SUPPORTING INFORMATION

Fluorescence Quenchers for Hydrazone and Oxime Orthogonal Bioconjugation

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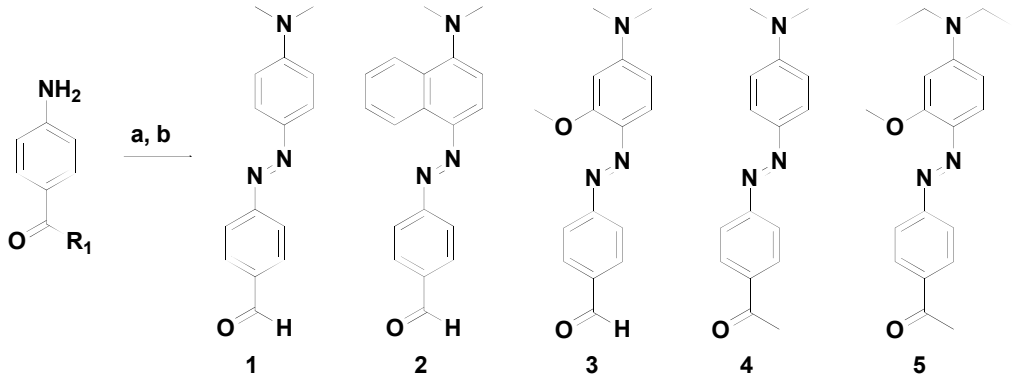
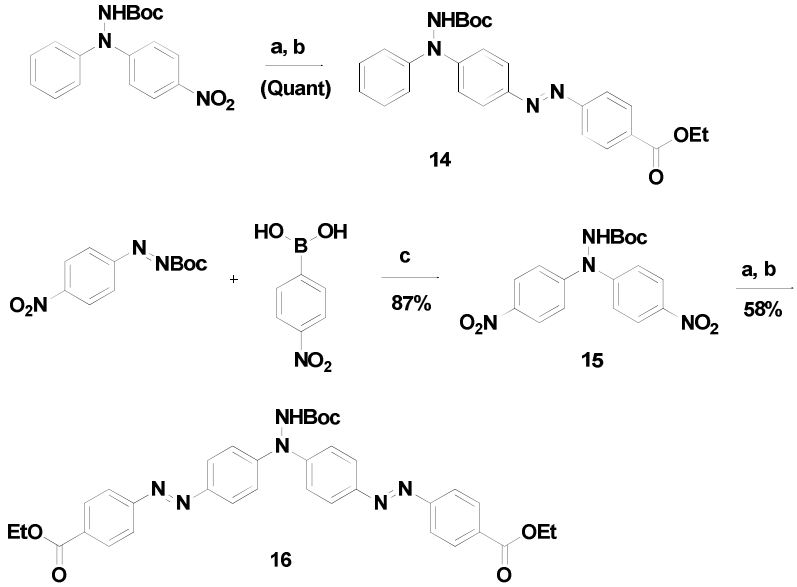
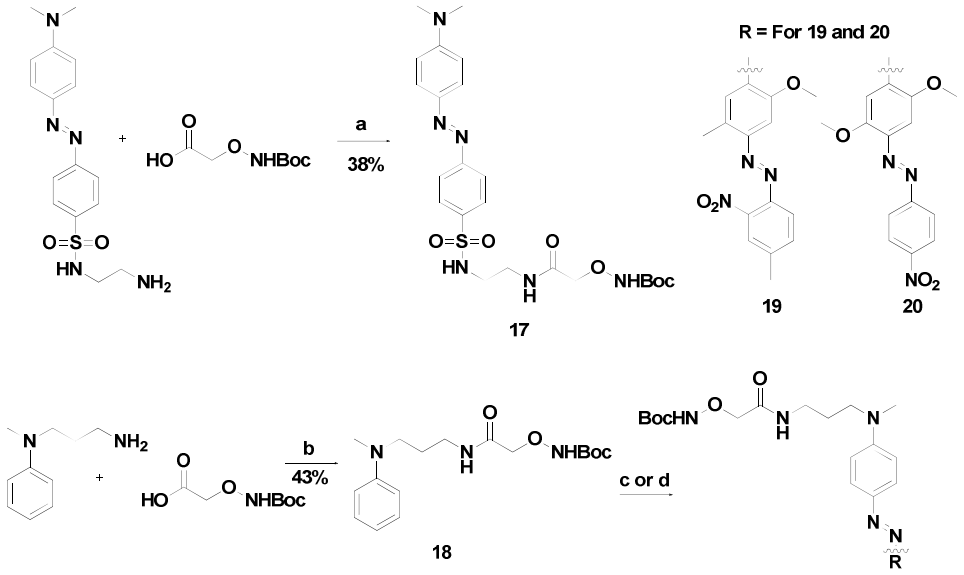


Scheme S1: Preparation of compounds **1 – 5**. R_1 = H (**1 – 3**) or R_1 = CH_3 (**4 – 5**). R_2 corresponds to the aniline used for each product. (a): $NaNO_2$, HCl, 0°C (b) Aniline derivative, NaOAc.



Scheme S2: Synthesis of boc-protected precursors for hydrazine-based quenchers. (a): H_2, Pd/C, MeOH (b): Ethyl 4-nitrosobenzoate, AcOH, Toluene (c): $Cu(Oac)_2 \cdot H_2O$, MeOH.



Scheme S3: Synthesis of boc-protected precursors for oxime-based quenchers. (a): EDCI, HOAt, Et_3N, CH_2Cl_2 (b): EDCI, Et_3N, CH_2Cl_2 (c): THF, MeOH, Fast Corinth V (13%) (d): THF, MeOH, Fast Black K (53%)

Table S1: MALDI-TOF and analytical HPLC data for oligodeoxynucleotides in this study.

DNA conjugate	MALDI Calc	MALDI Obs	Purity (HPLC)
Dabcyl	6541.2	6541.9	99.6%
1 oxime	6485.2	6486.7	99.5%
1 hydrazone	6554.2	6554.6	97.2%
2 oxime	6535.2	6536.1	99.7%
3 oxime	6515.2	6513.9	99.8%
3 hydrazone	6584.3	6581.9	98.2%
4 oxime	6499.2	6500.4	99.0%
5 oxime	6557.2	6558.2	97.1%
Bis (1) oxime	6765.3	6765.6	96.1%
Bis (2) oxime	6865.3	6870.5	99.8%
Bis (3) oxime	6825.3	6824.8	99.6%
10 hydrazone	6735.2	6734.6	99.5%
7 hydrazone	6690.2	6691.5	99.8%
8a hydrazone	6748.2	6752.7	99.8%
8b hydrazone	6747.3	6751.0	99.8%
9a hydrazone	6896.3	6899.3	99.7%
9b hydrazone	6894.3	6893.2	99.9%
6 hydrazone	6569.2	6572.3	98.8%
Fluorescein	6678.2	6677.7	98.9%
Fam 6 Beacon	8877.6	8878.8	99.6%
Fam 7 Beacon	8833.6	8834.2	99.8%
Aldehyde DNA	6434.1	6433.3	99.1%
BHQ2	6651.2	6652.1	99.9%
13 oxime	6966.3	6972.4	99.3%
12 oxime	6964.4	6964.9	98.7%
11 oxime	6836.3	6836.4	99.1%

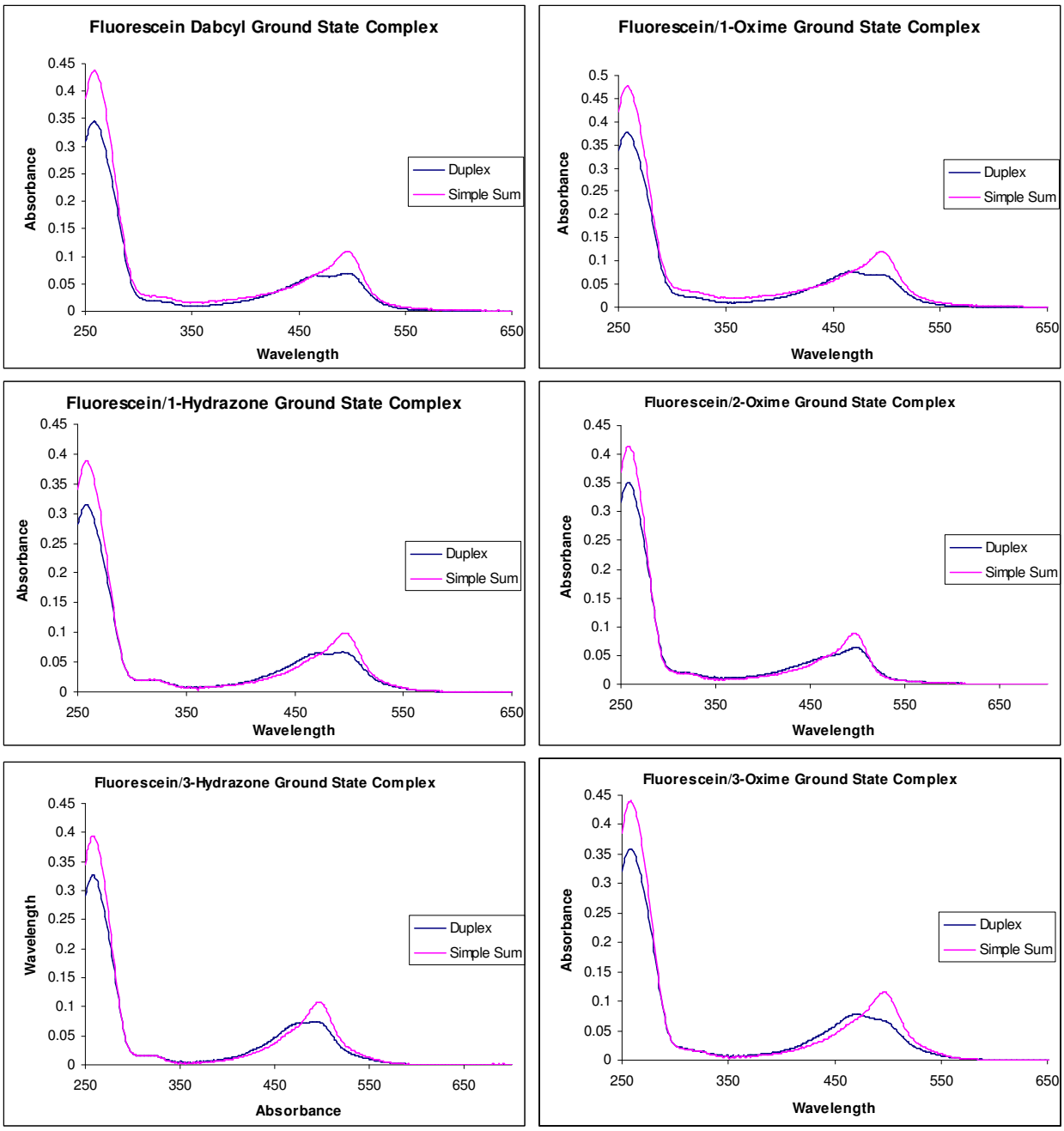


Figure S1: Spectral evidence of ground-state complex formation between quencher-conjugated 20mers and 3'-fluorescein 20mer. Duplexes were formed by annealing a solution 1 μM in each strand in hybridization buffer at 70 °C for 5 min, then allowing to cool to room temperature over 30 min. The simple sum was created by adding data for solutions containing only 1 μM of either quencher- or fluorophore-labeled oligonucleotide.

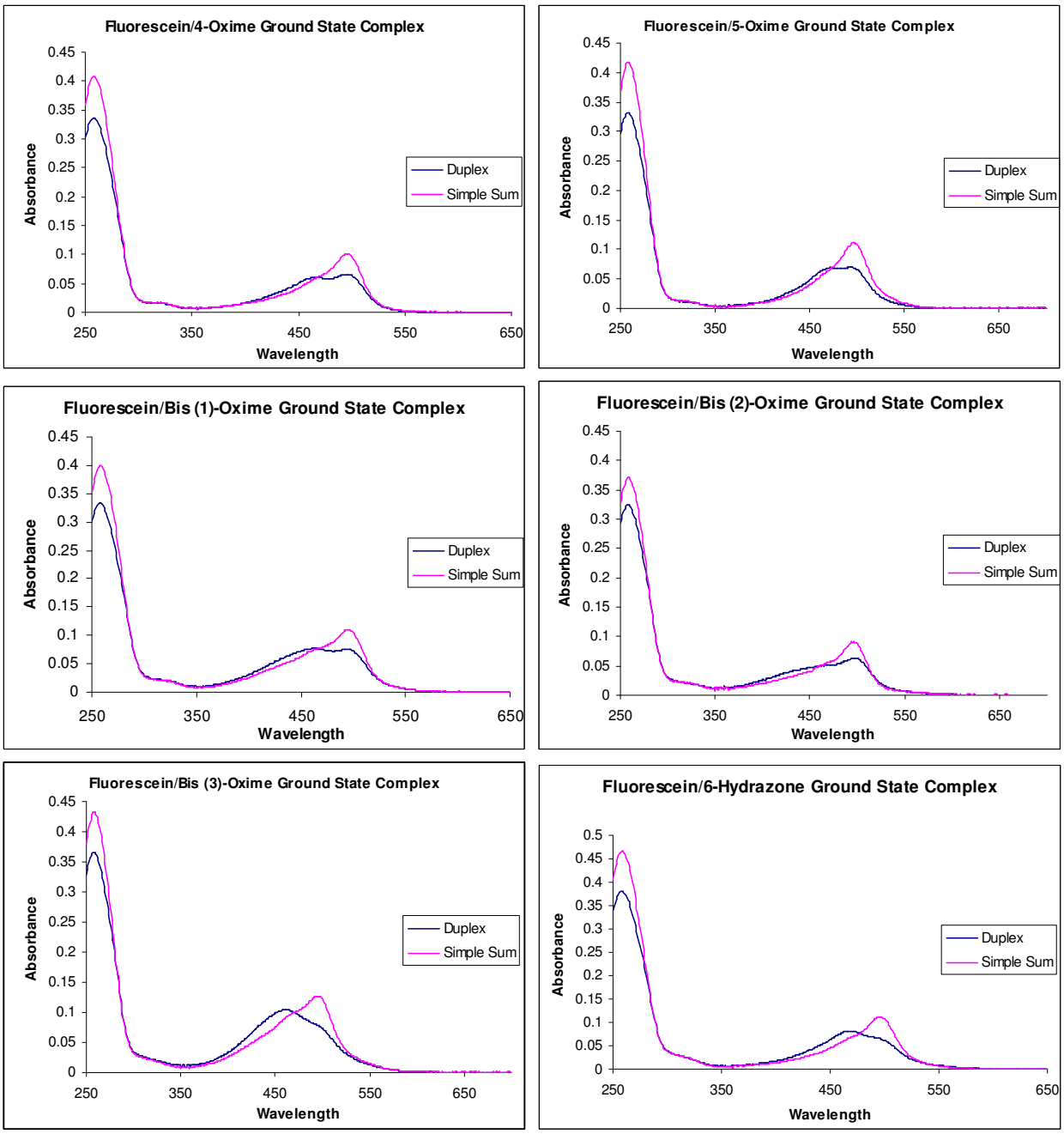


Figure S1 (continued)

