Efficient Synthesis of *S*-Adenosyl-L-Homocysteine Natural Product Analogues and Their Use to Elucidate the Structural Determinant for Cofactor Binding of the DNA Methyltransferase M·*Hha*I

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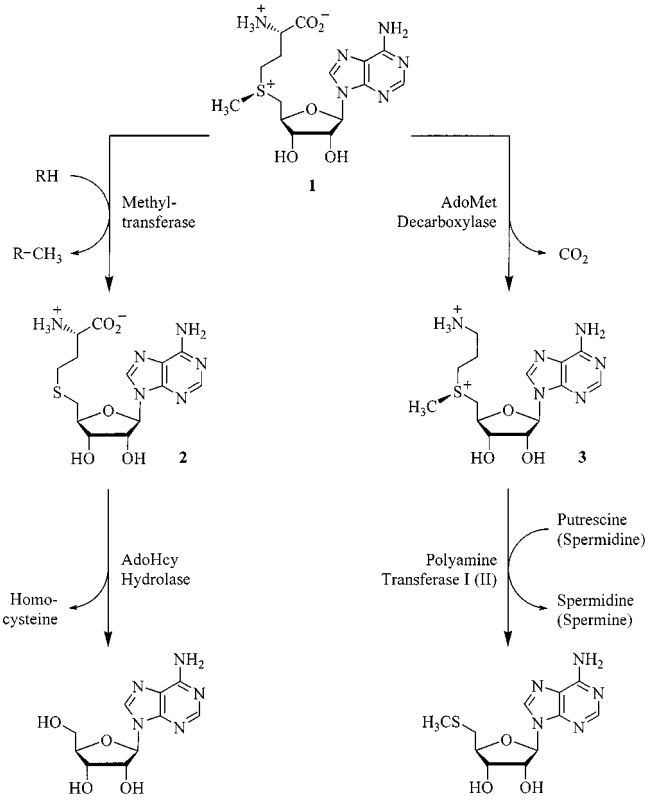
5′-Acetylthio-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**8**) was directly prepared from commercially available 2′,3′-*O*-isopropylideneadenosine (**7**) and thioacetic acid under Mitsunobu conditions in almost quantitative yield. In situ cleavage of the acetylthio function of **8** followed by coupling with different alkyl bromides proceeded with high yields. Deprotection of the obtained 5′-thionucleosides yielded the *S*-adenosyl-L-homocysteine analogues decarboxylated AdoHcy (**11**), deaminated AdoHcy (**14**) and 5′-[3-(cyano)propylthio]-5′-deoxyadenosine (**16**) in good overall yields. Direct deprotection of the thionucleoside **8** delivered 5′-thio-5′-deoxyadenosine (**18**) in excellent yield. In addition, binding constants of these AdoHcy analogues and the DNA methyltransferase M·*Hha*I were determined in a fluorescence assay.

Introduction

Nucleosides containing 5′-sulfur substituents are found in a number of natural products and serve important functions in the cell. The most prominent representative is the cofactor *S*-adenosyl-L-methionine (**1**, AdoMet), which is the major methyl group donor in a myriad of methyltransferase-catalysed reactions (Scheme 1).[1] The transmethylation reaction yields the cofactor product *S*-adenosyl-L-homocysteine (**2**, AdoHcy), which is a potent inhibitor of methyltransferases and is removed by *S*-adenosyl-L-homocysteine hydrolase.[2] In addition, **1** is involved in the polyamine biosynthesis. Decarboxylation of **1** by *S*-adenosyl-L-methionine decarboxylase leads to decarboxylated AdoMet (**3**), which serves as aminopropyl donor for the synthesis of spermidine and spermine.[3]

Because of the biological importance and the variety of the *S*-adenosyl-L-methionine metabolism, a great number of nucleosides containing 5′-sulfur atoms were synthesized. They could serve as pharmacologically valuable inhibitors for *S*-adenosyl-L-homocysteine hydrolase,[4] *S*-adenosyl-L-methionine decarboxylase,[5] and the aminopropyl transferases spermidine and spermine synthase (polyamine transferase I and II).[6] Among these compounds, structurally related analogues of AdoHcy showed significant inhibitory effects on *S*-adenosyl-L-methionine-dependent methyltransferases.[7] Recently, the human C5-cytosine DNA methyltransferase was suggested as a potential target for drug design, because hypermethylation of upstream regulatory sequences leads to an inactivation of tumor suppressor genes in numerous cancers.[8] Thus, inhibitors of the human DNA methyltransferase could reverse the effects of DNA methylation and could have therapeutic value in the treatment of cancer. For these important applications of AdoHcy analogues, efficient synthetic routes leading to this class of compounds are needed.



Scheme 1. Biological transformations of *S*-adenosyl-L-methionine (**1**)

In order to elucidate the structural determinant for cofactor binding of DNA methyltransferases we revisited the

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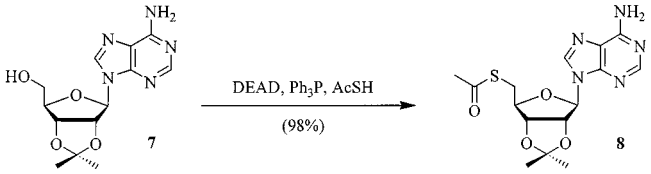
synthesis of AdoHcy analogues carrying different deletions in the amino acid portion and report a very efficient synthetic strategy.

In general, there are two synthetic routes towards the formation of the 5′-thioether bond in AdoHcy and its analogues (Scheme 2). Route A starts from 2′,3′-*O*-isopropylidene-5′-tosyladenosine (**4**)[9] or 5′-chloro-5′-deoxyadenosine (**5**),[10] in which the 5′-hydroxy group is converted into a good leaving group. The adenosine 5′-thioether is then formed by nucleophilic substitution with different thiols in the presence of aqueous sodium hydroxide or formed in situ with sodium in liquid ammonia from appropriate precursors. However, this route is particularly problematic with 5′-tosylates as starting material, because cycloadducts can form by an intramolecular attack of the N^3 nitrogen atom of the adenine ring.[11] In addition, the reaction conditions can lead to other side reactions like depurination.[12] All these factors result in mediocre yields in the coupling step. Route B starts with 5′-acetylthio-5′-deoxy-N^6-formyl-2′,3′-*O*-isopropylideneadenosine (**6**), which is prepared from the corresponding 5′-tosylate using potassium thioacetate.[13] The formation of the cycloadduct in this reaction is suppressed by acylation of the adenine amino group.[14] The 5′-thioacetate **6** is first cleaved in situ under basic conditions and then treated with an appropriate alkyl halide to yield the desired adenosine 5′-thioether. If oxygen is rigorously excluded from the reaction mixture, excellent yields can be obtained for the coupling reaction using this route.[15] Unfortunately, route B is longer than route A, because the 5′-thioacetate **6** needs to be prepared from 2′,3′-*O*-isopropylideneadenosine (**7**) in three steps.[16] Alternatively, 5′-acetylthio-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**8**), which was prepared from the protected adenosine derivative **7** in two steps, can be used in route B.[17]

