Electronic Supplementary Information (ESI) for manuscript entitled

Intrastrand Locks Increase Duplex Stability and Base Pairing Selectivity

by

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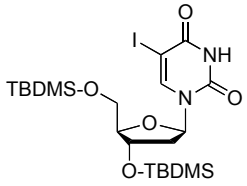
Contents

General Experimental Details

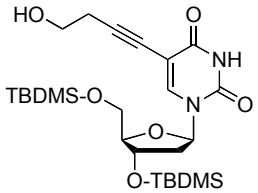
All chemicals and solvents were purchased from commercial suppliers and were used without further purification. Reactions were carried out under argon atmosphere, wherever anhydrous solvents were used. Thin layer chromatography (TLC) was performed with Merck silica gel 60 F254 plates with visualization by ultraviolet light or staining with phosphomolybdato cerium(IV) sulfate solution prepared using standard procedures (25 g phosphomolybdic acid hydrate, 10 g cerium(IV) sulfate tetrahydrate, 60 mL conc. sulfuric acid, diluted with water to 1 L). Column chromatography was carried out using Fluka silica gel 60 (particle size: 0.040 – 0.063 mm). ^{1}H NMR (300 MHz), ^{13}C NMR (75.5 MHz) and ^{31}P NMR (121.5 MHz) spectra were recorded on a Bruker Avance 300 MHz spectrometer in deuterated solvents. Chemical shifts are reported as δ values (ppm) relative to the solvent peak, and coupling constants (*J*) are given in Hz. Infrared spectra were recorded on a Bruker Vector 22 FT-IR Spectrometer. Selected absorption maxima (max) are reported in wave numbers (cm^{-1}). High-resolution mass spectrometry (HRMS) and Electrospray ionization mass spectrometry (ESI MS) were performed on a Bruker Daltonics micrOTOFQ spectrophotometer with a resolution of ±5 mDa.

Experimental Procedures



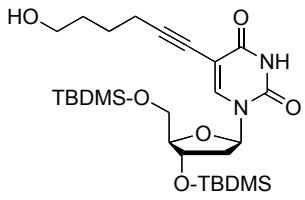
3

3',5'-*O*-Bis(*tert*-butyldimethylsilyl)-2'-deoxy-5-iodouridine (3).[1, 2] Imidazole (1.70 g, 25.0 mmol, 5 eq.) was added to a solution of *tert*-butyldimethylsilyl chloride (2.26 g, 15.1 mmol, 3 eq.) in dry DMF (10 mL). After stirring at room temperature for 10 min, 2'-deoxy-5-iodouridine (1.77 g, 5.1 mmol, 1 eq.) was added. The reaction mixture was stirred at room temperature for 18 h before ice water (100 mL) was carefully added. The product was extracted with diethyl ether (3 × 30 mL), and the combined organic layers were washed with demineralized water (2 × 50 mL) and saturated brine (50 mL). The organic phase was dried over Na_2SO_4, and the solvent was evaporated in vacuum. Purification of the crude product by column chromatography (petroleum ether/ethyl acetate, 8:2 (v/v)) gave the title compound (**3**) as an off-white foam (2.86 g, 4.9 mmol, 98%). TLC R_f = 0.30 (petroleum ether/ethyl acetate 8:2 (v/v)); IR: [cm^{-1}] = 2952 (CH, m, CH_2), 2929 (CH, m, CH_2), 2857 (CH, w, CH), 1700 (C=O, s, C=O), 1693 (C=O, s, C=O), 1600 (C=C, w, C=C), 1072 (C-I, m, C-I); ^{1}H NMR (300 MHz, DMSO): δ [ppm] = 11.64 (s, 1 H, *NH*), 7.88 (s, 1 H, *H5*), 6.00 (t, *J* = 6.1 Hz, 1 H, *H1'*), 4.27 (bs, 1 H, *H4'*), 3.76 -3.66 (m, 2 H, *H5'*, *H5''*), 3.65-3.61 (m, 1 H, *H3'*), 2.20-1.99 (m, 2 H, *H2'*, *H2''*), 0.82 (s, 9 H, *Si-C(CH$_3$)$_3$*), 0.79 (s, 9 H, *Si-C(CH$_3$)$_3$*), 0.04 (s, 6 H, *Si-CH$_3$*), 0.01 (s, 6 H, *Si-CH$_3$*); ^{13}C NMR (75 MHz, DMSO): δ [ppm] = 160.4, 150.0, 144.0, 87.0, 84.5, 71.9, 70.0, 25.9, 25.5, 18.1, 17.6, -3.4, -4.8, -5.0, -5.2; ESI MS: m/z = 583.15 Da [(M+H)$^+$]; HRMS calcd. for $C_{21}H_{39}IN_2O_5Si_2$ [(M+H)$^+$] 583.1515, found: 583.1505.



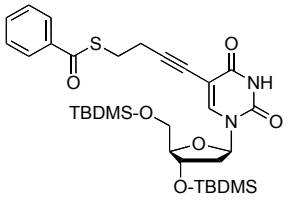
4

3',5'-*O*-Bis(*tert*-butyldimethylsilyl)-2'-deoxy-5-(4-hydroxybutynyl)uridine (4).[2] Nucleoside **3** (4.16 g, 7.2 mmol, 1 eq.) was dissolved in dry DMF (30 mL). Then, 3-butyn-1-ol (1.35 mL, 1.25 g, 17.9 mmol, 2.5 eq.), copper(I)iodide (0.27 g, 1.4 mmol, 0.2 eq.), triethylamine (2.49 mL, 1.81 g, 17.9 mmol, 2.5 eq.) and tetrakis(triphenylphosphino)palladium (0.83 g, 0.72 mmol, 0.1 eq.) were added. The reaction was stirred at room temperature under argon atmosphere for 16 h. Then, ethyl acetate (50 mL) was added, and the organic layer was washed with brine (30 mL), saturated $NHCO_3$ solution (30 mL) and 5% EDTA solution (15 mL). The organic layer was dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. Purification of the crude product by column chromatography (ethyl acetate/petroleum ether, 8:2 (v/v)) yielded titel compound **4** as an off-white foam (1.99 g, 3.798 mmol, 53%). TLC R_f = 0.50 (ethyl acetate/petroleum ether, 8:2 (v/v)); IR: [cm^{-1}] = 2929 (CH, m, CH_2), 2857 (CH, w, CH), 1691 (C=O, s, C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.20 (s, 1H, *NH*), 7.94 (s, 1H, *H5*), 6.27 (t, *J* = 5.9 Hz, 1H, *H1'*), 4.38 (t, *J* = 2.8 Hz, 1H, *H4'*), 3.95 (t, *J* = 2.3 Hz, 1H, *H5'*), 3.89 (dd, *J* = 11.5 Hz, *J* = 2.3 Hz, 1H, *H5''*), 3.78-3.72 (m, 3H, *H3'*, *CH$_2$-O*), 2.63 (t, *J* = 6.2 Hz, 2H, *CH$_2$*), 2.32-2.25 (m, 1H, *H2'*), 2.04-1.95 (m, 1H, *H2''*), 0.91 (s, 9H, *Si-C(CH$_3$)$_3$*), 0.87 (s, 9H, *Si-C(CH$_3$)$_3$*), 0.12 (d, *J* = 3.8 Hz, 6H, *Si-CH$_3$*), 0.05 (d, *J* = 2.2 Hz, 6H, *Si-CH$_3$*). ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 164.4, 149.1, 142.2, 127.4, 107.6, 88.6, 85.9, 72.4, 63.2, 60.9, 42.1, 26.2, 16.0, 24.3, 18.6, 18.2, 0.11, -4.6, -5.5; ESI MS: m/z = 525.28 Da [(M+H)$^+$]; HRMS calcd. for $C_{25}H_{44}N_2O_6Si_2$ [(M+H)$^+$] 525.2811, found: 525.2803.



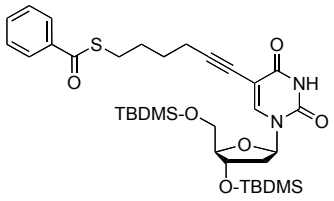
5

3',5'-*O*-Bis(*tert*-butyldimethylsilyl)-2'-deoxy-5-(6-hydroxyhexynyl)uridine (5).[3] To a solution of nucleoside **3** (2.83 g, 4.9 mmol, 1 eq.) in dry DMF (25 mL), 5-hexyn-1-ol (1.00 mL, 0.91 g, 9.2 mmol, 1.9 eq.), copper(I) iodide (0.19 g, 1.0 mmol, 0.2 eq.), triethylamine (1.35 mL, 0.98 g, 9.7 mmol, 2 eq.) and tetrakis(triphenylphosphine)palladium(0) (0.56 g, 0.5 mmol, 0.1 eq.) were added successively. After stirring at room temperature under argon atmosphere for 14 h, the same workup was performed as described for **4**. The crude product was purified *via* silica chromatography (ethyl acetate/hexane 7:3 (v/v)) to furnish the title product **5** as a white foam (1.93 g, 3.5 mmol, 72%). TLC R_f=0.38 (ethyl acetate/hexane 7:3 (v/v)); IR: [cm^{-1}] =3461 (OH, w, OH), 2930 (CH, m, CH_2), 2857 (CH, w, CH), 1693 (C=O, s, C=O); ^{1}H NMR (300 MHz, DMSO): δ [ppm] = 11.60 (s, 1 H, *NH*), 7.81 (s, 1 H, *H5*), 6.10 (t, *J* = 6.9 Hz, 1 H, *H1'*), 4.41-4.34 (m, 1 H, *H4'*), 3.82 (dd, *J* = 9.4 Hz, *J* = 3.0 Hz, 2 H, *H5'*, *H5''*), 3.71 (dd, *J* = 12.3 Hz, *J* = 3.8 Hz, 1 H, *H3'*), 3.40 (d, *J* = 4.9 Hz, 2 H, *CH_2-O*), 2.35 (s, 2 H, *CH_2*), 2.24-2.08 (m, 2 H, *H2'*, *H2''*), 1.53-1.49 (m, 4 H, *CH_2*, *CH_2*), 0.91 (s, 9 H, *Si-C(CH_3)$_3$*), 0.88 (s, 9 H, *Si-C(CH_3)$_3$*), 0.11 (s, 6 H, *Si-CH_3*), 0.09 (s, 6 H, *Si-CH_3*); ^{13}C NMR (75 MHz, DMSO): δ [ppm] = 161.6, 149.2, 141.8, 99.5, 93.4, 87.0, 84.5, 72.7, 72.7, 62.4, 60.0, 31.8, 26.0, 25.5, 24.7, 19.0, 18.0, 17.6, -4.8, -5.2, -5.6; ESI MS: m/z = 553.31 Da [$(M+H)^+$]; HRMS calcd. for $C_{27}H_{48}N_2O_6Si_2$ [$(M+H)^+$] 553.3124, found: 553.3129.



6

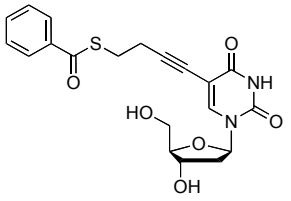
5-(4-Benzoylthiobutynyl)-3',5'-*O*-bis(*tert*-butyldimethylsilyl)-2'-deoxyuridine (6). Nucleoside **4** (1.98 g, 3.8 mmol, 1 eq.) was dissolved in dry DMF (20 mL). Triethylamine (1.31 mL, 0.95 g, 9.4 mmol, 2.5 eq.) was added. The mixture was cooled to -55 °C, and methanesulfonyl chloride (0.35 mL, 0.52 g, 4.5 mmol, 1.2 eq.) was carefully added. After stirring for 1 h at -55 °C, thiobenzoic acid (0.54 mL, 0.63 g, 4.5 mmol, 1.2 eq.) was added, and the mixture was allowed to warm to room temperature. When the TLC showed remaining mesylate [TLC R_f= 0.40 (ethyl acetate/petroleum ether 55:45 (v/v))] after 3 h at room temperature, triethylamine (0.70 mL, 0.51 g, 5.1 mmol, 1.3 eq.) and thiobenzoic acid (0.53 mL, 0.63 g, 4.5 mmol, 1.2 eq.) were again added, and the reaction was stirred at room temperature for another 16 h. Dichloromethane (35 mL) and water (25 mL) were added, and the layers were separated. The organic phase was washed with brine (20 mL), dried over Na_2SO_4, and the solvent was removed under reduced pressure. Purification of the crude by column chromatography (petroleum ether/ethyl acetate, 7:3 (v/v)) yielded the title compound (**6**) as a yellow foam (2.06 g, 3.2 mmol, 85%). TLC R_f=0.39 (petroleum ether/ethyl acetate, 7:3 (v/v)); IR: [cm^{-1}] = 3065 (C=C, w, aromatic), 2929 (CH, m, CH_2), 2856 (CH, w, CH), 1686 (C=O, s, C=O), 1666 (C=O, s, C=O), 1205 (CH_2-S-CO, m, CH_2-S-CO), 1101 (S-CO, m, S-CO); ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.20 (s, 1H, *NH*), 7.95-7.92 (m, 3H, *H5*, *CH_{ar}*, *CH_{ar}*), 7.55 (t, *J* = 7.5 Hz, 1 H, *CH_{ar}*), 7.43 (t, *J* = 7.3 Hz, 2 H, *CH_{ar}*, *CH_{ar}*), 6.26 (dd, *J* = 7.6 Hz, J= 5.8 Hz, 1H, *H1'*), 4.38 (t, *J* = 2.8 Hz, 1H, *H4'*), 3.95 (d, *J* = 2.3 Hz, 1H, *H5'*), 3.87 (dd, *J* = 11.4 Hz, *J* = 2.3 Hz, 1H, *H5''*), 3.73 (dd, *J* = 11.4 Hz, *J* = 2.2 Hz, 1H, *H3'*), 3.25 (t, *J* = 7.2 Hz, 2H, *CH_2-O*), 2.75 (t, J= 2 Hz, 2H, *CH_2*), 2.32-2.25 (m, 1H, *H2'*), 2.05-1.96 (m, 1H, *H2''*), 0.90 (s, 9H, *Si-C(CH_3)$_3$*), 0.87 (s, 9H, *Si-C(CH_3)$_3$*), 0.11 (d, J= 5.6 Hz, 6H, *Si-CH_3*), 0.05 (d, J= 2.4 Hz, 6H, *Si-CH_3*); ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 191.5, 161.6, 149.2, 142.3, 137.1, 133.7, 128.8, 127.5, 100.5, 92.8, 88.6, 86.0, 72.7, 63.2, 42.2, 28.2, 26.2, 26.0, 21.0, 18.6, 18.2, 0.2, -4.4, -5.1; ESI MS: m/z = 645.28 Da [$(M+H)^+$]; HRMS calcd. for $C_{32}H_{48}N_2O_6SSi_2$ [$(M+H)^+$] 645.2844, found: 645.2845.



