Synthesis of Two Stable Nitrogen Analogues of *S*-Adenosyl-L-methionine

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Homochiral syntheses of two stable nitrogen analogues of *S*-adenosyl-L-methionine (AdoMet) are described. In the first analogue, AzaAdoMet, the sulfonium center of AdoMet, is replaced by an *N*-methyl moiety whose pK_a is 7.08. This provides a charge-switchable analogue of AdoMet whose ionic state is a function of the pH. A second analogue, MeAzaAdoMet, has a quaternary dimethylammonium group in place of the methylsulfonium center of AdoMet: thus, its ionic state is independent of pH.

Introduction

S-Adenosyl-L-methionine (AdoMet, Figure 1) **1** is an essential coenzyme involved in many biochemical processes, most notably as a methyl group donor. Stable isosteric analogues of AdoMet having variable charge character at the 5′-locus and that cannot donate a methyl group should show a range of activities as inhibitors of AdoMet-dependent methyl-transfer enzymes and thereby support mechanistic and crystallographic studies of such proteins. Moreover, the stability of such analogues relative to AdoMet should contribute to an understanding of the hydrolytic lability of AdoMet.[1] In a previous paper,[2] we described the first homochiral synthesis of the azadesthio analogue of **1**, AzaAdoMet **2**, and demonstrated that in its protonated form, this analogue binds to the *E. coli* methionine repressor protein (MetJ) by 1 order of magnitude more tightly than does AdoMet itself. The unusually low pK_a of AzaAdoMet, 7.08, permits the compound to be employed as a charge-switchable analogue of **1** through control of pH. However, the switch to AzaAdoMet into its protonated, cationic form requires a pH well below 6.5, which may not be appropriate for some proteins. We now report full details of the homochiral synthesis of both AzaAdoMet **2** and the previously unreported quaternary dimethylammonium analogue MeAzaAdoMet **3**, which bears a positive charge at the 5′-position that is independent of pH.

Results and Discussion

Two previous syntheses[3,4] of **2** used the routes shown in Figure 2. The 5′-methylamino-5′-deoxyadenosine derivative **4** was alkylated with a protected iodide[3,4] **5** or **6** followed by removal of all protecting groups. However, because both iodides **5** and **6** were employed in racemic form, the final analogue **2** was obtained as a mixture of epimers. We sought to modify these syntheses by replacing **5** and **6** with a chiral iodide, leading to homochiral AzaAdoMet **2**.

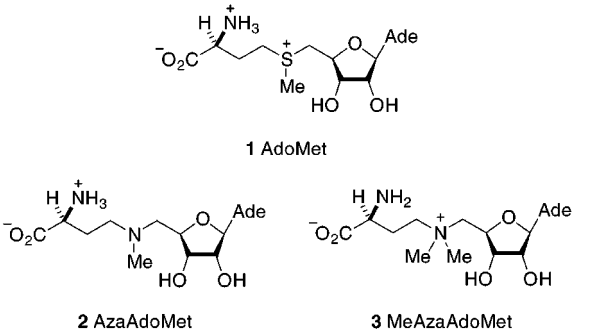

Figure 1. Structure of *S*-adenosyl-L-methionine (AdoMet) and its nitrogen analogues AzaAdoMet **2** and MeAzaAdoMet **3**.

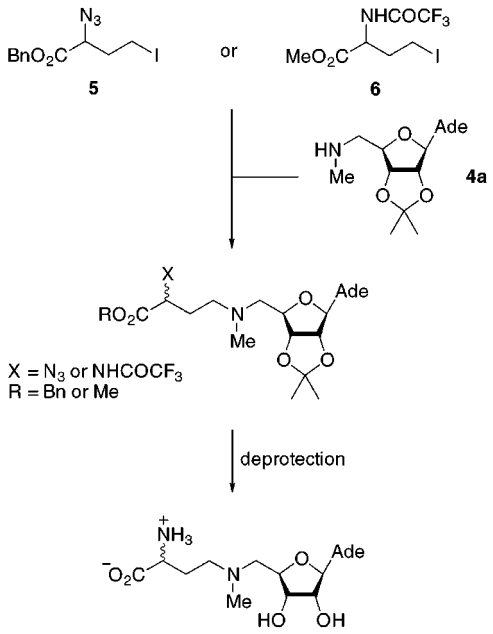

Figure 2. Previous preparations of AzaAdoMet as mixed epimers.[3,4]

Amine **4a** was prepared by a modification of existing procedures (Figure 3). D-Adenosine was protected[5] as the 2′,3′-*O*,*O*-(1-methylethylidene) derivative **7**. Conversion[6]

(1) (a) Parks, L. W.; Schlenk, F. *J. Biol. Chem.* **1958**, *230*, 295. (b) Schlenk, F. In *Biochemistry of Adenosylmethionine*; Salvatore, F., et al., Eds.; Columbia University Press: New York, 1977; p 7.

(2) Thompson, M. J.; Mekhalfia, A.; Jakeman, D. L.; Phillips, S. E. V.; Phillips, K.; Porter, J.; Blackburn, G. M. *J. Chem. Soc., Chem. Commun.* **1996**, 791.

(3) Minnick, A. A.; Kenyon, G. L. *J. Org. Chem.* **1988**, *53*, 4952.

(4) Davis, M.; Dudman, N. P. B.; White, H. F. *Aust. J. Chem.* **1983**, *36*, 1623.

(5) Tomasz, J. In *Synthetic Procedures in Nucleic Acid Chemistry (Vol. 2)*; Tipson, R. S., Zorbach, W. W., Eds.; Wiley-Interscience: New York, 1973; p 765.

(6) Jahn, W. *Chem. Ber.* **1965**, *98*, 1705.

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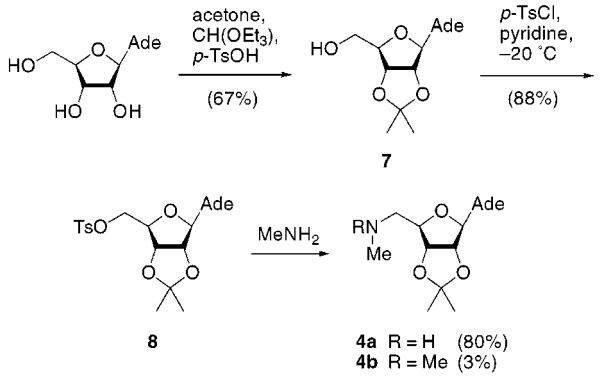

Figure 3. Preparation of 5′-methylamino-5′-deoxy-2′,3′-*O*,*O*-(1-methylethylidene) adenosine **4a** and coproduct **4b** from D-adenosine.

