Supporting Information

Reduction-triggered red fluorescent probes for dual-color detection of oligonucleotide sequences

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CONTENTS:

Supporting experimental section

Reverse phase HPLC separation of regioisomers of compound 1. The separation of regioisomers of compound 1 (5-carboxynaphthorhodamine and 6-carboxynaphthorhodamine) was carried out by reverse phase HPLC (0-80 % acetonitrile/50 mM triethylammonium acetate gradient) using hydrosphere C18 column (250×4.6 mm; YMC Co. Ltd, Japan). Fractions containing same isomer were combined, and the solvent was removed under reduced pressure. The buffer component, triethylammonium acetate, was removed by repeated desalination using C18 column. The position of carboxyl group was determined by analyzing ^{1}H NMR, ^{13}C NMR, and HMBC experiment.

^{1}H and ^{13}C NMR of 5-carboxynaphthorhodamine (isomer II). ^{1}H NMR (600 MHz, CD_3OD) δ 6.93 (2H, brs), 6.99 (2H, d, J = 9.0 Hz), 7.09 (2H, brd, J = 9.1 Hz), 7.48 (2H, d, J = 9.0 Hz), 7.61 (1H, d, J = 7.5 Hz), 8.50 (1H, brd, J = 7.5 Hz), 8.58 (2H, d, J = 9.1 Hz), 9.00 (1H, brs) ^{13}C NMR (150 MHz, CD_3OD) δ 109.96, 114.67, 118.57, 120.27, 123.87, 127.47, 128.60, 131.38, 133.08, 135.05, 141.89, 155.66, 167.81, 168.01

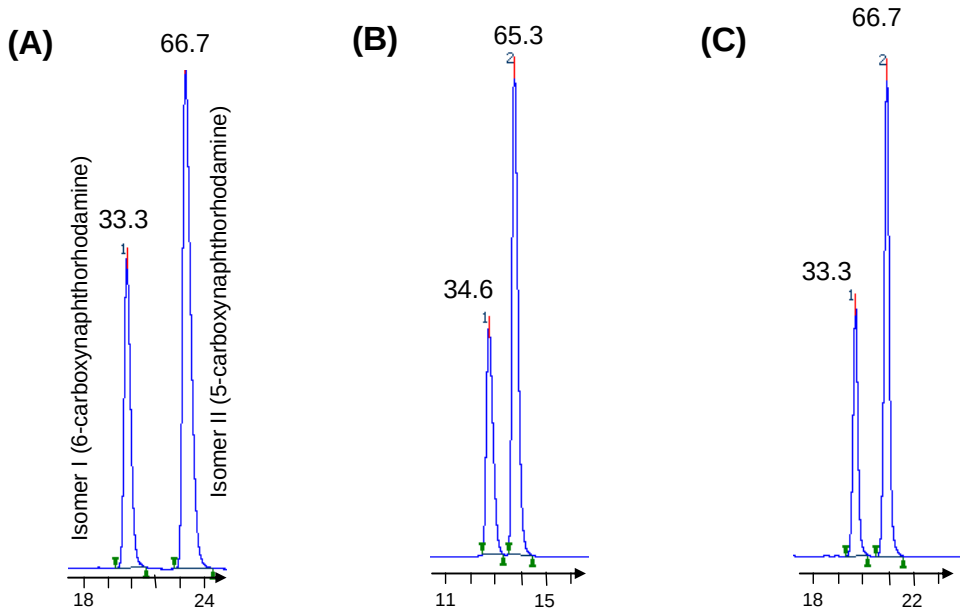


Figure S1. Reverse phase HPLC analysis of the compound 1 (A), 2 (B), and 3 (C). The values indicate that percentage of the area. The HPLC conditions are as follows: 30-30 % (A), 80-80 % (B), or 80-100 % (C), 50 mM triethylammonium acetate / acetonitrile gradient using hydrosphere C18 column (250×4.6 mm; YMC Co., Ltd, Japan). HMBC experiment revealed that isomer I was 6-carboxynaphthorhodamine derivatives and isomer II was 5-carboxynaphthorhodamine derivatives.

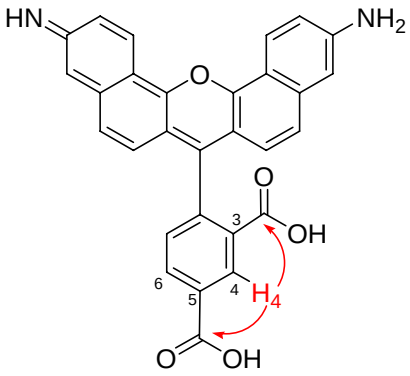
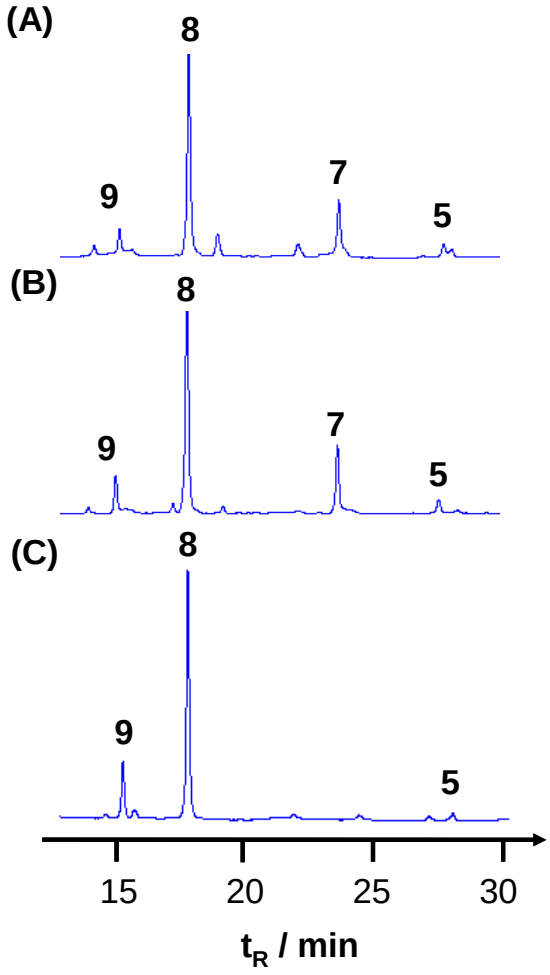