7

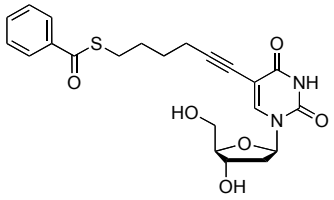
5-(6-Benzoylthiohexynyl)-3',5'-*O*-bis(*tert*-butyldimethylsilyl)-2'-deoxyuridine (7). Triethylamine (0.32 mL, 229 mg, 2.3 mmol, 2.5 eq.) was added to a solution of nucleoside **5** (500 mg, 0.9 mmol, 1 eq.) in dry DMF (10 mL). The mixture was cooled to -55 °C. Then, methansulfonyl chloride (0.08 mL, 124 mg, 1.1 mmol, 1.2 eq.) was added, and the mixture was allowed to react for 1 h at -55 °C. After addition of thiobenzoic acid (0.13 mL, 150 mg, 1.1 mmol, 1.2 eq.), the solution was

stirred for 3 h at room temperature. When TLC showed remaining mesylate [TLC R_f = 0.40 (ethyl acetate/petroleum ether, 55:45 (v/v))], triethylamine (0.20 mL, 146 mg, 1.4 mmol, 1.6 eq.) and thiobenzoic acid (0.13 mL, 150 mg, 1.1 mmol, 1.2 eq.) were again added, and the reaction was allowed to proceed for 18 h at room temperature. Work-up as described for **6** and purification by column chromatography (petroleum ether/ethyl acetate, 7:3 (v/v)) furnished the title compound (**7**) as a rose-colored foam (550 mg, 0.8 mmol, 90%). TLC R_f=0.35 (petroleum ether/ethyl acetate 7:3 (v/v)); IR: [cm^{-1}] = 3065 (C=C, w, aromatic), 2928 (CH, m, CH_2), 2856 (CH, w, CH), 1683 (C=O, s, C=O), 1205 (CH_2-S-CO, m, CH_2-S-CO), 1101 (S-CO, m, S-CO); ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.48 (s, 1 H, *NH*), 7.88 (d, *J* = 7.1 Hz, 2 H, CH_{ar}, CH_{ar}), 7.82 (s, 1 H, *H5*), 7.48 (t, *J* = 7.4 Hz, 1 H, CH_{ar}), 7.36 (t, *J* = 8.5 Hz, 2 H, CH_{ar}, CH_{ar}), 6.21 (t, *J* = 7.5 Hz, 1 H, *H1'*), 4.32 (bs, 1 H, *H4'*), 3.88 (bs, 1 H, *H3'*), 3.82 (d, J= 11.4 Hz, 1 H, *H5'*), 3.68 (d, J= 9.3 Hz, 1 H, *H5''*), 3.02 (t, J= 7.0 Hz, 2 H, CH_2-*S*), 2.35 (t, *J* = 6.8 Hz, 2 H, CH_2), 2.25-2.18 (m, 1 H, *H2'*), 1.99-1.90 (m, 1H, *H2''*), 1.78-161 (m, 4 H, CH_2, CH_2), 0.84 (s, 9 H, *Si*-CH_3), 0.81 (s, 9 H, *Si*-$C(CH_3)_3$), 0.06 (s, 6 H, *Si*-CH_3), 0.00 (s, 6 H, *Si*-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 192.4, 161.7, 149.3, 141.6, 137.7, 133.4, 128.8, 127.3, 100.9, 94.7, 88.5, 85.9, 72.7, 72.2, 63.0, 42.2, 29.1, 28.7, 27.9, 26.3, 25.9, 19.4, 18.6, 18.2, -4.3, -4.7, -5.2, -5.4; ESI MS: m/z = 673.31 Da [$(M+H)^+$]; HRMS calcd. for $C_{34}H_{52}N_2O_6SSi_2$ [$(M+H)^+$] 673.3157, found: 673.3144.



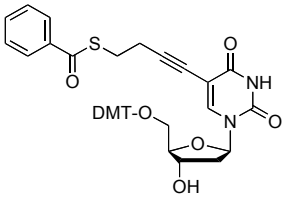
8

5-(4-Benzoylthiobutynyl)-2'-deoxyuridine (8). Nucleoside **6** (2.02 g, 3.1 mmol, 1 eq.) was dissolved in dry pyridine (5 mL). Tetrabutylammonium fluoride (TBAF, 1 M in THF, 7.84 mL, 7.8 mmol, 2.5 eq.) and HF · pyridine (CAUTION: HF is extremely hazardous) (3.8 M, 4.13 mL, 15.7 mmol, 5 eq.) were added, and the solution was stirred at room temperature for 16 h. Then, the mixture was filtered through a short silica column (ethyl acetate/methanol, 5:5 (v/v)), and the solvents were removed under vacuum. Purification of the crude by column chromatography (ethyl acetate/methanol, 97:3 (v/v)) gave the title product **8** as a white foam (1.02 g, 2.5 mmol, 78%). TLC R_f=0.43 (ethyl acetate/methanol 97:3 (v/v)); IR: [cm^{-1}] = 3044 (C=C, w, aromatic), 2918 (CH, w, CH_2), 1702 (C=O, s, C=O), 1669 (C=O, s, C=O), 1636 (C=O, s, C=O), 1207 (CH_2-S-CO, m, CH_2-S-CO), 1090 (S-CO, m, S-CO); ^{1}H NMR (300 MHz, DMSO): δ [ppm] = 11.56 (s, 1H, *NH*), 8.16 (s, 1H, *H5*), 7.93 (dd, *J* = 8.5 Hz, *J* = 1.2 Hz, 2H, CH_{ar}, CH_{ar}), 7.71 (t, *J* = 6.4 Hz, 1H, CH_{ar}), 7.57 (t, *J* = 7.3 Hz, 2H, CH_{ar}, CH_{ar}), 6.11 (t, *J* = 6.8 Hz, 1H, *H1'*), 5.24 (d, *J* = 4.2 Hz, 1H, *H4'*), 5.10 (t, *J* = 5.1 Hz, 1H, *H3'*), 4.23 (bs, 1H, *H5'*), 3.78 (d, *J* = 3.2 Hz, 1H, *H5''*), 3.65-3.52 (m, 2H, CH_2-*S*), 3.23 (t, *J* = 7.1 Hz, 2H, CH_2), 2.76-2.71 (m, 1H, *H2'*), 2.28-2.26 (m, 1H, *H2''*); ^{13}C NMR (75 MHz, DMSO): δ [ppm] =197.2, 166.1, 152.1, 143.9, 136.0., 130.1, 127.7, 123.1, 107.7, 92.6, 87.7, 85.8, 72.5, 69.5, 60.9, 38.4, 26.9, 19.6.; ESI MS: m/z = 417.11 Da [$(M+H)^+$]; HRMS calcd. for $C_{20}H_{20}N_2O_6S$ [$(M+Na)^+$] 439.0934, found: 439.0934. Spectral data are consistent with literature values. [4]



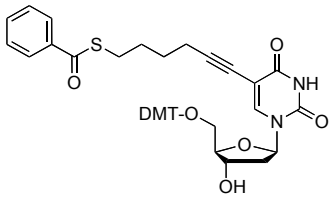
9

5-(6-Benzoylthiohexynyl)-2'-deoxyuridine (9). To a stirred solution of TBDMS-nucleoside **7** (200 mg, 0.3 mmol, 1 eq.) in dry pyridine (0.3 mL), TBAF (1 M in THF, 0.74 mL, 0.7 mmol, 2.5 eq.) and HF · pyridine (CAUTION: HF is extremely hazardous) (3.8 M, 0.39 mL, 1.5 mmol, 5 eq.) were added. The reaction was allowed to proceed for 17 h. Then, the mixture was filtered through a short silica column (ethyl acetate/methanol, 5:5 (v/v)), and the solvents were removed in vacuum. The crude product was purified by chromatography (silica, ethyl acetate/methanol, 97:3 (v/v)) to yield **9** as an off-white foam (108 mg, 0.2 mmol, 82%). TLC R_f=0.42 (ethyl acetate/methanol, 97:3 (v/v)); IR: [cm^{-1}] = 3392 (OH, w, OH), 3058 (C=C, w, aromatic), 2930 (CH, m, CH_2), 1683 (C=O, s, C=O), 1665 (C=O, s, C=O), 1206 (CH_2-S-CO, m, CH_2-S-CO), 1091 (S-CO, m, S-CO); ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm] = 8.33 (s, 1H, *NH*), 8.07 (s, 1H, *H5*), 7.90 (d, *J* = 7.2 Hz, 2 H, CH_{ar}, CH_{ar}), 7.62-7.54 (m, 1H, CH_{ar}), 7.44 (t, *J* = 7.9 Hz, 2 H, CH_{ar}, CH_{ar}), 6.22 (t, *J* = 6.5 Hz, 1 H, *H1'*), 4.60-4.56 (m, 1 H, *H4'*), 4.02-4.00 (m, 1 H, *H3'*), 3.93 (dd, *J* = 11.7 Hz, *J* = 2.7 Hz, 1 H, *H5'*), 3.82 (dd, *J* = 11.6 Hz, *J* = 2.6 Hz, 1 H, *H5''*), 3.09-3.04 (m, 2H, CH_2-*S*), 2,46 (t, *J* = 6.6 Hz, 2 H, CH_2), 2.36-2.31 (m, 2H, *H2'*, *H2''*), 1.88-1.78 (m, 2H, CH_2), 1.73-1.63 (m, 2H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] = 193.3, 170.9, 168.2, 162.3, 158.4, 137.5, 133.7, 128.9, 127.5, 121.9, 82.9, 66.6, 62.5, 60.6, 28.9, 27.6, 21.4, 19.3, 17.7, 14.3; ESI MS: m/z = 445.14 Da [$(M+H)^+$]; HRMS calcd. for $C_{22}H_{24}N_2O_6S$ [$(M+Na)^+$] 467.1247, found: 467.1244.



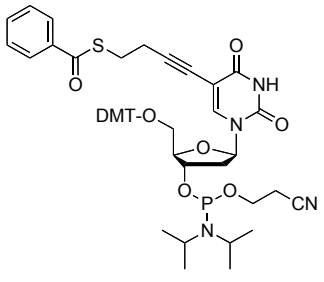
10

5-(4-Benzoylthiobutynyl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)uridine (10). Nucleoside **8** (1.02 g, 2.5 mmol, 1 eq.) was dissolved in dry pyridine (15 mL). 4,4'-Dimethoxytrityl chloride (0.91 g, 2.7 mmol, 1.1 eq.) was added, and the reaction was stirred at room temperature for 15 h. After removal of the solvent in vacuum, dichloromethane (20 mL) was added. The organic layer was washed with saturated sodium hydrogen carbonate solution (15 mL) and brine (15 mL). After drying of the organic phase over Na_2SO_4, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (dichloromethane/methanol 97:3 (v/v)) to give the title product **10** as a rose foam (1.52 g, 2.1 mmol, 86%). TLC R_f=0.36 (dichloromethane/methanol 97:3 (v/v)); IR: [cm^{-1}] = 3065 (C=C, w, aromatic), 2834 (CH, w, CH_2), 1661 (C=O, s, C=O), 1174 (CH_2-S-CO, m, CH_2-S-CO), 1088 (S-CO, w, S-CO); ^{1}H NMR (300 MHz, DMSO): δ [ppm] = 11.64 (s, 1H, *NH*), 7.93 (s, 1H, *H5*), 7.89 (d, *J* = 7.3 Hz, 2H, $CH_{ar,\ Bz}$), 7.69 (t, *J* = 7.4 Hz, 1H $CH_{ar\ Bz}$), 7.54 (t, *J* = 7.3 Hz, 2H, $CH_{ar\ Bz}$), 7.42—7.19 (m, 9H, $CH_{ar\ DMT}$), 6.88 (d, *J* = 8.4 Hz, 4H, $CH_{ar\ DMT}$), 6.12 (t, *J* = 6.9 Hz, 1H, *H1'*), 5.33 (d, *J* = 4.5 Hz, 1H, *OH*), 4.29 (bs, 1H, *H4'*), 3.92 (bs, 2H, *H3'*), 3.72 (s, 6H, *O-CH*$_3$), 3.31 (m, 2H, *H5'*, *H5''*), 3.26-3.09 (m, 2H, CH_2-*S*), 2.94 (t, *J* = 7.1 Hz, 2H, *H2'*, *H2''*), 2.31-2.17 (m, 2H, CH_2); ^{13}C NMR (75 MHz, CD_3CN): δ [ppm] =192.5, 163.0, 160.1, 151.1, 150.7, 146.3, 144.1, 138.1, 137.4, 137.1, 135.1, 131.4, 130.3, 129.4, 128.3, 125.2, 123.0, 114.5, 101.3, 100.5, 92.6, 87.8, 86.8, 74.3, 72.4, 64.7, 56.3, 42.1, 28.8, 21.4; ESI MS: m/z = 719.25 Da [$(M+H)^+$]; HRMS calcd. for $C_{41}H_{38}N_2O_8S$ [$(M+Na)^+$] 741.2241, found: 741.2234.



11

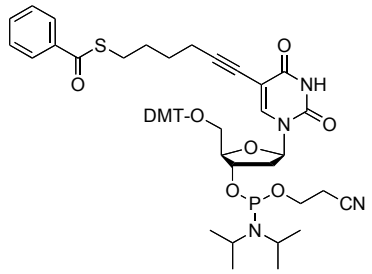
5-(6-Benzoylthiohexynyl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)uridine (11). 4,4'-Dimethoxytrityl chloride (401 mg, 1.2 mmol, 1.1 eq.) was added to a stirred solution of nucleoside **9** (478 mg, 1.1 mmol, 1 eq.) in dry pyridine (7 mL). The solution was allowed to react for 17 h. Then, the solvent was removed under vacuum, followed by addition of dichloromethane (15 mL). The organic solution was washed with saturated $NaHCO_3$ solution (10 mL) and brine (10 mL). The solvent was removed under reduced pressure after drying of the organic phase over Na_2SO_4. Purification of the crude by column chromatography (dichloromethane/methanol, 97:3 (v/v)) yielded **11** as an off-white foam (707 mg, 1.0 mmol, 88%). TLC R_f=0.25 (dichloromethane/methanol, 97:3 (v/v)); IR: [cm^{-1}] = 3065 (C=C, w, aromatic), 2932 (CH, w, CH_2), 1657 (C=O, s, C=O), 1174 (CH_2-S-CO, m, CH_2-S-CO); ^{1}H NMR (300 MHz, CD_3CN): δ [ppm] = 9.03 (s, 1H, *NH*), 7.93-7.89 (m, 3H, *H5*, $CH_{ar\ Bz}$), 7.64 (t, *J* = 7.5 Hz, 1H, $CH_{ar\ Bz}$), 7.52-7.20 (m, 11H, $CH_{ar\ Bz}$, $CH_{ar\ DMT}$), 6.86 (dd, *J* = 9.0 Hz, *J* = 1.9 Hz, 4H, $CH_{ar\ DMT}$), 6.14 (t, *J* = 6.4 Hz, 1 H, *H1'*), 4.52-4.46 (m, 1 H, *H4'*), 3.97-3.96 (m, 1 H, *H3'*), 3.76 (s, 6H, *O-CH*$_3$), 3.30 (dd, *J* = 10.8 Hz, *J* = 4.0 Hz, 1 H, *H5'*), 3.18 (dd, *J* = 10.8 Hz, *J* = 2.6 Hz, 1 H, *H5''*), 2.97 (t, *J* = 6.9 Hz, 2H, CH_2-*S*), 2.30-2.26 (m, 2H, CH_2), 2.16-2.11 (m, 2H, *H2'*, *H2''*), 1.65-1.55 (m, 2H, CH_2), 1.44-1.35 (m, 2H, CH_2); ^{13}C NMR (75 MHz, CD_3CN): δ [ppm] = 160.1, 151.1, 150.7, 146.3, 143.4, 137.3, 137.0, 134.9, 131.4, 131.3, 130.2, 129.3, 128.2, 125.1, 114.5, 101.1, 94, 6, 87.9, 87.8, 86.7, 72.4, 64.7, 56.3, 42.1, 35.5, 29.9, 29.5, 28.7, 19.9; ESI MS: m/z = 769.25 Da [$(M+Na)^+$]; HRMS calcd. for $C_{43}H_{42}N_2O_8S$ [$(M+Na)^+$] 769.2554, found: 769.2543.



1

[5-(4-Benzoylthiobutynyl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)urid-3'-*O*-yl]-*O*-(2-cyanoethyl)-*N*,*N*-di*iso*propyl-phosphoramidite (1). Nucleoside **10** (180 mg, 0.3 mmol, 1 eq.) was dissolved in dry acetonitrile (5 mL). *N*,*N*-Di*iso*propylethylamine (DIEA, 0.13 mL, 97 mg, 0.8 mmol, 3 eq.) and *O*-(2-cyanoethyl)-*N*,*N*-di*iso*propylchlorophosphoramidite (0.08 mL, 83 mg, 0.4 mmol, 1.4 eq.) were added, and the solution was stirred for 4 h at room temperature. Dichloromethane (20 mL) was added, and the organic layer was washed with saturated $NaHCO_3$ solution

(15 mL) and brine (10 mL). The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude was purified *via* silica column (dichloromethane/triethylamine, 98:2 (v/v)) to furnish the title product **1** as an off-white foam (227 mg, 0.2 mmol, 99%). TLC R_f=0.23, 0.27 (dichloromethane/triethylamine 98:2 (v/v)); IR: [cm^{-1}] = 2970 (CH, w, CH_2), 1697 (C=O, s, C=O), 1179 (CH_2-S-CO, m, CH_2-S-CO), 1034 (P-O, m, P-O); ^{1}H NMR (300 MHz, CD_3CN): δ [ppm] = 9.69 (s, 1H, *NH*), 7.96 (s, 1H, *H5*), 7.91 (d, *J* = 7.4 Hz, 2H, $CH_{ar\ Bz}$), 7.65 (t, *J* = 7.4 Hz, 1H, $CH_{ar\ Bz}$), 7.52-7.19 (m, 11H, $CH_{ar\ Bz}$, $CH_{ar\ DMT}$), 6.87 (dd, *J* = 6.7 Hz, *J* = 3.4 Hz, 4H, $CH_{ar\ DMT}$), 6.15 (q, *J* = 7.0 Hz, 1H, *H1'*), 4.63 (bs, 1H, *H4'*), 4.17-4.08 (m, 1H, *H3'*), 3.74 (s, 6H, *O*-CH_3), 3.70-3.50 (m, 4H), 3.32-3.27 (m, 2H), 2.91-2.82 (m, 4H), 1.29-1.23 (m, 2H), 1.18-1.13 (m, 12H, $C(CH_3)_2$), 1.08-1.04 (m, 2H); ^{31}P NMR (300 MHz, CD_3CN): δ [ppm] = 147.97, 147.94; ESI MS: m/z = 919.34 Da [$(M+H)^+$]; HRMS calcd. for $C_{50}H_{55}N_4O_9PS$ [$(M+Na)^+$] 941.3320, found: 941.2219.



