**An efficient metal-free synthesis of carbodiimide tethered amino acid conjugates**

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**Experimental section**

All chemicals were used as obtained from Sigma Aldrich, USA, and Spectrochem India. All the solvents were dried and purified using recommended procedures in the literature whenever necessary. High resolution mass spectra were recorded on a Micromass QTOF micromass spectrometer using electron spray ionization mode. 1H NMR and 13C NMR spectra were recorded on a Bruker AMX 400 MHz and 100 MHz spectrometer, respectively. The RP-HPLC analysis of isomers was carried out by using an Agilent instrument (1200 series Agilent Chem Station) at λ = 254 nm; column: Phenomenex Lux Amylose-2 and Cellulose-1, pore size-5 μm, diameter × length = 4.6 × 250 mm; method: gradient 0.1% TFA water-acetonitrile; Flow rate: 1 mL/min in 30 min and Eclipse, XDB-C18, pore size-5 μm, diameter x length = 4.6 x 150 nm). TLC experiments were done using MERCK TLC aluminum sheets (silica gel 60 F254) and chromatograms were visualized by exposing in iodine chamber and in UV-lamp. Column chromatography was performed on silica gel (100-200 mesh) using ethyl acetate and hexane mixture as eluent.

**General procedure for the synthesis of α-carbodiimide tethered amino acid conjugate**



To a solution of α-isocyanide ester (1.2 equiv., 1.21g) in 1,4-dioxane (10 mL) were added I2 (20 mol %, 0.5g) portions wise, tert-butyl hydroperoxide (2.0 equiv., 1.93g) and aniline (1.0 equiv., 1.0 g) derivatives. After addition, the mixture was allowed to reflux. When the reaction was complete (monitored by TLC), the mixture was cooled to room temperature, washed with 10% aq. Na2S2O3 (3 × 15 mL) and the resulting solution was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel 100-200 mesh) with 1% - 5% ethyl acetate and hexane to afford corresponding title compounds.

**General procedure for the synthesis of *Nβ*-Cbz protected carbodiimide tethered amino acid conjugate**



To a solution of *Nβ*-Cbz-protected amino alkyl isocyanide (1.2 equiv., 2.12g) in 1,4-dioxane (10 mL) were added I2 (30 mol %, 1.03g) portions wise, tert-butyl hydroperoxide (2.0 equiv., 1.46g) and aniline (1.0 equiv., 1.0 g) derivatives. After addition, the mixture was allowed to reflux for 4 h. After completion of reaction(monitored by TLC),the mixture was cooled to room temperature, washed with 10% aq. Na2S2O3 (3 × 15 mL) and the resulting solution was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel 100-200 mesh) with 8% - 12% ethyl acetate and hexane to afford corresponding title compounds.

Characterization data:

**(2S)-methyl 3-phenyl-2-(((phenylimino)methylene)amino)propanoate (3a)**

Light yellow oil; Yield 86%; IR (ν, cm-1): 2129 (-N=C=N-), 1746 (C=O); 1H NMR (400 MHz, DMSO-*d*6): δ 7.33-6.93 (m, 10H), 4.74 (dd, *J* = 8 Hz, 4 Hz, 1H), 3.75 (s, 3H), 3.18 (dd, *J* = 16 Hz, 4 Hz, 1H), 3.08 (dd, *J* = 16 Hz, 8 Hz, 1H); 13C NMR (100 MHz, DMSO-*d*6): δ 171.4, 139.7, 136.7, 130.6, 129.8, 128.8, 128.3, 127.3, 126.6, 125.8, 60.3, 53.1, 39.5; HRMS: calcd. For C17H16N2O2H [M+H]+: 281.1290, found: 281.1290.

**(2R)-methyl 3-phenyl-2-(((phenylimino)methylene)amino)propanoate (3a\*)**

Light yellow oil; Yield 84%; 1H NMR (400 MHz, DMSO-*d*6): δ 7.33-6.92 (m, 10H), 4.75 (dd, *J* = 8 Hz, 4 Hz, 1H), 3.75 (s, 3H), 3.18 (dd, *J* = 12 Hz, 4 Hz, 1H), 3.08 (dd, *J* = 12 Hz, 8 Hz, 1H); 13C NMR (100 MHz, DMSO-*d*6): δ 171.4, 139.7, 136.7, 130.6, 129.8, 128.8, 127.3, 126.6, 125.4, 124.1, 60.2, 53.1, 39.5; HRMS: calcd. For C17H16N2O2H [M+H]+: 281.1290, found: 281.1290.

**(2S)-methyl 2-((((4-bromophenyl)imino)methylene)amino)-3-methylbutanoate (3b)**

Light yellow oil; Yield 82%; IR (ν, cm-1): 2126 (-N=C=N-), 1740 (C=O); 1H NMR (400 MHz, DMSO-*d*6): δ 7.53-7.49 (m, 2H), 7.11-7.08 (m, 2H), 4.35 (d, *J* = 8 Hz, 1H), 3.73 (s, 3H), 2.24-2.16 (m, 1H), 0.98 (d, *J* = 8 Hz, 3H), 0.86 (d, *J* = 8 Hz, 3H); 13C NMR (100 MHz, DMSO-*d*6): δ 171.5, 139.8, 136.5, 132.8, 126.1, 117.4, 64.9, 53.2, 31.8, 19.8, 17.2; HRMS: calcd. for C13H15BrN2O2H [M+H]+: 311.0395, found: 311.0392.

**(2S)-methyl 2-((((4-methoxyphenyl)imino)methylene)amino)-3-methylbutanoate (3c)**

Light yellow oil; Yield 88%; IR (ν, cm-1): 2127 (-N=C=N-), 1741 (C=O); 1H NMR (400 MHz, DMSO-*d*6): δ 7.13-7.11 (m, 2H), 6.94-6.92 (m, 2H), 4.27 (d, *J* = 4 Hz, 1H), 3.75 (s, 6H), 2.25-2.19 (m, 1H), 1.02 (d, *J* = 4 Hz, 3H), 0.91 (d, *J* = 8 Hz, 3H); 13C NMR (100 MHz, DMSO-*d*6): δ 171.7, 157.1, 137.6, 132.3, 128.2, 127, 125.8, 125, 64.8, 55.7, 53, 31.9, 19.7, 17.3; HRMS: calcd. for C14H18N2O3H [M+H]+: 263.1396, found: 263.1396.

**(2S)-methyl 4-methyl-2-(((m-tolylimino)methylene)amino)pentanoate (3d)**

Light yellow oil; Yield 86%; IR (ν, cm-1): 2124 (-N=C=N-), 1744 (C=O); 1H NMR (400 MHz, DMSO-*d*6): δ 7.46-7.22 (m, 2H,), 7.00-6.96 (m, 2H), 4.39 (t, *J* = 8 Hz, 1H), 3.74 (s, 3H), 2.29 (s, 3H), 1.83-1.76 (m, 1H), 1.67 (t, *J* = 8 Hz, 2H), 0.94 (dd, *J* = 8 Hz, 1.2 Hz, 6H); 13C NMR (100 MHz, DMSO-*d*6): δ 172.4, 139.7, 139.4, 136.8, 129.7, 126.2, 124.6, 121.2, 57.8, 53.1, 42.7, 25.1, 23.1, 21.7, 21.2; HRMS: calcd. for C15H20N2O2H [M+H]+: 261.1603, found: 261.1601.

**(2S)-methyl 2-((((2-chlorophenyl)imino)methylene)amino)propanoate (3e)**

Light yellow oil; Yield 81%; IR (ν, cm-1): 2139 (-N=C=N-), 1743 (C=O); 1H NMR (400 MHz, DMSO-*d*6): δ 7.49-7.16 (m, 4H), 4.53 (qt, *J* = 8 Hz, 1H), 3.74 (s, 3H), 1.48 (d, *J* = 8 Hz, 3H); 13C NMR (100 MHz, DMSO-*d*6): δ 172.2, 137, 135, 130.4, 128.6, 126.6 (2C), 126.2, 54.9, 53.2, 20; HRMS: calcd. for C11H11ClN2O2Na [M+Na]+: 261.0407, found: 261.0407.

**(2S)-methyl 2-phenyl-2-(((p-tolylimino)methylene)amino)acetate (3f)**

Light yellow oil; Yield 84%; IR (ν, cm-1): 2108 (-N=C=N-), 1742 (C=O); 1H NMR (400 MHz, DMSO-*d*6): δ 7.45-7.37 (m, 5H), 7.17 (d, *J* = 8 Hz, 2H), 7.08 (t, *J* = 8 Hz, 2H), 5.57 (s, 1H), 3.70 (s, 3H), 2.29 (s, 3H); 13C NMR (100 MHz, DMSO-*d*6): δ 171, 138.1, 137.2, 136.5, 135.9, 130.5, 129.4, 129.1, 127.5, 124, 62.3, 53.5, 20.9; HRMS: calcd. for C17H16N2O2Na[M+Na]+: 303.1109, found: 303.1107.

**(2S,3R)-methyl 2-((((4-bromophenyl)imino)methylene)amino)-3-methylpentanoate (3g)**

Light yellow oil; Yield 87%; IR (ν, cm-1): 2126 (-N=C=N-), 1740 (C=O); 1H NMR (400 MHz, DMSO-*d*6): δ 7.52 (t, *J* = 8 Hz, 2H), 7.14-7.10 (m, 2H), 4.40 (d, *J* = 4 Hz, 1H), 3.75 (s, 3H), 2.0-1.94 (m, 1H), 1.27-1.17 (m, 2H), 0.98 (d, *J* = 8 Hz, 3H), 0.88 (t, *J* = 8 Hz, 3H); 13C NMR (100 MHz, DMSO-*d*6): 171.6, 139.8, 136.4, 132.8, 126.1, 117.4, 64.4, 53.1, 38.4, 24.6, 16.5, 11.8.

**(2S,3R)-methyl 3-methyl-2-(((m-tolylimino)methylene)amino)pentanoate (3h)**

Light yellow oil; Yield 82%; IR (ν, cm-1): 2126 (-N=C=N-), 1741 (C=O); 1H NMR (400 MHz, DMSO-*d*6): δ 7.45-6.95 (m, 4H), 4.34 (d, *J* = 4 Hz, 1H), 3.75 (s, 3H), 2.29 (s, 3H), 1.98-1.95 (m, 1H), 1.40-1.37 (m, 2H), 0.99 (d, *J* = 8 Hz, 3H), 0.89 (t, *J* = 8 Hz, 3H); 13C NMR (100 MHz, DMSO-*d*6): δ 171.6, 139.9, 139.4, 136.7, 129.7, 126, 124.5, 121.1, 64.2, 53, 38.6, 24.7, 21.2, 16.5, 11.8; HRMS: calcd. for C15H20N2O2Na[M+Na]+: 283.1422, found: 283.1422.

**(2S)-methyl 2-((((4-cyanophenyl)imino)methylene)amino)-2-phenylacetate (3i)**

Yellow oil; Yield 76%; 1H NMR (400 MHz, DMSO-*d*6): δ 7.65-7.39 (m, 9H), 5.21 (s, 1H), 3.73 (s, 3H); 13C NMR (100 MHz, DMSO-*d*6): δ 171.6, 144.3, 138.8, 137.7, 132.8, 129.2, 128.9, 128.8, 127, 119.3, 114.3, 69.2, 54.1; HRMS: calcd. for C17H13N3O2H [M+H]+: 292.1086, found: 292.0602.

benzyl ((2S)-1-phenyl-3-(((m-tolylimino)methylene)amino)propan-2-yl)carbamate **(6a)**

Yellow oil; Yield 82 %; IR (ν, cm-1): 3397 (-NH-), 2128 (-N=C=N-); 1H NMR (400 MHz, DMSO-*d*6): δ 7.91 (s, 1H), 7.70-7.22 (m, 14H), 5.26 (dd, *J* = 8 Hz, 8 Hz, 2H), 4.10-3.98 (m, 1H), 3.70 (dd, *J* = 8 Hz, 4 Hz, 1H), 3.61 (dd, *J* = 8 Hz, 4 Hz, 1H), 2.91 (dd, *J* = 8 Hz, 4 Hz, 1H), 2.75 (dd, *J* = 8 Hz, 4.5 Hz, 1H), 2.16 (s, 3H); 13C NMR (100 MHz, DMSO-*d*6): δ 156.3, 151, 140.3, 139.4, 138.7, 136.1, 129.7, 129.6, 128.7, 128.2, 127.9, 126.3, 125.9, 125, 124.5, 121, 65.6, 53.9, 49.8, 38.3, 21.3; HRMS: calcd. for C25H25N3O2H [M+H]+: 400.2025, found: 400.2024.

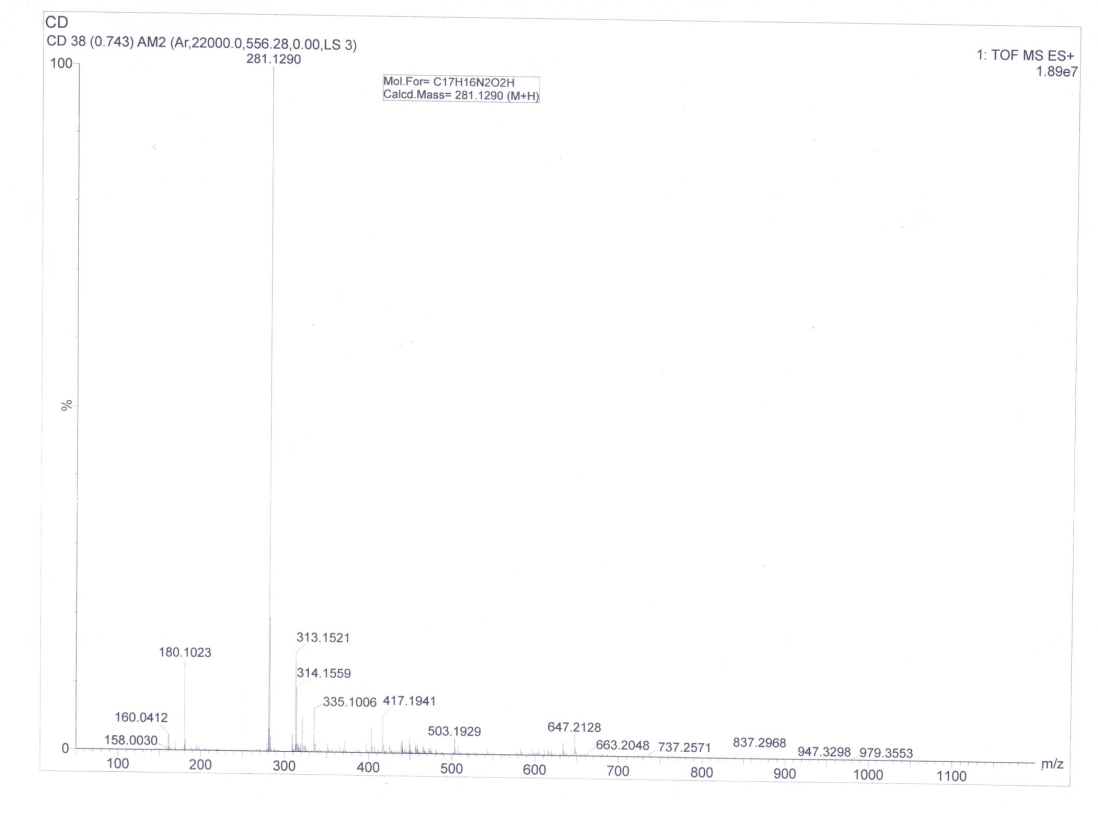
benzyl ((2S)-1-((((4-methoxyphenyl)imino)methylene)amino)propan-2-yl)carbamate **(6b)**

Yellow oil; Yield 86 %; IR (ν, cm-1): 3301 (-NH-), 2119 (-N=C=N-); 1H NMR (400 MHz, DMSO-*d*6): δ 7.42 (s, 1H), 7.34-6.84 (m, 9H), 4.94 (dd, *J* = 18 Hz, 12 Hz, 2H), 3.71 (s, 3H), 3.41-3.37 (m, 1H), 1.26 (d, *J* = 8 Hz, 2H), 1.11 (d, *J* = 8 Hz, 3H); 13C NMR (100 MHz, DMSO-*d*6): δ 156.9, 156.1, 137.4, 136.7, 132.8, 128.7, 128.2, 128.1, 124.7, 115.2, 65.7, 55.7, 51, 48, 18.7; HRMS: calcd. for C19H21N3O3H [M+H]+: 340.1661, found: 340.1659.

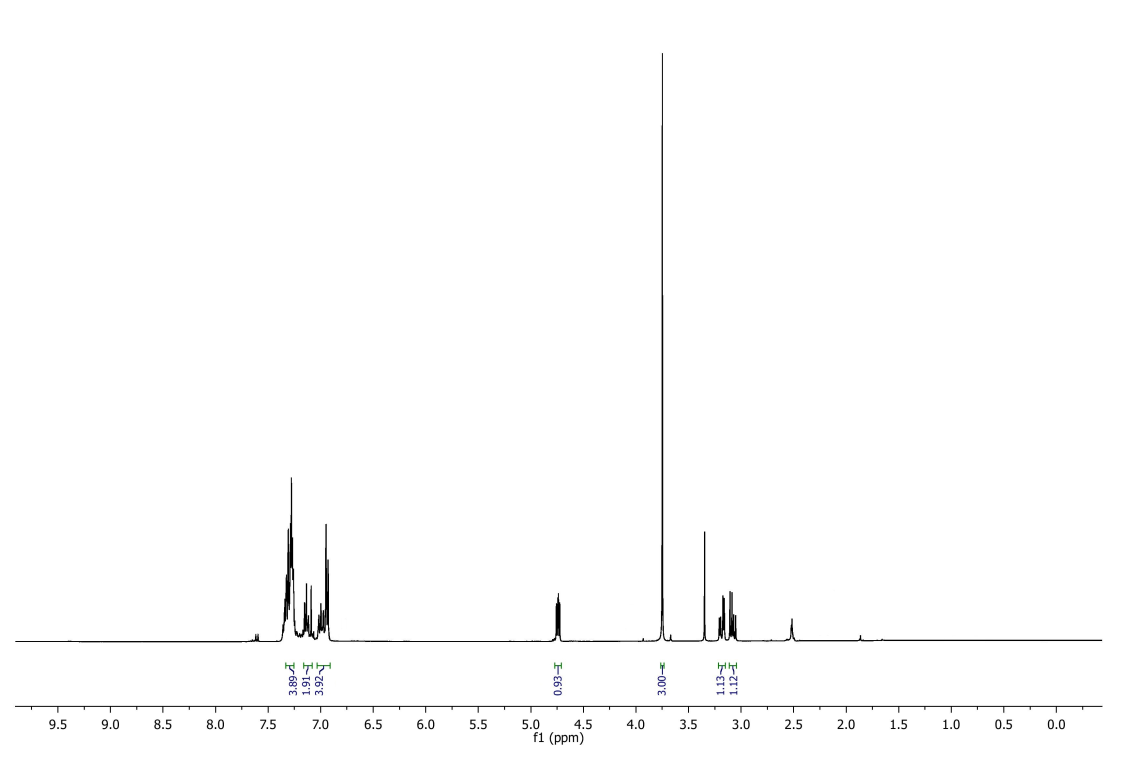
benzyl ((2S)-3-methyl-1-(((phenylimino)methylene)amino)butan-2-yl)carbamate **(6c)**

Yellow oil; Yield 81 %; 1H NMR (400 MHz, DMSO-*d*6): δ 8.23 (s, 1H), 7.75-7.28 (m, 10H), 5.24 (s, 2H), 3.53-3.48 (m, 1H), 2.98 (dd, *J* = 8 Hz, 8 Hz, 1H), 2.75 (dd, *J* = 12 Hz, 8 Hz, 1H), 1.77-1.69 (m, 1H), 0.86 (d, *J* = 8 Hz, 6H); 13C NMR (100 MHz, DMSO-*d*6): δ 158.6, 140.6, 137.6, 134.6, 128.1, 127.9, 127, 126.2, 125.8, 124.9, 67.8, 61.3, 50.2, 31.6, 19.4, 18.1; HRMS: calcd. for C20H23N3O2H [M+H]+: 338.1869, found: 338.1867.