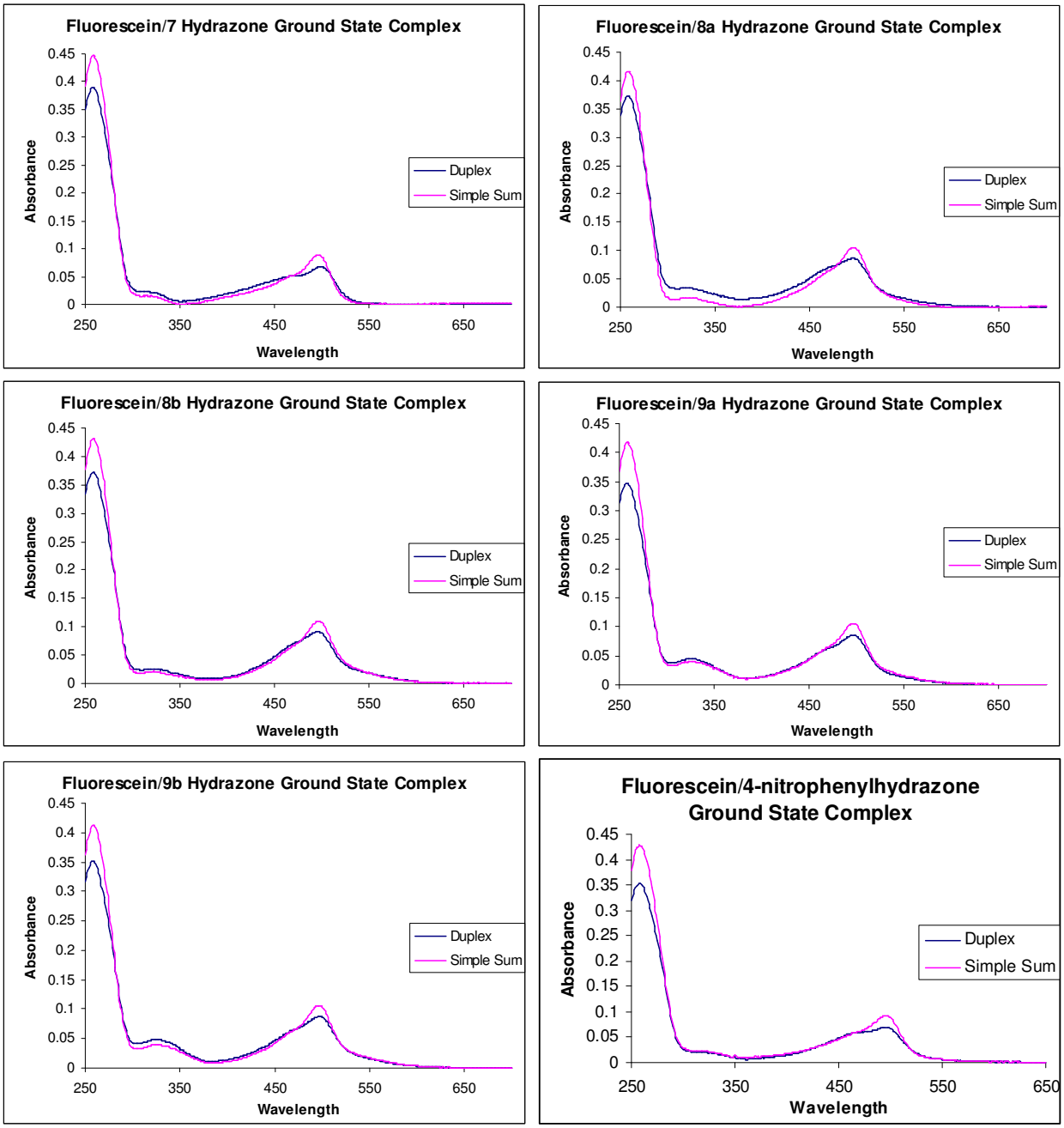


Figure S1 (continued)

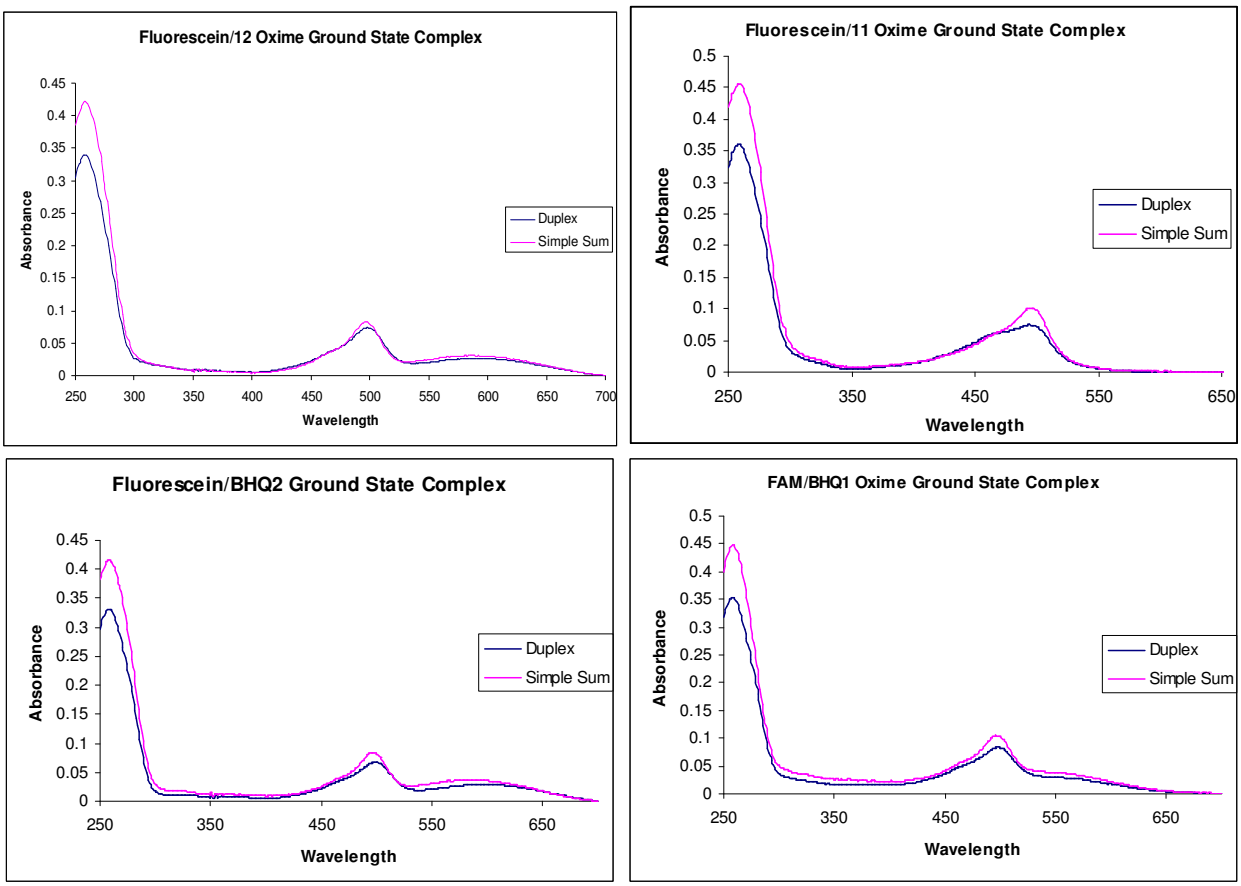
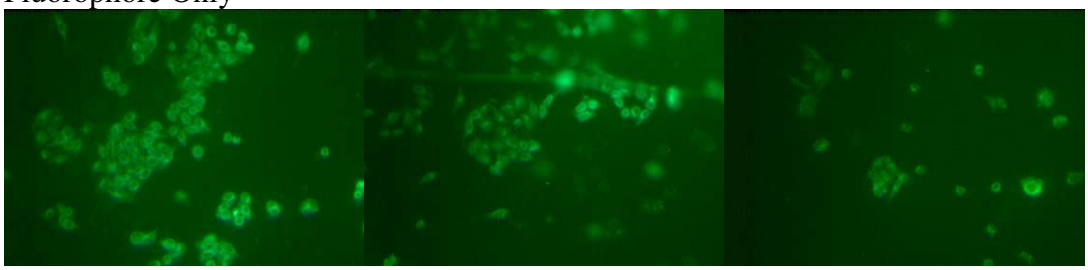


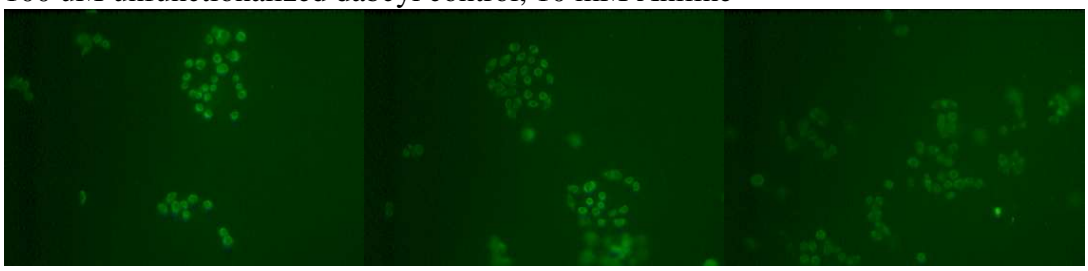
Figure S1 (continued)

Fluorophore Only



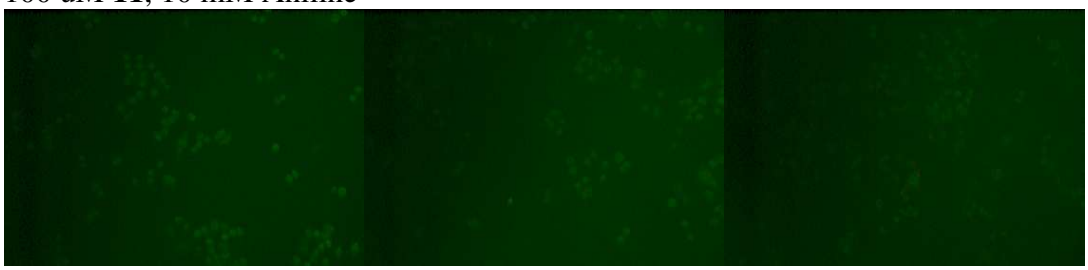
1 hr 2 hr 3 hr

100 uM unfunctionalized dabcyl control, 10 mM Aniline



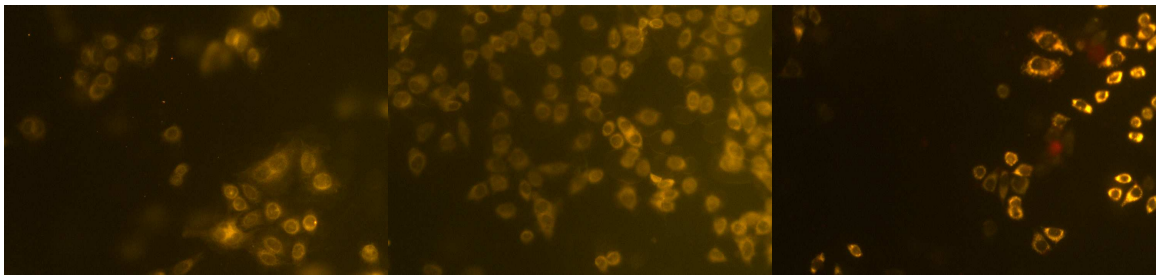
1 hr 2 hr 3 hr

100 uM **11**, 10 mM Aniline



1 hr 2 hr 3 hr

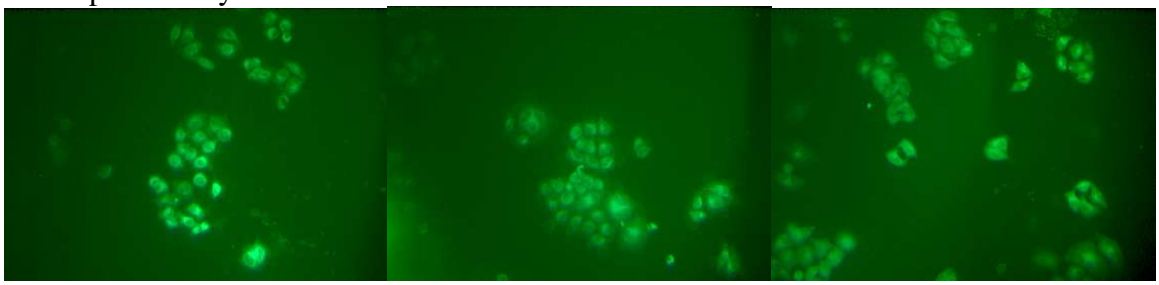
Figure S2: Time course experiments of cellular quenching. HeLa cells were incubated with 10 μM 7-diethylamino-3-formylcoumarin for 1 hour, then with dabcyl or quencher **11** and 10 mM aniline for 1, 2 or 3 hours and imaged (excitation 400 – 400 nm, emission > 470 nm). Identical camera settings were used in all cases.



No Quencher | Dabcyl control | Dye **11**

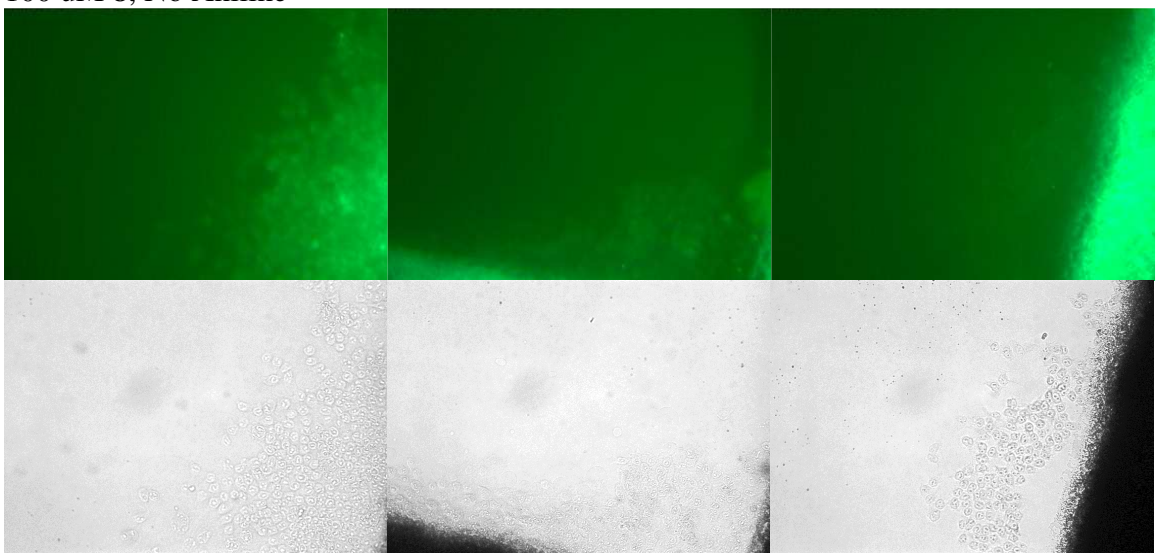
Figure S3: Cellular control experiments with rhodamine 6G (a non-aldehyde containing fluorophore). HeLa cells were incubated with 1 μM rhodamine 6G for 1 hour, then with 100 μM dabcyl or quencher **11** + 10 mM aniline for 3 hours, then imaged (excitation 450 – 490 nm, emission > 520 nm).

