Supplementary Information

Synthesis and Structure-Chirality Relationship Analysis of St*e*roidal Quinoxalines to Design and Develop New Chiral Drugs

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Experimental

Materials and instruments

Cholesterol (purity ≥99%), potassium sodium tartrate (purity; 99%), anhydrous magnesium sulphate, 1,2-diaminobenzene (purity 99.5%), aluminium isopropoxide (purity 98.0%), sodium carbonate (≥99.0%), 4-methyl-1, 2-diaminobenzene (purity ≥98.0%), 4-chloro-1, 2-diaminobenzene (purity 97%), sodium hydride (60% in mineral oil), petroleum ether, ethyl acetate methanol, chloroform, cyclohexanone, and toluene were purchased from Sigma-Aldrich. Characterisation of the synthesised compound was done by using varying analytical instruments including melting point apparatus (MP; Gallenkamp, Sanyo MPD350, UK), nuclear magnetic resonance spectrometer (NMR; Bruker Avance 300-400 MHz), Fourier transform infra-red spectrophotometer (FTIR; Perkin-Elmer, USA), mass spectrometer (MS; Varian MAT 312, USA and Bruker micrOTOF-Q™, USA), and circular dichroism spectropolarimeter (CD; JASCO J-810, Japan)

Synthesis, purification, and characterisation

Cholest-3-en-4-one (I)

Anhydrous toluene (500 mL) was introduced in 1 L double-neck round bottomed flask, which was sealed with reflux assembly setup under inert atmosphere. 40 g of cholesterol was added to flask followed by the addition of 200 ml of cyclohexanone. After this, in a separate beaker, a solution of aluminium isopropoxide (12 g) in anhydrous toluene (80 mL) was prepared and added dropwise to the flask by using dropping-funnel which was sealed with reflux assembly. Initially while magnetic stirring, the reaction mixture became cloudy and yellow colour, which was heated at 80^{o}C for 8 h. The progress of the reaction was monitored at different intervals. Reaction assembly was dismantled, and the mixture was cooled at room temperature for 1 h. After cooling, about 160 mL of saturated solution of potassium sodium tartrate was added to

the reaction mixture and the organic layer became clear and orange, then the mixture was steam distilled until 240 ml of toluene was distilled. The residue mixture was extracted with chloroform three times. Extract was washed with water, dried with anhydrous magnesium sulphate, and the solvent was removed by using rotary evaporator. The yellow oily residue was dissolved in 60 mL of anhydrous methanol and placed in an ice-salt bath for 24 h. The final white solid product was filtered, dried in a vacuum desiccator and stored in closed vessel for further use.

Yield (76%), MP (80^{o}C), Rf value (0.8; petroleum ether: ethyl acetate, 8:1), FTIR (1680 cm^{-1}; keto group), 2930 cm^{-1} (aliphatic methylene groups), MS (384 m/z; M^{+}), ^{1}H-NMR [$CDCl_3$; 5.737 ppm (s, 1H), 2.43~2.29 ppm (m, 2H), 2.01~1.91 ppm (m, 2H), 1.82 ppm (m, 1H), 1.59~1.31 ppm (m, 23H), 1.26 ppm (m, 6H), 1.11 ppm (m, 3H)], ^{13}C-NMR [$CDCl_3$; 199.8 ppm (carbonyl carbon), 171.9 ppm (α-carbon), 123.7 ppm (β-carbon)]

5α-cholest-3-eno-[3,4-b]-quinoxaline (II)

Anhydrous toluene (50 mL) was introduced in 250 mL double-neck round bottomed flask, which was sealed with reflux assembly setup under inert atmosphere. Equimolar (1.2 mmole) amount of cholest-3-en-4-one **(I)** and 1, 2-diaminobenzene was added to flask. The mixture was magnetically stirred and refluxed for 1 h. After this, 20 ml of toluene was distilled out and reaction was allowed to stir at 80^{o}C for 24 h. The progress of the reaction was monitored at different intervals. Reaction assembly was dismantled, and the mixture was cooled at room temperature for 1 h. After cooling, toluene was removed by using rotary evaporator. The dark brown residue was dissolved in 10 mL of anhydrous methanol and purified by column chromatography using petroleum ether: ethyl acetate (5:1) solvent system. The final product was dried in a vacuum desiccator and stored in closed vessel for further use.

Yield (57%), MP (165^{o}C), Rf value (0.45; petroleum ether: ethyl acetate, 5:1), FTIR (1538 cm^{-1} quinoxaline moiety C=C), MS (472 m/z, M^{+}), ^{1}H-NMR [$CDCl_3$; 7.68~ 7.92 ppm (m, 4H, C-6', C-7', C-8', C-9'), 2.33 ppm (m, 3H, C-5(α –H) and C-2) 2.00 ~0.70 ppm (m, cholestane nucleus peaks)], ^{13}C-NMR [$CDCl_3$; 130.86 ppm (C-3 and C-4), 128.8 ppm (C-5', C-10'), 126.61 ppm (C-6', C-7', C-8', C-9')]

5α-cholest-3-eno-[3, 4-b]-7′(8′)-methylquinoxaline (III)

Anhydrous toluene (50 mL) was introduced in 250 mL double-neck round bottomed flask, which was sealed with reflux assembly setup under inert atmosphere. After this, 1.2 mmole of 4-methyl-1, 2-diamino benzene and 1.2 mmole cholest-3-en-4-one **(I)** was added to flask and reaction was allowed to stir at 80^{o}C for 24 h. The progress of the reaction was monitored at different intervals. Reaction assembly was dismantled, and the mixture was cooled at room temperature for 1 h. After cooling, toluene was removed by using rotary evaporator. The dark brown residue was dissolved in 10 mL of anhydrous methanol and purified by column chromatography using petroleum ether: ethyl acetate (5:3) solvent system and reparative thin layer chromatography. The final product was dried in a vacuum desiccator and stored in closed vessel for further use.

Yield (35%), MP (195^{o}C), Rf value (0.39; petroleum ether: ethyl acetate, 5:3), FTIR (1535 cm^{-1} quinoxaline moiety C=C), MS (486 m/z, M^{+}), ^{1}H-NMR [$CDCl_3$; 7.53-7.51 ppm (d, 2H, C-8' and C-9' when CH_3 at C-7'), 7.06-7.04 ppm (d, 2H, C-6' and C-7' when CH_3 at C-8'), (d, 2H, C-7', C-8', C-9'), 6.554 ppm (s, 1H, C-6'or C-9') 2.48 ~2.46 ppm (s, 3H, C-11' when at C-7' or C-8'), 2.40 ppm (m, 3H, C-5(α –H) and C-2)], ^{13}C-NMR [$CDCl_3$; 135.4 and 135.6 ppm (C-3 and C-4), 131.7 and 134.9 ppm (C-5', C-10'), 109.9-108.5 ppm (C-6', C-9'), 123.5-123.6 ppm (C-7', C-8'), 22.54, and 22.81 ppm (C-11' when at C-7' or C-8')]

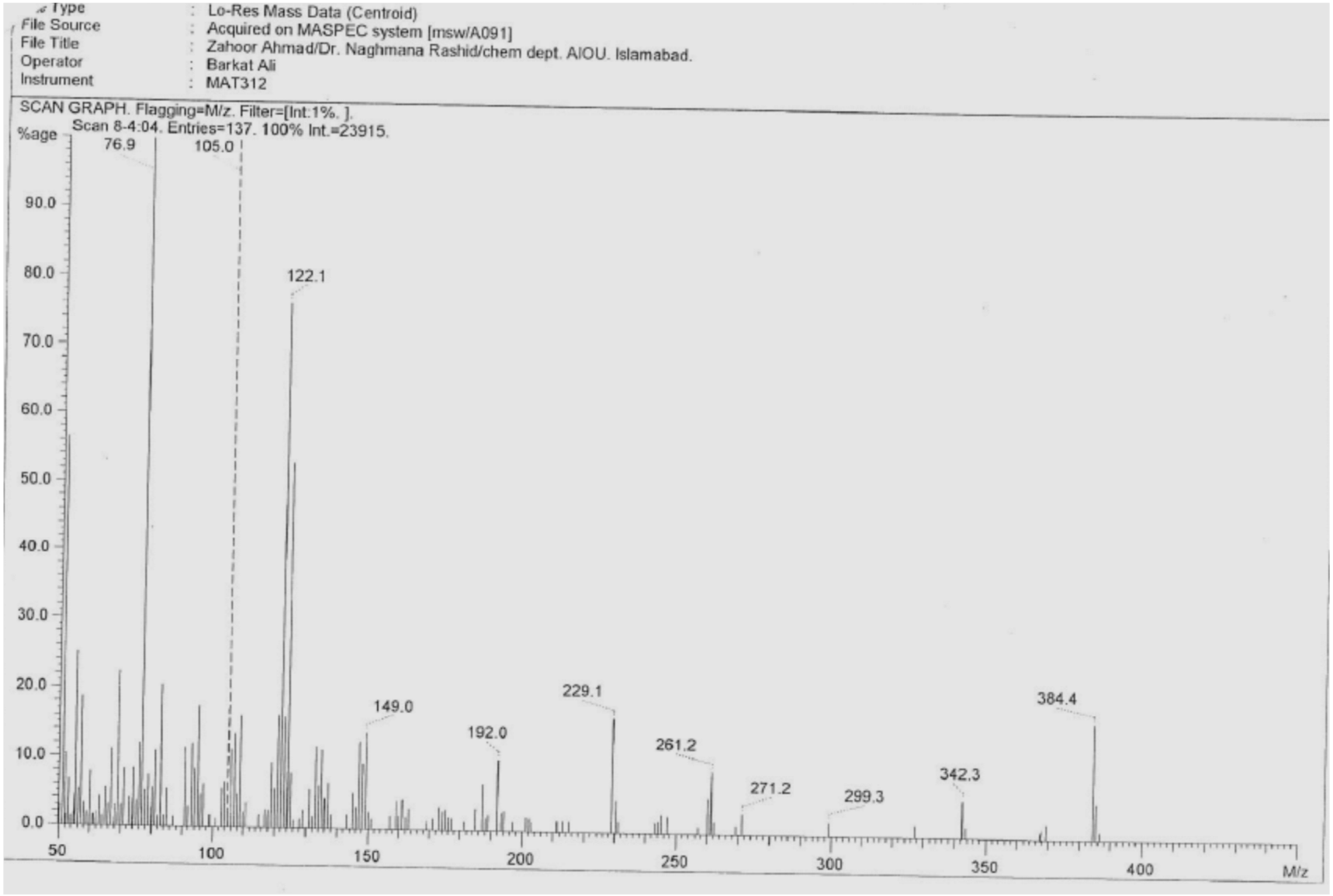
5α-cholest-3-eno-[3, 4-b]-7´(8´)-chloroquinoxaline (IV)

