Electronic Supplementary Material (ESI) for:

Amino acids attached to 2’-amino-LNA: Synthesis and increased duplex stability

Marie W. Johannsen, Lia Crispino, Michael C. Wamberg, Neerja Kalra, and Jesper Wengel*

Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark,
Campusvej 55, DK-5230 Odense M, Denmark.
E-mail: jwe@ifk.sdu.dk

Table S1 - MALDI TOF Mass spectrometry data for modified ONs

Instrumentation and conditions as described in journal article

Modification	Calculated mass [M+H]$^+$	Found mass [M+H]$^+$
2’-amino-LNA	2781.9	2780.7
gly	2838.9	2838.4
(gly)$_2$	2896.0	2893.8
(gly)$_3$	2953.0	2952.5
(gly)$_4$	3010.1	3006.8
(gly)$_5$	3067.1	3068.1
gly-pro	2936.0	2933.6
gly-lys	2967.1	2966.7
gly-gly-lys	3024.2	3023.3
gly-lys(lys)$_2$	3223.4	3224.0
acetyl	2823.9	2823.3
C16	3020.3	3018.5
3 x 2’-amino-LNA	2835.9	2836.7
3 x acetyl	2961.9	2960.8
3 x C16	3551.1	3550.0
3 x gly	3007.1	3005.6
Complementary strands with modifications		
2 x 2’-amino-LNA	2737.9	2736.8
2 x gly	2849.9	2847.7

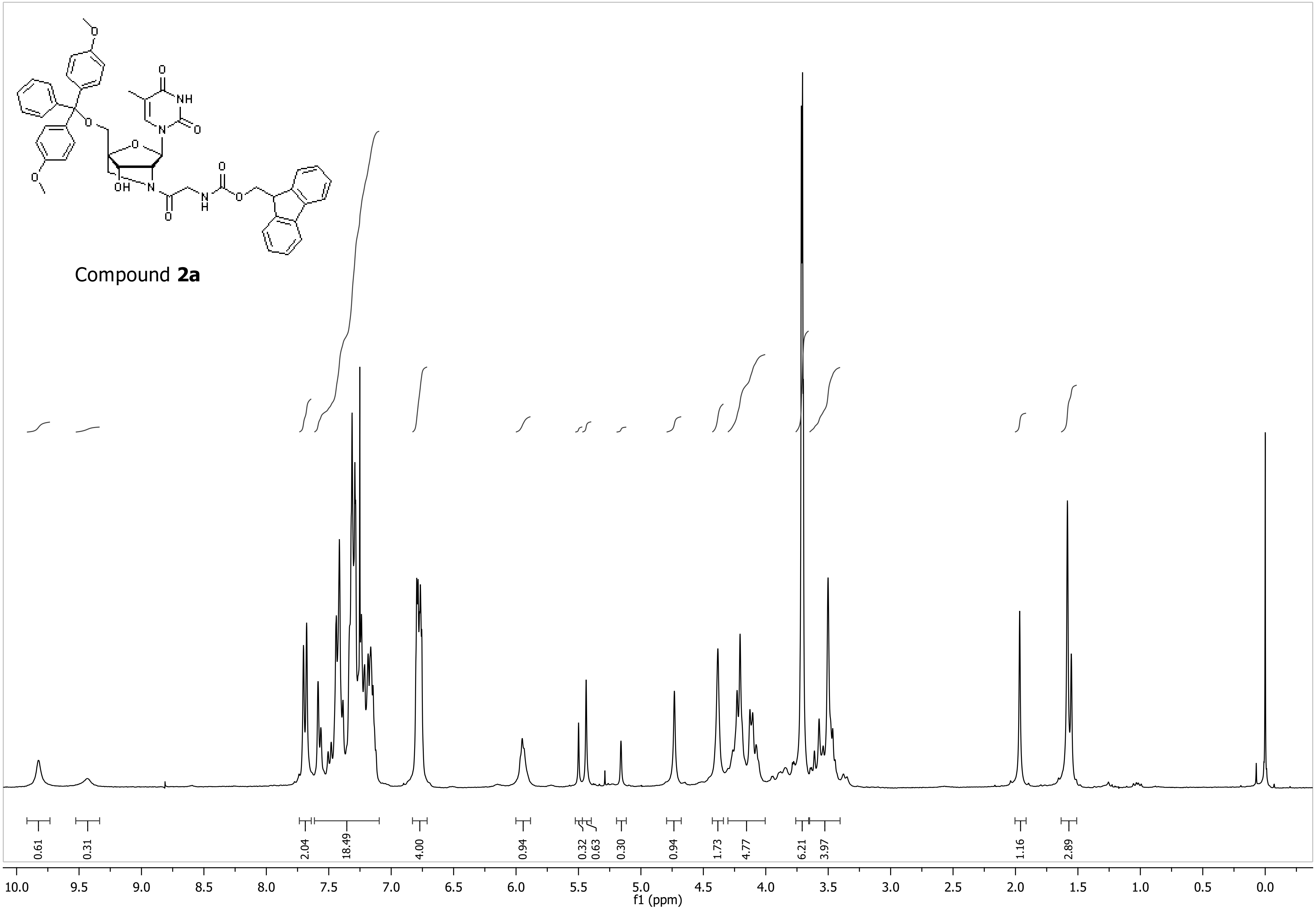
Table S2 - Overview of yields for synthesis of modified ONs

