SUPPORTING INFORMATION

Exploring the Limits of DNA Size: Naphtho-homologated DNA Bases and Pairs

Alex H. F. Lee and Eric T. Kool*

Department of Chemistry, Stanford University, Stanford CA 94305-5080

*to whom correspondence should be addressed: kool@stanford.edu

CONTENTS:

Preparation of phosphoramidite derivative of nucleoside 1

1'-β-[7-(benzoquinazoline2,4-dione)]-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxy-D-ribofuranose. The nucleoside **1** (0.29 g, 0.72 mmol) was coevaporated with distilled pyridine (3 × 5 mL), and the residue was dissolved in distilled pyridine (10 mL) under argon. 4,4'-dimethoxytrityl chloride (0.40 g, 1.10 mmol) was added to the solution in a portion. The mixture was stirred at room temperature for 12 h. The volatiles were removed *in vacuo*, and the residue was purified by silica column chromatography (EtOAc to EtOAc/ methanol 10:1) to afford 5'-DMT-**1** (0.45 g, 90%) as slightly yellow foam. ^{1}H NMR (DMSO-*d*6, 400 MHz): δ 8.54 (s, 1H), 8.04 (s, 1H), 7.86 (d, 1H, J = 7.2 Hz), 7.61 (d, 1H, J = 7.2 Hz), 7.52 (s, 1H), 7.41 (d, 2H, J = 7.2 Hz), 7.28 (m, 7H), 6.87 (d, 4H, J = 8.8 Hz), 5.19 (dd, 1H, J = 10.4, 5.2 Hz), 4.20 (m, 1H), 3.99 (bs, 1H), 3.71 (s, 6H), 3.16 (m, 2H), 2.20 (m, 1H), 1.99 (m, 1H). ^{13}C NMR (DMSO-*d*6, 100 MHz): δ 158.05, 150.36, 145.00, 139.07, 136.49, 135.86, 135.70, 129.78, 128.93, 128.68, 128.16, 127.84, 126.92, 125.83, 113.17, 112.78, 110.29, 94.00, 85.40, 79.24, 72.56, 55.02. HRFABMS *m/z*: $[M]^+$ calcd for $C_{38}H_{34}N_2O_7$, 630.2366 found 630.2365.

1'-β-[7-(benzoquinazoline2,4-dione)]-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxy-D-ribofuranose-3'-(2-cyanoethyl-N-diisopropylphosphoramidite). To a solution of 5'-DMT-protected nucleoside **1** (0.43 g, 0.63 mmol) and *N, N*-diisopropylammonium tetrazolide (106 mg, 0.62 mmol) in freshly distilled CH_2Cl_2 (15 mL) was added 2-cyanoethyl tetraisopropylphosphoramidite (0.30 mL, 0.98 mmol) in a portion. The reaction mixture was allowed to stir at room temperature for 6 h. The volatiles were removed *in vacuo* and the residue

was purified by silica column chromatography (hexanes/ EtOAc 1:1 initially, EtOAc subsequently) to yield 5'-DMT-3'-phosphoramidite **1** (0.45 g, 86%) as white foam containing two inseparable diasteromers due to the chiral center at phosphorus. ^{1}H NMR ($CDCl_3$, 400 MHz):δ 8.67 (d, 1H, J = 4.4 Hz), 7.94 (s, 1H), 7.75 (m, 1H), 7.65 (m, 1H), 7.50 (m, 3H), 7.38 (m, 4H), 7.20 (m, 3H), 6.83 (m, 4H), 5.32 (dd, 1H, J = 5.6, 4.8 Hz) 4.59 (m, 1H), 4.31 (m, 1H), 3.85 (m, 6H), 3.60 (m, 2H), 3.36 (m, 2H), 2.60 (t, 1H, J = 6.4 Hz), 2.49 (t, 1H, J = 6.4 Hz), 2.16 (m, 1H), 1.20 (m, 9H), 1.10 (m, 3H) . ^{31}P NMR ($CDCl_3$, 161 MHz): δ 149.15, 149.07. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.29, 158.32, 152.04, 144.77, 138.88, 136.45, 135.87, 135.09, 130.03, 129.76, 128.16, 128.12, 127.71, 127.12, 126.73, 126.13, 117.64, 117.52, 114.68, 112.99, 111.51, 86.04, 86.01, 85.77, 79.98, 77.32, 76.11, 75.52, 64.12, 60.31, 58.30, 58.11, 55.10, 45.46, 43.13, 43.08, 42.96, 25.13, 24.56, 24.49, 24.41, 24.35, 23.39, 22.73, 21.31, 20.96, 20.31, 20.24, 20.13, 20.07, 14.08. HRFABMS m/z: $[M]^+$ calcd for $C_{47}H_{47}N_4O_8P$, 826.3120 found 826.3101.

Preparation of phosphoramidite derivative of nucleoside 2

1'-β-{7-[*N*-(4-oxo-1,4-dihydrobenzoquinazolin-2-yl)]-isobutyramide}-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyribofuranose. The *N*-isobutyryl-nucleoside **16** (0.56 g, 1.41 mmol) was coevaporated with distilled pyridine (3 × 5 mL), and the residue was dissolved in distilled pyridine (12 mL) under argon. 4,4'-dimethoxytrityl chloride (0.60 g, 1.70 mmol) was added to the solution in a portion. The mixture was stirred at room temperature for 12 h. The volatiles were removed *in vacuo*, and the residue was purified by silica column chromatography (hexanes/ EtOAc 1:2 initially, EtOAc subsequently) to afford 5'-DMT-**16** (0.76 g, 78%) as a white foam. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.79 (s, 1H), 7.96 (s, 1H), 7.85 (d, 2H, J = 8.4 Hz), 7.61 (d, 1H, J = 8.8 Hz), 7.47 (d, 2H, J = 6.8 Hz), 7.35 (d, 4H, J = 9.2 Hz), 7.28 (m, 3H), 6.82 (d, 4H, J = 8.8 Hz), 5.36 (dd, 1H, J = 5.6, 4.4 Hz), 4.50 (m, 1H), 4.16 (m, 1H), 3.77 (s, 6H), 3.41 (m, 1H), 3.33

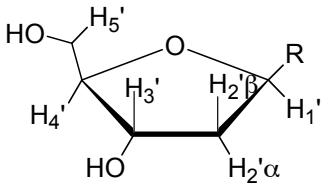
(m, 1H), 2.66 (bs, 1H), 2.35 (m, 1H), 2.15 (m, 1H), 1.26 (d, 6H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.22, 158.38, 158.28, 149.19, 144.67, 139.52, 139.44, 136.33, 135.81, 135.78, 130.29, 129.96, 129.93, 129.07, 128. 41, 128.05, 127.68, 127.65, 127.58, 127.29, 126.86, 126.66, 125.83, 123.83, 112.96, 112.94, 86.57, 86.09, 81.31, 79.79, 74.28, 64.45, 60.32, 55.02, 43.57, 36.23, 20.92, 18.89, 18.88, 14.03. HRFABMS *m/z*: $[M]^+$ calcd for $C_{42}H_{41}N_3O_7$, 700.3017 found 700.2991.

1'-β-{7-[*N*-(4-oxo-1,4-dihydrobenzoquinazolin-2-yl)]-isobutyramide}-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyribofuranose-3'-(2-cyanoethyl-N-diisopropylphosphoramidite).

To a solution of 5'-DMT-protected nucleoside **16** (0.50 g, 0.72 mmol) and *N, N*-diisopropylammonium tetrazolide (140 mg, 0.80 mmol) in freshly distilled CH_2Cl_2 (20 mL) was added 2-cyanoethyl tetraisopropylphosphoramidite (0.35 mL, 1.20 mmol) in one portion. The reaction mixture was allowed to stir at room temperature for 3.5 h. The volatiles were removed *in vacuo* and the residue was purified by silica column chromatography (hexanes/ EtOAc 1:1 initially, EtOAc subsequently) to yield 5'-DMT-3'-phosphoramidite **16** (0.45 g, 70%) as a white foam containing two inseparable diastereomers due to the chiral center at phosphorus. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.81 (s, 1H), 8.03 (d, 1H, J = 3.6 Hz), 7.84 (dd, 2H, J = 12.8, 2.4 Hz), 7.67 (d, 1H, J = 8.4 Hz), 7.50 (m, 2H), 7.37 (m, 4H), 7.29 (m, 2H), 7.20 (m, 1H), 6.82 (m, 4H), 5.36 (m, 1H), 4.60 (m, 1H), 4.34 (m, 1H), 3.89 (m, 1H), 3.74 (m, 6H), 3.60 (m, 2H), 3.37 (m, 2H), 2.64 (t, 1H, J = 6.4 Hz), 2.49 (t, 1H, J = 6.4 Hz), 2.18 (m, 1H), 1.21 (m, 18H). ^{31}P NMR ($CDCl_3$, 161 MHz): δ 149.12, 149.02. ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.36, 158.35, 144.75, 144.73, 135.88, 135.87, 135.86, 130.07, 130.03, 130.00, 128.17, 128.13, 127.71, 126.72, 126.69, 126.02, 117.55, 117.47, 112.98, 86.18, 86.07, 86.05, 85.85, 85.80, 80.12, 80.04, 75.63, 64.12,

58.31, 58.29, 58.12, 58.10, 55.12, 55.11, 45.49, 43.16, 43. 12, 43.03, 42.99, 36.33, 24.58, 24.57, 24.49, 24.35, 23.40, 22.67, 21.37, 20.36, 20.26, 20.16, 20.93, 18.99. HRFABMS *m/z*: $[M]^+$ calcd for $C_{51}H_{58}N_5O_8P$ 899.4009, found 899.3990.

Confirmation of anomeric geometry



β-anomer

yyT **8** Irradiation at :	H1' (5.22 ppm)	NOE observed at H4' (4.01 ppm), H2'α (2.29 ppm)
yyC **16** Irradiation at:	H1' (5.18 ppm)	NOE observed at H4' (3.85 ppm), H2'α (2.15 ppm)

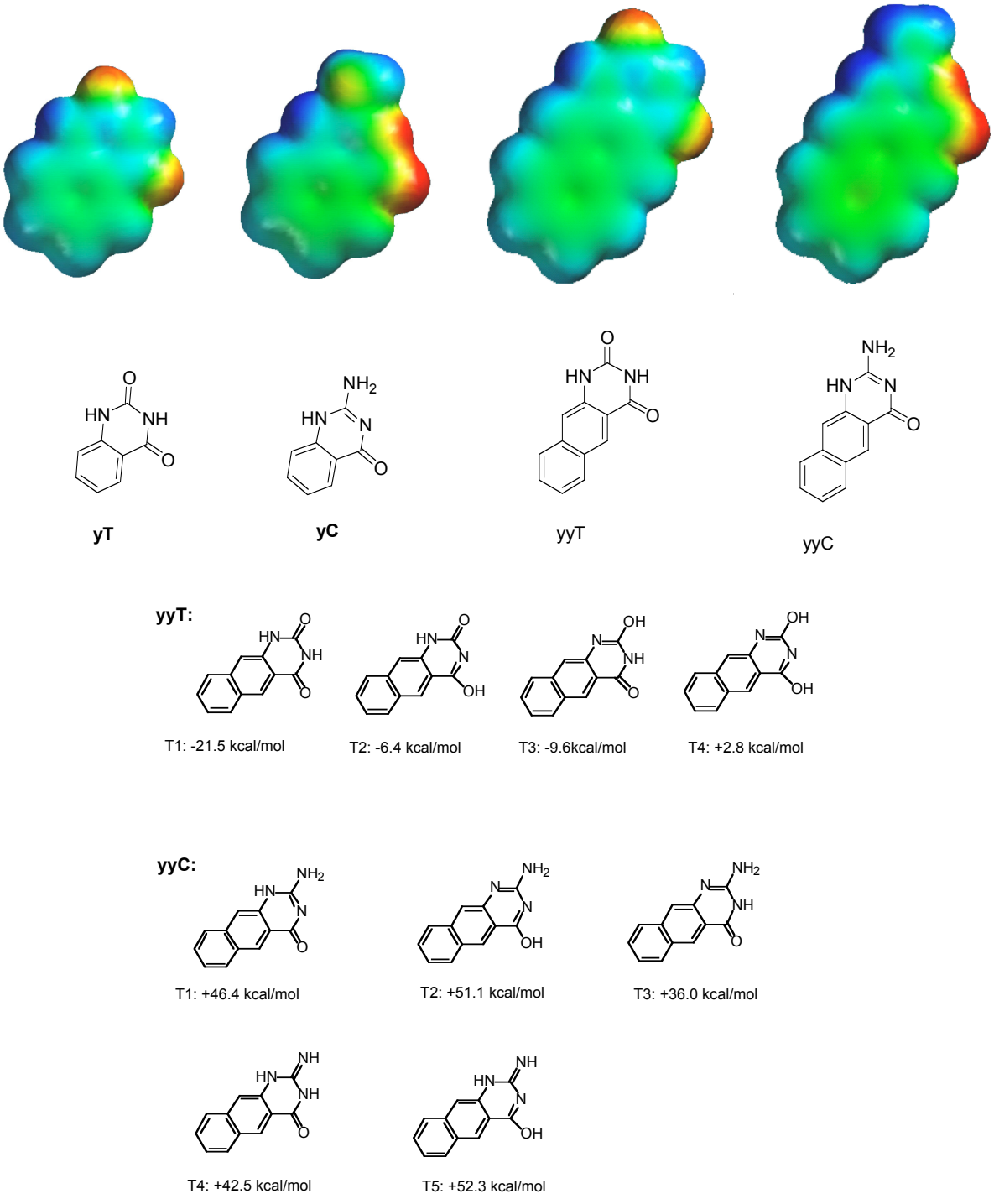


Figure S1. **(top)** Space-filling models of the yyT, yyC, yT and yC nucleobases with electrostatic potentials mapped on the surfaces. Natural T and C bases are shown for comparison. Calculated using AM1 with Spartan (Wavefunction, Inc.). **(below)** AM1-Calculated heats of formation in the gas phase (kcal/mol) for several tautomers of yyT and yyC.

Oligonucleotide characterization. Synthesized oligonucleotides containing modified deoxyribosides were characterized MALDI-TOF mass spectrometry. Data are given below.

Mass spectrometry data for oligonucleotides containing yyT and yyC deoxyribosides.

Oligonucleotide	Average MW calcd.	MW found
5'-yyTCGCGCG-3'	2183	2183
5'-yyCCGCGCG-3'	2183	2183
5'-CTTTTCyyTTTCTT-3'	3630	3631
5'-AAGAAyyTGAAAAG-3'	3822	3823
5'-CTTTTCyyCTTCTT-3'	3629	3631
5'-AAGAAyyCGAAAAG-3'	3821	3825
5'-yyCyyCyyTGAyyCG-3'	2468	2472
5'-yyCGyyTyyCAGG-3'	2408	2408
5'-yyCyyCGyyCGG-3'	2095	2098
5'-yyTAyyTyyTAyyTA-3'	2242	2238
5'-yyTAyyTAAyyTA-3'	2366	2369
5'-yyCGyyCyyCGyyCG-3'	2488	2490
5'-yyCGyyCGGyyCG-3'	2425	2429
5'-yyTAyyCAyyTG-3'	2064	2065
5'-yyCAyyTGyyTA-3'	2064	2064
5'-GyyTAyyTAyyTA-3'	2378	2379
5'-AyyTAyyTAyyTG-3'	2378	2379
5'-yyCyyCyyTyyTyyCyyTyyCyyC-3'	3055	3057

Table S1. Thermal melting data for 12mer DNA duplexes containing a size-expanded base pair or mismatch (X-Y) in a central position.[a,b,e]

base pair **X - Y**	T_m[c] (°C)	$\Delta G°_{37}$[d] (kcal/mol)	base pair **X - Y**	T_m[c] (°C)	$\Delta G°_{37}$[d] (kcal/mol)
A - T	40.4 ± 0.5	-9.4 ± 0.1	T - A	41.2 ± 0.5	-9.4 ± 0.1
A - yyT	33.9 ± 0.5	-7.7 ± 0.1	yyT - A	34.3 ± 0.5	-7.8 ± 0.1
G - yyT	36.3 ± 0.5	-8.0 ± 0.2	yyT - T	30.8 ± 0.5	-7.1 ± 0.1
T - yyT	25.3 ± 0.5	-7.7 ± 0.3	yyT - G	34.4 ± 0.5	-7.7 ± 0.1
C - yyT	26.3 ± 0.5	-7.9 ± 0.2	yyT - C	32.6 ± 0.5	-7.4 ± 0.1
G - C	46.5 ± 0.5	-11.1 ± 0.4	C - G	43.0 ± 0.5	-9.7 ± 0.1
A - yyC	32.5 ± 0.5	-7.3 ± 0.1	yyC - G	30.8 ± 0.5	-7.0 ± 0.3
G - yyC	33.3 ± 0.5	-7.4 ± 0.2	yyC - C	29.7 ± 0.5	-7.1 ± 0.2
C - yyC	33.1 ± 0.5	-7.9 ± 0.2	yyC - A	29.8 ± 0.5	-6.9 ± 0.3
T - yyC	28.2 ± 0.5	-7.8 ± 0.2	yyC - T	27.8 ± 0.5	-6.5 ± 0.4
ϕ - T	21.0 ± 0.5	nd	T - ϕ	22.4 ± 0.5	nd
ϕ - C	20.6 ± 0.5	nd	C - ϕ	23.5 ± 0.5	nd
ϕ - yyT	31.1 ± 0.5	-7.7 ± 0.4	yyT - ϕ	32.7 ± 0.5	-7.7 ± 0.2
ϕ - yyC	35.0 ± 0.5	-8.6 ± 0.2	yyC - ϕ	30.9 ± 0.5	-7.2 ± 0.1

[a] Conditions: 100 mM NaCl, 10 mM $MgCl_2$, 10 mM PIPES•Na (pH 7.0).
[b] Sequence is 5'-AAGAAXGAAAAG • 5'-CTTTTCYTTCTT.
[c] T_m values are for 5.0 μM oligonucleotide.
[d] Averages of values from van't Hoff and curve fitting methods.
[e] "ϕ" is tetrahydrofuran abasic analogue.

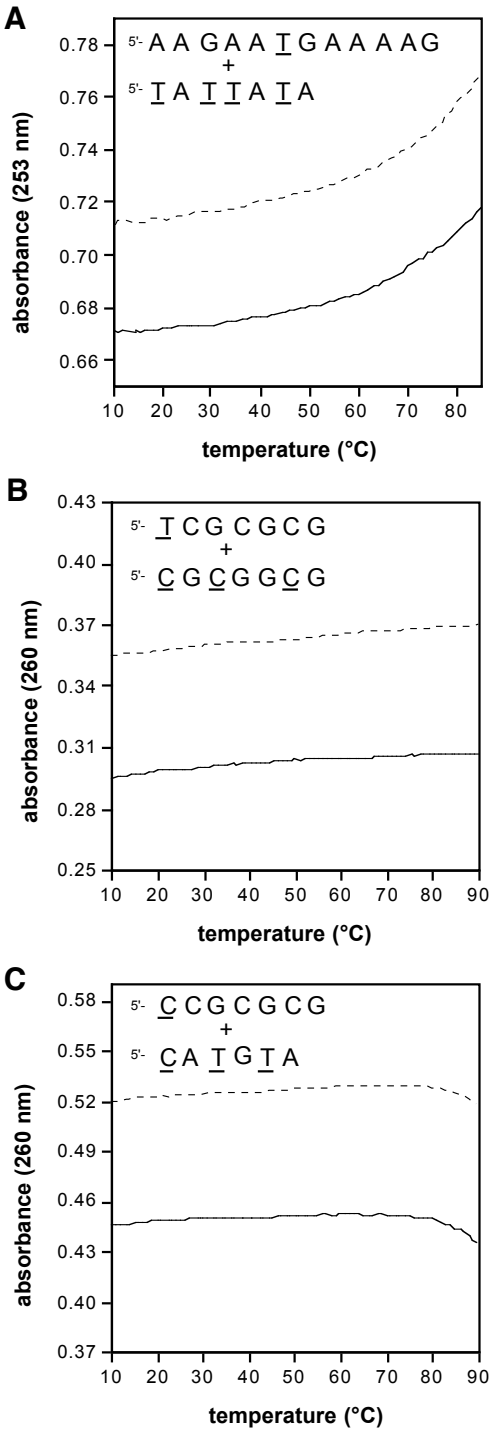


Figure S2. Thermal melting data for three noncomplementary sequences (A-C), showing little or no difference in curve shape between oligonucleotide mixtures (solid lines) and mathematical addition of data for single strands alone (dashed lines). Sequences are as shown in each plot, with yyDNA bases underlined. Compare to Figure 3, main text.

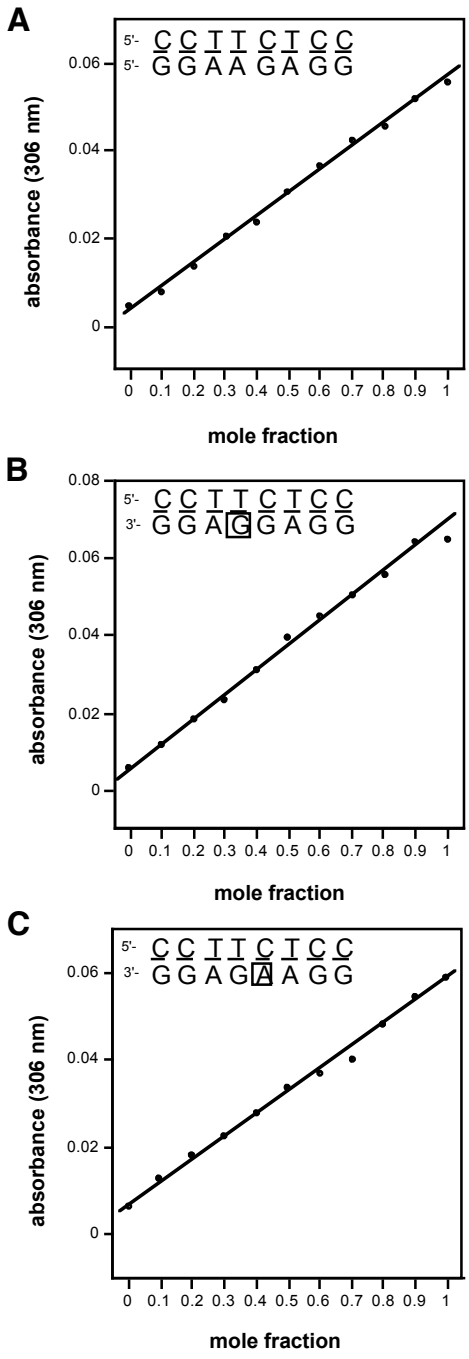


Figure S3. Mixing plots for three different yDNA sequences, testing strand orientation and sequence selectivity. All three cases are best fit by a single line, suggesting no complexation under the experimental conditions. (A) Parallel-oriented strands; (B) Strands containing a yyT – G mismatch; (C) Strands containing a yyC – A mismatch. The mixing plot for the antiparallel, fully complementary case is shown in Fig. 4A (main text). See Table 3 (main text) for all sequences. Doublewide nucleotides are underlined.

