Supporting Information

Structural Determinants for ERK5 (MAPK7) and Leucine Rich Repeat Kinase 2 Activities of benzo[e]pyrimido-[5,4-b]diazepine-6(11H)-ones.

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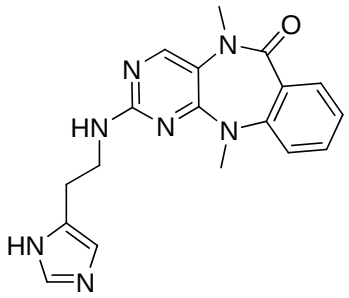
Contents

1. Spectral data of compounds 9, 10, 12 and 24.

Compounds **9**, **10**, **12** and **24** were synthesized by the previous procedures[1].

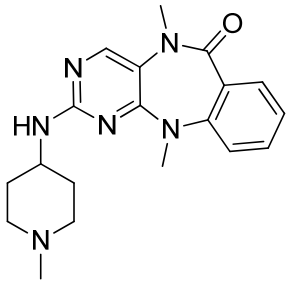
2-((2-(1H-imidazol-5-yl)ethyl)amino)-5,11-dimethyl-5H-benzo[e]pyrimido[5,4-b][1,4]-diazepin-6(11H)-one (9)



^{1}H NMR (600 MHz, CD_3OD) δ 8.07 (s, 1H), 7.70 (dd, *J* = 1.8, 7.8 Hz, 1H), 7.65 (s, 1H), 7.46 (dt, *J* = 1.8, 7.8 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 6.86 (s, 1H), 3.61 (t, *J* = 7.2 Hz, 2H), 3.41 (s, 3H), 3.31 (s, 3H), 2.88 (t, *J* = 7.2 Hz, 2H).

^{13}C NMR (100 MHz, DMSO-d6) δ 167.8, 159.3, 152.8, 149.9, 135.0, 132.7, 132.0, 116.6, 113.9, 108.0, 41.5, 38.0, 35.5, 26.6. MS (ESI) *m/z* 350 $(M+H)^+$, HRMS (ESI) *m/z* calc. 350.1729, measured 350.1721 $(M+H)^+$.

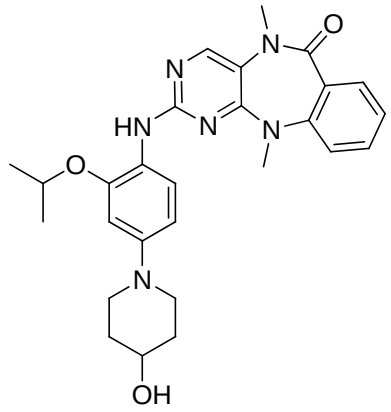
5,11-dimethyl-2-((1-methylpiperidin-4-yl)amino)-5H-benzo[e]pyrimido[5,4-b][1,4]-diazepin-6(11H)-one (10)



^{1}H NMR (600 MHz, CD_3OD) δ 8.14 (s, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.56 (t, *J* = 7.8 Hz, 1H), 7.28-7.24 (m, 2H), 4.16 (brs, 1H), 3.62 (d, *J* = 12.0 Hz, 2H), 3.47 (s, 3H), 3.44 (s, 3H), 3.20 (t, *J* = 12.6 Hz, 2H), 2.91 (s, 3H), 2.34 (d, *J* = 13.2 Hz, 2H), 1.87 (q, *J* = 13.2 Hz, 2H).

^{13}C NMR (100 MHz, DMSO-d6) δ 167.5, 158.8, 158.4, 158.1, 152.4, 149.7, 132.8, 132.1, 126.7, 123.8, 118.0, 53.3, 43.0, 40.9, 37.9, 35.7, 29.4. MS (ESI) *m/z* 353 $(M+H)^+$. HRMS (ESI) *m/z* calc. 353.2090, measured 353.2102 $(M+H)^+$.

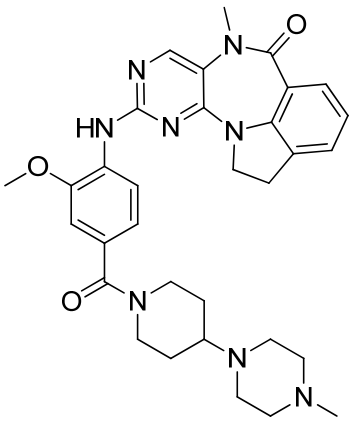
2-((4-(4-hydroxypiperidin-1-yl)-2-isopropoxyphenyl)amino)-5,11-dimethyl-5H-benzo[e] pyrimido[5,4-b][1,4]diazepin-6(11H)-one (12)



^{1}H NMR (600 MHz, CD_3OD) δ 8.17 (s, 1H), 8.12 (d, *J* = 9.0 Hz, 1H), 7.71 (dd, *J* = 1.2, 7.8 Hz, 1H), 7.45 (dt, *J* = 1.8, 7.8 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H), 6.60 (dd, *J* = 3.0, 9.0 Hz, 1H), 4.64-4.60 (m, 1H), 3.74-3.70 (m, 1H), 3.48-3.44 (m, 2H), 3.43 (s, 3H), 3.36 (s, 3H), 2.84-2.80 (m, 2H), 1.97-1.95 (m, 2H), 1.69-1.63 (m, 2H), 1.33 (d, *J* = 6.6 Hz, 6H).

^{13}C NMR (100 MHz, DMSO-d6) δ 167.5, 163.5, 156.6, 152.7, 149.8, 149.5, 148.1, 132.8, 132.1, 126.7, 123.8, 121.9, 121.6, 120.4, 118.2, 108.2, 103.6, 71.2, 66.5, 47.7, 39.9, 36.0, 34.4, 22.3. MS (ESI) *m/z* 489 $(M+H)^+$. HRMS (ESI) *m/z* calc. 489.2614, measured 489.2614 $(M+H)^+$.

2-((2-methoxy-4-(4-(4-methylpiperazin-1-yl)piperidine-1-carbonyl)phenyl)amino)-5-methyl-10,11-dihydropyrimido[4',5':2,3][1,4]diazepino[6,7,1-hi]indol-6(5H)-one (24)



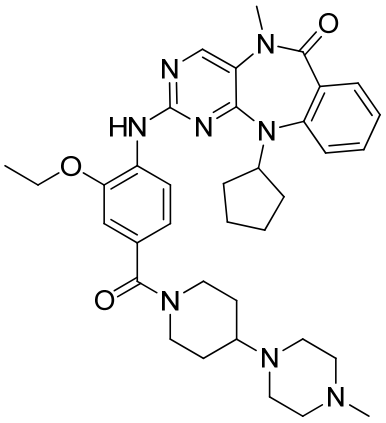
^{1}H NMR (600 MHz, CD_3OD) δ 8.11 (d, *J* = 8.4 Hz, 1H), 7.99 (s, 1H), 7.88 (d, *J* = 7.2 Hz, 1H), 7.44 (dd, *J* = 1.2, 7.8 Hz, 1H), 7.16 (d, *J* = 1.2 Hz, 1H), 7.14-7.11 (m, 2H), 4.70 (brs, 1H), 4.39

(t, *J* = 8.4 Hz, 2H), 3.96 (s, 3H), 3.55-3.45 (m, 4H), 3.41 (s, 3H), 3.40-3.32 (m, 6H), 3.25-3.21 (m, 4H), 2.65 (s, 3H), 2.20-1.95 (m, 2H), 1.75-1.65 (m, 2H).

