

ESI for

PEGylated Dmoc Phosphoramidites for Sensitive Oligodeoxynucleotide Synthesis

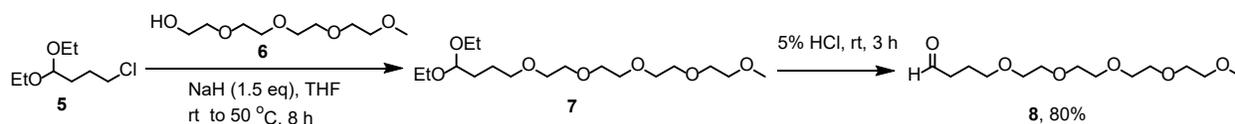
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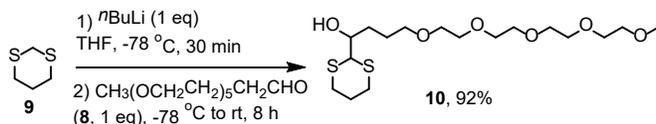
Experimental Details

General: All reactions were performed in oven-dried glassware under nitrogen using standard Schlenk techniques. Reagents and solvents from commercial sources were used as received with the following exceptions. DCM, pyridine, ACN, diethyl ether and diisopropylamine were distilled over CaH₂ under nitrogen. THF was distilled over CaH₂ and then Na/benzophenone under nitrogen. Sigma-Aldrich TLC plates, silica gel 60F-254 over glass support, 0.25 μm thickness, were used for TLC. Selecto Scientific silica gel, particle size 32-63 μm, was used for flash column chromatography. ¹H, ¹³C and ³¹P NMR spectra were measured on a Varian UNITYINOVA spectrometer at 400, 100 and 162 MHz, or Bruker's spectrometer at 500, 126 and 202 MHz, respectively. Chemical shifts (δ) were reported in reference to solvent peaks, residue CHCl₃ at 7.24 ppm for ¹H, CDCl₃ at 77.00 ppm for ¹³C, and H₃PO₄ at 0.00 ppm for ³¹P. ODN syntheses were performed on a MerMade 6 solid phase synthesizer. RP HPLC was performed on a JASCO LC-2000Plus System: pump, PU-2089Plus Quaternary Gradient; detector, UV-2075Plus. Column: C-18 reversed phase, analytical, 5 μm diameter, 100 Å, 250 × 4.60 mm. Solvent A: 0.1 M triethylammonium acetate in 5% ACN. Solvent B: 90% ACN. All profiles were generated by detecting absorbance at 260 nm using the linear gradient solvent system: solvent B (0%-45%) in solvent A over 60 min followed by solvent B (45%-100%) in solvent A over 20 min at a flow rate of 1.0 mL/min. Capillary electrophoresis (CE) was carried out on an Agilent 7100 CE system with UV-Visible diode-array detector (190–600 nm). Capillary: PVA coated 25 cm × 100 μm (40 cm was cut to 25 cm). Buffer solution: 200 mM Bis-Tris and 200 mM boric acid in CE water, pH 7.2. Sieving solution: 27% (w/v) PEG 35,000 in buffer solution. Sieving solution injection: -8.0 bar, 5 min. Sample injection: 0.02-0.07 μM ODNs in buffer solution, -10 KV for 10 sec. Run: -25 KV, 30 °C for 40 min. Detection: alignment interface for standard capillary with 75 μm ID, detected at 260 ± 8 nm. Sieving gel removal (cleaning capillary): -12 bar, 1 min. HRMS were obtained on Thermo HR-Orbitrap Elite or Waters G2-XS QToF Mass Spectrometers. MALDI-TOF MS were obtained on Bruker's microflex™ LRF MALDI-TOF System.

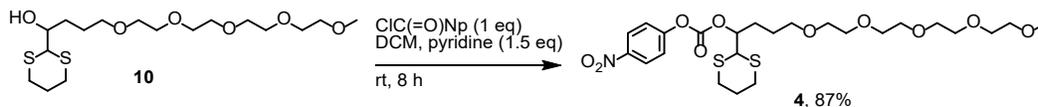


Compound 8: To NaH (2.16 g, 90.03 mmol, 1.5 equiv.) in dry THF (15 mL) was added the solution of tetraethylene glycol monomethyl ether (**6**, 10.0 g, 48.02 mmol, 0.8 equiv.) in dry THF (70 mL) dropwise at rt under nitrogen. The mixture was stirred at rt for 45 min, and then heated to 50 °C. The solution of **5** (10.843 g, 60.02 mmol, 1.0 equiv.) in dry THF (20 mL) was added via a cannula over 5 h under nitrogen. After stirring at 50 °C for 8 h, water (30 mL) was added slowly. The majority of THF was evaporated under reduced pressure. The mixture was partitioned between water (30 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (50 mL × 3). The combined organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated to dryness. The crude **7** was dissolved in suitable volume of 5% HCl. The reaction was allowed to proceed with stirring at rt for 3 h, and then quenched by adding 0.5 M NaOH until pH 7. The product was extracted with DCM (50 mL × 3), and the combined organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated. Product **8** was purified with flash column chromatography (SiO₂, EtOAc/MeOH 18:1): 12.95 g, 80%; colorless oil; TLC R_f = 0.3 (SiO₂, EtOAc/MeOH 18:1); ¹H NMR (400 MHz, CDCl₃) δ 1.27-1.34 (m, 2H), 1.95 (t, J = 7.0 Hz, 2H),

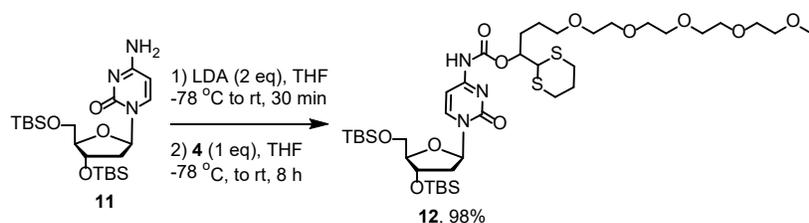
2.77 (s, 3H), 2.90-2.99 (m, 6H), 3.02-3.05 (m, 12H), 9.18 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 22.3, 22.3, 22.4, 40.3, 40.4, 40.5, 58.2, 58.4, 69.7, 69.8, 70.0, 70.1, 70.1, 70.2, 70.2, 70.3, 70.5, 71.6, 71.8, 201.3, 201.4; HRMS (ESI) m/z calcd for $\text{C}_{13}\text{H}_{26}\text{O}_6\text{Na}$ $[\text{M} + \text{Na}]^+$ 301.1627, found 301.1631.