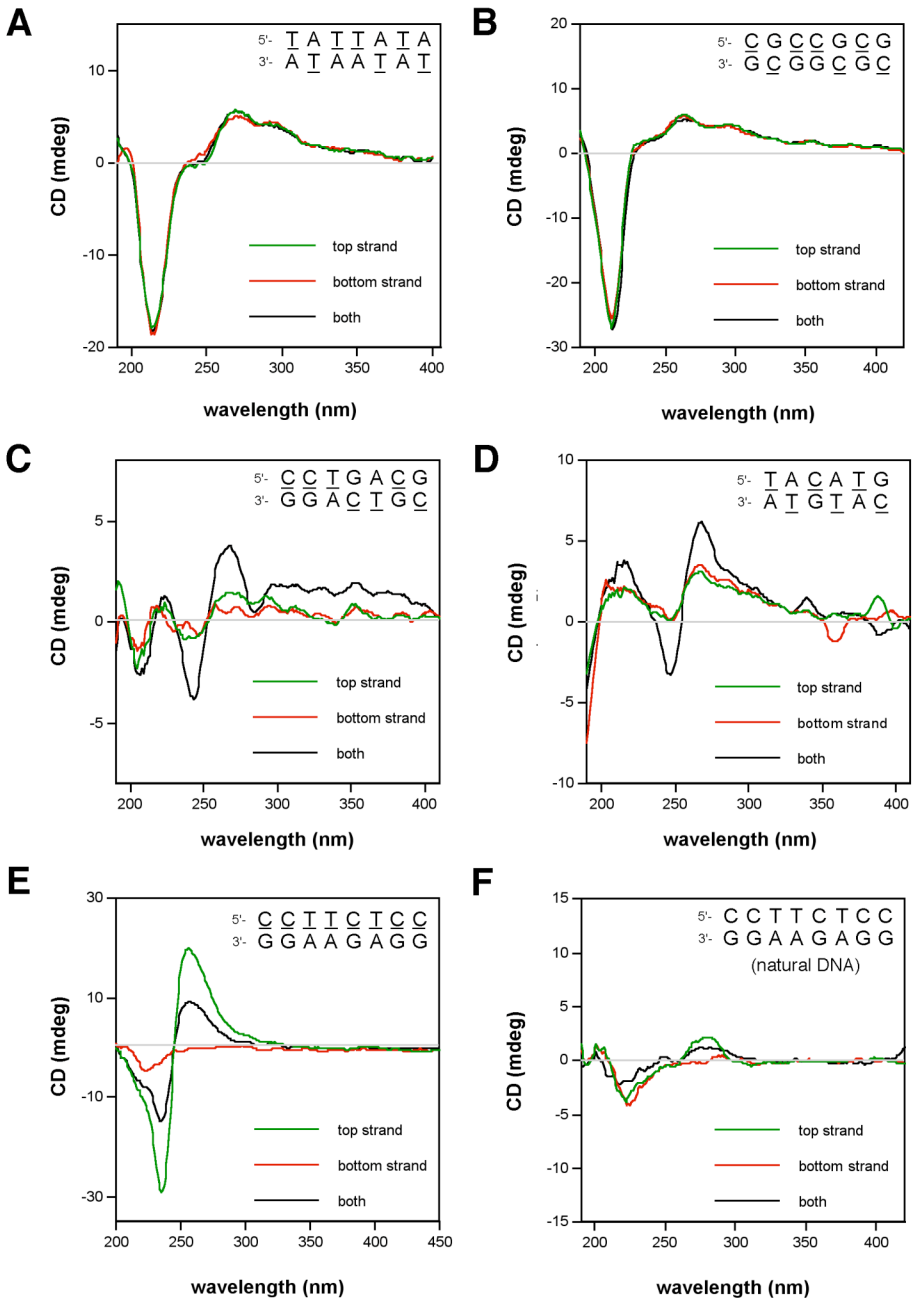


Figure S4. CD spectra of yyDNA strands and putative duplexes. Sequences are as shown; yyDNA nucleotides are underlined. (A,B) related sequences containing only one type of yyDNA base; (C,D) mixed sequences containing four different base pairs; (E) sequence in which all yyDNA bases are segregated to one strand; (F) all-DNA control for (E). Conditions: 2 μM [DNA]; 100 mM NaCl, 10 mM $MgCl_2$, 10 mM Na•PIPES (pH 7.0).

S11

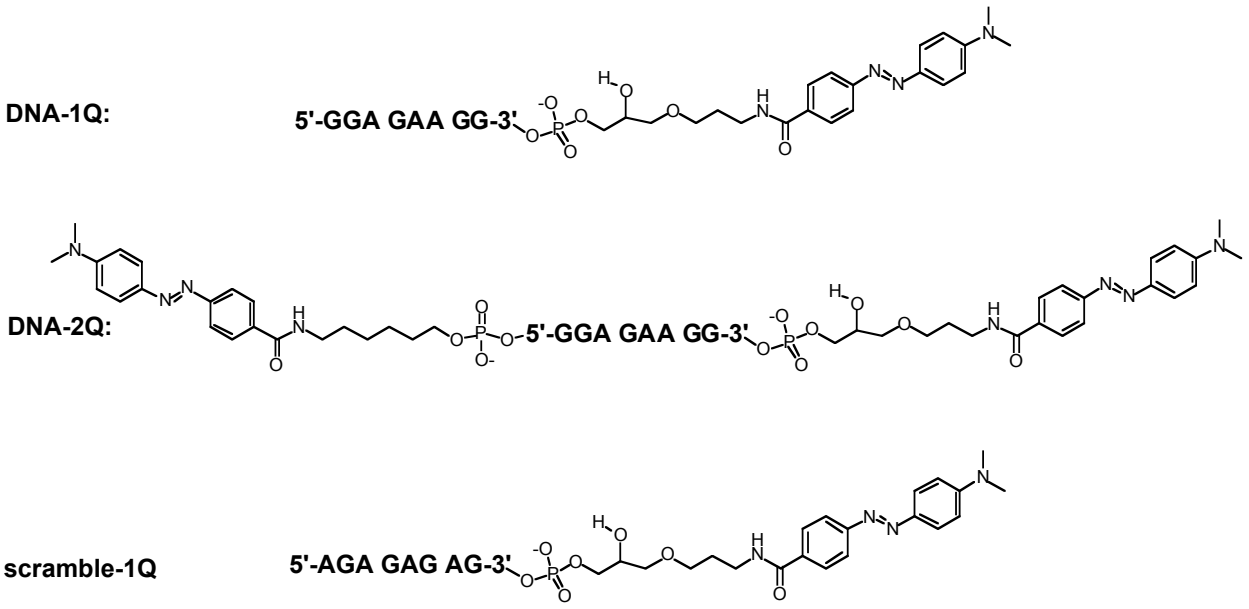
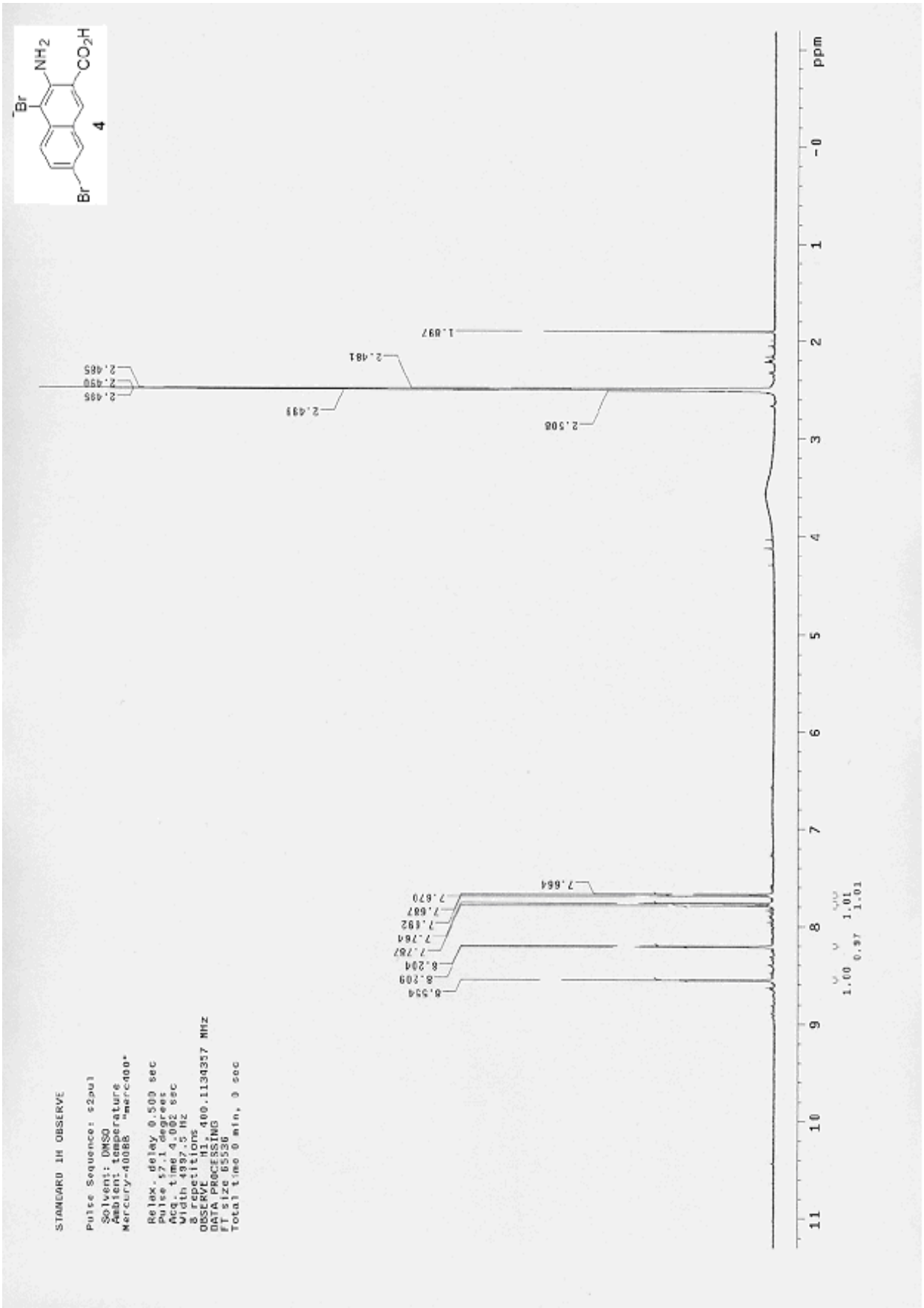
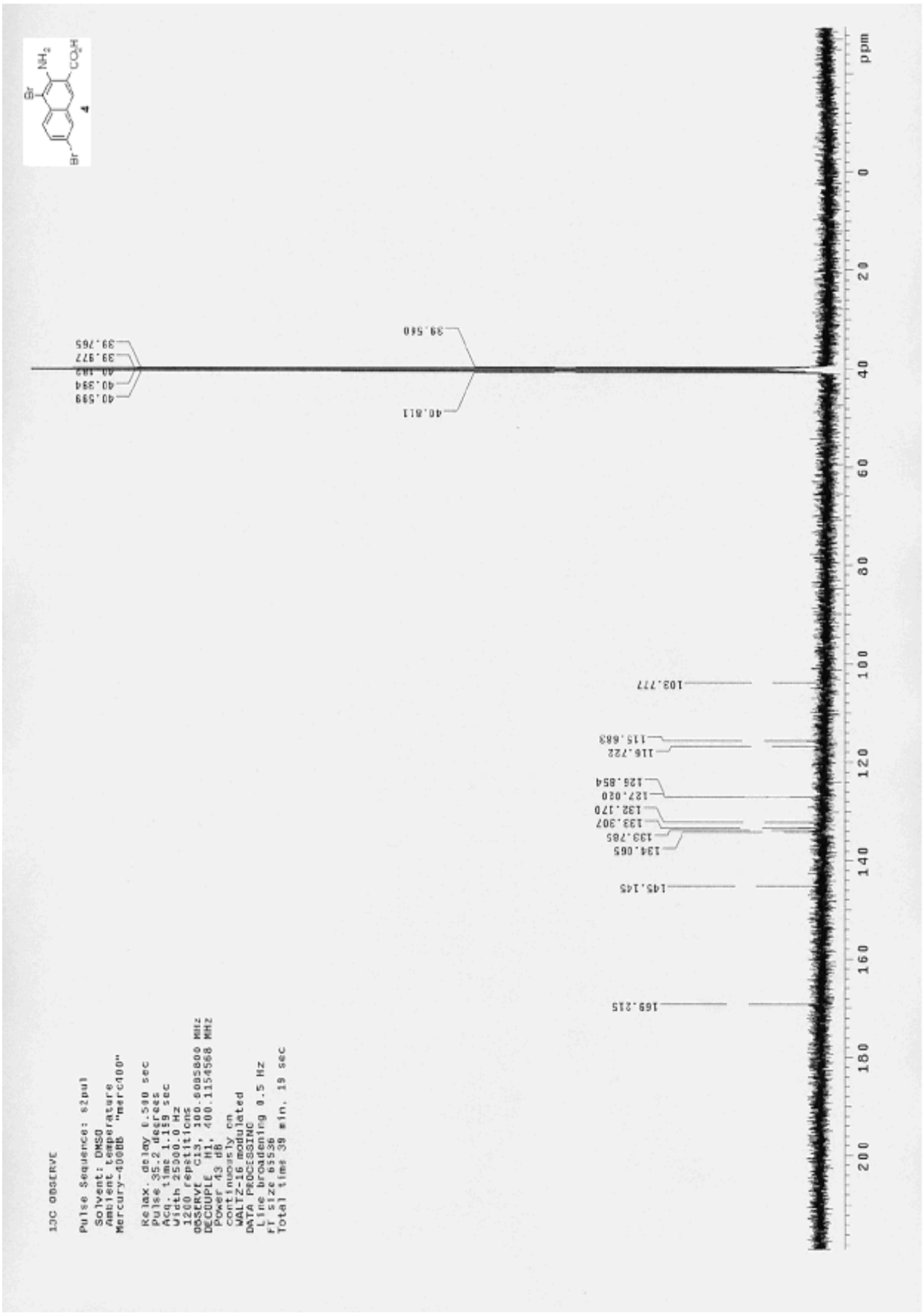
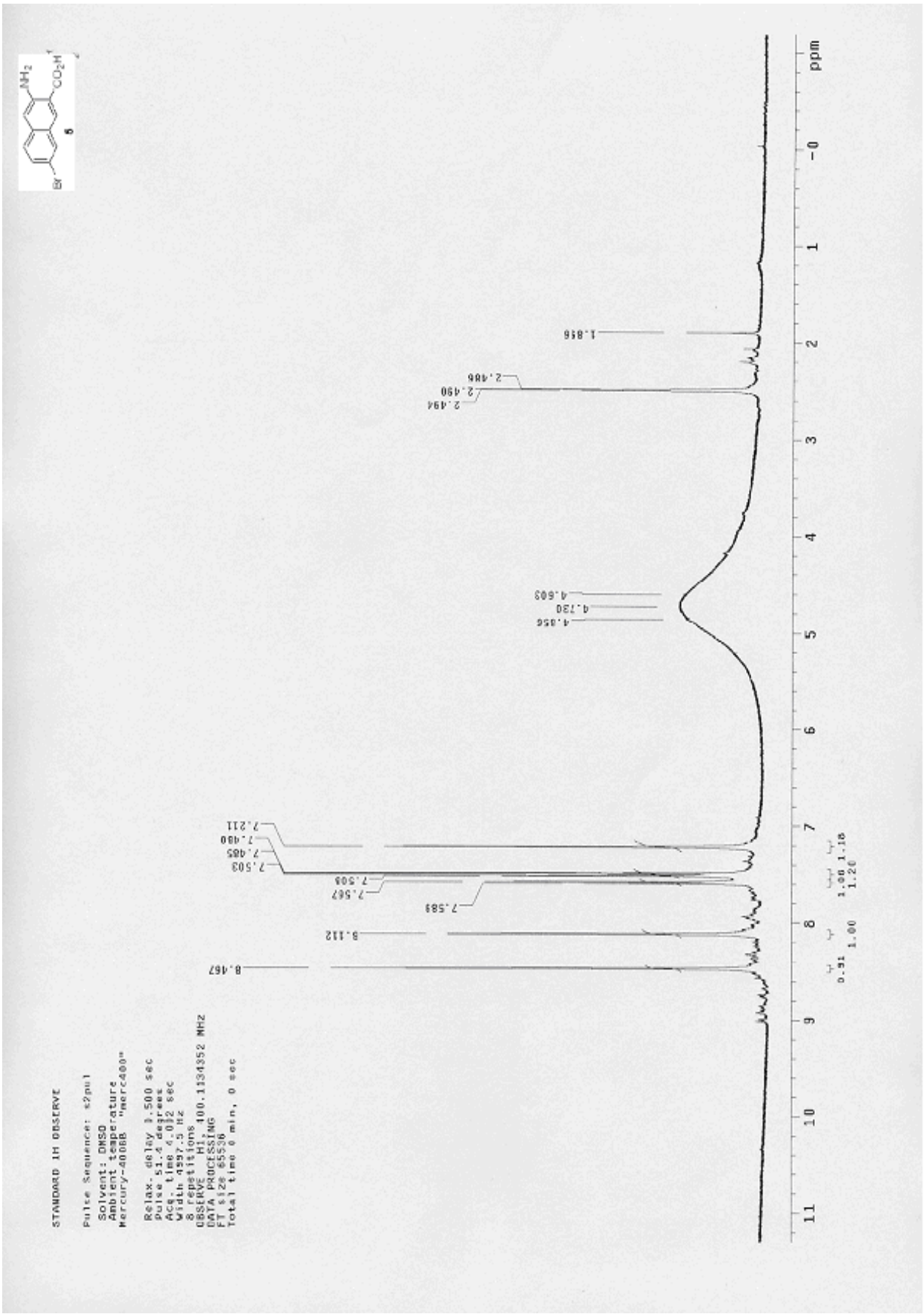
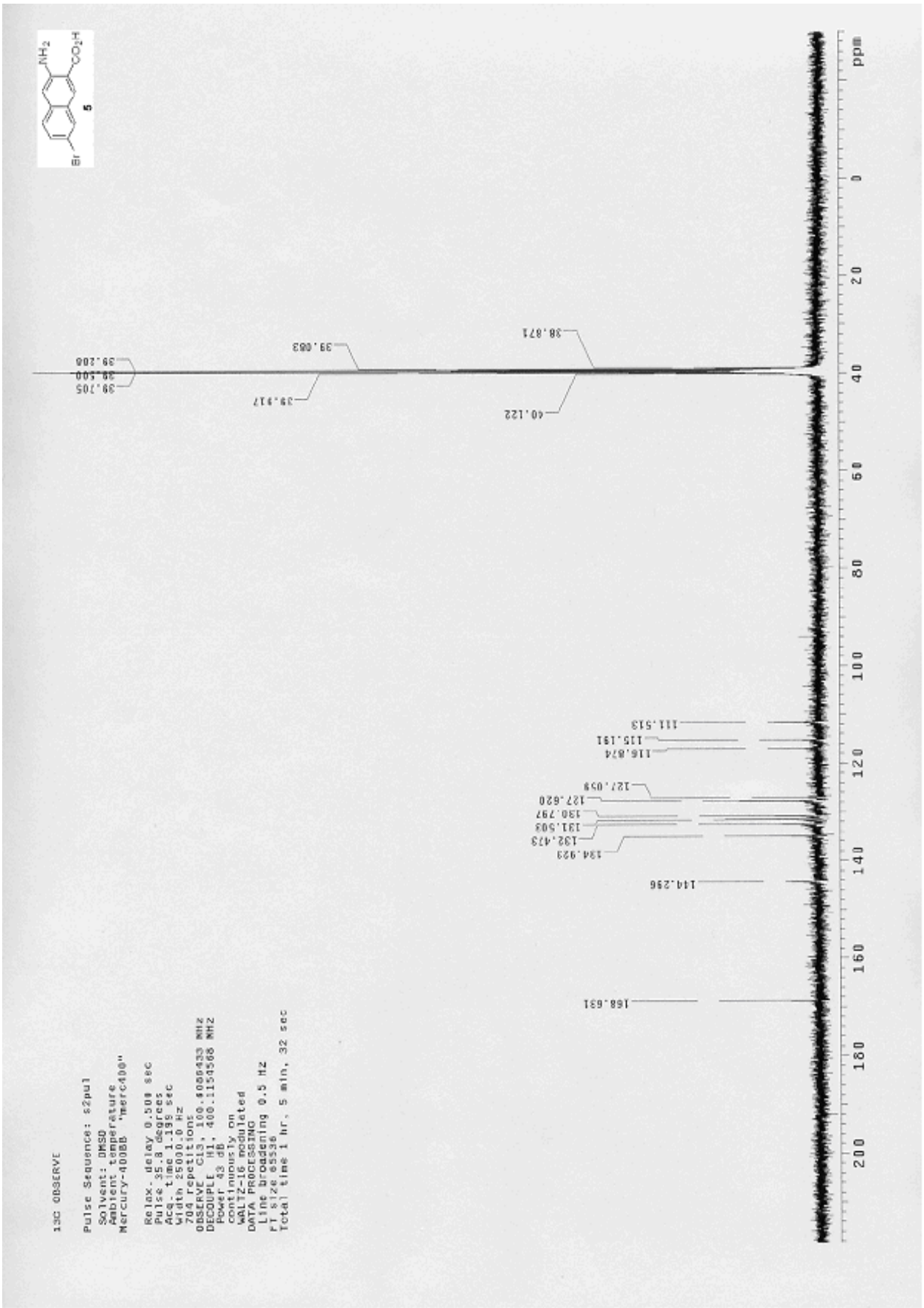


