Electronic Supplementary Information

Synthesis of oligonucleotides containing *N,N*-disubstituted 3-deazacytosine nucleobases by post-elongation modification and their triplex-forming ability with double-stranded DNA

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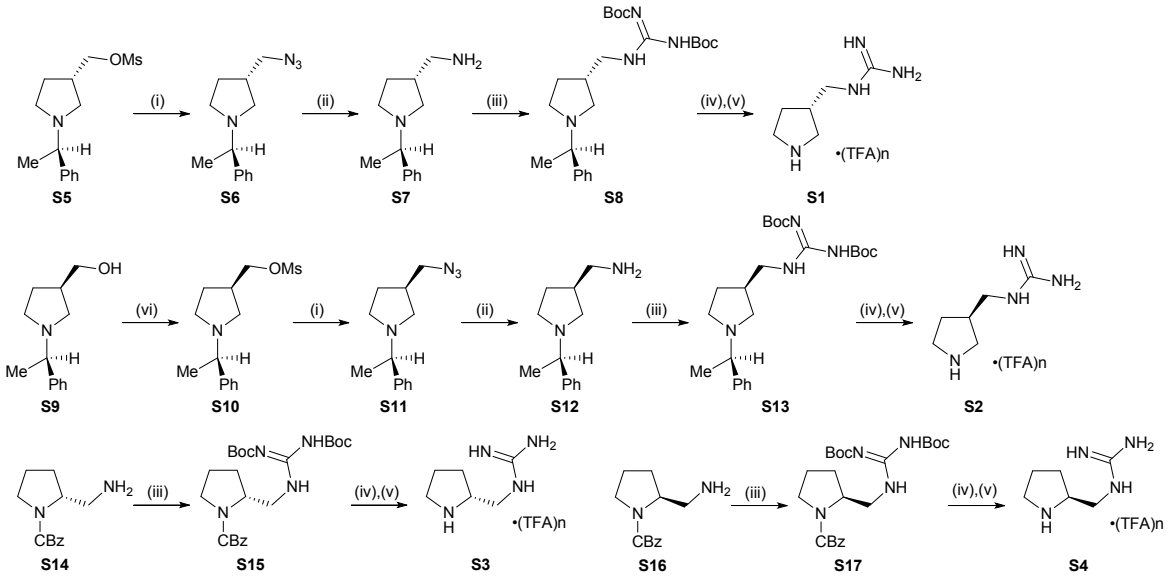
Contents

General: Melting points are uncorrected. All moisture-sensitive reactions were carried out in well-dried glassware under a N_2 atmosphere. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) were recorded on JEOL JNM-ECS-400 spectrometers. Chemical shifts are reported in parts per million downfield from an internal standard [tetramethylsilane (0.00 ppm) for ^{1}H NMR, or CD_3OD (49.00 ppm) or $CDCl_3$ (77.00 ppm) for ^{13}C NMR]. IR spectra were recorded on a JASCO FT/IR-4200 spectrometers. Optical rotations were recorded on a JASCO P-2200 instrument. Mass spectra were measured on a JEOL JMS-700 mass spectrometer. For silica gel flash column chromatography, Fuji Silysia PSQ-100B, FL-100D was used. For amine silica gel column chromatography, Fuji Silysia DM-1020 was used.

Synthesis of secondary amines: All new secondary amines **S1-S4** used in this study were synthesized in Scheme S1.



Scheme S1. Synthesis of guanidinomethylpyrrolidines. *Reagents and conditions*: (i) NaN_3, DMF, 60 °C, 10 h, 88% (**S6**), 95% (**S11**); (ii) *n*-Bu_3P, THF–H_2O, room temperature, 10 h, quant. (**S7**), quant. (**S12**); (iii) $(BocNH)_2CS$, DIPEA, EDCI•HCl, CH_2Cl_2, room temperature, 5–13 h, 71% (**S8**), 66% (**S13**), 64% (**S15**), 89% (**S17**); (iv) TFA, CH_2Cl_2, room temperature, 2–10 h; (v) H_2, 20% $Pd(OH)_2$-C, MeOH, room temperature, 10–13 h, 97% (**S1**), 79% (**S2**), 75% (**S3**), 87% (**S4**); (vi) MsCl, Et_3N, DMAP, CH_2Cl_2, room temperature, 3 h, quant..

(3*S*)-3-Azidomethyl-1-[(*R*)-1-phenylethyl]pyrrolidine (S6): Under a N_2 atmosphere, NaN_3 (459 mg, 7.06 mmol) was added to a solution of compound **S5**[1)] (1.0 g, 3.53 mmol) in anhydrous DMF (50 mL) and the resulting mixture was stirred at 60 °C for 10 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was extracted with Et_2O. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 5/1) to give compound **S6** (715 mg, 88%) as a yellow syrup.

$[\alpha]_D^{28}$ +38.3 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3061, 3028, 2971, 2930, 2872, 2784, 2095, 1492, 1452, 1368, 1280, 1150 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.36 (3H, d, *J* = 6.4 Hz), 1.41–1.53 (1H, m), 1.95–2.04

(1H, m), 2.26 (1H, dd, *J* = 6.4 and 13.2 Hz), 2.32–2.43 (2H, m), 2.55–2.67 (2H, m), 3.17 (2H, q, *J* = 6.4 Hz), 3.25 (2H, d, *J* = 7.3 Hz), 7.20–7.33 (5H, m). ^{13}C NMR ($CDCl_3$) δ 23.02, 28.12, 37.15, 52.23, 55.64, 56.52, 65.47, 126.85, 127.04, 128.24, 145.39. MS (FAB) *m/z* 231 ($M+H^+$). HRMS (FAB): Calcd for $C_{13}H_{19}N_4$ ($M+H^+$), 231.1604; found, 231.1610.

(3*R*)-3-Aminomethyl-1-[(*R*)-1-phenylethyl]pyrrolidine (S7): *n*-Bu_3P (1.52 mL, 6.08 mmol) was added to a solution of compound **S6** (700 mg, 3.04 mmol) in THF (30 mL) and H_2O (6 mL), and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was concentrated *in vacuo*, the residue was purified by amine silica gel column chromatography (CH_2Cl_2/MeOH = 100/1 to 20/1) to give compound **S7** (639 mg, quant.) as a yellow syrup.
$[\alpha]_D^{25}$ +53.2 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3335, 2969, 2785, 2596, 2158, 1750, 1491, 1452, 1372, 1309, 1219, 1148 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.08 (2H, brs), 1.37 (3H, d, *J* = 6.4 Hz), 1.38–1.46 (1H, m), 1.95–2.04 (1H, m), 1.93–2.03 (1H, m), 2.08–2.22 (2H, m), 2.34 (1H, ddd, *J* = 6.4, 8.3 and 13.6 Hz), 2.59–2.69 (3H, m), 2.75 (1H, ddd, *J* = 6.4, 8.3 and 13.6 Hz), 3.16 (2H, q, *J* = 6.4 Hz), 7.19–7.33 (5H, m). ^{13}C NMR ($CDCl_3$) δ 23.09, 28.28, 40.84, 47.03, 52.50, 57.35, 65.86, 126.73, 127.09, 128.18, 145.60. MS (FAB) *m/z* 205 ($M+H^+$). HRMS (FAB): Calcd for $C_{13}H_{21}N_2$ ($M+H^+$), 205.1699; found, 205.1709.

(3*R*)-3-[*N*,*N*′-bis(*tert*-buthoxycarbonyl)guanidinomethyl]-1-[(*R*)-1-phenylethyl]pyrrolidine (S8): Under a N_2 atmosphere, EDCI•HCl (141 mg, 0.734 mmol) was added to a solution of compound **S7** (100 mg, 0.489 mmol), $(BocNH)_2CS$[2)] (135 mg, 0.489 mmol), and DIPEA (0.256 mL, 1.47 mmol) in anhydrous CH_2Cl_2 (10 mL); the resulting mixture was stirred at room temperature for 8 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was extracted with CH_2Cl_2. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 1/1) to give compound **S8** (155 mg, 71%) as a colorless syrup.
$[\alpha]_D^{30}$ +10.3 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3330, 3280, 3129, 2975, 2931, 2876, 2783, 1795, 1722, 1639, 1415, 1366, 1318, 1133, 1056, 1027 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.37 (3H, d, *J* = 6.4 Hz), 1.50 (18H, s), 1.92–2.01 (1H, m), 2.37–2.53 (5H, m), 3.15 (1H, q, *J* = 6.4 Hz), 3.37 (1H, ddd, *J* = 7.0 , 7.0 and 13.3 Hz), 3.47 (1H, ddd, *J* = 7.0 , 7.0 and 13.3 Hz), 7.20–7.38 (5H, m), 8.51 (1H, s), 11.5 (1H, s). ^{13}C NMR ($CDCl_3$) δ 23.42, 28.26, 28.35, 28.42, 28.58, 36.31, 46.13, 52.98, 56.99, 65.93, 79.45, 83.14, 127.05, 127.51, 128.48, 145.91, 149.00, 153.34, 156.73, 163.92. MS (FAB) *m/z* 447 ($M+H^+$). HRMS (FAB): Calcd for $C_{24}H_{39}N_4O_4$ ($M+H^+$), 447.2966; found, 447.2970.

(3*R*)-3-Guanidinomethylpyrrolidine, TFA salt (S1): TFA (5 mL) was added to a solution of **S8** (1.0 g, 2.42 mmol) in CH_2Cl_2 (5 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was concentrated *in vacuo*, the crude product was dissolved in MeOH (5 mL). Under a H_2 atmosphere, the solution was added to a solution of 20% $Pd(OH)_2$-C (1.0 g) in MeOH (5 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction

mixture was filtered, the filtrate was concentrated *in vacuo*. The residue was purified by amine silica gel column chromatography ($CHCl_3$/MeOH = 1/1) to give compound **S1** (600 mg, 97%) as a yellow syrup.

$[\alpha]_D^{24}$ –6.22 (*c* 1.0, MeOH). IR ν_{max} (KBr) 3141, 2152, 1679, 1511, 1436, 1202, 1139 cm^{-1}. ^1H NMR (CD_3OD) δ 1.44 (1H, dddd, *J* = 5.0, 6.9, 6.9 and 14.7 Hz), 1.98 (1H, dddd, *J* = 5.0, 6.9, 6.9 and 14.7 Hz), 2.37 (1H, ddd, *J* = 6.9, 6.9 and 14.7 Hz), 2.55 (1H, dd, *J* = 7.3 and 11.4 Hz), 2.82–2.97 (2H, m), 3.02 (1H, dd, *J* = 7.3 and 11.4 Hz), 3.15 (2H, d, *J* = 7.3 Hz). ^{13}C NMR (CD_3OD) δ 30.85, 39.89, 45.78, 46.92, 50.93, 118.16 (q, *J* = 293 Hz), 158.84, 163.21 (q, *J* = 34.5 Hz). MS (FAB) *m/z* 143 ($M+H^+$). HRMS (FAB): Calcd for $C_6H_{15}N_4$ ($M+H^+$), 143.1291; found, 143.1299.

(3*R*)-3-Methanesulfonyloxymethyl-1-[(*R*)-1-phenylethyl]pyrrolidine (S10): Under a N_2 atmosphere, MsCl (0.566 mL, 7.31 mmol) was added to a solution of compound **S9**[3] (1.0 g, 4.87 mmol), DMAP (59.5 mg, 0.487 mmol), and Et_3N (2.04 mL, 14.6 mmol) in anhydrous CH_2Cl_2 (40 mL) at 0 °C; the resulting mixture was stirred at room temperature for 3 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 1/1) to give compound **S10** (1.43 mg, quant.) as a yellow syrup.

$[\alpha]_D^{25}$ +40.9 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3616, 3027, 2971, 2936, 2876, 2789, 1492, 1453, 1415, 1355, 1283, 1174 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.37 (3H, d, *J* = 6.4 Hz), 1.46–1.54 (1H, m), 1.93–2.02 (1H, m), 2.28 (1H, dd, *J* = 4.5 and 9.0 Hz), 2.36 (1H, ddd, *J* = 8.7, 8.7 and 8.7 Hz), 2.51–2.63 (2H. m), 2.71 (1H, ddd, *J* = 5.0, 8.7 and 8.7 Hz), 2.94 (3H, s), 3.20 (1 H, q, *J* = 6.4 Hz), 4.07–4.14 (2H, m), 7.20–7.30 (5H, m). ^{13}C NMR ($CDCl_3$) δ 23.06, 26.74, 36.50, 37.15, 51.84, 55.35, 65.19, 72.66, 126.92, 126.97, 145.29. MS (FAB) *m/z* 284 ($M+H^+$). HRMS (FAB): Calcd for $C_{14}H_{22}NO_3S$ ($M+H^+$), 284.1315; found, 284.1317.

(3*R*)-3-Azidomethyl-1-[(*R*)-1-phenylethyl]pyrrolidine (S11): Under a N_2 atmosphere, NaN_3 (642 mg, 9.88 mmol) was added to a solution of compound **S10** (1.4 g, 4.94 mmol) in anhydrous DMF (30 mL) and the resulting mixture was stirred at 60 °C for 10 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was extracted with Et_2O. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 2/1) to give compound **S11** (1.08 g, 95%) as a yellow syrup.

$[\alpha]_D^{21}$ +59.3 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3061, 3027, 2970, 2931, 2872, 2785, 2095, 1491, 1451, 1367, 1280, 1151 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.35 (3H, d, *J* = 6.4 Hz), 1.41–1.49 (1H, m), 1.90–1.99 (1H, m), 2.18 (1H, dd, *J* = 5.5 and 9.6 Hz), 2.59 (1H, ddd, 5.5, 9.2 and 9.2 Hz), 2.68 (1H, dd, 7.8 and 9.6 Hz), 3.14–3.25 (3H, m), 7.18–7.31 (5H, m). ^{13}C NMR ($CDCl_3$) δ 23.08, 28.07, 36.95, 52.08, 55.67, 56.44, 65.33, 126.71, 126.85, 128.16, 145.32. MS (FAB) *m/z* 231 ($M+H^+$). HRMS (FAB): Calcd for $C_{13}H_{19}N_4$ ($M+H^+$), 231.1604; found, 231.1609.

(3*S*)-3-Aminomethyl-1-[(*R*)-1-phenylethyl]pyrrolidine (S12): *n*-Bu_3P (2.17 mL, 8.68 mmol) was added to a solution of compound **S11** (1.0 g, 4.34 mmol) in THF (60 mL) and H_2O (12 mL), and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was concentrated *in vacuo*, the residue was purified by amine silica gel column chromatography (CH_2Cl_2/MeOH = 50/1) to give compound **S12** (887 mg, quant.) as a yellow syrup.

$[\alpha]_D^{23}$ +54.1 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3277, 2970, 2783, 2602, 2151, 1570, 1490, 1453, 1373, 1310, 1220, 1147 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.09 (1H, brs), 1.37 (3H, d, *J* = 6.4 Hz), 1.38–1.46 (1H, m), 1.88–1.97 (1H, m), 2.11 (1H, dd, *J* = 6.0 and 8.7 Hz), 2.14–2.25 (1H, m), 2.41 (1H, ddd, *J* = 6.0, 8.7 and 8.7 Hz), 2.52 (1H, ddd, *J* = 6.0, 8.7 and 8.7 Hz), 2.64 (1H, dd, *J* = 1.9 and 6.8 Hz), 2.64 (1H, dd, *J* = 1.9 and 6.8 Hz), 2.79 (1H, dd, *J* = 7.8 and 8.7 Hz), 3.16 (2H, q, *J* = 6.4 Hz), 7.19–7.33 (5H, m). ^{13}C NMR ($CDCl_3$) δ 23.44, 28.59, 40.91, 47.42, 52.83, 57.44, 66.07, 126.95, 127.26, 128.44, 145.92. MS (FAB) *m/z* 205 ($M+H^+$). HRMS (FAB): Calcd for $C_{13}H_{21}N_2$ ($M+H^+$), 205.1699; found, 205.1707.

(3*S*)-3-[*N*,*N*′-bis(*tert*-buthoxycarbonyl)guanidinomethyl]-1-[(*R*)-1-phenylethyl]pyrrolidine (S13): Under a N_2 atmosphere, EDCI•HCl (3.66 g, 19.1 mmol) was added to a solution of compound **S12** (3.0 g, 14.7 mmol), $(BocNH)_2CS$ (4.06 g, 14.7 mmol), and DIPEA (7.67 mL, 44.0 mmol) in anhydrous CH_2Cl_2 (30 mL); the resulting mixture was stirred at room temperature for 5 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was extracted with CH_2Cl_2. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 5/1 to 2/1) to give compound **S13** (4.02 g, 66%) as a colorless syrup.

$[\alpha]_D^{31}$ –13.7 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3330, 3288, 3128, 2975, 2932, 2877, 2785, 1795, 1722, 1639, 1415, 1366, 1314, 1134, 1055, 1027 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.38 (3H, d, *J* = 6.4 Hz), 1.44–1.58 (19 H, m), 1.98 (1H, dddd, *J* = 5.0, 5.0, 9.6 and 19.2 Hz), 2.22 (1H, dd, *J* = 5.0 and 9.6 Hz), 2.31–2.50 (2H, m), 2.61 (1H, dd, *J* = 7.8 and 9.2 Hz), 2.75 (1H, ddd, *J* = 5.0, 5.0 and 9.6 Hz), 3.16 (1H, q, *J* = 6.4 Hz), 3.32 (1H, ddd, *J* = 5.0 , 6.0 and 13.4 Hz), 3.46 (1H, ddd, *J* = 6.0 , 6.0 and 13.4 Hz), 7.19–7.38 (5H, m), 8.62 (1H, s), 11.5 (1H, s). ^{13}C NMR ($CDCl_3$) δ 23.05, 27.89, 27.97, 28.20, 35.90, 45.95, 52.16, 57.06, 65.66, 79.03, 82.67, 126.68, 128.16, 145.57, 152.91, 156.25, 163.54. MS (FAB) *m/z* 447 ($M+H^+$). HRMS (FAB): Calcd for $C_{24}H_{39}N_4O_4$ ($M+H^+$), 447.2966; found, 447.2972.

(3*S*)-3-Guanidinomethylpyrrolidine, TFA salt (S2): TFA (5 mL) was added to a solution of compound **S13** (800 mg, 1.93 mmol) in CH_2Cl_2 (5 mL) and the resulting mixture was stirred at room temperature for 3 h. After the reaction mixture was concentrated *in vacuo*, the crude product was dissolved in MeOH (5 mL). Under a H_2 atmosphere, the solution was added to a solution of 20% $Pd(OH)_2$-C (1.0 g) in MeOH (5 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was filtered, the filtrate was concentrated *in vacuo*. The residue was purified by amine silica gel column chromatography ($CHCl_3$/MeOH = 1/1 to 1/5) to give compound

S2 (390 mg, 79%) as a yellow syrup.
$[\alpha]_D^{24}$ +6.63 (*c* 1.0, MeOH). IR ν_{max} (KBr) 3158, 2494, 1681, 1511, 1430, 1201, 1136 cm^{-1}. ^1H NMR (CD_3OD) δ 1.40–1.49 (1H, m), 1.94–2.02 (1H, m), 2.36 (1H, ddd, *J* = 7.8, 7.8 and 14.8 Hz), 2.54 (1H, dd, *J* = 6.0 and 7.8 Hz), 2.83–2.97 (2H, m), 3.02 (1H, dd, *J* = 7.8 and 7.8 Hz), 3.15 (2H, d, *J* = 7.8 Hz). ^{13}C NMR (CD_3OD) δ 30.88, 39.93, 45.77, 46.95, 50.96, 118.16 (q, *J* = 293 Hz), 158.86, 163.21 (q, *J* = 34.5 Hz). MS (FAB) *m/z* 143 ($M+H^+$). HRMS (FAB): Calcd for $C_6H_{15}N_4$ ($M+H^+$), 143.1291; found, 143.1298.

(2*R*)-1-Benzyloxycarbonyl-2-[*N*,*N*′-bis(*tert*-buthoxycarbonyl)guanidinomethyl]pyrrolidine (S15): Under a N_2 atmosphere, EDCI•HCl (1.06 g, 5.55 mmol) was added to a solution of commercially available compound **14** (1.0 g, 4.27 mmol), $(BocNH)_2CS$ (1.18 g, 4.27 mmol), and DIPEA (2.23 mL, 12.8 mmol) in anhydrous CH_2Cl_2 (30 mL); the resulting mixture was stirred at room temperature for 5 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was extracted with CH_2Cl_2, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 5/1 to 3/1) to give compound **S15** (1.30 g, 64%) as a yellow syrup.
$[\alpha]_D^{27}$ +37.4 (*c* 0.5, $CHCl_3$). IR ν_{max} (KBr) 3327, 3287, 3136, 2936, 2887, 1706, 1639, 1575, 1450, 1413, 1369, 1329, 1137, 1056 cm^{-1}. ^1H NMR ($CDCl_3$, as a mixture of atropisomers) δ 1.47 (6H, s), 1.50 (12H, s), 1.78–2.04 (4H, m), 3.40–3.70 (4H, m), 4.07–4.12 (1H, m), 5.08–5.30 (2H, m), 7.28–7.38 (5H, m), 8.51 (0.5H, s), 8.59 (0.5H, s), 11.49 (1H, s). ^{13}C NMR ($CDCl_3$, as a mixture of atropisomers) δ 22.98, 23.81, 27.94, 28.21, 28.70, 29.47, 43.70, 44.15, 46.61, 46.88, 56.11, 56.96, 66.68, 66.77, 79.01, 79.09, 82.78, 82.97, 127.70, 127.78, 127.86, 128.33, 136.79, 152.87, 152.99, 154.98, 155.30, 156.43, 156.51, 163.50. MS (FAB) *m/z* 477 ($M+H^+$). HRMS (FAB): Calcd for $C_{24}H_{37}N_4O_7$ ($M+H^+$), 477.2708; found, 477.2717.

