

Supplementary information

Modified oligodeoxynucleotide primers for reverse-transcription of target RNAs that can discriminate among length variants at the 3'-terminus

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Experimental Section

General methods

Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. NMR spectra (^1H and ^{13}C NMR) were recorded on Varian inova-500 instruments at 500 MHz and 126 MHz, respectively, and calibrated using residual undeuterated solvent as an internal reference (CHCl_3 : 7.26 ppm for ^1H NMR, 77.0 ppm for ^{13}C NMR and DMSO-d_5 : 2.49 ppm for ^1H NMR, 39.5 ppm for ^{13}C NMR). NMR spectra (^{31}P) were recorded on Varian inova-500 instruments at 203 MHz and calibrated using an external reference (85% orthophosphoric acid, 0 ppm).

UV spectra were recorded with a U-2000 spectrometer. Column chromatography was performed with silica gel C-200 (purchased from Wako Co. Ltd.). Gel filtration column was performed with a combination of LC-908, UV detector 310, and JAIGEL GS-310 (Japan Analytical Industry Co, Ltd.). High performance liquid chromatography (HPLC) was performed using the following systems: Reversed exchange HPLC was done on a Waters Alliance system with a Waters 3D UV detector and a Waters XTerra MS C18 column (4.6 x 150 mm); a linear gradient (0–30%) of solvent I (CH_3CN) in solvent II [0.1 M ammonium acetate buffer (pH 7.0)] was used at 50 °C at a flow rate of 1.0 mL/min for 30 min; anion-exchange HPLC was done on a Shimadzu LC-10 AD VP with a Shimadzu 3D UV detector and a Gen-Pak FAX column (Waters, 4.6 100 mm); a linear gradient (10–67%) of solvent III [1 M NaCl in 25 mM phosphate buffer (pH 6.0)] in solvent IV [25 mM phosphate buffer (pH 6.0)] was used at 50 °C at a flow rate of 1.0 mL/min for 40 min. The synthesis of modified oligonucleotides was carried out by use of a DNA/RNA synthesizer 392 (Applied Biosystem).

DNA and RNA-oligonucleotide were purchased from Sigma-Aldrich Co. LTD. Reverse transcription, primer extension, and PCR were carried out in CFB-3120 MiniOpticon (Bio-Rad, USA). Water purified by using Direct-Q UV (Merck Millipore, USA) was used for the experiment with DNA and RNA.

Pharma Spec UV-1700 (Shimadzu Corp., Japan) was used for the T_m measurement.

5'-O-(4, 4'-dimethoxytrityloxy)propyl]-2'-deoxyuridine (1)

Compound **1** was synthesized according to a reported procedure (M. Sekine, O. Kurasawa, K. Shohda, K. Seio and T. Wada, *Eur. J. Org. Chem.* 2001, **10**, 1989.).

^1H NMR (CDCl_3 , 500 MHz) δ 1.55-1.60 (1H, m), 1.67-1.71 (1H, m), 1.76-1.82 (1H, m), 2.00-2.05 (1H, m), 2.18-2.23 (1H, m), 2.35-2.39 (1H, m), 2.78 (1H, dd, $J = 6.3$, 14.6 Hz), 2.85 (1H, dd, $J = 6.3$, 14.6 Hz), 3.34-3.40 (1H, m), 3.64-3.73 (13H, m), 4.06 (1H, d, $J = 2.5$ Hz), 4.49 (1H, t, $J = 2.5$ Hz), 6.39 (1H, t, $J = 7.0$ Hz), 6.73-6.81 (8H, m), 7.07-7.42 (19H, m); ^{13}C NMR (CDCl_3 , 126 MHz) δ 24.30, 29.24, 41.00, 53.79, 55.47, 63.29, 64.03, 72.77, 85.13, 85.96, 86.51, 87.06, 113.56, 115.77, 126.83, 127.38, 127.97, 128.26, 128.41, 128.43, 130.29, 130.37, 135.64, 135.71, 135.88, 136.82, 136.87, 144.66, 145.60, 151.02, 158.55, 158.92, 163.84; HRMS m/z calcd for $\text{C}_{54}\text{H}_{54}\text{N}_2\text{O}_9$ $[\text{M}+\text{Na}]$ 913.3721, found 913.3732.

5'-O-(4, 4'-Dimethoxytrityl)-5-[3-(4, 4'-dimethoxytrityloxy)propyl]-3'-O-*tert*-butyldimethylsilyl-2'-deoxyuridine (2)

1.0 g (1.1 mmol) of **1** was rendered anhydrous by co-evaporation three times each with dry pyridine and toluene, and finally dissolved in dry dichloromethane (2.2 mL). Triethylamine (230 μL , 1.7 mmol) was added and the resulting solution was stirred at -30°C . To the solution, trifluoromethanesulfonic acid *tert*-butyldimethylsilyl ester (309 μL , 1.3 mmol) was added dropwise. The solution was stirred at ambient temperature for 10 h. The reaction was quenched by addition of water (1.0 mL), and the solution was diluted with ethyl acetate (10 mL). The solution was washed with saturated aq. NaHCO_3 (10 mL) twice and brine (10 mL) once, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel column with hexane-ethyl acetate (8:2, v/v) to give **2** (845.8 mg, 75%). ^1H NMR (DMSO , 500 MHz) δ -0.03 (3H, s), 0.02 (3H, s), 1.84 (9H, s), 1.53-1.76 (2H, m), 1.78-1.86 (1H, m), 2.04-2.16 (2H, m), 2.24-2.29 (1H, m), 2.78 (1H, dd, $J = 8.0$, 15.1 Hz), 2.85 (1H, dd, $J = 8.0$, 15.1 Hz), 3.28 (1H, dd, $J = 2.9$, 10.5 Hz), 3.39 (1H, dd, $J = 2.9$, 10.5 Hz), 3.68 (3H, s), 3.69 (3H, s), 3.76 (6H, s), 3.96 (1H, d, $J = 2.7$ Hz), 4.45 (1H, t, $J = 3.1$ Hz), 6.31 (1H, t, $J = 6.7$ Hz), 6.74-6.77 (8H, m), 7.11 (1H, m), 7.14-7.26 (13H, m), 7.35 (4H, m), 7.46 (1H, s), 8.44 (1H, s); ^{13}C NMR (CDCl_3 , 126 MHz) δ -4.61, -4.43, 18.21, 24.30, 25.98, 29.34, 41.48, 55.43, 63.24, 63.33, 72.68, 85.22, 85.90, 86.97, 87.03, 113.19, 113.48, 113.51, 115.47, 126.76, 127.33, 127.91, 128.20, 128.31, 128.41, 130.22, 130.27, 130.30, 135.61, 135.65, 135.74, 136.77, 136.80, 144.59, 145.51, 150.36, 158.51, 158.90, 163.33; HRMS m/z calcd for $\text{C}_{60}\text{H}_{68}\text{N}_2\text{O}_{10}\text{Si}$ $[\text{M}+\text{Na}]$ 1027.4585, found 1027.4581.

5-(3-Hydroxypropyl)-3'-(*tert*-butyldimethylsilyl)-2'-deoxyuridine (3)

1.1 g (1.1 mmol) of **2** was dissolved in dichloromethane (5 mL). 2% trifluoroacetic acid in dichloromethane (20 mL) was added and the resulting solution was stirred at ambient temperature for 2 min. This reaction mixture was added to the 40 mL of methanol-pyridine (1:1, v/v) at 0 °C. The solution was concentrated under reduced pressure. The residue was chromatographed on a silica gel column with chloroform-methanol (94:6, v/v) to give **3** (300 mg, 70%). ¹H NMR (CDCl₃, 500 MHz) δ 0.08 (6H, s), 0.89 (9H, s), 1.73 (1H, s, br), 2.27 (1H, m), 2.43 (1H, m), 2.95 (1H, s, br), 3.62 (3H, m), 3.74 (1H, d, *J* = 11.0 Hz), 3.91 (2H, d, *J* = 12.2 Hz), 4.51 (1H, s), 6.24 (1H, t, *J* = 6.5 Hz), 7.65 (1H, s), 9.21 (1H, s, br); ¹³C NMR (CDCl₃, 126 MHz) δ -4.61, -4.46, 18.20, 22.91, 25.98, 30.92, 41.31, 61.22, 61.82, 71.95, 86.13, 88.10, 114.10, 137.94, 150.77, 164.70; HRMS *m/z* calcd for C₁₈H₃₂N₂O₆Si [M+H] 401.2102, found 401.2169.

5'-O-(4,

4'-Dimethoxytrityl)-6-*N*-[*N*-(*trans*-4-levulinyloxycyclohexyl)carbamoyl]-deoxyadenosine 3'-(*N*, *N*, *N'*, *N'*-tetraisopropyl phosphorodiamidite) (5)

2.0 g (2.5 mmol) of **4** was rendered anhydrous by co-evaporation three times each with dry pyridine and toluene, and finally dissolved in dry 1, 4-dioxane (25 mL). To this solution, triethylamine (527 μL, 3.8 mmol) and bis(diisopropylamino)chlorophosphine (807 mg, 3.0 mmol) were added, and the solution was stirred at ambient temperature for 5 h. The reaction was quenched by addition of water (1.0 mL), and the solution was diluted with ethyl acetate (10 mL). The solution was washed with saturated aq. NaHCO₃ (10 mL) twice and brine (10 mL) once, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-ethyl acetate (3:7, v/v, containing 1% pyridine) to give crude material. The crude material was dissolved in acetonitrile and chromatographed on a gel filtration column with acetonitrile to give **5** (1.9 g, 74%). ¹H NMR (CDCl₃, 500 MHz) δ 1.11-1.18 (24H, m), 1.24-1.27 (2H, m), 1.46-1.60 (4H, m), 2.02-2.04 (3H, m), 2.15-2.20 (5H, m), 2.57 (2H, t, *J* = 6.7 Hz), 2.69-2.71 (1H, m), 2.75 (2H, t, *J* = 6.6 Hz), 2.80-2.87 (1H, m), 3.31-3.39 (2H, m), 3.45-3.59 (4H, m), 3.77 (6H, s), 3.82-3.90 (1H, m), 4.39 (1H, s), 4.57-4.63 (1H, m), 4.76-4.83 (1H, m), 6.46 (1H, t, *J* = 6.7 Hz), 6.73-6.78 (4H, m), 7.15-7.24 (3H, m), 7.25-7.30 (4H, m), 7.37-7.41 (2H, m), 7.96 (1H, s, br), 8.12 (1H,

d, $J = 1.5$ Hz), 8.44 (1H, s), 9.43 (1H, d, $J = 7.6$ Hz); ^{13}C NMR (CDCl_3 , 126 MHz) δ 24.07, 24.12, 24.13, 24.18, 24.48, 24.55, 24.61, 28.42, 29.80, 29.99, 30.40, 38.08, 39.95, 39.99, 44.63, 44.73, 44.76, 44.86, 48.10, 64.20, 72.18, 74.16, 74.31, 85.26, 86.51, 86.67, 86.72, 113.15, 120.92, 126.89, 127.87, 128.19, 130.13, 135.72, 135.80, 141.28, 144.64, 150.06, 150.28, 151.00, 153.28, 158.54, 172.34, 206.78; ^{31}P NMR (CDCl_3 , 203 MHz) δ 117.30; ESI-MS m/z calcd for $\text{C}_{55}\text{H}_{75}\text{N}_8\text{O}_9\text{P}$ $[\text{M}+\text{Na}]$ 1045.5286, found 1045.5260.

