCCCGGCCCAGGCTC-3'. The infusion mix was transformed into Stellar cells (Takara Clontech, France) and the mutation V21G was confirmed by sequencing using the commercial T7 primer (GATC).

The plasmid encoding for M.TaqI WT or M.TaqI V21G were used to transform *E. coli* BL21Gold (DE3) competent cells (Agilent, Waldbronn, Germany). 2 L LB medium (+50 mg/L kanamycin) were inoculated with an overnight starter culture of transformed *E.coli* BL21 (DE3) and grown at 37 °C until OD_{600} reached 0.6. After cooling down to 20 °C protein expression was induced by adding 1 mM IPTG and the cell culture was incubated at 20 °C overnight. Cells were harvested by centrifugation at 4 °C (5000 x g), treated with Benzonase® Nuclease (0.25 µL, 250 µkat mL^{-1}, Sigma-Aldrich), resuspended in lysis buffer (50 mM Tris pH 8.0, 50 mM NaCl, 10 mM imidazole) including a protease inhibitor cocktail tablet (SigmaFast™, Sigma-Aldrich, München, Germany) and lysed by a microfluidizer (Fluidizer 110S, Microfluidics, Newton, USA). After centrifugation (20.000 rpm, 4 °C, 25 min) the soluble fraction was incubated for 1 h at 4 °C with 4 mL of Ni-NTA agarose® (MACHEREY-NAGEL) pre-equilibrated with 2 x 10 column bed volumes (CV) of wash buffer (50 mM Tris pH 8.0, 300 mM NaCl, 10 mM imidazole). After separating the resin from the supernatant by centrifugation (1000 x g, 4 °C, 2 min) it was transferred into a column and washed with 10 CV of wash buffer. Proteins were eluted with 3 CV of elution buffer (50 mM

Tris pH 8.0, 300 mM NaCl, 300 mM imidazole). The elution fraction was further purified by size exclusion chromatography on a Superose-12 column (GE Healthcare Life Sciences, München, Germany) connected to an Äkta pure 25 chromatography system (GE Healthcare Life Sciences) using gel filitration buffer (20 mM Tris.HCl pH 7.4, 20 mM NaCl, 5 mM DTE). Elution fractions were analysed by SDS-PAGE, appropriate fractions pooled and concentrated using centrifugal concentrators with a 10,000 MW cut-off (Sartorius Stedim Biotech, Göttingen, Germany).

Cosubstrate removal

Cosubstrate (AdoMet) still bound to the protein was removed through extensive washing. M.TaqI or M.TaqI V21G was loaded onto a HisTrap™ FF Column (1 mL, GE Healthcare Life Sciences) coupled to an Äkta pure 25 chromatography system and washed with 4.00 L Ni-NTA wash buffer. Finally, M.TaqI was eluted with 15 mL of Ni-NTA elution buffer. Fractions containing the protein were pooled and the buffer was exchanged to gel filtration buffer using a NAP™-25 column (GE Healthcare Life Sciences) and following the manufacturer's protocol. Removal of AdoMet was investigated by a modification-restriction assay. Aliquots were flash frozen in liquid nitrogen and stored at -80 °C.

Oligonucleotides used for enzymatic *in vitro* activity assays

Unmodified single-stranded HPLC-purified oligodeoxynucleotides (ODNs) were ordered from Sigma-Aldrich as freeze-dried pellets. Methylated ODN II^{Me} was obtained from IBA Lifesciences as freeze-dried pellet. The pellets were dissolved in H_2O to yield 100 µM stock solutions and stored at −20 °C. Duplex ODN III·ODN IV served as a control template with randomized M.TaqI recognition sequence.

ODN	Sequence
ODN I	5‘-CGCG**TCGAT**GCCGC -3‘
ODN II	5‘-GCGGCA**TCGA**CGCG -3‘
ODN II^{Me}	5‘-GCGGCA**TCGA^{Me}**CGCG -3‘
ODN III	5‘GGTCAATGCATCTAGG-3‘
ODN IV	5‘-CCTAGATGCATTGACC-3‘
ODN V	5‘-AAAAA-3‘

DNA Duplex Preparation

Hybridization of the single-stranded ODNs was carried with the help of a thermocycler (Tpersonal, Biometra™). Equimolar quantities of complementary ODNs were mixed in a PCR soft tube. The soft tube was placed in the thermocycler and the following program was started: 80 °C, 5 min; 40 °C, 1 min; 4 °C, pause. Subsequently, the mixture was purified by HPLC to separate the DNA duplex from single-stranded DNA and other impurities. Therefore, a Promincence HPLC system (Shimadzu) equipped with a Waters xBridge™ Peptide BEH C18 Column (300 Å, 3.5 µm, 4.6 x 250 mm) and a SPD-20AV UV/VIS detector for compound visualization was used.

A: triethylammonium acetate (TEAA) (100 mM) in H_2O. B: acetonitrile. Gradient of 5% B for 6 min, 5% B to 25% B over 20 min, 25% B to 100% B over 1min, 100% B for 6 min, 100% B to 5% B over 1min, and 5% B for 4min. Flow rate: 1.0 mL min^{-1}. Absorption at 260 and 350 nm was monitored.

The fractions containing double-stranded DNA were pooled and dried by lyophylization and finally dissolved in H_2O. The DNA concentration was determined spectroscopically using a NanoDrop 2000c UV-Vis Spectrophotometer. The purity of the isolated DNA duplex was verified via UHPLC-MS.

Enzymatic activity assay (methyltransferase-directed caging of DNA)

The purified DNA duplex (25.0 μM) and M.TaqI (2.00 μM) were presented in CutSmart® buffer (50 mM potassium acetate, 20mM Tris-acetate, 10 mM magnesium acetate, 100 μg mL^{-1} BSA, pH 7.9 at 25 °C, NEB) in an Eppendorf tube and heated to 37 °C using a thermomixer (Thermomixer comfort, Eppendorf). The reaction was started by addition of the respective cosubstrate derivative **1**–**5** (500 μM). Samples were taken at different points in time. Therefore, 10.0 μL of the assay solution were diluted with H_2O (20.0 μL) and mixed with a phenol/chloroform/isoamylalcohol solution (49.5:49.5:1 (v/v/v); saturated with 100 mM TRIS pH 8.0; 0.1% 8-hydroxyquinoline, 30.0 μL) to quench the reaction by phenol-chlorform extraction. Subsequently, 20.0 μL of the aqueous layer were taken off and placed on ice under exclusion of light until analysis by UHPLC-MS (solvent A: 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (300 mM), triethylamine (10.0 mM), pH 7.9; solvent B: methanol (70%), solvent A (30%); gradient: 5% B for 30 s, 5% B to 10% B over 30 s, 10% B to 30% B over 13 min, 30% B to 100% B over 30 s, 100% B for 1 min, 100% B to 5% B over 1 min; flow rate: 0.5 mL min^{-1}). The experiments were performed as triplicates. Analysis was performed using MS quantifying the decrease of unlabeled ODN spectroscopically (absorption at 260 nm) in the chromatogram using ODN II^{Me} as internal standard. In other cases, ODN V was added and utilized for normalization.

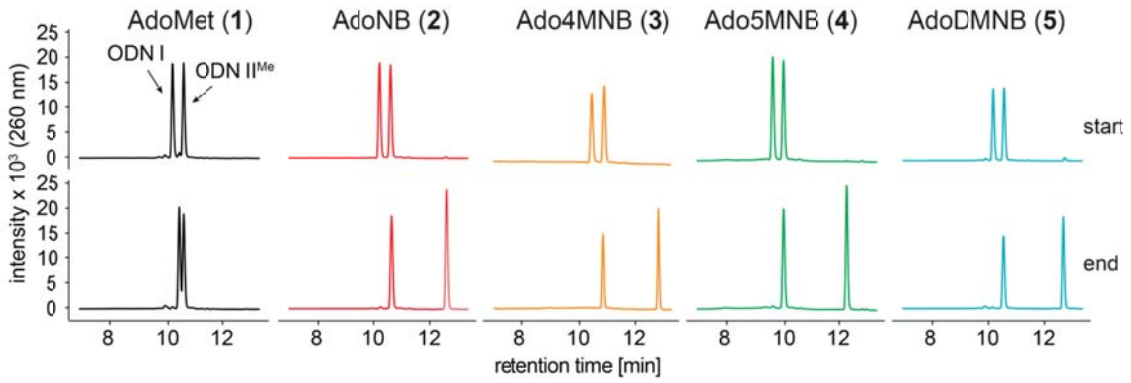


Fig. S6 Representative UHPLC-chromatograms (intensity at 260 nm) of samples taken 0 min and 15 min after starting the enzymatic labeling reaction. Peaks were identified by mass spectrometry (see Fig. S9).

