Electronic Supplementary Material (ESI) for Chemical Communications

Unique biocatalytic resolution of racemic tetrahydroberberrubine via kinetic glucosylation and enantio-selective sulphation

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Supporting Information

1. Instrumentation and general procedures

Thin-layer chromatography (TLC) was performed on silica gel GF254 plates of 0.5 mm of thickness for analysis. Layers were air dried and activated at 110°C for 0.5 h before use. The transformation mixtures were extracted with EtOAc, then were chromatographed by silica gel with $CHCl_3$-MeOH (V:V= 30:1). Chromatograms were visualized by spraying freshly prepared $KBiI_4$ reagent on the plate. The HPLC equipment used was an Agilent 1100 series HPLC system consisting of binary pump, autosampler, thermostated column compartment,UV system.A reversed phase ODS-2 column (Hedera; media 10nm 5μm,size 250mm×4.6mm, Hanbon Sci. & Tech., Jiangsu, China) was used for all chromatographic separations. A linear solvent gradient of solvents A（0.5% aqueous acetic acid and 20mmol/L ammonium acetate) and B (acetonitrile) was as follows: 20%-30% B at 0–5 min, 30%-35% B at 5-20 min, and the detection wavelength was set at 285nm. The HPLC chiral analytical separation was carried out on a chiral OJ-RH column (4.6 i.d. ×150 mm,Daicel Chemical Ltd.) using a solvent system, (A) 0.1 M NH_4OAc (0.05% TFA) / (B) MeCN (0.05% TFA) under the following gradient conditions: A/B, initial (80:20), 10 min (60:40), 20 min (60:40), 30 min (0:100) (flow rate: 0.8 ml/min, detection:285 nm). NMR spectra were recorded on a Bruker Avance 500 spectrometer with tetramethylsilane as the internal reference, and chemical shifts were expressed in δ (parts per million).Optical rotations were obtained using a JASCO P-1020 digital polarimeter. CD spectra were recorded on a JASCO J-810 spectropolarimeter. HRESITOFMS experiment was performed on a G1969A time of flight (TOF)-MS (Agilent Technologies, Santa Clara, CA, USA).
Sulfatase from Helix pomatia (Type H-1, CAS 9016-17-5 Cat. No. S9626-10ku), was purchased from Sigma Chemical (St Louis, MO, USA).

2. Microorganisms and chemicals

G. deliquescens NRRL1086 was obtained from a courtesy of Prof. J. P. N. Rosazza of University of Iowa, USA. Compound **1** and **2** were synthesised from berberine hydrochloride and palmatine hydrochloride via pyrolysis monodemethylation and reduction reaction respectively. Compound **3** was obtained by biotransformation of *Streptomyces griseus* YD 007 from *l*-tetrahydropalmatine. Compound **4** was obtained commercially and **5** was gifted by Prof. Yang from Institute of Basic Medical Science, Chinese Academy of Military Medical Sciences. Compound **6** was synthesized from berberine hydrochloride reacted with phloroglucinol in H_2SO_4 (60%) at 90-95℃and reduction reaction. The purity of these compounds was determined to be higher than 99% by normalization of the peak areas detected by HPLC.

3. Acid Hydrolysis of M1 and enzymatic hydrolysis of M3

M1(10mg) in 2% HCl-ethanol (1:1, 20 ml) were heated at 60□ for 2 days in a water bath. The PH value of reaction mixtures were transferred to 8 with Na_2CO_3, and then extracted with EtOAc, the $CHCl_3$ layer was evaporated, and then the residue was analyzed by HPLC.
Enzymatic hydrolysis was conducted by mixing 10 mg M3 with 10 ml 0.2M sodium acetate buffer (Ph 5.0) solution and sulfatase (30 units), then the mixtures were incubated for 2h at 37□ . After incubation, the samples were analyzed by HPLC.

4. Screening test of compound 1-6

Cultures were grown by a two-stage procedure in 30ml of potato medium (PD) held in 150-ml culture flasks. The PD medium was prepared as follows: 200g of peeled potatoes were cut into pieces, boiled in water for 20min and filtered. 20g of glucose, 3g of KH_2PO_4 and 1.5g of $MgSO_4 \cdot 7H_2O$ were added into the filtrate and diluted with distilled water to 1L before being autoclaved at 121°C for 15 min before use. Cultures were incubated with shaking at 180 rpm at 28 °C on rotary shakers. 1 ml of *G. deliquescens* NRRL1086 inoculum derived from 24 h old stage I cultures was used to initiate stage II cultures, which were incubated for 24 h before receiving 10 mg of substrate in 1ml of acetone, and incubations were conducted as before. Substrate controls consisted of sterile medium and substrates incubated under the same conditions but without microorganism. The cultures were extracted with EtOAc at 120 h after addition of substrate. The organic solvent layer was removed, evaporated to dryness, reconstituted in 0.5 ml of methanol and then spotted on TLC plates developed with $CHCl_3$ /

MeOH (V/V,10:1), and chromatograms were visualized by spraying developed plates with $KBiI_4$ reagent.

5. Preparative scale biotransformation of compound 1 and 2

Using 24h old stage II cultures of *G. deliquescens* NRRL1086, a total of 500 mg of compound **1** was distributed evenly among 50 150-mL culture flasks. Substrate-containing cultures were incubated for 120 h and then extracted with equal EtOAc. The pooled EtOAc solution was dried over anhydrous Na_2SO_4, and the solvent was removed in a vacuum, evaporated to dryness. The extract was subjected to Si gel column chromatography and the sephedex LH-20 chromatography to afford the product **M1** (308 mg, 41%), **M2** (15 mg, 2%), **M3** (224 mg, 36%). According to the HPLC quantitative analysis, the yield of **M1**, **M2** and **M3** was 48.3%, 2.78% and 43.3% respectively. The structures were identified based on its mass spectrometry, and nuclear magnetic resonance and 2D-NMR spectra data.

Similarly, a total of 500 mg of compound **2** was subjected to the biotransformation in the same procedure. Substrate-containing cultures were incubated for 120 h and then extracted with equal EtOAc. The pooled EtOAc solution was dried over anhydrous Na_2SO_4, and the solvent was removed in a vacuum, evaporated to dryness. The extract was subjected to the chromatographic separation to afford the glucosidic products **T1** (296mg, 40%) and **T2** (133mg, 18%). According to the HPLC quantitative analysis, the yield of **T1** and **T2** was 48.3%, and 27.1% respectively. (Fig. 1)

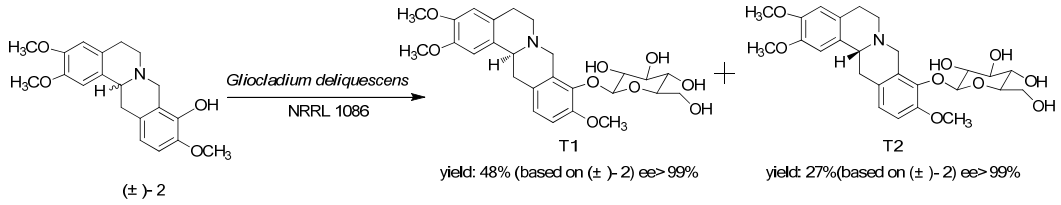
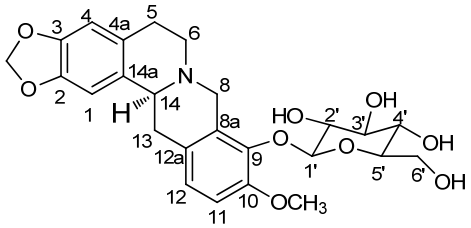


Fig.1 Biotransformation of compound 2

6. The spectrum data of substrate 1 and the metabolites M1, M2 and M3



M1

Compound **1** was obtained as white crystal (alcohol). It showed a positive $KBiI_4$ reaction. ^{13}CNMR showed 19 carbon signals, The ^{1}HNMR (CD_3OD, 500MHz) δ: 2.61 (m, 1H, 5-H), 2.65 (m, 1H, 6-H), 2.75 (m, 1H, 13-H), 3.06 (m, 1H, 5-H), 3.20 (m, 1H, 6-H), 3.34 (m, 1H, 13-H), 3.44 (d, J=16Hz, 1H, 8-H), 3.53 (m, 1H, 14-H), 3.84 (s, 3H, -OCH_3), 4.20 (d, *J*=16Hz, 1H, 8-H), 5.90 (s, 2H, -OCH_2O-), 6.60 (s, 1H, 4-H), 6.67 (d, *J*=8Hz, 1H, 11-H), 6.82 (s, 1H, 1-H), 6.81 (d, *J*=8Hz, 1H, 12-H). The ^{13}CNMR (CD_3OD, 500MHz) δ: 106.99 (C1), 146.99 (C2), 148.16 (C3), 109.64 (C4), 132.17 (C4a), 30.34 (C5), 53.09 (C6), 55.27 (C8), 129.07 (C8a), 144.04 (C9), 148.34 (C10), 111.68 (C11), 120.57 (C12), 122.67 (C12a), 37.33 (C13), 61.59 (C14), 128.96 (C14a), 102.61 (-OCH_2O-), 57.12 (10-OCH_3).

Compound **M1** showed a positive $KBiI_4$ reaction. $[\alpha]^{20}_D$ = -224.5(c 0.510, Pyridine). ^{13}CNMR showed 25 carbon signals. The ^{1}HNMR (DMSO-*d6*, 500MHz) δ: 2.46 (m, 1H, 6-H), 2.54 (m, 1H, 13-H), 2.60 (m, 1H, 5-H), 2.89 (m, 1H, 5-H), 3.06 (m, 1H, 6-H), 3.07 (m, 1H, 5′-H), 3.18 (m, 1H, 4′-H), 3.22 (m, 1H, 2′-H), 3.23 (m, 1H, 3′-H), 3.26 (m, 1H, 13-H), 3.36 (m, 1H, 14-H), 3.37 (d, *J*=16Hz, 1H, 8-H), 3.45 (m, 1H, 6′-H), 3.62 (m, 1H, 6′-H), 3.76 (s, 3H, -OCH_3), 4.32 (d, *J*=16Hz, 1H, 8-H), 4.33 (t, *J*=5.5Hz, 1H, 6′-OH), 4.92 (d, *J*=5Hz, 1H, 4′-OH), 4.92 (d, *J*=7.5Hz, 1H, 1′-H), 4.95 (d, *J*=4Hz, 1H, 3′-OH), 4.99 (d, *J*=4Hz, 1H, 2′-OH), 5.94, 5.93 (d, d, *J*=1Hz, 2H, -OCH_2O-), 6.66 (s, 1H, 4-H), 6.85 (d, *J*=8Hz, 1H, 12-H), 6.89 (s, 1H, 1-H), 6.89 (d, *J*=8Hz, 1H, 11-H). The ^{13}CNMR

(DMSO-*d6*, 500MHz) δ: 105.66 (C1), 145.35 (C2), 145.62 (C3), 108.00 (C4), 127.42 (C4a), 29.00 (C5), 50.29 (C6), 53.79(C8),127.83(C8a), 141.49(C9), 149.10(C10), 111.96(C11), 123.94(C12), 129.19(C12a), 35.61 (C13), 58.82(C14), 130.97 (C14a), 102.95 (C1′), 74.23 (C2′), 76.48 (C3′), 69.80 (C4′), 77.07 (C5′), 60.84 (C6′), 100.44 (-OCH_2O-), 56.36 (10-OCH_3).