^{13}C NMR (100 MHz, DMSO-d6) δ 169.2, 166.7, 159.3, 158.7, 158.3, 155.3, 151.4, 148.5, 147.6, 133.2, 130.3, 129.4, 123.0, 119.9, 119.4, 119.2, 118.7, 110.3, 61.5, 58.5, 56.7, 47.6, 46.4, 43.8, 43.2, 41.8, 38.9, 26.8. MS (ESI) *m/z* 583 $(M+H)^+$. HRMS (ESI) *m/z* calc. 583.3145, measured 583.3143 $(M+H)^+$.

Compound **26** was synthesized by the same procedures as the preparation of compound **25**.

11-cyclopentyl-2-((2-ethoxy-4-(4-(4-methylpiperazin-1-yl)piperidine-1-carbonyl)-phenyl)amino)-5-methyl-5H-benzo[e]pyrimido[5,4-b][1,4]diazepin-6(11H)-one (26)

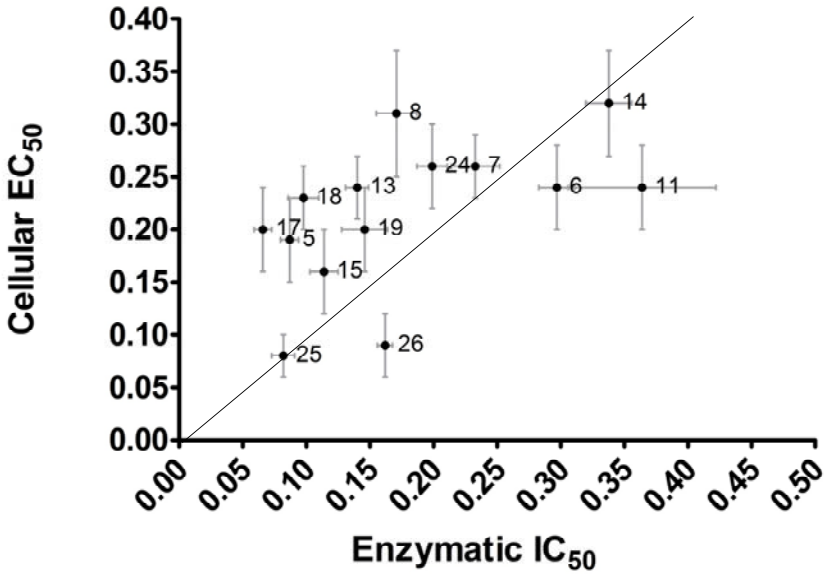


^{1}H NMR (600 MHz, CD_3OD) δ 8.49 (d, *J* = 8.4 Hz, 1H), 8.36 (s, 1H), 7.64 (dd, *J* = 1.8, 7.8 Hz, 1H), 7.48-7.45 (m, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 7.18 (t, *J* = 7.8 Hz, 1H), 7.08-7.07 (m, 2H), 4.82-4.78 (m, 1H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.55-3.48 (m, 6H), 3.45-3.30 (m, 9H), 3.29-3.25 (m, 2H), 2.93 (s, 3H), 2.36-2.33 (m, 1H), 2.15-2.10 (m, 3H), 1.70-1.57 (m, 6H), 1.56-1.54 (m, 1H), 1.47 (t, *J* = 7.2 Hz, 3H).

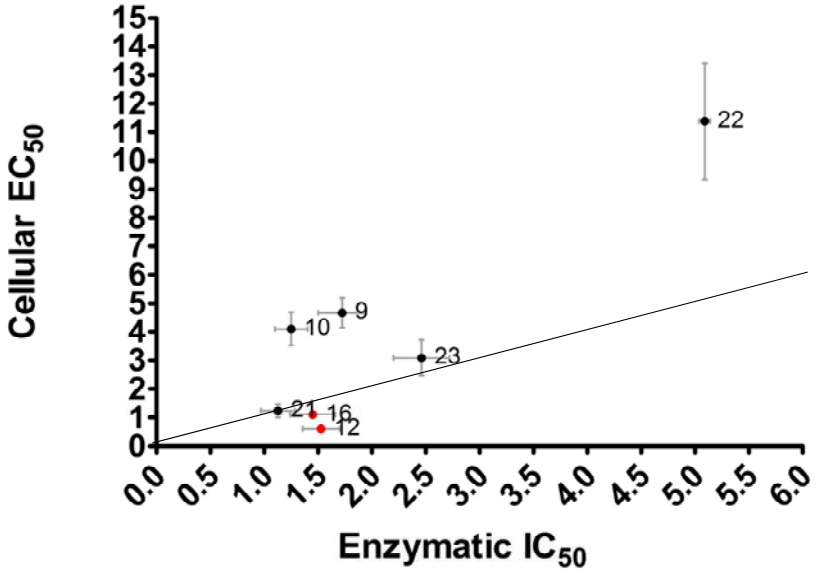
^{13}C NMR (100 MHz, DMSO-d6) δ 169.3, 167.7, 164.2, 158.7, 156.1, 152.6, 149.6, 147.8, 132.3, 131.2, 130.3, 128.7, 124.5, 123.7, 121.6, 119.7, 118.9, 110.9, 64.7, 61.3, 57.1, 52.6, 46.3, 42.5, 37.2, 33.1, 32.2, 24.7, 24.5, 15.1. MS (ESI) *m/z* 639 $(M+H)^+$. HRMS (ESI) *m/z* calc. 639.3771, measured 639.3770 $(M+H)^+$.

2. Scatter plot of the cellular results and the enzymatic results against MAPK7 (Fig. S1). Each dot represent the compound, the number correlate with the compound ID. The coordinate of X-axis means the *in vitro* enzymatic IC_{50} of ERK5, and the coordinate of Y-axis means the cellular EC_{50} value for inhibiting epidermal growth factor induced ERK5 autophosphorylation.

a)



