Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY DATA

Synthesis of Non Steroidal Anti-Inflammatory Drugs (NSAIDs) - Lantadene Prodrugs as Novel Lung Adenocarcinoma Inhibitors via Inhibition of Cyclooxygenase-2 (COX-2), Cyclin D1 and TNF-α-induced NF-κB Activation

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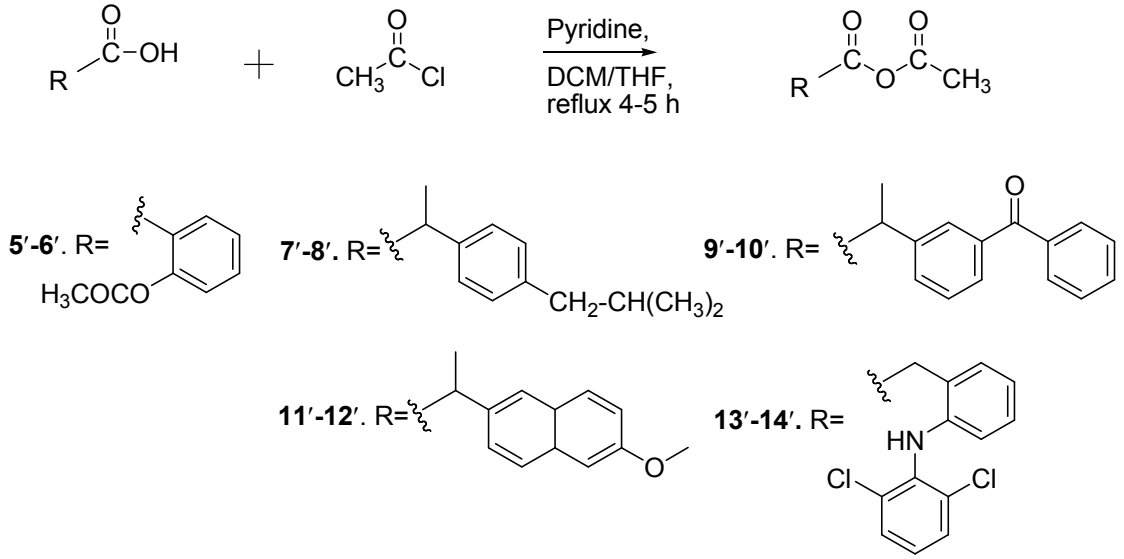
D. Reverse phase-HPLC purity chromatograms of compounds (1−14)

A. Synthetic scheme S1- Synthesis of anhydride derivatives of NSAIDs (5′–14′) for esterification step



Scheme S1. Synthesis of anhydrides derivatives of NSAIDs

B. Isolation, semi-synthesis and spectral data of compounds (1–4)

5.3. Extraction and isolation of lantadene A (1) and B (2)

1 kg of lantana leaves powder was extracted with 5 L ethyl acetate at room temperature for 24 h with intermittent shaking. The extract was filtered and 250 g of activated charcoal was added to it for 1 h. Extract was filtered again and filtrate was concentrated under reduced pressure in rotary evaporator. To the concentrated extract, 100 ml of chloroform was added and partitioned with 100 ml water. The aqueous layer was again washed with chloroform (100 ml×2). The organic portion was finally evaporated to dryness to yield a crude mixture of lantadenes, 0.448% w/w (4.48±0.216 g). Lantadene A and B were isolated from a mixture of crude lantadenes using column chromatography (14 cm silica gel bed height, 110.30 g silica gel of 100–200 mesh, and 4 cm column diameter) in a mobile phase of petroleum ether (60-80 °C): ethyl acetate (4:1). ($R_{f:}$ lantadene A: 0.40, lantadene B: 0.37).

5.3.1. 22*β*-Angeloyloxy-3-oxo-olean-12-en-28-oic acid (1)

Mp: 285–286 °C. Anal. calcd. for $C_{35}H_{52}O_5$ (552.38): %C, 76.05; H, 9.48. Found: %C, 76.10; H, 9.49. IR (KBr, cm^{-1}): 3308.77 (O–H), 2952.45, (C–H), 1736.06 (C=O keto), 1715.85 (C=O ester), 1702.14 (C=O acid), 1649.42 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 5.9759–6.0295 (1H, m, C-33-H), 5.3816 (1H, s, C-12-H), 5.0911 (1H, s, C-22-H), 3.0321–3.0734 (1H, dd, *J*= 13.76, 3.36 Hz, C-18-H), 2.5175–2.6028 (1H, m, C-2-Ha), 2.3396–2.4033 (1H, m, C-2-Hb), 1.1754 (3H, s, CH_3), 1.0920 (3H, s, CH_3), 1.0538 (6H, s, CH_3), 1.0032 (3H, s, CH_3), 0.8951 (3H, s, CH_3), 0.8271 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 217.70 (C-3), 179.28 (C-28), 166.27 (C-31), 143.11 (C-13), 139.07 (C-33), 127.59 (C-32), 122.50 (C-12), 75.85 (C-22), 55.30 (C-5), 50.59 (C-17), 47.45 (C-9), 46.88 (C-4), 45.94 (C-19) 42.00 (C-14), 39.22 (C-8), 39.11 (C-1), 38.46 (C-18), 37.72 (C-21), 36.78 (C-10), 34.14 (C-2), 33.70 (C-29), 32.19 (C-7), 30.05 (C-20), 27.57 (C-15), 26.45 (C-23), 26.15 (C-27), 25.79 (C-30), 24.19 (C-16), 23.51 (C-11), 21.49 (C-6), 20.59 (C-35), 19.48 (C-26), 16.85 (C-24), 15.68 (C-34), 15.11 (C-25). ESI-MS (*m/z*): 553.40 (M^++1).

5.3.2. 22*β*-Senecioyloxy-3-oxo-olean-12-en-28-oic acid (2)

Mp: 283–284 °C. Anal. calcd. for $C_{35}H_{52}O_5$ (552.38): %C, 76.05; H, 9.48. Found: %C, 76.13; H, 9.50. IR (KBr, cm^{-1}): 3289.29 (O–H), 2950.25, 2925.42, 2864.39 (C–H), 1738.61 (C=O keto), 1712.29 (C=O ester), 1693.62 (C=O acid), 1648.72 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 5.5577 (1H, s, C-32-H), 5.3785 (1H, s, C-12-H), 5.0404 (1H, s, C-22-H), 3.0072–3.0488 (1H, dd, *J*= 13.44, 3.48 Hz, C-18-H), 2.5190–2.6039 (1H, m, C-2-Ha), 2.3417–2.4022 (1H, m, C-2-Hb), 1.1754 (3H, s, CH_3), 1.0906 (3H, s, CH_3), 1.0656 (3H, s, CH_3), 1.0486 (3H, s, CH_3), 1.0027 (3H, s, CH_3), 0.8845 (3H, s, CH_3), 0.8388 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 217.77 (C-3), 178.84 (C-28), 165.33 (C-31), 157.16 (C-33), 143.09 (C-13), 122.37 (C-12), 115.96 (C-32), 75.20 (C-22), 55.30 (C-5), 50.57 (C-17), 47.45 (C-9), 46.87 (C-4), 45.97 (C-19) 42.05 (C-14), 39.24 (C-8), 39.17 (C-1), 38.54 (C-18), 37.63 (C-21), 36.77 (C-10), 34.16 (C-2), 33.75 (C-29), 32.26 (C-7), 30.07 (C-20), 27.59 (C-15), 27.46 (C-35), 26.44 (C-23), 26.28 (C-27), 25.77 (C-30), 24.13 (C-16), 23.56 (C-11), 21.50 (C-6), 20.25 (C-34), 19.52 (C-26), 16.85 (C-24), 15.16 (C-25). ESI-MS (*m/z*): 553.50 (M^++1).

5.4. Synthesis of 22*β*-hydroxy-3-oxo-olean-12-en-28-oic acid (3)

To 1 g mixture of **1** and **2**, 100 ml of 10% ethanolic KOH was added and the reaction mixture was refluxed for 6 h. After completion of reaction, dilute HCl solution was added to reaction mixture to neutralize the KOH and the product precipitated out was washed with water (100 ml×3), and purified through column chromatography to afford a compound **3** (651.17 mg, 76.47%).

5.4.1. 22*β*-Hydroxy-3-oxo-olean-12-en-28-oic acid (3)

Yield: 76.47%, Mp: 240–242 °C. Anal. calcd. for $C_{30}H_{46}O_4$ (470.34): %C, 76.55; H, 9.85. Found: %C, 76.62; H, 9.87. IR (KBr, cm^{-1}): 3439.83, 3261.69 (O–H), 2929.90, 2868.21 (C–H), 1730.93 (C=O keto), 1706.08 (C=O acid), 1622.39 (C=C). ^{1}H NMR (400 MHz, $CDCl_3$+DMSO-d_6 mixture, δ ppm): 5.2486–5.2637 (1H, t, *J*= 3.02 Hz, C-12-H), 3.7501–3.7670 (1H, t, *J*= 3.38 Hz, C-22-H), 3.5997 (1H, s (br), C-22-OH), 2.9251–2.9699 (1H, dd, *J*= 13.20, 4.28 Hz, C-18-H), 2.4448–2.5298 (1H, m, C-2-Ha), 2.2748–2.3405 (1H, m, C-2-Hb), 1.1238 (3H, s, CH_3), 1.0769 (3H, s, CH_3), 0.9908 (3H, s, CH_3), 0.8440 (3H, s, CH_3), 0.8330 (3H, s, CH_3), 0.7805 (3H, s,

CH_3), 0.7094 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6 mixture, δ ppm): 216.41 (C-3), 176.33 (C-28), 144.25 (C-13), 120.77 (C-12), 77.23 (C-22), 54.56 (C-5), 51.07 (C-17), 47.10 (C-9), 46.70 (C-4), 45.96 (C-19) 41.74 (C-14), 38.79 (C-8), 38.75 (C-1), 38.52 (C-18), 38.00 (C-21), 36.22 (C-10), 33.68 (C-2), 33.59 (C-29), 31.82 (C-7), 29.77 (C-20), 27.31 (C-15), 27.02 (C-23), 26.13 (C-27), 25.25 (C-30), 23.88 (C-16), 22.97 (C-11), 21.00 (C-6), 19.11 (C-26), 16.52 (C-24), 14.67 (C-25). ESI-MS (negative-ion mode, *m/z*): 470.32 (M^-) (469.29 (M^-–1).

5.5. Synthesis of 3*β*,22*β*-Dihydroxy-olean-12-en-28-oic acid (4)

1 mmol (470.68 mg) of compound **3** was stirred with 1 mmol (37.83 mg) of sodium borohydride in 50 ml solution of methanol (25 ml) and tetrahydrofuran (25 ml) for 7 h (Scheme 2). After completion of reaction, dilute HCl solution was added to the reaction mixture to quench the $NaBH_4$. The organic solvents were removed under reduced pressure and precipitated product was extracted with DCM. The solvent was removed under reduced pressure to afford a compound **4**, which was further purified by using column chromatography (silica gel of 100–200 mesh and gradient mobile phase of hexane-ethyl acetate).

5.5.1. 3*β*,22*β*-Dihydroxy-olean-12-en-28-oic acid (4)

Yield: 87.79%, Mp: 282–284 °C. Anal. calcd. for $C_{30}H_{48}O_4$ (472.36): %C, 76.23; H, 10.24. Found: %C, 76.29; H, 10.23. IR (KBr, cm^{-1}): 3435.07 (O–H), 2948.50, 2876.33 (C–H), 1705.76 (C=O), 1648.59 (C=C). ^1H NMR (400 MHz, $CDCl_3$+DMSO-d_6 mixture, δ ppm): 11.4773 (1H, s (br), C-28-H (COOH)), 5.2267–5.2441 (1H, t, *J*= 3.48 Hz, C-12-H), 4.1543 (1H, s (br), C-22-OH), 3.7499–3.7654 (1H, t, *J*= 3.10 Hz, C-22-H), 3.5768 (1H, s (br), C-3-OH), 3.0544–3.0934 (1H, t, *J*= 7.80 Hz, C-3-H), 2.9195–2.9626 (1H, dd,

J= 13.84, 3.56 Hz, C-18-H), 1.1270 (3H, s, CH_3), 1.0925 (3H, s, CH_3), 0.9397 (3H, s, CH_3), 0.8953 (3H, s, CH_3), 0.8513 (3H, s, CH_3), 0.7982 (3H, s, CH_3), 0.7250 (3H, s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6 mixture, δ ppm): 176.23 (C-28), 143.82 (C-13), 120.96 (C-12), 77.16 (C-3), 72.70 (C-22), 54.80 (C-5), 51.00 (C-17), 47.10 (C-9), 46.06 (C-19), 41.63 (C-14), 41.11 (C-8), 38.80 (C-4), 38.30 (C-18), 38.10 (C-1), 37.93 (C-21), 36.52 (C-10), 33.71 (C-29), 32.42 (C-7), 29.79 (C-20), 27.96 (C-2), 27.32 (C-15), 27.06 (C-23), 26.80 (C-27), 25.38 (C-30), 23.89 (C-16), 22.90 (C-11), 17.90 (C-6), 16.69 (C-26), 15.63 (C-24), 15.03 (C-25). ESI-MS (negative-ion mode, *m/z*): 472.30 (M^-) 471.20 (M^-–1).

5.6.1. 3*β*-(2-Acetoxybenzoyloxy)-22*β*-hydroxy-olean-12-en-28-oic acid (5) and

5.6.2. 3*β*,22*β*-Di(2-acetoxybenzoyloxy)-olean-12-en-28-oic acid (6)

Synthesis of 3*β*-substituted olean-12-en-28-oic acid prodrug (**5**) and 3*β*,22*β*-disubstituted olean-12-en-28-oic acid prodrug (**6**) was carried out in two steps. In the step-1, carboxylic function of aspirin was converted into anhydride function by the base catalyzed reaction of acid and acyl halide. Aspirin/acetylsalicylic acid (360.32 mg, 2 mmol) with an equimolar amount of acetyl chloride (142.20 µl, 2 mmol) in the presence of pyridine (161.76 µl, 2 mmol) were refluxed in tetrahydrofuran for 5 h. Organic solvent was removed in rotary evaporator and the reaction mixture was washed with chloroform (100 ml×3) under reduced pressure at 65 °C to yield solid anhydride product of aspirin, which was used in the subsequent step without further purification.

In the step-2, 3*β*,22*β*-dihydroxy substituted compound **4** (472.70 mg, 1 mmol) and anhydride derivative of aspirin (444.38 mg, 2 mmol), in the presence of 4-DMAP (366.51 mg, 3 mmol), were refluxed in pyridine for 14 h (Scheme 1). At the end of the reaction, the reaction mixture was transferred to the 10% HCl solution and precipitated product (mixture of 3*β*-(2-acetoxybenzoyloxy) substituted (**5**) and 3*β*,22*β*-di(2-acetoxybenzoyloxy) substituted (**6**) esters) was extracted with dichloromethane and washed further

three times with 10% HCl solution (100 ml×3). The organic layer was removed under reduced pressure and the crude product obtained was chromatographed over silica gel (100-200 mesh) and eluted with varying ratios of hexane-ethyl acetate to yield final purified products separated as **5** and **6**.

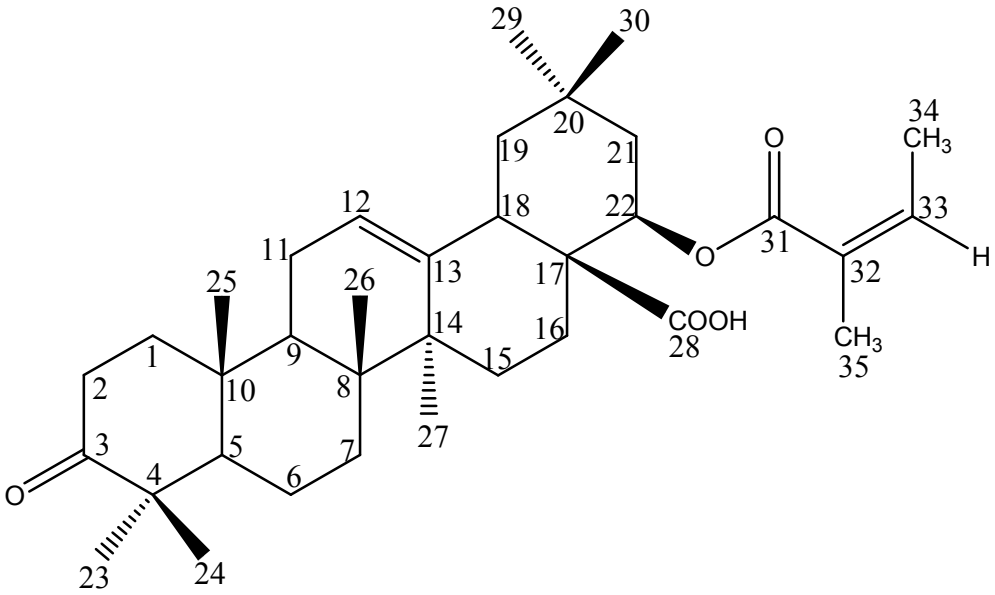
C. IR, NMR and Mass spectra of compounds (1−14)

FT-IR (KBr, cm^{-1})- Wave numbers respective to peaks visible in the IR spectra were recorded on a PerkinElmer spectrum 400 FT-IR and FT-NIR spectrometer using potassium bromide pellets with peak values assigned in auto detect mode.

NMR (δ ppm)- NMR spectra were recorded in $CDCl_3$, DMSO-d_6, and in a mixture of $CDCl_3$ and DMSO-d_6 with a BRUKER AVANCE II 400 NMR spectrometer.

ESI-MS (*m/z*)- Mass spectra of some compounds were recorded directly of reaction mixture, while for other compounds either crude product or purified product (after column chromatography) was used for mass spectrometric analysis. Mass spectra were recorded with a Waters Micromass Q-Tof micro Mass spectrometer.

1. 22*β*-Angeloyloxy-3-oxo-olean-12-en-28-oic acid (Lantadene A or LA) (1)



22β-Angeloyloxy-3-oxo-olean-12-en-28-oic acid

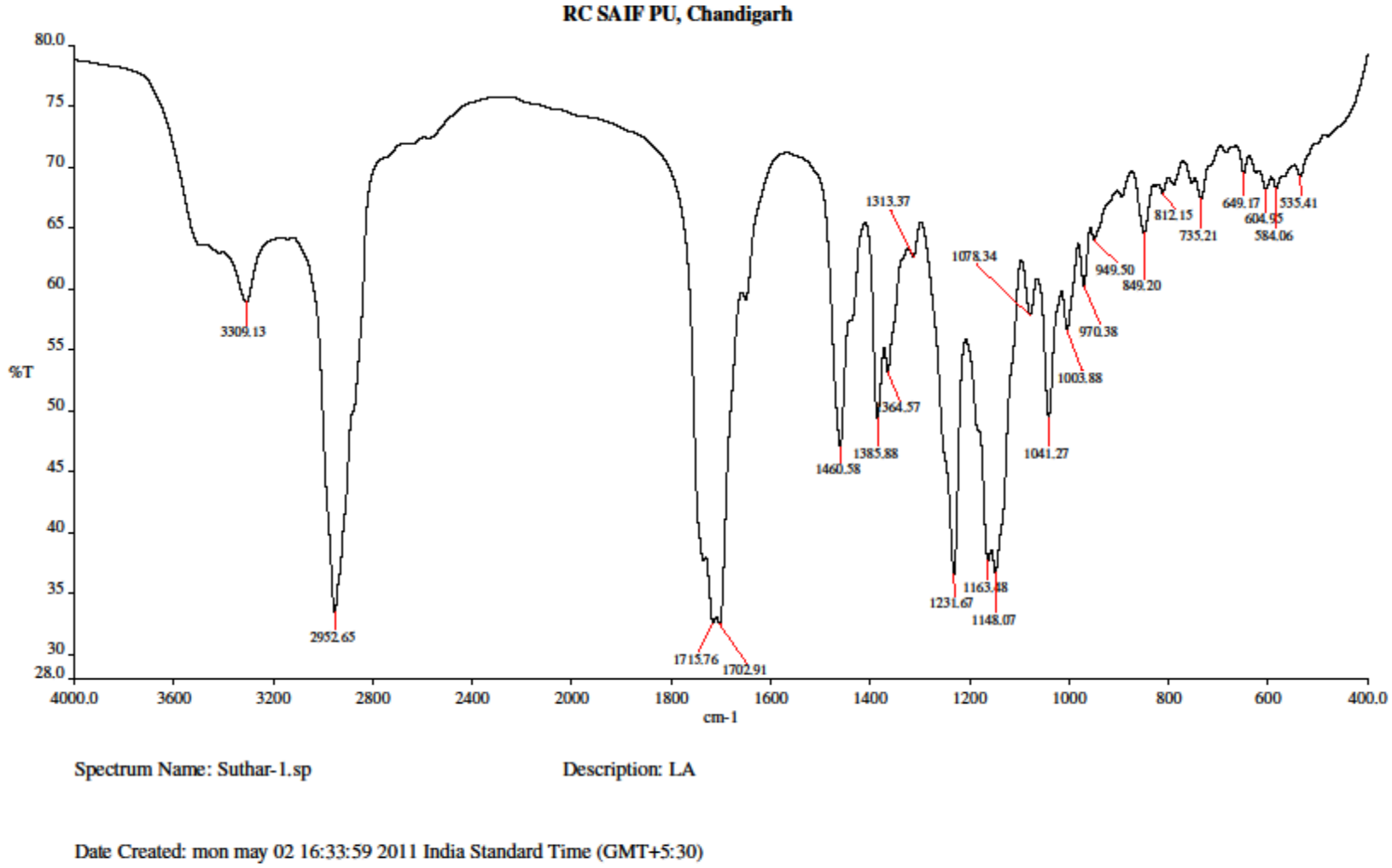


Fig. 1. FT-IR spectrum of compound **1**

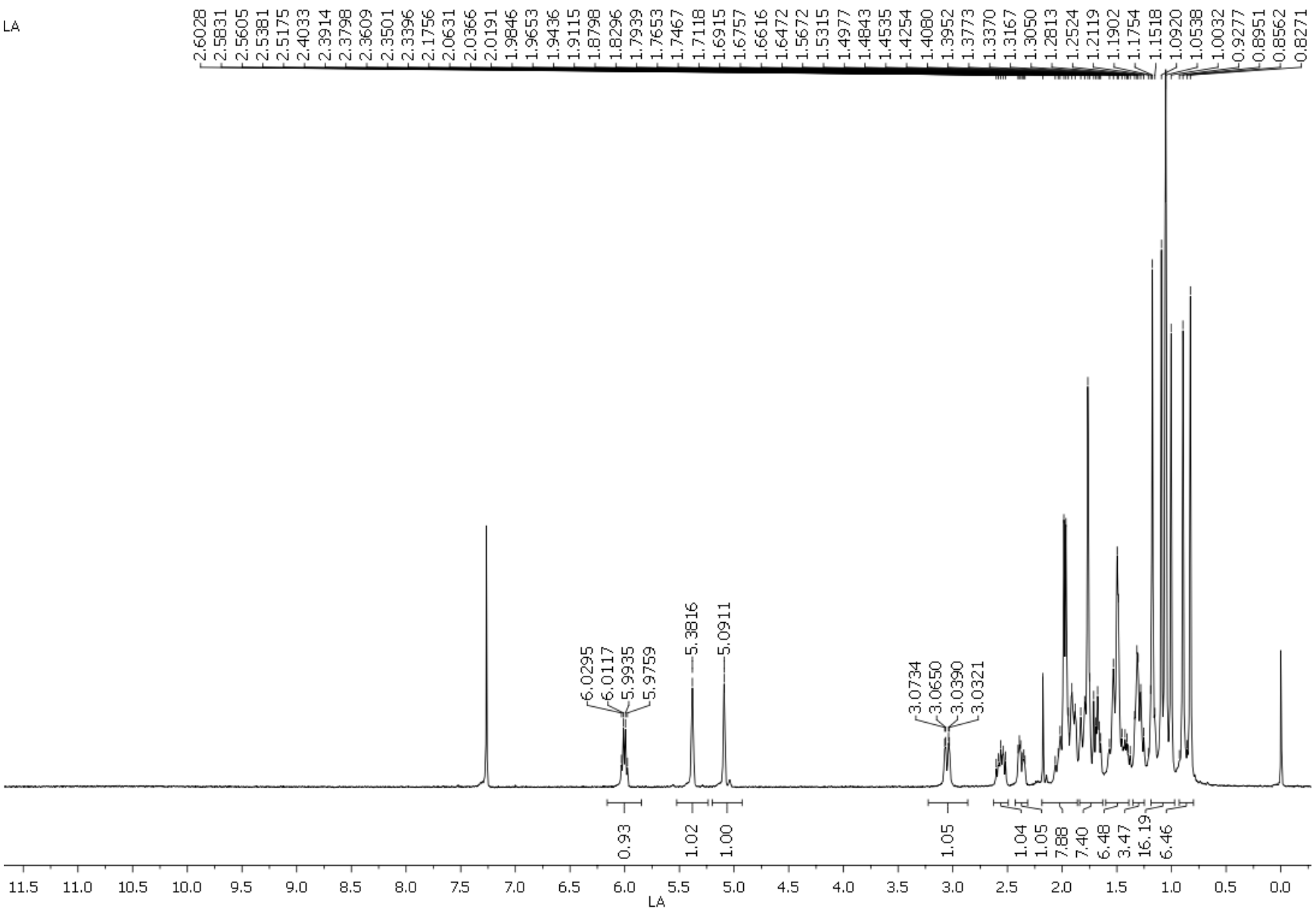


Fig. 2. ^{1}H NMR spectrum of compound **1** ($C_{35}H_{52}O_5$) in $CDCl_3$

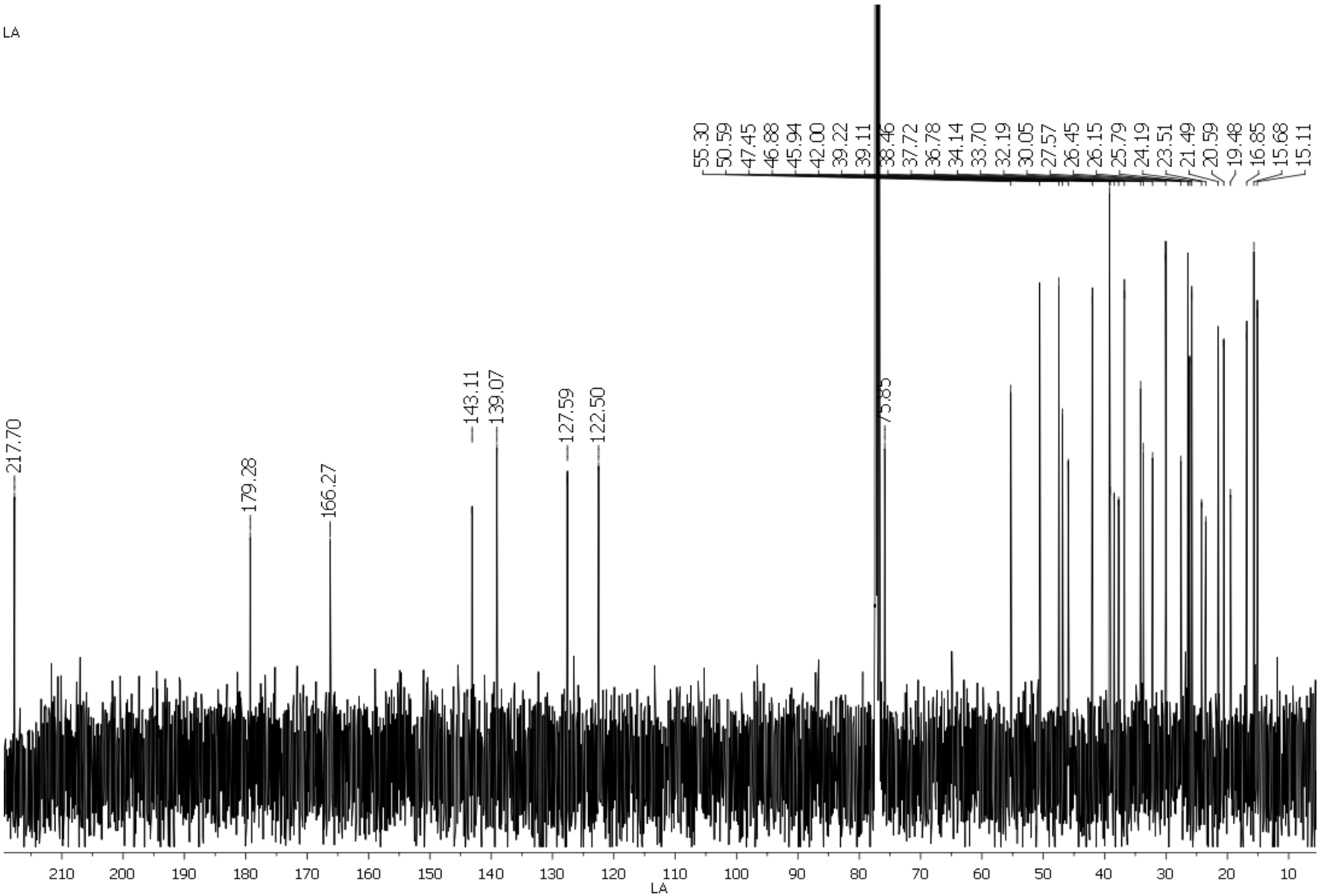


Fig. 3. ^{13}C NMR spectrum of compound **1** ($C_{35}H_{52}O_5$) in $CDCl_3$

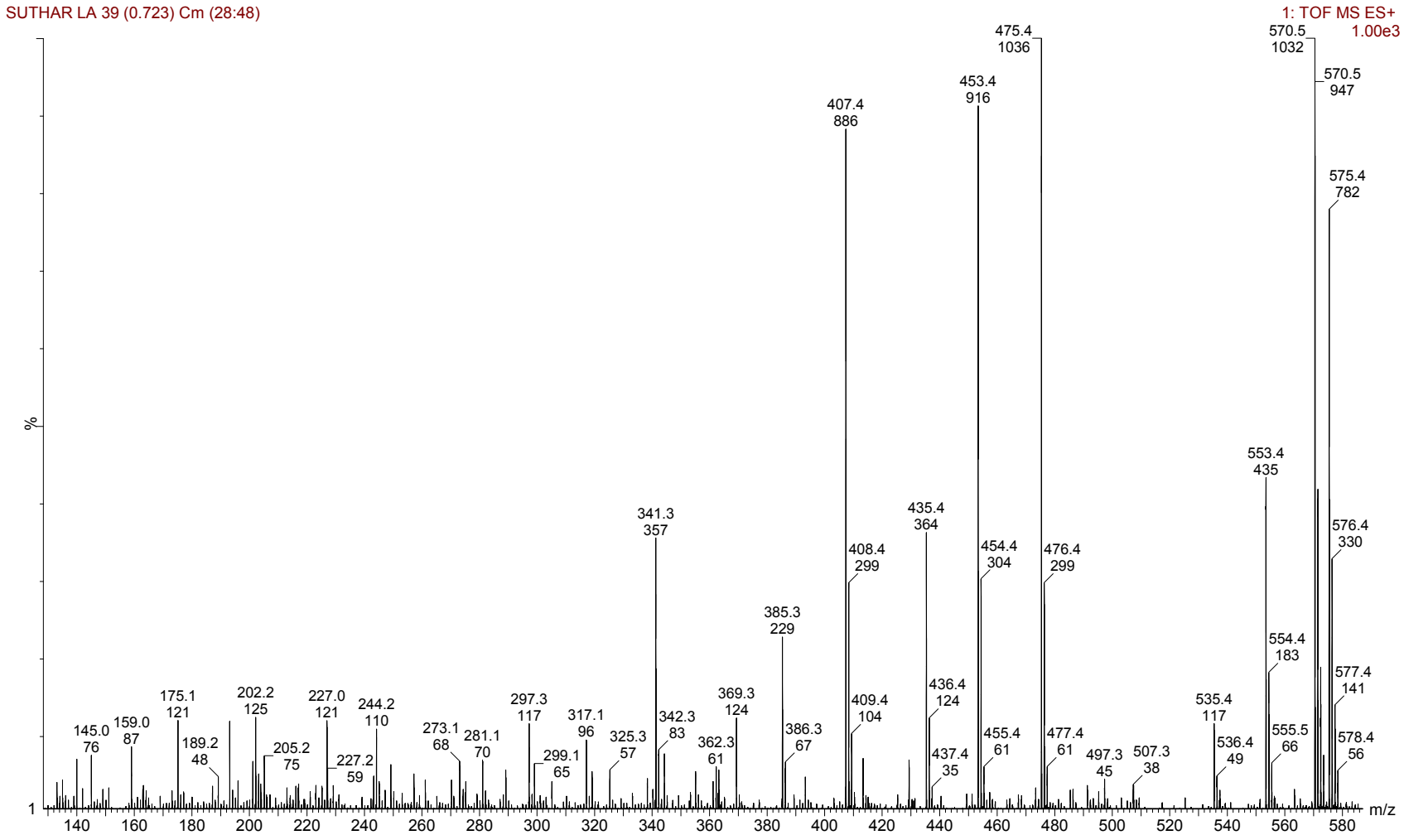
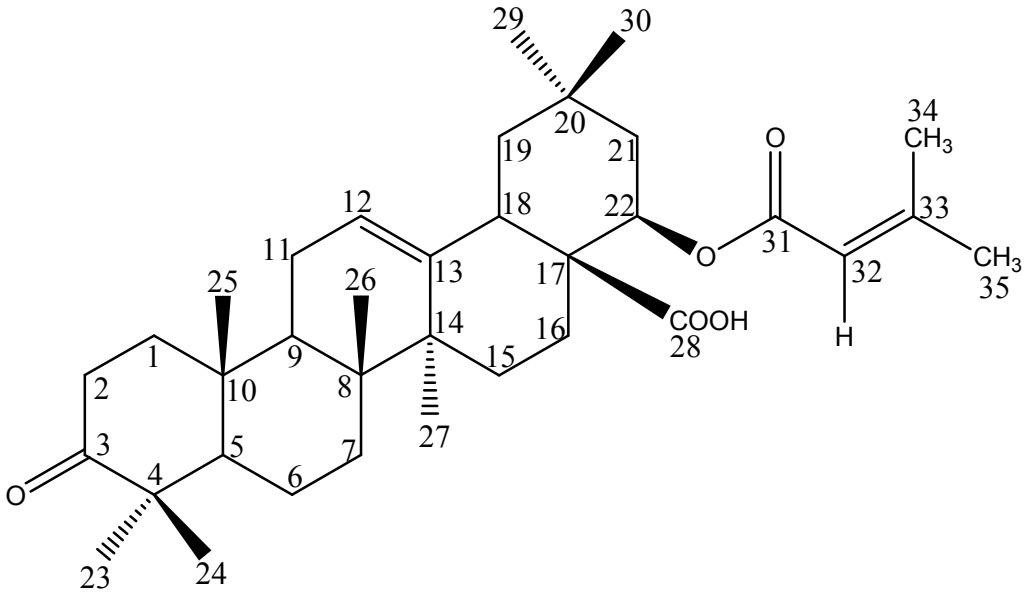


Fig. 4. ESI-MS spectrum of compound **1** (Exact Mass- 552.38)

2. 22*β*-Senecioyloxy-3-oxo-olean-12-en-28-oic acid (Lantadene B or LB) (2)



