Electronic Supplementary Material (ESI) for Chemical Communications
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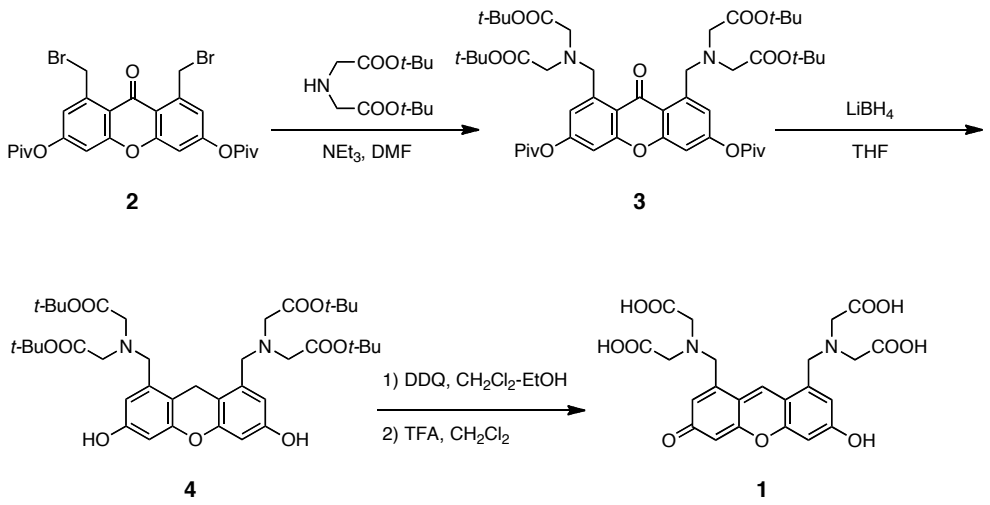
Electronic Supplementary Information for

Coordination Ligand Exchange of a Xanthene Probe-Ce(III) Complex for Selective Fluorescence Sensing of Inorganic Pyrophosphate

Ekkachai Kittiloespaisan, Ippei Takashima, Yuji, Kiatpathomchai, Jirarut Wongkongkatep*, and Akio Ojida*

Scheme S1



Synthesis of **3**

To a cooled (0 °C) solution of *tert*-butyl iminodiacetate (1.0 g, 4.08 mmol), TEA (0.125 mL, 0.90 mmol) in anhydrous DMF (10 ml), the crude **2** (485 mg containing 0.29 mmol of **2**) was added, and the mixture was stirred for 3 h at rt. After dilution with AcOEt, the organic layer was washed with saturated $NaHCO_3$ and brine followed by drying over Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel ($CHCl_3$ / MeOH / NH_3 = 300 / 10 / 1 → 200 / 10 / 1) to give **3** (173 mg, 66%) as a colorless oil. ^{1}H-NMR (400MHz, $CDCl_3$) δ 1.38 (18H, s), 1.46 (36H, s), 3.49 (8H, s), 4.60 (4H, s), 7.07 (2H, d, J = 2.4 Hz), 7.60 (2H, d, J = 2.0 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 179.1, 176.2, 157.3, 155.0, 145.7, 129.8, 128.4, 118.6, 117.7, 108.7, 80.9, 57.1, 56.2, 28.2, 27.1. IR (KBr) 2978, 1736, 1605, 1420, 1366, 1265, 1141, 1103 cm^{-1}. ESI-HRMS *m/e* calcd for $C_{49}H_{71}N_2O_{14}$ $[M+H]^+$ = 911.4900, observed 911.4906. The purity of **3** was confirmed by HPLC (> 90%, UV absorbance 220 nm).

Synthesis of **4**

A solution of **3** (83 mg, 91 μmol) in dry THF (5 ml) was added dropwise to a suspension of $LiBH_4$ (~ 50 mg) in THF at rt. The mixture was stirred at rt for 30 min. After cooling to 0 °C, the solution was acidified to pH 7 with 1N HCl. The mixture was extracted with AcOEt, and washed with saturated $NaHCO_3$ and brine followed by drying over Na_2SO_4. After removal of the solvent in vacuo, the residue was purified

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by flash column chromatography on silica gel ($CHCl_3$ / MeOH / NH_3 = 200 / 10 / 1) to give **4** (42.7 mg, 64%) as a pale yellow solid. ^{1}H-NMR (400MHz, CD_3OD) δ 1.45 (36H, s), 3.43 (8H, s), 3.89 (4H, s), 3.98 (2H, s), 6.40 (2H, d, *J* = 2.4 Hz), 6.65 (2H, d, *J* = 2.4 Hz). ^{13}C-NMR (126 MHz, CD_3OD) δ 172.3, 157.2, 153.6, 139.1, 113.5, 113.1, 103.1, 82.1, 57.1, 56.1, 28.5, 22.2. IR (KBr) 3418, 2978, 1728, 1450, 1366, 1149 cm^{-1}. MALDI-TOF-MS (CHCA, Linear, positive) *m/e* calcd for $C_{39}H_{57}N_2O_{11}$ $[M+H]^+$ = 729.3957, observed 729.3952. The purity of **3** was confirmed by HPLC (> 97%, UV absorbance 220 nm).

Synthesis of compound **1**

A solution of DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone, 14.0 mg, 61.6 μmol) in dry EtOH (3 ml) was added dropwise to a solution of **4** (46.0 mg, 63.1 μmol) in dry CH_2Cl_2 (4 mL) and dry EtOH (2 ml). The solution was stirred for 30 min at rt. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel ($CHCl_3$ / MeOH = 20 / 1). The obtained solid was washed with isopropyl ether. The residue was dissolved in dry CH_2Cl_2 (4 mL) and TFA (3 mL) was added. The solution was stirred for 6 h at rt. After removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ and evaporated (the procedure was repeated twice). The residue was purified by HPLC (column ; YMC-Pack, ODS-A, 250×10 mm, CH_3CN / Water (0.1 % TFA) = 13/87 (35 min)) to give **1** (12.8 mg, 40 %) as an orange solid. The purity of **1** was confirmed by HPLC (> 98%, UV absorbance 220 nm). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ 3.48 (8H, s), 4.26 (4H, s), 6.84 (2H, s), 7.00 (2H, s), 9.94 (1H, s). ^{13}C-NMR (126 MHz, DMSO-*d6*) δ 172.2, 172.1, 172.0, 157.5, 156.1, 152.8, 141.0, 102.1, 54.1, 53.6. IR (KBr) 3017, 1728, 1612, 1397, 1265, 1195 cm^{-1}. FAB-HRMS (triethanolamine) *m/e* calcd for $C_{23}H_{21}N_2O_{11}$ $[M-H]^-$ = 501.1145, observed 501.1146.