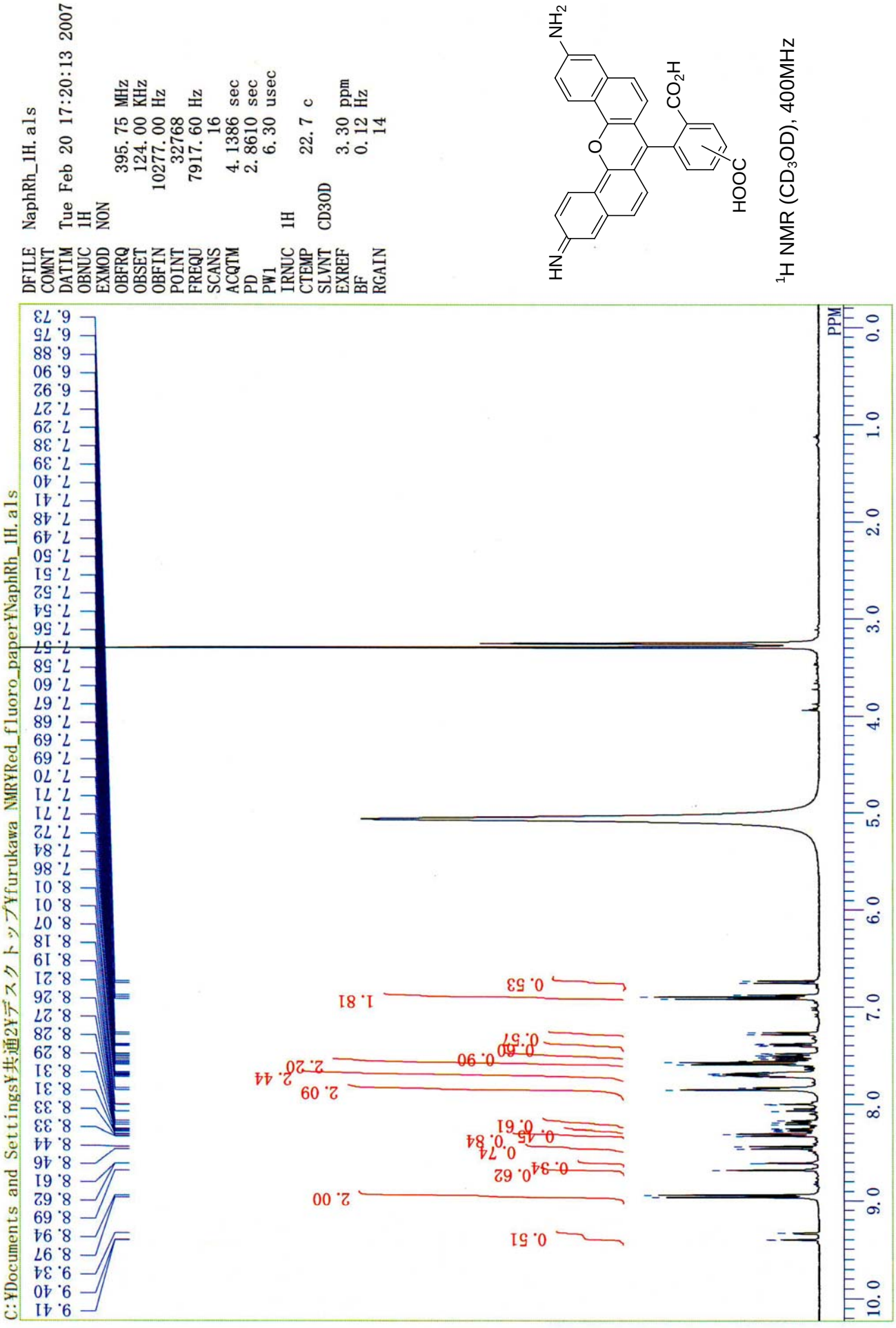
Figure S2. Partial HMBC (1H → 13C) correlations in Isomer II (5-carboxynaphthorhodamine).



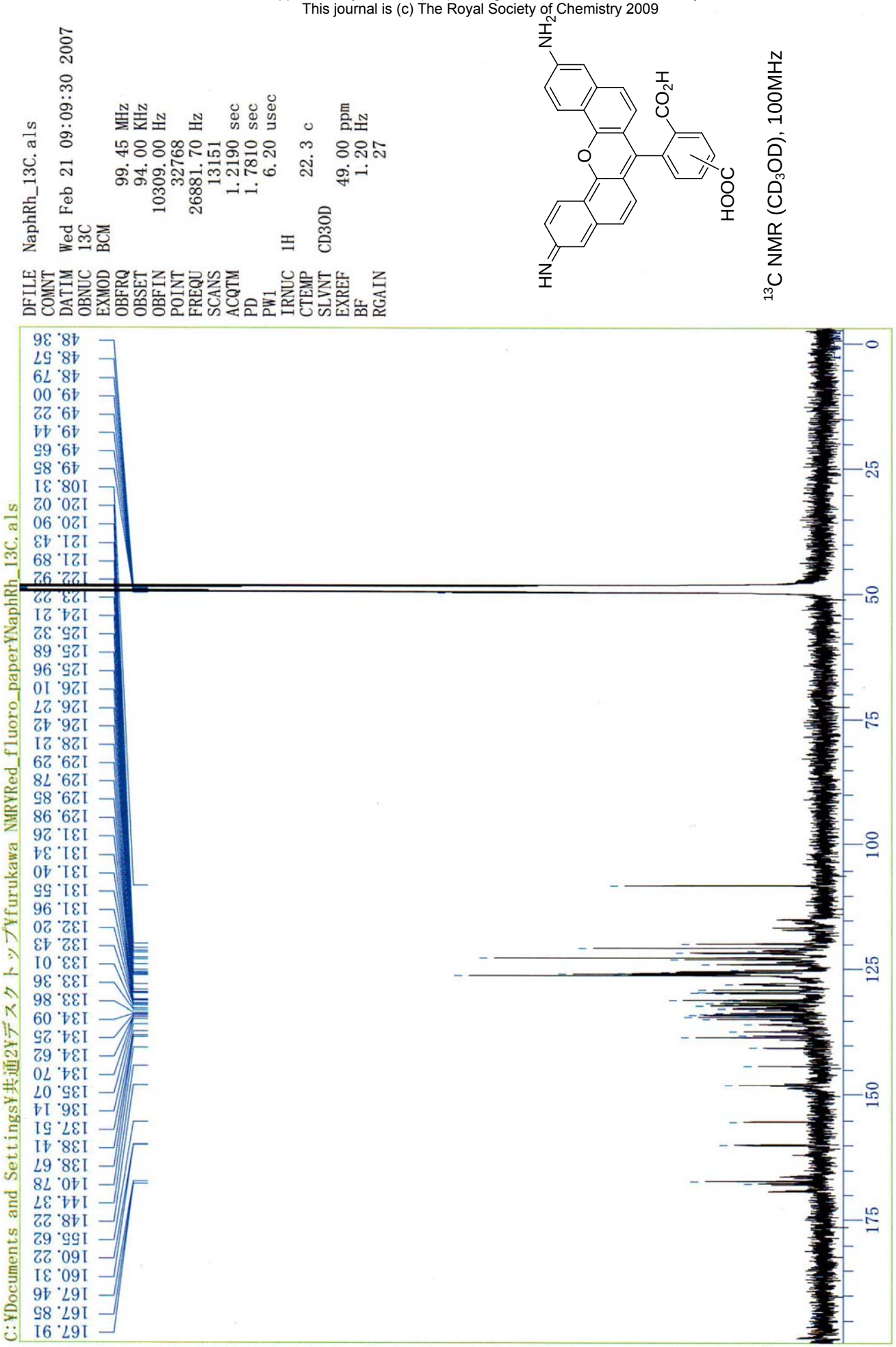
Relative peak area analysis (%)

