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o-Nitrobenzenesulfonamides in Nucleoside Synthesis: Efficient 5′-Aziridination of Adenosine

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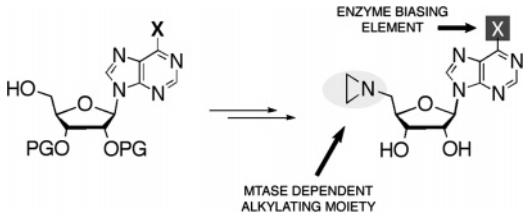

5′-Aziridinoadenylates of the form **1** and a related nitrogen mustard variant have been constructed using a novel variation of the Mitsunobu reaction. Such molecules allow conversion of biological methyltransferases into nucleoside transferases, thus providing powerful tools for investigating *S*-adenosyl-L-methionine (SAM)-dependent methylation. We present here a highly effective synthesis of such molecules that is amenable to aziridine diversification as well as elaboration of the base moiety so as to afford "bumped" cofactor mimics compatible with "hole"-bearing mutant proteins.

All human cells have self-contained programs that control levels of gene expression; proper gene expression is integrally linked to transcriptional control. Enzymes referred to as "coactivators" that are recruited to sites of transcription are often involved in methylation, phosphorylation, and acylation in a regulatory fashion in events that regulate transcription, thus conferring epigenetic transcriptional control. Covalent histone modifications in the N terminal domains of histones may be the product of signaling pathways leading to the silenced or active state of specific genes.[1] These modifications to chromatin structure have an impact upon transcriptional regulation, cell cycle progression, DNA replication, DNA repair mechanisms, recombination events, and overall chromosome stability. Errors in transcriptional regulation have been correlated to many different disease states, most notably carcinogenesis.[2] Coactivators responsible for controlling the status of histone methylation are particularly attractive targets for small-molecule drugs. Enzyme-mediated protein methylation events associated with transcriptional regulation, like their DNA counterparts, are dependent upon *S*-adenosyl-L-methionine (SAM).[3] Importantly, small-molecule cofactor mimics capable of interfering with coactivator functions may exert anticancer activity by averting an otherwise destructive transcriptional cascade or by inducing the expression of regulatory genes. We report here facile synthetic routes by which to generate aziridine-based cofactors and which also allow for designed enzyme specificity. Aziridine adenylate **1** is used by the DNA methyltransferases M.HhaI, M.TaqI, and M.EcoRI in lieu of SAM.[4] The net result is that **1** effectively converts these

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(1) (a) Gibbs, W. W. *Sci. Am.* **2003**, *289*, 106. (b) Grewal, S. I. S.; Moazed, D. *Science* **2003**, *5634*, 798. (c) Jenewein, T.; Allis, D. C. *Science* **2001**, *293*, 1074.

(2) Gu, H.; Park, S. H.; Park, G. H.; Lim, I. K.; Lee, H. W.; Paik, W. K.; Kim, S. *Life Sci.* **1999**, *65*, 737.

(3) (a) Jenuwein T. *Trends Cell Biol.* **2001**, *11,* 266. (b) Nakayama, J.; Rice, J. C.; Strahl, B. D.; Allis, C. D.; Grewal, S. I. S. *Science* **2001**, *292,* 110. (c) Tang J.; Frankel A.; Cook R. J.; Kim S.; Paik W. K.; Williams K. R.; Clarke S.; Herschman H. R. *J. Biol. Chem.* **2000**, *275,* 7723. (d) Qi, C.; Chang, J.; Zhu, Y.; Yeldandi, A. V.; Rao, S. M.; Zhu, Y. J. *J. Biol. Chem.* **2002**, *277*, 28624. (e) Tang, J.; Gary, J. D.; Clarke, S.; Herschman, H. R. *J. Biol. Chem.* **1998**, *273*, 16935. (f) Zhang, X.; Zhou, L.; Cheng, X. *EMBO J.* **2000**, *19*, 3509. (g) Wada, K.; Inoue, K.; Hagiwara, M. *Biochim. Biophys. Acta* **2002**, *1591*, 1. (h) Strahl, B. D.; Briggs, S. D.; Brame, C. J.; Caldwell, J. A.; Koh, S. S.; Ma, H.; Cook, R. G.; Shabonowitz, J.; Hunt, D. F.; Stallcup, M. R.; Allis, C. D. *Curr. Biol*. **2001**, *11*, 996. (i) Branscombe, T. L.; Frankel. A.; Lee, J. H.; Cook, J. R.; Yang, Z. H. Pestka, S.; Clarke, S. *J. Biol. Chem.* **2001**, *276*, 32971. (j) Pollack, B. P.; Kotenko, S. V.; He, W.; Izotova, L. S.; Barnoski, B. L.; Pestka, S. *J. Biol. Chem.* **1999**, *274*, 31531. (k) Rho, J.; Choi, S.; Seong, Y. R.; Cho, W. K.; Kim, S. H.; Im, D.-S. *J. Biol. Chem*. **2001**, *276*, 11393. (l) Xu, W.; Chen, H.; Du, K.; Asahara, H.; Tini, M.; Emerson, B. M.; Montminy, M.; Evans, R. M. *Science* **2001**, *294,* 2507. (m) Chen, S. L.; Leffler, K. A.; Chen, D.; Stallcup, M. R.; Muscat, G. E. O. *J. Biol. Chem.* **2002**, *277*, 4324. (n) Chen, D. G.; Ma, H.; Hong, H.; Koh, S. S.; Huang, S. M.; Schurter, B. T.; Aswad, D. W.; Stallcup, M. R. *Science* **1999**, *284*, 2174. (o) Koh, S. S.; Chen, D.; Lee, Y. H.; Stallcup, M. R. *J. Biol. Chem.* **2001**, *276,* 1089.