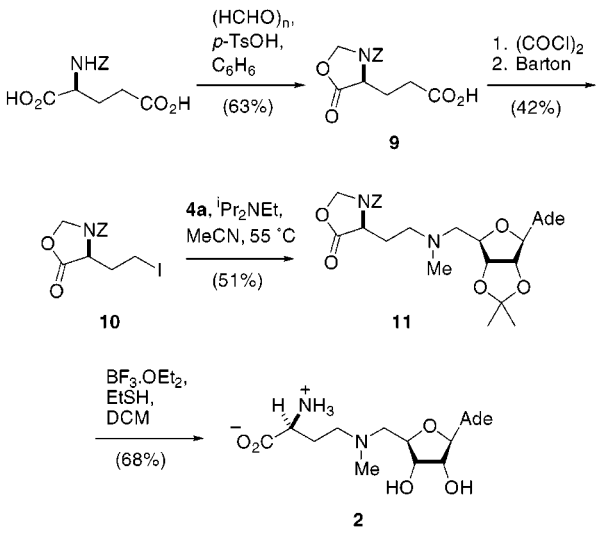

Figure 4. Synthesis of the chiral protected iodide **10** from L-glutamic acid and its use in the preparation of AzaAdoMet **2**.

into 5′-*O*-tosylate **8** was followed by reaction with neat liquid methylamine[7] to give amine **4a** in 46% overall yield. In the reaction of the 5′-*O*-tosylate **8** with methylamine, a 3% yield of the 5′-dimethylamino derivative **4b** was also isolated. This material was identified positively as **4b** by comparison with a sample prepared by the reaction of 5′-*O*-tosylate **8** with dimethylamine. The 5′-*O*-mesylate derivative was also explored but gave considerably lower yields of **4a**.

(*S*)-Glutamic acid was protected[8] as the oxazolidinone **9** (Figure 4), which was treated with oxalyl chloride to afford the corresponding acid chloride and subsequently converted into the alkyl iodide **10** via a Barton-type decarboxylative iodination[9] using 2-iodo-1,1,1-trifluoroethane as iodine donor.[10] While the transformation of (*S*)-glutamic acid into **10** uses procedures of reliable stereochemical integrity, the (*S*)-absolute configuration of **10** was confirmed by anomalous dispersion X-ray crystallography (Figure 5).[11] The yield of **10** was found to be

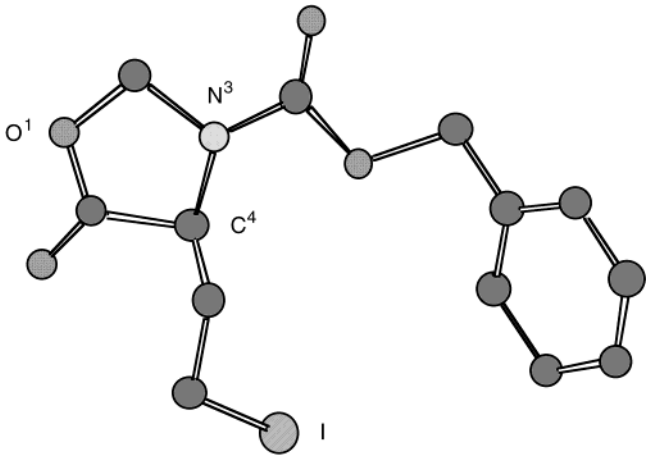

Figure 5. Structure of iodide **10** as determined by X-ray crystallography.

variable largely because of its instability on silica. Direct conversion of **9** into the iodide **10** was attempted using iodobenzene diacetate (IBDA) and iodine.[12] Although this reaction proceeded well, separation of iodide **10** from the iodobenzene formed in the reaction proved difficult and low yields were obtained.

The key intermediates **4a** and **10** were heated together in acetonitrile solution in the presence of *N*,*N*-diisopropylethylamine to give the fully protected analogue **11**. Attempts to remove the benzyloxycarbonyl group from **11** by catalytic hydrogenation with 10% Pd−C proved unsuccessful; reactions were incomplete after several days, and mixtures of products were detected by TLC. Use of freshly prepared Pd black[13] as the catalyst drove the reaction to completion, but these forcing conditions resulted in fragmentation of the molecule. Catalytic transfer hydrogenation using cyclohexene,[14] 1,4-cyclohexadiene,[15] or formic acid[16] as hydrogen donor also afforded mixtures of products. However, treatment of **11** with BF_3−EtSH complex[17] was found to effect complete removal of all protecting groups. The final product AzaAdoMet **2** was purified on Dowex 50WX4-400 ion-exchange resin.[3]

The pK_a of **2** was determined by NMR titration by monitoring the NMe ^{1}H signal (singlet at δ 2.3 at high pH changing to a doublet at δ 2.9 at low pH). The data so obtained (Figure 6) reveal a major shift response to the titration of the tertiary MeN group (pH 5.5−8.5) and a smaller response to a second ionization (pH 8.5−10.5), which we assign to the α-amino function. These data were analyzed using[18] Kaleidagraph to give pK_a values of 7.08 ± 0.07 (>NMe) and 9.66 ± 0.31 (−NH_2). Thus, analogue **2** may be employed either in its protonated (net cationic) form or in its unprotonated (net zwitterionic) form by controlling pH in the range 5.5−8.5. The NMR solutions of **2** (H_2O with 10% D_2O, ionic strength 10 mM, 25 °C) used in these titrations showed no change in the signals

(7) Murayama, A.; Jastorff, B.; Cramer, F.; Hettler, H. *J. Org. Chem.* **1971**, *36*, 3029.
(8) Itoh, M. *Chem. Pharm. Bull.* **1969**, *17*, 1679.
(9) Barton, D. H. R.; Crich, D.; Motherwell, W. B. *Tetrahedron Lett.* **1983**, *24*, 4979.
(10) Eaton, P. E.; Tsanaktsidis, J. *Tetrahedron Lett.* **1989**, *30*, 6967.
(11) Coordinates for this structure are provided in the Supporting Information.
(12) Concepción, J. I.; Francisco, C. G.; Friere, R.; Hernández, R.; Salazar, J. A.; Suárez, E. *J. Org. Chem.* **1986**, *51*, 402.
(13) Jung, M. E. *J. Org. Chem.* **1992**, *57*, 3528.
(14) Jackson, A. E.; Johnstone, R. A. W. *Synthesis* **1976**, 685.
(15) Felix, A. M.; Heimer, E. P.; Lambros, T. J.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1978**, *43*, 4194.
(16) ElAmin, B.; Anantharamaiah, G. M.; Royer, G. P.; Means, G. E. *J. Org. Chem.* **1979**, *44*, 3442.
(17) Bose, D. S.; Thurston, D. E. *Tetrahedron Lett.* **1990**, *31*, 6903.
(18) KaleidaGraph 3.0.5, Abelbeck Software, Reading, PA, 1986−1994.

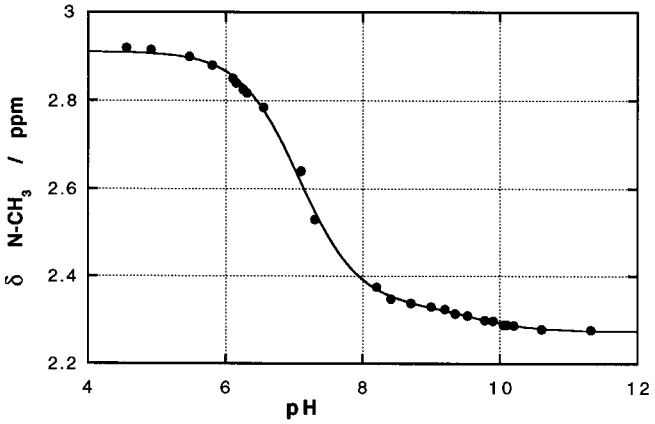

Figure 6. Plot of N-Me ^{1}H chemical shift for **2** vs pH at 25 °C ionic strength 10 mM in H_2O/D_2O (9:1 v/v). Curve calculated for overlapping pK_{a^1} 7.0756 ± 0.076 (step 0.5228 ppm) and pK_{a^2} 9.662 ± 0.259 (step 0.0566 ppm).

either for the nucleoside or for the diaminobutyrate moieties over the course of these titrations or for several months afterward. It thus appears that AzaAdoMet exhibits none of the hydrolytic instability of AdoMet itself either with respect to glycosidic hydrolysis or to homoserine lactone formation,[19] both of which must therefore be attributable to the activity of the sulfonium center in AdoMet.

