Production of a recyclable nanobiocatalyst to synthesize quinazolinone derivatives

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General procedures

❖ **Procedure to find enzyme loading over functionalized magnetic support:** The amount of lipase adsorbed on magnetic support was estimated using Bradford assay using BSA as standard.[1]

$$q = \frac{(C_i - C_f)V}{W}\left(\frac{mg}{g}\right) \qquad \text{---------- equation 1.}$$

Where q = amount of protein loaded over magnetic support (mg/g), C_i = initial concentration of protein in original solution before immobilization (mg/ml), C_f = final concentration of protein in supernatant after immobilization (mg/ml), V= volume of buffer added during immobilization (ml), W = weight of magnetic support (g). All the readings are recorded in triplicate and then averaged to find the amount of protein load on magnetic support.

The immobilization efficiency (IE) was determined from the ratio of enzymes attached to MrGO surface to the total volume of added enzymes, as given by equation 2:

$$IE = \frac{Ci - Cf}{Ci} \times 100\% \qquad \text{---------- equation 2.}$$

Where, Ci = initial concentration of protein in original solution before immobilization (mg/ml), Cf = final concentration of protein in supernatant after immobilization (mg/ml).

❖ **Procedure for Scale-up of DHQ derivative (3a):**

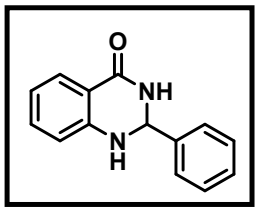
The gram scale reaction was set by adding 2-aminobenzamide (1.0 g, 0.0073 mol, 1.0 equiv.) and benzaldehyde (0.973g, 0.0087mol, 1.25 equiv.) in a round bottom flask followed by addition of CALB@MrGO (0.6 g) in EtOH (25 mL) at 55°C and stirred for 10 hrs on a magnetic stirrer. The progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate in hexane (30:70). After completion of the reaction, the reaction mixture was cooled to room temperature and water (50 mL) was added. Next, the catalyst CALB@MrGO was easily separated from the reaction mixture by encompassing an external magnet. The obtained product was then filtered and washed 3-4 times with 15 mL of EtOH to get the pure product. Finally obtained 2,3-dihydroquinazolin-4(1H)-one derivative (3a) in 87.1% (1.43 g) isolated yield.

❖ **Reaction procedure for studying Kinetics parameters:**

In a round bottom flask, equipped with a stir bar, added 2-aminobenzamide (1a) (1.0 equiv.) and varying concentration of benzaldehyde from (0.25mM to 1.50mM) (2a). Then, 60 mg of CALB@MrGO / 20 mg of Pure *Candida antarctica* lipase in 5 mL of ethanol was added and stirred the resulting mixture gently at 55°C for stipulated time. The progress of the reaction was monitored by thin-layer chromatography (TLC) using ethyl acetate in hexane (30:70). Upon completion of the reaction as indicated by TLC, the catalyst was separated from the reaction mixture using an external magnet. The reaction mixture was cooled to room temperature and water (10 mL) was added. The organic layer was separated using ethyl acetate (15 mL) and dried over anhydrous Na_2SO_4. Further, the volatiles were evaporated under

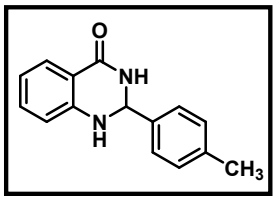
reduced pressure and the residue was purified by column chromatography (mobile phase: silica gel, eluent: hexane–ethyl acetate) to obtain the corresponding product (3a).

^{1}H & ^{13}C Nmr Data of synthesized derivatives



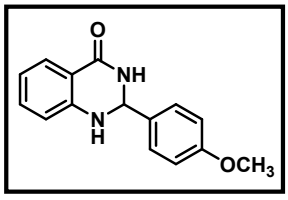
2-phenyl-2,3-dihydroquinazolin-4(1H)-one (3a) [2]

^{1}H NMR (400 MHz, DMSO-D6) δ = 8.30 (s, 1H), 7.62 (d, *J*=6.4, 1H), 7.50 (d, *J*=6.4, 2H), 7.41 – 7.35 (m, 3H), 7.26 – 7.22 (m, 1H), 7.11 (s, 1H), 6.75 (d, *J*=7.6, 1H), 6.68 (t, *J*=6.8, 1H), 5.75 (s, 1H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 164.11, 148.39, 142.14, 133.83, 128.98, 128.85, 127.87, 127.39, 117.63, 115.47, 114.91, 67.07.



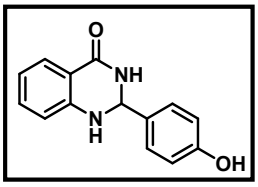
2-(p-tolyl)-2,3-dihydroquinazolin-4(1H)-one (3b) [2]

^{1}H NMR (500 MHz, DMSO-D6) δ = 8.21 (s, 1H), 7.61 (d, *J*=6.5, 1H), 7.37 (d, *J*=8.0, 2H), 7.24 (t, *J*=8.5, 1H), 7.19 (d, *J*=8.0, 2H), 7.04 (s, 1H), 6.74 (d, *J*=8.0, 1H), 6.67 (t, *J*=7.5, 1H), 5.70 (s, 1H), 3.32 (s, 3H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 164.16, 148.44, 139.18, 138.24, 133.78, 129.33, 127.86, 127.32, 117.58, 115.52, 114.92, 66.89, 21.25.



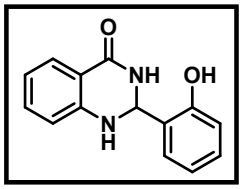
2-(4-methoxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (3c) [2]

^{1}H NMR (500 MHz, DMSO-D6) δ = 8.17 (s, 1H), 7.61 (d, *J*=9.2, 1H), 7.42 (d, *J*=8.5, 2H), 7.24 (t, *J*=8.0, 1H), 7.00 (s, 1H), 6.95 (d, *J*=8.5, 2H), 6.74 (d, *J*=7.5, 1H), 6.68 (t, *J*=7.5, 1H), 5.71 (s, 1H), 3.75 (s, 3H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 164.21, 159.95, 148.54, 133.98, 133.76, 128.74, 127.86, 117.60, 115.52, 114.93, 114.15, 66.81, 55.68.



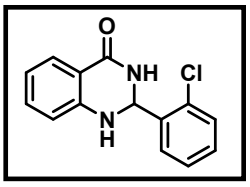
2-(4-hydroxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (3d) [2]

^{1}H NMR (400 MHz, DMSO-D6) δ = 9.50 (s, 1H), 8.09 (s, 1H), 7.60 (d, *J*=6.8, 1H), 7.29 (d, *J*=8.8, 2H), 7.22 (t, *J*=8.4, 1H), 6.93 (s, 1H), 6.76-7.671 (m, 3H), 6.66 (t, *J*=7.2, 1H), 5.64 (s, 1H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 164.27, 158.21, 148.69, 133.71, 132.10, 128.83, 127.86, 117.55, 115.44, 114.89, 67.16.



2-(2-hydroxyphenyl)-2,3-dihydroquinazolin-4(1H)-one (3e) [3]

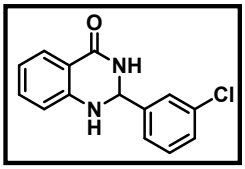
^{1}H NMR (500 MHz, DMSO-D6) δ = 9.84 (s, 1H), 7.92 (s, 1H), 7.62 (d, J=7.5, 1H), 7.35 (d, J=7.5, 1H), 7.22 (t, J=8.5, 1H), 7.15 (t, J=8.5, 1H), 6.87 (d, J=8.0, 1H), 6.81-6.74 (m, 3H), 6.67 (t, J=7.5, 1H), 6.01 (s, 1H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 164.51, 155.09, 148.62, 133.70, 129.78, 127.83, 127.71, 119.28, 117.51, 115.84, 115.30, 115.05, 61.68.



2-(2-chlorophenyl)-2,3-dihydroquinazolin-4(1H)-one (3f) [3]

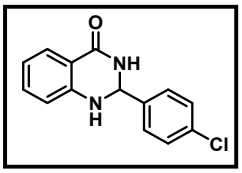
^{1}H NMR (400 MHz, DMSO-D6) δ = 8.34 (s, 1H), 7.61 (d, *J*=7.2, 1H), 7.51 (d, *J*=8.4, 2H), 7.46 (d, *J*=8.4, 2H), 7.25 (t, *J*=7.2, 1H), 7.15 (s, 1H), 6.75 (d, *J*=8.1, 1H), 6.68 (t, *J*=7.6, 1H), 5.77 (s, 1H).

^{13}C NMR (100 MHz, DMSO-D6) δ = 163.79, 149.85, 147.93, 147.76, 134.09, 128.55, 127.92, 124.11, 117.98, 115.41, 115.06, 65.78.