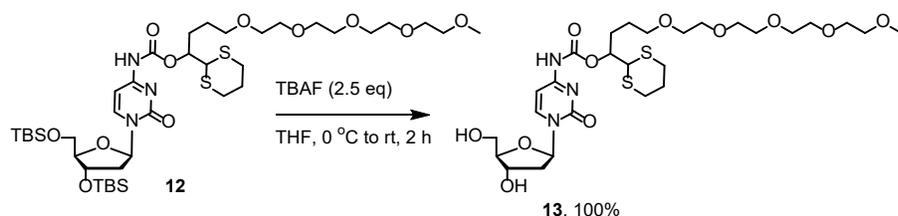
Compound 10: To a solution of 1,3-dithiane (**9**, 2.23 g, 18.55 mmol, 1.0 equiv.) in dry THF (50 mL) was slowly added $n\text{BuLi}$ (2.5 M in hexanes, 7.42 mL, 62.5 mmol, 1.0 equiv.) at $-78\text{ }^\circ\text{C}$ under nitrogen. After stirring for 30 min, compound **8** (5.15 g, 18.55 mmol, 1.0 equiv.) dissolved in THF (20 mL) was added to the reaction mixture via a cannula over 15 min at $-78\text{ }^\circ\text{C}$ under nitrogen. The mixture was stirred for 8 h while warming to rt gradually. The reaction was quenched with sat. NH_4Cl (20 mL). The majority of THF was evaporated under reduced pressure. The mixture was partitioned between sat. NH_4Cl (50 mL) and EtOAc (50 mL). The aqueous layer was extracted with EtOAc (50 mL \times 3). The combined organic layer was dried over anhydrous Na_2SO_4 , filtered, and concentrated. Product **10** was purified with flash column chromatography (SiO_2 , EtOAc/MeOH 18:1): 6.78 g, 92%; colorless oil; TLC R_f = 0.5 (SiO_2 , EtOAc/MeOH 18:1); ^1H NMR (500 MHz, CDCl_3) δ 1.60-1.68 (m, 1H), 1.71-1.85 (m, 2H), 1.88-2.00 (m, 2H), 2.06-2.13 (m, 1H), 2.72-2.2.85 (m, 2H), 2.90-2.95 (m, 1H), 3.39 (s, 3H), 3.51-3.70 (m, 18H), 3.86-3.91 (m, 1H), 3.99 (d, J = 6.2 Hz, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ 25.9, 26.1, 28.6, 29.0, 31.6, 53.0, 59.0, 70.1, 70.5, 70.56, 70.61, 71.3, 71.9, 72.5; HRMS (ESI) m/z calcd for $\text{C}_{17}\text{H}_{34}\text{O}_6\text{S}_2\text{Na}$ $[\text{M} + \text{Na}]^+$ 421.1694, found 421.1684.



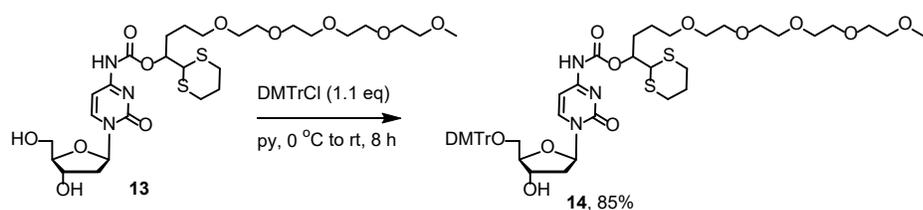
Compound 4: To a solution of **10** (5.56 g, 14.0 mmol, 1.0 equiv.) in dry DCM (100 mL) under nitrogen was added distilled pyridine (1.7 mL, 21.0 mmol, 1.5 equiv.). After cooling to $0\text{ }^\circ\text{C}$, 4-nitrophenol chloroformate (2.82 g, 14.0 mmol, 1.0 equiv.) was added. The mixture was stirred overnight while warming to rt gradually. The reaction was quenched with sat. NH_4Cl (75 mL) and extracted with DCM (50 mL \times 3). The combined extract was dried over anhydrous Na_2SO_4 , filtered, and concentrated to dryness. Product **4** was precipitated from DCM with hexanes: 6.85 g, 87%; light yellow oil; TLC R_f = 0.65 (SiO_2 , EtOAc/MeOH 5:1); ^1H NMR (500 MHz, CDCl_3) δ 1.35-1.88 (m, 6H), 2.41-2.50 (m, 2H), 2.62-2.72 (m, 2H), 3.03 (s, 3H), 3.19-3.35 (m, 18 H), 3.76 (d, J = 7.0 Hz, 1H), 4.84-4.89 (m, 1H), 7.12 (d, J = 9.2 Hz, 2H), 7.96 (d, J = 9.2 Hz, 2H); ^{13}C NMR (126 MHz, CDCl_3) δ 25.4, 27.9, 28.2, 28.7, 48.3, 58.9, 70.2, 70.4, 70.5, 71.8, 79.5, 121.8, 125.2, 145.3, 152.2, 155.5; HRMS (ESI) m/z calcd for $\text{C}_{24}\text{H}_{37}\text{NO}_{10}\text{S}_2\text{Na}$ $[\text{M} + \text{Na}]^+$ 586.1757, found 586.1756.



