Supporting information for

Synthesis and properties of DNA oligomers containing stereopure phosphorothioate linkages and C-5 modified deoxyuridine derivatives

Rintaro Iwata Hara,[a,b] Reijiro Yoshino,[a] Yohei Nukaga, [a] Yusuke Maeda, [a] Kazuki Sato [a] and Takeshi Wada [a,*]

(a) Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan.

(b) Department of Neurology and Neurological Science, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-Ku, Tokyo, 113-8519, Japan.

* E-mail: twada@rs.tus.ac.jp

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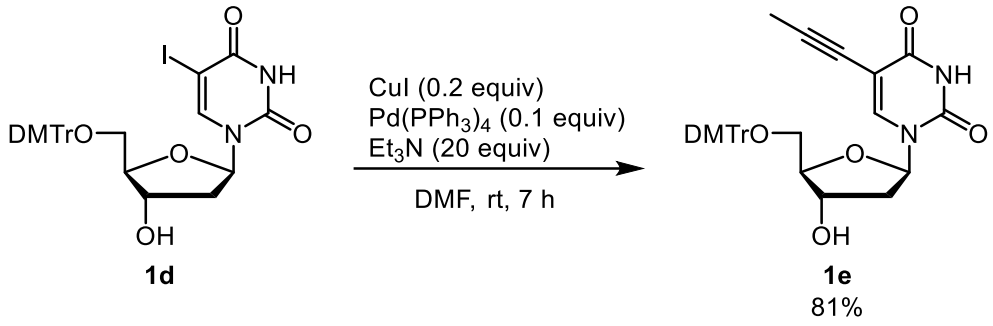

Figure S1. The synthetic scheme of compound 1e

Compound **1e**

5′-*O*-(4,4′-Dimethoxytrityl)-5-iodo-2′-deoxyuridine (**1d**) (0.658 g, 1.0 mmol) was dissolved in dry DMF (2.2 mL). Triethylamine (TEA) (2.8 mL, 20 mmol), CuI (38 mg, 0.2 mmol), tetrakis(triphenylphosphine)palladium (0.117 g, 0.1 mmol) and a 1 M solution of propyne in DMF (10 mL) were successively added to the solution, which was stirred for 7 h at room temperature. The mixture was diluted with toluene–ethyl acetate (30 mL, 1:1, v/v) and washed with a saturated aqueous solution of ammonium chloride (20 mL × 2) and an aqueous solution of sodium chloride (20 mL). The water layers were then combined and extracted with toluene–ethyl acetate (20 mL, 1:1, v/v). The combined organic layers were dried over Mg_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography [neutral silica, hexane–ethyl acetate (13:7–15:5, v/v), 0.5% pyridine] to afford **1e** as slightly yellow foam (0.462 g, 0.81 mmol, 81%).

^{1}H NMR (400 MHz, $CHCl_3$): δ 8.89 (br, 1H), 7.98 (s, 1H), 7.45–7.42 (m, 2H), 7.37–7.19 (m, 7H), 6.85 (dd, *J* = 8.9, 0.9 Hz, 4H), 6.31 (dd, *J* = 7.6, 5.9 Hz, 1H), 4.57–4.53 (m, 1H), 4.09 (q, *J* = 3.0 Hz, 1H), 3.79 (s, 6H), 3.37 (d, *J* = 3.2 Hz, 2H), 2.59 (d, *J* = 3.7 Hz, 1H), 2.49 (ddd, *J* = 13.7, 5.7, 2.7 Hz, 1H), 2.32–2.24 (m, 1H), 1.72 (s, 3H); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): δ 161.9, 158.6, 149.3, 144.5, 141.7, 135.5, 135.4, 130.0, 128.0, 127.9, 126.9, 113.3, 101.0, 90.9, 87.0, 86.4, 85.5, 72.3, 69.9, 63.5, 55.2, 41.4, 4.4; ESI-HRMS: m/z calcd for $C_{33}H_{32}ClN_2O_7^-$ [M + Cl] $^-$; 603.1904. found; 603.1913.

Compound **(*R*p)-3a** (*R*p-dU)

5′-*O*-(4,4′-Dimethoxytrityl)-2′-deoxyuridine (**1a**, 1.06 g, 2.0 mmol) was dried by repeated co-evaporation with pyridine and toluene and then dissolved in THF (9 mL). TEA (2.78 mL, 20 mmol) was added and the resulting solution was stirred and cooled to –78 °C. Afterwards, a solution of (4*S*,5*R*)-2-chloro-3-phenyl-1,3,2-oxazaphospholidine ((4*S*,5*R*)-**2**) (0.5 M) in toluene (8.0 mL) was added dropwise at –78 °C. The mixture was warmed to rt and stirred for 1 h. After the reaction completion, the mixture was cooled to –20 °C and diluted with ethyl acetate and washed with a saturated aqueous solution of $NaHCO_3$ and saturated aqueous solutions of NaCl (twice). The organic layer was then dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography [NH-silica, toluene–ethyl acetate (7:3, v/v), 0.1% TEA] to afford **(*R*p)-3a** (0.66 g, 0.90 mmol, 45%) as colorless foam.

^{1}H NMR (400 MHz, $CHCl_3$): δ 7.82 (d, *J* = 8.0 Hz, 1H), 7.38–7.22 (m, 14H), 6.80 (dd, J = 9.0, 3.2 Hz, 4H), 6.33 (t, *J* = 6.2 Hz, 1H), 5.74 (d, *J* = 6.4 Hz, 1H), 5.30 (d, *J* = 8.4 Hz, 1H), 4.97–4.91 (m, 1H), 4.10 (dd, *J* = 6.8, 2.8 Hz, 1H), 3.90–3.86 (m, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.65–3.54 (m, 1H), 3.50-3.42 (m, 2H), 3.23–3.15 (m, 1H), 2.65-2.59 (m, 1H), 2.37-2.30 (m, 1H), 1.69–1.62 (m, 2H), 1.22–1.16 (m, 1H), 0.97–0.90 (m, 1H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 162.9, 158.7, 158.6, 150.0, 144.3, 140.2, 138.1 (d, $^{3}J_{PC}$ = 3.9 Hz), 135.2, 135.2, 130.1, 130.0, 128.3, 128.1, 128.0, 127.5, 127.1, 125.4, 113.2, 102.1, 87.0, 85.4 (d, $^{3}J_{PC}$ = 1.9 Hz), 84.9, 82.2 (d, $^{2}J_{PC}$ = 9.6 Hz), 72.3 (d, $^{2}J_{PC}$ = 14.5 Hz), 67.4 (d, $^{2}J_{PC}$ = 2.9 Hz), 62.2, 55.2, 47.2 (d, $^{2}J_{PC}$ = 34.7 Hz), 40.4 (d, $^{3}J_{PC}$ = 4.8 Hz), 28.1, 26.0 (d, $^{3}J_{PC}$ = 3.9 Hz) ^{31}P {^{1}H} NMR (161 MHz, $CDCl_3$): *δ* 157.2; FAB-HRMS: m/z calcd for $C_{41}H_{43}N_3O_8P^+$ $[M + H]^+$ 736.2782. found; 736.2791.

Compound **(*S*p)-3a** (*S*p-dU)

5′-*O*-(4,4′-Dimethoxytrityl)-2′-deoxyuridine (**1a**, 1.06 g, 2.0 mmol) was dried by repeated co-evaporation with pyridine and toluene, and THF, and then dissolved in THF (10 mL). TEA (1.98 mL, 14 mmol) was added and the resulting solution was stirred and cooled to –78 °C. Afterwards, a solution of (4*S*,5*R*)-2-chloro-3-phenyl-1,3,2-oxazaphospholidine ((4*S*,5*R*)-**2**) (0.5 M) in THF (12 mL) was added dropwise at –78 °C. The mixture was warmed to rt and stirred for 2.5 h. The mixture was cooled to –78 °C and a solution of (4*S*,5*R*)-2-chloro-3-phenyl-1,3,2-oxazaphospholidine ((4*S*,5*R*)-**2**) (0.5 M) in THF (1 mL) was further added to the mixture. The mixture was warmed to rt and stirred for 1 h. After the reaction completion, the mixture was diluted with chloroform (400 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (150 mL). The water layer was extracted with chloroform (30 mL × 2) and the combined organic layers were then dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography [NH-silica, toluene–ethyl acetate (7:3, v/v), 0.1% TEA] to afford **(*S*p)-3a** (0.84 g, 1.14 mmol, 57%) as colorless foam.

^{1}H NMR (400 MHz, $CHCl_3$): δ 8.40–8.15 (br, 1H) 7.87 (d, *J* = 8.0 Hz, 1H), 7.41–7.20 (m, 14H), 6.84 (d, *J* = 8.8 Hz, 4H), 6.31 (t, *J* = 6.2 Hz, 1H), 5.73 (d, *J* = 6.4 Hz, 1H), 5.33 (d, *J* = 7.6 Hz, 1H), 4.97-4.91 (m, 1H), 4.15 (dd, *J* = 6.4, 2.4 Hz, 1H), 3.95–3.85 (m, 1H), 3.79 (s, 6H), 3.61–3.44 (m, 3H), 3.23–3.13 (m, 1H), 2.53–2.46 (m, 1H), 2.36–2.29 (m, 1H), 1.67–1.60 (m, 2H), 1.26–1.17 (m, 1H), 1.03–0.93 (m, 1H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 163.2, 158.6, 158.6, 150.1, 144.2, 140.1, 137.9 (d, $^{3}J_{PC}$ = 3.9 Hz), 135.2, 135.1, 130.1, 128.2, 128.1, 128.0, 127.6, 127.1, 126.0, 125.5, 113.2, 102.1, 87.0, 85.1 (d, $^{3}J_{PC}$ = 5.9 Hz), 84.7, 82.2 (d, $^{2}J_{PC}$ = 9.6 Hz), 71.6 (d, $^{2}J_{PC}$ = 13.5 Hz), 67.4 (d, $^{2}J_{PC}$ = 2.9 Hz), 62.1, 55.2, 47.2 (d, $^{2}J_{PC}$ = 34.7 Hz), 40.5, 28.0, 25.9 (d, $^{3}J_{PC}$ = 3.9 Hz; ^{31}P {^{1}H} NMR (161 MHz, $CDCl_3$) *δ* 156.9; FAB-HRMS: m/z calcd for $C_{41}H_{43}N_3O_8P^+$ $[M + H]^+$; 736.2782. found; 736.2785.

Compound **(*R*p)-3c** (*R*p-d^{Br}U)

5′-*O*-(4,4′-Dimethoxytrityl)-5-bromo-2′-deoxyuridine (**1c**, 0.61 g, 1.0 mmol) was dried by repeated co-evaporation with pyridine and toluene, and then dissolved in THF (5.0 mL). TEA (0.97

mL, 7.0 mmol) was added and the solution was stirred and cooled to –78 °C. a solution of (4*S*,5*R*)-2-chloro-3-phenyl-1,3,2-oxazaphospholidine ((4*S*,5*R*)-**2**) (0.5 M) in THF (6.0 mL) was then added dropwise at –78 °C. The mixture was warmed to rt and stirred for 4 h. After the reaction completion, the mixture was diluted with chloroform (300 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (100 mL × 3). The water layers were combined and extracted with chloroform (30 mL × 3) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography [NH-silica, toluene–ethyl acetate (8:2 to 0:10, v/v), 0.1% TEA] to afford **(*R*p)-3c** (0.33 g, 0.41 mmol, 41%) as colorless foam.

^{1}H NMR (400 MHz, $CHCl_3$): δ 8.06 (s, 1H), 7.21–7.43 (m, 14H), 6.81 (d, J = 9.2 Hz, 4H), 6.35 (dd, J = 7.8, 6.0 Hz, 1H), 5.73 (d, J = 6.4 Hz, 1H), 4.93–4.50 (m, br, 2H), 4.16–4.15 (dd, J = 5.2, 2.8 Hz 1H), 3.88–3.80 (m, 1H), 3.77 (s×2, 6H), 3.59–3.53 (m, 1H), 3.43–3.35 (m, 2H), 3.22–3.10 (m, 1H), 2.68–2.62 (m, 1H), 2.37–2.30 (m, 1H), 1.68–1.61 (m, 2H), 1.21–1.14 (m, 1H), 0.99–0.89 (m, 1H); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): δ 159.7, 158.6, 149.9, 144.3, 139.2, 138.0 (d, $^3J_{PC}$ = 3.9 Hz), 135.4, 135.3, 130.0, 128.2, 128.0, 127.5, 127.0, 125.7, 125.4, 113.3, 97.2, 87.1, 85.9 (d, $^3J_{PC}$ = 1.9 Hz), 85.5, 82.3 (d, $^2J_{PC}$ = 9.6 Hz), 73.3 (d, $^2J_{PC}$ = 13.5 Hz), 67.4 (d, $^2J_{PC}$ = 2.9 Hz), 62.9, 55.2, 47.2 (d, $^2J_{PC}$ = 34.7 Hz), 40.6 (d, $^3J_{PC}$ = 4.8 Hz), 28.1, 26.0 (d, $^3J_{PC}$ = 3.9 Hz); ^{31}P {^{1}H} NMR (161 MHz, $CDCl_3$): δ 156.5; FAB-HRMS: m/z calcd for $C_{41}H_{41}BrN_3NaO_8P^+$ $[M + Na]^+$; 836.1707. found; 836.1715.

Compound **(*S*p)-3c** (*S*p-d^{Br}U)

Compound **(*S*p)-3c** was synthesized following the same procedure for **(*R*p)-3c** using **1c** (0.61 g, 1.0 mmol) and (4*R*,5*S*)-**2** (3.0 mmol). The reaction was performed at rt for 2 h. The crude product was purified with silica gel column chromatography [NH-silica, toluene–ethyl acetate (8:2 to 0:10, v/v), 0.5% TEA] to afford **(*S*p)-3c** (0.40 g, 0.49 mmol, 49%) as colorless foam.