Compound **M2** showed a positive $KBiI_4$ reaction. $[\alpha]^{20}_D$=216.8(c 0.545, Pyridine). ^{13}CNMR showed 25 carbon signals. The ^{1}HNMR (DMSO-*d6*, 500MHz) δ: 2.46 (m, 1H, 6-H), 2.53 (m, 1H,13-H), 2.59 (m, 1H, 5-H), 2.89 (m, 1H, 5-H), 3.05 (m, 1H, 6-H), 3.06 (m, 1H, 5′-H), 3.14 (m, 1H, 4′-H), 3.20 (m, 1H, 2′-H), 3.21 (m, 1H, 3′-H), 3.28 (m, 1H, 13-H), 3.40 (m, 1H, 14-H), 3.44 (m, 1H, 6′-H), 3.50 (d, *J*=16Hz, 1H, 8-H), 3.62 (m, 1H, 6′-H), 3.76 (s, 3H, -OCH_3), 4.12 (d, *J*=16Hz, 1H, 8-H), 4.29 (t, *J*=5.5Hz, 1H, 6′-OH), 4.78 (d, *J*=7.5Hz, 1H, 1′-H), 4.87 (d, *J*=5Hz, 1H, 4′-OH), 4.94 (d, J=4.5Hz, 1H, 3′-OH), 4.96 (d, *J*=4Hz, 1H, 2′-OH), 5.95, 5.94 (d, d, *J*=1Hz, 2H, -OCH_2O-), 6.66 (s, 1H, 4-H), 6.85 (d, *J*=8.5Hz, 1H, 12-H), 6.89 (s, 1H, 1-H), 6.89 (d, *J*=8.5Hz, 1H, 11-H). The ^{13}CNMR (DMSO-*d6*, 500MHz) δ: 105.59 (C1), 145.31 (C2), 145.60 (C3), 107.97 (C4), 127.42 (C4a), 28.98 (C5), 50.45 (C6), 53.86 (C8), 127.74 (C8a), 141.65 (C9), 148.89 (C10), 111.76 (C11), 123.94 (C12), 129.59 (C12a), 35.60 (C13), 58.84 (C14), 131.01 (C14a), 103.49 (C1′), 74.16 (C2′), 76.50 (C3′), 69.94 (C4′), 77.06 (C5′), 60.98 (C6′), 100.43 (-OCH_2O-), 56.30 (10-OCH_3).

Compound **M3** showed a positive $KBiI_4$ reaction. ^{13}CNMR showed 19carbon signals. The HR-ESI-MS showed a quasi-molecular ion at *m/z* 406.0974 ($[M+H]^+$). The ^{1}HNMR (CD_3OD, 500MHz) δ: 2.72 (m, 1H, 5-H), 2.75 (m, 1H, 6-H), 2.80 (m, 1H, 13-H), 3.09 (m, 1H, 5-H), 3.30 (m, 1H, 6-H), 3.38 (m, 1H, 13-H), 3.71 (m, 1H, 14-H), 3.84 (s, 3H, -OCH_3), 3.86 (m, 1H, 8-H), 4.48 (d, *J*=16Hz, 1H, 8-H), 5.90 (m, 2H, -OCH_2O-), 6.61 (s, 1H, 4-H), 6.83 (s, 1H, 1-H), 6.89 (d, *J*=8Hz, 1H, 11-H), 6.92 (d, *J*=8Hz, 1H, 12-H). The ^{13}CNMR (CD_3OD, 500MHz) δ: 106.99 (C1), 148.33 (C2), 148.44 (C3), 109.69 (C4), 131.64 (C4a), 30.01 (C5), 52.78 (C6), 55.71 (C8), 129.26 (C8a), 140.94 (C9), 152.01 (C10), 113.25 (C11), 125.44 (C12), 128.40 (C12a), 36.87 (C13), 61.49 (C14), 128.85 (C14a), 102.68 (-OCH_2O-), 57.17 (10-OCH_3).

7. The spectrum data of substrate 2 and the metabolites T1 and T2

Compound **2** was obtained as white crystal (alcohol). It showed a positive $KBiI_4$ reaction. ESI-MS m/z: 342.2 $[M+H]^+$. ^{1}HNMR ($CDCl_3$, 500MHz) δ: 2.68 (m, 2H, 6-H, 13-H), 2.86 (m, 1H, 5-H), 3.22 (m, 3H, 13-H, 6-H, 5-H), 3.54 (d, J=15Hz, 1H, 8-H), 3.58 (m, 1H, 14-H), 3.87 (s, 6H, -OCH_3), 3.89 (s, 3H, -OCH_3), 4.25 (d, J=15Hz, 1H, 8-H), 5.68 (s, 1H, -OH), 6.68 (d, J=8Hz, 1H, 12-H), 6.74 (d, J=8Hz, 1H, 11-H), 6.62 (s, 1H, 4-H), 6.74 (s, 1H, 1-H). ^{13}CNMR ($CDCl_3$, 125MHz) δ: 108.70 (C1), 147.54 (C2), 147.48 (C3), 109.00 (C4), 121.29 (C4a), 29.31 (C5), 51.46 (C6), 53.51 (C8), 126.88 (C8a), 141.56 (C9), 144.06 (C10), 111.42 (C11), 119.28 (C12), 128.08 (C12a), 36.41 (C13), 59.30 (C14), 129.81 (C14a), 56.17 (2-OCH_3), 56.11 (3-OCH_3), 55.87 (10-OCH_3).

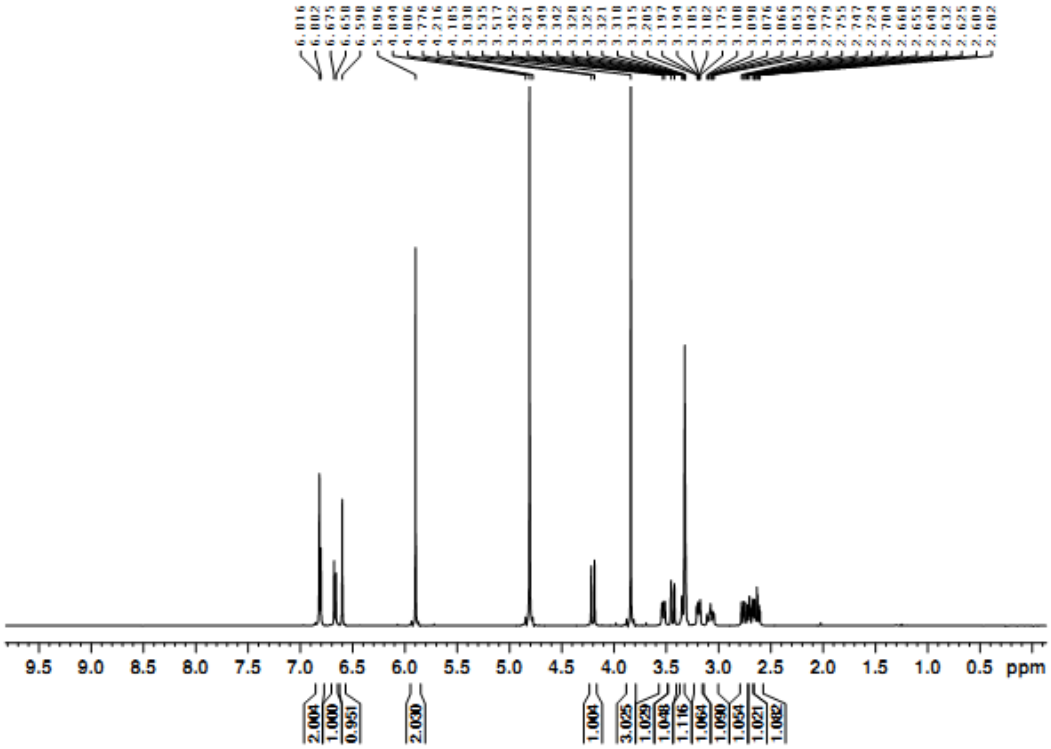
Compound **T1** showed a positive $KBiI_4$ reaction and the CD spectra exhibited negative Cotton effects around 210 nm (similar to 14*S* protoberberine). $[\alpha]^{20}_D$= -221.3(c 0.501, Pyridine). ESI-MS m/z: 504.3 $[M+H]^+$. ^{1}HNMR (DMSO-*d6*, 500 MHz) δ: 2.45 (m, 1H, 6-H), 2.56 (m, 1H, 13-H), 2.61 (m, 1H, 5-H), 2.91 (m, 1H, 5-H), 3.07 (m, 1H, 6-H), 3.08 (m, 1H, 5′-H), 3.20 (m, 1H, 4′-H), 3.23 (m, 1H, 2′-H), 3.24 (m, 1H,3′-H), 3.34 (m, 1H, 13-H), 3.37 (d, 1H, J=16Hz, 8-H), 3.39 (m, 1H, 14-H), 3.46 (m, 1H, 6′-H), 3.62 (m, 1H, 6′-H), 3.73 (s, 3H, -OCH_3), 3.74 (s, 3H, -OCH_3), 3.76 (s, 3H, -OCH_3), 4.33 (d, 1H, J=16Hz, 8-H), 4.35 (t, 1H, J=5.5Hz, 6′-OH), 4.93 (d, 1H, J=7.5Hz, 1′-H), 4.93 (s, 1H, 4′-OH), 4.99 (s, 1H, 3′-OH), 4.99 (s, 1H, 2′-OH), 6.68 (s, 1H, 4-H)，6.86 (s, 1H, 1-H), 6.88 (d, 1H, J=11Hz, 12-H), 6.89 (d, 1H, J=11Hz, 11-H). ^{13}CNMR (DMSO-*d6*, 125MHz) δ: 109.49 (C1), 149.06 (C2), 147.14 (C3), 111.74 (C4), 126.33 (C4a), 28.57 (C5), 50.51 (C6), 53.90 (C8), 127.97 (C8a), 141.48 (C9), 147.13 (C10), 111.93 (C11), 123.88 (C12), 129.29 (C12a), 35.57 (C13), 58.59 (C14), 129.79 (C14a), 102.92 (C1′), 74.25 (C2′), 76.48 (C3′), 69.79 (C4′),77.10 (C5′), 60.82 (C6′), 55.39 (10-OCH_3), 55.71 (3-OCH_3), 56.34 (2-OCH_3).

Compound **T2** showed a positive $KBiI_4$ reaction and the CD spectra exhibited positive Cotton effects around 210 nm (similar to 14*R* protoberberine). $\mathbf{[\alpha]^{20}_D}$=235.1(c 0.521, Pyridine). ESI-MS m/z: 504.3 $[M+H]^+$. ^{1}HNMR (DMSO-*d6*, 500MHz) δ: 2.45 (m, 1H, 6-H), 2.56 (m, 1H, 13-H), 2.60 (m, 1H, 5-H), 2.91 (m, 1H, 5-H), 3.04 (m, 1H, 6-H), 3.06 (m, 1H, 5′-H), 3.15 (m, 1H, 4′-H), 3.20 (m, 1H, 2′-H), 3.22 (m, 1H, 3′-H), 3.34 (m, 1H, 13-H), 3.42 (m, 1H, 14-H), 3.45 (m, 1H, 6′-H), 3.51 (d, 1H, J=16Hz, 8-H), 3.63 (m, 1H,6′-H), 3.73 (s, 3H, -OCH_3), 3.74 (s, 3H, -OCH_3), 3.76 (s, 3H, -OCH_3), 4.13 (d, 1H, J=16Hz,

