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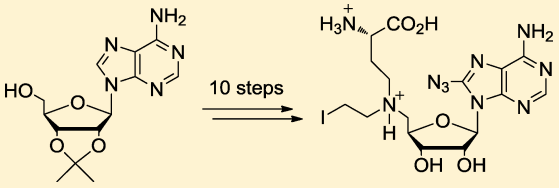
Synthesis of an Azide-Bearing *N*-Mustard Analogue of *S*-Adenosyl-L-methionine

Van Mai and Lindsay R. Comstock*

Department of Chemistry, Wake Forest University, Winston-Salem, North Carolina 27109 United States

Supporting Information

ABSTRACT: The synthesis of an azide-bearing *N*-mustard *S*-adenosyl-L-methionine (SAM) analogue, 8-azido-5′-(diaminobutyric acid)-*N*-iodoethyl-5′-deoxyadenosine, has been accomplished in 10 steps from commercially available 2′,3′-isopropylidene adenosine. Critical to this success was executing C8 azidation prior to derivatizing the 5′-position of the ribose sugar and the late stage alkylation of the 5′ amino group with bromoethanol, which was necessitated by the reactivity of the aryl azide moiety. The azide-bearing *N*-mustard is envisioned as a useful biochemical tool by which to probe DNA and protein methylation patterns.



S-adenosyl-L-methionine (SAM)-dependent methylation of nucleic acids and proteins is now recognized as playing an absolutely vital role in the careful regulation of gene transcription.[1−3] Changes in the activity or expression levels of the eukaryotic DNA methyltransferase DNMT1 have been integrally linked to various diseases, including carcinogenesis and obesity.[4−6] Although agents capable of exploiting such flawed traits of DNMT1 may serve as a launching point for the development of new therapeutics, substances capable of undergoing transfer to nucleic acids and proteins in an enzyme-dependent fashion also hold tremendous promise as biochemical tools by which to dissect and understand biological methylation. Our interests in developing these tools are reflected in the synthesis and biochemical study of SAM-based analogues containing reactive functionalities capable of undergoing chemoselective ligations.

The design of SAM analogues containing these functionalities was inspired by Weinhold et al. Originally exploiting an aziridine-containing SAM mimic (**1**, Figure 1) to alkylate DNA

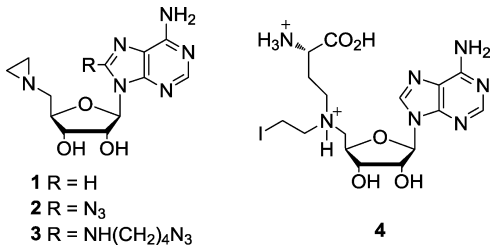


Figure 1. First-generation SAM analogues.

in a sequence-specific fashion,[7] his more recent work has demonstrated that derivatives of **1** bearing large functional probes at the C8 and N^6 positions of the adenine base are capable of labeling DNA.[8−10] As an alternative to Weinhold's approach incorporating a large, bulky probe on the adenine base, Rajski simplified and increased the versatility of **1** by synthesizing analogues containing azides.[11] It was envisioned that such analogues would be capable of undergoing the Staudinger ligation or copper-catalyzed azide−alkyne cycloaddition (CuAAC or Click) chemistry, to covalently label DNA and protein in a methyltransferase-dependent fashion. As a result, first-generation azide-bearing SAM analogues **2** and **3** (Figure 1) were generated and shown to be transferred to both oligonucleotides and plasmid DNA using a variety of DNA methyltransferases.[12,13] It was also demonstrated that these azide-modified DNAs undergo efficient Staudinger ligation with functionalized triarylphosphines[13,14] and Click chemistry with a fluorophore-alkyne conjugate.[15]

Although it was demonstrated that **2** and **3** can be transferred to DNA substrates in an enzyme-dependent fashion and undergo subsequent ligation, reaction conditions required a 100-fold excess of analogue.[13] This was easily attributed to the reduced binding of **2** and **3** to the methyltransferase due to their core structures lacking the amino acid recognition moiety present in SAM for optimal binding and enzymatic activity. Thus, an *N*-mustard analogue bearing this recognition moiety (**4**, Figure 1) was synthesized and has been shown to generate a highly reactive aziridinium at physiological pH. Incorporating this amino acid functionality at the 5′-position of the ribose sugar generated a more effective SAM analogue compared to **1**−**3**, as demonstrated by its enzymatic incorporation onto both DNA and peptide substrates.[16,17] Recent reports indicate that alternate SAM analogues bearing reactive functionalities, such as ketones[18] and alkynes,[19−22] at the sulfonium center are also capable of labeling small molecule, DNA, and protein substrates. The utility of the alkyne-containing SAM analogues was further demonstrated through successful protein labeling studies via Click chemistry. Although these alkyne-bearing

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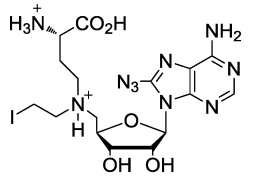
analogues show promise for future biochemical studies, their utility is currently limited to a small number of protein lysine methyltransferases.[20−22]

In this work, we describe the further advancement of compounds **2** and **4** by synthesizing a second-generation azide-bearing analogue of SAM, 8-azido-5′-(diaminobutyric acid)-*N*-iodoethyl-5′-deoxyadenosine, **5**. As shown in Figure 2, this new



5

Figure 2. 8-Azido-5′-(diaminobutyric acid)-*N*-iodoethyl-5′-deoxyadenosine, **5**.

