

Efficient Synthesis of Azide-Bearing Cofactor Mimics

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Abstract: 8-Azido-5'-aziridino-5'-deoxyadenosine (**6**), a novel cofactor mimic, was synthesized in nine steps from commercially available 2',3'-isopropylideneadenosine in ~4% overall yield. Crucial to this success was a very unorthodox phthalimide cleavage procedure, C8 azidation prior to aziridination and late stage alkylation of the 5' amino group with iodoethanol necessitated by the high degree of lability endowed by the aryl azide moiety. Aziridine **6** is envisioned as a useful biochemical tool by which to probe DNA and protein methylation patterns.

S-Adenosyl-L-methionine (SAM, **2**)-dependent methylation of nucleic acids and proteins is now recognized as playing an absolutely vital role in the careful regulation of gene transcription.^{1,2} Notably, flaws in activity and expression levels of the eukaryotic DNA methyltransferase (Mtase) DNMT1 have been integrally linked to oncogenic potential.² Thus, the design and synthesis of agents capable of exploiting such flawed traits of DNMT1 offers an attractive new means by which chemotherapeutics might be devised. Substances capable of undergoing transfer to nucleic acids and proteins in an Mtase-dependent way also hold tremendous promise as biochemical tools by which to dissect and understand biological methylation. The advent of proteomics and its goal of understanding how post-translational protein modifications alter function lends significant credence to the pursuit of substances that undergo Mtase-dependent delivery to biomolecules, specifically components of the transcriptional machinery. Our interests in the area of cofactor mimicry are reflected by investigations into the synthesis and biochemical study of **4** (Scheme 1) and related congeners.

The 5' aziridine adenylate **4** is a substitute for SAM (**2**) in the *M. TaqI*-catalyzed alkylation of adenine within the recognition sequence 5'TCGA3' (Scheme 1).^{3,4} Instead of generating the N⁶-methylated dA residue, substrate adenylation is accomplished via ring-opening of the aziridine. This chemistry is tolerant of cofactor C8 modification and has been successfully used to fluorescently tag short oligonucleotides and large plasmid substrates in *M. TaqI*-dependent fashion.⁴ Cofactor **4** also undergoes *M. HhaI*-dependent DNA attachment within the *M. HhaI* recognition sequence 5'GCGC3'.^{5,6} Studies in which the

"target" cytosine (bold) of 5'GCGC3' is methylated prior to reaction with **4** revealed no enzyme-specific alkylation by **4**.⁶ Thus, *M. HhaI*-mediated alkylation of 5'GCGC3' with SAM and **4** share the same regiochemistry. These findings provide clear evidence of the importance of 5' aziridine adenylates as "cofactor mimics" of SAM as tools for biology in the short term and potential therapeutic agents in the long term. As a first step in developing these agents as useful and broadly applicable biochemical tools, we wished to equip **4** with a handle through which any desired molecule (DNA damaging moiety, affinity matrix handle, fluorophore, etc.) could be appended following Mtase-dependent anchoring. Such a handle would need to present a minimal disruption to cofactor-Mtase interactions and be highly reactive in a chemoselective sense following the enzymatic reaction. Most importantly, this handle would need to be abiotic and capable of rapid couplings to other reagents orthogonal to typical cellular constituents.

An extensive body of literature now supports the importance of the azido group as an abiotic handle capable of undergoing highly efficient chemoselective couplings.^{7–20} Alkyl azides have been reported to react with *o*-methoxycarbonyl-functionalized triarylphosphines to afford amide-linked coupling products in what is now termed the Staudinger ligation,^{7–16,20} and a wide array of azides have been shown to be highly active participants in [2 + 3] Huisgen cycloadditions^{17–19,21} when presented with alkynes. The latter reaction is representative of "click chemistry", a class of reactions nicely outlined by Sharpless and co-workers.²¹ Importantly, both reaction types are amenable to complex biological mixtures.