22β-Senecioyloxy-3-oxo-olean-12-en-28-oic acid

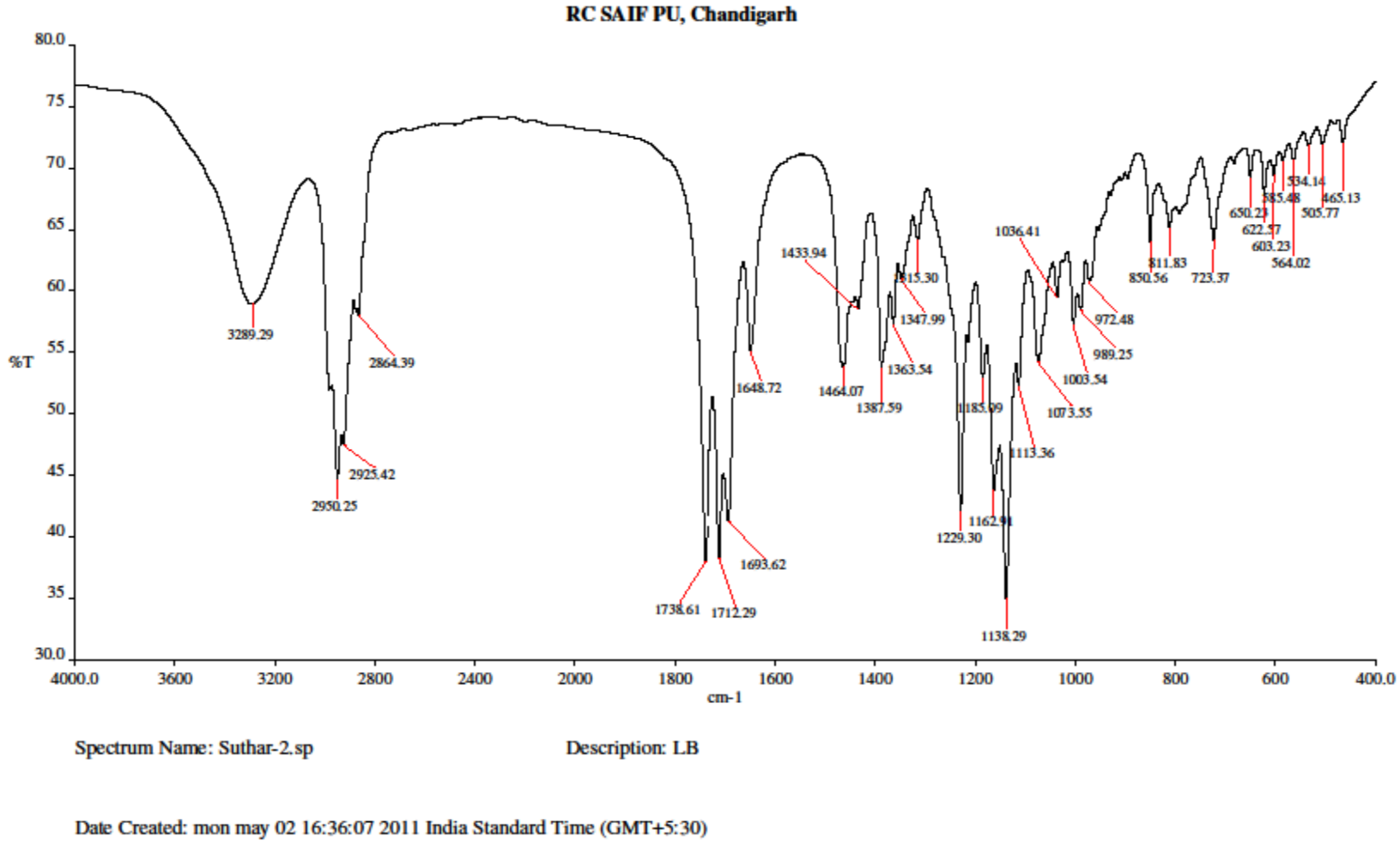


Fig. 5. FT-IR spectrum of compound **2**

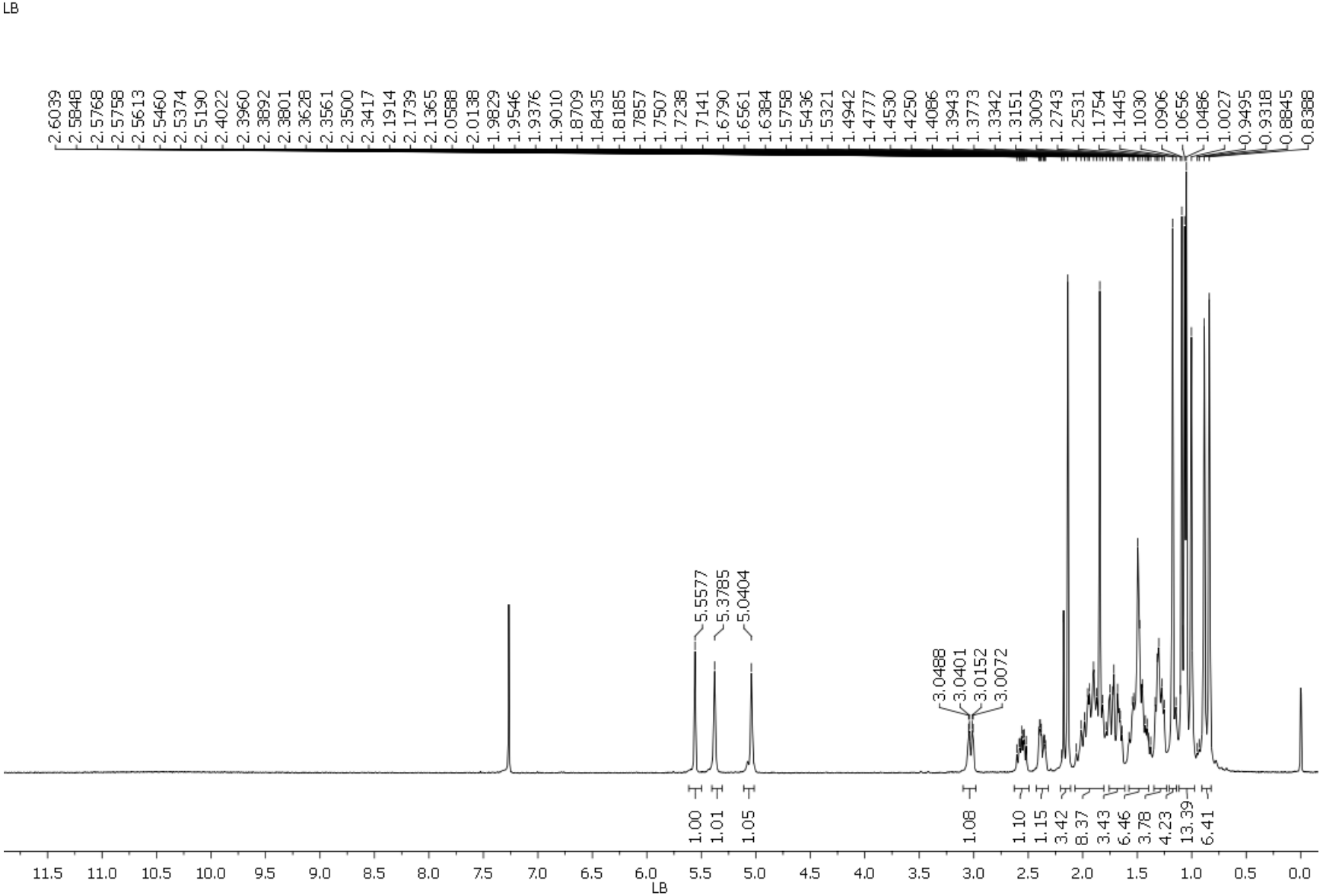


Fig. 6. ^{1}H NMR spectrum of compound **2** ($C_{35}H_{52}O_5$) in $CDCl_3$

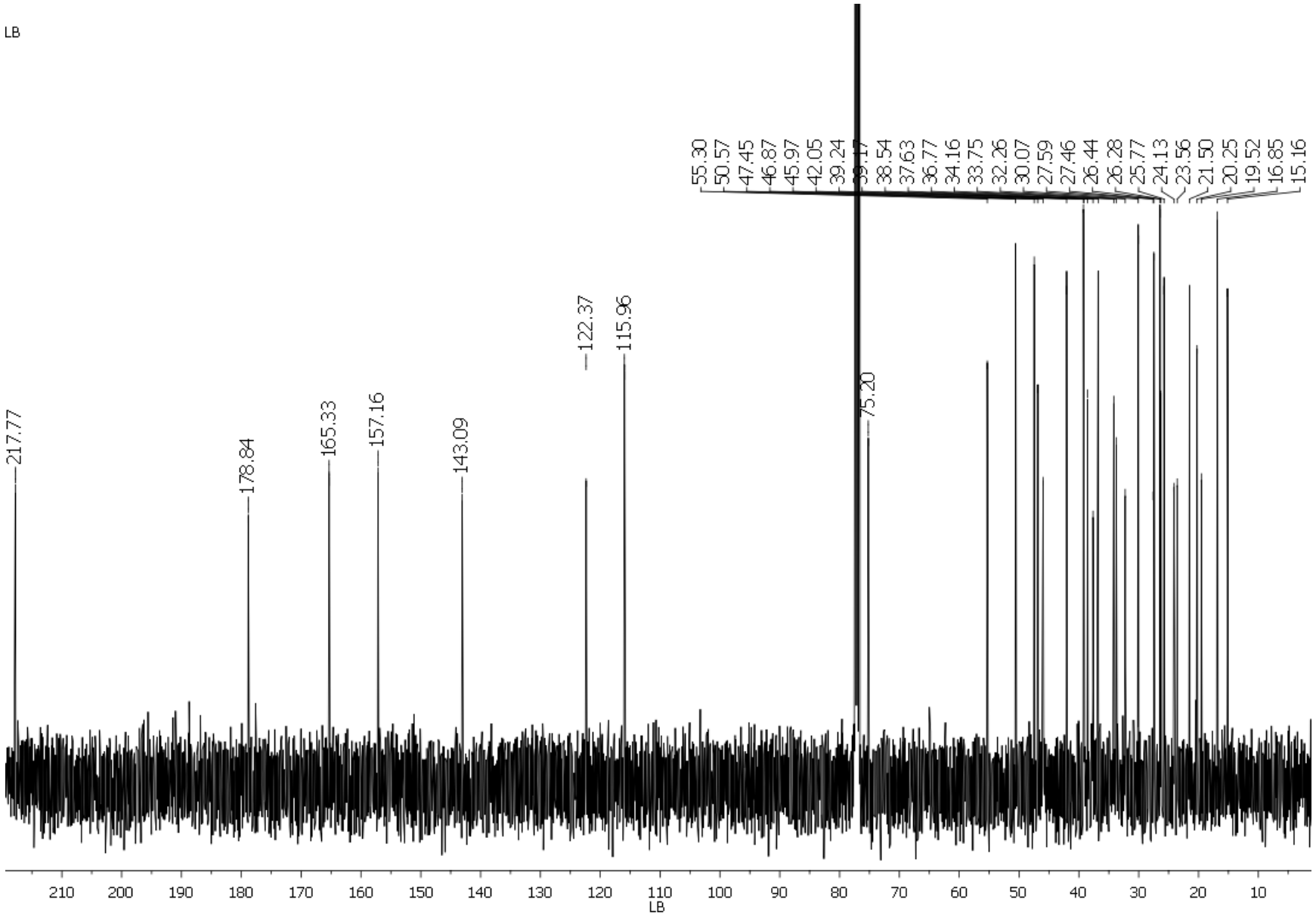


Fig. 7. ^{13}C NMR spectrum of compound **2** ($C_{35}H_{52}O_5$) in $CDCl_3$

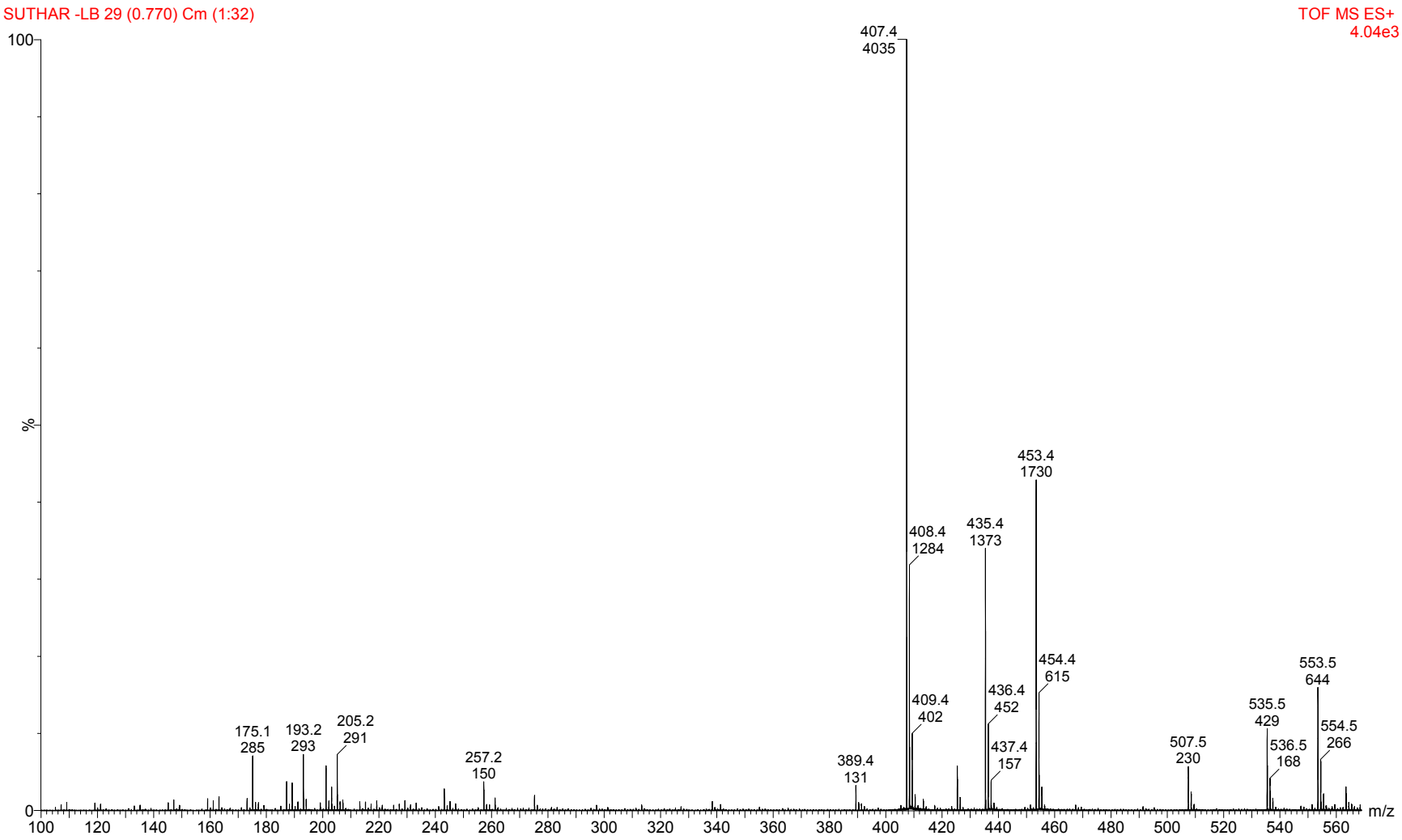
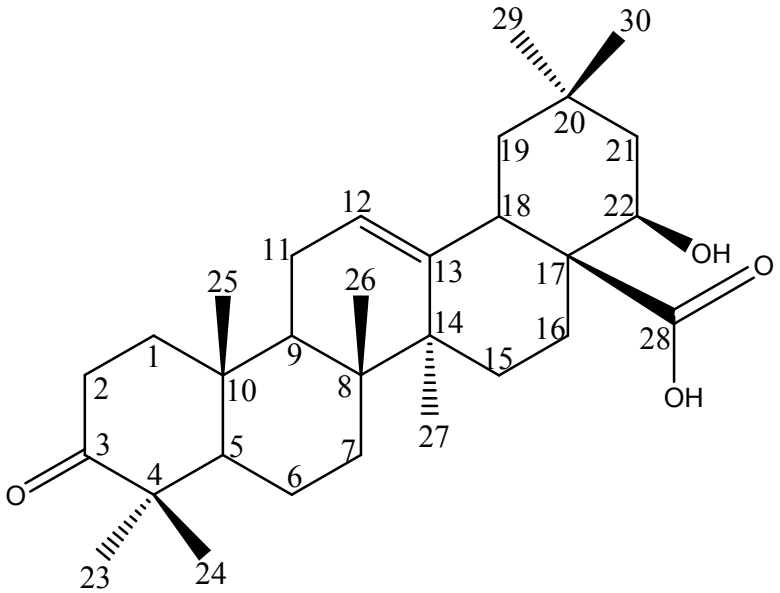
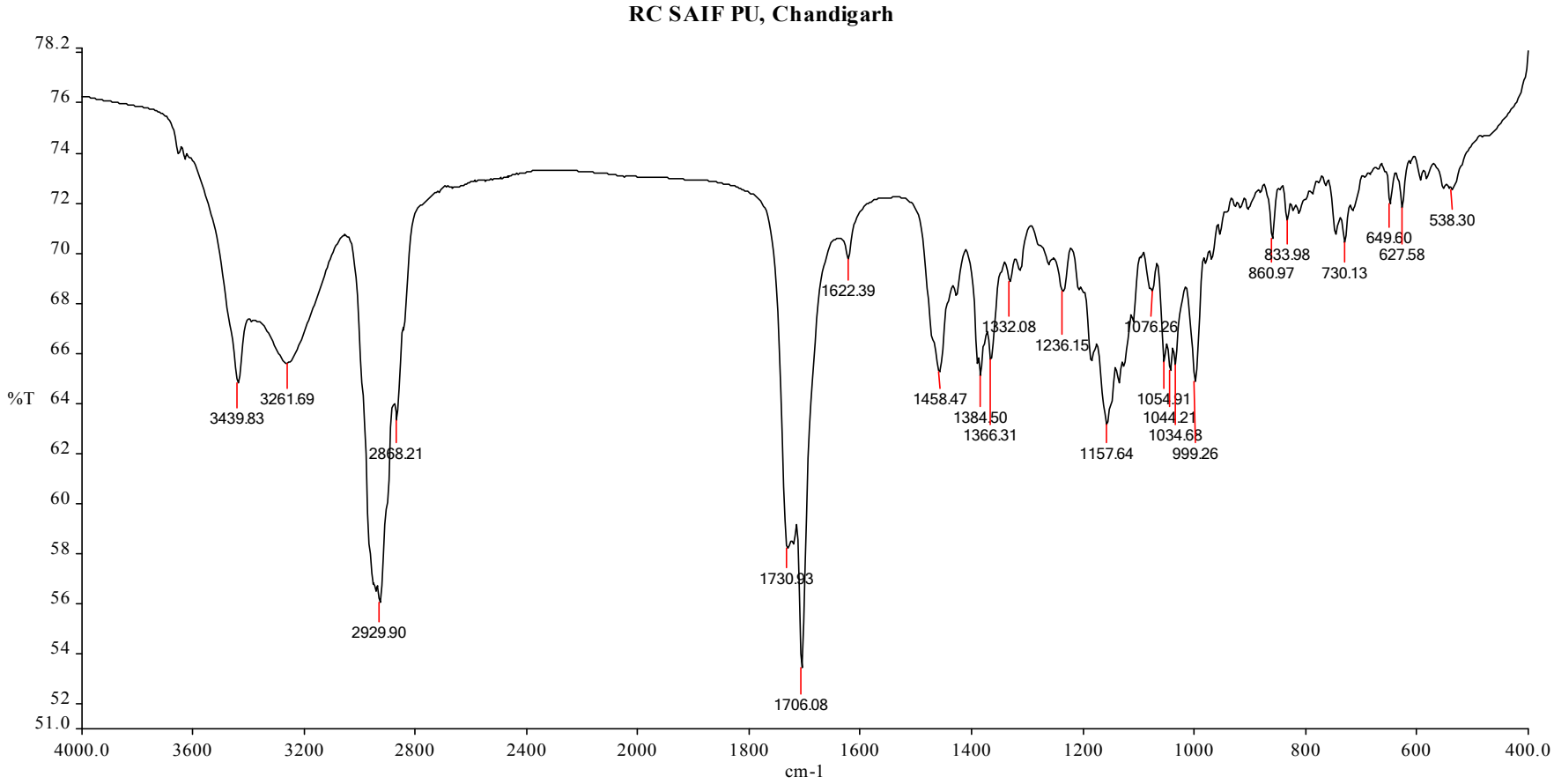


Fig. 8. ESI-MS spectrum of compound **2** (Exact Mass- 552.38)

3. 22*β*-Hydroxy-3-oxo-olean-12-en-28-oic acid (3)



22β-Hydroxy-3-oxo-olean-12-en-28-oic acid



Spectrum Name: Sharad Kumar-12.sp Description: 3CO 22OH

Date Created: mon apr 09 15:51:23 2012 India Standard Time (GMT+5:30)

Fig. 9. FT-IR spectrum of compound **3**

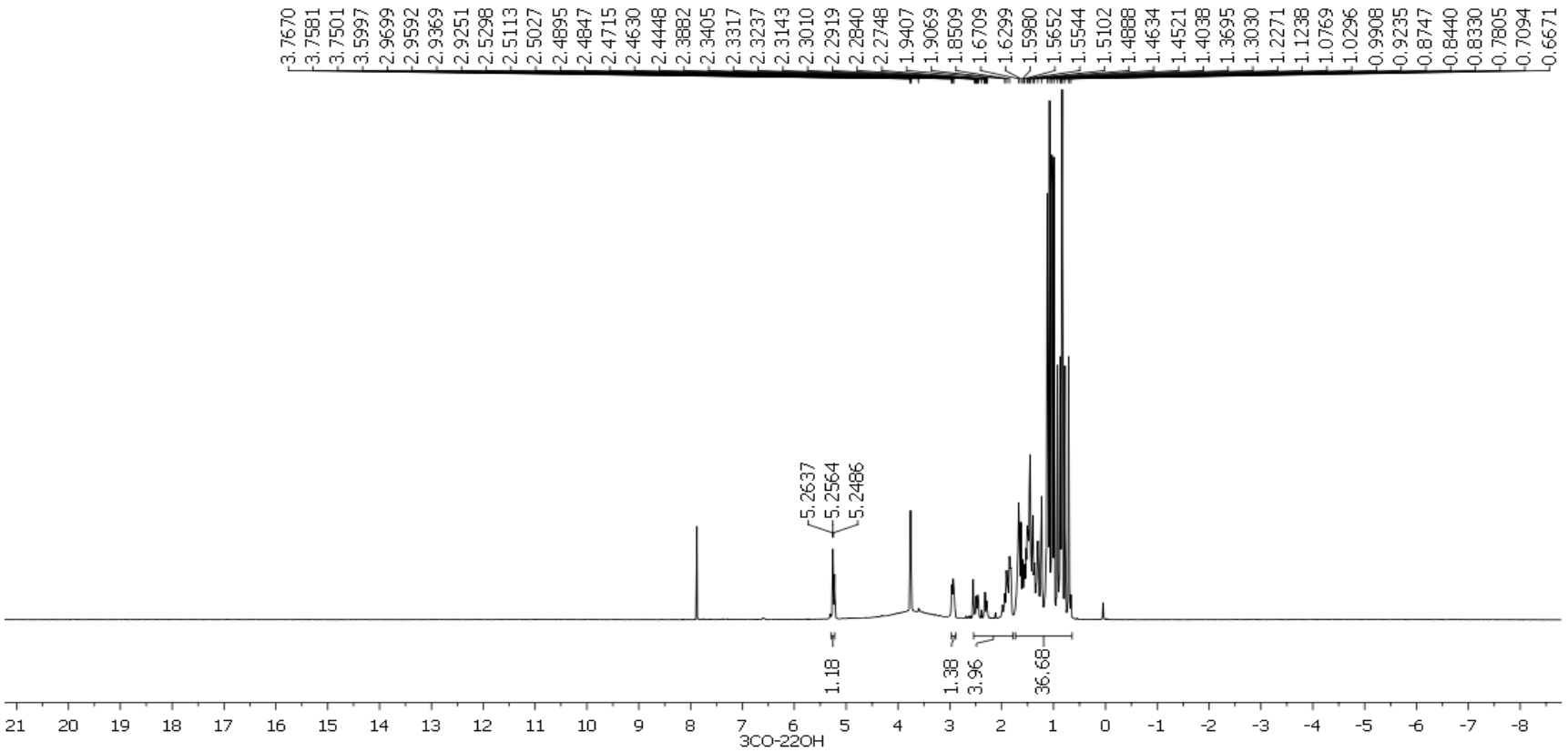


Fig. 10. ^{1}H NMR spectrum of compound **3** ($C_{30}H_{46}O_4$) in a mixture of $CDCl_3$ and DMSO-d_6

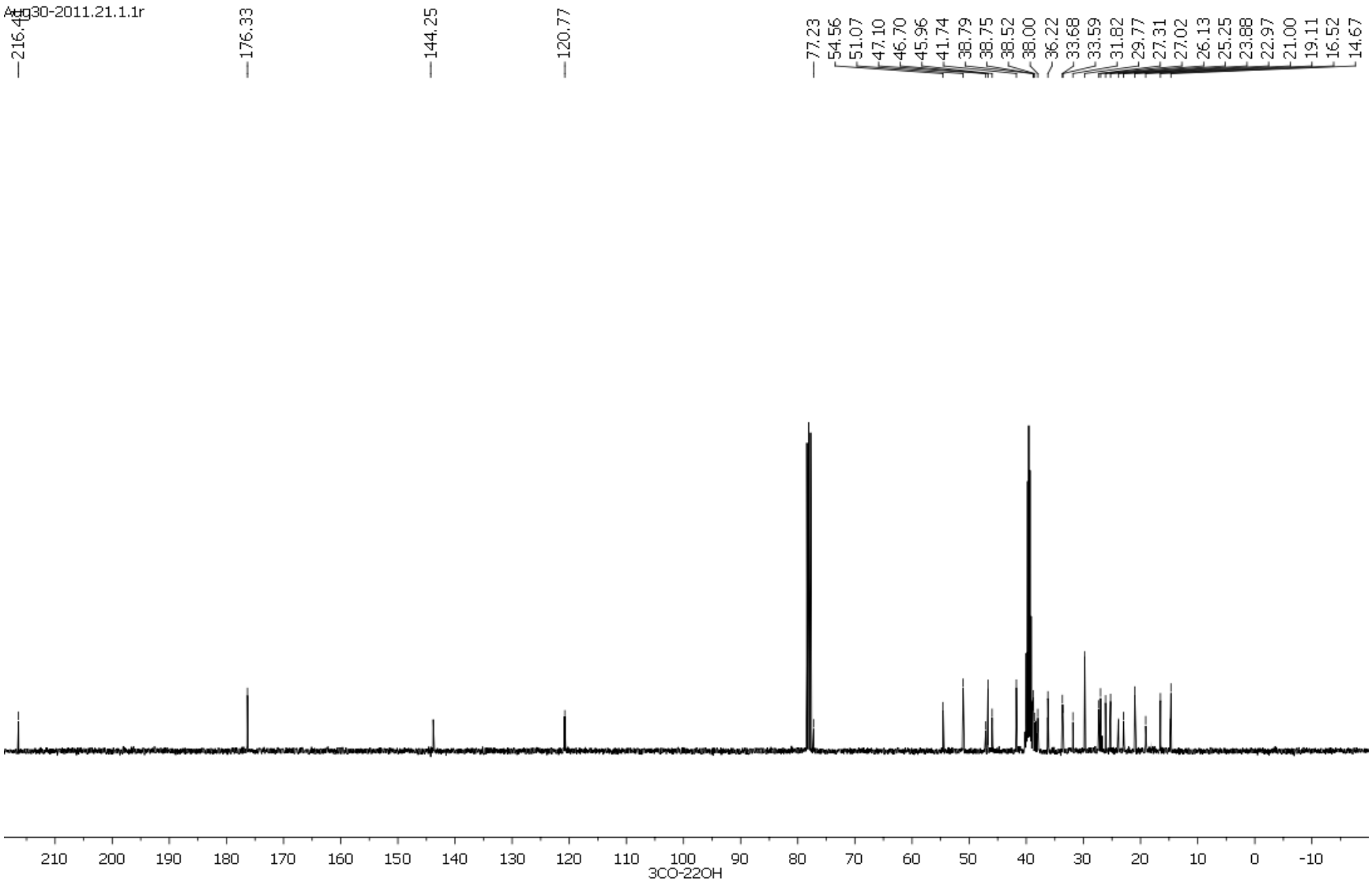


Fig. 11. ^{13}C NMR spectrum of compound **3** ($C_{30}H_{46}O_4$) in a mixture of $CDCl_3$ and DMSO-d_6

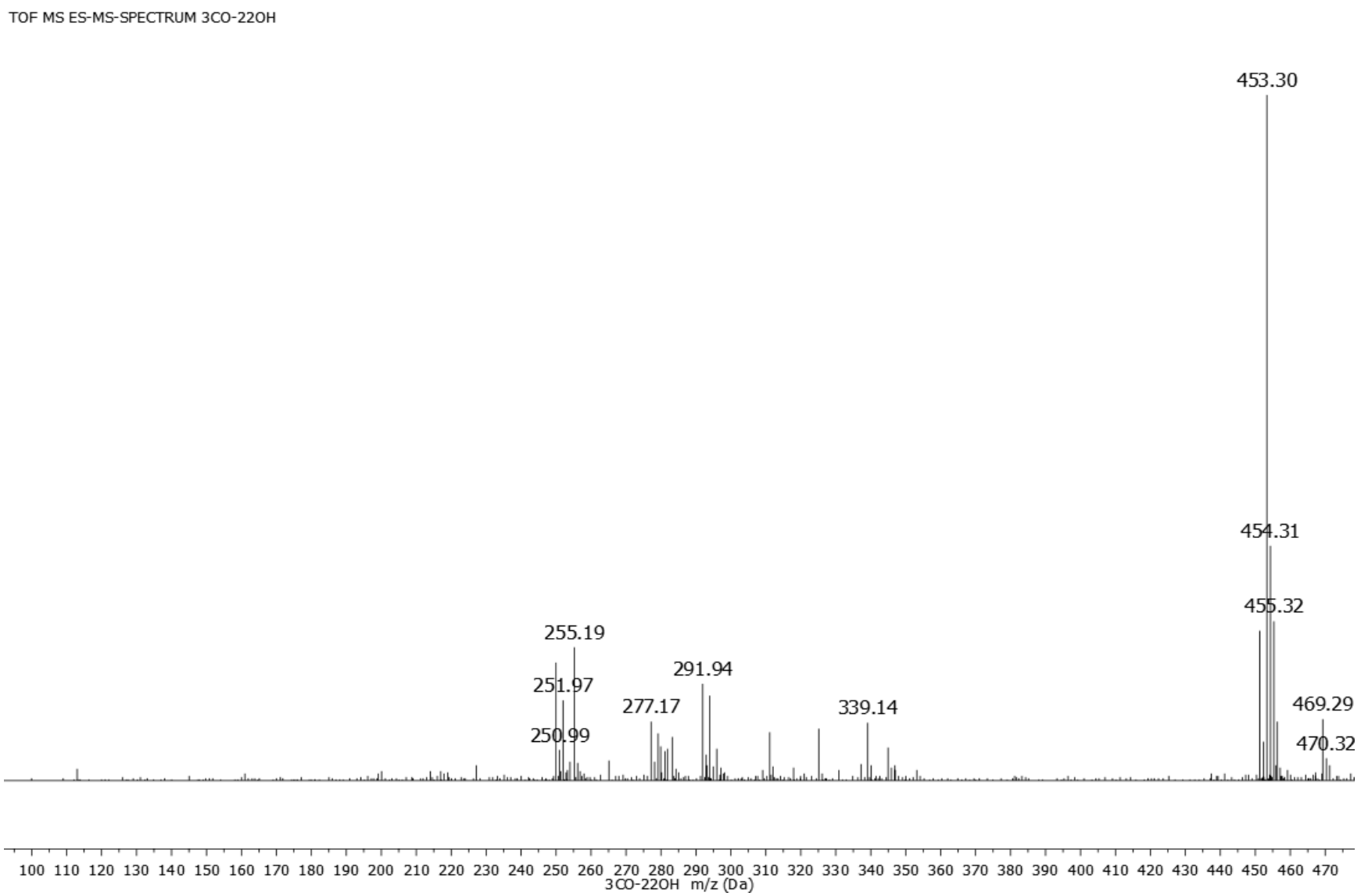
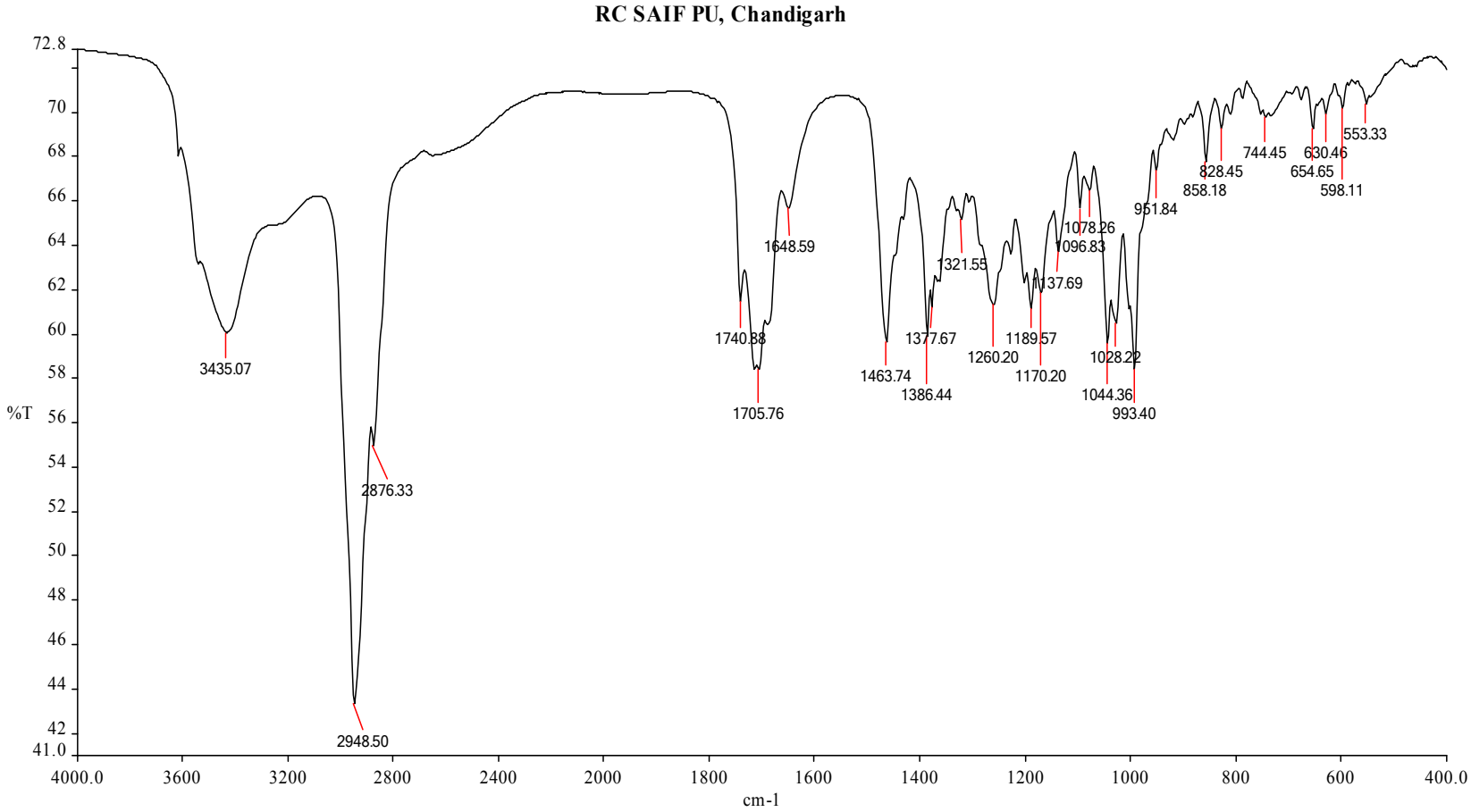
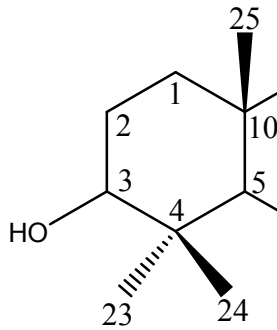


Fig. 12. ESI-MS negative-ion mode spectrum of compound **3** (Exact Mass- 470.34)

4. 3*β*,22*β*-Dihydroxy-olean-12-en-28-oic acid (4)





3β,22β-Dih

Spectrum Name: Sharad Kumar-13.sp Description: 3OH 22OH

Date Created: mon apr 09 15:53:11 2012 India Standard Time (GMT+5:30)