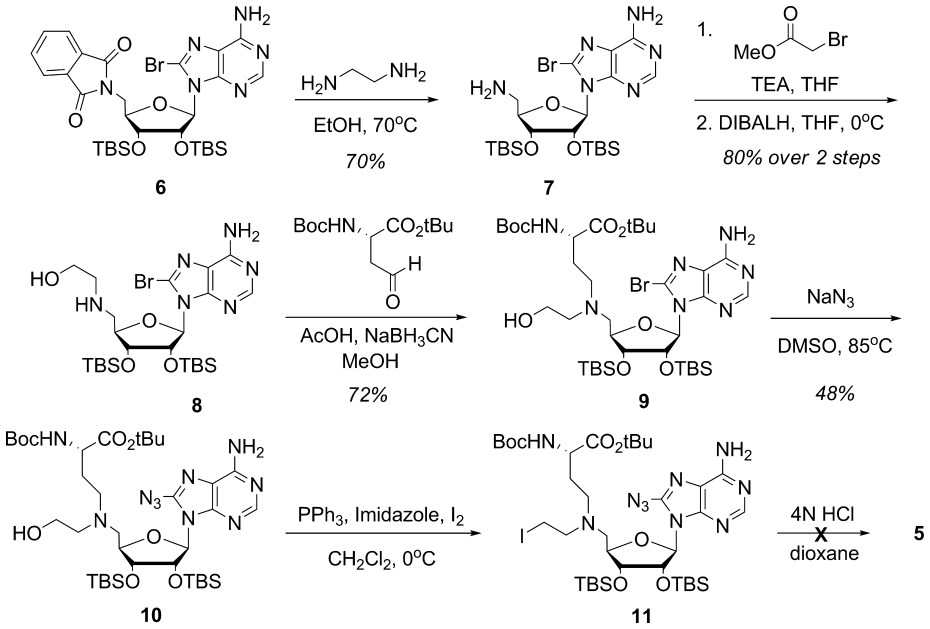
SAM-based analogue was engineered to bring together the advantage of containing an azide on the adenine base for subsequent chemoselective ligation reactions in addition to incorporating the amino acid moiety for improved enzyme interactions. Ultimately, **5** is hypothesized to serve as a biochemical tool for future studies in identifying both sites and substrates of biological methylation.

On the basis of the synthetic steps that were used to generate **2** and **4**,[12,16] a pathway to synthesize **5** was developed and relied on literature precedence that derivatized the *C*8 position of the adenine base prior to incorporating the amino acid functionality at the 5′ position of the ribose sugar (Scheme 1). It was determined that installation of the azide functionality would be best carried out following the requisite reductive amination to incorporate the amino acid functionality, as the use of $NaBH_3CN$ was foreseen to reduce the azide functional group to a primary amine. Thus, our synthesis began with previously reported **6**, and phthalimide removal easily afforded **7** using previously described methods.[12] The resulting amine underwent facile S_N2 reaction with methylbromoacetate, followed by its reduction with DIBALH, to generate amino alcohol **8** in 80% yield over two steps. Reductive amination with *tert*-butyl (*S*)-2-[*N*-(*tert*-butoxycarbonyl)amino]-4-oxobutanoate,[23] in the presence of $NaCNBH_3$ and acetic acid, provided the fully *N*-protected amino alcohol **9**. The azide functionality was easily introduced using NaN_3 to generate **10** in moderate yield.

Completion of the synthetic pathway required iodination of the primary alcohol, followed by a global deprotection. On the basis of precedence to generate **4**,[16] both of these steps were expected to proceed smoothly in providing the desired azide-containing *N*-mustard **5**. Although iodination of **10** proceeded smoothly to obtain **11**, its subsequent deprotection using 4 N HCl in dioxane failed to completely remove the TBS protecting groups. In an attempt to determine whether complete deprotection of the TBS protecting groups could be achieved, several methodologies were explored. The use of other acids (10% HCl in acetone,[24] 50% TFA in CH_2Cl_2,[25] and Dowex-H^+ in ACN[26]) failed to carry out the global deprotection. Attempts to employ TBAF to remove the TBS groups from **11** appeared promising following TLC, but NMR and MS analysis failed to confirm the presence of the diol and only indicated residual TBAF salt along with several side products that could not be identified. Although additional deprotection strategies could have been explored, switching to the more labile triethylsilyl (TES) protecting group was deemed to be most productive at this stage in generating **5**.

Literature precedence and previous preparation of **4** in our laboratory have demonstrated that TES ethers on the ribose sugar can be completely removed using HCl in dioxane.[16] Thus, an alternate synthetic route incorporating the TES protecting group was developed, following the synthetic route shown in Scheme 1. Preparation of the TES-protected intermediates of **6**−**9** was facile and proceeded in similar yields as compared to their TBS-protected analogues. Following the successful reductive amination to yield the TES-protected version of **9**, the next step required the installation of the azide

Scheme 1. Proposed Synthetic Pathway to 5



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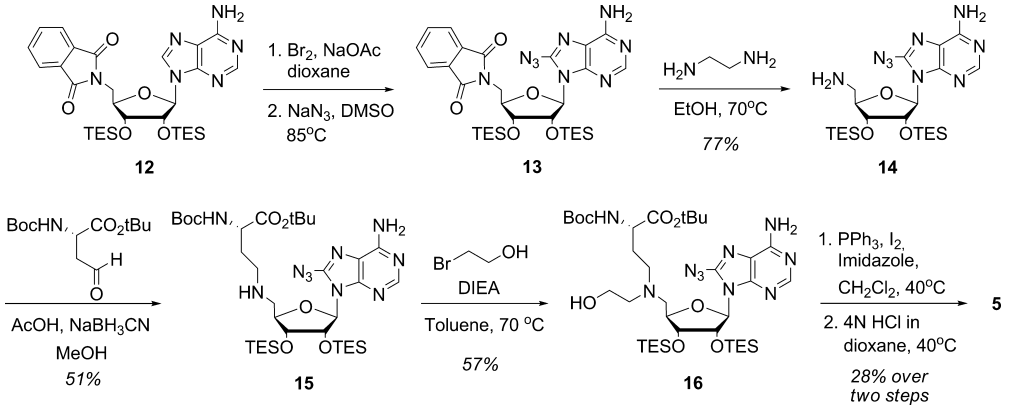
Scheme 2. Alternate Synthetic Pathway Incorporating Azide Earlier in Pathway