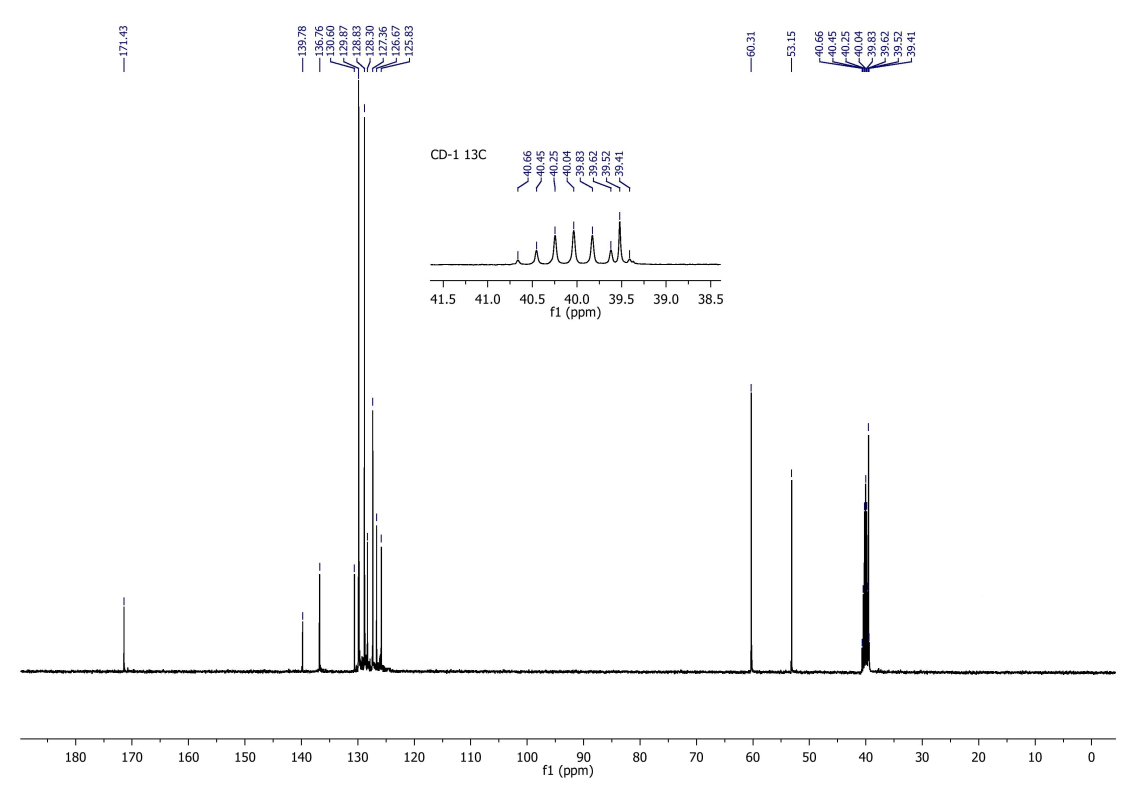
**HRMS, 1H NMR, 13C NMR and IR spectra for the title compounds**



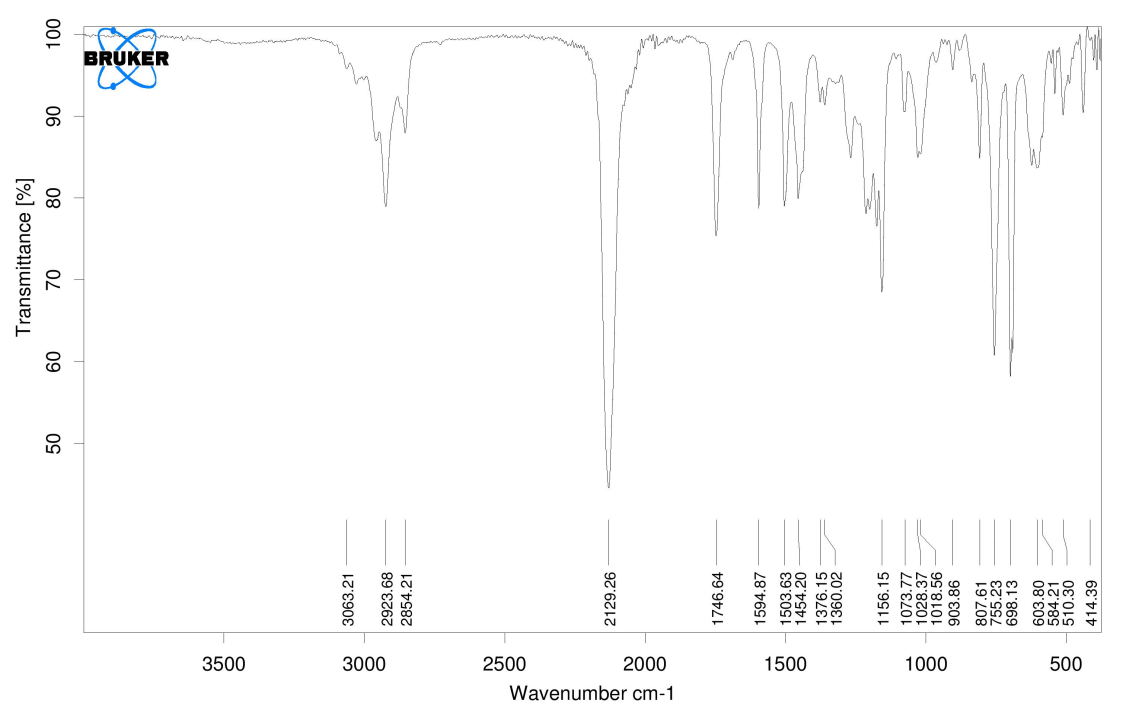
**HRMS spectrum of compound 3a**



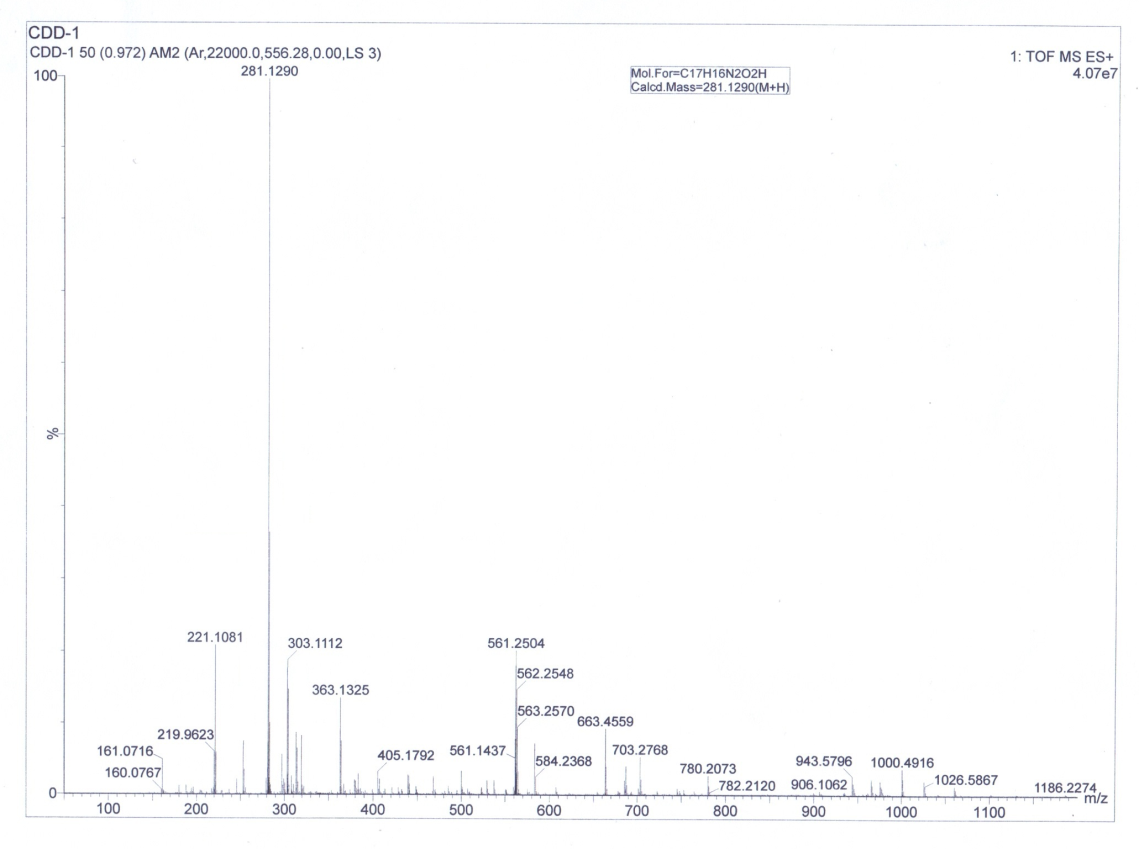
**1H NMR spectrum of compound 3a**

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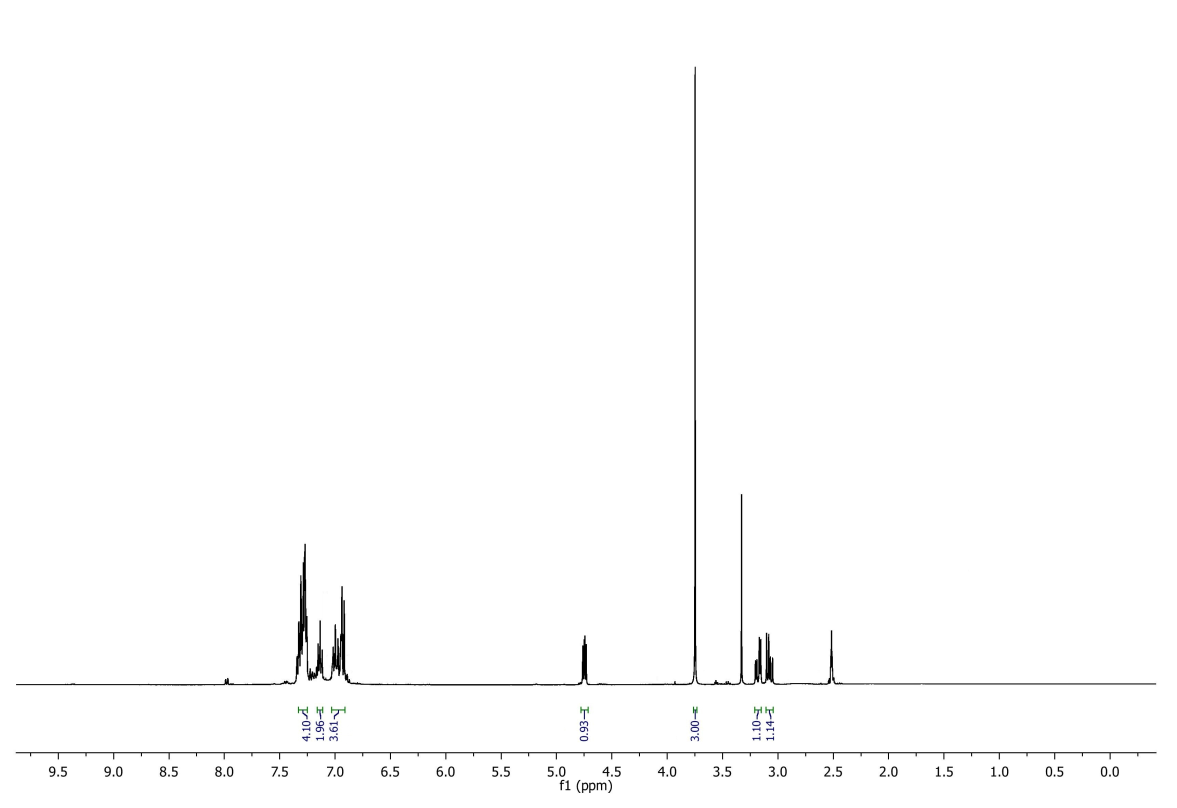
**13C NMR spectrum of compound 3a**

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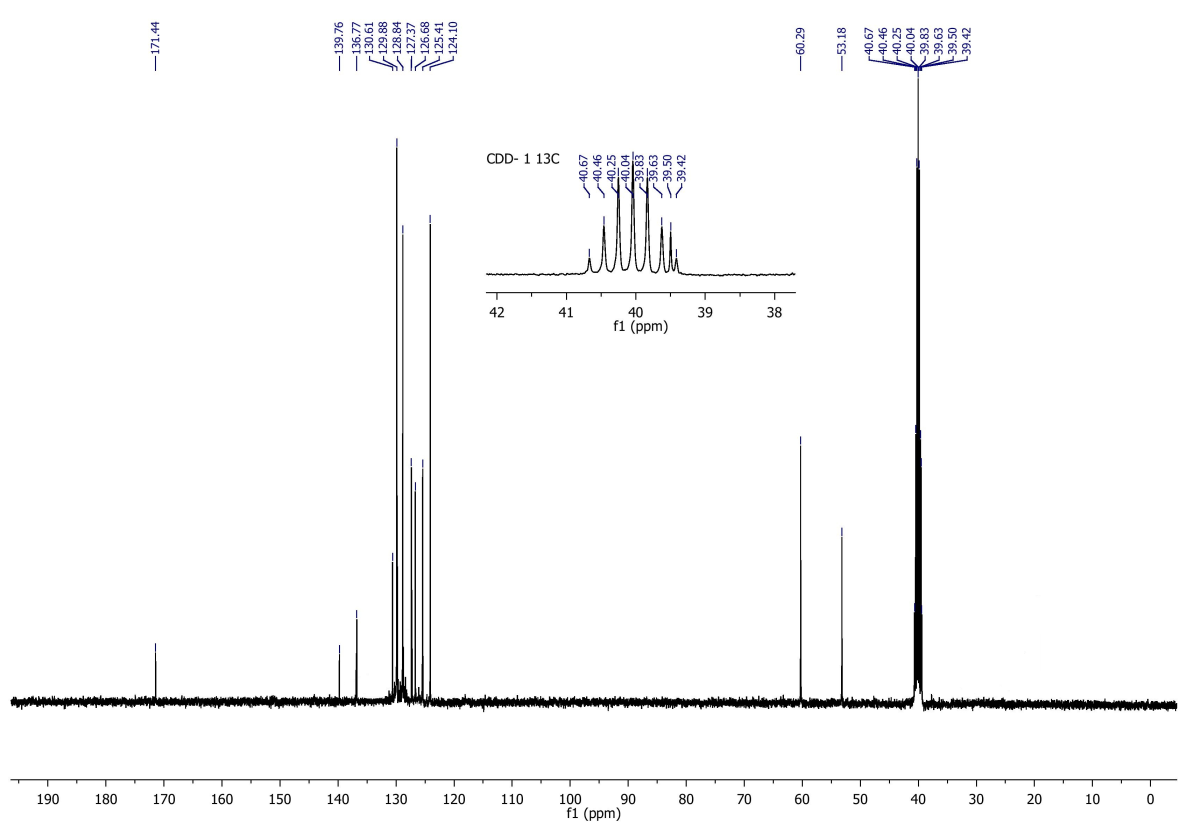
**IR Spectrum of compound 3a**



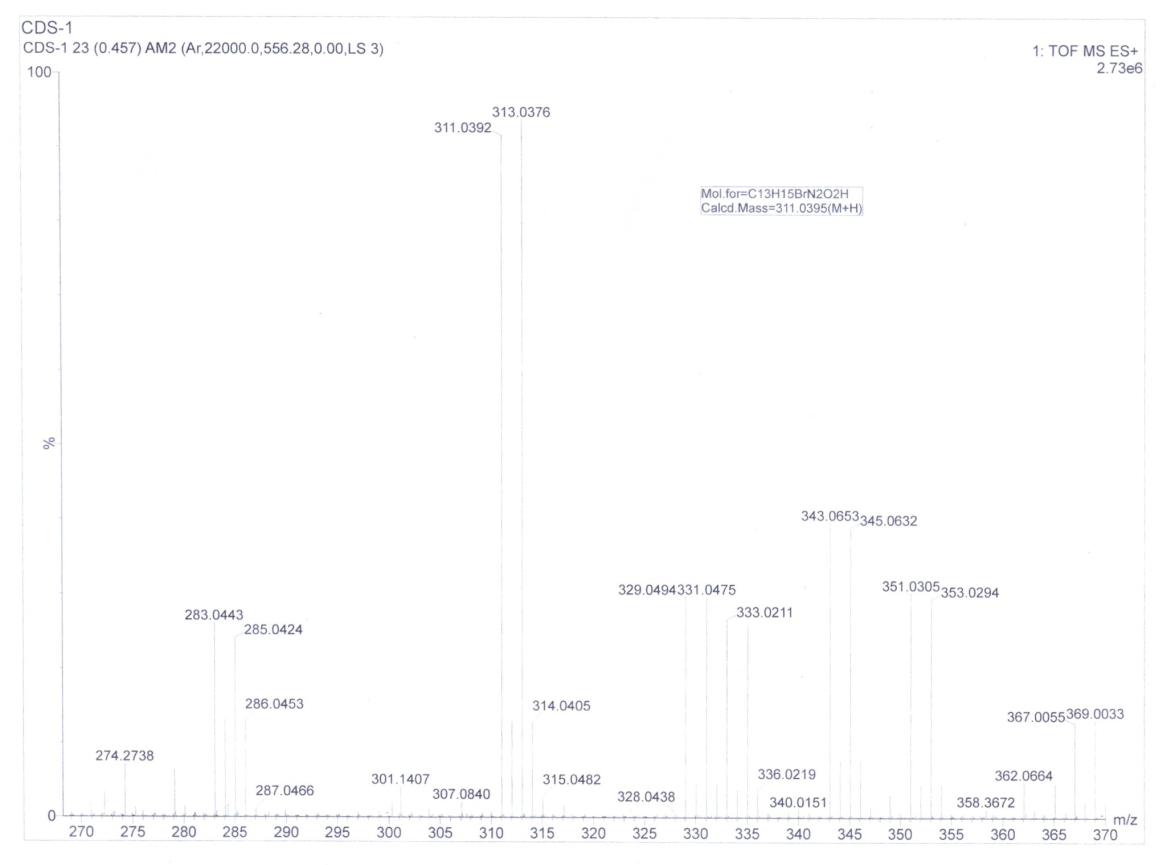
**HRMS spectrum of compound 3a\***



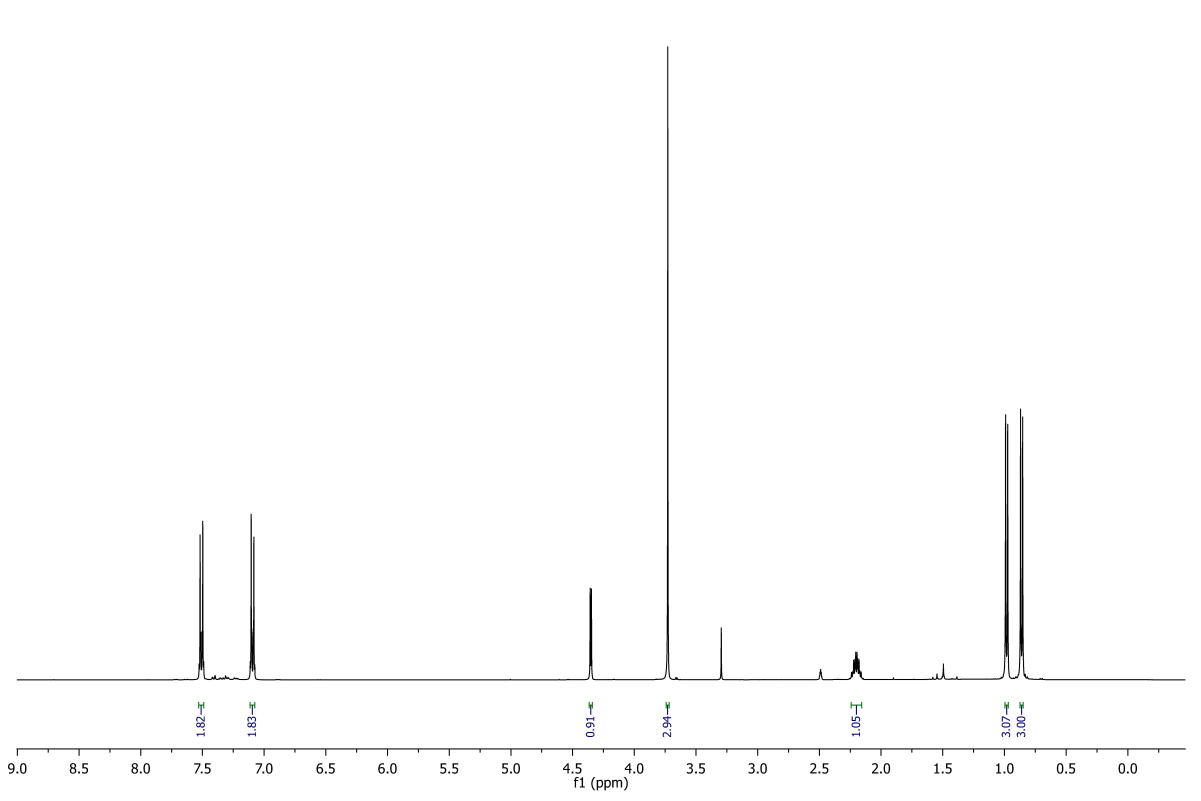
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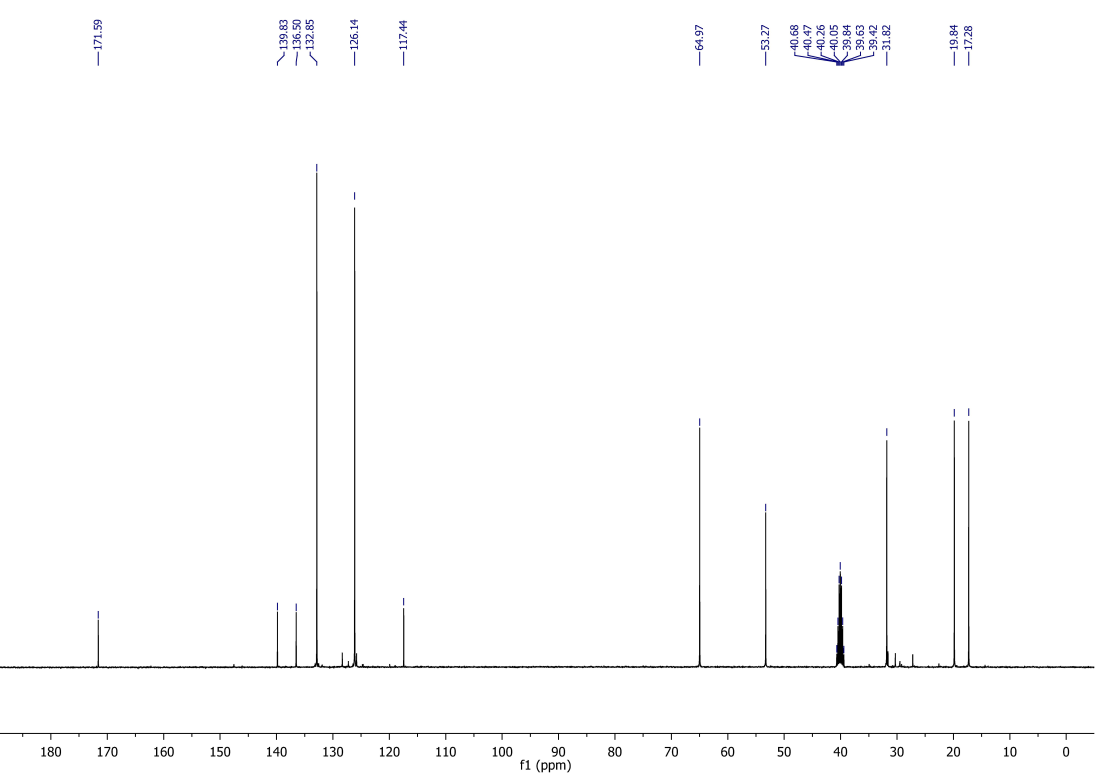
**13C NMR spectrum of compound 3a\***



