SUPPLEMENTARY MATERIAL

A new acylated flavonoid tetraglycoside with anti-inflammatory activity from *Tipuana tipu* leaves

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Abstract

A new acylated kaempferol glycoside, kaempferol 3-*O*-α-L-rhamnopyranosyl (1→6)-*O*-[β-D-glucopyranosyl (1→2)-4-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside, has been isolated from the leaves of *Tipuana tipu* (Benth.) Lillo growing in Egypt, along with three known flavonol glycosides, kaempferol 3-*O*-rutinoside, quercetin 3-*O*-rutinoside (rutin) and kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1→6)]-[α-L-rhamnopyranosyl-(1→2]-β-D-glucopyranoside. Structure elucidation was achieved through different spectroscopic methods. Structure relationship to anti-inflammatory activity using carrageenin-induced rat paw edema model, is discussed.

Key Words: kaempferol tetraglycoside, *Tipuana tipu*, Fabaceae, anti-inflammatory.

NMR data of known compounds

Compound 1: yellow needles, m.p. 182-184 °C, UV λ_{max} (nm) MeOH: 267, 351; $NaOCH_3$ 275, 327sh, 400; $AlCl_3$ 269, 394; $AlCl_3$/HCl 274, 393; NaOAc 274, 378; NaOAc/H_3BO_3 267, 353; ^{1}H-NMR (400 MHz, CD_3OD): 6.20 (1H, d, *J* = 2.0 Hz, H-6), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 8.06 (2H, d, *J* = 8.8 Hz, H-2',6'), 6.88 (2H, d, *J* = 8.8 Hz, H-3',5'), 5.12 (1H, d, *J* = 7.2 Hz, H-1''), 4.50 (1H, br s, H-1'''), 1.11 (3H, d, *J* = 6.4 Hz, H-6'''); ^{13}C-

NMR (100 MHz, CD_3OD): 158.5 (C-2), 135.5 (C-3), 179.4 (C-4), 163.0 (C-5), 100.0 (C-6), 166.1 (C-7), 95.0 (C-8), 159.4 (C-9), 105.6 (C-10), 122.7 (C-1'), 132.4 (C-2',6'), 116.1 (C-3',5'), 161.5 (C-4'), 104.6 (C-1''), 75.8 (C-2''), 78.1 (C-3''), 71.4 (C-4''), 77.2 (C-5''), 68.6 (C-6''), 102.4 (C-1'''), 72.1 (C-2'''), 72.3 (C-3'''), 73.9 (C-4'''), 69.7 (C-5'''), 17.9 (C-6''').

compound 2: yellow needles, m.p. 192-194 °C, UV λ_{max} (nm) MeOH: 259, 357; $NaOCH_3$ 271, 415; $AlCl_3$ 272, 430; $AlCl_3$/HCl 269, 405; NaOAc 271, 393; NaOAc/H_3BO_3 259, 387; ^{1}H-NMR (400 MHz, CD_3OD): 6.11 (1H, d, *J* = 2.0 Hz, H-6), 6.37 (1H, d, *J* = 2.0 Hz, H-8), 7.67 (1H, d, *J* = 2.4 Hz, H-2'), 6.87 (1H, d, *J* = 8.4 Hz, H-5'), 7.63 (1H, dd, *J* = 2.4, 8.4 Hz, H-6'), 5.11 (1H, d, *J* = 7.6 Hz, H-1''), 4.53 (1H, d, *J* = 0.8 Hz, H-1'''), 1.12 (3H, d, *J* = 6.0 Hz, H-6'''); ^{13}C-NMR (100 MHz, CD_3OD): 158.3 (C-2), 135.5 (C-3), 179.2 (C-4), 162.7 (C-5), 99.8 (C-6), 165.8 (C-7), 94.7 (C-8), 159.2 (C-9), 105.7 (C-10), 123.4 (C-1'), 115.9 (C-2'), 145.5 (C-3'), 149.6 (C-4'), 117.5 (C-5'), 122.9 (C-6'), 104.6 (C-1''), 75.5 (C-2''), 77.9 (C-3''), 71.2 (C-4''), 77.0 (C-5''), 68.4 (C-6''), 102.2 (C-1'''), 71.8 (C-2'''), 71.9 (C-3'''), 73.7 (C-4'''), 70.0 (C-5'''), 17.8 (C-6''').

Compound 3: yellowish brown needles, m.p. 174-176 °C, IR (KBr, υ_{max}): 3475, 2926, 1666, 1613, 1513, 1452, 1050 cm^{-1}. UV λ_{max} (nm) MeOH: 265, 345; $NaOCH_3$ 275, 321sh, 402; $AlCl_3$ 266, 405; $AlCl_3$/HCl 266, 403; NaOAc 271, 386; NaOAc/H_3BO_3 265, 349; EI-MS *m/z* (%) 741 (25.4) $[M+H]^+$, *m/z* 448 (18.6) $[740 - 2\ rha]^+$ and *m/z* 286 (29.6) $[448 - glu]^+$; ^{1}H-NMR (400 MHz, CD_3OD): 6.19 (1H, br s, H-6), 6.38 (1H, br s, H-8), 8.02 (2H, d, *J* = 8.8 Hz, H-2',6'), 6.90 (2H, d, *J* = 8.4 Hz, H-3',5'), 5.60 (1H, d, *J* = 7.2 Hz, H-1''), 3.58 (1H, m, H-2''), 3.55 (1H, m, H-3''), 3.25 (1H, m, H-4''), 3.35 (1H, m, H-5''), 3.37 (1H, m, H-6''α), 3.81 (1H, m, H-6''β), 4.50 (1H, br s, H-1'''), 3.58 (1H, m, H-2'''), 3.48 (1H, m, H-3'''), 3.24 (1H, m, H-4'''), 3.53 (1H, m, H-5'''), 1.08 (3H, *J* = 6.0 Hz, H-6'''), 5.23 (1H, br s, H-1''''), 4.01 (1H, m, H-2''''), 3.82 (1H, m, H-3''''), 3.35 (1H, m, H-4''''), 4.07 (1H, m, H-5''''), 0.98 (3H, d, *J* = 6.4 Hz, H-6''''); ^{13}C-NMR (100 MHz, CD_3OD): 158.9 (C-2), 134.2 (C-3), 179.2 (C-4), 163.0 (C-5), 99.7 (C-6), 165.5 (C-7), 94.7 (C-8), 158.4 (C-9), 105.8 (C-10), 123.1 (C-1'), 132.1 (C-2',6'), 116.1 (C-3',5'), 161.1 (C-4'), 100.4 (C-1''), 79.8 (C-2''), 78.8 (C-3''), 71.8 (C-4''), 76.9 (C-5''), 68.2 (C-6''), 102.2 (C-1'''), 72.0 (C-2'''), 72.2 (C-3'''), 73.7 (C-4'''), 69.6 (C-5'''), 17.8 (C-6'''), 102.5 (C-1''''), 72.3 (C-2''''), 72.2 (C-3''''), 73.9 (C-4''''), 69.8 (C-5''''), 17.5 (C-6'''').

NMR data of the new compound

Compound 4: yellow needles, m.p. 210-212 °C, IR (KBr, υ_{max}): 3455, 2929, 1723, 1660, 1618, 1511, 1458, 1042 cm^{-1}. UV λ_{max} (nm) MeOH: 267, 351; $NaOCH_3$ 274, 325sh, 404; $AlCl_3$