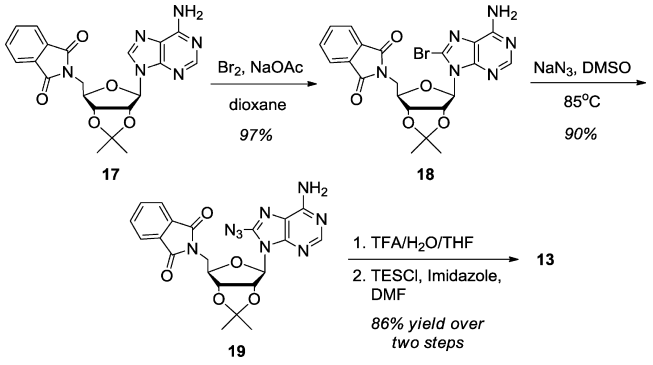
at the C8 position via bromide displacement. Interestingly, reaction analysis indicated the formation of a small percentage of the desired azide-containing product, in addition to several side products identified as either the mono-TES-protected product or the corresponding diol. This finding was confirmed upon repeating the azidation, and all attempts to determine if the resulting bromide or heat was the cause failed to provide any conclusive results.

On the basis of these difficulties incorporating the azide, an alternative approach was envisioned to incorporate the azide earlier in the synthetic pathway. Although this reaction was a feasible option, our major concern was whether or not the use of $NaBH_3CN$ would reduce the azide to a primary amine during the reductive amination. Model studies investigating this conversion using 1.5 equiv of hydride indicated a 1:1 ratio of azide to amine. Reaction optimization found that reducing the number of equivalents of $NaBH_3CN$ to 1.1 allowed for a marked increase in product yield. Having achieved control over the formation of the amine byproduct, we developed an alternative synthetic pathway (Scheme 2) that incorporated the azide on the adenine base prior to the reductive amination. In re-engineering our pathway to **5**, we relied on the previously reported procedures[12] in generating **13**. We began with previously reported **12**,[27] and bromination of the adenine base occurred readily. Subsequent incorporation of the azide afforded **13**, but yields fluctuated between 39 and 68% and were attributed to removal of the TES groups, as indicated by TLC analysis and mass spectrometry. Reducing the reaction time did not improve reaction yield, as unreacted starting material remained and comigrated with **13** by TLC. Under these conditions, it was hypothesized that retention of the isopropylidene on the ribose sugar would be a more robust protecting group.

To test the viability of the isopropylidene group during the bromination and azidation steps, a pathway that involved the formation of 8-azido-5′-phthalimide-2′,3′-*O*-isopropylidene adenosine (**19**) was developed (Scheme 3). We began with previously described **17**,[27] and bromination and azidation were easily carried out using the chemistry described above to generate **18** and **19**, respectively, in high yields. Subsequent isopropylidene cleavage and reprotection as the TES ethers produced **13** in a yield of 86%, which was significantly higher compared to the conversion of **12** to **13**.

After obtaining **13** in sufficient quantity, the synthetic pathway (see Scheme 2) was continued upon removal of the phthalimide to primary amine **14**. Introduction of the amino

Scheme 3. Improved Synthesis of 13



acid moiety via reductive amination was then conducted via treatment with *tert*-butyl (*S*)-2-[*N*-(tert-butoxycarbonyl)-amino]-4-oxobutanoate and $NaBH_3CN$ to yield **15** in 51% yield after the elimination of the contaminating primary amine byproduct. Subsequent 5′-alkylation was carried out most efficiently using bromoethanol in the presence of *N*,*N*-diisopropylethylamine. It is interesting to note that the use of iodoethanol produced a low yield of **16** (approximately 20%), as the formation of a secondary alkylated product (addition of a second ethyl alcohol to the 5′-nitrogen) was prevalent.[12]

Having successfully synthesized **16**, iodination and subsequent global deprotection was carried out. Although the literature indicated that iodination should be carried out at 0 °C,[16] we consistently observed unreacted starting material that was independent of reaction time. While raising the temperature to ambient conditions failed to yield complete conversion, it was found that a gentle reflux in CH_2Cl_2 facilitated iodination in a near quantitative yield. Having the penultimate product in hand, initial attempts to globally deprotect **16** using 4 N HCl in dioxane failed to go to completion. Although **5** was the major product obtained, a derivative of **5** retaining the *tert*-butyl ester was also present. Further reaction optimization to increase the overall yield of **5** indicated that complete global deprotection was achieved under a gentle reflux in CH_2Cl_2 for 3 h.

In summary, 8-azido-5′-(diaminobutyric acid)-*N*-iodoethyl-5′-deoxyadenosine, **5**, was successfully synthesized on the basis of the two previously reported SAM analogues, **2** and **4**. The use of suitable protecting groups was found to be a key factor in several transformations to successfully obtain the final azide-bearing *N*-mustard SAM analogue. Preliminary work in our laboratory has indicated that **5** can be enzymatically transferred

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by methyltransferase *Taq*I to plasmid DNA using a restriction/protection assay in comparable yields to *N*-mustard analogue **4**.[28] On the basis of these results, we anticipate that **5** will be useful to a variety of DNA and protein methyltransferases and serve as a unique biochemical tool to detect sites of biological methylation.[29] Further biochemical analysis of **5** is ongoing and will be reported shortly.