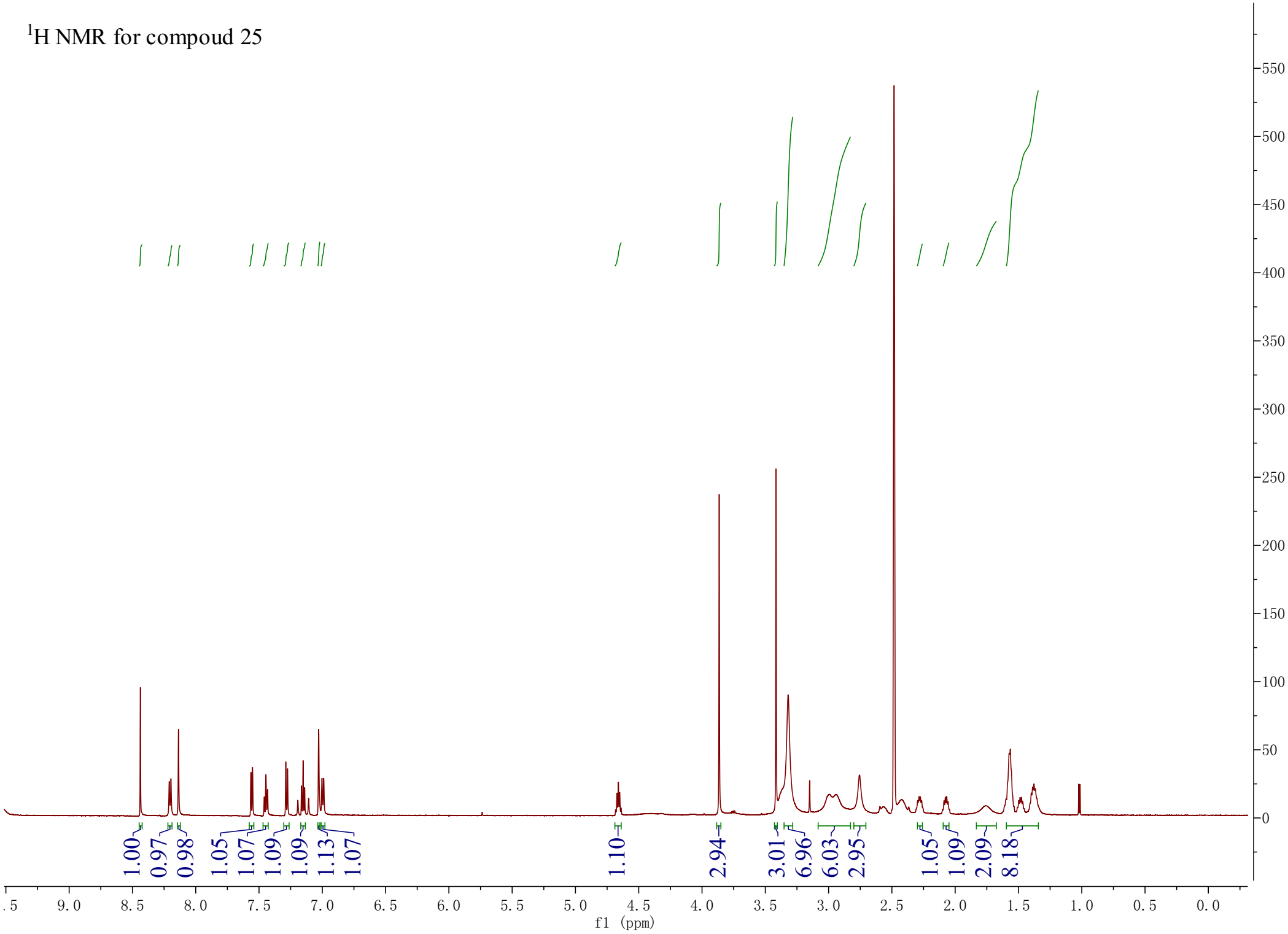
b)



References

[1] Deng, X.; Yang, Q.; Kwiatkowski, N.; Sim, T.; McDermott, U.; Settleman, J. E.; Lee, J. D.; Gray, N. S. Discovery of a benzo[e]pyrimido-[5,4-b][1,4]diazepin-6(11H)-one as a Potent and Selective Inhibitor of Big MAP Kinase 1. *ACS Med. Chem. Lett.* **2011**, *2*, 195-200.

