Electronic Supplementary Information

Synthesis and duplex-forming ability of oligonucleotides containing 4'-carboxythymidine analogs

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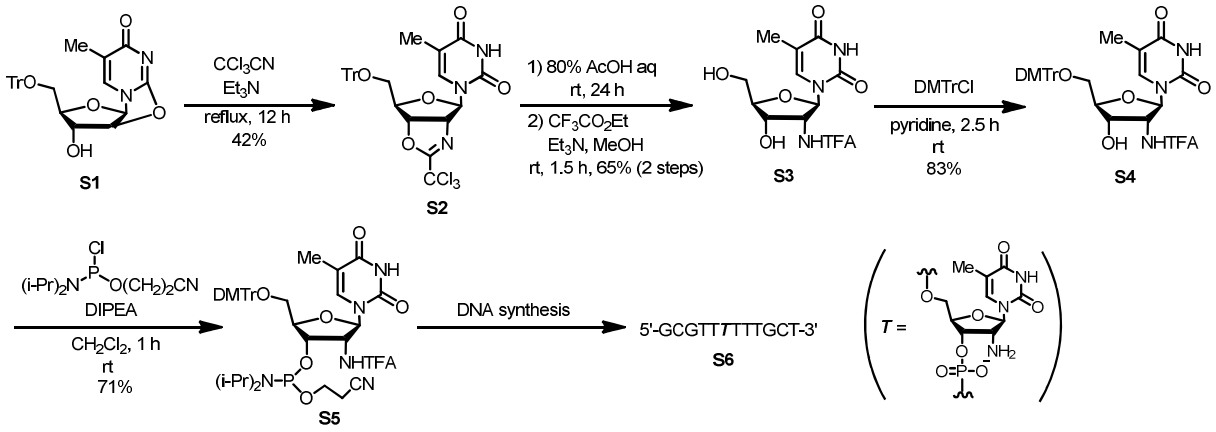


Scheme S1 Synthetic route of oligonucleotide **S6**.

(2'*R*)-2'-Deoxy-2'-*N*,3'-*O*-2-(Trichloromethyl)oxazolino-5'-*O*-trityl-5-methyluridine (S2): Under a nitrogen atmosphere, Et_3N (58 μL, 0.41 mmol) was added to a solution of compound **S1**[1] (1.00 g, 2.07 mmol) in CCl_3CN (4.0 mL) at room temperature. The reaction mixture was refluxed for 12 h. The resulting mixture was concentrated *in vacuo* and the residue (1.60 g) was purified by column chromatography (silica gel, 30 g, *n*-hexane:EtOAc = 3:2) to give compound **S2** (556 mg, 42%) as a white foam. Mp: 104–108 ˚C. $[\alpha]_D^{22}$ +12.2 (c 1.00, $CHCl_3$). IR: ν_{max} (KBr): 3168, 3055, 2927, 1695, 1478, 1448, 1368, 1306, 1263 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.82 (d, *J* = 1.0 Hz, 3H), 3.46 (dd, *J* = 4.0, 10.5 Hz, 1H), 3.59 (dd, *J* = 7.0, 10.5 Hz, 1H), 4.31 (ddd, *J* = 4.0, 4.5, 7.0 Hz, 1H), 5.14 (dd, *J* = 2.5, 8.5 Hz, 1H), 5.38 (dd, *J* = 4.5, 8.5 Hz, 1H), 5.68 (d, *J* = 2.5 Hz, 1H), 7.15–7.46 (m, 16H), 9.58 (brs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.10, 63.91, 85.76, 86.83, 86.86, 86.98, 95.19, 111.43, 127.15, 127.40, 127.80, 127.97, 128.51, 128.61, 138.61, 143.14, 143.37, 149.98, 163.12, 163.93. MS (EI): *m/z* = 625 (M^+, 0.3), 384 (3.1), 382 (3.2), 368 (4.8), 331 (3.1), 366 (5.1), 243 (100). HRMS (EI): Calcd for $C_{31}H_{26}Cl_3N_3O_5$ [M^+], 625.0938, found, 625.0963.

(2'*R*)-2'-Trifluoroacetamidothymidine (S3): A solution of compound **S2** (507 mg, 0.809 mmol) in 80% AcOH (10 mL) was stirred for 24 h at room temperature. The resulting mixture was concentrated in vacuo. The obtained residue (515 mg) was dissolved in anhydrous MeOH (10 mL), then Et_3N (56 μL, 0.40 mmol) and CF_3CO_2Et (70 μL, 0.89 mmol) were added to this solution at 0 ˚C. The reaction mixture was stirred for 1.5 h at room temperature. The resulting mixture was concentrated *in vacuo* and the residue (540 mg) was purified by column chromatography (silica gel, 15 g, $CHCl_3$:MeOH = 15:1 to 5:1) to give compound **S3** (185 mg, 65%, 2 steps) as a white powder. Mp: 172–177 ˚C. $[\alpha]_D^{21}$ +1.1 (c 1.00, MeOH). IR: ν_{max} (KBr): 3494, 3250, 2989, 2699, 2506, 1682, 1474, 1428, 1270, 1205 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ: 1.86 (d, *J* = 1.0 Hz, 3H), 3.79 (dd, *J* = 4.0, 12.0 Hz, 1H), 3.85 (dd, *J* = 3.0, 12.0 Hz, 1H), 4.11–4.12 (m, 1H), 4.37 (dd, *J* = 2.5, 6.0 Hz, 1H), 4.64 (dd, *J* = 6.0, 8.0 Hz, 1H), 6.13 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 1.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.56, 57.34, 62.93, 87.70, 88.82, 112.00, 117.67 (q, *J* = 290 Hz), 137.85, 152.65, 162.10 (q, *J* = 39 Hz), 166.06. MS (EI): *m/z* = 353 (M^+, 2.9), 264 (2.1), 240 (10.1), 228 (30.6), 203

(10.1), 180 (86.4), 168 (21.6), 152 (31.9), 138 (15.3), 126 (55.5), 110 (24.8), 95 (19.8), 84 (38.1), 69 (100). HRMS (EI): Calcd for $C_{12}H_{14}F_3N_3O_6$ [M^+], 353.0835, found, 353.0824.

(2'*R*)-5'-*O*-(4,4'-Dimethoxytrityl)-2'-trifluoroacetamidothymidine (S4): Under a nitrogen atmosphere, DMTrCl (253 mg, 0.747 mmol) was added to a solution of compound **S3** (132 mg, 0.374 mmol) in anhydrous pyridine (2.0 mL) at 0 ˚C. The reaction mixture was stirred at room temperature for 2.5 h. The reaction was quenched with sat. $NaHCO_3$ at 0 ˚C, and the product was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue (422 mg) was purified by column chromatography (silica gel, 15 g, $CHCl_3$:MeOH = 30:1) to give compound **S4** (202 mg, 83%) as a white powder. Mp: 242–243 ˚C. $[\alpha]_D^{22}$ +7.8 (c 1.00, MeOH). IR: ν_{max} (KBr): 3444, 3263, 3062, 2932, 2838, 1728, 1702, 1607, 1508, 1469, 1382, 1302, 1252 cm^{-1}. ^1H NMR (300 MHz, CD_3OD) δ: 1.32 (s, 3H), 3.41–3.44 (m, 2H), 3.80 (s, 6H), 4.21 (m, 1H), 4.46 (dd, *J* = 5.5, 6.0 Hz, 1H), 4.97 (dd, *J* = 5.5, 8.5 Hz, 1H), 6.29 (d, *J* = 8.5 Hz, 1H), 6.87–7.57 (m, 13H), 8.31 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, CD_3OD) δ: 10.64, 54.45, 55.46, 63.22, 70.56, 84.50, 85.39, 86.83, 111.28, 112.71, 115.27 (q, *J* = 286 Hz), 120.84, 126.66, 127.48, 127.70, 129.64, 134.48, 134.72, 135.27, 136.60, 143.44, 151.05, 158.28, 157.82 (q, *J* = 37 Hz), 164.25. MS (EI): *m/z* = 655 (M^+, 7.8), 336 (2.1), 303 (100), 288 (8.8), 228 (16.5), 180 (27.3). HRMS (EI): Calcd for $C_{33}H_{32}F_3N_3O_8$ [M^+], 655.2141, found, 655.2133.

(2'*R*)-3'-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5'-*O*-(4,4'-dimethoxytrityl)-2'-trifluoroacetamidothymidine (S5): Under a nitrogen atmosphere, DIPEA (0.28 mL, 1.6 mmol) and *i*-$Pr_2NP(Cl)OCH_2CH_2CN$ (81 μL, 0.37 mmol) were added to a solution of compound **S4** (202 mg, 0.310 mmol) in anhydrous CH_2Cl_2 (2.0 mL) at 0 ˚C. The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with sat. $NaHCO_3$ at 0 ˚C, and the product was extracted with CH_2Cl_2. The combined organic layer was washed with sat. $NaHCO_3$, water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue (280 mg) was chromatographed (silica gel, 15 g, $CHCl_3$:MeOH = 30:1) to give **S5** with a small amount of impurity (201 mg), which was reprecipitated to give compound **S5** (188 mg, 71%) as a white powder. Mp: 118–123 ˚C. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.05–1.19 (m, 12H), 1.32 (s, 1.5H), 1.34 (s, 1.5H), 2.31–2.62 (m, 2H), 3.34–3.97 (m, 12H), 4.27 (m, 0.5H), 4.45–4.50 (m, 1H), 4.66 (dd, *J* = 5.5, 9.5 Hz, 0.5H), 4.94–5.11 (m, 1H), 6.39 (d, *J* = 9.5 Hz, 0.5H), 6.42 (d, *J* = 9.5 Hz, 0.5H), 6.85–7.44 (m, 13H), 7.62 (s, 0.5H), 7.68 (s, 0.5H), 7.84 (d, *J* = 8.5 Hz, 0.5H), 7.95 (d, *J* = 8.0 Hz, 0.5H), 9.67 (brs, 1H). ^{31}P NMR (161 MHz, $CDCl_3$) δ: 150.32, 151.67. MS (FAB): *m/z* = 856 $[M+H]^+$. HRMS (FAB): Calcd for $C_{42}H_{50}F_3N_5O_9P$ $[M+H]^+$, 856.3298, found, 856.3301.

Synthesis of oligonucleotide S6: Phosphoramidite **S5** was used and the 0.2 μmol scale synthesis of oligonucleotides was performed on an automated DNA synthesizer (Applied Biosystems Expedite™ 8909) using a standard phosphoramidite protocol (DMTr-ON mode). After treatment with 28% NH_3 aq., rt, 1.5 h then 55 ˚C, 12 h, removal of ammonia was carried out *in vacuo*. The

crude **S6** was purified with Sep-Pak® Plus C18 cartridges (Waters) followed by reversed-phase HPLC (Waters XBridge® MS C_{18} 2.5 μm, 10 x 50 mm). The composition of **S6** was confirmed by MALDI-TOF mass analysis. Yield and MALDI-TOF-MS data ($[M-H]^-$) for **S6**; 26% yield, found 3646.68 (calcd 3647.38).

References

(1) J. F. Codington, I. L. Doerr and J. J. Fox, *J. Org. Chem.*, **1964**, 29, 558; I. A. Mikhailopulo, G. V. Zaitseva, E. V. Vaaks, J. Balzarini, E. De Clercq, H. Rosemeyer and F. Seela, *Liebigs Ann. Chem.*, **1993**, 513.

ON1a

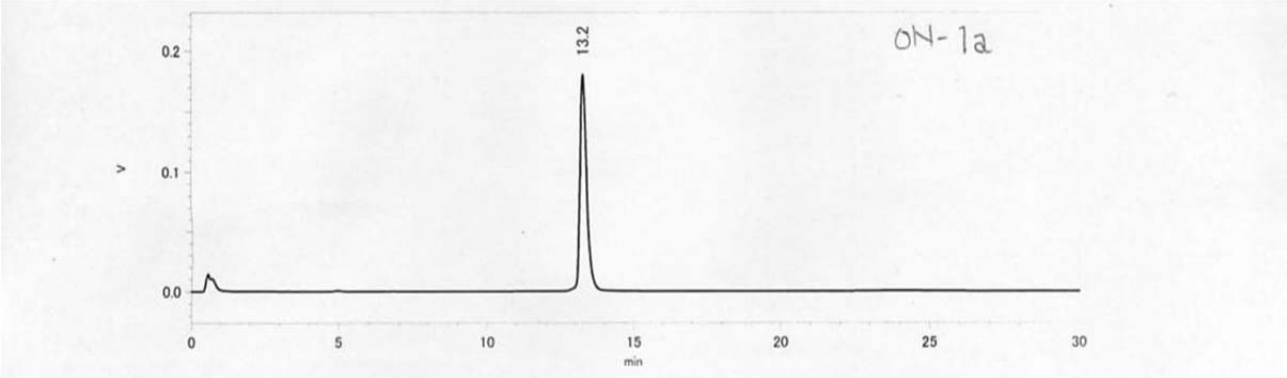
HPLC

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

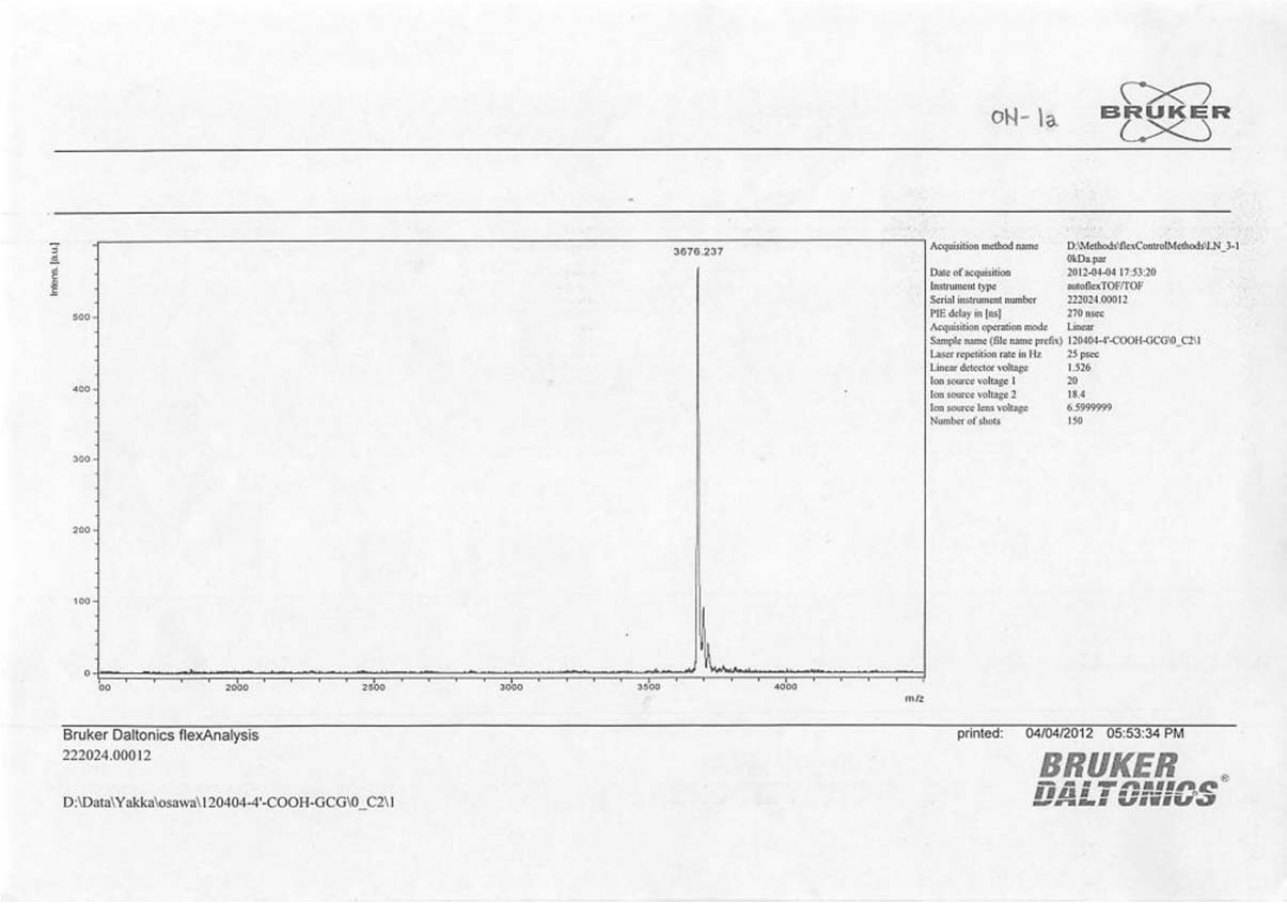
Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



ON1b

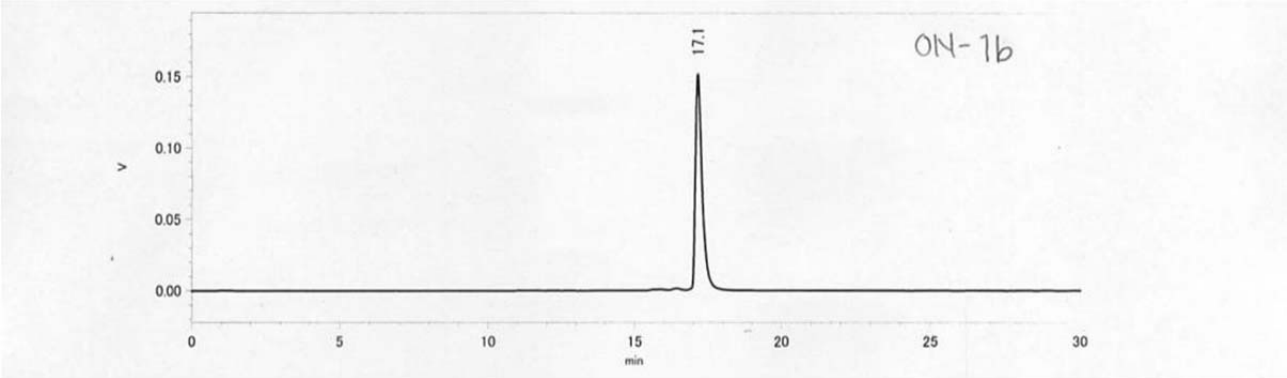
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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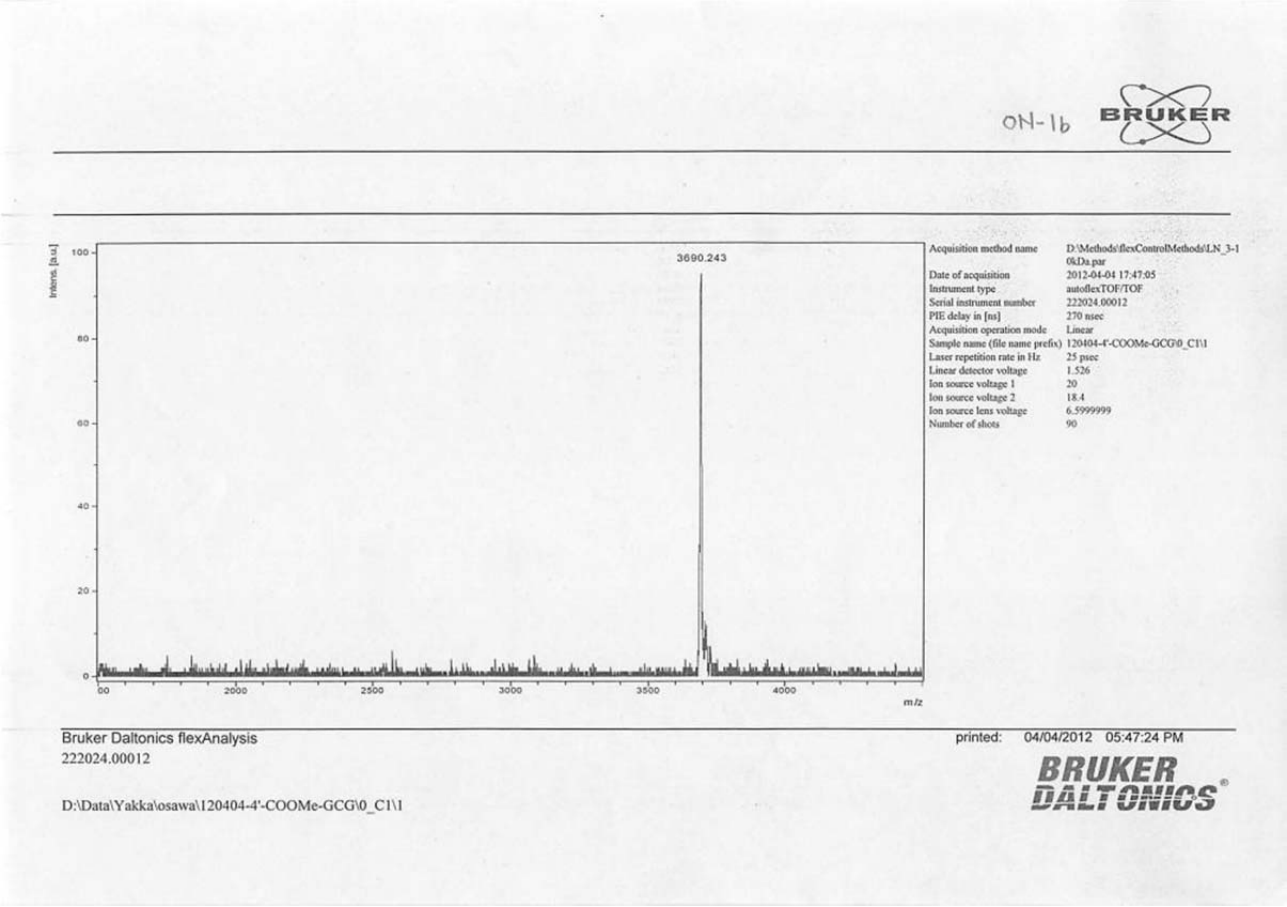


ON1c

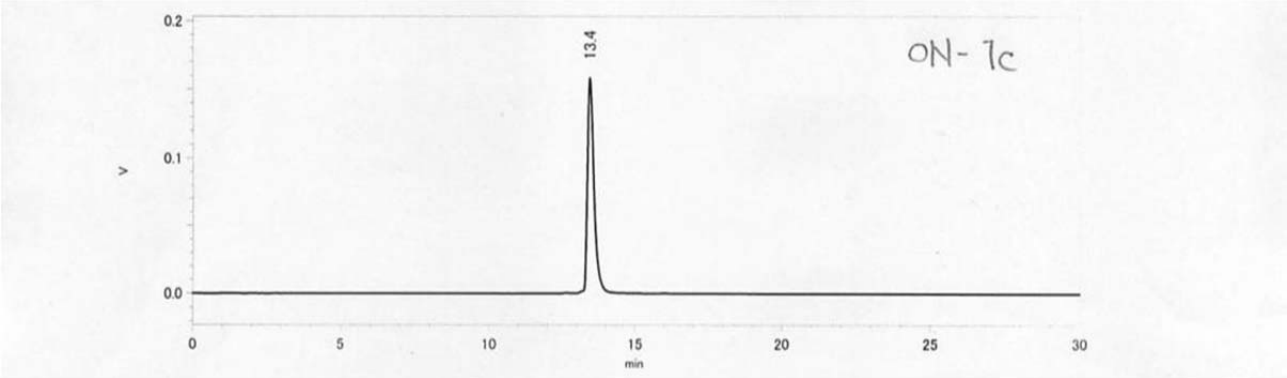
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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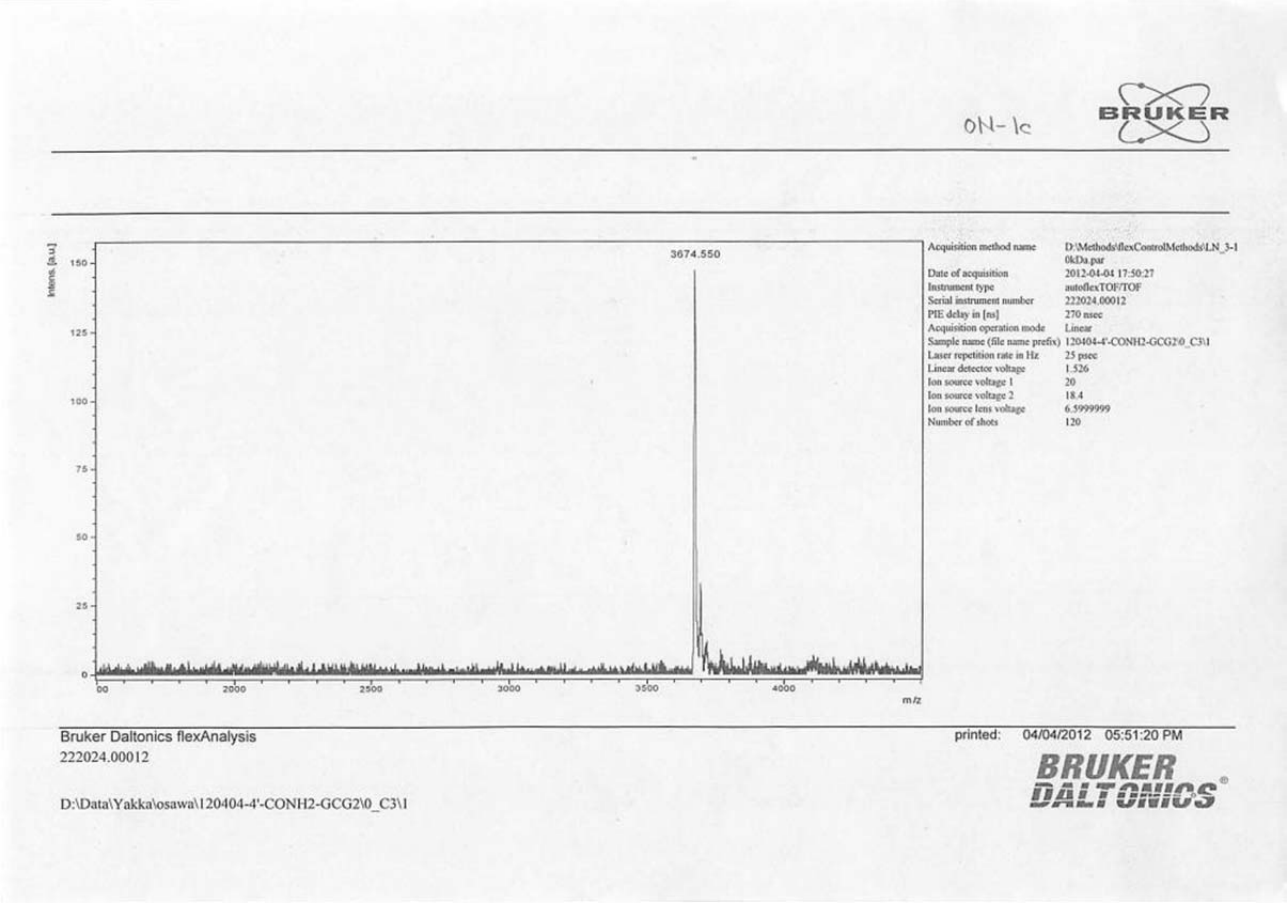


ON1d

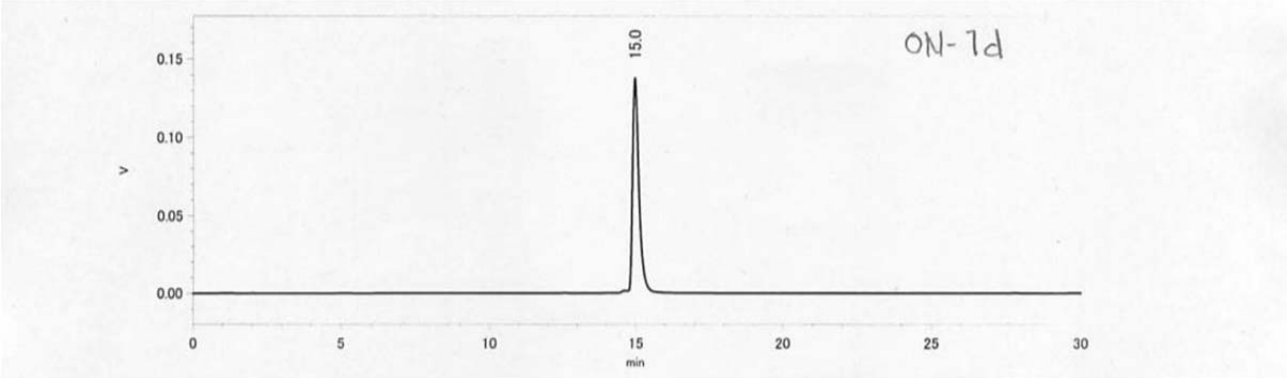
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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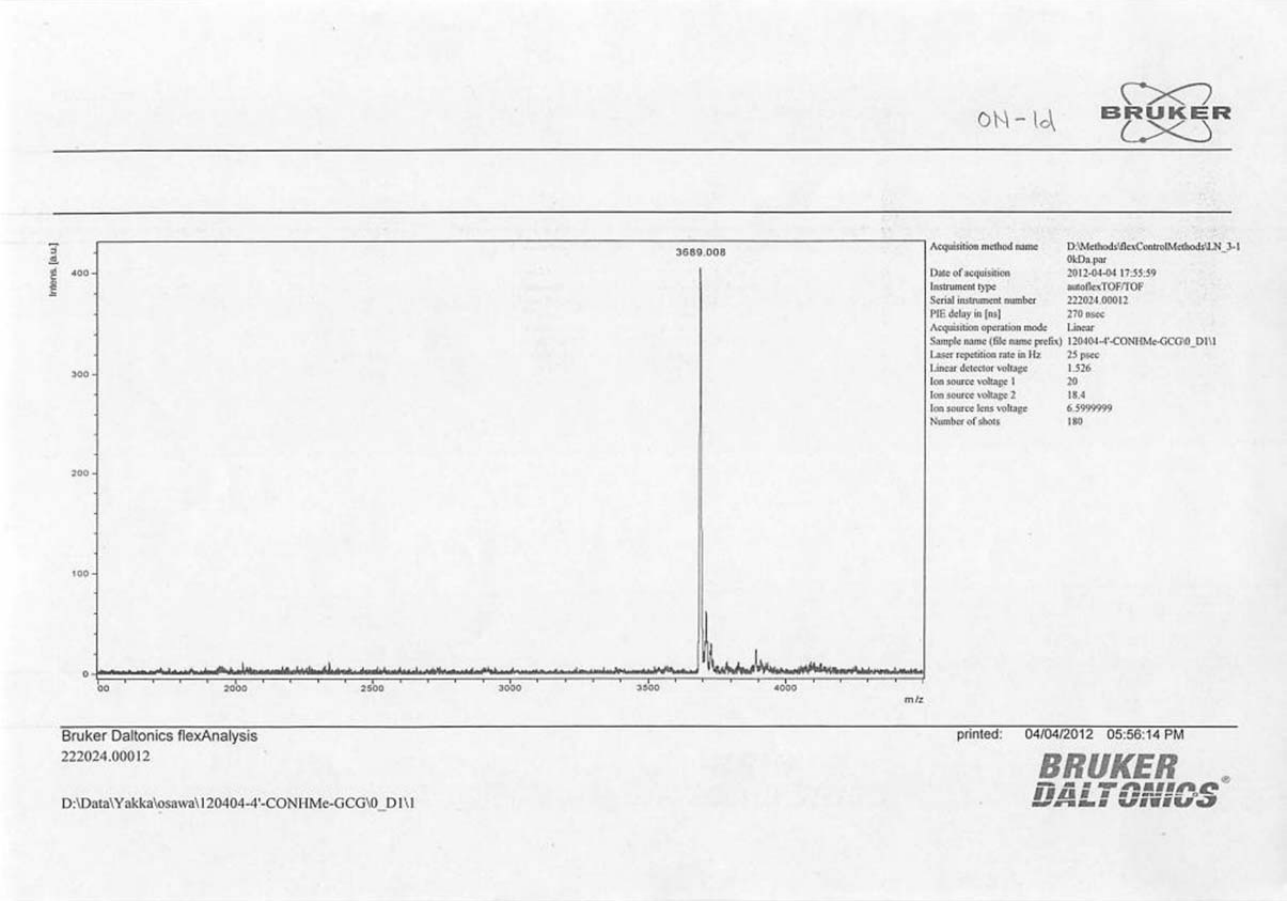


ON2a

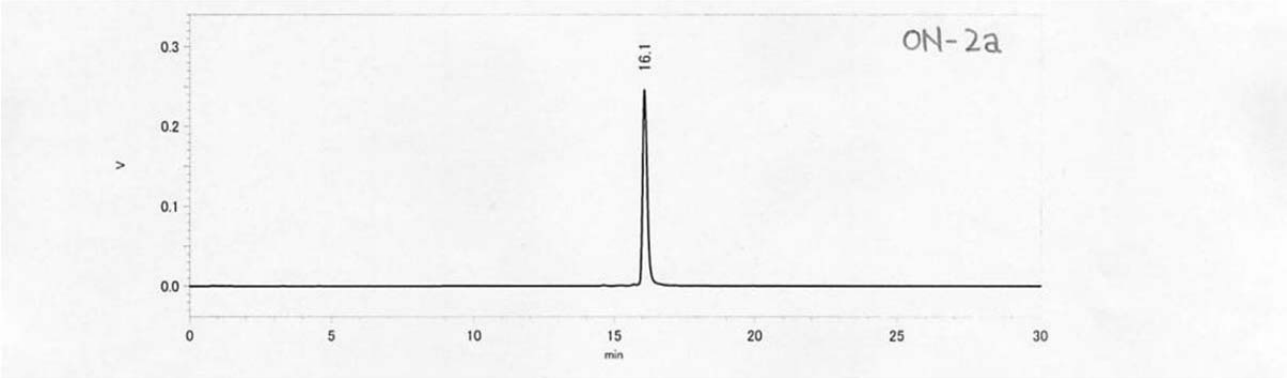
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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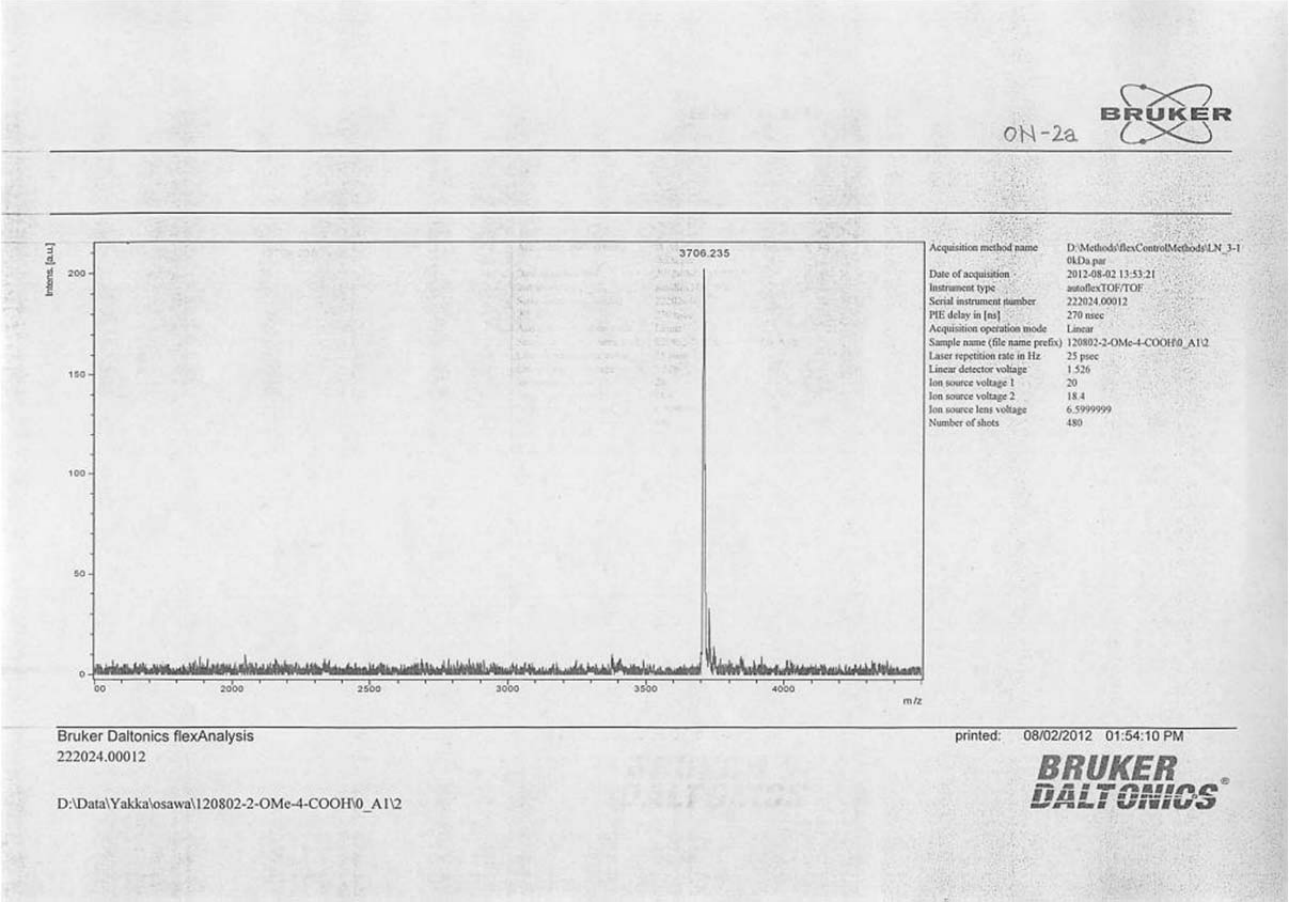