Synthesis was carried out on either 0.2 µmol or 1 µmol scale (A). Intermediate numbers represent approximate scale of synthesis where a 1 µmol scale was divided into several portions. The UV absorbance at 260 nm (OD$_{260}$) was measured for 1.00 mL solutions (C). Final yield of all modified oligonucleotides (D) was calculated from (C) using a calculated extinction coefficient of 92.3 OD$_{260}$ µmol^{-1} for the sequences 5’-G**X**G A**X**A **X**GC-3’ and a calculated extinction coefficient of 90.6 OD$_{260}$ µmol^{-1} for the complementary sequences 5’-GCA **X**A**X** CAC-3’ where **X** = DNA-T or modified monomers. Extinction coefficients were found using the oligo calculator at www.ribotask.com. Percentage yields of the total synthesis of both the ONs and peptide couplings (F) was calculated from (A) and (D). The yield of on-column peptide couplings, where these were carried out, was calculated from (D) and an initial synthetic scale which takes into account the yield of the oligonucleotide synthesis (B). These percentage yields are recorded in column (E).

Modification	A: ON synthetic scale / µmol	B: Yield of ON synthesis (DMT cation measurements)	C: Final yield after peptide coupling and purification /OD$_{260}$mL^{-1}	D Final yield after peptide coupling and purification / µmol	E: Yield of peptide coupling	F: Overall yield of whole synthesis / %
2’-amino-LNA	0.20	>100%	9.2	0.10		50%
gly	0.20	94%	4.3	0.05		23%
(gly)$_2$	0.25	>100%	4.2	0.05	18%	18%
(gly)$_3$	0.25	>100%	7.4	0.08	32%	32%
(gly)$_4$	0.46	55%	5.4	0.06	23%	13%
(gly)$_5$	0.46	55%	2.2	0.02	9%	5%
gly-pro	0.48	>100%	10.8	0.12	25%	25%
gly-lys	0.46	>100%	3.8	0.04	9%	9%
gly-gly-lys	0.60	>100%	2.7	0.03	5%	5%
gly-lys(lys)$_2$	0.46	>100%	3.2	0.04	8%	8%
acetyl	0.20	85%	12.6	0.16		68%
C16	0.20	95%	8.0	0.09		43%
Multiple modifications						
3 x 2’-amino-LNA	0.20	78%	1.4	0.02		8%
3 x acetyl	0.20	76%	8.8	0.10		48%
3 x C16	0.20	91%	15.4	0.17		83%
3 x gly	0.20	77%	3.4	0.04		18%
Complementary strands with modifications						
2 x 2’-amino-LNA	0.20	77%	3.0	0.03		17%
2 x gly	0.20	46%	2.9	0.03		16%