(2*R*)-2-Guanidinomethylpyrrolidine, TFA salt (S3): TFA (2 mL) was added to a solution of compound **S15** (1.3 g, 2.73 mmol) in CH_2Cl_2 (2 mL) and the resulting mixture was stirred at room temperature for 3 h. After the reaction mixture was concentrated *in vacuo*, the crude product was dissolved in MeOH (5 mL). Under a H_2 atmosphere, the solution was added to a solution of 20% $Pd(OH)_2$-C (1.0 g) in MeOH (5 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was filtered, the filtrate was concentrated *in vacuo*. The residue was purified by amine silica gel column chromatography ($CHCl_3$/MeOH = 2/1 to 1/5) to give compound **S3** (526 mg, 75%) as a yellow syrup.
$[\alpha]_D^{24}$ –0.34 (*c* 1.0, MeOH). IR ν_{max} (KBr) 3143, 1676, 1523, 1420, 1200, 1137 cm^{-1}. ^1H NMR (CD_3OD) δ 1.43 (1H, dddd, *J* = 5.0, 6.8, 6.8 and 13.6 Hz),1.67-1.84 (2H, m), 1.91–2.00 (1H, m), 2.84 (1H, ddd, *J* = 6.8, 6.8 and 13.6 Hz), 2.93 (1H, ddd, *J* = 5.0, 6.8 and 13.6 Hz), 3.12 (1H, dd, *J* = 6.8 and 13.6 Hz), 3.20 (1H, dd, *J* = 5.0 and 13.6 Hz). ^{13}C NMR (CD_3OD) δ 26.74, 29.93, 47.06, 47.72, 58.83, 118.19 (q, *J* = 292 Hz), 159.71, 163.18 (q, *J* = 34.5 Hz). MS (FAB) *m/z* 143 ($M+H^+$). HRMS (FAB): Calcd for $C_6H_{15}N_4$ ($M+H^+$), 143.1291; found, 143.1288.

(2*S*)-1-Benzyloxycarbonyl-2-[*N*,*N*′-bis(*tert*-buthoxycarbonyl)guanidinomethyl]pyrrolidine (S17): Under a N_2 atmosphere, EDCI•HCl (1.23 g, 6.41 mmol) was added to a solution of commercially available compound **S16** (1.0 g, 4.27 mmol), $(BocNH)_2CS$ (1.18 g, 4.27 mmol), and DIPEA (2.23 mL, 12.8 mmol) in anhydrous CH_2Cl_2 (30 mL); the resulting mixture was stirred at room temperature for 13 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was extracted with CH_2Cl_2. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel flash column chromatography (*n*-hexane/AcOEt = 10/1 to 5/1) to give compound **S17** (1.81 g, 89%) as a yellow syrup.

$[\alpha]_D^{31}$ –39.4 (*c* 1.0, $CHCl_3$). IR ν_{max} (KBr) 3328, 3288, 3127, 2935, 2887, 1707, 1639, 1576, 1447, 1412, 1367, 1327, 1138, 1056 cm^{-1}. ^1H NMR ($CDCl_3$, as a mixture of atropisomers) δ1.47 (6H, s), 1.50 (12H, s), 1.77-2.04 (4H, m), 3.40–3.71 (4H, m), 4.07–4.12 (1H, m), 5.08–5.30 (2H, m), 7.28–7.38 (5H, m), 8.51 (0.5H, s), 8.59 (0.5H, s), 11.49 (1H, s). ^{13}C NMR ($CDCl_3$, as a mixture of atropisomers) δ 22.75, 23.58, 27.71, 27.98, 28.47, 29.24, 43.49, 43.92, 46.37, 46.64, 55.84, 56.71, 66.43, 66.51, 78.71, 78.79, 82.49, 82.68, 127.45, 127.55, 127.60, 128.11, 136.55, 152.62, 152.73, 154.70, 155.05, 156.20, 156.27, 163.27. MS (FAB) *m/z* 477 ($M+H^+$). HRMS (FAB): Calcd for $C_{24}H_{37}N_4O_7$ ($M+H^+$), 477.2708; found, 477.2707.

(2*S*)-2-Guanidinomethylpyrrolidine, TFA salt (S4): TFA (5 mL) was added to a solution of compound **S17** (900 mg, 1.89 mmol) in CH_2Cl_2 (5 mL) and the resulting mixture was stirred at room temperature for 2 h. After the reaction mixture was concentrated *in vacuo*, the crude product was dissolved in MeOH (5 mL). Under a H_2 atmosphere, the solution was added to a solution of 20% $Pd(OH)_2$-C (1.0 g) in MeOH (5 mL) and the resulting mixture was stirred at room temperature for 13 h. After the reaction mixture was filtered, the filtrate was concentrated *in vacuo*. The residue was purified by amine silica gel column chromatography ($CHCl_3$/MeOH = 1/1) to give compound **S4** (421 mg, 87%) as a yellow syrup.

$[\alpha]_D^{25}$ +1.12 (*c* 1.0, MeOH). IR ν_{max} (KBr) 3143, 1680, 1517, 1426, 1202, 1137 cm^{-1}. ^1H NMR (CD_3OD) δ 1.34 (1H, dddd, *J* = 5.0, 6.8, 6.8 and 13.6 Hz), 1.59–1.77 (2H, m), 1.81–1.90 (1H, m), 2.74 (1H, ddd, *J* = 6.8, 6.8 and 13.6 Hz), 2.81 (1H, ddd, *J* = 5.0, 6.8 and 13.6 Hz), 3.03 (1H, dd, *J* = 6.8 and 13.6 Hz), 3.10 (1H, dd, *J* = 5.0 and 13.6 Hz). ^{13}C NMR (CD_3OD) δ 26.75, 29.93, 47.06, 47.73, 58.85, 119.06 (q, *J* = 292 Hz), 159.73, 163.19 (q, *J* = 34.5 Hz). MS (FAB) *m/z* 143 ($M+H^+$). HRMS (FAB): Calcd for $C_6H_{15}N_4$ ($M+H^+$), 143.1291; found, 143.1298.

References

(1) C. Fava, R. Galeazzi, G. Mobbili, M. Orena, *Heterocycles*, **1999**, *51*, 2463.

(2) S. Robinson, E. J. Roskamp, *Tetrahedron*, **1997**, *53*, 6697; B. R. Linton, A. J. Carr, B. P. Orner, A. D. Hamilton, *J. Org. Chem.*, **2000**, *65*, 1566.

(3) L. Nielsen, L. Brehm, P. Krogsgaard-Larsen, *J. Med. Chem.,* **1992**, *33*, 71.

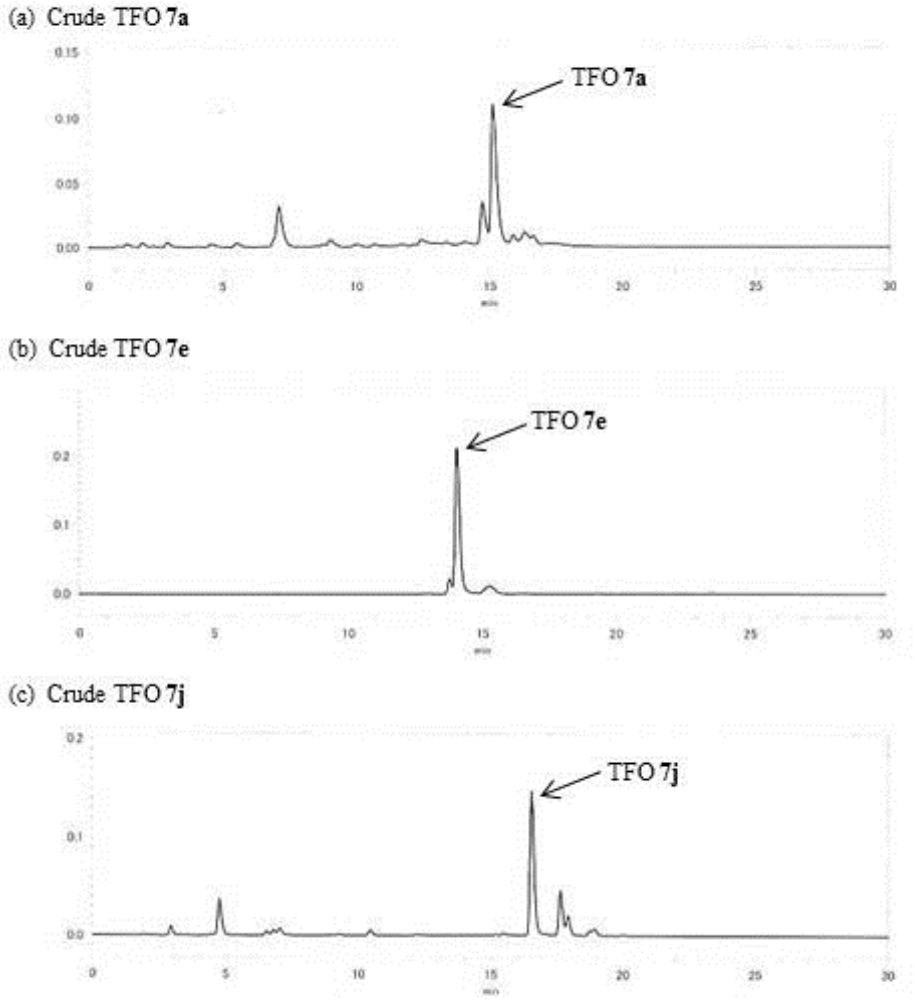


Fig. S1. Representative HPLC charts of crude TFOs before HPLC purification.

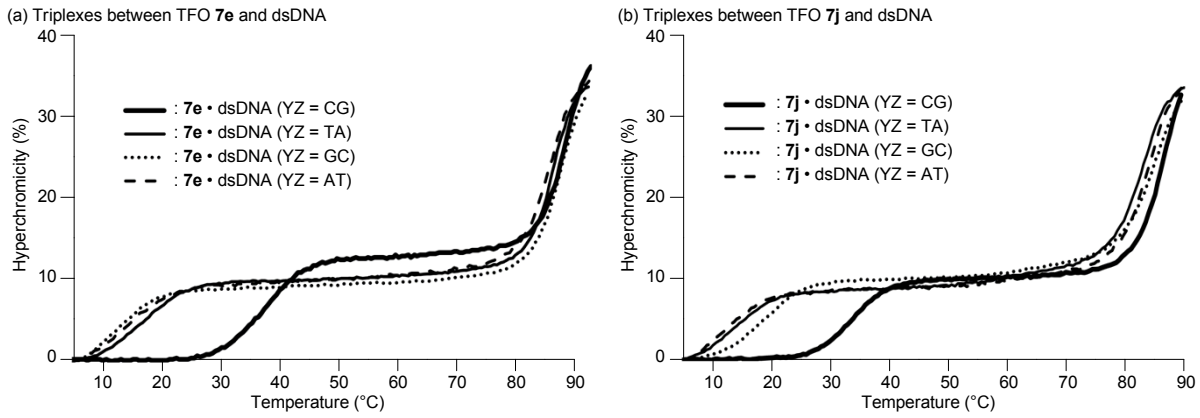
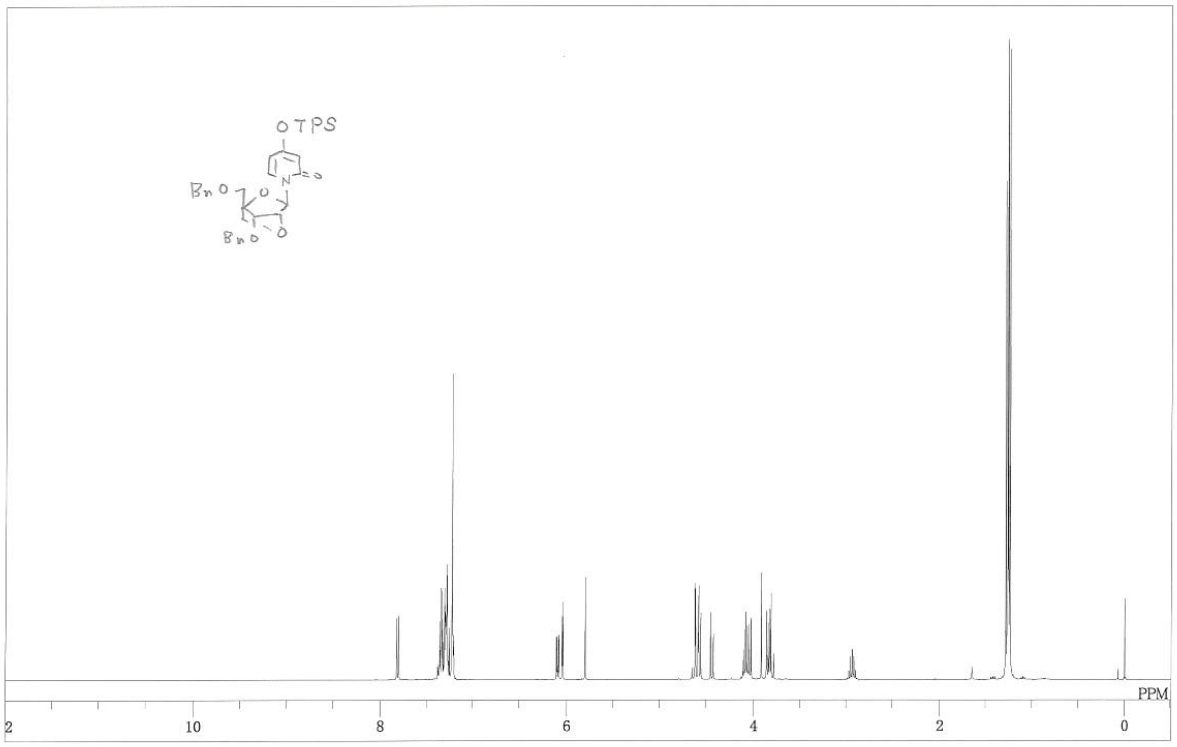


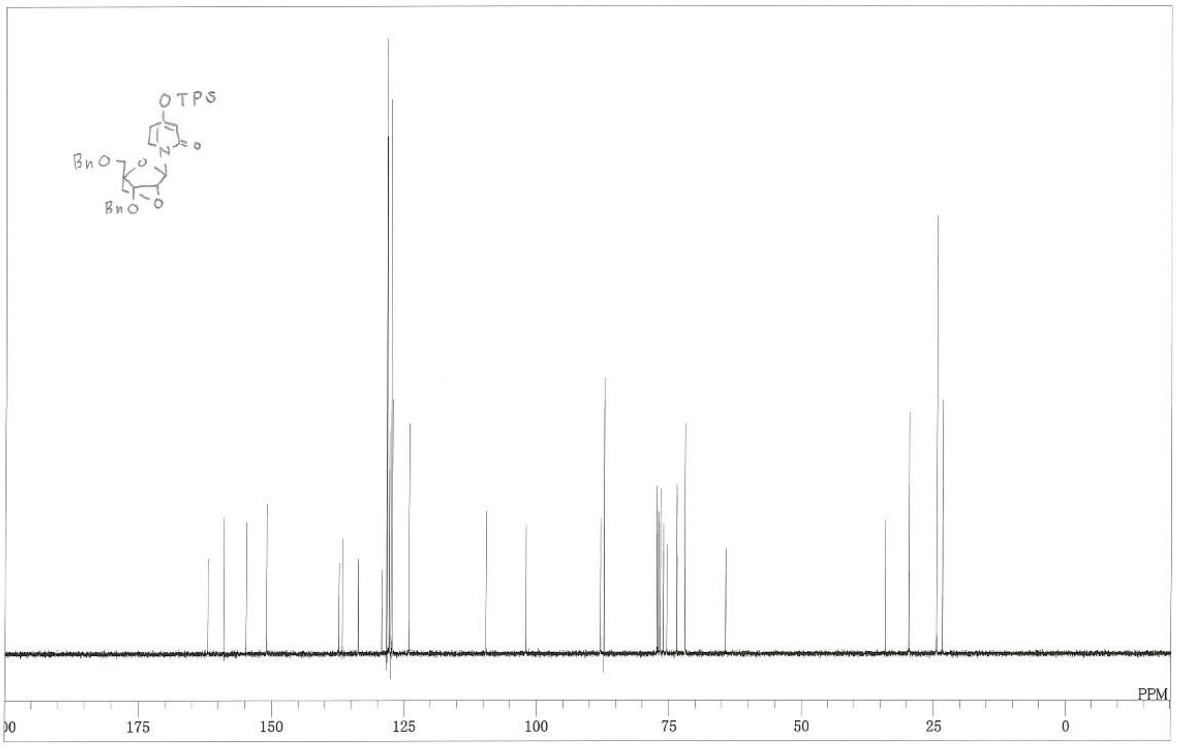
Fig. S2. Representative UV-melting curves of triplexes. Conditions: 10 mM sodium cacodylate buffer (pH 6.8), 100 mM KCl and 50 mM $MgCl_2$. The concentration of each oligonucleotide used was 1.89 μM.

Compound **3**

1H NMR (CDCl3)

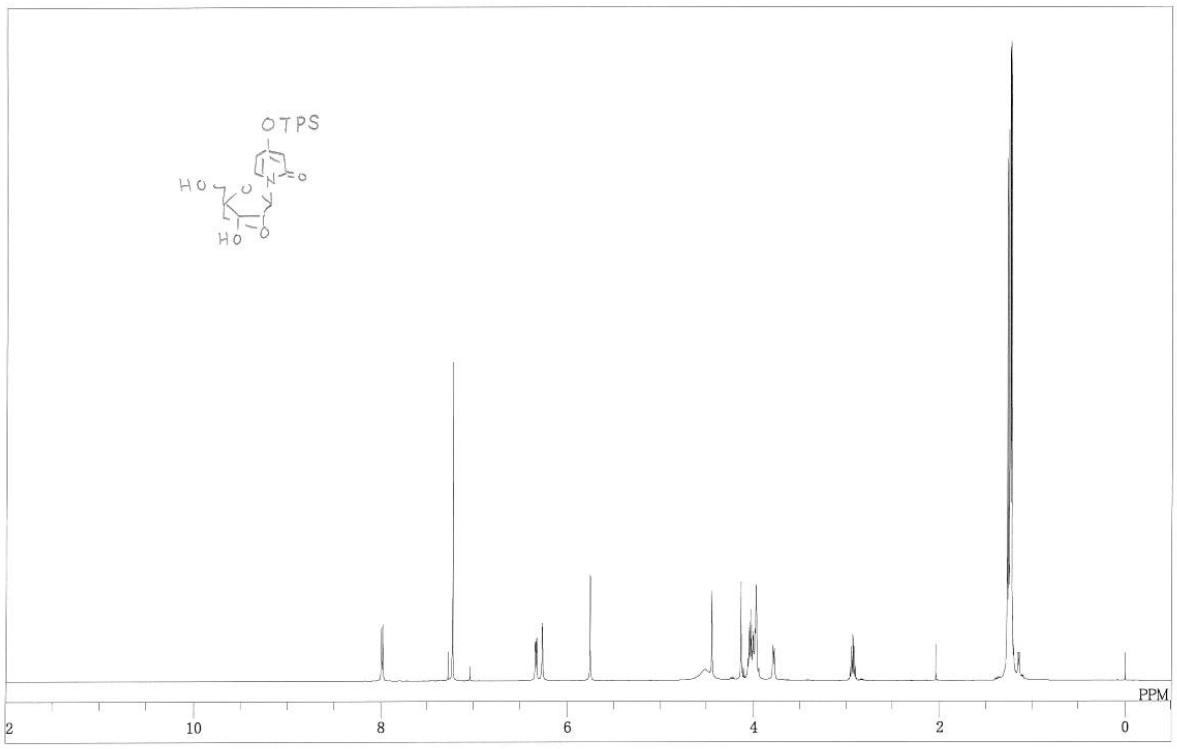


13C NMR (CDCl3)

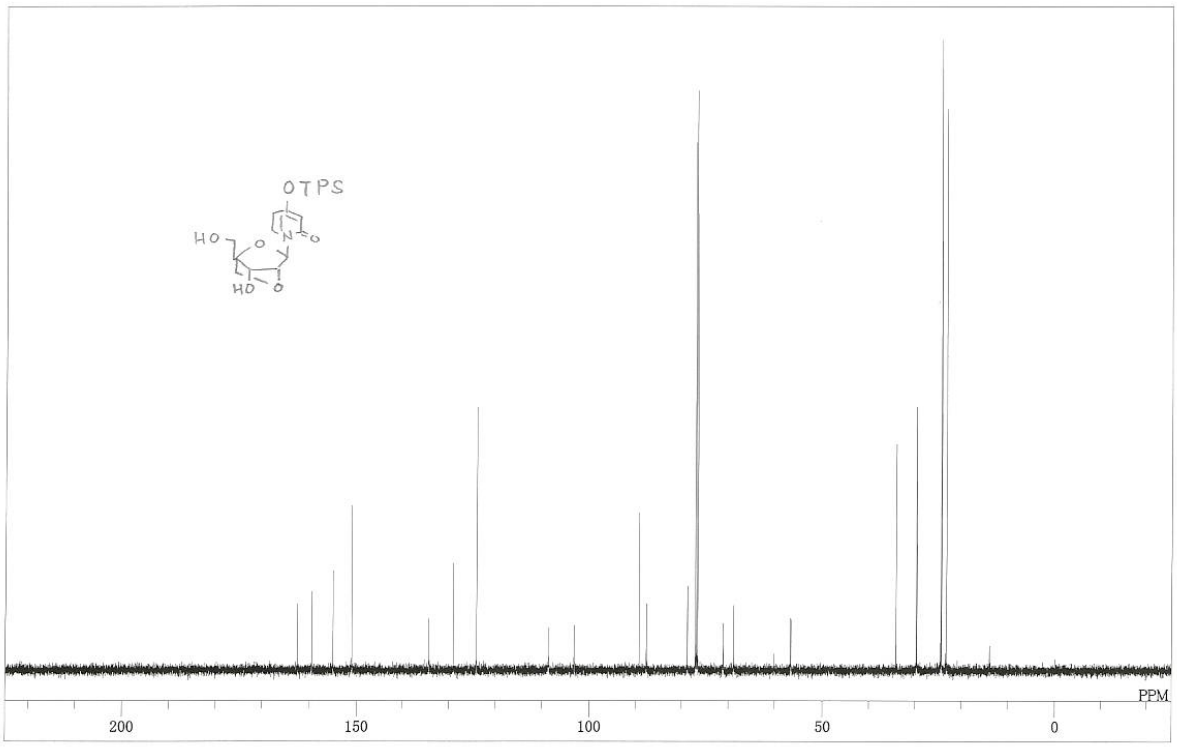


Compound **4**

1H NMR (CDCl3)