266, 400; $AlCl_3$/HCl 272, 400; NaOAc 275, 385; NaOAc/H_3BO_3 267, 352; HRESIMS *m/z* 945.2866 (100%); ^{1}H-NMR (400 MHz, CD_3OD): 6.20 (1H, br s, H-6), 6.39 (1H, br s, H-8), 8.04 (2H, d, *J* = 8.8 Hz, H-2',6'), 6.90 (2H, d, *J* = 8.8 Hz, H-3',5'), 5.48 (1H, d, *J* = 7.6 Hz, H-1''), 3.94 (1H, m, H-2''), 3.71 (1H, m, H-3''), 3.78 (1H, m, H-4''), 3.63 (1H, m, H-5''), 3.42 (1H, m, H-6''α), 3.72 (1H, m, H-6''β), 5.25 (1H, br s, H-1'''), 4.14 (1H, dd, *J* = 2.4, 2.8 Hz, H-2'''), 4.31 (1H, m, H-3'''), 5.06 (1H, d, *J* = 10.0 Hz, H-4'''), 4.31 (1H, m, H-5'''), 0.88 (3H, d, *J* = 6.0 Hz, H-6'''), 4.52 (1H, br s, H-1''''), 3.36 (1H, m, H-2''''), 3.60 (1H, m, H-3''''), 3.28 (1H, m, H-4''''), 3.50 (1H, m, H-5''''), 1.17 (3H, d, *J* = 6.4 Hz, H-6''''), 4.45 (1H, d, *J* = 7.6 Hz, H-1'''''), 3.20 (1H, m, H-2'''''), 3.31 (1H, m, H-3'''''), 3.50 (1H, m, H-4'''''), 3.31 (1H, m, H-5'''''), 3.77 (1H, m, H-6'''''α), 3.88 (1H, m, H-6'''''β), $\underline{CH_3}CO$ (3H, s); ^{13}C-NMR (100 MHz, CD_3OD): 158.9 (C-2), 134.6 (C-3), 179.4 (C-4), 163.2 (C-5), 99.9 (C-6), 165.8 (C-7), 94.8 (C-8), 158.4 (C-9), 105.8 (C-10), 122.9 (C-1'), 132.2 (C-2',6'), 116.2 (C-3',5'), 161.4 (C-4'), 101.4 (C-1''), 77.6 (C-2''), 75.6 (C-3''), 70.7 (C-4''), 75.3 (C-5''), 67.3 (C-6''), 102.4 (C-1'''), 79.9 (C-2'''), 72.1 (C-3'''), 74.1 (C-4'''), 67.8 (C-5'''), 17.4 (C-6'''), 101.9 (C-1''''), 71.1 (C-2''''), 72.1 (C-3''''), 73.9 (C-4''''), 69.7 (C-5''''), 18.0 (C-6''''), 106.1 (C-1'''''), 74.9 (C-2'''''), 77.9 (C-3'''''), 69.7 (C-4'''''), 77.8 (C-5'''''), 62.4 (C-6'''''), 21.2 ($\underline{CH_3}CO$), 172.9 ($CH_3\underline{CO}$).

Acid hydrolysis

5 mg of compound **4** were dissolved in 5 ml MeOH, mixed with equal volume of 5% HCl (v/v) and the solution was refluxed for two hours on boiling water bath. Methanol was distilled off and the hydrolysate was shaken with CH_2Cl_2 to separate the aglycone. The CH_2Cl_2 extract was dried over anhydrous sodium sulfate and the solvent was distilled off. The residue was dissolved in methanol and kept for identification of the aglycone moiety. The acidic mother liquor was neutralized with silver oxide and the formed precipitate was filtered off and washed several times with few mls of distilled water. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was dissolved in few drops of methanol and used for identification of the sugar moiety by comparison with authentic sugar samples.

Figures

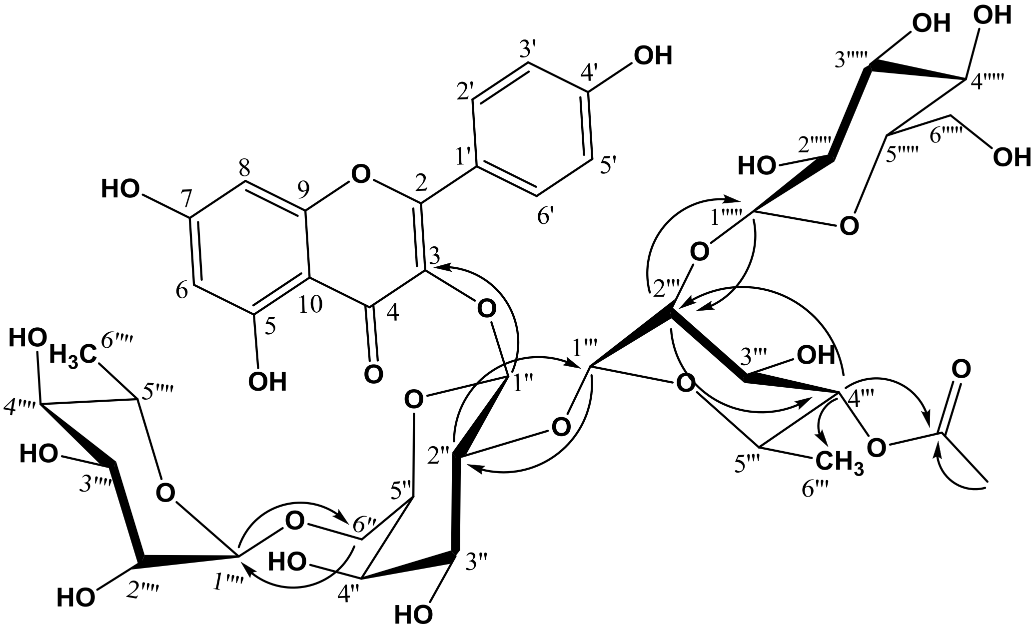
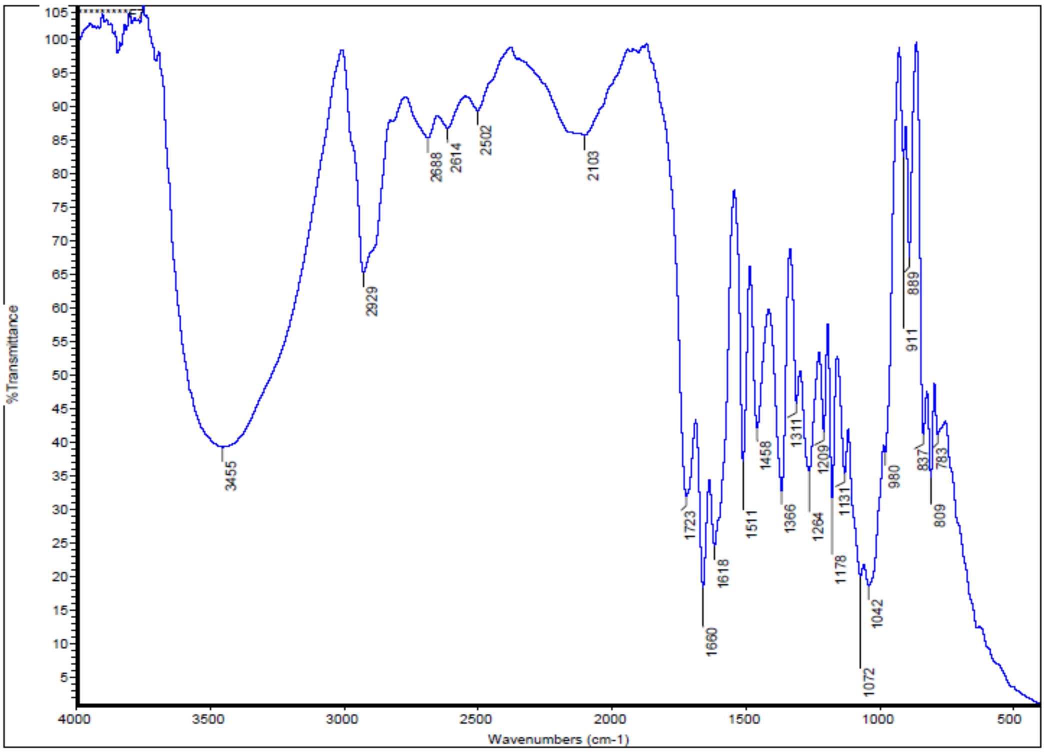
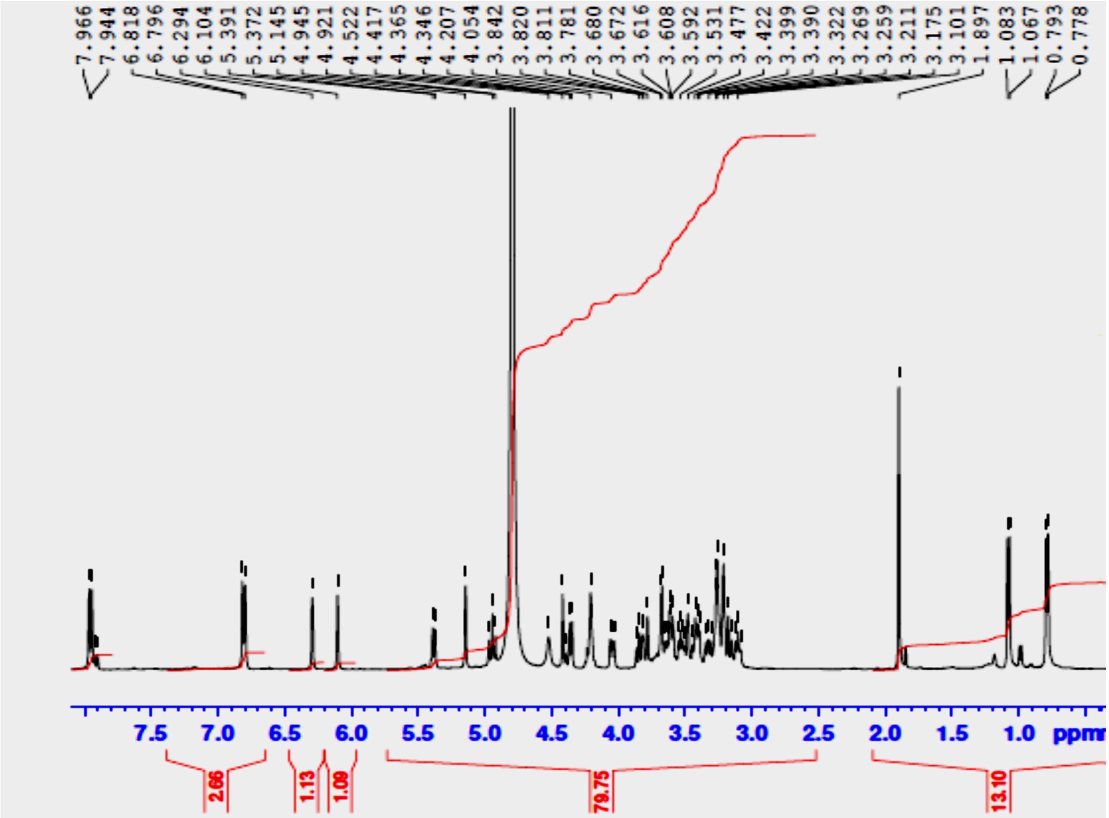