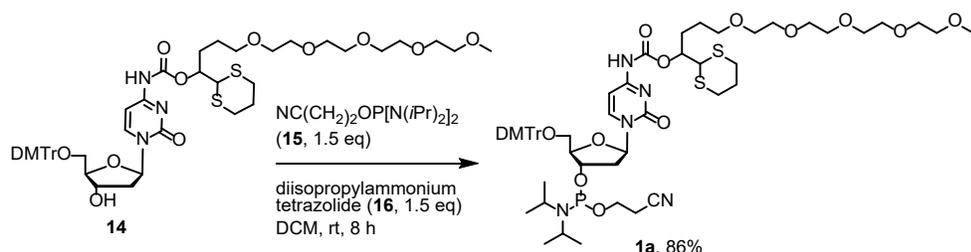
Compound 12: To a solution of diisopropylamine (1.27 mL, 9.06 mmol, 2.0 equiv.) in THF (50 mL) at $-78\text{ }^{\circ}\text{C}$ was added *n*BuLi (2.5 M in hexanes, 3.62 mL, 9.06 mmol, 2.0 equiv.). After stirring for 30 min, the freshly prepared LDA solution was added via a cannula to the solution of **11** (2.061 g, 4.53 mmol, 1.0 equiv.) in THF (50 mL) at $-78\text{ }^{\circ}\text{C}$. The mixture was stirred at the same temperature for 30 min, and compound **4** (2.552 g, 16.01 mmol, 1.0 equiv.) was then added under positive nitrogen pressure. The mixture was stirred for 8 h while warming to rt gradually. The reaction was quenched with sat. NaCl (20 mL). The majority of THF was evaporated under reduced pressure. The mixture was partitioned between EtOAc (50 mL) and sat. NaCl (50 mL). The aqueous layer was extracted with EtOAc (40 mL \times 3). The combined organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated. Product **12** was purified with flash column chromatography (SiO_2 , EtOAc/MeOH 18:1): 3.91 g, 98%; yellow oil; TLC $R_f = 0.55$ (SiO_2 , EtOAc/MeOH 18:1); ^1H NMR (500 MHz, CDCl_3) δ 0.06 (s, 3H), 0.07 (s, 3H), 0.12 (s, 3H), 0.13 (s, 3H), 0.88 (s, 9H), 0.94 (s, 9H), 1.56-2.14 (m, 7H), 2.46-2.55 (m, 1H), 2.71-2.82 (m, 2H), 2.88-2.99 (m, 2H), 3.39 (s, 3H), 3.46-3.71 (m, 18H), 3.78 (d, $J = 9.5$ Hz, 1H), 3.91-4.01 (m, 2H), 4.10 (t, $J = 4.3$ Hz, 1H), 4.35-4.45 (m, 1H), 5.12-5.21 (m, 1H), 6.24 (t, $J = 5.2$ Hz, 1H), 7.16 (d, $J = 7.3$ Hz, 1H), 8.37 (d, $J = 7.3$ Hz, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ -5.51, -5.45, -4.9, -4.6, 17.9, 18.3, 25.6, 25.7, 25.9, 28.6, 28.8, 29.0, 31.5, 42.2, 49.4, 53.1, 59.0, 61.7, 69.8, 70.1, 70.2, 70.4, 70.5, 70.6, 71.2, 71.9, 72.4, 76.2, 86.6, 87.7, 94.4, 100.0, 144.3, 152.0, 154.9, 162.1; HRMS (ESI) m/z calcd for $\text{C}_{39}\text{H}_{73}\text{N}_3\text{O}_{11}\text{S}_2\text{Si}_2\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 902.4123, found 902.4107.



Compound 13: To a solution of **12** (3.18 g, 3.61 mmol, 1.0 equiv.) in THF (50 mL) at $0\text{ }^{\circ}\text{C}$ was added TBAF (1 M in THF, 9.02 mL, 9.02 mmol, 2.5 equiv.). The mixture was stirred for 2 h while warming to rt. THF was evaporated under reduced pressure and the residue was loaded onto a column for flash column chromatography (SiO_2 , EtOAc/MeOH 8:2). Compound **13**: 2.454 g, 100%; pale yellow foam; TLC $R_f = 0.3$ (SiO_2 , EtOAc/MeOH 8:2); ^1H NMR (500 MHz, CD_3OD) δ 1.59-1.75 (m, 2H), 1.76-1.94 (m, 2H), 2.01-2.09 (m, 2H), 2.18-2.226 (m, 1H), 2.47-2.255 (m, 1H), 2.76-2.88 (m, 2H), 2.89-3.00 (m, 2H), 3.36 (s, 3H), 3.57-3.69 (m, 18H), 3.75-3.89 (m, 2H), 4.02-4.07 (m, 1H), 4.20 (d, $J = 6.2$ Hz, 1H), 4.43-4.46 (m, 1H), 5.15-5.21 (m, 1H), 6.25 (t, $J = 6.2$ Hz, 1H), 7.29 (d, $J = 7.4$ Hz, 1H), 8.50 (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ 25.3, 25.6, 28.0, 28.3, 28.5, 41.2, 49.1, 57.9, 61.1, 69.9, 70.0, 70.16, 70.19, 70.20, 70.29, 71.6, 75.7, 87.2, 88.1, 95.4, 144.7, 152.9, 156.2, 163.4; HRMS (ESI) m/z calcd for $\text{C}_{27}\text{H}_{45}\text{N}_3\text{O}_{11}\text{S}_2\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 674.2393, found 674.2376.

