Supporting Information

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Synthesis and Characterization of Oligonucleotides Containing the C4'-Oxidized Abasic Site Produced by Bleomycin and Other DNA Damaging Agents

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Contents:
1. Description of oligonucleotide synthesis, deprotection, purification, quantitation.
2. Description of oligonucleotide photolysis procedure.
3. Description of UV-melting experiments, representative melt of 17b, Table of UV-melting temperatures, HPLC traces of 14b before and after 3 melting/annealing cycles (Supporting Information Figures 1, 2; Supporting Information Table 1).
4. Mass spectra (ESI; MALDI-TOF) of 14-16 (Supporting Information Figures 3-8).

3-(tert-Butyl-diphenyl-silanyloxy)-2-(tert-butyl-diphenyl-silanyloxymethyl)-5-methoxy-tetrahydrofuran (8)

To a solution of 2-deoxy-D-ribose (4.02 g, 30.0 mmol) in MeOH (70 mL) under an Ar atmosphere was added a solution of Me₂SiCl₂ (53 mg, 0.45 mmol) in MeOH (4.0 mL) at room temperature. After 30 min, the reaction mixture was quenched with Ag₂CO₃ (2.0 g) and then filtered through celite pad washed with methanol. The filtrate was concentrated and dissolved in DMF (60 mL). TBDSCl (16.49 g, 60 mmol) and imidazole (10.21 g, 0.15 mol) were added successively at room temperature. After stirring overnight, the solution was poured into ether, washed with NaHCO₃ (sat), washed with H₂O, brine, and dried over Na₂SO₄. The residue was purified by chromatography (10 % EtOAc in hexanes) to give an anomeric mixture of 3-(tert-butyl-diphenyl-silanyloxy)-2-(tert-butyl-diphenyl-silanyloxymethyl)-5-methoxy tetrahydrofuran.
8 (17.54 g, 93 % for 2 steps): \( R_f \) 0.48 (1:9 EtOAc/Hexanes); IR (film) 3071, 2931, 1472, 1589, 1112, 822 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \( \delta \) 7.74-7.64 (m, 16H), 7.50-7.28 (m, 24H), 5.20 (dd, \( J = 3.3, 5.7 \) Hz, 1H), 5.07 (dd, \( J = 2.7, 5.4 \) Hz, 1H), 4.49-4.39 (m, 2H), 4.26-4.17 (m, 2H), 3.72 (dd, \( J = 2.7, 11.1 \) Hz, 1H), 3.58 (dd, \( J = 5.4 \) Hz, 2H), 3.50 (s, 3H), 3.46 (dd, \( J = 4.2, 11.1 \) Hz, 1H), 3.34 (s, 3H), 2.23–1.88 (m, 4H), 1.13 (s, 18H), 1.08 (s, 9H), 1.05 (s, 9H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 13.59, 135.8, 135.82, 135.7, 135.6, 133.9, 133.8, 133.6, 133.5, 129.8, 129.7, 129.6, 127.7, 105.6, 105.1, 87.4, 85.9, 73.9, 73.1, 65.2, 64.1, 55.4, 55.0, 42.2, 41.9, 27.2, 27.0, 19.5, 19.4; HRMS (FAB) M + H\(^+\) calc. for C\(_{38}\)H\(_{47}\)O\(_4\)Si\(_2\) 623.3013, found 623.3012.

1,3-Bis-(tert-butyl-diphenyl-silanyloxy)-4-[1,3]dithian-2-yl-butan-2-ol (9)

![Chemical Structure](image)

