

Electronic Supplementary Information

Synthesis and properties of novel 2'-C,4'-C-ethyleneoxy-bridged 2'-deoxyribonucleic acids with exocyclic methylene groups

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1. General

All moisture-sensitive reactions were conducted in well-dried glassware under an N₂ atmosphere. Anhydrous CH₂Cl₂, DMF, THF, Et₂O, toluene and pyridine were used as purchased. ¹H NMR spectra were recorded at 300, 400 and 500 MHz, ¹³C NMR spectra were recorded at 75, 100 and 125 MHz, and ³¹P spectra were recorded at 160 and 200 MHz. Chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as an internal standard, and residual solvents for ¹H NMR, and CHCl₃ (δ = 77.00 ppm) for ¹³C NMR, and 5% H₃PO₄ (δ = 0 ppm) for ³¹P NMR. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Optical rotations were recorded on a JASCO P-2200 instrument. MALDI-TOF mass spectra were recorded on Bruker Daltonics Autoflex II or JEOL JMS-S3000 mass spectrometers. For column chromatography, silica gel PSQ 100B was used. The progress of the reaction was monitored by analytical thin-layer chromatography (TLC) on pre-coated aluminum sheets. For high performance liquid chromatography (HPLC), JASCO PU-4180, CO-4060 and UV-4075 were used.

2. Synthesis of methylene-EoDNA phosphoramidite 10a

1-[2-*O*-*tert*-Butyldimethylsilyl-4,5-dehydro-5-deoxy-β-D-ribofuranosyl]thymine (2) and 1-[3-*O*-*tert*-butyldimethylsilyl-4,5-dehydro-5-deoxy-β-D-ribofuranosyl]thymine (3)

Under N₂ atmosphere, DABCO (21.7 g, 194 mmol), TBSCl (7.00, 46.5 mmol), AgNO₃ (7.90 g 46.5 mmol) were added to a solution of compound **1**¹ (9.30 g, 38.7 mmol) in anhydrous DMF (100 mL) at 0 °C. The reaction mixture was stirred at room temperature for 12 h. The resulting mixture was filtered through Celite® and the filtrate was diluted with Et₂O. This solution was washed with water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The obtained crude residue (14.8 g) was purified by column chromatography (silica gel 300 g, *n*-hexane:EtOAc = 3:1 to 3:2) to give **2** (5.35 g, 39%), compound **3** (5.90g, 43%) and **4**² (1.08 g, 6%) as a white foam, respectively.

Compound 2: [α]_D²² -27.6 (c 1.00, CHCl₃). IR ν_{max} (KBr): 3429, 3188, 3069, 2953, 2895, 2859, 2712, 1696, 1471 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ: 0.20 (s, 6H), 0.95 (s, 9H), 1.95 (s, 3H), 2.97 (d, *J* = 7.5 Hz, 1H), 4.25 (dd, *J* = 4.5, 5.5, 7.5 Hz, 1H), 4.30 (d, *J* = 2.5 Hz, 1H), 4.61 (d, *J* = 2.5 Hz, 1H), 4.71 (d, *J* = 5.5 Hz, 1H), 5.93 (d, *J* = 4.5 Hz, 1H), 6.98 (s, 1H), 8.02 (brs, 1H). ¹³C NMR (125 MHz, CDCl₃) δ: -4.78, -4.40, 12.53, 18.16, 25.71, 70.79, 73.68, 87.72, 91.71, 111.76, 135.79, 149.94, 160.39, 162.95. HRMS (MALDI): calcd for C₁₆H₂₆N₂NaO₅Si [MNa⁺] 377.1509, found 377.1503.

Compound 3: [α]_D¹⁵ +35.6 (c 1.00, CHCl₃). IR ν_{max} (KBr): 3463, 3396, 3192, 3064, 2953, 2930, 2896, 2858, 1691, 1470, 1415, 1389, 1364, 1322 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ: 0.15 (s, 3H), 0.20 (s, 3H), 0.92 (s, 9H), 1.94 (d, *J* = 1.0 Hz, 3H), 2.58 (d, *J* = 7.0 Hz, 1H), 4.30 (d, *J* = 3.0, 5.5 Hz, 1H), 4.46–4.51 (m, 2H), 4.69 (t, *J* = 1.5 Hz, 1H), 5.92 (d, *J* = 3.0 Hz, 1H), 6.95 (d, *J* = 1.5 Hz, 1H), 9.53 (brs, 1H). ¹³C NMR (75 MHz, CDCl₃) δ: -5.20, -4.72, 12.66, 18.00, 25.63, 69.48, 74.14, 87.08, 90.55, 111.57, 134.30, 150.04, 160.78, 163.74. HRMS (MALDI): calcd for C₁₆H₂₇N₂O₅Si [MH⁺] 355.1689, found 355.1684.

1-[3-*O*-*tert*-Butyldimethylsilyl-4-*C*-(2-propyn-1-yloxy)-β-D-ribofuranosyl]thymine (5a)

Conditions using mCPBA: Under N₂ atmosphere, mCPBA (75w/w%, 1.22 g, 7.03 mmol), which was dried over MgSO₄, was added to a solution of compound **2** (2.08 g, 5.86 mmol) in anhydrous 2-propyn-1-ol (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The reaction was then quenched with sat. NaHCO₃ and the mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na₂SO₄ and concentrated *in vacuo*. The obtained crude residue (3.09 g) was purified by column chromatography (silica gel 100 g, *n*-hexane:EtOAc = 2:1 to 2:3) to give compound **5a** as a white foam (1.00

g, 40%).

Conditions using ZnCl₂: Acetone (30 mL) and sat. NaHCO₃ (150 mL) were added to a solution of compound **2** (3.00 g, 8.46 mmol) in CH₂Cl₂ (45 mL). Then, Oxone[®] (15.6 g, 25.4 mmol) in H₂O (100 mL) was dropwise added to this solution at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The resulting solution was extracted with CH₂Cl₂. The combined organic layer was washed with water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The obtained crude residue (2.88 g) was dissolved in anhydrous Et₂O (40 mL) under N₂ atmosphere, then propargyl alcohol (5.0 mL, 84.6 mmol) and ZnCl₂ (1M in Et₂O, 8.5 mL, 8.5 mmol) was added to this solution at -40 °C. The reaction mixture was stirred at 0 °C for 1 h. The reaction was then quenched with sat. NaHCO₃ at 0 °C and the mixture was filtered through Celite[®], and the filtrate was diluted with EtOAc. The solution was washed with water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The obtained crude residue (4.82 g) was purified by column chromatography (silica gel 50 g, *n*-hexane:EtOAc = 2:1 to 2:3) to give compound **5a** as a white foam (1.89 g, 52%, 2 steps from **2**).

$[\alpha]_D^{25}$ -45.8 (c 1.00, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ: 0.18 (s, 3H), 0.20 (s, 3H), 0.95 (s, 9H), 1.93 (d, *J* = 1.0 Hz, 3H), 2.46 (t, *J* = 2.5 Hz, 1H), 2.63 (dd, *J* = 4.5, 8.0 Hz, 1H), 3.14 (d, *J* = 8.0 Hz, 1H), 3.70 (dd, *J* = 8.0, 12.0 Hz, 1H), 3.86 (dd, *J* = 4.5, 12.0 Hz, 1H), 4.33 (d, *J* = 2.5 Hz, 2H), 4.42 (ddd, *J* = 4.0, 6.5, 8.0 Hz, 1H), 4.69 (d, *J* = 6.5 Hz, 1H), 5.59 (d, *J* = 4.0 Hz, 1H), 7.12 (d, *J* = 1.0 Hz, 1H), 8.84 (brs, 1H). ¹³C NMR (100 MHz, CDCl₃) δ: -5.12, -4.66, 12.34, 18.30, 25.76, 50.94, 62.61, 72.05, 72.70, 74.08, 80.34, 95.88, 107.26, 111.35, 138.67, 150.24, 163.50. HRMS (MALDI): calcd for C₁₉H₃₀N₂NaO₇Si [MNa⁺] 449.1720, found 449.1714.

1-[3-*O*-*tert*-Butyldimethylsilyl-5-*O*-(4,4'-dimethoxytrityl)-4-*C*-(2-propyn-1-yloxy)-β-*D*-ribofuranosyl]thymine (**6a**)

Under N₂ atmosphere, 2,6-lutidine (0.54 mL, 4.68 mmol) and DMTrOTf (1M in CH₂Cl₂, 2.6 mL, 2.6 mmol) were added to a solution of compound **5a** (1.00 g, 2.34 mmol) in anhydrous CH₂Cl₂ (20 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The reaction was then quenched with MeOH and the mixture was diluted with CH₂Cl₂. This solution was washed with water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The obtained crude residue (1.77 g) was purified by column chromatography (silica gel 50 g, *n*-hexane:EtOAc = 2:1 to 1:1) to give compound **6a** as a white foam (1.38 g, 81%).

$[\alpha]_D^{25}$ -27.5 (c 1.00, CHCl₃). IR ν_{\max} (KBr): 3532, 3293, 3200, 3060, 2931, 2856, 1695, 1608, 1580, 1509, 1463, 1411, 1372, 1298, 1254 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ: 0.03 (s, 3H), 0.05 (s, 3H), 0.88 (s, 9H), 1.45 (d, *J* = 1.0 Hz, 3H), 2.34 (t, *J* = 2.5 Hz, 1H), 3.06 (d, *J* = 9.0 Hz, 1H), 3.19 (d, *J* = 10.0 Hz, 1H), 3.58 (d, *J* = 10.0 Hz, 1H), 3.80 (s, 6H), 4.20 (dd, *J* = 2.5, 15.5 Hz, 1H), 4.23-4.54 (m, 2H), 4.53 (d, *J* = 6.0 Hz, 1H), 6.14 (d, *J* = 4.5 Hz, 1H), 6.83-7.38 (m, 13H), 7.51 (d, *J* = 1.0 Hz, 1H), 8.43 (brs, 1H). ¹³C NMR (100 MHz, CDCl₃) δ: -5.09, -4.64, 11.75, 18.31, 25.76, 50.58, 51.17, 55.26, 63.46, 72.60, 74.16, 74.33, 79.97, 87.47, 90.18, 106.61, 111.60, 113.31, 113.35, 127.31, 128.06, 128.12, 130.04, 130.07, 130.09, 134.88, 135.47, 143.82, 150.24, 158.83, 163.31. HRMS (MALDI): calcd for C₄₀H₄₈N₂O₉Si [MNa⁺] 751.3027, found 751.3021.

1-[3-*O*-*tert*-Butyldimethylsilyl-5-*O*-(4,4'-dimethoxytrityl)-2-*O*-(1-imidazolylthiocarbonyl)-4-*C*-(2-propyn-1-yloxy)-β-*D*-ribofuranosyl]thymine (**7a**)

Under N₂ atmosphere, TCDI (1.04 g, 5.82 mmol) was added to a solution of compound **6a** (2.12 g, 2.91 mmol) in anhydrous THF (20 mL) at 0 °C. After being refluxed for 3 h, the reaction mixture was concentrated *in vacuo*. The obtained crude residue (3.09 g) was purified by column chromatography (silica gel 50 g, *n*-hexane:EtOAc = 1:1 to 1:2) to give compound **7a** as a white foam (2.20 g, 90%).

$[\alpha]_D^{26}$ -25.4 (c 1.00, CHCl₃). IR ν_{\max} (KBr): 3276, 3168, 3055, 3004, 2952, 2931, 2856, 1692, 1608, 1582, 1509, 1466, 1392, 1333, 1288, 1250 cm⁻¹. ¹H NMR (400 MHz, CDCl₃) δ: -0.09 (s, 3H), -0.08 (s, 3H), 0.72 (s, 9H), 1.51 (s, 3H), 2.36 (t, *J* = 2.5 Hz, 1H), 3.26 (d, *J* = 10.0 Hz, 1H), 3.60 (d, *J* = 10.0 Hz, 1H), 3.80 (s, 6H), 4.26 (dd, *J* = 2.5, 16.0 Hz, 1H), 4.34 (dd, *J* = 2.5, 16.0 Hz, 1H),

4.95 (d, $J = 6.5$ Hz, 1H), 6.06 (dd, $J = 4.5, 6.5$ Hz, 1H), 6.42 (d, $J = 4.5$ Hz, 1H), 6.83–6.86 (m, 4H), 7.04 (t, $J = 1.0$ Hz, 1H), 7.26–7.44 (m, 9H), 7.47 (d, $J = 1.0$ Hz, 1H), 7.69 (t, $J = 1.0$ Hz, 1H), 8.43 (s, 1H), 8.73 (brs, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ : -5.15, -4.69, 11.79, 17.87, 25.44, 51.53, 55.26, 63.60, 71.78, 74.27, 79.91, 81.09, 87.21, 87.70, 106.63, 112.29, 113.31, 113.35, 118.15, 127.32, 128.05, 128.21, 130.16, 130.18, 131.11, 134.77, 134.84, 135.46, 137.42, 149.93, 158.84, 163.27, 183.13. HRMS (MALDI): calcd for $\text{C}_{44}\text{H}_{50}\text{N}_2\text{NaO}_9\text{SiS}$ [MNa^+] 861.2965, found 861.2960.

(1R,5R,6R,8S)-1-[8-*tert*-Butyldimethylsiloxy-1-(4,4'-dimethoxytrityloxy)methyl-4-methylene-2,7-dioxabicyclo[3.2.1]octan-6-yl]thymine (8a)

Under N_2 atmosphere, $(\text{Me}_3\text{Si})_3\text{SiH}$ (2.4 mL, 7.86 mmol) and AIBN (86.1 mg, 0.524 mmol) were added to a solution of compound **7a** (2.20 g, 2.62 mmol) in anhydrous toluene (20 mL) at 90 °C. After being stirred at 90 °C for 1 h, the reaction mixture was concentrated *in vacuo*. The obtained crude residue (2.41 g) was purified by column chromatography (silica gel 50 g, *n*-hexane:EtOAc = 3:1 to 3:2) to give compound **8a** as a white foam (1.07 g, 57%).

$[\alpha]_{\text{D}}^{25}$ -14.7 (c 1.00, CHCl_3). IR ν_{max} (KBr): 3168, 3065, 3018, 2953, 2929, 2856, 1692, 1608, 1581, 1508, 1464, 1414, 1361, 1275, 1252 cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ : -0.04 (s, 3H), 0.06 (s, 3H), 0.76 (s, 9H), 1.19 (s, 3H), 3.22 (d, $J = 10.0$ Hz, 1H), 3.23 (d, $J = 4.5$ Hz, 1H), 3.58 (d, $J = 10.0$ Hz, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 4.28 (d, $J = 14.0$ Hz, 1H), 4.44 (d, $J = 14.0$ Hz, 1H), 4.53 (d, $J = 4.5$ Hz, 1H), 5.02 (s, 2H), 5.87 (s, 1H), 6.81–7.40 (m, 13H), 8.08 (s, 1H), 9.03 (brs, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ : -4.84, -4.15, 11.77, 17.79, 25.49, 52.35, 55.23, 61.57, 65.10, 67.49, 87.13, 87.64, 105.93, 110.42, 112.34, 113.18, 113.25, 127.31, 127.95, 128.40, 130.29, 134.87, 134.95, 135.16, 138.71, 143.71, 150.16, 158.83, 158.84, 164.13. HRMS (MALDI): calcd for $\text{C}_{40}\text{H}_{48}\text{N}_2\text{NaO}_8\text{Si}$ [MNa^+] 735.3078, found 735.3072.

(1R,5R,6R,8S)-1-[1-(4,4'-Dimethoxytrityloxy)methyl-8-hydroxy-4-methylene-2,7-dioxabicyclo[3.2.1]octan-6-yl]thymine (9a)

TBAF (1M in THF, 0.50 mL, 0.50 mmol) was added to a solution of compound **8a** (321 mg, 0.450 mmol) in THF (20 mL). After being stirred at room temperature for 15 h, the reaction mixture was concentrated *in vacuo*. The obtained crude residue (388 mg) was purified by column chromatography (silica gel 10 g, *n*-hexane:EtOAc = 1:1 to 1:2) to give compound **9a** as a white foam (234 mg, 87%).

$[\alpha]_{\text{D}}^{25}$ +10.3 (c 1.00, CHCl_3). IR ν_{max} (KBr): 3399, 3182, 3069, 3016, 2953, 2837, 1684, 1607, 1581, 1509, 1464, 1414, 1355, 1251 cm^{-1} . ^1H NMR (400 MHz, CDCl_3) δ : 1.27 (d, $J = 1.0$ Hz, 3H), 2.06 (d, $J = 10.5$ Hz, 1H), 3.34 (d, $J = 5.0$ Hz, 1H), 3.40 (d, $J = 10.0$ Hz, 1H), 3.42 (d, $J = 10.0$ Hz, 1H), 3.78 (s, 3H), 3.79 (s, 3H), 4.21 (d, $J = 14.0$ Hz, 1H), 4.43 (d, $J = 14.0$ Hz, 1H), 4.58 (dd, $J = 5.0, 10.5$ Hz, 1H), 5.21 (s, 2H), 5.91 (s, 1H), 6.82–7.44 (m, 13H), 7.87 (d, $J = 1.0$ Hz, 1H), 9.06 (brs, 1H). ^{13}C NMR (100 MHz, CDCl_3) δ : 11.86, 52.25, 55.21, 61.36, 65.46, 67.78, 86.74, 87.22, 105.72, 110.42, 113.35, 115.80, 127.18, 128.09, 128.11, 130.10, 130.12, 134.81, 134.94, 135.02, 136.61, 143.89, 150.07, 158.71, 158.75, 163.96. HRMS (MALDI): calcd for $\text{C}_{34}\text{H}_{34}\text{N}_2\text{NaO}_8$ [MNa^+] 621.2213, found 621.2207.

(1R,5R,6R,8S)-1-[8-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4'-dimethoxytrityloxy)methyl-4-methylene-2,7-dioxabicyclo[3.2.1]octan-6-yl]thymine (10a)

Under N_2 atmosphere, DIPEA (0.21 mL, 1.18 mmol) and *i*-Pr₂NP(Cl)OCH₂CH₂CN (0.11 mL, 0.472 mmol) were added to a solution of compound **9a** (230 mg, 0.393 mmol) in anhydrous CH_2Cl_2 (3.0 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The reaction was then quenched with sat. NaHCO_3 and extracted with EtOAc. The combined organic layer was washed with

sat. NaHCO₃, water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The obtained crude residue (312 mg) was purified by column chromatography (silica gel 10 g, *n*-hexane:EtOAc = 1:1 to 1:2) to give compound **10a** as a white foam (256 mg, 78%).

¹H NMR (400 MHz, CDCl₃) δ: 0.99 (d, *J* = 7.0 Hz, 3.6H), 1.09 (d, *J* = 7.0 Hz, 3.6H), 1.12–1.13 (m, 5.4H), 1.18 (d, *J* = 7.0 Hz, 2.4H), 2.36 (dt, *J* = 6.0, 9.5 Hz, 0.8H), 2.60 (t, *J* = 6.0 Hz, 1.2H), 3.32–3.85 (m, 13H), 4.26 (d, *J* = 14.0 Hz, 0.4H), 4.27 (d, *J* = 14.0 Hz, 0.6H), 4.42–4.48 (m, 1H), 4.65 (d, *J* = 5.0, 8.0 Hz, 0.6H), 4.72 (d, *J* = 5.0, 9.5 Hz, 0.4H), 5.04–5.10 (m, 2H), 5.88 (s, 0.4H), 5.91 (s, 0.6H), 6.79–7.49 (m, 13H), 7.96–7.99 (m, 2H). ³¹P NMR (160 MHz, CHCl₃) δ: 149.00, 149.67. HRMS (MALDI): calcd for C₄₃H₅₁N₄NaO₉P [MNa⁺] 821.3291, found 821.3286.

3. Synthesis of (*R*)-Me-methylene-EoDNA phosphoramidite **10b**

1-[3-*O*-*tert*-Butyldimethylsilyl-4-*C*-[(2*R*)-3-butyn-2-yloxy]-β-*D*-ribofuranosyl]thymine (**5b**)

Acetone (50 mL) and sat. NaHCO₃ (500 mL) were added to a solution of compound **2** (2.53 g, 7.13 mmol) in CH₂Cl₂ (50 mL). Then, Oxone[®] (13.1 g, 21.4 mmol) in H₂O (50 mL) was dropwise added to this solution at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The resulting solution was extracted with CH₂Cl₂. The combined organic layer was washed with water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The obtained crude residue (2.66 g) was dissolved in anhydrous Et₂O (10 mL) under N₂ atmosphere, then (*R*)-3-butyn-2-ol (4.5 g, 64.2 mmol) and ZnCl₂ (1M in Et₂O, 7.1 mL, 7.1 mmol) was added to this solution at –40 °C. The reaction mixture was stirred at 0 °C for 1 h. The reaction was then quenched with sat. NaHCO₃ at 0 °C and the mixture was filtered through Celite[®], and the filtrate was diluted with EtOAc. The solution was washed with water and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The obtained crude residue (2.88 g) was purified by column chromatography (silica gel 50 g, *n*-hexane:EtOAc = 2:1 to 1:2) to give compound **5b** as a white foam (1.86 g, 59%, 2 steps from **2**).

[α]_D²⁷ +4.1 (c 1.00, CHCl₃). IR ν_{max} (KBr): 3431, 3286, 3062, 2958, 2932, 2889, 2857, 1686, 1467, 1373, 1343, 1260 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ: 0.16 (s, 3H), 0.18 (s, 3H), 0.95 (s, 9H), 1.44 (d, *J* = 7.0 Hz, 3H), 1.92 (d, *J* = 1.0 Hz, 3H), 2.34 (dd, *J* = 3.0, 9.0 Hz, 1H), 2.50 (d, *J* = 2.0 Hz, 1H), 3.23 (d, *J* = 7.5 Hz, 1H), 3.57 (dd, *J* = 9.0, 11.5 Hz, 1H), 3.82 (dd, *J* = 3.0, 11.5 Hz, 1H), 4.39 (ddd, *J* = 3.0, 6.5, 7.5 Hz, 1H), 4.59 (dt, *J* = 2.0, 7.0 Hz, 1H), 4.76 (d, *J* = 6.5 Hz, 1H), 5.69 (d, *J* = 3.0 Hz, 1H), 7.06 (d, *J* = 1.0 Hz, 1H), 8.23 (brs, 1H). ¹³C NMR (75 MHz, CDCl₃) δ: –4.99, –4.86, 12.26, 18.16, 23.23, 25.67, 58.77, 61.27, 71.39, 72.34, 73.06, 85.13, 97.16, 106.95, 111.08, 139.18, 150.30, 163.97. HRMS (MALDI): calcd for C₂₀H₃₂N₂NaO₇Si [MNa⁺] 463.1876, found 463.1871.

1-[3-*O*-*tert*-Butyldimethylsilyl-4-*C*-[(2*R*)-3-butyn-2-yloxy]-5-*O*-(4,4'-dimethoxytrityl)-β-*D*-ribofuranosyl]thymine (**6b**)

Under N₂ atmosphere, 2,6-lutidine (0.96 mL, 8.26 mmol) and DMTrOTf (1M in CH₂Cl₂, 4.5 mL, 4.5 mmol) were added to a solution of compound **5b** (1.82 g, 4.23 mmol) in anhydrous CH₂Cl₂ (25 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The reaction was then quenched with MeOH and diluted with CH₂Cl₂. This solution was washed with water and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The obtained crude residue (3.49 g) was purified by column chromatography (silica gel 100 g, *n*-hexane:EtOAc = 3:1 to 1:1) to give compound **6b** as a white foam (2.96 g, 96%).

[α]_D²⁵ +5.9 (c 1.00, CHCl₃). IR ν_{max} (KBr): 3549, 3277, 3065, 2952, 2933, 2856, 2837, 1693, 1607, 1580, 1509, 1465, 1446, 1371, 1298, 1253 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ: –0.02 (s, 3H), 0.03 (s, 3H), 0.84 (s, 9H), 1.28 (d, *J* = 6.5 Hz, 3H), 1.47 (s, 3H), 2.50 (d, *J* = 2.0 Hz, 1H), 3.07 (d, *J* = 10.0 Hz, 1H), 3.18 (d, *J* = 9.5 Hz, 1H), 3.49 (d, *J* = 10.0 Hz, 1H), 3.80 (s, 6H), 4.17–4.20 (m, 1H), 4.47–4.53 (m, 2H), 6.24 (d, *J* = 3.5 Hz, 1H), 6.83–7.39 (m, 13H), 7.50 (s, 1H), 8.39 (brs, 1H). ¹³C NMR (125 MHz, CDCl₃) δ: –4.86, –4.77, 11.82, 18.19, 23.23, 25.67, 55.26, 59.13, 62.36, 72.11, 72.68, 74.64, 84.64, 87.28, 91.81, 106.51, 111.19, 113.27, 113.33,

127.19, 128.03, 128.14, 130.05, 130.09, 134.92, 134.93, 135.94, 143.84, 149.98, 158.81, 163.43. HRMS (MALDI): calcd for $C_{41}H_{50}N_2NaO_9Si$ [MNa^+] 765.3183, found 765.3178.

1-[3-*O*-*tert*-Butyldimethylsilyl-4-*C*-[(2*R*)-3-butyn-2-yloxy]-5-*O*-(4,4'-dimethoxytrityl)-2-*O*-(1-imidazolylthiocarbonyl)- β -D-ribofuranosyl]thymine (7b)

Under N_2 atmosphere, TCDI (1.40 g, 7.86 mmol) was added to a solution of compound **6b** (2.92 g, 3.93 mmol) in anhydrous THF (30 mL) at 0 °C. After being refluxed for 3 h, the reaction mixture was concentrated *in vacuo*. The obtained crude residue (4.41 g) was purified by column chromatography (silica gel 100 g, *n*-hexane:EtOAc = 3:2 to 2:3) to give compound **7b** as a white foam (2.68 g, 80%).

$[\alpha]_D^{28}$ -23.5 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3276, 3162, 3135, 3062, 2952, 2933, 2856, 2837, 1695, 1607, 1581, 1509, 1466, 1392, 1287, 1251, 1232 cm^{-1} . 1H NMR (300 MHz, $CDCl_3$) δ : -0.18 (s, 3H), -0.10 (s, 3H), 0.66 (s, 9H), 1.35 (d, $J = 7.0$ Hz, 3H), 1.54 (d, $J = 1.0$ Hz, 3H), 2.41 (d, $J = 2.0$ Hz, 1H), 3.12 (d, $J = 10.0$ Hz, 1H), 3.56 (d, $J = 10.0$ Hz, 1H), 3.80 (s, 6H), 4.56 (dt, $J = 2.0, 7.0$ Hz, 2H), 4.95 (d, $J = 6.5$ Hz, 1H), 6.08 (dd, $J = 3.0, 6.5$ Hz, 1H), 6.38 (d, $J = 3.0$ Hz, 1H), 6.82–6.86 (m, 4H), 7.03 (dd, $J = 1.0, 1.5$ Hz, 1H), 7.26–7.42 (m, 10H), 7.73 (t, $J = 1.5$ Hz, 1H), 8.28 (brs, 1H), 8.47 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ : -4.98, -4.94, 11.82, 17.71, 23.24, 25.23, 55.15, 59.15, 62.48, 71.56, 72.51, 81.39, 84.96, 87.34, 89.40, 106.05, 111.84, 113.16, 113.21, 118.25, 127.18, 127.91, 128.20, 130.07, 130.11, 130.73, 134.82, 134.86, 136.25, 137.34, 143.71, 149.98, 158.72, 163.99, 183.24. HRMS (MALDI): calcd for $C_{45}H_{52}N_4NaO_9SiS$ [MNa^+] 875.3122, found 875.3116.

(1*R*,3*R*,5*R*,6*R*,8*S*)-1-[8-*tert*-Butyldimethylsiloxy-1-(4,4'-dimethoxytrityloxy)methyl-3-methyl-4-methylene-2,7-dioxabicyclo[3.2.1]octan-6-yl]thymine (8b)

Under N_2 atmosphere, $(Me_3Si)_3SiH$ (1.9 mL, 6.18 mmol) and AIBN (102 mg, 0.619 mmol) were added to a solution of compound **7b** (2.64 g, 3.09 mmol) in anhydrous toluene (30 mL) at 90 °C. After being stirred at 90 °C for 1 h, the reaction mixture was concentrated *in vacuo*. The obtained crude residue (4.01 g) was purified by column chromatography (silica gel 50 g, *n*-hexane:EtOAc = 5:1 to 2:1) to give compound **8b** (1.23 g, 55%) as a white foam.