^{1}H NMR (400 MHz, $CHCl_3$): δ 8.12 (s, 1H), 7.44-7.23 (m, 14H), 6.85 (dd, J = 9.2, 1.2 Hz, 4H), 6.31 (dd, J = 7.2, 6.0 Hz, 1H), 5.71 (d, J = 6.4 Hz, 1H), 4.93–4.89 (m, 1H), 4.21 (d, J = 2.8, 1H), 3.92–3.85 (m, 1H), 3.79 (s, 6H), 3.61–3.38 (m, 3H), 3.22–3.10 (m, 1H), 2.56–2.50 (m, 1H), 2.37–2.30 (m, 1H), 1.66–1.59 (m, 2H), 1.26–1.18 (m, 1H), 1.03–0.94 (m, 1H); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): δ 159.2, 158.7, 149.6, 144.3, 139.2, 137.9 (d, $^3J_{PC}$ = 3.9 Hz), 135.4, 135.3, 130.1, 130.1, 128.3, 128.1, 128.0, 127.6, 127.1, 125.5, 113.3, 97.1, 87.1, 85.8 (d, $^3J_{PC}$ = 5.8 Hz), 85.5, 82.4 (d, $^2J_{PC}$ = 9.6 Hz), 72.7 (d, $^2J_{PC}$ = 12.5 Hz), 67.4 (d, $^2J_{PC}$ = 2.9 Hz), 62.8, 55.2, 47.2 (d, $^2J_{PC}$ = 34.7 Hz), 40.7, 28.1, 26.0 (d, $^3J_{PC}$ = 2.9 Hz); ^{31}P {^{1}H} NMR (161 MHz, $CDCl_3$): δ 156.1; FAB-HRMS: m/z calcd for $C_{41}H_{42}BrN_3O_8P^+$ $[M + H]^+$; 814.1887. found; 814.1887.

Compound **(*R*p)-3d** (*R*p-d^{I}U)

Compound **(*R*p)-3d** was synthesized following the same procedure for **(*R*p)-3c** using 5′-*O*-(4,4′-Dimethoxytrityl)-5-iodo-2′-deoxyuridine (**1d**) (0.66 g, 1.0 mmol) and (4*S*,5*R*)-**2** (3.0 mmol). The reaction was performed at rt for 1.5 h. The crude product was purified by silica gel column

chromatography [NH-silica, toluene–ethyl acetate−acetone (80:20:0 to 0:100:0 to 0:95:5, v/v/v), 0.5% TEA] to afford **(*R*p)-3d** (0.36 g, 0.42 mmol, 42%) as colorless foam.

^{1}H NMR (400 MHz, $CHCl_3$): δ 8.13 (s, 1H), 7.43–7.21 (m, 14H), 6.82 (d, J = 8.8 Hz, 4H), 6.34 (dd, J = 7.8, 5.2, Hz, 1H), 5.72 (d, J = 6.0 Hz, 1H), 4.92–4.87 (m, 1H), 4.17–4.14 (m, 1H), 3.86–3.76 (m, 7H), 3.63–3.53 (m, 1H), 3.42–3.35 (m, 2H), 3.22–3.13 (m, 1H), 2.67–2.62 (m, 1H), 2.36–2.29 (m, 1H), 1.67–1.60 (m, 2H), 1.21–1.14 (m, 1H), 0.99–0.89 (m, 1H); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): δ 159.9, 158.7, 149.7, 144.3, 138.1 (d, $^{3}J_{PC}$ = 3.9 Hz), 135.4, 135.4, 130.1, 128.3, 128.1, 127.6, 127.1, 125.7, 125.4, 113.3, 87.0, 85.9, 85.5, 82.4 (d, $^{2}J_{PC}$ = 10.6 Hz), 73.4 (d, $^{2}J_{PC}$ = 13.5 Hz), 68.4, 67.4 (d, $^{2}J_{PC}$ = 3.9 Hz), 62.9, 55.2, 47.2 (d, $^{2}J_{PC}$ = 34.7 Hz), 40.6 (d, $^{3}J_{PC}$ = 3.9 Hz), 28.1, 26.0 (d, $^{3}J_{PC}$ = 2.9 Hz); ^{31}P {^{1}H} NMR (161 MHz, $CDCl_3$) δ 156.2; FAB-HRMS: m/z calcd for $C_{41}H_{42}IN_3O_8P^+$ $[M + H]^+$; 862.1749. found; 862.1750.

Compound **(*S*p)-3d** (*S*p-d^{I}U)

5′-*O*-(4,4′-Dimethoxytrityl)-5-iodo-2′-deoxyuridine (**1d**, 1.32 g, 2.0 mmol) was dried by repeated co-evaporation with pyridine and toluene, and then dissolved in THF (10 mL). TEA (1.95 mL, 14 mmol) was added and the solution was stirred and cooled to −75 °C. A 0.5 M of (4*R*,5*S*)-2-chloro-3-phenyl-1,3,2-oxazaphospholidine ((4*R*,5*S*)-**2**) in THF (12 mL) was then added dropwise at −75 °C. The mixture was warmed to rt and stirred for 3.5 h. After the reaction completion, the mixture was diluted with chloroform (400 mL) and washed with a saturated aqueous $NaHCO_3$ solution (150 mL × 3). The water layers were combined and extracted with chloroform (30 mL × 2) and the resulting organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography [NH-silica, toluene–ethyl acetate (7:3, v/v), 0.1% TEA to ethyl acetate, 0.5% triethylamine] to afford **(*S*p)-3d** (0.86 g, 1.0 mmol, 50%) as colorless foam.

^{1}H NMR (400 MHz, $CHCl_3$): δ 8.18 (s, 1H), 7.45–7.25 (m, 14H), 6.86 (d, J = 8.8 Hz, 4H), 6.31 (dd, J = 8.4, 6.0 Hz, 1H), 5.69 (d, J = 6.4 Hz, 1H), 4.91–4.87 (m, 1H), 4.21 (d, J = 2.8 Hz, 1H), 3.90–3.85 (m, 1H), 3.79 (s, 6H), 3.60–3.45 (m, 2H), 3.40–3.36 (m, 1H), 3.21–3.08 (m, 1H), 2.55–2.49 (m, 1H), 2.35–2.29 (m, 1H), 1.66–1.56 (m, 2H), 1.26–1.19 (m, 1H), 1.01–0.94 (m, 1H); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): δ 159.9, 158.7, 149.7, 144.3, 144.2, 137.9 (d, $^{3}J_{PC}$ = 3.9 Hz), 135.3, 135.3, 130.1, 128.3, 128.1, 127.6, 127.1, 125.5, 113.4, 113.3, 87.0, 85.8 (d, $^{3}J_{PC}$ = 5.8 Hz), 85.4, 82.4 (d, $^{2}J_{PC}$ = 8.7 Hz), 72.8 (d, $^{2}J_{PC}$ = 13.5 Hz), 68.4, 67.4 (d, $^{2}J_{PC}$ = 2.9 Hz), 62.8, 55.2, 47.2 (d, $^{2}J_{PC}$ = 34.7 Hz), 40.7, 28.0, 26.0 (d, $^{3}J_{PC}$ = 3.9 Hz); ^{31}P {^{1}H} NMR (161 MHz, $CDCl_3$): δ 155.9; FAB-HRMS: m/z calcd for $C_{41}H_{42}IN_3O_8P^+$ $[M + H]^+$; 862.1749. found; 862.1755.

Compound **(*R*p)-3e** (*R*p-d^{pr}U)

5′-*O*-(4,4′-Dimethoxytrityl)-5-(1-propynyl)-2′-deoxyuridine (**1e**, 0.53g, 0.93 mmol) was dried with repeated co-evaporation with pyridine and toluene, and then dissolved in THF (5 mL). TEA (0.97 mL, 7.0 mmol) was added and the solution was stirred and cooled to –78 °C. A solution of (4*S*,5*R*)-2-chloro-3-phenyl-1,3,2-oxazaphospholidine ((4*S*,5*R*)-**2**) (0.5 M) in THF (6.0 mL) was added dropwise

at –78 °C. The mixture was then warmed to rt and stirred for 2.5 h. Afterwards, the mixture was cooled to –78 °C and a solution of (4*S*,5*R*)-2-chloro-3-phenyl-1,3,2-oxazaphospholidine ((4*S*,5*R*)-**2**) (0.5 M) in THF (1.0 mL) was added dropwise to the mixture. The mixture was then warmed again to rt and stirred for 30 min. After the reaction completion, the mixture was diluted with chloroform (300 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (× 3). The water layers were combined and extracted with chloroform (× 3) and the obtained organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography [NH-silica, toluene–ethyl acetate (8:2 to 0:10, v/v), 0.1% TEA] to afford **(*R*p)-3e** (0.29 g, 0.40 mmol, 42%) as colorless foam.

^{1}H NMR (400 MHz, $CHCl_3$): δ 7.99 (s, 1H), 7.45–7.18 (m, 14H), 6.81 (d, J = 8.8 Hz, 4H), 6.31 (dd, J = 7.6, 5.2 Hz, 1H), 5.73 (d, J = 6.4 Hz, 1H), 4.92–4.87 (m, 1H), 4.15 (dd, J = 5.2, 2.8 Hz, 1H), 3.89–3.82 (m, 1H), 3.77 (s × 2, 6H), 3.65–3.50 (m, 1H), 3.41–3.34 (m, 2H), 3.23–3.16 (m, 1H), 2.67–2.62 (m, 1H), 2.37–2.30 (m, 1H), 1.71–1.61 (m, 5H), 1.26–1.14 (m, 1H), 0.99–0.92 (m, 1H); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): δ 162.1, 158.5, 149.2, 144.5, 141.7, 138.1 (d, $^3J_{PC}$ = 3.9 Hz), 135.5, 135.5, 130.0, 128.2, 128.2, 127.9, 127.9, 127.5, 126.9, 125.4, 113.2, 100.9, 90.7, 87.0, 85.8, 85.4, 82.3 (d, $^2J_{PC}$ = 9.6 Hz), 73.2 (d, $^2J_{PC}$ = 13.5 Hz), 70.0, 67.4 (d, $^2J_{PC}$ = 2.9 Hz), 62.9, 55.2, 47.2 (d, $^2J_{PC}$ = 34.7 Hz), 40.6 (d, $^3J_{PC}$ = 4.8 Hz), 28.1, 26.0 (d, $^3J_{PC}$ = 3.9 Hz), 4.4; ^{31}P {^{1}H} NMR (161 MHz, $CDCl_3$): δ 156.7; FAB-HRMS: m/z calcd for $C_{44}H_{45}N_3O_8P^+$ $[M + H]^+$; 774.2939. found; 774.2947.

Compound **(*S*p)-3e** (*S*p-d^{pr}U)

5′-*O*-(4,4′-Dimethoxytrityl)-5-propynyl-2′-deoxyuridine (**1e**) (0.51 g, 0.90 mmol) was dried by repeated co-evaporation with pyridine and toluene, and then dissolved in THF (5.0 mL). TEA (0.97 mL, 7.0 mmol) was added and the solution was stirred and cooled to −78 °C. Afterwards, a 0.5 M solution of (4*R*,5*S*)-2-chloro-3-phenyl-1,3,2-oxazaphospholidine ((4*R*,5*S*)-**2**) in THF (6.0 mL) was added dropwise at −78 °C. The mixture was warmed to rt and stirred for 3.5 h. After the reaction completion, chloroform was added (300 mL) and the organic layer was washed with a saturated aqueous $NaHCO_3$ solution (100 mL × 3). The water layers were then combined and extracted with chloroform (30 mL × 3). The obtained organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography [NH-silica, toluene–ethyl acetate (8:2 to 0:10, v/v), 0.1% TEA] to afford **(*S*p)-3e** (0.35 g, 0.47 mmol, 53%) as colorless foam.

^{1}H NMR (400 MHz, $CHCl_3$): δ 8.04 (s, 1H), 7.47−7.22 (m, 14H), 6.85 (d, J = 8.4 Hz, 4H), 6.30 (dd, J = 7.2, 6.0 Hz, 1H), 5.71 (d, J = 6.4 Hz, 1H), 4.92−4.88 (m, 1H), 4.10 (d, J = 2.8 Hz, 1H), 3.94−3.87 (m, 1H), 3.79 (s, 6H), 3.61−3.51 (m, 1H), 3.45−3.37 (m, 2H), 3.22−3.14 (m, 1H), 2.55−2.50 (m, 1H), 2.37−2.30 (m, 1H), 1.69−1.59 (m, 5H), 1.26−1.18 (m, 1H), 1.03−0.94 (m, 1H); ^{13}C {^{1}H} NMR (100 MHz, $CHCl_3$): δ 161.6, 158.6, 148.9, 144.5, 141.7, 137.9 (d, $^3J_{PC}$ = 3.9 Hz), 135.5, 135.4, 130.0, 128.3, 128.0, 127.9, 127.6, 126.9, 125.5, 113.3, 100.9, 90.9, 87.0, 85.8 (d, $^3J_{PC}$ = 4.8 Hz), 85.3, 82.3 ($^2J_{PC}$ = 9.6 Hz), 72.7 (d, $^2J_{PC}$ =12.5 Hz), 69.9, 67.4 (d, $^2J_{PC}$ = 2.9 Hz), 62.9, 55.2, 47.2 (d, $^2J_{PC}$ = 34.7 Hz),

40.7, 28.1, 26.0 (d, $^{3}J_{PC}$ = 2.9 Hz), 4.4; ^{31}P {^{1}H} NMR (161 MHz, $CDCl_3$): δ 156.1; FAB-HRMS: m/z calcd for $C_{44}H_{45}N_3O_8P^+$ $[M+H]^+$; 774.2939. found; 774.2946.