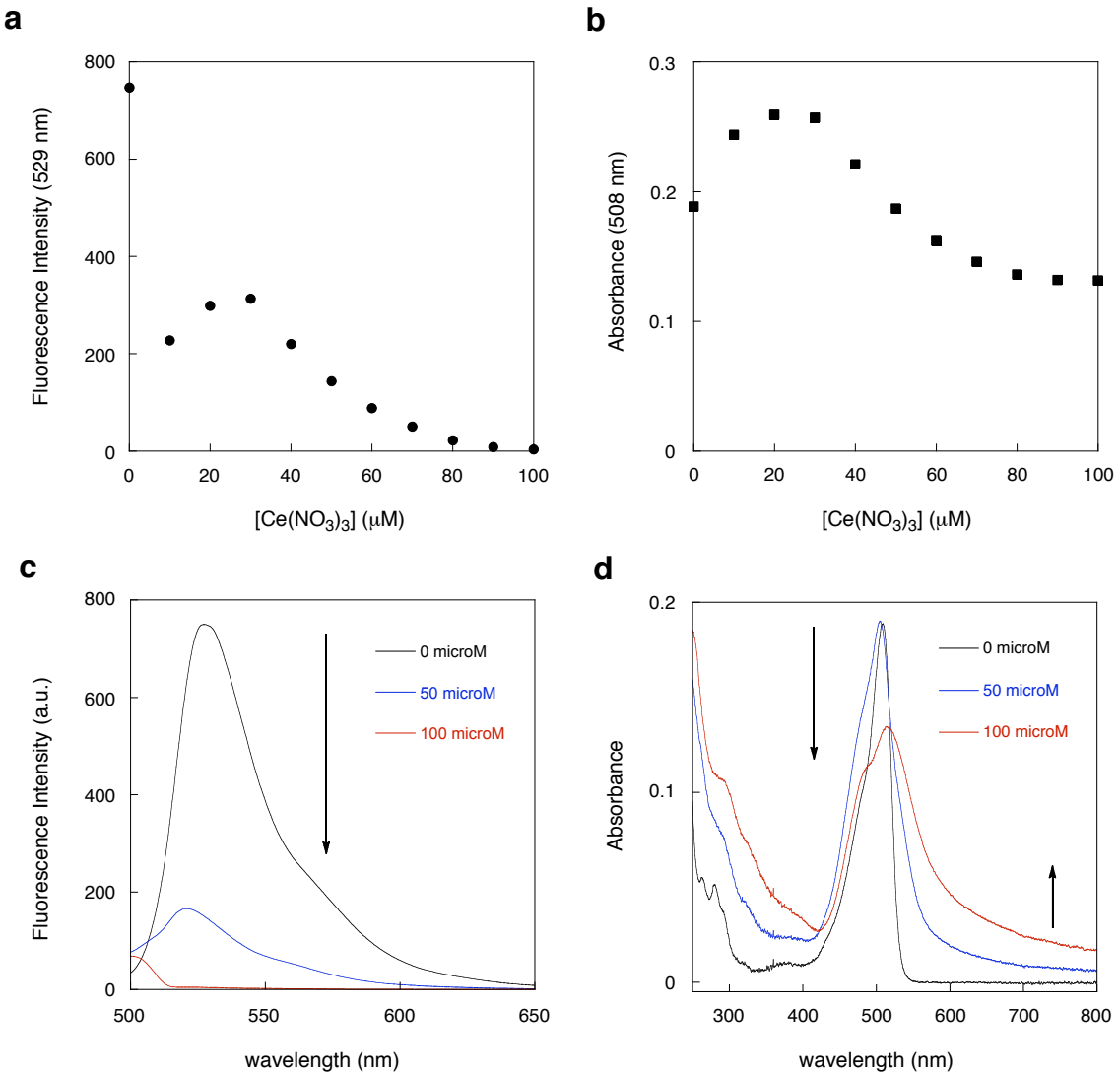

Fig. S1 UV absorbance (a, c) and fluorescence (b, d) spectral change of **1** upon addition of $Ce(NO_3)_3$. Conditions: [**1**] = 5 μM, 25 mM MES (pH 6.8)-MeOH (1 : 1), 25 °C, λ_{ex} = 500 nm.

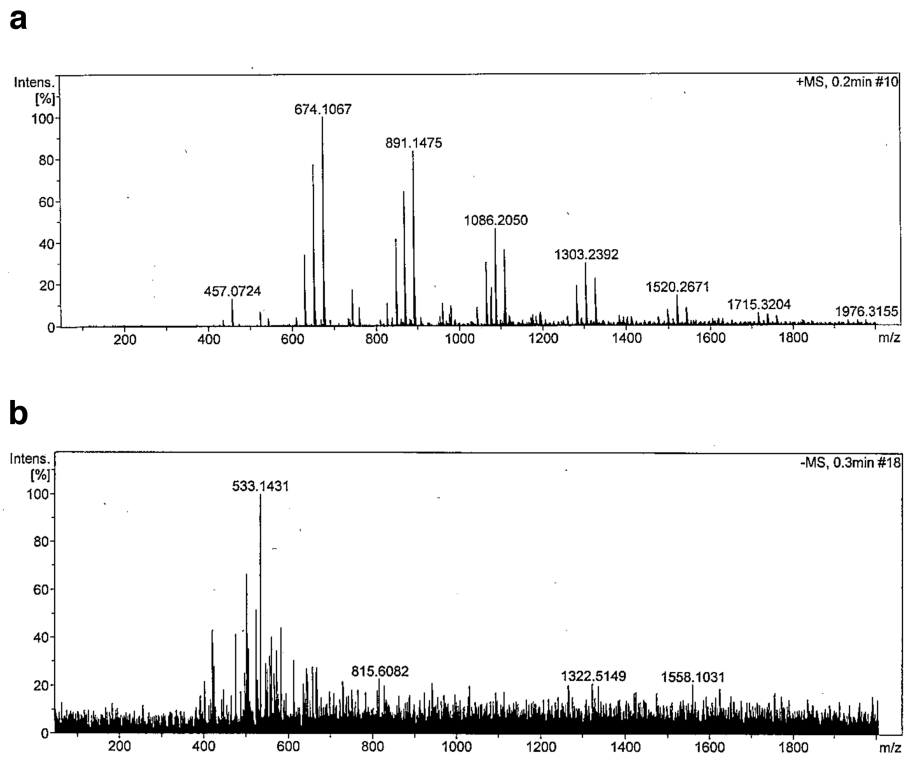

Fig. S2 ESI spectra of **1**-Ce(III) complex in the absence (a) and presence (b) of PPi.

