Nucleoside $H$-boranophosphonates: a new class of boron-containing nucleotide analogues

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Supporting Information

General
Dry organic solvents were prepared by appropriate procedures prior to use. The other organic solvents were reagent grade and used as received. Analytical TLC was performed on Merck Kieselgel 60-F254 plates. Silica gel column chromatography was carried out using Kanto silica gel 60N (spherical, neutral, 63–210 μm). Medium-pressure liquid chromatography (MPLC) was performed on a pre-packed column (Yamazen ODS-S-50B, 26 × 300 mm, 40 mm, 60 Å) at a flow rate of 6 mL/min. All NMR spectra were recorded on a Varian Mercury 300. $^1$H NMR spectra were obtained at 300 MHz with tetramethylsilane (TMS) (δ 0.0) as an internal standard in CDCl$_3$ or with 3-(trimethylsilyl)propionic acid-d$_4$ sodium salt (δ 0.0) as an external standard in D$_2$O. $^{13}$C NMR spectra were obtained at 75.5 MHz with CDCl$_3$ as an internal standard (δ 77.0) in CDCl$_3$ or with 3-(trimethylsilyl)propionic acid-d$_4$ sodium salt (δ 0.0) as an external standard in D$_2$O. $^{31}$P NMR spectra were obtained at 121.5 MHz with 85% H$_3$PO$_4$ (δ 0.0) as an external standard in CDCl$_3$ or in D$_2$O. ESI mass spectra were recorded on an Applied Biosystems QSTAR.

Pyridinium $H$-boranophosphonate (7)

Phosphinic acid solution in water (50 wt%, 5.2 mL, 50 mmol) was concentrated under reduced pressure and the residue was dried by repeated coevaporation with dry pyridine (15 × 10 mL) and dry toluene (3 × 10 mL). The residue was then dissolved in dry MeCN (50 mL) under argon. N$_2$O-Bis(trimethylsilyl)benzamide (42.9 mL, 150 mmol) was added dropwise to the mixture over 5 min, and the mixture was stirred for 1 h at rt. The mixture was then cooled to 0 °C and a 1.01 M solution of BH$_3$·THF in dry THF (59.4 mL, 60 mmol) was added dropwise over 5 min. Dry MeOH (50 mL) and dry pyridine (20 mL) were then successively added, and the mixture was stirred for 5 min at 0 °C and overnight at rt. The mixture was concentrated under reduced pressure and any residual volatile solvents were removed by coevaporation with dry toluene (3 × 20 mL). The residue was dissolved in H$_2$O-pyridine (1:1, v/v) (200 mL) and washed with CHCl$_3$ (7 × 50 mL). The aqueous layer was concentrated under reduced pressure, dried by repeated coevaporation with dry pyridine (3 × 5 mL) and dry toluene (3 × 5 mL), and in vacuo to afford 7 (3.87 g, 24 mmol, 49%) as a white waxy solid. The organic layers were combined and back-extracted with H$_2$O (3 × 100 mL). The
aqueous layers were combined and concentrated under reduced pressure. The residue was dried by repeated coevaporation with dry pyridine (3 × 5 mL) and dry toluene (3 × 5 mL). The residue was dissolved in H2O (100 mL) and washed with CHCl3 (7 × 50 mL). The aqueous layer was concentrated under reduced pressure, dried by coevaporation with dry pyridine (3 × 5 mL) and dry toluene (3 × 5 mL), and in vacuo to afford 7 (3.39 g, 21 mmol, 43%, total yield 92%) as a white waxy solid. 1H NMR (300 MHz, D2O) δ 8.55 (m, 2H), 8.30 (m, 1H), 7.78 (m, 2H), 7.03 (brd, 1JPH = 399 Hz, 1H), 0.17 (dq, 2JPB = 88.8 Hz, 3H). 31P NMR (121.5 MHz, D2O) δ 94.2 (q, 1JPB = 109.1 Hz). ESI-HRMS: m/z calcd for BH5O2P− [(M−H+)−] 79.0126, found 79.0121.

Triethylammonium 3′-O-dimethoxytrityl-N3-benzoylthymidine-5′-H-boranophosphonate (9a)

