Supplementary Information

Enhancement of affinity of 2′-*O*-Me-oligonucleotides for complementary RNA by incorporating a stereoregulated boranophosphate backbone

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General information.

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 $CDCl_3$. ^{13}C NMR spectra were obtained at 75.5 MHz with $CDCl_3$ as an internal standard (δ 77.0) in $CDCl_3$. ^{31}P NMR spectra were obtained at 121.5 MHz with 85% H_3PO_4 (δ 0.0) as an external standard. Silica gel column chromatography was carried out using Kanto silica gel 60N (spherical, neutral, 63−210 μm) or NH Silica gel (Fuji Silysia Chemical). Analytical TLC was performed on Merck Kieselgel 60-F254 plates. Dry organic solvents were prepared by appropriate procedures prior to use. The other organic solvents were reagent grade and used as received. Manual solid-phase synthesis was performed by using a glass filter (10 mm × 50 mm) with a stopper at the top and a stopcock at the bottom as a reaction vessel. Highly cross-linked polystyrene (HCP) were purchased from Applied Biosystems. UV quantitation at 260 nm for 2′-*O*-Me-PB-ORNs was performed with a JASCO V-550 UV/VIS spectrophotometer. RP-HPLC was carried out using a μBondasphere (C18) (100 Å, 3.9 mm × 150 mm) (Waters). Melting curves of ORN duplexes were recorded on a Shimadzu UV-1650PC UV-Visible spectrophotometer.

(*S*p)-U-Monomer [(*S*p)-5].

5′-*O*-MMTr-2′-*O*-Me-uridine (0.53 g, 1.0 mmol) was dried by repeated coevaporations with dry pyridine and dry toluene and dissolved in freshly distilled THF (5.0 mL). Triethylamine (1.0 mL, 7.0 mmol) and a 0.167 M solution of the 2-chloro-1,3,2-oxazaphospholidine derivative L-**3** in freshly distilled THF (12 mL, 2.0 mmol) were successively added at –78 °C, and the mixture was stirred for 4 h at rt. The mixture was then diluted with $CHCl_3$ (100 ml) and washed with a saturated $NaHCO_3$ aqueous solutions (3 × 100 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was then purified by silica gel column chromatography [NH silica gel, hexane–ethyl acetate–triethylamine (30:70:0.05, v/v/v)]. The fractions containing (*S*p)-**5** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure to afford (*S*p)-**5** (0.44 g, 0.59 mmol, 59%) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 9.16 (1H, brs), 8.08 (1H, d, J = 7.8 Hz), 7.42–7.22 (17H, m), 6.81 (2H, d, J = 9.0 Hz), 6.01 (1H, d, J = 2.1 Hz), 5.20 (1H, d, J = 8.4 Hz), 4.86–4.79 (1H, m), 4.28 (1H, d, J = 7.5 Hz), 3.97 (1H, dd, J = 4.8, 2.1 Hz), 3.81–3.77 (1H, m), 3.72 (3H, s), 3.62–3.59 (5H, m), 3.50–3.39 (1H, m), 3.09–2.98 (1H, m), 1.75 (3H, s), 1.58–1.35 (2H, m), 1.28–1.15 (1H, m), 1.02–0.91 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.2, 159.0, 150.2, 144.0 (d, $^{3}J_{PC}$ = 4.4 Hz), 143.7, 140.5, 134.7, 130.8, 128.8, 128.7, 128.3, 127.6, 127.5, 127.3, 125.6, 125.6, 113.5, 102.2, 91.9 (d, $^{2}J_{PC}$ = 11.9 Hz), 88.0, 87.6, 83.7, 82.5, 76.8, 73.3, 69.7 (d, $^{2}J_{PC}$ = 12.8 Hz), 61.1, 58.9, 55.4, 46.7 (d, $^{2}J_{PC}$ = 34.1 Hz), 30.4, 29.9, 25.9 (d, $^{3}J_{PC}$ = 3.5 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ 159.1.

(*R*p)-U-Monomer [(*R*p)-5].

5′-*O*-MMTr-2′-*O*-Me-uridine (0.53 g, 1.0 mmol) was dried by repeated coevaporations with pyridine and toluene and dissolved in freshly distilled THF (5.0 mL). Triethylamine (1.0 mL, 7.0 mmol) and a 0.4 M solution of the 2-

chloro-1,3,2-oxazaphospholidine derivative D-**3** in freshly distilled THF (5.0 mL, 2.0 mmol) were successively added at –78 °C, and the mixture was stirred for 3 h at rt. The mixture was then diluted with $CHCl_3$ (100 ml) and washed with a saturated $NaHCO_3$ aqueous solutions (3 × 100 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was then purified by silica gel column chromatography [NH silica gel, hexane–ethyl acetate–triethylamine (30:70:0.05, v/v/v)]. The fractions containing (*R*p)-**5** were collected, washed with a saturated $NaHCO_3$ aqueous solution (100 mL), dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure to afford (*R*p)-**5** (0.38 g, 0.51 mmol, 51%) as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 8.21 (1H, d, J = 8.1 Hz), 7.46–7.26 (17H, m), 6.87 (2H, d, J = 9.0 Hz), 5.95 (1H, s), 5.23 (1H, d, J = 8.1 Hz), 4.93–4.85 (1H, m), 4.27 (1H, d, J = 8.4 Hz), 3.85–3.76 (5H, m), 3.65–3.53 (5H, m), 3.43–3.32 (1H, m), 3.08–2.95 (1H, m), 1.88 (3H, s), 1.59–1.39 (2H, m), 1.34–1.21 (1H, m), 1.01–0.90 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 159.1, 150.2, 144.0 (d, $^3J_{PC}$ = 6.2 Hz), 143.9, 143.7, 140.4, 134.7, 130.9, 128.8, 128.4, 128.3, 127.6, 127.5, 127.3, 126.9, 125.5, 125.4, 125.1, 113.5, 102.1, 92.2 (d, $^2J_{PC}$ = 11.8 Hz), 87.9, 87.6, 84.1, 81.7 ($^2J_{PC}$ = 5.2 Hz), 76.8, 73.3, 73.3, 68.9 ($^2J_{PC}$ = 15.9 Hz), 68.1, 60.2, 58.6, 55.5, 46.6 (d, $^2J_{PC}$ = 33.8 Hz), 30.4, 30.0, 29.7, 26.8, 26.0 (d, $^3J_{PC}$ = 3.8 Hz), 25.6. ^{31}P NMR (121 MHz, $CDCl_3$) δ 159.8.