$[\alpha]_D^{26}$ -10.5 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3181, 3065, 3035, 2952, 2933, 2895, 2856, 2837, 1693, 1608, 1581, 1509, 1464, 1445, 1298, 1280, 1253, 1200 cm^{-1} . 1H NMR (300 MHz, $CDCl_3$) δ : -0.04 (s, 3H), 0.05 (s, 3H), 0.75 (s, 9H), 1.16 (s, 3H), 1.33 (d, $J = 6.5$ Hz, 3H), 3.19 (d, $J = 5.0$ Hz, 1H), 3.24 (d, $J = 10.0$ Hz, 1H), 3.57 (d, $J = 10.0$ Hz, 1H), 3.79 (s, 6H), 4.44–4.49 (m, 2H), 4.99 (brs, 2H), 5.87 (s, 1H), 6.80–7.40 (m, 13H), 8.07 (s, 1H), 8.28 (brs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ : -4.89, -4.09, 11.72, 17.66, 17.73, 25.48, 53.40, 55.18, 61.49, 67.47, 69.21, 87.00, 87.43, 106.36, 110.34, 112.42, 113.14, 113.21, 127.22, 127.90, 128.38, 130.27, 130.34, 134.94, 135.01, 135.22, 143.22, 143.78, 150.34, 158.75, 158.77, 164.51. HRMS (MALDI): calcd for $C_{41}H_{50}N_2NaO_8Si$ [MNa^+] 749.3234, found 749.3229.

(1*R*,3*R*,5*R*,6*R*,8*S*)-1-[1-(4,4'-Dimethoxytrityloxy)methyl-8-hydroxy-3-methyl-4-methylene-2,7-dioxabicyclo[3.2.1]octan-6-yl]thymine (9b)

TBAF (1M in THF, 1.8 mL, 1.8 mmol) was added to a solution of compound **8b** (1.18 g, 1.62 mmol) in THF (10 mL). After being stirred at room temperature for 12 h, the reaction mixture was concentrated *in vacuo*. The obtained crude residue (1.95 g) was purified by column chromatography (silica gel 30 g, *n*-hexane:EtOAc = 1:1 to 1:2) to give compound **9b** as a white foam (889 mg, 89%).

$[\alpha]_D^{25}$ +10.6 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 3453, 3192, 3063, 3033, 2983, 2951, 2936, 2836, 1686, 1607, 1580, 1509, 1466, 1446,

1416, 1296, 1279, 1253 cm^{-1} . ^1H NMR (300 MHz, CDCl_3) δ : 1.25 (d, $J = 1.0$ Hz, 3H), 1.36 (d, $J = 6.0$ Hz, 3H), 1.75 (d, $J = 11.5$ Hz, 1H), 3.33 (d, $J = 5.5$ Hz, 1H), 3.39 (d, $J = 10.0$ Hz, 1H), 3.42 (d, $J = 10.0$ Hz, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 4.40–4.50 (m, 1H), 4.55 (dd, $J = 5.5, 11.5$ Hz, 1H), 5.18 (d, $J = 2.0$ Hz, 1H), 5.23 (d, $J = 1.5$ Hz, 1H), 5.88 (s, 1H), 6.82–7.45 (m, 13H), 7.89 (d, $J = 1.0$ Hz, 1H), 8.28 (brs, 1H). ^{13}C NMR (75 MHz, CDCl_3) δ : 11.84, 17.07, 53.15, 55.21, 61.27, 67.74, 69.21, 86.78, 87.17, 106.09, 110.38, 113.35, 115.39, 127.15, 128.06, 128.16, 130.13, 130.15, 134.89, 135.04, 135.10, 141.20, 143.95, 150.12, 158.72, 158.75, 163.97. HRMS (MALDI): calcd for $\text{C}_{35}\text{H}_{36}\text{N}_2\text{NaO}_8$ [MNa^+] 635.2369, found 635.2364.

(1R,3R,5R,6R,8S)-1-[8-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4'-dimethoxytrityloxy)methyl-3-methyl-4-methylene-2,7-dioxabicyclo[3.2.1]octan-6-yl]thymine (10b) Under N_2 atmosphere, DIPEA (0.61 mL, 3.42 mmol) and $i\text{-Pr}_2\text{NP}(\text{Cl})\text{OCH}_2\text{CH}_2\text{CN}$ (0.31 mL, 1.37 mmol) were added to a solution of compound **9b** (700 mg, 1.14 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 $^\circ\text{C}$. The reaction mixture was stirred at room temperature for 3 h. The reaction was then quenched with sat. NaHCO_3 and the mixture was extracted with EtOAc. The combined organic layer was washed with sat. NaHCO_3 , water and brine, dried over Na_2SO_4 , and concentrated *in vacuo*. The obtained crude residue (815 mg) was purified by column chromatography (silica gel 20 g, $n\text{-hexane}:\text{EtOAc} = 2:1$ to $2:3$) to give compound **10b** as a white foam (656 mg, 71%).

^1H NMR (500 MHz, CDCl_3) δ : 1.01 (d, $J = 6.5$ Hz, 3.6H), 1.11–1.35 (m, 14.4H), 2.39–2.42 (m, 0.8H), 2.61 (t, $J = 6.0$ Hz, 1.2H), 3.35–3.81 (m, 13H), 4.49–4.52 (m, 1H), 4.63 (dd, $J = 5.0, 7.5$ Hz, 0.6H), 4.70 (dd, $J = 5.0, 9.0$ Hz, 0.4H), 5.02 (s, 1H), 5.09 (s, 0.4H), 5.11 (s, 0.6H), 5.88 (s, 0.4H), 5.91 (s, 0.6H), 6.84–7.47 (m, 13H), 7.98 (s, 0.4H), 7.99 (s, 0.6H), 8.15 (brs, 0.6H), 8.19 (brs, 0.4H). ^{31}P NMR (200 MHz, CHCl_3) δ : 148.36, 150.32. HRMS (MALDI): calcd for $\text{C}_{44}\text{H}_{53}\text{N}_4\text{NaO}_9\text{P}$ [MNa^+] 835.3448, found 835.3442.

4. Synthesis of (S)-Me-methylene-EoDNA phosphoramidite 10c

1-[3-O-tert-Butyldimethylsilyl-4-C-[(2S)-3-butyn-2-yloxy]- β -D-ribofuranosyl]thymine (5c)

Acetone (50 mL) and sat. NaHCO_3 (500 mL) were added to a solution of compound **2** (2.53 g, 7.13 mmol) in CH_2Cl_2 (50 mL). Then, Oxone[®] (13.1 g, 21.4 mmol) in H_2O (50 mL) was dropwise added to this solution at 0 $^\circ\text{C}$. The reaction mixture was stirred at room temperature for 3 h. The resulting solution was extracted with CH_2Cl_2 . The combined organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The obtained crude residue (2.66 g) was dissolved in anhydrous Et_2O (10 mL) under N_2 atmosphere, then (S)-3-butyn-2-ol (4.5 g, 64.2 mmol) and ZnCl_2 (1M in Et_2O , 7.1 mL, 7.1 mmol) was added to this solution at -40 $^\circ\text{C}$. The reaction mixture was stirred at 0 $^\circ\text{C}$ for 1 h. The reaction was then quenched with sat. NaHCO_3 at 0 $^\circ\text{C}$, and filtered through Celite[®] and the filtrate was diluted with EtOAc. The solution was washed with water and brine, dried over Na_2SO_4 , and concentrated *in vacuo*. The obtained crude residue (3.25 g) was purified by column chromatography (silica gel 50 g, $n\text{-hexane}:\text{EtOAc} = 2:1$ to $2:3$) to give compound **5c** as a white foam (2.42 g, 77%, 2 steps from **2**).

$[\alpha]_D^{28} -68.4$ (c 1.00, CHCl_3). IR ν_{max} (KBr): 3431, 3260, 3065, 2953, 2891, 2858, 1697, 1608, 1472, 1408, 1390, 1373, 1254 cm^{-1} . ^1H NMR (300 MHz, CDCl_3) δ : 0.18 (s, 3H), 0.20 (s, 3H), 0.95 (s, 9H), 1.46 (d, $J = 6.5$ Hz, 3H), 1.93 (d, $J = 1.0$ Hz, 3H), 2.45 (d, $J = 2.0$ Hz, 1H), 2.59 (dd, $J = 5.5, 7.0$ Hz, 1H), 3.16 (d, $J = 7.5$ Hz, 1H), 3.84 (dd, $J = 5.5, 12.0$ Hz, 1H), 3.89 (dd, $J = 7.0, 12.0$ Hz, 1H), 4.25–4.35 (m, 1H), 4.62–4.68 (m, 2H), 5.60 (d, $J = 4.5$ Hz, 1H), 7.12 (d, $J = 1.0$ Hz, 1H), 8.94 (brs, 1H). ^{13}C NMR (75 MHz, CDCl_3) δ : $-5.11, -4.58, 12.39, 18.31, 23.47, 25.75, 57.99, 62.64, 71.69, 72.58, 72.85, 93.99, 106.99, 111.32, 137.86, 150.29, 163.57$. HRMS (MALDI): calcd for $\text{C}_{20}\text{H}_{32}\text{N}_2\text{NaO}_7\text{Si}$ [MNa^+] 463.1876, found 463.1871.

1-[3-*O*-*tert*-Butyldimethylsilyl-4-*C*-[(2*S*)-3-butyn-2-yloxy]-5-*O*-(4,4'-dimethoxytrityl)- β -D-ribofuranosyl]thymine (6c)

Under N₂ atmosphere, 2,6-lutidine (1.3 mL, 10.9 mmol) and DMTrOTf (1M in CH₂Cl₂, 6.0 mL, 6.0 mmol) were added to a solution of compound **5c** (2.40 g, 5.45 mmol) in anhydrous CH₂Cl₂ (25 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The reaction was then quenched with MeOH and the mixture was diluted with CH₂Cl₂. This solution was washed with water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The obtained crude residue (4.08 g) was purified by column chromatography (silica gel 100 g, *n*-hexane:EtOAc = 3:1 to 3:2) to give compound **6c** as a white foam (3.45 g, 85%).

$[\alpha]_D^{26}$ -61.9 (c 1.00, CHCl₃). IR ν_{\max} (KBr): 3535, 3404, 3280, 3173, 3064, 2953, 2933, 2905, 2856, 1697, 1607, 1582, 1509, 1465, 1392, 1332, 1286, 1252 cm⁻¹. ¹H NMR (500 MHz, CDCl₃) δ : -0.01 (s, 3H), 0.04 (s, 3H), 0.86 (s, 9H), 1.39 (d, *J* = 7.0 Hz, 3H), 1.41 (s, 3H), 2.18 (d, *J* = 2.0 Hz, 1H), 3.11 (d, *J* = 8.5 Hz, 1H), 3.27 (d, *J* = 10.0 Hz, 1H), 3.80 (s, 6H), 3.84 (d, *J* = 10.0 Hz, 1H), 4.18 (ddd, *J* = 4.0, 6.5, 8.5 Hz, 1H), 4.50 (dt, *J* = 2.0, 7.0 Hz, 1H), 4.58 (d, *J* = 6.5 Hz, 1H), 6.07 (d, *J* = 4.0 Hz, 1H), 6.81–7.38 (m, 13H), 7.55 (s, 1H), 8.13 (brs, 1H). ¹³C NMR (125 MHz, CDCl₃) δ : -5.03, -4.59, 11.70, 18.27, 23.43, 25.70, 55.27, 58.26, 63.24, 71.58, 72.33, 74.55, 84.63, 87.43, 89.74, 106.82, 111.49, 113.24, 113.28, 127.31, 128.00, 128.22, 130.07, 130.11, 135.06, 135.08, 135.24, 143.89, 150.13, 158.81, 163.23. HRMS (MALDI): calcd for C₄₁H₅₀N₂NaO₉Si [MNa⁺] 765.3183, found 765.3178.

1-[3-*O*-*tert*-Butyldimethylsilyl-4-*C*-[(2*S*)-3-butyn-2-yloxy]-5-*O*-(4,4'-dimethoxytrityl)-2-*O*-(1-imidazolylthiocarbonyl)- β -D-ribofuranosyl]thymine (7c)

Under N₂ atmosphere, TCDI (1.63 g, 9.15 mmol) was added to a solution of compound **6c** (3.40 g, 4.58 mmol) in anhydrous THF (30 mL) at 0 °C. After being refluxed for 3 h, the reaction mixture was concentrated *in vacuo*. The obtained crude residue (5.01 g) was purified by column chromatography (silica gel 100 g, *n*-hexane:EtOAc = 3:2 to 2:3) to give compound **7c** as a white foam (3.06 g, 78%).

$[\alpha]_D^{30}$ -57.5 (c 1.00, CHCl₃). IR ν_{\max} (KBr): 3270, 3152, 3066, 2953, 2932, 2895, 2856, 2837, 1695, 1607, 1581, 1509, 1466, 1446, 1393, 1332, 1286, 1250, 1233 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ : -0.10 (s, 3H), -0.09 (s, 3H), 0.71 (s, 9H), 1.41 (d, *J* = 6.5 Hz, 3H), 1.44 (d, *J* = 1.0 Hz, 1H), 2.21 (d, *J* = 2.0 Hz, 1H), 3.36 (d, *J* = 10.5 Hz, 1H), 3.80 (s, 6H), 3.87 (d, *J* = 10.5 Hz, 1H), 4.51 (dt, *J* = 2.0, 6.5 Hz, 1H), 4.97 (d, *J* = 6.5 Hz, 1H), 6.00 (dd, *J* = 5.5, 6.5 Hz, 1H), 6.40 (d, *J* = 5.5 Hz, 1H), 6.83–6.86 (m, 4H), 7.04 (dd, *J* = 0.5, 1.0 Hz, 1H), 7.26–7.41 (m, 9H), 7.43 (d, *J* = 2.0 Hz, 1H), 7.51 (d, *J* = 1.0 Hz, 1H), 7.66 (dd, *J* = 1.0, 2.0 Hz, 1H), 8.39 (brs, 1H). ¹³C NMR (75 MHz, CDCl₃) δ : -5.13, -4.57, 11.68, 17.88, 23.55, 25.41, 55.25, 58.68, 63.71, 70.92, 72.58, 81.40, 84.44, 85.82, 87.74, 106.94, 112.38, 113.26, 113.30, 118.01, 127.32, 128.00, 128.29, 130.14, 130.20, 131.06, 134.87, 134.91, 135.00, 137.33, 143.77, 150.11, 158.85, 163.47, 183.09. HRMS (MALDI): calcd for C₄₅H₅₂N₄NaO₉SiS [MNa⁺] 875.3122, found 875.3116.

(1*R*,3*S*,5*R*,6*R*,8*S*)-1-[8-*tert*-Butyldimethylsiloxy-1-(4,4'-dimethoxytrityloxy)methyl-3-methyl-4-methylene-2,7-dioxabicyclo[3.2.1]octan-6-yl]thymine (8c)

Under N₂ atmosphere, (Me₃Si)₃SiH (2.2 mL, 7.04 mmol) and AIBN (115 mg, 0.703 mmol) were added to a solution of compound **7c** (3.00 g, 3.52 mmol) in anhydrous toluene (30 mL) at 90 °C. The reaction mixture was stirred at 90 °C for 2 h. The resulting was concentrated *in vacuo*. The obtained crude residue (4.48 g) was purified by column chromatography (silica gel 50 g, *n*-hexane:EtOAc = 5:1 to 2:1) to give compound **8c** (1.05 g, 41%) as a white foam.

$[\alpha]_D^{25}$ -7.3 (c 1.00, CHCl₃). IR ν_{\max} (KBr): 3173, 3065, 3037, 2952, 2933, 2900, 2857, 1690, 1607, 1580, 1509, 1465, 1447, 1362, 1297, 1276, 1253 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ : -0.05 (s, 3H), 0.04 (s, 3H), 0.75 (s, 9H), 1.18 (d, *J* = 1.0 Hz, 3H), 1.35 (d, *J* = 6.5 Hz, 3H), 3.20 (d, *J* = 10.0 Hz, 1H), 3.23 (d, *J* = 5.0 Hz, 1H), 3.63 (d, *J* = 10.0 Hz, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 4.56 (d, *J* = 5.0 Hz, 1H), 4.67–4.71 (m, 1H), 5.02 (d, *J* = 2.0 Hz, 1H), 5.18 (d, *J* = 2.0 Hz, 1H), 5.63 (s, 1H), 6.80–7.40 (m, 13H), 8.06 (brs, 2H). ¹³C

NMR (75 MHz, CDCl₃) δ : -5.03, -4.58, 11.75, 17.65, 23.70, 25.38, 51.96, 55.14, 61.94, 66.68, 70.49, 86.92, 88.81, 105.69, 110.16, 113.10, 113.18, 127.18, 127.85, 128.36, 130.09, 130.24, 134.95, 135.07, 135.31, 143.81, 145.48, 150.54, 158.72, 158.74, 164.66. HRMS (MALDI): calcd for C₄₁H₅₀N₂NaO₈Si [MNa⁺] 749.3234, found 749.3229.

(1R,3S,5R,6R,8S)-1-[1-(4,4'-Dimethoxytrityloxy)methyl-8-hydroxy-3-methyl-4-methylene-2,7-dioxabicyclo[3.2.1]octan-6-yl]thymine (9c)

TBAF (1M in THF, 1.5 mL, 1.5 mmol) was added to a solution of compound **8c** (1.00 g, 1.38 mmol) in THF (10 mL). After being stirred at room temperature for 12 h, the reaction mixture was concentrated *in vacuo*. The obtained crude residue (1.80 g) was purified by column chromatography (silica gel 30 g, *n*-hexane:EtOAc = 1:1 to 1:2) to give compound **9c** as a white foam (485 mg, 58%).

$[\alpha]_D^{25}$ -29.8 (c 1.00, CHCl₃). IR ν_{\max} (KBr): 3437, 3177, 3067, 2980, 2934, 2836, 1687, 1607, 1581, 1509, 1465, 1446, 1296, 1271, 1251 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) δ : 1.30 (s, 3H), 1.42 (d, *J* = 7.0 Hz, 3H), 3.34 (d, *J* = 5.0 Hz, 1H), 3.43 (d, *J* = 10.0 Hz, 1H), 3.53 (d, *J* = 10.0 Hz, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 4.45–4.46 (m, 1H), 4.68–4.75 (m, 1H), 5.12 (s, 1H), 5.27 (s, 1H), 5.76 (s, 1H), 6.83–7.42 (m, 13H), 7.81 (s, 1H), 8.01 (brs, 1H). ¹³C NMR (75 MHz, CDCl₃) δ : 11.84, 24.99, 52.25, 55.20, 62.52, 66.58, 71.40, 87.25, 87.73, 105.48, 110.09, 113.33, 115.26, 127.18, 128.06, 128.16, 128.26, 130.12, 130.14, 134.97, 135.07, 135.16, 142.70, 143.96, 150.28, 158.72, 158.75, 163.14. HRMS (MALDI): calcd for C₃₅H₃₆N₂NaO₈ [MNa⁺] 635.2369, found 635.2364.

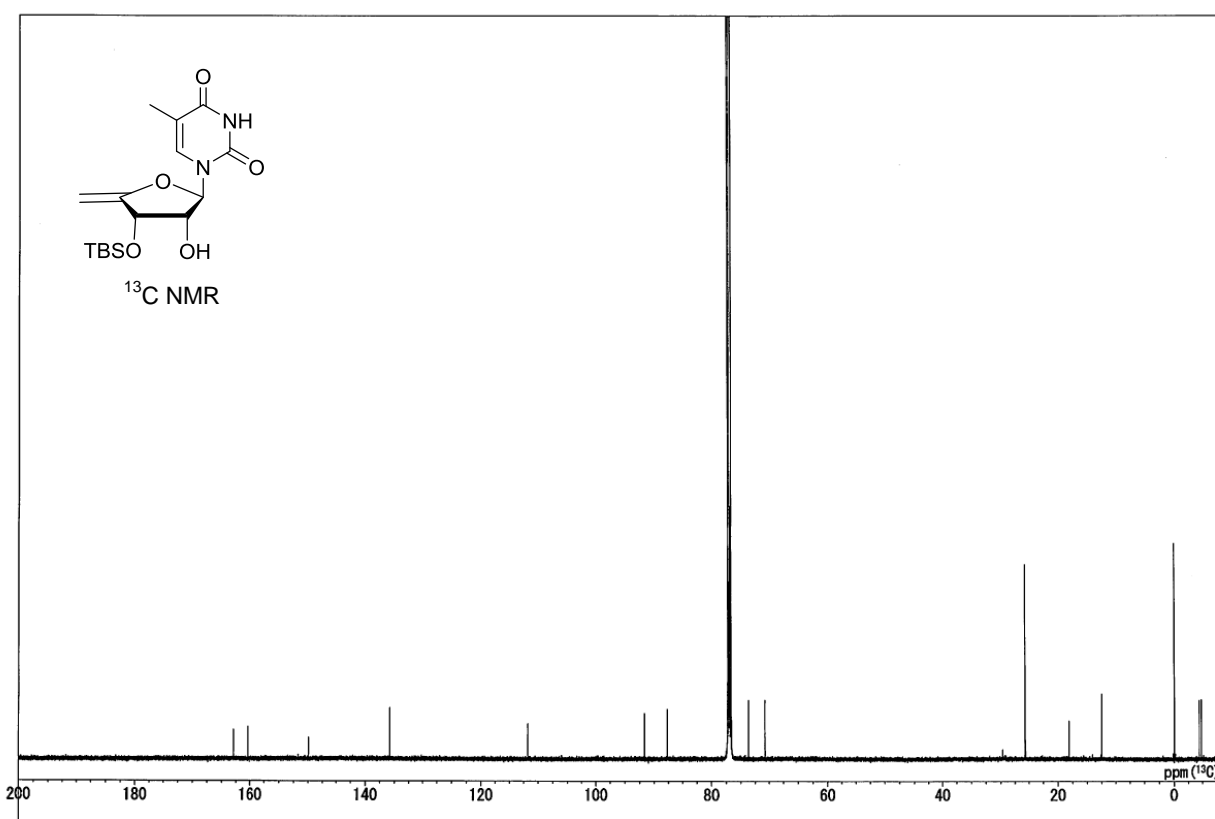
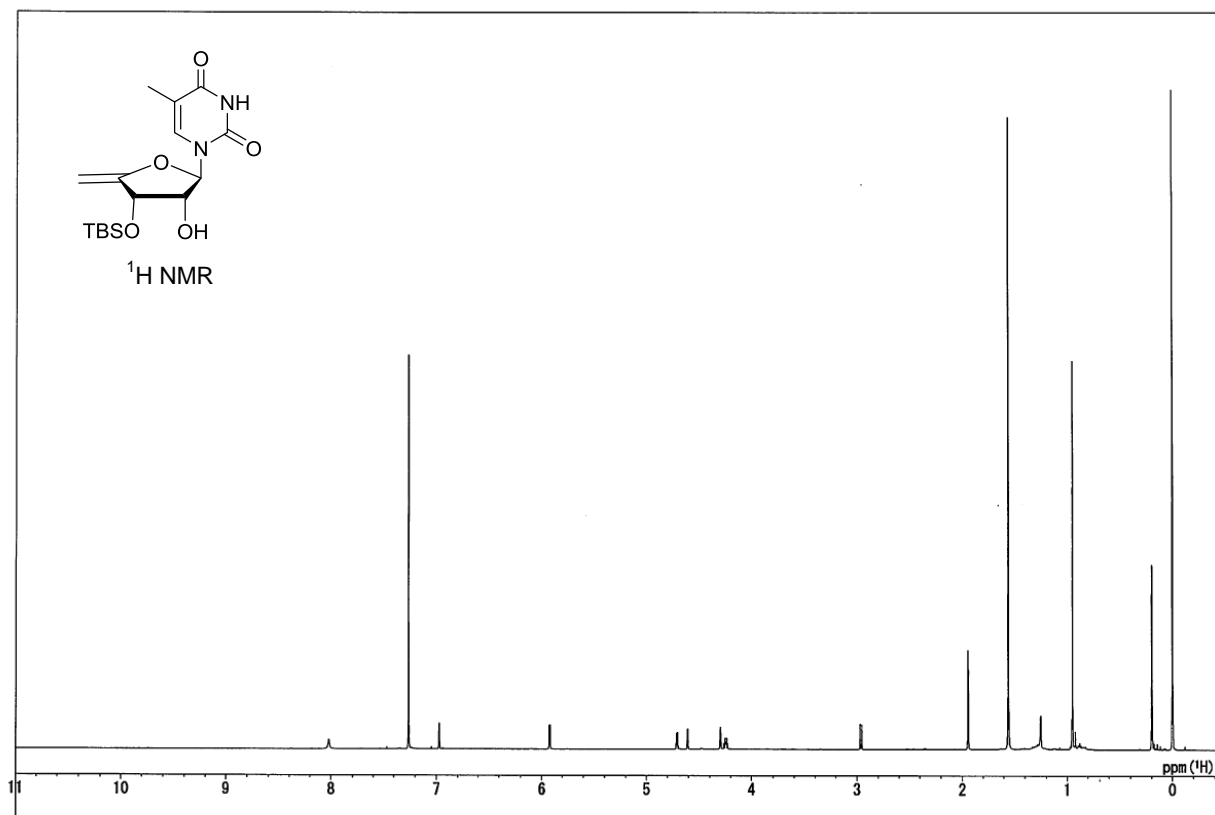
(1R,3S,5R,6R,8S)-1-[8-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-(4,4'-dimethoxytrityloxy)methyl-3-methyl-4-methylene-2,7-dioxabicyclo[3.2.1]octan-6-yl]thymine (10c)

Under N₂ atmosphere, DIPEA (0.35 mL, 1.96 mmol) and *i*-Pr₂NP(Cl)OCH₂CH₂CN (0.17 mL, 0.783 mmol) were added to a solution of compound **9c** (400 mg, 0.653 mmol) in anhydrous CH₂Cl₂ (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The reaction was then quenched with sat. NaHCO₃ and extracted with EtOAc. The combined organic layer was washed with sat. NaHCO₃, water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The obtained crude residue (528 mg) was purified by column chromatography (silica gel 15 g, *n*-hexane:EtOAc = 2:1 to 2:3) to give compound **10c** as a white foam (332 mg, 63%).