3′-O-Dimethoxytrityl-N3-benzoylthymidine 8a (0.649 g, 1.0 mmol) and 7 (0.191 g, 1.2 mmol) were dried by repeated coevaporation with dry pyridine (3 × 5 mL) and dissolved in dry pyridine (10 mL) under argon. Bop-Cl (0.305 g, 1.2 mmol) was added, and the mixture was stirred for 2 h at rt. 0.5 M triethylammonium bicarbonate (TEAB) buffer (pH 7.0) (10 mL) was added, and the mixture was extracted with CHCl3 (3 × 50 mL). The organic layers were combined, washed with 0.5 M TEAB buffer (50 mL), dried over Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (25 g of silica gel, gradient elution of 1–4% MeOH–CH2Cl2 with 1% Et3N) to afford 9a (0.771 g, 0.95 mmol, 95%) as a colorless foam. A 56:44 mixture of P-diastereomers (1H NMR). 1H NMR (300 MHz, CDCl3) δ 12.8 (br, 1H), 7.92 (m, 2H), 7.77 (d, J = 11.7 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 7.48–7.17 (m, 11H), 7.12, 7.01 (brd, 1JPH = 386 Hz, brd, 1JPH = 379 Hz, 1H), 6.81 (d, J = 6.9 Hz, 4H), 6.54 (m, 1H), 4.42, 4.35 (d, J = 4.8 Hz, d, J = 5.1 Hz, 1H), 3.97, 3.62, 3.31 (m, m, m, 2H), 3.86, 3.70 (m, m, 1H), 3.77 (s, 6H), 2.92 (q, J = 7.1 Hz, 6H), 2.10–1.87 (m, 2H), 2.00, 1.98 (s, s, 3H), 1.20 (t, J = 7.1 Hz, 9H), 1.00 to −0.9 (br, 3H). 13C NMR (75.5 MHz, CDCl3) δ 169.2, 162.9, 158.6, 149.7, 149.6, 145.0, 136.0, 136.0, 136.0, 134.9, 131.6, 130.2, 130.1, 129.1, 128.5, 128.2, 128.2, 128.0, 127.4, 127.0, 113.3, 111.6, 111.2, 87.2, 87.1, 85.5, 85.1, 85.0, 75.4, 66.7 (d, 2JPC = 8.9 Hz), 66.1 (d, 2JPC = 8.9 Hz), 55.2, 45.4, 39.4, 39.3, 12.4, 8.5. 31P NMR (121.5 MHz, CDCl3) δ 109.4–102.6 (m). ESI-HRMS: m/z calcd for C34H28N2O9P− [(M−H+)−] 709.2492, found 709.2513.

Triethylammonium 3′-O-dimethoxytrityl-thymidine-5′-H-boranophosphonate (9b)
3′-O-Dimethoxytrityl-thymidine 8b (0.545 g, 1.0 mmol) and 7 (0.250 g, 1.6 mmol) were dried by repeated coevaporation with dry pyridine (3 × 5 mL) and dissolved in dry pyridine (10 mL) under argon. Bop-Cl (0.404 g, 1.6 mmol) was added, and the mixture was stirred for 1 h at rt. The mixture was then diluted with CHCl3 (50 mL) and washed with 1 M TEAB buffer (pH 7.0) (20 mL). The aqueous layer was back-extracted with CHCl3 (3 × 50 mL). The organic layers were combined, dried over Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (25 g of silica gel, gradient elution of 1–3% MeOH–CH2Cl2 with 0.5% Et3N) to afford 9b (0.671 g, 0.95 mmol, 95%) as a colorless foam. A 54:46 mixture of P-diastereomers (1H NMR). 1H NMR (300 MHz, CDCl3) δ 8.54 (br, 1H), 7.65, 7.62 (s, s, 1H), 7.45 (d, J = 7.5 Hz, 2H), 7.35–7.19 (m, 7H), 7.11, 7.00 (brd, 1JPH = 388 Hz, brd, 1JPH = 385 Hz, 1H), 6.83 (d, 2JPH = 9.0 Hz, 4H), 6.54 (m, 1H), 4.41, 4.34 (d, 2JPC = 4.8 Hz, 1H), 3.95, 3.62, 3.31 (m, m, m, 2H), 3.90, 3.75 (m, m, 1H), 3.79 (s, 6H), 2.91 (q, J = 7.5 Hz, 6H), 2.01–1.78 (m, 2H), 1.96, 1.94 (s, s, 3H), 1.30 (t, J = 7.5 Hz, 9H), 1.00 to –0.9 (br, 3H). 13C NMR (75.5 MHz, CDCl3) δ 163.9, 158.6, 150.6, 145.1, 145.0, 136.3, 136.2, 136.0, 130.2, 130.1, 128.3, 128.2, 127.9, 127.0, 126.9, 113.2, 111.5, 111.1, 87.2, 87.1, 85.5, 85.4, 84.6, 75.4, 75.3, 66.7 (d, 2JPC = 9.5 Hz), 66.0 (d, 2JPC = 9.5 Hz), 55.2, 45.3, 39.2, 39.0, 12.3, 9.0. 31P NMR (121.5 MHz, CDCl3) δ 109.8–102.6 (m).

ESI-HRMS: m/z calcd for C31H35BN2O8P– [(M – H+)–] 605.2230, found 605.2205.