The synthesis procedure for **(IV)** is identical to the preceding **(II)** and **(III)** except the use of different starting material 4-chloro-1, 2-diamino benzene and use of sodium hydride to deprotonate amine of the starting materials prior to condensation with **(I)**. Yield (23%), Rf value (0.5; petroleum ether: ethyl acetate, 5:3), FTIR [1540 cm^{-1} (quinoxaline moiety C=C), ^{1}H-NMR [$CDCl_3$; 7.68-7.66 ppm (d, 2H, C-8' and C-9' when Cl at C-7'), 7.42-7.40 ppm (d, 2H, C-6' and C-7' when Cl at C-8'), 5.9 ppm (s, 1H, C-6' or C-9'), 4.02 ppm (m, 3H, C-5(α –H) and C-2)], ^{13}C-NMR [$CDCl_3$; 123.63 and 123.46 ppm (C-3 and C-4), 119.1 and 118.6 ppm (C-7', C-10'), 109.9-108.5 ppm (C-6', C-9'), 112.4-112.3 ppm (C-7', C-8'), 2.54, and 22.81 ppm (C-11' when at C-7' or C-8')]

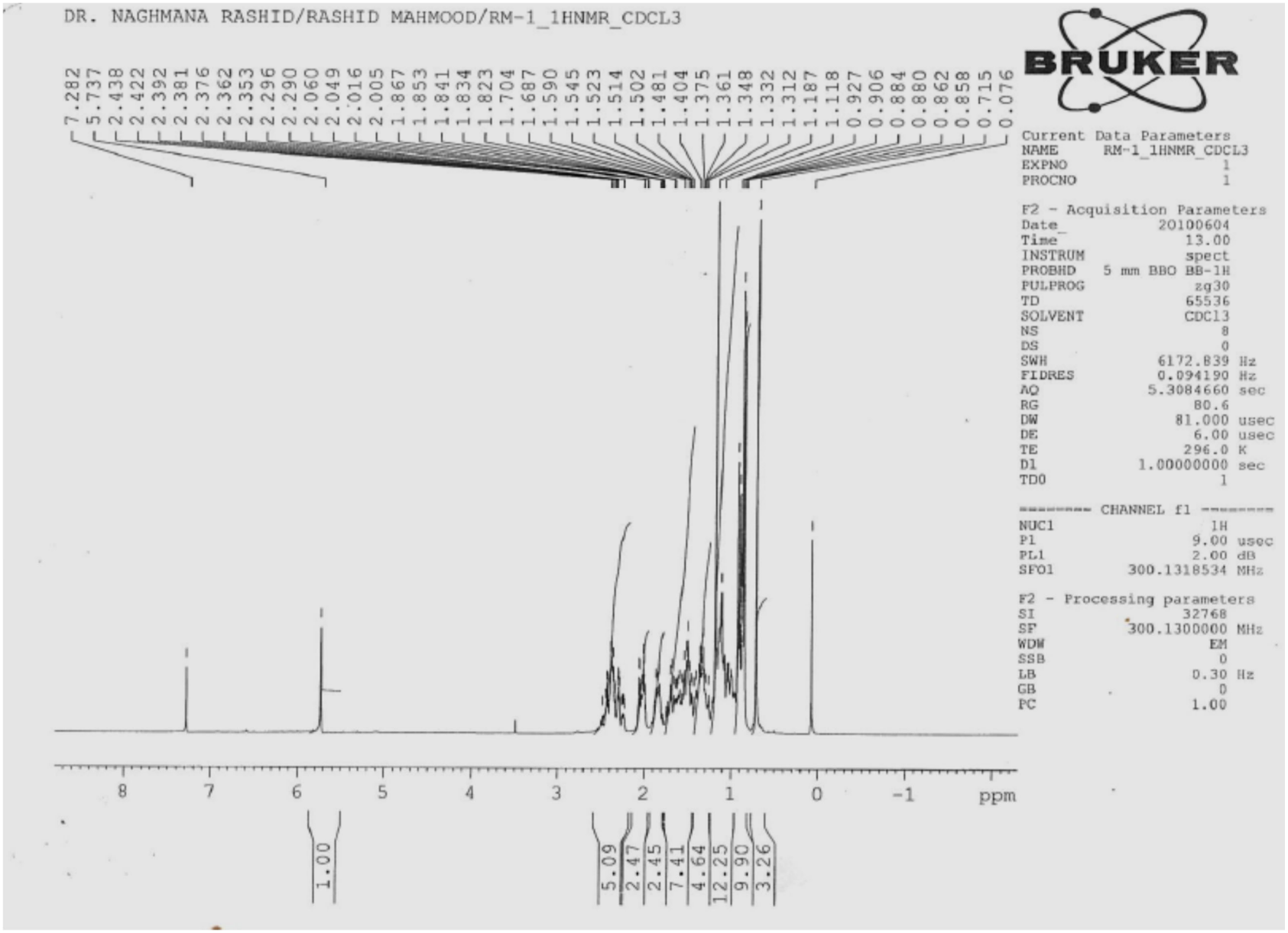
CD analysis

Samples **(II)**, **(III)**, and **(IV)** were prepared in acetonitrile at a concentration of 2.05 x 10^{-4} mol/L, 1.91 x 10^{-4} mol/L, and 3.28 x 10^{-4} mol/L, respectively. 1-mm and 0.5-mm rectangular cells were used to measure near UV-CD analysis at wavelength range of 200-400 nm.

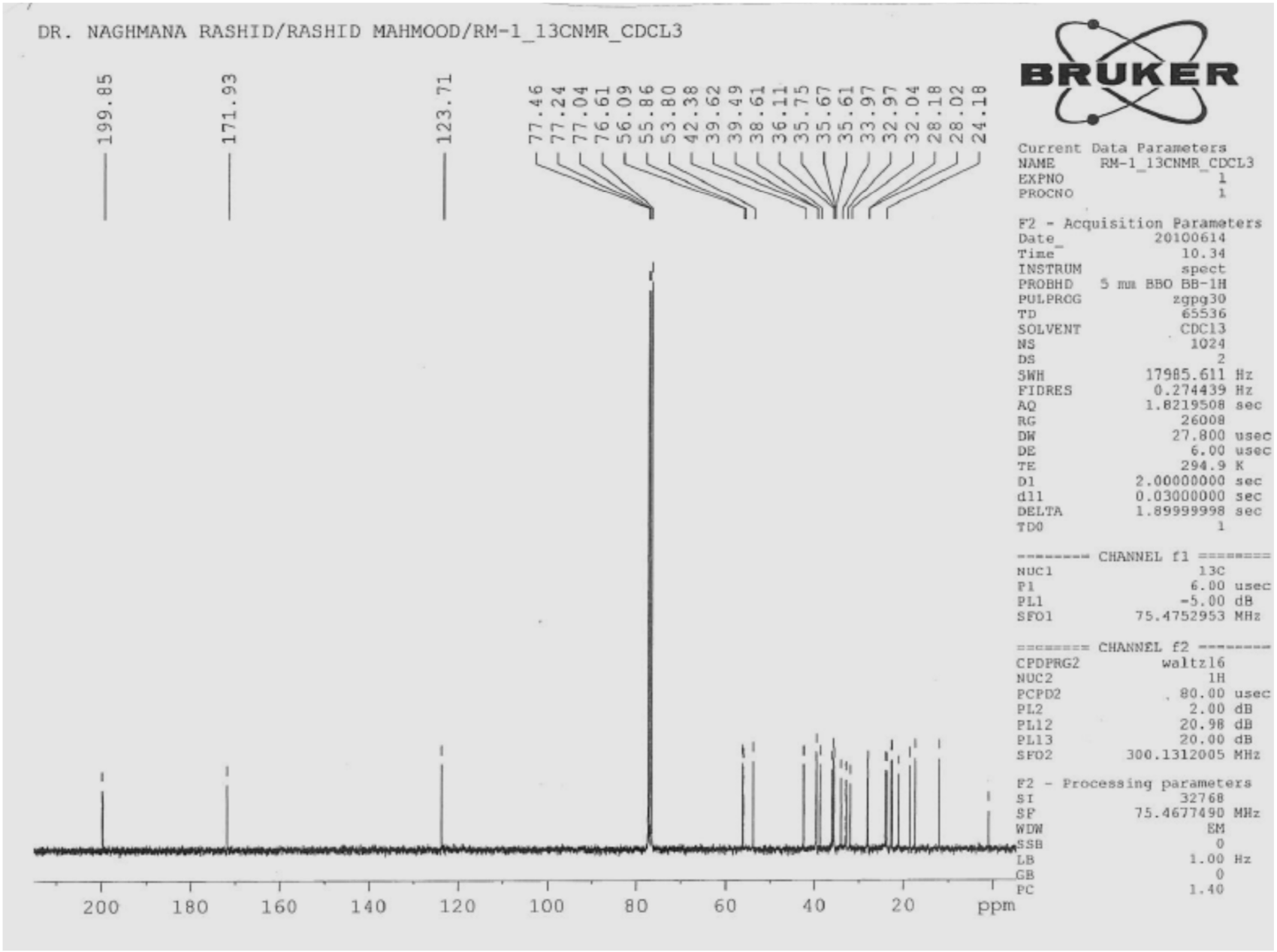
Supplementary Figure 1: Mass spectrum of cholest-3-en-4-one (I)