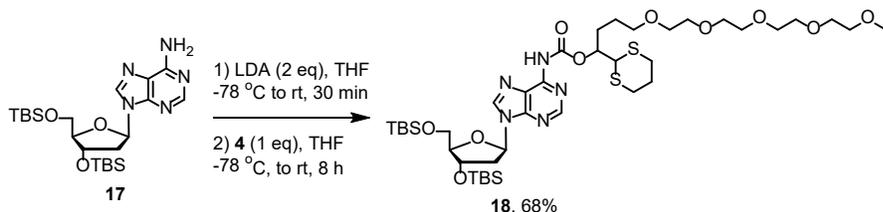


Compound 14: To a solution of **13** (1.6 g, 2.71 mmol, 1.0 equiv.) in pyridine (50 mL) at 0 °C was added DMTrCl (1.01 g, 2.98 mmol, 1.1 equiv.) under positive nitrogen pressure. The mixture was stirred for 8 h while warming to rt. The volume of the mixture was reduced to about 2 mL under vacuum from an oil pump (the remaining pyridine can help to retain DMTr on the product). The mixture was partitioned between 5% Na₂CO₃ (20 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (30 mL × 3). The combined organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated to dryness. Product **14** was purified with flash column chromatography (SiO₂, EtOAc/MeOH 9:1 with 5% Et₃N): 2.20 g, 85%; pale yellow foam; TLC *R_f* = 0.45 (SiO₂, EtOAc/MeOH 9:1 with 5% Et₃N); ¹H NMR (500 MHz, CDCl₃) δ 1.55-1.91 (m, 5H), 1.92-2.01 (m, 2H), 2.11-2.19 (m, 1H), 2.61-2.69 (m, 2H), 2.70-2.88 (m, 2H), 3.29 (s, 3H), 3.3.29-3.65 (m, 20H), 3.71 (s, 6H), 4.01 (dd, *J* = 17.9, 6.45 Hz, 1H), 4.13-4.14 (m, 1H), 4.44-4.47 (m, 1H), 5.05-5.11 (m, 1H), 6.19-6.21 (m, 0.8H), 6.28-6.32 (m, 0.2H), 6.77 (d, *J* = 7.9 Hz, 4H), 6.88-6.89 (m, 1H), 7.15 (t, *J* = 7.2 Hz, 1H), 7.18-7.27 (m, 6H), 7.34 (d, *J* = 7.8 Hz, 2H), 8.17-8.21 (m, 1H); ¹³C NMR (126 MHz, CDCl₃) δ 25.5, 25.6, 28.5, 28.6, 28.7, 42.0, 49.2, 49.4, 55.2, 58.9, 62.7, 70.1, 70.4, 70.8, 71.8, 75.8, 76.0, 86.5, 86.7, 87.2, 95.1, 113.2, 127.0, 127.9, 128.1, 130.0, 135.4, 135.5, 144.2, 152.2, 155.2, 158.5, 162.4; HRMS (ESI) *m/z* calcd for C₄₈H₆₃N₃O₁₃S₂Na [M + Na]⁺ 976.3700, found 976.3685.

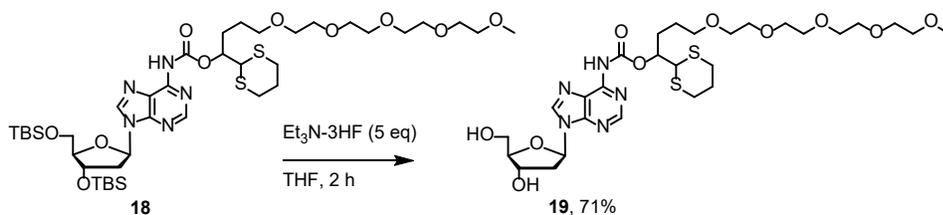


Compound 1a: To a solution of **14** (330 mg, 0.35 mmol, 1.0 equiv.) in dry DCM (10 mL) was added diisopropylammonium tetrazolide (**16**, 89.7 mg, 0.525 mmol, 1.5 equiv.) and 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite (**15**, 0.17 mL, 0.525 mmol, 1.5 equiv.) at rt under nitrogen. After stirring overnight, the mixture was concentrated to dryness. Product **1a** was purified by dissolving the sample in the solvent mixture of EtOAc/MeOH 9:1 with 5% Et₃N, loading onto a column (SiO₂), and eluting with the same solvent mixture: mixture of diastereomers; 0.340 g, 86%; pale yellow oil; TLC *R_f* = 0.40 and 0.45 (SiO₂, EtOAc/MeOH 9:1 with 5% Et₃N); ¹H NMR (500 MHz, CDCl₃) δ 0.94-1.19 (m, 12H), 1.47-1.99 (m, 6H), 2.14-2.22 (m, 1H), 2.32 (t, *J* = 6.1 Hz, 1H), 2.48 (t, *J* = 6.2 Hz, 1H), 2.41-2.50 (m, 1H), 2.54-2.81 (m, 6H), 3.23 (s, 3H), 3.22-3.75 (m, 22H), 3.66 (s, 3H), 3.67 (s, 3H), 3.92-3.94 (m, 1H), 4.09-4.13 (m, 1H), 4.48-4.54 (m, 1H), 5.00-5.06 (m, 1H), 6.11-6.16 (m, 1H), 6.72 (d, *J* = 7.6 Hz, 2H), 6.74 (d, *J* = 8.4 Hz), 7.12 (t, *J* = 7.4 Hz, 1H), 7.12-7.25 (m, 6H), 7.22-7.7.35 (m, 2H), 8.08 (d, *J* = 7.4 Hz, 0.5H), 8.18 (d, *J* = 7.1

Hz, 0.5H); ^{13}C NMR (126 MHz, CDCl_3) δ 19.9, 20.0, 20.07, 20.13, 20.3, 22.8, 22.9, 23.0, 24.4, 24.5, 24.6, 25.5, 28.3, 28.4, 28.5, 28.7, 40.7, 41.1, 43.1, 43.2, 45.2, 45.4, 45.9, 49.1, 49.2, 55.1, 58.2, 58.3, 58.4, 58.82, 58.83, 60.2, 61.9, 62.3, 70.1, 70.3, 70.5, 71.8, 75.7, 75.9, 85.5, 86.7, 95.1, 113.2, 117.3, 117.5, 117.6, 127.0, 127.9, 128.1, 130.0, 135.2, 135.3, 144.0, 158.6, 162.4; ^{31}P NMR (202 MHz, CDCl_3) δ 148.47, 148.98, 149.03; HRMS (ESI) m/z calcd for $\text{C}_{57}\text{H}_{80}\text{N}_5\text{O}_{14}\text{PS}_2\text{H}$ [$\text{M} + \text{H}$] $^+$ 1154.4959, found 1154.4972.