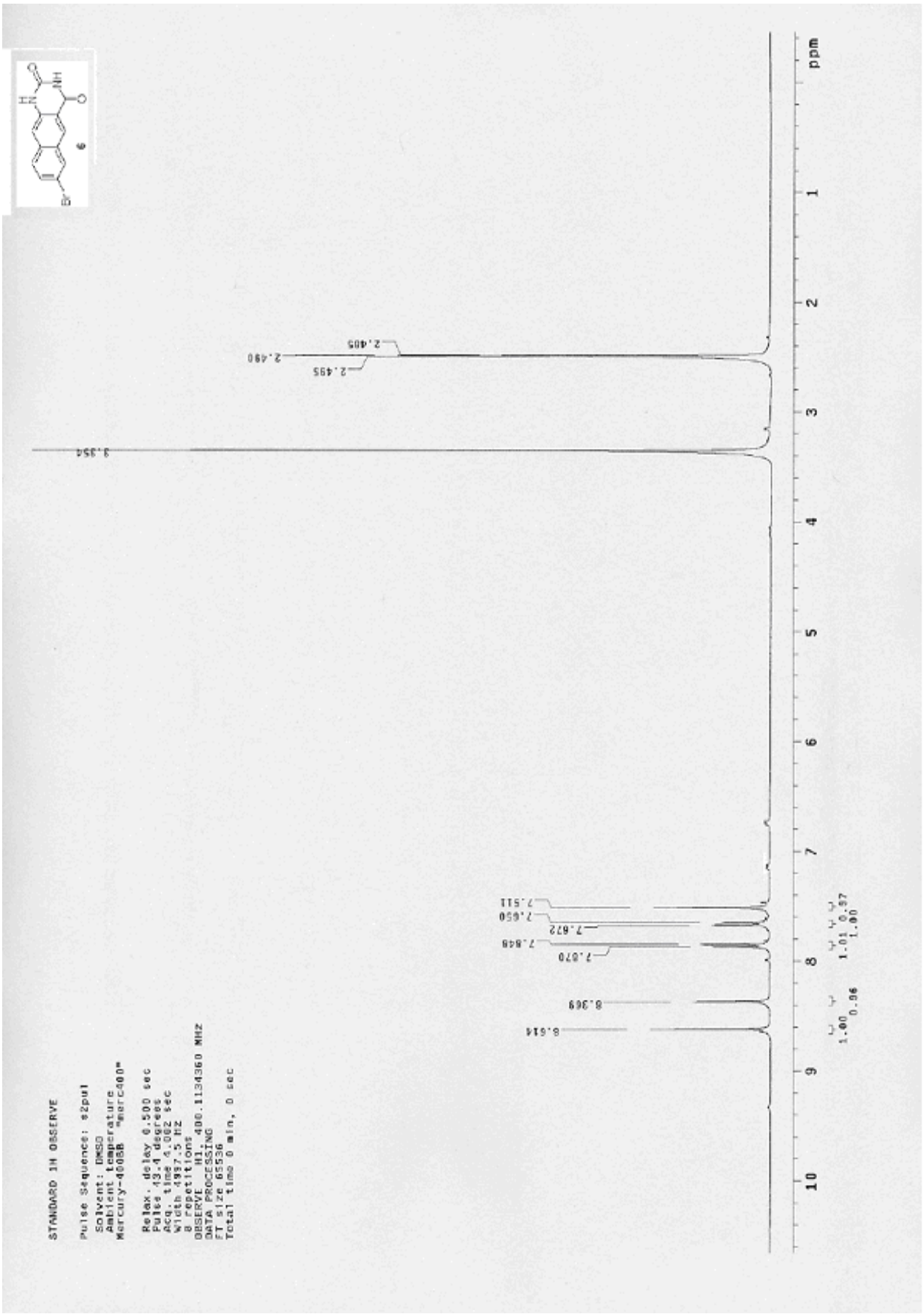
Figure S5. Structures of quencher-oligonucleotide conjugates used in experiments to test hybridization and quenching of a yyDNA strand. See Fig. 6 (main text) for fluorescence data. 5' and 3' Dabcyl conjugates were added during oligonucleotide synthesis, using commercial reagents (Glen Research).

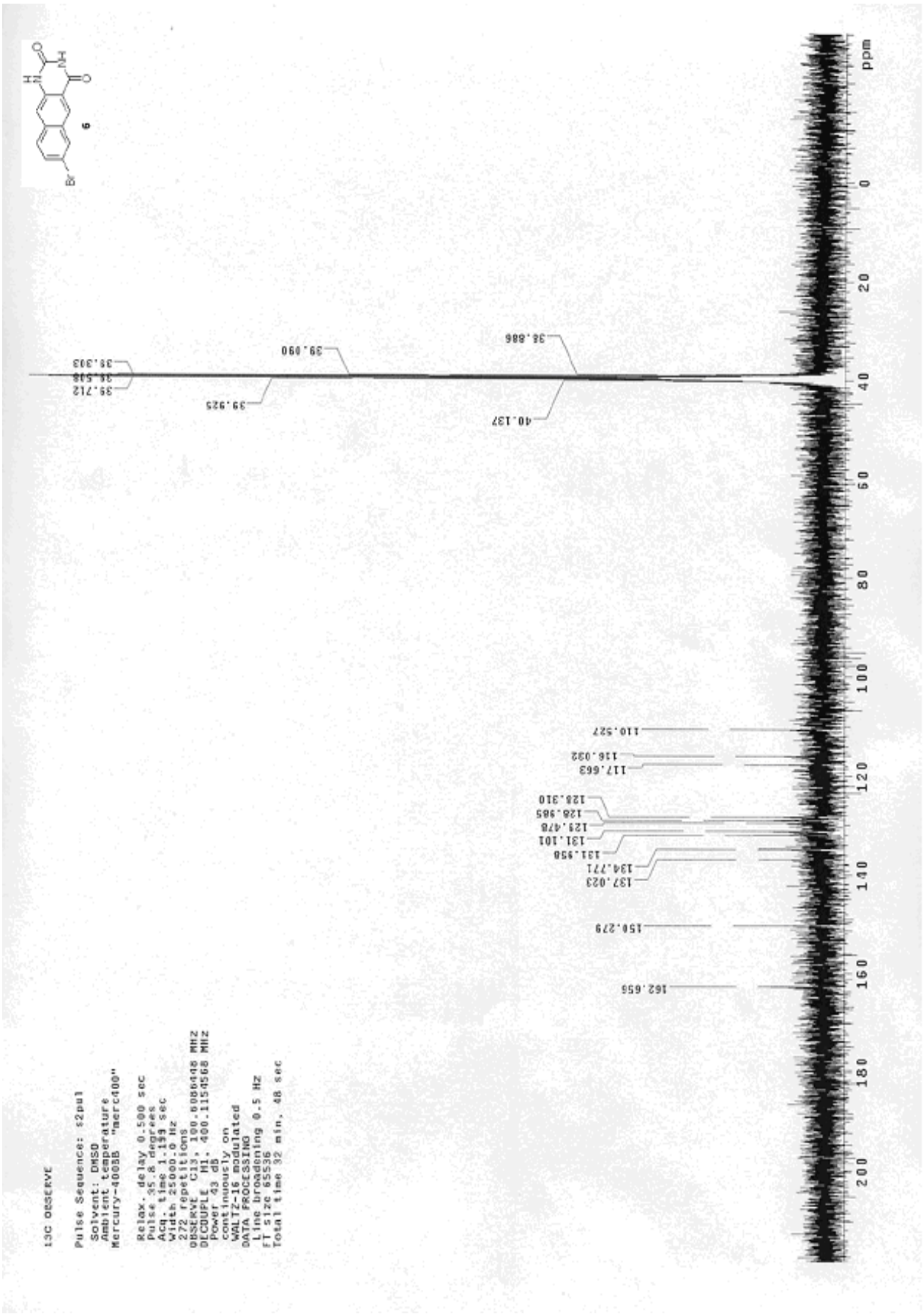

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 57.1 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
8 repetitions
OBSERVE H1, 400.1134357 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 3 sec
Br
NH2
CO2H
Br
4
8.554
8.209
8.204
7.787
7.764
7.692
7.687
7.670
7.664
2.508
2.499
2.485
2.481
1.897
1.00
0.97
1.01
1.01
ppm

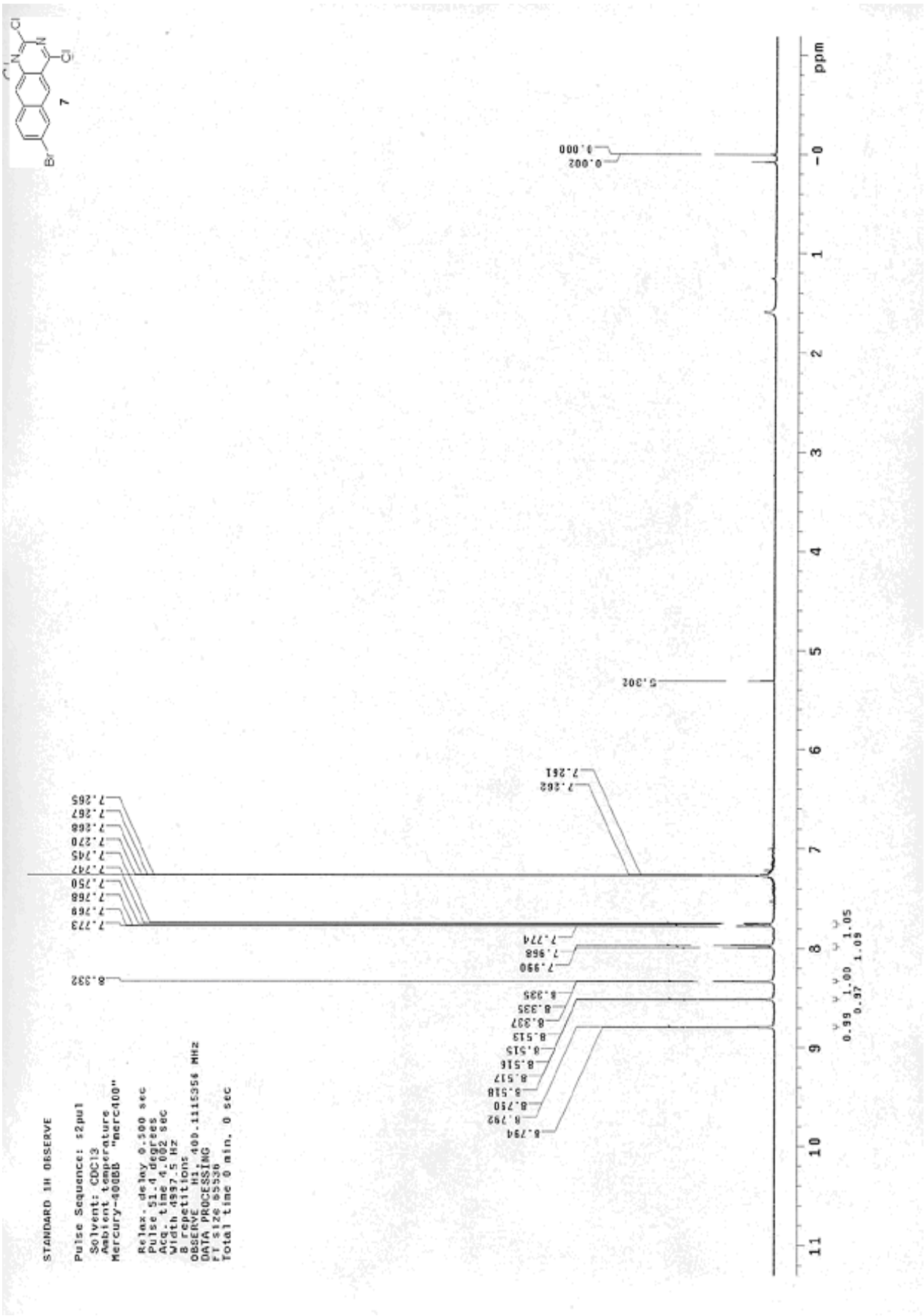

13C OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc100"
Relax. delay 0.500 sec
Pulse 35.2 degrees
Acq. time 1.119 sec
Width 25000.0 Hz
1200 repetitions
OBSERVE C13, 100.6085800 MHz
DECOUPLE H1, 400.1154568 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 39 min, 19 sec
39.765
39.977
40.189
40.394
40.599
39.560
40.811
103.777
115.683
116.722
126.854
127.020
132.170
133.307
133.785
134.065
145.145
169.215
ppm

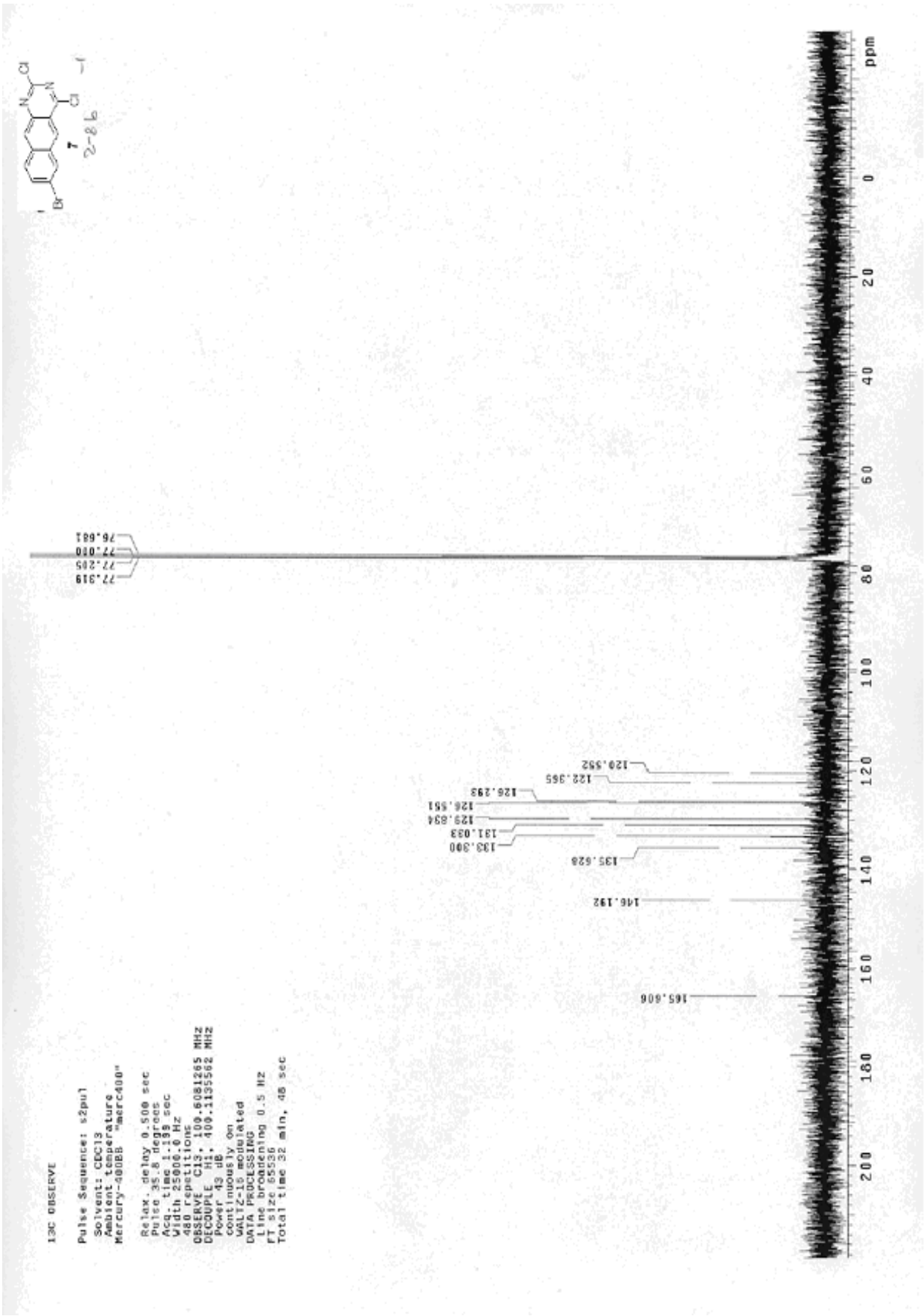

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 3.500 sec
Pulse 51.4 degrees
Acq. time 4.092 sec
Width 4597.5 Hz
8 repetitions
OBSERVE H1, 100.1334552 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
8.467
8.112
7.589
7.567
7.508
7.503
7.485
7.480
7.211
4.656
4.730
4.603
2.494
2.490
2.486
1.816
0.91
1.00
1.00
1.18
1.20
ppm

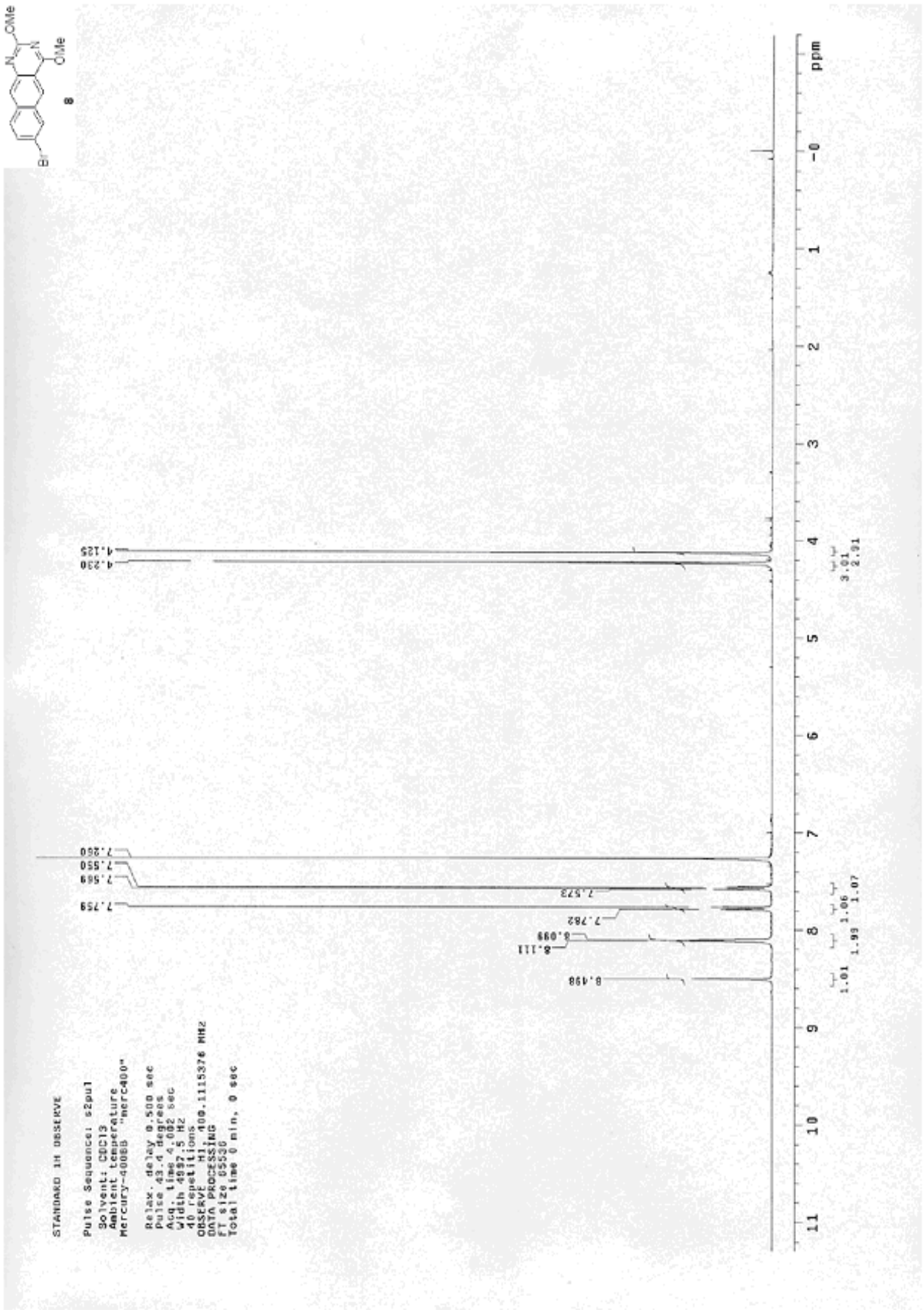

13C OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.8 degrees
Acq. time 1.135 sec
Width 25010.0 Hz
704 repetitions
OBSERVE C13, 100.4086433 MHz
DECOUPLE H1, 400.1154568 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 5 min, 32 sec
NH2
CO2H
Br
5
ppm
0
20
40
60
80
100
120
140
160
180
200
39.205
39.500
39.206
39.083
39.917
38.871
40.122
111.513
115.191
116.874
127.059
127.620
130.797
131.503
132.473
134.923
144.296
168.631