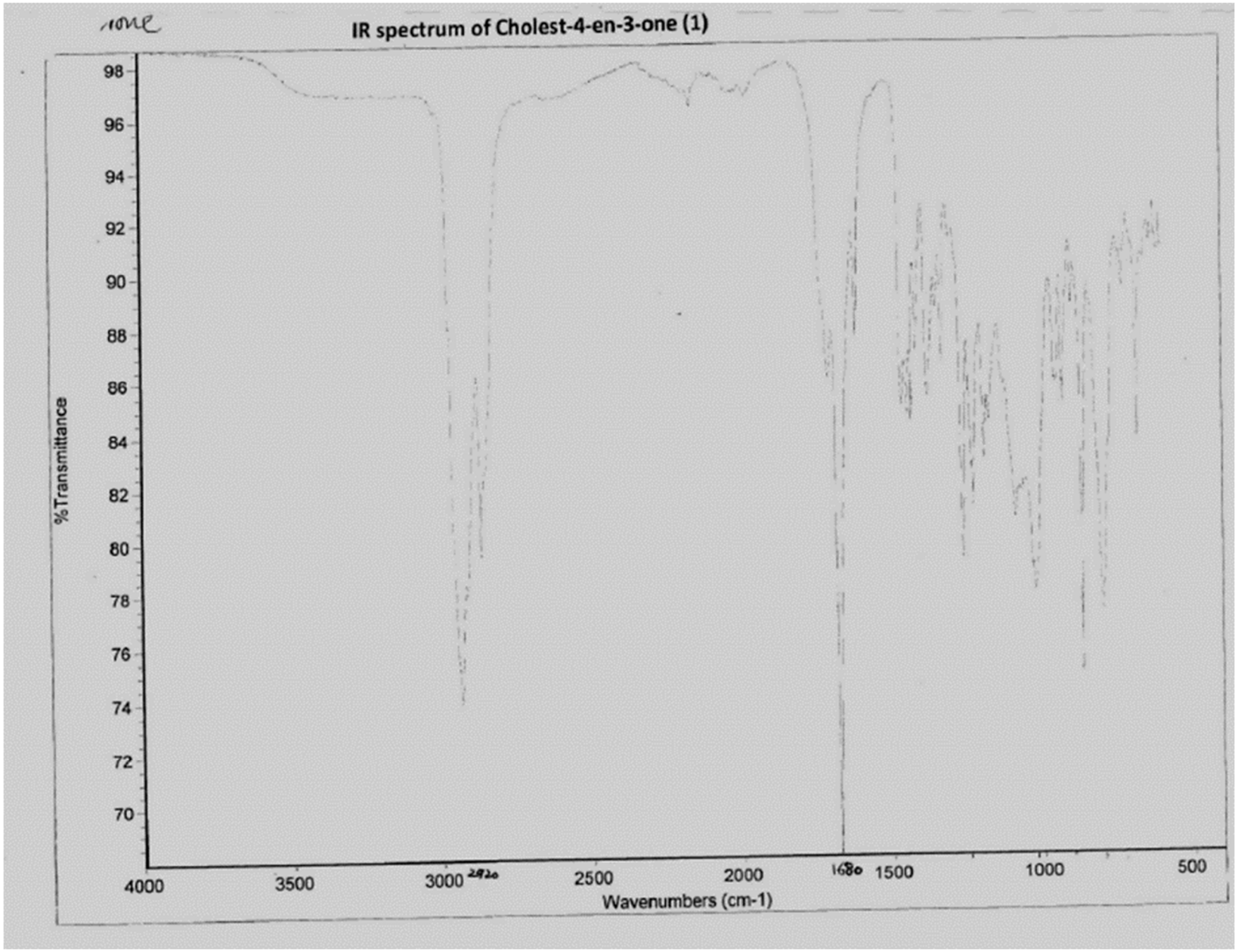
Supplementary Figure 2: ^{1}H-NMR spectrum of cholest-3-en-4-one (I)



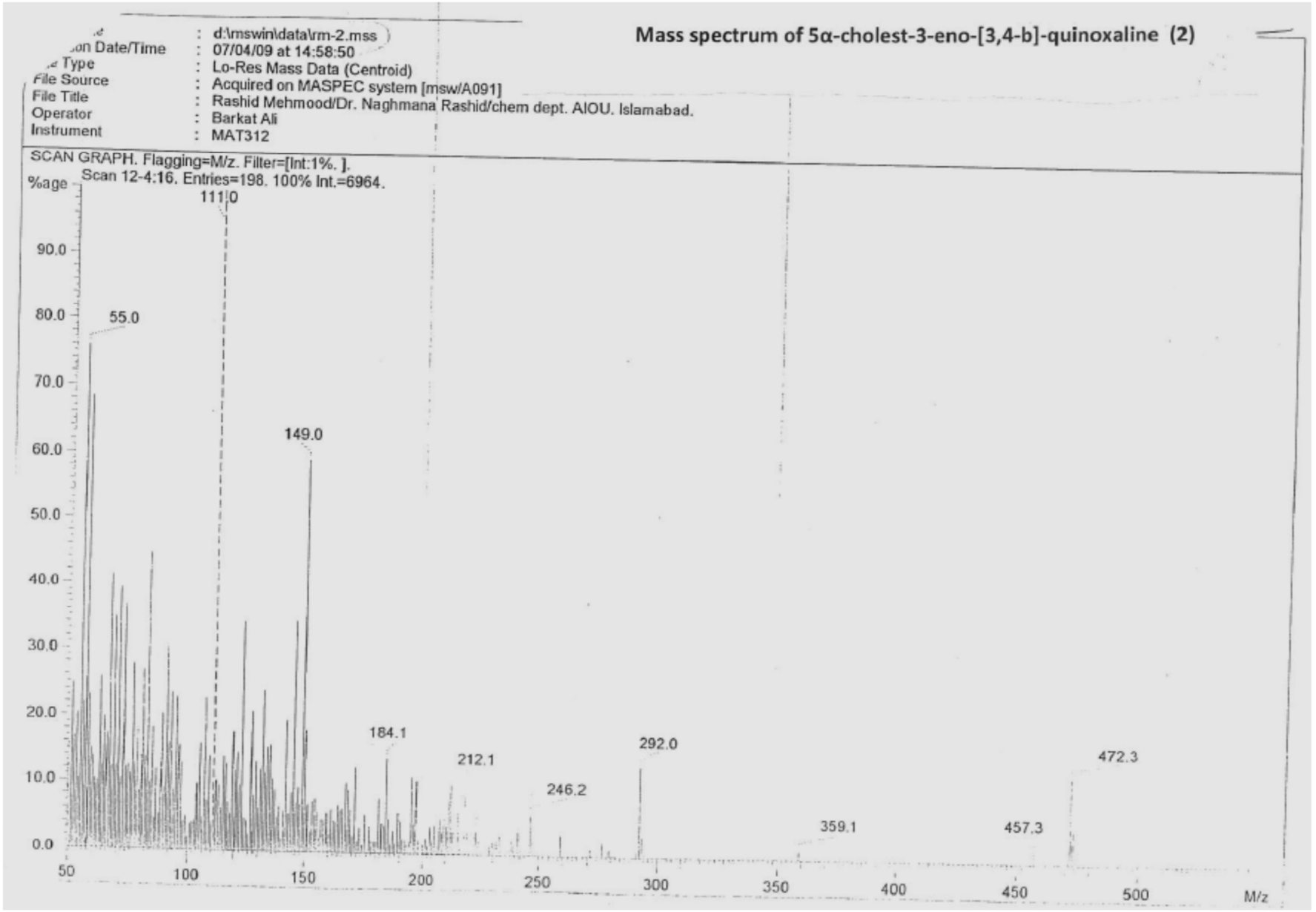
Supplementary Figure 3: ^{13}C-NMR spectrum of cholest-3-en-4-one (I)



