Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

A 4-[(3*R*,4*R*)-dihydroxypyrrolidino]pyrimidin-2-one nucleobase for a CG base pair in triplex DNA

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Contents

TFO-2a

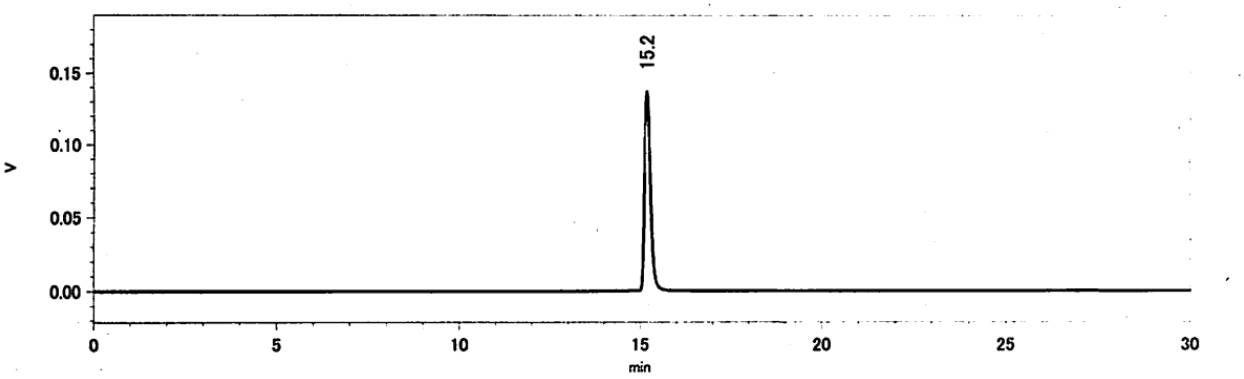
HPLC

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

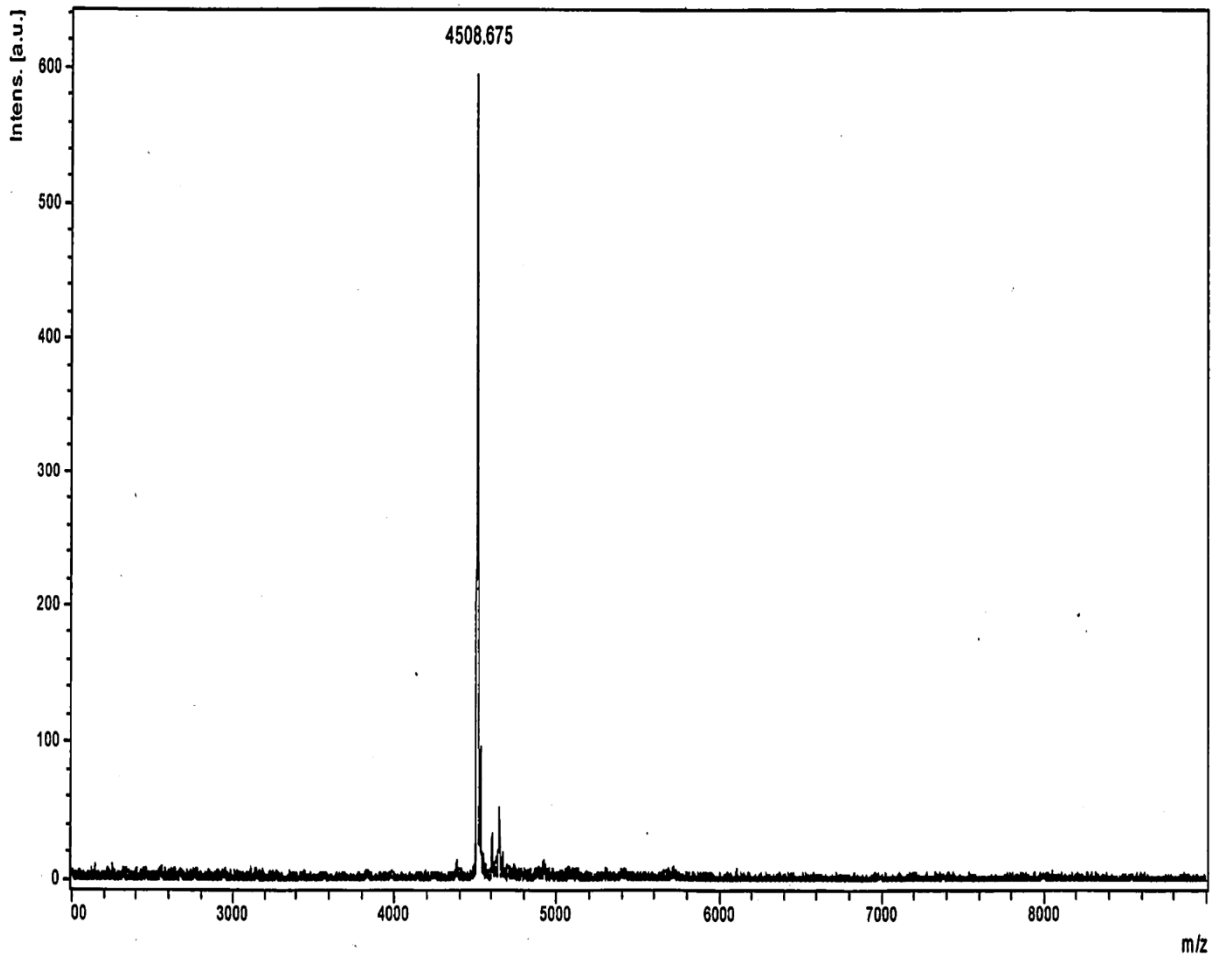
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2b

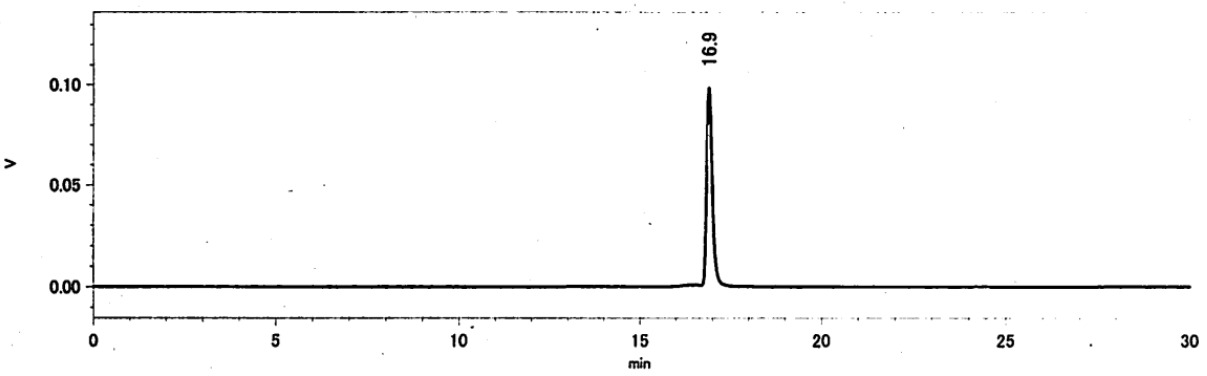
HPLC

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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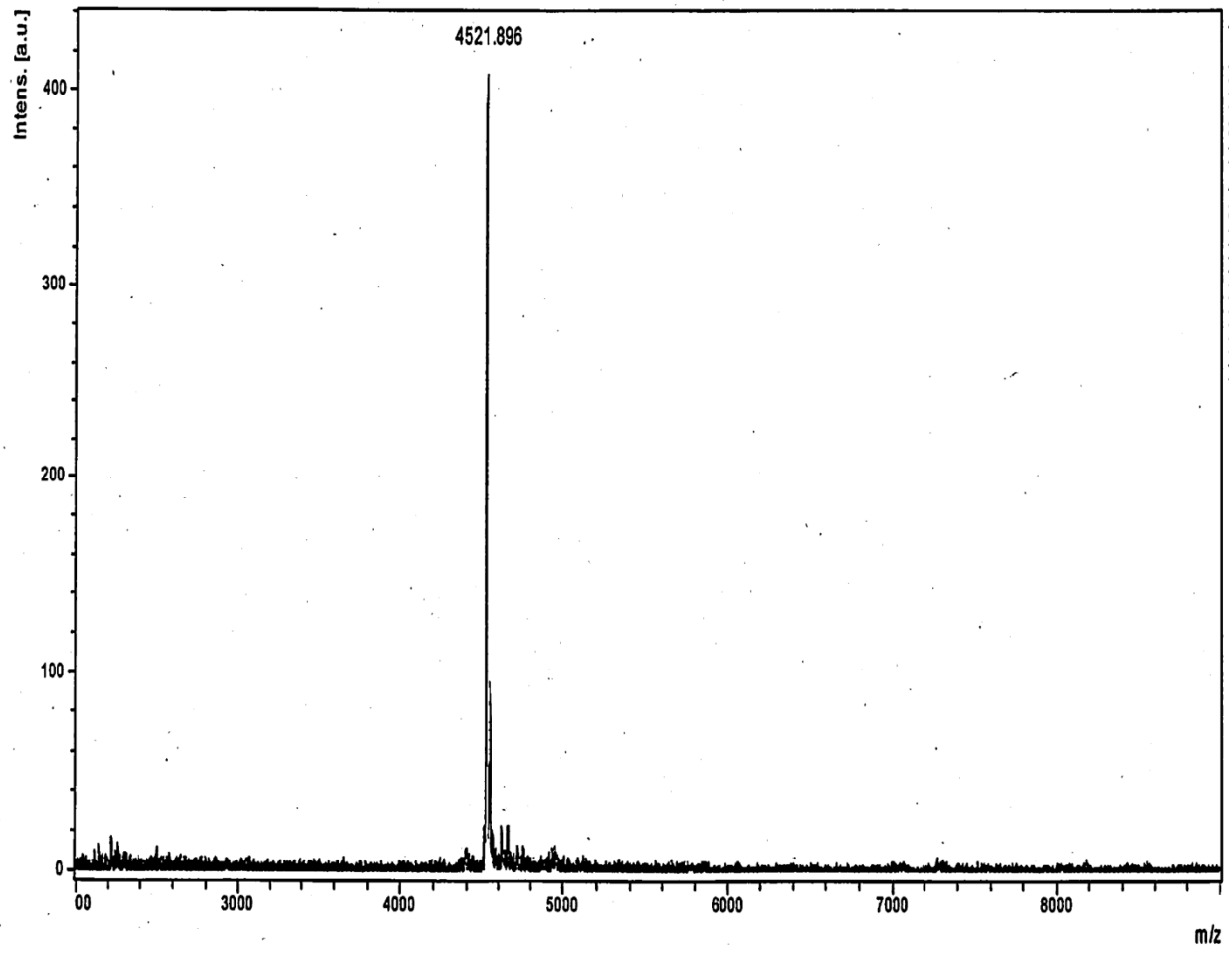


TFO-2c

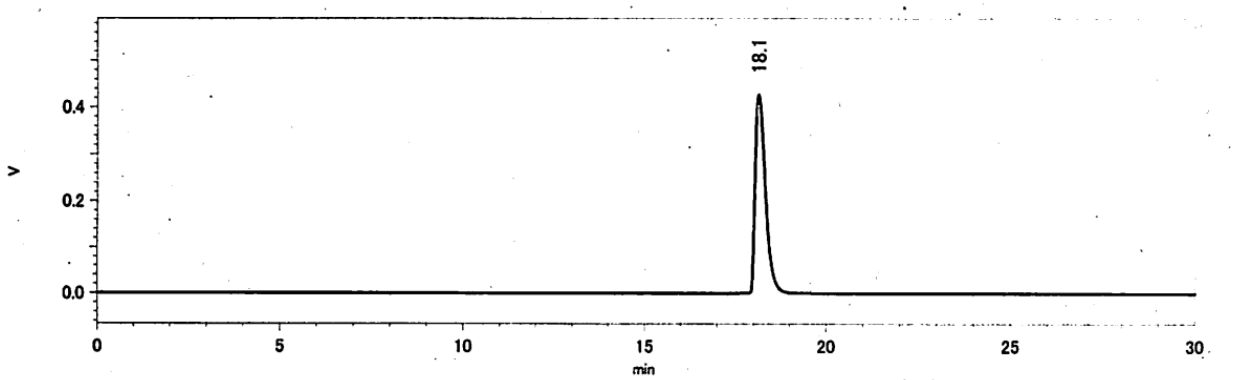
HPLC

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

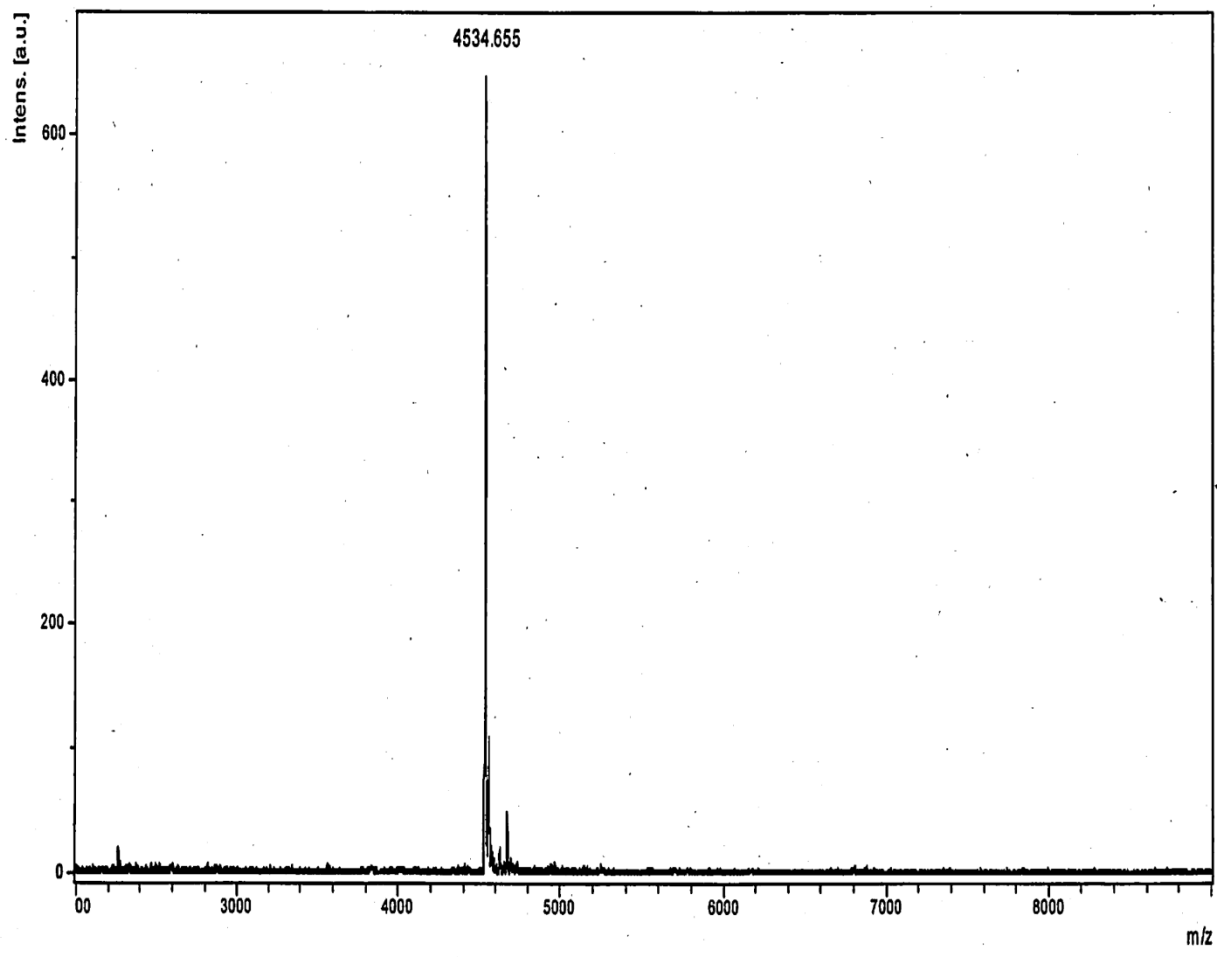
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2d