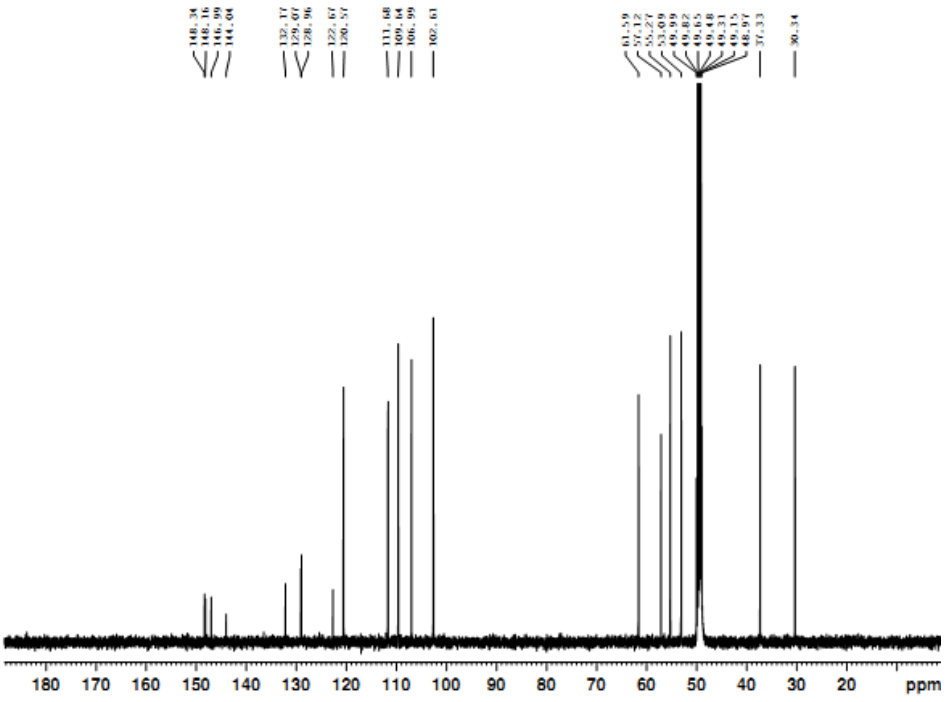
8-H), 4.30 (s, 1H, 6′-OH), 4.78 (d, J=7.5Hz, 1H, 1′-H), 4.92 (s, 1H, 4′-OH), 4.98 (s, 1H, 3′-OH), 4.98 (s, 1H, 2′-OH), 6.68 (s, 1H, 4-H), 6.86 (s, 1H, 1-H), 6.88 (d, 1H, J=9Hz, 12-H), 6.89 (d, 1H, J=9Hz, 11-H). ^{13}CNMR (DMSO-*d6*, 125 MHz) δ: 109.44 (C1), 148.88 (C2), 147.14 (C3), 111.72 (C4), 126.35 (C4a), 28.56 (C5), 50.66 (C6), 53.97 (C8), 127.86 (C8a), 141.65 (C9), 147.11 (C10), 111.74 (C11), 123.90 (C12), 129.71 (C12a), 35.54 (C13), 58.59 (C14), 129.85 (C14a), 103.47 (C1′), 74.17 (C2′), 76.50 (C3′), 69.95 (C4′), 77.05 (C5′), 61.01 (C6′), 55.38 (10-OCH_3), 55.71 (3-OCH_3), 56.28 (2-OCH_3).

8. The spectrum of substrate 1 and the metabolites M1, M2 and M3

8.1 The ^{1}HNMR and ^{13}CNMR spectrum of compound 1

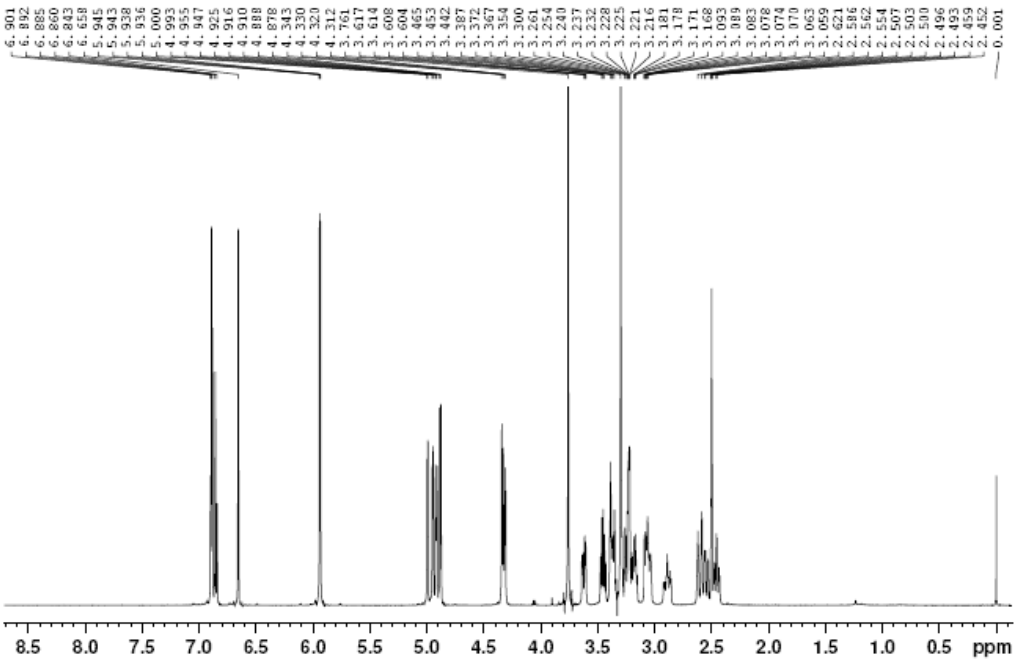


^{1}HNMR spectrum of compound 1 (CD_3OD)

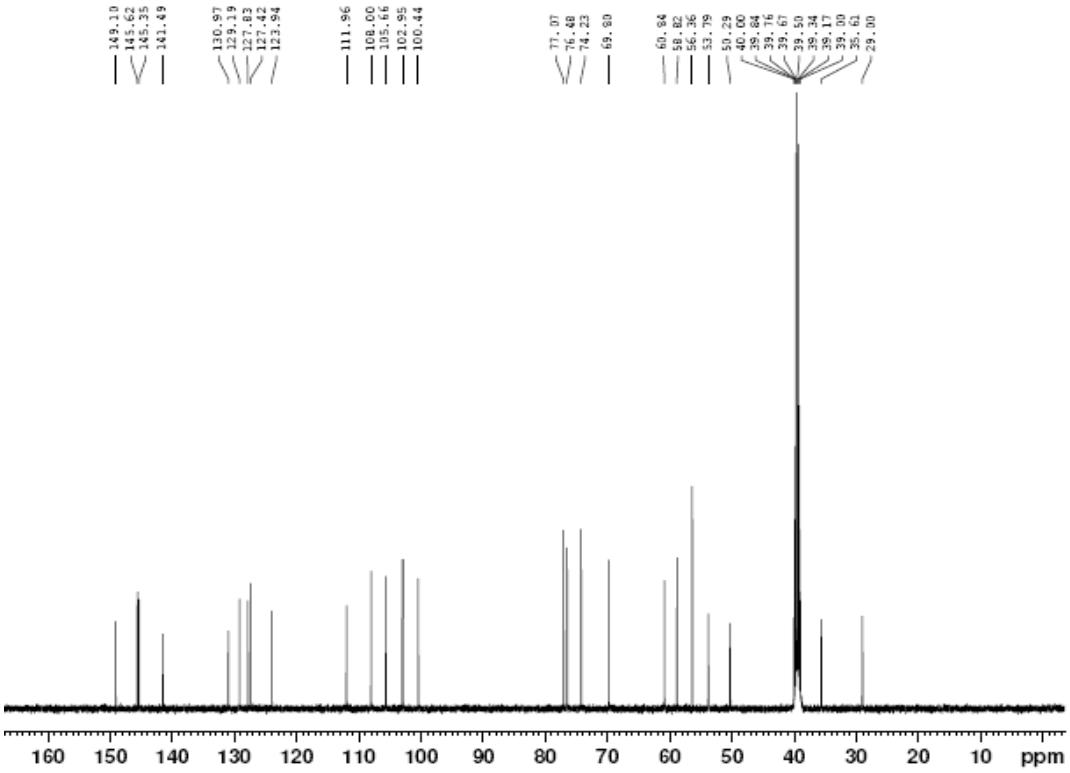


^{13}CNMR spectrum of compound 1(CD_3OD)

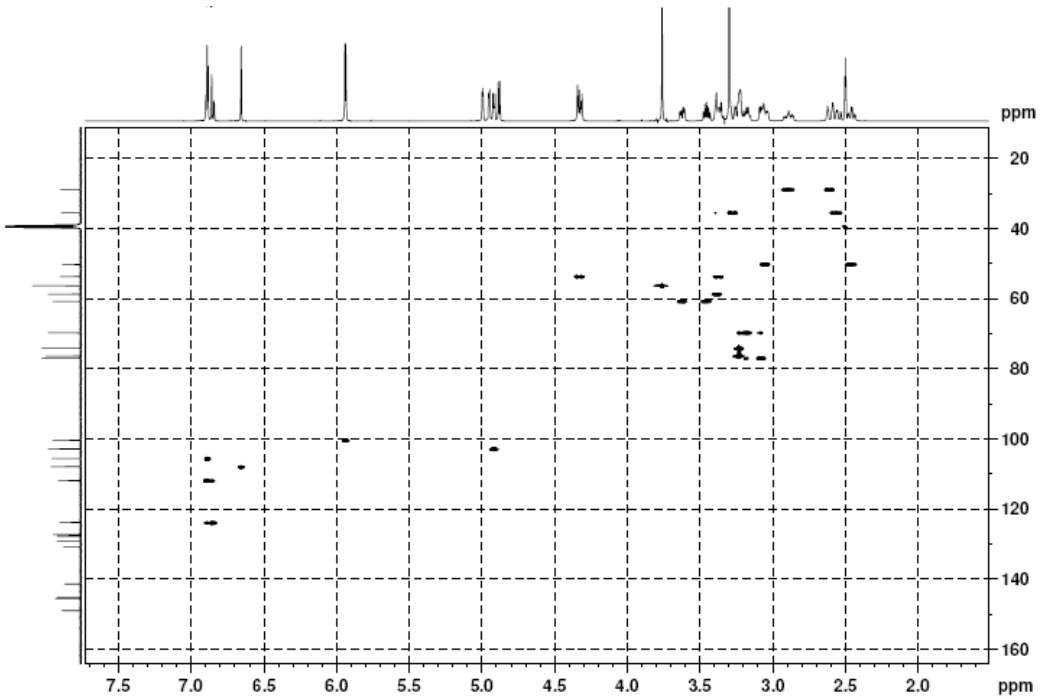
8.2 The ^{1}HNMR, ^{13}CNMR, HSQC, HMBC, DEPT of compound M1