Fig. 13. FT-IR spectrum of compound **4**

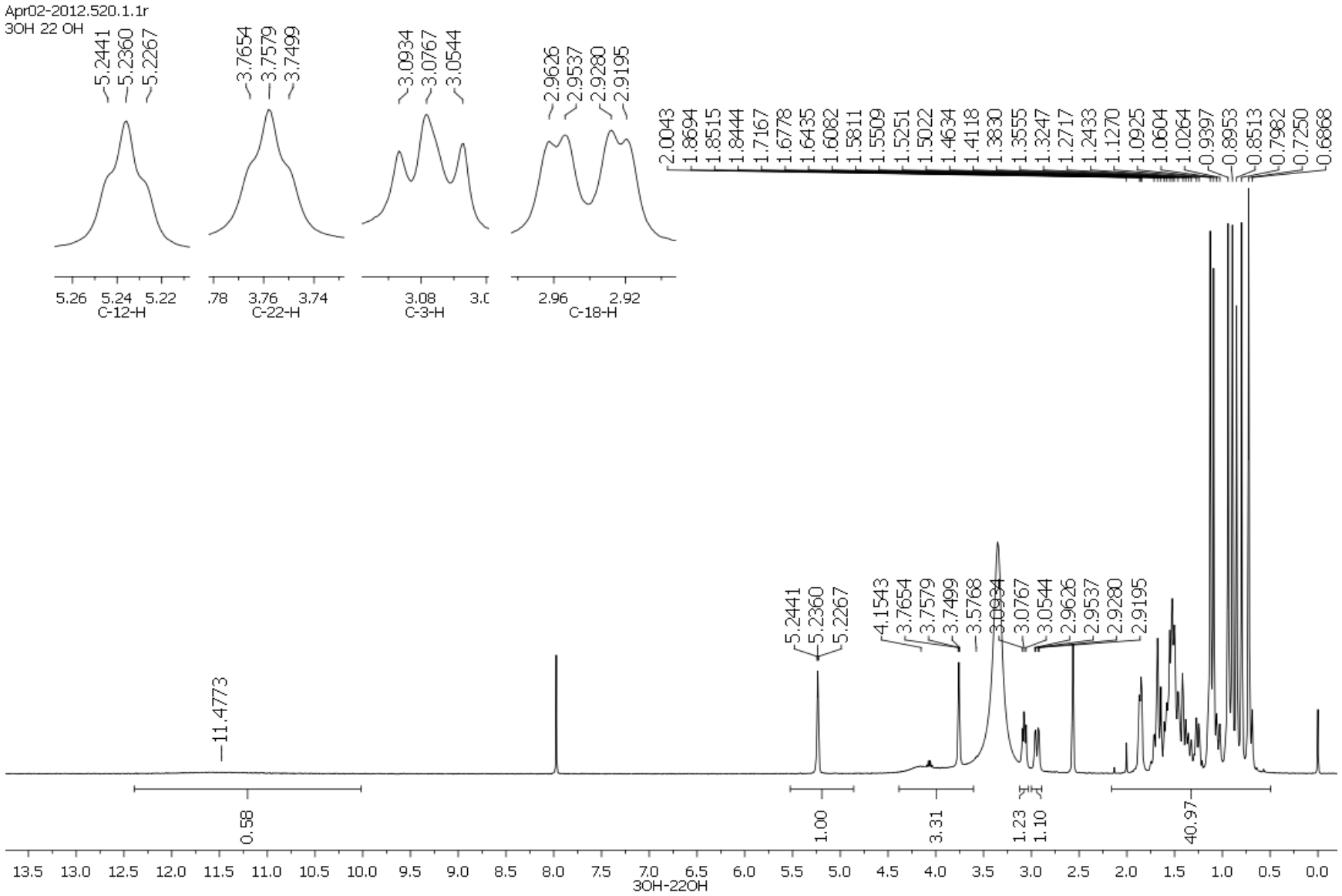


Fig. 14. ^{1}H NMR spectrum of compound **4** ($C_{30}H_{48}O_4$) in a mixture of $CDCl_3$ and DMSO-d_6

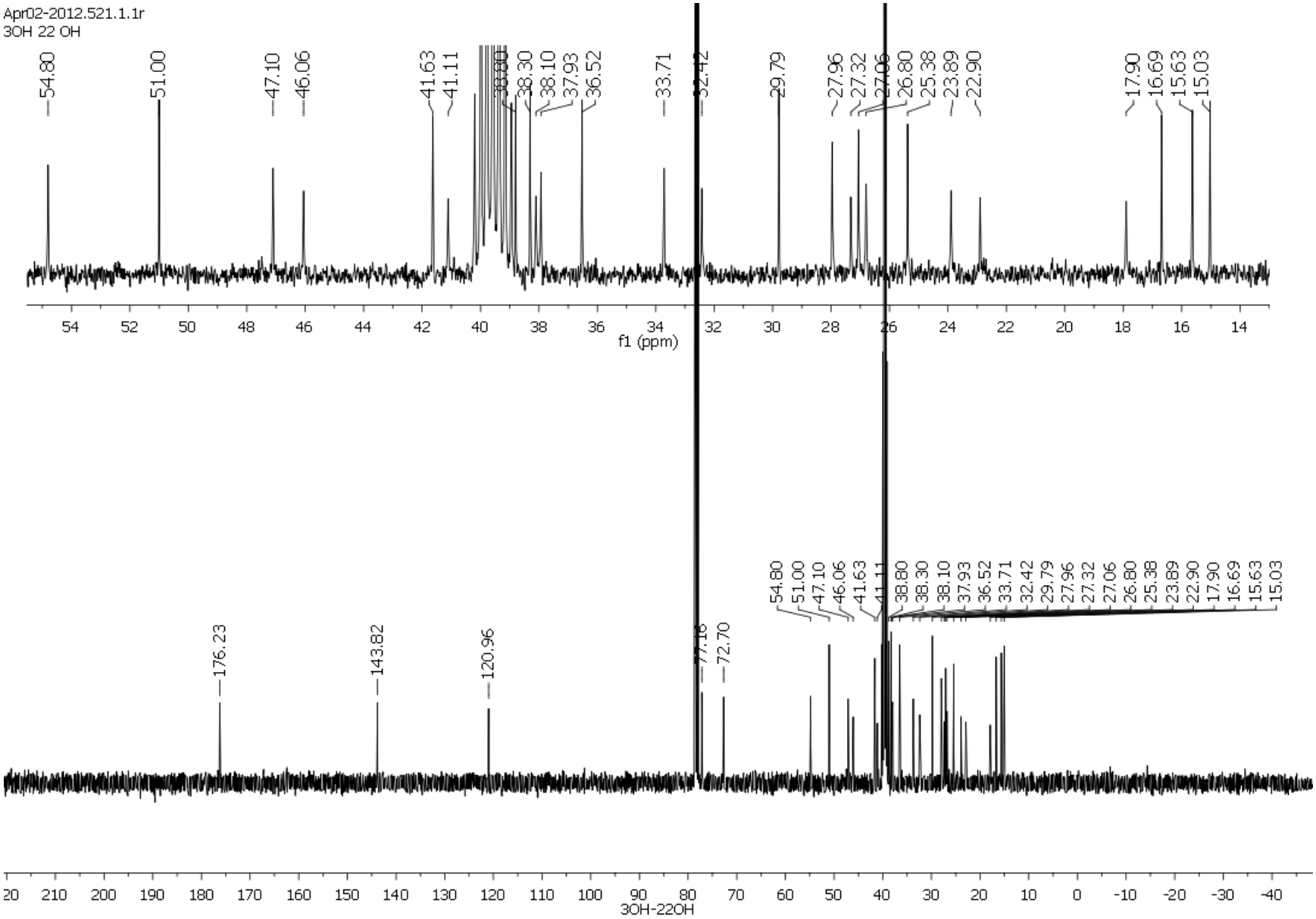


Fig. 15. ^{13}C NMR spectrum of compound **4** ($C_{30}H_{48}O_4$) in a mixture of $CDCl_3$ and DMSO-d_6

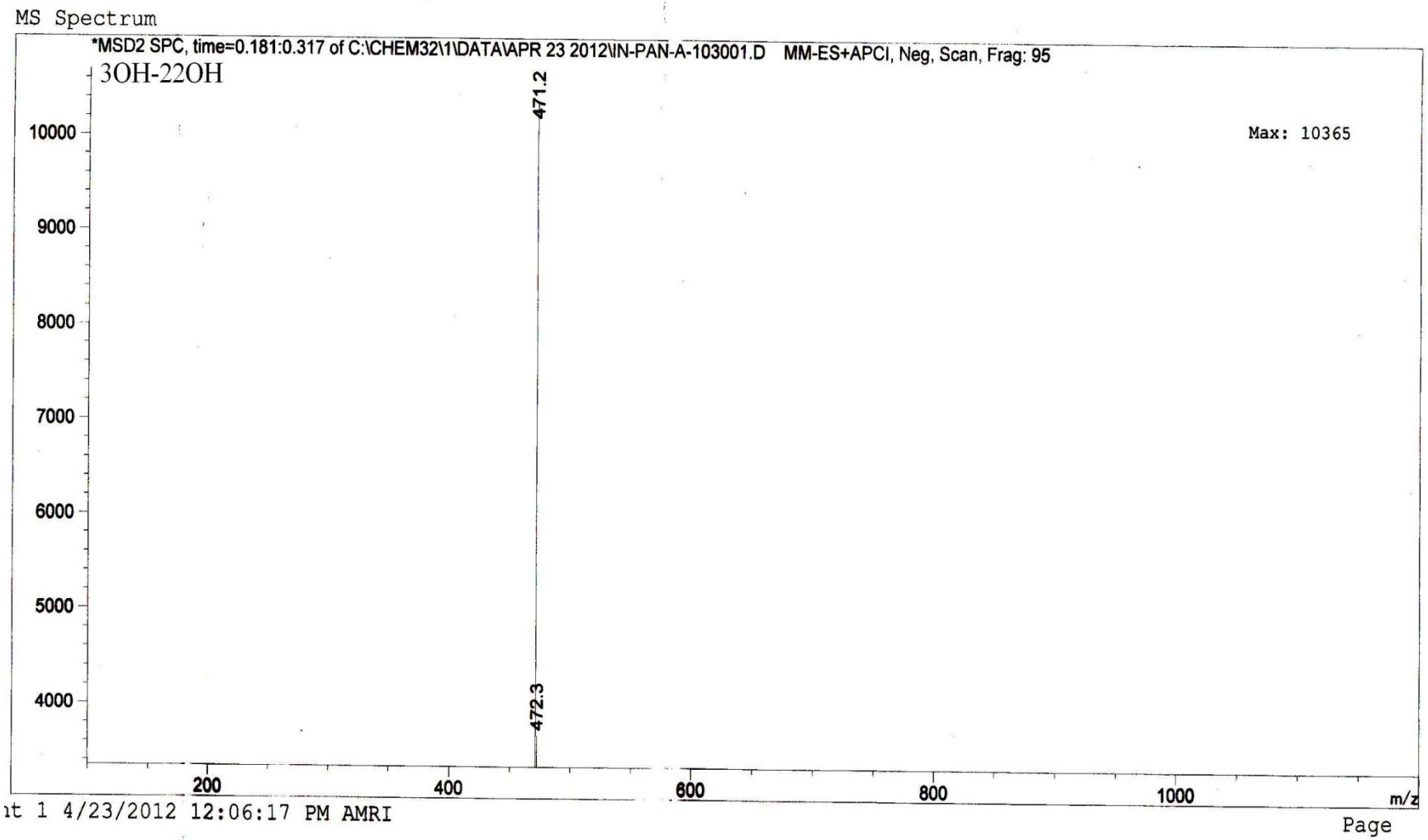
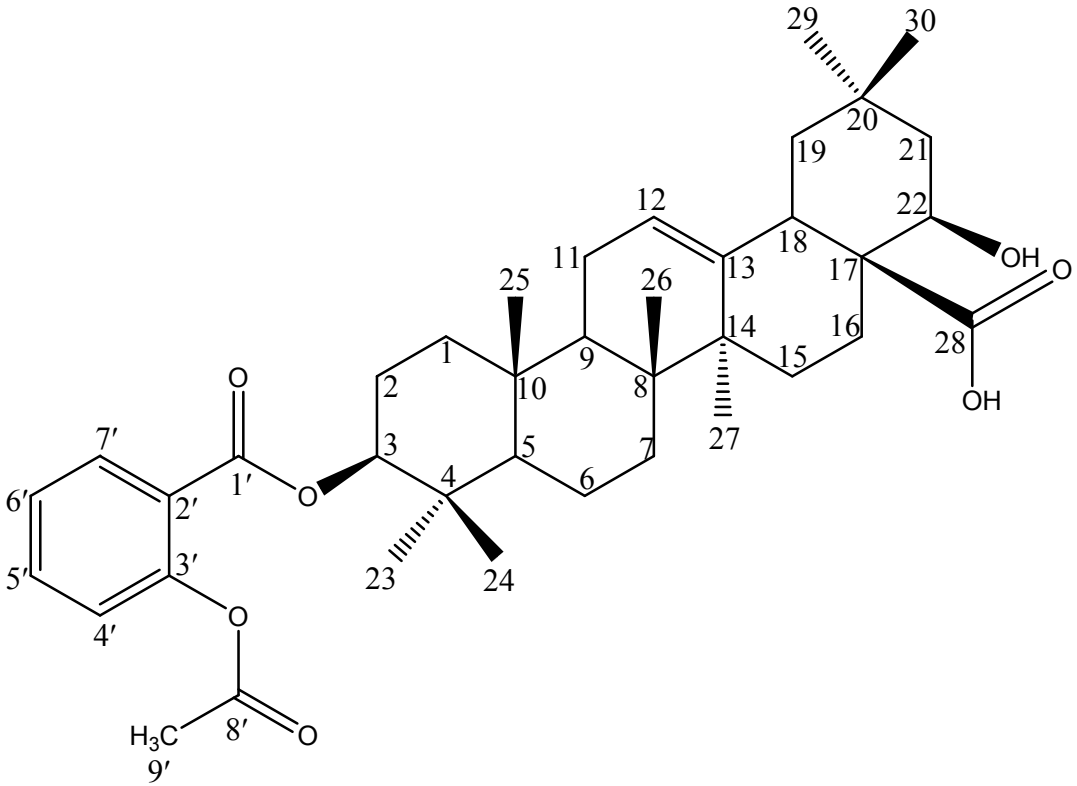
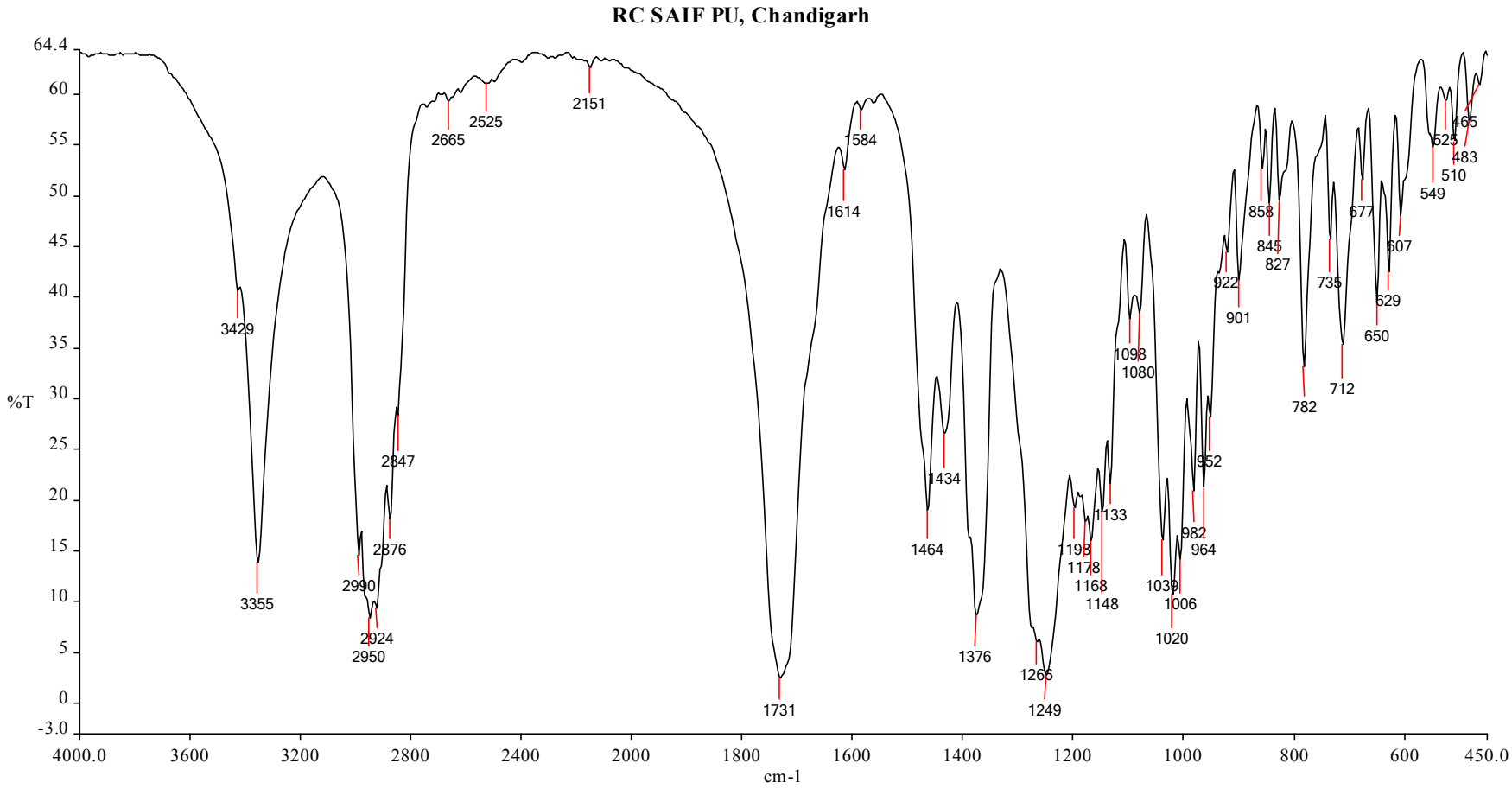


Fig. 16. ESI-MS negative-ion mode spectrum of compound **4** (Exact Mass- 472.36)

5. 3*β*-(2-Acetoxybenzoyloxy)-22*β*-hydroxy-olean-12-en-28-oic acid (5)



3β-(2-Acetoxybenzoyloxy)-22β-hydroxy-olean-12-en-28-oic acid



Spectrum Name: SharadKumar-18.sp Description: 3 ASP-22OH

Date Created: fri dec 21 12:33:24 2012 India Standard Time (GMT+5:30)

Fig. 17. FT-IR spectrum of compound **5**

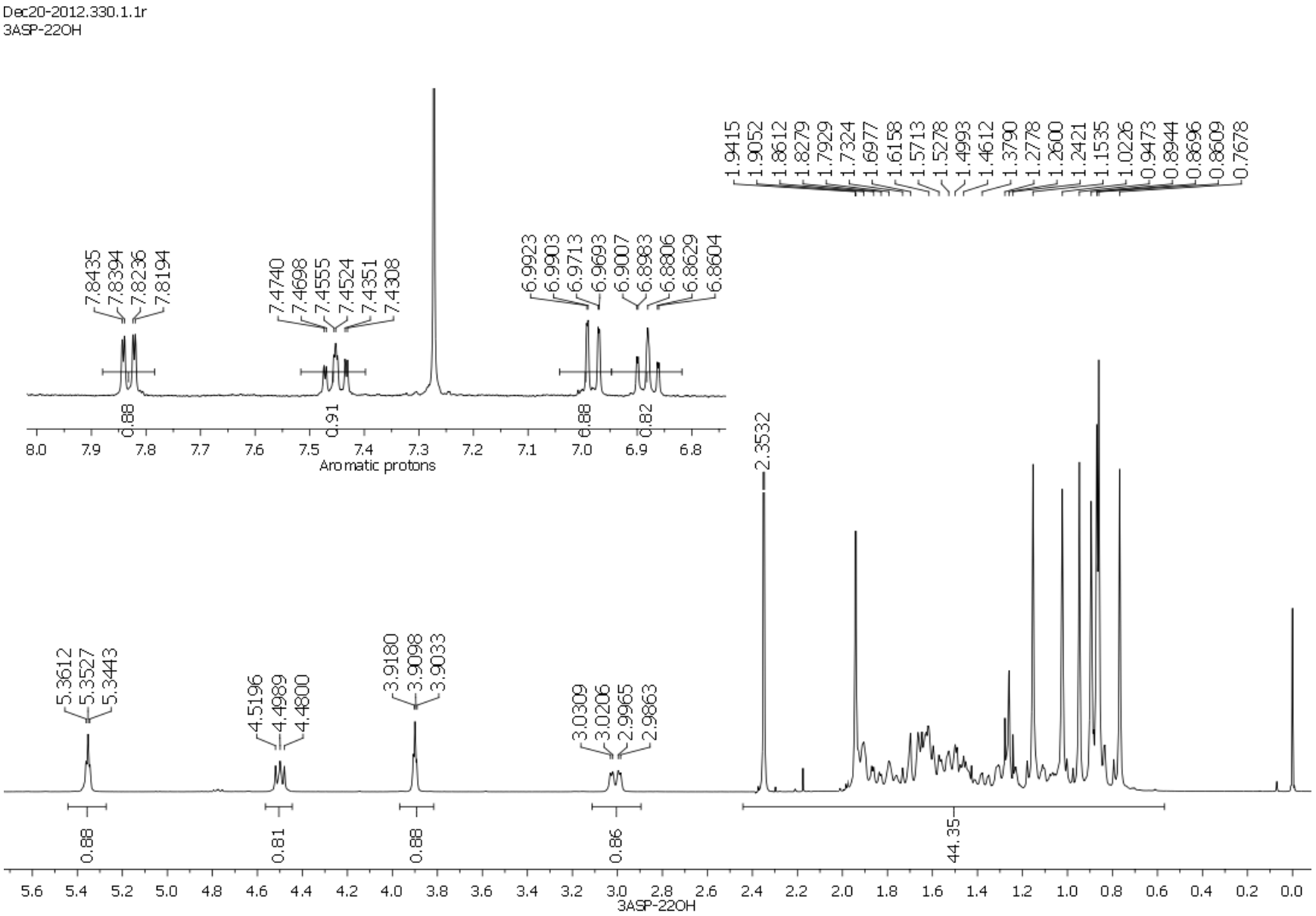


Fig. 18. ^{1}H NMR spectrum of compound **5** ($C_{39}H_{54}O_7$) in $CDCl_3$

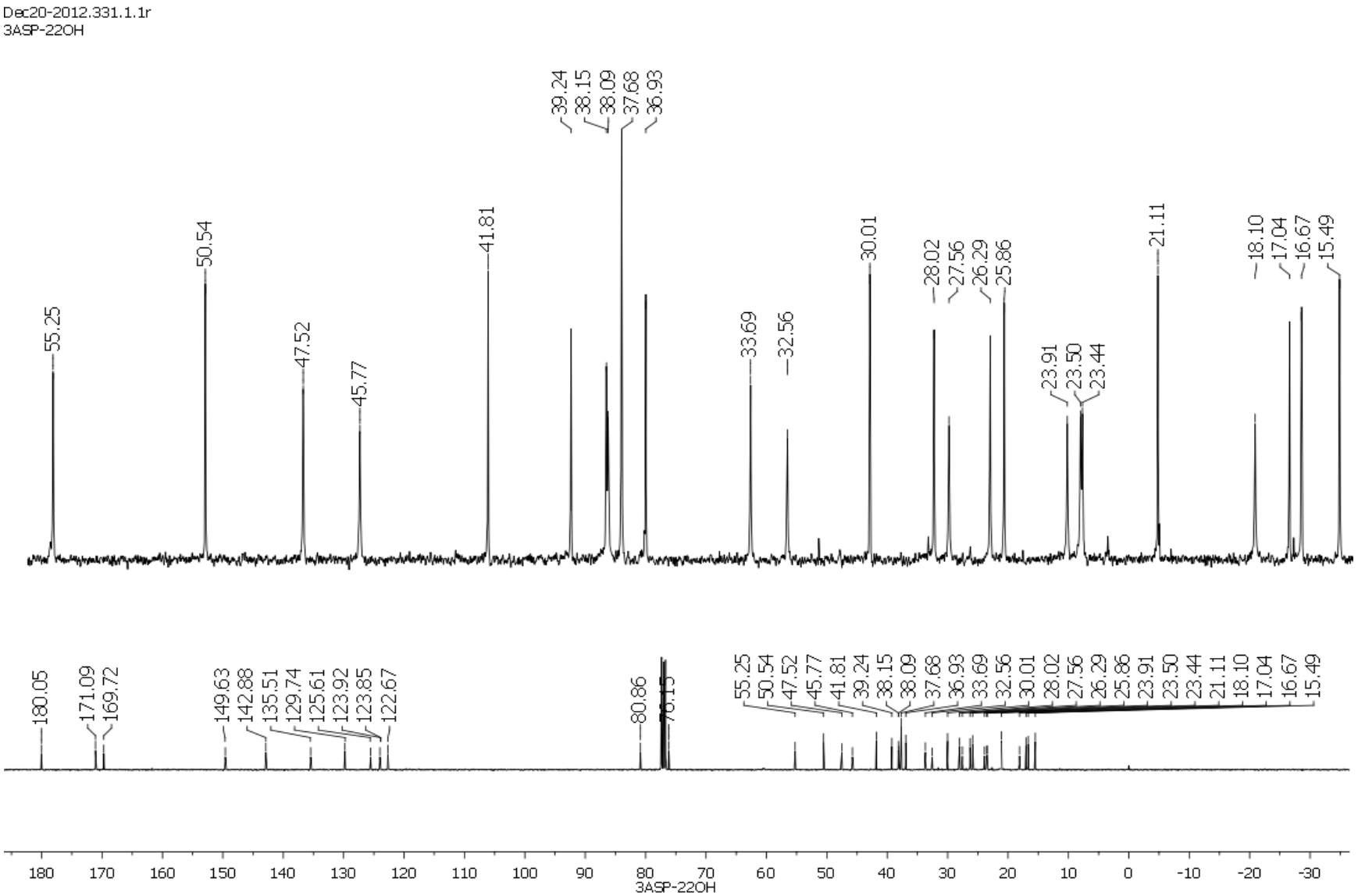


Fig. 19. ^{13}C NMR spectrum of compound **5** ($C_{39}H_{54}O_7$) in $CDCl_3$. The C-4 & C-21 appeared at 37.68 ppm. Hence, 38 peaks of compound are visible in the spectrum.

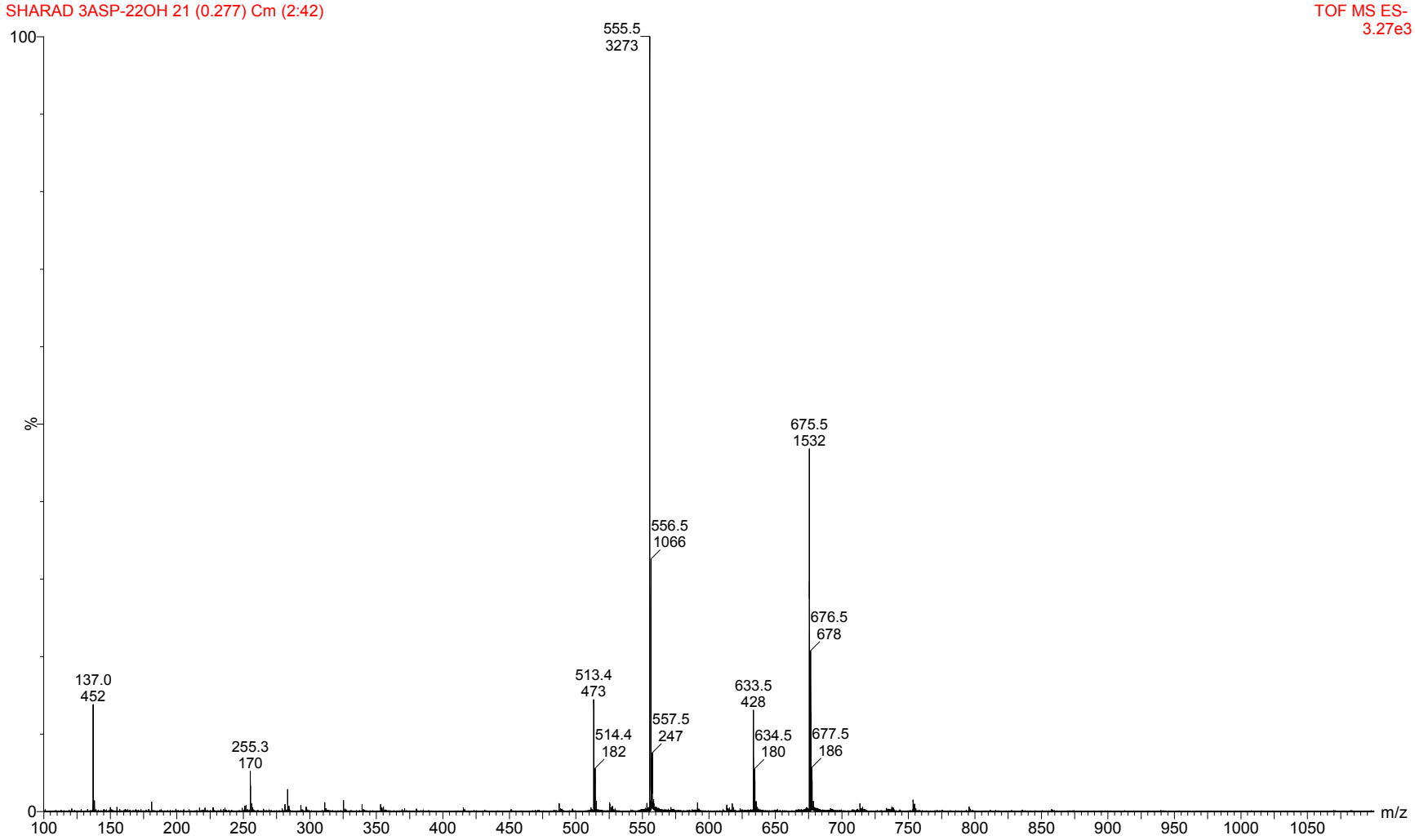
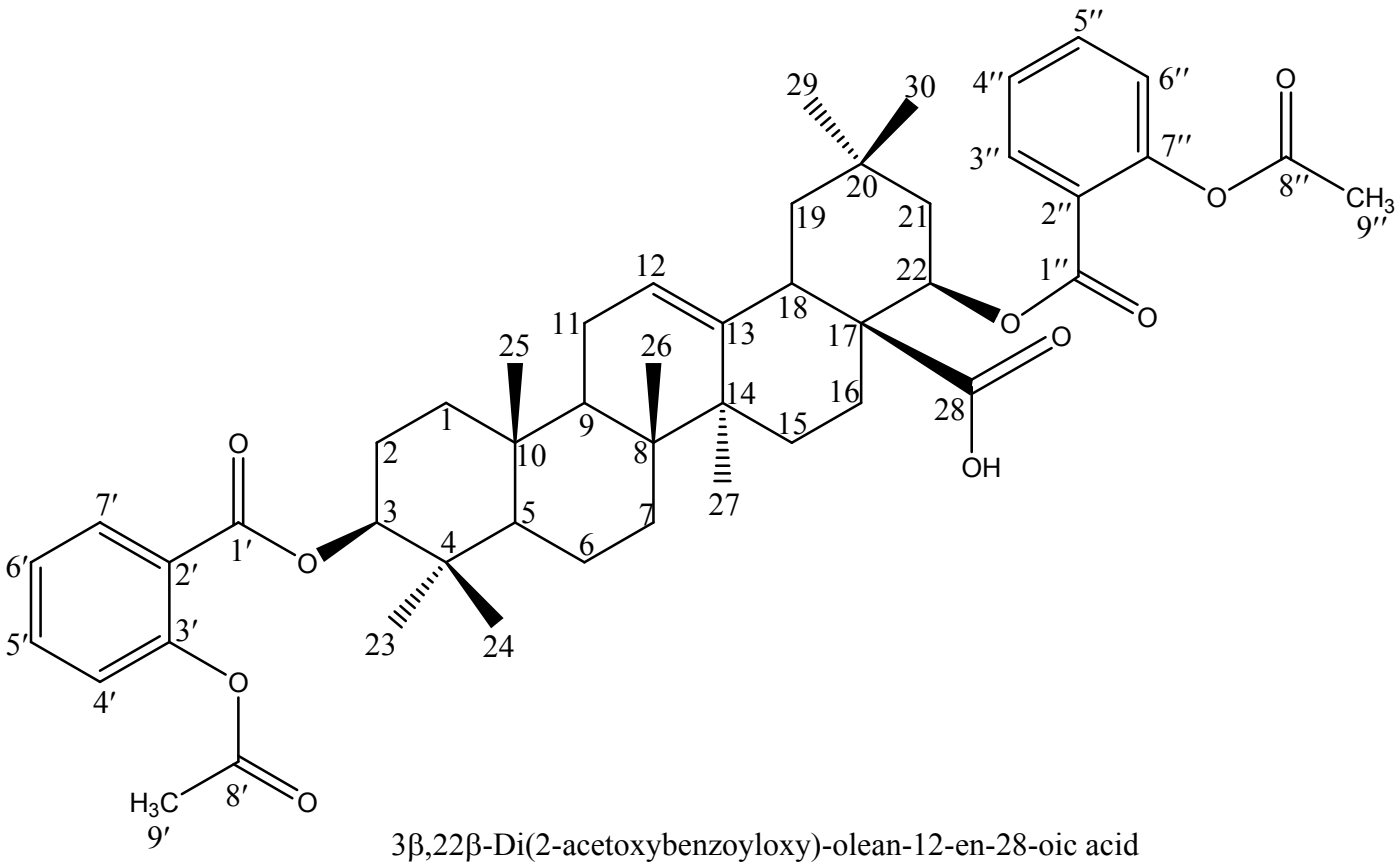


Fig. 20. ESI-MS negative-ion mode spectrum of compound **5** (Exact Mass- 634.39)

6. 3*β*,22*β*-Di(2-acetoxybenzoyloxy)-olean-12-en-28-oic acid (6)



3β,22β-Di(2-acetoxybenzoyloxy)-olean-12-en-28-oic acid

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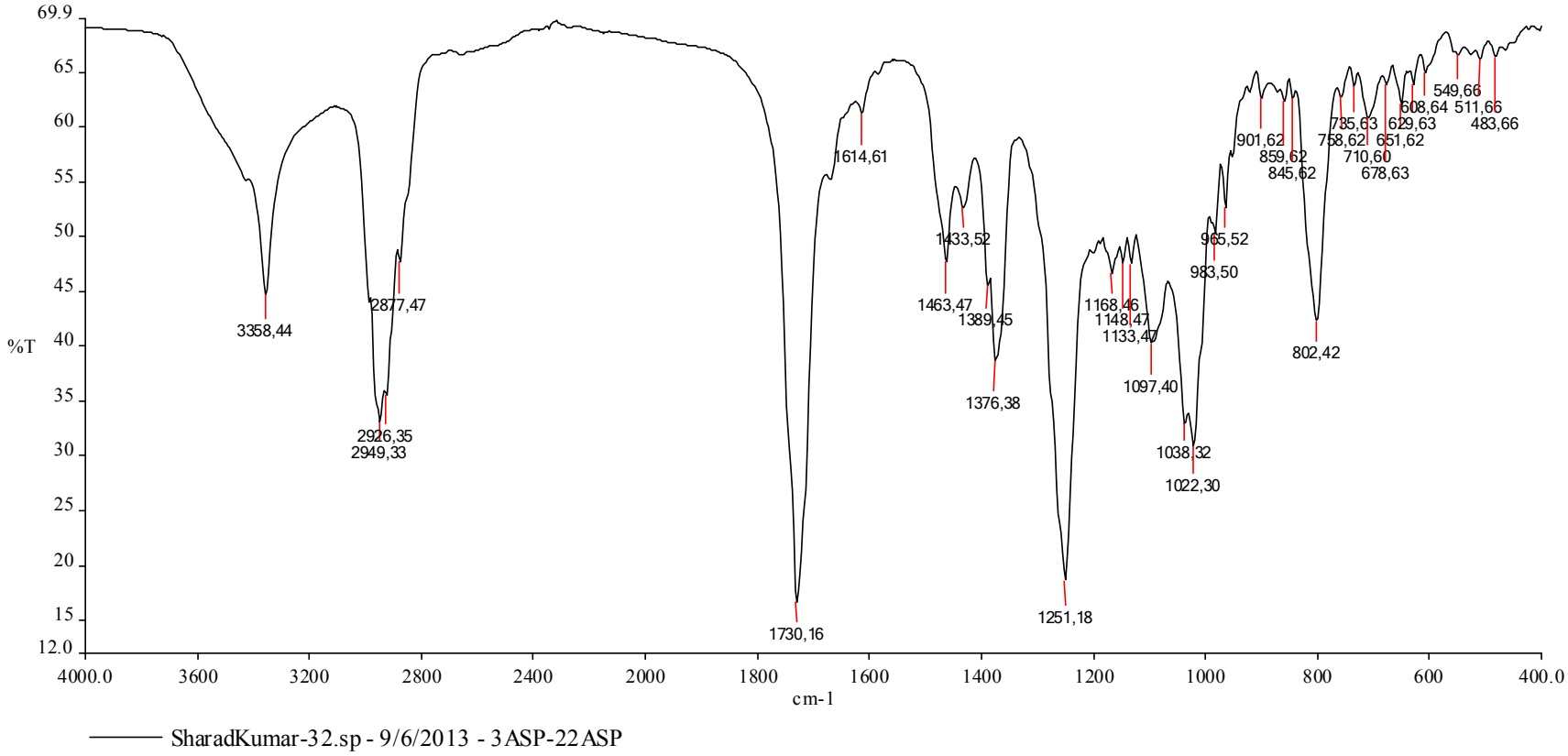