This charge-switchable nature of AzaAdoMet **2** has been utilized in an investigation of the MetJ−DNA−AdoMet complex,[20] where Phillips proposed[2] that the positive charge at the 5′-position of the corepressor (AdoMet) is the major feature responsible for the enhanced stability of the ternary repressor/corepressor/operator complex relative to the binary repressor/operator complex.[21,22] At pH 7.4, in its uncharged form, the binding of AzaAdoMet to the MetJ protein was found to be very weak. However, at pH 5.5 when the protonated form predominates, the binding of AzaAdoMet to MetJ was found to be 10 times stronger than for AdoMet itself at the same pH.[22] This result amply demonstrates the charge-switchable nature of AzaAdoMet.

An X-ray crystal structure[2] of the resulting binary complex provides independent confirmation of the absolute configuration of **2**. The details of this structure show that for both the natural co-repressor, AdoMet (Figure 7a), and the isosteric analogue, AzaAdoMet **2** (Figure 7b), the glycosylic bond of the bound nucleoside adopts the higher energy *syn*-conformation (+*synclinal*) with torsion angle χ +76° for AdoMet (+77° for AzaAdoMet). The ribose ring pucker is $^0T_4$, with largely O4′-*endo* character. In the case of purine nucleosides adopting the syn conformation, the observed sugar pucker of O4′-*endo* lies at the energy maximum of the interconversion between C2′-*endo* and C3′-*endo*, the C3′-*endo* conformation being 1−2 kcal mol^{-1} higher in energy; hence, C2′-*endo* rather than C3′-*endo* pucker is more often observed in these cases.[23] The O4′-*endo* pucker seen here for protein-bound

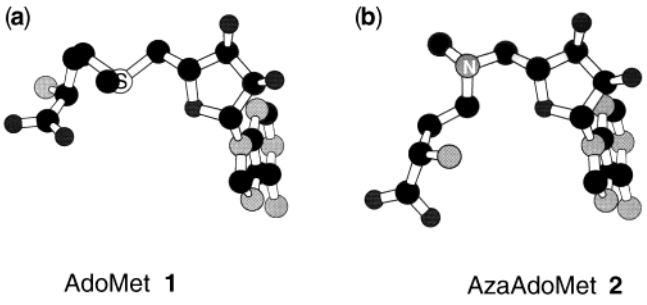

Figure 7. Syn conformations of (**a**) AdoMet **1** and (**b**) AzaAdoMet **2** excised from X-ray structures of binary complexes with MetJ repressor protein.[2]

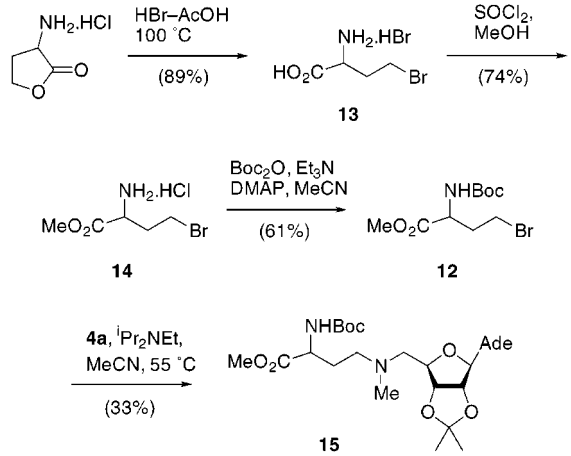

Figure 8. Bromoaminobutyrate **12** was prepared and investigated as an alternative to iodide **10** for synthesis of **2**. This was used to prepare protected AzaAdoMet **15**.

AdoMet and AzaAdoMet **2** lies approximately 6 kcal mol^{-1} above the favored C2′-*endo* conformation, which shows that the MetJ protein binds the corepressor in a high-energy conformation.

In a study of the binding of AzaAdoMet to cobalt precorrin-4-methyltransferase (CbiF), preliminary X-ray analysis of the binary complex shows the nucleoside adopts the more favored *anti* conformation.[24] The observed pucker of CbiF-bound AzaAdoMet is C2′-*exo*-C3′-*endo* ($^3T_2$), very close in energy to the C3′-*endo* minimum. C2′-*endo* and C3′-*endo* are equally populated in *anti*-purine nucleosides, unlike the *syn* case where C2′-*endo* is preferred.

We next sought to improve our synthesis of **2** because of difficulties encountered in the deprotection of **11**, so we explored alternative protecting group strategies. We envisaged that iodide **10** might be replaced by a bromide such as **12** (Figure 8), derived from homoserine lactone (α-amino-γ-butyrolactone). The synthesis[25] of **12** was carried out with racemic lactone in order to evaluate the viability of this route. Ring opening of DL-homoserine lactone under strongly acidic conditions gave α-(2-bromoethyl)glycine[25,26] **13**. Esterification[25,26] to give **14** followed by *N*-Boc protection[25] gave bromide **12** in moderate yield. This compound was then used in the alkylation of amine **4a** under the same conditions as those described above. However, the maximum yield of alkylated product

(19) Hoffman, J. L. *Biochemistry* **1986**, *25*, 4444.
(20) The coordinates of both the MetJ−DNA−AdoMet and MetJ−DNA−AzaAdoMet complexes have been deposited in the Brookhaven Protein Database.
(21) Phillips, K.; Phillips, S. E. V. *Structure* **1994**, *2*, 309.
(22) Parsons, I. D.; Persson, B.; Mekhalfia, M. A.; Blackburn, G. M.; Stockley, P. G. *Nucleic Acids Res.* **1995**, *23*, 211.
(23) Saenger, W. *Principles of Nucleic Acid Structure*; Verlag: New York, 1983; p 69.
(24) Schubert, H. Unpublished results.
(25) Bajgrowicz, J. A.; El Hallaoui, A.; Jacquier, R.; Pigière, C.; Viallefont, P. *Tetrahedron Lett.* **1984**, *25*, 2231.
(26) Koch, T.; Buchardt, O. *Synthesis* **1993**, 1065.

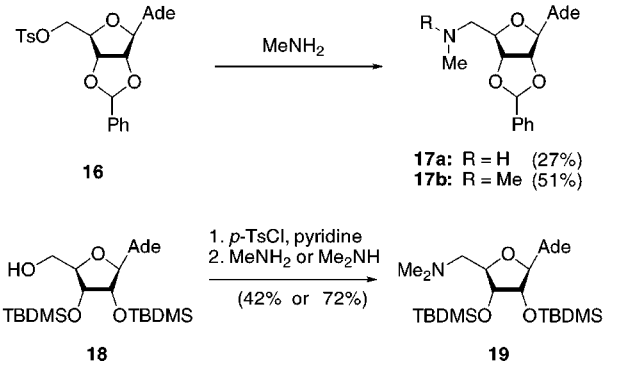

Figure 9. Use of alternative 2′,3′-*O*-ribose protecting groups in the synthesis of 5′-amino derivatives of adenosine.