ON2b

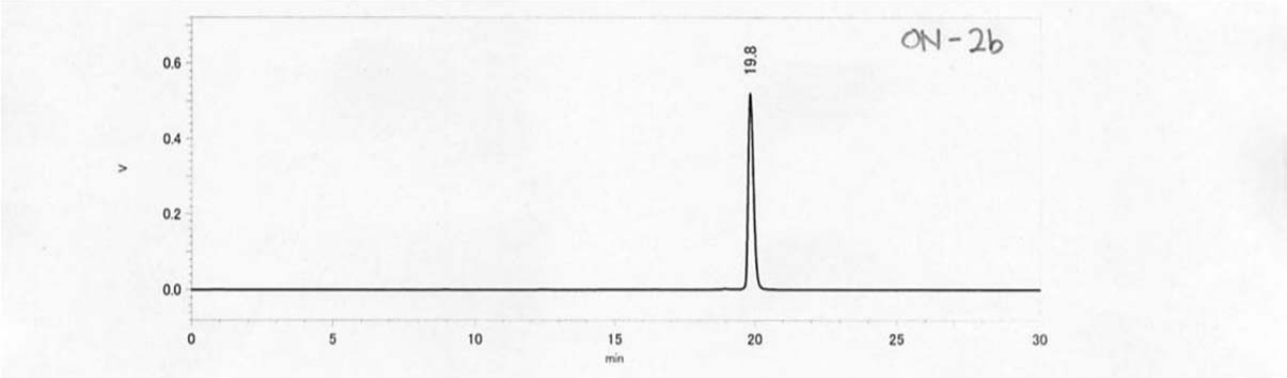
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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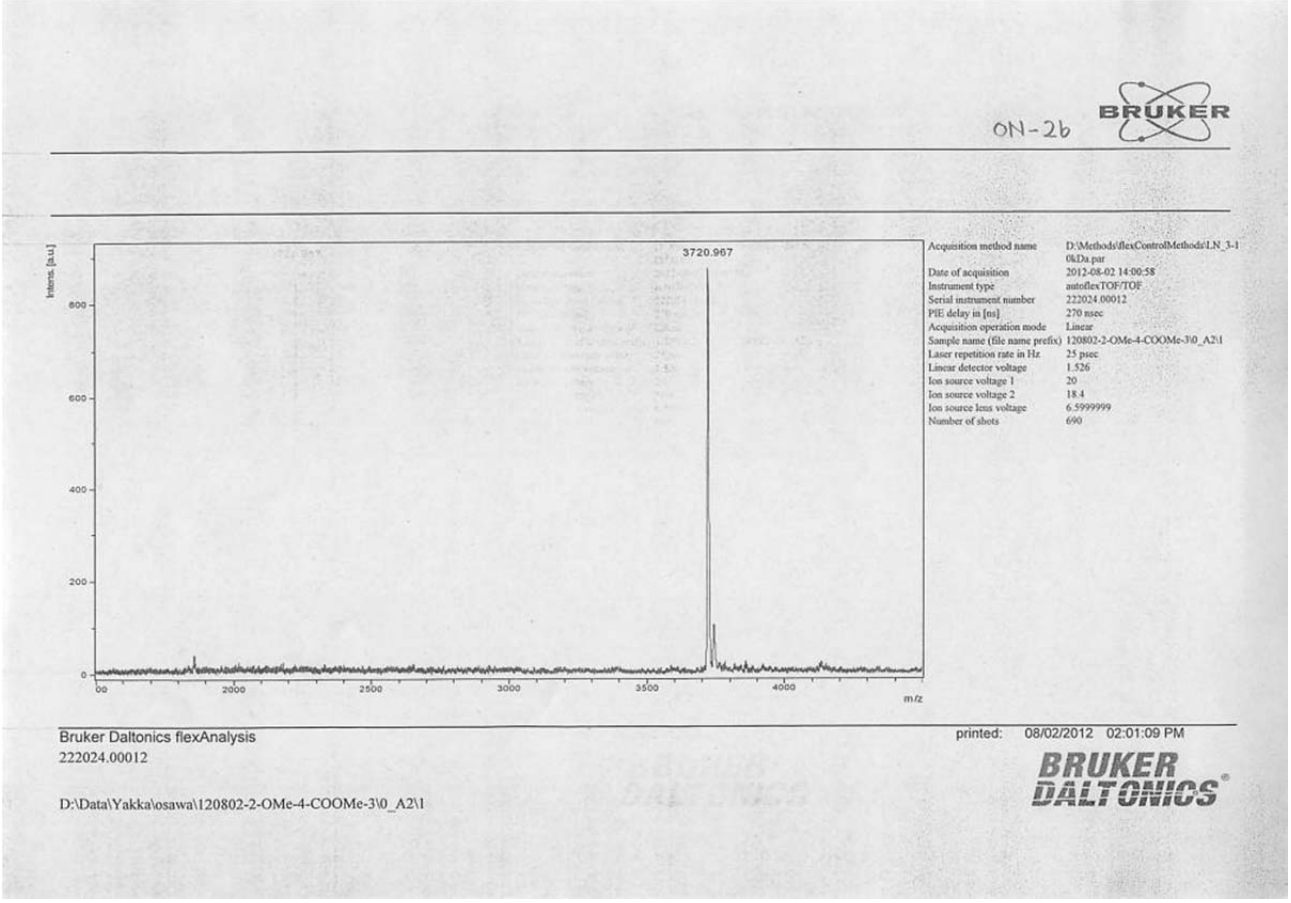


ON2c

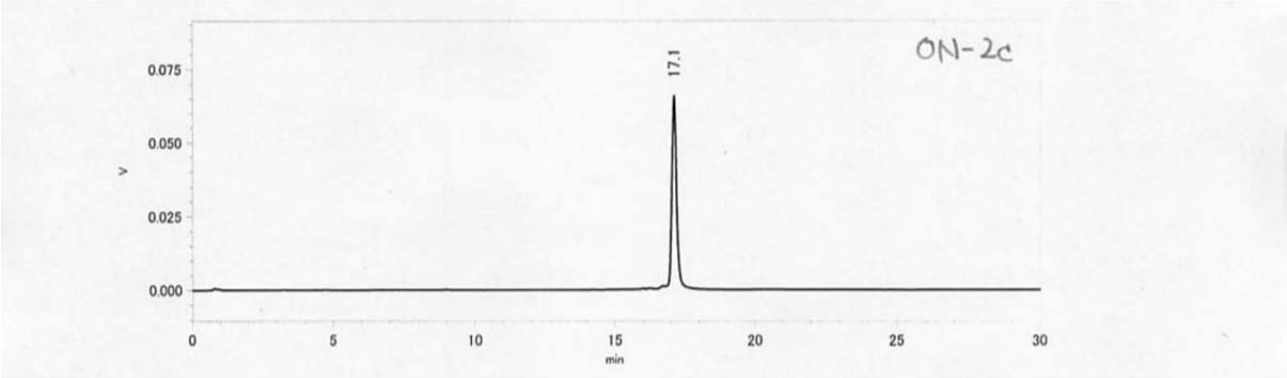
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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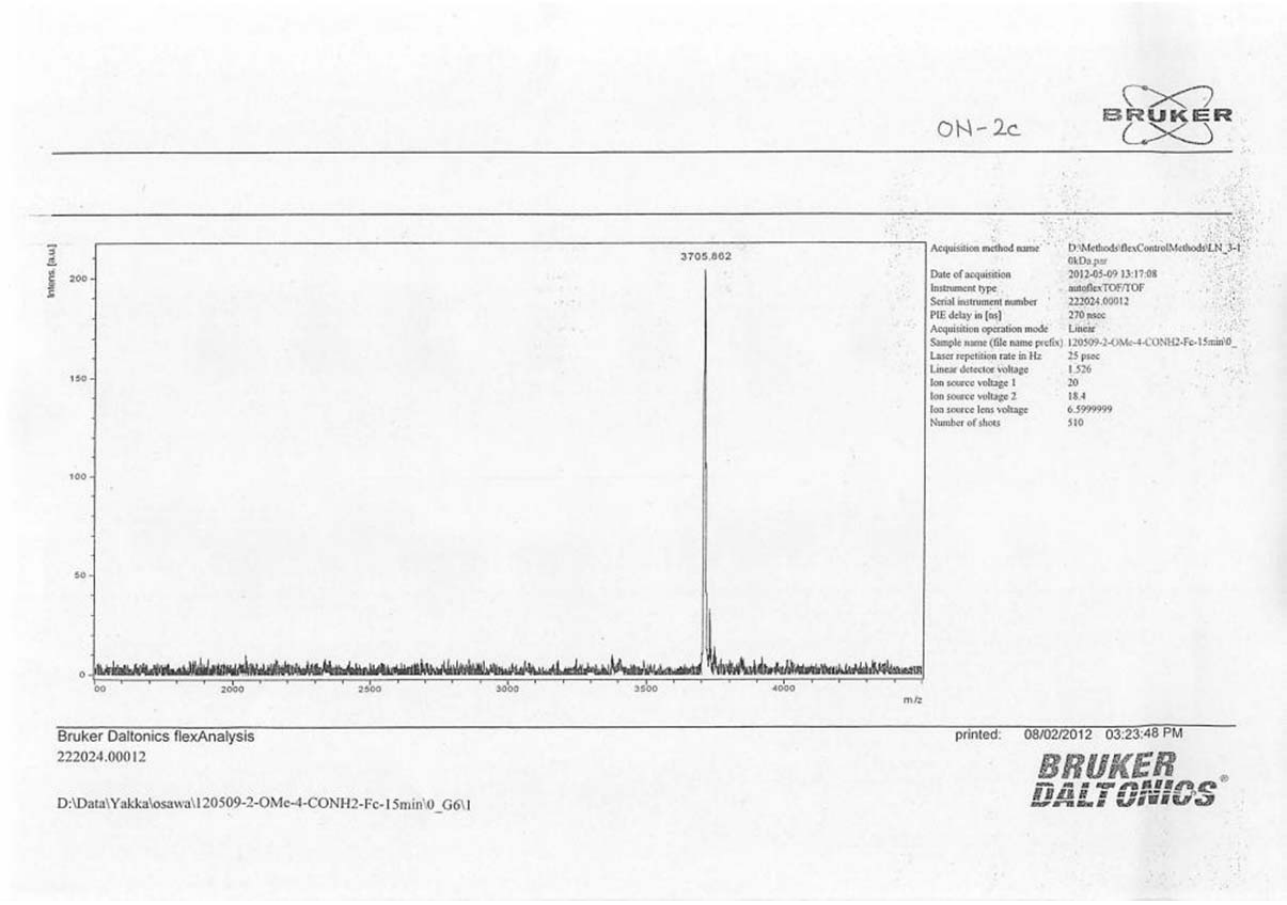


ON2d

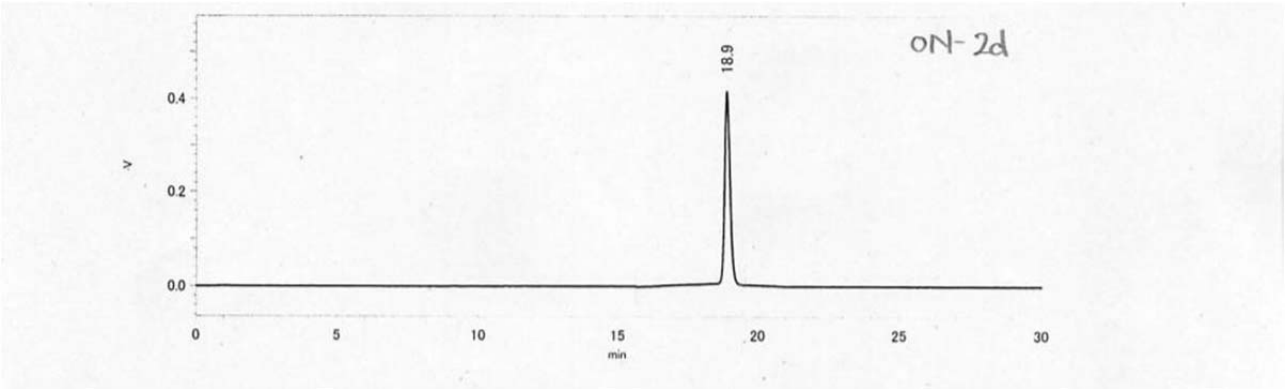
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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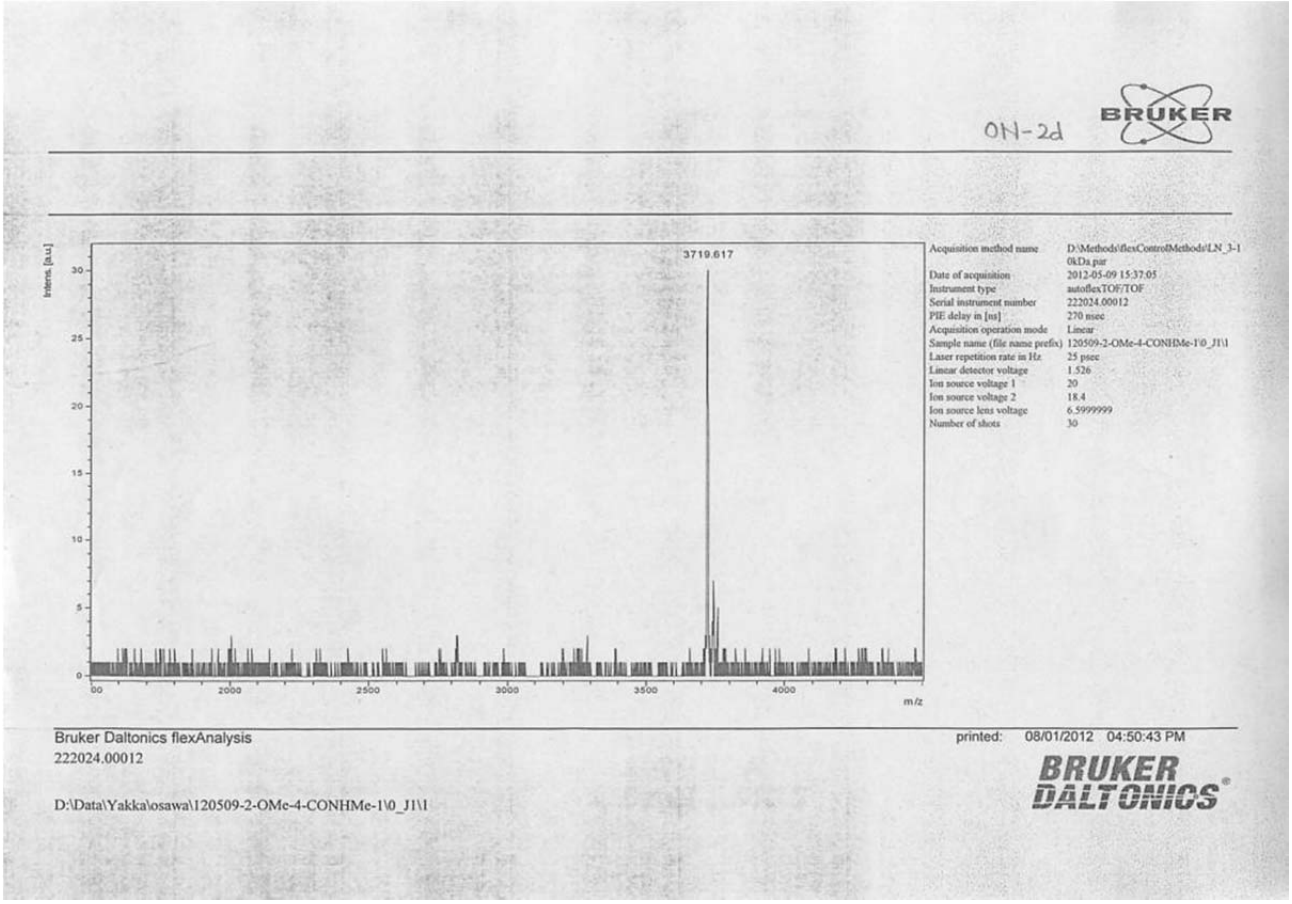


ON3a

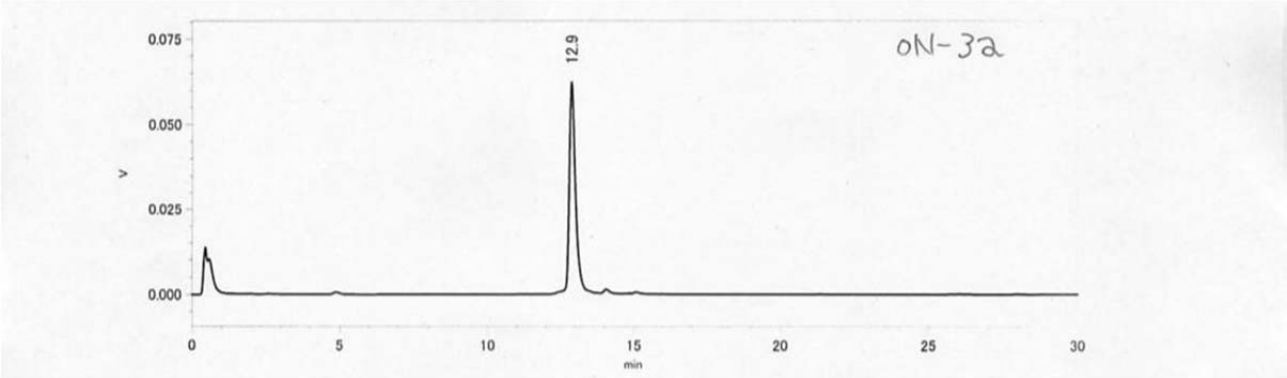
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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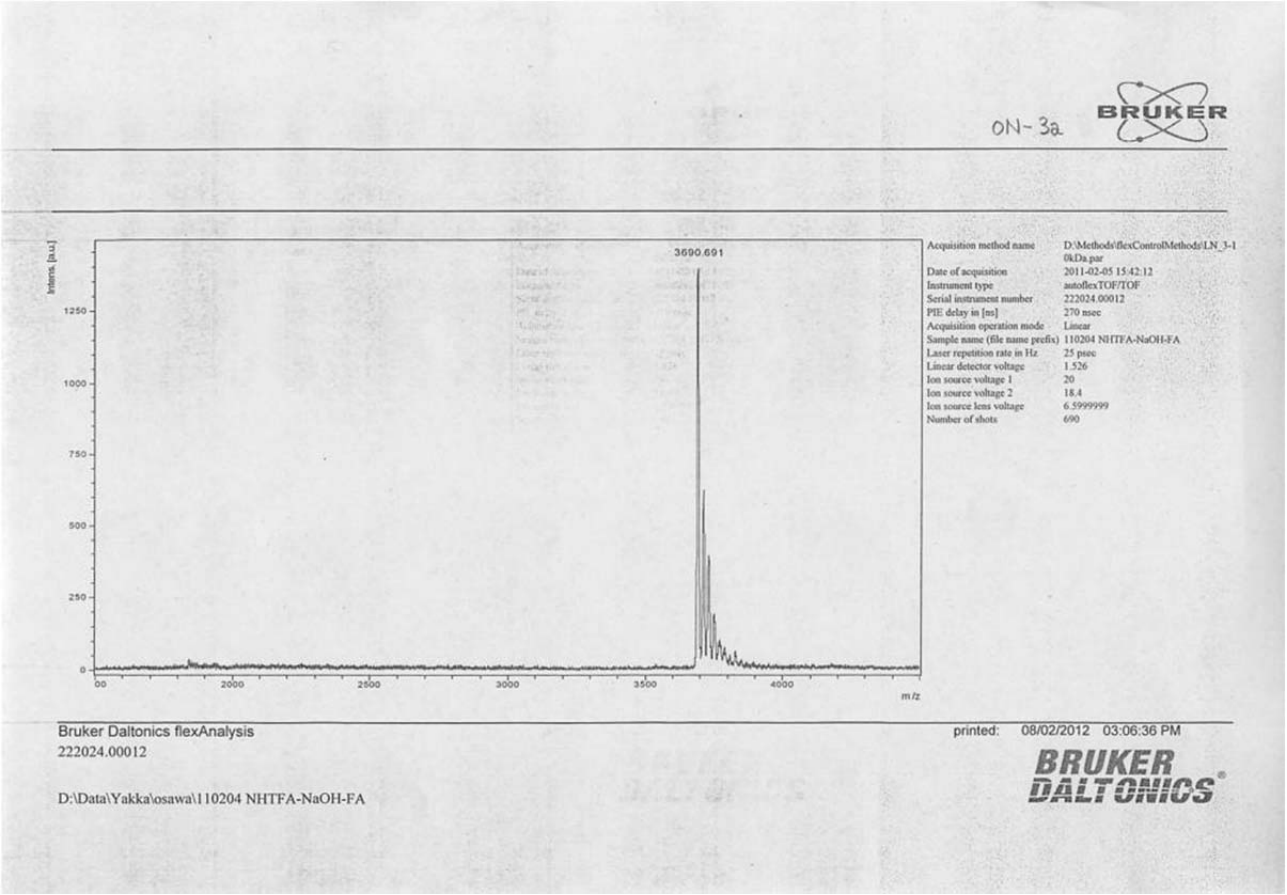


ON3b

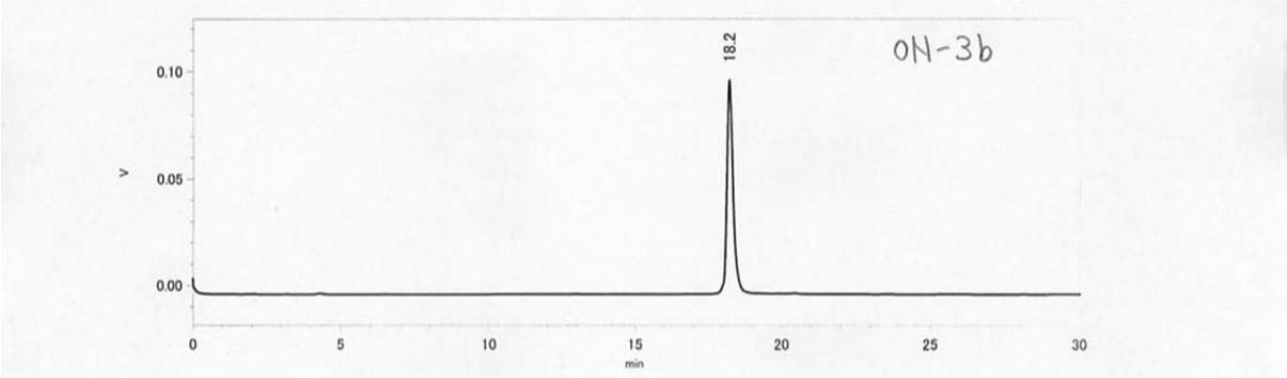
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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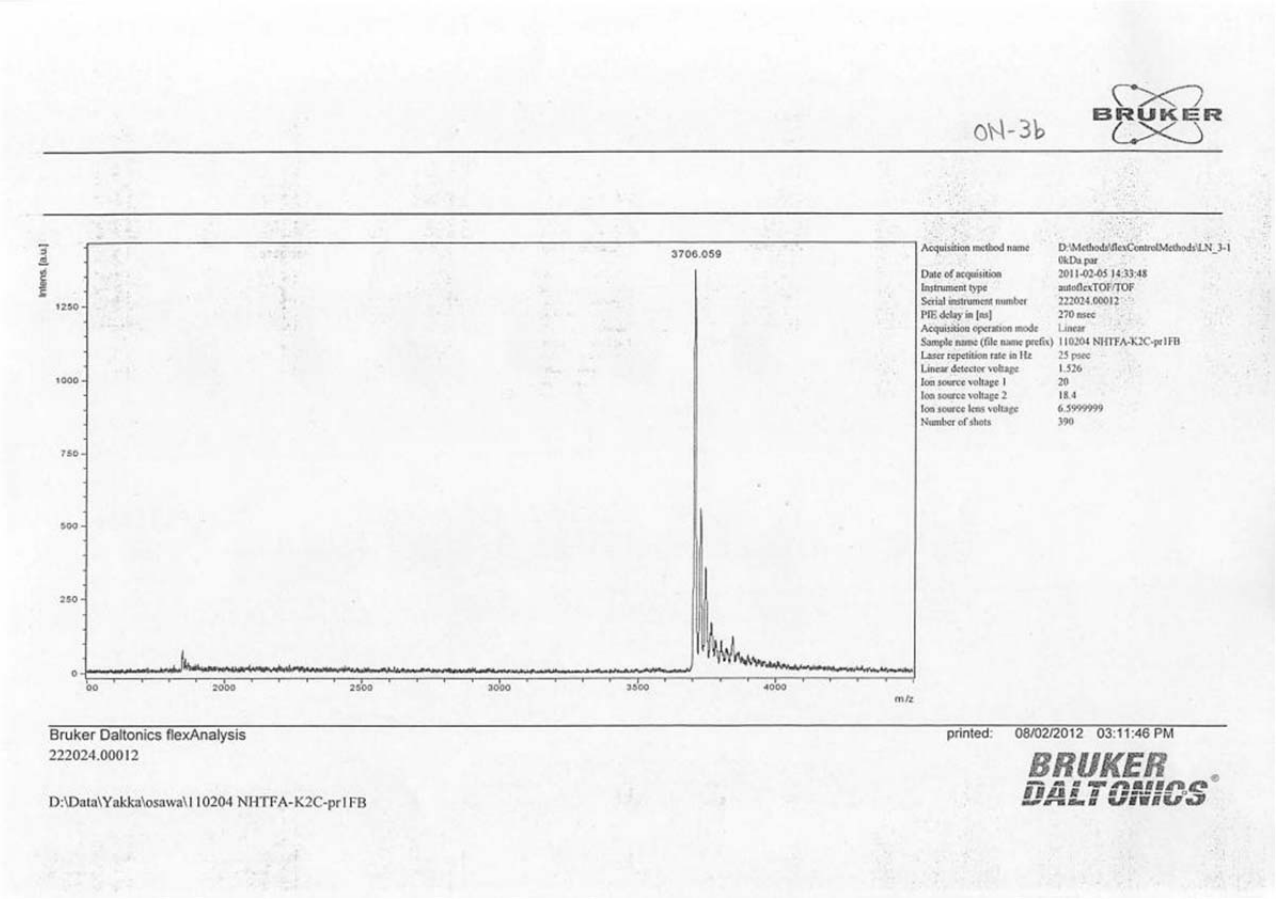


ON3c

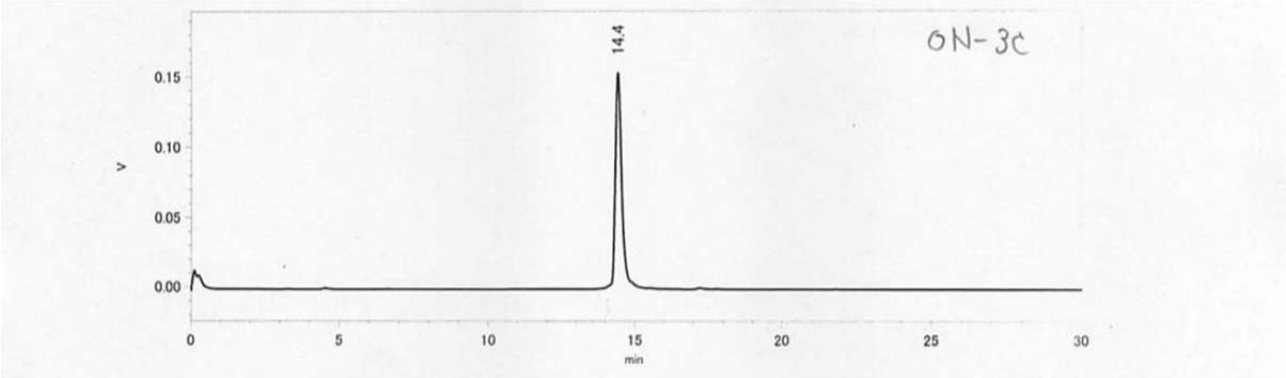
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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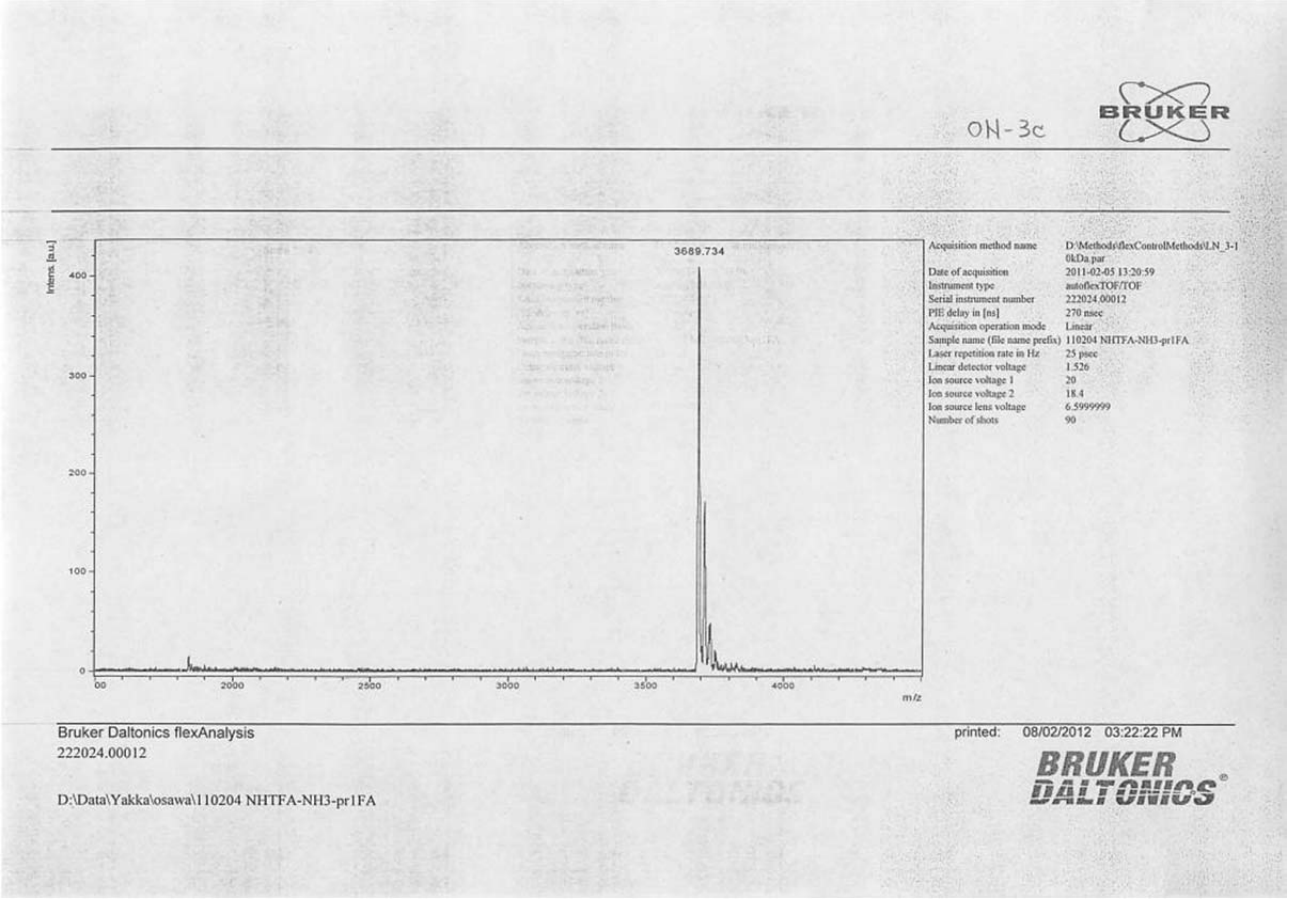


ON3d

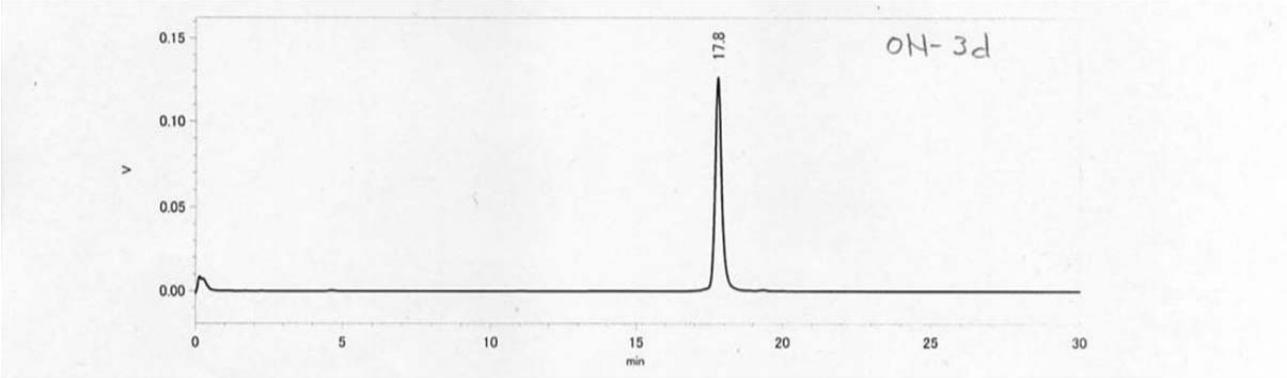
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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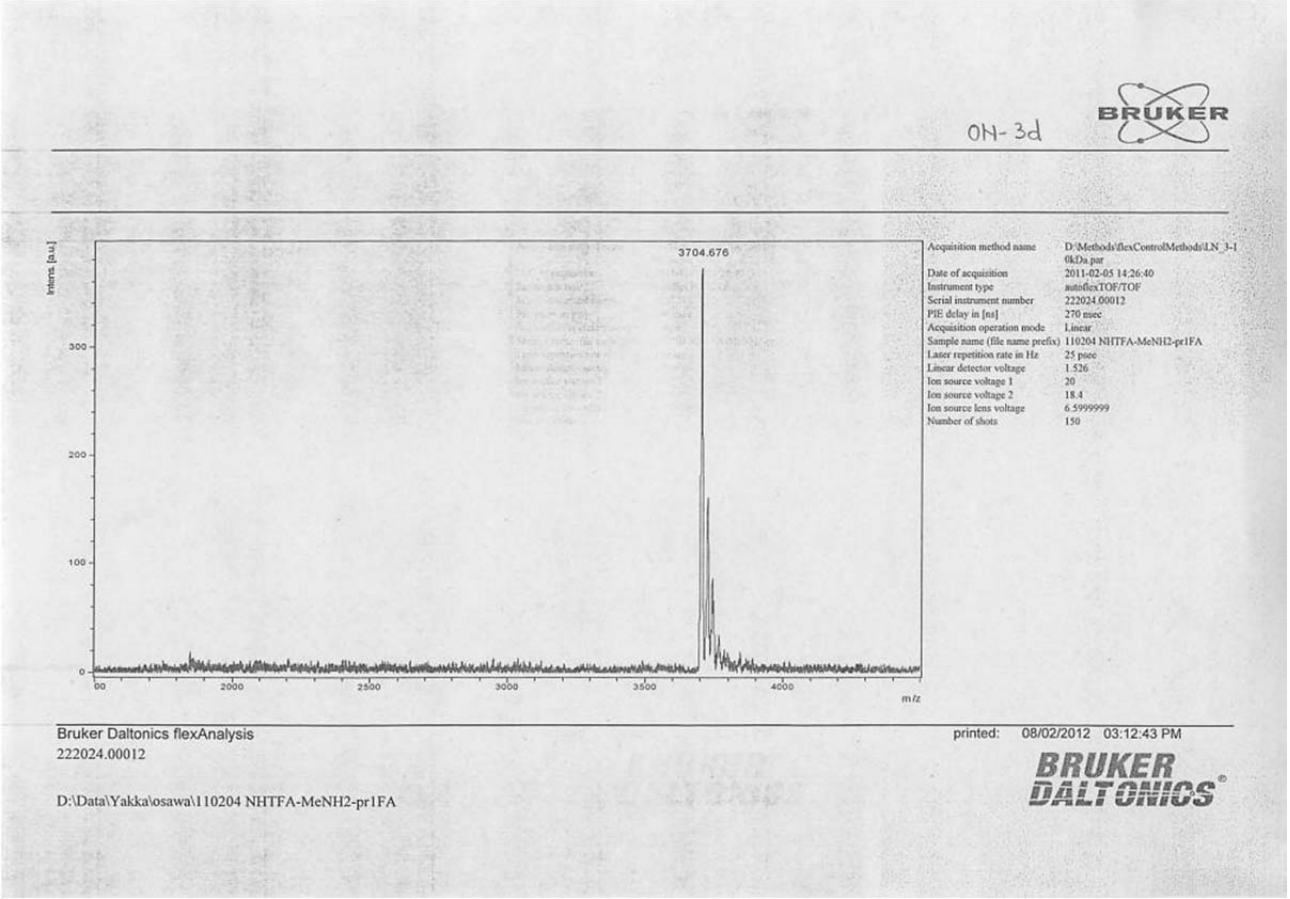