	1eq. of TPP		2eq. of TPP
	30 min	24 hr	24 hr
Probe **5**	19.2	13.0	9.5
Probe **7**	61.6	57.8	0
Probe **9**	19.2	29.2	90.5

Figure S3. HPLC analysis of the DNA-templated reaction mixture. A) 30 min reaction with 1 equivalent of probe **6**, B) 24 hours reaction with 1 equivalent of probe **6**, C) 24 hours reaction with 2 equivalent of probe **6**. Peak 1, probe **9** (reduced probe **5** with diamino group), calculated: 3042.0, observed: 3041.6; Peak 2, probe **8** (oxidized probe **6**), calculated: 3499.5, observed: 3499.7; Peak 3, probe **7** (reduced probe **5** with monoamino group), calculated: 3067.4, observed: 3067.6; Peak 4, probe **5** with bisazide group, calculated: 3093.3, observed: 3093.6. The reaction products were analyzed by reverse-phase HPLC (0-80 % acetonitrile/50 mM triethylammonium acetate gradient) using hydrosphere C18 column (250×4.6 mm; YMC Co., Ltd, Japan). Each peak was characterized by ESI-mass spectrometry.



^{1}H NMR (CD_3OD), 400MHz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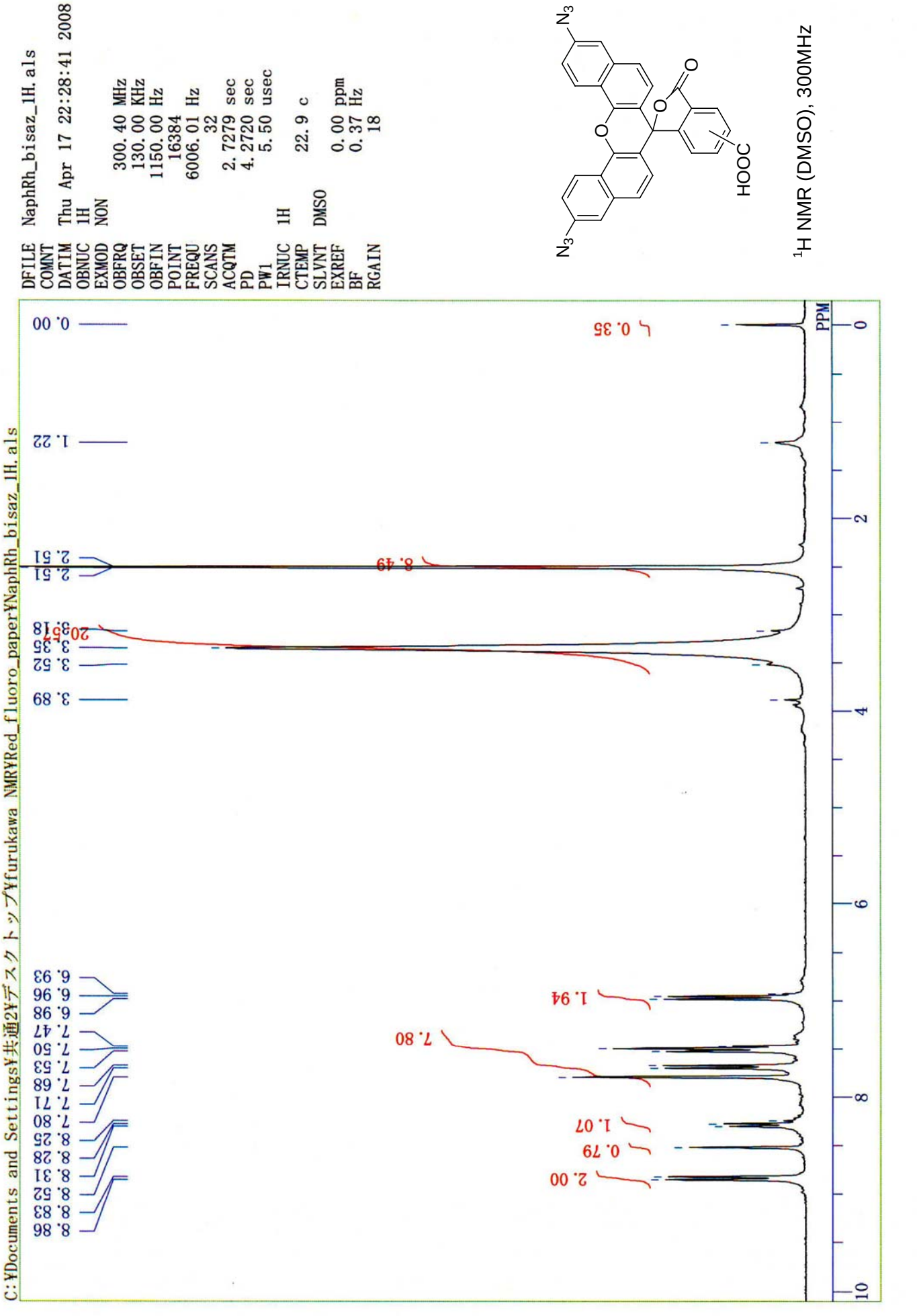
HN
O
CO2H
HOOC
13C NMR (CD3OD), 100MHz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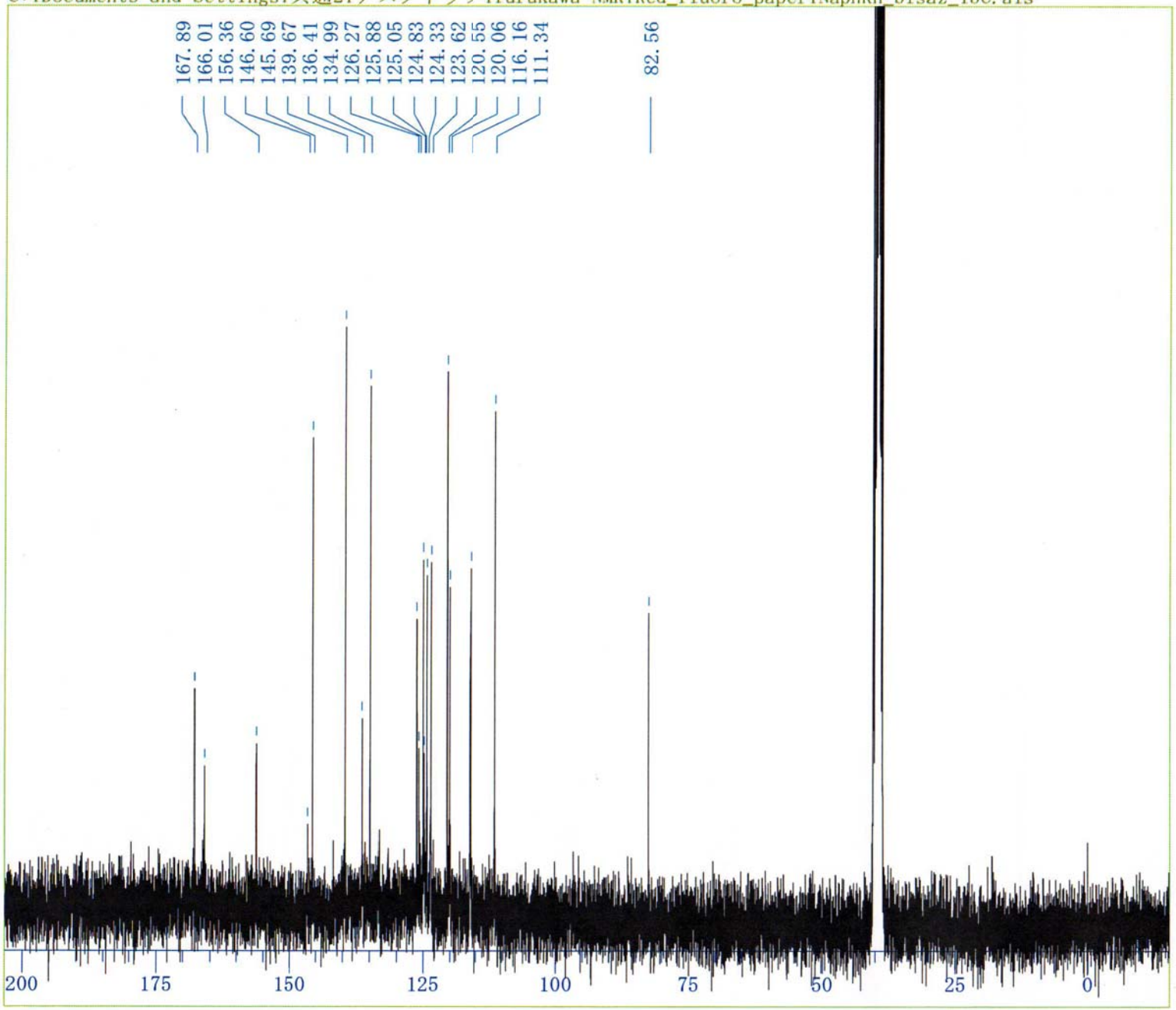


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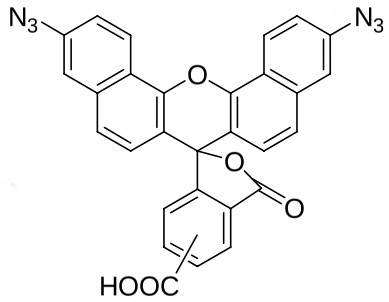

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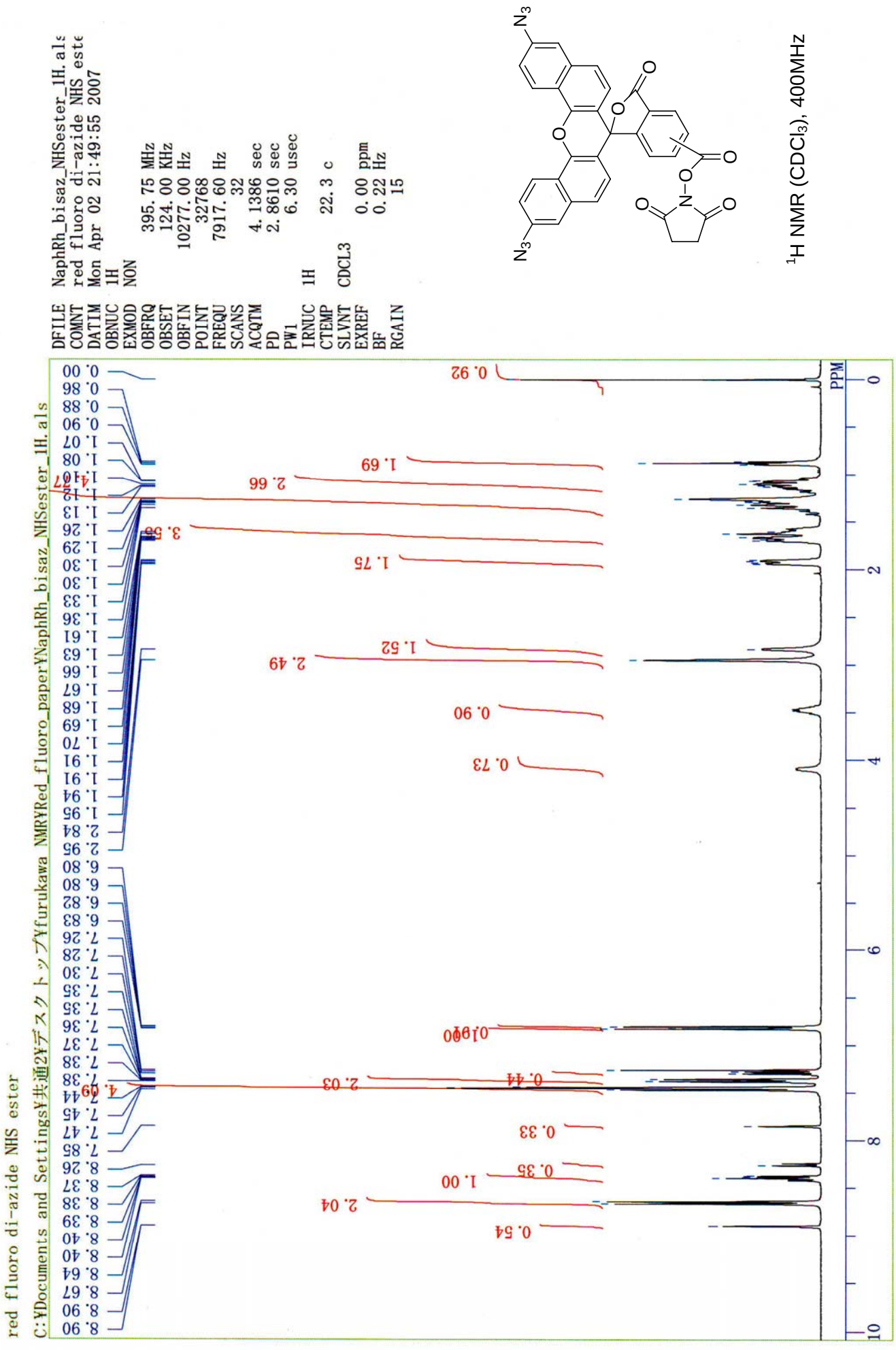