2

[5-(6-Benzoylthiohexynyl)-2'-deoxy-5'-*O*-(4,4'-dimethoxytrityl)-urid-3'-*O*-yl]-*O*-(2-cyanoethyl)-*N*,*N*-di*iso*propyl-phosphoramidite (2). Nucleoside **11** (175 mg, 0.2 mmol, 1 eq.) was dissolved in dry acetonitrile (6 mL). Then, *N*,*N*-di*iso*propylethylamine (DIEA) (0.09 mL, 68 mg, 0.5 mmol, 3 eq.) and *O*-(2-cyanoethyl)-*N*,*N*-di*iso*propylchlorophosphoramidite (0.06 mL, 58 mg, 0.2 mmol, 1.4 eq.) were added. After stirring at room temperature for 4 h, dichloromethane (20 mL) was added and the organic layer was washed with saturated $NaHCO_3$ solution (15 mL) and brine (15 mL). The organic phase was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Column chromatography (dichloromethane/triethylamine, 98:2 (v/v)) gave **2** as an off-white foam (157 mg, 0.2 mmol, 94%). TLC R_f=0.40, 0.26 (dichloromethane/triethylamine, 98:2 (v/v)); IR: [cm^{-1}] = 2969 (CH, w, CH_2), 1692 (C=O, s, C=O), 1177 (CH_2-S-CO, m, CH_2-S-CO), 1003 (P-O, m, P-O); ^{1}H NMR (300 MHz, CD_3CN): δ [ppm] = 7.93-7.89 (m, 3H, *H5*, $CH_{ar\ Bz}$), 7.67-7.62 (m, 1H, $CH_{ar\ Bz}$), 7.52-7.20 (m, 11H, $CH_{ar\ Bz}$, $CH_{ar\ DMT}$), 6.88-6.83 (m, 4H, $CH_{ar\ DMT}$), 6.15 (q, *J* = 6.6 Hz, 1H, *H1'*), 4.69-4.59 (m, 1H, *H4'*), 4.16-4.08 (m, 1H, *H3'*), 3.76 (s, 6H, *O*-CH_3), 3.71-3.48 (m, 4H), 3.32-3.26 (m, 2H), 2.95 (t, *J* = 6.9 Hz, 2H), 2.75 (t, *J* = 6.0 Hz, 1H), 2.64 (t, *J* = 6.3 Hz, 1H) 2.17-2.07 (m, 2H), 1.64-1.54 (m, 2H), 1.41-1.34 (m, 2H), 1.25-1.15 (m, 12H, $C(CH_3)_2$), 1.06 (d, *J* = 6.8 Hz, 2H); ^{31}P NMR (300 MHz, CD_3CN): δ [ppm] = 147.94, 147.91; ESI MS: m/z = 969.36 Da [$(M+Na)^+$]; HRMS calcd. for $C_{52}H_{59}N_4O_9PS$ [$(M+Na)^+$] 969.3633, found: 969.3623.

Oligodeoxynucleotide Synthesis

Unmodified oligodeoxynucleotides were purchased from biomers GmbH (Ulm, Germany) in HPLC-purified form. The locked oligodeoxynucleotides were synthesized by biomers GmbH (Ulm, Germany) on a 0.2 µmol scale, using the phosphoramidites **1** and/or **2** for the introduction of the modified residues. The resulting oligodeoxynucleotide-bearing controlled pore glass was subjected to deprotection and cleavage from the support using General Protocol A (*vide infra*). The crude oligonucleotides were purified *via* reversed phase HPLC on C4 Nucleosil columns (250 mm × 4.6 mm by Macherey-Nagel, Düren, Germany) with a gradient of acetonitrile in 0.1 M triethylammonium acetate (pH= 7.0) with detection at 260 nm. Yields of oligodeoxynucleotides are based on the isolated amount of oligodeoxynucleotide and the loading of the controlled pore glass, as specified by the supplier. Extinction coefficients for oligodeoxynucleotides were calculated through linear combination of the extinction coefficients of the single nucleotides and the covalently linked chromophores. MALDI-TOF mass spectra were acquired on a Bruker REFLEX IV spectrometer in negative, linear mode or positive reflectron mode. The software used was XACQ 4.0.4 and XTof 5.1. The MALDI matrix consisted of a 2:1 (v/v) mixture of 2,4,6-trihydroxyacetophenone solution (THAP, 0.2 M in ethanol) and diammonium citrate (0.1 M in water). The m/z found values are those of the unresolved isotope envelope of the pseudomolecular ions ($[M-H]^-$), except for spectra acquired in reflectron mode and with internal standard. UV melting curves were measured on a Perkin-Elmer Lambda 10 spectrometer with a gradient of 1 °C/min and detection at 260 nm. The melting temperatures were determined with UV Winlab 2.0 (Perkin-Elmer), as the extrema of the first derivative of the respective melting curve. Circular Dicroism spectra were measured on a JASCO J-710 Spectropolarimeter with JASCO CD Explorer software. Samples were dissolved in PBS buffer with 9 µM strand concentration and 9 µM target concentration. The samples were renatured prior to the acquisition of spectra at 20 °C. The wavelength range scanned was 400 nm to 200 nm with 1 nm step size, 2 nm bandwidth and 1.0 s signal averaging time. The curves shown are an average over 3 scans and slightly smoothed with Savitzky-Golay function using a convolution factor of 15.

Cleavage of Oligodeoxynucleotides from Solid Support and Formation of Intrastrand Lock (General Protocol A).
The controlled pore glass (cpg) loaded with the modified oligodeoxynucleotide in a polypropylene vessel was first treated with triethylamine/DMF (1:9 (v/v), 300 µL) for 20 min. After brief drying under vacuum, the cpg was treated with AMA (40% methylamine in water/25% NH_3 in water 1:1 (v/v), 300 µL) and dithiothreitol (DTT, 1 M in water, 8 µL) for 1.5 h. Then, the supernatant was aspired, and the cpg was washed with phosphate buffer (10 mM, pH 5, 2 × 400 µL). The aqueous solutions were combined, and the disulfide lock was allowed to form over 20 h at room temperature with the solution exposed to air. The solution was lyophilized, the product was redissolved in water/acetonitrile (160 µL, 95:5 (v/v)), filtered (pore size 0.45 µm), and subjected to HPLC purification.

5'-U^{S4}U^{S4}GCGCAA-3' (12). Synthesized *via* automated DNA synthesis involving phosphoramidite **2**, followed by cleavage (1.3 mg cpg, 40 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% to 30% in 60 min): t_R = 46.6 min (2.3 nmol, 6%). MALDI-TOF mass: m/z: calcd. for $C_{88}H_{109}N_{30}O_{46}P_7S_2$ 2601.5 ($[MH]^-$), found 2601.3.

5'-U^{S4}U^{S2}GCGCAA-3' (13). Synthesized *via* automated DNA synthesis involving phosphoramidites **1** and **2**, followed by cleavage (1.3 mg cpg, 40 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 37.6 min (2.7 nmol, 7%). MALDI-TOF mass: m/z: calcd. for $C_{86}H_{105}N_{30}O_{46}P_7S_2$ 2573.4 ($[MH]^-$), found 2574.3.

5'-U^{S2}U^{S2}GCGCAA-3' (14). Synthesized *via* automated DNA synthesis involving phosphoramidite **1**, followed by cleavage (5.2 mg cpg, 160 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 33.5 min (2.0 nmol, 1%). MALDI-TOF mass: m/z: calcd. for $C_{84}H_{101}N_{30}O_{46}P_7S_2$ 2545.4 ($[MH]^-$), found 2547.1.

5'-U^{S4}U^{S4}TTCCAC-3' (15). Synthesized *via* automated DNA synthesis involving phosphoramidite **2**, followed by cleavage (5.2 mg cpg, 160 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 41.6 min (4.2 nmol, 3%). MALDI-TOF mass: m/z: calcd. for $C_{87}H_{111}N_{22}O_{49}P_7S_2$ 2527.4 ($[MH]^-$), found 2528.7.

5'-U^{S4}U^{S2}TTCCAC-3' (16). Synthesized *via* automated DNA synthesis involving phosphoramidites **1** and **2**, followed by cleavage (5.2 mg cpg, 160 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 36.7 min (3.6 nmol, 2%). MALDI-TOF mass: $C_{85}H_{107}N_{22}O_{49}P_7S_2$ 2499.4 ($[MH]^-$), m/z: found 2500.1.

5'-U^{S2}U^{S2}TTCCAC-3' (17). Synthesized *via* automated DNA synthesis involving phosphoramidite **1**, followed by cleavage (1.3 mg cpg, 40 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 30.6 min (4.6 nmol, 12%). MALDI-TOF mass: m/z: $C_{83}H_{103}N_{22}O_{49}P_7S_2$ 2471.4 ($[MH]^-$), found 2472.4.

5'-U^{S4}TU^{S4}TCCAC-3' (18). Synthesized *via* automated DNA synthesis involving phosphoramidite **2**, followed by cleavage (1.3 mg cpg, 40 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 43.6 min (4.7 nmol, 12%). MALDI-TOF mass: m/z: $C_{87}H_{111}N_{22}O_{49}P_7S_2$ 2527.4 ($[MH]^-$), found 2526.3.

5'-TU^{S4}TU^{S4}CCAC-3' (19). Synthesized *via* automated DNA synthesis involving phosphoramidite **2**, followed by cleavage (3.9 mg cpg, 120 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 31.6 min (6.3 nmol, 5%). MALDI-TOF mass: m/z: $C_{87}H_{111}N_{22}O_{49}P_7S_2$ 2527.4 ($[MH]^-$), found 2528.4.

5'-TTU^{S4}U^{S4}CCAC-3' (20). Synthesized *via* automated DNA synthesis involving phosphoramidite **2**, followed by cleavage (3.9 mg cpg, 120 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 32.2 min (9.8 nmol, 8%). MALDI-TOF mass: m/z: $C_{87}H_{111}N_{22}O_{49}P_9S_2$ 2527.4 ($[MH]^-$), found 2525.4.

5'-CCTU^{S4}U^{S4}TAC-3' (21). Synthesized *via* automated DNA synthesis involving phosphoramidite **2**, followed by cleavage (3.9 mg cpg, 120 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 33.3 min (26.7 nmol, 22%). MALDI-TOF mass: m/z: $C_{87}H_{111}N_{22}O_{49}P_7S_2$ 2527.4 ($[MH]^-$), found 2526.8.

5'-CCTU^{S2}U^{S2}TAC-3' (22). Synthesized *via* automated DNA synthesis involving phosphoramidite **1**, followed by cleavage (3.9 mg cpg, 120 nmol loading) according to General Protocol A. HPLC (MeCN gradient: 5% for 5 min to 30% in 50 min): t_R = 18.4 min (12.1 nmol, 10%). MALDI-TOF mass: m/z: $C_{83}H_{103}N_{22}O_{49}P_7S_2$ 2471.4 ($[MH]^-$), found 2472.2.

NMR Spectra of Nucleosides

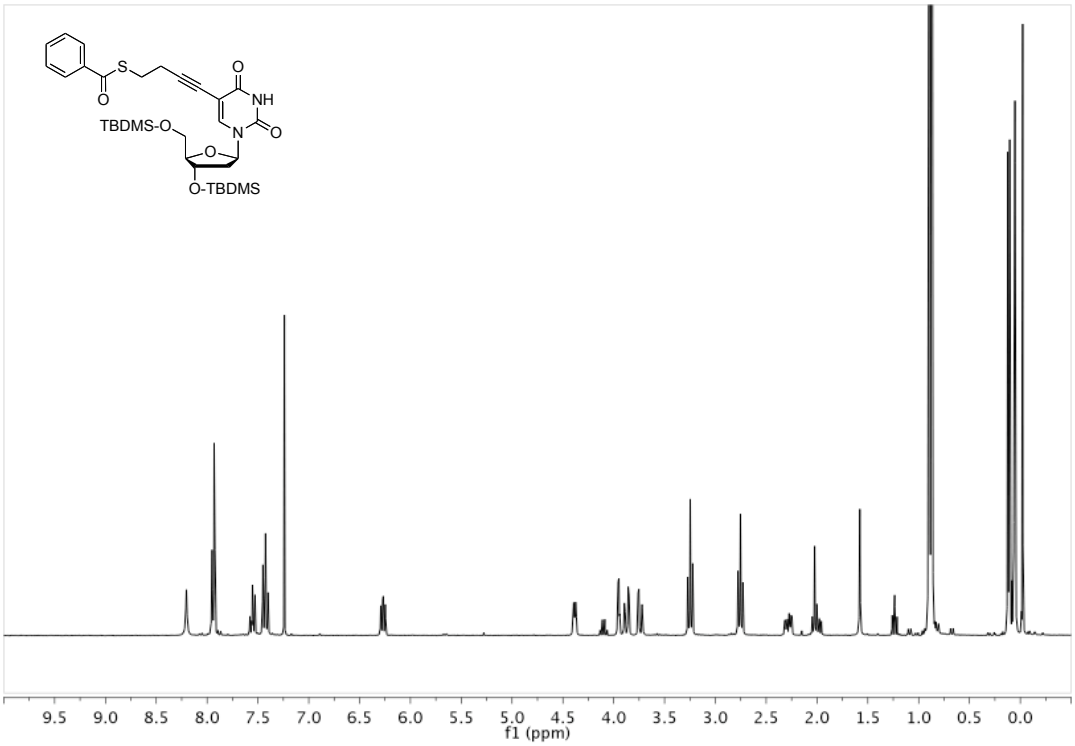


Figure S1. ^{1}H NMR spectrum of **compound 6**, $CDCl_3$, 300 MHz.

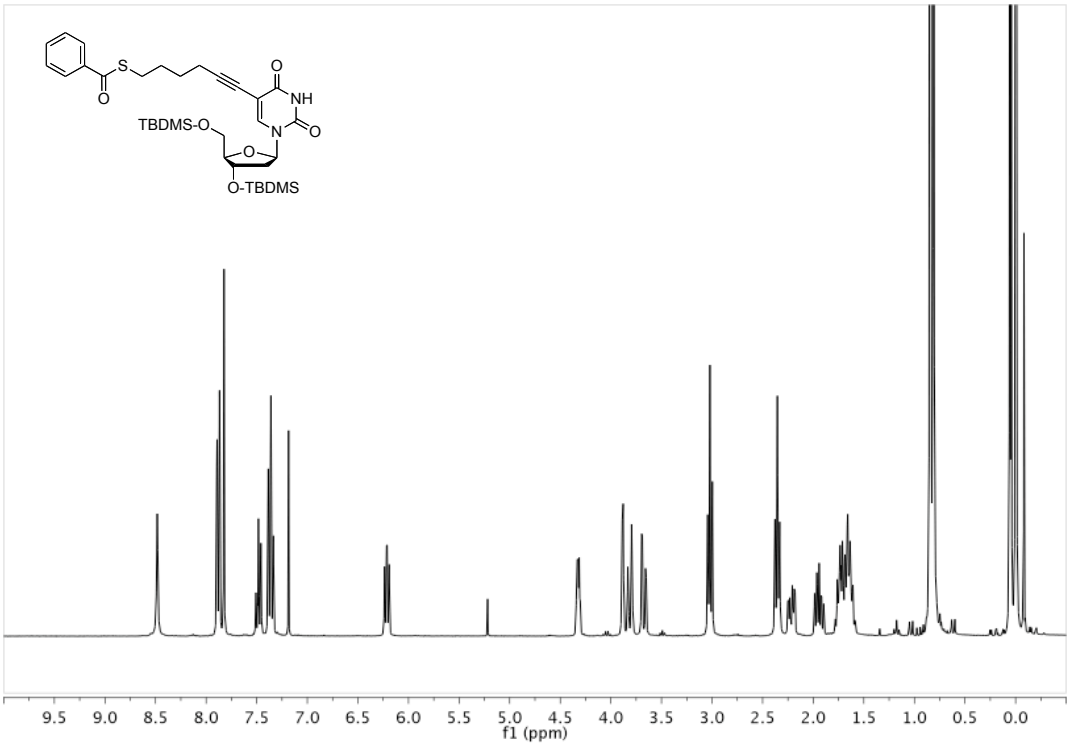


Figure S2. ^{1}H NMR spectrum of **compound 7**, $CDCl_3$, 300 MHz.