Figure S1. Key HMBC correlations of compound 4

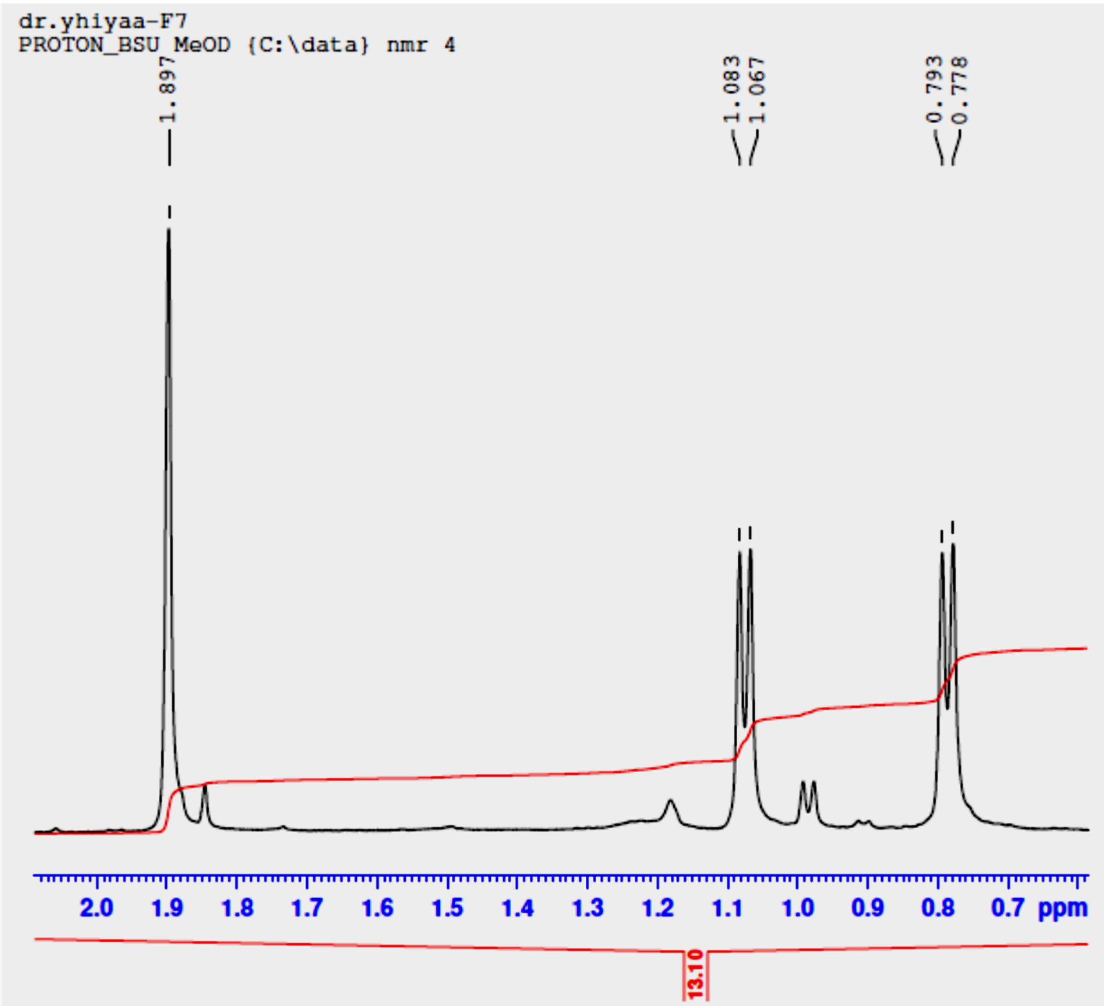
Original NMR spectra of compound 4

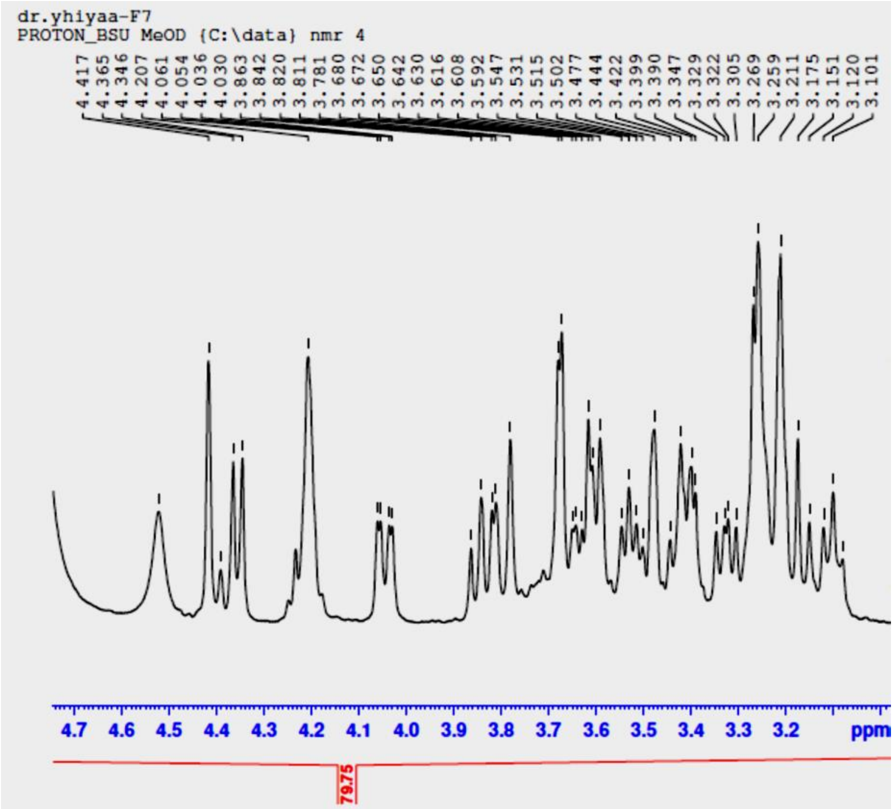


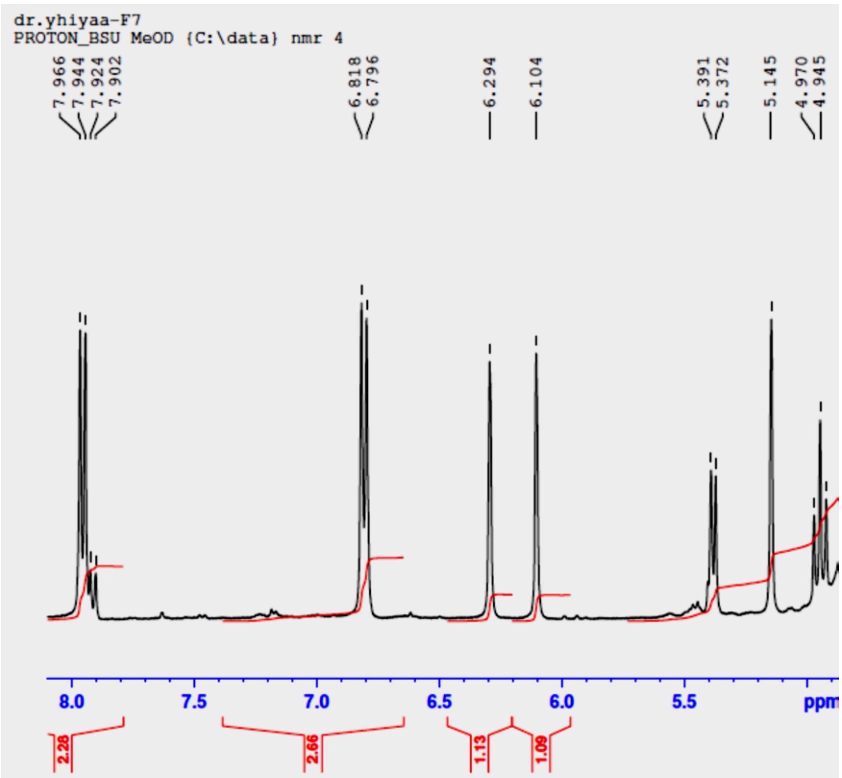