Results and Discussion

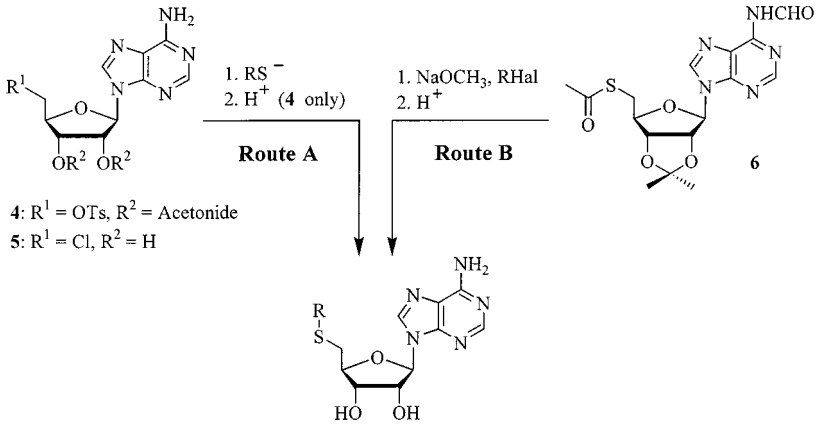
We present a generally applicable synthesis for AdoHcy analogues modified in their amino acid portion, improving route B. The key step of the synthesis is the direct formation of the thionucleoside **8** from commercially available, protected adenosine derivative **7** and thioacetic acid under standard Mitsunobu conditions[18] without previous protection of the amino group of the adenine ring and activation of the 5′-hydroxy group (Scheme 3). This one-step procedure is shorter than the reported syntheses of the 5′-thioacetates **6** or **8** and leads, after chromatographic purification, to a nearly quantitative yield.



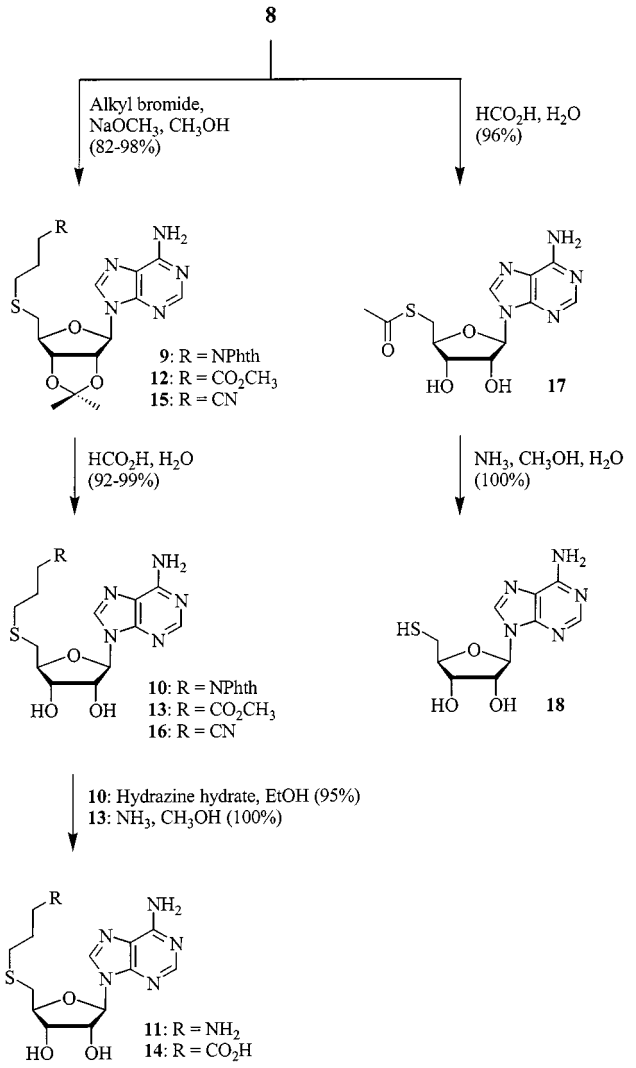
Scheme 3. One-step synthesis of the protected thionucleoside **8**

Coupling of the protected thionucleoside **8** with *N*-(3-bromopropyl)phthalimide was achieved after in situ cleavage of the acetylthio function with $NaOCH_3$ in MeOH under rigorous exclusion of oxygen (Scheme 4). Exclusion of oxygen is necessary, because traces of oxygen can lead to disulfide bond formation of the thiol produced in situ.[19] The protected AdoHcy analogue **9** was obtained in 82% yield. Acid-catalysed hydrolysis of the isopropylidene acetal was accomplished with aqueous formic acid to yield the protected AdoHcy analogue **10**. Decarboxylated AdoHcy (**11**) was then obtained by hydrazinolysis of the phthalimide function. Our overall yield of the AdoHcy analogue **11** from the protected thionucleoside **8** (72%) is considerably higher than the yields reported for route A from the tosylate **4** (48%)[20] or from the chloride **5** (37%),[13b] and is comparable to the yield reported for route B starting from the thioacetate **6** (80%).[15]

The versatility of the protected thionucleoside **8** as an intermediate in the synthesis of AdoHcy analogues is readily demonstrated by the excellent coupling yields with methyl 4-bromobutyrate and 4-bromobutyronitrile in addition to *N*-(3-bromopropyl)phthalimide (Scheme 4). The coupling products **12** and **15** were obtained in 89% and 98% yield, respectively. Removal of the isopropylidene protecting groups in aqueous formic acid delivered the nucleosides **13** and **16** in nearly quantitative yields. Deaminated AdoHcy



Scheme 2. Synthetic routes towards *S*-adenosyl-L-homocysteine (**2**) and analogues with different side chains



Scheme 4. Synthesis of *S*-adenosyl-L-homocysteine analogues with different side chains

(**14**) was then obtained quantitatively by hydrolysis of the methyl ester **13** in a mixture of aqueous ammonia and methanol. The overall yield of the AdoHcy analogue **14** from protected thionucleoside **8** was 85%, which is better than the yield obtained by route A from the tosylate **4** (72%).[20] An additional advantage of our strategy is that methyl 4-bromobutyrate is commercially available, while *S*-benzyl-4-thiobutyric acid used in the preparation of the AdoHcy analogue **14** by route A has to be synthesized first. The AdoHcy analogue **16** was prepared for the first time and can be regarded as an analogue of deaminated AdoHcy (**14**) in which the carboxylate group, a strong hydrogen bond acceptor, is replaced by a nitrile group, a weak hydrogen bond acceptor.

The protected thionucleoside **8** is also a very useful intermediate for the synthesis of 5′-deoxy-5′-thioadenosine (**18**). The known synthesis procedures are either awkward[21] or lead to poor yields of the thionucleoside **18**.[22] The synthesis sequence presented in this publication (Scheme 4) allows for the synthesis of the thionucleoside **18** in a simple way with nearly quantitative overall yield. In our approach, the acetonide group of the protected thionucleoside **8** was first removed in aqueous formic acid, and the resulting 5′-acetylthio-5′-deoxyadenosine (**17**) was then hydrolyzed under oxygen-free conditions using a mixture of methanol and water saturated with ammonia. After removal of the solvent by lyophilisation, the thionucleoside **18** was obtained as a white foam, which does not undergo disulfide bond formation under exposure to air, as verified by analytical reversed-phase HPLC.