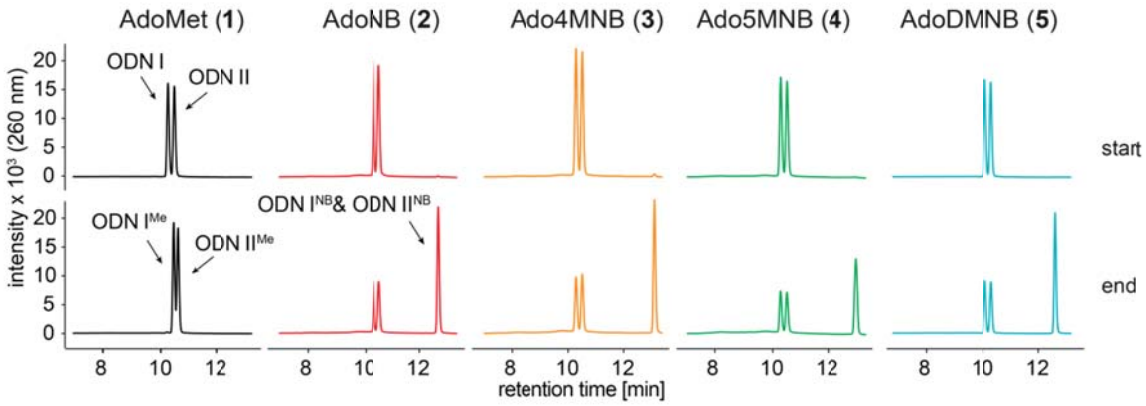


Fig. S7 Chemo-enzymatic labeling of the unmethylated duplex ODN I·ODN II: representative UHPLC-chromatograms (intensity at 260 nm) of samples taken 0 min and 30 min after starting the enzymatic labeling reaction of duplex (25 µM) using M.TaqI (2 µM) and the respective cosubstrate derivative (500 µM). Labeling with bulky AdoMet derivatives **2**- **5** results only in hemialkylation of duplex ODN I·ODN II. Alkylated strands ODNI^{R} and ODNII^{R} do not elute separately, but were identified by mass spectrometry (see. Fig. S10).

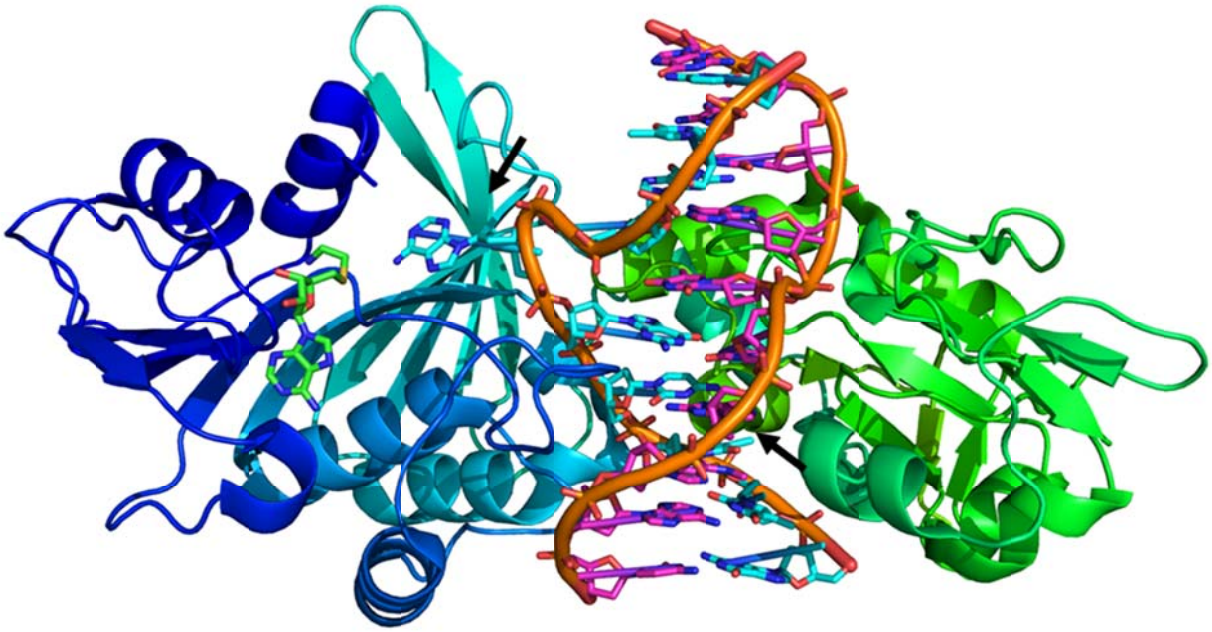


Fig. S8 Structure of a ternary complex of M.TaqI: The complex consists of the methyltransferase, a double-stranded deoxyoligonucleotide containing the enzymes palindromic recognition sequence and the cosubstrate analogue AETA. Blue, N-terminal domain; green, C-terminal domain. PDB, 1G38.[5] One adenosine of the recognition sequence is flipped out for methylation, while the other adenosine is in close proximity to a C-terminal helix, which probably prevents binding of already hemialkylated DNA for further alkylation (both adenosines are marked with an arrow).

Table S1: Mass table for compound identification via UHPLC-MS (negative mode)

ODN	Exact Mass [Da]	Monitored ion	Calculated ion mass [Da]	Monitored mass (SIM) [Da]
ODN I	4238.729	$[M-4H]^{4-}$	1058.674	1058.60
ODN I^{Me}	4253.753	$[M-4H]^{4-}$	1062.430	1062.20
ODN I^{NB}	4374.769	$[M-4H]^{4-}$	1092.684	1092.45
ODN I^{4MNB}	4404.780	$[M-4H]^{4-}$	1100.187	1100.20
ODN I^{5MNB}	4404.780	$[M-4H]^{4-}$	1100.187	1100.20
ODN I^{DMNB}	4434.790	$[M-4H]^{4-}$	1107.689	1107.45
ODN II	4287.747	$[M-4H]^{4-}$	1070.929	1058.60
ODN II^{Me}	4302.770	$[M-4H]^{4-}$	1074.684	1074.45
ODN II^{NB}	4423.786	$[M-4H]^{4-}$	1104.939	1104.70
ODN II^{4MNB}	4453.797	$[M-4H]^{4-}$	1112.441	1112.45
ODN II^{5MNB}	4453.797	$[M-4H]^{4-}$	1112.441	1112.45
ODN II^{DMNB}	4483.807	$[M-4H]^{4-}$	1119.944	1119.70
ODN III	4921.268	$[M-4H]^{4-}$	1229.309	1229.30
ODN IV	4841.218	$[M-4H]^{4-}$	1209.296	1209.30
ODN III^{NB}	5057.308	$[M-4H]^{4-}$	1263,319	1263,10
ODN IV^{NB}	4977.258	$[M-4H]^{4-}$	1243,306	1243,10
ODN V	1504.085	$[M-H]^{-}$	1503.077	1502.35