2′-*O*-Me-PB-ORN 2mers (9, 10).

5′-*O*-DMTr-2′-*O*-Me-uridine-loaded HCP resin (0.5 μmol) via a succinyl linker was treated 3% DCA in CH_2Cl_2 (4 × 15 s) (1 mL) for the removal of the 5′-*O*-DMTr group, washed with CH_2Cl_2 (4 × 1 mL) and CH_3CN (3 × 1 mL) and dried *in vacuo*. Coupling reaction was performed by using a solution containing the corresponding nucleoside 3′-*O*-oxazaphospholidine monomer **5** (0.2 M) and CMMT **6** (1.0 M) in CH_3CN (0.1 mL) (3 min). After the condensation, the solid-support was washed with CH_3CN (3 × 1 mL) and CH_2Cl_2 (3 × 1 mL) and dried *in vacuo*. The 5′-*O*-MMTr group and the chiral auxiliary were then removed by treatment with 1% TFA in CH_2Cl_2 (4 × 5 s), (1 mL) and following washings with CH_2Cl_2 (4 × 1 mL) and CH_3CN (4 × 1 mL) and dried *in vacuo*. The residue was treated with a mixture of DMAc (0.8 mL), BSA (0.1 mL) and $BH_3{\cdot}S(CH_3)_2$ (0.1 mL) at rt for 15 min, and the resin was successively washed with DMAc (4 × 1 mL), CH_3CN (4 × 1 mL) and dried *in vacuo*. The resin was then treated with a saturated solution of NH_3 in EtOH (5 mL) at rt for 13 h. The resin was filtered off and washed with H_2O (5 × 1 mL). The filtrates were combined and concentrated to dryness under reduced pressure. The residue was analyzed by RP-HPLC.

A general procedure for manual solid-phase synthesis.

5′-*O*-DMTr-2′-*O*-Me-uridine-loaded HCP resin (0.5 μmol) via a succinyl linker was treated 3% DCA in CH_2Cl_2 (4 × 15 s) (1 mL) for the removal of the 5′-*O*-DMTr group, and washed with CH_2Cl_2 (4 × 1 mL) and CH_3CN (3 × 1 mL) and dried *in vacuo*. Chain elongation was performed by repeating the following steps (i) and (ii). (i) Coupling reaction using a solution containing the corresponding nucleoside 3′-*O*-oxazaphospholidine monomer **5** (0.2 M) and CMMT **6** (1.0 M) in CH_3CN (0.1 mL) (3 min), and following washings with CH_3CN (3 × 1 mL) and CH_2Cl_2 (3 × 1

mL) and dried *in vacuo*. (ii) removal of the 5′-*O*-MMTr group and the chiral auxiliary by treatment with 1% TFA in CH_2Cl_2–Et_3SiH (1:1, v/v) (4 × 5 s) (1 mL), and subsequent washing with CH_2Cl_2 (4 × 1 mL) and CH_3CN (4 × 1 mL) and drying *in vacuo*. The resultant *H*-phosphonate oligonucleotides on the resin were converted to boranophosphate, phosphorothioate, or phosphoramidate oligonucleotides as described below.

2′-*O*-Me-PB-ORN 4mers (11 and 12).

The corresponding *H*-phosphonate oligonucleotide 4mer assembled on an HCP resin as above was treated with a mixture of DMAc (0.8 mL), BSA (0.1 mL) and $BH_3 \cdot S(CH_3)_2$ (0.1 mL) at rt for 15 min, and the resin was successively washed with DMAc (4 × 1 mL) and CH_3CN (4 × 1 mL), and dried *in vacuo*. The resin was then treated with a saturated solution of NH_3 in EtOH (5 mL) at rt for 13 h. The resin was filtered off and washed with H_2O (5 × 1 mL). The filtrates were combined and concentrated to dryness under reduced pressure. The residue was analyzed and characterized by RP-HPLC and MALDI-TOF-MS. **11**, 75% HPLC yield, MALDI-TOF-MS: Calcd. For $[M–H]^–$; 1211.31. Found; 1211.61. **12**, 62% HPLC yield, MALDI-TOF-MS: Calcd. For $[M–H]^–$; 1211.31. Found; 1211.70.

2′-*O*-Me-PS-ORN 4mers (13 and 14).

The corresponding *H*-phosphonate oligonucleotide 4mer assembled on an HCP resin as above was treated with a solution of 3-phenyl-1,2,4-dithiazoline-5-one (POS) (0.1 M) and BSA (0.5 M) in CH_3CN (0.2 mL) at rt for 30 min, and the resin was washed with CH_3CN (3 × 1 mL) and dried *in vacuo*. The resin was then treated with a 25% NH_3 aqueous solution–EtOH (5:1, v/v) (5 mL) at rt for 3 h. The resin was filtered off and washed with H_2O (5 × 1 mL). The filtrates were combined and concentrated to dryness under reduced pressure. The residue was analyzed and characterized by RP-HPLC and MALDI-TOF-MS. **13**, 82% HPLC yield, MALDI-TOF-MS: Calcd. For $[M–H]^–$; 1265.13. Found; 1265.52. **14**, 85% HPLC yield, MALDI-TOF-MS: Calcd. For $[M–H]^–$; 1265.13. Found; 1265.52.

2′-*O*-Me-PN-ORN 4mers bearing 2-dimethylaminoethylamino groups on the phosphorus atoms (15 and 16).

