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

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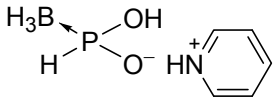
Supporting Information

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-F_{254} 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_2O. ^{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_2O. ^{31}P NMR spectra were obtained at 121.5 MHz with 85% H_3PO_4 (δ 0.0) as an external standard in $CDCl_3$ or in D_2O. ESI mass spectra were recorded on an Applied Biosystems QSTAR.

Pyridinium *H*-boranophosphonate (**7**)

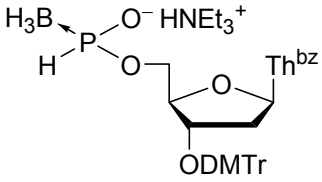


7

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*,*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_2O–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_2O (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 H_2O (100 mL) and washed with $CHCl_3$ (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. ^{1}H NMR (300 MHz, D_2O) δ 8.55 (m, 2H), 8.30 (m, 1H), 7.78 (m, 2H), 7.03 (brd, $^1J_{PH}$ = 399 Hz, 1H), 0.17 (dq, $^1J_{PB}$ = 88.8 Hz, $^2J_{PH}$ = 19.8 Hz, 3H). ^{31}P NMR (121.5 MHz, D_2O) δ 94.2 (q, $^1J_{PB}$ = 109.1 Hz). ESI-HRMS: *m*/*z* calcd for $BH_5O_2P^-$ [(M – H^+)$^-$] 79.0126, found 79.0121.

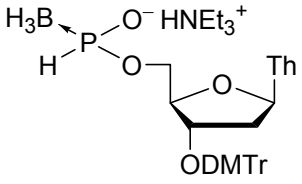
Triethylammonium 3′-*O*-dimethoxytrityl-N^3-benzoylthymidine-5′-*H*-boranophosphonate (**9a**)



9a

3′-*O*-Dimethoxytrityl-N^3-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 $CHCl_3$ (3 × 50 mL). The organic layers were combined, washed with 0.5 M TEAB buffer (50 mL), dried over Na_2SO_4, 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–CH_2Cl_2 with 1% Et_3N) to afford **9a** (0.771 g, 0.95 mmol, 95%) as a colorless foam. A 56:44 mixture of *P*-diastereomers (^{1}H NMR). ^{1}H NMR (300 MHz, $CDCl_3$) 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, $^1J_{PH}$ = 386 Hz, brd, $^1J_{PH}$ = 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). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.2, 162.9, 158.6, 149.7, 149.6, 145.0, 136.3, 136.1, 136.0, 134.9, 131.6, 130.4, 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, $^2J_{PC}$ = 8.9 Hz), 66.1 (d, $^2J_{PC}$ = 8.9 Hz), 55.2, 45.4, 39.4, 39.3, 12.4, 8.5. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 109.4–102.6 (m). ESI-HRMS: *m*/*z* calcd for $C_{38}H_{39}BN_2O_9P^-$ [(M – H^+)$^-$] 709.2492, found 709.2513.

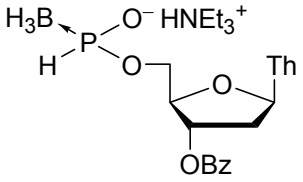
Triethylammonium 3′-*O*-dimethoxytrityl-thymidine-5′-*H*-boranophosphonate (**9b**)



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 $CHCl_3$ (50 mL) and washed with 1 M TEAB buffer (pH 7.0) (20 mL). The aqueous layer was back-extracted with $CHCl_3$ (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, 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–CH_2Cl_2 with 0.5% Et_3N) to afford **9b** (0.671 g, 0.95 mmol, 95%) as a colorless foam. A 54:46 mixture of *P*-diastereomers (^{1}H NMR). ^{1}H NMR (300 MHz, $CDCl_3$) 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, $^1J_{PH}$ = 388 Hz, brd, $^1J_{PH}$ = 385 Hz, 1H), 6.83 (d, J = 9.0 Hz, 4H), 6.54 (m, 1H), 4.41, 4.34 (d, J = 4.8 Hz, d, J = 5.7 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). ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $^2J_{PC}$ = 9.5 Hz), 66.0 (d, $^2J_{PC}$ = 9.5 Hz), 55.2, 45.3, 39.2, 39.0, 12.3, 9.0. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 109.8–102.6 (m). ESI-HRMS: *m*/*z* calcd for $C_{31}H_{35}BN_2O_8P^-$ [$(M - H^+)^-$] 605.2230, found 605.2205.

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

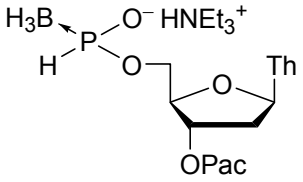


9c

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 $CHCl_3$ (40 mL) and washed with 0.5 M TEAB buffer (pH 7.0) (40 mL). The aqueous layer was back-extracted with $CHCl_3$ (3 × 80 mL). The organic layers were combined, dried over Na_2SO_4, 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–CH_2Cl_2 with 0.5% Et_3N). The fractions containing **9c** were combined and concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (30 mL), washed with 0.5 M TEAB buffer (pH 7.0) (30 mL), dried over Na_2SO_4, 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 (^{1}H NMR). ^{1}H NMR (300 MHz, $CDCl_3$) 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, $^1J_{PH}$ = 396 Hz, brd, $^1J_{PH}$ = 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 H, 9H), 1.06 to –0.1 (br, 3H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 166.0, 163.8, 150.7, 135.9, 135.7, 133.5, 129.7, 129.7, 129.2, 128.5, 112.2, 111.7, 84.6, 84.4, 84.2, 84.1, 66.6 (d, $^2J_{PC}$ = 9.5 Hz), 66.4 (d, $^2J_{PC}$ = 10.9 Hz), 45.4, 37.5, 37.2, 12.5, 8.5. ^{31}P

NMR (121.5 MHz, $CDCl_3$) δ 110.8–103.5 (m). ESI-HRMS: *m/z* calcd for $C_{17}H_{21}BN_2O_7P^-$ $[(M - H^+)^-]$ 407.1185, found 407.1187.

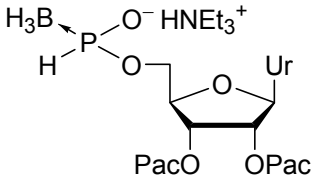
Triethylammonium 3′-*O*-phenoxyacetyl-thymidine-5′-*H*-boranophosphonate (**9d**)



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 CH_2Cl_2 (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, 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–CH_2Cl_2 with 0.5% Et_3N). The fractions containing **9d** were combined and concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (30 mL), washed with 0.5 M TEAB buffer (pH 7.0) (30 mL), dried over Na_2SO_4, 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 (^{1}H NMR). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.50 (br, 1H), 7.78, 7.75 (s, s, 1H), 7.34–7.29 (m,2H), 7.28 (brd, $^1J_{PH}$ = 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). ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $^2J_{PC}$ = 9.8 Hz), 64.9, 45.2, 37.1, 36.8, 12.3, 8.6. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 109.8–101.2 (m). ESI-HRMS: *m/z* calcd for $C_{18}H_{23}BN_2O_8P^-$ $[(M - H^+)^-]$ 437.1291, found 437.1312.

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

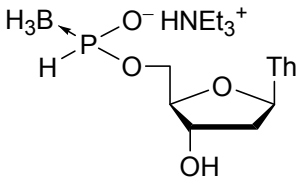


9e

2′,3′-*O*,*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 $CHCl_3$ (20 mL) and washed with 0.5 M TEAB buffer (pH 7.0) (20 mL). The aqueous layer was back-extracted with $CHCl_3$ (3 × 20 mL). The organic layers were combined, dried over Na_2SO_4, 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–CH_2Cl_2 with 0.5% Et_3N). The fractions containing **9e** were combined and concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (30 mL), washed with 0.5 M TEAB buffer (pH 7.0) (30 mL), dried over Na_2SO_4, 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 (^{1}H NMR). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (br, 1H), 8.05, 7.98 (d, J = 8.4 Hz, d, J = 8.1 Hz, 1H), 7.32–7.21 (m, 4H), 7.01–6.81 (m, 6H), 7.31, 7.26 (brd, $^1J_{PH}$ = 391 Hz, brd, $^1J_{PH}$ = 386 Hz, 1H), 6.38, 6.29 (d, J = 7.5 Hz, d, J = 6.9 Hz, 1H), 5.78 (m, 1H), 5.63–5.53 (m, 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). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.0, 167.9, 167.5, 163.3, 163.3, 157.2, 157.2, 157.2, 150.9, 150.7, 140.0, 129.4, 129.3, 129.3, 121.8, 121.7, 121.6, 121.5, 114.4, 114.3, 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, $^2J_{PC}$ = 9.5 Hz), 65.0 (d, $^2J_{PC}$ = 7.8 Hz), 64.5, 64.2, 64.1, 45.1, 8.2. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 108.0–104.0 (m). ESI-HRMS: *m*/*z* calcd for $C_{25}H_{27}BN_2O_{11}P^-$ [(M – H^+)$^-$] 573.1451, found 573.1444.

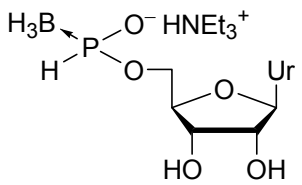
Triethylammonium thymidine-5′-*H*-boranophosphonate (**10a**)



10a

Triethylammonium 3′-*O*-phenoxyacetyl-thymidine-5′-*H*-boranophosphonate **9d** (0.207 g 0.38 mmol) was treated with saturated NH_3/MeOH (15 mL) for 50 min at rt. The mixture was then concentrated under reduced pressure. The residue was dissolved in H_2O (30 mL), washed with $CHCl_3$ (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 (^{1}H NMR). ^{1}H NMR (300 MHz, D_2O) δ 7.73, 7.63 (s, s, 1H), 7.14 (brd, $^1J_{PH}$ = 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). ^{13}C NMR (75.5 MHz, D_2O) 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, $^2J_{PC}$ = 9.8 Hz), 65.9 (d, $^2J_{PC}$ = 10.3 Hz), 46.8, 39.2, 12.0, 8.4. ^{31}P NMR (121.5 MHz, D_2O) δ 106.3–101.2 (m). ESI-HRMS: *m*/*z* calcd for $C_{10}H_{17}BN_2O_6P^-$ [(M – H^+)$^-$] 303.0923, found 303.0913.

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

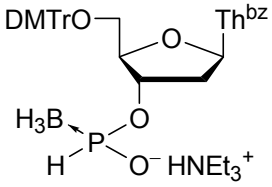


10b

Triethylammonium 2′,3′-*O*,*O*-diphenoxyacetyl-uridine-5′-*H*-boranophosphonate **9e** (0.135 g 0.20 mmol)

was treated with saturated NH_3/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 $CHCl_3$ (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 (^{1}H NMR). ^{1}H NMR (300 MHz, D_2O) δ 8.03, 7.98 (d, J = 8.1 Hz, d, J = 7.8 Hz, 1H), 7.18 (brd, $^1J_{PH}$ = 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). ^{13}C NMR (75.5 MHz, D_2O) 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. ^{31}P NMR (121.5 MHz, D_2O) δ 106.3–101.1 (m). ESI-HRMS: m/z calcd for $C_9H_{15}BN_2O_7P^-$ [$(M - H^+)^-$] 305.0715, found 305.0719.

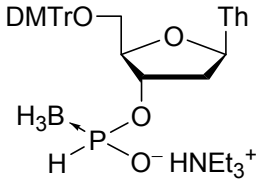
Triethylammonium 5′-*O*-dimethoxytrityl-N^3-benzoylthymidine-3′-*H*-boranophosphonate (**12a**)



12a

5′-*O*-Dimethoxytrityl-N^3-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 $NaHCO_3$ aqueous solution (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were combined, washed with 0.5 M TEAB buffer (pH 7.0) (50 mL), dried over Na_2SO_4, 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–CH_2Cl_2 with 1% Et_3N) to afford **12a** (2.31 g, 2.8 mmol, 95%) as a colorless foam. A 51:49 mixture of *P*-diastereomers (^{1}H NMR). ^{1}H NMR (300 MHz, $CDCl_3$) 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, $^1J_{PH}$ = 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). ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, 128.1, 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, $^2J_{PC}$ = 8.4 Hz), 75.3 (d, $^2J_{PC}$ = 5.4 Hz), 63.5, 63.1, 55.2, 45.3, 40.6, 40.0, 11.6, 11.6, 8.5. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 107.4 (brq, $^1J_{PB}$ = 97.2 Hz), 104.0 (brq, $^1J_{PB}$ = 92.6 Hz). ESI-HRMS: m/z calcd for $C_{38}H_{39}BN_2O_9P^-$ [$(M - H^+)^-$] 709.2492, found 709.2516.

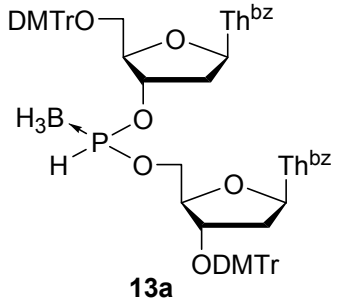
Triethylammonium 5′-*O*-dimethoxytrityl-thymidine-3′-*H*-boranophosphonate (**12b**)



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 $CHCl_3$ (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, 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–CH_2Cl_2 with 0.5% Et_3N) to afford **12b** (2.90 g, 4.1 mmol, 82%) as a colorless foam. A 51:49 mixture of *P*-diastereomers (^{1}H NMR). ^{1}H NMR (300 MHz, $CDCl_3$) 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, $^1J_{PH}$ = 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). ^{13}C NMR (75.5 MHz, $CDCl_3$) 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, $^2J_{PC}$ = 9.5 Hz), 75.4 (d, $^2J_{PC}$ = 6.3 Hz), 63.5, 63.2, 55.2, 45.3, 40.3, 39.7, 11.6, 11.6, 8.7. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 109.5–101.8 (m). ESI-HRMS: *m*/*z* calcd for $C_{31}H_{35}BN_2O_8P^-$ $[(M - H^+)^-]$ 605.2230, found 605.2215.