5'-O-(4,

4'-Dimethoxytrityl)-6-N-[N-(trans-4-levulinyloxycyclohexyl)carbamoyl]-deoxyadenylyl(3'-5')3'-(tert-butyldimethylsilyl)-2'-deoxyuridine derivative, possessing a cyclic structure with a propylene bridge (6)

720 mg (1.8 mmol) of **3** and 1.8 g (1.8 mmol) of **5** was separately rendered anhydrous by co-evaporation three times each with dry pyridine and toluene, and finally dissolved in each 90 mL of dry acetonitrile-1, 4-dioxane (1:1, v/v). To the solution of **3**, 1H-tetrazol (756.6 mg, 10.8 mmol) was added. The solution of **5** was added dropwise to the mixture over a period of 5 min and the solution was stirred at ambient temperature for 18 h. I_2 (1.4 g, 5.4 mmol) in water-pyridine-acetonitrile (5.4 mL, 1:9:10, v/v/v) was added to the reaction mixture. After being stirred for 10 min, the reaction was quenched by addition of aq. $\text{Na}_2\text{S}_2\text{O}_3$ (50 mL). The solution was diluted with ethyl acetate (100 mL). The solution was washed with saturated aq. NaHCO_3 (100 mL) twice and brine (100 mL) once, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-ethyl acetate (3:7, v/v, containing 1% pyridine) to give crude material. The crude material was dissolved with acetonitrile and chromatographed on a gel filtration column with acetonitrile to give diastereomixture **6** (1.2 g, 53%). ^1H NMR (CDCl_3 , 500 MHz) δ 0.11-0.13 (6H, m), 0.87-0.90 (9H, m), 1.24-1.27 (2H, m), 1.46-1.60 (4H, m), 2.02-2.04 (3H, m), 2.15-2.20 (5H, m), 2.32-2.60 (6H, m), 2.72-2.80 (3H, m), 3.01-3.11 (1H, m), 3.38-3.48 (2H, m), 3.74-3.80 (7H, m), 3.82-3.90 (1H, m), 3.94-4.04 (2H, m), 4.09-4.18 (1H, m), 4.24 (1H, s, br), 4.43 (1H, s, br), 4.47-4.60 (1H, m), 4.76-4.83 (1H, m), 5.27 (1H, s, br), 6.27-6.29 (0.7H, m), 6.37-6.41 (0.3H, m), 6.44-6.48 (1H, m), 6.77-6.80 (4H, m), 7.19-7.28 (8H, m), 7.35-7.39 (2H, m), 7.49 (0.3H, s), 7.65 (0.7H, s), 8.00-8.12 (2H, m), 8.41-8.42 (1H, m), 9.00 (1H, s, br), 9.38-9.40 (1H, m); ^{13}C NMR (CDCl_3 , 126 MHz) δ -5.19, -5.09, -5.06, -4.95, -4.88, 13.90, 17.42, 17.49, 17.60, 20.70, 21.08, 21.93, 22.97, 23.13, 25.31,

25.37, 25.44, 26.61, 28.01, 29.28, 29.46, 29.52, 29.86, 37.56, 37.63, 40.73, 47.57, 53.31, 53.35, 54.80, 59.98, 62.89, 63.75, 65.50, 68.64 68.91, 71.68, 78.66, 84.67, 86.22, 86.29, 110.24, 110.63, 112.79, 120.48, 126.55, 127.49, 127.73, 129.68, 135.11, 137.57, 142.01, 144.16, 149.69, 150.31, 150.44, 150.60, 153.25, 158. 19, 163.46, 163.63, 171.88, 206.34; ^{31}P NMR (CDCl_3 , 203 MHz) δ -1.94, -0.05; ESI-MS m/z calcd for $\text{C}_{61}\text{H}_{77}\text{N}_8\text{O}_{16}\text{PSi}$ $[\text{M}+\text{Na}]$ 1237.5037, found 1237.4986.

5'-O-(4,

4'-Dimethoxytrityl)-6-N-[N-(trans-4-levulinyloxycyclohexyl)carbamoyl]-deoxyadenosyl(3'-5')2'-deoxyuridine derivative, possessing a cyclic structure with a propylene bridge (fast-eluted product: **7 and slow-eluted product: **8**)**

107 mg (0.09 mmol) of the diastereomixture of **6** was rendered anhydrous by co-evaporation three times each with dry pyridine and toluene, and finally dissolved in 13 mL of dry THF. 106.0 μL (0.65 mmol) of TEA•3HF and TEA (91.0 μL) were added, and the solution was stirred at ambient temperature. After 12 h, dichloromethane (10 mL) was added and washed with saturated aq. NaHCO_3 (10 mL) twice and brine (10 mL) once, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with dichloromethane-methanol (97:3, v/v, containing 1% pyridine) to give **7** (fast, 50.3 mg, 50%), with dichloromethane-methanol (96:4, v/v, containing 1% pyridine) to give **8** (slow, 20.9 mg, 21%).

7 (fast): ^1H NMR (CDCl_3 , 500 MHz) δ 1.45-1.61 (2H, m), 1.80 (1H, s), 1.91 (1H, s), 2.01-2.14 (2H, m), 2.15-2.21 (5H, m), 2.42-2.44 (2H, m), 2.49-2.59 (4H, m), 2.76 (3H, t, $J = 6.3$ Hz), 3.06-3.11 (1H, m), 3.40 (1H, dd, $J = 4.0, 10.3$ Hz), 3.46 (1H, dd, $J = 4.3, 10.3$ Hz), 3.65 (1H, s), 3.77 (6H, s), 3.84-3.92 (2H, m), 4.04 (1H, s), 4.10 (1H, s), 4.24-2.9 (1H, m), 4.31-4.35 (1H, m), 4.39 (1H, s), 4.56 (1H, dd, $J = 7.8, 16.1$ Hz), 4.79 (1H, m), 5.27 (1H, s), 6.18 (1H, d, $J = 6.6$ Hz), 6.46 (1H, t, $J = 6.6$ Hz), 6.79 (4H, d, $J = 8.3$ Hz), 7.18-7.27 (7H, m), 7.36 (2H, d, $J = 7.6$ Hz), 7.74 (1H, s), 8.02 (1H, s), 8.10 (1H, s), 8.43 (1H, s), 8.95 (1H, s, br), 9.37 (1H, d, $J = 7.3$ Hz); ^{13}C NMR (CDCl_3 , 126 MHz) δ 21.68, 26.90, 28.45, 29.82, 30.04, 30.43, 38.12, 38.50, 40.02, 48.19, 55.38, 63.09, 64.14, 66.36, 67.29, 70.69, 72.18, 79.06, 84.48, 84.57, 84.96, 85.01, 85.21, 86.93, 110.18, 113.34, 120.92, 127.18, 128.04, 128.16, 130.16, 130.18, 135.41, 135.46, 138.11, 141.14, 144. 40, 150.03, 150.41, 150.54, 151.28, 153.25, 158.74, 163.61, 172.41,

206.88; ^{31}P NMR (CDCl_3 , 203 MHz) δ -0.96; ESI-MS m/z calcd for $\text{C}_{55}\text{H}_{63}\text{N}_8\text{O}_{16}\text{P}$ $[\text{M}+\text{H}]$ 1123.4172, found 1123.4191.

8 (slow): ^1H NMR (CDCl_3 , 500 MHz) δ 1.44-1.85 (6H, m), 2.01-2.04 (2H, m), 2.14-2.21 (5H, m), 2.34-2.39 (1H, m), 2.47-2.66 (5H, m), 2.74-2.82 (3H, m), 2.97-3.01 (1H, m), 3.40-3.44 (3H, m), 3.65 (1H, s), 3.78 (6H, s), 3.81-3.85 (1H, m), 3.98-4.05 (1H, m), 4.26 (1H, d, J = 11.2 Hz), 4.40 (1H, s), 4.62 (1H, d, J = 12.0 Hz), 4.64-4.70 (1H, m), 4.76-4.81 (1H, m), 5.28 (1H, s), 6.38 (1H, d, J = 6.3 Hz) 6.46 (1H, t, J = 7.1 Hz), 6.80 (4H, d, J = 8.3 Hz), 7.20-7.28 (7H, m), 7.37 (2H, d, J = 7.3 Hz), 7.55 (1H, s), 8.07 (1H, s), 8.40 (1H, s), 8.59 (1H, s, br), 9.35 (1H, d, J = 7.57 Hz); ^{13}C NMR (CDCl_3 , 126 MHz) δ 14.26, 21.29, 22.82, 25.06, 26.78, 26.86, 28.45, 29.33, 29.49, 29.62, 29.78, 29.82, 30.04, 30.39, 32.04, 34.13, 38.12, 38.82, 40.77, 48.24, 55.38, 63.33, 65.53, 65.68, 68.85, 70.63, 72.13, 79.13, 84.50, 84.54, 84.60, 84.67, 85.12, 86.99, 111.02, 113.36, 120.83, 127.23, 128.08, 128.13, 130.15, 135.35, 135.40, 138.33, 141.11, 144.37, 149.90, 150.45, 150.53, 151.24, 153.28, 158.79, 163.29, 172.43, 206.95; ^{31}P NMR (CDCl_3 , 203 MHz) δ -0.77; ESI-MS m/z calcd for $\text{C}_{55}\text{H}_{63}\text{N}_8\text{O}_{16}\text{P}$ $[\text{M}+\text{H}]$ 1123.4172, found 1123.4217.

Phosphoramidite of fast-isomer (**9**)

120 mg (0.10 mmol) of **7** (the fast derivative) was rendered anhydrous by repeated co-evaporation three times each with dry pyridine, dry toluene and finally dissolved in dry dichloromethane (1.0 mL). To this solution, diisopropylethylamine (26.1 μL , 0.15 mmol) and chloro-2-cyanoethoxy-diisopylaminophosphine (24.5 μL , 0.11 mmol) were added, and the solution was stirred at ambient temperature for 6 h. The reaction was quenched with addition of water (1 mL), and the solution was diluted with dichloromethane (10 mL). The solution was washed with saturated aq. NaHCO_3 (10 mL) twice and brine (10 mL) once, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with dichloromethane-methanol (98:2, v/v, containing 1% pyridine) to give **9** (98.5 mg, 79%). ^1H NMR (CDCl_3 , 500 MHz) δ 1.17-1.20 (12H, m), 1.23-1.27 (2H, m), 1.47-1.58 (4H, m), 1.83-2.00 (2H, m), 2.01-2.04 (2H, m), 2.15-2.18 (2H, m), 2.21 (3H, s), 2.43-2.78 (8H, m), 2.92-3.09 (1H, m), 3.42-3.46 (2H, m), 3.57-3.60 (4H, m), 3.78 (6H, s), 3.81-3.97 (2H, m), 4.02-4.17 (3H, m), 4.20-4.32 (1H, m), 4.40-4.49 (1H, m), 4.72-4.83 (2H, m), 5.20-5.30 (1H, m), 6.32-6.40 (1H, m), 6.43-6.50 (1H, m), 6.75-6.82

(4H, m), 7.13-7.31 (7H, m), 7.34-7.40 (2H, m), 7.48-7.52 (1H, m), 8.00 (1H, s, br), 8.08 (1H, d, $J = 2.7$ Hz), 8.44 (1H, d, $J = 8.1$ Hz), 8.97 (1H, s, br), 8.38 (1H, d, $J = 7.6$ Hz); ^{13}C NMR (CDCl_3 , 126 MHz) δ 19.22, 20.38, 20.53, 20.59, 21.87, 22.32, 22.77, 24.65, 26.84, 28.37, 29.44, 29.76, 30.03, 30.36, 31.99, 38.05, 38.72, 39.49, 43.25, 43.31, 43.41, 47.45, 48.13, 55.25, 55.32, 57.65, 63.33, 64.39, 66.36, 70.59, 70.93, 72.08, 72.17, 79.13, 84.08, 84.59, 84.76, 85.08, 86.83, 111.25, 111.34, 113.18, 117.77, 118.02, 118.47, 120.80, 127.06, 127.97, 128.11, 130.10, 135.34, 137.82, 138.19, 141.19, 144.35, 144.40, 149.92, 150.44, 150.64, 150.80, 151.12, 153.23, 158.63, 163.46, 163.58, 172.34, 206.88; ^{31}P NMR (CDCl_3 , 203 MHz) δ -2.28, -2.20, 150.20, 150.28; ESI-MS m/z calcd for $\text{C}_{64}\text{H}_{80}\text{N}_{10}\text{O}_{17}\text{P}_2$ $[\text{M}+\text{H}]$ 1323.5256, found 1323.5242.

