Electronic Supplementary Information

2',4'-BNA bearing a chiral guanidinopyrrolidine-containing nucleobase with potent ability to recognize the CG base pair in parallel-motif DNA triplex

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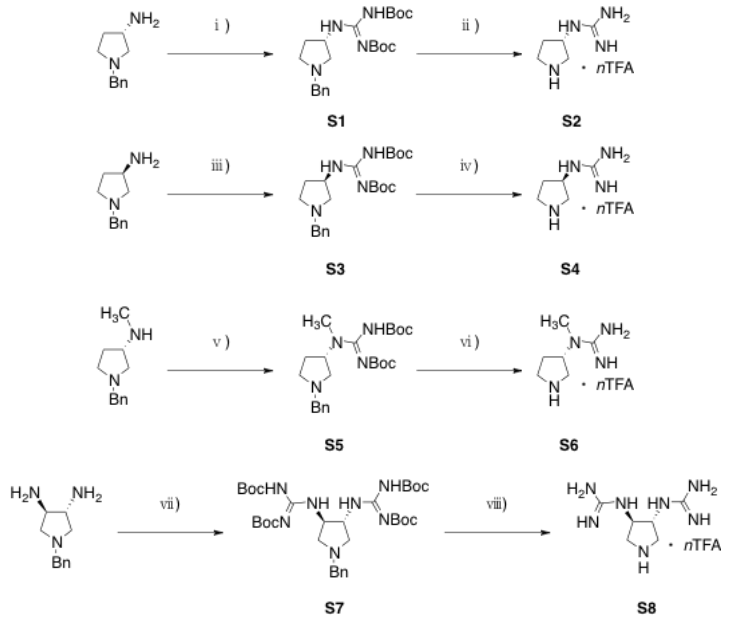
Contents

General: Melting points are uncorrected. All moisture-sensitive reactions were carried out in well-dried glassware under a N_2 atmosphere. ^{1}H NMR (400.00 MHz), ^{13}C NMR (100.53 MHz), and ^{31}P NMR (161.84 MHz) were recorded on JEOL JNM-ECS-400 spectrometers. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane (0.00 ppm) for ^{1}H NMR, CD_3OD (49.00 ppm) or $CDCl_3$ (77.00 ppm) for ^{13}C NMR, or external H_3PO_4 (0.00 ppm) for ^{31}P NMR. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer. Optical rotations were recorded on a JASCO P-2200 instrument. Mass spectra were measured on a JEOL JMS-600, JEOL JMS-700, Bruker Daltonics Autoflex II TOF/TOF or JEOL JMS-S3000 mass spectrometer. For silica gel column chromatography, Fuji Silysia PSQ-100B, FL-60D and FL-100D were used. For amine silica gel column chromatography, Fuji Silysia DM-1020 was used.



Scheme S1. Synthesis of guanidinopyrrolidines. *Reagent and conditions*: (i) (3*S*)-(+)-1-benzyl-3-aminopyrrolidine, $(BocNH)_2CS$[1)], DIPEA, EDCI, CH_2Cl_2, rt, 8 h, 88%; (ii) (1) TFA, CH_2Cl_2, rt, 3 h; (2) 20% $Pd(OH)_2$-C, MeOH, rt, 12 h, 86%; (iii) (3*R*)-(–)-1-benzyl-3-aminopyrrolidine, $(BocNH)_2CS$, DIPEA, EDCI, CH_2Cl_2, rt, 3 h, 91%; (iv) (1) TFA, CH_2Cl_2, rt, 1 h; (2) 20% $Pd(OH)_2$-C, MeOH, rt, 8 h, 80%; (v) (3*S*)-(+)-1-benzyl-3-(methylamino)pyrrolidine, $(BocNH)_2CS$, DIPEA, EDCI, CH_2Cl_2, rt, 24 h, 51%; (vi) (1) TFA, CH_2Cl_2, rt, 3 h; (2) 20% $Pd(OH)_2$-C, MeOH, rt, 5 h, quant.; (vii) (3*R*,4*R*)-1-benzyl-3,4-diaminopyrrolidine, $(BocNH)_2CS$, DIPEA, EDCI, CH_2Cl_2, rt, 3 h, 61%; (viii) (1) TFA, CH_2Cl_2, rt, 3 h; (2) 20% $Pd(OH)_2$-C, MeOH, rt, 10 h, 80%.

All guanidine derivatives used in this study were synthesized in Scheme S1.

(3*S*)-1-Benzyl-3-{*N*,*N*'-bis-[(2-*tert*-buthoxy)carbonyl]guanidino}pyrrolidine (S1): Under a N_2 atmosphere, EDCI (104 mg, 0.543 mmol) was added to a solution of (3*S*)-(+)-1-benzyl-3-aminopyrrolidine (63.8 mg, 0.362 mmol), *N*,*N*'-bis-(2-*tert*-buthoxy)carbonyl-thiourea [$(BocNH)_2CS$][1)] (63.8 mg, 0.362 mmol) and DIPEA (0.189 mL, 1.09 mmol) in anhydrous CH_2Cl_2 (20 mL) and the resulting mixture was stirred at room temperature for 8 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was

purified by silica gel column chromatography (*n*-hexane/AcOEt = 5/1) to give compound **S1** (134 mg, 88%) as a white amorphous powder.

Mp 95–96°C. $[\alpha]_D^{22}$ +4.02 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 3326, 3114, 2978, 2792, 1721, 1638, 1612, 1564, 1415, 1327, 1158, 1056, 1027 cm^{-1}. ^1H NMR ($CDCl_3$) : δ 1.49 (9 H, s), 1.51 (9 H, s), 1.65-1.74 (1 H, m), 2.24-2.34 (2 H, m), 2.59 (2 H, d, *J* = 4.6 Hz), 2.81-2.88 (1 H, m), 3.60 (1 H, AB, *J* = 12.8 Hz), 3.64 (1 H, AB, *J* = 12.8 Hz), 4.68 (1 H, ddt, *J* = 4.6, 8.5 and 8.5 Hz), 7.21-7.35 (5 H, m), 8.67 (1 H, d, *J* = 8.3 Hz), 11.5 (1 H, s). ^{13}C NMR ($CDCl_3$): δ 27.97, 28.20, 32.42, 49.63, 52.31, 59.58, 60.30, 78.98, 82.78, 126.80, 128.11, 128.44, 138.91, 152.98, 155.24, 163.53. HRMS (MALDI): Calcd for $C_{22}H_{44}N_4O_4Na$ ($M+Na^+$): 441.2472. Found: 441.2474. *Anal.* Calcd for $C_{22}H_{34}N_4O_4$: C, 63.13; H, 8.19; N, 13.39. Found: C, 62.74; H, 7.97; N, 13.24.

(3*S*)-3-Guanidinopyrrolidine-TFA (S2): TFA (20 mL) was added to a solution of **S1** (500 mg, 1.19 mmol) in CH_2Cl_2 (20 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 (10 mL). Under a H_2 atmosphere, the solution was added to a solution of 20% $Pd(OH)_2$-C (300 mg) in MeOH (10 mL) and the resulting mixture was stirred at room temperature for 12 h. After the reaction mixture was filtered, the solution was concentrated *in vacuo*. The residue was purified by amine silica gel column chromatography (CH_2Cl_2/MeOH = 1/1) to give compound **S2** (247 mg, 86%) as a white amorphous powder.

Mp 138–140°C. $[\alpha]_D^{23}$ –9.40 (c 1.0, MeOH). IR ν_{max} (KBr): 3339, 1675, 1523, 1428, 1344, 1201, 1133, 1034 cm^{-1}. ^1H NMR (CD_3OD) δ 2.05–2.13 (1 H, m), 2.39 (1 H, dddd, *J* = 6.4, 7.3, 7.3 and 14.4 Hz), 3.28–3.33 (1 H, m), 3.38–3.52 (2 H, m), 3.58 (1 H, dd, *J* = 6.4 and 12.4 Hz), 4.34–4.39 (1 H, m). ^{13}C NMR ($CDCl_3$) δ 31.69, 45.24, 50.90, 51.91, 118.08 (q, *J* = 292 Hz, TFA), 158.44, 163.39 (q, *J* = 34.5 Hz, TFA). MS (FAB) *m/z* 129 ($M+H^+$). HRMS (FAB): Calcd for $C_5H_{13}N_4$ ($M+H^+$): 129.1135. Found: 129.1142.

(3*R*)-1-Benzyl-3-{*N*,*N*'-bis-[(2-*tert*-buthoxy)carbonyl]guanidino}pyrrolidine (S3): Under a N_2 atmosphere, EDCI (104 mg, 0.543 mmol) was added to a solution of (3*R*)-(–)-1-benzyl-3-aminopyrrolidine (63.8 mg, 0.362 mmol), $(BocNH)_2CS$ (63.8 mg, 0.362 mmol) and DIPEA (0.189 mL, 1.09 mmol) in anhydrous CH_2Cl_2 (20 mL) and the resulting mixture was stirred at room temperature for 8 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 5/1) to give compound **S3** (134 mg, 88%) as a white amorphous powder.

Mp 96–97°C. $[\alpha]_D^{23}$ –4.49 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 3326, 3114, 2978, 2792, 1721, 1638, 1612, 1563, 1415, 1327, 1158, 1056, 1027 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.49 (9 H, s), 1.51 (9 H, s), 1.65–1.74 (1 H, m), 2.24–2.36 (2 H, m), 2.60 (2 H, d, *J* = 4.6 Hz), 2.82–2.88 (1 H, m), 3.60 (1 H, AB, *J* = 12.9 Hz), 3.64 (1 H, AB, *J* = 12.9 Hz), 4.68 (1 H, ddt, *J* = 4.6, 6.5 and 6.5 Hz), 7.22–7.36 (5 H, m), 8.67 (1 H, d, *J* = 8.2 Hz), 11.5 (1 H, s). ^{13}C NMR ($CDCl_3$) δ 28.07, 28.30, 32.52, 49.71,

52.41, 59.69, 60.41, 79.11, 82.89, 126.88, 128.20, 128.54, 139.04, 153.09, 155.54, 163.63. HRMS (MALDI): Calcd for $C_{22}H_{44}N_4O_4Na$ ($M+Na^+$): 441.2472. Found: 441.2460. *Anal.* Calcd for $C_{22}H_{34}N_4O_4$: C, 63.13; H, 8.19; N, 13.39. Found: C, 62.93; H, 8.09; N, 13.14.

(3*R*)-3-Guanidinylpyrrolidine-TFA (S4): TFA (10 mL) was added to a solution of **S3** (200 mg, 0.478 mmol) in CH_2Cl_2 (10 mL) and the resulting mixture was stirred at room temperature for 1 h. After the reaction mixture was concentrated *in vacuo*, the crude product was dissolved in MeOH (10 mL). Under a H_2 atmosphere, the solution was added to a solution of 20% $Pd(OH)_2$-C (400 mg) in MeOH (10 mL) and the resulting mixture was stirred at room temperature for 8 h. After the reaction mixture was filtered, the solution was concentrated *in vacuo*. The residue was purified by amine silica gel column chromatography (CH_2Cl_2/MeOH = 1/1) to give compound **S4** (88.2 mg, 80%) as a white amorphous powder.
Mp 138–140°C. $[\alpha]_D^{24}$ –9.37 (c 1.0, MeOH). IR ν_{max} (KBr): 3345, 1675, 1523, 1428, 1344, 1201, 1133, 1034 cm^{-1}. ^1H NMR (CD_3OD) δ 1.69–1.77 (1 H, m), 2.16 (1 H, dddd, *J* = 7.8, 7.8, 7.8 and 15.6 Hz), 2.77 (1 H, dd, *J* = 4.1 and 11.9 Hz), 2.87–2.93 (1 H, m), 2.99–3.05 (1 H, m), 3.11 (1 H, dd, *J* = 6.4 and 11.9 Hz), 3.98–4.03 (1 H, m). ^{13}C NMR ($CDCl_3$) δ 33.73, 46.05, 53.41, 53.61, 118.13 (q, *J* = 292 Hz, TFA), 158.33, 163.29 (q, *J* = 34.5 Hz, TFA). MS (FAB) *m/z* 129 ($M+H^+$). HRMS (FAB): Calcd for $C_5H_{13}N_4$ ($M+H^+$): 129.1135. Found: 129.1140.

(3*S*)-1-Benzyl-3-{*N*,*N*'-bis-[(2-*tert*-buthoxy)carbonyl]-1-methylguanidino}pyrrolidine (S5): Under a N_2 atmosphere, EDCI (4.03 g, 21.0 mmol) was added to a solution of (3*S*)-(+)-1-benzyl-3-(methylamino)pyrrolidine (2.0 g, 10.5 mmol), $(BocNH)_2CS$ (5.81 g, 21.0 mmol) and DIPEA (5.49 mL, 31.5 mmol) in anhydrous CH_2Cl_2 (50 mL) and the resulting mixture was stirred at room temperature for 24 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 2/1) to give compound **S5** (2.30 g, 51%) as colorless syrup.
$[\alpha]_D^{25}$ –25.5 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 3156, 2977, 2931, 2792, 1747, 1632, 1604, 1496, 1451, 1393, 1295, 1234, 1144, 1082 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.38–1.48 (19 H, m), 1.82–1.91 (1 H, m), 2.17–2.29 (2 H, m), 2.46 (1 H, dd, *J* = 8.3 and 11.0 Hz), 2.79–2.82 (1 H, m), 2.91–2.95 (1 H, m), 3.07 (3 H, s), 3.48 (1 H, AB, *J* = 12.8 Hz), 3.66 (1 H, AB, *J* = 12.8 Hz), 7.22–7.33 (5H, m), 10.1 (1 H, brs). ^{13}C NMR ($CDCl_3$) δ 28.09, 29.01, 32.52, 53.62, 57.44, 59.94, 79.10, 81.59, 126.96, 128.23, 128.43, 138.77, 150.67, 155.36, 162.58. HRMS (MALDI): Calcd for $C_{23}H_{36}N_4O_4Na$ ($M+Na^+$): 455.2629. Found: 455.2633.

(3*S*)-3-(1-Methylguanidino)pyrrolidine-TFA (S6): TFA (10 mL) was added to a solution of **S5** (1.0 g, 2.31 mmol) in CH_2Cl_2 (10 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 (10 mL). Under a H_2 atmosphere, the solution was added to a solution of 20% $Pd(OH)_2$-C

(1.0 g) in MeOH (30 mL) and the resulting mixture was stirred at room temperature for 5 h. After the reaction mixture was filtered, the solution was concentrated *in vacuo*. The residue was purified by amine silica gel column chromatography (CH_2Cl_2/MeOH = 1/1) to give compound **S6** (610 mg, quant.) as a white amorphous powder.

Mp 180–181°C. $[\alpha]_D^{25}$ –2.07 (c 0.5, MeOH). IR ν_{max} (KBr): 3346, 3156, 1670, 1625, 1469, 1435, 1205, 1133 cm^{-1}. ^1H NMR (CD_3OD) δ 1.94 (1 H, dddd, *J* = 5.5, 6.4, 6.4 and 12.2 Hz), 2.21 (1 H, dddd, *J* = 5.5, 6.4, 6.4 and 12.2 Hz), 2.39 (3 H, s), 3.26 (1 H, dd, *J* = 4.6 and 10.6 Hz), 3.34–3.48 (2 H, m), 3.51–3.60 (2 H, m). ^{13}C NMR ($CDCl_3$) δ 31.19, 34.21, 46.42, 52.64, 59.81, 118.02 (q, *J* = 292 Hz, TFA), 156.26, 162.83 (q, *J* = 34.5 Hz, TFA). MS (FAB) *m/z* 143 ($M+H^+$). HRMS (FAB): Calcd for $C_5H_{13}N_4$ ($M+H^+$): 143.1291. Found: 143.1293.

(3*R*,4*R*)-1-Benzyl-3,4-di{*N*,*N*'-bis-[(2-*tert*-buthoxy)carbonyl]guanidino}pyrrolidine (S7): Under a N_2 atmosphere, EDCI (3.75 g, 19.6 mmol) was added to a solution of (3*R*,4*R*)-1-benzyl-3,4-diaminopyrrolidine[2)] (1.10 g, 5.75 mmol), $(BocNH)_2CS$ (3.60 g, 13.0 mmol) and DIPEA (6.81 mL, 39.1 mmol) in anhydrous CH_2Cl_2 (100 mL) and the resulting mixture was stirred at room temperature for 8 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 10/1) to give compound **S7** (2.37 g, 61%) as a white amorphous powder.

Mp 93–95°C. $[\alpha]_D^{25}$ –15.7 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 3321, 3126, 2979, 2796, 1722, 1613, 1413, 1335, 1154, 1056 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.47 (18 H, s), 1.50 (18 H, s), 2.44 (2 H, dd, *J* = 4.6 and 9.6 Hz), 3.07 (2 H, dd, *J* = 6.4 and 9.6 Hz), 3.59 (1 H, AB, *J* = 13.3 Hz), 3.64 (1 H, AB, *J* = 13.3 Hz), 4.51–4.57 (2 H, m), 7.22–7.34 (5 H, m), 8.67 (2 H, d, *J* = 7.3 Hz), 11.4 (2 H, s). ^{13}C NMR ($CDCl_3$) δ 28.00, 28.22, 56.21, 59.17, 59.21, 78.93, 82.90, 126.96, 128.18, 128.41, 138.38, 152.88, 155.69, 163.42. MS (FAB) *m/z* 676 ($M+H^+$). HRMS (FAB): Calcd for $C_{33}H_{54}N_7O_8$ ($M+H^+$): 676.4028. Found: 676.4022.

(3*R*,4*R*)-3,4-Diguanidinylpyrrolidine-TFA (S8): TFA (30 mL) was added to a solution of **S7** (1.00 g, 1.48 mmol) in CH_2Cl_2 (60 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 (50 mL). Under a H_2 atmosphere, the solution was added to a solution of 20% $Pd(OH)_2$-C (500 mg) in MeOH (50 mL) and the resulting mixture was stirred at room temperature for 10 h. After the reaction mixture was filtrated, the solution was concentrated *in vacuo*. The residue was purified by amine silica gel column chromatography (MeOH) to give compound **S8** (489 mg, 80%) as a white amorphous powder.

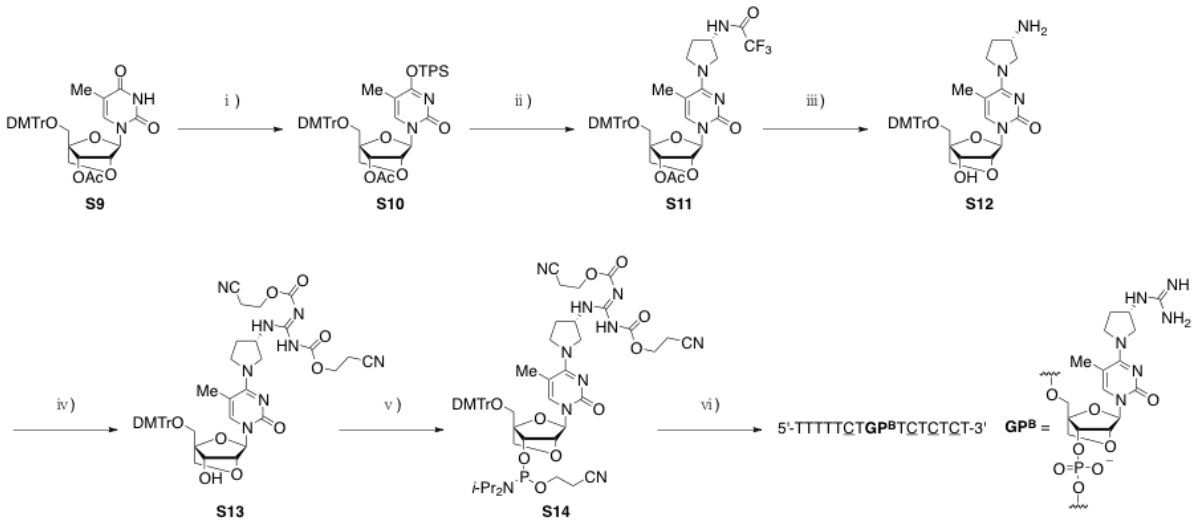
Mp 89–91°C. $[\alpha]_D^{30}$ –9.74 (c 1.0, MeOH). IR ν_{max} (KBr): 3366, 3175, 1675, 1433, 1202, 1136 cm^{-1}. ^1H NMR (CD_3OD) δ 2.79 (2 H, dd, *J* = 6.0 and 6.0 Hz), 3.35 (2 H, dd, *J* = 6.0 and 11.0 Hz), 3.98 (2 H, dd, *J* = 6.0 and 11.0 Hz). ^{13}C NMR ($CDCl_3$) δ 51.49, 58.77, 117.92 (q, *J* = 293 Hz, TFA), 158.39, 163.17 (q, *J* = 34.5 Hz, TFA). HRMS (MALDI): Calcd for $C_6H_{15}N_7Na$ ($M+Na^+$): 208.1281.

Found: 208.1280.

Synthesis of TFOs by using the PEM method: TFOs were synthesized on a 0.2-μmol scale on an automated DNA synthesizer (GeneDesign nS-8) using the common phosphoramidite protocol (Synthesis mode: DMTr-ON). The CPG resin-supported oligonucleotides were treated with 10% aqueous guanidinopyrrolidines solution at room temperature for 2 h for conversion of the triazolylated nucleobase into the desired guanidinopyrrolidine-containing nucleobases. Then, additional treatment with 28% aqueous NH_3 solution at room temperature for 5–6 h resulted in complete removal of the acetyl groups of the 5-methylcytosine bases and the complete cleavage of oligonucleotides from the CPG resin. After the two solutions were combined, the solvent was removed *in vacuo*. The crude TFOs obtained were purified with Nap™-10 columns (GE Healthcare) for removal of the excess amount of pyrrolidines and then treated with Sep-Pak® Plus C18 cartridges (Waters) followed by reversed-phase HPLC (Waters XBridge® MS C_{18} 2.5 μm, 10 × 50 mm). The composition of the TFOs was confirmed by MALDI-TOF-MS analysis (Table S1).