In addition, the AdoHcy analogues **11**, **14**, **16** and **18** with different side chains were used to investigate the thermodynamic contribution of the amino acid side chain of AdoHcy to the cofactor binding of a methyltransferase. Methyltransferases share a conserved catalytic domain structure and bind the cofactor in a very similar way.[23] For our cofactor binding studies, we chose the C5-cytosine DNA methyltransferase from *Haemophilus haemolyticus* (M·*Hha*I). This enzyme contains a single tryptophan residue (Trp 41), which interacts with the adenine ring of the bound cofactor.[24] Interestingly, its fluorescence intensity is highly quenched upon binding of the cofactor, which offers a convenient assay to measure cofactor binding of M·*Hha*I. Fluorescence titrations with AdoHcy (**2**) and its truncated analogues **11**, **14**, **16** and **18** are shown in Figure 1, and the obtained binding constants are summarised in Table 1. Decarboxylated AdoHcy (**11**) binds to M·*Hha*I with a 78-fold reduced affinity compared to AdoHcy (**2**). This corresponds to a loss in free binding energy of 10.8 kJ mol^{-1} and indicates that the carboxylate group of AdoHcy forms multiple hydrogen bonds with M·*Hha*I. In fact, three hydrogen bonds were found between the carboxylate group of the cofactor and M·*Hha*I in the three-dimensional structure of the binary complex.[24] In contrast to the decarboxylated AdoHcy (**11**), deaminated AdoHcy (**14**) binds only with a 17-fold reduced affinity compared to AdoHcy (**2**). This smaller reduction in affinity is also in agreement with the binary structure of M·*Hha*I in complex with the cofactor, where the amino group is only hydrogen-bonded to the enzyme through two water molecules.[24] Further replacement of the carboxylate group by a nitrile group in the AdoHcy analogue **16** does lead to a similar binding affinity. Since the nitrile group should be a weaker hydrogen bond acceptor compared to the carboxylate group, this result is surprising. However, in contrast to deaminated AdoHcy (**14**), the AdoHcy analogue **16** should not be charged under the assay conditions (pH = 7.4) and thus the similar binding constants could result from a compensating hydrophobic effect.[25] Most interestingly, the adenosine derivative **18** is also bound with quite good affinity. Although the fact that its affininty is only 43-fold reduced, compared to AdoHcy (**2**), could also be partly explained by a hydrophobic effect, this result suggests that the molecular anchor for cofactor binding of M·*Hha*I is the adenosyl part of the cofactor. Due to the high structural homology of the catalytic domains of methyltransferases, it is expected that the adenosyl part of the cofactor also functions as the struc-

tural determinant for cofactor binding of other methyltransferases.

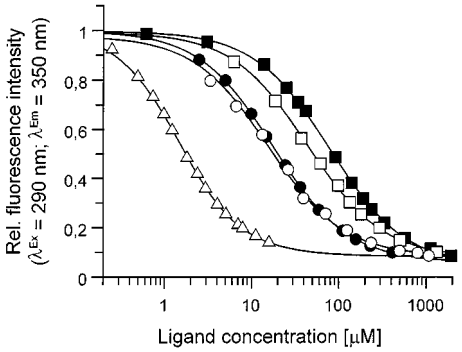


Figure 1. Fluorescence titrations of the DNA methyltransferase M·*Hha*I (1 μM) with increasing amounts of AdoHcy (**2**) (open triangles) and the AdoHcy analogues **16** (open circles), **14** (closed circles), **18** (open squares) and **11** (closed squares)

Table 1. Binding of AdoHcy (**2**) and its analogues **11**, **14**, **16**, and **18** to the DNA methyltransferase M·*Hha*I

Ligand	Dissociation constant K_D[a] [μM]	$\Delta\Delta G$[b] [kJ/mol]
2	1.0 ± 0.1	
16	15 ± 2.0	6.7
14	17 ± 0.3	7.0
18	43 ± 2.4	9.3
11	78 ± 3.0	10.8

[a] Titration data from Figure 1 were fitted to the real solution of the quadratic binding equation for one binding site and errors are given as 2σ standard deviations. – [b] Calculated from $\Delta\Delta G = RT\ln[K_D(\text{AdoHcy analogue})/K_D(\text{AdoHcy})]$.

Conclusion

A variety of AdoHcy analogues with different side chains was prepared in good overall yields from 5′-acetylthio-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**8**), which is accessible from commercially available 2′,3′-*O*-isopropylideneadenosine (**7**) in a single step with excellent yield. The described synthetic strategy of coupling a 5′-deoxy-5′-thioadenosine derivative with an alkyl halide (route B) results in better yields compared to other strategies starting with 5′-activated adenosine derivatives and alkylthiols (route A), and should be most useful for the synthesis of AdoHcy analogues, for which a corresponding halide of the side chain component is commercially available. In addition, binding of the synthesised AdoHcy analogues to the DNA methyltransferase M·*Hha*I was investigated using a fluorescence assay. From these binding studies we conclude that the molecular anchor for cofactor binding of M·*Hha*I and most likely of other methyltransferases as well is the adenosyl part of the cofactor.

Experimental Section

General Remarks: All chemicals were of reagent quality and were used without further purification. Solvents were dried and distilled according to literature procedures.[26] 2′,3′-*O*-Isopropylideneadenosine, *N*-(3-bromopropyl)phthalimide, 4-bromobutyronitrile and methyl 4-bromobutyrate were purchased from Aldrich. – NMR: Bruker AX 500 (500 MHz and 125.7 MHz, for ^{1}H and ^{13}C, respectively). For ^{1}H NMR, $CDCl_3$ as solvent, tetramethylsilane (TMS) as internal standard, D_2O as solvent, 3-trimethylsilyl-[2,2,3,3-D_4]propionic acid sodium salt (TSP) as internal standard, [D_6]DMSO as solvent δ_H = 2.49; for ^{13}C NMR, $CDCl_3$ as solvent, TMS as internal standard, D_2O as solvent, TSP as internal standard, [D_6]DMSO as solvent δ_C = 39.5. Assignment of ^{13}C signals are based on ^{1}H,^{13}C-correlated 2D-NMR and on ^{13}C-DEPT spectra. All NMR spectra were recorded at ambient probe temperature. The nomenclature used to denote side chain atoms follows the nomenclature of AdoHcy (**2**).[27] – Electrospray MS: Finnigan LCQ connected to a nanoelectrospray ion source.[28] For measurements in the positive ion mode samples were dissolved in CH_3OH/water (1:1 v/v) containing 5% formic acid; for measurements in the negative ion mode a mixture of CH_3OH/water (1:1 v/v) was used. FAB-MS: Finnigan MAT 8200 (70 eV, thioglycolic acid matrix). – TLC: DC–aluminia plates Sil–G60/UV_{254} (Merck). Compounds were visualized by treatment with a solution of ammonium molybdate (15 g), cerium(IV) sulfate (0.4 g) in 10% sulfuric acid (300 mL) and heating with a hot air blower. – Flash chromatography: Merck silica gel 60 (40–63 μm). – Reversed-phase HPLC: Analytical HPLC analysis was carried out using a C-18 column (ODS Hypersil, 5 μm, 120 Å, 250 × 4.6 mm, Bischoff), flow rate 1 mL min^{-1}, UV detection at 259 nm. Preparative runs were performed with a C-18 column (ODS YMC-Pack, 5 μm, 120 Å, 250 × 20 mm, YMC), flow rate 10 mL min^{-1}, UV detection at 259 and 290 nm. Standard buffer conditions: Buffer A [triethylammonium acetate (0.1 M, pH = 7.0)] and buffer B [CH_3CN containing buffer A (30%)]. Standard gradient conditions: Gradient 1: 20% buffer B (0–10 min), 20–100% buffer B (10–30 min); gradient 2: 40% buffer B (0–10 min), 40–60% buffer B (10–15 min), 60% buffer B (15–25 min), 60–100% buffer B (25–35 min); gradient 3: 10% buffer B (0–10 min), 10–20% buffer B (10–30 min), 20–100% buffer B (30–45 min); gradient 4: 0% buffer B (0–10 min), 0–20% buffer B (10–30 min), 20–100% buffer B (30–45 min), 100% buffer B (45–50 min); gradient 5: 20% buffer B (0–5 min), 20–40% buffer B (5–15 min), 40% buffer B (15–25 min), 40–100% buffer B (25–45 min); gradient 6: 7% buffer B (0–10 min), 7–40% buffer B (10–40 min), 40–100% buffer B (40–50 min), 100% buffer B (50–60 min). – Elemental analyses: LECO CHNS-932. Since the accuracy of the standards (4-aminobenzenesulfonic acid, *N*-phenylacetamide; Merck) in the elemental analyses was 0.1%, the analyses of compounds are given with the same accuracy. – Fluorescence titrations: Titrations were performed with an SLM-Aminco 8100 fluorescence spectrometer. The excitation and emission wavelengths were set to 290 nm and 350 nm, respectively, and the excitation and emission bandwidths were adjusted to 1 and 16 nm, respectively.