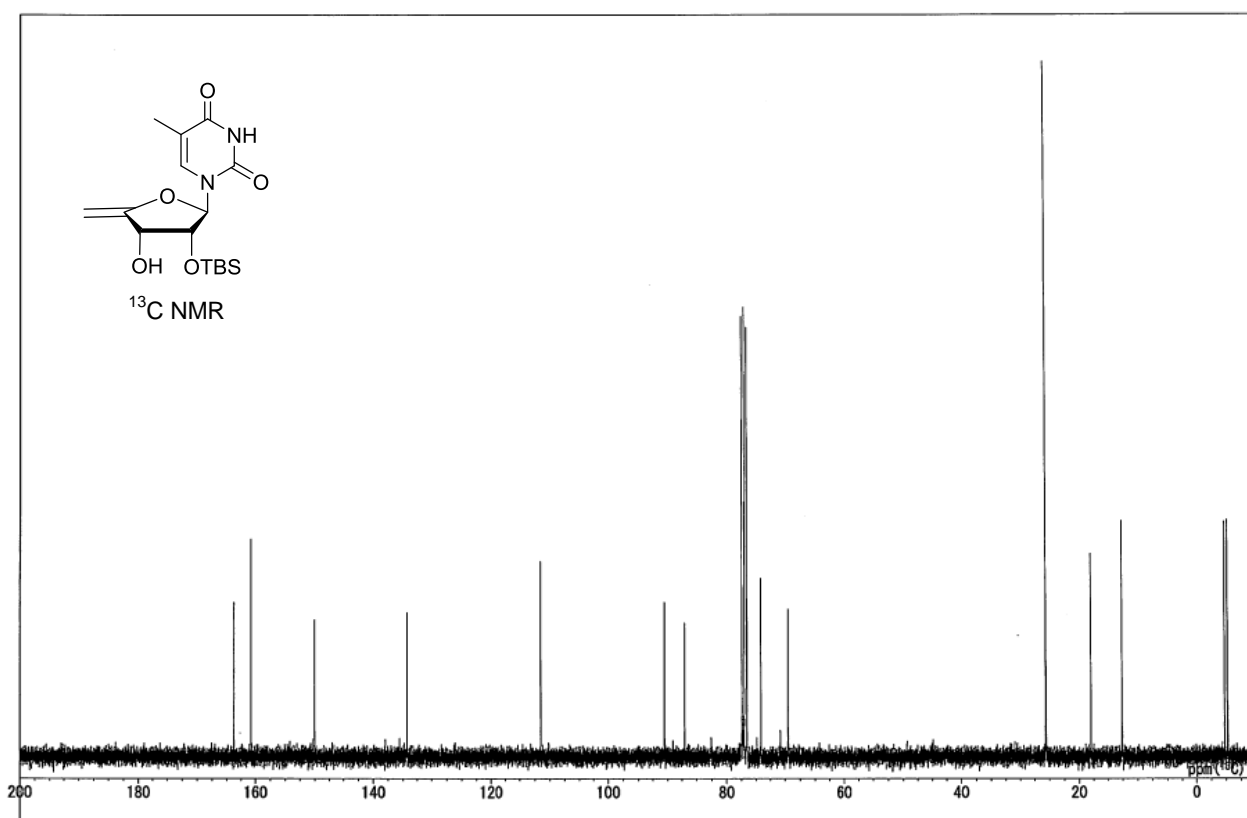
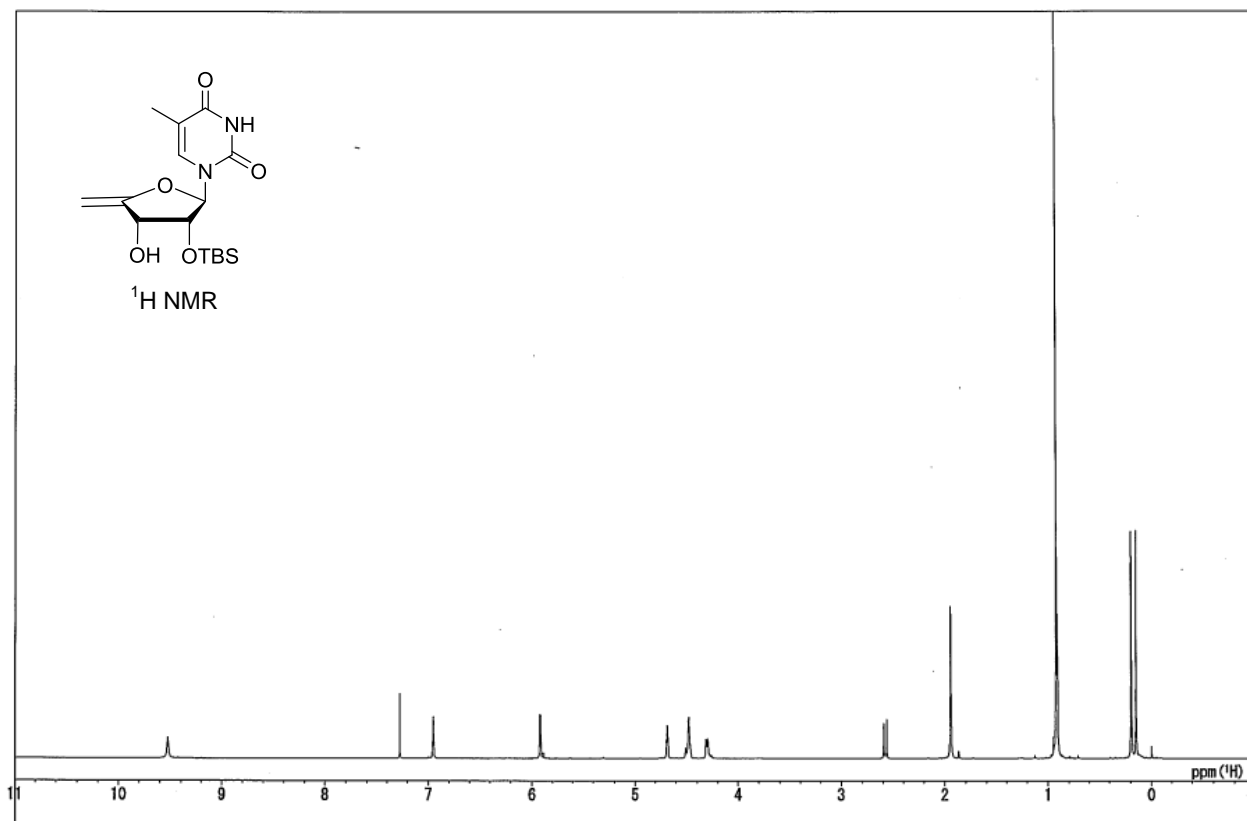
¹H NMR (500 MHz, CDCl₃) δ : 0.98 (d, *J* = 7.0 Hz, 3H), 1.09–1.31 (m, 15H), 2.32–2.36 (m, 1H), 2.58 (t, *J* = 6.0 Hz, 1H), 3.20–3.80 (m, 13H), 4.67–4.70 (m, 1.5H), 4.76 (dd, *J* = 5.0, 9.5 Hz, 0.5H), 5.03 (d, *J* = 1.5 Hz, 1H), 5.23 (d, *J* = 1.0 Hz, 0.5H), 5.24 (d, *J* = 2.0 Hz, 0.5H), 5.68 (s, 0.5H), 5.70 (s, 0.5H), 6.81–7.44 (m, 13H), 7.98 (s, 0.5H), 8.00 (s, 0.5H), 8.06 (brs, 0.5H), 8.07 (brs, 0.5H). ³¹P NMR (200 MHz, CHCl₃) δ : 149.73, 149.97. HRMS (MALDI): calcd for C₄₅H₅₃N₄NaO₉P [MNa⁺] 835.3448, found 823.3442.

5. ^1H , ^{13}C and ^{31}P spectra for new compounds

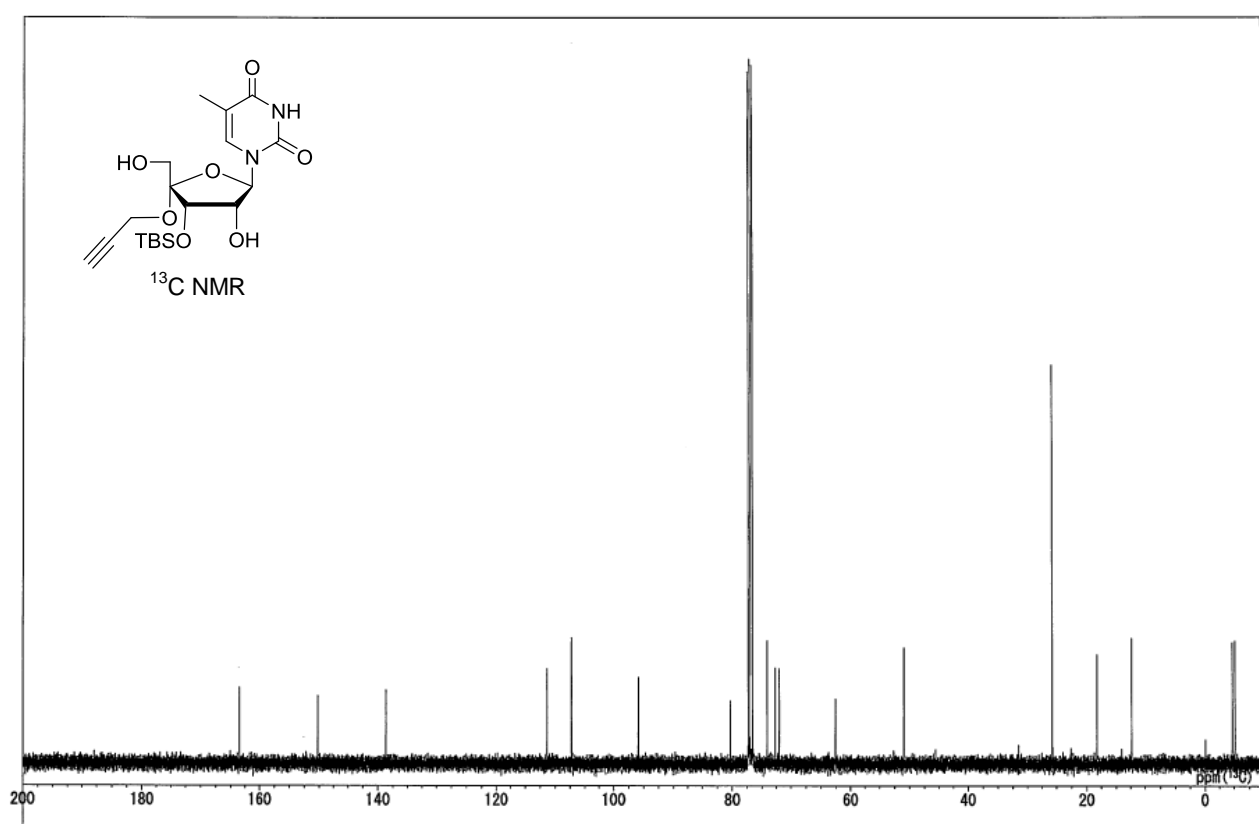
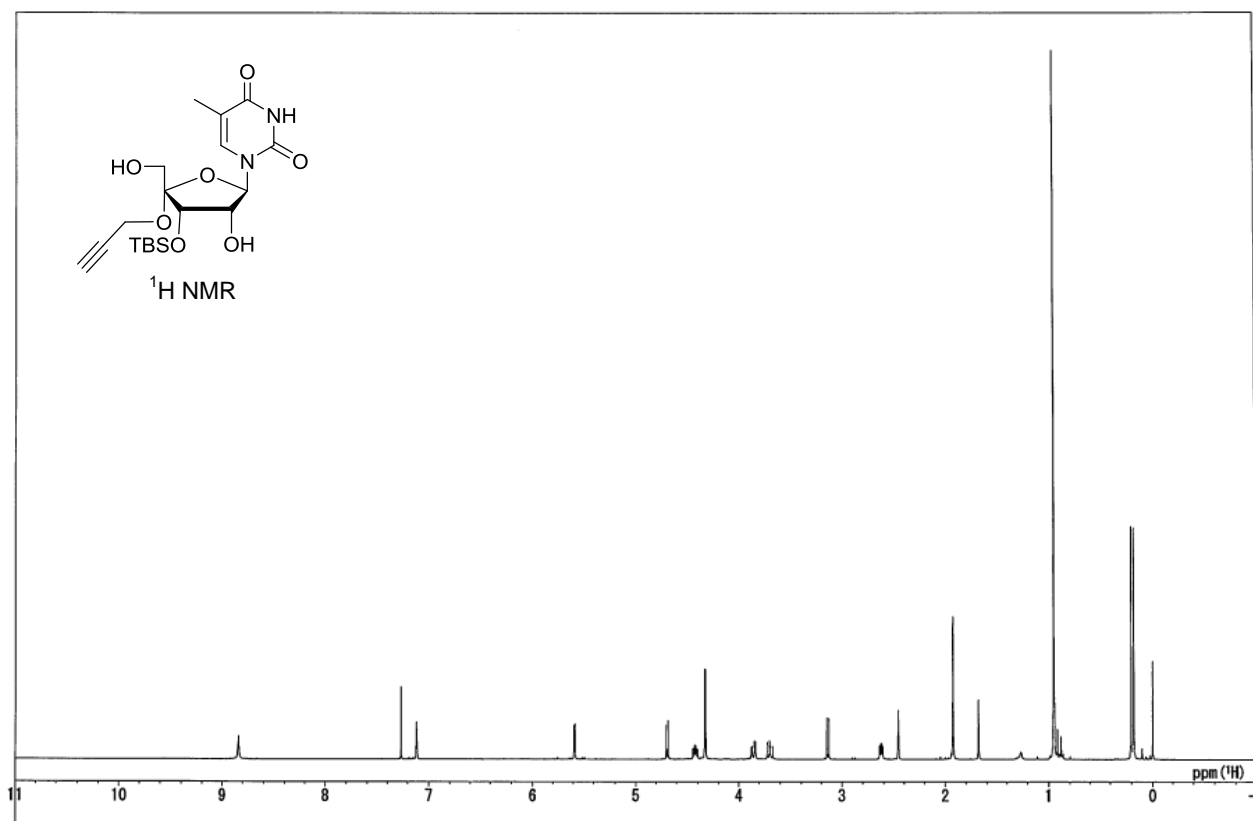
(Compound **2** in CDCl_3)



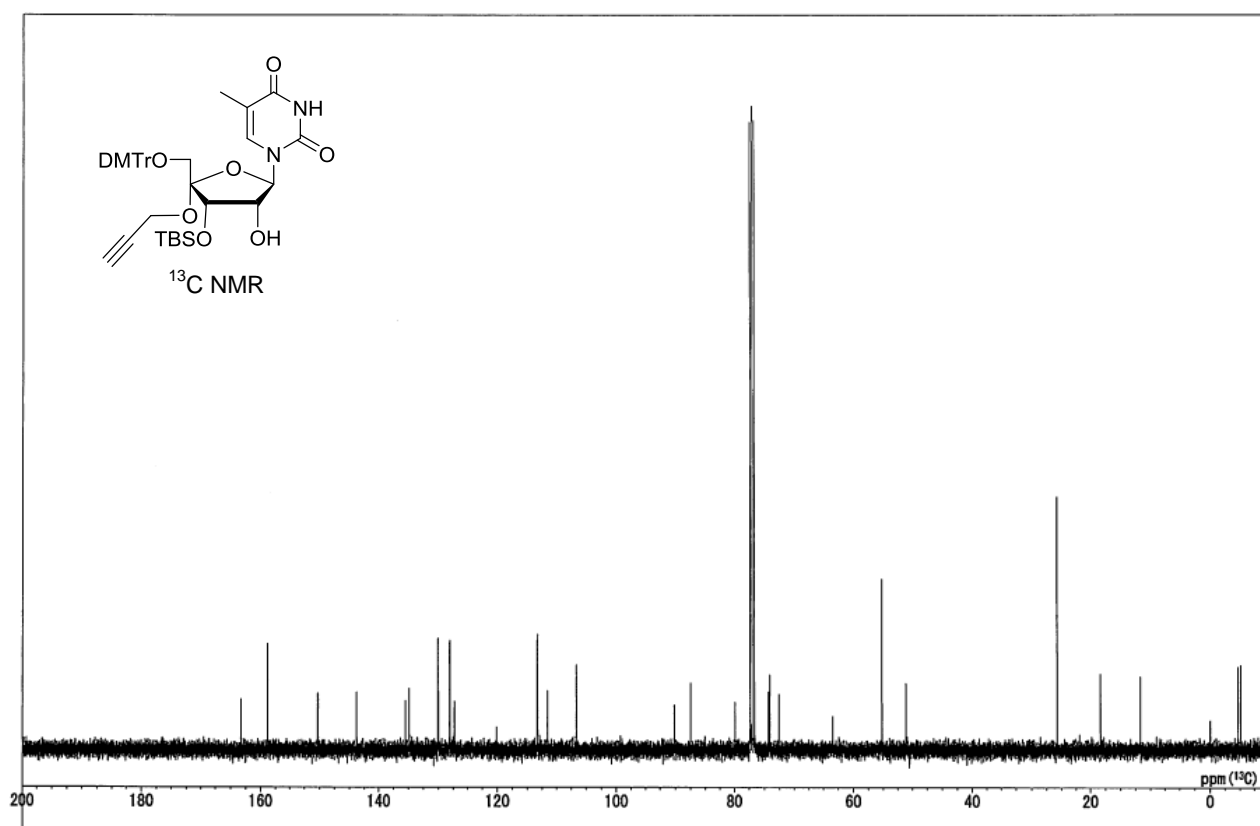
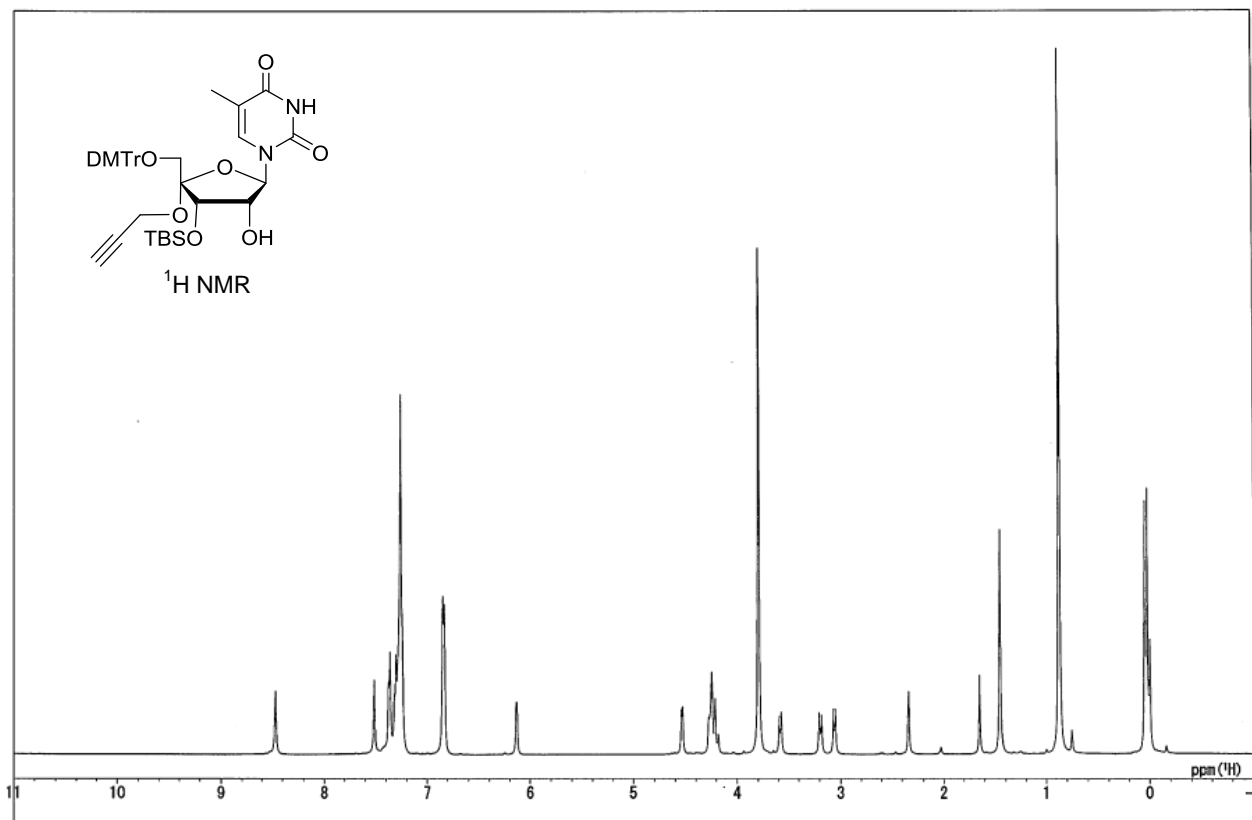
(Compound **3** in CDCl₃)



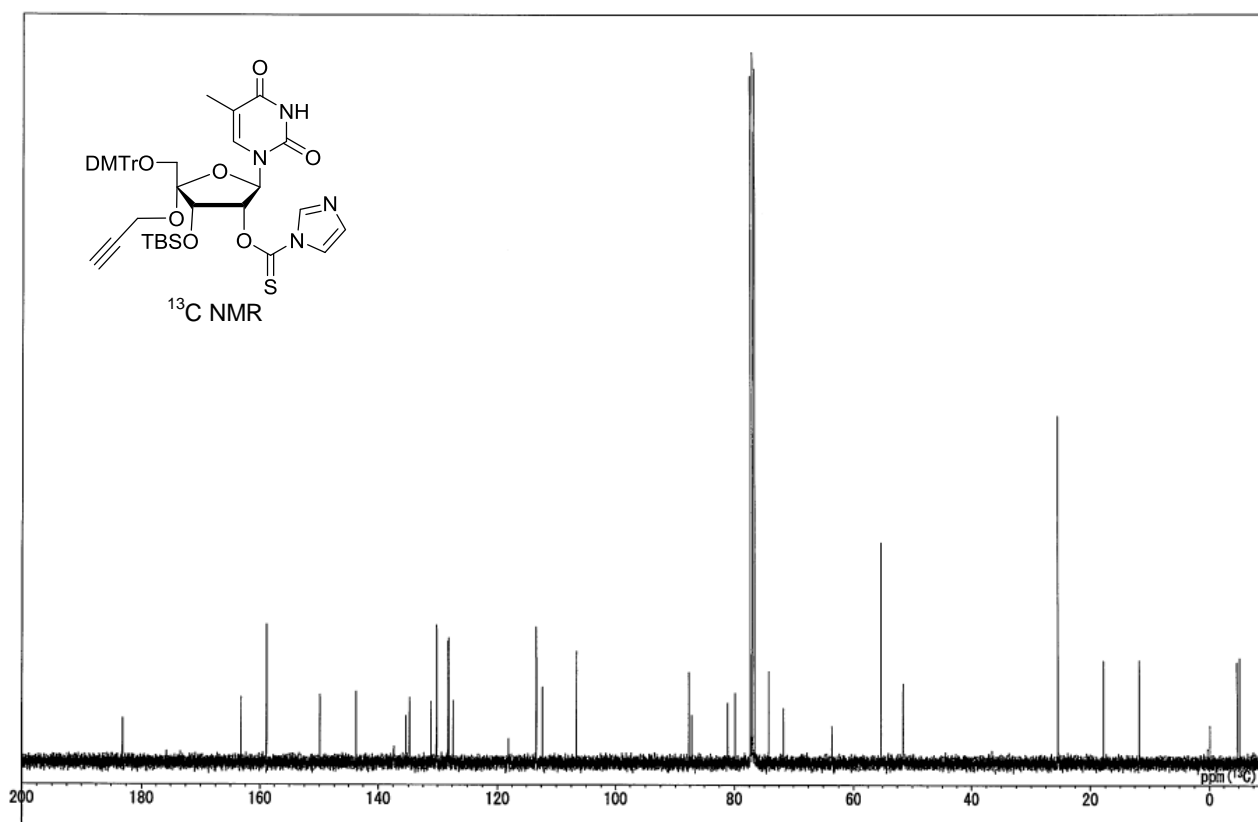
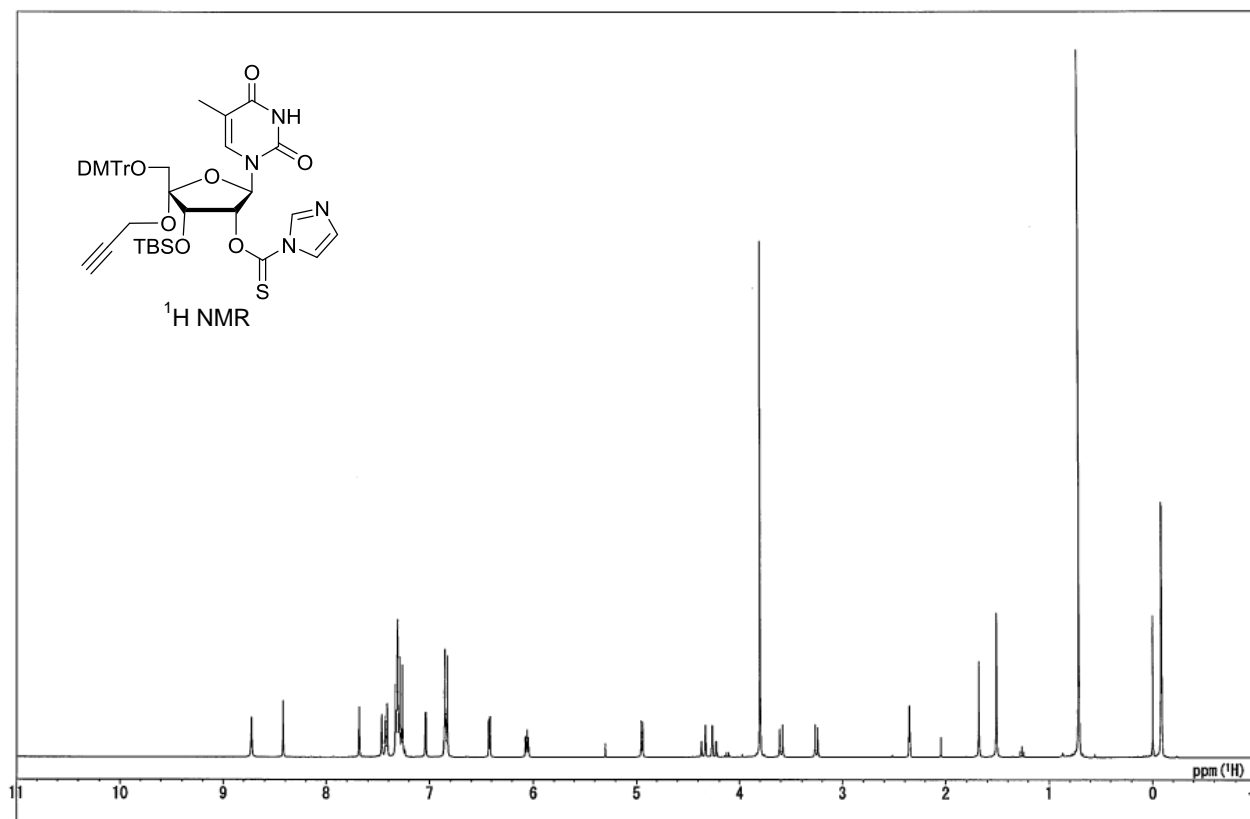
(Compound **5a** in CDCl₃)