Table S1. MALDI-TOF-MS data of TFOs

	MALDI-TOF-MS	
Sequence of TFOs	Calcd. $[M-H]^-$	Found $[M-H]^-$
5'-TTTTTCT**GP**TCTCTCT-3'	4606.15	4606.82
5'-TTTTTCT**GP'**TCTCTCT-3'	4606.15	4606.46
5'-TTTTTCT**mGP**TCTCTCT-3'	4620.17	4620.73
5'-TTTTTCT**diGP**TCTCTCT-3'	4663.20	4662.85
5'-TTTTTCT**GE**TCTCTCT-3'	4580.11	4579.15
5'-TTTTTCT**GP^{OMe}**TCTCTCT-3'	4636.17	4635.59
5'-TTTTTCT**GP^{B}**TCTCTCT-3'	4634.16	4633.57
5'-TTTTTTC**GP^{B}**TCTCTCT-3'	4634.16	4634.13
5'-TTTTTCT**GP^{B}**CTTCTCT-3'	4634.16	4634.91
5'-TTTTTTC**GP^{B}**CTTCTCT-3'	4634.16	4633.80
5'-TTTTT**GP^{B}**T**GP^{B}**T**GP^{B}**TCTCT-3'	4912.47	4911.62
5'-TTTT**GP^{B}**TT**GP^{B}**TT**GP^{B}**TTCT-3'	4913.45	4913.66
5'-TTT**GP^{B}**TCT**GP^{B}**TCT**GP^{B}**TCT-3'	4911.48	4911.15



Scheme S2. Synthesis of TFO containing **GP^{B}**. C = 2'-deoxy-5-methylcytidine. *Reagent and conditions*: (i) 2,4,6-triisopropylphenylsulfonyl chloride (TPS-Cl), Et_3N, DMAP, CH_2Cl_2, rt, 12 h, 70%; (ii) (3*S*)-(–)-3-(trifluoroacetamido)pyrrolidine hydrochloride, Et_3N, CH_2Cl_2, rt, 3 h, 90%; (iii) 1N NaOHaq., THF, rt, 2 h, 91%; (iv) *N,N'-bis*-[(2-cyanoethoxy)carbonyl]-*S*-methylisothiourea[3], DMF, rt, 15 h, 85%; (v) *i*-$Pr_2NP(Cl)OCH_2CH_2CN$, *i*-Pr_2NEt, CH_2Cl_2, 0ºC, 3 h, 83%; (vi) oligonucleotide synthesis.

TFO containing **GP^{B}** was synthesized as shown in Scheme S2. The oligonucleotide was synthesized using the common phosphoramidite protocol. The synthesis of **S14** from **S9**[4] was carried out as described below.

1-(3-*O*-Acetyl-5-*O*-dimethoxytrityl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-4-*O*-(2,4,6-triisopropylphenyl)sulfonylthymine (S10): Under a N_2 atmosphere, 2,4,6-triisopropylbenzenesulfonyl chloride (197 mg, 0.651 mmol) was added to a solution of compound **S9**[4] (200 mg, 0.325 mmol), DMAP (3.97 mg, 0.0325 mmol) and Et_3N (0.136 mL, 0.976 mmol) in anhydrous CH_2Cl_2 (10 mL) and the resulting mixture was stirred at room temperature for 12 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was diluted 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 column chromatography (*n*-hexane/AcOEt = 10/1) to give compound **S10** (200 mg, 70%) as a white amorphous powder.

Mp 99–101°C. $[\alpha]_D^{23}$ +64.8 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 2959, 1751, 1683, 1605, 1510, 1379, 1252, 1175, 1071 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.26 (6 H, d, *J* = 6.8 Hz), 1.29 (6 H, d, *J* = 6.8 Hz), 1.33 (6 H, d, *J* = 6.8 Hz), 1.80 (3 H, s), 2.00 (3 H, s), 2.91 (1 H, sept, *J* = 6.8 Hz), 3.31 (1 H, AB, *J* = 11.0 Hz), 3.55 (1 H, AB, *J* = 11.0 Hz), 3.80–3.84 (8 H, m), 4.32 (2 H, sept, *J* = 6.8 Hz), 4.68 (1 H, s), 5.08 (1 H, s), 5.65 (1 H, s), 6.82–6.85 (4 H, m), 7.21–7.42 (9 H, m), 8.01 (1 H, s). ^{13}C NMR ($CDCl_3$) δ 12.40, 20.64, 23.40, 23.46, 24.38, 24.53, 29.59, 55.21, 57.53, 70.30, 72.11, 77.22, 86.79, 87.14, 88.23, 104.34, 113.26, 113.30, 124.04, 127.16, 127.99, 128.08, 129.98, 130.02, 130.63, 134.98, 135.02, 142.04, 143.98, 151.21, 153.25, 154.41, 158.71, 166.83, 169.26. MS (FAB) *m/z* 881 ($M+H^+$). HRMS (FAB): Calcd for $C_{49}H_{57}N_2O_{11}S$ ($M+H^+$): 881.3678. Found: 881.3691.

1-(3-*O*-Acetyl-5-*O*-dimethoxytrityl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-4-[(3*S*)-3-(trifluoro acetamido)pyrrolidino]-5-methylpyrimidin-2-one (S11): Under a N_2 atmosphere, (3*S*)-(–)-3-(trifluoroacetamido)pyrrolidine hydrochloride (54.6 mg, 0.250 mmol) was added to a solution of compound **S10** (200 mg, 0.227 mmol) and Et_3N (94.9 μL, 0.681 mmol) in CH_2Cl_2 (10 mL) and the resulting mixture was stirred at room temperature for 3 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was diluted 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 column chromatography (AcOEt) to give compound **S11** (158 mg, 90%) as a white amorphous powder.
Mp 141–143°C. $[\alpha]_D^{30}$ +4.01 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 3201, 2956, 1748, 1714, 1653, 1611, 1505, 1471, 1302, 1249, 1078, 1050 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.97 (3 H, s), 1.97 (3 H, s), 2.17–2.22 (2 H, m), 3.35 (1 H, AB, *J* = 11.0 Hz), 3.52 (1 H, AB, *J* = 11.0 Hz), 3.76–4.01 (11 H, m), 4.28–4.32 (1 H, m), 4.60–4.64 (2 H, m), 5.05 (1 H, s), 5.68 (1 H, s), 6.80–6.82 (4 H, m), 7.18–7.32 (7 H, m), 7.42–7.44 (2 H, m), 7.53 (1 H, s), 9.73 (1 H, brs). ^{13}C NMR ($CDCl_3$) δ 17.90, 20.51, 31.39, 47.04, 48.55, 53.29, 55.01, 57.84, 70.65, 72.13, 77.70, 86.48, 86.56, 87.53, 102.57, 113.05, 113.08, 115.88 (q, *J* = 288 Hz), 126.89, 127.81, 129.88, 134.92, 135.03, 137.85, 144.11, 154.63, 157.71 (q, *J* = 137 Hz), 158.48, 162.42, 169.33. MS (FAB) *m/z* 779 ($M+H^+$). *Anal.* Calcd for $C_{40}H_{41}F_3N_4O_9$: C, 61.69; H, 5.31; N, 7.19. Found: C, 61.67; H, 5.36; N, 6.91.

1-(5-*O*-Dimethoxytrityl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-4-[(3*S*)-3-aminopyrrolidino]-5-methylpyrimidin-2-one (S12): 1N aqueous NaOH (5 mL) was added to a solution of compound **S11** (200 mg, 0.257 mmol) in THF (5 mL) and the resulting mixture was stirred at room temperature for 2 h. After the reaction mixture was concentrated *in vacuo*, was dissolved in CH_2Cl_2. The solution was washed with saturated aqueous Na_2CO_3, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt /MeOH = 1/3) to give compound **S12** (150 mg, 91%) as a white amorphous powder.
Mp 153–156°C. $[\alpha]_D^{24}$ +20.8 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 3280, 2949, 1650, 1607, 1505, 1467, 1302, 1251, 1177, 1050 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.66–1.74 (1 H, m), 1.96–2.07 (4 H, m), 3.44–3.53 (3 H, m), 3.60 (1 H, dddd, *J* = 4.6, 4.6, 5.0 and 5.0 Hz), 3.70–3.96 (11 H, m), 4.23 (1 H, s), 4.49 (1 H, s), 5.66 (1 H, s), 6.84 (4 H, dd, *J* = 2.8 and 8.9 Hz), 7.20–7.37 (7 H, m), 7.48 (2 H, d, *J* = 7.8 Hz), 7.55 (1 H, s). ^{13}C NMR ($CDCl_3$) δ 18.35, 33.95, 47.70, 50.44, 55.43, 57.70, 59.12, 70.14, 71.96, 79.65, 86.57, 87.65, 87.93, 102.25, 113.41, 127.12, 128.13, 128.33, 130.32, 130.36, 139.09, 144.91, 155.08, 158.74, 163.18. MS (FAB) *m/z* 641 ($M+H^+$). HRMS (FAB): Calcd for $C_{36}H_{41}N_4O_7$ ($M+H^+$): 641.2970. Found: 641.2969.

4-[(3*S*)-3-{*N*,*N*'-Bis-[(2-cyanoethoxy)carbonyl]guanidinyl}pyrrolidino]-1-(5-*O*-dimethoxytrityl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-5-methylpyrimidin-2-one (S13): Under a N_2 atmosphere, *N*,*N*'-bis-[(2-cyanoethoxy)carbonyl]-*S*-methylisothiourea[3] was added to a solution of compound **S12** (100 mg, 0.156 mmol) in DMF (2 mL) and the resulting mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated *in vacuo*. The residue was purified by

silica gel column chromatography (AcOEt to AcOEt/MeOH = 10/1) to give compound **S13** (116 mg, 91%) as a white amorphous powder.

Mp 193–195°C. $[\alpha]_D^{30}$ +9.18 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 3330, 3185, 2959, 2252, 1743, 1648, 1505, 1469, 1294, 1254, 1209, 1085, 1052 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.97–2.07 (4 H, m), 2.22-2.28 (1 H, m), 2.75 (2 H, t, *J* = 6.0 Hz), 2.77 (2 H, t, *J* = 6.0 Hz), 3.43 (1 H, AB, *J* = 11.0 Hz), 3.51 (1 H, AB, *J* = 11.0 Hz), 3.59 (1 H, d, *J* = 5.0 Hz), 3.72–3.88 (11 H, m), 3.96–4.03 (1 H, m), 4.24 (1 H, d, *J* = 5.0 Hz), 4.30 (2 H, t, *J* = 6.4 Hz), 4.37 (2 H, t, *J* = 6.4 Hz), 4.47 (1 H, s), 4.63–4.70 (1 H, m), 6.83 (4 H, dd, *J* = 2.3 and 8.7 Hz), 7.20–7.31 (3 H, m), 7.36 (4 H, dd, *J* = 2.3 and 8.7 Hz), 7.48 (2 H, d, *J* = 7.3 Hz), 7.60 (1 H, s), 8.45 (1 H, d, *J* = 6.8 Hz), 11.65 (1 H, s). ^{13}C NMR ($CDCl_3$) δ 17.86, 17.96, 30.67, 47.05, 50.18, 54.26, 55.13, 58.63, 59.72, 60.80, 70.03, 71.56, 79.21, 86.32, 87.25, 87.66, 101.98, 113.10, 116.24, 116.97, 126.88, 127.84, 127.98, 130.01, 135.36, 135.42, 139.25, 144.55, 152.84, 154.64, 155.41, 158.43, 162.61, 163.14. MS (FAB) *m/z* 877 ($M+H^+$). HRMS (FAB): Calcd for $C_{45}H_{49}N_8O_{11}$ ($M+H^+$): 877.3515. Found: 877.3521.

4-[(3*S*)-3-{*N*,*N*'-Bis-[(2-cyanoethoxy)carbonyl]guanidinyl}pyrrolidino]-1-{3-*O*-[2-cyanoethoxy (diisopropylamino)phosphino]-5-*O*-dimethoxytrityl-2-*O*,4*C*-methylene-β-D-ribofuranosyl}-5-methylpyrimidin-2-one (S14): Under a N_2 atmosphere, *i*-$Pr_2NP(Cl)OCH_2CH_2CN$ (0.458 mL, 2.05 mmol) was added to a solution of **S13** (1.50 g, 1.71 mmol) and *i*-Pr_2NEt (0.894 mL, 5.13 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0°C and the resulting mixture was stirred at 0°C for 3 h. After addition of saturated aqueous $NaHCO_3$ solution, the reaction mixture was diluted 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 column chromatography (CH_2Cl_2/MeOH = 20/1) to give compound **S14** (1.53 g, 83%) as a white amorphous powder.

Mp 107–109°C. ^1H NMR ($CDCl_3$) δ 0.96–1.28 (12 H, m), 1.87 (1.8 H, s), 2.05 (1.2 H, s), 2.00–2.08 (1 H, m), 2.23–2.32 (1 H, m), 2.38 (1.2 H, t, *J* = 6.4 Hz), 2.55 (0.8 H, t, *J* = 6.4 Hz), 2.77 (2 H, t, *J* = 6.4 Hz), 2.79 (2 H, t, *J* = 6.4 Hz), 3.35–4.12 (23 H, m), 4.65–4.72 (2 H, m), 5.76 (1 H, s), 6.82-6.88 (4 H, m), 7.22–7.38 (7 H, m), 7.46–7.49 (2 H, m), 7.65 (0.4 H, s), 7.67 (0.6 H, s), 8.45 (1 H, d, *J* = 6.9 Hz), 11.7 (1 H, s). ^{31}P NMR ($CDCl_3$) δ 148.6, 149.1. MS (FAB) *m/z* 1077 ($M+H^+$). HRMS (FAB): Calcd for $C_{54}H_{66}N_{10}O_{12}$ ($M+H^+$): 1077.4594. Found: 1077.4592.

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TFO: 5'-TTTTTCT**GP**TCTCTCT-3'

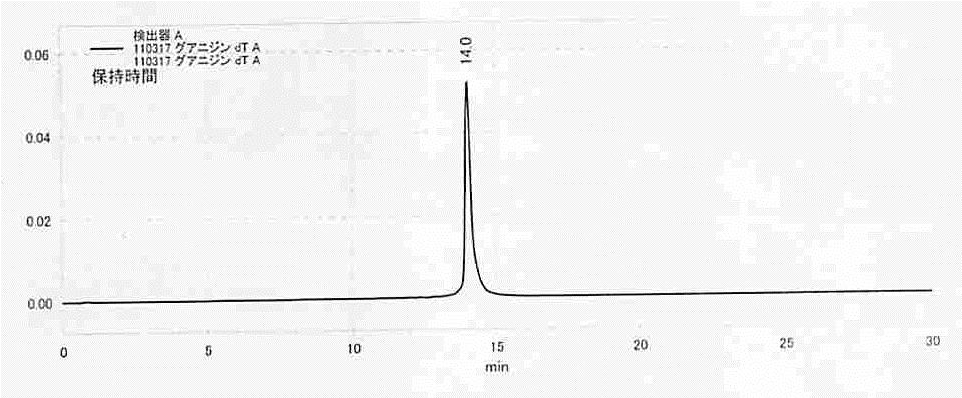
HPLC

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. : 50ºC.



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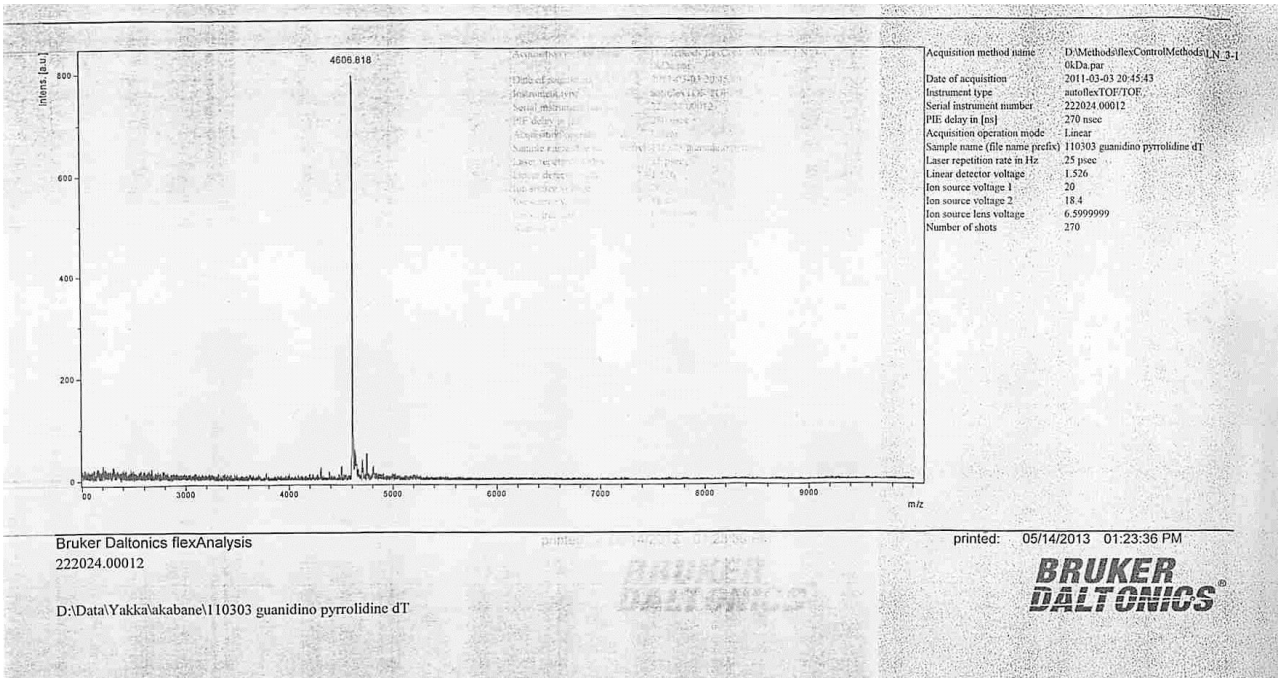


TFO: 5’-TTTTTCT**GP’**TCTCTCT-3’

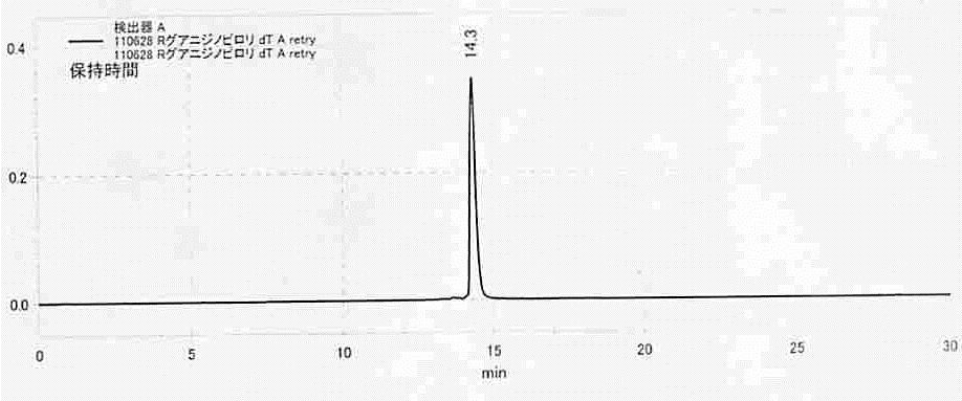
HPLC

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. : 50ºC.



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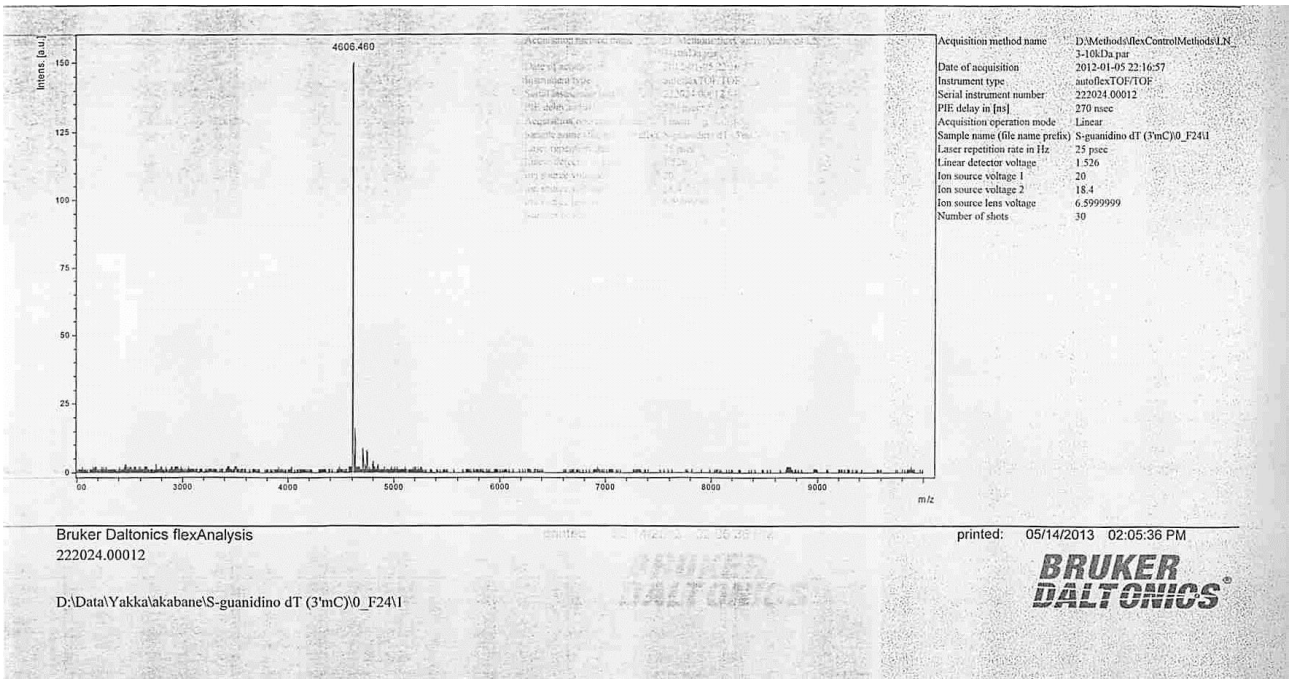


TFO: 5’-TTTTTCT**mGP**TCTCTCT-3’

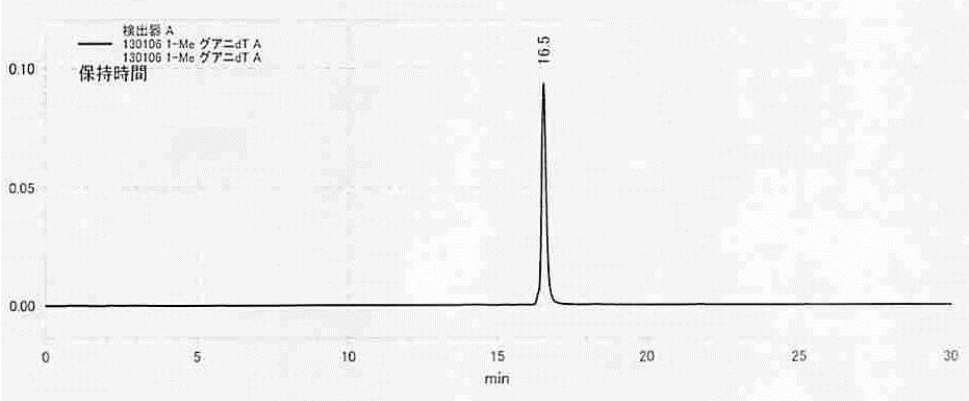
HPLC

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. : 50ºC.