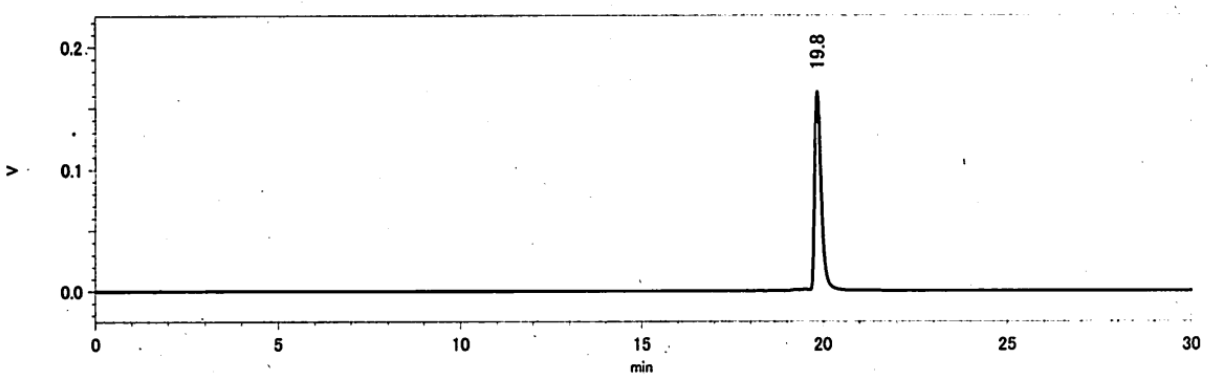
HPLC

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

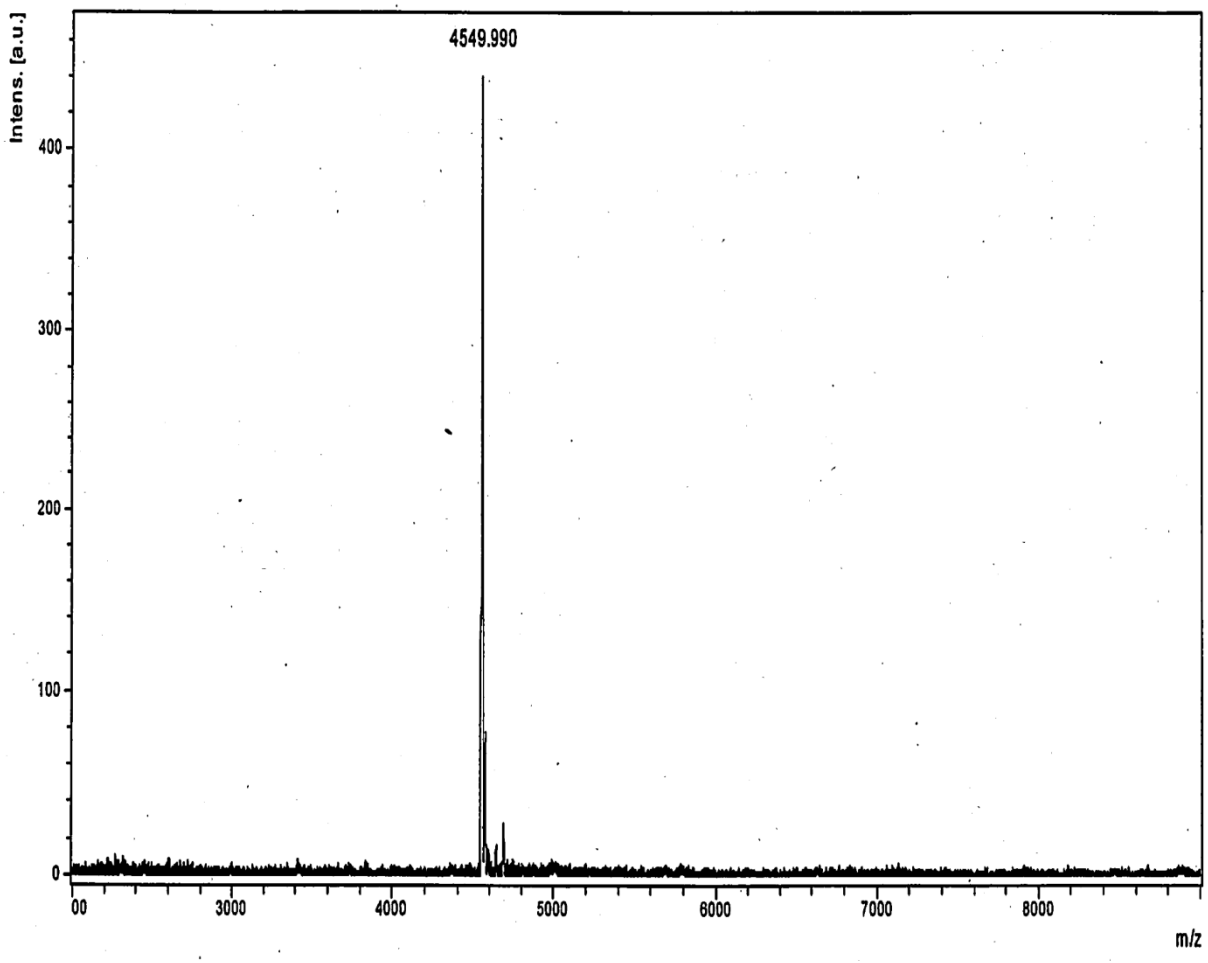
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2e

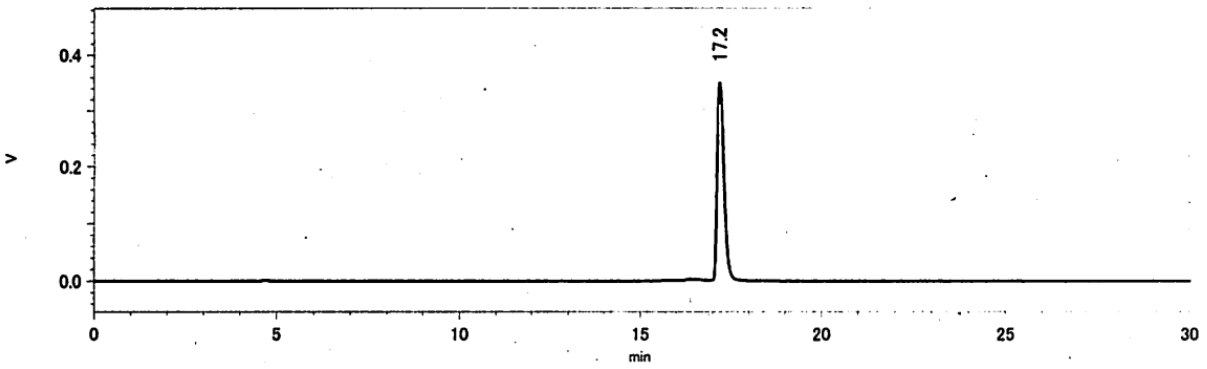
HPLC

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

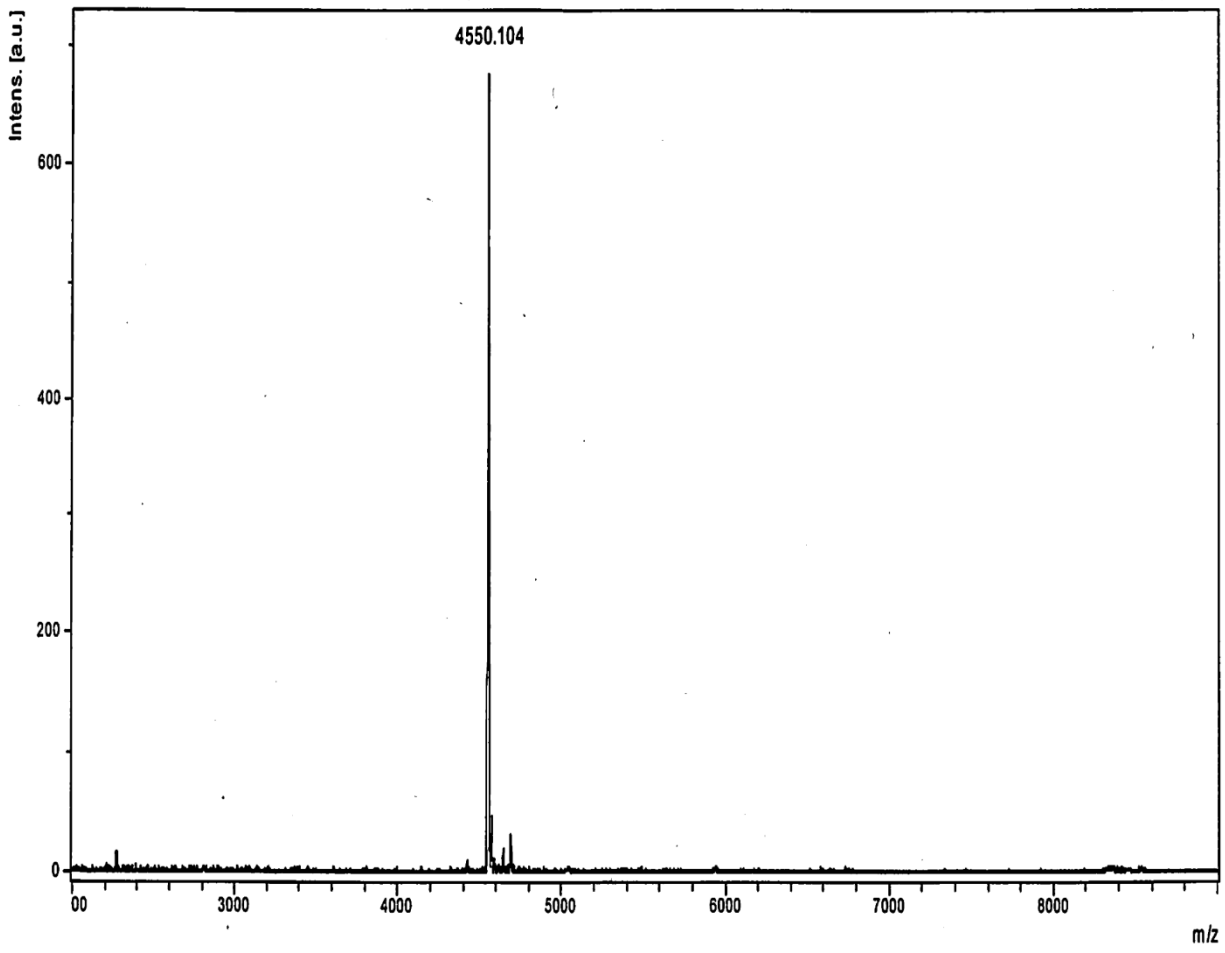
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2f

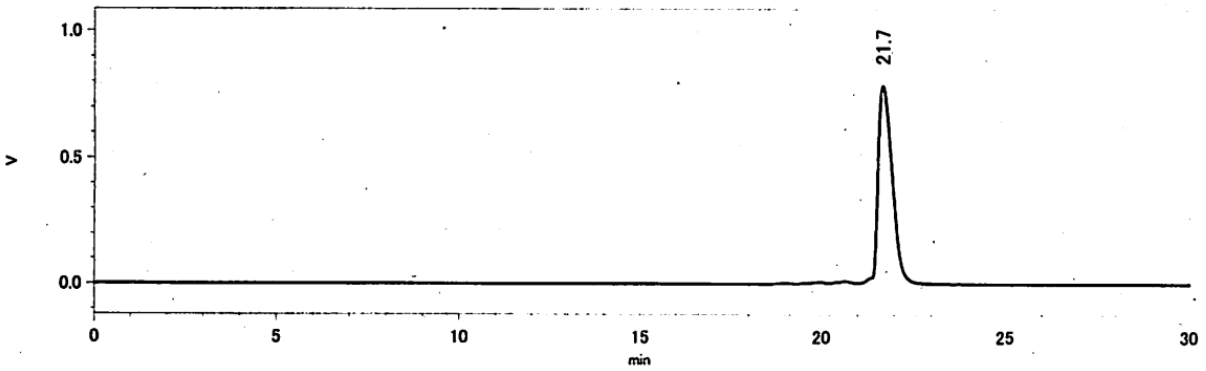
HPLC

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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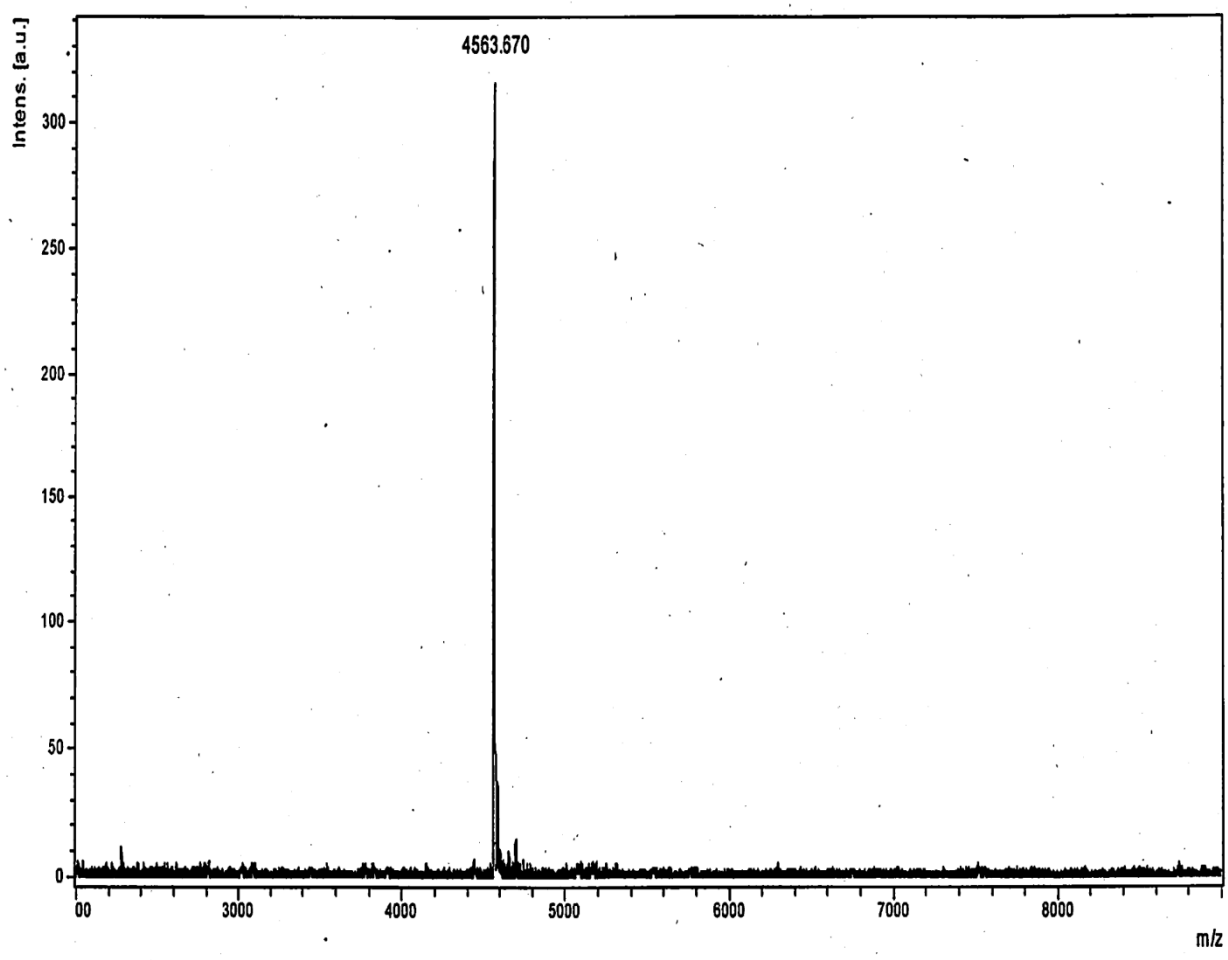