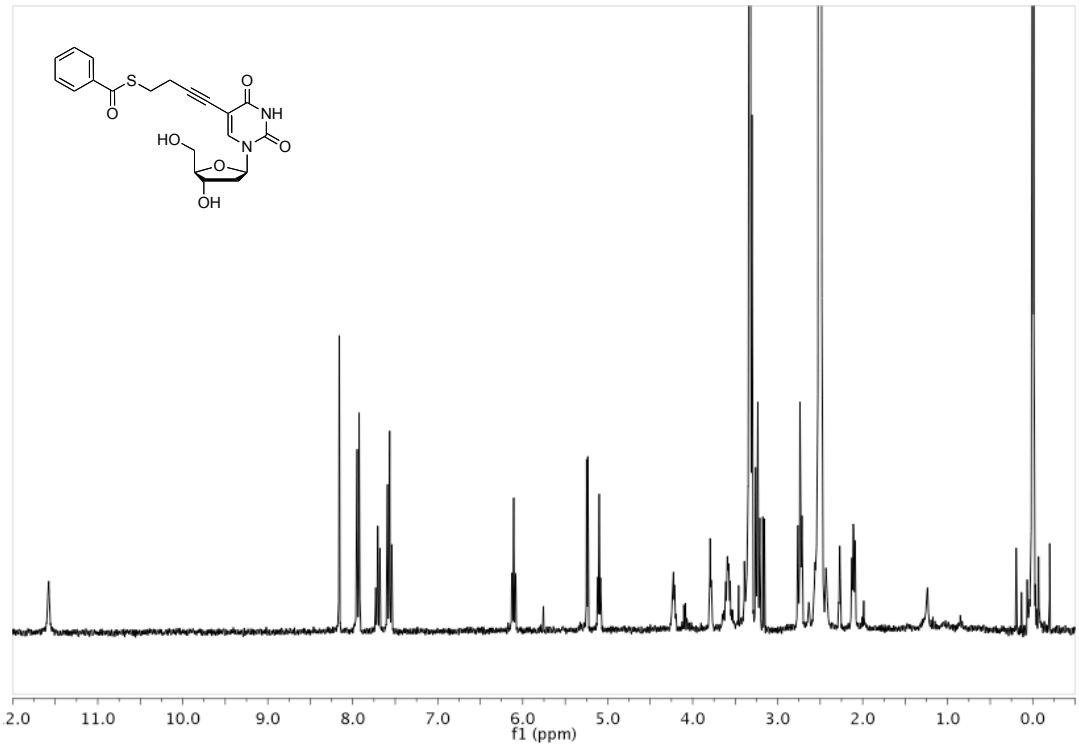


Figure S3. ^{1}H NMR spectrum of **compound 8**, $CDCl_3$, 300 MHz.

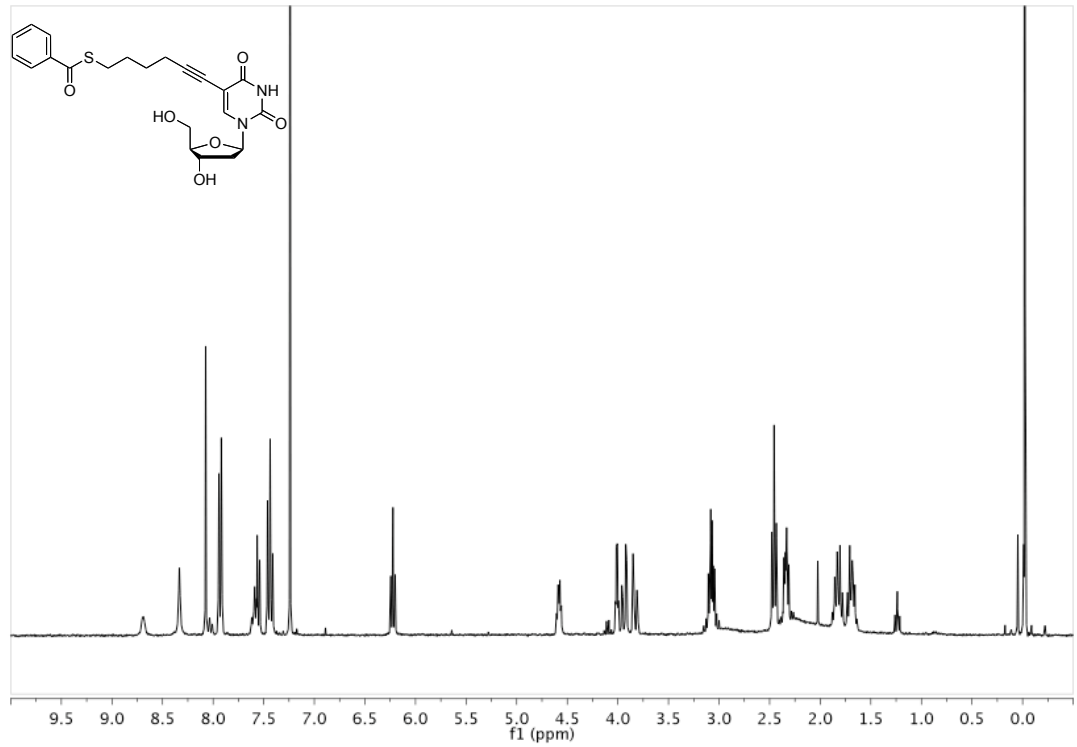


Figure S4. ^{1}H NMR spectrum of **compound 9**, $CDCl_3$, 300 MHz.

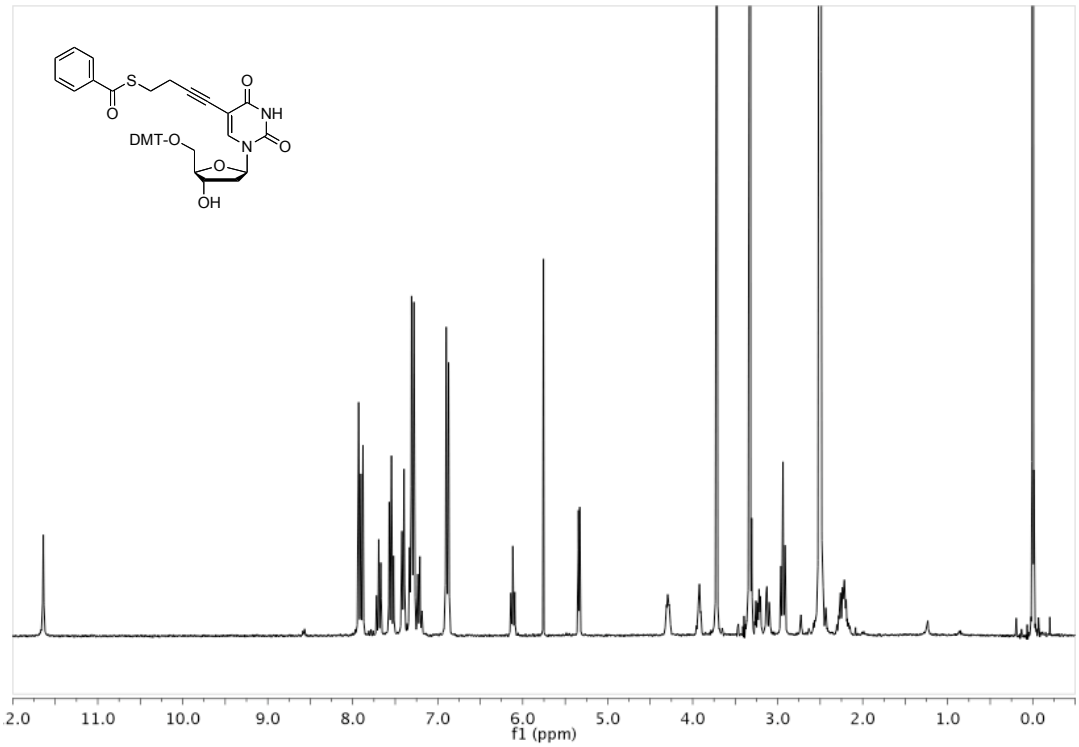


Figure S5. ^{1}H NMR spectrum of **compound 10**, DMSO, 300 MHz.

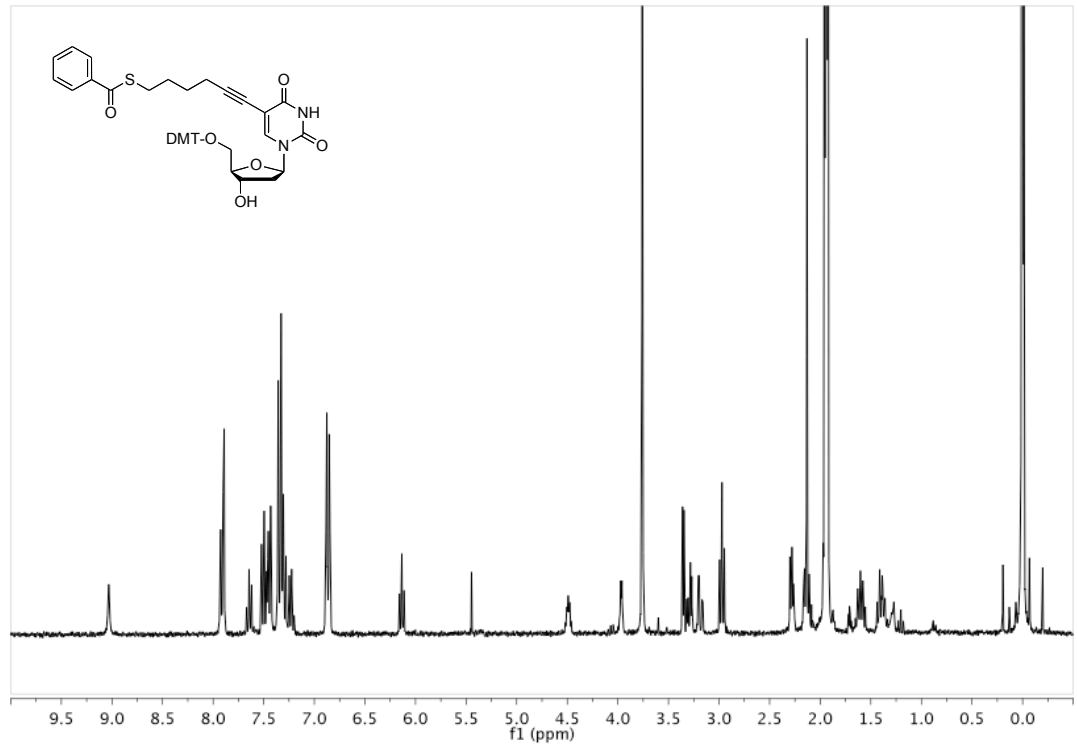


Figure S6. ^{1}H NMR spectrum of **compound 11**, CD_3CN, 300 MHz.

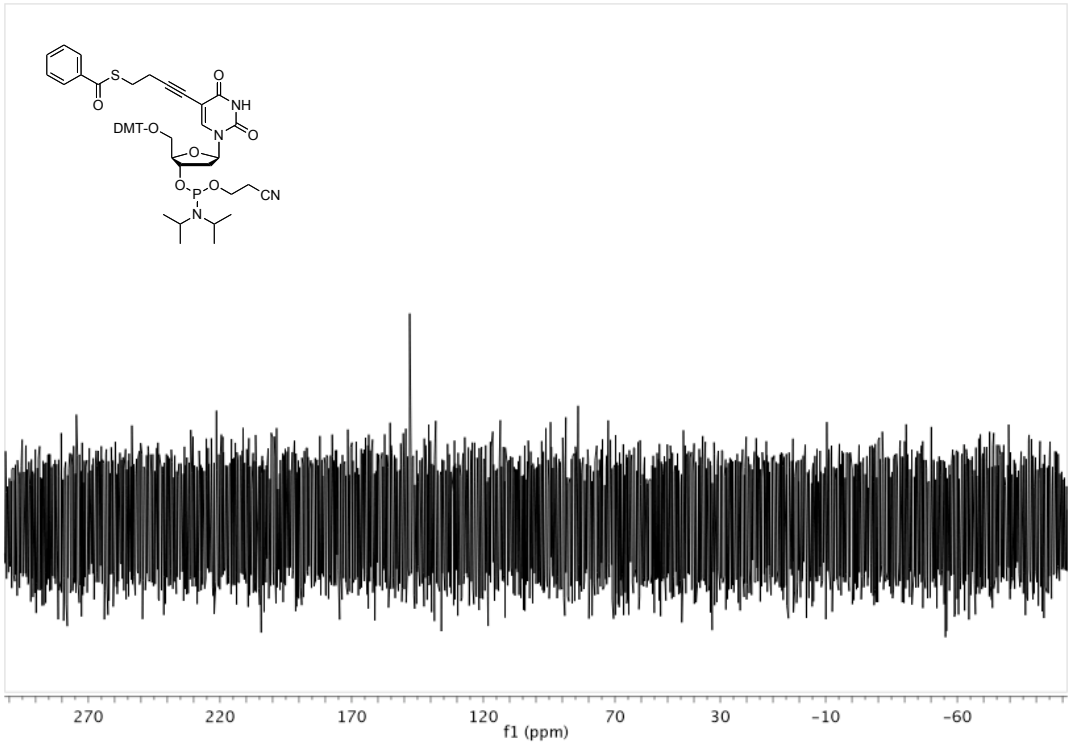


Figure S7. ^{31}P NMR spectrum of **compound 1**, CD_3CN: NEt_3 97:3,121 MHz.

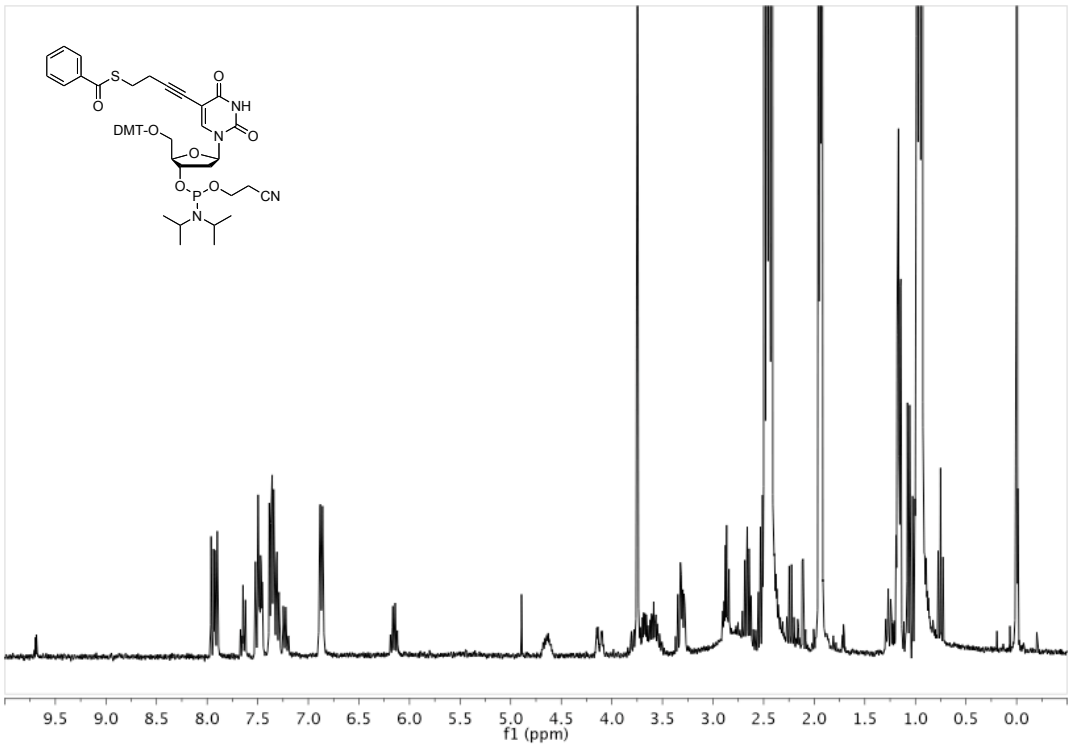


Figure S8. ^{1}H NMR spectrum of **compound 1**, CD_3CN: NEt_3 97:3, 300 MHz.