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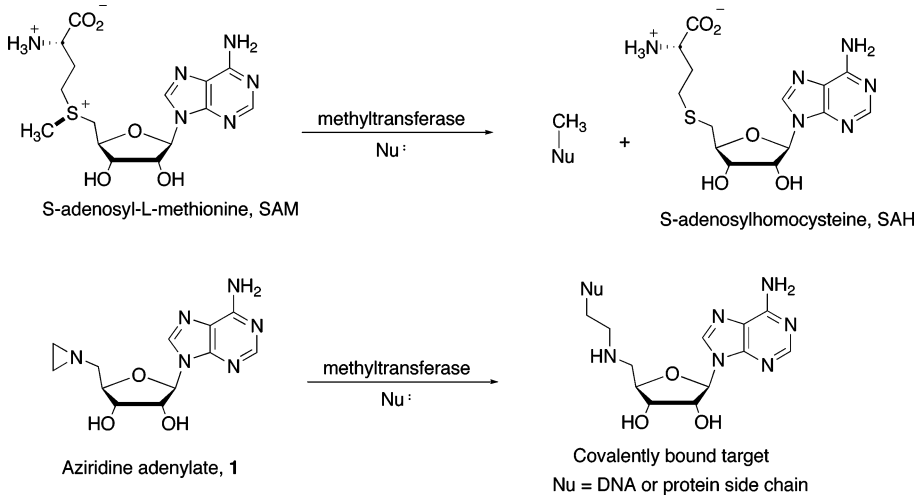
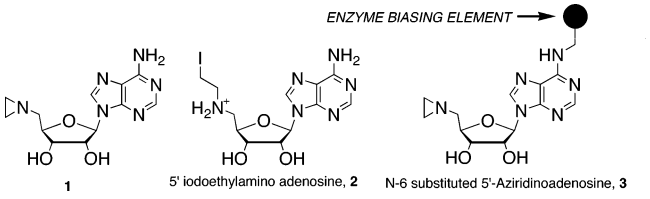


FIGURE 1. Natural versus synthetic cofactor products.

methyltransferases into nucleoside transferases; the sites ordinarily methylated by these enzymes are anchored to **1** and a related fluorophoric material via nucleophilic attack upon the 5′-aziridine moiety.[4a−c] Azides and alkynes have also been delivered sequence selectively in a similar fashion.[4d,e] We have hypothesized that **1** and related congeners may also be used by protein methyltransferases and that these synthetic cofactors might prove to be powerful new tools by which to identify as yet unknown but highly important protein methyltransferase substrates. However, such interests require more efficient and readily diversifiable syntheses of these substances.



The previously reported synthesis of **1** exploits 5′-tosylation or mesylation followed by S_N2 coupling with ethyleneimine (aziridine).[4a] We found this synthesis to be impractical due to a wide array of unproductive side reactions. Moreover, this approach called for tremendous excesses of aziridine, and the reported purification procedures required that the aziridine be of suitable volatility so as to be partially removed prior to chromatography. Finally, the massive excess of aziridine required was deemed to be a liability in the context of structure diversification.

We report here two means by which **1** can be rapidly constructed with consistently high to moderate yields. More significantly, we demonstrate the utility of *o*-nitrobenzenesulfonamide (*o*-NBS)-protected amino acids in the Mitsunobu condensation of 5′-unmasked adenosine derivatives. This reaction proceeds in high yields and with little evidence of base-modified materials, which are common adducts obtained with purines subjected to Mitsunobu conditions. To our knowledge, this is the first report of Mitsunobu-type condensations of *o*-NBS-protected amines with purines and thus represents a complement to the efforts of Van Boom and co-workers on pyrimidine modifications.[5] Importantly, the disclosed condensation is expected to allow aziridine diversification by simply changing the nosylated α-amino acid precursor. This methodology is tolerant of the unprotected native adenine base and is also compatible with adenine base N6 modification. Aziridine **3** is a "bumped" analogue of **1** for which complementary "hole"-bearing methyltransferases will be generated en route to achieving enzyme specificity.[6]

Results and Discussion

Unsubstituted Aziridine. Aziridine adenylate **1** cannot be readily derived via Gabriel Cromwell chemistry or on the scale necessary for rigorous biochemical study.[7] Although Gabriel Cromwell aziridination of 5′-amino adenosine derivatives proceeds with high yields, the obligate aziridine substitution pattern prohibits installation of unsubstituted aziridines. We believed, however, that an alternative synthesis of **1** might still exploit the unique 5′-amino adenylate previously exploited for Cromwell-based syntheses.[7] Scheme 1 highlights key steps to a simple but structurally nondiverse approach to aziridine-based cofactor mimics bearing the core structure **1**. Although this approach to **1** requires multiple steps, it is noteworthy that lessons learned from this approach are translatable to analogues of **1** that are not readily achievable via Weinholds' one-step synthesis of **1**. Phthalimidoadenylate **4**, generated using Mitsunobu chemistry described by Kolb and co-workers, was readily amenable

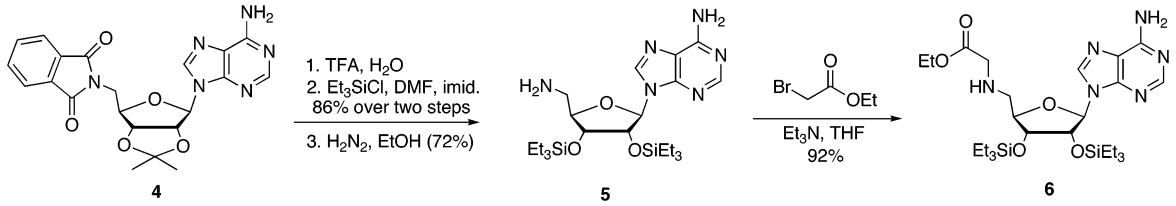
(4) (a) Pignot, M.; Siethoff, C.; Linscheid, M.; Weinhold, E., *Angew. Chem., Int. Ed.* **1998**, *37*, 2888. (b) Pljevaljcic, G.; Pignot, M.; Weinhold, E. *J. Am. Chem. Soc.* **2003**, *125,* 3486. (c) Pljevaljcic, G.; Schmidt, F.; Weinhold, E. *ChemBioChem* **2004**, *5,* 265. (d) Comstock, L. R.; Rajski, S. R., *Nucleic Acids Res.* **2005**, *33*, 1644. (e) Weller, R. L.; Rajski, S. R. *Org. Lett.* **2005**, *7*, 2141−2144.

(5) Turner, J.; Filippov, D.; Overhand, M.; van der Marel, G.; Van Boom, J. *Tetrahedron Lett.* **2002**, *42*, 5763.

(6) (a) Lin, Q.; Jiang, F.; Schultz, P. G.; Gray, N. S. *J. Am. Chem. Soc.* **2001**, *123*, 11608. (b) Bishop, A.; Buzko, O.; Heyeck-Dumas, S.; Jung, I.; Kraybill, B.; Liu, Y.; Shah, K.; Ulrich, S.; Witucki, L.; Yang, F.; Zhang, C.; Shokat, K. M. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 577.

(7) (a) Comstock, L. R.; Rajski, S. R. *Tetrahedron* **2002**, *58*, 6019. (b) Weller, R. L.; Rajski, S. R. *Tetrahedron Lett.* **2004**, *45*, 5807.