TFO-2g

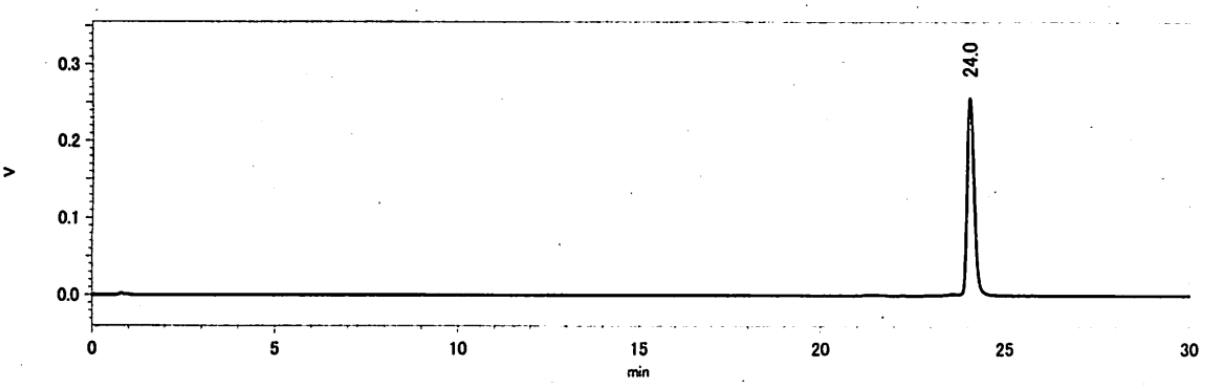
HPLC

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

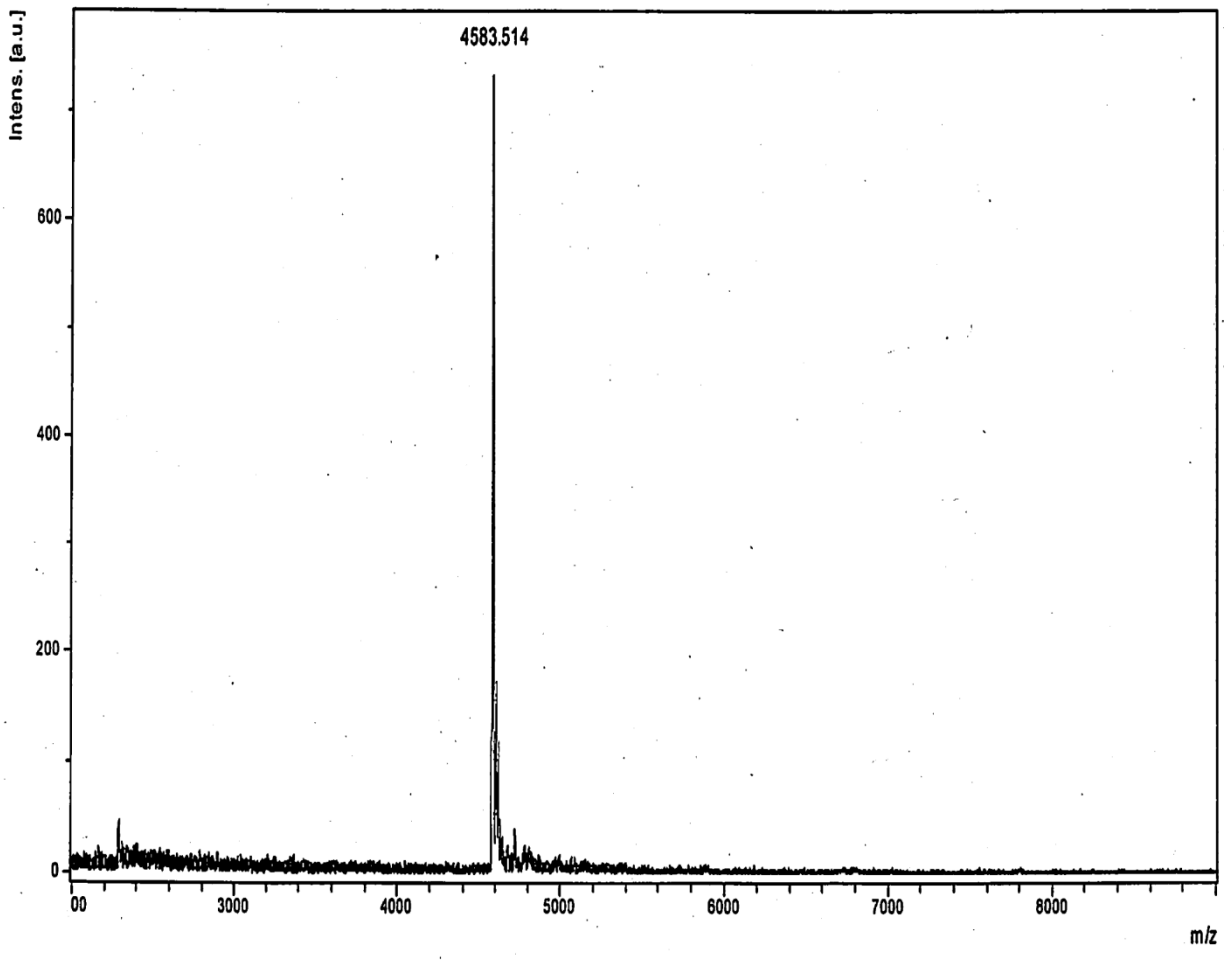
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2h

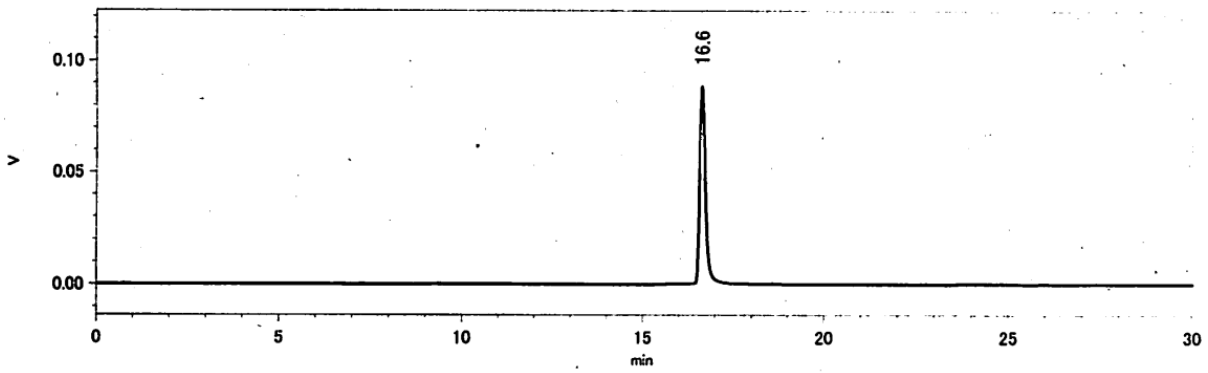
HPLC

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

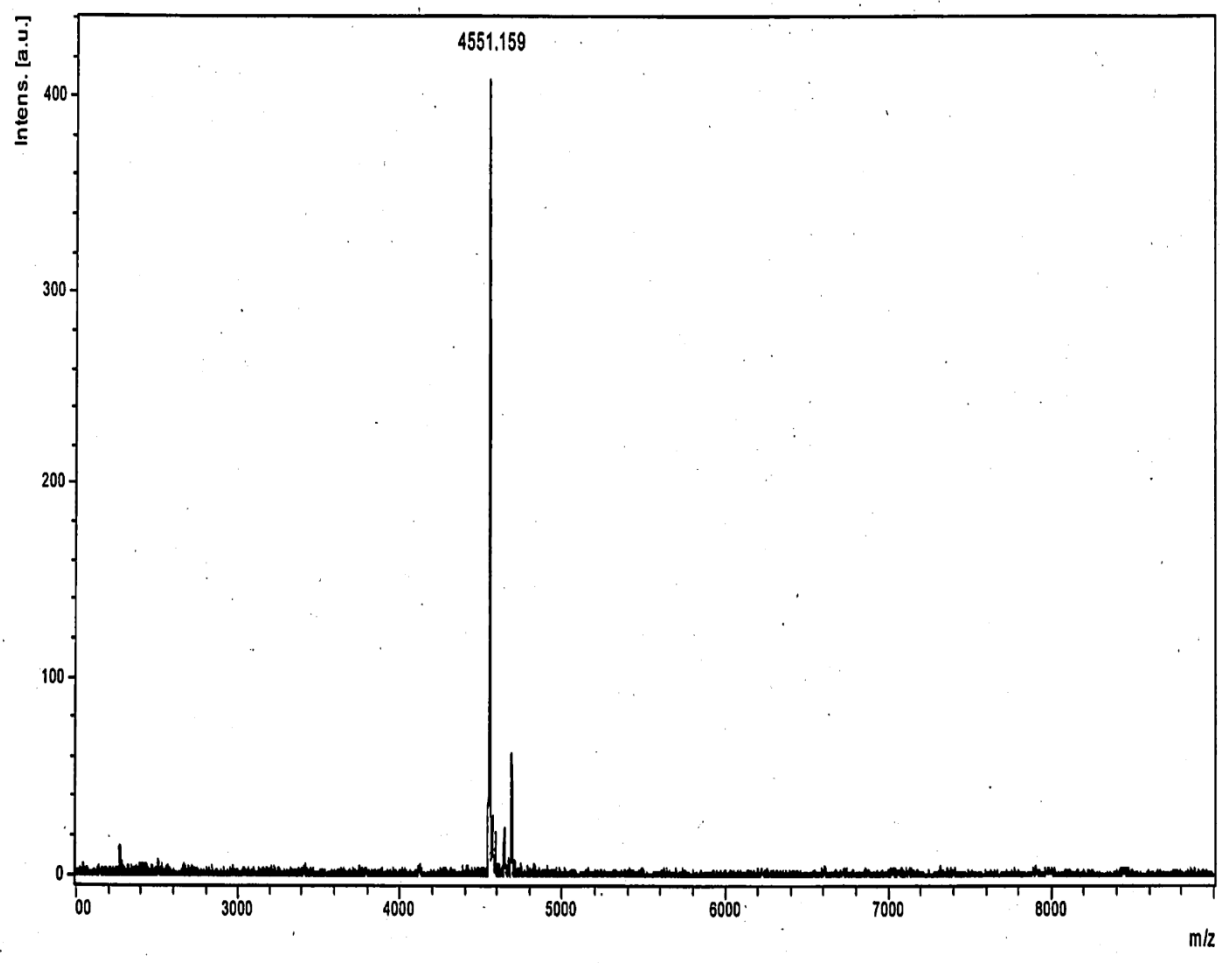
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2i

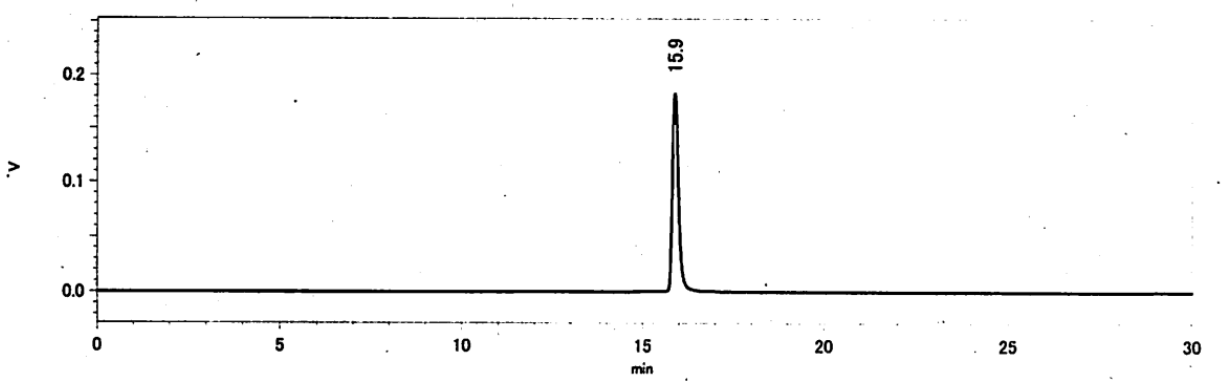
HPLC

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

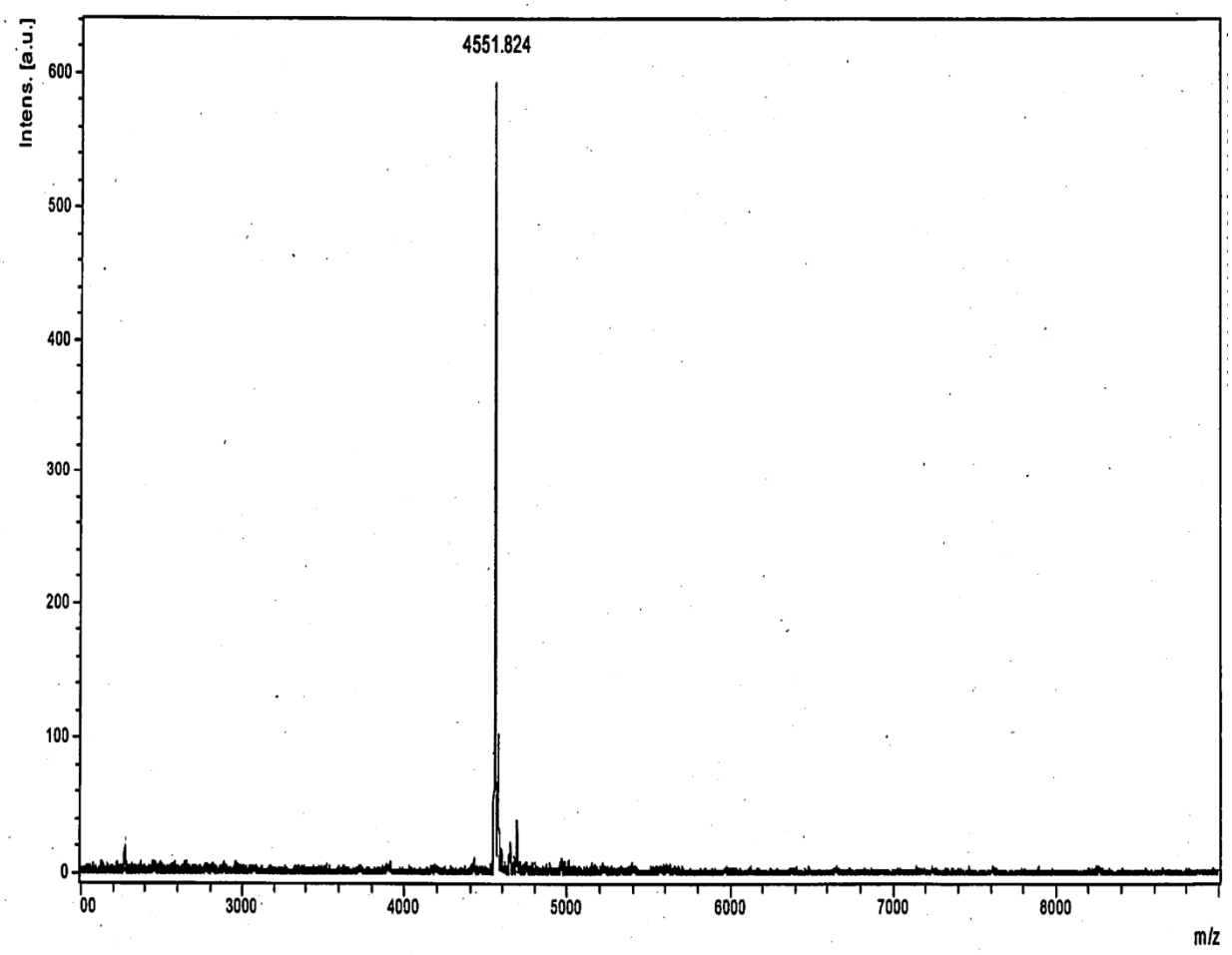
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2j