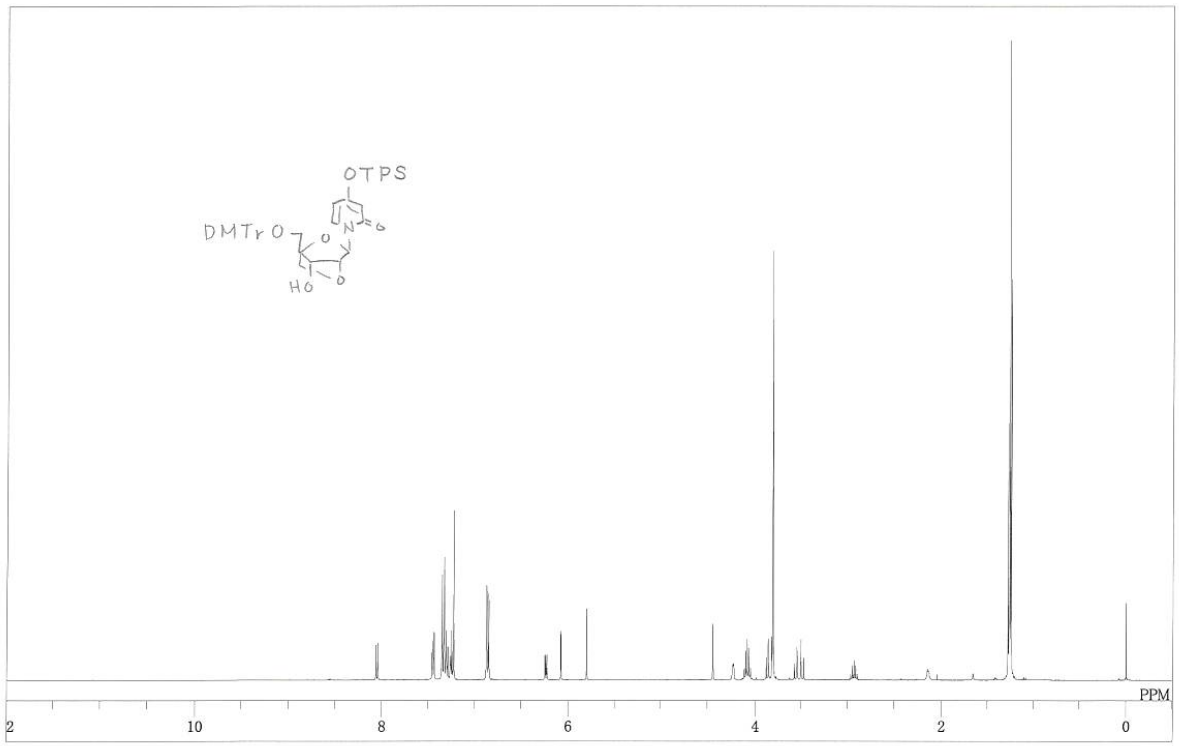


13C NMR (CDCl3)

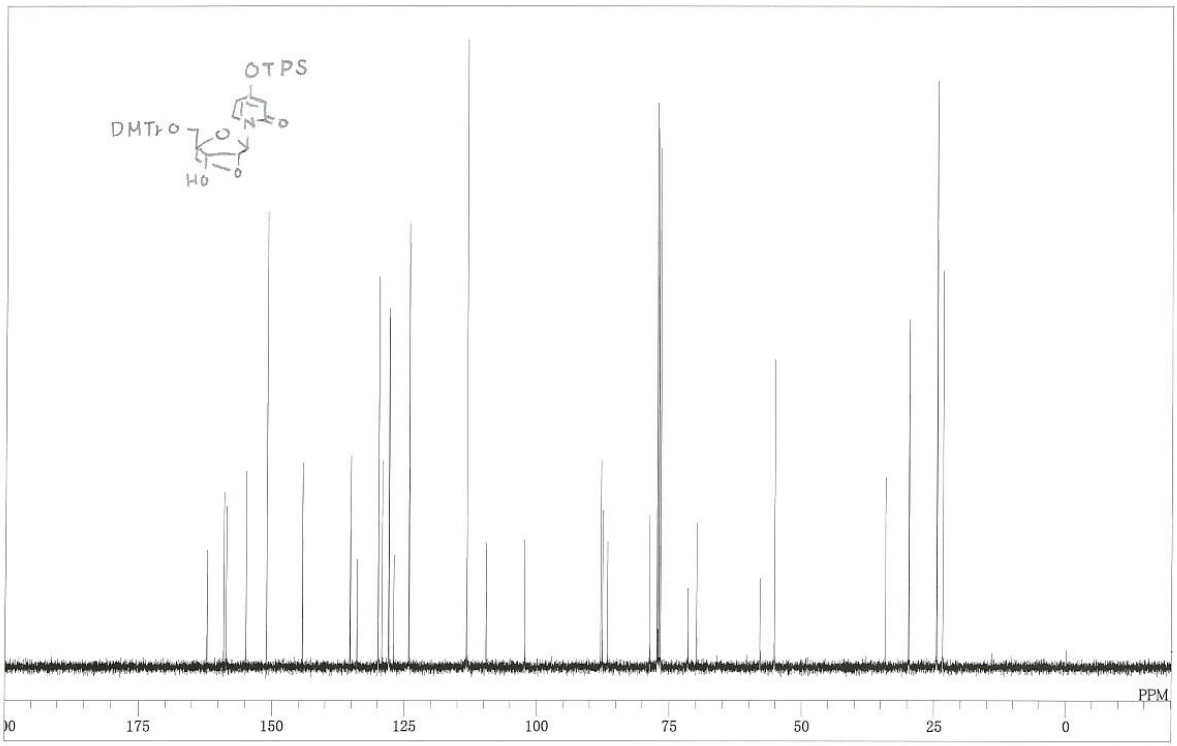


Compound **5**

1H NMR (CDCl3)

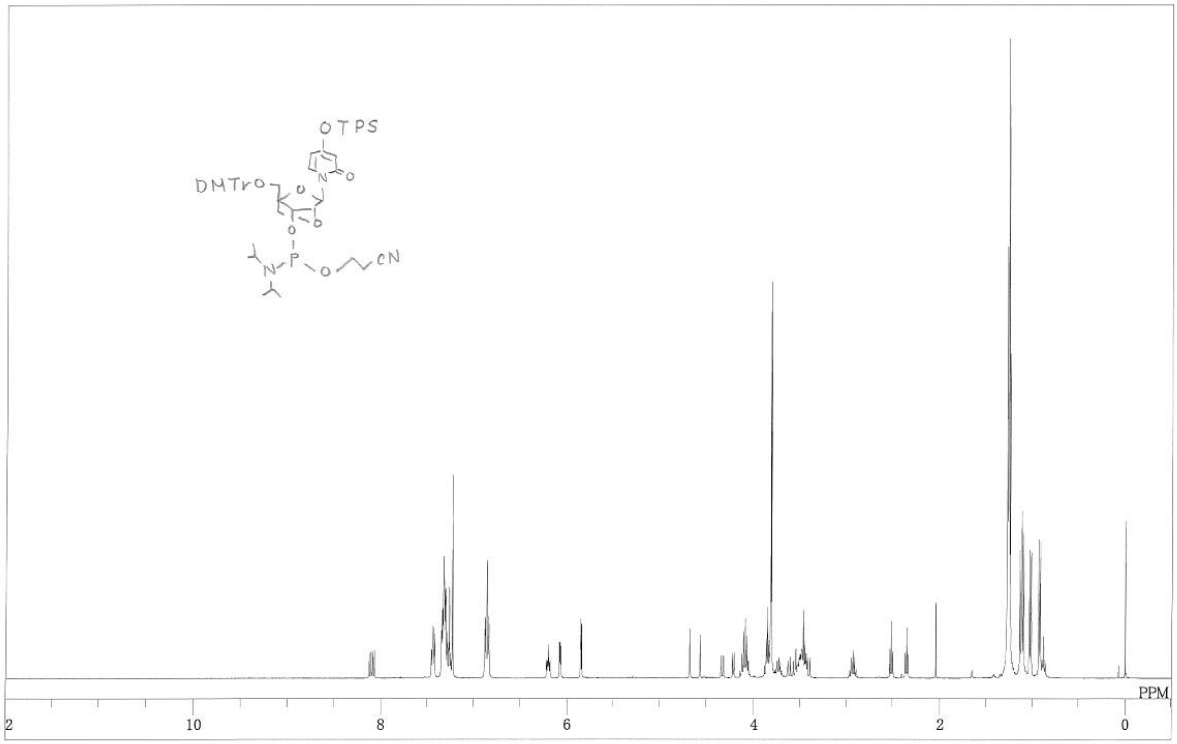


13C NMR (CDCl3)

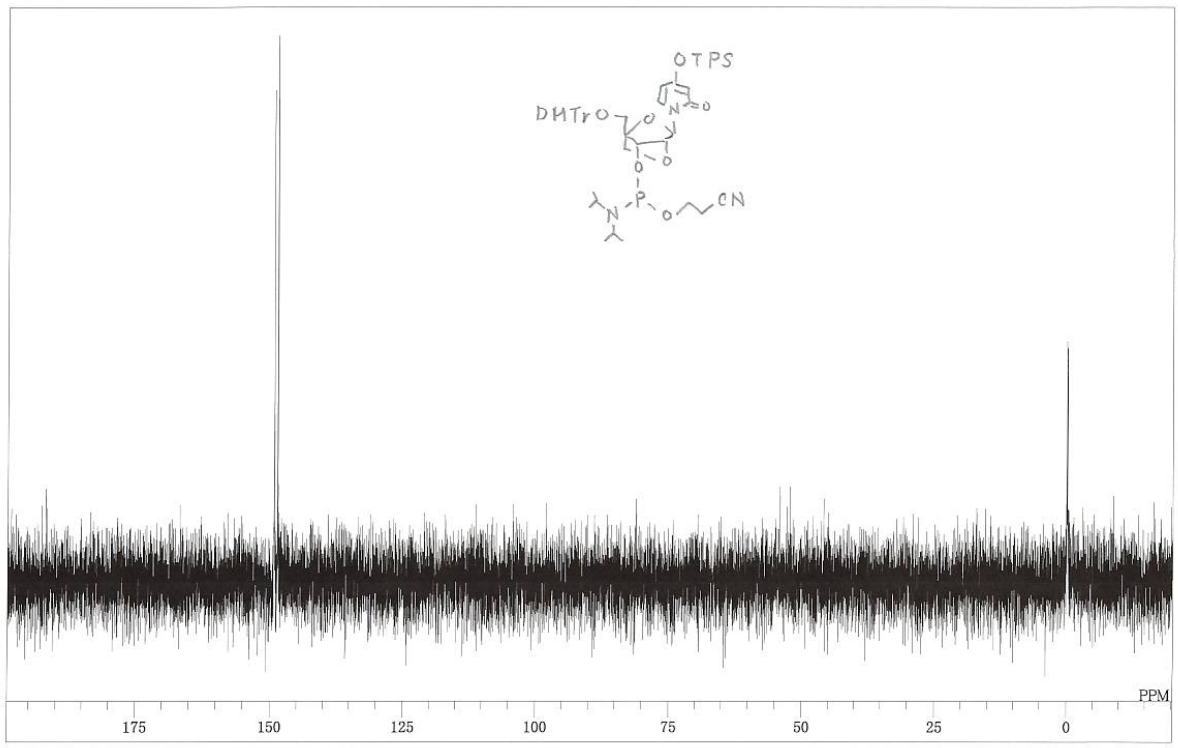


Compound **1**

1H NMR (CDCl3)

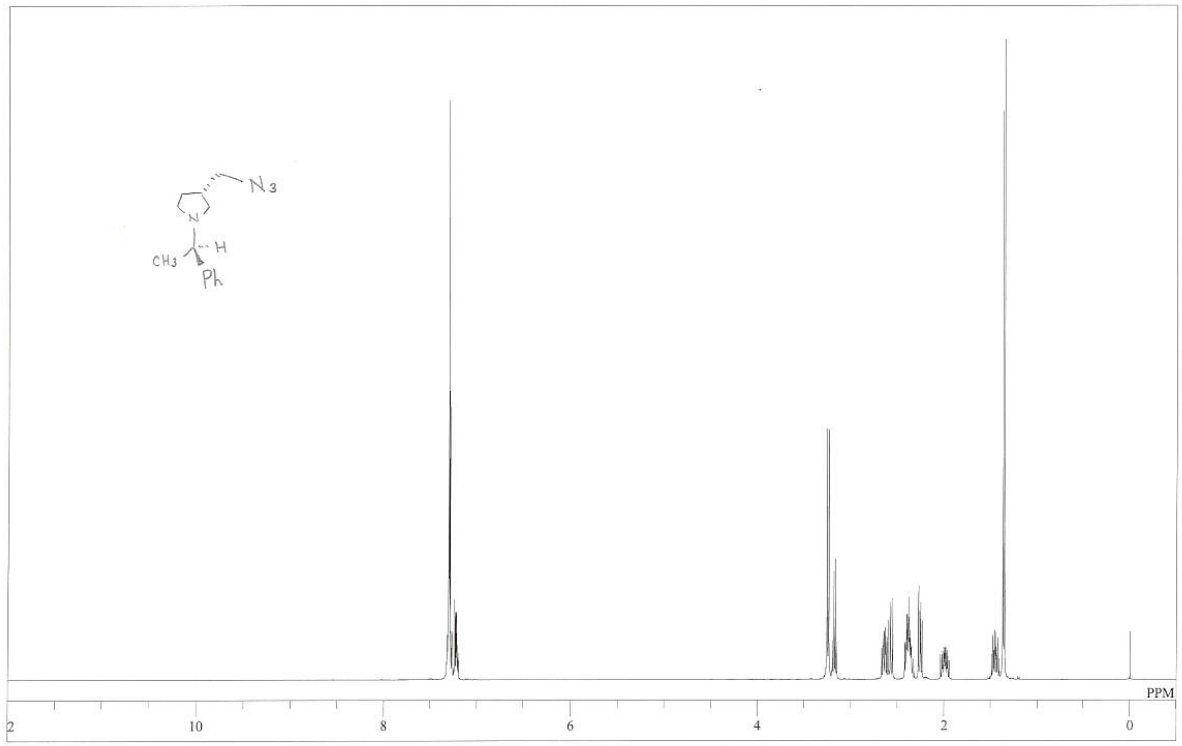


31P NMR (CDCl3)

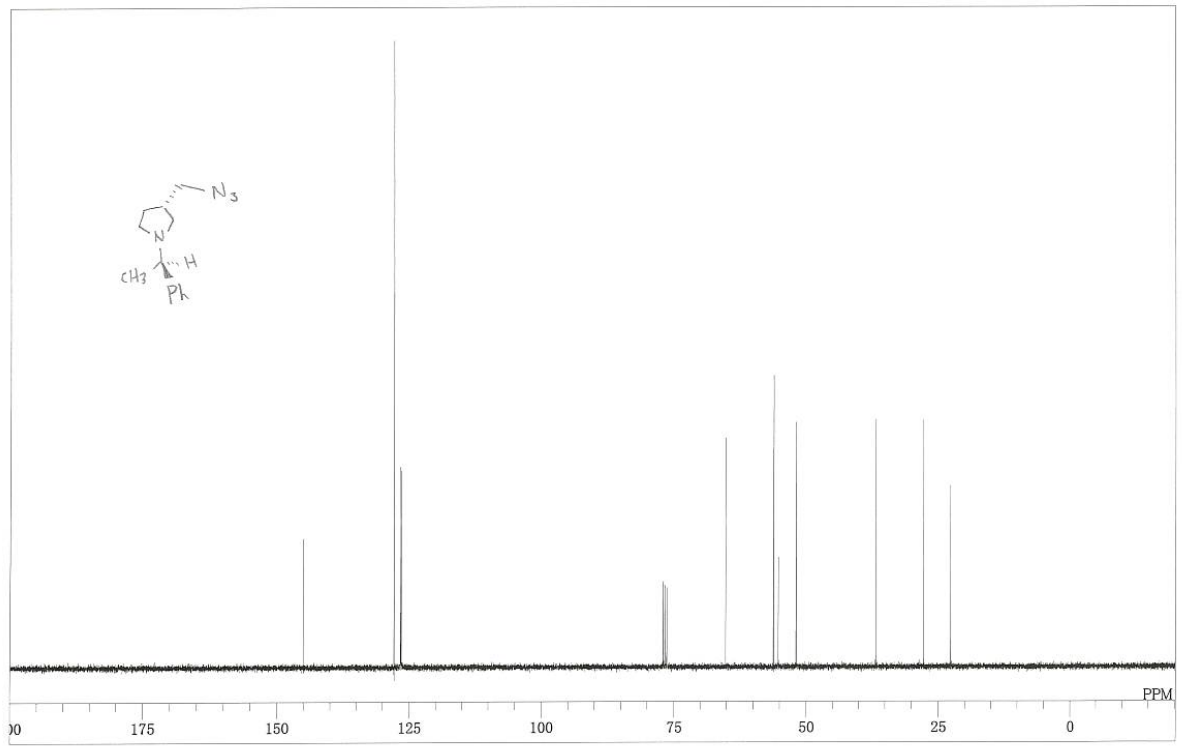


Compound **S6**

1H NMR (CDCl3)

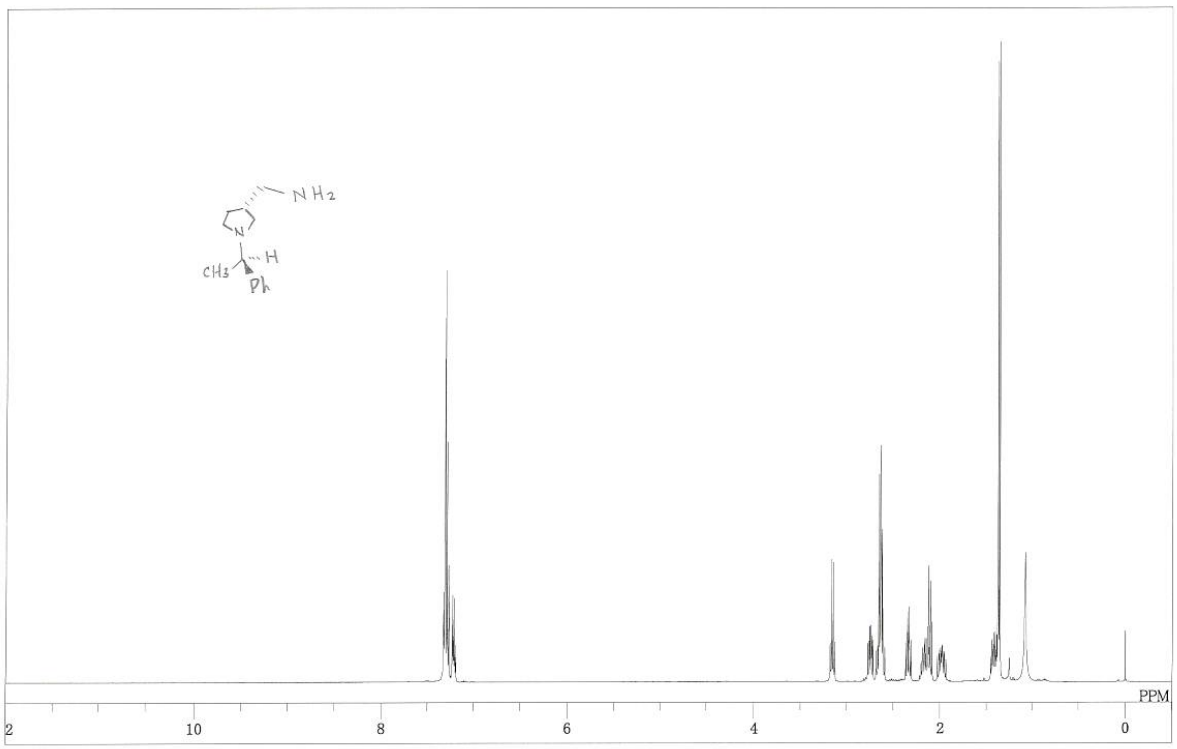


13C NMR (CDCl3)