SCHEME 1



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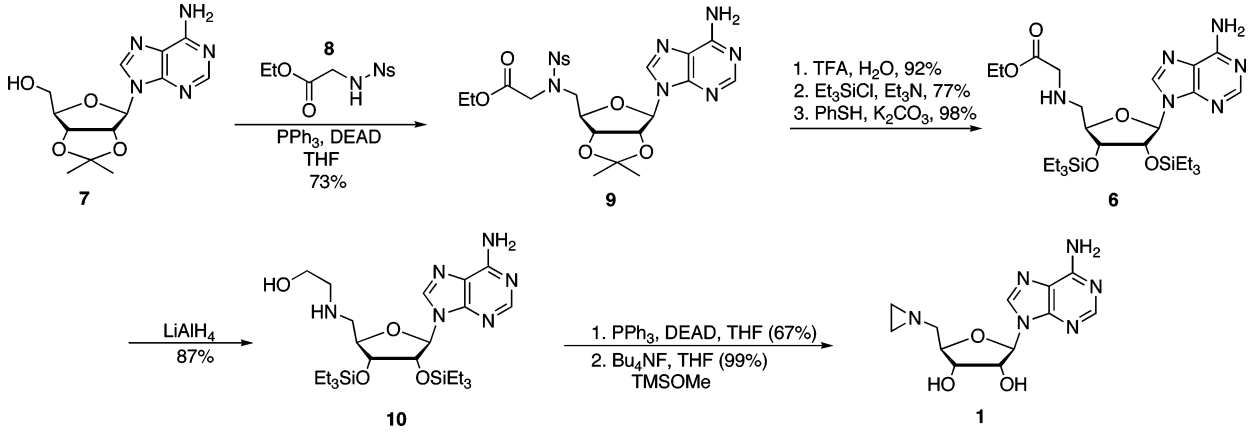


to 2′,3′-protecting group exchange via acid-catalyzed acetonide cleavage and subsequent silylation to afford a bis-triethylsilyl (TES) ether.[8] Phthalimide cleavage was effected by subjection of the newly formed di-TES ether to hydrazine in refluxing ethanol. The resulting amine **5** readily condenses with bromoacetates to afford materials such as **6**. We envisioned that the ester moiety of **6** would undergo reduction to afford the 5′-ethanolamine, which would segue to aziridination and ultimate deprotection.

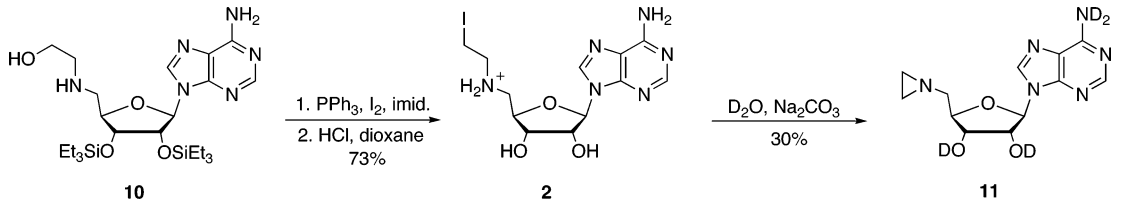
Amination at the 5′-center of adenosine has been described by Kolb and co-workers via phthalimide installation and subsequent hydrazine-mediated phthalimide cleavage.[8] We and others have also described diphenylphosphoryl azide (DPPA)-mediated azidation and subsequent reduction.[7a,9] These O → N exchange methods often proved to be cumbersome and significantly reduced the efficiency desired in obtaining **1** and related analogues. Moreover, 5′-amino adenylates such as **5** tend to be rather poor nucleophiles relative to other primary amines and, once alkylated with materials other than bromoacetates, readily undergo conversion to the unproductive tertiary amine.

The *o*-NBS moiety represents a novel protecting group for amines that is base and acid stable yet readily cleaved upon subjection to thiols. Fukuyama and co-workers have extended the use of this protecting group by demonstrating that nosylated amines are extremely effective nucleophiles in Mitsunobu condensations.[10] Unlike their *N*-tosyl counterparts, the Mitsunobu adducts obtained can be deprotected under very mild conditions. We thus predicted the use of *o*-NBS-protected α-amino esters as an effective way to perform the otherwise troublesome O → N exchange while also introducing the hydroxyethyl moiety necessary for ultimate aziridination. Chemical diversity ancillary to the aziridine might ultimately arise from the use of suitably protected amino acids. Such diversity could have a significant impact upon biological specificities of these agents. Additionally, one might readily envision that ligatable moieties could be arranged in such a fashion as to allow isolation and identification of biomolecules modified with such cofactors.

The application of *o*-NBS-protected glycine ethyl ester to the construction of **1** is depicted within Scheme 2. Commercially available 2′,3′-isopropylidene adenosine was coupled with nosylate **8** in the presence of triphenylphosphine (PPh_3) and diethylazodicarboxylate (DEAD) in THF. Ethyl ester **9** was obtained in purified form in 73% yield. Acetonide cleavage followed by 2′,3′-reprotection as the di-TES ether and almost quantitative cleavage of the *o*-NBS moiety provided **6**, which was amenable to ester reduction with $LiAlH_4$. Aziridination was accomplished with triphenylphosphine and DEAD in THF in modest yields, followed by deprotection of the silyl groups with Bu_4NF. Although the sequence of ester reduction and aziridination proceeded as predicted, silyl ether cleavage presented some difficulties in terms of isolation and purification of **1**. Following desilylation, crude **1** is extremely water soluble. This solubility issue, in combination with the hygroscopic nature of Bu_4NF, vastly complicated purification procedures. Ultimately, the use of anhydrous TBAF in THF dried over molecular sieves and used as the limiting reagent facilitated purification of **1**. Although simple, what is remarkable about this synthesis is the effectiveness of the initial Mitsunobu condensation and that adenine base protection is not required. This is clearly a viable means by which to produce large quantities of **1** for biological evaluation and

(8) Kolb, M.; Danzin, C.; Barth, J.; Claverie, N. *J. Med. Chem.* **1982**, *25*, 550.
(9) Liu, F.; Austin, D. J. *Org. Lett.* **2001**, *3*, 2273.
(10) (a) Fukyama, T.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373. (b) Kan, T.; Kobayashi, H.; Fukuyama, T. *Synlett* **2002**, 697. (c) Fujiwara, A.; Kan, T.; Fukuyama, T. *Synlett* **2000**, 1667.

SCHEME 3



10 **2** **11**

studies necessary for the development of **1** and related analogues as biochemical tools. *Although requiring multiple steps, this approach presents a number of opportunities for analogue* development that are not available using the original Weinhold procedure.