^{1}H NMR for compoud 25



XMD17-26

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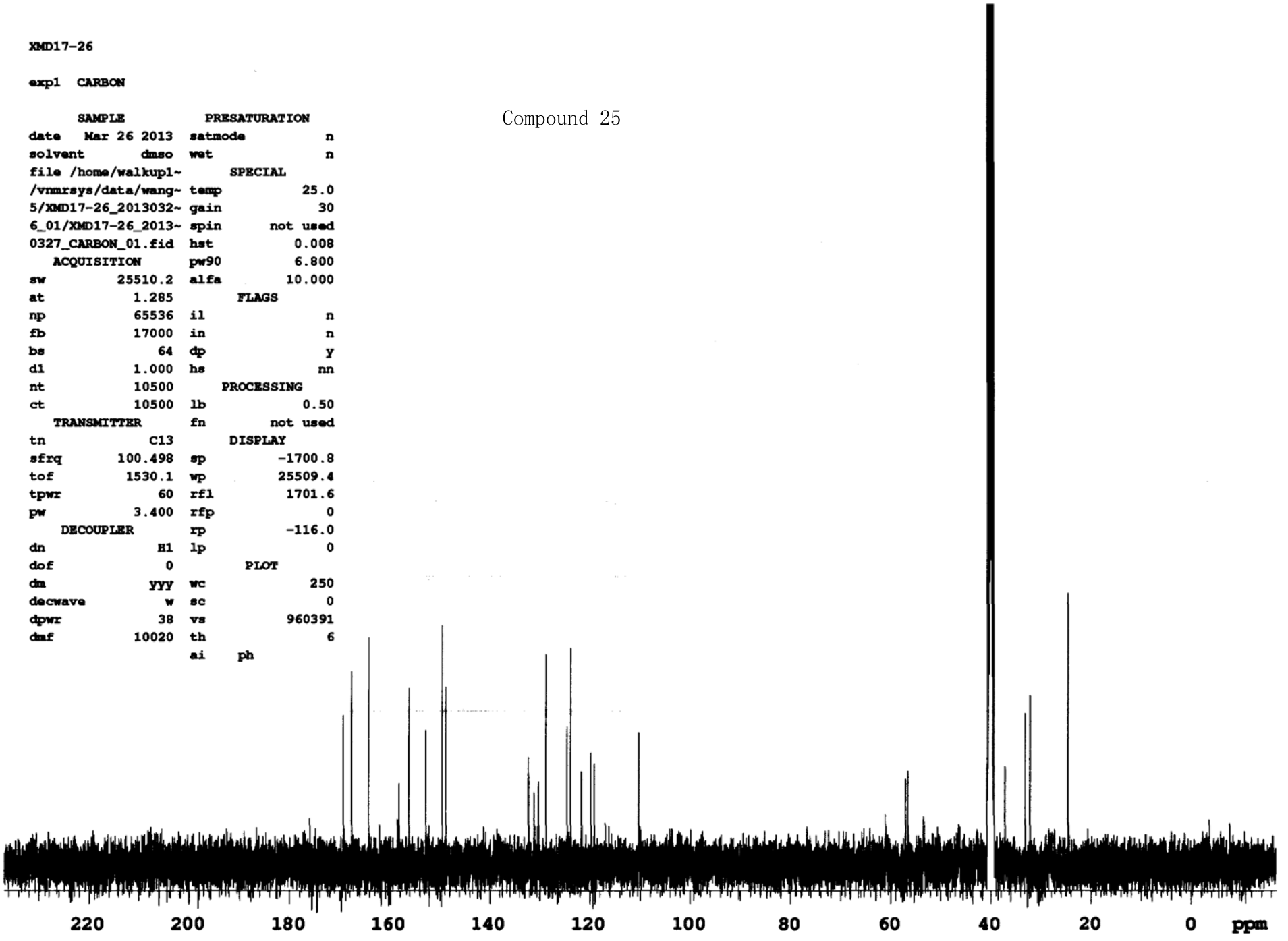
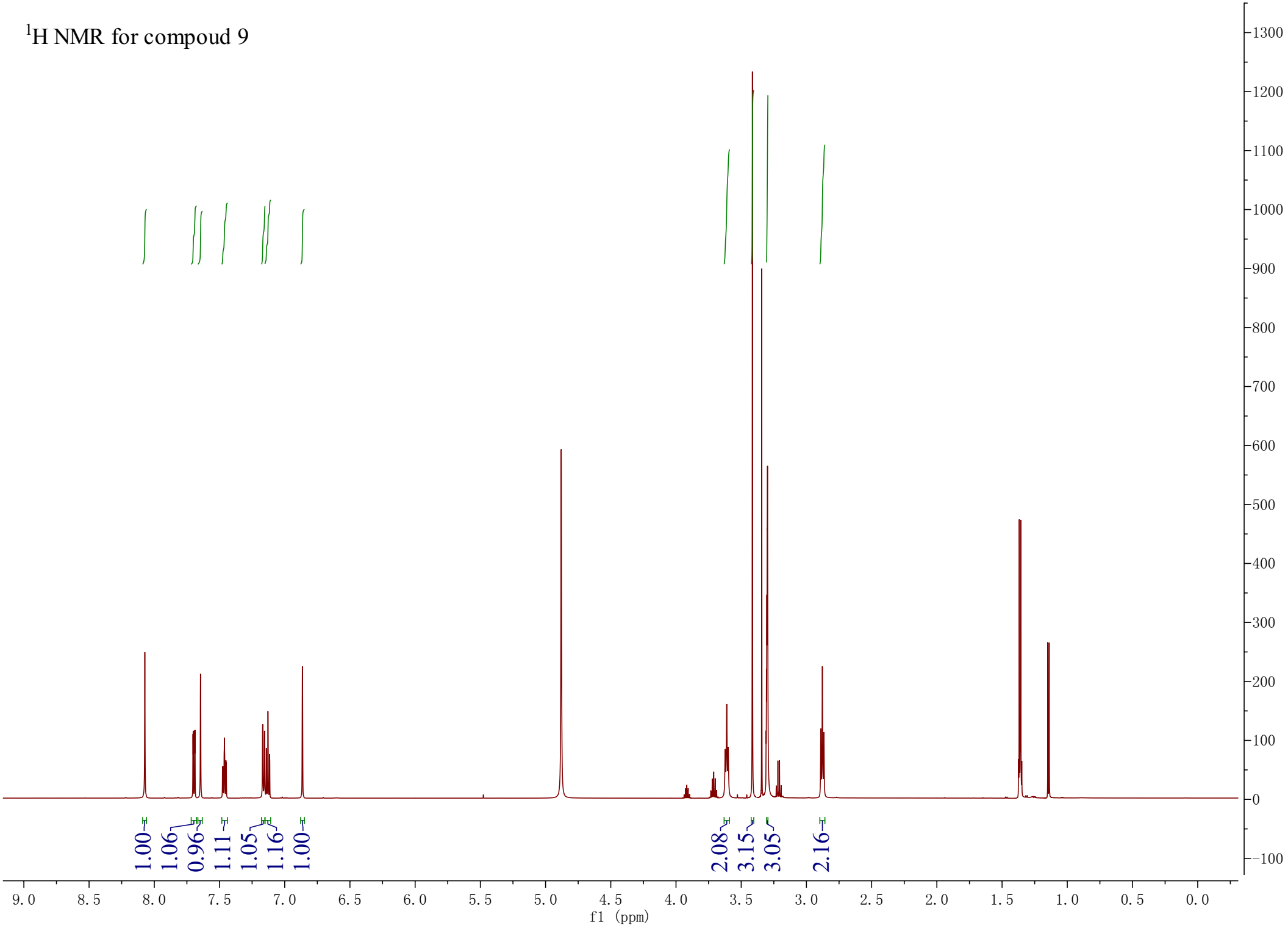
Compound 25