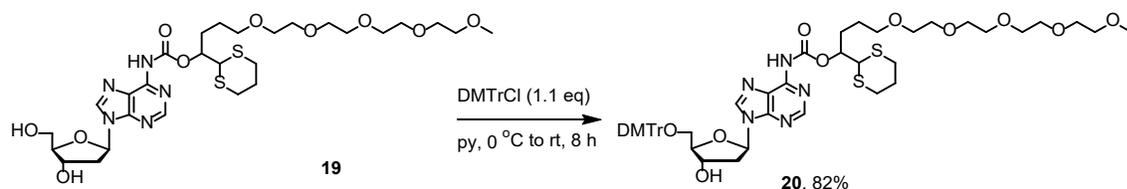


Compound 18: Synthesized using the procedure for **12**. Diisopropylamine (2.25 mL, 16.0 mmol, 2.0 equiv.), THF (100 mL), *n*BuLi (2.5 M in hexanes, 6.4 mL, 16.0 mmol, 2.0 equiv.), **17** (3.83 g, 8.0 mmol, 1.0 equiv.), THF (20 mL) and **4** (4.50 g, 8.0 mmol, 1.0 equiv.) were used. Product **18** was purified with flash column chromatography (SiO_2 , EtOAc/MeOH 18:1): 4.89 g, 68%; pale yellow oil; TLC R_f = 0.4 (SiO_2 , EtOAc/MeOH 18:1); ^1H NMR (500 MHz, CDCl_3) δ 0.04 (s, 6H), 0.06 (s, 6H), 0.82-0.89 (s, 18H), 1.54-2.10 (m, 6H), 2.37-2.99 (m, 6H), 3.32 (s, 3H), 3.32-3.62 (m, 18H), 3.72 (dd, J = 11.2, 3.10 Hz, 1H), 3.83 (dd, J = 11.1, 3.85 Hz, 1H), 3.94-3.99 (m, 1H), 4.12 (t, J = 5.9 Hz, 1H), 4.56-4.58 (m, 1H), 5.20-5.23 (m, 1H), 6.45 (t, J = 6.7 Hz, 1H), 8.28 (s, 1H), 8.71 (s, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ -5.5, -5.4, -4.8, -4.7, 17.9, 18.4, 25.6, 25.7, 25.86, 25.91, 26.0, 28.5, 28.6, 28.7, 29.0, 30.9, 31.5, 41.2, 49.6, 53.1, 59.0, 62.7, 70.1, 70.4, 70.5, 71.2, 71.9, 72.5, 75.3, 84.6, 88.0, 122.3, 141.3, 149.4, 150.5, 150.7, 152.6; HRMS (ESI) m/z calcd for $\text{C}_{40}\text{H}_{73}\text{N}_5\text{O}_{10}\text{S}_2\text{Si}_2\text{H}$ [$\text{M} + \text{H}$] $^+$ 904.4416, found 904.4413.

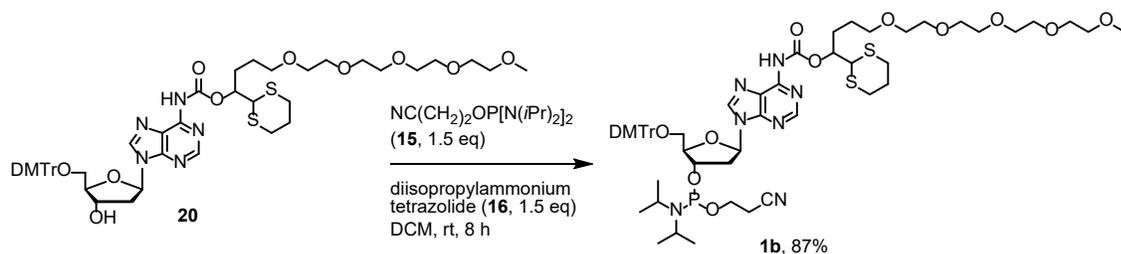


Compound 19: To the solution of **18** (3.0 g, 3.32 mmol, 1.0 equiv.) in THF (50 mL) was added triethylamine trihydrogen fluoride (2.7 mL, 16.6 mmol, 5.0 equiv.) at rt. After stirring for 2 h, the reaction was quenched with methoxytrimethylsilane (2.29 mL, 16.6 mmol, 5.0 equiv.).¹ Volatiles were evaporated under reduced pressure. Product **19** was purified with flash chromatography by dissolving the crude product in the solvent mixture of EtOAc/MeOH 8:2, loading onto a column (SiO_2) and eluting with the same solvent mixture: 1.590 g, 71%; white foam; TLC R_f = 0.3 (SiO_2 , EtOAc/MeOH 8:2); ^1H NMR (500 MHz, CD_3OD) δ 1.67-1.80 (m, 2H), 1.84-1.93 (m, 2H), 2.05-2.10 (m, 2H), 2.40-2.51 (m, 1H), 2.78-3.02 (m, 5H), 3.36 (s, 3H), 3.52-3.69 (m, 18H), 3.74-3.78 (m, 1H), 3.84 (t, J = 2.8 Hz, 0.7H), 3.87 (t, J = 2.8 Hz, 0.3H), 4.07-4.09 (m, 1H), 4.27 (d, J = 6.3 Hz, 1H), 4.58-4.62 (m, 1H), 5.26-5.30 (m, 1H), 6.44 (dd, J = 7.9, 6.10 Hz, 0.3H), 6.54 (t, J = 6.8 Hz, 0.7H), 8.19 (s, 0.7H), 8.33 (s, 0.3H), 8.57 (s, 0.3H), 8.60 (s, 0.7H); ^{13}C NMR (126 MHz, CD_3OD) δ 25.3, 25.7, 28.1, 28.4, 28.5, 40.1, 40.2, 49.3, 57.7, 62.0, 62.3, 69.8, 69.9, 70.1, 71.4,