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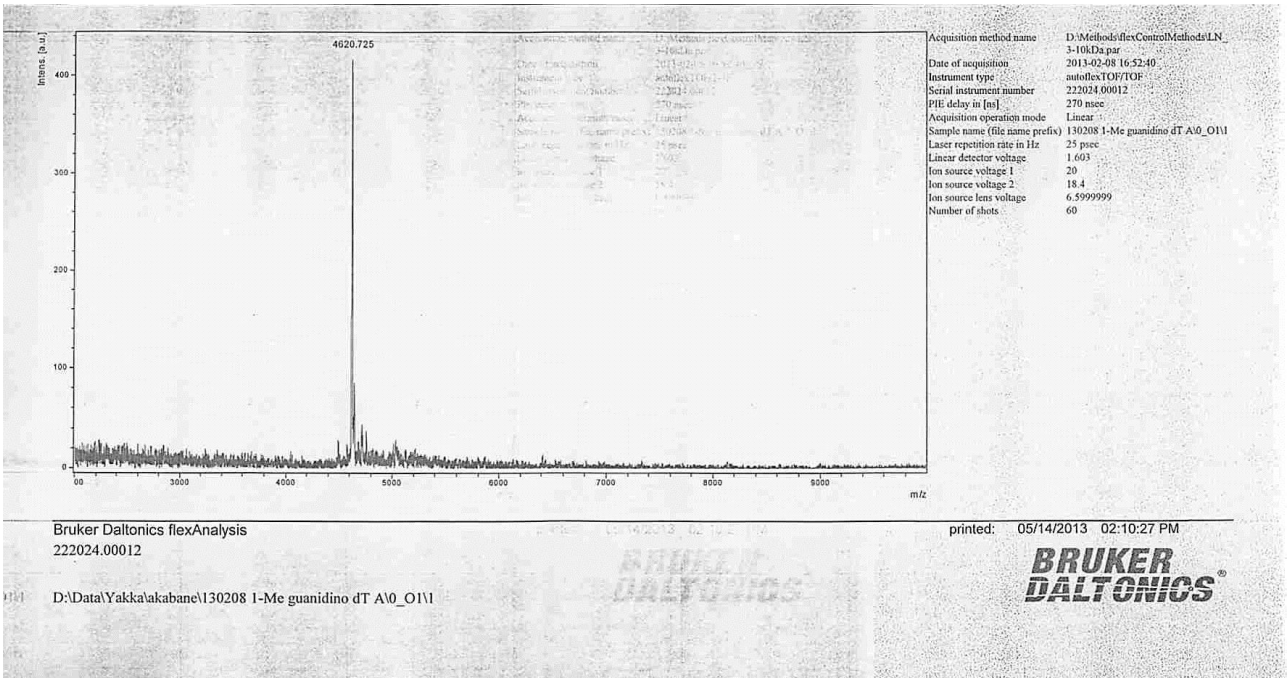


TFO: 5'-TTTTTCT**diGP**TCTCTCT-3'

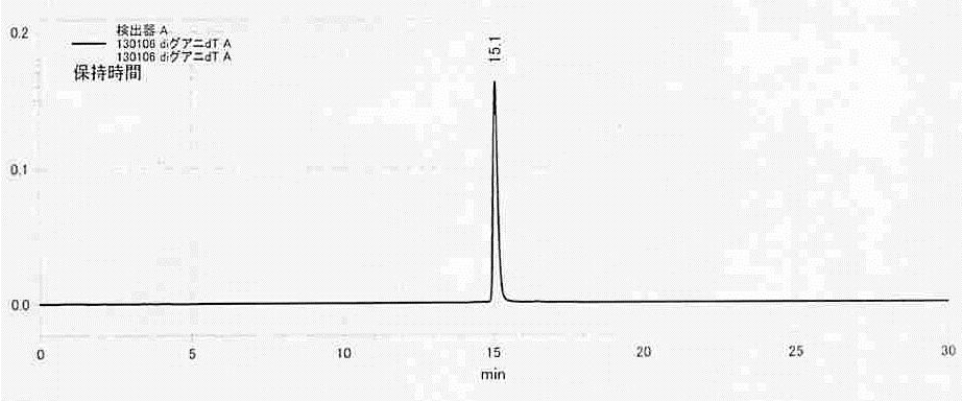
HPLC

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. : 50ºC.



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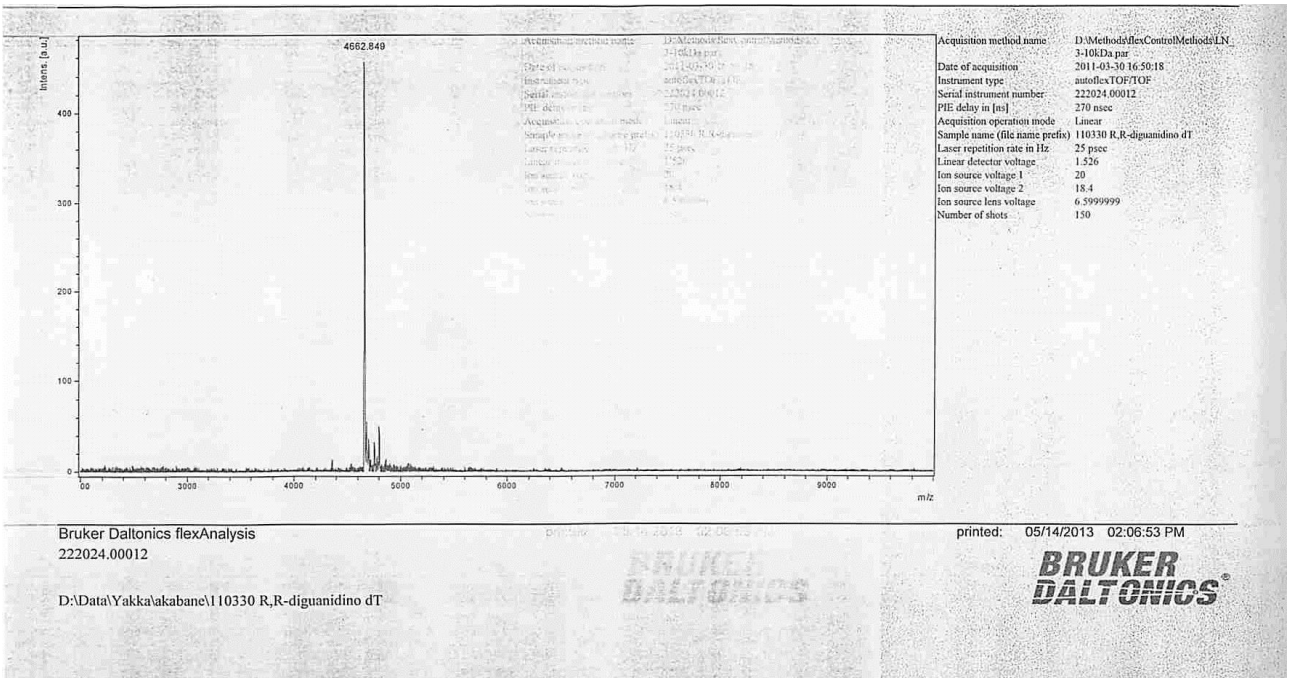


TFO: 5’-TTTTTCT**GE**TCTCTCT-3’

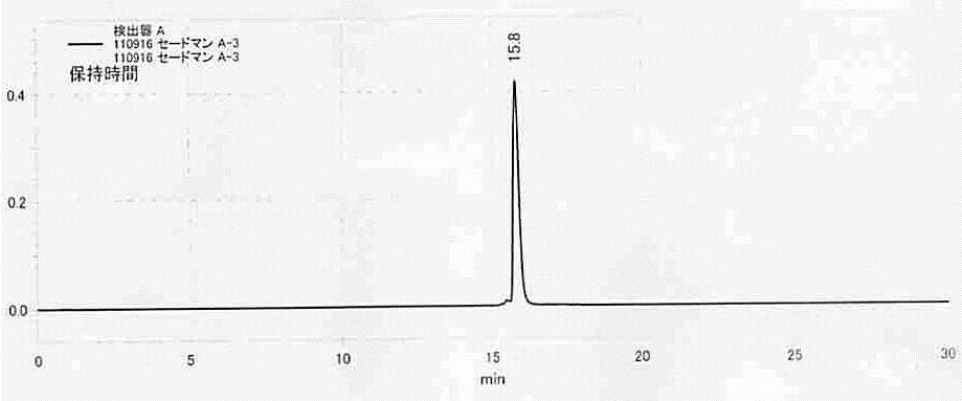
HPLC

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. : 50ºC.



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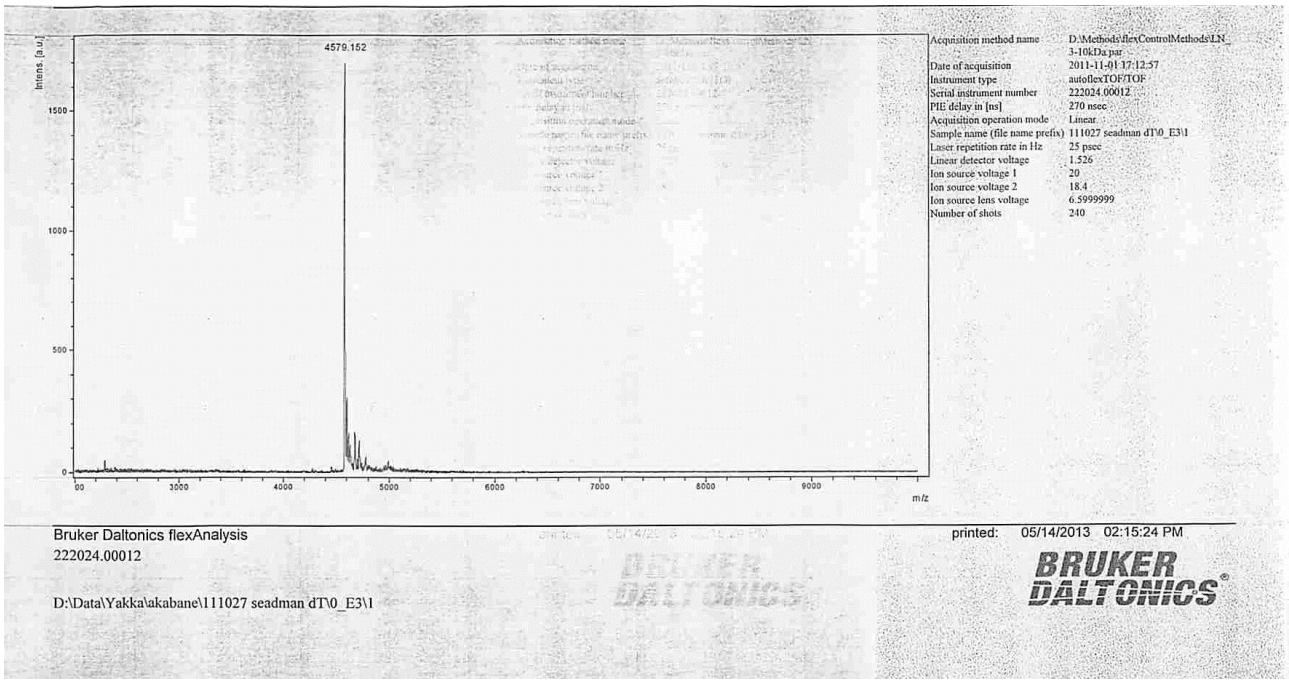


TFO: 5'-TTTTTCTC**GP^{OMe}**TCTCTCT-3'

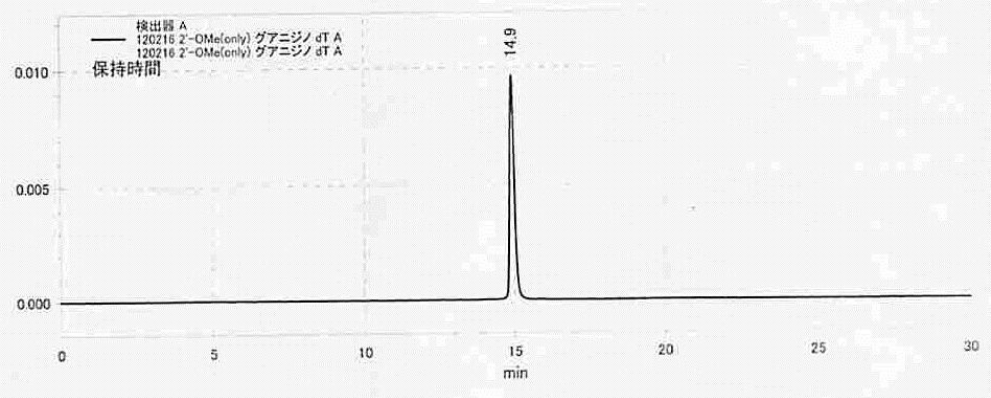
HPLC

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. : 50ºC.



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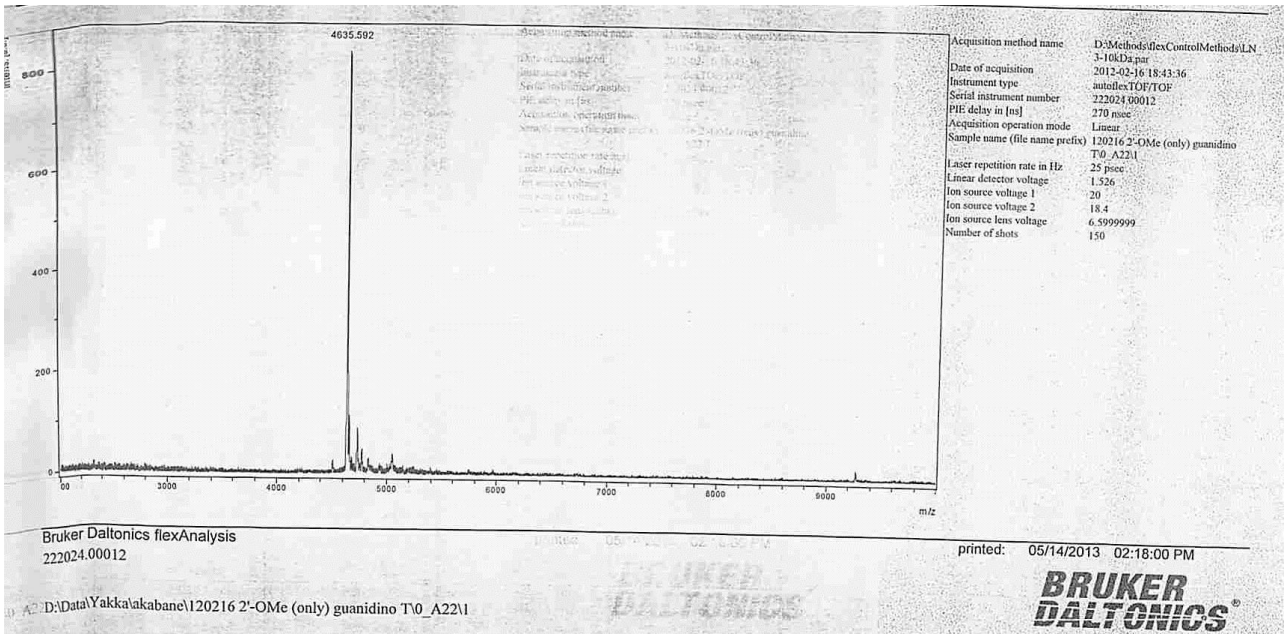


TFO: 5'-TTTTTCT**GP^{B}**TCTCTCT-3'

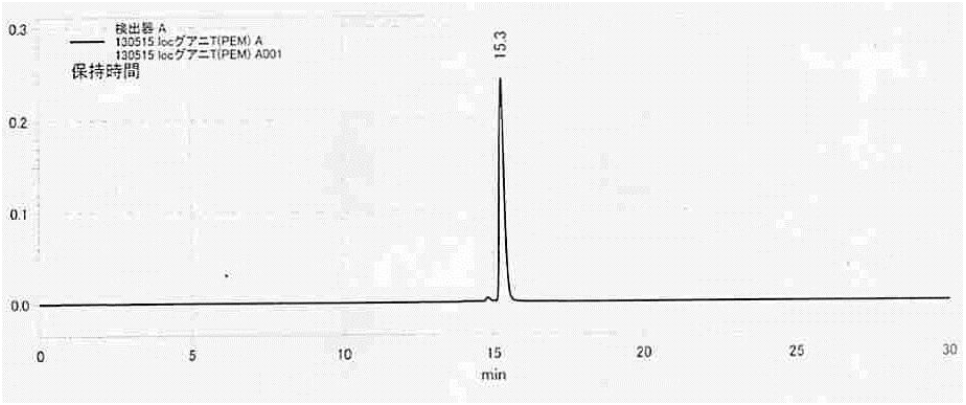
HPLC

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. : 50ºC.



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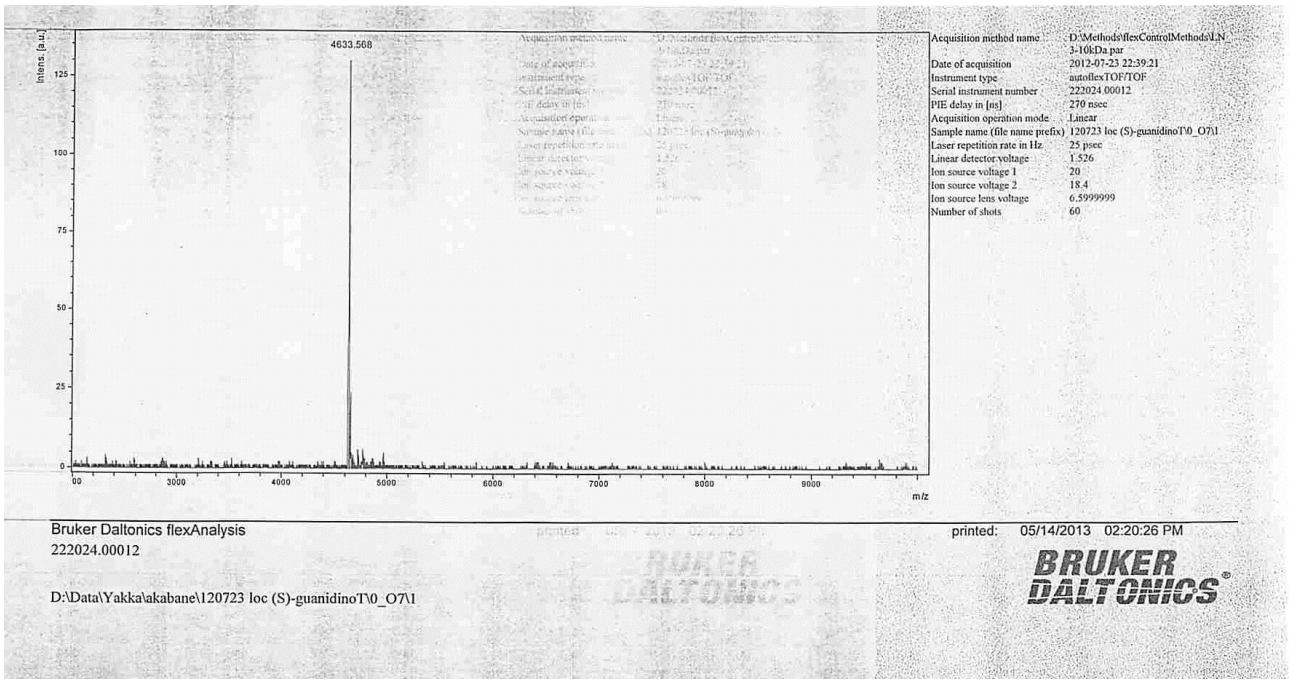


TFO: 5'-TTTTTTC**GP**$^{\mathbf{B}}$TCTCTCT-3'

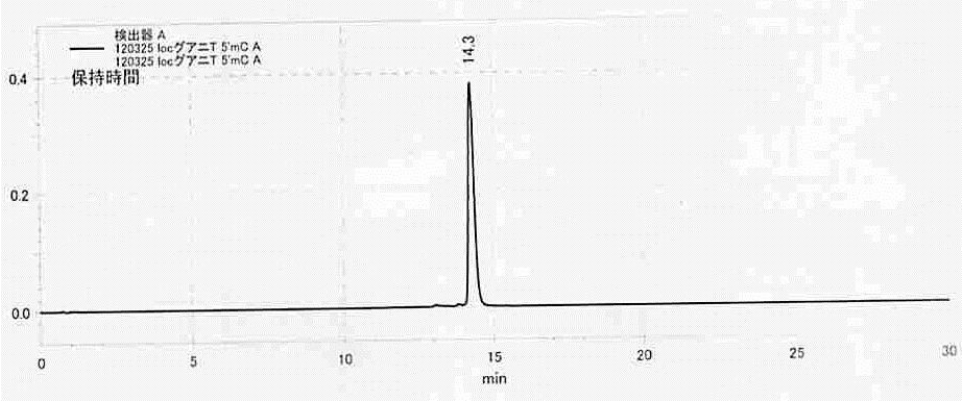
HPLC

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. : 50ºC.