To a -10 ~ -15 °C solution of 3-(tert-butyl-diphenyl-silanyloxy)-2-(tert-butyl-diphenyl-silanyloxy methyl)-5-methoxy-tetrahydrofuran 8 (9.23 g, 14.8 mmol) in CH\(_2\)Cl\(_2\) (80 mL) under an Ar atmosphere was added 1,3-propanedithiol (1.92 g, 17.7 mmol) and then BF\(_3\)-Et\(_2\)O (4.48 g, 31.6 mmol) over 5 min. After 1.5 h, the reaction mixture was quenched with NaHCO\(_3\) (sat), extracted with ether, washed with H\(_2\)O, brine, and dried over Na\(_2\)SO\(_4\). The residue was purified by chromatography (10 % EtOAc in hexanes) to give a 1,3-bis-(tert-butyl-diphenyl-silanyloxy)-4-[1,3]dithian-2-yl-butan-2-ol 9 (9.22 g, 89 %): \( R_f \) 0.28 (1:9 EtOAc/Hexanes); IR (film) 3571, 3071, 2931, 1585, 1427, 1113, 822 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \( \delta \) 7.72-7.56 (m, 8H), 7.44-7.35 (m, 12H), 4.20-4.15 (m, 1H), 3.98 (dd, \( J = 4.2, 9.3 \) Hz, 1H), 3.79 – 3.76 (m, 1H), 3.64 (dd, \( J = 5.7, 7.2 \) Hz, 1H), 3.52 (dd, \( J = 9.9, 7.2 \) Hz, 1H), 2.68-2.57 (m, 2H), 2.46-2.38 (m, 2H), 2.07-1.74 (m,
4H), 1.05 (s, 9H), 1.02 (s, 9H); $^{13}$C NMR (CDCl$_3$) $\delta$ 136.1, 135.6, 133.3, 133.1, 129.8, 129.7, 127.7, 127.6, 74.6, 71.5, 64.7, 43.9, 38.3, 30.4, 29.8, 27.3, 27.1, 26.0, 19.8, 19.4; HRMS (FAB) M + H$^+$ calc. for C$_{40}$H$_{51}$O$_3$Si$_2$S$_2$ 699.2818, found 699.2823.

1,3-Bis-(tert-butyl-diphenyl-silanyloxy)-4-[1,3]dithian-2-yl-butan-2-one (10)

To a -78 °C solution of DMSO (0.5 mL, 7.05 mmol) in CH$_2$Cl$_2$ (20 mL) under an Ar atmosphere was added an oxalyl chloride (0.47 g, 3.67 mmol). After 1.5 h, a solution of 1,3-bis-(tert-butyl-diphenyl-silanyloxy)-4-[1,3]dithian-2-yl-butan-2-ol 9 (2.05 g, 2.92 mmol) in CH$_2$Cl$_2$ (50 mL) was added to the reaction mixture. After 1.5 h TEA (1.48 g, 14.6 mmol) was added and the solution was allowed to stir for 1.5 h at -78 °C. The solution was warmed to 25 °C and NH$_4$Cl (sat, 50 mL) was added and stirring for 15 min. The layers were separated and the aqueous phase was extracted with ether (3 x 40 mL). The combined organic layers were washed with 50 mL of saturated NaCl solution, and dried with Na$_2$SO$_4$. The solution was then concentrated on a rotary evaporator. The crude compound was purified by column chromatography using a gradient of 5%-10% EtOAc in Hexanes to afford 1,3-bis-(tert-butyl-diphenyl-silanyloxy)-4-[1,3]dithian-2-yl-butan-2-one 10 (1.63 g, 79 %) as an oil: $R_f$ 0.56 (1:4 EtOAc/ Hexanes); IR (film) 3084, 2931, 1735, 1428, 1113, 823 cm$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 7.68-7.57 (m, 8H), 7.46-7.28 (m, 12H), 4.64-4.58 (m, 2H), 4.35 (d, $J = 18.3$ Hz, 1H), 3.94 (t, $J = 6.9$ Hz, 1H), 2.73-2.52 (m, 4H), 2.24-2.17 (m, 1H), 2.05-1.96 (m, 2H), 1.85-1.82 (m, 1H), 1.09 (s, 9H), 1.04 (s, 9H); $^{13}$C NMR (CDCl$_3$) $\delta$ 208.2, 135.9, 135.6, 133.0, 132.4, 130.1, 129.9, 127.8,
74.7, 68.3, 41.7, 40.1, 29.1, 29.0, 27.2, 27.0, 25.8, 19.6, 19.5; HRMS (FAB) M + Na⁺ calc. 
C₄₀H₅₀O₃NaSi₂S₂ 721.2638, found 721.2630.