71.5, 71.7, 75.5, 85.3, 85.8, 88.3, 88.5, 100.0, 119.5, 122.5, 140.2, 142.7, 148.5, 149.7, 150.8, 151.5, 151.7, 152.1, 156.1; HRMS (ESI) m/z calcd for $C_{28}H_{45}N_5O_{10}S_2H [M + H]^+$ 676.2686, found 676.2688.

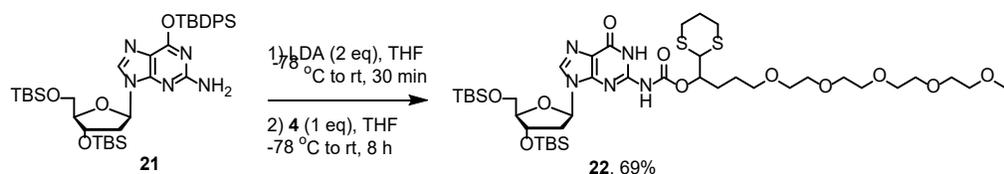


Compound 20: Synthesized using the procedure for **14**. Compound **19** (1.06 g, 1.60 mmol, 1.0 equiv.), pyridine (20 mL) and DMTrCl (596 mg, 1.76 mmol, 1.1 equiv.) were used. Product **20** was purified with flash column chromatography (SiO_2 , EtOAc/MeOH 9:1 with 5% Et_3N): 1.26 g, 82%; pale white foam; TLC R_f = 0.55 (SiO_2 , EtOAc/MeOH 9:1 with 5% Et_3N); 1H NMR (500 MHz, $CDCl_3$) δ 1.66-1.76 (m, 2H), 1.79-1.94 (m, 2H), 2.02-2.036 (m, 2H), 2.55- 2.61 (m, 1H), 2.70-2.78 (m, 2H), 2.83-2.91 (m, 2H), 2.94-2.98 (m, 1H), 3.35 (s, 3H), 3.40-3.69 (m, 20H), 3.74 (s, 6H), 4.16 (d, J = 6.1 Hz, 1H), 4.10-4.23 (m, 1H), 4.69-4.71 (m, 1H), 5.20-5.28 (m, 1H), 6.49 (t, J = 6.5 Hz, 1H), 6.76 (d, J = 8.8 Hz, 4H), 7.16 (t, J = 7.2 Hz, 1H), 7.21 (t, J = 7.8 Hz, 2H), 7.26 (d, J = 8.9 Hz, 4H), 7.36 (d, J = 8.1 Hz, 2H), 8.10 (s, 0.2H), 8.14 (s, 0.8H), 8.66 (s, 0.2H), 8.68 (s, 0.8H), 9.05 (brs, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 25.6, 25.7, 28.5, 28.7, 29.0, 29.1, 40.3, 49.7, 55.2, 59.0, 63.7, 67.8, 70.2, 70.5, 70.6, 71.9, 72.3, 75.5, 84.7, 86.3, 86.6, 113.2, 122.3, 126.9, 127.9, 128.1, 129.5, 130.0, 135.6, 141.4, 144.5, 149.4, 150.5, 150.7, 152.7, 158.5; HRMS (ESI) m/z calcd for $C_{49}H_{63}N_5O_{12}S_2Na [M + Na]^+$ 1000.3812, found 1000.3788.

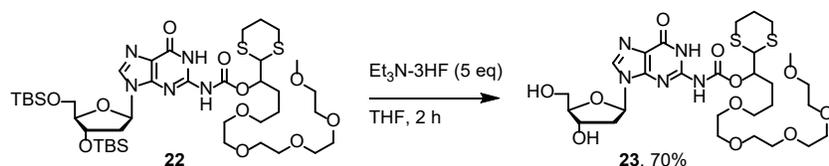


Compound 1b: Synthesized using the procedure for **1a**. Compound **20** (2.215 g, 2.27 mmol, 1.0 equiv.), DCM (30 mL), diisopropylammonium tetrazolide (**16**, 582.25 mg, 3.40 mmol, 1.5 equiv.) and 2-cyanoethyl N,N,N',N' -tetraisopropylphosphorodiamidite (**15**, 1.08 mL, 3.40 mmol, 1.5 equiv.) were used. Product **1b** was purified by dissolving the sample in the solvent mixture of EtOAc/MeOH 18:1 with 5% Et_3N , loading onto a column (SiO_2), and eluting with the same solvent mixture: mixture of diastereomers; 2.33 g, 87%; pale yellow oil; TLC R_f = 0.4 and 0.45 (SiO_2 , EtOAc/MeOH 18:1 with 5% Et_3N); 1H NMR (500 MHz, $CDCl_3$) δ 0.90-1.25 (m, 12H), 1.55-1.81 (m, 4H), 1.94-1.98 (m, 2H), 2.39 (t, J = 6.1 Hz, 1H), 2.50-2.89 (m, 9H), 3.27 (s, 3.5H) 3.10-3.88 (m, 21H), 3.55 (s, 6H), 3.99-4.13 (m, 2H), 4.21-4.24 (m, 1H), 4.66-4.72 (m, 1H), 5.15-5.20 (m, 1H), 6.35-6.46 (m, 1H), 6.60 (m, 4H), 7.08-7.20 (m, 7H), 7.30 (d, J = 7.2 Hz, 2H), 7.50 (s, 0.2H), 8.15 (s, 0.4H), 8.17 (s, 0.4 H), 8.22 (s, 0.2H), 8.61 (s, 0.8H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 19.8,, 20.0, 20.2, 22.7, 22.8, 22.9, 24.4, 25.4, 25.6, 28.3, 28.6, 28.93, 28.92, 38.7, 38.9, 43.0, 43.1, 45.0, 49.6, 49.7, 55.0, 58.1, 58.2, 58.3, 58.7, 63.2, 63.3, 69.9, 70.2, 70.3, 71.7, 75.2, 84.8, 86.2, 112.9,