5′-*O*-Dimethoxytrityl-N^3-benzoylthymidin-3′-yl 3′-*O*-dimethoxytrityl-N^3-benzoylthymidin-5′-yl *H*-boranophosphonate (**13a**)

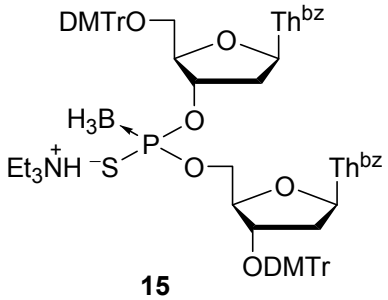


13a

3′-*O*-Dimethoxytrityl-N^3-benzoylthymidine **8a** (0.270 g, 0.42 mmol) and triethylammonium 5′-*O*-dimethoxytrityl-N^3-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 $NaHCO_3$ aqueous solution (50 mL) was then added, and the mixture was extracted with $CHCl_3$ (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, 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 (^{1}H NMR). ^{1}H NMR (300 MHz,

$CDCl_3$) δ 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), 6.90–6.76 (m, 8H), 6.42–6.26 (m, 2H), 5.21 (m, 1H), 4.18 (m, 1H), 4.02–3.89 (m, 2H), 3.80–3.72 (m, 12H), 3.60–3.35 (m, 4H), 2.61–2.42 (m, 2H), 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, $CDCl_3$) δ 168.9, 168.8, 168.8, 168.7, 162.6, 162.4, 162.4, 158.7, 158.7, 149.2, 149.2, 149.1, 144.6, 144.5, 143.8, 143.8, 135.7, 135.6, 135.6, 135.5, 135.2, 135.0, 135.0, 134.9, 134.8, 134.7, 131.3, 130.3, 130.3, 130.1, 130.0, 129.9, 129.0, 128.1, 128.0, 127.9, 127.8, 127.3, 127.2, 127.2, 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, $CDCl_3$) δ 135.1 (br), 133.7 (br). ESI-HRMS: *m*/*z* calcd for $C_{76}H_{74}BN_4NaO_{16}P^+$ (M + Na^+) 1363.4823, found 1363.4845.

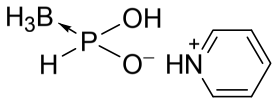
Triethylammonium 5′-*O*-dimethoxytrityl-N^3-benzoylthymidin-3′-yl 3′-*O*-dimethoxytrityl-N^3-benzoylthymidin-5′-yl boranophosphorothioate (**15**)



15

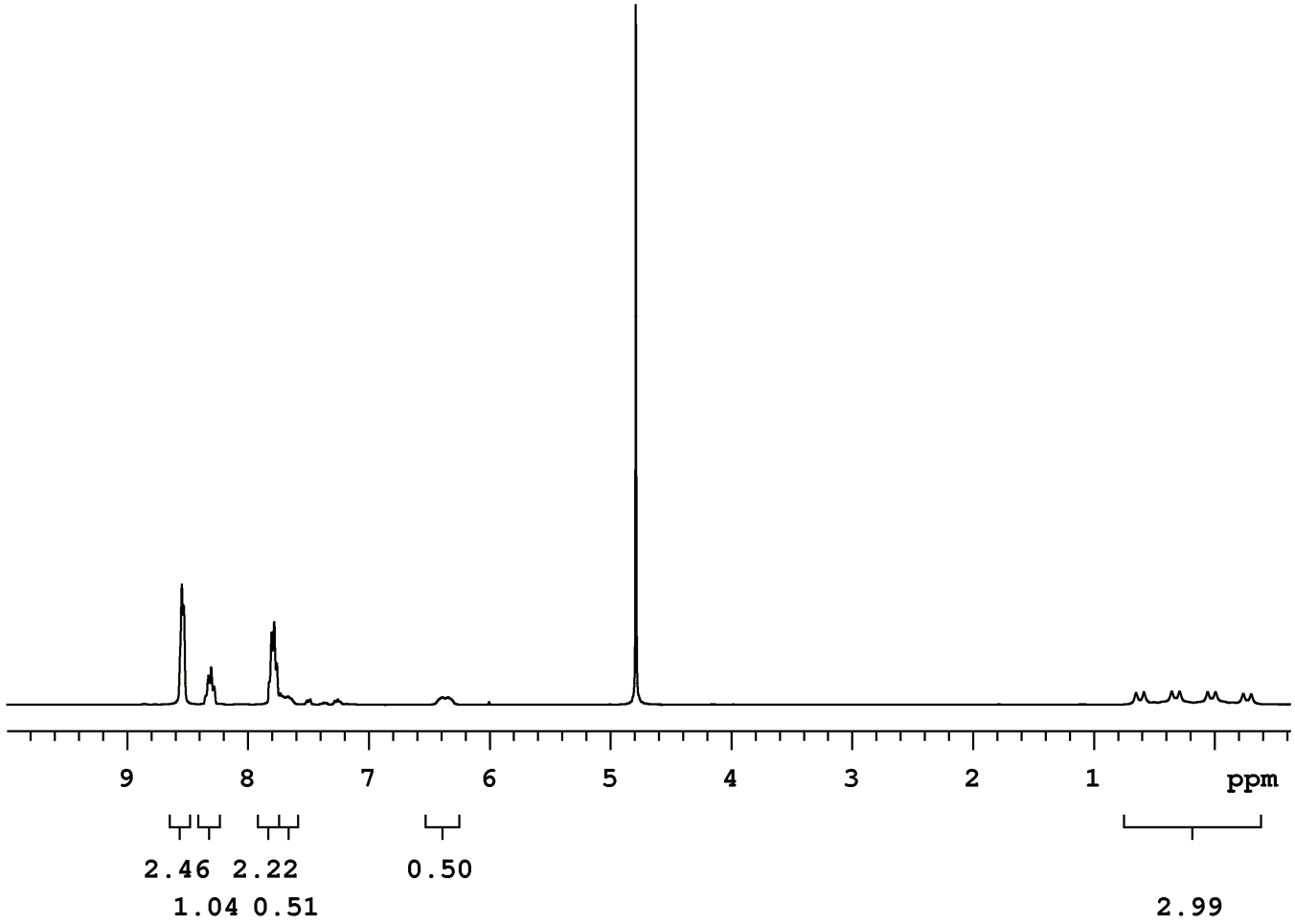
5′-*O*-Dimethoxytrityl-N^3-benzoylthymidin-3′-yl 3′-*O*-dimethoxytrityl-N^3-benzoylthymidin-5′-yl *H*-boranophosphonate **13a** (0.100 g, 75 μmol) was dissolved in dry MeCN (2.0 mL) under argon. Sulfur powder (7.9 mg, 0.25 mmol) and distilled Et_3N (34 μL, 0.24 mmol) were added, and the mixture was stirred 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_2SO_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, $CDCl_3$) δ 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), 3.11 (q, *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, $CDCl_3$) δ 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, 136.2, 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, 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, $^2J_{PC}$

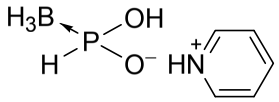
= 5.8 Hz), 75.6, 75.5, 74.7 (d, $^{2}J_{PC}$ = 6.3 Hz), 64.8, 64.7, 63.5, 63.4, 55.2, 55.2, 46.2, 40.0, 39.3, 39.2, 12.5, 12.4, 11.6, 11.5, 8.5. ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 162.5 (br), 161.0 (br). ESI-HRMS: *m*/*z* calcd for $C_{76}H_{73}BN_4O_{16}PS^-$ [$(M - H^+)^-$] 1371.4578, found 1371.4548.



7

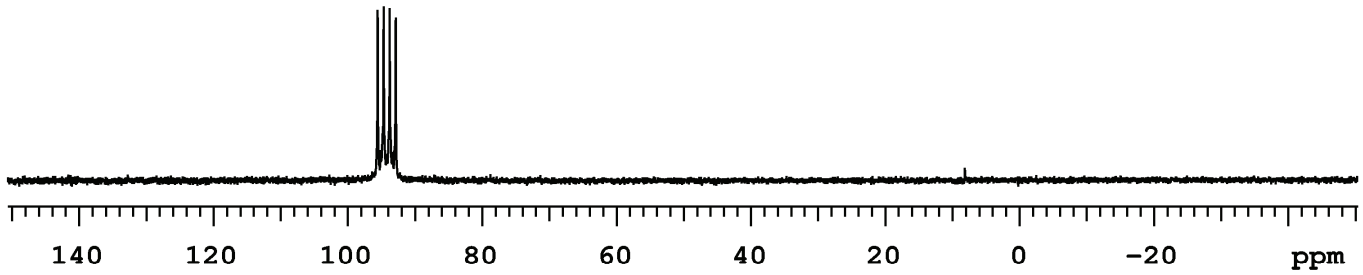
(^{1}H, 300 MHz, D_2O)

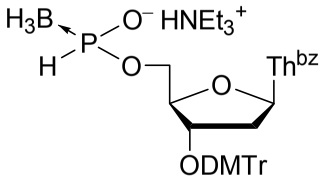




7

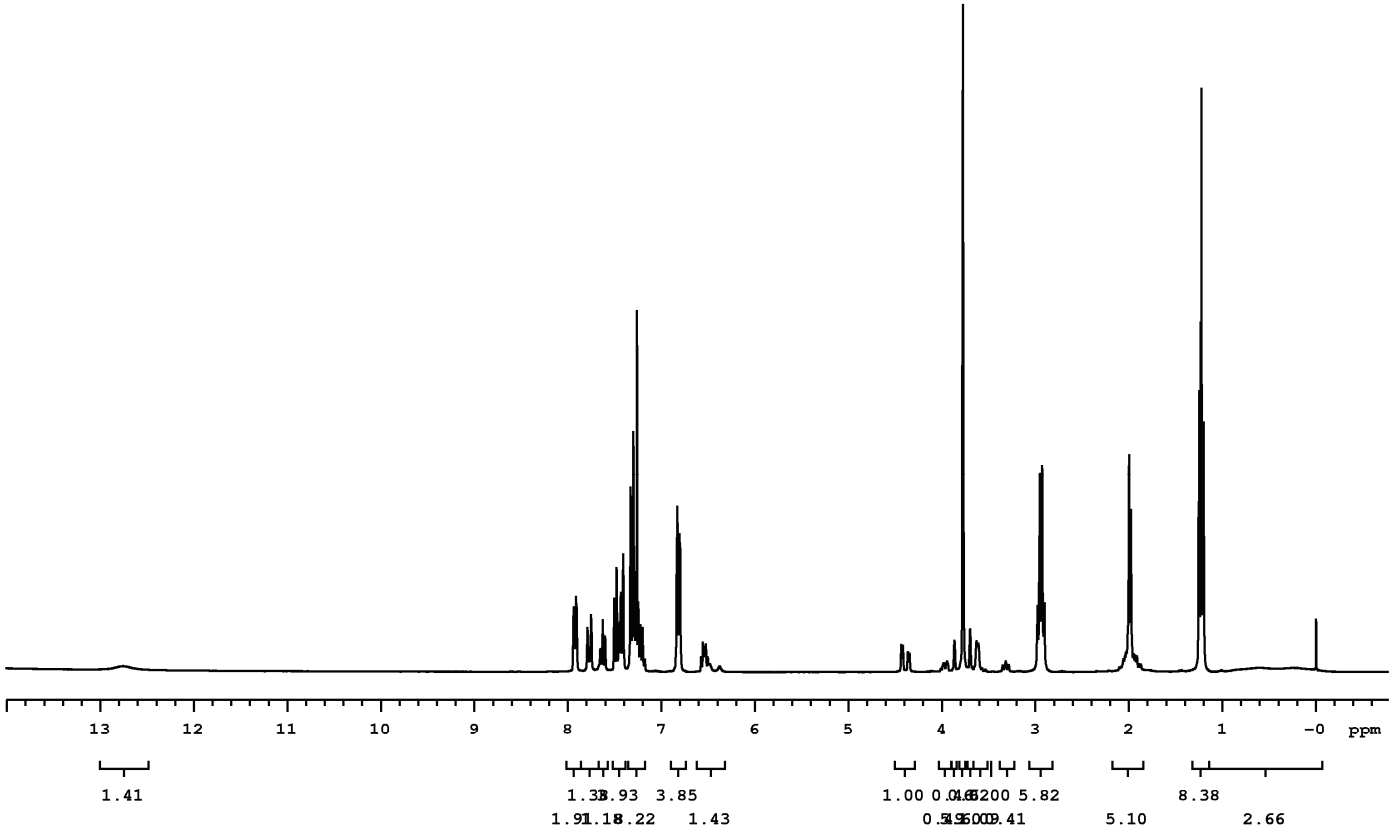
(^{31}P, 121.5 MHz, D_2O)

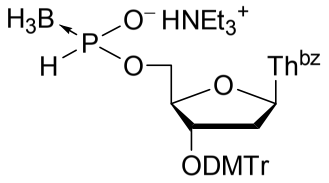




9a

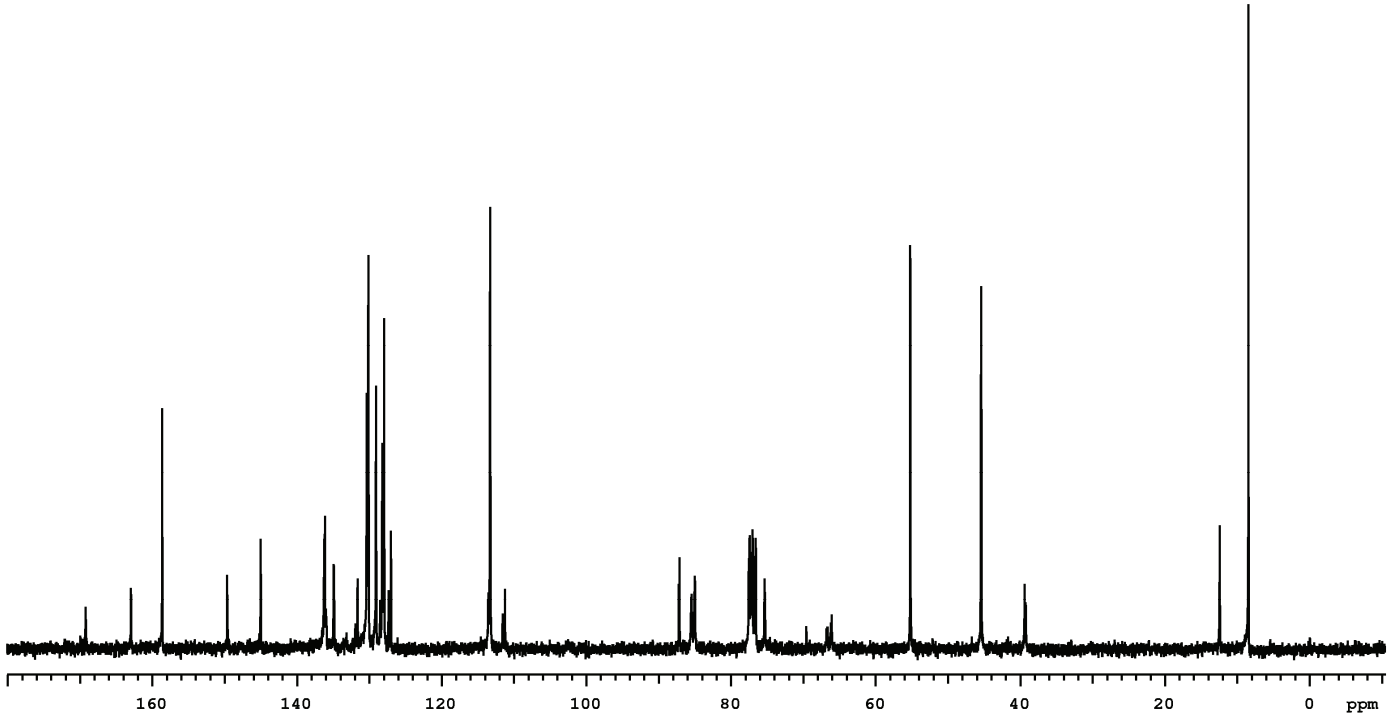
(^{1}H, 300 MHz, $CDCl_3$)