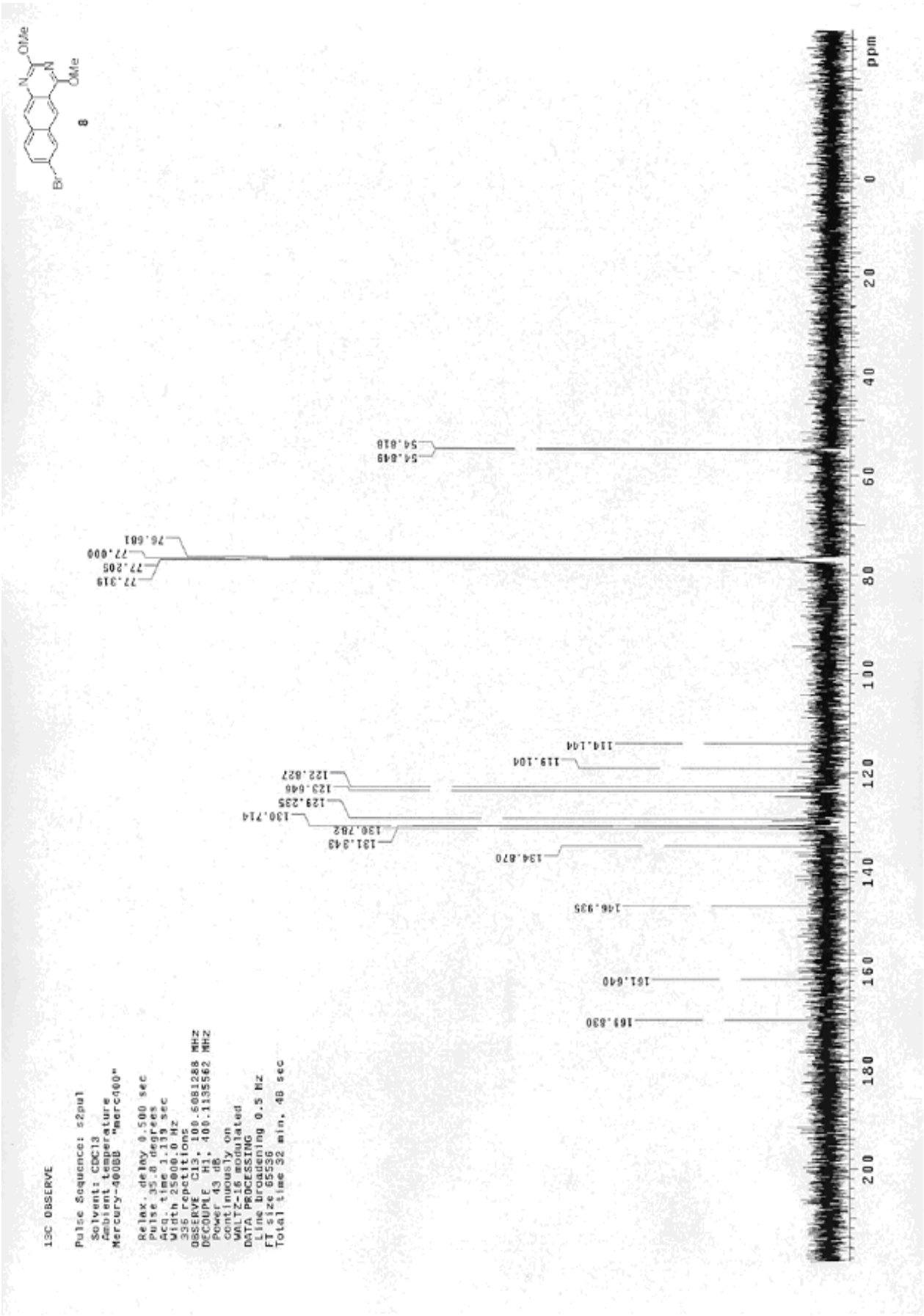

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 43.4 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
8 repetitions
OBSERVE H1, 400.1134360 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
8.614
8.369
7.870
7.848
7.672
7.650
7.511
3.354
2.495
2.490
2.485
1.00
0.96
1.01
0.97
1.00
ppm

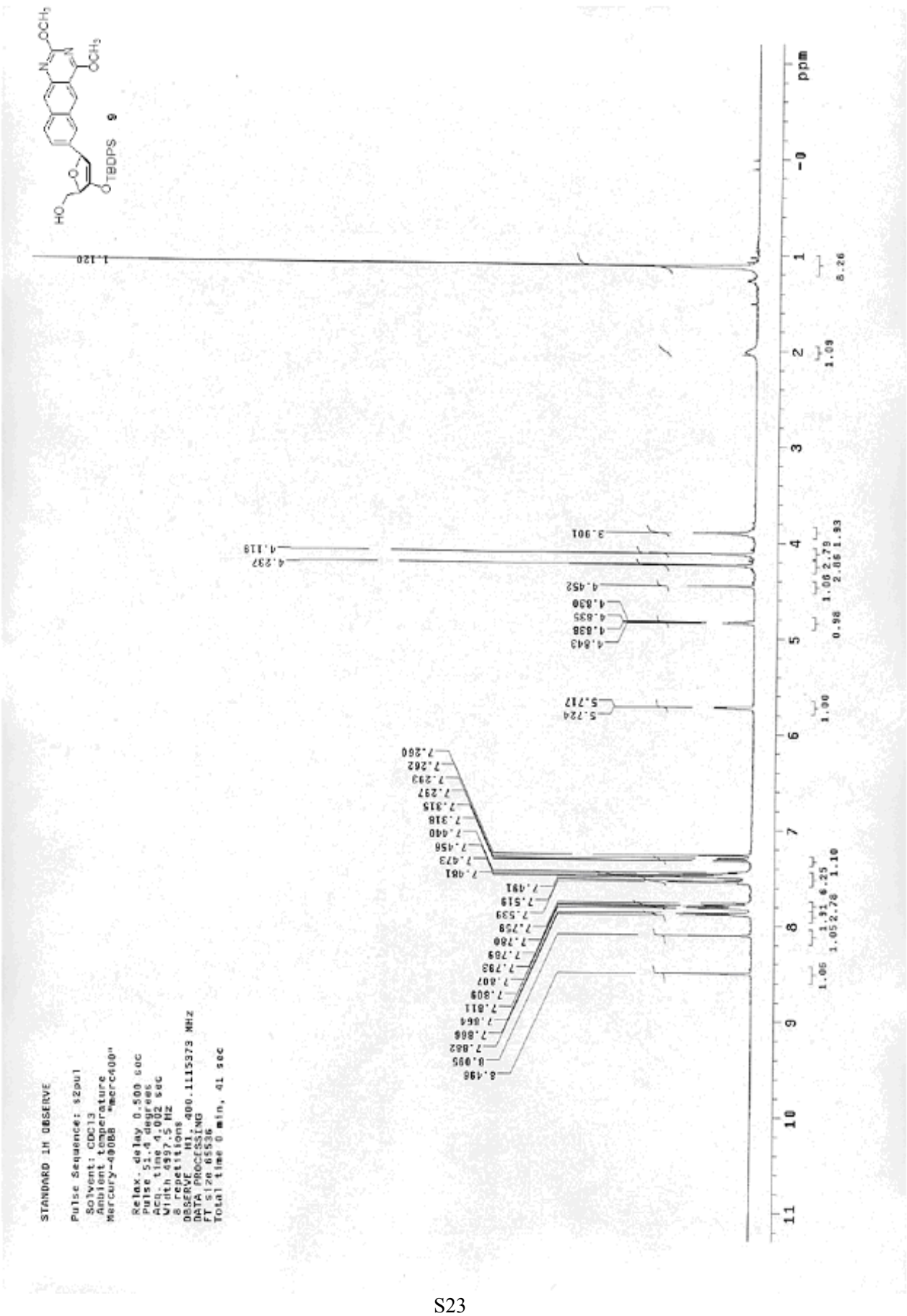

6
Br
ppm
0
20
40
60
80
100
120
140
160
180
200
39.712
39.518
39.303
39.090
38.886
39.925
40.137
110.527
116.032
117.663
126.310
126.985
129.478
131.101
131.958
134.771
137.023
150.279
162.656
13C OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.8 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
272 repetitions
OBSERVE C13, 100.6086448 MHz
DECOUPLE H1, 400.1154568 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 32 min, 48 sec

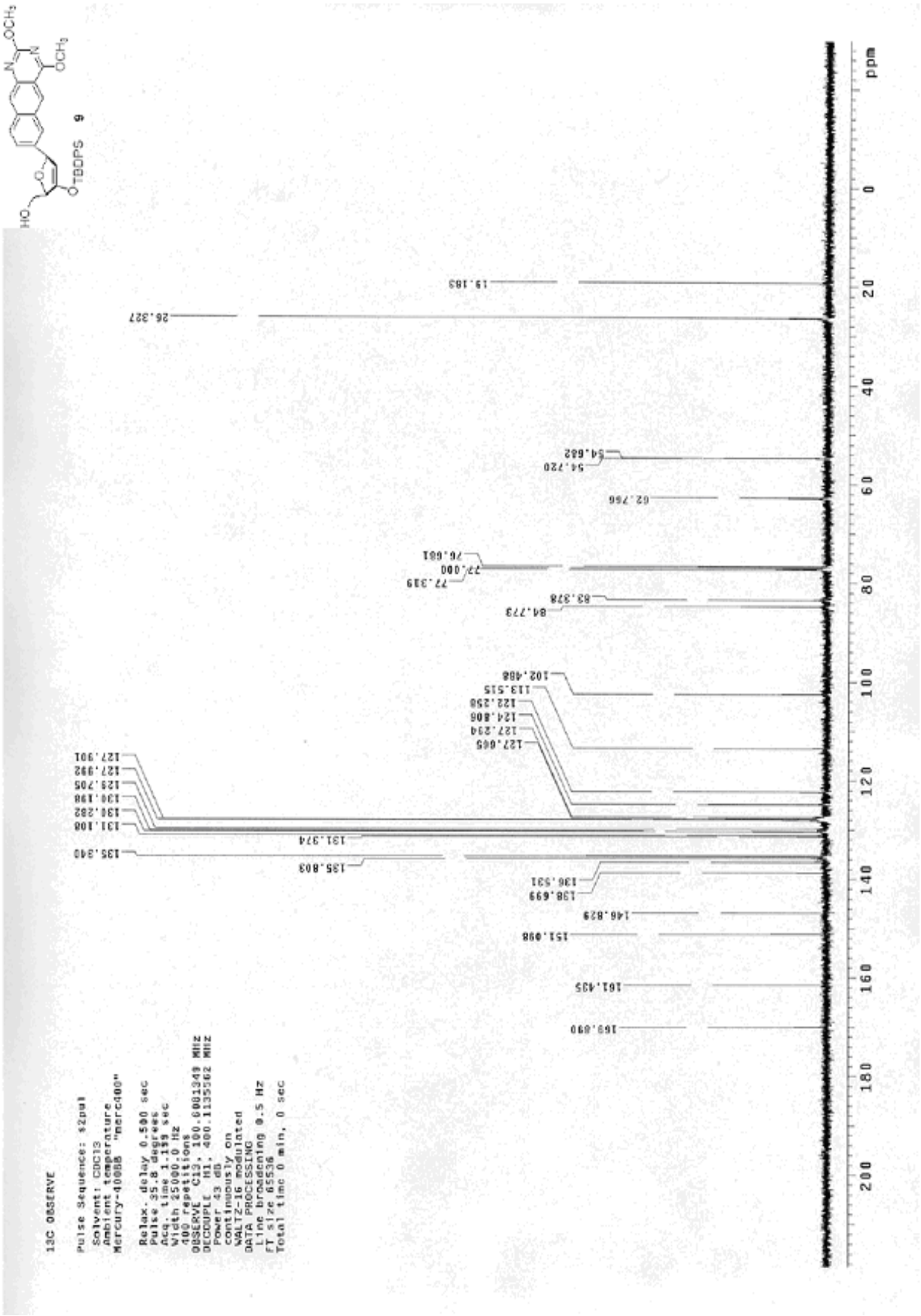

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 51.4 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
8 repetitions
OBSERVE H1, 400.1115354 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
7
Cl
N
N
Cl
Br
0.002
0.000
5.302
7.262
7.261
7.773
7.769
7.768
7.750
7.747
7.745
7.720
7.668
7.667
7.665
8.392
7.774
7.968
7.990
8.335
8.335
8.337
8.513
8.515
8.516
8.517
8.518
8.710
8.792
8.794
0.99
1.00
1.05
0.97
1.09
ppm

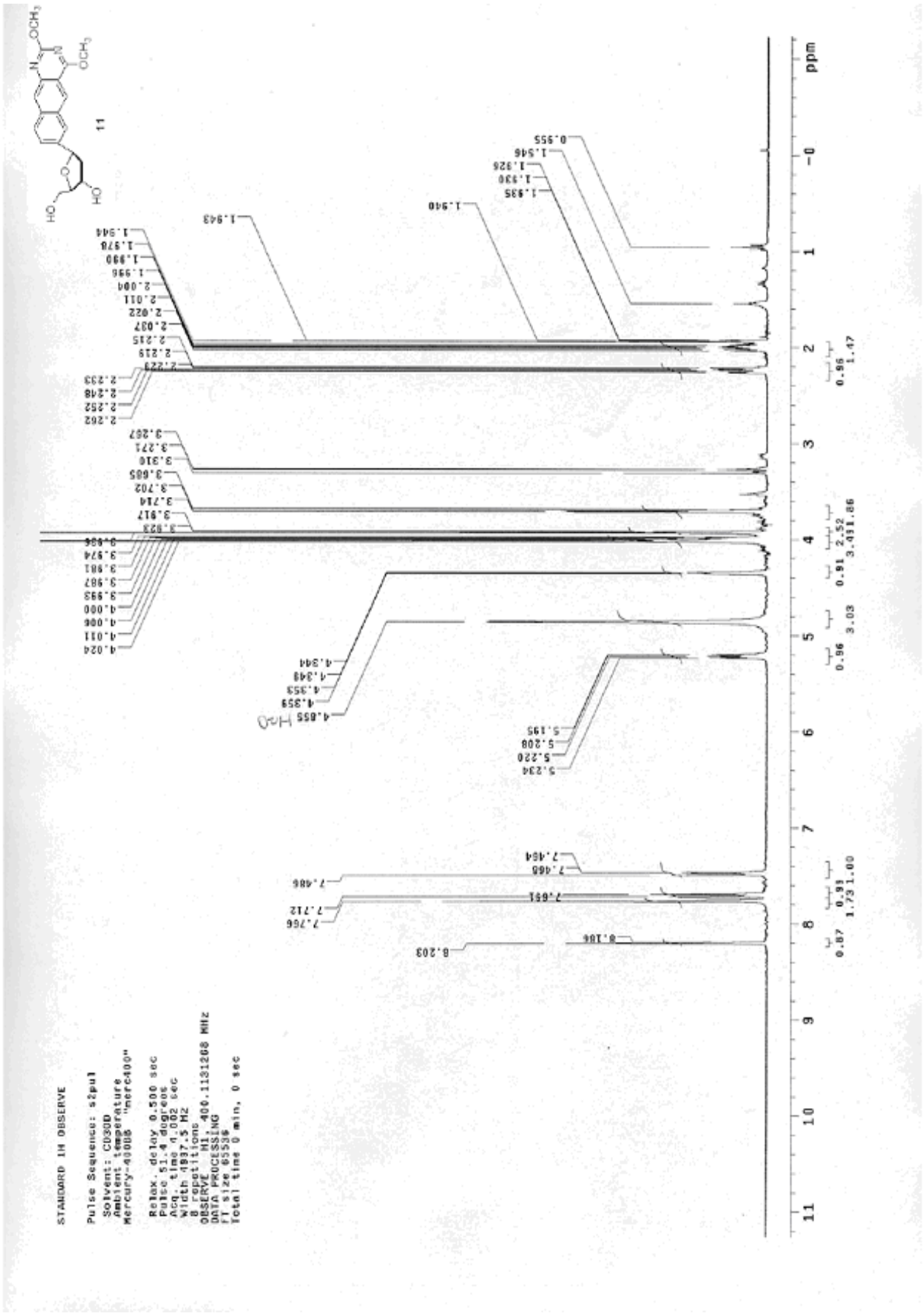

13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.8 degrees
Acq. time 1.188 sec
Width 25000.0 Hz
480 repetitions
OBSERVE C13, 100.6081265 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 32 min, 48 sec
77.319
77.285
77.010
76.681
165.606
146.192
135.628
133.300
131.033
129.634
126.551
126.293
122.365
120.582
ppm

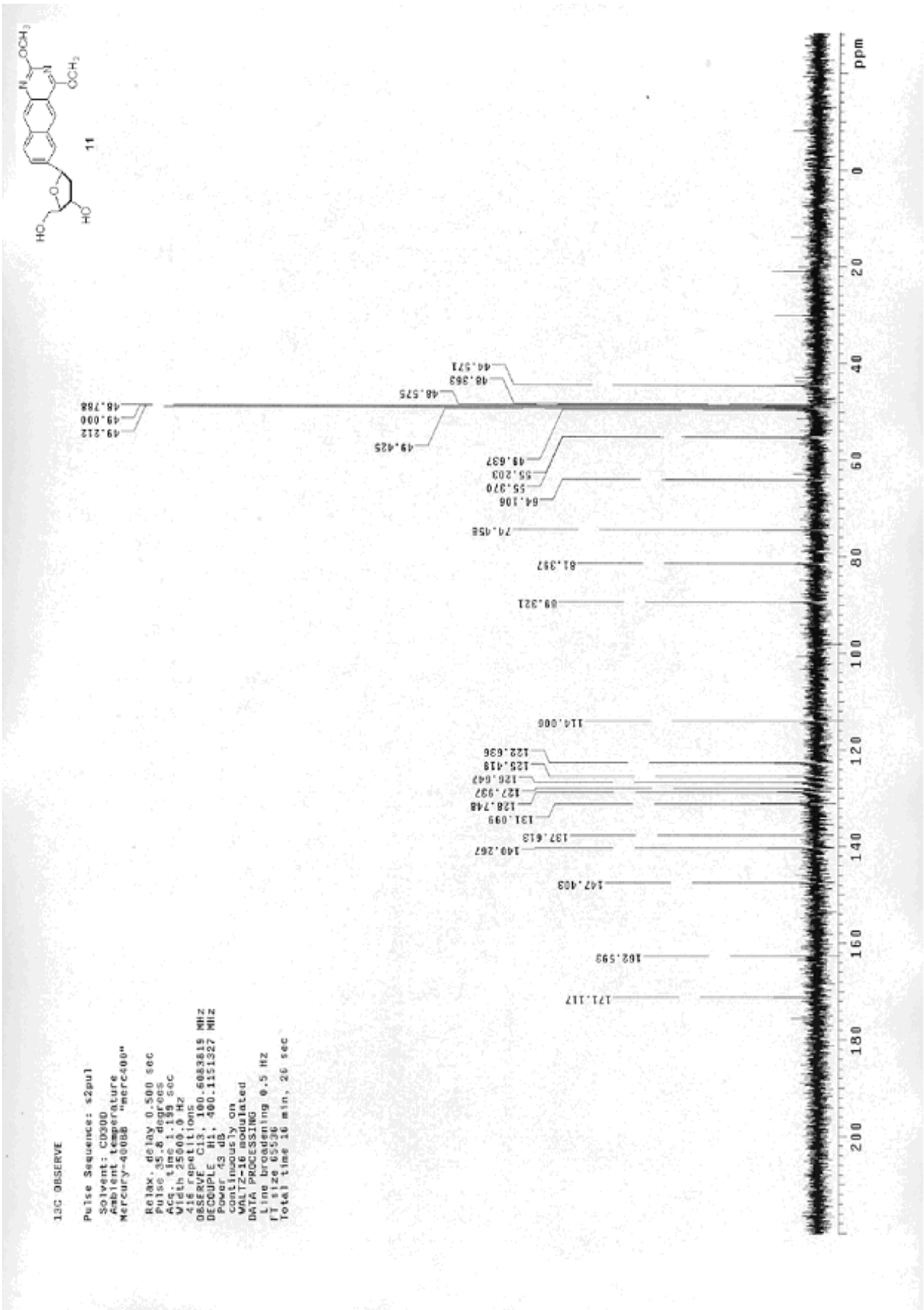

OMe
OMe
Br
8
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 43.4 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
40 repetitions
OBSERVE H1, 400.1115376 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
4.125
4.230
7.260
7.550
7.569
7.759
7.573
7.782
8.099
8.111
8.498
3.01
2.01
1.01
1.06
1.93
1.07
ppm

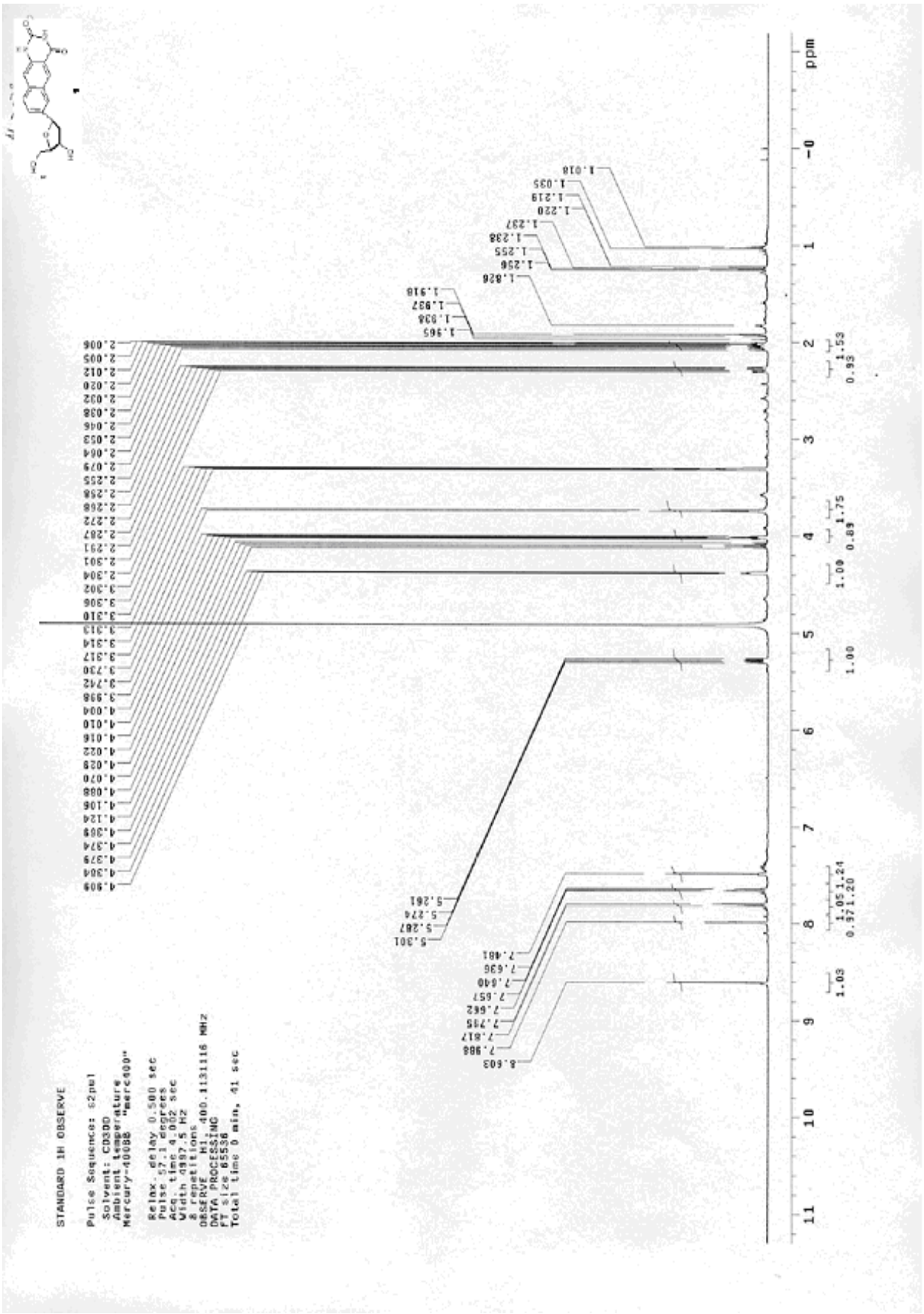

OMe
N
N
OMe
Br
8
ppm
0
20
40
60
80
100
120
140
160
180
200
54.049
54.818
76.601
77.000
77.205
77.319
114.104
119.104
122.827
123.646
129.235
130.714
130.782
131.343
134.870
146.935
161.640
169.830
13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.0 degrees
Acq. time 1.139 sec
Width 25000.0 Hz
336 repetitions
OBSERVE C13, 100.6081288 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 32 min, 48 sec