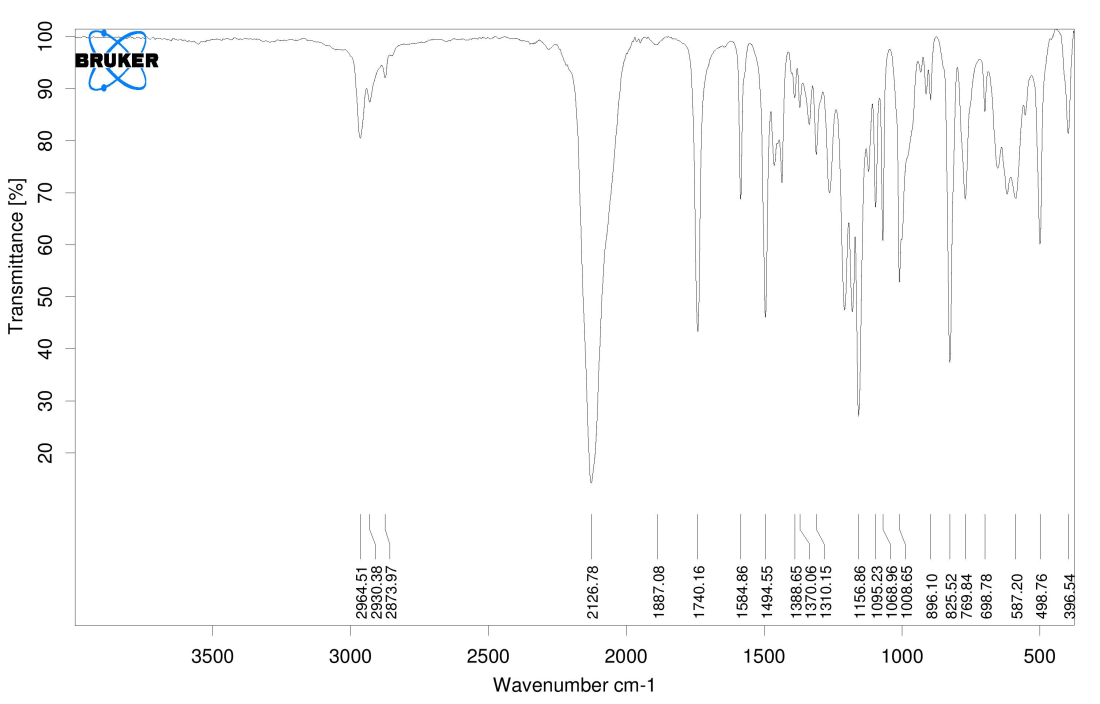
**HRMS spectrum of compound 3b**



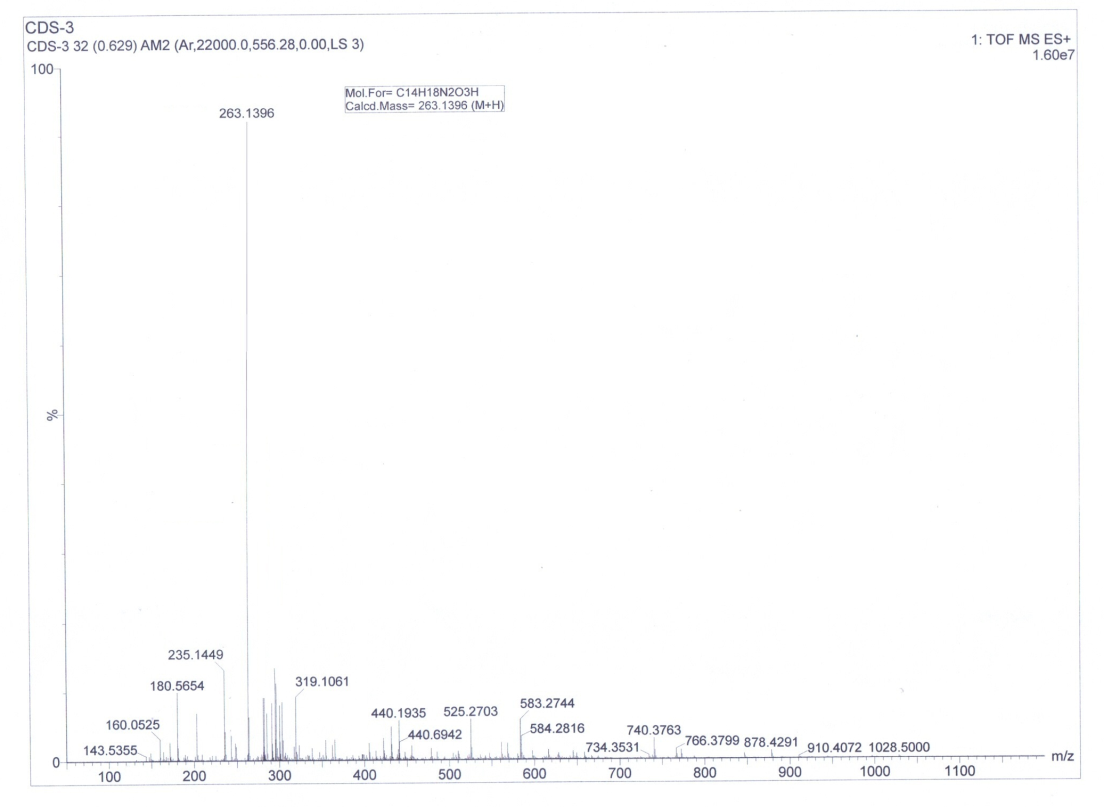
**1H NMR spectrum of compound 3b**

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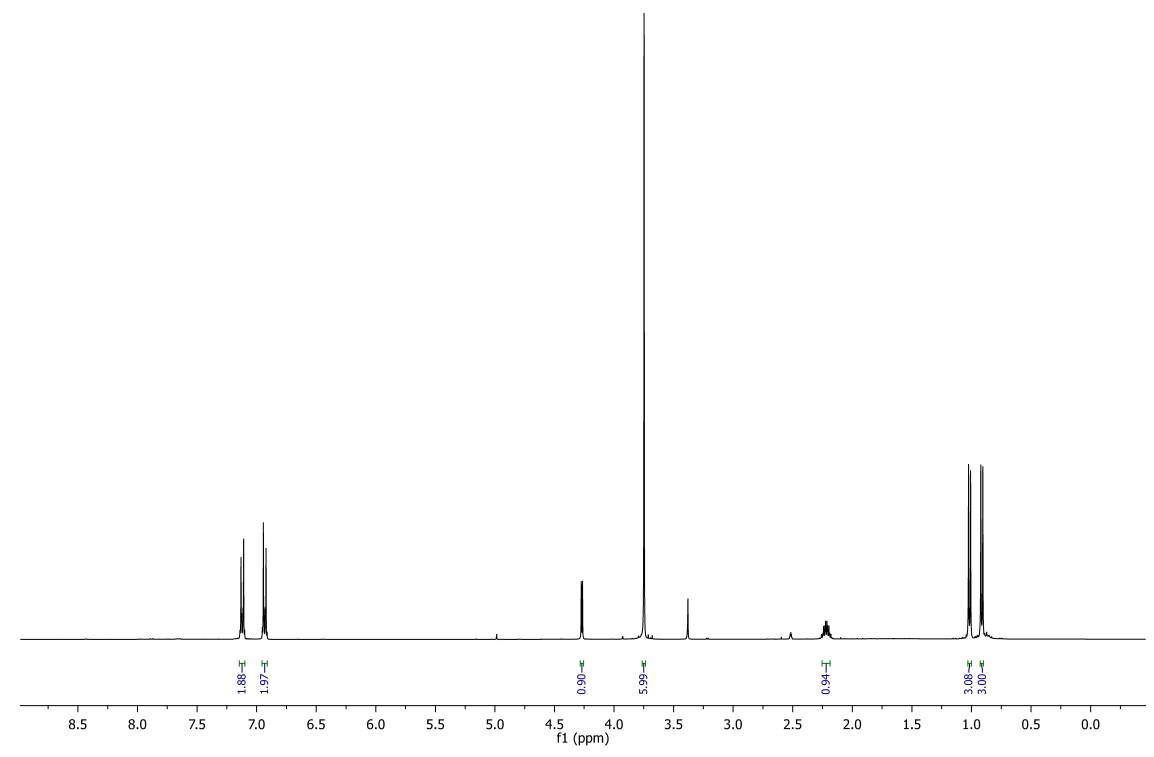
**13C NMR spectrum of compound 3b**

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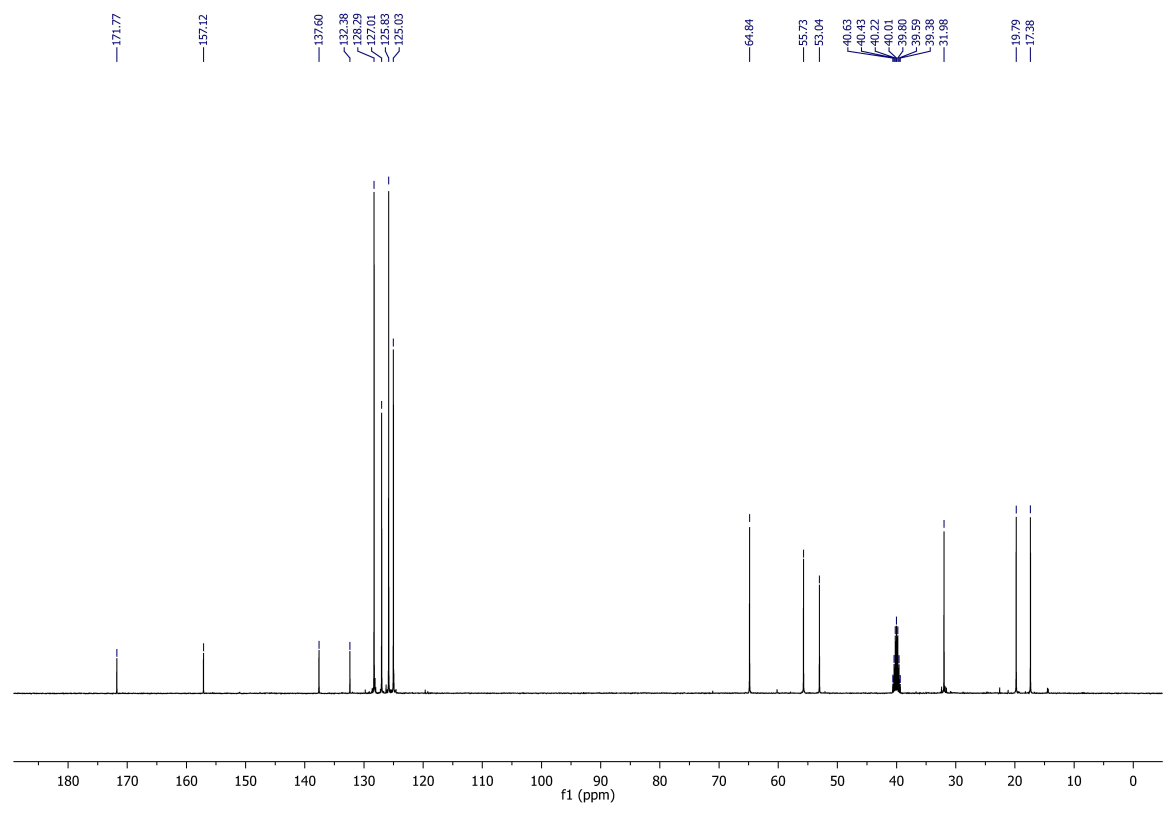
IR Spectrum of compound 3b

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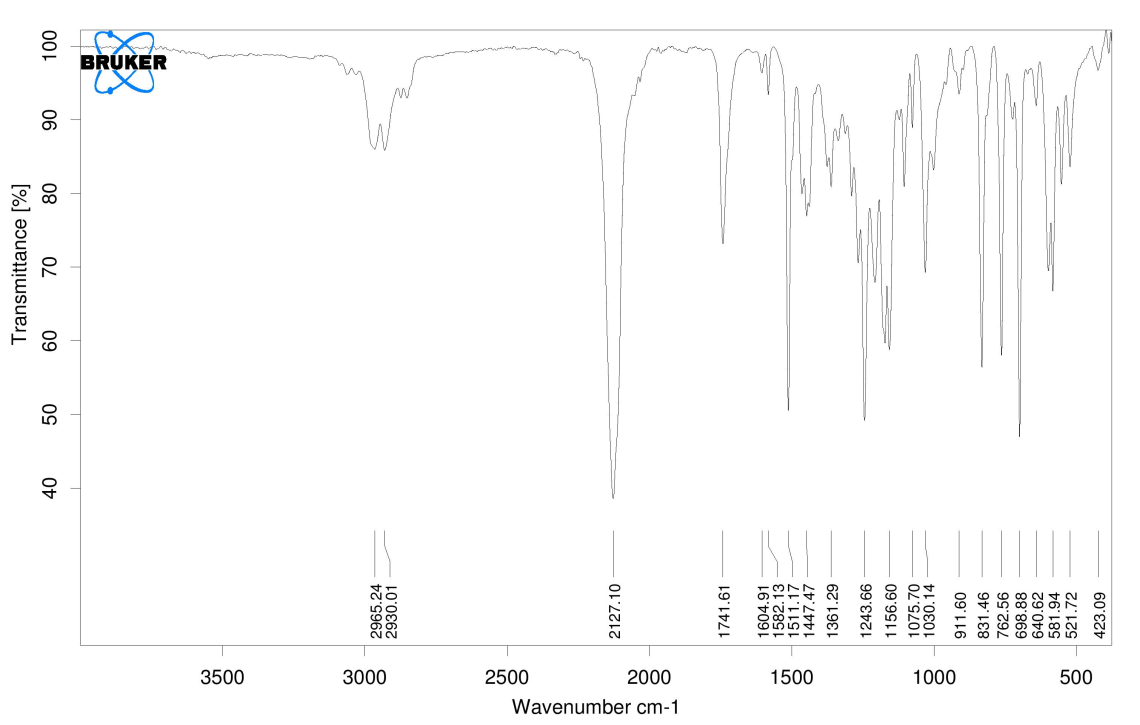
**HRMS spectrum of compound 3c**