Fluorescence Measurement

Fluorescence spectra were recorded on a Perkin-Elmer LS55 spectrometer. A solution (3 mL) of **1** (5 μM) and $Ce(NO_3)_3$ (100 μM) in 25 mM MES (pH 6.8)-MeOH (1 : 1) was stirred for 1 h at rt in a quartz cell. The titration experiments (Figure 2 and 3) were carried out at 25 °C using this solution. The fluorescence emission spectral change (excitation wavelength λ_{ex} = 500 nm) was monitored at 1~3 min later after addition of aqueous anion solution, at which time the fluorescence change completed and the solution reached an equilibrium state. The aqueous anion solution was freshly prepared before the titration and added to the cell with a micro syringe. Abbreviations of the analytes listed in Figure 3 are as follows; PPi = inorganic pyrophosphate, ATP = adenosine-5'-triphosphate, GTP = guanosine-5'-triphosphate, CTP = cytidine-5'-triphosphate, UDP = uridine-5'-diphosphate, ADP = adenosine-5'-diphosphate, AMP = adenosine-5'-monophosphate, cAMP = adenosine-3',5'-cyclic monophosphate, Pi = inorganic phosphate, $NaHCO_3$ = sodium bicarbonate, AcONa = sodium acetate Na_2SO_4 = sodium sulfate, $K_2S_2O_7$ = potassium pyrosulfate.

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Fluorescent LAMP assay

The fluorescent LAMP assay was performed using the serially diluted samples of WSSV recombinant plasmid (0, 2, 20, 200, and 1000 copies). The amplification reaction was conducted at 63 °C for 30 min under the reaction conditions shown in Table S1. After reaction termination at 92 °C for 2 min, @ mL of the reaction mixture was added to the solution of **1**-Ce(III) complex (100 μL containing 15 mM of **1**, 270 mM $Ce(NO_3)_3$, pH 6.8, 25 °C in 25 mM MES (pH 6.8)-MeOH (1 : 1)). Fluorescence intensity of each sample was measured by microplate reader (EnSpire, Perkin-Elmer).

Table S1. Conditions of the fluorescent LAMP assay

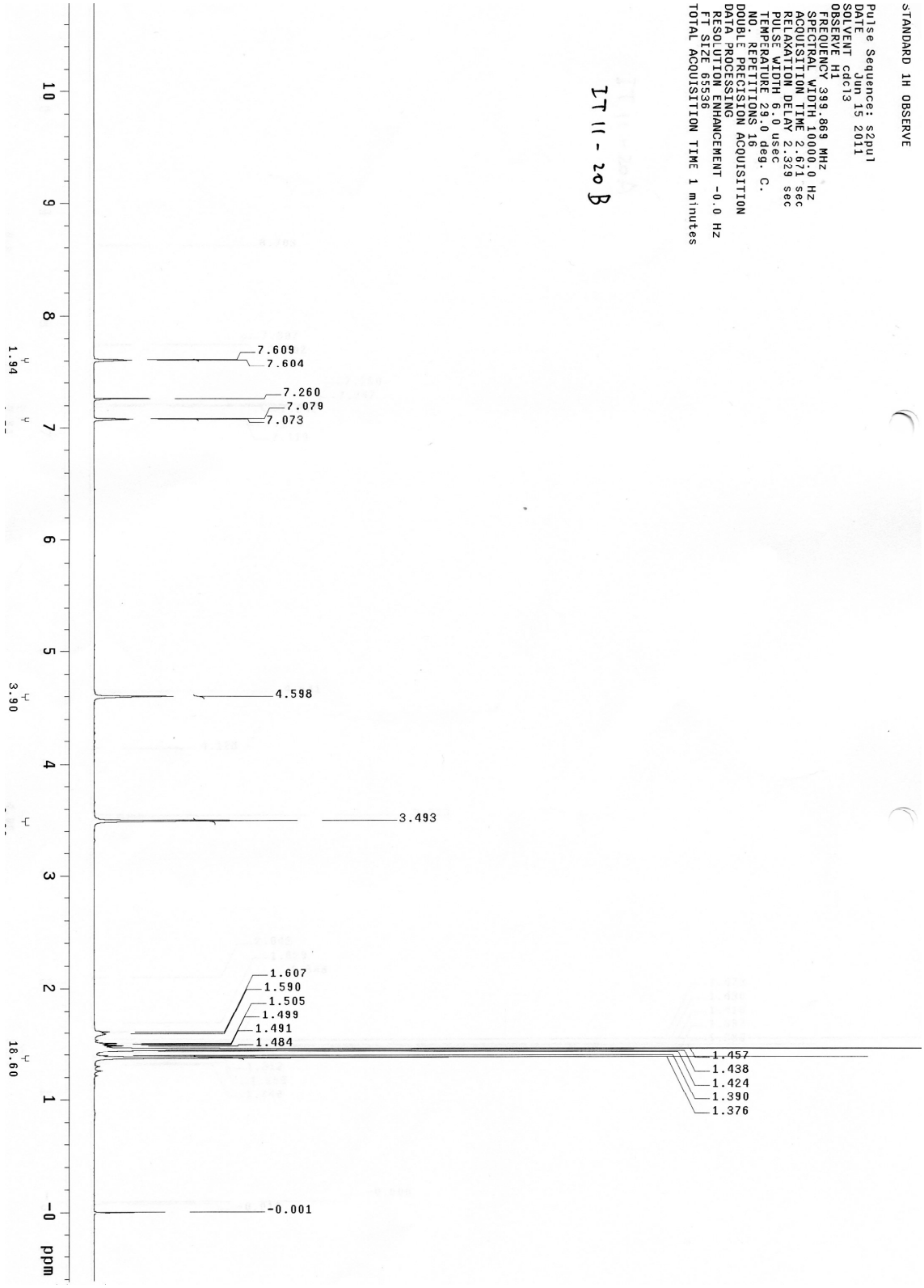
Component	Final concentration
inner primers (FIP and BIP)[a]	2 μM (each)
loop primers (LF and LB)[a]	2 μM (each)
outer primers (F3 and B3)[a]	0.2 μM (each)
betaine	0.4 M
dNTP mix	0.5 mM
$MgSO_4$	2 mM
Bst DNA polymerase	0.32 μU
Bst DNA polymerase supplied buffer	1x