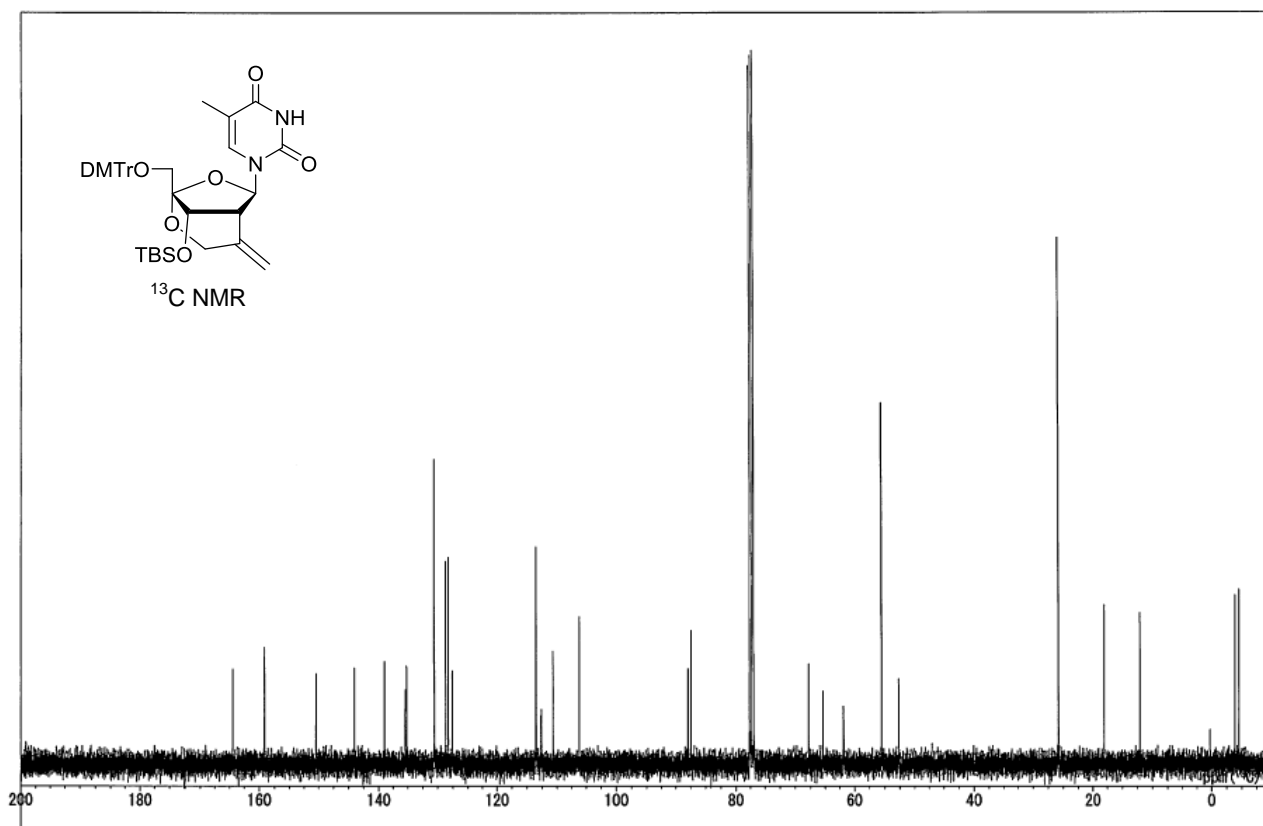
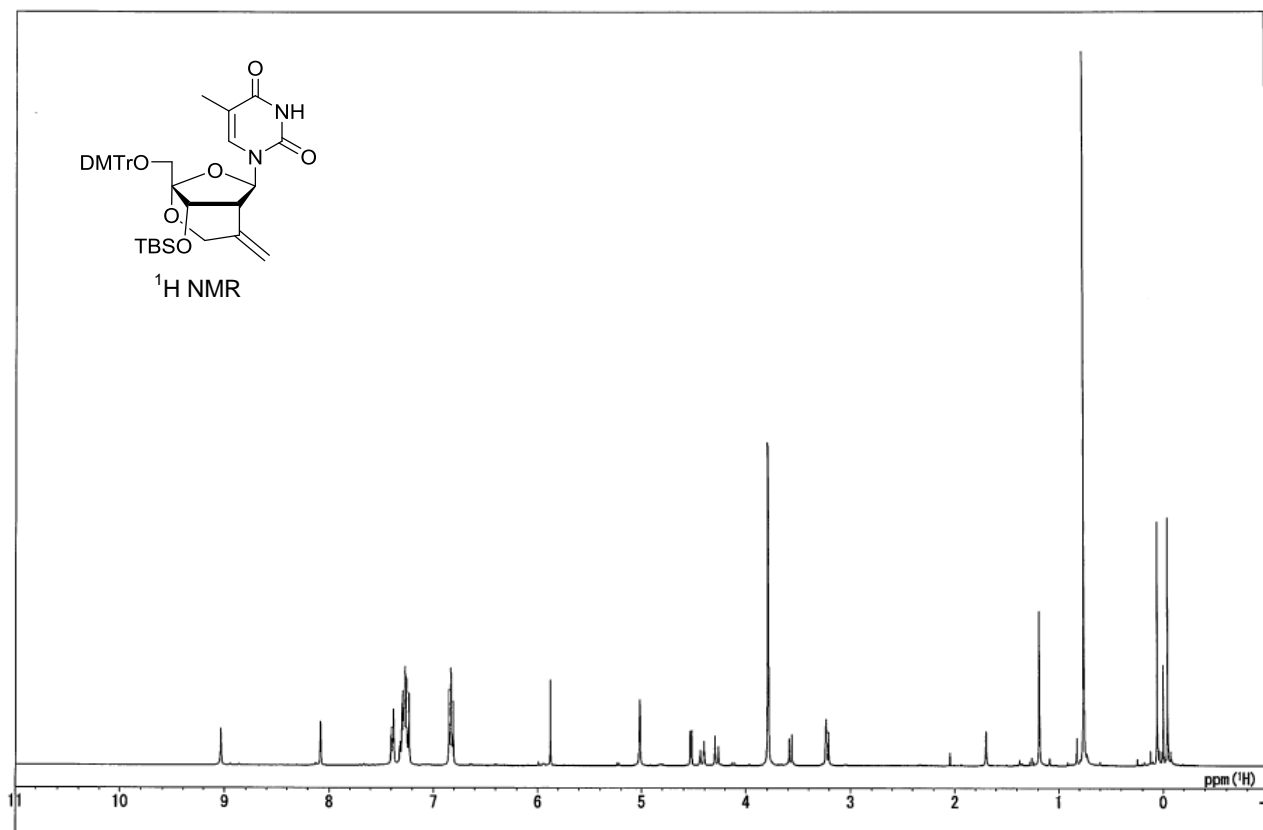
(Compound **6a** in CDCl₃)



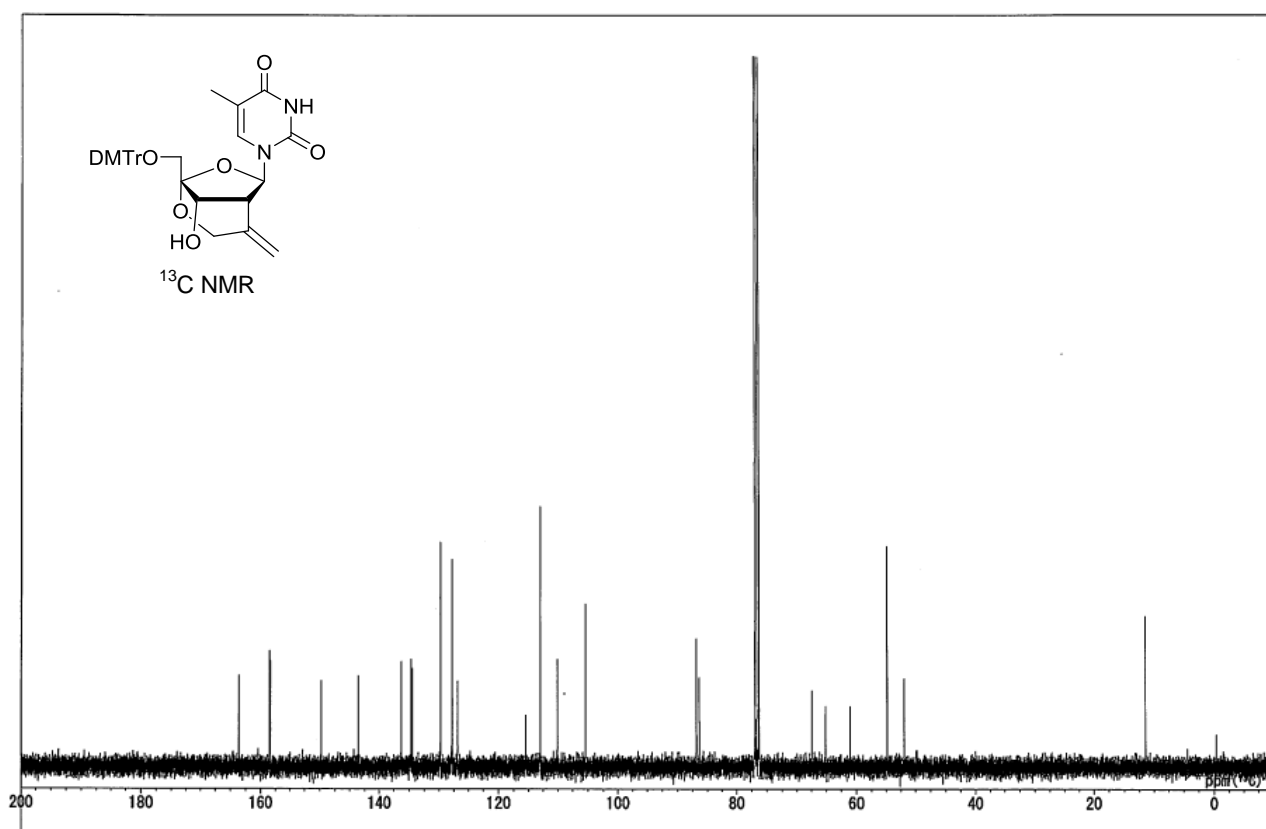
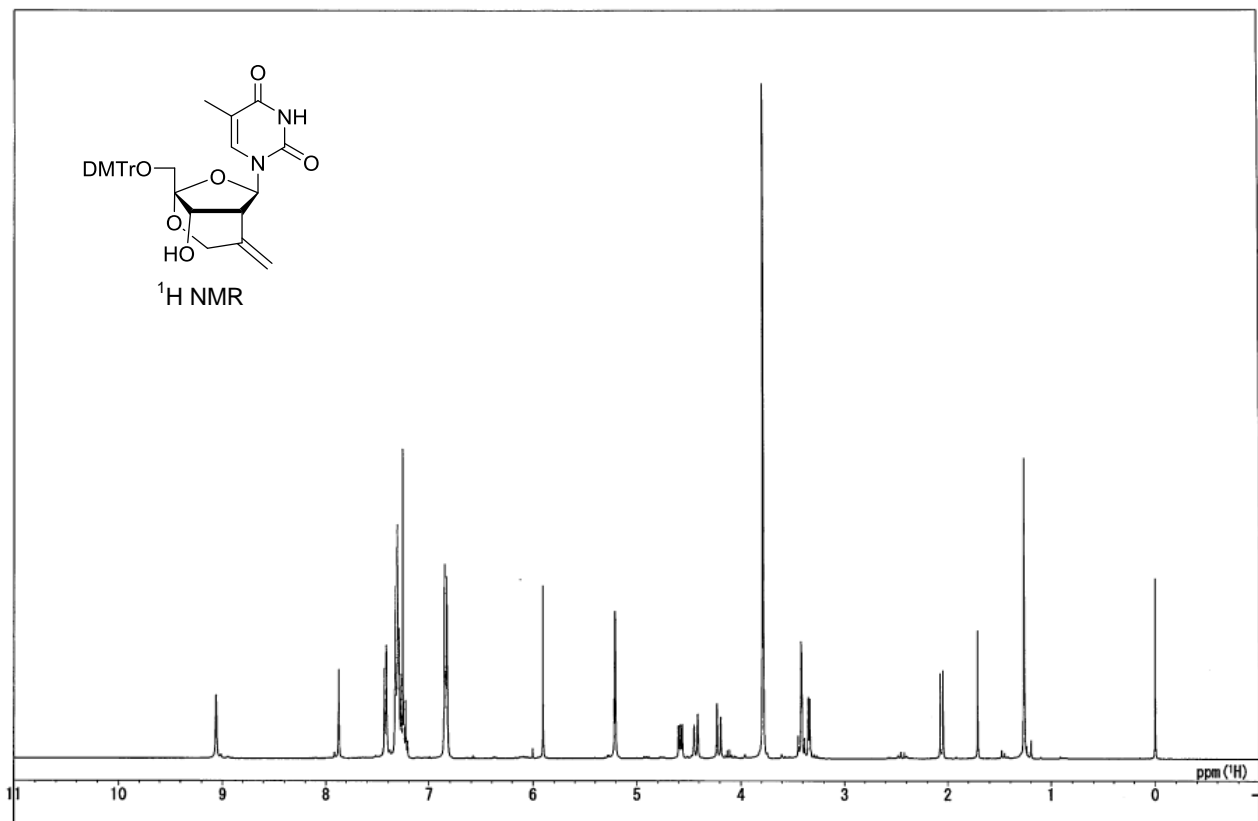
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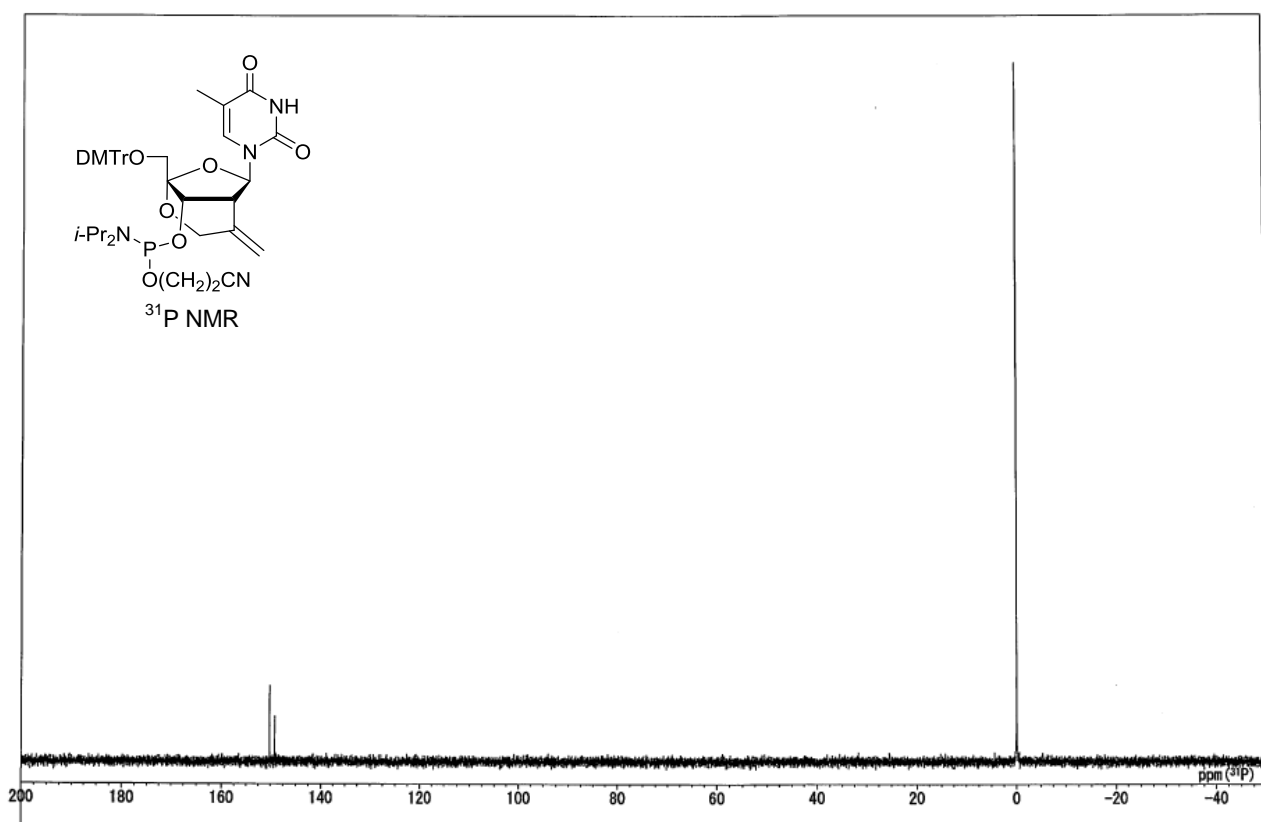
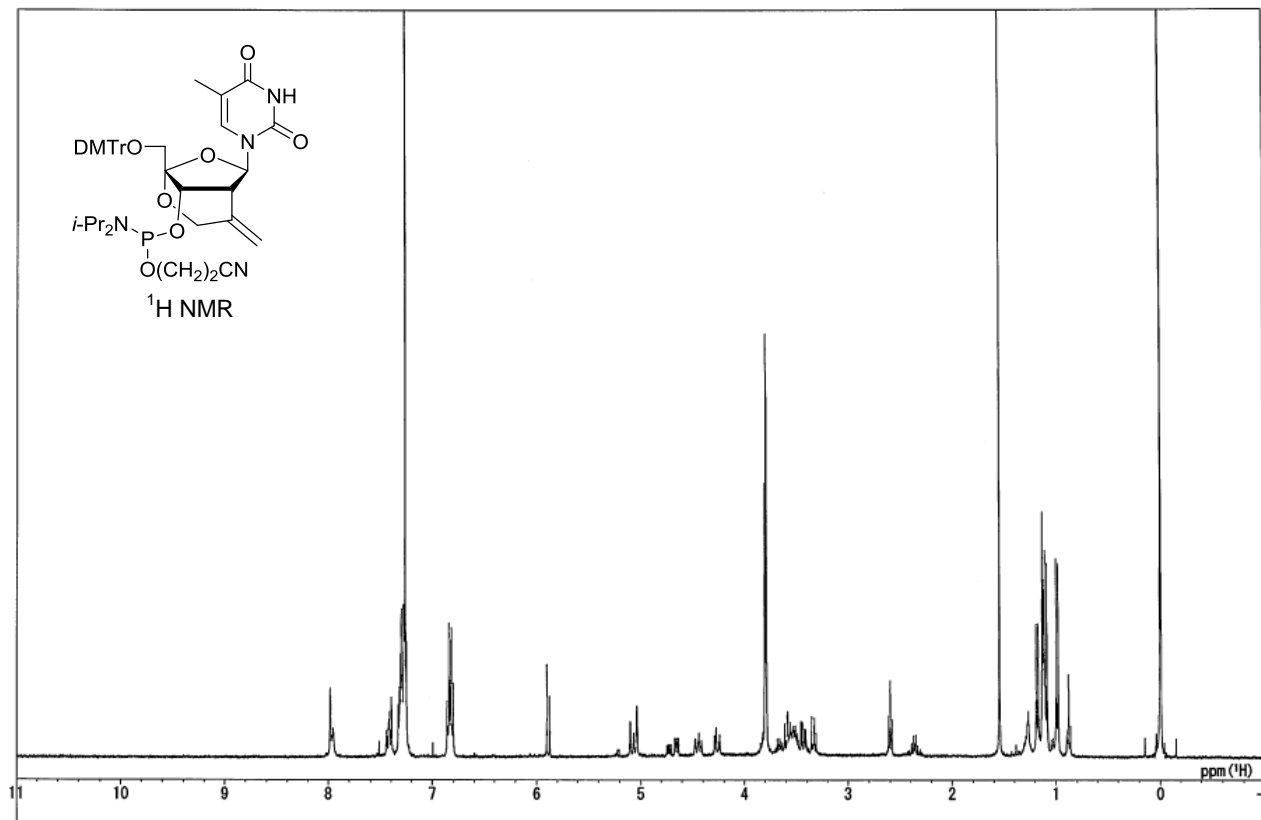
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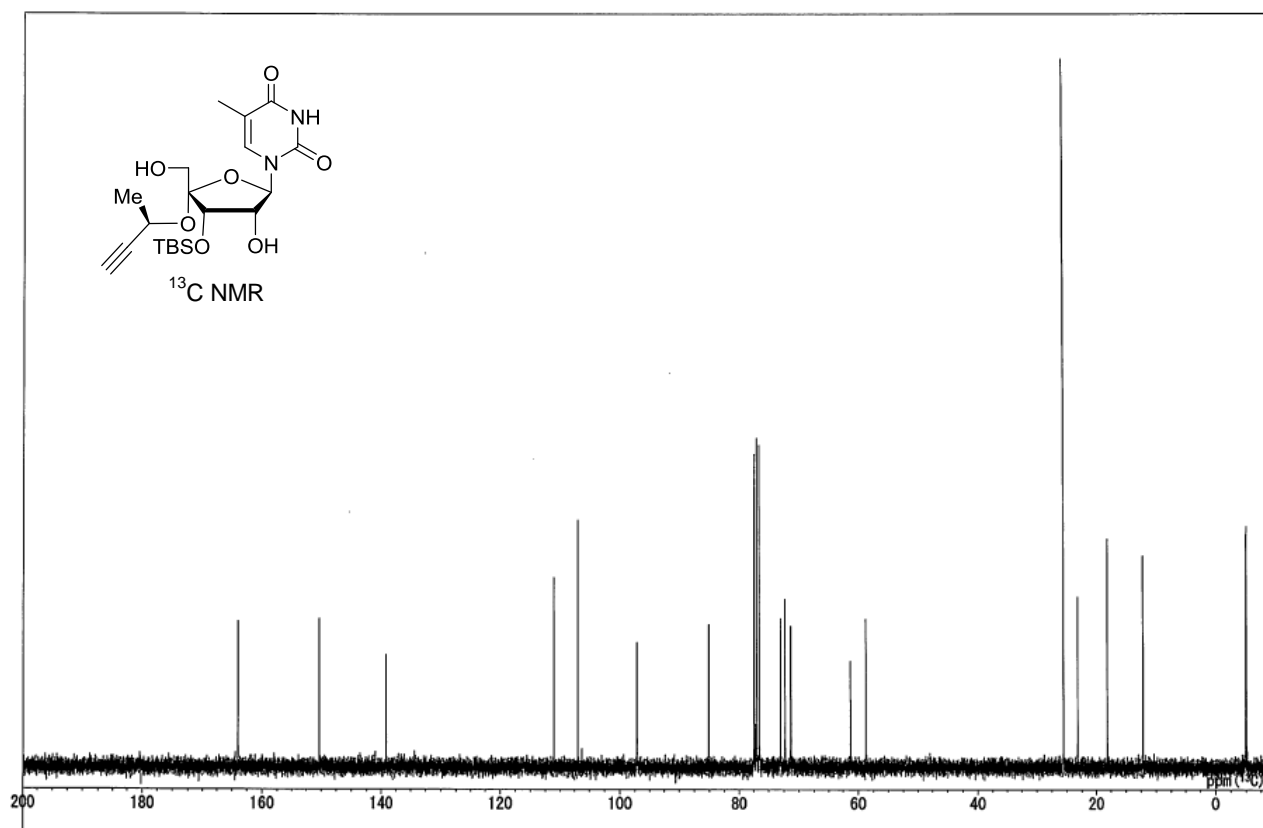
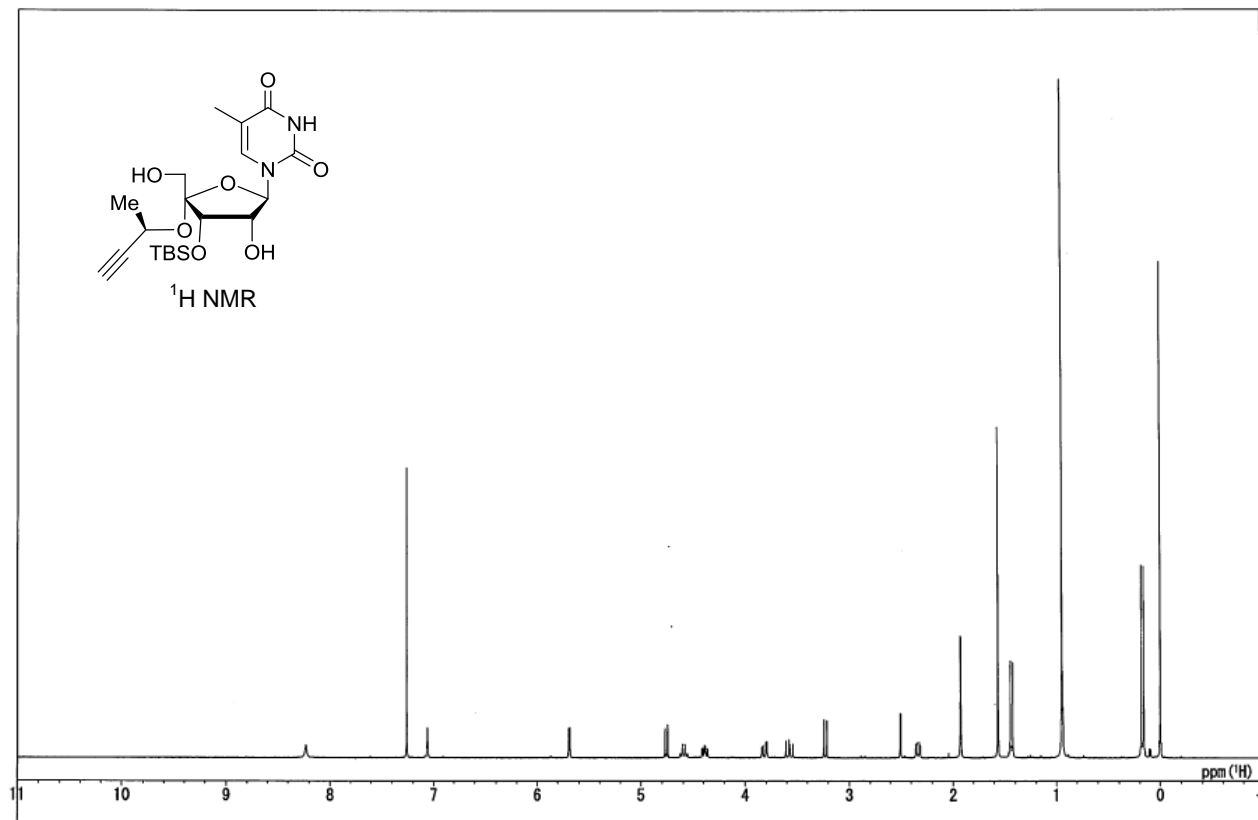
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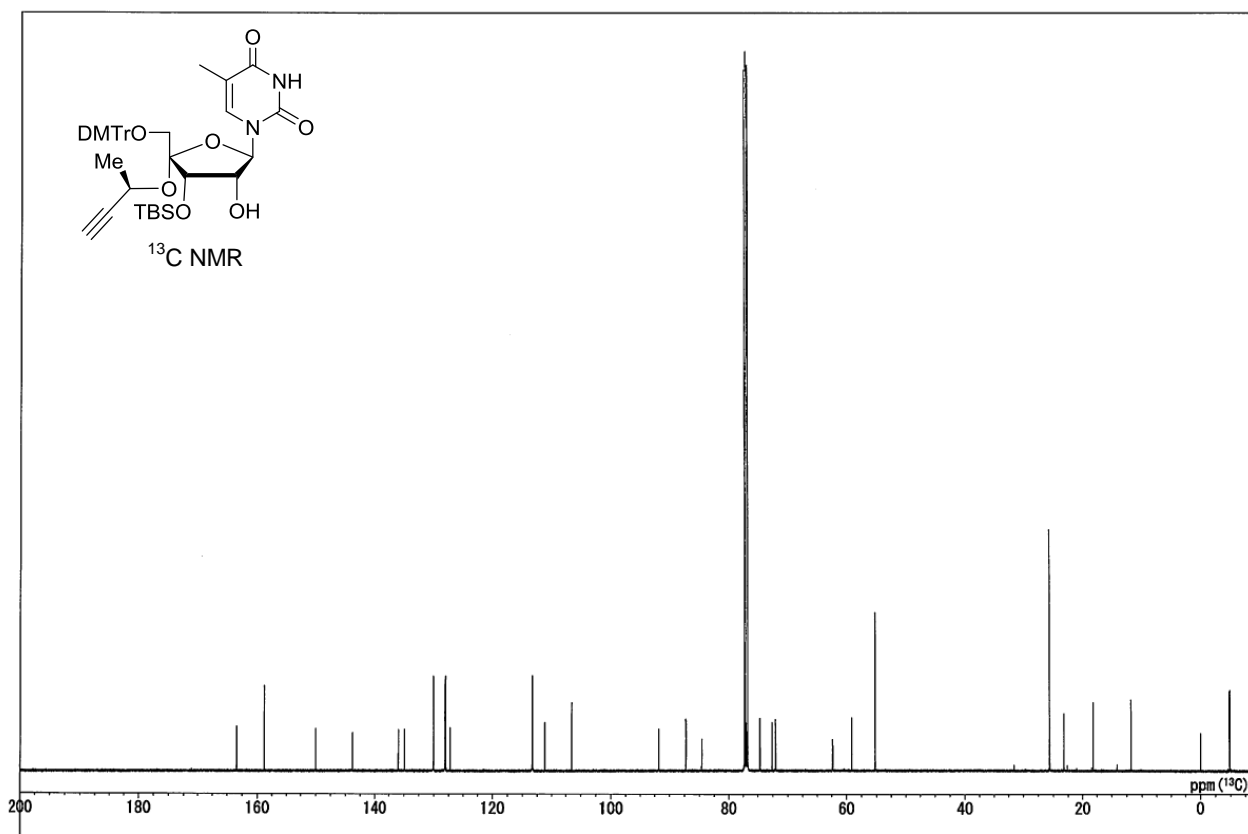
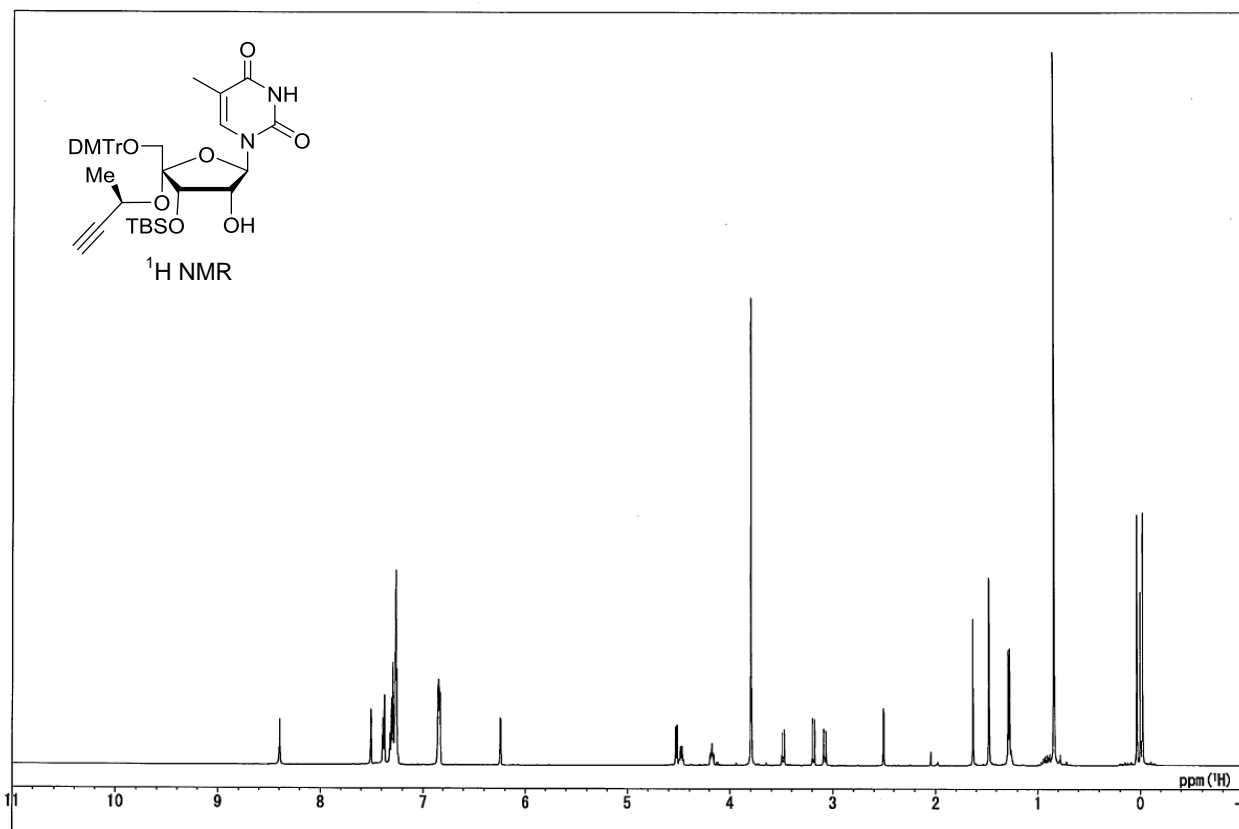
(Compound **10a** in CDCl₃)