Alternative Aziridination Method. We initially examined a number of methods for aziridination of **10**, ultimately settling on the disclosed intramolecular Mitsunobu reaction of Scheme 2. Typically, such reactions involve some form of N-protection resulting in enhanced acidity of the NH proton. That aziridination of **10** proceeds at all represents an interesting example of an unprotected amine taking part in such a cyclization. The relatively low yield of this transformation facilitated a survey of other cyclization methods. Moreover, we were intrigued at the possibility of generating masked analogues of **1** that might undergo activation events to afford the aziridine within biological samples. Construction of the *N*-mustard **2** (Scheme 3) outlines an alternative closure system. Iodide installation was effected by subjecting **10** to I_2, PPh_3, and imidazole followed by washing with acidic water to remove water-soluble contaminants.[11] The resulting oil was then stirred at 0 °C in 3.32 M HCl/dioxane so as to cleave the silyl ether linkages with concomitant formation of ammonium hydrochloride **2**.

This methodology offers several advantages over the triphenylphosphine-mediated coupling. The workup conditions for this preparation conveniently remove side products giving **2** in respectable yields, providing a stable precursor to the aziridine, which can readily be converted to the aziridine under mildly basic conditions. Indeed, we have observed aziridination during the course of NMR experiments where **2** was treated with Na_2CO_3 in D_2O. Under these suboptimal conditions (>1 mM concentration of **2**), **11** was formed in 30% yield after 15 min at room temperature. In addition, aziridination via the agency of **2** affords **1** or **11** in a fashion that does not rely on a fluoride source such as Bu_4NF to effect 2′,3′-unmasking. This is important because removal of all byproducts of aziridine deprotection is very problematic due to solubility factors. Finally, we envision the prospect of masking the amine of **10** so as to generate "triggerable" analogues of **2** for use in chemical biological studies where **1** can be selectively generated in situ.

Bumped Cofactors for Use by Mutant Methyltransferases. Having established the importance of *o*-NBS-protected amino esters in construction of **1** and iodoethyl precursor **2**, we wished to adapt these advances to base-modified or "bumped" aziridines. In achieving aziridine-based cofactors orthogonal to other SAM-binding enzymes and specific to given proteins of interest, we foresaw the placement of bulky moieties attached to the adenine N6. Such bumps have been incorporated into SAM and SAH and afford significant enzyme selectivities by small-molecule ligands.[6a] Shokat and co-workers have similarly incorporated ancillary groups off the adenine portion of ATP in order to unveil the intricate workings of numerous protein kinases.[6b] Bumped ligands are most apt to bind to, and be used by, engineered enzymes with a complementary hole in the ligand binding domain.[6b] Cofactor specificity is conferred via the bump, which sterically prohibits these modified ligands from fitting into the active sites of wild-type enzymes and restricts activity to hole-containing mutant methyltransferases.[6a] Given progress on the synthesis of **1**, several opportunities existed for bump incorporation. However, structural information on a number of DNA and protein methyltransferases strongly supports the notion that N6 modification affords the highest measure of predictability in generating chemical complementarity between cofactor mimics and methyltransferases.[6a,12]

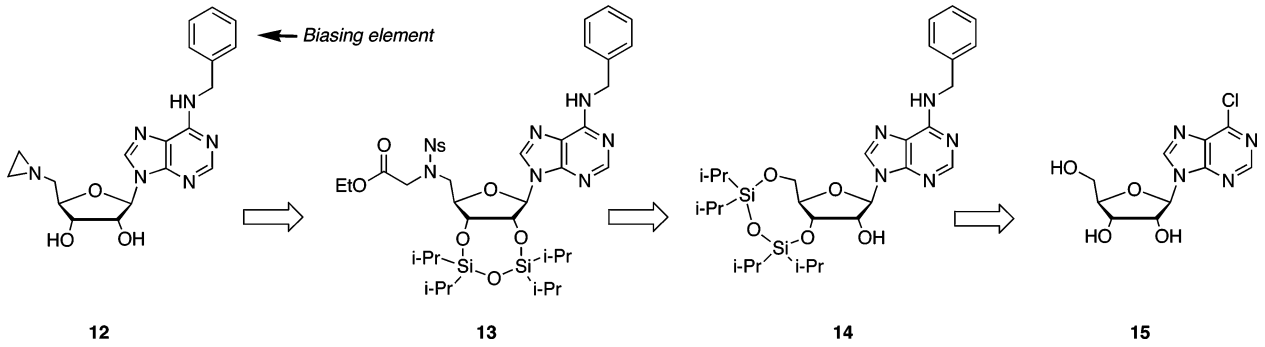
In formulating a synthesis of N6-modified cofactors (of which **12** is representative), we envisioned the use of SNAr reactions of primary amines with **15**, diprotection of the 3′,5′-diol as the corresponding disiloxane and isomerization to the 2′,3′ disiloxane, and Mitsunobu condensation. Processing of the 5′ amino acid moiety to an ethanolamine moiety would allow access to **12** and related congeners. Indeed, disiloxane **14** proved to be easily accessible and was amenable to protecting group isomerization to its corresponding 2′,3′ disiloxane using methanesulfonic acid (Supporting Information). By analogy to the transformation **7** → **9**, Mitsunobu condensation with *o*-NBS-protected glycine ethyl ester **8** (Scheme 2) proceeded very efficiently with the now free 5′ center to afford **13**, which could then be readily denosylated with previously established conditions. Disappointingly, and in contrast to the di-TES counterpart, subjection of the denosylated analogue of **13** to $LiAlH_4$ returned only unproductive intermediates. Numerous efforts to control the reaction via alteration of reductants and other conditions failed. The reductive lability of the 2′,3′ disiloxane moiety (Supporting Information) ultimately proved to be fatal to this approach, thus forcing an alternative strategy by which to derive N6-modified analogues of **1**. In formulating an alternative route to "bumped" analogues of **1**, we were inspired by the work of Maruyama and co-workers describing selective 2′,3′-diprotection of chlororiboside **15** in aqueous benzoyl chloride.[13] We adapted this chemistry to generate chlororiboside **16**. 5′-Amination was then effected with 79% yield to afford chromatographically pure **17**, which was

(11) Hoffmann, R. W.; Koberstein, R.; Harms, K. *J. Chem. Soc., Perkin Trans. 2* **1999**, 183.

(12) Zhang, X.; Cheng, X. *Structure* **2003**, *11*, 509.

(13) Kozai, S.; Takamatsu, S.; Izawa, K.; Maruyama, T. *Tetrahedron Lett.* **1999**, *40*, 4355.