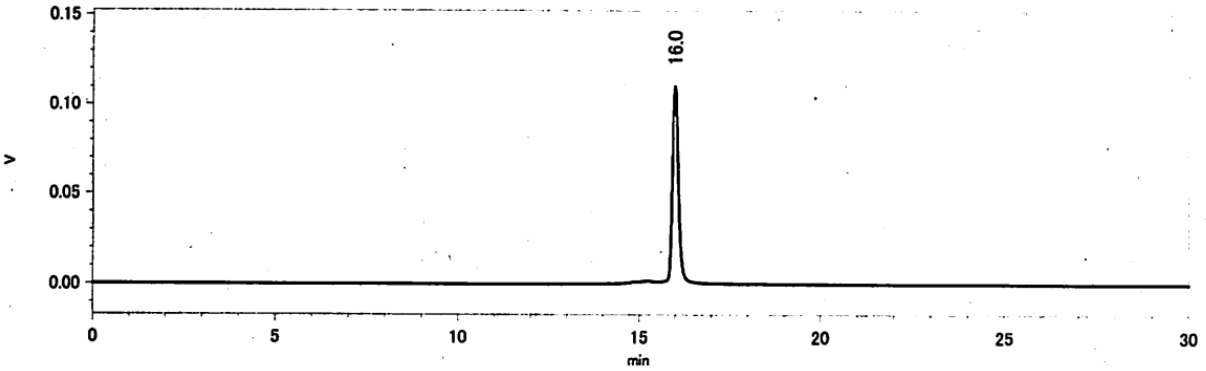
HPLC

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

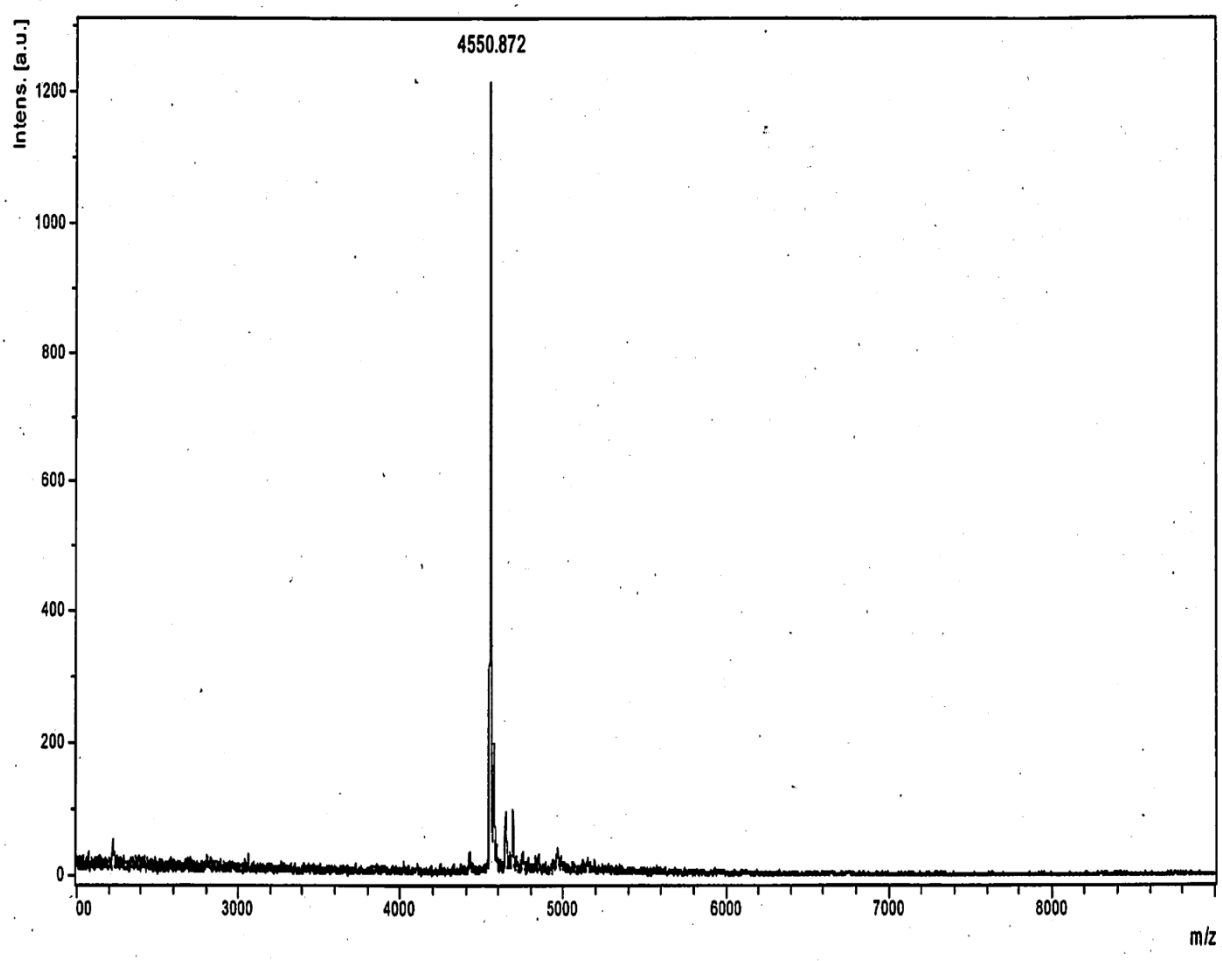
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2k

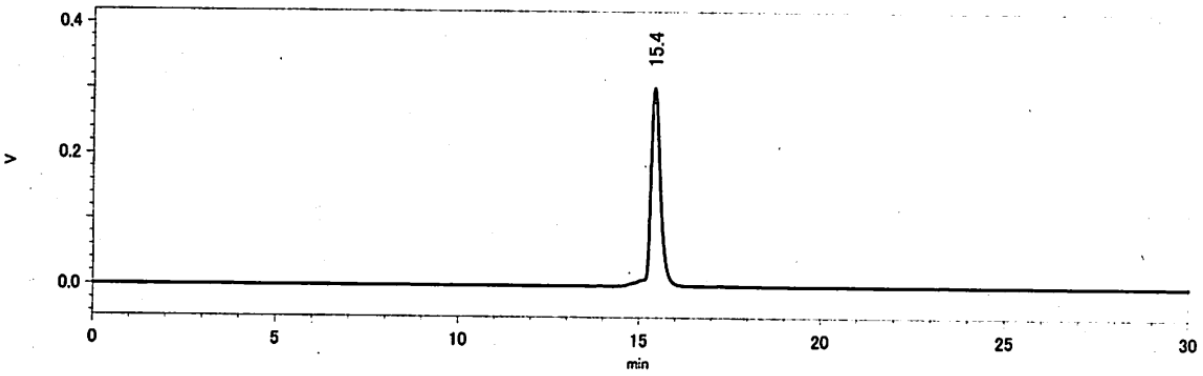
HPLC

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

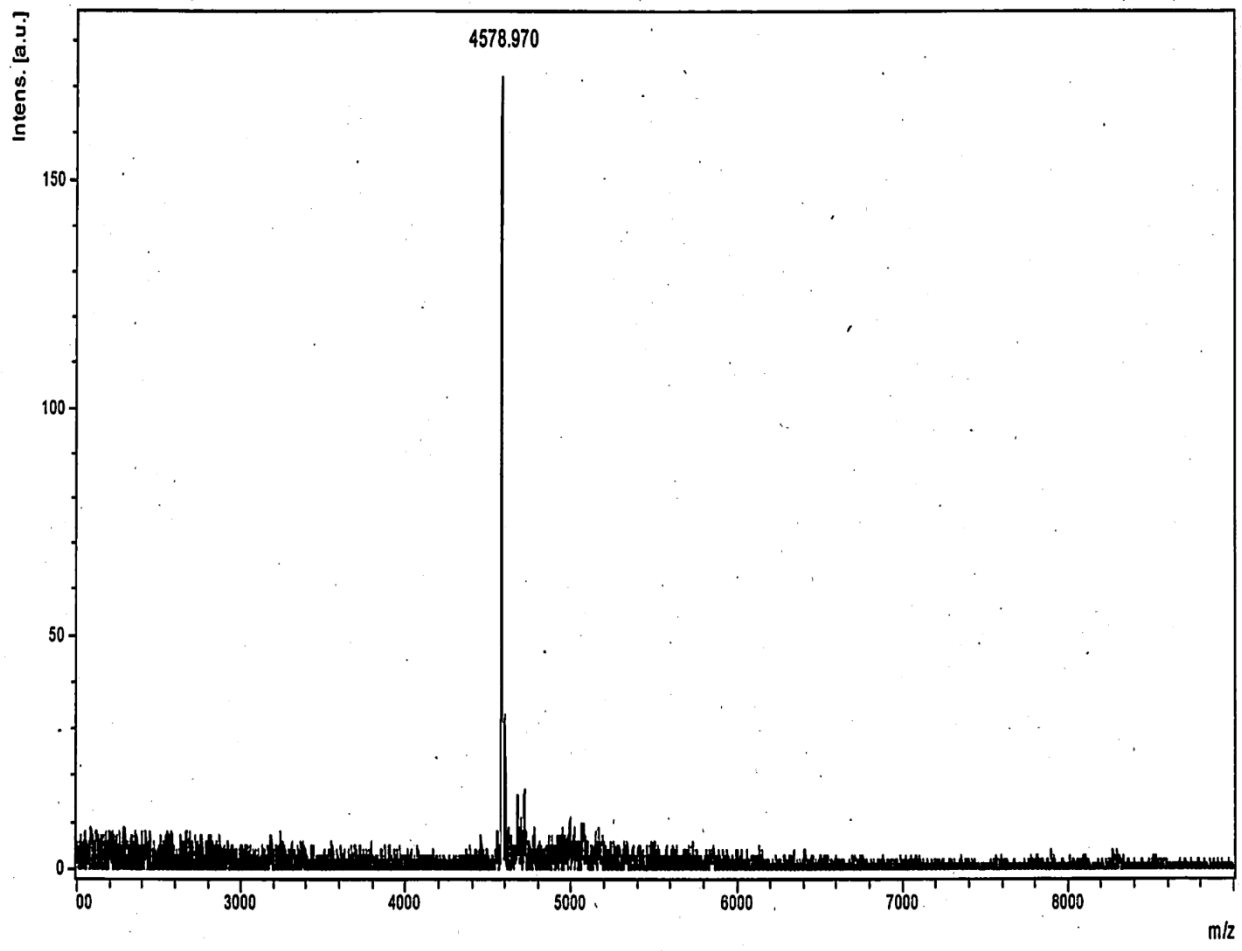
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2l

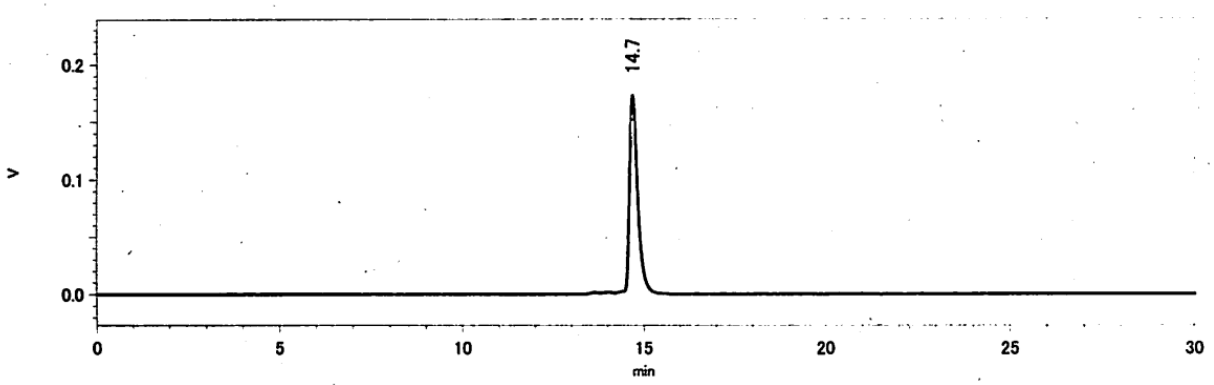
HPLC

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

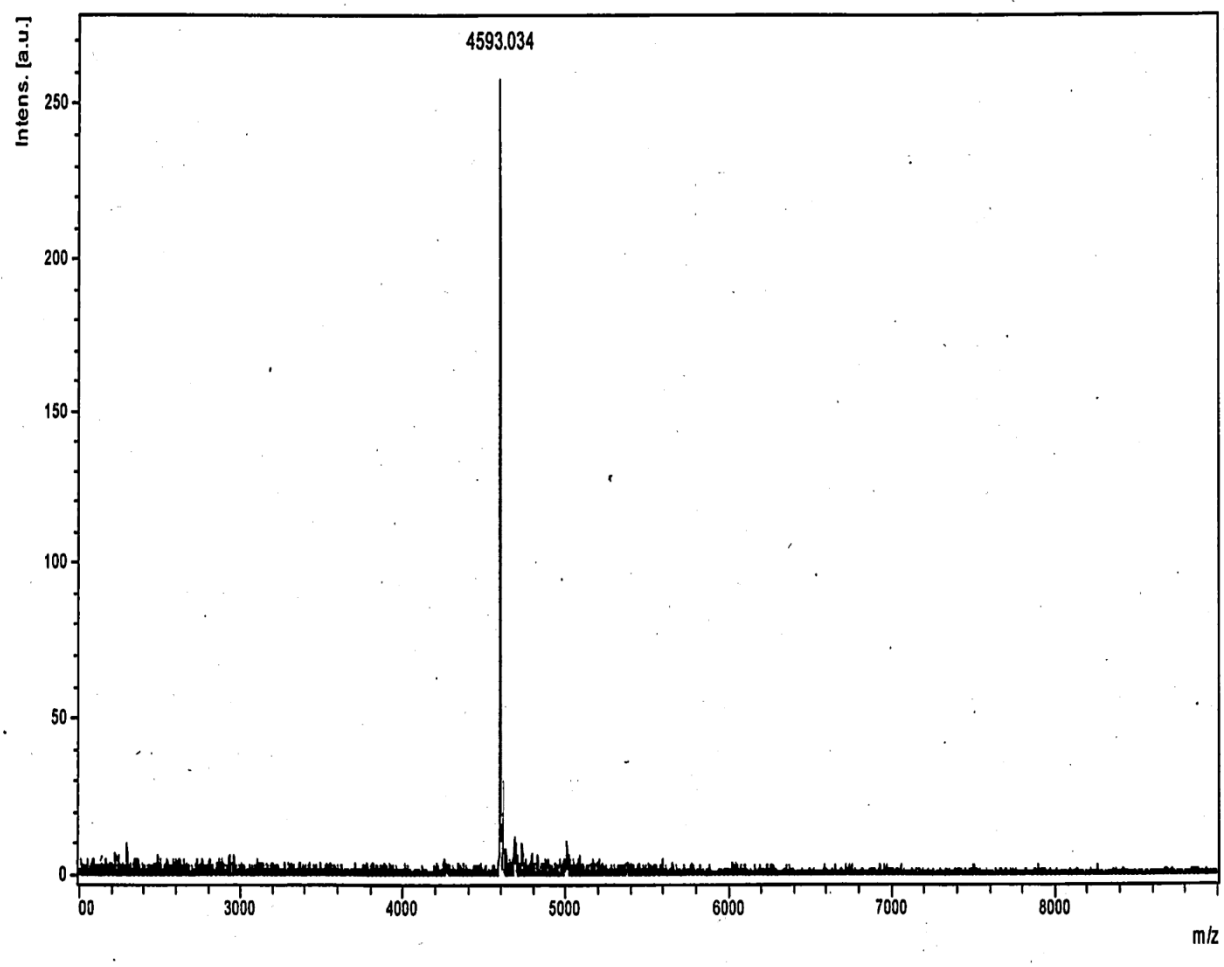
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2m