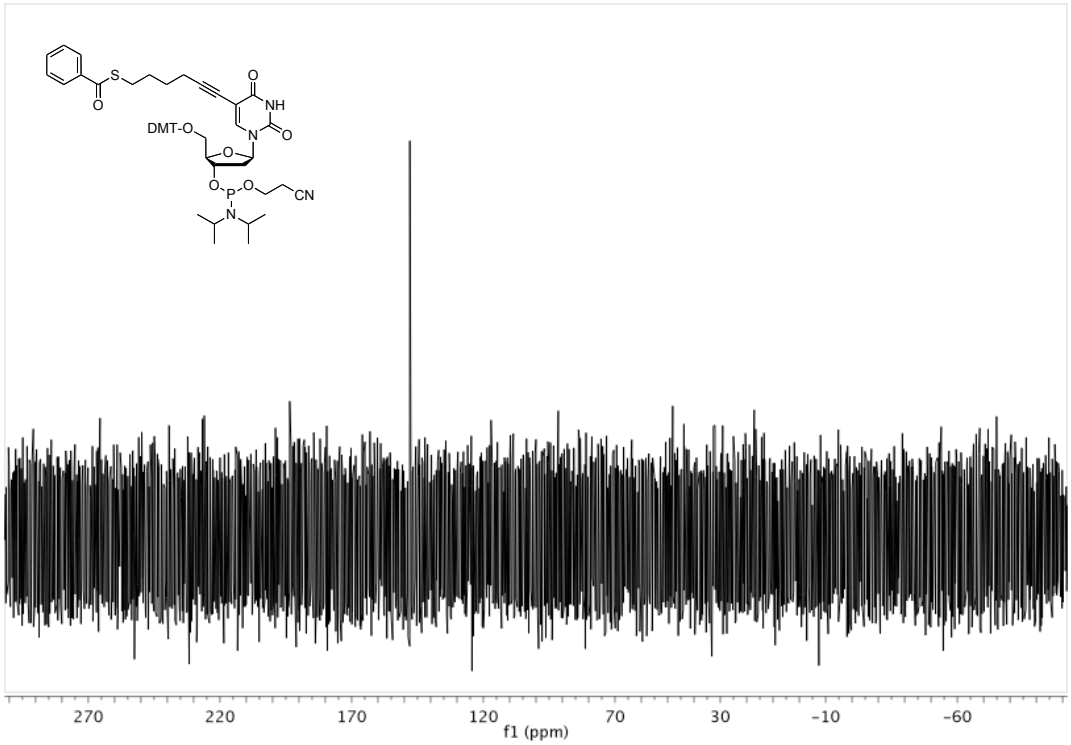


Figure S9. ^{31}P NMR spectrum of **compound 2**, CD_3CN: NEt_3 97:3, 121 MHz.

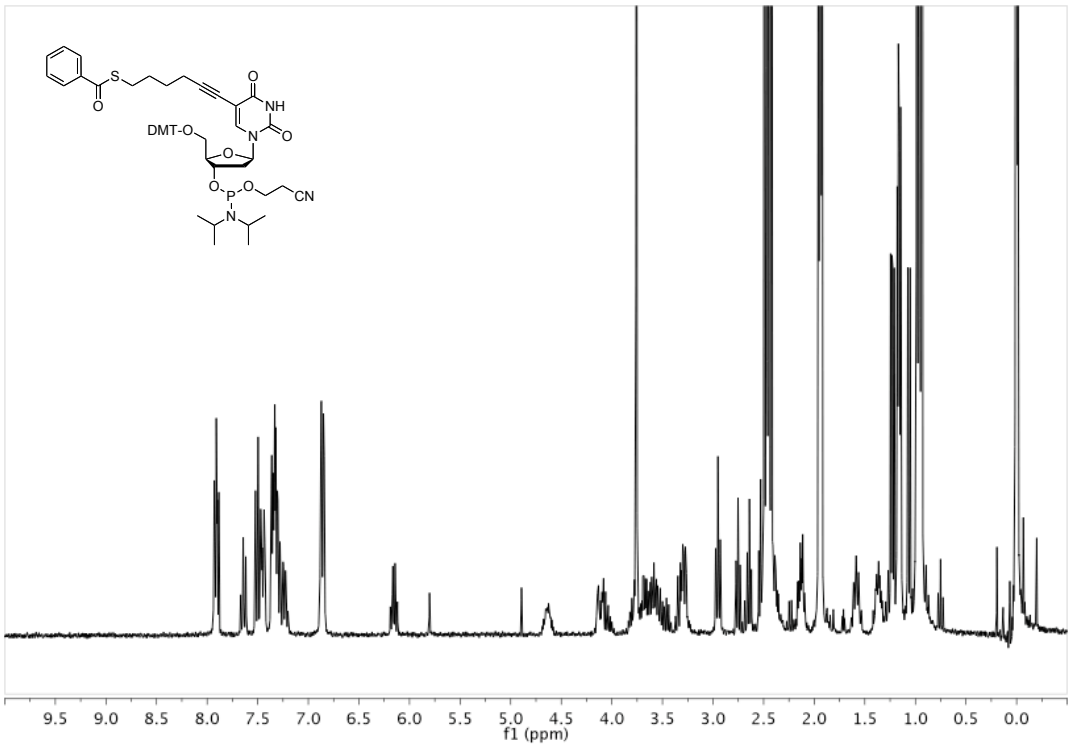


Figure S10. ^{1}H NMR spectrum of **compound 2**, CD_3CN: NEt_3 97:3, 300 MHz.

MALDI-TOF Mass Spectra of Purified Oligodeoxynucleotides.

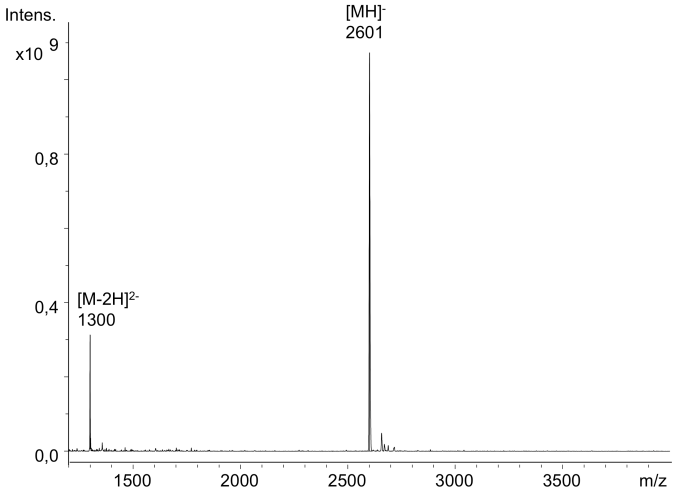


Figure S11. MALDI-TOF mass spectrum of 5'-$U^{S4}U^{S4}$GCGCAA-3' (**12**).

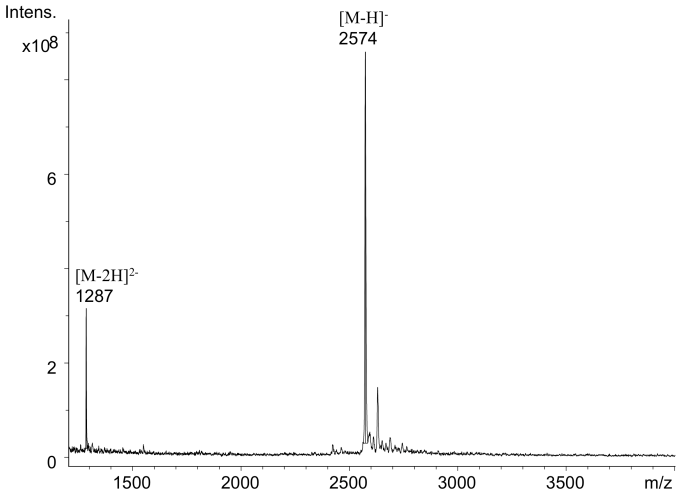


Figure S12. MALDI-TOF mass spectrum of 5'-$U^{S4}U^{S2}$GCGCAA-3' (**13**).

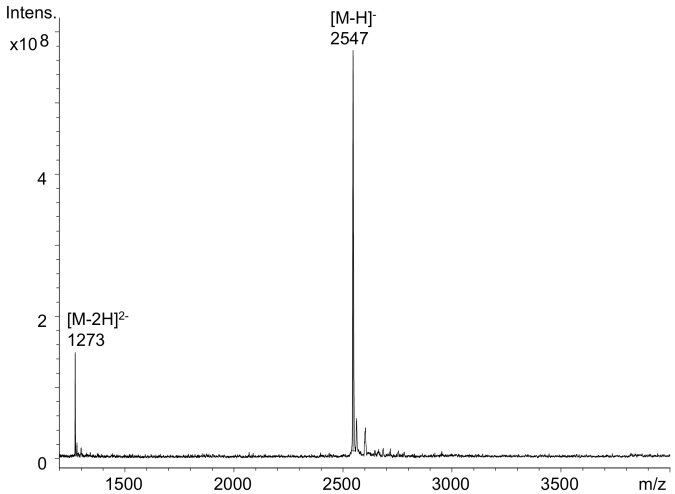


Figure S13. MALDI-TOF mass spectrum of 5'-$U^{S2}U^{S2}$GCGCAA-3' (**14**).

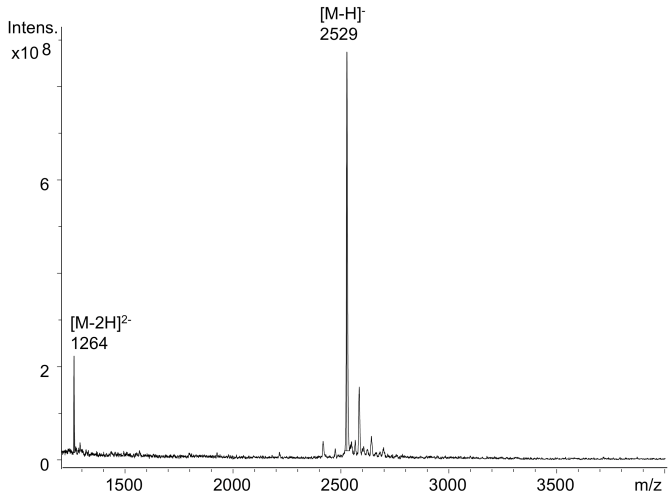


Figure S14. MALDI-TOF mass spectrum of 5'-U^{S4}U^{S4}TTCCAC-3' (**15**).

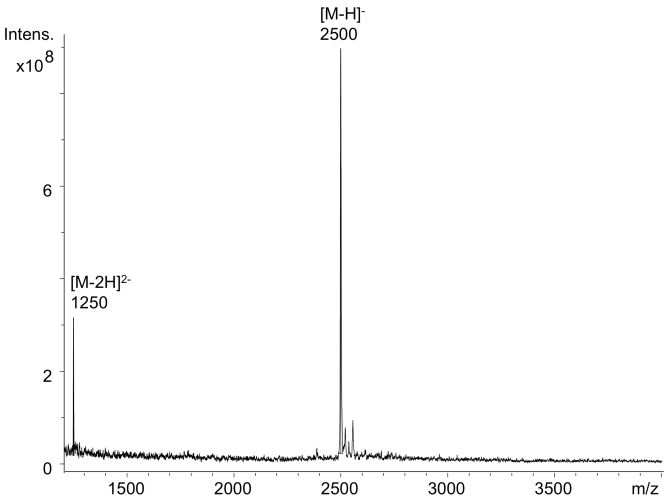


Figure S15. MALDI-TOF mass spectrum of 5'-U^{S4}U^{S2}TTCCAC-3' (**16**).

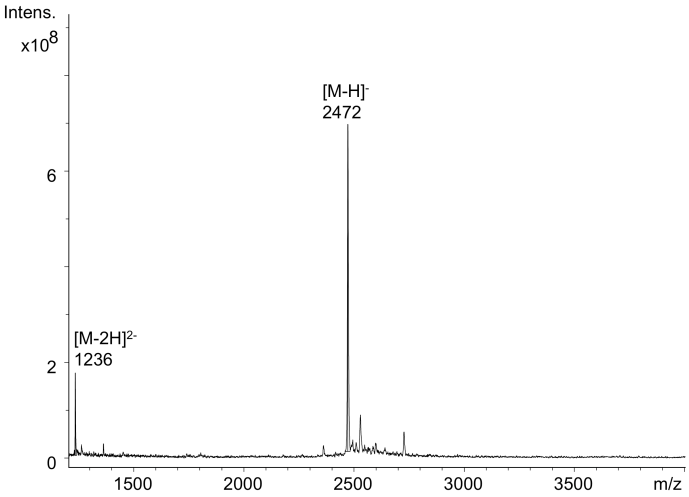


Figure S16. MALDI-TOF mass spectrum of 5'-U^{S2}U^{S2}TTCCAC-3' (**17**).

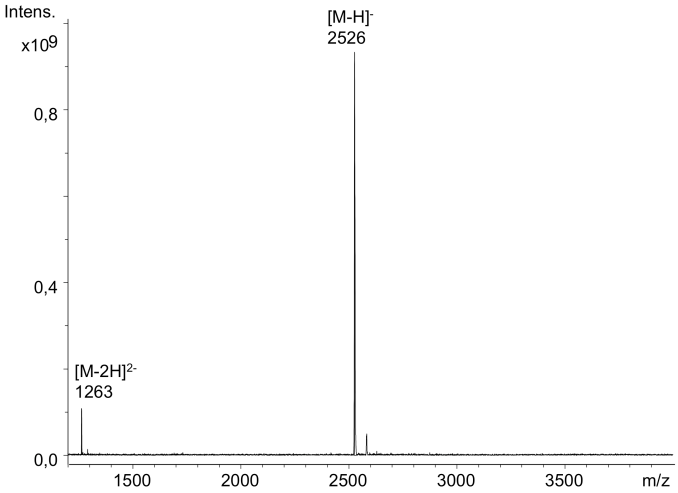


Figure S17. MALDI-TOF mass spectrum of 5'-U^{S4}TU^{S4}TCCAC-3' (**18**).

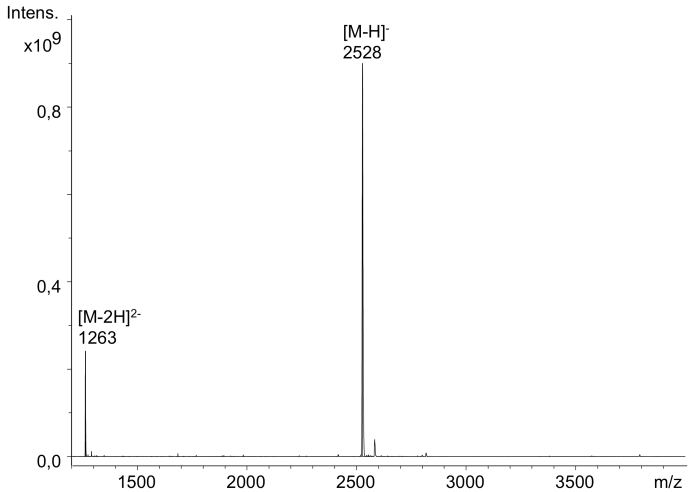


Figure S18. MALDI-TOF mass spectrum of 5'-TU^{S4}TU^{S4}CCAC-3' (**19**).