In hypothesizing where best to equip **4** with an azide moiety, we were struck by three realizations. First, 8-azidoadenosine (and aryl azides in general) undergoes

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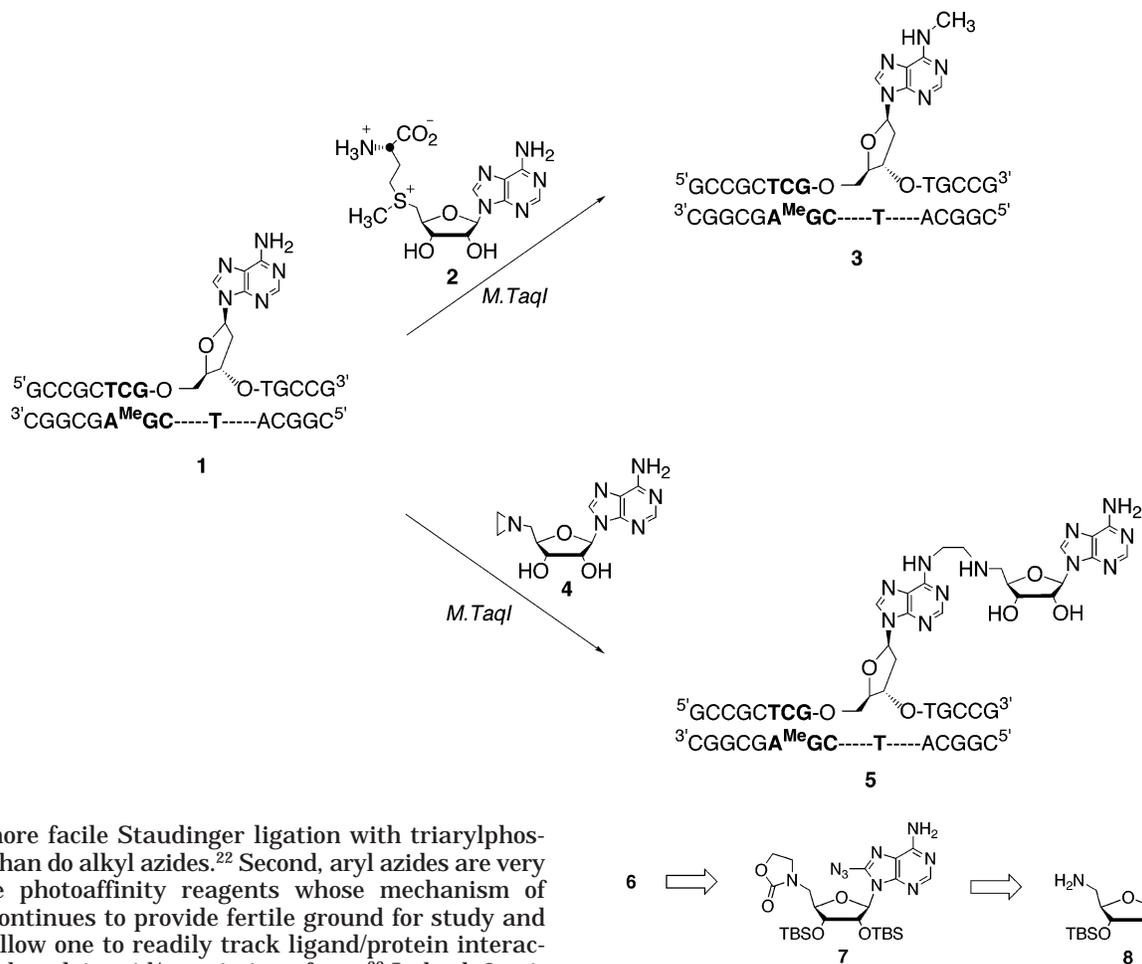
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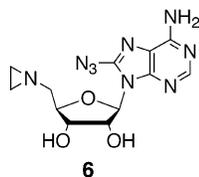
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SCHEME 1



much more facile Staudinger ligation with triarylphosphines than do alkyl azides.²² Second, aryl azides are very effective photoaffinity reagents whose mechanism of action continues to provide fertile ground for study and which allow one to readily track ligand/protein interactions and nucleic acid/protein interfaces.²³ Indeed, 8-azidoadenosine and related adenylates represent one of the most extensively studied classes of photoaffinity reagents known.²⁴ Finally, a small, but not insignificant amount of work has demonstrated that C8 azido-SAM retains its cofactor function.²⁵ These considerations led us to undertake the construction of **6**, an azide bearing cofactor mimic for applications in chemical biology to understand SAM-dependent methylation.