[a]Sequence of the primer was reported in the previous manuscript.[S1]

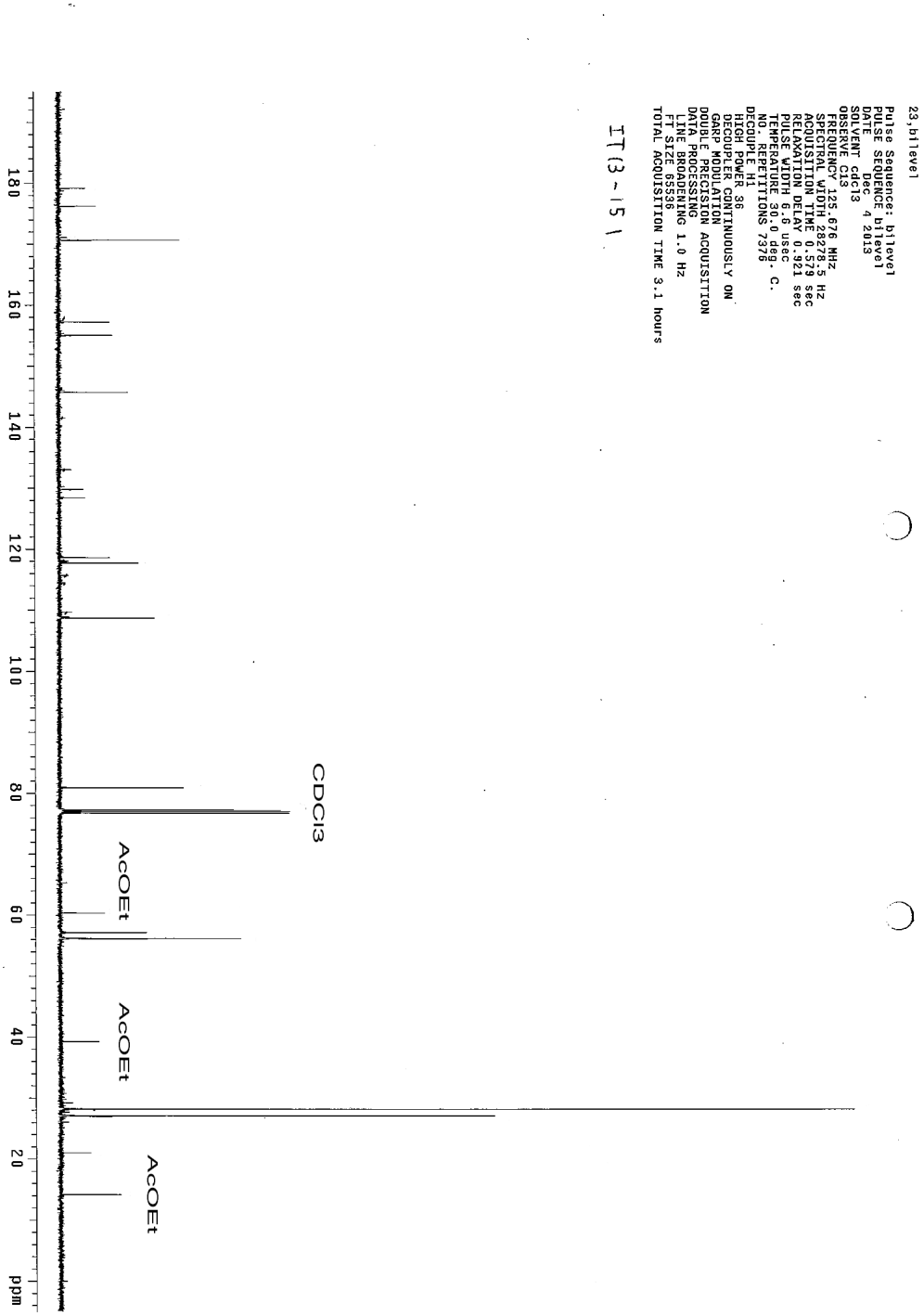
References

S1. E. Kittiloespaisan, A. Ojida, I. Hamachi, Y. S.eetang-Nun, W. Kiatpathomchai, J. Wongkongkatep, *Chem. Lett.*, **2012**, *41*, 1666.