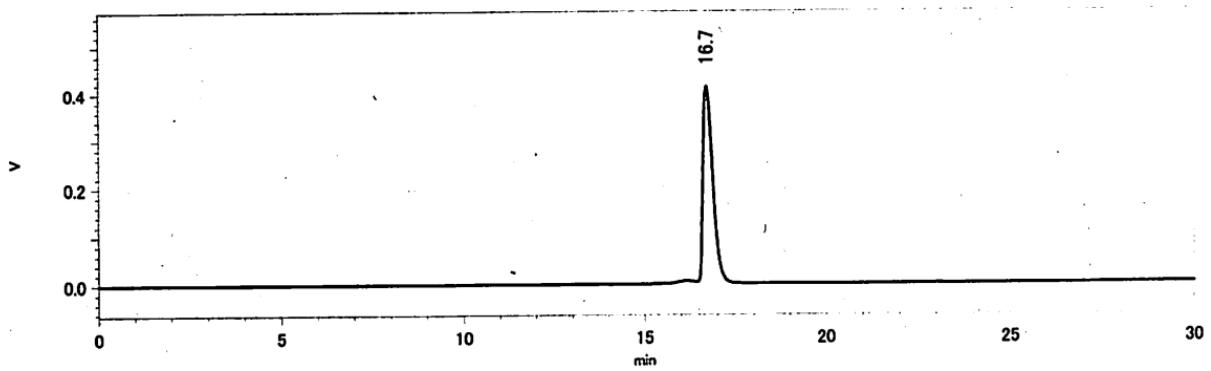
HPLC

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

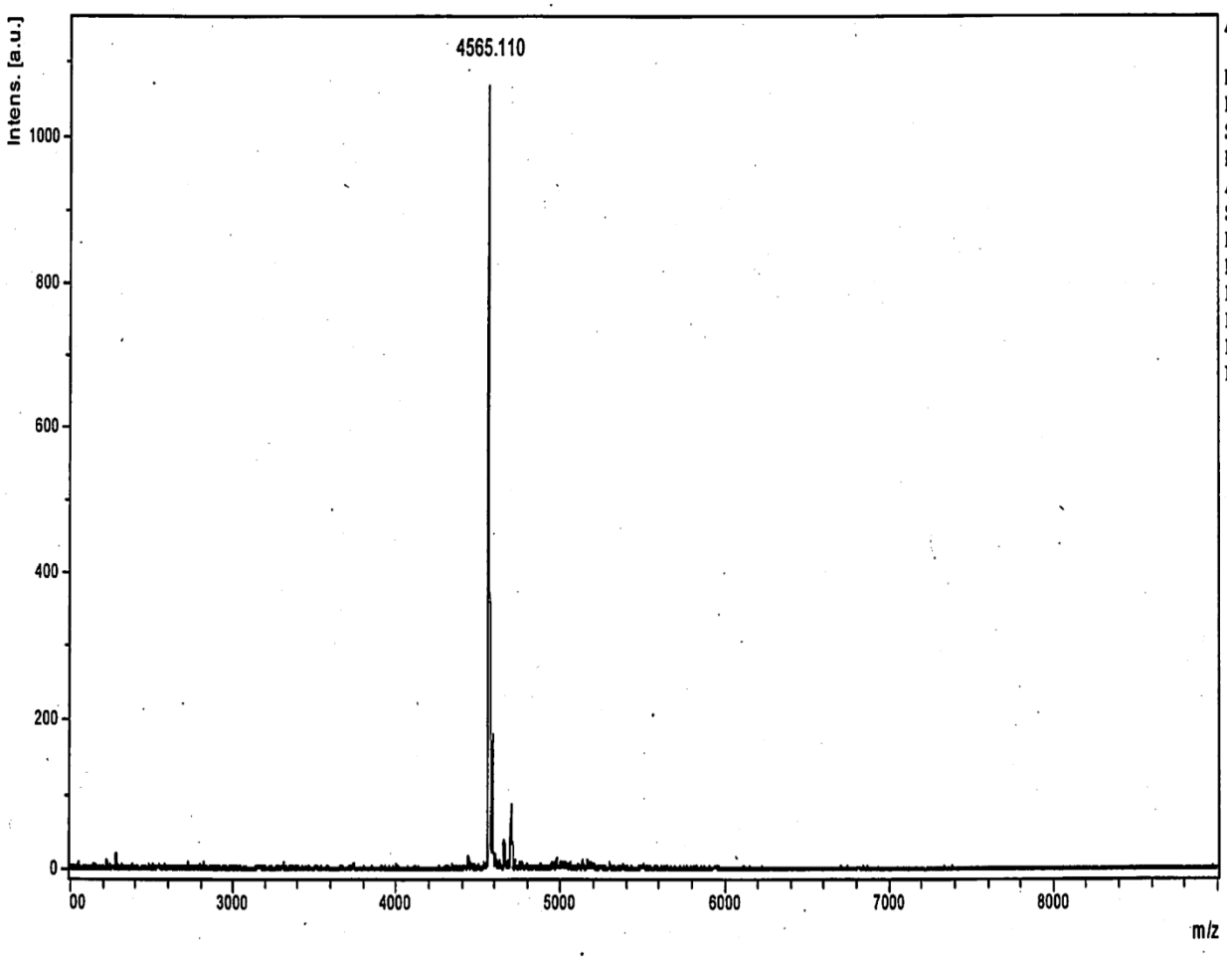
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2n

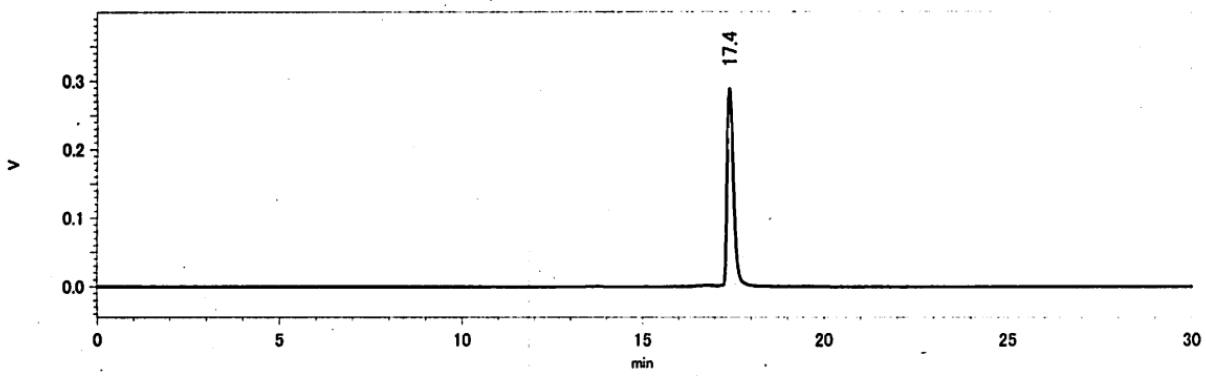
HPLC

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

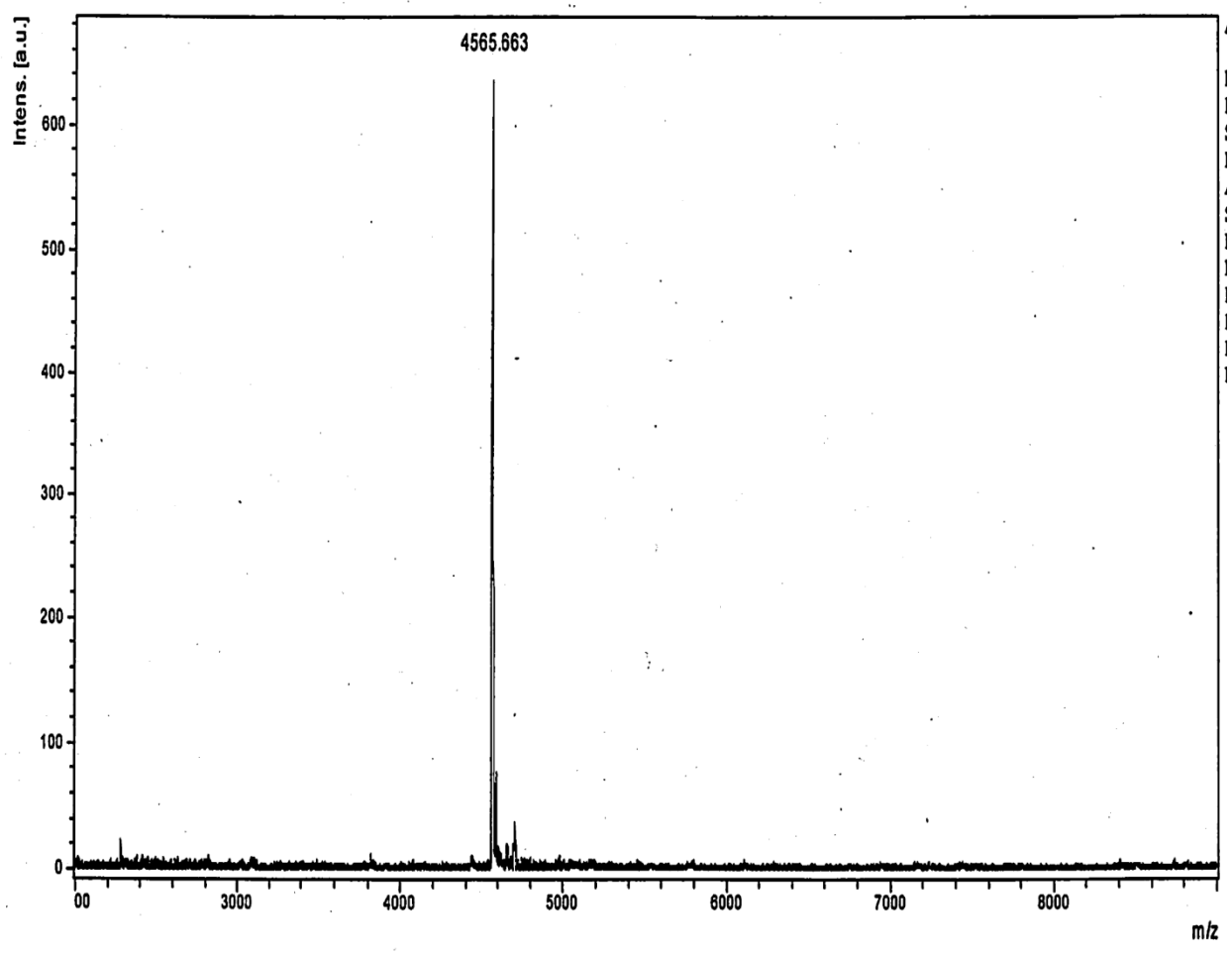
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2o

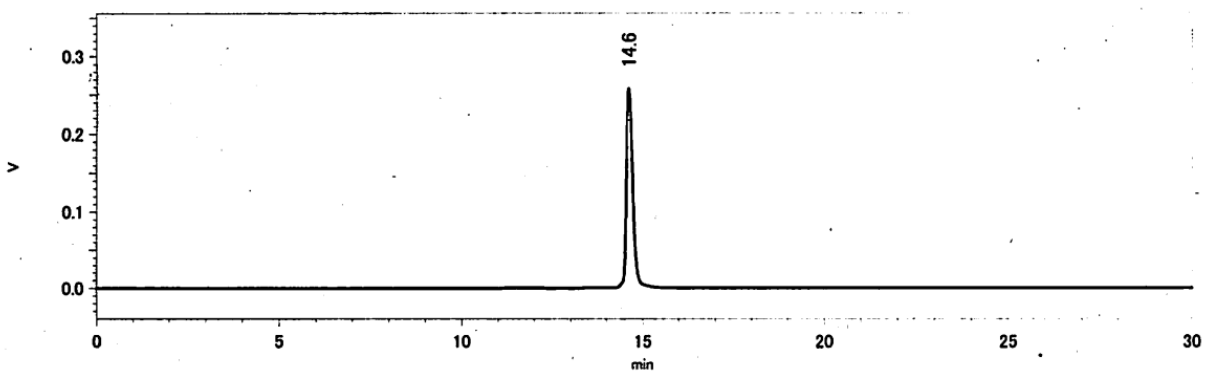
HPLC

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

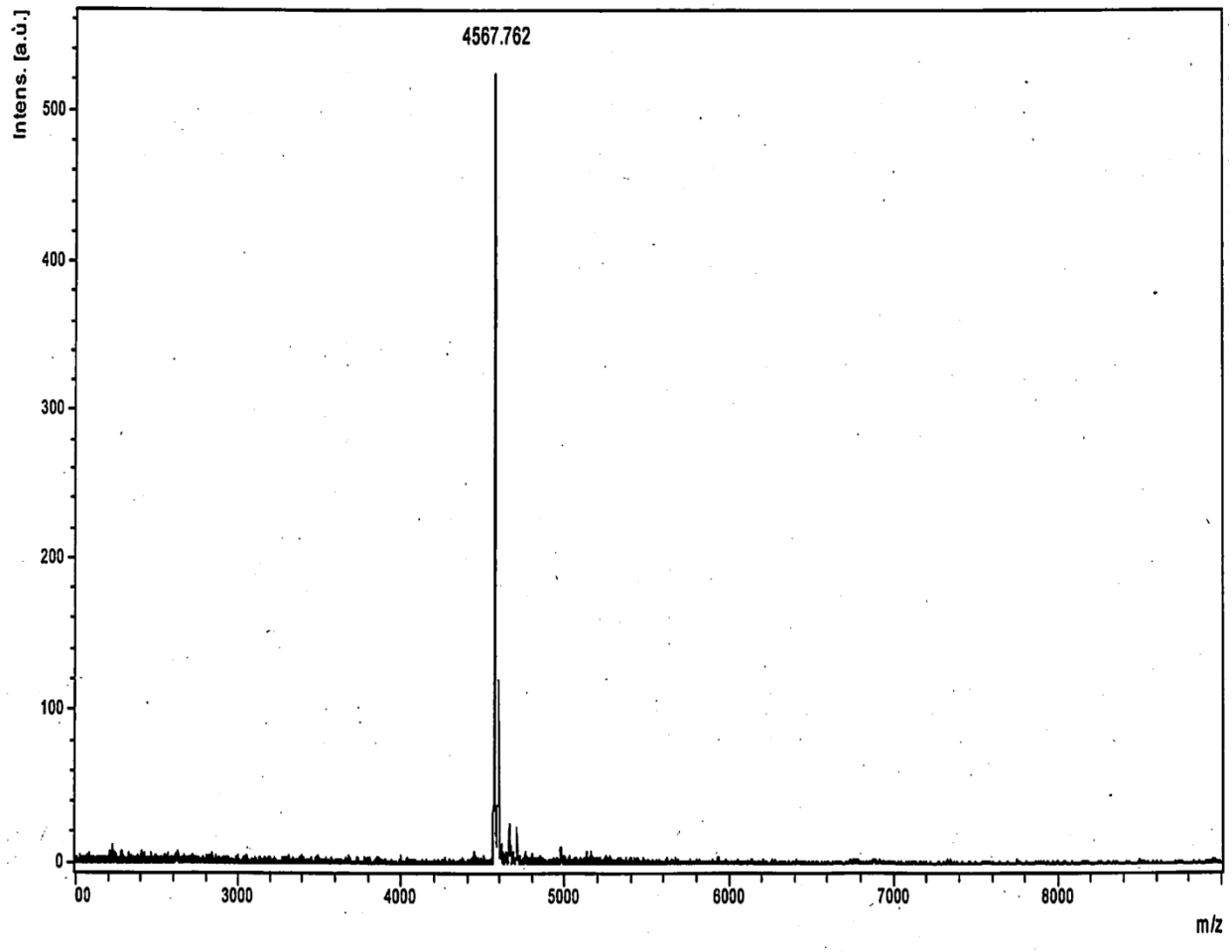
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass