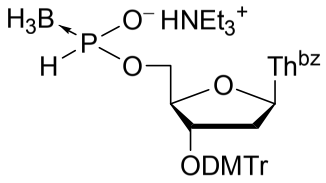




9a

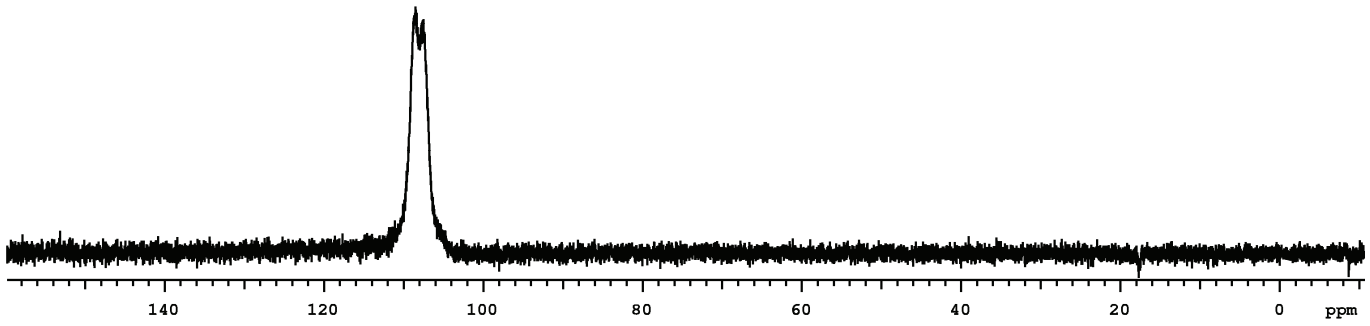
(^{13}C, 75.5 MHz, $CDCl_3$)

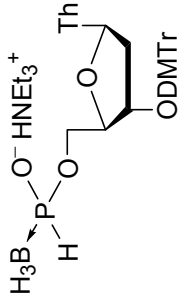




9a

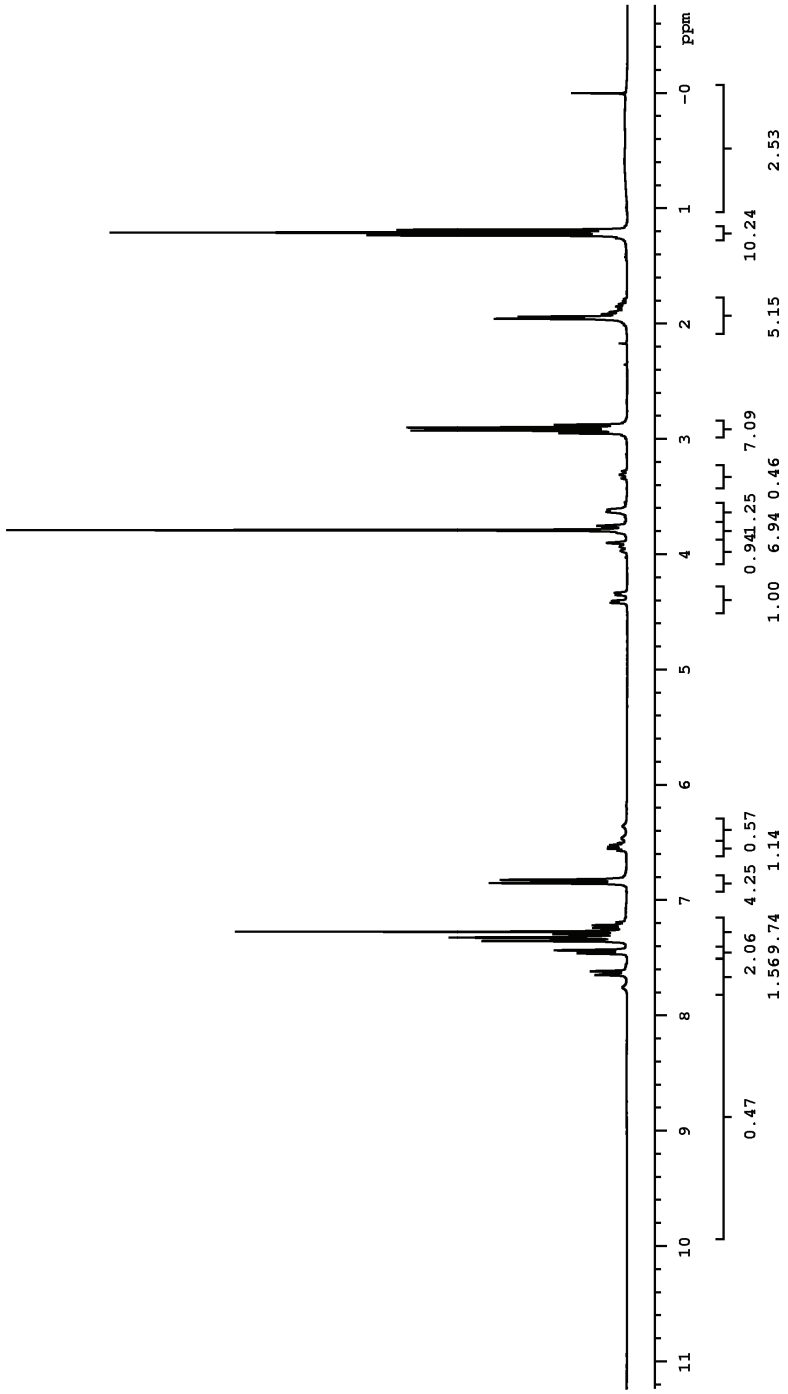
(^{31}P, 121.5 MHz, $CDCl_3$)

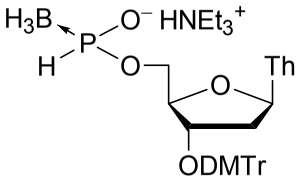




9b

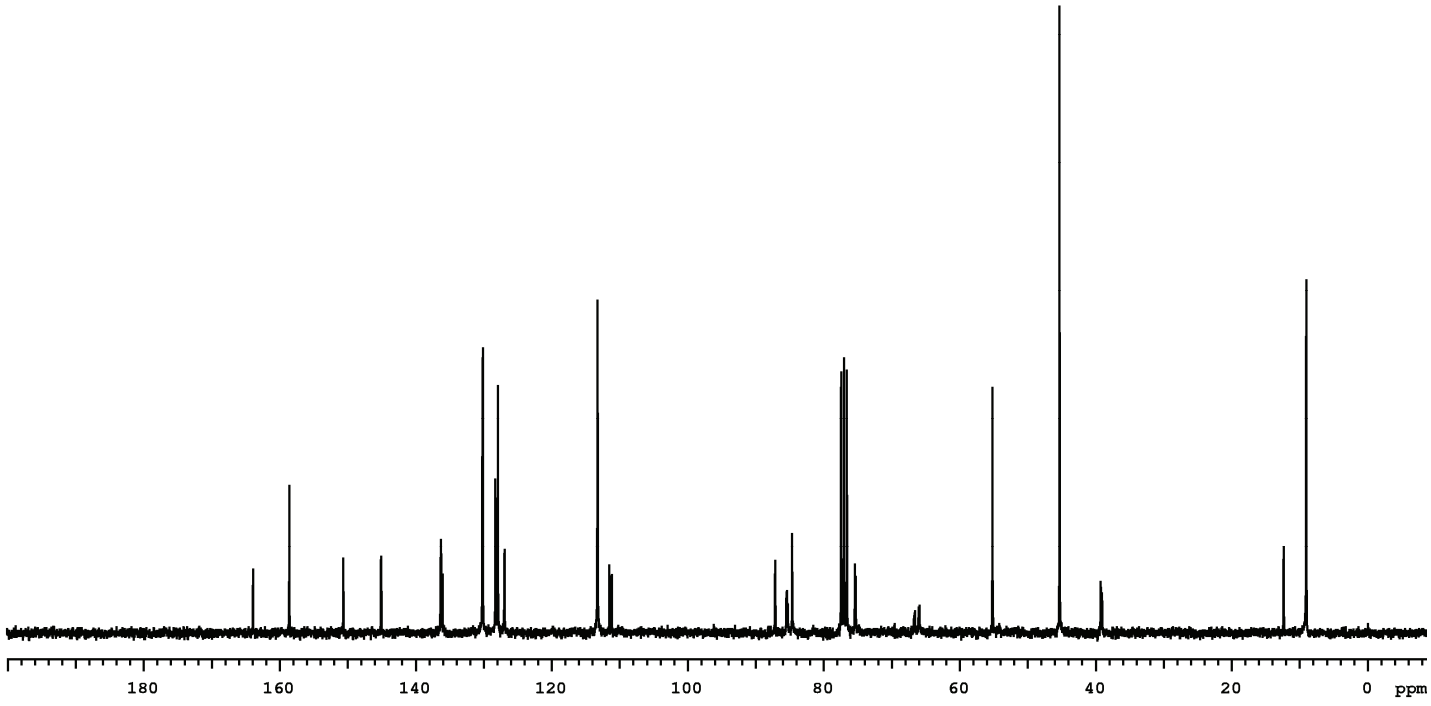
(^{1}H, 300 MHz, $CDCl_3$)

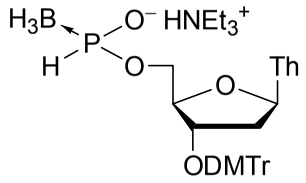




9b

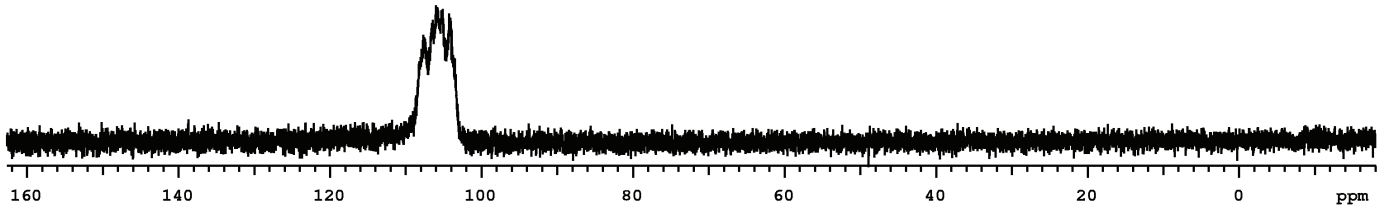
(^{13}C, 75.5 MHz, $CDCl_3$)

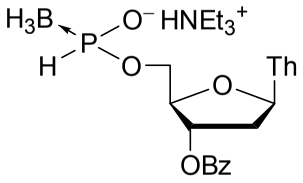




9b

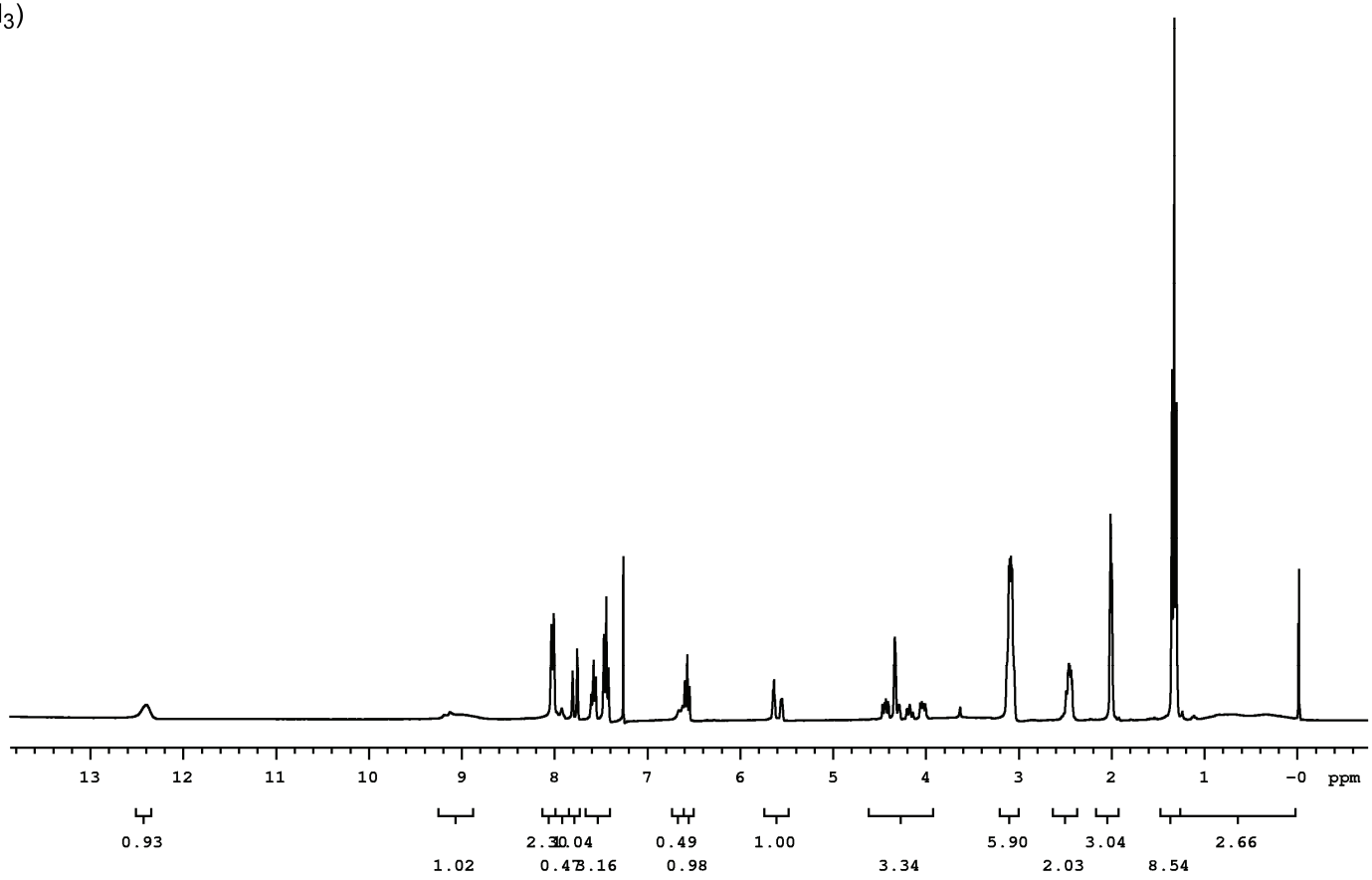
(^{31}P, 121.5 MHz, $CDCl_3$)