15 was found to be only 33%, suggesting that bromide **12** may be unstable at the reaction temperature (55 °C). Furthermore, attempts to remove the *N*-Boc and 1-methylethylidene protecting groups from **15** using TMSBr or TMSI proved unsuccessful.

Alternative protecting groups were then considered for the ribose moiety. 2′,3′-*O*,*O*-Benzylidene adenosine[27] was converted into the 5′-*O*-tosylate **16**, which was treated with liquid methylamine (Figure 9), but the desired 5′-methylamino compound **17a** was only isolated as minor product. Unexpectedly, the major product (2:1 ratio) from this reaction (51% yield) was found to be a 5′-dimethylamino derivative, shown to be **17b** by NMR analysis and unambiguous synthesis. 2′,3′-Di-*O*-TBDMS adenosine **18** was synthesized by the method of Ogilvie et al.[28] and then treated with *p*-toluenesulfonyl chloride followed by liquid methylamine. The only product isolated after chromatography was again found to be 5′-dimethylamino compound **19**, shown as previously to be identical by ^{1}H NMR and MS to a sample prepared by using liquid dimethylamine in place of liquid methylamine. This formation of the 5′-dimethylamino compounds **17b** and **19** from the corresponding 5′-*O*-tosylates and pure methylamine[29] appears unprecedented. While such a methyl transfer might involve intermediate formation of an N^1-methyladenosine species via a Dimroth-like transamination[30] followed by transmethylation from N^1 to $N^{5'}$, such a process might well be expected to lead to some N^6-methylation product and none was observed. An alternative possibility is that toluenesulfonic acid produced in the reaction catalyzes the formation of some dimethylamine in situ (by disproportionation of methylamine), which can then react with 5′-*O*-tosyladenosine.[31]

(27) Ponpipom, M. M.; Hanessian, S. *Can. J. Chem.* **1972**, *50*, 246.
(28) Ogilvie, K. K.; Beaucage, S. L.; Schifman, A. L.; Theriault, N. Y.; Sadana, K. L. *Can. J. Chem.* **1978**, *56*, 2768.
(29) The possibility of a Dimroth-type rearrangement to give N^6-methyl-5′-(dimethylamino)-5′-deoxy-2′,3′-*O*,*O*-(1-methylethylidine)adenosine was considered, but no substitution of the adenine ring could be identified. ^{1}H NMR (400 MHz, DMSO-d_6) of **4a** and **4b** showed the two methyl groups to be equivalent (s, 6H in both cases). Mass spectroscopy data (CI$^+$) showed peaks at 321 (MH$^+$), 186 (MH − Ade$^+$), and 136 (AdeH$^+$), indicating that no substitution of the adenine ring had occurred. The purity of the methylamine used in the reaction was determined by GC and its t_R (5.31 min) compared with that of pure dimethylamine (7.80 min). We found that the methylamine used in the reactions described was greater than 99% pure and contained no dimethylamine.
(30) For a recent review, see: Fujii, T.; Itaya, T. *Heterocycles* **1998**, *48*, 359.
(31) A reviewer has suggested that dimethylamine might arise through methyl transfer from *N*,*N*-ditosylmethylamine. Experiments to explore these alternative possibilities are in progress.

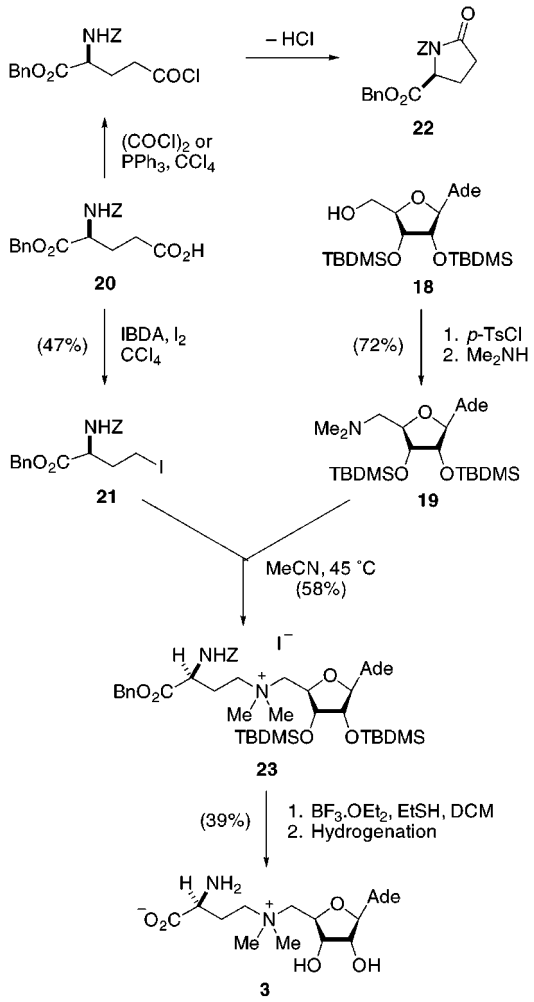

Figure 10. Synthesis of the MeAzaAdoMet **3** and side-reaction generating pyrrolidone **22**.

The synthesis of MeAzaAdoMet **3** used 2′,3′-di-*O*-TBDMS protection for the adenosine ribose moiety. For the amino acid, protecting groups removable by hydrogenation were selected, the *N*-Z-group being preferred. 2′,3′-Di-*O*-TBDMS adenosine **18** was treated with *p*-toluenesulfonyl chloride in dry pyridine (Figure 10), and the resulting crude 5′-*O*-tosylate was stirred in liquid dimethylamine to afford the 5′-(dimethylamino)-5′-deoxy compound **19** in excellent overall yield. *N*-Z-(*S*)-glutamic acid α-benzyl ester **20** was treated with oxalyl chloride to generate the acid chloride and the crude product reacted under the Barton decarboxylative iodination conditions previously described for the synthesis of **10**. However, the product from this procedure was not iodide **21** but rather the γ-lactam product **22** resulting from intramolecular cyclization of the acid chloride. Synthesis of iodide **21** from the acid **20** was achieved directly in moderate yield by use of IBDA-I_2.[12]

The tertiary amine **19** was reacted with iodide **21** to form the quaternary dimethylammonium compound, fully protected analogue **23**. This reaction was initially attempted at reflux, but at this temperature the major process was that of benzyl transfer onto the 5′-nitrogen. At lower temperature, the reaction was slower but more selective and gave the quaternary dimethylammonium salt **23** as the major product, purified by column chromatography.

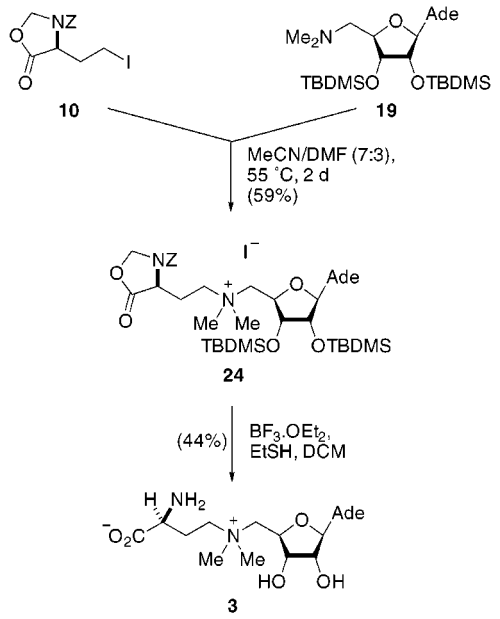

Figure 11. Improved synthesis of AzaAdoMeMet **3**.