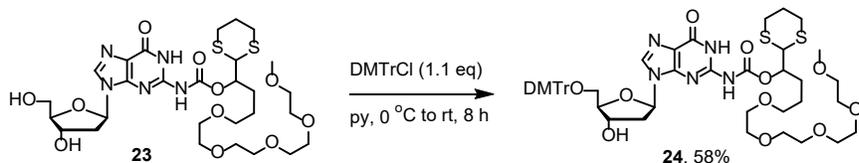
117.1, 117.5, 117.6, 122.6, 126.6, 127.6, 127.9, 129.8, 135.5, 144.5, 149.6, 150.9, 152.2, 158.3; ^{31}P NMR (202 MHz, CDCl_3) δ 148.74, 148.78, 148.88, 148.93; HRMS (ESI) m/z calcd for $\text{C}_{58}\text{H}_{80}\text{N}_7\text{O}_{13}\text{PS}_2\text{H}$ $[\text{M} + \text{H}]^+$ 1178.5071, found 1178.5088.



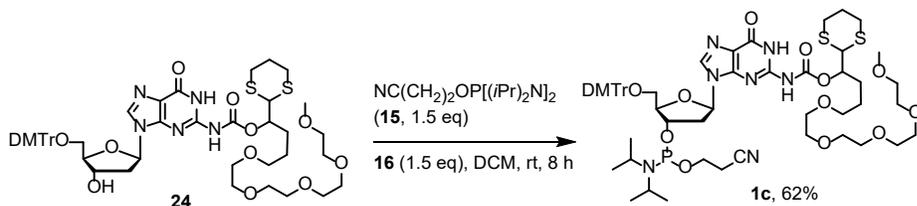
Compound 22: Synthesized using the procedure for **12**. Diisopropylamine (4.70 mL, 33.4 mmol, 2.0 equiv.), THF (100 mL), *n*BuLi (2.5 M in hexanes, 13.36 mL, 33.4 mmol, 2.0 equiv.), **21**² (12.247 g, 16.7 mmol, 1.0 equiv.), THF (50 mL) and **4** (9.405 g, 16.7 mmol, 1.0 equiv.) were used. Product **22** was purified with flash column chromatography (SiO_2 , EtOAc/MeOH, 19:1): 10.62 g, 69%; light brown oil; TLC R_f = 0.5 (SiO_2 , EtOAc/MeOH 19:1); ^1H NMR (500 MHz, CDCl_3) δ -0.06-0.15 (m, 12H), 0.70-1.00 (m, 18H), 1.59-2.11 (m, 6H), 2.31-2.48 (m, 2H), 2.71-2.85 (m, 2H), 2.91-3.05 (m, 2H), 3.39 (s, 3H), 3.44-4.01 (m, 21H), 4.57 (brs, 1H), 5.27 (brs, 1H), 6.24 (t, J = 6.2 Hz, 1H), 8.00 (s, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ -5.6, -5.4, -4.8, -4.7, 17.9, 18.3, 25.3, 25.4, 25.6, 25.7, 25.8, 25.9, 27.7, 28.0, 28.4, 28.6, 28.7, 29.0, 31.3, 41.6, 48.5, 53.1, 58.8, 58.9, 62.7, 70.0, 70.37, 70.39, 70.4, 70.5, 71.1, 71.75, 71.76, 72.4, 78.5, 83.6, 87.7, 120.7, 136.5, 147.1, 148.1, 154.8, 155.6; HRMS (ESI) m/z calcd for $\text{C}_{40}\text{H}_{73}\text{N}_5\text{O}_{11}\text{S}_2\text{Si}_2\text{Na}$ $[\text{M} + \text{Na}]^+$ 942.4184, found 942.4151.



Compound 23: Synthesized using the procedure for **19**. Compound **22** (7.66 g, 11.08 mmol, 1.0 equiv.), THF (70 mL), triethylamine trihydrogen fluoride (9.03 mL, 55.4 mmol, 5.0 equiv.), and trimethylmethoxy silane (7.66 mL, 55.4 mmol, 5.0 equiv.) were used. Product **23** was purified with flash chromatography by dissolving the crude product in the solvent mixture of EtOAc/MeOH 8:2, loading onto a column (SiO_2) and eluting with the same solvent mixture: 4.00 g, 70%; yellow oil; TLC R_f = 0.3 (SiO_2 , EtOAc/MeOH 8:2); ^1H NMR (500 MHz, CD_3OD . Some peaks were buried in the peaks of Et_3NH^+ and H_2O . Attempts to remove these impurities were unsuccessful.) δ 1.83-1.96 (m, 2H), 2.24-2.27 (m, 2H), 2.40-2.45 (m, 1H), 2.69-2.74 (m, 1H), 2.80-2.91 (m, 2H), 2.94-3.06 (m, 4H), 3.51-3.52 (m, 4H), 3.62 (m, 10H), 3.76-3.81 (m, 2H), 3.89-3.95 (m, 1H), 4.01-4.4.11 (m, 1H), 5.27-5.31 (m, 1H), 5.50 (brs, 1H), 6.32 (t, J = 6.9 Hz, 1H), 7.10-7.24 (m, 1H), 8.10 (s, 1H); ^{13}C NMR (126 MHz, CD_3OD) δ 21.6, 29.2, 29.6, 29.8, 38.2, 40.0, 58.0, 62.4, 63.16, 63.17, 69.9, 70.2, 71.7, 72.5, 84.4, 86.5, 88.2, 88.4, 115.4, 116.5, 136.9, 151.2, 152.4, 153.0, 153.3, 157.8, 157.9; HRMS (ESI) m/z calcd for $\text{C}_{28}\text{H}_{45}\text{N}_5\text{O}_{11}\text{S}_2\text{Na}$ $[\text{M} + \text{Na}]^+$ 714.2455, found 714.2456.