The corresponding *H*-phosphonate oligonucleotide 4mer assembled on an HCP resin as above was treated with CCl_4–2-dimethylaminoethylamine (9:1, v/v) (5 mL) at rt for 2 h, and the resin was washed with CH_3CN (3 × 1 mL) and dried *in vacuo*. The resin was then treated with a 25% NH_3 aqueous solution–EtOH (4:1, v/v) (5 mL) at rt for 2 h. The resin was filtered off and washed with H_2O (5 × 1 mL). The filtrates were combined and concentrated to dryness under reduced pressure. The residue was analyzed and characterized by RP-HPLC and MALDI-TOF-MS. **15**, 62% HPLC yield, MALDI-TOF-MS: Calcd. For $[M+H]^+$; 1429.48. Found; 1427.61. **16**, 67% HPLC yield, MALDI-TOF-MS: Calcd. For $[M+H]^+$; 1429.48. Found; 1428.89.

2′-*O*-Me-PB-ORN 10mers (17 and 18).

The corresponding *H*-phosphonate oligonucleotide assembled on an HCP resin as above was treated with a

mixture of DMAc (0.8 mL), BSA (0.1 mL) and $BH_3 \cdot S(CH_3)_2$ (0.1 mL) at rt for 15 min, and the resin was successively washed with DMAc (4 × 1 mL), CH_3CN (4 × 1 mL) and dried *in vacuo*. The resin was then treated with a saturated solution of NH_3 in EtOH (5 mL) at rt for 12 h. The resin was filtered off and washed with H_2O (5 × 1 mL). The filtrates were combined and concentrated to dryness under reduced pressure. The residue was purified and characterized by RP-HPLC and MALDI-TOF-MS. **17**, 5% isolated yield, LC-MS: Calcd. For $[M–H]^–$; 3119.8. Found; 3119.7. **18**, 2% isolated yield, MALDI-TOF-MS: Calcd. For $[M–H]^–$; 3119.8. Found; 3119.7.

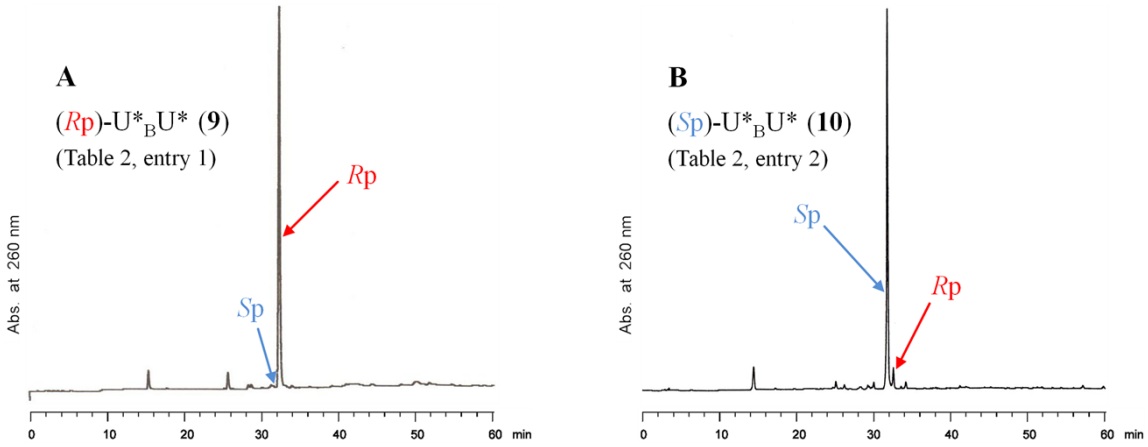


Figure S1. RP-HPLC profiles of crude **9** and **10** (Table 2, entries 1–2). RP-HPLC was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min at a rate of 0.5 mL/min.

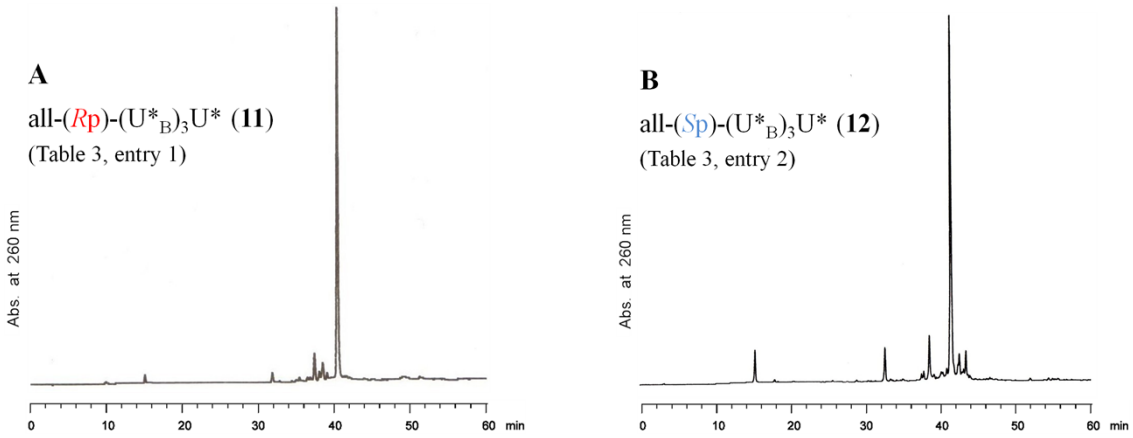


Figure S2. RP-HPLC profiles of crude **11** and **12** (Table 3, entries 1–2). RP-HPLC was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min at a rate of 0.5 mL/min.