SCHEME 4



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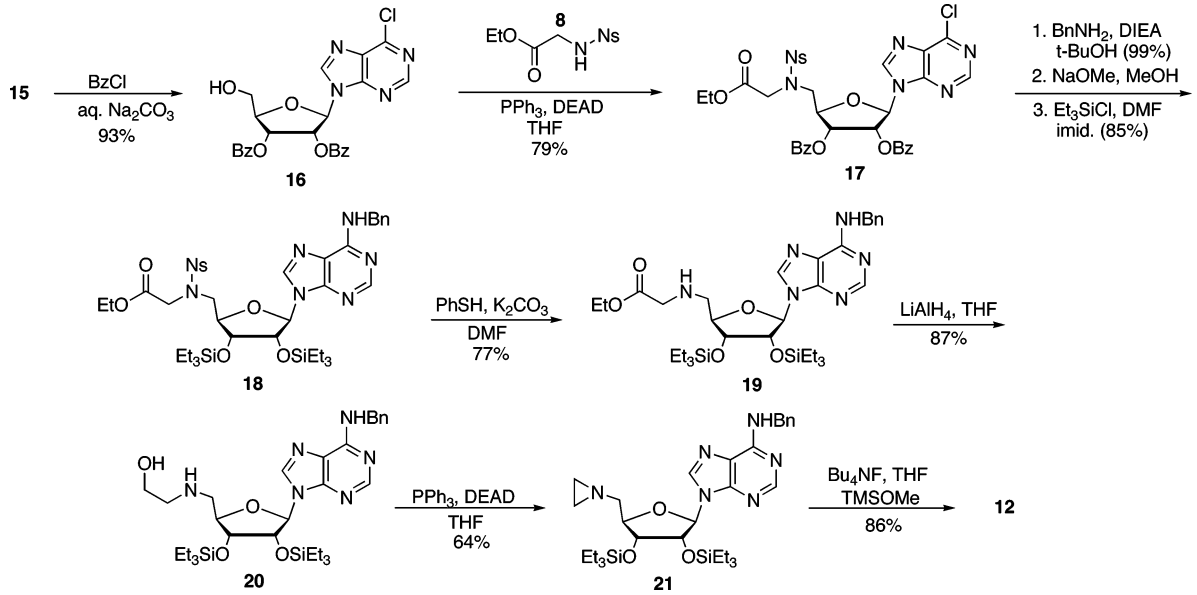

then subjected to the highly efficient sequence of bump installation and 2′,3′-protecting group exchange, thus rendering **18**.[14] It is significant to note that protecting group conversion proceeded smoothly in one pot via initial treatment with catalytic NaOMe followed by an ammonium chloride quench and coevaporation with DMF prior to standard disilylation procedures. Predictably, ethyl ester **18** was readily amenable to the sequence of denosylation, reduction,[15] and aziridination to render **21** in 43% yield over the three steps.[10a] Finally, silyl ether cleavage was effected with Bu_4NF in cold THF to afford **12**. *In a significant departure from our initial findings with **1** (Scheme 2), desilylation of **21** with TBAF only afforded isolable material devoid of rapid decomposition when performed in tandem with a TMSOMe quench to remove excess fluoride ion*.[16]

In sum, we have shown that *o*-NBS-protected amino acids can be readily coupled to suitably protected adenylates to ultimately render 5′-aziridine adenylates. This variation of the Mitsunobu reaction is applicable to the synthesis of iodo mustard precursors to **1** and also to the construction of cofactor mimics potentially orthogonal to native methyltransferases but compatible with mutant enzymes. Such cofactor mimics capable of extending the chemistry of naturally occurring enzymes hold tremendous promise in the context of biosynthetic drug discovery, transcription therapies, and biochemical tool development, yet efforts to devise efficient and flexible syntheses of these agents have been lacking. Thus, the synthetic advances highlighted here are sure to hasten the development of these aziridine-based cofactor mimics as therapeutic and mechanistic tools.

Experimental Section

5′-Amino-5′-deoxy-2′,3′-bis(*O*-triethylsilyl)adenosine (5). 5′-Phthalimidoisopropylidene adenosine (2.84 g, 6.5 mmol) was slowly added to 15 mL of a 10:1 mixture of TFA/H_2O at 0 °C, thus forming a clump that dissolved over the course of the reaction. Completion occurred at approximately 3 h as determined by the TLC. The resulting solution was coevaporated with anhydrous ethanol, dissolved in water, and lyophilized. The resulting lyophilate was dissolved in THF; Et_3N was added, and Et_3SiCl was added dropwise. No reaction was observed in the first 24 h. A second 4 mL portion of Et_3N and then a 2.4 mL portion of Et_3SiCl, 20 mL of DMF, and 10 mL of acetonitrile were added to the reaction to effect complete conversion. The crude mixture was evaporated and washed

(14) Iwamura, H.; Masuda, N.; Koshimizu, K.; Matsubara, S. *J. Med. Chem*. **1983**, *26*, 838.
(15) Morimoto, Y.; Matsuda, F.; Shirahama, H. *Synlett* **1991**, *3*, 201.
(16) (a) Liao, Y.; Fathi, R.; Reitman, M.; Zhang, Y.; Yang, Z. *Tetrahedron Lett.* **2001**, *42*, 1818. (b) Kudasheva, I. A.; Musavirov, R. S.; Nedogrei, E. P.; Akhmatdinov, R. T.; Kantor, E. A.; Rakhmankulov, D. L. *Zhurnal Obshchei Khimii* **1986**, *56*, 617. (c) Mayr, M.; Buchmeiser, M. R.; Wurst, K. *Adv. Synth. Catal.* **2002**, *344*, 712. (d) Pace, S. C.; Elkaim, J. C.; Riess, J. G. *J. Organomet. Chem.* **1973**, *56*, 157.

with a 10% citric acid solution, water, sodium bicarbonate, and then brine, dried over sodium sulfate, and evaporated to give an off-white solid. Trituration of the crude solid with diethyl ether afforded 3.5 g of pure 5′-amino-5′-deoxy-2′,3′-bis(*O*-triethylsilyl)adenosine as a white powder in 86% yield: mp 153.5−155 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (1H, s), 8.08 (1H, s), 6.64 (2H, s), 5.89 (1H, d, J = 5.9 Hz), 5.25 (1H, dd, J = 6.0, 4.24 Hz), 4.40−4.30 (2H, m), 4.23 (1H, dd, J = 14.1, 6.6 Hz), 3.94 (1H, dd, J = 14.2, 6.2 Hz), 0.93 (9H, t, J = 8.0 Hz), 0.80 (9H, t, J = 7.9 Hz), 0.61 (6H, q, J = 7.9 Hz), 0.41 (3H, decet, J = 8.0 Hz), 0.37 (3H, decet, J = 7.8 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 155.5, 152.0, 149.8, 141.2, 134.4, 132.2, 123.6, 120.7, 89.3, 83.3, 74.2, 73.6, 69.1, 6.9, 6.7, 6.1, 5.1, 4.6 ppm; *m/z* 214 (M^+, 100); HRMALDI calcd for $C_{30}H_{45}N_6O_5Si_2$ $(M + H)^+$ 625.2990, found 625.2910.