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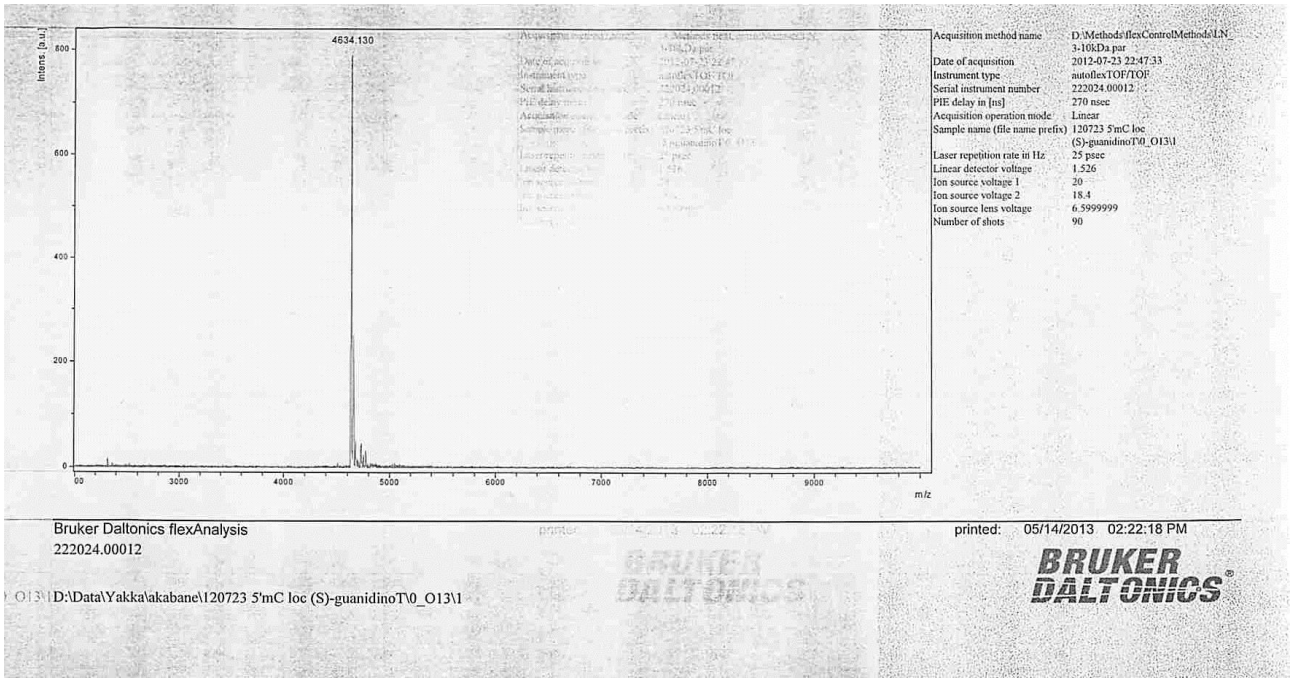


TFO: 5'-TTTTTCTG**P**$^{\mathbf{B}}$CTTCTCT-3'

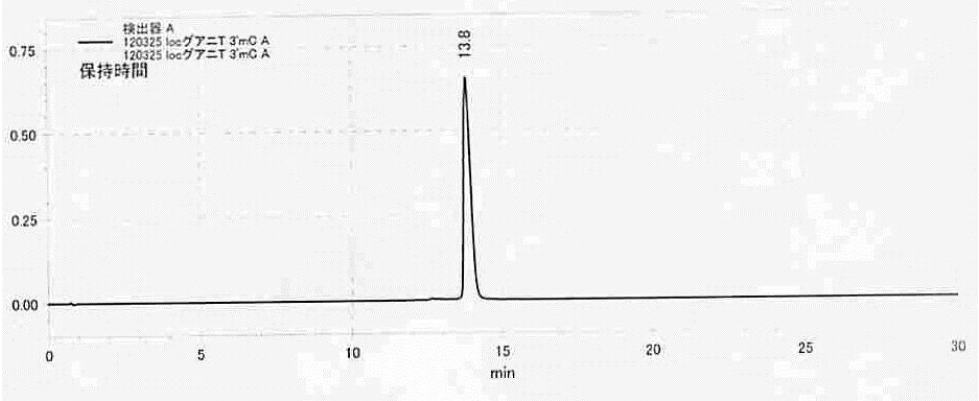
HPLC

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. : 50ºC.



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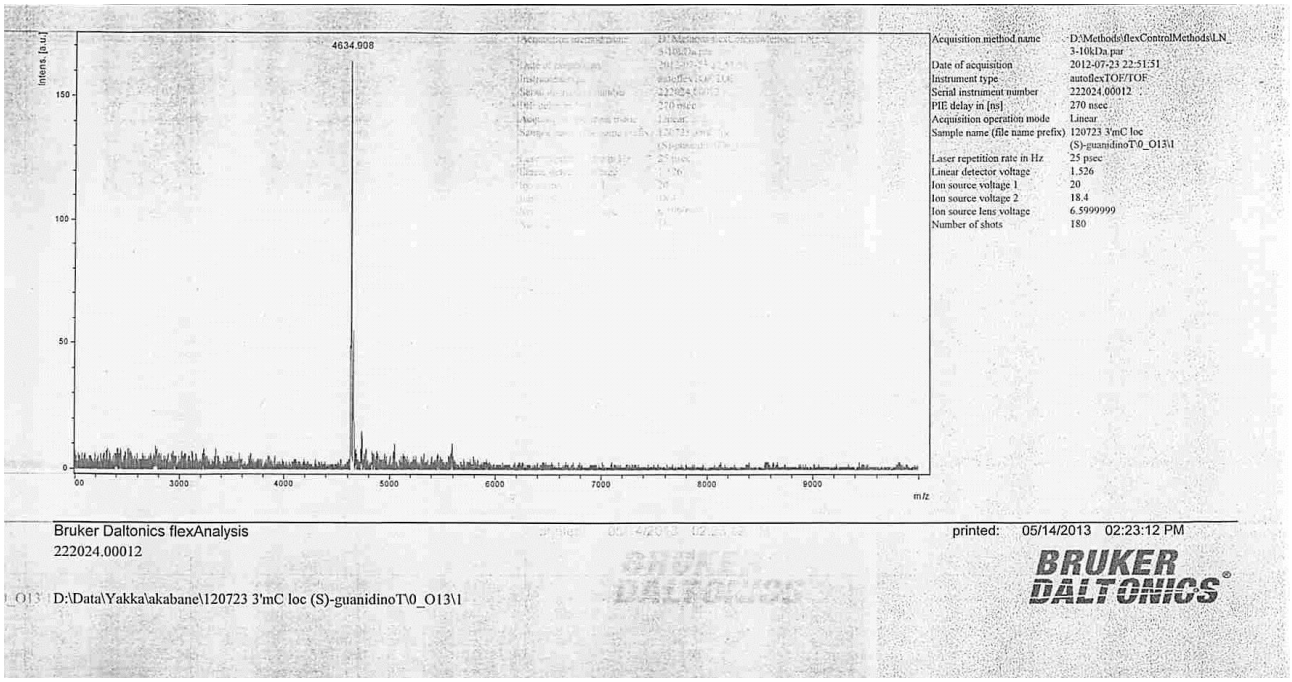


TFO: 5’-TTTTTTC**GP**$^{\mathbf{B}}$CTTCTCT-3’

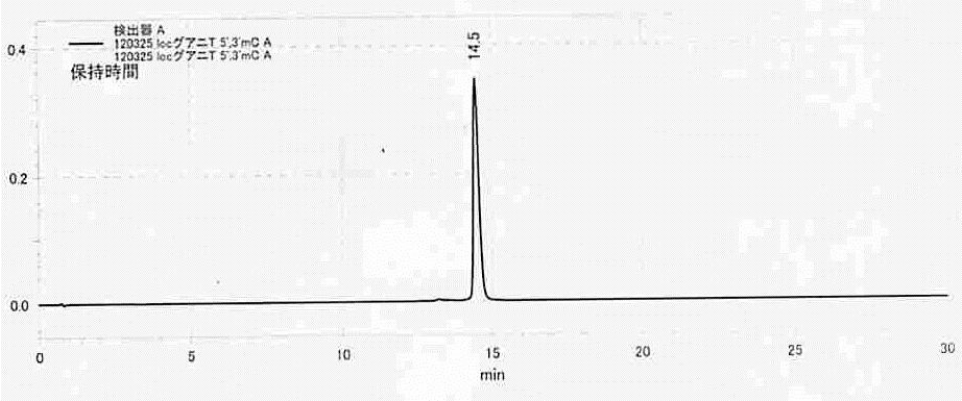
HPLC

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

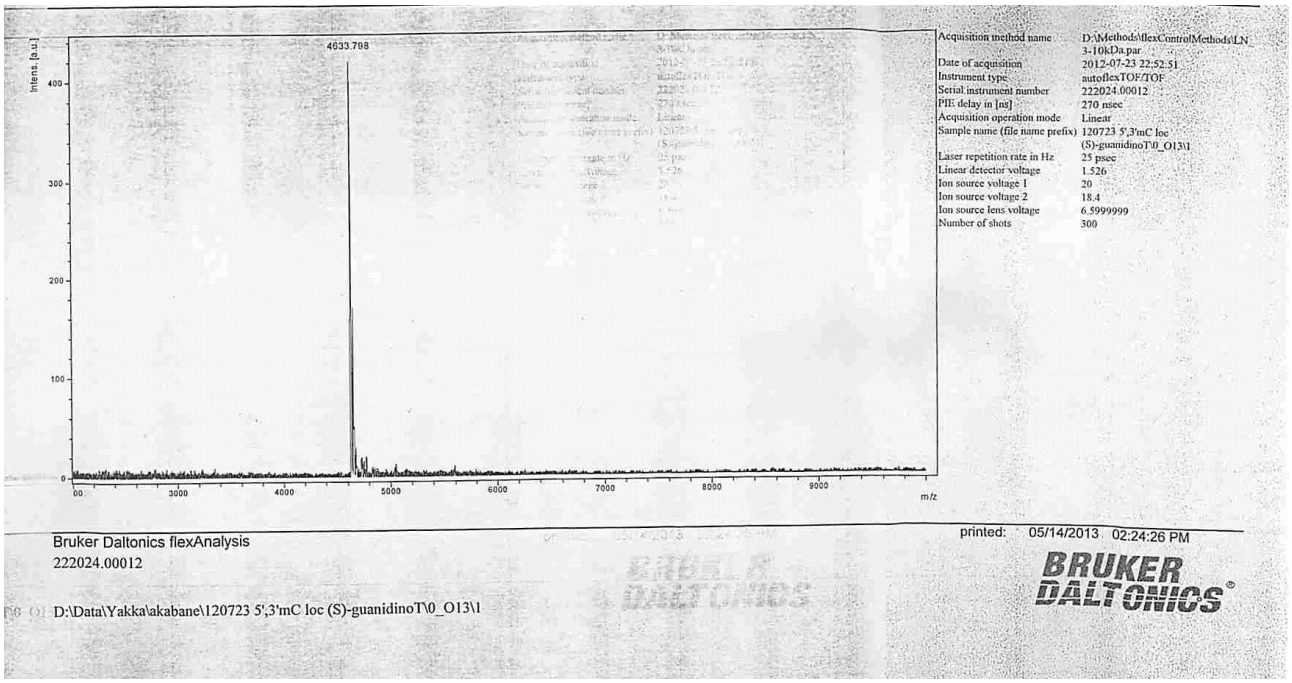
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO: 5'-TTTTT**GP^{B}**T**GP^{B}**T**GP^{B}**TCTCT-3'

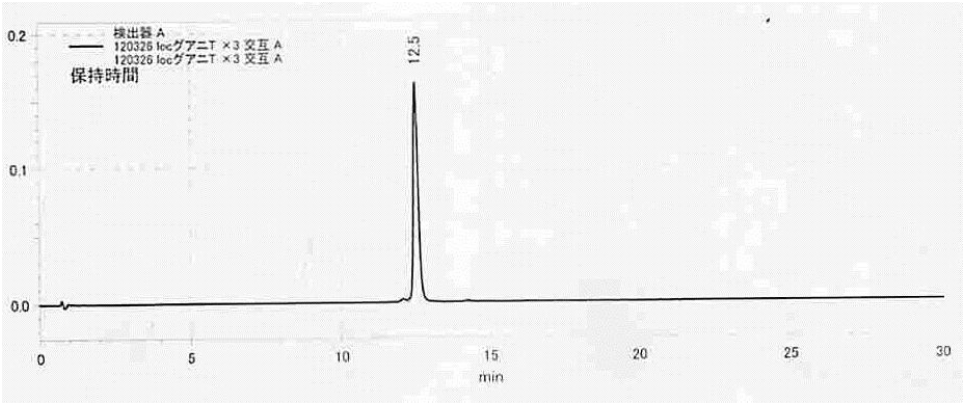
HPLC

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. : 50ºC.



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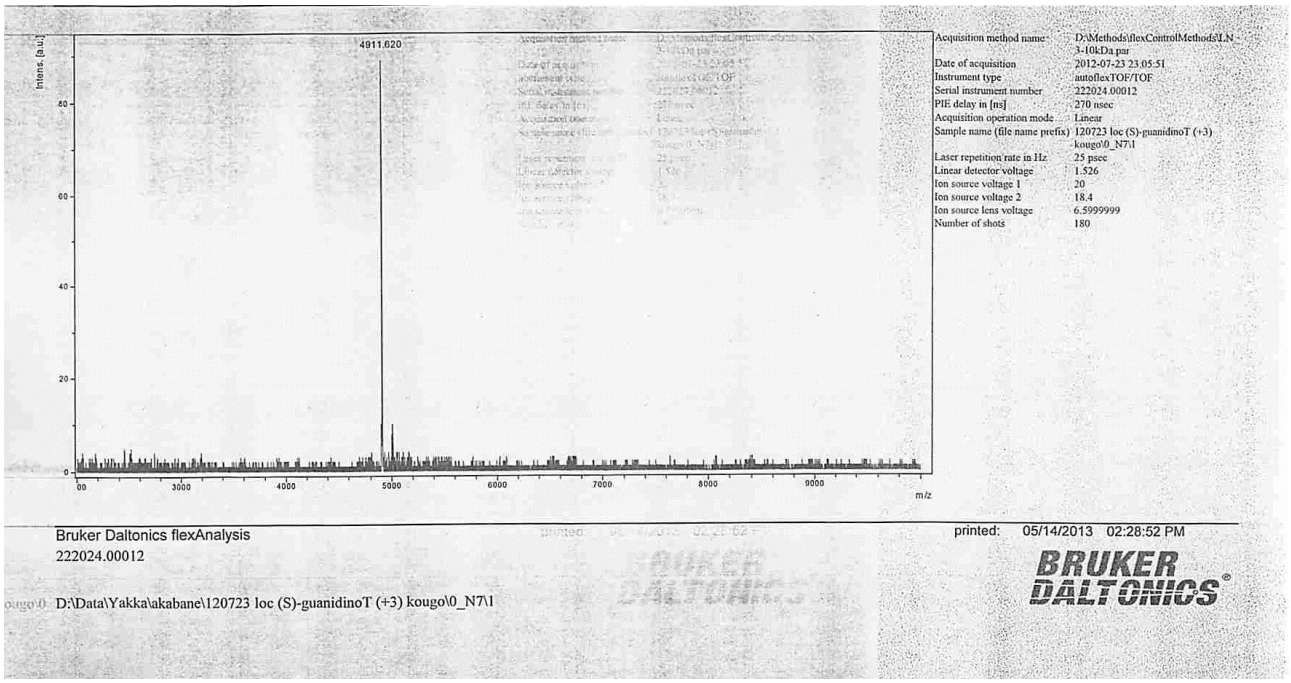


TFO: 5'-TTTT**GP^{B}**TT**GP^{B}**TT**GP^{B}**TTCT-3'

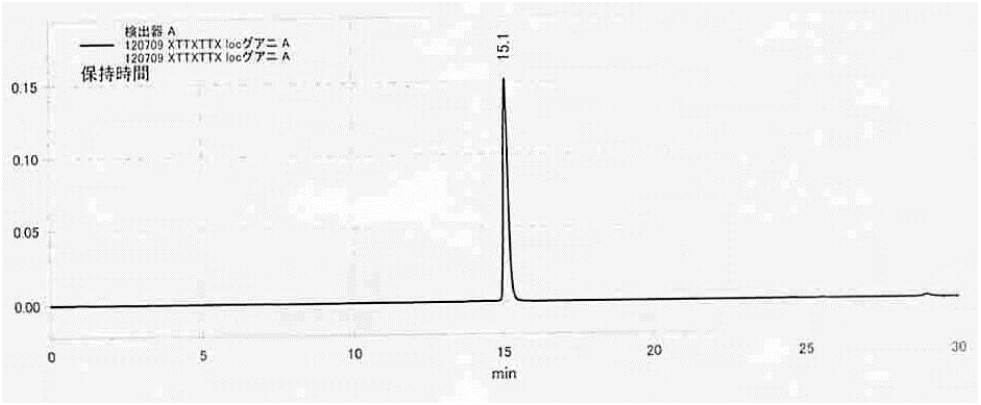
HPLC

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

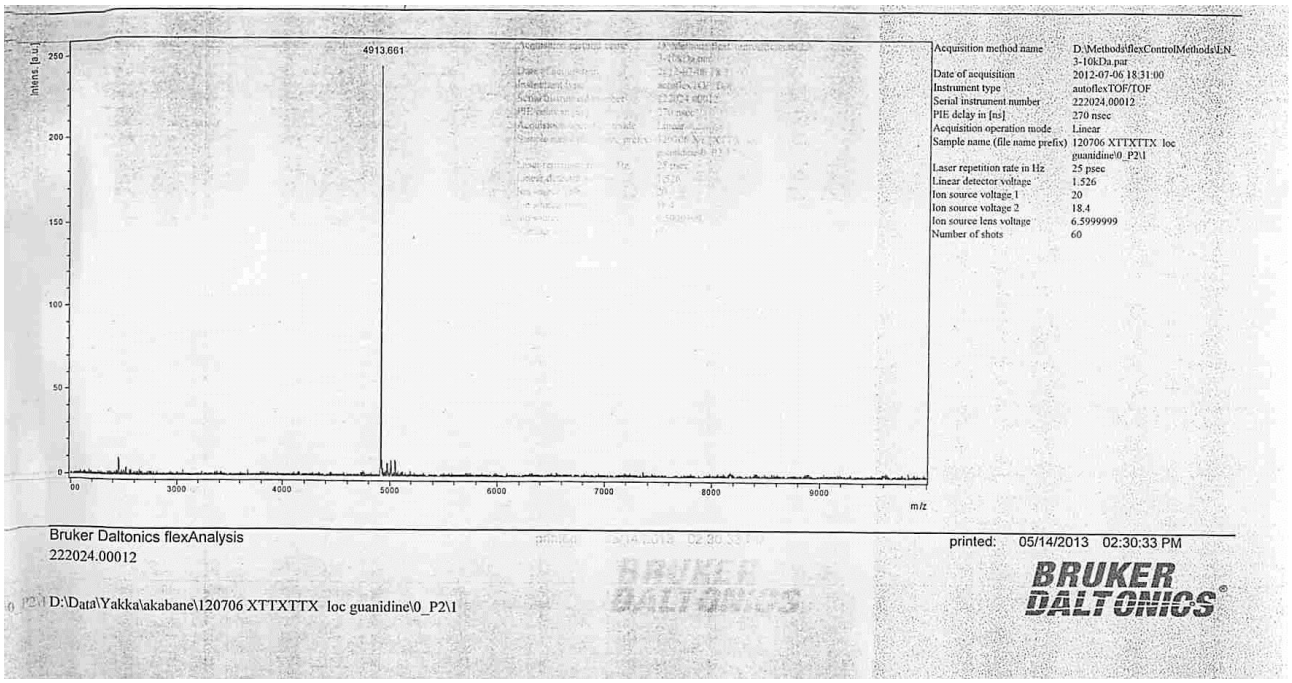
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO: 5’-TTT**GP^{B}**TCT**GP^{B}**TCT**GP^{B}**TCT-3’

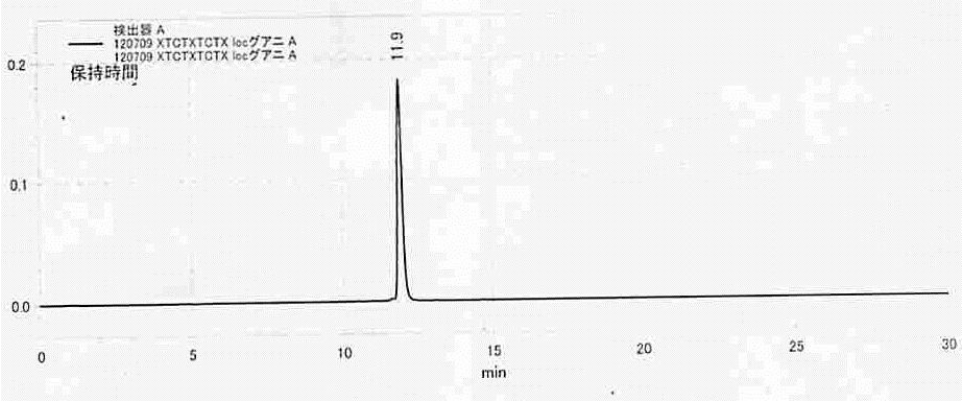
HPLC

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

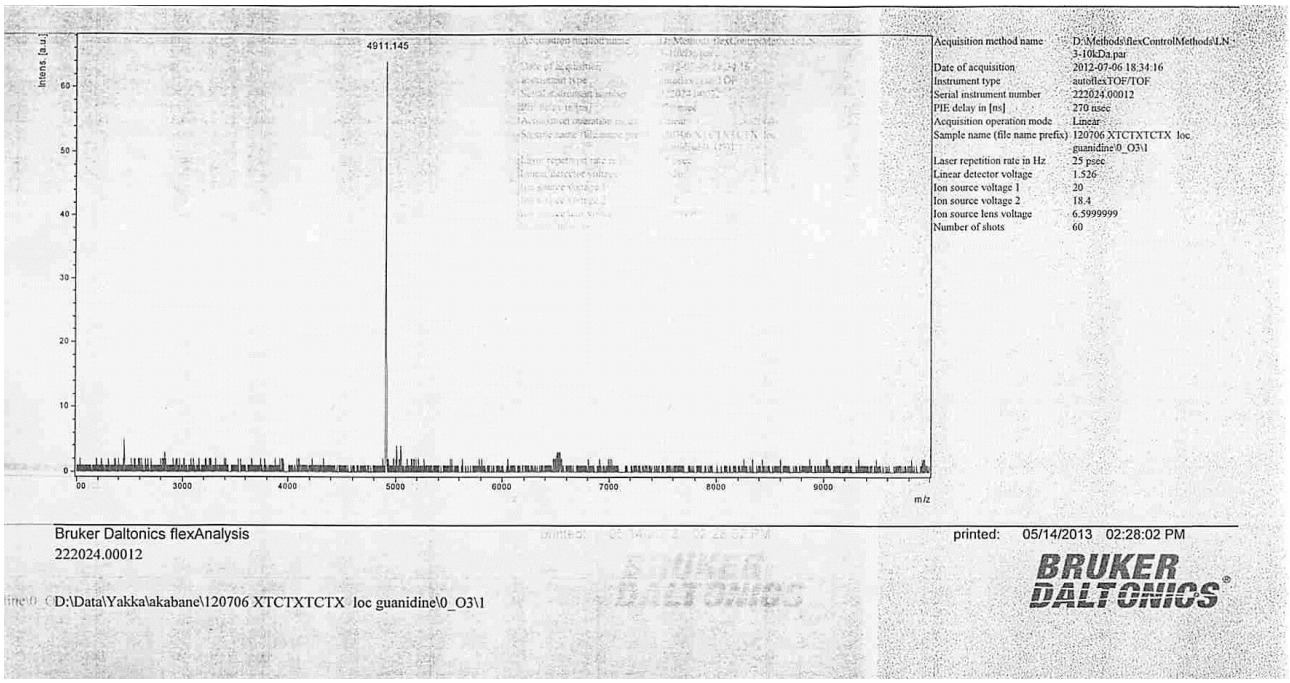
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