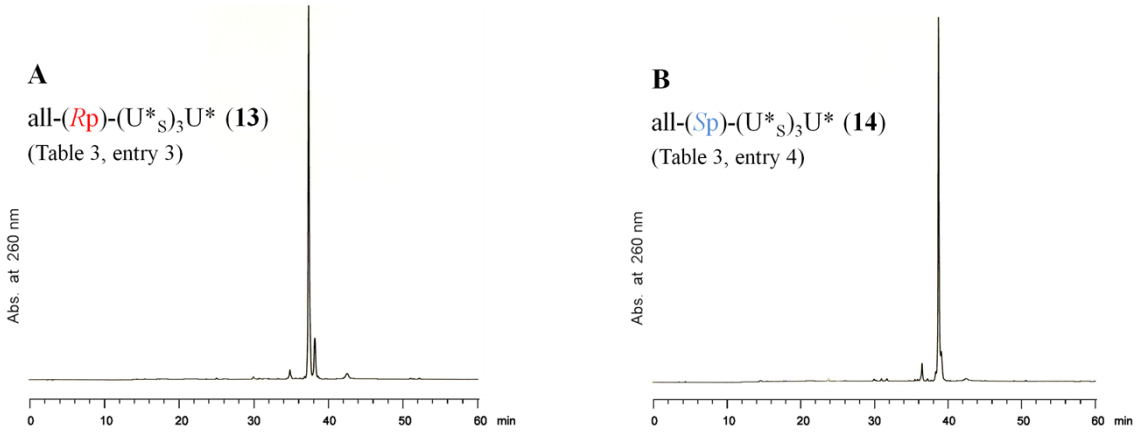


Figure S3. RP-HPLC profiles of crude **13** and **14** (Table 3, entries 3–4). RP-HPLC was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min at a rate of 0.5 mL/min.

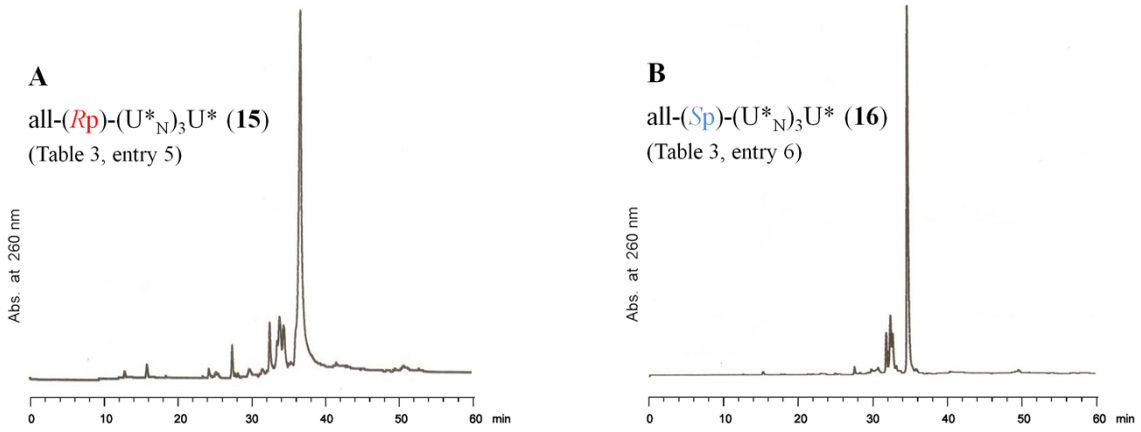


Figure S4. RP-HPLC profiles of crude **15** and **16** (Table 3, entries 5–6). RP-HPLC was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min at a rate of 0.5 mL/min.

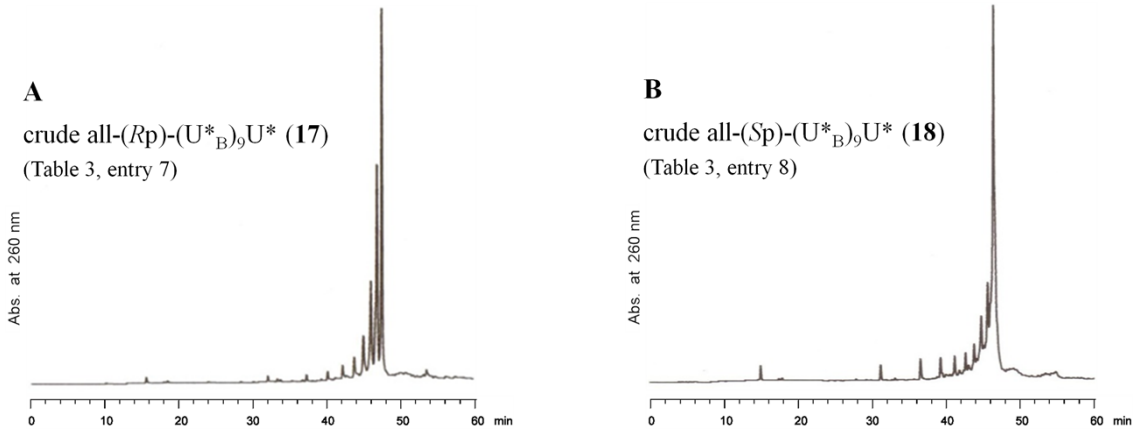


Figure S5. RP-HPLC profiles of crude **17** and **18** (Table 3, entries 7–8). RP-HPLC was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min at a rate of 0.5 mL/min.