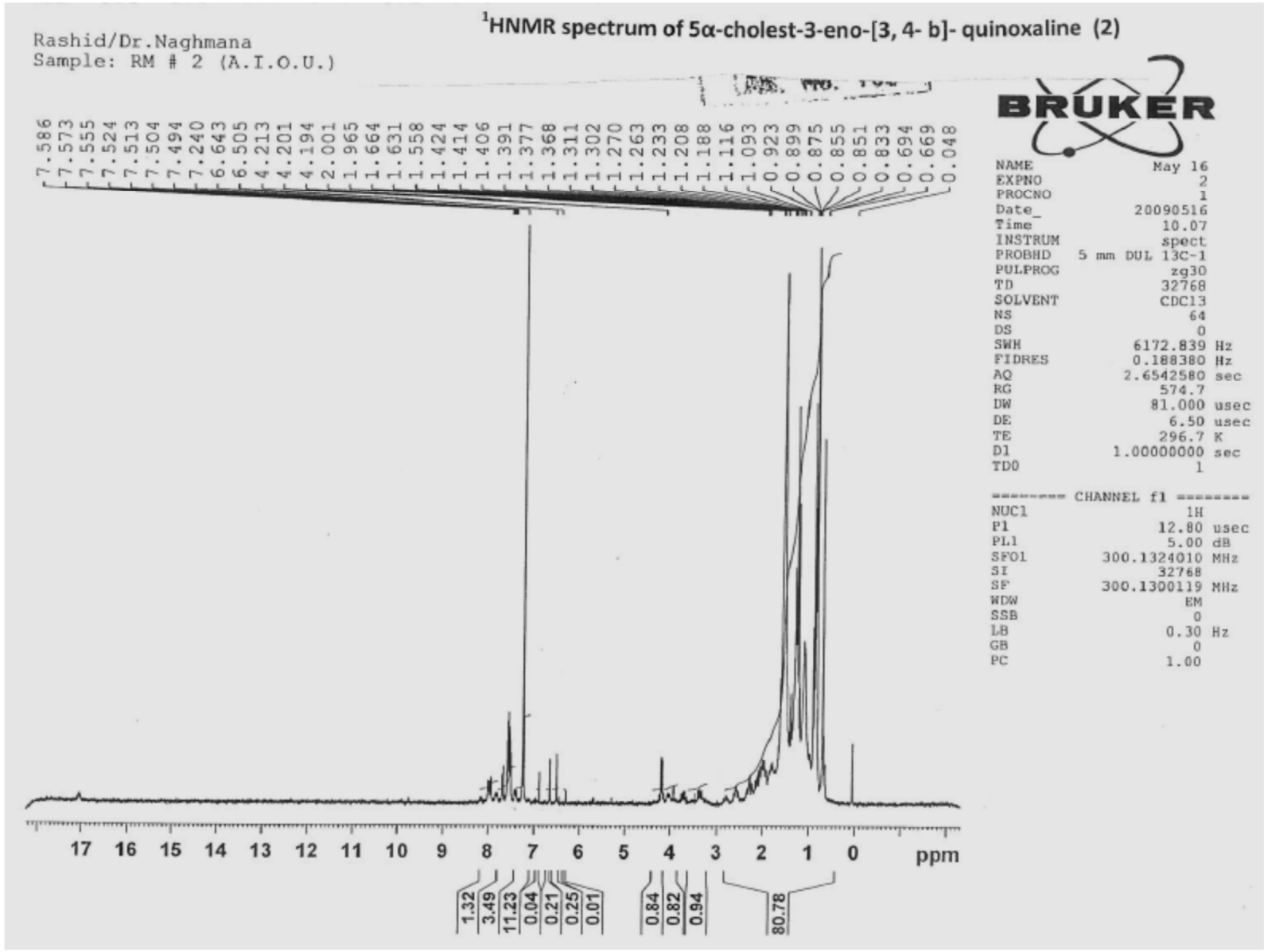
Supplementary Figure 4: FTIR spectrum of cholest-3-en-4-one (I)



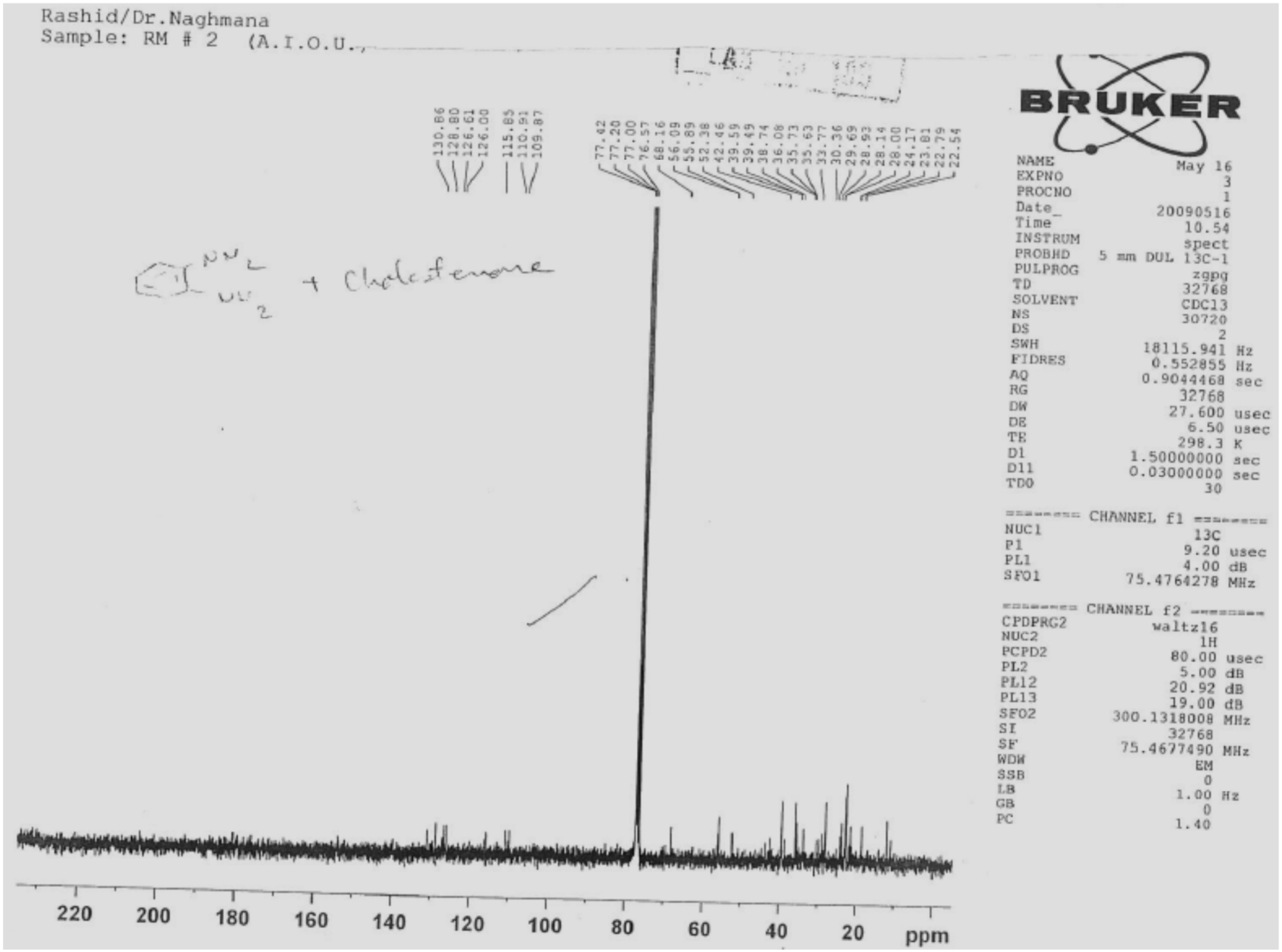
Supplementary Figure 5: Mass spectrum of 5α-cholest-3-eno-[3,4-b]-quinoxaline (II)



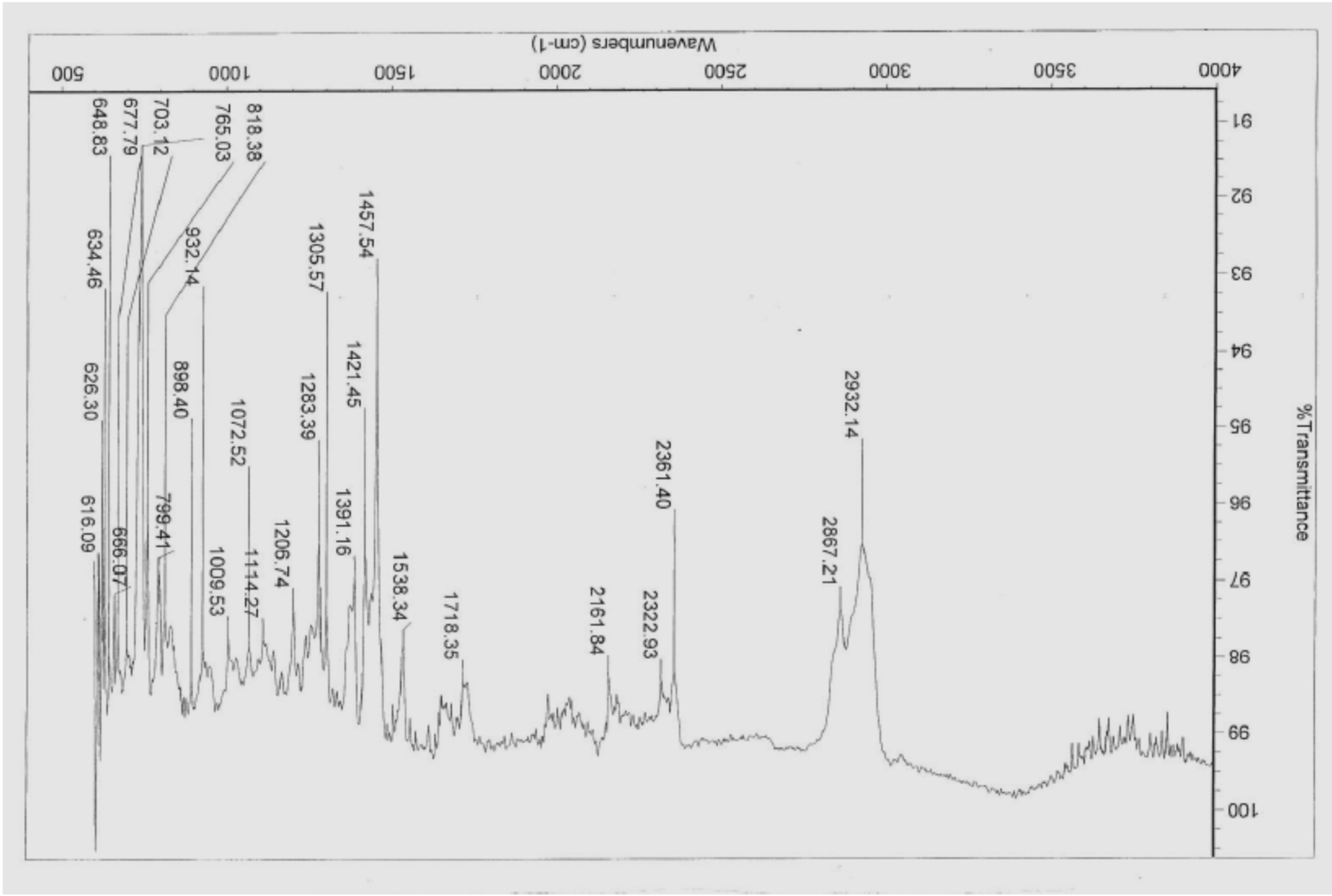
Supplementary Figure 6: ^{1}H-NMR spectrum of 5α-cholest-3-eno-[3,4-b]-quinoxaline (II)