EXPERIMENTAL SECTION

General Experimental Methods. All reagents and solvents were purchased from commercial sources and used without additional purification. Anhydrous solvents were obtained from a Meyer solvent system except for DMSO and toluene, which were purchased. Reactions were performed at room temperature under argon, unless otherwise indicated. ^{1}H and ^{13}C NMR spectra were recorded on a 300 or 500 MHz spectrometer using solvent as the internal reference. Chemical shifts are reported in δ (ppm). Analytical and semi-preparative HPLC was performed with the UV−Vis detector set at 254 nm. An Alltima C18 analytical column (100 Å, 5 μm, 250 × 4.6 mm) and Alltima C18 preparative column (100 Å, 5 μm, 250 × 10.0 mm) were used for analysis and isolation of **5**. All mobile phases were filtered through a 0.22 μm membrane filter prior to use.

Experimental Procedures. *8-Bromo-5′-amine-5′-deoxy-2′,3′-bis-(O-tert-butyldimethylsilyl) Adenosine (**7**).* Ethylenediamine (668 μL, 9.984 mmol) was added to 8-bromo-5′-phthalimide-5′-deoxy-2′,3′-bis-(*O-tert*-butyldimethylsilyl) adenosine (**6**) (1.405 g, 1.997 mmol) in 65.5 mL of EtOH. The reaction was heated at 70 °C and stirred for 5 h. The solvent was evaporated in vacuo and chromatographed on silica pretreated with 2% TEA (4:2:1 EtOAc/CH_2Cl_2/MeOH) to yield **7** (0.807 g, 70%) as a solid: ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1H), 5.99 (d, J = 6.4 Hz, 1H), 5.57 (bs, 2H), 5.26 (dd, J = 6.6, 4.7 Hz, 1H), 4.41 (dd, J = 4.6, 2.4 Hz, 1H), 4.11−4.07 (m, 1H), 3.06−3.04 (m, 2H), 1.65 (bs, 2H), 0.96 (s, 9H), 0.80 (s, 9H), 0.14 (s, 3H), 0.14 (s, 3H), −0.08 (s, 3H), −0.43 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 154.7, 152.6, 150.6, 128.1, 120.4, 90.4, 87.3, 73.1, 72.6, 43.7, 25.8, 25.6, 18.0, 17.7, −4.5, −4.6, −4.7, −5.4; HRMS-ESI calcd for $C_{22}H_{41}BrN_6O_3Si_2$ (M + Na^+) 595.1853, obsd 595.1858.

8-Bromo-5′-amino-acetic acid methyl ester-5′-deoxy-2′,3′-bis-(O-tert-butyldimethylsilyl) Adenosine. To 8-bromo-5′-amine-5′-deoxy-2′,3′-bis-(*O-tert*-butyldimethylsilyl) adenosine (**7**) (0.825 g, 1.439 mmol) in 7.9 mL of dry THF was added TEA (240 μL, 1.727 mmol). Methylbromoacetate (164 μL, 1.727 mmol) in 4 mL of dry THF was then added dropwise to the solution. The reaction mixture was stirred overnight. The resulting precipitate was filtered off and washed with anhydrous Et_2O; the resulting organic was evaporated in vacuo. Column chromatography (3:1 EtOAc/CH_2Cl_2 to 15:5:1 EtOAc/CH_2Cl_2/MeOH) yielded 8-bromo-5′-amino-acetic acid methyl ester-5′-deoxy-2′,3′-bis-(*O-tert*-butyldimethylsilyl) adenosine (0.770 g, 83%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.28 (s, 1H), 5.98 (d, J = 6.7 Hz, 1H), 5.48 (bs, 2H), 5.30 (dd, J = 6.7, 4.7 Hz, 1H), 4.38 (dd, J = 4.7, 2.2 Hz, 1H), 4.20−4.16 (m, 1H), 3.70 (s, 3H), 3.46 (d, J = 2.2 Hz, 2H), 2.99 (dd, J = 12.3, 3.7 Hz, 1H), 2.92 (dd, J = 12.3, 6.3 Hz, 1H), 2.64 (bs, 1H), 0.95 (s, 9H), 0.79 (s, 9H), 0.13 (s, 3H), 0.13 (s, 3H), −0.10 (s, 3H), −0.45 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.5, 154.6, 152.6, 150.6, 128.1, 120.4, 90.4, 85.8, 73.6, 72.4, 51.5, 51.0, 50.8, 25.7, 25.6, 17.9, 17.6, −4.6, −4.6, −4.7, −5.5; HRMS-ESI calcd for $C_{25}H_{45}BrN_6O_5Si_2$ (M + Na^+) 667.2066, obsd 667.2071.

*8-Bromo-5′-ethanolamine-5′-deoxy-2′,3′-bis-(O-tert-butyldimethylsilyl) Adenosine (**8**).* To 8-bromo-5′-amino-acetic acid methyl ester-5′-deoxy-2′,3′-bis-(*O-tert*-butyldimethylsilyl) adenosine (0.770 g, 1.192 mmol) in 25.6 mL of dry THF at 0 °C was slowly added 1 M DIBALH in hexanes (5.96 mmol). The reaction was kept cold for a 10 min, warmed to rt, and stirred for an additional 5 h. Saturated potassium sodium tartrate tetrahydrate (Rochelle's salt, 25.6 mL) was added to the reaction, and the mixture was stirred vigorously overnight. The organic was washed (saturated Rochelle's salt, H_2O, EtOAc, brine), dried over Na_2SO_4, and evaporated in vacuo to obtain **8** (0.714 g, 97%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1H), 6.00 (d, J = 7.0 Hz, 1H), 5.72 (bs, 2H), 5.29 (dd, J = 6.9, 4.7 Hz, 1H), 4.35 (dd, J = 4.6, 1.9 Hz, 1H), 4.25−4.21 (m, 1H), 3.79−3.69 (m, 2H), 3.02−2.91 (m, 3H), 2.84−2.77 (m, 2H), 0.96 (s, 9H), 0.78 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H), −0.11 (s, 3H), −0.44 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 154.8, 152.9, 150.6, 128.4, 120.6, 90.6, 85.3, 73.9, 72.7, 60.5, 51.2, 50.8, 26.0, 25.8, 18.2, 17.9, −4.4, −4.4, −4.5, −5.3; HRMS-ESI calcd for $C_{24}H_{45}BrN_6O_4Si_2$ (M + Na^+) 639.2116, obsd 639.2128.