Fluorophore Only



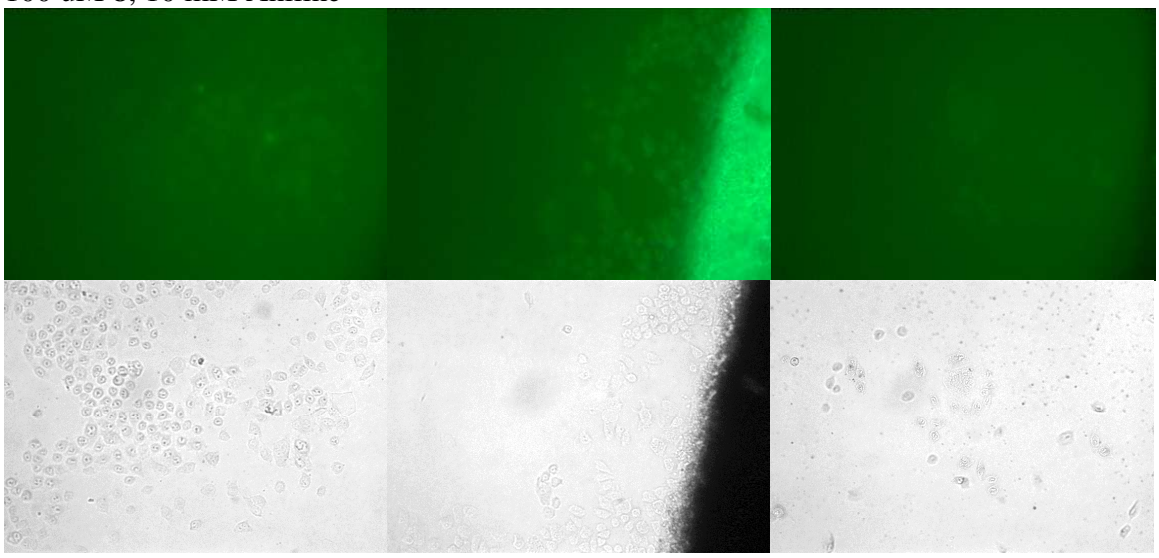
1 hr | 2 hr | 3 hr

100 uM **8**, No Aniline



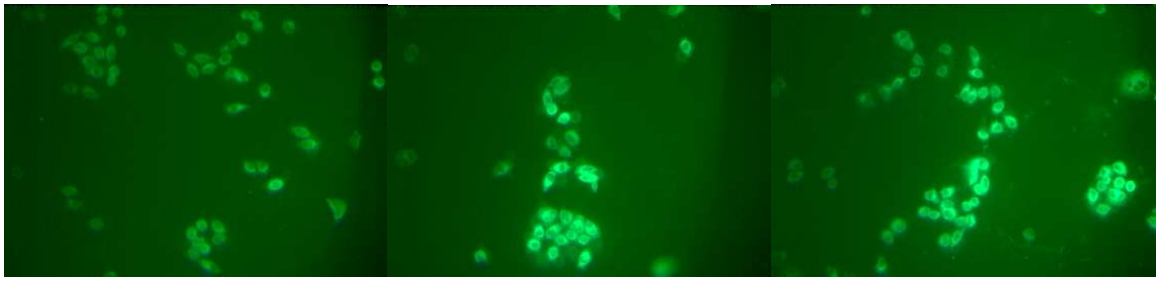
1 hr | 2 hr | 3 hr

100 uM **8**, 10 mM Aniline



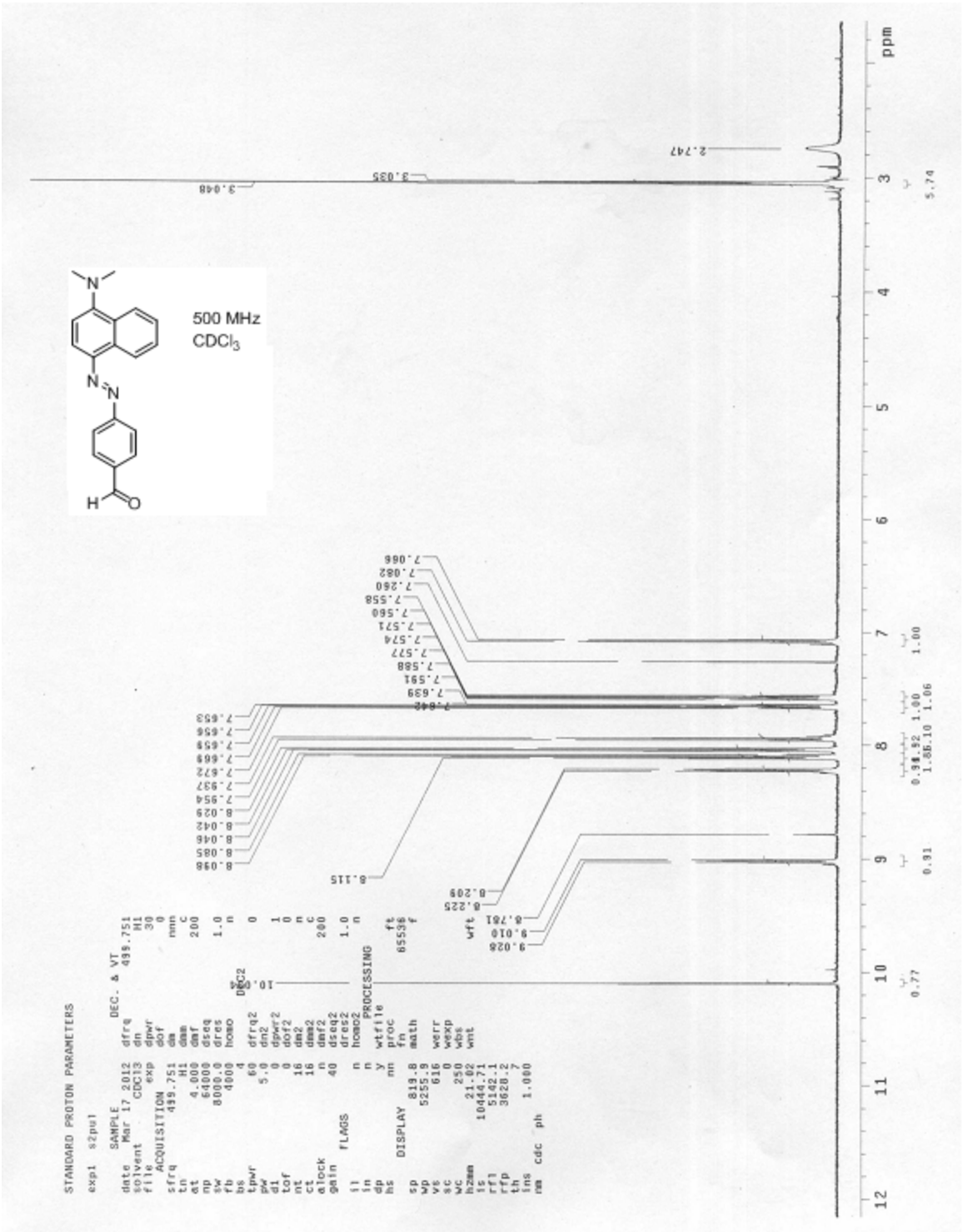
1 hr 2 hr 3 hr

10 mM Aniline

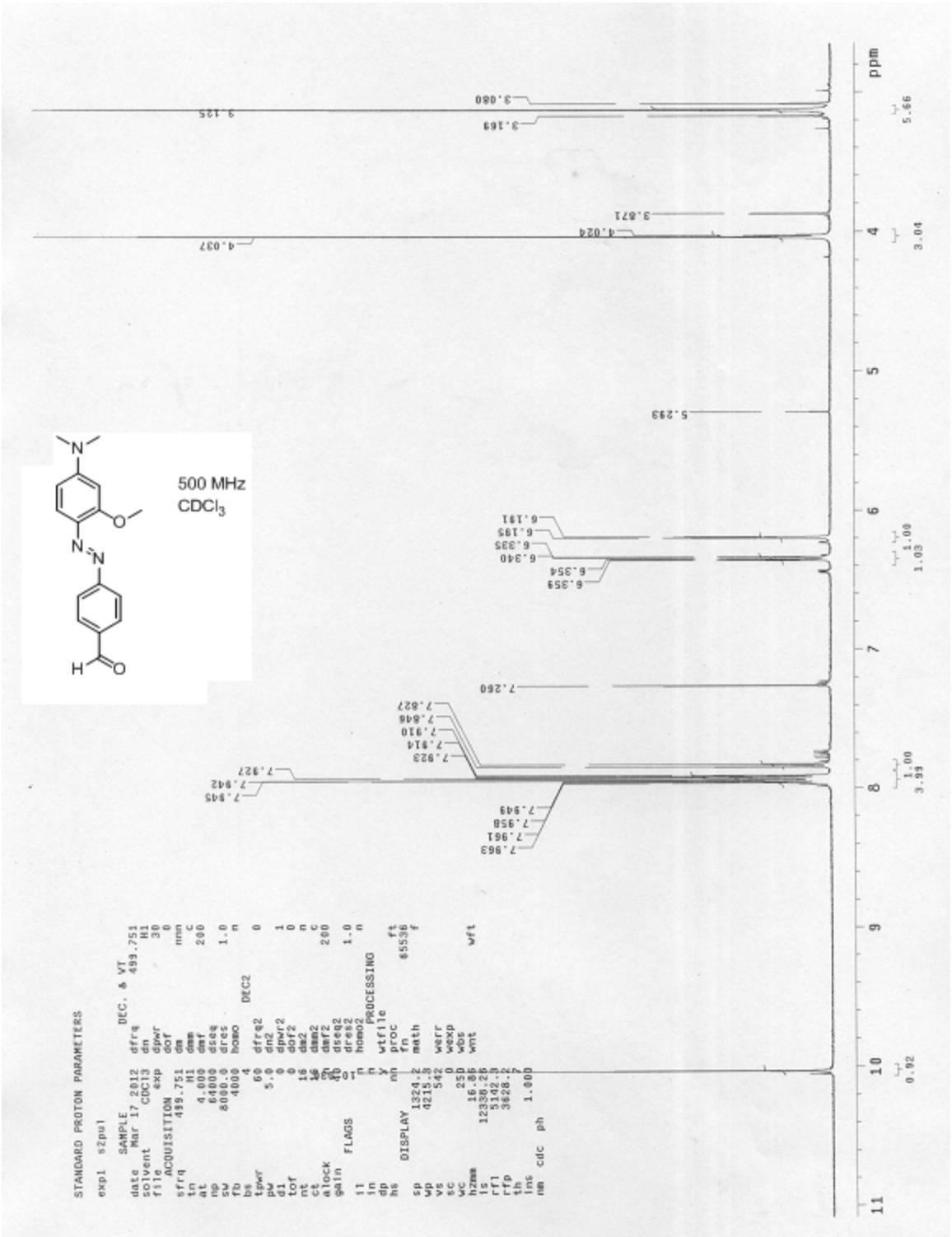


1 hr 2 hr 3 hr

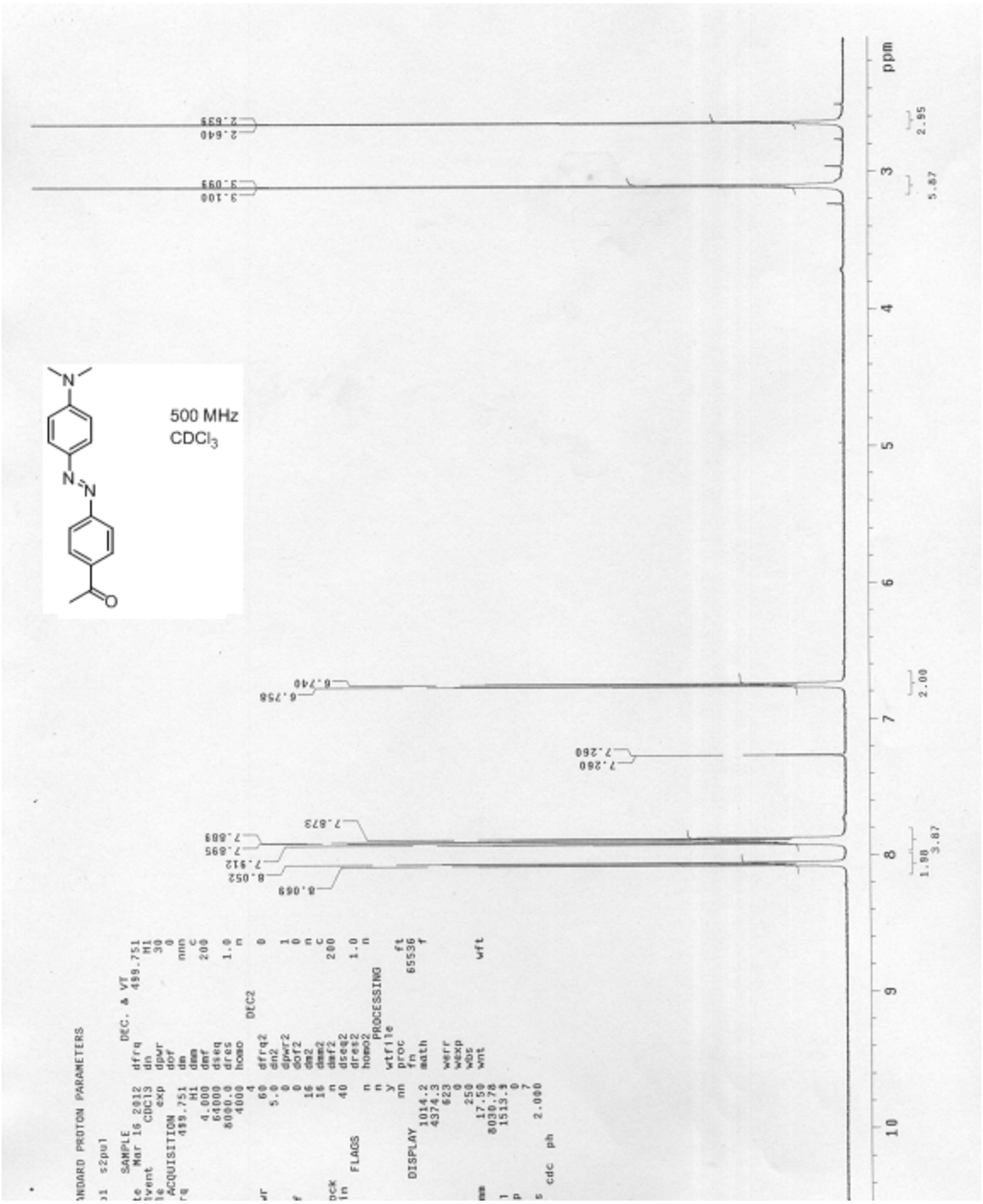
Figure S4: Time course of cellular quenching experiments. Cells were incubated with 10 μM 7-diethylamino-3-formylcoumarin for 1 hour then with quencher **8**, 10 mM aniline or quencher **8** and 10 mM aniline for 1, 2 or 3 hours and imaged (excitation 400 – 400 nm, emission greater than 470 nm). Brightfield images for treatment of cells with quencher **8** are also provided, the same areas are shown in the fluorescence and brightfield images for comparison.



