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Supporting Information

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Supporting Information

for

Synthesis of Light-Responsive Bridged Nucleic Acid and Changes in Affinity with Complementary ssRNA

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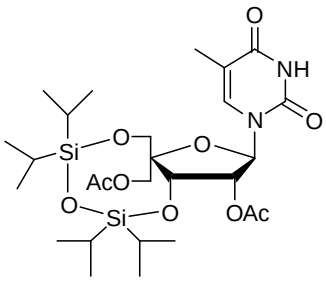
Contents

1. General

All moisture-sensitive reactions were carried out in well-dried glassware under a N_2 atmosphere. Acetonitrile, DMF and pyridine were distilled from CaH_2. ^{1}H NMR (270 MHz), ^{13}C NMR (67.8 MHz), ^{31}P NMR spectra (202 MHz), HMBC spectra (400 MHz), and NOESY spectra (400 MHz) were recorded on JEOL JNM-EX270 and JEOL JNM-ECS400 and JEOL JNM-LA500 spectrometers. Chemical shifts are reported in ppm in reference to $CHCl_3$ (δ 7.26) and DMSO (δ 2.50) for ^{1}H NMR spectra and $CDCl_3$ (δ 77.0) and [D_6]DMSO (δ 39.5) for ^{13}C NMR, respectively. IR spectra were recorded on a JASCO FT/IR-200 and JASCO FT/IR-4200 spectrometer. Optical rotations were recorded on a JASCO DIP-370 instrument. FAB Mass spectra were measured on JEOL JMS-600 or JEOL JMS-700 mass spectrometer. MALDI-TOF Mass spectra were recorded on a Bluker Daltonics Autoflex II TOF/TOF mass spectrometer. For column chromatography, Fuji Silysia silica gel PSQ-100B (0.100 mm) and FL-100D (0.100 mm) was used. For flash column chromatography, silica gel PSQ-60B (0.060 mm) and FL-60D (0.060 mm) was used. For high performance liquid chromatography (HPLC), SHIMADZU LC-10AT$_{VP}$ and SHIMADZU SPD-10A$_{VP}$ and SHIMADZU CTO-10$_{VP}$ was used.

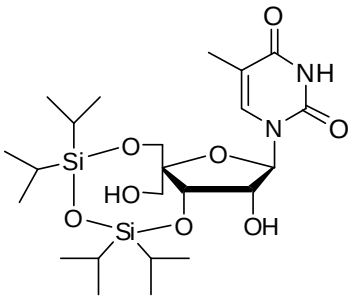
2. Synthesis of Light-Responsive BNA Monomer and Phosphoroamidites: 1-[4-*C*-Acetoxymethyl-2-*O*-acetyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribo- furanosyl]thymine (2).



Under H_2 atmosphere, a mixture of compound **1**[1)] (10.0 g, 18.6 mmol) and 20% Pd $(OH)_2$-C (5.0 g) in AcOEt (100 mL) was stirred at room temperature for 9 h. The reaction mixture was filtered and concentrated to give a crude diol (6.95 g) as white foam, to a solution of the crude diol in DMF (200 mL) was added imidazole (5.62 g, 93.5 mmol) and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (8.99 mL, 28.1 mmol) and the resultant mixture was stirred at room temperature for 3.5 h. After addition of water at 0 ˚C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude was purified by column chromatography (*n*-hexane/AcOEt = 3/2) to give compound **2** (8.16 g, 72% from compound **1**) as a white foam: m.p. 75－78 ℃. $[\alpha]_D^{23}$ －28.8 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 1372, 1465, 1724,

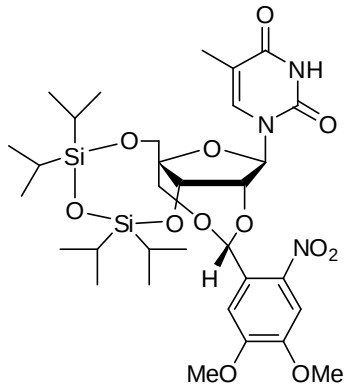
2869, 2947, 3192 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 1.01-1.15 (28H, m), 1.90 (3H, d, *J* = 1 Hz), 2.13 (3H, s), 2.16 (3H, s), 3.87 (1H, d, *J* = 11 Hz), 3.96 (1H, d, *J* = 11 Hz), 4.17 (1H, d, *J* = 12 Hz), 4.83 (1H, d, *J* = 12 Hz), 4.97 (1H, d, *J* = 7 Hz), 5.46 (1H, d, *J* = 1 Hz), 5.69 (1H, dd, *J* = 1, 7 Hz), 7.04 (1H, d, *J* = 1 Hz), 8.04 (1H, brs). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 12.3, 12.5, 12.8, 13.0, 13.1, 17.0, 17.0 17.1, 17.1, 17.3, 17.4, 17.5, 20.8, 21.1, 61.5, 64.5, 73.1, 75.4, 85.2, 92.6, 110.7, 138.6, 149.3, 163.8, 169.3, 171.0. MS (FAB): *m/z* 615 $[M+H]^+$. HRMS (FAB): calcd for $C_{27}H_{47}N_2O_{10}Si_2$ $[M+H]^+$: 615.2769; found: 615.2764.

1-[4-*C*-Hydroxymethyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribo-furanosyl]thy-mine (3).



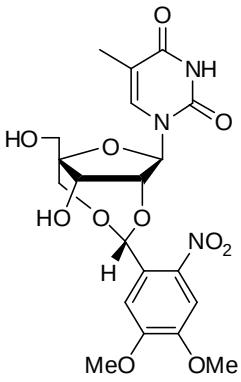
To a solution of compound **2** (721 mg, 1.17 mmol) in THF (12 mL) was added aqueous 40% $MeNH_2$ (10.1 mL, 117 mmol) at 0 ˚C and the resultant mixture was stirred at 0 ˚C for 3 h. The reaction mixture was concentrated and diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude was purified by column chromatography (*n*-hexane/AcOEt 2:3) to give compound **3** (522 mg, 85%) as a white form: m.p. 94－96 ℃. $[\alpha]_D^{23}$ －24.4 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 1265, 1387, 1467, 1694, 2869, 2947, 3382 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 1.04-1.14 (28H, m), 1.91 (3H, d, *J* = 1 Hz), 2.32 (1H, dd, *J* = 4, 8 Hz), 3.54 (1H, d, *J* = 4 Hz), 3.68 (1H, d, *J* = 12 Hz), 3.71 (1H, d, *J* = 12 Hz), 3.88 (1H, d, *J* = 12 Hz), 3.94 (1H, d, *J* = 12 Hz), 4.00 (1H, d, *J* = 4 Hz), 4.05 (1H, d, *J* = 4 Hz), 4.44-4.48 (1H, m), 4.92 (1H, d, *J* = 7 Hz), 5.54 (1H, s), 7.16 (1H, d, *J* = 1 Hz), 8.14 (1H, brs). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 12.5, 12.7, 12.9, 13.4, 17.1, 17.1, 17.2, 17.3, 17.4, 17.4, 17.5, 62.3, 64.8, 71.8, 75.4, 88.0, 94.6, 110.6, 137.5, 150.0, 163.8. MS (FAB): *m/z* 531 $[M+H]^+$. HRMS (FAB): calcd for $C_{23}H_{43}N_2O_8Si_2$ $[M+H]^+$: 531.2558; found: 531.2574.

1-[2-*O*,4-*C*-{(1*R*)-1-(2-Nitro-4,5-dimethoxyphenyl)-2-oxapropylene}-3,5-*O*-(1,1,3,3-tetraisopro-pyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]thymine (4).



To a solution of compound **3** (600 mg, 1.13 mmol) in 1,1,1,3,3,3-hexafluoroisopropanol (5.5 mL) was added 6-nitroveratraldehyde (1.16 g, 5.5 mmol) and zinc chloride (185 mg, 1.36 mmol) and the resultant mixture was stirred at room temperature for 21 h. After addition of saturated $NaHCO_3$ (aq.) at 0 ˚C, the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude was purified by column chromatography (*n*-hexane/AcOEt 2:1 to 1:1 to 2:3) to give compound **4** (439 mg, 54%) as a yellow foam: m.p. 142－144 ℃. $[\alpha]_D^{24}$ －14.0 (c 1.00, $CHCl_3$). IR ν_{max} (KBr): 1280, 1464, 1523, 1693, 2869, 3173, 3614 cm^{-1}. ^1H NMR (270 MHz, $CDCl_3$): δ 1.07-1.14 (28H, m), 1.93 (3H, s), 3.67 (1H, d, *J* = 7 Hz), 3.72 (1H, d, *J* = 7 Hz), 3.95 (3H, s), 4.04 (1H, d, *J* = 12 Hz), 4.05 (3H, s), 4.11 (1H, d, *J* = 12 Hz), 4.36 (1H, d, *J* = 7 Hz), 4.60 (1H, d, *J* = 7 Hz), 6.29 (1H, s), 6.98 (1H, s), 7.60-7.64 (3H, m), 8.16 (1H, brs). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 12.4, 12.6, 12.7, 13.4, 16.9, 17.0, 17.1, 17.1, 17.2, 17.2, 17.4, 56.4, 56.6, 59.5, 68.0, 70.6, 79.5, 89.5, 91.5, 98.3, 107.8, 109.5, 110.2, 129.0, 134.5, 140.0, 148.6, 150.0, 153.3, 164.0. MS (FAB): *m/z* 724 $[M+H]^+$. HRMS (FAB): calcd for $C_{23}H_{43}N_2O_8Si_2$ $[M+H]^+$: 724.2933; found: 724.2936.