ON4a

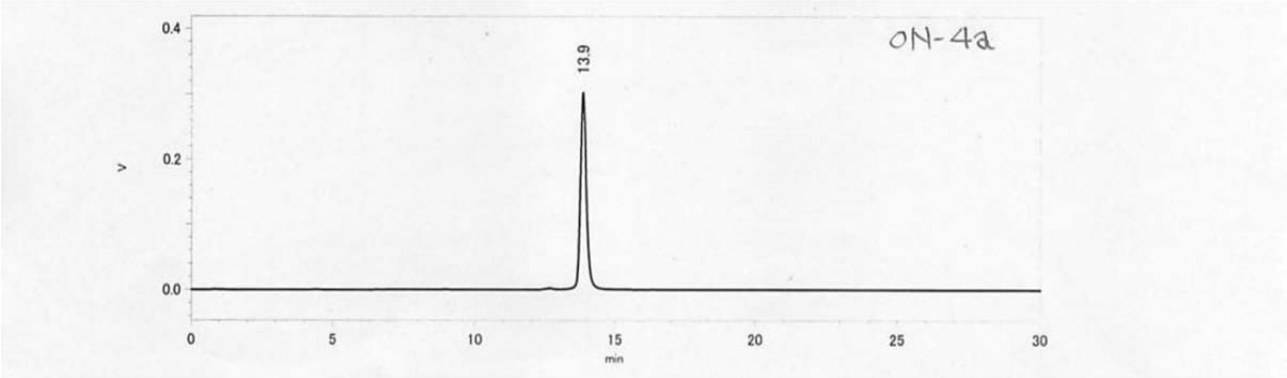
HPLC

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

Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



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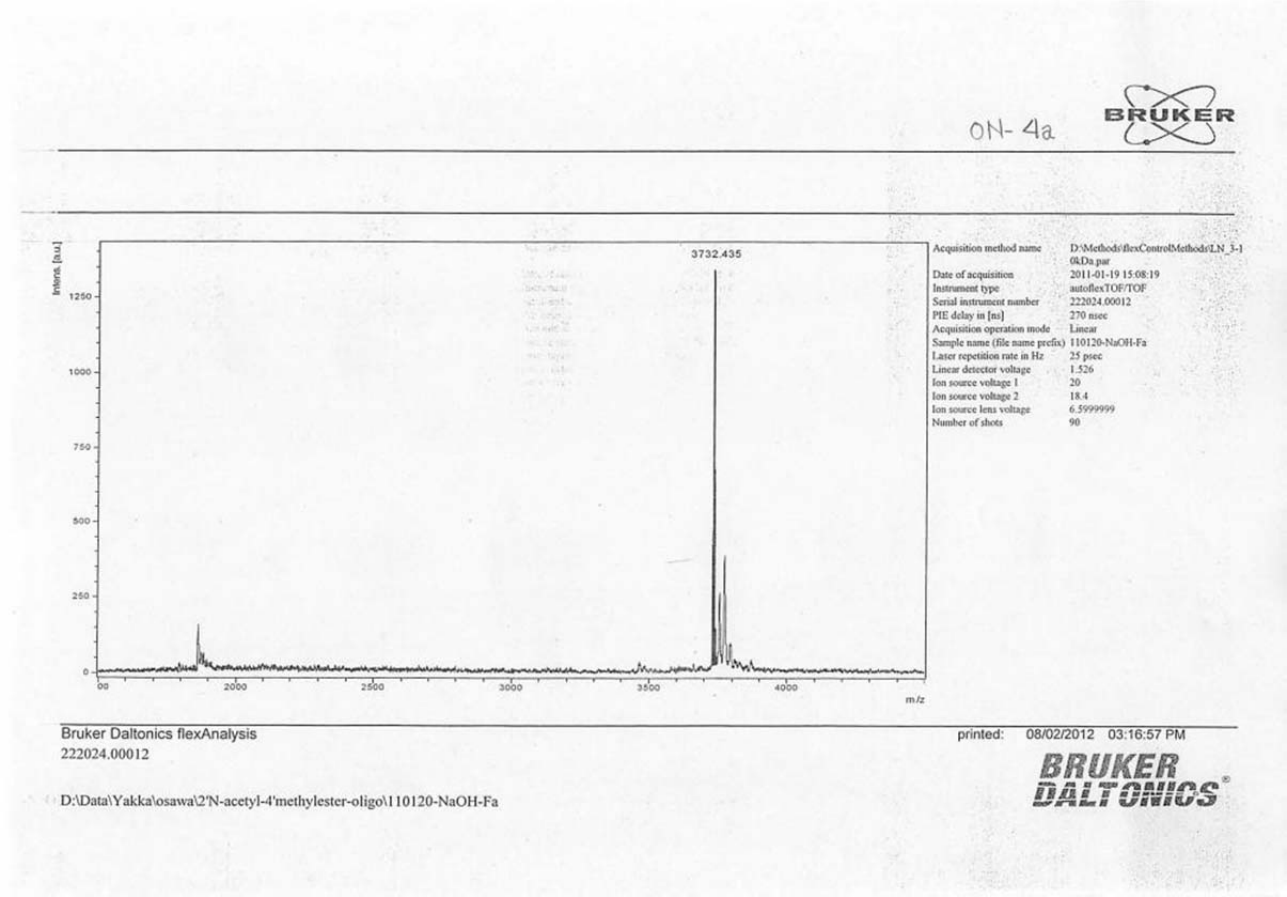


ON4b

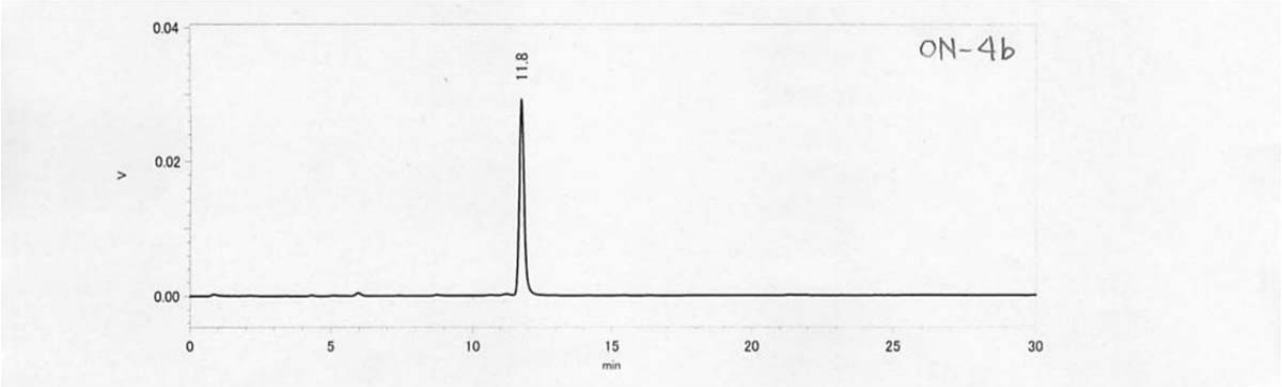
HPLC

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

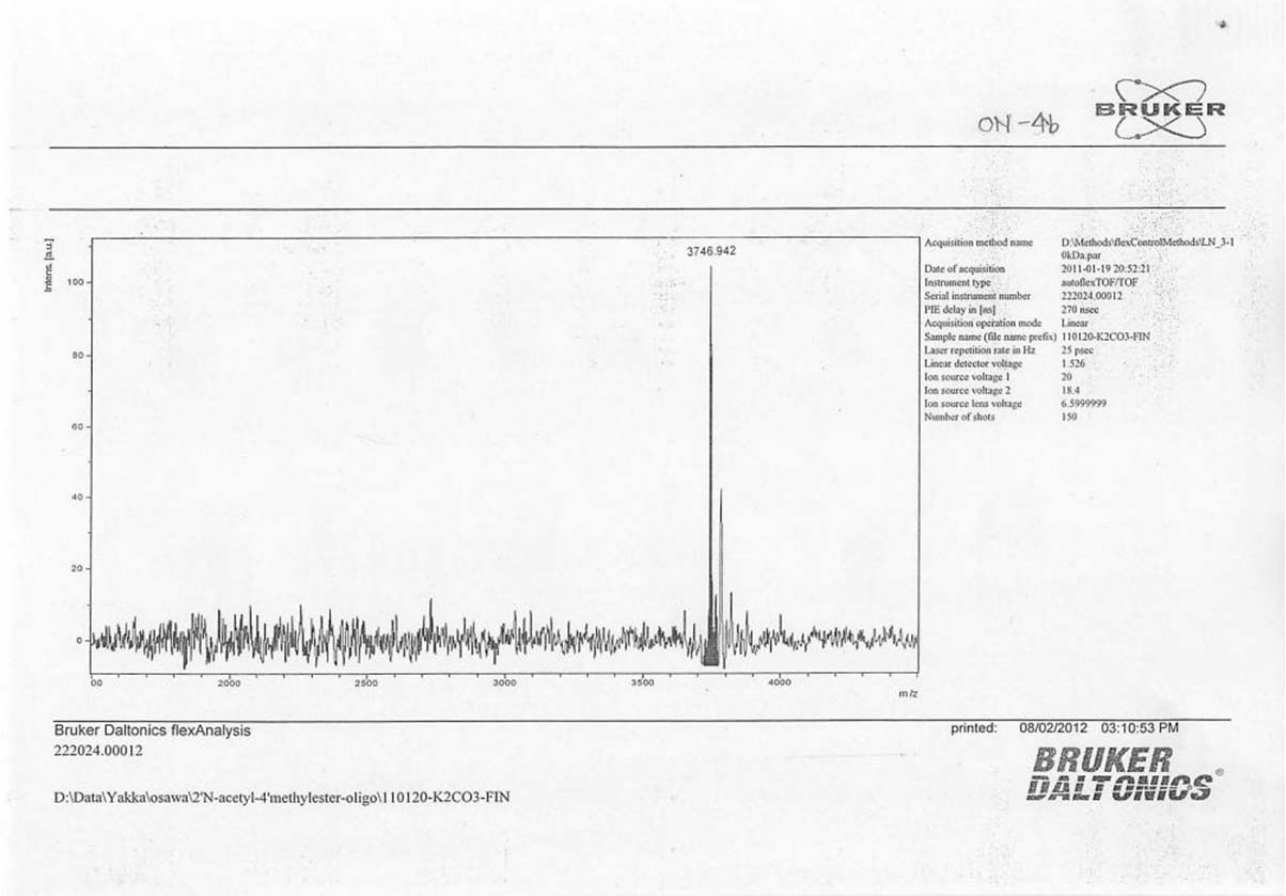
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



ON4c

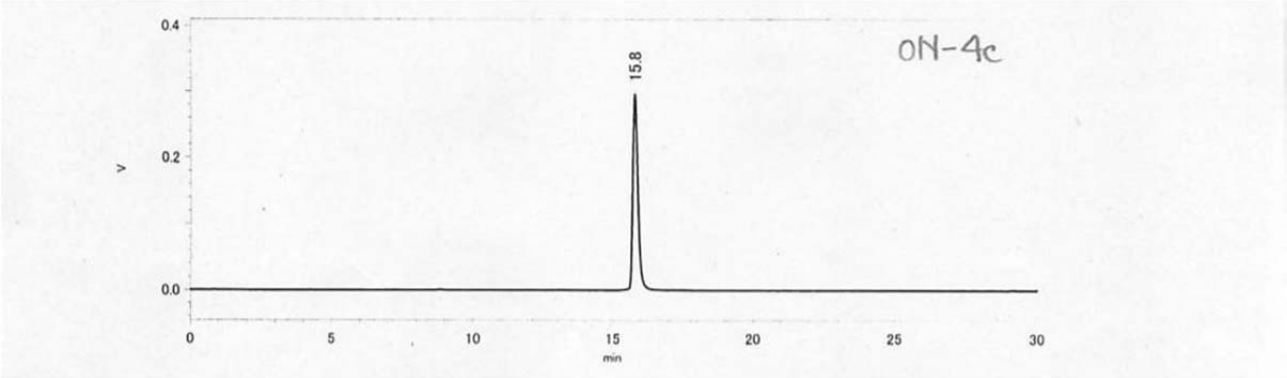
HPLC

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

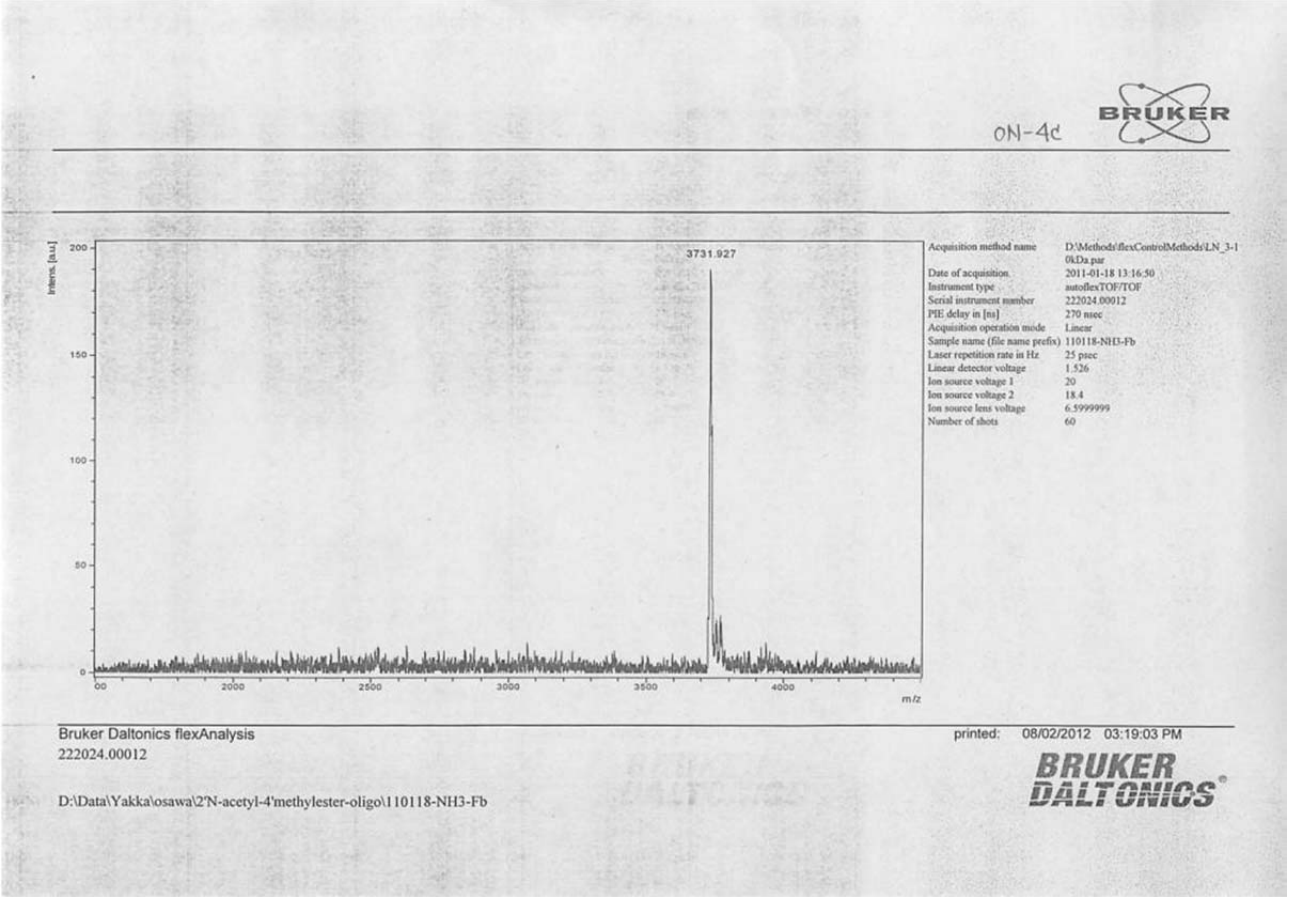
Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



ON4d

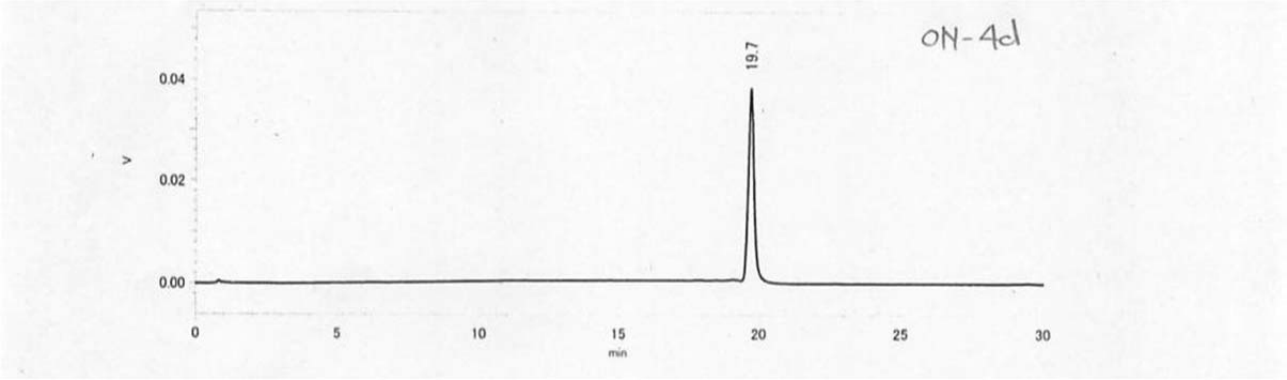
HPLC

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

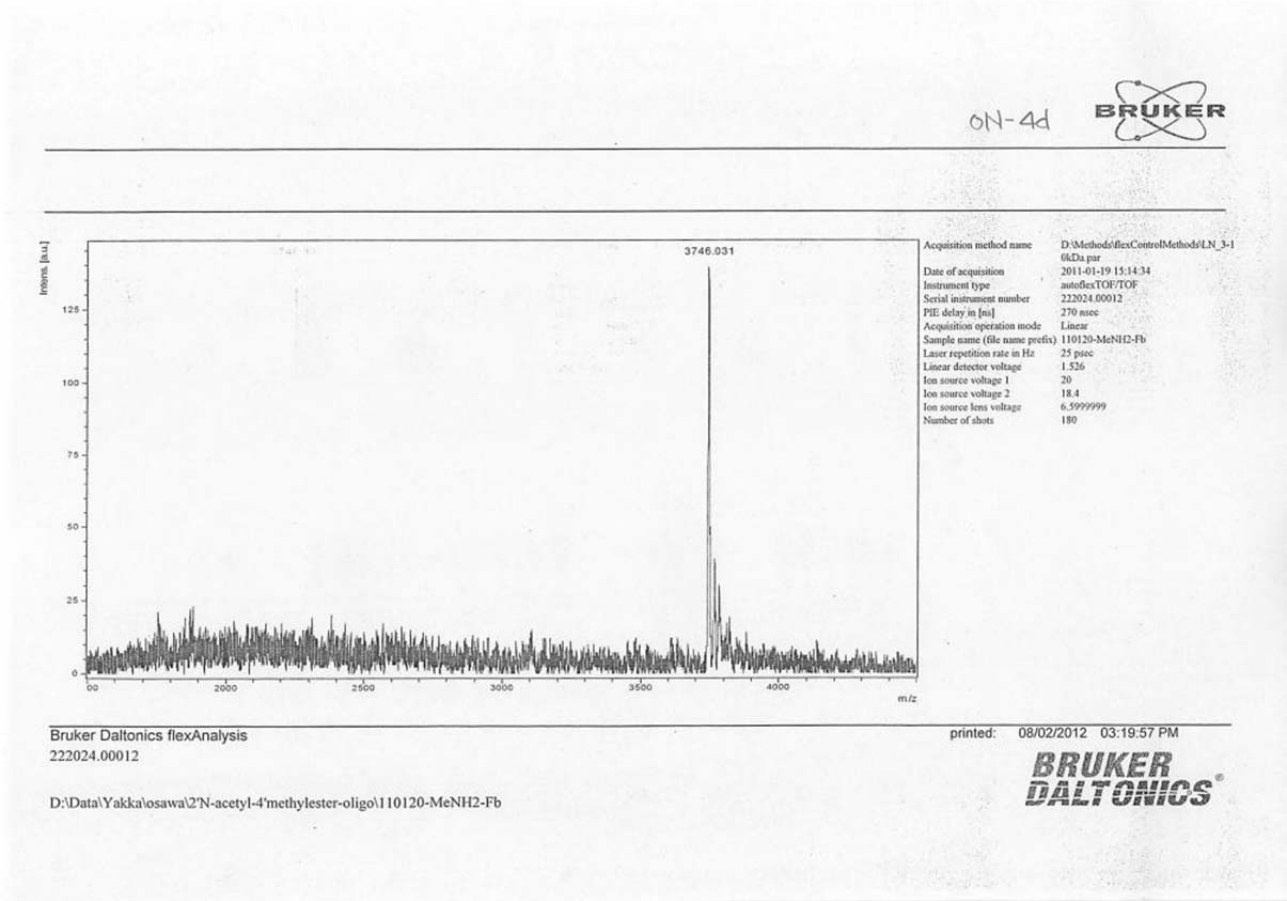
Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



ON5a

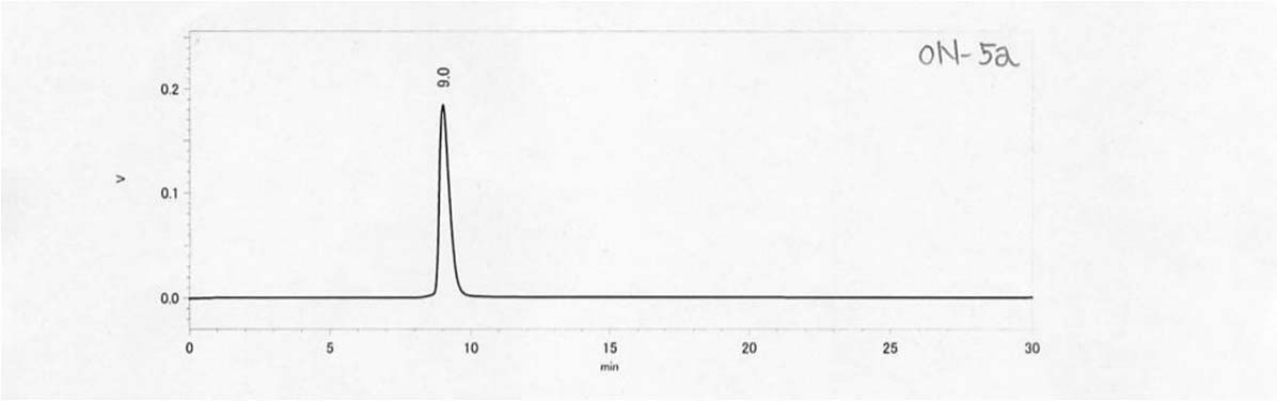
HPLC

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

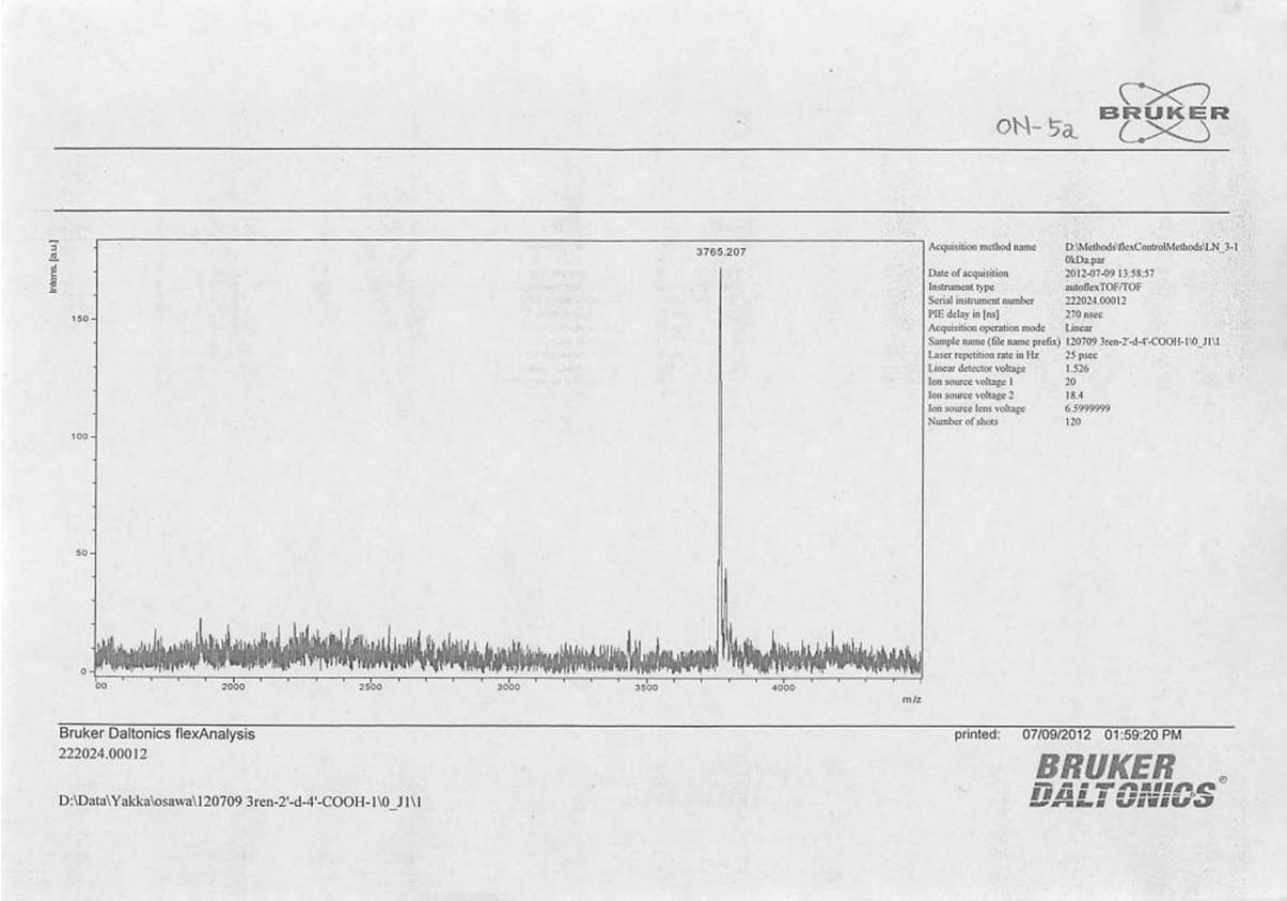
Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



ON6a

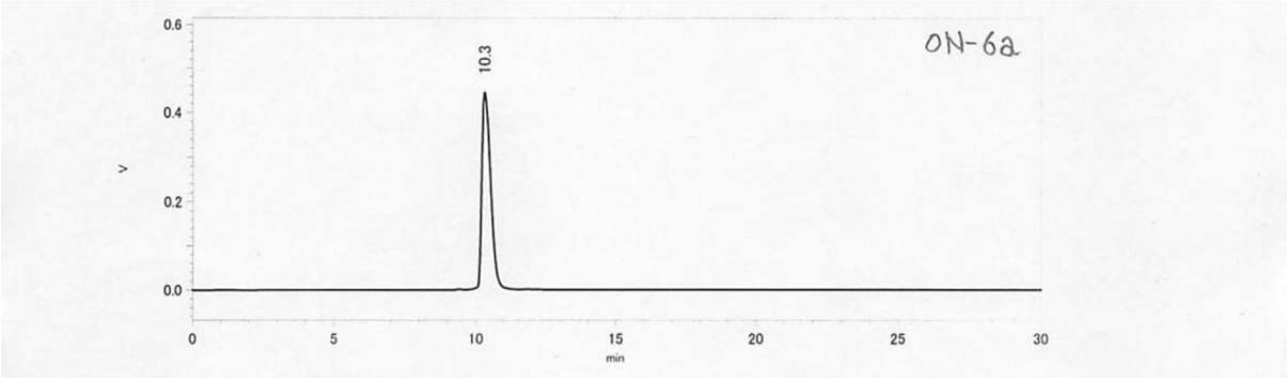
HPLC

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

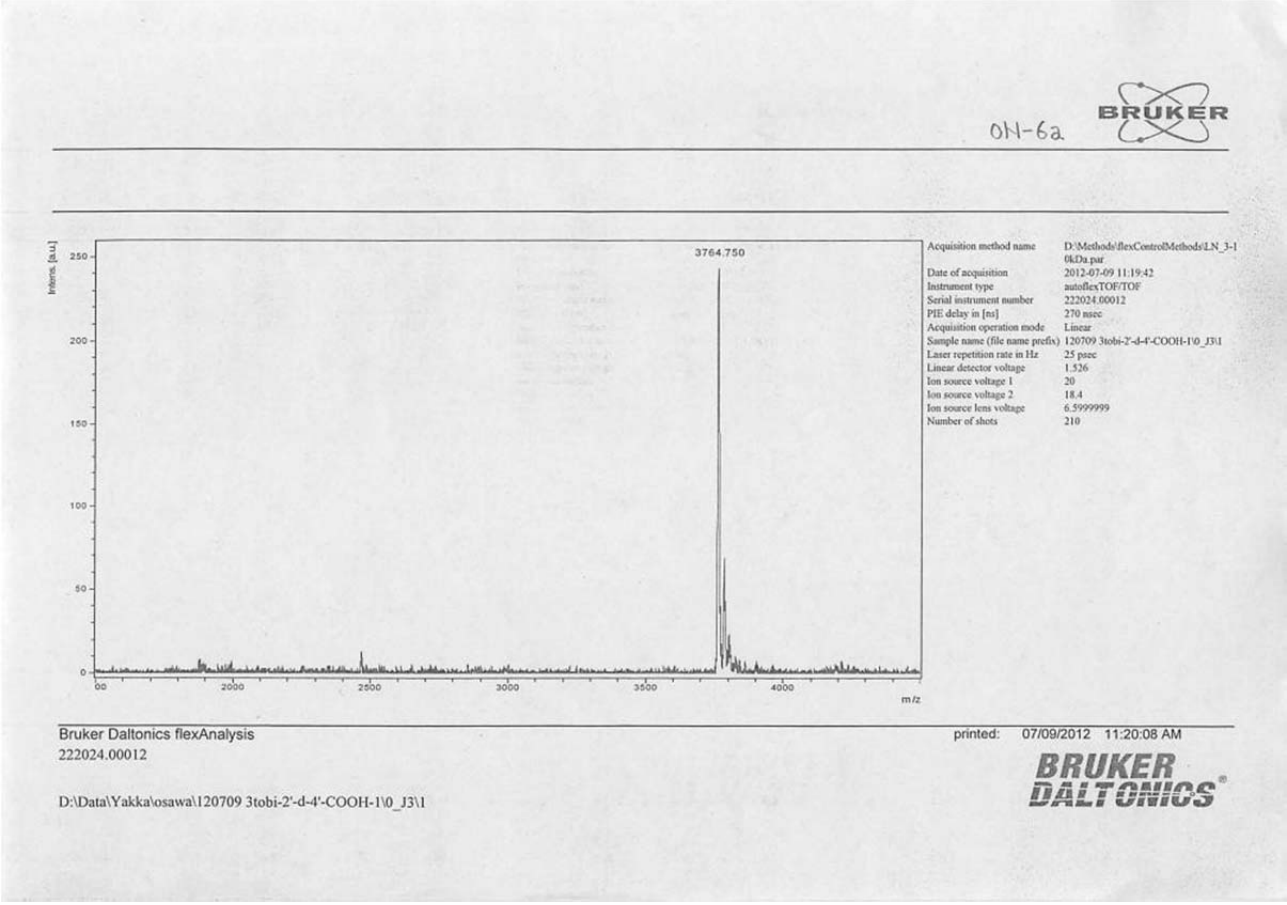
Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



S6

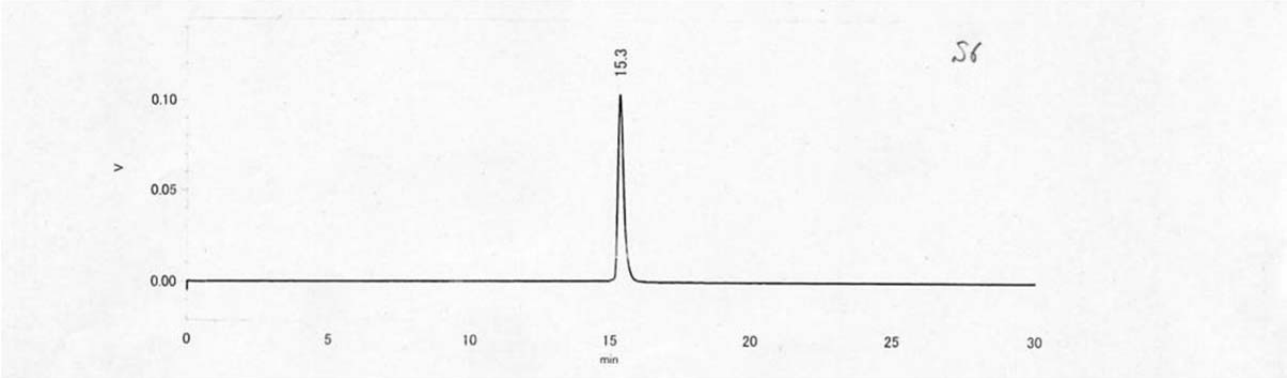
HPLC

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

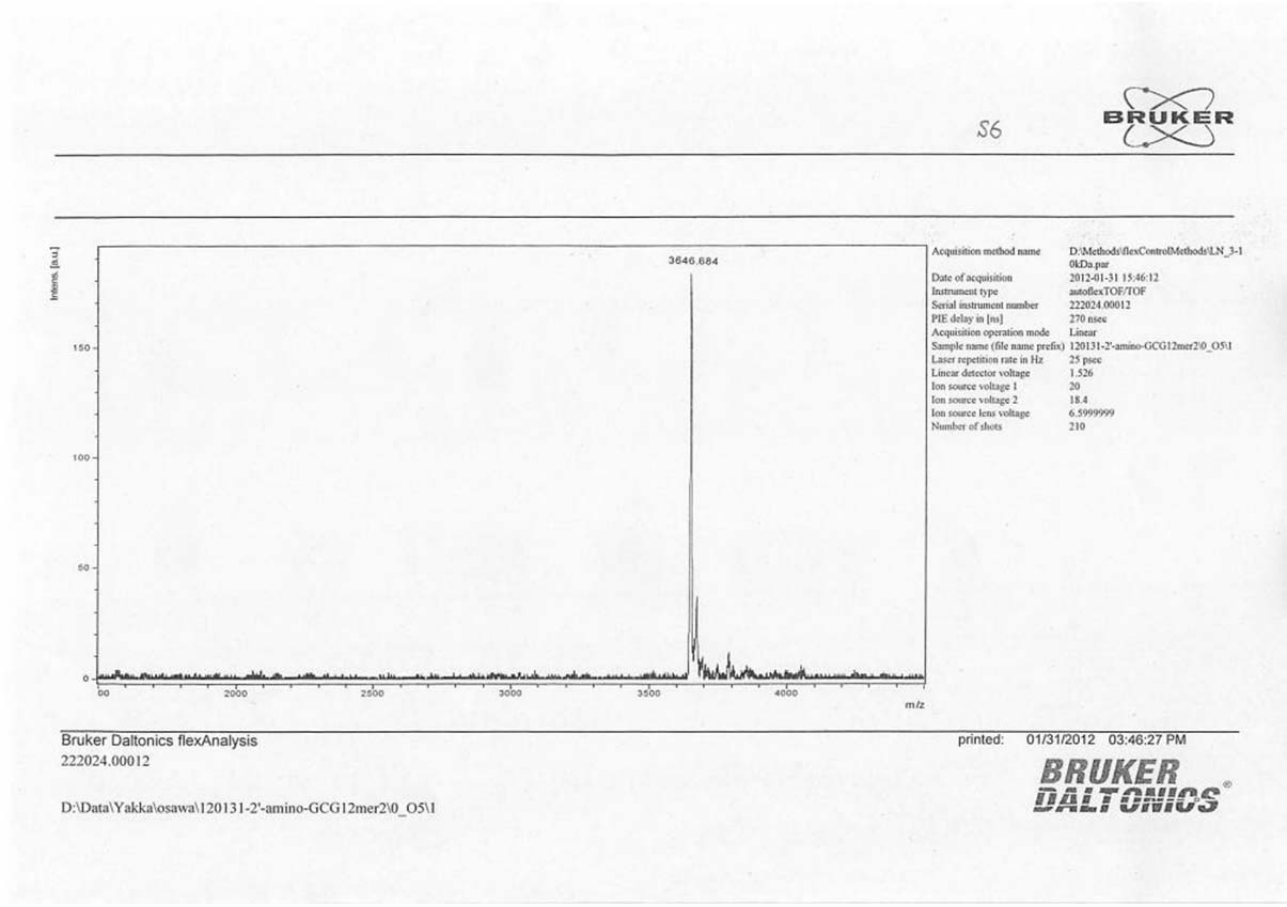
Gradient : 5-11% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

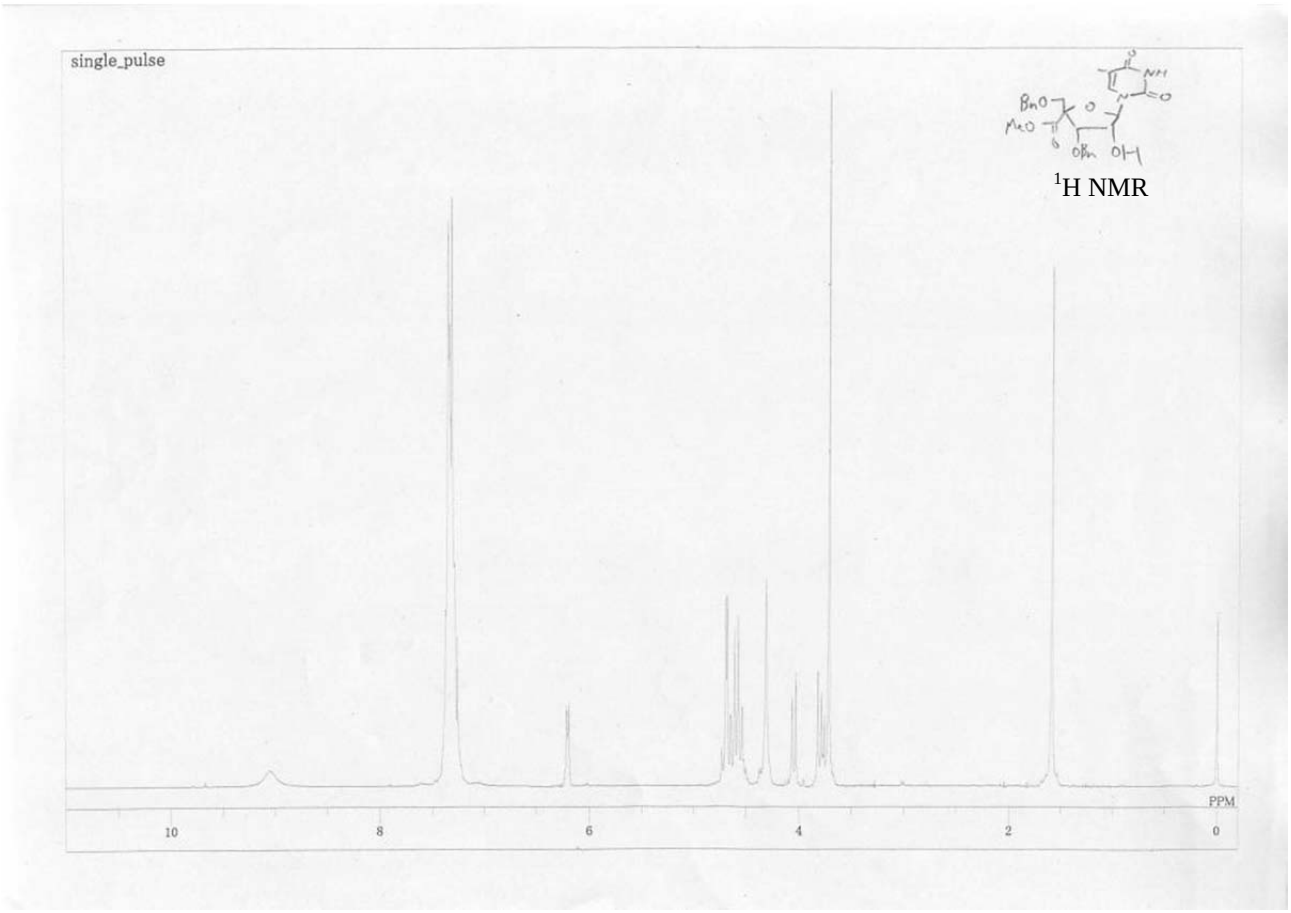
Column temp. : 50ºC.