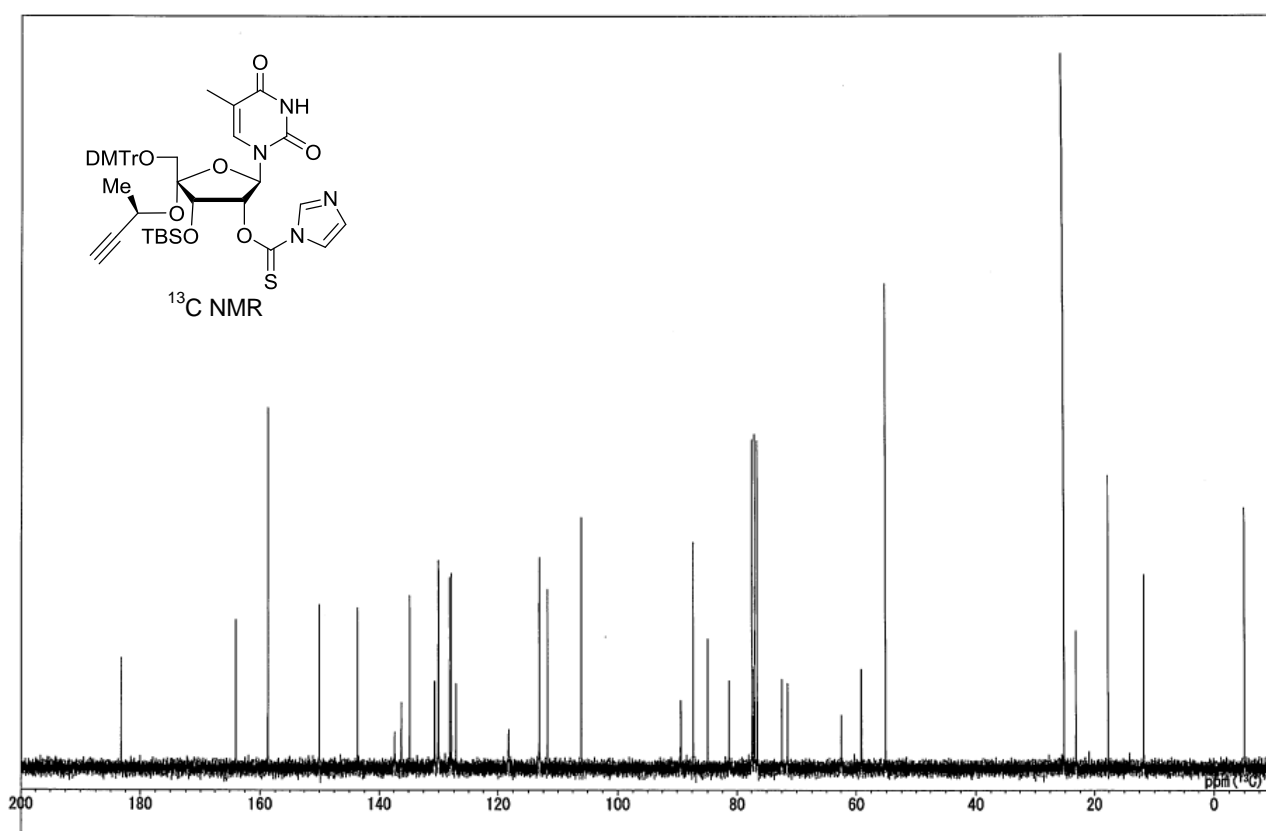
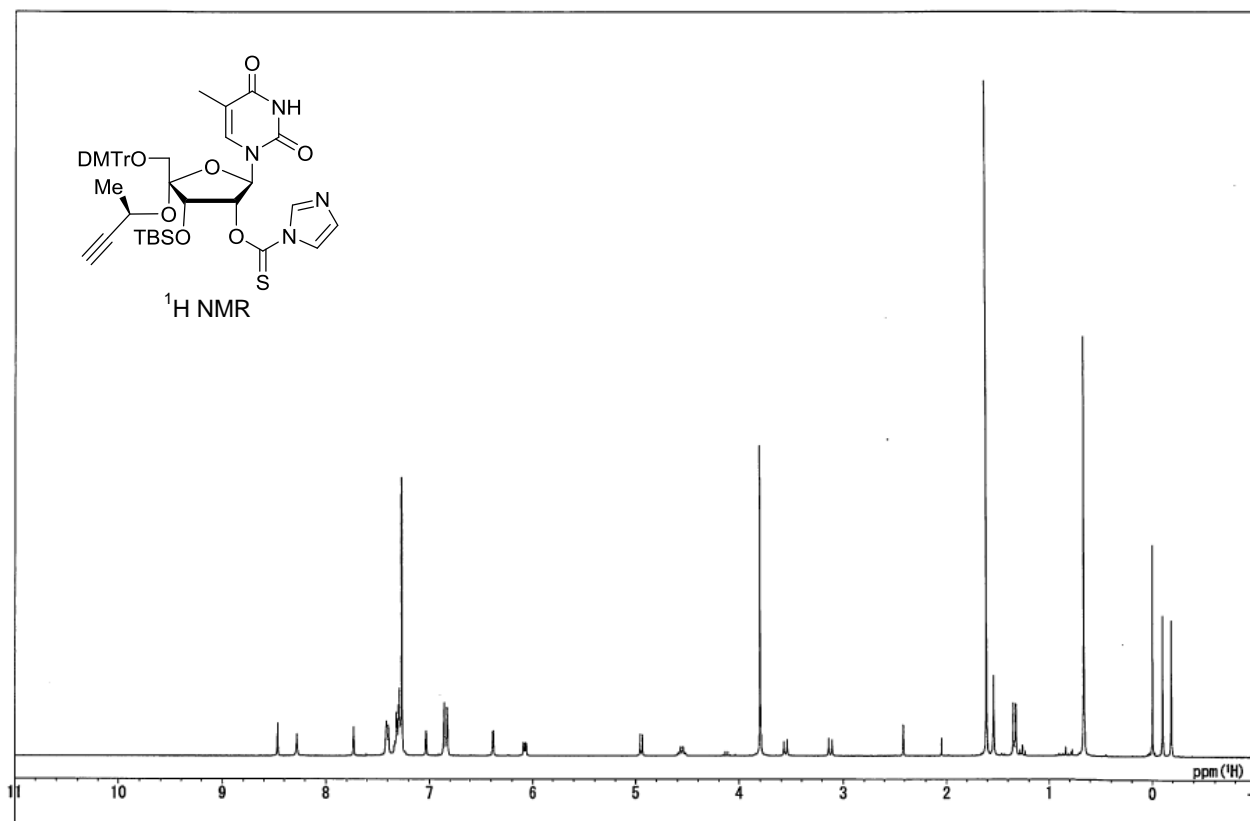
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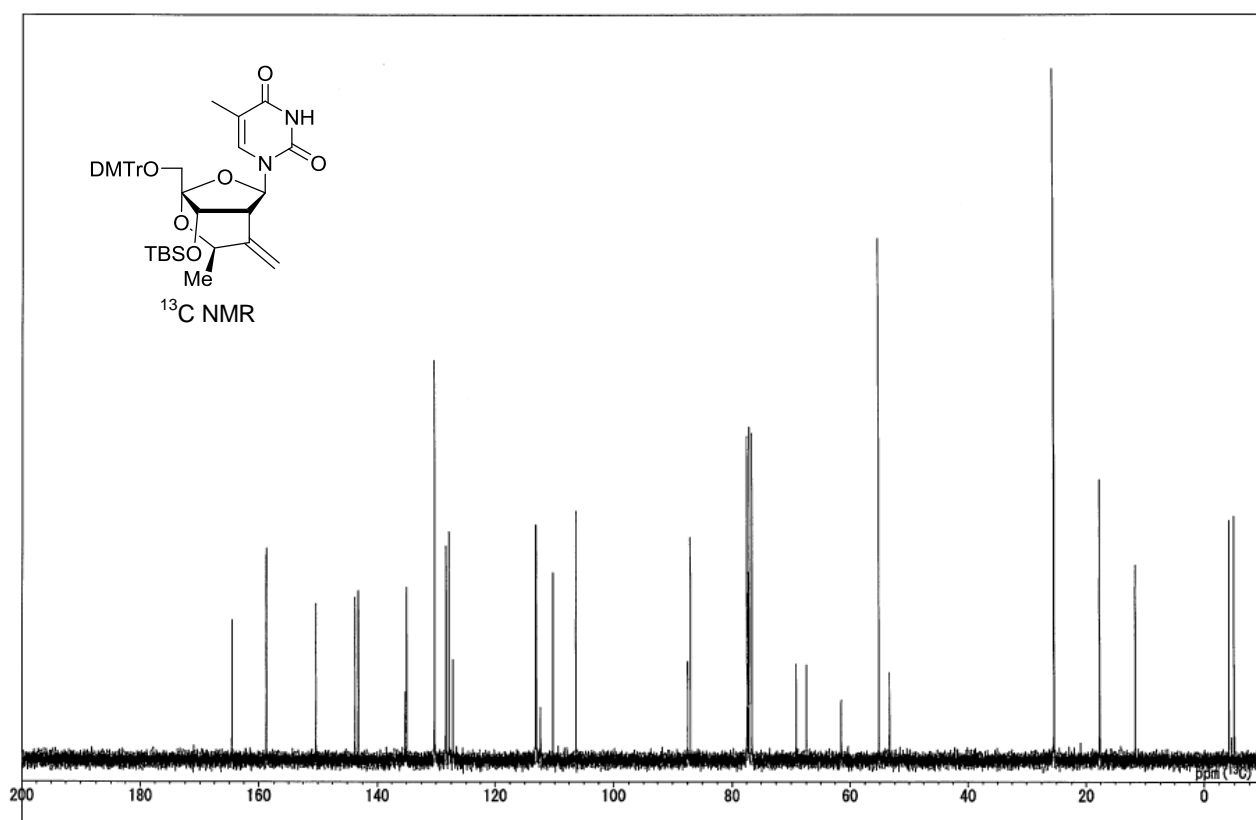
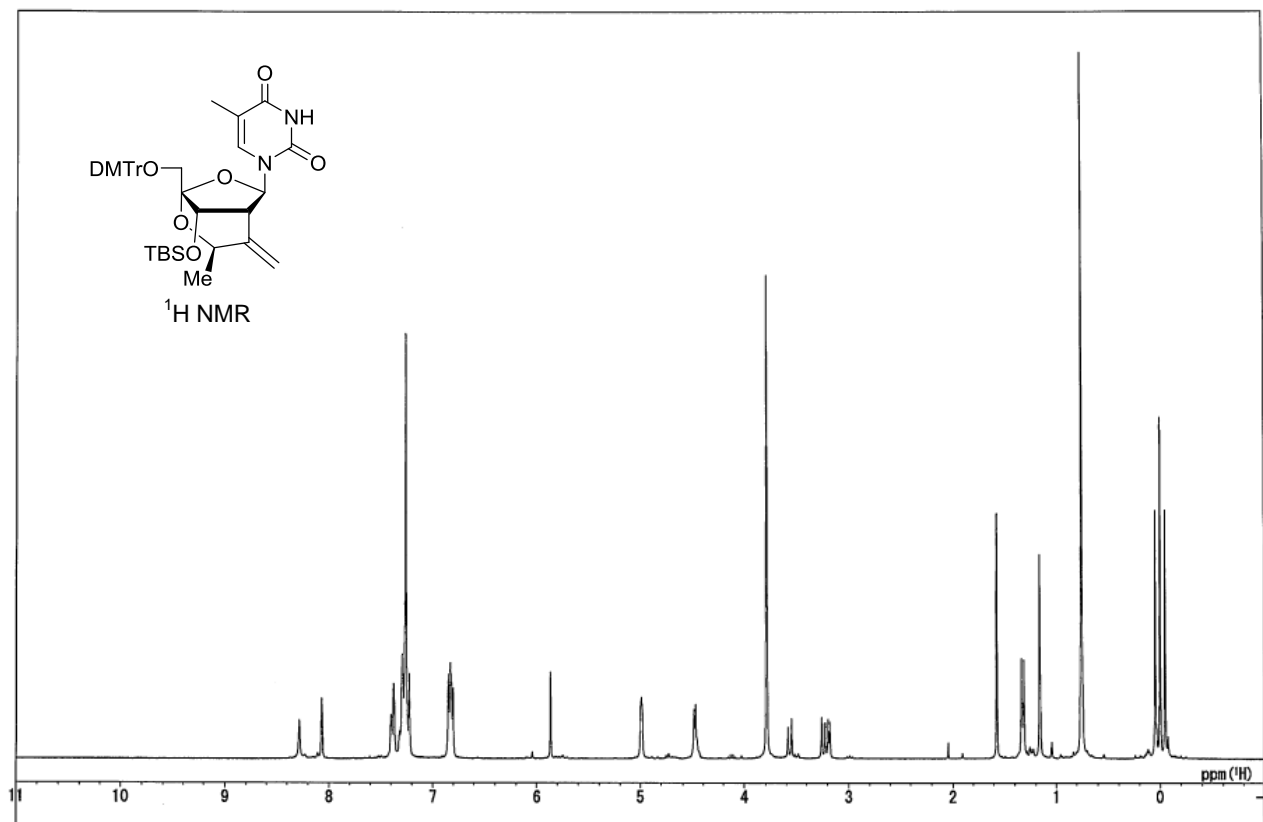
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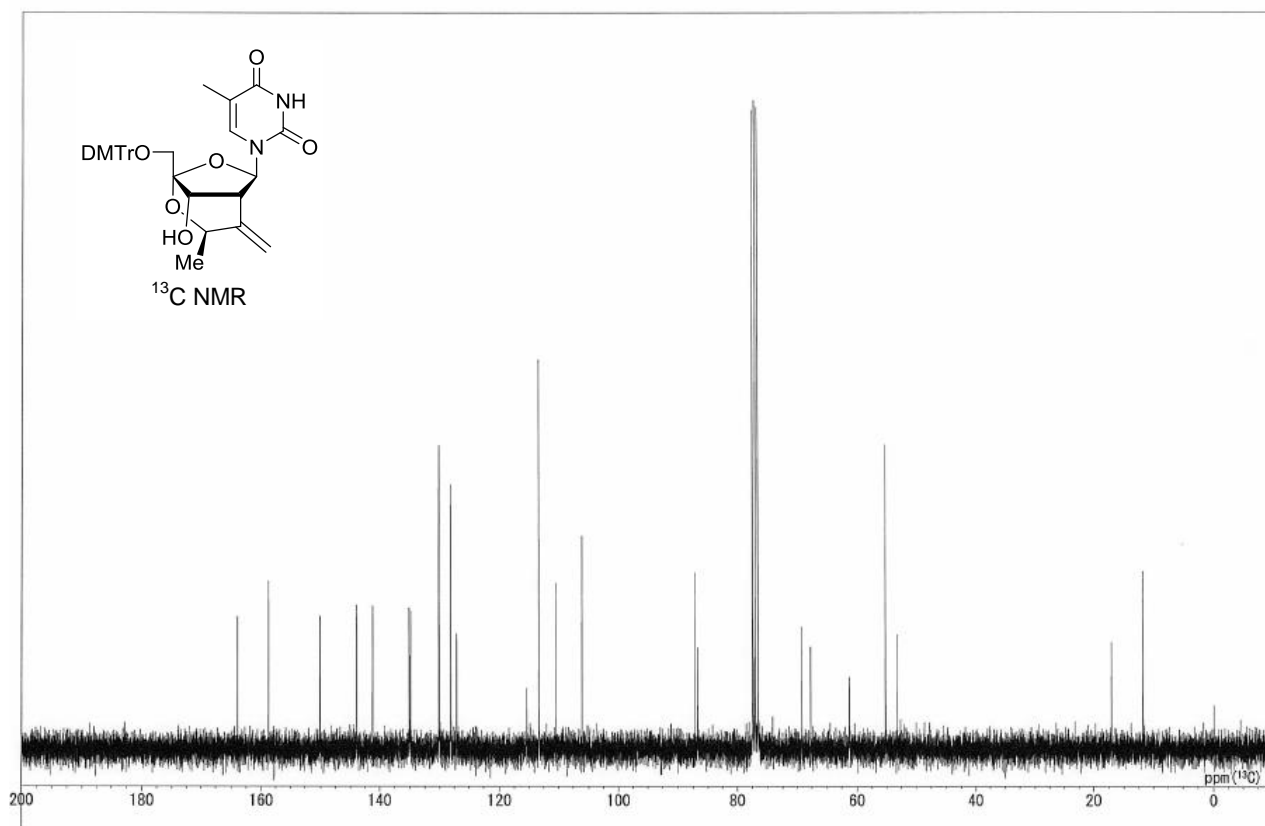
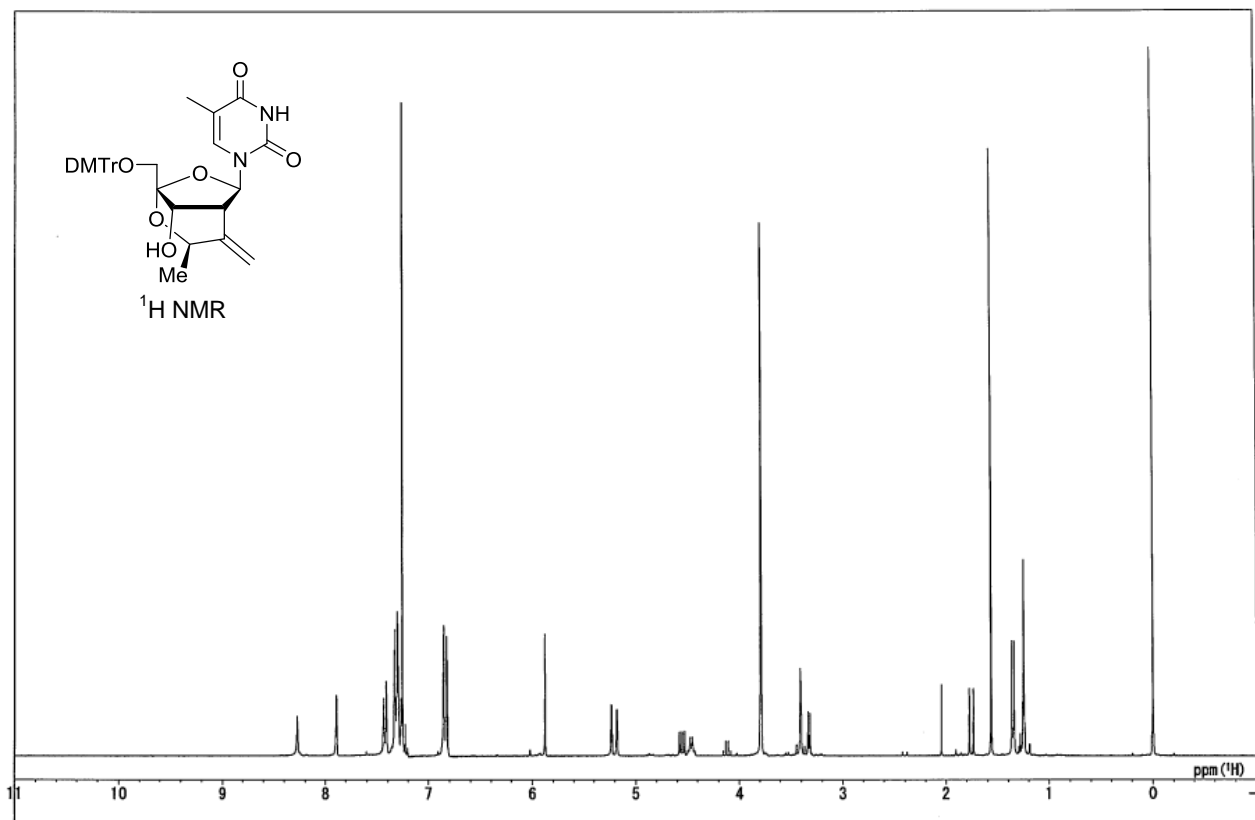
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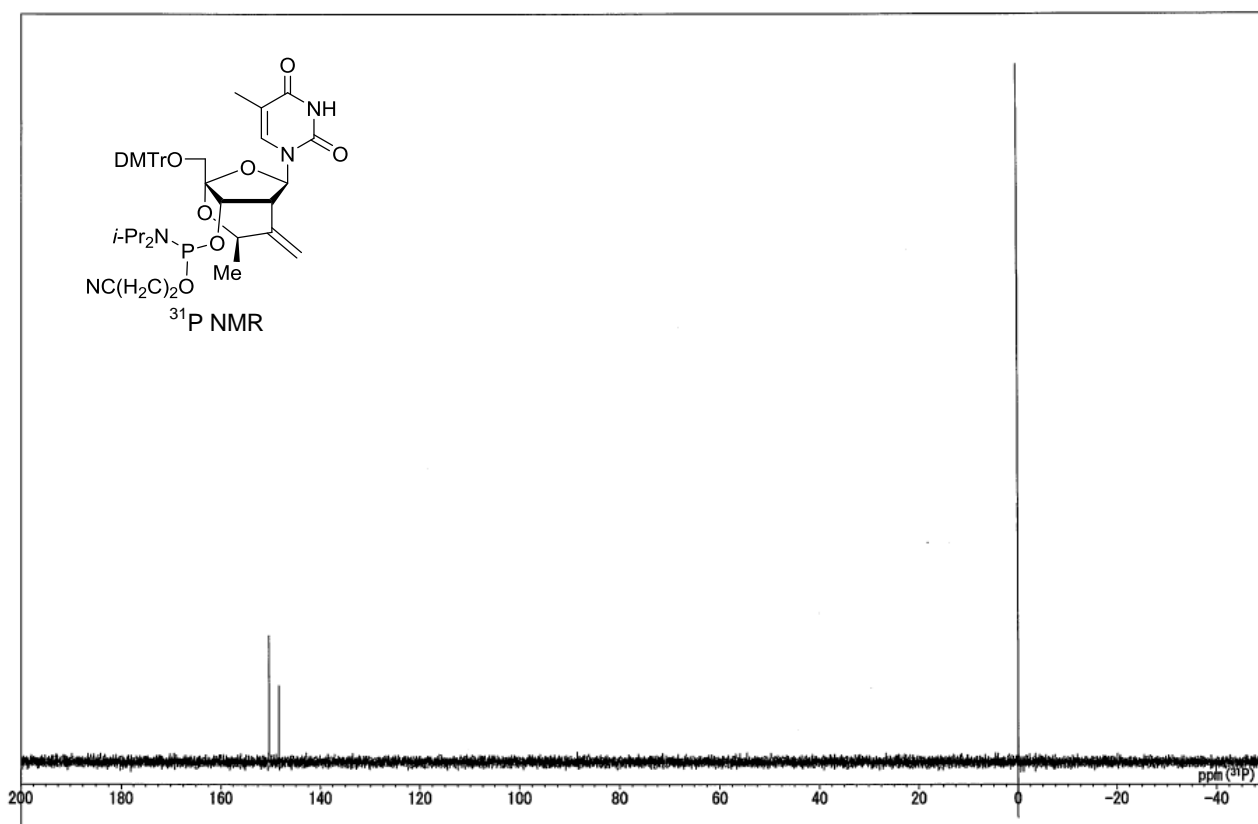
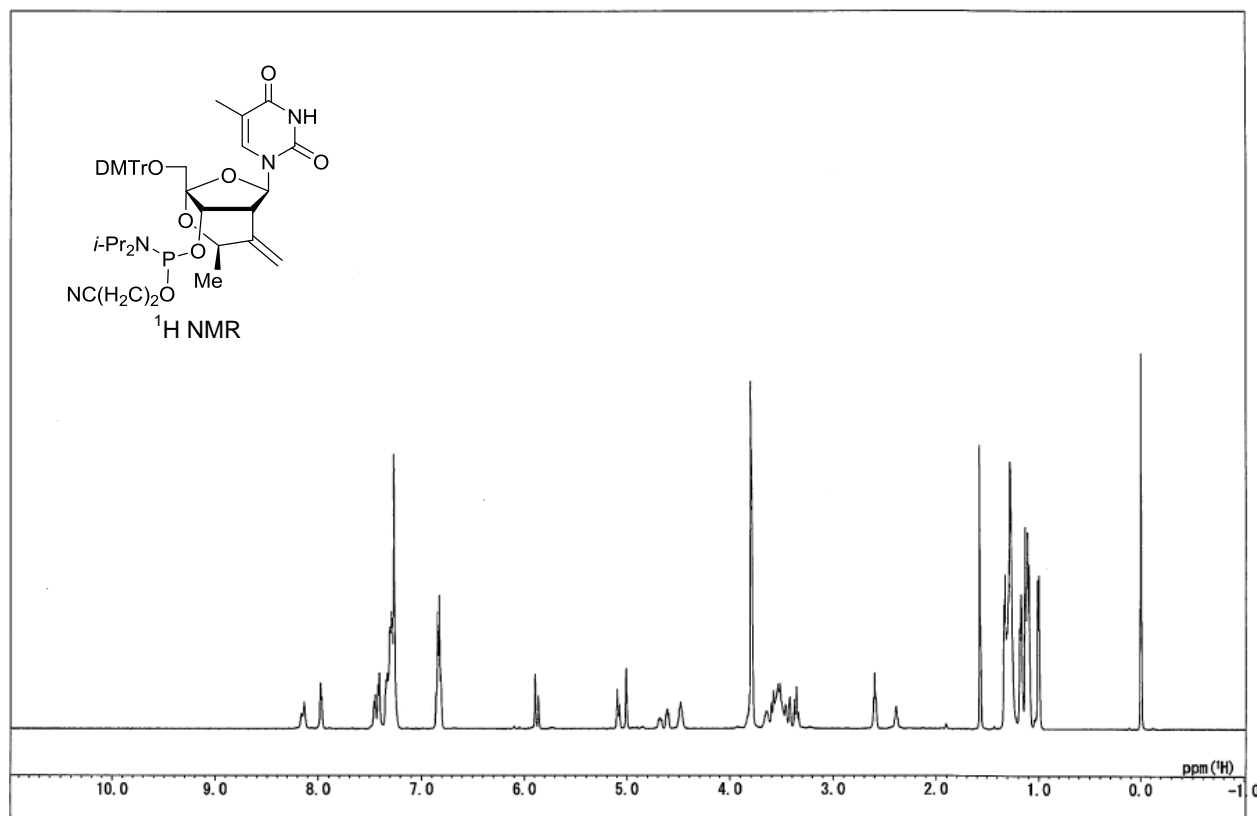
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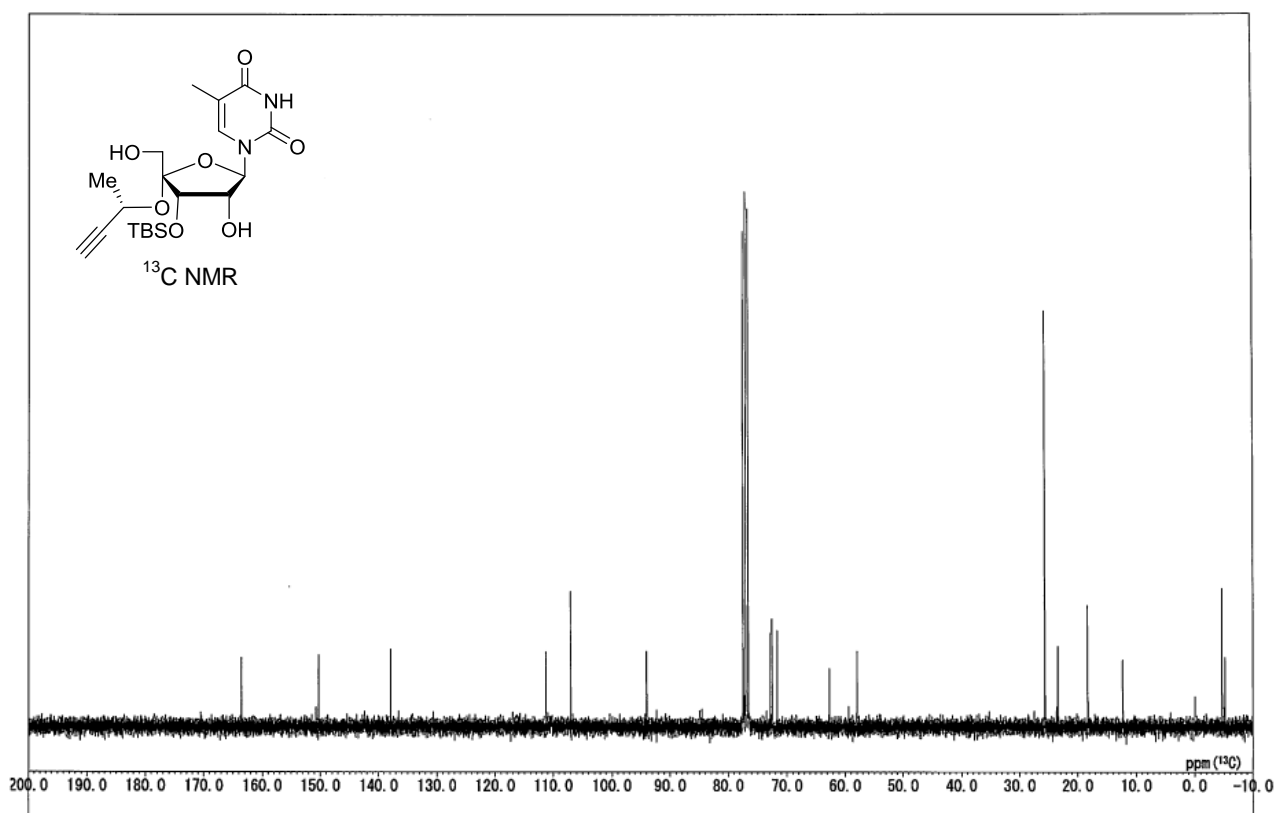
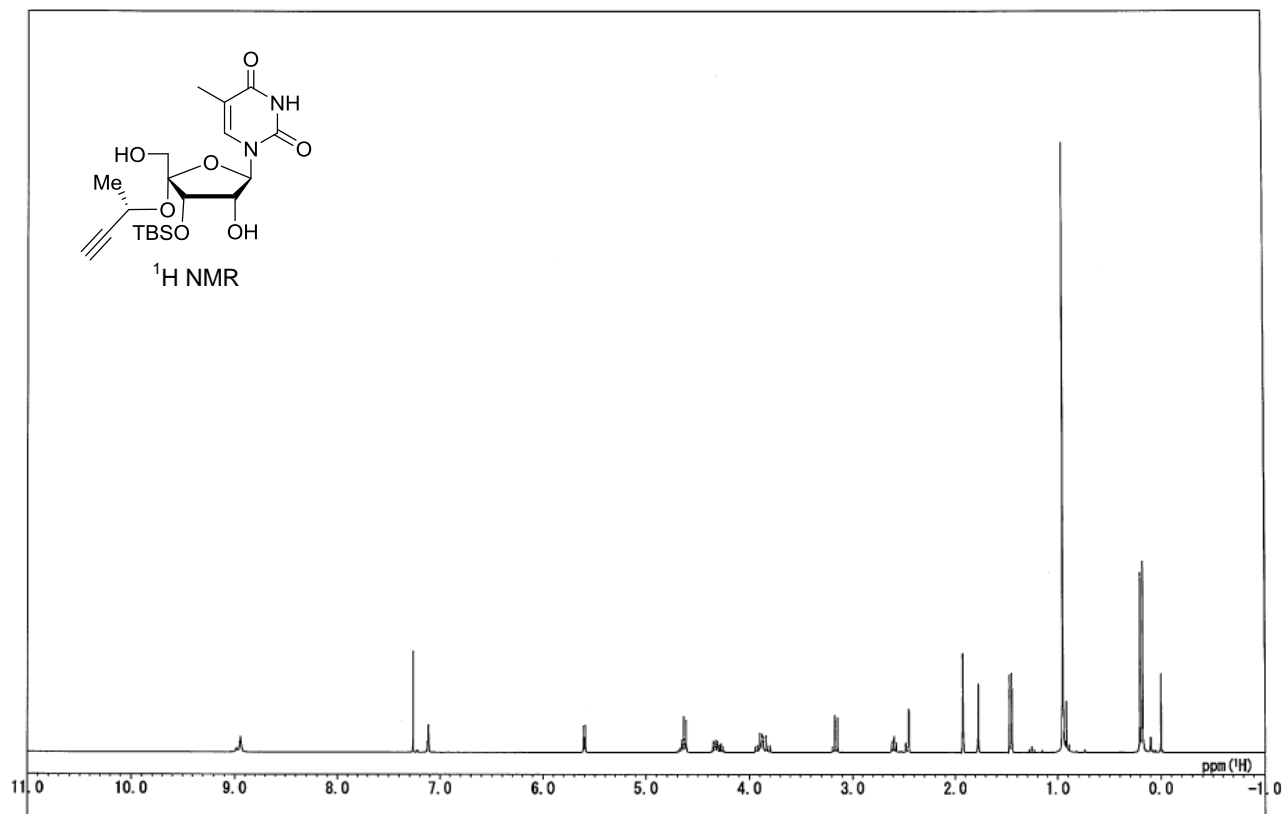
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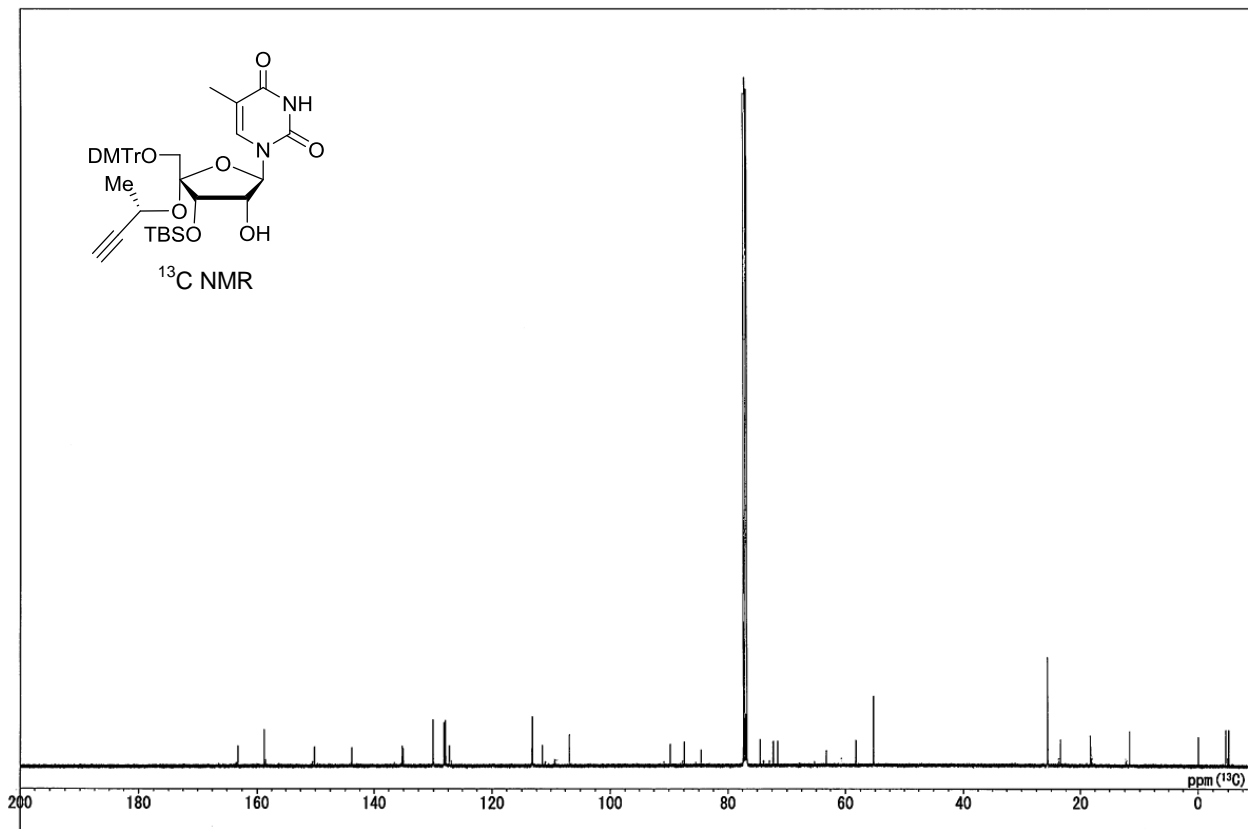
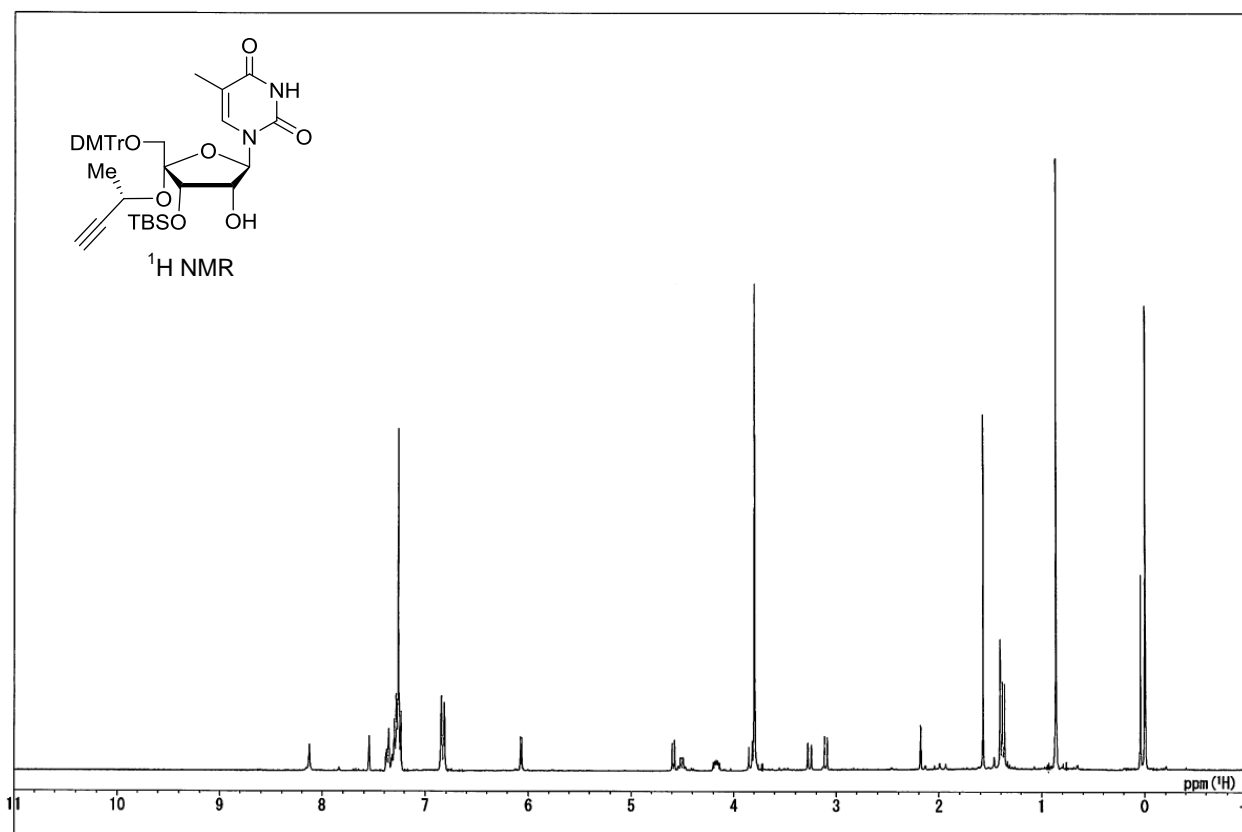
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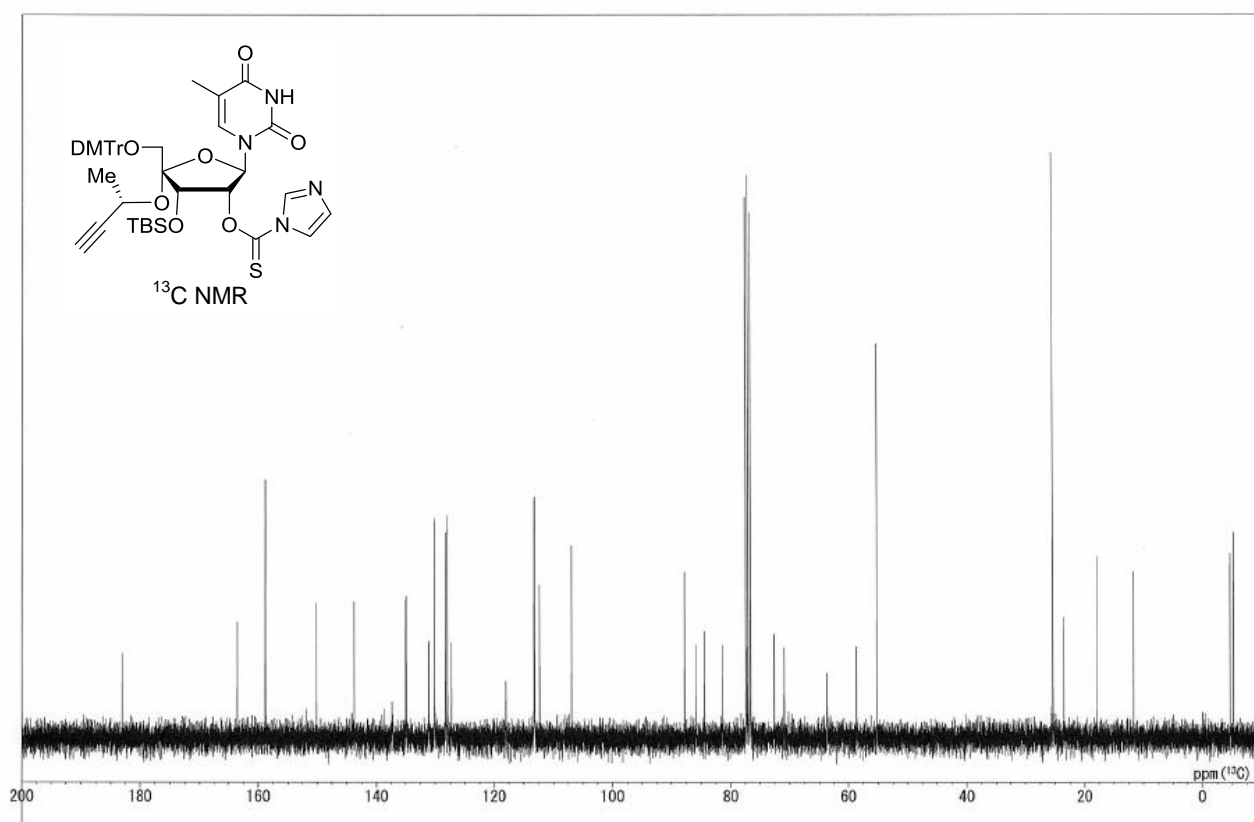
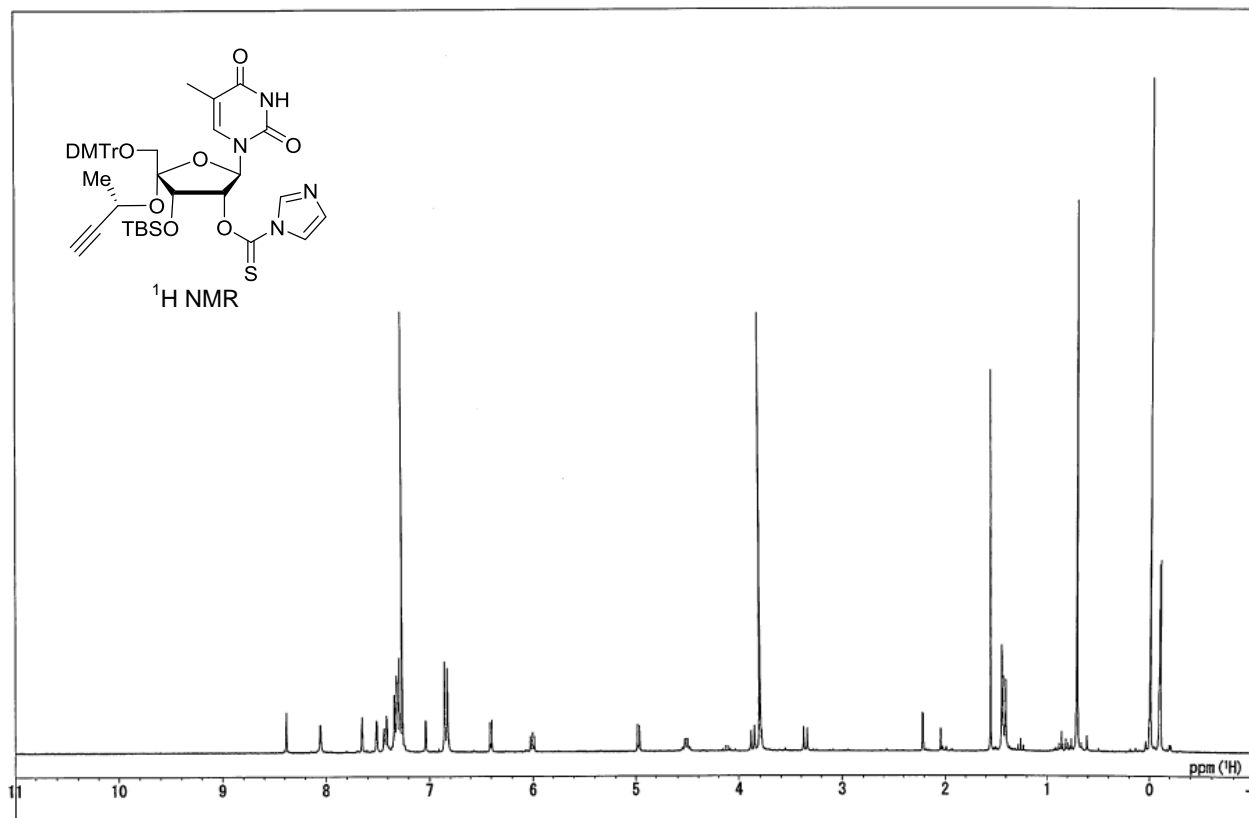
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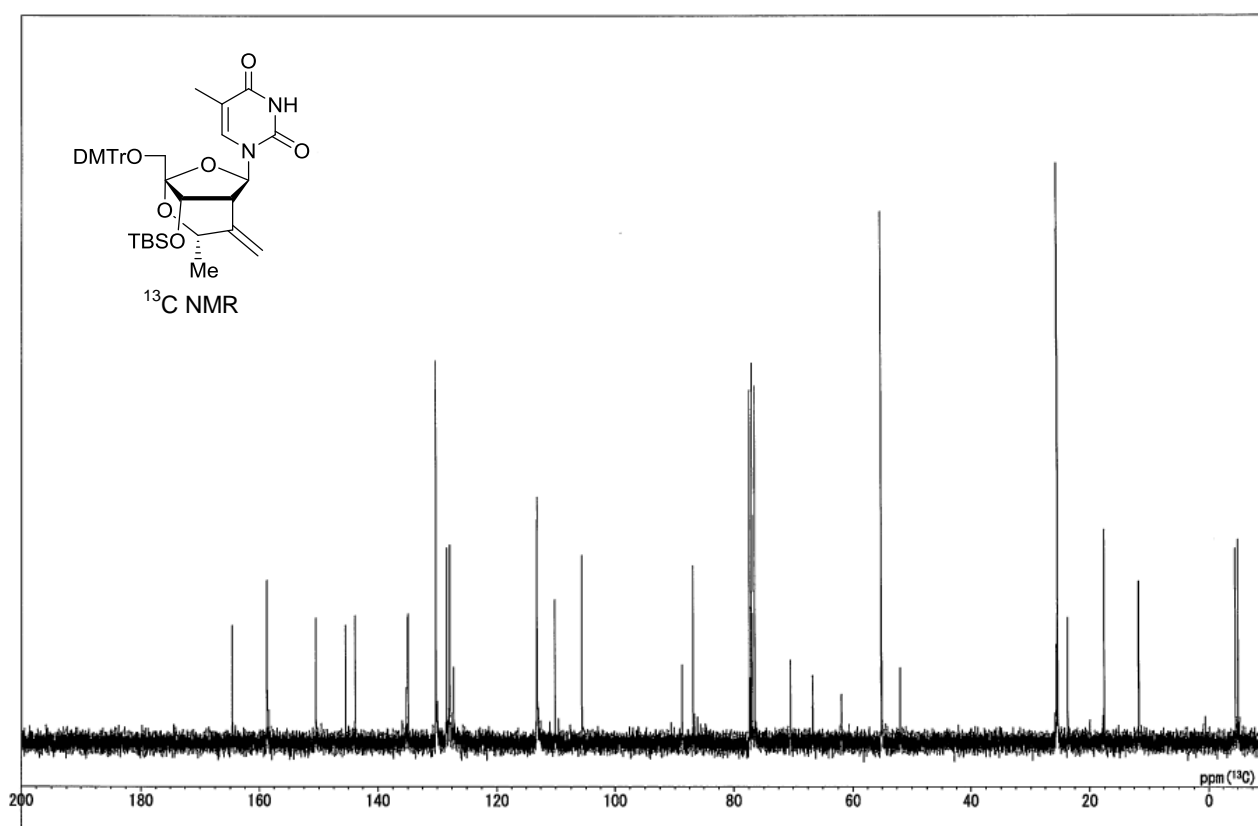
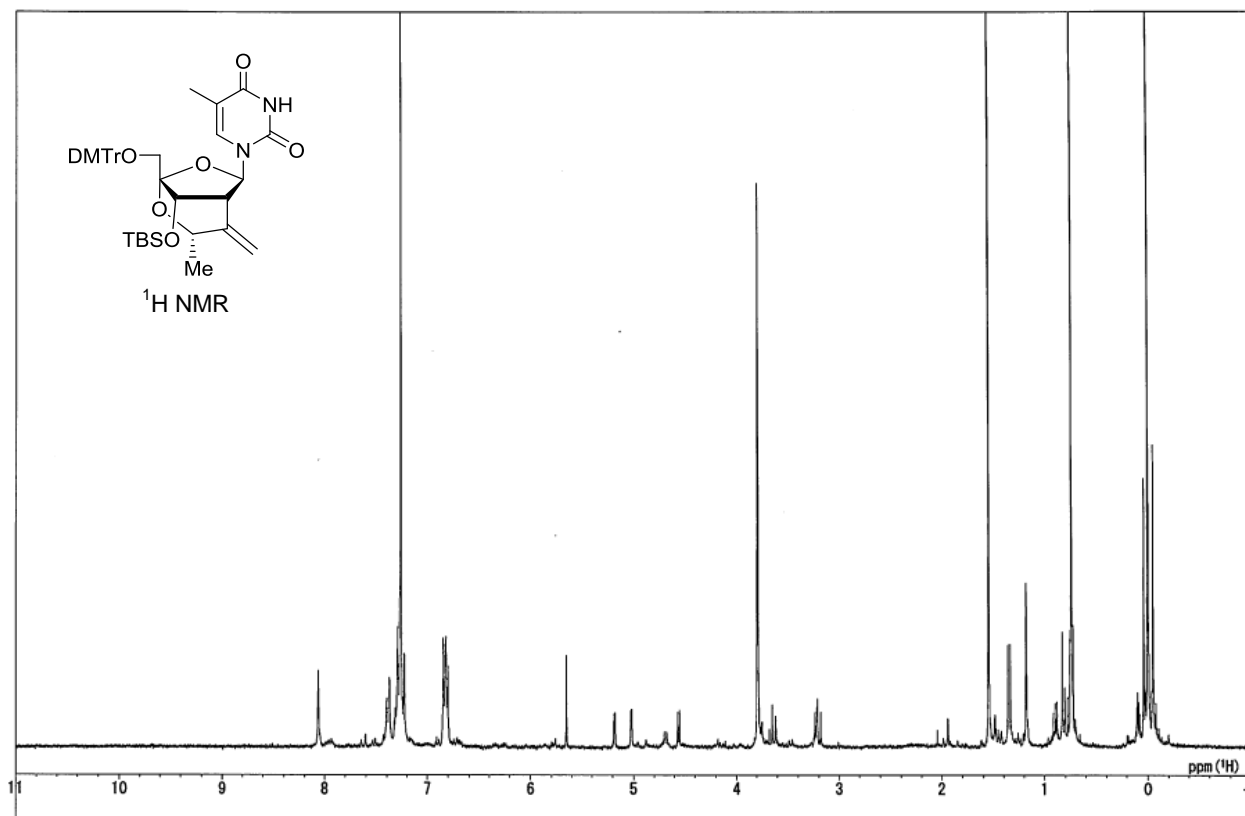
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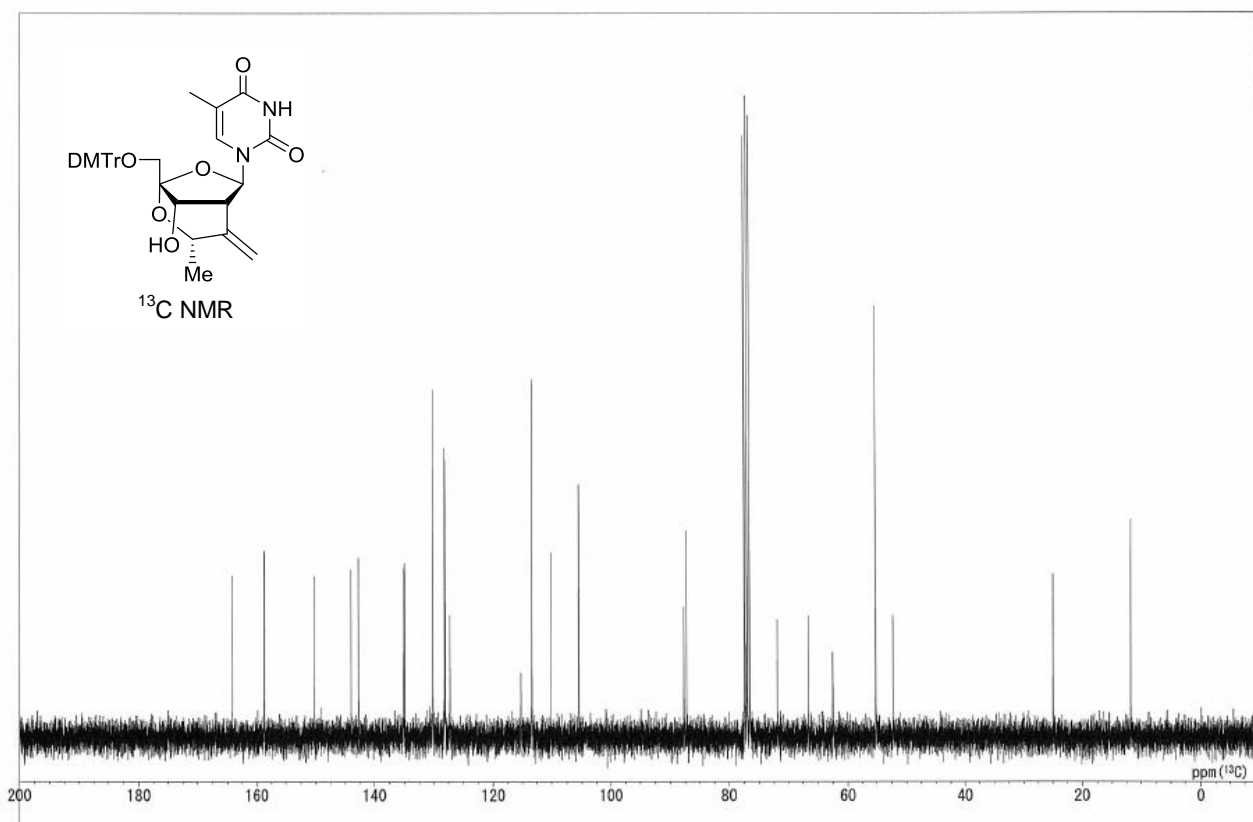
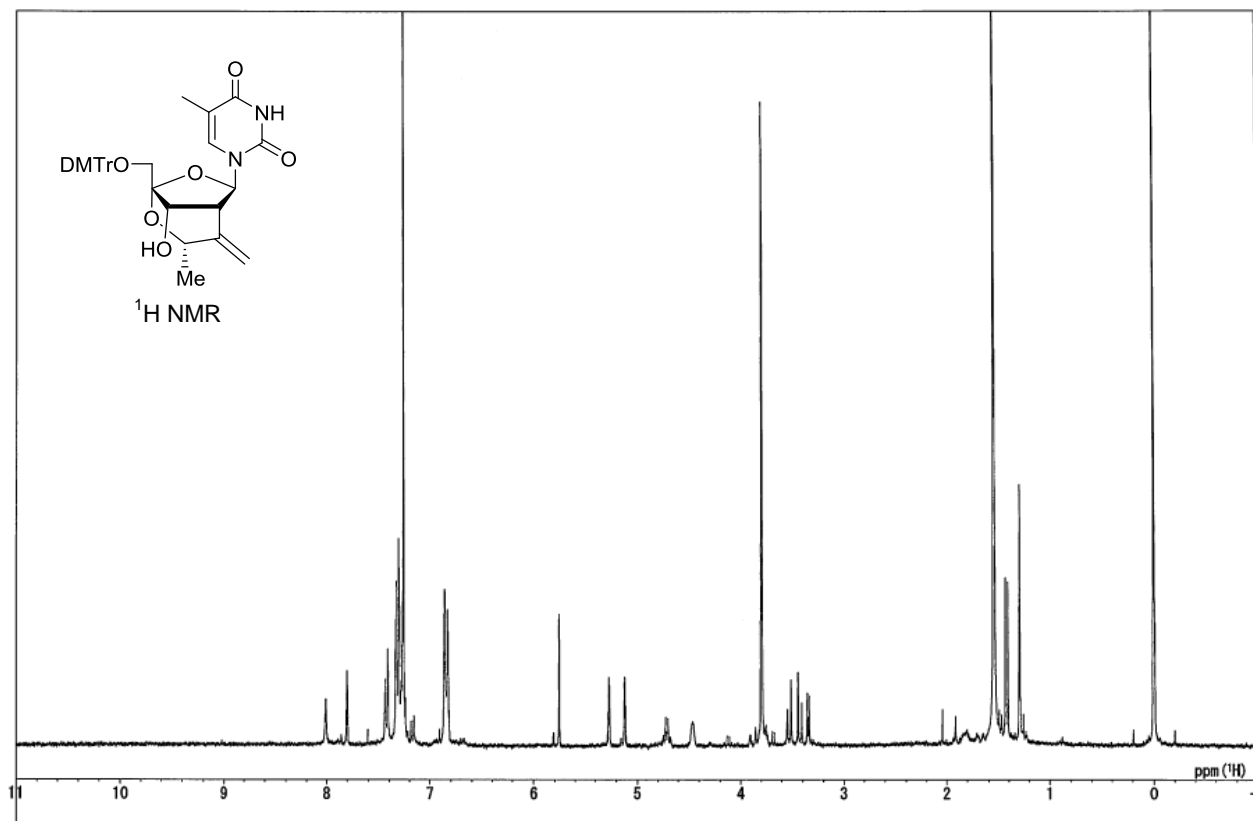
(Compound 7c in CDCl₃)