3-(tert-Butyl-diphenyl-silanyloxy)-2-(tert-butyl-diphenyl-silanyloxymethyl)-5-(4,5-
dimethoxy-2-nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxymethyl)-tetrahydrofuran
(11a,b)

To a -10 °C solution of 1,3-bis-(tert-butyl-diphenyl-silanyloxy)-4-[1,3]dithian-2-yl-butan-
2-one 10 (1.80 g, 2.57 mmol) and nitrobenzylalcohol (2.47 g, 11.6 mmol) in a mixture of 
CH₃CN/CH₂Cl₂ (2:1, 72 mL) was added a NBS (2.52 g, 14.2 mmol). After 1 h, the solution was 
warmed to 25 °C and quenched with NaSO₃ (sat, 20 mL). The layers were separated and the 
aqueous phase was extracted with ether (3 x 50 mL), washed with NaHCO₃ (sat, 20 mL), and 
NaCl (sat, 20 mL). The combined organic layers were dried with Na₂SO₄. The crude compound 
was purified by column chromatography using a gradient of 5%-50% Et₂O in Hexanes to afford 
3-(tert-butyl-diphenyl-silanyloxy)-2-(tert-butyl-diphenyl-silanyloxymethyl)-5-(4,5-dimethoxy-2-
nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxymethyl)-tetrahydrofuran 11 (1.44 g, 56%) as 
a mixture of isomers (foam). Analytical samples of the two major diastereomers were obtained 
by partial purification of the mixture.
11a (Higher $R_f$): $R_f$ 0.15 (1:4 EtOAc/Hexanes); IR (KBr) 2934, 1522, 1327, 1276, 1167, 1113, 1069, 703 cm$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 7.72-7.28 (m, 23H), 7.01 (s, 1H), 5.21 (d, $J = 0.8$ Hz, 2H), 5.05 (dd, $J = 4.8$, 6.0 Hz, 1H), 4.83 (dd, $J = 15.2$, 28.4 Hz, 2H), 4.68 (t, $J = 8.0$ Hz, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.73 (t, $J = 12.0$ Hz 2H), 3.71 (s, 3H), 2.31-2.24 (m, 1H), 2.16-2.10 (m, 1H), 1.01 (s, 9H), 0.89 (s, 9H); $^{13}$C NMR (CDCl$_3$) $\delta$ 153.53, 153.48, 147.5, 146.9, 138.9, 138.1, 135.69, 135.61, 133.4, 133.2, 133.1, 132.7, 132.1, 129.9, 129.88, 129.76, 129.67 127.7, 127.64, 127.6, 109.5, 109.4, 107.8, 107.5, 106.9, 102.4, 72.2, 67.4, 64.4, 62.9, 56.28, 56.18, 56.14, 56., 40.0, 26.9, 26.6, 19.2, 19.1; HRMS (FAB) M + Na$^+$ calc. C$_{55}$H$_{64}$N$_2$O$_{13}$NaSi$_2$ 1039.3839, found 1039.3864.

11b (Lower $R_f$): $R_f$ 0.13 (1:4 EtOAc/Hexanes); IR (KBr) 2934, 1520, 1456, 1348, 1276, 1113, 1069, 703 cm$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 7.75-7.67 (m, 7H), 7.60 (s, 1H), 7.49 (s, 1H), 7.42-7.28 (m, 2H), 7.09 (s, 1H), 6.81 (s, 1H), 5.30 (dd, $J = 2.8$, 5.6 Hz, 1H), 4.79 (d, $J = 2.4$ Hz, 2H),4.66 (d, $J = 16.4$ Hz, 2H), 4.28 (d, $J = 10.8$ Hz, 1H), 3.99 (t, $J = 11.6$ Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.68 (s, 3H), 3.66 (s, 3H), 2.37-2.32 (m, 1H), 2.08-1.98 (m, 1H), 1.10 (s, 9H), 1.05 (s, 9H); $^{13}$C NMR (CDCl$_3$) $\delta$ 153.33, 153.30, 147.5, 147.0, 139.0, 138.3, 135.82, 135.76, 133.8, 133.2, 133.1, 132.7, 132.1, 129.9, 129.8, 129.7, 129.4, 127.7, 127.61, 127.60, 110.6, 109.5, 109.3, 107.8, 107.2, 103.5, 75.6, 67.0, 63.9, 61.1, 56.27, 56.20, 56.1, 56.0, 40.3, 26.9, 26.8, 19.3, 19.2; HRMS (FAB) M + Na$^+$ calc. C$_{55}$H$_{64}$N$_2$O$_{13}$NaSi$_2$ 1039.3839, found 1039.3852
5-(4,5-Dimethoxy-2-nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxy-methyl)-2-hydroxymethyl-tetrahydrofuran-3-ol (12a,b)