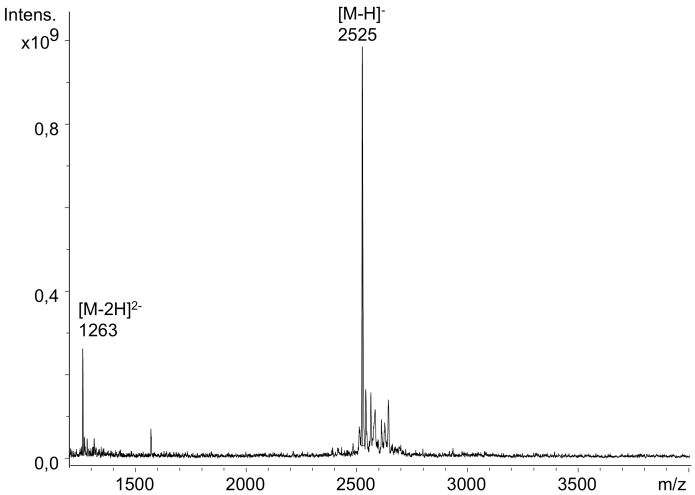


Figure S19. MALDI-TOF mass spectrum of 5'-TTU^{S4}U^{S4}CCAC-3' (**20**).

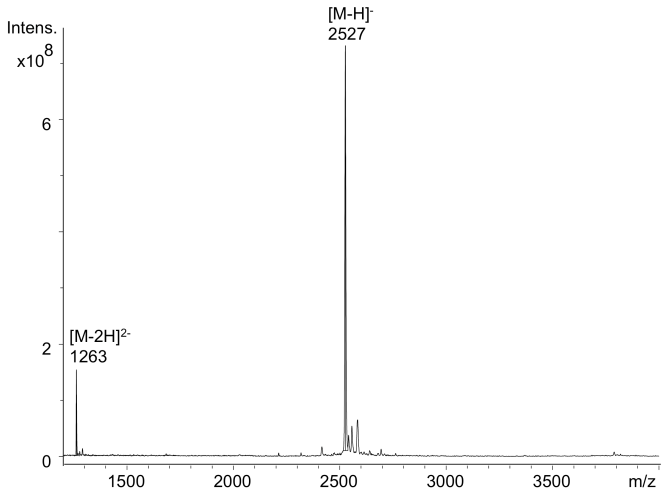


Figure S20. MALDI-TOF mass spectrum of 5'-CCTU^{S4}U^{S4}TAC-3' (**21**).

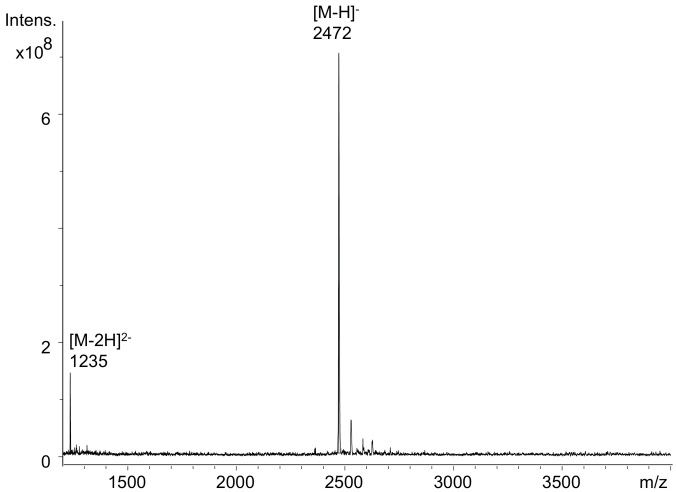


Figure S21. MALDI-TOF mass spectrum of 5'-CCTU^{S2}U^{S2}TAC-3' (**22**).

MALDI-TOF Mass Spectra with Internal Standard

Standard mass accuracy of MALDI-TOF MS with external calibration is insufficient to unambiguously determine the oxidation status of the locked oligodeoxynucleotides (dithiol or disulfide). Shown below are MALDI-TOF mass spectra with internal calibration, acquired in reflectron mode (isotopic resolution). No attempt was made to correct absolute masses.

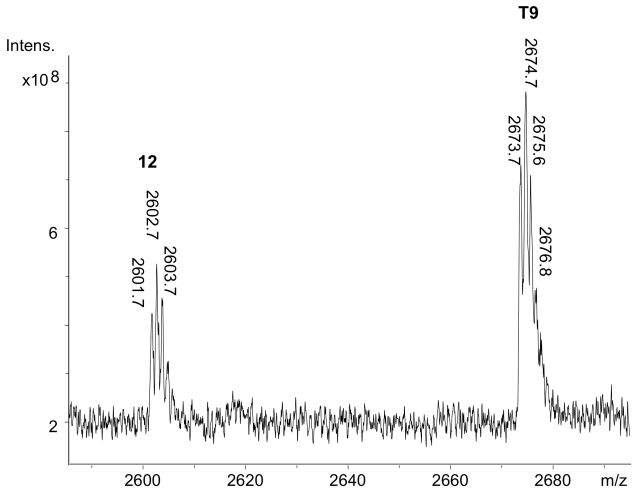
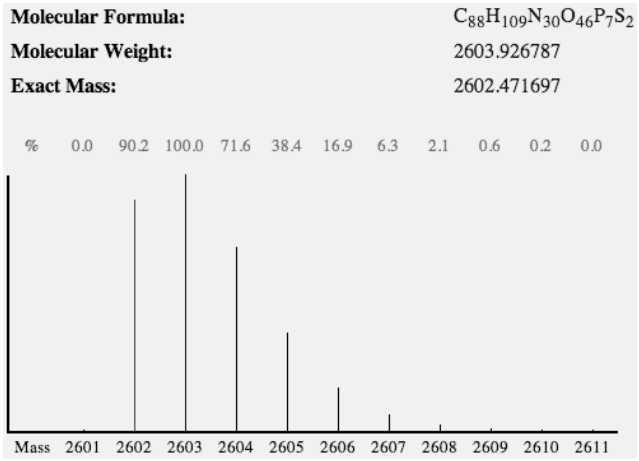


Figure S22. MALDI-TOF mass spectrum of 5'-U^{S4}U^{S4}GCGCAA-3' **12** (monoisotopic mass: 2602 g/mol) and the internal standard 5'-TTTTTTTTT-3' (monoisotopic mass: 2674 g/mol), positive reflectron mode.

a)



b)

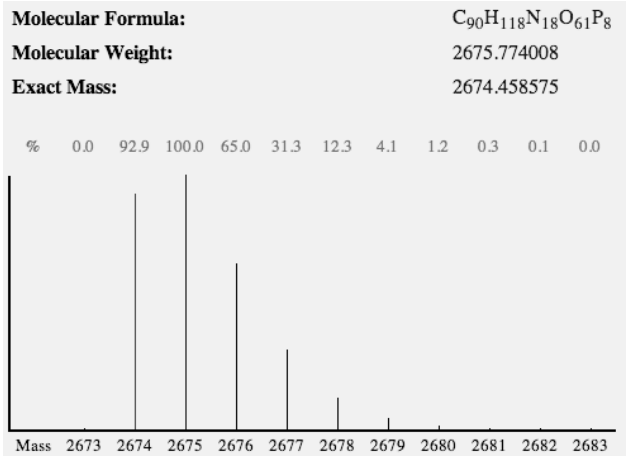


Figure S23. Calculated isotope pattern for the pseudomolecular ions ([M-H]$^-$) a) of disulfide-locked oligodeoxynucleotide **12**, and b) internal standard 5'-TTTTTTTTT-3'. Calculated with Isotope Pattern Calculator v4.0.[‡]

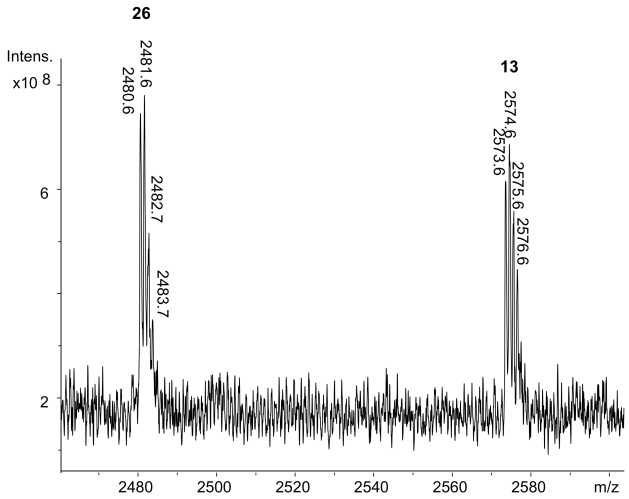
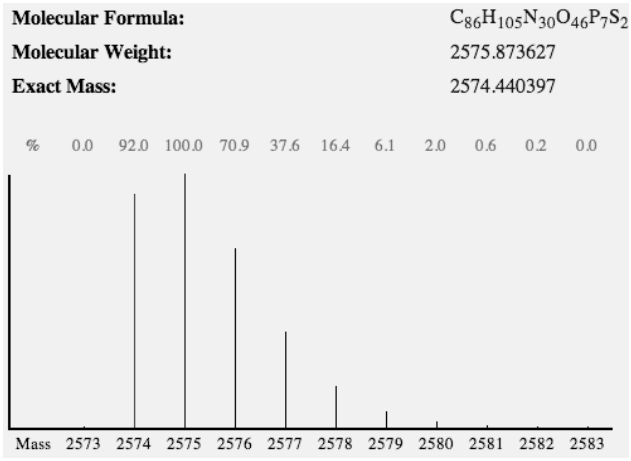


Figure S24. MALDI-TOF mass spectrum of 5'-U^{42}U^{S2}GCGCAA-3' **13** (monoisotopic mass: 2574 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

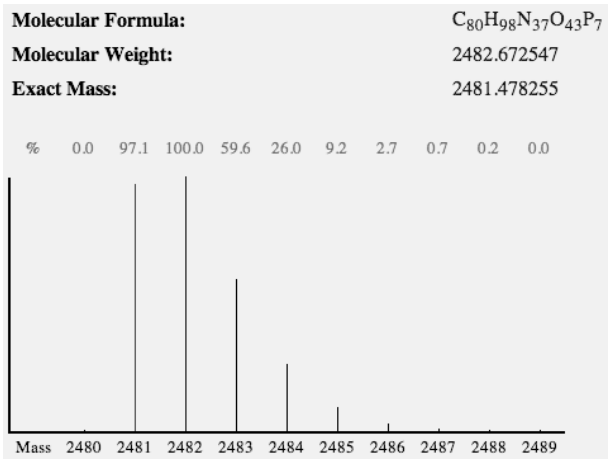


Figure S25. Calculated isotope pattern for the pseudomolecular ions ([M-H]$^-$) a) of disulfide-locked oligodeoxynucleotide **13**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

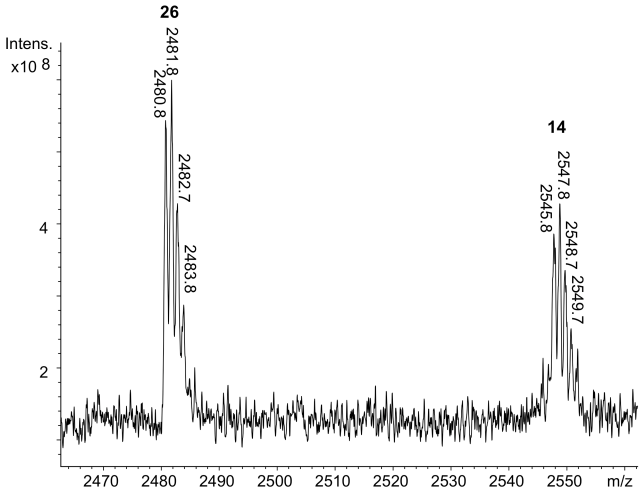
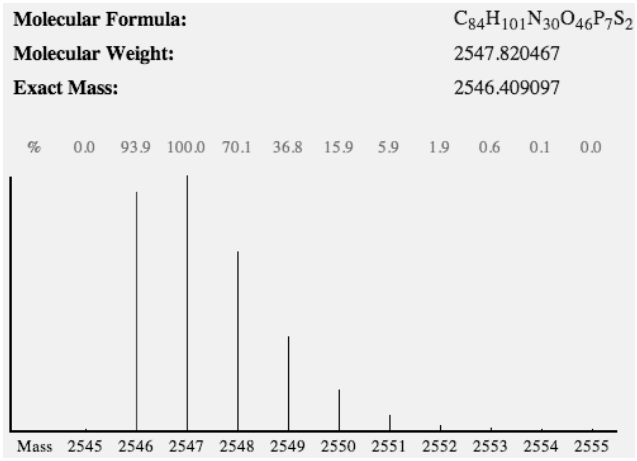


Figure S26. MALDI-TOF mass spectrum of 5'-$U^{S2}U^{S2}$GCGCAA-3' **14** (monoisotopic mass: 2546 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

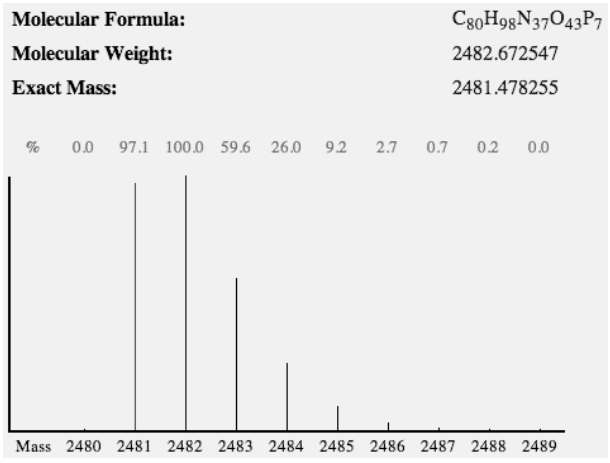


Figure S27. Calculated isotope pattern for the pseudomolecular ions ($[M-H]^-$) a) of disulfide-locked oligodeoxynucleotide **14**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

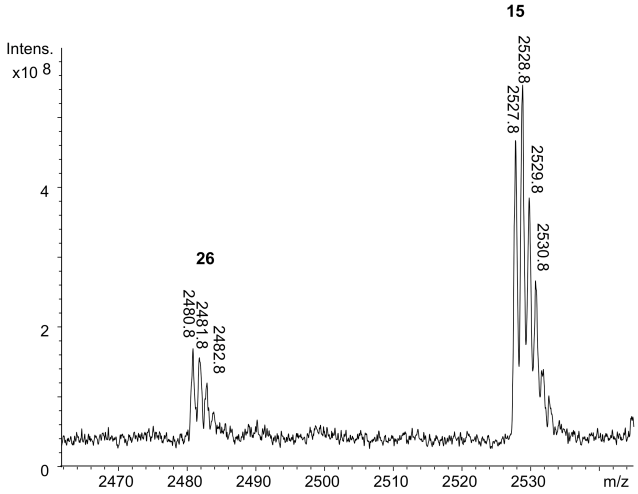
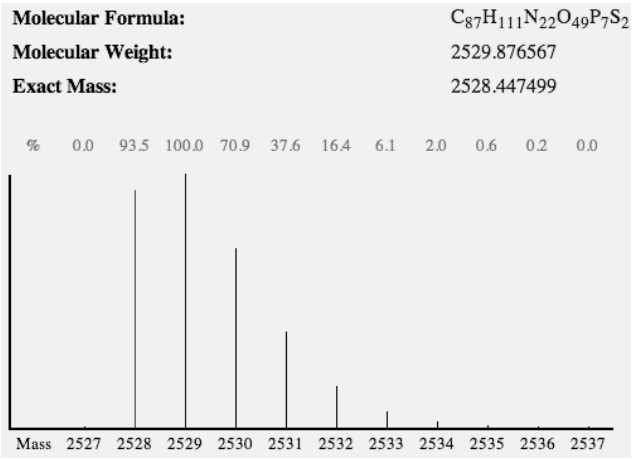


Figure S28. MALDI-TOF mass spectrum of 5'-$U^{S4}U^{S4}$TTCCAC-3' **15** (monoisotopic mass: 2528 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

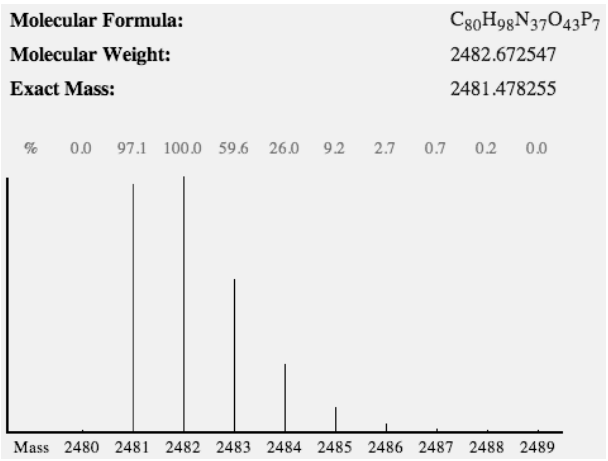


Figure S29. Calculated isotope pattern for the pseudomolecular ions ($[M-H]^-$) a) of disulfide-locked oligodeoxynucleotide **15**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