*8-Bromo-5′-(N-Boc-diaminobutyric acid-O-tert-butyl ester)-N-ethanolamine-5′-deoxy-2′,3′-bis-(O-tert-butyldimethylsilyl) Adenosine (**9**).* To a solution of 8-bromo-5′-ethanolamine-5′-deoxy-2′,3′-bis-(*O-tert*-butyldimethylsilyl) adenosine (**8**) (0.400 g, 0.647 mmol) and *tert*-butyl (*S*)-2-[*N*-(*tert*-butoxycarbonyl)amino]-4-oxobutanoate (0.161 g, 0.588 mmol) in 2.82 mL of dry MeOH were added $NaBH_3CN$ (0.055 g, 0.882 mmol) and AcOH (33.7 μL, 0.588 mmol). The reaction mixture was stirred overnight. After the reaction was diluted with EtOAc and saturated $NaHCO_3$, the organic layer was washed with saturated $NaHCO_3$, dried over Na_2SO_4, and evaporated in vacuo. Column chromatography (10:4:2:1 petroleum ether/EtOAc/CH_2Cl_2/MeOH) yielded **9** (0.353 g, 72%) as a solid: ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1H), 5.94 (d, J = 5.2 Hz, 1H), 5.64 (bs, 2H), 5.43 (bd, J = 7.8 Hz, 1H), 5.31 (t, J = 4.9 Hz, 1H), 4.39 (t, J = 4.1 Hz, 1H), 4.20−4.15 (m, 2H), 3.55−3.51 (m, 2H), 2.96−2.90 (m, 2H), 2.69−2.56 (m, 4H), 1.99−1.92 (m, 1H), 1.73−1.64 (m, 1H), 1.42 (s, 9H), 1.42 (s, 9H), 0.96 (s, 9H), 0.81 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H), −0.06 (s, 3H), −0.32 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.0, 155.6, 154.6, 152.8, 150.8, 128.7, 120.6, 91.0, 85.8, 82.0, 79.8, 74.4, 72.4, 59.6, 56.6, 56.2, 52.9, 50.5, 29.7, 28.5, 28.1, 26.0, 25.8, 18.2, 18.0, −4.2, −4.3, −4.5, −5.0; HRMS-ESI calcd for $C_{37}H_{68}BrN_7O_8Si_2$ (M + Na^+) 896.3743, obsd 896.3715.

*8-Azido-5′-(N-Boc-diaminobutyric acid-O-tert-butyl ester)-N-ethanolamine-5′-deoxy-2′,3′-bis-(O-tert-butyldimethylsilyl) Adenosine (**10**).* NaN_3 (0.056 g, 0.858 mmol) was added to a solution of 8-bromo-5′-(*N*-Boc-diaminobutyric acid-*O-tert*-butyl ester)-*N*-ethanolamine-5′-deoxy-2′,3′-bis-(*O-tert*-butyldimethylsilyl) adenosine (**9**) (0.188 g, 0.215 mmol) in 1.82 mL of DMSO. The reaction mixture was heated to 85 °C and stirred overnight. Upon cooling to rt, an aqueous workup was performed (saturated $NaHCO_3$, CH_2Cl_2, brine). The resulting organic was dried over Na_2SO_4, evaporated in vacuo, and column chromatographed (8:4:2:1 petroleum ether/EtOAc/CH_2Cl_2/MeOH) to yield **10** (0.087 g, 48%) as a light yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1H), 5.73 (d, J = 5.0 Hz, 1H), 5.45 (bd, J = 7.9 Hz, 1H), 5.35 (bs, 2H), 5.08 (t, J = 4.4 Hz, 1H), 4.40 (t, J = 3.6 Hz, 1H), 4.16−4.11 (m, 2H), 3.55−3.48 (m, 2H), 2.93−2.87 (m, 2H), 2.66−2.59 (m, 4H), 2.00−1.89 (m, 1H), 1.73−1.60 (m, 1H), 1.41 (s, 18H), 0.94 (s, 9H), 0.81 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H), −0.06 (s, 3H), −0.28 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.0, 155.7, 153.7, 151.9, 150.3, 146.2, 128.9, 118.4, 88.4, 83.1, 82.0, 79.8, 74.3, 72.8, 59.3, 56.7, 56.3, 53.0, 50.6, 34.3, 29.7, 28.5, 28.1, 26.1, 25.8, 18.2, 18.0, −4.1, −4.3, −4.4, −4.9; HRMS-ESI calcd for $C_{37}H_{68}N_{10}O_8Si_2$ (M + Na^+) 859.4652, obsd 859.4667.