To a solution of 3-(tert-butyl-diphenyl-silanyloxy)-2-(tert-butyl-diphenyl-silanyloxy-methyl)-5-(4,5-dimethoxy-2-nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxy-methyl)-tetrahydrofuran 11 (463 mg, 0.455 mmol) in THF (8.0 mL) under an Ar atmosphere was added an 1.0 M solution of TBAF in THF (2.50 mL, 2.50 mmol) at 25 °C. After 3 h, the solution was then concentrated on a rotary evaporator. The crude compound was purified by column chromatography using a gradient of 0%-2% MeOH in CH₂Cl₂ to afford 5-(4,5-dimethoxy-2-nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxy-methyl)-2-hydroxymethyl-tetrahydro-furan-3-ol 12 (186 mg, 76 %) as a mixture of diastereomers (foam): Analytical samples of the two major diastereomers were prepared from single isomers, 11a and 11b.

12a: R₇ 0.49 (10 % MeOH/CH₂Cl₂); IR (KBr) 3524, 2933, 1522, 1328, 1276, 1221, 1113, 796, 703 cm⁻¹; ¹H NMR (CDCl₃) δ 7.58 (s, 1H), 7.50 (s, 1H), 7.20 (s, 1H), 6.88 (s, 1H), 5.30 (t, J = 4.4 Hz, 1H), 5.03 (s, 2H), 4.95 (dd, J = 14.0, 20.8 Hz, 2H), 4.41 (t, J = 8.0 Hz, 1H), 3.91 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.83-3.71 (m, 2H), 3.81 (s, 3H), 2.72-2.65 (m, 1H), 2.13-2.07 (m, 1H); ¹³C NMR (CDCl₃) δ 153.24, 153.17, 147.7, 147.3, 139.4, 130.0, 110.0, 109.8, 107.8, 107.4, 104.8, 103.1, 72.8, 67.6, 61.7, 61.4, 56.21, 56.18, 56.12, 39.3; HRMS (FAB) M + Na⁺ calc. C₂₃H₂₈N₂O₁₃Na 563.1489, found 563.1481.
12b: Rf 0.49 (10% MeOH/CH2Cl2); IR (KBr) 3524, 2941, 1618, 1524, 1456, 1328, 1274, 1221, 1106, 1067, 796, 701 cm⁻¹; ¹H NMR (CDCl3) δ 7.60 (s, 1H), 7.52 (s, 1H), 7.15 (s, 1H), 6.89 (s, 1H), 5.57 (dd, J = 4.0, 5.6 Hz, 1H), 4.92-4.82 (m, 4H), 4.58 (dd, J = 2.8, 6.0 Hz, 1H), 4.04-3.95 (m, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H), 3.82 (s, 3H), 2.49-2.43 (m, 1H), 2.34-2.29 (m, 1H); ¹³C NMR (CDCl3) δ 153.3, 153.2, 147.7, 147.4, 139.5, 139.0, 130.1, 128.6, 110.1, 110.0, 109.9, 107.9, 107.5, 105.5, 76.6, 67.7, 61.0, 60.7, 56.3, 56.2, 56.1, 40.5; HRMS (FAB) M + Na⁺ calc. C23H28N2O13Na 563.1489, found 563.1498.

5-(4,5-Dimethoxy-2-nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxymethyl)-2-silyloxymethyl-tetrahydrofuran-3-ol (13)