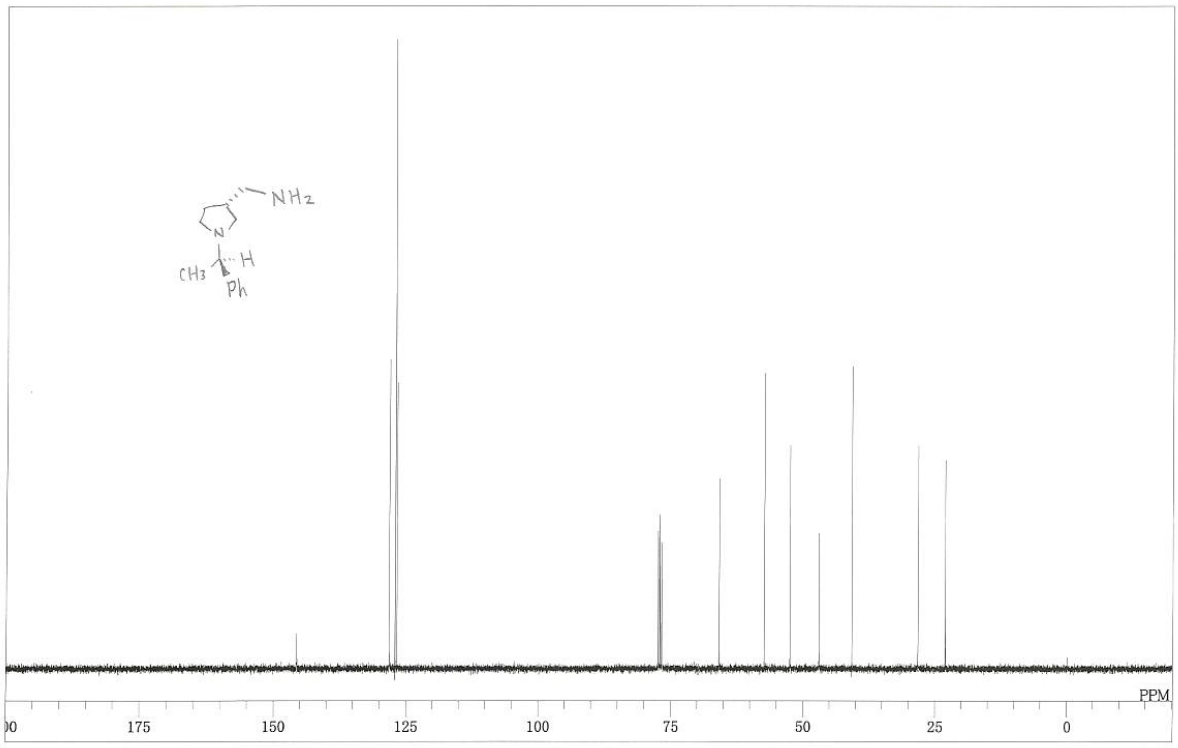


Compound **S7**

1H NMR (CDCl3)

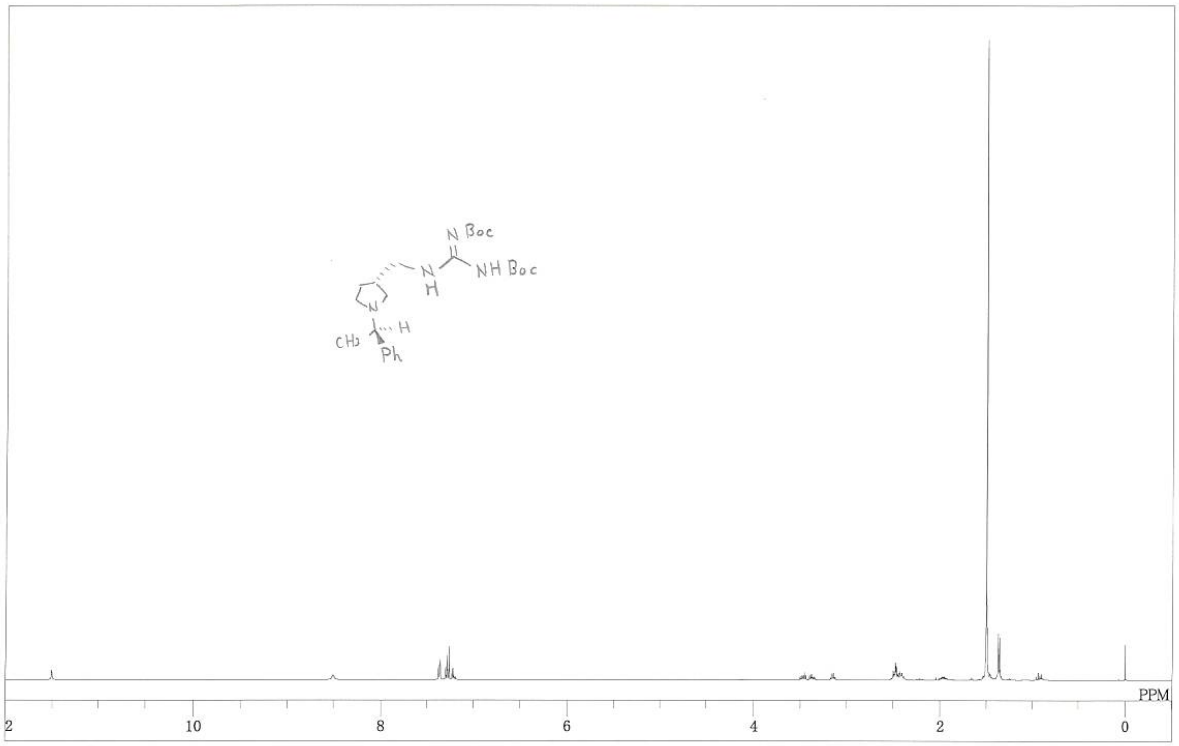


13C NMR (CDCl3)

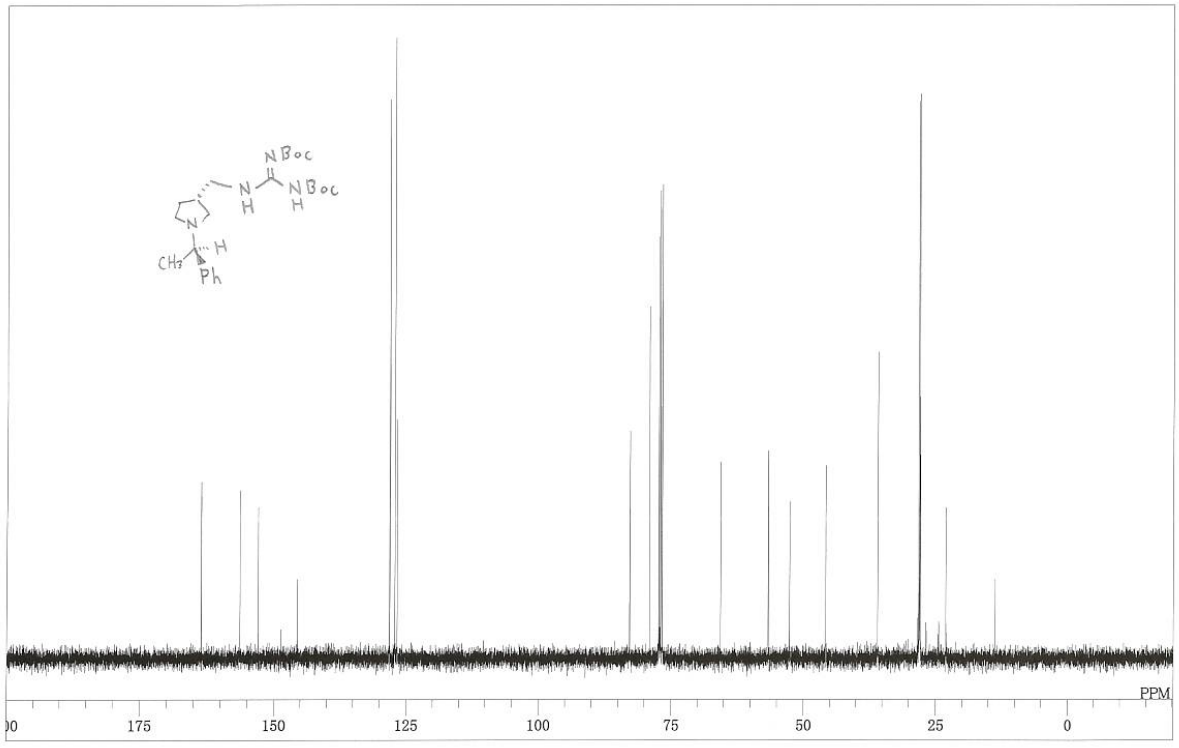


Compound **S8**

1H NMR (CDCl3)

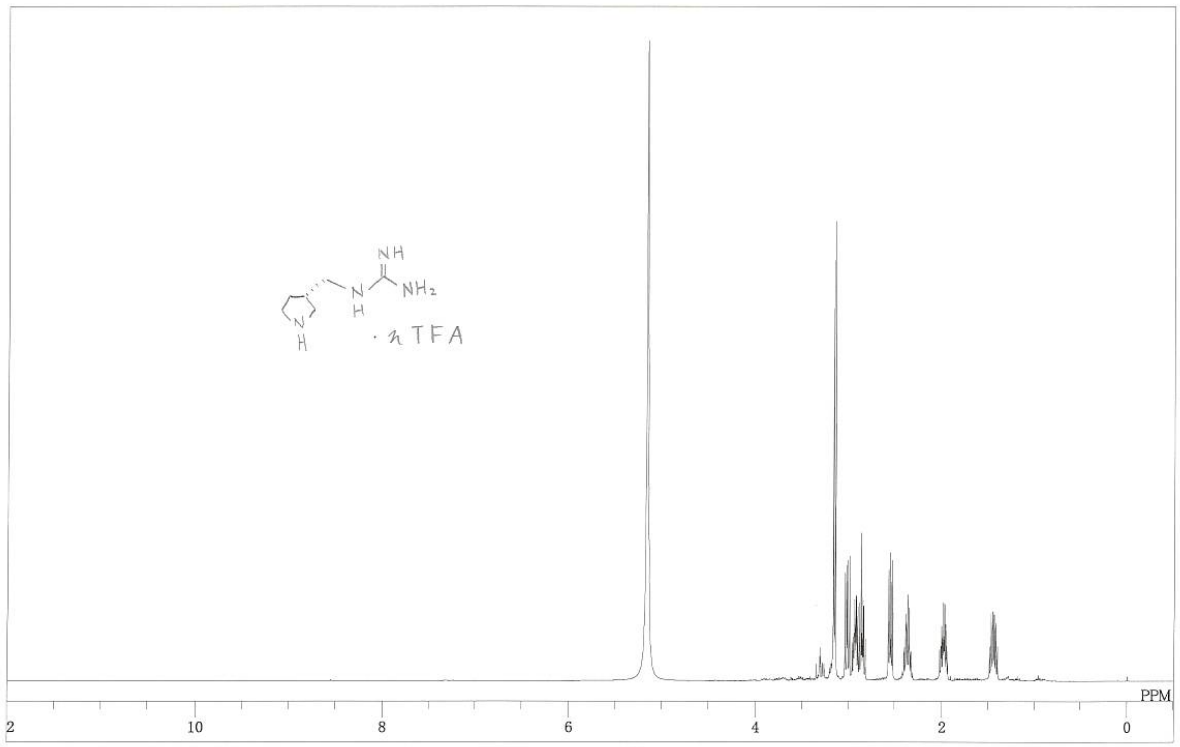


13C NMR (CDCl3)

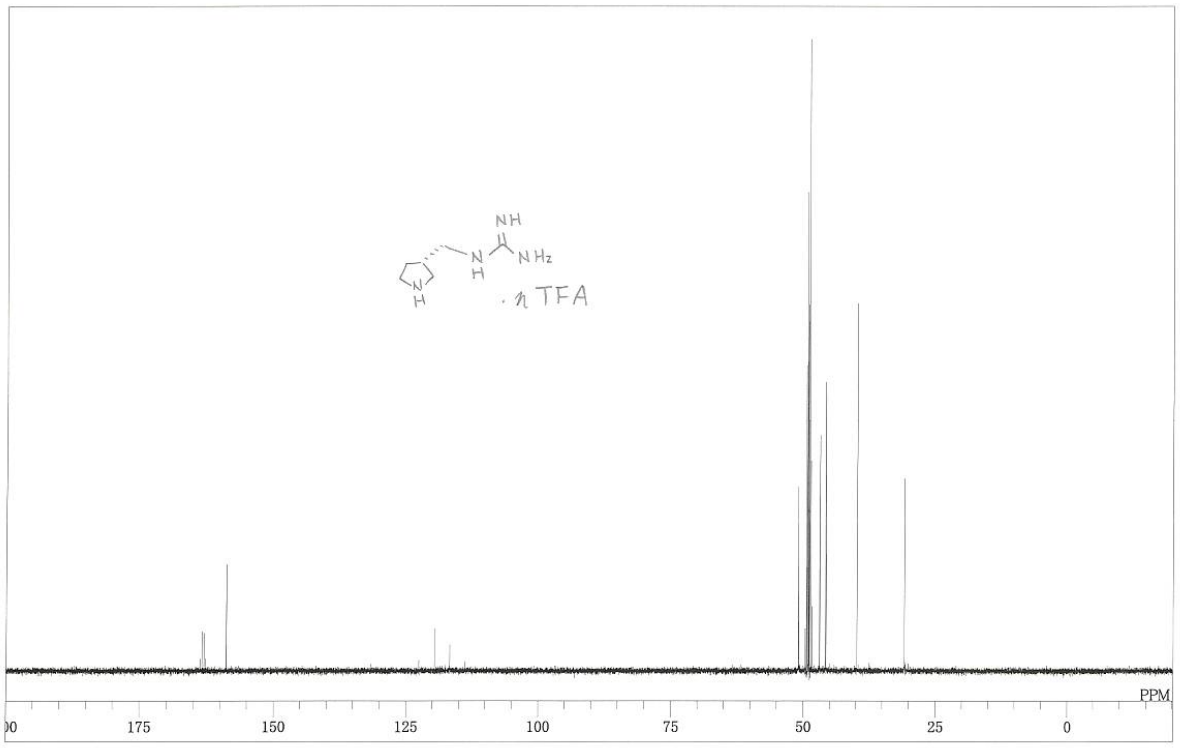


Compound **S1**

1H NMR (CD3OD)

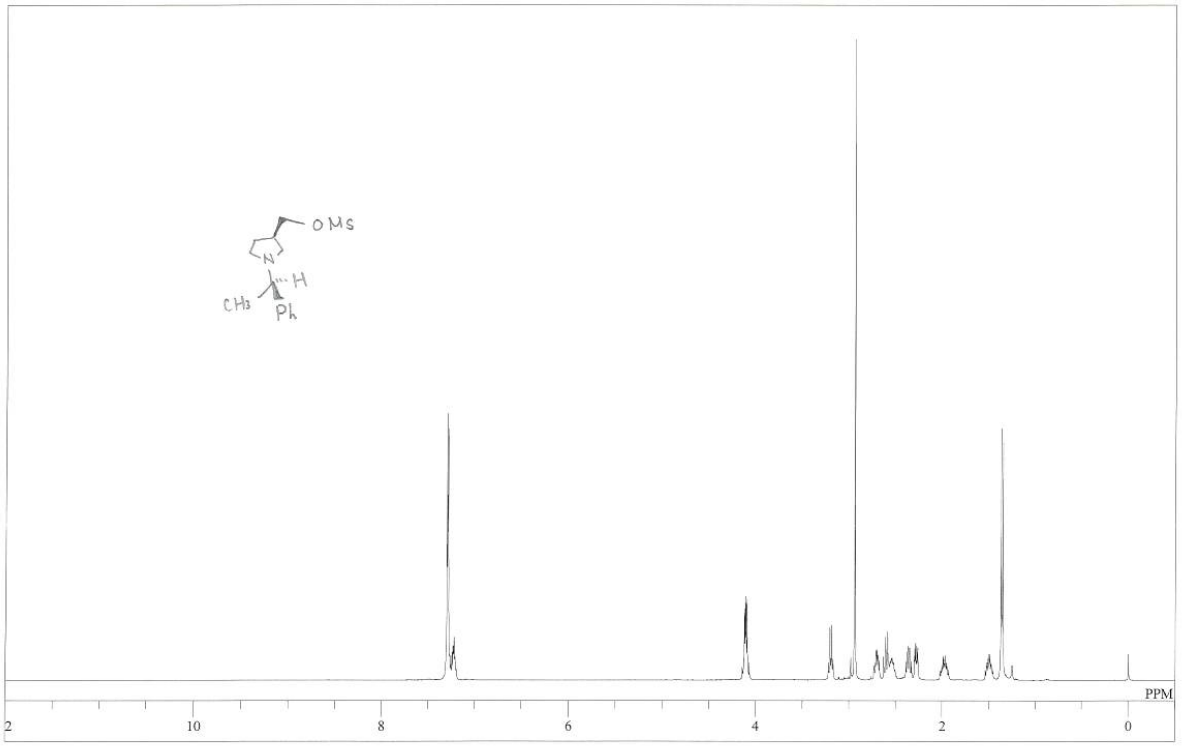


13C NMR (CD3OD)

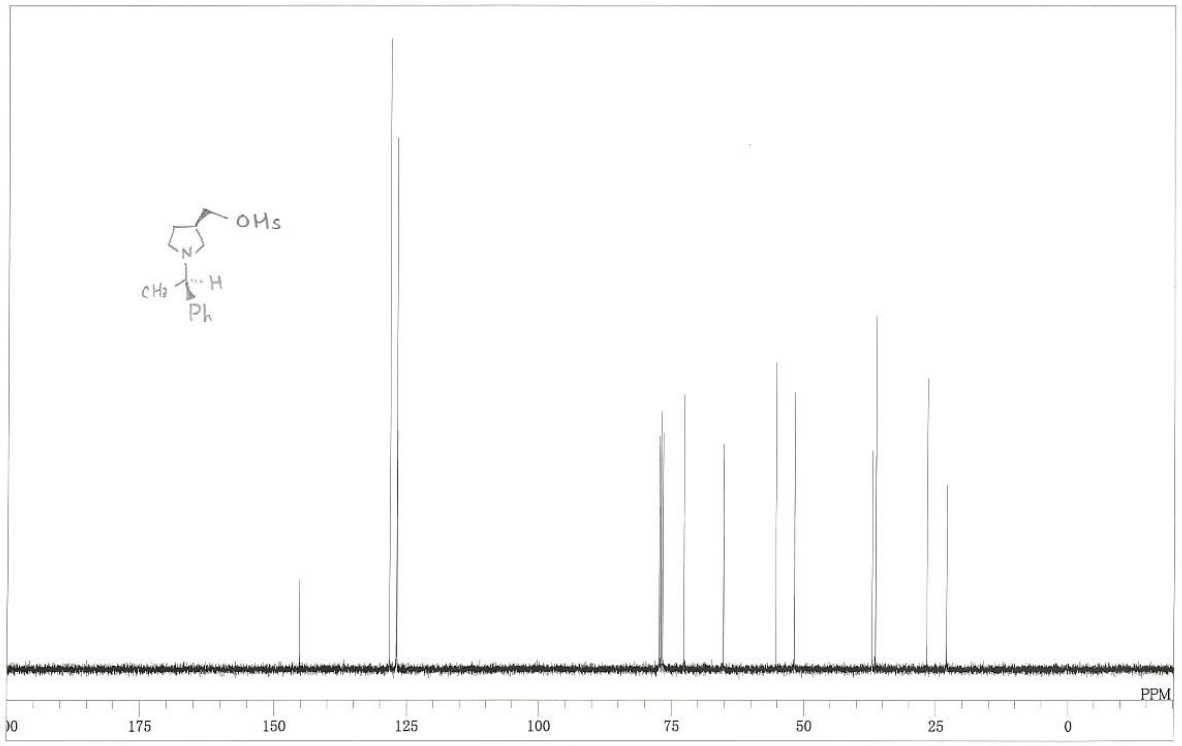


Compound **S10**

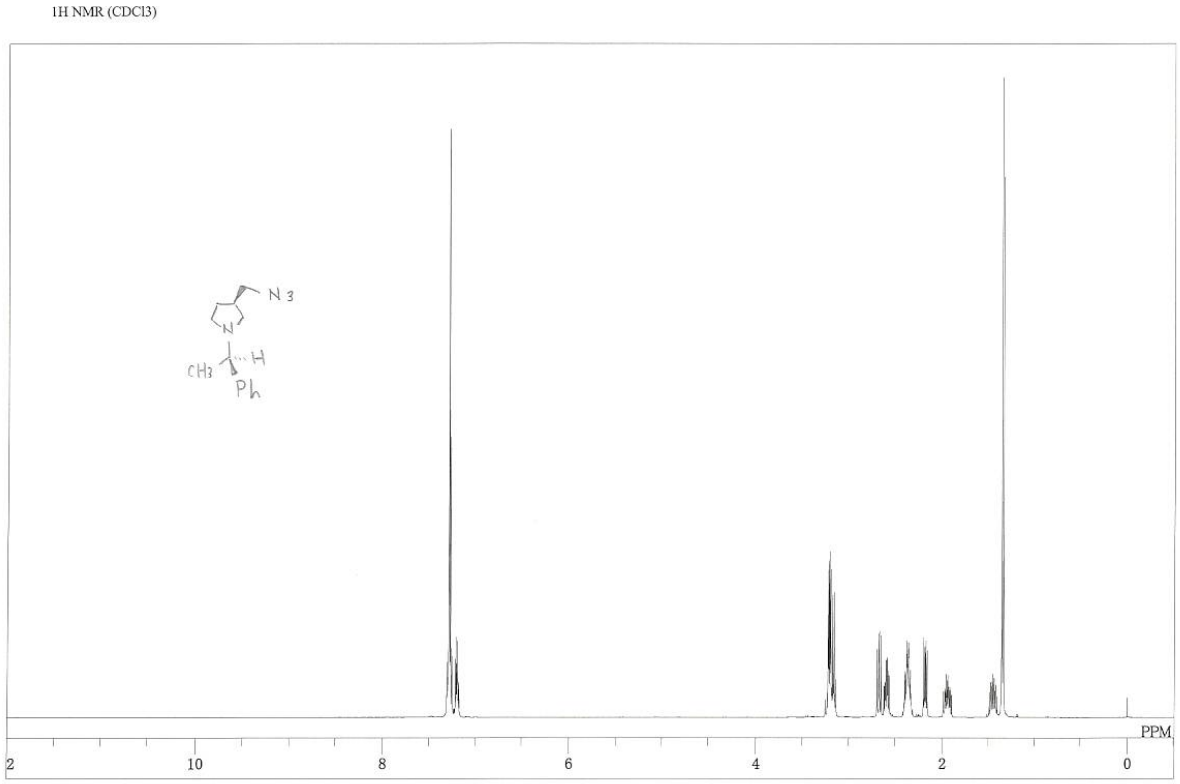
1H NMR (CDCl3)