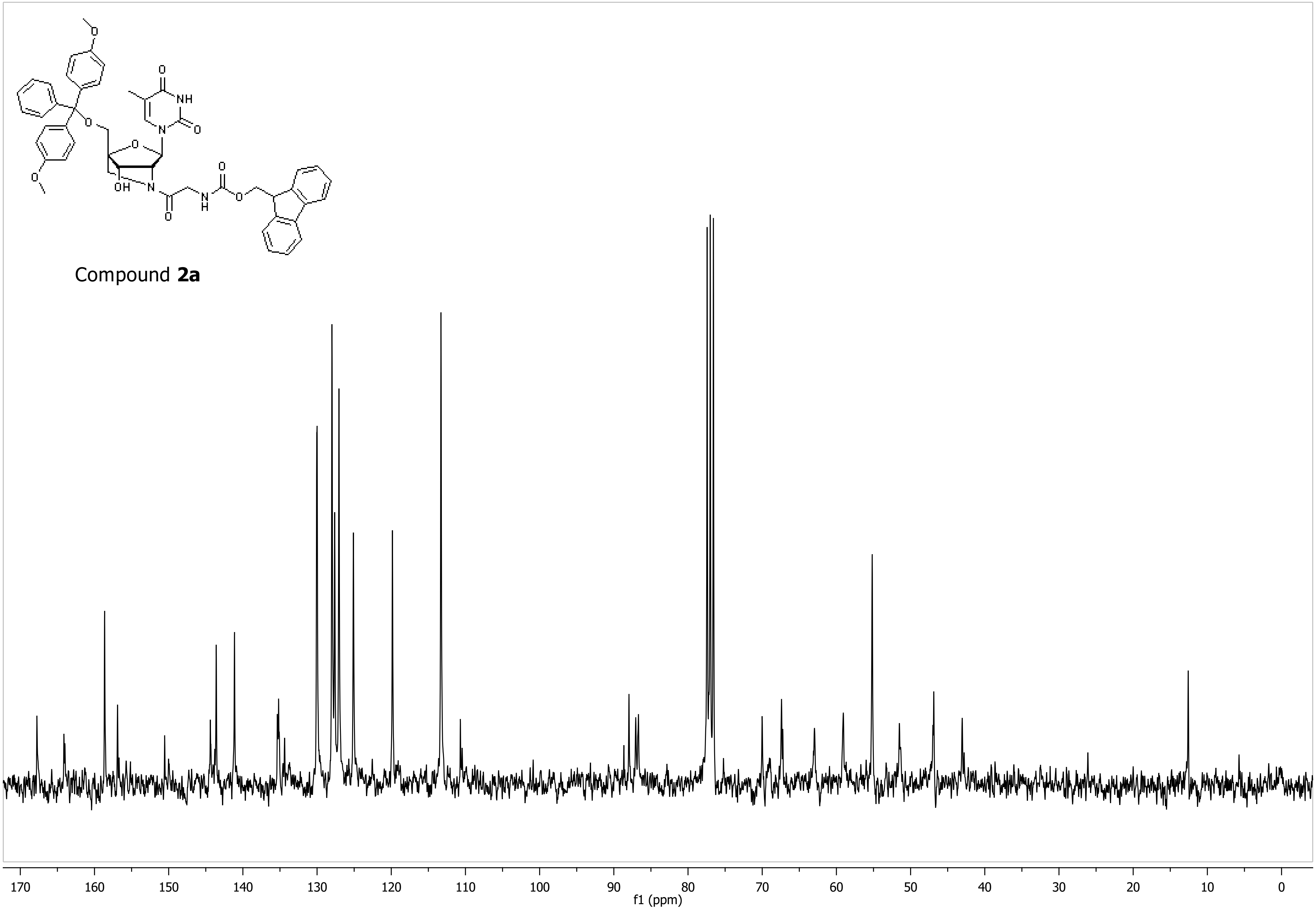
S3 – NMR spectra

Conditions as described in journal article



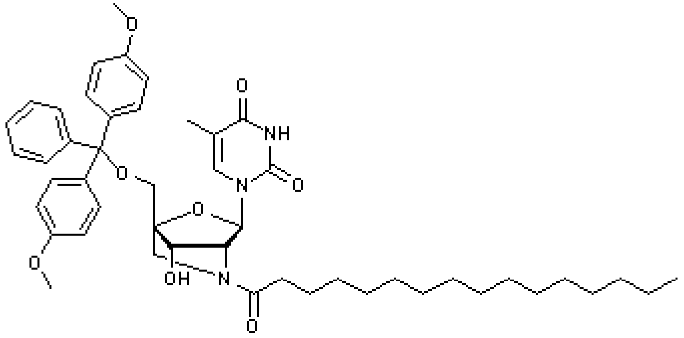
Compound **2a**

0.61
0.31
2.04
18.49
4.00
0.94
0.32
0.63
0.30
0.94
1.73
4.77
6.21
3.97
1.16
2.89
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
O
NH
N
O
OH
H