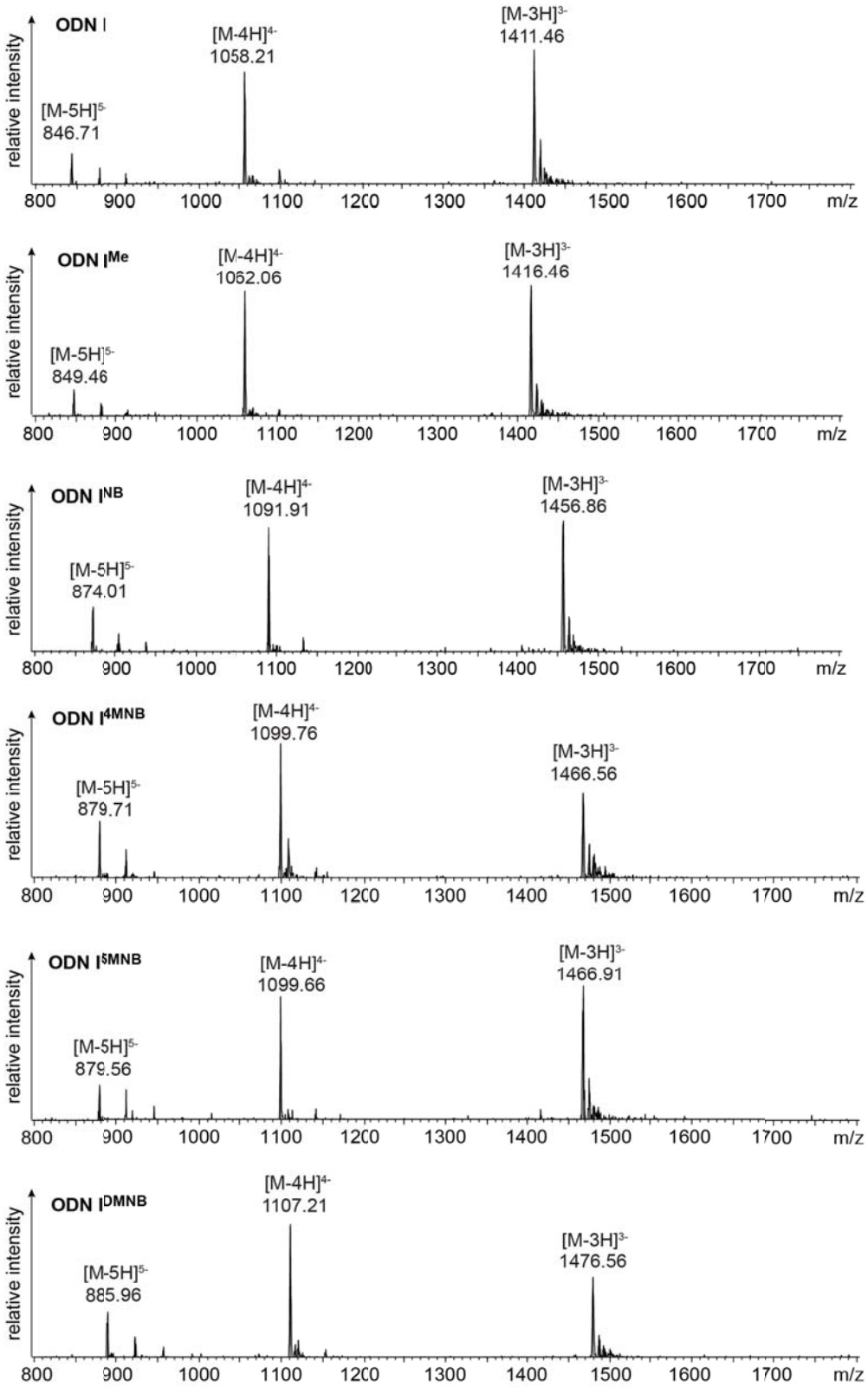


Fig. S9 Mass spectra of ODN I and alkylated ODN I^R, determined by UHPLC-MS (negative mode, electron spray ionisation).

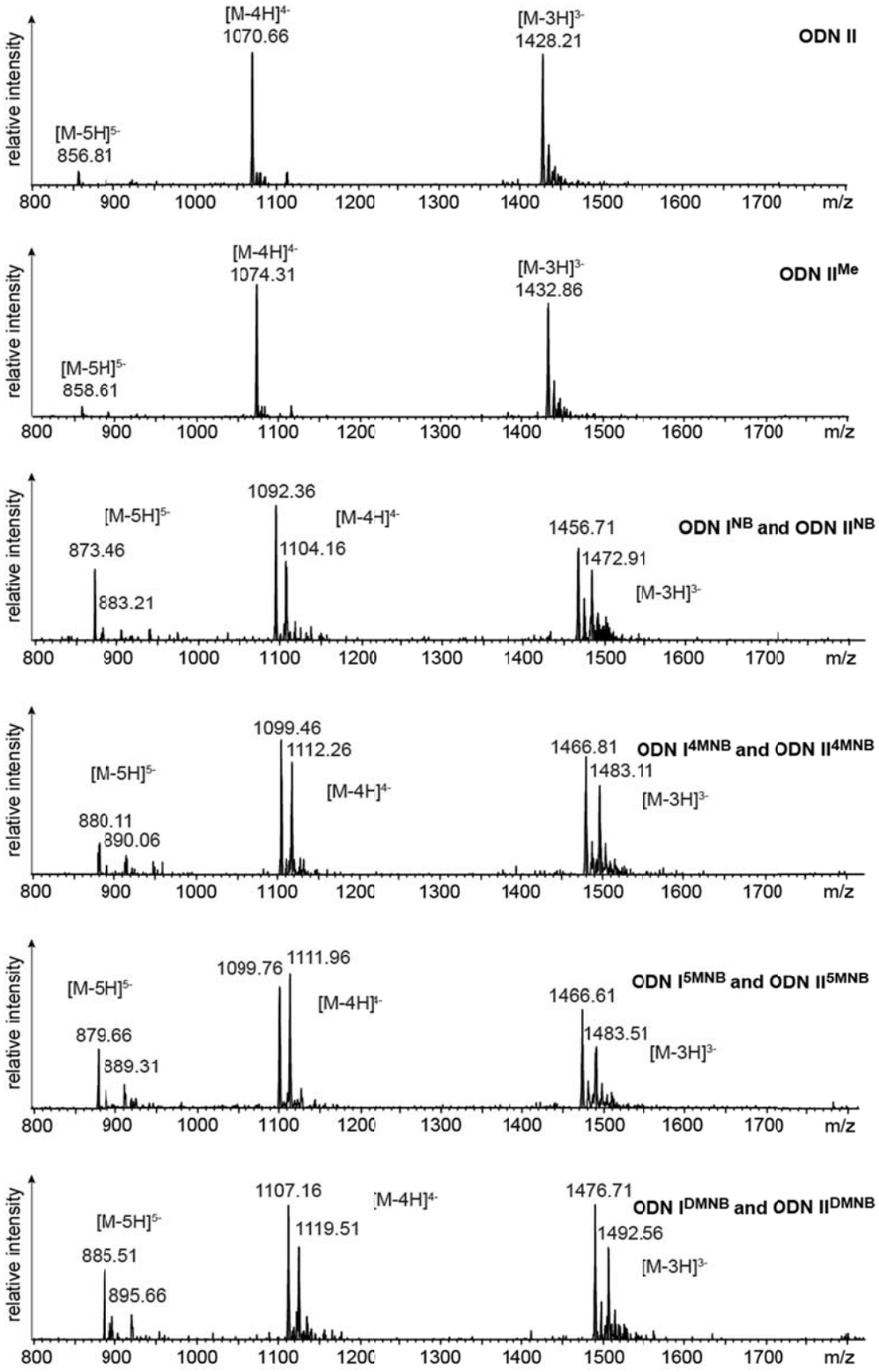


Fig. S10 Mass spectra of ODN II and alkylated ODN I^{R}/ODN II^{R} determined by UHPLC-MS (negative mode, electron spray ionisation).

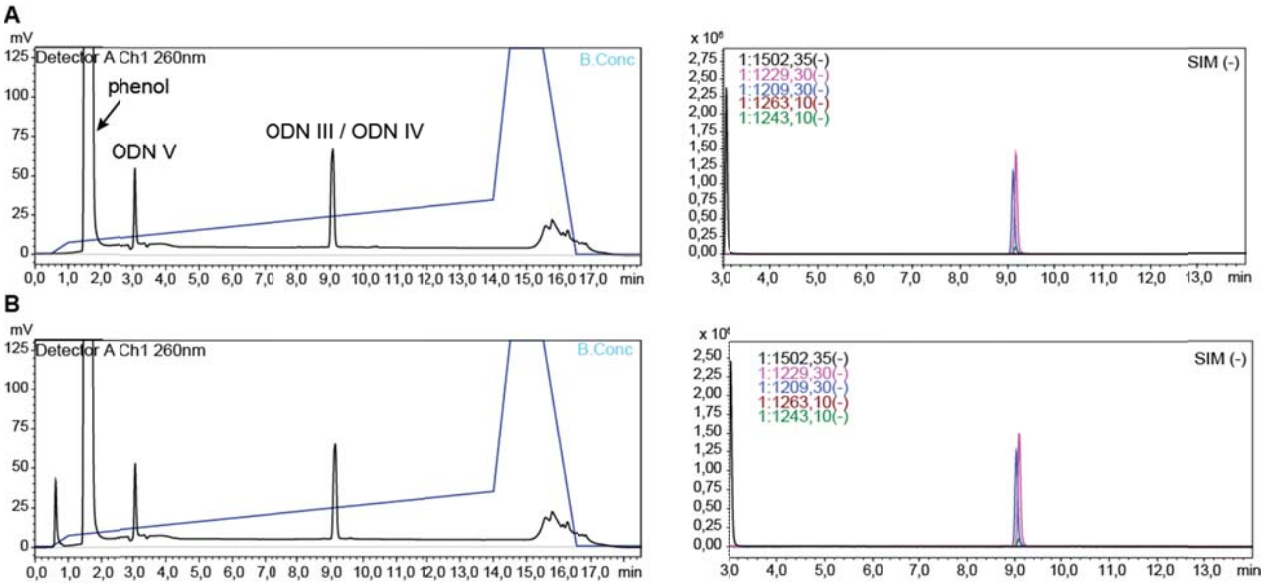


Fig. S11 M.TaqI is highly specific towards its recognition sequence 5'-TCGA-3'. UHPLC-UV/VIS (left side) and single ion monitoring (right side, monitored using single ion detection of fourfold charged species of ODN III and ODN IV in negative mode) traces of the M.TaqI activity assay described above using the randomized duplex ODN III:ODN IV with AdoNB: At t = 0 min (A) and t = 30 min (B): no alkylation can be detected demonstrating the high sequence specificity of M.TaqI. ODN V serves as an internal standard, the large peak eluting at the front of the runs (R_t = 1.7 min) is due to remaining phenol from workup (MS monitoring starts at 3 min run time).