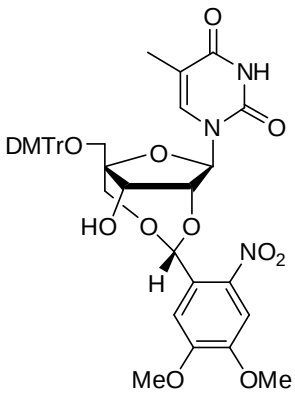
1-[2-*O*-4-*C*-{(1*R*)-1-(2-Nitro-4,5-dimethoxyphenyl)-2-oxapropylene}-β-D-ribofu-ranosyl]-thymi-ne (5).



To a solution of compound **4** (864 mg, 1.19 mmol) in THF (30 mL) was added tetrabutylammonium fluoride (1.0 M in THF, 2.38 mL, 2.38 mmol) and the resultant mixture was stirred at 0 ˚C for 0.5 h. The reaction mixture was concentrated and the crude was purified by column chromatography (AcOEt/MeOH = 15/1) to give compound **5** (509 mg, 89%) as a yellow powder: m.p. 241－243 ℃. $[\alpha]_D^{25}$ －52.9 (c 1.00, DMSO). IR ν_{max} (KBr): 1279, 1335, 1468, 1521, 1692, 3075, 3284 cm^{-1}. ^1H NMR (270 MHz, $[D_6]$DMSO): δ 1.76 (3H, s), 3.55 (1H, dd, *J* = 6, 12 Hz), 3.63 (1H, dd, *J* = 6, 12 Hz), 3.73 (1H, d, *J* = 12 Hz), 3.86 (1H,

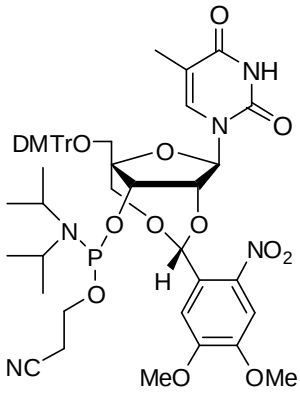
d, *J* = 12 Hz), 3.86 (3H, s), 3.90 (3H, s), 4.33 (1H, d, *J* = 5 Hz), 4.43 (1H, t, *J* = 6 Hz), 5.36 (1H, t, *J* = 5 Hz), 6.16 (1H, s), 6.25 (1H, d, *J* = 5 Hz), 6.80 (1H, s), 7.44 (1H, s), 7.56 (1H, s), 7.76 (1H, s), 11.3 (1H, s). ^{13}C NMR (67.8 MHz, [D_6]DMSO): δ 12.4, 56.0, 56.2, 59.2, 67.4, 70.6, 79.3, 88.8, 90.5, 97.4, 107.7, 108.6, 109.2, 128.3, 135.6, 140.2, 148.3, 150.3, 152.4, 163.9. MS (FAB): *m/z* 482 $[M+H]^+$. HRMS (FAB): calcd for $C_{20}H_{24}N_3O_{11}$ $[M+H]^+$: 482.1411; found: 482.1428.

1-[5-*O*-(4,4'-Dimethoxytrityl)-2-*O*-4-*C*-{(1*R*)-1-(2-nitro-4,5-dimethoxyphenyl)-2-oxapropylene}-β-D-ribofuranosyl]-thymine (6).



To a solution of compound **5** (508 mg, 1.06 mmol) in pyridine (27 mL) was added 4,4'-dimethoxytrityl chloride (468 mg, 1.38 mmol) and the resultant mixture was stirred at room temperature for 13 h. After addition of saturated $NaHCO_3$ (aq.), the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude was purified by column chro-matography (0.5% triethylamine in *n*-hexane/AcOEt 1:3) to give compound **6** (734 mg, 89%) as a yellow foam: m.p. 155－157 ℃. $[\alpha]_D^{26}$ －1.52 (c 1.00, MeOH). IR ν_{max} (KBr): 2935, 1696, 1515, 1464, 1280 cm^{-1}. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.48 (3H, s), 3.17 (1H, brs), 3.30 (1H, d, *J* = 11 Hz), 3.36 (1H, d, *J* = 11 Hz), 3.74 (6H, s), 3.85 (3H, s), 3.95 (3H, s), 3.74-3.95 (1H, m), 4.02 (1H, d, *J* = 13 Hz), 4.41 (1H, d, *J* = 6 Hz), 4.70 (1H, brs), 6.23 (1H, s), 6.79-7.52 (17H, m), 8.85 (1H, brs). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 12.2, 55.2, 56.3, 56.6, 61.6, 69.4, 71.3, 79.8, 87.0, 88.7, 92.0, 98.6, 107.7, 109.5, 110.4, 113.4, 127.2, 128.0, 128.1, 129.0, 130.0, 135.0, 140.0, 144.1, 148.6, 149.9, 153.3, 158.7, 163.9. MS (FAB): *m/z* 784 $[M+H]^+$. HRMS (FAB): calcd for $C_{41}H_{42}N_3O_{13}$ $[M+H]^+$: 784.2718; found: 784.2756.

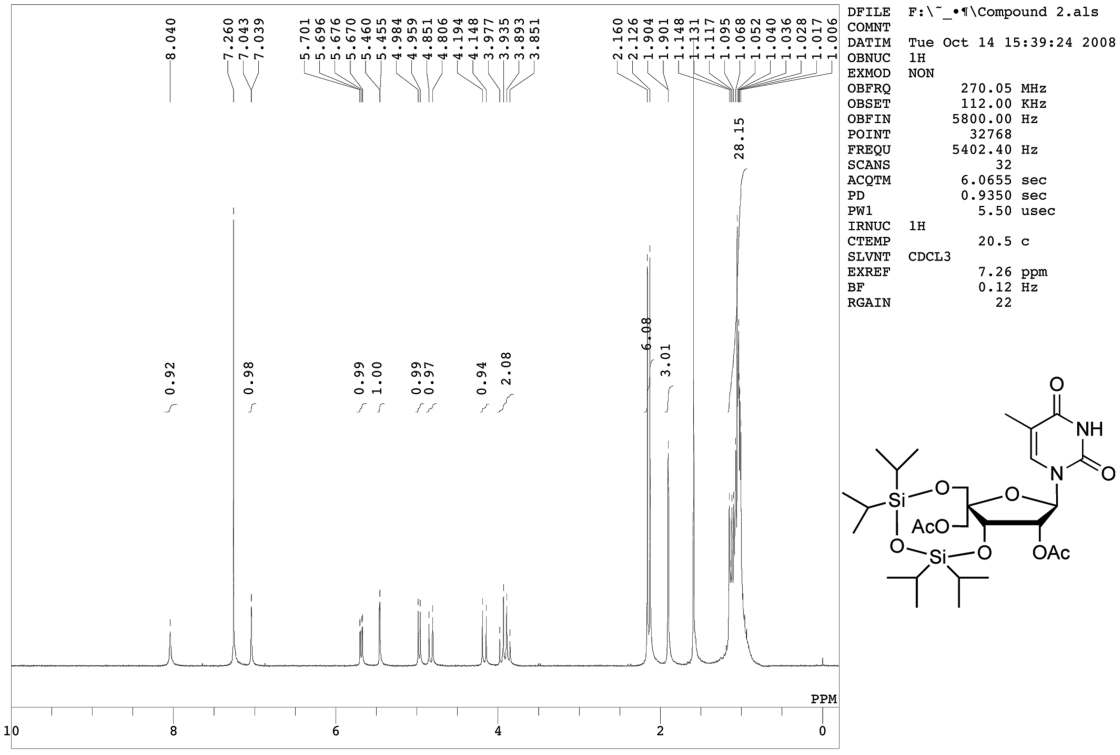
1-[3-*O*-{2-Cyanoethoxy(diisopropylamino)phosphino}-5-*O*-(4,4'-dimethoxytrityl)-2-*O*-4-*C*-{(1*R*)-1-(2-nitro-4,5-dimethoxyphenyl)-2-oxapropylene}-β-D-ribofuranosyl]-thymine (7).