TFO-2p

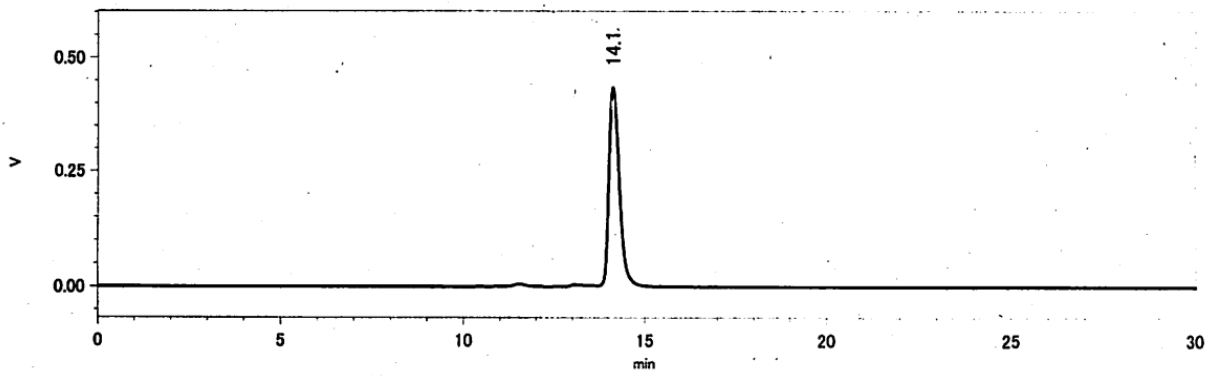
HPLC

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

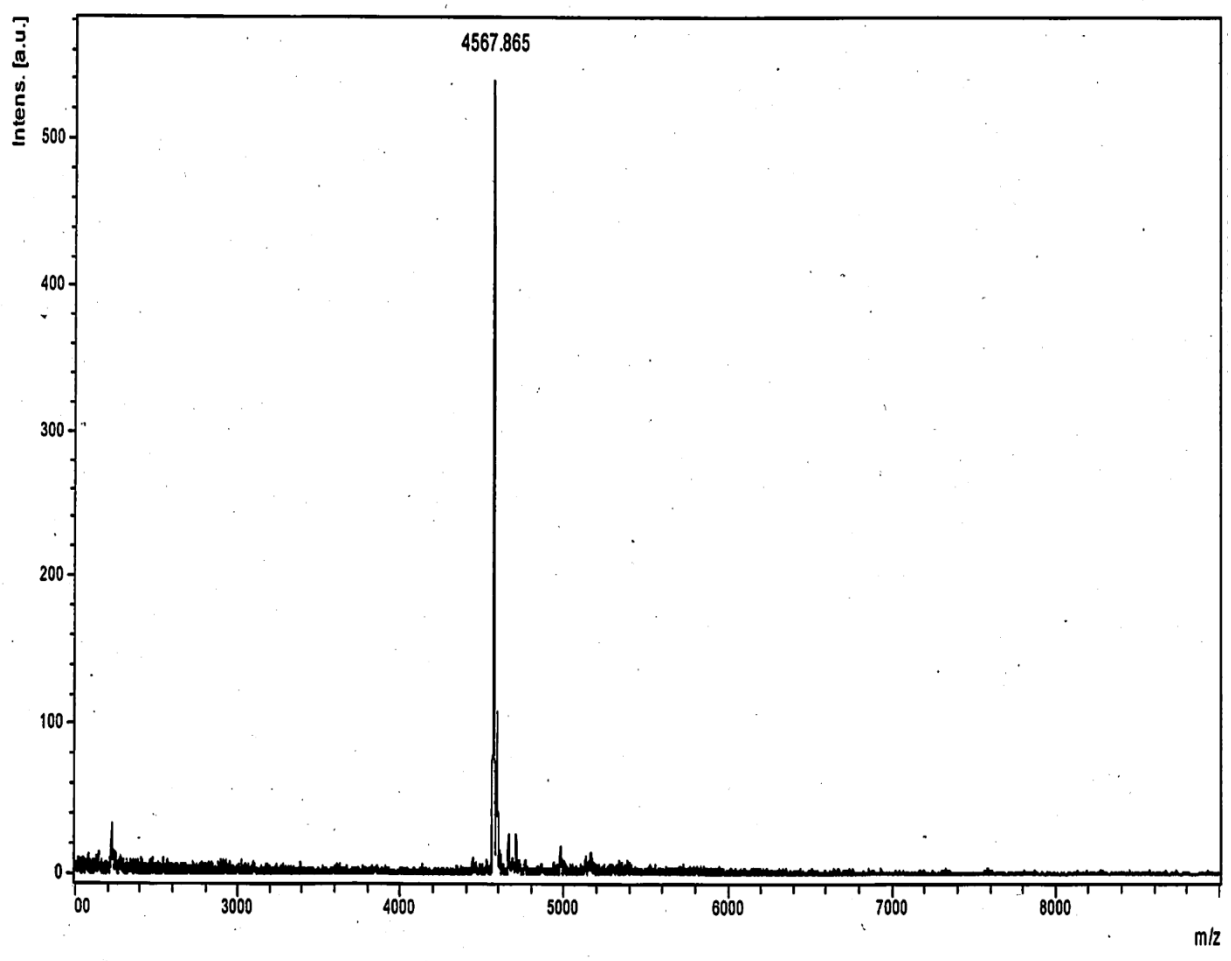
Gradient : 7-13% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer.

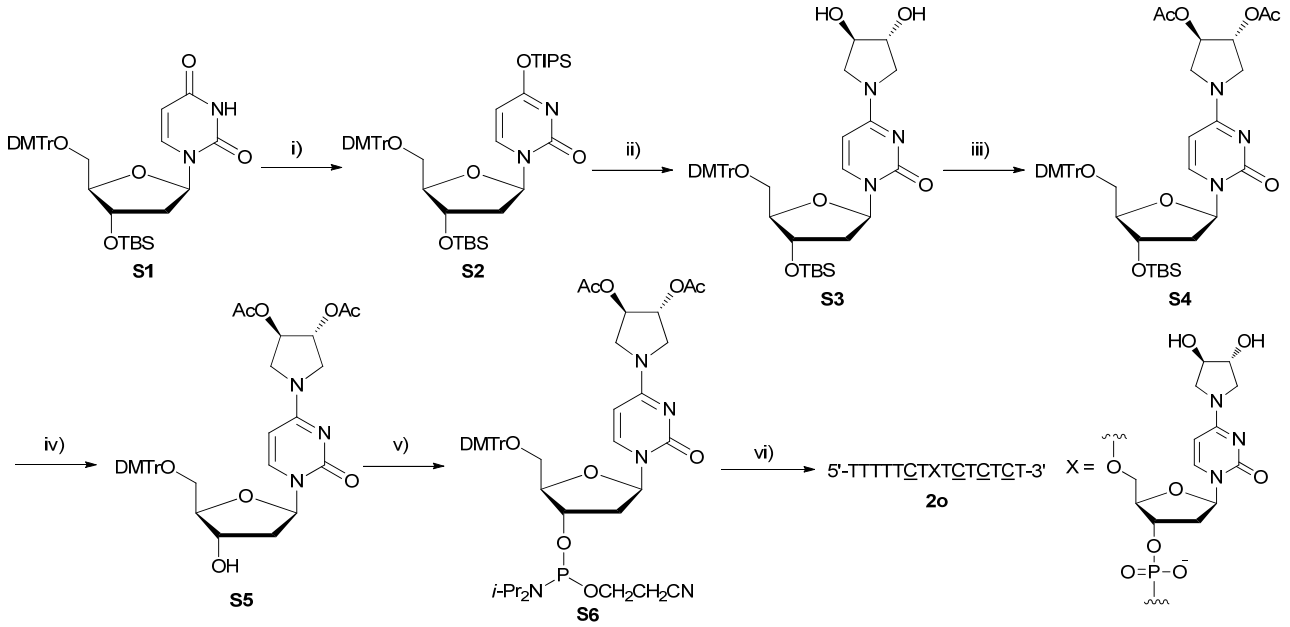
Flow rate : 1.0 mL/min.

Column temp. : 50ºC.



MALDI-TOF-Mass





Scheme S1. Synthesis of TFO **2o**. C = 2'-deoxy-5-methylcytidine. *Reagent and conditions*: (i) 2,4,6-triisopropylphenylsulfonyl chloride (TIPS-Cl), Et_3N, DMAP, CH_2Cl_2, rt, 2 h, 93%; (ii) (3*S*,4*S*)-dihydroxypyrrolidine, Et_3N, CH_2Cl_2-MeOH, rt, 6 h, 85%; (iii) Ac_2O, DMAP, pyridine, rt, 2 h, quant.; (iv) TBAF, AcOH, THF, rt, 24 h, quant.; (v) *i*-$Pr_2NP(Cl)OCH_2CH_2CN$, *i*-Pr_2NEt, CH_2Cl_2, 0ºC, 3 h, 90%; (vi) oligonucleotide synthesis.

TFO **2o** was synthesized as shown in Scheme S1. The oligonucleotide synthesis was achieved by using the common phosphoramidite protocol. The synthesis of **S6** from **S1**[1] was carried out as below.

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, $CDCl_3$ (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 spectrometers. Optical rotations were recorded on a JASCO P-2200 instrument. For column chromatography, Fuji Silysia PSQ-100B, FL-60D and FL-100D was used as silica gel.

3'-*O*-*tert*-Butyldimethylsilyl-2'-deoxy-5'-*O*-dimethoxytrityl-4-*O*-(2,4,6-triisopropylphenyl)sulfonyluridine (S2): Under a N_2 atmosphere, 2,4,6-triisopropylbenzenesulfonyl chloride (423 mg, 1.40 mmol) was added to a solution of compound **S1**[1] (750 mg, 1.16 mmol), DMAP (14 mg, 0.11 mmol) and Et_3N (0.81 mL, 5.82 mmol) in anhydrous CH_2Cl_2 (7.0 mL) and the resulting mixture was stirred at room temperaure for 2 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 column chromatography (*n*-hexane/AcOEt =

5/1) to give compound **S2** (991 mg, 93%) as a white amorphous powder.

Mp 73－74°C. $[\alpha]_D^{30}$ +91.3 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 2957, 1684, 1542, 1509, 1362, 1281, 1253, 1181, 1108, 1036 cm^{-1}. ^1H NMR ($CDCl_3$): δ –0.07 (3H, s), –0.01 (3H, s), 0.79 (9H, s), 1.26 (12H, d, *J* = 6.9 Hz), 1.31 (6H, d, *J* = 6.9 Hz), 2.23 (1H, ddd, *J* = 4.1, 6.4 and 13.6 Hz), 2.50 (1H, ddd, *J* = 6.4, 6.4 and 13.6 Hz), 2.91 (1H, sept, *J* = 6.9 Hz), 3.32 (1H, dd, *J* = 2.8 and 11.0 Hz), 3.55 (1H, dd, *J* = 2.8 and 11.0 Hz), 3.81 (6H, s), 3.92 (1H, ddd, *J* = 2.8, 2.8 and 6.4 Hz), 4.25 (2H, sept, *J* = 6.9 Hz), 4.40 (1H, q, *J* = 6.4 Hz), 5.69 (1H, d, *J* = 7.3 Hz), 6.08 (1H, dd, *J* = 4.1 and 6.4 Hz), 6.82-6.85 (4H, m), 7.20-7.35 (11H, m), 8.50 (1H, d, *J* = 7.3 Hz). ^{13}C NMR ($CDCl_3$): δ –5.08, –4.64, 17.82, 23.43, 23.44, 24.35, 24.62, 25.59, 29.62, 34.22, 41.90, 55.21, 61.23, 69.70, 86.58, 86.86, 87.29, 94.53, 113.20, 113.23, 124.01, 127.19, 127.94, 128.14, 130.06, 130.08, 130.62, 134.95, 135.05, 143.99, 146.06, 151.17, 153.80, 154.42, 158.71, 166.92. ^{13}C NMR ($CDCl_3$): δ –5.1, –4.6, 17.8, 23.4, 23.4, 24.4, 24.6, 25.6, 29.6, 34.2, 41.9, 55.2, 61.2, 69.7, 86.6, 86.9, 87.3, 94.5, 113.2, 113.2, 124.0, 127.2, 127.9, 128.1, 130.1, 130.1, 130.6, 135.0, 135.0, 143.99, 146.1, 151.2, 153.8, 154.42, 158.71, 166.9. MS (FAB): *m/z* 933 ($M+Na^+$). HRMS (FAB): Calcd for $C_{51}H_{66}N_2NaO_9SSi$ ($M+Na^+$): 933.4156. Found: 933.4153.

1-[3-*O*-*tert*-Butyldimethylsilyl-2-deoxy-5-*O*-dimethoxytrityl-β-D-ribosyl]-4-[(3*R*,4*R*)-dihydroxy pyrrolidino]pyrimidin-2-one (S3): Under a N_2 atmosphere, a solution of (3*R*,4*R*)-dihydroxypyrrolidine[2] (339 mg, 3.29 mmol) in MeOH (1.0 mL) was added to a solution of compound **S2** (3.00 g, 3.29 mmol) and Et_3N (2.3 mL, 16.5 mmol) in CH_2Cl_2 (50 mL) and the resulting mixture was stirred at room temperature for 6 h. After addition of water, 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 column chromatography (CH_2Cl_2/MeOH = 20/1) to give compound **S3** (2.0 g, 85%) as a white amorphous powder.

Mp 114–115°C. $[\alpha]_D^{24}$ –12.2 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 3339, 2931, 2857, 1638, 1506, 1462, 1293, 1253, 1177, 1106, 1036 cm^{-1}. ^1H NMR ($CDCl_3$): δ –0.06 (3H, s), 0.00 (3H, s), 0.80 (9H, s), 2.16-2.19 (1H, m), 2.40 (1H, ddd, *J* = 6.0, 6.0 and 13.1 Hz), 3.27-3.33 (2H, m), 3.54-3.56 (1H, m), 3.66-3.67 (1H, m), 3.77 (6H, s), 3.77-3.82 (1H, m), 3.89-3.90 (1H, m), 3.99-4.01 (1H, m), 4.40-4.50 (3H, m), 5.33-5.38 (2H, m), 5.59 (1H, brs), 6.23 (1H, t, *J* = 6.0 Hz), 6.83-6.86 (4H, m), 7.22–7.32 (7H, m), 7.41 (2H, d, *J* = 7.3 Hz), 8.07 (1H, d, *J* = 7.3 Hz). ^{13}C NMR ($CDCl_3$): δ –5.1, –4.7, 17.7, 25.5, 41.8, 52.4, 52.6, 55.1, 61.5, 69.8, 74.3, 74.8, 85.6, 85.7, 86.6, 93.2, 113.0, 113.1, 126.9, 127.8, 128.1, 130.1, 135.1, 135.2, 140.0, 144.3, 156.0, 158.5, 161.6. MS (FAB) *m/z* 730 ($M+H^+$). HRMS (FAB): Calcd for $C_{40}H_{52}N_3O_8Si$ ($M+H^+$): 730.3518. Found: 730.3548.