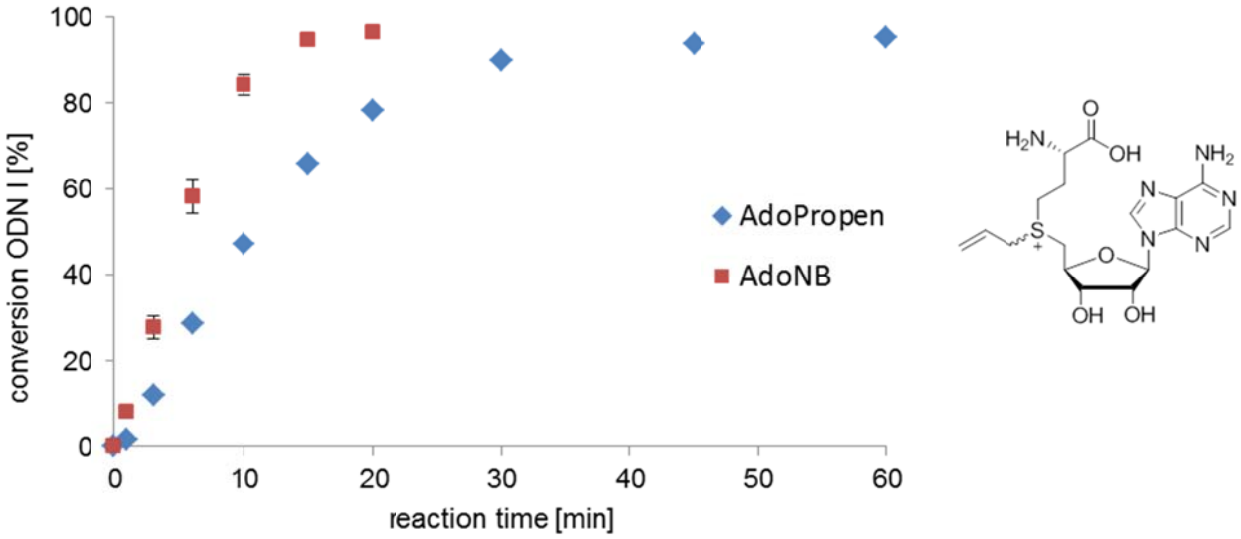


Fig S12 Time course experiments for enzymatic transfer of AdoPropen (1 mM, diastereomeric mixture, structure shown on the right side)[6,7] onto ODN I·ODN II^{Me} (25 µM) by M.TaqI (2 µM) at 37 °C in comparison to AdoNB. Experiments were performed in triplicates.

Irradiation experiments of ODN I·ODN II^{R} samples

Nitrobenzyl-modified DNA was prepared as described above. Samples of 20.0 µL were illuminated with UV light at 365 nm using a VL-6.LC lamp (VILBER, 6 W) for different periods (0 to 30 min). Subsequently, the samples were analyzed via UHPLC-MS as described above. The experiments were performed as triplicates.

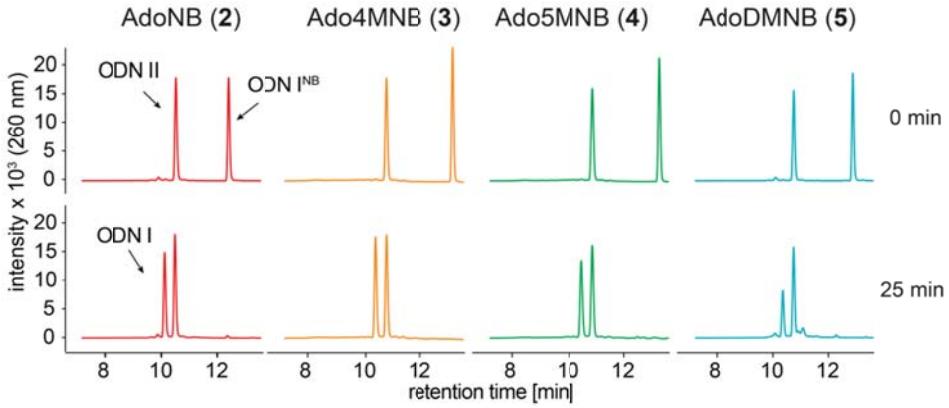


Fig. S13 Representative UHPLC chromatograms of samples (ODN I^{R}: ODN II^{Me}) illuminated with UV light at 365 nm for 0 and 20 min. Irradiation causes a decrease of the concentration of the caged species (ODN II^{R}) and an increase of the concentration of unmodified ODN I.

Competition activity assay

The purified DNA duplex ODN I·ODN II^{Me} (25 µM) and M.TaqI or M.TaqI V21G (2.00 µM) in CutSmart® buffer were presented in an Eppendorf tube and heated to 37 °C using a thermomixer (Thermomixer comfort, Eppendorf). AdoMet analogue **2**–**5** (500 µM / 1.00mM / 2.50mM / 4.00mM) as well as AdoMet (**1**) (500 µM) were added simultaneously to start the reaction. After 30 min, the reaction was quenched as described above and the samples were analyzed via UHPLC-MS as described above. The experiments were performed as triplicates.

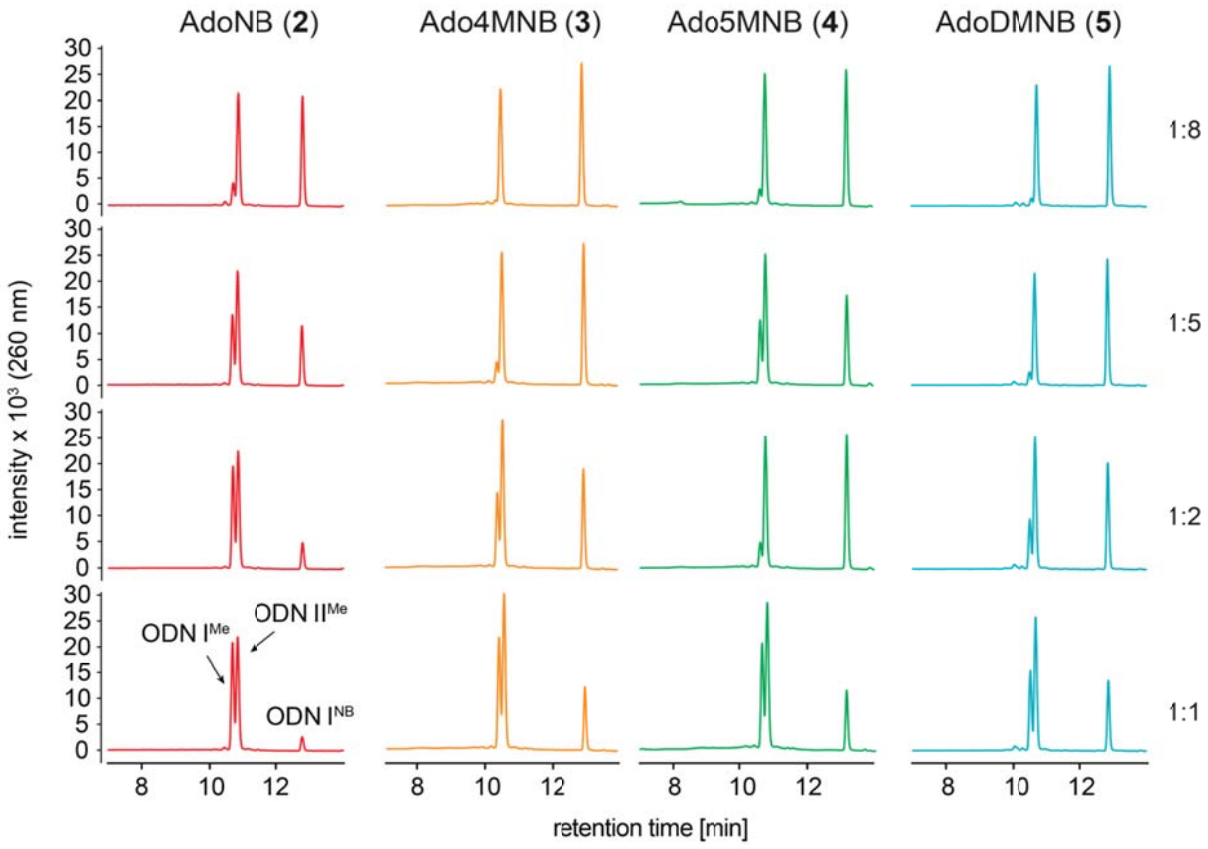


Fig. S14 Representative UHPLC chromatograms of competition activity assays using different ratios of AdoMet to cosubstrate **2**- **5** (ratio is specified on the right side).

