Supplementary Material (ESI) for Chemical Communications

Ligand-Based Backbone Modifications for Metal-Chelating Nucleic Acids

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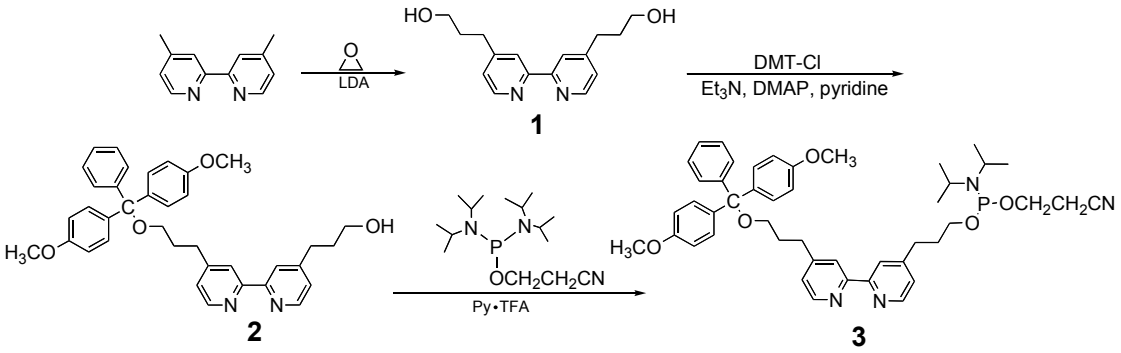
Section I. Synthesis of Protected Ligands

General Procedures

Proton and phosphorous NMR spectra were recorded on a Varian Inova 300 spectrometer. MALDI and ESI mass spectrometry were performed at Purdue University in-house facilities. All reactions were carried out under argon.

Scheme S1. Bpy synthesis



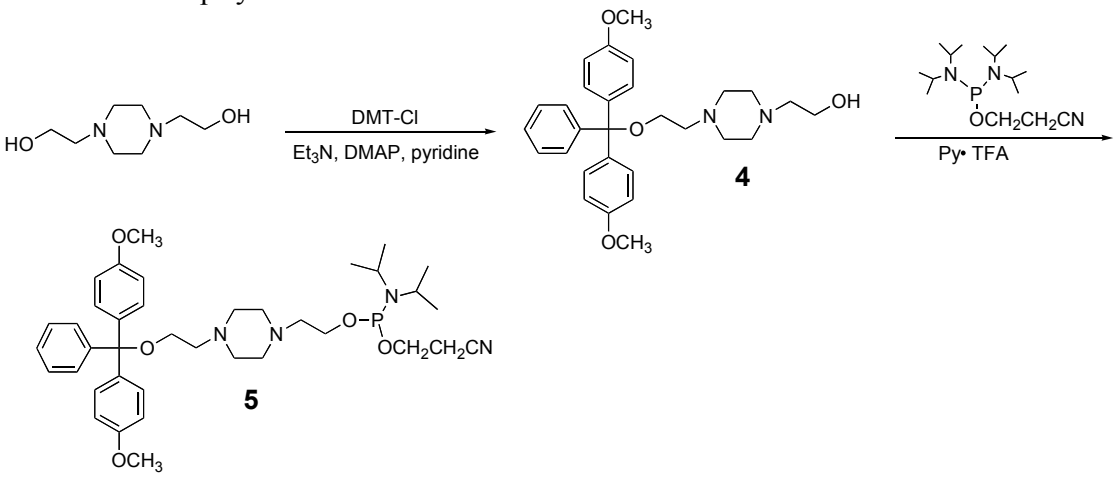
Compound 1. 4,4'-Dimethyl-2,2'-dipyridyl (12.0, 65.2 mmol) was dissolved in dry THF (250 mL) with heating. In a separate flask, diisopropylamine (21.0 mL, 0.150 mol) was dissolved in anhydrous THF (50 mL) and chilled to –80 ºC. Butyllithium (2.5 M solution in hexanes, 60.0 mL, 0.150 mol) was added slowly to the diisopropylamine solution. The reaction mixture was warmed to 0 ºC. After stirring for 20 minutes, the reaction was returned to -80 ºC. The dipyridyl solution was then added dropwise to the freshly prepared lithium diisopropylamide solution. After stirring for 2 hours at -80ºC, the reaction was warmed to 0 ºC. Ethylene oxide (81.0 mL, 1.62 mol) was added slowly. The reaction was warmed to room temperature and stirred overnight. The reaction was then quenched with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed twice with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography using a solvent gradient of 2% to 15% methanol in CH_2Cl_2. Yield 6.5 g (37%). ^{1}H NMR ($CDCl_3$, ppm): 1.92-2.01 (m, 6H), 2.79-2.84 (t, J = 13.48 Hz, 6H), 3.67-3.71 (t, J = 12.59 Hz, 6H), 7.16-7.18 (d, J = 10.4 Hz, 2H), 8.25 (s, 2H), 8.55-8.57 (d, J = 4.8 Hz, 2H). MALDI-TOF (*m/z*): $[M+H]^+$ calc'd for **1**, 273.17, found, 273.07.