^{1}H NMR for compoud 9



XMD17-88

Compound 9

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bs	64	dp	y
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ct	10500	lb	0.50
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tn	C13	DISPLAY	
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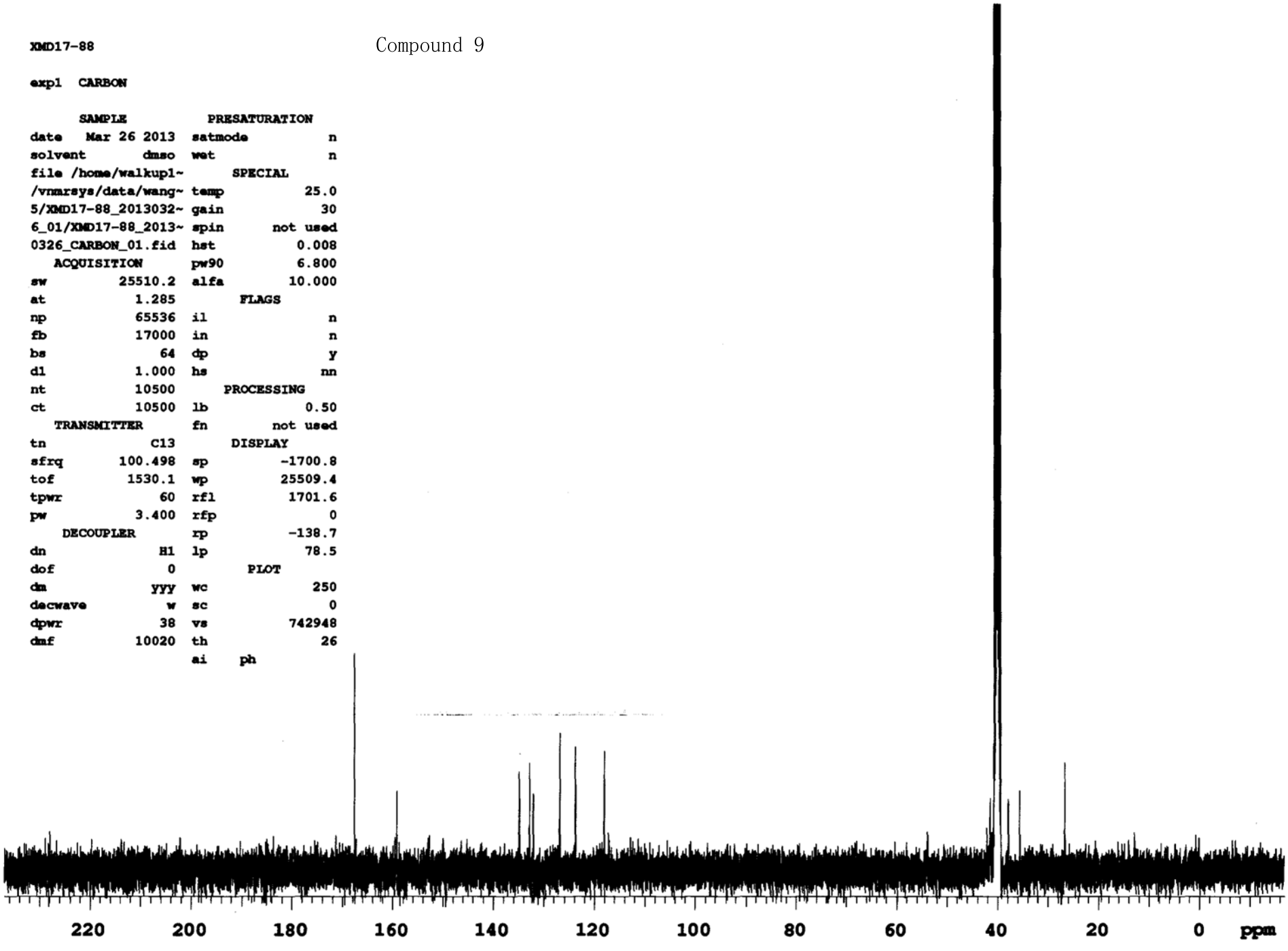
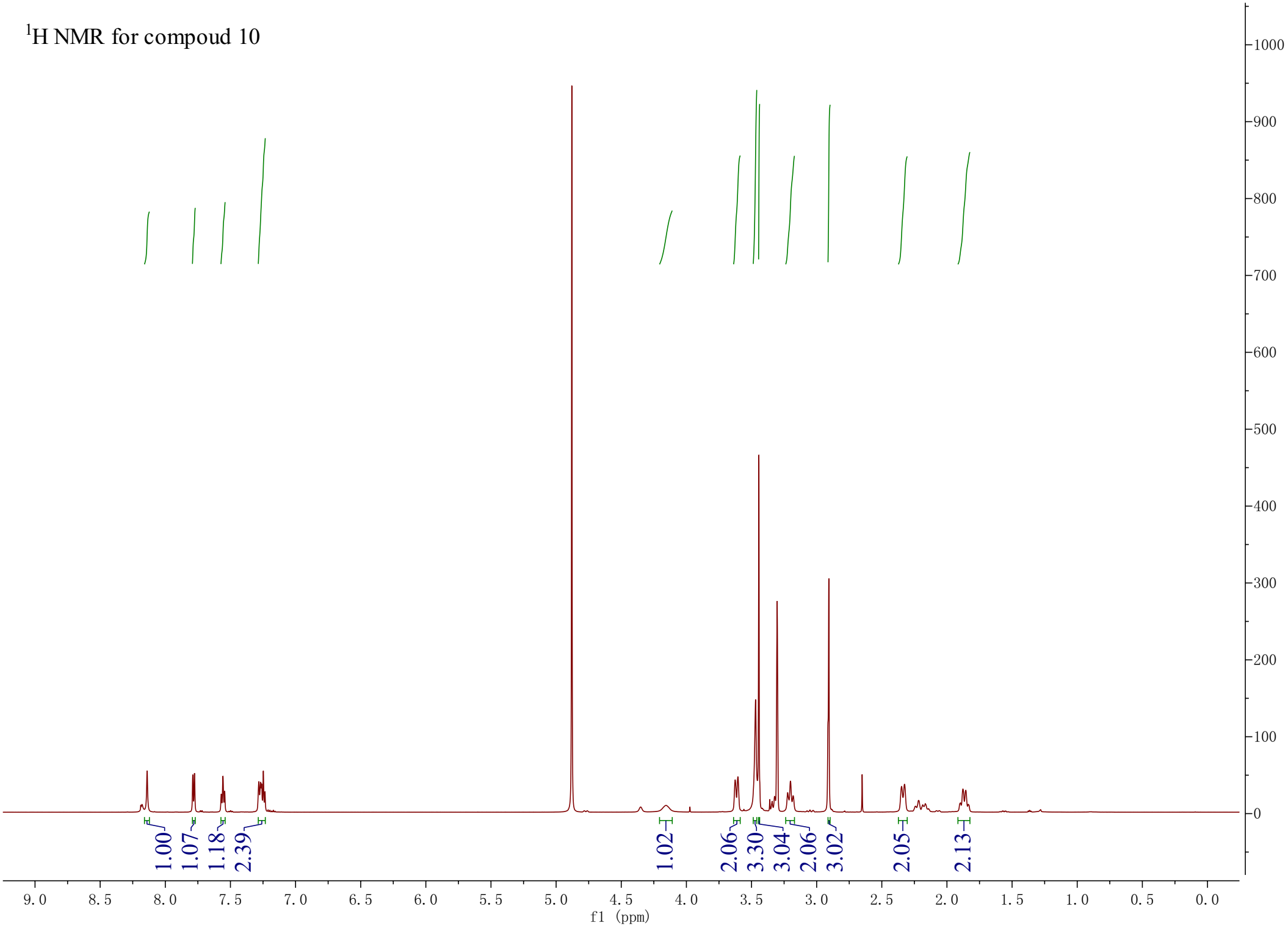
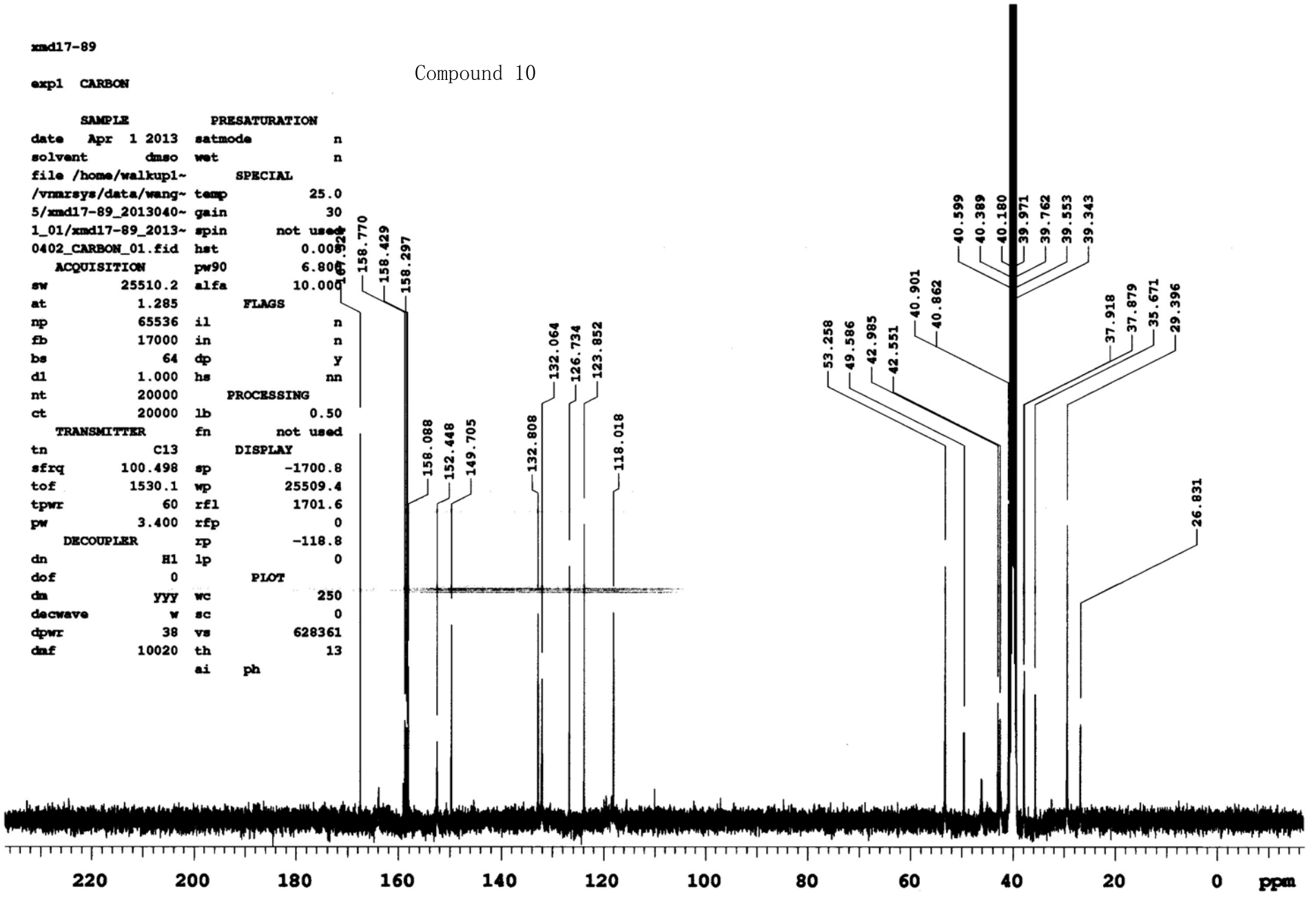