IR (KBr, υ_{max}) spectrum



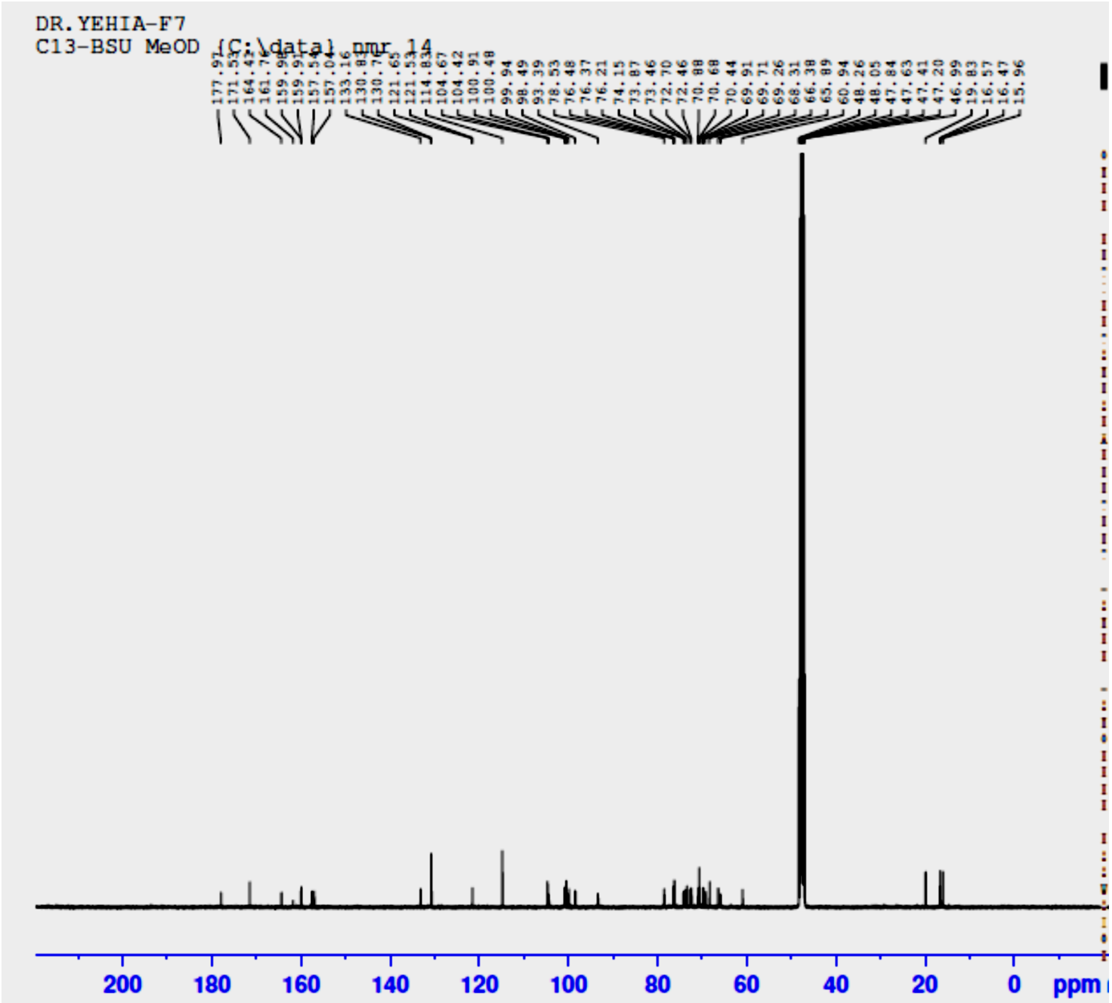
[1]H-NMR spectrum (CD_3OD, 400 MHz)



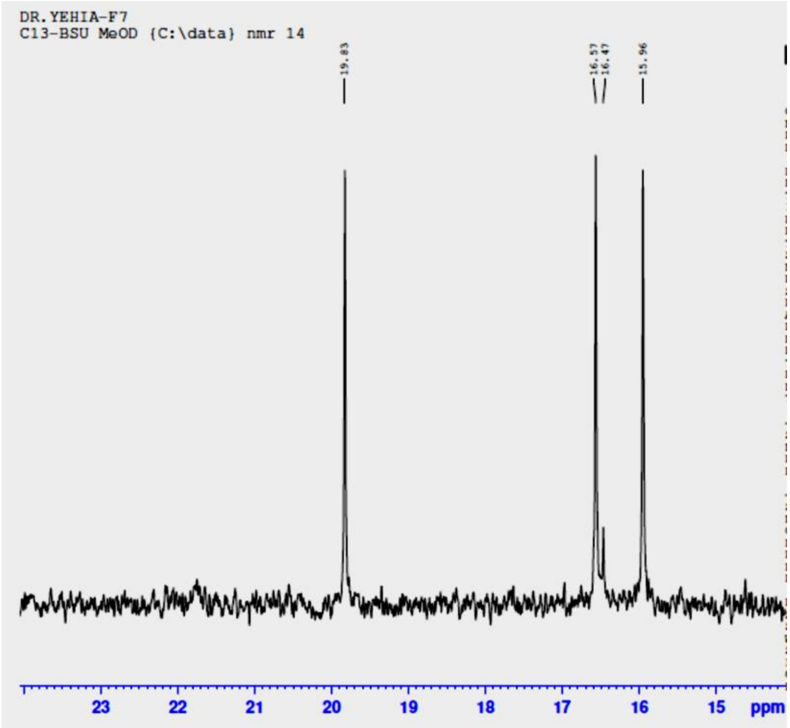


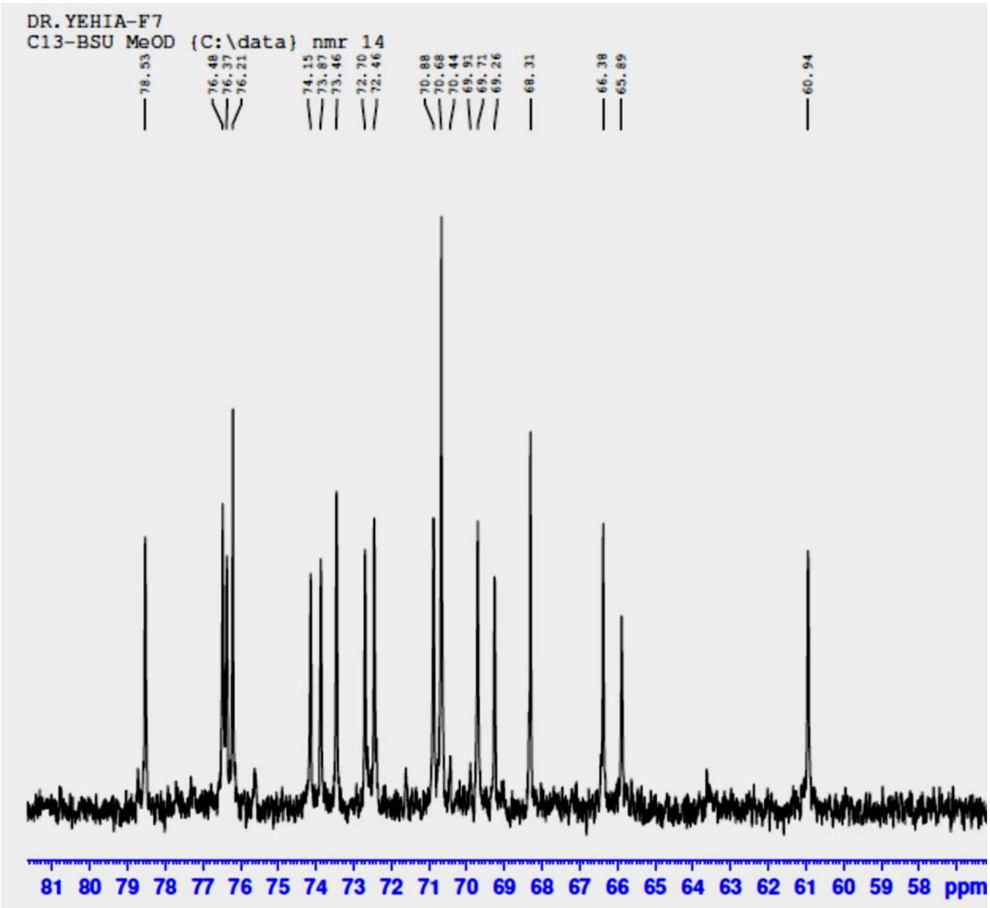


^{1}H-NMR spectrum (expansion)