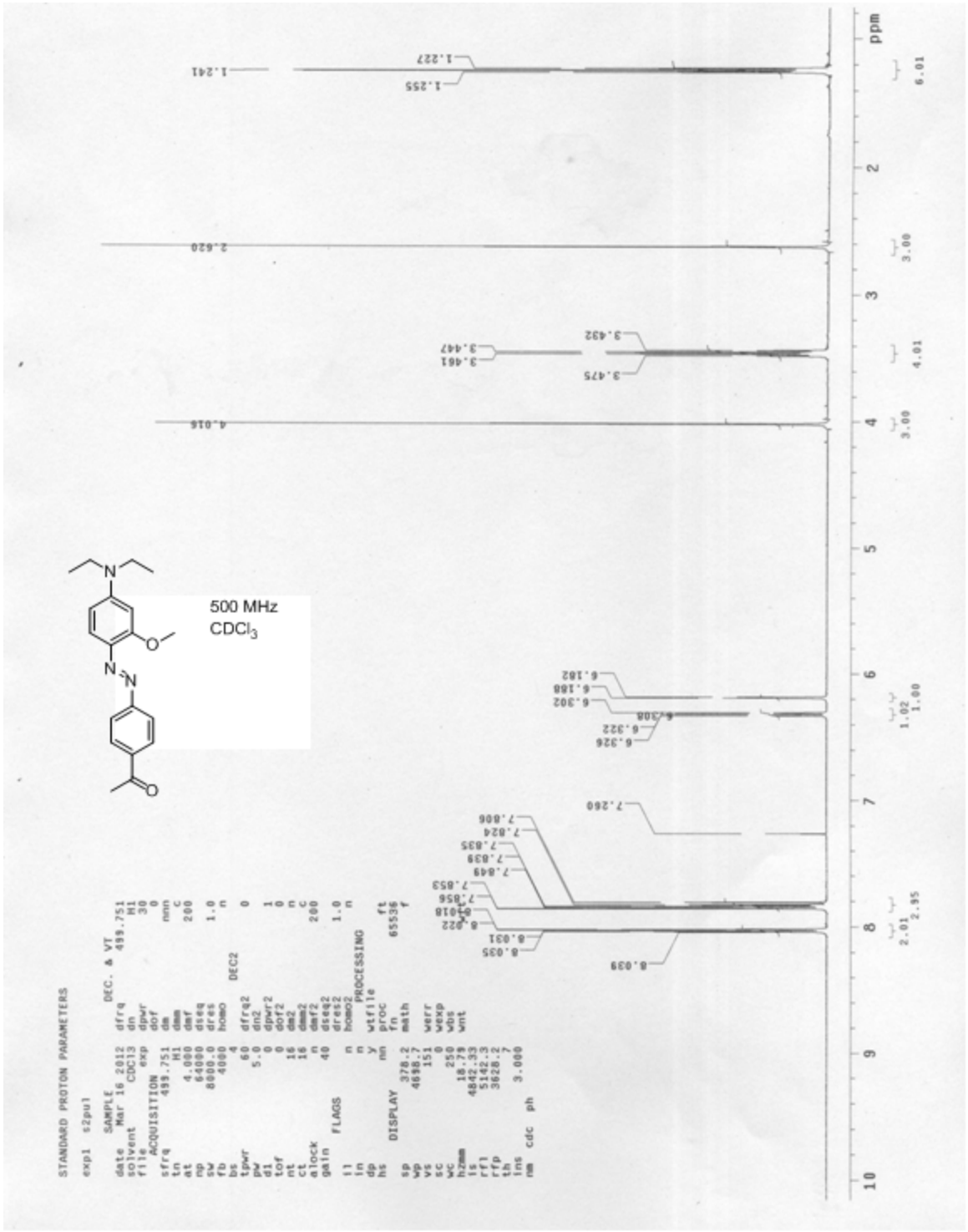
Proton NMR spectrum of **2**



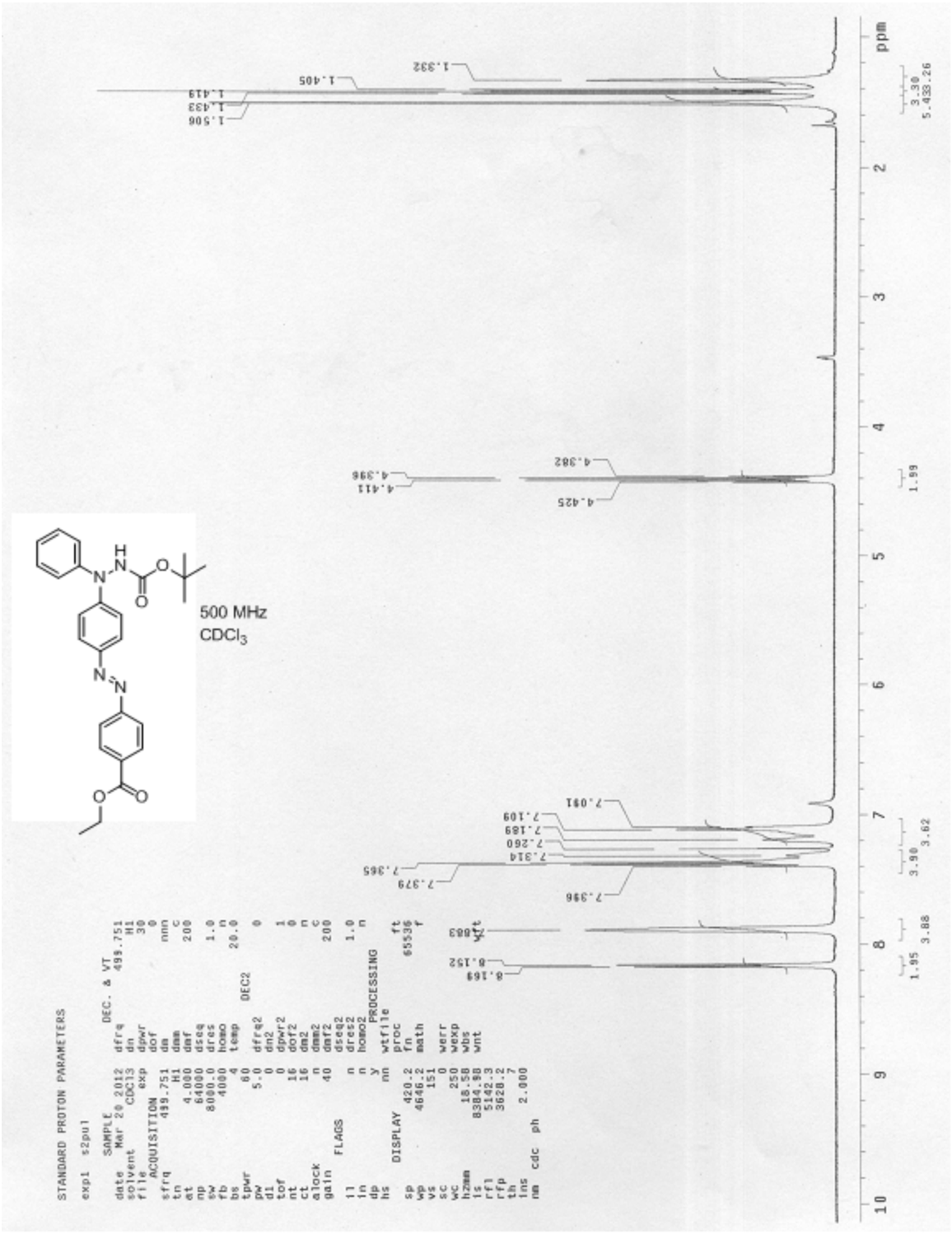
Proton NMR spectrum of **3**



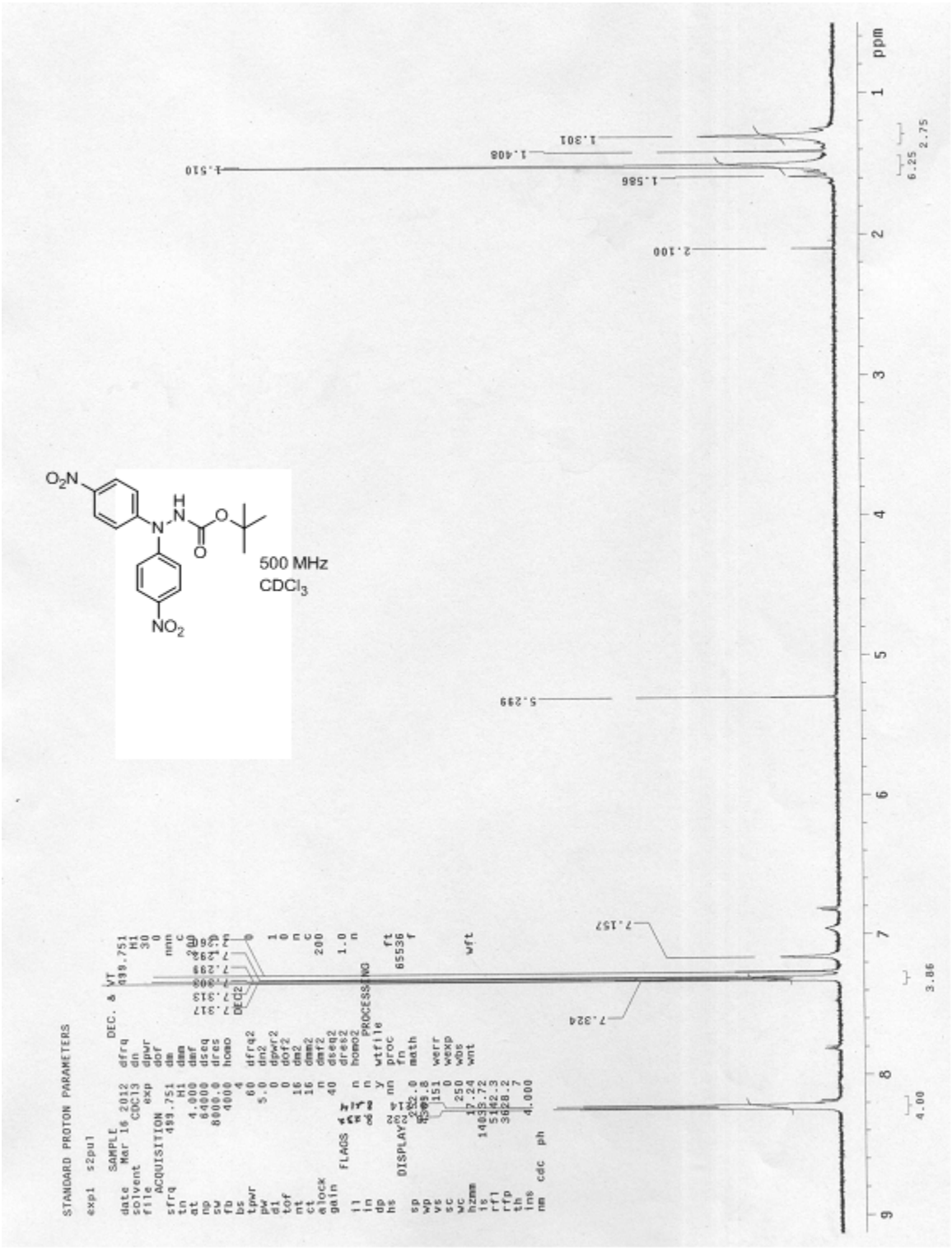
Proton NMR spectrum of **4**