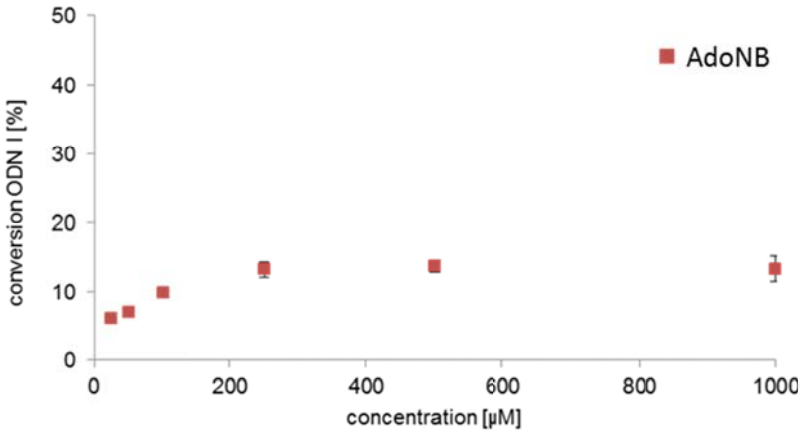


Fig. S15 Enzymatic conversion of ODN I·ODN II^{Me} (25 µM) by M.TaqI (2 µM) after 2 min reaction time using increasing concentrations of AdoNB confirms that DNA labelling experiments are conducted under saturating conditions. Experiments were performed in triplicates.

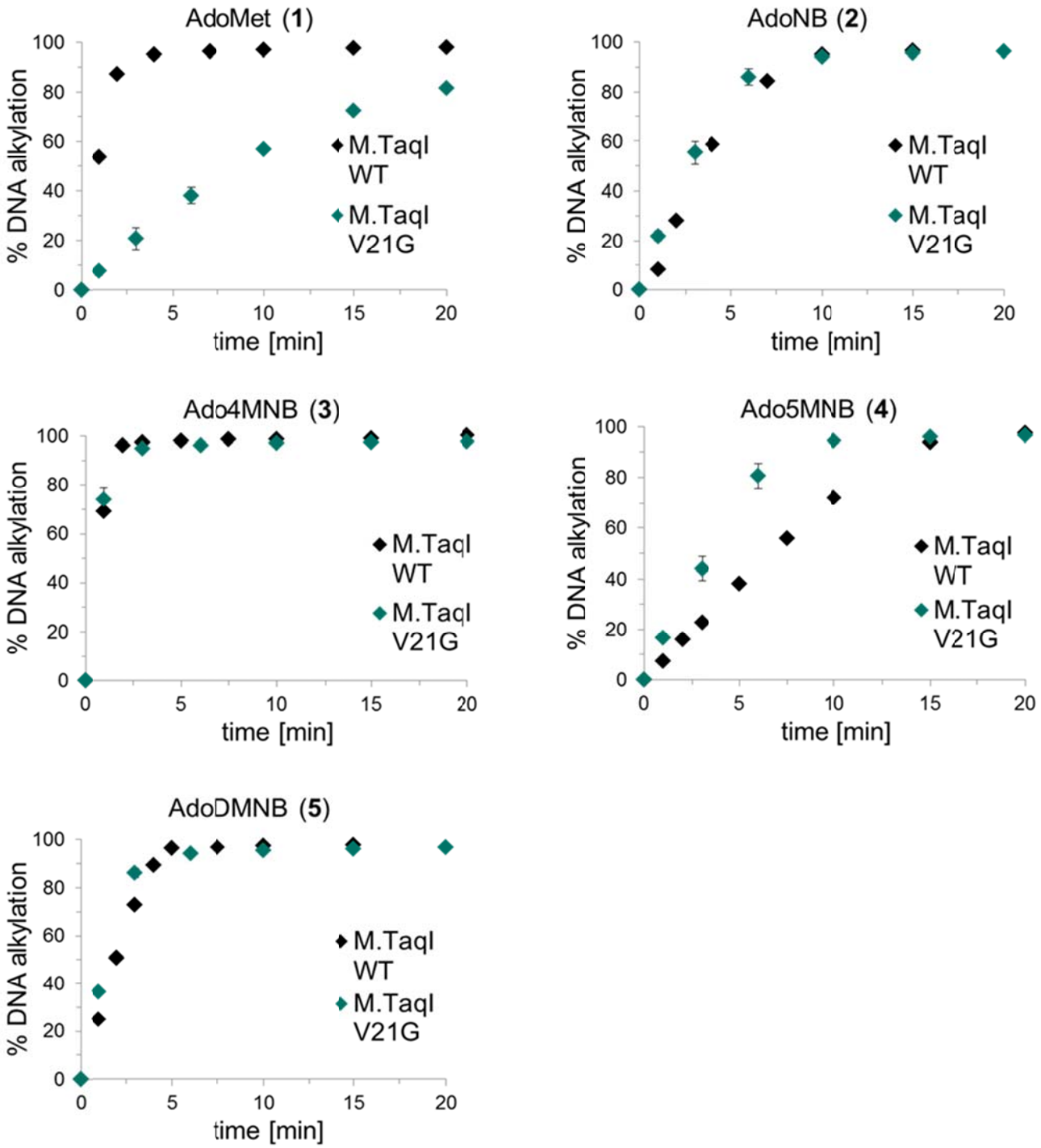


Fig. S16 Comparison of time course experiments for enzymatic transfer of **1**-**5** (500 mM) onto ODN I·ODN II^{Me} (25 µM) by M.TaqI or M.TaqI V21G (2 µM) at 37 °C. Experiments were performed in triplicates.

In vitro gene expression assays

Cloning of constructs used for *in vitro* transcription and *in vitro* transcription/translation assays

pET28a-sfYFP_**V1** was a kind gift from Robert Lindner (MPI Heidelberg). Construct pET28a-sfYFP_**V2** carrying two additional M.TaqI recognition sites directly downstream to the T7 promoter was generated with the In-Fusion® HD Cloning Kit (Takara Clontech, France) and transformed into Stellar cells (Takara Clontech, France). Constructs pET28a-sfYFP_**V3** with one additional M.TaqI regognition site after the T7 promoter was also generated with the In-Fusion® HD Cloning Kit (Takara Clontech, France) using pET28a-sfYFP_**V2** as template. All primers are listed in Table S2. Isolated plasmids of all constructs were sequenced using the commercial pBR3 primer (GATC).

Table S2. List of primers

Plasmid	Primers
pET28a-sfYFP_**V2**	fwd: 5‘-GGGAAT**TCGA**TC**TCGA**GCGGATAACAATTCCCCTCTAGAAATAAT TTTG-3‘
	rvd: 5‘-CCGC**TCGA**GA**TCGA**ATTCCCCTATAGTGAGTCGTATTAATTTCGC-3‘
pET28a-sfYFP_**V3**	fwd: 5‘-GGAAT**TCGA**TCACGAGCGGATAACAATTCCC-3‘
	rvd: 5‘-GCTCGTGA**TCGA**ATTCCCCTATAGTGAGTCG-3‘

Plasmid amplification and purification (MidiPrep)

Purification of plasmid DNA from over-night cultures (150 mL, LB medium) inoculated with a single colony of *E. coli* (BL21-Gold (D3)) cells and grown at 37 °C (120 rpm) was carried out using the QIAGEN Plasmid Midi Kit (QIAGEN) according to the manufacturer’s protocol. The air-dried pellet was redissolved in 200 µL H_2O and plasmid concentrations were determined spectroscopically using a NanoDrop 2000c UV-Vis Spectrophotometer.

pET28a Caging and Linearization

A 500 µL solution containing NEBuffer™ 4 (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM DTT, pH 7.9 at 25 °C, NEB), M.TaqI (1.14 µM), Ado4MNB (456 µM) and pET28a-sfYFP_**V1** – **V3** (12.0 nM, 22.2 µg) was incubated at 37 °C for 2.5 h. Psi I (5,000 U ml^{-1}) was added and the solution was incubated further for 1.5 h. Subsequently, the plasmid was purified by phenol-chlorform extraction and ethanol precipitation. The air-dried pellet was redissolved in 20 µL H_2O and plasmid concentrations were determined spectroscopically using a NanoDrop 2000c UV-Vis Spectrophotometer. Successful caging and linearization of the plasmids were confirmed using a restriction based assay as described above. To produce the linearized plasmid without further modifications, which acts as a control in subsequent experiments, the assay was performed without addition of M.TaqI and Ado4MNB.

pET28a Restriction

The following procedure was carried out under exclusion of light. Taq$^{\alpha}$I (0.5 µL, 20 000 U mL^{-1}, NEB) was added to the samples prepared as described above. Incubation at 65 °C was carried out for 30 min. Subsequently, the samples were kept on ice until analysis using agarose gel electrophoresis was carried out.

