

SUPPORTING INFORMATION

Multi-Path Quenchers: Efficient Quenching of Common Fluorophores

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Strand	Mass Calc	Mass Observed	Purity (HPLC)
Alexa350 20mer	6543.110	6544.614	98.86%
FAM 20mer	6678.201	6678.831	99.55%
Cy3 20mer	6618.312	6617.014	99.86%
TAMRA 20mer	6734.274	6734.328	99.30%
ATTO590 20mer	6821.371	6822.563	98.02%
Quasar670 20mer	6743.396	6742.522	99.94%
MPQ1 15mer	5156.014	5156.707	98.54%
MPQ1 20mer	6676.244	6675.056	99.52%
MPQ2 15mer	5206.029	5205.537	97.74%
MPQ2 20mer	6726.259	6713.450	98.53%
MPQ3 15mer	5200.040	5200.674	99.70%
MPQ3 20mer	6720.270	6720.037	99.03%
MPQ4 15mer	5053.971	5052.607	87.30%
MPQ4 20mer	6576.191	6576.154	99.13%
MPQ5 15mer	5233.040	5233.804	N/A*
MPQ5 20mer	6753.270	6754.076	96.04%
MPQ6 15mer	5206.041	5205.809	99.29%
MPQ6 20mer	6726.271	6724.371	99.15%
DABCYL 15mer	5021.018	5021.461	98.55%
DABCYL 20mer	6541.248	6541.436	98.19%
BHQ2 15mer	5130.993	5129.682	98.55%
BHQ2 20mer	6651.223	6650.192	99.18%

Table S1: MALDI-TOF calculated and observed masses of DNA sequences used.

*An insufficient amount of labelled DNA was prepared to obtain an analytical HPLC

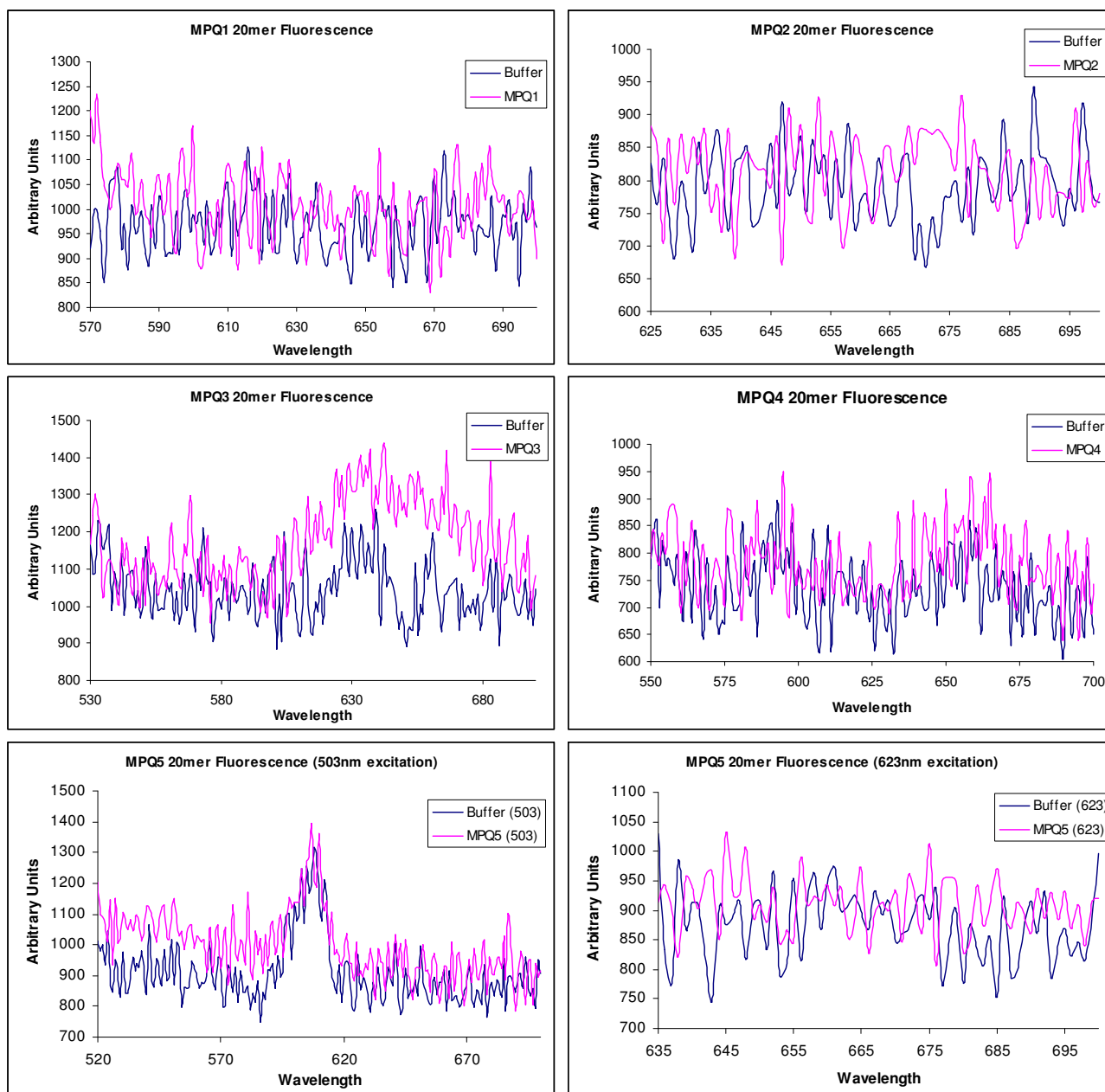


Figure S1: Fluorescence spectra of quencher 20mers (---) compared to buffer alone (---). Samples (100nM) were excited at the λ_{max} values indicated in Table XXXX.

Quencher	λ_{\max}	ϵ_{\max}	ϵ_{260}
MPQ1	512	36400	25100
MPQ2	516	19200	10300
MPQ3	517	50800	15000
MPQ4	522	22600	10800
MPQ5	617	8700	18200
MPQ6	620	28700	N.D.*

Table S2: Absorbance maxima and extinction coefficients (in $\text{L M}^{-1} \text{cm}^{-1}$) for multiple pathway quenchers in ethanol. ϵ_{260} values are provided for quenchers in 9:1 PBS:DMF

* Value could not be determined as a result of aggregation. A value of $8000 \text{ L M}^{-1} \text{cm}^{-1}$ was used in DNA concentration calculations (the value of BHQ1 at 260 nm).

Figure S2: Ground state complex formation between MPQ 20mers and 3'-Fluorescein 20mer. Duplexes were formed by annealing a solution 1 μ M of each strand in hybridization buffer at 70 $^{\circ}$ C for 5 minutes then allowing to cool to room temperature over 30 minutes. The simple sum was created by adding solutions containing only 1 μ M of either quencher or fluorophore labelled strand.