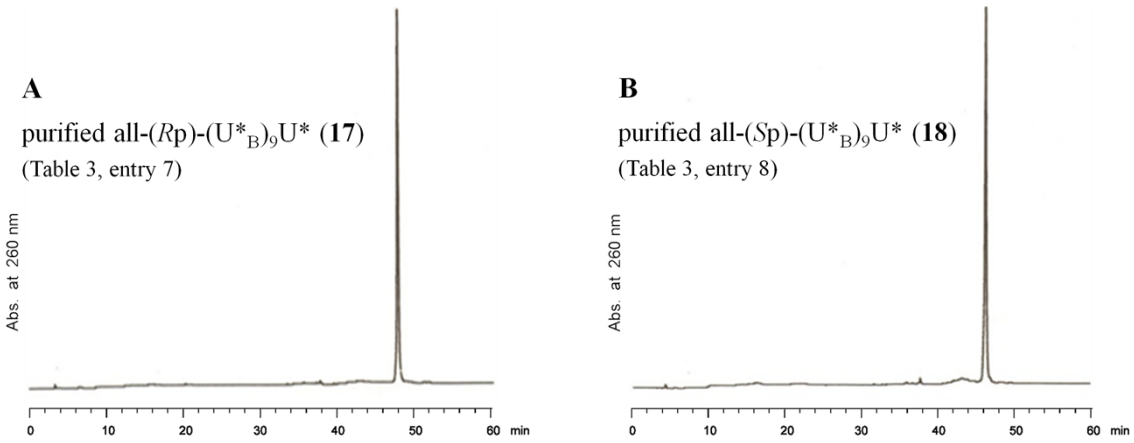
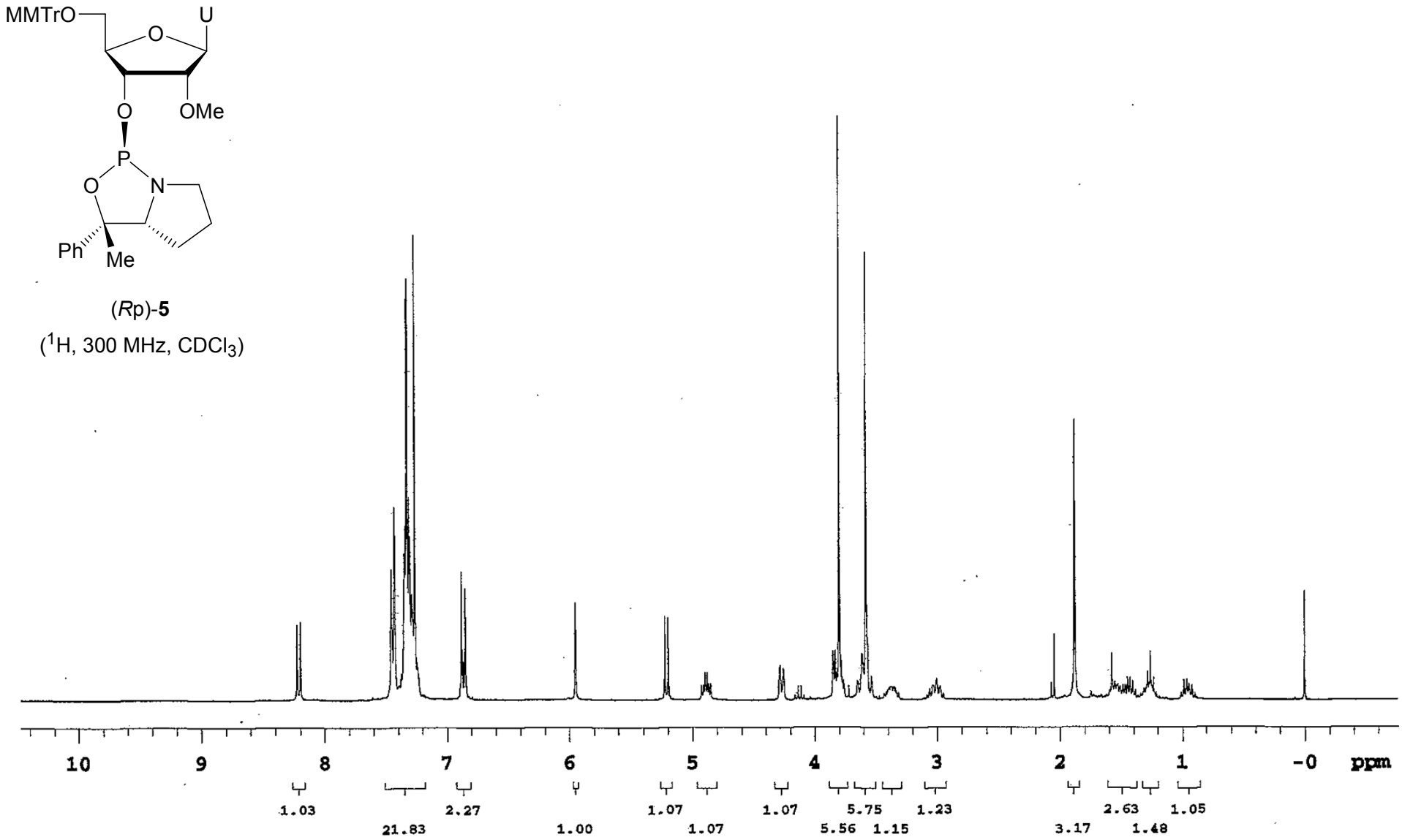
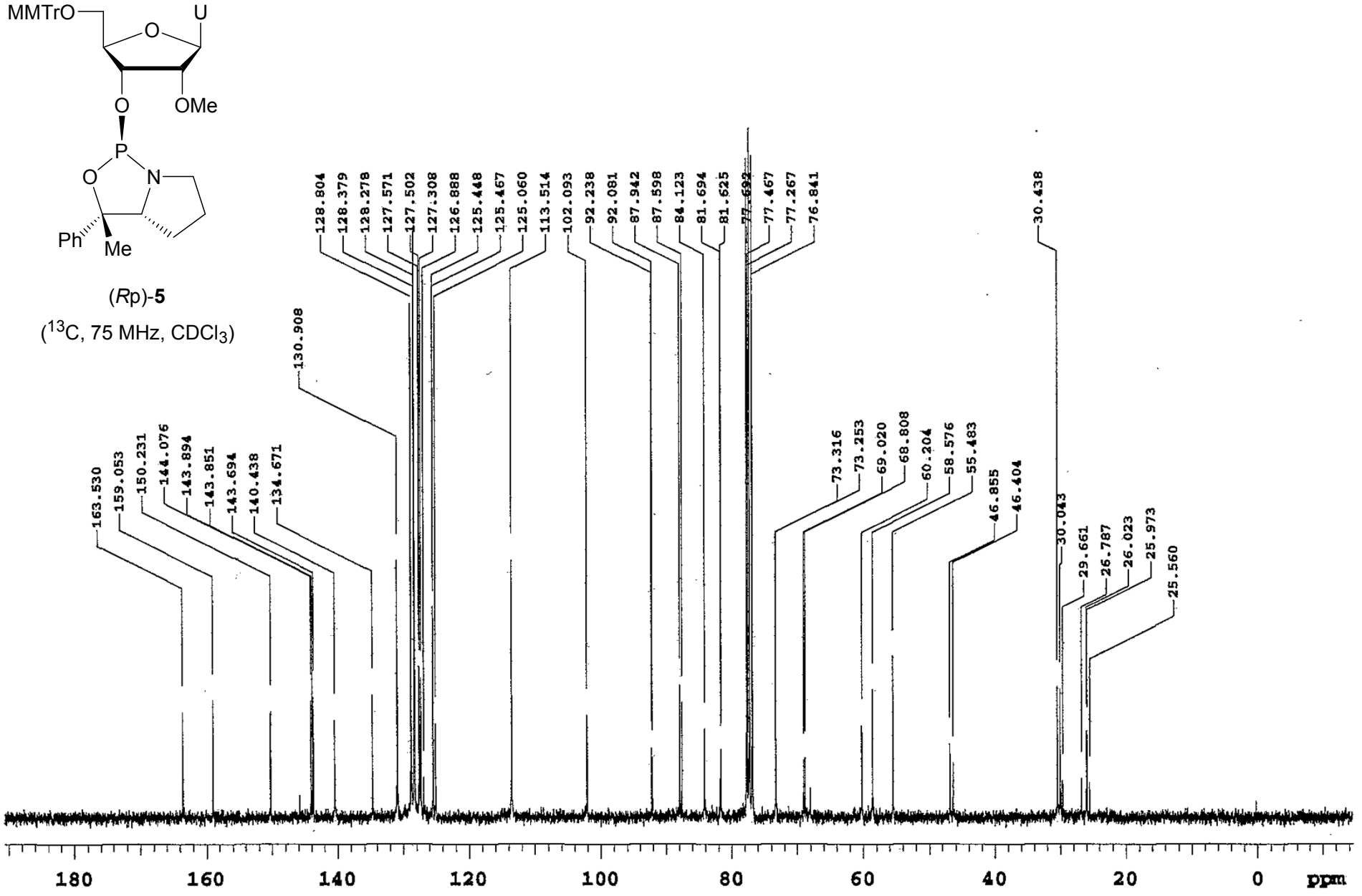