5′-Acetylthio-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (8): To an ice-cold solution of triphenylphosphane (5.7 g, 21.6 mmol) in absol. THF (30 mL), diethyl azodicarboxylate (3.4 mL, 21.6 mmol) was added over 5 min. After stirring for 30 min, 2′,3′-*O*-isopropylideneadenosine (**7**) (3.0 g, 9.8 mmol) was added, and stirring was continued for 10 min. To the resulting yellow suspension a solution of thioacetic acid (1.6 mL, 21.6 mmol) in absol. THF (5 mL) was added dropwise and stirring was continued for another 1 h at 0 °C. During this time the yellow suspension cleared, and an orange solution was obtained. At the end of the reaction the solvent was removed under reduced pressure, and the resulting yellowish residue was purified by flash chromatography on silica gel [350 g, $CHCl_3$/

THF (4:1 v/v) and then $CHCl_3/CH_3OH$ (9:1 v/v)]. The fractions containing the product were combined, the solvent was removed under reduced pressure, and the residue was dried in vacuo (0.01 mbar) to yield pure protected thionucleoside **8** (3.5 g, 98%) as a yellowish foam. − R_f (CH_2Cl_2/CH_3OH, 9:1 v/v) = 0.57. − ^{1}H NMR ($CDCl_3$): δ = 1.39 (s, 3 H, CH_3), 1.60 (s, 3 H, CH_3), 2.34 (s, 3 H, $COCH_3$), 3.19 and 3.30 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H} = J_{5'b\text{-}H,4'\text{-}H}$ = 6.9 Hz, J_{gem} = 13.7 Hz, 2 H, 5′a-H, 5′b-H), 4.35 (dt, $J_{4'\text{-}H,3'\text{-}H}$ = 2.9 Hz, $J_{4'\text{-}H,5'a\text{-}H} = J_{4'\text{-}H,5'b\text{-}H}$ = 6.9 Hz, 1 H, 4′-H), 4.99 (dd, $J_{3'\text{-}H,4'\text{-}H}$ = 2.9 Hz, $J_{3'\text{-}H,2'\text{-}H}$ = 6.2 Hz, 1 H, 3′-H), 5.53 (dd, $J_{2'\text{-}H,1'\text{-}H}$ = 2.0 Hz, $J_{2'\text{-}H,3'\text{-}H}$ = 6.4 Hz, 1 H, 2′-H), 6.09 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 2.2 Hz, 1 H, 1′-H), 6.36 (s, br., 2 H, 6-NH_2), 7.92 (s, 1 H, 8-H), 8.36 (s, 1 H, 2-H). − ^{13}C NMR ($CDCl_3$): δ = 25.38 (q, CH_3), 27.10 (q, CH_3), 30.57 (q, $CO\mathit{C}H_3$), 31.31 (t, C-5′), 83.75 (d, C-3′), 84.22 (d, C-2′), 86.16 (d, C-4′), 90.93 (d, C-1′), 114.50 (s, $\mathit{C}(CH_3)_2$), 120.30 (s, C-5), 139.92 (d, C-8), 149.18 (s, C-4), 153.20 (d, C-2), 155.92 (s, C-6), 194.57 (s, CO). − ESI-MS; *m/z* (%): 366.2 (100) $[M + H]^+$. − $C_{15}H_{19}N_5O_4S$ (365.4): calcd. C 49.3, H 5.2, N 19.2; found C 49.5, H 5.3, N 18.8.

5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[3-(phthalimido)propylthio]-adenosine (9): Protected thionucleoside **8** (500 mg, 1.37 mmol) and *N*-(3-bromopropyl)phthalimide (536 mg, 1.99 mmol) were added to oxygen-free absol. CH_3OH (30 mL) under Ar. The suspension was cooled to −20°C, $NaOCH_3$ (165 mg, 3.06 mmol) was added, and the mixture was allowed to warm up slowly to room temperature. After 30 min, all material had dissolved, and the resulting pale yellow solution was stirred for about 12 h. The solvent was removed under reduced pressure, and the resulting residue was extracted with $CHCl_3$/water (3 × 30 mL). The organic layers were combined, the solvent was removed under reduced pressure, and the resulting white foam was purified on silica gel [100 g, CH_2Cl_2/CH_3OH (9:1 v/v)]. The fractions containing the product were combined, the solvent was removed under reduced pressure, and the residue was dried in vacuo (0.01 mbar) to yield **9** (571 mg, 82%) as a white foam with a purity of 98% according to reversed-phase HPLC analysis. For the analytical HPLC an aliquot of **9** was dissolved in buffer B (100 μL), injected onto the column and eluted using gradient 1. Nucleoside **9** eluted with a retention time of 27.4 min. − R_f = (CH_2Cl_2/CH_3OH, 9:1 v/v) = 0.62. − ^{1}H NMR ($CDCl_3$): δ = 1.34 (s, 3 H, CH_3), 1.55 (s, 3 H, CH_3), 1.86 (quint, $J_{H\beta,H\gamma} = J_{H\beta,H\alpha}$ = 7.2 Hz, 2 H, Hβ), 2.51 (t, $J_{H\gamma,H\beta}$ = 7.3 Hz, 2 H, Hγ), 2.75 and 2.83 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H} = J_{5'b\text{-}H,4'\text{-}H}$ = 6.8 Hz, J_{gem} = 13.6 Hz, 2 H, 5′a-H, 5′b-H), 3.68 (t, $J_{H\alpha,H\beta}$ = 7.0 Hz, 2 H, Hα), 4.33 (dt, $J_{4'\text{-}H,3'\text{-}H}$ = 3.3 Hz, $J_{4'\text{-}H,5'a\text{-}H} = J_{4'\text{-}H,5'b\text{-}H}$ = 6.7 Hz, 1 H, 4′-H), 5.02 (dd, $J_{3'\text{-}H,4'\text{-}H}$ = 3.3 Hz, $J_{3'\text{-}H,2'\text{-}H}$ = 6.4 Hz, 1 H, 3′-H), 5.45 (dd, $J_{2'\text{-}H,1'\text{-}H}$ = 2.2 Hz, $J_{2'\text{-}H,3'\text{-}H}$ = 6.2 Hz, 1 H, 2′-H), 6.05 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 2.2 Hz, 1 H, 1′-H), 6.48 (s, br., 2 H, 6-NH_2), 7.62−7.64 (m, 2 H, arom. H), 7.76−7.78 (m, 2 H, arom. H), 7.91 (s, 1 H, 8-H), 8.28 (s, 1 H, 2-H). − ^{13}C NMR ($CDCl_3$): δ = 25.36 (q, CH_3), 27.11 (q, CH_3), 28.37 (t, Cβ), 29.92 (t, Cγ), 34.34 (t, C-5′), 36.94 (t, Cα), 83.78 (d, C-3′), 84.06 (d, C-2′), 86.71 (d, C-4′), 90.82 (d, C-1′), 114.59 [s, $\mathit{C}(CH_3)_2$], 120.35 (s, C-5), 123.29 (d, 2 × arom. C), 132.07 (s, 2 × arom. C), 133.98 (d, 2 × arom. C), 140.05 (d, C-8), 149.25 (s, C-4), 153.20 (d, C-2), 155.55 (s, C-6), 168.30 (s, 2 × CO). − FAB-MS (70 eV); *m/z* (%): 511 (75) $[M^+ + H]$, 376 (25) $[M^+ - B]$, 188 (100) $[PhthN(CH_2)_3]$, 164 (22) $[B + CH_2O]$, 136 (88) $[BH_2^+]$; B = deprotonated adenine.