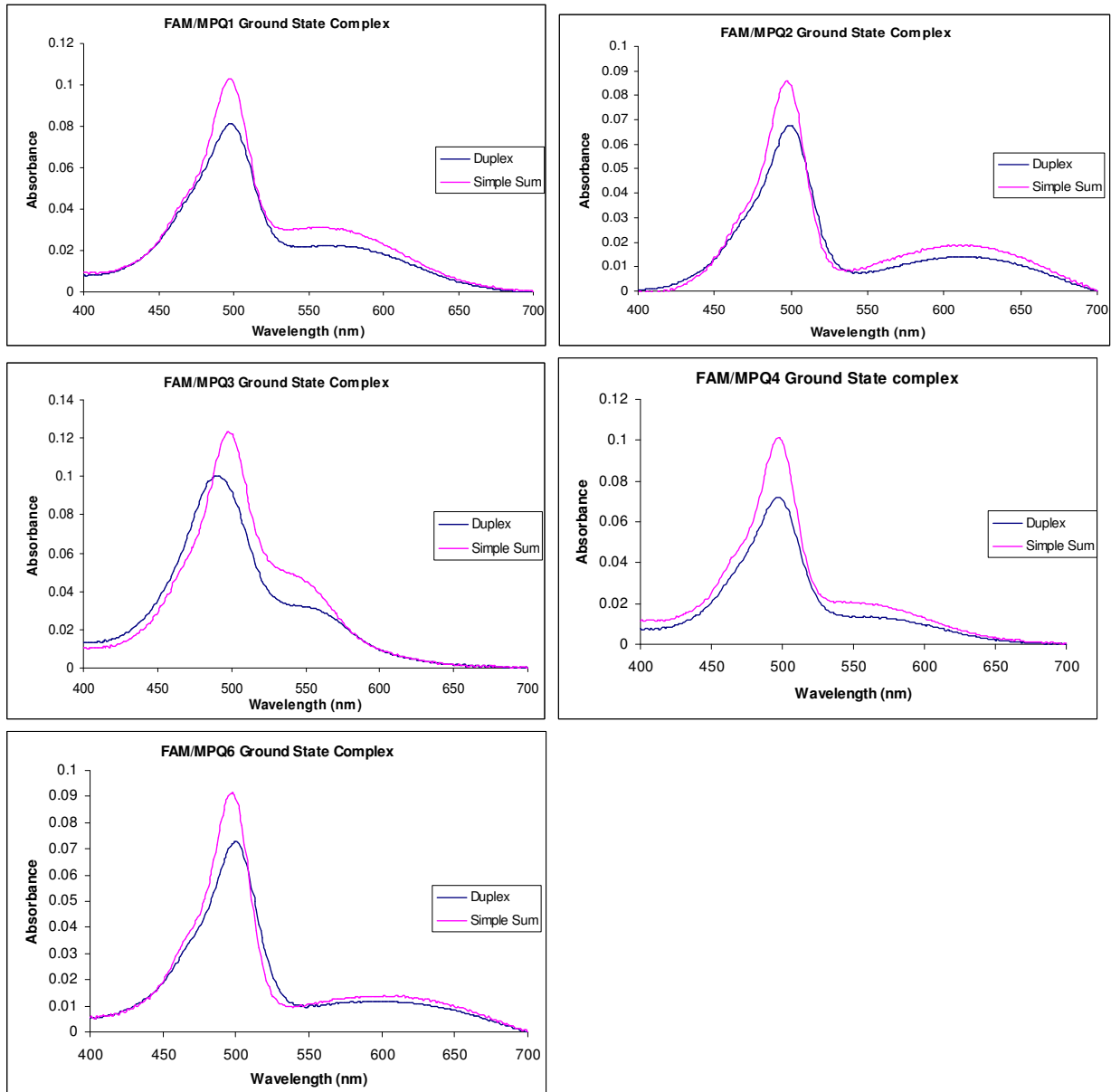
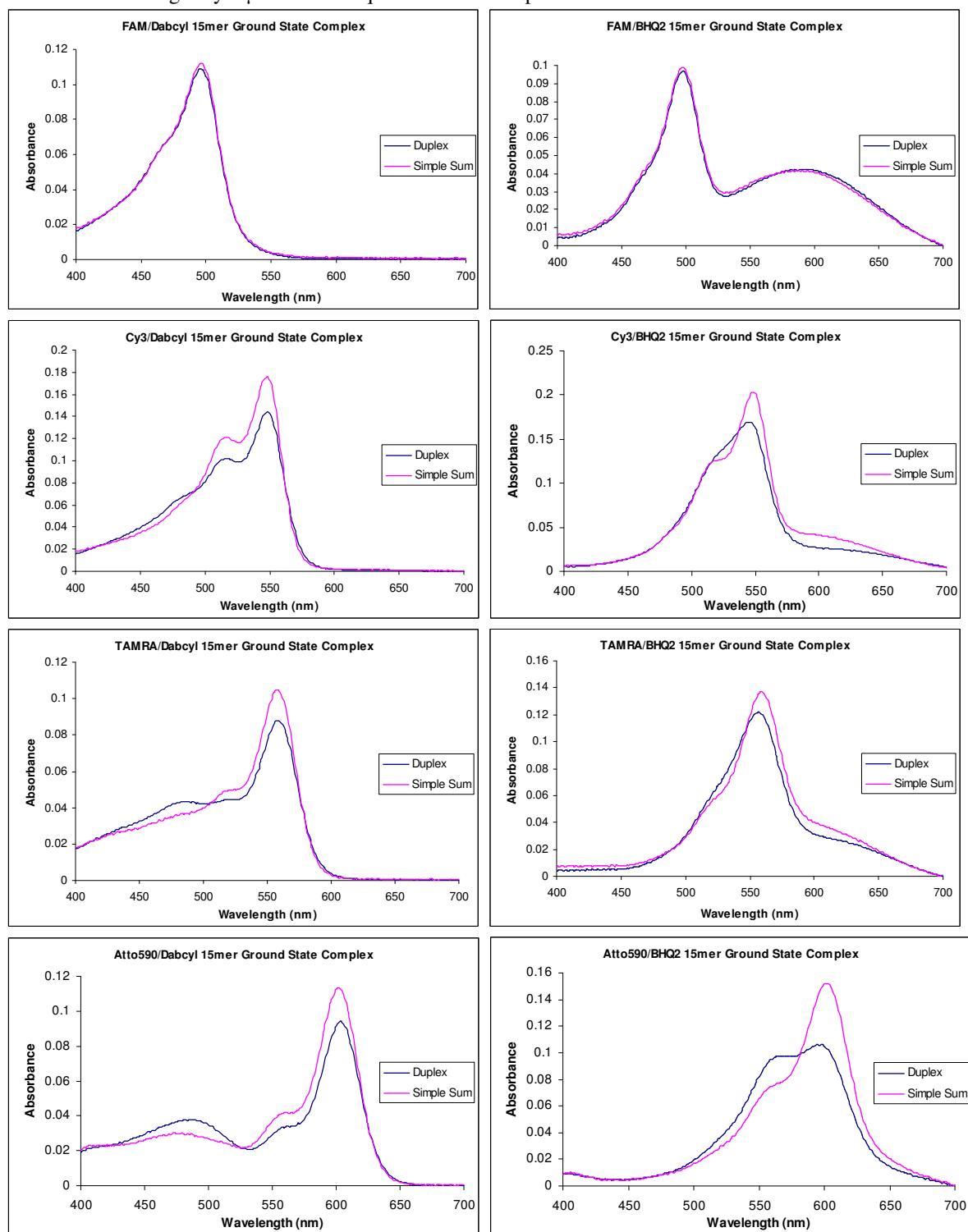
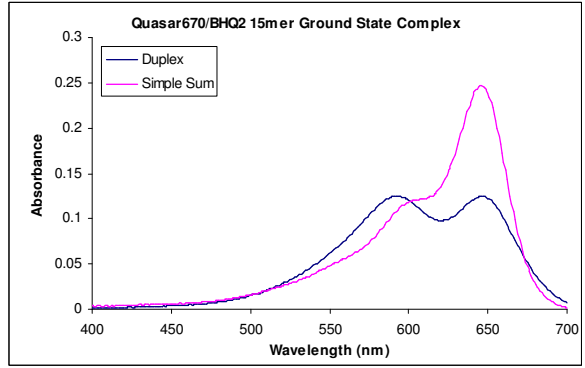
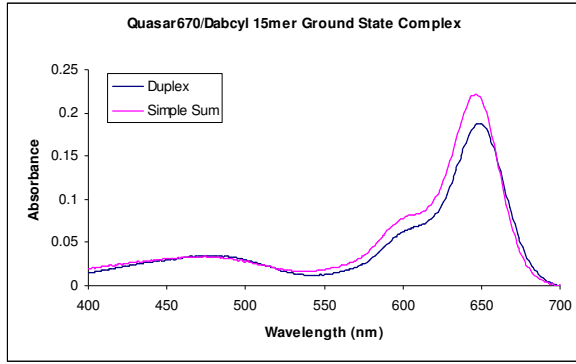