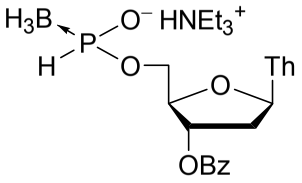




9c

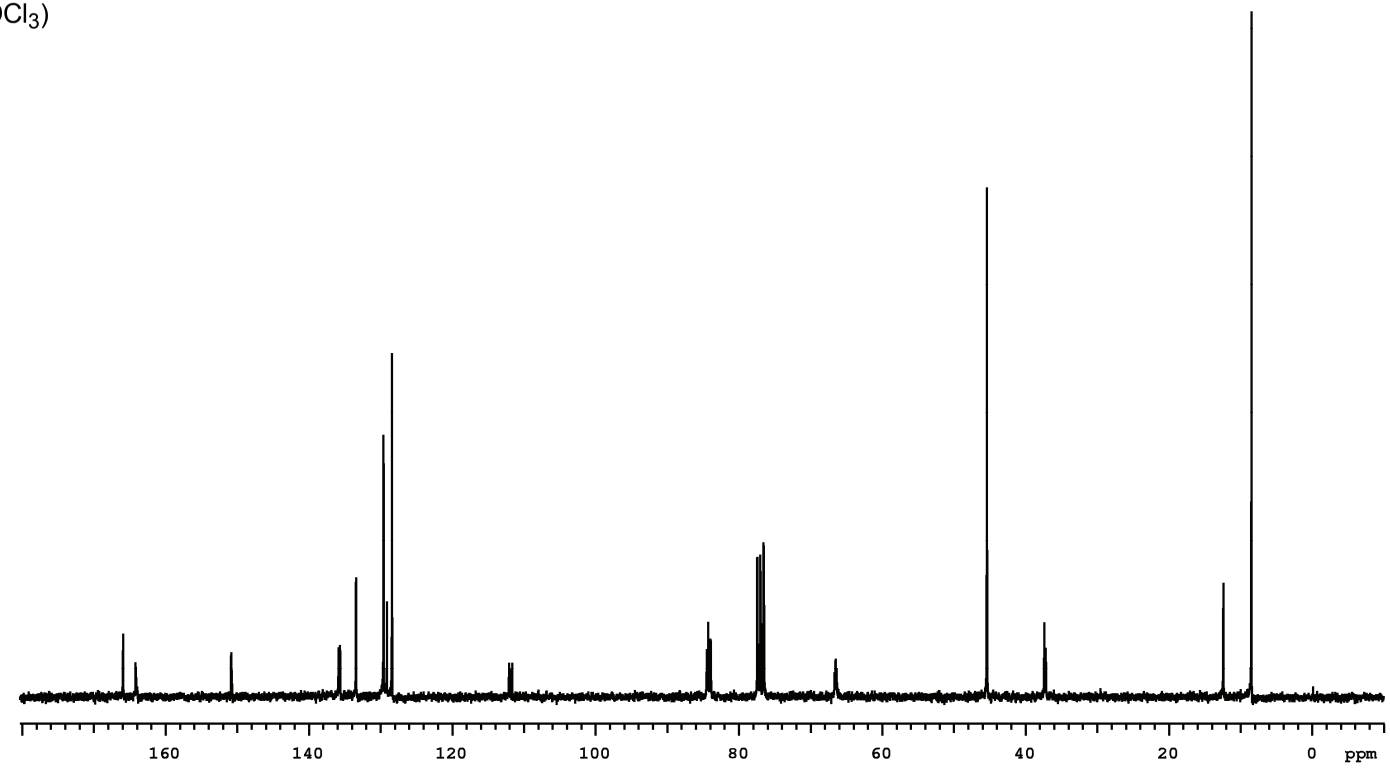
(^{1}H, 300 MHz, $CDCl_3$)

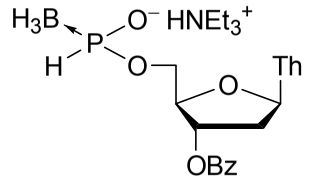




9c

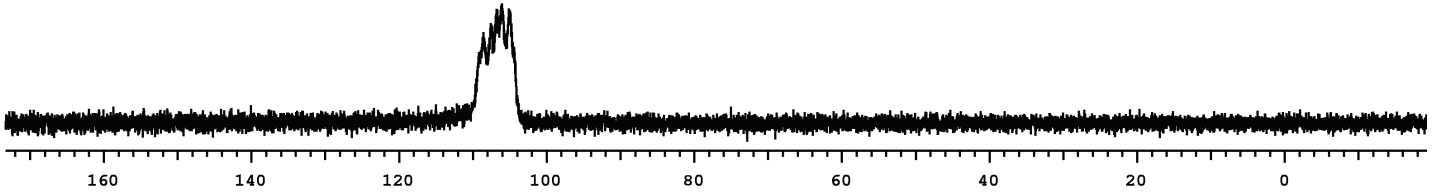
(^{13}C, 75.5 MHz, $CDCl_3$)

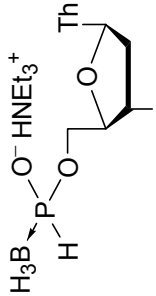




9c

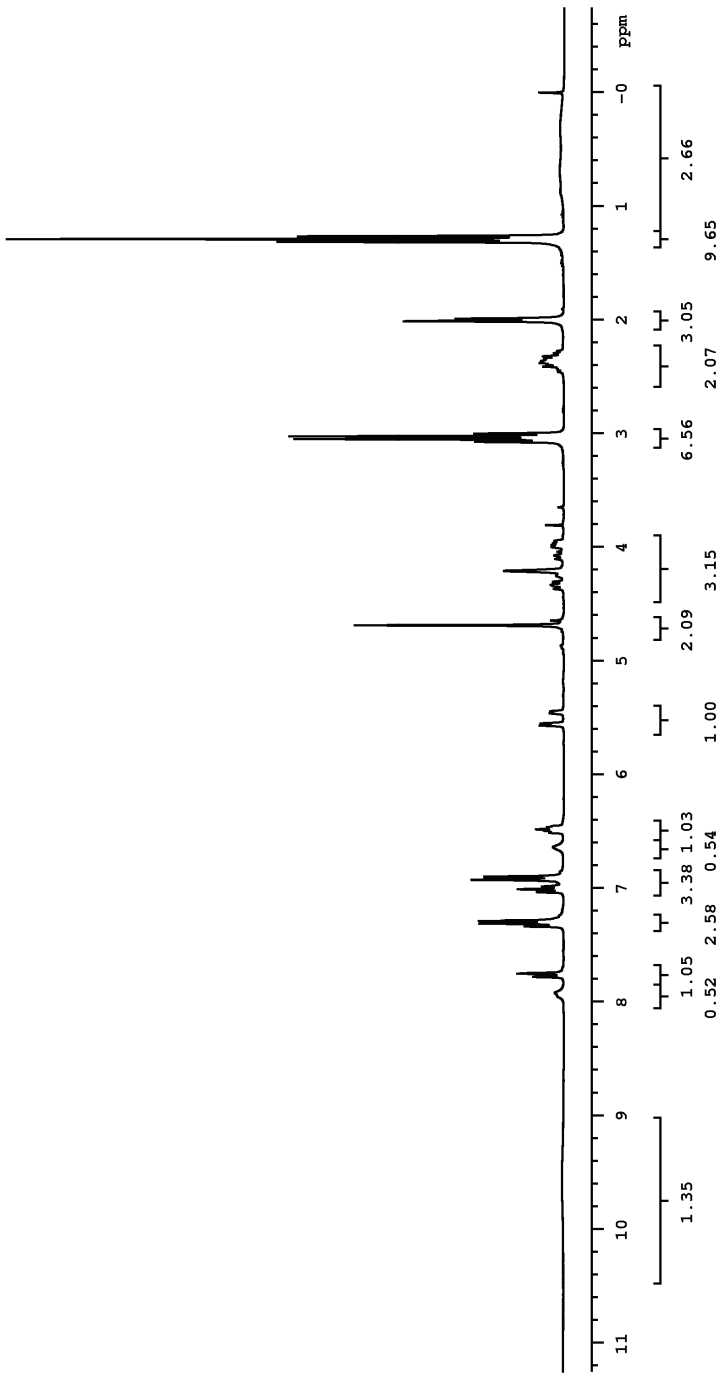
(^{31}P, 121.5 MHz, $CDCl_3$)

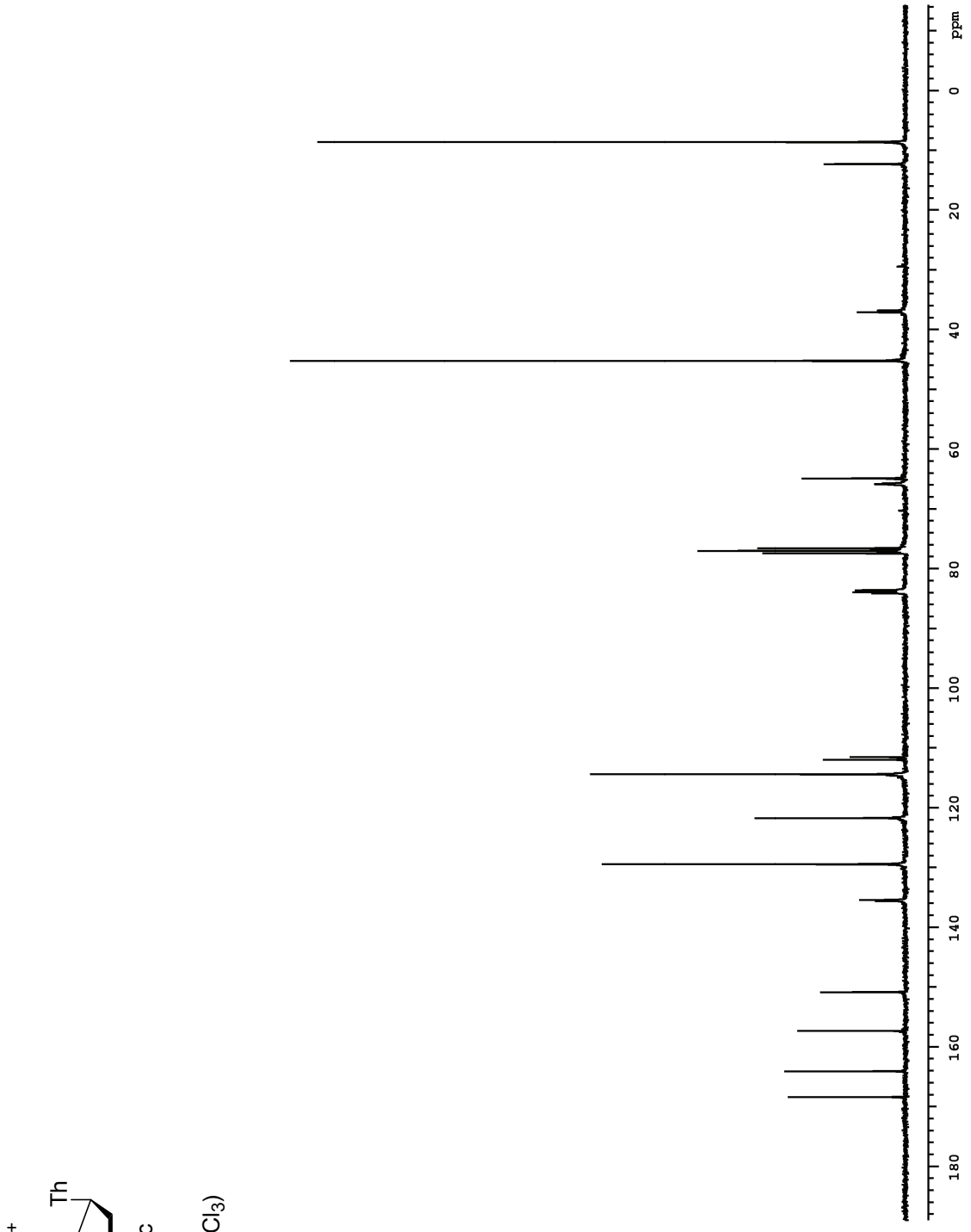




9d

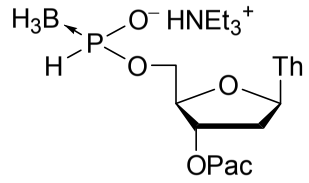
(^{1}H, 300 MHz, $CDCl_3$)





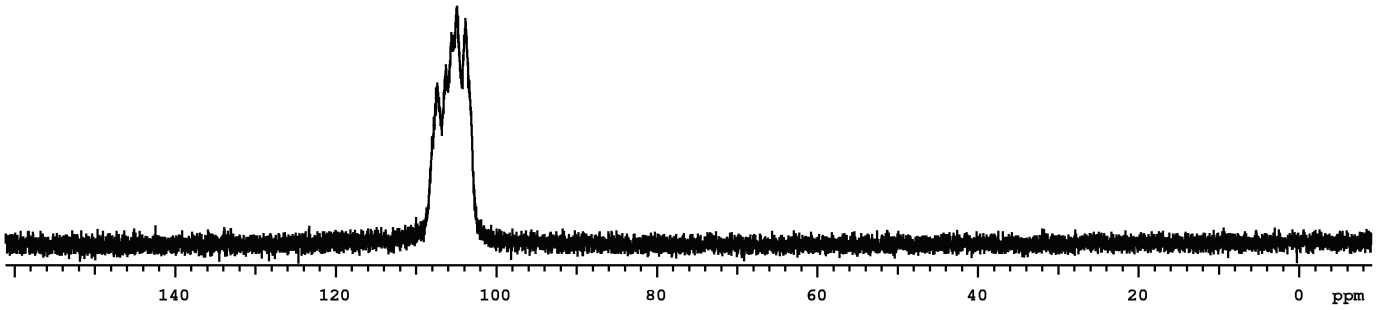
9d

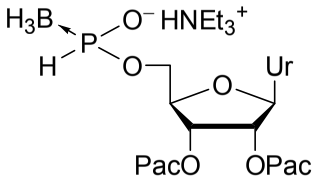
(^{13}C, 75.5 MHz, $CDCl_3$)