Figure S6. RP-HPLC profiles of purified **17** and **18** (Table 3, entries 7–8). RP-HPLC was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min at a rate of 0.5 mL/min.



(*R*p)-**5**

(^{1}H, 300 MHz, $CDCl_3$)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U
O
OMe
O
P
N
Ph
Me
(Rp)-5
(13C, 75 MHz, CDCl3)
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125.060
113.514
102.093
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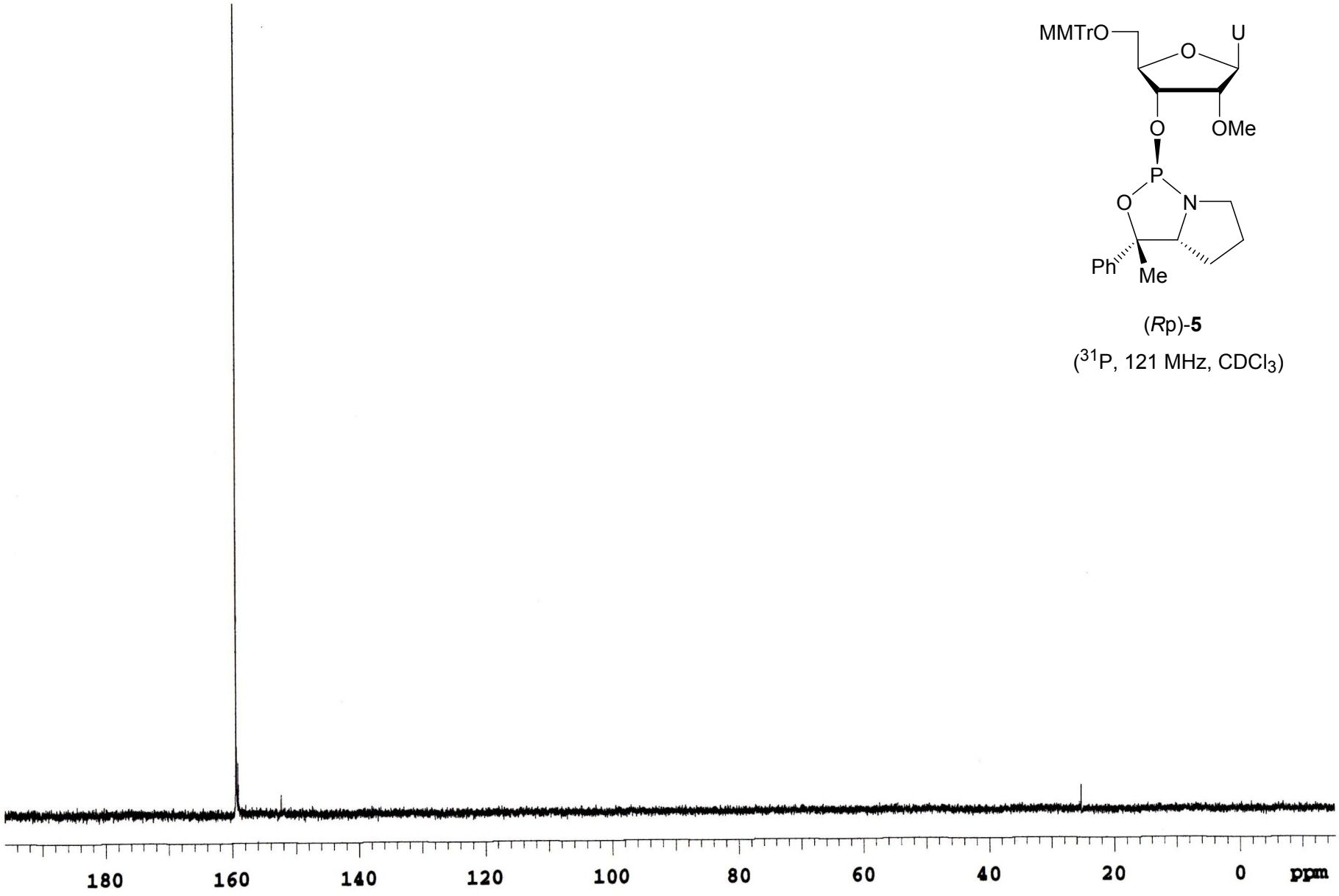
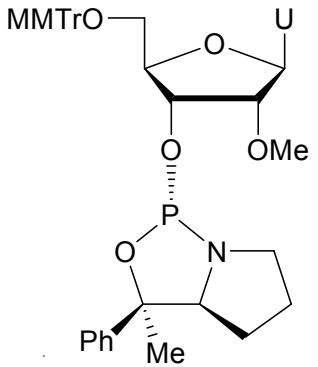


