Synthesis and Application of a 5′-Aldehyde Phosphoramidite for Covalent Attachment of DNA to Biomolecules

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General Experimental Procedures. Reagents were commercial grade and used without purification unless otherwise indicated. Dry solvents were obtained from sealed bottles (pyridine, MeOH) or by passage through activated neutral alumina under positive argon pressure (CH_2Cl_2, THF). All reactions were performed at room temperature under argon or nitrogen unless otherwise noted. A reaction temperature of 0 °C was maintained with an ice-water bath. Thin-layer chromatography (TLC) was performed on silica gel plates pre-coated with fluorescence indicator with visualization by UV light (254 nm). Organic solutions that were dried over Na_2SO_4 were filtered through paper to remove the drying agent, and the paper was rinsed into the collection flask with an appropriate organic solvent. All chromatographic purifications used 230-400 mesh silica gel. NMR spectra were obtained at the indicated frequencies. ^{1}H and ^{13}C chemical shifts in parts per million (δ) were referenced to internal tetramethylsilane (TMS). The designation "ABq" for a ^{1}H NMR peak indicates that the particular peak was one partner of an AB quartet; if additional splittings were evident, they are noted following the ABq designation (e.g., ABqd). In the ^{1}H NMR spectra, peaks not assigned to the indicated compound are labeled as follows: w = water; n = NMR solvent; s = common solvent (e.g., CH_2Cl_2 or EtOAc). ^{31}P chemical shifts in δ were referenced to external H_3PO_4 at 0 ppm.

Tabulation of ^{1}H and ^{13}C NMR spectral peaks for compounds 3–7

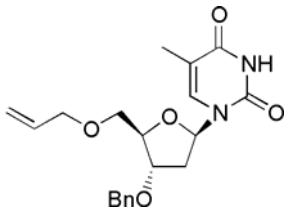
5′-*O*-Allyl-3′-*O*-benzylthymidine (3): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (br s, 1H), 7.63 (br q, 1H, J = 1.0 Hz), 7.39-7.28 (m, 5H), 6.38 (dd, 1H, J = 6.0 Hz), 5.89 (ddt, 1H, J = 15.0, 10.5, 5.0 Hz), 5.27 (dq, 1H, J = 17.0, 1.5 Hz), 5.22 (dq, 1H, J = 10.2, 1.5 Hz), 4.51 and 4.57 (ABq, 1H, J = 11.6 Hz), 4.25 (dt, 1H, J = 6.0, 2.5 Hz), 4.23 (q, 1H, J = 2.5 Hz), 4.04 (m, 2H), 3.71 and 3.62 (ABqd, 2H, J = 10.6, 2.0 Hz), 2.47 (ddd, 1H, J = 13.2, 5.6, 2.4 Hz), 2.10 (ddd, 1H, J = 13.2, 7.6, 5.2 Hz), 1.89 (d, 3H, J = 1.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.8, 150.3, 137.5, 135.8, 133.9, 128.5, 127.6, 117.5, 110.8, 85.1, 83.9, 79.2, 72.3, 71.3, 70.4, 37.9, 12.5.

5′-*O*-(2,3-Dihydroxypropyl)-3′-*O*-benzylthymidine (4): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (br s, 1H), 7.41-7.29 (m, 6H), 6.25 (m, 1H), 4.54 (ABq, 2H, J = 11.6 Hz), 4.21 (dt, 1H, J = 6.0, 2.5 Hz), 4.19 (q, 1H, J = 3.0 Hz), 3.89 (m, 1H), 3.78-3.52 (m, 6H), 2.73 (br s, 1H), 2.46 (m, 1H), 2.28 (br s, 1H), 2.14 (m, 1H), 1.92 (d, 3H, J = 1.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.4, 151.0, 137.0, 136.0, 128.6, 128.0, 127.7, 110.0, 85.8, 83.6, 78.6, 72.9, 71.5, 71.3, 70.7, 64.0, 38.2, 12.7.