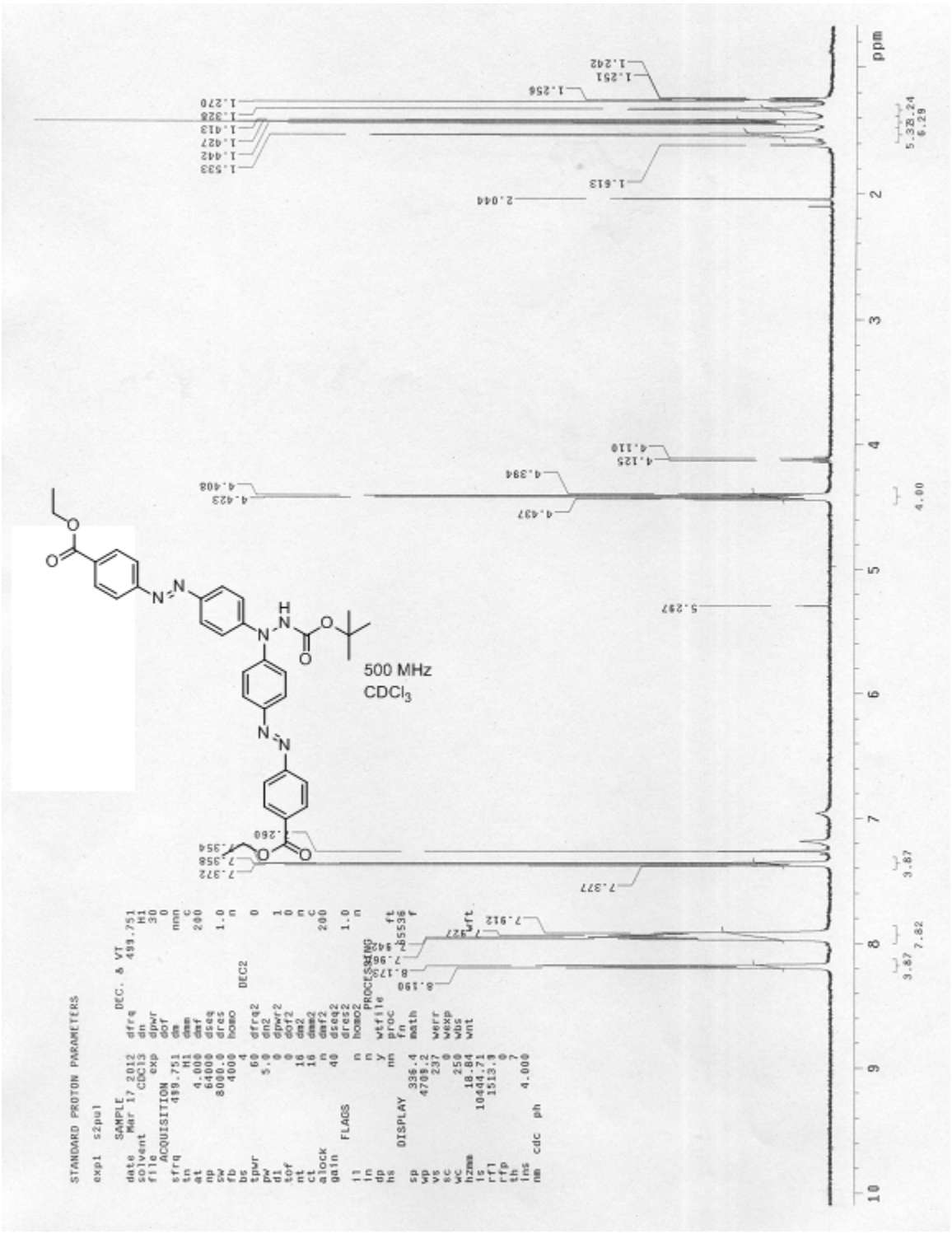
Proton NMR spectrum of **5**



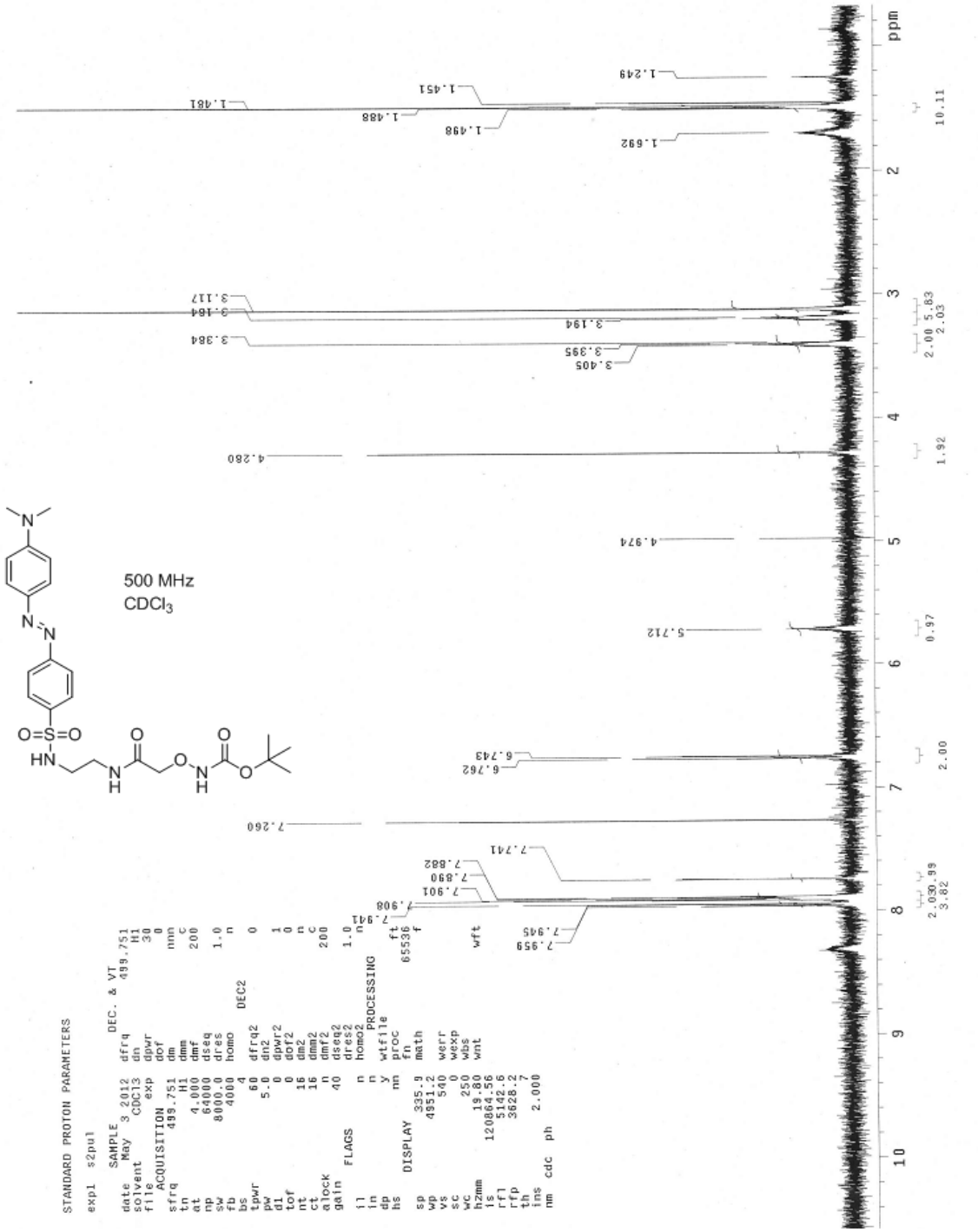
Proton NMR spectrum of **14**



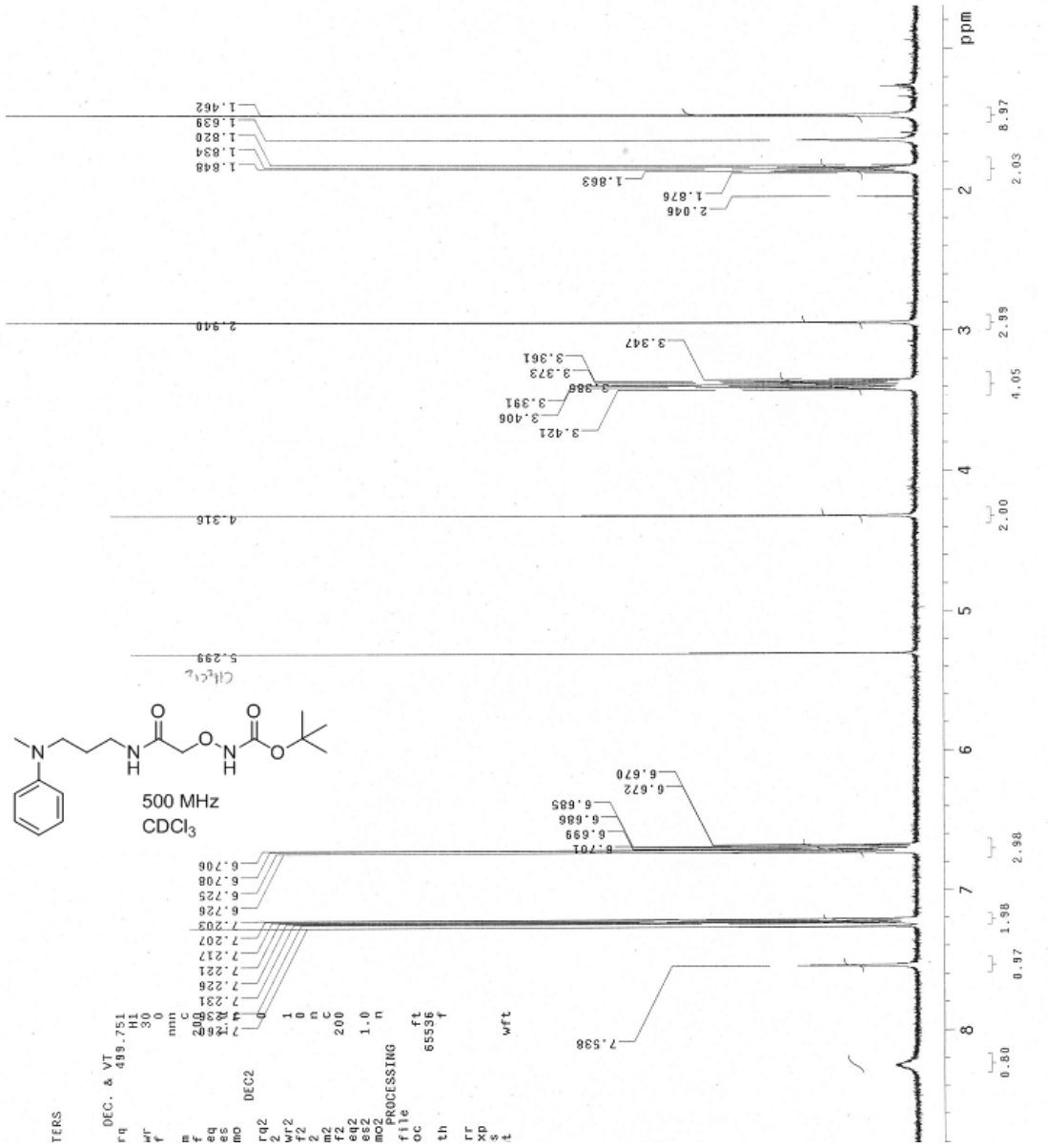
Proton NMR spectrum of **15**