^{1}H NMR for compoud 10




xmd17-89
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Compound 10
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pw 3.400
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alfa 10.00
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in n
dp y
hs nn
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wp 25509.4
rfl 1701.6
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sc 0
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th 13
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158.770
158.429
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152.448
149.705
132.808
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37.918
37.879
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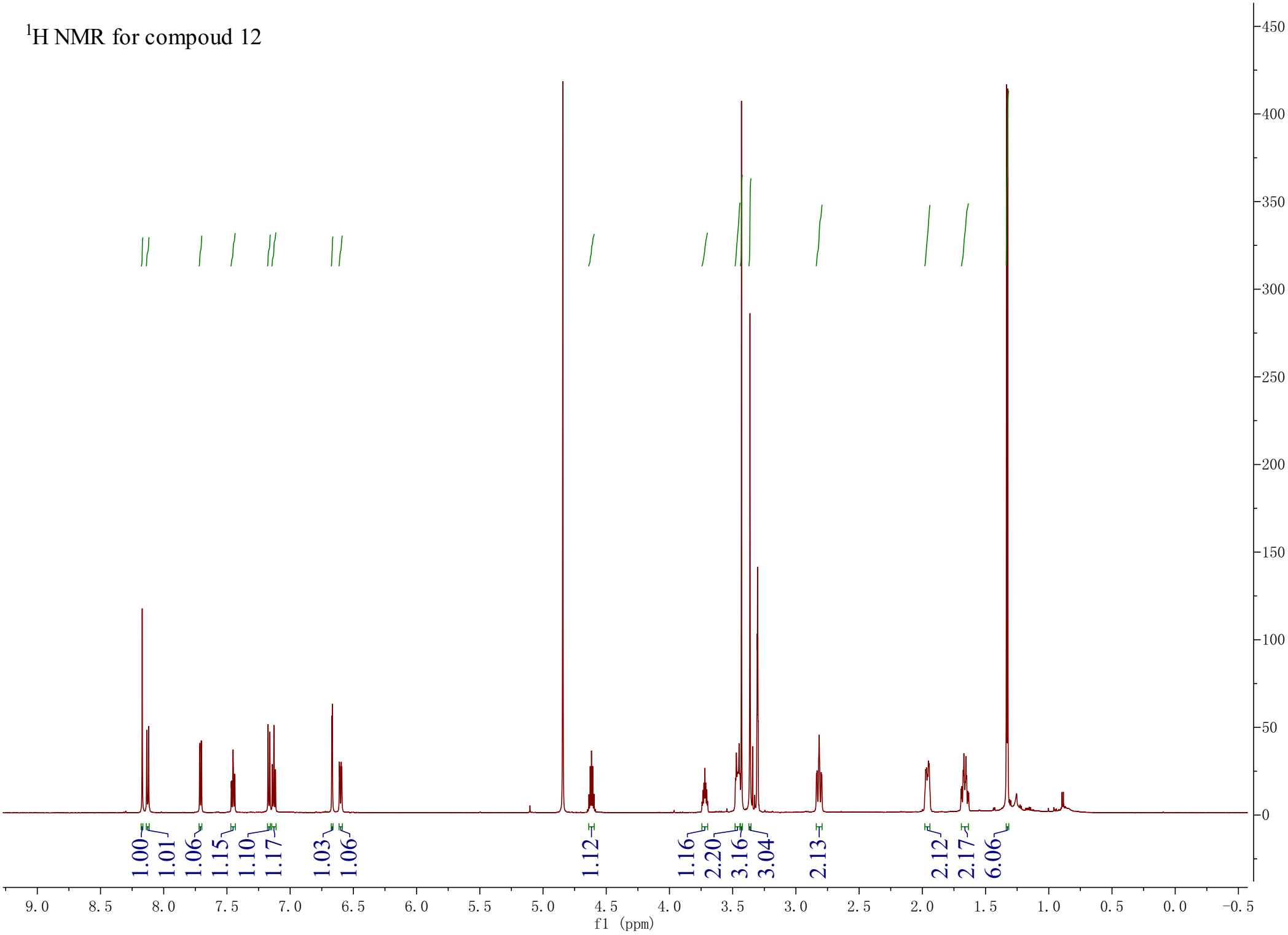
ppm