Triethylammonium 3′-O-benzoylthymidine-H-boranophosphonate (9c)

3′-O-Benzoylthymidine 8c (0.692 g, 2.0 mmol) and 7 (0.380 g, 2.4 mmol) were dried by repeated coevaporation with dry pyridine (3 × 10 mL) and dissolved in dry pyridine (20 mL) under argon. Bop-Cl (0.611 g, 2.4 mmol) was added, and the mixture was stirred for 30 min at rt. The mixture was then diluted with CHCl3 (40 mL) and washed with 0.5 M TEAB buffer (pH 7.0) (40 mL). The aqueous layer was back-extracted with CHCl3 (3 × 80 mL). The organic layers were combined, dried over Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (20 g of silica gel, gradient elution of 5–10% MeOH–CH2Cl2 with 0.5% Et3N). The fractions containing 9c were combined and concentrated under reduced pressure. The residue was dissolved in CHCl3 (30 mL), washed with 0.5 M TEAB buffer (pH 7.0) (30 mL), dried over Na2SO4, filtered and concentrated to dryness under reduced pressure to afford 9c (0.937 g, 1.8 mmol, 92%) as a colorless foam. A 59:41 mixture of P-diastereomers (1H NMR). 1H NMR (300 MHz, CDCl3) δ 12.4 (br, 1H), 9.01 (br,1H), 8.04–8.00 (m, 2H), 7.80, 7.75 (s, s, 1H), 7.61–7.55 (m, 1H), 7.48–7.41 (m, 2H), 7.32, 7.27 (brd, 1JPH = 396 Hz, brd, 1JPH = 391 Hz, 1H), 6.57 (t, J = 7.5 Hz, 1H), 5.64, 5.55 (m, m, 1H), 4.44, 4.28 (m, m, 1H), 4.33 (m, 1H), 4.17, 4.03 (m, m, 1H), 3.09 (m, 6H), 2.49–2.45 (m, 2H), 2.01, 1.99 (s, s, 3H), 1.32 (t, J = 7.2 Hz, 9H), 1.06 to –0.1 (br, 3H). 13C NMR (75.5 MHz, CDCl3) δ 166.0, 163.8, 150.7, 135.9, 135.7, 133.5, 129.7, 129.7, 128.5, 128.5, 112.2, 111.7, 84.6, 84.4, 84.2, 84.1, 66.6 (d, 2JPC = 9.5 Hz), 66.4 (d, 2JPC = 10.9 Hz), 45.4, 37.5, 37.2, 12.5, 8.5. 31P
NMR (121.5 MHz, CDCl₃) δ 110.8–103.5 (m). ESI-HRMS: m/z calcd for C₁₇H₂₁BN₂O₇P⁻ [(M – H⁺)] 407.1185, found 407.1187.

Triethylammonium 3′-O-phenoxyacetyl-thymidine-5′-H-boranophosphonate (9d)

3′-O-Phenoxyacetyl-thymidine 8d (0.753 g, 2.0 mmol) and 7 (0.380 g, 2.4 mmol) were dried by repeated coevaporation with dry pyridine (3 × 2 mL) and dissolved in dry pyridine (20 mL) under argon. Bop-Cl (0.611 g, 2.4 mmol) was added, and the mixture was stirred for 40 min at rt. The mixture was then diluted with 0.5 M TEAB buffer (pH 7.0) (40 mL) and extracted with CH₂Cl₂ (3 × 50 mL). The organic layers were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (20 g of silica gel, gradient elution of 5–10% MeOH–CH₂Cl₂ with 0.5% Et₃N). The fractions containing 9d were combined and concentrated under reduced pressure. The residue was dissolved in CHCl₃ (30 mL), washed with 0.5 M TEAB buffer (pH 7.0) (30 mL), dried over Na₂SO₄, filtered and concentrated to dryness under reduced pressure to afford 9d (0.970 g, 1.8 mmol, 90%) as a colorless foam. A 59:41 mixture of P-diastereomers (1H NMR). ¹H NMR (300 MHz, CDCl₃) δ 9.50 (br, 1H), 7.78, 7.75 (s, s, 1H), 7.34–7.29 (m, 2H), 7.28 (brd, J₉₈ = 385 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 6.91 (d, J = 8.4 Hz, 2H), 6.48 (m, 1H), 5.56, 5.45 (d, J = 5.1 Hz, d, J = 5.1 Hz, 1H), 4.69 (s, 2H), 4.37–4.22 (m, 1H), 4.21 (m, 1H), 4.11–3.95 (m, 1H), 3.06 (q, J = 7.5 Hz, 6H), 2.39 (m, 2H), 2.01, 1.99 (s, s, 3H) 1.29 (t, J = 7.5 Hz, 9H), 1.04 to –0.1 (br, 3H). ¹³C NMR (75.5 MHz, CDCl₃) δ 168.4, 164.1, 157.3, 150.8, 150.8, 135.6, 135.4, 129.4, 121.7, 114.4, 112.0, 111.5, 84.1, 83.9, 83.7, 83.6, 65.8 (d, J₉₈ = 9.8 Hz), 64.9, 45.2, 37.1, 36.8, 12.3, 8.6. ³¹P NMR (121.5 MHz, CDCl₃) δ 109.8–101.2 (m). ESI-HRMS: m/z calcd for C₁₈H₂₃BN₂O₈P⁻ [(M – H⁺)] 437.1291, found 437.1312.