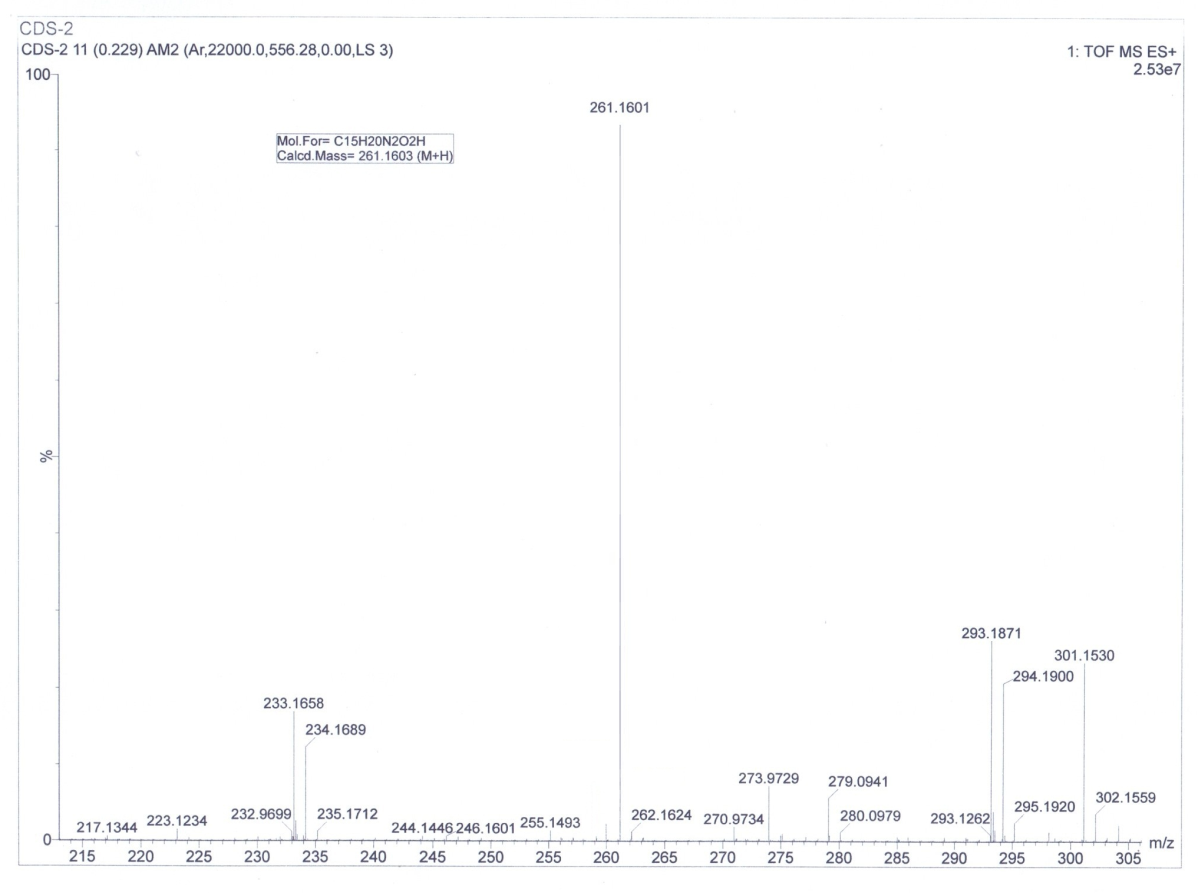
**1H NMR spectrum of compound 3c**



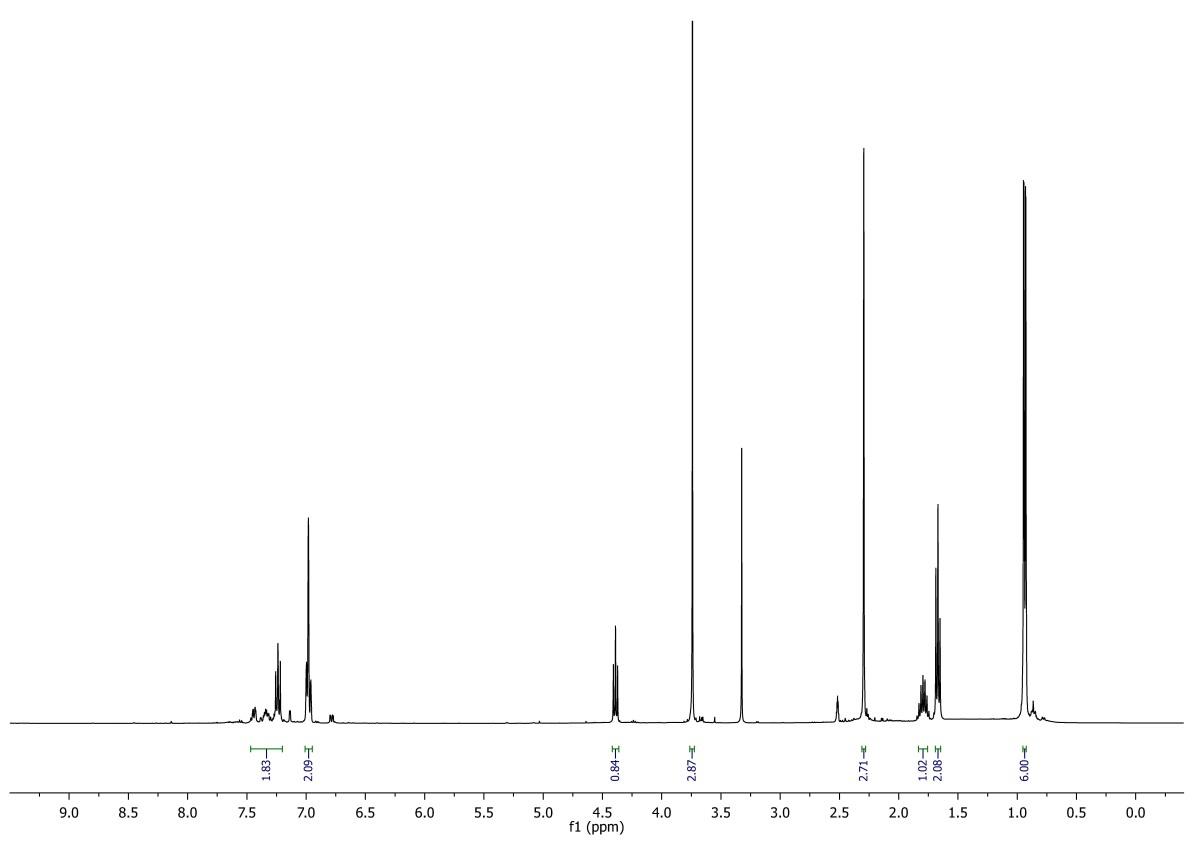
**13C NMR spectrum of compound 3c**

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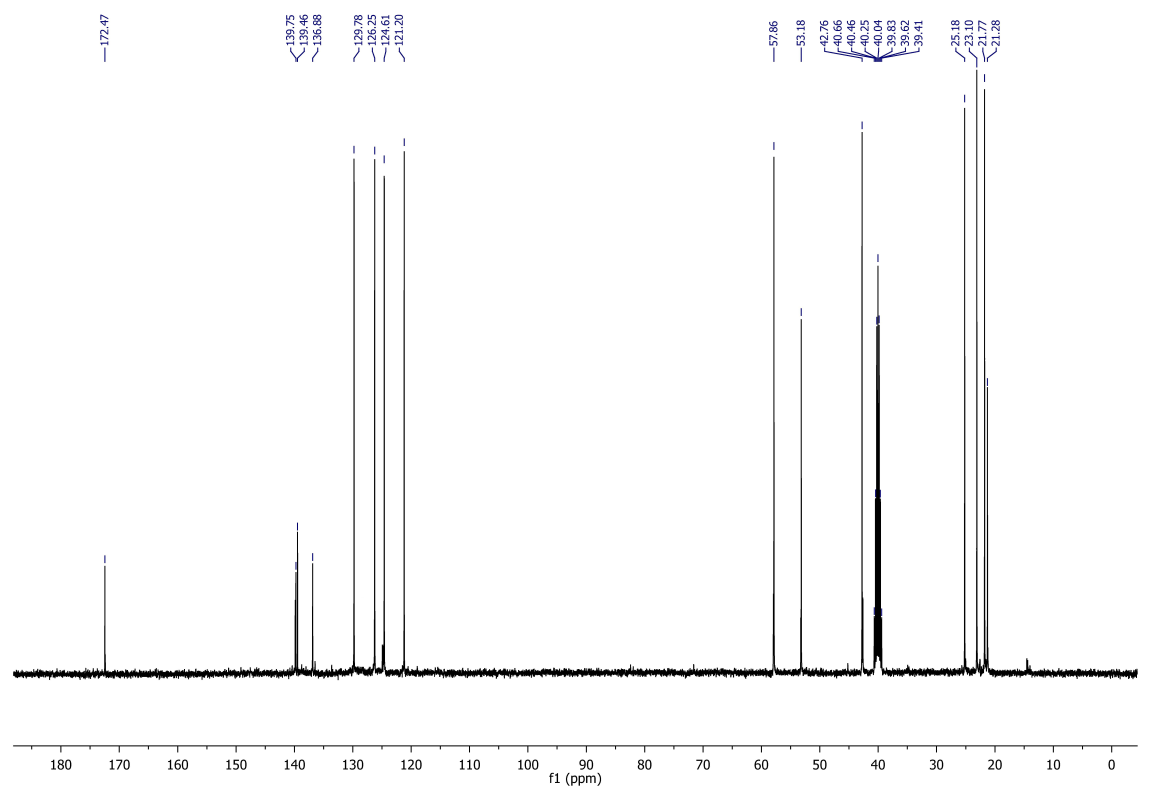
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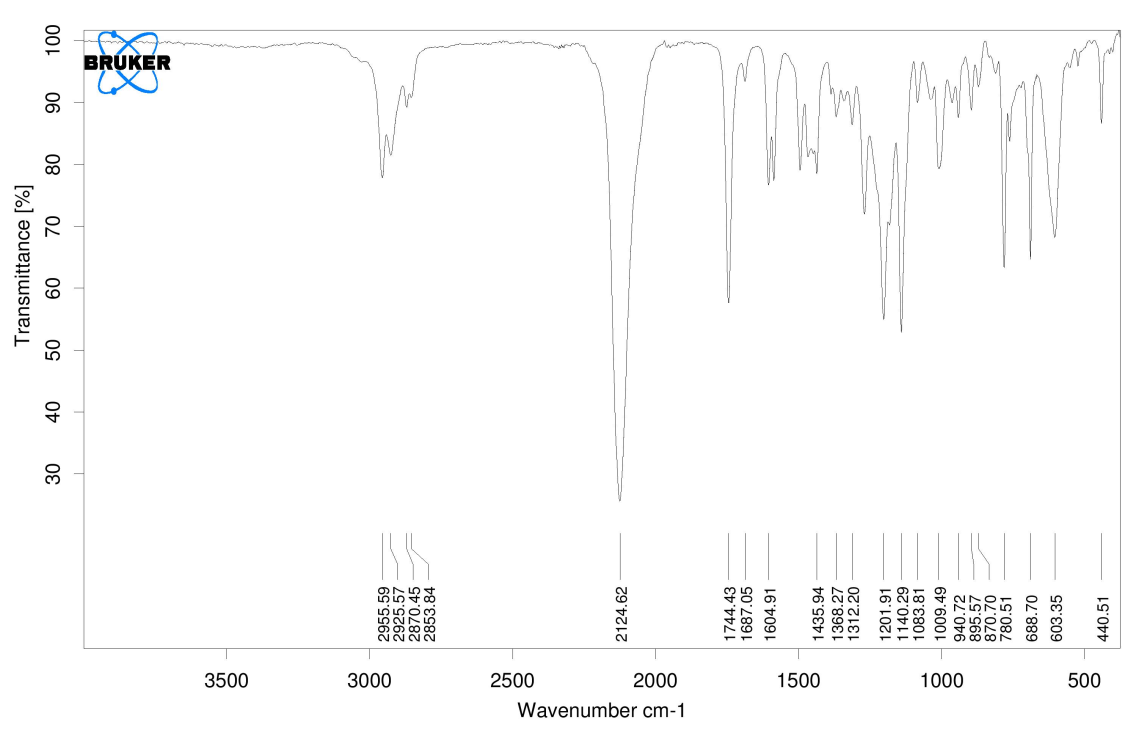
**HRMS spectrum of compound 3d**

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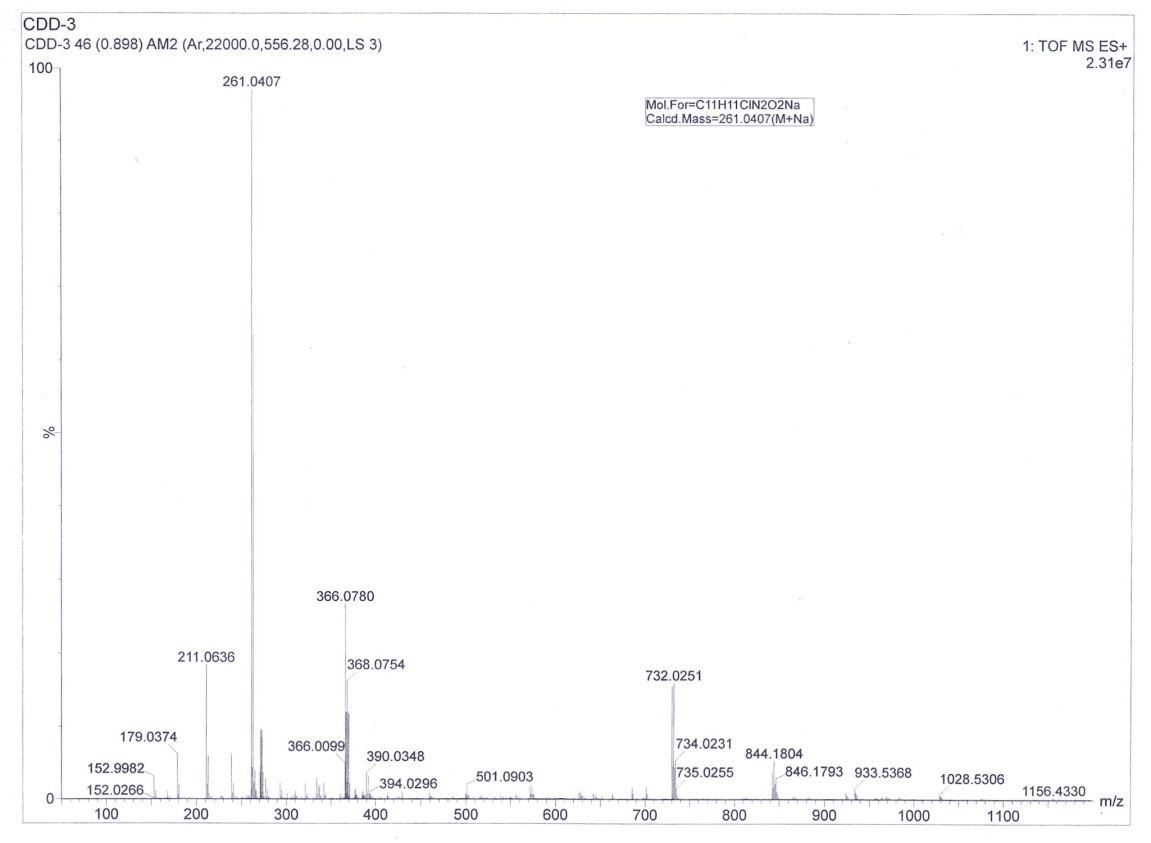
**1H NMR spectrum of compound 3d**

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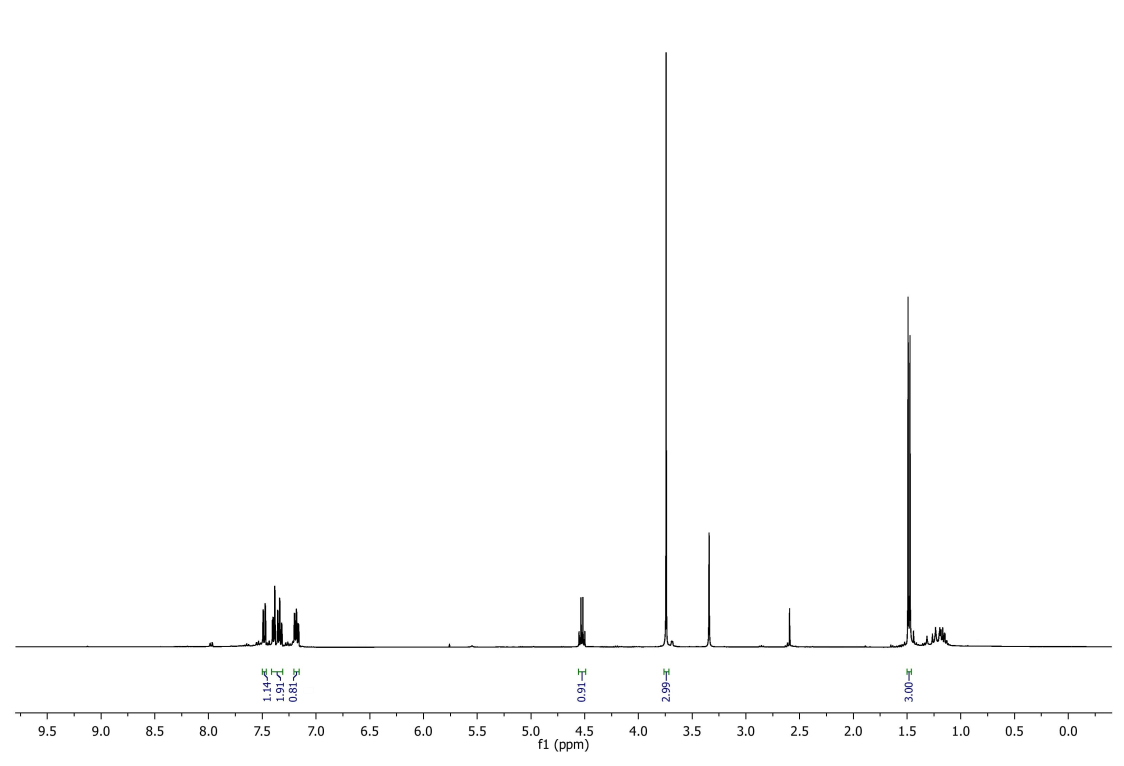
**13C NMR spectrum of compound 3d**