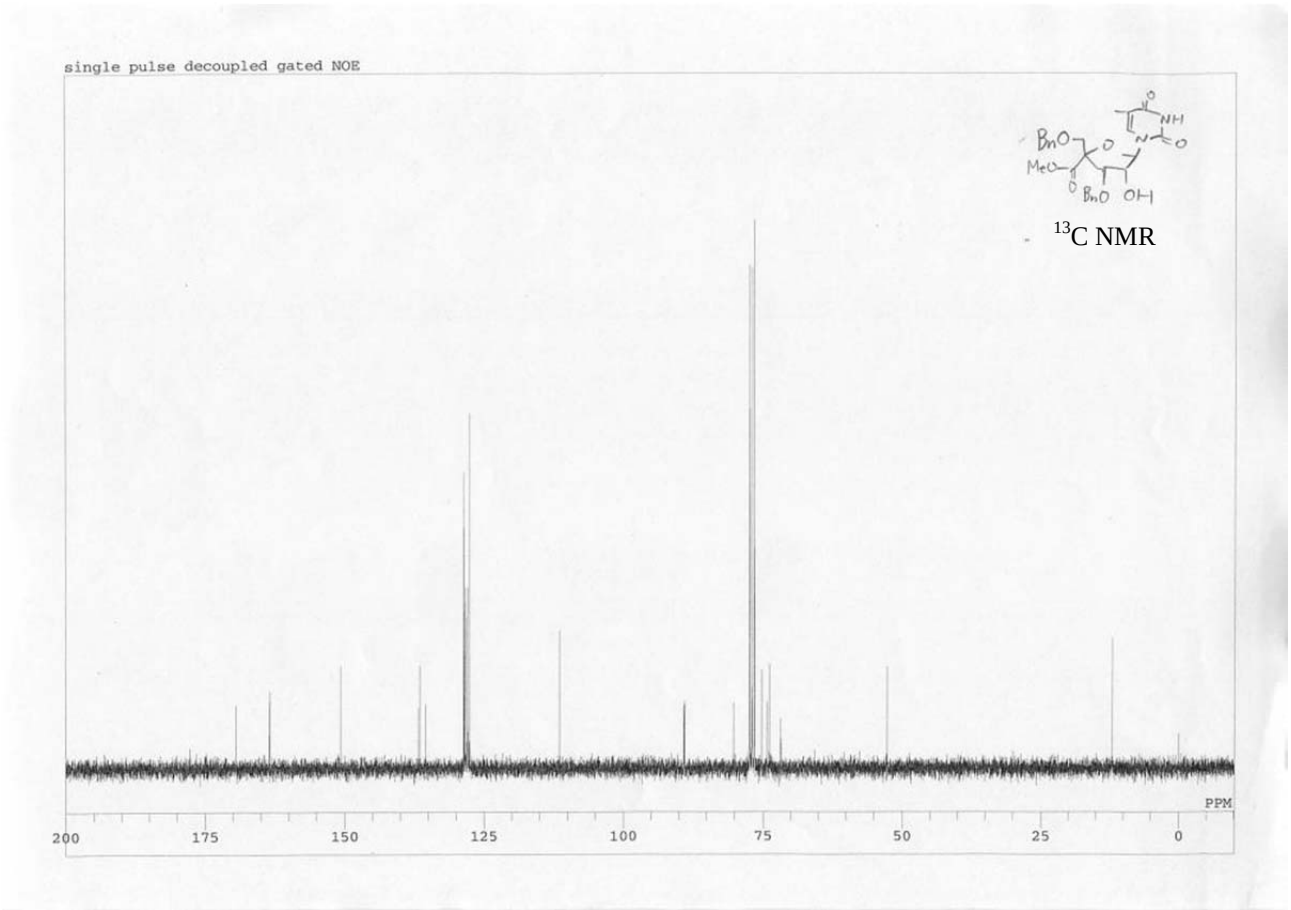
MALDI-TOF-Mass



Compound **6**

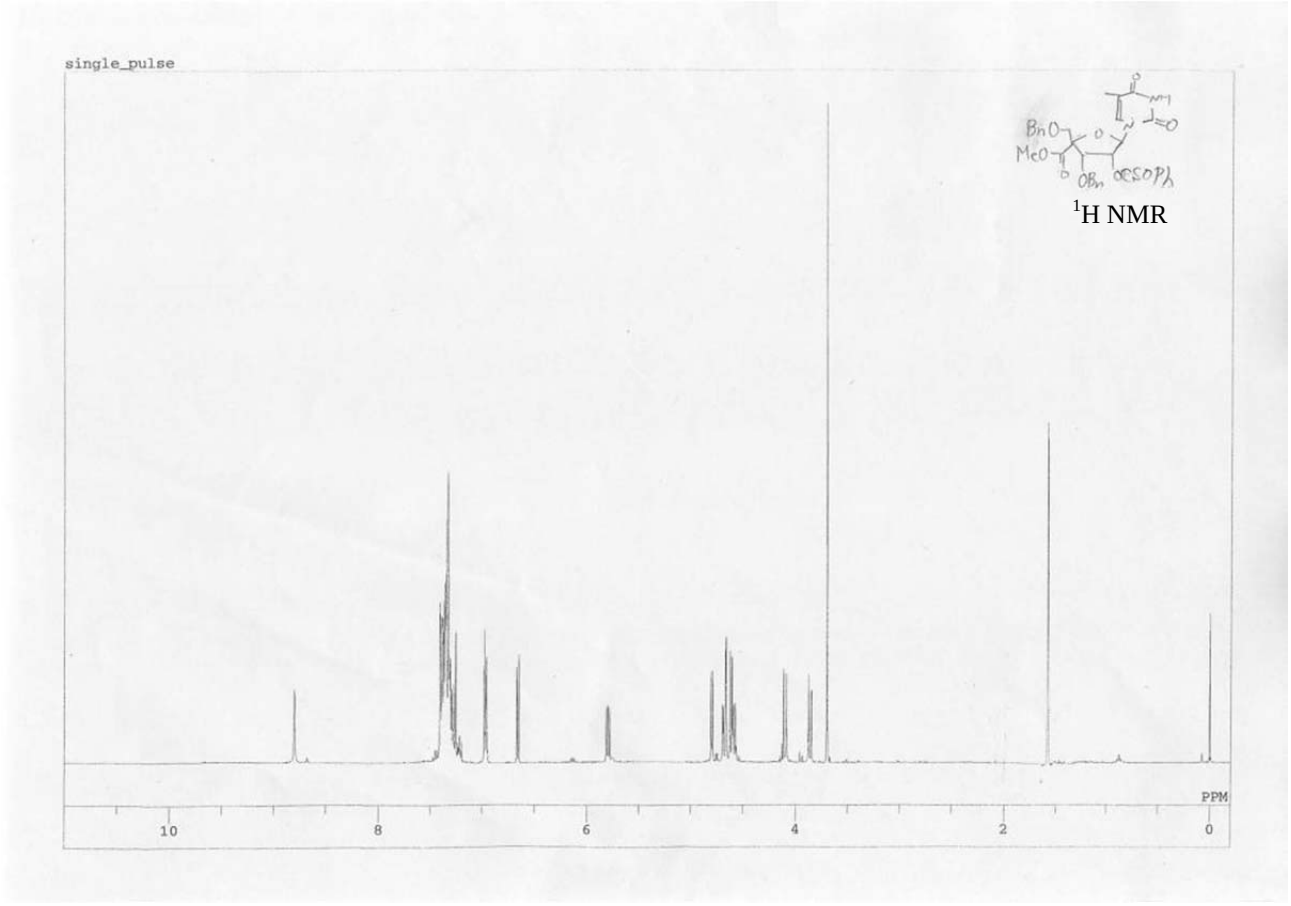


^{1}H NMR

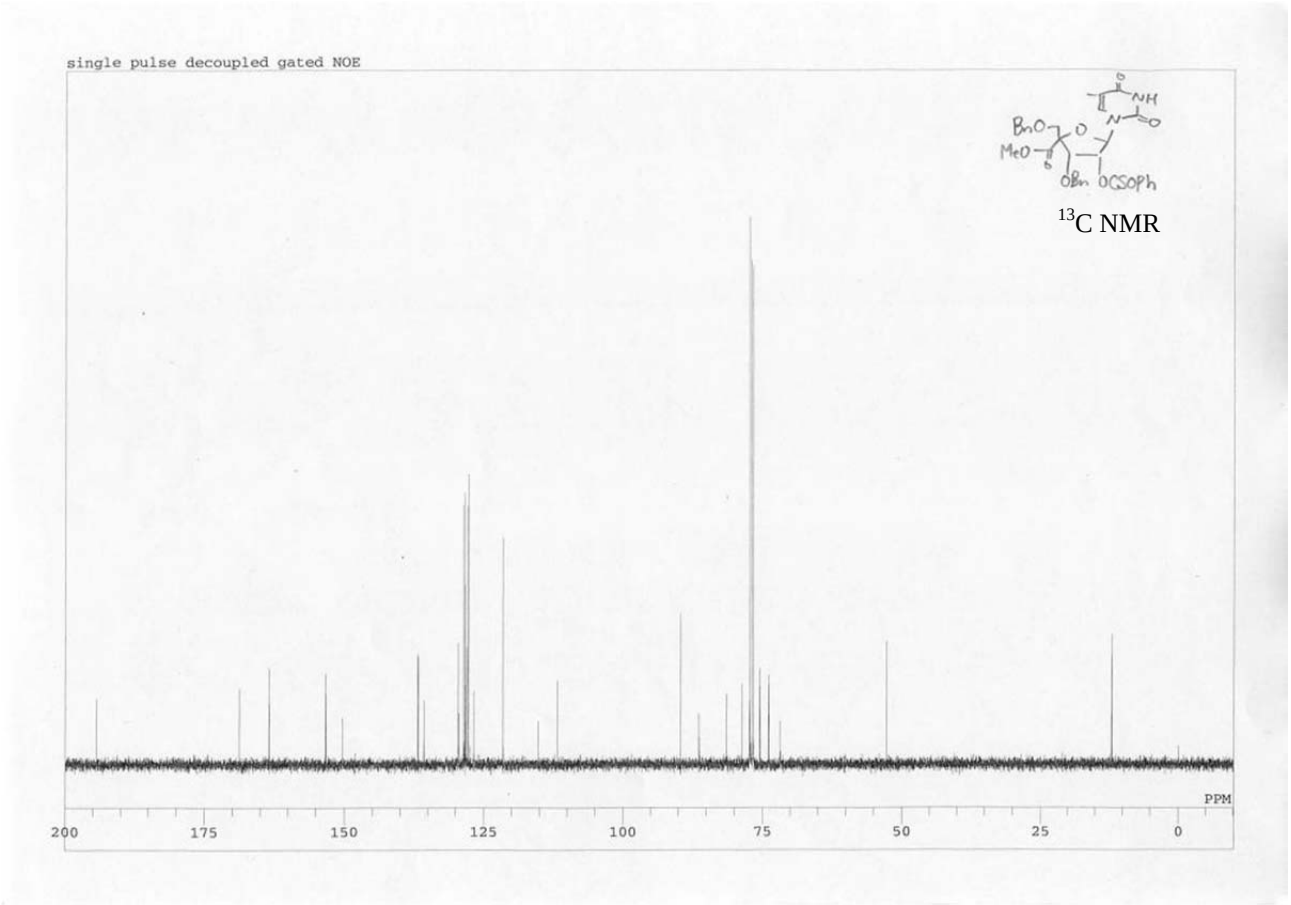


^{13}C NMR

Compound **7**

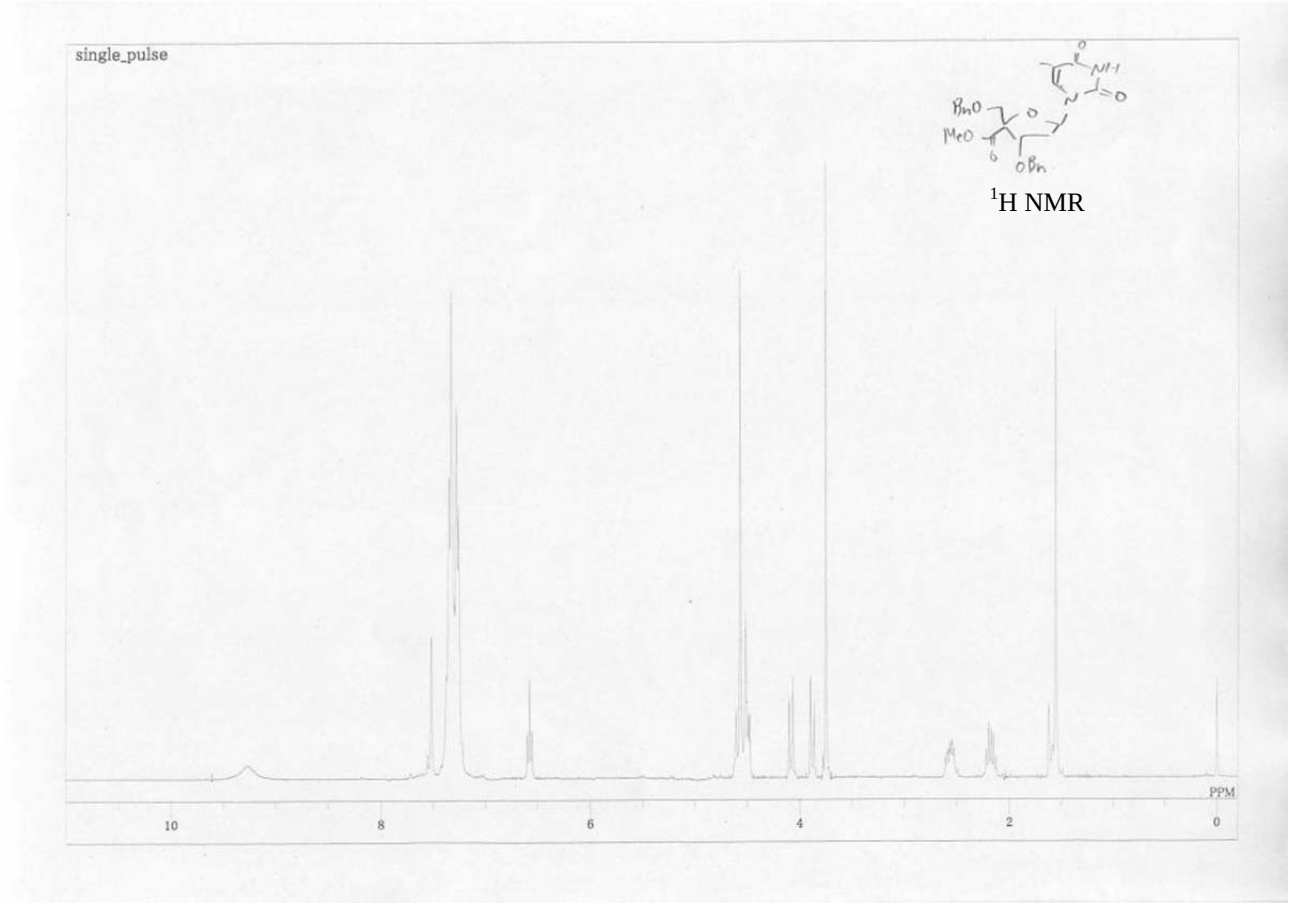


^{1}H NMR

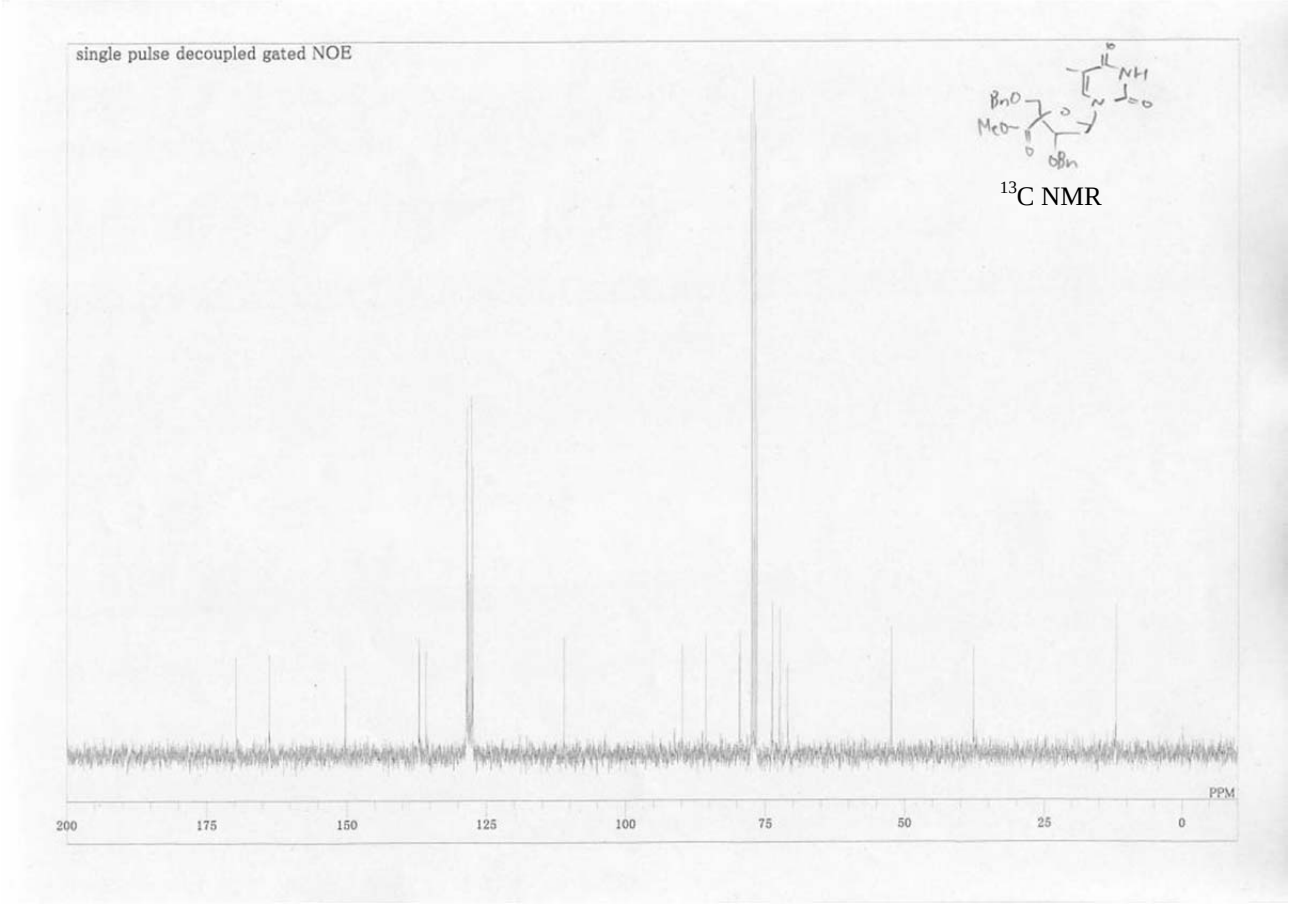


^{13}C NMR

Compound **8**

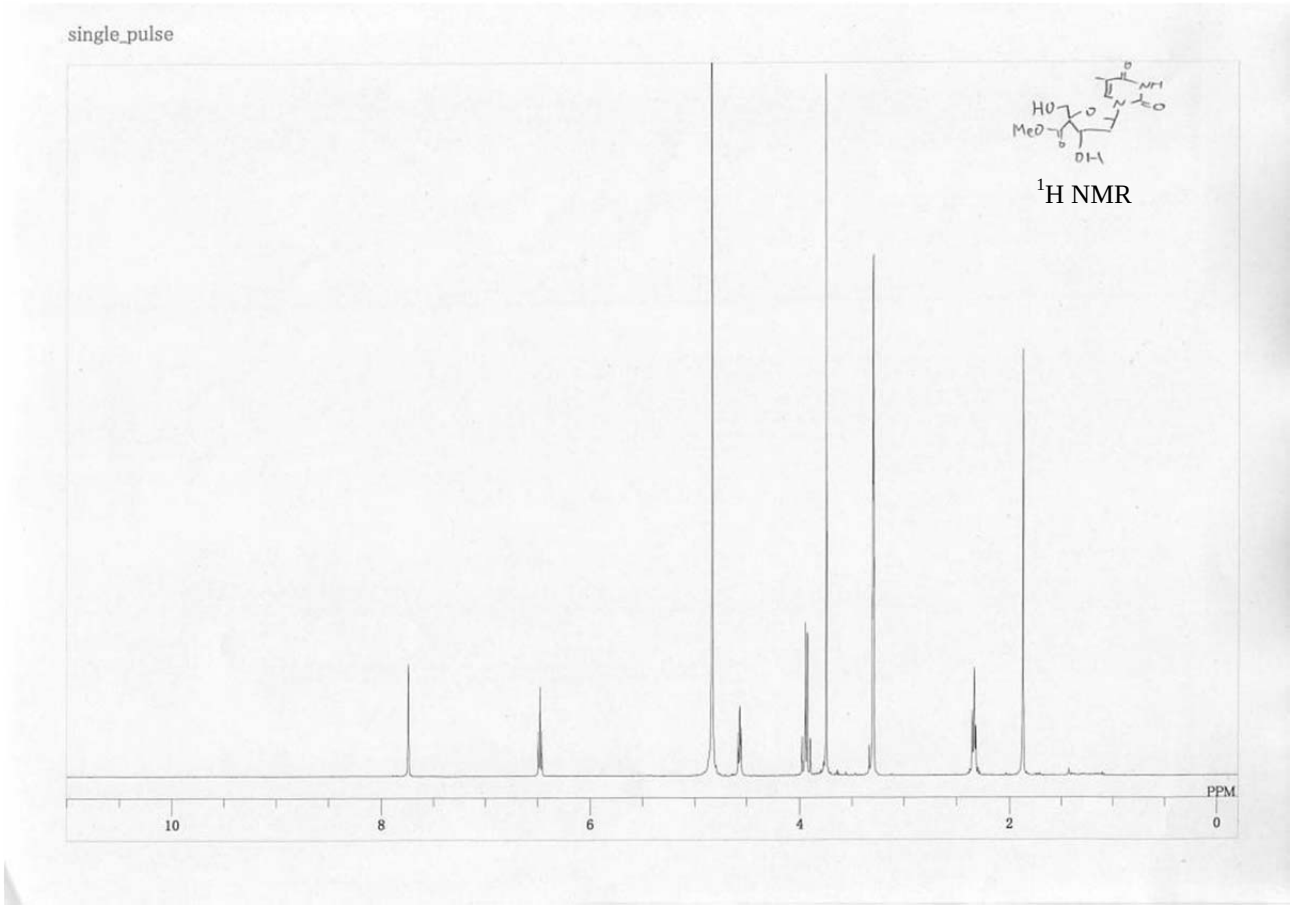


^{1}H NMR

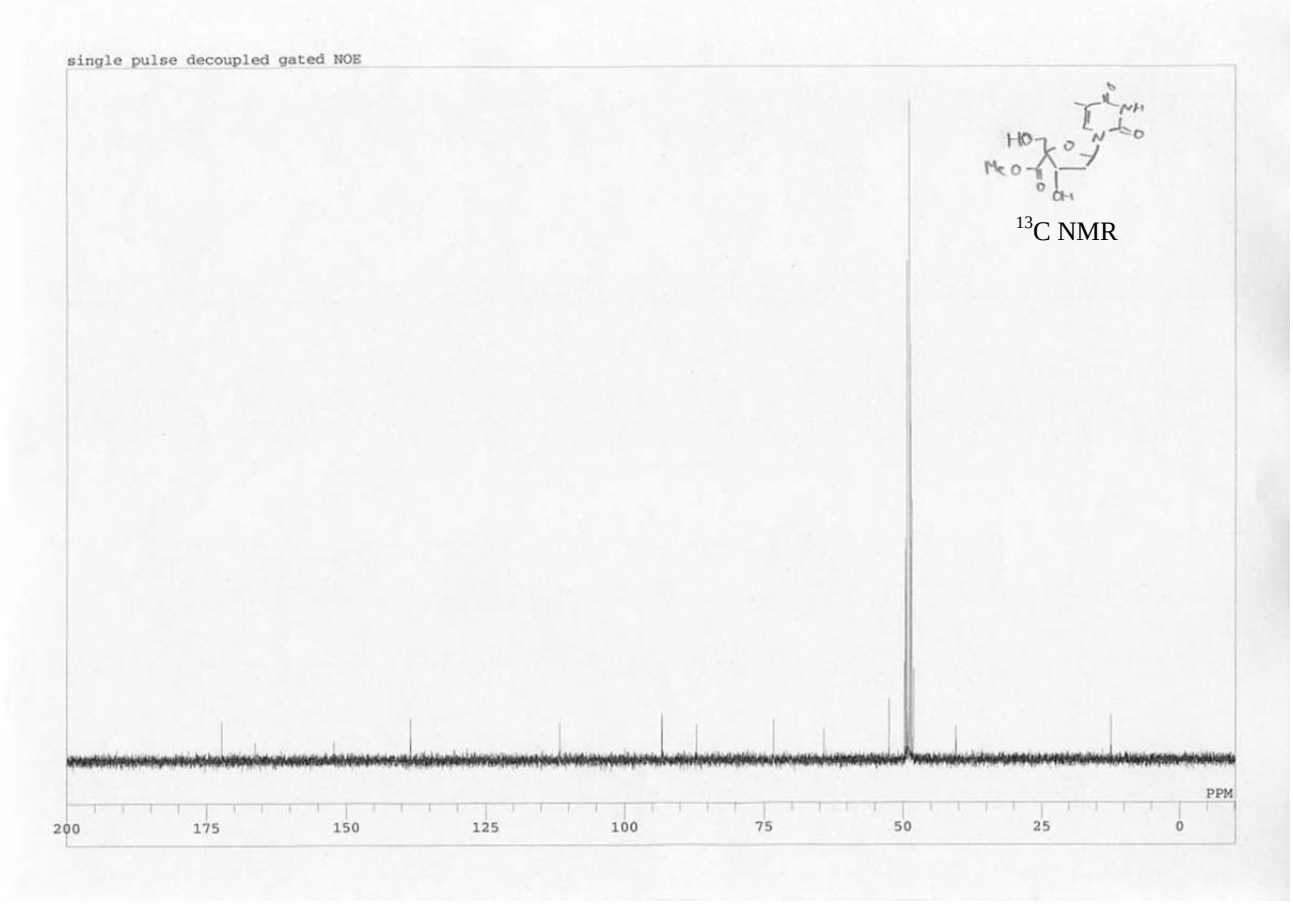


^{13}C NMR

Compound **9**

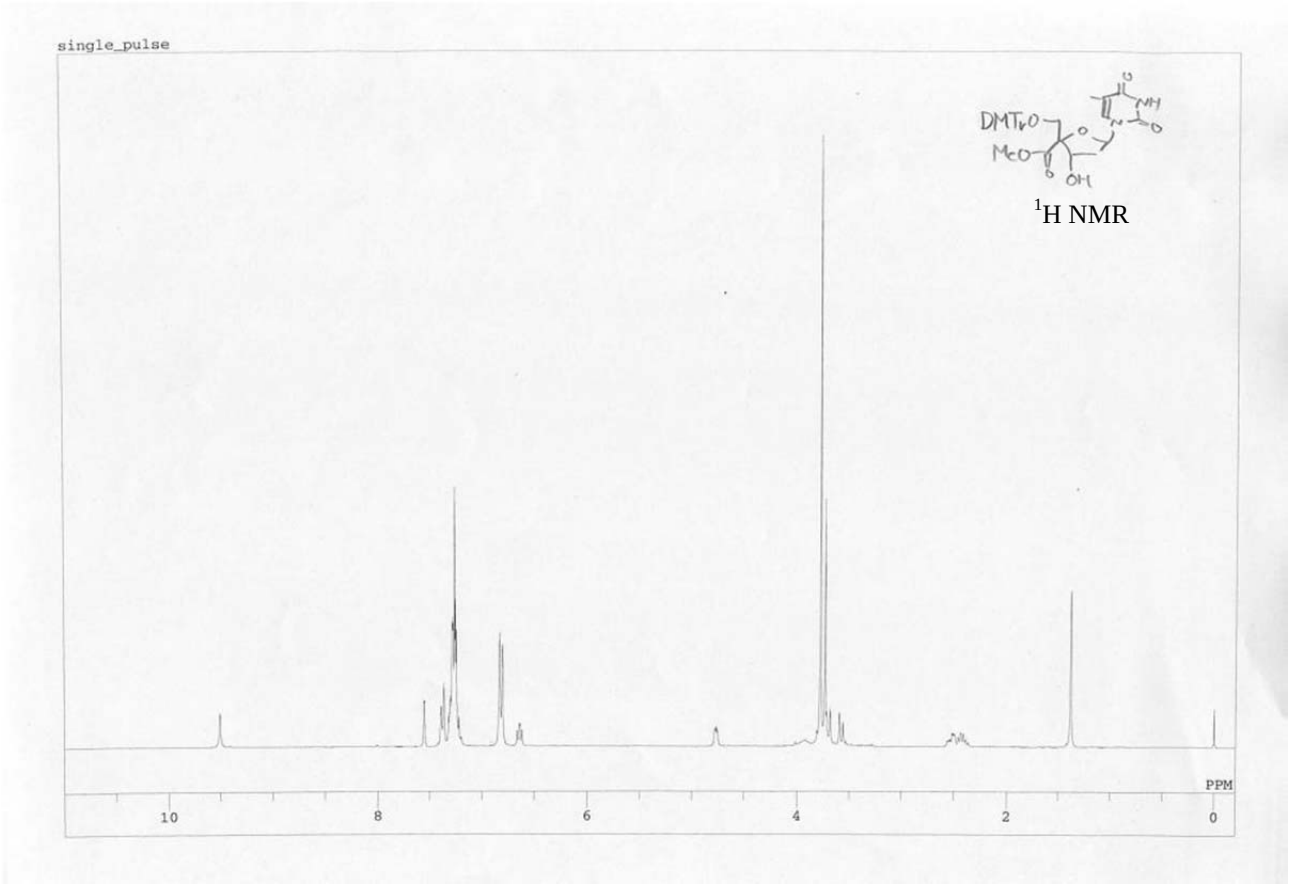


^{1}H NMR

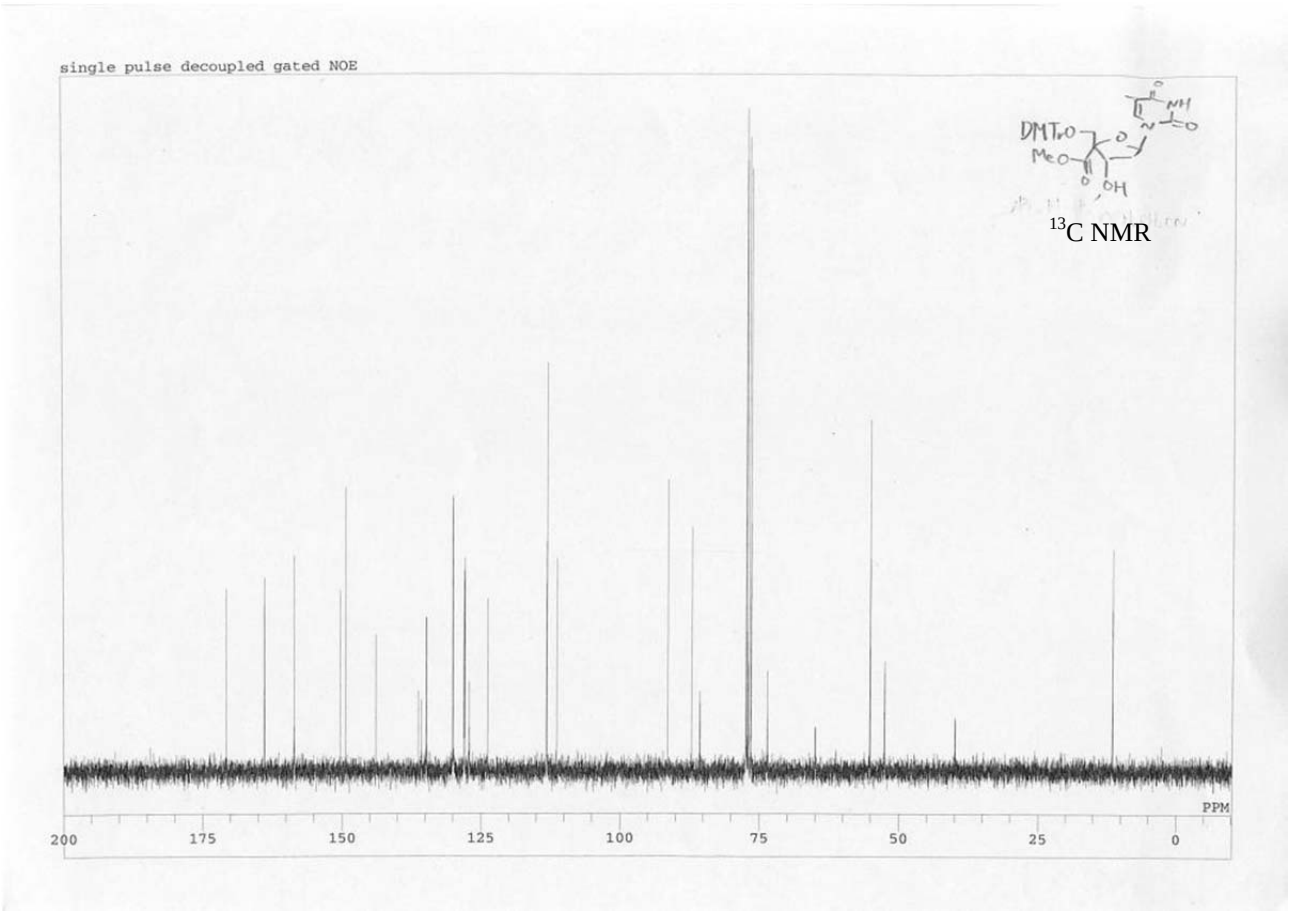


^{13}C NMR

Compound **10**

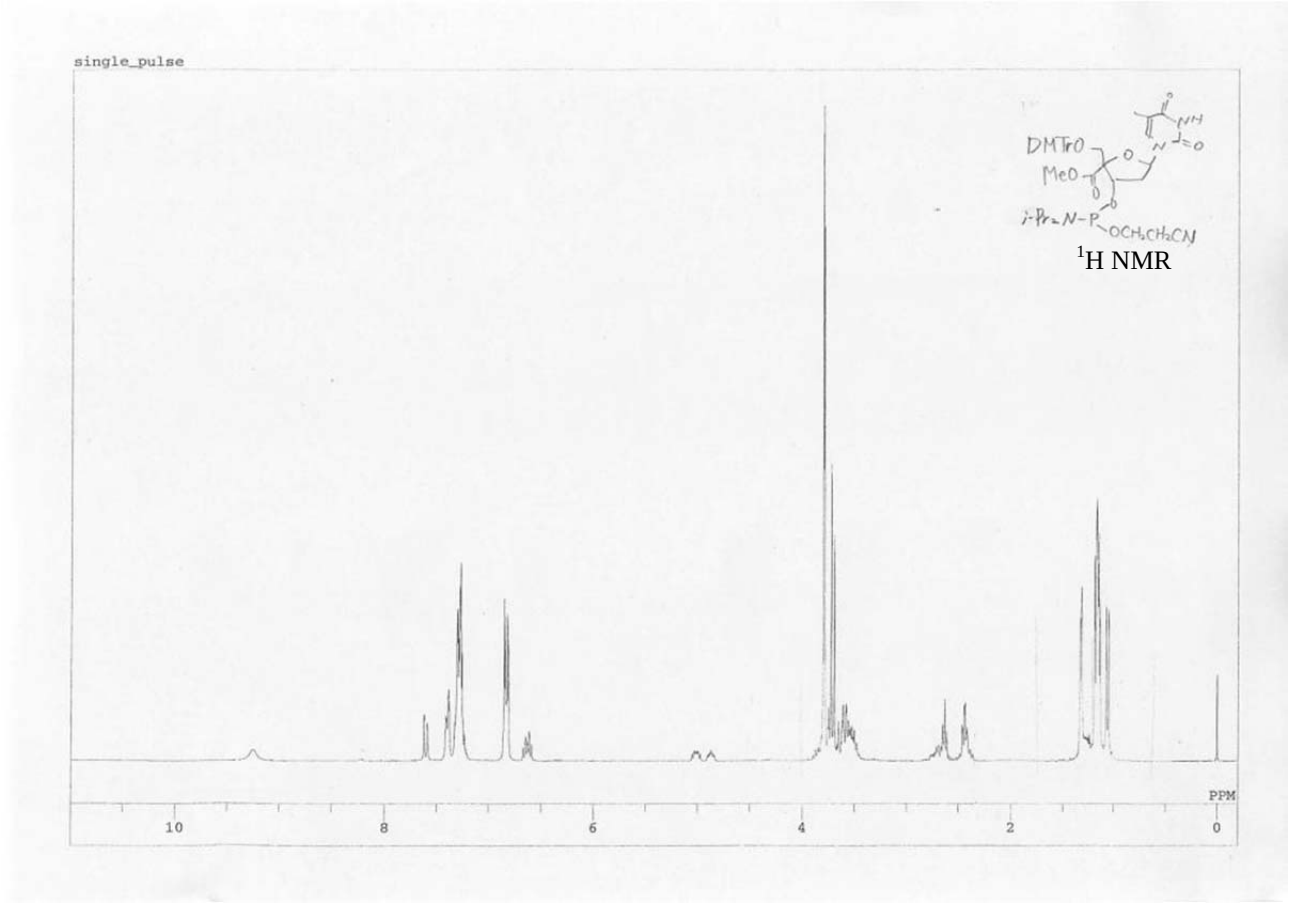


^{1}H NMR

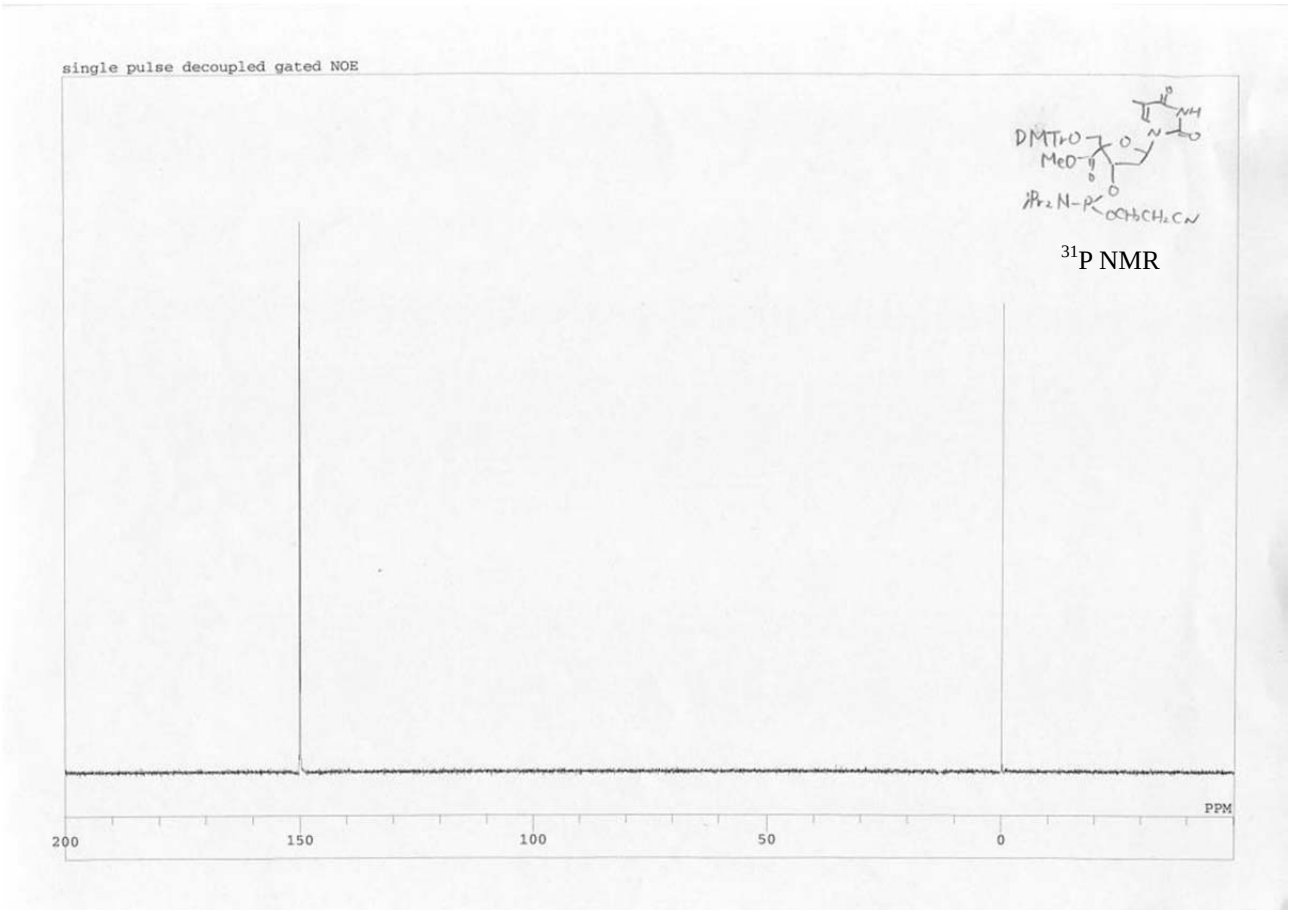


^{13}C NMR

Compound **1** (diastereoisomers on the basis of chirality at the phosphorus)