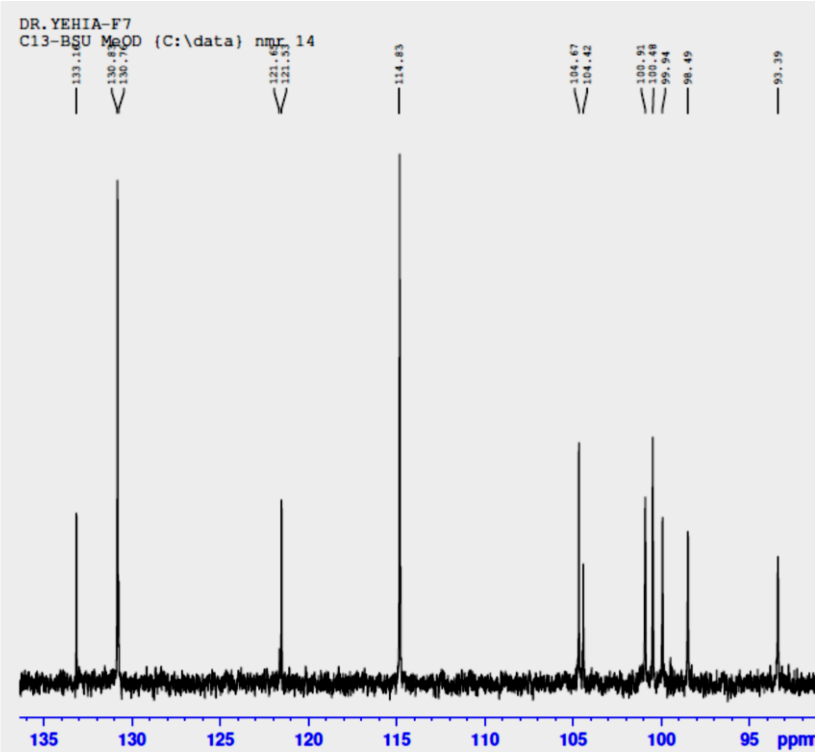


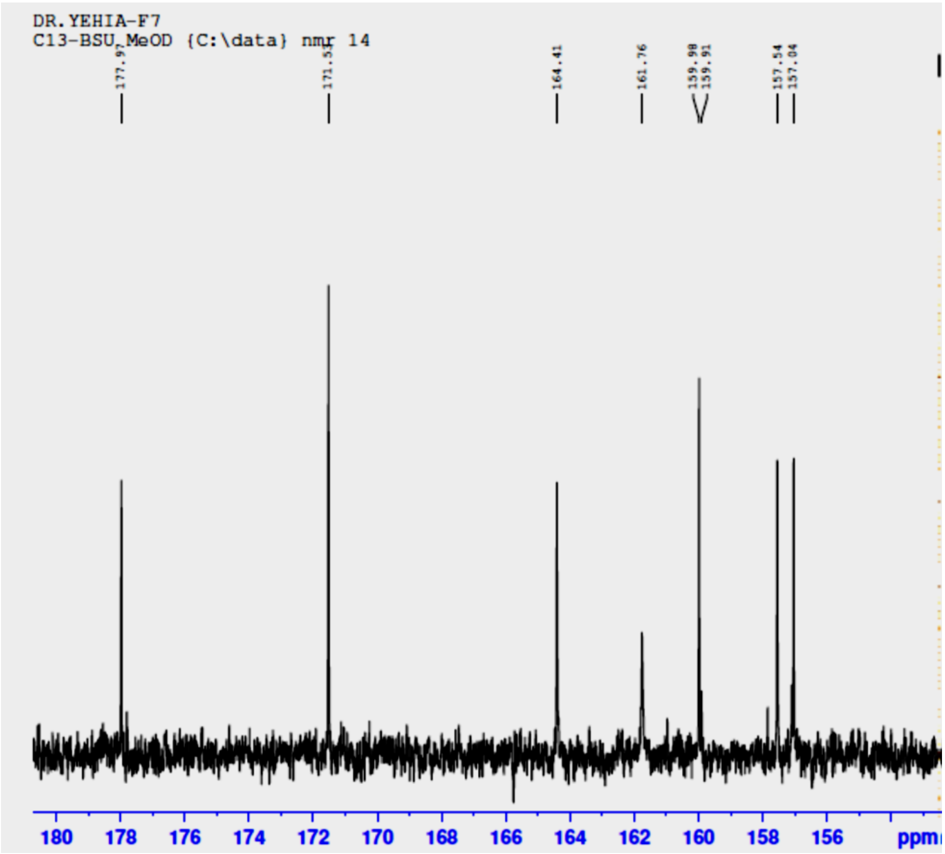
^{13}C-NMR spectrum (CD_3OD, 100 MHz)



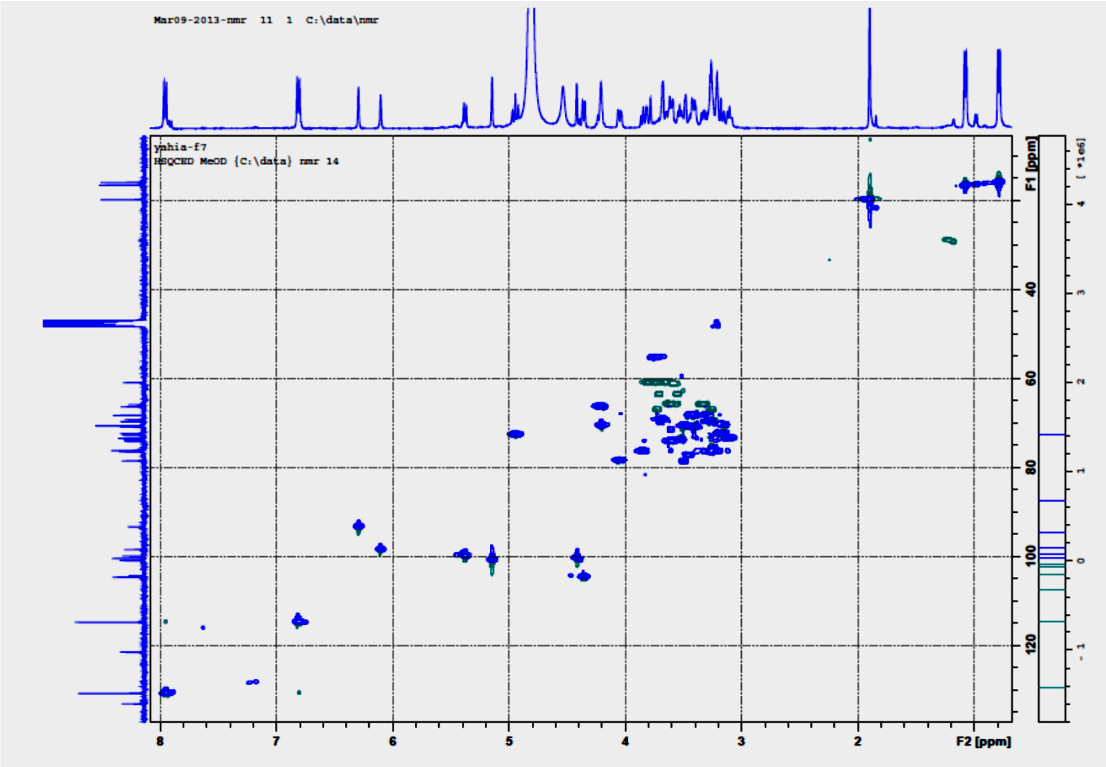


^{13}C-NMR spectrum (expansion)