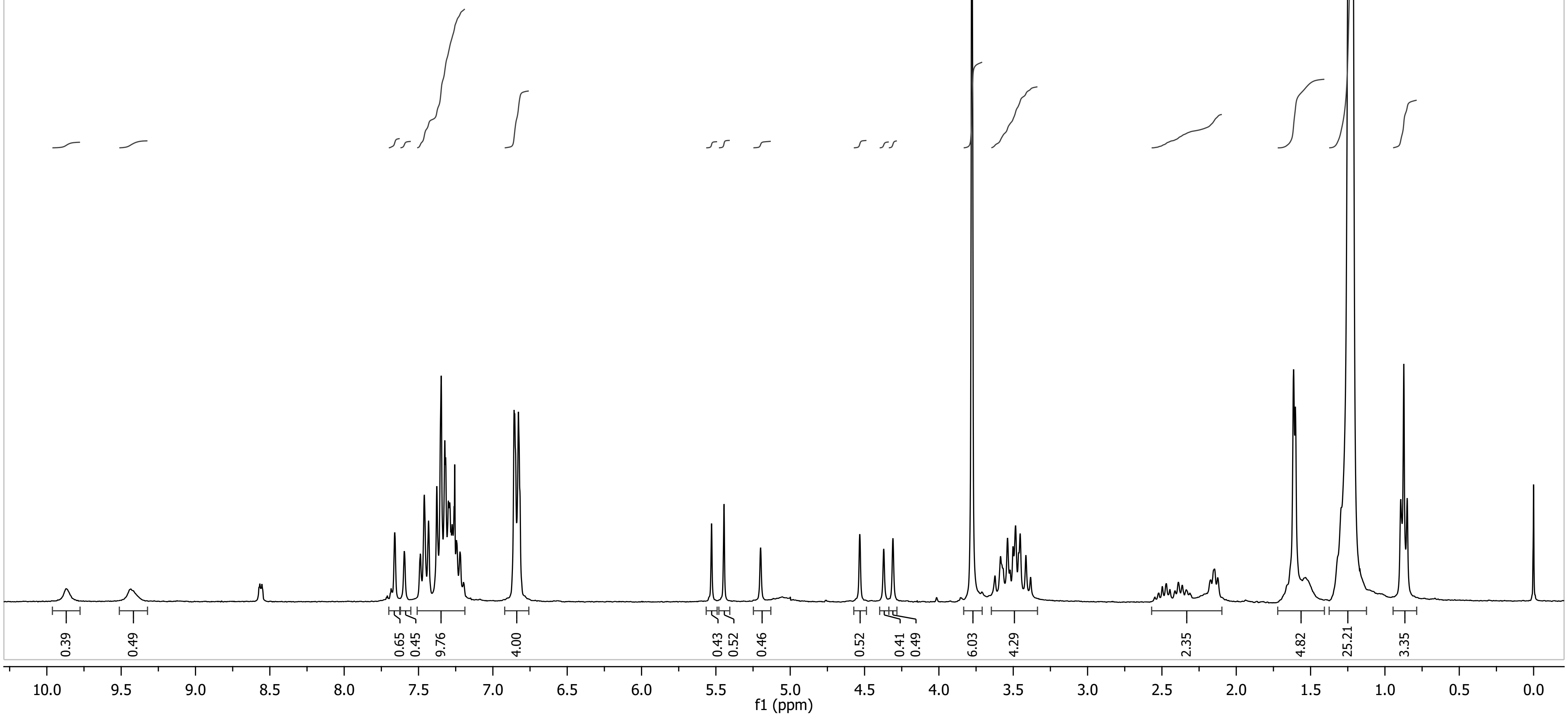


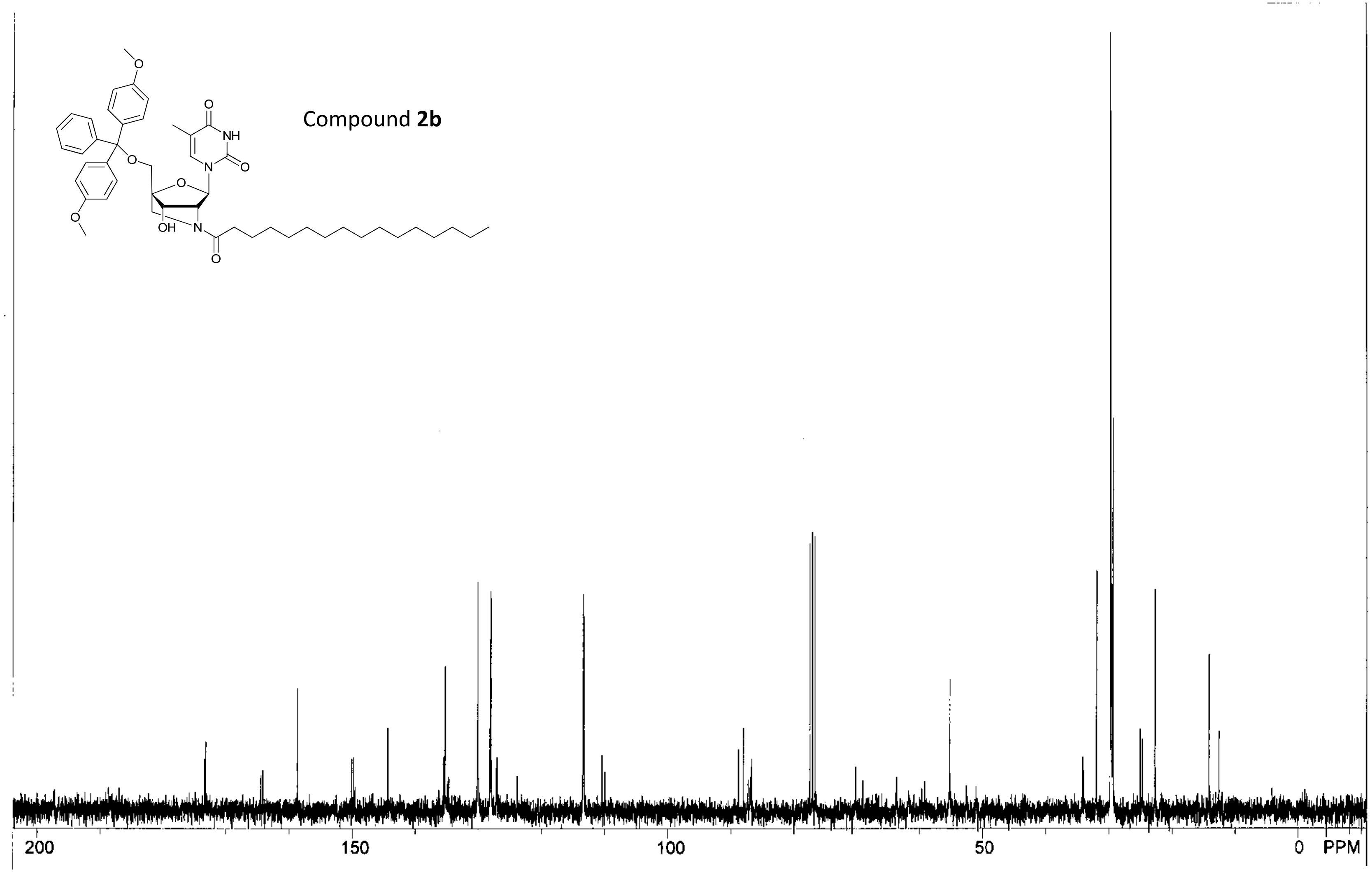