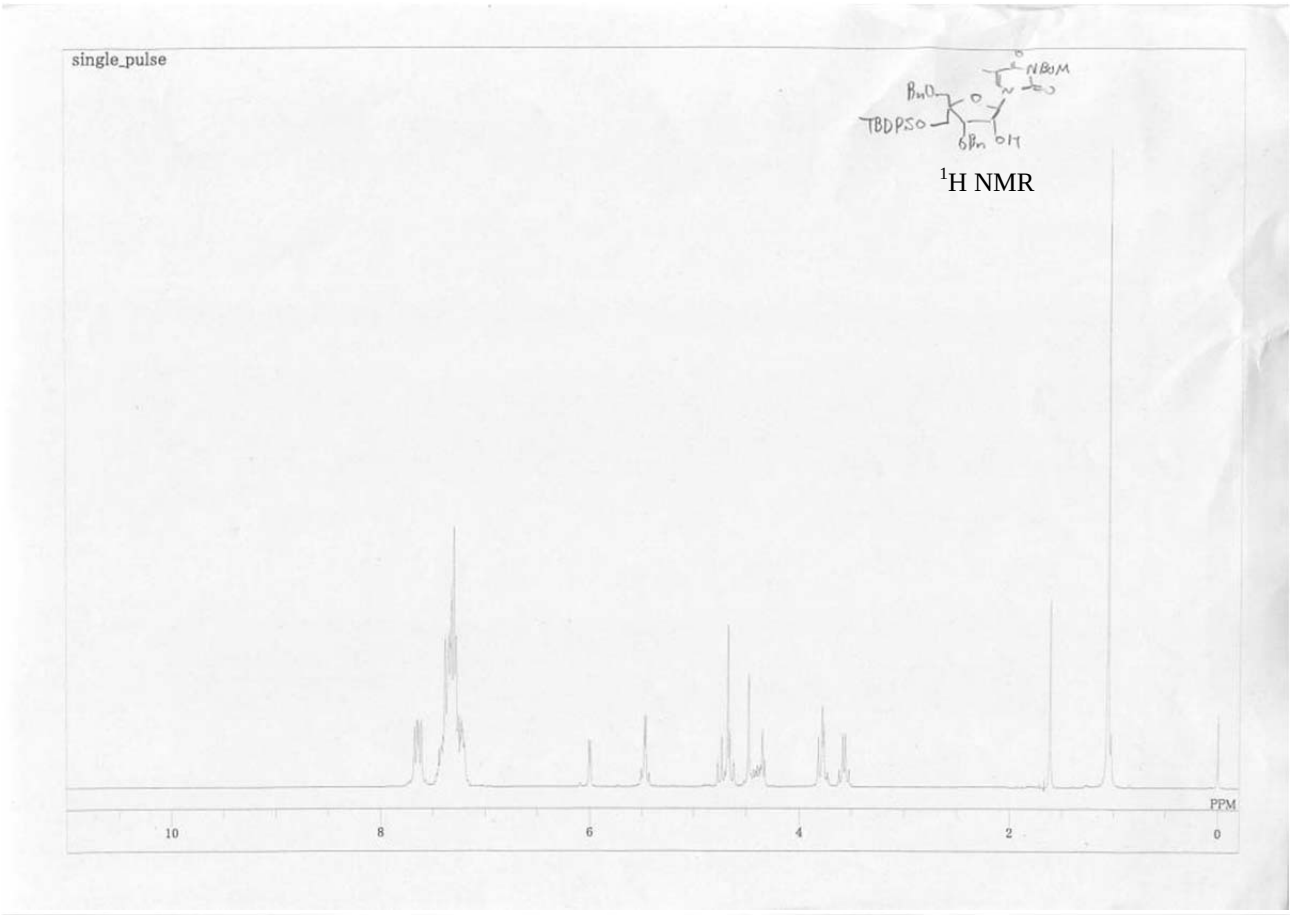


^{1}H NMR

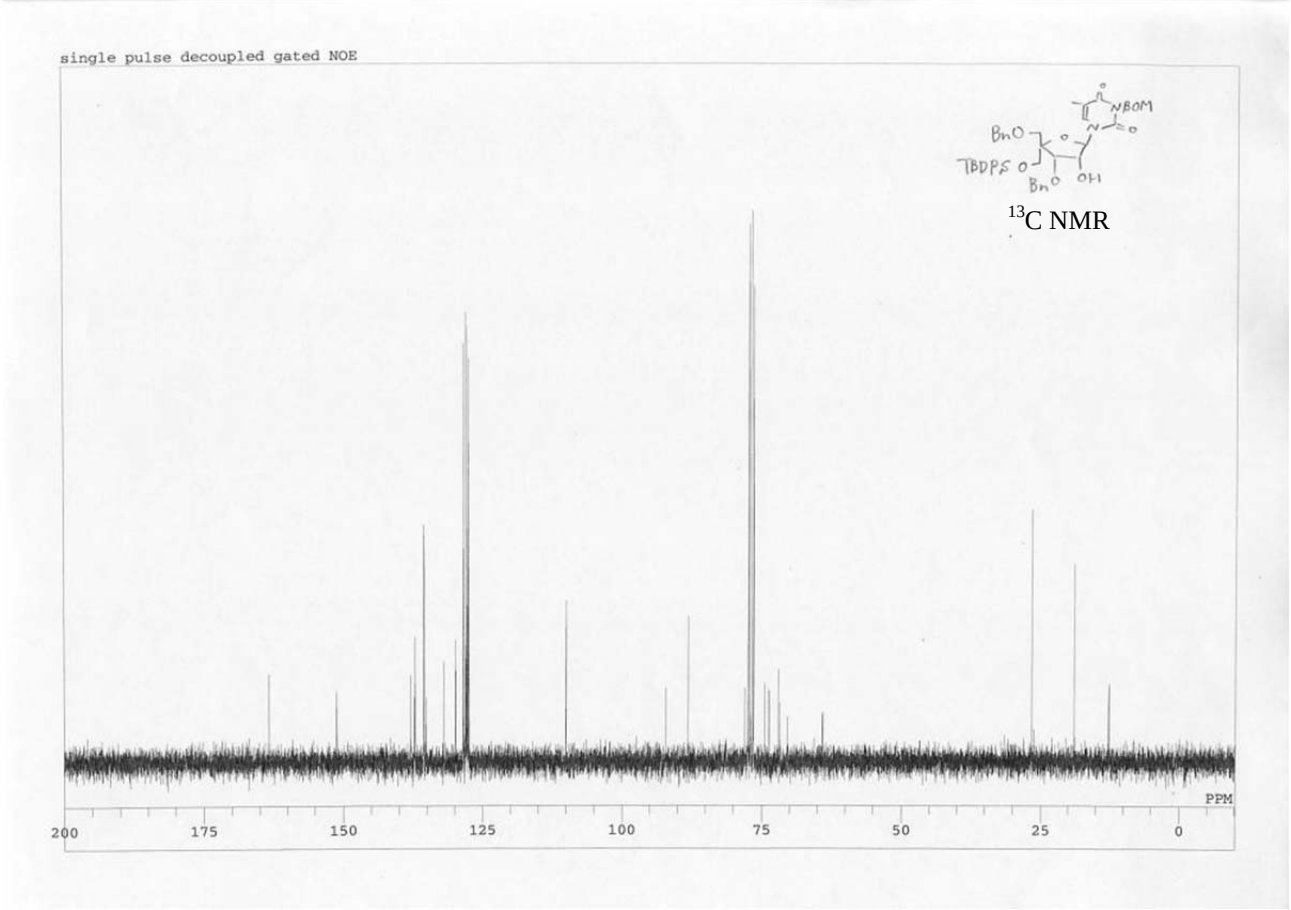


^{31}P NMR

Compound **12**

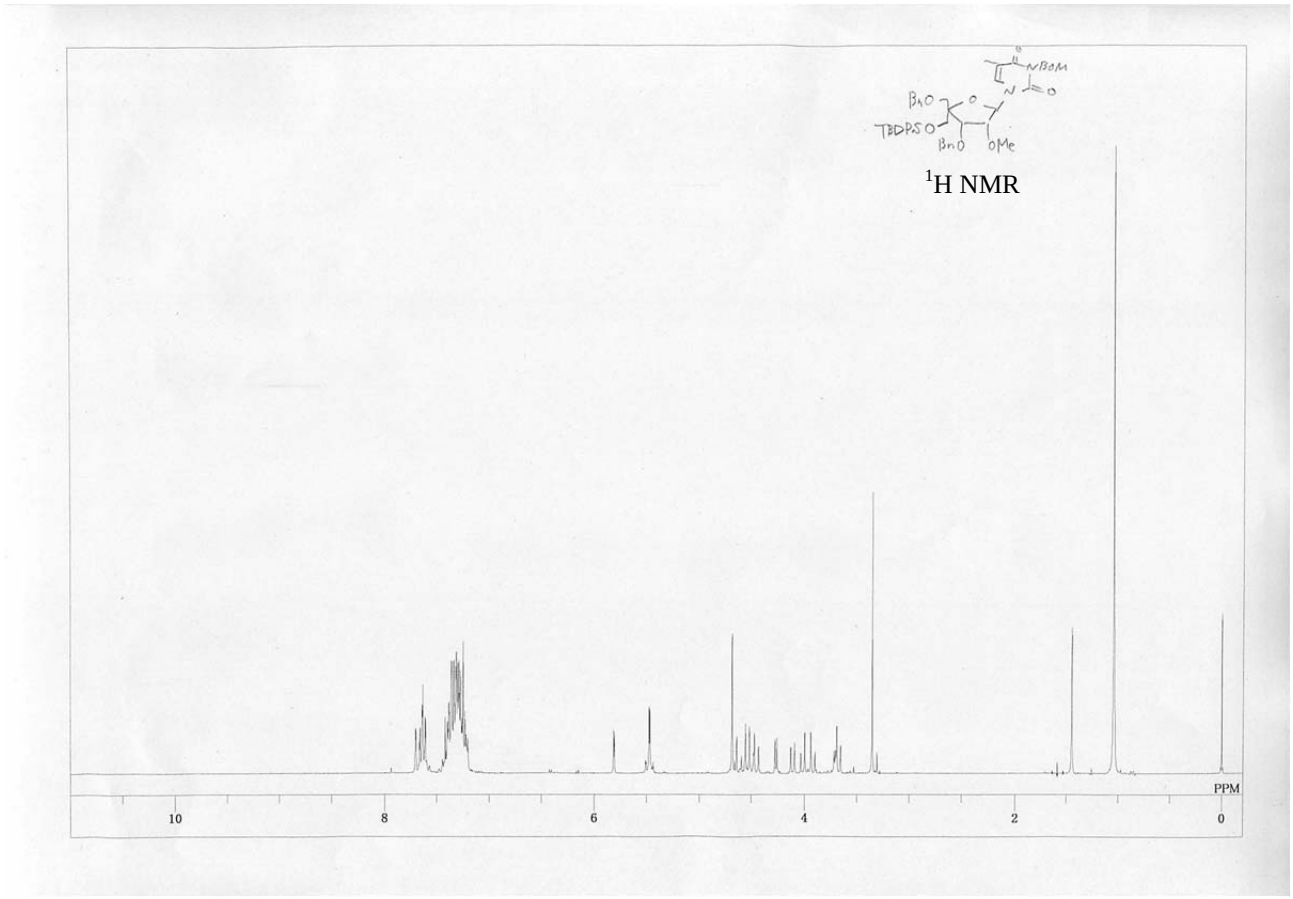


^{1}H NMR

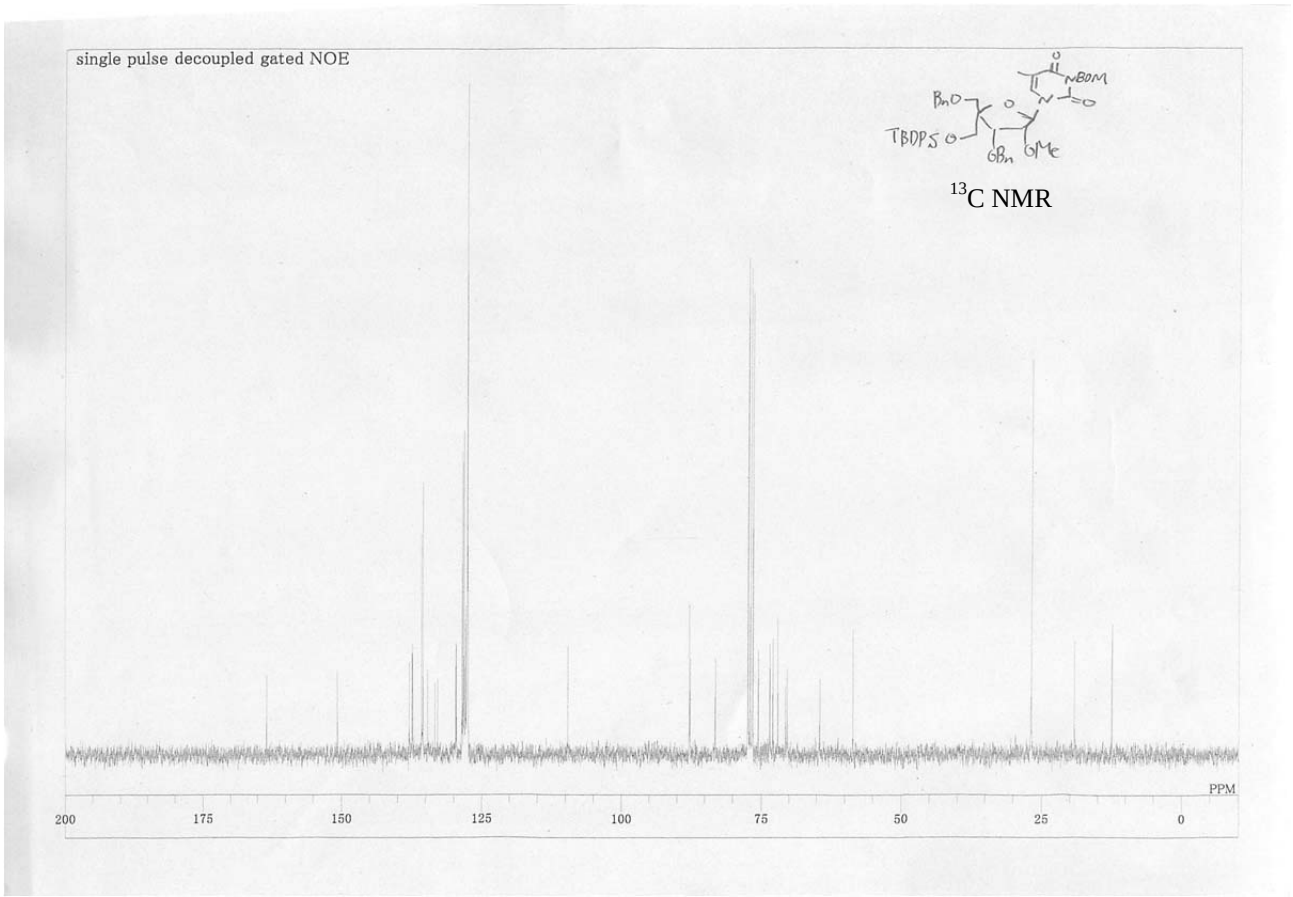


^{13}C NMR

Compound **13**

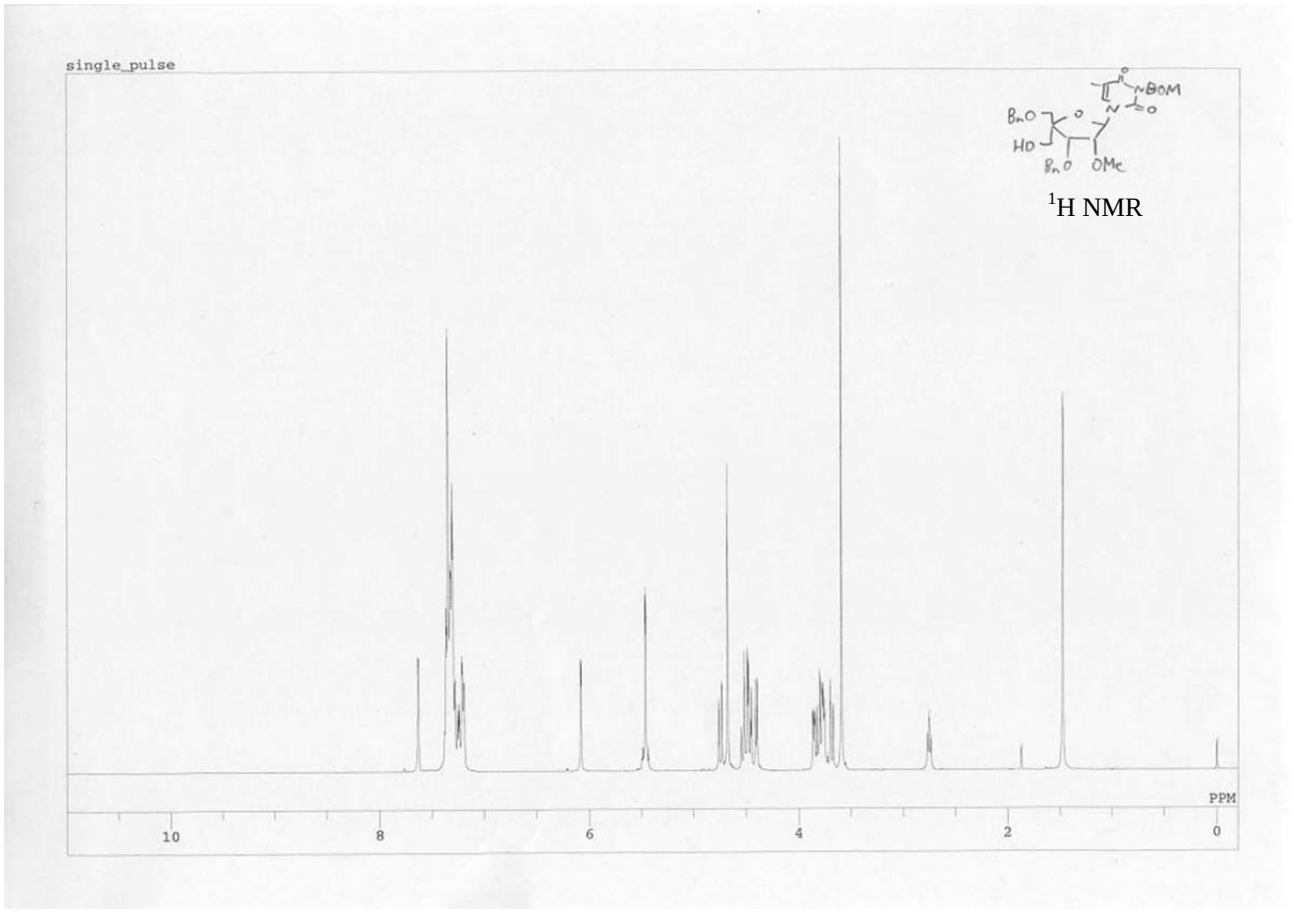


^{1}H NMR

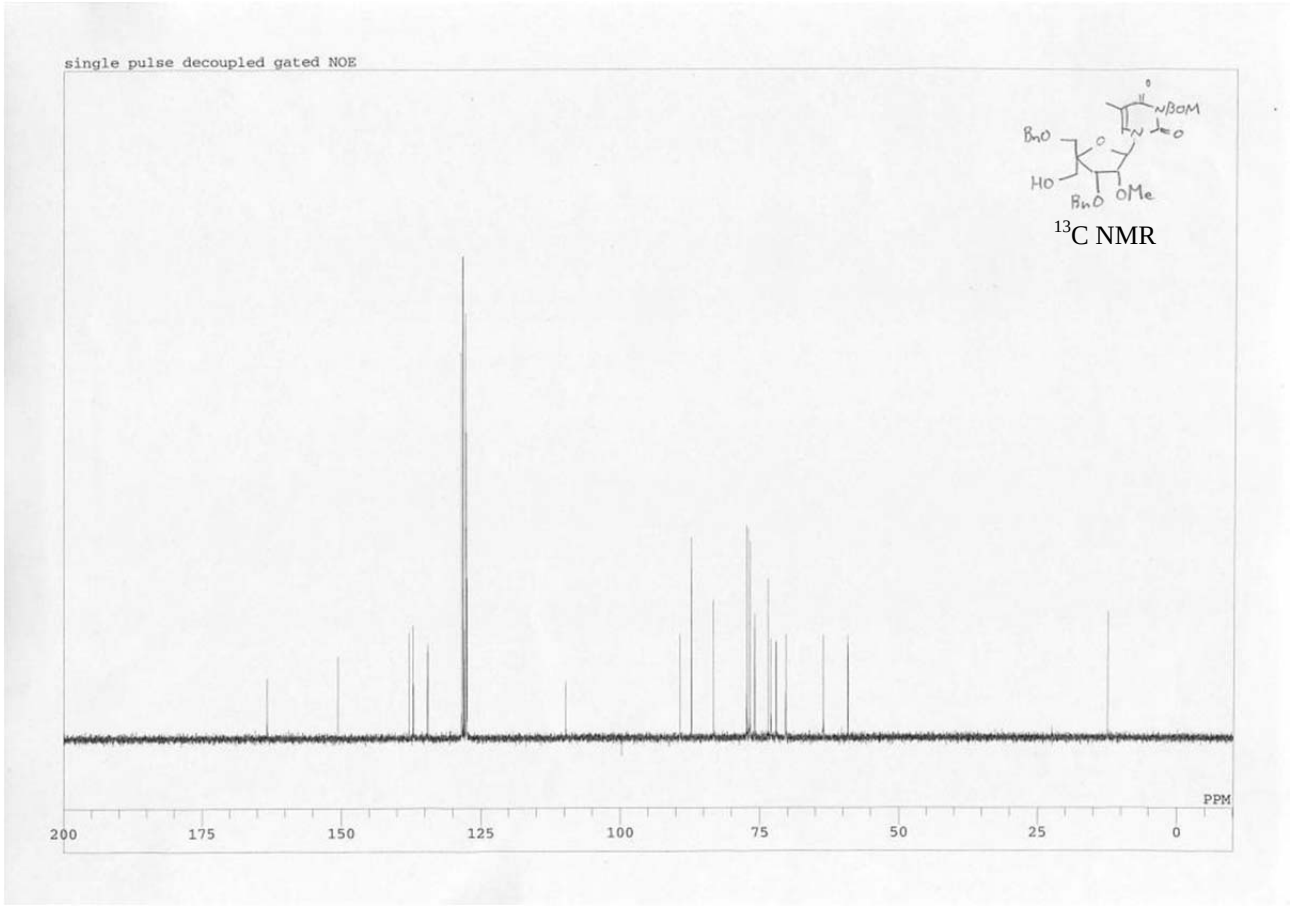


^{13}C NMR

Compound **14**

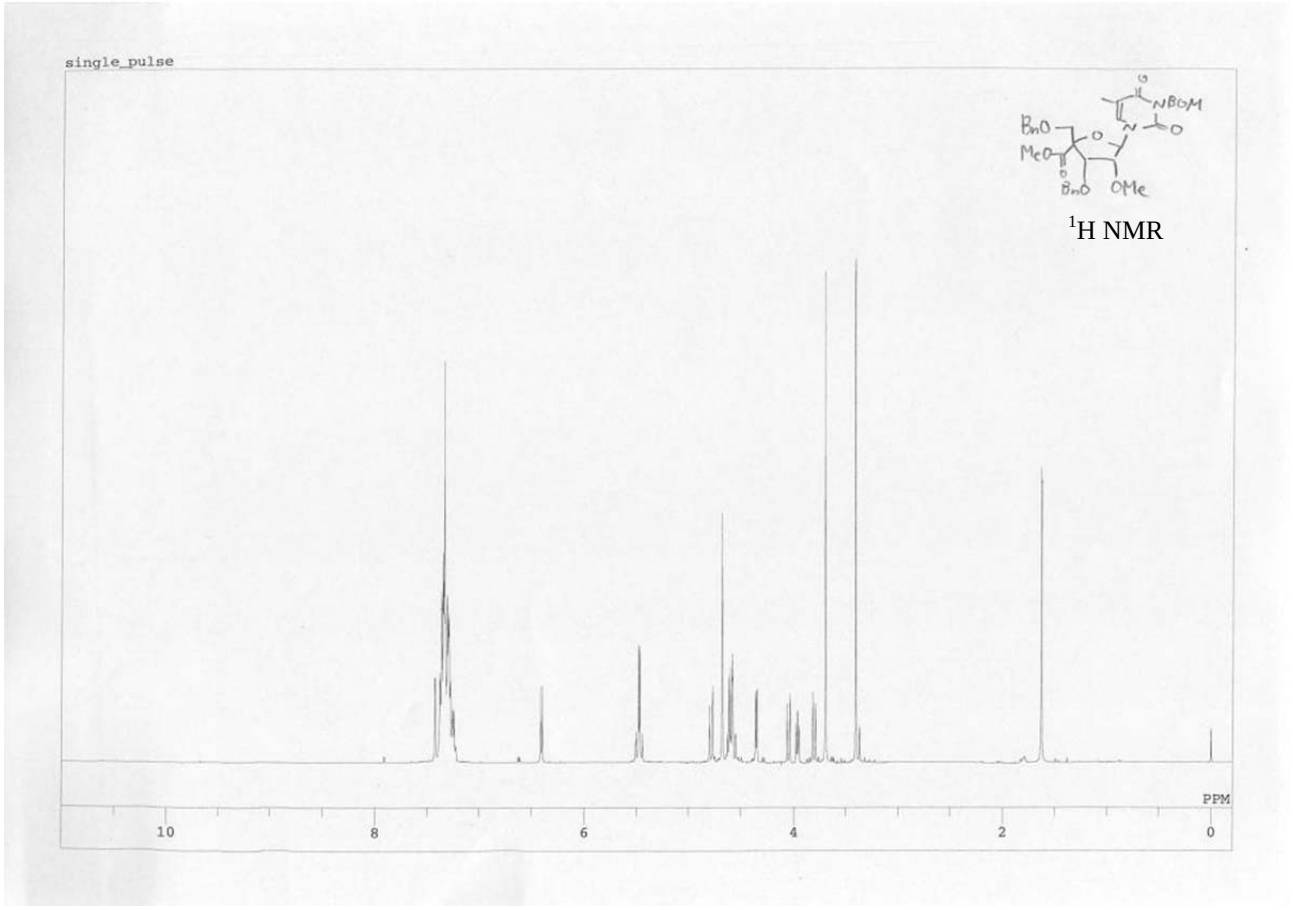


^{1}H NMR

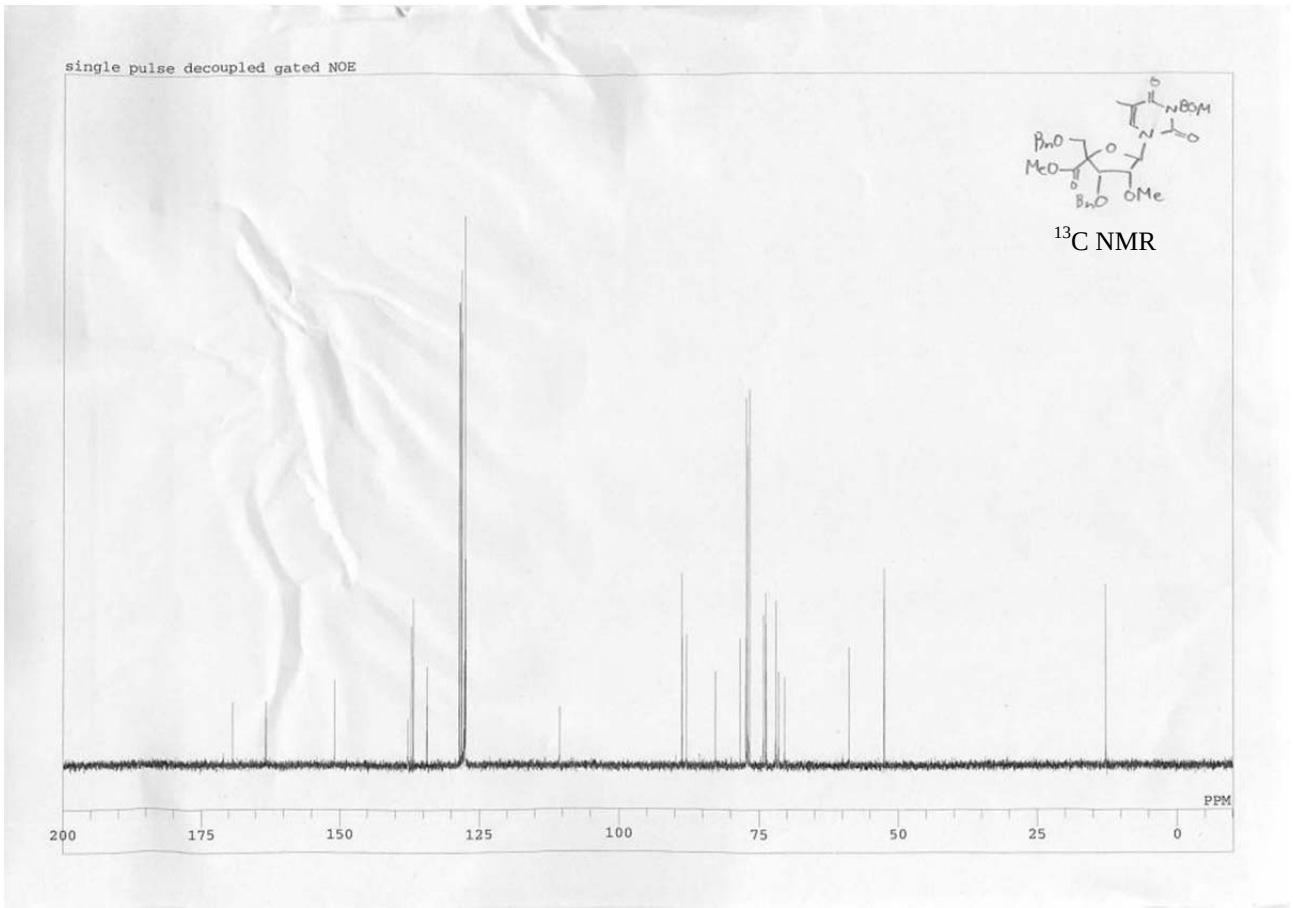


^{13}C NMR

Compound **15**

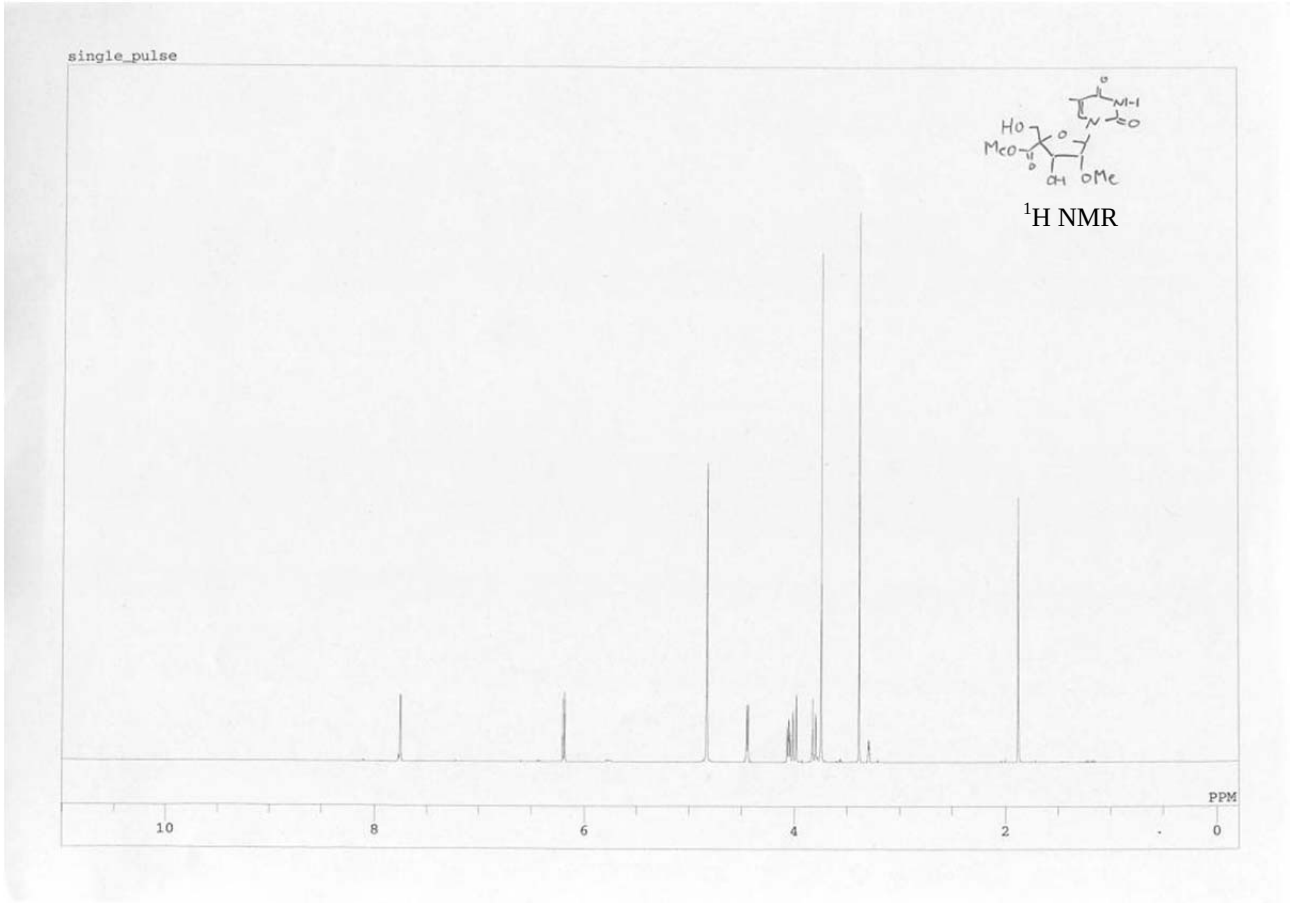


^{1}H NMR

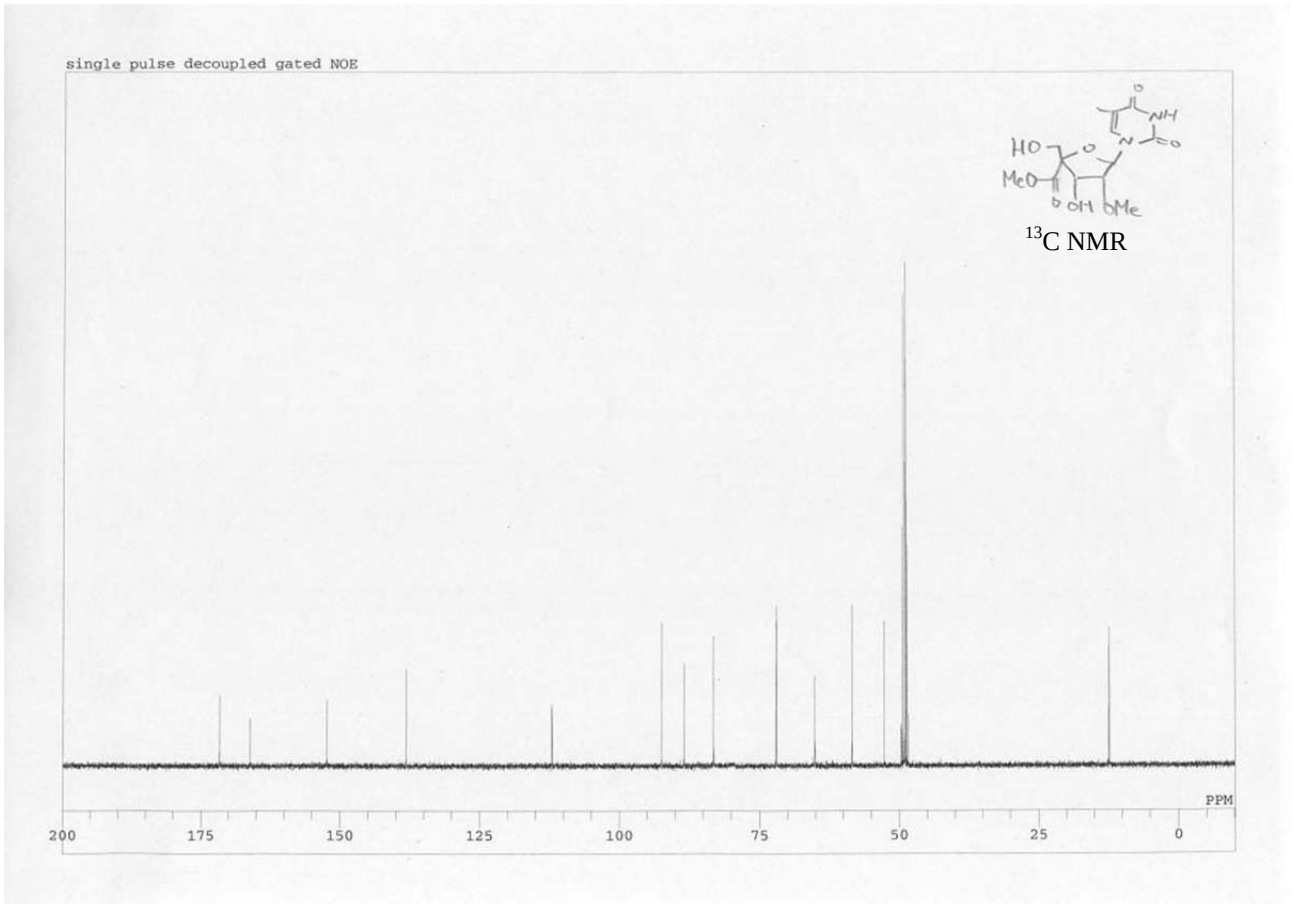