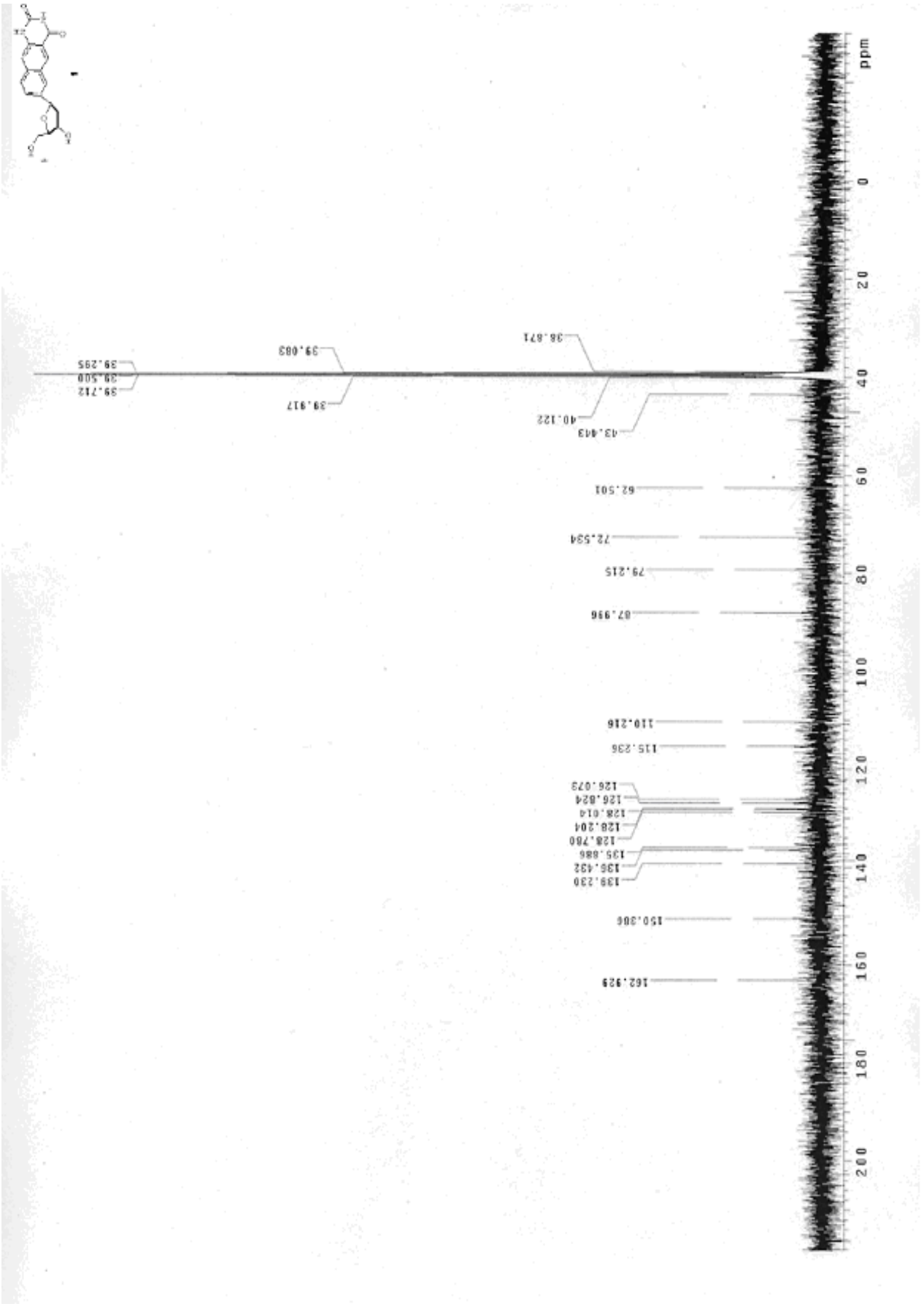

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 51.4 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
8 repetitions
OBSERVE H1, 400.1115379 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 41 sec
TBDPS
9
ppm

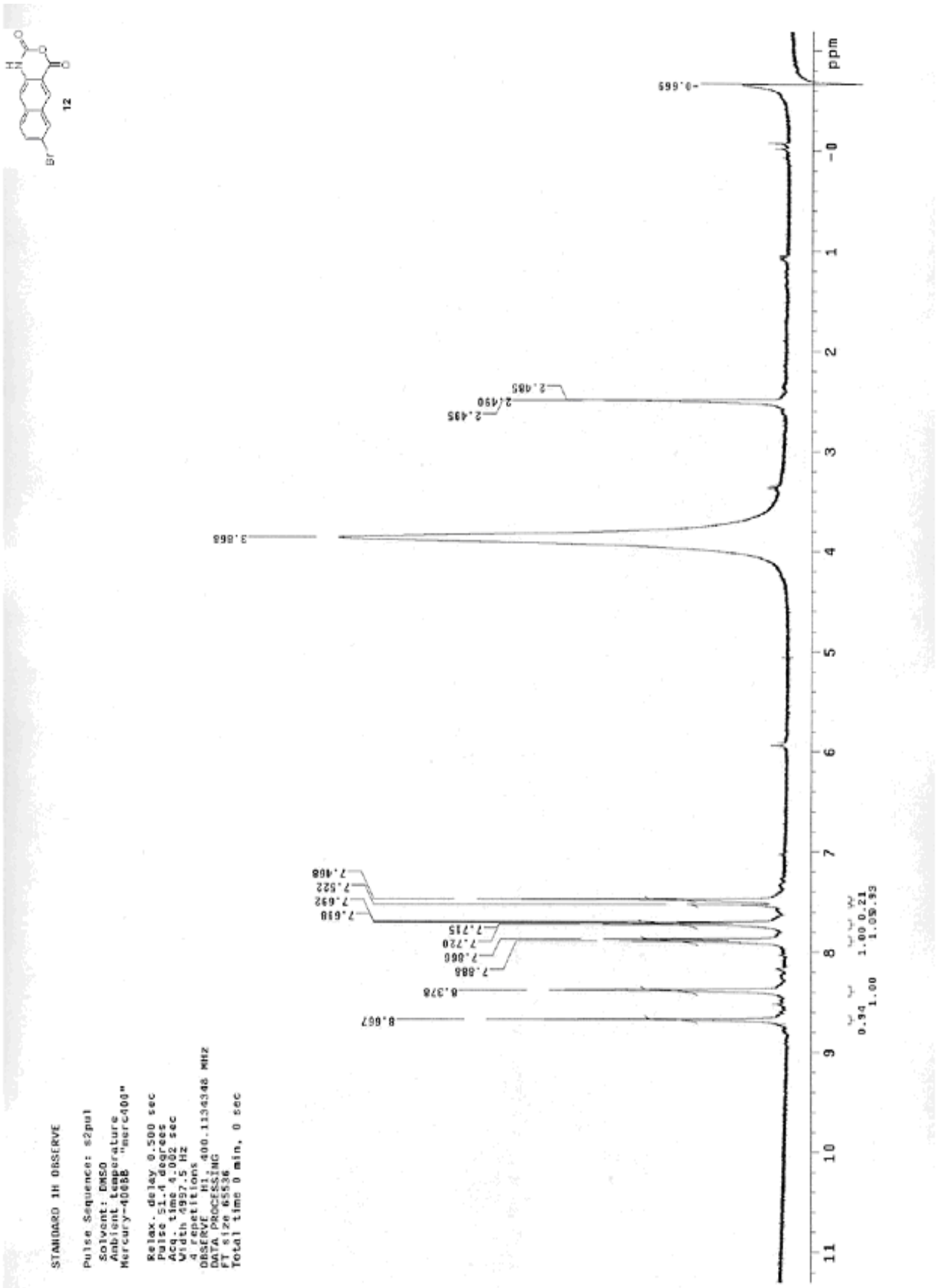

9
TBDPS
HO
OCH3
OCH3
13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.8 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
400 repetitions
OBSERVE C13, 100.0803349 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 0 min, 0 sec
19.183
26.327
54.682
54.720
62.766
76.681
77.000
77.319
83.378
84.773
102.488
113.515
122.258
124.806
127.294
127.665
127.901
127.992
129.705
130.198
130.282
131.100
131.374
135.803
135.840
136.531
138.699
146.829
151.098
161.435
169.090
ppm

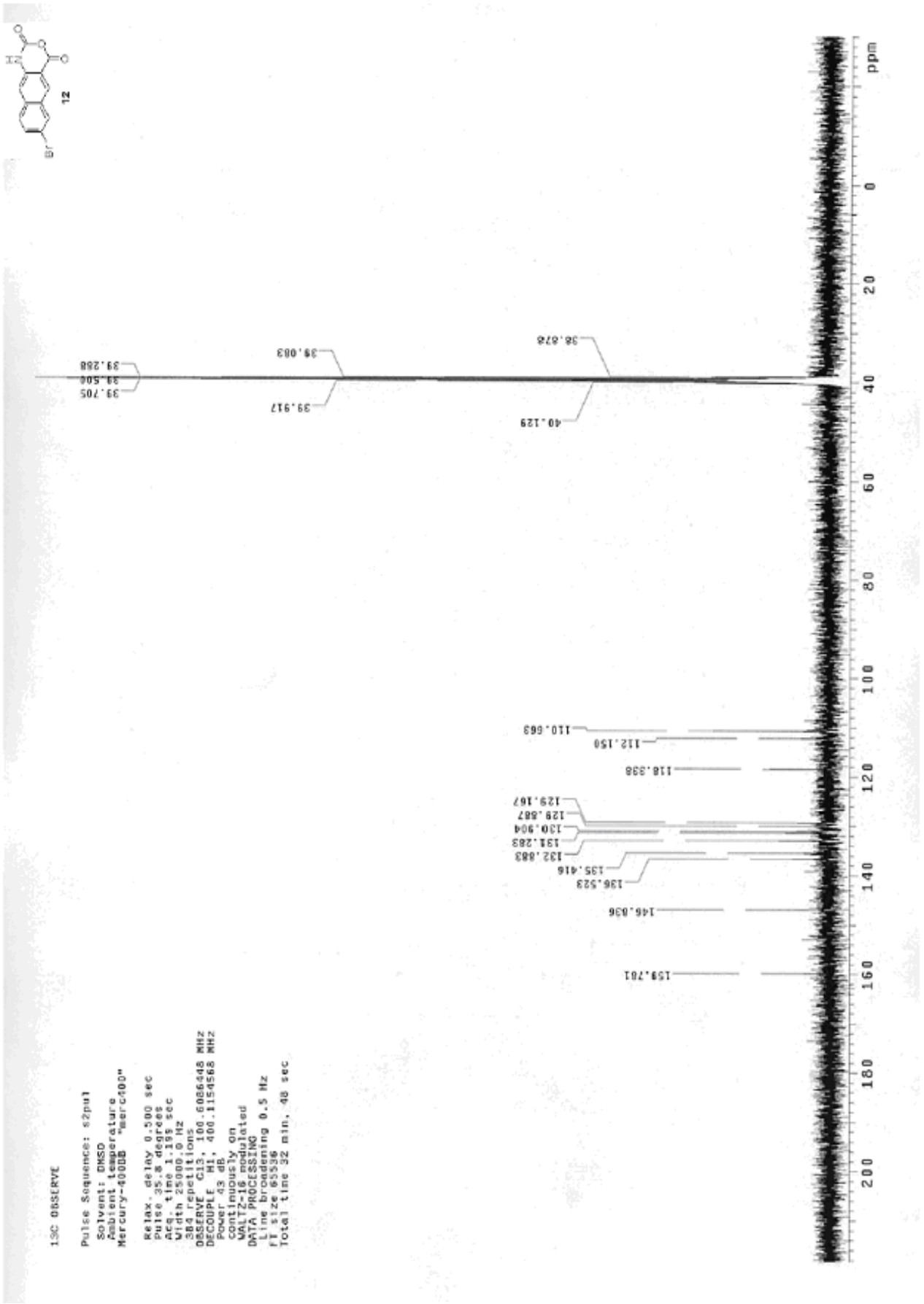

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CD3OD
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 51.4 degrees
Acq. time 4.002 sec
Width 4937.5 Hz
8 repetitions
OBSERVE H1, 400.1131268 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
11
ppm

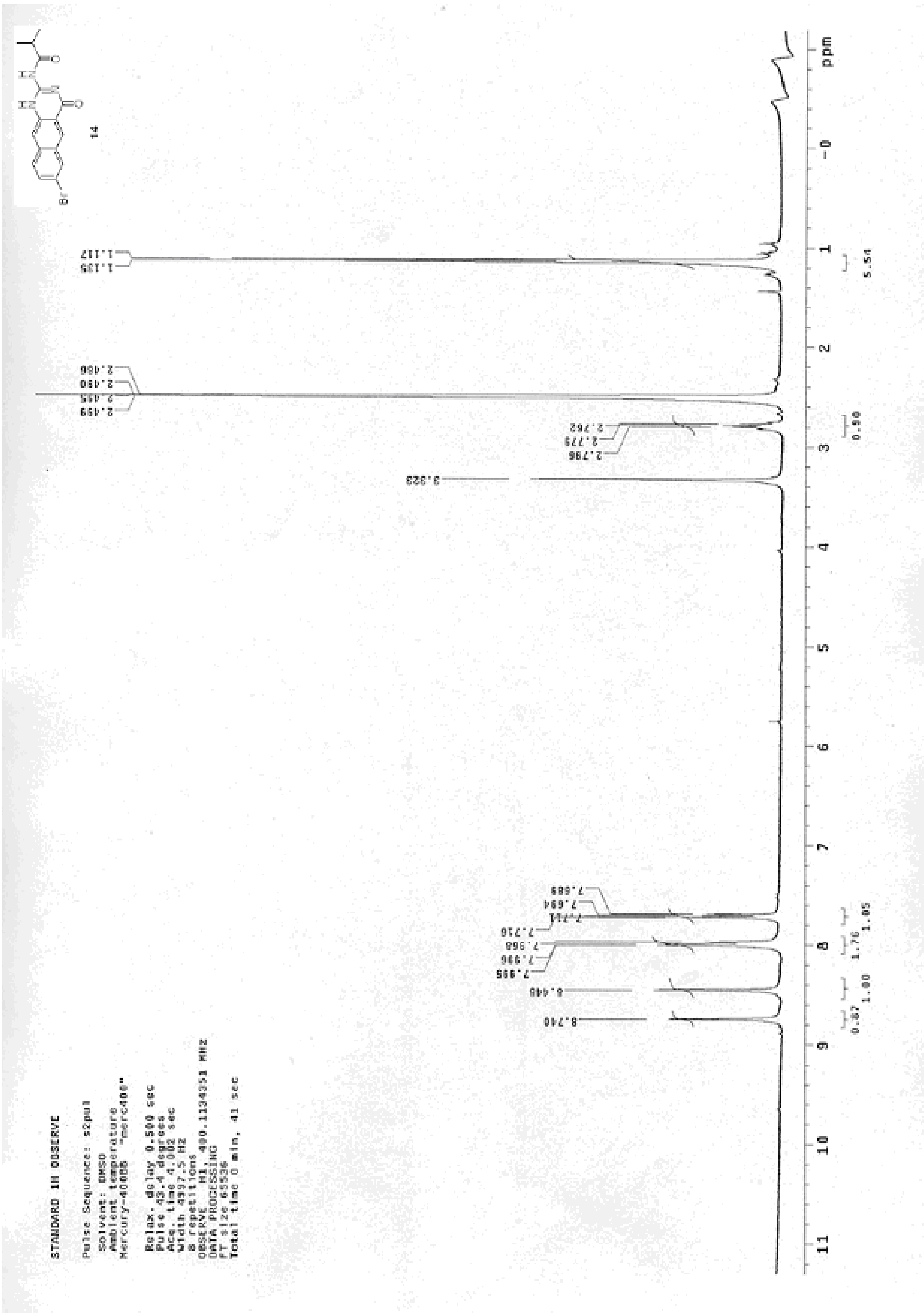

11
13C OBSERVE
Pulse Sequence: s2pul
Solvent: CD3OD
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 55.8 degrees
Acq. time 1.195 sec
Width 25000.0 Hz
4116 repetitions
OBSERVE C13, 100.6083815 MHz
DECOUPLE H1, 400.1151327 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 16 min, 26 sec
ppm
0
20
40
60
80
100
120
140
160
180
200
44.571
48.393
48.575
48.788
49.000
49.212
49.425
49.637
55.203
55.370
64.106
74.458
81.397
89.321
114.006
122.636
125.418
126.647
127.997
128.748
131.099
137.613
140.267
147.409
162.599
171.117

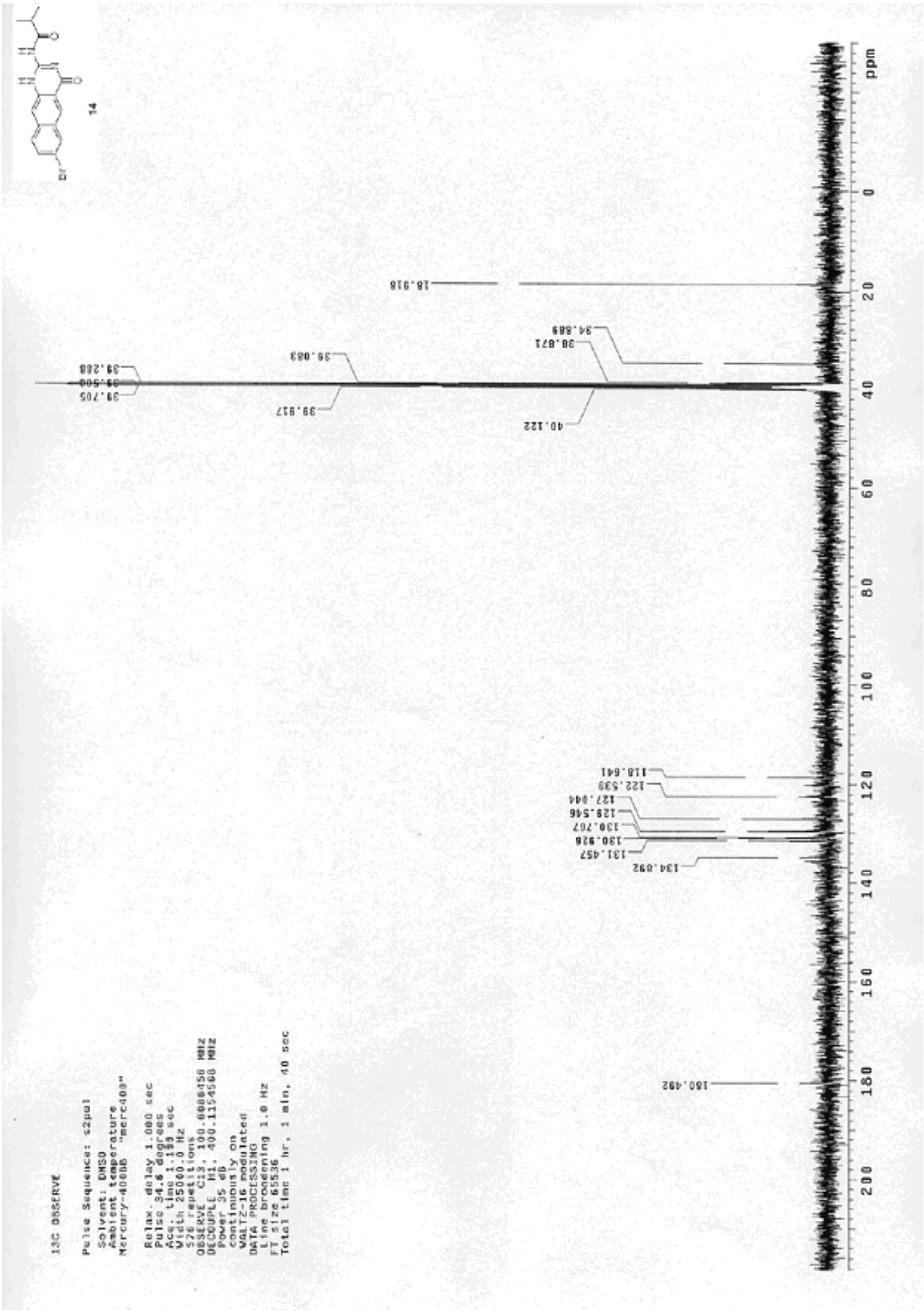

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CD3OD
Ambient temperature
Mercury-400BB "mere400"
Relax. delay 0.500 sec
Pulse 57.1 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
8 repetitions
OBSERVE H1, 400.1131116 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 41 sec
ppm