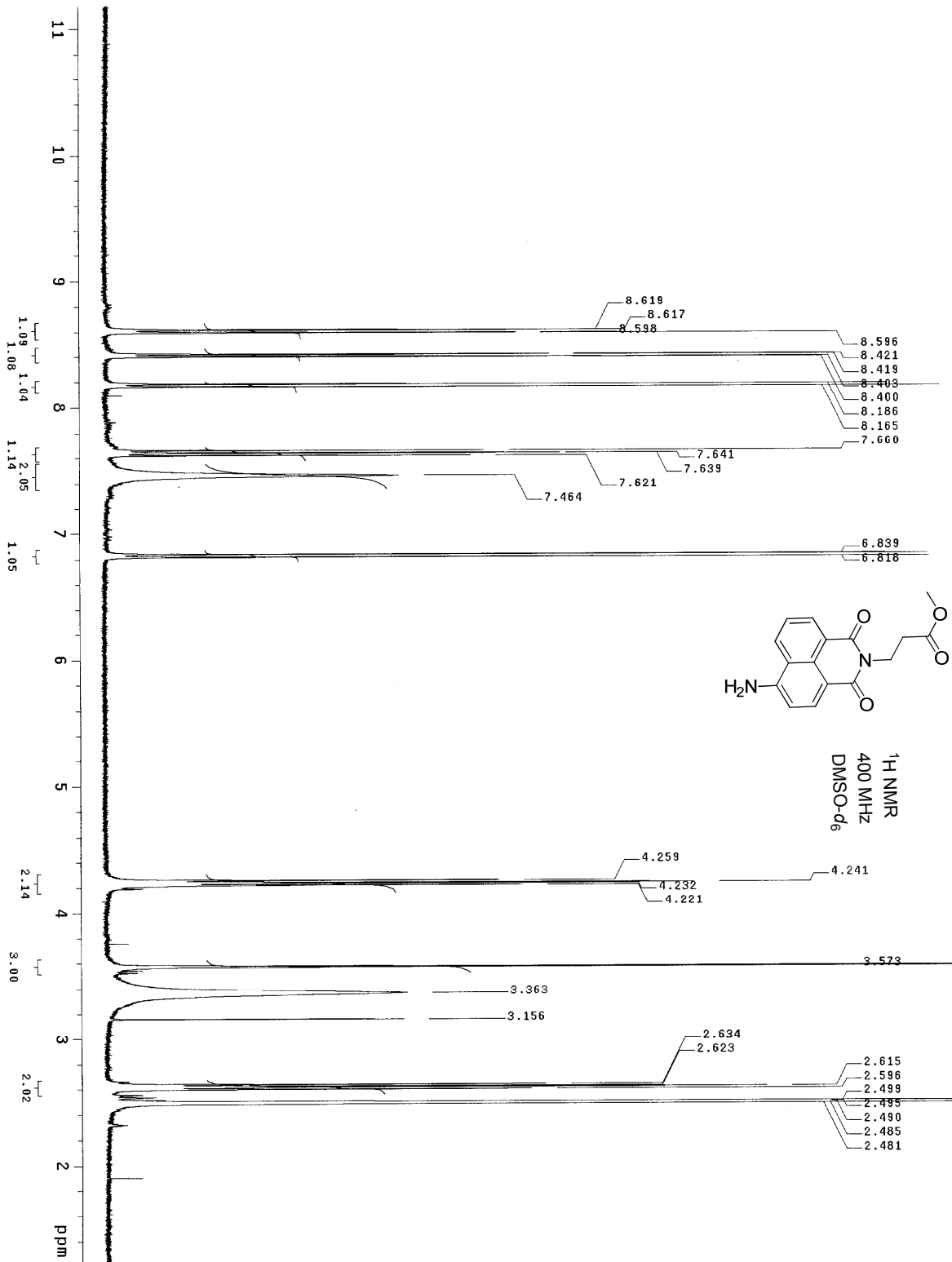
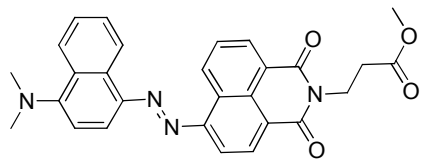
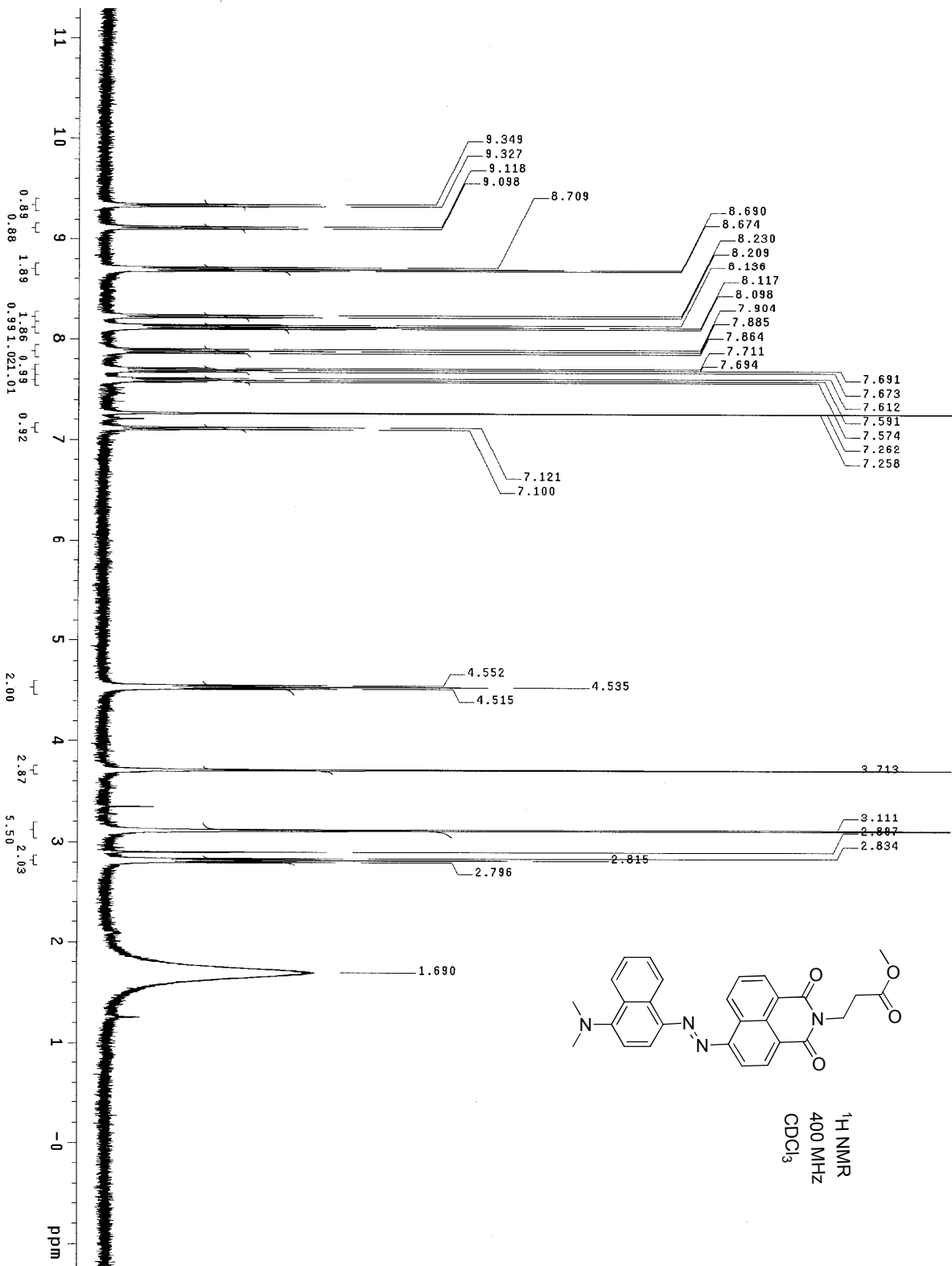