^{13}C NMR

Compound **16**

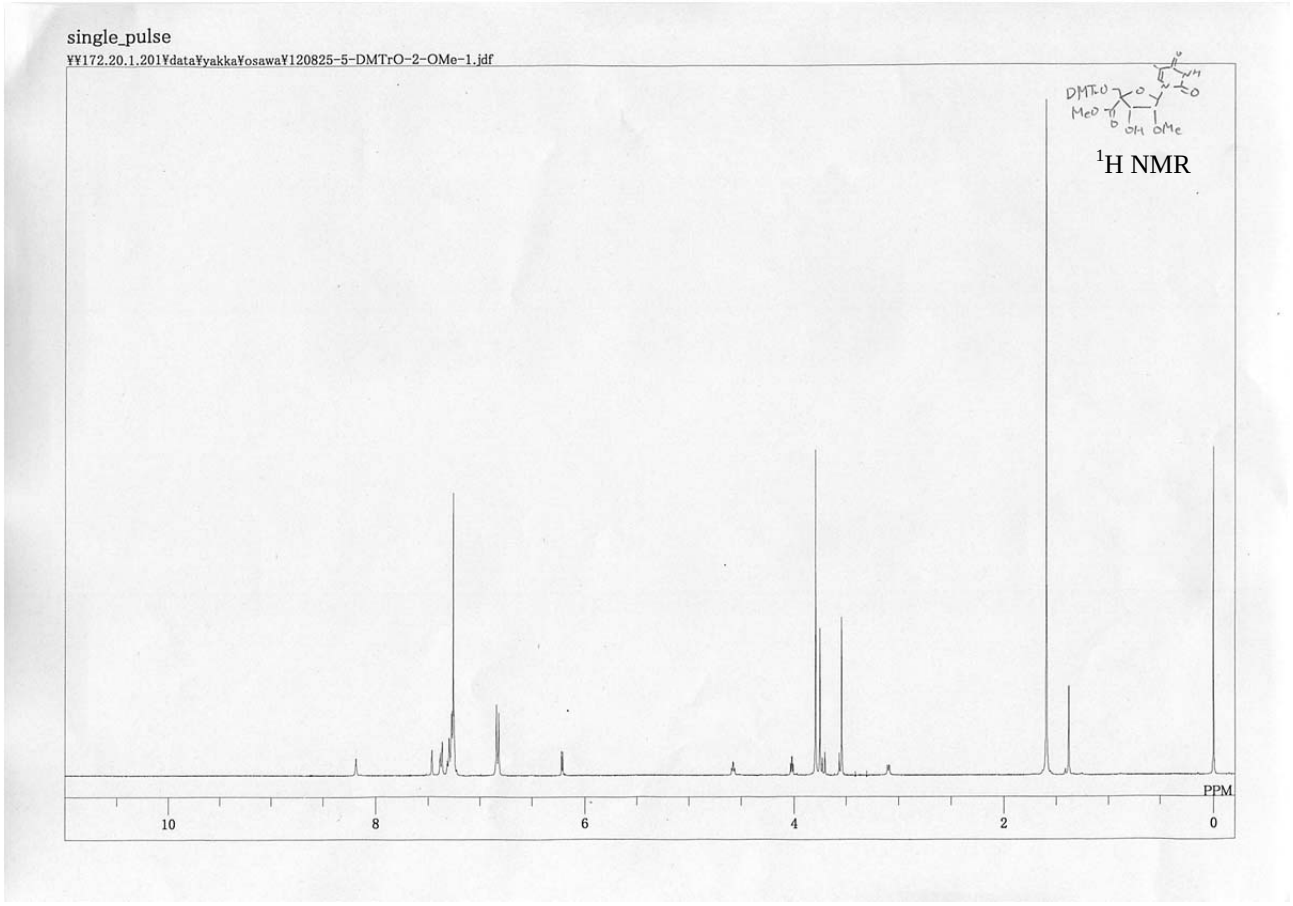


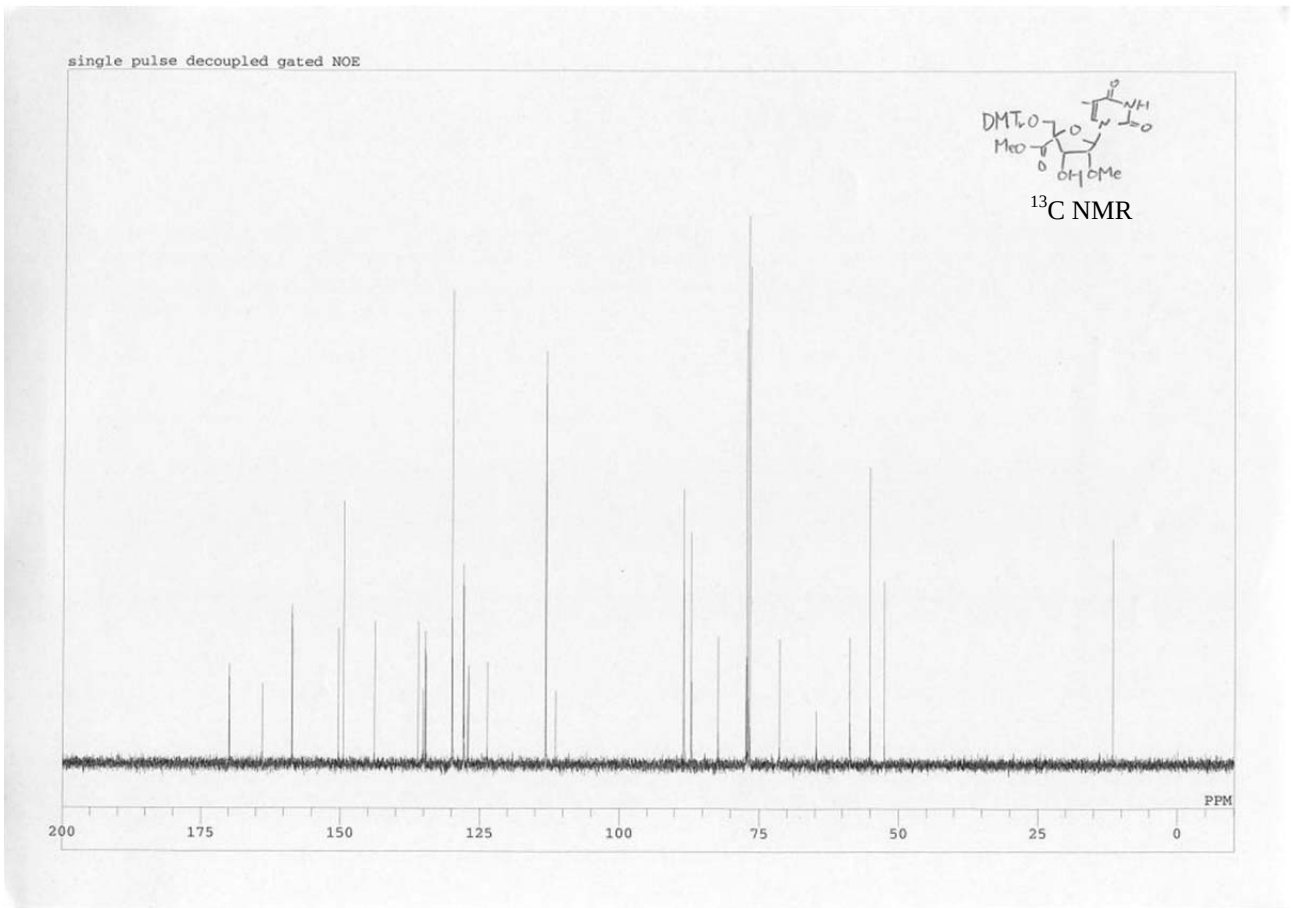
^{1}H NMR



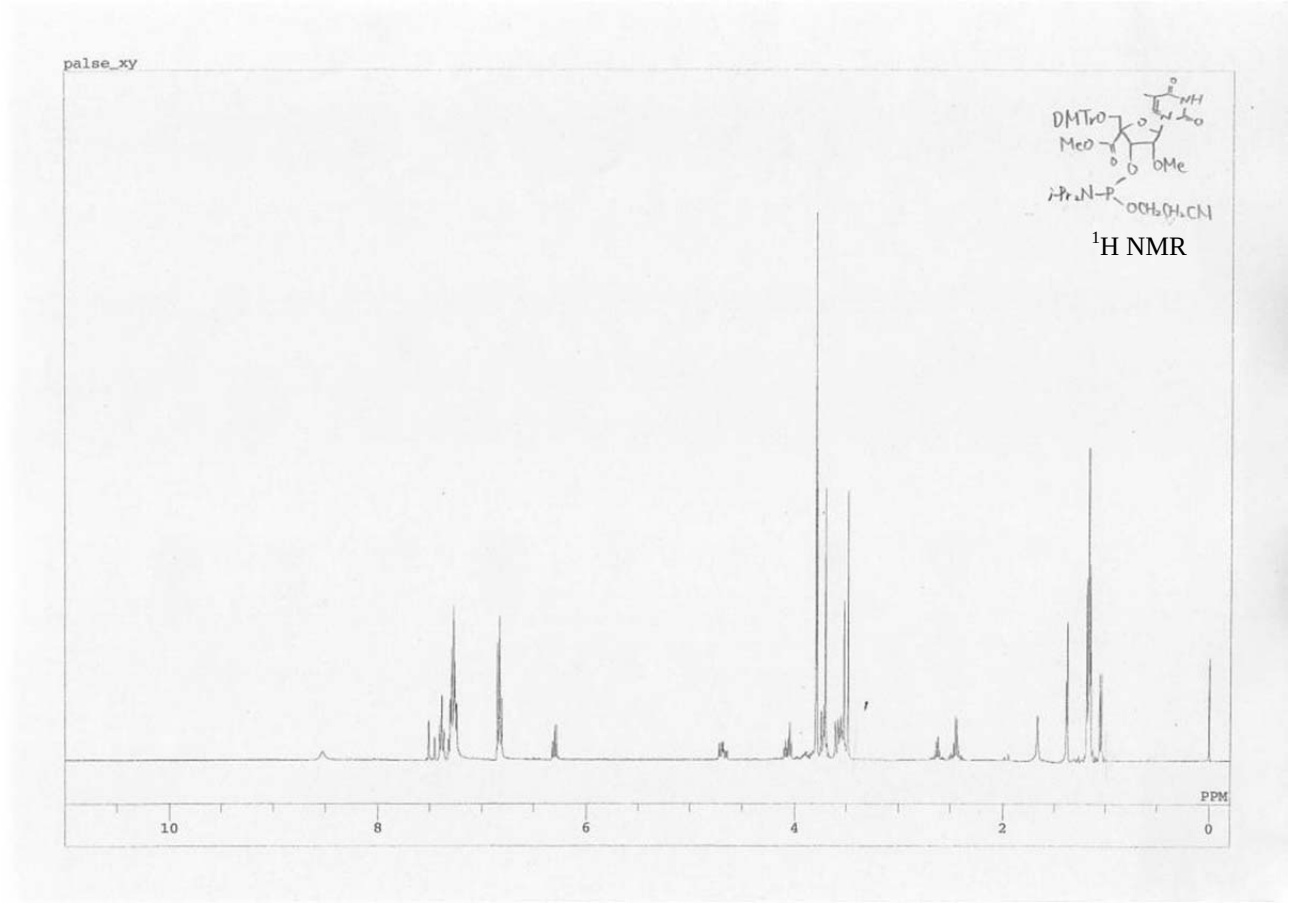
^{13}C NMR

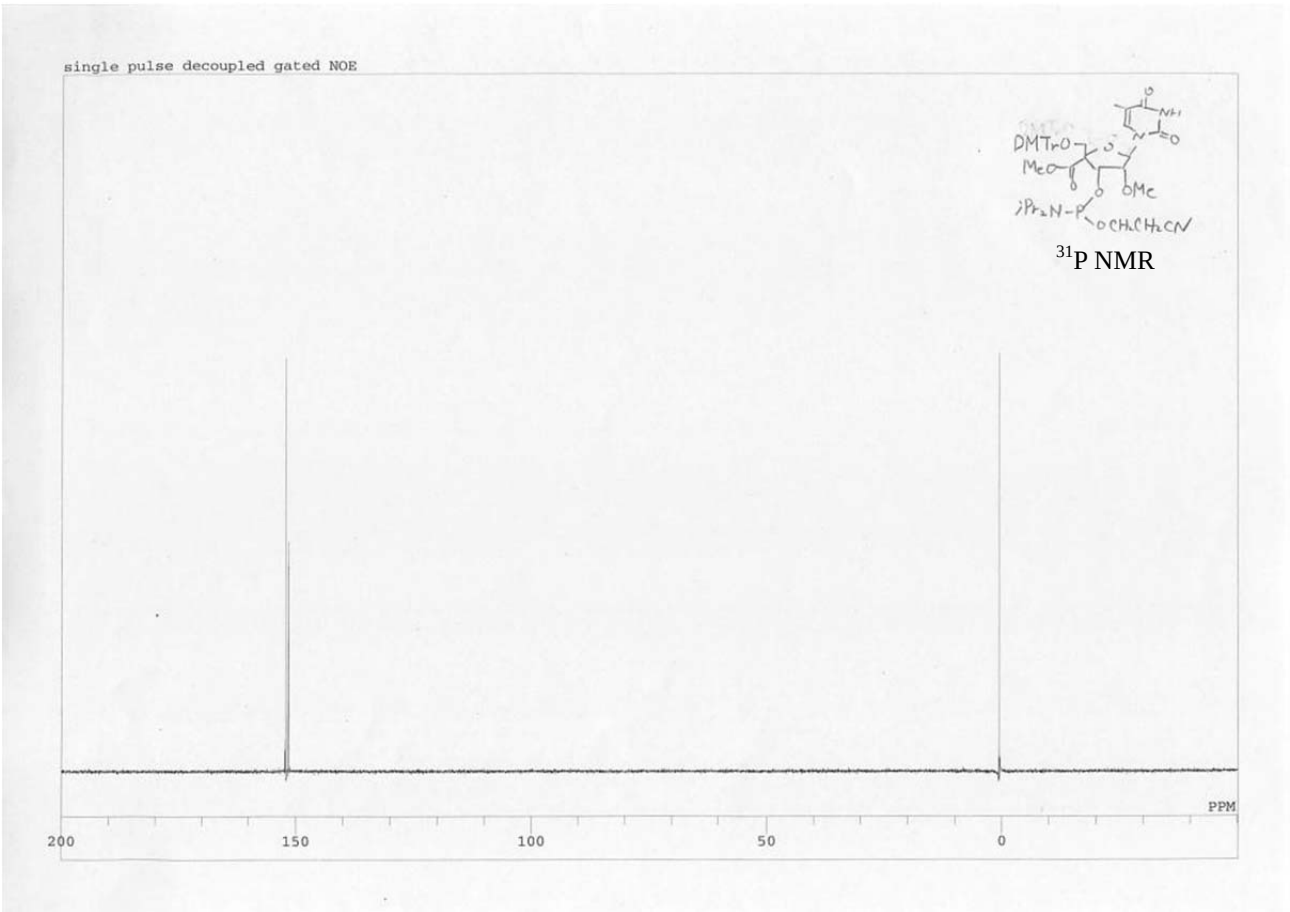
Compound **17**


single_pulse
¥¥172.20.1.201¥data¥yakka¥osawa¥120825-5-DMTrO-2-OMe-1.jdf
DMTrO
MeO
OH
OMe
^{1}H NMR
PPM
10
8
6
4
2
0

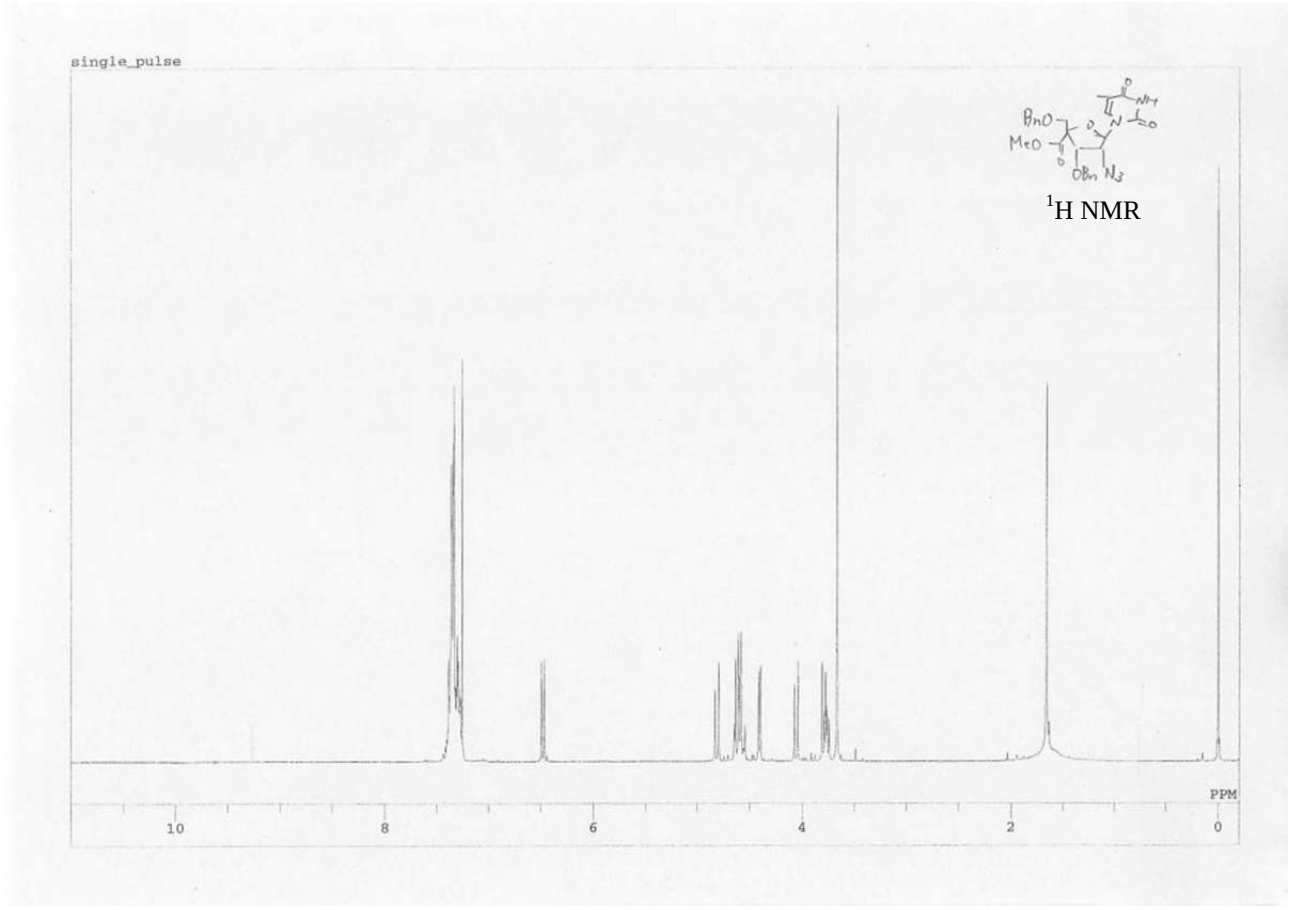

single pulse decoupled gated NOE
DMTrO
MeO
OH
OMe
^{13}C NMR
PPM
200
175
150
125
100
75
50
25
0

Compound **2** (diastereoisomers on the basis of chirality at the phosphorus)