5′-Deoxy-5′-[3-(phthalimido)propylthio]adenosine (10): Nucleoside **9** (200 mg, 0.39 mmol) was dissolved in an ice-cold formic acid/water mixture (4 mL, 1:1) and the solution was allowed to warm up slowly to room temperature. The reaction was followed by analytical reversed-phase HPLC. Aliquots of the reaction solution (2 μL) were diluted with buffer B (18 μL) and injected onto the column. Compounds were eluted with gradient 1. The hydrolysis product **10** eluted with a retention time of 21.2 min. After stirring at room temperature for 19 h the reaction was complete, and the solvent was evaporated under reduced pressure. Traces of formic acid were removed by coevaporation with absol. ethanol (5 × 5 mL). The resulting solid material was purified by preparative reversed-phase HPLC using gradient 2. After purification, **10** (168 mg, 92%) was obtained as a white powder. − R_f (CH_2Cl_2/CH_3OH, 9:1 v/v) = 0.37. − ^{1}H NMR ([D_6]DMSO): δ = 1.81 (quint, $J_{H\beta,H\gamma} = J_{H\beta,H\alpha}$ = 7.0 Hz, 2 H, Hβ), 2.52−2.55 (m, 2 H, Hγ), 2.81 and 2.89 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H} = J_{5'b\text{-}H,4'\text{-}H}$ = 6.4 Hz, J_{gem} = 13.7 Hz, 2 H, 5′a-H, 5′b-H), 3.60 (t, $J_{H\alpha,H\beta}$ = 6.9 Hz, 2 H, Hα), 3.97−4.00 (m, 1 H, 4′-H), 4.12 (q, $J_{3'\text{-}H,4'\text{-}H} = J_{3'\text{-}H,2'\text{-}H} = J_{3'\text{-}H,3'\text{-}OH}$ = 4.5 Hz, 1 H, 3′-H), 4.72 (q, $J_{2'\text{-}H,1'\text{-}H} = J_{2'\text{-}H,3'\text{-}H} = J_{2'\text{-}H,2'\text{-}OH}$ = 5.5 Hz, 1 H, 2′-H), 5.31 (d, $J_{3'\text{-}OH,3'\text{-}H}$ = 4.8 Hz, 1 H, D_2O-exchangeable, 3′-OH), 5.48 (d, $J_{2'\text{-}OH,2'\text{-}H}$ = 6.0 Hz, 1 H, D_2O-exchangeable, 2′-OH), 5.86 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 6.0 Hz, 1 H, 1′-H), 7.26 (s, br., 2 H, D_2O-exchangeable, 6-NH_2), 7.78−7.83 (m, 4 H, arom. H), 8.14 (s, 1 H, 2-H), 8.32 (s, 1 H, 8-H). − ^{13}C NMR ([D_6]DMSO): δ = 28.51 (t, Cβ), 29.71 (t, Cγ), 34.37 (t, C-5′), 37.05 (t, Cα), 73.06 (d, C-3′), 73.09 (d, C-2′), 84.40 (d, C-4′), 87.86 (d, C-1′), 119.63 (s, C-5), 123.45 (d, 2 × arom. C), 132.13 (s, 2 × arom. C), 134.78 (d, 2 × arom. C), 140.31 (d, C-8), 149.93 (s, C-4), 153.14 (d, C-2), 156.53 (s, C-6), 168.44 (s, 2 × CO). − ESI-MS; *m/z* (%): 471.1 (100) $[M + H]^+$.

5′-[3-(Amino)propylthio]-5′-deoxyadenosine (11): Nucleoside **10** (50 mg, 106 μmol) was suspended in absol. ethanol (2 mL), and the mixture was heated to 50°C until **10** was completely dissolved. Then, hydrazine hydrate (16.1 μL, 330 μmol) was added dropwise, and the reaction mixture was stirred at 80°C for 3.5 h. The hydrazinolysis was followed by analytical reversed-phase HPLC. Water (8 μL) was added to aliquots of the reaction mixture (2 μL), the solution was injected onto the column, and compounds were eluted with gradient 3. The deprotected nucleoside **11** eluted after 30.7 min. After complete deprotection, the solution was kept at room temperature for about 12 h, the formed precipitate was removed by filtration, and the filtrate was concentrated in vacuo (10 mbar). The resulting yellow oil was dissolved in water (3 mL) and purified by preparative reversed-phase HPLC using gradient 4. After lyophilisation, **11** (34 mg, 95%) was obtained as a white fluffy solid. − ^{1}H NMR (D_2O): δ = 1.91 (quint, $J_{H\beta,H\gamma} = J_{H\beta,H\alpha}$ = 7.4 Hz, 2 H, Hβ), 2.60−2.66 (m, 2 H, Hγ), 2.96 and 3.05 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H} = J_{5'b\text{-}H,4'\text{-}H}$ = 6.1 Hz, J_{gem} = 14.3 Hz, 2 H, 5′a-H, 5′b-H), 3.03 (t, $J_{H\alpha,H\beta}$ = 8.0 Hz, 2 H, Hα), 4.34 (dt, $J_{4'\text{-}H,3'\text{-}H}$ = 5.1 Hz, $J_{4'\text{-}H,5'a\text{-}H} = J_{4'\text{-}H,5'b\text{-}H}$ = 6.3 Hz, 1 H, 4′-H), 4.44 (t, $J_{3'\text{-}H,4'\text{-}H} = J_{3'\text{-}H,2'\text{-}H}$ = 5.1 Hz, 1 H, 3′-H), 4.88 (t, $J_{2'\text{-}H,1'\text{-}H} = J_{2'\text{-}H,3'\text{-}H}$ = 5.1 Hz, 1 H, 2′-H), 6.08 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 5.1 Hz, 1 H, 1′-H), 8.24 (s, 1 H, 2-H), 8.34 (s, 1 H, 8-H). − ^{13}C NMR (D_2O): δ = 26.56 (t, Cβ), 28.82 (t, Cγ), 33.60 (t, C-5′), 38.35 (t, Cα), 72.36 (d, C-3′), 73.33 (d, C-2′), 83.40 (d, C-4′), 87.59 (d, C-1′), 118.76 (s, C-5), 140.10 (d, C-8), 148.90 (s, C-4), 152.93 (d, C-2), 155.56 (s, C-6). − ESI-MS; *m/z* (%): 341.2 (100) $[M + H]^+$.