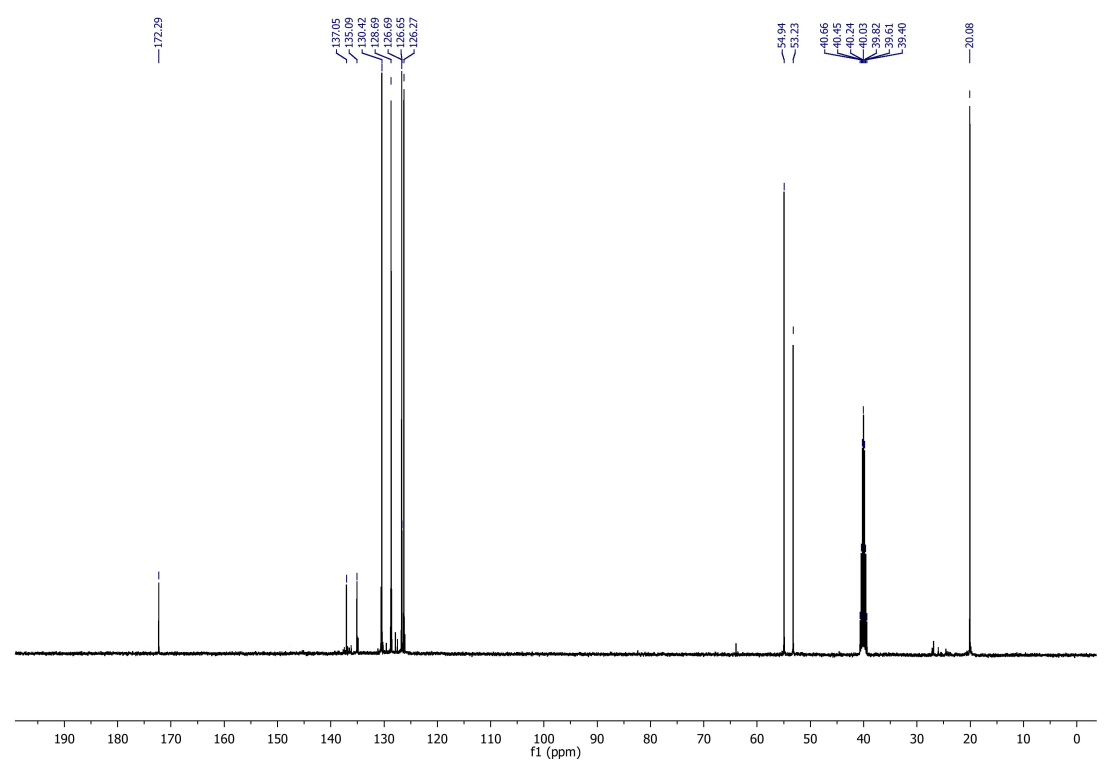
IR Spectrum of compound 3d



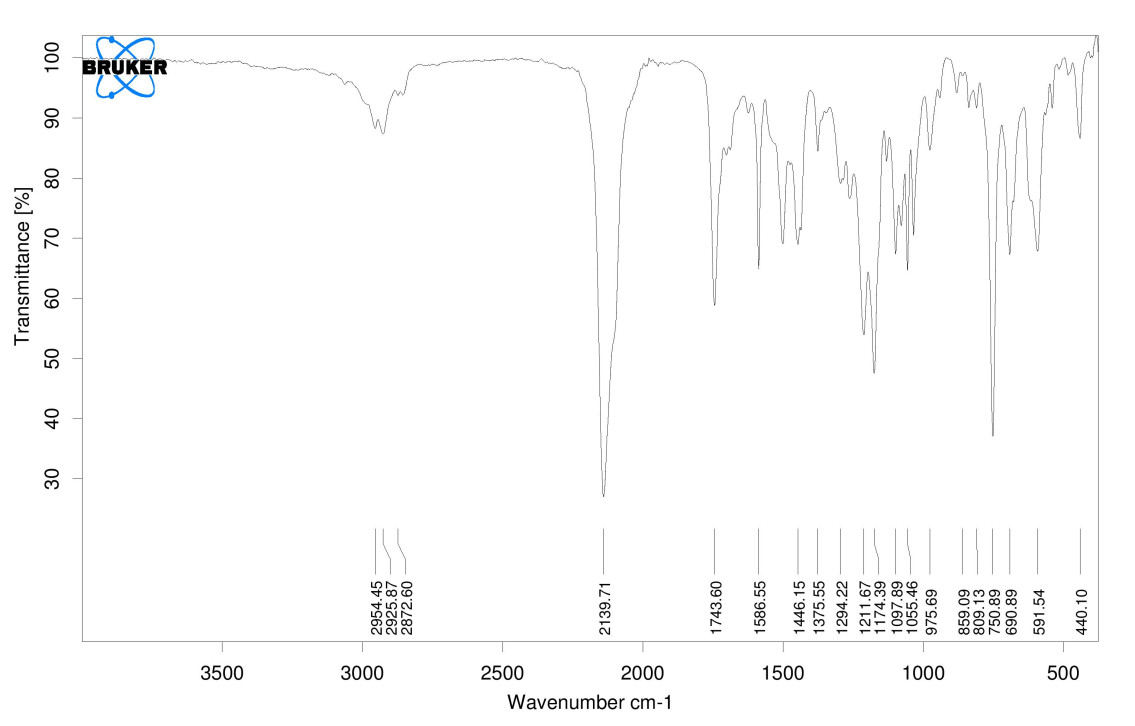
**HRMS spectrum of compound 3e**



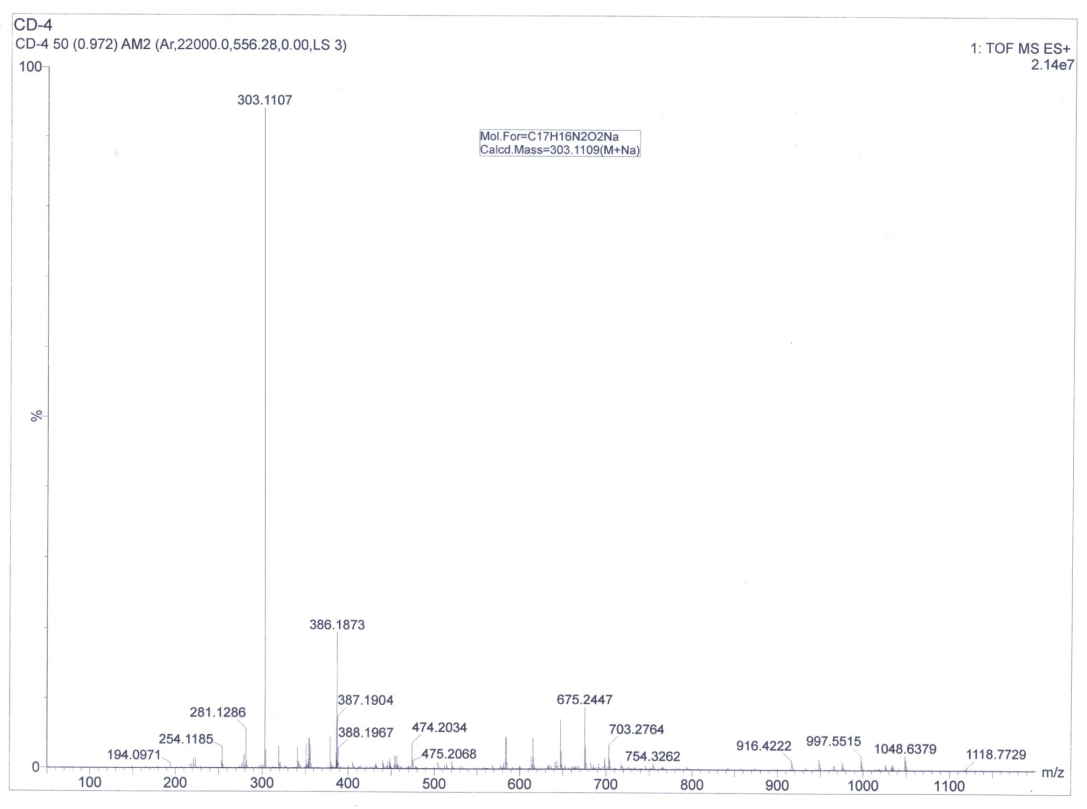
**1H NMR spectrum of compound 3e**



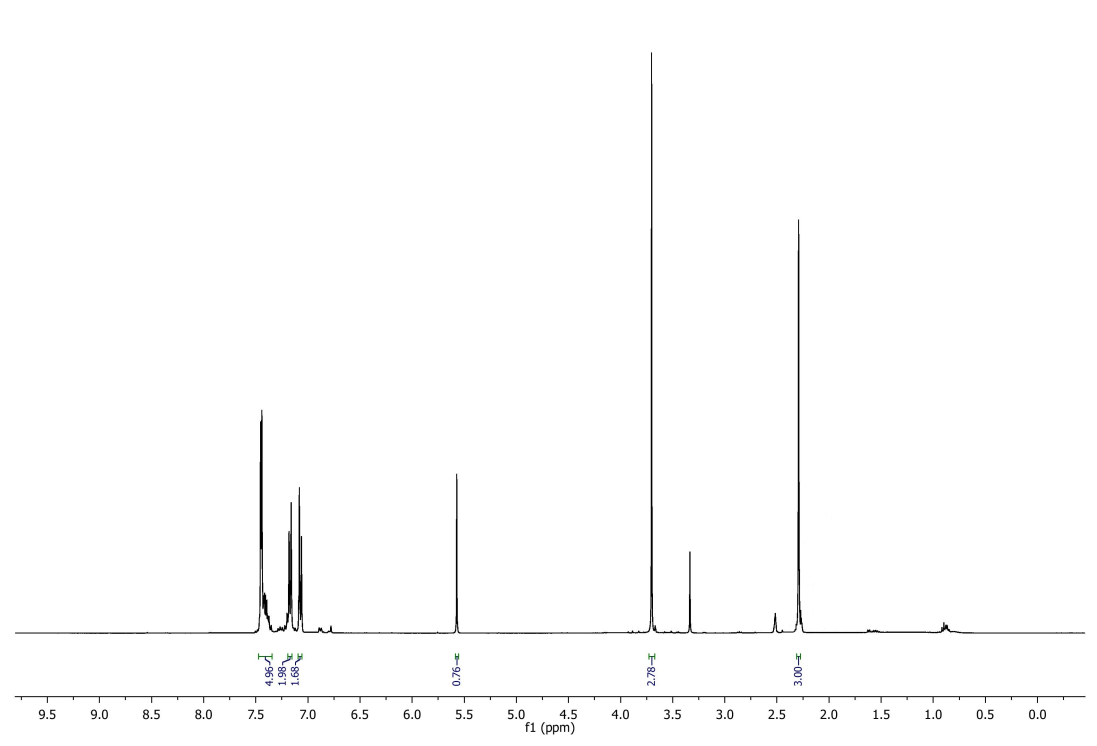
**13C NMR spectrum of compound 3e**

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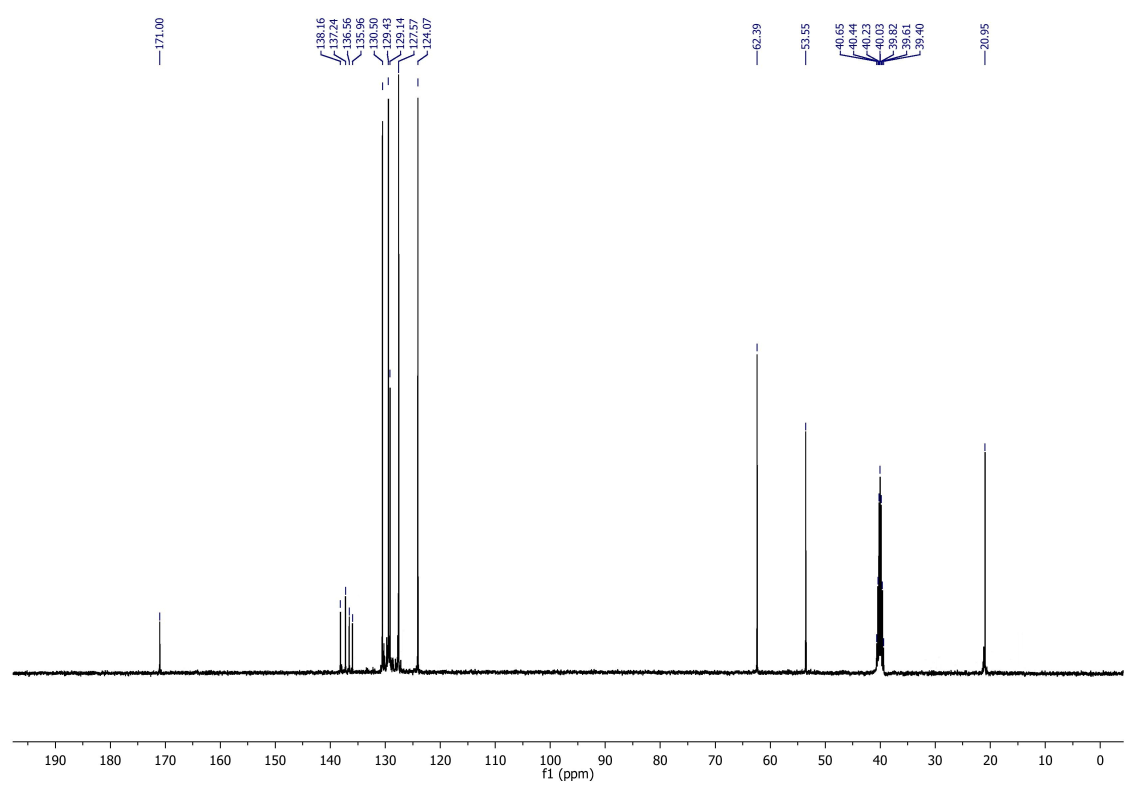
**IR Spectrum of compound 3e**



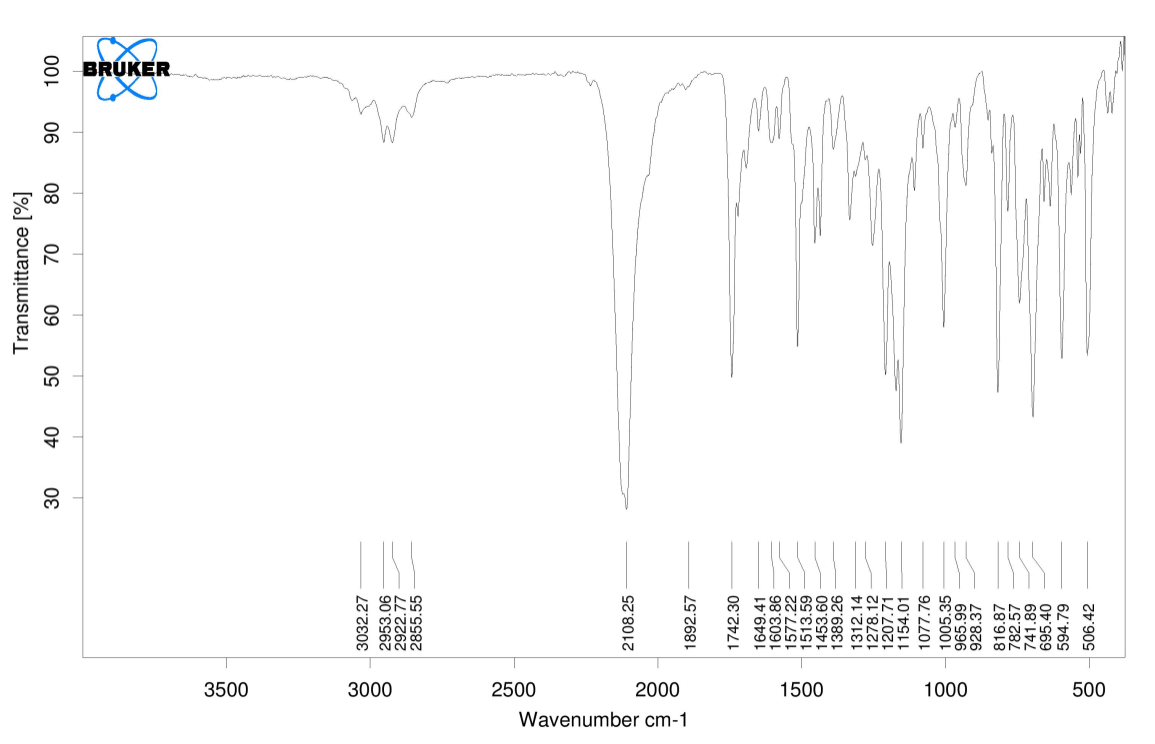
**HRMS spectrum of compound 3f**



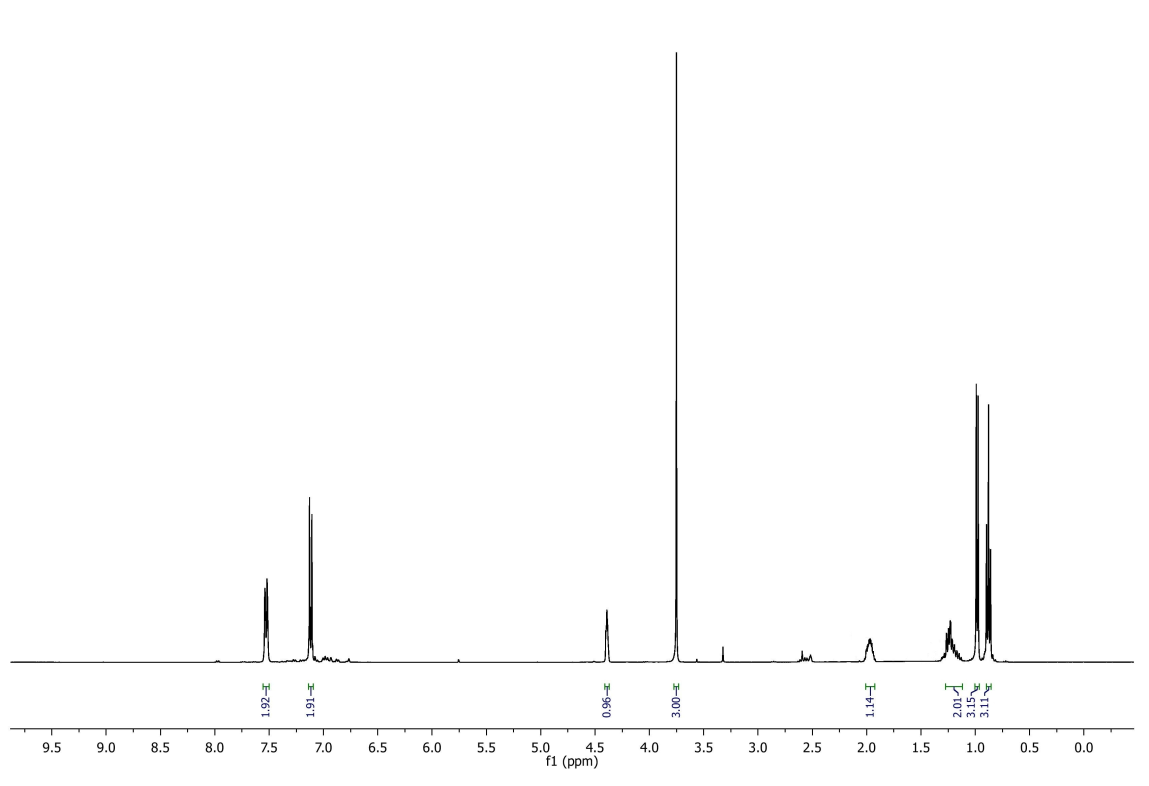
**1H NMR spectrum of compound 3f**



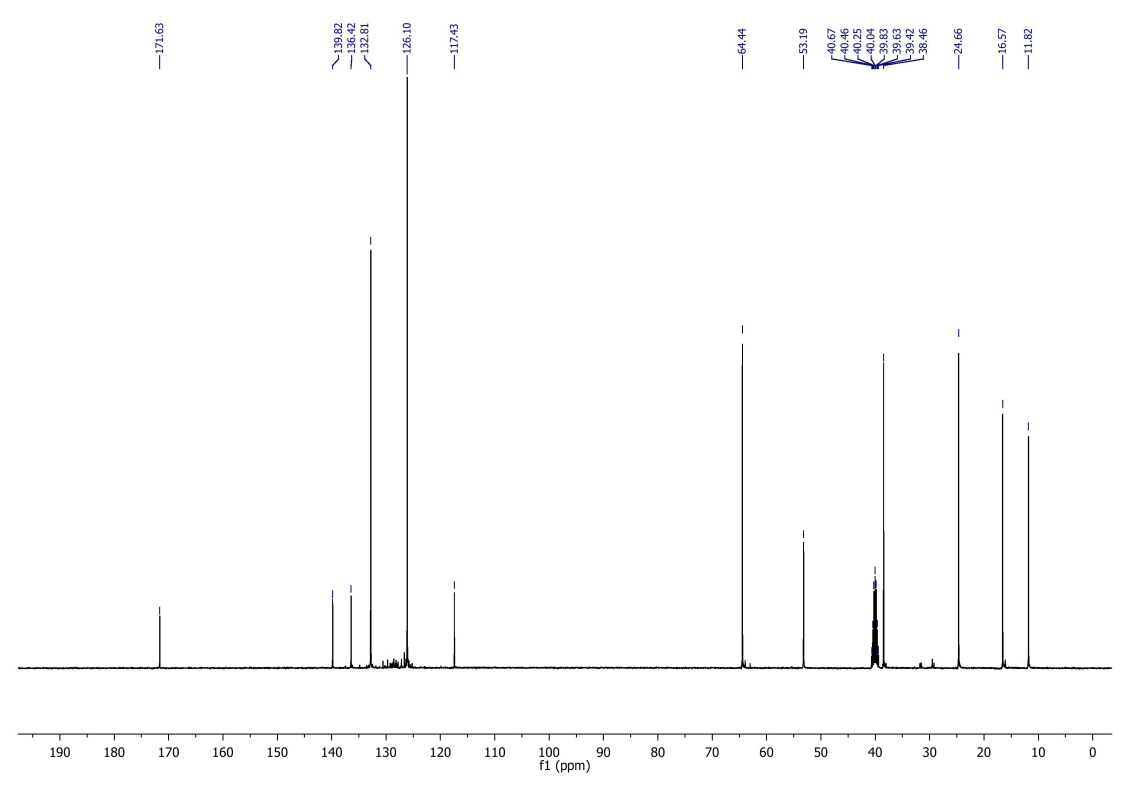
**13C NMR spectrum of compound 3f**

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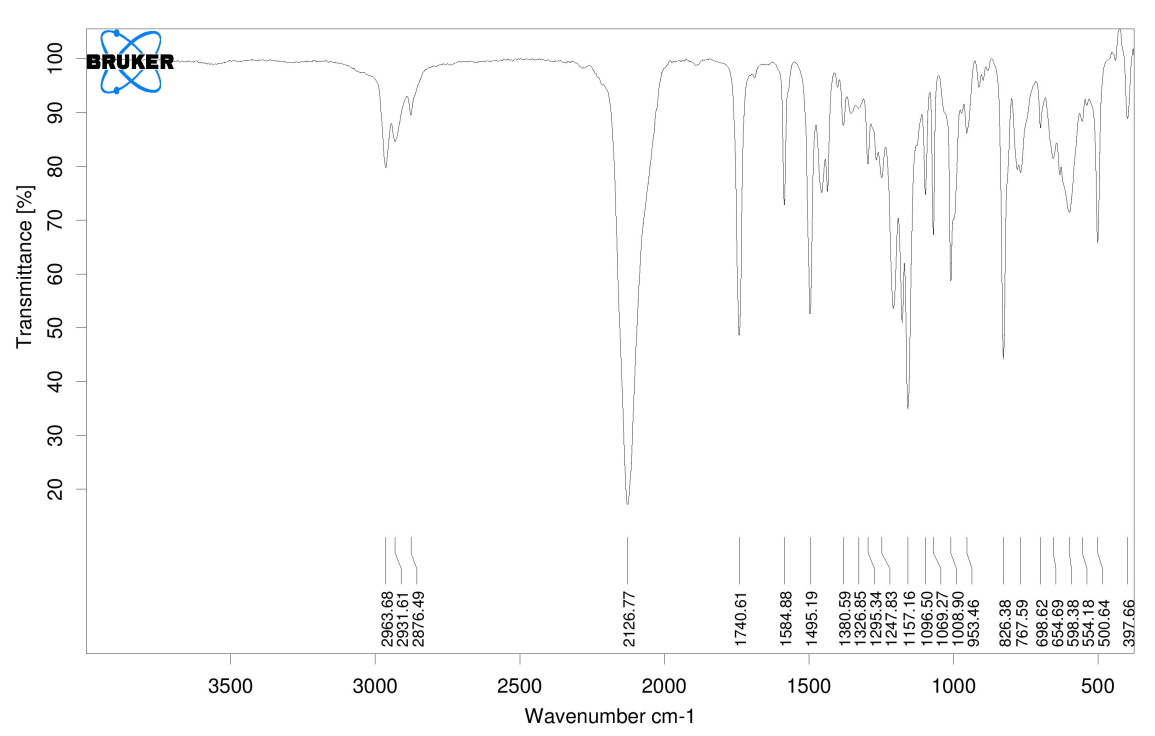
**IR Spectrum of compound 3f**

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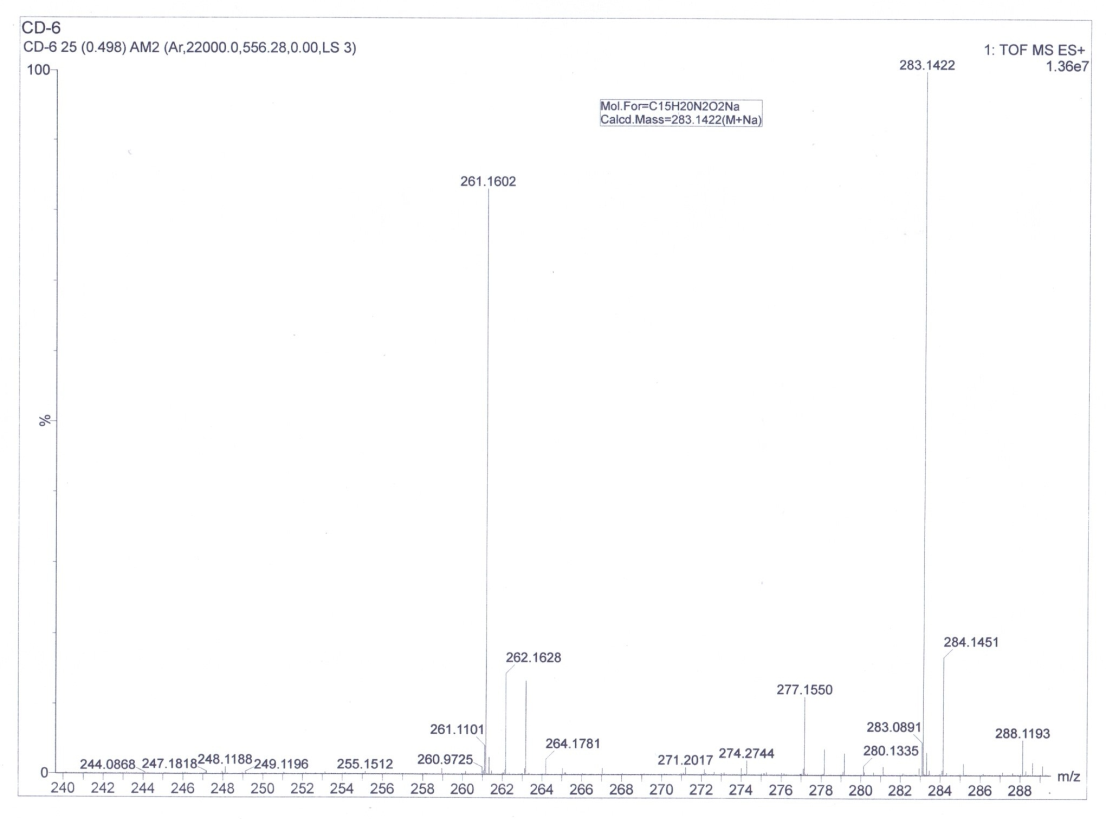
**1H NMR spectrum of compound 3g**