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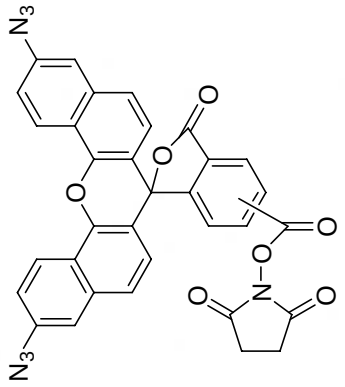

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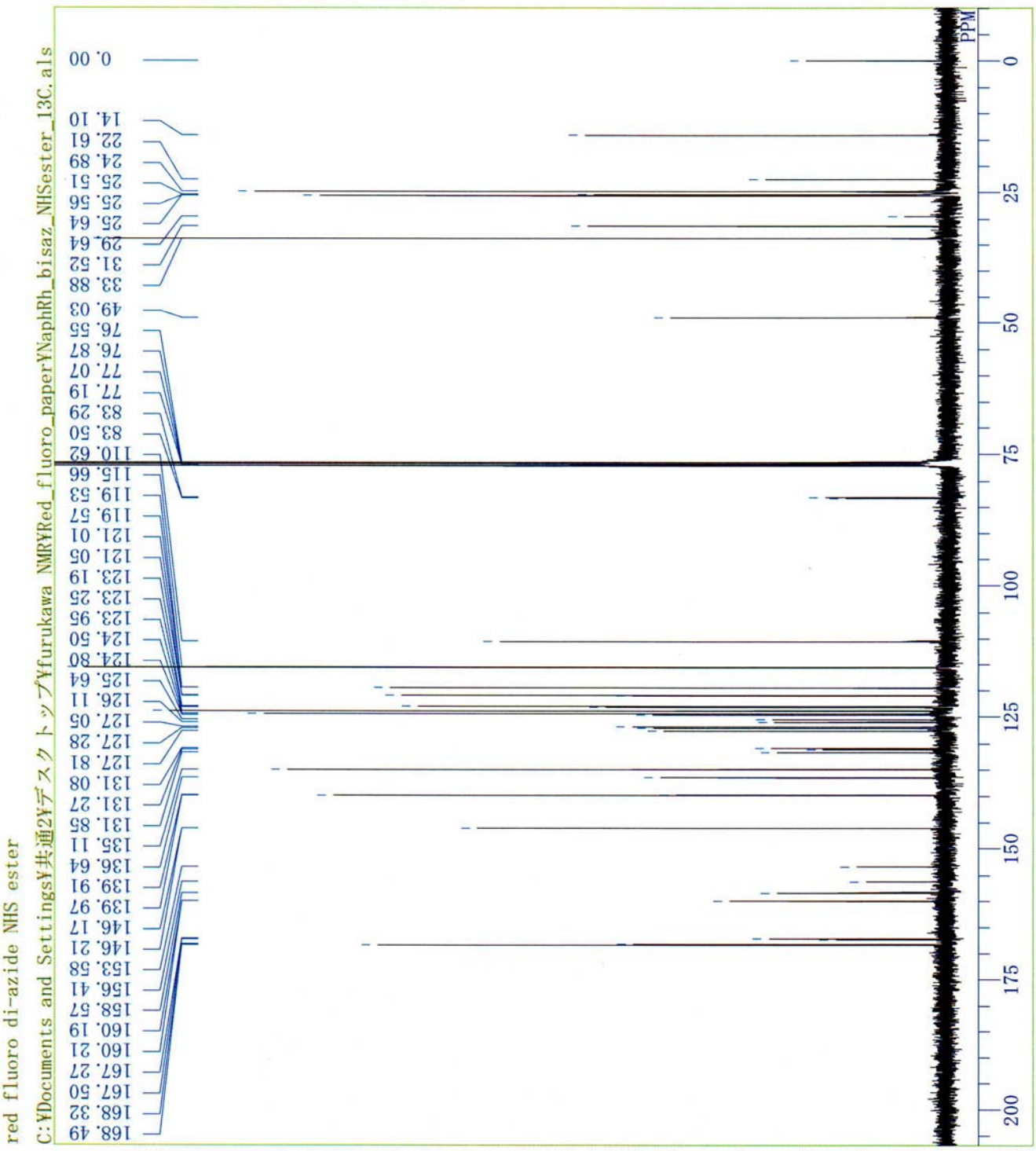
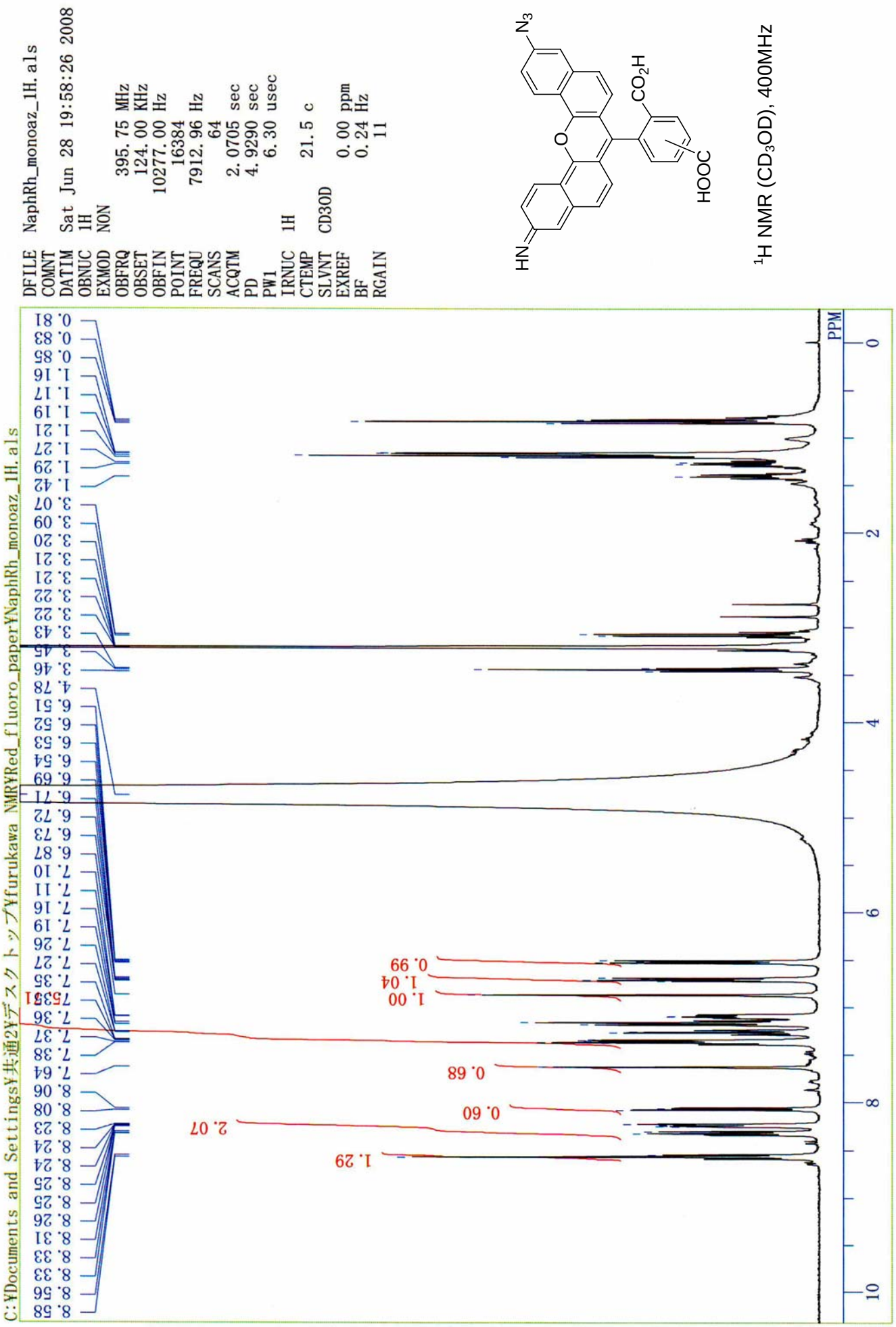
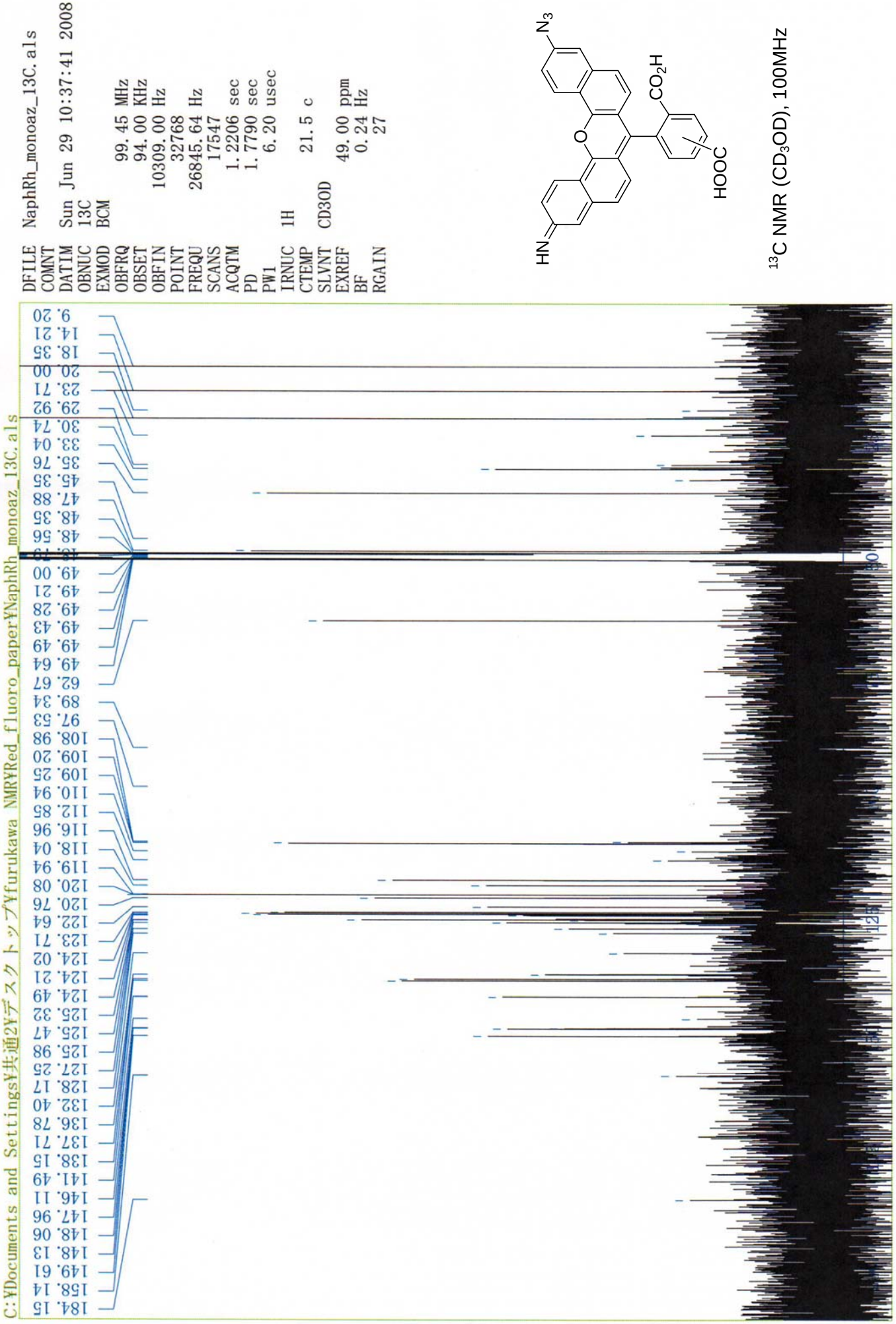