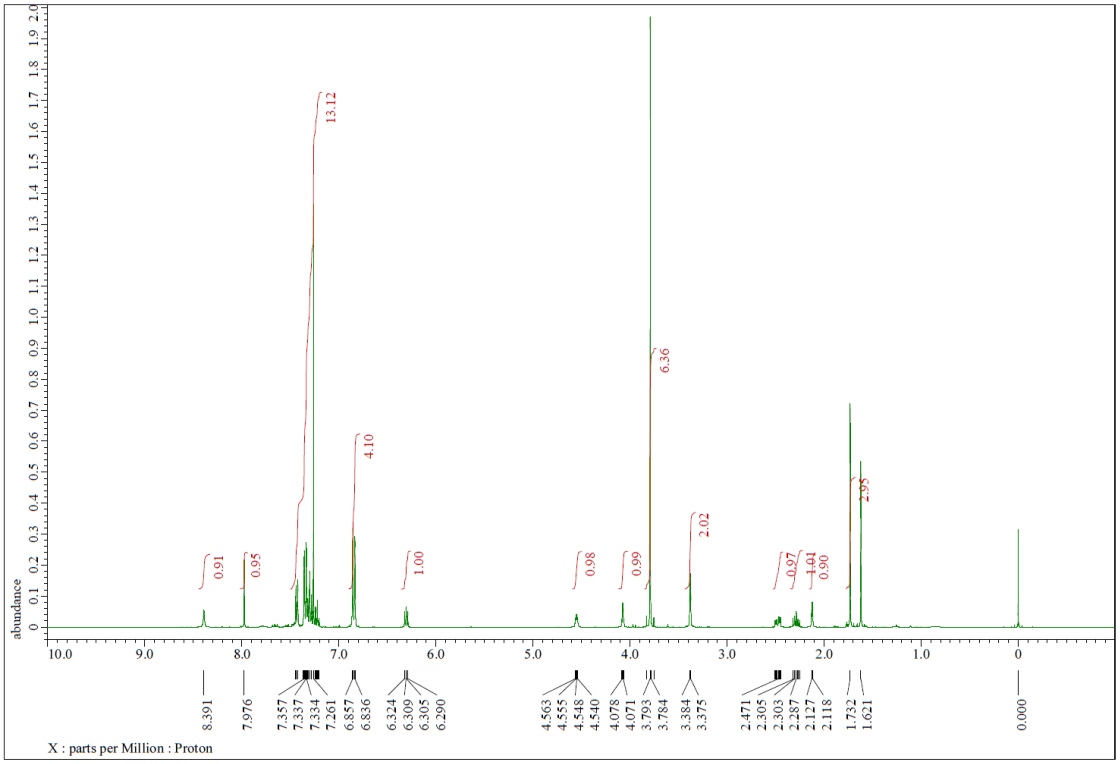

Figure S2. ^{1}H NMR spectra of compound 1e

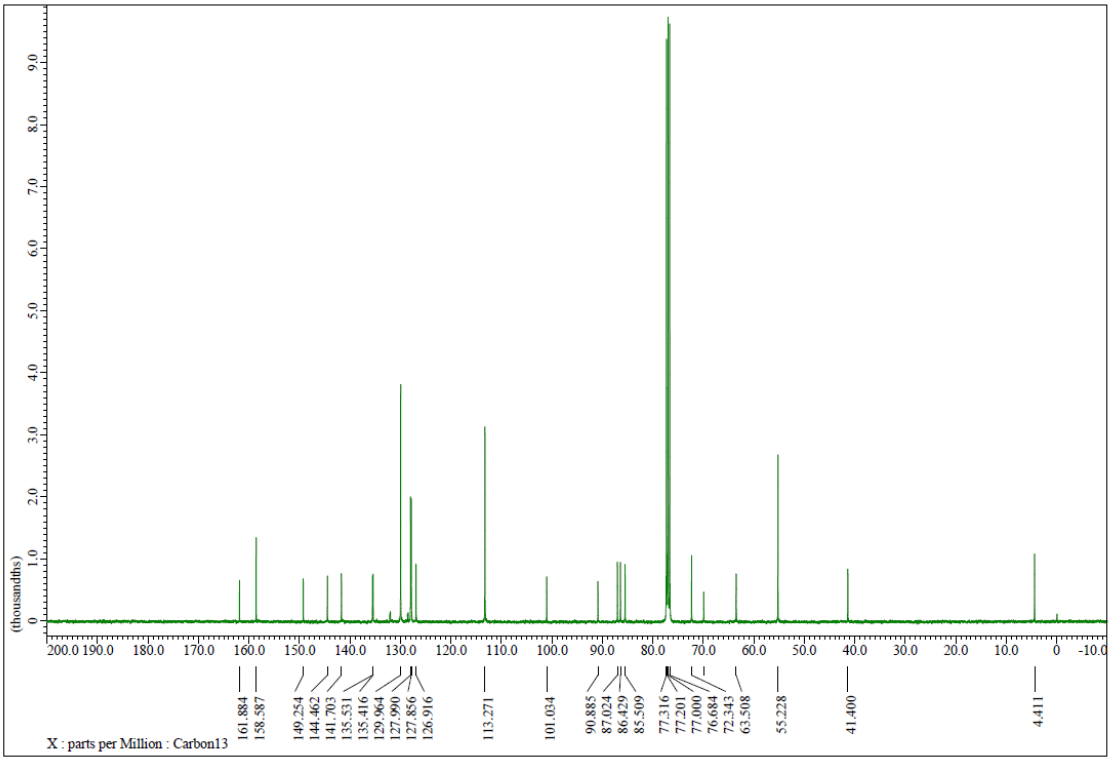

Figure S3. ^{13}C NMR spectra of compound 1e

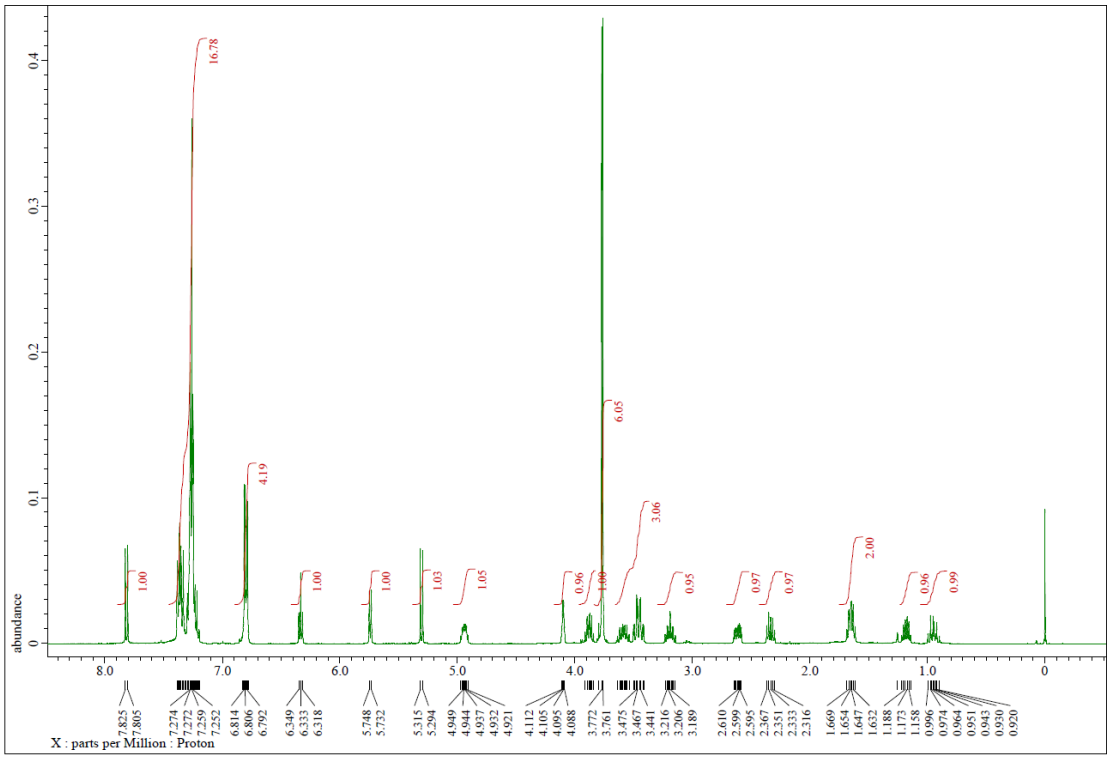

Figure S4. ^{1}H NMR spectra of compound (*R*p)-3a

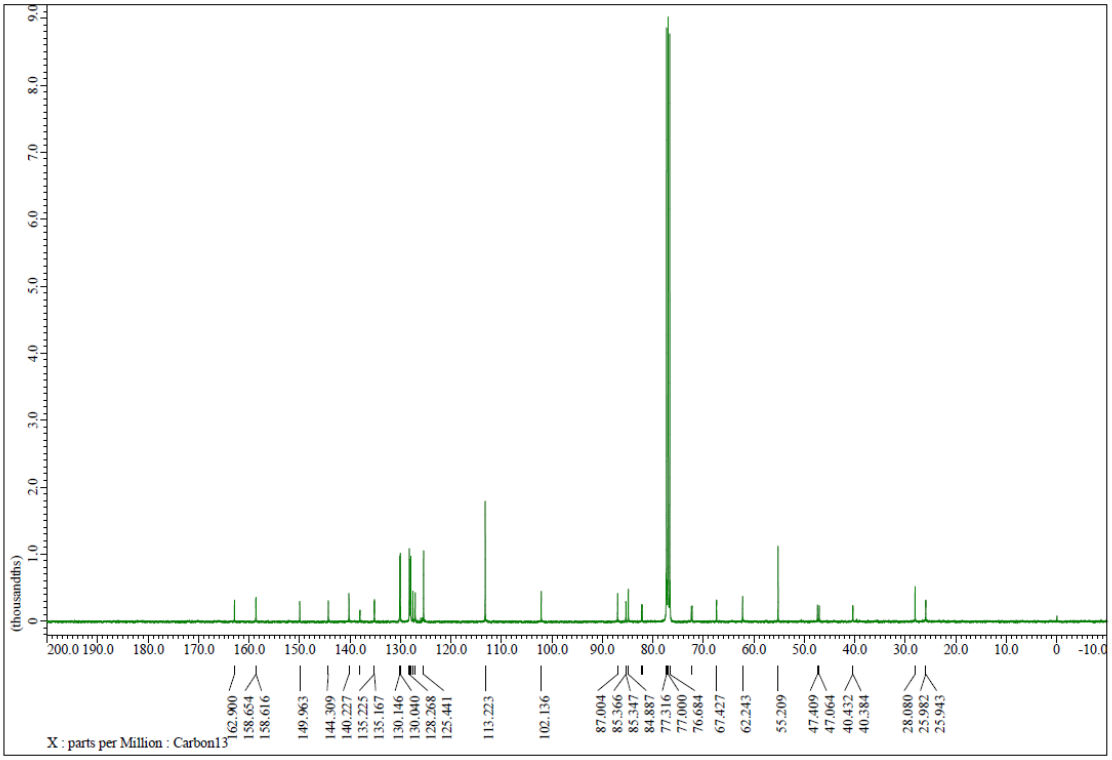

Figure S5. ^{13}C NMR spectra of compound (*R*p)-3a

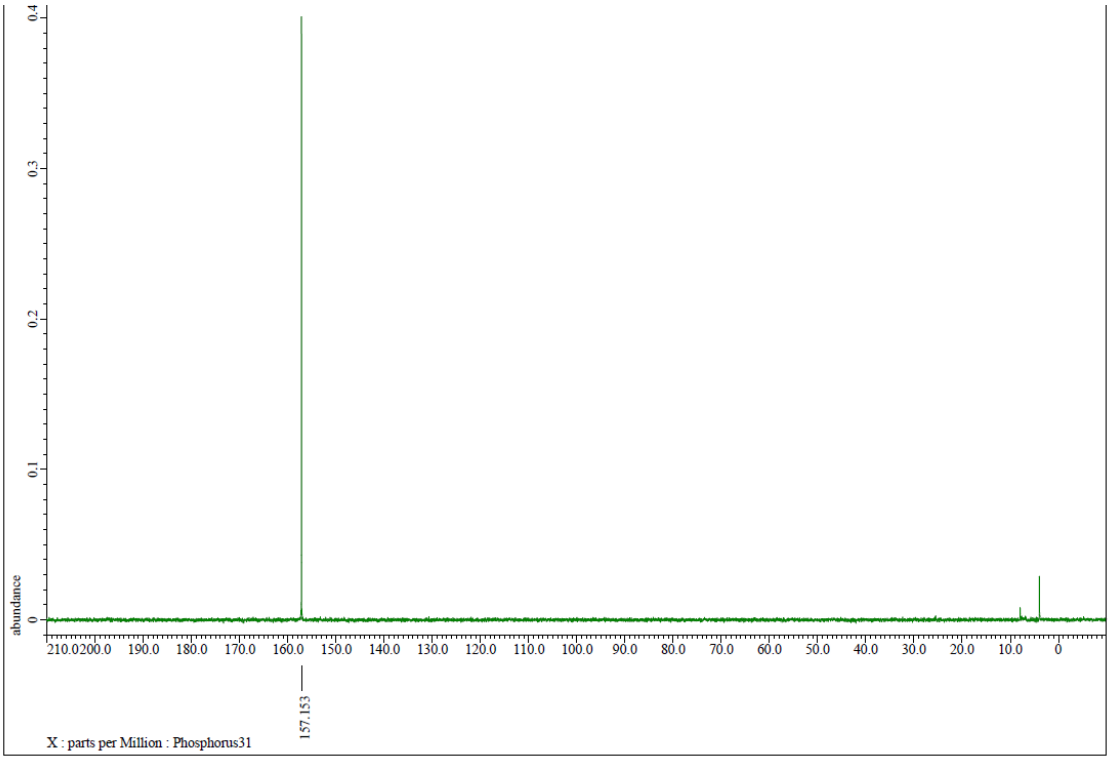

Figure S6. ^{31}P NMR spectra of compound (*R*p)-3a

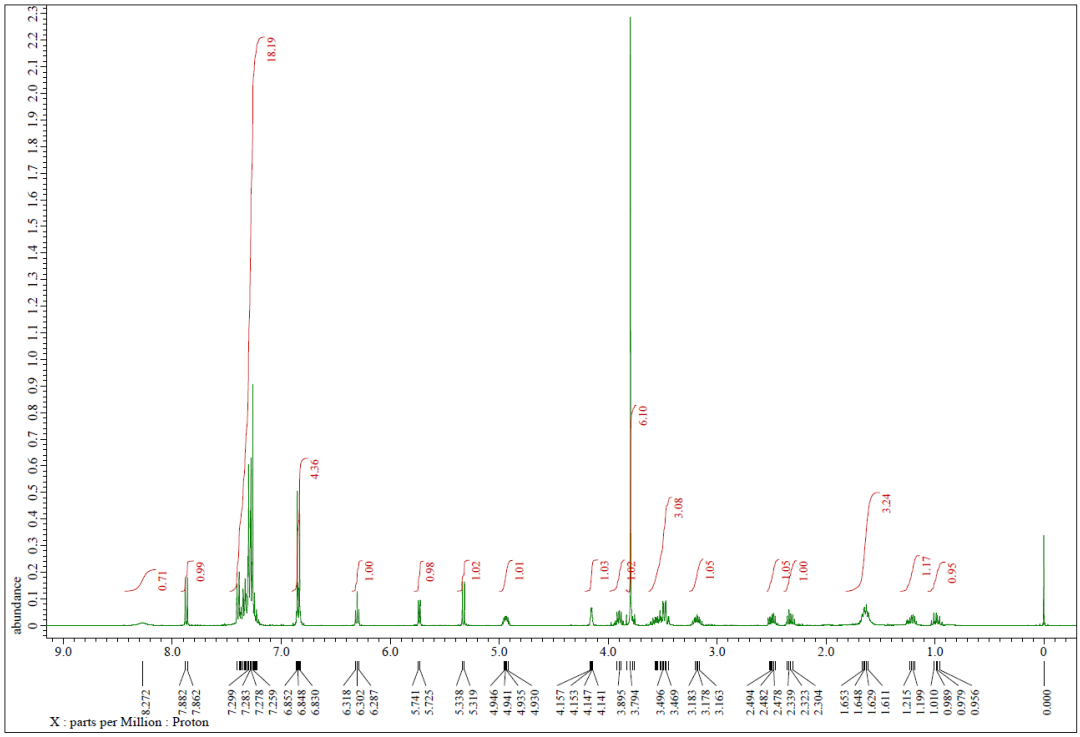

Figure S7. ^{1}H NMR spectra of compound (*S*p)-3a