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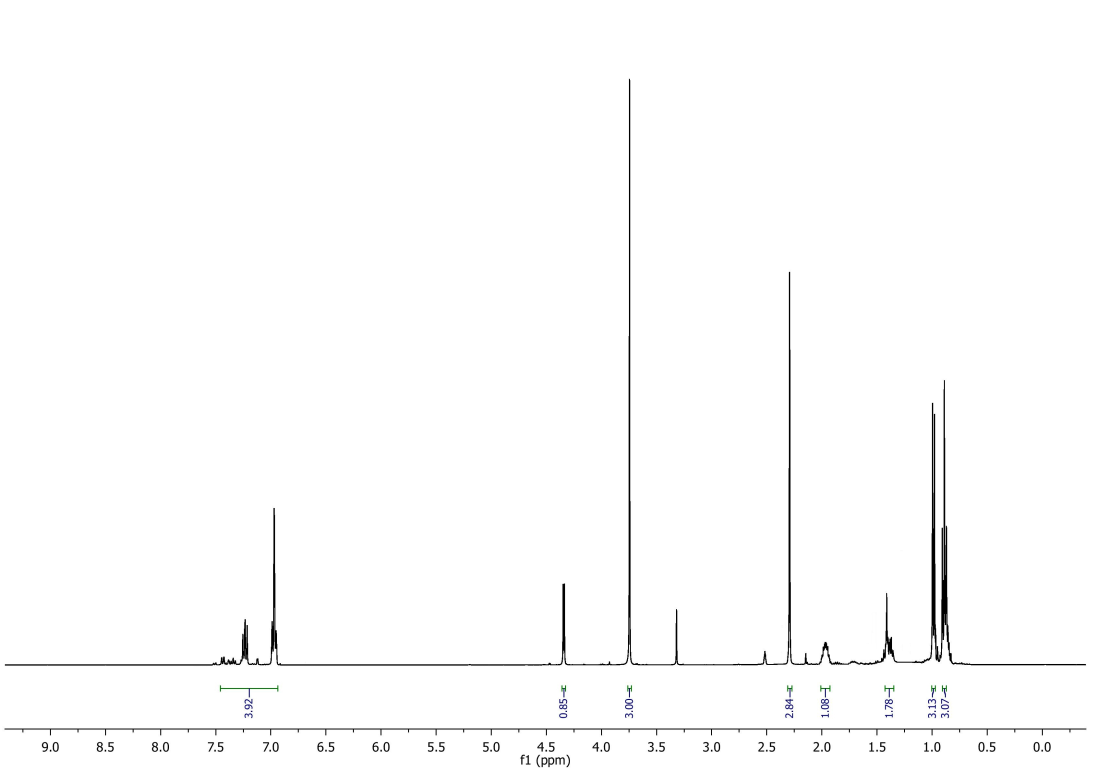
**13C NMR spectrum of compound 3g**



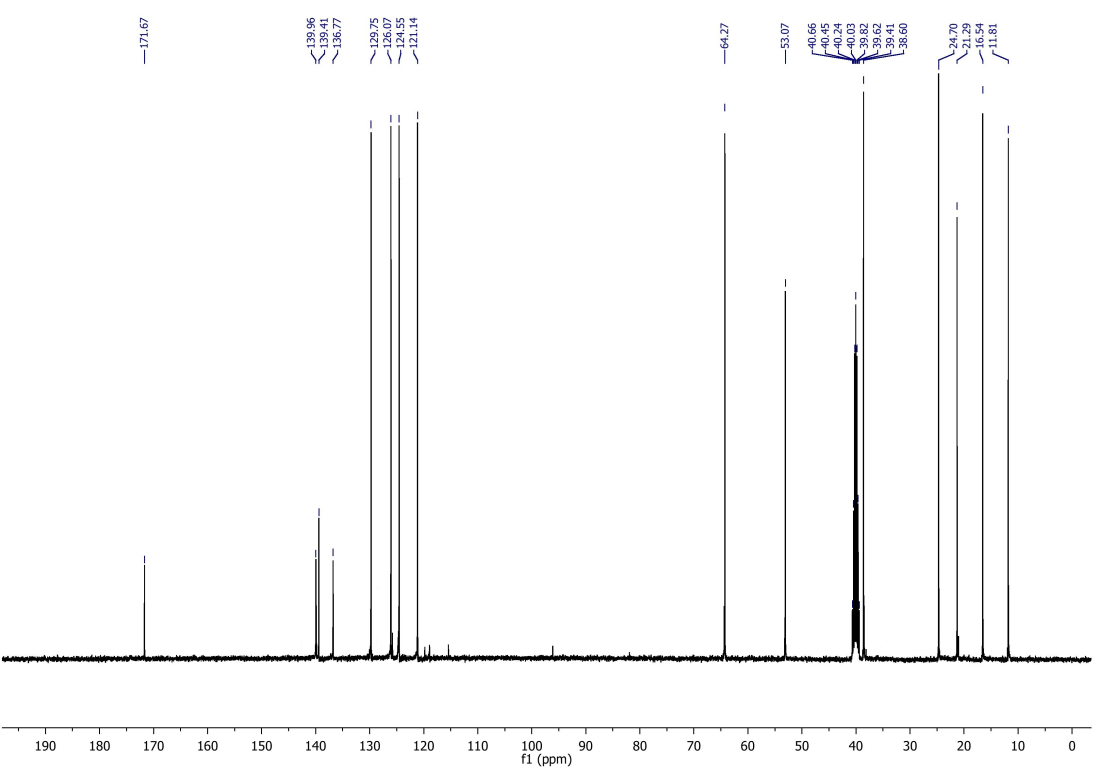
**IR Spectrum of compound 3g**



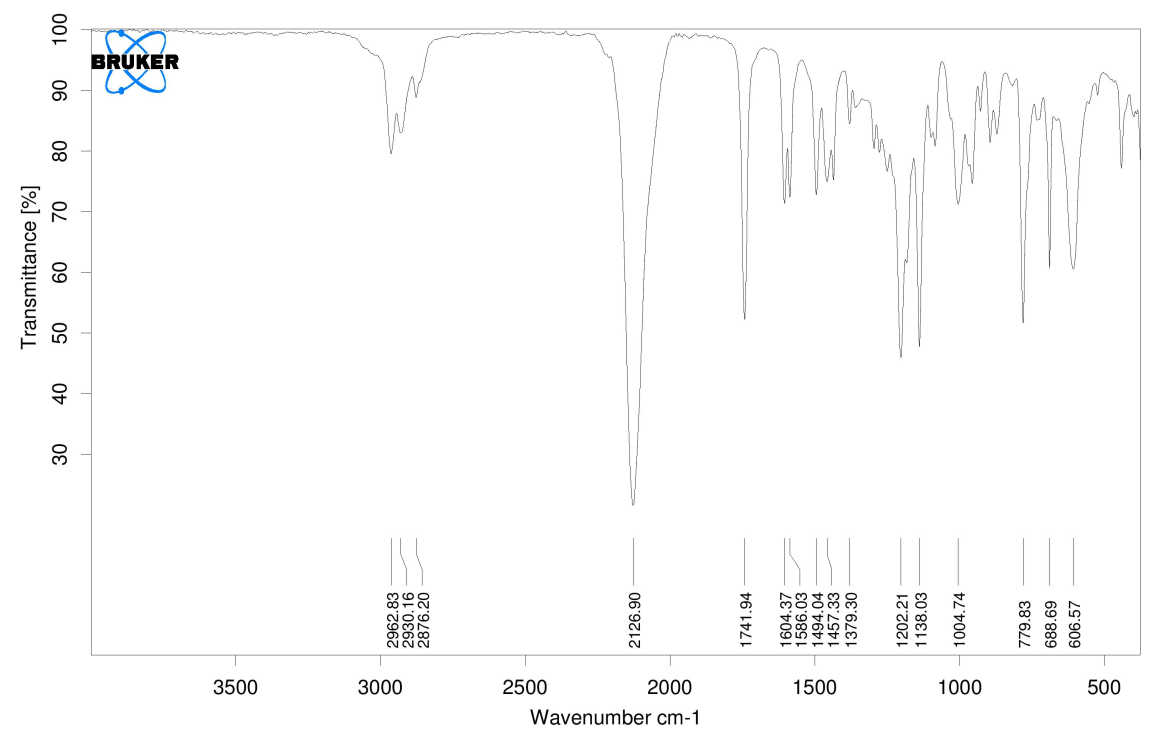
**HRMS spectrum of compound 3h**

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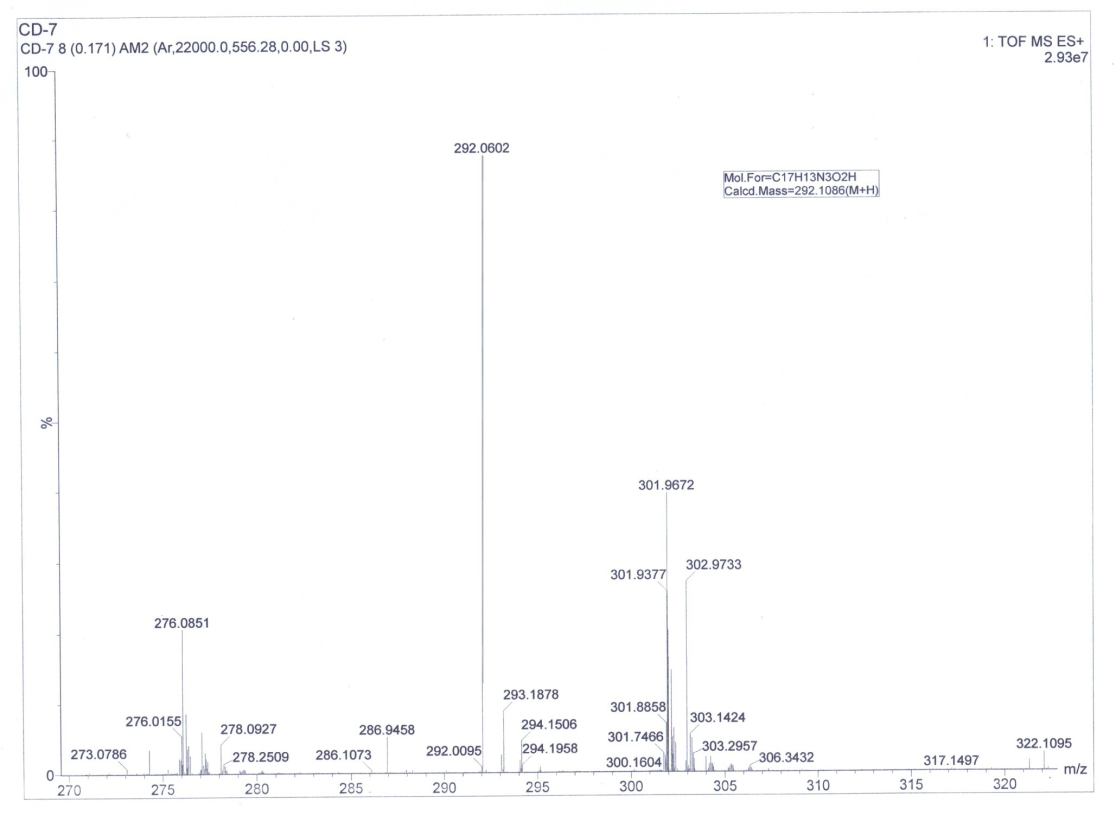
**1H NMR spectrum of compound 3h**

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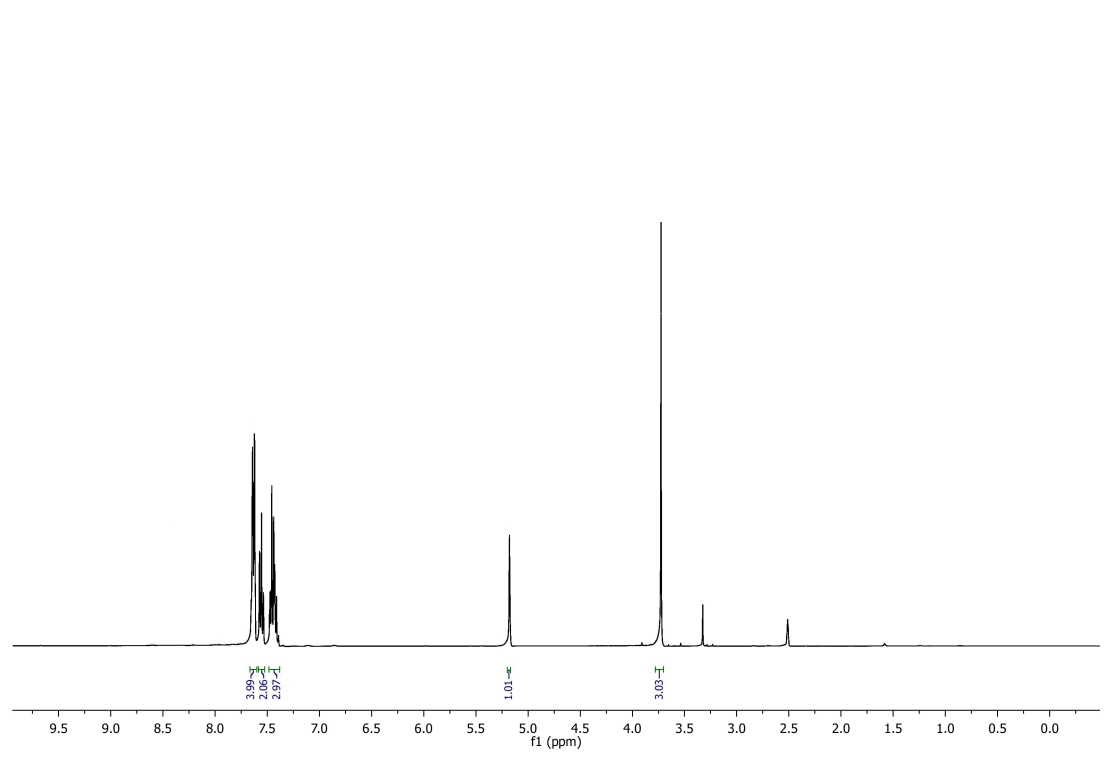
**13C NMR spectrum of compound 3h**



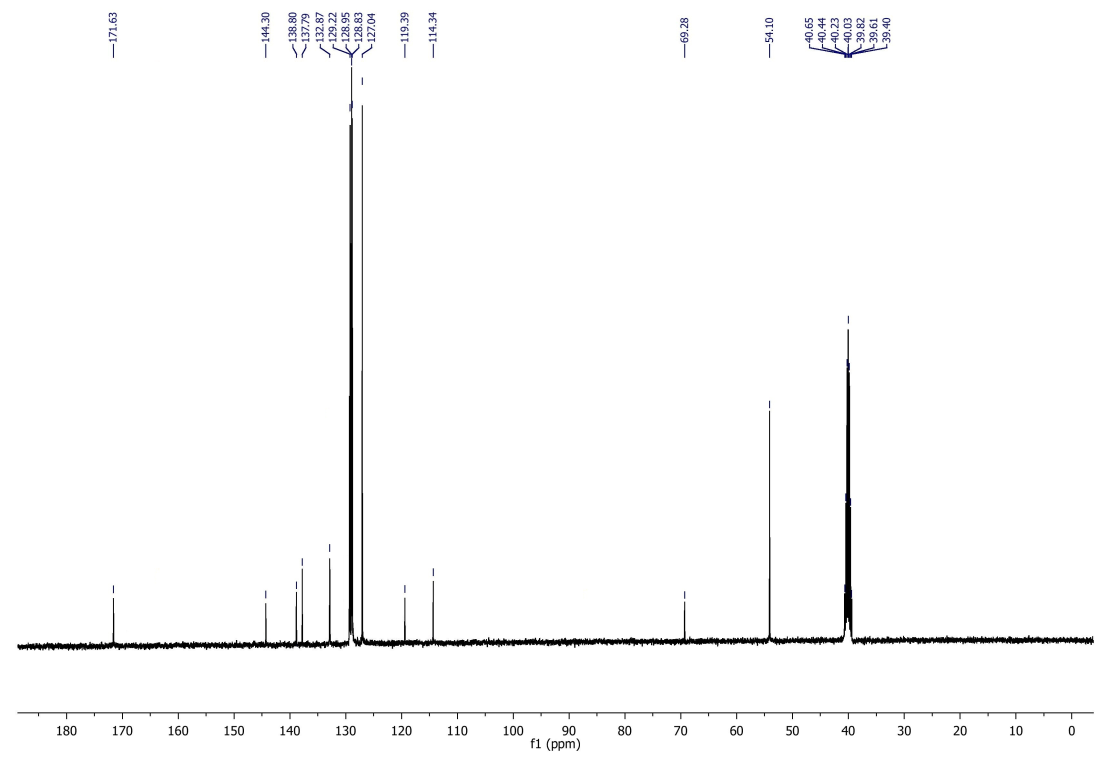
IR Spectrum of compound 3h

****

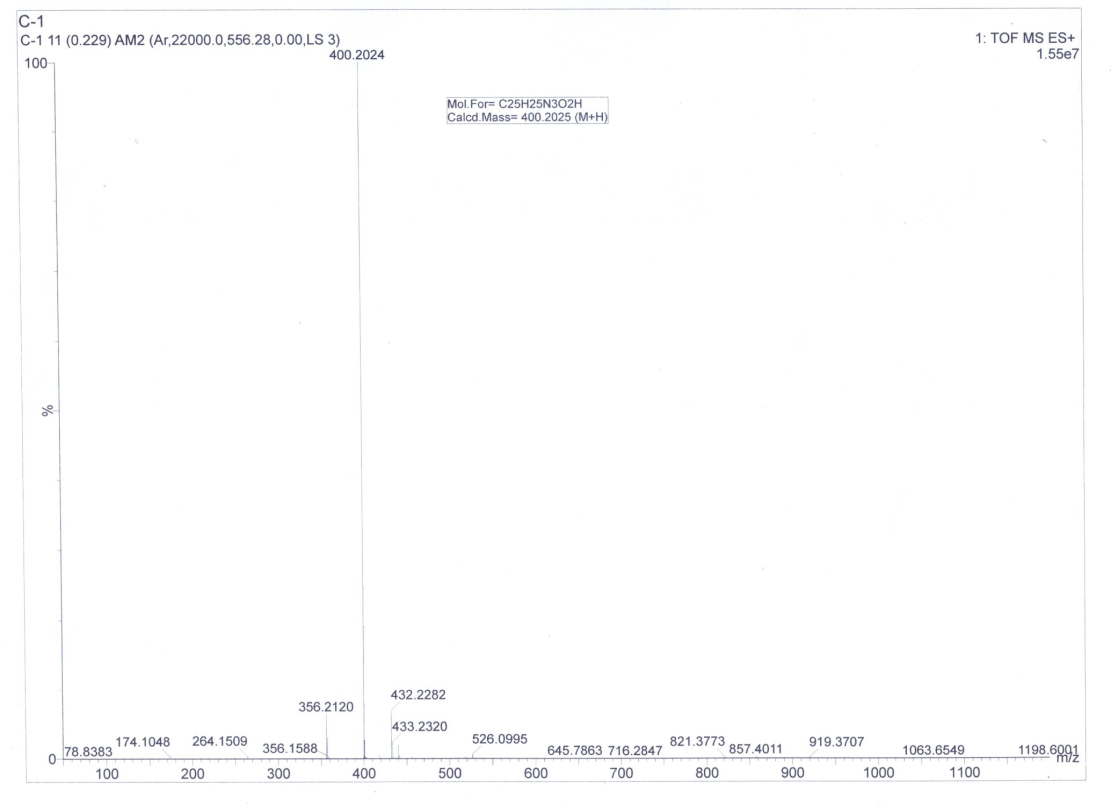
**HRMS spectrum of compound 3i**

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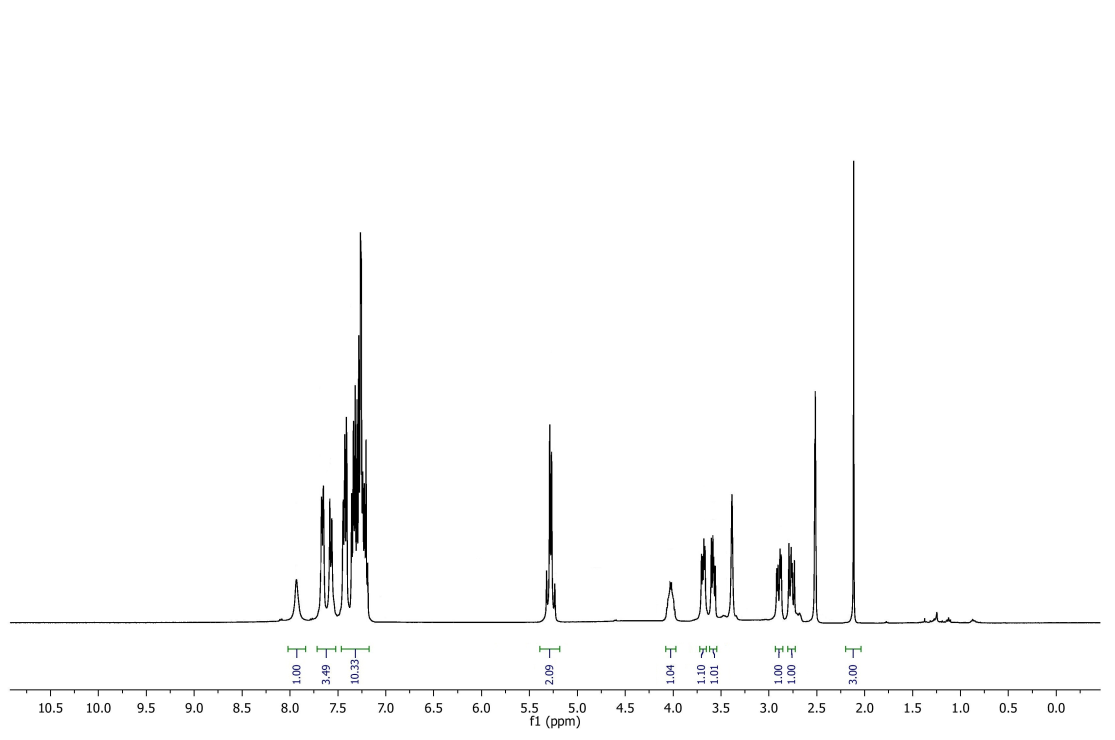
**1H NMR spectrum of compound 3i**