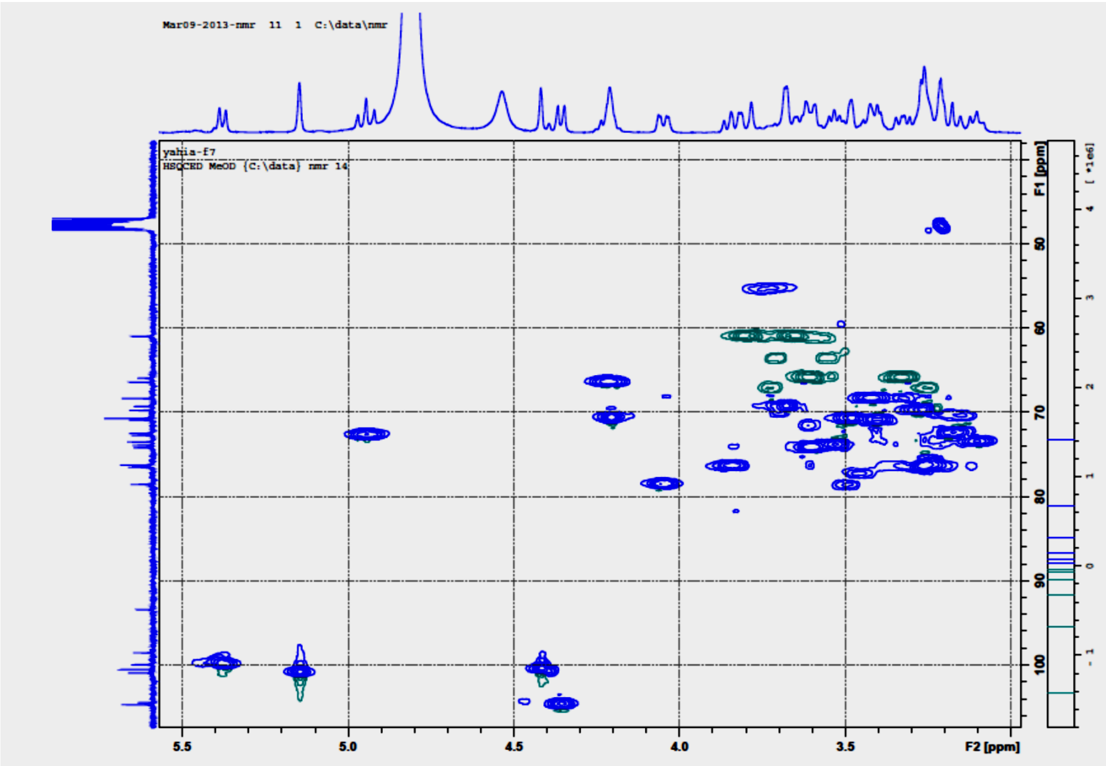




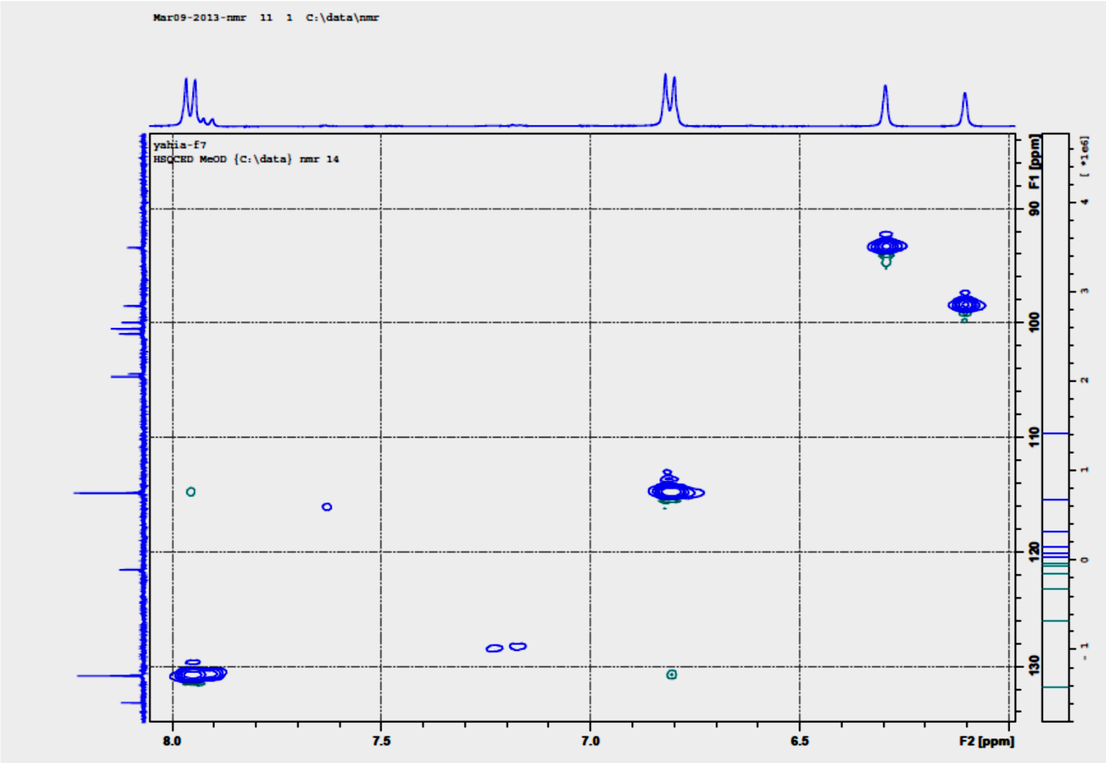
^{13}C-NMR spectrum (expansion)

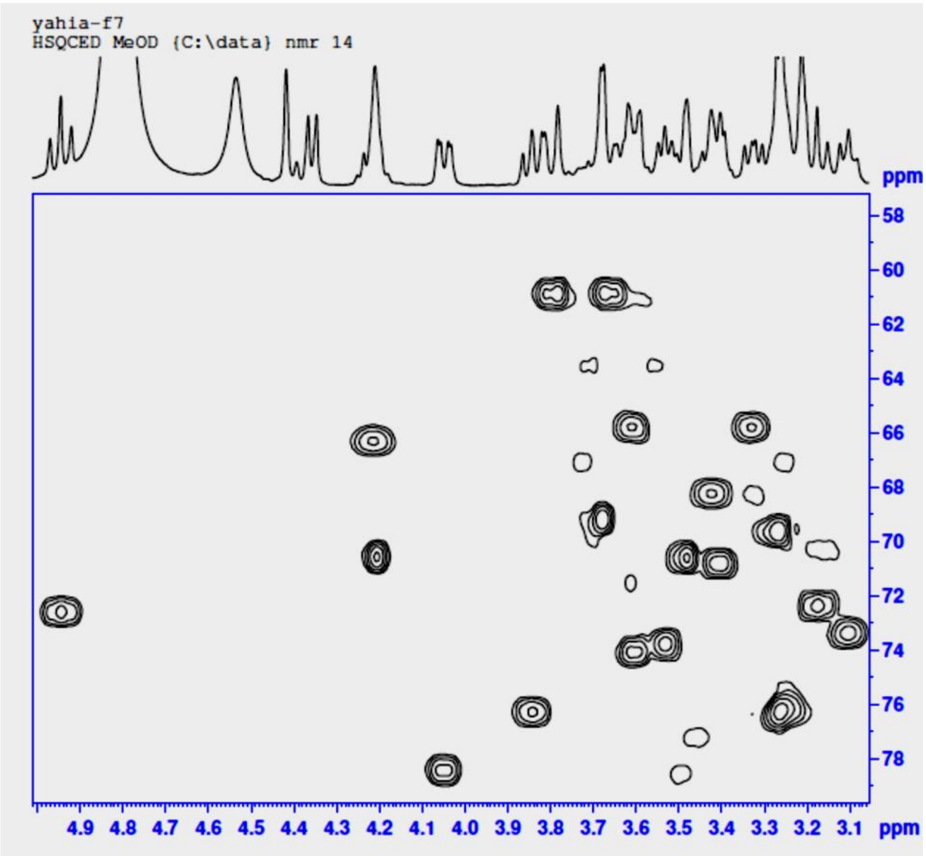


HSQC spectrum



HSQC expansion





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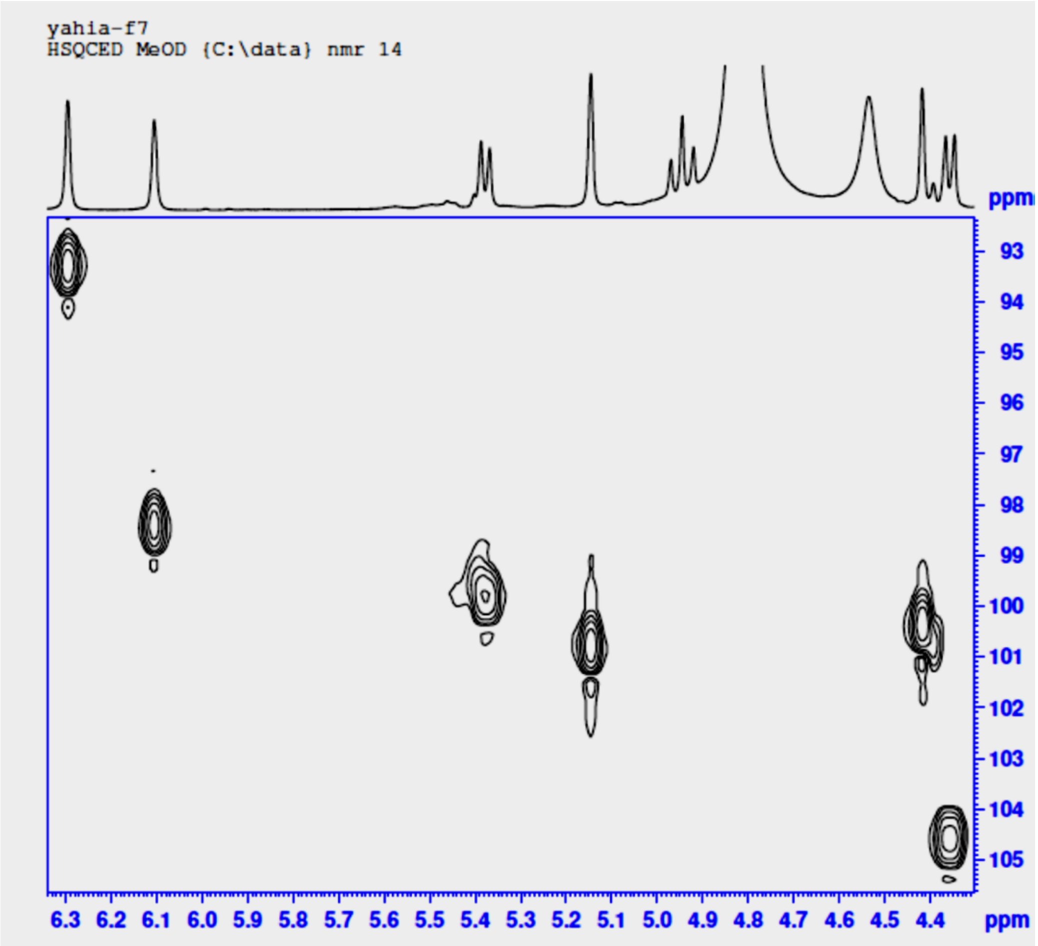