Fig. 21. FT-IR spectrum of compound **6**

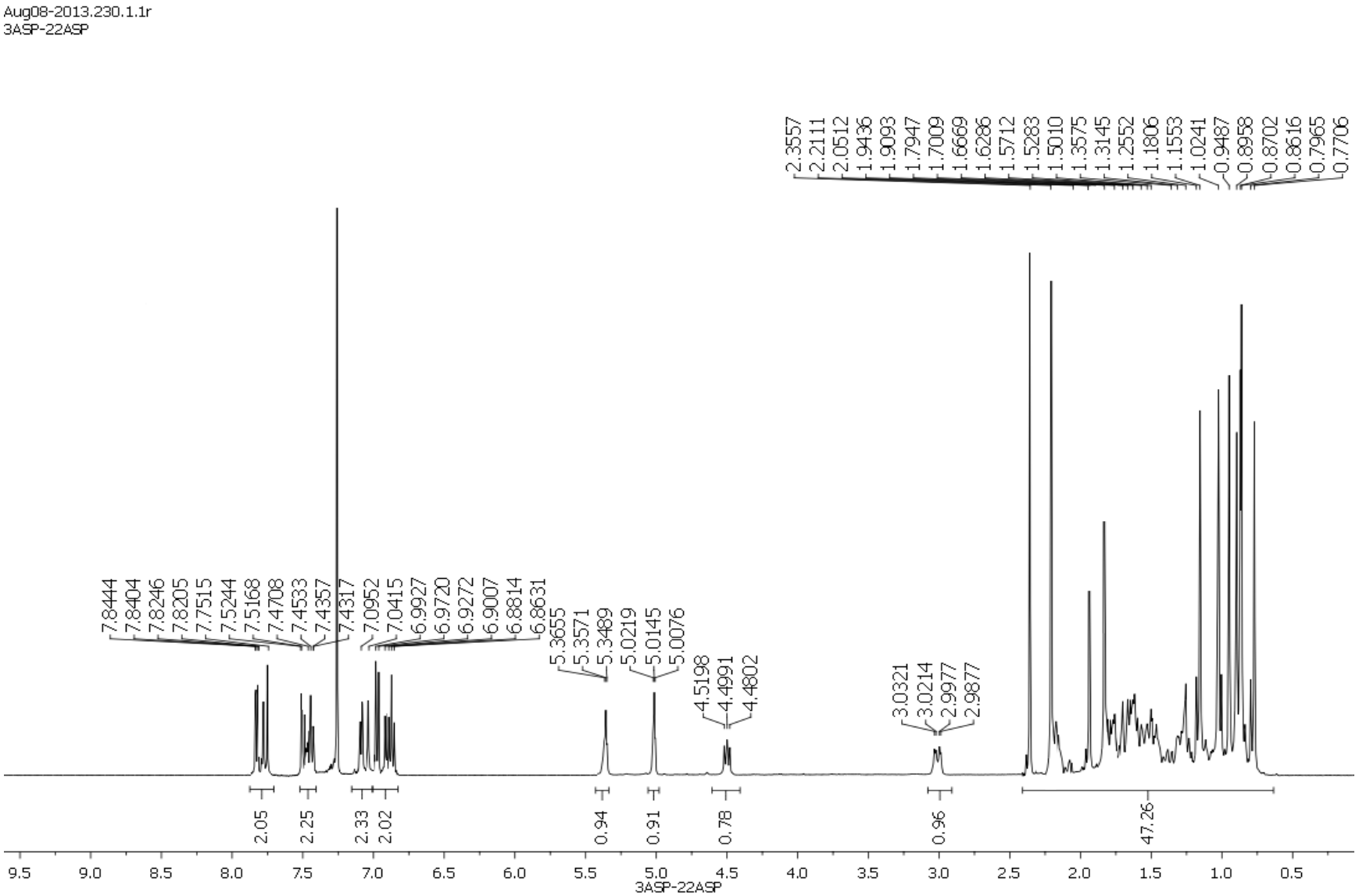


Fig. 22. ^{1}H NMR spectrum of compound **6** ($C_{48}H_{60}O_{10}$) in $CDCl_3$

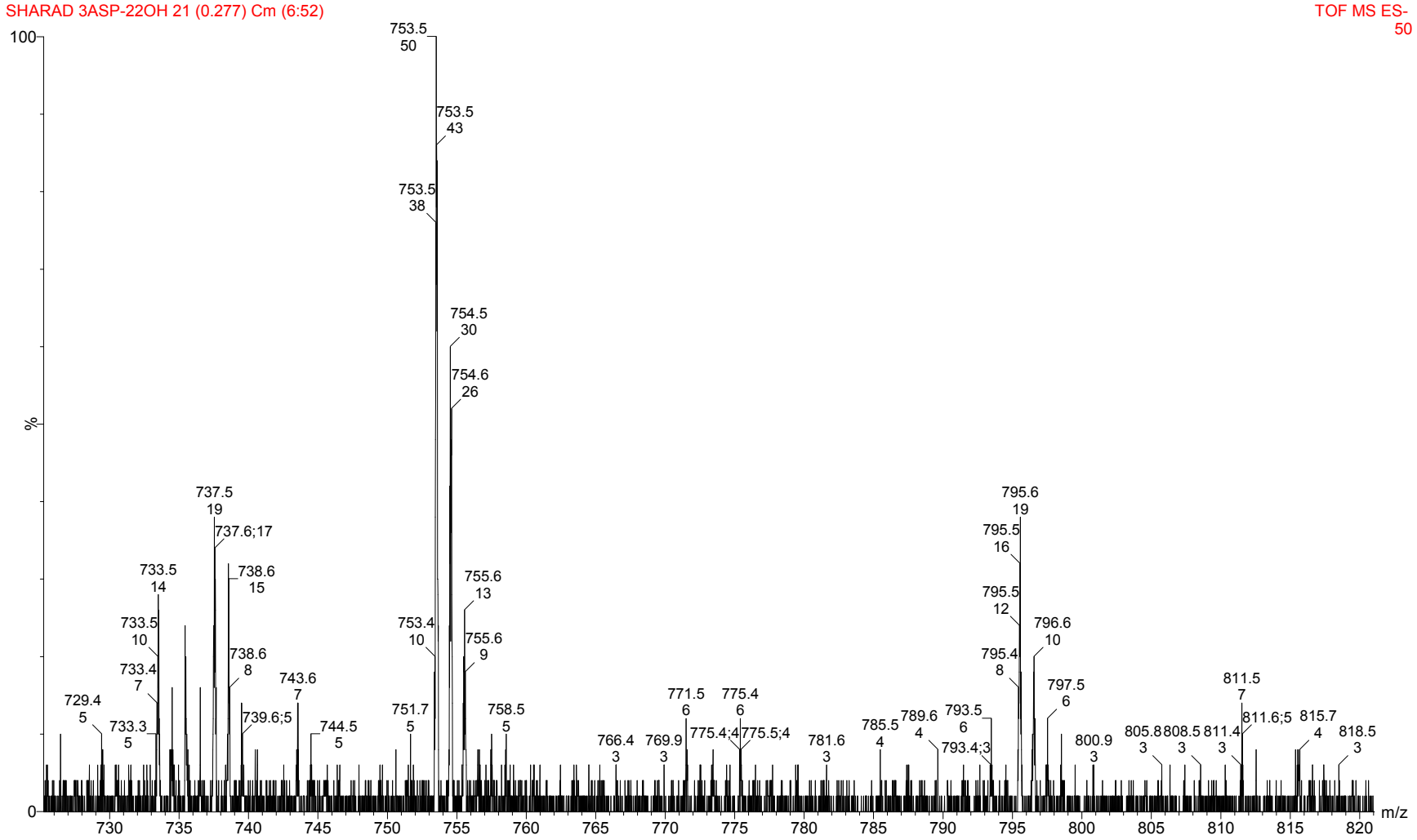
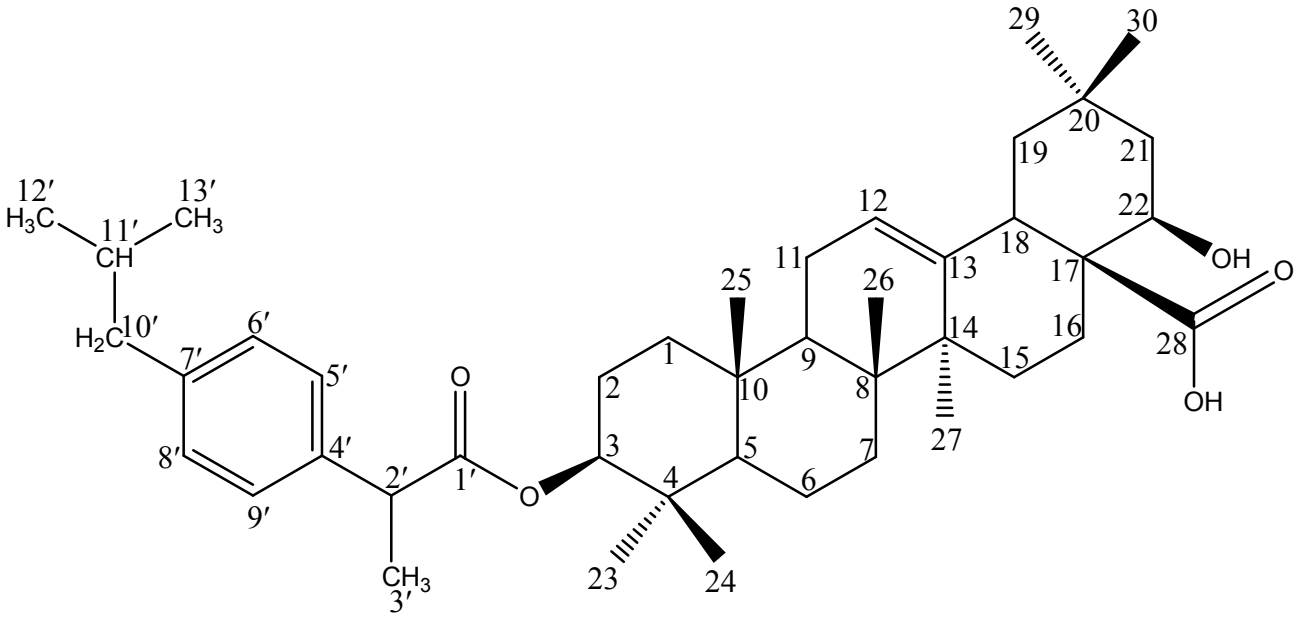
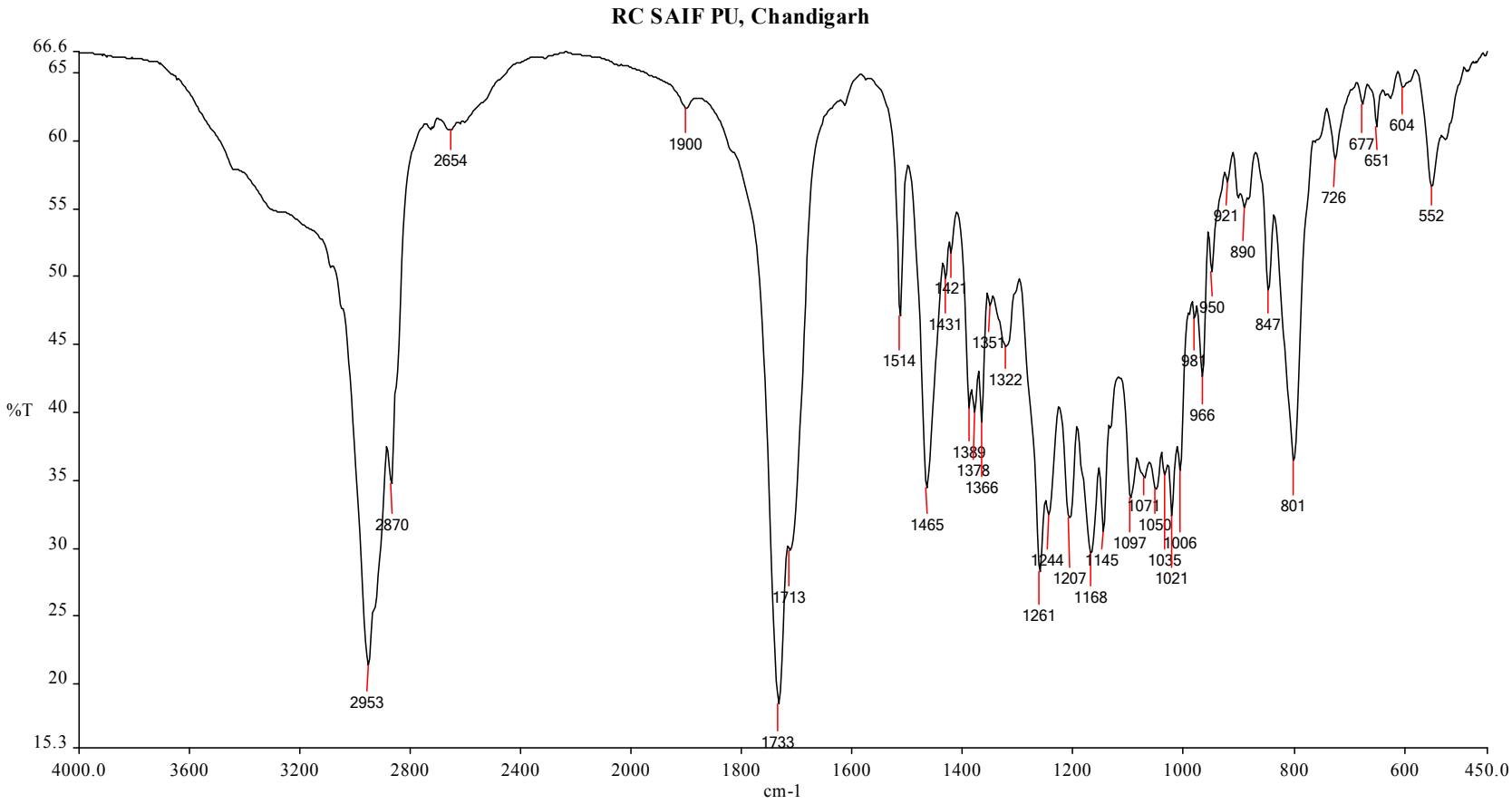


Fig. 23. ESI-MS negative-ion mode spectrum of compound **6** (Exact Mass- 796.42)

7. 3*β*-((*RS*)-2-(4-Isobutylphenyl)propanoyloxy)-22*β*-hydroxy-olean-12-en-28-oic acid (7)



3β-((*RS*)-2-(4-Isobutylphenyl)propanoyloxy)-22β-hydroxy-olean-12-en-28-oic acid



Spectrum Name: SharadKumar-20.sp Description: 3 IBU-22OH

Date Created: fri dec 21 12:37:10 2012 India Standard Time (GMT+5:30)

Fig. 24. FT-IR spectrum of compound **7**

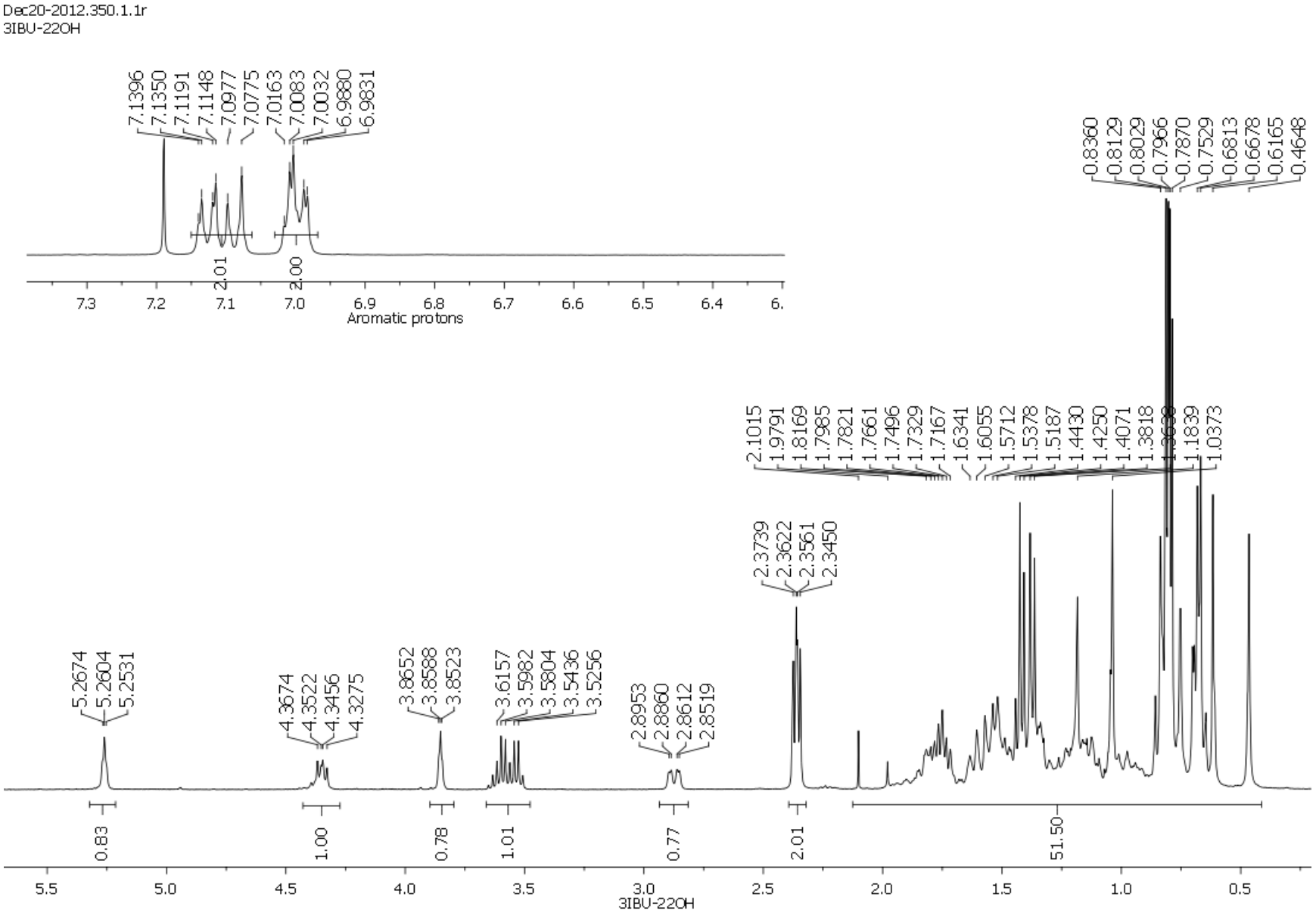


Fig. 25. ^{1}H NMR spectrum of compound **7** ($C_{43}H_{64}O_5$) in $CDCl_3$

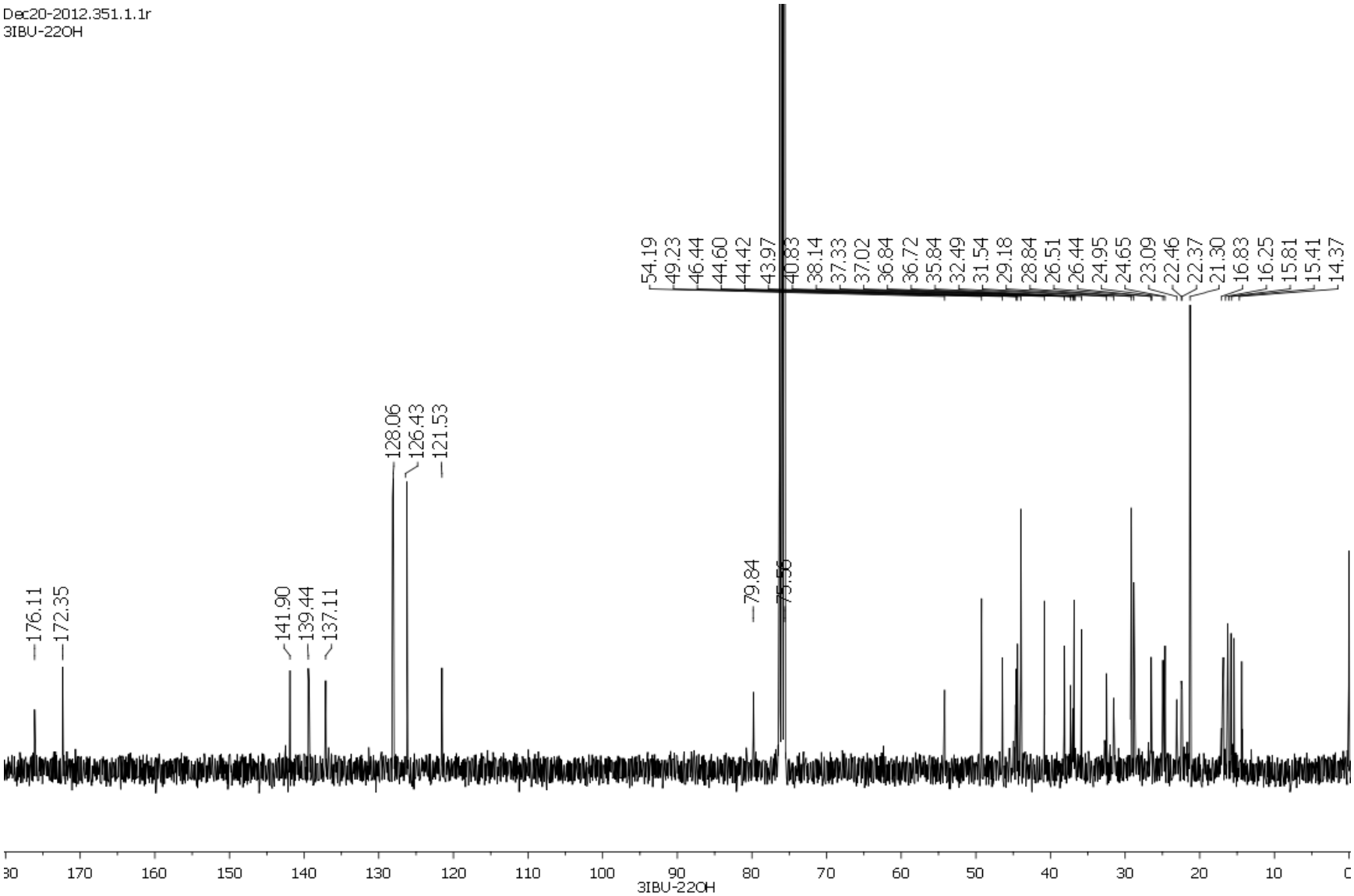


Fig. 26. ^{13}C NMR spectrum of compound **7** ($C_{43}H_{64}O_5$) in $CDCl_3$. The C-5′ & C-9′ appeared at 128.06 ppm, C-6′ & C-8′ appeared at 126.43 ppm, and C-12′ & C-13′ appeared at 21.30 ppm. Hence, 40 peaks of compound are visible in the spectrum.

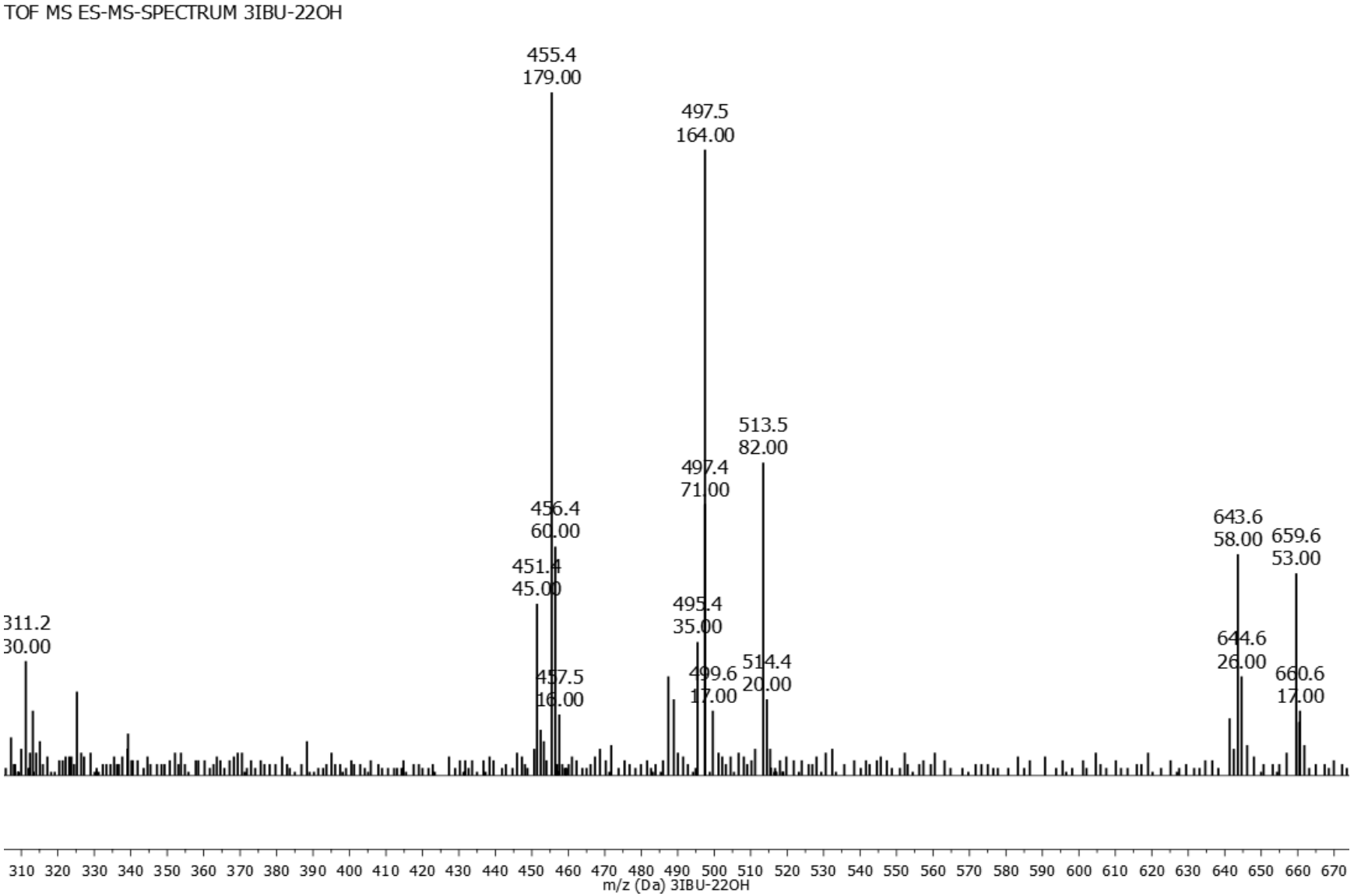
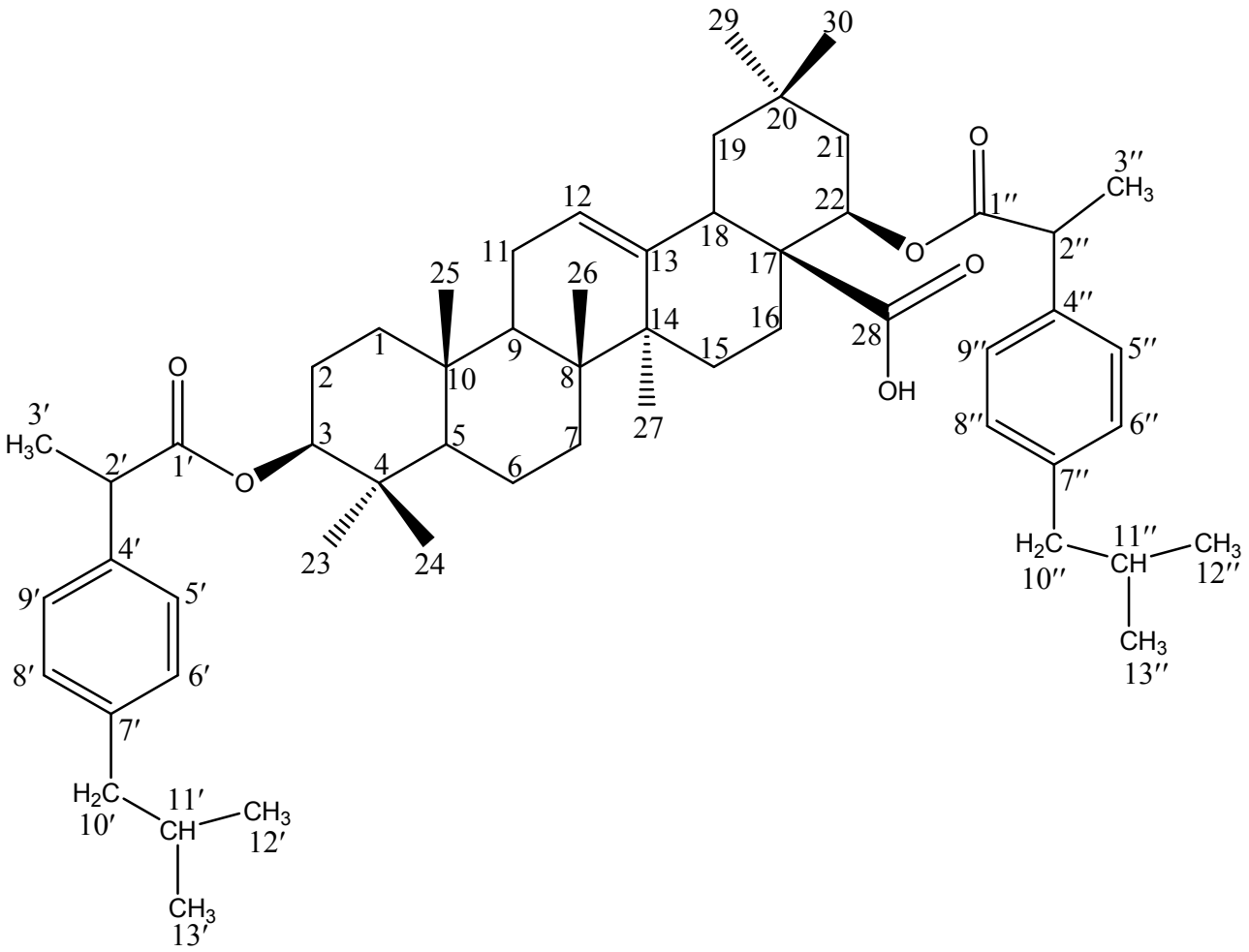


Fig. 27. ESI-MS negative-ion mode spectrum of compound **7** (Exact Mass- 660.48)

8. 3*β*,22*β*-Di((*RS*)-2-(4-isobutylphenyl)propanoyloxy)-olean-12-en-28-oic acid (8)



3β,22β-Di((*RS*)-2-(4-isobutylphenyl)propanoyloxy)-olean-12-en-28-oic acid

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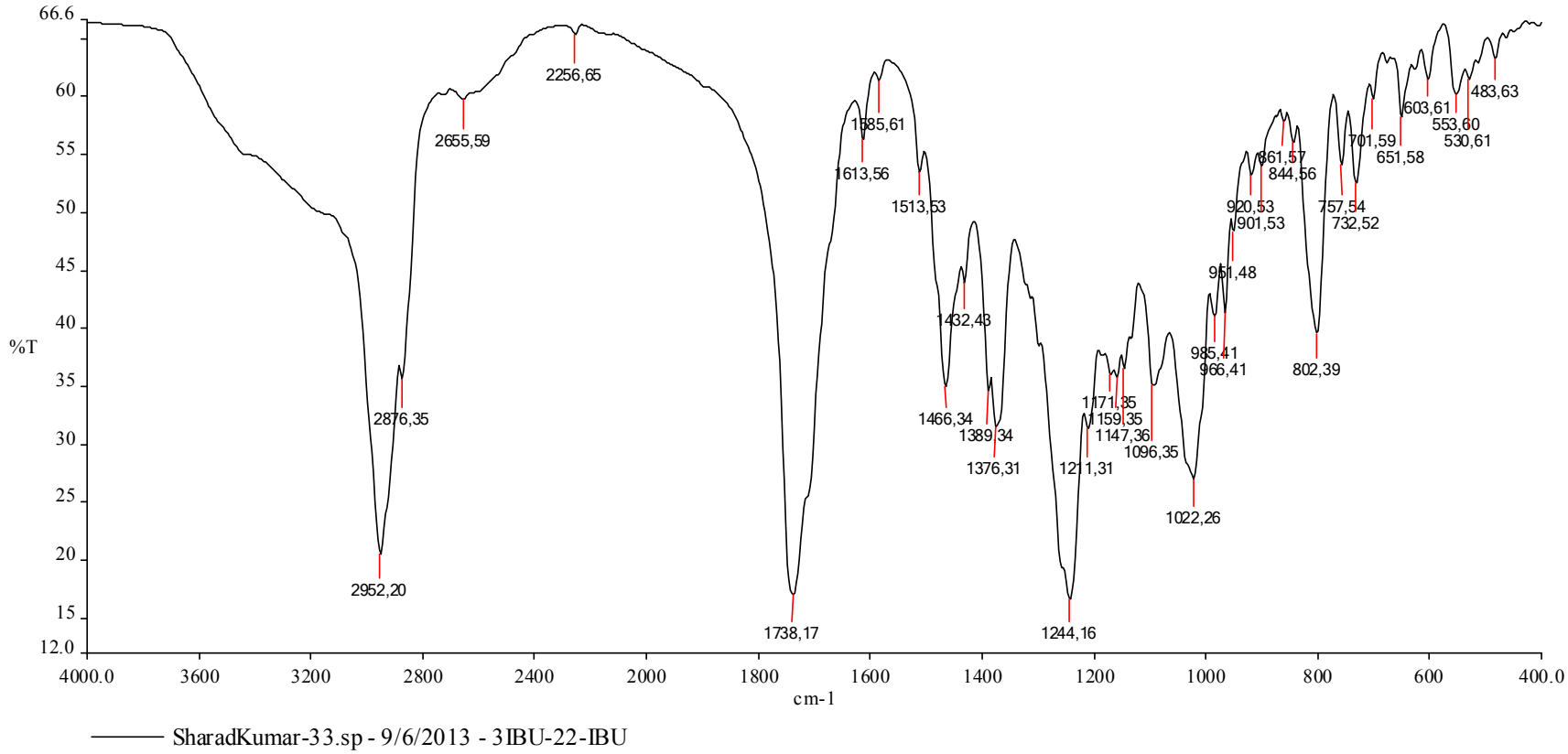