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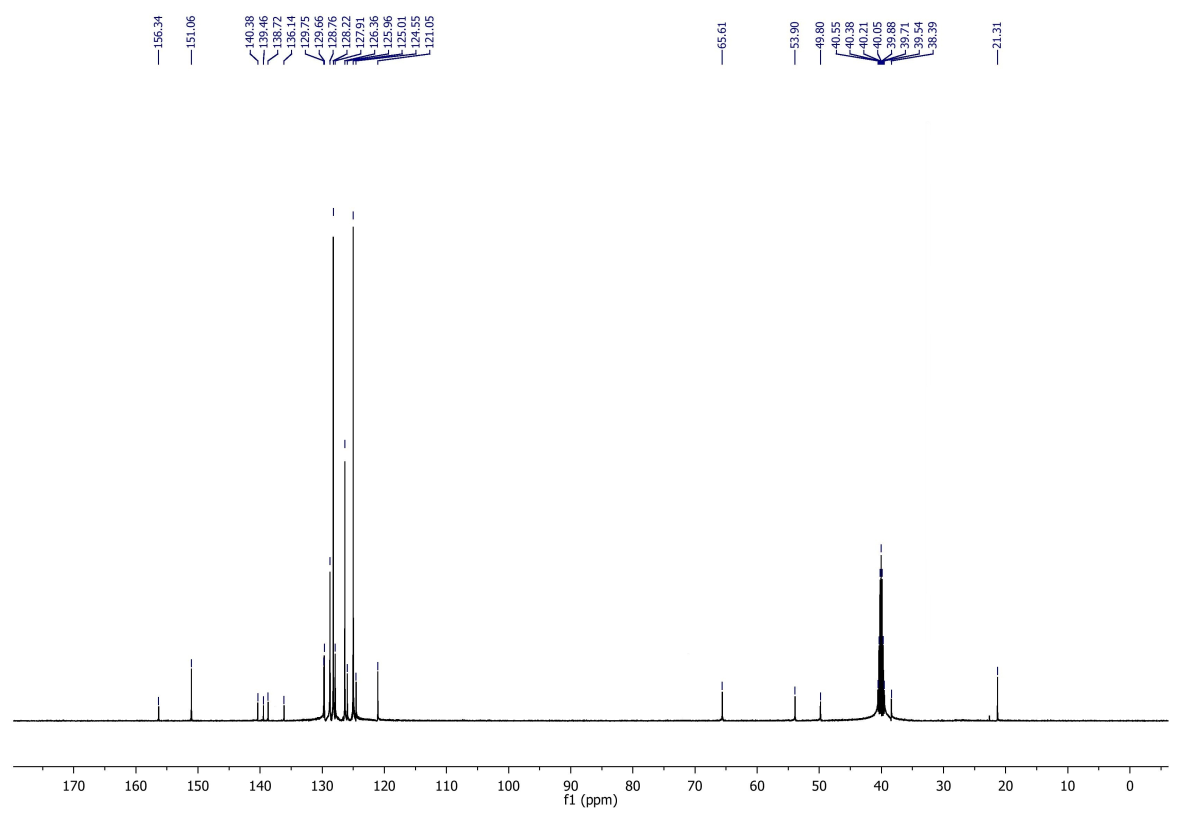
**13C NMR spectrum of compound 3i**