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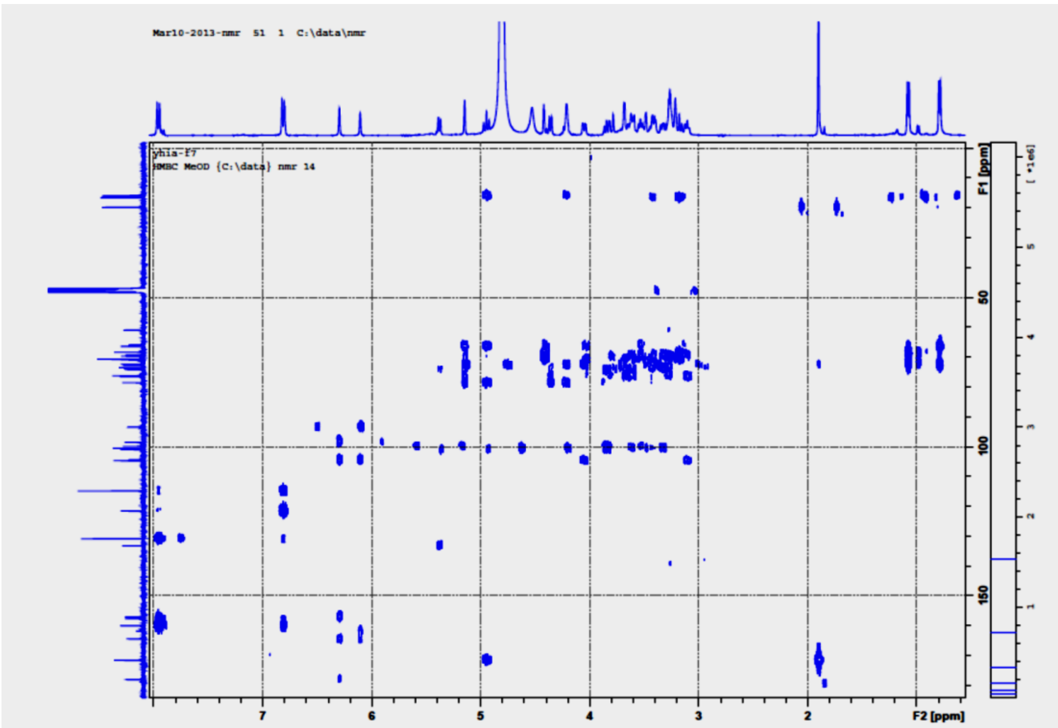
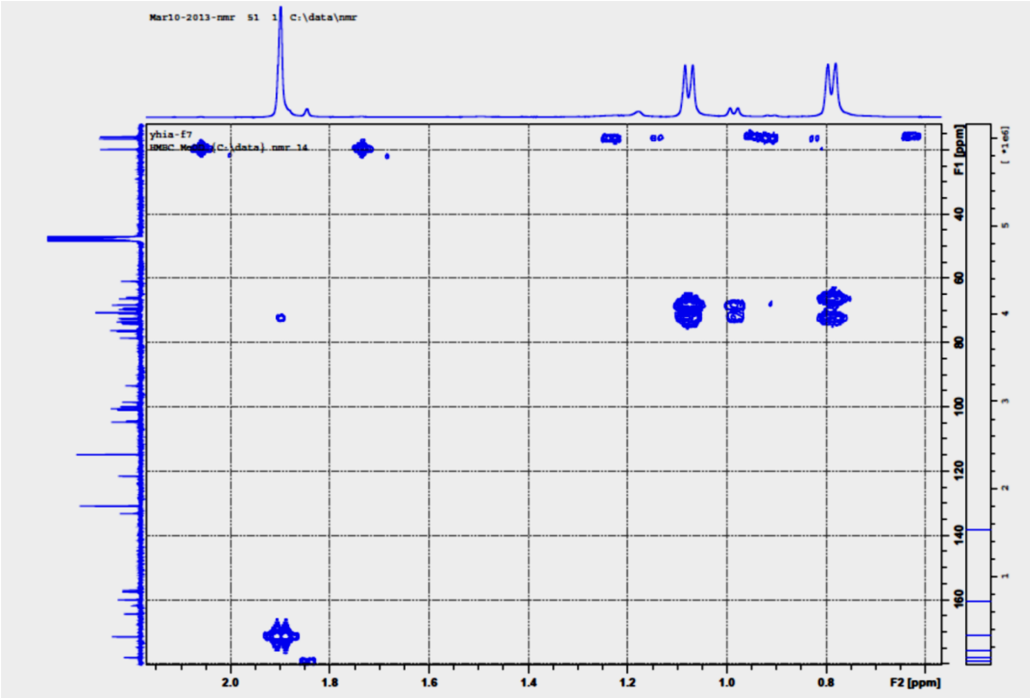
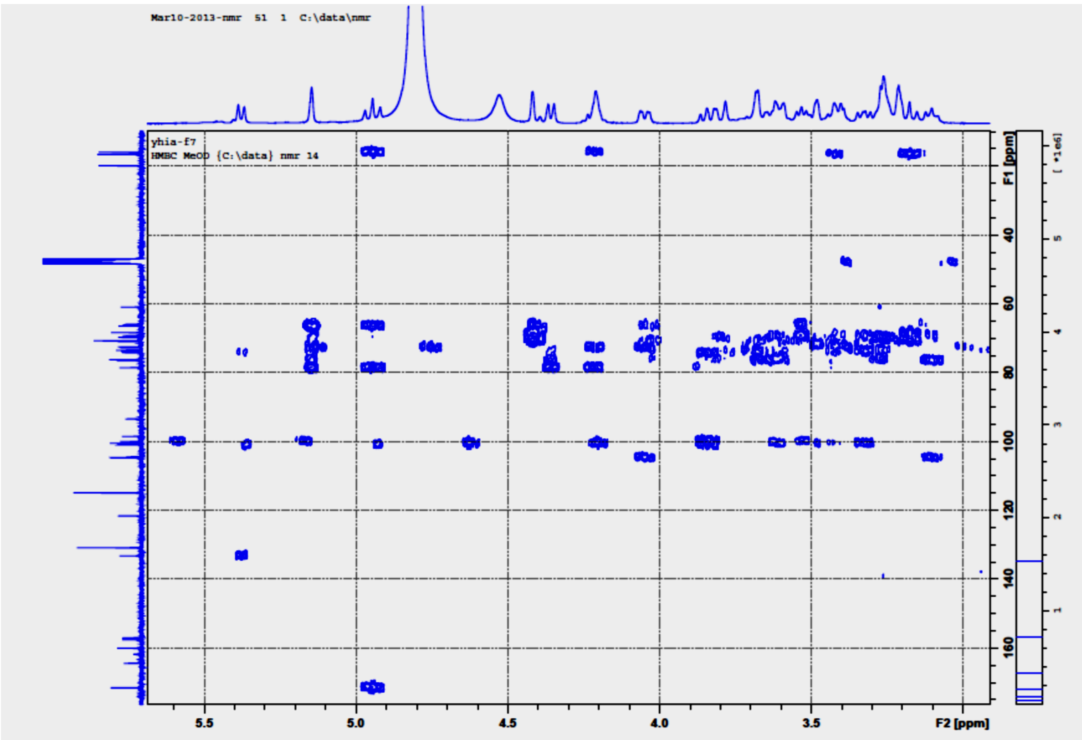