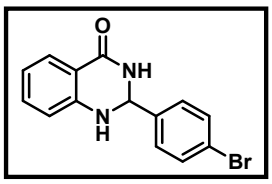
2-(3-chlorophenyl)-2,3-dihydroquinazolin-4(1H)-one (3g) [4]

^{1}H NMR (400 MHz, DMSO-D6) δ = 8.40 (s, 1H), 7.61 (d, *J*=7.6, 1H), 7.53 (s, 1H), 7.44 – 7.40 (m, 3H), 7.27 – 7.22 (m, 2H), 6.76 (d, *J*=8.0, 1H), 6.69 (t, *J*=7.2, 1H), 5.78 (s, 1H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 163.95, 148.03, 144.91, 133.99, 133.49, 130.84, 128.80, 127.89, 127.28, 125.94, 117.85, 115.42, 115.00, 66.08.



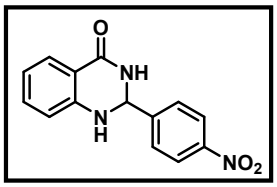
2-(4-chlorophenyl)-2,3-dihydroquinazolin-4(1H)-one (3h) [2]

^{1}H NMR (400 MHz, DMSO-D6) δ = 8.22 (s, 1H), 7.66 (d, J=6.4, 2H), 7.50-7.48 (m, 1H), 7.41-7.38(m, 2H), 7.26 (t, J=6.9, 1H), 7.02 (s, 1H), 6.77 – 6.69 (m, 2H), 6.14 (s, 1H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 164.16, 148.18, 138.38, 133.97, 132.38, 130.83, 130.11, 129.27, 128.00, 127.90, 117.98, 115.20, 115.09, 64.21.



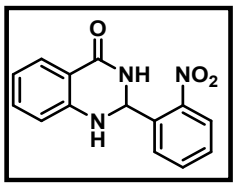
2-(4-bromophenyl)-2,3-dihydroquinazolin-4(1H)-one (3i) [5]

^{1}H NMR (500 MHz, DMSO-D6) δ = 8.17 (s, 1H), 7.62 – 7.60 (m, 1H), 7.42 (d, J=8.5, 2H), 7.24 (t, J=8.0, 1H), 7.0 (s, 1H), 6.95 (d, J=8.5, 1H), 6.67 (t, J=7.5, 1H), 5.76 (s, 1H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 163.99, 148.15, 141.63, 133.93, 131.75, 129.61, 127.88, 122.08, 117.80, 115.45, 114.98, 66.29.



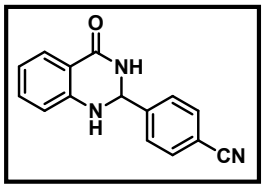
2-(4-nitrophenyl)-2,3-dihydroquinazolin-4(1H)-one (3j) [2]

^{1}H NMR (500 MHz, DMSO-D6) δ = 8.51 (s, 1H), 8.26 (d, J=8.5, 2H), 7.75 (d, J=8.5, 2H), 7.62 (d, J=7, 1H), 7.32 (s, 1H), 7.27 (t, J=7.0, 1H), 6.77 (d, J=8.0, 1H), 6.70 (t, J=7.5, 1H), 5.92 (s, 1H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 163.79, 149.85, 147.93, 147.76, 134.09, 128.55, 127.92, 124.11, 117.98, 115.41, 115.06, 65.78.



2-(2-nitrophenyl)-2,3-dihydroquinazolin-4(1H)-one (3k) [2]

^{1}H NMR (500 MHz, DMSO-D6) δ = 8.20 (s, 1H), 8.07 (d, J=8.0, 1H), 7.86 (d, J=8.0, 1H), 7.80 (t, J=8.0, 1H), 7.64 (m, 2H), 7.26 (t, J=8.5 1H), 7.00 (s, 1H), 6.77 (d, J=8.0, 1H), 6.73 (t, J=8.0, 1H), 6.33 (s, 1H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 163.87, 148.17, 147.64, 136.42, 134.44, 134.07, 130.42, 129.45, 127.82, 125.24, 118.19, 115.42, 115.03, 62.70.

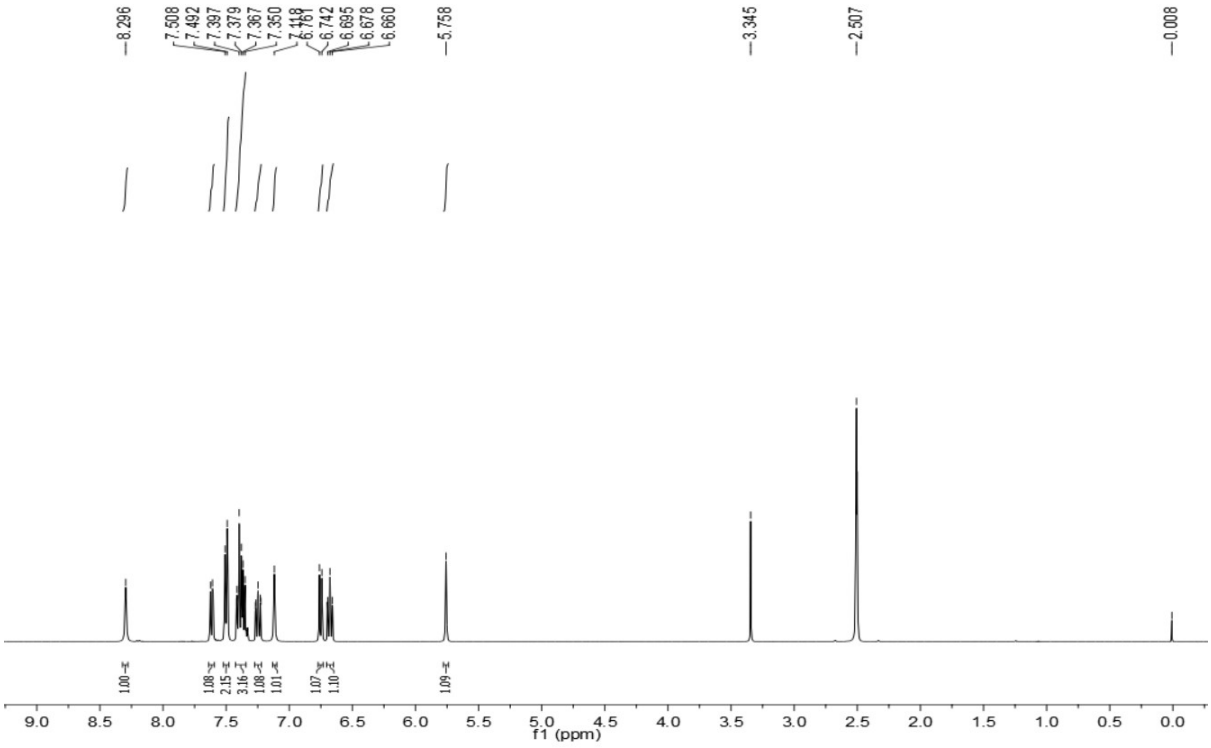


4-(4-oxo-1,2,3,4-tetrahydroquinazolin-2-yl)benzonitrile (3l) [6]

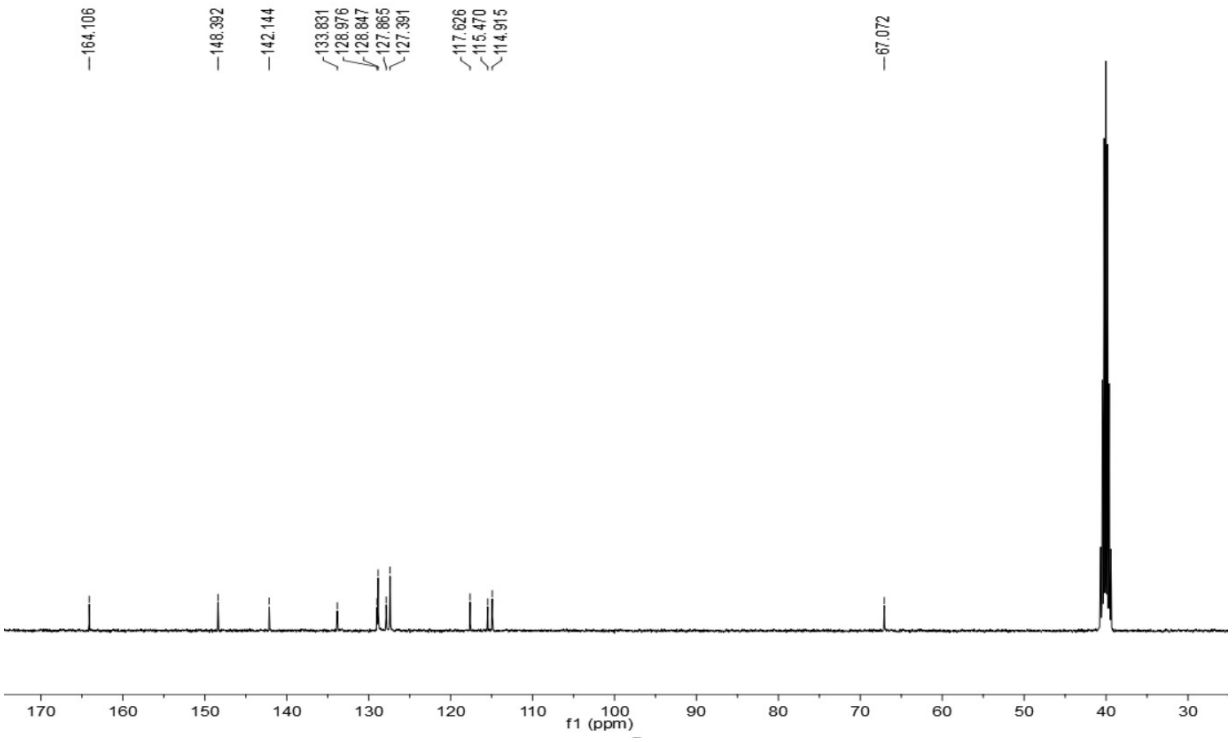
^{1}H NMR (500 MHz, DMSO-D6) δ = 8.46 (s, 1H), 7.87 (d, *J*=8.5, 2H), 7.66 (d, *J*=8.5, 2H), 7.61 (d, *J*=8, 1H), 7.28 – 7.24 (m, 2H), 6.76 (d, *J*=7.5, 1H), 6.69 (t, *J*=8, 1H), 5.85 (s, 1H).
^{13}C NMR (100 MHz, DMSO-D6) δ = 163.83, 147.84, 134.06, 132.94, 128.20, 127.90, 119.18, 117.93, 115.41, 115.02, 111.56, 66.01.