5′-Phthalimide-5′-deoxy-2′,3′-bis(*O*-triethylsilyl)adenosine (625 mg, 1.0 mmol) and a 16-fold excess of hydrazine hydrate were dissolved in 30 mL of ethanol and gently refluxed overnight. The obtained solution was allowed to cool to room temperature and then filtered. The filtrate was evaporated to dryness in vacuo. The residue was treated with 5 mL of water; glacial acetic acid was added to achieve pH 4, and the mixture was then filtered. The filtrate was adjusted to pH 10 with aqueous 4 N NaOH. Extraction with chloroform, drying of the combined organic layers with $MgSO_4$, and removal of the solvents by evaporation gave the 353 mg of amino adenylate **5** at 72%: mp 164−166.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (1H, s), 7.94(1H, s), 6.12−5.84 (2H, br), 5.86 (1H, d, J = 6.0 Hz), 5.11 (1H, dd, J = 6.0, 4.8 Hz), 4.29 (1H, dd, J = 4.0, 2.8 Hz), 4.12−4.08 (1H, m), 3.14 (1H, dd, J = 13.6, 3.2 Hz), 3.02 (1H, dd, J = 13.2, 5.6 Hz), 1.80 (2H, s, br), 1.00 (9H, t, J = 7.8 Hz), 0.78 (9H, t, J = 7.8 Hz), 0.67 (6H, q, J = 7.8 Hz), 0.41 (3H, decet, J = 8.0 Hz), 0.37 (3H, decet, J = 7.8 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 153.6, 150.1, 141.1, 121.0, 90.0, 74.0, 73.8, 44.0, 7.2, 6.9, 5.8, 5.5 ppm; *m/z* 214 (M^+, 100); HRMALDI calcd for $C_{22}H_{42}O_3N_6Si_2$ $(M + H)^+$ 495.2935, found 495.2943.

5′-Amino Glycyl Ethyl Ester-5′-deoxy-2′,3′-bis(*O*-triethylsilyl)adenosine (6) [Bromoacetate Alkylation Method]. To **5** (4.95 g, 10.0 mmol) was added Et_3N (4.5 mL, 32 mmol) and 20 mL of anhydrous THF at 0 °C. Ethylbromoacetate (1.12 mL, 10 mmol) was added dropwise to the stirring solution and allowed to warm to room-temperature overnight. The resulting mixture was filtered to remove the triethylamine hydrobromide salt and evaporated. The resulting oil was diluted with diethyl ether to precipitate unreacted starting material, and the filtrate was evaporated. The crude material was dissolved in ethanol on ∼100 mL of silica and evaporated. The dried powder was added to a flash column containing wet packed silica 4:4:0.5 CH_2Cl_2/EtOAc/MeOH. The non-UV-active initial fractions were discarded, and the first 100 mL of UV-active eluant was discarded (removing dialkylated contaminant). Fractions were collected until UV activity disappeared, and solvent was evaporated, giving 5.08 g of product **6** in 92% yield: mp 117−121 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (1H, s), 7.95(1H, s), 6.27 (2H, br), 5.86 (1H, d, J = 6.2 Hz), 5.10 (1H, dd, J = 6.0, 4.5 Hz), 4.26 (1H, dd, J = 4.4, 2.9 Hz), 4.16 (2H, q, 7.2 Hz),4.15 (1H, d, 10.3 Hz), 3.44 (2H, s), 2.99 (1H, dd, J = 12.5, 3.2 Hz), 2.88 (1H, dd, J = 12.5, 6.2 Hz), 2.84−2.76 (1H, br), 1.23 (3H, t, J = 7.0 Hz) 0.97 (9H, t, J = 7.9 Hz), 0.76 (9H, t, J = 7.9 Hz), 0.64 (6H, q, J = 7.8 Hz), 0.41 (3H, decet, J = 8.0 Hz), 0.39 (3H, decet, J = 7.8 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.5, 156.1, 153.1, 149.9, 140.9, 121.0, 89.7, 85.6, 74.2, 74.1, 60.9, 51.5, 51.1, 14.4, 7.05, 6.7, 5.1, 4.7 ppm; *m/z* 214 (M^+, 100); HRMALDI calcd for $C_{26}H_{48}O_5N_6Si_2$ $(M + H)^+$ 603.3122, found 603.3081.

5′-*o*-Nitrobenzenesulfonyl-*N*-glycyl Ethyl Ester 2′,3′-*O*-Isopropylidene Adenosine (9). 2′,3′-Isopropylideneadenosine (3.07 g, 10.0 mmol), triphenylphosphine (3.15 g, 12 mmol), and nosylated glycine ethyl ester **8** (3.46 g, 12 mmol) were dissolved in 35 mL of anhydrous THF. DEAD (1.89 mL, 12 mmol) was added to the reaction mixture slowly at 0 °C. The reaction solution was stirred at room temperature for 2.5 h. The mixture was filtered, washed with saturated $NaHCO_3$, water, and then brine, and evaporated. The product was recrystallized from MeOH/hexanes to give pure **9** in 62% yield. Product retained in the mother liquor was isolated by column chromatography. Conditions: 2.5 L of a 1:1 mixture of DCM/EtOAc was used to elute all byproducts. Product started to elute after passage of 300 mL of 4.5:4.5:1 DCM/EtOAc/MeOH and was collected in the following 1.5 L. Collected fractions and recrystallized compound were evaporated to give 3.86 g of product in 73% overall yield: mp 178.5−180 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (1H, s), 8.00(1H, s), 7.92 (1H, dd, J = 8.1, 1.1 Hz), 7.80 (1H, dd, J = 8.1, 1.1 Hz), 7.73 (1H, dt, J = 7.7, 0.7 Hz), 7.65 (1H, dt, J = 7.7, 0.7 Hz), 6.11 (1H, d, J = 2.2 Hz), 5.36 (1H, dd, J = 6.2, 2.2 Hz), 4.94 (1H, dd, J = 6.2, 1.9 Hz), 4.28−4.24 (1H, m), 4.03 (1H, d, 18.7 Hz), 3.78−3.64 (4H, m), 3.52 (1H, dd, J = 15.38, 9.15 Hz), 3.23 (1H, s), 1.42 (3H, s), 1.21 (3H, s) 0.85 (3H, t, J = 7.3 Hz) ppm; ^{13}C NMR (125 MHz, d_6-DMSO) δ 168.6, 156.9, 153.4, 149.0, 148.0, 141.1, 135.3, 132.9, 132.2, 131.0, 124.8, 120.0, 114.0, 90.3, 86.6, 83.6, 82.6, 61.4, 50.3, 49.7, 27.6, 25.8, 14.3 ppm; *m/z* 214 (M^+, 100); HRMALDI calcd for $C_{23}H_{27}O_9N_7S$ $(M + H)^+$ 578.1669, found 578.1631.