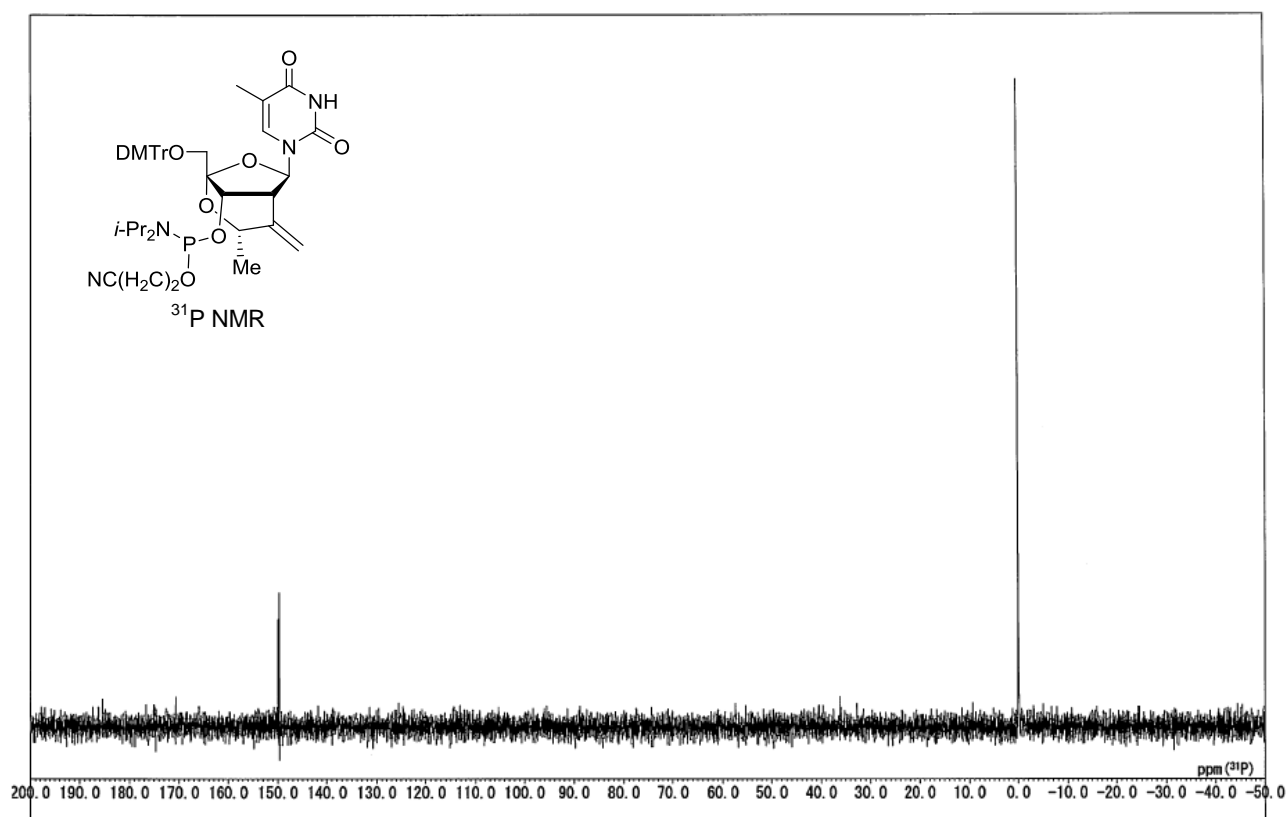
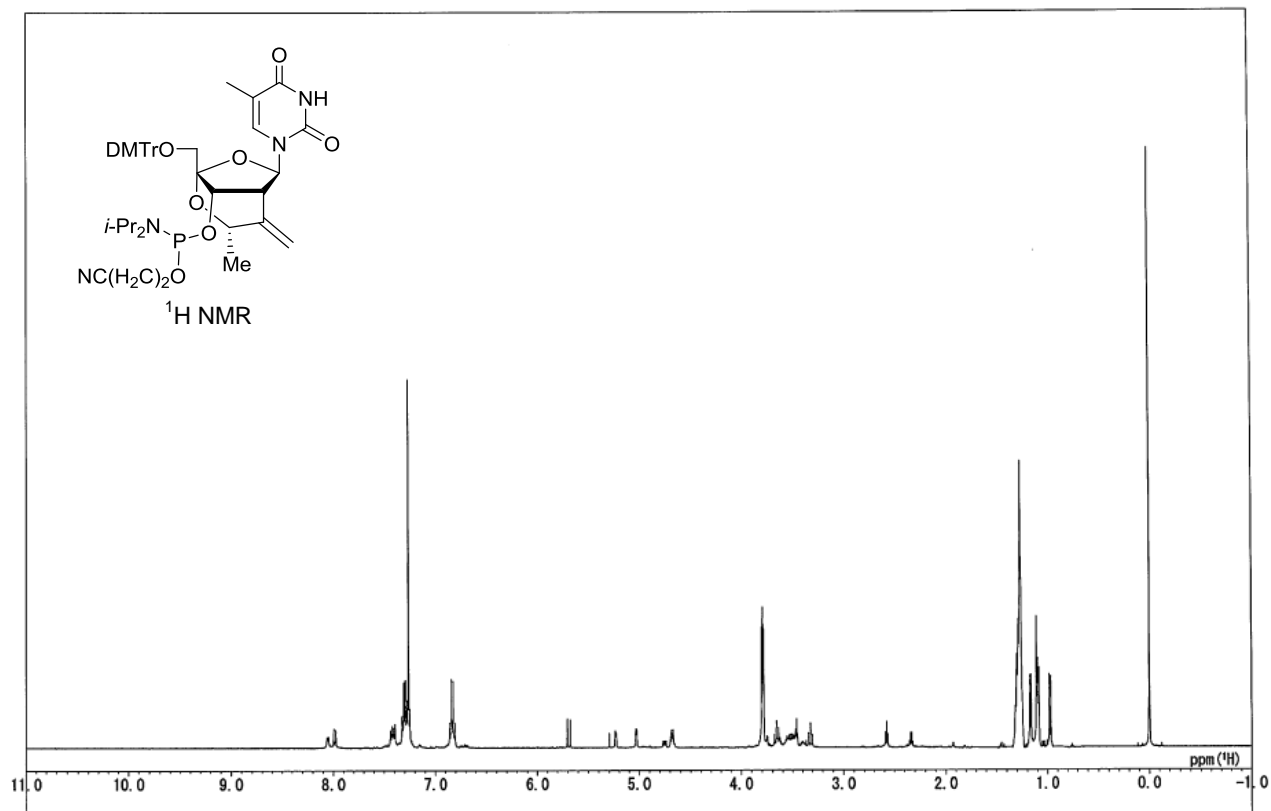
(Compound **8c** in CDCl₃)