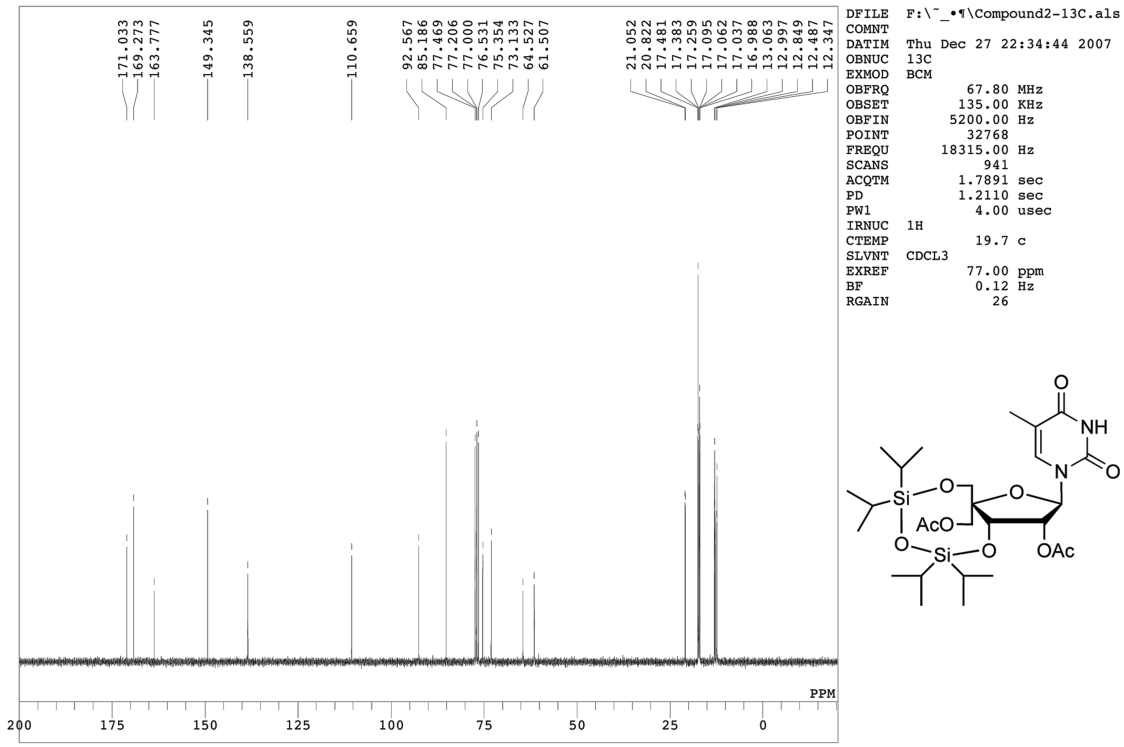


To a solution of **6** (113 mg, 0.144 mmol) in acetonitlile (2 mL) was added 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphane (0.137 ml, 0.432 mmol) and 4,5-dicyanoimidazole (0.25 M in acetonitlile, 0.632 mL, 0.158 mmol) and the resultant mixture was stirred at room temperature for 18 h. After addition of saturated $NaHCO_3$ (aq.), the reaction mixture was diluted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude was purified by column chromatography (0.5% triethylamine in *n*-hexane/AcOEt 1:1) followed by the precipitation from hexane/AcOEt to give compound **7** (77.4 mg, 55%): m.p. 118－121 ℃. ^{31}P NMR (202 MHz, $CDCl_3$): δ 149.8, 150.6. MS (FAB): *m/z* 984 $[M+H]^+$. HRMS (FAB): calcd for $C_{50}H_{59}N_5O_{14}P$ $[M+H]^+$: 984.3796; found: 984.3809.

3. ^{1}H and ^{13}C and ^{31}P Spectra of New Compounds:

*3-I. ^{1}H and ^{13}C Spectra of Compound **2***



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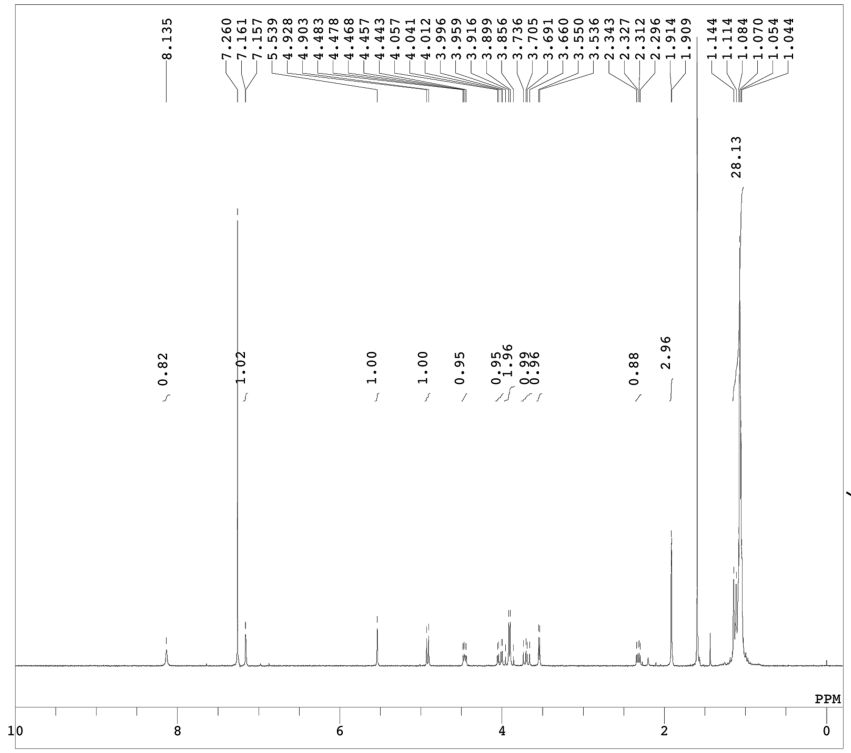
*3-II. ^{1}H and ^{13}C Spectra of Compound **3***

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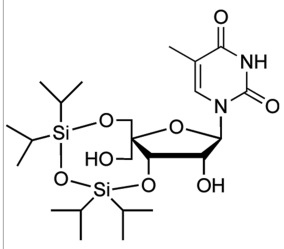

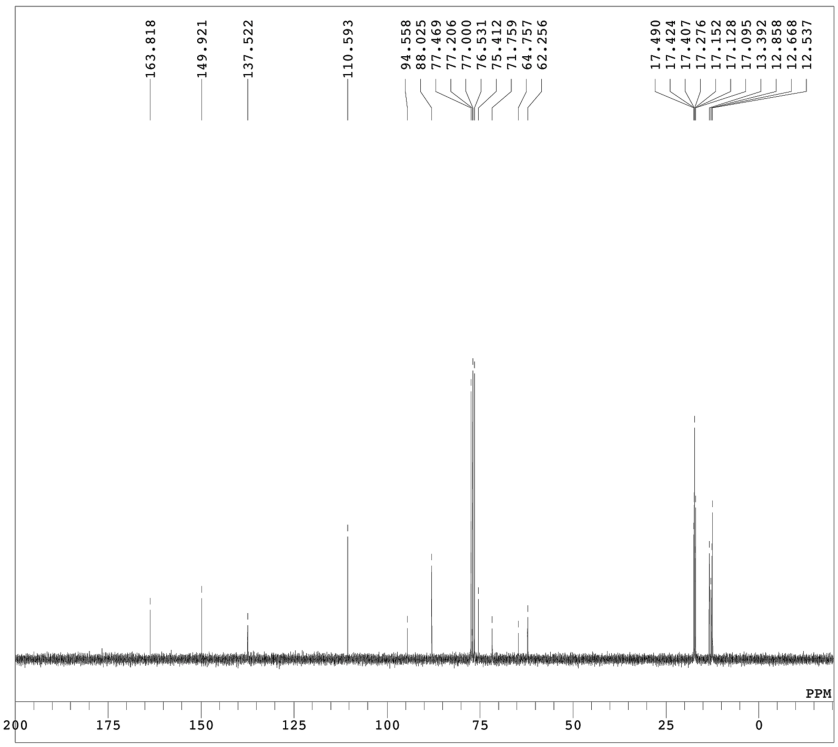


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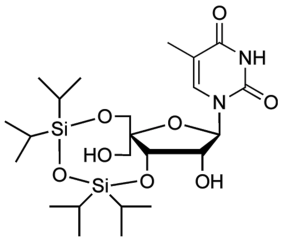
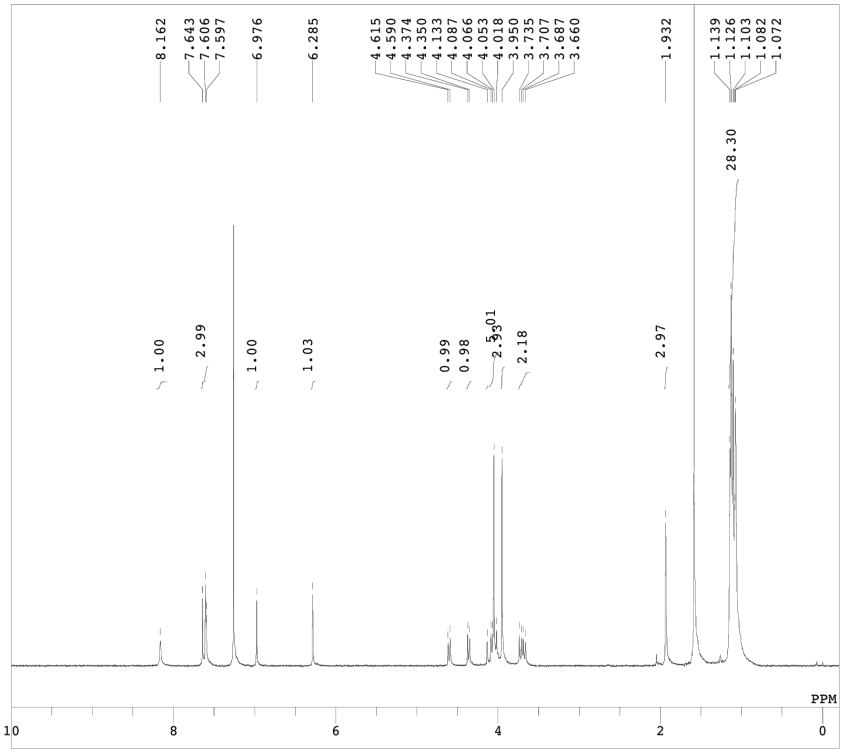


*3-III. ^{1}H and ^{13}C Spectra of Compound **4***

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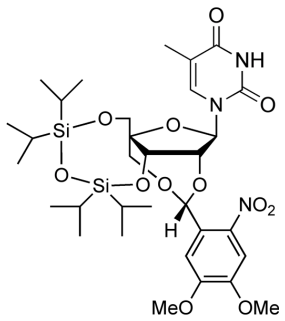