^{1}H NMR (CD_3OD), 400MHz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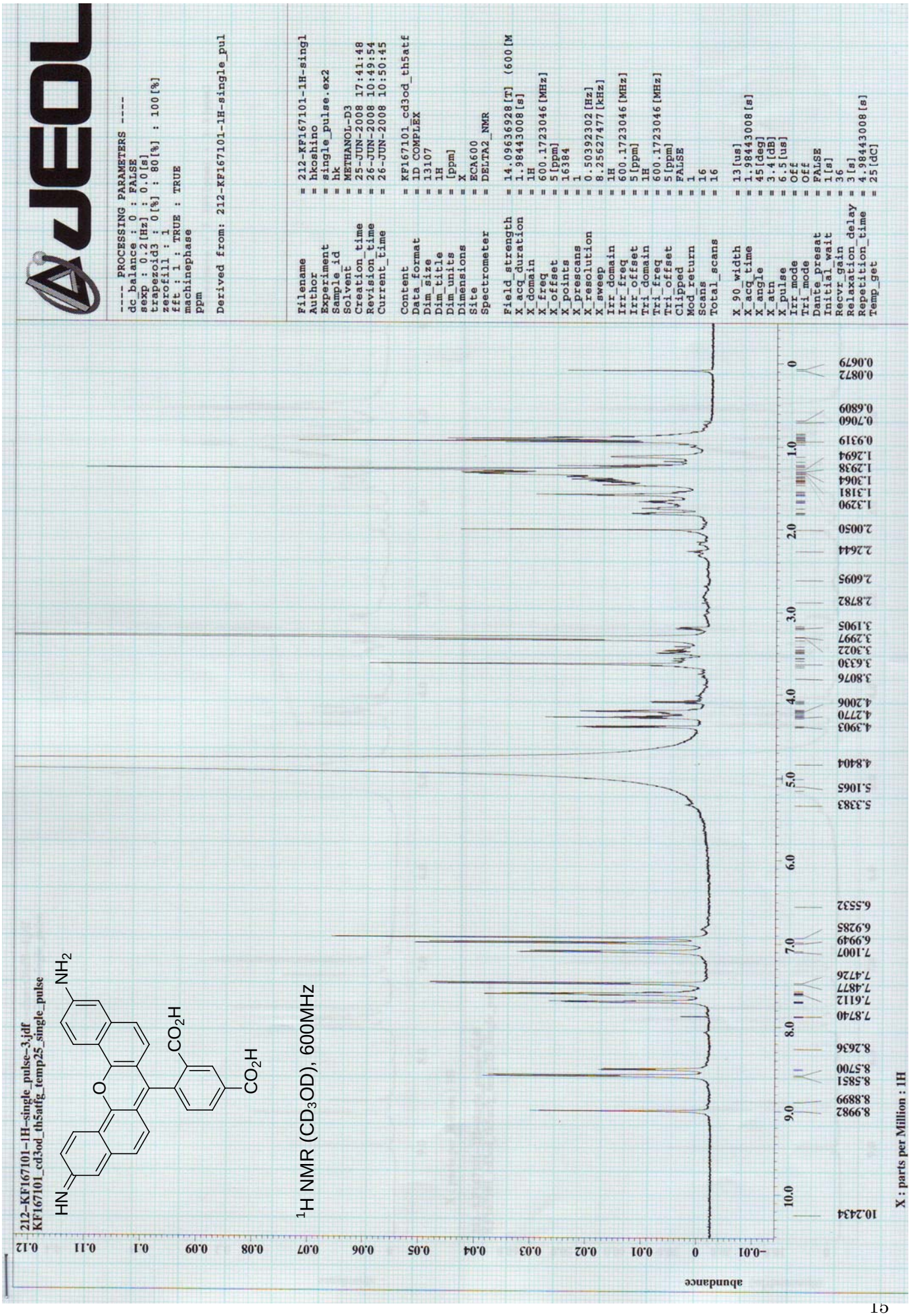
^{13}C NMR (CD_3OD), 100MHz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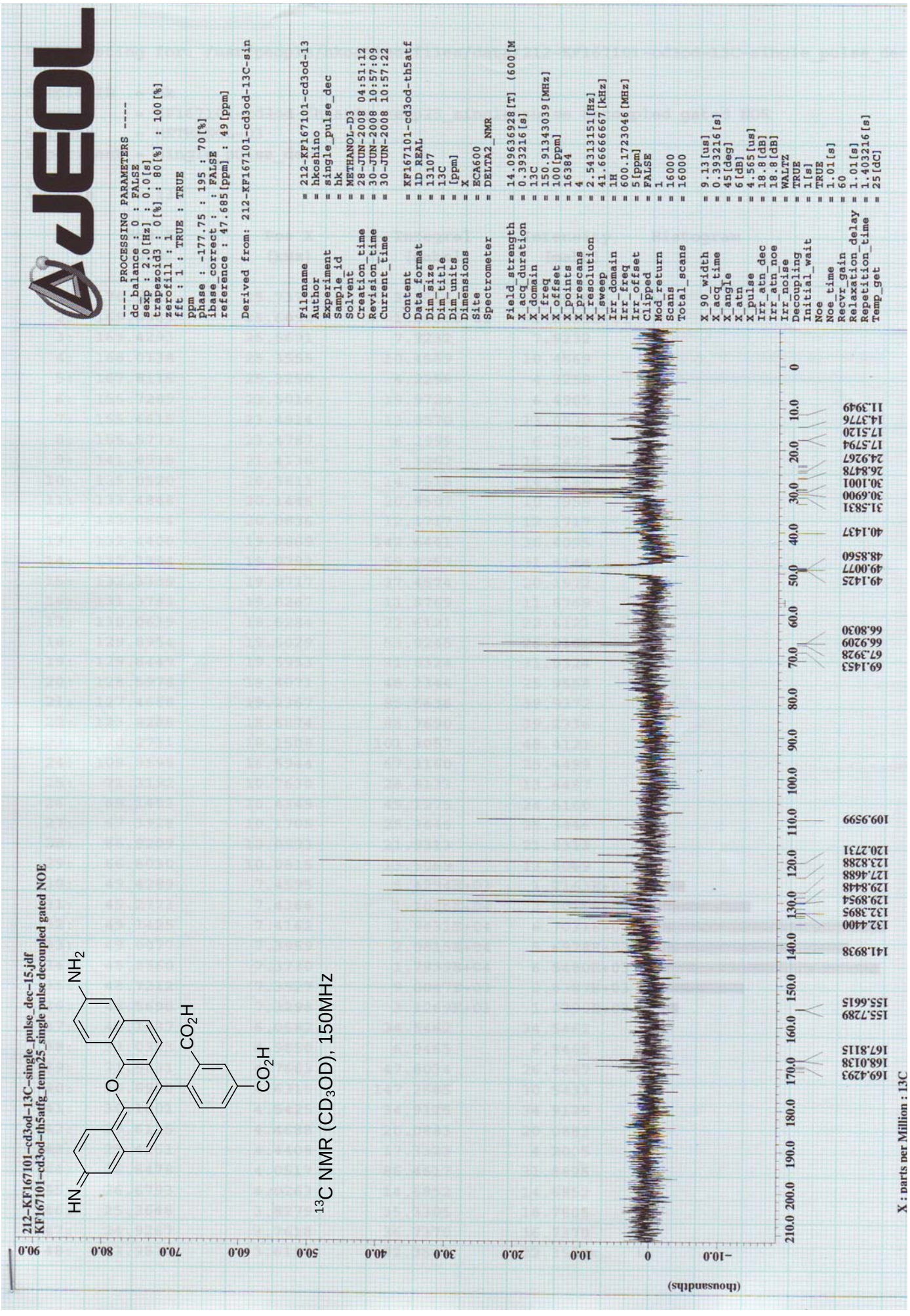