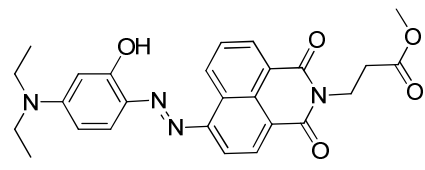
Figure S2: Ground state complex formation between 5'-Dabcyl or 5'-BHQ2 15mers and 3' Fluorophore labelled 20mers. Duplexes were formed by annealing a solution 1 μ M of each strand in hybridization buffer at 70 $^{\circ}$ C for 5 minutes then allowing to cool to room temperature over 30 minutes. The simple sum was created by adding solutions containing only 1 μ M of either quencher or fluorophore labelled strand.



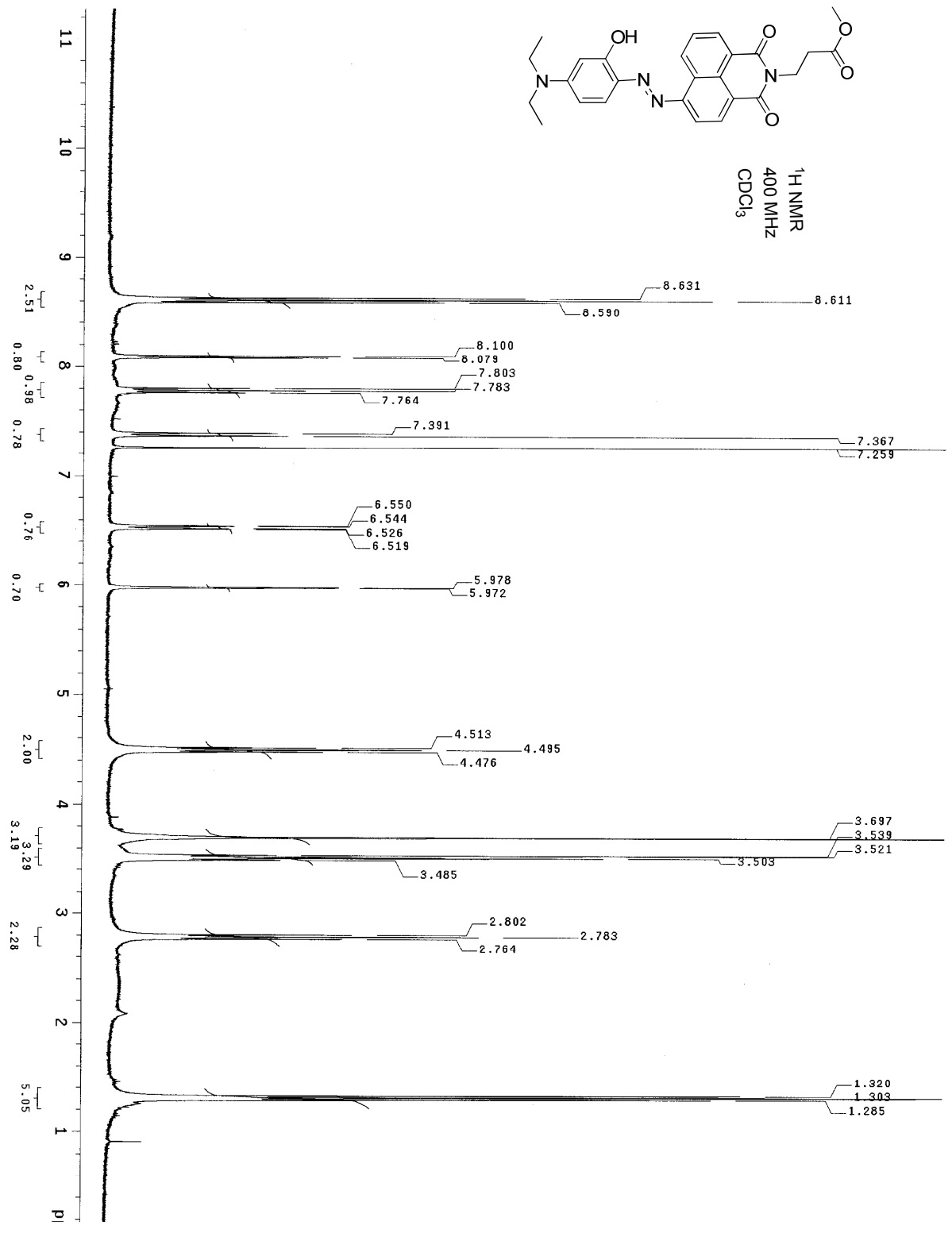


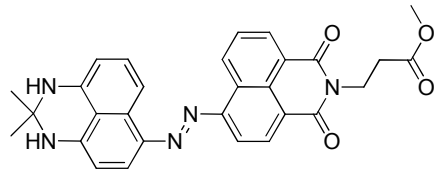
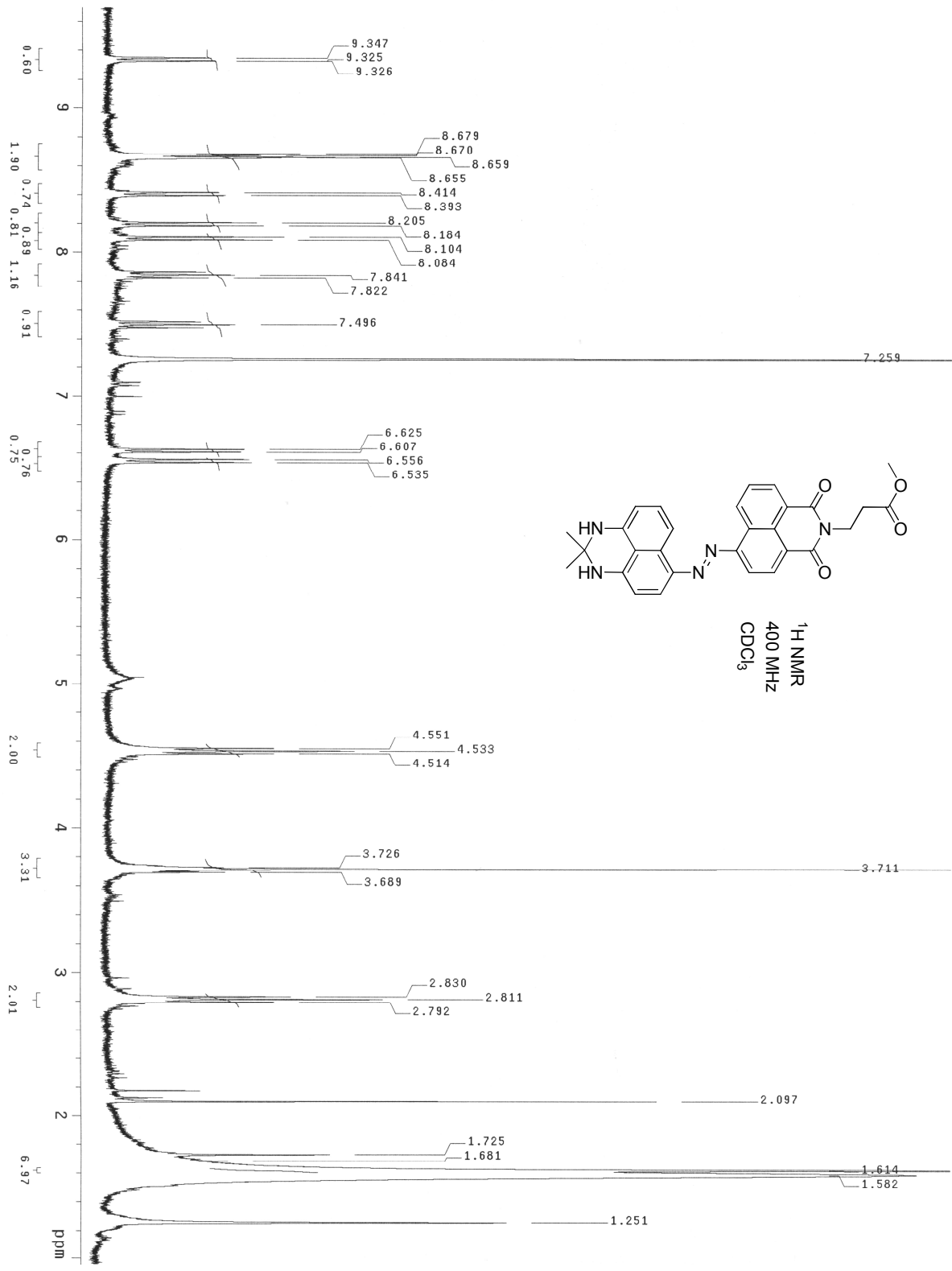