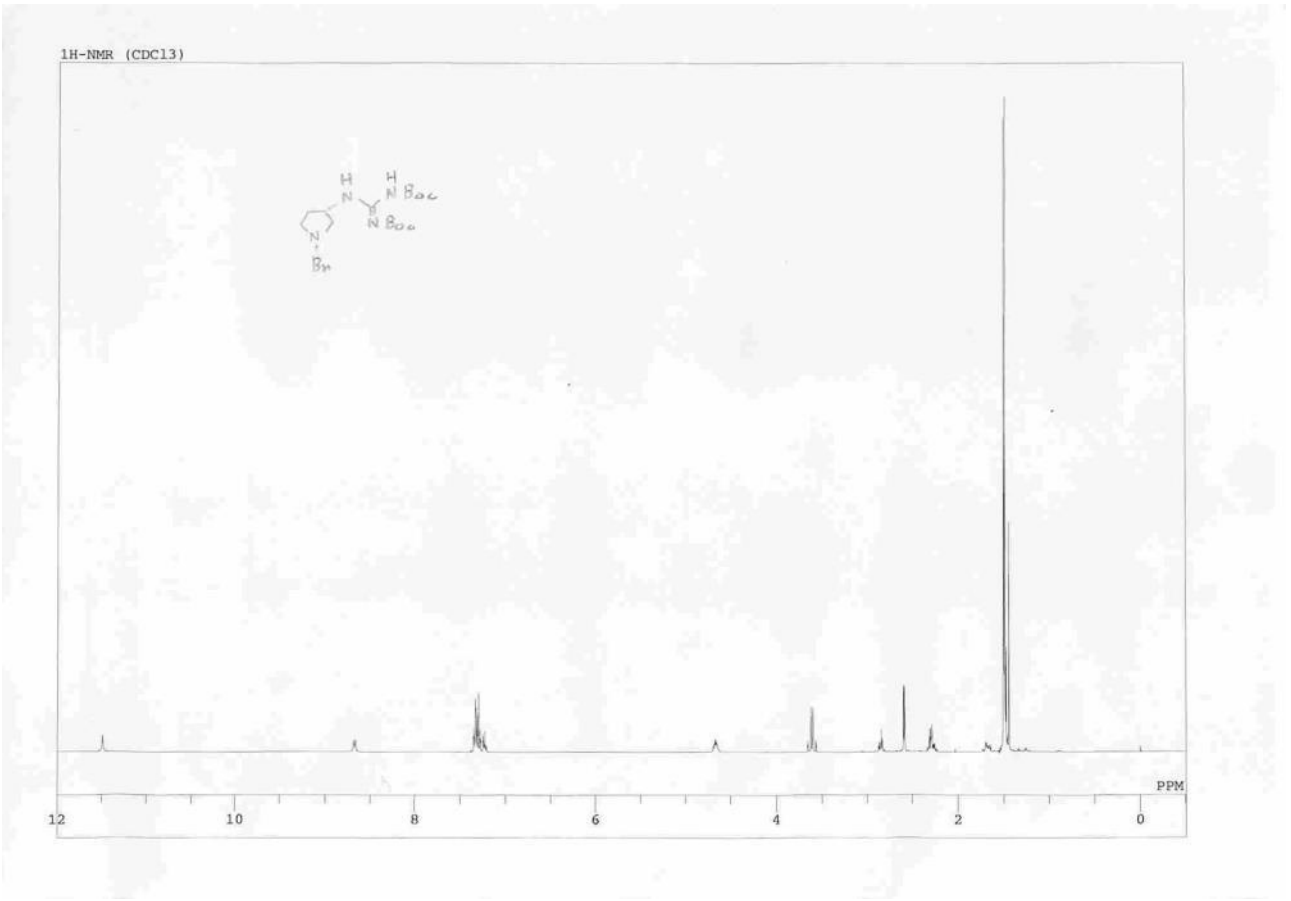
Column temp. : 50ºC.