Triethylammonium 2′,3′-O,Diphenoxyacetyl-uridine-5′-H-boranophosphonate (9e)

2′,3′-O-Diphenoxyacetyl-uridine 8e (0.171 g, 0.33 mmol) and 7 (0.105 g, 0.66 mmol) were dried by repeated coevaporation with dry pyridine (3 × 5 mL) and dissolved in dry pyridine (17 mL). Bop-Cl (0.168 g, 0.66 mmol) was added, and the mixture was stirred for 2 h at rt. The mixture was then diluted with CHCl₃ (20 mL) and washed with 0.5 M TEAB buffer (pH 7.0) (20 mL). The aqueous layer was back-extracted with CHCl₃ (3 × 20 mL). The organic layers were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel.
(15 g of silica gel, gradient elution of 3–9% MeOH–CH₂Cl₂ with 0.5% Et₃N). The fractions containing 9e were combined and concentrated under reduced pressure. The residue was dissolved in CHCl₃ (30 mL), washed with 0.5 M TEAB buffer (pH 7.0) (30 mL), dried over Na₂SO₄, filtered and concentrated to dryness under reduced pressure to afford 9e (0.220 g, 0.33 mmol, 98%) as a colorless foam. A 61:39 mixture of P-diastereomers (¹H NMR). ¹H NMR (300 MHz, CDCl₃) δ 8.75 (br, 1H), 8.05, 7.98 (d, J = 8.4 Hz, 1H), 7.32–7.21 (m, 4H), 7.01–6.81 (m, 6H), 7.31, 7.26 (brd, Jₚₙ = 391 Hz, brd, Jₚₙ = 386 Hz, 1H), 6.38, 6.29 (d, J = 7.5 Hz, 1H), 4.66–4.44 (m, 4H), 4.34 (m, 1H), 4.29–4.19 (m, 1H), 4.06–3.97 (m, 1H), 2.98 (q, J = 7.3 Hz, 6H), 1.22 (t, J = 7.3 Hz, 9H), 0.98 to –0.1 (br, 3H). ¹³C NMR (75.5 MHz, CDCl₃) δ 168.0, 167.9, 167.5, 163.3, 157.2, 150.9, 150.7, 140.0, 129.4, 129.3, 121.8, 121.7, 121.5, 121.4, 114.4, 114.3, 103.7, 103.2, 85.2, 84.8, 82.4, 82.0, 73.2, 73.1, 72.9, 72.5, 65.1 (d, Jₚₙ = 9.5 Hz), 65.0 (d, Jₚₙ = 7.8 Hz), 64.5, 64.2, 64.1, 45.1, 8.2. ³¹P NMR (121.5 MHz, CDCl₃) δ 108.0–104.0 (m). ESI-HRMS: m/z calcd for C₂₃H₂₇BN₂O₁₁P− [(M – H⁺)−] 573.1451, found 573.1444.

Triethylammonium thymidine-5′-H-boranophosphonate (10a)

Triethylammonium 3′-O-phenoxyacetyl-thymidine-5′-H-boranophosphonate 9d (0.207 g 0.38 mmol) was treated with saturated NH₃/MeOH (15 mL) for 50 min at rt. The mixture was then concentrated under reduced pressure. The residue was dissolved in H₂O (30 mL), washed with CHCl₃ (3 × 30 mL) and lyophilized. The residue was purified by MPLC [20% MeCN in 0.1 M triethylammonium acetate buffer (pH 7.0)] to afford 10a (0.109 g, 0.27 mmol, 71%) as a white amorphous solid. A 58:42 mixture of P-diastereomers (¹H NMR). ¹H NMR (300 MHz, D₂O) δ 7.73, 7.63 (s, s, 1H), 7.14 (brd, Jₚₙ = 393 Hz, 1H), 6.31 (m, 1H), 4.57–4.49 (m, 1H), 4.25–4.15 (m, 2H), 4.00–3.91 (m, 1H), 3.18 (q, J = 7.3 Hz 6H), 2.38–2.27 (m, 2H), 1.90, 1.89 (s, s, 3H), 1.26 (t, J = 7.3 Hz, 9H), 0.96 to –0.2 (bq, 3H). ¹³C NMR (75.5 MHz, D₂O) δ 166.4, 151.7, 137.5, 137.3, 111.6, 86.0, 85.9, 85.4, 85.2, 71.6, 71.4, 66.4 (d, Jₚₙ = 9.8 Hz), 65.9 (d, Jₚₙ = 10.3 Hz), 46.8, 39.2, 12.0, 8.4. ³¹P NMR (121.5 MHz, D₂O) δ 106.3–101.2 (m). ESI-HRMS: m/z calcd for C₁₀H₁₇BN₂O₆P− [(M – H⁺)−] 303.0923, found 303.0913.