^{1}H & ^{13}C Nmr Spectra of synthesized derivatives

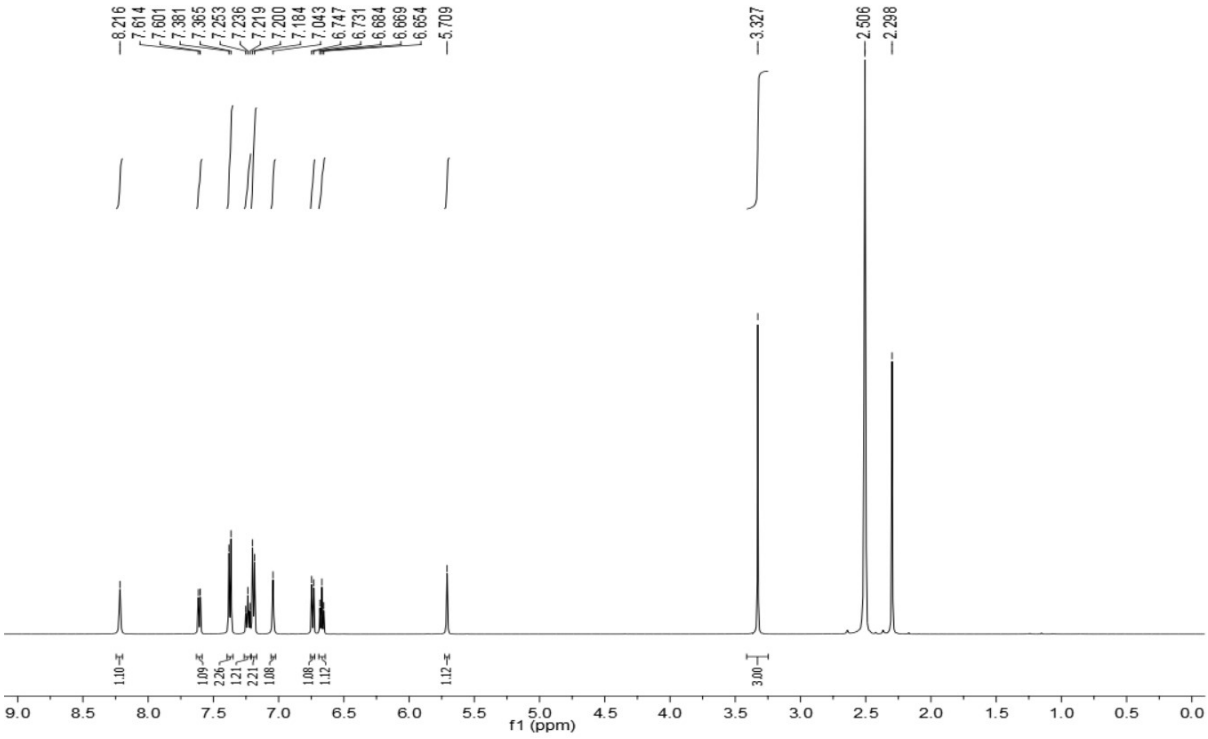
^{1}H spectra of (3a)



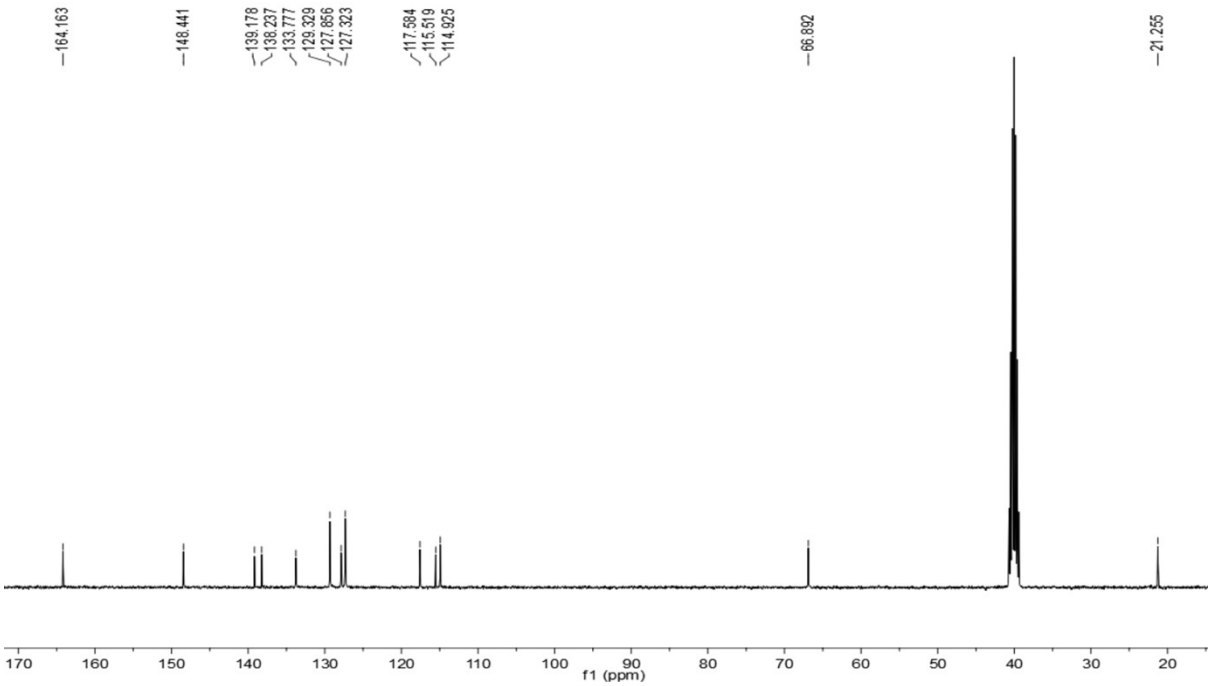
^{13}C spectra of (3a)



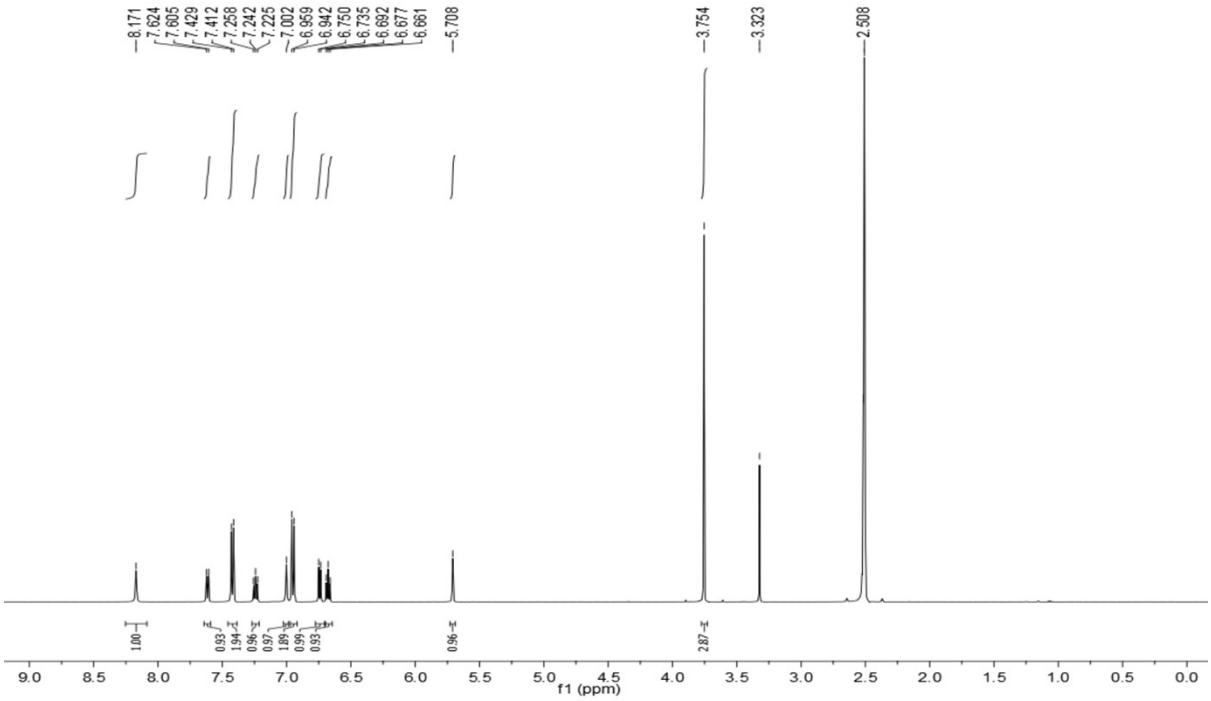
^{1}H spectra of (3b)