Fig. 28. FT-IR spectrum of compound **8**

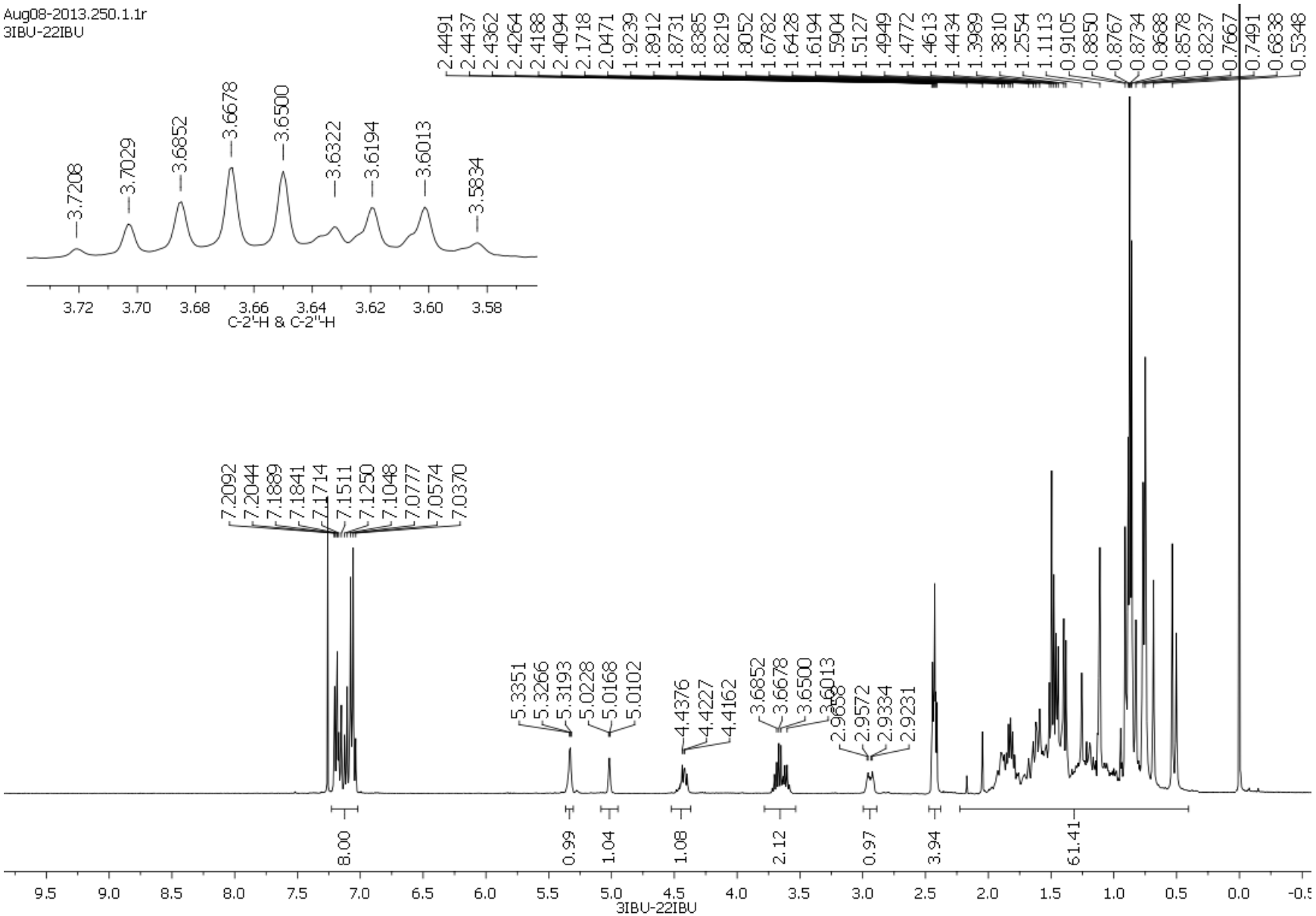


Fig. 29. ^{1}H NMR spectrum of compound **8** ($C_{56}H_{80}O_6$) in $CDCl_3$

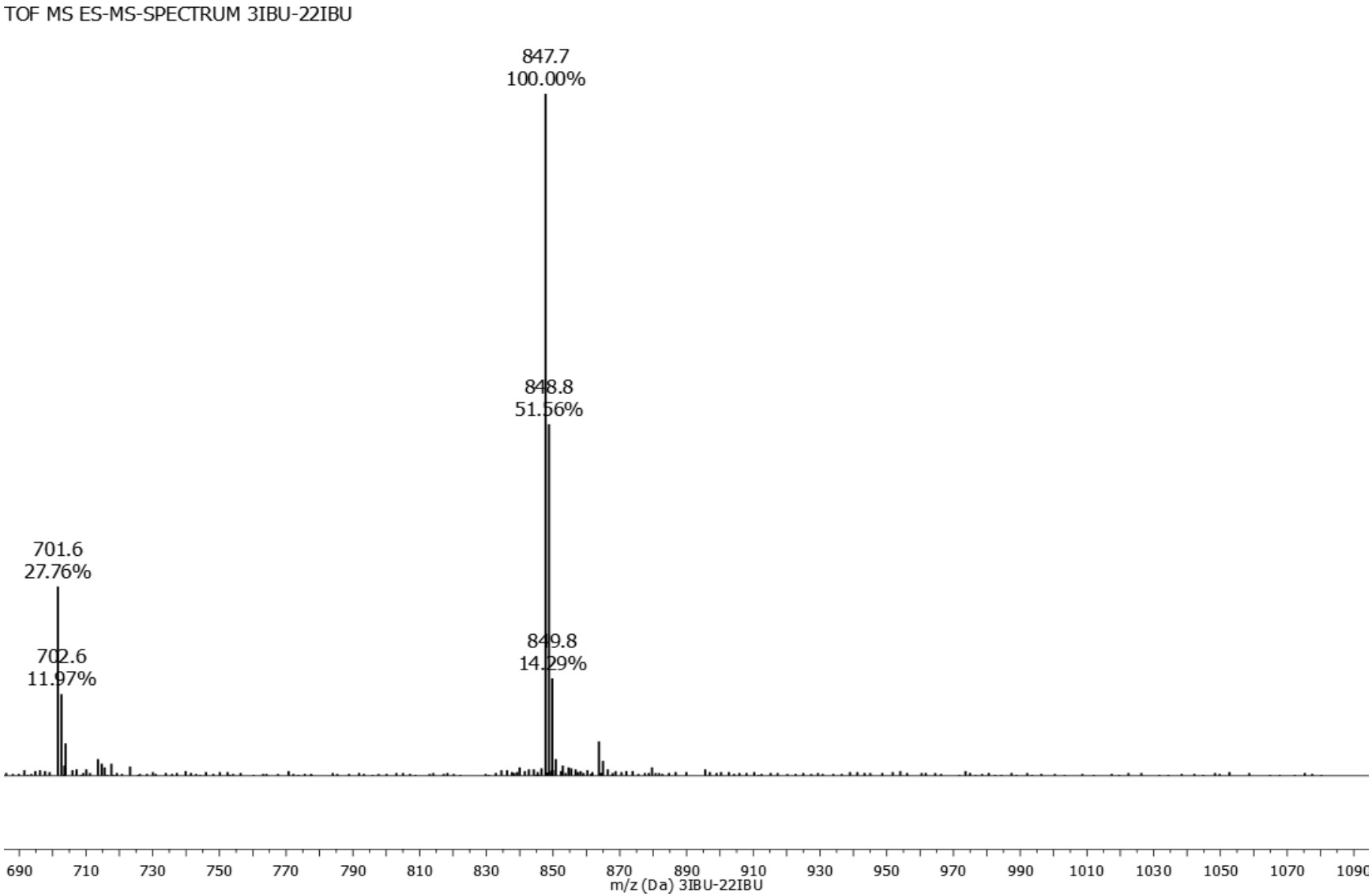
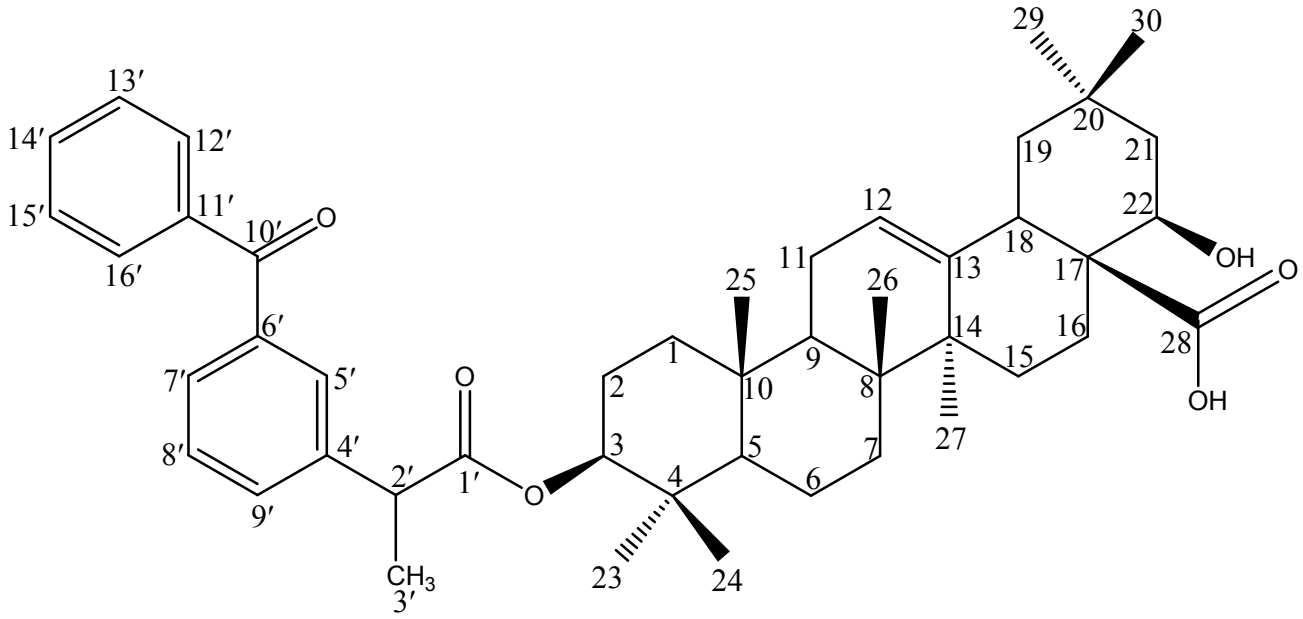


Fig. 30. ESI-MS negative-ion mode spectrum of compound **8** (Exact Mass- 848.60)

9. 3*β*-((*RS*)-2-(3-Benzoylphenyl)propanoyloxy)-22*β*-hydroxy-olean-12-en-28-oic acid (9)



3β-((*RS*)-2-(3-Benzoylphenyl)propanoyloxy)-22β-hydroxy-olean-12-en-28-oic acid

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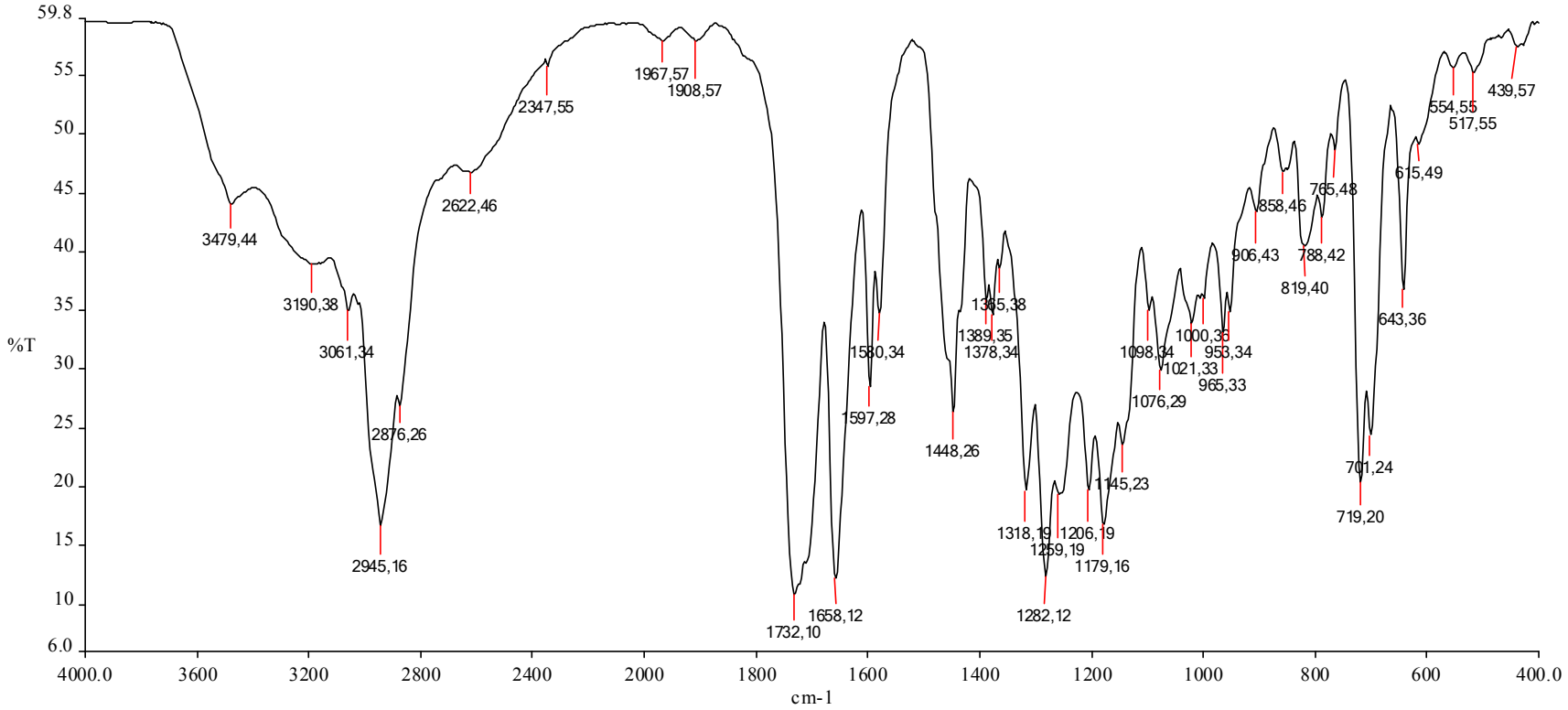


Fig. 31. FT-IR spectrum of compound **9**

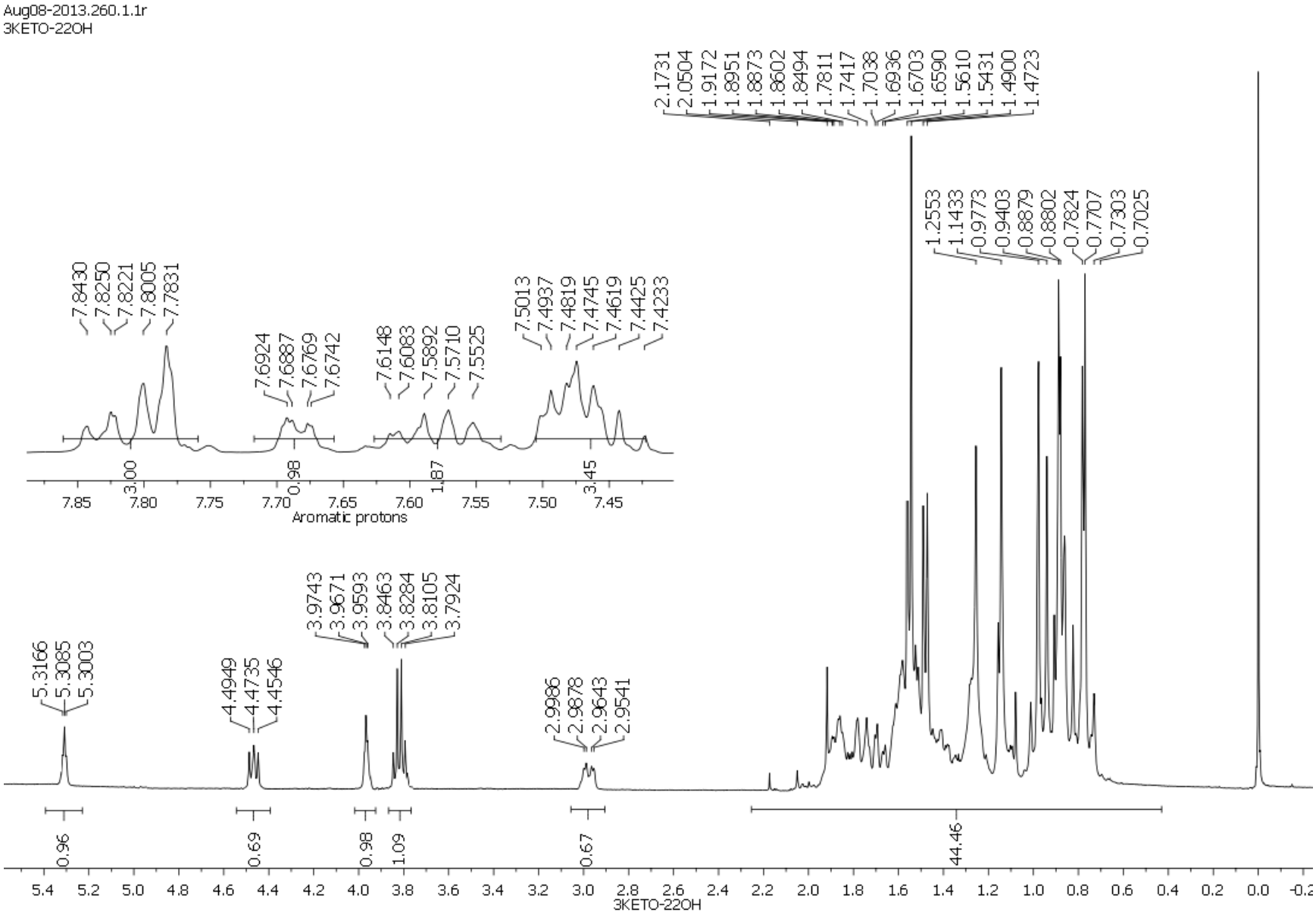


Fig. 32. ^{1}H NMR spectrum of compound **9** ($C_{46}H_{60}O_6$) in $CDCl_3$

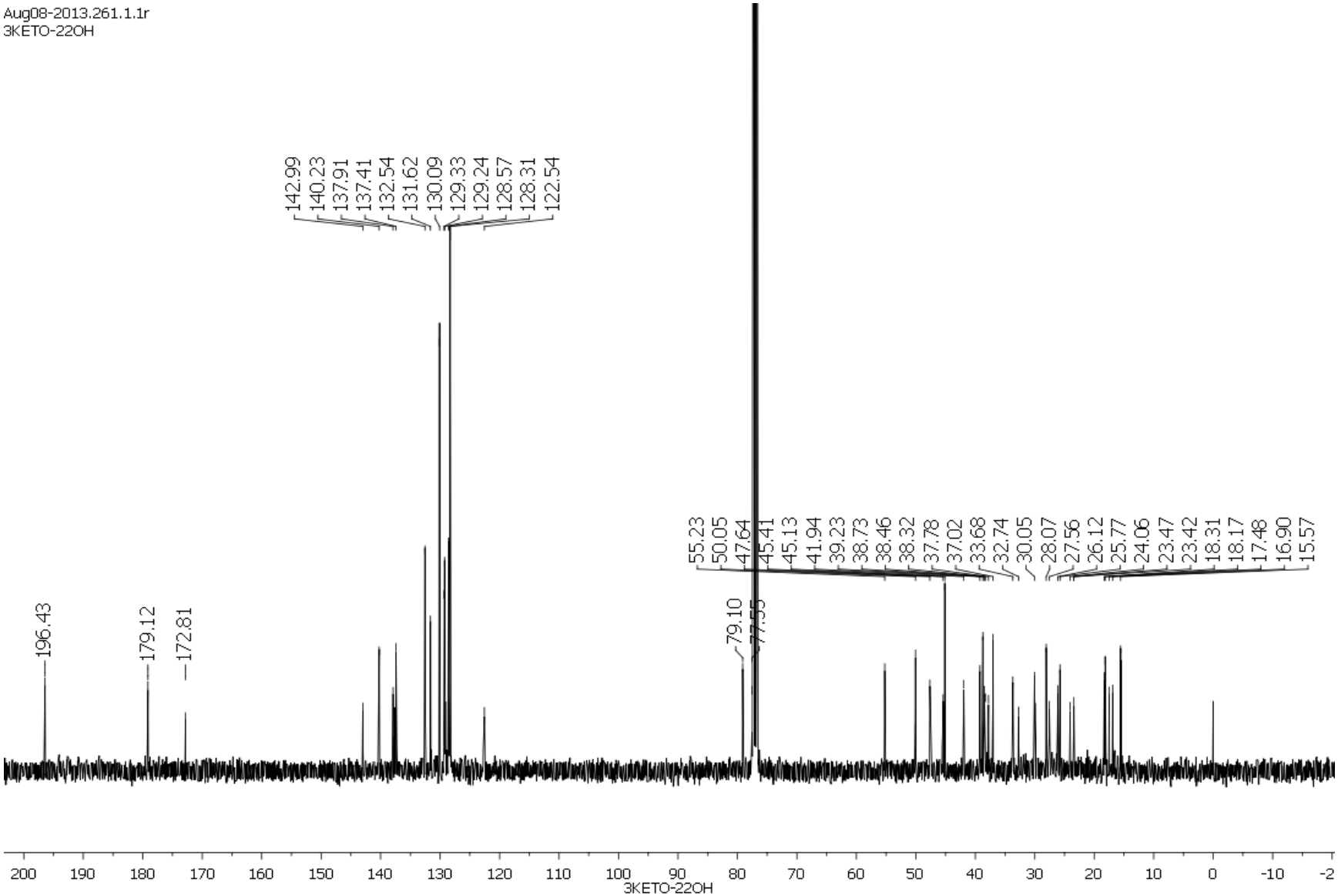


Fig. 33. ^{13}C NMR spectrum of compound **9** ($C_{46}H_{60}O_6$) in $CDCl_3$. The C-12′ & C-16′ appeared at 130.09 ppm and C-13′ & C-15′ appeared at 128.31 ppm. Hence, 44 peaks of compound are visible in the spectrum.

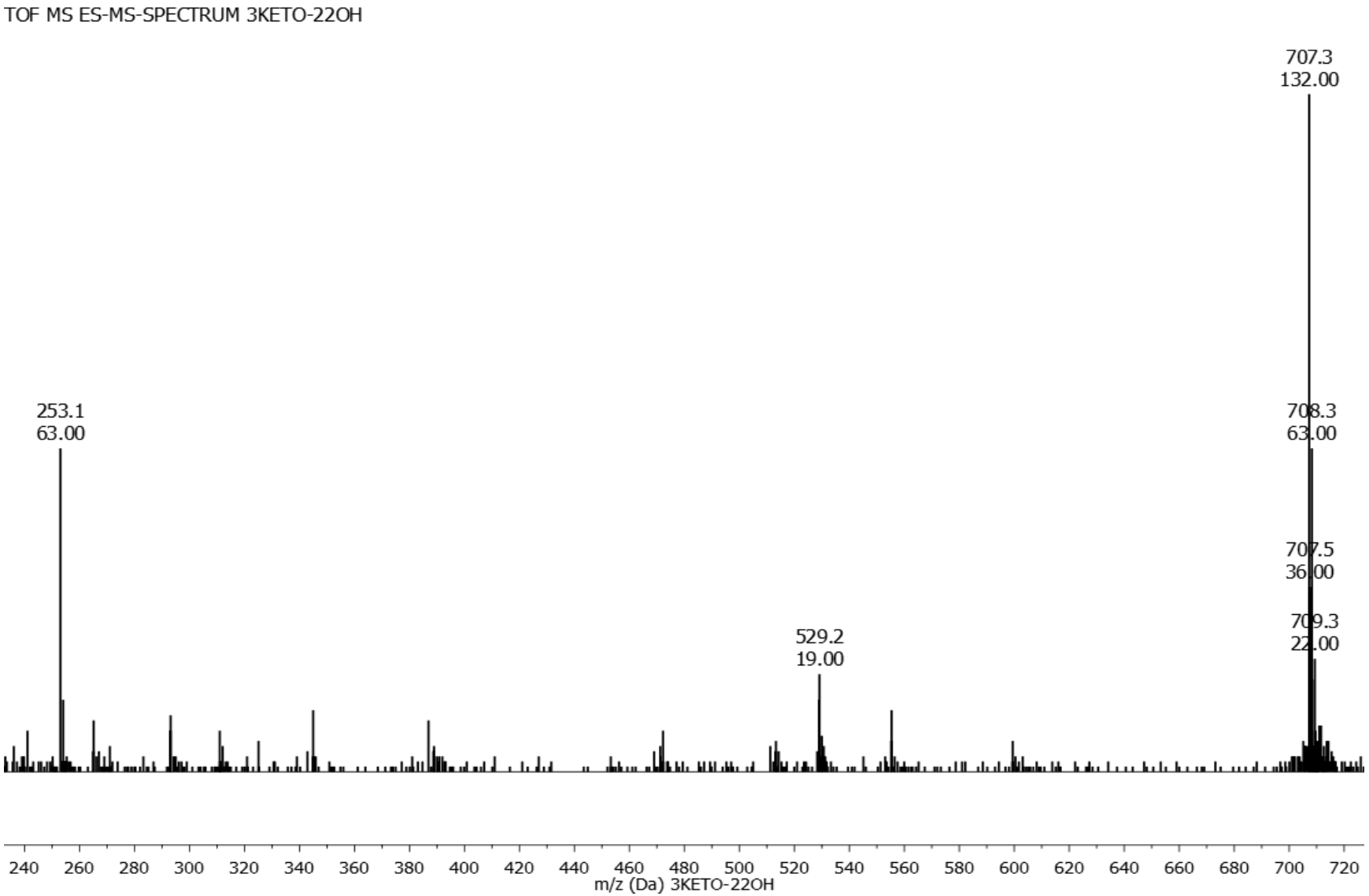
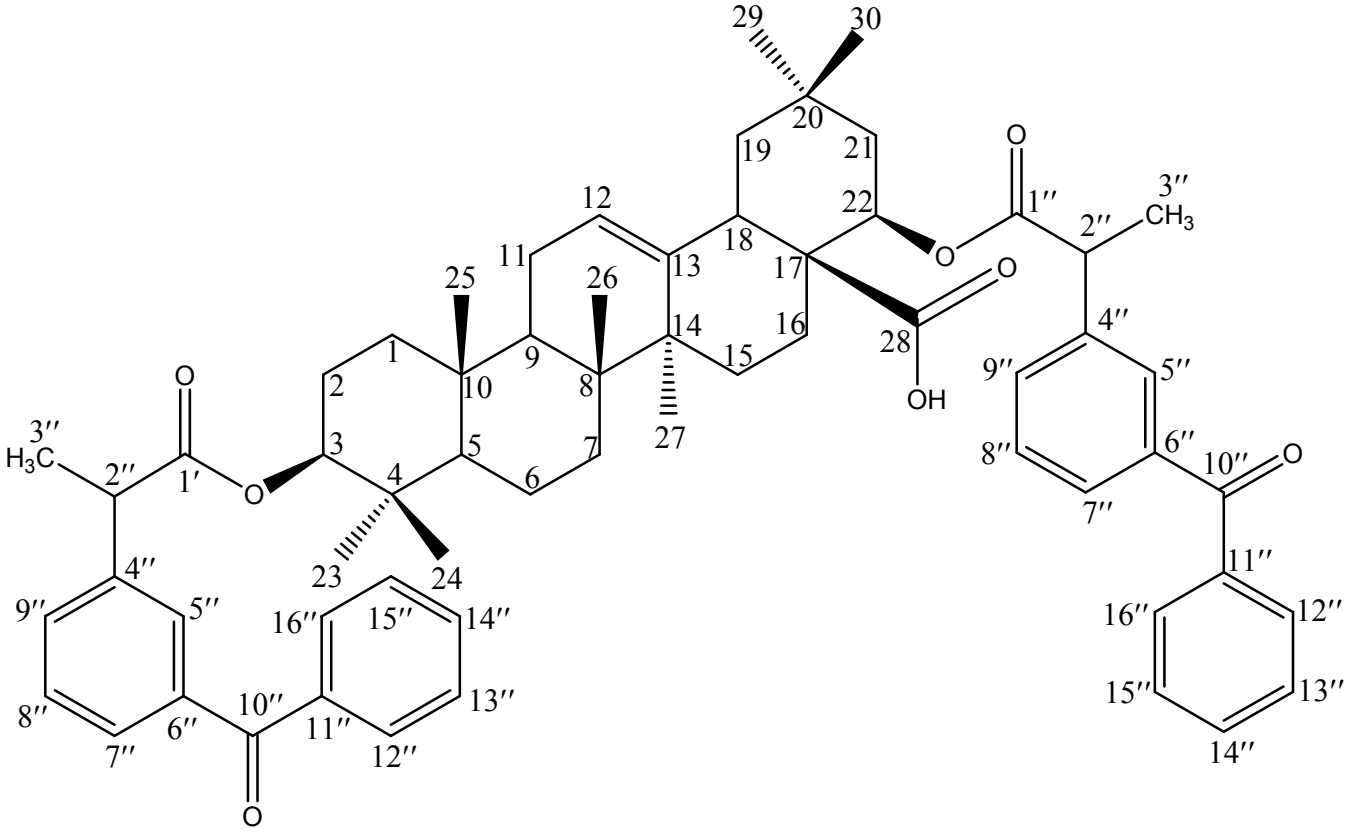


Fig. 34. ESI-MS negative-ion mode spectrum of compound **9** (Exact Mass- 708.44)

10. 3*β*,22*β*-Di((*RS*)-2-(3-benzoylphenyl)propanoyloxy)-olean-12-en-28-oic acid (10)



3β,22β-Di((*RS*)-2-(3-benzoylphenyl)propanoyloxy)-olean-12-en-28-oic acid

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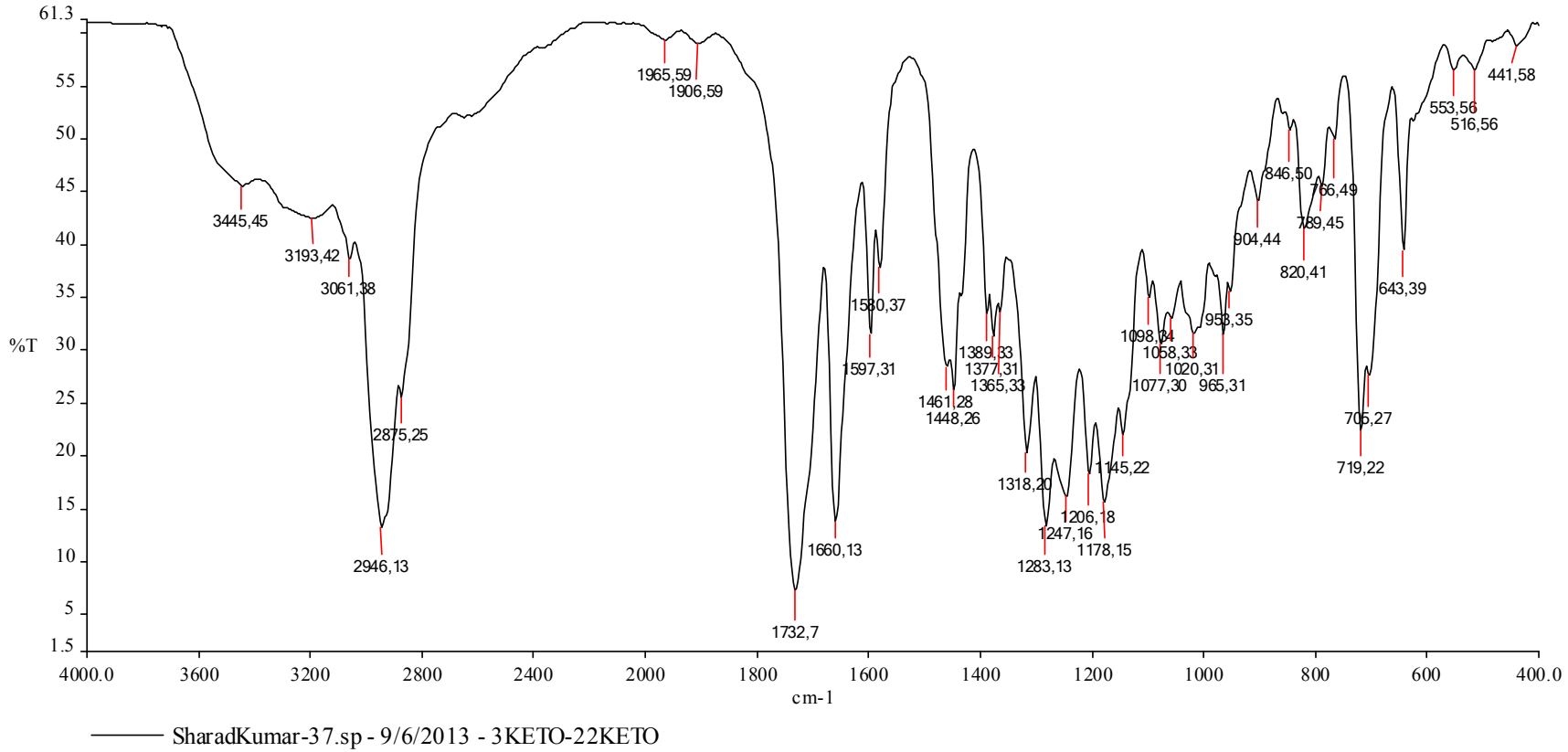