We have previously reported on the utility of the Gabriel Cromwell reaction in forming the critical 5'-aziridine of analogues of **4**.²⁶ An unfortunate limitation of this chemistry is the absolute requirement for functionalization of the α,β -dibromide starting material. This abrogates unsubstituted aziridine generation and thus would not prove useful for generation of **6**. In our laboratories, we have reconciled the difficulty of 5'-aziridination via alkylation of the 5'-aminoadenylate **8** (Figure 1) with various bromoacetates followed by LiAlH_4 reduction and

FIGURE 1.

aziridination. Thus, at the outset we envisioned construction of **6** through the agency of **7**, which was readily accessible by condensation of primary amine **8** with methyl bromoacetate, reduction, oxazolidinone generation, and the sequence of C8 bromination followed by azidation. Having generated gram quantities of **7**, we sought to unmask the 5'-ethanolamine moiety in preparation for aziridination and 2',3'-deprotection. Model studies of the same transformation on material lacking the C8 azido substituent showed 10% ethanolic NaOH ²⁷ to be very effective at oxazolidinone cleavage. However, subsection of **7** to these and a wide variety of related conditions, led to significant decomposition processes.²⁷⁻³⁴ These difficulties, compounded by complications involved with alternative protection strategies, ultimately proved insurmountable, thus forcing a reevaluation of synthetic approach.

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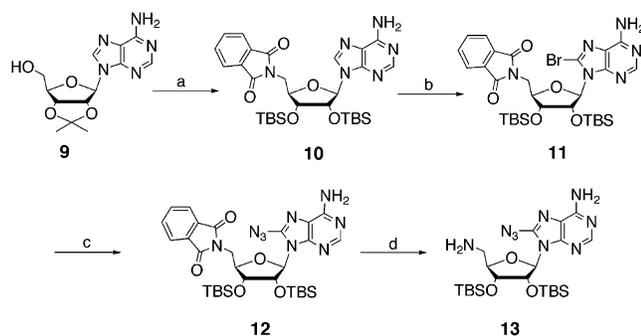
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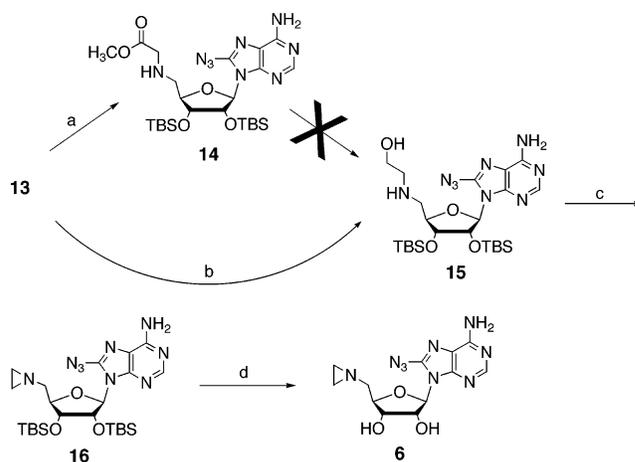
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SCHEME 2^a

^a Reagents and conditions: (a) (1) phthalimide, PPh₃, DEAD, THF, rt, (2) 3:1:1 TFA/H₂O/THF, rt, (3) TBSCl, imidazole, DMF, rt, 60% three steps; (b) Br₂, 0.5M NaOAc (pH 5.3), dioxane, rt, 79%; (c) NaN₃, DMSO, 70 °C, 90%; (d) ethylenediamine, EtOH, 70 °C, 81%.

In re-engineering our path to **6**, we again postulated the importance of 5'-amine **8**. Might the C8 azido analogue of **8** permit 5'-amine alkylation and subsequent ester reduction en route to ethanolamine incorporation? To investigate this, commercially available 2',3'-isopropylideneadenosine was converted to the bis-silyl ether **10** using Mitsunobu chemistry reported by Kolb and co-workers³⁵ and subsequent isopropylidene cleavage with aqueous TFA³⁶ followed by reaction with TBSCl and imidazole.³⁷ Phthalimide **10** was derived from **9** in 60% overall yield in a fashion not requiring chromatography (Scheme 2). Bromination at the 8-position was accomplished with Br₂³⁸ under mildly acidic conditions in 79% yield to render **11**, which was then converted to azide³⁹ **12** in 90% yield. Interestingly, phthalimide cleavage efforts using hydrazine hydrate and other well-known procedures failed to produce primary amine **13** with suitable efficiencies. An alternative procedure utilizing ethylenediamine⁴⁰ afforded the versatile C8 azido 5'-amine **13** in 81% yield.