(Compound **9c** in CDCl₃)



(Compound **10c** in CDCl₃)

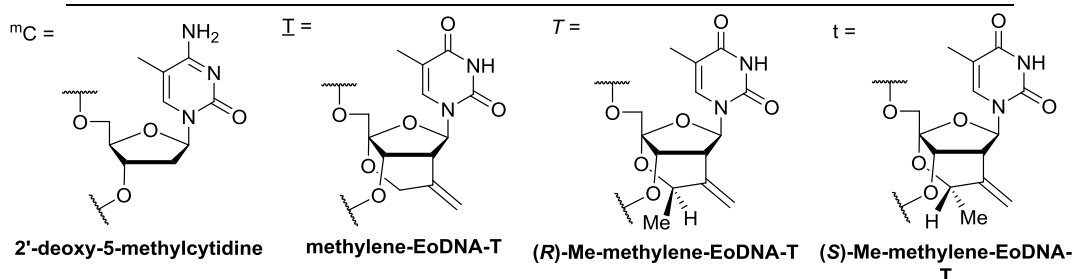


6. Synthesis and purification of oligonucleotides ON1–15

Phosphoramidites (**10a**, **10b** and **10c**), dT-phosphoramidite (Proligo) and d^mC(Ac)-phosphoramidite (Proligo) were used and the 0.2 μmol scale synthesis of oligonucleotides was performed on an automated DNA synthesizer (Gene Design nS-8) using a standard phosphoramidite protocol (DMTr-ON mode). For introduction of all methylene-EoDNA phosphoramidites **10a**, **10b** and **10c**, coupling time and waiting time for detritylation were prolonged to 10 min and 30 sec × 2, respectively. 1M *t*-BuOOH in toluene³ was used as an oxidizing agent. Sequences of synthesized oligonucleotides (**ON1–15**) are shown in Table S1. Then, **ON1–15** were prepared by cleavage from CPG supports, deprotection of the nucleobase and phosphate moieties [28% NH₃ aq, rt, 1.5 h]. Removal of ammonia was carried out *in vacuo*. The crude **ON1–15** were purified with Sep-Pak[®] Plus C18 cartridges (Waters), followed by reversed-phase HPLC (Waters XBridge[®] MS C₁₈ 2.5 μm, 10×50 mm). The compositions of **ON1–15** were confirmed by MALDI-TOF mass analysis (Table S1).

Table S1. Sequences of synthesized oligonucleotides (**ON1–15**) and MALDI-TOF mass spectra data for **ON1–15**

Oligonucleotides (5'-3')	Calcd. [M-H] ⁻	Found [M-H] ⁻
d(T ^m CTT ^m CTTTT ^m CT ^m CT) (ON1)	4245.84	4235.47
d(T ^m CTT ^m CTTTT ^m CT ^m CT) (ON2)	4253.94	4253.06
d(T ^m CTT ^m CITTTT ^m CT ^m CT) (ON3)	4253.94	4253.78
d(T ^m CTT ^m CTTTT ^m CT ^m CT) (ON4)	4462.03	4461.99
d(T ^m CTT ^m CTTTT ^m CT ^m CT) (ON5)	4259.89	4260.74
d(T ^m CTT ^m CTTTT ^m CT ^m CT) (ON6)	4396.04	4395.06
d(T ^m CTT ^m CTTTT ^m CT ^m CT) (ON7)	4396.04	4397.33
d(T ^m CTT ^m CTTTT ^m CT ^m CT) (ON8)	4532.19	4532.43
d(T ^m CTT ^m CTTtT ^m CT ^m CT) (ON9)	4259.89	4260.56
d(T ^m CTT ^m CTttT ^m CT ^m CT) (ON10)	4396.04	4395.78
d(T ^m CTT ^m CtTtT ^m CT ^m CT) (ON11)	4396.04	4396.92
d(T ^m CTt ^m CtTtT ^m Ct ^m CT) (ON12)	4532.19	4532.04
TTTTTTTTT (ON13)	3032.65	3032.87
TTTTTTTTT (ON14)	3047.05	3047.69
TTTTTTTTt (ON15)	3047.05	3046.34



7. HPLC charts of oligonucleotides 1-15.

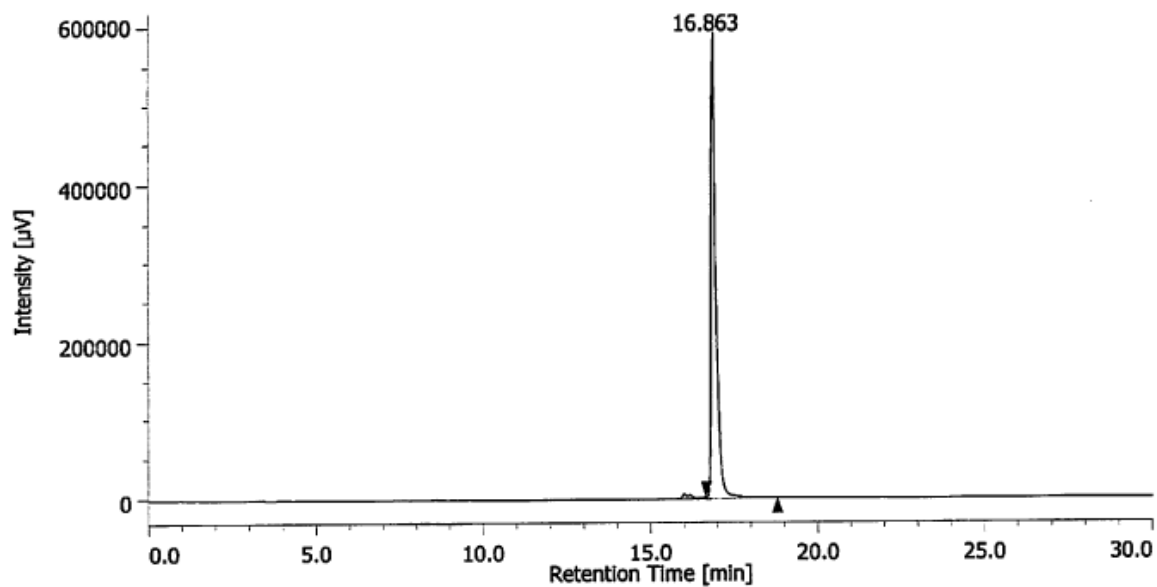
(ON1)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



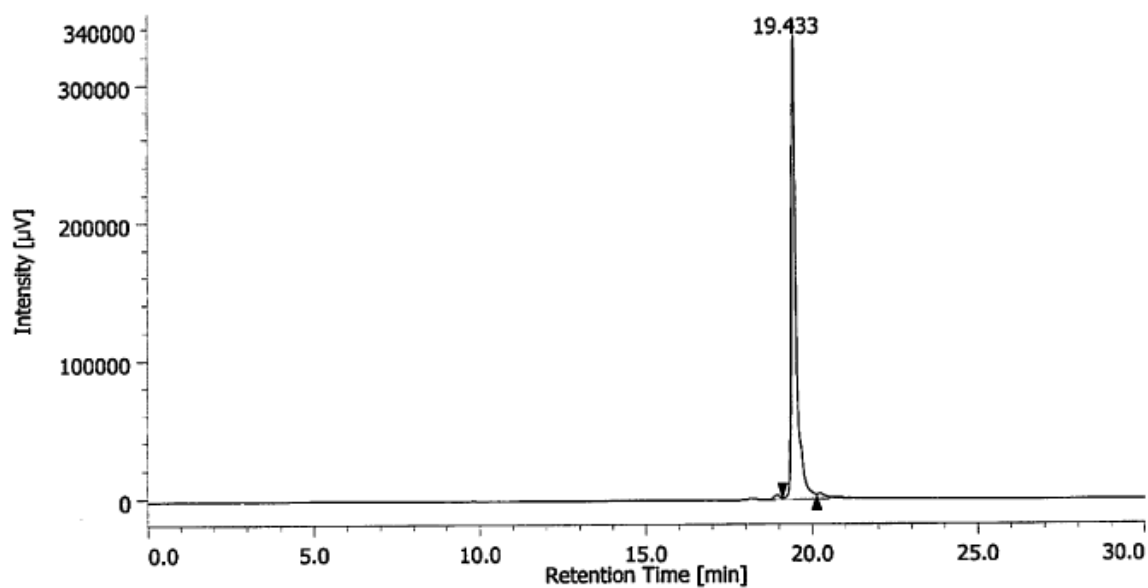
(ON2)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



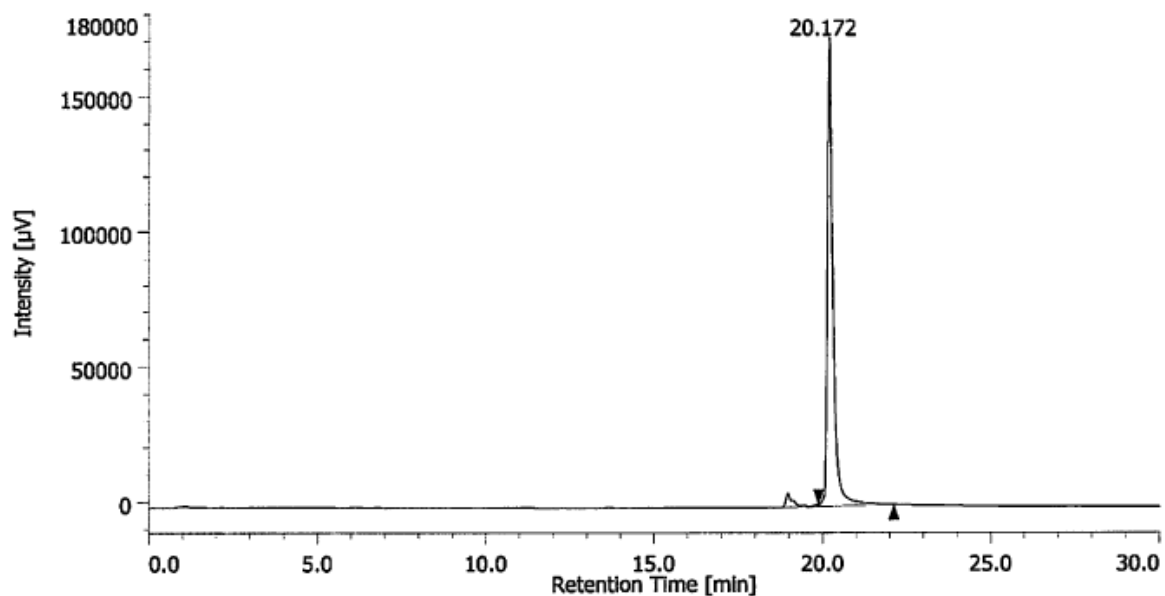
(ON3)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



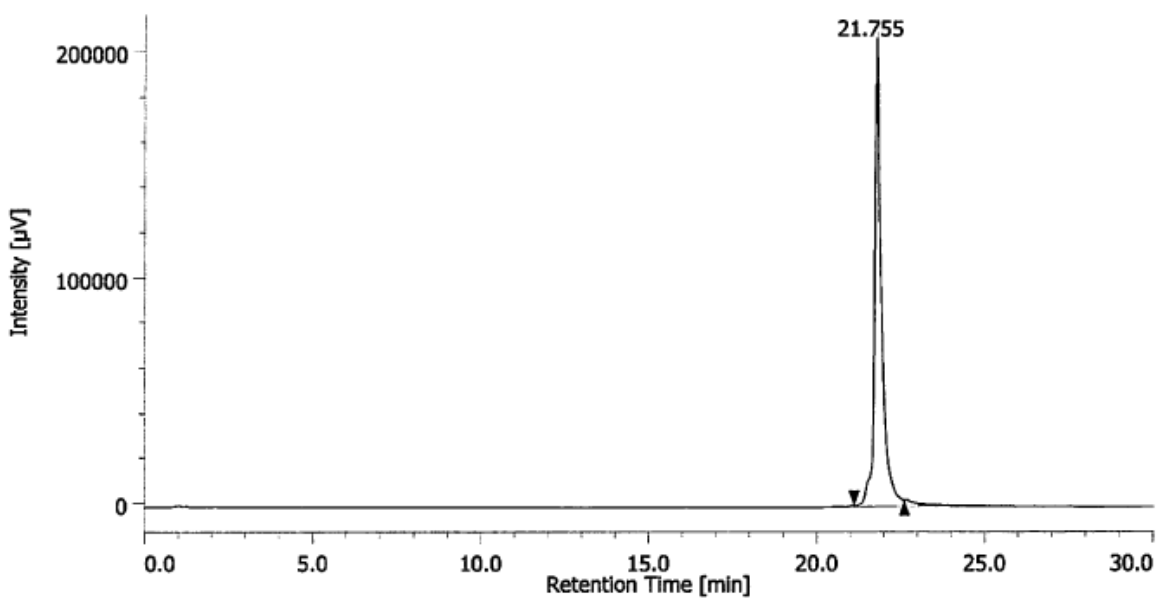
(ON4)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



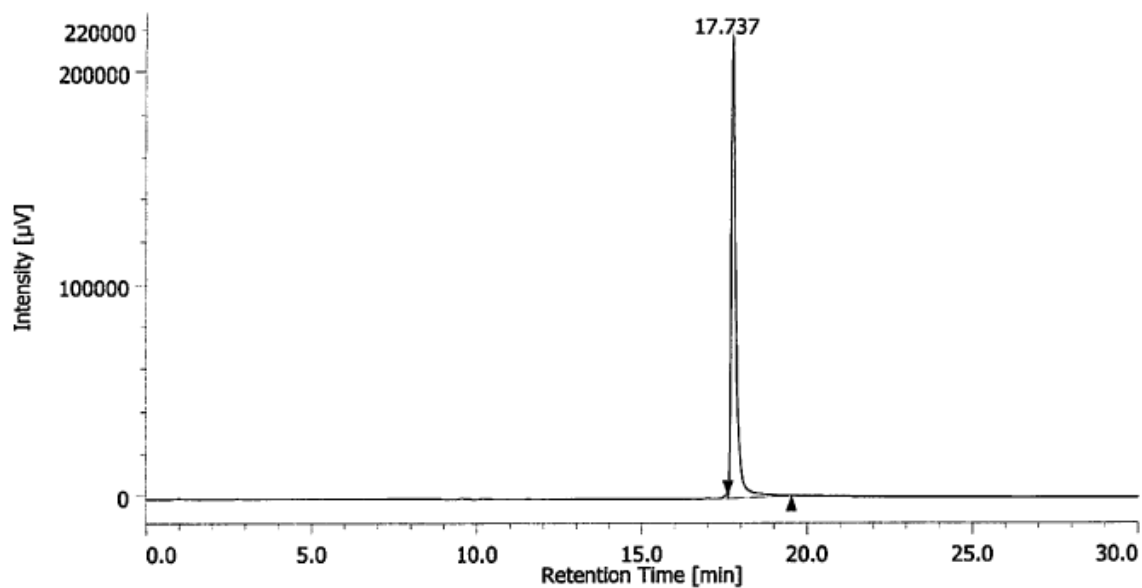
(ON5)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



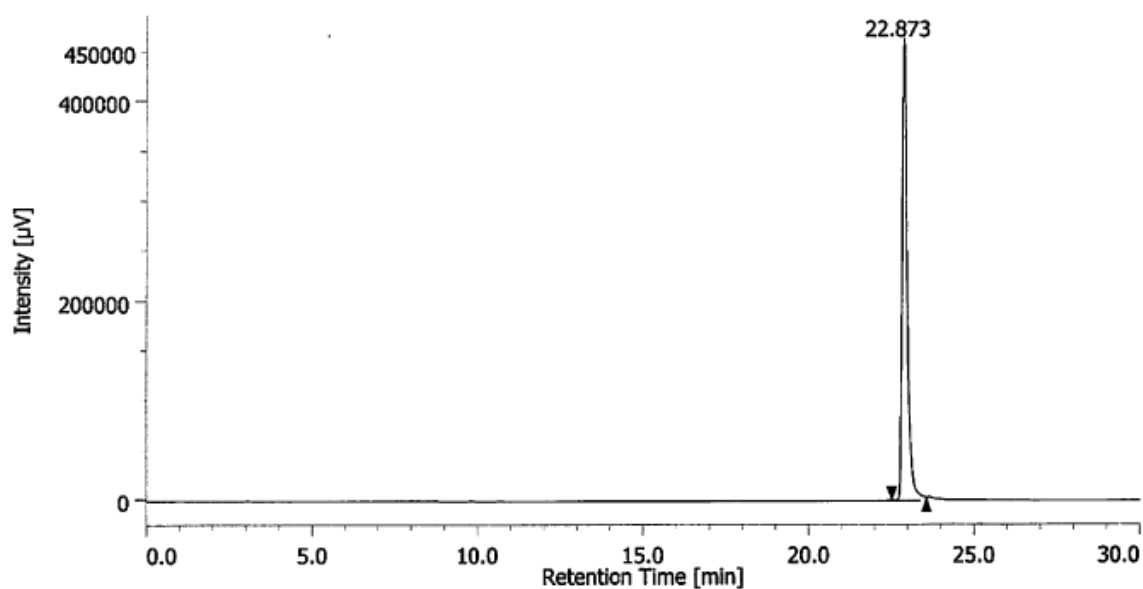
(ON6)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

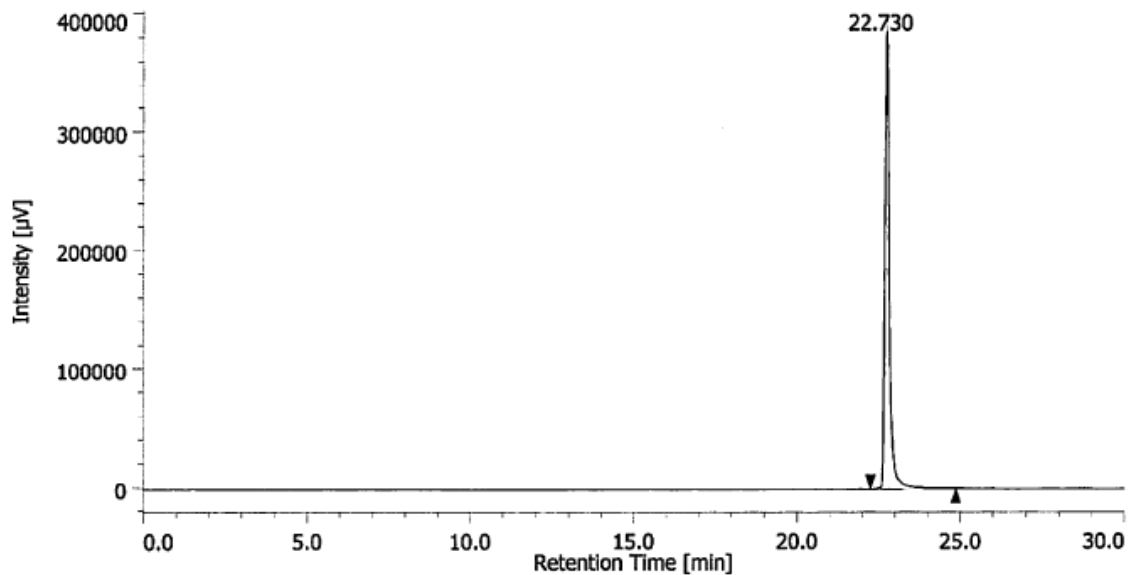
Flow rate : 1.0 mL/min.