^{1}H NMR for compoud 12



XMD8-86

exp1 CARBON

Compound 12

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d1	1.000	hs	nn
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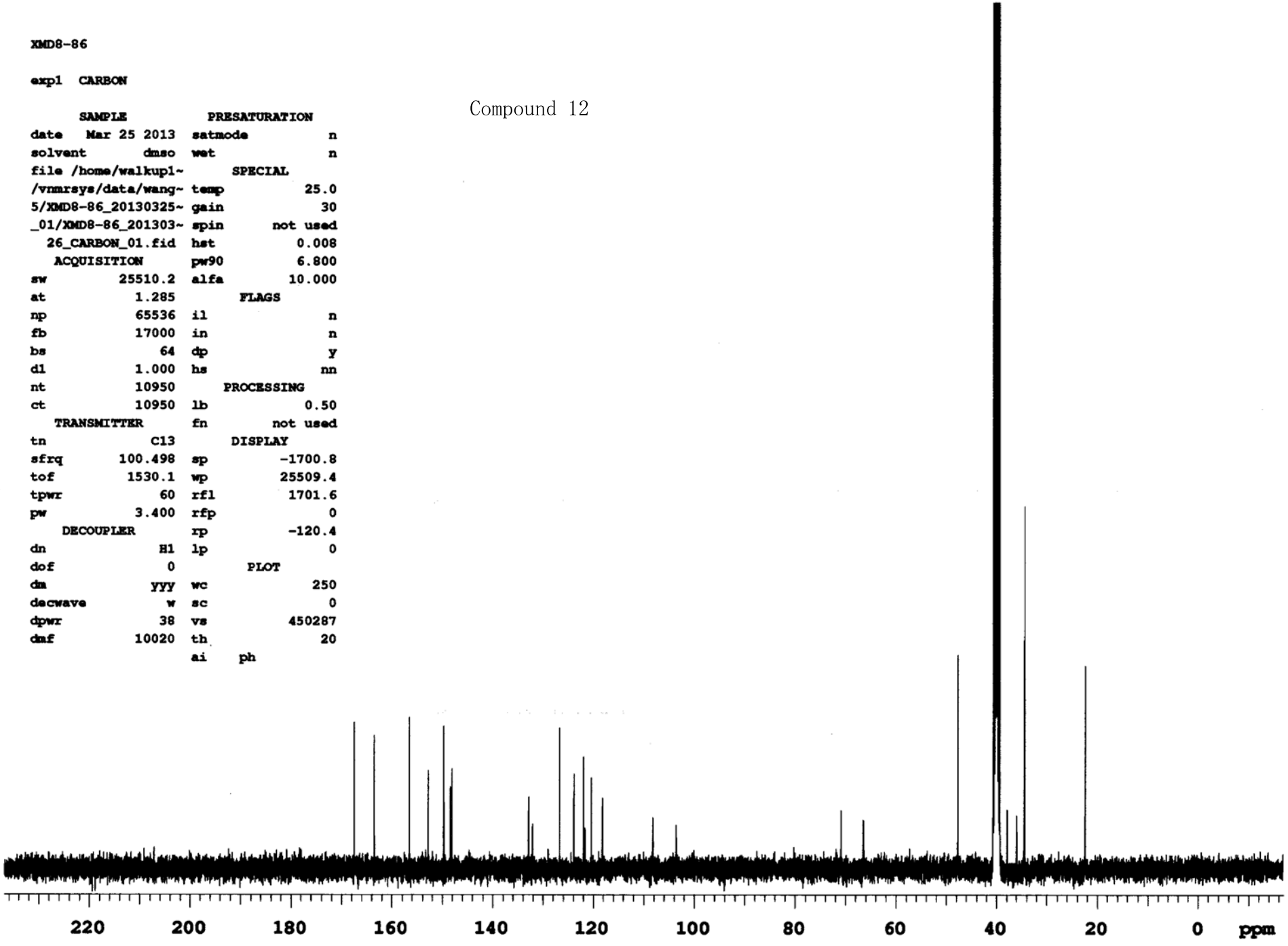
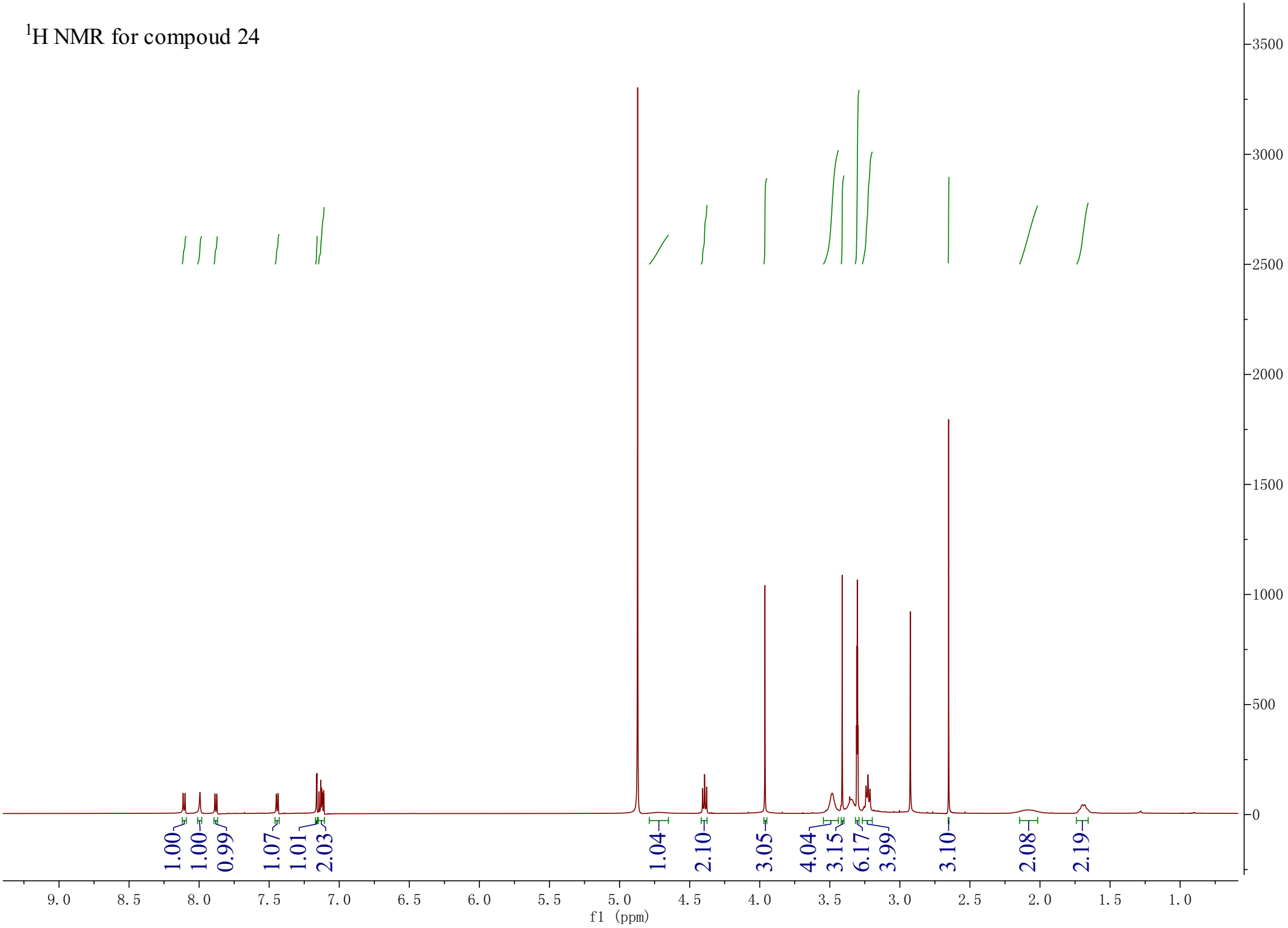