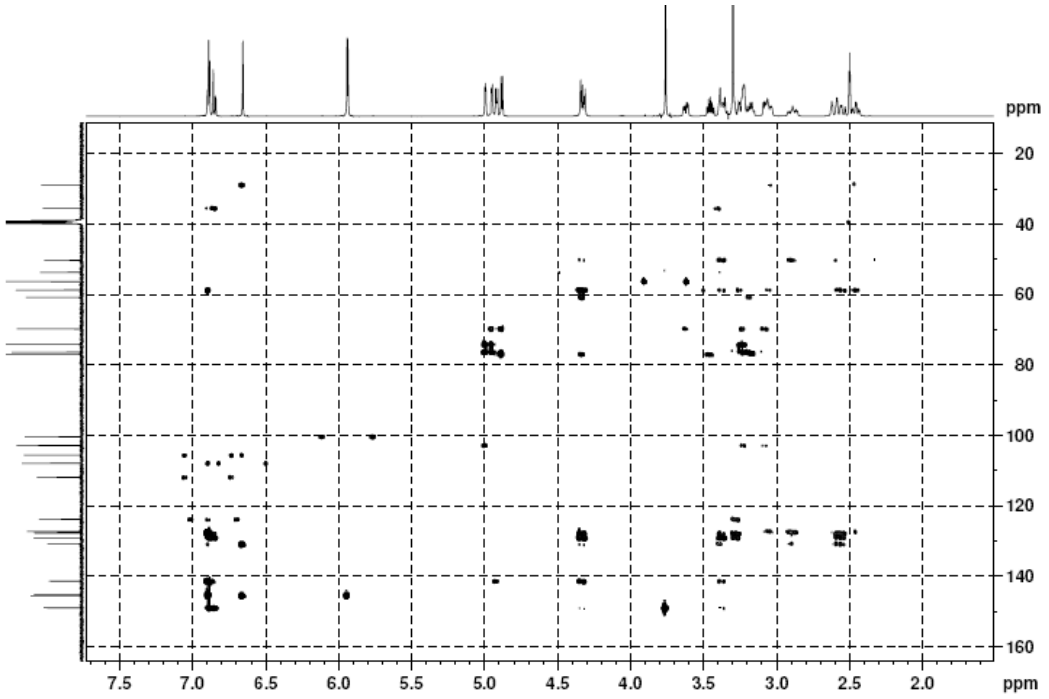
^{1}HNMR of compound M1



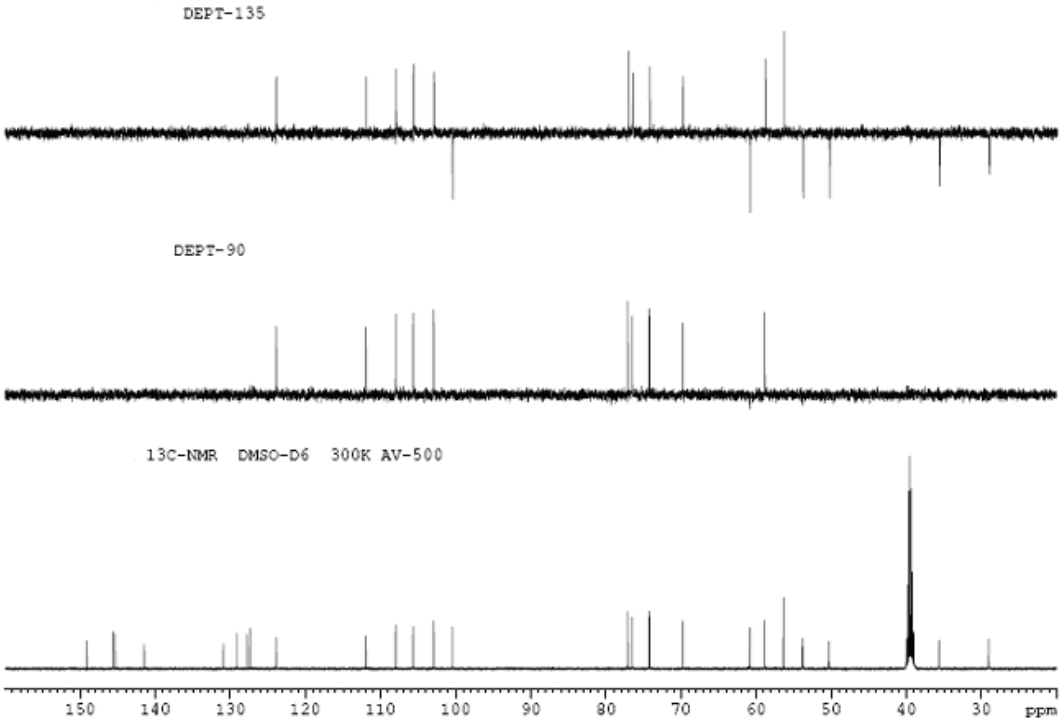
^{13}CNMR of compound M1



HSQC of compound M1

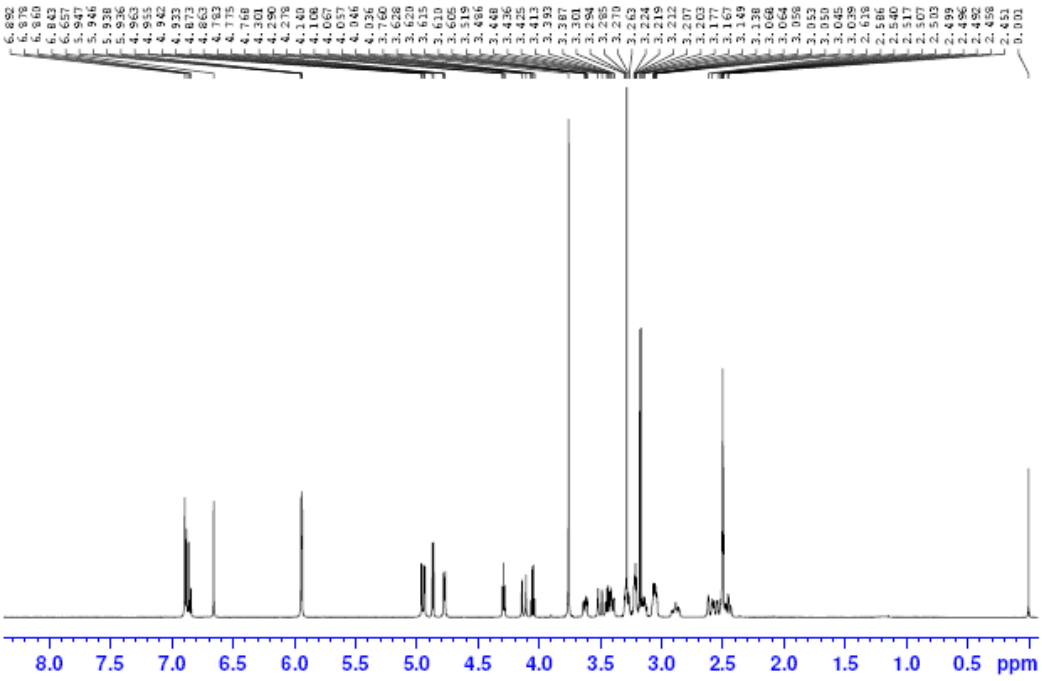


HMBC of compound M1

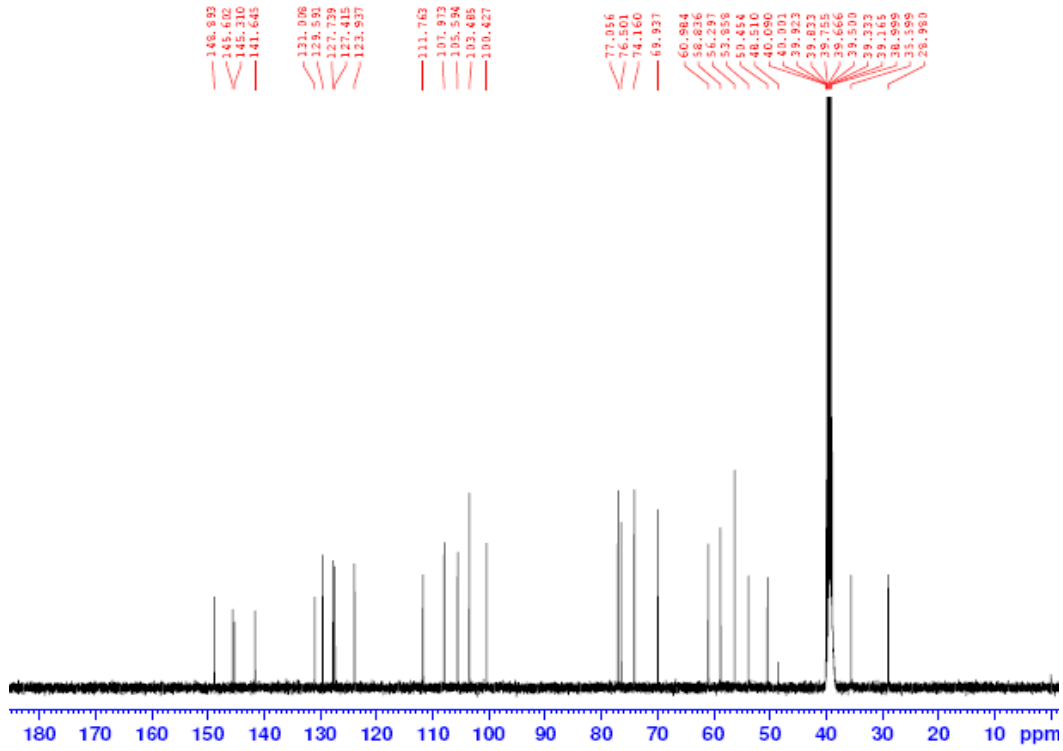


DEPT of compound M1

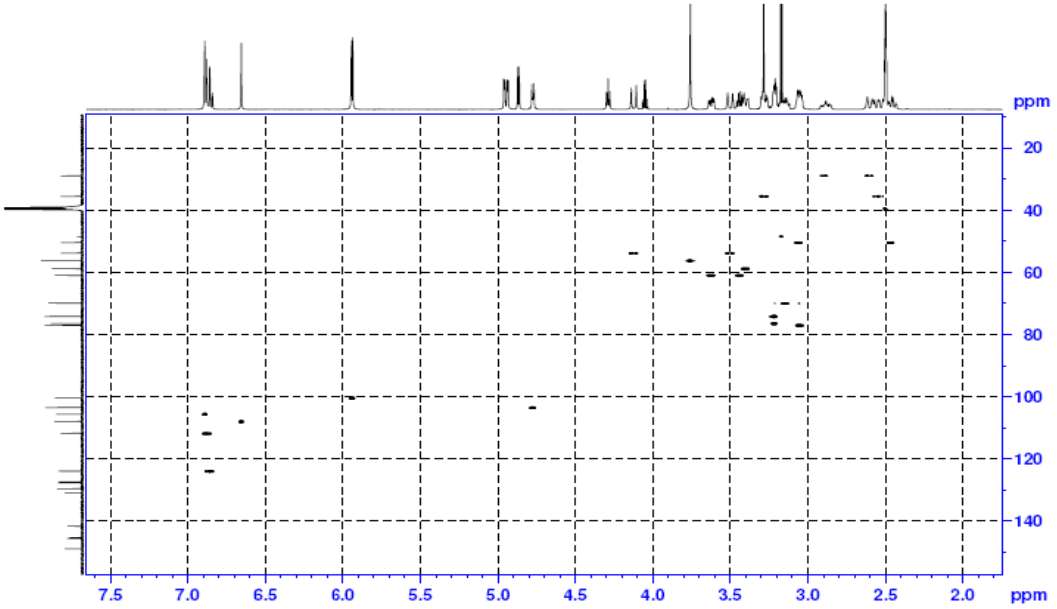
8. 3 The ^{1}HNMR, ^{13}CNMR,HSQC,HMBC,DEPT and CD spectrum of compound M2



