TITLE: Conversion of DNA Methyltransferases into Azidases

Author Affiliation: Lindsay R. Comstock
Scott R. Rajski
School of Pharmacy
University of Wisconsin-Madison
777 Highland Ave.
Madison, WI 53705

Corresponding Author: Scott R. Rajski
School of Pharmacy
University of Wisconsin-Madison
777 Highland Ave.
Madison, WI 53705
(608)262-7582
(608)262-5345 (fax)
srrajski@pharmacy.wisc.edu

SUPPLEMENTARY MATERIALS

General Experimental Methods. All reactions were carried out under an inert atmosphere of argon unless indicated otherwise. All reagents were obtained from commercial suppliers and used as received unless otherwise noted. The silica gel used in column flash chromatography was Merck no. 9385, 60 Å, 230–400 mesh. Analytical TLC was conducted on EM Science silica gel plates with detection by ninhydrin and/or UV light. Tetrahydrofuran (THF) was distilled over Na and benzophenone. TEA was dried over KOH and then distilled; 2,6-lutidine was distilled from $AlCl_3$; DIEA was distilled from ninhydrin, and then from KOH; Br_2 was distilled from KBr, and then from P_2O_5. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian ui400 and ui500 spectrometers using solvent as the internal reference. Chemical shifts are reported in ppm, in δ units. High-resolution matrix-assisted desorption/ionization (MALDI) were obtained from the University of Wisconsin-Madison, School of Pharmacy.

Synthesis and characterization of Azide-linked intermediates and final product:

5'-Amino-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (7). ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H), 7.95 (s, 1H), 5.99 (bs, 2H), 5.84 (d, J= 6.0 Hz, 1H), 5.00 (dd, J= 6.0, 4.8 Hz, 1H), 4.30 (dd, J= 4.8, 3.2 Hz, 1H), 4.13 (m, 1H), 3.11 (dd, J= 13.6, 3.6 Hz, 1H), 3.01 (dd, J= 13.6, 5.6 Hz, 1H), 0.95 (s, 9H), 0.79 (s, 9H), 0.12 (s, 6H), -0.06 (s, 3H), -0.36 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 155.9, 153.0, 149.8, 141.0, 121.1, 90.0, 87.3, 74.2, 73.3, 43.8, 26.0, 25.9, 18.3, 18.0, -4.2, -4.4, -4.4, -5.1. HRMALDI: calcd for $C_{22}H_{42}N_6O_3Si_2$ ($M + H^+$) 495.29, obsd 495.285.

5'-Amino-acetic acid methyl ester-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (8). To 5'-Amino-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (6.664 g, 13.48 mmol) in 66.3 mL THF at 0ºC was added triethylamine (1.637 g, 16.17 mmol). To this solution was added methyl bromoacetate (2.474 g,

16.17 mmol) in 38.0 mL THF, drop wise. The reaction was stirred at rt for 16 h. The resulting precipitate was filtered off and triturated with anhydrous diethyl ether. The organic was evaporated *in vacuo*. Column chromatography (4:2:1 EtOAc/CH_2Cl_2/MeOH) gave **8** as a white solid (6.47 g, 85 %). ^{1}H NMR ($CDCl_3$) δ 8.26 (s, 1H), 7.94 (s, 1H), 6.45 (bs, 2H), 5.81 (d, J= 6.0 Hz, 1H), 4.94 (dd, J= 6.0, 4.8 Hz, 1H), 4.22 (dd, J= 4.8, 3.2 Hz, 1H), 4.17 (m, 1H), 3.67 (s, 3H), 3.45 (s, 2H), 2.97 (dd, J= 12.8, 3.2 Hz, 1H), 2.87 (dd, J= 12.8, 6.0 Hz, 1H), 0.88 (s, 9H), 0.73 (s, 9H), 0.05 (s, 6H), -0.13 (s, 3H), -0.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.7, 156.1, 152.9, 149.6, 140.5, 120.8, 89.8, 85.4, 74.2, 73.7, 51.8, 51.1, 50.9, 25.9, 25.8, 18.1, 17.9, -4.3, -4.6, -4.6, -5.2. HRMALDI: calcd for $C_{25}H_{46}N_6O_5Si_2$ ($M + H^+$) 567.31, obsd 567.309.