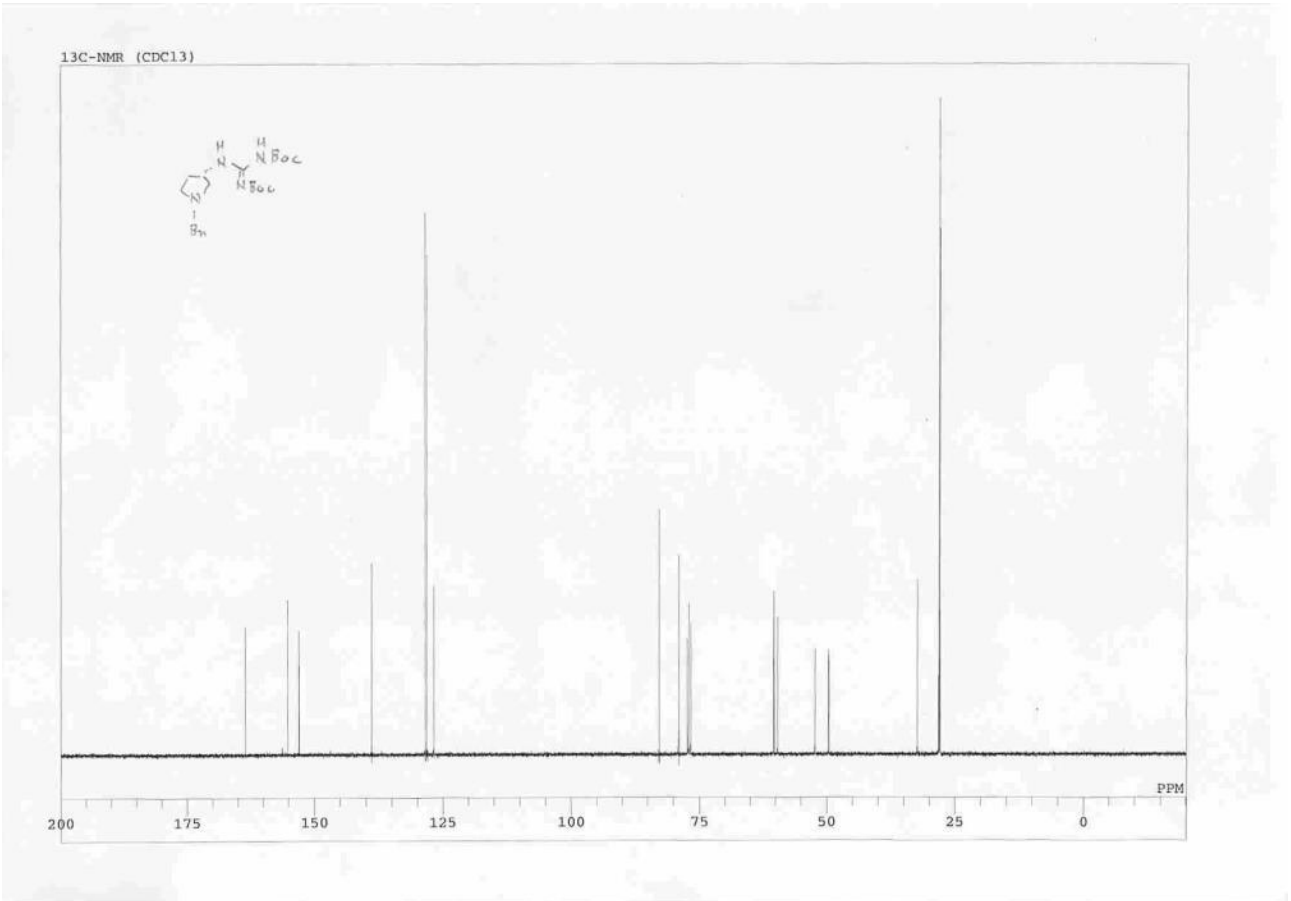


MALDI-TOF-Mass

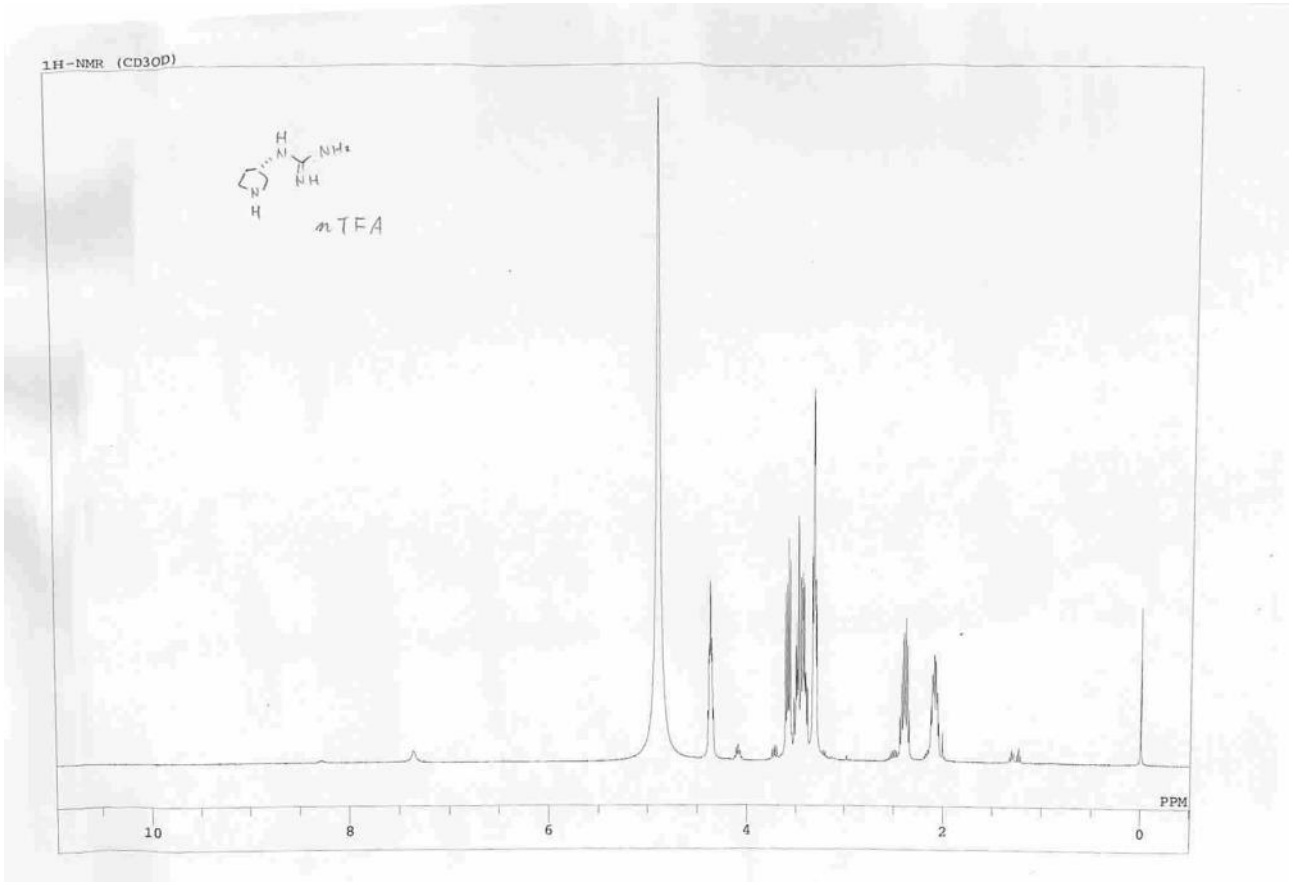


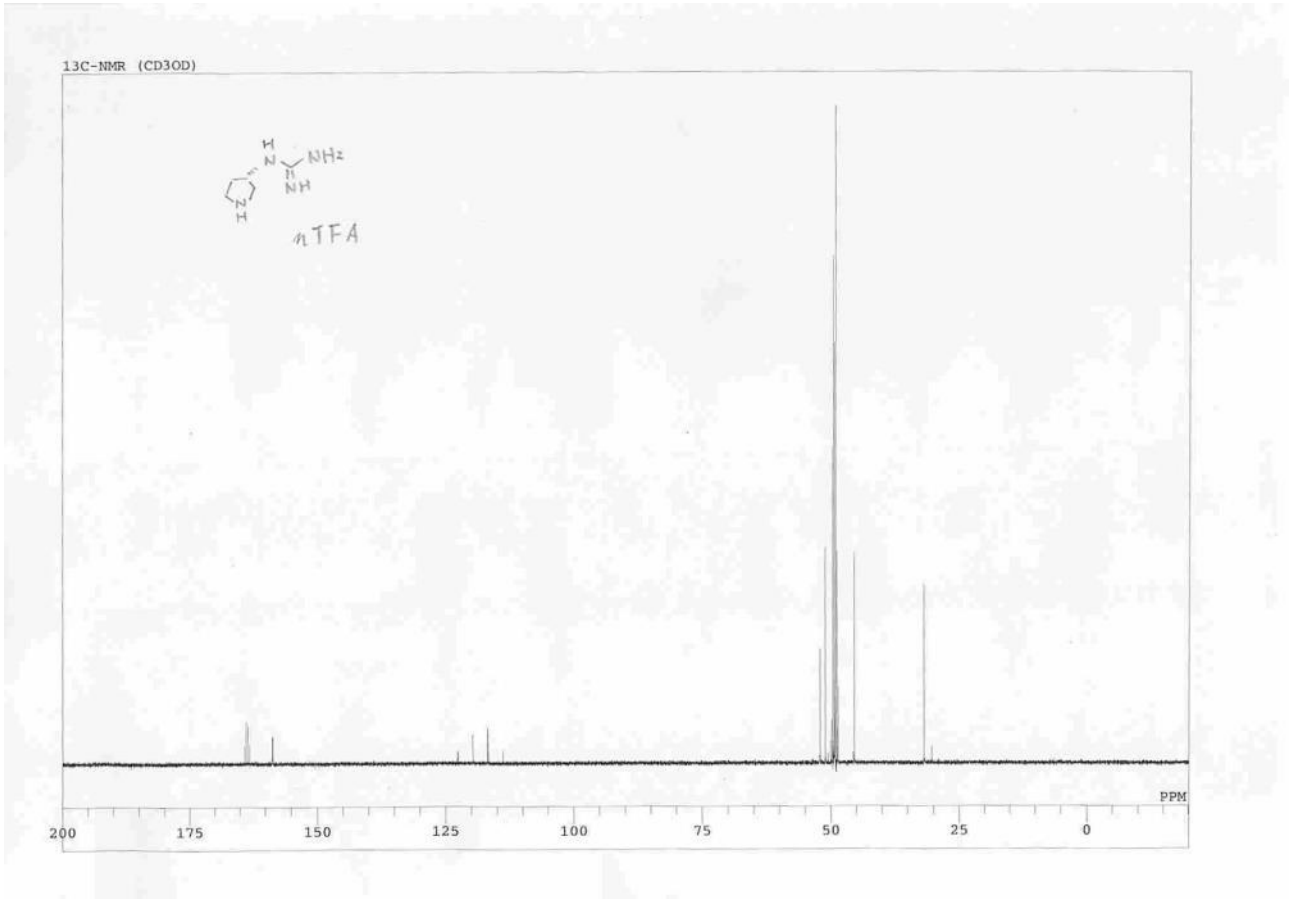
Compound **S1**


1H-NMR (CDCl3)
PPM
12
10
8
6
4
2
0

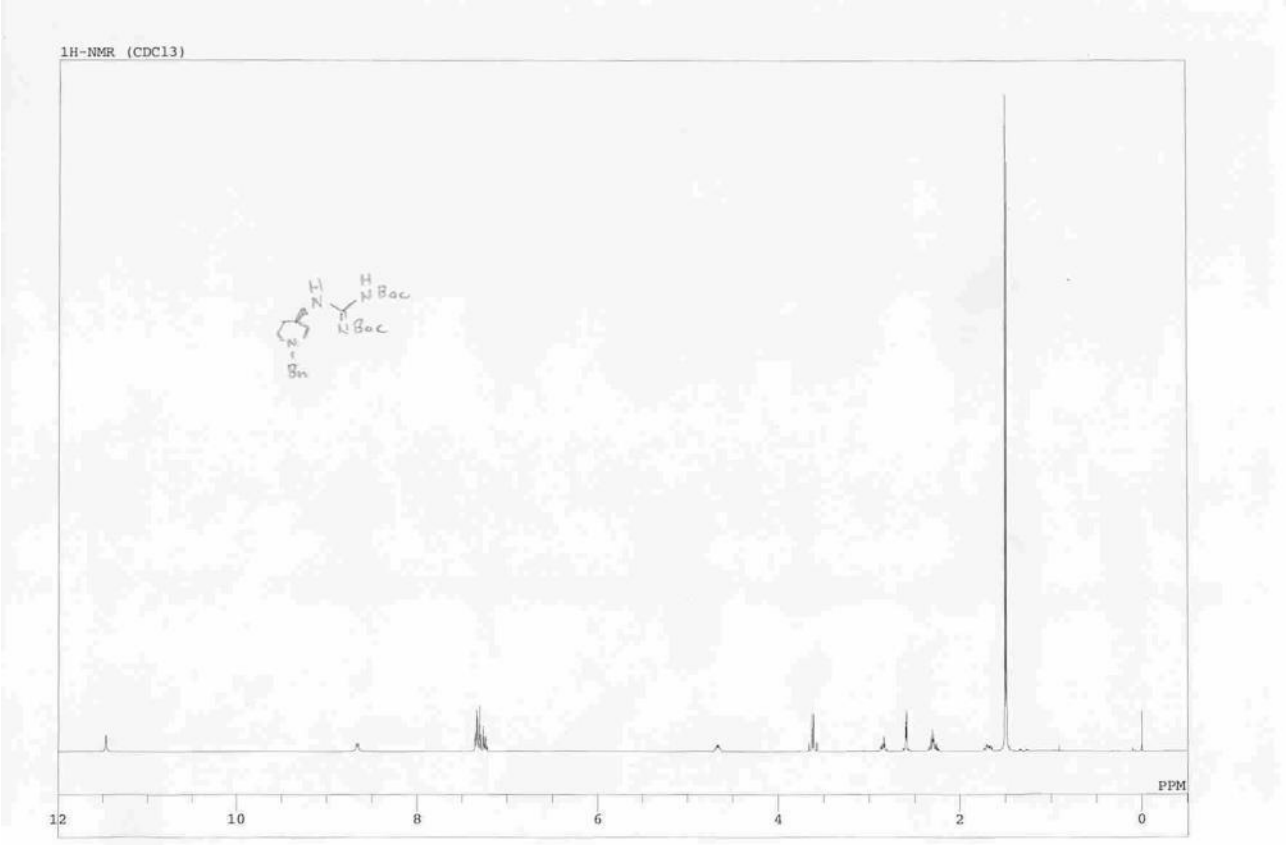

13C-NMR (CDCl3)
PPM
200
175
150
125
100
75
50
25
0

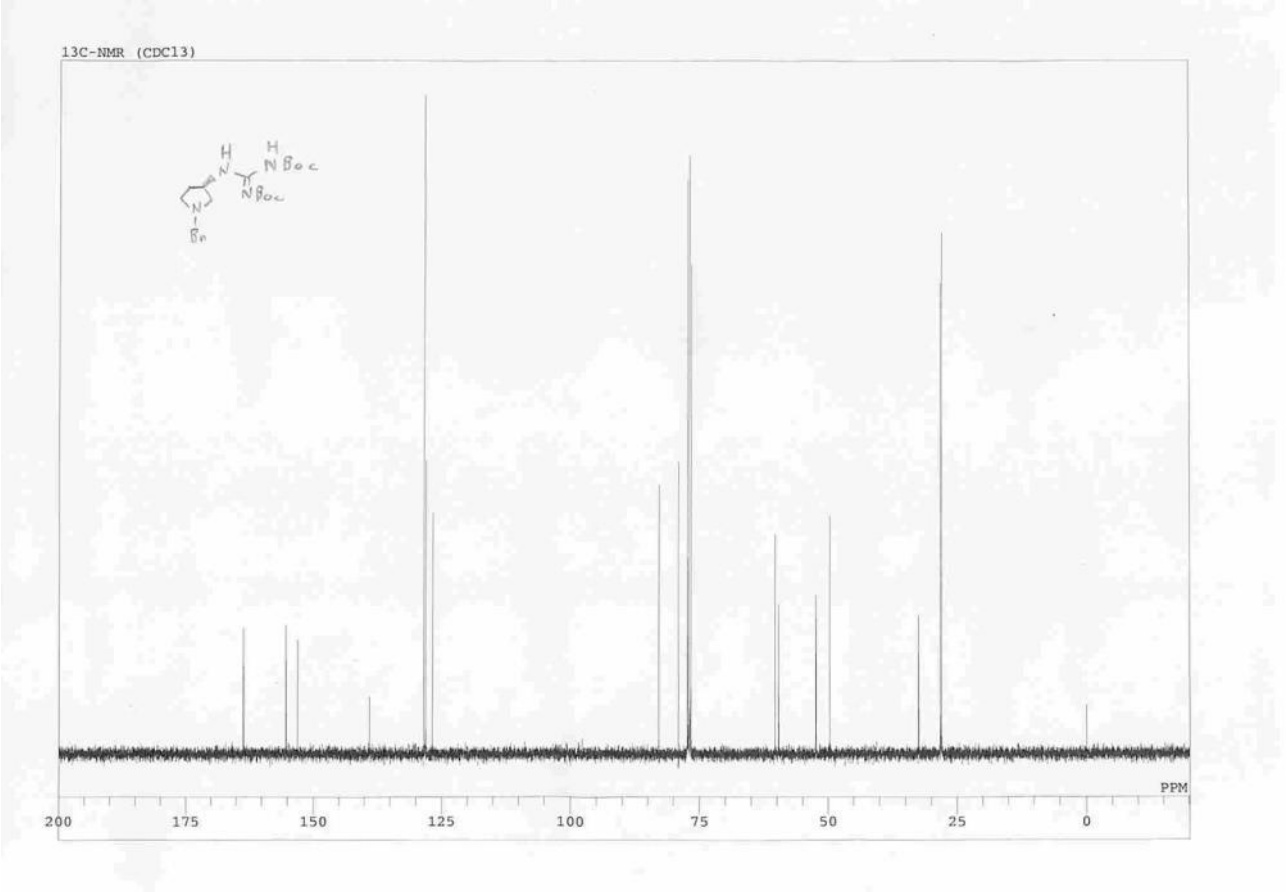
Compound **S2**


1H-NMR (CD3OD)
n TFA
PPM
10
8
6
4
2
0

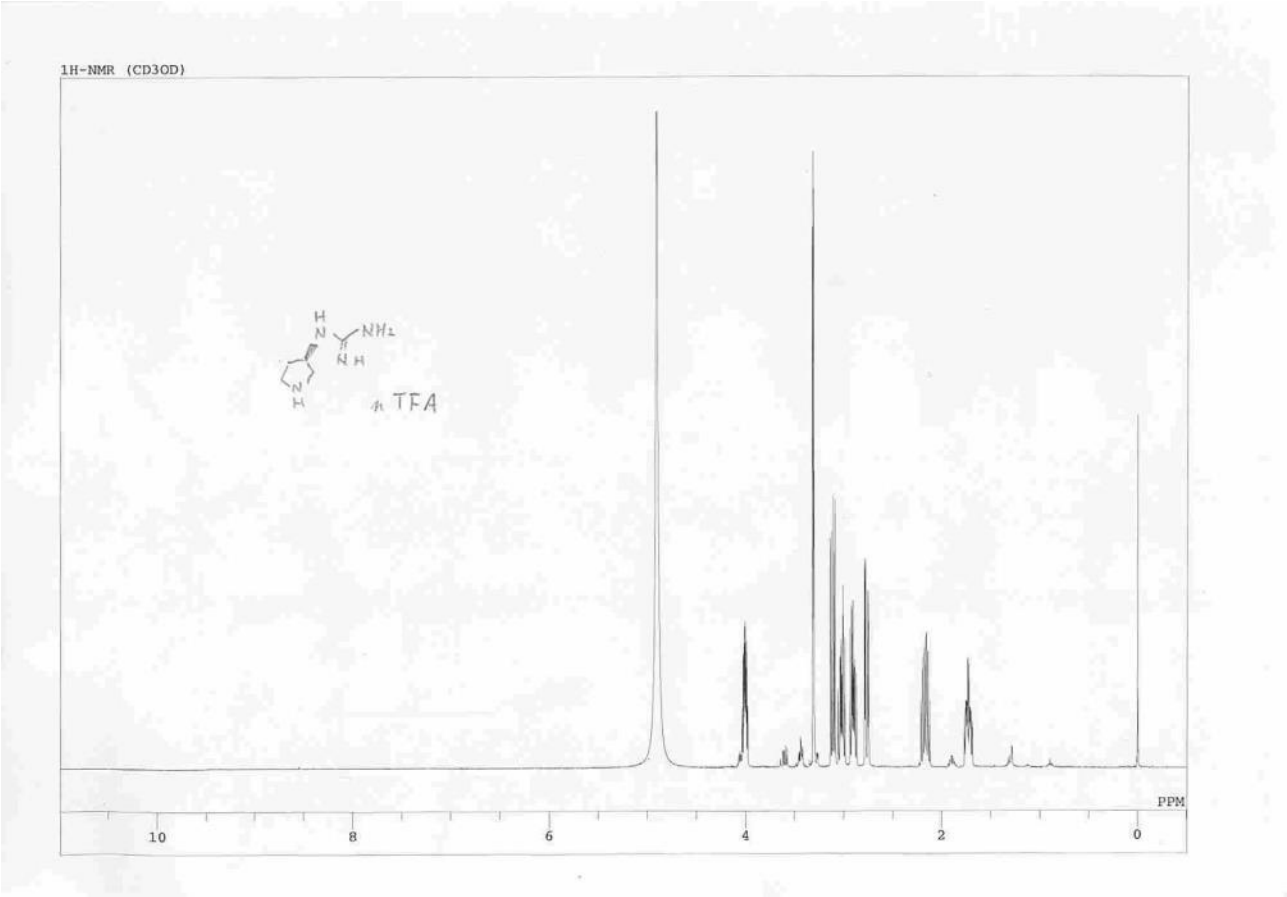

13C-NMR (CD3OD)
n TFA
PPM
200
175
150
125
100
75
50
25
0

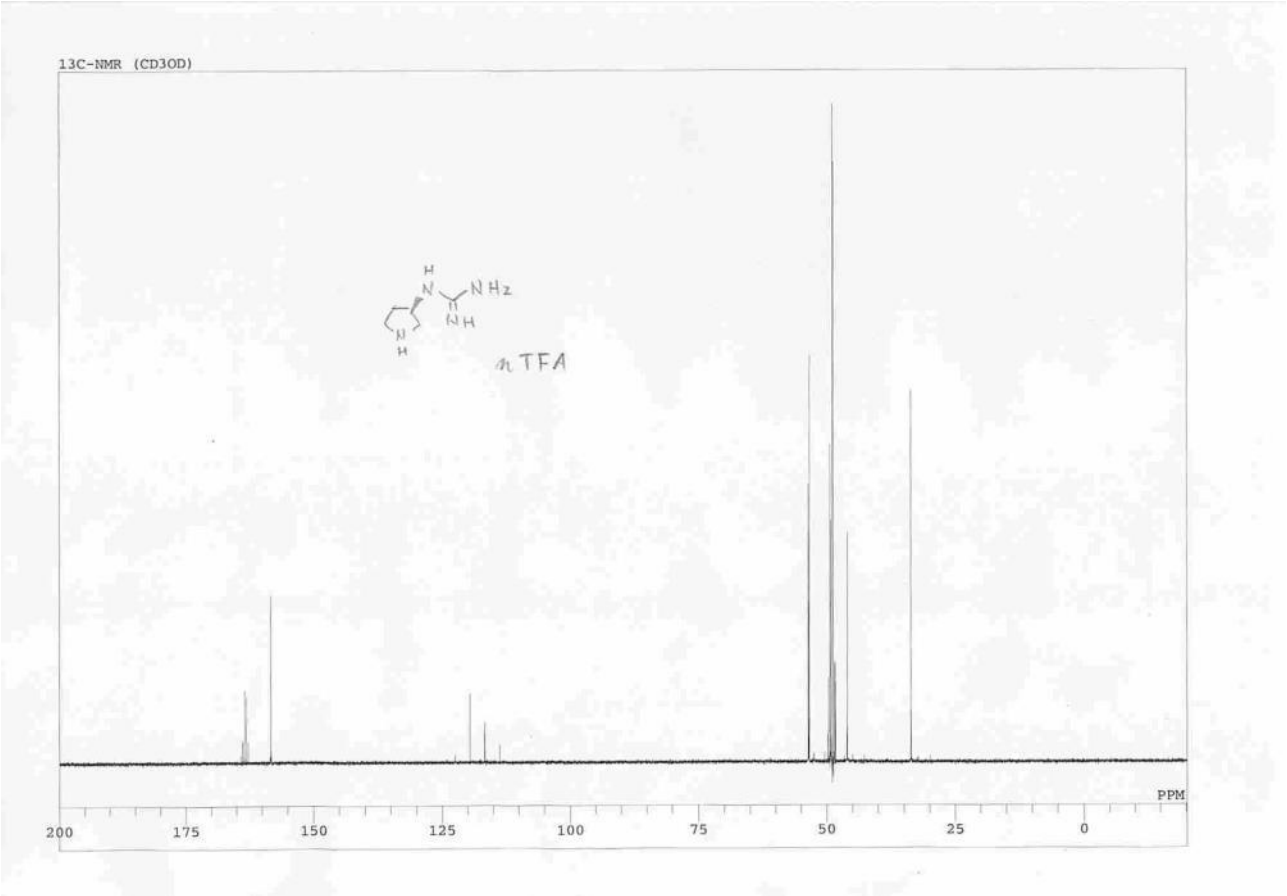
Compound **S3**


1H-NMR (CDCl3)
PPM
12
10
8
6
4
2
0

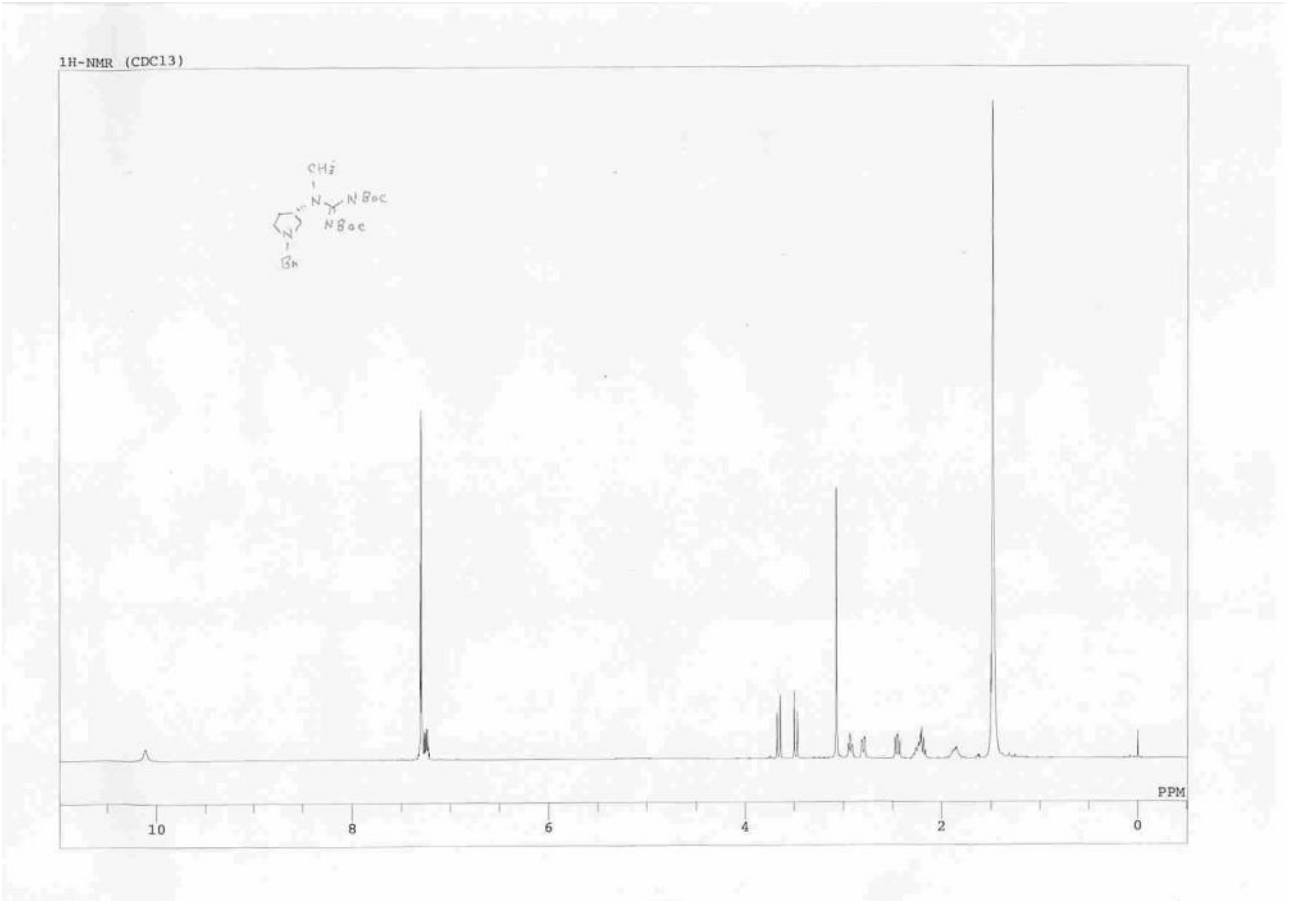

13C-NMR (CDCl3)