Fig. 35. FT-IR spectrum of compound **10**

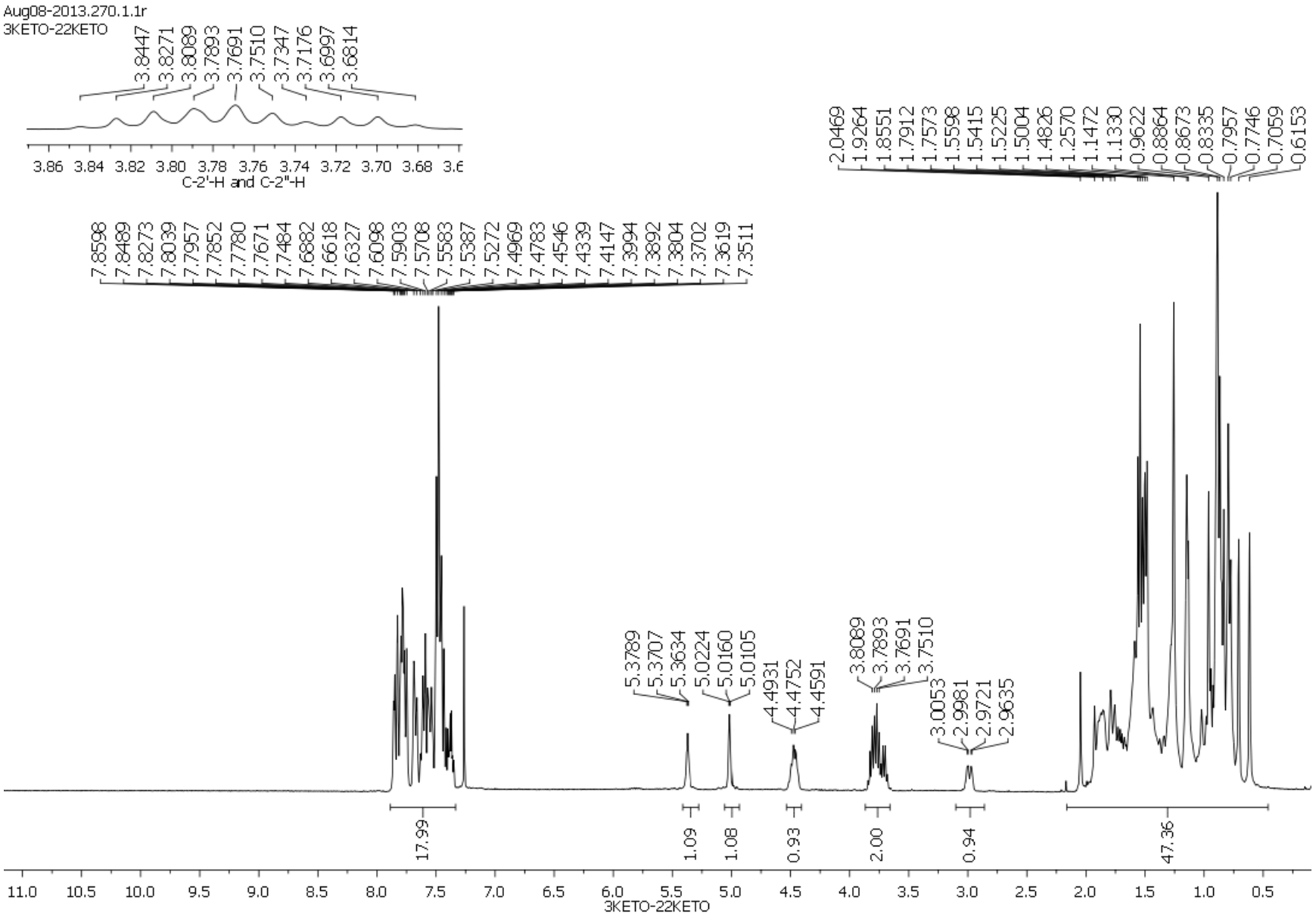


Fig. 36. ^{1}H NMR spectrum of compound **10** ($C_{62}H_{72}O_8$) in $CDCl_3$

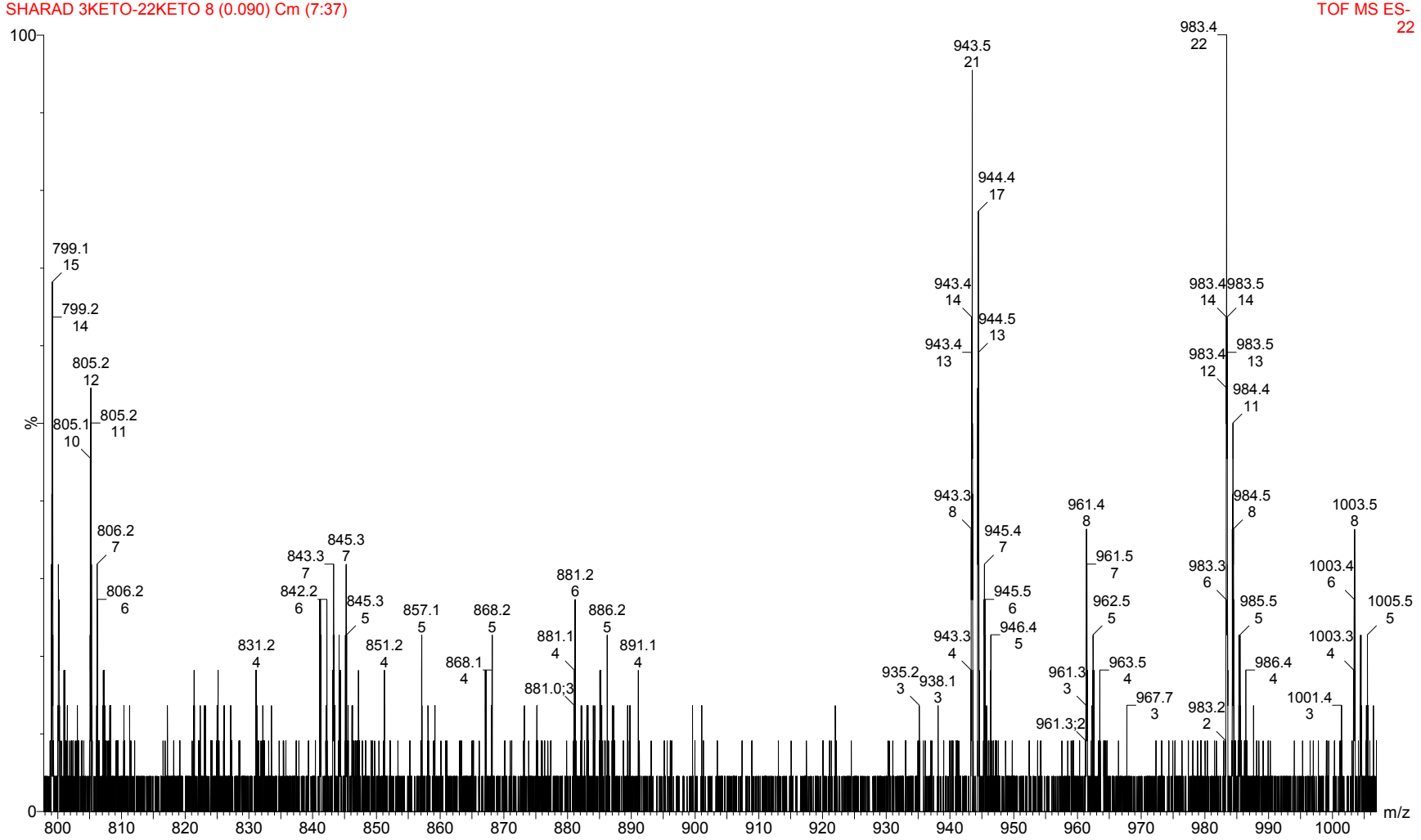
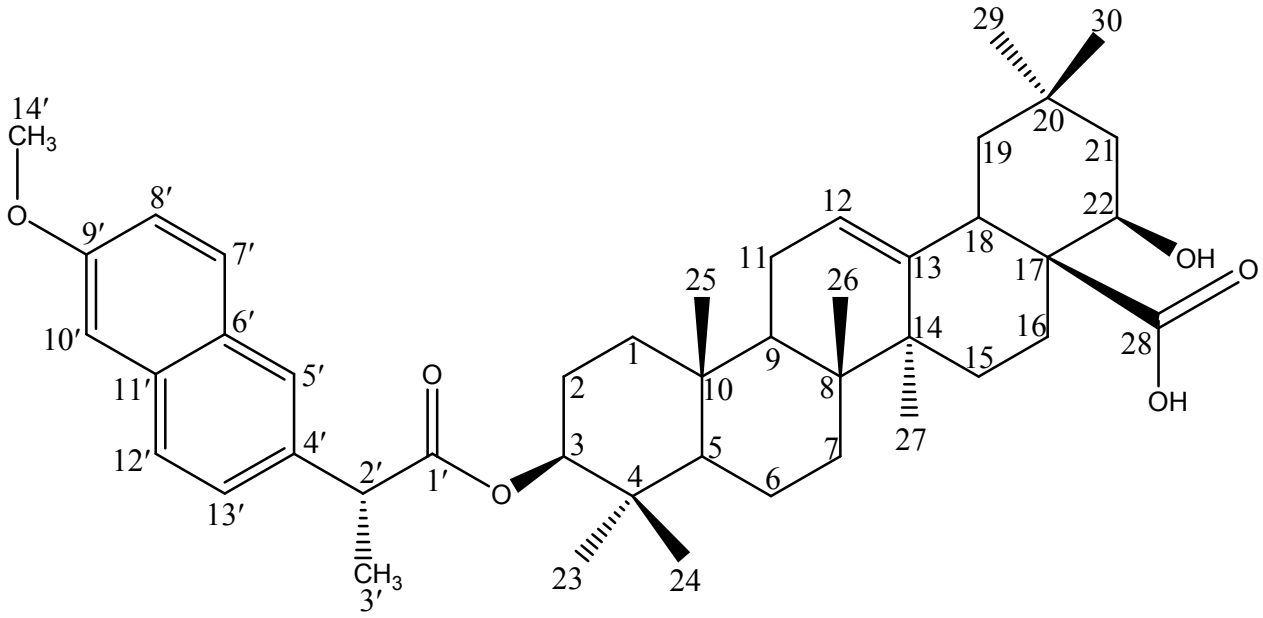
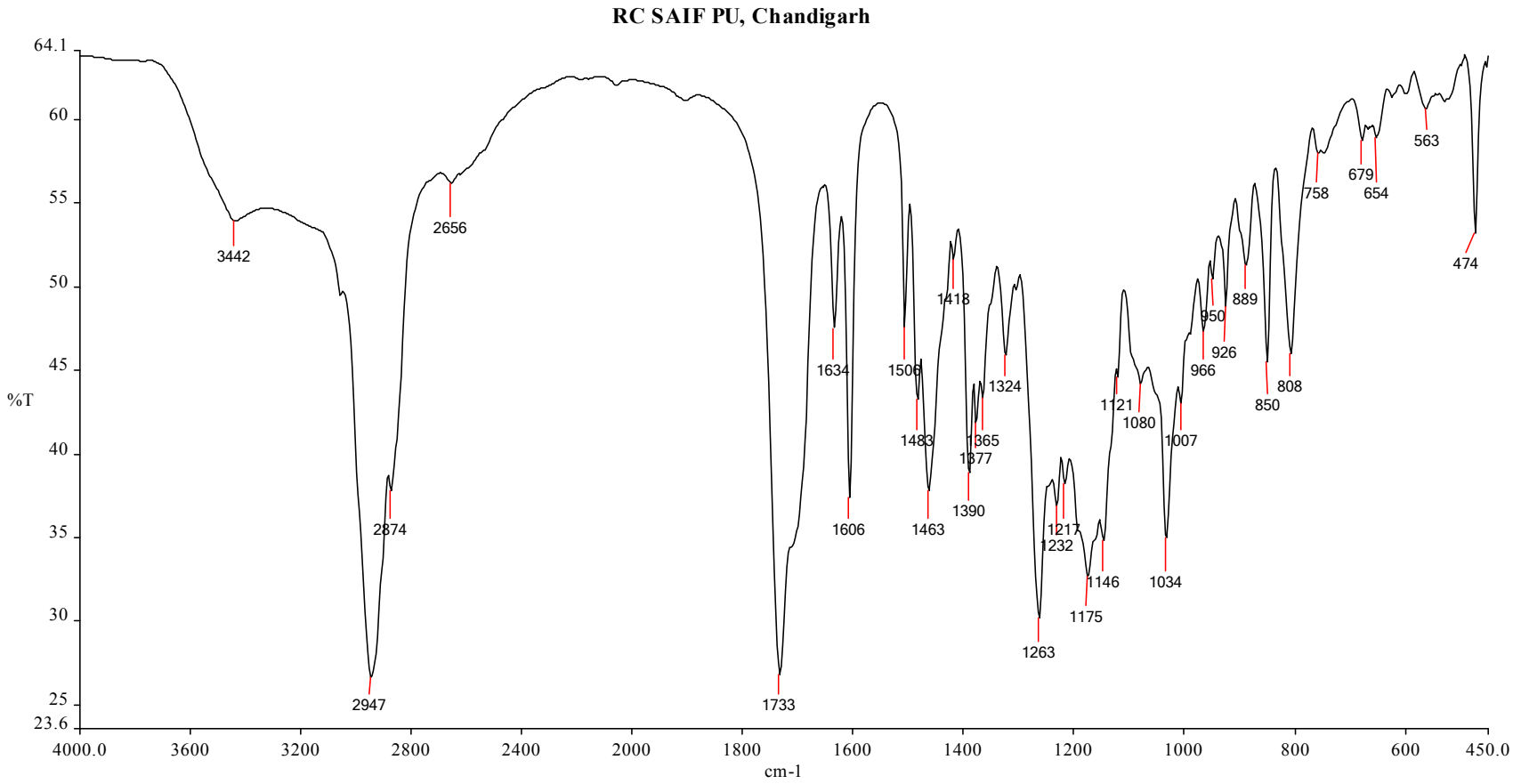


Fig. 37. ESI-MS negative-ion mode spectrum of compound **10** (Exact Mass- 944.52)

11. 3*β*-((+)-(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoyloxy)-22*β*-hydroxy-olean-12-en-28-oic acid (11)



3β-((+)-(*S*)-2-(6-Methoxynaphthalen-2-yl)propanoyloxy)-22β-hydroxy-olean-12-en-28-oic acid



Spectrum Name: SharadKumar-19.sp Description: 3 NPR-22OH

Date Created: fri dec 21 12:35:27 2012 India Standard Time (GMT+5:30)

Fig. 38. FT-IR spectrum of compound **11**

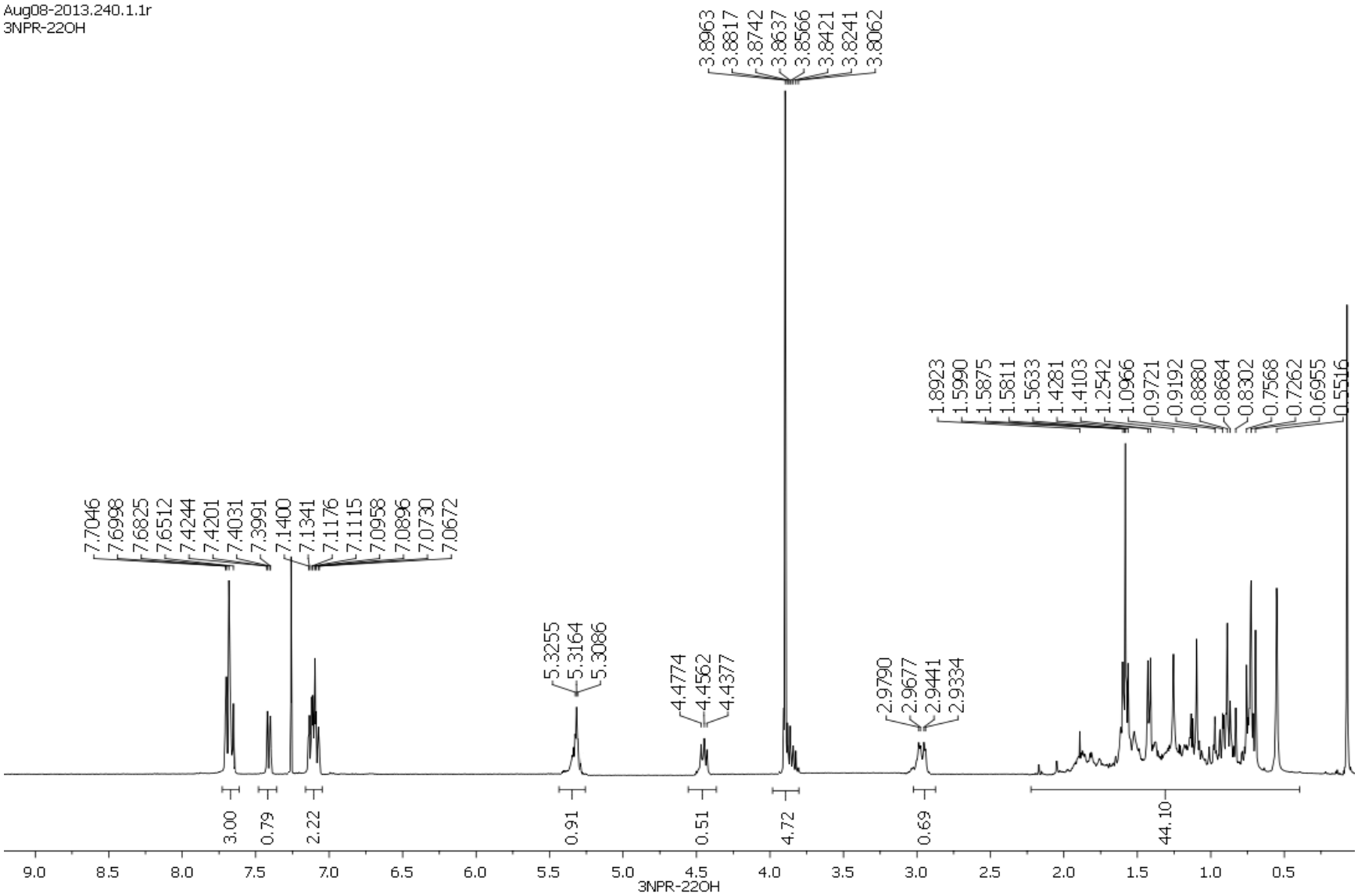


Fig. 39. ^{1}H NMR spectrum of compound **11** ($C_{44}H_{60}O_6$) in $CDCl_3$

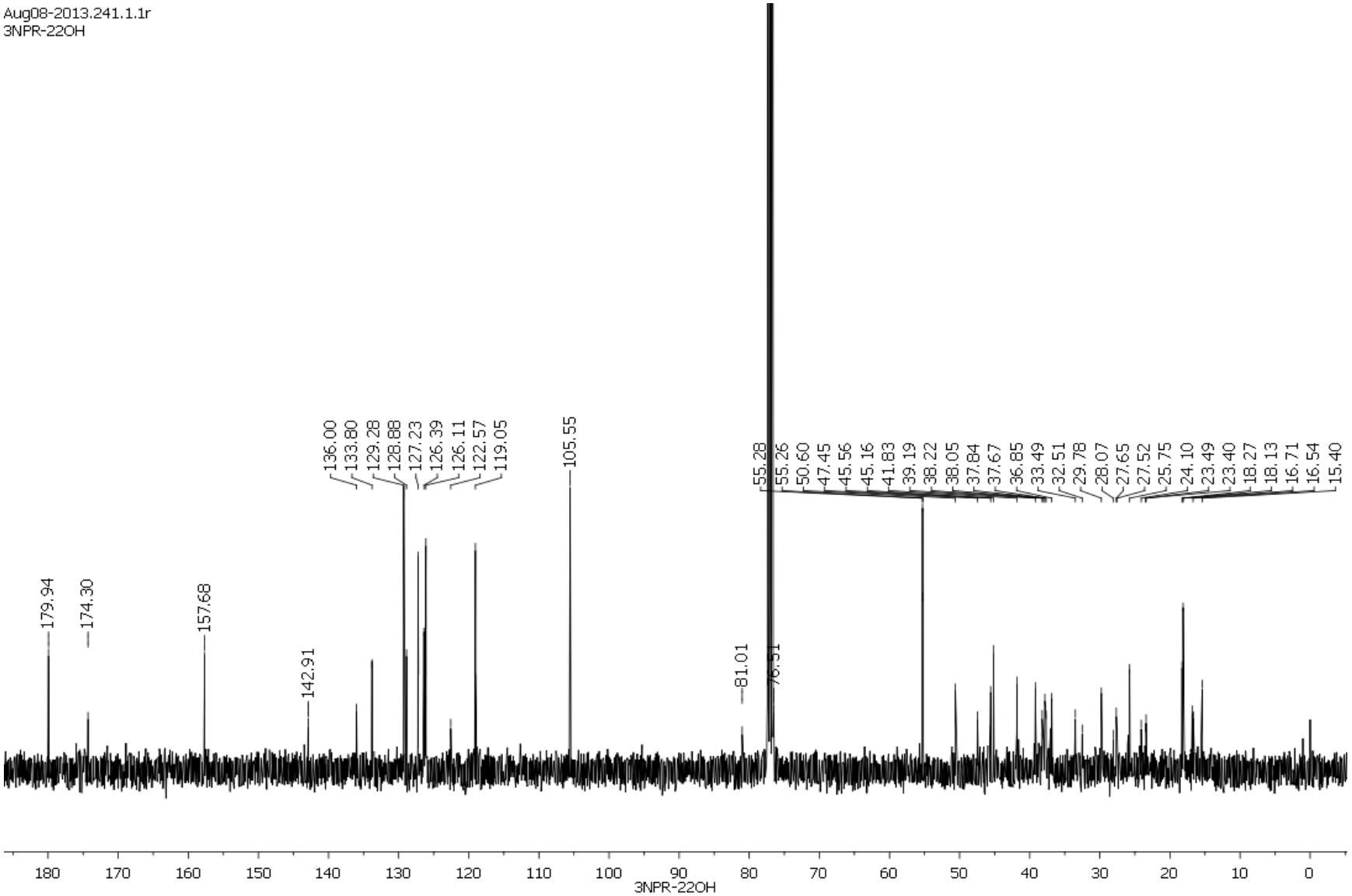


Fig. 40. ^{13}C NMR spectrum of compound **11** ($C_{44}H_{60}O_6$) in $CDCl_3$

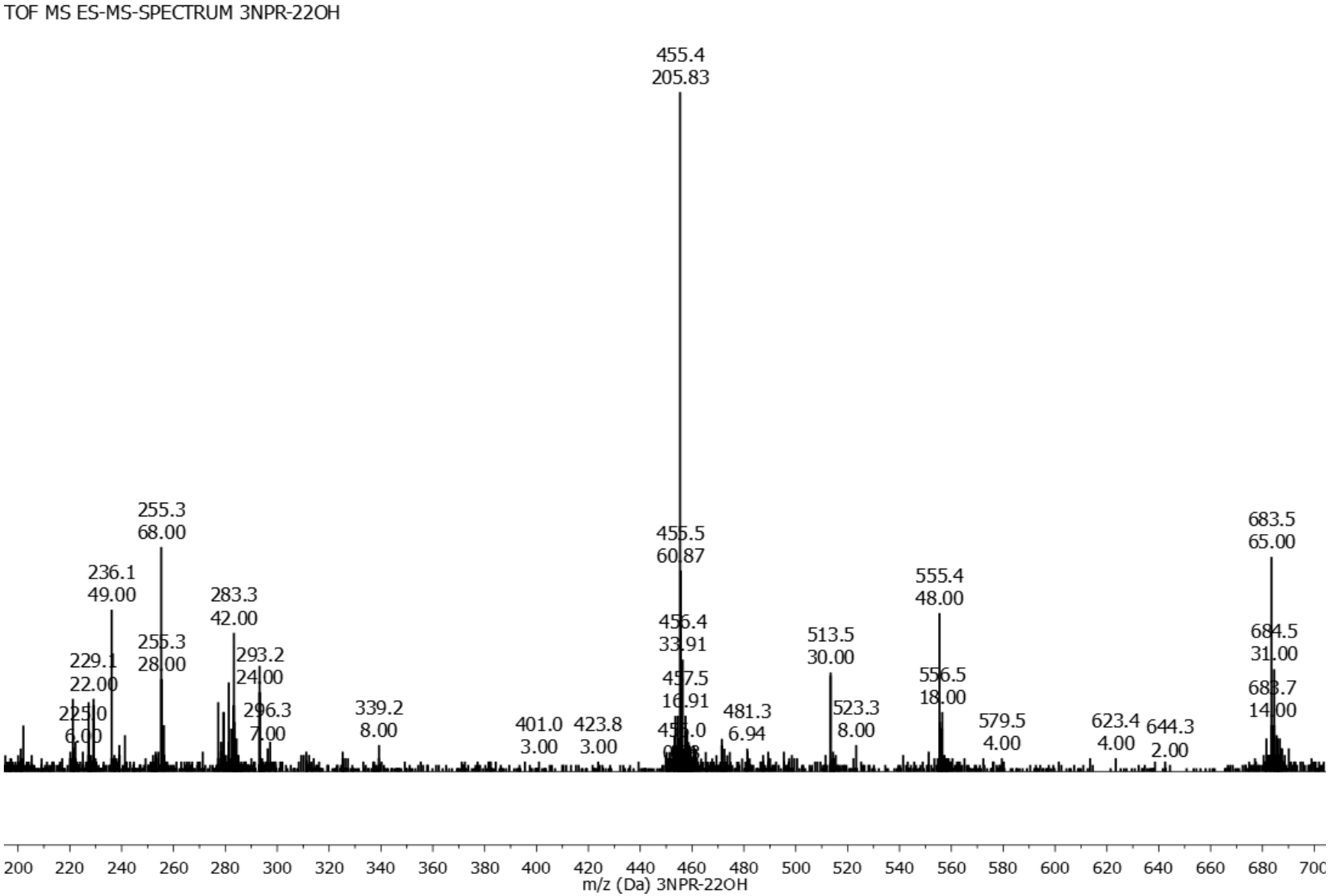
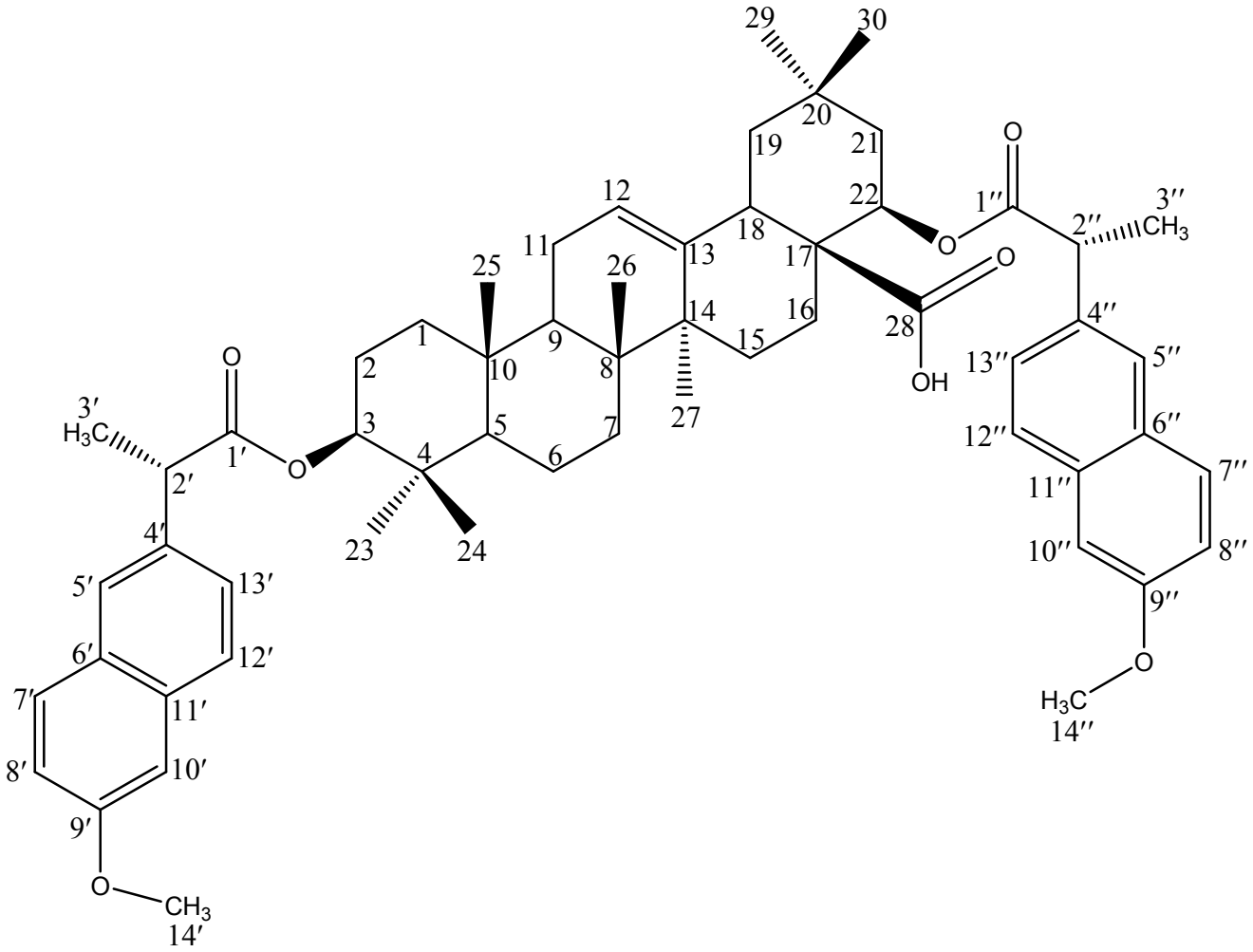


Fig. 41. ESI-MS negative-ion mode spectrum of compound **11** (Exact Mass- 684.44)

12. 3*β*,22*β*-Di((+)-(*S*)-2-(6-methoxynaphthalen-2-yl)propanoyloxy)-olean-12-en-28-oic acid (12)



3β,22β-Di((+)-(*S*)-2-(6-methoxynaphthalen-2-yl)propanoyloxy)-olean-12-en-28-oic acid

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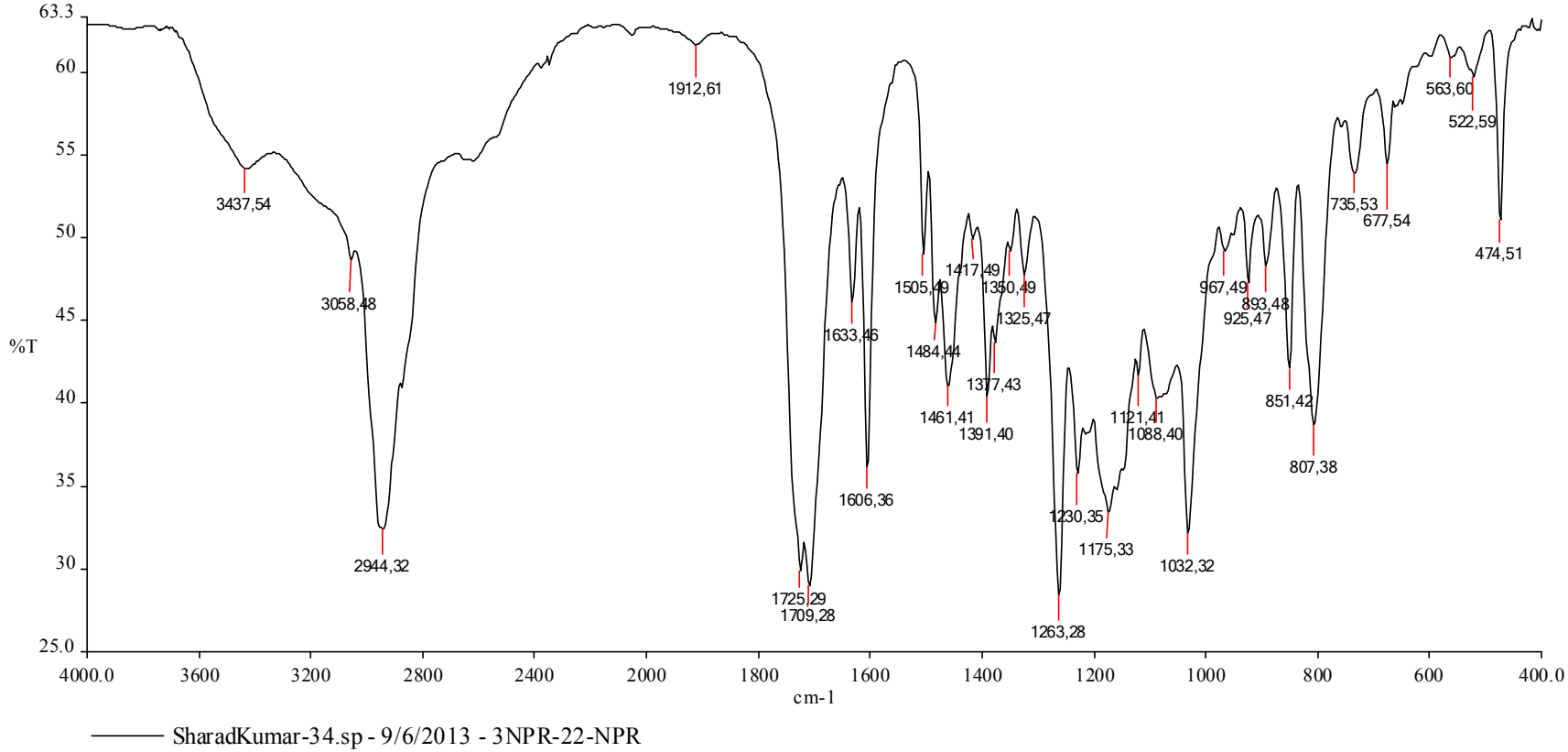


Fig. 42. FT-IR spectrum of compound **12**

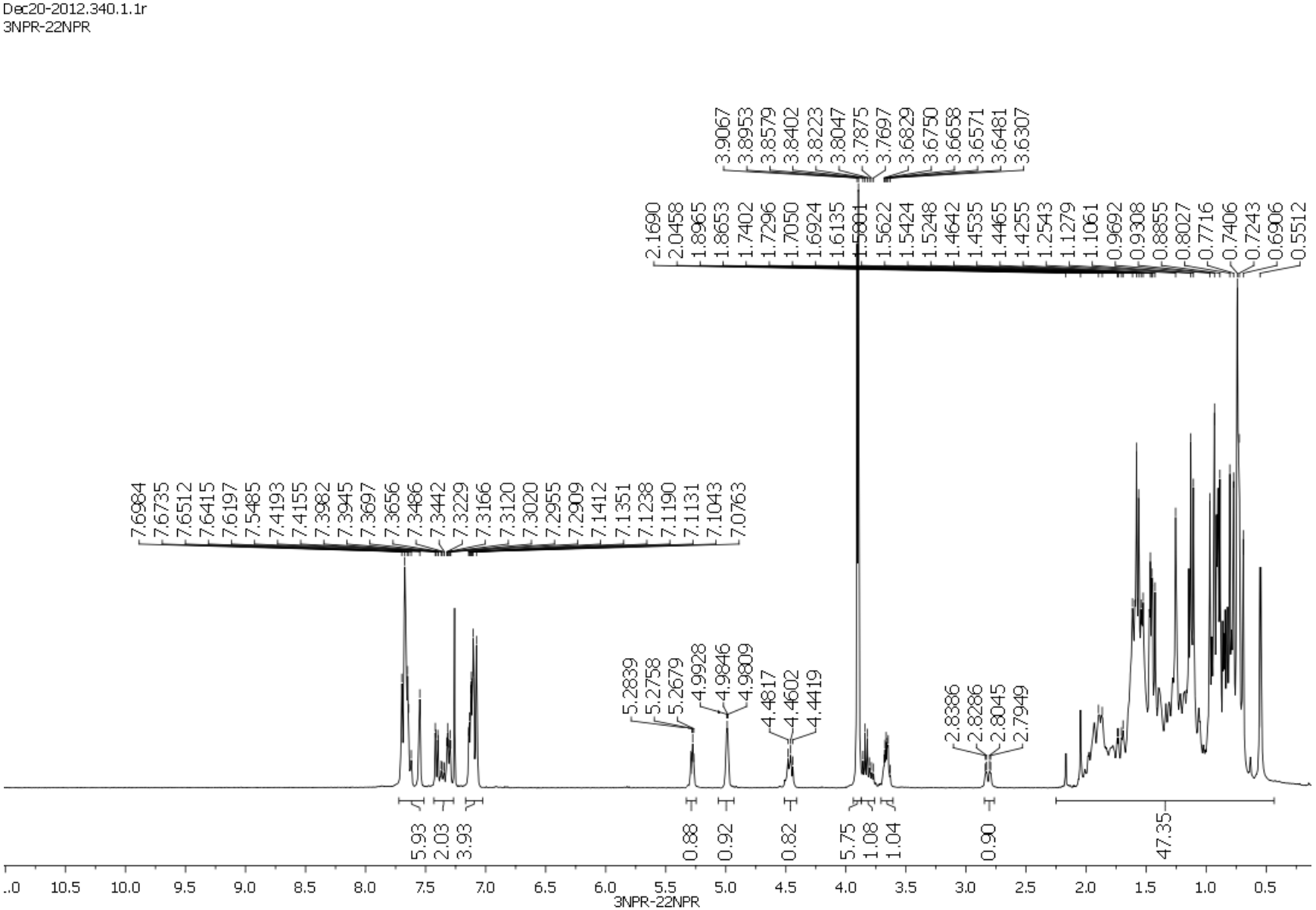


Fig. 43. ^{1}H NMR spectrum of compound **12** ($C_{58}H_{72}O_8$) in $CDCl_3$

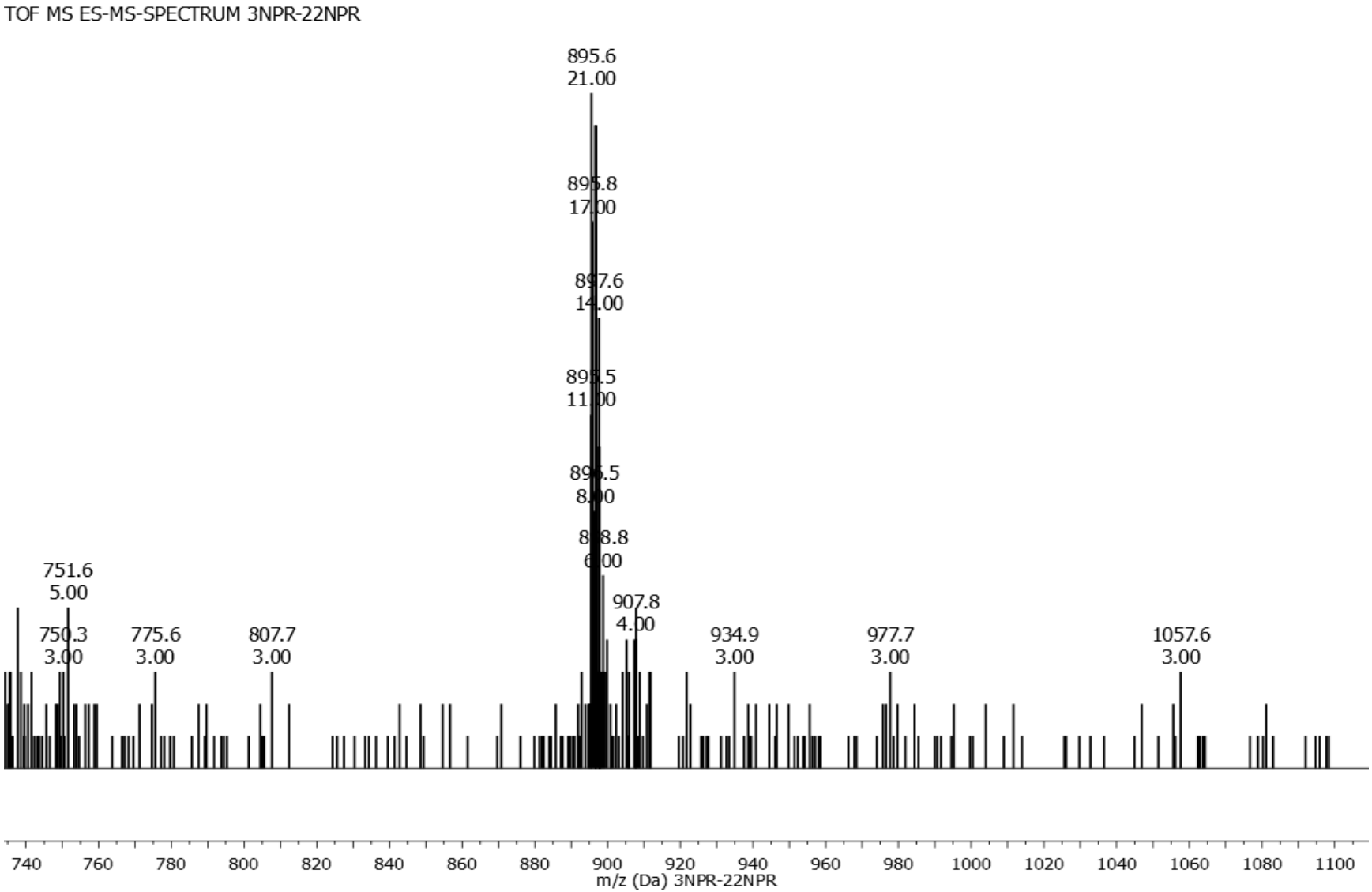
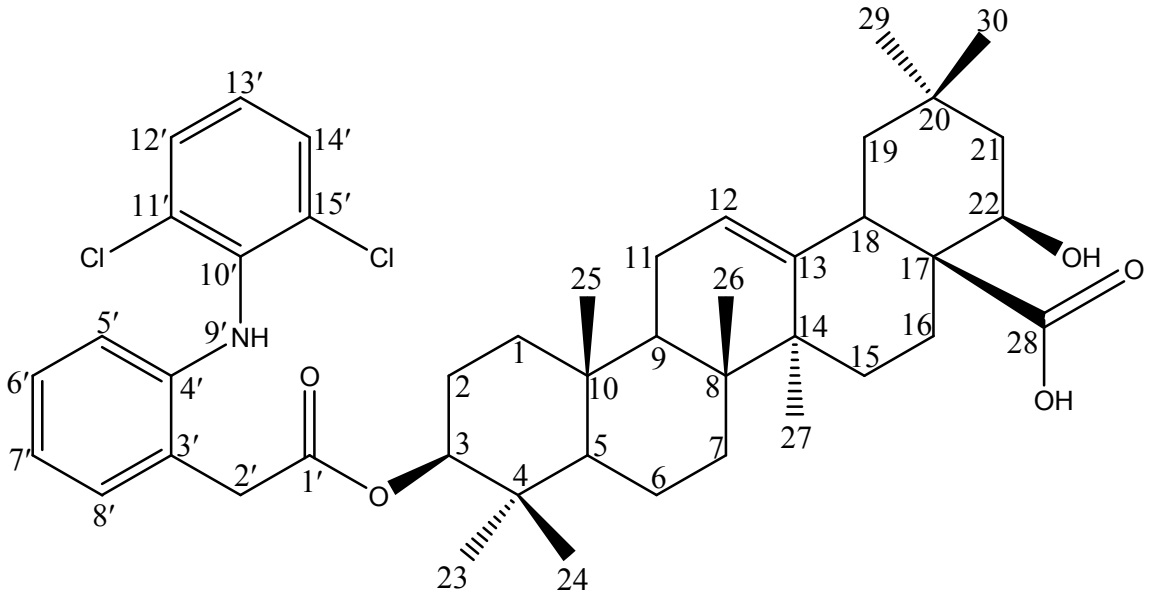