5′-Deoxy-2′,3′-*O*-isopropylidene-5′-[3-(methoxycarbonyl)-propylthio]adenosine (12): To a solution of the protected thionucleoside **8** (267 mg, 0.73 mmol) and methyl 4-bromobutyrate (199 mg, 1.02 mmol) in oxygen-free absol. CH_3OH (15 mL) was added $NaOCH_3$ (87 mg, 1.61 mmol). The mixture was stirred at room temperature for about 12 h. The solvent was removed under reduced pressure, and the resulting yellow oil was extracted with $CHCl_3$/water (3 × 50 mL). The organic layers were combined, the solvent was removed under reduced pressure, and the resulting crude product was purified on silica gel [30 g, CH_2Cl_2/CH_3OH (9:1 v/v)]. The

fractions containing the product were combined and yielded, after evaporation of the solvent and subsequent drying in vacuo (0.01 mbar), **12** (275 mg, 89%) as a glassy foam. – R_f = (CH_2Cl_2/CH_3OH, 9:1 v/v) = 0.59. – ^1H NMR ($CDCl_3$): δ = 1.34 (s, 3 H, CH_3), 1.56 (s, 3 H, CH_3), 1.79 (quint, $J_{H\beta,H\gamma} = J_{H\beta,H\alpha}$ 7.2 Hz, 2 H, Hβ), 2.32 (t, $J_{H\alpha,H\beta}$ = 7.2 Hz, 2 H, Hα), 2.49 (t, $J_{H\gamma,H\beta}$ = 7.2 Hz, 2 H, Hγ), 2.72 and 2.80 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H} = J_{5'b\text{-}H,4'\text{-}H}$ = 6.8 Hz, J_{gem} = 13.5 Hz, 2 H, 5′a-H, 5′b-H), 3.60 (s, 3 H, OCH_3), 4.33 (dt, $J_{4'\text{-}H,3'\text{-}H}$ = 3.1 Hz, $J_{4'\text{-}H,5'a\text{-}H} = J_{4'\text{-}H,5'b\text{-}H}$ = 6.7 Hz, 1 H, 4′-H), 5.02 (dd, $J_{3'\text{-}H,4'\text{-}H}$ = 3.1 Hz, $J_{3'\text{-}H,2'\text{-}H}$ = 6.7 Hz, 1 H, 3′-H), 5.47 (dd, $J_{2'\text{-}H,1'\text{-}H}$ = 2.1 Hz, $J_{2'\text{-}H,3'\text{-}H}$ = 6.7 Hz, 1 H, 2′-H), 6.05 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 2.1 Hz, 1 H, 1′-H), 6.51 (s, br., 2 H, 6-NH_2), 7.91 (s, 1 H, 8-H), 8.28 (s, 1 H, 2-H). – ^{13}C NMR ($CDCl_3$): δ = 24.41 (t, Cβ), 25.13 (q, CH_3), 26.88 (q, CH_3), 31.54 (t, Cγ), 32.37 (t, Cα), 33.91 (t, C-5′), 51.38 (q, OCH_3), 83.62 (d, C-3′), 83.84 (d, C-2′), 86.59 (d, C-4′), 90.62 (d, C-1′), 114.17 [s, $C(CH_3)_2$], 120.02 (s, C-5), 139.77 (d, C-8), 148.92 (s, C-4), 152.92 (d, C-2), 155.86 (s, C-6), 173.14 (s, CO). – ESI-MS; *m/z* (%): 424.1 (100) $[M + H]^+$.

5′-Deoxy-5′-[3-(methoxycarbonyl)propylthio]adenosine (13): Nucleoside **12** (161 mg, 0.38 mmol) was dissolved in a formic acid/water mixture (4 mL, 1:1), and the mixture was stirred at room temperature for 19 h. The solvent was evaporated under reduced pressure and traces of formic acid were removed by coevaporation with absol. EtOH (4 × 5 mL). The resulting white solid was purified by preparative reversed-phase HPLC using gradient 5 to yield **13** (138 mg, 95%) as a white powder. – R_f = (CH_2Cl_2/CH_3OH, 8:2 v/v) = 0.73. – ^1H NMR (D_2O): δ = 1.59–1.66 (m, 2 H, Hβ), 2.20 (t, $J_{H\alpha,H\beta}$ = 7.4 Hz, 2 H, Hα), 2.36–2.42 (m, 2 H, Hγ), 2.80 and 2.88 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H} = J_{5'b\text{-}H,4'\text{-}H}$ = 6.5 Hz, J_{gem} = 14.3 Hz, 2 H, 5′a-H, 5′b-H), 3.51 (s, 3 H, OCH_3), 4.20 (dt, $J_{4'\text{-}H,3'\text{-}H}$ = 4.9 Hz, $J_{4'\text{-}H,5'a\text{-}H} = J_{4'\text{-}H,5'b\text{-}H}$ = 6.5 Hz, 1 H, 4′-H), 4.31 (t, $J_{3'\text{-}H,4'\text{-}H} = J_{3'\text{-}H,2'\text{-}H}$ = 5.3 Hz, 1 H, 3′-H), 4.77 (t, $J_{2'\text{-}H,1'\text{-}H} = J_{2'\text{-}H,3'\text{-}H}$ = 5.1 Hz, 1 H, 2′-H), 5.93 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 5.1 Hz, 1 H, 1′-H), 8.09 (s, 1 H, 2-H), 8.20 (s, 1 H, 8-H). – ^{13}C NMR (D_2O): δ = 24.06 (t, Cβ), 31.56 (t, Cγ), 32.48 (t, Cα), 33.48 (t, C-5′), 52.14 (q, OCH_3), 72.35 (d, C-3′), 73.24 (d, C-2′), 83.74 (d, C-4′), 87.53 (d, C-1′), 119.15 (s, C-5), 140.15 (d, C-8), 148.93 (s, C-4), 152.82 (d, C-2), 155.50 (s, C-6), 176.41 (s, CO). – ESI-MS; *m/z* (%): 384.1 (100) $[M + H]^+$.

5′-[3-(Carboxy)propylthio]-5′-deoxyadenosine (14): Nucleoside **13** (147 mg, 0.38 mmol) was dissolved in CH_3OH (2.8 mL), and ice-cold aqueous ammonia (3 mL, 33%) was added. The solution was stirred at 0°C for 2 h and then at room temperature for about 12 h. The solvent was removed under reduced pressure to yield **14** (140 mg, 100%) as a white solid. – ^1H NMR ([D_6]DMSO): δ = 1.80 (quint, $J_{H\beta,H\gamma} = J_{H\beta,H\alpha}$ = 7.4 Hz, 2 H, Hβ), 2.20 (t, $J_{H\alpha,H\beta}$ = 7.4 Hz, 2 H, Hα), 2.58–2.61 (m, 2 H, Hγ), 2.90 and 3.00 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H} = J_{5'b\text{-}H,4'\text{-}H}$ = 6.5 Hz, J_{gem} = 13.9 Hz, 2 H, 5′a-H, 5′b-H), 4.10 (dt, $J_{4'\text{-}H,3'\text{-}H}$ = 3.7 Hz, $J_{4'\text{-}H,5'a\text{-}H} = J_{4'\text{-}H,5'b\text{-}H}$ = 6.2 Hz, 1 H, 4′-H), 4.23–4.25 (m, 1 H, 3′-H), 4.85 (q, $J_{2'\text{-}H,3'\text{-}H} = J_{2'\text{-}H,1'\text{-}H} = J_{2'\text{-}H,2'\text{-}OH}$ = 5.6 Hz, 1 H, 2′-H), 5.42 (d, $J_{3'\text{-}OH,3'\text{-}H}$ = 5.1 Hz, 1 H, D_2O-exchangeable, 3′-OH), 5.59 (d, $J_{2'\text{-}OH,2'\text{-}H}$ = 6.0 Hz, 1 H, D_2O-exchangeable, 2′-OH), 5.99 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 5.6 Hz, 1 H, 1′-H), 7.37 (s, br., 2 H, D_2O-exchangeable, 6-NH_2), 8.25 (s, 1 H, 2-H), 8.45 (s, 1 H, 8-H). – ^{13}C NMR ([D_6]DMSO): δ = 25.33 (t, Cβ), 31.67 (t, Cγ), 34.08 (t, Cα, C-5′), 72.80 (d, C-3′, C-2′), 84.08 (d, C-4′), 87.52 (d, C-1′), 119.34 (s, C-5), 140.03 (d, C-8), 149.68 (s, C-4), 152.87 (d, C-2), 156.26 (s, C-6), 173.88 (s, CO). – ESI-MS; *m/z* (%): 368.3 (100) $[M - H]^-$.