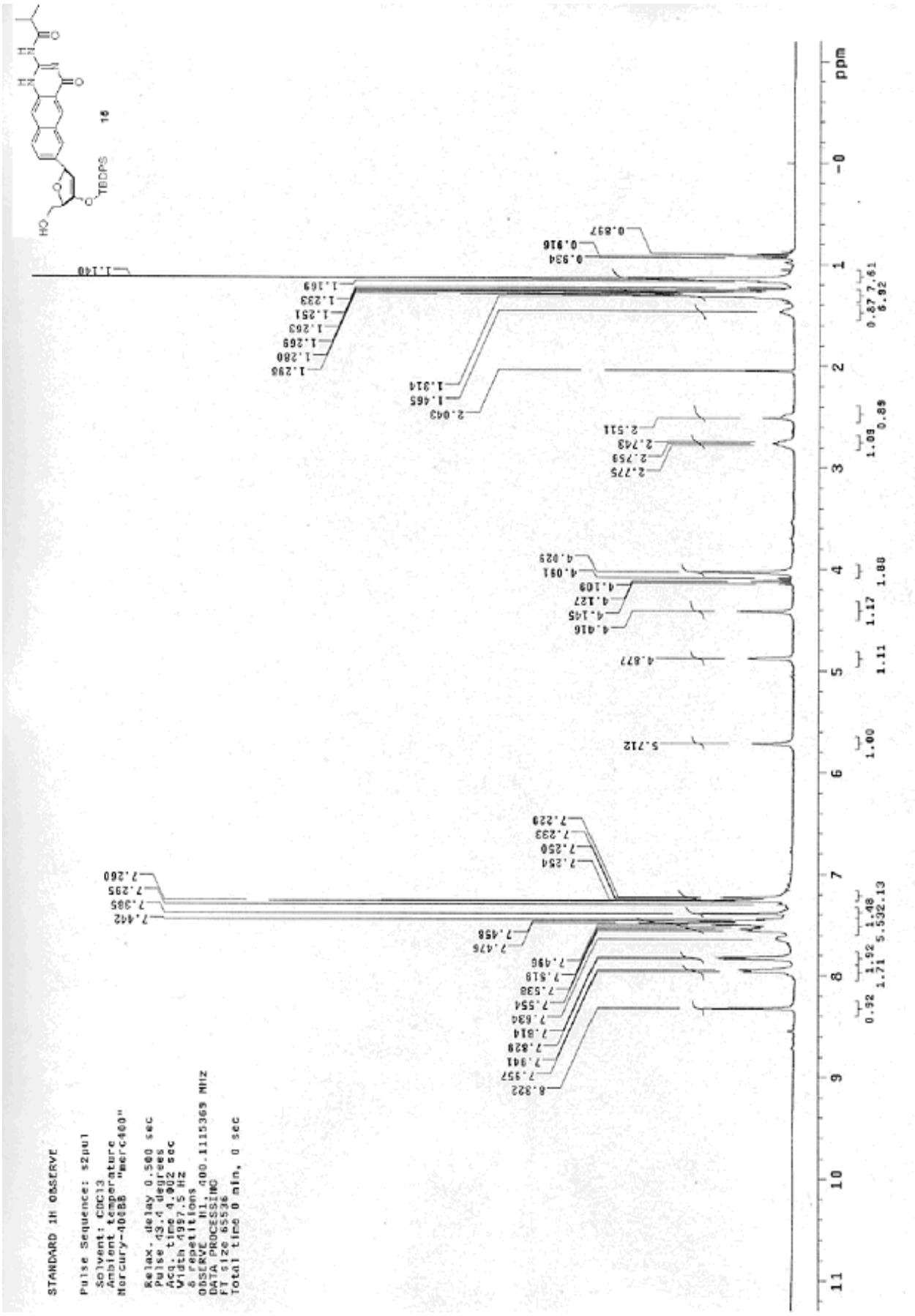

ppm
0
20
40
60
80
100
120
140
160
180
200
39.712
39.500
39.295
39.083
38.871
39.917
40.122
43.443
62.501
72.594
79.215
87.996
110.216
115.236
126.079
126.824
128.014
128.204
128.760
135.886
136.432
139.230
150.306
162.929

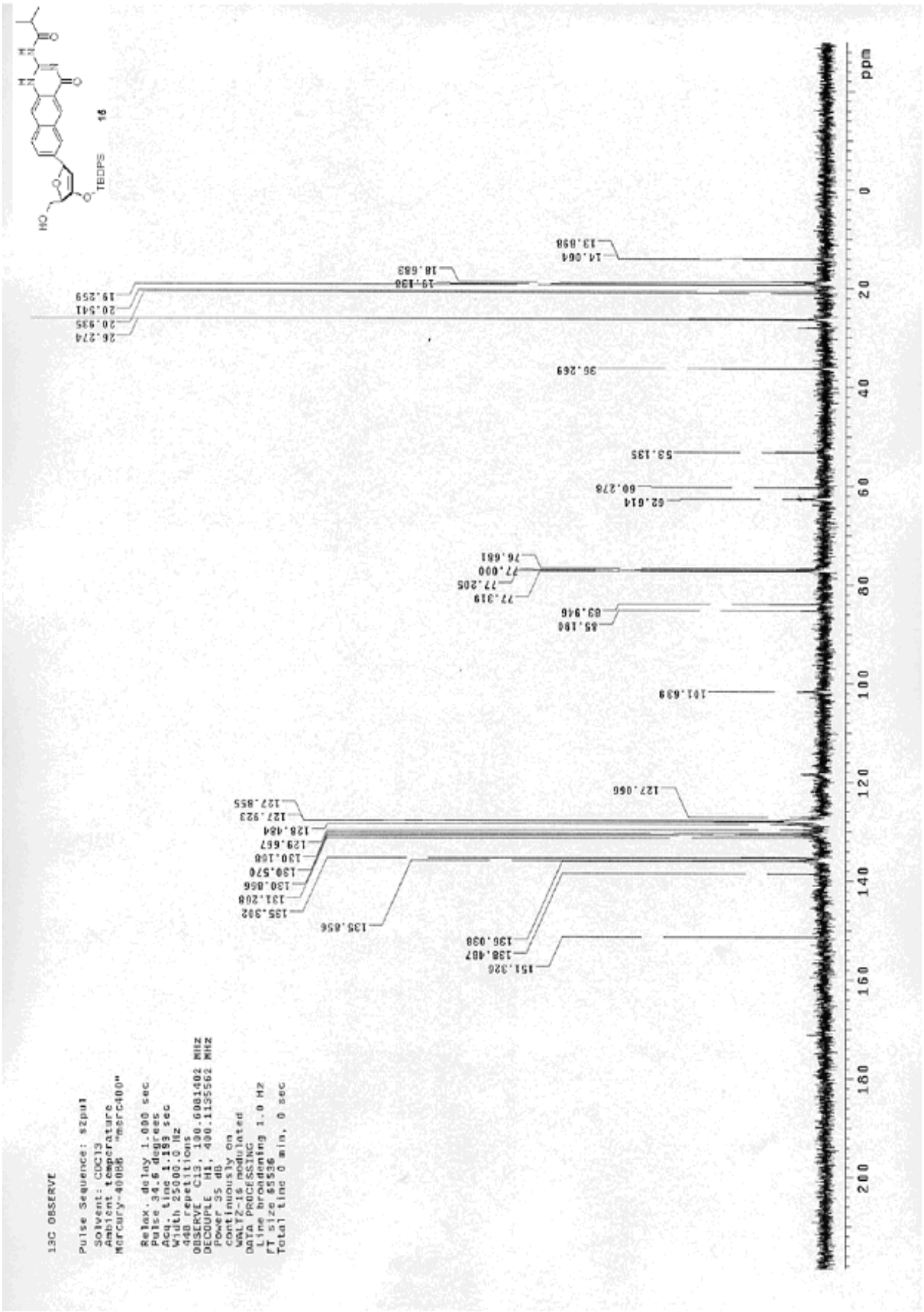

12
Br
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 51.4 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
4 repetitions
OBSERVE H1, 400.1134348 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
8.667
8.378
7.886
7.866
7.720
7.715
7.638
7.692
7.522
7.408
3.868
2.495
2.490
2.485
-0.669
ppm
0.94
1.00
1.00
0.21
1.09.93

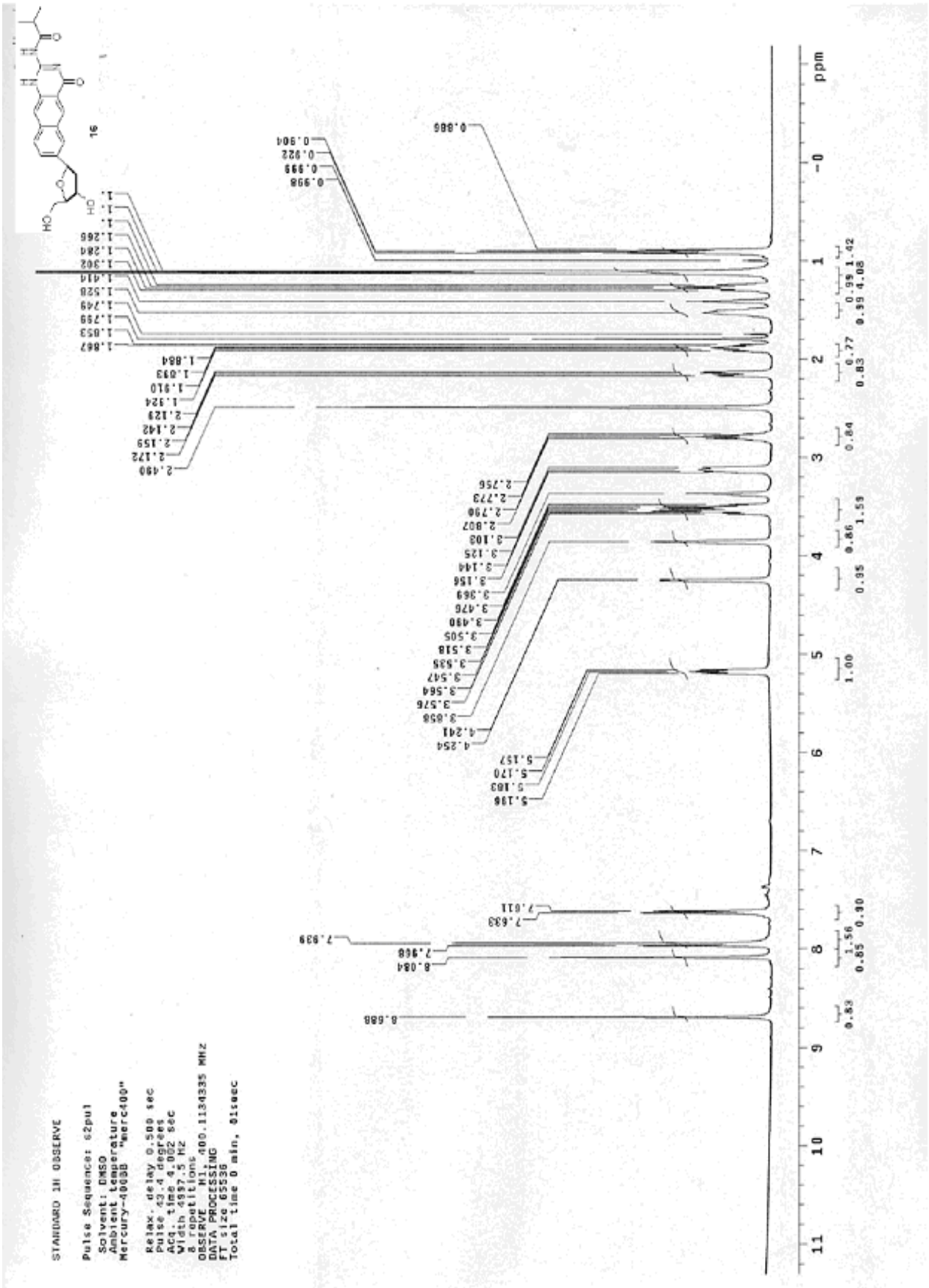

12
Br
ppm
0
20
40
60
80
100
120
140
160
180
200
39.288
39.506
39.705
39.083
39.917
36.678
40.129
110.663
112.150
118.338
129.187
129.887
130.904
131.283
132.883
135.416
136.523
146.036
159.761
13C OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.8 degrees
Acq. time 1.185 sec
Width 25000.0 Hz
384 repetitions
OBSERVE C13, 100.6086448 MHz
DECOUPLE H1, 400.1154568 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 32 min, 48 sec

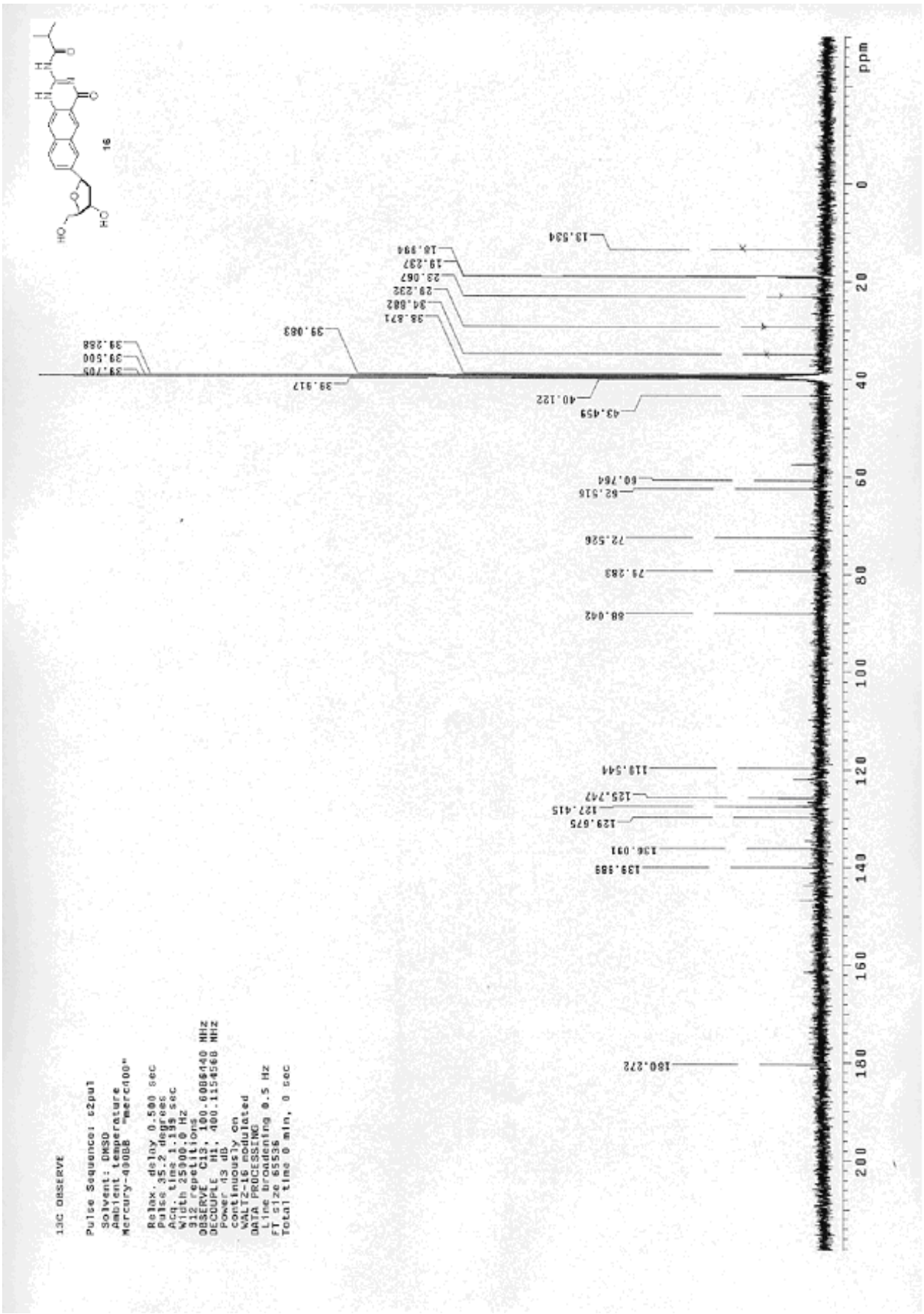

14
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 43.4 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
8 repetitions
OBSERVE H1, 400.1134351 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 41 sec
ppm

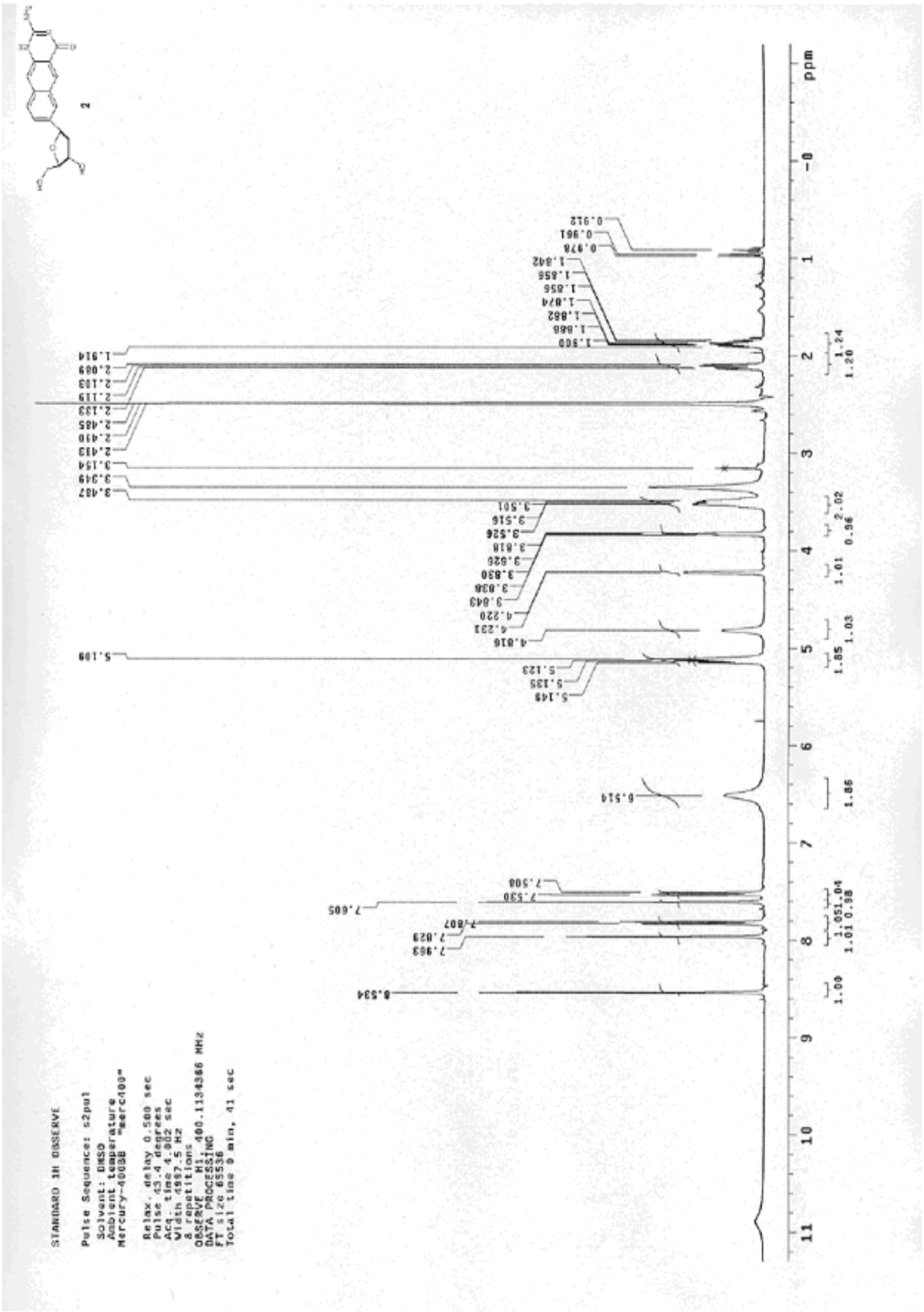

14
Br
18.918
34.889
38.871
39.083
39.705
39.500
39.288
39.917
40.122
118.641
122.538
127.044
128.546
130.767
130.926
131.457
134.092
180.492
ppm
0
20
40
60
80
100
120
140
160
180
200

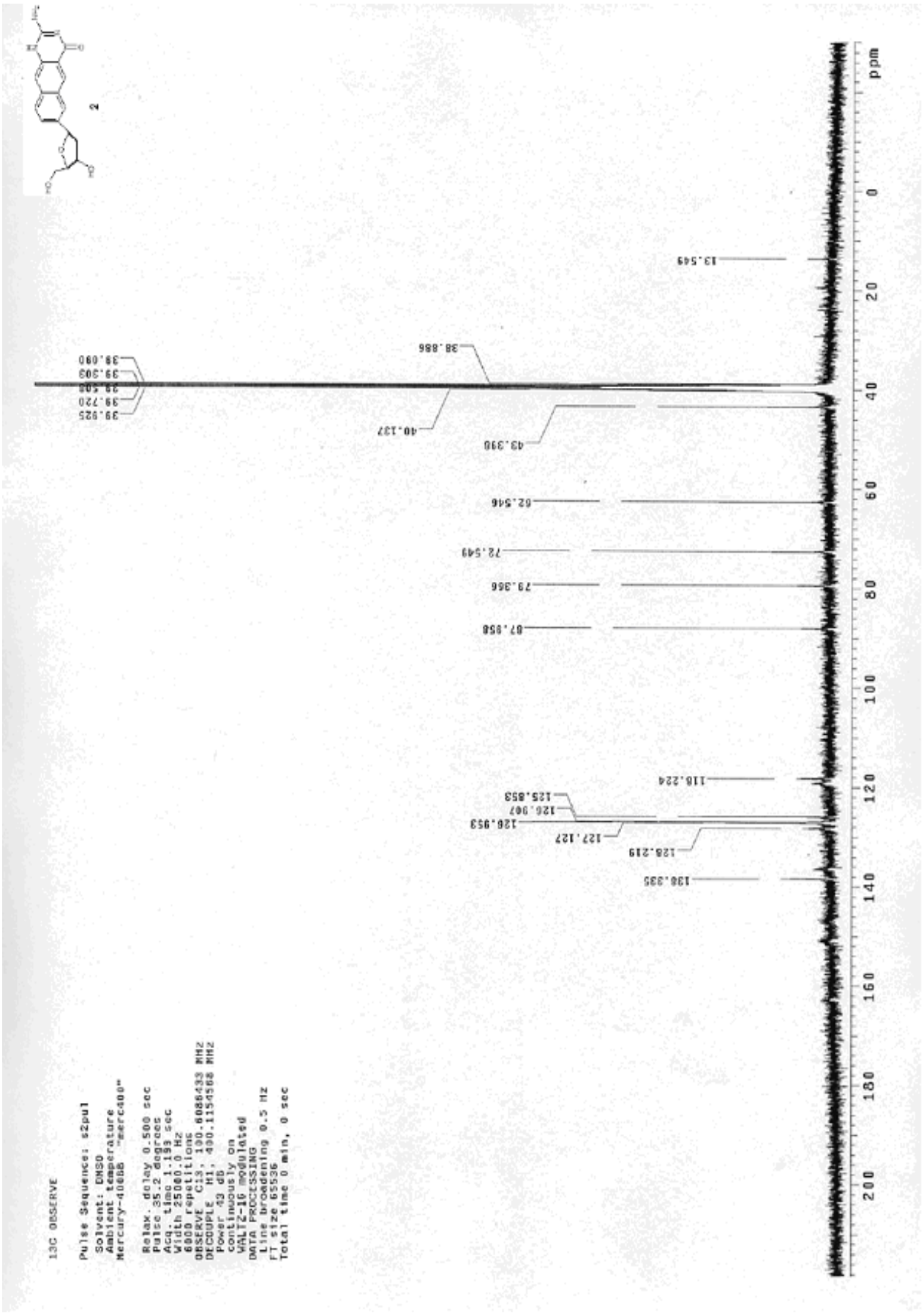

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 43.4 degrees
Acq. time 4.902 sec
Width 4997.5 Hz
8 repetitions
OBSERVE H1, 400.1115369 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
TBDPS
16
ppm