¹H NMR
400 MHz
CDCl₃

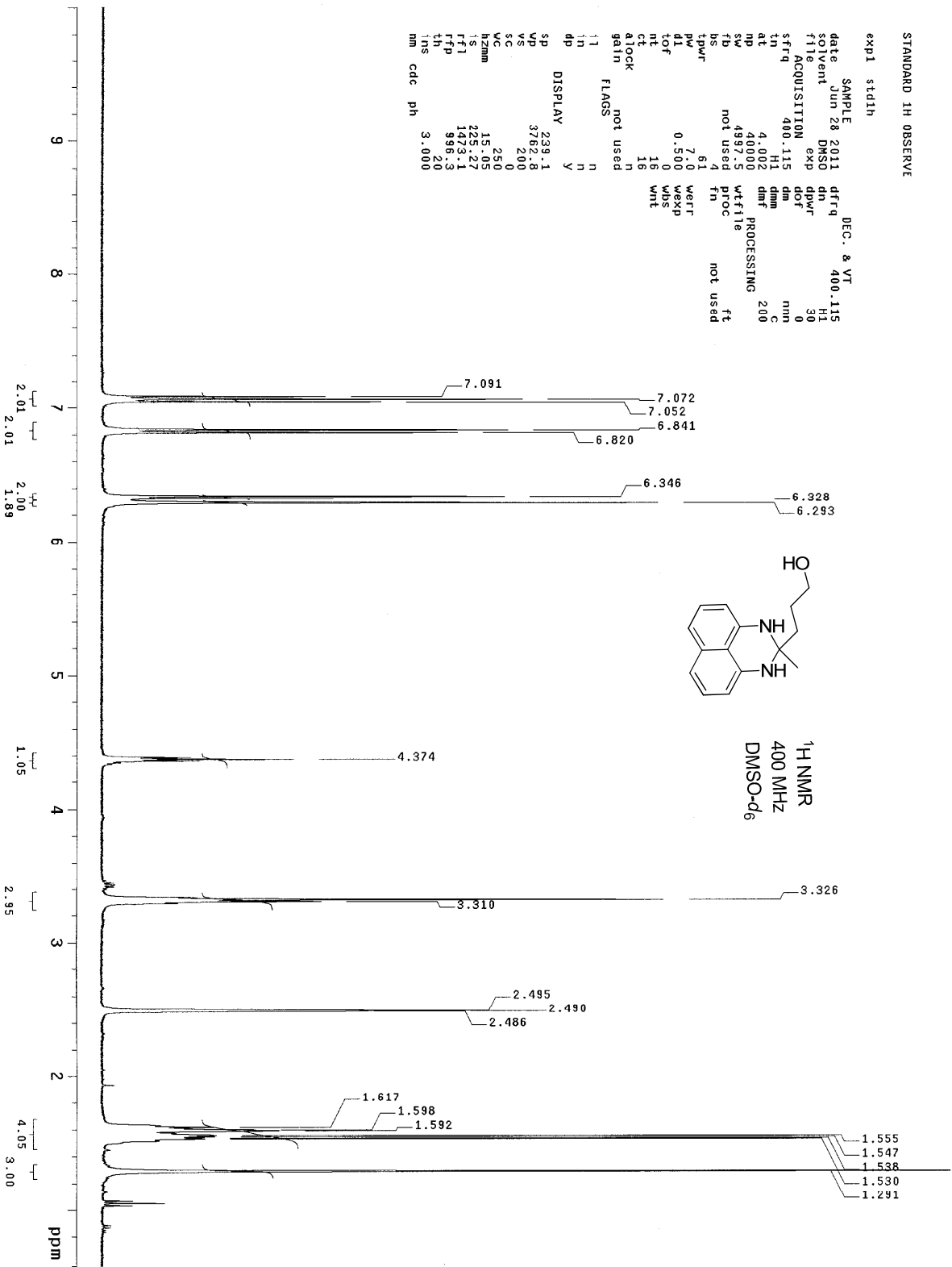


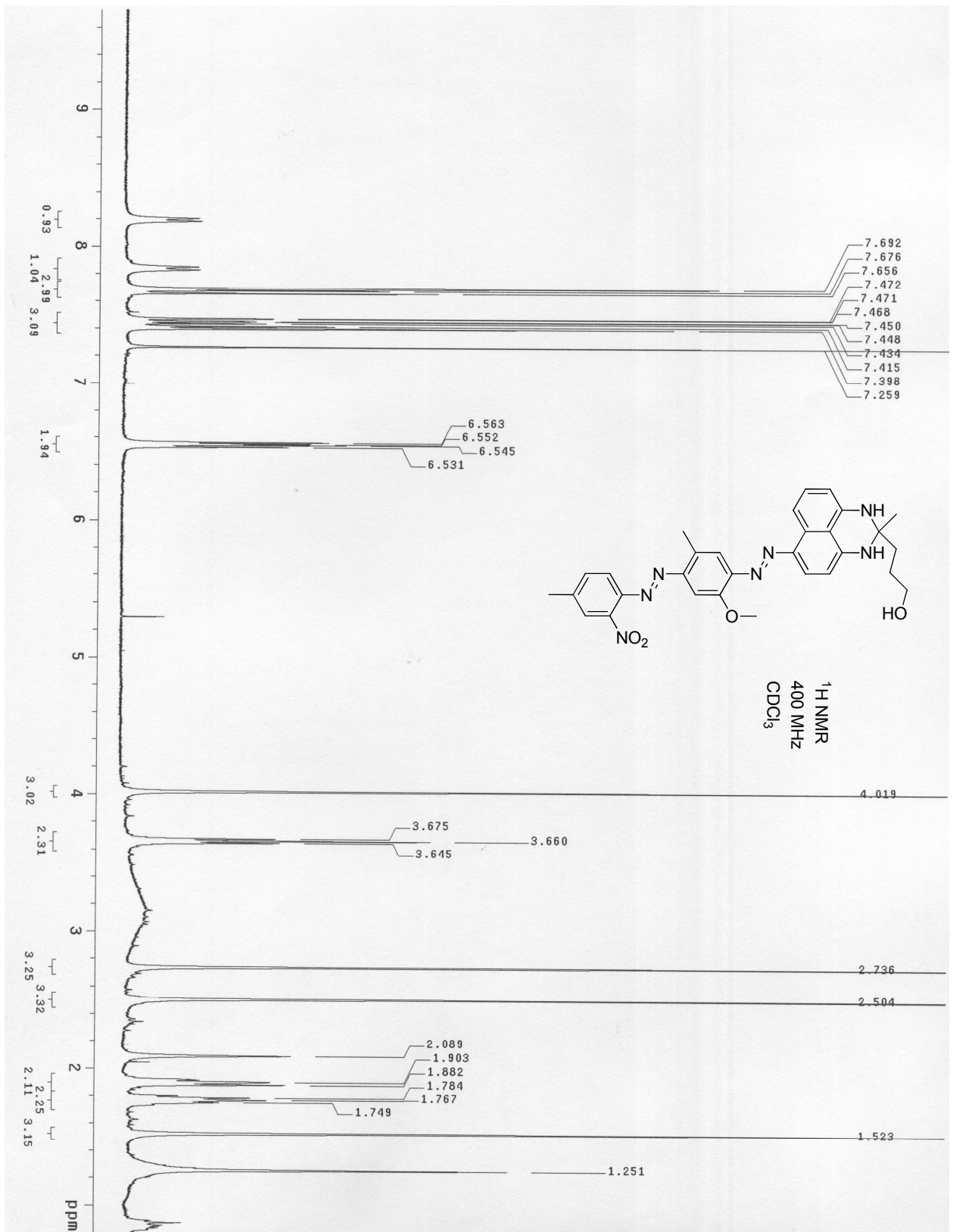


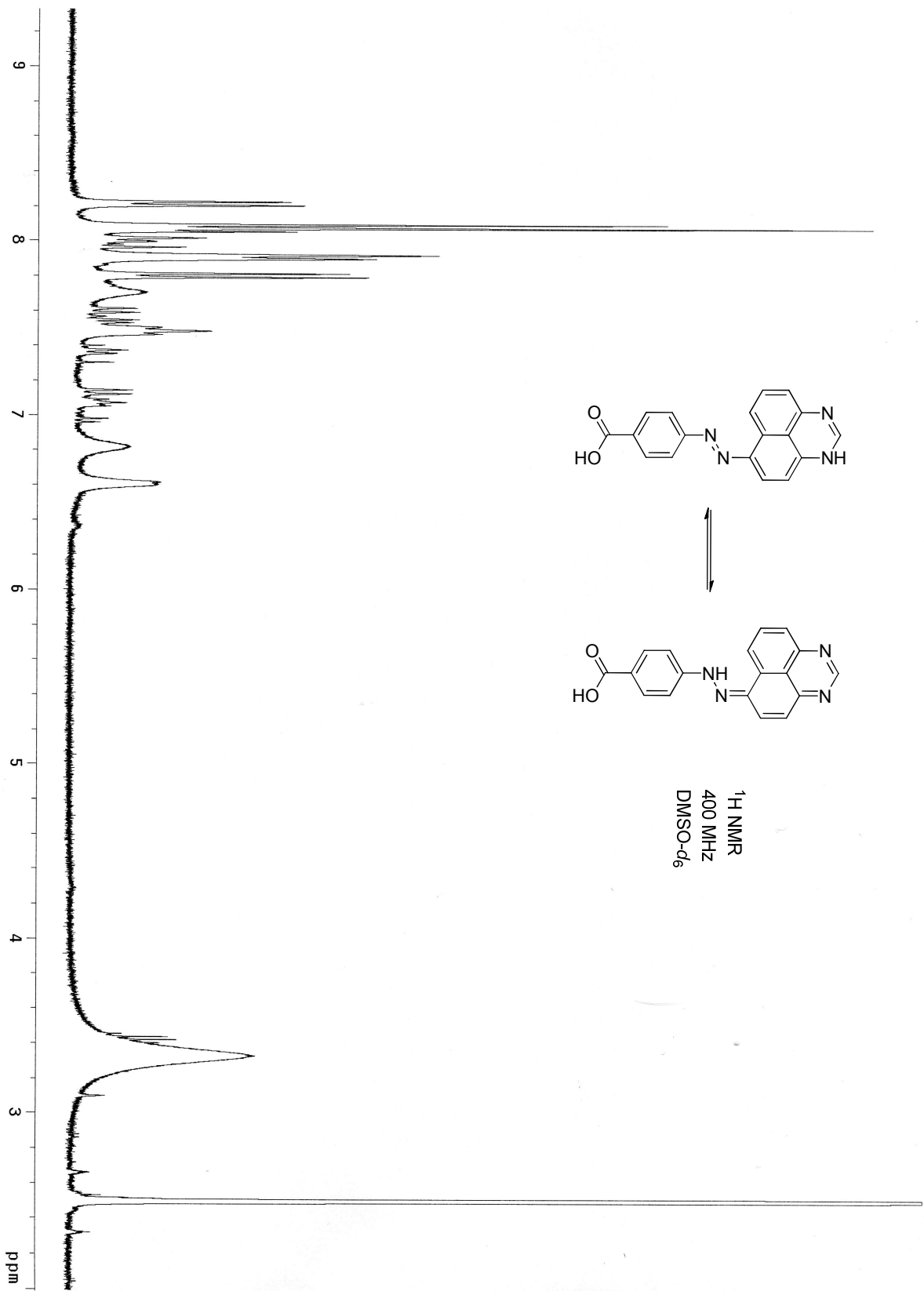
STANDARD 1H OBSERVE

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sfrq 400.115 dof 30
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at 4.002 dmf 200
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sw 4897.5 wfile
fb not used proc
bs 4 fn
tpwr 61
pw 7.0 werr
d1 0.500 wexp
tof 0 wds
nt 18 wnt
s 10
atlock not used
gain not used
flags not used
i1 n
in n
dp Y
DISPLAY
sp 239.1
vp 3762.8
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th 20
ins 3.000
nm cdc ph