Compound **2a**

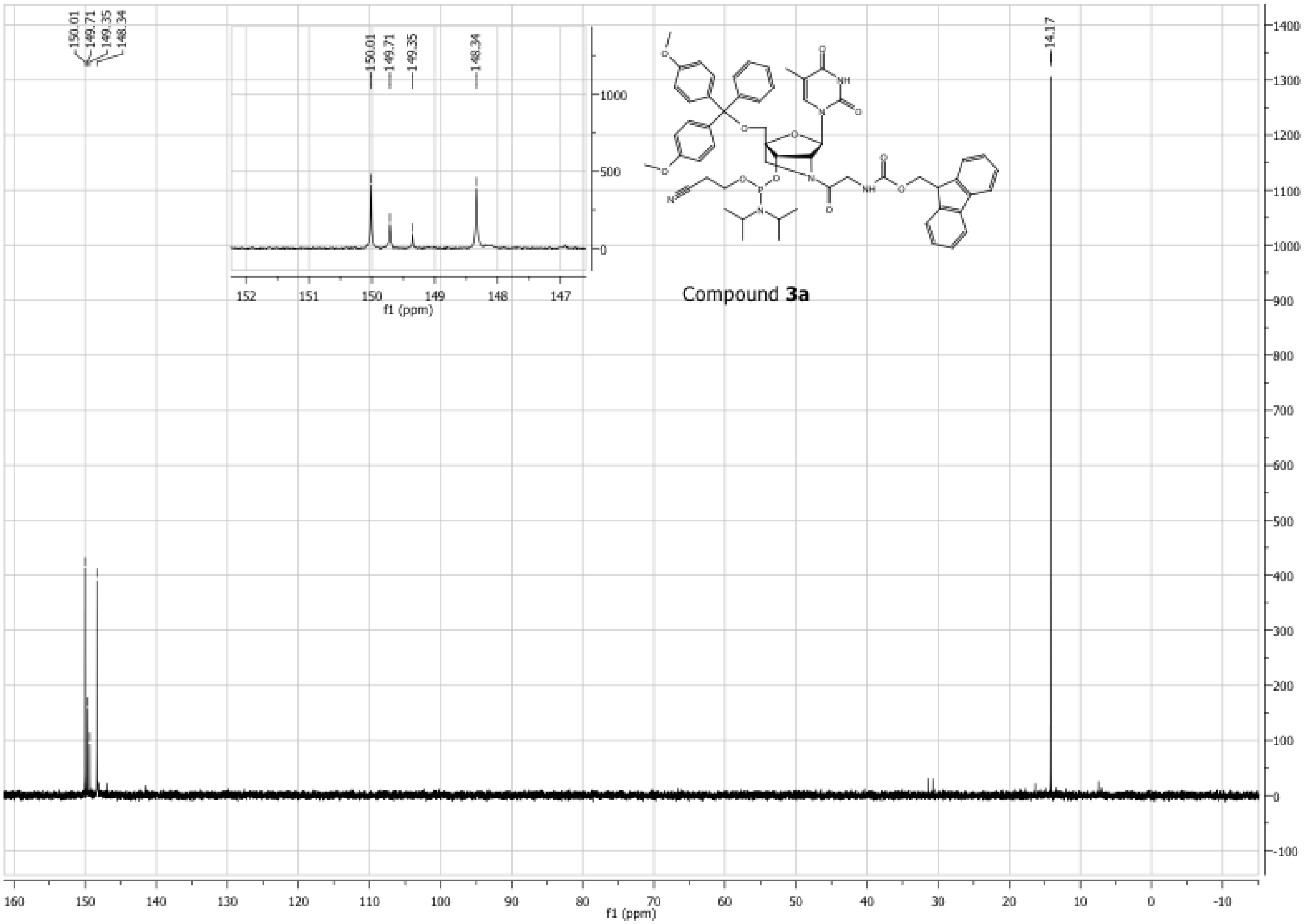
O
NH
N
O
OH
H
f1 (ppm)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