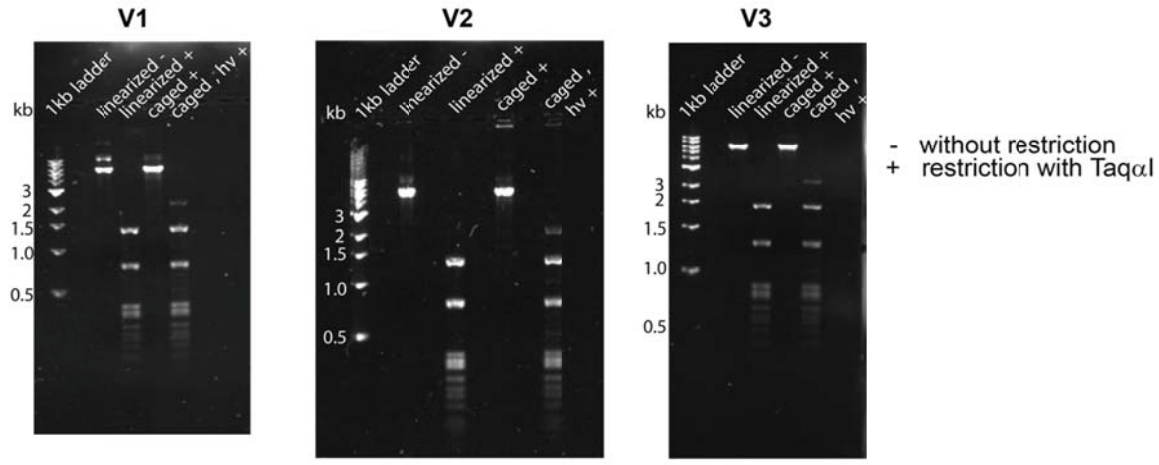


Fig. S17 Modification-restriction assay confirms successful caging and linearization of the plasmid. Uncaging was carried out by illuminating the sample with ligh at 365 nm for 5 min using a LED (M365L2-C1, Thorlabs). Agarose gel electrophoresis (85 V, 60 min) was performed using 1% (w/w) agarose gels (TBE buffer). 1kb DNA Ladder (NEB) was used in order to estimate the molecular weight of the DNA fragments. Bands were visualized by fluorescence through staining with GelRed™ (Biotium).

Table S3. List of TaqαI restriction sites in pET28a-sfYFP (5989 bps); restriction sites highlighted in red are located in the sfYFP gene.

Start	Ende	Size
208	573	**366**
574	976	**403**
977	1250	**274**
1251	2043	**793**
2044	3463	**1420**
3464	4284	**821**
4285	4306	**22**
4307	4665	**359**
4666	4683	**18**
4684	4961	**278**
4692	4969	**278**
4970	6098	**1129**
5099	5435	**337**
5436	5462	**27**
5463	5477	**15**
5478	5707	**230**
5708	5716	**9**
5717	5828	**112**
5829	207	**368**

Fig. S18 Sequence of the sfYFP gene encode in pET28a_sfYFP_**V1** with TaqαI restriction sites marked. T7 promoter and terminator regions are highlighted in red.

Fig. S19 Sequence of the sfYFP gene encode in pET28a_sfYFP_**V2** with Taqᵅ restriction sites marked. T7 promoter and terminator regions are highlighted in red.

Fig. S20 Sequence of the sfYFP gene encode in pET28a_sfYFP_**V3** with Taqᵅ restriction sites marked. T7 promoter and terminator regions are highlighted in red.

In vitro Transcription

PsiI-linearized plasmids pET28a-sfYFP_**V1**–**V3** were used as templates for the HiScribe™ T7 High Yield RNA Synthesis Kit (NEB). *In vitro* transcription was carried out according to the manufacturer's protocol (1000 ng template, 40 µL reaction). Ado4MNB-labeled and unmodified control plasmids were incubated with the enzyme mixture supplied with the kit for 2 h at 37 °C. Prior to incubation, one fraction of the caged as well as of the control plasmid were illuminated with UV-light at 365 nm for 5 min (LED, M365L2-C1, Thorlabs), while the other fraction was excluded from light. After 2 h, the volume of each reaction was adjusted to 100 µL and the transcripts were analyzed by denaturing agarose gel electrophoresis. Therefore, 1 µL of each reaction was mixed 1:1 with RNA Loading Dye (NEB) and applied to a 1 % (w/w) agarose gel containing 6.5 % formaldehyde as well as GelRed™ (Biotium, 10000x in water) for nucleic acid staining to undergo electrophoresis (70 V, 80 min) in 1x MOPS buffer. The bands were visualized by fluorescence using the ChemiDoc™ MP Imaging System (Bio-Rad Laboratories, Inc) and ssRNA Ladder (500 – 9000 bases, NEB) was used in order to estimate the molecular weight of the RNA fragments.

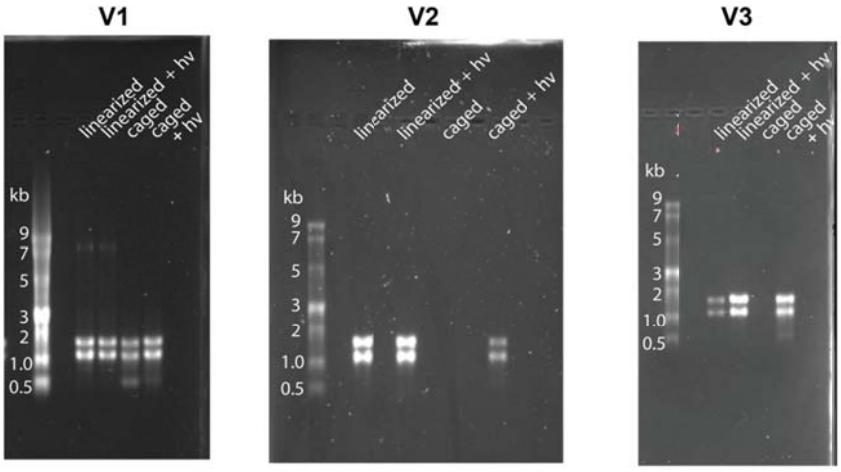


Fig. S21 *In vitro* transcription assay: Introduction of protecting groups by methyltransferease directed transfer of photoactivatable groups reversibly interferes with transcription depending on the position of caging. 4MNB groups distributed over the whole gene (V1) only partly block transcription and since the T7 RNAP can efficiently transcribe past 4MNB-modified sites thereby primarily producing full-length transcripts with some

truncated RNA. However, caging close to the promoter site (V2 & V3) efficiently blocks transcription. (Upper band, run-off transcript. Lower band, termination at T7 terminator.)

In vitro Expression

PsiI-linearized plasmids pET28a-sfYFP_**V1**–**V3** were used as templates for the PurExpress® *In Vitro* Protein Synthesis Kit (NEB). *In vitro expression* was carried out according to the manufacturer's protocol (500 ng template, 50 µL reaction). Ado4MNB-caged and unmodified control plasmids were incubated with the enzyme mixture supplied with the kit for 2 h at 37 °C. Prior to the incubation, a fraction of the caged as well as a fraction of the control plasmid were illuminated with UV-light at 365 nm for 5 min (LED, M365L2-C1, Thorlabs), while the other fractions were excluded from light. After 2 h, a sample of 4.0 µL was taken from each reaction mixure, 2.5 µL 5x SDS loading dye and 8.0 µL H_2O were added. The mixture was heated to 95 °C for 45 s. 7.5 µL of the solution were applied to precasted gels (Mini-PROTEAN TGX, 4-20%, Bio-Rad) to undergo gel electrophoresis (45 mV, 40 min) in SDS running buffer. The bands were visualized by fluorescence using the ChemiDoc™ MP Imaging System (Bio-Rad Laboratories, Inc). Gel band intensities were quantified using Image Lab™ 5.0 Quantity Tools (Bio-Rad Laboratories, Inc) and normalized to the fluorescence of the linearized (non-illuminated) control sample.