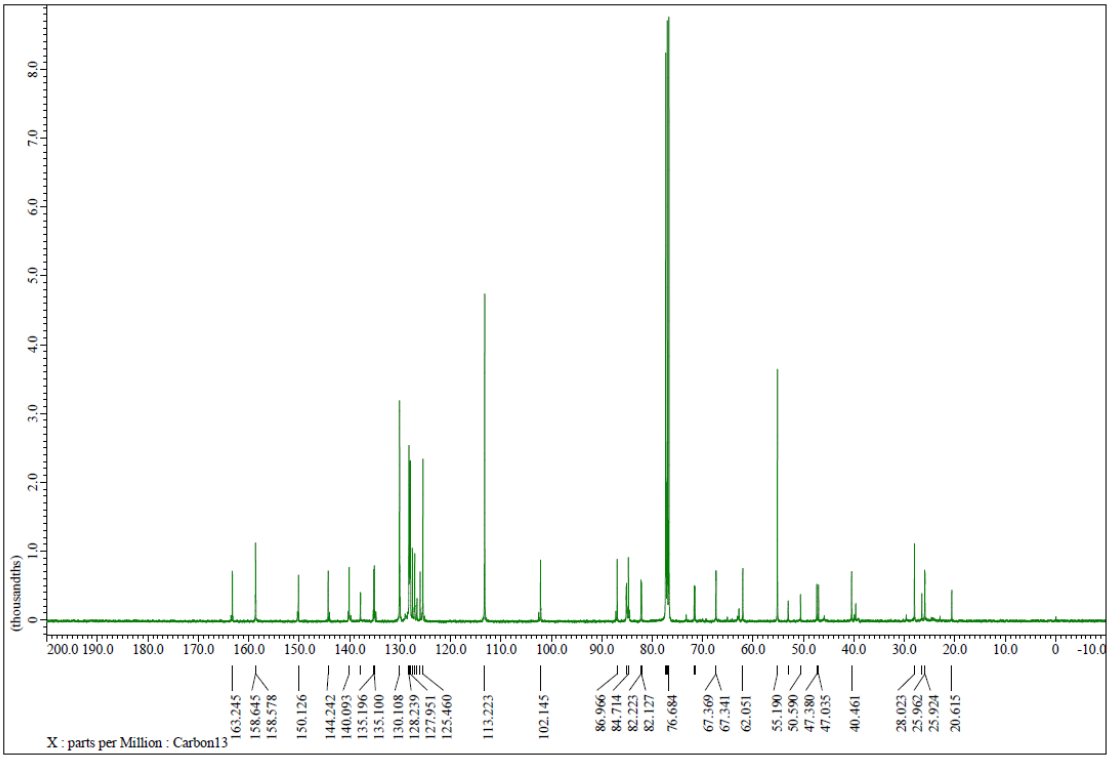

Figure S8. ^{13}C NMR spectra of compound (*S*p)-3a

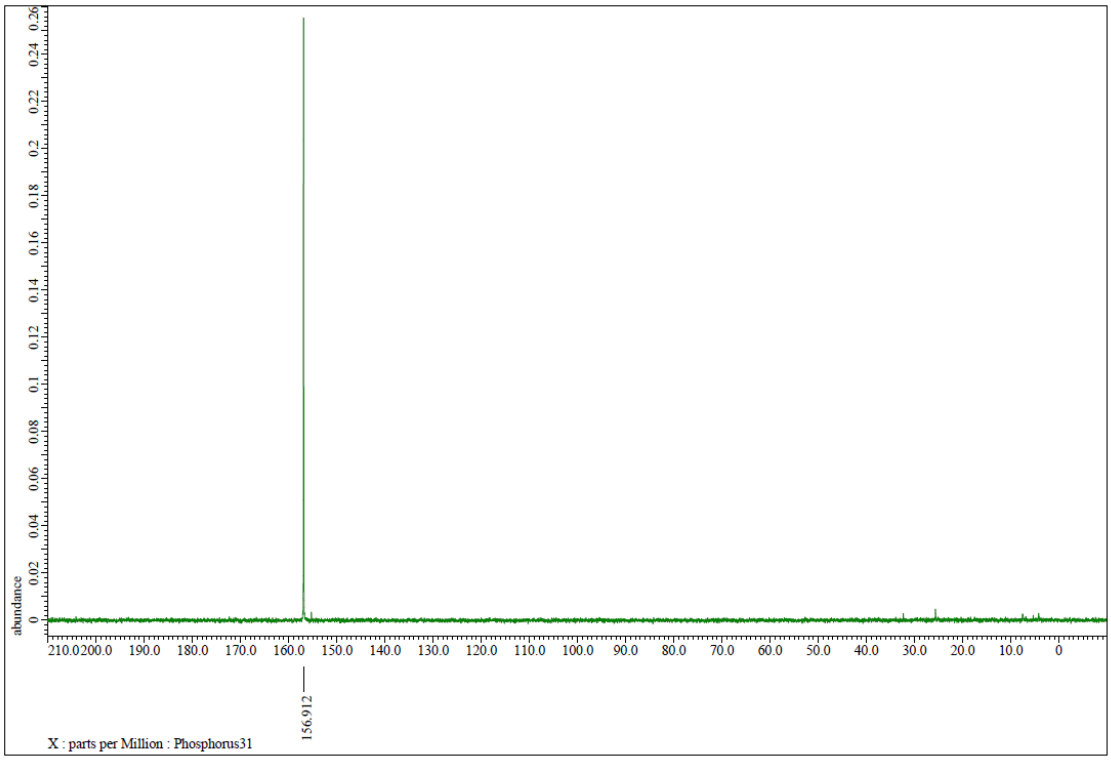

Figure S9. ^{31}P NMR spectra of compound (*S*p)-3a

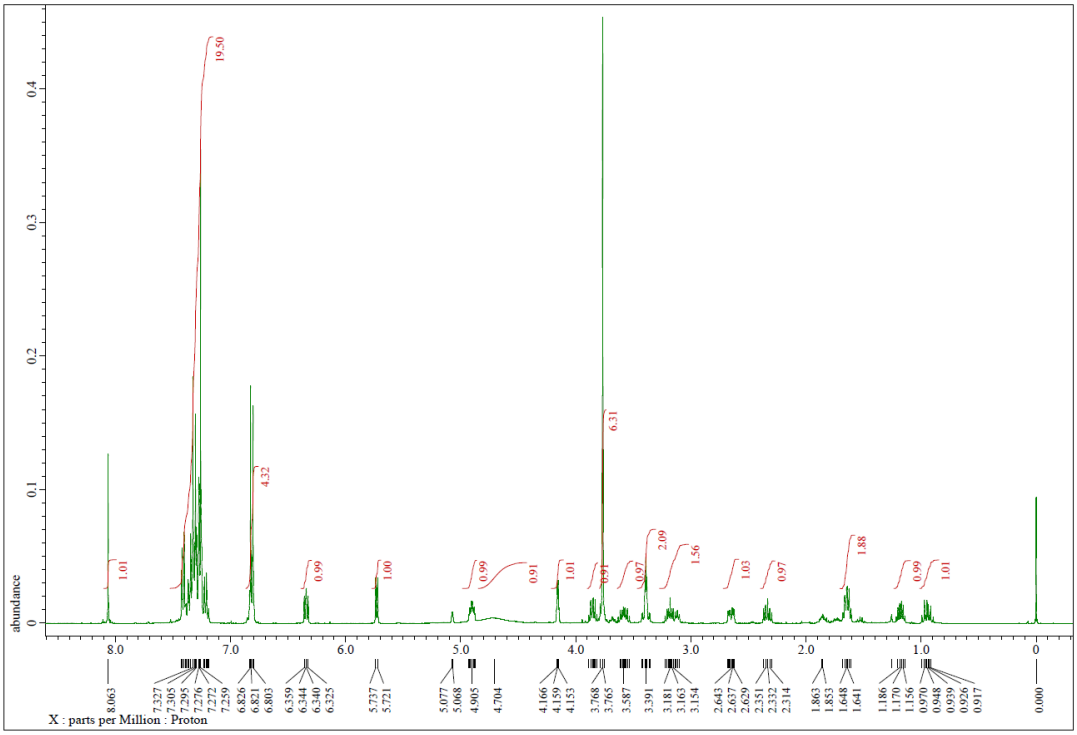

Figure S10. ^{1}H NMR spectra of compound (*R*p)-3c

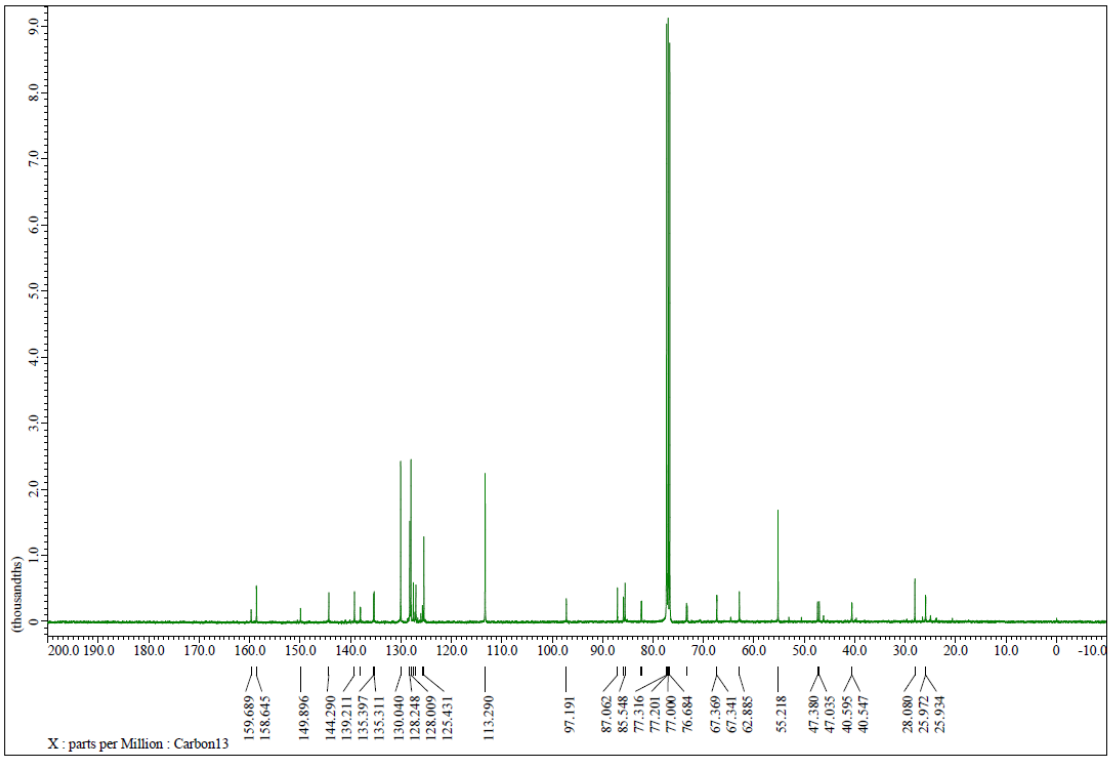

Figure S11. ^{13}C NMR spectra of compound (*R*p)-3c

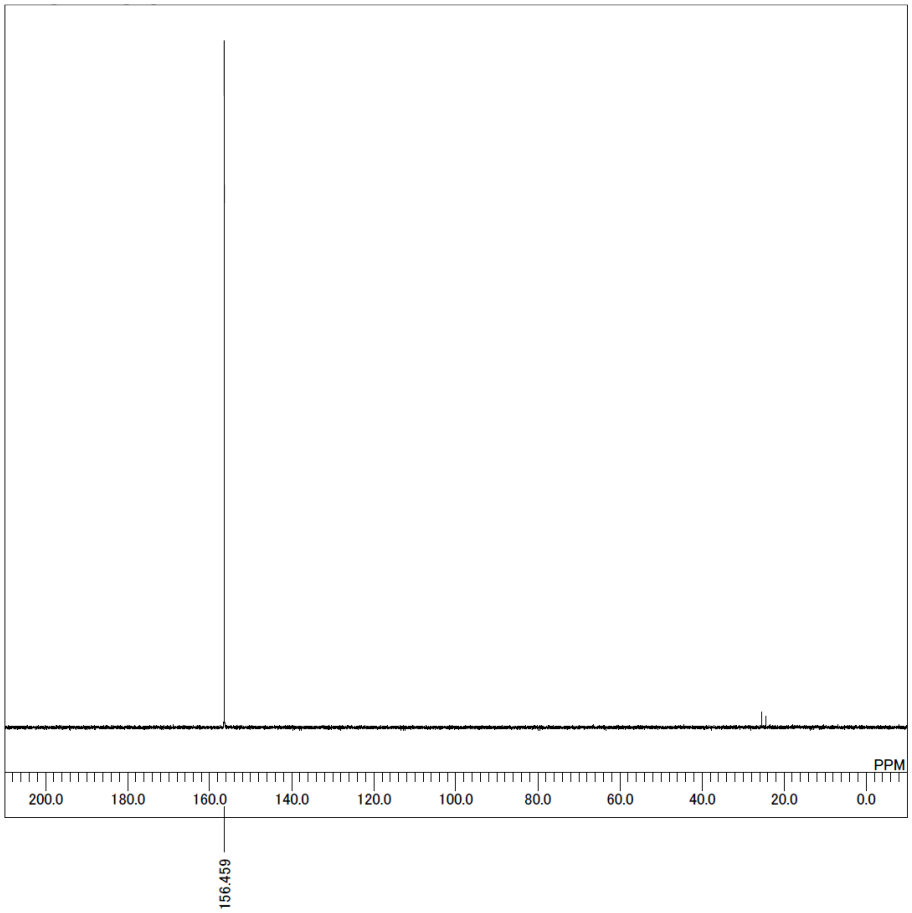

Figure S12. ^{31}P NMR spectra of compound (*R*p)-3c

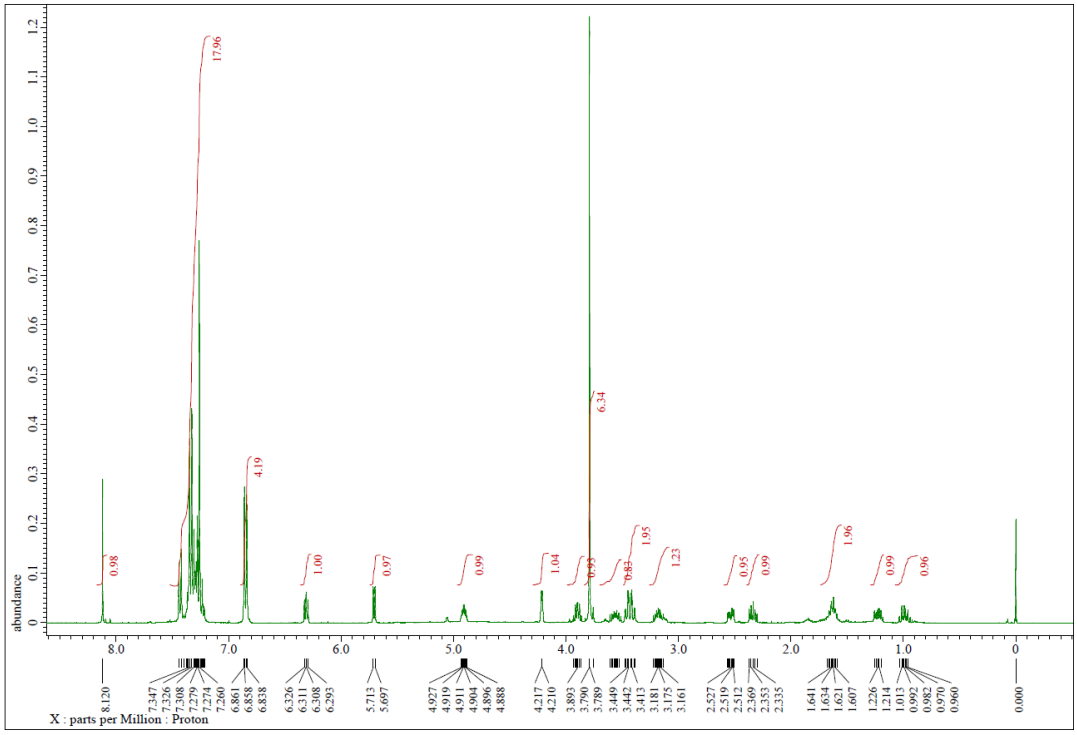

Figure S13. ^{1}H NMR spectra of compound (*S*p)-3c