Treatment of **23** with TBAF removed both TBDMS groups and the benzyl ester, but this crude product could not be separated from TBAF itself. Use of ammonium fluoride in methanol at 50 °C resulted in a mixture of both TBDMS deprotection and transesterification of the benzyl ester. Catalytic hydrogenation using 10% Pd−C catalyst again proved slow, taking 3 d for complete disappearance of starting material while giving a mixture of products, and indeed, electrospray MS of the crude mixture showed that the required product **23** was not present. Catalytic transfer hydrogenation of **23** was found to result in transesterification of the benzyl ester leaving the Z group intact.

Finally, the fully protected species **23** was treated BF_3−EtSH complex, which effected complete removal of the Z group and both TBDMS groups together with partial removal of the benzyl ester (Figure 10). This crude mixture of analogue **3** and its benzyl ester was treated under catalytic transfer hydrogenation conditions in aqueous methanol to avoid transesterification, and the final product was purified by ion exchange. Catalytic (Pd−$BaSO_4$ in MeOH−H_2O, 14%) and transfer (MeOH−H_2O, 27%) hydrogenation conditions were not as effective as treatment with tetra-*n*-butylammonium hydroxide[32] (MeOH−H_2O, 42%), although it could not be established whether debenzylation was complete.

Because of these difficulties, iodide **21** was replaced by **10** to give a different quaternary dimethylammonium, fully protected product **24** (Figure 11) which was deprotected by BF_3−EtSH treatment alone. Although purification of **3** proved problematic, it was isolated in a final yield of 44%. The X-ray crystal structure of a ternary MetJ−DNA−MeAzaAdoMet complex has recently been solved[33] to 2.0 Å, which confirms the absolute configuration of analogue **3** and establishes its binding conformation as close to that of AdoMet itself.

In preliminary experiments, MeAzaAdoMet **3** has been confirmed as a nanomolar inhibitor of the DNA methylase HhaI and is competitive with AdoMet **1**.[34] The affinities of the analogues AzaAdoMet **2** and MeAzaAdoMet **3** for a range of proteins that utilize AdoMet and crystal structures of their complexes are under investigation by a number of research groups, and results of such studies will be reported in due course.

Experimental Section

DCM was refluxed over P_2O_5 or CaH_2 and distilled prior to use. Pyridine was refluxed over CaH_2 and distilled. Anhydrous grade benzene, carbon tetrachloride, DMF, and acetonitrile and HPLC grade methanol and acetone were purchased from Aldrich Chemical Co. and used as supplied. *J* values are given in Hz. $[\alpha]_D$ values are given in deg cm^2 g^{-1}. Melting points are uncorrected.

5′-*p*-Toluenesulfonyl-2′,3′-*O*,*O*-(1-methylethylidene)adenosine[6] 8. 2′,3′-*O*,*O*-(1-Methylethylidene)adenosine **12** (10.05 g, 32.7 mmol)[5] was dissolved in dry pyridine (60 mL) with warming, and then the solution was cooled to −20 °C. *p*-Toluenesulfonyl chloride (7.06 g, 37.0 mmol, 1.1 equiv) was added and then the solution stored at −20 °C for 3 d with occasional shaking. This mixture was poured into chloroform (200 mL) and extracted with cold 3 M sulfuric acid (360 mL). The chloroform solution was concentrated to 50 mL by rotary evaporation at room temperature and then the product precipitated by addition of light petroleum (500 mL). The solid was collected by filtration, dissolved in methanol (60 mL), and placed in the freezer for 2 h. The product was again filtered, washed with cold methanol and then light petroleum, and transferred to a 1 L round-bottom flask. Chloroform (15 mL) was added followed by light petroleum (300 mL) and the suspension allowed to stand in the freezer for 3 h. The solid was collected by filtration, washed with light petroleum and then ether, and dried thoroughly under high vacuum giving **8** as an off-white to pale yellow powder (13.26 g, 88%): ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.51 (s, 1H), 8.39 (s, 1H), 7.62 (d, 2H, J = 8.6), 7.30 (d, 2H, J = 8.2), 6.25 (d, 1H, J = 2.1), 5.35 (dd, 1H, J = 2.1, 6.1), 4.97 (dd, 1H, J = 3.4, 6.1), 4.45−4.37 (m, 1H), 4.37−4.16 (m, 2H), 2.38 (s, 3H), 1.52 (s, 3H), 1.30 (s, 3H); *m*/*z* (FAB) 462 (MH^+).

(4*S*)-*N*-Benzyloxycarbonyl-4-(2-iodoethyl)oxazolidin-5-one 10. The oxazolidin-5-one **9** (3.73 g, 12.7 mmol) was dissolved in dry DCM (125 mL) under nitrogen. Distilled oxalyl chloride (1.69 g, 1.16 mL, 13.3 mmol, 1.05 equiv) was added followed by DMF (2 drops) and the mixture stirred for 1 h. TLC (MeOH/DCM 1:19) showed complete disappearance of starting material so the solvent was evaporated under vacuum. The residue was twice redissolved in DCM and evaporated and then dried under high vacuum to give a pale yellow solid (3.74 g, 94%). This crude acid chloride was used in the next step without further purification. Dry *N*-hydroxypyridine-2-thione sodium salt (1.50 g, 10.1 mmol, 1.1 equiv), DMAP (65 mg, 0.53 mmol), and 2,2,2-trifluoro-1-iodoethane (8.6 g, 4.05 mL, 41 mmol, 5.0 equiv) were added to dry DCM (55 mL) under argon. The mixture was brought to reflux (heated with an aluminum ring), and then a solution of the crude acid chloride (2.56 g, 8.2 mmol) in dry DCM (29 mL) was added over 10 min. As addition was started the mixture was irradiated with two tungsten filament lamps (100 and 150 W), and after addition was complete, reflux was continued under irradiation for a further 1 h. The reaction mixture was then washed with water (50 mL), concentrated HCl (25 mL), and water (50 mL) and evaporated under vacuum. The product was purified by flash column chromatography on silica (DCM) and crystallized from dry ether to give **10** as a white solid (1.33 g, 42%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.34−7.28 (br s, 5H), 5.54−5.46 (s, 1H), 5.20 (d, 1H, J = 4.0), 5.14 (m, 2H), 4.31 (t, 1H, J = 6.0), 3.15 (t, 2H, J = 7.3), 2.5−2.3 (br m, 2H); ^{13}C NMR (60 MHz, $CDCl_3$) δ 171.2, 153.0, 137.5, 135.2, 130.3, 128.8, 128.5, 127.5, 78.0, 68.2, 55.3, 34.8, −2.3; *m*/*z* (FAB) 375 (M^+).

(32) Abdel-Magid, A.; Cohen, J. M.; Maryanoff, C. A.; Shah, R. D.; Villani, F. J.; Zhang, F. D. *Tetrahedron Lett.* **1998**, *39*, 3391.
(33) Porter, J. Uunpublished results.
(34) Hornby, D. P. Personal communication.