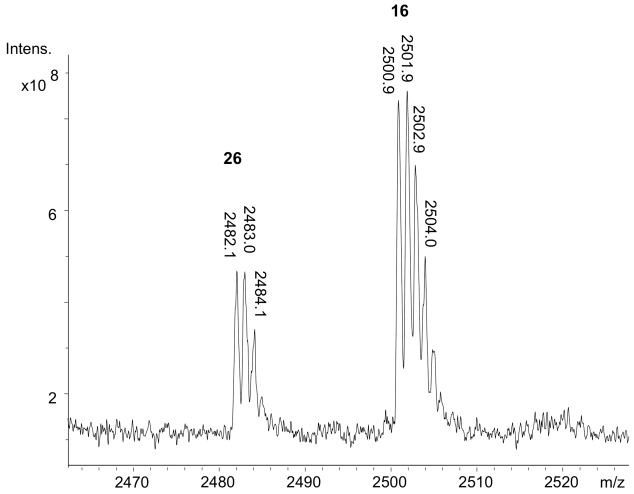
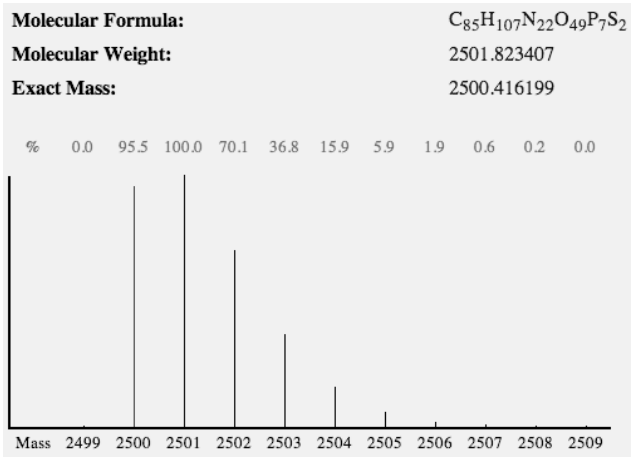


Figure S30. MALDI-TOF mass spectrum of 5'-U^{4S}U^{2S}TTCCAC-3' **16** (monoisotopic mass: 2500 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

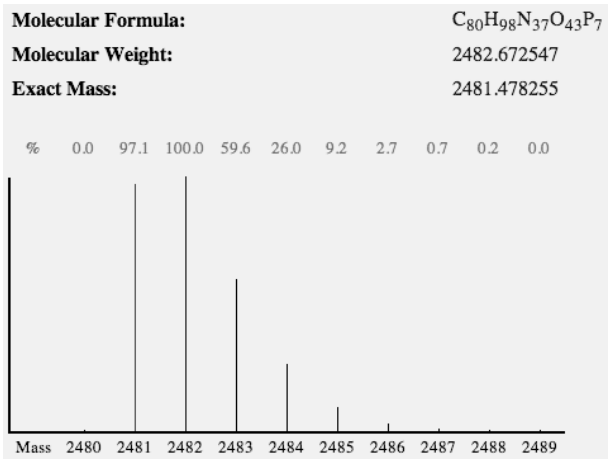


Figure S31. Calculated isotope pattern for the pseudomolecular ions ([M-H]$^-$) a) of disulfide-locked oligodeoxynucleotide **16**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

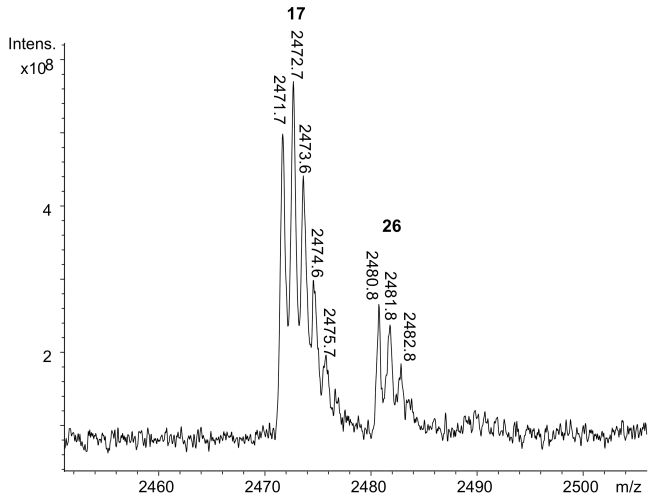
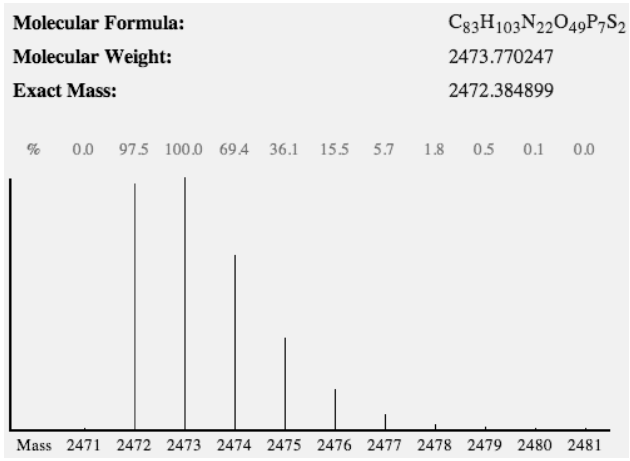


Figure S32. MALDI-TOF mass spectrum of 5'-$U^{S2}U^{S2}$TTCCAC-3' **17** (monoisotopic mass: 2472 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

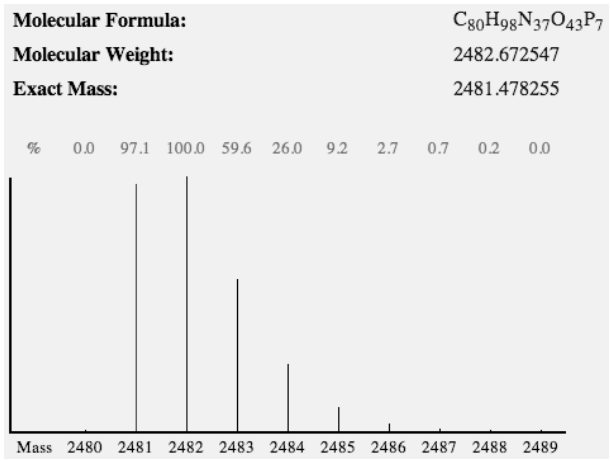


Figure S33. Calculated isotope pattern for the pseudomolecular ions ($[M-H]^-$) a) of disulfide-locked oligodeoxynucleotide **17**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

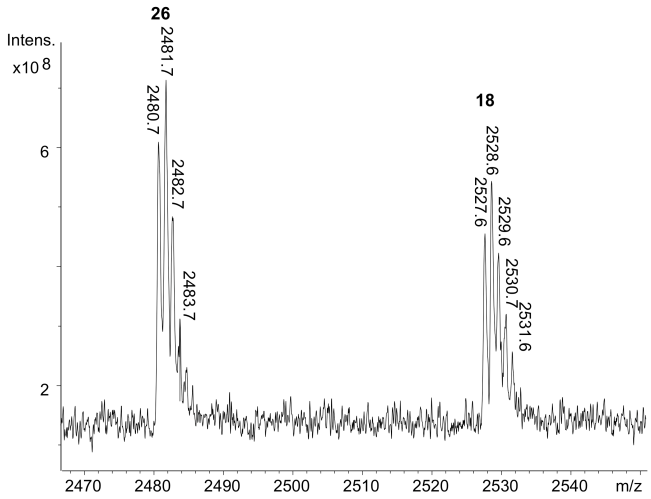
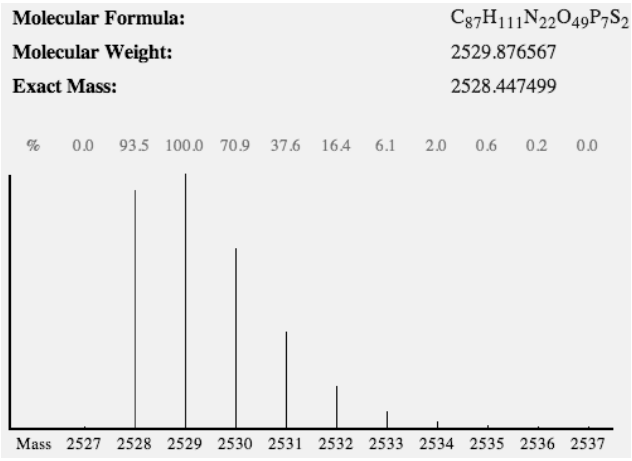


Figure S34. MALDI-TOF mass spectrum of 5'-U^{S4}TU^{S4}TCCAC-3' **18** (monoisotopic mass: 2528 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

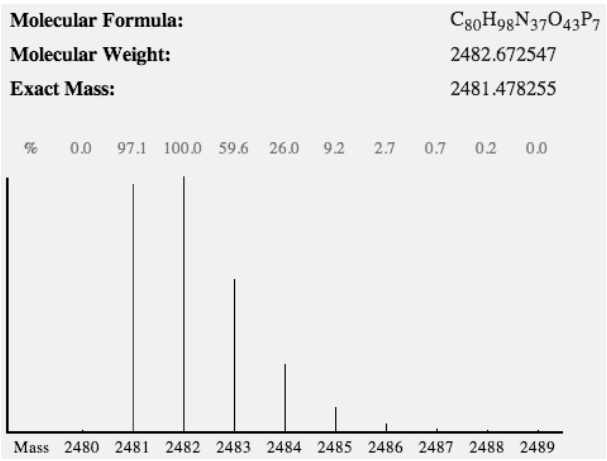


Figure S35. Calculated isotope pattern for the pseudomolecular ions ([M-H]$^-$) a) of disulfide-locked oligodeoxynucleotide **18**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

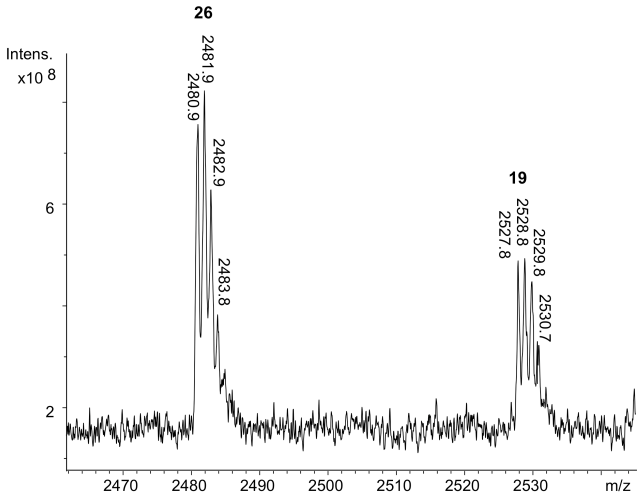
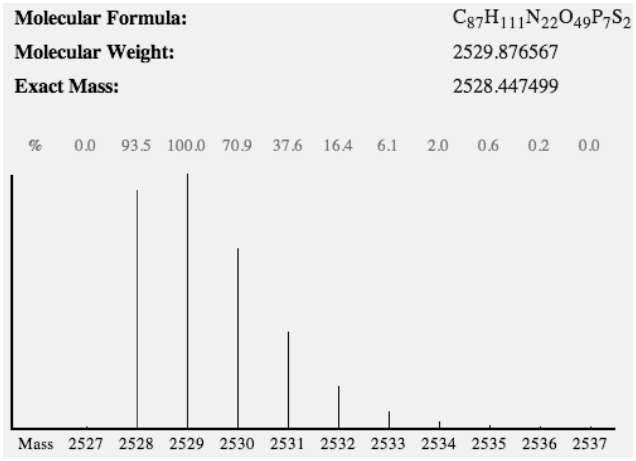


Figure S36. MALDI-TOF mass spectrum of 5'-TU^{S4}TU^{S4}CCAC-3' **19** (monoisotopic mass: 2528 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

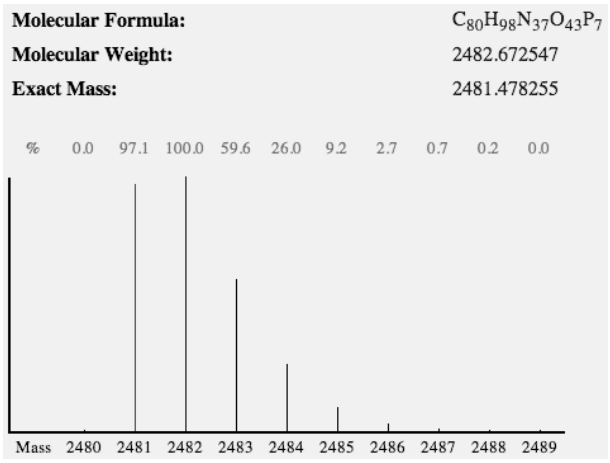


Figure S37. Calculated isotope pattern for the pseudomolecular ions ([M-H]$^-$) a) of disulfide-locked oligodeoxynucleotide **19**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

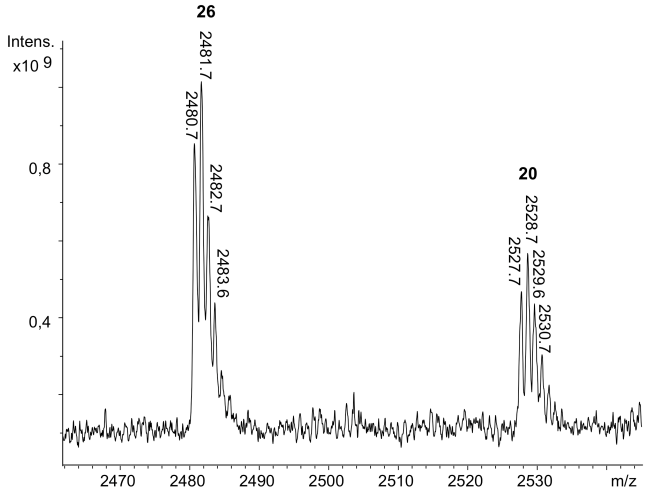
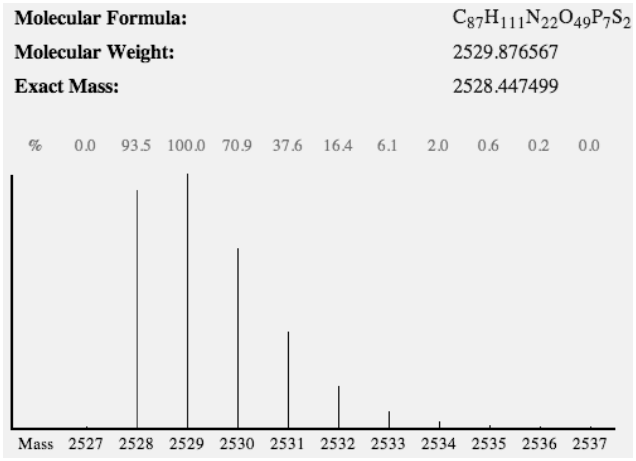


Figure S38. MALDI-TOF mass spectrum of 5'-TTU^{S4}U^{S4}CCAC-3' **20** (monoisotopic mass: 2528 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

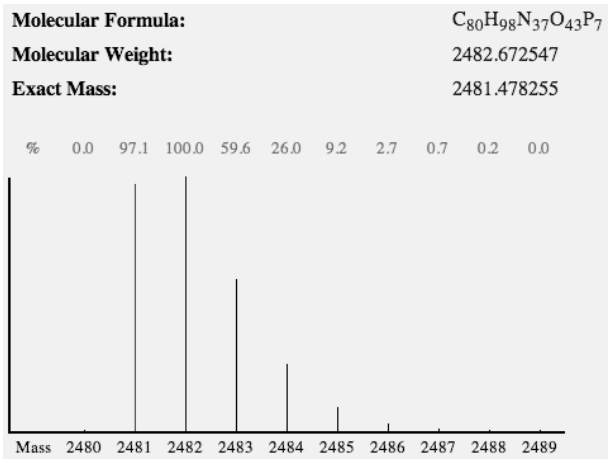


Figure S39. Calculated isotope pattern for the pseudomolecular ions ([M-H]$^-$) a) of disulfide-locked oligodeoxynucleotide **20**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