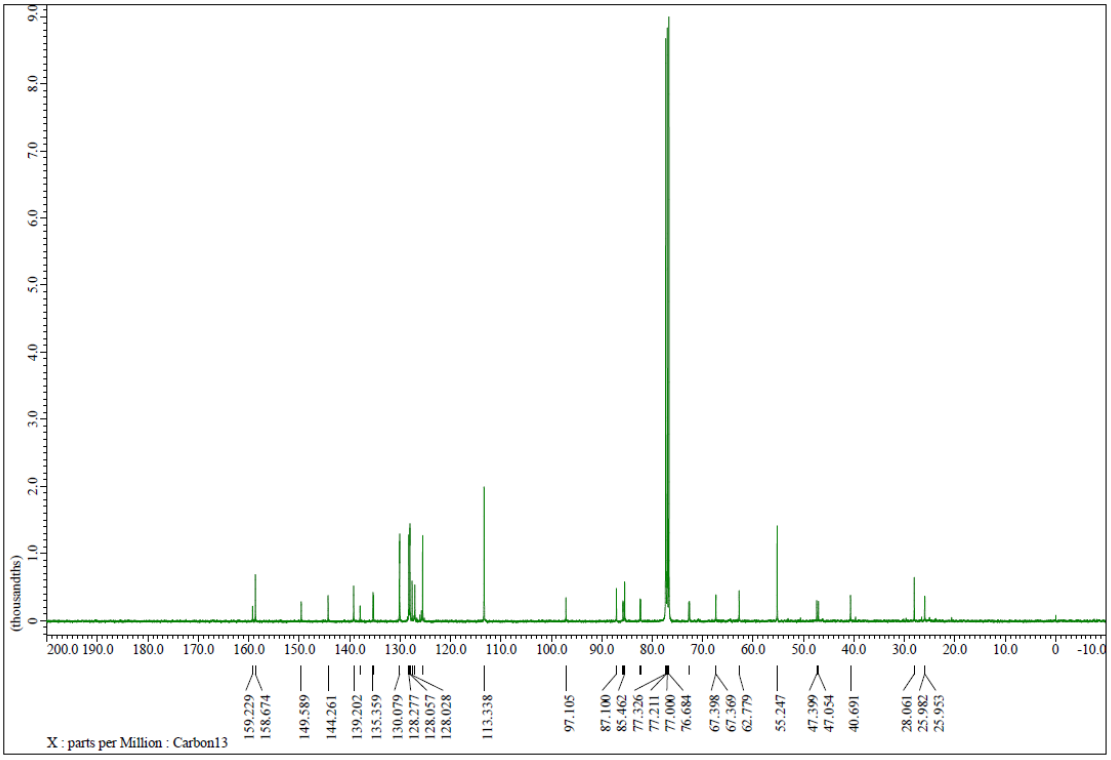

Figure S14. ^{13}C NMR spectra of compound (*S*p)-3c

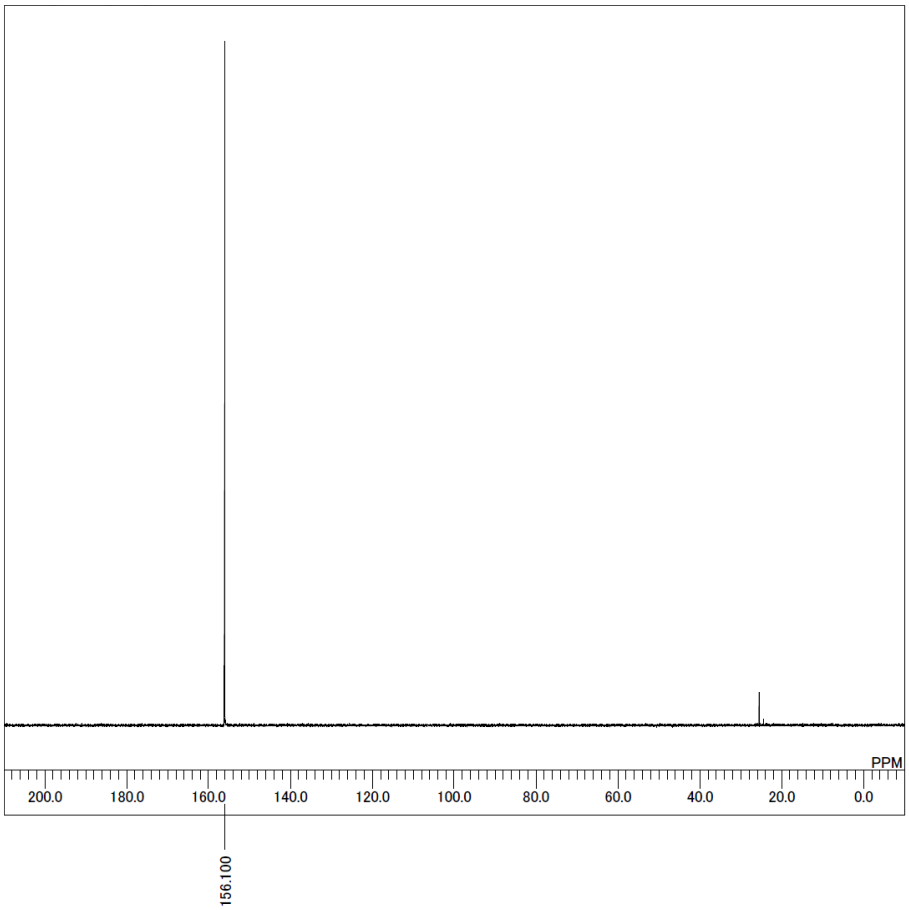

Figure S15. ^{31}P NMR spectra of compound (*S*p)-3c

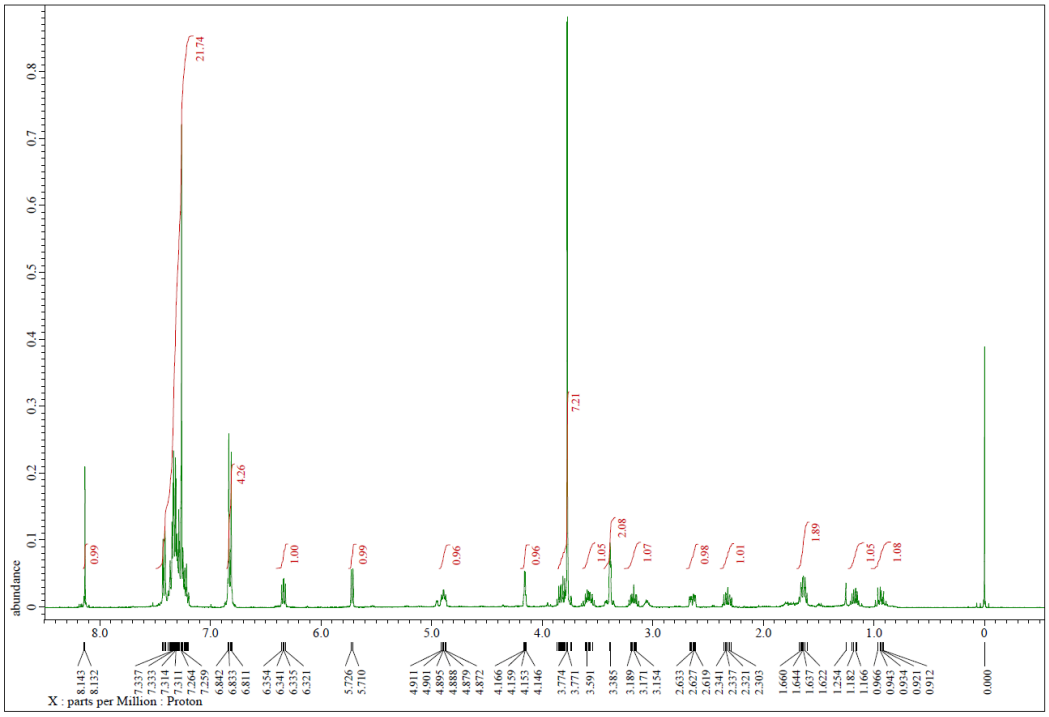

Figure S16. ^{1}H NMR spectra of compound (*R*p)-3d

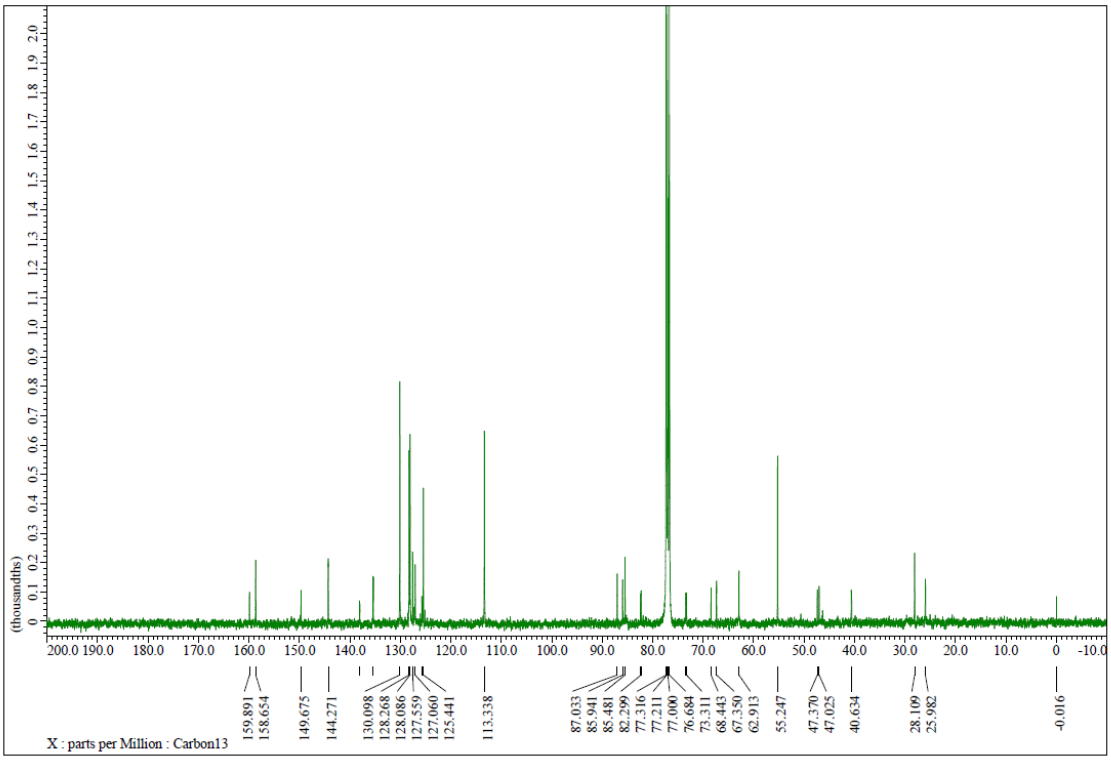

Figure S17. ^{13}C NMR spectra of compound (*R*p)-3d

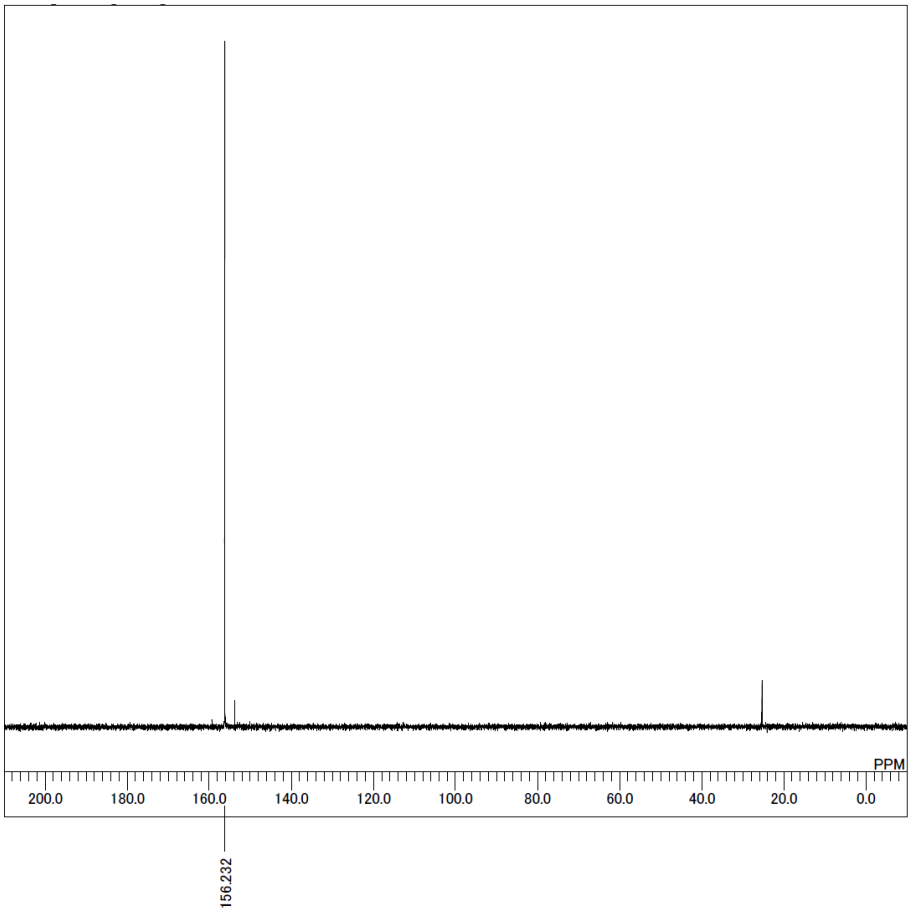

Figure S18. ^{31}P NMR spectra of compound (*R*p)-3d

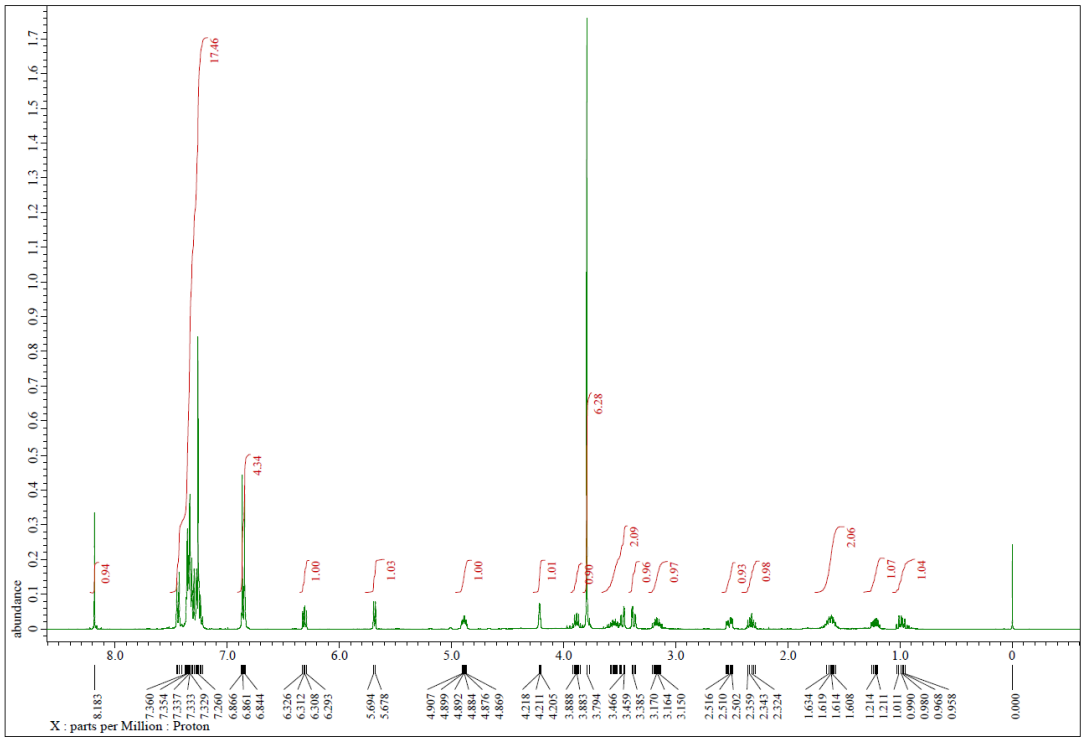

Figure S19. ^{1}H NMR spectra of compound (*S*p)-3d