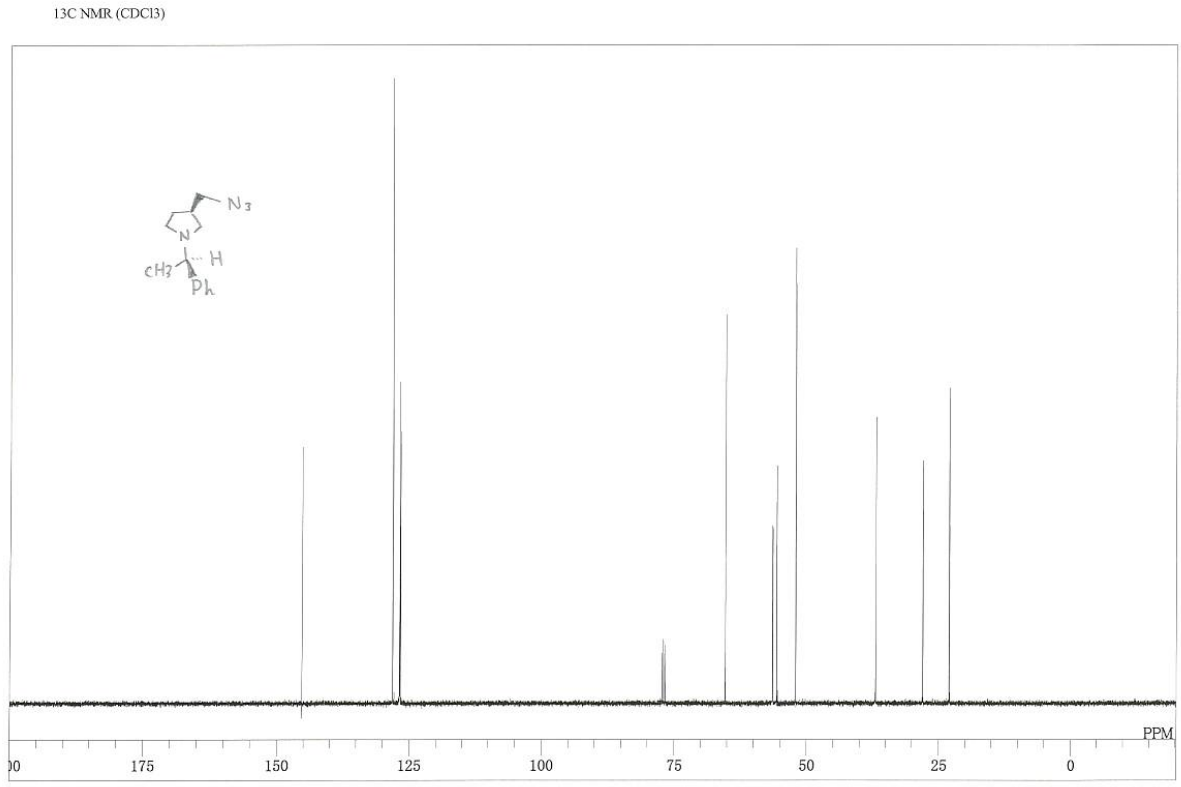


13C NMR (CDCl3)



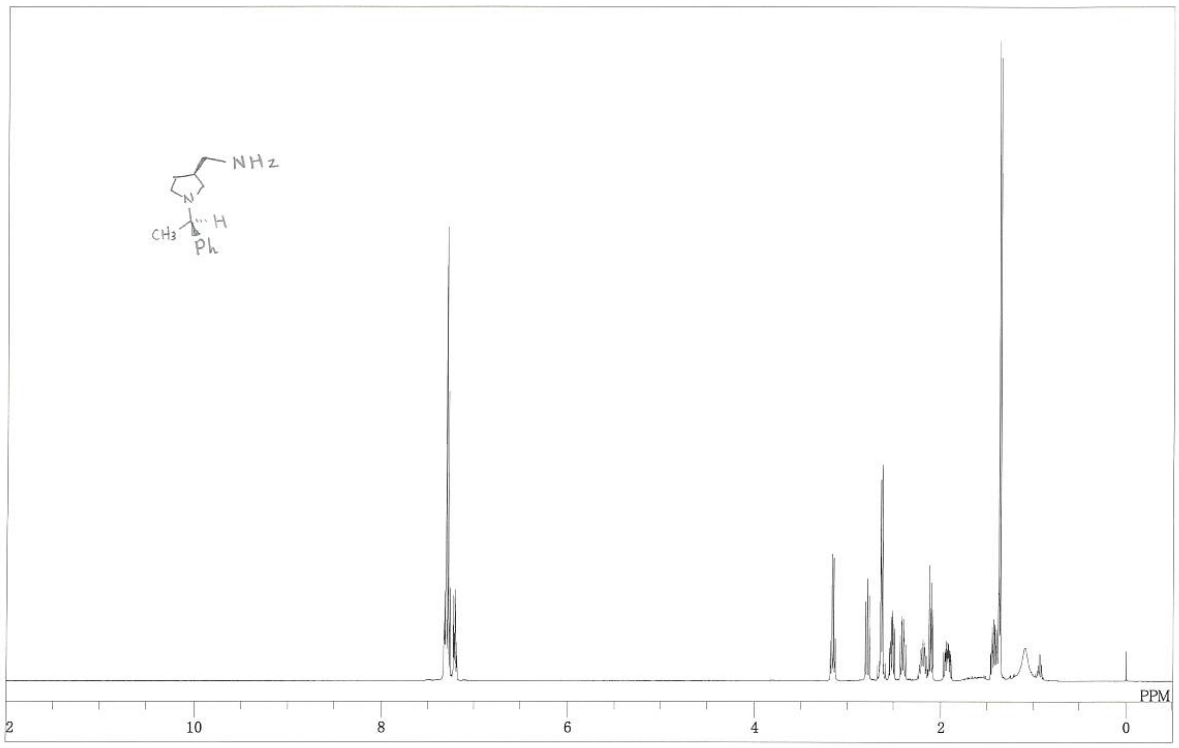
Compound **S11**



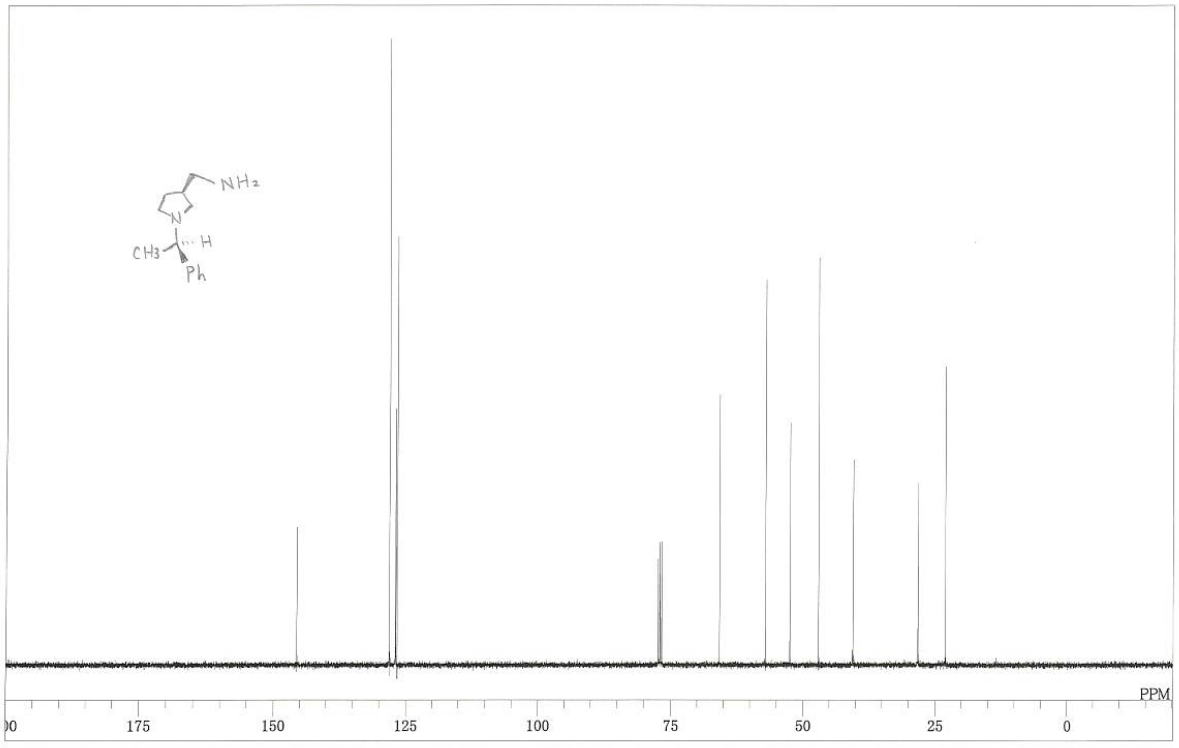


Compound **S12**

1H NMR (CDCl3)

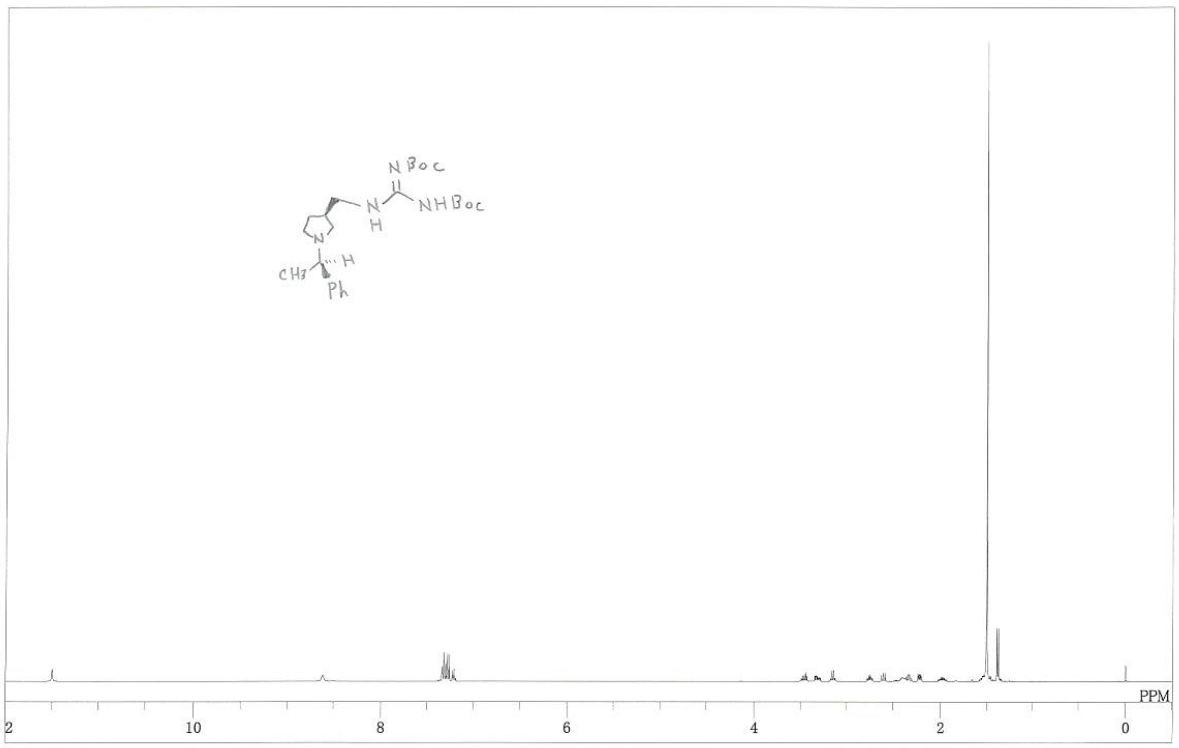


13C NMR (CDCl3)

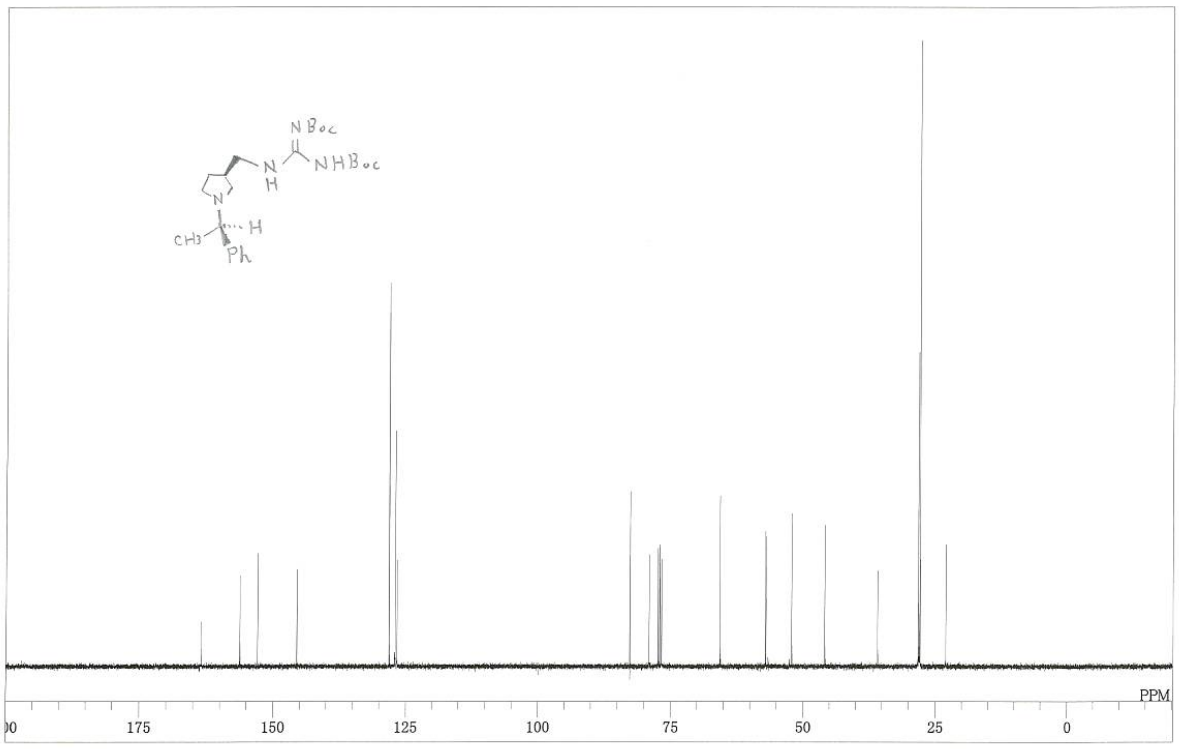


Compound **S13**

1H NMR (CDCl3)

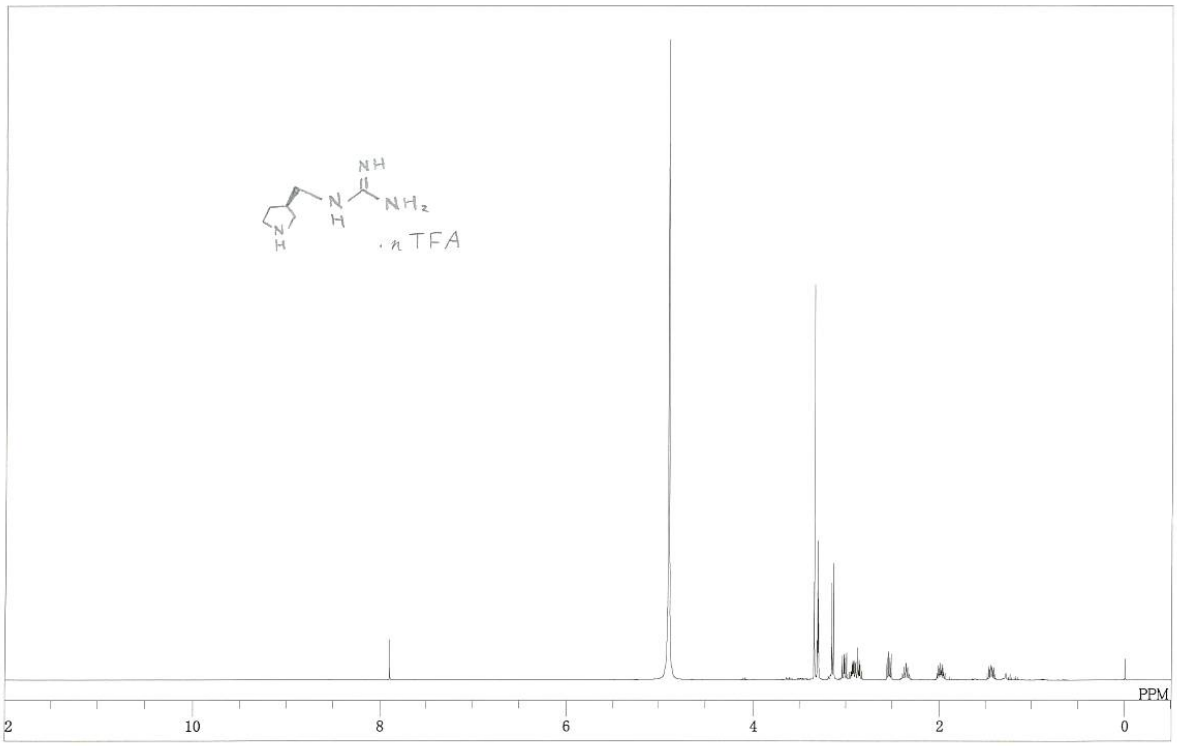


13C NMR (CDCl3)

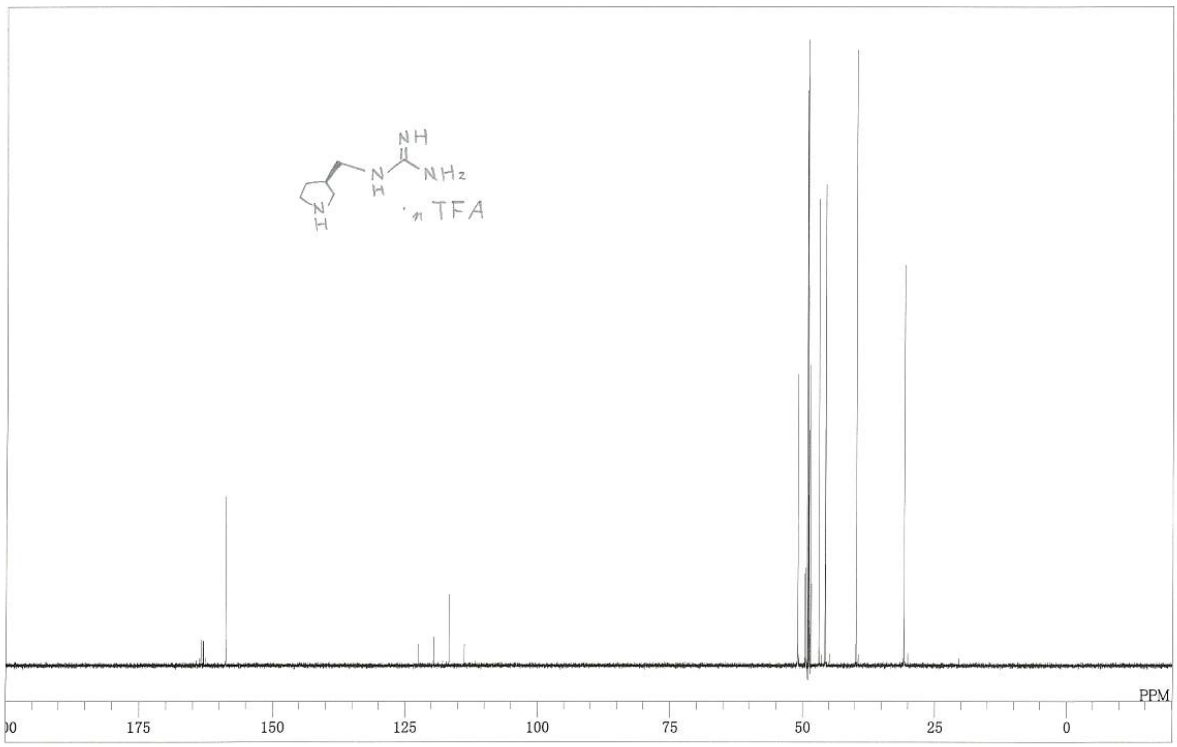


Compound **S2**

1H NMR (CD3OD)