1-[3-*O*-*tert*-Butyldimethylsilyl-2-deoxy-5-*O*-dimethoxytrityl-β-D-ribosyl]-4-[(3*R*,4*R*)-diacetoxy pyrrolidino]pyrimidin-2-one (S4): Under a N_2 atmosphere, Ac_2O (77 μL, 0.82 mmol) was added to a solution of compound **S3** (200 mg, 0.27 mmol), DMAP (3.3 mg, 0.027 mmol) in pyridine (20 mL) and the resulting mixture was stirred at room temperature for 2 h. After addition of water, the reaction mixture was diluted with Et_2O, washed with water and brine, dried over Na_2SO_4, and

concentrated *in vacuo*. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1/3 to 1/5) to give compound **S4** (232 mg, quant.) as a white amorphous powder.

Mp 88–91˚C. $[\alpha]_D^{24}$ +16.2 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 2931, 2857, 1745, 1656, 1506, 1462, 1369, 1283, 1248, 1178, 1107, 1068, 1035. ^{1}H NMR ($CDCl_3$): δ –0.07 (3H, s), –0.01 (3H, s), 0.80 (9H, s), 2.05 (3H, s), 2.11 (3H, s), 2.22 (1H, ddd, *J* = 3.7, 6.9 and 13.7 Hz), 2.47 (1H, ddd, *J* = 6.9, 6.9 and 13.7 Hz), 3.33 (1H, dd, *J* = 2.8 and 10.6 Hz), 3.38 (1H, d, *J* = 11.9 Hz), 3.57 (1H, dd, *J* = 2.8 and 10.6 Hz), 3.69-3.73 (1H, m), 3.80 (6H, s), 3.87-4.03 (3H, m), 4.48 (1H, q, *J* = 6.9 Hz), 5.21-5.25 (3H, m), 6.29 (1H, dd, *J* = 3.7 and 6.9 Hz), 6.83-6.86 (4H, m), 7.25-7.32 (7H, m), 7.40 (2H, d, *J* = 6.9 Hz), 8.18 (1H, d, *J* = 7.3 Hz). ^{13}C NMR ($CDCl_3$): δ –5.1, –4.7, 17.8, 20.7, 20.8, 25.6, 42.0, 50.6, 50.7, 55.1, 61.5, 69.8, 73.6, 74.6, 85.8, 86.6, 91.7, 113.1, 113.1, 127.0, 127.8, 128.2, 130.1, 130.2, 135.2, 135.3, 141.3, 144.2, 155.3, 158.5, 161.8, 169.3, 169.6. MS (FAB) *m/z* 814 ($M+H^+$). HRMS (FAB): Calcd for $C_{44}H_{56}N_3O_{10}Si$ ($M+H^+$): 814.3729. Found: 814.3763.

1-[2-Deoxy-5-*O*-dimethoxytrityl-β-D-ribosyl]-4-[(3*R*,4*R*)-diacetoxypyrrolidino]pyrimidin-2-one (S5): Under a N_2 atmosphere, TBAF (1.0 M in THF, 0.81 mL, 0.81 mmol) and AcOH (8.1 uL) were added to a solution of compound **S4** (550 mg, 0.68 mmol) in THF (30 mL) at 0˚C 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 column chromatography (AcOEt/MeOH = 20/1) to give compound **S5** (476 mg, quant.) as a white amorphous powder.

Mp 101–103˚C. $[\alpha]_D^{25}$ +14.2 (c 1.0, $CHCl_3$). IR ν_{max} (KBr): 3311, 2999, 2932, 2838, 1744, 1639, 1505, 1460, 1370, 1290, 1245, 1178, 1036 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.04 (3H, s), 2.09 (3H, s), 2.19 (1H, ddd, *J* = 6.0, 6.0 and 12.5 Hz), 2.67 (1H, ddd, *J* = 6.0, 6.0 and 12.5 Hz), 3.38-3.48 (3H, m), 3.72-3.98 (4H, m), 3.78 (6H, s), 4.11-4.14 (1H, m), 4.57 (1H, brs), 5.24 (2H, brs), 5.31 (1H, d, *J* = 7.8 Hz), 6.34 (1H, t, *J* = 6.0 Hz), 6.83 (4H, d, *J* = 9.2 Hz), 7.20–7.31 (7H, m), 7.40 (2H, d, *J* = 7.3 Hz), 7.99 (1H, d, *J* = 7.8 Hz). ^{13}C NMR ($CDCl_3$): δ 20.7, 20.8, 42.1, 50.7, 50.8, 55.1, 62.9, 71.0, 73.6, 74.6, 85.9, 86.2, 86.6, 92.0, 113.1, 126.8, 127.8, 128.1, 130.1, 135.4, 135.6, 141.3, 144.4, 155.5, 158.5, 161.7, 169.4, 169.6. MS (FAB) *m/z* 700 ($M+H^+$). HRMS (FAB): Calcd for $C_{38}H_{42}N_3O_{10}$ ($M+H^+$): 700.2865. Found: 700.2906.

1-[3-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-2-deoxy-5-*O*-dimethoxytrityl-β-D-ribosyl]-4-[(3*R*,4*R*)-diacetoxypyrrolidino]pyrimidin-2-one (S6): Under a N_2 atmosphere, *i*-$Pr_2NP(Cl)OCH_2CH_2CN$ (0.14 ml, 0.64 mmol) was added to a solution of **S5** (300 mg, 0.43 mmol) and *i*-Pr_2NEt (0.37 mL, 2.2 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0˚C 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 AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1/5) to give compound **S6** (350 mg, 90%) as a white amorphous powder.

Mp 109–113˚C. ^{1}H NMR ($CDCl_3$): δ 1.04 (3H, d, *J* = 6.4 Hz), 1.14-1.17 (9H, m), 2.05-2.12 (6H, m),

2.21-2.31 (1H, m), 2.41 (1H, t, J = 6.4 Hz), 2.57-2.69 (2H, m), 3.34-3.40 (2H, m), 3.49-3.61 (4H, m), 3.69-4.12 (5H, m), 3.80 (3H, s), 3.80 (3H, s), 4.57-4.68 (1H, m), 5.22-5.25 (3H, m), 6.33 (0.5H, dd, J = 5.0 and 6.4 Hz), 6.37 (0.5H, t, J = 6.0 Hz), 6.82-6.86 (4H, m), 7.24–7.33 (7H, m), 7.39–7.42 (2H, m), 7.97 (0.5H, d, J = 7.8 Hz), 8.07 (0.5H, d, J = 7.3 Hz). ^{31}P NMR ($CDCl_3$): δ 148.51, 149.09. MS (FAB) *m/z* 900 ($M+H^+$). HRMS (FAB): Calcd for $C_{47}H_{59}N_5O_{11}P$ ($M+H^+$): 900.3943. Found: 900.3990.

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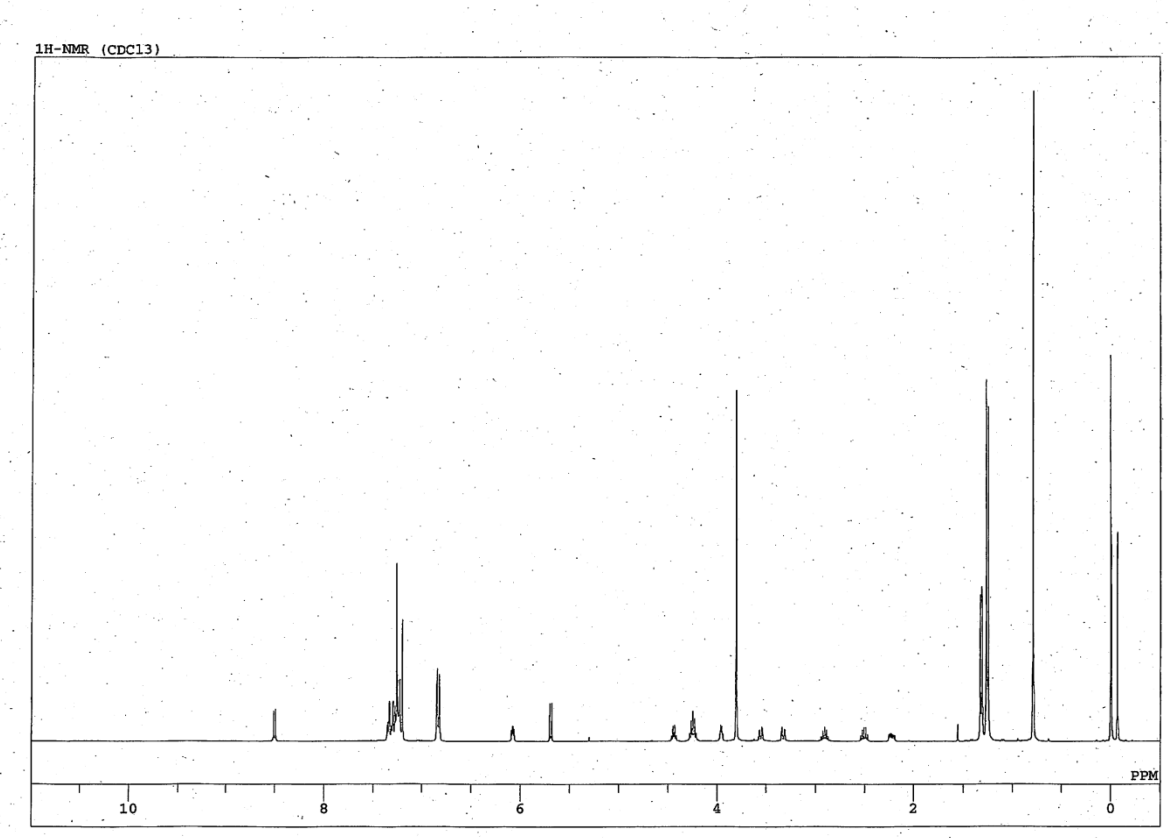
Compound **S2**


1H-NMR (CDCl3)
PPM
10
8
6
4
2
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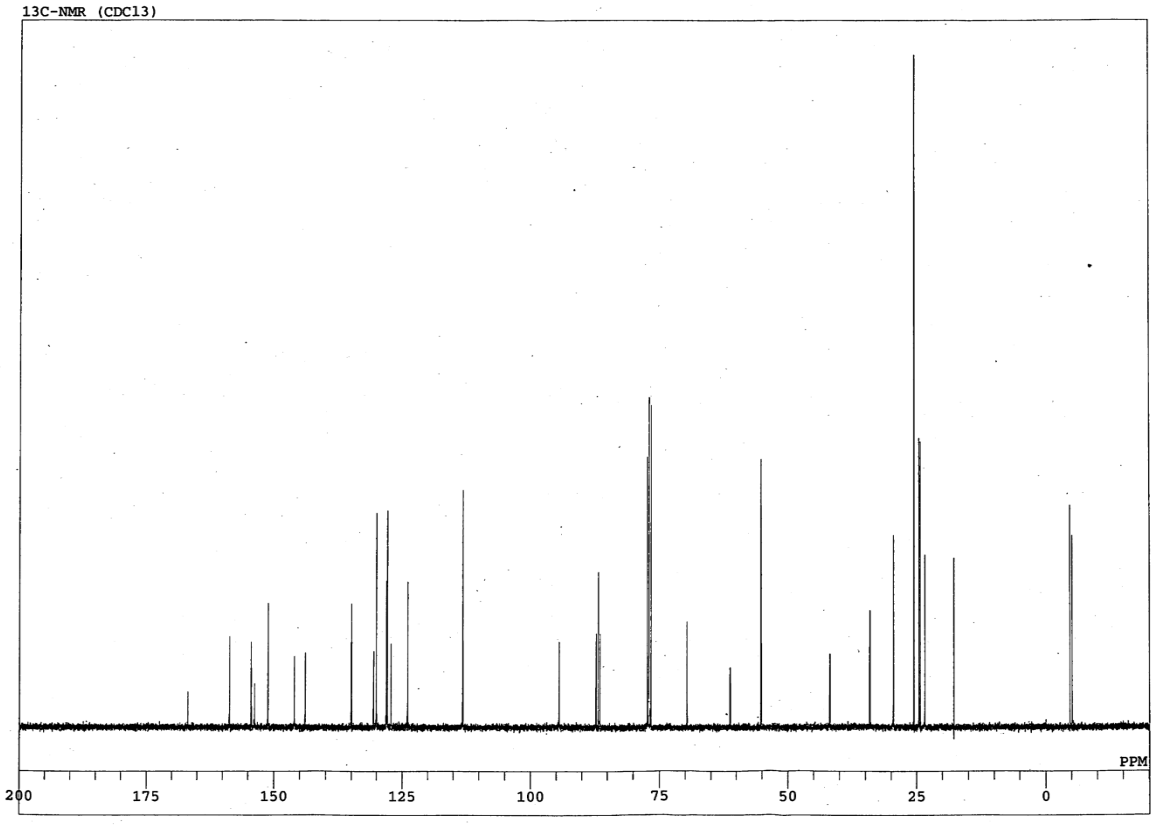
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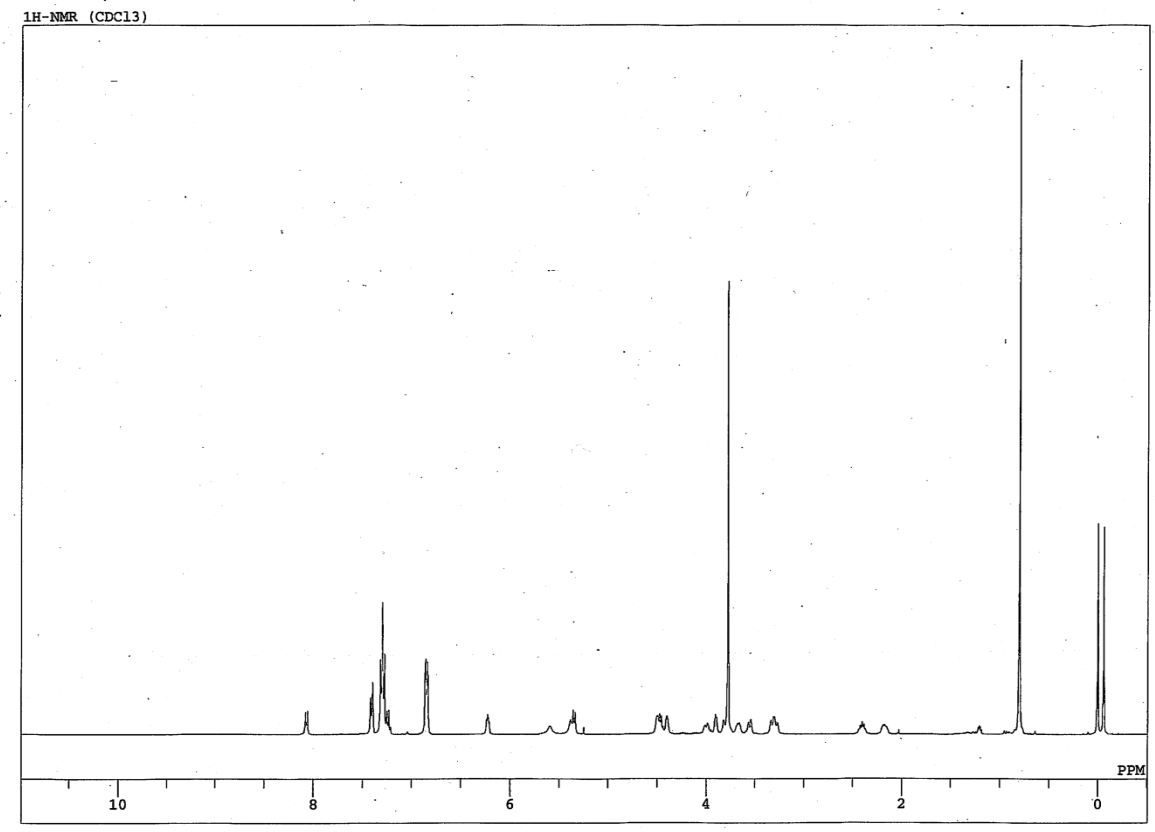
PPM
200
175
150
125
100
75
50
25
0