5′-Amino Glycyl Ethyl Ester 5′-Deoxy-2′,3′-Bis(*O*-triethylsilyl)adenosine (6) [Mitsunobu Method]. To a stirring 10:1 TFA/water mixture at 0 °C was added **9** (1.15 g, 2 mmol) portion-wise. The reaction was stirred until complete disappearance of starting material, which was observed at 3.5 h. The resulting suspension was evaporated in vacuo followed by three coevaporations with anhydrous EtOH to remove all traces of TFA. Crude **6** precursor was triturated with water and then $CHCl_3$ to afford 990 mg of diol in 92% yield. Crude precursor to **6** (950 mg, 1.77 mmol) and TEA (1.5 mL, 10.8 mmol) were dissolved in THF and cooled to 0 °C. Et_3SiCl (1.07 mL, 6.4 mmol) was added dropwise and the reaction stirred for 8 h. The resulting crude reaction contents were filtered, evaporated, and redissolved in 50 mL of EtOAc. The organic portion was washed with saturated $NaHCO_3$, water, and brine, dried over Na_2SO_4, and evaporated. Silica chromatography (8:4:1 Hex/EtOAc/MeOH) afforded 1.04 g of di-TES-protected compound in 77% yield: mp 158−160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (1H, s), 8.05(1H, d, J = 7.98 Hz), 7.81 (1H, s), 7.68−7.57(3H, m), 5.79 (1H, d, J = 6.5 Hz), 5.60 (2H, br), 5.27 (1H, dd, J = 6.5, 4.4 Hz), 4.31−4.28 (1H, m), 4.27−4.24 (2H, m), 4.16 (1H, d, 18.7 Hz), 4.08−4.0 (2H, m), 3.98−3.90 (1H, m), 3.84 (1H, dd, J = 15.1, 9.3 Hz), 1.26 (1H, s), 1.1 (3H, t, J = 7.1 Hz) 0.99 (9H, t, J = 7.9 Hz), 0.75 (9H, t, J = 7.9 Hz), 0.67 (6H, q, J = 7.9 Hz), 0.35 (3H, decet, J = 7.7 Hz), 0.28 (3H, decet, J = 7.7 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 158.6, 152.8, 150.1, 150.1, 141.0, 133.6, 131.7, 131.1, 127.9, 124.2, 90.1, 86.0, 74.3, 72.6, 61.4, 50.6, 49.8, 14.1, 7.1, 6.6, 5.1, 4.4 ppm; *m/z* 214 (M^+, 100); HRMALDI calcd for $C_{32}H_{51}O_9N_7SSi_2$ $(M + Na)^+$ 788.2905, found 788.2860.

To 577 mg (0.753 mmol) of silyl ether was added K_2CO_3 (312 mg, 2.26 mmol) and 50 mL DMF. To the stirring solution was added thiophenol (92 μL, 0.904 nmol) dropwise. The mixture was stirred for 12 h. The crude mixture was filtered and evaporated. The resulting yellow oil was resuspended in 4 mL of CH_2Cl_2 and extracted with saturated $NaHCO_3$. The organic layer was subsequently chromatographed using 100% DCM until all traces of a UV-active yellow band had eluted. The eluent was then changed to 85% CH_2Cl_2/MeOH, which facilitated isolation of 428 mg of **6** at 98% yield: mp 119−121.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (1H, s), 7.95(1H, s), 6.27 (2H, br), 5.86 (1H, d, J = 6.2 Hz), 5.10 (1H, dd, J = 6.0, 4.5 Hz), 4.26 (1H, dd, J = 4.4, 2.9 Hz), 4.16 (2H, q, 7.2 Hz), 4.15 (1H, d, 10.3 Hz), 3.44 (2H, s), 2.99 (1H, dd, J = 12.5, 3.2 Hz), 2.88 (1H, dd, J = 12.5, 6.2 Hz), 2.84−2.76 (1H, br), 1.23 (3H, t, J = 7.0 Hz) 0.97 (9H, t, J = 7.9 Hz), 0.76 (9H, t, J = 7.9 Hz), 0.64 (6H, q, J = 7.8 Hz), 0.41 (3H, decet, J = 8.0 Hz), 0.39 (3H, decet, J = 7.8 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 155.8, 153.1, 150.1, 141.1, 121.1, 89.8, 85.6, 74.1, 74.1,

60.9, 51.5, 51.2, 14.5, 7.1, 6.7, 5.2, 4.7. ppm; *m/z* 214 (M^+, 100); HRMALDI calcd for $C_{26}H_{48}O_5N_6Si_2$ $(M + H)^+$ 603.3122, found 603.3081.

5′-Aminoethylhydrin-5′-deoxy-2′,3′-bis(*O*-triethylsilyl)-adenosine (10). To ester **6** (2.9 g, 5.0 mmol) was added 100 mL of anhydrous THF, and the mixture was cooled to −15 °C. $LiAlH_4$ 1 M/THF (12 mL, 12 mmol) was added to the reaction mixture slowly over 15 min. The reaction was allowed to warm to room temperature over 2.5 h, giving quantitative conversion of starting material. The reaction was cooled to 0 °C, and saturated $NaHCO_3$ was added very slowly to the stirring mixture to quench the reducing agent. The crude reaction was diluted in 500 mL of EtOAc, extracted, and washed with saturated $NaHCO_3$, water, and brine. The resulting organic layer was dried over sodium sulfate, evaporated, and placed on a short silica plug to remove the aluminum salts. Compound was eluted with 85% $CHCl_3$/MeOH, and UV-active fractions were pooled to give 2.35 g of a white solid after evaporation (87% yield): mp 143−145.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (1H, s), 7.80(1H, s), 6.65 (2H, br), 5.76 (1H, d, J = 6.6 Hz), 5.19 (1H, dd, J = 6.6, 4.5 Hz), 4.24 (1H, d, J = 3.7 Hz), 4.19 (1H, d, 2.7 Hz), 3.77−3.69 (2H, m), 3.01 (1H, dd, J = 11.3, 7.2 Hz), 2.90 (1H, dd, J = 11.9, 3.1 Hz), 2.83−2.80 (2H, m), 0.98 (9H, t, J = 8.0 Hz), 0.71 (9H, t, J = 8.0 Hz), 0.64 (6H, q, J = 8.0 Hz), 0.32 (3H, decet, J = 8.0 Hz), 0.26 (3H, decet, J = 7.9 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 153.0, 150.1, 141.4, 120.6, 90.0, 86.0, 74.6, 73.4, 61.4, 52.0, 51.5, 7.1, 6.7, 5.2, 4.6 ppm; *m/z* 214 (M^+, 100); HRMALDI calcd for $C_{24}H_{46}O_4N_6Si_2$ $(M + H)^+$ 561.30168, found 561.2906.