Compound 2. *N,N*-Dimethylaminopyridine (DMAP) (72 mg, 0.59 mmol) and Et_3N (2.0 mL, 14 mmol) were added to a solution of **1** (2.8 g, 0.010 mol) in dry pyridine (40 mL) at room temperature. 4,4'-Dimethoxytrityl chloride (DMT-Cl) (0.73 g, 2.2 mmol) was added to the reaction mixture slowly. The reaction was stirred for 36 hours at room temperature. The solvent was removed in vacuo and the residue dissolved in CH_2Cl_2 (100 mL). The solution was poured into water and extracted with CH_2Cl_2 (3 x 50 mL). The organic layers were combined and washed twice with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography using a solvent gradient of 10% hexanes in EtOAc to 100% EtOAc to 0.5% triethylamine in 100% EtOAc. Yield 0.31 g (29%). ^{1}H NMR ($CDCl_3$, ppm): 1.88-

1.98 (m, 4H), 2.72-2.79 (m, 4H), 3.03-3.07 (t, J = 12.31 Hz, 2H), 3.62-3.66 (t, J = 12.31 Hz, 2H), 3.71 (s, 6H), 6.72-6.75 (d, J = 8.79 Hz, 4H), 6.99-7.00 (d, J = 1.76 Hz, 2H), 7.02-7.20 (m, 3H), 7.23-7.26 (d, J = 8.79 Hz, 4H), 7.35-7.37 (d, J = 7.03 Hz, 2H), 8.18 (s, 2H), 8.42-8.51 (d, J = 24.6 Hz, 2H). MALDI-TOF (*m/z*): $[M+H]^+$ calc'd for **2**, 575.29, found, 575.15.

Compound 3. (2-Cyanoethyl)-*N,N,N′,N′*-tetraisopropylphosphorodiamidite (0.26 g, 0.81 mmol) was added to a solution of **2** (0.33 g, 0.58 mmol) in anhydrous CH_2Cl_2 (20 mL). Pyridinium trifluoroacetate (0.13 g, 0.81 mmol) was co-evaporated with CH_2Cl_2 (2 x 20 mL) and added to the reaction mixture. The reaction was stirred for 3 hours at room temperature. The reaction mixture was poured into water and extracted with CH_2Cl_2 (2 x 30 mL). The organic layers were combined, washed twice with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography using a solvent gradient of 1:1 EtOAc/hexanes to 7:1 EtOAc/hexanes. Yield 0.18 g (40%), ^{1}H NMR ($CDCl_3$, ppm): 1.09-1.13 (m, 12H), 1.89-1.94 (m, 4H), 2.55-2.59 (t, J = 12.74 Hz, 2H), 2.71-2.78 (m, 4H), 3.02-3.06 (t, J = 11.86 Hz, 2H), 3.50-3.64 (m, 4H), 3.70 (s, 6H), 3.79-3.83 (m, 2H), 6.72-6.75 (d, J = 8.35 Hz, 4H), 6.99-7.01 (d, J = 4.84 Hz, 2H), 7.01-7.20 (m, 3H) 7.24-7.26 (d, J = 8.5 Hz, 4H), 7.35-7.38 (d, J = 7.91 Hz, 2H), 8.17 (s, 2H), 8.42-8.50 (d, J = 24.46 Hz, 2H). ^{31}P NMR ($CDCl_3$, ppm): 148.05. ESI (*m/z*): $[M+H]^+$ calc'd for **3**, 775.40, found, 774.96.