Triethylammonium uridine-5′-H-boranophosphonate (10b)

Triethylammonium 2′,3′-O,O-diphenoxacyl-uridine-5′-H-boranophosphonate 9e (0.135 g 0.20 mmol)
was treated with saturated NH₃/MeOH (10 mL) for 3 h at rt. The mixture was then concentrated under reduced pressure. The residue was dissolved in 0.5 M TEAB buffer (pH 7.0) (20 mL), washed with CHCl₃ (20 mL) and lyophilized. The residue was purified by MPLC [20% MeCN in 0.1 M triethylammonium acetate buffer (pH 7.0)] to afford 10b (55.1 mg, 0.14 mmol, 68%) as a white amorphous solid. A 60:40 mixture of P-diastereomers (¹H NMR). ¹H NMR (300 MHz, D₂O) δ 8.03, 7.98 (d, J = 8.1 Hz, d, J = 7.8 Hz, 1H), 7.18 (brd, JPH = 396 Hz, 1H), 5.96 (m, 1H), 5.92–5.88 (m, 1H), 4.35–4.21 (m, 4H), 4.09–3.97 (m, 1H), 3.19 (q, J = 7.3 Hz, 6H), 1.27 (t, J = 7.3 Hz, 9H), 0.99–0.0 (bq, 3H). ¹³C NMR (75.5 MHz, D₂O) δ 166.3, 166.2, 151.8, 142.0, 141.9, 102.6, 102.5, 89.0, 88.8, 83.6, 83.4, 74.2, 69.9, 65.5, 65.4, 46.8, 8.4. ³¹P NMR (121.5 MHz, D₂O) δ 106.3–101.1 (m). ESI-HRMS: m/z calcd for C₉H₁₅BN₂O₇P− [(M – H −)−] 305.0715, found 305.0719.

Triethylammonium 5′-O-dimethoxytrityl-N³-benzoylthymidine-3′-H-boranophosphonate (12a)

5′-O-Dimethoxytrityl-N³-benzoylthymidine 11a (1.95 g, 3.0 mmol) and 7 (0.570 g, 3.6 mmol) were dried by repeated coevaporation with dry pyridine (3 × 10 mL) and dissolved in dry pyridine (30 mL) under argon. Bop-Cl (0.916 g, 3.6 mmol) was added, and the mixture was stirred for 1 h at rt. Saturated NaHCO₃ aqueous solution (10 mL) was added, and the mixture was extracted with CH₂Cl₂ (3 × 50 mL). The organic layers were combined, washed with 0.5 M TEAB buffer (pH 7.0) (50 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (30 g of silica gel, gradient elution of 1–5% MeOH–CH₂Cl₂ with 1% Et₃N) to afford 12a (2.31 g, 2.8 mmol, 95%) as a colorless foam. A 51:49 mixture of P-diastereomers (¹H NMR). ¹H NMR (300 MHz, CDCl₃) δ 12.6 (br, 1H), 7.92 (d, J = 7.8 Hz, 2H), 7.80, 7.75 (s, s, 1H), 7.63 (t, J = 7.4 Hz, 1H), 7.50–7.23 (m, 11H), 7.25 (brd, JPH = 396 Hz, 1H), 6.85 (d, J = 8.7 Hz, 4H), 6.42 (m, 1H), 5.12, 4.97 (m, m, 1H), 4.30 (m, 1H), 3.79 (s, 6H), 3.46 (m, 2H), 2.98 (q, J = 7.2 Hz, 6H), 2.76–2.61 (m, 1H), 2.49–2.34 (m, 1H), 1.40, 1.38 (s, s, 3H), 1.25 (t, J = 7.2 Hz, 9H), 1.05–0.0 (br, 3H). ¹³C NMR (75.5 MHz, CDCl₃) δ 169.2, 169.2, 162.9, 158.7, 149.2, 144.2, 135.6, 135.6, 135.4, 135.3, 135.2, 135.2, 134.9, 131.9, 131.6, 130.5, 130.1, 130.0, 129.0, 128.5, 128.1, 128.0, 128.0, 127.3, 127.1, 113.3, 113.3, 111.1, 111.1, 87.0, 87.0, 85.7, 85.6, 85.0, 78.1 (d, JPC = 8.4 Hz), 75.3 (d, JPC = 5.4 Hz), 63.5, 63.1, 55.2, 45.3, 40.6, 40.0, 11.6, 11.6, 8.5. ³¹P NMR (121.5 MHz, CDCl₃) δ 107.4 (brq, JPB = 97.2 Hz), 104.0 (brq, JPB = 92.6 Hz). ESI-HRMS: m/z calcd for C₃₈H₃₉BN₂O₃P− [(M – H −)−] 709.2492, found 709.2516.

Triethylammonium 5′-O-dimethoxytrityl-thymidine-3′-H-boranophosphonate (12b)
5′-O-Dimethoxytrityl-thymidine 11b (2.72 g, 5.0 mmol) and 7 (1.59 g, 10 mmol) were dried by repeated coevaporation with dry pyridine (3 × 10 mL) and dissolved in dry pyridine (30 mL) under argon. Bop-Cl (2.54 g, 10 mmol) was added, and the mixture was stirred for 3 h at rt. 0.5 M TEAB buffer (pH 7.0) (40 mL) was added, and the mixture was extracted with CHCl₃ (3 × 50 mL). The organic layers were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (90 g of silica gel, gradient elution of 2–3% MeOH–CH₂Cl₂ with 0.5% Et₃N) to afford 12b (2.90 g, 4.1 mmol, 82%) as a colorless foam. A 51:49 mixture of P-diastereomers (¹H NMR).