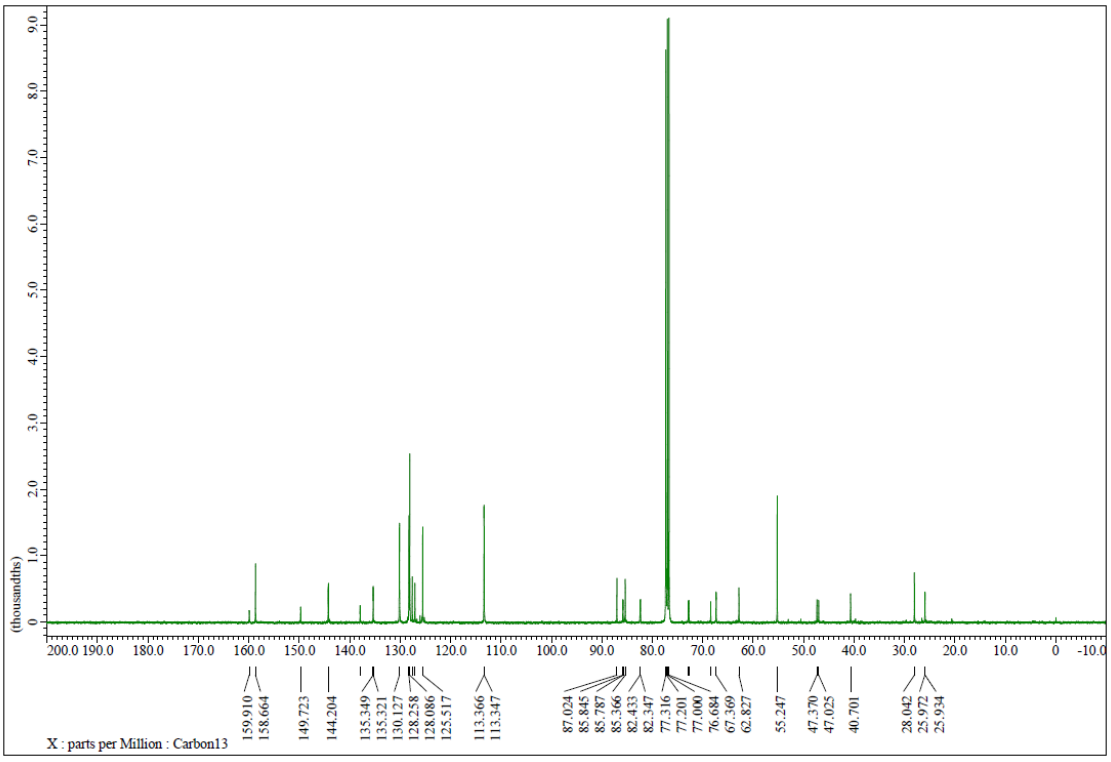

Figure S20. ^{13}C NMR spectra of compound (*S*p)-3d

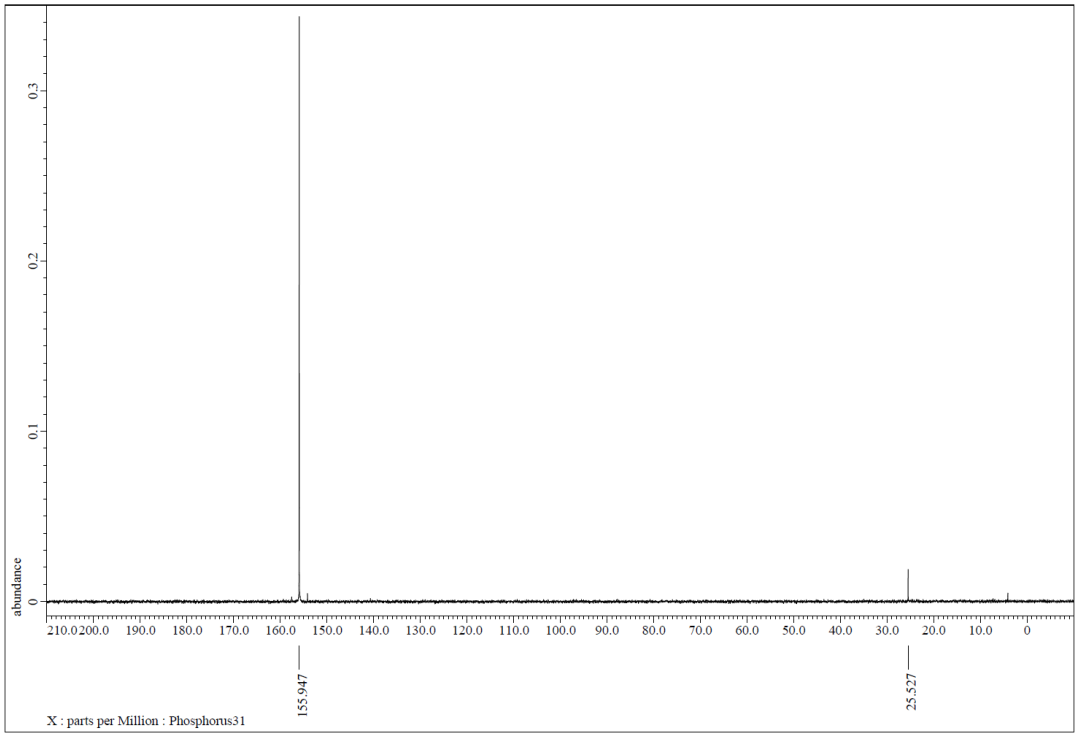

Figure S21. ^{31}P NMR spectra of compound (*S*p)-3d

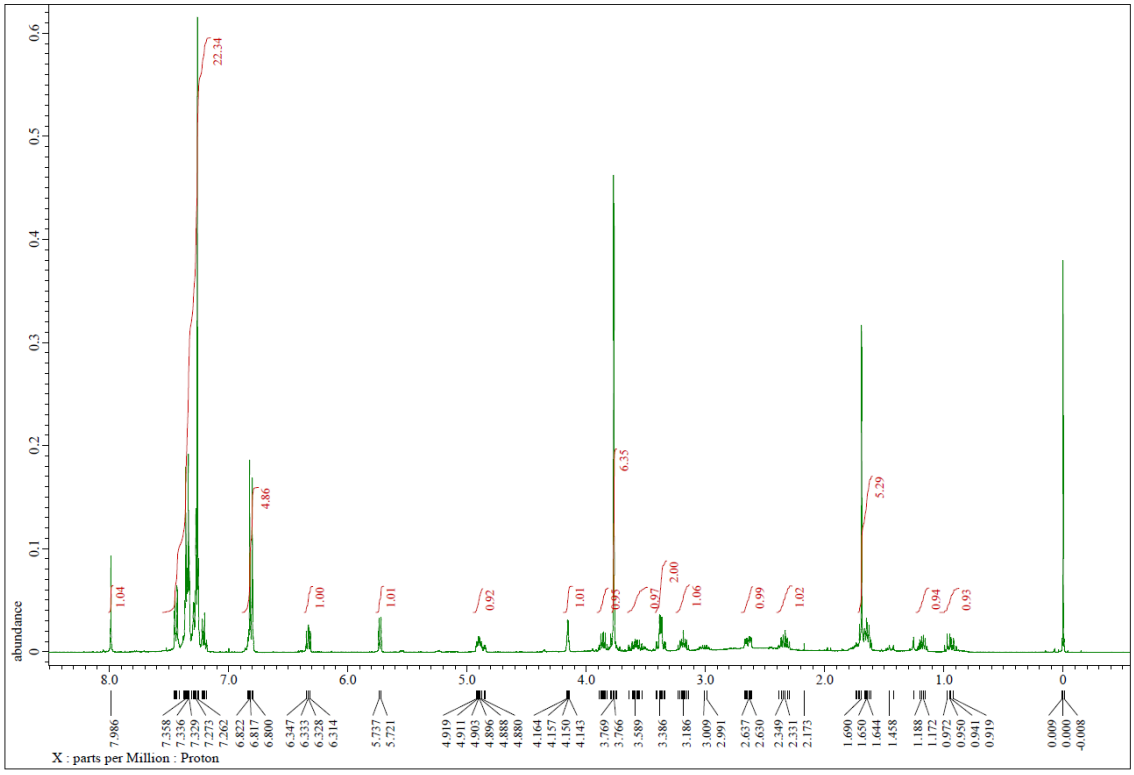

Figure S22. ^{1}H NMR spectra of compound (*R*p)-3e

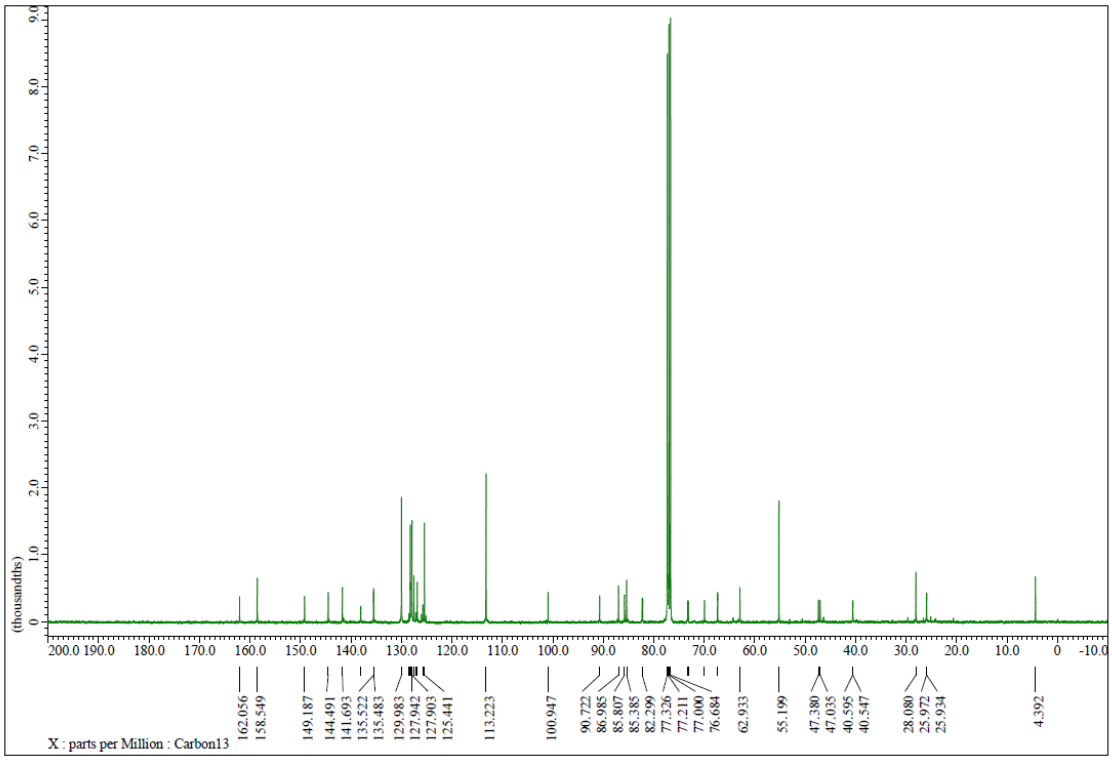

Figure S23. ^{13}C NMR spectra of compound (*R*p)-3e

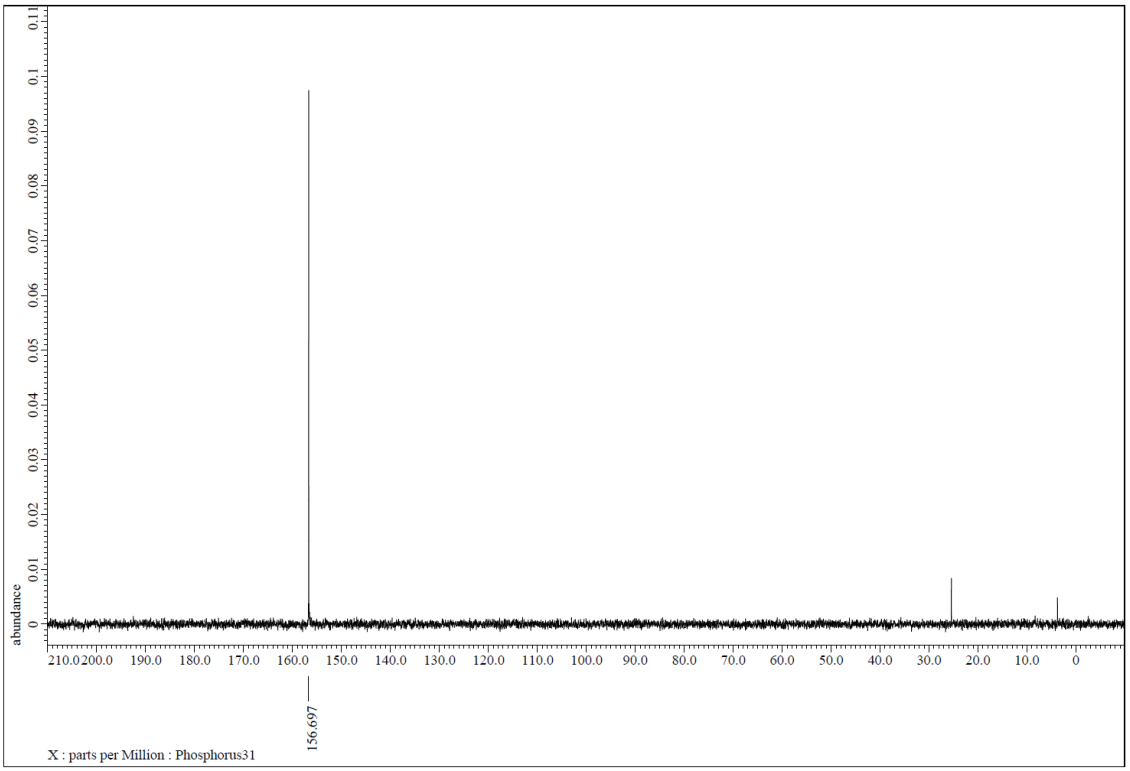

Figure S24. ^{31}P NMR spectra of compound (*R*p)-3e

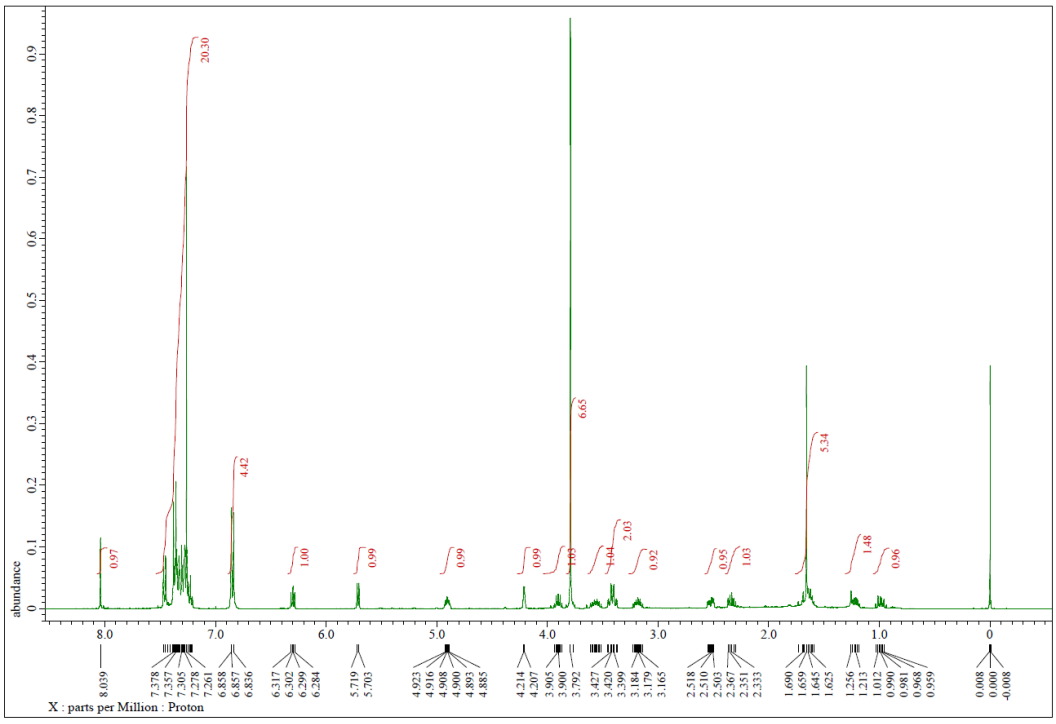