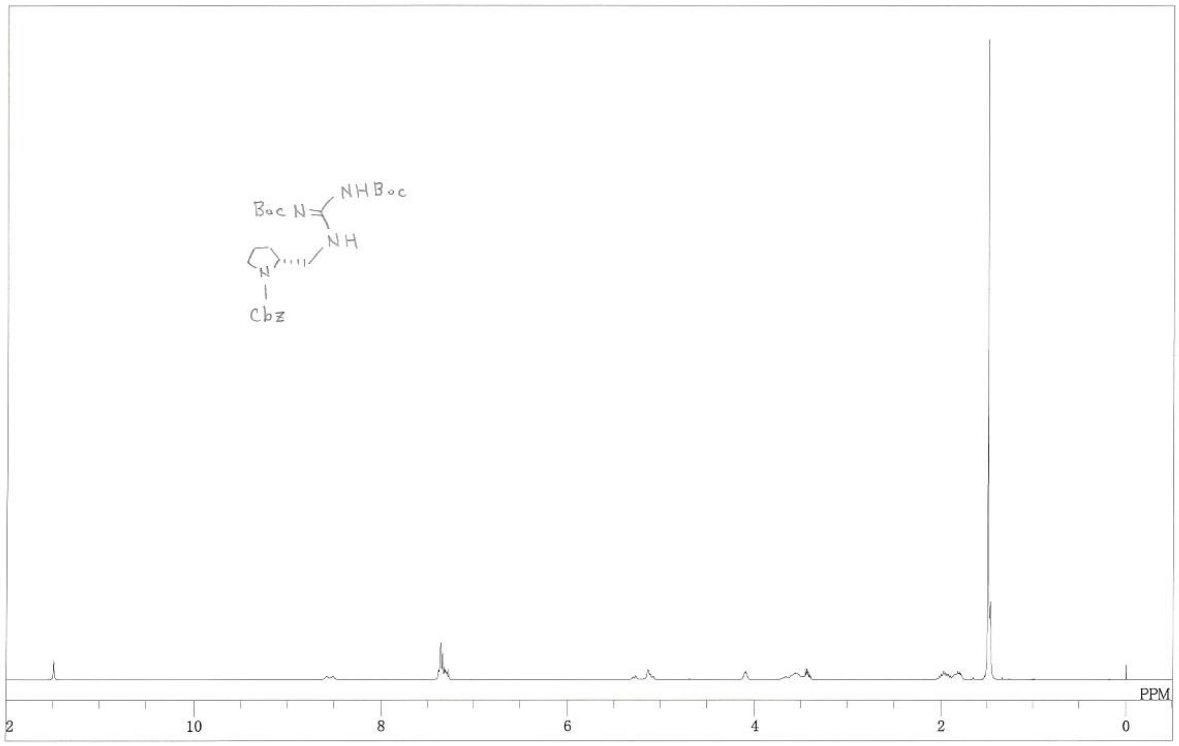


13C NMR (CD3OD)

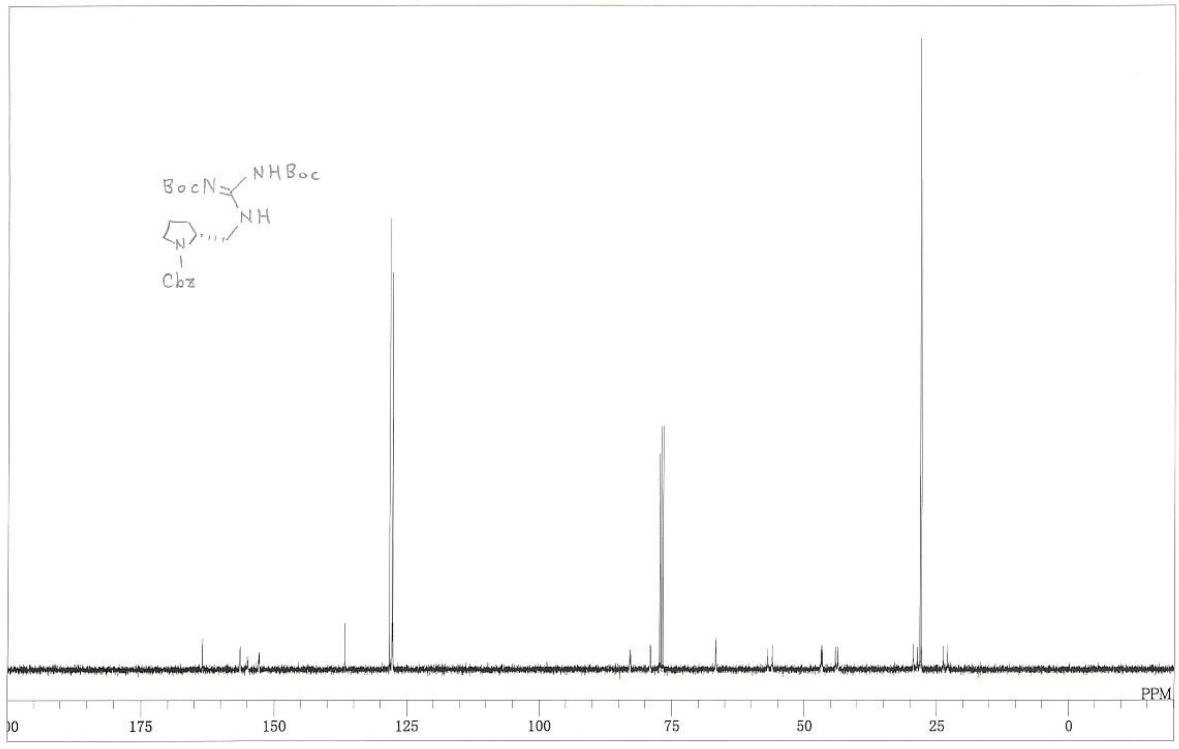


Compound **S15**

1H NMR (CDCl3)

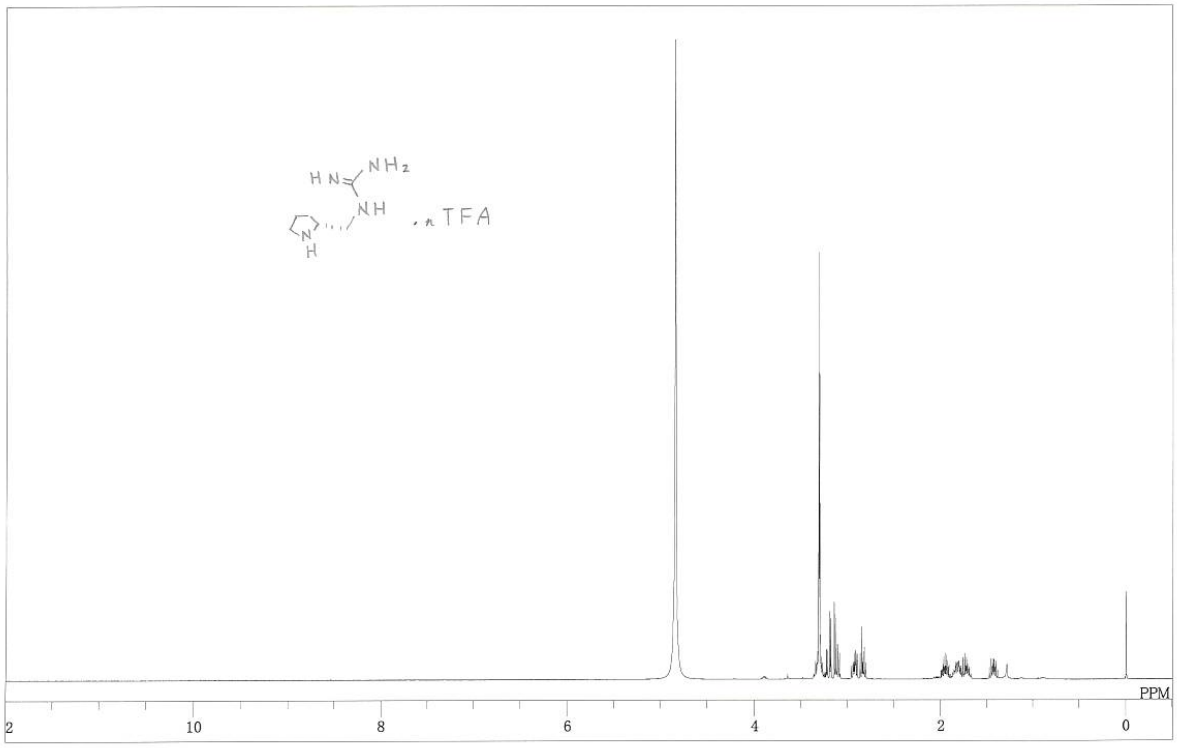


13C NMR (CDCl3)

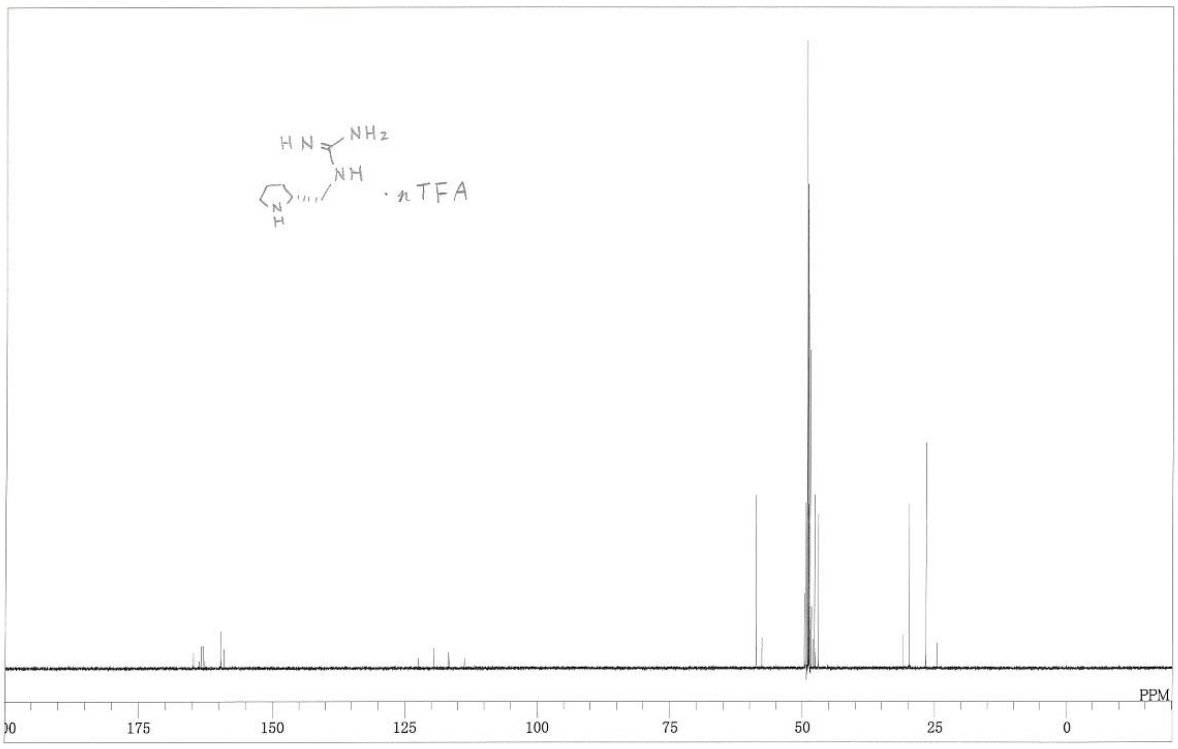


Compound **S3**

1H NMR (CD3OD)

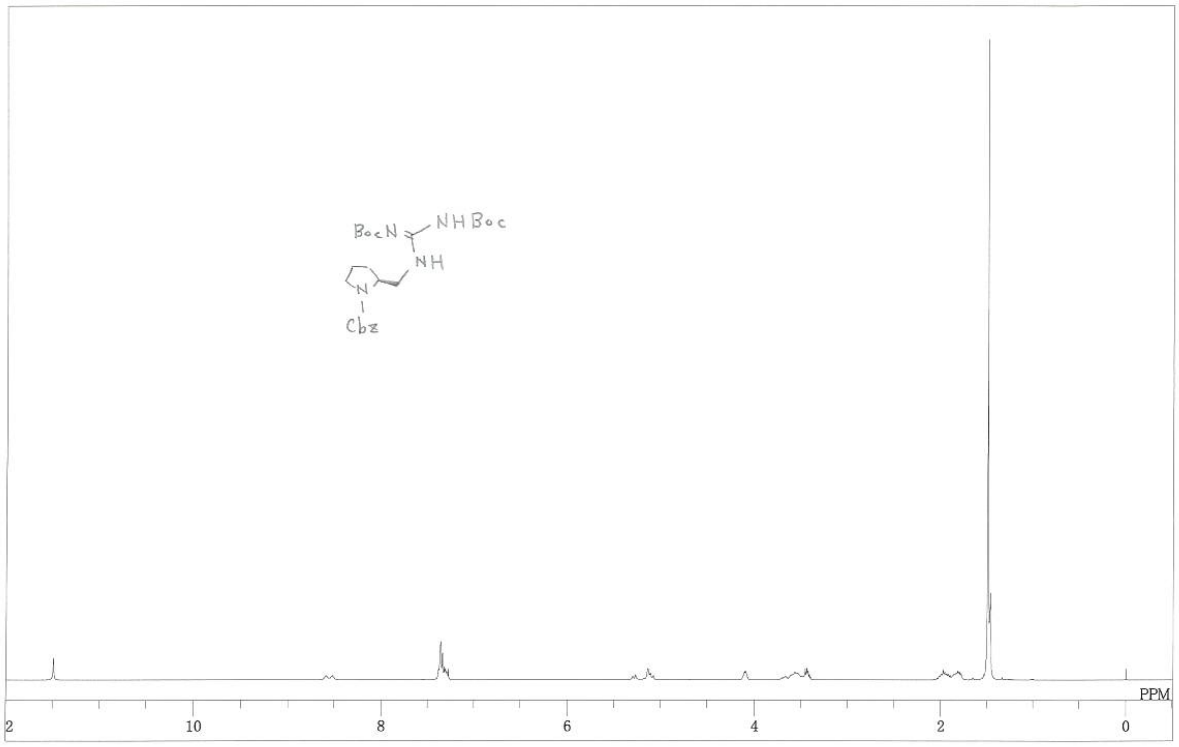


13C NMR (CDCl3)

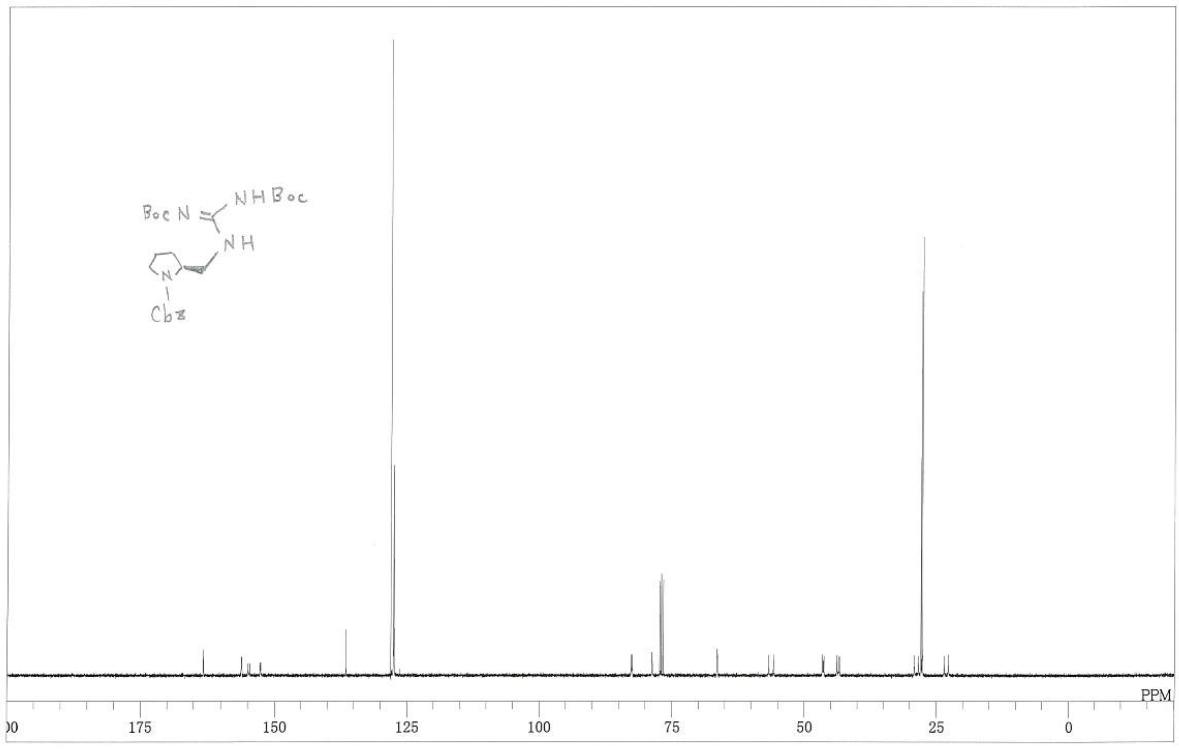


Compound **S17**

1H NMR (CDCl3)

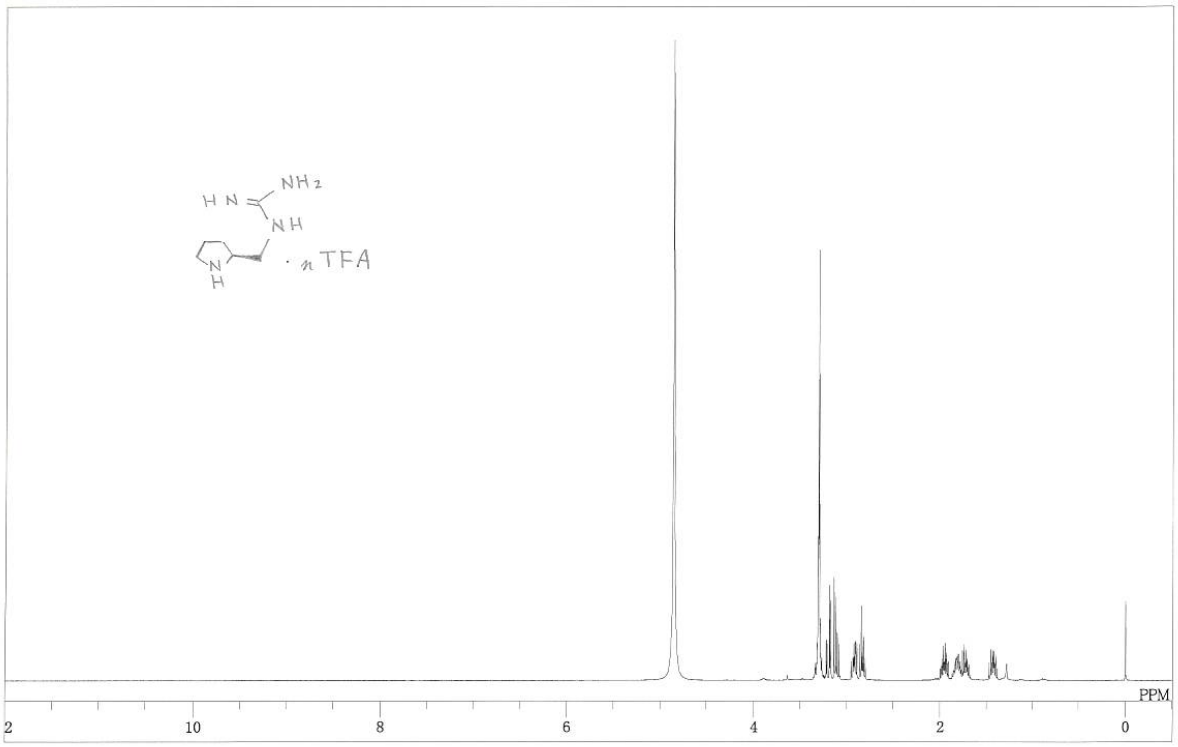


13C NMR (CDCl3)

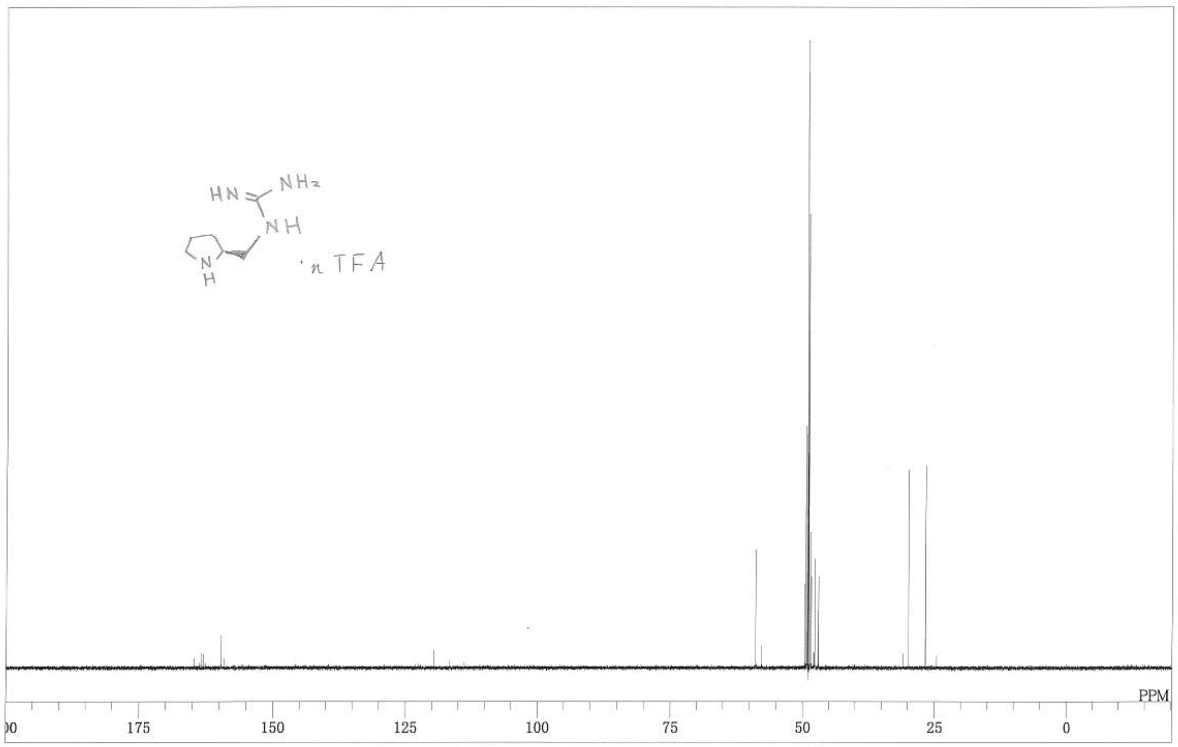


Compound **S4**

1H NMR (CD3OD)



13C NMR (CD3OD)