Phosoramidite of slow-isomer (10)

120 mg (0.10 mmol) of **8** (the slow derivative) was rendered anhydrous by repeated co-evaporation three times each with dry pyridine, dry toluene and finally dissolved in dry dichloromethane (1.0 mL). To this solution, diisopropylethylamine (26.1 μL , 0.15 mmol) and chloro-2-cyanoethoxy-diisopropylaminophosphine (24.5 μL , 0.11 mmol) were added, and the solution was stirred at ambient temperature for 6 h. The reaction was quenched with addition of water (1 mL), and the solution was diluted with dichloromethane (10 mL). The solution was washed with saturated aq. NaHCO_3 (10 mL) twice and brine (10 mL) once, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with dichloromethane-methanol (98:2, v/v, containing 1% pyridine) to give **10** (82.8 mg, 66%). ^1H NMR (CDCl_3 , 500 MHz) δ 1.15-1.22 (12H, m), 1.23-1.28 (2H, m), 1.44-1.62 (4H, m), 1.68-1.84 (2H, m), 1.97-2.09 (2H, m), 2.14-2.20 (2H, m), 2.21 (6H, s), 2.11-2.81 (9H, m), 3.00-3.12 (1H, m), 3.36-3.49 (2H, m), 3.56-3.68 (2H, m), 3.69-3.77 (2H, m), 3.78 (6H, s), 3.82-3.99 (3H, m), 4.00-4.08 (1H, m), 4.09-4.37 (2H, m), 4.42-4.49 (1H, m), 4.60-4.69 (1H, m), 4.75-4.85 (2H, m), 5.26 (1H, s), 6.42-6.52 (2H, m), 6.75-6.83 (4H, m), 7.18-7.32 (7H, m), 7.36-7.40 (2H, m), 7.46-7.50 (1H, m), 8.04 (1H, s, br), 8.08 (1H, s), 8.40 (1H, d, $J = 3.4$ Hz), 8.87 (1H, s, br), 9.38 (1H, d, $J = 7.6$ Hz); ^{13}C NMR (CDCl_3 , 126 MHz) δ 20.53, 20.58, 21.07, 24.64, 24.68, 26.66, 26.74, 28.35, 29.73, 29.99, 30.34, 38.02, 39.52, 39.70, 43.25, 43.35, 48.04, 55.27, 57.82, 57.90, 57.97, 58.06, 63.18, 65.36, 65.96, 70.59, 71.07, 71.13, 71.19, 71.25, 72.10, 76.91, 77.16, 77.41, 78.66, 78.70, 78.82, 78.86, 83.96, 84.06, 84.19, 84.54, 84.62, 85.03, 85.08,

86.74, 111.21, 111.36, 113.19, 117.80, 117.86, 120.76, 120.80, 127.06, 127.94, 128.06, 130.05, 135.38, 138.31, 138.42, 141.27, 141.39, 144.37, 150.06, 150.46, 150.64, 150.70, 151.06, 153.28, 158.61, 163.44, 163.49, 172.34, 206.88; ^{31}P NMR (CDCl_3 , 203 MHz) δ -0.01, 0.53, 149.98, 150.42; ESI-MS m/z calcd for $\text{C}_{64}\text{H}_{80}\text{N}_{10}\text{O}_{17}\text{P}_2$ $[\text{M}+\text{Na}]$ 1345.5075, found 1345.5047.

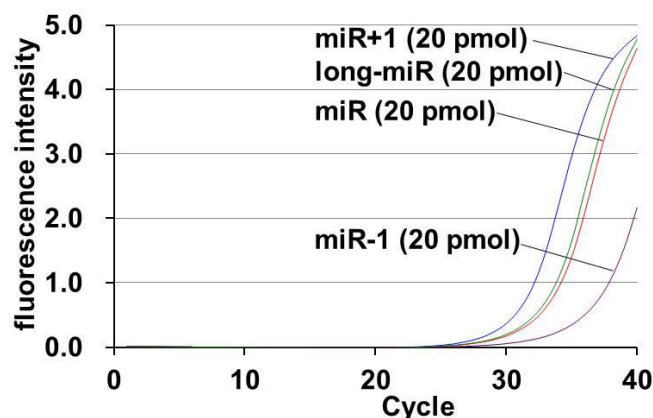


Fig. S1 PCR following reverse transcription of miRNAs using **primer-2** and primer extension.

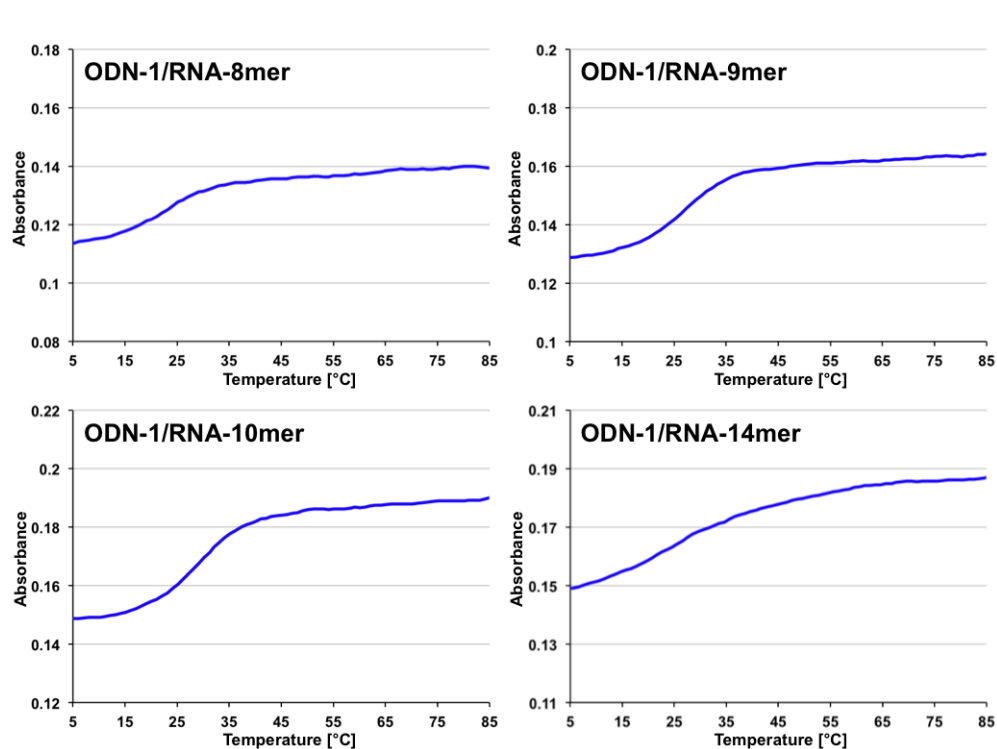


Fig. S2 Thermal melting of the duplex of **ODN-1** with RNAs.

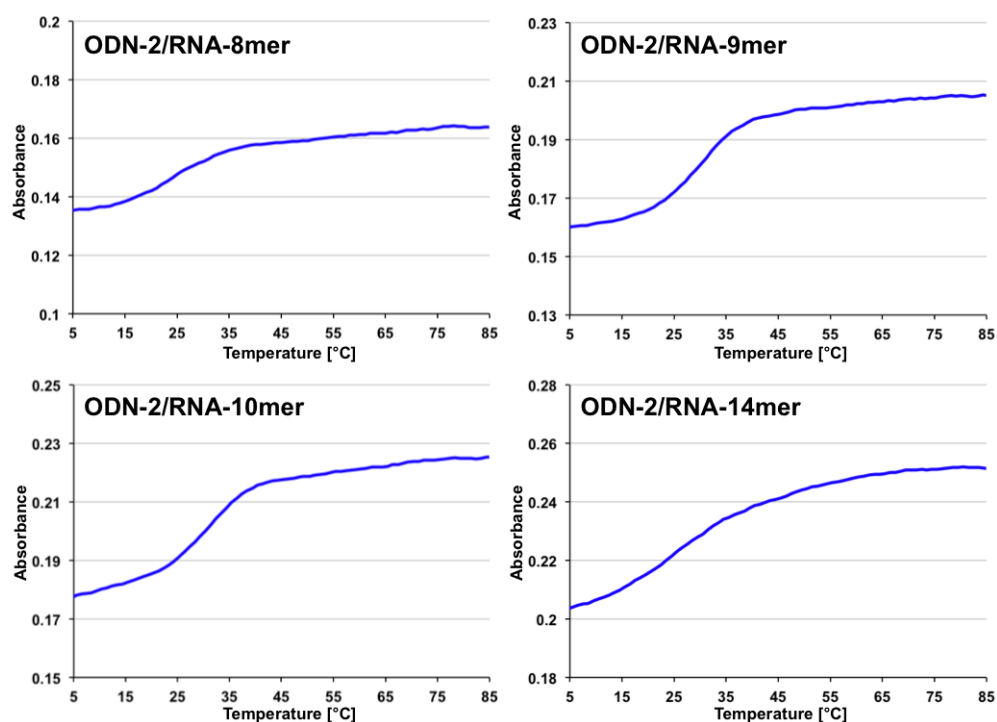


Fig. S3 Thermal melting of the duplex of **ODN-2** with RNAs.

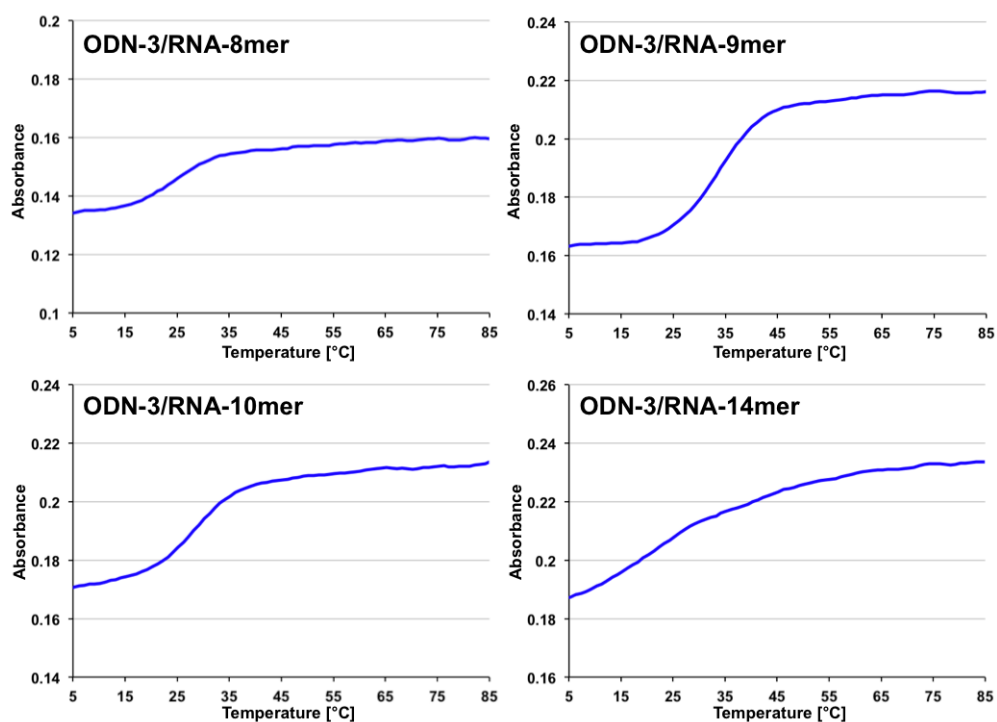


Fig. S4 Thermal melting of the duplex of **ODN-3** with RNAs.