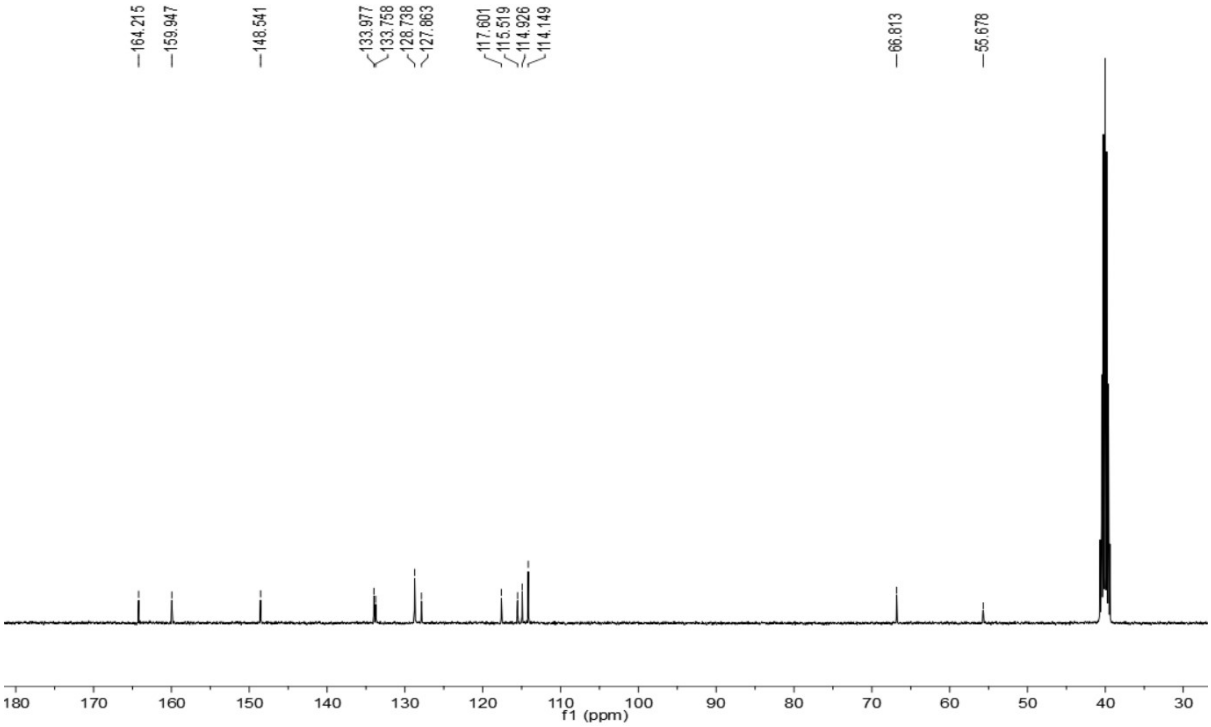
^{13}C spectra of (3b)



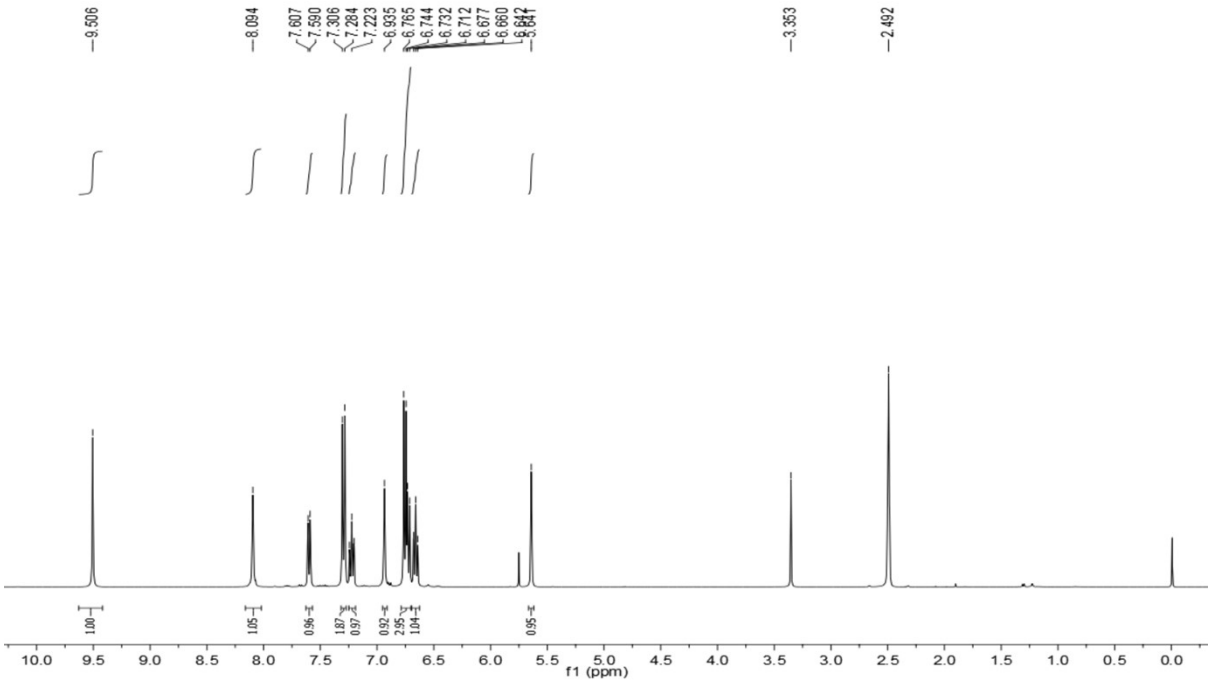
^{1}H spectra of (3c)



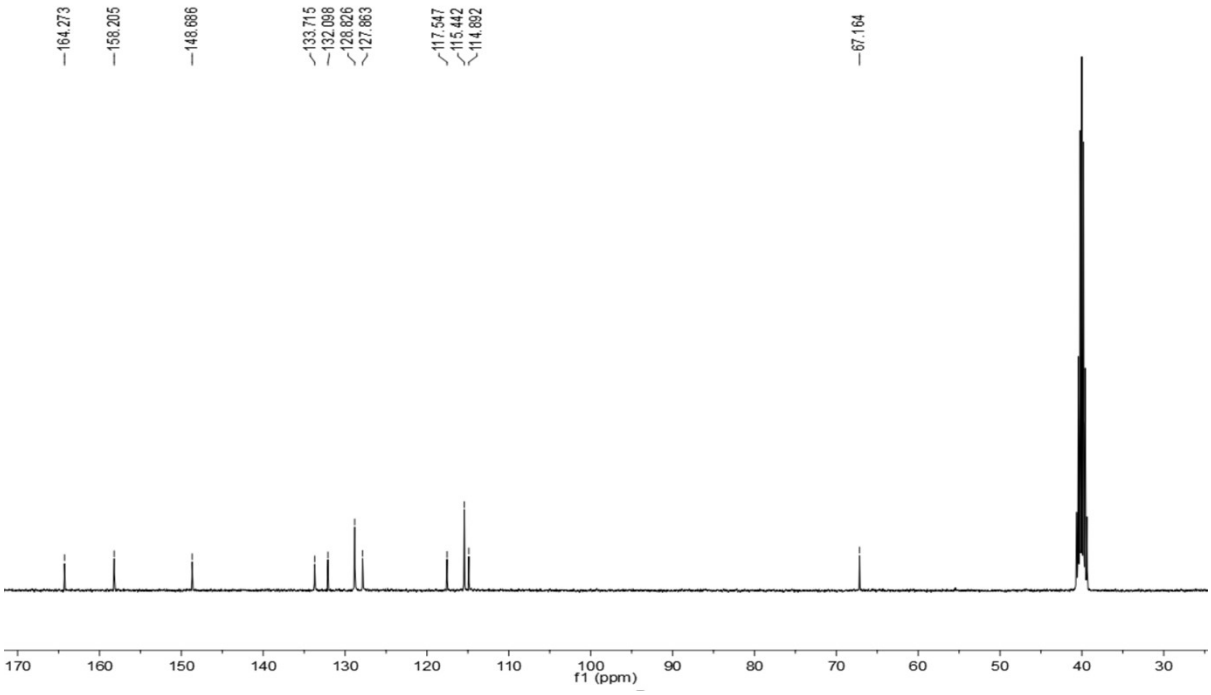
^{13}C spectra of (3c)