Proton NMR spectrum of **16**



Proton NMR spectrum of **17**



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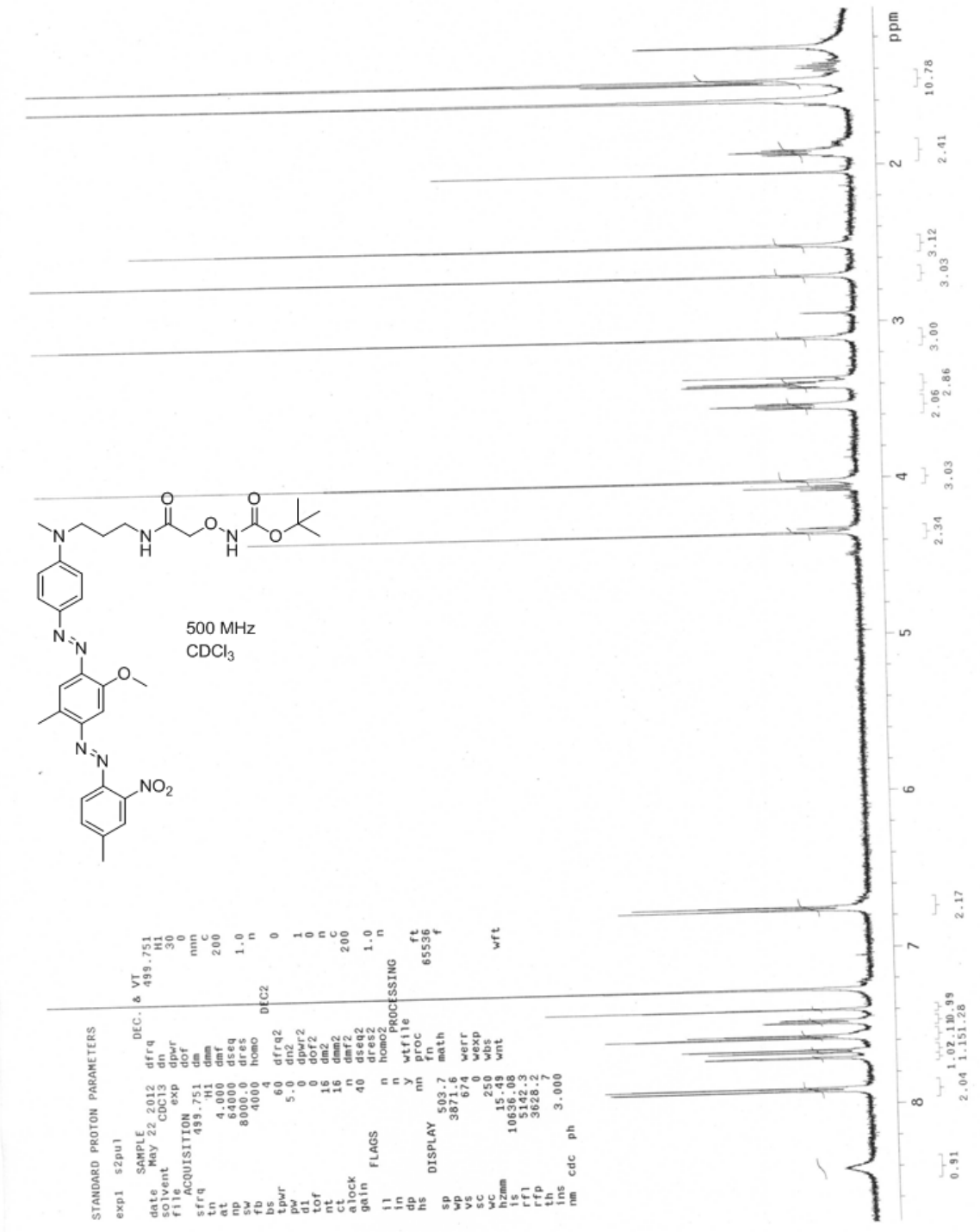
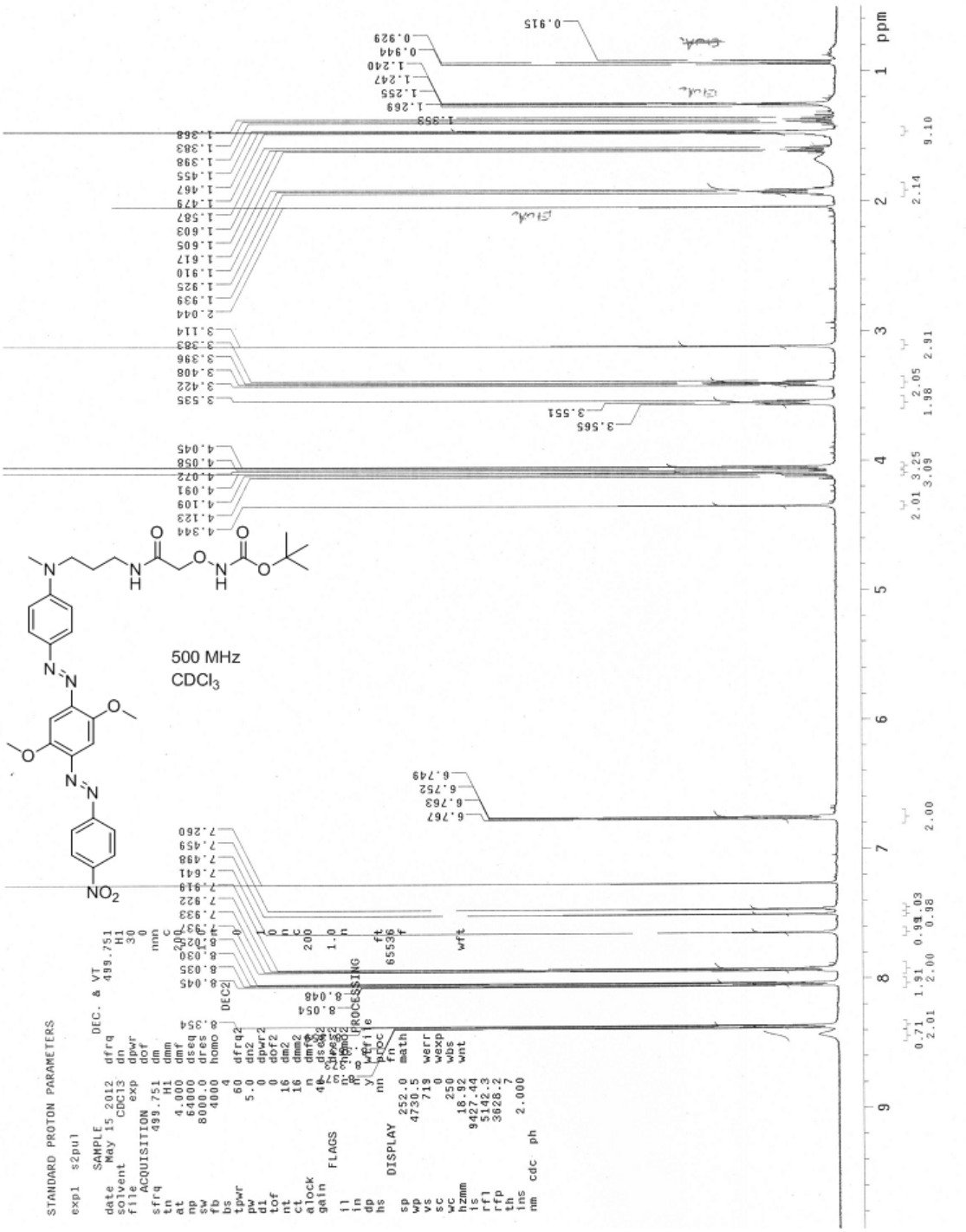
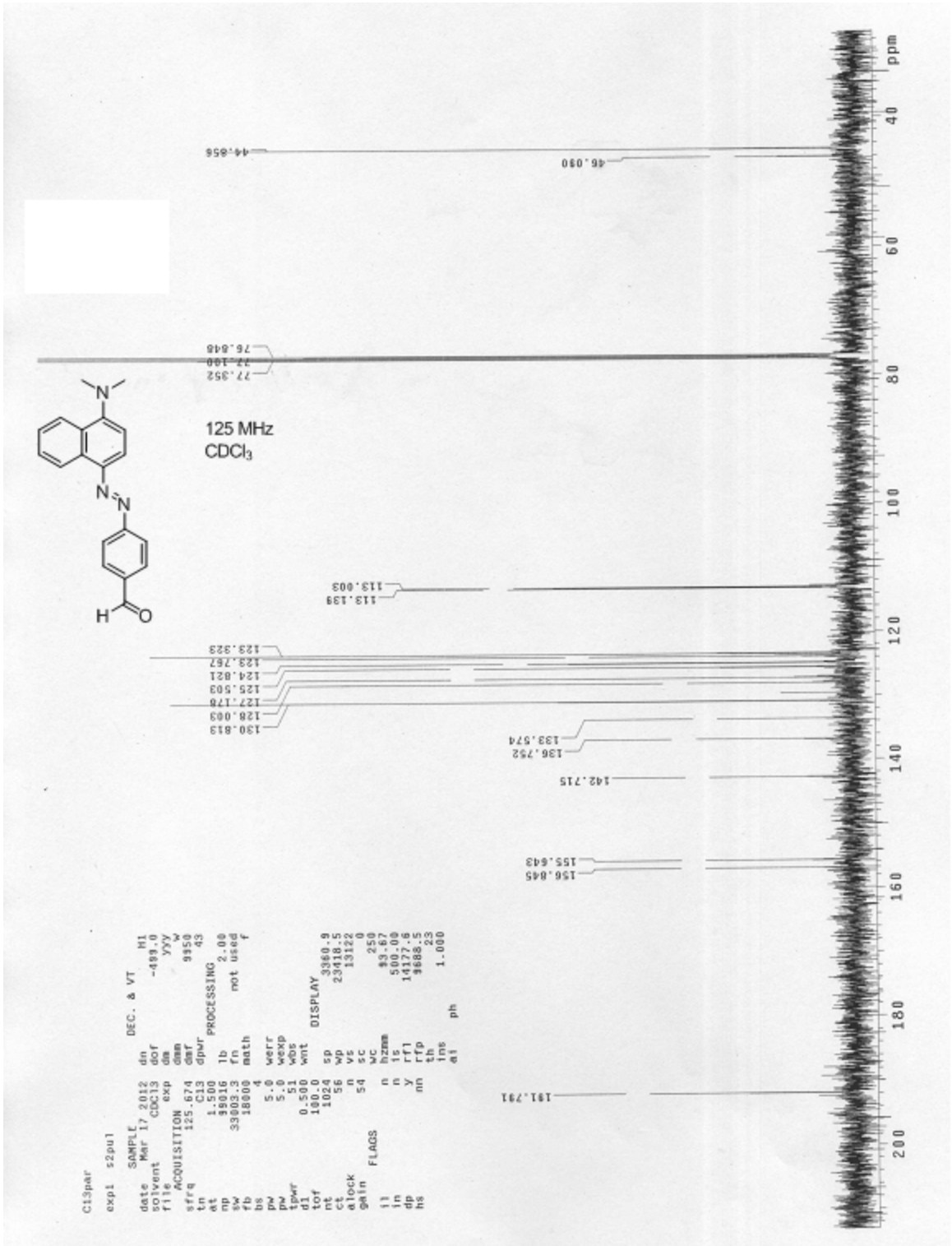