Column temp. : 40°C.



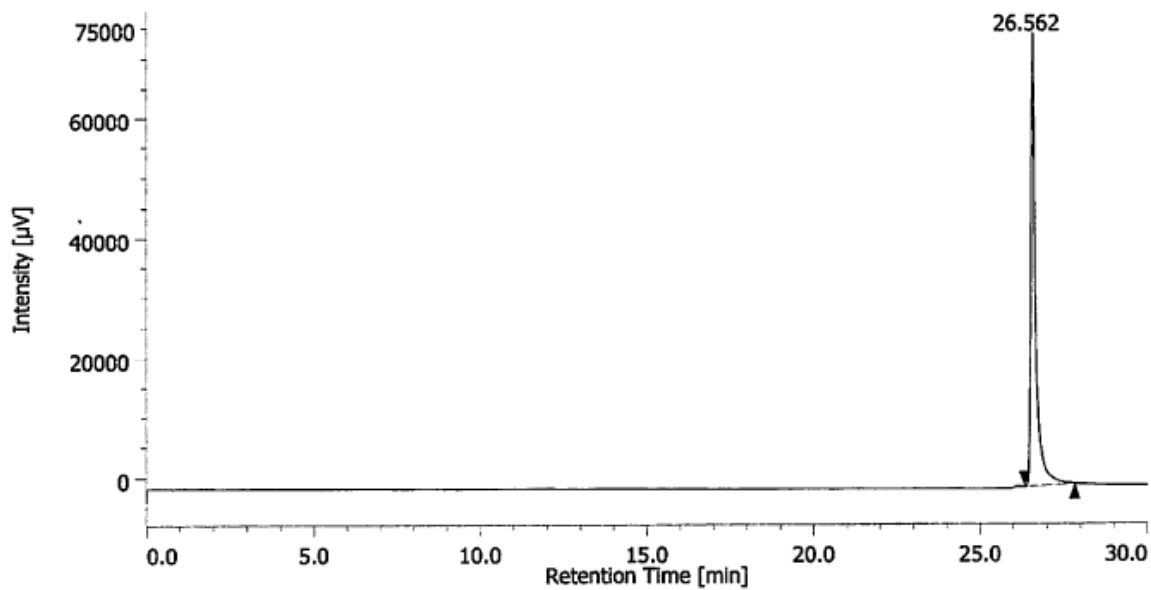
(ON7)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40°C.



(ON8)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40°C.



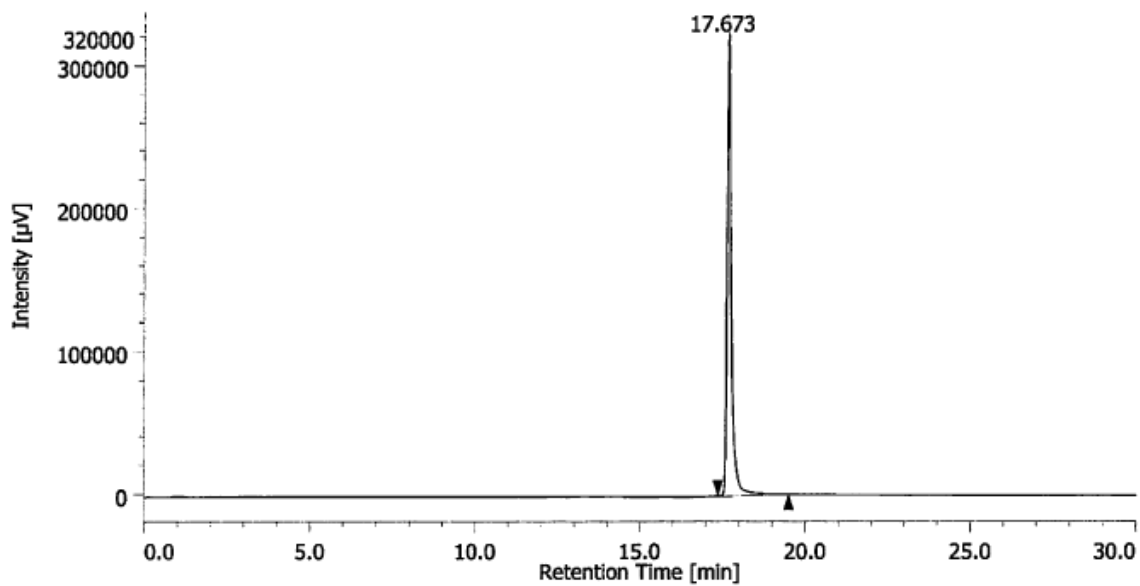
(ON9)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



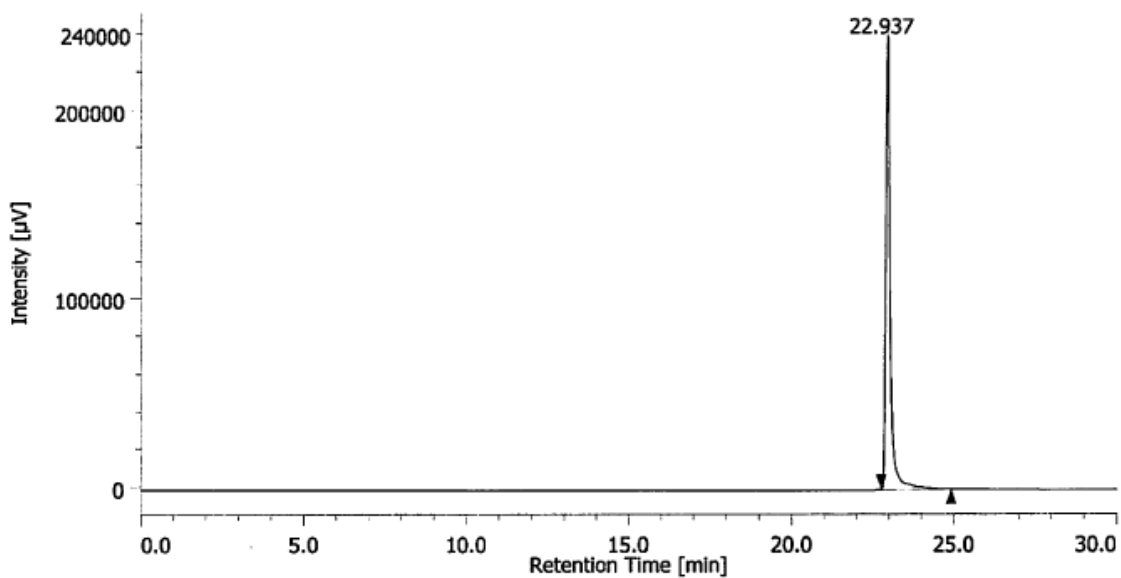
(ON10)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



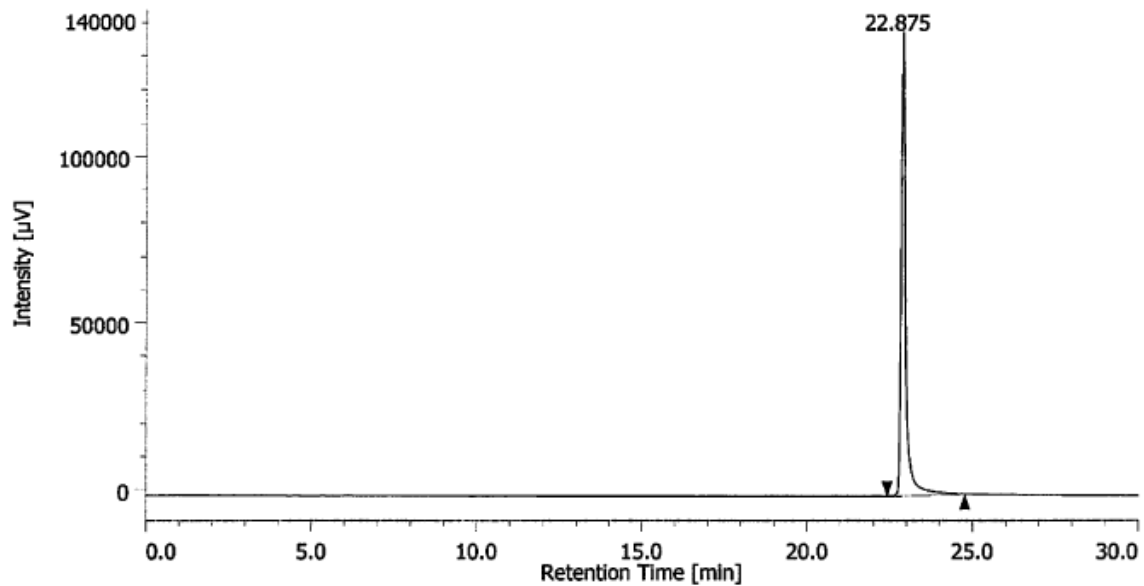
(ON11)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



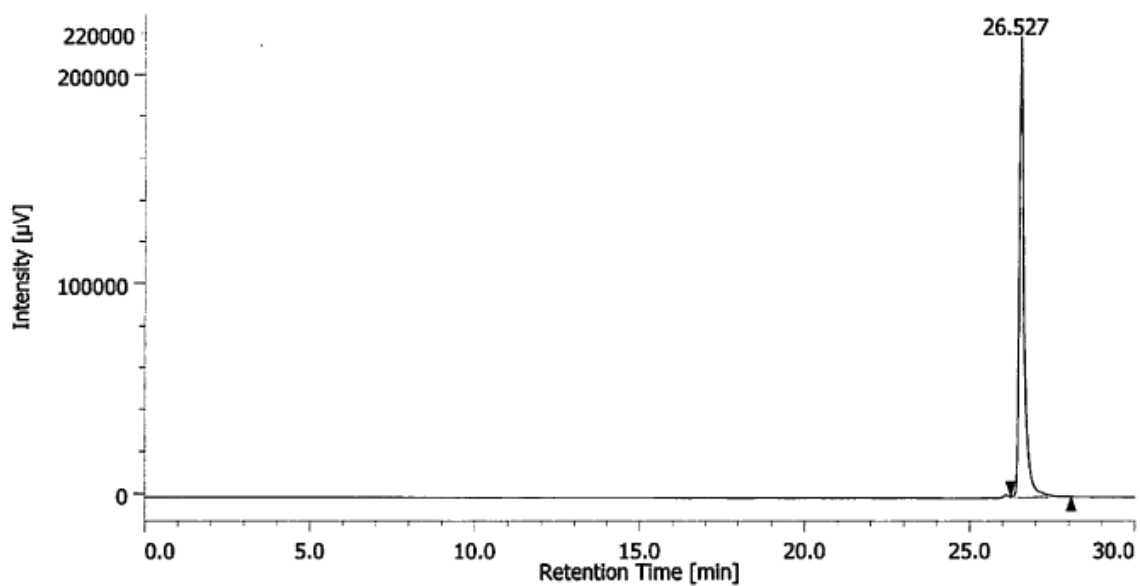
(ON12)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



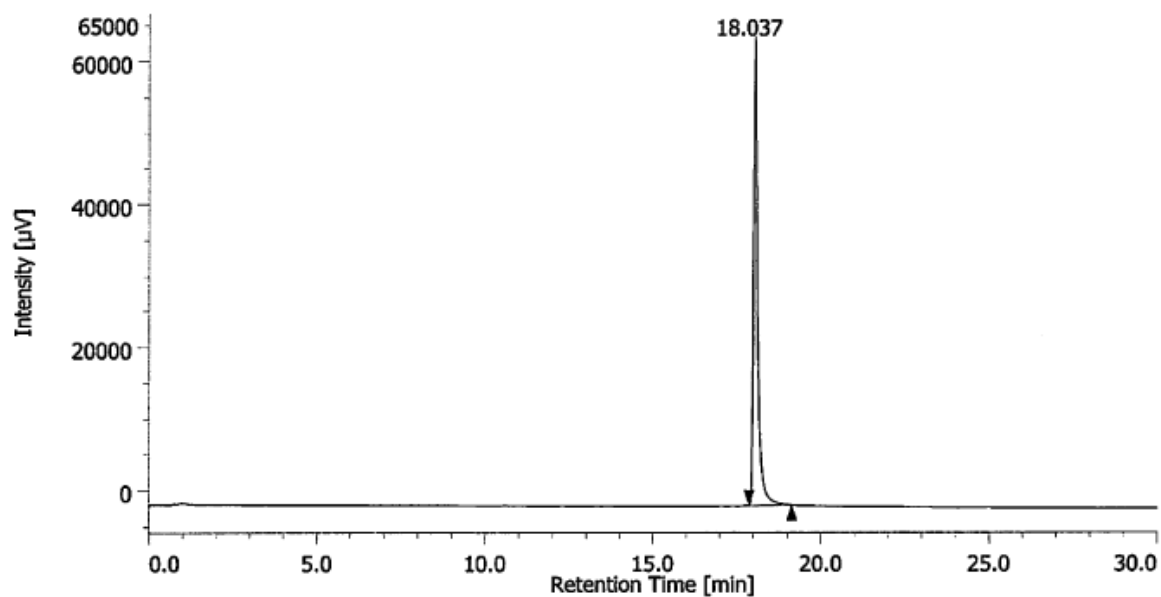
(ON13)

Column : Waters XBridge® MS C₁₈ 2.5 µm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



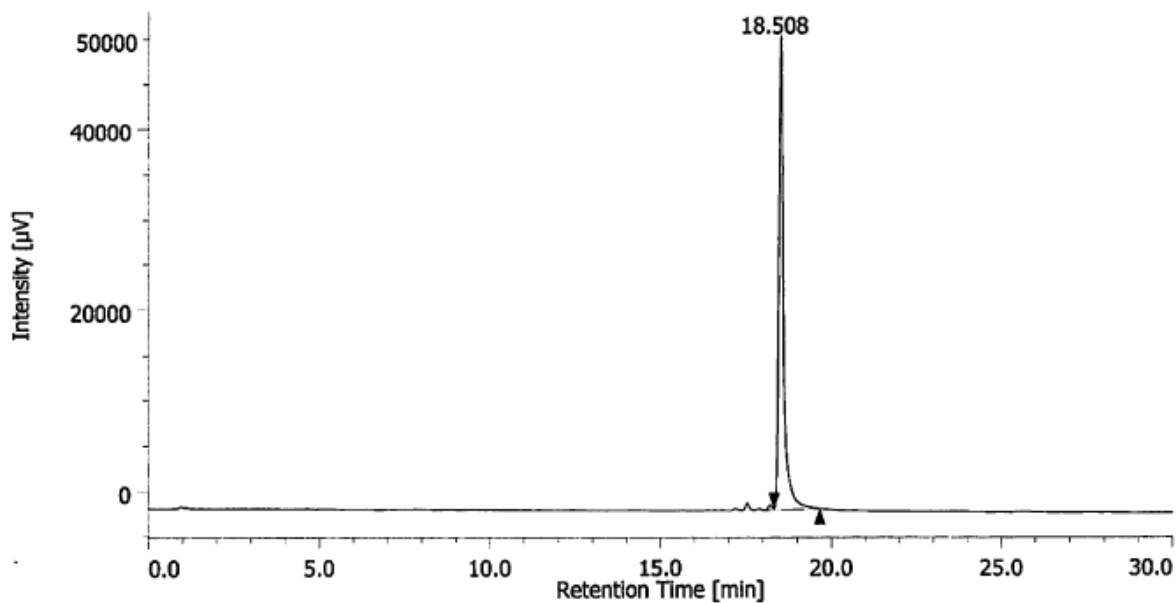
(ON14)

Column : Waters XBridge® MS C₁₈ 2.5 µm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



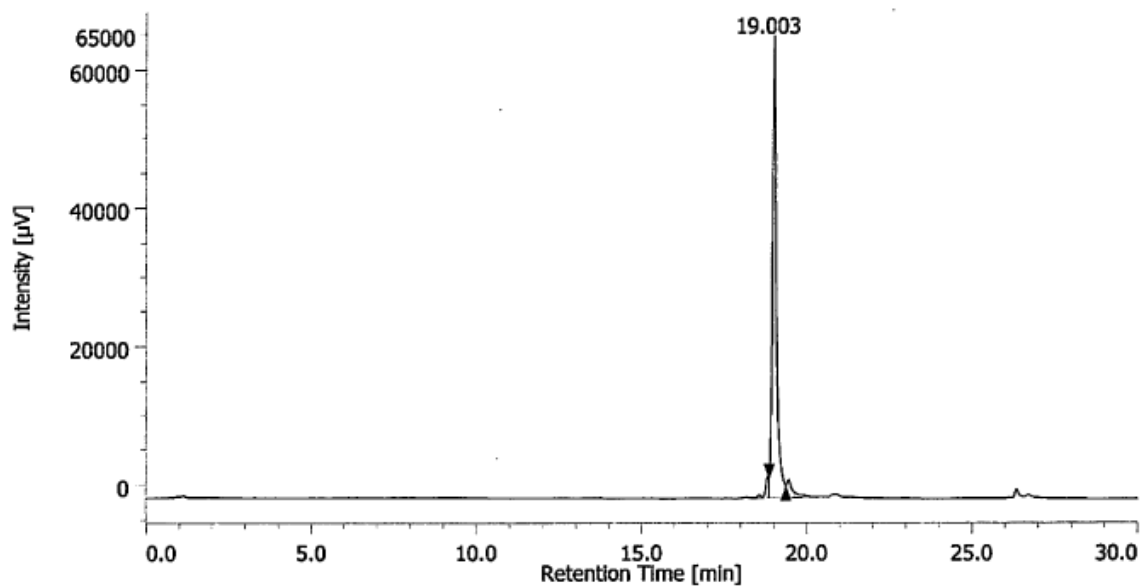
(ON15)

Column : Waters XBridge® MS C₁₈ 2.5 μm, 4.6 × 50 mm.

Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.

Flow rate : 1.0 mL/min.

Column temp. : 40°C.



8. UV-melting experiments.

UV-melting experiments were carried out using SHIMADZU UV-1650 and SHIMADZU UV-1800 spectrophotometers equipped with a T_m analysis accessory. Oligonucleotides and ssDNA or ssRNA were dissolved in 10 mM sodium cacodylate buffer (pH 7.2) containing 140 mM KCl to give a final concentration of each strand of 4 μ M. The samples were annealed by heating at 100 °C followed by slow cooling to 20 °C. The melting profiles were recorded at 260 nm from 20 °C to 100 °C at a scan rate of 0.5 °C/min. The two-point average method was employed to obtain the T_m values and the final values were determined by averaging three independent measurements, which were accurate to within 1 °C. Determined T_m values are listed in Table S2, and the representative melting data are shown in Figure S1.

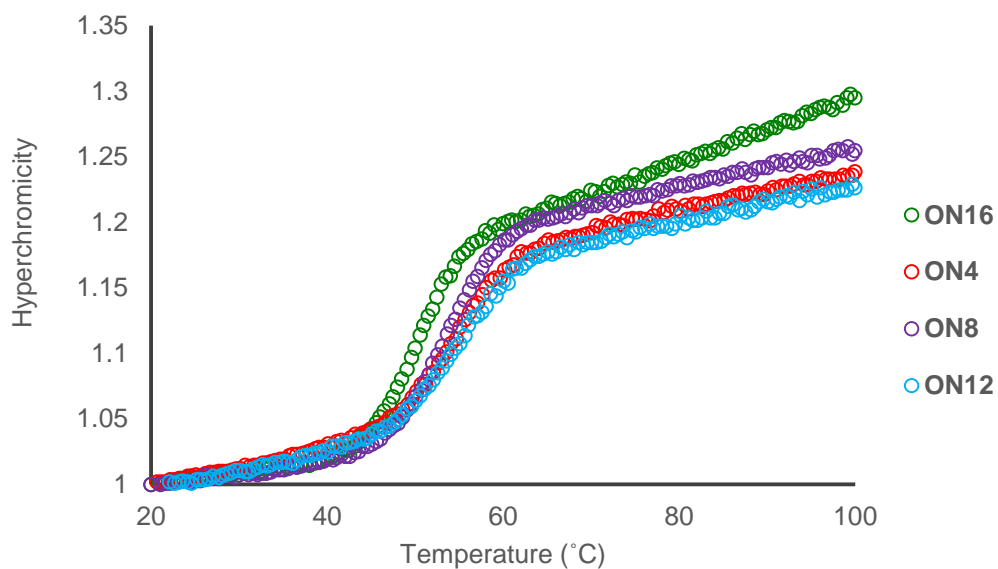
Table S2. T_m (°C) values of duplex formed by methylene-EoDNA derivatives (**ON1–12**) with complementary ssRNA and ssDNA^a

Oligonucleotides (5'-3')	T_m (°C) (ΔT_m /mod.)	
	ssDNA	ssRNA
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON16)	50	51
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON1)	48 (-2.0)	54 (+3.0)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON2)	51 (+0.3)	62 (+3.7)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON3)	50 (\pm 0.0)	65 (+4.7)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON4)	55 (+1.0)	75 (+4.8)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON5)	46 (-4.0)	53 (+2.0)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON6)	49 (-0.3)	62 (+3.7)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON7)	49 (-0.3)	65 (+4.7)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON8)	54 (+0.8)	73 (+4.4)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON9)	47 (-3.0)	54 (+3.0)
d(T ^m C ^m T ^m C ^m T ^m t ^m T ^m C ^m T ^m CT) (ON10)	48 (-0.7)	63 (+4.0)
d(T ^m C ^m T ^m C ^m T ^m t ^m T ^m C ^m T ^m CT) (ON11)	49 (-0.3)	66 (+5.0)
d(T ^m C ^m T ^m C ^m T ^m t ^m T ^m C ^m T ^m CT) (ON12)	54 (+0.8)	76 (+5.0)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON17)	48 (-2.0)	52 (+1.0)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON18)	47 (-1.0)	59 (+2.7)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON19)	47 (-1.0)	60 (+3.0)
d(T ^m C ^m T ^m C ^m T ^m T ^m T ^m T ^m C ^m T ^m CT) (ON20)	48 (-0.4)	69 (+3.6)

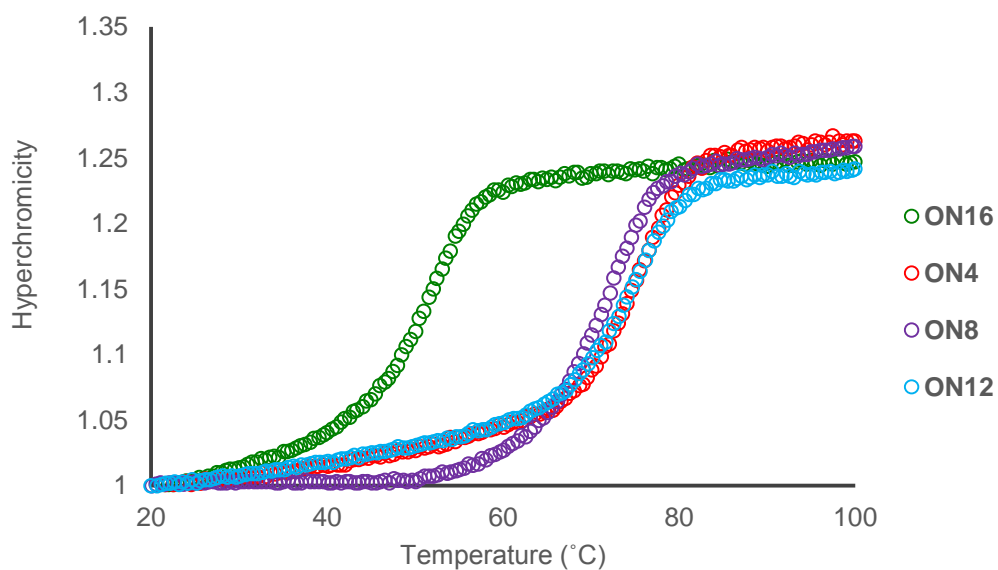
^a Conditions: 10 mM sodium cacodylate buffer (pH 7.2), 140 mM KCl, and 4 μ M of each oligonucleotide. ^mC = 2'-deoxy-5-methylcytidine, T = methylene-EoDNA-T, T = (R)-Me-methylene-EoDNA-T, t = (S)-Me-methylene-EoDNA-T, T = EoNA-T.¹ The sequences of target ssDNA and ssRNA are 5'-d(AGAGAAAAGAAGA)-3' and 5'-r(AGAGAAAAGAAGA)-3', respectively. ΔT_m /mod.: the change in T_m value (ΔT_m) per modification compared to the unmodified natural strand (**ON16**).

Figure S1. Representative UV-melting data (methylene-EoDNA-modified oligonucleotides **ON4**, **8**, **12** and natural oligonucleotide **ON16**) with ssDNA (A) and with ssRNA (B).

(A) With ssDNA



(B) With ssRNA

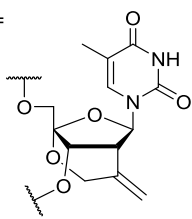
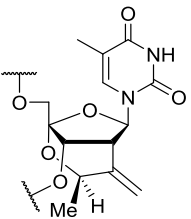
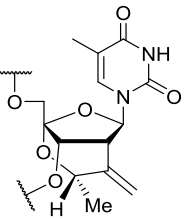
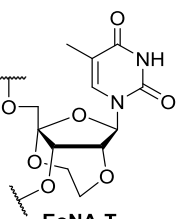


9. Enzymatic degradation experiments.

Enzymatic degradation experiments were carried out under conditions of 2.5 $\mu\text{g/mL}$ *Crotalus adamanteus* venom phosphodiesterase (CAVP), 10 mM MgCl_2 , 50 mM Tris-HCl (pH 8.0) and 7.5 μM each oligonucleotide **ON13–15**, **21** and **22** at 37 $^\circ\text{C}$. The amount of intact oligonucleotides was determined by reversed-phase HPLC (Waters XBridge[®] MS C_{18} 2.5 μm , 3.0×50 mm).

Table S3. Sequences of oligonucleotides used in this enzymatic degradation experiments

Oligonucleotides (5'-3')			
TTTTTTTTTT (ON13)			
TTTTTTTTTT (ON14)			
TTTTTTTTtT (ON15)			
TTTTTTTTTT (ON21)			
TTTTTTTTTT (ON22)			

I =	T =	t =	T =
			

10. Reference

- 1) Hari, Y.; Morikawa, T.; Osawa, T.; Obika, S. *Org. Lett.* **2013**, *15*, 3702–3705.
- 2) Osawa, T.; Hari, Y.; Dohi, M.; Matsuda, Y.; Obika, S. *J. Org. Chem.* **2015**, *80*, 10474-10481.
- 3) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191–4194.