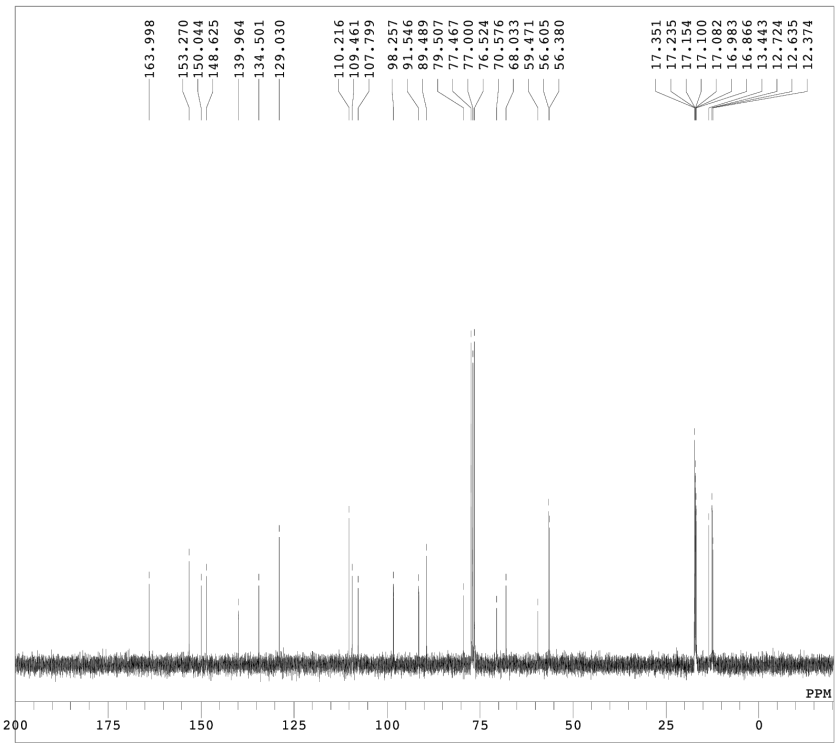


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RGAIN            26
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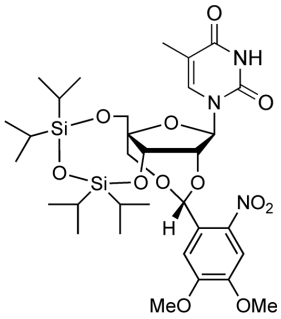
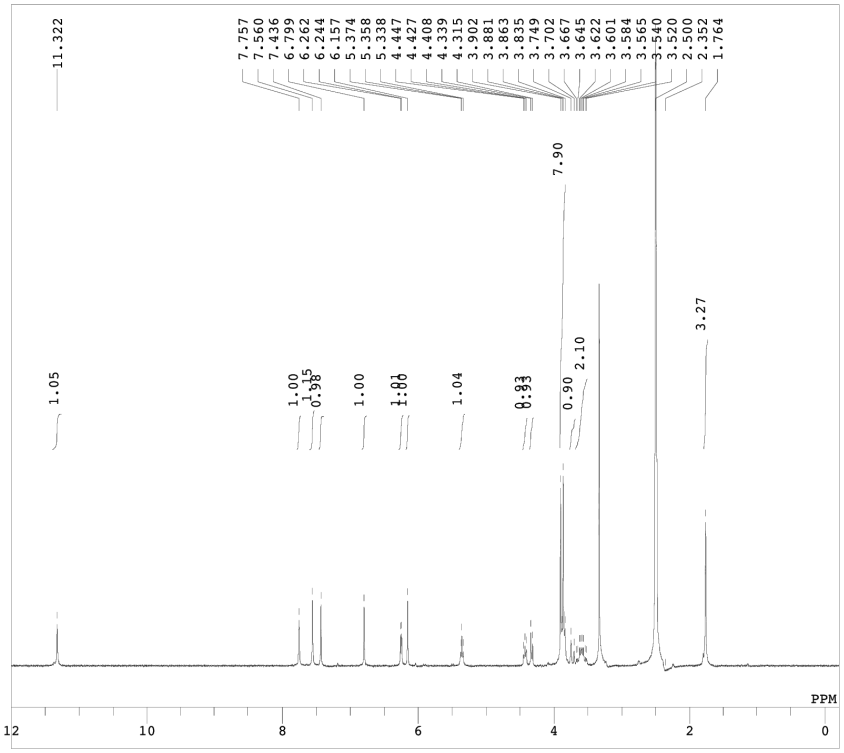


*3-IV. ^{1}H and ^{13}C Spectra of Compound **5***

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RGAIN            23
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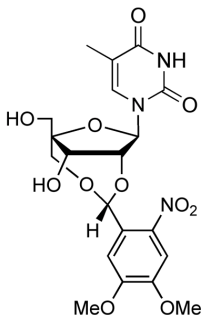
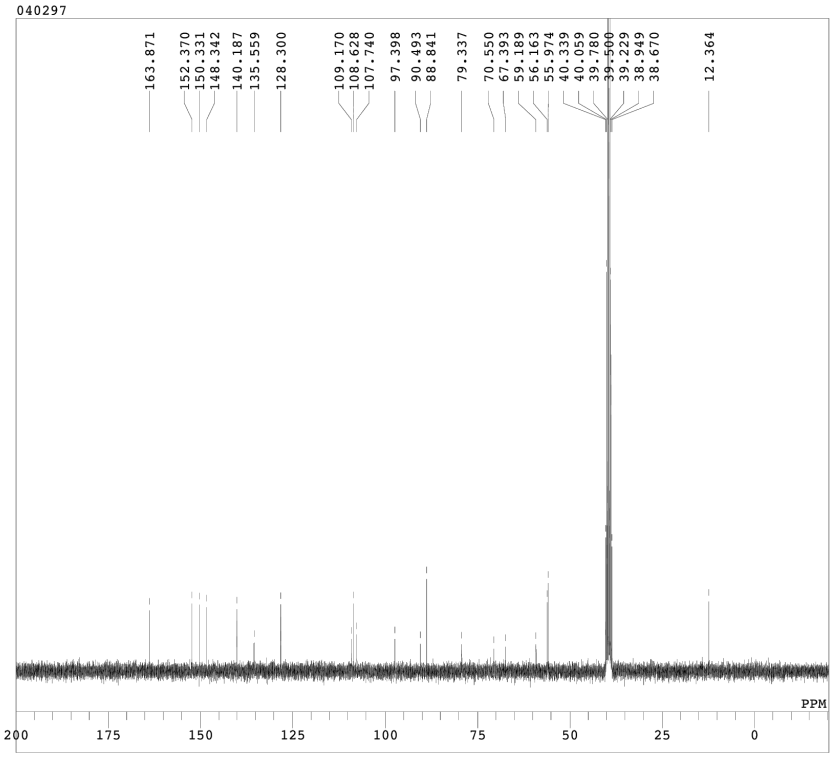
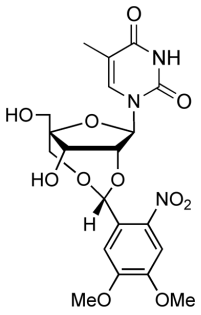
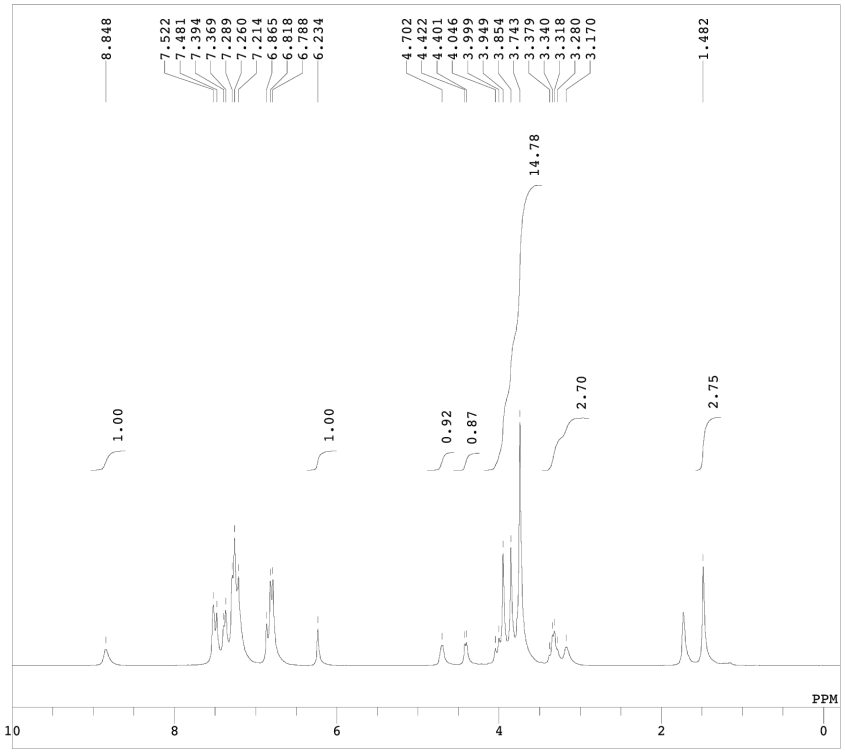




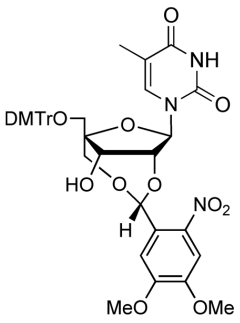

```
DFILE  F:\~_•¶\Compound5-13C.als
COMNT  040297
DATIM  Sat Dec 20 10:38:38 2008
OBNUC  13C
EXMOD  BCM
OBFRQ         75.45 MHz
OBSET        124.00 KHz
OBFIN       1840.00 Hz
POINT         32768
FREQU      20356.23 Hz
SCANS           901
ACQTM        1.6097 sec
PD           1.3900 sec
PW1            4.50 usec
IRNUC  1H
CTEMP         21.6 c
SLVNT  DMSO
EXREF         39.50 ppm
BF             0.12 Hz
RGAIN            24
```