(4*S*)-*N*-Benzyloxycarbonyl-4-[2-[*N*-2′,3′-*O*,*O*-(1-methylethylidene)adenosyl-*N*-methyl]aminoethyl]oxazolidin-5-one 11. A solution of iodide **10** (1.32 g, 3.52 mmol, 1.1 equiv) in anhydrous acetonitrile (25 mL) was added to a stirred solution of amine **4** (1.03 g, 3.22 mmol) and *N*,*N*-diisopropylethylamine (0.45 g, 0.61 mL, 3.5 mmol, 1.1 equiv) in the same solvent (40 mL) under argon. The solution was heated at 55 °C for 2 d and then the solvent removed under vacuum and the residue twice purified by flash column chromatography on silica using (Et_3N/MeOH/EtOAc, 1:1:98) and then (propan-2-ol/DCM, 7.5:92.5) as eluent to give a pale yellow oil. This product was twice dissolved in DCM and evaporated to yield the *title compound* as a white foam (0.93 g, 51%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.87 (s, 1H), 7.40−7.20 (br s, 5H), 5.96 (d, 1H, J = 2.1), 5.84 (s, 2H), 5.50−5.35 (br m, 2H), 5.20 (d, 1H, J = 4.0), 5.13 (m, 2H), 4.83 (dd, 1H, J = 4.0, 6.4), 4.35−4.19 (br m, 2H), 2.62−1.78 (br m, 9H), 1.54 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (60 MHz, $CDCl_3$) δ 172.1, 155.7, 149.2, 128.7, 128.4, 128.3, 128.0, 127.7, 120.2, 114.5, 90.8, 84.9, 83.9, 83.3, 81.7, 67.5, 60.1, 59.3, 57.6, 57.0, 54.5, 52.9, 42.2, 41.0, 27.1, 25.3, 14.1; HRMS calcd for $C_{27}H_{33}N_7O_7$ 568.2520, found 568.2511.

5′-[*N*-[(3*S*)-3-Amino-3-carboxypropyl]-*N*-methylamino]-5′-deoxyadenosine (AzaAdoMet) 2. A solution of **11** (1.17 g, 2.06 mmol) in dry DCM (16 mL) was added via syringe to a stirred mixture of boron trifluoride etherate (3.0 g, 2.6 mL, 21 mmol, 10 equiv) and ethanethiol (4.0 g, 4.8 mL, 62 mmol, 30 equiv) under nitrogen at 0 °C (ice bath). After 30 min, the ice bath was removed and the reaction was continued at room temperature for a further 23 h. Solvent was removed under vacuum (using a $KMnO_4$ trap) and the residue coevaporated with methanol (3 × 10 mL) and then taken up in water (10 mL). This suspension was filtered and then lyophilized. The sticky gum formed was taken up in water, filtered again, and then loaded to a column of Dowex 50WX4-400 ion-exchange resin (100 g). The column was washed with water (250 mL) and then eluted with a gradient of NH_4HCO_3 (0−1.2 M in 3.0 L). Fractions from the major UV-active peak were collected and evaporated under vacuum and then coevaporated with water. The residue was redissolved in a small volume of water and lyophilized to give pure analogue **2** as a white powder (535 mg, 68%): ^{1}H NMR (400 MHz, D_2O) δ 8.10 (s, 1H), 7.98 (s, 1H), 5.88 (d, 1H, J = 5), 4.61 (t, 1H, J = 5), 4.25−4.19 (m, 1H), 4.11 (t, 1H, J = 5), 3.60 (dd, 1H), 2.88−2.76 (m, 2H), 2.73−2.61 (m, 2H), 2.27 (s, 3H), 2.01−1.91 (m, 1H), 1.88−1.78 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 174.0, 154.7, 152.0, 147.9, 139.4, 118.1, 87.5, 79.9, 72.6, 71.3, 58.5, 53.8, 53.5, 40.2, 25.7; HRMS calcd for $C_{15}H_{24}N_7O_5$ 382.1839, found 382.1933.

pK_a Determination for AzaAdoMet 2. AzaAdoMet **2** (20 mg) was dissolved in 10% D_2O in H_2O (500 mm^3) at pH 3.0 ionic strength 10 mM (KCl) and acetone (2 mm^3) added as the NMR internal reference (δ = 2.214). Proton spectra were recorded at 500.13 MHz on a Bruker AMX 500 spectrometer at 298 K. An aliquot of KOH (0.01 M) was added, the solution homogenized, and the pH determined at 298 K in the NMR tube using a Russell RL150 pH meter model fitted with a combination glass electrode (error ± 0.03 pH unit). This process was repeated stepwise to give 26 data points in the range 3 < pH < 11.4. The chemical shift for the *N*-methyl signal of **2** was plotted as a function of pH (adjusted to compensate for the D_2O-H_2O mixture)[35] (Figure 6) and analyzed for two overlapping pK_a values using[18] Kaleidagraph to give values of 7.08 ± 0.07 and 9.66 ± 0.31 (Figure 6).

Methyl [4-[*N*-2′,3′-*O*,*O*-(1-methylethylidene)adenosyl-*N*-methyl]amino]-2-(*tert*-butyloxycarbonylamino)butanoate 15. Amine **4** (0.23 g, 0.72 mmol) was dissolved in dry acetonitrile (15 mL) under nitrogen and then *N*,*N*-diisopropylethylamine (0.15 g, 0.20 mL, 1.16 mmol, 1.6 equiv) added followed by a solution of bromide **12** (0.34 g, 1.15 mmol, 1.6 equiv) in dry acetonitrile (10 mL). The solution was heated at 55 °C for 3 d, and then the solvent was removed under vacuum and the residue purified by flash column chromatography (MeOH/EtOAc, 1:19) giving an oil. Ether was twice added and evaporated to give the title compound as a white foam (125 mg, 33%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.90 (s, 1H), 6.01 (d, 1H, J = 1.8), 5.90 (s, 2H), 5.85−5.74 (br m, 1H), 5.44 (t, 1H, J = 6.7), 4.4−4.2 (br m, 1H), 3.65 (d, 3H, J = 3.7), 2.62−2.21 (br m, 4H), 2.16 (s, 3H), 1.94−1.66 (br m, 2H), 1.55 (s, 3H), 1.39−1.24 (br m, 12H); HRMS calcd for $C_{24}H_{37}N_7O_7$ 536.2827, found 536.2833.