Compound 24: Synthesized using the procedure for **14**. Compound **23** (3.60 g, 5.21 mmol, 1.0 equiv.), pyridine (40 mL) and DMTrCl (1.94 g, 5.73 mmol, 1.1 equiv.) were used. Product **24** was purified with flash column chromatography (SiO₂, EtOAc/MeOH 8:2 with 5% Et₃N): 3.00 g, 58%; pale white foam; TLC *R_f* = 0.45 (SiO₂, EtOAc/MeOH 8:2 with 5% Et₃N); ¹H NMR (500 MHz, CDCl₃) δ 1.55-1.63 (m, 2H), 1.75-1.80 (m, 1H), 1.86-1.92 (m, 1H), 1.97-2.05 (m, 2H), 2.51-2.74 (m, 3H), 2.84-2.99 (m, 2H), 3.07-3.15 (m, 1H), 3.34 (s, 3H), 3.34-3.80 (m, 20H), 3.98-4.22 (m, 3H), 4.70-4.77 (m, 1H), 5.19-5.29 (m, 1H), 6.17-6.32 (m, 1H), 6.23-6.28 (m, 1H), 6.72 (d, *J* = 8.3 Hz, 4H), 7.05-7.45 (m, 9H), 7.85 (d, *J* = 7.3 Hz, 1H); ¹³C NMR (126 MHz, CDCl₃) δ 25.5, 25.6, 27.9, 28.0, 28.2, 28.3, 28.6, 28.7, 40.5, 48.4, 48.6, 55.2, 59.0, 60.4, 64.2, 70.09, 70.12, 70.39, 70.45, 70.48, 70.49, 71.77, 71.84, 76.5, 84.2, 86.27, 86.29, 86.5, 113.1, 120.6, 120.7, 126.8, 127.7, 128.1, 129.96, 130.00, 135.7, 137.6, 144.6, 146.85, 146.87, 148.4, 148.5, 153.89, 153.99, 155.8, 158.40, 158.42; HRMS (ESI) *m/z* calcd for C₄₉H₆₃N₅O₁₃S₂Na [M + Na]⁺ 1016.3761, found 1016.3763.



Compound 1c: Synthesized using the procedure for **1a**. Compound **24** (2.455 g, 2.47 mmol, 1.0 equiv.), DCM (40 mL), diisopropylammonium tetrazolide (**16**, 633.55 mg, 3.70 mmol, 1.5 equiv.) and 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodiamidite (**15**, 1.17 mL, 3.70 mmol, 1.5 equiv.) were used. Product **1c** was purified by dissolving the sample in the solvent mixture of EtOAc/MeOH 9:1 with 5% Et₃N, loading onto a column (SiO₂), and eluting with the same solvent mixture: mixture of diastereomers; 1.81 g, 62%; pale yellow foam; TLC *R_f* = 0.4 and 0.5 (SiO₂, EtOAc/MeOH 9:1 with 5% Et₃N); ¹H NMR (500 MHz, CDCl₃) δ 1.16-1.24 (m, 12H), 1.56-2.15 (m, 6H), 2.37-3.03 (m, 8H), 3.25-3.40 (m, 2H), 3.39 (s, 3H), 3.48-3.78 (m, 20H), 3.79 (s, 6H) 3.80-3.88 (m, 1H), 3.90-3.99 (m, 1H), 4.15-4.32 (m, 1.5H), 4.50-4.54 (m, 0.5H), 4.65-4.79 (m, 1H), 5.20-5.28 (m, 1H), 6.22 (t, *J* = 6.9 Hz, 0.5H), 6.24 (t, *J* = 6.6 Hz, 0.5H), 6.71-6.82 (m, 4H), 7.15-7.35 (m, 7H), 7.35-7.46 (m, 2H), 7.80 (s, 0.5H), 7.81 (s, 0.5H); ¹³C NMR (126 MHz, CDCl₃) δ 20.12, 20.18, 20.27, 20.31, 20.37, 20.45, 20.50, 24.57, 24.60, 24.62, 24.66, 25.46, 25.47, 27.8, 28.2, 28.73, 28.74, 43.2, 43.27, 43.33, 48.45, 55.21, 55.23, 55.25, 55.27, 58.13, 58.25, 58.40, 59.0, 63.6, 70.2, 70.3, 70.4, 70.5, 70.6, 70.7, 71.9, 76.3, 76.5, 84.0, 84.5, 84.7, 85.9, 86.0, 86.3, 86.4, 86.5, 113.0, 113.1, 113.2, 113.4, 117.4, 117.5, 121.4, 121.57, 121.64, 127.0, 127.83, 127.88, 128.10, 128.15, 128.22, 129.97, 130.01, 130.06, 130.08, 13.60, 135.63, 135.67, 135.69, 135.78, 137.01, 137.02, 137.05, 144.46, 144.48, 144.51, 146.49, 146.50, 148.14, 148.21, 150.1, 151.3,

153.4, 155.7, 158.5; ^{31}P NMR (202 MHz, CDCl_3) δ 147.63, 148.22, 148.41, 148.75; HRMS (ESI) m/z calcd for $\text{C}_{58}\text{H}_{80}\text{N}_7\text{O}_{14}\text{PS}_2\text{H}$ $[\text{M} + \text{H}]^+$ 1194.5020, found 1194.5052.

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