8-Bromo-5'-(N-Boc)-amino-acetic acid methyl ester-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (9). To 5'-Amino-acetic acid methyl ester-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (3.276 g, 5.779 mmol) in 23.4 mL 1:1 $CHCl_3$/H_2O was added $KHCO_3$ (0.579 g, 5.779 mmol) and $(Boc)_2O$ (1.262 g, 5.779 mmol). The reaction was stirred at rt for 5 h and followed by an aqueous workup (H_2O, $CHCl_3$). The organic was dried over Na_2SO_4 and evaporated *in vacuo*. To the crude material in 110 mL 9:4 dioxane/0.5 M NaOAc (pH 5.2) was added Br_2 (1.85 g, 11.558 mmol). After stirring at rt for 3 h, the reaction was washed ($Na_2S_2O_3$, EtOAc, brine). The organic was dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography (2:1 EtOAc/Pet Ether) afforded **9** as a light yellow solid (3.83 g, 89 %). The resulting NMR spectra indicated the resulting product as a mixture of rotomers.* ^{1}H NMR ($CDCl_3$) δ 8.12 (s, 1H), 8.10 (s, 1H), 6.81 (bs, 4H), 5.93 (d, J= 6.4 Hz, 1H), 5.91 (d, J= 6.4 Hz, 1H), 5.38 (m, 2H), 4.17 (bs, 4H), 4.06 (m, 2H), 3.90 (m, 2H), 3.78 (m, 2H), 3.58 (s, 3H), 3.57 (s, 3H), 3.39 (m, 2H), 1.40 (s, 9H), 1.33 (s, 9H), 0.89 (s, 9H), 0.88 (s, 9H), 0.70 (s, 9H), 0.68 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H), 0.07 (s, 3H), 0.06 (s, 3H), -0.17 (s, 3H), -0.18 (s, 3H), -0.52 (s, 3H), -0.53 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 175.8, 170.72, 170.70, 166.6, 155.4, 155.2, 154.9, 154.8, 152.4, 150.6, 150.5, 128.6, 120.5, 90.5, 90.3, 86.4, 86.0, 80.6, 80.2, 73.9, 71.8, 71.7, 68.6, 66.3, 62.6, 51.7, 50.1, 50.0, 49.2, 28.5, 28.2, 25.9, 25.7, 25.6, 25.4, 18.08, 18.06, 17.8, 17.7, -4.42, -4.45, -4.49, -4.5, -4.6, -4.7, -5.3, -5.4. HRMALDI: calcd for $C_{30}H_{53}BrN_6O_7Si_2$ ($M + H^+$) 744.27, obsd 747.270.

8-Bromo-5'-(N-Boc)-ethanolamine-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (10). To 8'-Bromo-5'-(N-Boc)-amino-acetic acid methyl ester-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (0.710 g, 0.952 mmol) in 16.2 mL dry THF at 0ºC was added DIBALH (4.760 mmol). The reaction was warmed to rt and stirred for an additional 5 h. 10 mL saturated potassium sodium tartrate tetrahydrate (Rochelle's salt) was added to the reaction and stirred vigorously overnight. The organic was washed (sat. Rochelle's salt, H_2O, EtOAc, brine), dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography (6:1 EtOAc/CH_2Cl_2) provided **10** (0.550 g, 81 %). The resulting NMR spectra indicated the resulting product as a mixture of rotomers.* The peaks were very broad and often overlapping. The correct number of protons (at an appropriate chemical shift) was present in the spectra. HRMALDI: calcd for $C_{29}H_{53}BrN_6O_6Si_2$ ($M + H^+$) 717.27, obsd 717.272.

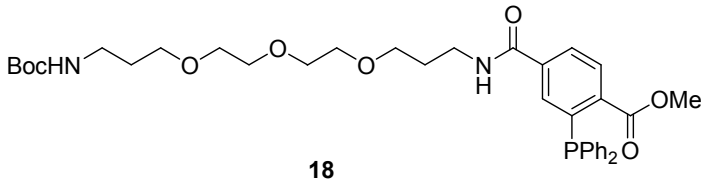
8-(4''-Azido-butylamino)-5'-(N-Boc)-ethanolamine-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (11). To 8'-Bromo-5'-(N-Boc)-ethanolamine-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (0.546 g, 0.761 mmol) in 9.36 mL dioxane at 70ºC was added 4-Azido-butylamine (0.869 g, 7.610 mmol). The reaction was stirred at this temperature for 2 d. The organic was washed ($NaHCO_3$, EtOAc, brine), dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography (6:1 EtOAc/CH_2Cl_2 → 1:1 Pet Ether/4:2:1 EtOAc/CH_2Cl_2/MeOH) yielded **11** (0.279 g, 49 %). The resulting NMR spectra indicated the resulting product as a mixture of rotomers.* The peaks were very broad and often overlapping. The correct number of protons (at an appropriate chemical shift) was present in the spectra. HRMALDI: calcd for $C_{33}H_{62}N_{10}O_6Si_2$ ($M + H^+$) 751.44, obsd 751.445.