5′-Methylamino-5′-deoxy-2′,3′-*O*,*O*-benzylideneadenosine 17a and 5′-Dimethylamino-5′-deoxy-2′,3′-*O*,*O*-benzylideneadenosine 17b. 2′,3′-*O*,*O*-Benzylidene adenosine (0.31 g, 0.87 mmol) was dissolved in dry pyridine (4 mL) with warming. This solution was cooled to −20 °C (freezer), and then *p*-toluenesulfonyl chloride (183 mg, 0.96 mmol, 1.1 equiv) was added. The mixture was kept at −20 °C for 3 d with occasional shaking and then diluted with DCM (20 mL) and washed with cold 3 M sulfuric acid (30 mL). The 5′-*O*-tosylate **16** (161 mg, 36%) was precipitated by addition of light petroleum, filtered off, recrystallized from methanol, and used directly in the next step without further purification. 5′-*O*-Tosylate **16** (161 mg, 0.32 mmol) was transferred to a bomb, and liquid methylamine (8 mL) was condensed onto it. The bomb was sealed and the mixture stirred at room temperature for 3 d. The excess amine was allowed to evaporate, and then the residue was taken up in DCM (30 mL) and washed with aqueous NaOH (0.75 M, 10 mL). The aqueous washings were back-extracted with DCM (30 mL) and the combined organic solutions evaporated. The crude mixture was then purified by flash column chromatography (MeOH/DCM, 1:4). The major product **17b** eluted first (59 mg, 51%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.93 (s, 1H), 7.55−7.47 (m, 2H), 7.43−7.32 (m, 3H), 6.16 (d, 1H, J = 3), 5.98 (s, 1H), 5.65−5.53 (m, 3H), 5.02 (dd, 1H, J = 2, 6), 4.57−4.48 (m, 1H), 2.70−2.48 (m, 2H), 2.23 (s, 6H); *m*/*z* (FAB) 383 (MH^+). The anticipated product **17a** eluted later (31 mg, 27%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.28(s, 1H), 7.75 (s, 1H), 7.56−7.45 (m, 2H), 7.43−7.30 (m, 3H), 6.08 (d, 1H, J = 3), 5.99 (s, 1H), 5.90 (s, 2H), 5.62 (dd, 1H, J = 3, 6.5), 5.11 (dd, 1H, J = 2.1, 6.4), 4.51 (dt, 1H, J = 2.1, 5), 2.95−2.82 (m, 2H), 2.40 (s, 3H); HRMS calcd for $C_{18}H_{20}N_6O_3$ 369.1675, found 369.1695.

5′-Dimethylamino-5′-deoxy-2′,3′-bis(*tert*-butyldimethylsilyl)adenosine 19. 2′,3′-Bis(*tert*-butyldimethylsilyl)adenosine **12** (230 mg, 0.46 mmol) was dissolved in dry pyridine (5 mL), and the solution was cooled to −20 °C. *p*-Toluenesulfonyl chloride (133 mg, 0.70 mmol, 1.5 equiv) was added and the mixture kept at −20 °C for 3 d with occasional shaking. The solution was then diluted with DCM (40 mL) and washed with cold 3 M sulfuric acid (30 mL). The DCM layer was transferred to a bomb and the solvent evaporated under a stream of nitrogen. Dimethylamine (5 mL) was then condensed onto the crude 5′-*O*-tosylate and the bomb sealed. The mixture was allowed to stir for 3 d at room temperature then the excess amine allowed to evaporate. The residue was taken up in DCM (30 mL) and washed with aqueous NaOH (0.75 M, 10 mL). The aqueous layer was back-extracted with further DCM (10 mL), and then the combined DCM extracts were evaporated. This crude product was purified by flash column chromatography (MeOH/DCM, 1:9) to give the *title compound* as an off-white solid (174 mg, 72% from **12**): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.21 (s, 1H,), 7.79 (s, 1H), 5.73 (d, 1H, J = 5.2), 5.54 (s, 2H), 4.86 (t, 1H, J = 4.4), 4.14−4.05 (m, 2H), 2.81−2.68 (m, 1H), 2.52−2.40 (m, 1H), 2.20 (s, 6H), 0.82 (s, 9H), 0.69 (s, 9H), 0.00 (s, 3H), −0.01 (s, 3H), −0.16 (s, 3H), −0.36 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 152.8, 149.6, 140.6, 120.8, 90.0, 82.7, 74.3, 73.9, 61.7, 46.0, 25.9, 25.7, 18.1, 17.9, −4.3, −4.7, −5.0; HRMS calcd for $C_{24}H_{47}N_6O_3Si_2$ 523.3248, found 523.3255.

Benzyl *N*-Benzyloxycarbonyl-L-α-(2-iodoethyl)glycinate 21. *N*-Benzyloxycarbonyl-L-glutamic acid α-benzyl ester **14** (0.37 g, 1.0 mmol) was dissolved in anhydrous carbon tetrachloride (40 mL) under argon, and then iodobenzene diacetate (0.18 g, 0.56 mmol, 0.55 equiv) and iodine (0.13 g, 0.51 mmol, 1.0 equiv) were added. The mixture was heated (using an aluminum ring) at reflux for 90 min under irradiation from two tungsten filament lamps (100 and 150 W). Further portions of iodobenzene diacetate (0.18 g, 0.56 mmol,

(35) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188.

0.55 equiv) and iodine (0.13 g, 0.51 mmol, 1.0 equiv) were added, and then the mixture was refluxed under irradiation for a further 90 min. The solution was allowed to cool to room temperature, washed with aqueous sodium thiosulfate until colorless, and evaporated under vacuum. The residue was purified by flash column chromatography (EtOAc/light petroleum, 1:4) to give the *title compound* as a white solid (181 mg, 40%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.37−7.15 (br m, 10H), 5.30 (s, 1H, J = 12), 5.12 (s, 2H), 5.05 (s, 2H), 4.45−4.36 (m, 1H), 3.13−2.98 (m, 2H), 2.47−2.29 (m, 1H), 2.23−2.05 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 155.8, 135.9, 134.9, 128.69, 128.63, 128.56, 128.33, 128.28, 128.15, 67.6, 67.2, 54.7, 36.8, −1.1; HRMS (CI) calcd for $C_{19}H_{21}INO_4$ 454.0515, found 454.0496.

***N*^{1},5-Bis(benzyloxycarbonyl)-2-pyrrolidone 22.** *N*-Benzyloxycarbonyl-L-glutamic acid α-benzyl ester (2.0 g, 5.39 mmol) was dissolved in dry DCM (50 mL) under nitrogen. Distilled oxalyl chloride (0.75 g, 0.52 mL, 13.3 mmol, 5.91 mmol, 1.1 equiv) was added followed by DMF (2 drops) and the mixture stirred at room temperature for 2 h. The solvent was evaporated, and then the yellowish oil was dried under high vacuum give a pale yellow solid used in the next step without further purification. DMAP (165 mg, 1.35 mmol) and 2,2,2-trifluoro-1-iodoethane (5.7 g, 2.66 mL, 27 mmol, 5.0 equiv) were added to a suspension of dry *N*-hydroxypyridine-2-thione sodium salt (0.89 g, 5.9 mmol, 1.1 equiv) in dry DCM (30 mL) under nitrogen. The mixture was brought to reflux (heated using an aluminum ring), and then a solution of the above crude acid chloride in dry DCM (12 mL) was added dropwise over 10 min under irradiation with two tungsten filament lamps (100 and 150 W). Reflux was continued under irradiation for a further 90 min after addition was complete. The reaction mixture was then washed with water (50 mL), 1 M HCl (30 mL), and water (30 mL) and then evaporated under vacuum. The product was purified by flash column chromatography (EtOAc/light petroleum, 1:1) to give an off-white, crystalline solid (1.44 g, 76%) identified as the γ-lactam product **22** of intramolecular cyclization of the acid chloride: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.37−7.24 (br s, 5H), 5.21 (s, 2H), 5.12 (s, 2H), 4.71 (dd, 1H, J = 2.7, 9.2), 2.71−2.25 (m, 3H), 2.11−1.99 (m, 1H); m/z (EI) 353 (M^+); R_f 0.18 (EtOAc/light petroleum, 1:2); $[\alpha]_D$ −0.11° (c 6.6 × 10^{-3} in DCM) (lit.[36] $[\alpha]_D$ −12.6° (c 0.9 in $CHCl_3$)).