To a 0 °C solution of 5-(4,5-dimethoxy-2-nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxymethyl)-2-hydroxymethyl-tetrahydro-furan-3-ol 12 (153 mg, 0.283 mmol) and diisopropylethylamine (0.11 g, 0.86 mmol) in CH₂Cl₂ (3 mL) under an Ar atmosphere was added a solution of silylchloride (682 mg, 1.53 mmol) and diisopropylethylamine (0.24 g, 1.86 mmol) in CH₂Cl₂ (2 mL) in 0.5 eq. portion (0.2 mL) every hour. After 7 h, the solution was warmed to 25 °C and quenched with NaHCO₃ (sat, 2.0 mL). The layers were separated and the aqueous phase was extracted with CH₂Cl₂ (3 x 5.0 mL), washed with H₂O (3.0 mL), and NaCl (sat, 2.0 mL). The combined organic layers were dried with Na₂SO₄. The solution was then concentrated on a rotary evaporator. The crude compound was purified by column
chromatography using a gradient of 5%-30% EtOAc in Hexanes (containing 1 % TEA) to afford
5-(4,5-dimethoxy-2-nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxy)methyl)-2-
silyloxyethyl-tetrahydro-furan-3-ol 13 (13a, 37 mg, 14 %; 13b, 67 mg; 25 %) as oils:

13a: Rf 0.15 (1:2 EtOAc/ Hexanes); IR (CHCl₃) 3506, 2930, 1522, 1328, 1276, 1071,
845 cm⁻¹; ¹H NMR (CDCl₃) δ 7.60 (s, 1H), 7.56 (s, 1H), 7.36 (s, 1H), 6.89 (s, 1H), 5.30 (dd, J =
4.0, 5.6 Hz, 1H), 5.10 (d, J = 16.0 Hz, 1H), 5.06 (d, J = 16.0 Hz, 1H), 4.97 (d, J = 16 Hz, 1H),
4.90 (d, J = 16 Hz, 1H), 4.40 (t, J = 6.8 Hz, 1H), 4.01 (d, J = 10.8 Hz , 1H), 4.00-3.92 (m, 1H),
3.90 (s, 3H), 3.88 (s, 3H), 3.79 (s, 3H), 3.77 (d, J = 11.2 Hz , 1H ), 2.90 (br s, 1H), 2.70-2.62 (m,
1H), 2.19-2.13 (m, 1H), 1.62-1.59 (m, 2H), 1.43-1.25 (m, 20H), 0.13 (s, 9H), 0.12 (s, 9H); ¹³C
NMR (CDCl₃) δ 153.3, 153.2, 147.6, 147.2, 139.5, 138.8, 131.0, 128.9, 110.0, 109.8, 107.9,
107.4, 104.9, 103.0, 73.3, 70.9, 67.4, 62.7, 61.1, 56.3, 56.2, 56.1, 39.2, 31.93 31.91, 24.4, 24.0,
23.2, 23.1, 20.8, 1.54, 1.52; HRMS (FAB) M + Na⁺ calc. C₄₁H₆₈N₂O₁₆NaSi₃ 951.3774, found
951.3820.

13b: Rf 0.20 (1:2 EtOAc/ Hexanes); IR (film) 3498, 2933, 1581, 1522, 1466, 1327, 1277,
1221, 1070, 845 cm⁻¹; ¹H NMR (CDCl₃) δ 7.58 (s, 1H), 7.54 (s, 1H), 7.33 (s, 1H), 6.82 (s, 1H),
5.58 (t, J = 5.2 Hz, 1H), 5.01 (d, J = 16 Hz, 1H), 4.94 (d, J = 15.6 Hz, 1H), 4.93 (s, 2H), 4.45 (t,
J = 2.8 Hz, 1H), 4.07 (d, J = 12.0 Hz, 1H), 4.03-3.99 (m, 1H), 3.98 (d, J = 11.6 Hz, 1H), 3.92 (s,
3H), 3.90 (s, 3H), 3.89 (s, 3H), 3.74 (s, 3H), 2.43-2.31 (m, 2H), 1.65-1.64 (m, 2H), 1.45-1.24 (m,
1H), 0.14 (s, 9H), 0.10 (s, 9H); ¹³C NMR (CDCl₃) δ 153.3, 153.0, 147.6, 147.0, 139.6, 138.6,
131.3, 128.8, 110.6, 110.0, 109.7, 107.8, 107.2, 105.7, 75.4, 71.4, 67.6, 60.2, 59.4, 56.22, 56.14,
56.0, 39.6, 31.8, 31.7, 24.2, 23.8, 23.3, 23.2, 23.1, 20.8, 20.7, 1.51, 1.43; HRMS (FAB) M + Na⁺
calc. C₄₁H₆₈N₂O₁₆NaSi₃ 951.3774, found 951.3774.
To a 0 °C solution of 5-(4,5-dimethoxy-2-nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxymethyl)-2-silyloxyethyl-tetrahydro-furan-3-ol (13b, 60 mg, 65 µmol) and diisopropylethylamine (41 mg, 0.32mmol) in CH₂Cl₂ (1.5 mL) under an Ar atmosphere was added bis(N,N-diisopropylamine)chloromethoxyphosphine (26 mg, 0.13 mmol). After 30 min, the solution was warmed to 25 °C, stirred overnight, and quenched with NaHCO₃ (sat, 0.5 mL). The layers were separated and the aqueous phase was extracted with CH₂Cl₂ (3 x 2.0 mL), washed with H₂O (1.0 mL), and NaCl (sat, 0.5 mL). The combined organic layers were dried with Na₂SO₄. The crude compound was purified by silica gel (oven dried) column chromatography using a gradient of 5%-10% EtOAc in Hexanes (containing 1 % TEA) to afford an inseparable mixture of two diastereomers 5-(4,5-dimethoxy-2-nitro-benzyloxy)-2-(4,5-dimethoxy-2-nitro-phenoxymethyl)-2-silyloxyethyl-tetrahydro-furan (6a,b) (53 mg, 75 %) as oil:

6b (a mixture of two diastereomers): Rf 0.36 (1:1:4 EtOAc/Ether/Hexanes); IR (film) 2933, 1582, 1521, 1464, 1328, 1277, 1221, 1069, 846 cm⁻¹; ¹H NMR (CDCl₃) δ 7.61 (s, 1H), 7.60 (s, 1H), 7.52 (s, 2H), 7.37 (s, 2H), 6.86 (s, 1H), 6.84 (s, 1H), 5.30 (dd, J = 4.8, 6.0 Hz, 2H), 5.03 (s, 2H), 5.01 (s, 2H), 4.94 (dd, J = 14.4, 22.8 Hz, 4H), 4.64-4.53 (m, 2H), 4.11-3.97 (m,
4H), 3.93 (s, 14H), 3.90 (s, 6H), 3.74 (s, 3H), 3.73 (s, 3H), 3.64-3.57 (m, 4H), 3.44 (d, \( J_P = 12.8 \) Hz, 3H), 3.38 (d, \( J_P = 13.2 \) Hz, 3H), 2.59-2.46 (m, 2H), 2.38-2.32 (m, 2H), 1.53-1.46 (m, 4H), 1.33-1.13 (m, 64H), 0.093 (s, 9H), 0.090 (s, 9H), 0.084 (s, 9H), 0.082 (s, 9H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 153.3, 153.1, 147.5, 146.9, 139.4, 138.5, 132.1, 131.9, 129.2, 129.0, 110.23, 110.16, 109.94, 109.9, 109.8, 107.9, 107.2, 105.0, 104.8, 76.2, 76.0, 70.77, 70.73, 67.61, 67.56, 60.6, 60.5, 60.2, 56.25, 56.21, 56.15, 56.0, 50.8, 50.1, 43.03, 42.97, 42.91, 42.85, 39.7, 31.9, 31.5, 24.79, 24.75, 24.71, 24.64, 24.58, 24.50, 24.42, 24.38, 24.30, 23.0, 22.97, 20.46, 20.40, 1.58, 1.57; \(^{31}\)P NMR (CDCl\(_3\)) \( \delta \) 150.2, 148.9; HRMS (FAB) \( M + Na^+ \) calc. C\(_{48}H_{84}N_3O_{17}NaSi_3P \) 1112.4744, found 1112.4727.

6a was prepared from 13a in a similar manner to synthesis of 6b.