With **13** in hand, the stage was now set for ultimate aziridination. As predicted based on earlier experiences, alkylation of **13** with methyl bromoacetate proceeded smoothly in 80% yield. Disappointingly, however, reduction of **14** with DIBALH, as well as numerous other milder reductants, failed to yield the obligate ethanolamine moiety. As with previous attempts to deprotect oxazolidinone **7**, the azide proved to be a clear liability. Motivated by the reductive liability posed by the C8 azide of **14**, we next pursued 5'-alkylation of **13** with an

SCHEME 3^a

^a Reagents and conditions: (a) methyl bromoacetate, TEA, THF, rt, 80%; (b) 2-iodoethanol, toluene, 70 °C, 34%; (c) PPh₃, DEAD, THF, reflux, 58%; (d) Bu₄NF, THF, 0 °C, 60%.

assortment of haloethanols. Previous efforts had shown that alkylation of 5'-aminoadenylates with oxirane is very difficult to control leading most often to dialkylated materials. This, in combination with reports by Brown and Chen of effective amine alkylations with haloethylhydriens, deterred us from investigating epoxide aminolysis as a means of ethanolamine installation.^{41,42} We ultimately chose to alkylate **13** with 2-iodoethanol to yield ethanolamine adenylate **15** (Scheme 3). Optimization of the reaction was necessary to avoid undesired bis-alkylated material, and careful control of time, solvent, and number of equivalents of iodoethanol proved vital to the success of this transformation. The yield for alkylation is low (34%) but is partly offset by facile recovery and recycling of **13** (37%) following column chromatography. Having successfully obtained **15**, aziridination was accomplished using Mitsunobu chemistry to yield **16** in a 58% yield.⁴³ Finally, TBS ether cleavage was effected using Bu₄NF to yield the 8-azido-5'-aziridino-5'-deoxyadenosine (**6**) in 60% yield.⁴⁴

In conclusion, we have completed the synthesis of the novel 8-azido-5'-aziridino-5'-deoxyadenosine. By virtue of the known biochemistry of **4**, we propose that **6** and related substances will allow the conversion of biological methyltransferases into azidas. In light of the abiotic nature of the azido group and its utility as a photoaffinity agent, participation in Staudinger ligation chemistries and [2 + 3] cycloadditions with acetylenes, **6** represents a potentially important new tool for those at the chemistry–biology interface. The methodology reported here is highly amenable to generation of analogues of **6**. Such synthetic efforts, as well as those to investigate the biochemistry of **6**, are ongoing and will be reported shortly.

Experimental Section

5'-Phthalimide-5'-deoxy-2',3'-bis(*O*-*tert*-butyldimethylsilyl)adenosine (10). To 2',3'-isopropylideneadenosine (**9**) (24.46 g, 79.64 mmol) in 275 mL of dry THF were added phthalimide