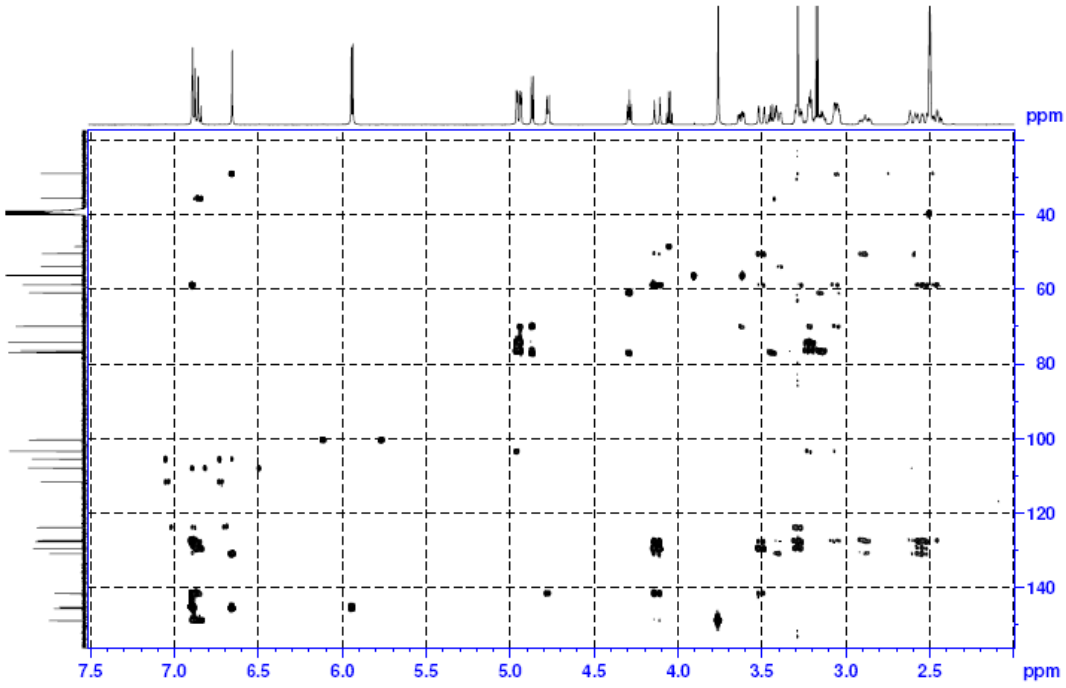
^{1}HNMR of compound M2



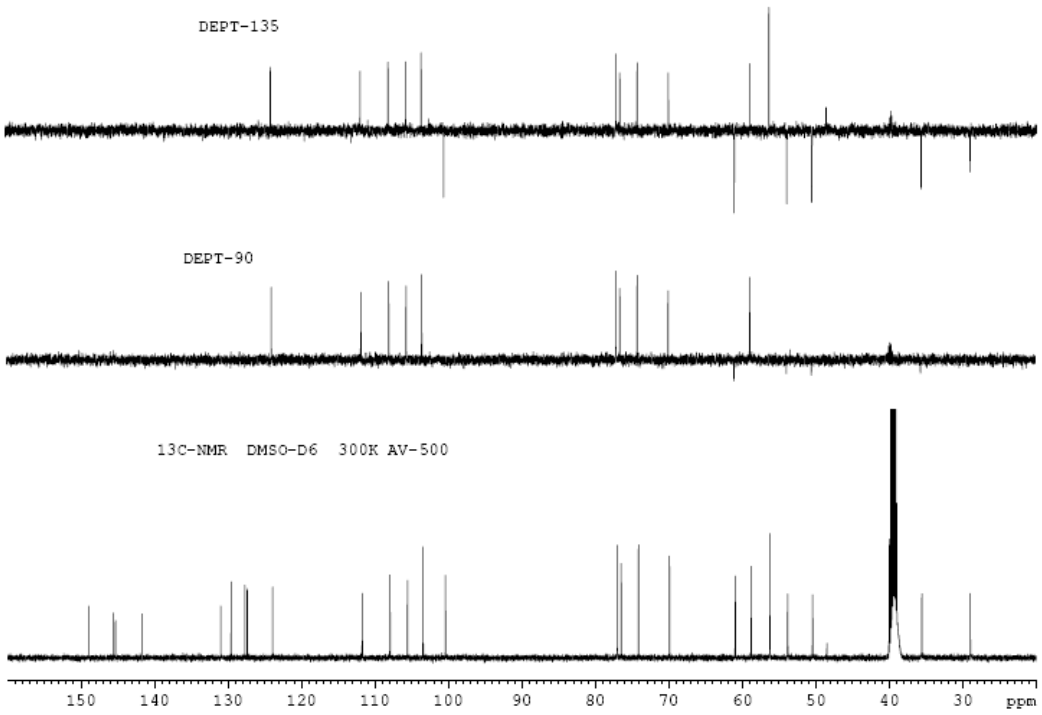
^{13}CNMR of compound M2



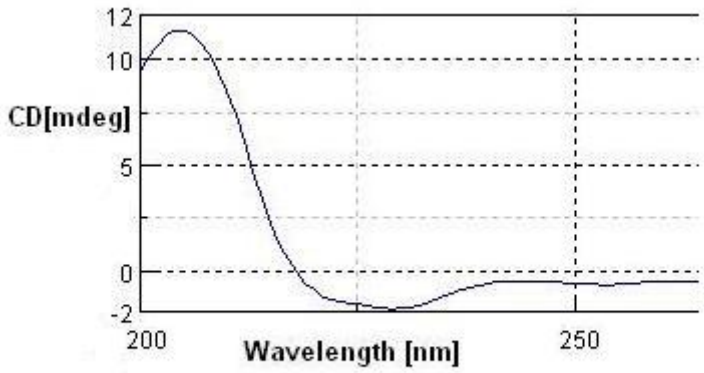
HSQC of compound M2



HMBC of compound M2

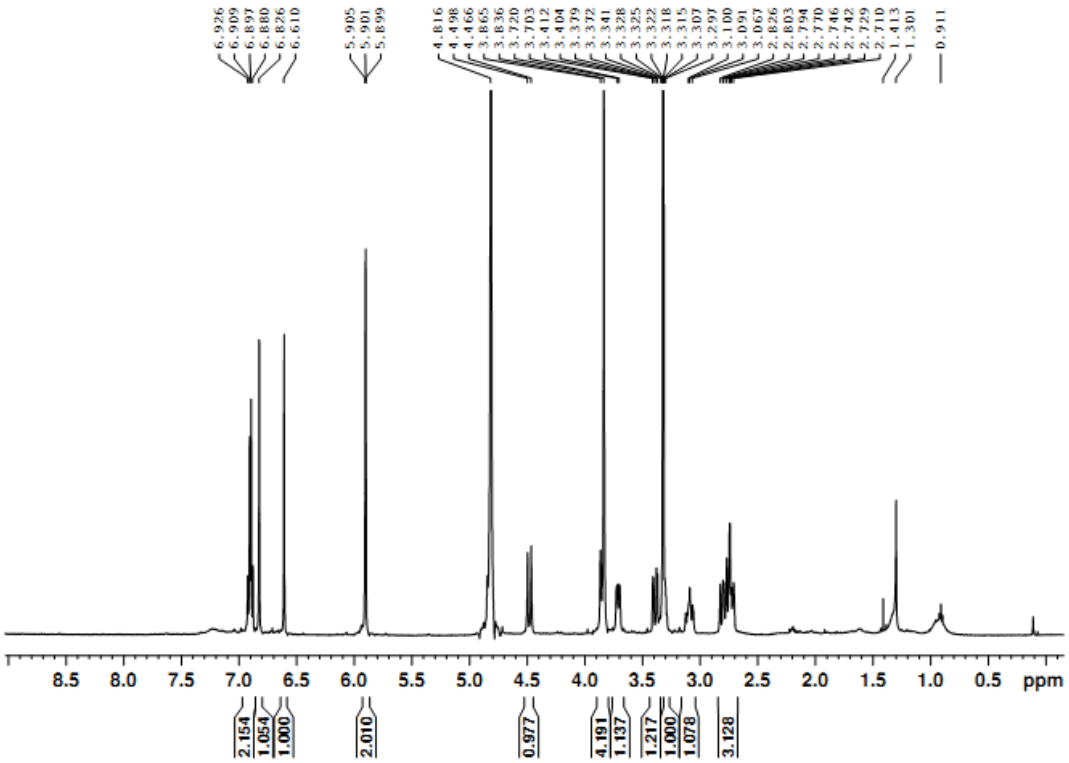


DEPT of compound M2

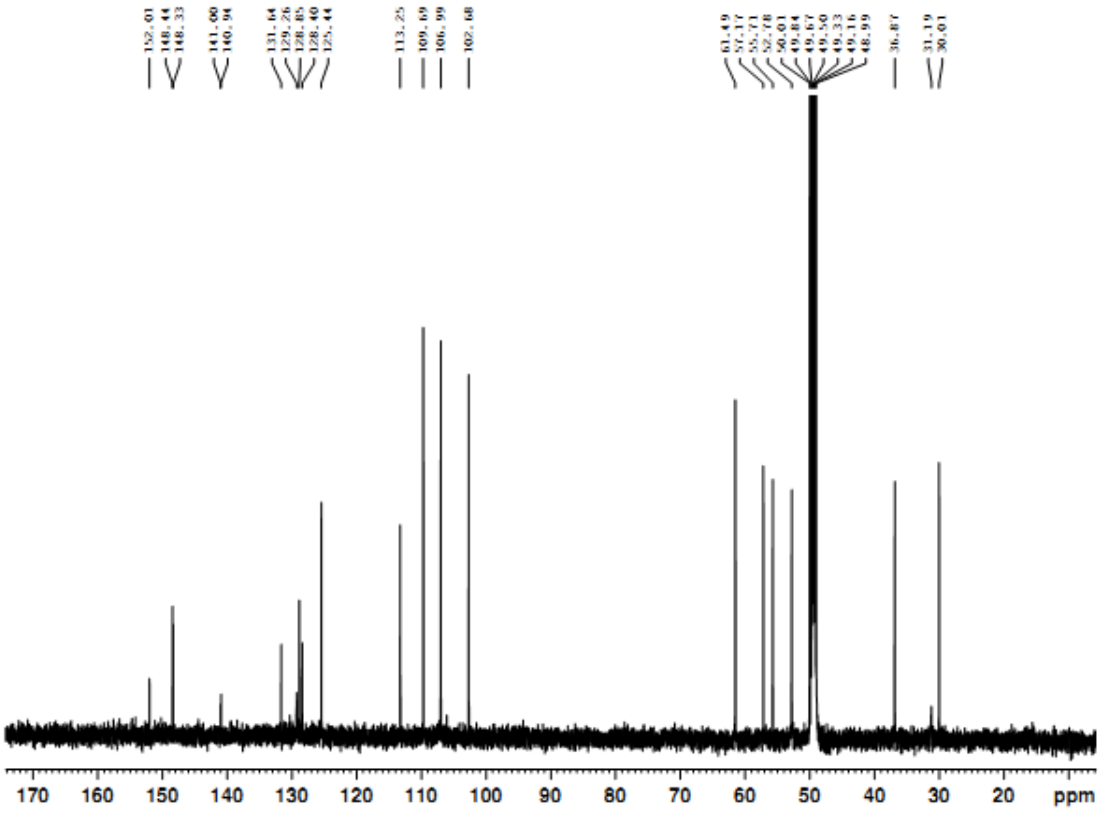


CD spectrum of Compound M2

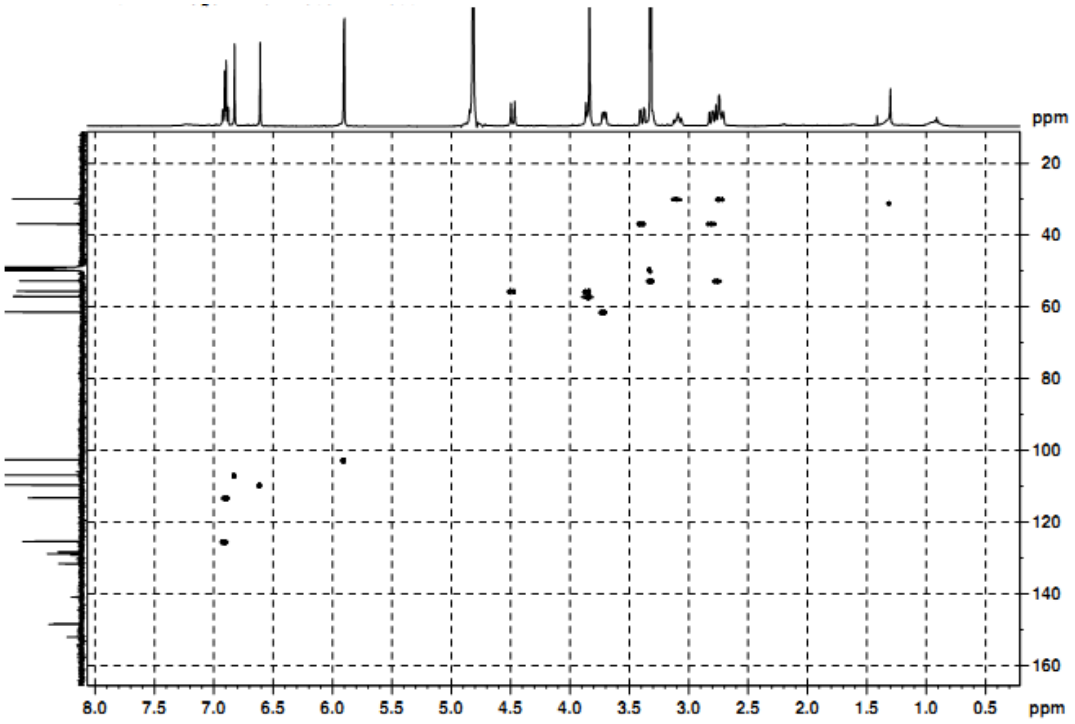
8. 4 The ^{1}HNMR, ^{13}CNMR, HSQC, HMBC, DEPT and CD spectrum of compound M3