6a (a mixture of two diastereomers 56 mg, 80%): \( R_f \) 0.36 (1:1:4 EtOAc/Ether/Hexanes); IR (film) 2937, 1581, 1522, 1466, 1327, 1277, 1226, 1069, 845 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)) \( \delta \) 7.64 (s, 1H), 7.63 (s, 1H), 7.54 (s, 1H), 7.50 (s, 1H), 7.49 (s, 1H), 7.46 (s, 1H), 7.05 (s, 1H), 6.98 (s, 1H), 5.30 (t, \( J = 5.6 \) Hz, 2H), 5.23-5.12 (m, 4H), 5.01-4.82 (m, 4H), 4.70-4.57 (m, 2H), 4.01-3.97 (m, 4H), 3.95 (s, 3H), 3.93 (s, 9H), 3.89 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 3.81-3.78 (m, 2H), 3.62-3.56 (m, 4H), 3.41 (d, \( J_P = 10.8 \) Hz, 3H), 3.38 (d, \( J_P = 10.8 \) Hz, 3H), 2.75-2.62 (m, 2H), 2.35-2.24 (m, 2H), 1.66-1.63 (m, 4H), 1.45-1.25 (m, 40H), 1.18-1.14 (m, 24H), 0.15 (s, 18H), 0.14 (s, 18H); \(^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 153.47, 153.43, 153.40, 147.5, 147.4, 146.9, 146.89, 139.8, 139.0, 138.28, 138.25, 132.8, 130.9, 129.6, 128.8, 109.8, 109.7, 109.4, 107.79, 107.74, 107.18, 107.14, 106.6, 102.64, 102.57, 71.3, 71.26, 71.2, 71.1, 68.1, 67.47, 67.42, 63.0, 62.8, 62.1, 61.9, 56.3, 56.2, 50.7, 50.5, 50.4, 50.2, 42.94, 42.87, 42.82, 42.7, 38.8, 38.7, 32.0, 30.3, 28.9, 24.71, 24.64, 24.55, 24.49, 24.1, 23.7, 23.3, 23.2, 23.0, 20.8, 14.0,
10.9, 1.61; $^{31}$P NMR (CDCl$_3$) $\delta$ 149.6, 149.4; HRMS (FAB) M + Na$^+$ calc. C$_{48}$H$_{84}$N$_3$O$_{17}$NaSi$_3$P 1112.4744, found 1112.4742.

**Oligonucleotide synthesis, deprotection, and purification.** Standard cycles (ABI 394) were used for synthesizing oligonucleotides until the point in the sequence where 6 is incorporated was reached. All silylated nucleosides (obtained from Dharmacon Research, Inc., Co) were incorporated using cycles identical to those described by Scaringe (*JACS* 1998, 120, 11820.) with two exceptions. Phosphoramidite coupling times were increased to 120 sec. Also, desilylations were carried out using Et$_3$N•HF solutions prepared in anhydrous DMF by combining Et$_3$N•3HF and Et$_3$N. Phosphoramidite 6 was coupled using a 5 min wait time.

Deprotections were carried out as described by Scaringe, except that demethylation was effected using Na$_2$S$_2$ (0.25 M, 45 min) in DMF/H$_2$O (9:1). Demethylation was followed by conc. aqueous ammonia (10 h, 55 °C). Oligonucleotides containing 7 were purified by 20% denaturing PAGE.

**General procedure of determination of concentration of oligonucleotides (14a,b-16a,b).** Extinction coefficients were calculated using the nearest neighbor analysis. The oligonucleotides on the 5'- and 3'-sides of 1 and 7 were calculated independently and summed. The independently measured extinction coefficient for 12 ($\varepsilon_{260} = 5.3 \times 10^3$ M$^{-1}$cm$^{-1}$) was added to this value to determine the contribution of 7 in 14a-16a. The extinction coefficients (10$^5$ M$^{-1}$cm$^{-1}$) are 1.14 , 1.38, and 2.61 for 14a–16a, respectively. The extinction coefficients (10$^5$ M$^{-1}$cm$^{-1}$) are 1.09 , 1.33, and 2.56 for 14b–16b, respectively.
General procedure of precipitation of oligonucleotides (14a-16a) for ESI-MS or MALDI-TOF MS analysis. From a stock solution, 4-6 nmol of sample was removed and diluted to 150 µL. The solution was treated with 5.0 M solution of NH₄OAc (pH=5.6, 50 µL) and let it sit for 12 min at RT. Absolute EtOH (600 µL) was added to the solution and frozen at -78 ºC for over 20 min. The sample was centrifuged at 4 ºC for 12 min, the supernatant was removed, and the sample dried in a speed-vac. The dried solid was dissolved in H₂O (55 µL) and an aliquot removed to determine its concentration by UV absorption.