9d

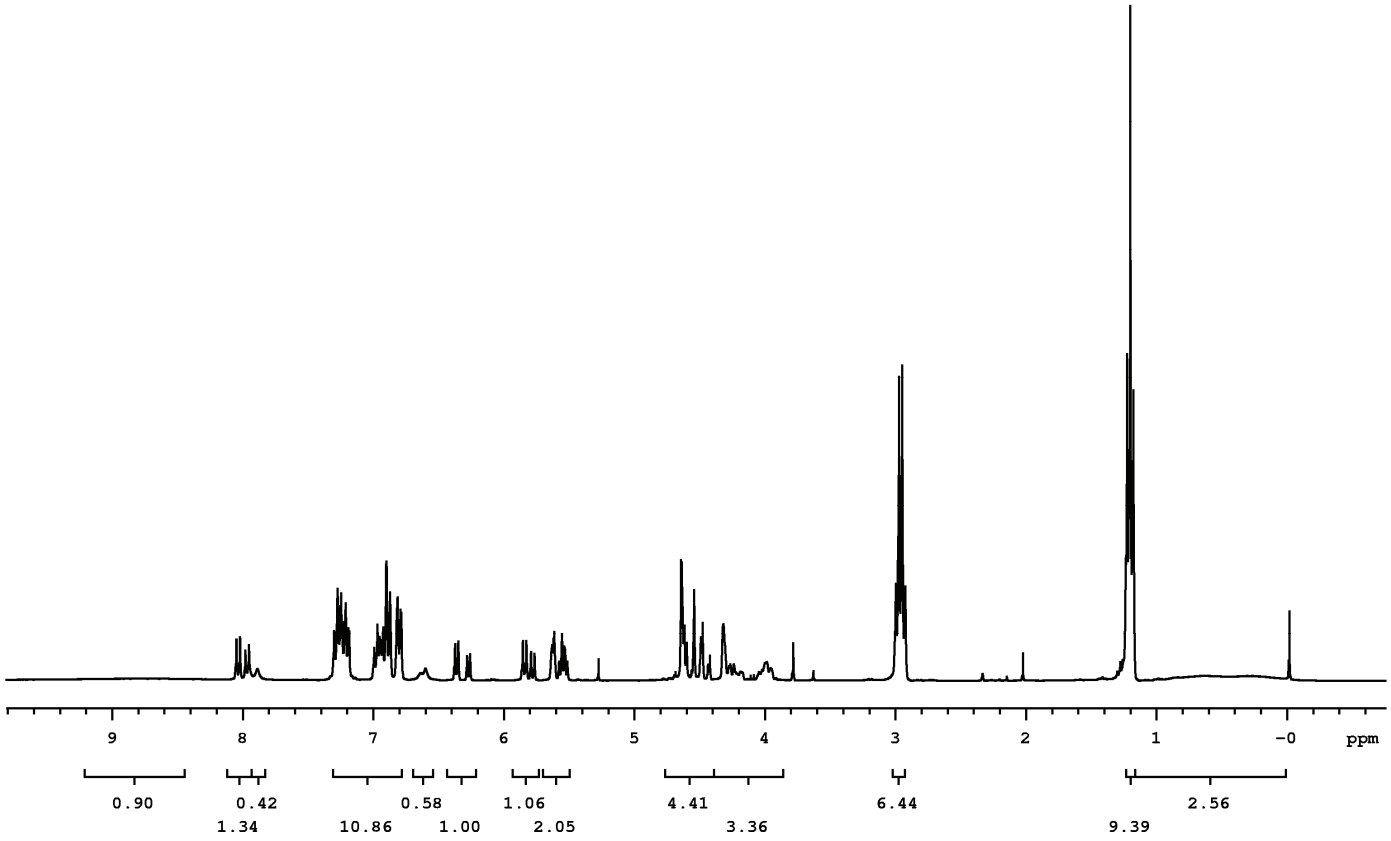
(^{31}P, 121.5 MHz, $CDCl_3$)

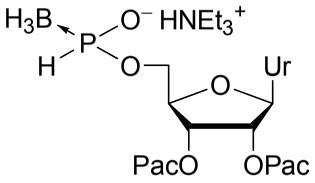




9e

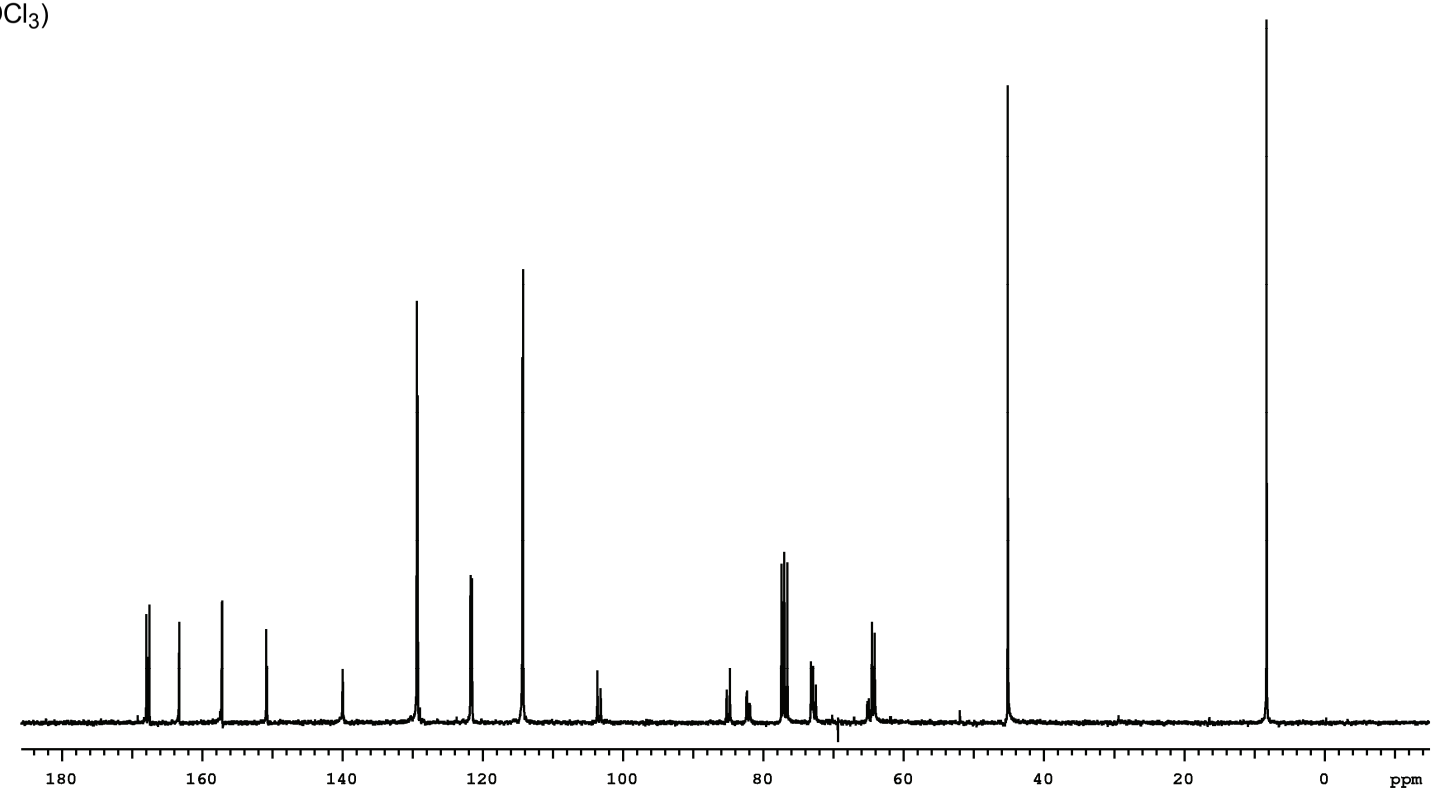
(^{1}H, 300 MHz, $CDCl_3$)

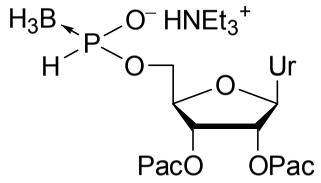




9e

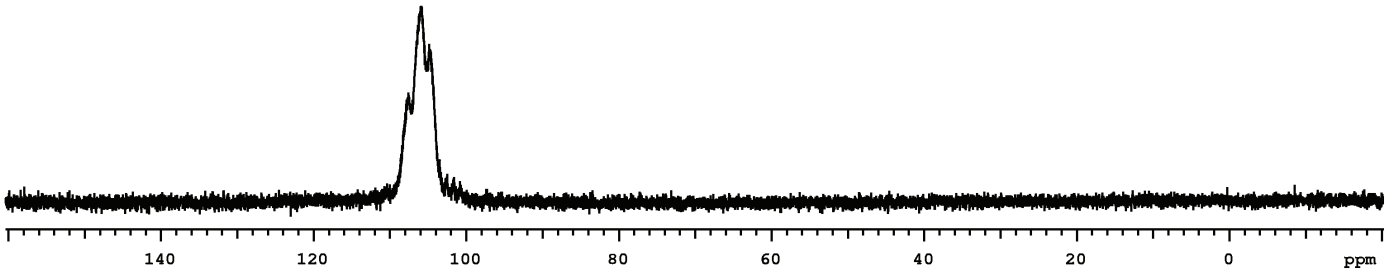
(^{13}C, 75.5 MHz, $CDCl_3$)

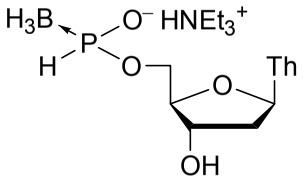




9e

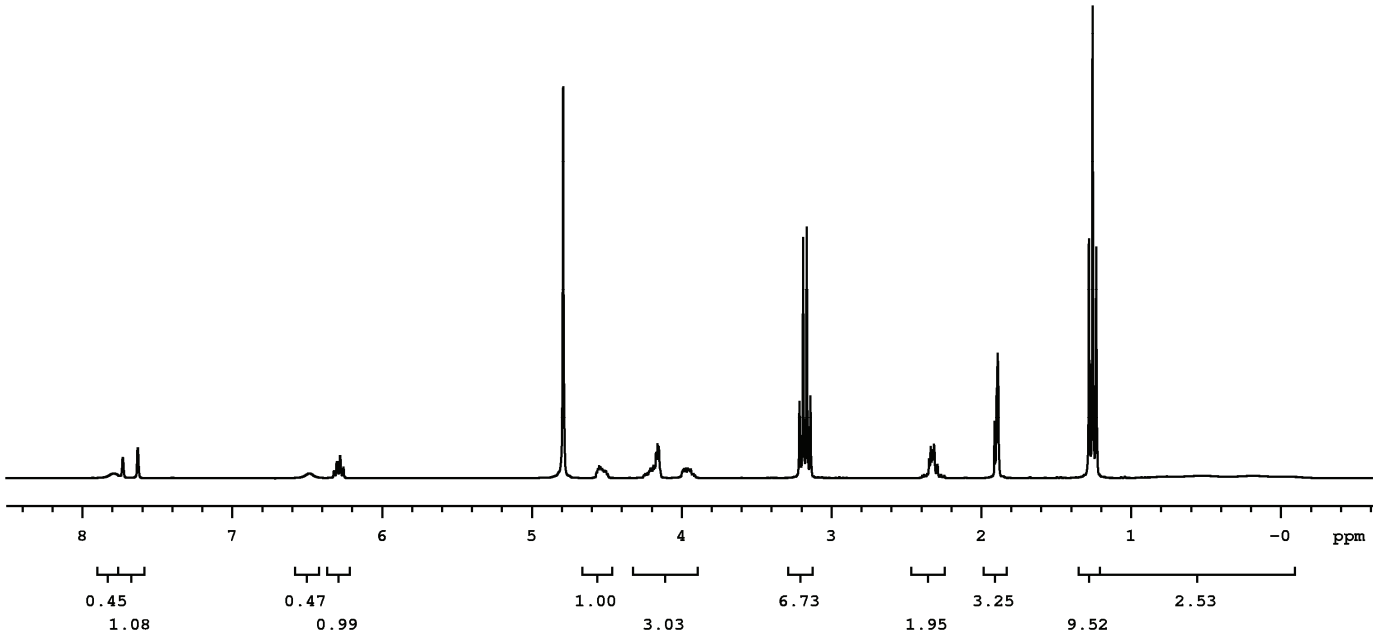
(^{31}P, 121.5 MHz, $CDCl_3$)

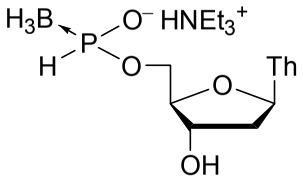




10a

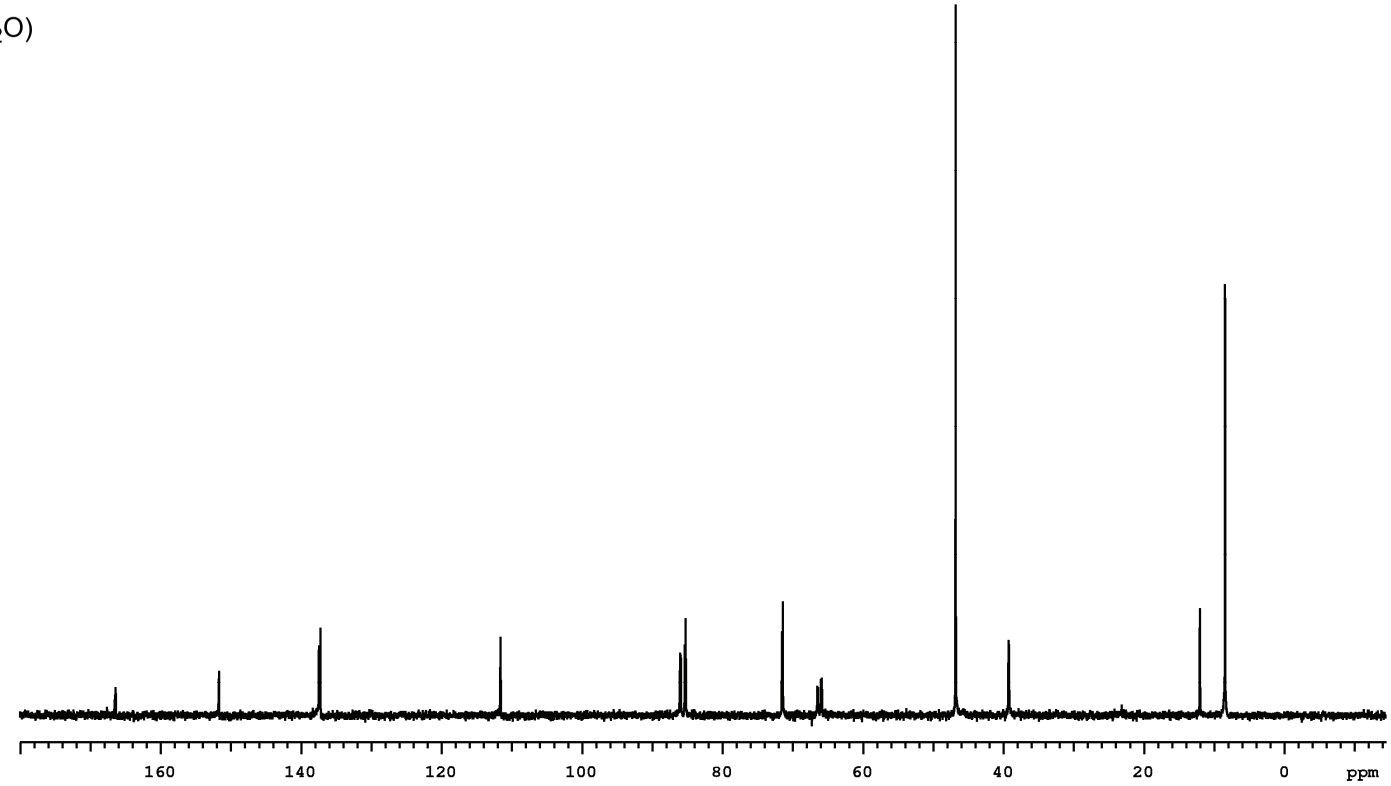
(^{1}H, 300 MHz, D_2O)