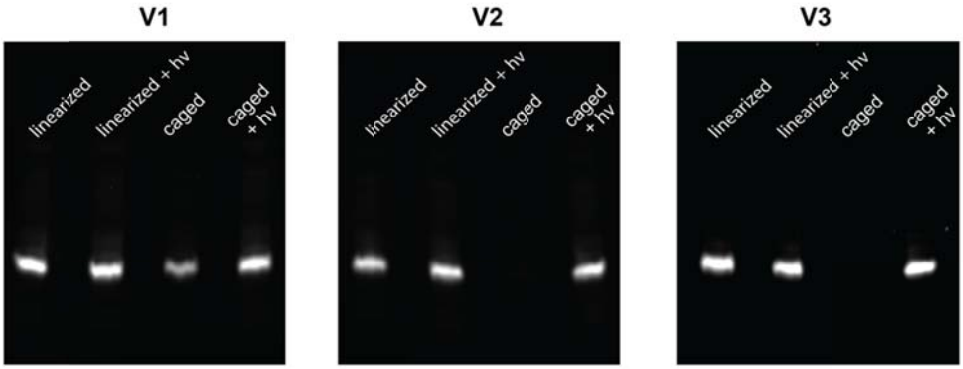


Fig. S22 *In vitro* expression assay: In-gel YFP fluorescence was used as a reporter for gene expression. Introduction of protecting groups by methyltransferease directed transfer of photoactivatable groups reversibly interferes with gene expression. Depending on the plasmid used, caging allows to shut down the of the reporter gene partly (V1) or completely (V2 and V3). Uncaging restores the level of YFP expression to its initial value.

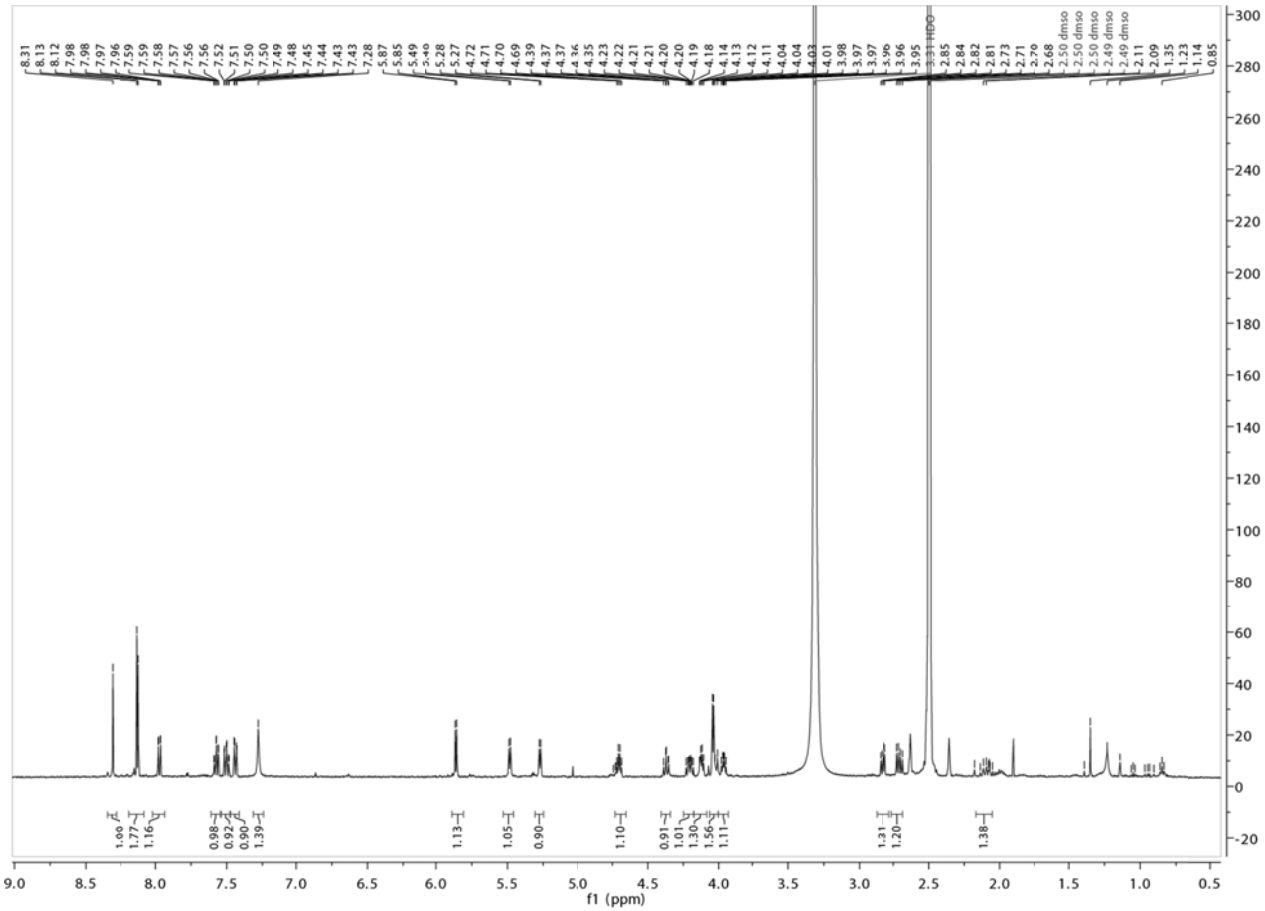


Fig. S23 ^{1}H-NMR (500 MHz) of AdoNB (**2**).

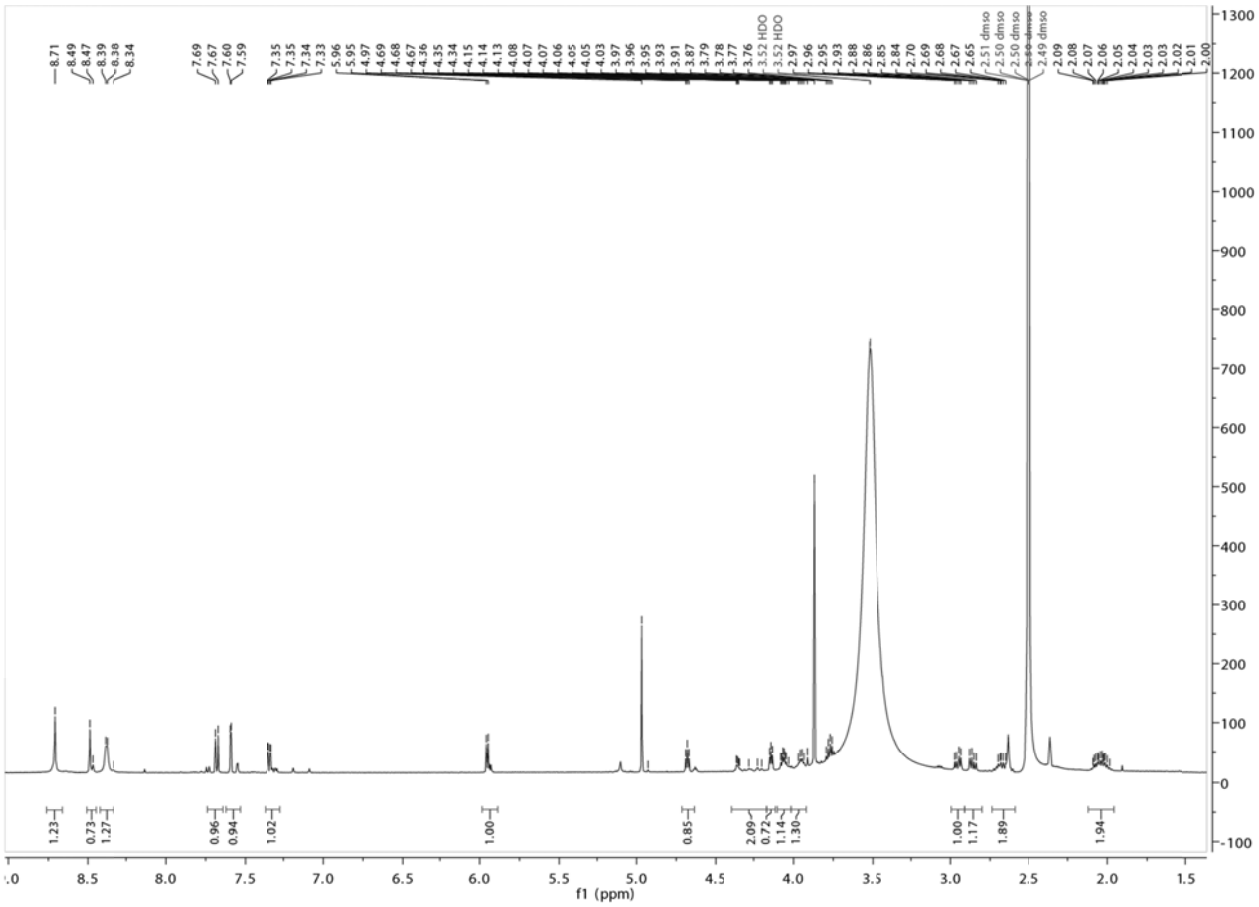


Fig. S24 ^{1}H-NMR (500 MHz) of Ado4MNB (**3**).