5′-*O*-(2,3-Di-*O*-benzoylpropyl)-N^3-benzoyl-3′-*O*-benzylthymidine (5): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60-8.05 and 7.95-7.91 (m, 6H) (mixture), 7.65-7.42 (m, 7H) (mixture), 7.34-7.20 (m, 5H) (mixture), 6.31 (dd, 1H, J = 6.0 Hz) (minor), 6.29 (dd, 1H, J = 6.0 Hz) (major), 5.73 (m, 1H) (minor), 5.66 (m, 1H) (major), 4.69-4.55 (m, 2H) (mixture), 4.46 (ABq, 2H, J = 11.6 Hz) (major), 4.35 (ABq, 2H, J = 11.6 Hz) (minor), 4.21 (m, 2H) (major), 4.17 (m, 1H, J = 3.0 Hz) (minor), 4.09 (m, 1H) (minor), 3.98-3.86 (m, 1H), 3.86-3.52 (m, 3H), 2.46 (ddd, 1H, J = 13.2, 5.6, 2.4 Hz) (major), 2.39 (ddd, 1H, J = 13.2, 5.6, 2.4 Hz) (minor), 2.06 (m, 1H) (mixture of diastereomers), 1.93 (d, 3H, J = 1.0 Hz) (minor diastereomer), 1.92 (d, 3H, J = 1.0 Hz) (major diastereomer); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.0, 166.1, 162.8, 150.0, 135.2, 135.0, 133.6, 133.4, 130.5, 129.70, 129.67, 129.1, 127.9, 127.7, 128.6, 128.5, 110.5, 85.4, 83.8, 78.9, 71.6, 71.5, 70.5, 69.9, 63.2, 37.8, 12.7.

5′-*O*-(2,3-Di-*O*-benzoylpropyl)-N^3-benzoylthymidine (6): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06-8.00 and 7.94-7.88 (m, 6H) (mixture), 7.65-7.54 and 7.50-7.41 (m, 7H) (mixture), 6.30 (t, 1H, J = 6.0 Hz) (minor), 6.25 (t, 1H, J = 6.0 Hz) (major), 5.71 (m, 1H) (mixture), 4.66 (d, 2H, J = 4.8 Hz) (mixture), 4.43 (m, 1H) (mixture), 4.08-3.68 (m, 5H) (mixture), 2.71 (br s, 1H) (major), 2.42 (br s, 1H) (minor), 2.35-2.22 (m, 1H) (mixture), 2.20-2.12 (m, 1H) (mixture of diastereomers), 1.92 (d, 3H, J = 1.2 Hz) (minor diastereomer), 1.90 (d, 3H, J = 1.2 Hz) (major diastereomer); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.0, 166.2, 162.8, 149.2, 135.4, 135.0, 133.7, 133.5, 131.5, 130.4, 129.7, 129.3, 129.1, 128.6, 128.5, 111.0, 85.1, 84.9, 70.7, 70.4, 70.3, 69.8, 63.0, 40.4, 12.7.

5′-*O*-(2,3-Di-*O*-benzoylpropyl)-N^3-benzoylthymidine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)-phosphoramidite (7): ^{1}H NMR (400 MHz, acetone-d_6) δ 8.10-7.98 (m, 6H) (mixture), 7.83, 7.81, 7.79 and 7.78 (br d, 1H, J = 1.0 Hz) (4 diastereomers), 7.76-7.72 and 7.68-7.48 (m, 8H) (mixture), 6.31 (m, 1H) (mixture), 5.86-5.71 (m, 1H) (mixture), 4.82-4.58 (m, 3H) (mixture), 4.32, 4.29, 4.23, 4.22 (q, 1H, J = 3.0 Hz) (4 diastereomers), 4.14-3.72 (m, 6H) (mixture), 3.63 (m, 2H) (mixture), 2.76 (m, 2H) (mixture), 2.52-2.30 (m, 2H) (mixture), 1.88 (m, 3H) (mixture), 1.17 (m, 12H) (mixture of diastereomers); ^{13}C NMR (125 MHz, acetone-d_6) δ 169.7, 165.9, 162.7, 149.7, 136.4, 135.2, 133.6, 133.5, 132.3, 130.5, 129.7, 129.6, 129.4, 128.8, 119.0, 110.5, 85.7, 85.4, 74.3, 71.2, 70.4, 67.9, 63.6, 58.6, 43.2, 39.5, 24.1, 20.0, 12.1.



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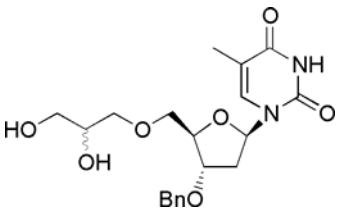

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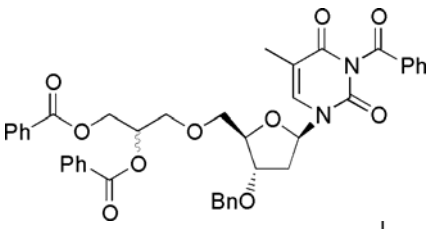