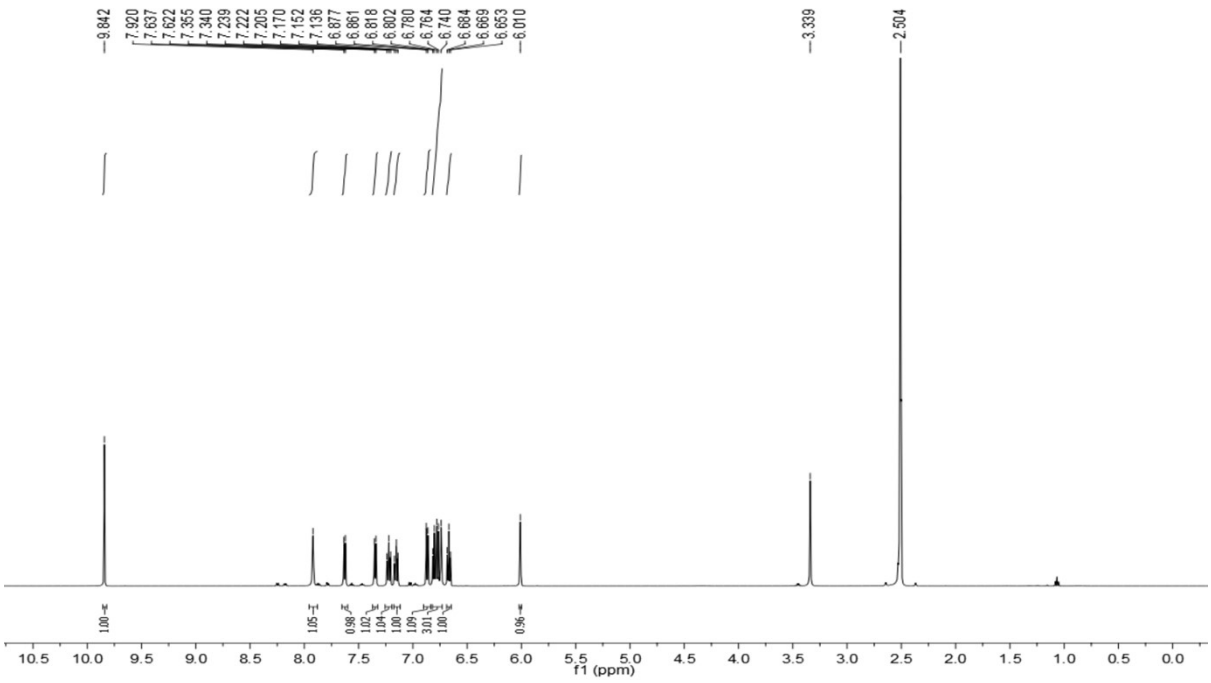
[1]H spectra of (3d)



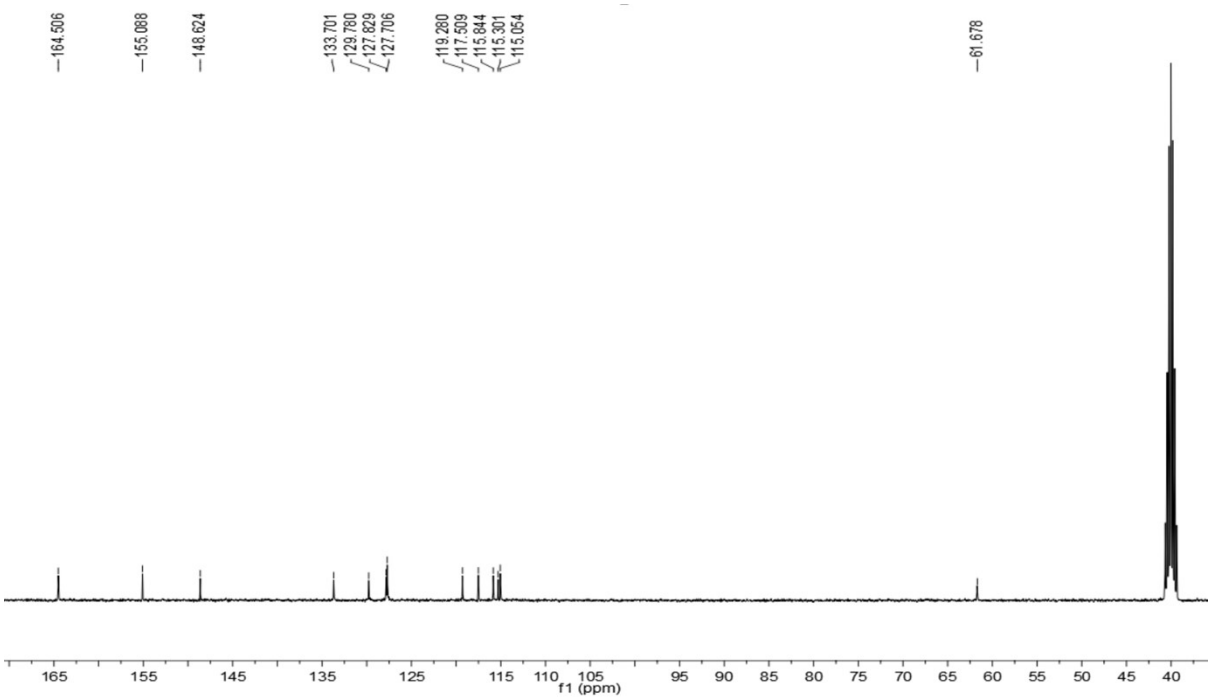
[13]C spectra of (3d)



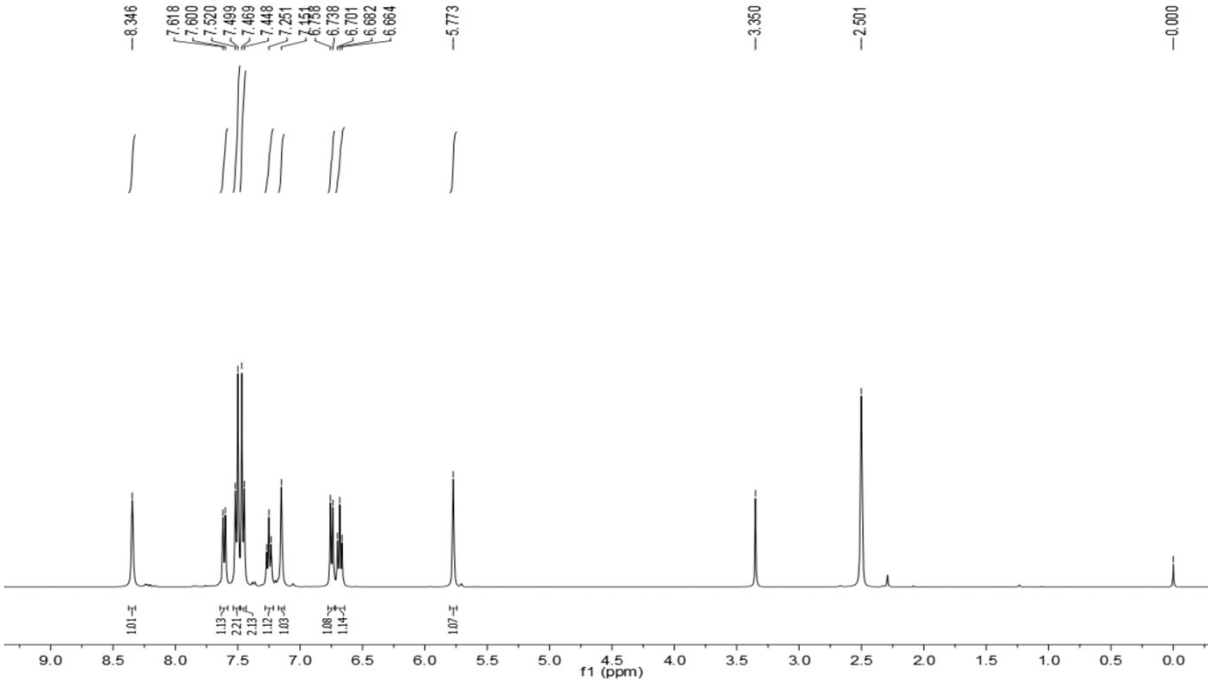
^{1}H spectra of (3e)