PPM
200
175
150
125
100
75
50
25
0

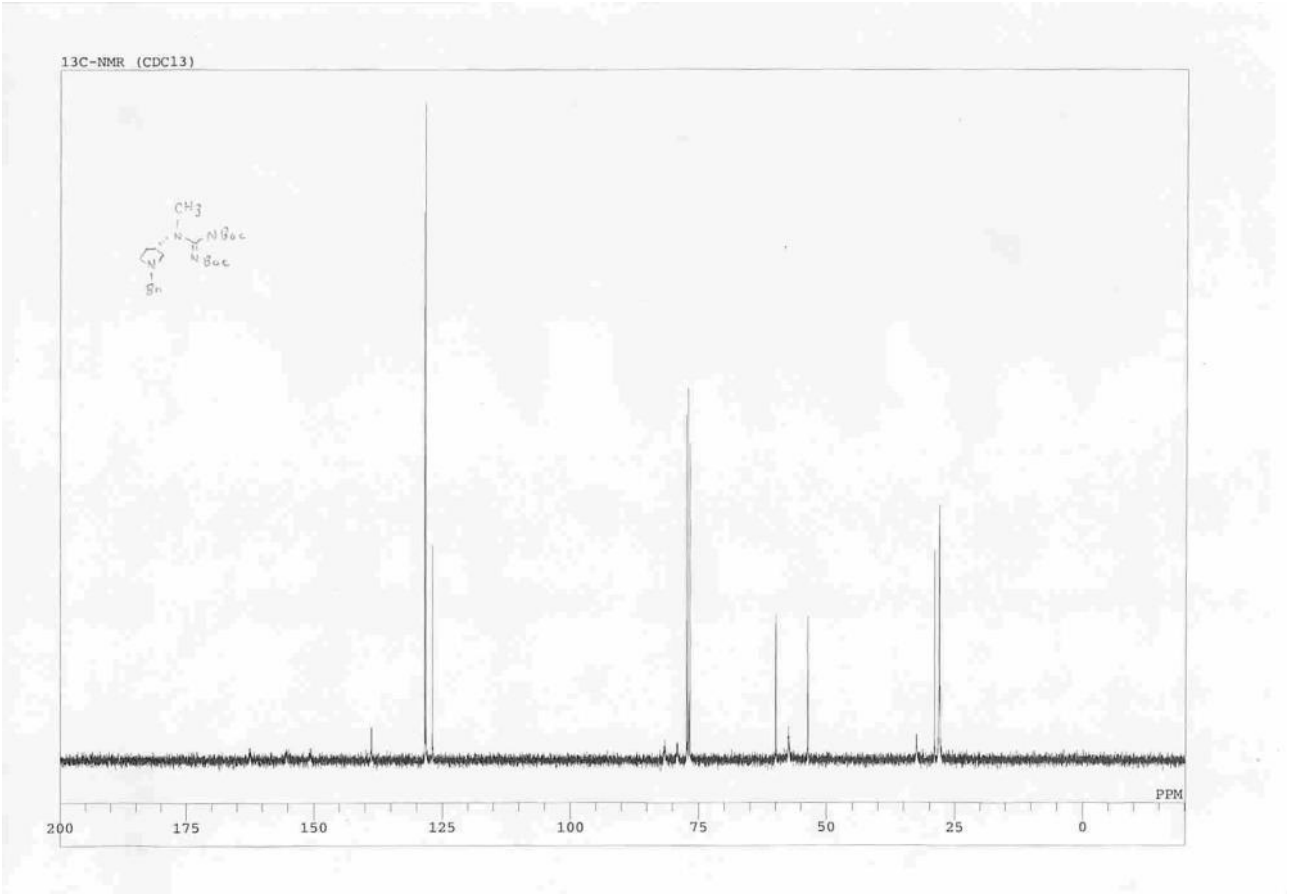
Compound **S4**


1H-NMR (CD3OD)
NH2
NH
n TFA
PPM
10
8
6
4
2
0

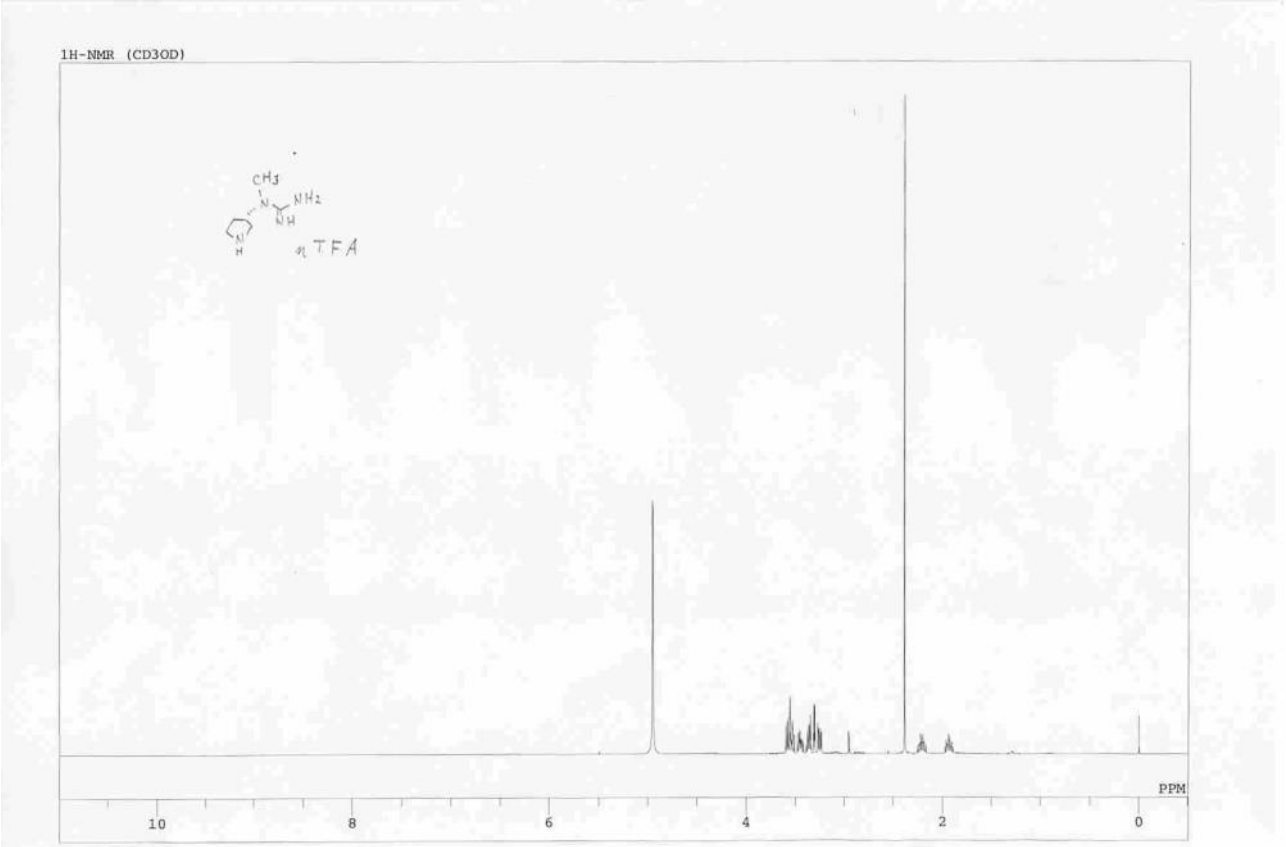

13C-NMR (CD3OD)
NH2
NH
n TFA
PPM
200
175
150
125
100
75
50
25
0

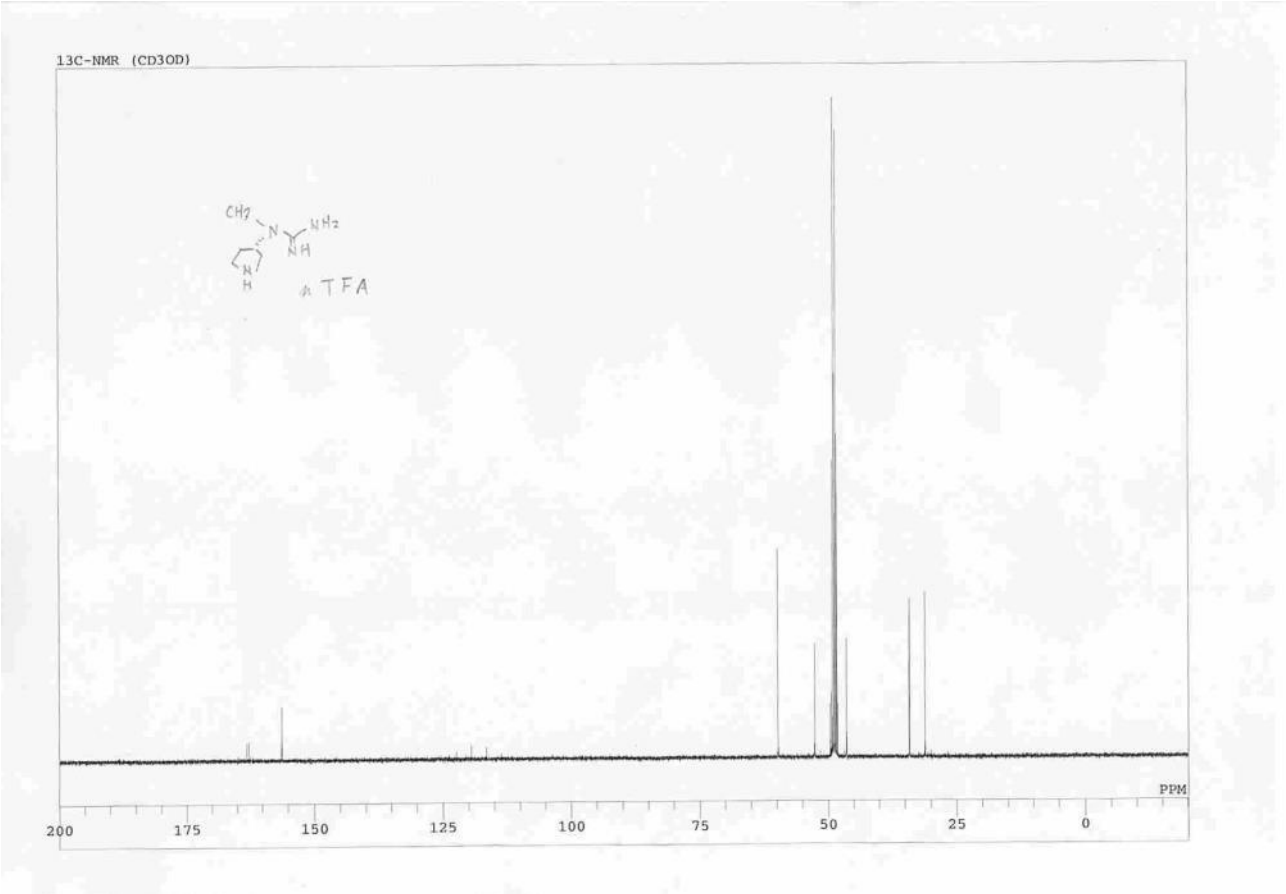
Compound **S5**



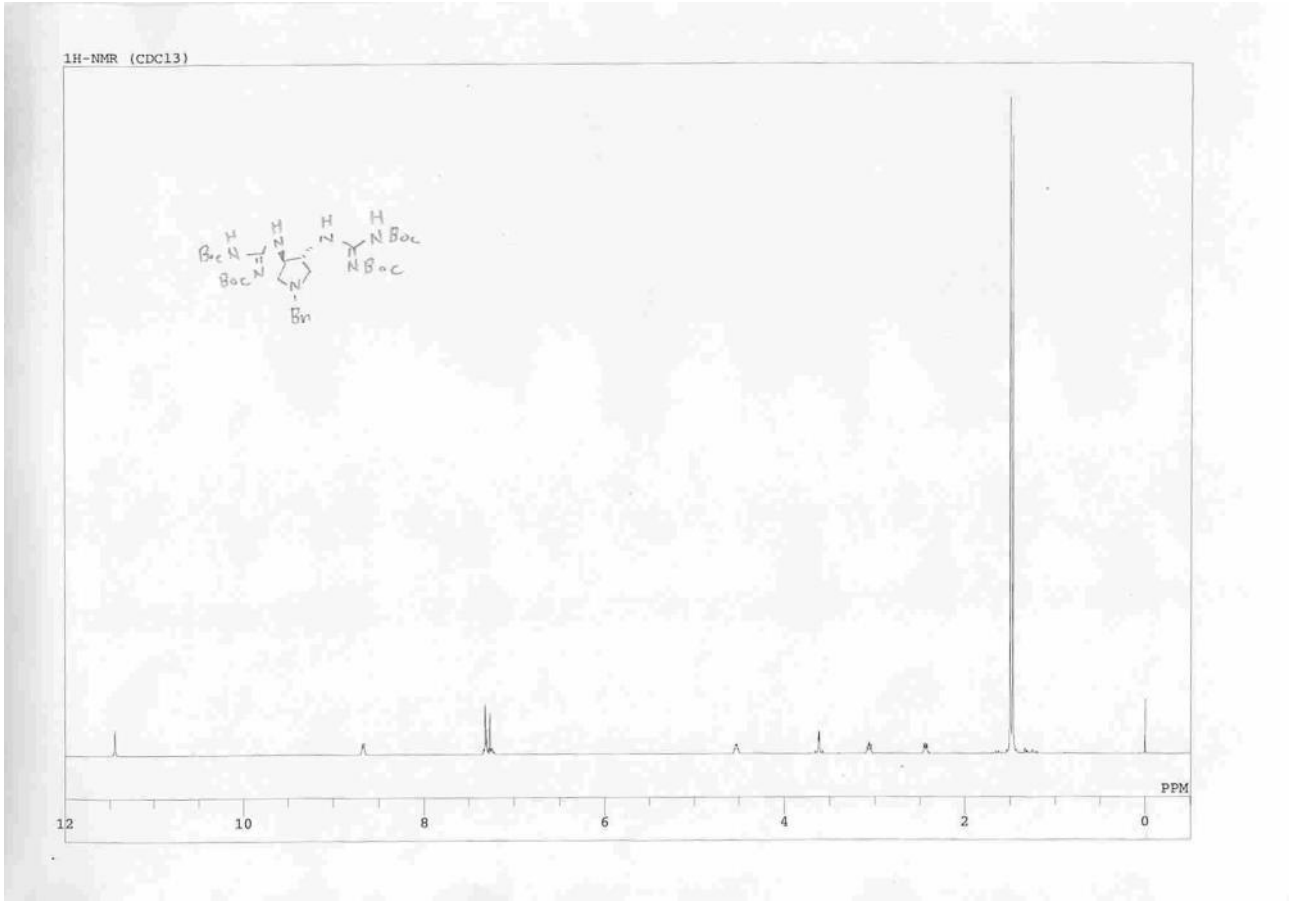


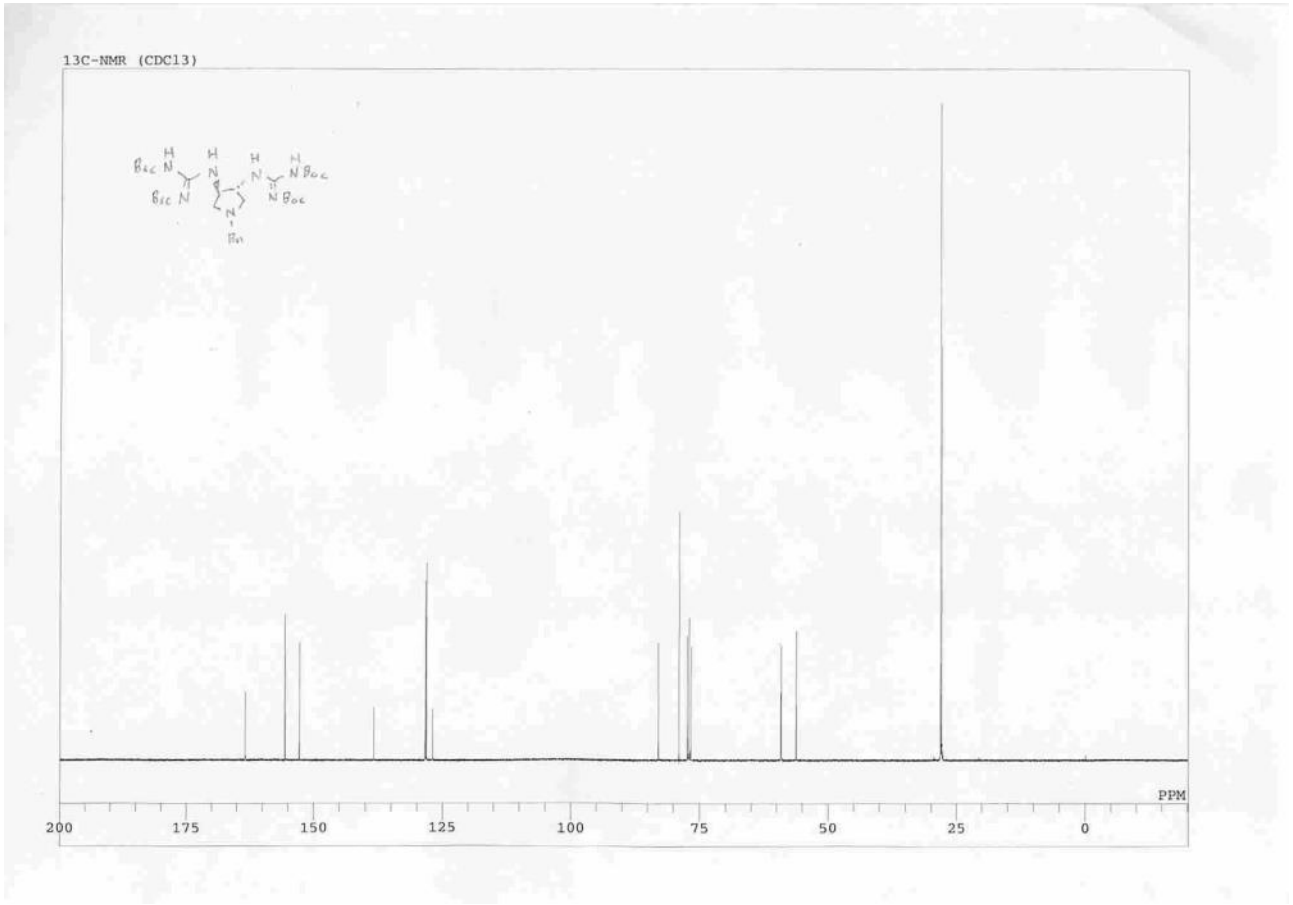
Compound **S6**


1H-NMR (CD3OD)
CH3
N
NH2
NH
H
TFA
PPM
10
8
6
4
2
0

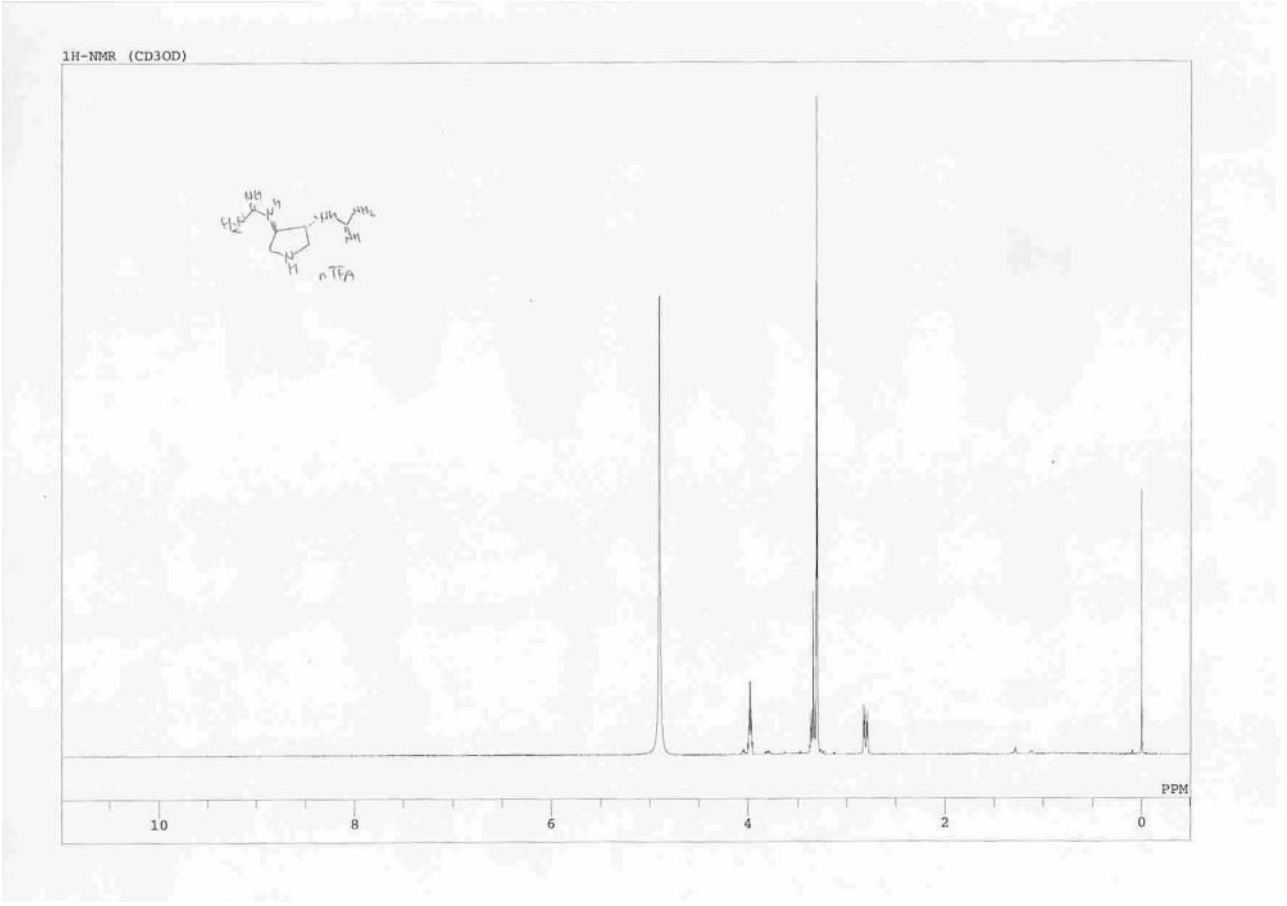

13C-NMR (CD3OD)
CH3
N
NH2
NH
H
TFA
PPM
200
175
150
125
100
75
50
25
0

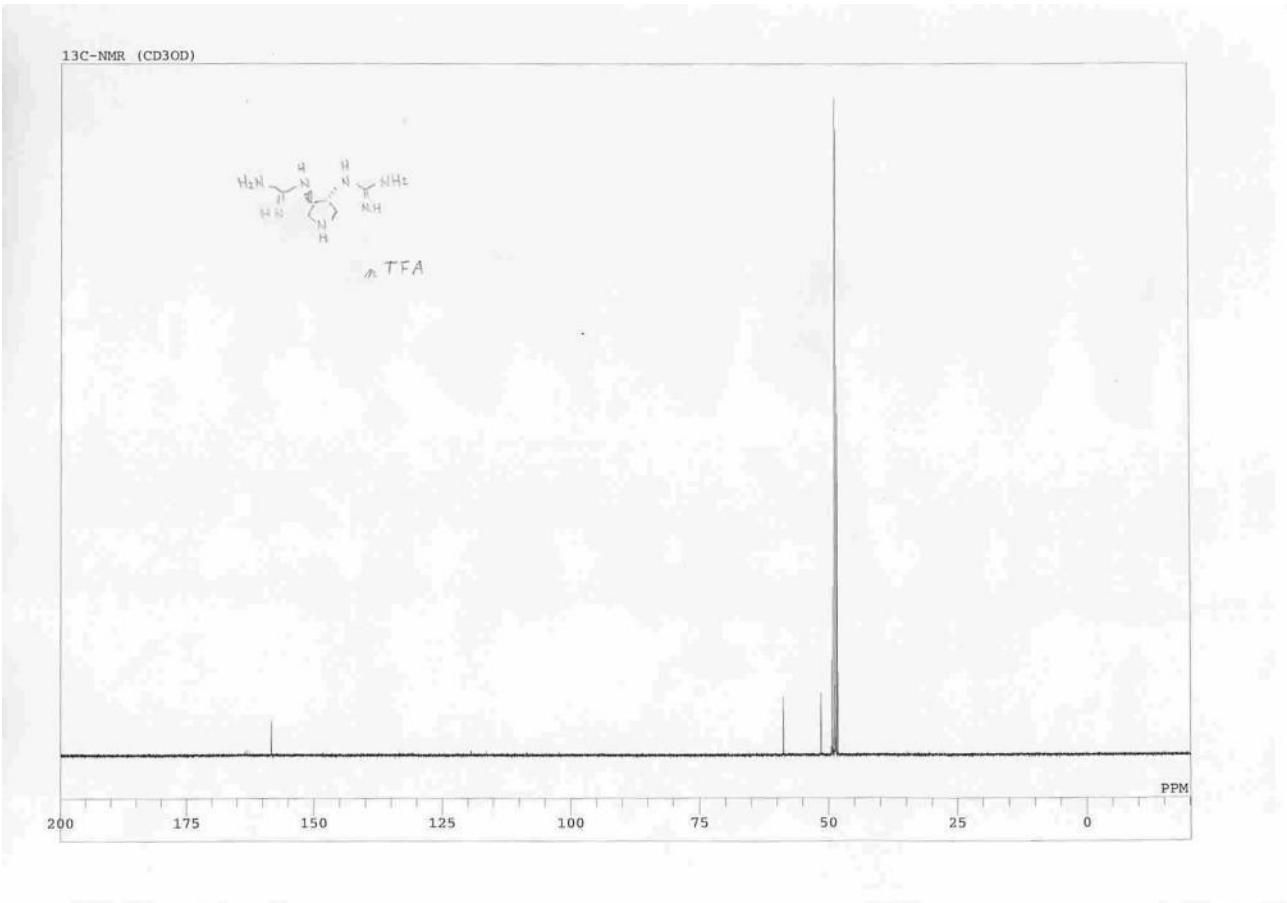
Compound **S7**