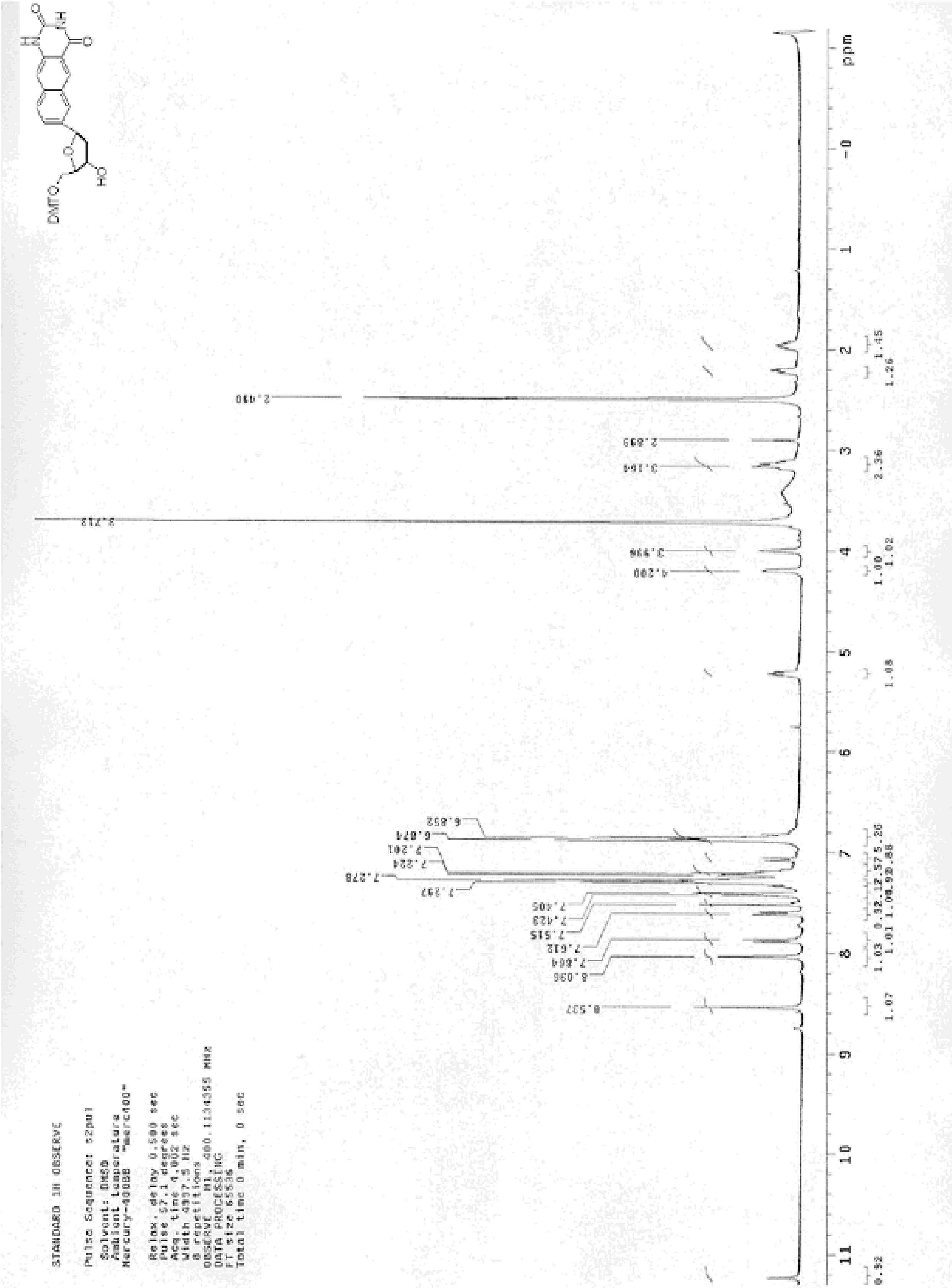

13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 1.000 sec
Pulse 34.6 degrees
Acq. time 1.193 sec
Width 25000.0 Hz
448 repetitions
OBSERVE C13, 100.6081402 MHz
DECOUPLE H1, 400.1135562 MHz
Power 35 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 0 min, 0 sec
TBDPS
16
ppm
13.898
14.064
18.683
19.136
19.259
20.341
20.935
26.274
36.269
53.135
60.278
62.614
76.681
77.000
77.205
77.319
83.946
85.184
101.639
127.050
127.855
127.923
128.484
129.667
130.166
130.570
130.856
131.208
135.302
135.856
136.038
136.407
151.326

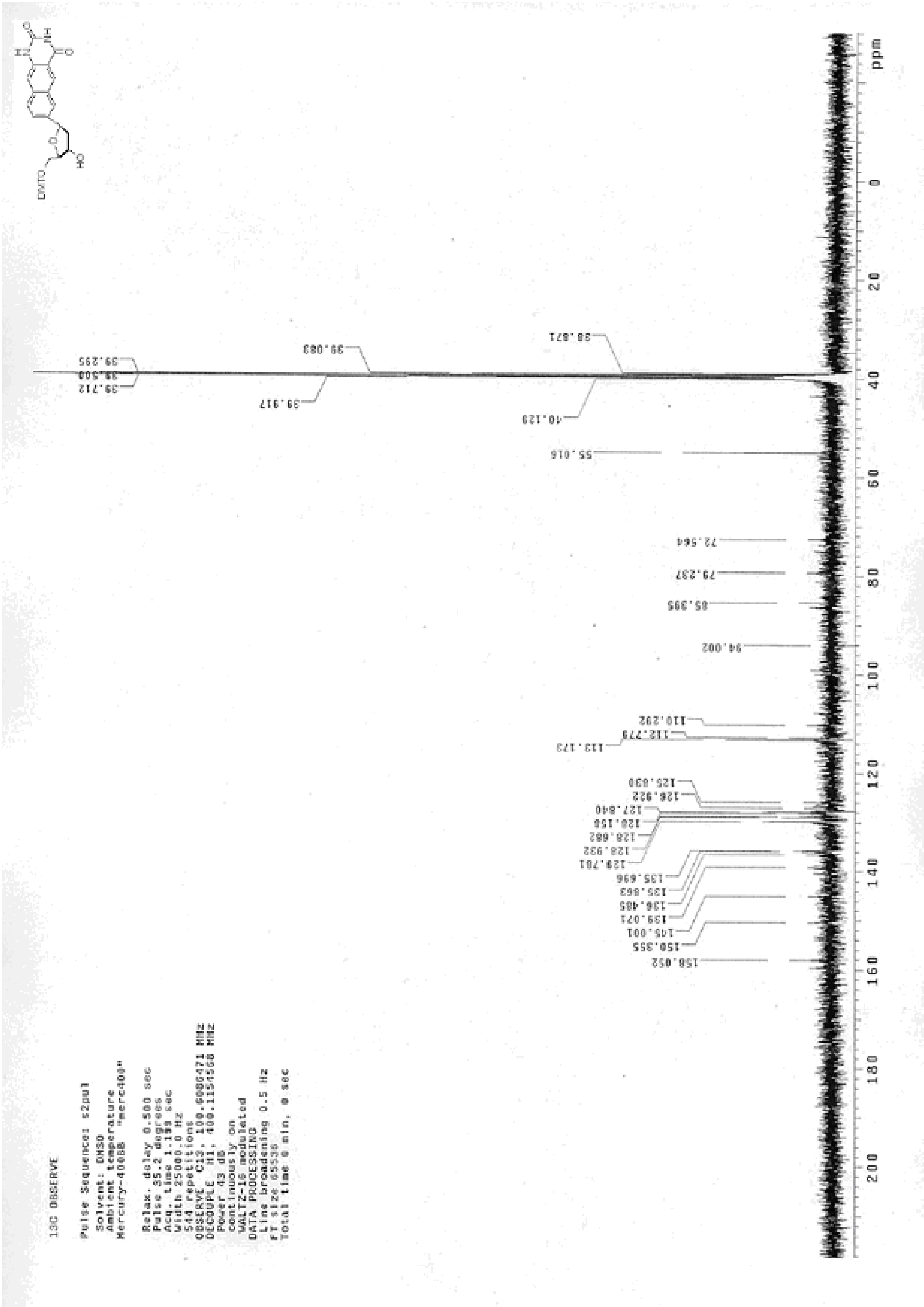

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 43.4 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
8 repetitions
OBSERVE H1, 400.1184335 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0sec
ppm

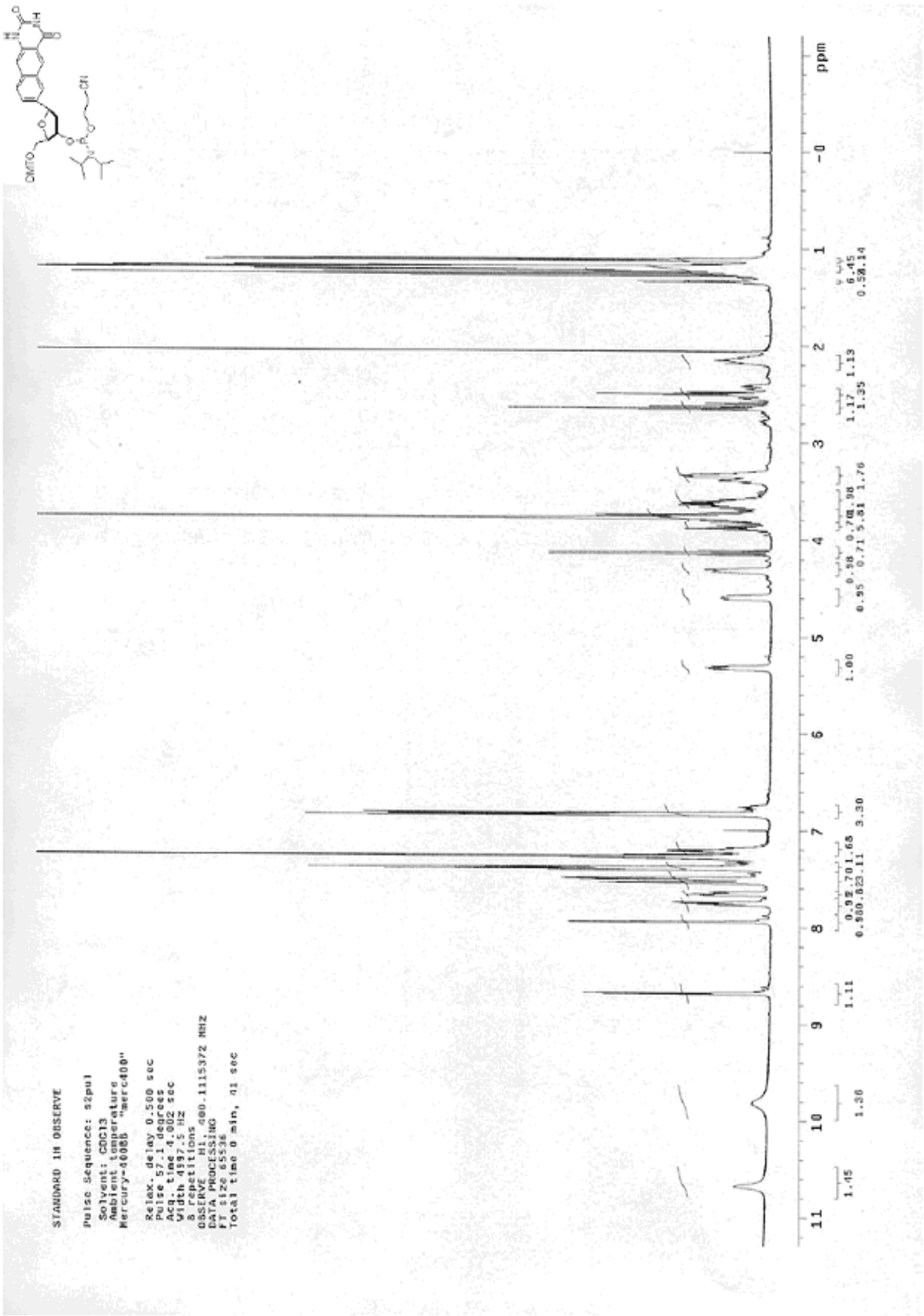

16
13C OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.2 degrees
Acq. time 1.199 sec
Width 25900.0 Hz
312 repetitions
OBSERVE C13, 100.6086140 MHz
DECOUPLE H1, 400.1154568 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 0 min, 0 sec
13.534
18.994
19.237
23.067
29.232
34.882
38.871
39.083
39.258
39.500
39.705
39.917
40.122
43.459
60.764
62.516
72.526
79.283
88.042
118.544
125.747
127.415
129.675
136.093
139.389
180.272
ppm

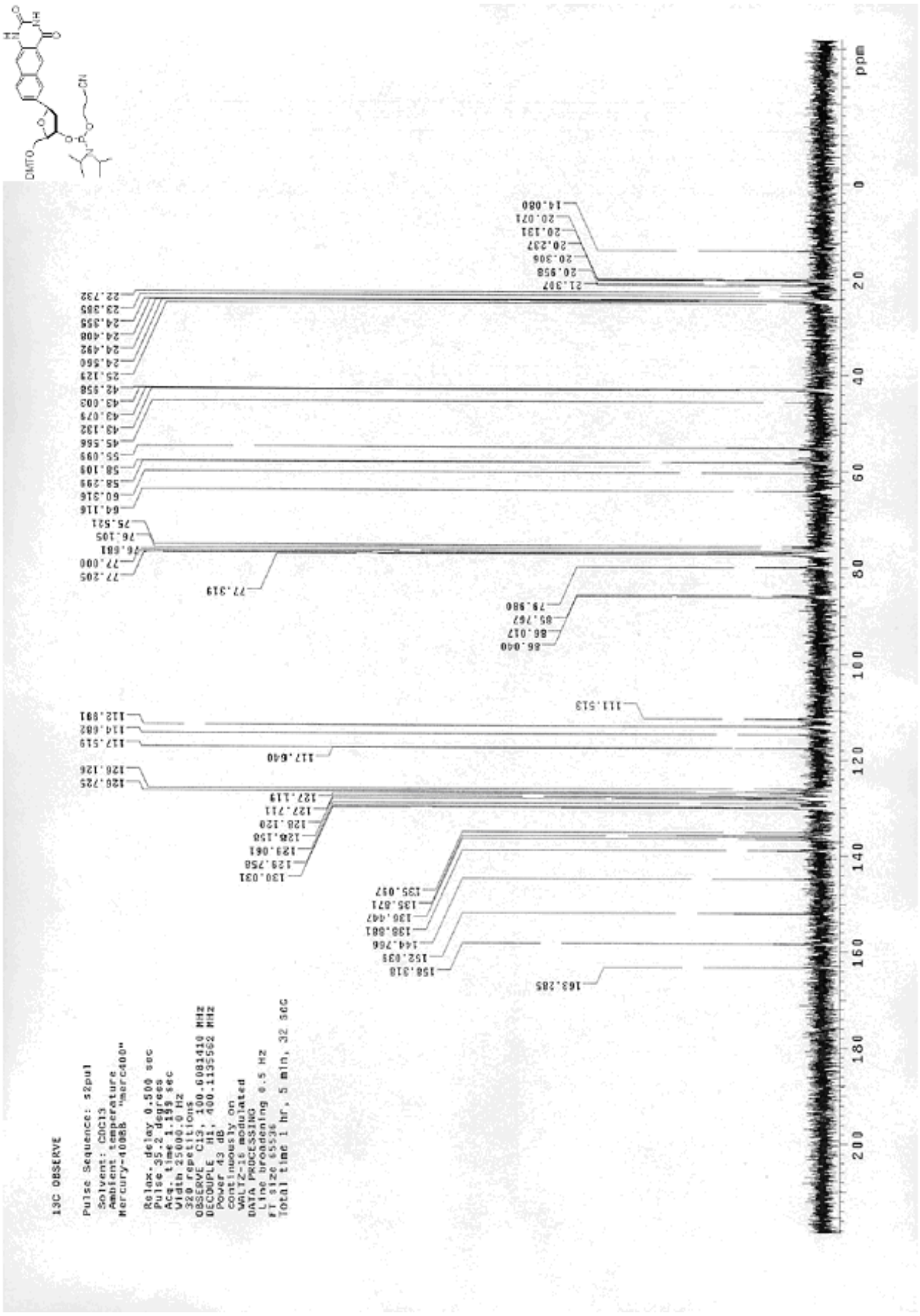

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 43.4 degrees
Acq. time 4.802 sec
Width 4997.5 Hz
8 repetitions
OBSERVE H1, 400.1134866 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 41 sec
2
ppm
8.534
7.605
7.963
7.829
7.807
7.530
7.508
6.514
5.189
5.149
5.135
5.123
4.616
4.231
4.220
3.843
3.838
3.830
3.825
3.818
3.526
3.516
3.501
3.487
3.319
3.154
2.433
2.430
2.425
2.133
2.119
2.133
2.089
1.914
1.900
1.888
1.862
1.874
1.856
1.856
1.842
0.978
0.961
0.912

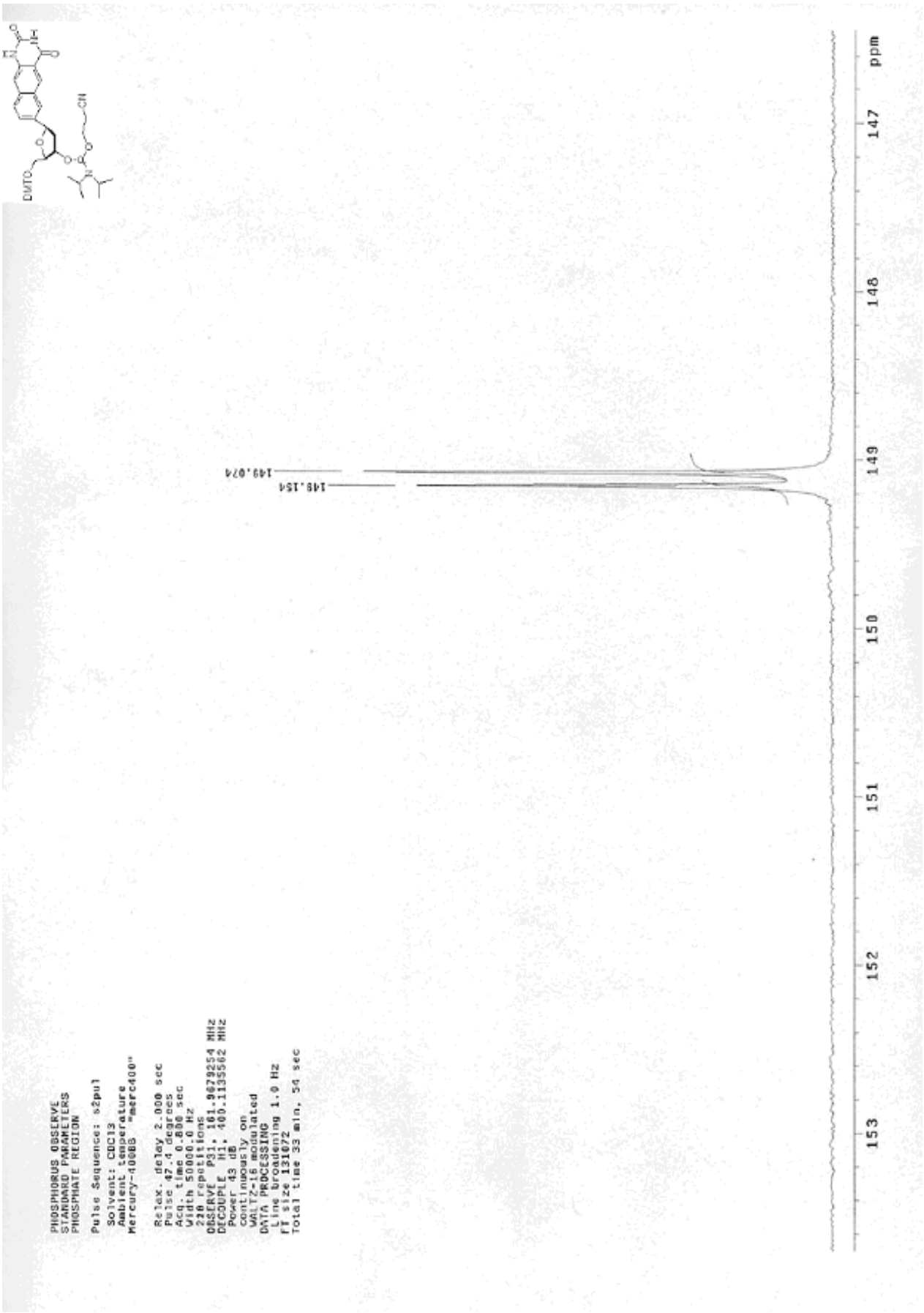

2
13.549
38.886
40.137
43.390
62.546
72.549
73.366
87.858
110.224
125.853
126.907
126.953
127.127
128.219
136.335
39.925
39.720
39.509
39.080
ppm
0
20
40
60
80
100
120
140
160
180
200
13C OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.2 degrees
Acq. time 1.193 sec
Width 25000.0 Hz
6000 repetitions
OBSERVE C13, 100.6086433 MHz
DECOUPLE H1, 400.1154568 MHz
Power 53 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 0 min, 0 sec