*5′-Phthalimide-5′-deoxy-2′,3′-bis-(O-triethylsilyl) Adenosine (**12**).* *Note*: Although this product has been previously described in the literature,[27] incorrect ^{1}H NMR data was reported and is corrected here. Additionally, our procedure deviated from that reported and was based on the previously described *tert*-butyldimethylsilyl-protected version of **12**.[12] To commercially available 2′,3′-isopropylidene adenosine (1.005 g, 3.257 mmol) in 11 mL of dry THF were added phthalimide (0.495 g, 3.366 mmol) and PPh_3 (0.852 g, 3.250 mmol). After the mixture was stirred for 10 min, DIAD (640 μL, 3.250 mmol) was added to the mixture, which was stirred an additional 2.5 h. The white precipitate was filtered off and washed with cold Et_2O to yield the product that was taken forward. 5′-Phthalimide-5′-deoxy-2′,3′-isopropylidene adenosine (1.175 g, 2.693 mmol) was dissolved in 27 mL of 3:1:1 TFA/H_2O/THF, and the mixture was stirred for 2 h. The solvent was evaporated in vacuo and coevaporated with EtOH (× 3). The resulting material was dissolved in 5.93 mL of dry DMF, followed by the addition of imidazole (0.917 g, 13.47 mmol) and TESCl (994 μL, 5.925 mmol). The reaction was stirred overnight, followed by an aqueous workup (saturated NH_4Cl (× 2), EtOAc, brine), dried over Na_2SO_4, and evaporated in vacuo. Column chromatography (4:4:2:1

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hexanes/EtOAc/CH_2Cl_2/MeOH) yielded **12** (1.148 g, 68%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.12 (s, 1H), 8.00 (s, 1H), 7.88−7.85 (m, 2H), 7.75−7.72 (m, 2H), 5.86 (d, J = 6.4 Hz, 1H), 5.67 (bs, 2H), 5.28 (dd, J = 6.3, 4.1 Hz, 1H), 4.35−4.30 (m, 2H), 4.24 (dd, J = 13.7, 7.2 Hz, 1H), 3.93 (dd, J = 13.7, 5.4 Hz, 1H), 0.93 (t, J = 7.8 Hz, 9H), 0.80 (t, J = 7.9 Hz, 9H), 0.61 (q, J = 8.0 Hz, 6H), 0.38 (qq, J = 15, 7.6 Hz, 6H); ^{13}C NMR data matched that previously described.[27]

*8-Bromo-5′-phthalimide-5′-deoxy-2′,3′-bis-(O-isopropylidene) Adenosine (**18**).* To 5′-phthalimide-5′-deoxy-2′,3′-bis-(*O*-isopropylidene) adenosine (**17**) (5.123 g, 11.74 mmol) in 176 mL of 7:4 dioxane/0.5 M NaOAc (pH 5.2) was added Br_2 (1.21 mL, 23.48 mmol). The reaction was stirred for 3.5 h and followed by an aqueous workup (saturated $Na_2S_2O_3$, CH_2Cl_2, brine), dried over Na_2SO_4, and evaporated in vacuo. Column chromatography (32:4:1 EtOAc/CH_2Cl_2/ MeOH) yielded **18** (5.879 g, 97%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 1H), 7.78−7.75 (m, 2H), 7.69−7.65 (m, 2H), 6.16 (d, J = 1.3 Hz, 1H), 5.97 (bs, 2H), 5.74 (dd, J = 6.2, 1.3 Hz, 1H), 5.34 (dd, J = 6.2, 3.2 Hz, 1H), 4.57−4.51 (m, 1H), 3.99 (dd, J = 14.1, 5.7 Hz, 1H), 3.87 (dd, J = 14.1, 6.8 Hz, 1H), 1.57 (s, 3H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.9, 154.4, 152.7, 150.2, 133.8, 131.8, 127.5, 123.2, 120.0, 114.0, 91.1, 85.7, 83.5, 82.6, 39.3, 27.0, 25.3; HRMS-ESI calcd for $C_{21}H_{19}BrN_6O_5$ (M + Na^+) 537.0493, obsd 537.0485.

*8-Azido-5′-phthalimide-5′-deoxy-2′,3′-bis-(O-isopropylidene) Adenosine (**19**).* NaN_3 (1.602 g, 24.62 mmol) was added to a solution of 8-bromo-5′-phthalimide-5′-deoxy-2′,3′-bis-(*O*-isopropylidene) adenosine (**18**) (3.172 g, 6.155 mmol) in 51.9 mL of DMSO. The reaction mixture was heated to 85 °C and stirred for 10.5 h. The organic was washed (saturated $NaHCO_3$, CH_2Cl_2, brine), dried over Na_2SO_4, and evaporated in vacuo. Column chromatography (16:2:1 EtOAc/CH_2Cl_2/MeOH) yielded **19** (2.632 g, 90%) as a light yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.01 (s, 1H), 7.78−7.74 (m, 2H), 7.70−7.66 (m, 2H), 5.98 (d, J = 1.5 Hz, 1H), 5.64 (bs, 2H), 5.60 (dd, J = 6.3, 1.5 Hz, 1H), 5.25 (dd, J = 6.3, 3.3 Hz, 1H), 4.52−4.47 (m, 1H), 3.99 (dd, J = 14.1, 5.7 Hz, 1H), 3.89 (dd, J = 14.1, 6.7 Hz, 1H), 1.55 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.9, 153.6, 151.7, 149.6, 145.1, 133.8, 131.8, 123.1, 117.7, 114.1, 88.4, 85.2, 83.4, 82.5, 39.4, 27.0, 25.4; HRMS-ESI calcd for $C_{21}H_{19}N_9O_5$ (M + Na^+) 500.1401, obsd 500.1395.