1H-NMR (CDCl3)
PPM
12
10
8
6
4
2
0

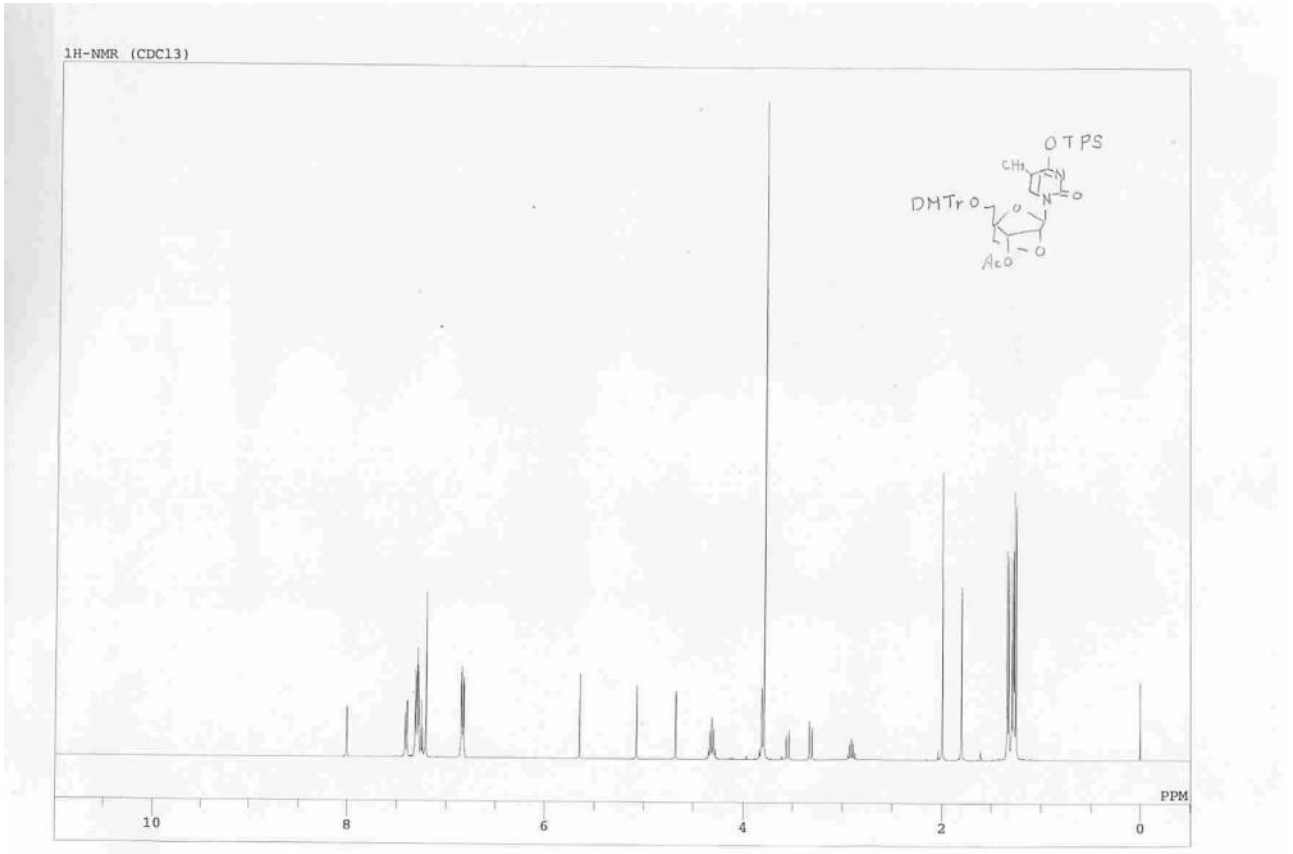

13C-NMR (CDCl3)
PPM
200
175
150
125
100
75
50
25
0

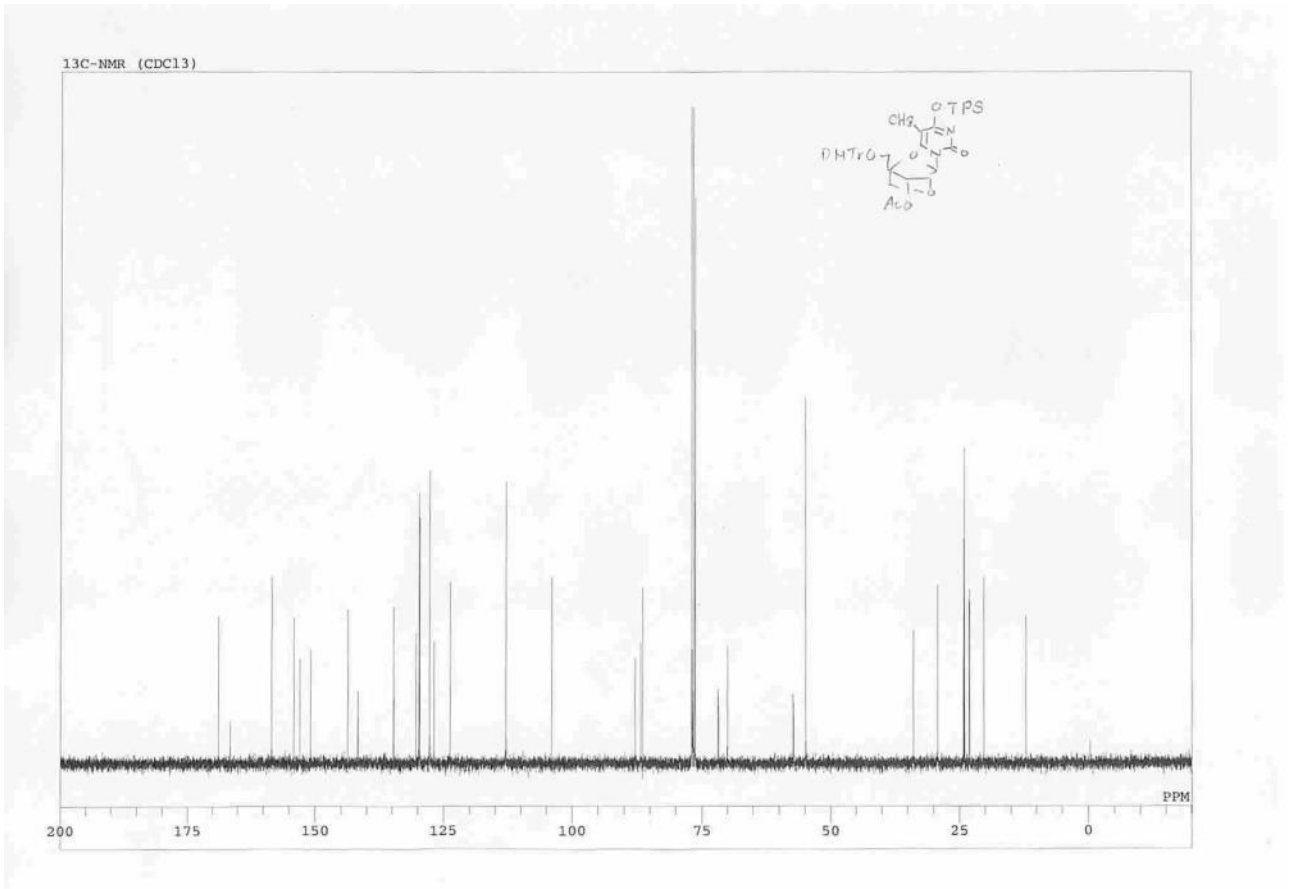
Compound **S8**


1H-NMR (CD3OD)
nTFA
PPM
10
8
6
4
2
0

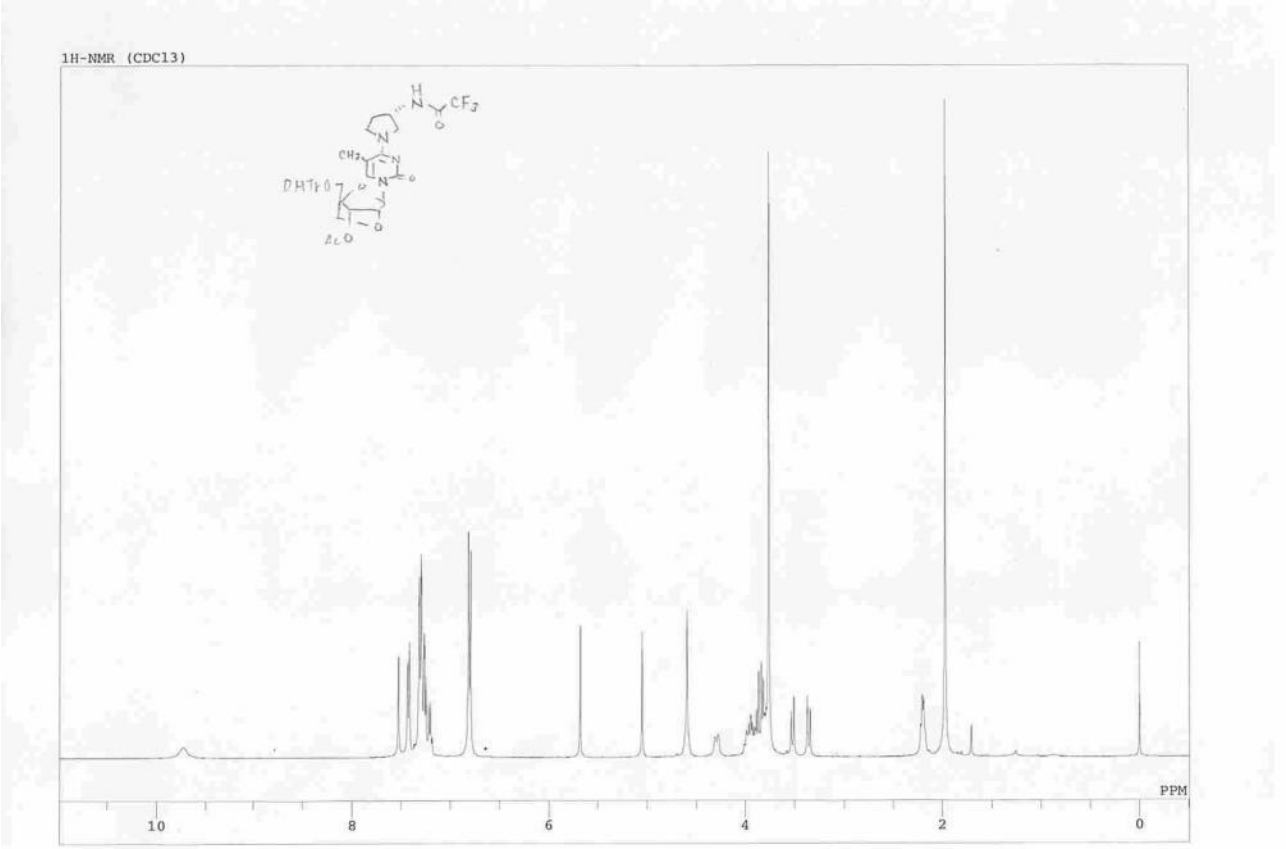

13C-NMR (CD3OD)
nTFA
PPM
200
175
150
125
100
75
50
25
0

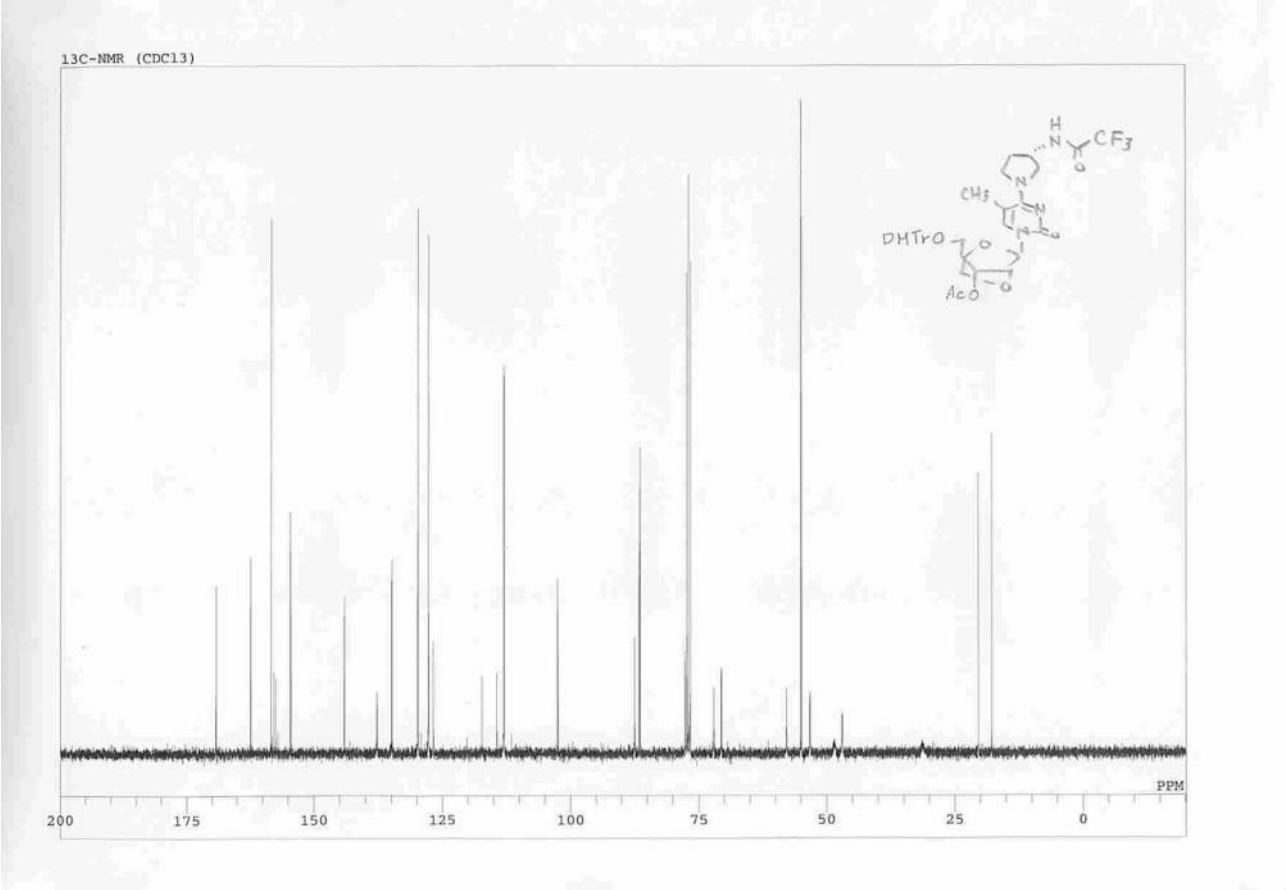
Compound **S10**



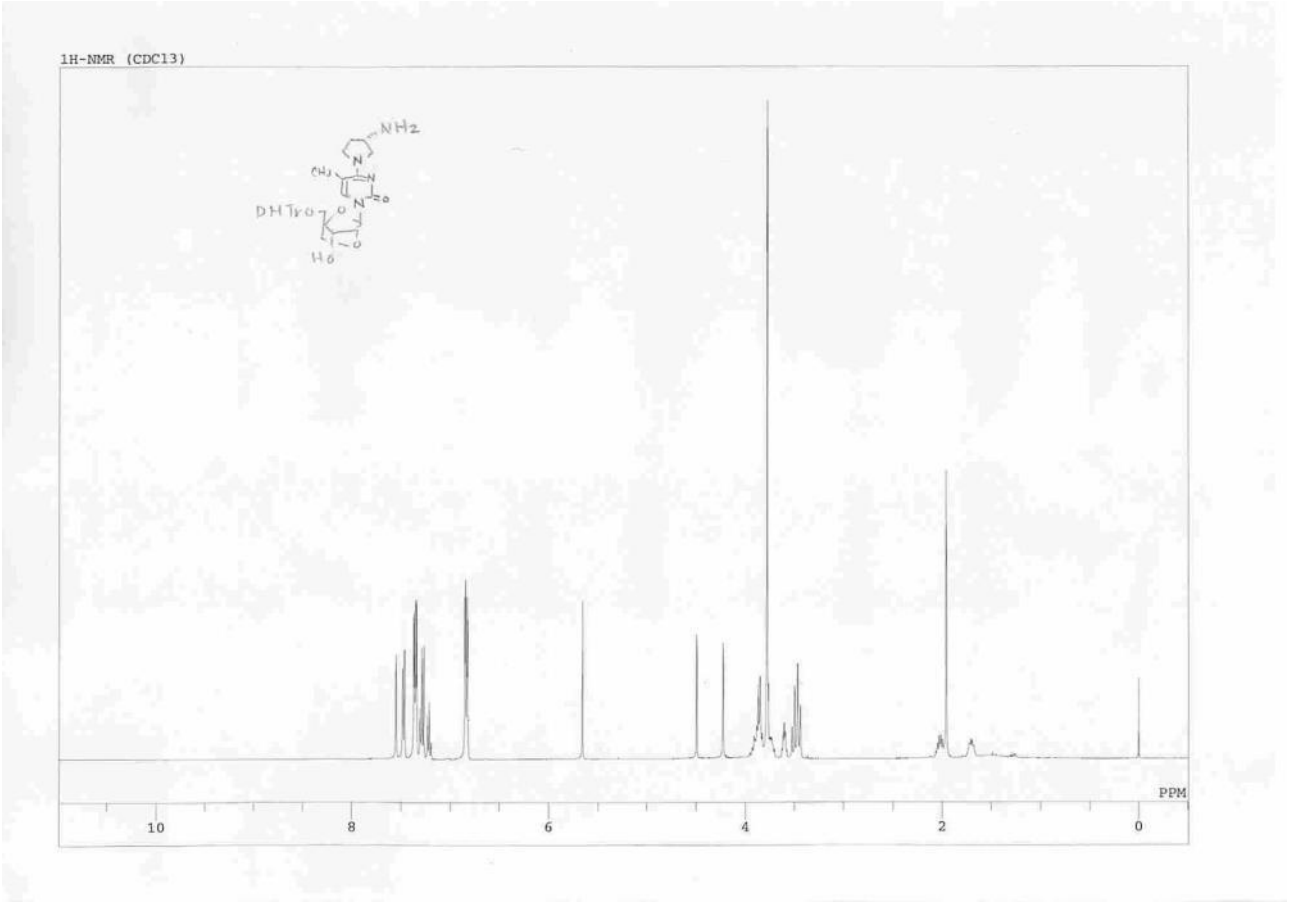


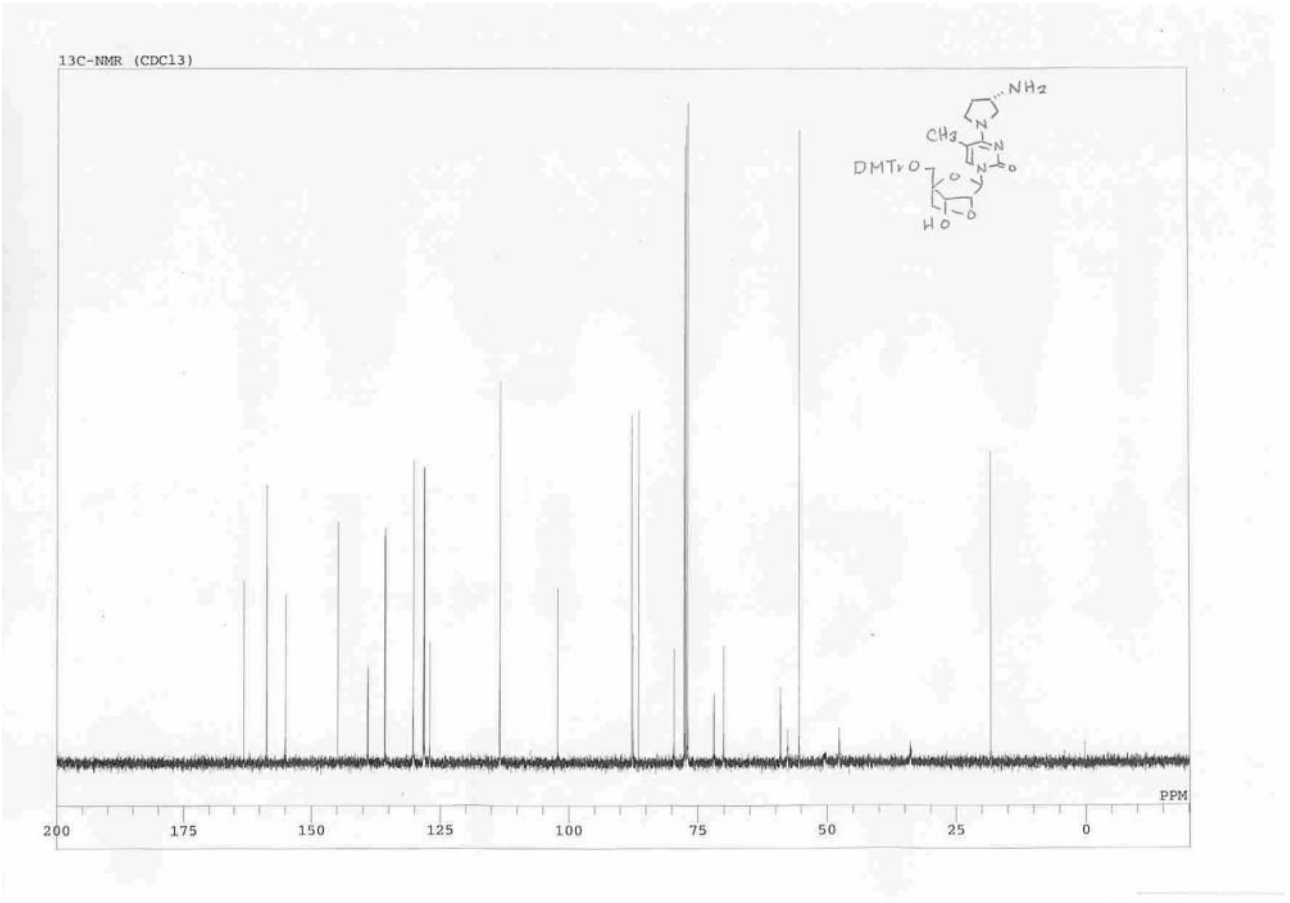
Compound **S11**



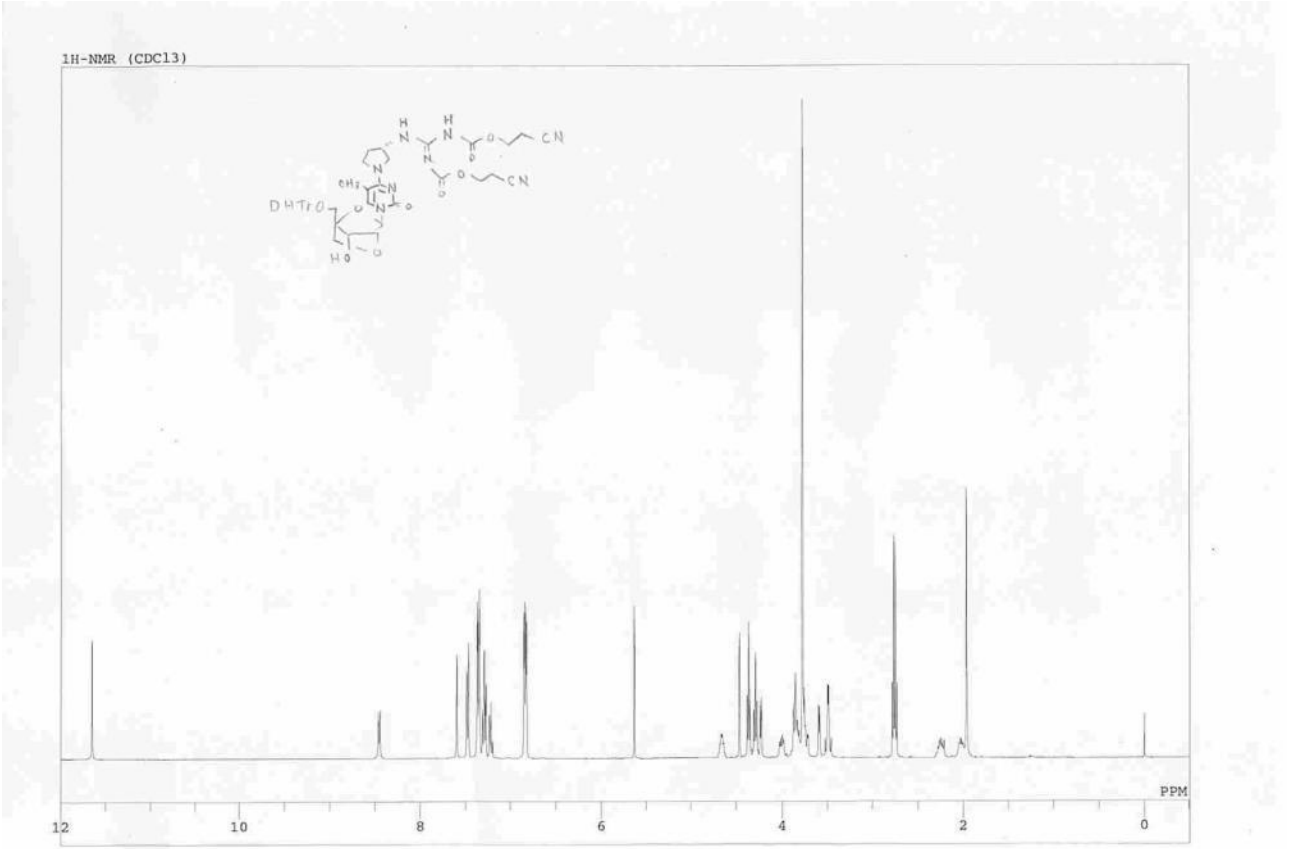


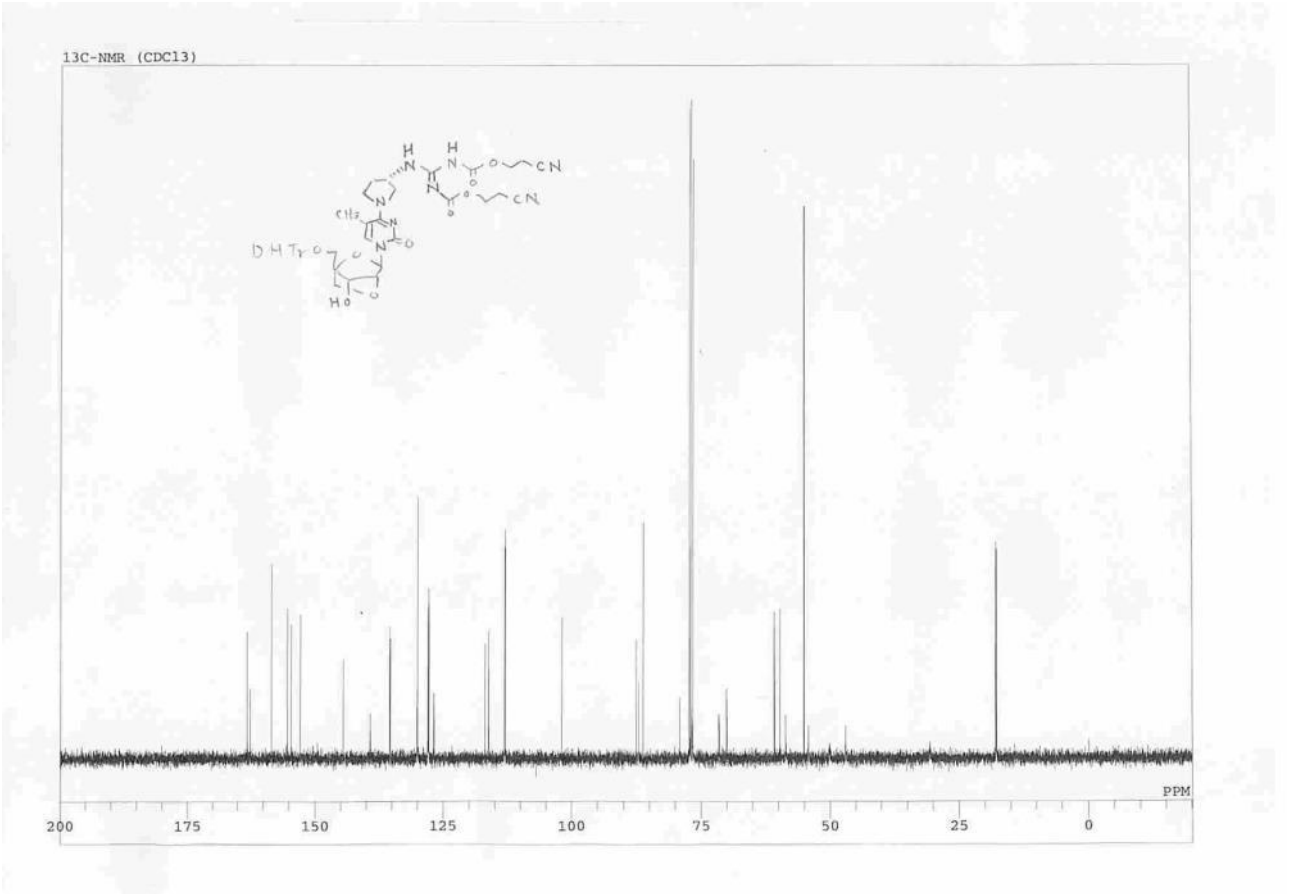
Compound **S12**





Compound **S13**





Compound **S14**