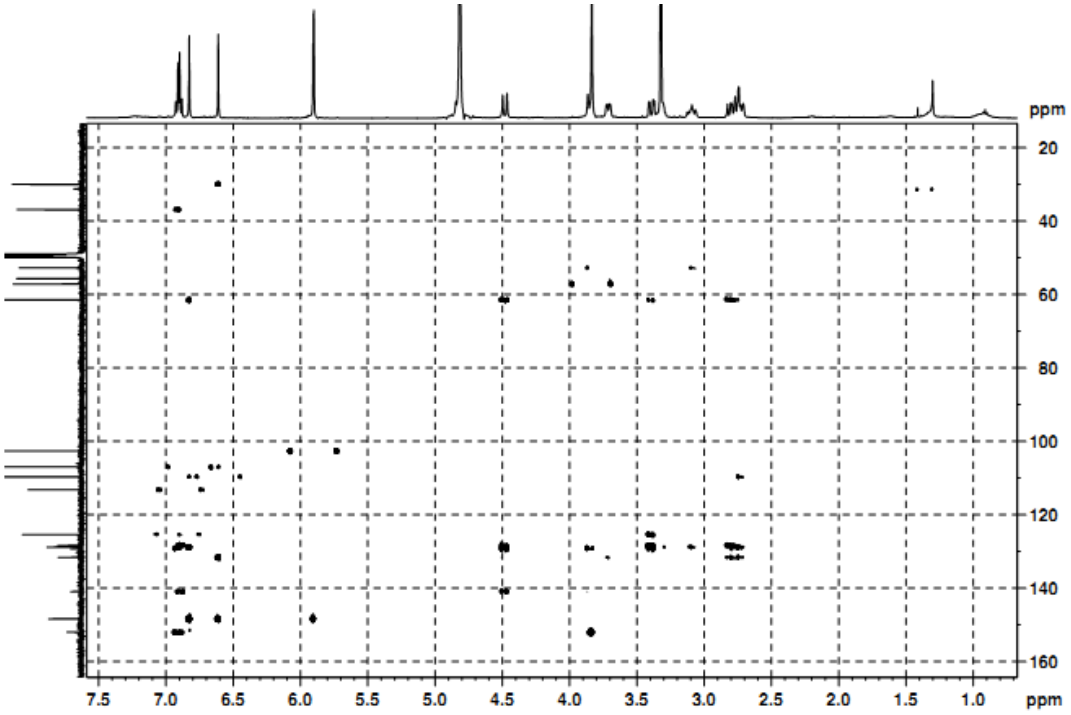
[1]HNMR of compound M3



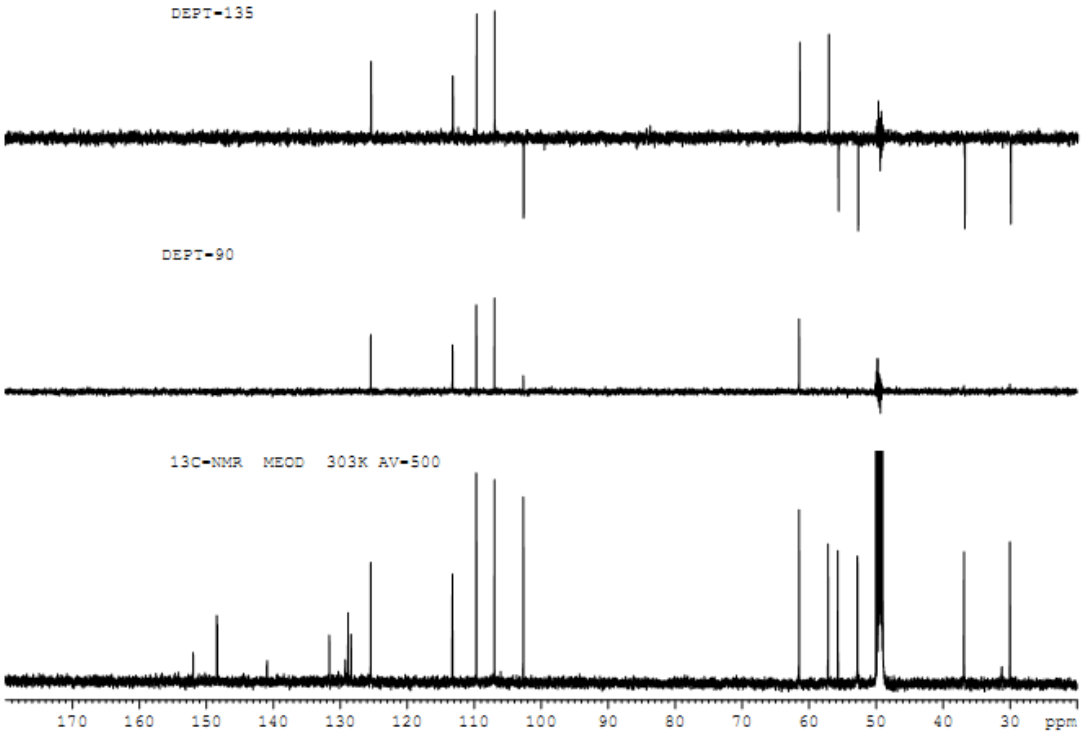
[13]CNMR of compound M3



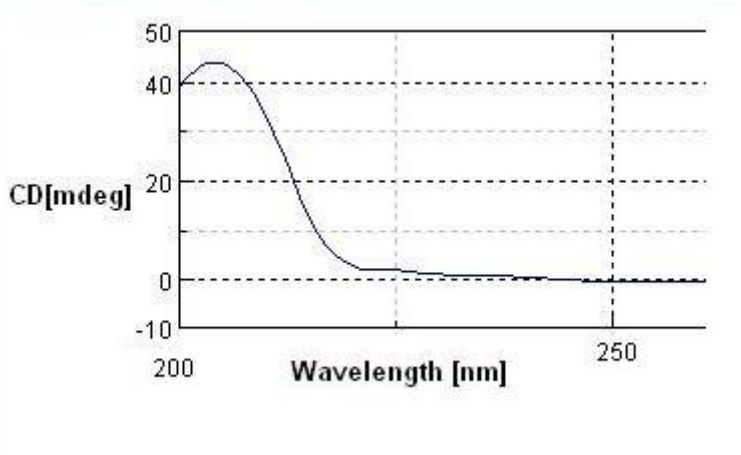
HSQC of compound M3



HMBC of compound M3



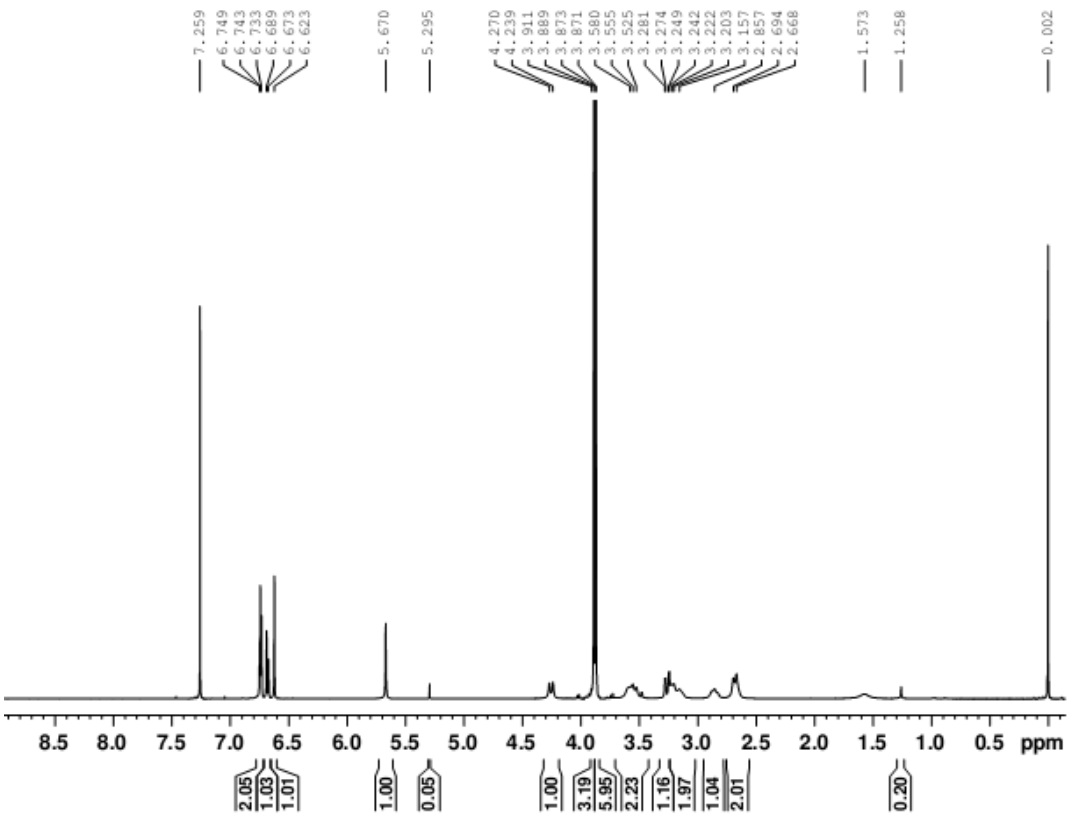
DEPT of compound M3



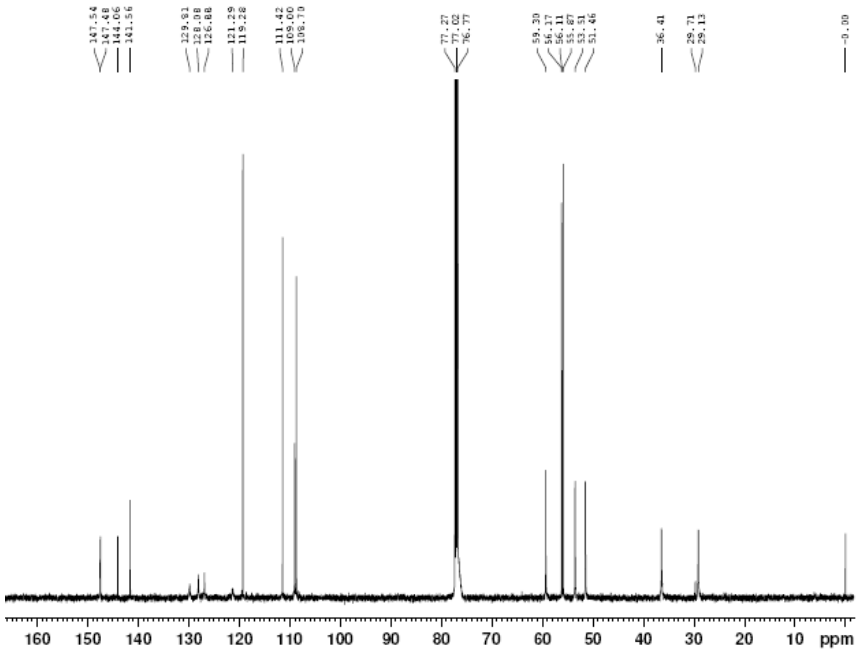
CD spectrum of compound M3