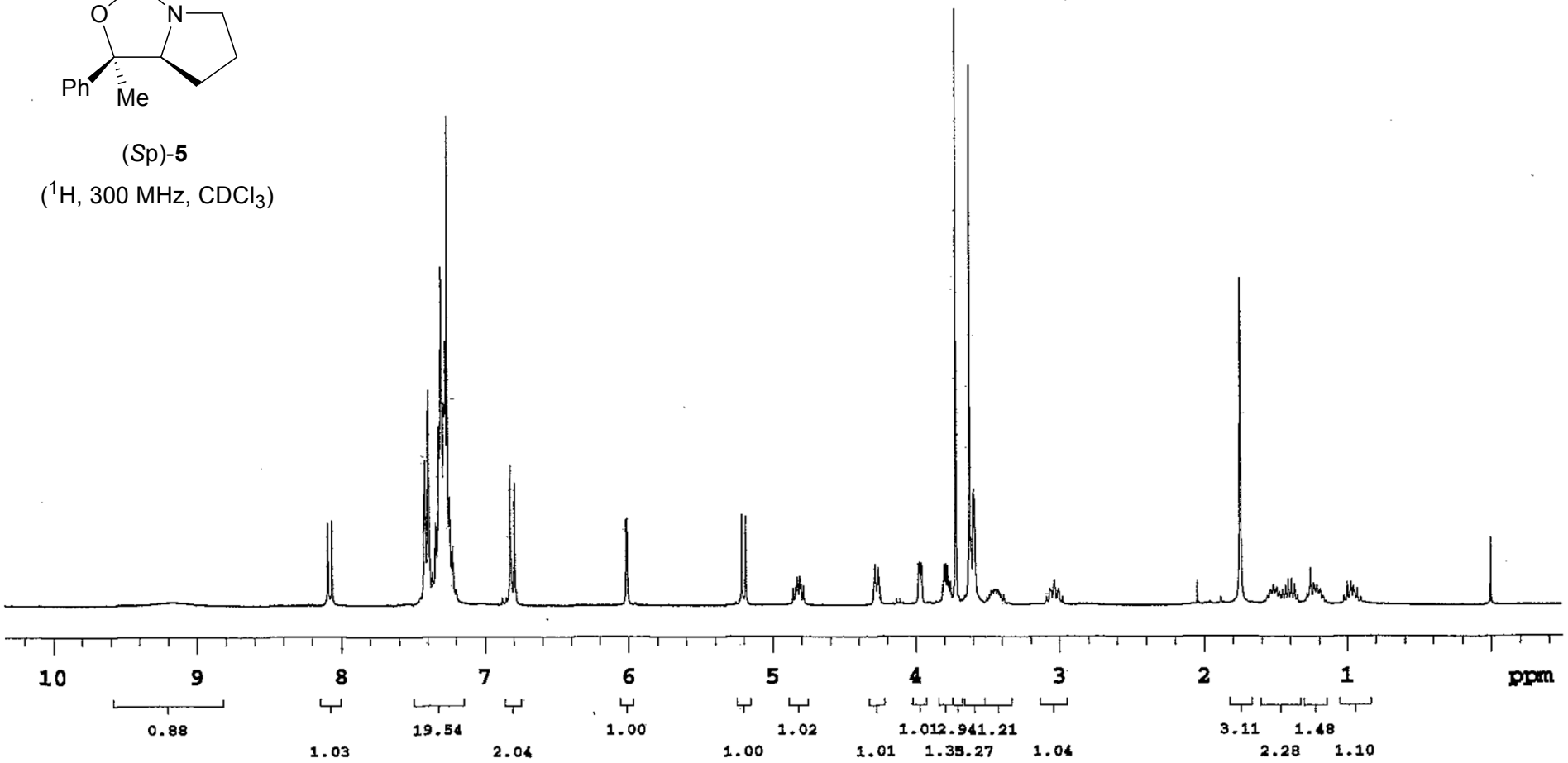
(*R*p)-**5**

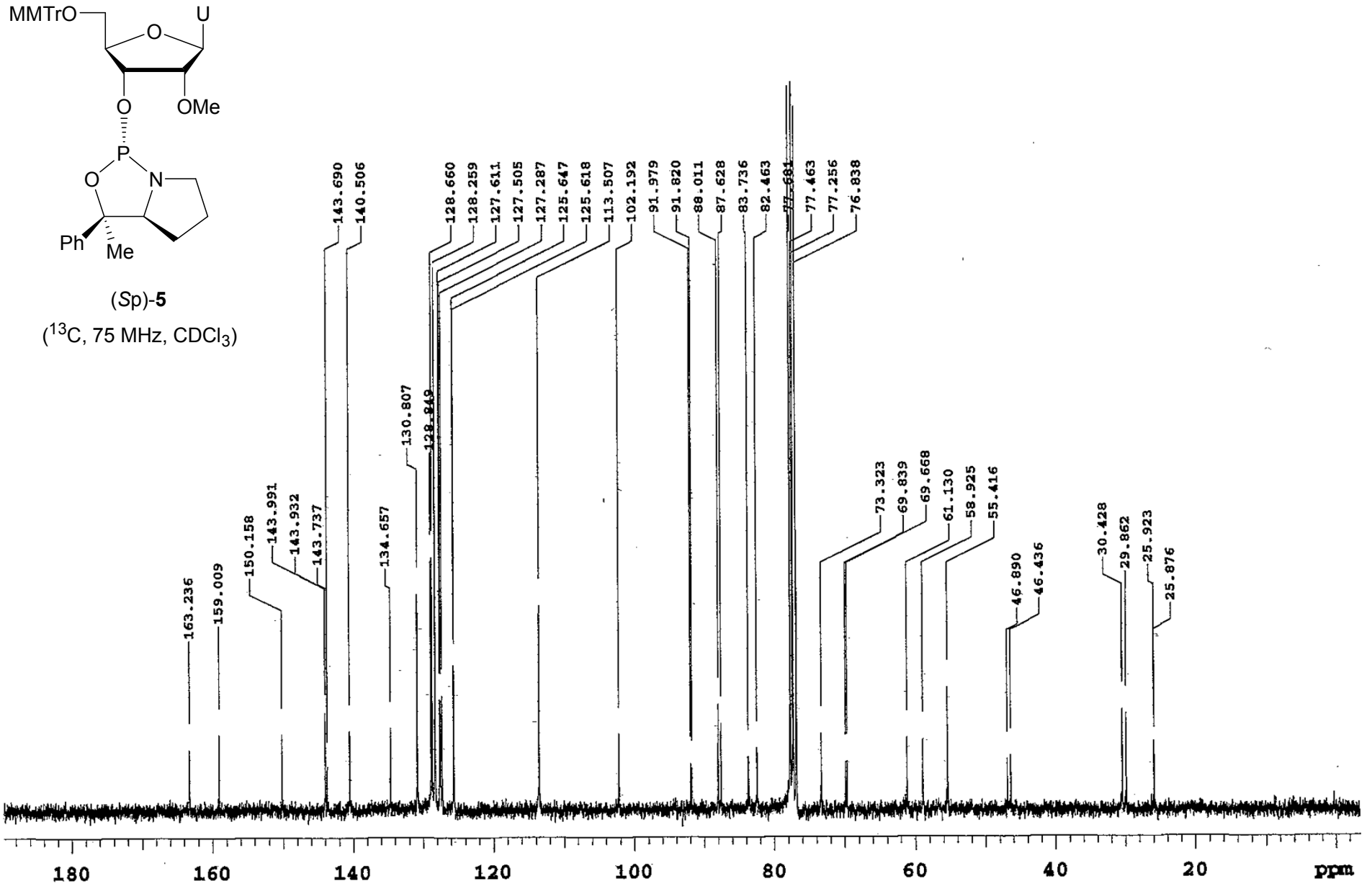
(^{31}P, 121 MHz, $CDCl_3$)



(*Sp*)-**5**

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