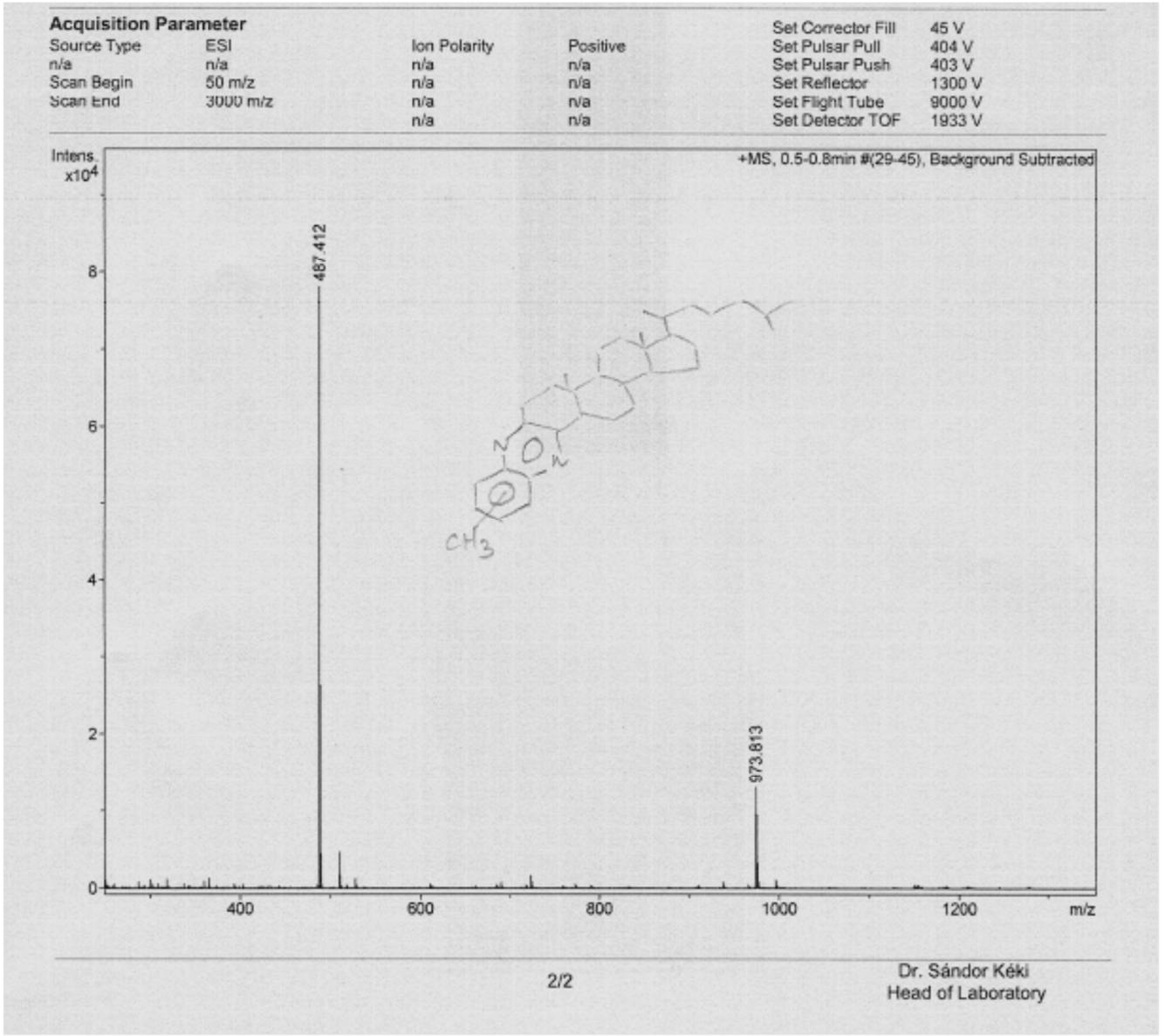
Supplementary Figure 7: ^{13}C-NMR spectrum of 5α-cholest-3-eno-[3,4-b]-quinoxaline (II)



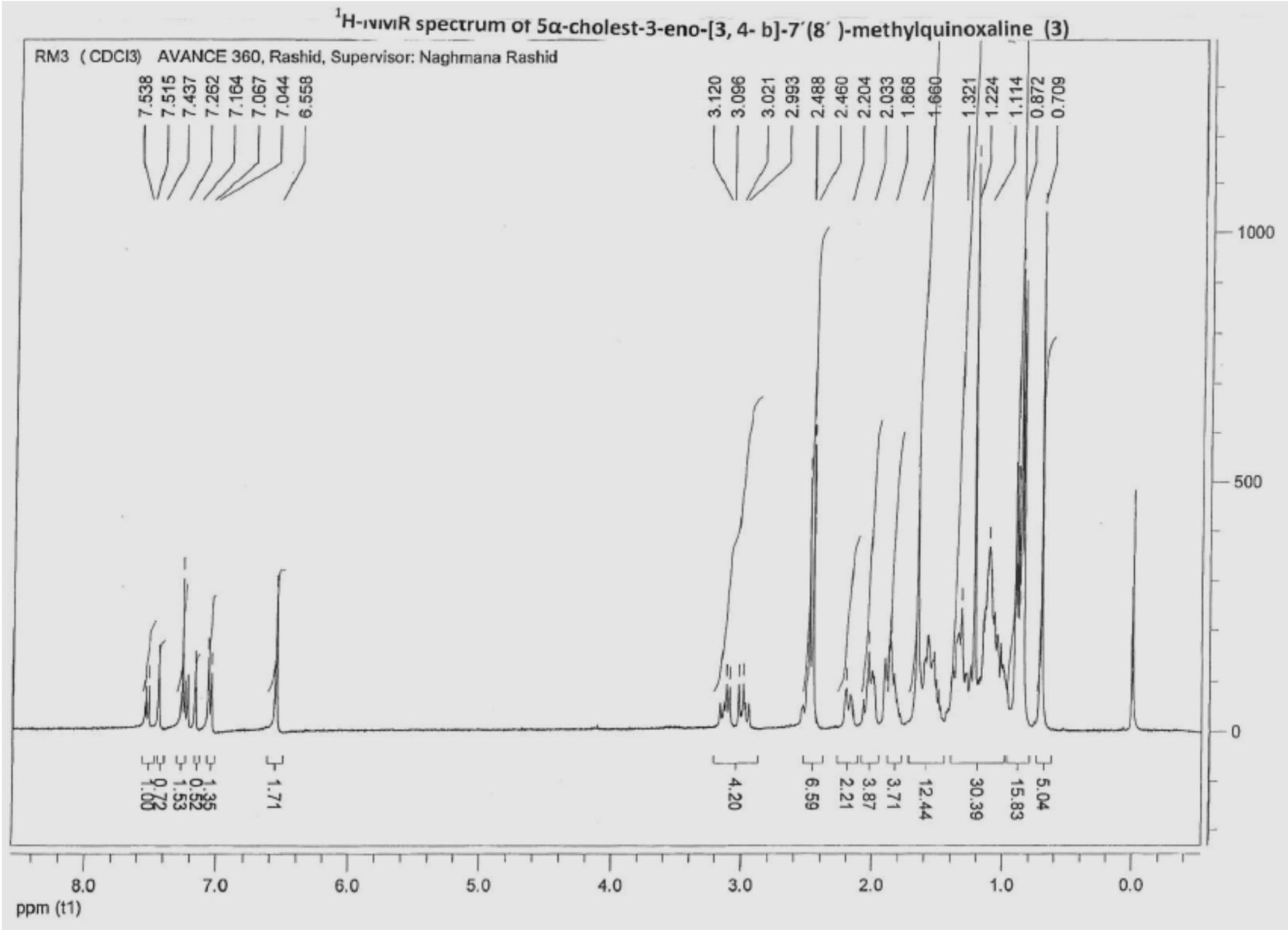
Supplementary Figure 8: FTIR spectrum of 5α-cholest-3-eno-[3,4-b]-quinoxaline (II)