5′-[3-(Cyano)propylthio]-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (15): Protected thionucleoside **8** (220 mg, 0.60 mmol) and 4-bromobutyronitrile (114 mg, 0.77 mmol) were suspended under a continuous stream of Ar in oxygen-free absol. CH_3OH (10 mL). The suspension was frozen with liquid nitrogen, and $NaOCH_3$ (66 mg, 1.21 mmol) was added. To eliminate traces of oxygen, the reaction mixture was repeatedly (5 ×) thawed under vacuum (0.01 mbar) and finally allowed to warm up to room temperature. The suspension cleared after 15 min, and stirring was continued for 3.5 h. The solvent was removed under reduced pressure and the obtained material was purified on silica gel [25 g, CH_2Cl_2/CH_3OH (9:1 v/v)] at 4°C. The fractions containing the product were combined and yielded, after removal of the solvent and drying in vacuo (0.01 mbar), **15** (230 mg, 98%) as a glassy foam. – ^1H NMR ($CDCl_3$): δ = 1.37 (s, 3 H, CH_3), 1.59 (s, 3 H, CH_3), 1.81 (quint, $J_{H\beta,H\gamma} = J_{H\beta,H\alpha}$ = 7.2 Hz, 2 H, Hβ), 2.40 (t, $J_{H\alpha,H\beta}$ = 7.2 Hz, 2 H, Hα), 2.60 (t, $J_{H\gamma,H\beta}$ = 7.2 Hz, 2 H, Hγ), 2.77 and 2.84 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H} = J_{5'b\text{-}H,4'\text{-}H}$ = 7.0 Hz, J_{gem} = 13.9 Hz, 2 H, 5′a-H, 5′b-H), 4.35 (dt, $J_{4'\text{-}H,3'\text{-}H}$ = 3.7 Hz, $J_{4'\text{-}H,5'a\text{-}H} = J_{4'\text{-}H,5'b\text{-}H}$ = 6.8 Hz, 1 H, 4′-H), 5.04 (dd, $J_{3'\text{-}H,4'\text{-}H}$ = 3.4 Hz, $J_{3'\text{-}H,2'\text{-}H}$ = 6.5 Hz, 1 H, 3′-H), 5.49 (dd, $J_{2'\text{-}H,1'\text{-}H}$ = 2.4 Hz, $J_{2'\text{-}H,3'\text{-}H}$ = 6.4 Hz, 1 H, 2′-H), 6.04–6.06 (m, 3 H, 1′-H, 6-NH_2), 7.90 (s, 1 H, 8-H), 8.32 (s, 1 H, 2-H). – ^{13}C NMR ($CDCl_3$): δ = 15.87 (t, Cα), 25.10 (t, Cβ), 25.32 (q, CH_3), 27.07 (q, CH_3), 31.09 (t, Cγ), 34.28 (t, C-5′), 83.77 (d, C-3′), 83.99 (d, C-2′), 86.98 (d, C-4′), 90.83 (d, C-1′), 114.60 [s, $C(CH_3)_2$], 118.93 (s, CN), 120.32 (s, C-5), 140.27 (d, C-8), 149.10 (s, C-4), 152.61 (d, C-2), 155.43 (s, C-6). – ESI-MS; *m/z* (%): 391.1 (100) $[M + H]^+$. – $C_{17}H_{22}N_6O_3S$ (390.5): calcd. C 52.3, H 5.7, N 21.5; found C 51.9, H 5.8, N 21.3.

5′-[3-(Cyano)propylthio]-5′-deoxyadenosine (16): Nucleoside **15** (52 mg, 0.13 mmol) was dissolved in a formic acid/water mixture (2 mL, 1:1), and the solution was stirred at room temperature for 17 h. The solvent was evaporated under reduced pressure, and traces of formic acid were removed by coevaporation with absol. EtOH (3 × 4 mL). Preparative reversed-phase HPLC purification using gradient 5 yielded **16** (46.2 mg, 99%) as a white powder. – ^1H NMR ([D_6]DMSO): δ = 1.78 (quint, $J_{H\beta,H\gamma} = J_{H\beta,H\alpha}$ = 7.2 Hz, 2 H, Hβ), 2.50 (t, $J_{H\alpha,H\beta}$ = 7.2 Hz, 2 H, Hα), 2.55–2.60 (m, 2 H, Hγ), 2.87 and 2.90 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H} = J_{5'b\text{-}H,4'\text{-}H}$ = 6.5 Hz, J_{gem} = 13.9 Hz, 2 H, 5′a-H, 5′b-H), 3.99 (dt, $J_{4'\text{-}H,3'\text{-}H}$ = 3.9 Hz, $J_{4'\text{-}H,5'a\text{-}H} = J_{4'\text{-}H,5'b\text{-}H}$ = 6.3 Hz, 1 H, 4′-H), 4.13–4.15 (m, 1 H, 3′-H), 4.73–4.75 (m, 1 H, 2′-H), 5.32 (s, br., 1 H, D_2O-exchangeable, 3′-OH), 5.50 (s, br., 1 H, D_2O-exchangeable, 2′-OH), 5.88 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 5.7 Hz, 1 H, 1′-H), 7.33 (s, br., 2 H, D_2O-exchangeable, 6-NH_2), 8.16 (s, 1 H, 2-H), 8.35 (s, 1 H, 8-H). – ^{13}C NMR ([D_6]DMSO): δ = 15.43 (t, Cα), 25.10 (t, Cβ), 30.69 (t, Cγ), 33.89 (t, C-5′), 72.80 (d, C-3′, C-2′), 84.13 (d, C-4′), 87.62 (d, C-1′), 119.36 (s, CN), 120.36 (s, C-5), 140.23 (d, C-8), 149.63 (s, C-4), 152.65 (d, C-2), 156.11 (s, C-6). – ESI-MS; *m/z* (%): 351.2 (100) $[M + H]^+$. – $C_{14}H_{18}N_6O_3S$ (350.4): calcd. C 48.0, H 5.2, N 24.0; found C 47.5, H 5.3, N 23.5.