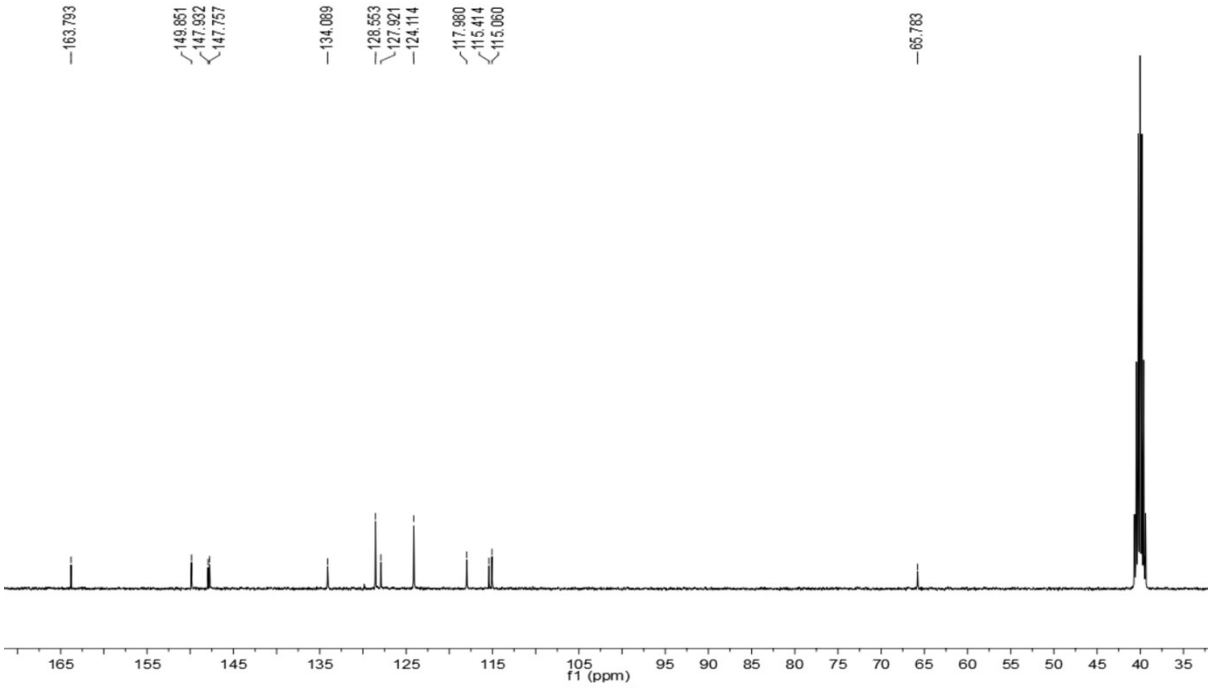
^{13}C spectra of (3e)



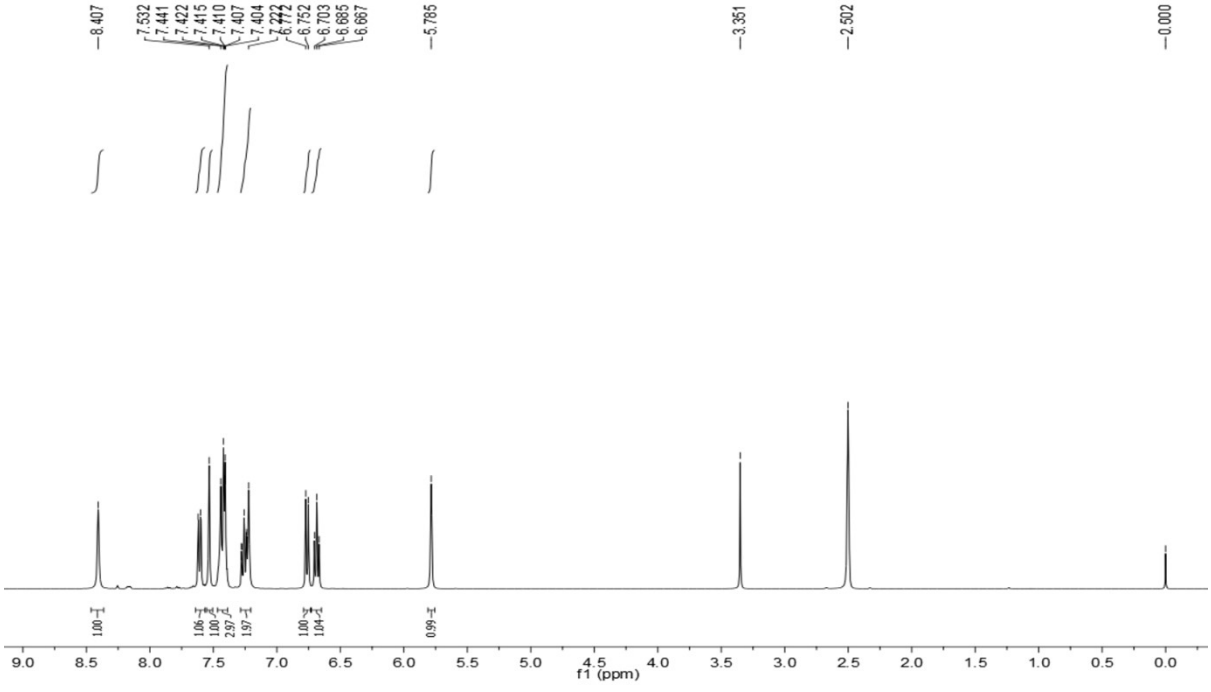
^{1}H spectra of (3f)



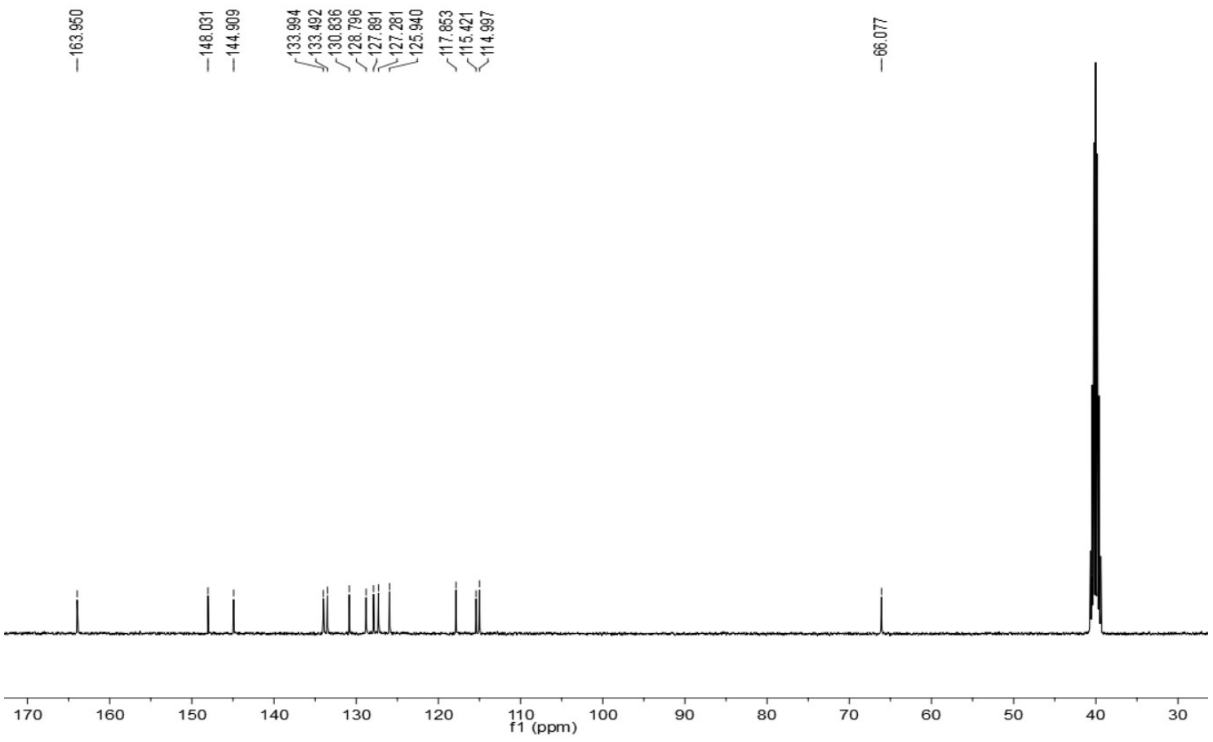
^{13}C spectra of (3f)



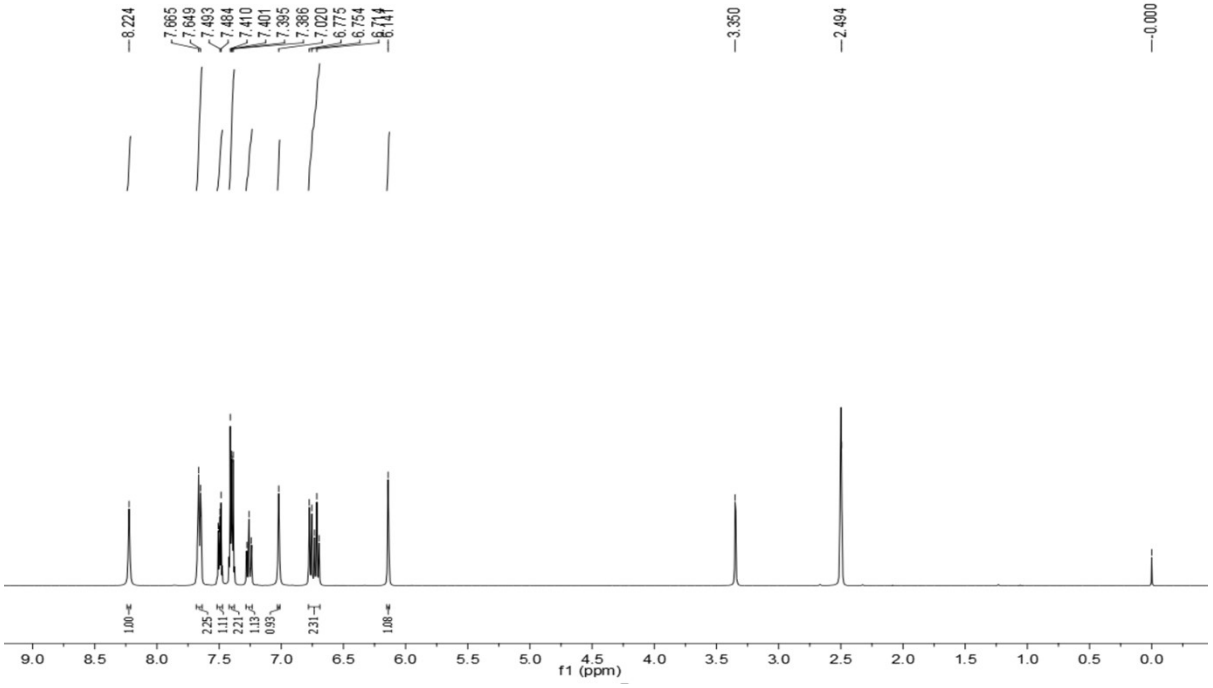
^{1}H spectra of (3g)