Fig. 44. ESI-MS negative-ion mode spectrum of compound **12** (Exact Mass- 896.52)

13. 3*β*-(2-(2-(2,6-Dichlorophenylamino)phenyl)acetoyloxy)-22*β*-hydroxy-olean-12-en-28-oic acid (13)



3β-(2-(2-(2,6-Dichlorophenylamino)phenyl)acetoyloxy)-22β-hydroxy-olean-12-en-28-oic acid

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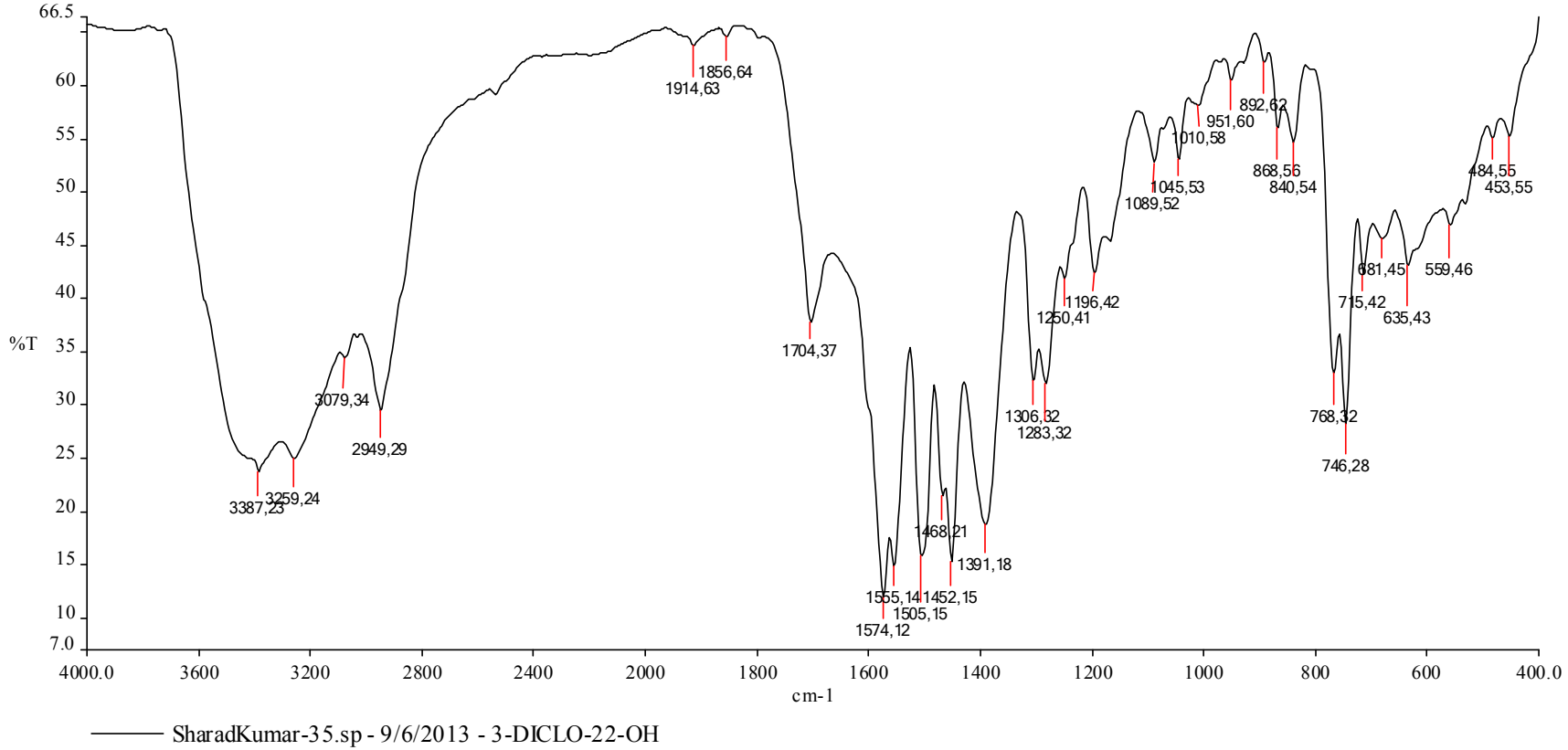


Fig. 45. FT-IR spectrum of compound **13**

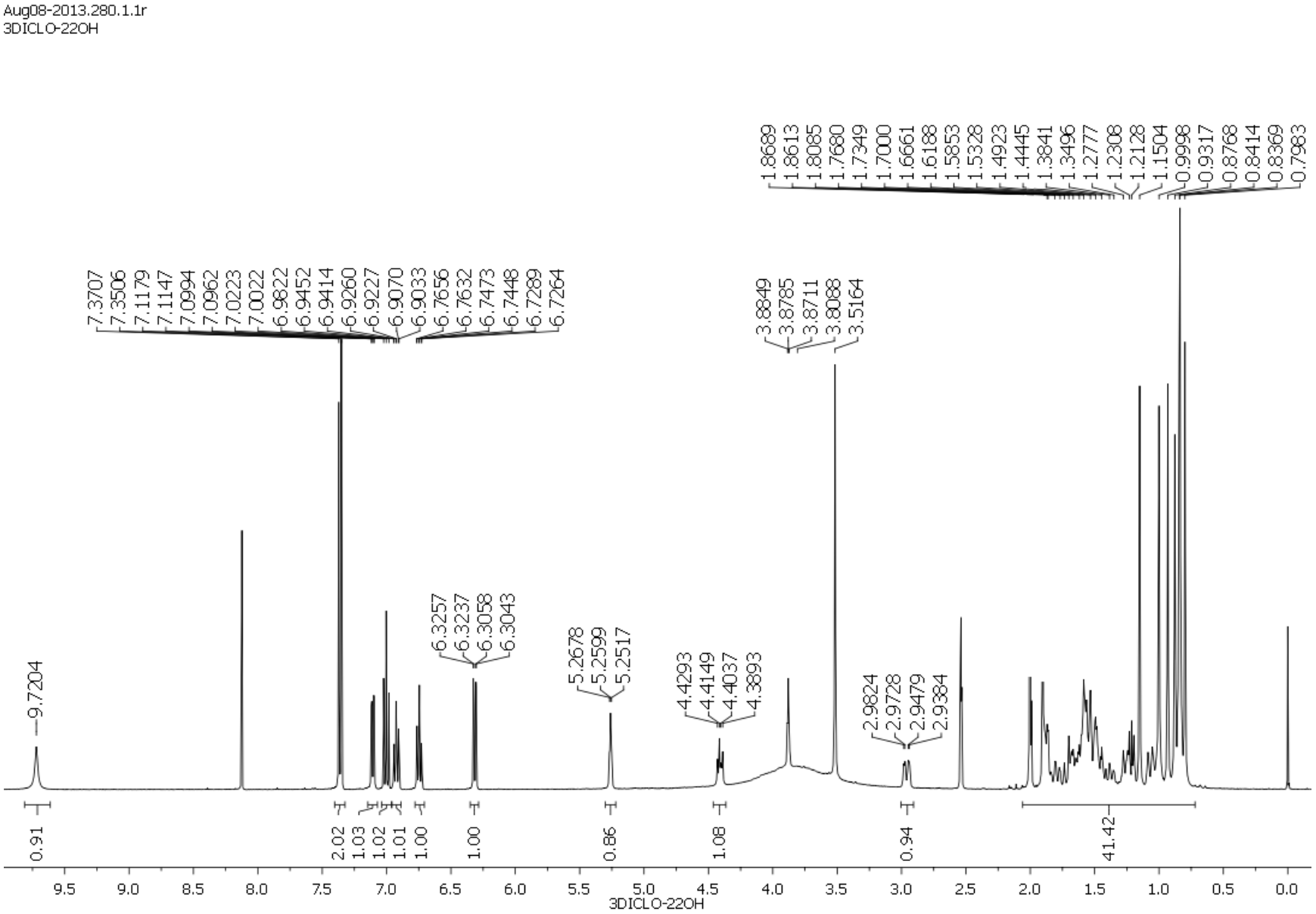


Fig. 46. ^{1}H NMR spectrum of compound **13** ($C_{44}H_{57}Cl_2NO_5$) in a mixture of $CDCl_3$ and DMSO-d_6

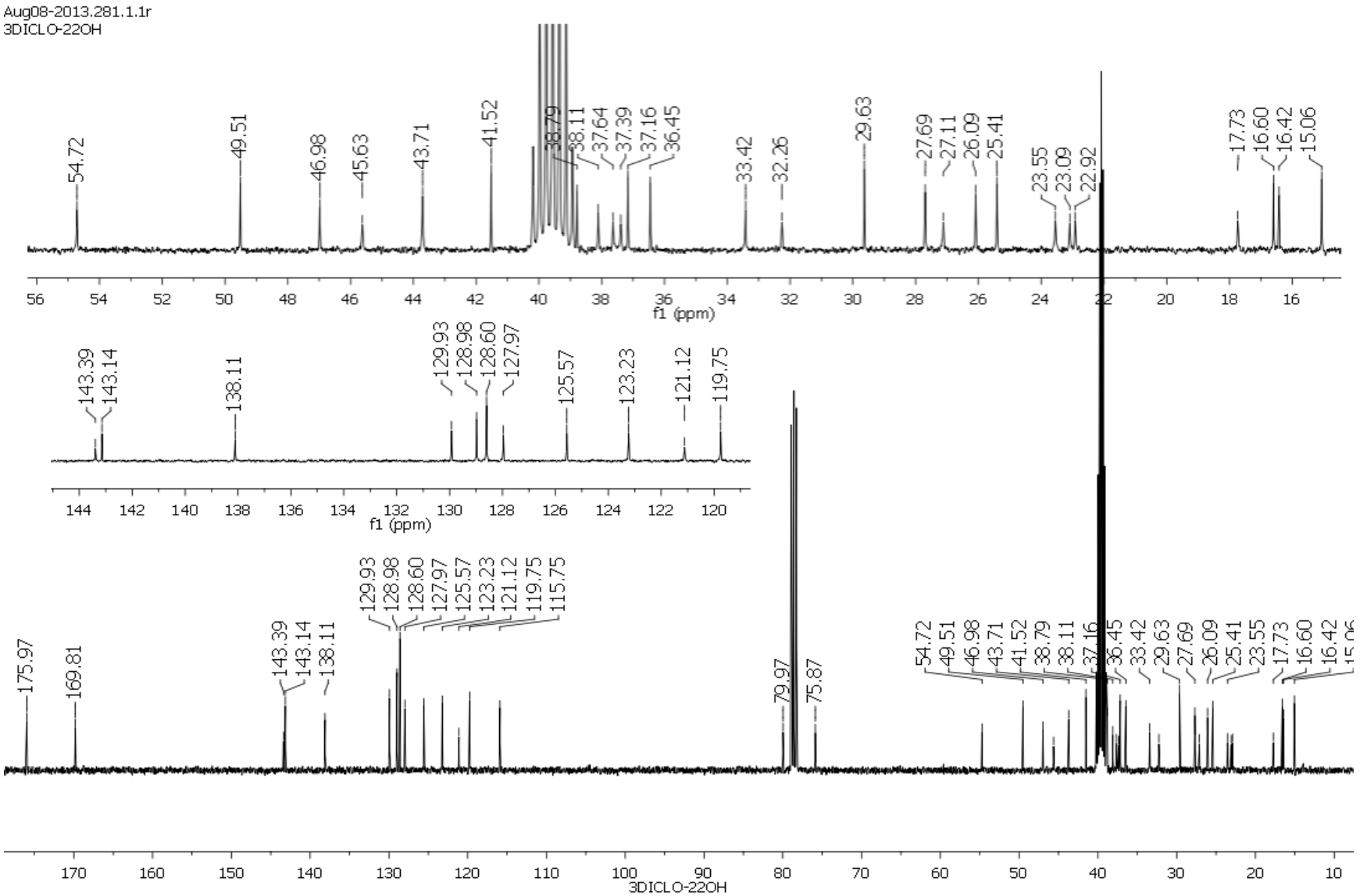


Fig. 47. ^{13}C NMR spectrum of compound **13** ($C_{44}H_{57}Cl_2NO_5$) in a mixture of $CDCl_3$ and DMSO-d_6. The C-11′ & C-15′ appeared at 128.98 ppm and C-12′ & C-14′ appeared at 128.60 ppm. Hence, 42 peaks of compound are visible in the spectrum.

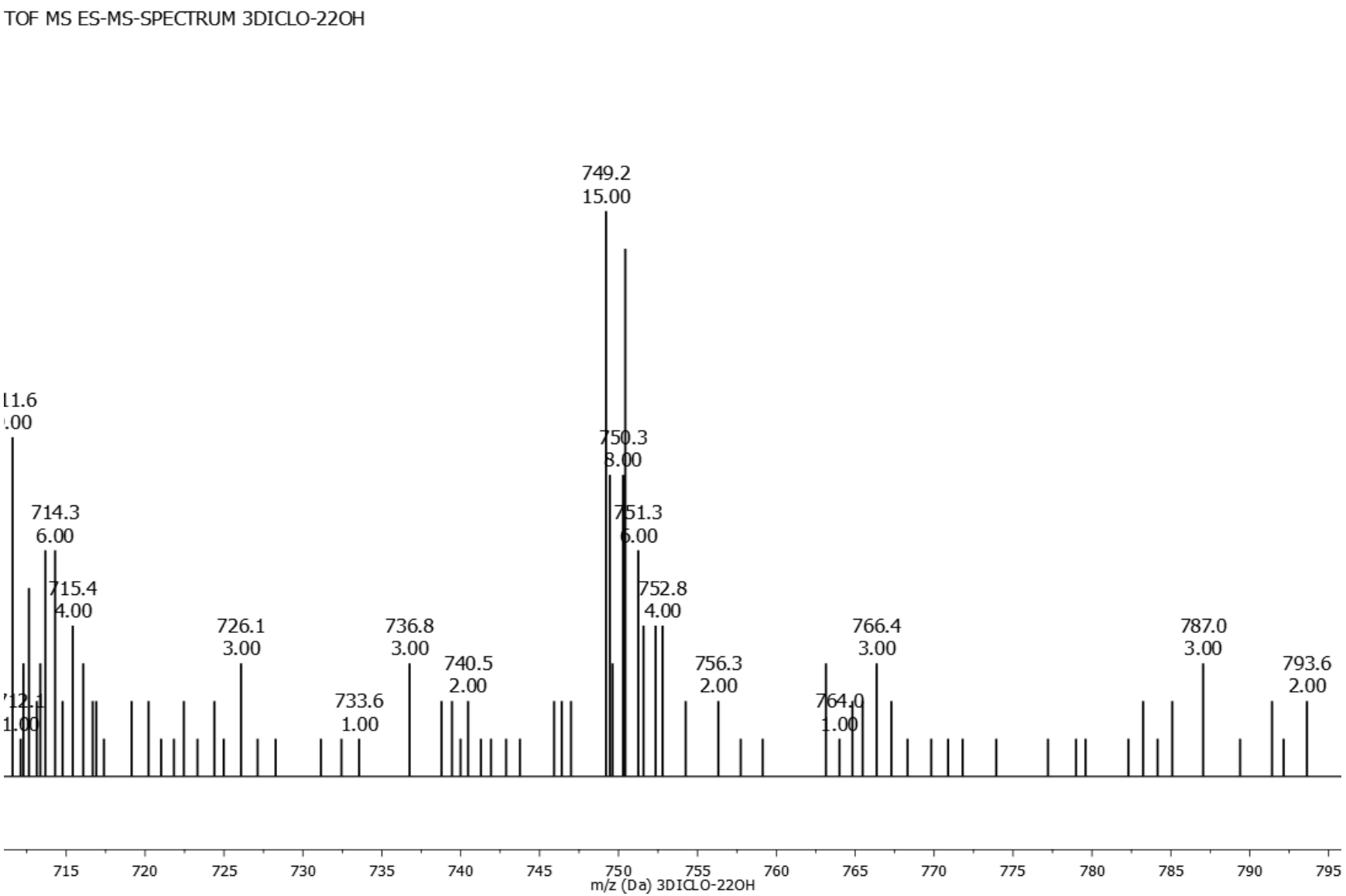
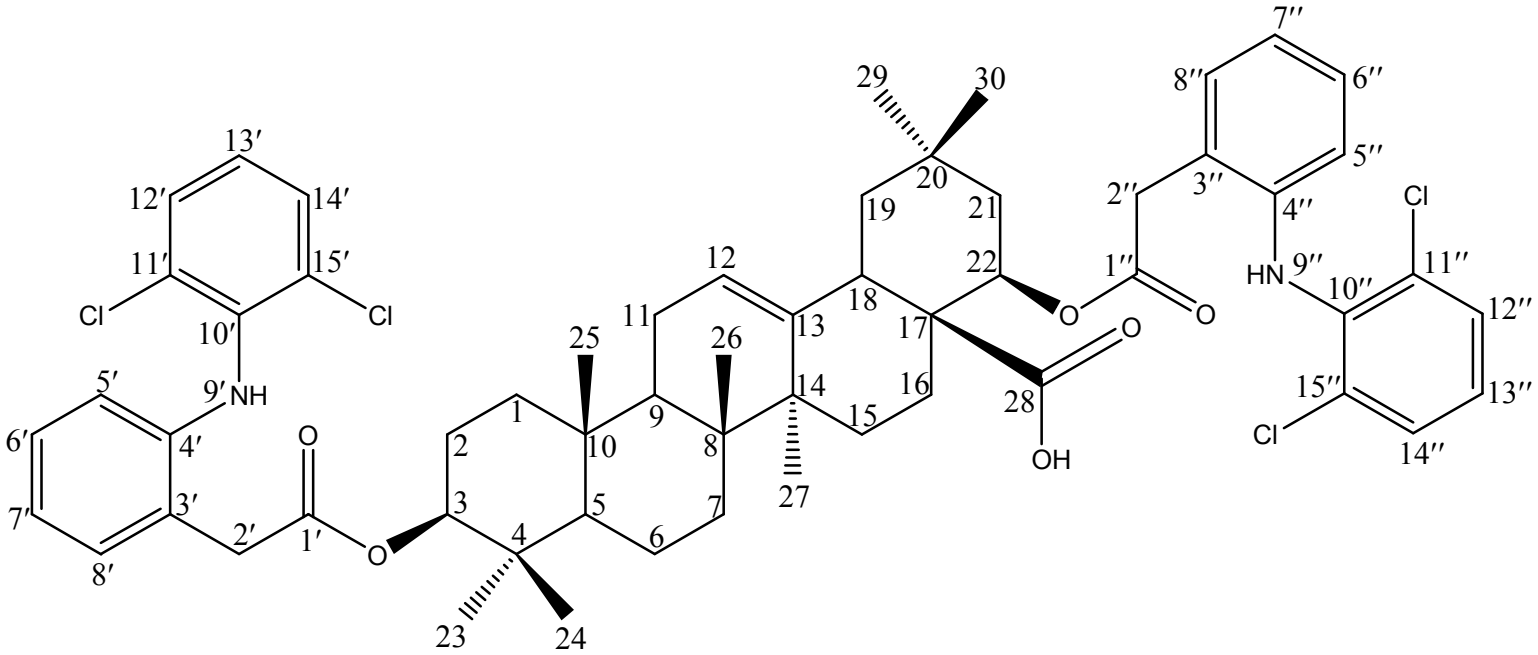


Fig. 48. ESI-MS negative-ion mode spectrum of compound **13** (Exact Mass- 749.36)

14. 3*β*,22*β*-Di(2-(2-(2,6-dichlorophenylamino)phenyl)acetoyloxy)-olean-12-en-28-oic acid (14)



3β,22β-Di(2-(2-(2,6-dichlorophenylamino)phenyl)acetoyloxy)-olean-12-en-28-oic acid

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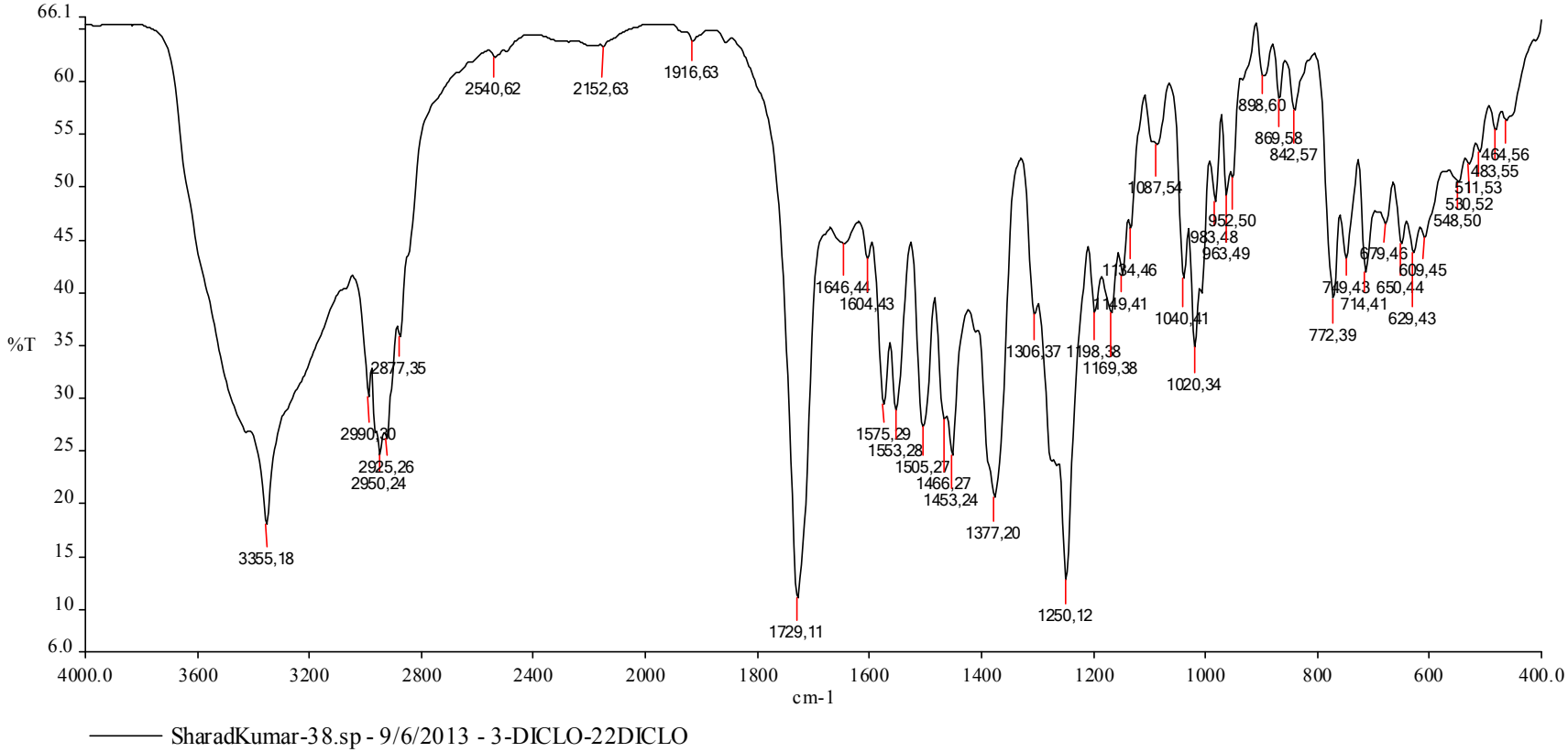

Fig. 49. FT-IR spectrum of compound **14**

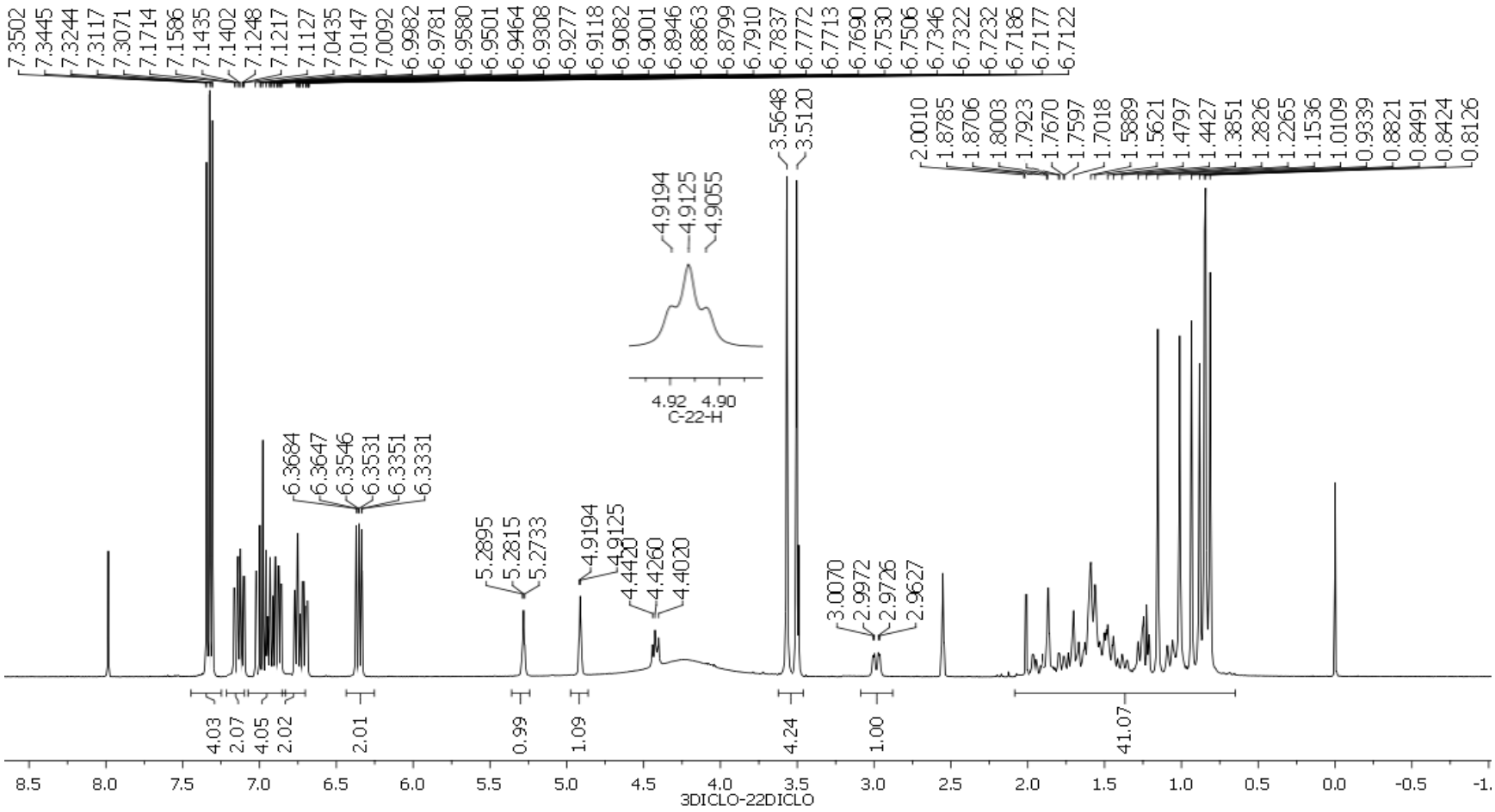

Fig. 50. ^{1}H NMR spectrum of compound **14** ($C_{58}H_{66}Cl_4N_2O_6$) in a mixture of $CDCl_3$ and DMSO-d_6

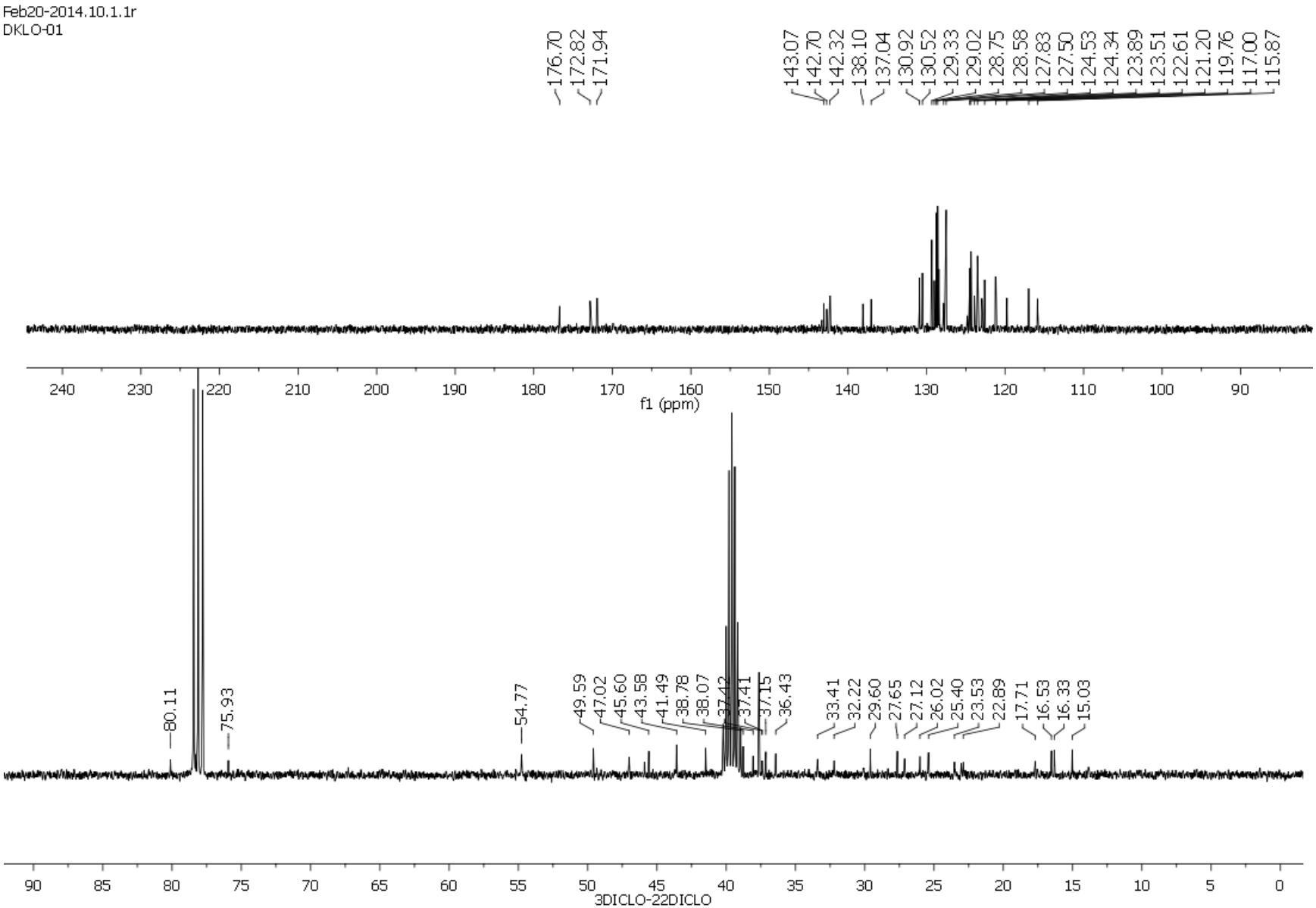

Fig. 51. ^{13}C NMR spectrum of compound **14** ($C_{58}H_{66}Cl_4N_2O_6$)) in a mixture of $CDCl_3$ and DMSO-d_6.