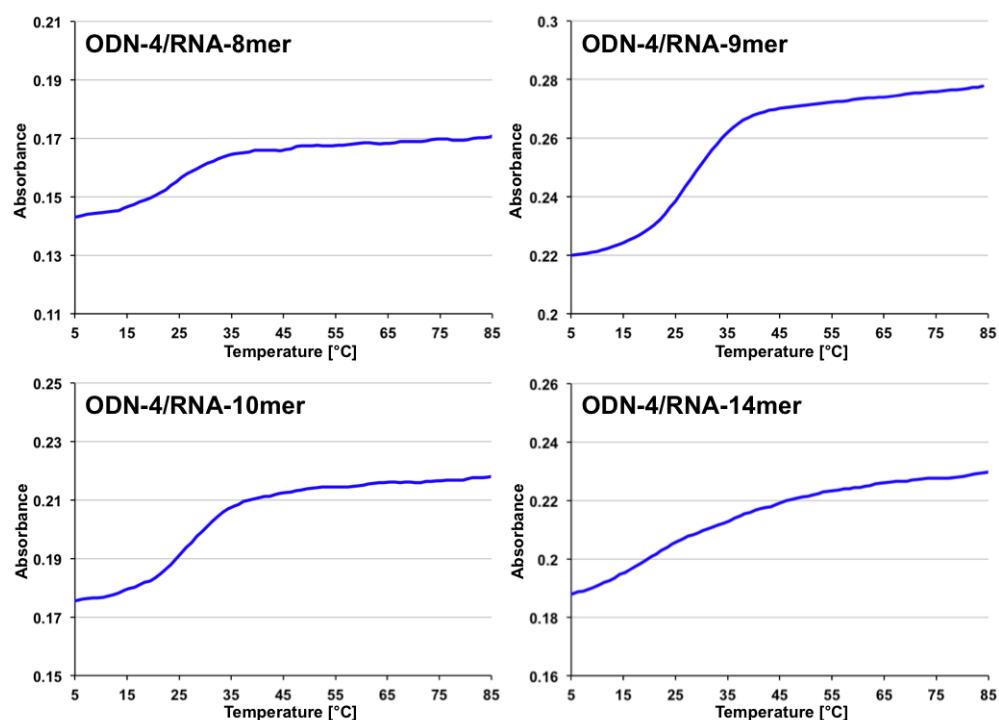


Fig. S5 Thermal melting of the duplex of **ODN-4** with RNAs.

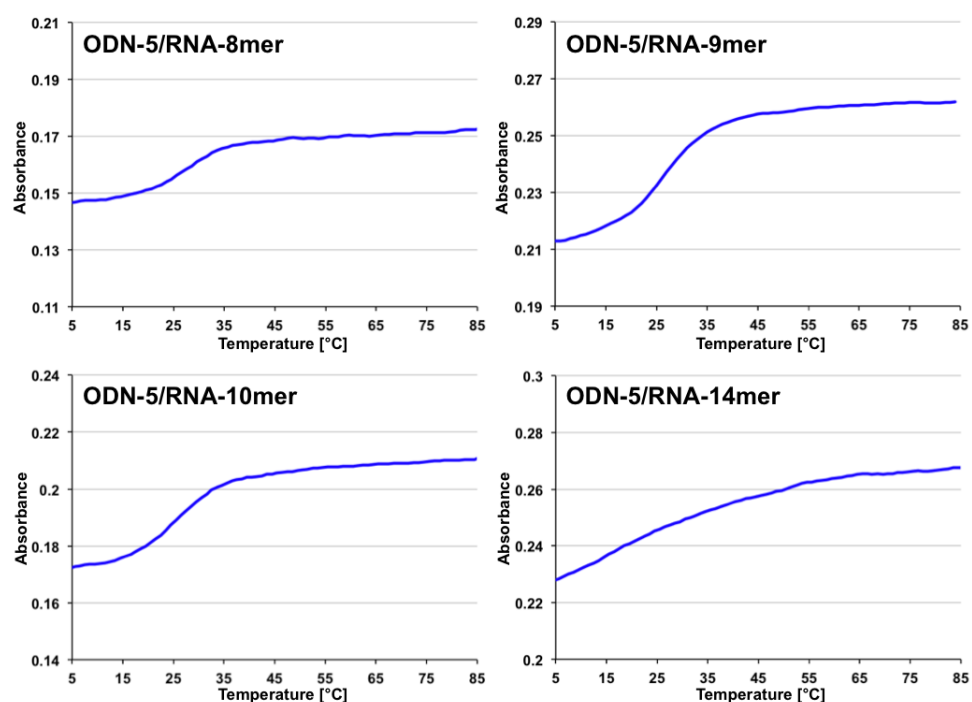
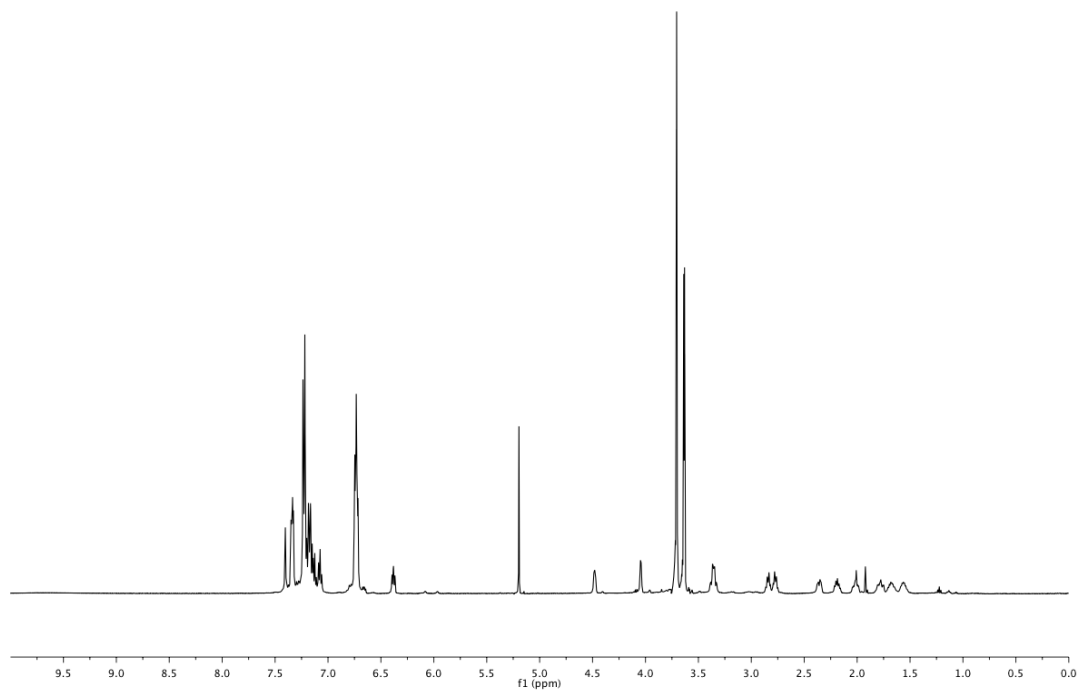
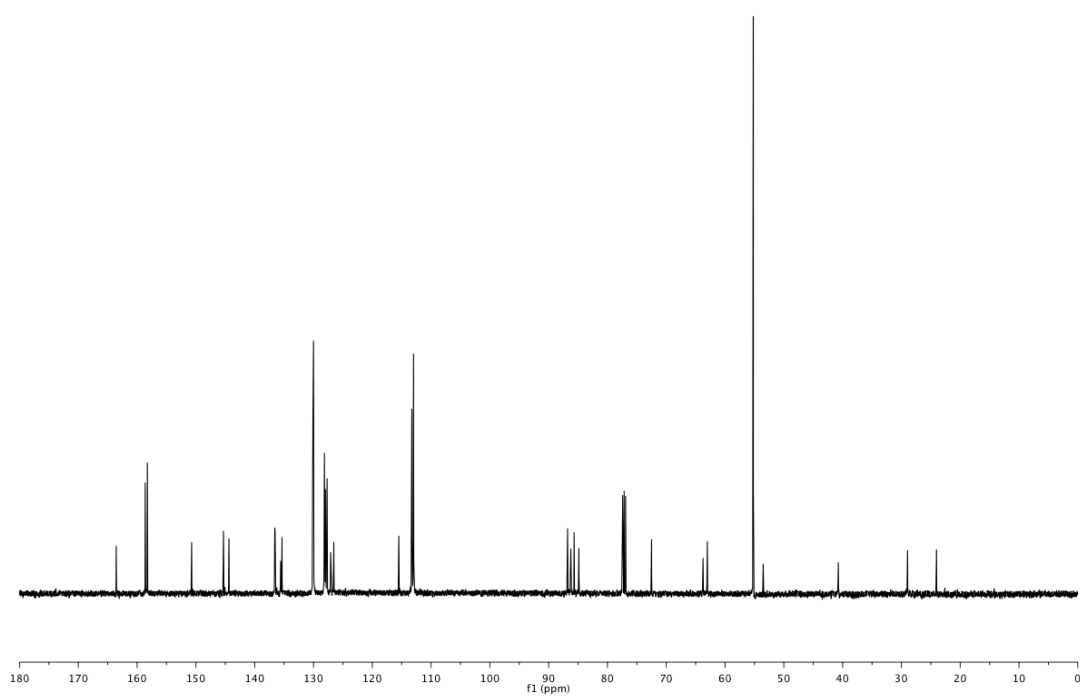


Fig. S6 Thermal melting of the duplex of **ODN-5** with RNAs.