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(11.68 g, 81.91 mmol) and PPh_3 (20.89 g, 79.64 mmol). After the mixture was stirred at rt for 10 min, DEAD (13.87 g, 79.64 mmol) was added to the slurry and the mixture stirred an additional 2.5 h. The white precipitate was filtered off and washed with 200 mL of cold Et_2O to yield product that was taken forward. 5'-Phthalimide-5'-deoxy-2',3'-isopropylideneadenosine (22.0 g, 50.41 mmol) was dissolved in 500 mL of 3:1:1 TFA/ H_2O /THF and stirred at rt for 2 h. The solvent was evaporated in vacuo and co-stripped with EtOH ($\times 3$). The resulting material was brought up in a 1:1:1 EtOAc/ CH_2Cl_2 /PE (petroleum ether) mixture, and the product was filtered off as a solid and washed to yield product (20.0 g). The resulting product was dissolved in 110 mL of dry DMF, followed by the addition of imidazole (34.4 g, 505.3 mmol) and TBSCl (16.7 g, 111.0 mmol). The reaction was stirred at rt for 3 h, followed by aqueous workup (NH_4Cl ($\times 2$), EtOAc, brine). The organic layer was dried over Na_2SO_4 and evaporated in vacuo, which provided **10** (29.86 g, 60%): mp 163–166 °C; ^1H NMR (CDCl_3) δ 8.14 (s, 1H), 8.08 (s, 1H), 7.83 (m, 2H), 7.68 (m, 2H), 6.53 (bs, 2H), 5.85 (d, $J = 5.6$ Hz, 1H), 5.12 (dd, $J = 5.6, 4.4$ Hz, 1H), 4.32 (td, $J = 6.4, 2.8$ Hz, 1H), 4.24 (m, 1H), 4.20 (dd, $J = 14.0, 6.4$ Hz, 1H), 3.92 (dd, $J = 14.0, 6.4$ Hz, 1H), 0.85 (s, 9H), 0.73 (s, 9H), 0.02 (s, 3H), -0.01 (s, 3H), -0.06 (s, 3H), -0.33 (s, 3H); ^{13}C NMR (CDCl_3) δ 168.3, 160.0, 152.9, 149.8, 140.5, 134.3, 132.0, 123.5, 120.7, 89.3, 82.9, 74.0, 73.8, 40.3, 25.84, 25.79, 18.1, 17.9, -4.4 , -4.6 , -4.8 , -5.1 ; HRMALDI calcd for $\text{C}_{30}\text{H}_{44}\text{N}_6\text{O}_5\text{Si}_2$ ($\text{M} + \text{H}^+$) 625.29, obsd 625.283.

8-Bromo-5'-phthalimide-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (11). To 5'-phthalimide-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (**10**) (4.150 g, 6.641 mmol) in 100 mL of 7:4 dioxane/0.5 M NaOAc (pH 5.3) was added Br_2 (2.122 g, 13.282 mmol). The reaction was stirred at rt for 3 h, followed by aqueous workup ($\text{Na}_2\text{S}_2\text{O}_3$, CH_2Cl_2 , brine). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. Column chromatography (1:1 hexanes/EtOAc) provided **11** (3.680 g, 79%): mp 89–93 °C; ^1H NMR (CDCl_3) δ 8.03 (s, 1H), 7.81 (m, 2H), 7.69 (m, 2H), 6.31 (bs, 2H), 5.96 (d, $J = 6.8$ Hz, 1H), 5.63 (dd, $J = 6.8, 4.4$ Hz, 1H), 4.47 (dd, $J = 4.4, 1.6$ Hz, 1H), 4.36 (dd, $J = 14.0, 8.4$ Hz, 1H), 4.27 (m, 1H), 3.93 (dd, $J = 14.0, 4.4$ Hz, 1H), 0.85 (s, 9H), 0.75 (s, 9H), 0.04 (s, 3H), -0.02 (s, 3H), -0.06 (s, 3H), -0.42 (s, 3H); ^{13}C NMR (CDCl_3) δ 168.4, 154.7, 152.7, 150.9, 134.2, 132.1, 128.7, 123.4, 120.6, 90.3, 84.1, 74.0, 71.4, 39.7, 25.9, 25.8, 18.2, 17.9, -4.4 , -4.7 , -5.1 , -5.2 ; HRMALDI calcd for $\text{C}_{30}\text{H}_{43}\text{BrN}_6\text{O}_5\text{Si}_2$ ($\text{M} + \text{H}^+$) 703.20, obsd 703.200.