9. The spectrum of substrate 2 and the metabolites T1 and T2

9.1 The ^{1}HNMR and ^{13}CNMR spectrum of compound 2

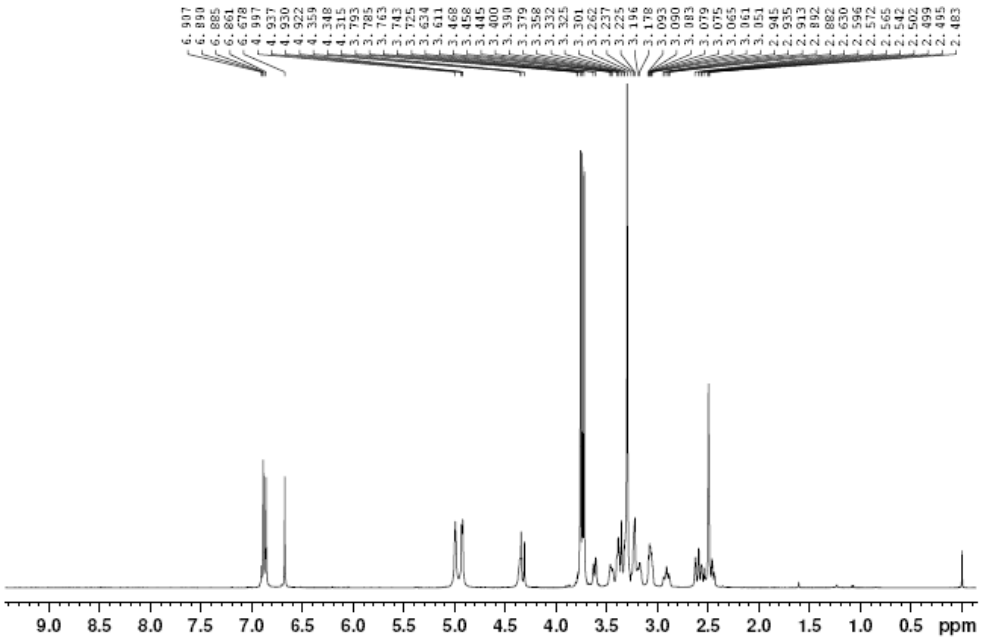


[1]HNMR of compound 2

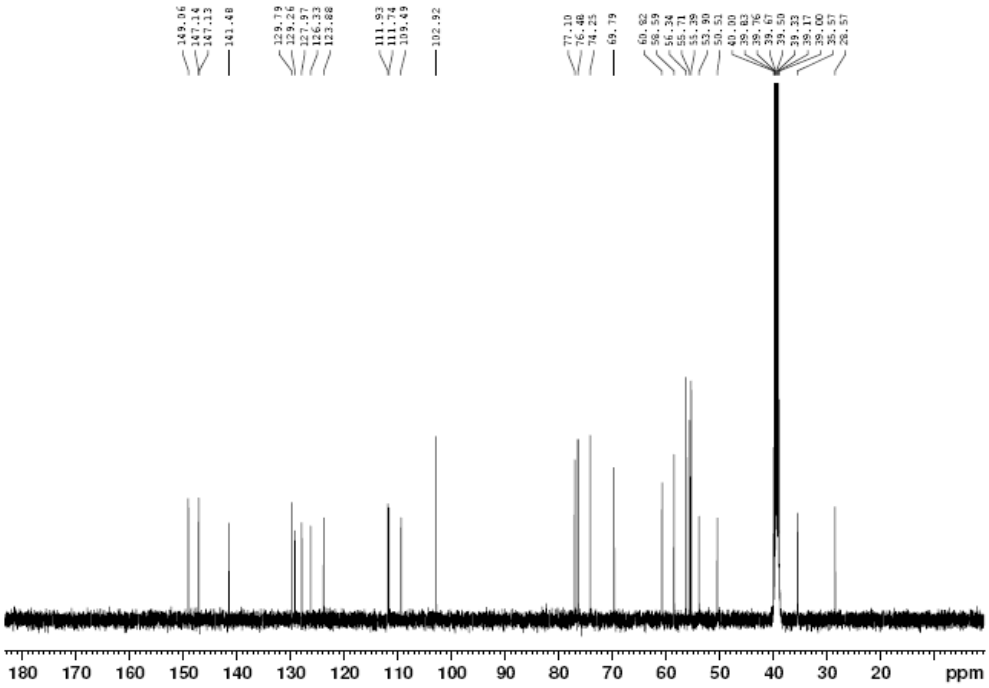


[13]CNMR of compound 2

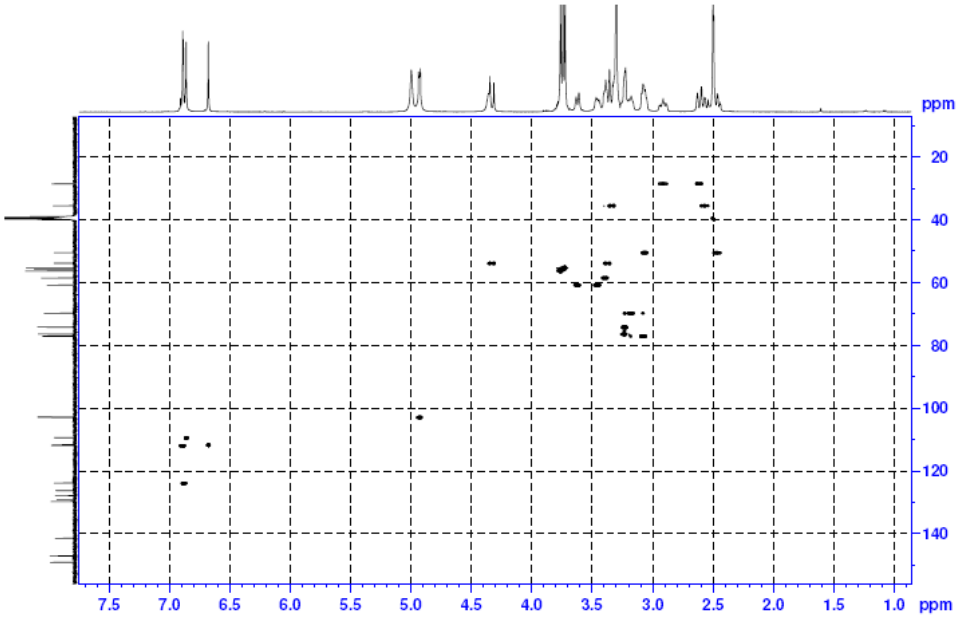
9.2 The [1]HNMR, [13]CNMR, HSQC, HMBC, DEPT of compound T1