1H NMR (300 MHz, CDCl₃) δ 9.12 (br, 1H), 7.66, 7.61 (s, s, 1H), 7.40 (d, J = 7.5 Hz, 2H), 7.30–7.20 (m, 7H), 7.29 (brd, J₁PΗ = 386 Hz, 1H), 6.83 (d, J = 8.7 Hz, 4H), 6.44 (m, 1H), 5.09, 4.93 (m, m, 1H), 4.28 (m, 1H), 3.79 (s, 6H), 3.43 (m, 2H), 3.02 (q, J = 7.3 Hz, 6H), 2.72–2.57 (m, 1H), 2.44–2.32 (m, 1H), 1.40, 1.38 (s, s, 3H), 1.28 (t, J = 7.3 Hz, 9H), 1.05–0.0 (br, 3H). ¹³C NMR (75.5 MHz, CDCl₃) δ 163.9, 158.6, 150.5, 150.4, 144.3, 135.7, 135.6, 135.4, 135.4, 135.3, 135.2, 130.1, 130.0, 128.1, 128.1, 127.9, 127.9, 127.0, 113.2, 113.2, 111.1, 111.0, 86.9, 86.9, 85.5, 85.4, 84.6, 77.8 (d, J₂PC = 9.5 Hz), 75.4 (d, J₂PC = 6.3 Hz), 63.5, 63.2, 55.2, 45.3, 40.3, 39.7, 11.6, 11.6, 8.7. ³¹P NMR (121.5 MHz, CDCl₃) δ 109.5–101.8 (m). ESI-HRMS: m/z calcd for C₃₁H₃₅BN₂O₈P– [(M – H⁺)–] 605.2230, found 605.2215.

5′-O-Dimethoxytrityl-N³-benzoylthymidin-3′-yl 3′-O-dimethoxytrityl-N³-benzoylthymidin-5′-yl H-boranophosphonate (13a)

3′-O-Dimethoxytrityl-N³-benzoylthymidine 8a (0.270 g, 0.42 mmol) and triethylammonium 5′-O-dimethoxytrityl-N³-benzoylthymidine-3′-H-boranophosphonate 12a (0.455 g, 0.56 mmol) were dried by repeated coevaporation with dry MeCN (3 × 10 mL) and dissolved in dry MeCN (5 mL) under argon. Distilled 2,2,6,6-tetramethylpiperidine (0.567 mL, 3.4 mmol) and Bop-Cl (0.356 g, 1.4 mmol) were added, and the mixture was stirred for 1 h at rt. Saturated NaHCO₃ aqueous solution (50 mL) was then added, and the mixture was extracted with CHCl₃ (3 × 50 mL). The organic layers were combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel [10 g of silica gel, isocratic elution of hexane–ethyl acetate (1:2, v/v)] to afford 13a (0.383 g, 0.29 mmol, 68%) as a colorless foam. A 52:48 mixture of P-diastereomers (¹H NMR). ¹H NMR (300 MHz,
CDCl$_3$ $\delta$ 7.96–7.89 (m, 4H), 7.68–7.61 (m, 3H), 7.51–7.14 (m, 23H), 6.97, 6.78 (brd, $^1J_{PH}$ = 452 Hz, brd, $^1J_{PH}$ = 458 Hz, 1H), 7.68–7.61 (m, 3H), 7.51–7.14 (m, 23H), 6.97, 6.78 (brd, $^1J_{PH}$ = 458 Hz, 1H), 1.98–1.87 (m, 1H), 1.91, 1.87 (s, s, 3H), 1.74–1.58 (m, 1H), 1.49, 1.47 (s, s, 3H), 0.94–0.1 (br, 3H). $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 168.9, 168.8, 168.8, 168.7, 162.6, 162.4, 162.4, 158.7, 158.7, 149.2, 149.2, 144.6, 144.5, 143.8, 143.8, 135.7, 135.6, 135.6, 135.5, 135.2, 135.0, 134.9, 134.8, 134.7, 131.3, 130.3, 130.1, 130.0, 129.9, 129.0, 128.0, 127.9, 127.8, 127.3, 127.2, 127.1, 127.1, 113.4, 113.3, 111.7, 111.6, 111.6, 111.4, 87.4, 87.3, 85.5, 85.2, 85.0, 84.6, 84.4, 83.9, 83.8, 83.7, 80.5, 79.5, 73.7, 73.5, 69.7 (d, $^2J_{PC}$ = 9.8 Hz), 69.5 (d, $^2J_{PC}$ = 10.1 Hz), 63.2, 63.1, 55.1, 55.1, 39.4, 38.8, 38.6, 12.4, 12.4, 11.7, 11.7. $^{31}$P NMR (121.5 MHz, CDCl$_3$) $\delta$ 135.1 (br), 133.7 (br). ESI-HRMS: $m/z$ calcd for C$_{76}$H$_{74}$BN$_4$NaO$_{16}$P$^+$ (M + Na$^+$) 1363.4823, found 1363.4845.