Photochemical generation of 1. Oligonucleotides containing 7 (e.g. 14a) were dissolved in H₂O (1 nmol/50 µL; smaller quantities were photolyzed in 50 µL), purged with Ar (20 min), and sealed with a rubber septum covered tightly with parafilm. The samples were photolyzed in Pyrex tubes (5 mm i.d.) inside a Rayonet photoreactor (λ_max = 350 nm, 16 lamps, 4.2 mW/cm² @ 360 nm) for ≤ 20 min. Photolysates were analyzed by HPLC on a Microsorb C-18 column (4.6 × 25 mm). Solvent A: 50 mM ammonium carbonate (pH 6.2); Solvent B: 50 mM ammonium carbonate (pH 6.2) + CH₃CN (50%). Gradient: Initial, 90% A for 10 min, then linear to 40% B at 50 min. Detection was carried out at 260 and 354 nm.

UV-Melting experiments and HPLC analysis following 3 annealing/melting cycles. Samples of 17b (total volume = 300 µL) contained a one-to-one ratio of complementary oligodeoxynucleotides. Duplex concentrations were 2.2 µM. Solutions were prepared by the addition of appropriate volumes of stock solutions of the two complementary oligodeoxynucleotides to 150 µl of 2x PIPES (1,4-piperazine bis-(ethanesulfonate) sesquisodium salt) buffer (20 mM PIPES (pH 7.0), 20 mM MgCl₂, 200 mM NaCl), followed by dilution with distilled, deionized water to a volume of 300 µL. The complementary oligodeoxynucleotides were hybridized by heating the samples to 37 ºC (30 min, followed by 2 h at 25 ºC). Melting studies were carried out in 1 cm pathlength quartz cells. Absorbance was monitored while the temperature was ramped at a rate of 1.0 ºC/min (in order to minimize time at which oligonucleotides containing 14b were heated) from 25 ºC to 55 ºC. Melting temperatures were calculated by computer fit of the first derivative of absorbance with respect to 1/T. Experiments were carried out in triplicate. A fourth sample containing only 14b was handled in an identical
manner. Aliquots (50 µL) of this were analyzed by HPLC before any heating and after three hybridization/melting cycles (see above for conditions).

Supporting Information Table 1. Reproducibility of Melting Temperature (°C) of 17b.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Sample cell 1</th>
<th>Sample cell 2</th>
<th>Sample cell 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>36.6</td>
<td>37.6</td>
<td>37.6</td>
<td>37.2 ± 0.6</td>
</tr>
<tr>
<td>2nd</td>
<td>36.3</td>
<td>36.3</td>
<td>36.9</td>
<td>36.5 ± 0.3</td>
</tr>
<tr>
<td>3rd</td>
<td>36.5</td>
<td>37.6</td>
<td>37.7</td>
<td>37.3 ± 0.7</td>
</tr>
</tbody>
</table>
Figure 1. Sample T_m of 17b (2.2 µM). Hybridization and melting carried out as described above.

Figure 2. HPLC chromatograms of 14b before hybridization and after 3 UV-melting cycles (as described above). Black trace (before heating); Red trace (after heating as described above).
Supporting Information Figure 3. ESI-MS of 14a.

5'-d(CGCA TG 5 GT AGT)  
14a  
Calc'd m/z: 3973.3
Supporting Information Figure 4. MALDI-TOF MS of 14b.

5'-d(CGC ATG 1GT AGT)
14b
Calc'd m/z: 3583.3
(M - H₂O: 3563.3)
Supporting Information Figure 5. ESI-MS of 15a.

5'-d(GAA GAC CC\textsubscript{5} GGC GCC)

$15a$

Calc'd m/z: 4859.9

Calc'd m/z: 4861.
Supporting Information Figure 6. MALDI-TOF MS of 15b.
Supporting Information Figure 7. ESI-MS of 16a.

5’-d(GTC ACG TGC TGC A5A CGA CGT GCT GAG CCT)

16a
Calc’d m/z: 9495.9
Supporting Information Figure 8. ESI-MS of 16b.

5'-d(GTC ACG TGC A1A CGA CGT GCT GAG CCT)