O
O
NH
O
O
N
O
O
OH
N
O

Compound **2b**

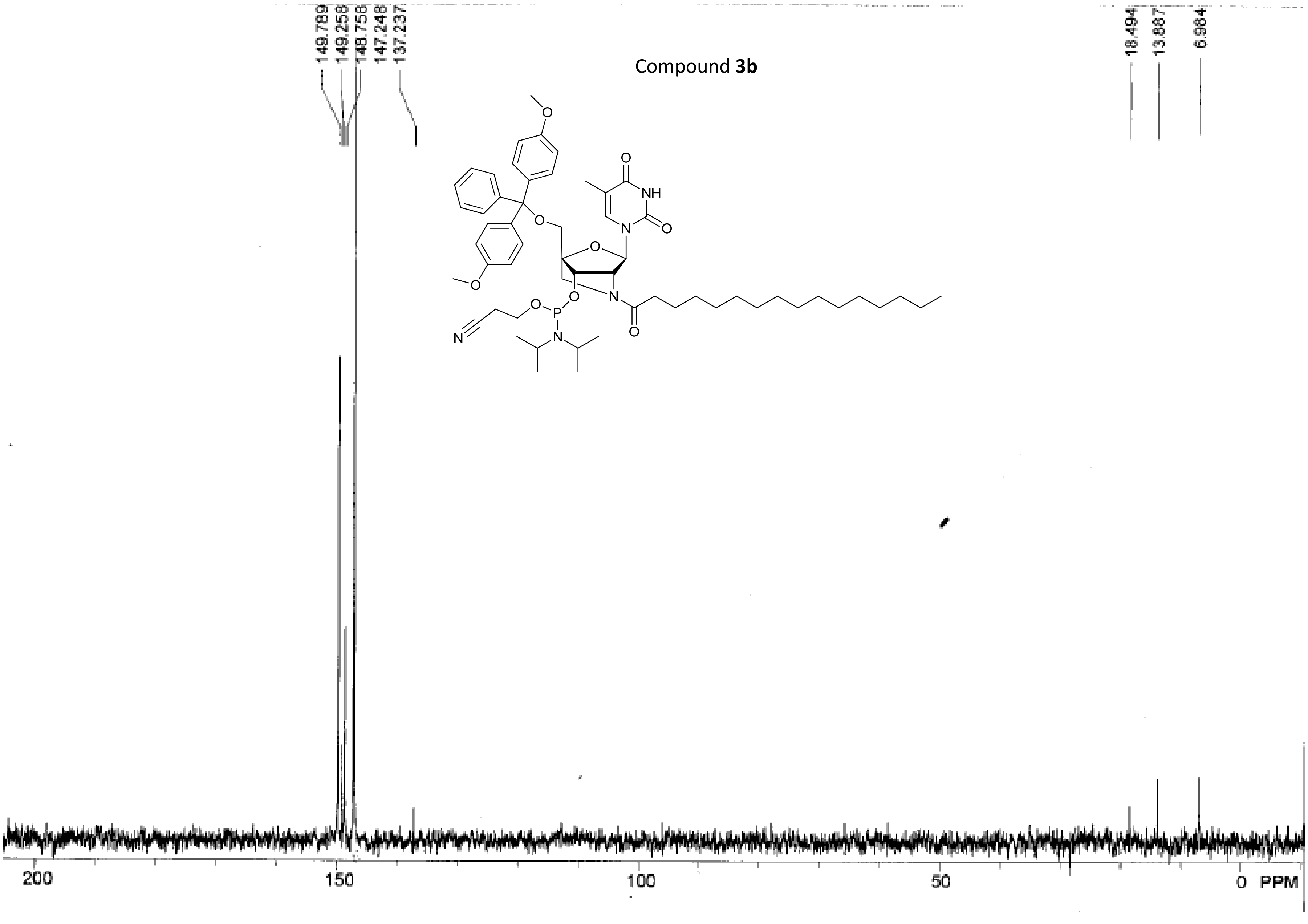

0.39
0.49
0.65
0.45
9.76
4.00
0.43
0.52
0.46
0.52
0.41
0.49
6.03
4.29
2.35
4.82
25.21
3.35
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Compound **2b**


150.01
149.71
149.35
148.34
14.17
152
151
150
149
148
147
f1 (ppm)
1000
500
0
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

Compound **3a**

Compound **3b**


149.789
149.258
148.758
147.248
137.237
18.494
13.887
6.984
O
NH
O
N
O
O
O
O
N
O
P
N
N
O
200
150
100
50
0 PPM