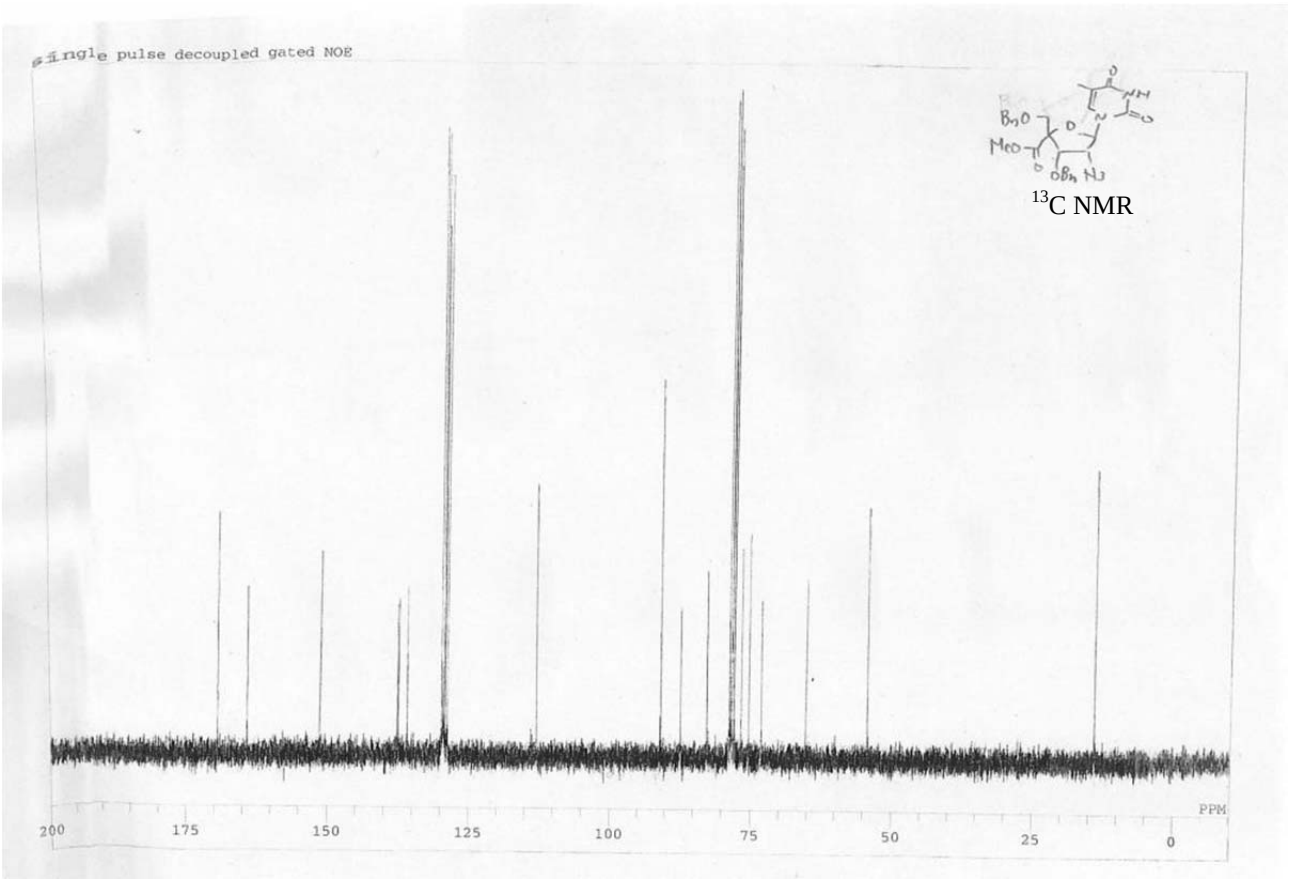




Compound **19**

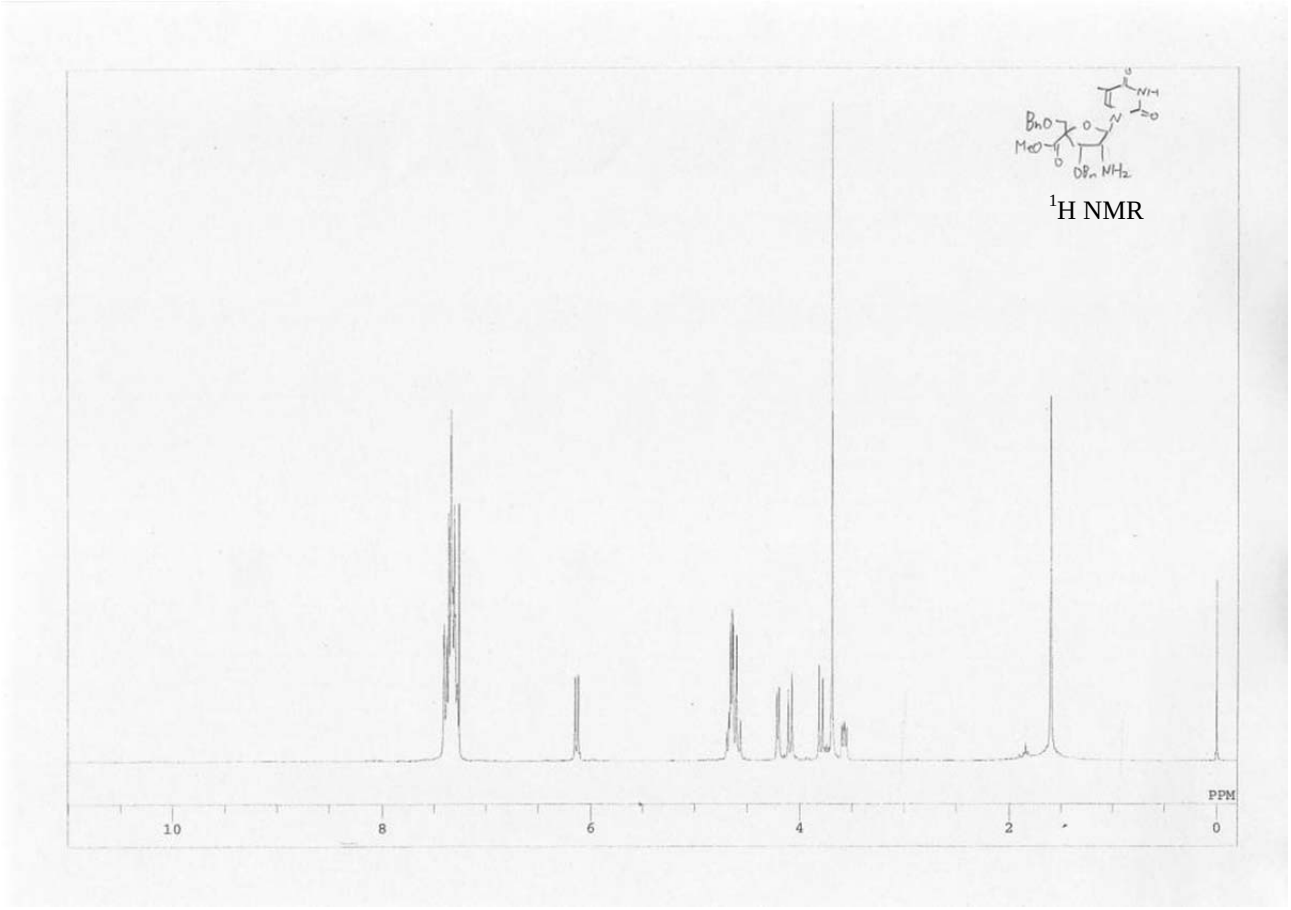


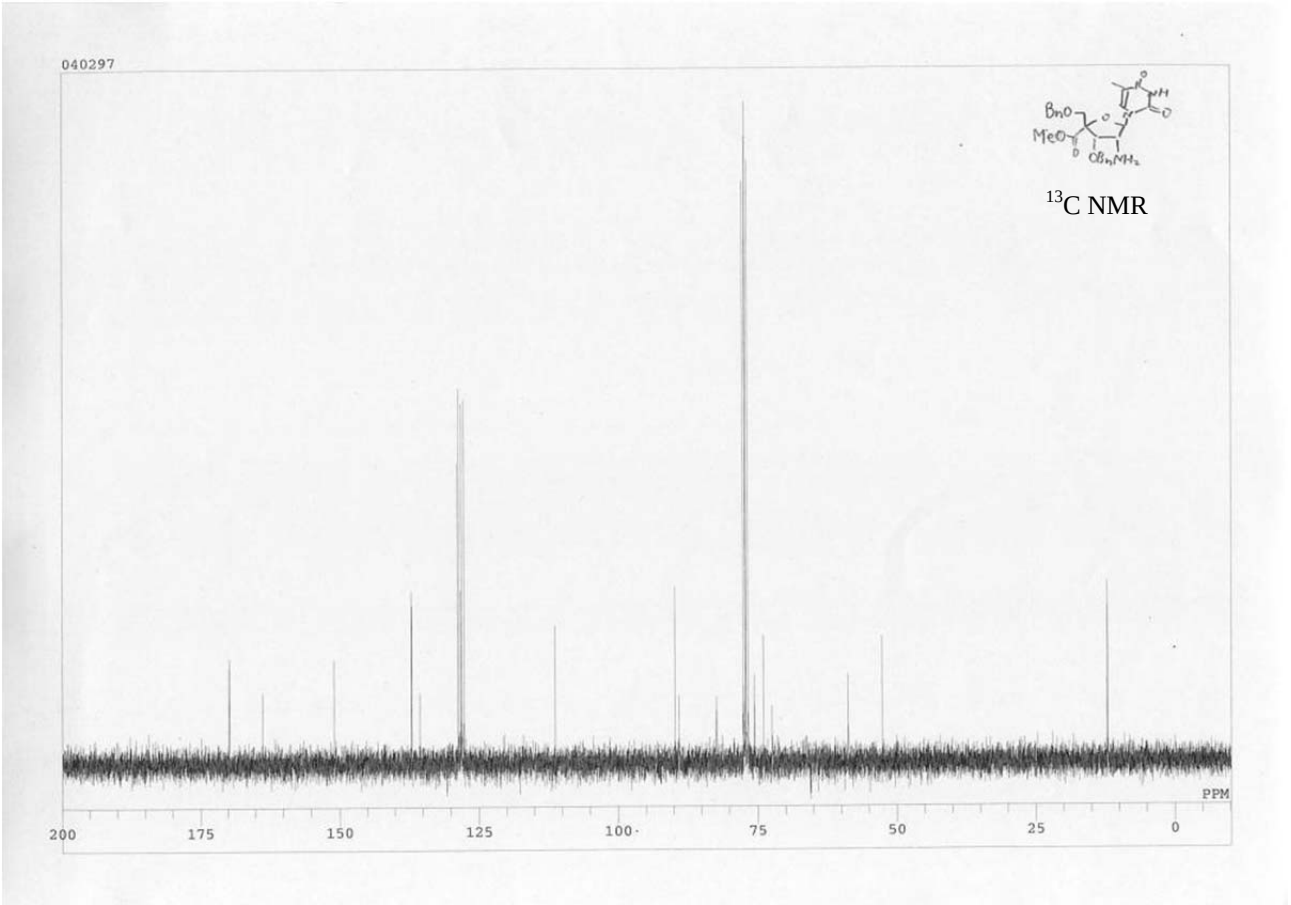
^{1}H NMR



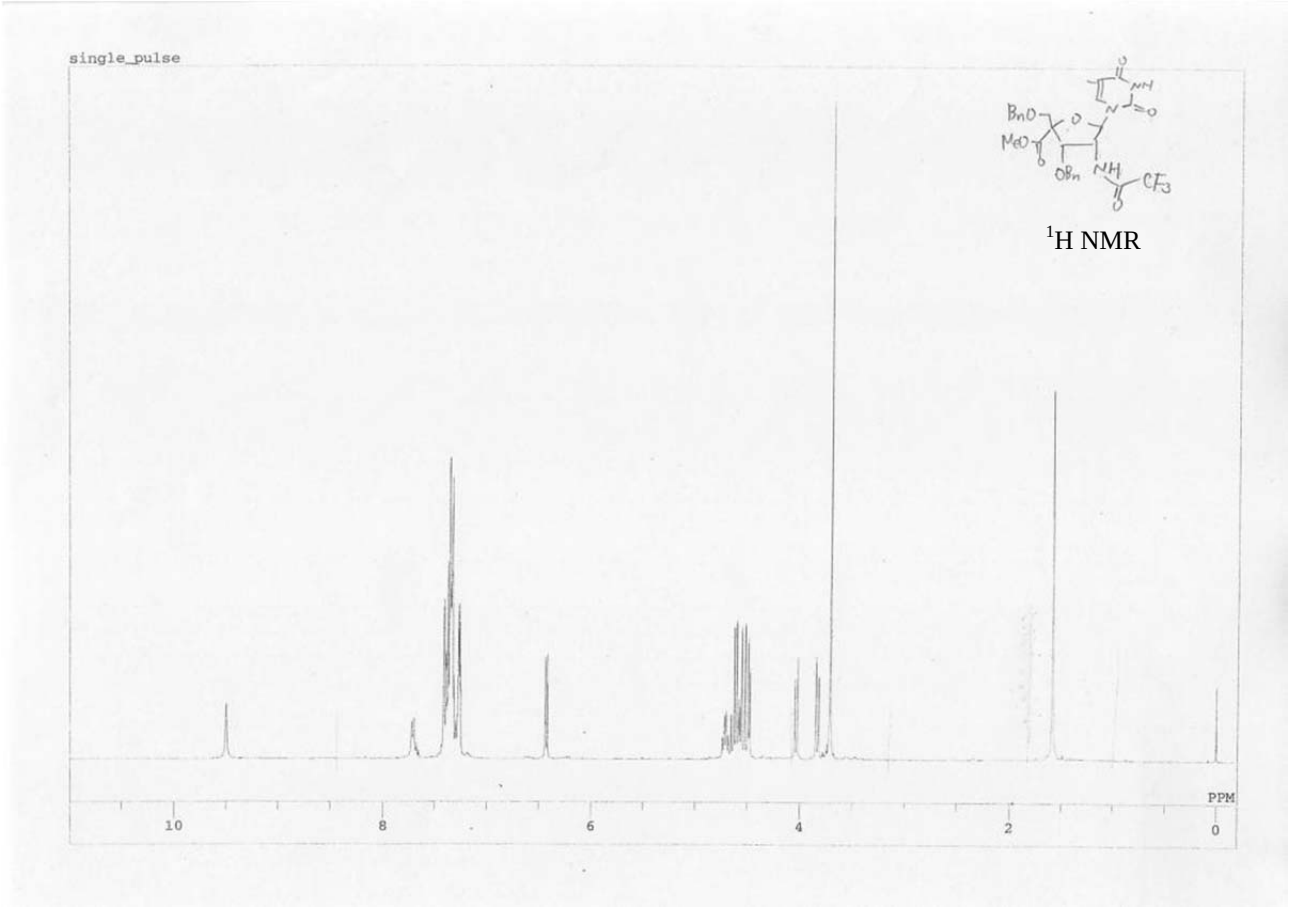
^{13}C NMR

Compound **20**

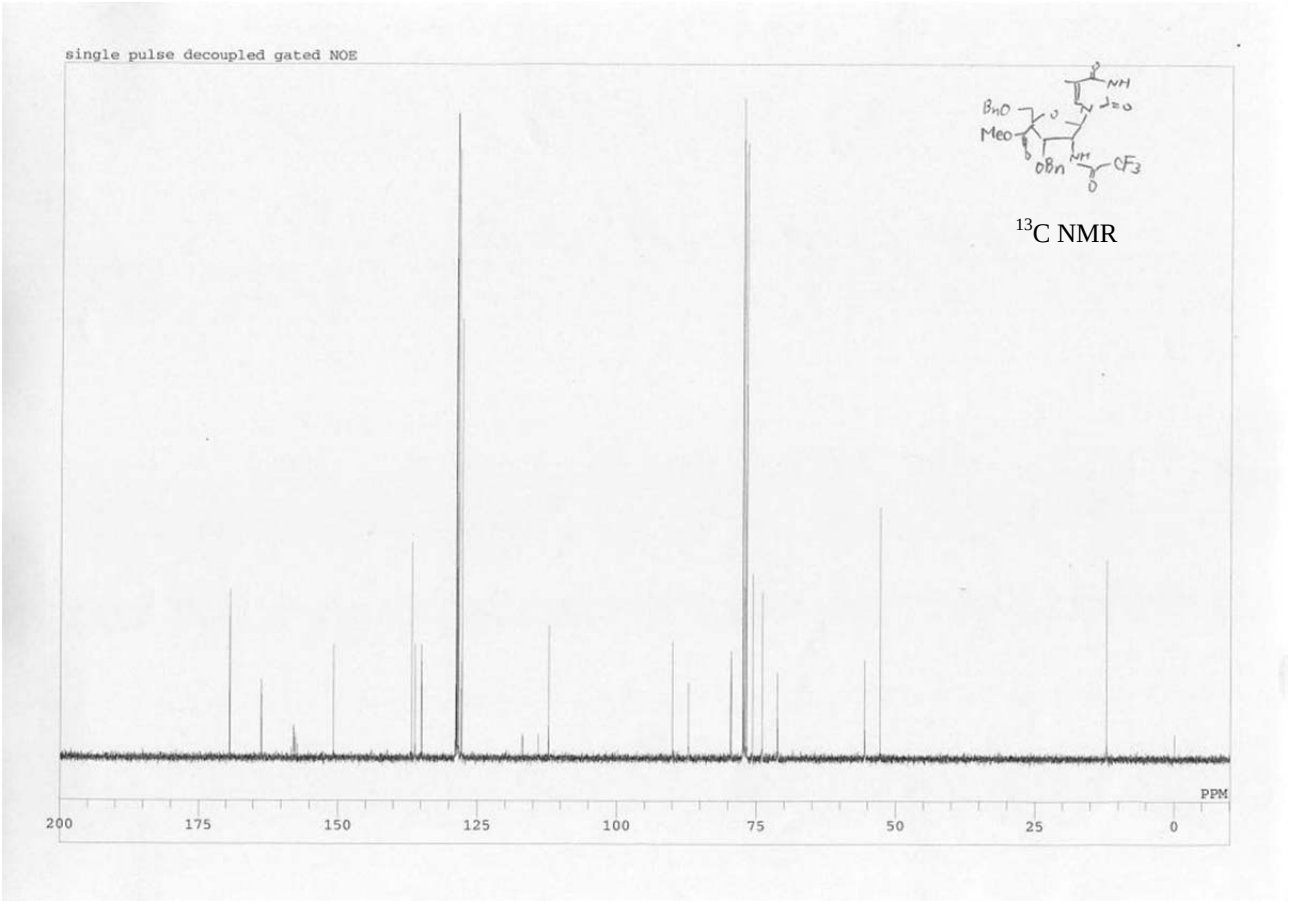

^{1}H NMR
PPM
10
8
6
4
2
0


040297
^{13}C NMR
PPM
200
175
150
125
100
75
50
25
0

Compound **21**

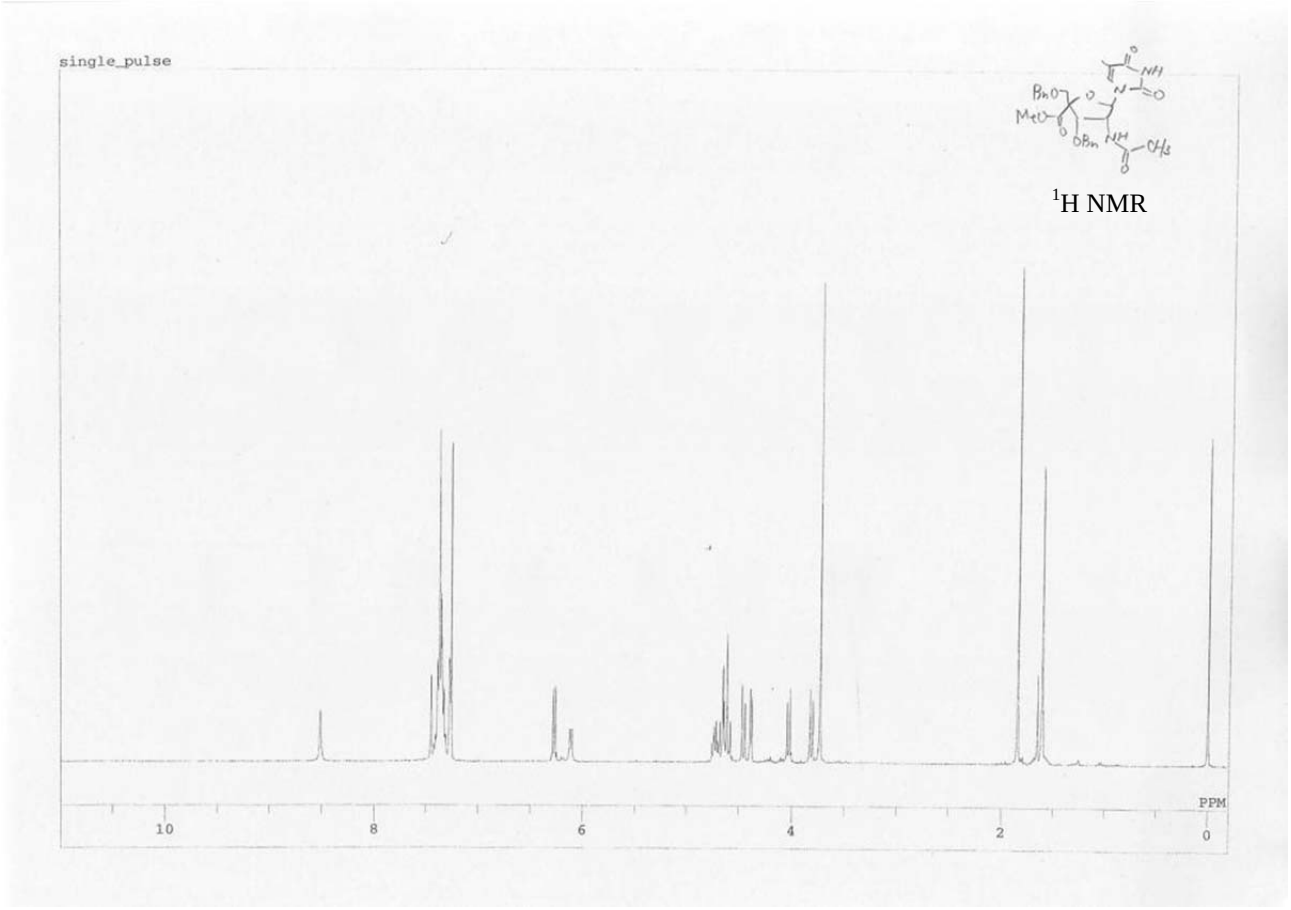


^{1}H NMR

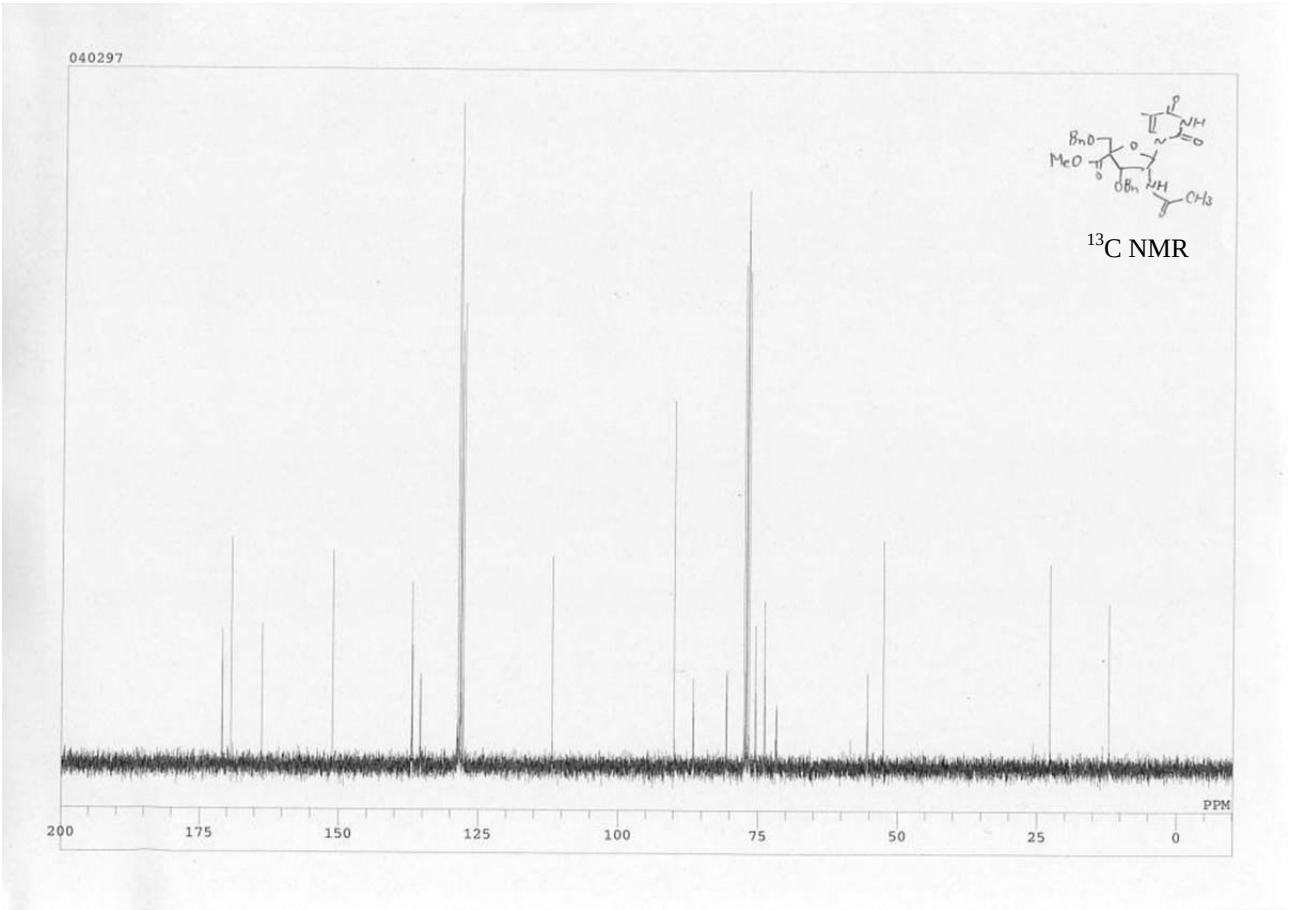


^{13}C NMR

Compound **22**

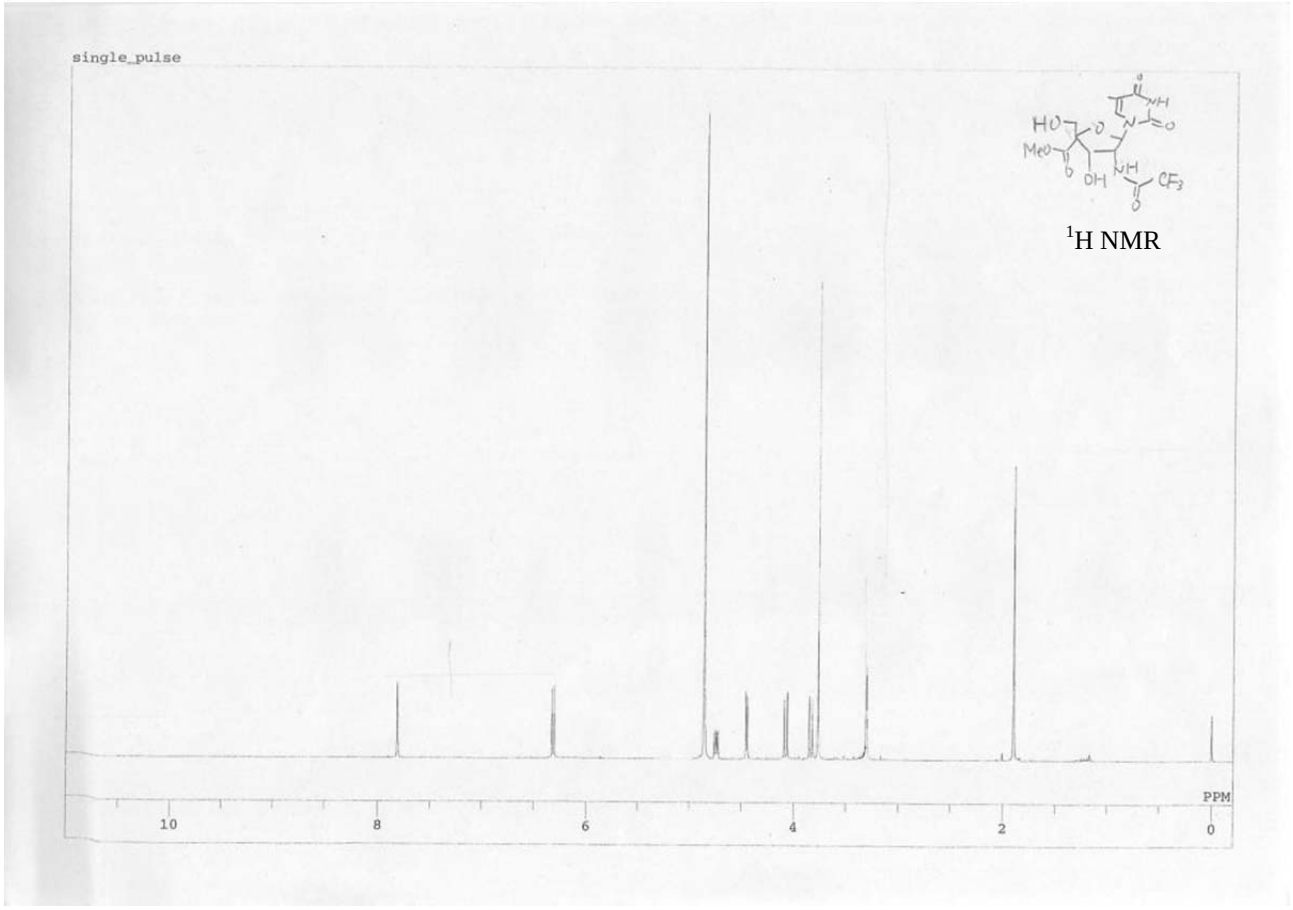


^{1}H NMR

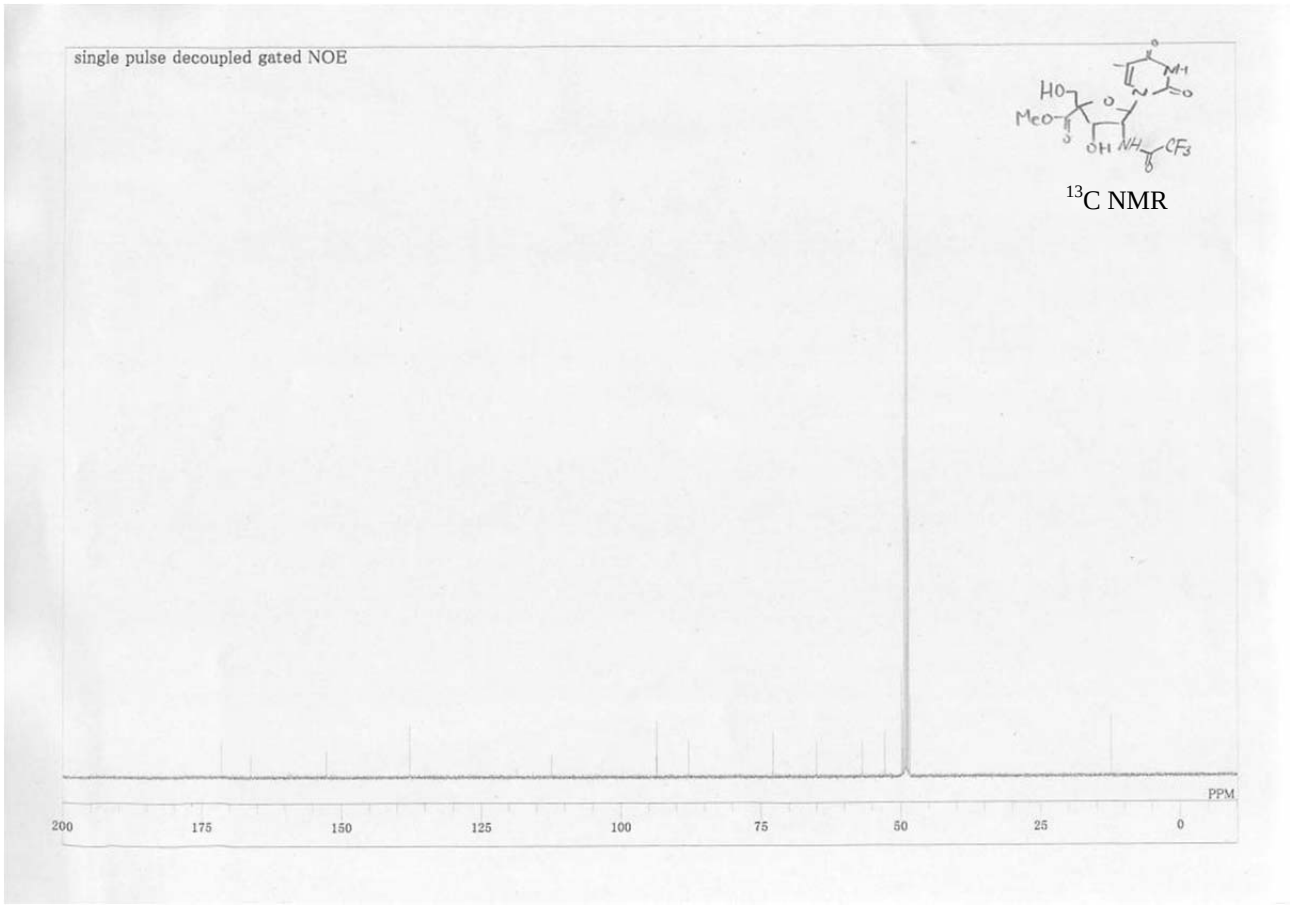


^{13}C NMR

Compound **23**

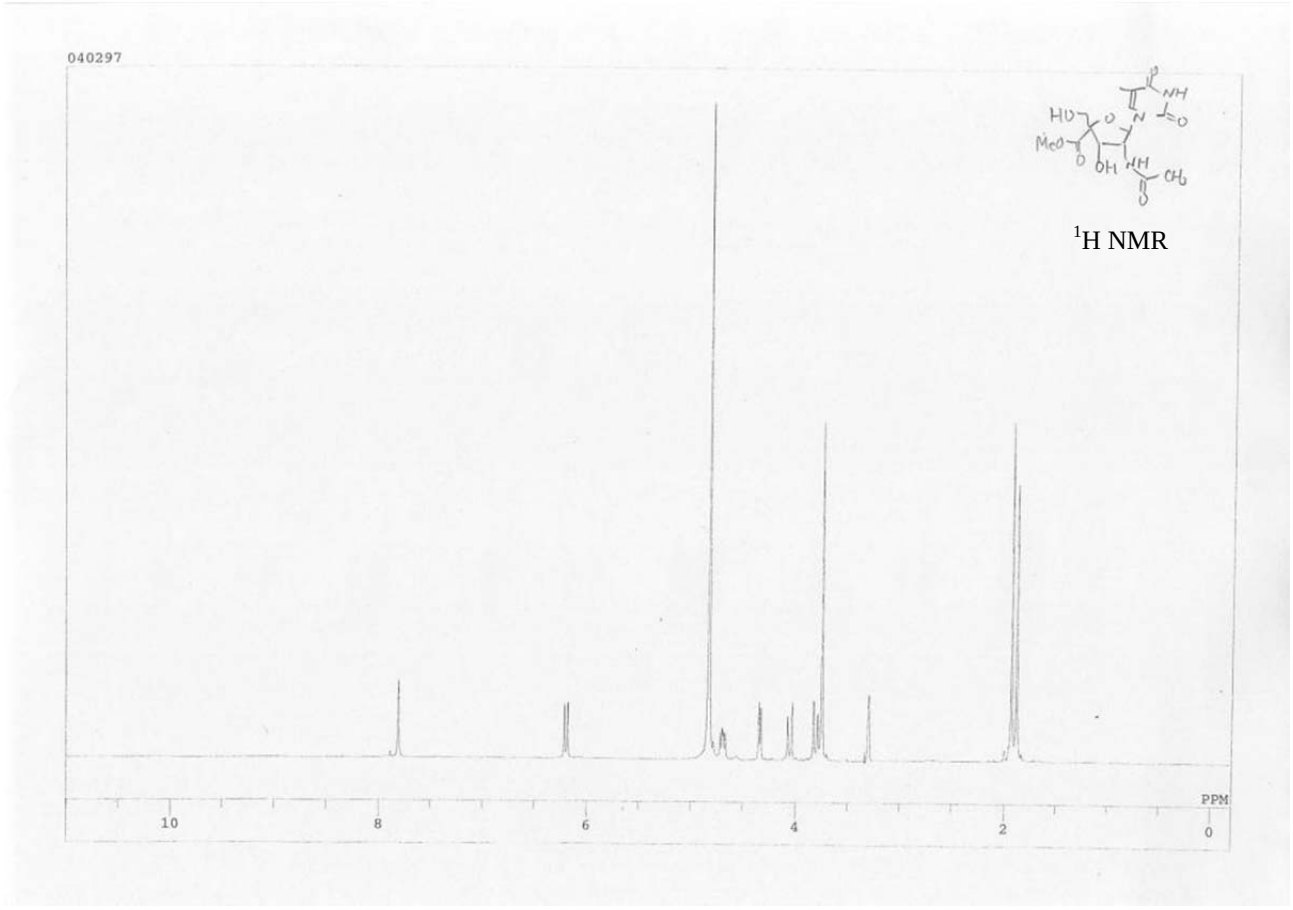


^{1}H NMR

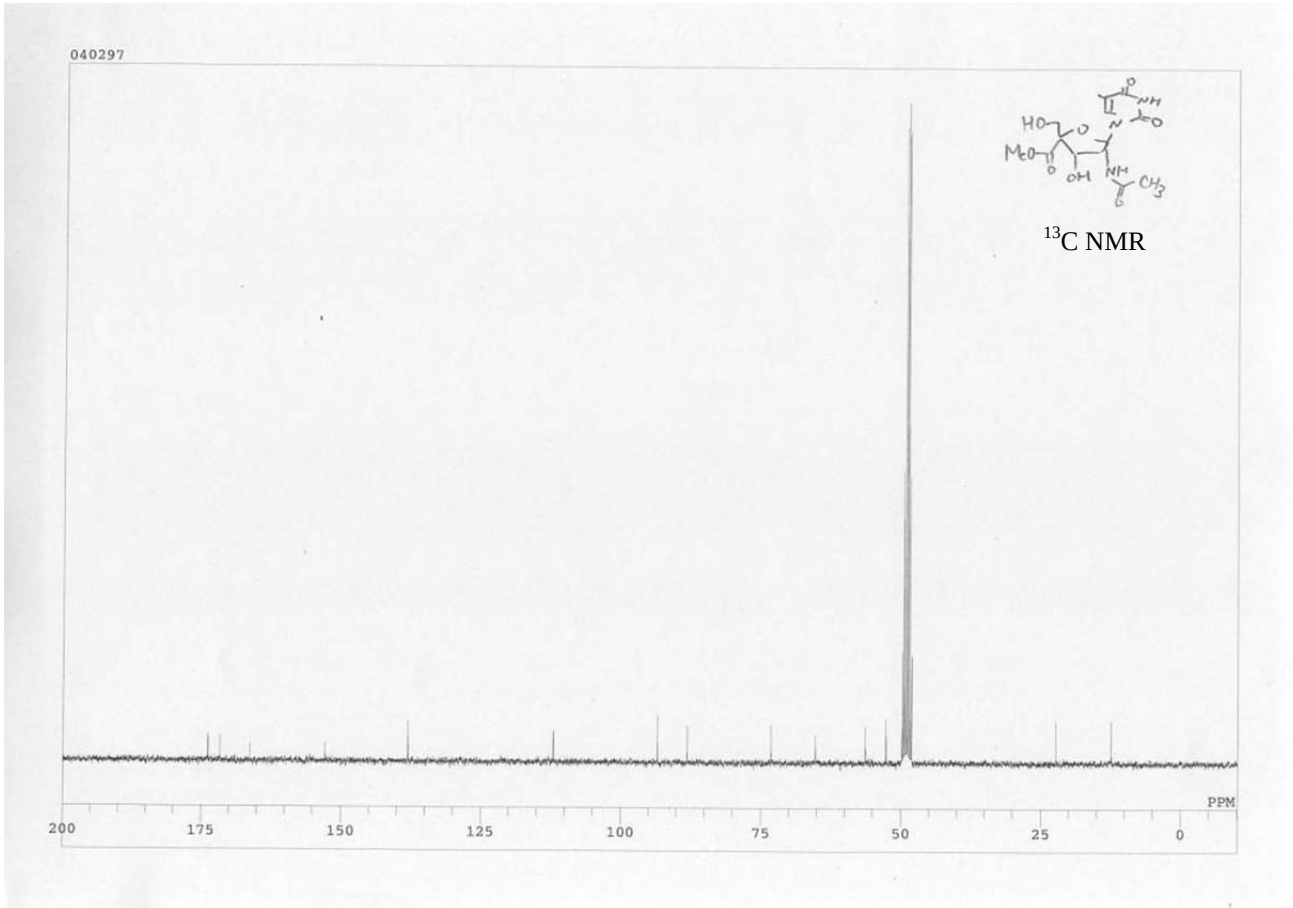


^{13}C NMR

Compound **24**

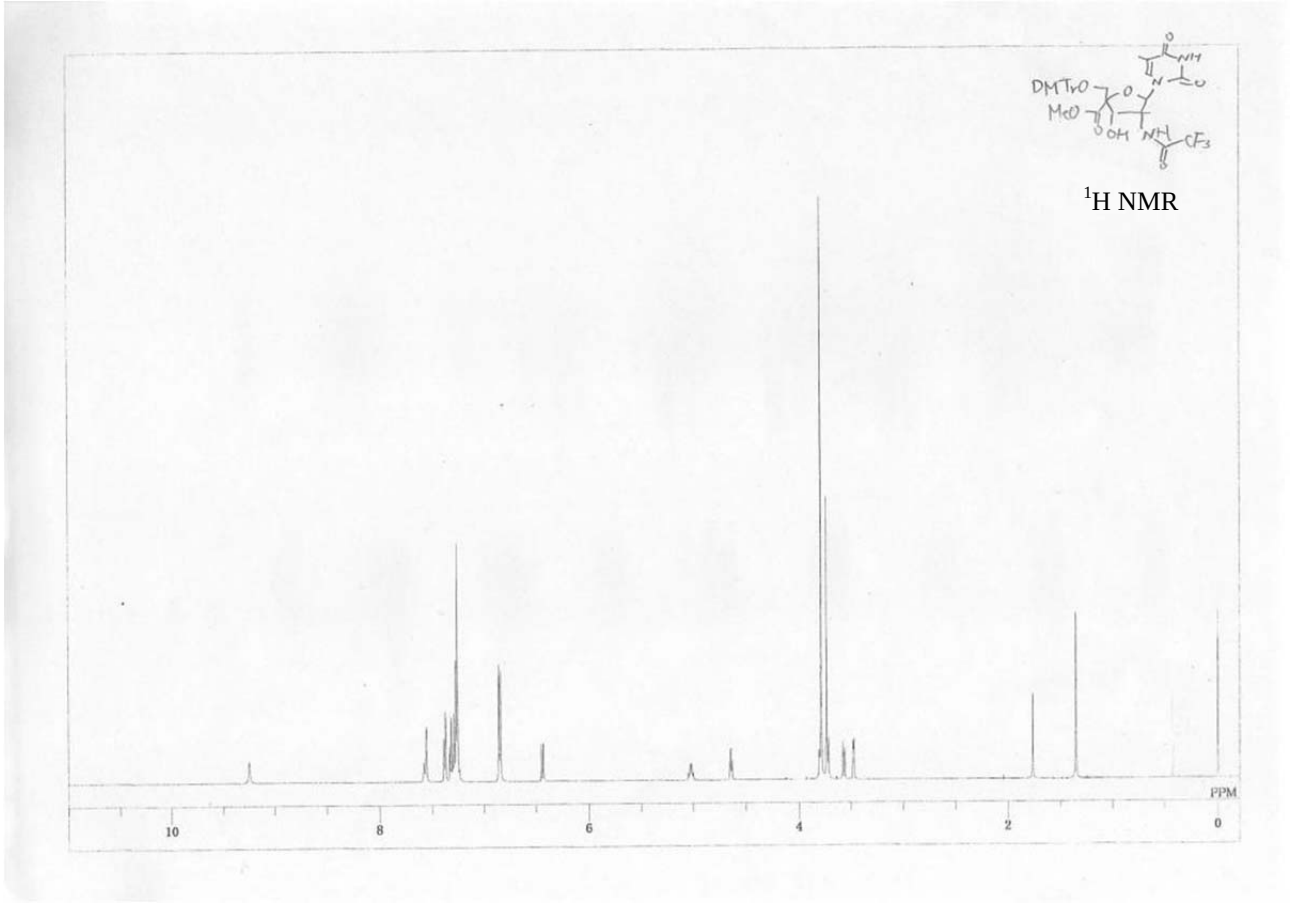


^{1}H NMR

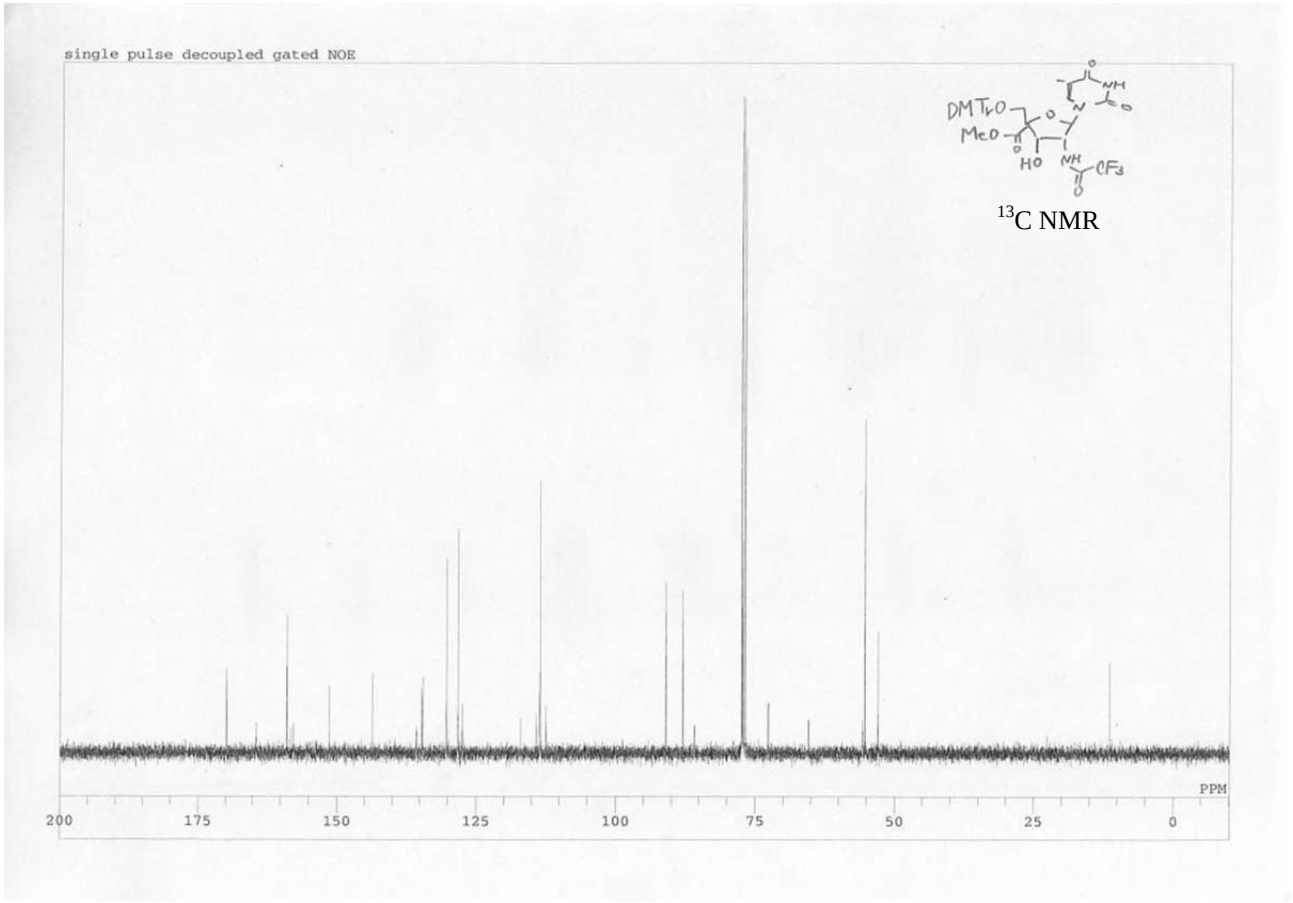


^{13}C NMR

Compound **25**

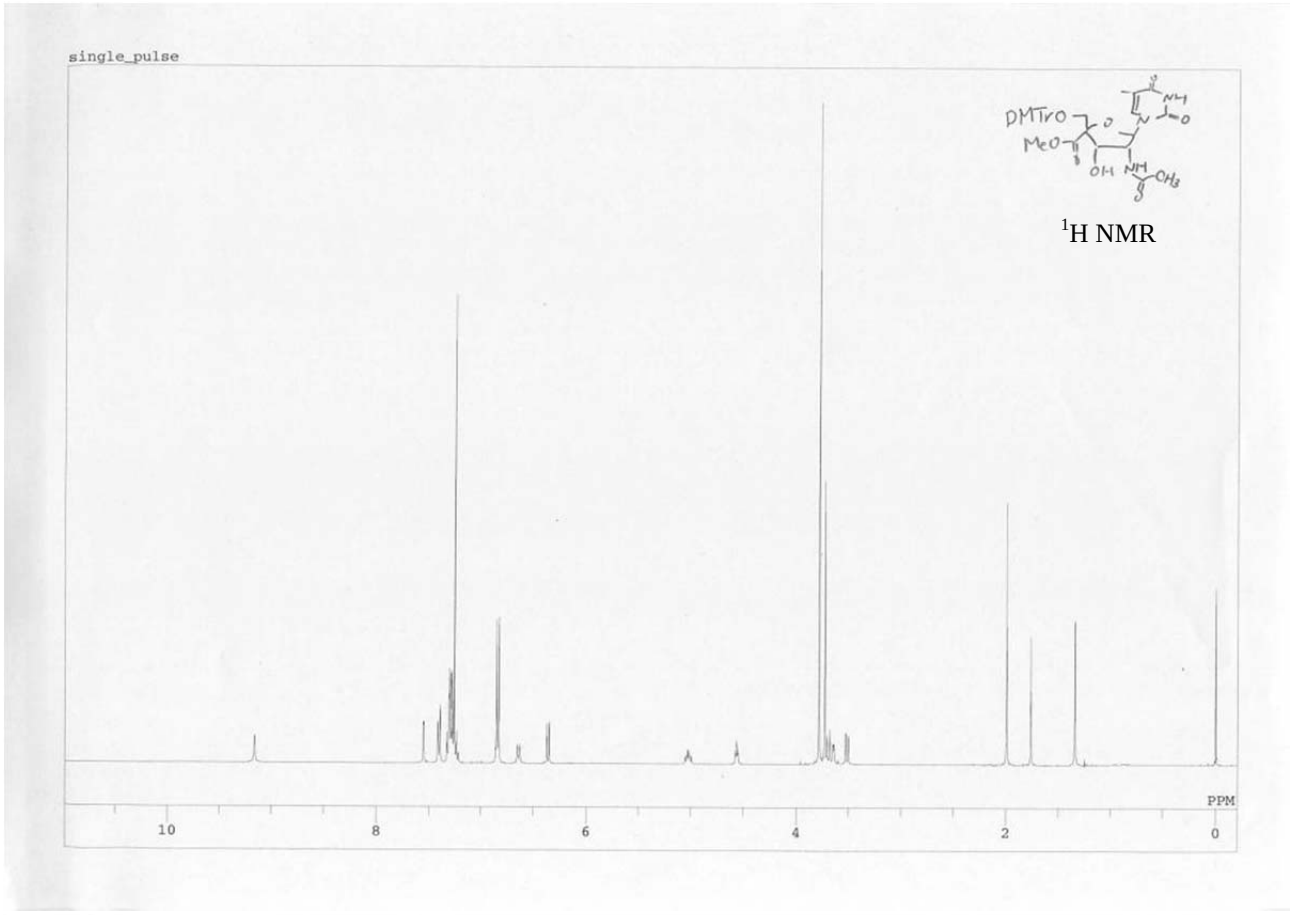


^{1}H NMR