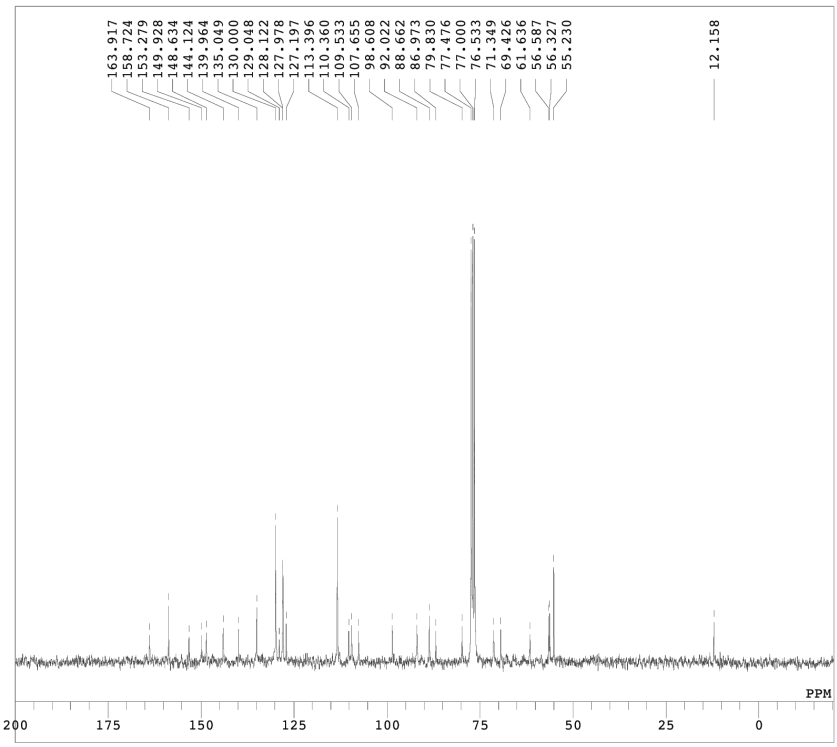


*3-V. ^{1}H and ^{13}C Spectra of Compound **6***

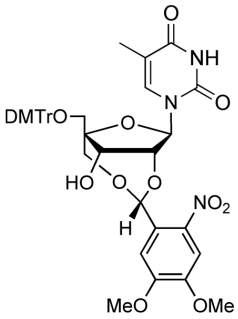



```
DFILE  F:\~_•¶\Compound 6.als
COMNT
DATIM  Mon Dec 01 20:06:11 2008
OBNUC  1H
EXMOD  NON
OBFRQ        270.05 MHz
OBSET        112.00 KHz
OBFIN       5800.00 Hz
POINT         32768
FREQU       5402.40 Hz
SCANS            21
ACQTM         6.0655 sec
PD            0.9350 sec
PW1             5.50 usec
IRNUC  1H
CTEMP          20.4 c
SLVNT  CDCL3
EXREF          7.26 ppm
BF             4.20 Hz
RGAIN            19
```

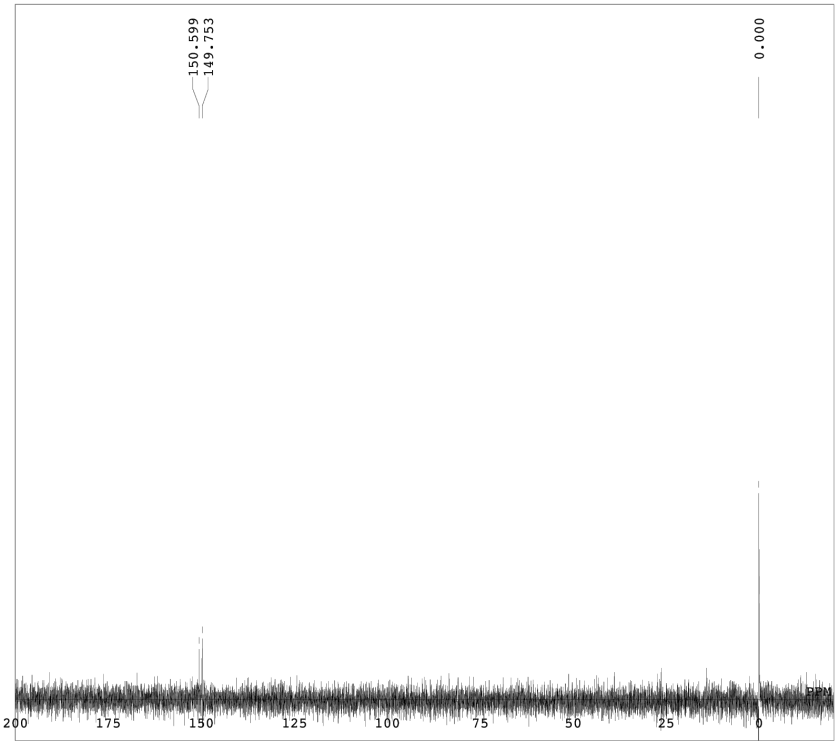




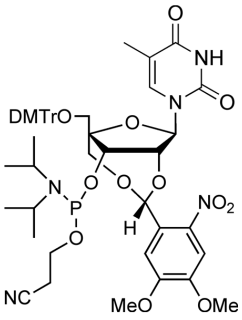

```
DFILE   F:\~_•¶\Compound6-13C.als
COMNT
DATIM   Mon Dec 01 21:12:32 2008
OBNUC   13C
EXMOD   BCM
OBFRQ         67.80 MHz
OBSET        135.00 KHz
OBFIN       5200.00 Hz
POINT         32768
FREQU      20000.00 Hz
SCANS          1125
ACQTM        1.6384 sec
PD           1.0000 sec
PW1            4.00 usec
IRNUC   1H
CTEMP          20.1 c
SLVNT   CDCL3
EXREF         77.00 ppm
BF             4.20 Hz
RGAIN            26
```



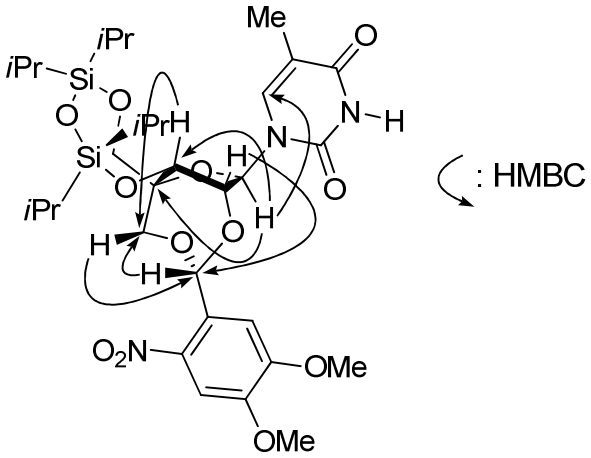
*3-VI. ^{31}P Spectrum of Compound **7***