Scheme S2. Pip synthesis

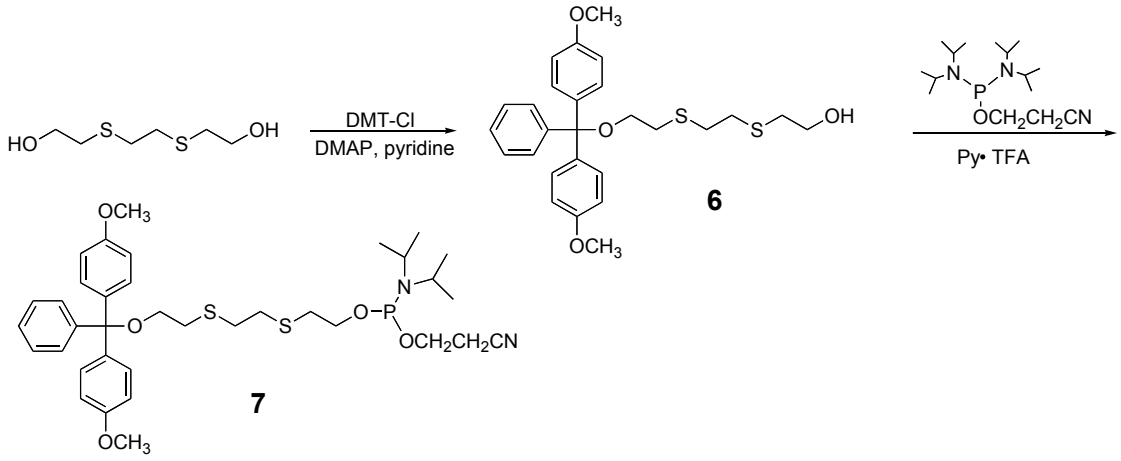


Compound 4. *N,N′*-Bis(hydroxyethyl)piperazine (12.3 g, 70.5 mmol), Et_3N (13.7 mL, 98.7 mmol), and DMAP (0.4 g, 4 mmol) were dissolved in dry pyridine (150 mL). To this reaction mixture, DMT-Cl (11.9 g, 35.3 mmol) was slowly added. This reaction was stirred overnight at room temperature. The reaction mixture was then poured into aqueous 5% $NaHCO_3$ and extracted with CH_2Cl_2 (2 x 100 mL). The organic layers were combined and washed with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. This crude product was purified by flash chromatography using a solvent gradient from 100% CH_2Cl_2 to 5% methanol in CH_2Cl_2. Yield 2.1 g (13%). ^{1}H NMR ($CDCl_3$, ppm): 2.65 (s, 8H), 2.70-2.74 (t, J = 12.01 Hz, 4H), 3.28-3.32 (t, J = 11.86 Hz, 2H), 3.68-3.72 (t, J = 10.54 Hz, 2H), 3.86 (s, 6H), 6.88-6.91 (d, J = 8.79 Hz, 4H), 7.27-

7.35 (m, 3H), 7.39-7.42 (d, J = 8.79 Hz, 4H), 7.50-7.53 (d, J = 7.18 Hz, 2H). MALDI-TOF (*m/z*): $[M+Na]^+$ calc'd for **4**, 499.26, found, 499.09.

Compound 5. To a mixture of (2-cyanoethyl)-*N,N*-diisopropylchlorophosphoramidite (0.63 mL, 2.82 mmol)) and **4** (1.08 g, 2.26 mmol) in anhydrous CH_2Cl_2 (15 mL) was added diisopropylethylamine (1.97 mL, 11.3 mmol). The reaction was stirred at room temperature for 75 minutes. The reaction mixture was then poured into aqueous 5% $NaHCO_3$ and extracted with CH_2Cl_2 (2 x 25 mL). The organic layers were then combined and washed twice with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography in 8:1 EtOAc/hexanes with 0.5% Et_3N. Yield, 0.23 g (13%). ^1H NMR ($CDCl_3$, ppm): 1.12-1.23 (m, 12H), 2.47-2.59 (m, 14H), 3.14-3.16 (t, J = 13.33 Hz, 2H), 3.31-3.36 (m, 2H), 3.72 (s, 6H), 3.91-4.12 (m, 4H), 6.73-6.76 (d, J = 8.79 Hz, 4H), 7.12-7.21 (m, 3H), 7.24-7.27 (d, J = 8.79 Hz, 4H), 7.35-7.38 (d, J = 7.03 Hz, 2H). ^{31}P NMR ($CDCl_3$, ppm): 147.97. ESI (*m/z*): $[M+H]^+$ calc'd for **5**, 677.38, found, 676.85.