5′-Acetylthio-5′-deoxyadenosine (17): A solution of **8** (200 mg, 0.54 mmol) in a mixture of formic acid and water (10 mL, 1:1) was stirred at room temperature. The progress of the reaction was monitored by analytical reversed-phase HPLC. Aliquots of the reaction solution (5 μL) were diluted with buffer B (100 μL), and 5 μL of the dilution were injected onto the column. Using gradient 6 the hydrolysis product **17** eluted with a retention time of 22.4 min. After 44 h reaction time the solvent was evaporated under reduced pressure, and traces of formic acid were removed by coevaporating five times with absol. ethanol. The obtained white powder was purified on silica gel [30 g, CH_2Cl_2/CH_3OH (4:1 v/v)]. The fractions containing the product were combined, the solvent was removed

under reduced pressure, and the product was dried in vacuo (0.01 mbar) for 2 d to yield **17** (169 mg, 96%) as a white powder. – R_f (CH_2Cl_2/CH_3OH, 9:1 v/v) = 0.26. – ^{1}H NMR ([D$_6$]DMSO): δ = 2.32 (s, 3 H, $COCH_3$), 3.15 and 3.34 (AB part of ABX spectrum, $J_{5'a\text{-}H,4'\text{-}H}$ = 5.6 Hz, $J_{5'b\text{-}H,4'\text{-}H}$ = 7.4 Hz, J_{gem} = 13.9 Hz, 2 H, 5′a-H, 5′b-H), 3.91 (ddd, $J_{4'\text{-}H,3'\text{-}H}$ = 3.7 Hz, $J_{5'a\text{-}H,4'\text{-}H}$ = 5.8 Hz, $J_{5'b\text{-}H,4'\text{-}H}$ = 7.5 Hz, 1 H, 4′-H), 4.08–4.10 (m, 1 H, 3′-H), 4.78 (q, $J_{2'\text{-}H,1'\text{-}H}$ = $J_{2'\text{-}H,3'\text{-}H}$ = $J_{2'\text{-}H,2'\text{-}OH}$ = 6.1 Hz, 1 H, 2′-H), 5.37 (d, $J_{3'\text{-}OH,3'\text{-}H}$ = 5.1 Hz, 1 H, D$_2$O-exchangeable, 3′-OH), 5.51 (d, $J_{2'\text{-}OH,2'\text{-}H}$ = 6.1 Hz, 1 H, D$_2$O-exchangeable, 2′-OH), 5.86 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 5.6 Hz, 1 H, 1′-H), 7.29 (s, br., 2 H, D$_2$O-exchangeable, 6-NH_2), 8.14 (s, 1 H, 2-H), 8.28 (s, 1 H, 8-H). – ^{13}C NMR ([D$_6$]DMSO: δ = 30.83 (q, CO*C*H$_3$), 31.59 (t, C-5′), 72.91 (d, C-3′), 72.97 (d, C-2′), 83.23 (d, C-4′), 87.84 (d, C-1′), 119.57 (s, C-5), 140.33 (d, C-8), 149.79 (s, C-4), 153.02 (d, C-2), 156.45 (s, C-6), 195.20 (s, CO). – ESI-MS; *m/z* (%): 326.2 (100) [M + H]$^+$. – $C_{12}H_{15}N_5O_4S \bullet 0.5\ H_2O$ (334.4): calcd. C 43.1, H 4.8, N 20.9; found C 42.9, H 4.9, N 20.5.

5′-Deoxy-5′-thioadenosine (18): To eliminate traces of oxygen, a mixture of CH_3OH/water (5:2) was repeatedly (7 ×) frozen with liquid nitrogen and allowed to thaw under vacuum (0.01 mbar). Ammonia was then passed through this mixture for 5 min. Nucleoside **17** (50 mg, 0.16 mmol) was dissolved in this ammonia-saturated CH_3OH/water mixture (7 mL) under Ar, and the reaction mixture was stirred at 0°C. The progress of the reaction was monitored by analytical reversed-phase HPLC. Aliquots of the reaction solution (5 μL) were diluted with buffer B (45 μL), and 7 μL of the dilution were injected onto the column. Using gradient 6, the deprotected nucleoside **17** eluted with a retention time of 19.2 min. After 1.5 h the reaction mixture was frozen with liquid nitrogen, and the solvent was removed by lyophilisation to yield **18** (45 mg, 100%) as a white foam with a purity of 99% according to reversed-phase HPLC analysis. – R_f (CH_2Cl_2/CH_3OH, 7:3 v/v) = 0.85. – ^{1}H NMR ([D$_6$]DMSO): δ = 2.52 (s, br., 1 H, D$_2$O-exchangeable, 5′-SH), 2.77–2.86 (m, 2 H, 5′a-H, 5′b-H), 3.95 (dt, $J_{4'\text{-}H,3'\text{-}H}$ = 3.3 Hz, $J_{4'\text{-}H,5'a\text{-}H}$ = $J_{4'\text{-}H,5'b\text{-}H}$ = 6.1 Hz, 1 H, 4′-H), 4.16 (q, $J_{3'\text{-}H,2'\text{-}H}$ = $J_{3'\text{-}H,4'\text{-}H}$ = $J_{3'\text{-}H,3'\text{-}OH}$ = 4.7 Hz, 1 H, 3′-H), 4.76 (q, $J_{2'\text{-}H,1'\text{-}H}$ = $J_{2'\text{-}H,3'\text{-}H}$ = $J_{2'\text{-}H,2'\text{-}OH}$ = 5.8 Hz, 1 H, 2′-H), 5.28 (d, $J_{3'\text{-}OH,3'\text{-}H}$ = 4.9 Hz, 1 H, D$_2$O-exchangeable, 3′-OH), 5.46 (d, $J_{2'\text{-}OH,2'\text{-}H}$ = 6.1 Hz, 1 H, D$_2$O-exchangeable, 2′-OH), 5.87 (d, $J_{1'\text{-}H,2'\text{-}H}$ = 6.1 Hz, 1 H, 1′-H), 7.26 (s, br., 2 H, D$_2$O-exchangeable, 6-NH_2), 8.14 (s, 1 H, 2-H), 8.34 (s, 1 H, 8-H). – ^{13}C NMR ([D$_6$]DMSO): δ = 26.44 (t, C-5′), 71.85 (d, C-3′), 72.52 (d, C-2′), 85.36 (d, C-4′), 87.26 (d, C-1′), 119.17 (s, C-5), 139.92 (d, C-8), 149.41 (s, C-4), 152.57 (d, C-2), 156.03 (s, C-6). – ESI-MS; *m/z* (%): 284.1 (100) [M + H]$^+$. – $C_{10}H_{13}N_5O_3S \bullet 0.5\ H_2O$ (292.3): calcd. C 41.1, H 4.8, N 23.9; found C 41.0, H 4.8, N 23.5.

Fluorescence Titrations: The DNA methyltransferase M·*Hha*I was kindly provided by Dr. Saulius Klimašauskas, Vilnius, Lithuania, and prepared as previously described.[29] Titrations of M·*Hha*I (1 μM) with increasing amounts AdoHcy (**2**) and its analogues **11**, **14**, **16**, and **18** were performed in 10 mM Tris hydrochloride buffer (pH = 7.4) containing 50 mM sodium chloride, 0.5 mM ethylenediaminetetraacetic acid and 2 mM β-mercaptoethanol at 25°C. Titration data (fluorescence intensities as a function of total AdoHcy or analogue concentrations) were fitted to the real solution of the quadratic binding equation for one binding site[30] using the data analysis program GraFit.[31]

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