TFO **7a**

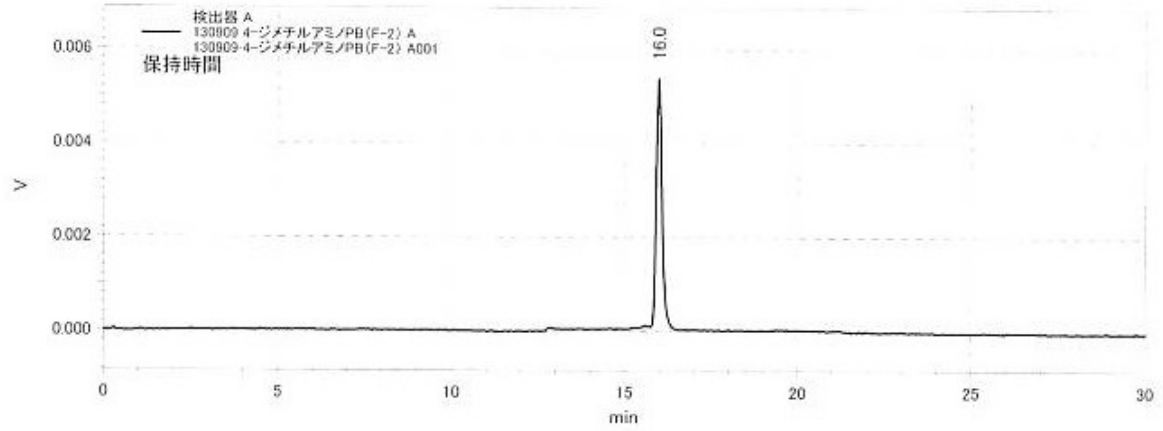
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

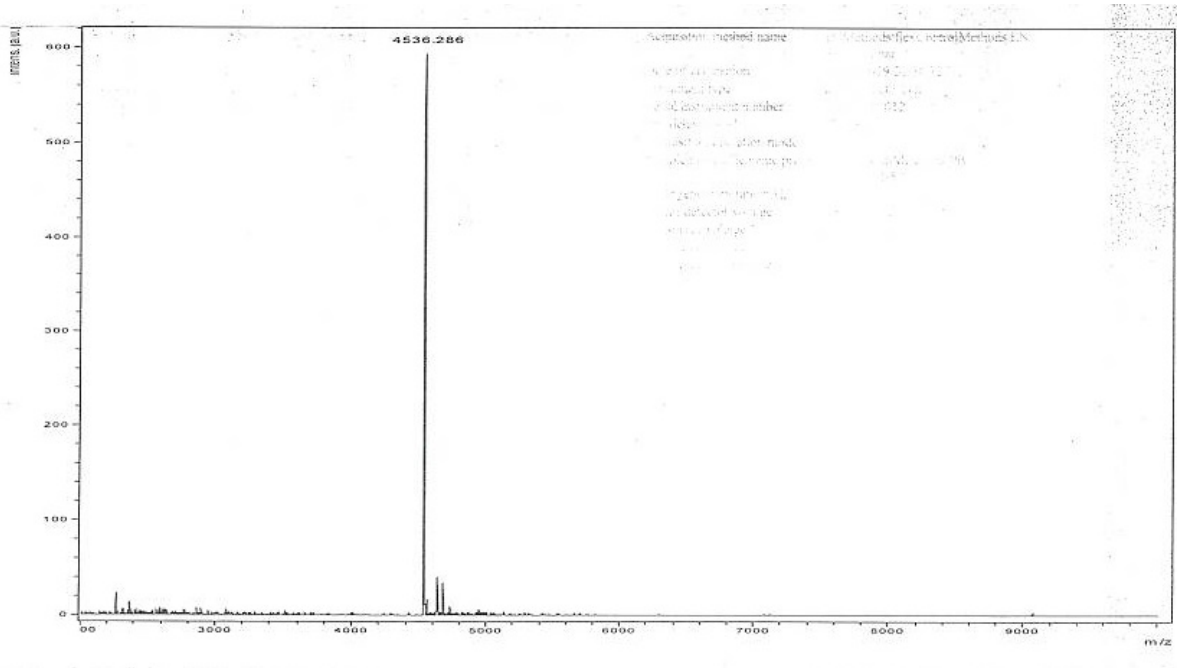
Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass



TFO **7b**

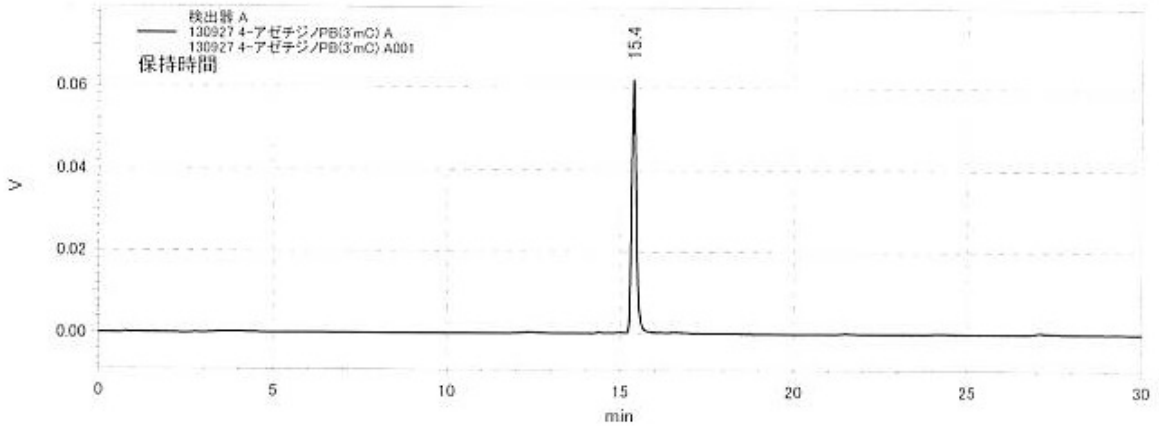
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



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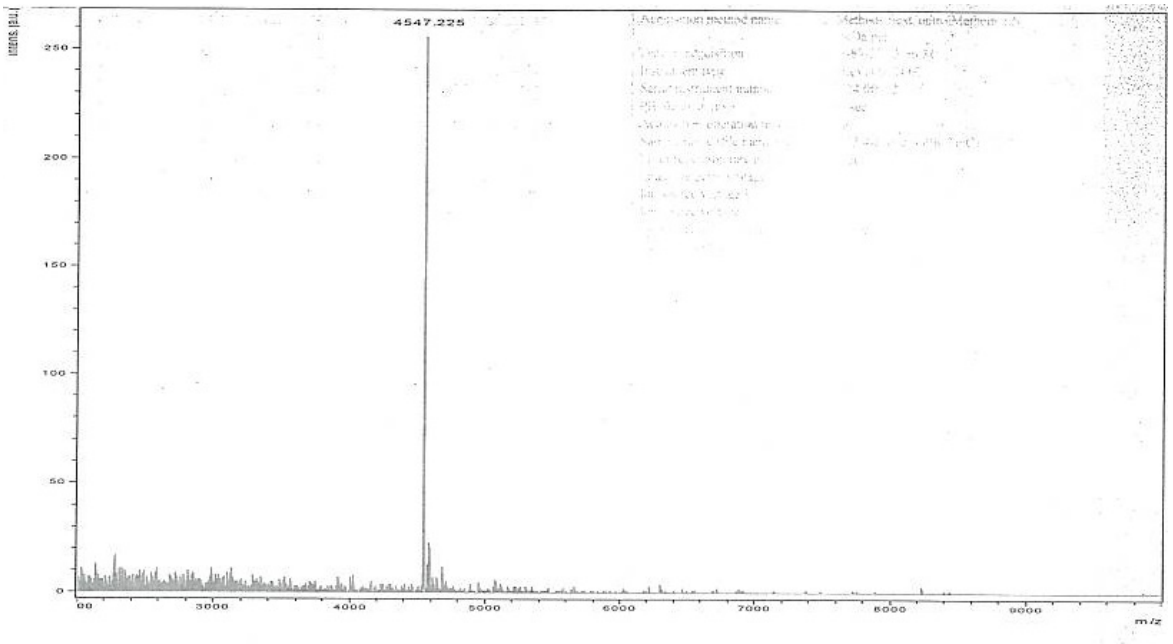


TFO **7c**

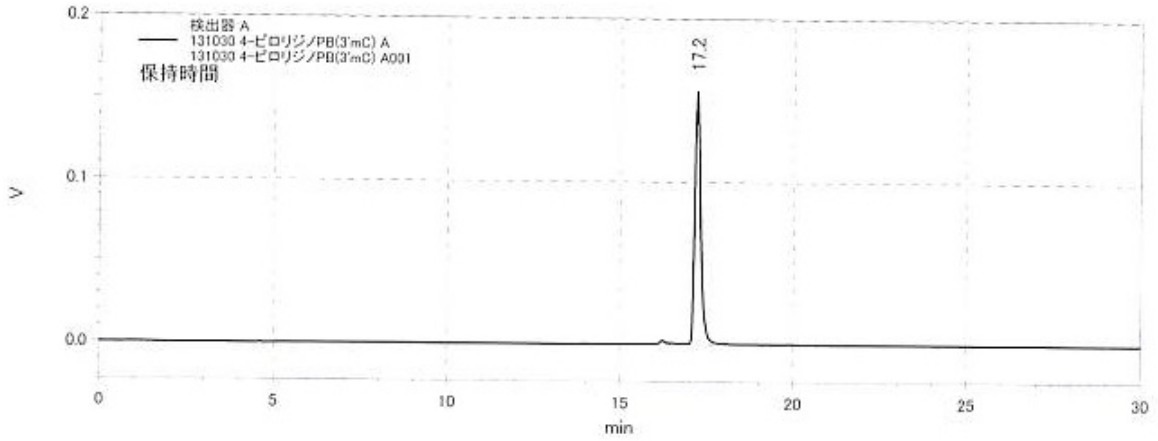
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

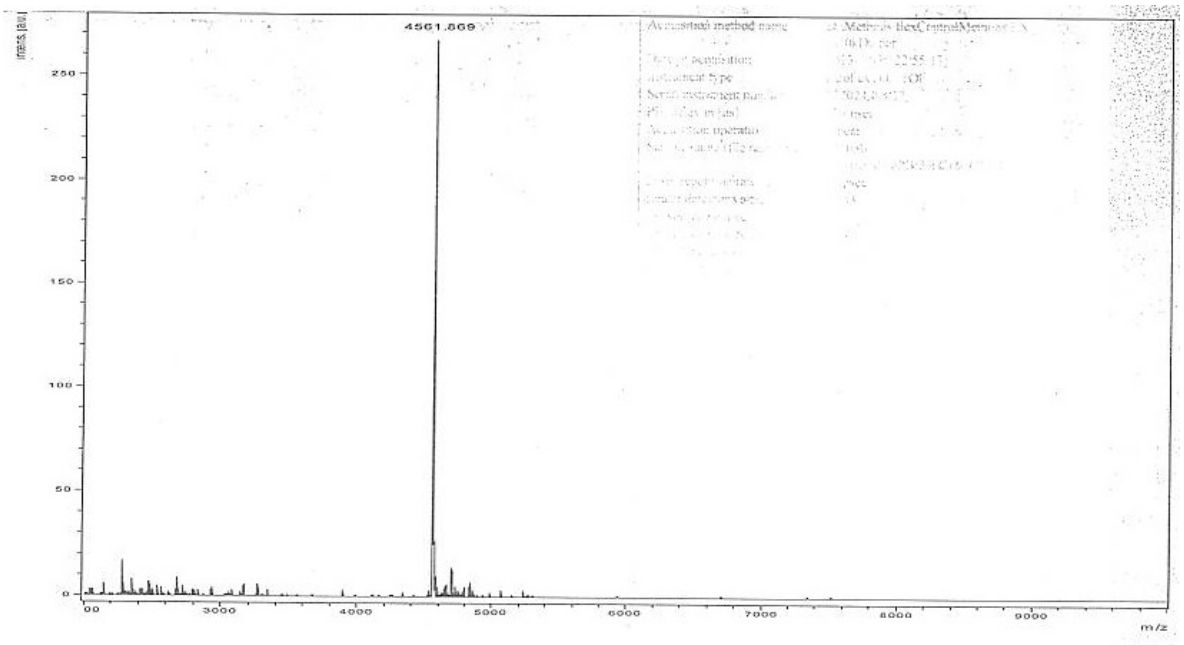
Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass



TFO **7d**

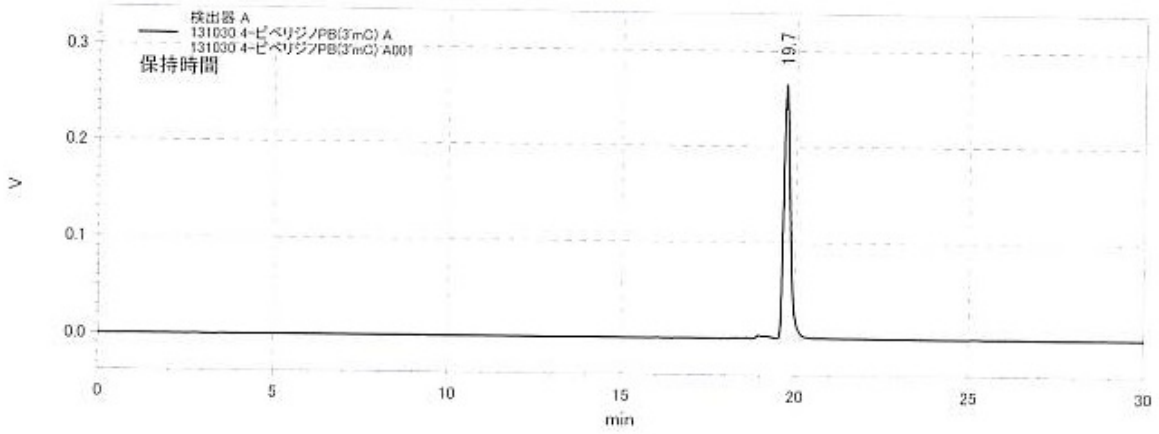
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

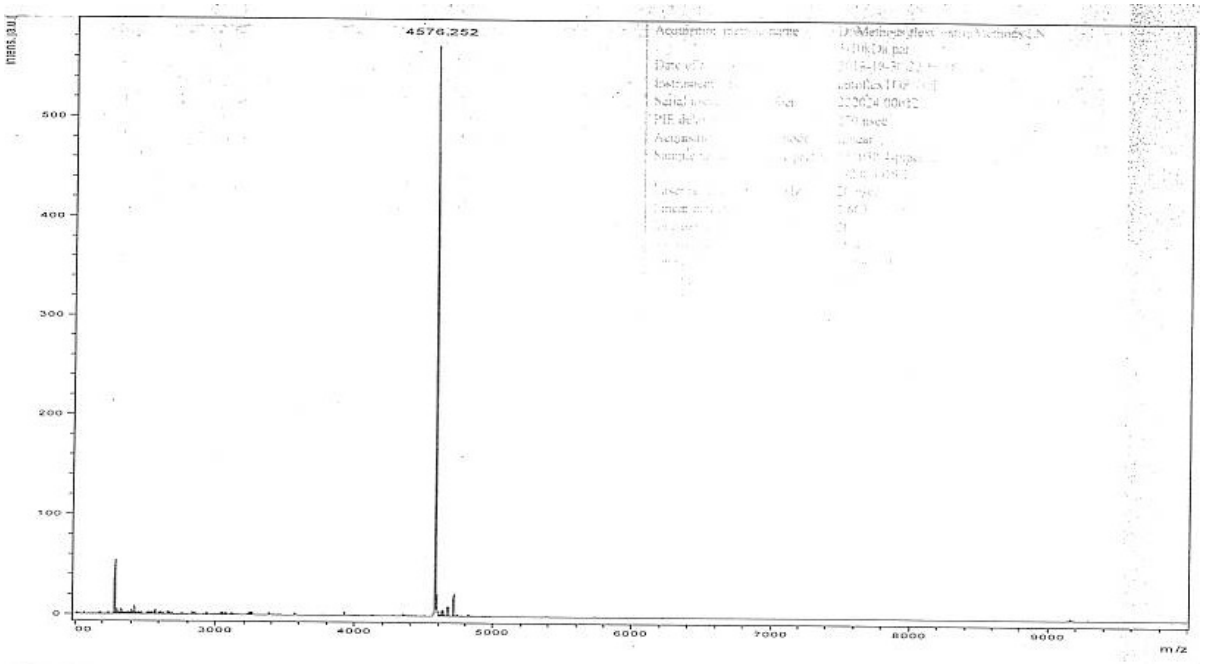
Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass



TFO **7e**

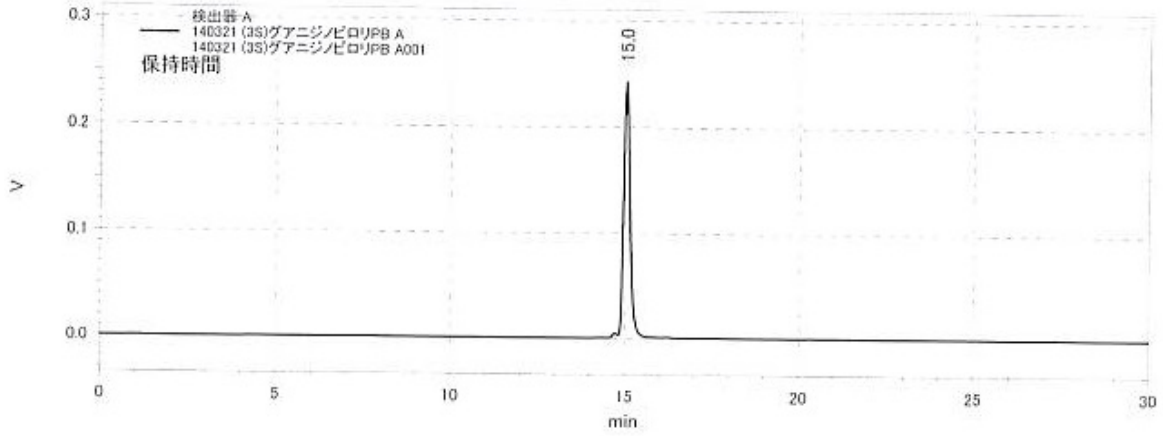
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

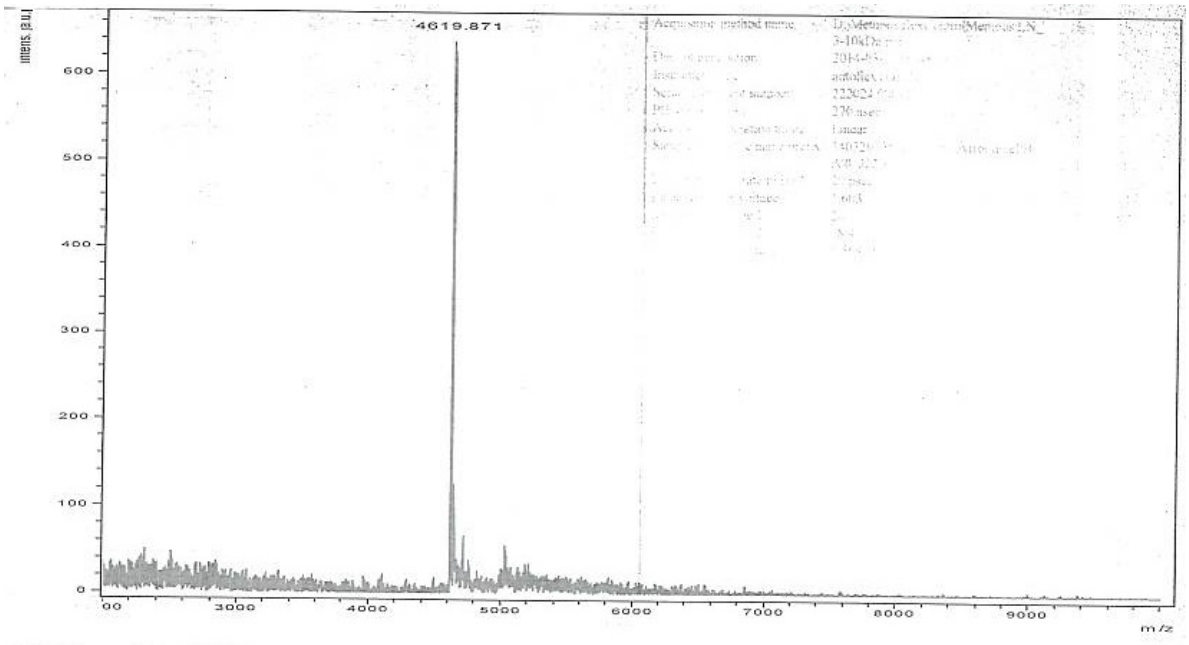
Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass



TFO **7f**

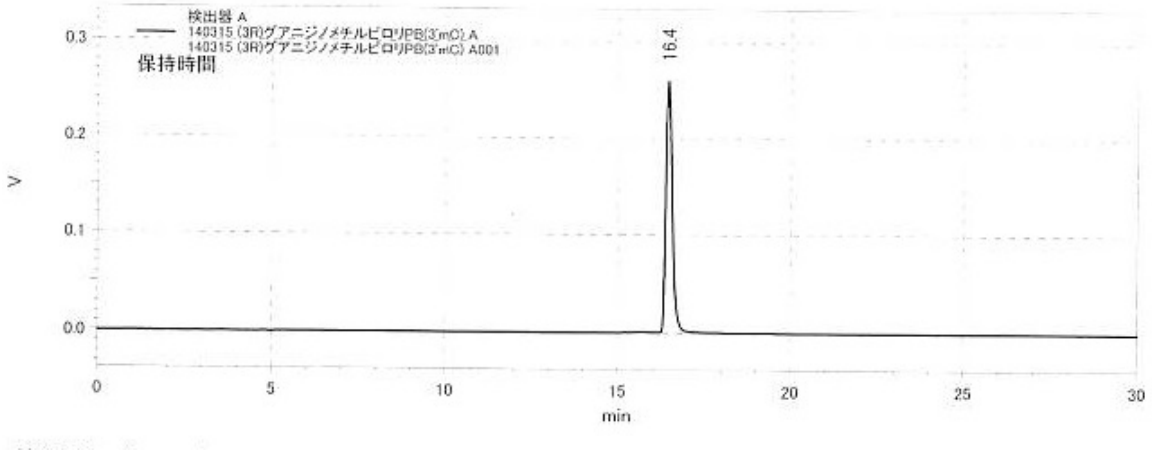
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