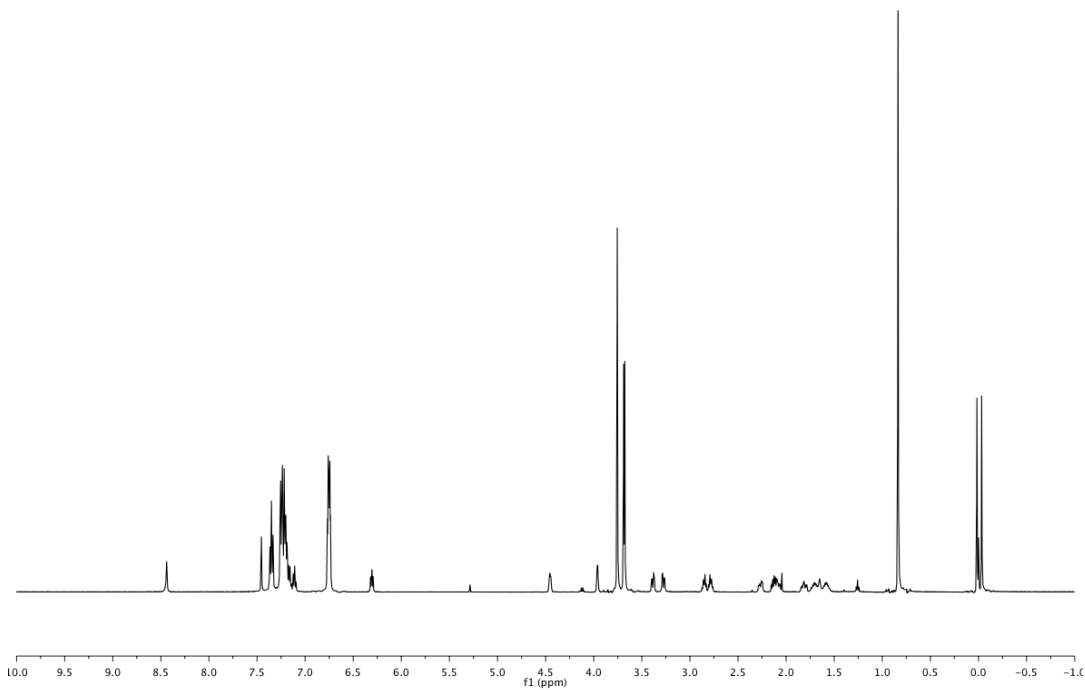
^1H NMR of Compound **1**



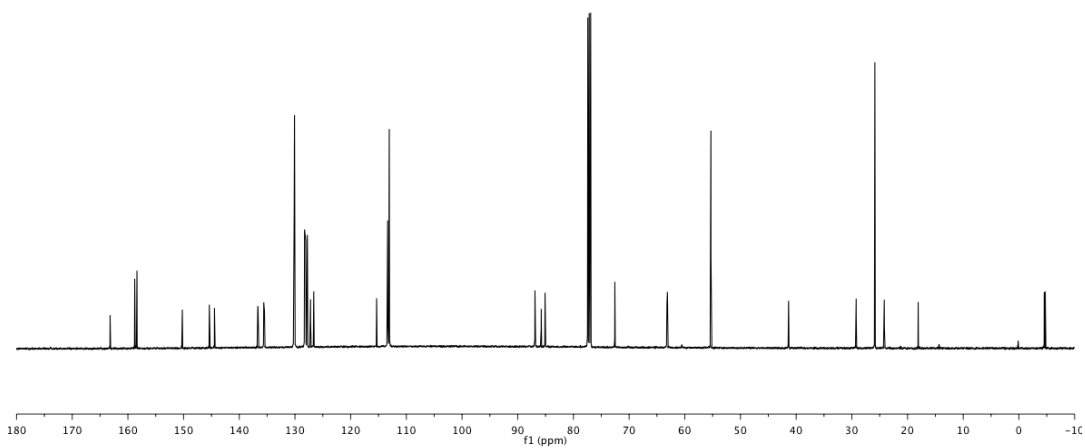
^{13}C NMR of Compound **1**



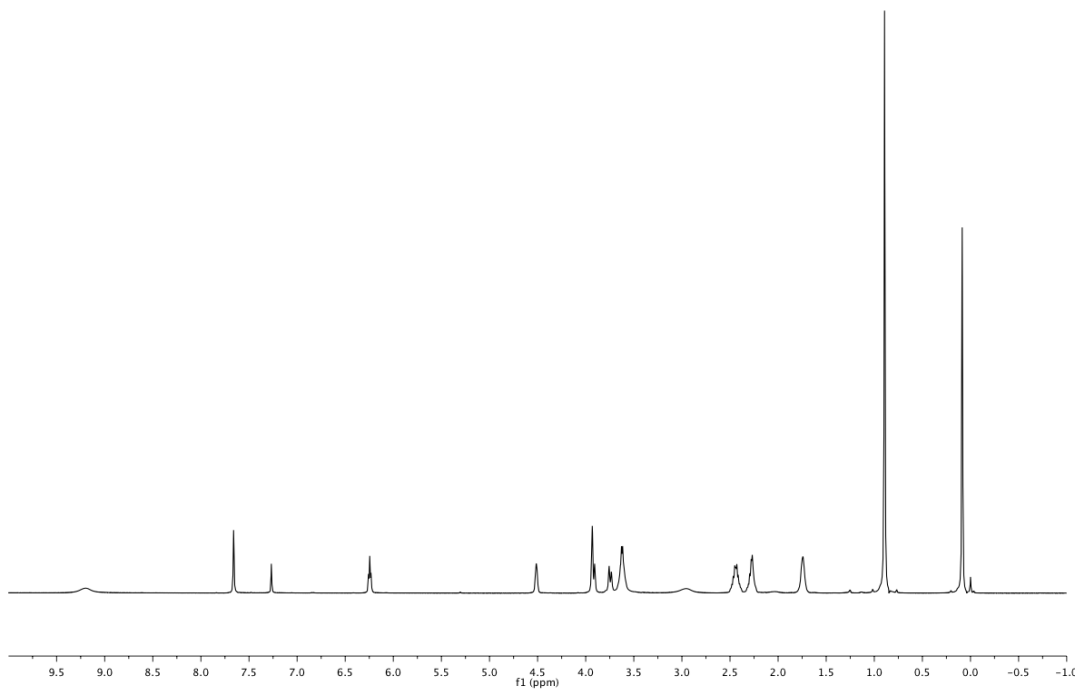
¹H NMR of Compound **2**



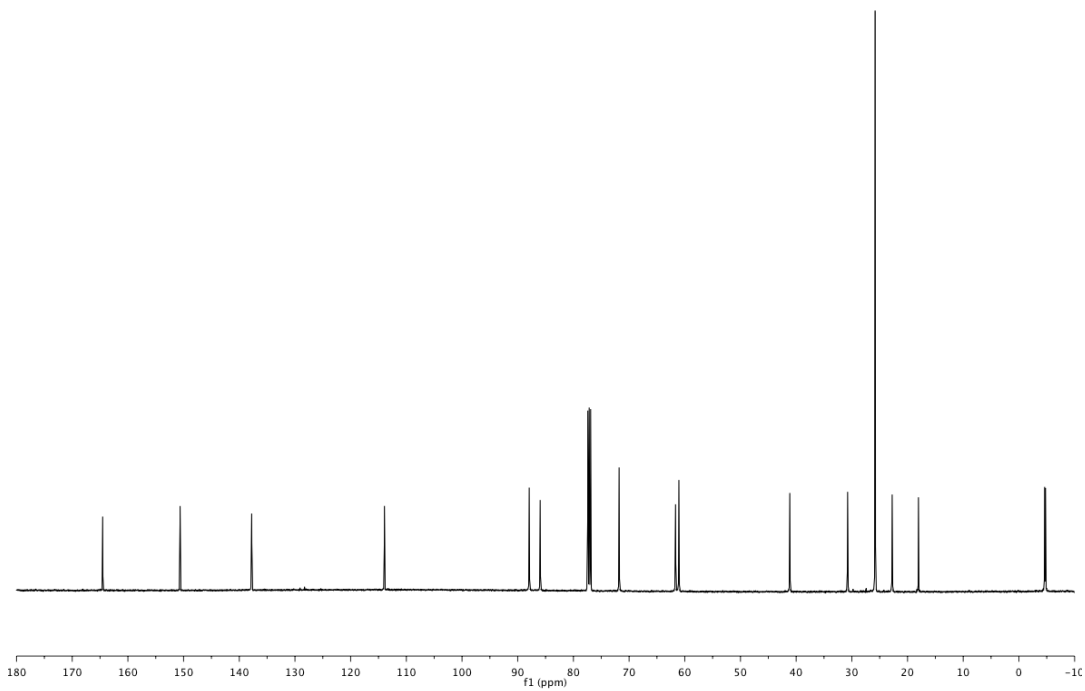
¹³C NMR of Compound **2**



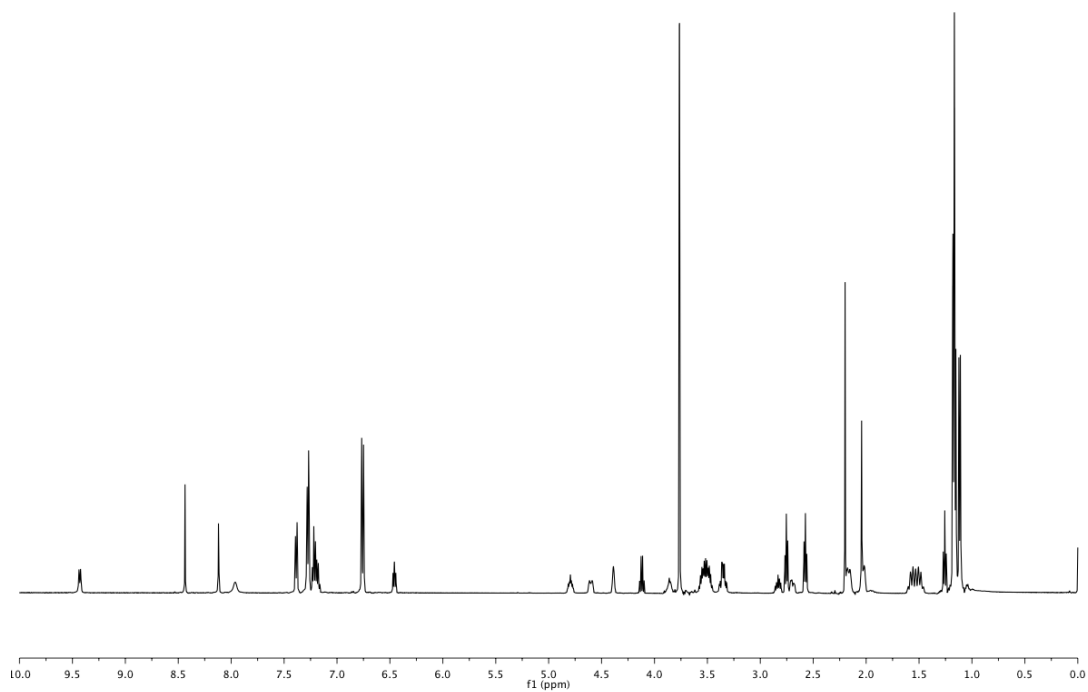
¹H NMR of Compound **3**



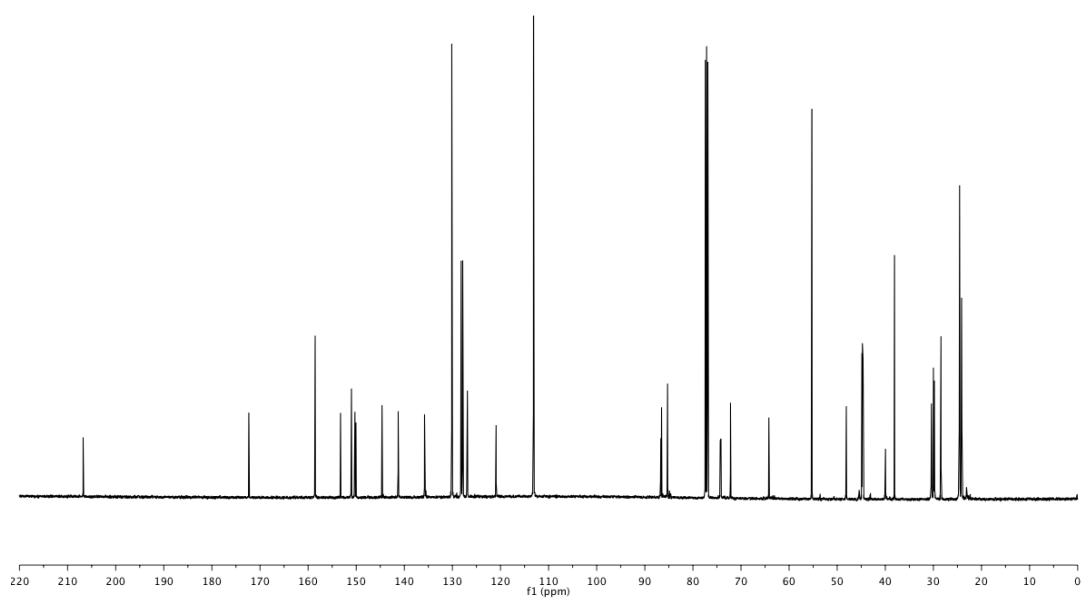