8-(4''-Azido-butylamino)-5'-aziridino-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (12). To 8'-(4''-Azido-butylamino)-5'-(N-Boc)-ethanolamine-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (0.118 g, 0.158 mmol) in 1.6 mL anhydrous CH_2Cl_2 was added 2,6-lutidine (0.084 g, 0.788 mmol) and TMSOTf (0.140 g, 0.630 mmol). The reaction was stirred for 45 min. and quenched via addition of 2 mL MeOH and 2 mL 200 mM Citric Acid (pH 4); stirring at room temperature for an additional hour ensued. The organic was washed ($NaHCO_3$, CH_2Cl_2), dried over Na_2SO_4 and evaporated *in vacuo*. The crude material was taken directly forward. To PPh_3 (0.062 g, 0.236 mmol) in 2.36 mL dry THF at 0ºC was added DEAD (0.041 g, 0.236 mmol). The components were stirred until TLC indicated complete consumption of PPh_3. The mixture was added to the crude adenosine in 2.1 mL dry THF. The reaction was warmed to rt and heated at reflux for 2.5 hours. An aqueous workup was performed ($NaHCO_3$, EtOAc, brine), the organic was dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography (6:1 EtOAc/CH_2Cl_2 → 1:1 Pet Ether/4:2:1 EtOAc/CH_2Cl_2/MeOH) afforded **12** (0.054 g, 54 %). ^{1}H NMR ($CDCl_3$) δ 8.12 (s, 1H), 7.41 (t, J= 5.6 Hz, 1H), 6.20 (d, J= 7.6 Hz, 1H), 5.31 (bs, 2H), 4.77 (dd, J= 7.6, 4.8 Hz, 1H), 4.26 (m, 1H), 4.10 (m, 1H), 3.35 (m, 2H), 3.28 (m, 2H), 3.21 (dd, J= 13.2, 3.2 Hz, 1H), 1.90 (dd, J= 13.2, 2.4 Hz, 1H), 1.85 (m, 2H), 1.62 (m, 4H), 1.38 (m, 1H), 1.27 (m, 1H), 0.90 (s, 9H), 0.69 (s, 9H), 0.11 (s, 3H), 0.06 (s, 3H), -0.13 (s, 3H), -0.40 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 152.1, 151.6, 151.3, 149.4, 117.9, 85.9, 85.6, 73.3, 71.3, 62.5, 51.3, 42.5, 30.0, 26.9, 26.6, 26.3, 26.0, 25.8, 18.3, 17.9, -4.2, -4.52, -4.55, -5.3. HRMALDI: calcd for $C_{28}H_{52}N_{10}O_3Si_2$ ($M + H^+$) 633.38, obsd 633.381.

8-(4''-Azido-butylamino)-5'-aziridino-5'-deoxy adenosine (4b). To 8'-(4''-Azido-butylamino)-5'-aziridino-5'-deoxy-2',3'-bis-(O-tert-butyldimethylsilyl) adenosine (0.076 g, 0.120 mmol) in 3.3 mL THF at 0ºC was added nBu_4NF (0.263 mmol). The reaction was stirred at 0°C for 1 h and evaporated *in vacuo*. Immediate chromatography (2:1:1 EtOAc/CH_2Cl_2/MeOH) yielded product (0.0290 g, 60 %). ^{1}H NMR (DMSO-d_6) δ 7.90 (s, 1H), 7.65 (t, J= 4.8 Hz, 1H), 6.41 (bs, 2H), 5.94 (d, J= 5.2 Hz, 1H), 5.33 (bs, 1H), 5.21 (bs, 1H), 4.72 (bs, 1H), 4.26 (m, 1H), 4.00 (m, 1H), 3.36 (m, 2H), 3.30 (m, 2H), 2.99 (dd, J= 10.4, 2.4 Hz, 1H), 2.25 (dd, J= 10.4, 2.4 Hz, 1H), 1.72 (m, 2H), 1.56 (m, 4H), 1.41 (m, 1H), 1.31 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 152.1, 151.2, 150.2, 148.5, 116.9, 85.9, 84.0, 71.0, 69.9, 61.7, 50.5, 41.7, 28.7, 26.2, 25.8, 25.6. HRMALDI: calcd for $C_{16}H_{24}N_{10}O_3$ ($M + H^+$) 405.20, obsd 405.209.

* These compounds were analyzed in DMSO-d_6 at 90ºC. The obtained signals in the spectra were broad and very little splitting was observed. Thus, we concluded that the mixture observed in $CDCl_3$ at 25ºC was due to a rotomeric mixture.