^{1}H NMR for compoud 24



XMD16-44

Compound 24

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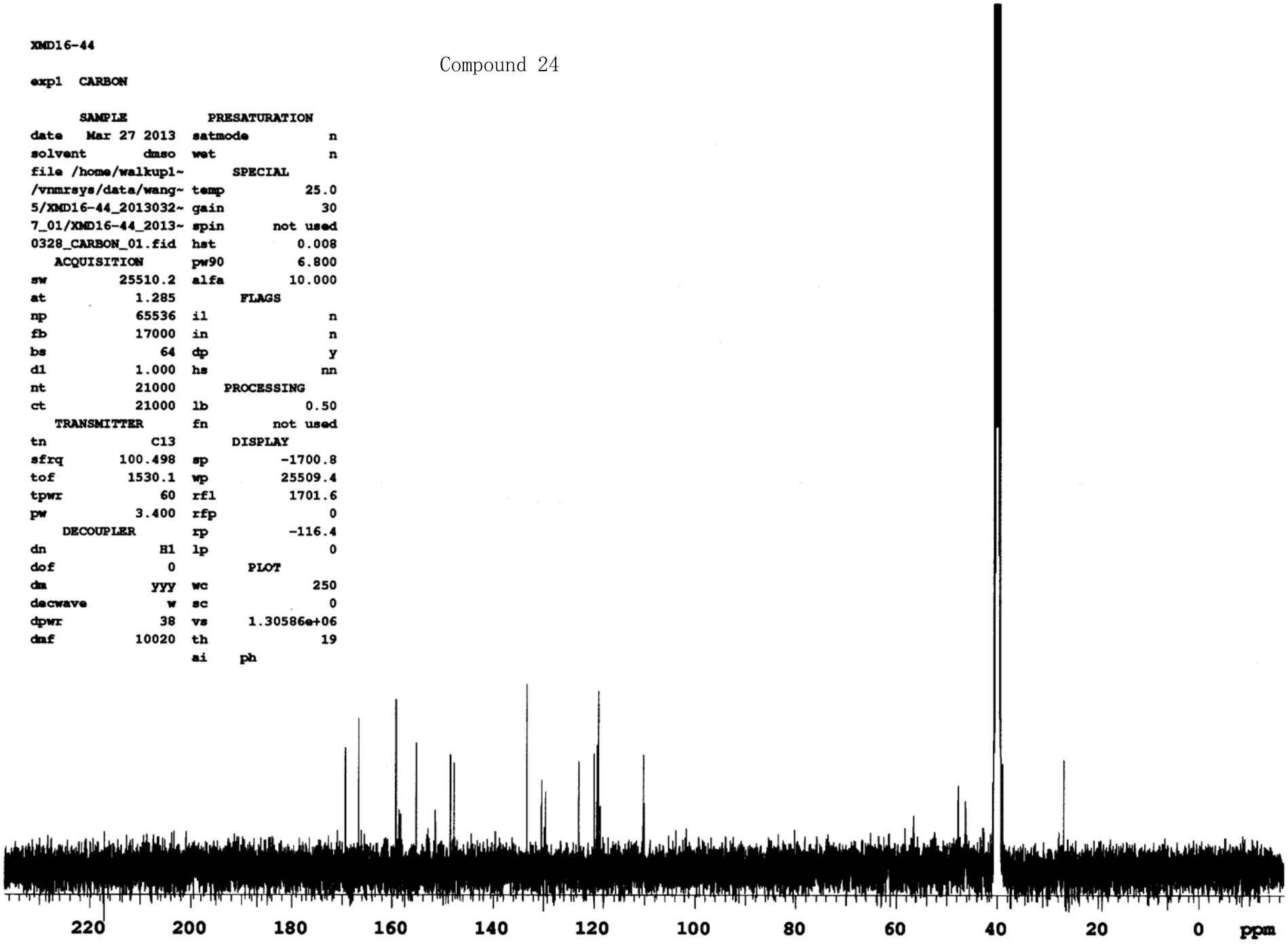
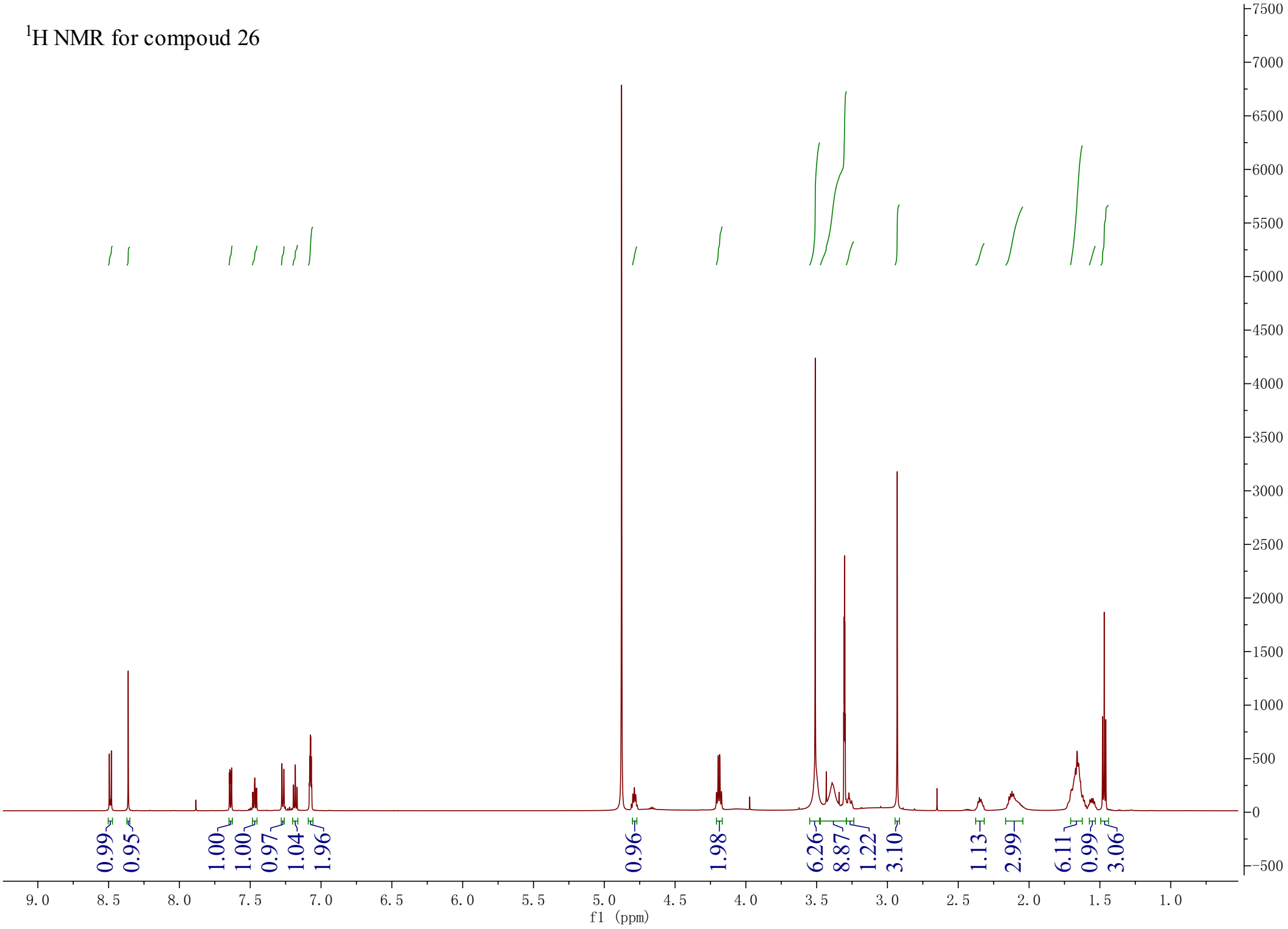


^{1}H NMR for compoud 26



xmd17-109

exp1 CARBON

Compound 26

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tpwr	60	rfp	0
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DECOUPLER		lp	0
dn	H1	PLOT	
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