HMBC spectrum





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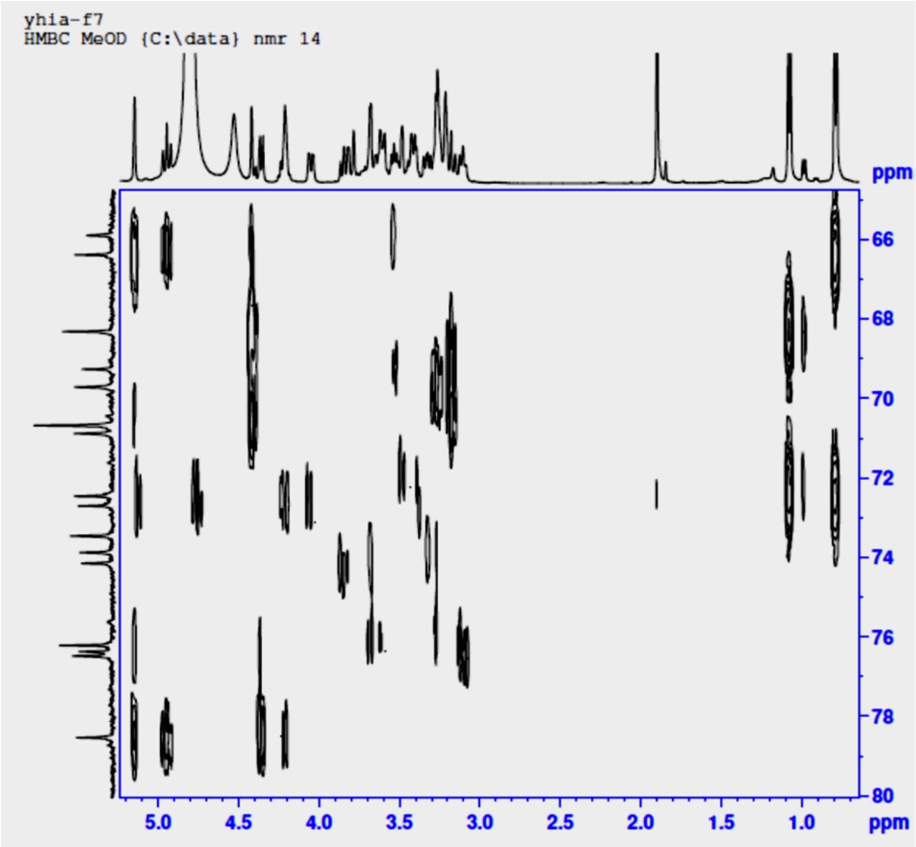

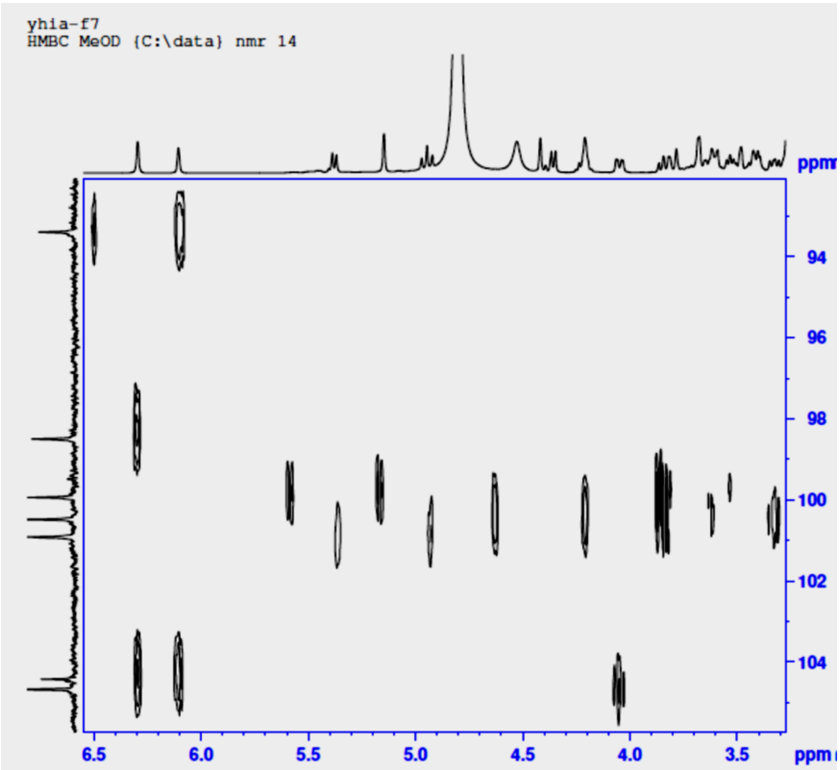

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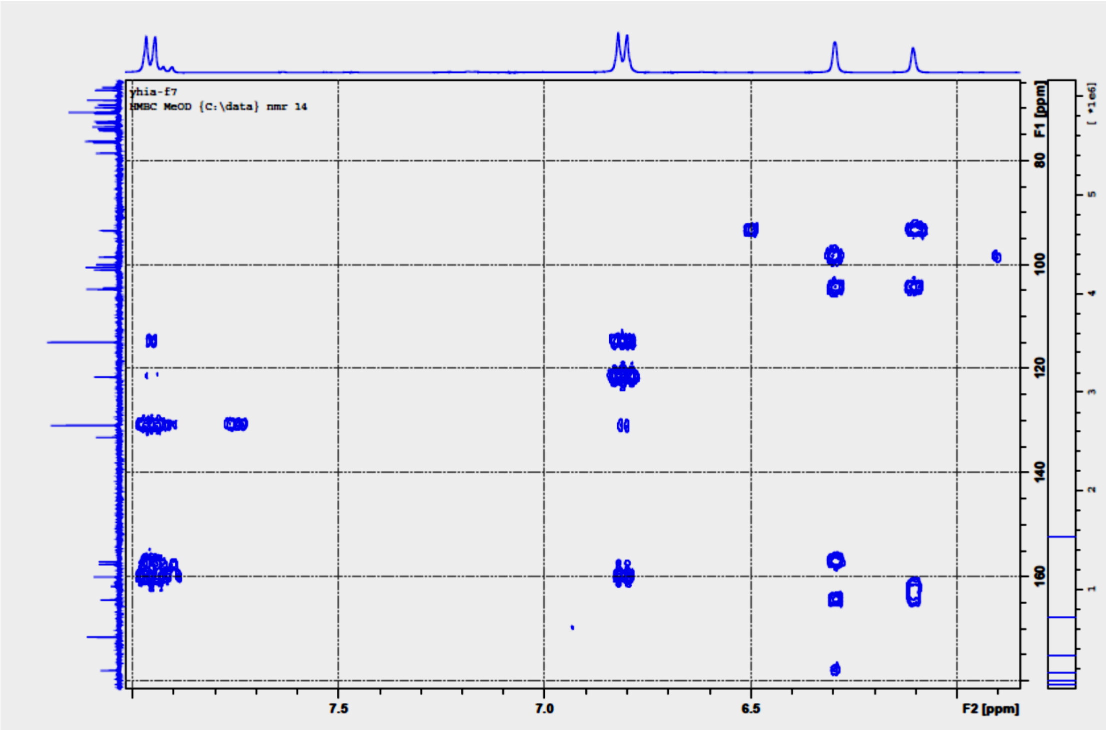

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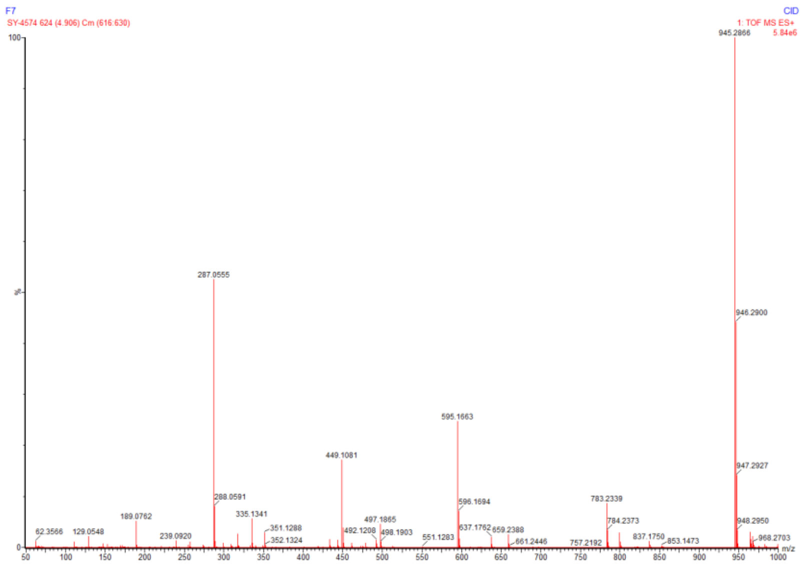

HRESI-MS