8-Azido-5'-phthalimide-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (12). To 8-bromo-5'-phthalimide-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (**11**) (2.138 g, 3.038 mmol) in 25.7 mL of DMSO was added NaN_3 (0.790 g, 12.152 mmol). The reaction was stirred at 70 °C for 7 h, followed by aqueous workup (NaHCO_3 , EtOAc, brine). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. Column chromatography (5:1 PE/EtOAc) yielded **12** (1.820 g, 90%): mp 95–97 °C; ^1H NMR (CDCl_3) δ 8.03 (s, 1H), 7.82 (m, 2H), 7.70 (m, 2H), 5.78 (dd, $J = 6.8$ Hz, 1H), 5.43 (dd, $J = 6.8, 4.4$ Hz, 1H), 5.33 (bs, 2H), 4.43 (dd, $J = 4.4, 0.8$ Hz, 1H), 4.27 (dd, $J = 18.4, 7.6$ Hz, 1H), 4.26 (m, 1H), 3.93 (dd, $J = 18.4, 8.8$ Hz, 1H), 0.85 (s, 9H), 0.75 (s, 9H), 0.04 (s, 3H), -0.02 (s, 3H), -0.06 (s, 3H), -0.36 (s, 3H); ^{13}C NMR (CDCl_3) δ 168.4, 153.8, 151.9, 150.6, 146.2, 134.2, 132.1, 123.5, 118.2, 87.5, 83.7, 74.0, 71.7, 39.8, 25.9, 25.8, 18.2, 18.0, -4.4 , -4.4 , -4.7 , -5.2 ; HRFAB calcd for $\text{C}_{30}\text{H}_{43}\text{N}_9\text{O}_5\text{Si}_2$ ($\text{M} + \text{H}^+$) 666.300, obsd 666.2991.

8-Azido-5'-amino-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (13). To 8-azido-5'-phthalimide-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (**12**) (1.820 g, 2.733 mmol) in 84.7 mL of EtOH was added ethylenediamine (0.821 g, 13.665 mmol). The reaction was stirred at 70 °C for 6 h and solvent evaporated in vacuo. Column chromatography on silica pretreated with 1% TEA (4:2:1 EtOAc/ CH_2Cl_2 /MeOH) yielded **13** (1.190 g, 81%): mp 150 °C dec; ^1H NMR (CDCl_3) δ 8.23 (s, 1H), 5.75 (d, $J = 6.4$ Hz, 1H), 5.59 (bs, 2H), 5.09 (dd, $J = 6.0, 4.4$ Hz, 1H), 4.40 (dd, $J = 4.4, 2.4$ Hz, 1H), 4.05 (m, 1H), 3.93 (m, 2H), 2.70 (bs, 2H), 0.95 (s, 9H), 0.79 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H),

-0.07 (s, 3H), -0.39 (s, 3H); ^{13}C NMR (CDCl_3) δ 154.1, 151.9, 150.2, 146.0, 118.3, 88.0, 87.0, 73.3, 73.1, 43.8, 26.0, 25.7, 18.2, 17.9, -4.3 , -4.4 , -4.5 , -5.2 ; HRFAB calcd for $\text{C}_{22}\text{H}_{41}\text{N}_9\text{O}_3\text{Si}_2$ ($\text{M} + \text{H}^+$) 536.295, obsd 536.2970.

8-Azido-5'-ethanolamine-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (15). To 8-azido-5'-amino-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (**13**) (0.479 g, 0.8940 mmol) in 9.78 mL of toluene was added 2-iodoethanol (0.261 g, 1.5198 mmol). The reaction was stirred at 70 °C for 7.5 h. An aqueous workup was performed (NaHCO_3 , EtOAc, brine), and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. Column chromatography (2:1:1 EtOAc/ CH_2Cl_2 /MeOH) yielded **15** (0.178 g, 34%) and recovered starting material (0.179 g, 37%): mp 134 °C dec; ^1H NMR (CDCl_3) δ 8.18 (s, 1H), 6.03 (bs, 2H), 5.73 (d, $J = 6.4$ Hz, 1H), 5.08 (dd, $J = 6.4, 4.8$ Hz, 1H), 4.35 (dd, $J = 4.8, 2.4$ Hz, 1H), 4.18 (m, 1H), 3.70 (t, $J = 5.6$ Hz, 2H), 3.64 (m, 2H), 3.01 (dd, $J = 12.0, 6.4$ Hz, 1H), 2.94 (dd, $J = 12.0, 3.2$ Hz, 1H), 2.84 (ddt, $J = 36.0, 12.4, 5.6$ Hz, 2H), 0.93 (s, 9H), 0.76 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H), -0.12 (s, 3H), -0.43 (s, 3H); ^{13}C NMR (CDCl_3) δ 153.9, 151.9, 150.2, 146.2, 118.4, 88.0, 85.4, 73.9, 72.9, 60.8, 51.4, 50.9, 26.1, 25.8, 18.3, 18.0, -4.2 , -4.35 , -4.37 , -5.2 ; HRFAB calcd for $\text{C}_{24}\text{H}_{45}\text{N}_9\text{O}_4\text{Si}_2$ ($\text{M} + \text{H}^+$) 580.321, obsd 580.3210.