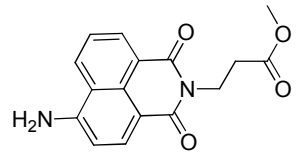


13C OBSERVE

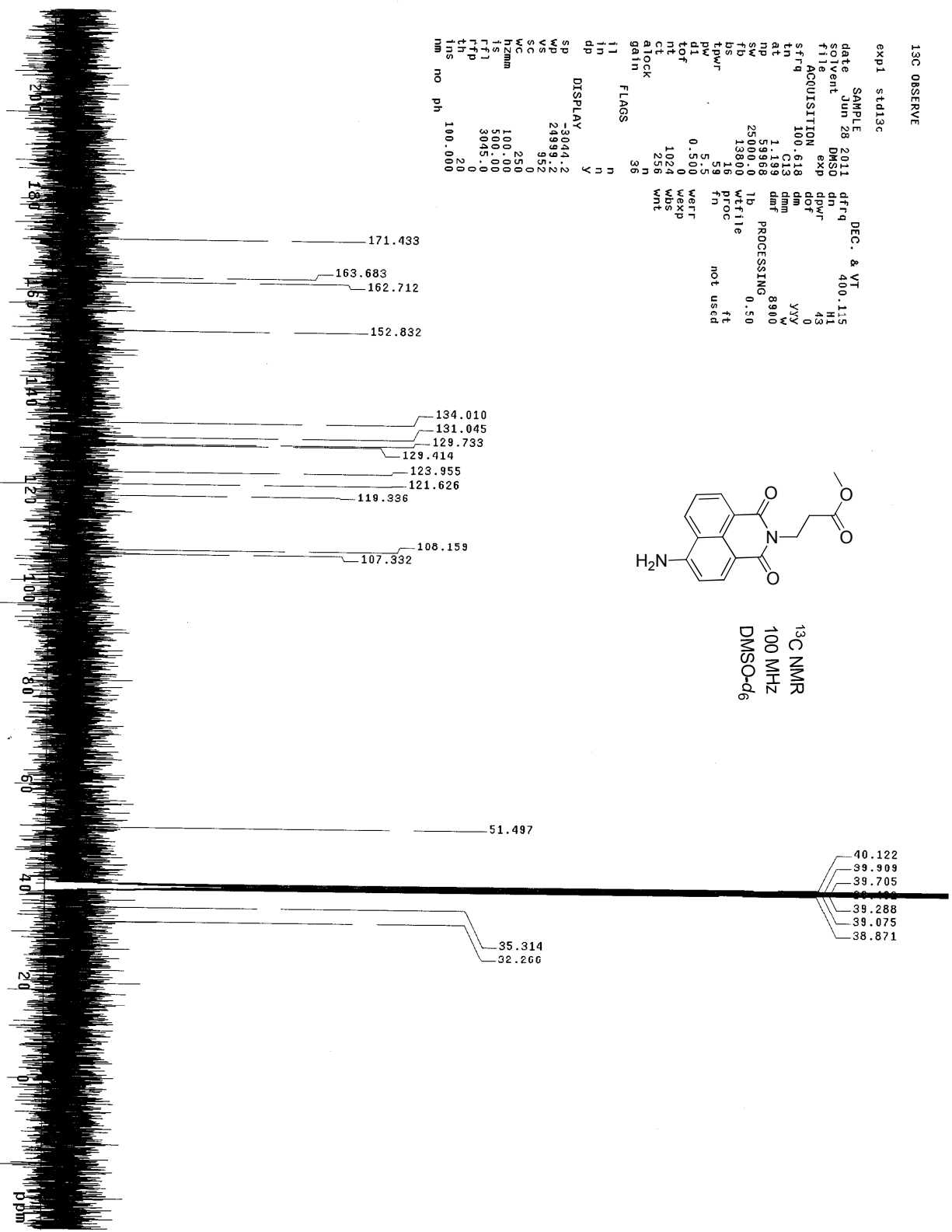
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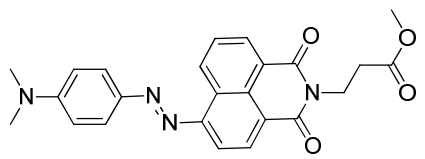
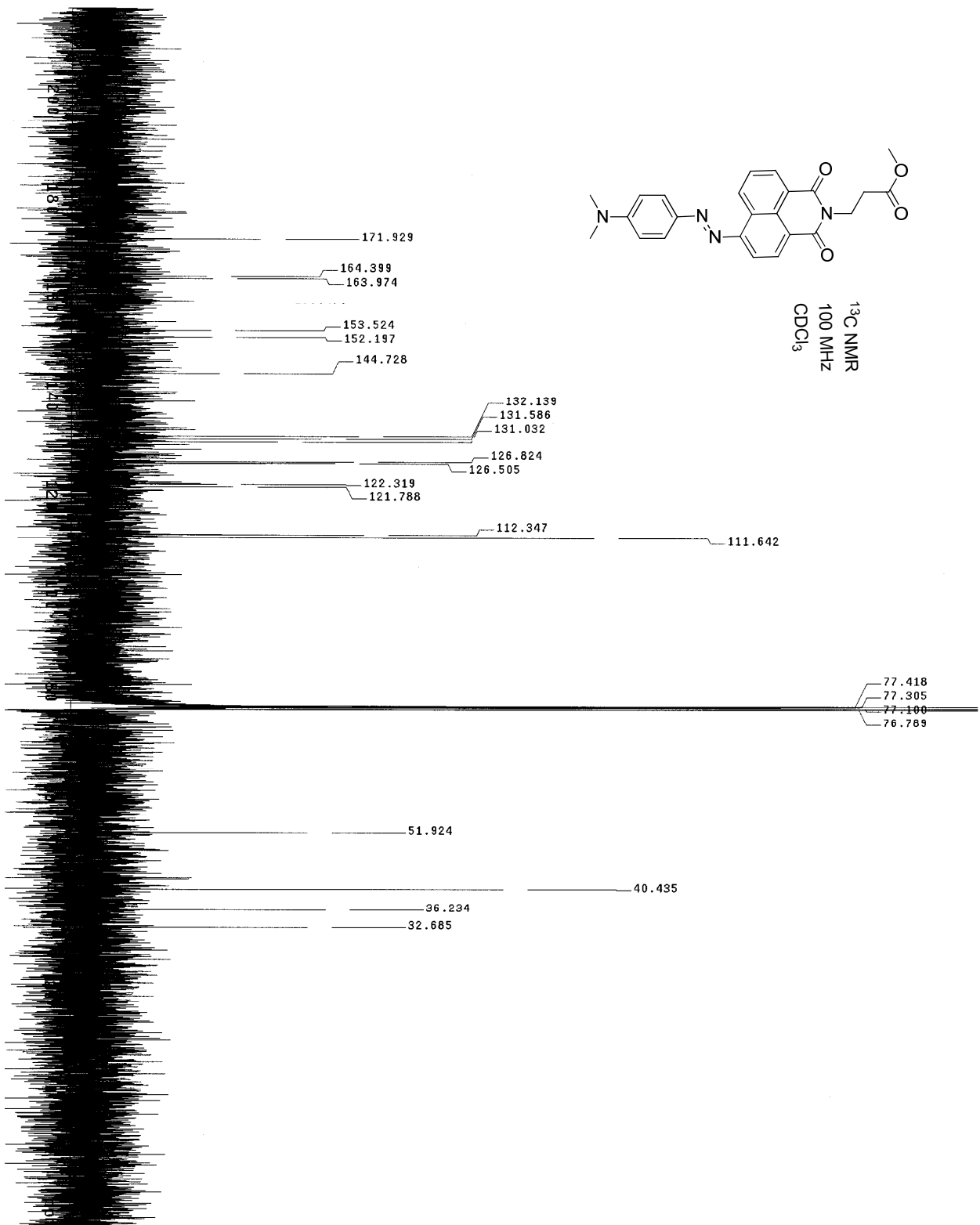
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 np 25000.0 W
 sw 13800 lb PROCESSING 0.50
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 tpwr 59 fn not used
 pw 5.5 werr
 d1 0.500 wexp
 nt 1024 wbs
 ct 256 wnt
 atlock n
 gain 36
 flags

DISPLAY
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 wd 24999.2
 vs 952
 sc 0
 wc 250
 hzmm 100.00
 ts 500.00
 rftl 3045.0
 rfp 0
 th 20
 tns 100.000
 nm no ph