Triethylammonium 5'-O-dimethoxytrityl-$N^3$-benzoylthymidin-3'-yl 3'-O-dimethoxytrityl-$N^3$-benzoylthymidin-5'-yl boranophosphorothioate (15)

5'-O-Dimethoxytrityl-$N^3$-benzoylthymidin-3'-yl 3'-O-dimethoxytrityl-$N^3$-benzoylthymidin-5'-yl $H$-boranophosphonate 13a (0.100 g, 75 μmol) was dissolved in dry MeCN (2.0 mL) under argon. Sulfur powder (7.9 mg, 0.25 mmol) and distilled Et$_3$N (34 μL, 0.24 mmol) were added, and the mixture was stirred for 3 h at rt. The mixture was then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (3 g of silica gel, gradient elution of 3–5% MeOH–AcOEt with 0.5% pyridine). The fractions containing 15 were combined and concentrated under reduced pressure. The residue was dissolved in CHCl$_3$ (30 mL) and washed with 0.5 M TEAB buffer (pH 7.0) (10 mL). The aqueous layer was extracted with CHCl$_3$ (2 × 30 mL). The organic layers were combined, dried over Na$_2$SO$_4$, filtered and concentrated to dryness under reduced pressure to afford 15 (94 mg, 64 μmol, 85%) as a colorless foam. A 53:47 mixture of $P$-diastereomers ($^1$H NMR). $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.63, 7.96–7.92 (m, 4H), 7.84, 7.81 (s, s, 1H), 7.74, 7.72 (s, s, 1H), 7.66–7.60 (m, 2H), 7.51–7.16 (m, 22H), 6.85–6.76 (m, 8H), 6.59–6.50 (m, 1H), 6.42–6.31 (m, 1H), 5.42–5.34, 5.27–5.21 (m, m, 1H), 4.51, 4.35 (m, m, 1H), 4.23, 4.02 (m, m, 1H), 4.02–3.93 (m, 1H), 3.78–3.71 (m, 12H), 3.62–3.33 (m, 4H), 1.96, 1.91 (s, s, 3H), 1.40, 1.36 (s, s, 3H), 1.28 (t, $J = 7.3$ Hz, 6H), 2.55–2.30 (m, 2H), 2.04–1.90 (m, 2H), 1.96, 1.91 (s, s, 3H), 1.40, 1.36 (s, s, 3H), 1.28 (t, $J = 7.3$ Hz, 9H), 1.05–0.0 (br, 3H). $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 169.3, 169.2, 162.9, 162.9, 158.7, 158.6, 158.5, 149.7, 149.7, 149.3, 149.2, 145.1, 145.0, 144.3, 144.3, 136.3, 136.3, 136.2, 136.1, 135.6, 135.4, 135.3, 135.2, 135.1, 135.0, 134.9, 131.7, 131.6, 131.6, 130.5, 130.4, 130.3, 130.1, 129.1, 128.4, 128.2, 128.1, 128.0, 127.9, 127.1, 126.9, 113.3, 111.4, 111.3, 111.2, 111.1, 87.1, 87.0, 85.7, 85.6, 85.5, 85.0, 75.7 (d, $^2J_{PC}$...
= 5.8 Hz), 75.6, 75.5, 74.7 (d, $J_{PC} = 6.3$ Hz), 64.8, 64.7, 63.5, 63.4, 55.2, 55.2, 46.2, 40.0, 39.3, 39.2, 12.5, 12.4, 11.6, 11.5, 8.5. $^{31}$P NMR (121.5 MHz, CDCl$_3$) $\delta$ 162.5 (br), 161.0 (br). ESI-HRMS: $m/z$ calcd for C$_{76}$H$_{73}$BN$_4$O$_{16}$PS$^- [(M – H$^+$)] 1371.4578, found 1371.4548.
\[ \text{H}_3\text{B} \rightarrow \text{P} \rightarrow \text{O}^{-} - \text{HNEt}_3^+ \]

\[ \text{ODMTr} \]

9a

\((^{13}\text{C}, 75.5 \text{ MHz, CDCl}_3)\)
$\text{H}_3\text{B} \overset{\text{P}}{\text{O}} \overset{\text{O}^-}{\text{HNEt}_3^+}$

$\text{H}^+ \overset{\text{O}}{\text{ODMTr}} \overset{\text{O}}{\text{Th}^{\text{bz}}} \overset{\text{ODMTr}}{\text{O}}$

9a

$^{31}\text{P}, 121.5 \text{ MHz, CDCl}_3$
$\text{H}_3\text{B} \rightarrow \text{P} \rightarrow \text{O} \rightarrow \text{HNEt}_3^+$