Figure S25. ^{1}H NMR spectra of compound (*S*p)-3e

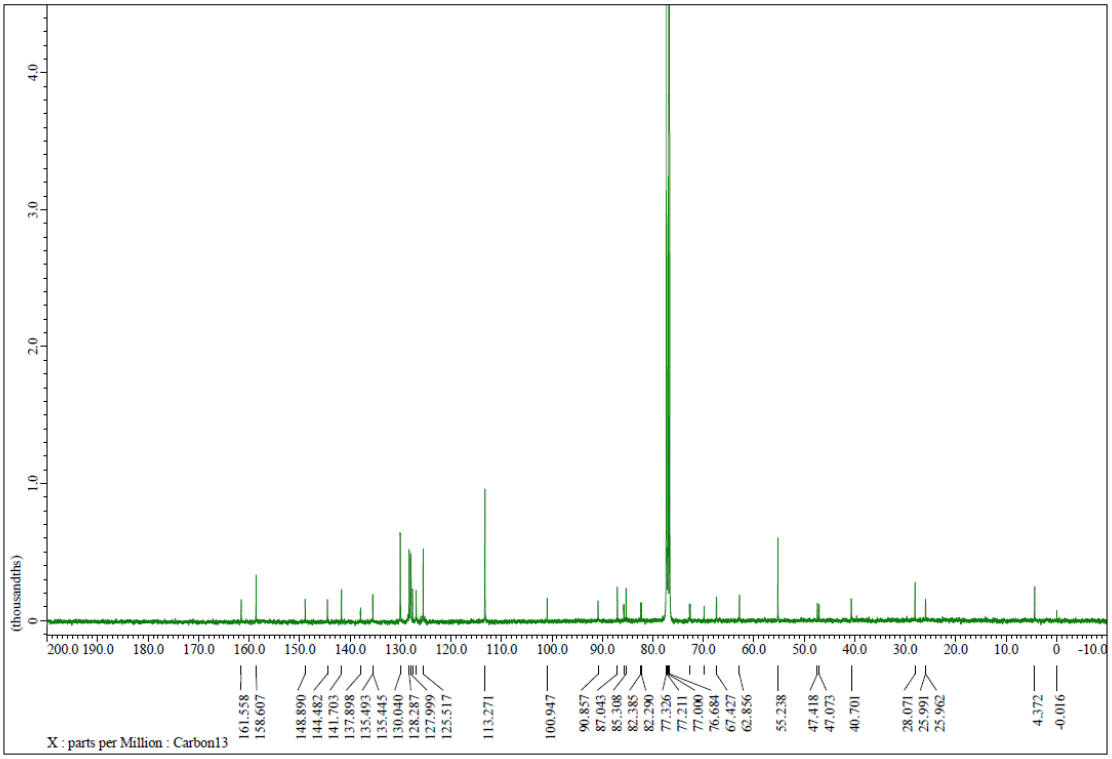

Figure S26. ^{13}C NMR spectra of compound (*S*p)-3e

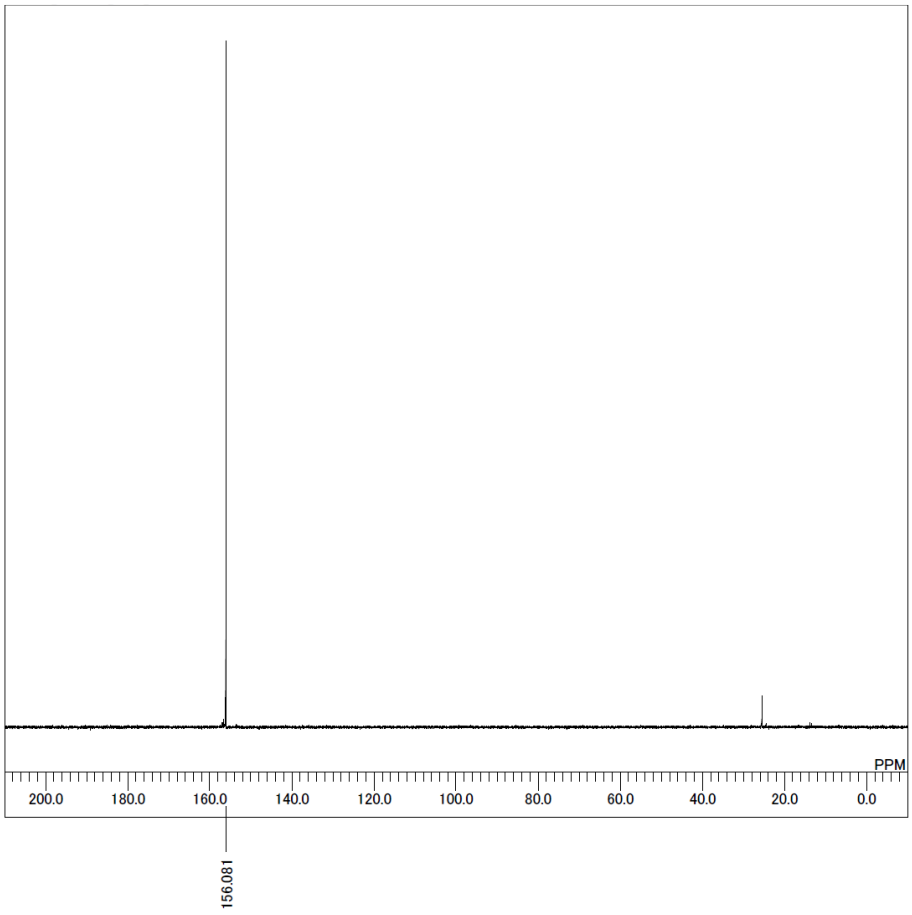

Figure S27. ^{31}P NMR spectra of compound (*S*p)-3e

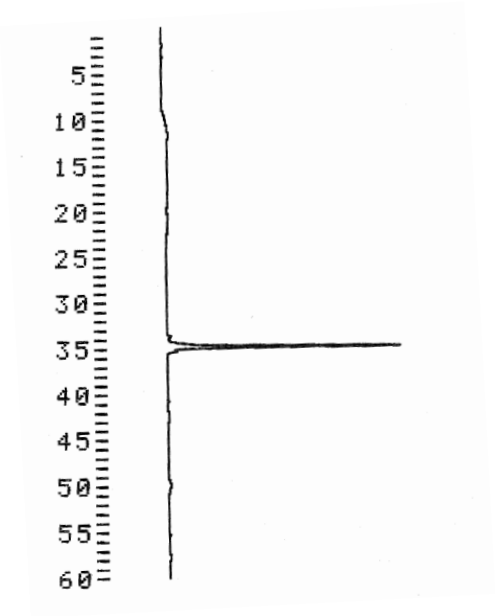

Figure S28. RP-HPLC profile of purified **(*R*p)-4a** ((*R*p)-dCG(U_{PS})$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

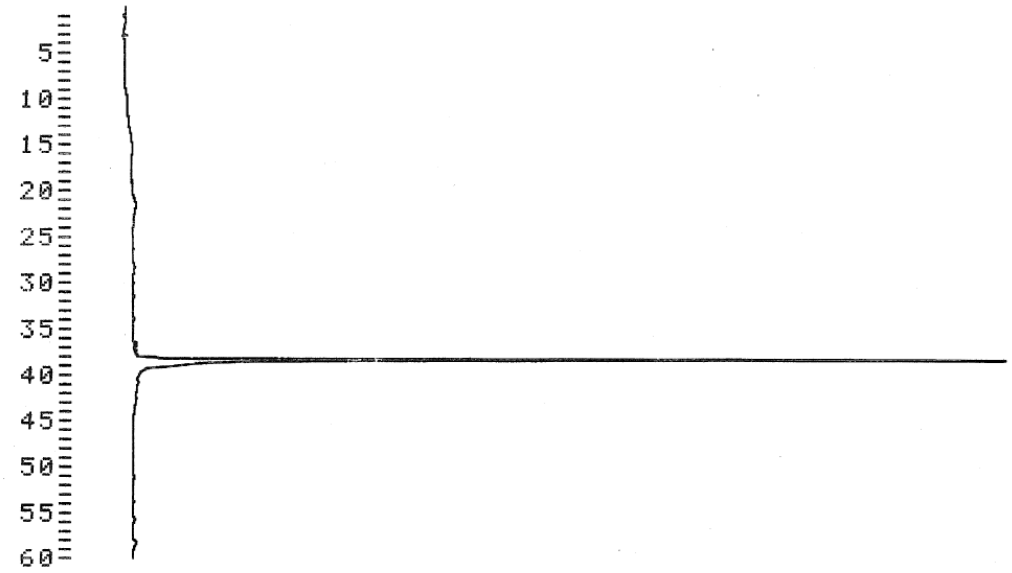

Figure S29. RP-HPLC profile of purified **(*S*p)-4a** ((*S*p)-dCG(U_{PS})$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