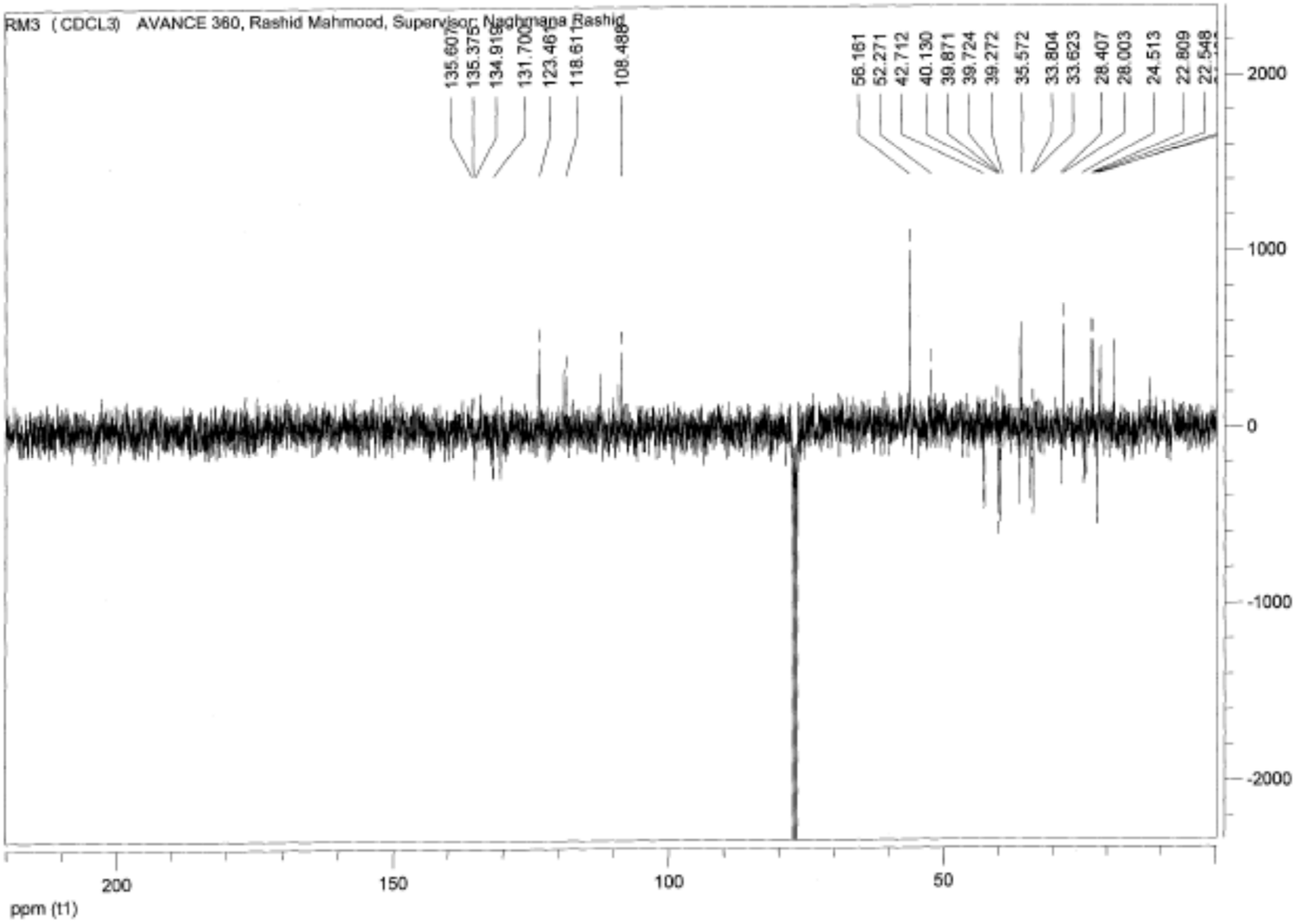
Supplementary Figure 9: MS spectrum of 5α-cholest-3-eno-[3, 4-b]-7′(8′)-methylquinoxaline (III)



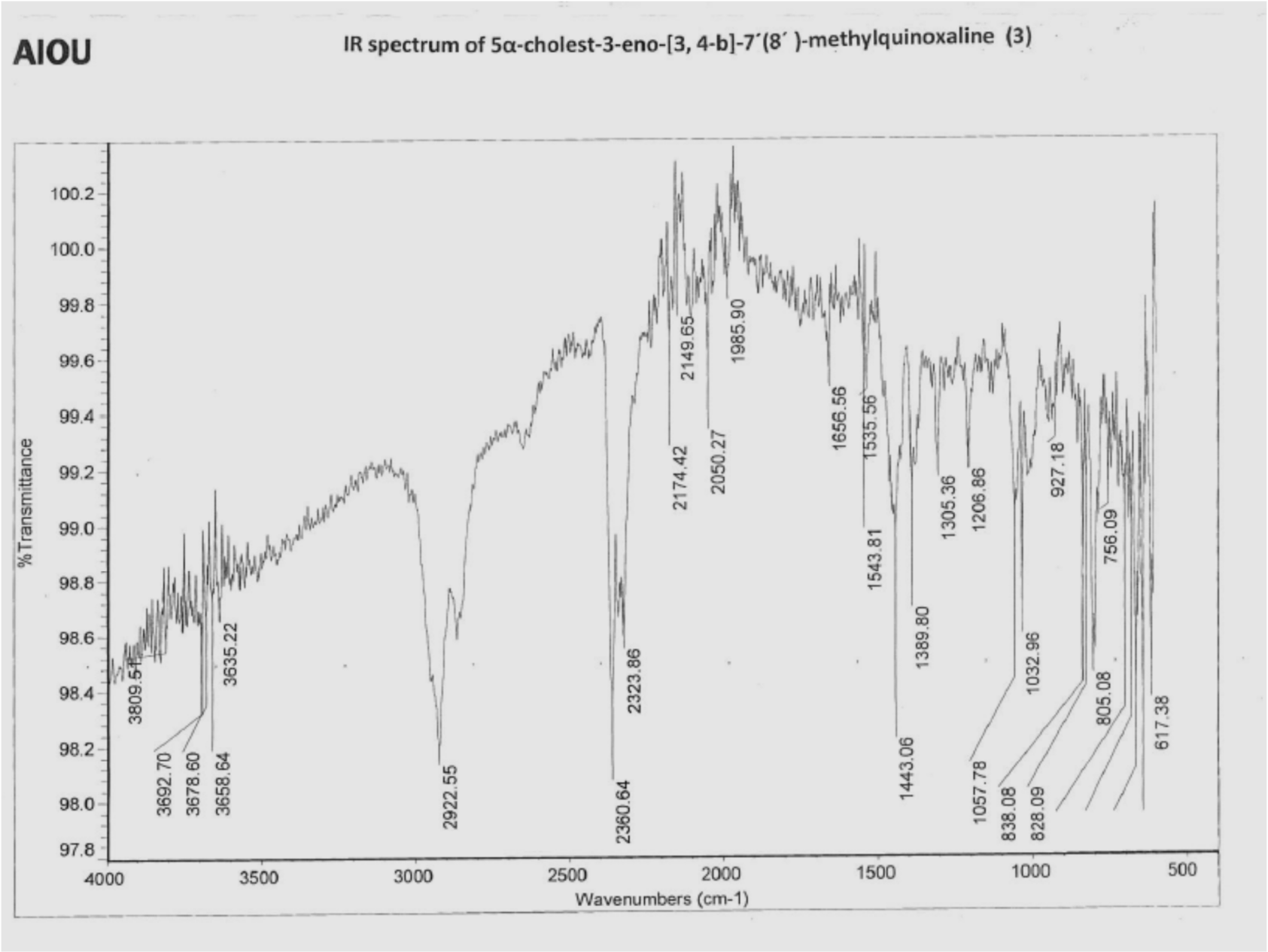
Supplementary Figure 10: ^{1}H-NMR spectrum of 5α-cholest-3-eno-[3, 4-b]-7′(8′)-methylquinoxaline (III)