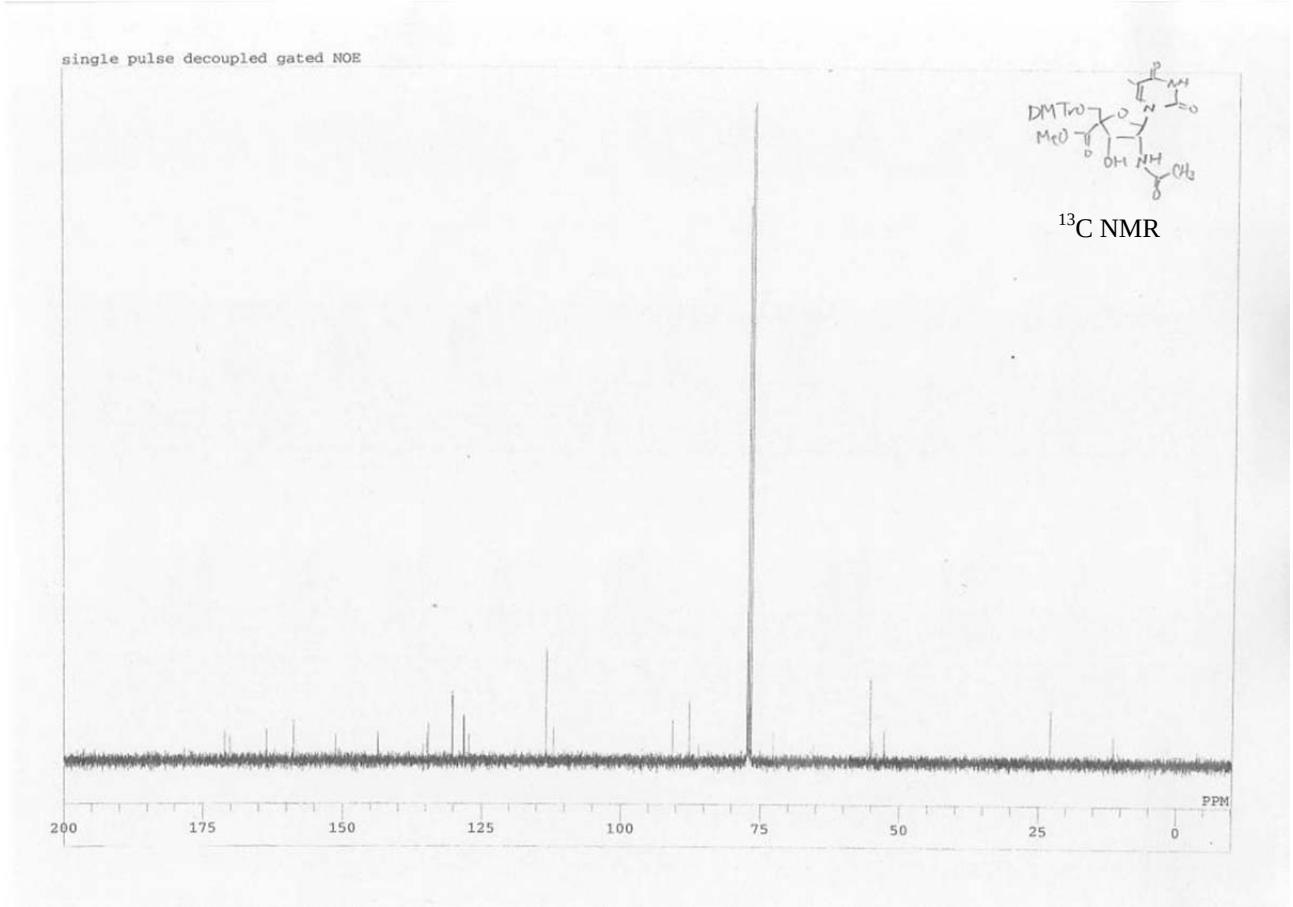


^{13}C NMR

Compound **26**

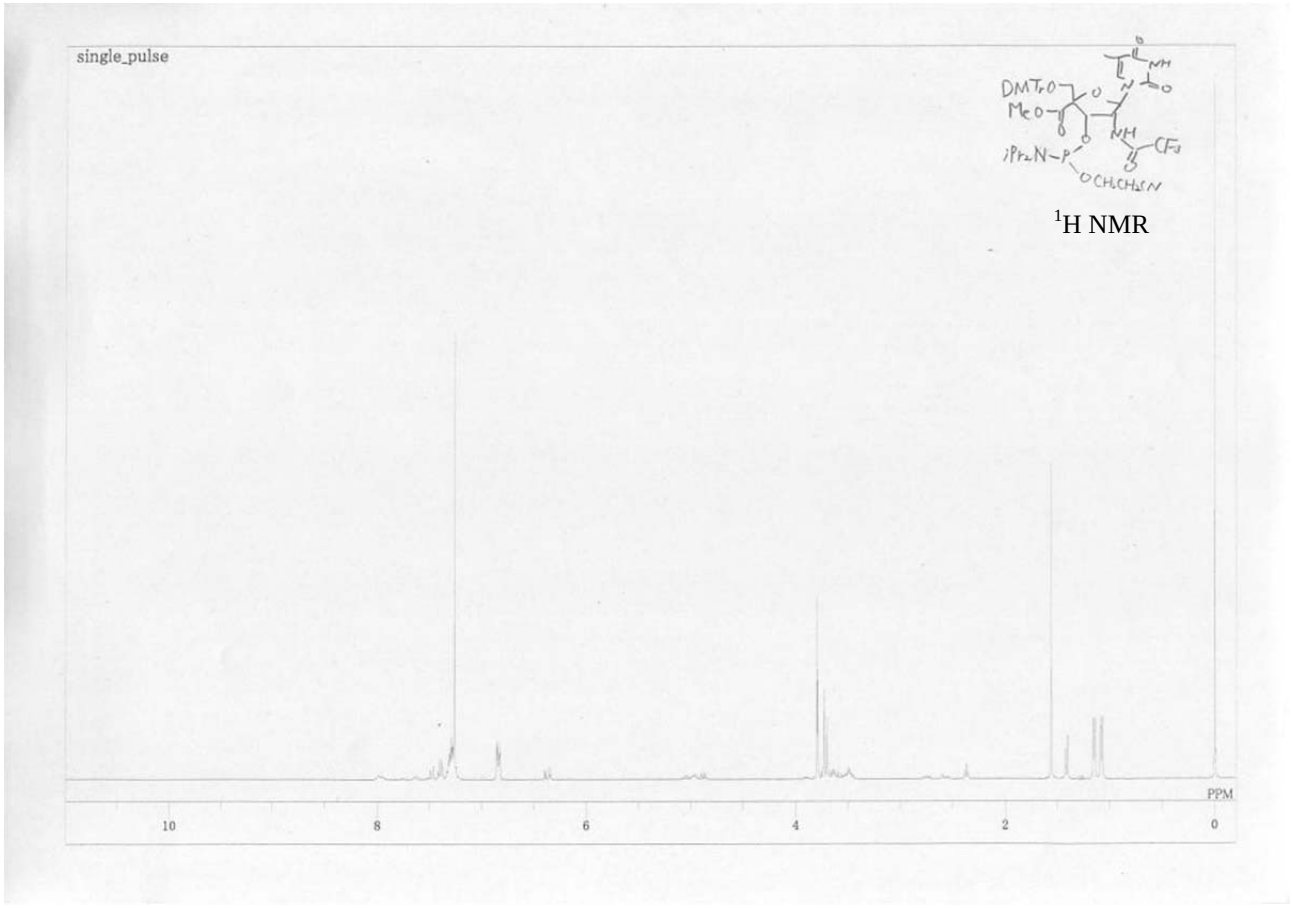


^{1}H NMR

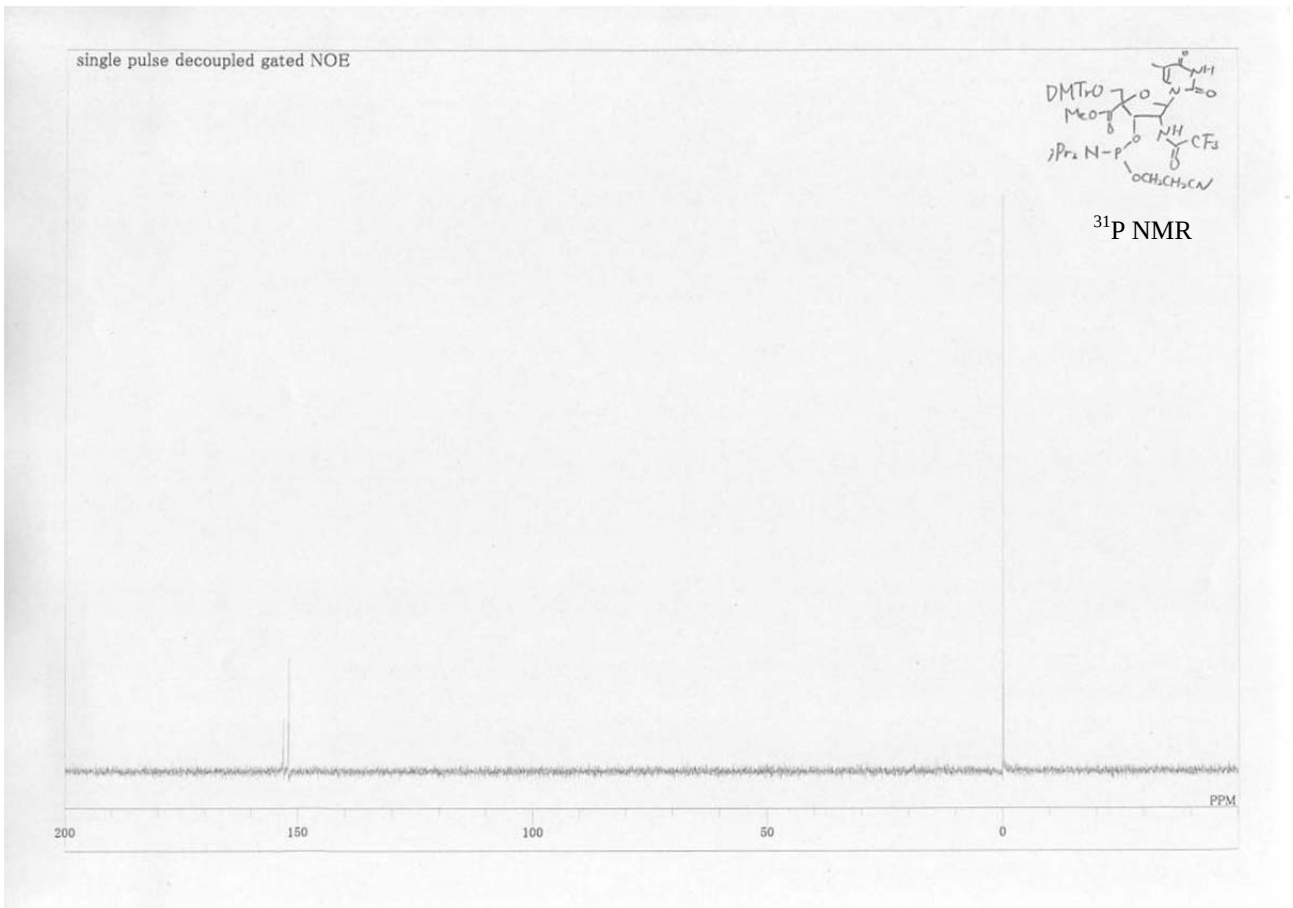


^{13}C NMR

Compound **3** (diastereoisomers on the basis of chirality at the phosphorus)

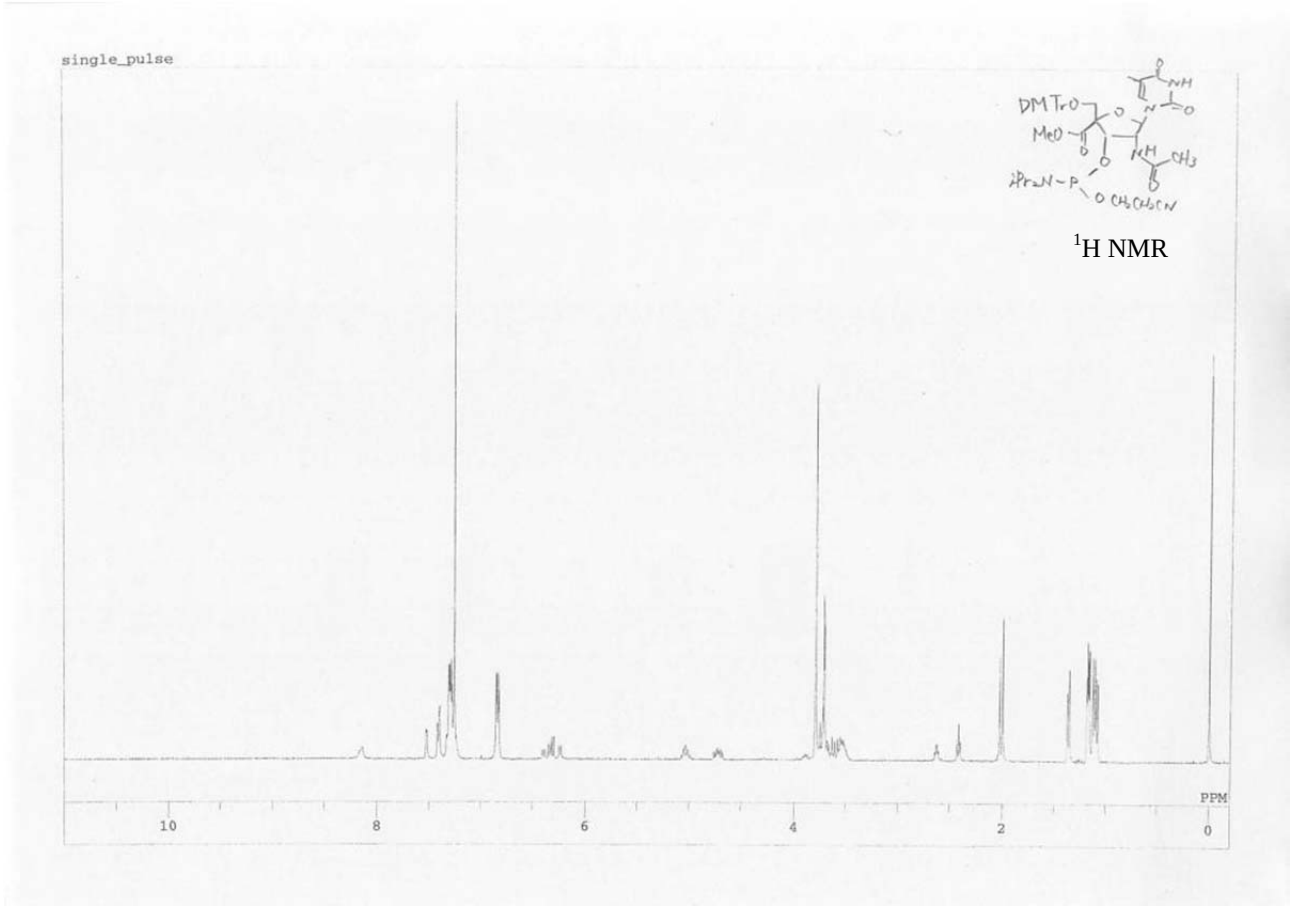


^{1}H NMR

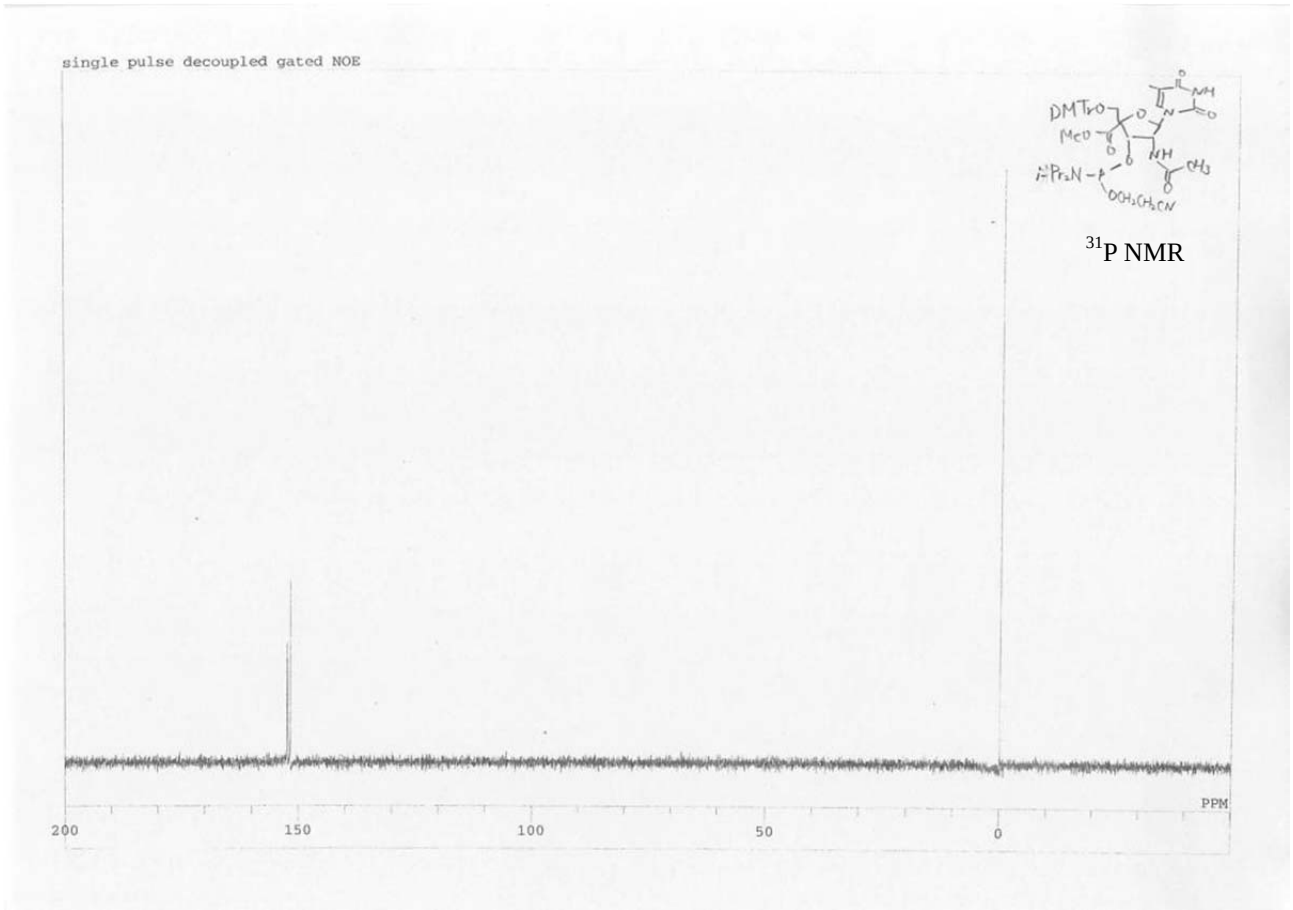


^{31}P NMR

Compound **4** (diastereoisomers on the basis of chirality at the phosphorus)

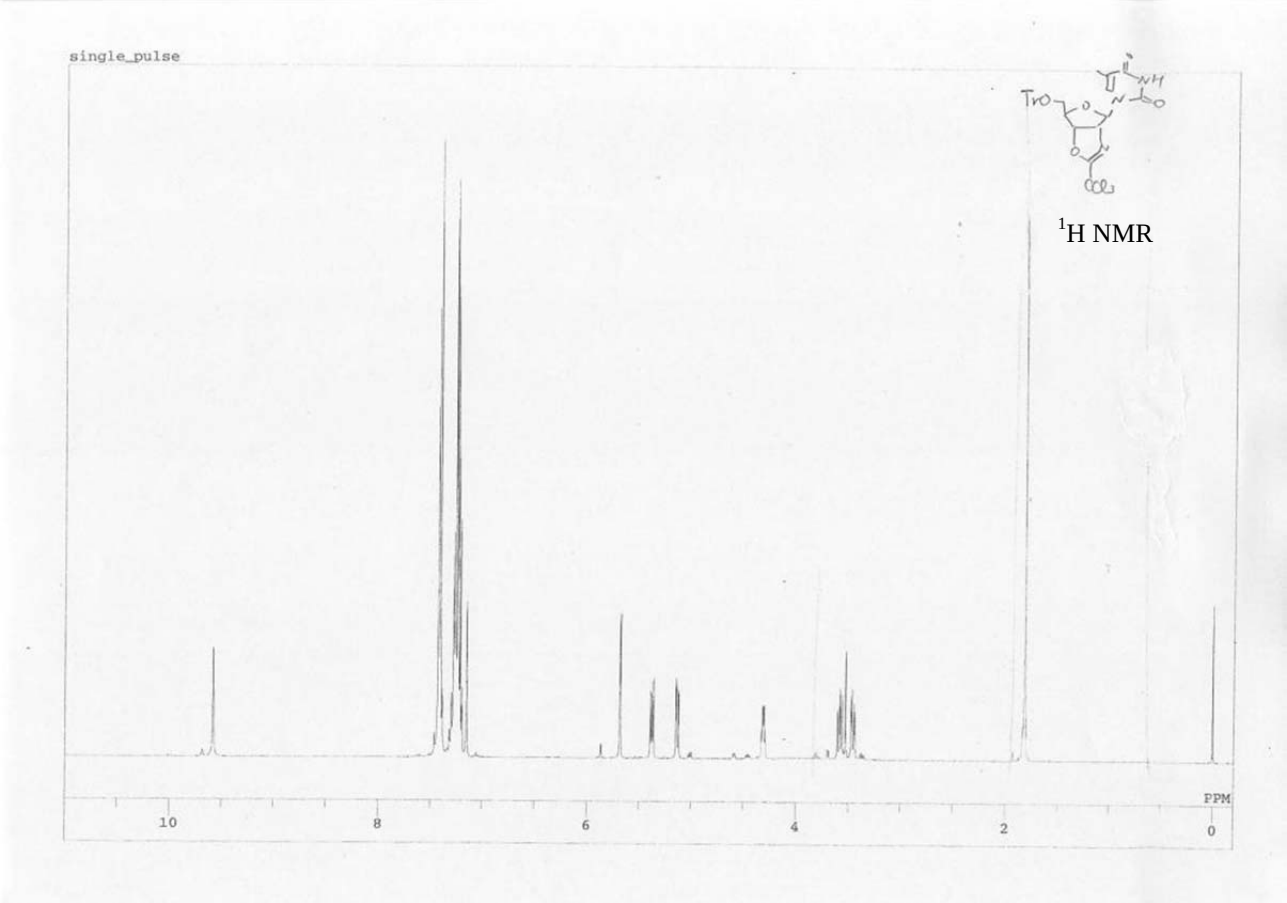


^{1}H NMR

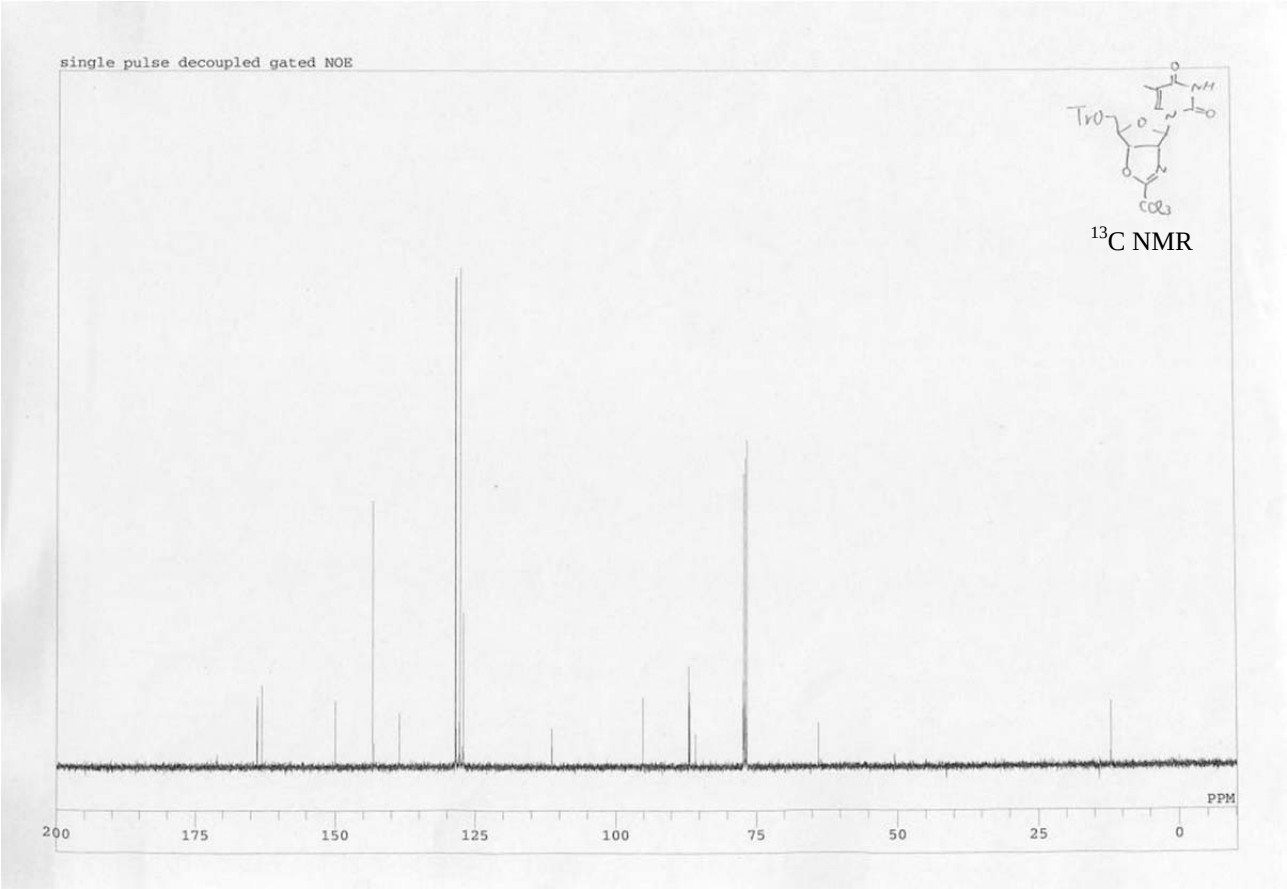


^{31}P NMR

Compound **S2**

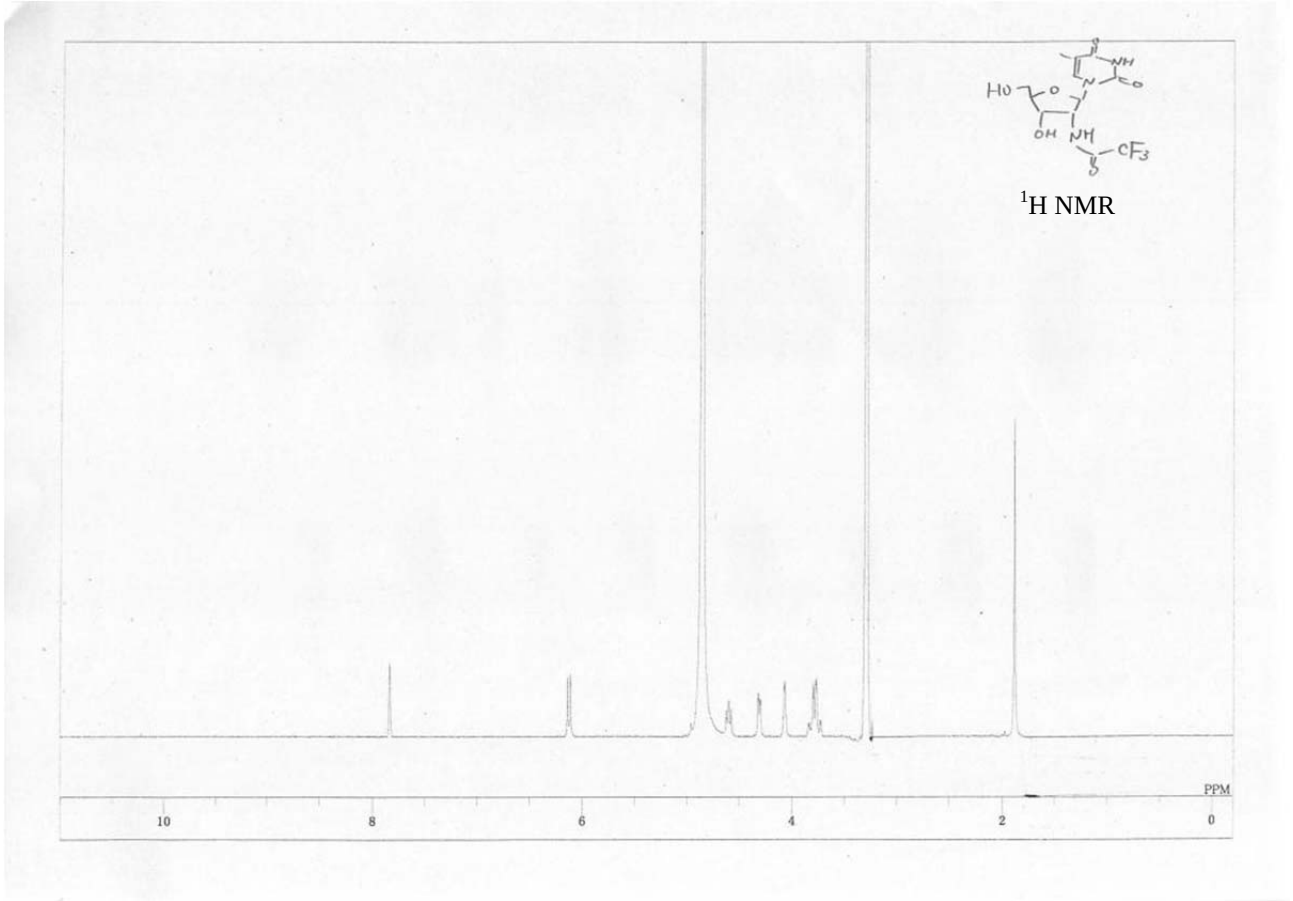


^{1}H NMR

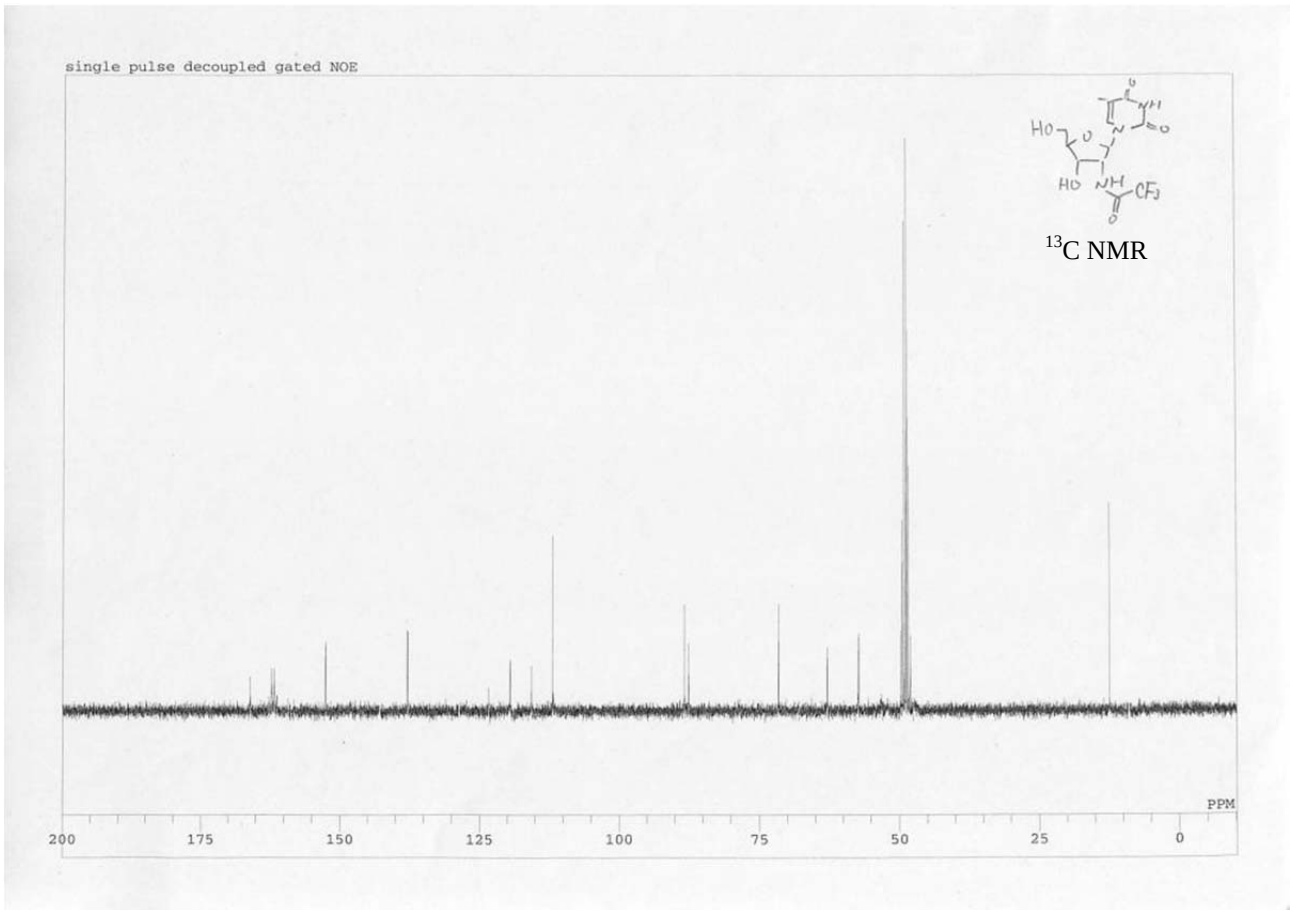


^{13}C NMR

Compound **S3**

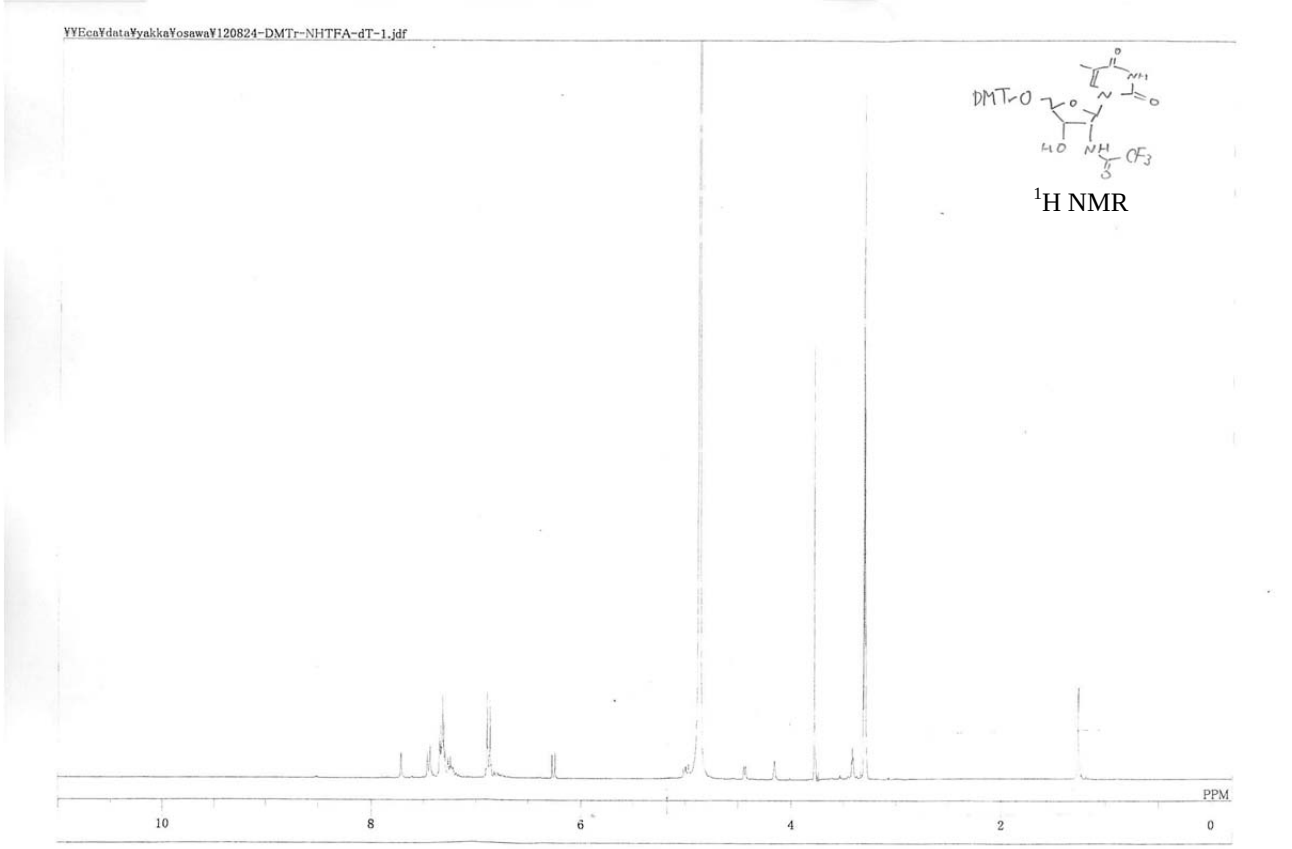


^{1}H NMR

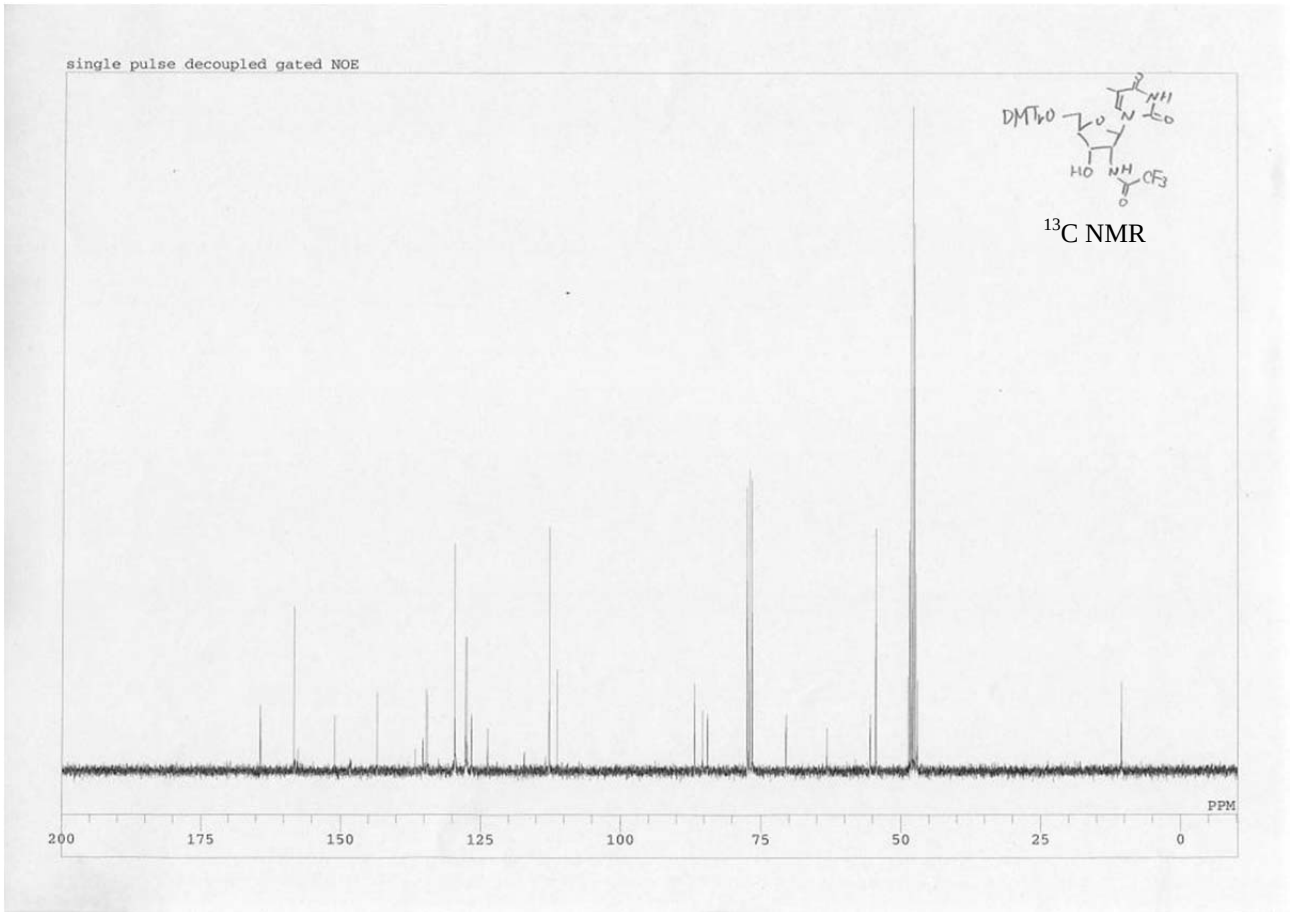


^{13}C NMR

Compound **S4**



^{1}H NMR



^{13}C NMR

Compound **S5** (diastereoisomers on the basis of chirality at the phosphorus)