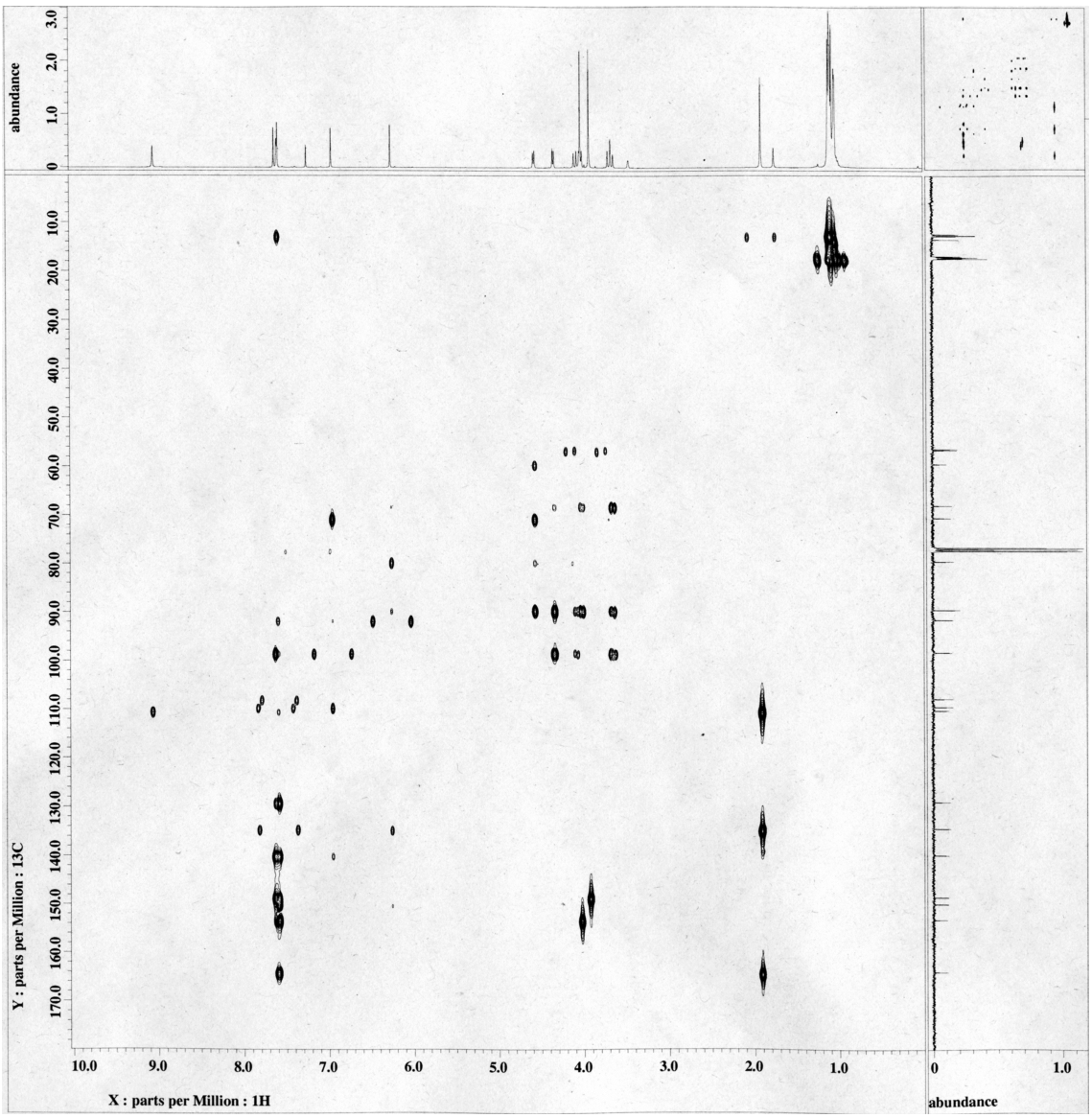


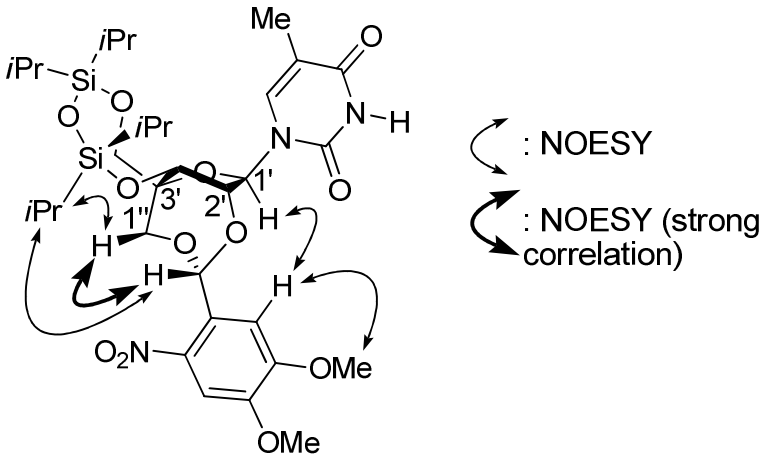

```
DFILE   F:\~_•¶\Compound7-31P.als
COMNT
DATIM   Thu May 29 23:07:52 2008
OBNUC   31P
EXMOD   bcm
OBFRQ        202.35 MHz
OBSET          0.00 KHz
OBFIN     140000.00 Hz
POINT         32768
FREQU     121951.22 Hz
SCANS           513
ACQTM        0.2687 sec
PD           2.7310 sec
PW1            6.00 usec
IRNUC   1H
CTEMP          22.6 c
SLVNT   CDCL3
EXREF          0.00 ppm
BF             0.12 Hz
RGAIN            25
```

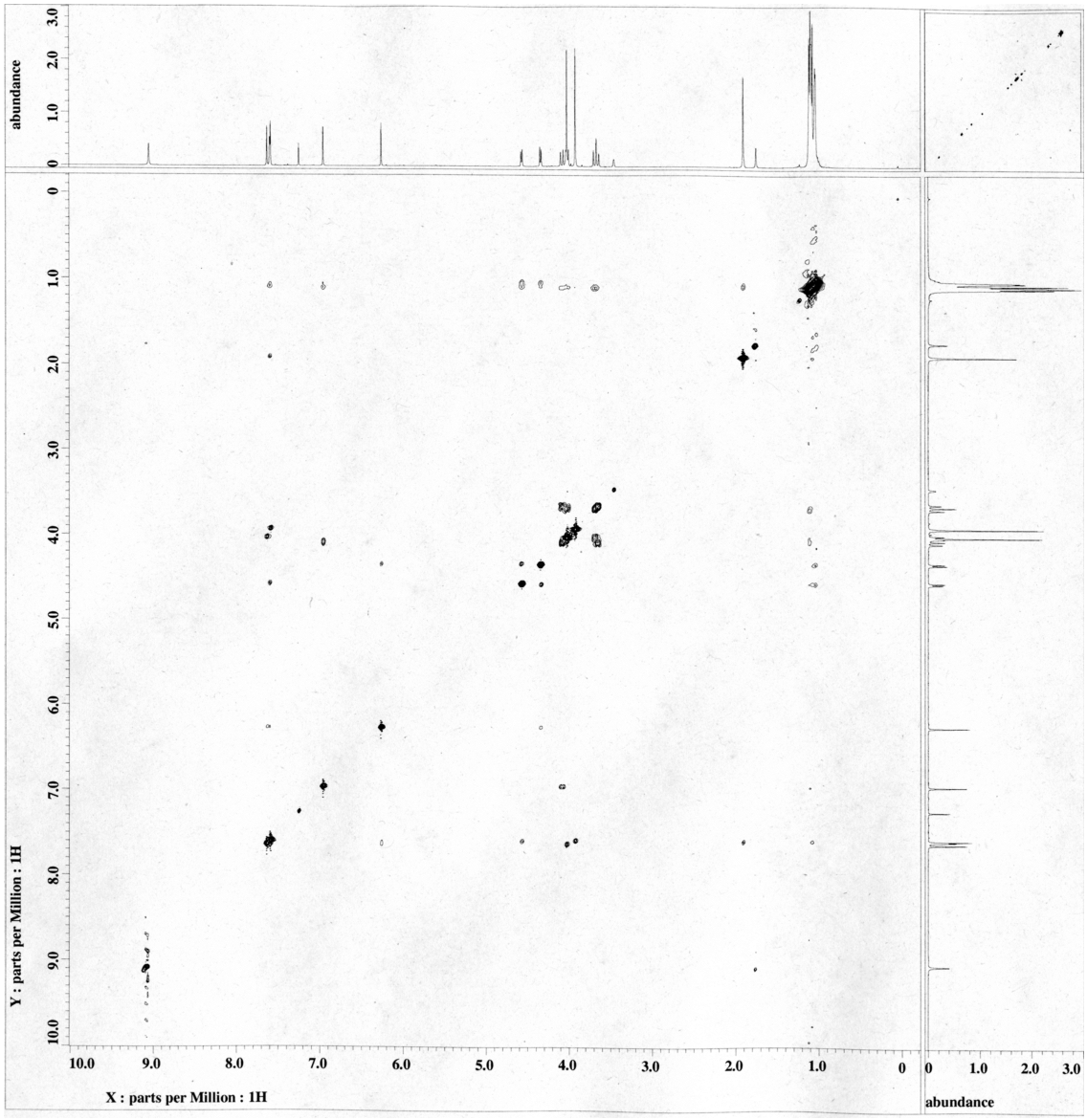


4. HMBC and NOESY Spectra of Compound 4:

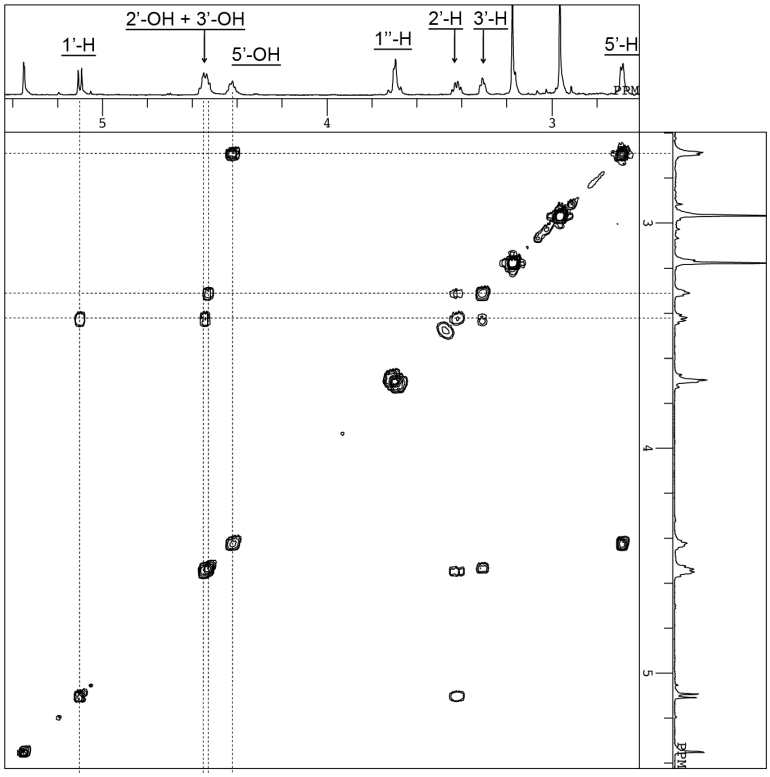




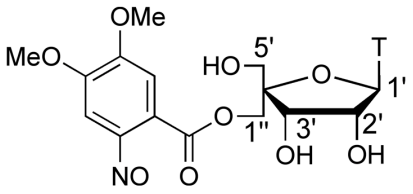

Me
O
iPr
iPr
Si
O
O
iPr
N
H
Si
O
O
1'
3'
2'
N
O
iPr
1''
H
O
H
H
H
O2N
OMe
OMe
: NOESY
: NOESY (strong correlation)