***N*-[2′,3′-Bis(*tert*-butyldimethylsilyl)adenosyl]-*N*-[3-(*S*)-3-*N*-benzyloxycarbonylamino-3-(benzyloxycarbonyl)propyl]-*N*,*N*-dimethylammonium Iodide 23.** Iodide **15** (0.86 g, 1.90 mmol, 1.5 equiv) in dry acetonitrile (10 mL) was added to a suspension of the tertiary amine **13** (0.66 g, 1.26 mmol) in the same solvent (5 mL) under nitrogen. This mixture was heated at 45−50 °C for 4 d and then allowed to cool to room temperature. Solvent was evaporated under vacuum and the residue purified by flash column chromatography (MeOH/DCM, 1:9) to give the *title compound* as an off-white foam (0.72 g, 58%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.83 (s, 1H), 7.19−7.02 (m, 10H), 6.22−6.10 (1H), 6.03−5.75 (2H), 5.67 (d, 1H, J = 6.7), 5.06−4.72 (6H), 4.52 (t, 1H, J = 13.1), 4.26−4.09 (4H), 3.75−3.41 (m, 2H), 3.07 (s, 3H), 2.99 (s, 3H), 2.33−2.08 (2H), 2.06−1.80 (2H), 0.77 (s, 9H), 0.55 (s, 9H), 0.04 (s, 3H), 0.00 (s, 3H), −0.24 (s, 3H), −0.63 (s, 3H$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 156.2, 155.7, 153.0, 149.0, 141.2, 136.1, 135.0, 128.6, 128.53, 128.47, 128.12, 128.07, 120.7, 90.0, 80.2, 74.7, 72.8, 67.9, 67.0, 65.0, 63.1, 52.1, 51.9, 51.5, 25.8, 25.6, 25.2, 17.9, 17.7, −1.3, −2.3, −2.8; HRMS calcd for $C_{43}H_{66}N_7O_7Si_2$ 848.4562, found 848.4616.

***N*-(2′,3′-Di-*O*-*tert*-butyldimethylsilyladenosin-5′-yl)-*N*-[2-(3′-benzyloxycarbonyloxazolidin-5-one)-4-yl]-ethyl-*N*,*N*-dimethylammonium Iodide 24.** Amine **13** (252 mg, 0.48 mmol) was suspended in dry acetonitrile (7 mL) under argon, and then dry DMF (3 mL) was added. Iodide **10** (281 mg, 0.75 mmol, 1.5 equiv) was then introduced and the mixture heated at 57 °C for 2 d. Solvent was evaporated and the residue purified by flash column chromatography (MeOH/DCM, 8:92) to give the quaternary dimethylammonium salt **24** as a white powder (254 mg, 59%): ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.17 (s, 1H), 7.48−7.24 (m, 6H), 6.79−6.53 (br m, 1H), 5.93 (d, 1H, J = 6.4), 5.29−5.06 (m, 3H), 4.90 (d, 1H, J = 11.3), 4.77 (t, 1H, J = 12.8), 4.64−4.48 (m, 2H), 4.48−4.40 (br m, 1H), 4.17−3.98 (m, 2H), 3.33 (s, 3H), 3.15 (s, 3H), 2.7−2.3 (br m, 4H), 1.01 (s, 9H), 0.81 (s, 9H), 0.28 (s, 3H), 0.22 (s, 3H), 0.00 (s, 3H), −0.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 155.6, 155.3, 153.0, 149.0, 141.2, 136.1, 128.7, 128.6, 128.4, 128.1, 128.0, 120.9, 90.6, 80.0, 74.9, 72.7, 72.1, 67.8, 65.2, 64.5, 57.0, 52.8, 51.5, 51.4, 25.8, 25.6, 23.6, 18.0, 17.7; HRMS calcd for $C_{37}H_{60}N_7O_7Si_2$ 771.4171, found 771.4117.

***N*-(5′-Adenosyl)-*N*-[(3*S*)-3-amino-3-carboxypropyl]-*N*,*N*-dimethylammonium iodide (MeAzaAdoMet) 3, from 24.** A solution of **24** (318 mg, 0.35 mmol) in dry DCM (13 mL) was added dropwise to a stirred mixture of boron trifluoride etherate (0.45 mL, 0. 51 g, 3.6 mmol, 10 equiv) and ethanethiol (0.80 mL, 0.68 g, 10.9 mmol, 30 equiv) under argon at 0 °C. The ice bath was removed 10 min after addition was complete and then the mixture stirred at room temperature overnight. Solvent was evaporated under vacuum (using a $KMnO_4$ trap) and the residue coevaporated with methanol (3 × 10 mL). The crude residue was dissolved in a small volume of water and loaded onto a column of Dowex 50WX4-400 (20 g, NH_4^+ form). The column was washed with water (100 mL) and then eluted with 1.0M NH_4OH (1.5 l), and the UV-active fractions were collected and evaporated to give 46 mg of product. Further product remained on the column so it was eluted with 1.0 M NaOH (100 mL), which was immediately neutralized with 1.0 M HCl and evaporated to dryness. The first 46 mg portion of product was loaded onto a second Dowex 50WX4-400 (100 g, NH_4^+ form) column and eluted with 0−1.5 M NH_4OH (1.0 L). The major peak (0.7−1.0 M) was evaporated to give a pale yellow solid, redissolved in water, and decolorized by passing through Dowex 1X4-400 (10 g, Cl^- form). The UV-active fractions were evaporated, and the solid was redissolved in a small volume of water and lyophilized to give **3** (34 mg). The portion of product from the NaOH wash was dissolved in water and loaded onto the Dowex 50WX4-400 column (100 g, NH_4^+ form), desalted by washing with 1.0 M NH_4Cl (300 mL) and then water (200 mL), and then eluted with the same gradient of NH_4OH used above and further purified on Dowex 1X4-400 in the same manner to give **3** (28 mg): a combined yield of **3** of (62 mg, 44%); ^{1}H NMR (400 MHz, D_2O) δ 8.10 (s, 2H), 5.92 (d, 1H, J = 3), 4.63−4.55 (br m, 1H), 4.40 (dd, 1H, J = 8, 6), 4.27 (dd, 1H, J = 6), 3.76 (dd, 1H, J = 10, 14), 3.62 (d, 1H, J = 14), 3.46−3.31 (m, 2H), 3.25 (t, 1H, J = 6), 3.04 (s, 6H), 2.09−1.97 (m, 1H), 1.97−1.85 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 177.1, 155.5, 152.8, 148.5, 140.2, 118.9, 89.4, 76.4, 72.1, 71.6, 65.6, 62.4, 52.6, 51.7, 51.4, 26.0; HRMS calcd for $C_{16}H_{26}N_7O_5$ 396.1995, found 396.1998. This procedure could be improved by treating the crude reaction product with base (aqueous Bu_4NOH followed by neutralization with AcOH) before loading onto the ion-exchange column. This would cleave the boron complex of **3**, which seemed to have been formed in the reaction.

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Supporting Information Available: Experimental details and characterization data for compounds **3** (alternative methods used), **7**, **9**, **12**−**14**, **18**, **19** (using methylamine), and 2′,3′-*O*,*O*-benzylidene adenosine. NMR spectra and/or additional characterization data for compounds **2**, **10**, **11**, **15**, **17a**,**b**, and **21**−**24**. X-ray structural data for compound **10**, including tables of atomic coordinates, bond lengths and bond angles. This material is available free of charge via the Internet at http://pubs.acs.org.

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(36) Baldwin, J. E.; Miranda, T.; Moloney, M. *Tetrahedron* **1989**, *45*, 7459.