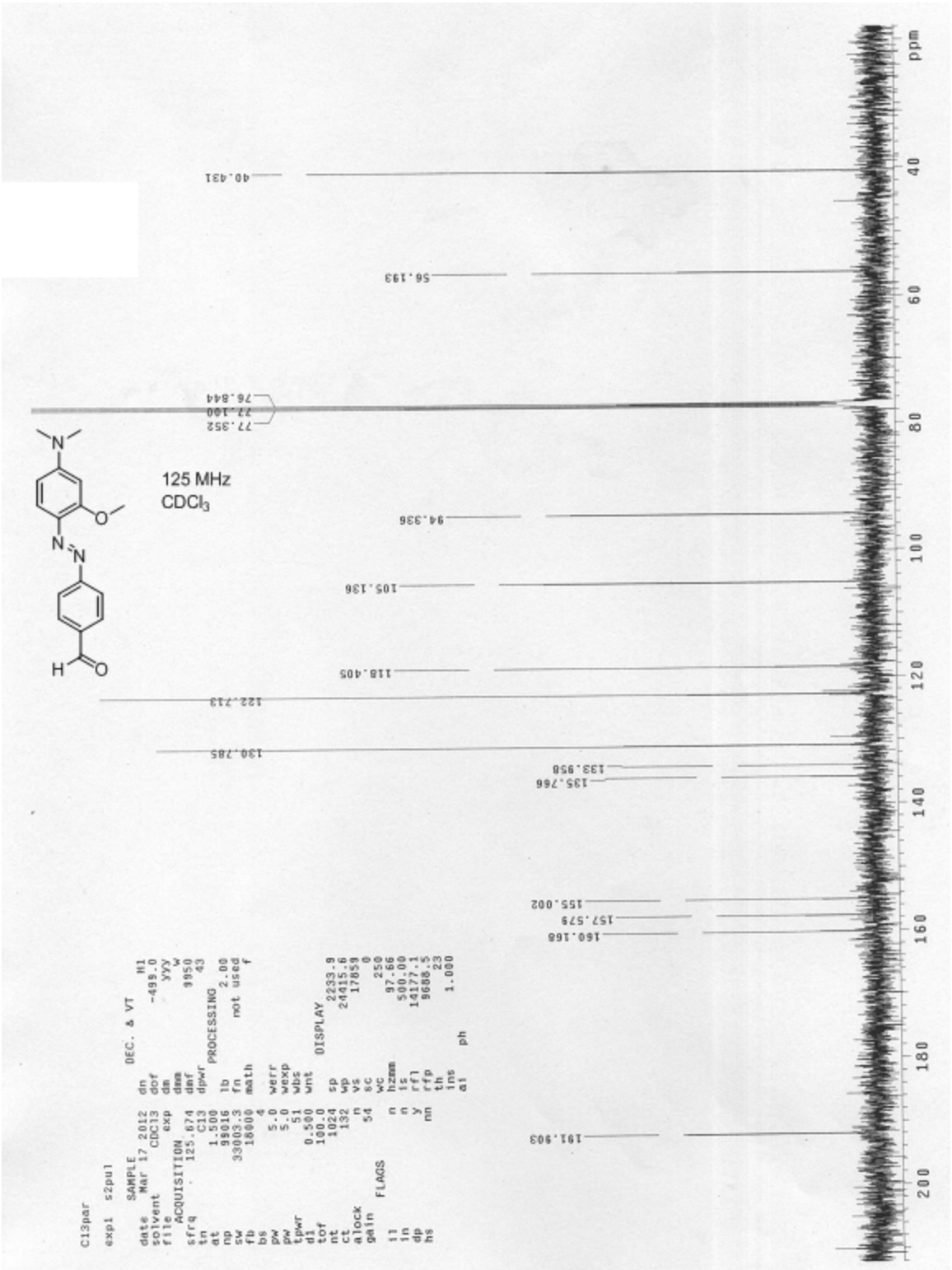
Proton NMR spectrum of **19**



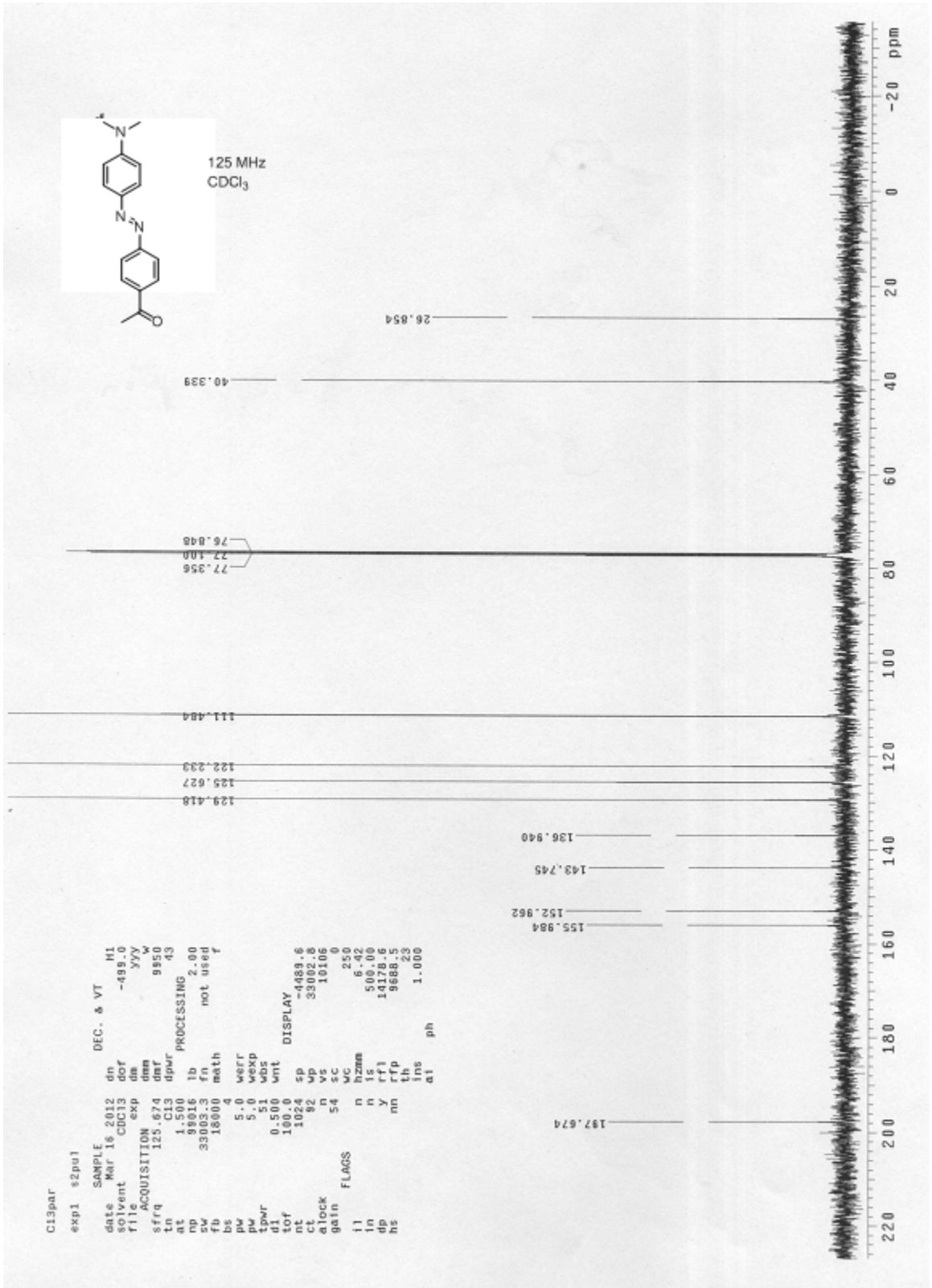
Proton NMR spectrum of **20**



Carbon NMR spectrum of **2**



Carbon NMR spectrum of **3**



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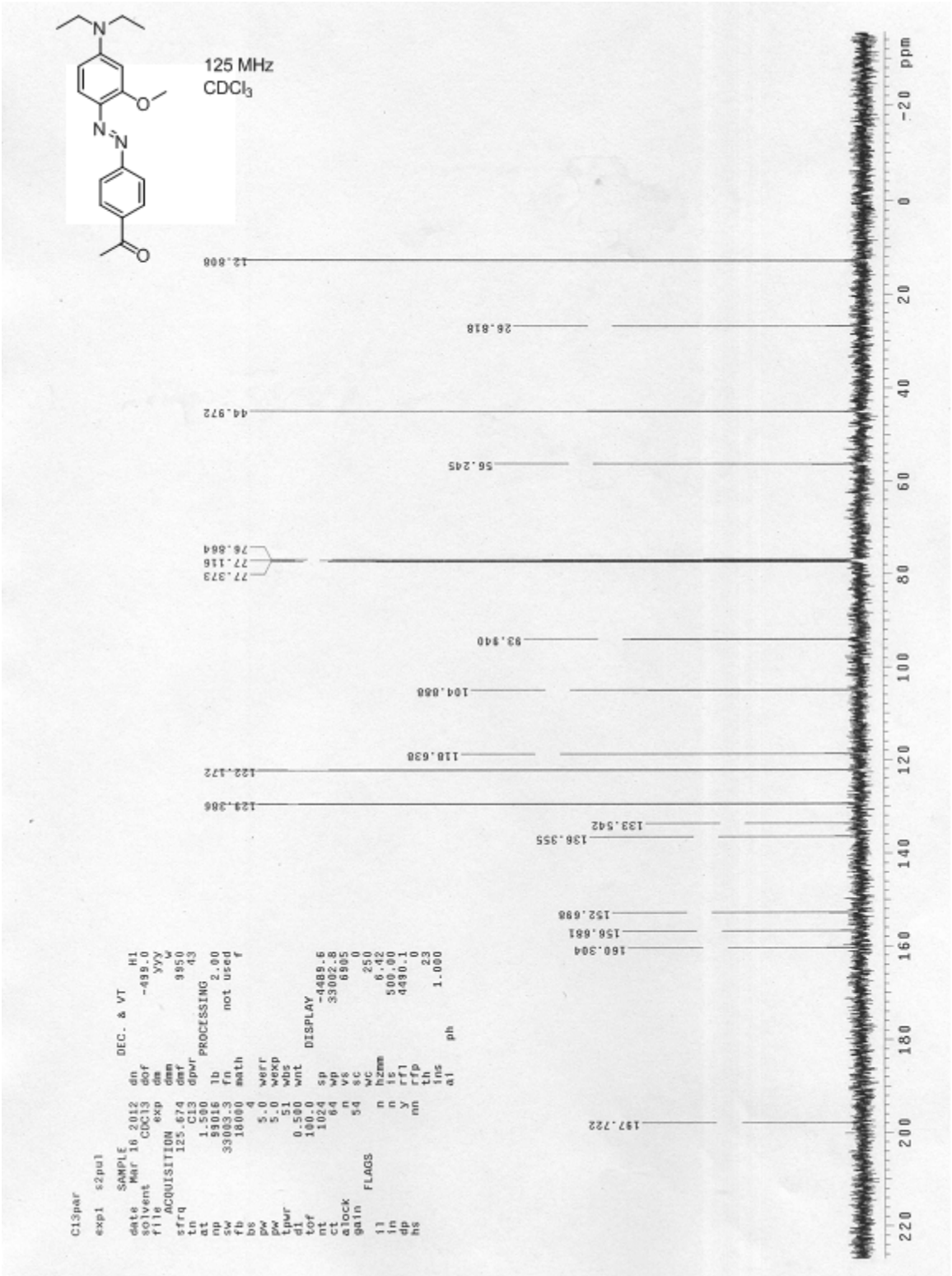

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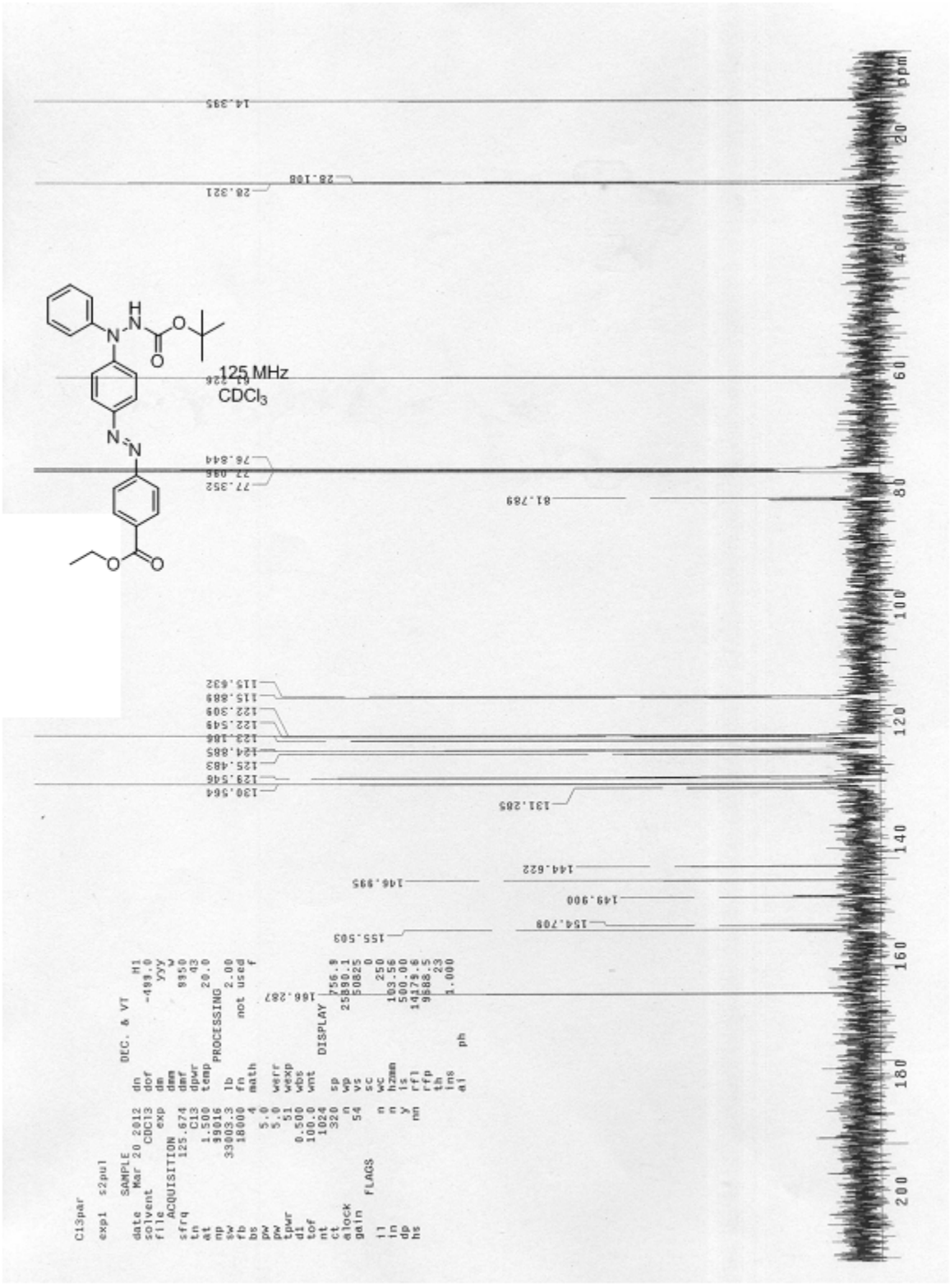
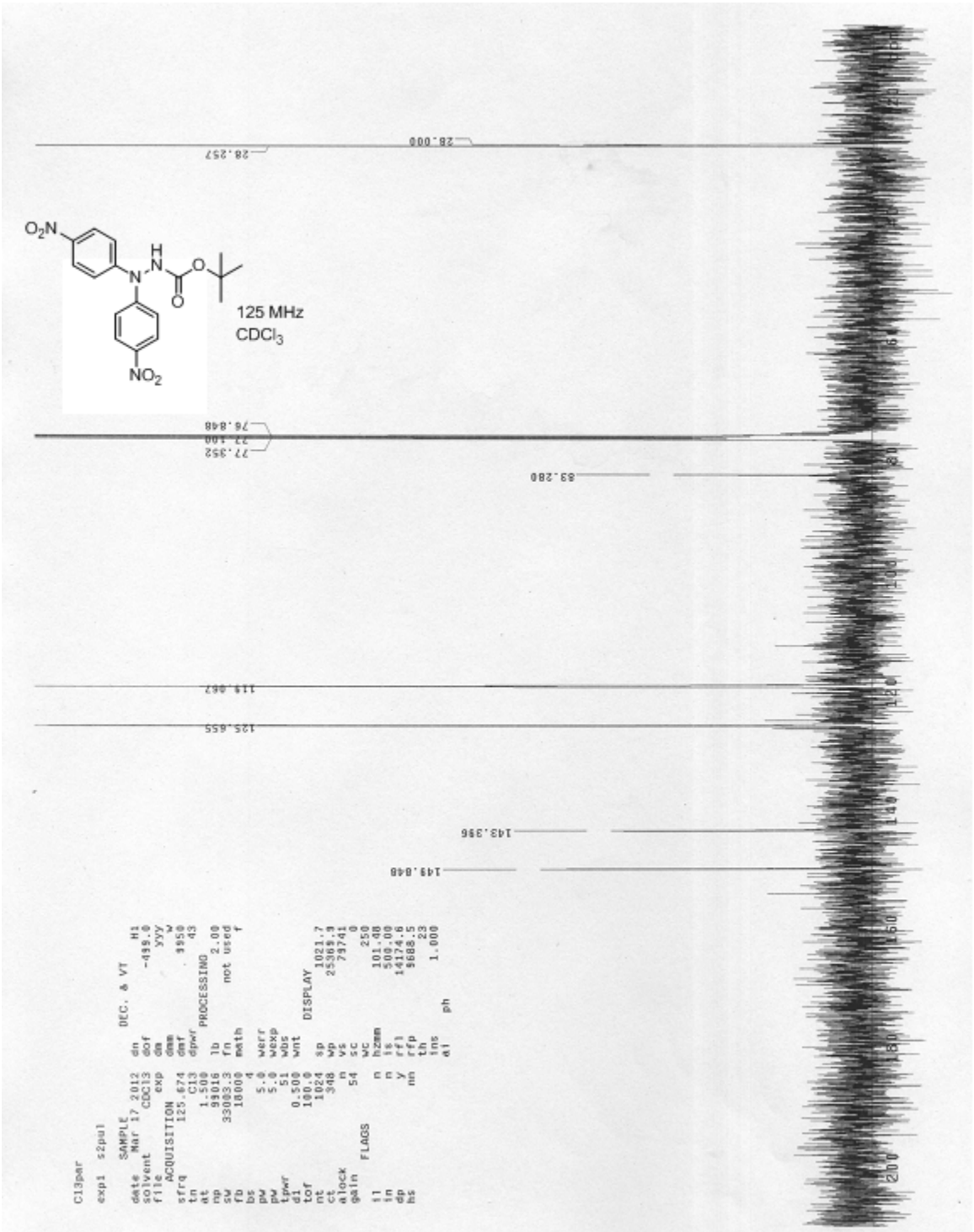