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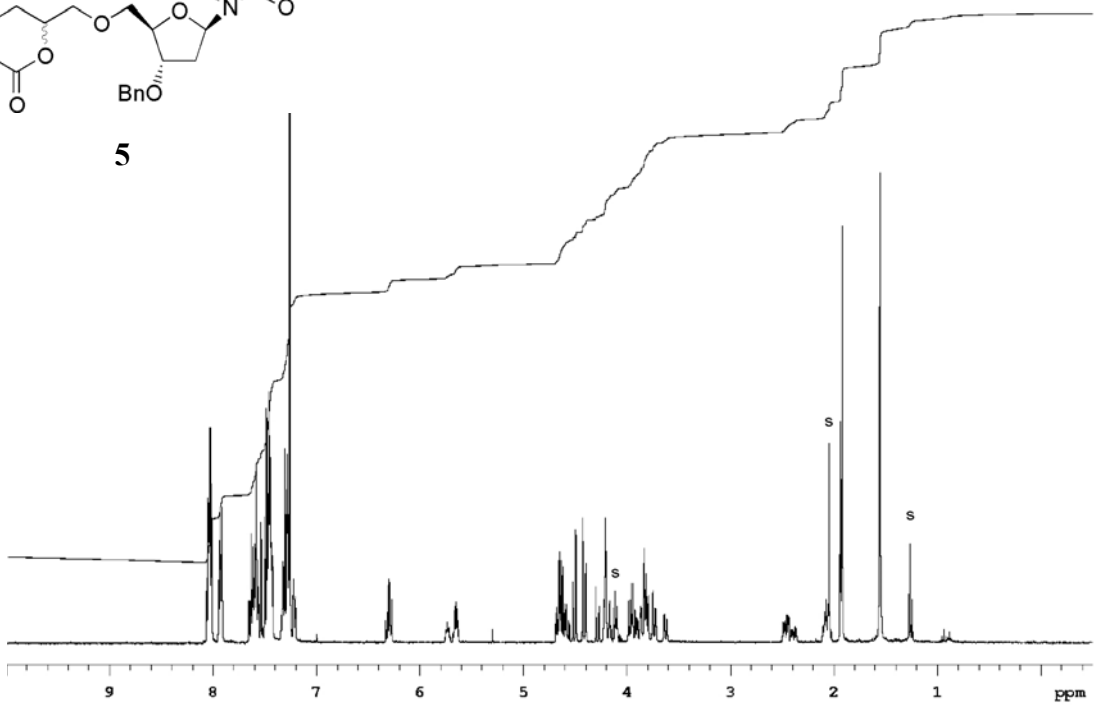

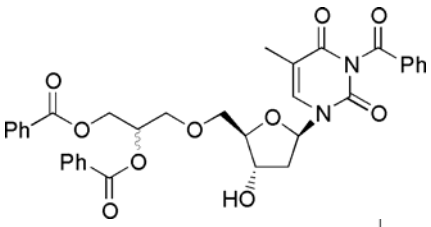

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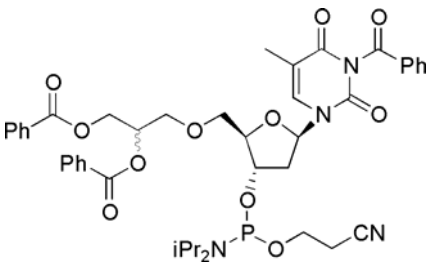

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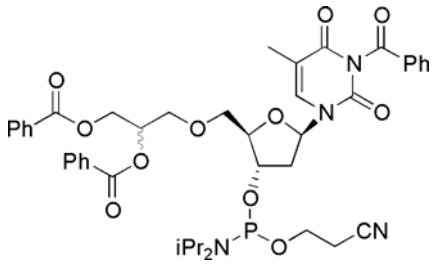
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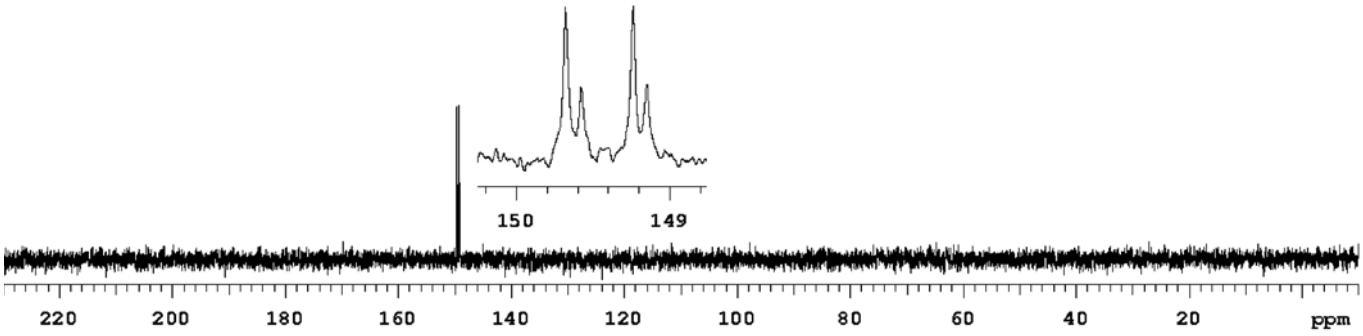
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