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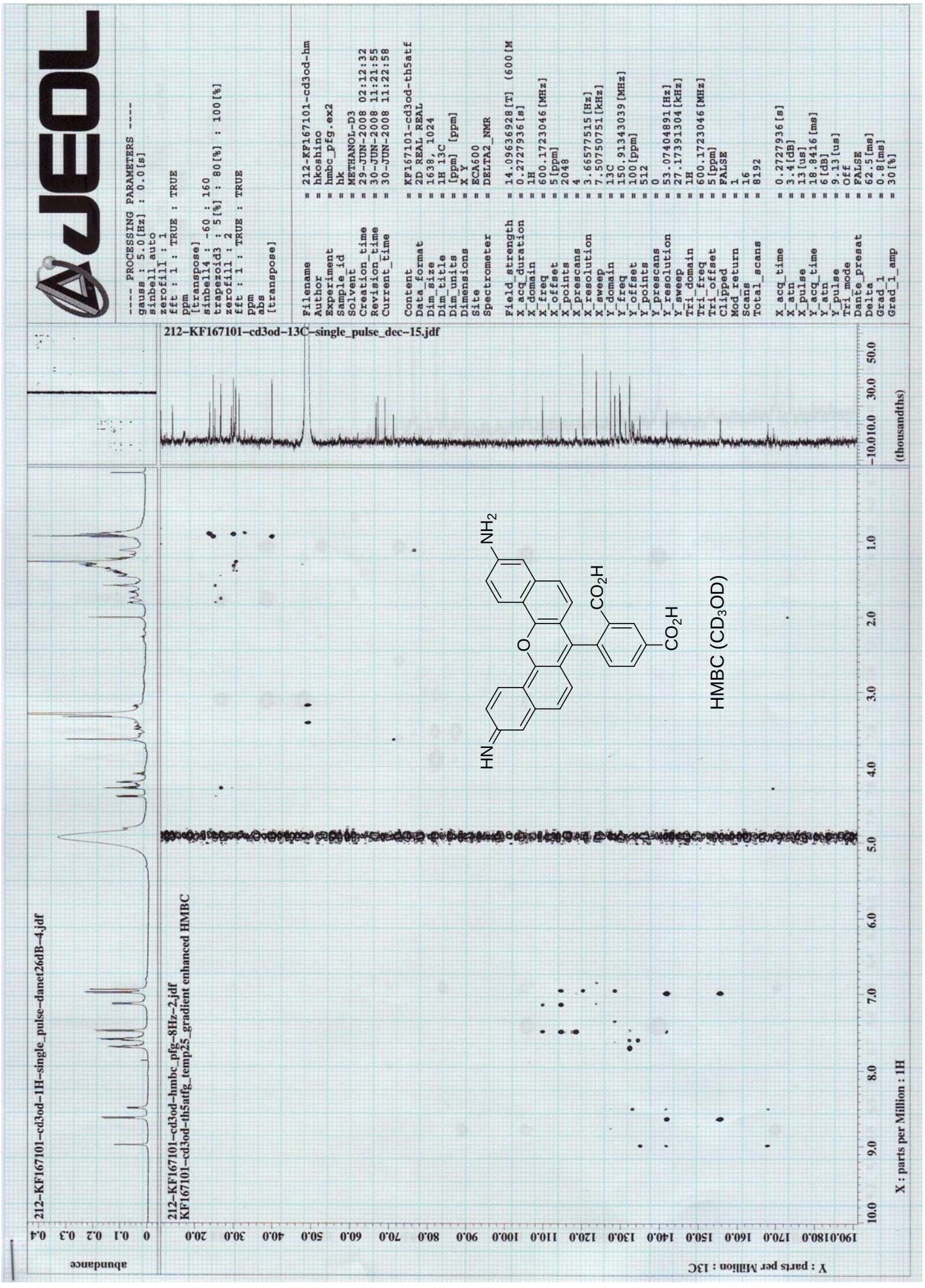
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Y : parts per Million : 13C
(thousandths)