Scheme S3. Dithio synthesis



Compound 6. 3,6-Dithia-1,8-octanediol (25.6 g, 0.140 mol) and DMAP (0.9 g, 7.0 mmol) were dissolved in dry pyridine (200 mL). DMT-Cl (9.5 g, 28 mmol) was added slowly and the reaction stirred at room temperature for 60 hours. The reaction mixture was poured into aqueous 5% $NaHCO_3$ and extracted with CH_2Cl_2 (2 x 125 mL). The organic layers were combined, washed twice with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography with a gradient of solvent from 100% CH_2Cl_2 to 5% methanol in CH_2Cl_2. Yield 3.1 g (23%). ^1H NMR ($CDCl_3$, ppm): 2.63 (s, 4H), 2.70-2.74 (t, J = 12.01 Hz, 2H), 3.27-3.31 (t, J = 11.87 Hz, 2H), 3.67-3.71 (t, J = 10.4 Hz, 2H), 3.86 (s, 6H), 6.88-6.91 (d, J = 8.79 Hz, 4H), 7.27-7.35 (m, 3H), 7.39-7.42 (d, J = 8.79 Hz, 4H), 7.50-7.53 (d, J = 7.77 Hz, 2H). MALDI-TOF (*m/z*): $[M+Na]^+$ calc'd for **6**, 507.16, found, 507.07.

Compound 7. To a mixture of (2-cyanoethyl)-*N,N,N′,N′*-tetraisopropyl-phosphorodiamidite (0.85 mL, 2.7 mmol) and **6** (1.03 g, 2.14 mmol) in anhydrous CH_2Cl_2 was added pyridinium trifluoroacetate (0.56 g, 2.9 mmol). The reaction was stirred for 3 hours at room temperature. The reaction mixture was then poured into aqueous 5% $NaHCO_3$ and extracted with CH_2Cl_2 (2 x 30 mL). The organic layers were combined and washed twice with saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography in 1:8 EtOAc/hexanes with 0.25% Et_3N. Yield, 0.21 g (14%). ^{1}H NMR ($CDCl_3$): 1.25-1.28 (m, 12H), 2.68-2.72 (t, J = 12.9 Hz, 2H), 2.75-2.84 (m, 8H), 3.34-3.39 (t, J = 13.48, 2H), 3.64-3.72 (m, 2H), 3.87 (s, 6H), 3.91-3.96 (m, 4H), 6.90-6.92 (d, J = 8.79 Hz, 4H), 7.29-7.37 (m, 3H), 7.40-7.43 (d, J = 8.79 Hz, 4H), 7.52-7.54 (d, J = 7.62 Hz, 2H). ^{31}P NMR ($CDCl_3$, ppm): 147.99. ESI (*m/z*): $[M+Na]^+$ calc'd for **7**, 707.27, found, 707.00.

Section II. Oligonucleotide Synthesis

All oligonucleotides were synthesized and purified by reversed phase high performance liquid chromatography (RP-HPLC) at Midland Certified Reagent Company, Inc. Table S1 shows mass spectral data for the six oligonucleotides synthesized.

Table S1. Mass spectral data for oligos

	single nucleotide replacement		triple nucleotide replacement	
compound	calc'd	found	calc'd	found
dithioether	6987.49	6985.42	6847.07	6848.97
piperazine	6979.58	6976.84	6823.35	6823.52
bipyridine	7077.50	7078.18	7117.30	7168.11[a]

[a]mass corresponds to M + 2 Na^+

Section III. UV-Vis Melting Curves

All melting curves were performed on a Cary 100 UV-Vis equipped with a Cary peltier thermostattable multi-cell holder and a Cary temperature controller. The samples were prepared at 1 μM per oligo concentration in 1.0 mL of 50 mM tris buffer with 50 mM NaCl (pH = 7.4). All samples were treated for 3 hours with Chelex resin to remove trace metals. For melting curves with added metals, the concentration of the metal was 1 μM for the single-nucleotide replacement oligo melting curves, and 3 μM for the three-nucleotide replacement oligo melting curves. The Pt^{2+} was generated from $PtCl_4^{2-}$ treated with 3.8 equiv $AgNO_3$, Pt^{4+} was from $PtCl_6^{2-}$ treated with 5.8 equiv $AgNO_3$, Pd^{4+} was from $PdCl_6^{2-}$ treated with 5.8 equiv $AgNO_3$, Os^{4+} was from $OsCl_6^{2-}$ treated with 5.8 equiv $AgNO_3$, and Cu^{2+} was from $Cu(NO_3)_2$. All samples were degassed with a stream of helium and topped with a layer of silicon oil prior to data collection. The absorbance was monitored at 260 nm as a function of temperature from 85 ˚C to 5 ˚C followed by reheating to 85 ˚C at 0.5 ˚C per minute. Melting temperatures were determined from the 85 ˚C to 5 ˚C curve.