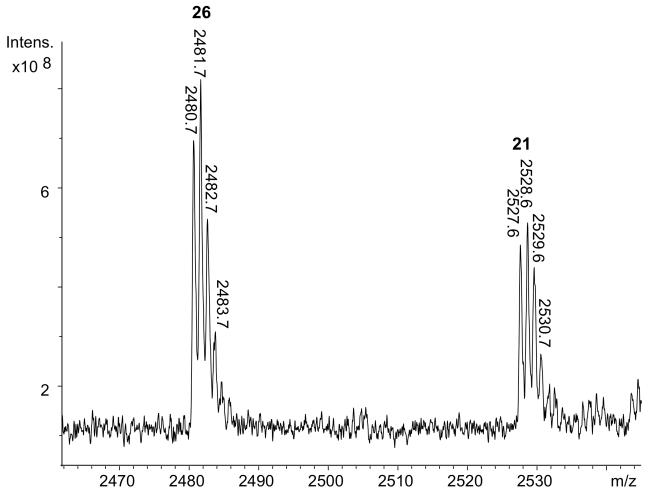
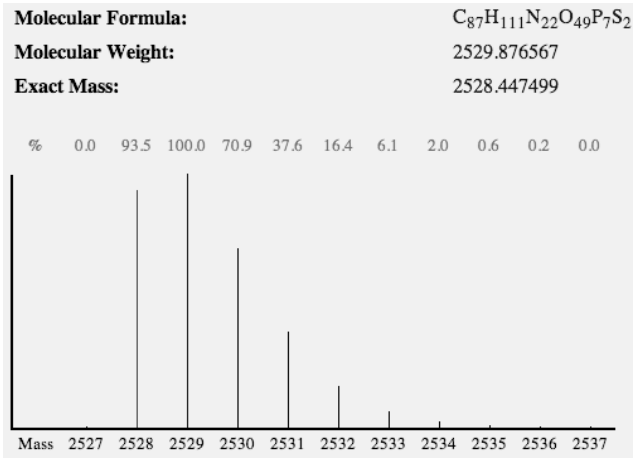


Figure S40. MALDI-TOF mass spectrum of 5'-CCTU^{S4}U^{S4}AC-3' **21** (monoisotopic mass: 2528 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

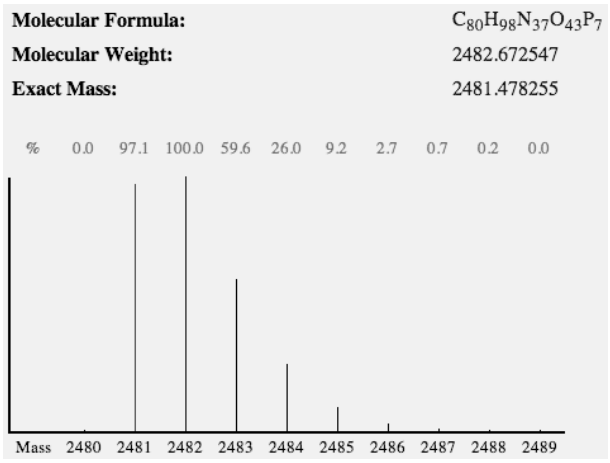


Figure S41. Calculated isotope pattern for the pseudomolecular ions ([M-H]$^-$) a) of disulfide-locked oligodeoxynucleotide **21**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

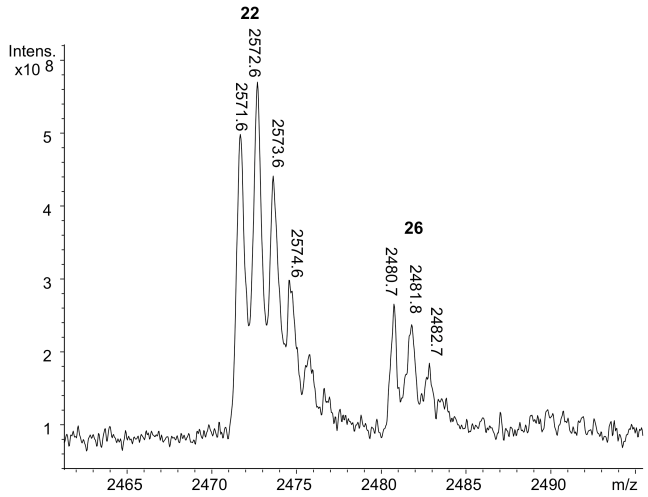
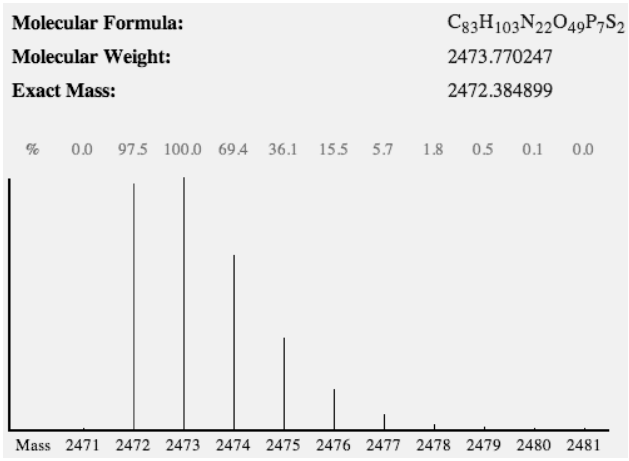


Figure S42. MALDI-TOF mass spectrum of 5'-CCTU^{S2}U^{S2}TAC-3' **22** (monoisotopic mass: 2472 g/mol) and the internal standard 5'-GTGGAAAA-3' **26** (monoisotopic mass: 2481 g/mol), positive reflectron mode.

a)



b)

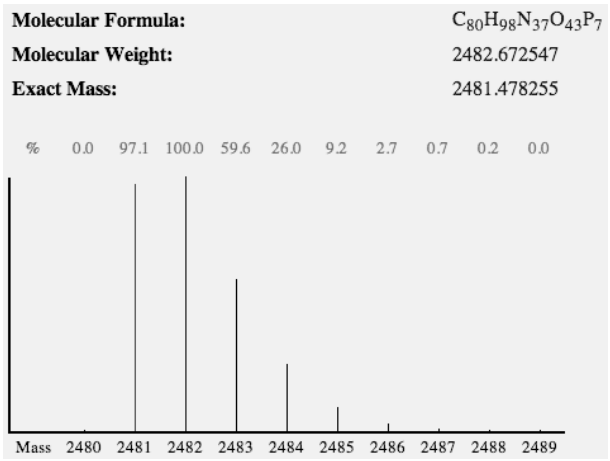


Figure S43. Calculated isotope pattern for the pseudomolecular ions ([M-H]$^-$) a) of disulfide-locked oligodeoxynucleotide **22**, and b) internal standard **26**. Calculated with Isotope Pattern Calculator v4.0.[‡]

UV-Melting Curves

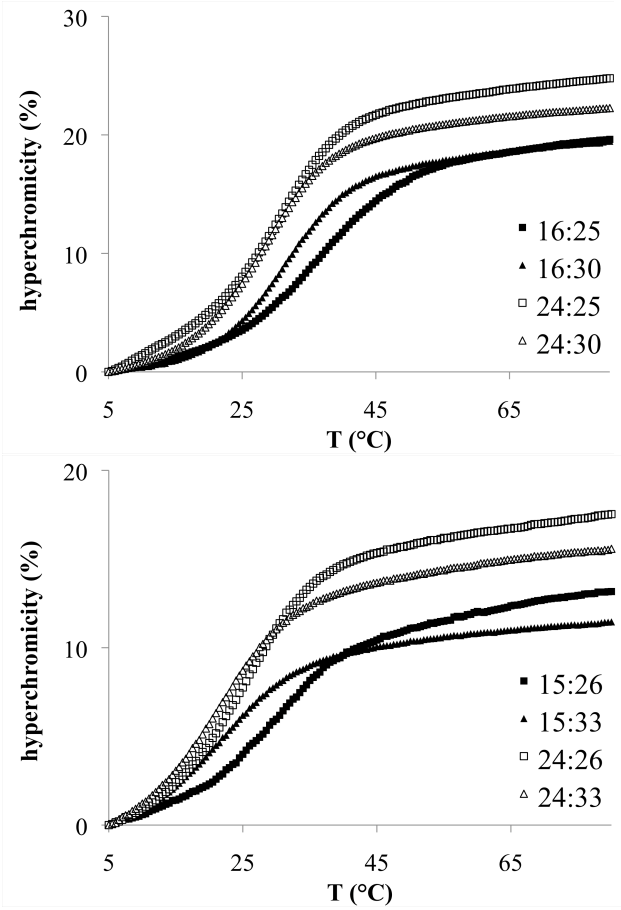


Figure S44. UV-melting curves of duplexes with or without mismatch. Top: RNA targets, with lock (5'-U^{S4}U^{S2}TTCCAC-3': 5'-*r*(GUGGAAAA)-3' **16**:**25** and 5'-U^{S4}U^{S2}TTCCAC-3':5'-*r*(GUGGAAAU)-3' **16**:**30**) or without lock (5'-TTTTCCAC-3': 5'-*r*(GUGGAAAA)-3' **24**:**25** and 5'-TTTTCCAC-3':5'-*r*(GUGGAAAU)-3' **24**:**30**); Bottom: with DNA targets, with lock (5'-U^{S4}U^{S4}TTCCAC-3':5'-GTGGAAAA-3' **15**:**26** and 5'-U^{S4}U^{S4}TTCCAC-3':5'-GTGGAAAT-3' **15**:**33**) or without lock (5'-TTTTCCAC-3':5'-GTGGAAAA-3' **24**:**26** and 5'-TTTTCCAC-3':5'-GUGGAAAT-3' **24**:**33**). Conditions: Average of 4 curves, 1.5 μM strand concentration, 1 M NaCl, 10 mM phosphate buffer, pH 7.0.

Representative Thermodynamics of Duplex Formation

Table S1. Thermodynamic Parameters for Duplex Dissociation, derived from Fits to Melting Curve Data[a] using Meltwin 3.5.

Target strand Probe strand	ΔH° (kcal/mol)	ΔS° (cal/mol K)	ΔG° (kcal/mol)
5'-*r*(GUGGAAAA)-3' (**25**)			
5'-TTTTCCAC-3' (**24**)	-54	-149	-7.5
5'-$U^{S4}U^{S2}$TTCCAC-3' (**16**)	-50	-135	-8.4
5'-GTGGAAAA-3' (**26**)			
5'-TTTTCCAC-3' (**24**)	-45	-123	-6.6
5'-$U^{S2}U^{S2}$TTCCAC-3' (**17**)	-43	-114	-7.6

[a] melting points were determined at 1.5 μM strand concentration and 1 M NaCl, 10 mM sodium phosphate buffer, pH 7.0.

Circular Dicroism Spectra

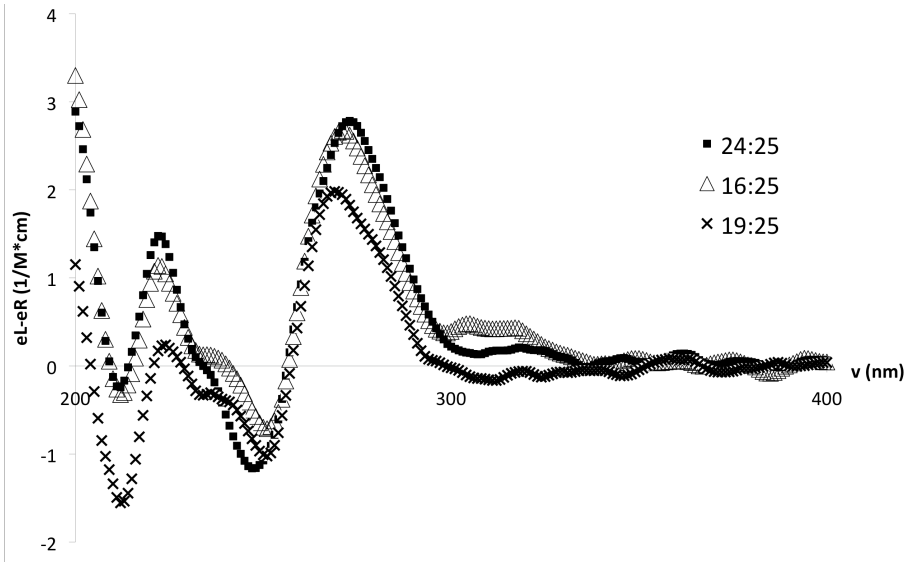


Figure S45. CD spectra of duplexes with RNA target strand. The duplex of unmodified 5'-TTTTCCAC-3' (**24**) with its perfect match target RNA strand **25** shows the Cotton effects characteristic for A-form duplexes, with a positive maximum at 272 nm, a negative maximum at 249 nm, and positive bands at 236 nm and 223 nm, as well as a negative signal at 214 nm. An almost identical spectrum is found for the duplex of locked 5'-$U^{S4}U^{S2}$TTCCAC-3' (**16**) with **25** (extrema at 271 nm, 251 nm, 240 nm, 224 nm and 214 nm) suggesting that the duplex is not disturbed by the intrastrand lock. As a negative control, the duplex of 5'-$TU^{S4}TU^{S2}$CCAC-3' (**19**) with **25**, believed to have a too short lock, is also shown (extrema at 269 nm, 252 nm, 239 nm, 226 nm, and 213 nm), which shows minor changes in the curve.

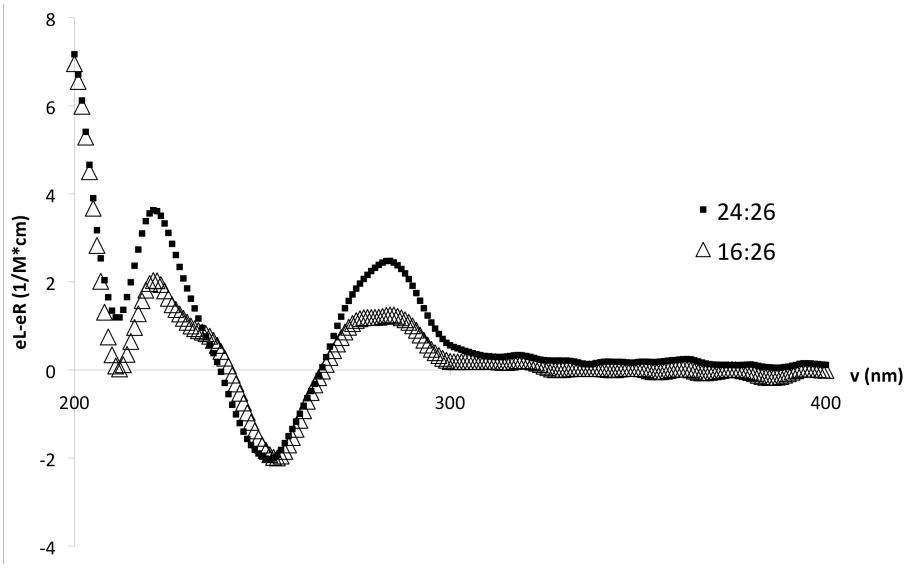


Figure S46. CD spectra of duplexes with DNA target strands. The perfect match duplex of **24** with **26** shows characteristic signals for a B DNA form duplex, with a large positive signal at 285 nm, a strong minimum at 256 nm, a positive signal at 222 nm and a negative band at 208 nm. The modified DNA duplex **16**:**26** shows an almost identical shape, with an additional local maximum at 236 nm. (other peaks: 287–279 nm, 258 nm, 236 nm, 223 nm and 210 nm).

References for Supplementary Information

[1] N. C. Chaudhuri and E. T. Kool, *J. Am. Chem. Soc.* 1995, **117**, 10434–10442.
[2] S. E. Osborne, R. J. Cain and G. D. Glick, *J. Am. Chem. Soc.* 1997, **119**, 1171–1182.
[3] J. D. Kahl and M. M. Greenberg, *J. Am. Chem. Soc.* 1999, **121**, 597–604.
[4] H. A. Held, A. Roychowdhury and S. A. Benner, *Nucleosides, Nucleotides Nucl. Acids* 2003, **22**, 391–404.
‡ This tool is available at: http://yanjunhua.tripod.com/pattern.htm