abundance
Y : parts per Million : 1H
X : parts per Million : 1H
abundance

5. H,H COSY Spectra of Compound 5 after Photoirradiation.



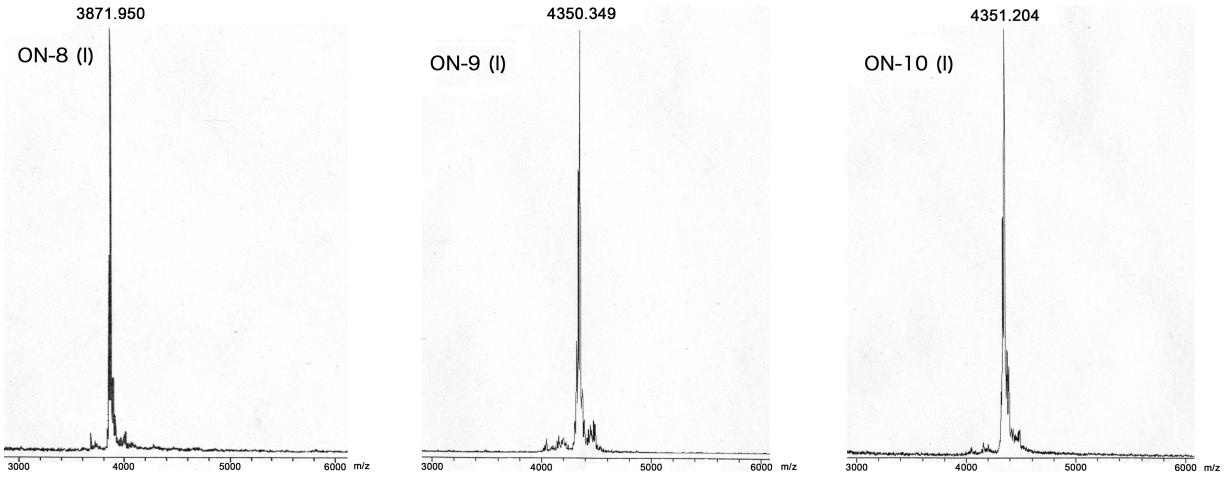

```
DATIM  Tue Jan 13 19:34:04 2009
DFILE  C:\Documents and Settings\yakka\ffXfNfgft
OBNUC  1H
EXMOD  cosy
OFR          500.00 MHz
OBSET          0.00 KHz
OBFIN     162269.30 Hz
POINT          1024
FREQU       3257.33 Hz
SCANS            32
ACQTM         0.1572 sec
PD            0.9490 sec
PW1           10.80 usec
IRN
CTEMP           30.0 c
SLVNT  DMSO
EXREF           2.50 ppm
BF              1.00 Hz
RGAIN             20
```



6. Yields and MALDI-TOF MS Data for the Oligonucleotides Containing Light-Responsive BNA:

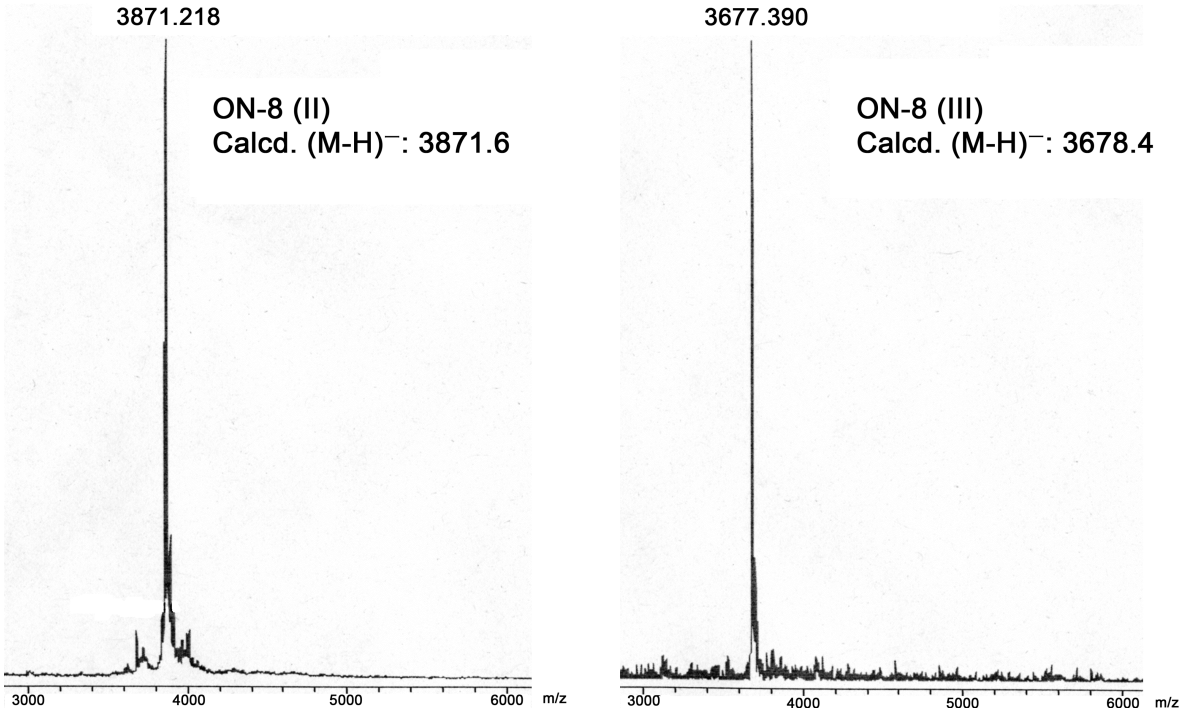
Oligonucleotide	Yields / %	MALDI-TOF MS	
		Calcd [*M*-H]$^-$	Found [*M*-H]$^-$
5'-d(GCGTT**T**TTTGCT)-3' (**ON-8**)	49	3871.6	3872.0
5'-d(GCGTT**TTT**TGCT)-3' (**ON-9**)	48	4349.9	4350.3
5'-d(GCG**T**T**T**T**T**TGCT)-3' (**ON-10**)	47	4349.9	4351.2

[a] Underlined bold characters indicate light-responsive BNA modified residues.

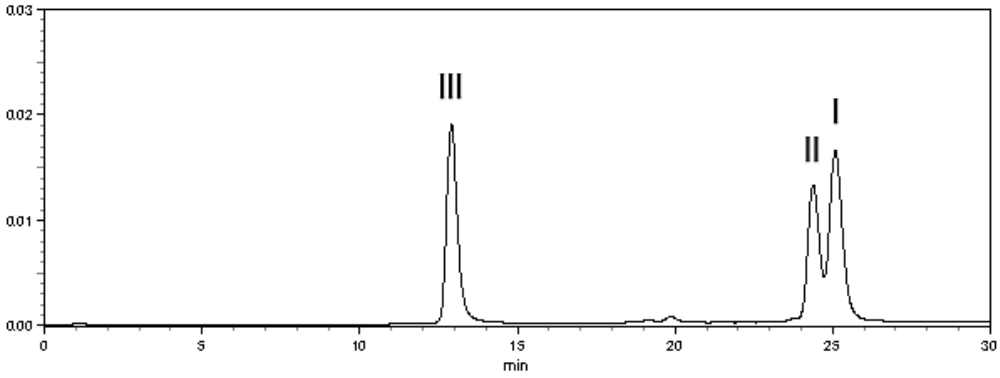


7. RP-HPLC Analysis and MALDI-TOF MS Spectra of the Oligonucleotides Containing Light-Responsive BNA:

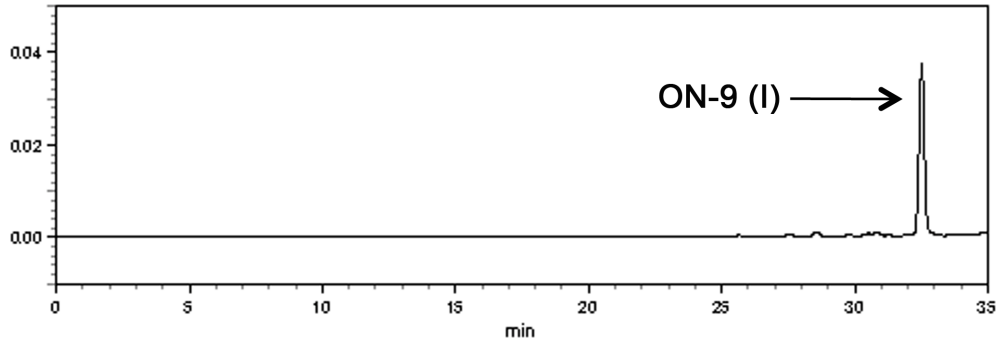
*7-I. MALDI-TOF-MS Spectra of ON-**8***

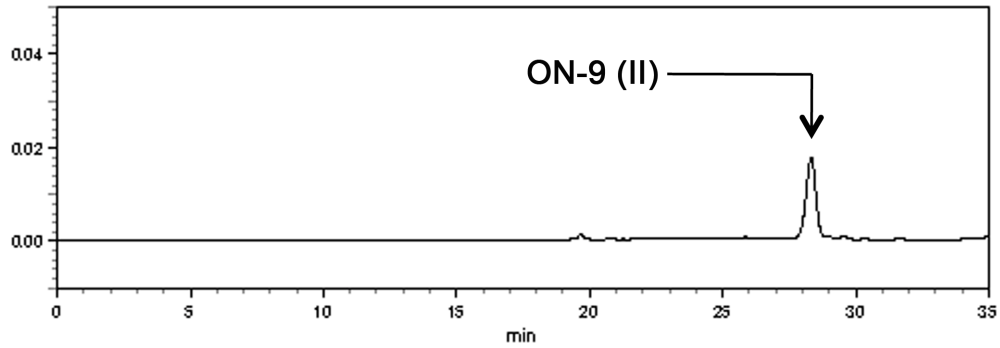


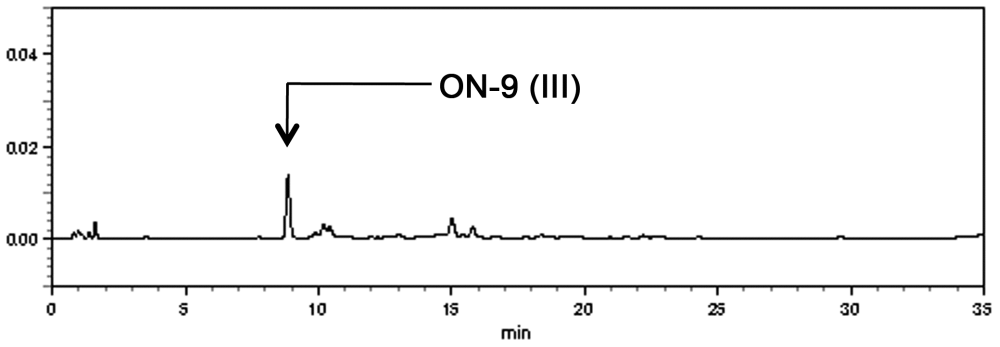
*7-II. RP-HPLC Analysis of ON-**8** (I-III; co-injection)*