¹³C NMR of Compound **3**



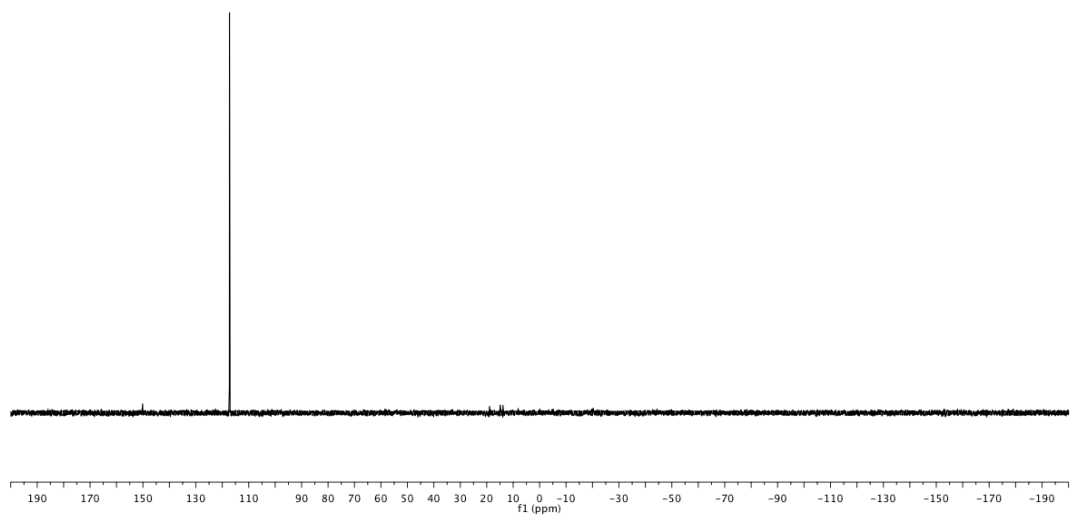
^1H NMR of Compound **5**



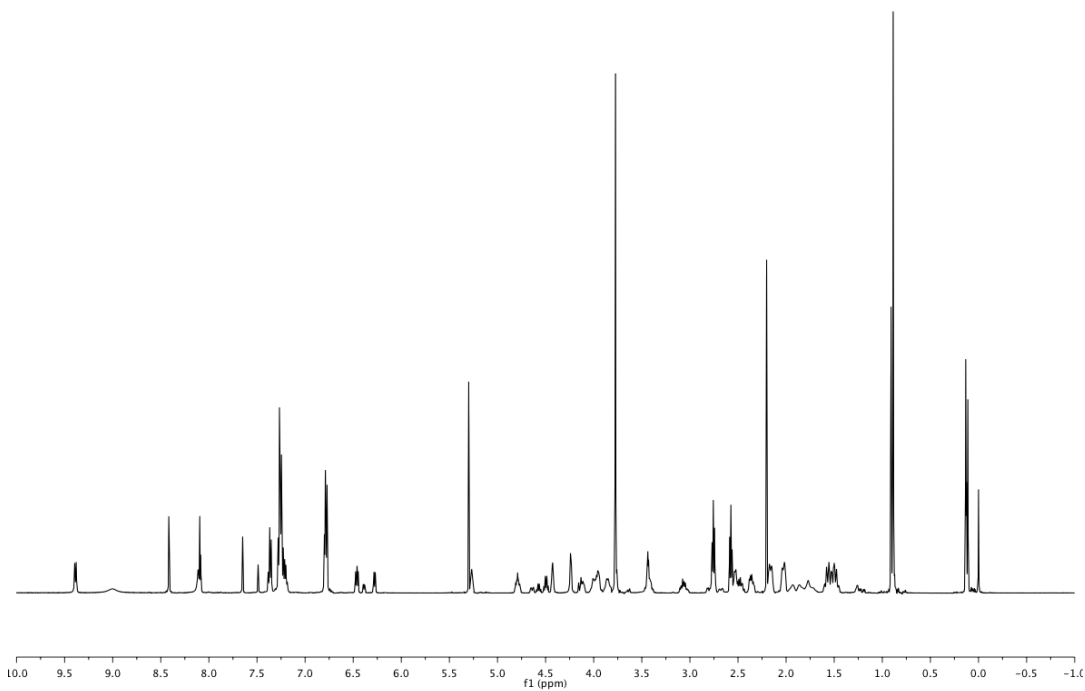
^{13}C NMR of Compound **5**



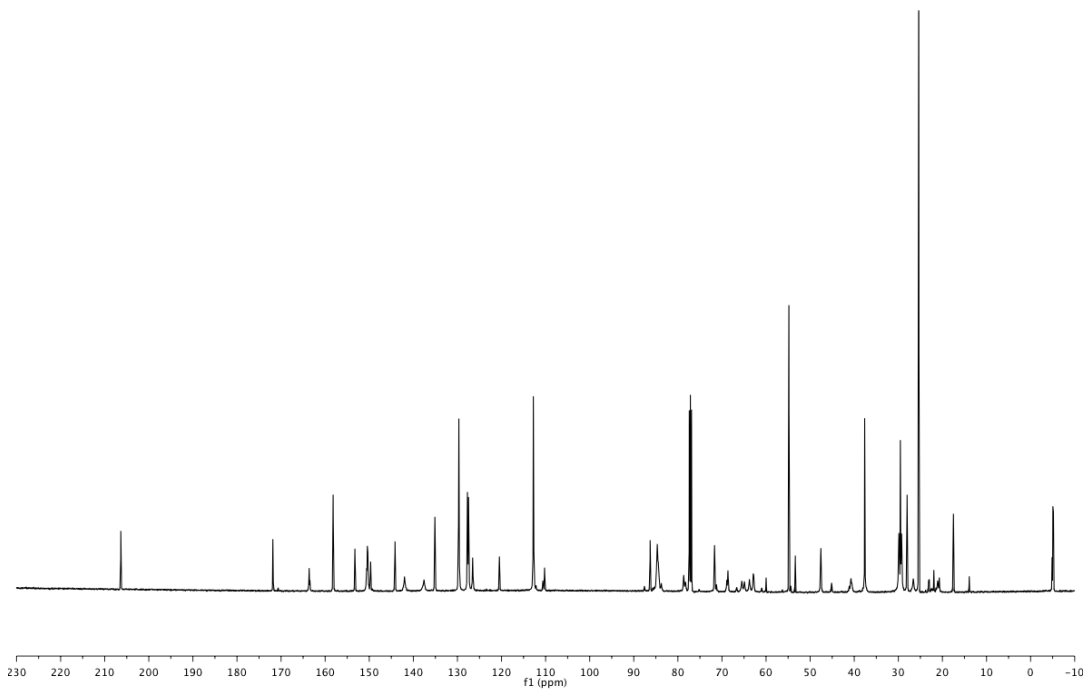
³¹P NMR of Compound **5**



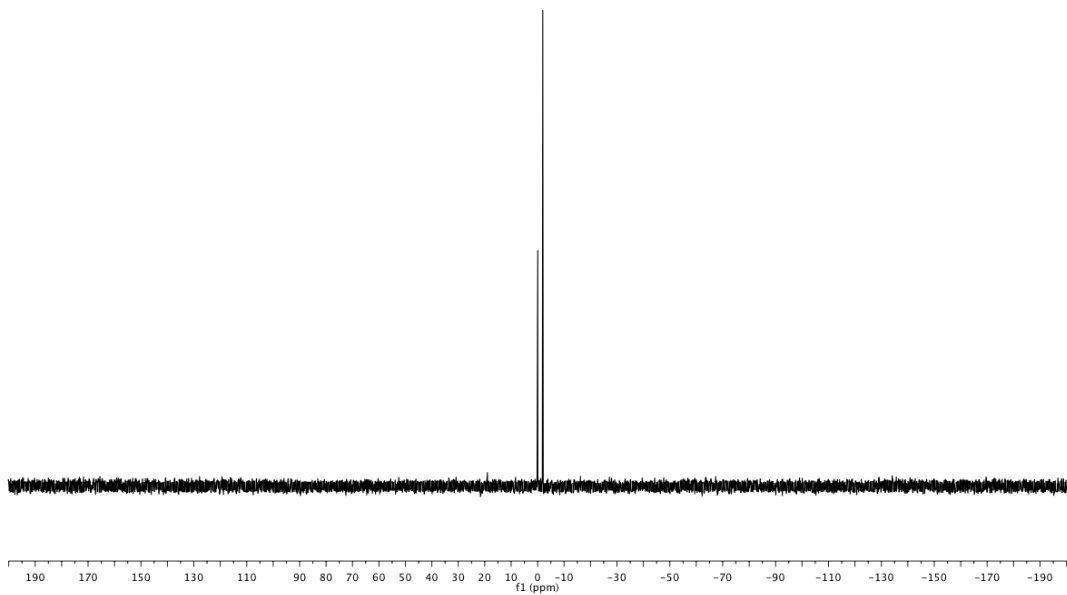
¹H NMR of Compound **6**



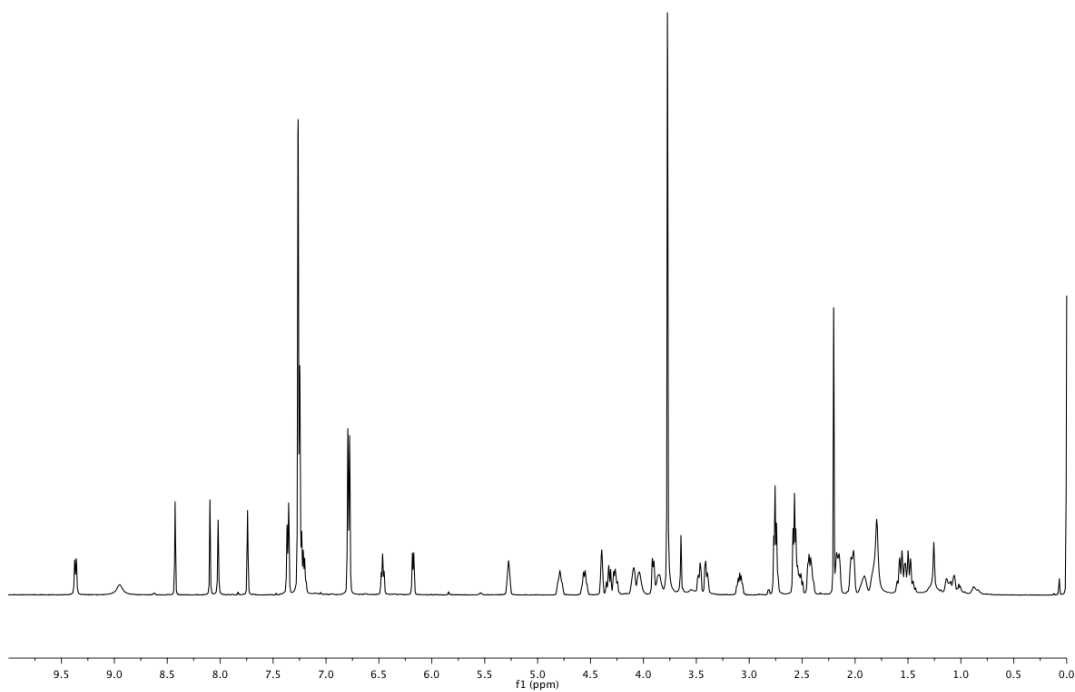
¹³C NMR of Compound **6**