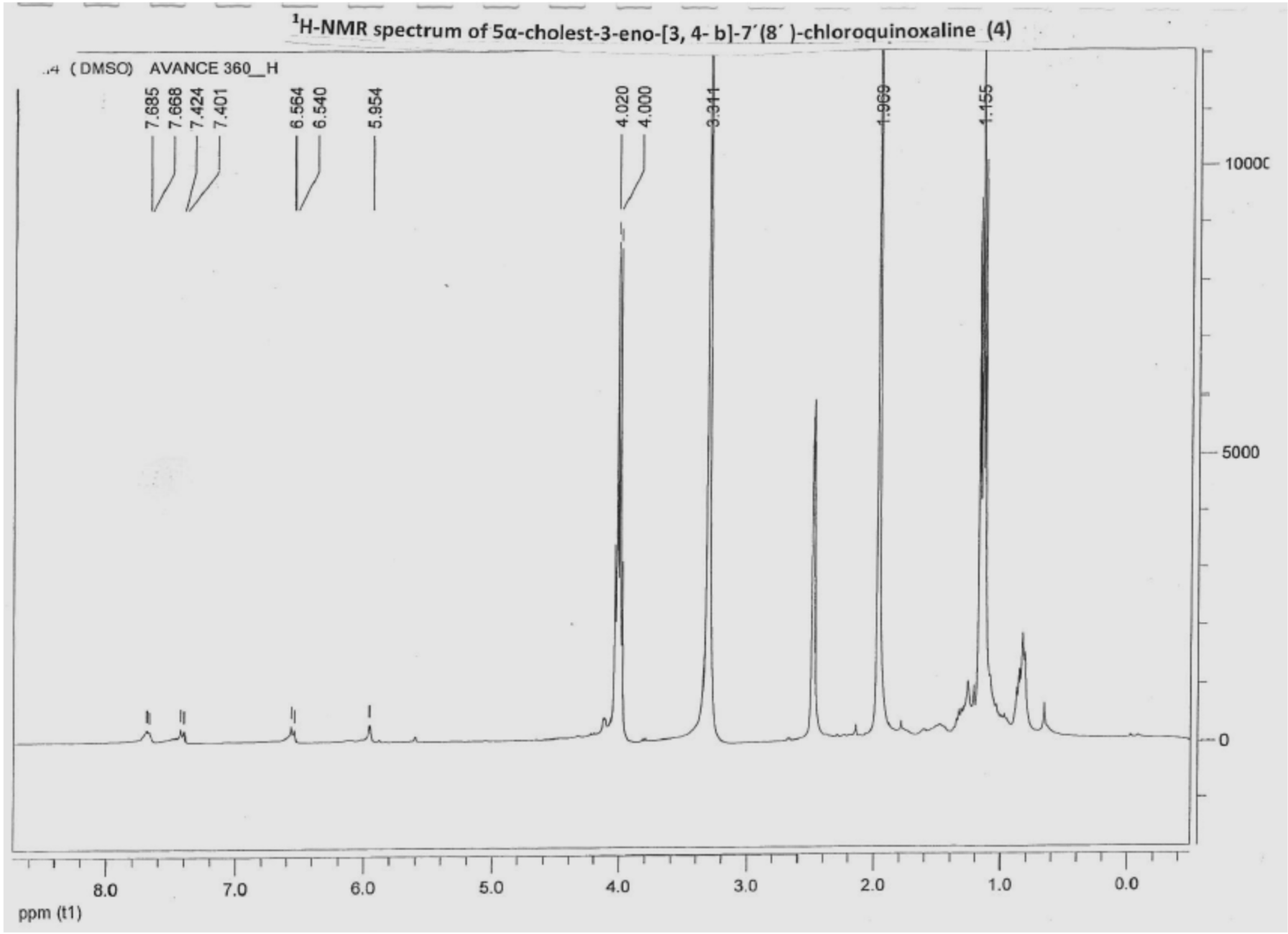
Supplementary Figure 11: ^{13}C-NMR spectrum of 5α-cholest-3-eno-[3, 4-b]-7´(8´)-methylquinoxaline (III)



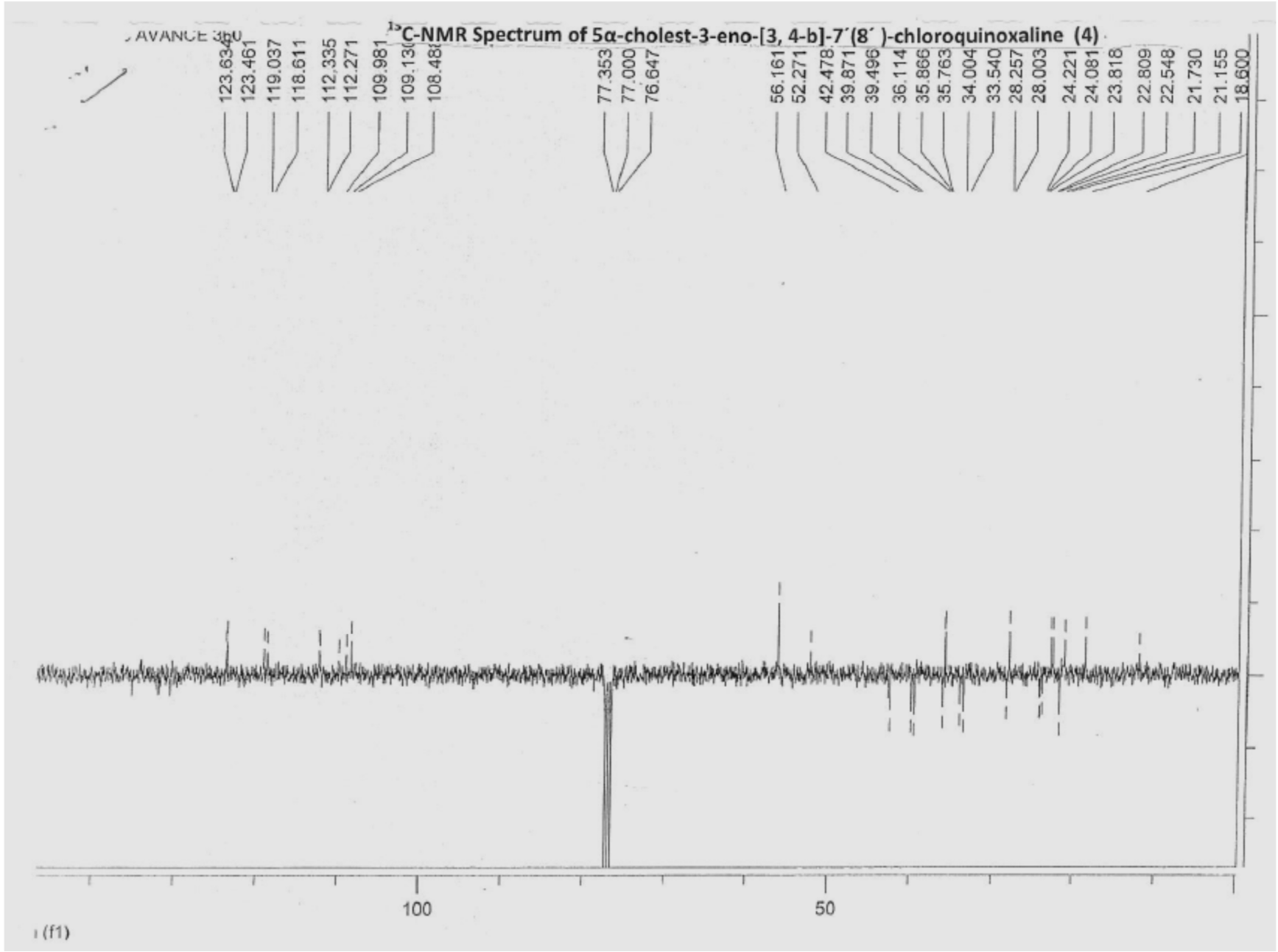
Supplementary Figure 12: FTIR spectrum of 5α-cholest-3-eno-[3, 4-b]-7′(8′)-methylquinoxaline (III)



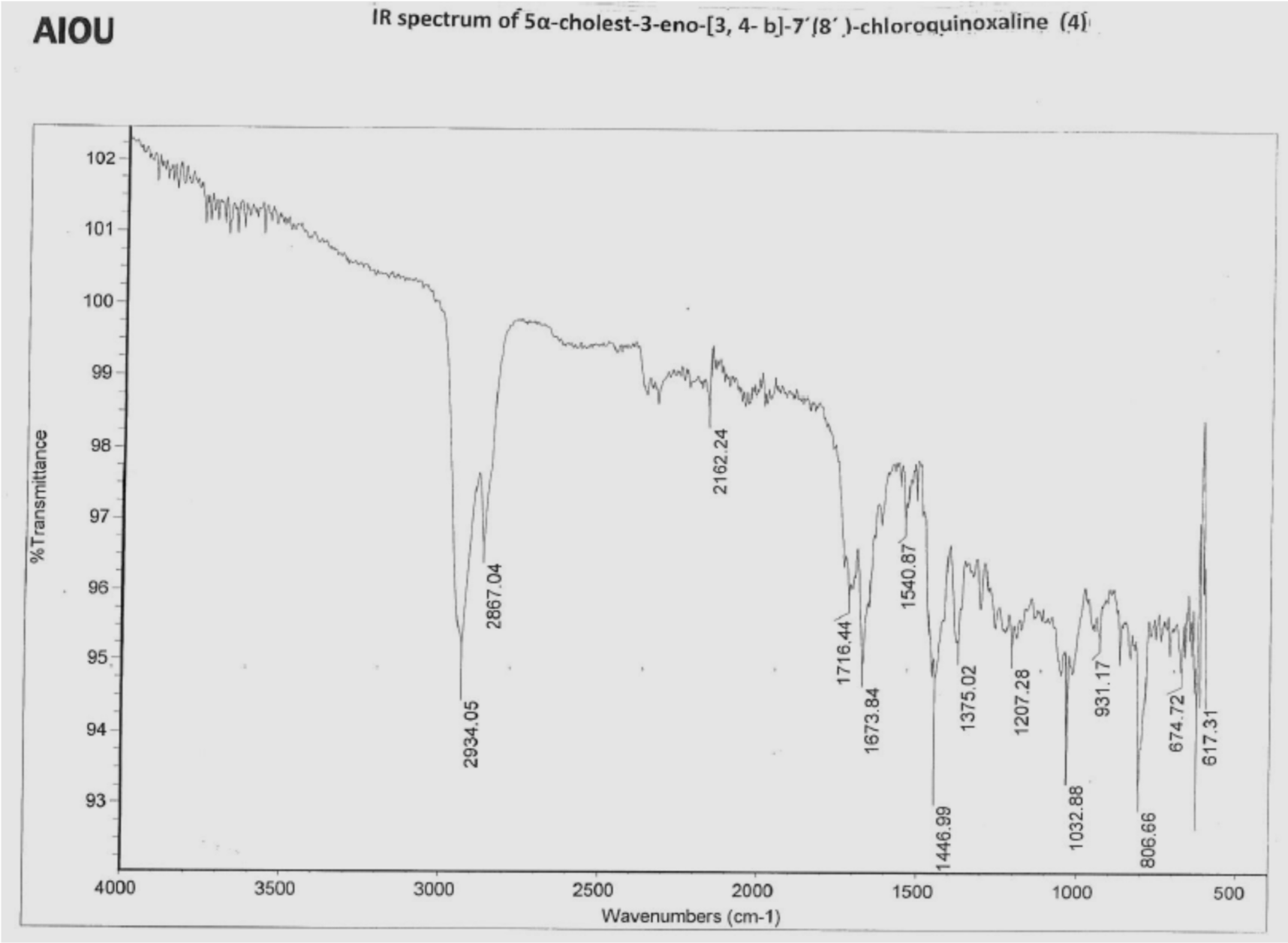
Supplementary Figure 13: ^{1}H-NMR spectrum of 5α-cholest-3-eno-[3, 4-b]-7′(8′)-chloroquinoxaline (IV)