$\text{H} \rightarrow \text{O} \rightarrow \text{Th}$

$\text{ODMTr}$

$9b$

($^1\text{H}, 300 \text{ MHz, CDCl}_3$)

S15
$H_3B\overset{\text{PO}}{\text{O}}\overset{\text{OEt}_3^+}{\text{O}}\overset{\text{Th}}{\text{ODMTr}}$

9b

($^{31}\text{P}, 121.5\text{ MHz}, \text{CDCl}_3$)
\[ \text{H}_3\text{B} \overset{\text{P}}{\text{O}} \text{O}^{-} \text{HNEt}_3^+ \]

\[
\begin{array}{c}
\text{O} \\
\text{OBz} \\
\end{array}
\]

\(9c\)

\(\left(^1\text{H}, 300\text{ MHz, CDCl}_3\right)\)
$\text{H}_3\text{P} - \text{O}^+ - \text{HNEt}_3^+$

$\text{H}^+ - \text{O} - \text{Obz}$

$\text{Th}$

9c

$^{13}\text{C}, 75.5$ MHz, CDCl$_3$
H₃B⁻P⁻O⁻HNEt₃⁺

O⁻Pac

9d

(¹H, 300 MHz, CDCl₃)
9d

\((^{13}\text{C}, 75.5 \text{ MHz, CDCl}_3)\)
$\text{H}_3\text{B\text{PO}_3^-\text{HNEt}_3^+}$

$\text{Th}$

$\text{OPac}$

$9d$

($^{31}\text{P, 121.5 MHz, CDCl}_3$)
H₃B-P-O⁻HNEt₃⁺
H
O
Ur
Paco
OPac
9e
(¹H, 300 MHz, CDCl₃)
H$_3$B$^+$\(\text{O}^\cdot\text{HNEt}_3\)\(\text{OPac}\)

(\(\text{\(^{31}\)P, 121.5 MHz, CDCl}_3\))
$\text{H}_3\text{B} \text{P}^{\text{O}^-} \text{HNEt}_3^+$

$\text{H} \text{P} \text{O} \text{O} \text{Th} \text{OH}$

**10a**

($^1\text{H}, 300 \text{ MHz}, \text{D}_2\text{O}$)
10a

$^{13}\text{C}, 75.5\text{ MHz}, \text{D}_2\text{O}$
H3B\cdot\text{O}+HNEt3+\xrightleftharpoons{}\text{Th}

(31P, 121.5 MHz, D2O)
$\text{H}_3\text{B}^+\text{O}^-\text{HNEt}_3^+$

$10\text{b}$

($\text{H}, 300 \text{ MHz}, \text{D}_2\text{O}$)
$^{13}$C, 75.5 MHz, D$_2$O)
$\text{H}_3\text{B} \rightarrow \text{P} - \text{O} \rightarrow \text{HNET}_3^+$

$\text{H} \rightarrow \text{O} \rightarrow \text{Ur}$

$\text{OH} \rightarrow \text{OH}$

$10b$

$^{31}\text{P}$, 121.5 MHz, D$_2$O
DMTr\text{O} - \text{O}^{\text{Th bz}}\text{O}

H_3B\text{P}_\text{O}^- \text{O}^- \text{HNEt}_3^+

\textbf{12a}

(^1\text{H}, 300 MHz, CDCl$_3$)
$\text{H}_3\text{B} - \text{P} - \text{O} - \text{HNE}_3^+$

$\text{DMTrO}$

$\text{Th}_{12a}^\text{P}$

$(\text{P}, 121.5 \text{MHz, CDCl}_3)$
DMT-\(\text{O}^+\) - Th

\[\text{H}_3\text{B} - \text{P} - \text{O}^- - \text{HNEt}_3^+\]

\(1\text{H, 300 MHz, CDCl}_3\)

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DMTrO\textsubscript{2}Th

\[
\text{H}_3\text{B}\xrightarrow{\text{O}}\text{P}\xrightarrow{\text{O}}\text{HNET}_3^+
\]

12b

\[
\left(^{13}\text{C}, 75.5 \text{ MHz}, \text{CDCl}_3\right)
\]
$\text{DMTrO} \begin{array}{c} \text{O} \\ \text{O} \end{array} \text{Th}$

$\text{H}_3\text{B} \begin{array}{c} \text{O} \\ \text{O} \end{array} \text{HNEt}_3^+$

$\text{12b}$

$^{31}\text{P}$, 121.5 MHz, CDCl$_3$
13a

$\left( ^{13}C, 75.5 \text{ MHz, CDCl}_3 \right)$
(3)P, 121.5 MHz, CDC$_3$
DMTrO

H₃B

Et₃NH−S

ODMTr

^{1}H, 300 MHz, CDCl₃

Supplementary Material (ESI) for Chemical Communications
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(\textsuperscript{13}C, 75.5 MHz, CDCl\textsubscript{3})
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Chemical structure: DMT-DO-OS-Thbz
Et3NH−S−O−Thbz
ODMT

(31P, 121.5 MHz, CDCl3)