5′-Aziridino-5′-deoxyadenosine (1). To ethanolamine **10** (269 mg, 0.5 mmol) in 10 mL of anhydrous THF was added PPh_3 (197 mg, 0.75 mmol). To the solution of **10** and PPh_3 at room temperature was added DEAD (118uL, 0.75 mmol). After 30 min, the mixture was heated to 45 °C and aged for 6 h. The reaction was quenched with saturated $NaHCO_3$ and then diluted with EtOAc. Extraction with $NaHCO_3$, brine, and evaporation gave a yellow oil, which was applied directly to a silica gel column (1:2:5:1 Hex/CH_2Cl_2/CH_3CN/PE), affording after elution 175 mg of colorless solid with 67% yield: mp 173.5−177 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (1H, s), 8.04 (1H, s), 6.18 (2H, br), 5.94 (1H, d, J = 5.4 Hz), 5.02 (1H, dd, J = 4.8, 4.5 Hz), 4.39 (1H, d, J = 3.8 Hz), 4.19 (1H, dt, 3.8, 5.0 Hz), 2.68 (1H, dd, 12.8, 5.0 Hz), 2.47 (1H, dd, 12.8, 6.0 Hz), 1.74 (1H, ddd, 20.0, 5.6, 3.7 Hz), 1.22−1.16 (2H, m), 0.98 (9H, t, J = 7.9 Hz), 0.79 (9H, t, J = 8.1 Hz), 0.66 (6H, q, J = 7.9 Hz), 0.42 (3H, decet, J = 7.9 Hz), 0.39 (3H, decet, J = 8.1 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 150.3, 140.8, 120.7, 89.2, 85.1, 74.5, 73.9, 63.0, 28.0, 27.6, 7.0, 6.8, 5.1, 4.8 ppm; *m/z* 214 (M^+, 100); HRMALDI calcd for $C_{24}H_{44}O_3N_6Si_2$ $(M + H)^+$ 521.3092, found 521.3071.

To 91 mg of crude aziridine (0.175 mmol) was added 2 mL of anhydrous THF. The solution was stirred at −10 °C for 10 min followed by addition of 3.5 mL of 0.1 M anhydrous Bu_4NF in THF. The solution became turbid and then gummed up so as to prohibit stirring. The reaction was briefly warmed to ambient temperature, and 500 μL of MeOH was added to allow effective stirring. The reaction was then immediately returned to a low temperature again at which point TLC analysis (1:1 $CHCl_3$/MeOH) revealed complete consumption of starting material and generation of a new UV-active spot with R_f = 0.35. The residual Bu_4NF was quenched by addition of 192 μL of TMSOMe (1.4 mmol). The solution was stirred for an additional 15 min followed by addition of 10 mL of MeOH. Solvent was partially removed (to about 1/3 volume), and a second cold coevaporation from anhydrous MeOH was performed to yield ∼100 μL of sample. The crude reaction was then chromatographed over silica that had been pretreated with excess methylaziridine (Aldrich) in MeOH. Silica gel chromatography to isolate pure **1** was performed with 2:1 $CHCl_3$/MeOH. The resulting colorless glass was suspended in D_2O, frozen in liquid N_2, and subjected to lyophilization for 12 h to ultimately afford 51 mg of analytically pure **1** (99% yield): mp 95.5−97 °C; ^{1}H NMR (500 MHz, D_2O) δ 8.25 (s, 1H), 8.15 (s, 1H),6.03 (d, 1H, J = 5.1 Hz), 4.74 (t, 1H, J = 5.2 Hz), 4.39 (t, 1H, J = 5.1 Hz), 4.28 (td, 1H, J = 4.6, 6.7 Hz), 2.64 (ddd, 2H, J = 5.4, 13.3, 17.3 Hz), 1.74 (ddd, 2H, J = 4.5, 6.1, 32.2 Hz), 1.38 (ddd, 2H, J = 4.5, 7.4, 20.7 Hz) ppm; ^{13}C NMR (125 MHz, D_2O) δ 155.7, 153.1, 149.1, 119.0, 87.7, 84.0, 73.7, 71.5, 61.1, 27.2, 26.8 ppm; HRMALDI calcd for $C_{10}H_{15}O_5$ $(M + H)^+$ 293.1362, found: 293.1365.

5′-Aminoethyliodo-5′-deoxyadenosine Ammonium Hydrochloride (2). Imidazole (78 mg, 1.15 mmol), iodine (292 mg, 1.15 mmol), and triphenyl phosphine (302 mg, 1.15 mmol) were stirred in 25 mL of THF for 15 min. Ethanolamine adenylate **10** (350 mg, 1.0 mmol) dissolved in 5 mL of anhydrous THF was added slowly to the stirring solution. The reaction solution was stirred at room temperature for 2.5 h. The mixture was then filtered, evaporated, and washed with saturated $NaHCO_3$, water, and brine. The organic layer was evaporated and immediately suspended in 4 mL of 3.32 M HCl/dioxane. After evaporation, the resulting solid was dissolved in water and washed sequentially with CH_2Cl_2 and hexane. The resulting solution was evaporated, giving 299 mg of diol **2** as the HCl salt in quantitative yield: mp 142.5 °C dec; ^{1}H NMR (500 MHz, D_2O) δ 8.52 (s, 1H), 8.51 (s, 1H), 6.20 (d, 1H, J = 4.9 Hz), 4.93 (t, 1H, J = 5.0 Hz), 4.54 (t, 1H, J = 5.0 Hz), 4.50 (ddd, 1H, J = 3.1, 4.6, 9.5 Hz), 3.75−3.64 (m, 2H), 3.62−3.58 (t, 2H, J = 7.0 Hz), 3.49−3.39 (m, 2H) ppm; ^{13}C (125 MHz, D_20) δ 155.7, 153.0, 149.0, 140.0, 119.0, 87.7, 83.9, 73.7, 71.5, 61.1, 27.2, 26.8 ppm; m/*z* 214 (M^+, 100); HRMALDI calcd for $C_{12}H_{18}H_6O_3$ $(M + H)^+$ 421.048, found 421.044.

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Supporting Information Available: General experimental methods and detailed experimental methods describing the synthesis depicted in Schemes 4 and 5. This material is available free of charge via the Internet at http://pubs.acs.org.

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