*7-III. RP-HPLC Analysis of ON-**9***

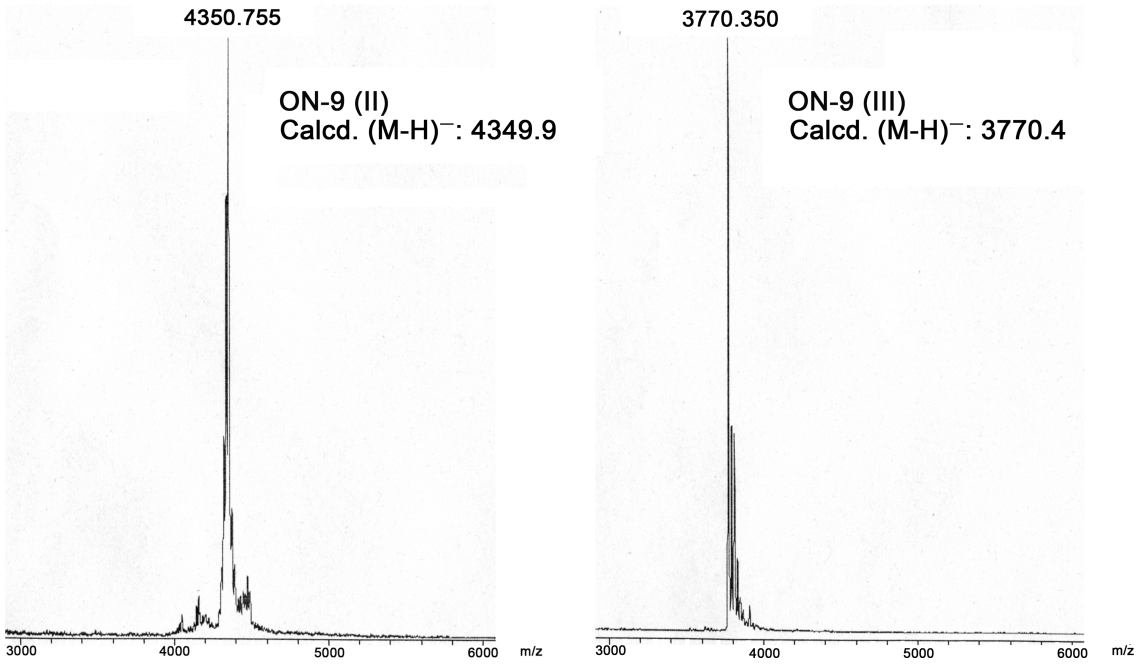




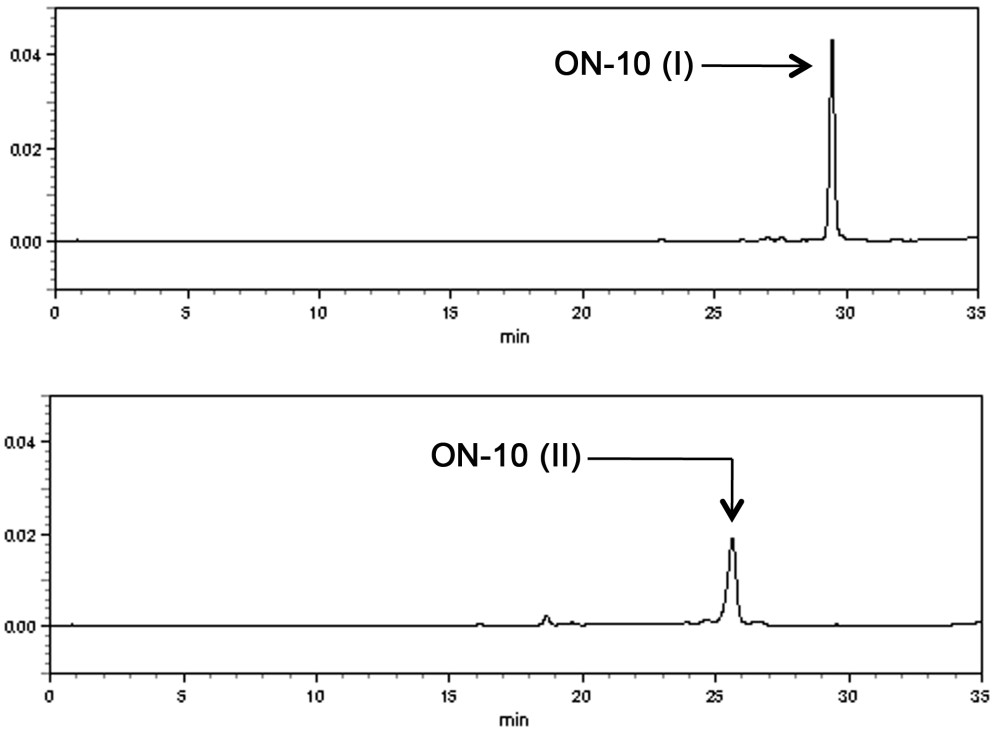


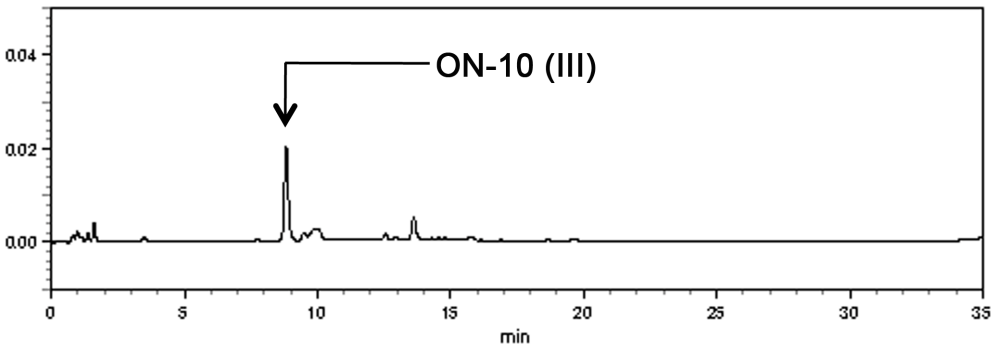
Analyzed by RP-HPLC using Waters XTerra MS C_{18} column 2.5 μm (4.6 x 50 mm) with a linear gradient of MeCN (from 8% to 20% over 30 min) in 0.1 M triethylammonium acetate (pH 7.0).

*7-IV. MALDI-TOF-MS Spectra of ON-**9***



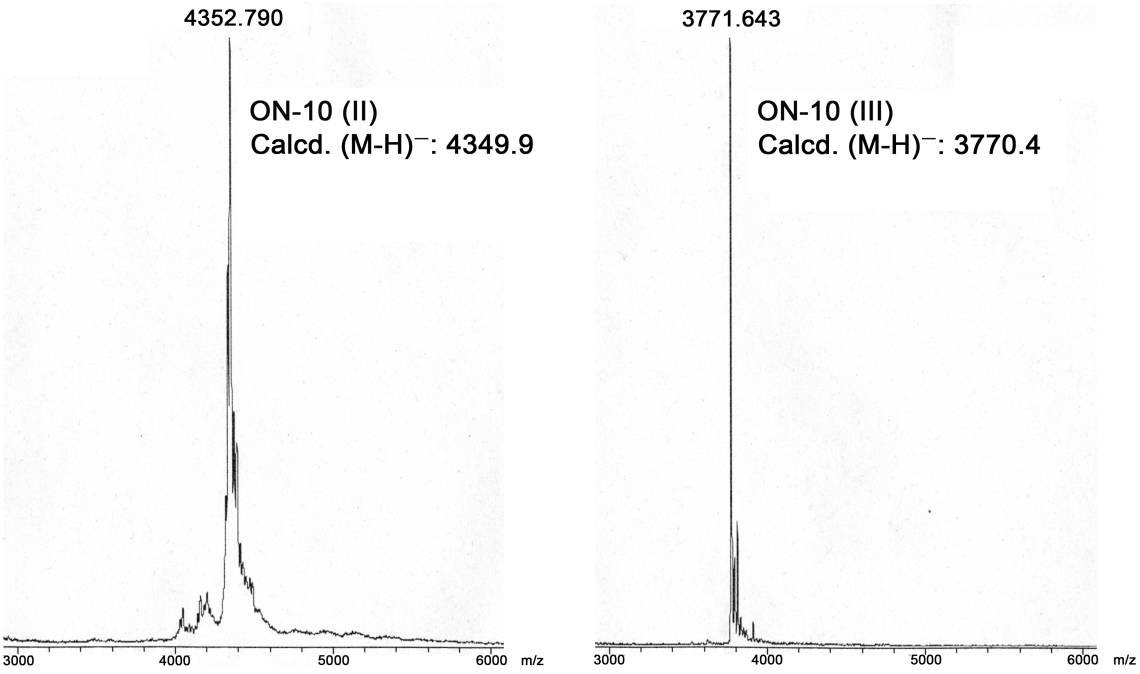
*7-V. RP-HPLC Analysis of ON-**10***





Analyzed by RP-HPLC using Waters XTerra MS C_{18} column 2.5 μm (4.6 x 50 mm) with a linear gradient of MeCN (from 8% to 20% over 30 min) in 0.1 M triethylammonium acetate (pH 7.0).

*7-VI. MALDI-TOF-MS Spectra of ON-**10***



8. References

1. a) S. K. Singh, P. Nielsen, A. A. Koshkin, J. Wengel, *Chem. Commun.* **1998**, 455; b) R. D. Youssefyeh, J. P. H. Verheyden, J. G. Moffatt, *J. Org. Chem.* **1979**, *44*, 1301.