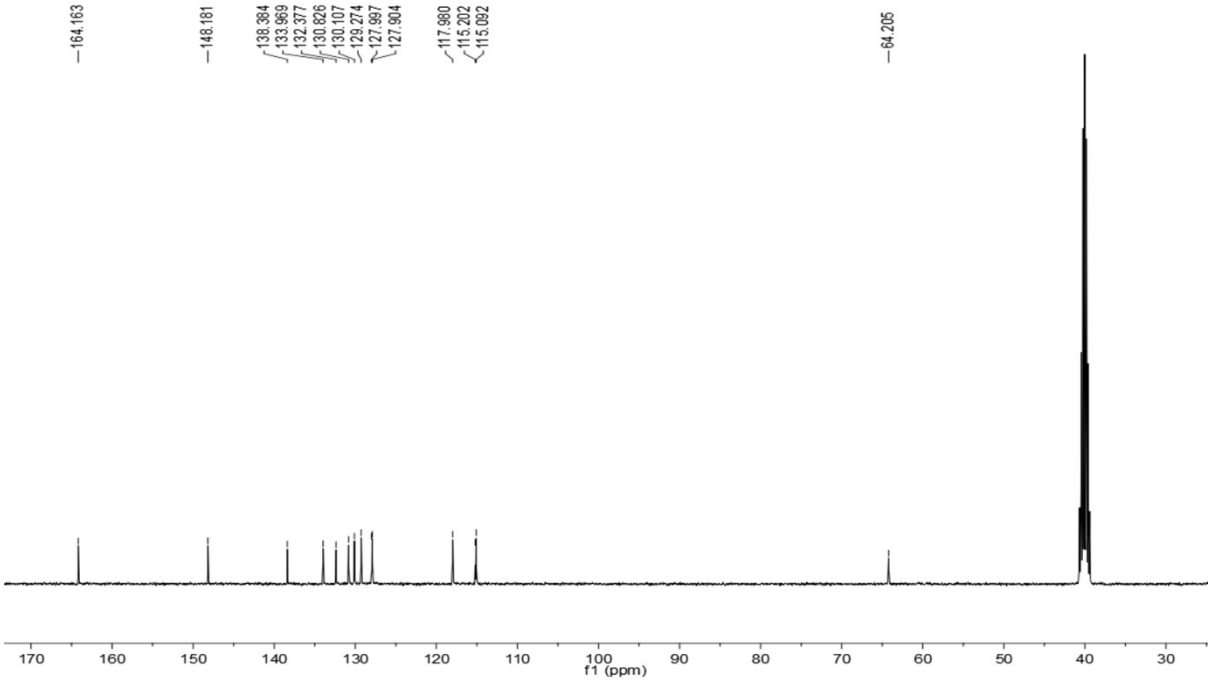
^{13}C spectra of (3g)



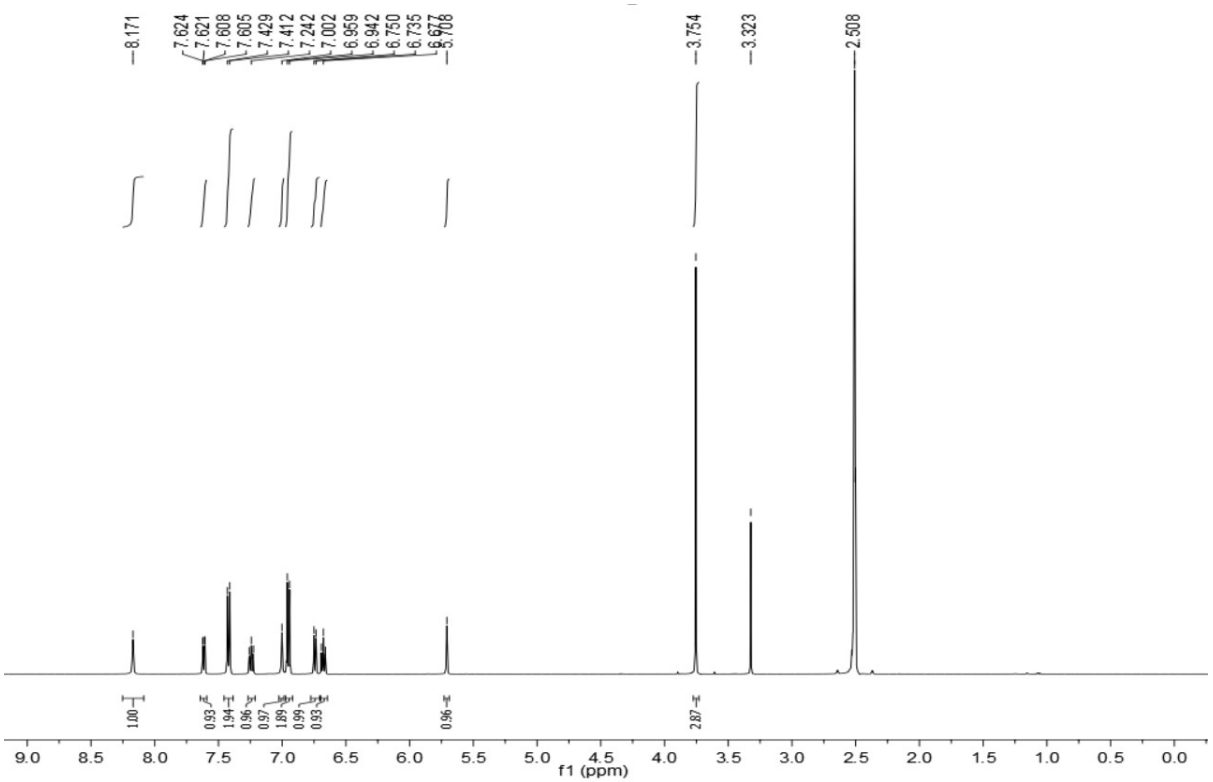
^{1}H spectra of (3h)



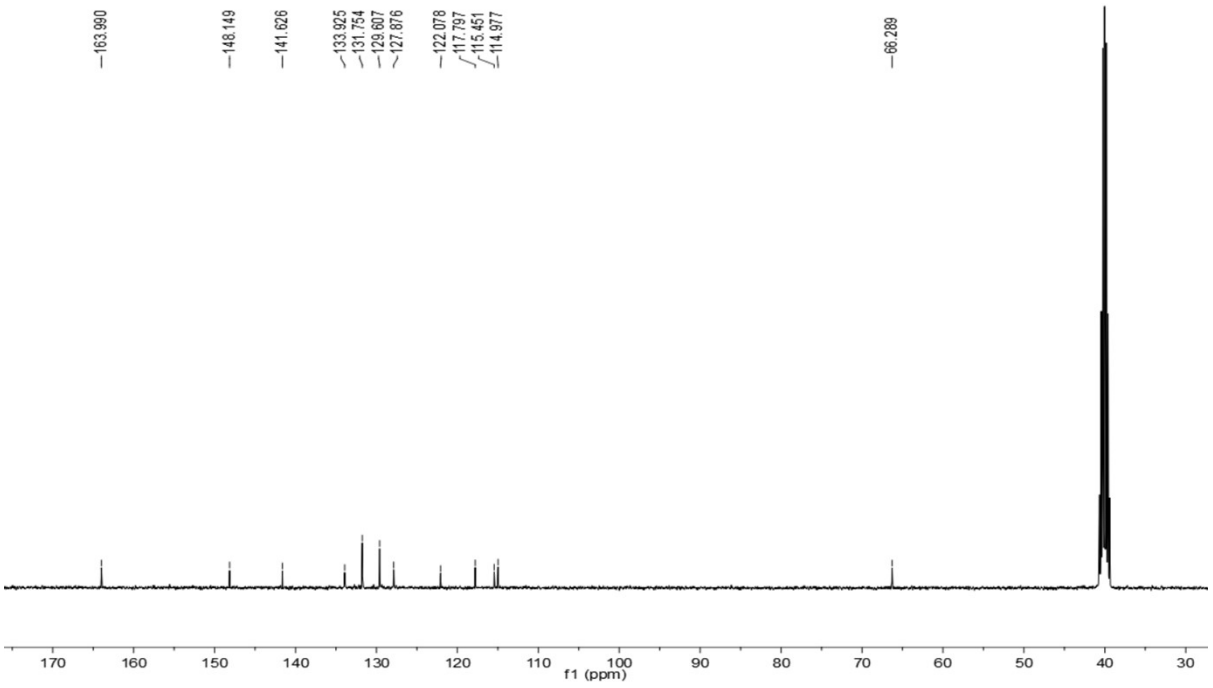
^{13}C spectra of (3h)