^{1}H-NMR of Compound **3**



^{13}C-NMR of Compound **3**



ESI-HRMS of Compound **3**

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active			Set Dry Heater	170 °C
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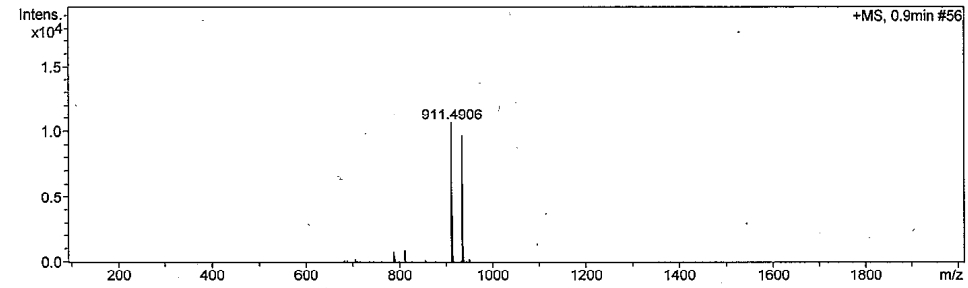
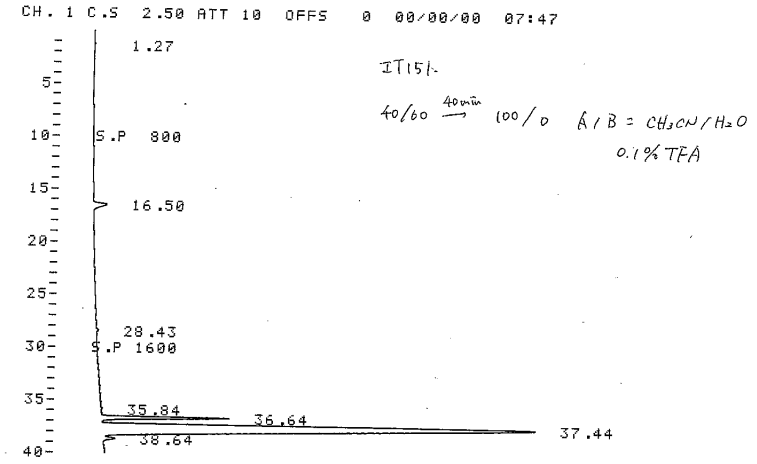
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
911.4906	1	C 49 H 71 N 2 O 14	100.00	911.4900	-0.6	-0.7	83.6	15.5	even	ok
	2	C 50 H 67 N 6 O 10	35.05	911.4913	0.7	0.8	105.2	20.5	even	ok
	3	C 54 H 71 O 12	4.02	911.4940	3.4	3.7	106.5	19.5	even	ok
	4	C 52 H 59 N 14 O 2	2.62	911.4940	3.4	3.7	115.1	30.5	even	ok
	5	C 51 H 63 N 10 O 6	8.59	911.4927	2.1	2.3	115.8	25.5	even	ok
	6	C 58 H 59 N 10 O	0.39	911.4868	-3.8	-4.2	140.7	34.5	even	ok
	7	C 61 H 67 O 7	1.39	911.4881	-2.5	-2.7	141.8	28.5	even	ok
	8	C 62 H 63 N 4 O 3	1.70	911.4895	-1.1	-1.2	153.6	33.5	even	ok
	9	C 67 H 63 N 2 O	0.08	911.4935	2.9	3.2	177.8	37.5	even	ok

HPLC of Compound **3**



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FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
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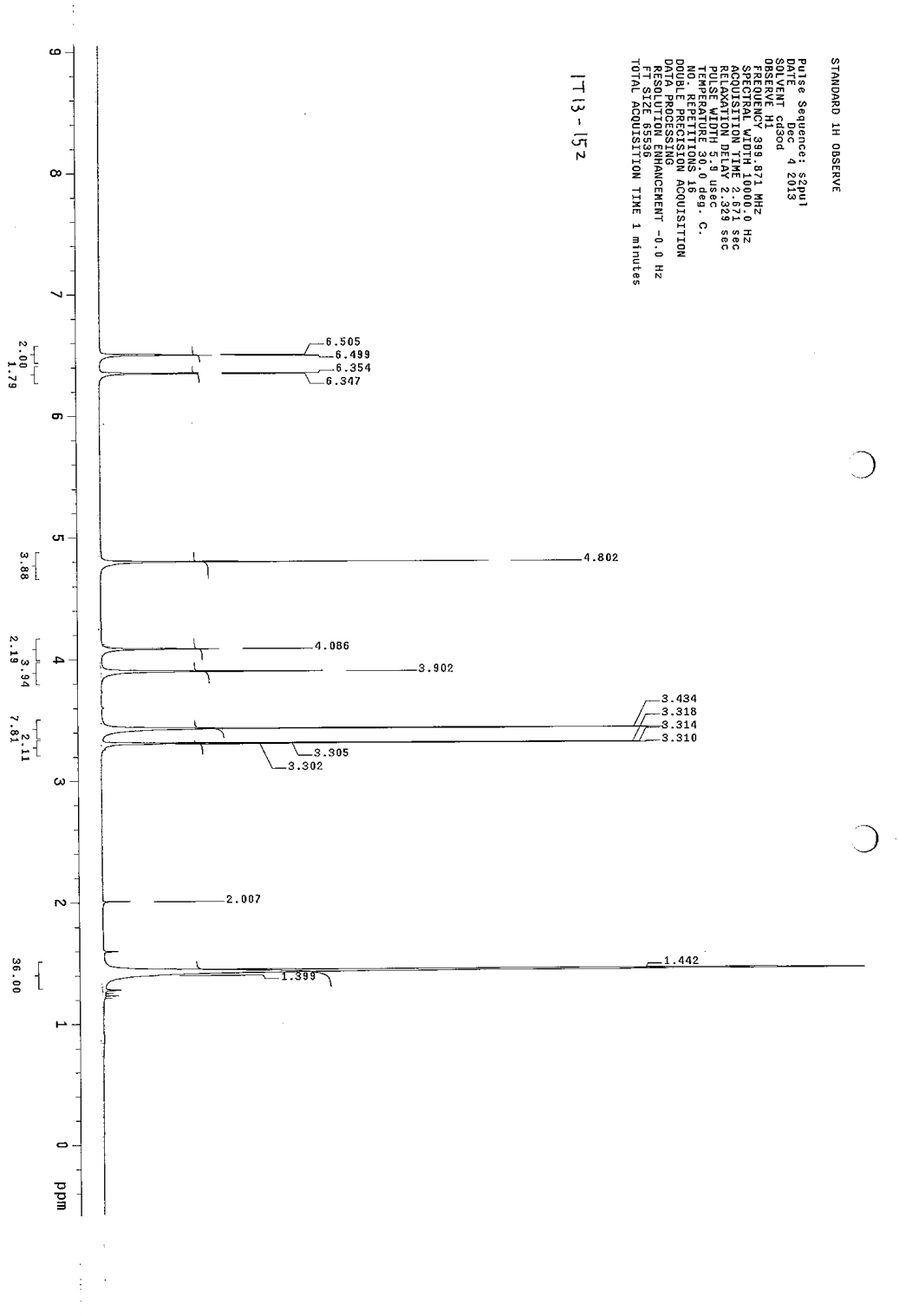
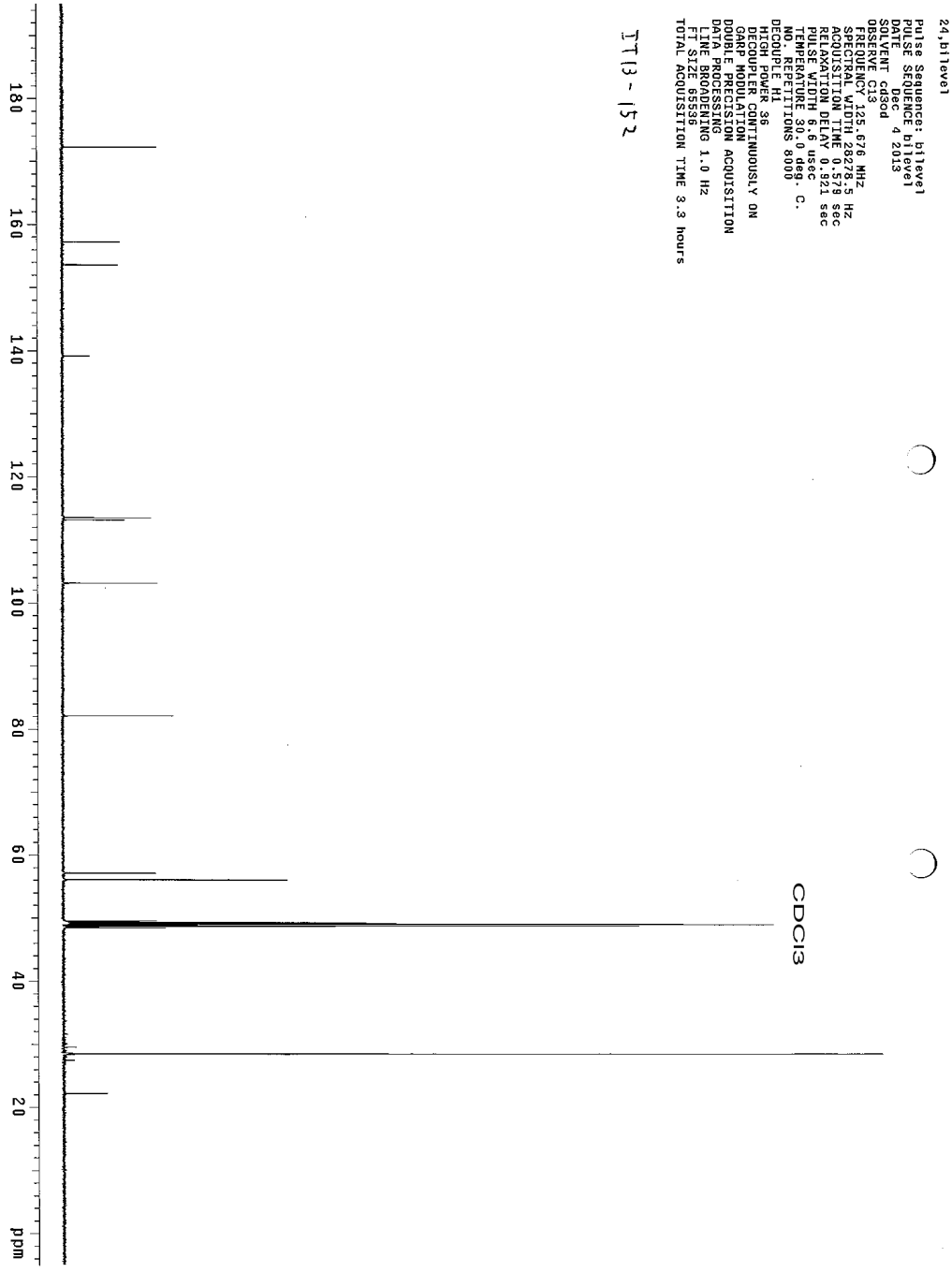
6	37.44	24050966	90.371	VV
TOTAL		26613655	100.000	
PEAK REJ :		500000		