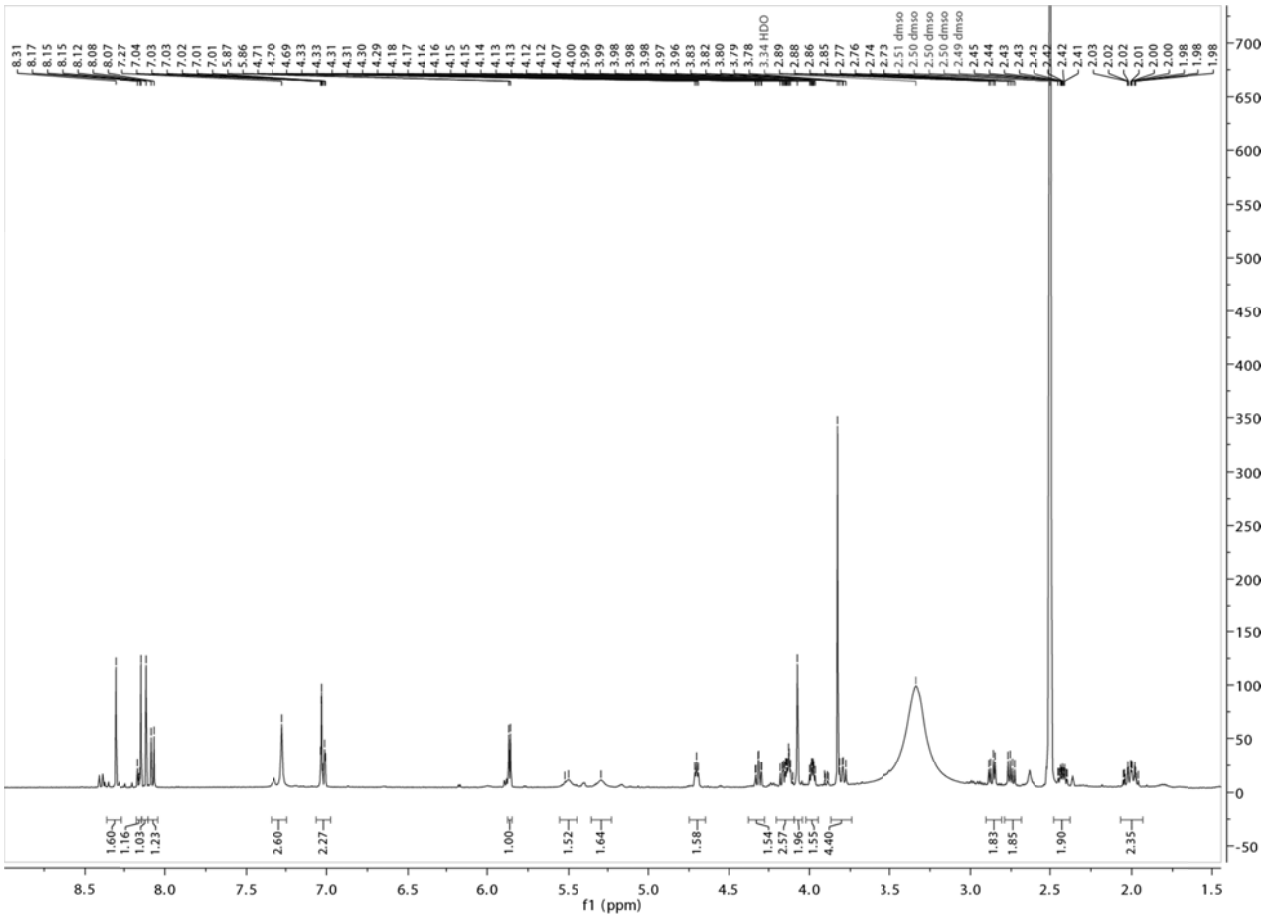


Fig. S25 ^{1}H-NMR (500 MHz) of Ado5MNB (**4**).

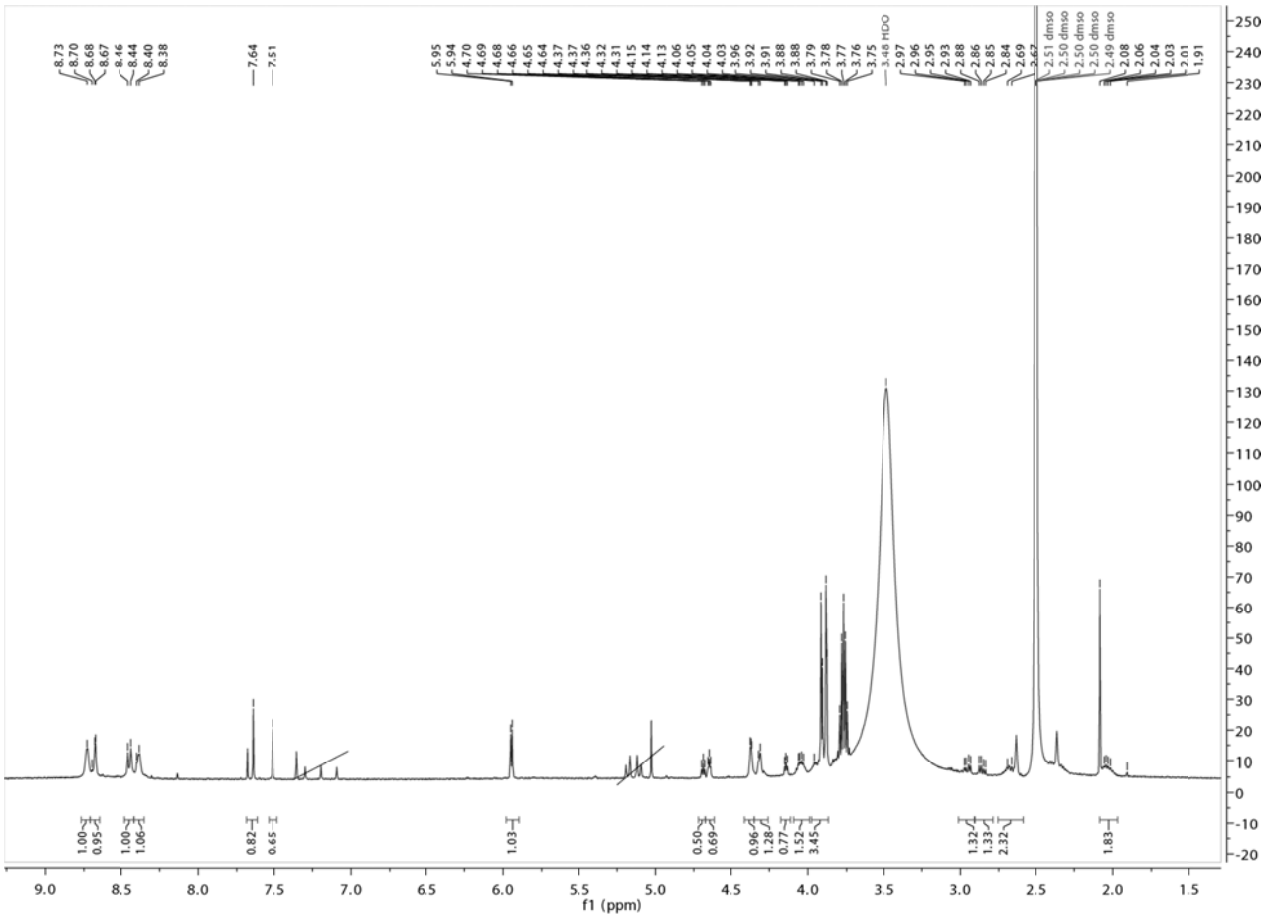


Fig. S26 ^{1}H-NMR (500 MHz) of AdoDMNB (**5**).

References:

[1] Y. Ju, D. Miao, R. Yu, S. Koo, *Org. Biomol. Chem.* **2015**, *13*, 2588–2599.
[2] C. Dalhoff, G. Lukinavičius, S. Klimašauskas, E. Weinhold, *Nat. Protoc.* **2006**, *1*, 1879–1886.
[3] I. R. Bothwell, M. Luo, *Org. Lett.* **2014**, *16*, 3056–3059.
[4] D. F. Iwig, S. J. Booker, *Biochemistry (Mosc.)* **2004**, *43*, 13496–13509.
[5] K. Goedecke, M. Pignot, R. S. Goody, A. J. Scheidig, E. Weinhold, *Nat. Struct. Mol. Biol.* **2001**, *8*, 121–125.
[6] C. Dalhoff, G. Lukinavičius, S. Klimasauskas, E. Weinhold, *Nat. Chem. Biol.* **2006**, *2*, 31–32.
[7] C. Brieke, G. Yim, M. Peschke, G. D. Wright, M. J. Cryle, *Chem. Commun.* **2016**, *52*, 13679–13682.