8-Azido-5'-aziridino-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (16). To PPh_3 (0.1242 g, 0.4737 mmol) in 4.74 mL of THF at 0 °C was added DEAD (0.0825 g, 0.4737 mmol). The components were stirred until TLC indicated complete consumption of PPh_3 . The mixture was added to 8-azido-5'-ethanolamine-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (**15**) (0.1831 g, 0.3158 mmol) in 4.22 mL of THF. The reaction was warmed to rt and heated at reflux for 4 h. An aqueous workup was performed (NaHCO_3 , EtOAc, brine), and the organic layer was dried over Na_2SO_4 and evaporated in vacuo. Column chromatography (6:1 EtOAc/ CH_2Cl_2) yielded **16** (0.1026 g, 58%): mp 151 °C dec; ^1H NMR (CDCl_3) δ 8.21 (s, 1H), 5.77 (d, $J = 6.0$ Hz, 1H), 5.73 (s, 2H), 5.22 (d, $J = 6.0, 4.8$ Hz, 1H), 4.46 (dd, $J = 4.8, 3.2$ Hz, 1H), 4.15 (m, 1H), 2.80 (dd, $J = 12.0, 5.6$ Hz, 1H), 2.31 (dd, $J = 12.0, 3.6$ Hz, 1H), 1.70 (m, 2H), 1.16 (m, 2H), 0.94 (s, 9H), 0.78 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H), -0.08 (s, 3H), -0.35 (s, 3H); ^{13}C NMR (CDCl_3) δ 153.8, 151.9, 150.6, 146.4, 118.4, 88.0, 85.2, 74.1, 72.4, 63.3, 27.6, 27.3, 26.1, 25.8, 18.3, 18.0, -4.2 , -4.3 , -4.4 , -5.0 ; HRFAB calcd for $\text{C}_{24}\text{H}_{43}\text{N}_9\text{O}_3\text{Si}_2$ ($\text{M} + \text{H}^+$) 562.311, obsd 562.3100.

8-Azido-5'-aziridino-5'-deoxyadenosine (6). To 8-azido-5'-aziridino-5'-deoxy-2',3'-bis(*O*-tert-butylidimethylsilyl)adenosine (**16**) (0.0593 g, 0.1055 mmol) in 3.5 mL of THF at 0 °C was added Bu_4NF (0.0552 g, 0.2110 mmol). The reaction was stirred cold for 1 h and evaporated in vacuo. Column chromatography (2:1:1 EtOAc/ CH_2Cl_2 /MeOH) yielded **6** (0.0211 g, 60%): mp 155 °C dec; ^1H NMR ($\text{DMSO}-d_6$) δ 8.10 (s, 1H), 7.23 (bs, 2H), 5.66 (d, $J = 5.6$ Hz, 1H), 5.40 (bs, 1H), 5.20 (bs, 1H), 4.98 (m, 1H), 4.24 (m, 1H), 3.95 (m, 1H), 2.60 (dd, $J = 12.4, 4.8$ Hz, 1H), 2.25 (dd, $J = 12.4, 7.2$ Hz, 1H), 1.52 (m, 2H), 1.09 (m, 2H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 154.3, 151.9, 149.8, 144.4, 116.8, 87.3, 83.9, 71.5, 70.6, 62.7, 26.7, 26.1; HRFAB calcd for $\text{C}_{12}\text{H}_{15}\text{N}_9\text{O}_3$ ($\text{M} + \text{H}^+$) 334.138, obsd 334.1377.

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Supporting Information Available: General experimental methods and ^1H and ^{13}C NMR spectra for compounds **6**, **10–13**, **15**, **16**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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