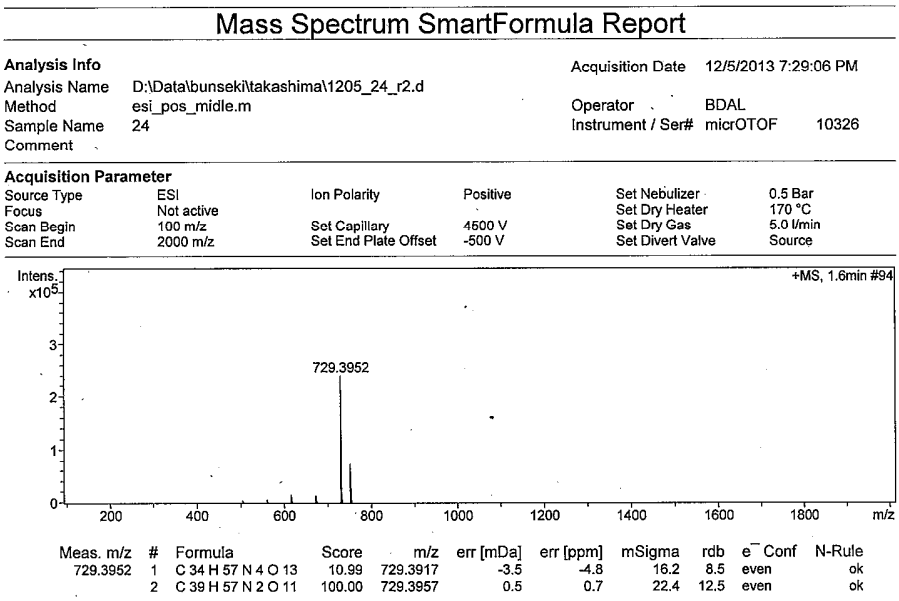
^{1}H-NMR of Compound **4**



^{13}C-NMR of Compound **4**



ESI-HRMS of Compound **4**



Mass Spectrum SmartFormula Report

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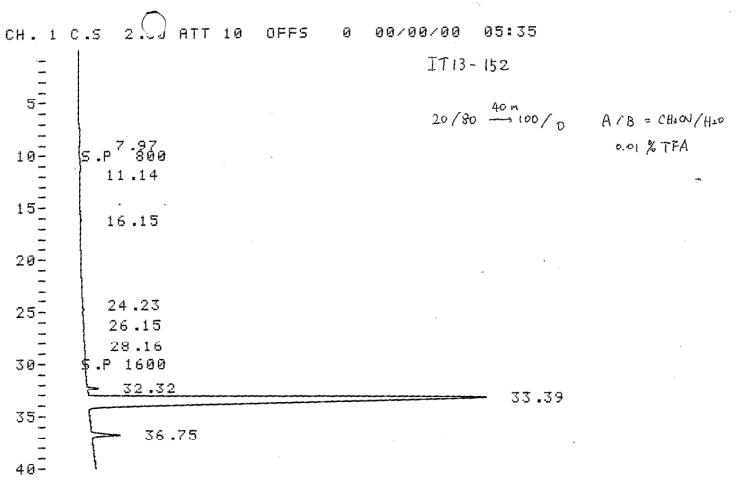
Sample Name	24	Instrument / Ser#	micrOTOF 10326
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active			Set Dry Heater	170 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	5.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
729.3952	1	C 34 H 57 N 4 O 13	10.99	729.3917	-3.5	-4.8	16.2	8.5	even	ok
	2	C 39 H 57 N 2 O 11	100.00	729.3957	0.5	0.7	22.4	12.5	even	ok