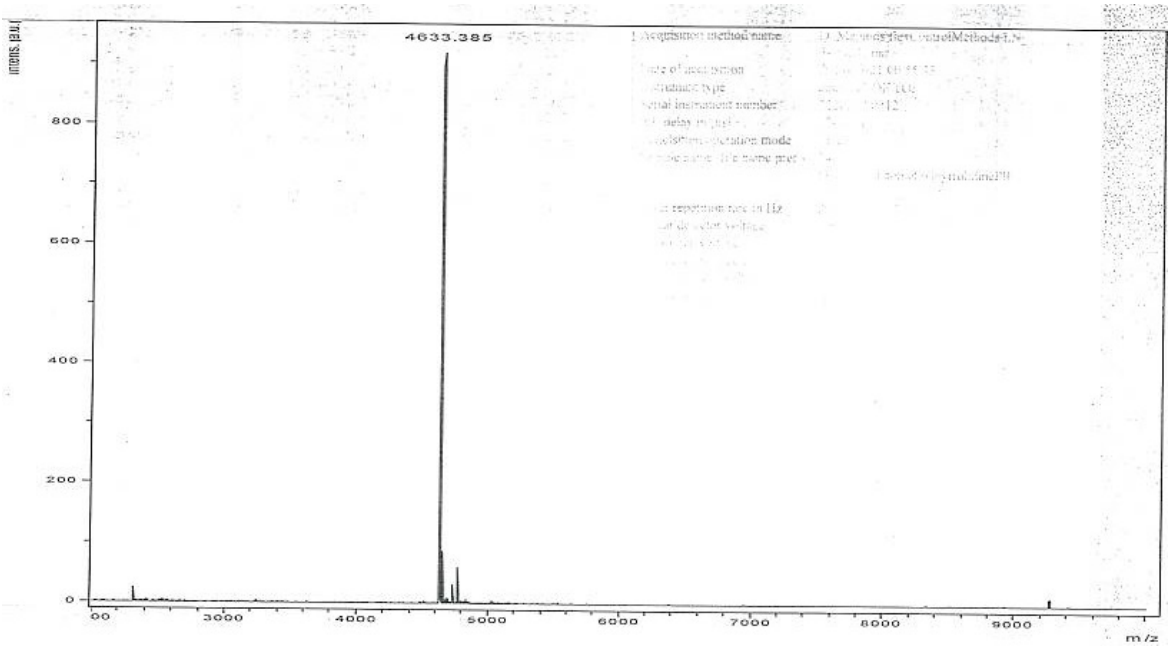
Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass



TFO **7g**

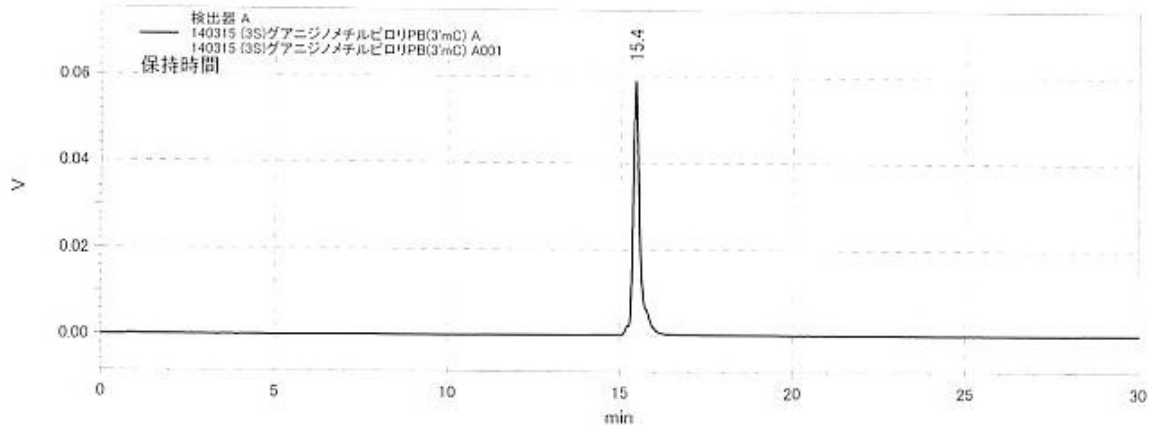
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

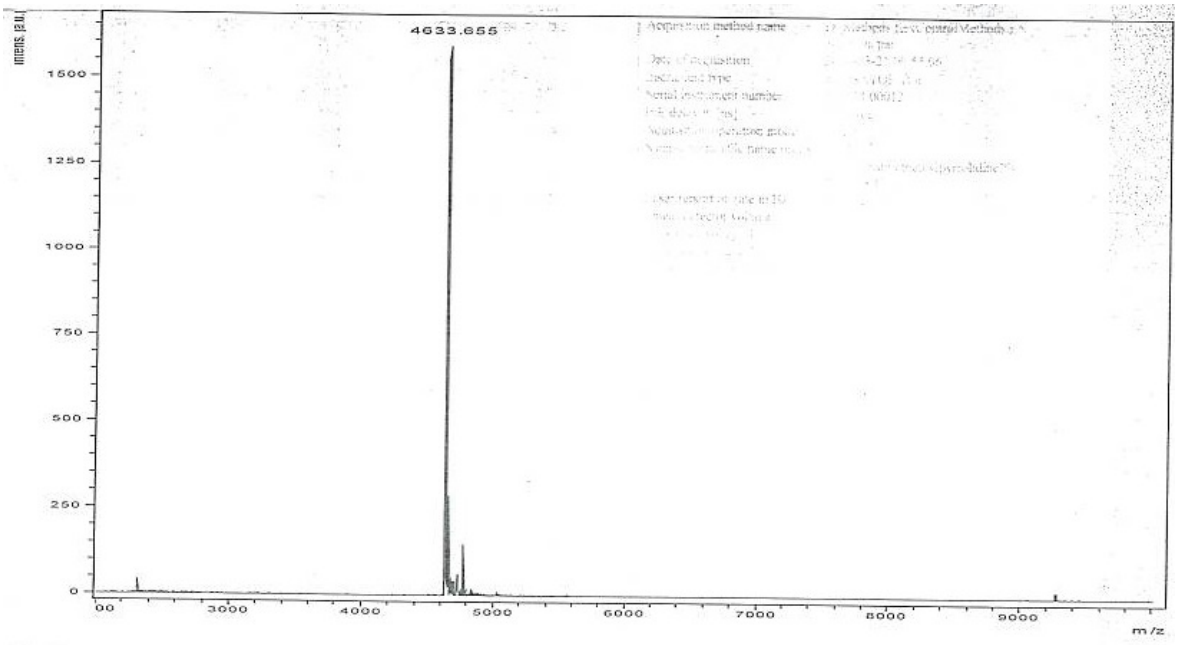
Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass



TFO **7h**

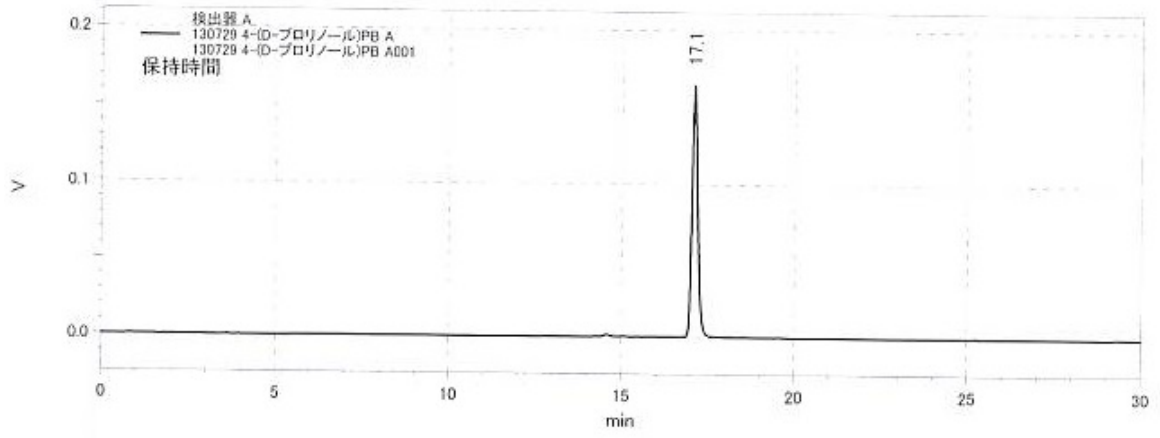
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

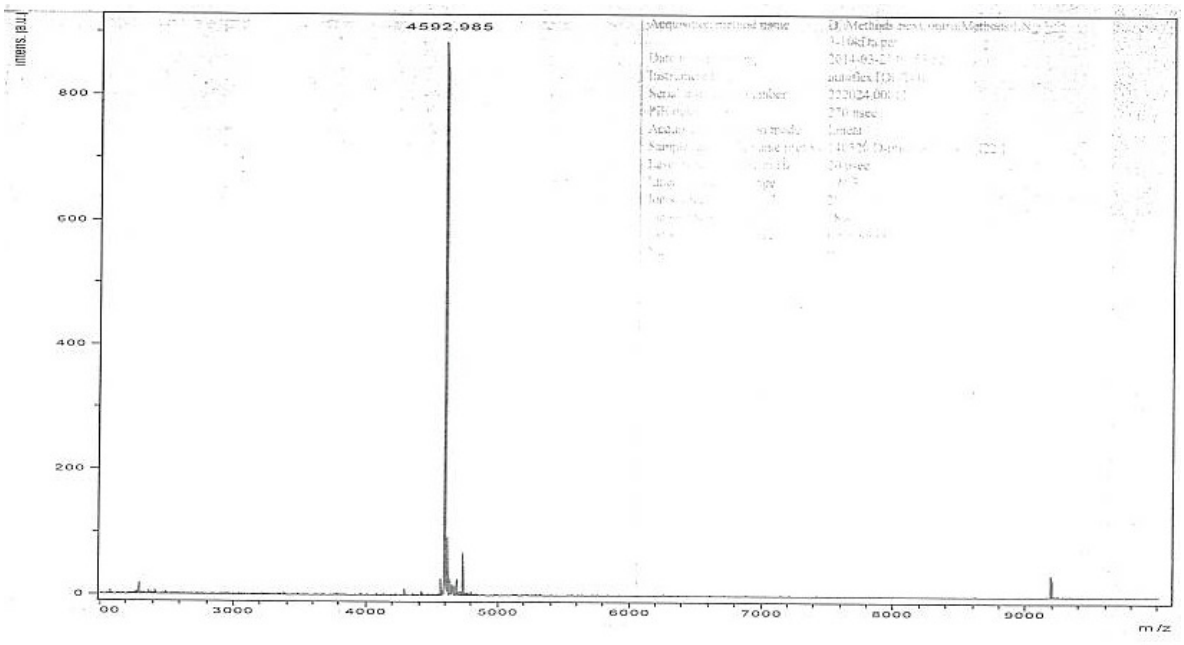
Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass



TFO **7i**

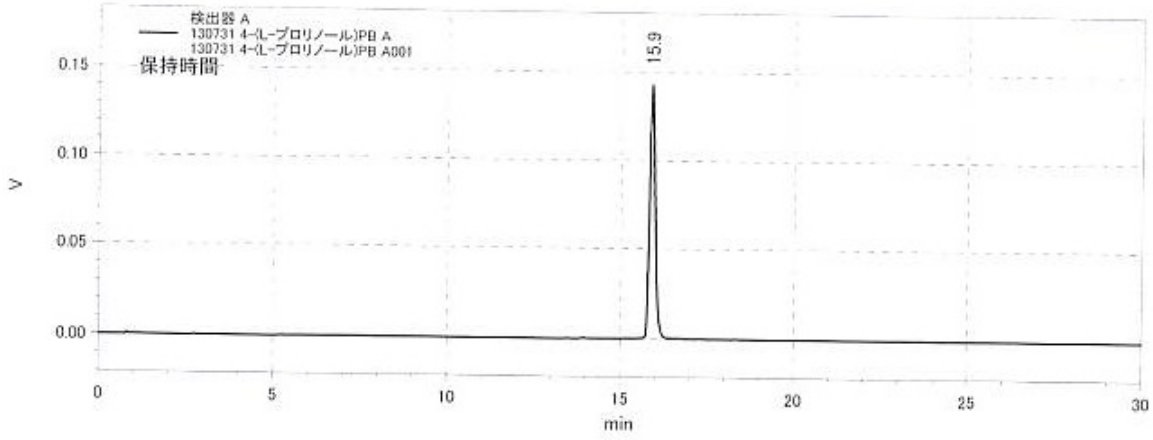
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

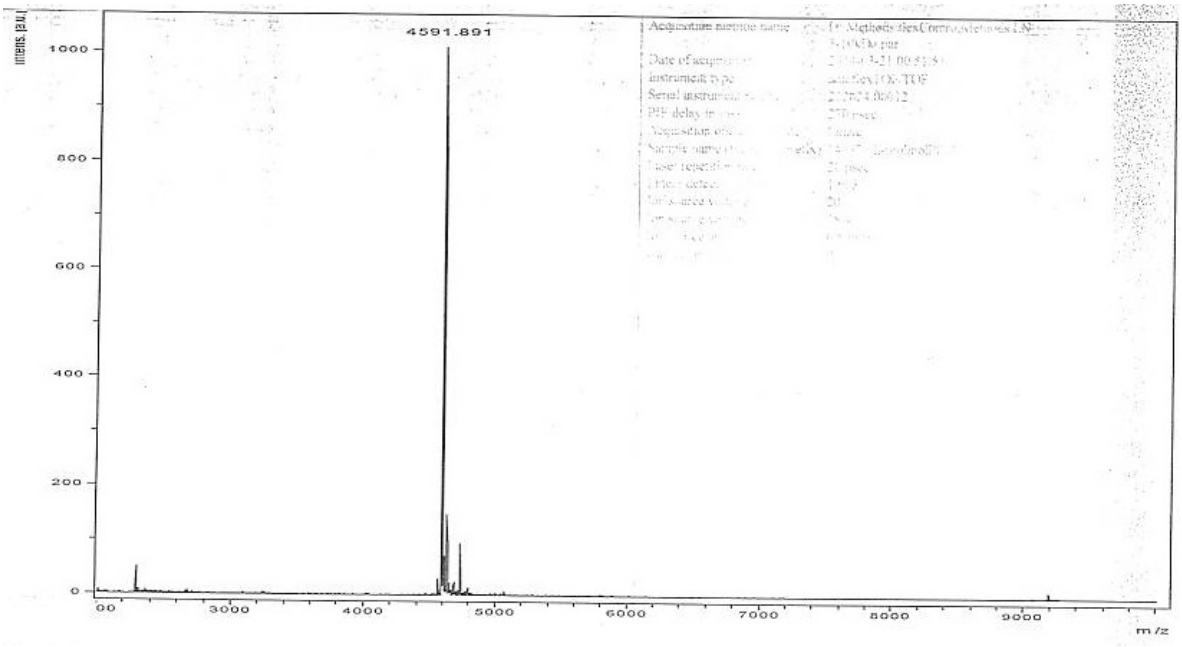
Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass



TFO **7j**

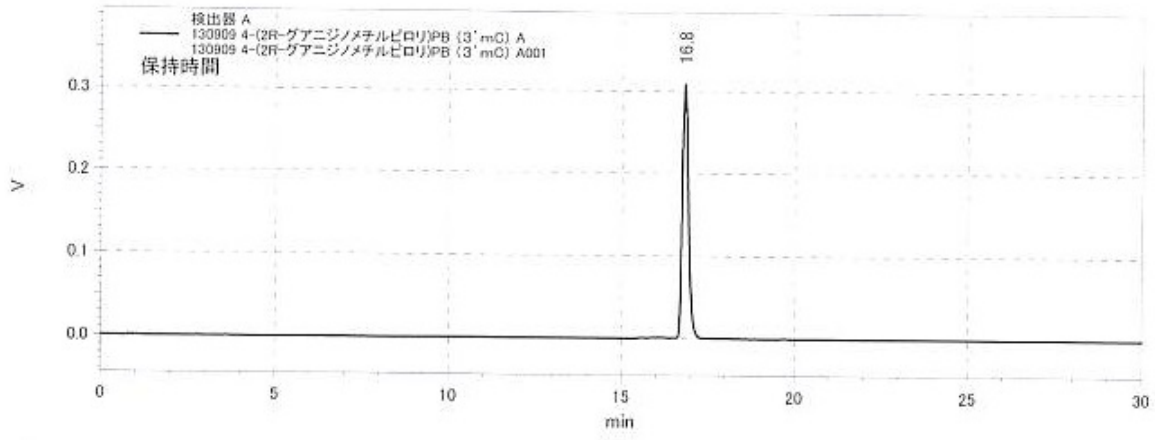
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

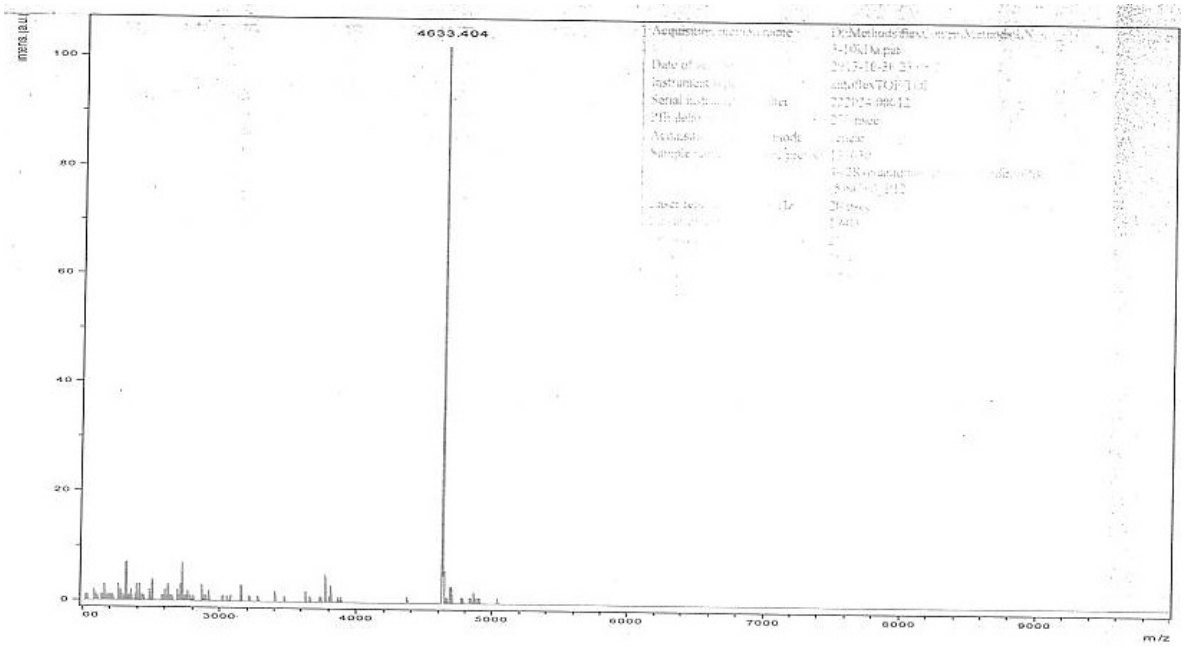
Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass



TFO **7k**

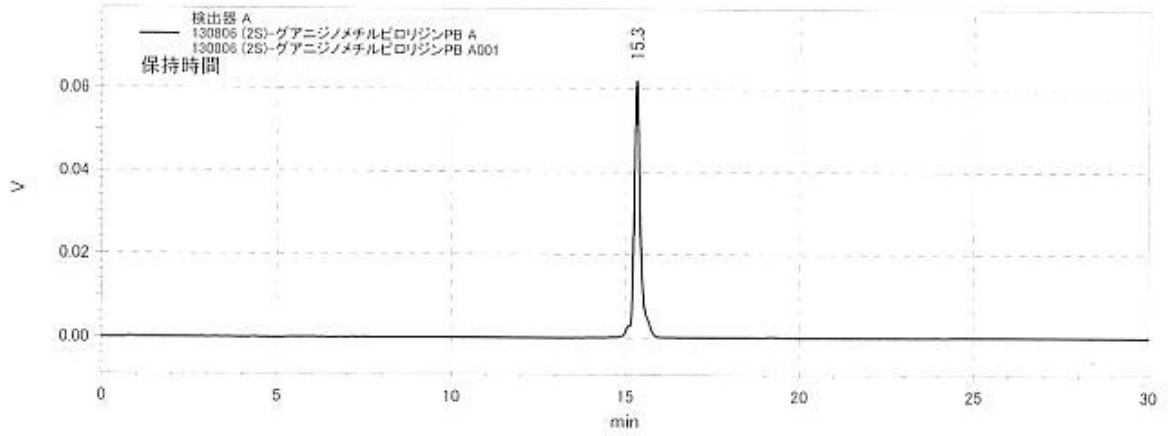
HPLC

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm

Gradient : 7–13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer

Flow rate : 1.0 mL/min

Column temp. : 50 °C



MALDI-TOF-Mass