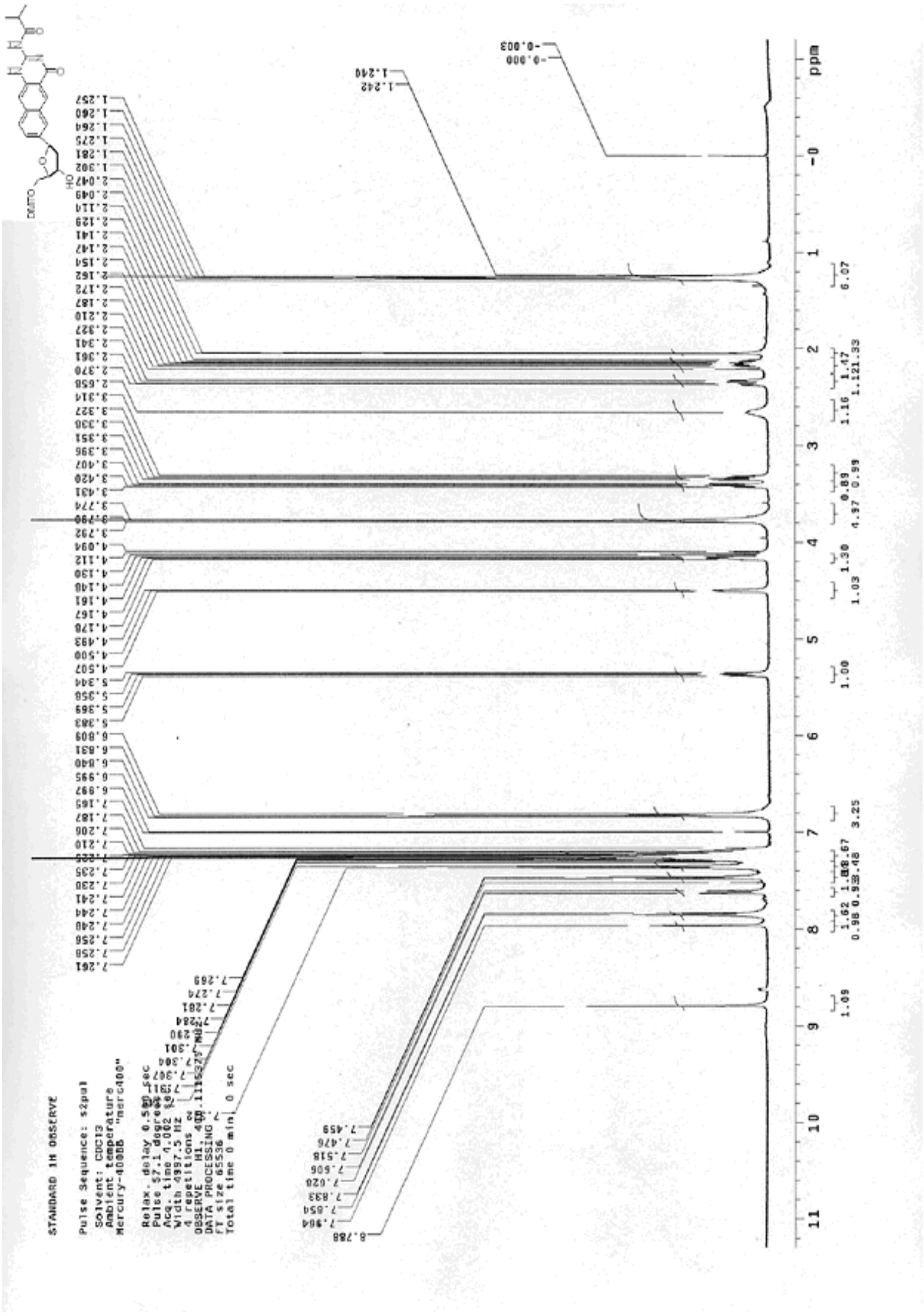

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
ppm
DMTO
HO
NH

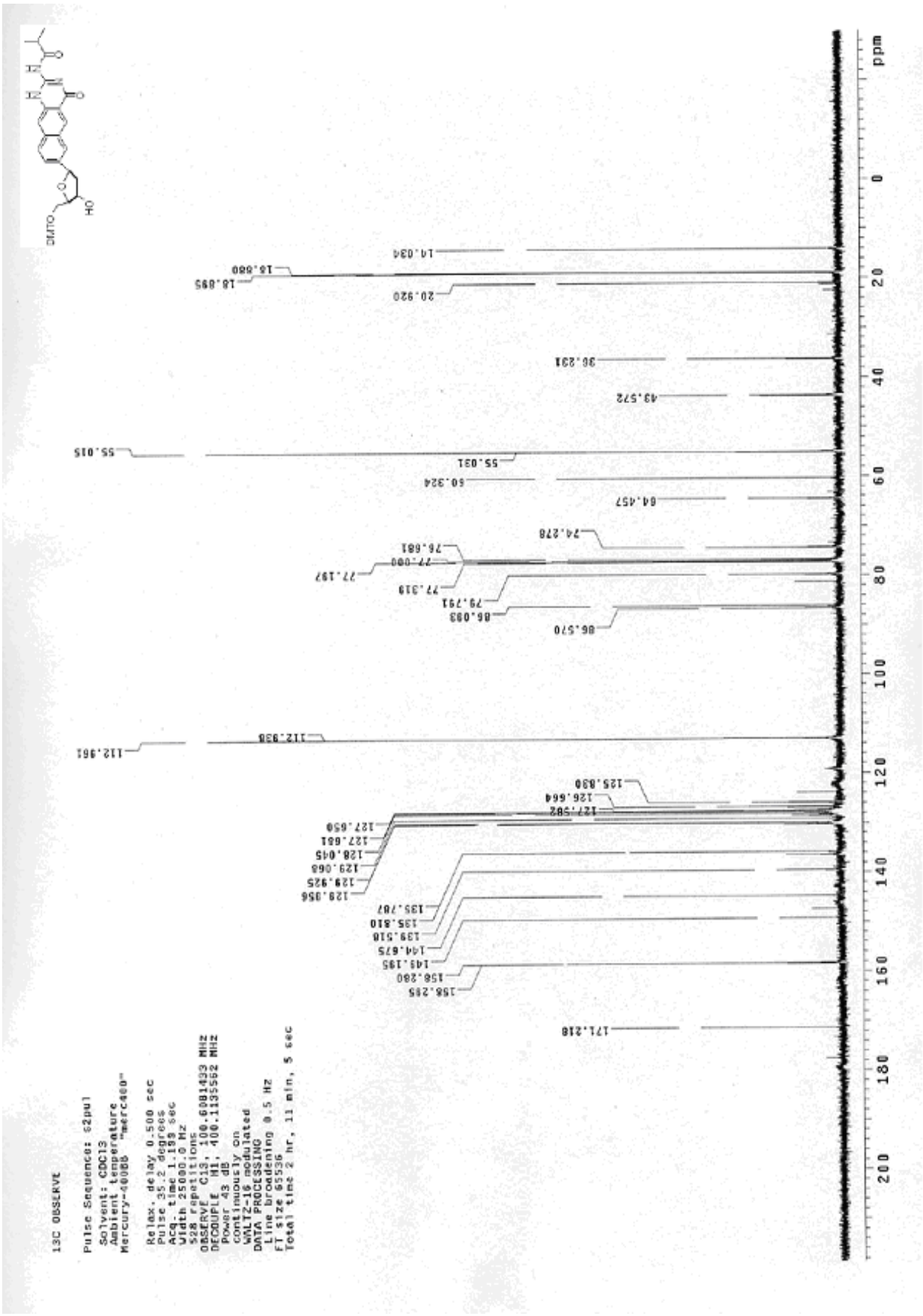

13C OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.2 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
544 repetitions
OBSERVE C13, 100.6086471 MHz
DECOUPLE H1, 400.1154560 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 6 min, 0 sec
DMTO
HO
HN
NH
O
39.295
39.500
39.712
39.083
39.917
38.871
40.129
55.016
72.564
79.237
85.395
94.002
110.292
112.779
113.173
125.830
126.922
127.840
128.150
128.682
128.932
129.701
135.696
135.863
136.485
139.071
145.001
150.355
158.052
ppm
0
20
40
60
80
100
120
140
160
180
200

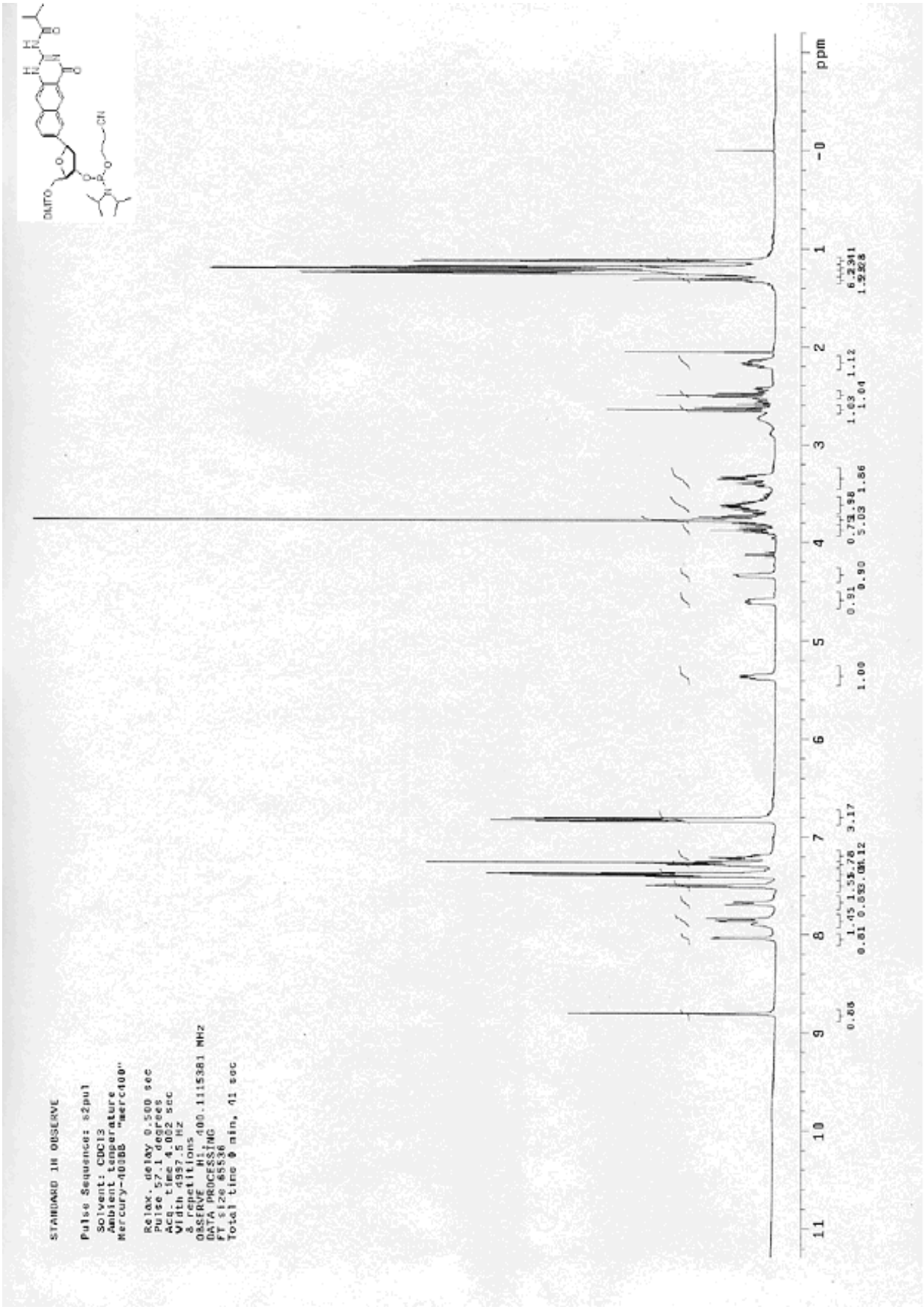

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 57.1 degrees
Acq. time 4.002 sec
Width 4597.5 Hz
8 repetitions
OBSERVE H1, 400.1115372 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 41 sec
DMTO
CN
ppm

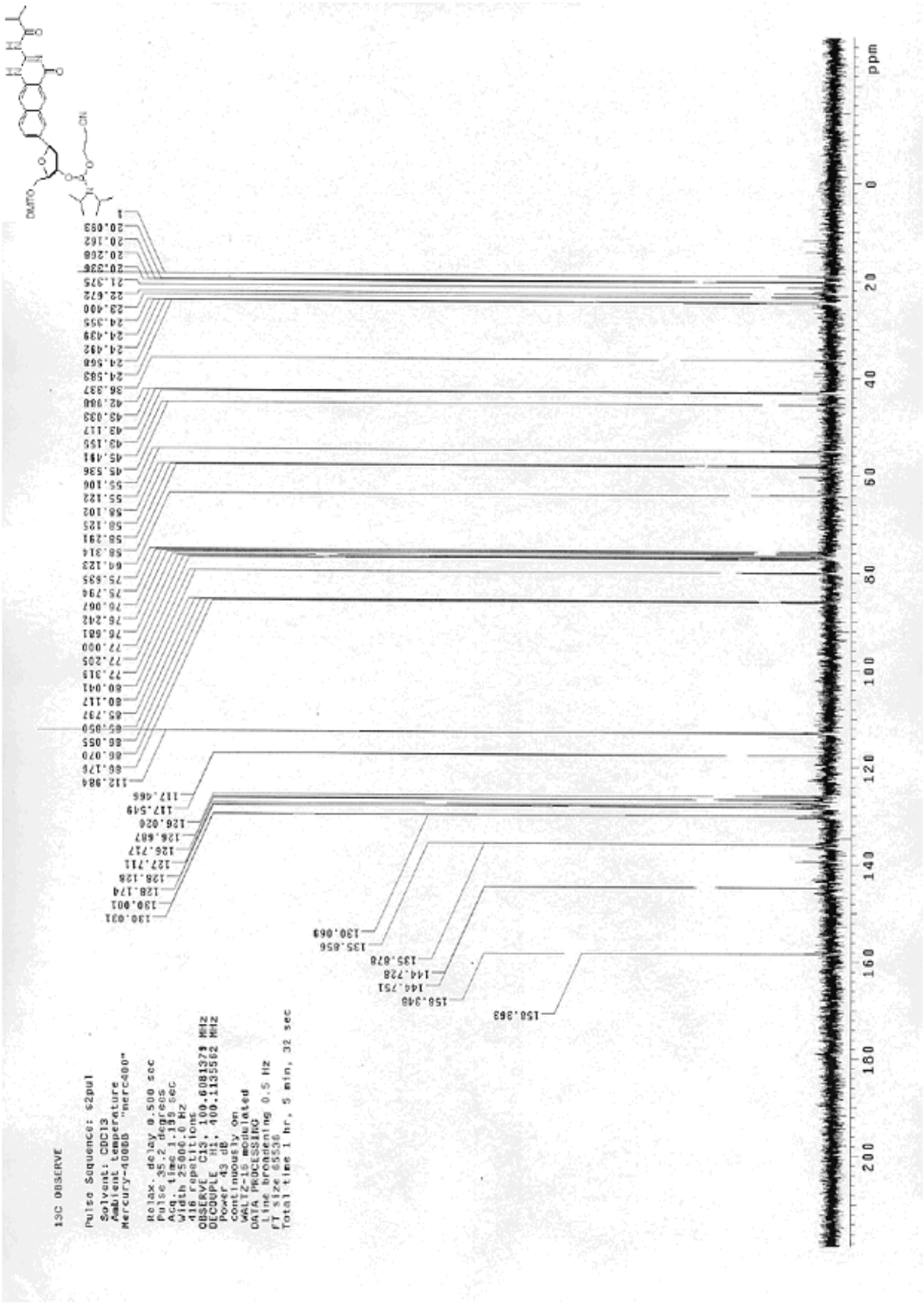

DMTO
CN
13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.2 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
320 repetitions
OBSERVE C13, 100.6084410 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 5 min, 32 sec
14.080
20.074
20.131
20.237
20.306
20.958
21.307
22.732
23.385
24.358
24.408
24.492
24.560
25.129
42.958
43.003
43.079
43.132
45.566
55.099
58.109
58.299
60.316
64.116
75.521
76.105
76.681
77.000
77.205
77.319
79.980
85.767
86.017
86.040
111.513
112.981
114.682
117.519
117.640
126.126
126.725
127.119
127.711
128.120
128.150
129.061
129.758
130.031
135.037
135.821
136.447
138.861
144.766
152.035
158.318
163.285
ppm

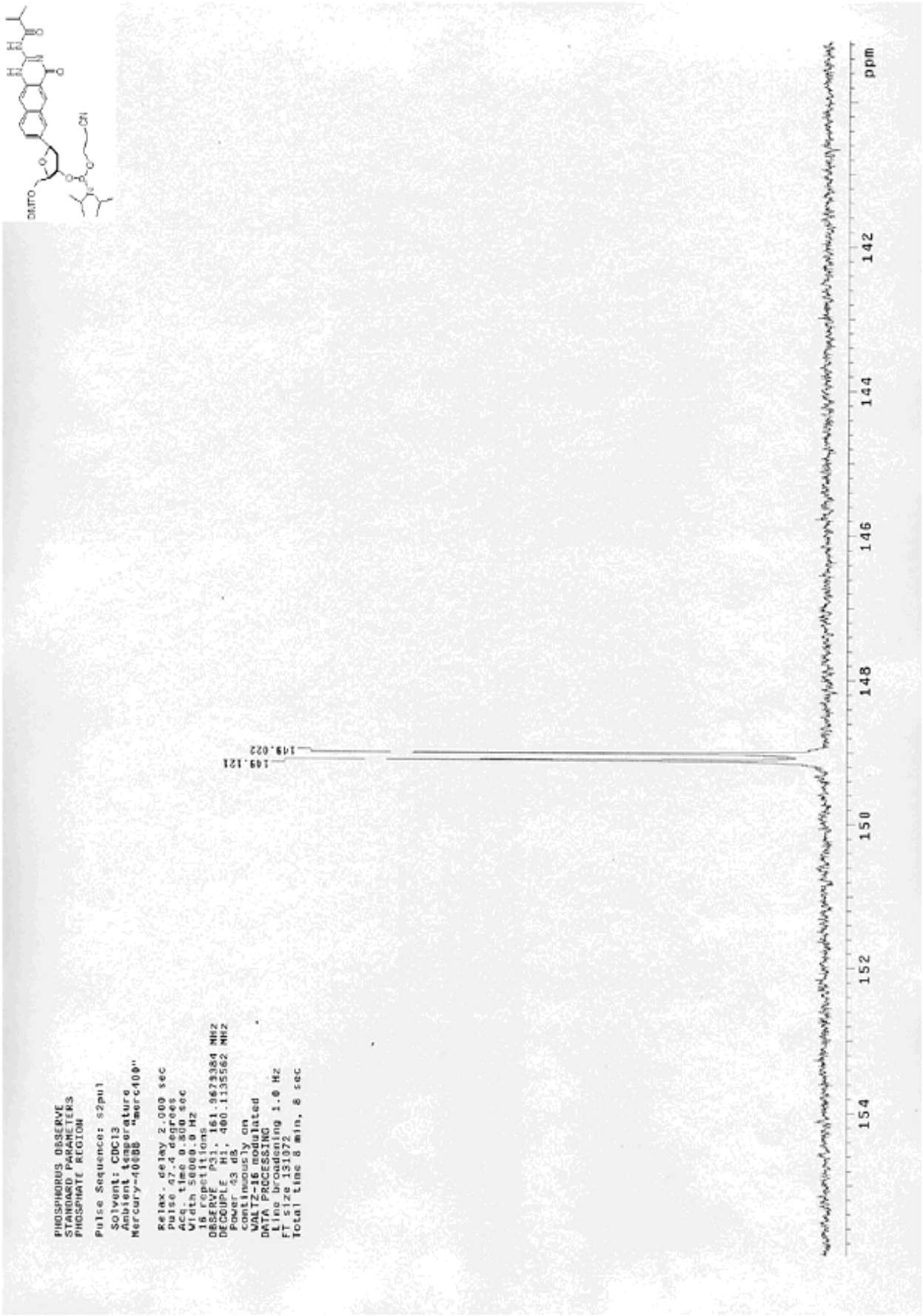

DMTO
CN
149.074
148.154
153
152
151
150
149
148
147
ppm
PHOSPHORUS OBSERVE
STANDARD PARAMETERS
PHOSPHATE REGION
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 2.000 sec
Pulse 47.4 degrees
Acq. time 0.800 sec
Width 50000.0 Hz
210 repetitions
OBSERVE P31, 161.9879254 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 33 min, 54 sec


DMTO
HO
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 57.1 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
4 repetitions
OBSERVE H1, 400.1115372 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
ppm


13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.2 degrees
Acq. time 1.188 sec
Width 25000.0 Hz
528 repetitions
OBSERVE C13, 100.6081433 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 2 hr, 11 min, 5 sec
DMTO
OH
14.034
18.680
18.895
20.920
36.231
43.572
55.015
55.031
60.324
64.457
74.278
76.681
77.000
77.192
77.319
79.791
86.083
86.570
112.961
112.938
125.830
126.664
127.582
127.650
127.681
128.045
129.060
129.925
129.956
135.787
135.810
139.518
144.675
149.195
158.280
158.285
171.218
ppm
0
20
40
60
80
100
120
140
160
180
200


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 57.1 degrees
Acq. time 4.022 sec
Width 4957.5 Hz
8 repetitions
OBSERVE H1, 400.1115381 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 41 sec
DMTO
CN
ppm


13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 35.2 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
416 repetitions
OBSERVE C13, 100.6081379 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 5 min, 32 sec
DMTO
CN
ppm
150.363
158.348
144.751
144.728
135.870
135.856
130.069
130.021
130.001
128.174
128.128
127.711
126.717
126.607
126.020
117.549
117.466
112.984
86.176
86.070
86.055
85.850
85.797
80.117
80.041
77.319
77.205
77.000
76.681
76.242
76.067
75.794
75.695
64.123
58.314
58.291
58.125
56.102
55.122
55.106
45.536
45.491
43.155
43.117
43.033
42.908
36.337
24.583
24.568
24.492
24.439
24.355
23.400
22.672
21.375
20.336
20.268
20.182
20.083
1


DMTO
CN
149.022
149.121
154
152
150
148
146
144
142
ppm
PHOSPHORUS OBSERVE
STANDARD PARAMETERS
PHOSPHATE REGION
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 2.000 sec
Pulse 47.4 degrees
Acq. time 0.500 sec
Width 50000.0 Hz
16 repetitions
OBSERVE P31, 161.9673384 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 8 min, 8 sec