HPLC of Compound **4**



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FILE: 1 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	CONC	BC
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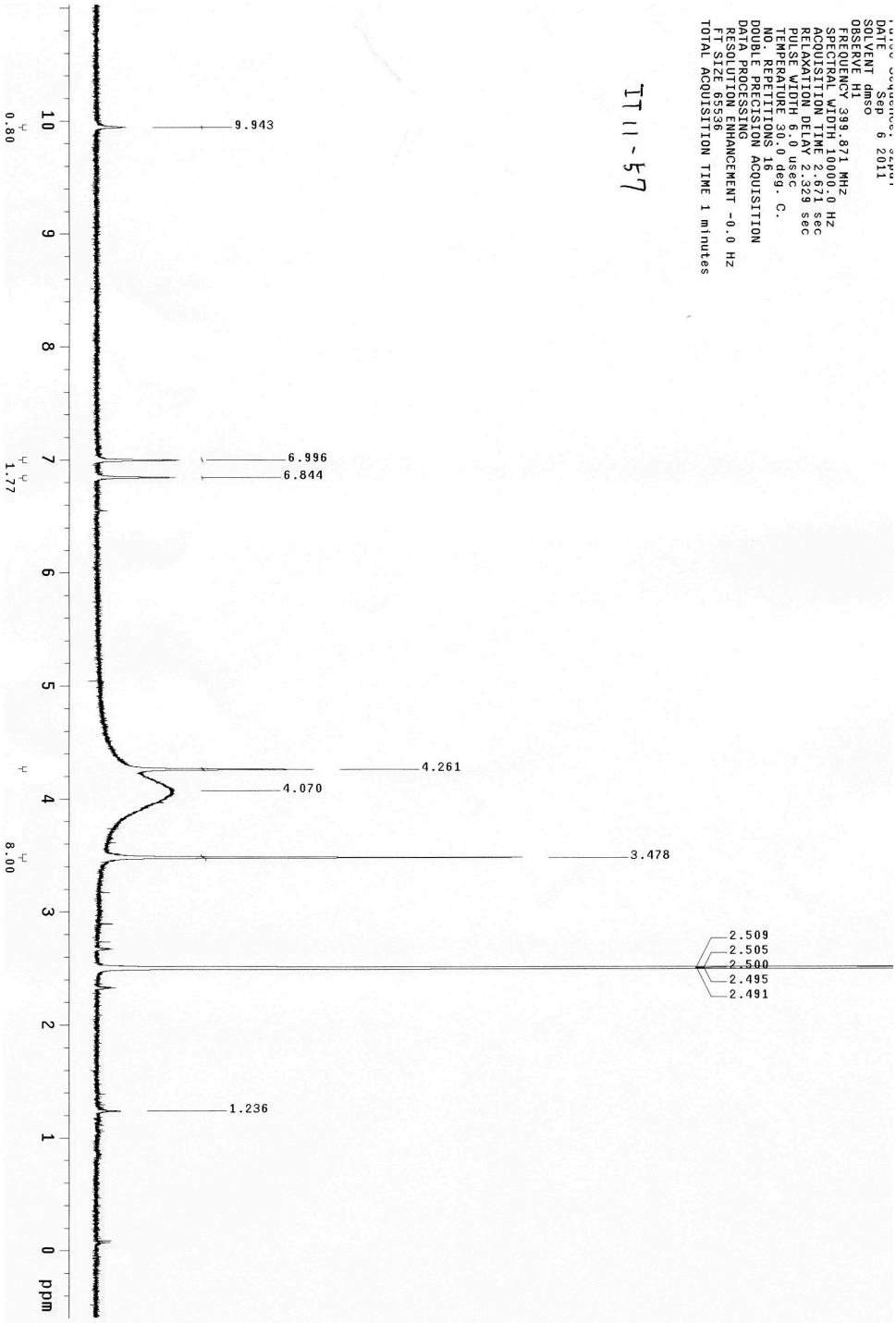
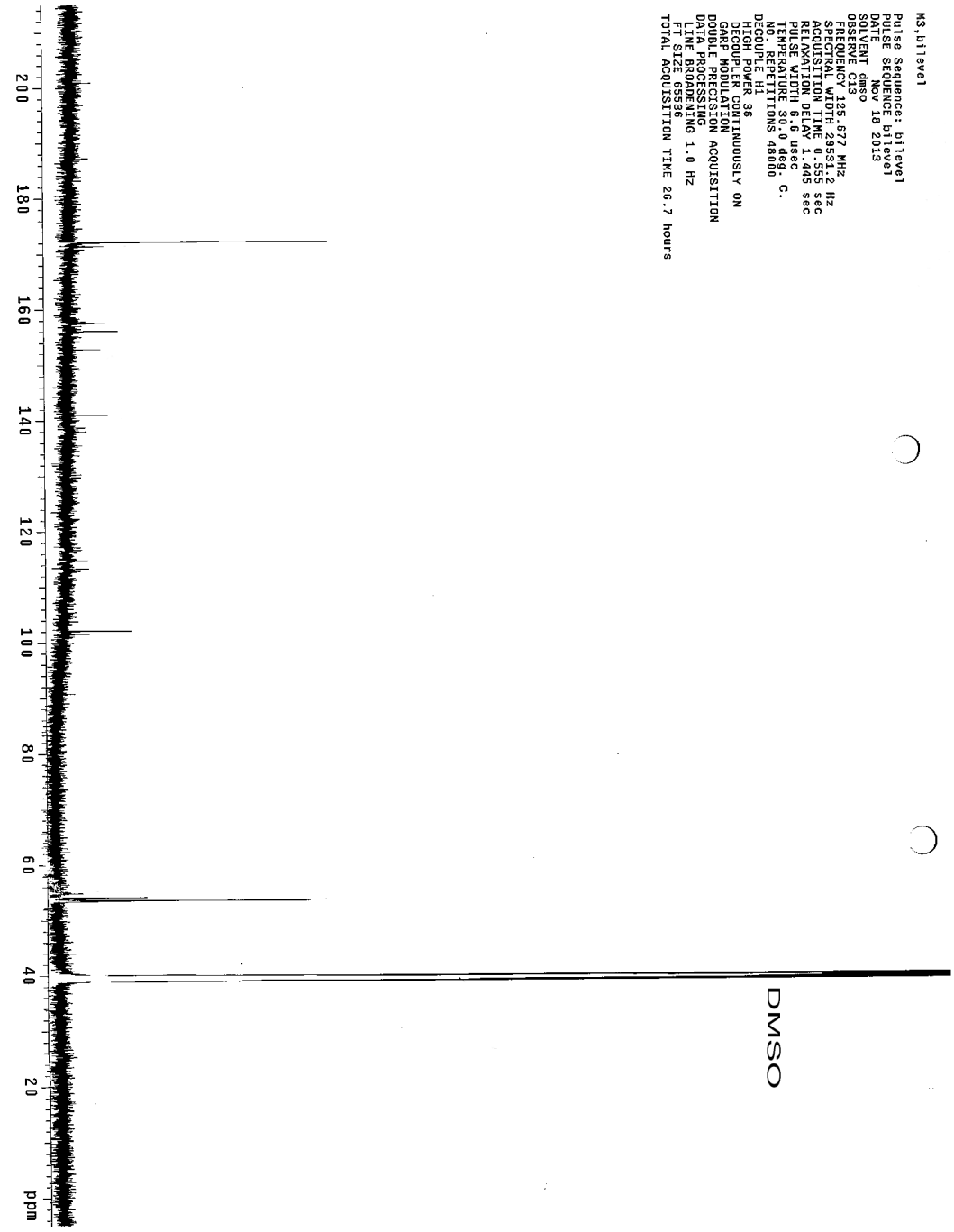
PEAK REJ :		500000		