¹³C NMR
 100 MHz
 DMSO-d₆





¹³C NMR
100 MHz
CDCl₃

171.929

164.399
163.974

153.524
152.197

144.728

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131.032

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126.505

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121.788

112.347

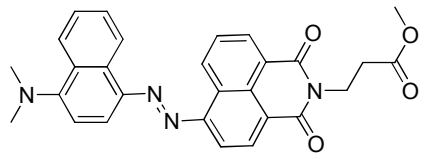
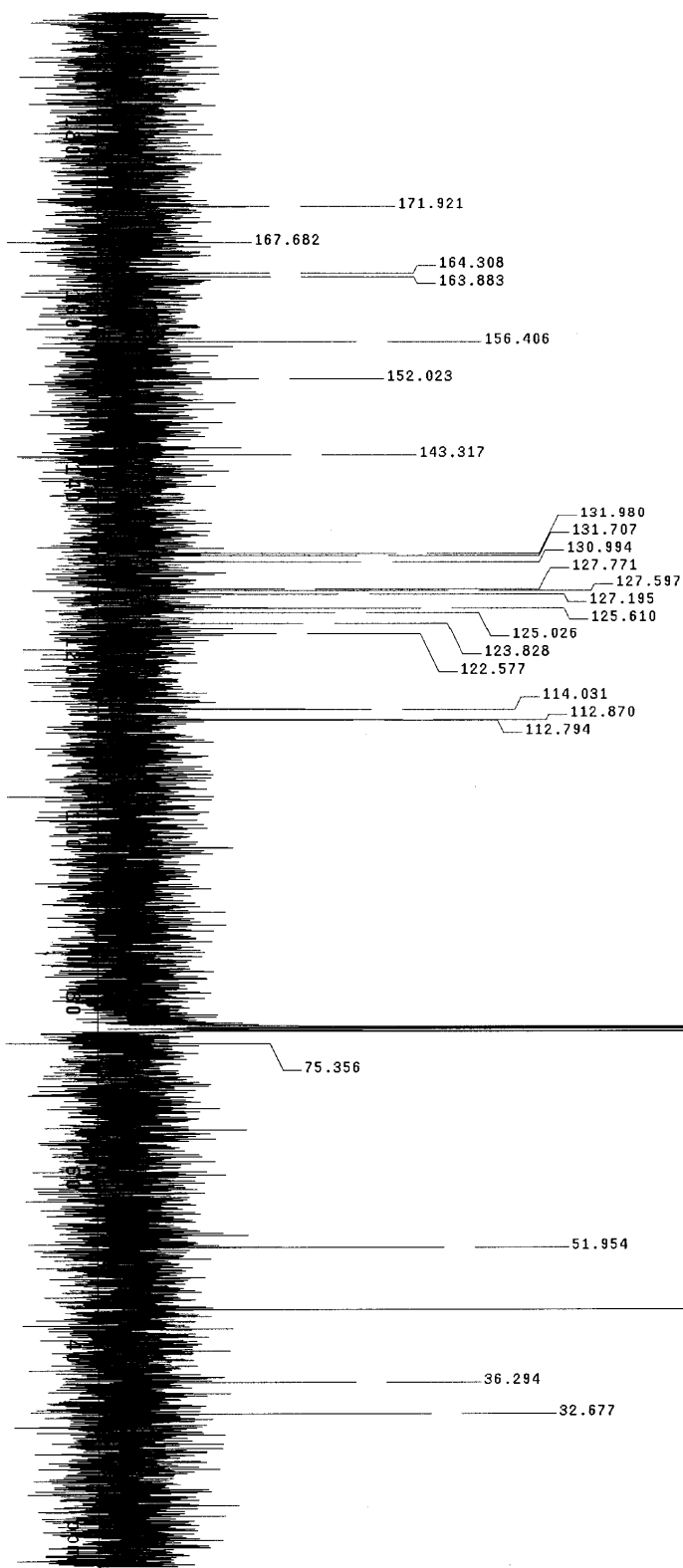
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77.100
76.789

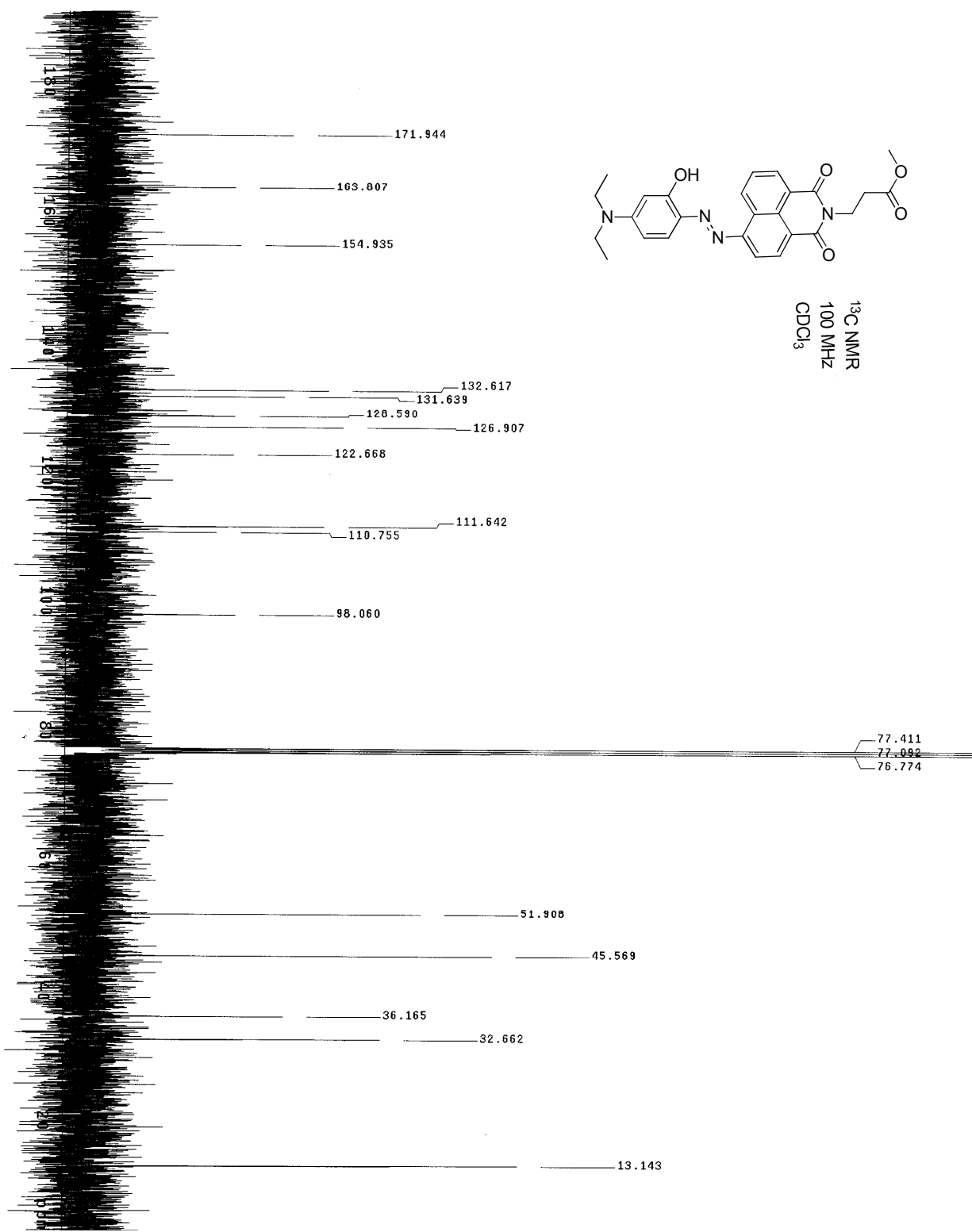
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¹³C NMR
100 MHz
CDCl₃

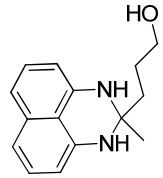


13C OBSERVE

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 fb 13800 16 proc
 bs 16 fn not used
 tavr 5.5
 pw 0.500 weff
 dl 0 wexp
 tof 1024 wbs
 nt 176 wht
 ct 176
 alock n
 gain n
 i1 36
 in n
 dp n

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 vs 558
 sc 0
 wc 250
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 f1 50.15
 f2 701.90
 rfd 3974.0
 tlb 20
 lns 100.000
 mm no ph



¹³C NMR
 100 MHz
 DMSO-d₆