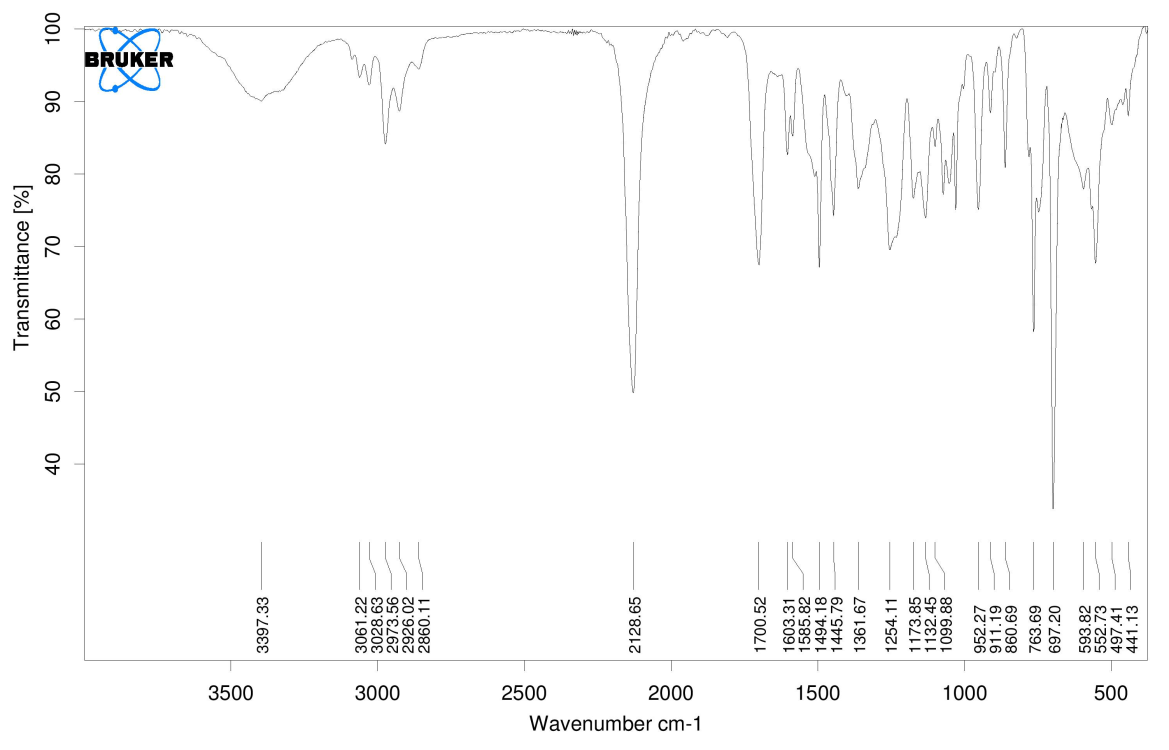
**HRMS spectrum of compound 6a**



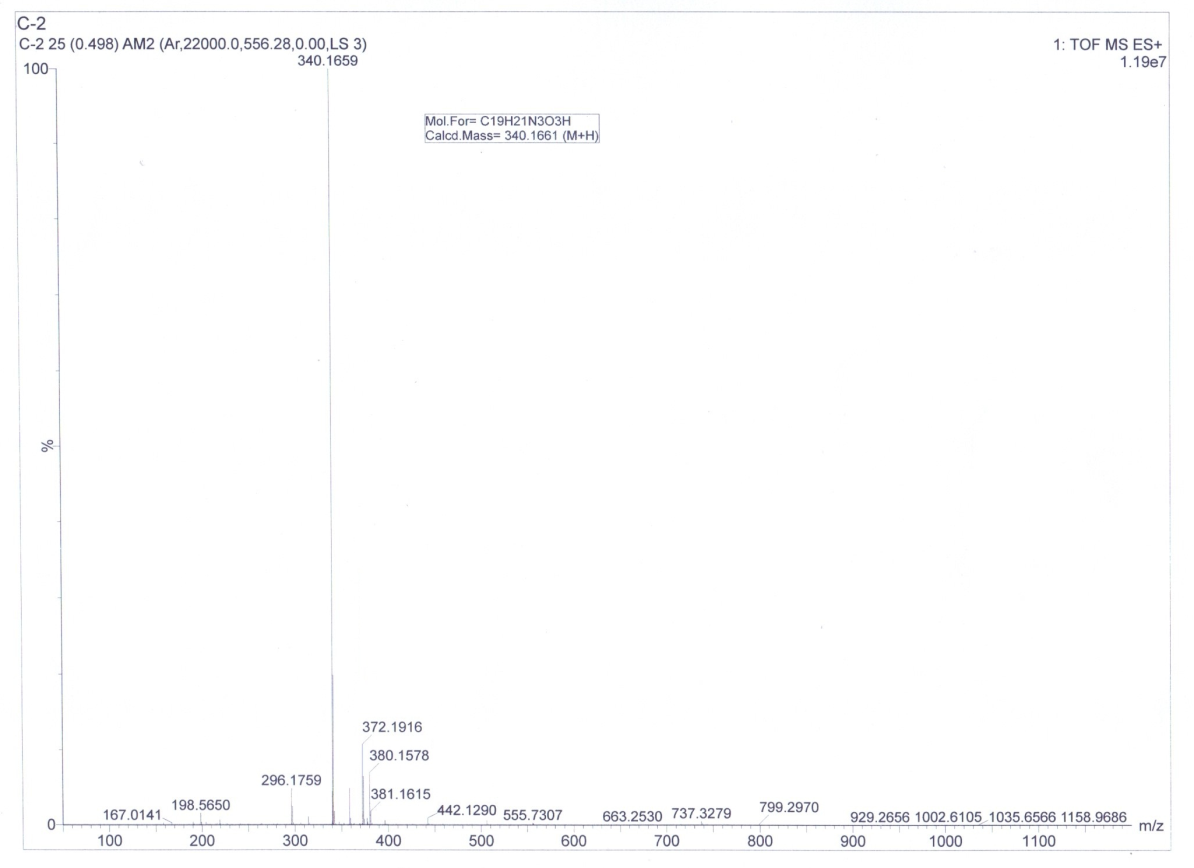
**1H NMR spectrum of compound 6a**



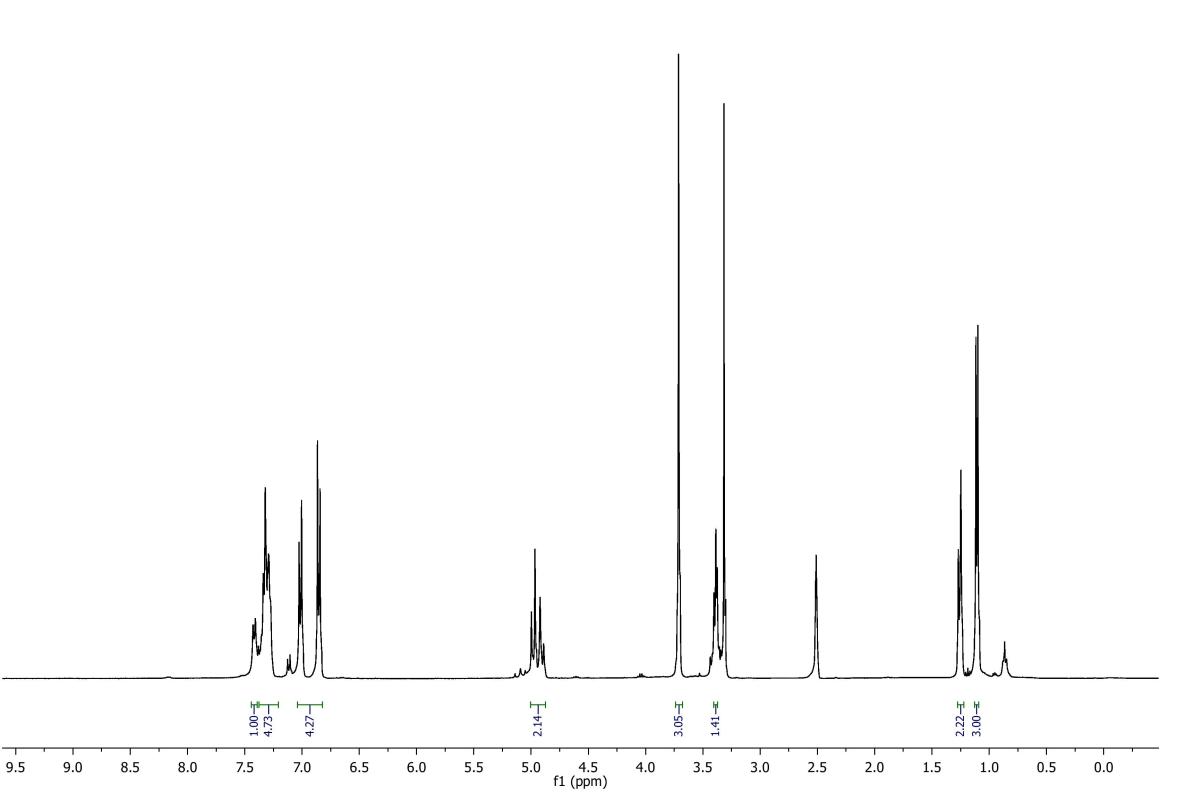
**13C NMR spectrum of compound 6a**



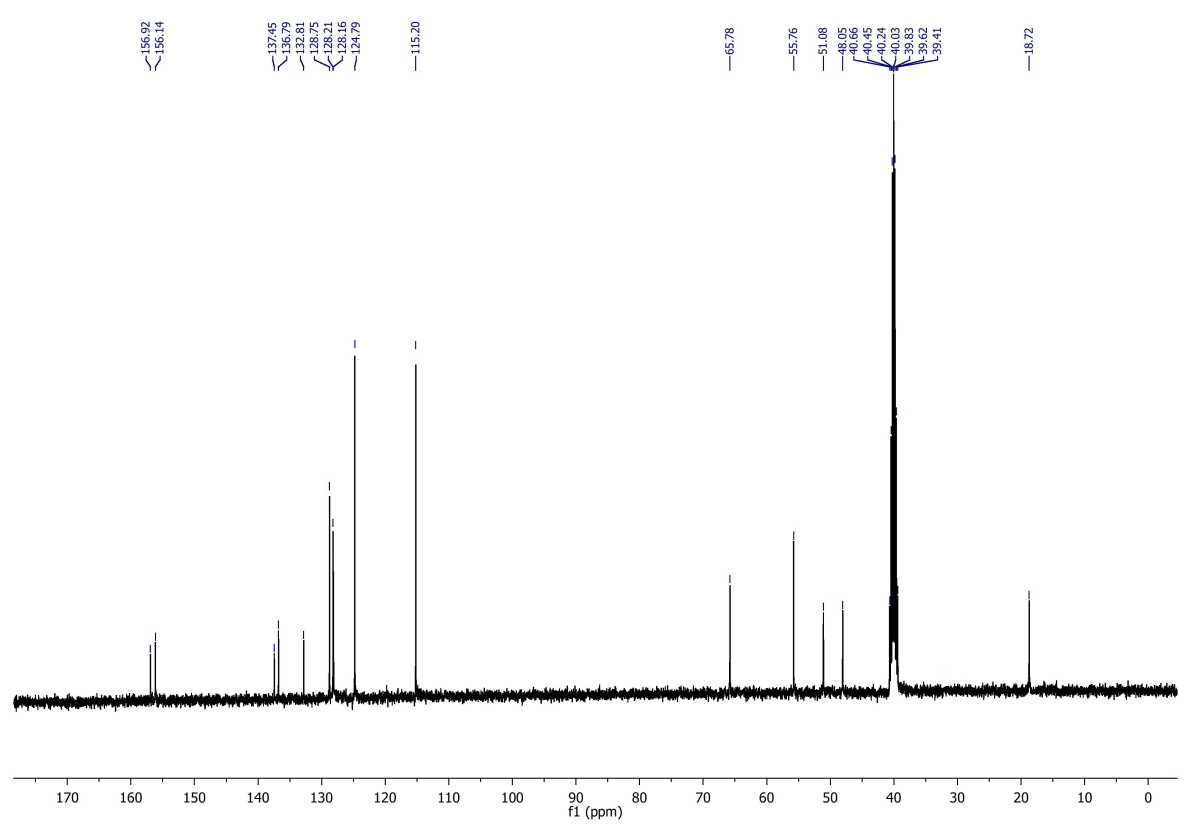
**IR Spectrum of compound 6a**



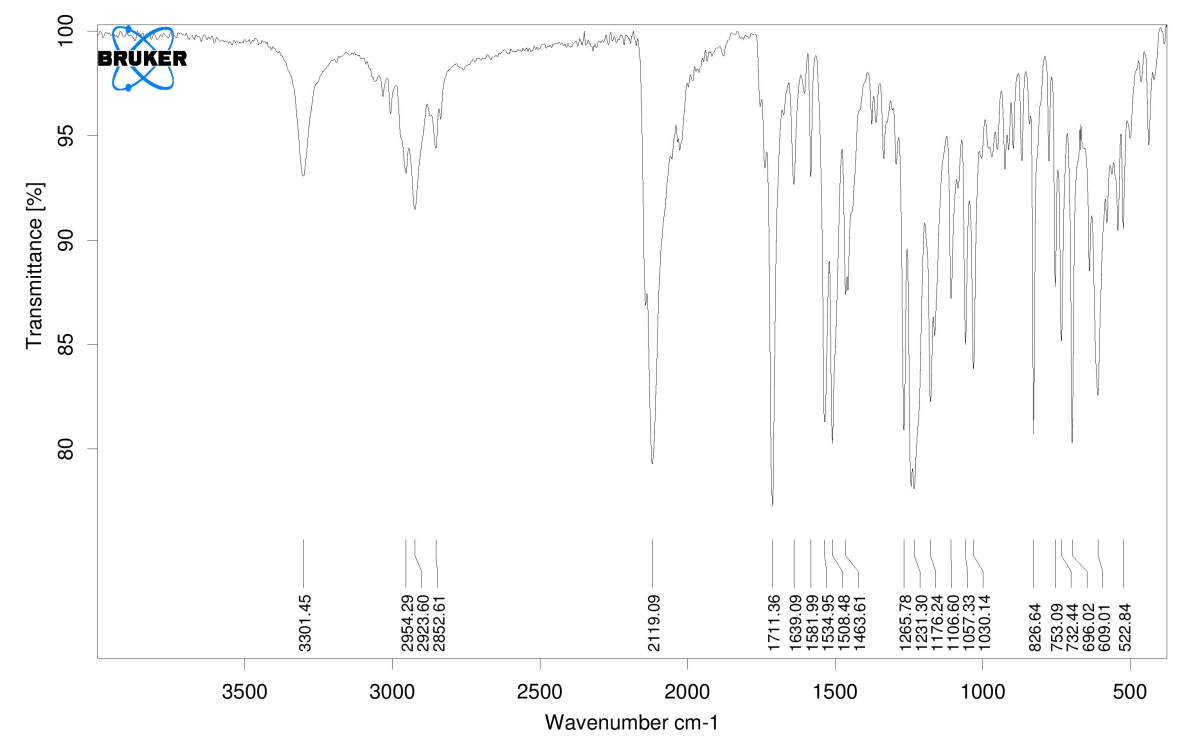
**HRMS spectrum of compound 6b**



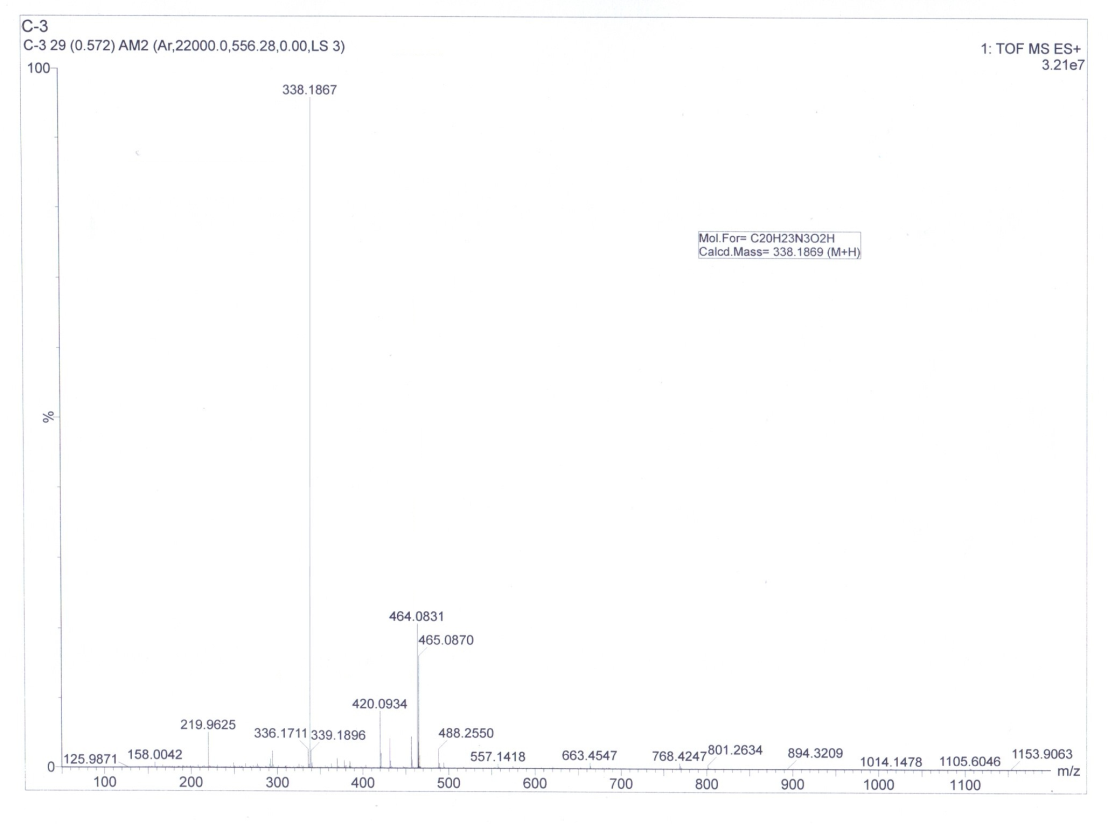
**1H NMR spectrum of compound 6b**



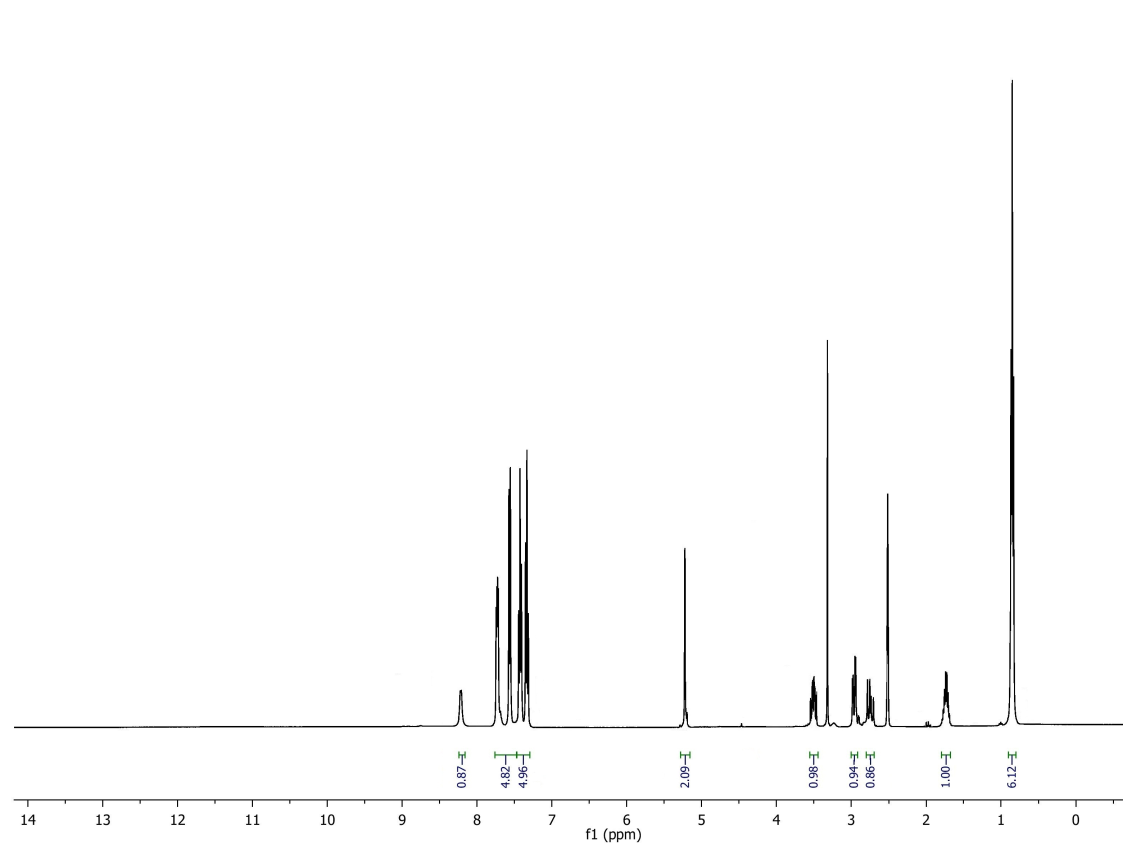
**13C NMR spectrum of compound 6b**



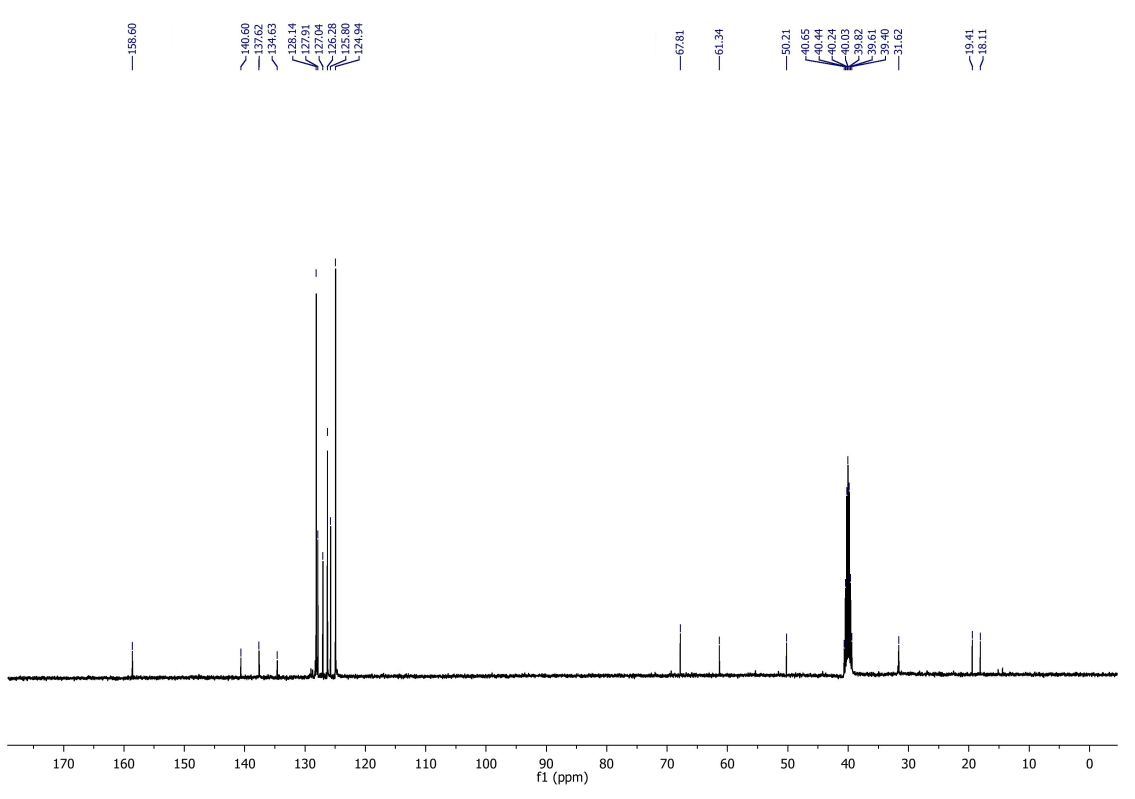
**IR Spectrum of compound 6b**



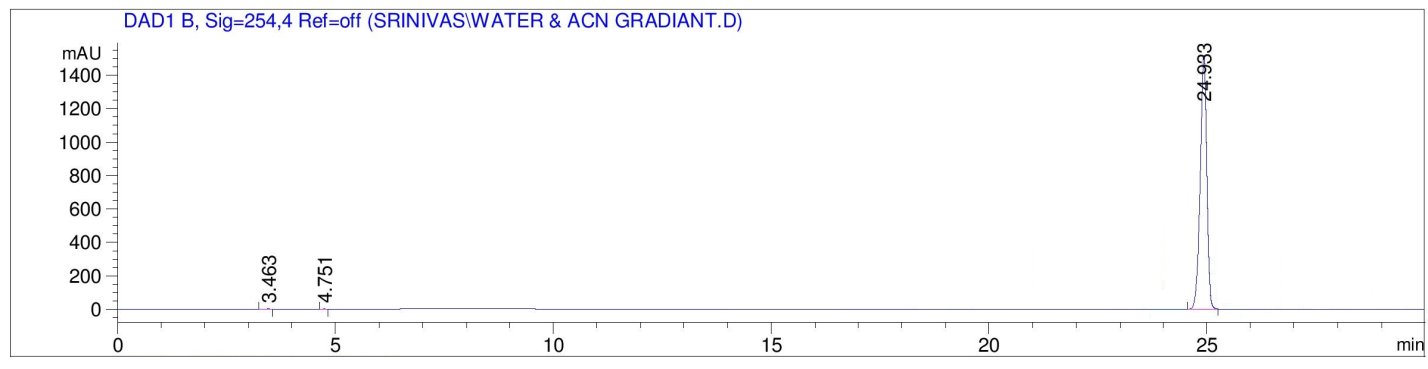
**HRMS spectrum of compound 6c**



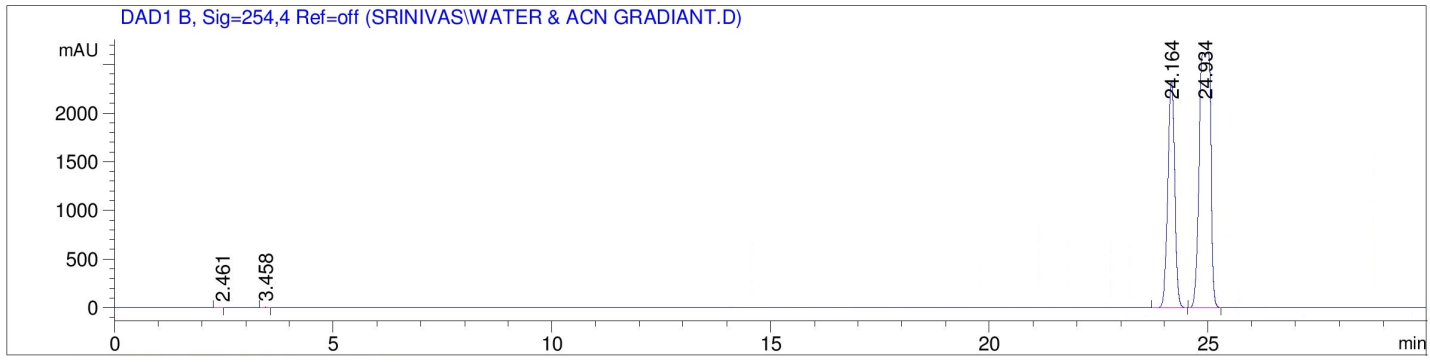
**1H NMR spectrum of compound 6c**



**13C NMR spectrum of compound 6c**

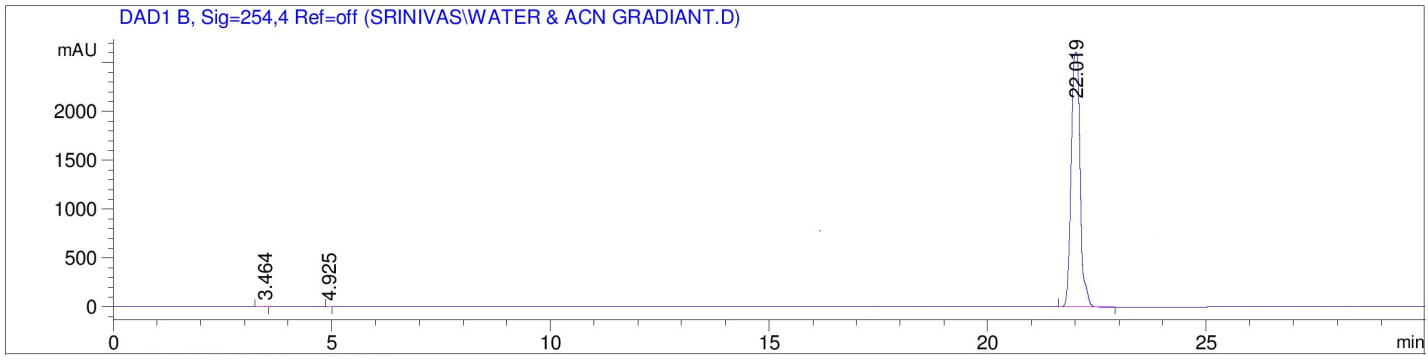


**RP-HPLC Chromatogram of 3a**



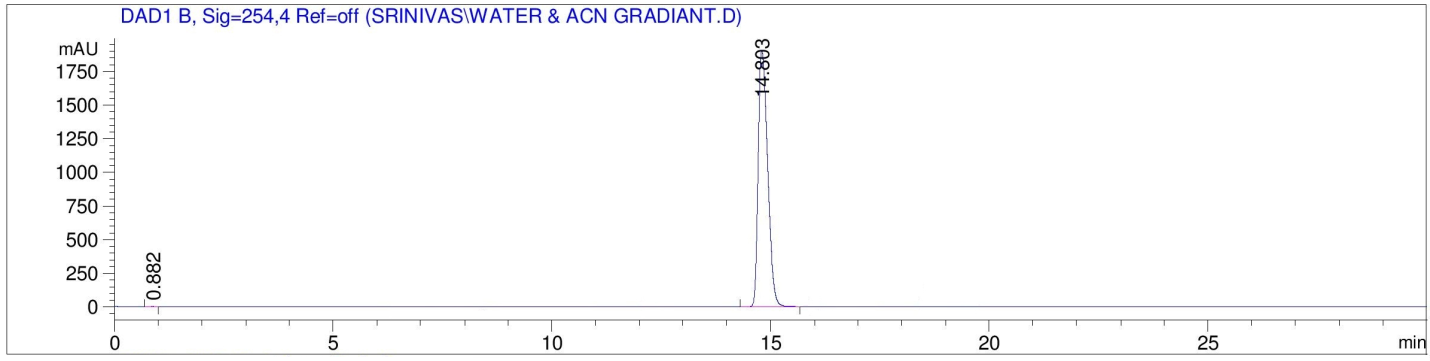
**RP-HPLC Chromatogram of 3a & 3a\***

RP-HPLC profiles of 3a and 3a\* (method: gradient 0.1% TFA, water-acetonitrile (0-100%) in 30 min; VWD at *λ* = 254 nm; flow rate: 1.0 mL/min; column: phenominex made Lux, pore size-5μ, Amylose-2, diameter x length = 250 x 4.6 mm).

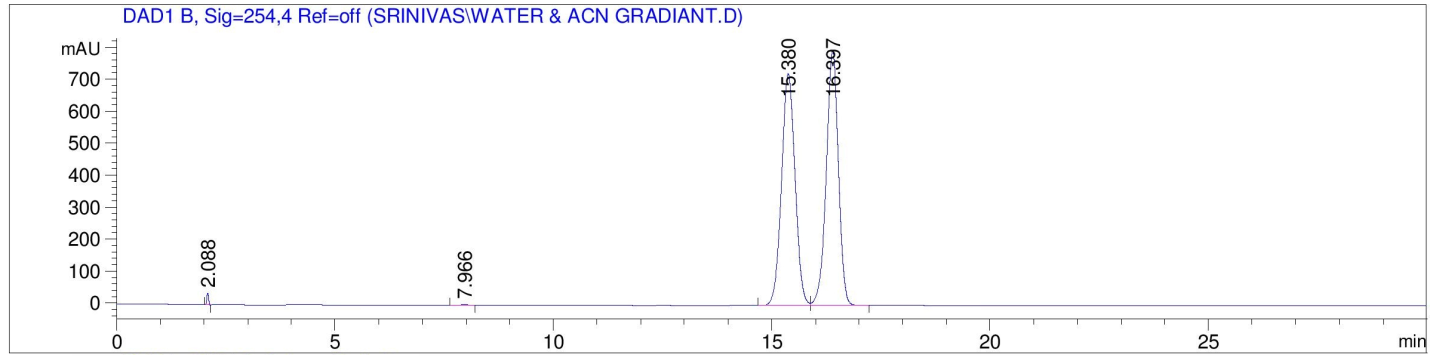


**RP-HPLC Chromatogram of 3e**

RP-HPLC profiles of 3e, (method: gradient 0.1% TFA, water-acetonitrile (0-100%) in 30 min; VWD at *λ* = 254 nm; flow rate: 1.0 mL/min; column: phenominex made Lux, pore size-5μ, Amylose-2, diameter x length = 250 x 4.6 mm).

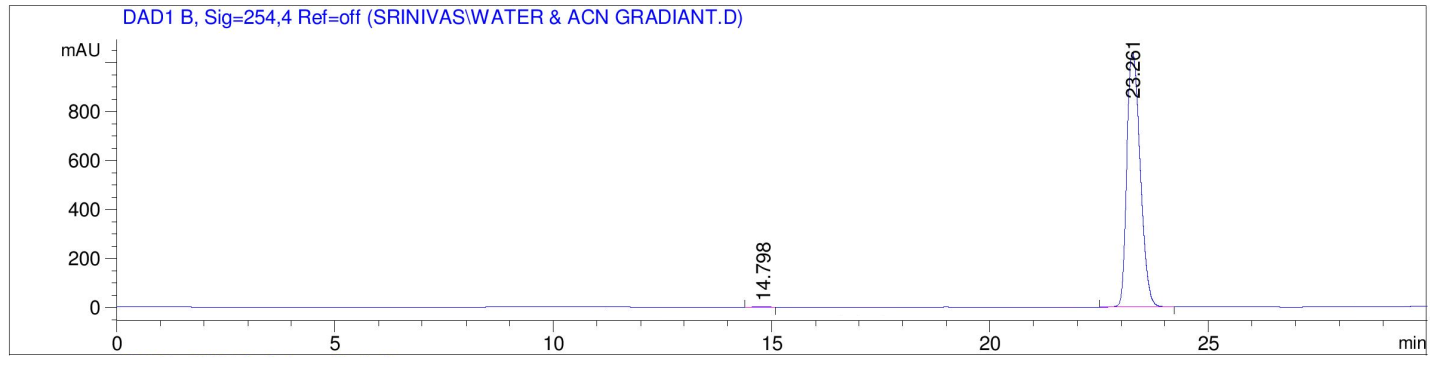


**RP-HPLC Chromatogram of 6b**



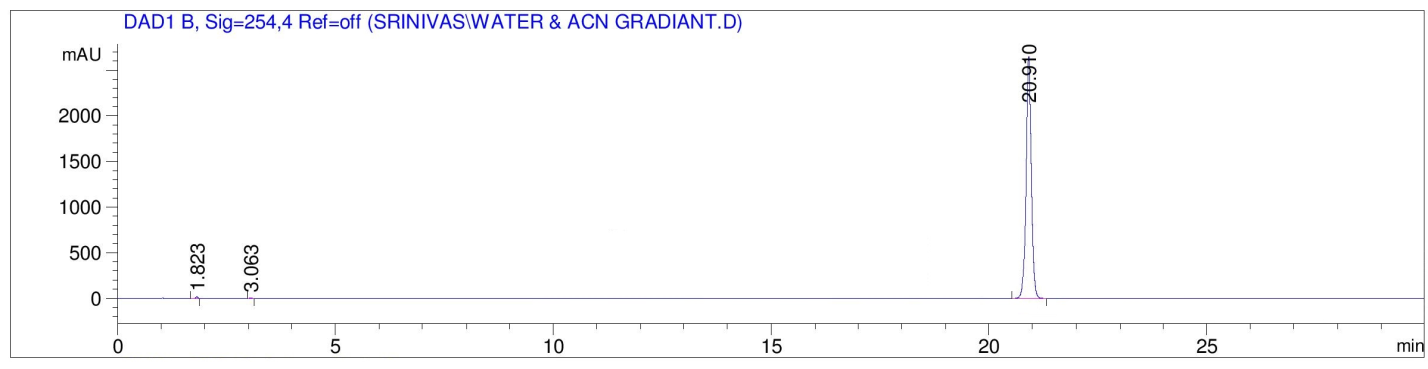
**RP-HPLC Chromatogram of 6b &6b\***

RP-HPLC profiles of 6b and6b\* (method: gradient 0.1% TFA, water-acetonitrile (0-100%) in 30 min; VWD at *λ* = 254 nm; flow rate: 1.0 mL/min; column: phenominex made Lux, pore size-5μ, Cellulose-1, diameter x length = 250 x 4.6 mm).



**RP-HPLC Chromatogram of 6a**

RP-HPLC profiles of 6a (method: gradient 0.1% TFA, water-acetonitrile (0-100%) in 30 min; VWD at *λ* = 254 nm; flow rate: 1.0 mL/min; column: phenominex made Lux, pore size-5μ, Cellulose-1, diameter x length = 250 x 4.6 mm).

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**RP-HPLC Chromatogram of 3a**



**RP-HPLC Chromatogram of 3e**

RP-HPLC profiles of 3a and 3e (method: gradient 0.1% TFA water-acetonitrile (0-100%) in 30 min; VWD at *λ* = 254 nm; flow rate: 1.0 mL/min; column: Agilent Eclipse, XDB-C18, pore size-5 μm, diameter x length = 4.6 x 150 nm).