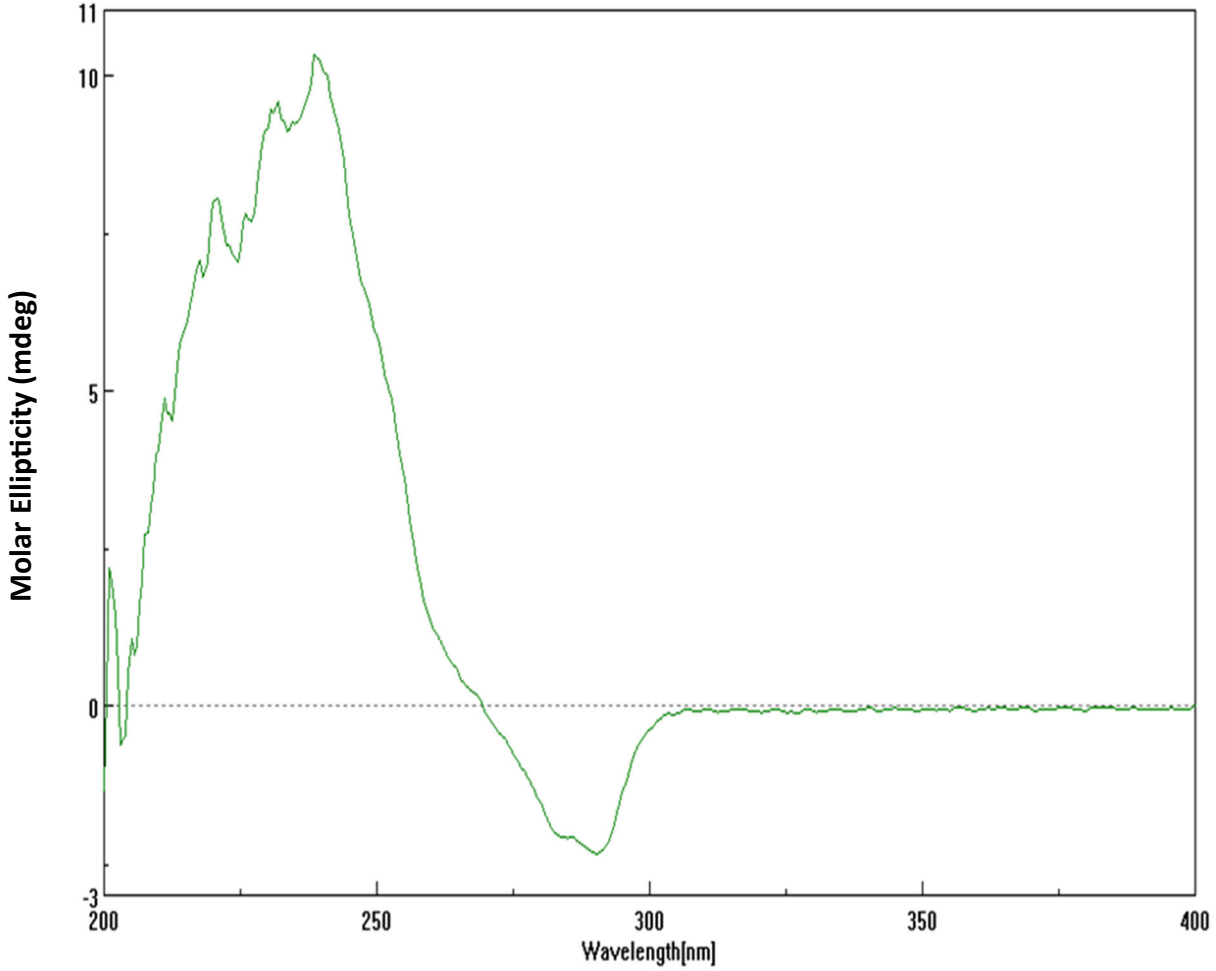
Supplementary Figure 14: ^{13}C-NMR spectrum of 5α-cholest-3-eno-[3, 4-b]-7′(8′)-chloroquinoxaline (IV)



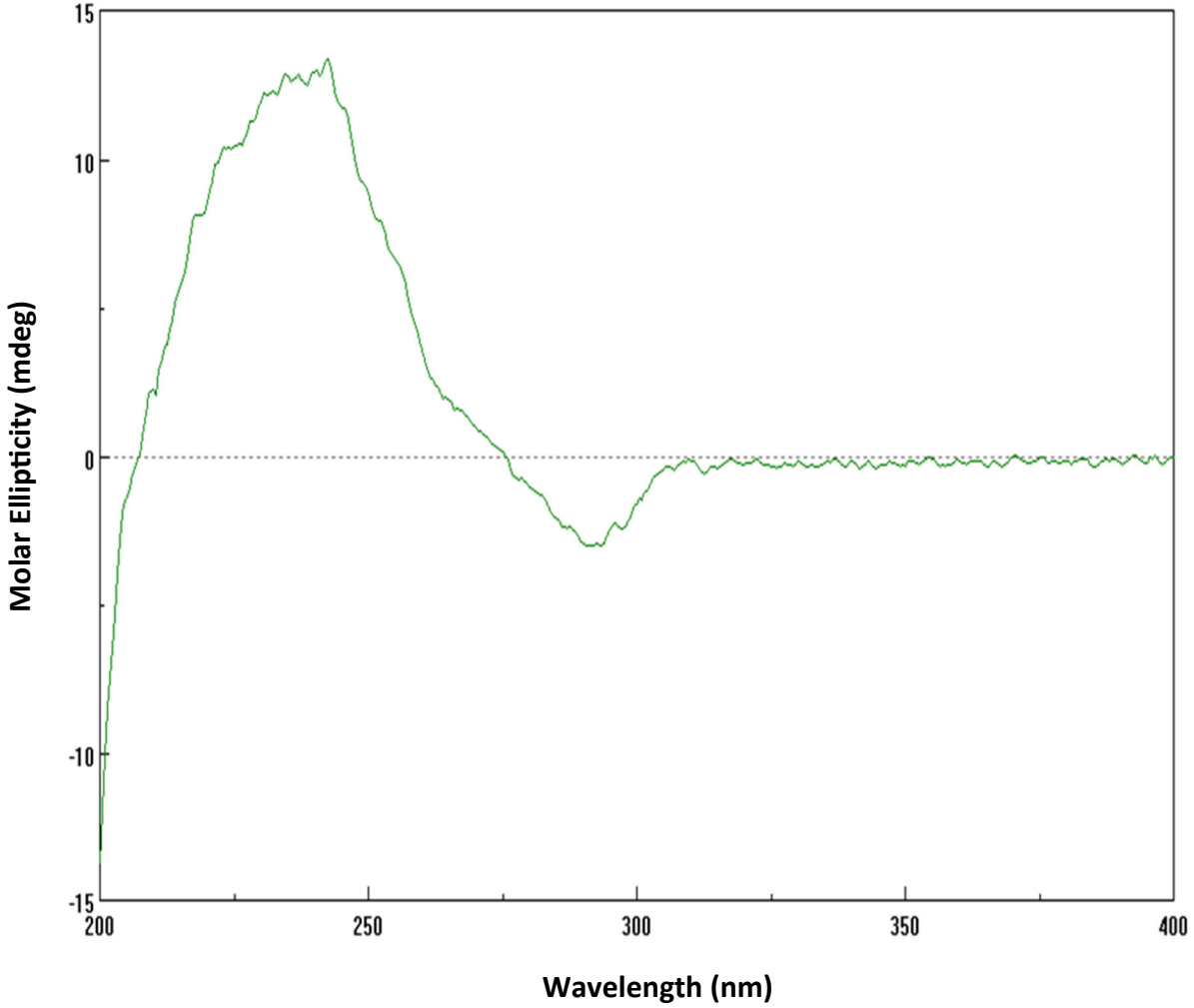
Supplementary Figure 15: FTIR spectrum of 5α-cholest-3-eno-[3, 4-b]-7′(8′)-chloroquinoxaline (IV)



Supplementary Figure 16: CD spectrum of 5α-cholest-3-eno-[3,4-b]-quinoxaline (II)



Supplementary Figure 18: CD spectrum of 5α-cholest-3-eno-[3, 4-b]-7´(8´)-methylquinoxaline (III)



Supplementary Figure 18: CD spectrum of 5α-cholest-3-eno-[3, 4-b]-7′(8′)-chloroquinoxaline (IV)