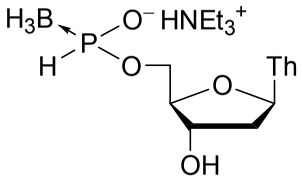




10a

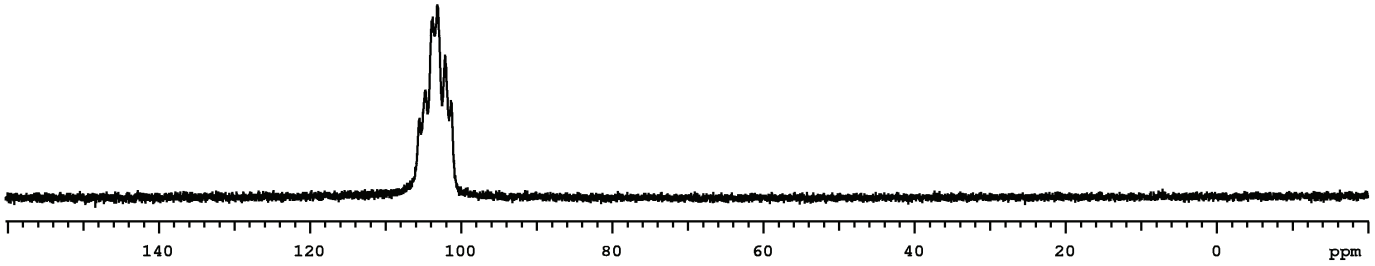
(^{13}C, 75.5 MHz, D_2O)

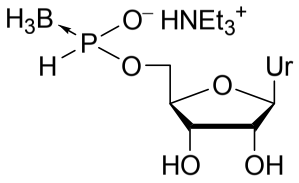




10a

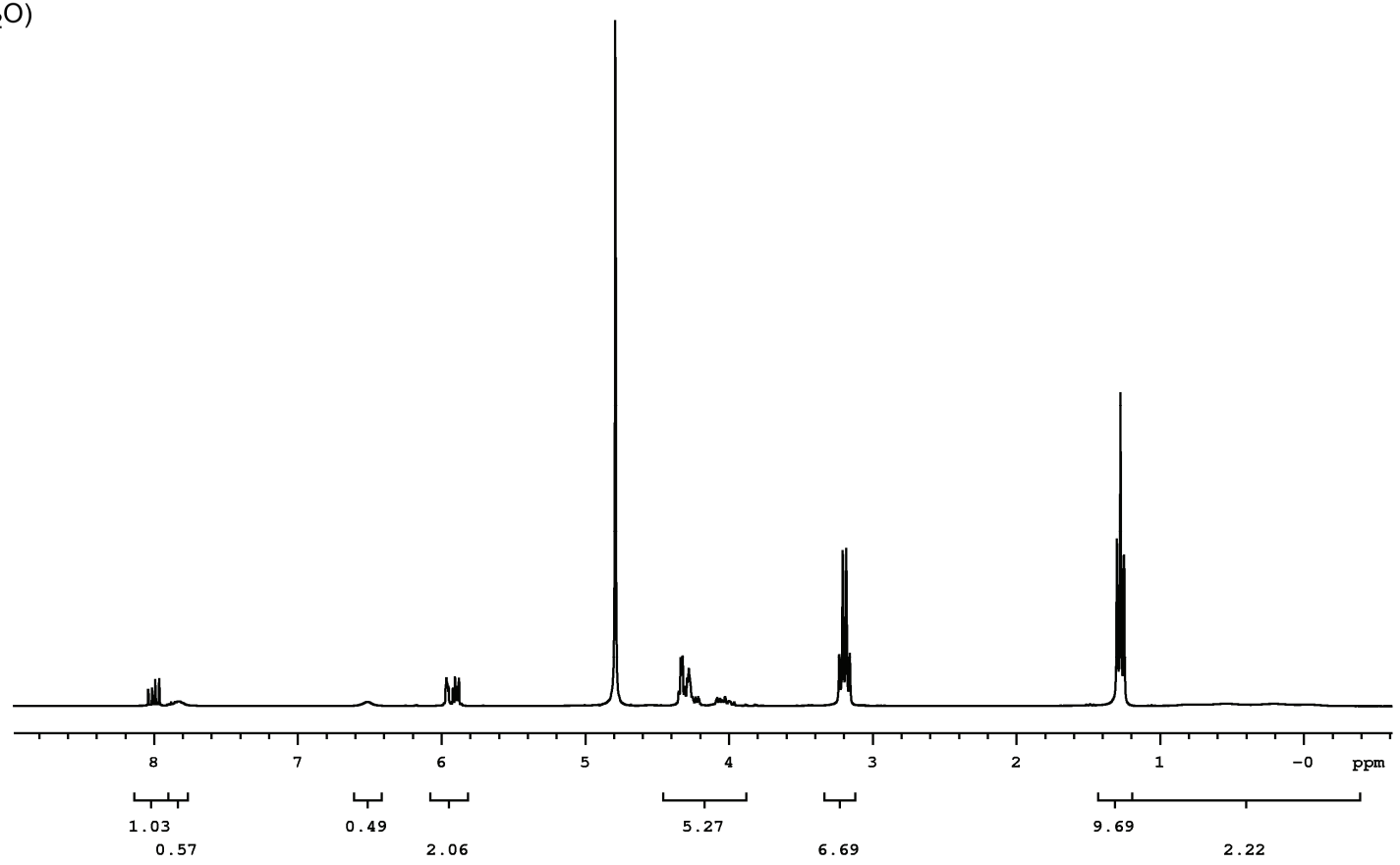
(^{31}P, 121.5 MHz, D_2O)

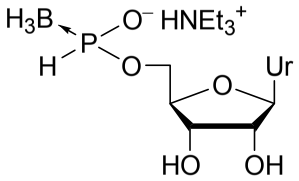




10b

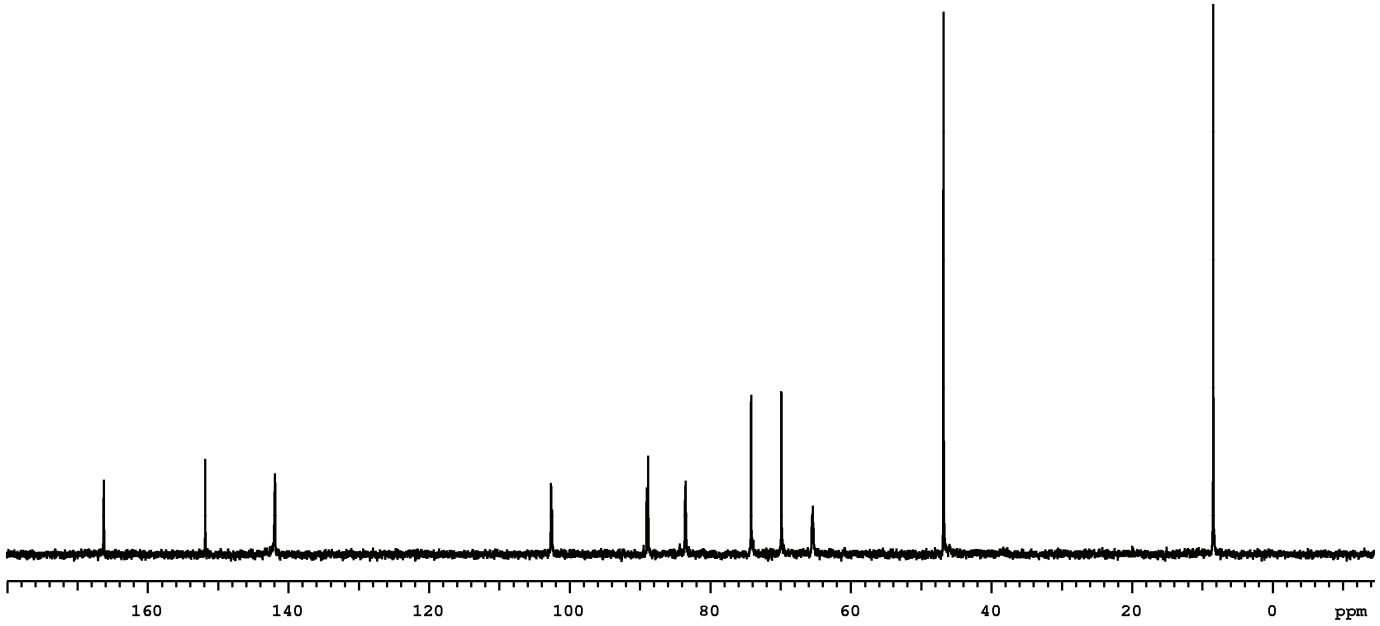
(^{1}H, 300 MHz, D_2O)

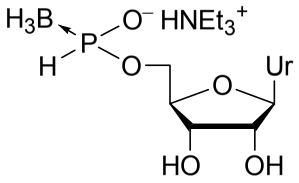




10b

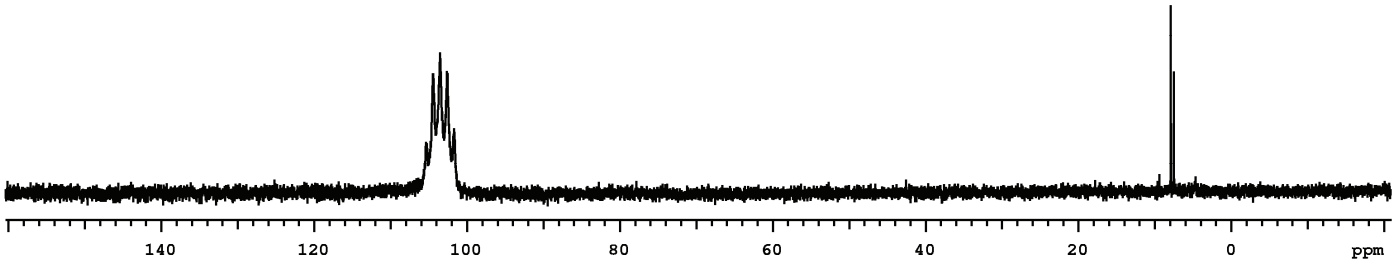
(^{13}C, 75.5 MHz, D_2O)

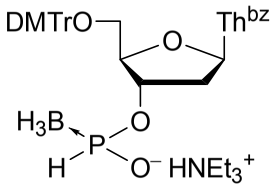




10b

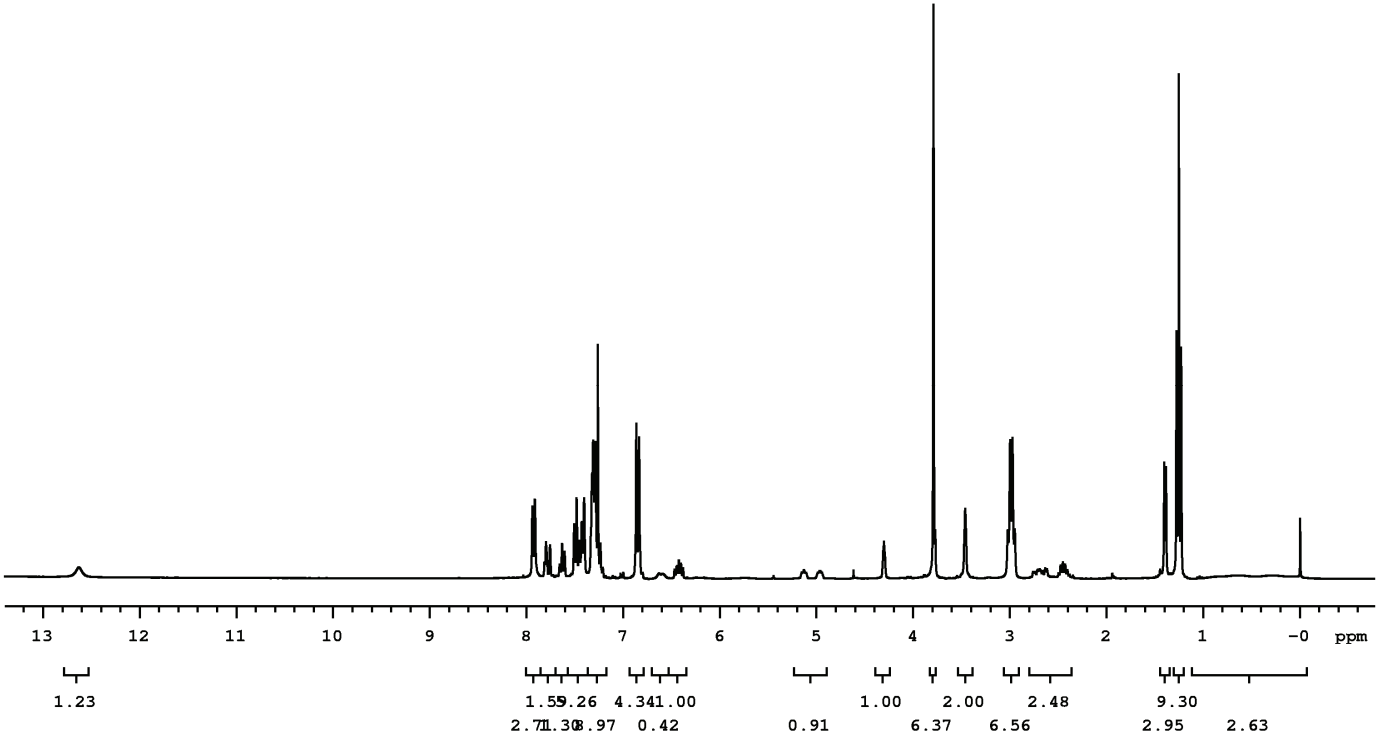
(^{31}P, 121.5 MHz, D_2O)