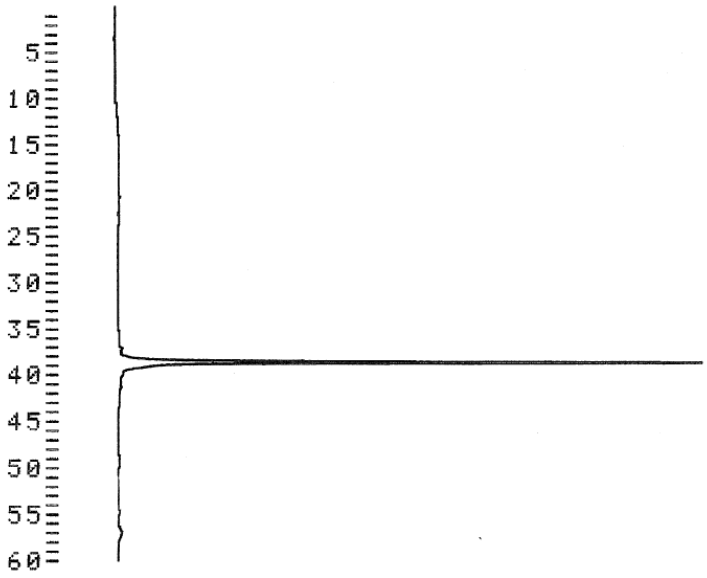

Figure S30. RP-HPLC profile of purified **(*R*p)-4b** ((*R*p)-dCG(T_{PS})$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

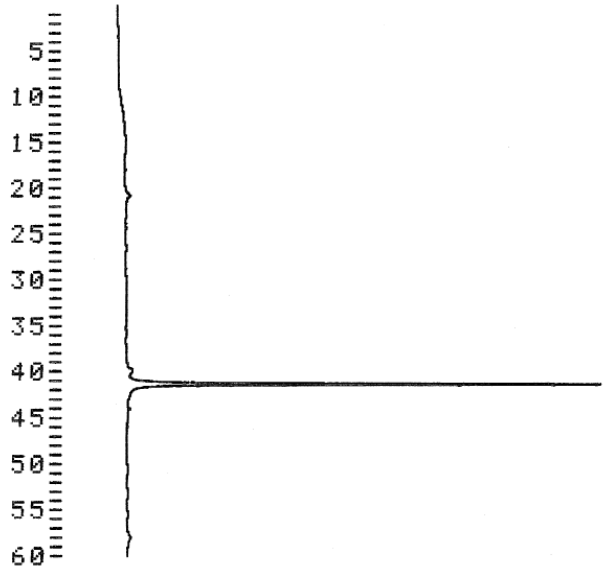

Figure S31. RP-HPLC profile of purified **(*S*p)-4b** (all-(*S*p)-PS-dCG(T_{PS})$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

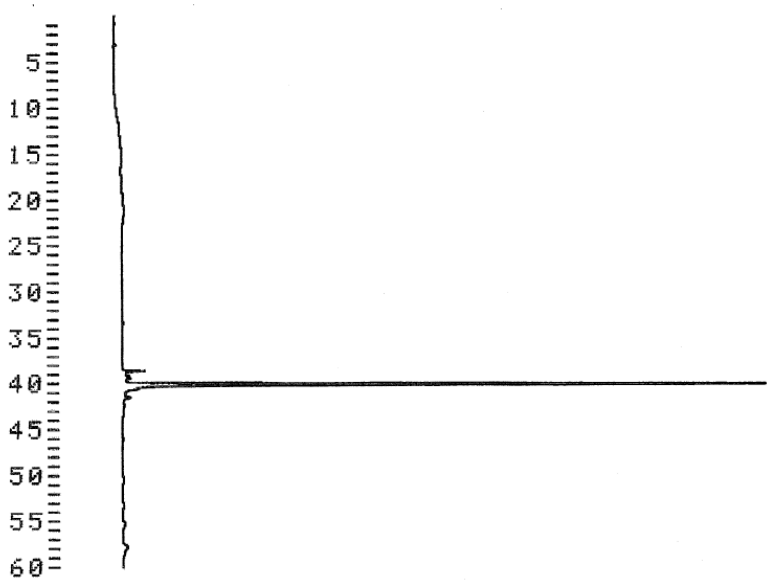

Figure S32. RP-HPLC profile of purified **(*R*p)-4c** ((*R*p)-dCG($^{Br}U_{PS}$)$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

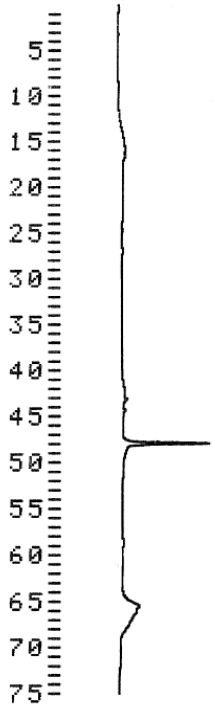

Figure S33. RP-HPLC profile of purified **(*S*p)-4c** ((*S*p)-dCG($^{Br}U_{PS}$)$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

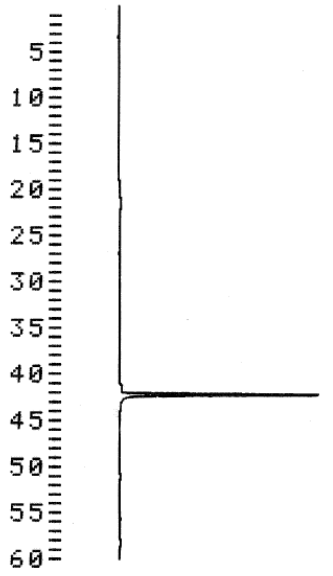

Figure S34. RP-HPLC profile of purified **(*R*p)-4d** ((*R*p)-dCG($^{I}U_{PS}$)$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

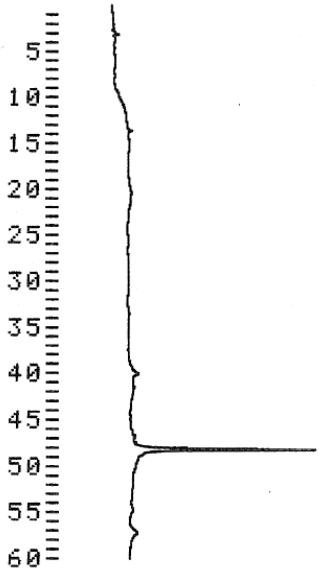

Figure S35. RP-HPLC profile of purified **(*S*p)-4d** ((*S*p)-dCG($^{I}U_{PS}$)$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

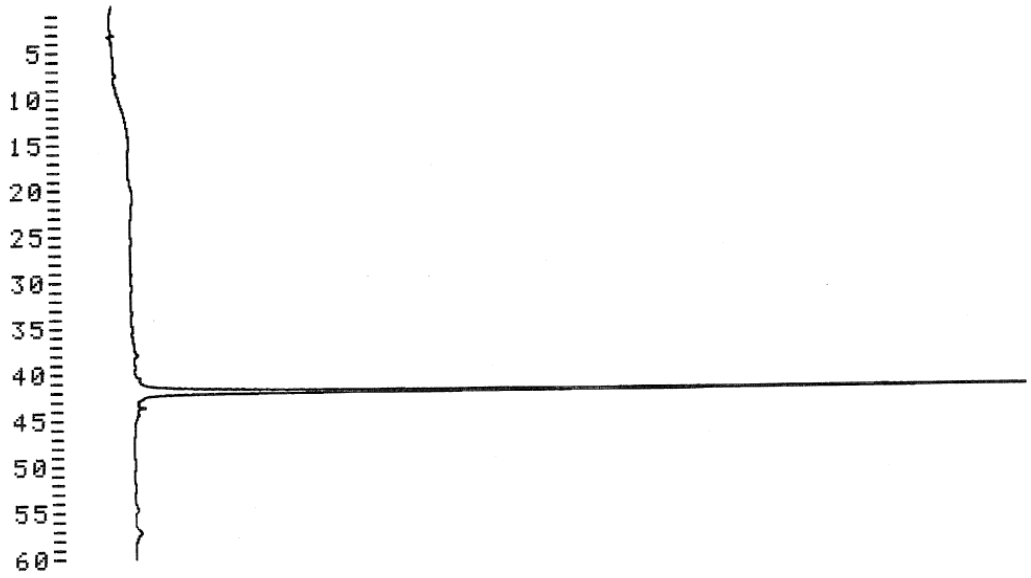

Figure S36. RP-HPLC profile of purified **(*R*p)-4e** ((*R*p)-dCG($^{pr}U_{PS}$)$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

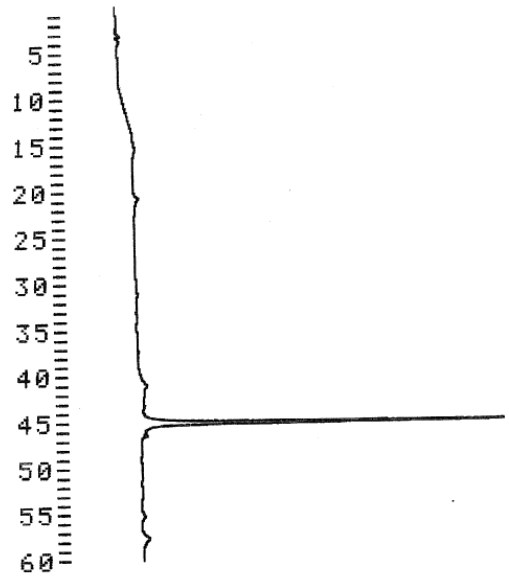

Figure S37. RP-HPLC profile of purified **(*S*p)-4e** ((*S*p)-dCG($^{pr}U_{PS}$)$_8$CG). RP-HPLC analysis (UV detection at 260 nm) was performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 30 °C for 60 min with a flow rate of 0.5 mL/min.

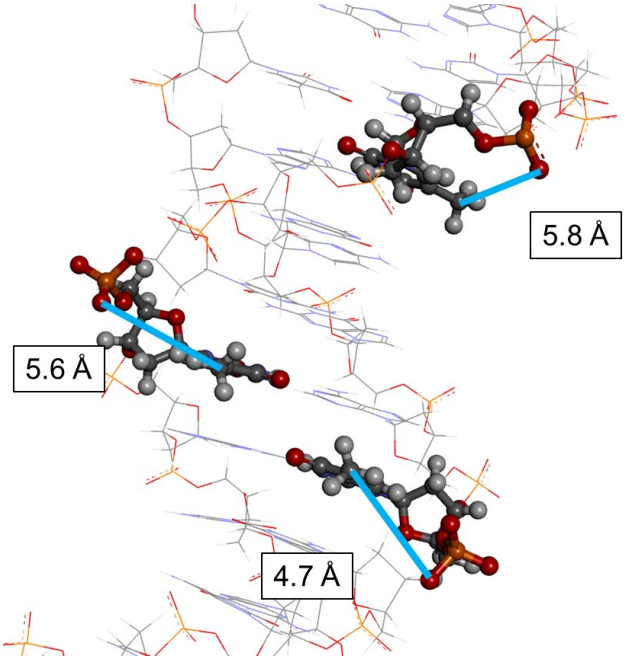

Figure S38. 3-D view of an DNA/DNA duplex, dCGCGTAGCATGCGC/dGCGCATGCTACGCG (PDB 2M2C, NMR solution structure). The underlined T ($_{PO}$T) residues are displayed in ball and stick style. The distances between the carbon atom of the methyl group of each thymine and a pro-*R*p oxygen atom in a phophodiester linkage were 4.7, 5.6, and 5.8 Å, respectively.

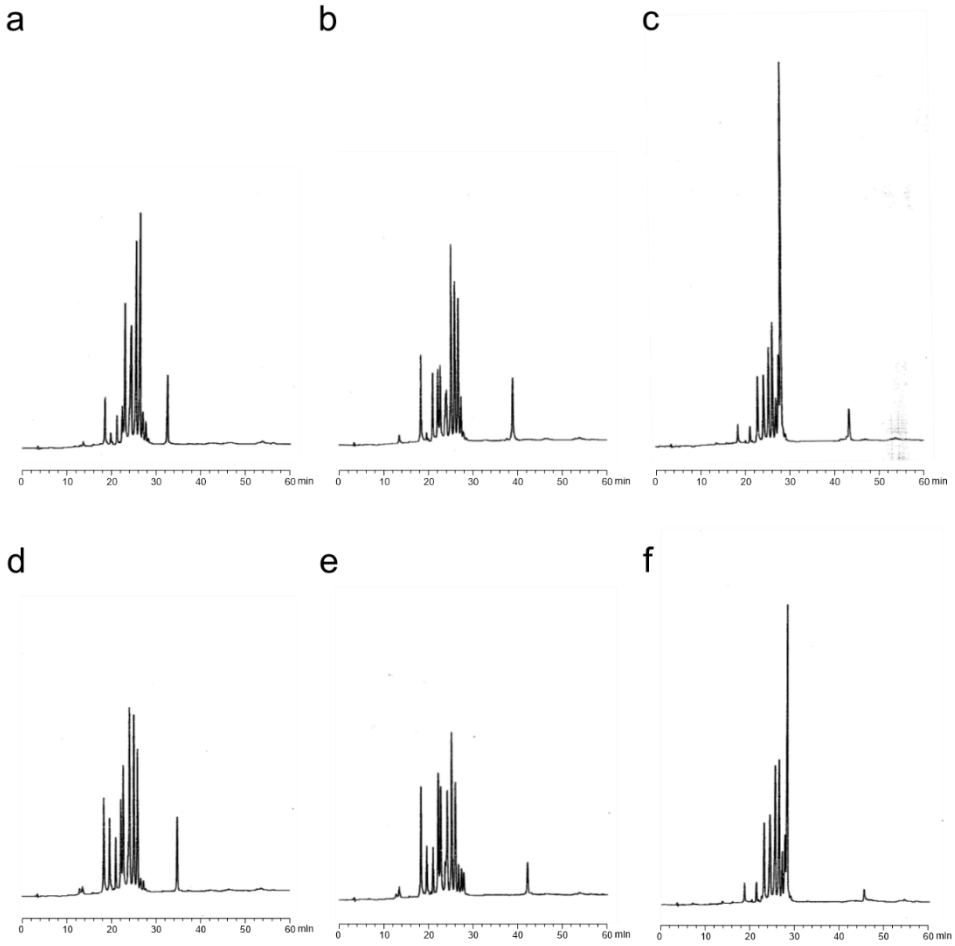

Figure S39. RP-HPLC profiles of the mixture of PO- or PO/PS chimeric-DNAs and their complementary RNA (rCGA$_8$CG) after treatment with 20U/100 μL RNase H at 20 °C for 30 min: (a) PO-dCGT$_8$CG (b) (*R*p)-dCG(T$_{PS}$)$_8$CG (***R*p-(4b)**) (c) (*S*p)-dCG(T$_{PS}$)$_8$CG (***S*p-(4b)**) (d) PO-dCG(^{pr}U)$_8$CG (e) (*R*p)-dCG(^{pr}U$_{PS}$)$_8$CG (***R*p-(4e)**) (f) (*S*p)-dCG(^{pr}U$_{PS}$)$_8$CG (***S*p-(4e)**). RP-HPLC analyses (UV detection at 260 nm) were performed with a linear gradient of 0–30% CH_3CN in 0.1 M TEAA buffer (pH 7.0) at 20 °C for 60 min with a flow rate of 0.5 mL/min.