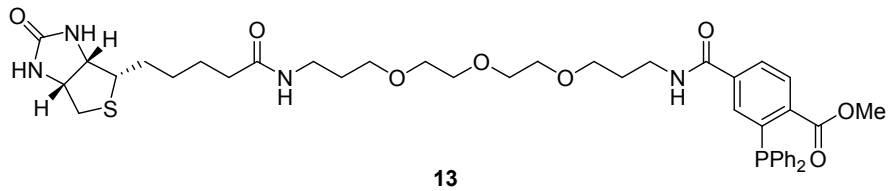
Characterization of Biotinylated triarylphosphine and intermediates



18

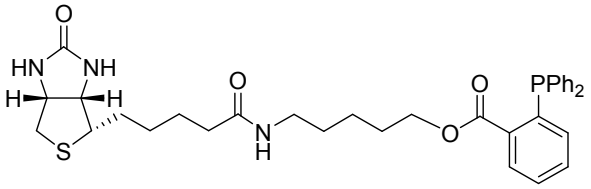
N-(3-{2-[2-(3-tert-Butoxycarbonylamino-propoxy)-ethoxy]-ethoxy}-propyl)-2-diphenylphosphanyl-terephthalamic acid methyl ester (18). To 2-Diphenyl-phosphanyl-terephthalic acid 1-methyl ester (1) (0.493 g, 1.352 mmol) in 4.75 mL THF was added CDI (0.263 g, 1.623 mmol). After stirring for 1 h, Boc-(4,7,10-trioxa-1,13-tridecanediamine) (2) (0.520 g, 1.623mmol) in 4.9mL THF was added and the mixture allowed to stir at ambient temperature for an additional hour. The organic was washed (NH_4Cl, EtOAc, brine), dried over Na_2SO_4, and evaporated *in vacuo*. Column chromatography (5:1 EtOAc/Pet Ether) yielded **18** as a yellow solid (0.733 g, 81 %). ^{1}H NMR ($CDCl_3$) δ 8.01 (dd, J= 8, 3.6 Hz, 1H), 7.71 (dd, J= 8, 1.2 Hz, 1H), 7.37 (dd, J= 3.6,

1.2 Hz, 1H), 7.30-7.23 (m, 10H), 6.88 (bs, 1H), 4.99 (bs, 1H), 3.69 (s, 3H), 3.58-3.45 (m, 8H), 3.45-3.40 (m, 6H), 3.14 (m, 2H), 1.75 (p, J= 6 Hz, 2H), 1.67 (p, J= 6 Hz, 2H), 1.39 (s, 9H); ^{31}P NMR ($CDCl_3$) δ -2.66; ^{13}C NMR ($CDCl_3$) δ 166.7, 166.2, 156.0, 141.55, 141.3, 137.7, 137.3, 137.2, 136.5, 136.3, 134.0, 133.8, 133.1, 130.7, 129.0, 128.7, 128.6, 126.4, 70.5, 70.3, 70.1, 69.5, 52.2, 38.7, 38.5, 29.7, 28.8, 28.5. HRMALDI: calcd for $C_{36}H_{47}N_2O_8P$ (M + H^+), 667.31, obsd 667.303.