*8-Azido-5′-phthalimide-5′-deoxy-2′,3′-bis-(O-triethylsilyl) Adenosine (**13**).* 8-Azido-5′-phthalimide-5′-deoxy-2′,3′-bis-(*O*-isopropylidene) adenosine (**19**) (2.632 g, 5.512 mmol) was dissolved in 55.3 mL of 3:1:1 TFA/H_2O/THF and stirred for 2.5 h. The solvent was evaporated in vacuo and coevaporated with EtOH (× 3). The resulting material was dissolved in 12.1 mL of dry DMF, followed by the addition of imidazole (1.876 g, 27.56 mmol) and chlorotriethylsilane (TESCl) (2.04 mL, 12.13 mmol). The reaction was stirred overnight, followed by an aqueous workup (saturated NH_4Cl (× 2), EtOAc, brine). The organic was dried over Na_2SO_4 and evaporated in vacuo. Column chromatography (8:4:2:0.5 hexanes/EtOAc/CH_2Cl_2/MeOH to 8:4:2:1 hexanes/EtOAc/CH_2Cl_2/MeOH) yielded **13** (3.139 g, 86%) as a light yellow solid: ^{1}H NMR ($CDCl_3$) δ 7.99 (s, 1H), 7.85−7.82 (m, 2H), 7.73−7.70 (m, 2H), 5.79 (d, J = 6.9 Hz, 1H), 5.54 (dd, J = 6.9, 4.5 Hz, 1H), 5.47 (bs, 2H), 4.47 (dd, J = 4.5, 1.3 Hz, 1H), 4.29−4.20 (m, 2H), 3.94−3.86 (m, 1H), 0.92 (t, J = 7.9 Hz, 9H), 0.81 (t, J = 7.9 Hz, 9H), 0.60 (q, J = 7.7 Hz, 6H), 0.37 (qq, J = 15, 7.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 167.0, 152.6, 150.7, 149.4, 145.3, 133.3, 131.2, 122.6, 117.5, 87.0, 83.5, 73.9, 71.3, 39.9, 7.5, 7.4, 5.7, 5.3; HRMS-ESI calcd for $C_{30}H_{43}N_9O_5Si_2$ (M + Na^+) 688.2818, obsd 688.2805.

*8-Azido-5′-amine-5′-deoxy-2′,3′-bis-(O-triethylsilyl) Adenosine (**14**).* To 8-azido-5′-phthalimide-5′-deoxy-2′,3′-bis-(*O*-triethylsilyl) adenosine (**13**) (0.917 g, 1.377 mmol) in 45 mL of EtOH was added ethylenediamine (460 μL, 6.887 mmol). The reaction was heated to 70 °C and stirred for 5 h. The solvent was evaporated in vacuo, and the product was chromatographed on silica pretreated with 2% TEA (4:2:1 EtOAc/CH_2Cl_2/MeOH) to yield **14** (0.566 g, 77%) as a light yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1H), 5.77 (d, J = 6.3 Hz, 1H), 5.55 (bs, 2H), 5.24 (dd, J = 6.3, 4.8 Hz, 1H), 4.37 (dd, J = 4.7, 2.8 Hz, 1H), 4.03−3.98 (m, 1H), 3.02 (dd, J = 13, 4.0 Hz, 1H), 2.97 (dd, J = 13, 5.8 Hz, 1H), 1.72 (bs, 2H), 1.02 (t, J = 7.9 Hz, 9H), 0.81 (t, J = 7.9 Hz, 9H), 0.69 (q, J = 7.7 Hz, 6H), 0.38 (qq, J = 15, 7.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 152.9, 150.8, 149.3, 145.1, 117.6, 87.5, 86.9, 73.3, 72.7, 44.2, 7.6, 7.3, 5.8, 5.3; HRMS-ESI calcd for $C_{22}H_{41}N_9O_3Si_2$ (M + Na^+) 558.2763, obsd 558.2763.

*8-Azido-5′-N-Boc-diaminobutyric acid-O-tert-butyl ester-5′-deoxy-2′,3′-bis-(O-triethylsilyl) Adenosine (**15**).* In an adaptation of a reported procedure,[16] $NaBH_3CN$ (0.066 g, 1.056 mmol) and AcOH (55 μL, 0.960 mmol) were added to a solution of 8-azido-5′-amine-5′-deoxy-2′,3′-bis-(*O*-triethylsilyl) adenosine (**14**) (0.566 g, 1.056 mmol) and *tert*-butyl (*S*)-2-[*N*-(*tert*-butoxycarbonyl)amino]-4-oxobutanoate (0.262 g, 0.960 mmol) in 5.6 mL of dry MeOH. The reaction mixture was stirred for 2 h. After diluting the reaction with EtOAc and saturated $NaHCO_3$, the organic layer was washed with saturated $NaHCO_3$, dried over Na_2SO_4, and evaporated in vacuo. Column chromatography (12:4:2:0.6 petroleum ether/EtOAc/CH_2Cl_2/MeOH) yielded **15** (0.431 g, 51%) as a light yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 1H), 5.76 (d, J = 6.7 Hz, 1H), 5.56 (bd, J = 6.5 Hz, 2H), 5.43 (bd, J = 8.0 Hz, 1H), 5.25 (dd, J = 6.5, 4.8 Hz, 1H), 4.35 (dd, J = 4.6, 2.2 Hz, 1H), 4.27−4.22 (m, 1H), 4.12−4.08 (m, 1H), 2.87 (d, J = 4.9 Hz, 2H), 2.74−2.58 (m, 2H), 2.16 (bs, 1H), 2.02−1.89 (m, 1H), 1.80−1.69 (m, 1H), 1.45 (s, 9H), 1.44 (s, 9H), 1.01 (t, J = 7.9 Hz, 9H), 0.80 (t, J = 7.9 Hz, 9H), 0.68 (q, J = 7.8 Hz, 6H), 0.35 (qq, J = 15, 7.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.8, 154.4, 152.8, 150.7, 149.3, 145.2, 117.7, 87.4, 85.2, 81.5, 79.3, 74.0, 72.5, 53.0, 51.8, 46.3, 33.4, 28.8, 28.5, 7.6, 7.2, 5.8, 5.3; HRMS-ESI calcd for $C_{35}H_{64}N_{10}O_7Si_2$ (M + Na^+) 815.4390, obsd 815.4390.