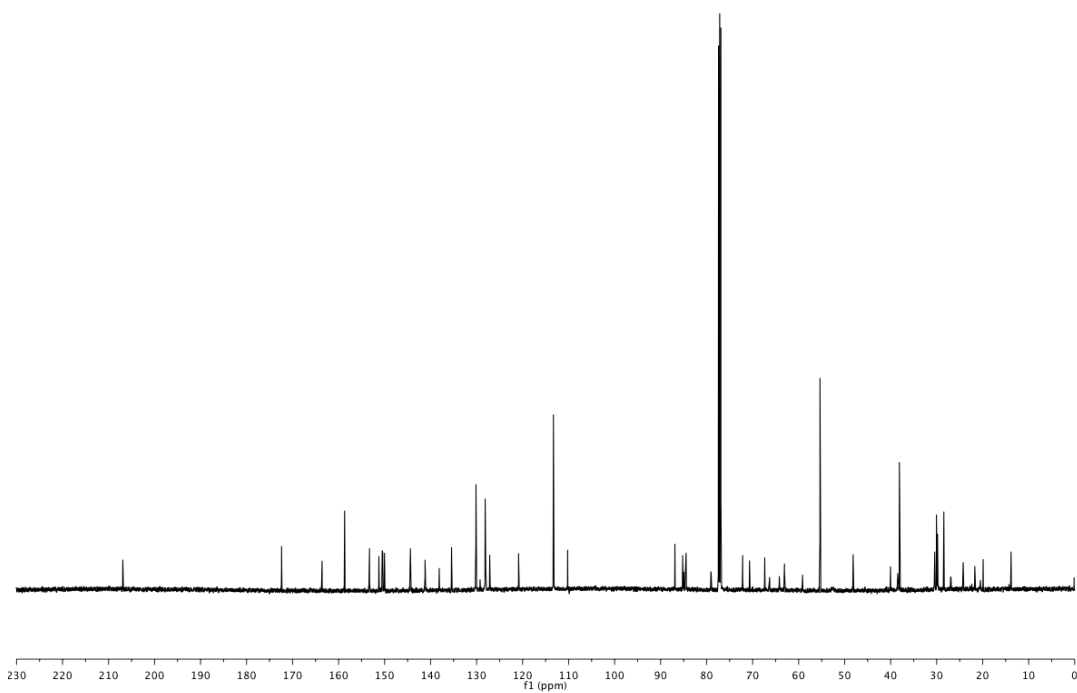
³¹P NMR of Compound **6**



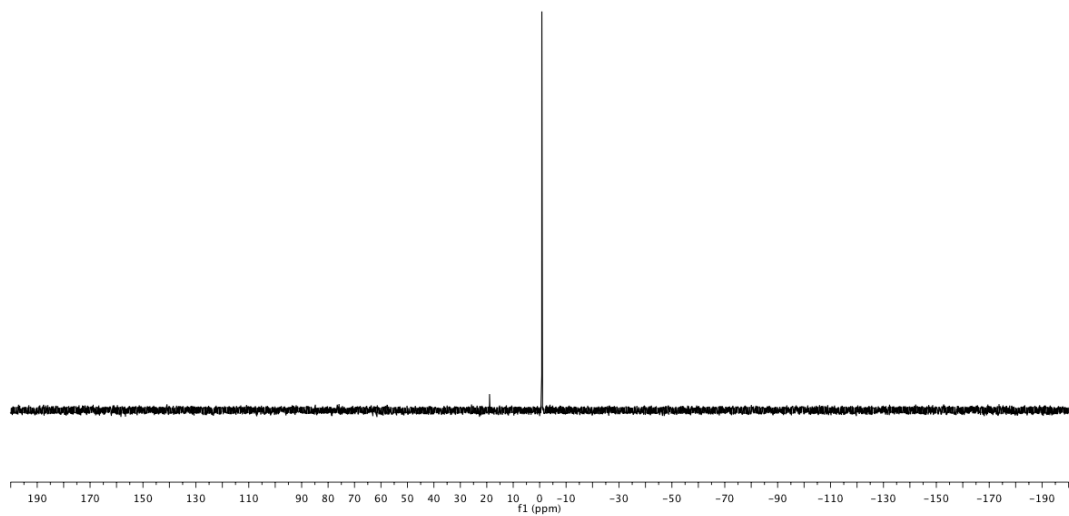
¹H NMR of Compound 7



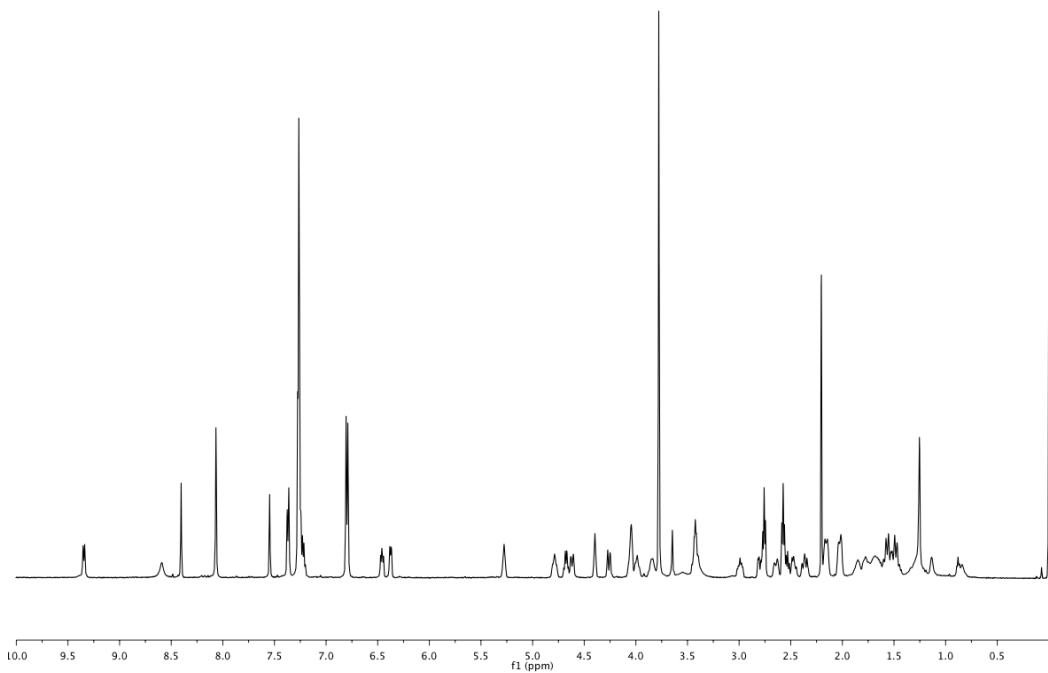
¹³C NMR of Compound 7



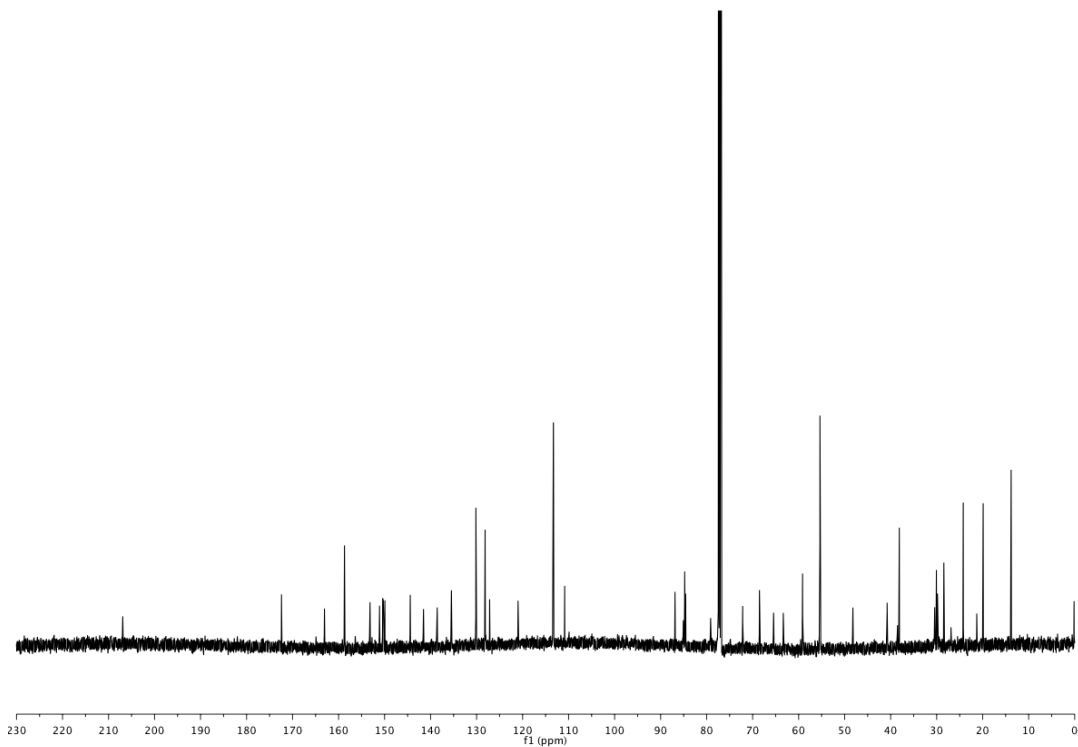
³¹P NMR of Compound **7**



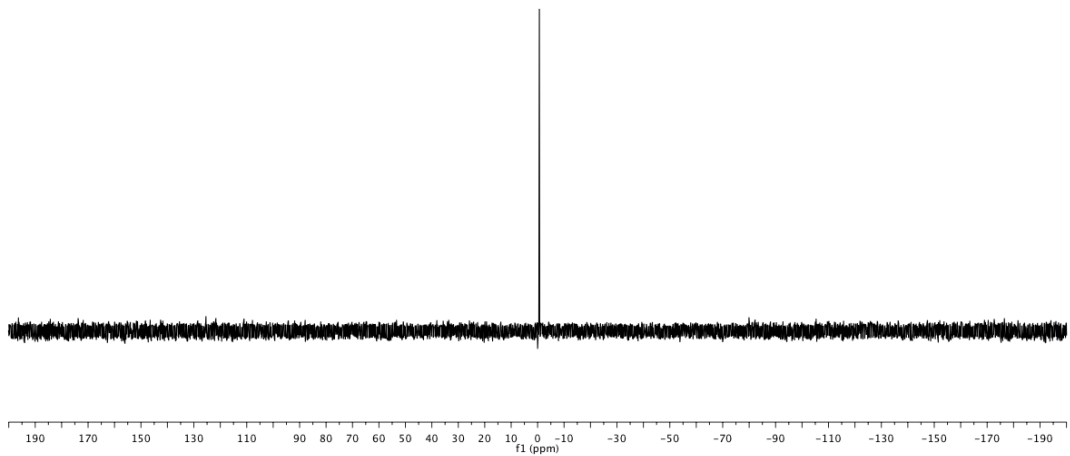
¹H NMR of Compound **8**



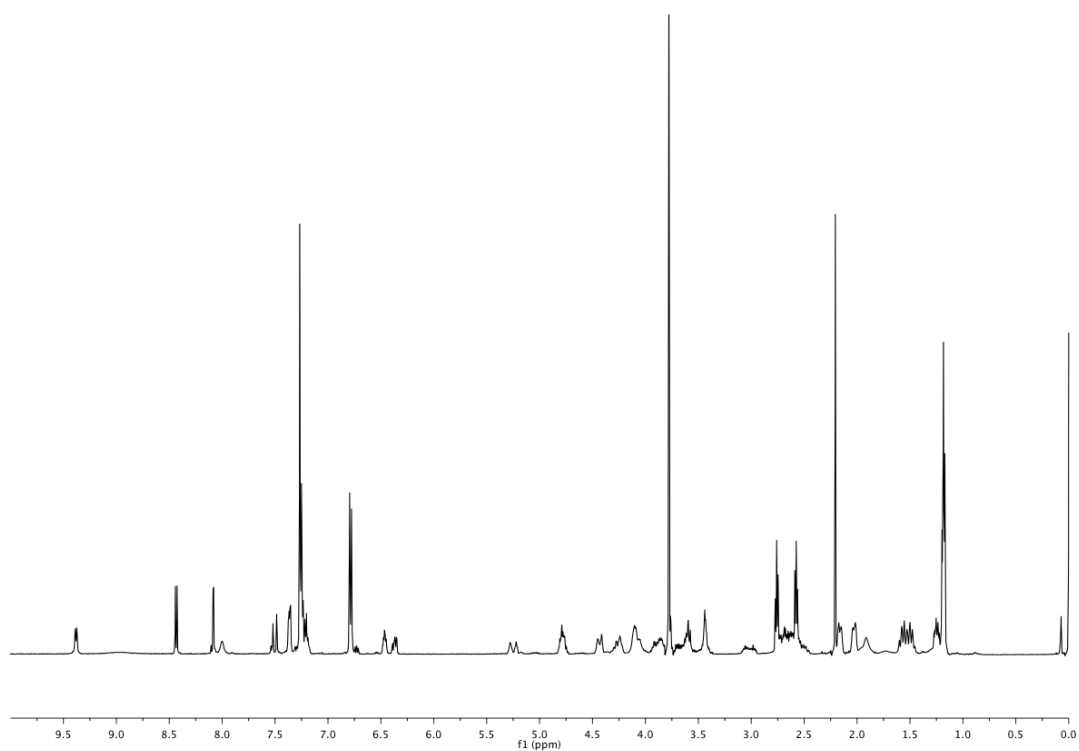
¹³C NMR of Compound **8**