Carbon NMR spectrum of **14**



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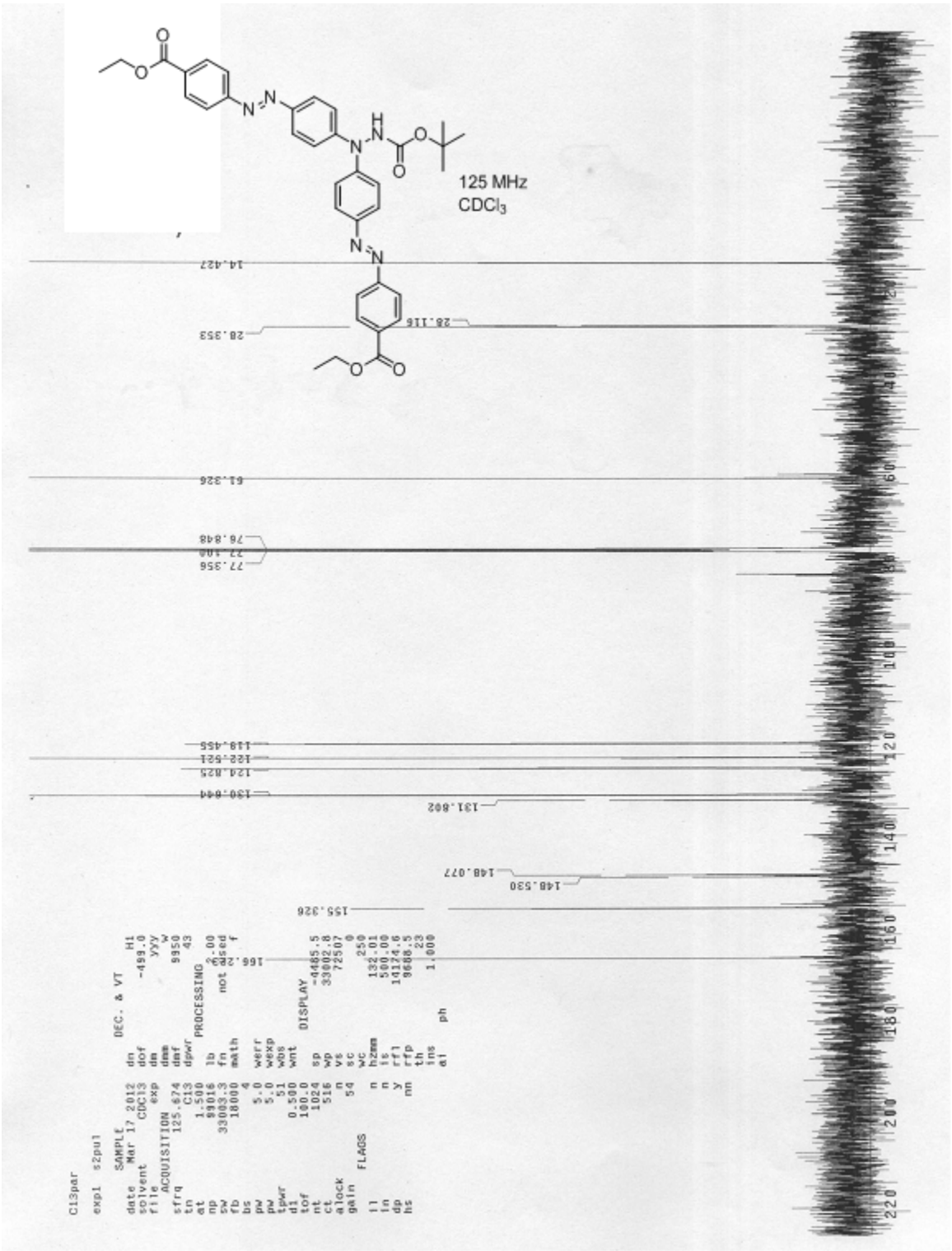
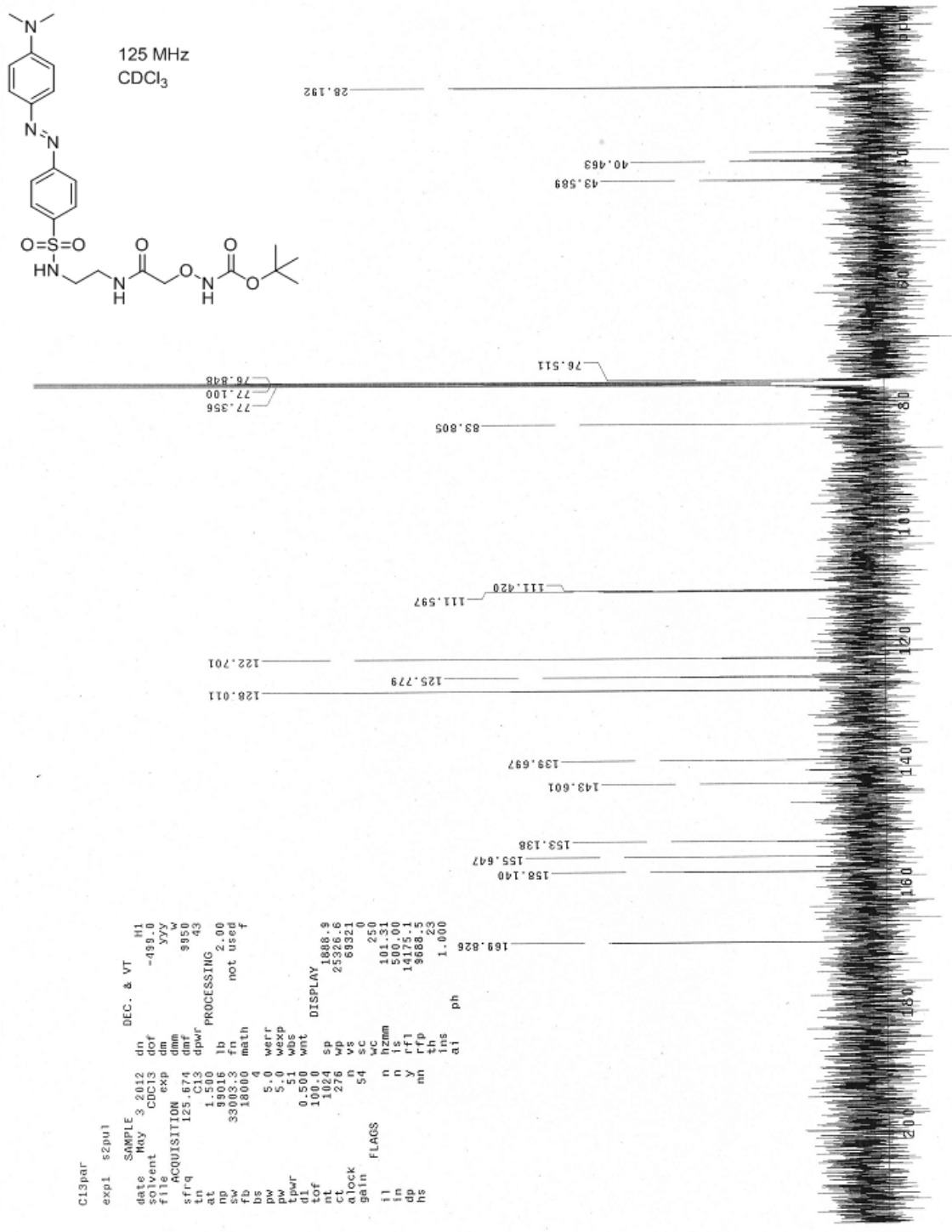
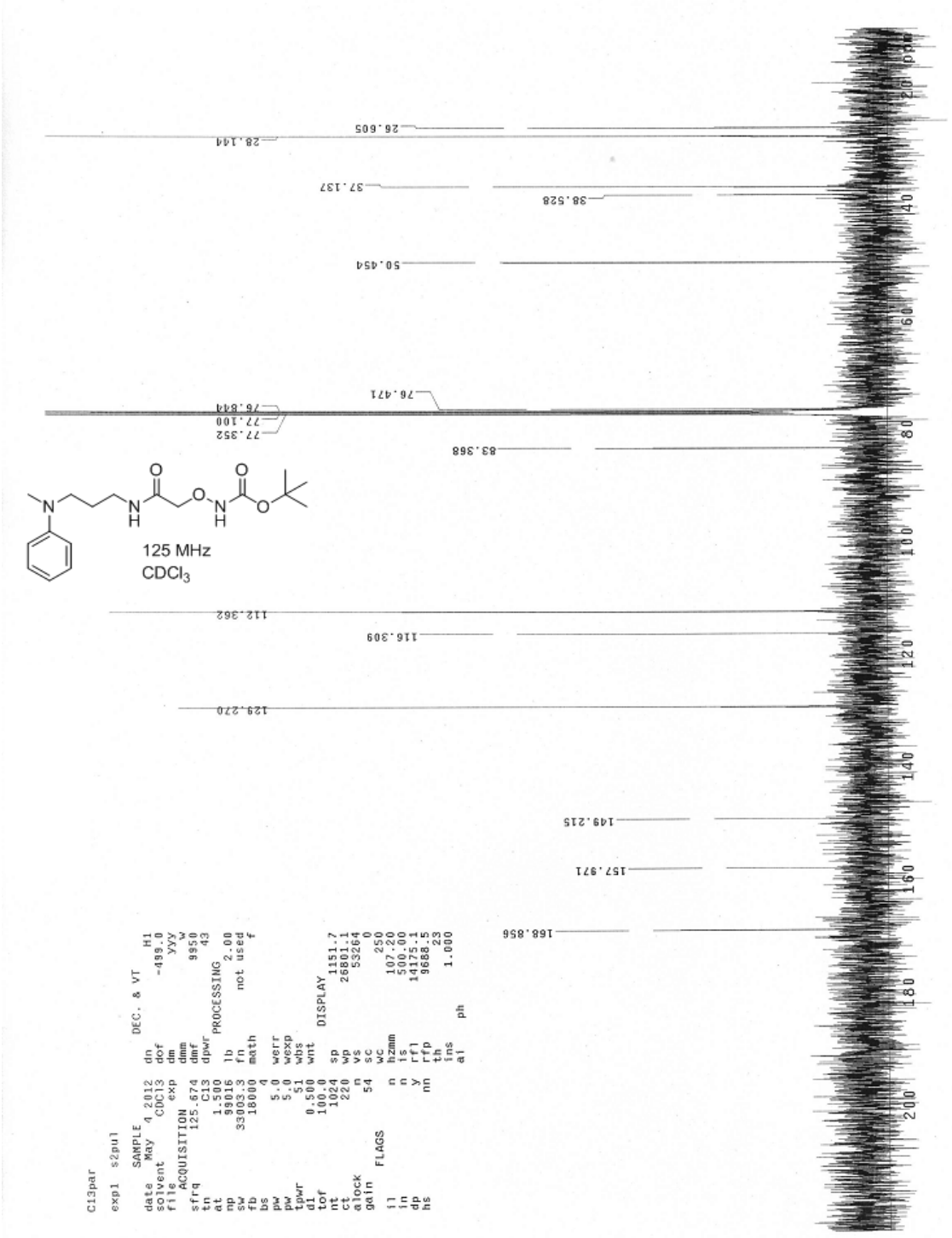


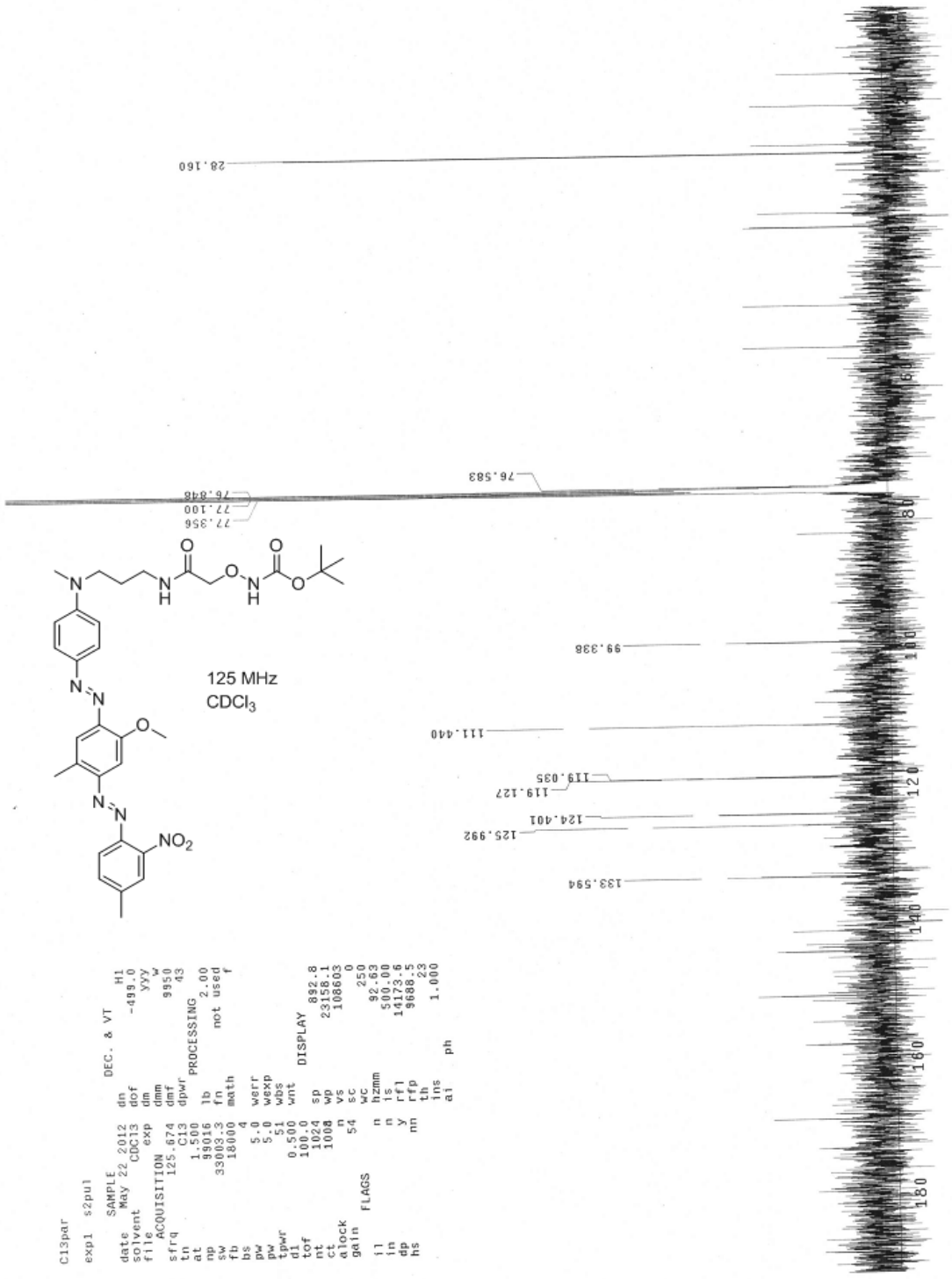
Carbon NMR spectrum of **16**



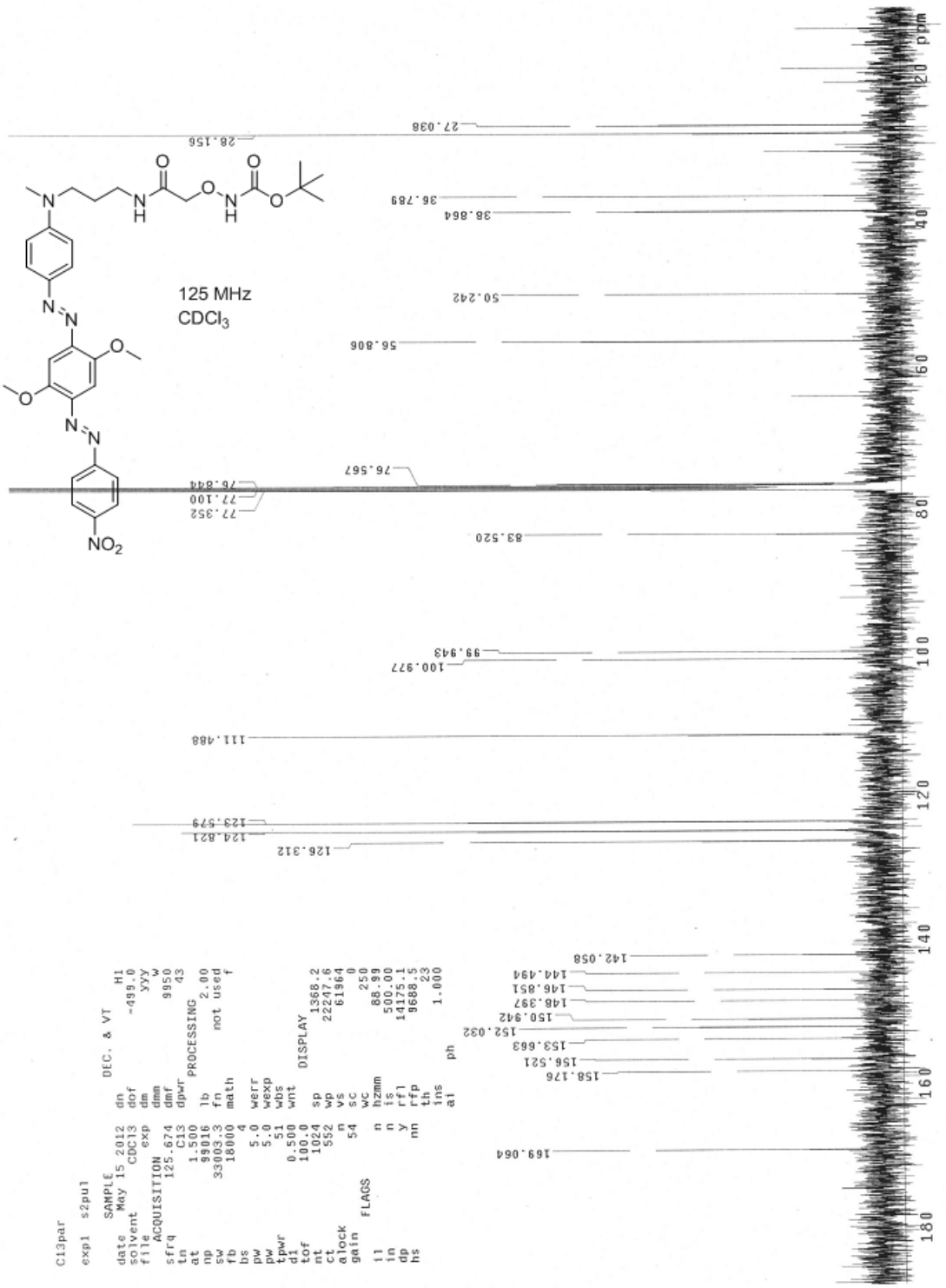
Carbon NMR spectrum of **17**



Carbon NMR spectrum of **18**



Carbon NMR spectrum of **19**



Carbon NMR spectrum of **20**