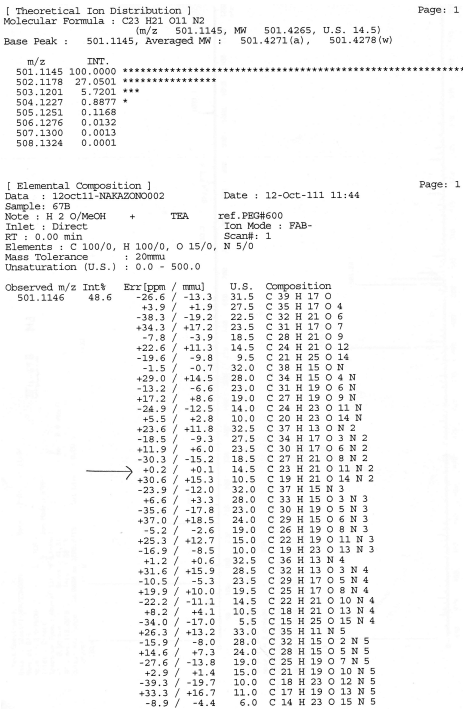
[1]H-NMR of Compound **1**



^{13}C-NMR of Compound **1**



FAB-HRMS of Compound **1**



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[ Theoretical Ion Distribution ]                                    Page: 1
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                    (m/z   501.1145, MW   501.4265, U.S. 14.5)
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    m/z       INT.
  501.1145 100.0000 ************************************************************
  502.1178  27.0501 ***************
  503.1201   5.7201 ***
  504.1227   0.8877 *
  505.1251   0.1168
  506.1276   0.0132
  507.1300   0.0013
  508.1324   0.0001


[ Elemental Composition ]                                           Page: 1
Data  : 12oct11-NAKAZONO002          Date : 12-Oct-111 11:44
Sample: 67B
Note : H 2 O/MeOH     +      TEA      ref.PEG#600
Inlet : Direct                        Ion Mode : FAB-
RT : 0.00 min                         Scan#: 1
Elements : C 100/0, H 100/0, O 15/0, N 5/0
Mass Tolerance          : 20mmu
Unsaturation (U.S.) : 0.0 - 500.0

Observed m/z Int%   Err[ppm / mmu]    U.S.  Composition
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                      +3.9 /  +1.9    27.5  C 35 H 17 O 4
                     -38.3 / -19.2    22.5  C 32 H 21 O 6
                     +34.3 / +17.2    23.5  C 31 H 17 O 7
                      -7.8 /  -3.9    18.5  C 28 H 21 O 9
                     +22.6 / +11.3    14.5  C 24 H 21 O 12
                     -19.6 /  -9.8     9.5  C 21 H 25 O 14
                      -1.5 /  -0.7    32.0  C 38 H 15 O N
                     +29.0 / +14.5    28.0  C 34 H 15 O 4 N
                     -13.2 /  -6.6    23.0  C 31 H 19 O 6 N
                     +17.2 /  +8.6    19.0  C 27 H 19 O 9 N
                     -24.9 / -12.5    14.0  C 24 H 23 O 11 N
                      +5.5 /  +2.8    10.0  C 20 H 23 O 14 N
                     +23.6 / +11.8    32.5  C 37 H 13 O N 2
                     -18.5 /  -9.3    27.5  C 34 H 17 O 3 N 2
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                     +37.0 / +18.5    24.0  C 29 H 15 O 6 N 3
                      -5.2 /  -2.6    19.0  C 26 H 19 O 8 N 3
                     +25.3 / +12.7    15.0  C 22 H 19 O 11 N 3
                     -16.9 /  -8.5    10.0  C 19 H 23 O 13 N 3
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                     +19.9 / +10.0    19.5  C 25 H 17 O 8 N 4
                     -22.2 / -11.1    14.5  C 22 H 21 O 10 N 4
                      +8.2 /  +4.1    10.5  C 18 H 21 O 13 N 4
                     -34.0 / -17.0     5.5  C 15 H 25 O 15 N 4
                     +26.3 / +13.2    33.0  C 35 H 11 N 5
                     -15.9 /  -8.0    28.0  C 32 H 15 O 2 N 5
                     +14.6 /  +7.3    24.0  C 28 H 15 O 5 N 5
                     -27.6 / -13.8    19.0  C 25 H 19 O 7 N 5
                      +2.9 /  +1.4    15.0  C 21 H 19 O 10 N 5
                     -39.3 / -19.7    10.0  C 18 H 23 O 12 N 5
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                      -8.9 /  -4.4     6.0  C 14 H 23 O 15 N 5
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HPLC of Compound **1**