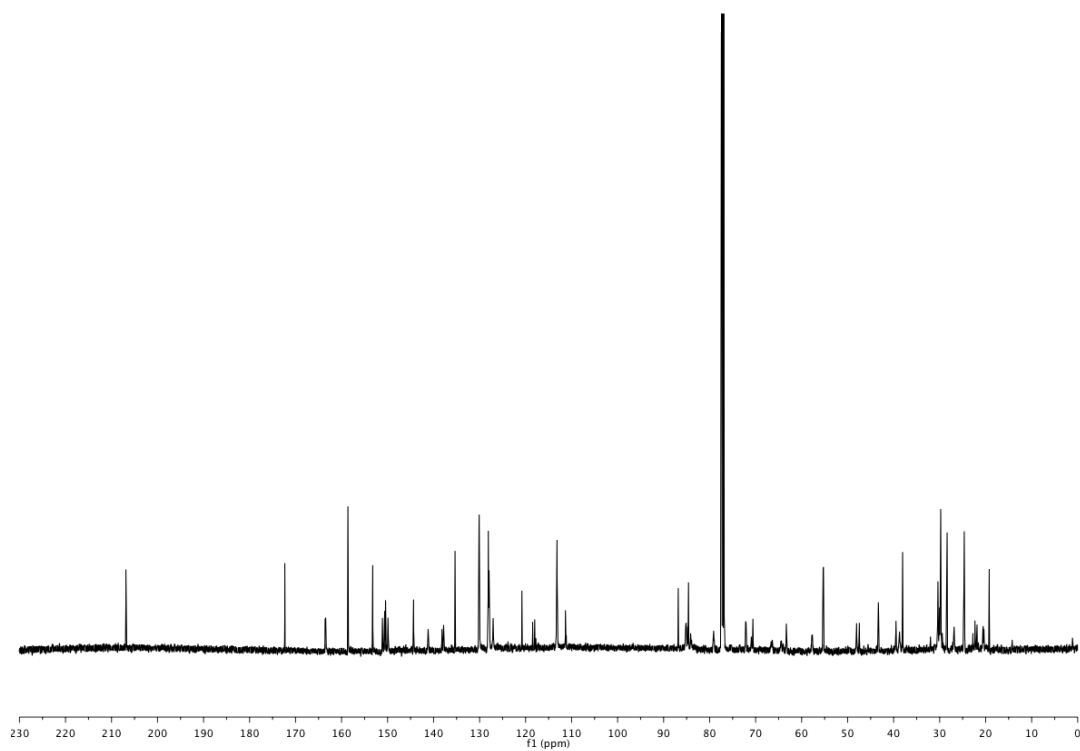
³¹P NMR of Compound **8**



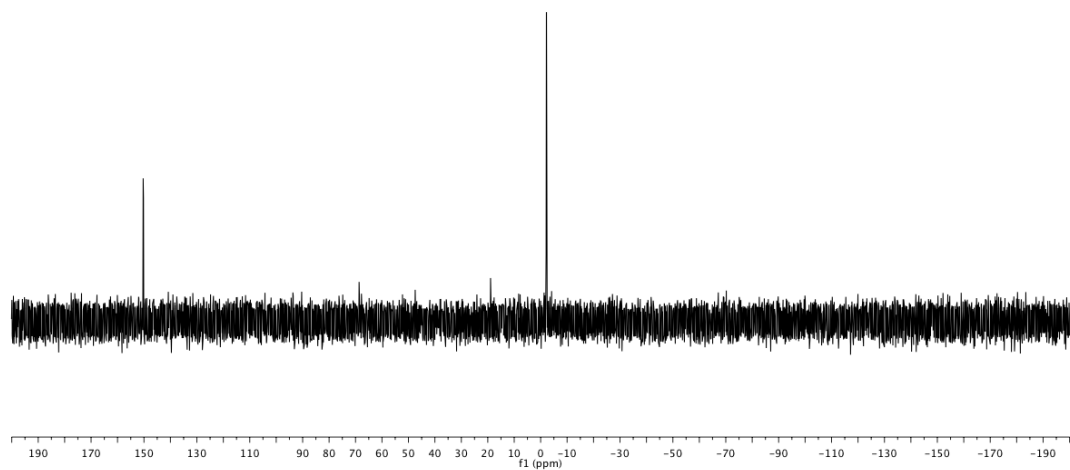
^1H NMR of Compound **9**



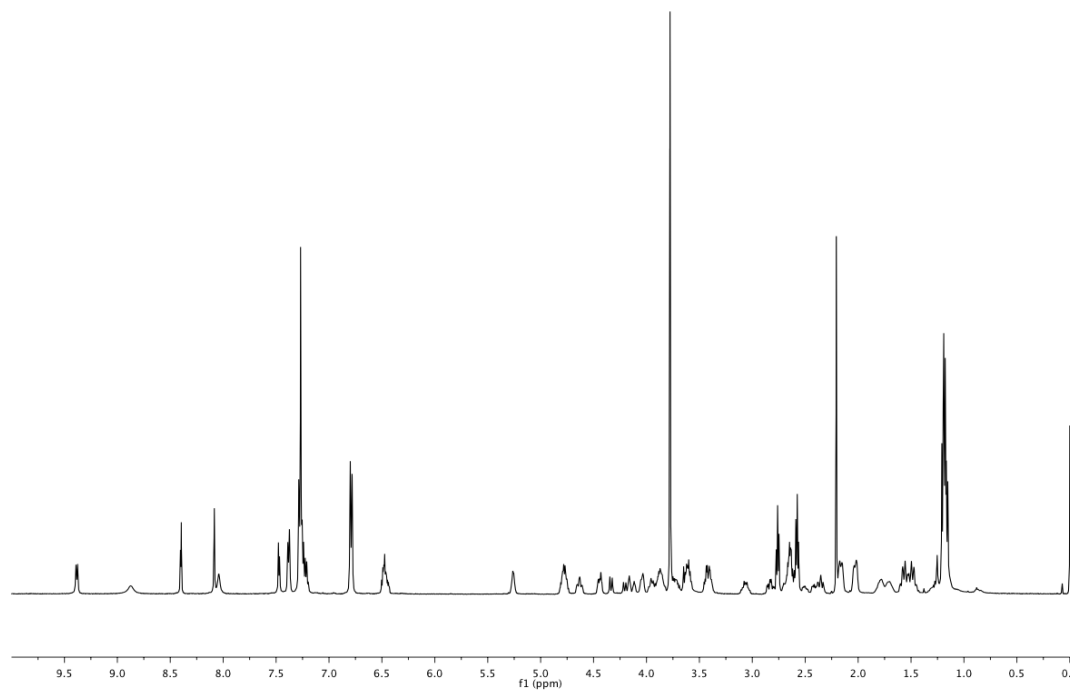
^{13}C NMR of Compound **9**



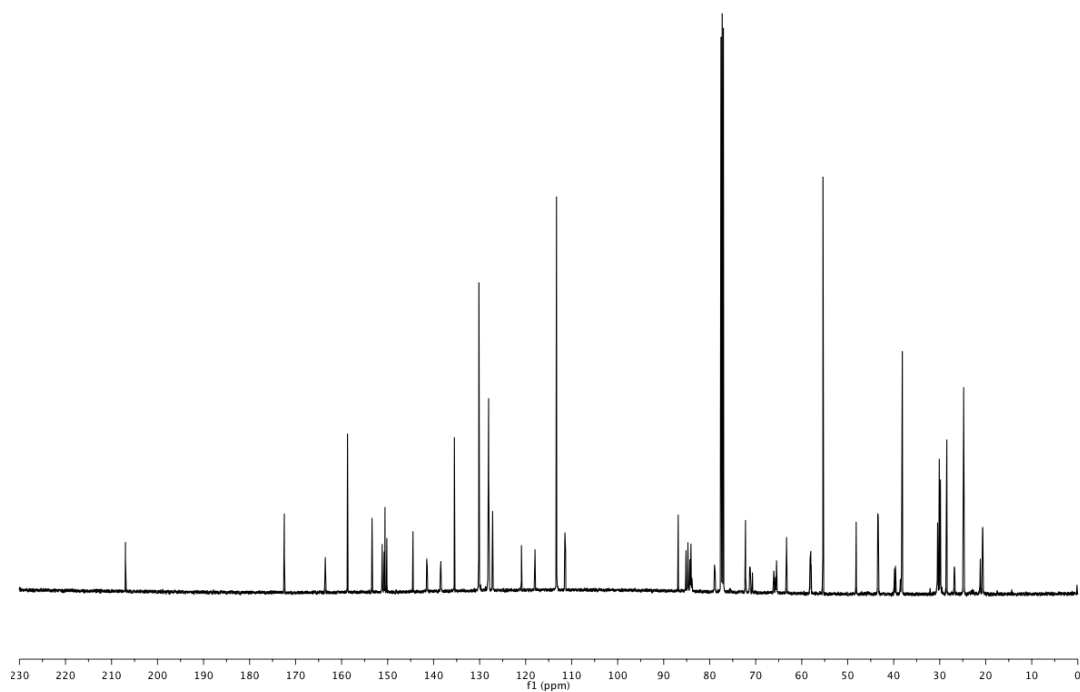
³¹P NMR of Compound **9**



¹H NMR of Compound **10**



^{13}C NMR of Compound **10**



^{31}P NMR of Compound **10**