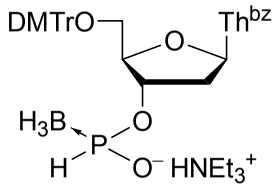




12a

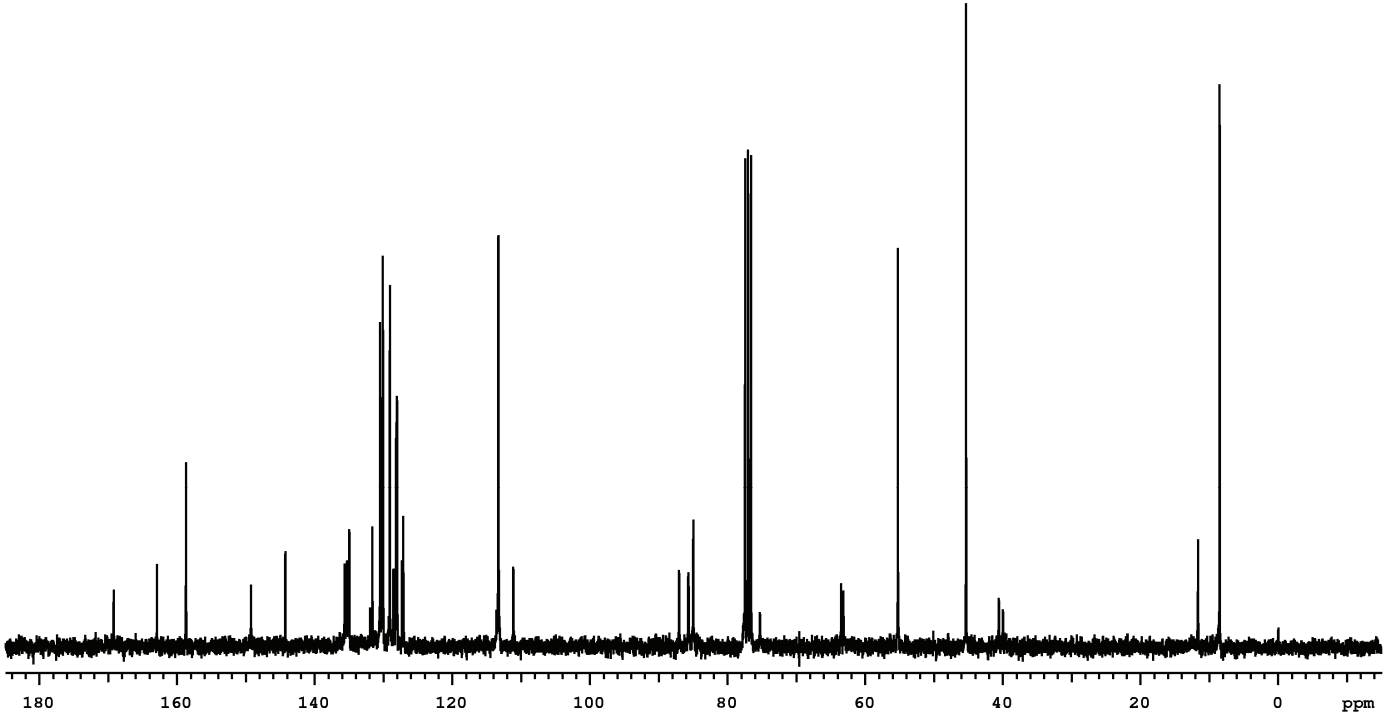
(^{1}H, 300 MHz, $CDCl_3$)

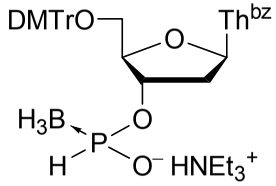




12a

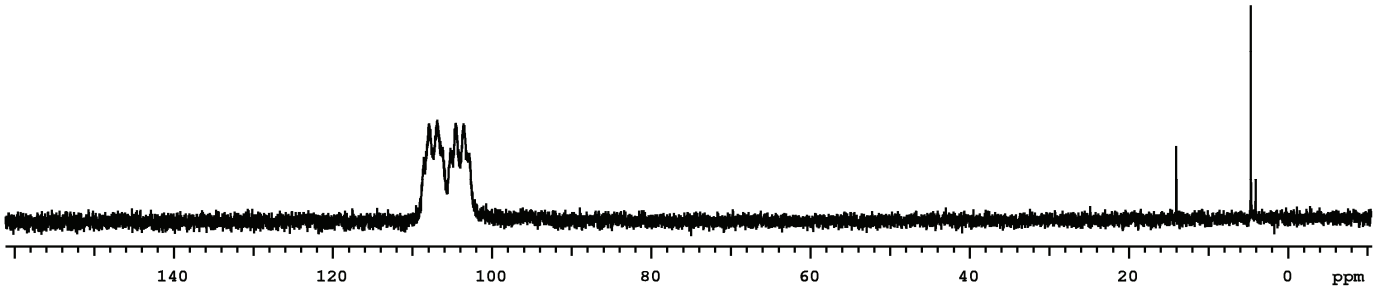
(^{13}C, 75.5 MHz, $CDCl_3$)

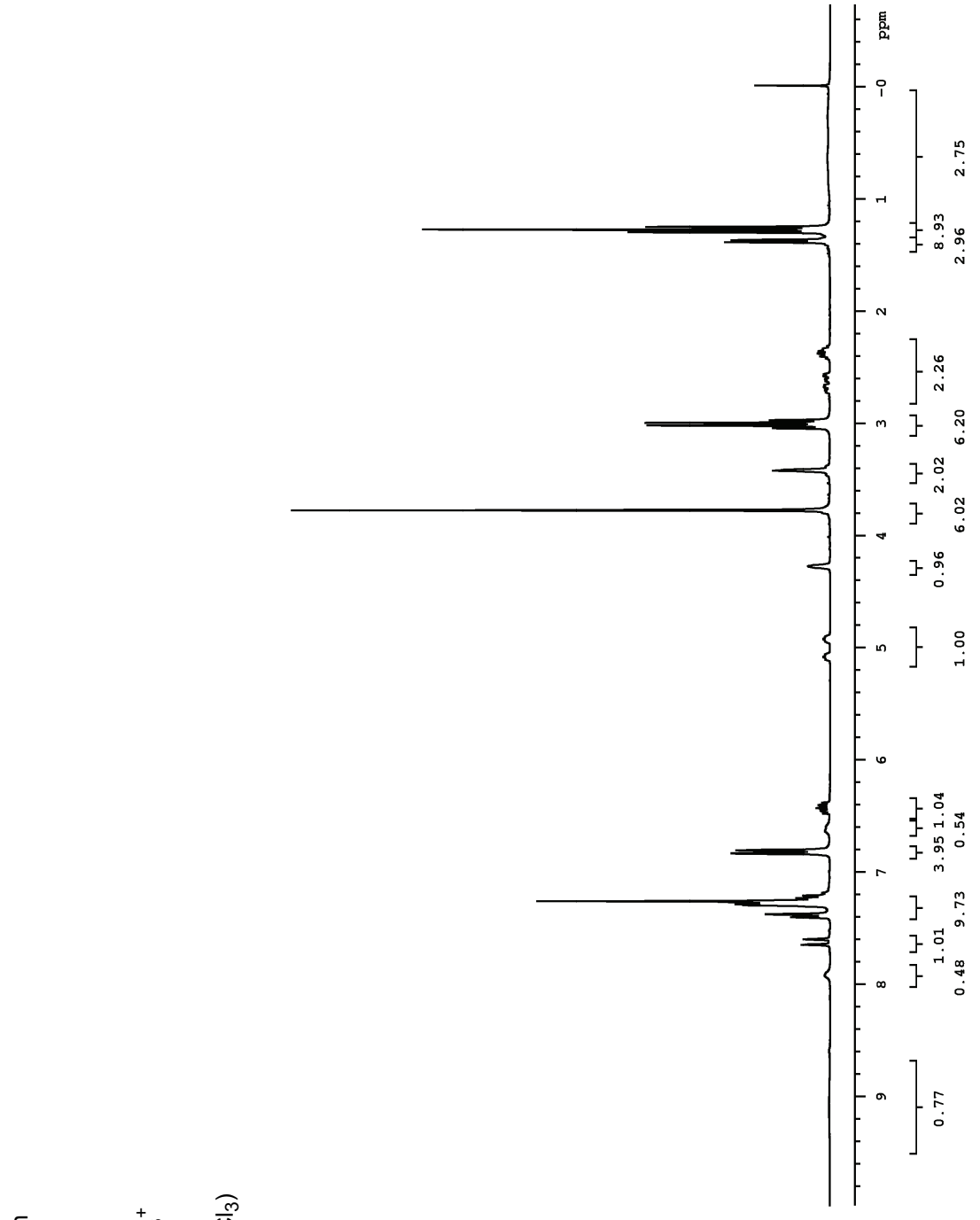




12a

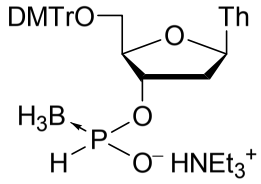
(^{31}P, 121.5 MHz, $CDCl_3$)





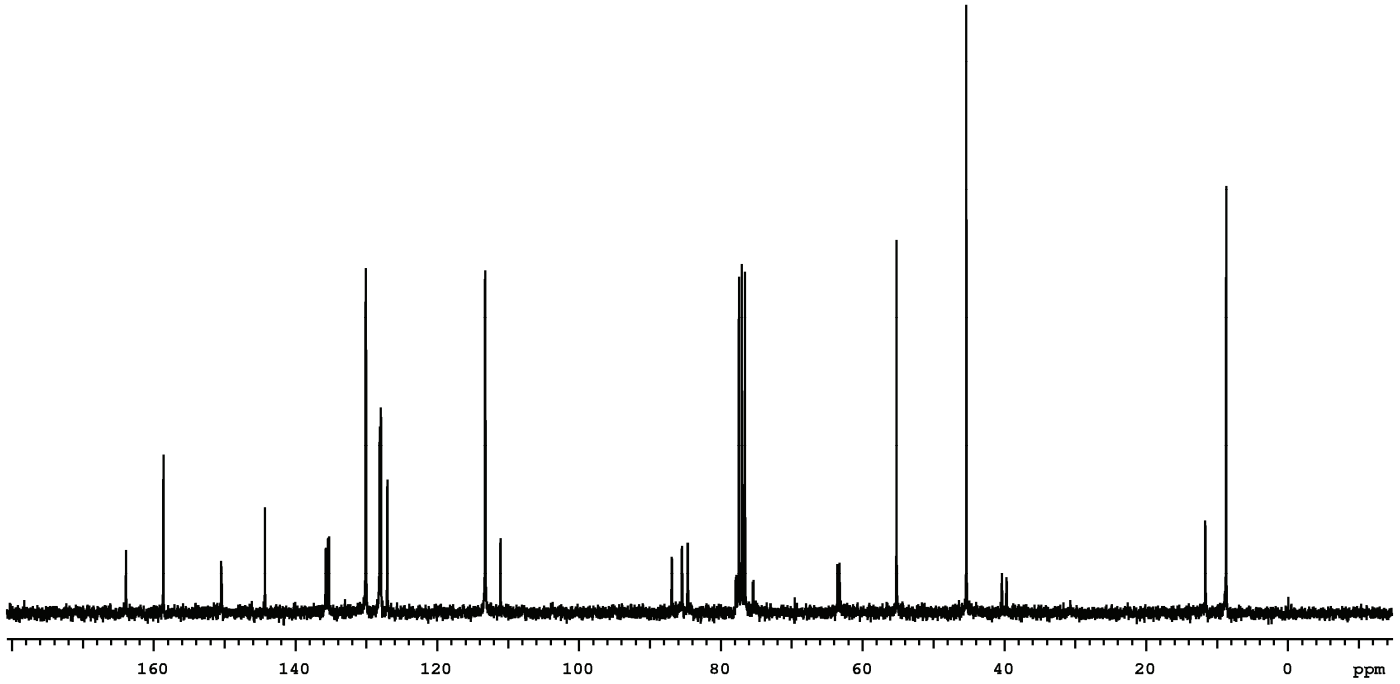
12b

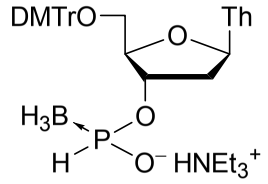
(^{1}H, 300 MHz, $CDCl_3$)



12b

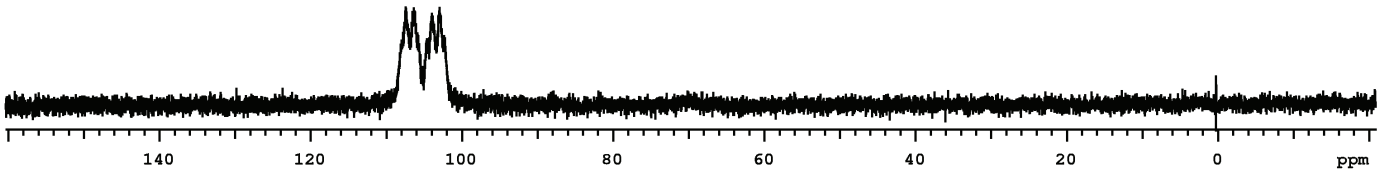
(^{13}C, 75.5 MHz, $CDCl_3$)

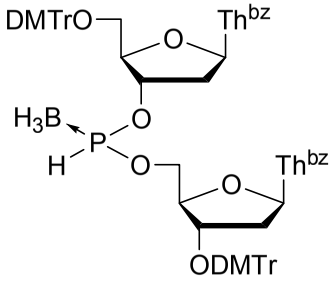




12b

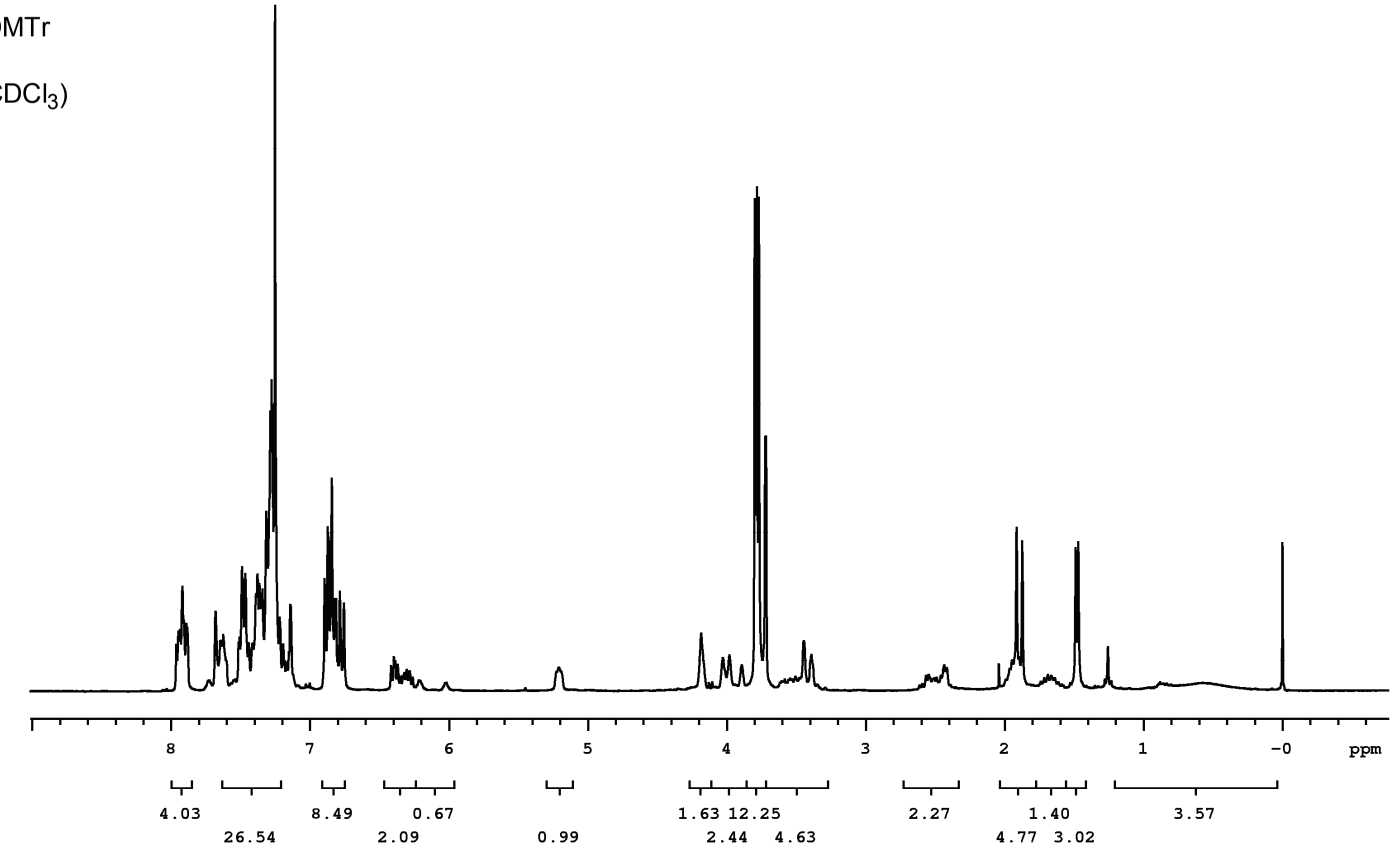
(^{31}P, 121.5 MHz, $CDCl_3$)