13

2-Diphenylphosphanyl-N-(3-{2-[2-(3-Biotin)-propoxy)-ethoxy]-ethoxy}-propyl)- terephthalamic acid methyl ester (13). To **18** (0.234 mmol) was added 2.4 mL 4N HCl/dioxane. After stirring at rt for 2 h, the solvent was evaporated and co-stripped with 1:1 EtOAc/MeOH twice. The resulting crude material was dried under vacuum before further use and taken directly forward without purification. Biotin (0.048 g, 0.195 mmol) was dissolved in 650 µL dry DMF by heating and magnetic stirring (3). The solution was cooled to ambient temperature and CDI (0.038 g, 0.234 mmol) in 65 µL dry DMF was added to give a white precipitate. After stirring at rt for 1 h, DIEA (0.076 g, 0.584 mmol) and deprotected **18** (0.133 g, 0.234 mmol) in 530 µL dry DMF was added and stirred overnight. The organic was evaporated and dried under vacuum. The crude material was brought up in CH_2Cl_2, washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. Column chromatography (10:1 CH_2Cl_2/MeOH) afforded **13** (0.110 g, 71%). ^{1}H NMR ($CDCl_3$) δ 8.05 (dd, J= 8, 3.6 Hz, 1H), 7.78 (dd, J= 8, 1 Hz, 1H), 7.42 (dd, J= 3.6, 1 Hz, 1H), 7.34-7.25 (m, 10H), 7.23 (t, J= 5.6 Hz, 1H), 6.74 (t, J= 5.6 Hz, 1H), 6.41 (s, 1H), 5.61 (s, 1H), 4.45 (dd, J= 7.6, 4.8 Hz, 1H), 4.25 (dd, J= 7.6, 5.2 Hz, 1H), 3.72 (s, 3H), 3.60-3.42 (m, 14H), 3.28 (q, J= 6.4 Hz, 2H), 3.12-3.08 (m, 1H), 2.86 (dd, J= 12.8, 4.8 Hz, 1H), 2.70 (d, J= 12.8 Hz, 1H), 2.15 (t, J= 7.6 Hz, 2H), 1.79 (p, J= 6.4 Hz, 2H), 1.72 (p, J= 6.4 Hz, 2H), 1.66-1.57 (m, 4H), 1.40 (p, J= 6.4 Hz, 2H); ^{31}P NMR ($CDCl_3$) δ -2.63; ^{13}C NMR ($CDCl_3$) δ 173.3, 166.9, 166.4, 164.2, 141.5, 141.2, 137.6, 137.3, 137.2, 136.6, 136.4, 134.0, 133.8, 133.2, 130.7, 129.0, 128.7, 128.6, 126.5, 70.45, 70.39, 70.2, 70.0, 69.96, 69.7, 61.8, 60.2, 55.8, 52.3, 40.5, 38.5, 37.5, 36.1, 29.7, 29.1, 28.9, 28.4, 28.2, 25.8. HRMALDI: calcd for $C_{41}H_{53}N_4O_8PS$ (M + H^+), 793.33, obsd 793.325.



15

2-Diphenylphosphanyl-benzoic acid 5-(biotin)-pentyl ester (15). To 2-Diphenylphosphanyl-benzoic acid 5-(Boc-amino)-pentyl ester (4) (0.295 g, 0.689 mmol) was added 10 mL 4N HCl/dioxane. After stirring at rt for 2 h, the solvent was evaporated and co-stripped with 1:1 EtOAc/MeOH twice. The resulting crude material was dried under vacuum before further use and taken directly forward without purification. Biotin (0.160 g, 0.655 mmol) was dissolved in 4 mL of a 5:1:1 THF/H_2O/DMF solution, along with DIEA (0.237 g, 1.837 mmol) and

O-(N-Succinimidyl)-N,N,N',N'-tetramethyl-uronium tetrafluroborate (TSTU) (0.240 g, 0.797 mmol). After stirring for 2 h, an additional aliquot of DIEA (0.519 g, 4.02 mmol) was added, followed by the crude phosphine (0.689 mmol) in 10 mL THF. The mixture was stirred for an additional 12 h and the organic was evaporated. The crude material was resuspended in $CHCl_3$ and washed (NH_4Cl, $NaHCO_3$, $CHCl_3$, brine), the organic was dried over Na_2SO_4 and evaporated *in vacuo*. Column chromatography (4:4:1 EtOAc/CH_2Cl_2/ MeOH) provided **15** (0.200 g, 49%). ^{1}H NMR (DMSO-d_6) δ 7.98-7.94 (m, 1H), 7.73 (t, J= 5.6 Hz, 1H), 7.52-7.50 (m, 2H), 7.38-7.37 (m, 6H), 7.19-7.15 (m, 4H), 6.86-6.82 (m, 1H), 6.42 (s, 1H), 6.35 (s, 1H), 4.28 (dd, J= 6.4, 5.2 Hz, 1H), 4.10 (dd, J= 6.4, 5.2 Hz, 1H), 4.04 (t, J= 6.4 Hz, 2H), 3.10-3.05 (m, 1H), 3.00 (q, J= 6.4 Hz, 2H), 2.80 (dd, J= 12.0, 4.8 Hz, 1H), 2.57 (d J= 12.0 Hz, 1H), 2.04 (t, J= 7.2 Hz, 2H), 1.63-1.56 (m, 1H), 1.52-1.42 (m, 5H), 1.38-1.15 (m, 6H); ^{31}P NMR ($CDCl_3$) δ -3.74; ^{13}C NMR ($CDCl_3$) δ 173.3, 166.9, 146.4, 140.1, 139.8, 137.9, 134.6, 134.4, 134.3, 133.8, 133.6, 131.9, 130.5, 128.6, 128.44, 128.38, 128.2, 65.1, 61.7, 60.3, 60.2, 55.8, 40.4, 39.2, 35.9, 29.1, 28.2, 28.1, 25.8, 23.3. HRMALDI: calcd for $C_{34}H_{40}N_3O_4PS$ ($M + H^+$), 618.25, obsd 618.248.

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