*8-Azido-5′-(N-Boc-diaminobutyric acid-O-tert-butyl ester)-N-ethanolamine-5′-deoxy-2′,3′-bis-(O-triethylsilyl) Adenosine (**16**).* *N*,*N*-diisopropylethylamine (1.09 mL, 6.235 mmol) and 2-bromoethanol (443 μL, 6.235 mmol) were added to a solution of 8-azido-5′-*N*-Boc-diaminobutyric acid-*O*-*tert*-butyl ester-5′-deoxy-2′,3′-bis-(*O*-triethylsilyl) adenosine (**15**) (0.380 g, 0.480 mmol) in 2.51 mL of dry toluene. The reaction was heated to 70 °C and stirred for 27 h. Upon cooling, an aqueous workup (saturated $NaHCO_3$, EtOAc, brine) was performed. The resulting organic was dried over Na_2SO_4 and evaporated in vacuo. Column chromatography on silica (1:0.5 petroleum ether/EtOAc to 1:2 petroleum ether/EtOAc) yielded **16** (0.229 g, 57%) as a light yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H), 5.73 (d, J = 5.3 Hz, 1H), 5.69 (bs, 2H), 5.43 (bd, J = 8.2 Hz, 1H), 5.19 (t, J = 4.9 Hz, 1H), 4.34 (t, J = 4.1 Hz, 1H), 4.19−4.11 (m, 2H), 3.55−3.47 (m, 2H), 2.96−2.82 (m, 2H), 2.70−2.56 (m, 4H), 1.97−1.90 (m, 1H), 1.76−1.66 (m, 1H), 1.42 (s, 9H), 1.42 (s, 9H), 1.00 (t, J = 7.9 Hz, 9H), 0.81 (t, J = 7.9 Hz, 9H), 0.66 (q, J = 7.8 Hz, 6H), 0.39 (qq, J = 15, 7.5 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 171.9, 155.4, 153.6, 151.6, 150.0, 145.9, 118.1, 87.9, 83.0, 81.7, 79.5, 74.4, 72.4, 59.1, 56.5, 56.1, 52.8, 50.5, 29.6, 28.2, 27.9, 6.8, 6.5, 4.9, 4.5; HRMS-ESI calcd for $C_{37}H_{68}N_{10}O_8Si_2$ (M + H^+) 837.4833, obsd 837.4810.

*8-Azido-5′-(diaminobutyric acid)-N-iodoethyl-5′-deoxyadenosine Ammonium Hydrochloride (**5**).* I_2 (0.0623 g, 0.2453 mmol) was added to triphenylphosphine (0.0623 g, 0.2375 mmol) and imidazole (0.0162 g, 0.2375 mmol) in 623 μL of CH_2Cl_2 at 0 °C. The components were stirred until TLC indicated complete consumption of PPh_3. 8-Azido-5′-(*N*-Boc-diaminobutyric acid-*O*-*tert*-butyl ester)-*N*-ethanolamine-5′-deoxy-2′,3′-bis-(*O*-triethylsilyl) adenosine (**16**) (0.1308 g, 0.1562 mmol) in CH_2Cl_2 (623 μL) was then added and warmed to 40 °C slowly. After stirring for 30 min, the reaction was cooled and diluted with ice-chilled CH_2Cl_2 and H_2O. The organic layer was washed with H_2O (× 3) and evaporated in vacuo. HCl/dioxane (4N, 898 μL) was added to the iodinated product in CH_2Cl_2 (1.80 mL), and the mixture was heated to 40 °C for 3 h. Upon cooling, ice-chilled H_2O was added, and the aqueous layer was extracted with CH_2Cl_2 (× 3) prior to lyophilization to afford a light yellow solid. This was dissolved in minimal MeOH, and EtOAc was then added dropwise to precipitate a light yellow solid, which was then further purified by reverse-phase HPLC. Compound separation utilized a gradient system comprising of 0.1% TFA in water (solvent A) and HPLC grade ACN (solvent B) using a flow rate of 1.0 mL/min (analytical scale) or 4.0 mL/min (preparative scale). The gradient was run isocratically with 5% B for 2 min followed by a linear gradient of 5−10% B over a 16 min period. The gradient was then increased to 100% B over the next

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1 min and run isocratically for an additional 6 min. Under these conditions, the azide-bearing *N*-mustard adenosine (**5**) eluted at 16 min. **5** was obtained following product lyophilization (0.025 g, 28%): ^{1}H NMR (1% TFA in CD_3OD) δ 8.39 (s, 1H), 5.96 (d, J = 3.8 Hz, 1H), 4.80 (dd, J = 5.4, 4.0 Hz, 1H), 4.49 (t, J = 5.7 Hz, 1H), 4.42−4.38 (m, 1H), 4.08 (dd, J = 7.4, 5.6 Hz, 1H), 3.73 (dd, J = 14, 10 Hz, 1H), 3.64−3.59 (m, 3H), 3.54−3.49 (m, 1H), 3.47−3.41 (m, 3H), 2.43−2.35 (m, 1H), 2.29−2.22 (m, 1H); ^{13}C NMR (1% TFA in CD_3OD) δ 171.1, 150.6, 150.2, 149.4, 145.3, 119.1, 90.9, 80.3, 73.6, 73.5, 57.4, 55.9, 52.0, 51.7, 26.2, 7.7; HRMS-ESI calcd for $C_{16}H_{24}IN_{10}O_5^+$ (M^+) 563.0970, obsd 563.0969. *Note*: The ^{13}C NMR spectrum of **5** contains minor TFA signals in the range of δ 116−117 and δ 161−163.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra for new compounds **5**, **7**−**10**, **13**−**16**, **18**, and **19** and HPLC chromatogram of **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: comstolr@wfu.edu.

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