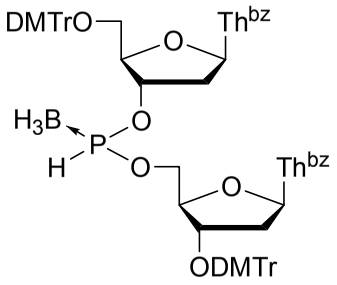




13a

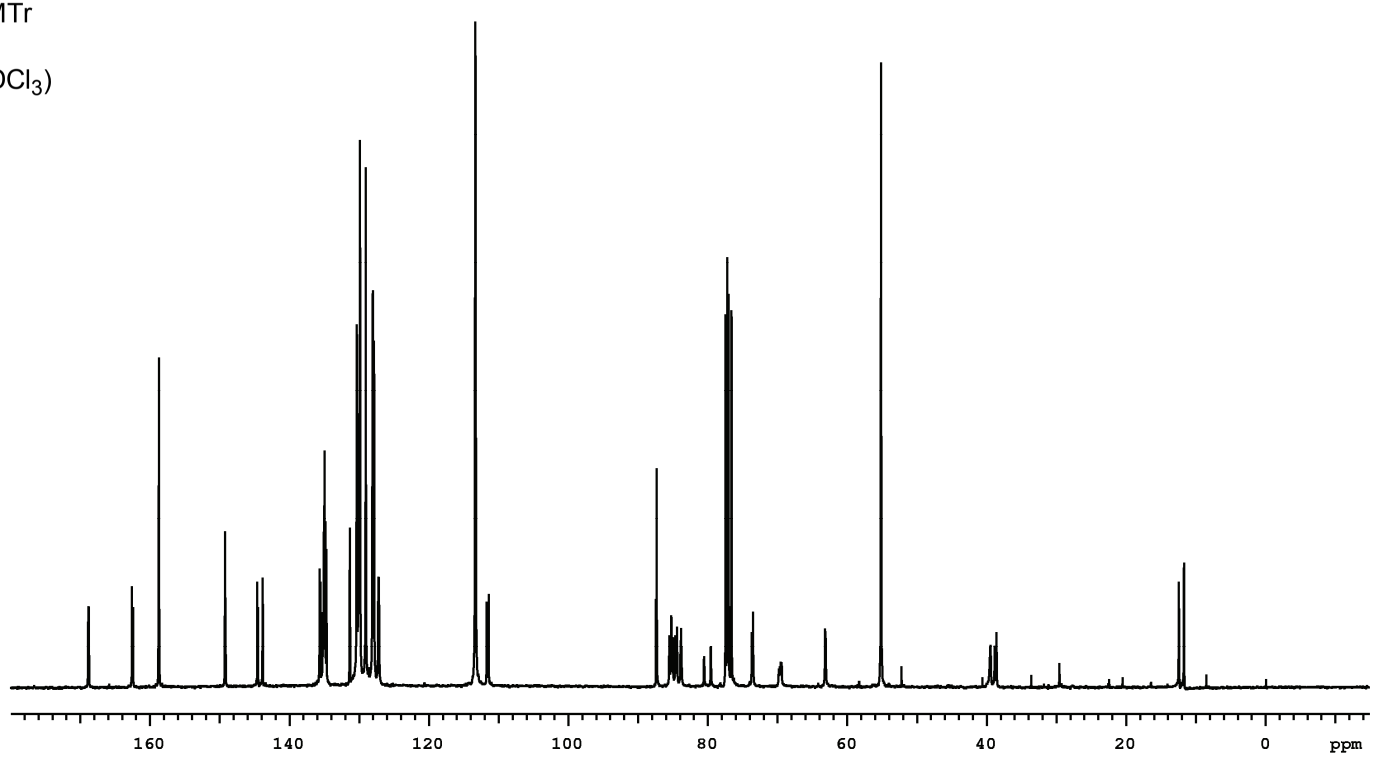
(^{1}H, 300 MHz, $CDCl_3$)

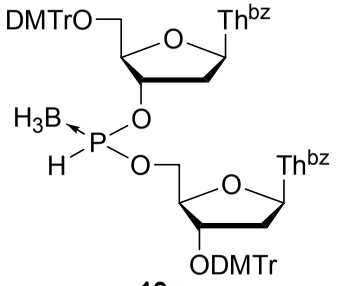




13a

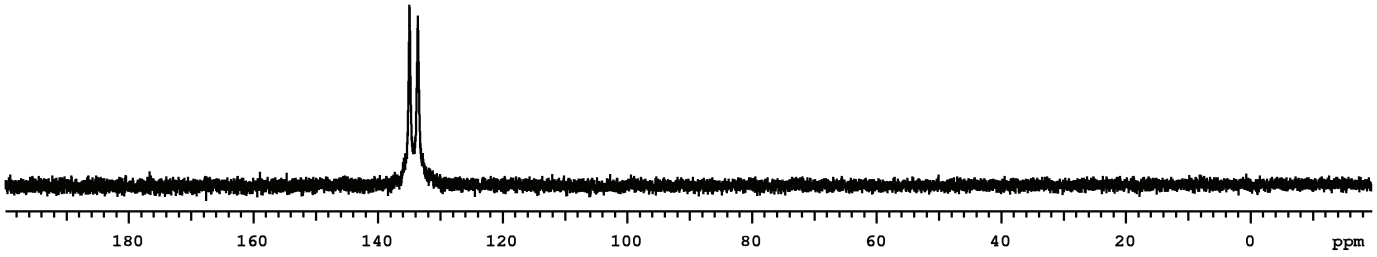
(^{13}C, 75.5 MHz, $CDCl_3$)

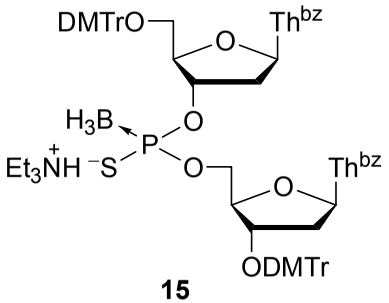




13a

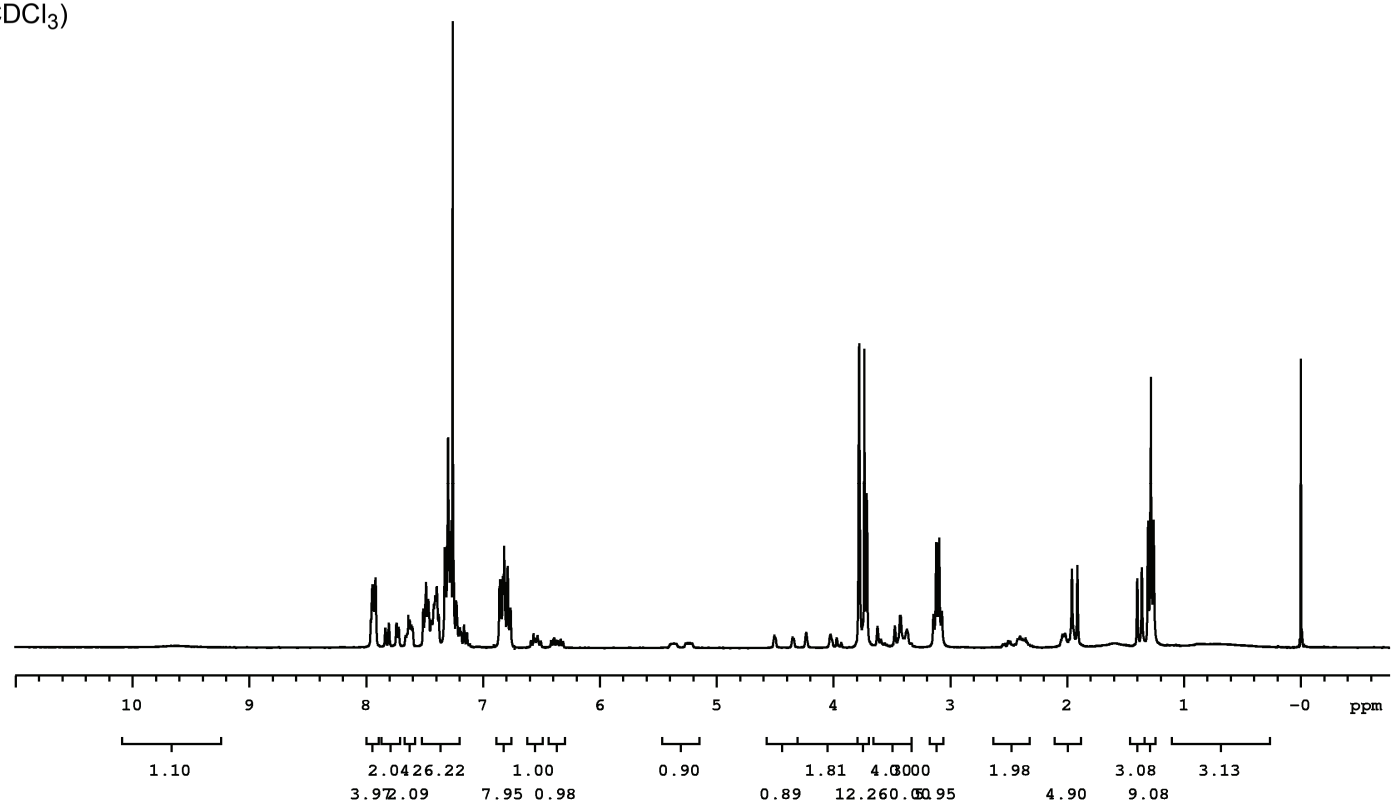
(^{31}P, 121.5 MHz, $CDCl_3$)

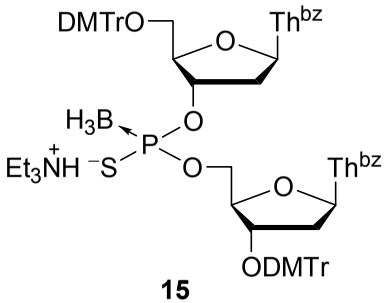




15

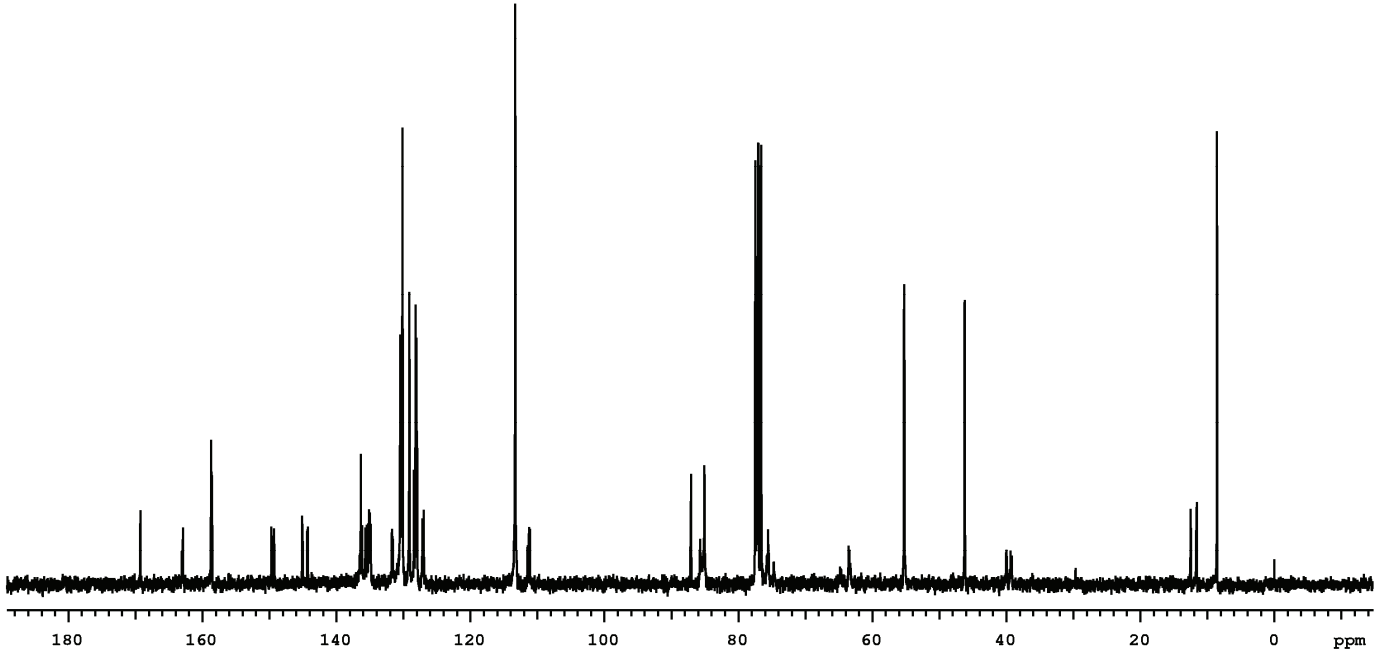
(^{1}H, 300 MHz, $CDCl_3$)

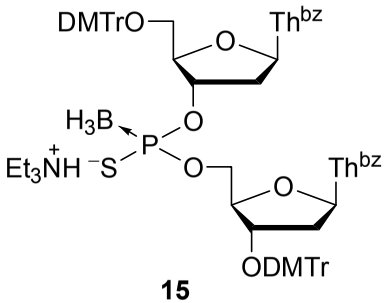




15

(^{13}C, 75.5 MHz, $CDCl_3$)





15

(^{31}P, 121.5 MHz, $CDCl_3$)