Compound **S3**


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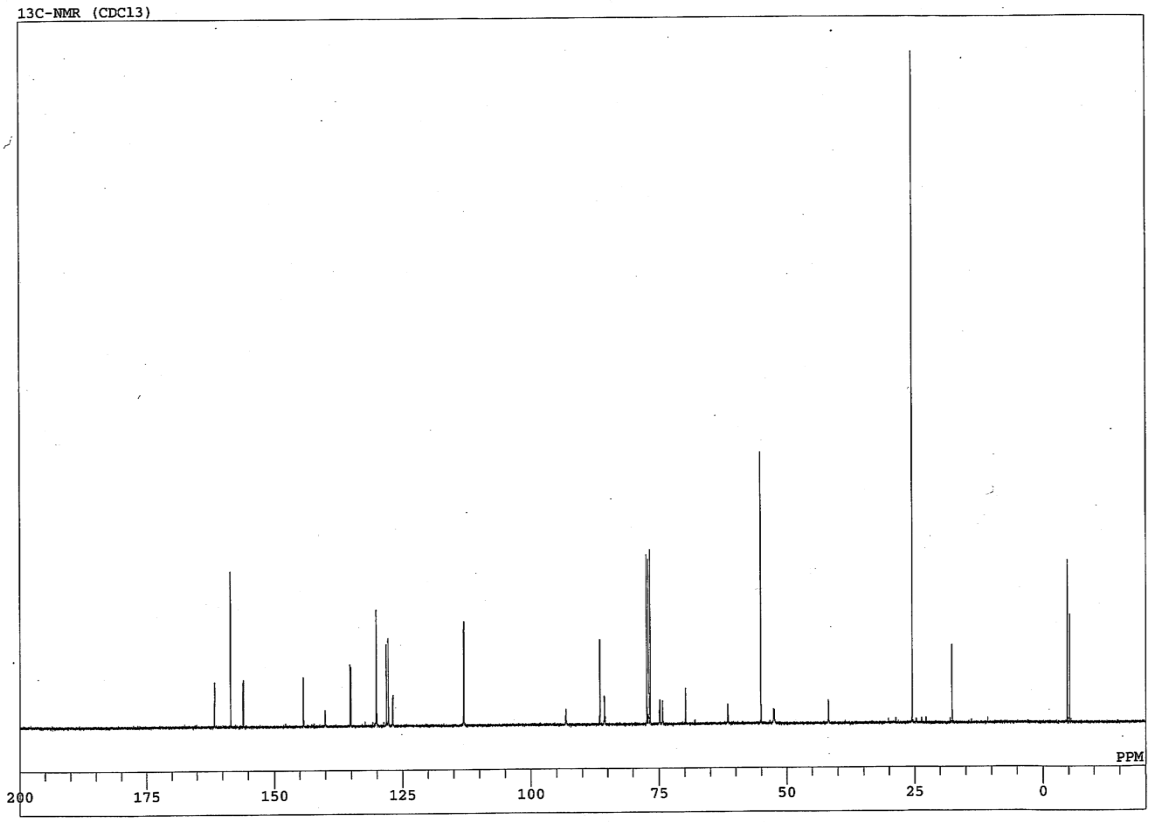
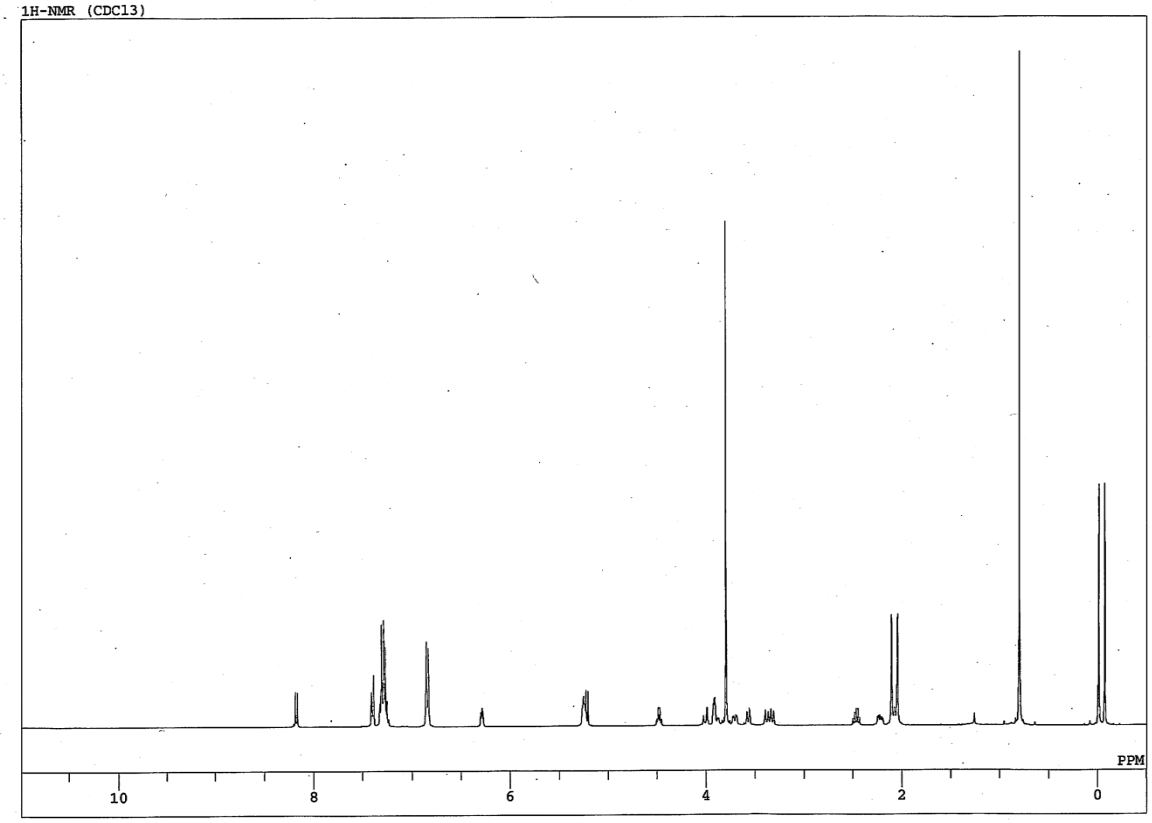

13C-NMR (CDCl3)
PPM
200
175
150
125
100
75
50
25
0

Compound **S4**


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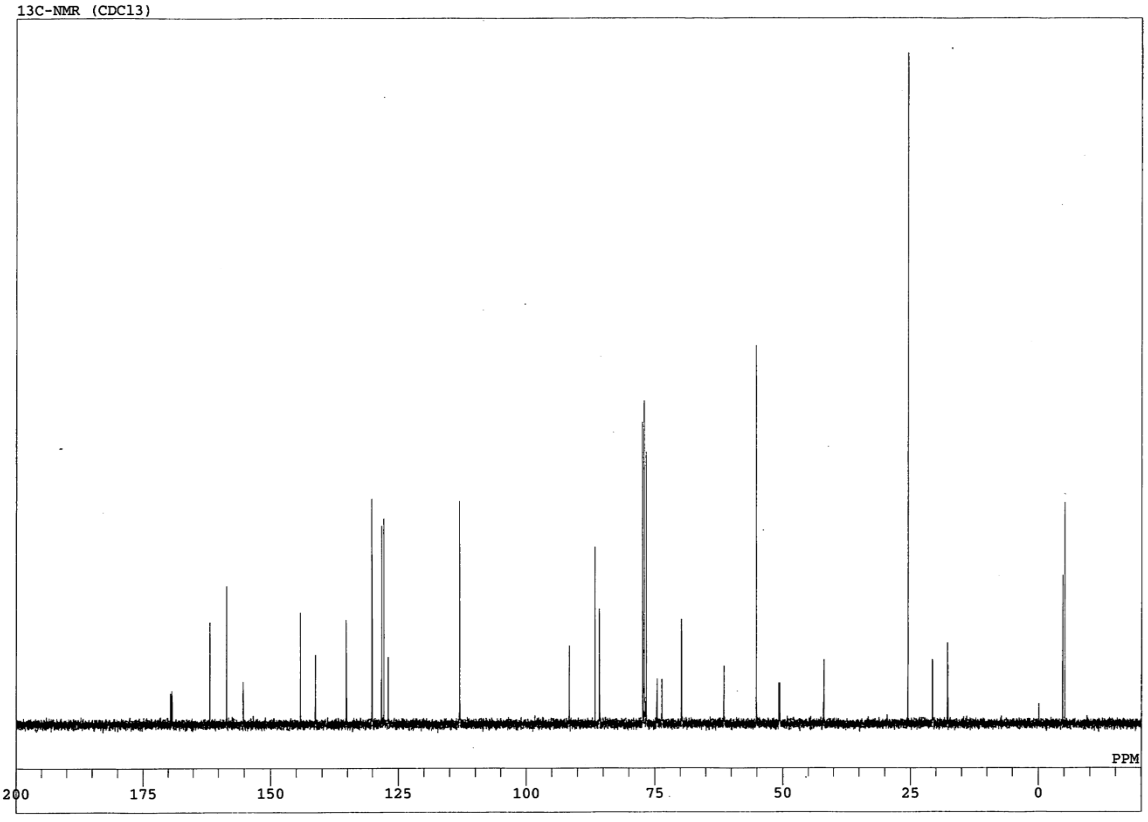
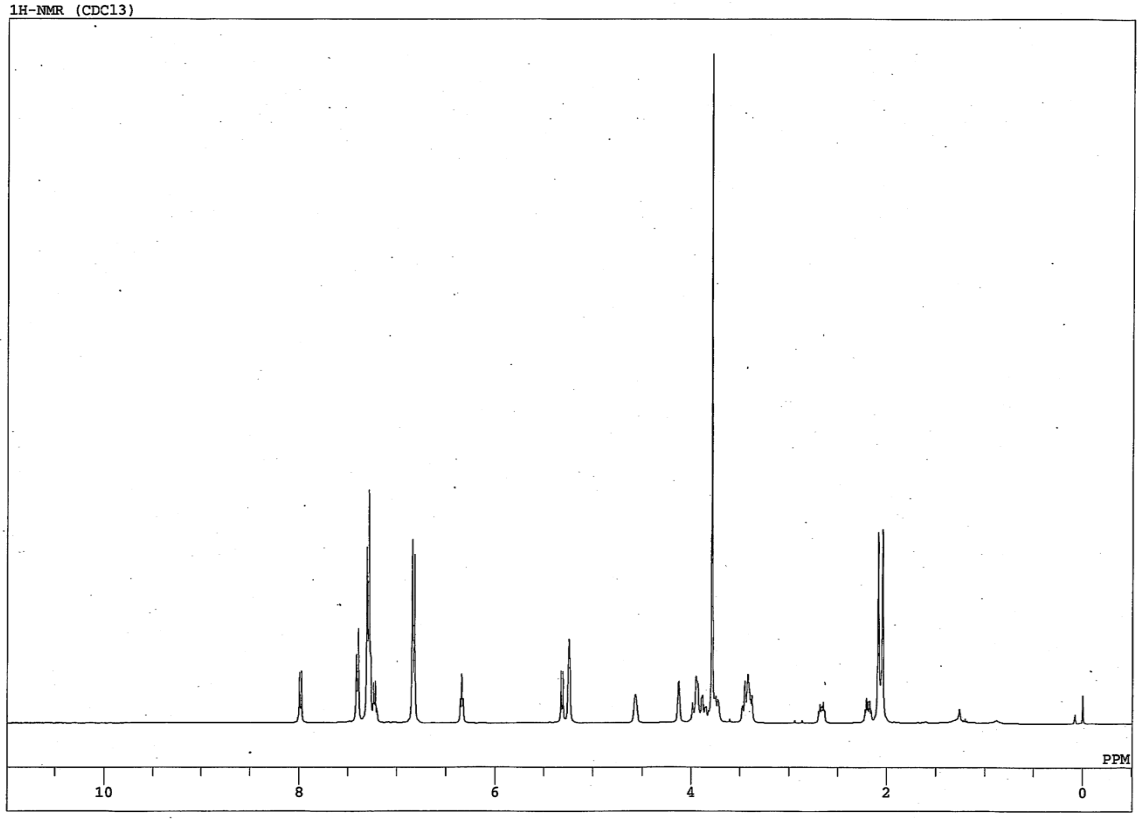
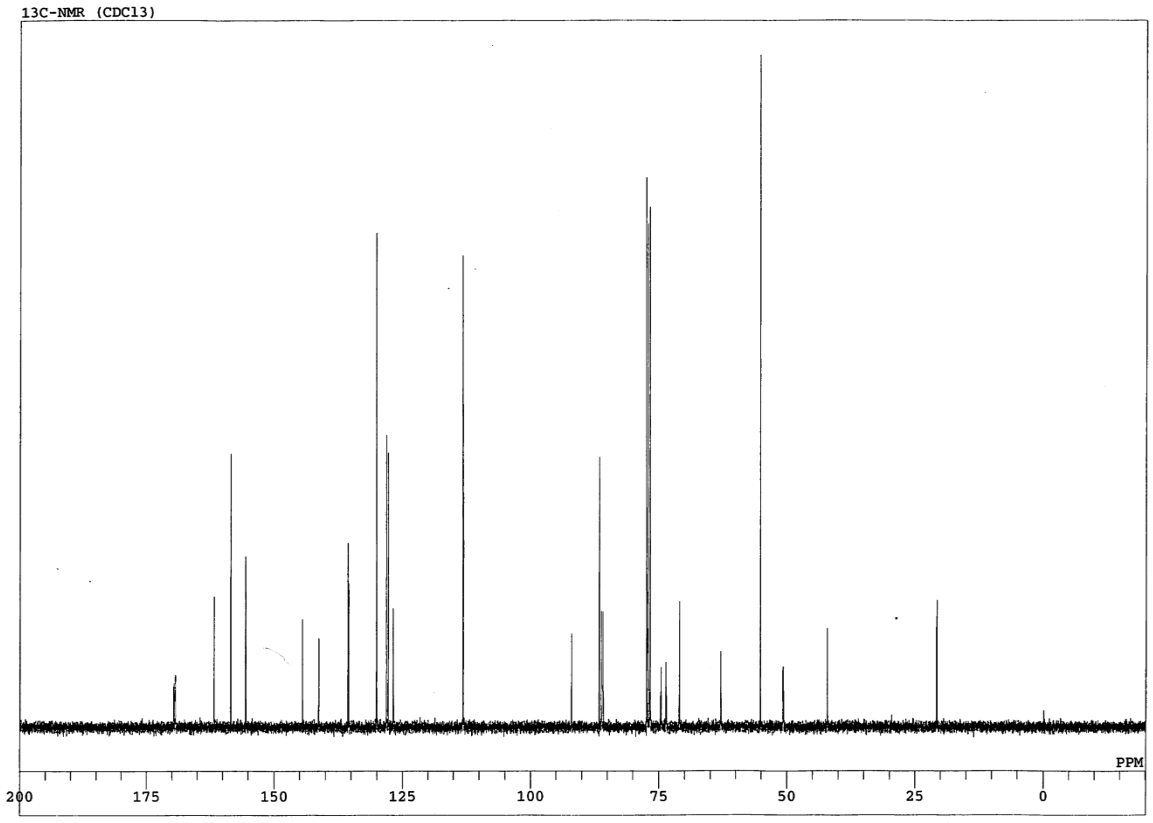

13C-NMR (CDCl3)
PPM
200
175
150
125
100
75
50
25
0

Compound **S5**


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


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

Compound **S6** (diastereoisomers on the basis of chirality at the phosphorus)