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U
O
OMe
O
P
N
Ph
Me
(Sp)-5
(13C, 75 MHz, CDCl3)
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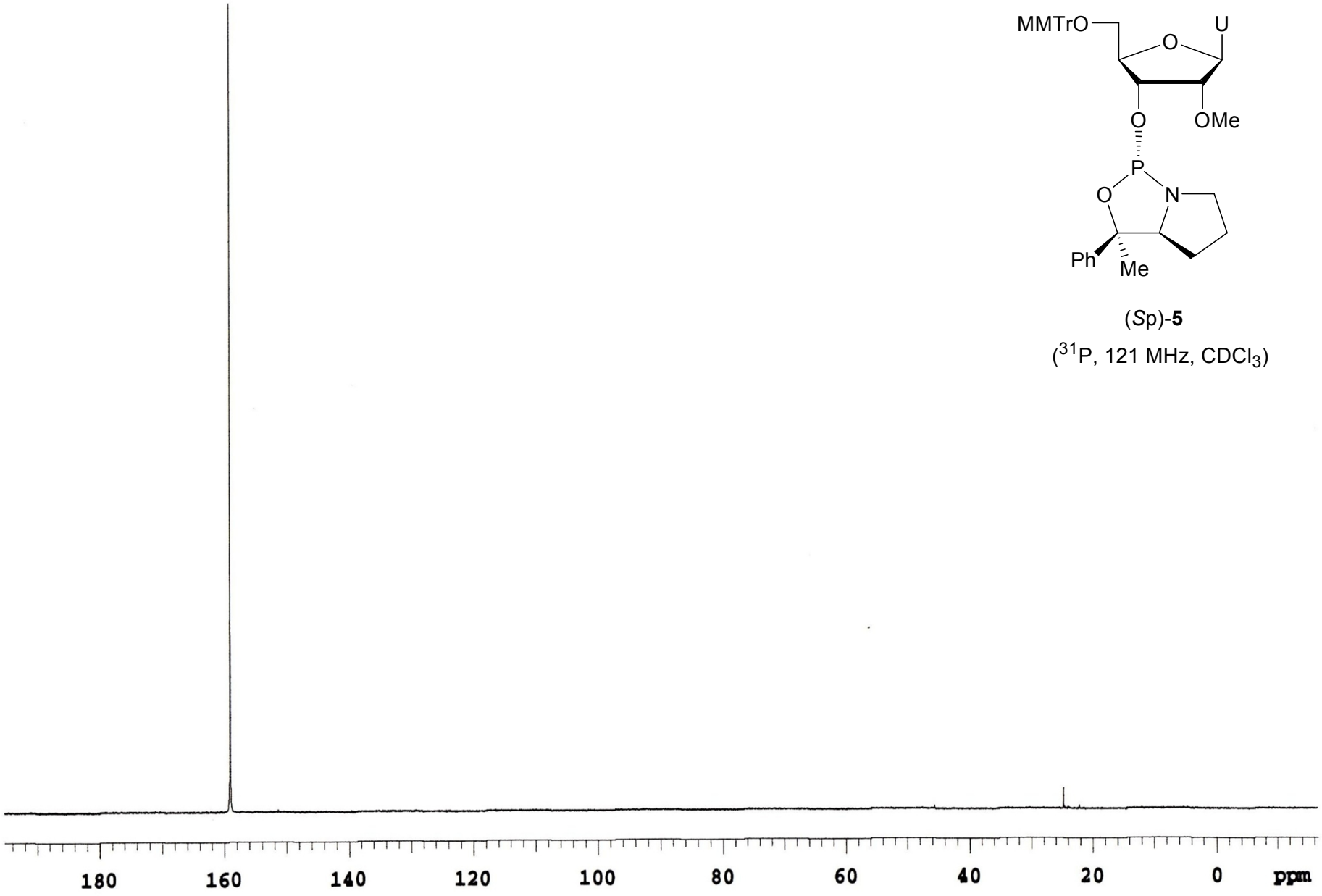

(*Sp*)-**5**

(^{31}P, 121 MHz, $CDCl_3$)