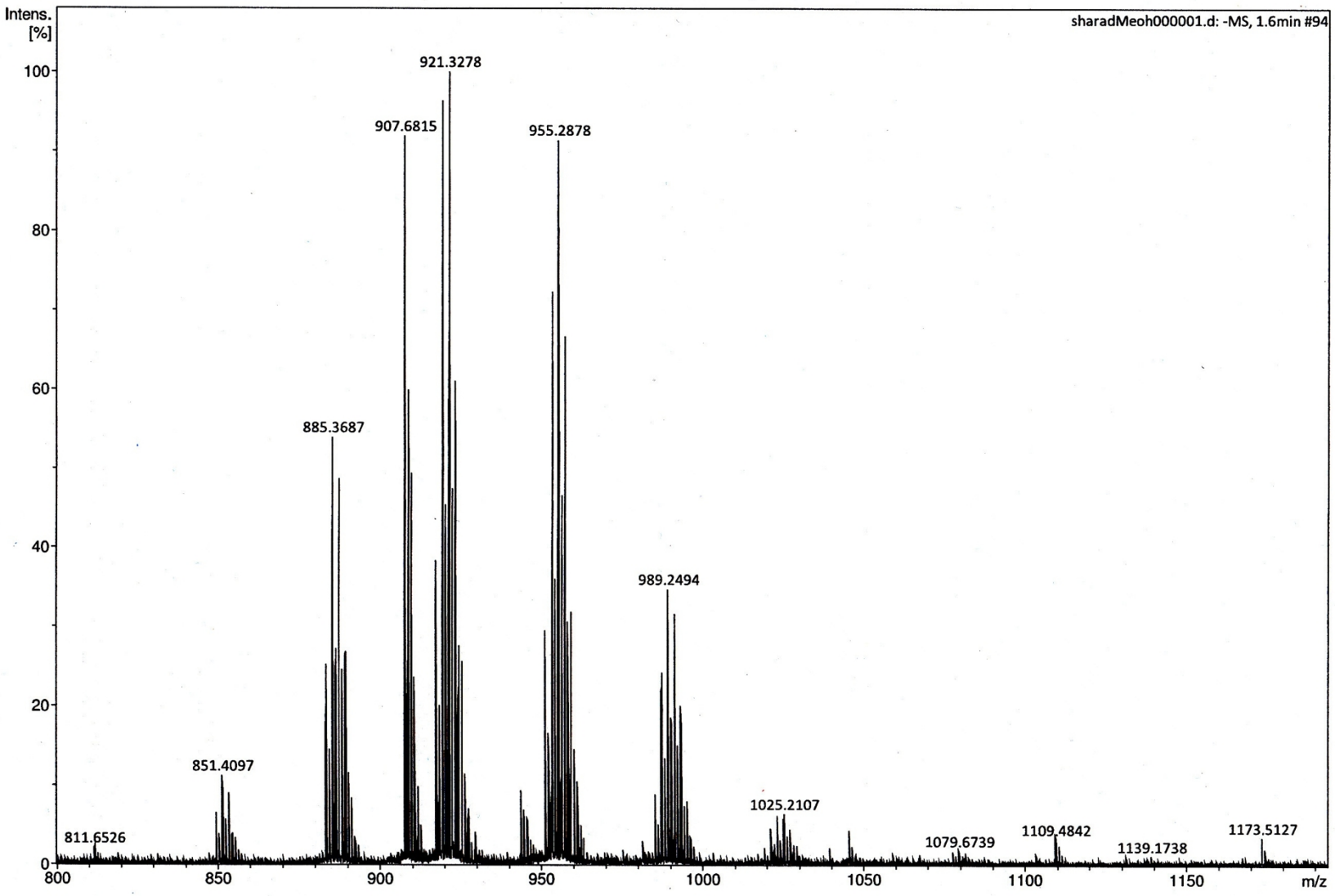

Fig. 52. ESI-MS negative-ion mode spectrum of compound **14** (Exact Mass- 1026.37)

C. REVERSE PHASE- HPLC PURITY CHROMATOGRAMS OF COMPOUNDS 1−14

Compounds were dissolved into methanol or tetrahydrofuran or methanol-THF or in the methanol-acetonitrile solutions. Chromatograms presented here were taken at different time periods, and hence, retention time (t_R) of compounds may vary slightly when comparing the t_R of one compound with another based on their structure or polarity. Chromatograms presented here do not represent the comparative concentrations of compounds.

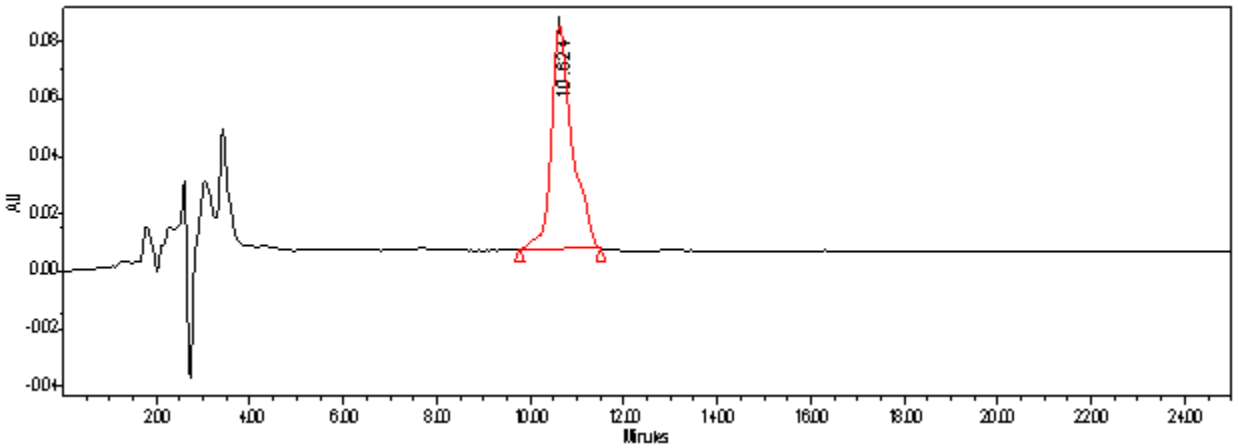

Fig. 53. HPLC chromatogram of isolated compound **1** (t_R 10.624 min)

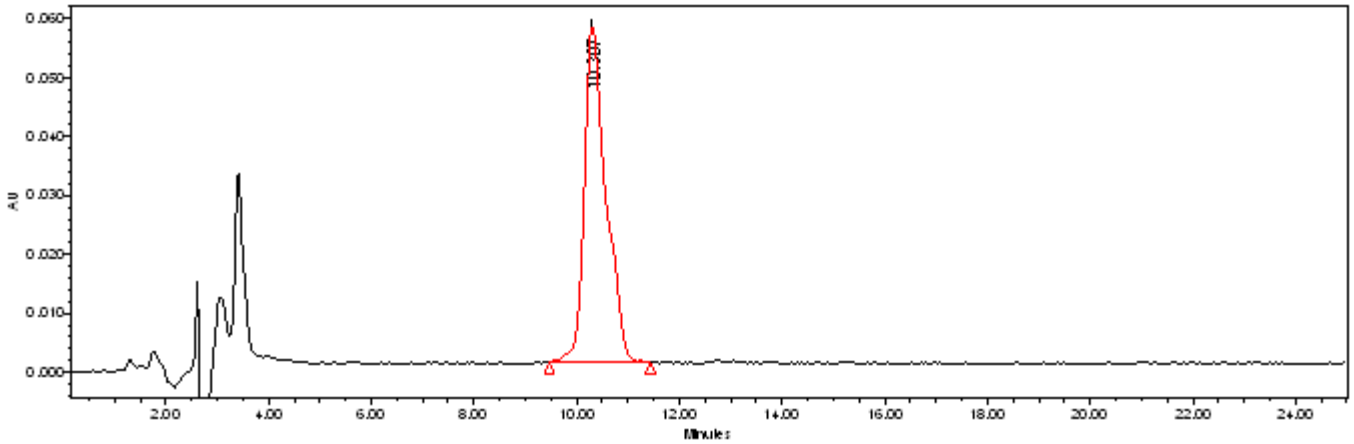

Fig. 54. HPLC chromatogram of isolated compound **2** (t_R 10.307 min)

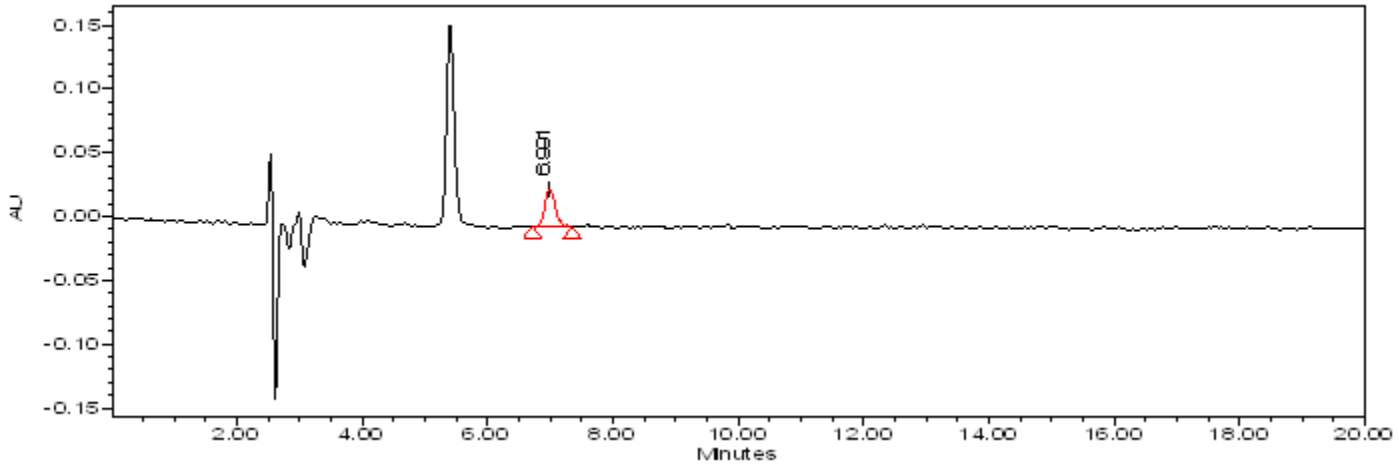

Fig. 55. HPLC chromatogram of compound **3** (t_R 6.991 min)

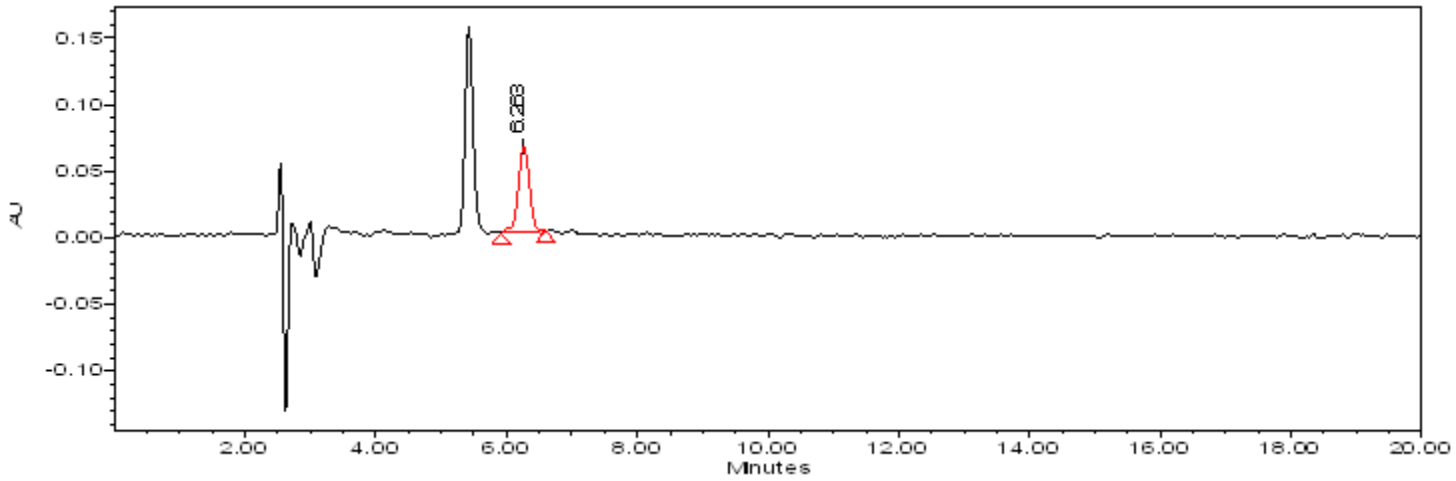

Fig. 56. HPLC chromatogram of compound **4** (t_R 6.263 min)

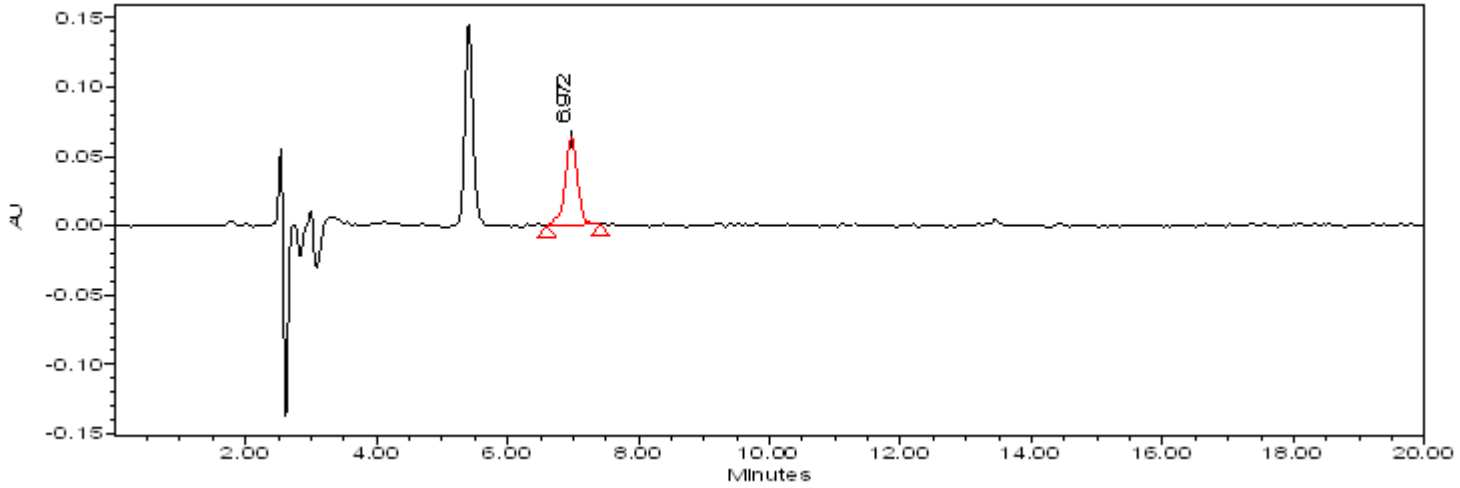

Fig. 57. HPLC chromatogram of compound **5** (t_R 6.972 min)

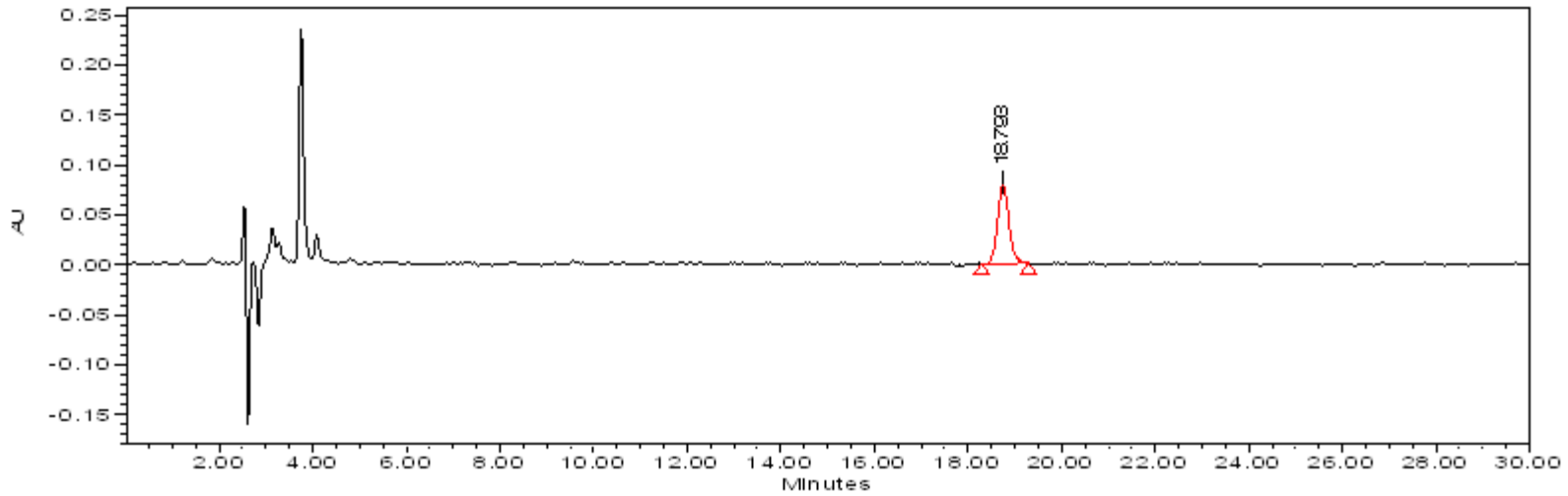

Fig. 58. HPLC chromatogram of compound **6** (t_R 18.793 min)

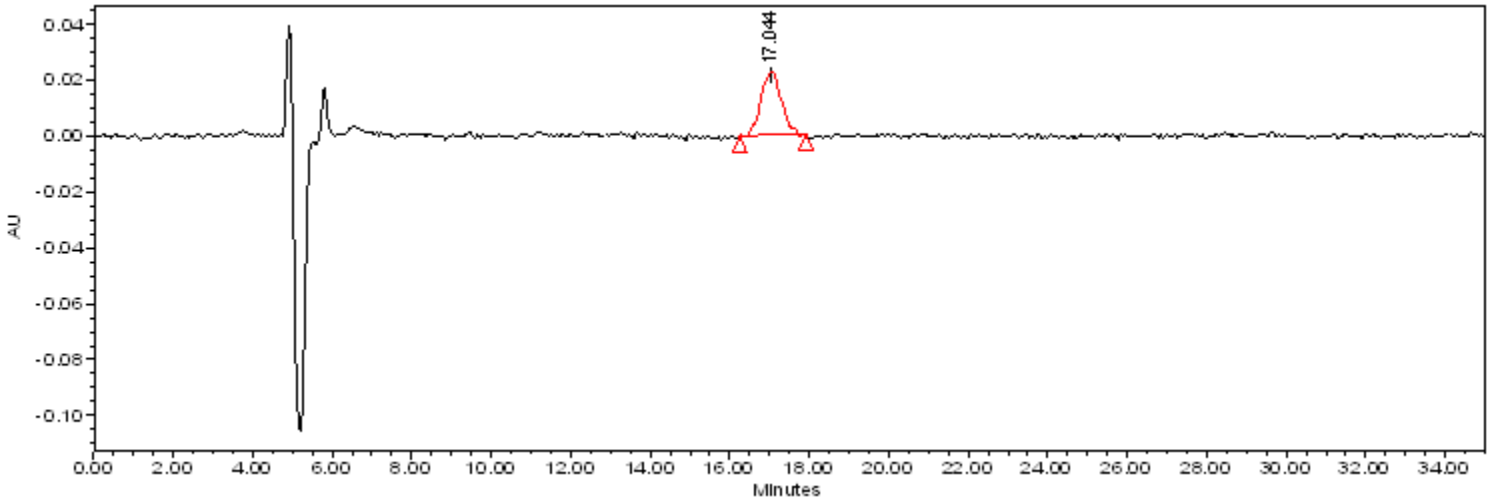

Fig. 59. HPLC chromatogram of compound **7** (t_R 17.044 min)

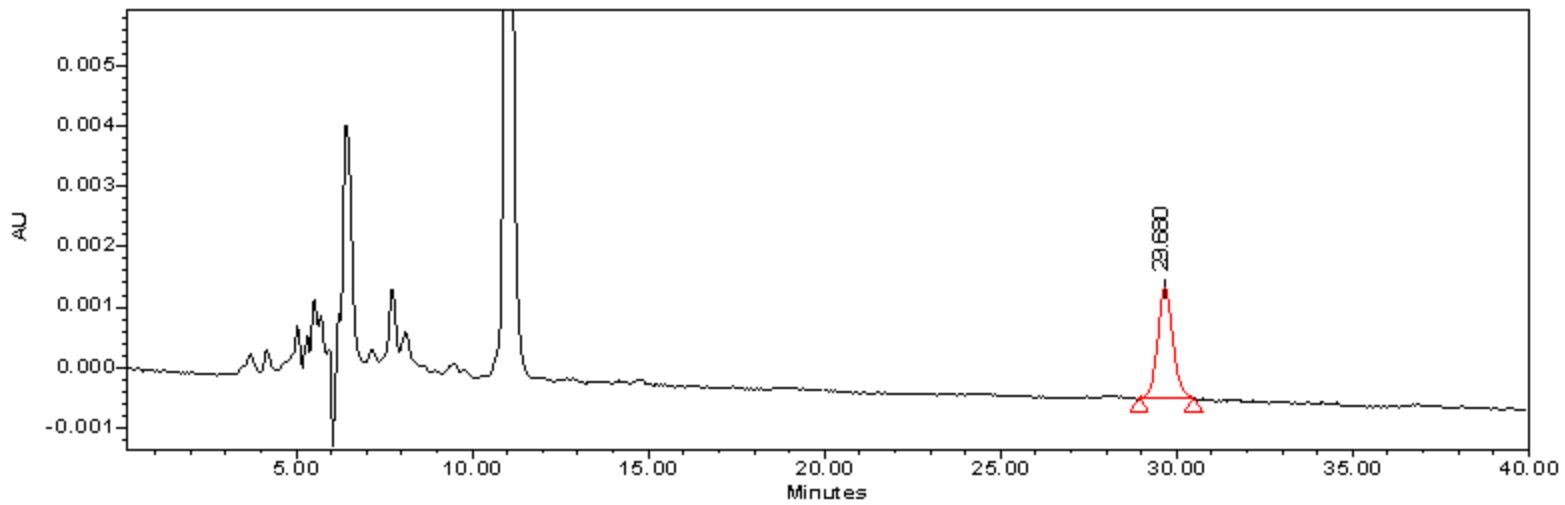

Fig. 60. HPLC chromatogram of compound **8** (t_R 29.680 min)

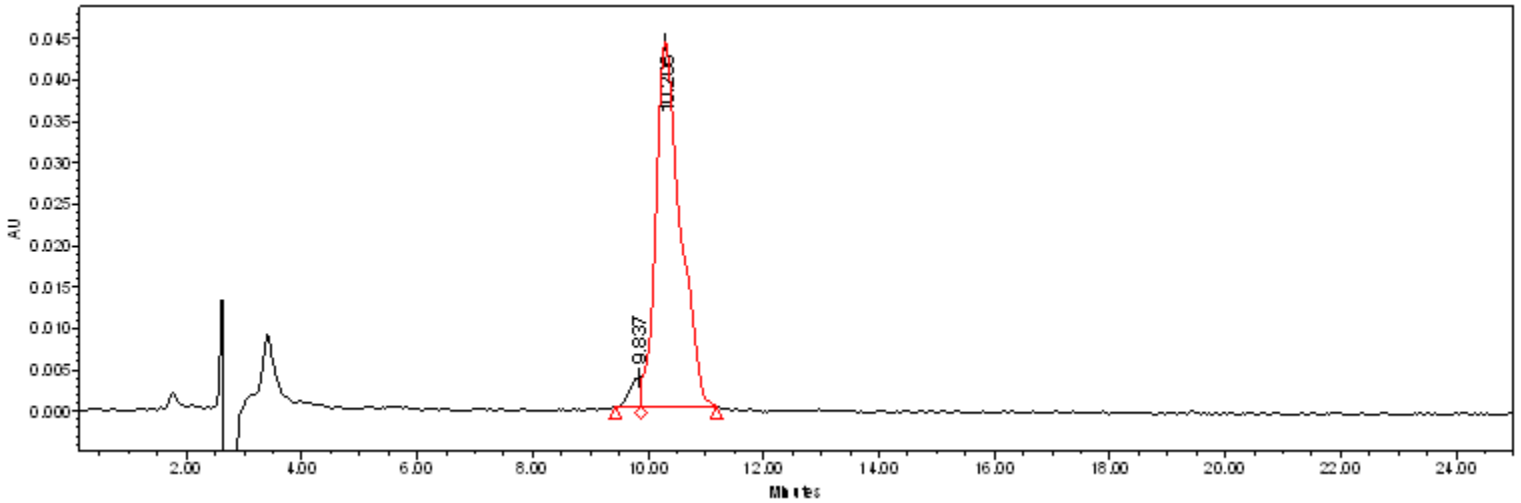

Fig. 61. HPLC chromatogram of compound **9** (t_R 10.296 min)

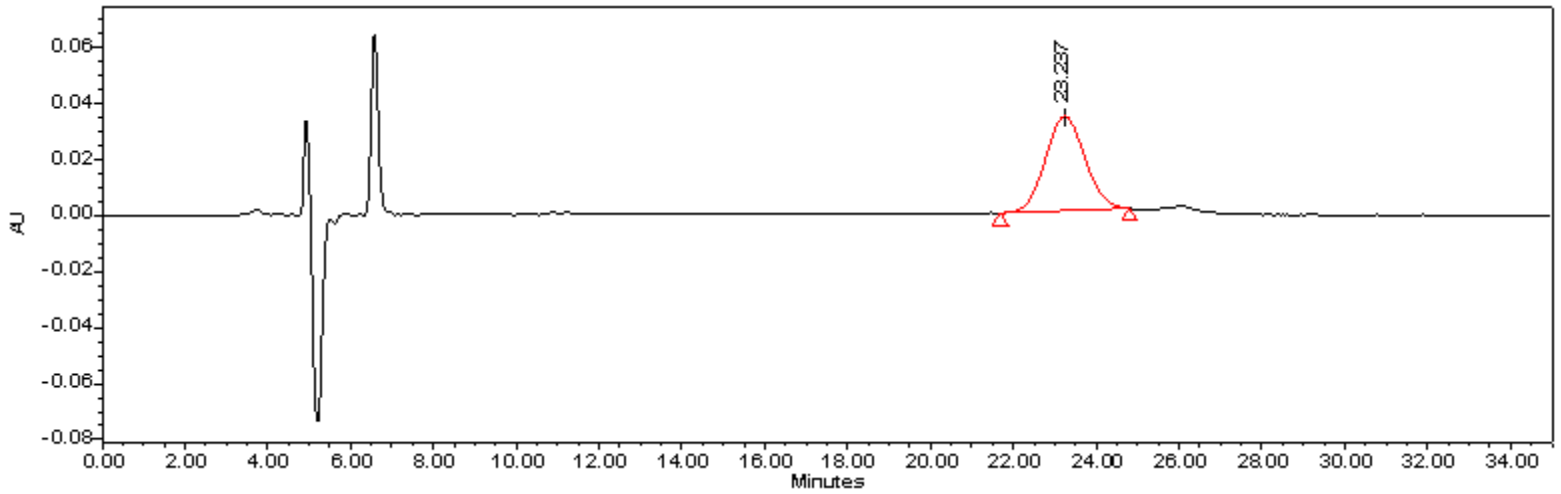

Fig. 62. HPLC chromatogram of compound **10** (t_R 23.237 min)

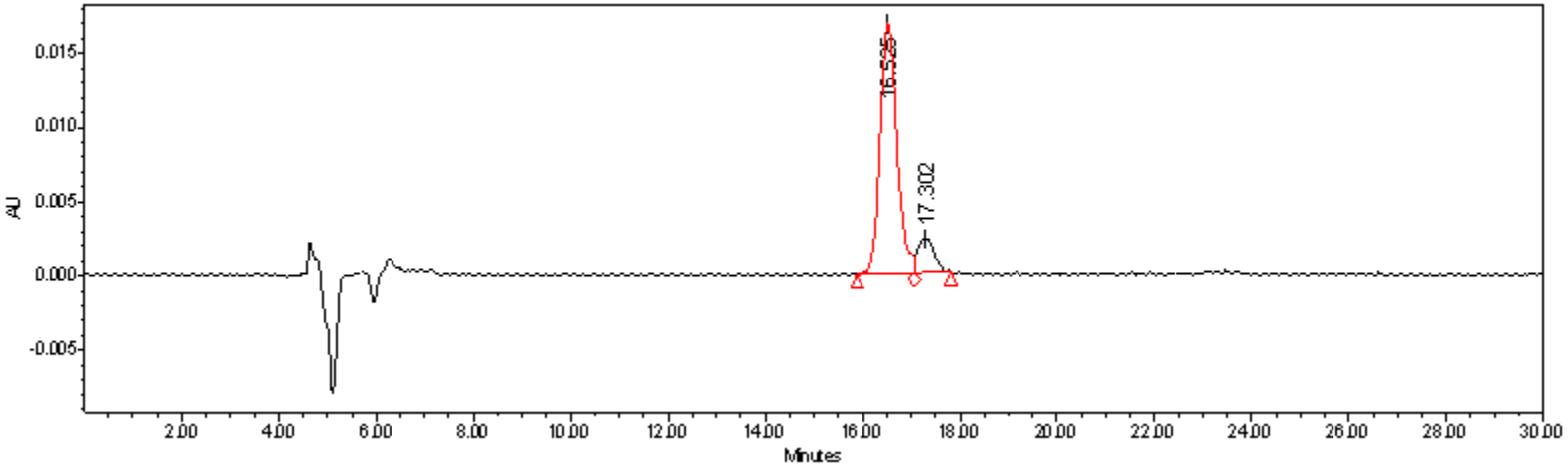

Fig. 63. HPLC chromatogram of compound **11** (t_R 16.525 min)

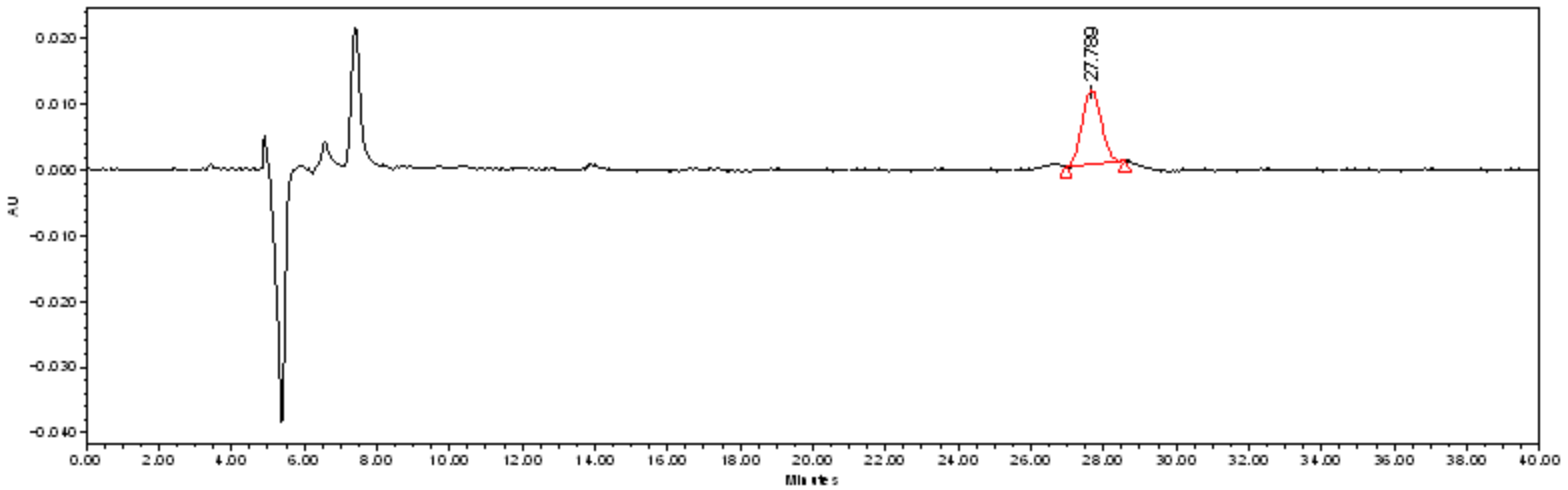

Fig. 64. HPLC chromatogram of compound **12** (t_R 27.789 min)

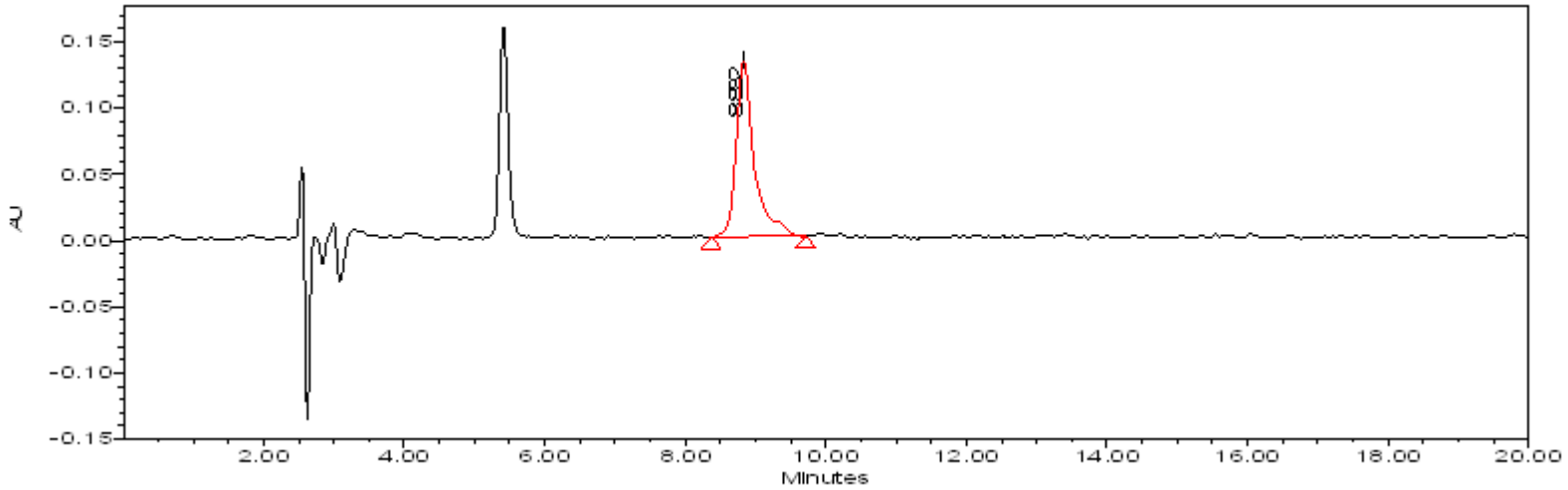

Fig. 65. HPLC chromatogram of compound **13** (t_R 8.832 min)

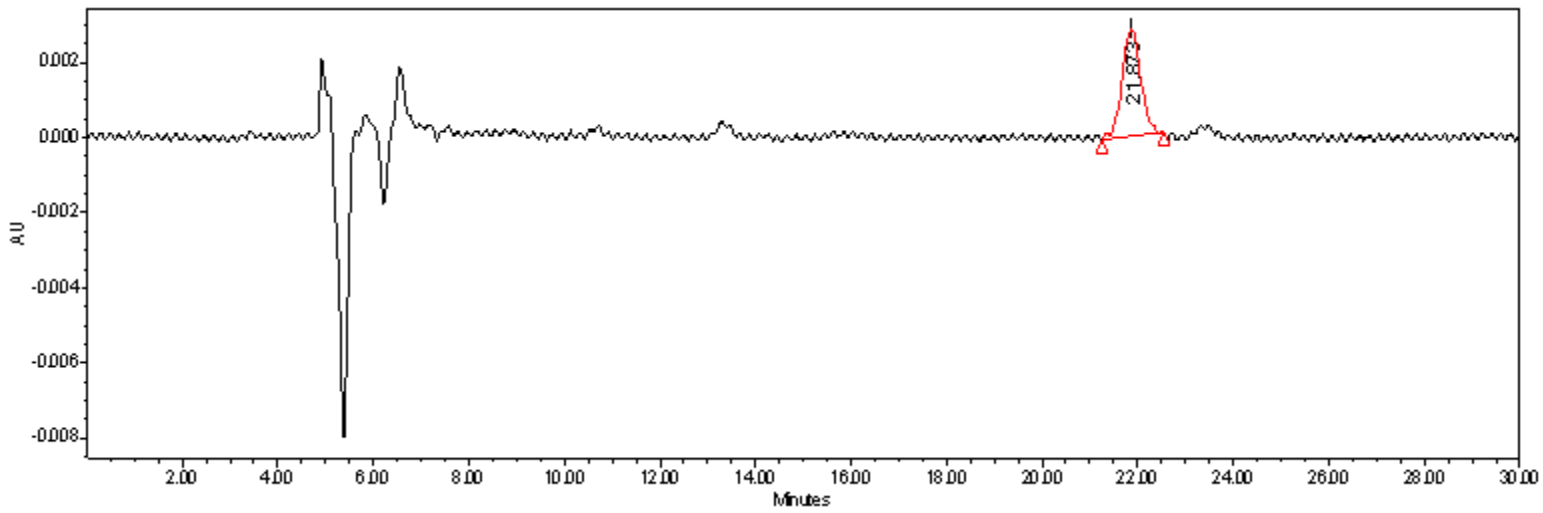

Fig. 66. HPLC chromatogram of compound **14 (t_R** 21.873 min**)**