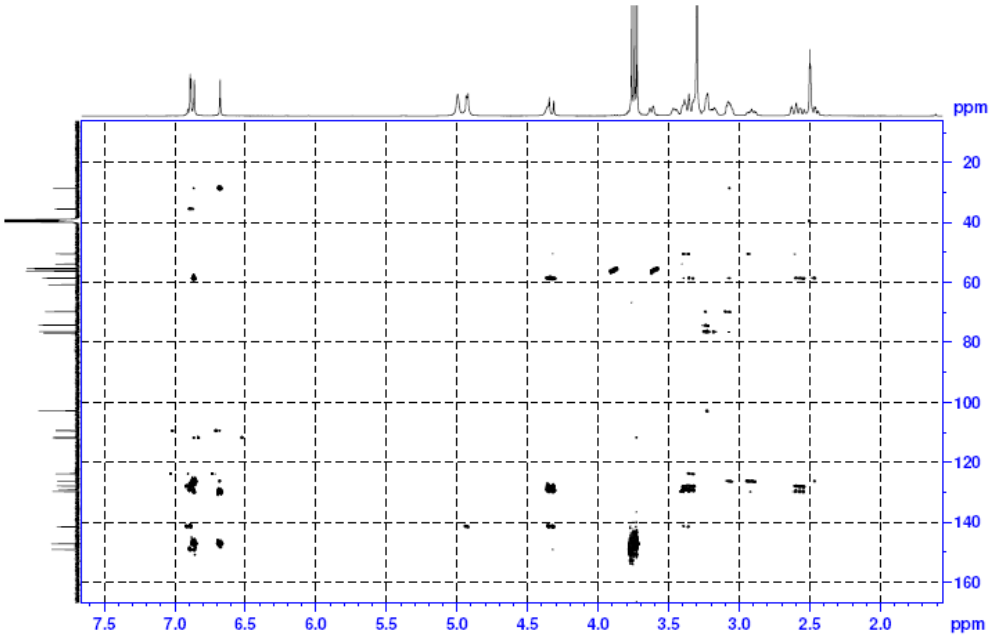
^{1}HNMR of compound T1



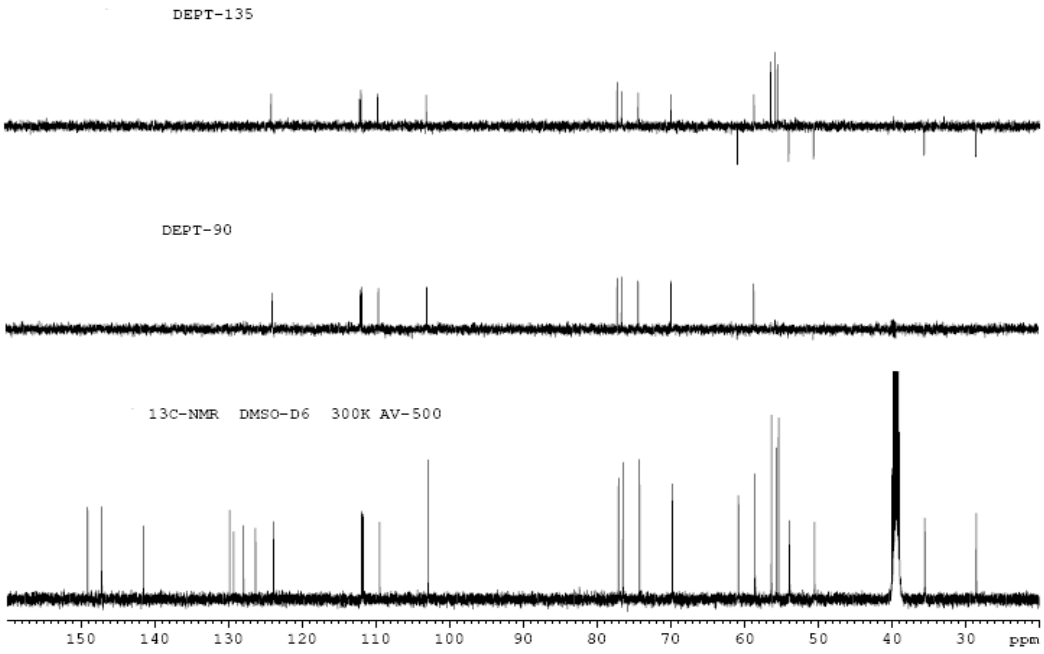
^{13}CNMR of compound T1



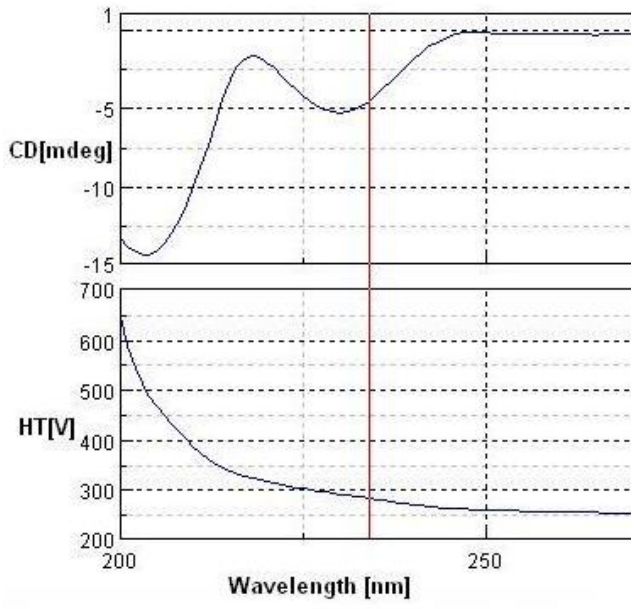
HSQC of compound T1



HMBC of compound T1

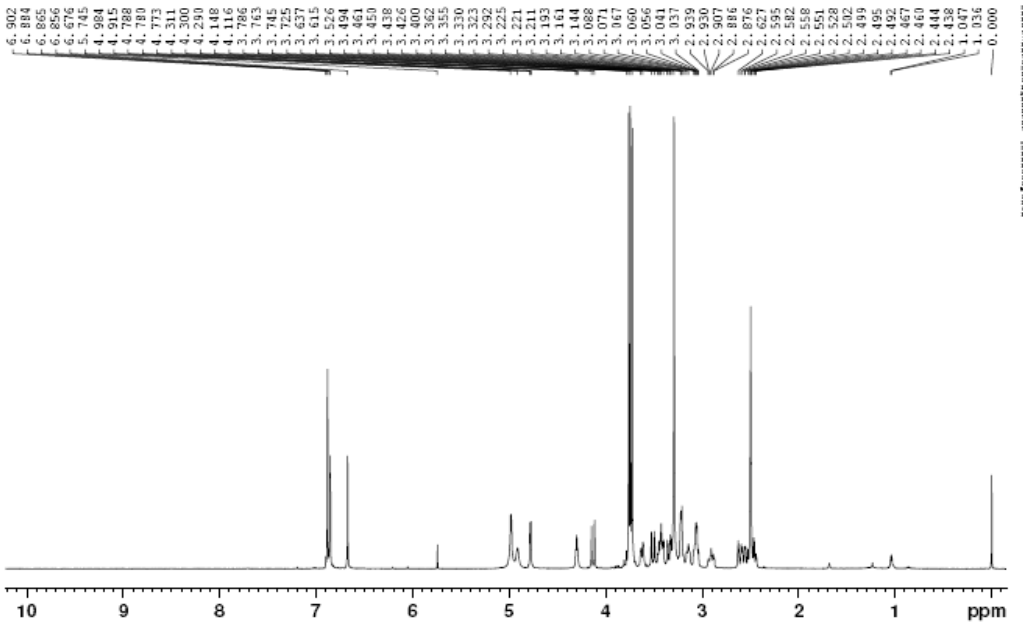


DEPT of compound T1

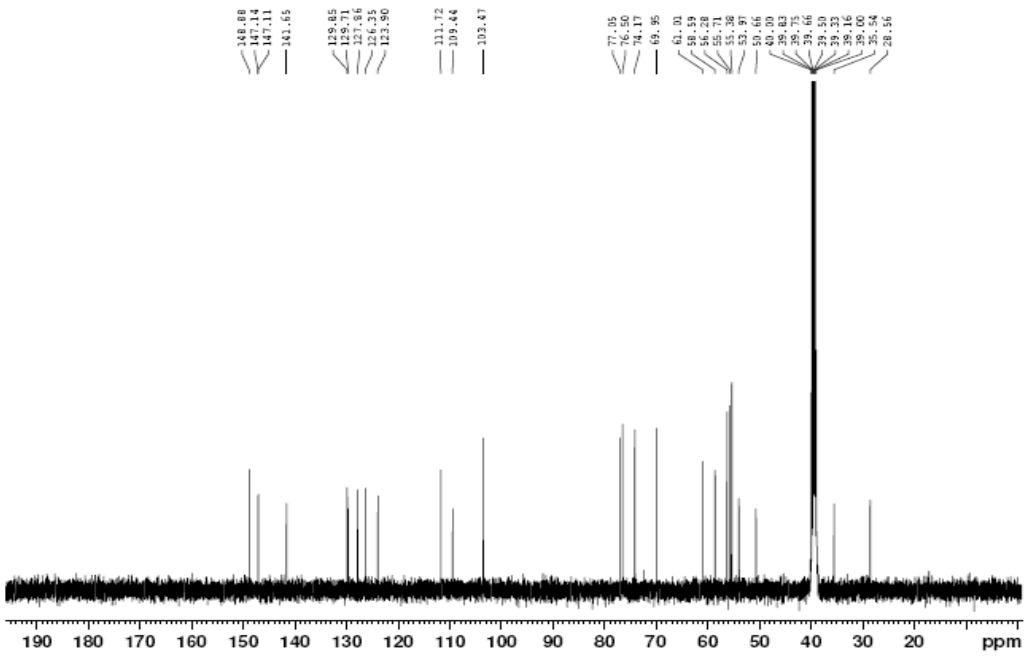


CD spectrum of compound T1

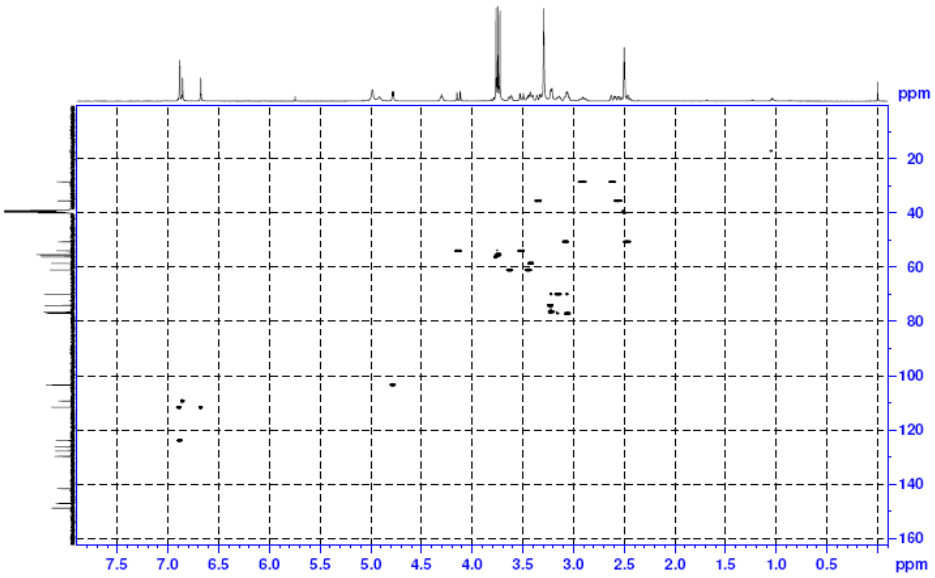
9. 3 The ^{1}HNMR, ^{13}CNMR, HSQC, HMBC, DEPT and CD spectrum of compound T2



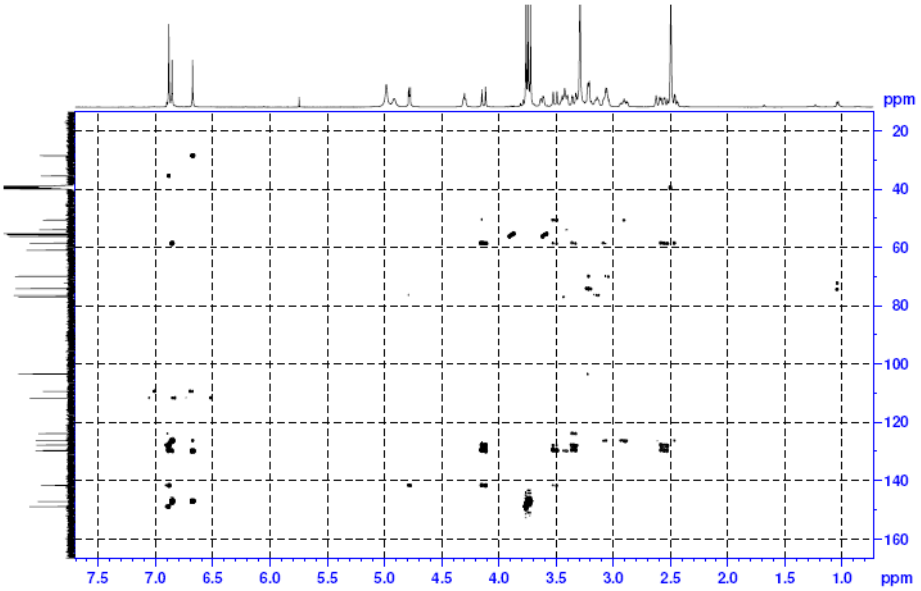
[1]HNMR of compound T2



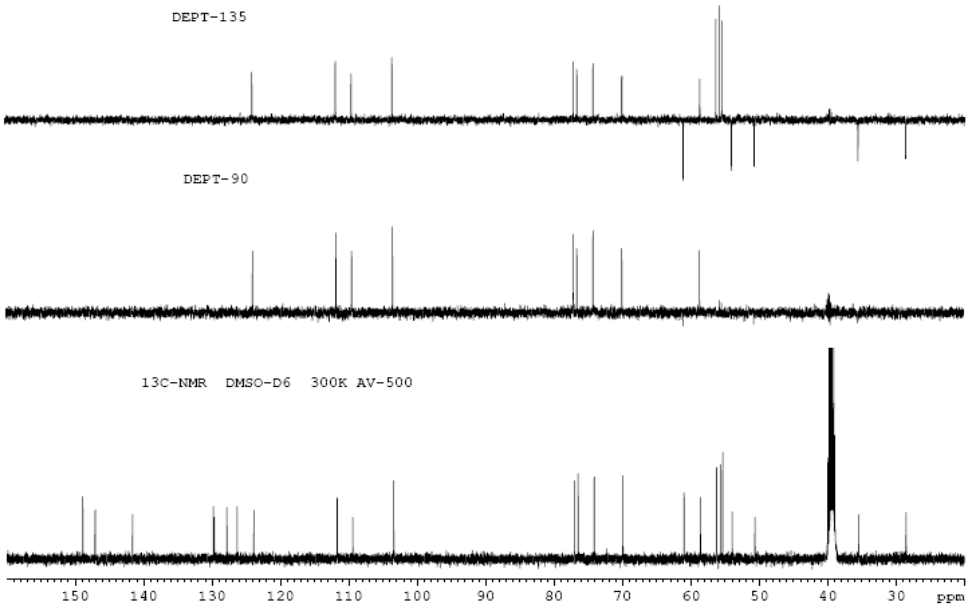
[13]CNMR of compound T2



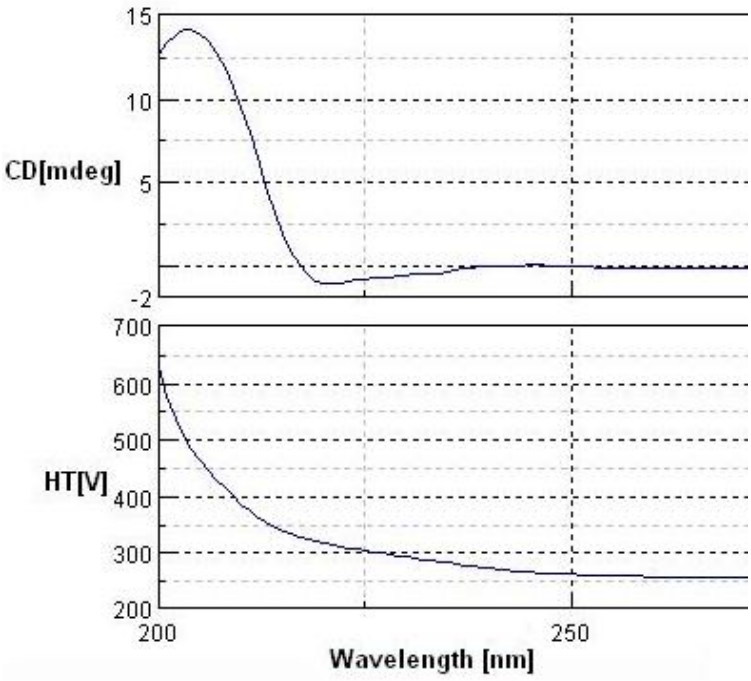
HSQC of compound T2



HMBC of compound T2



DEPT of compound T2



CD spectrum of compound T2