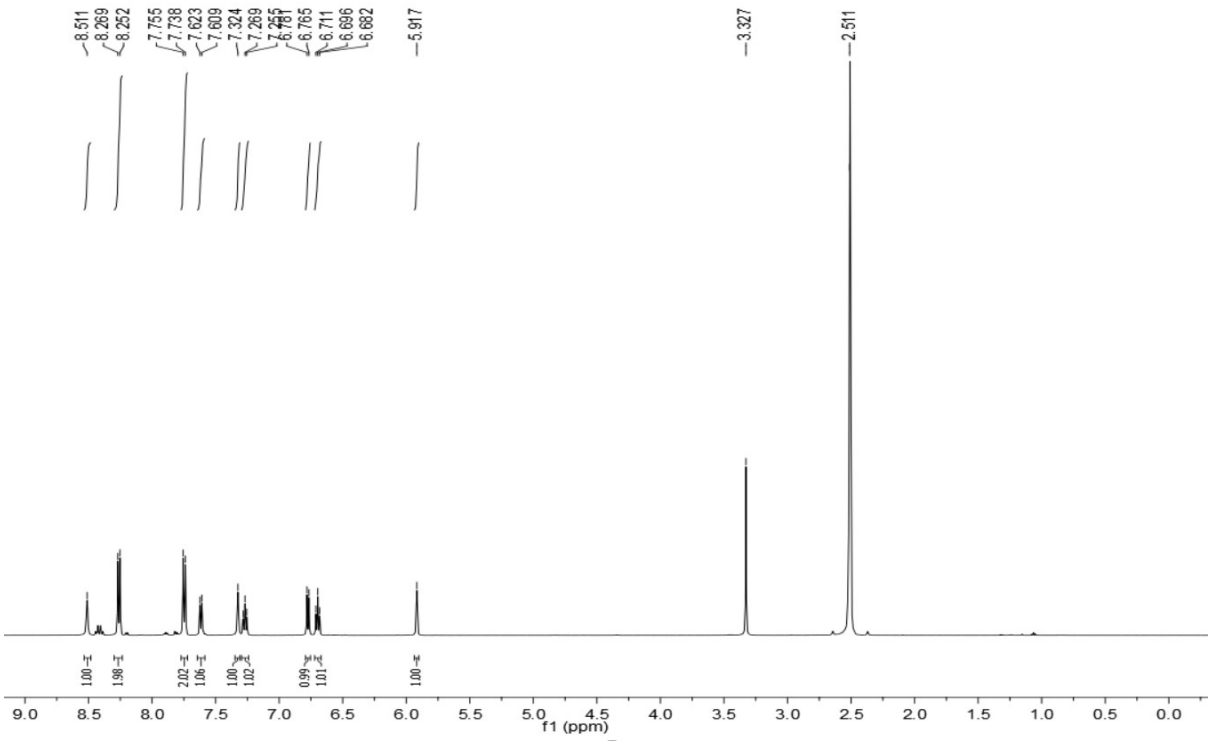
^{1}H spectra of (3i)



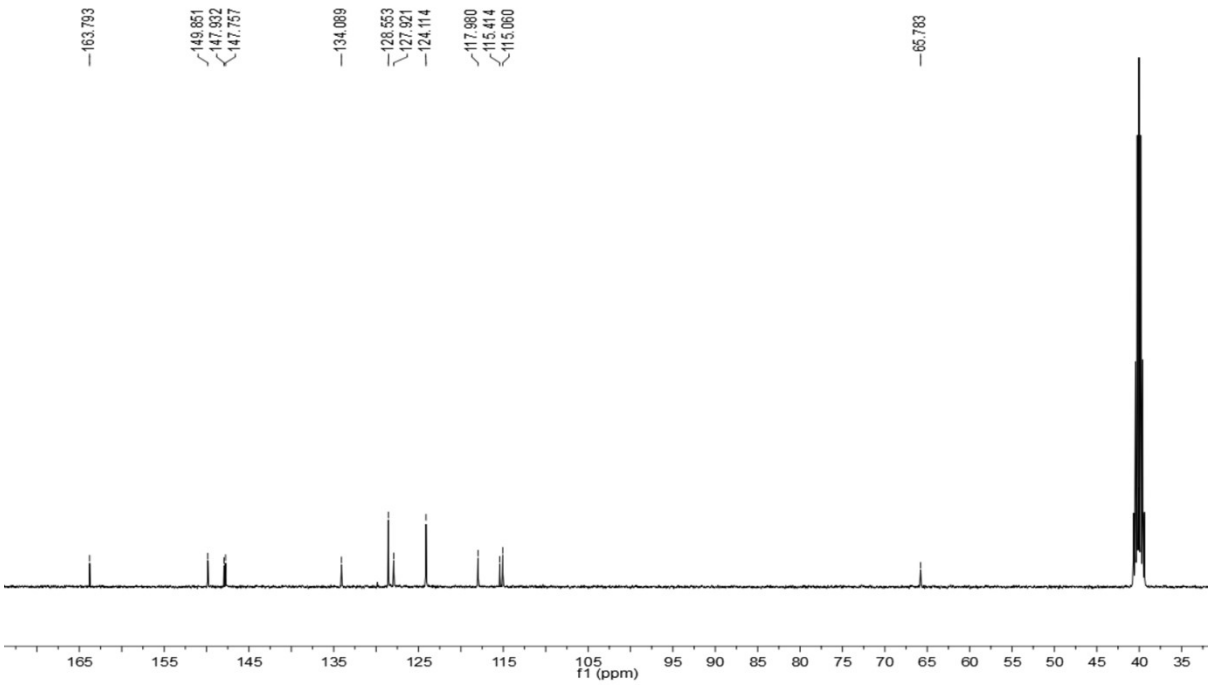
^{13}C spectra of (3i)



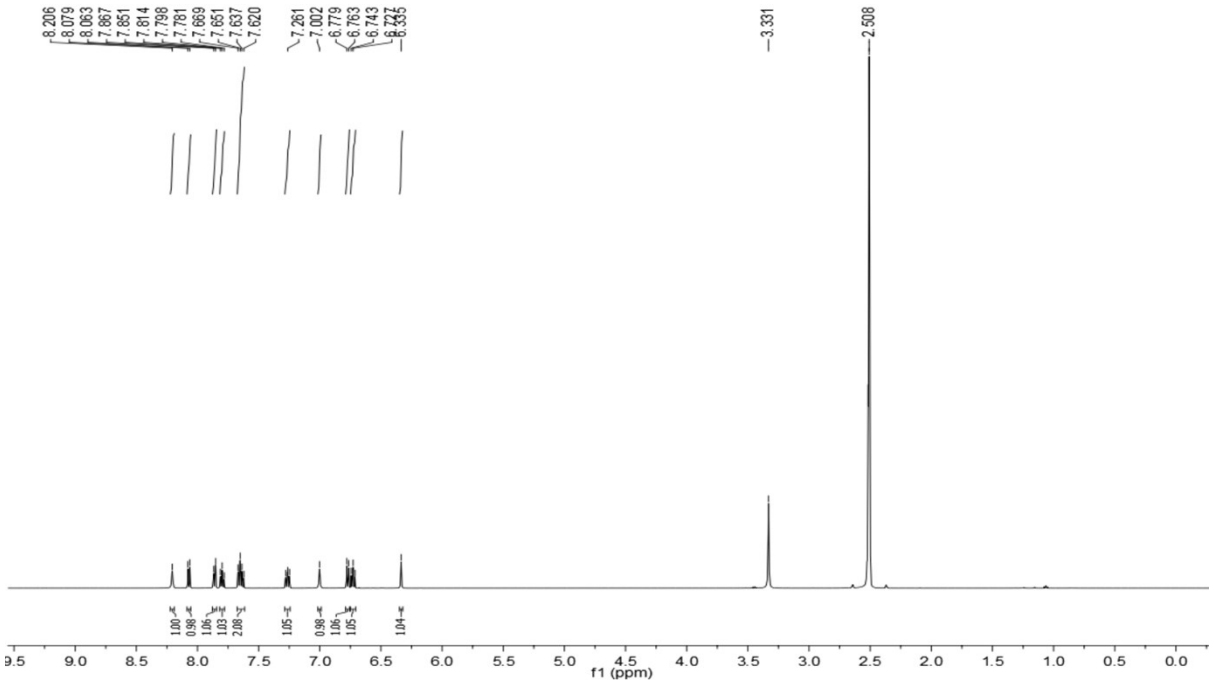
^{1}H spectra of (3j)



^{13}C spectra of (3j)



^{1}H spectra of (3k)



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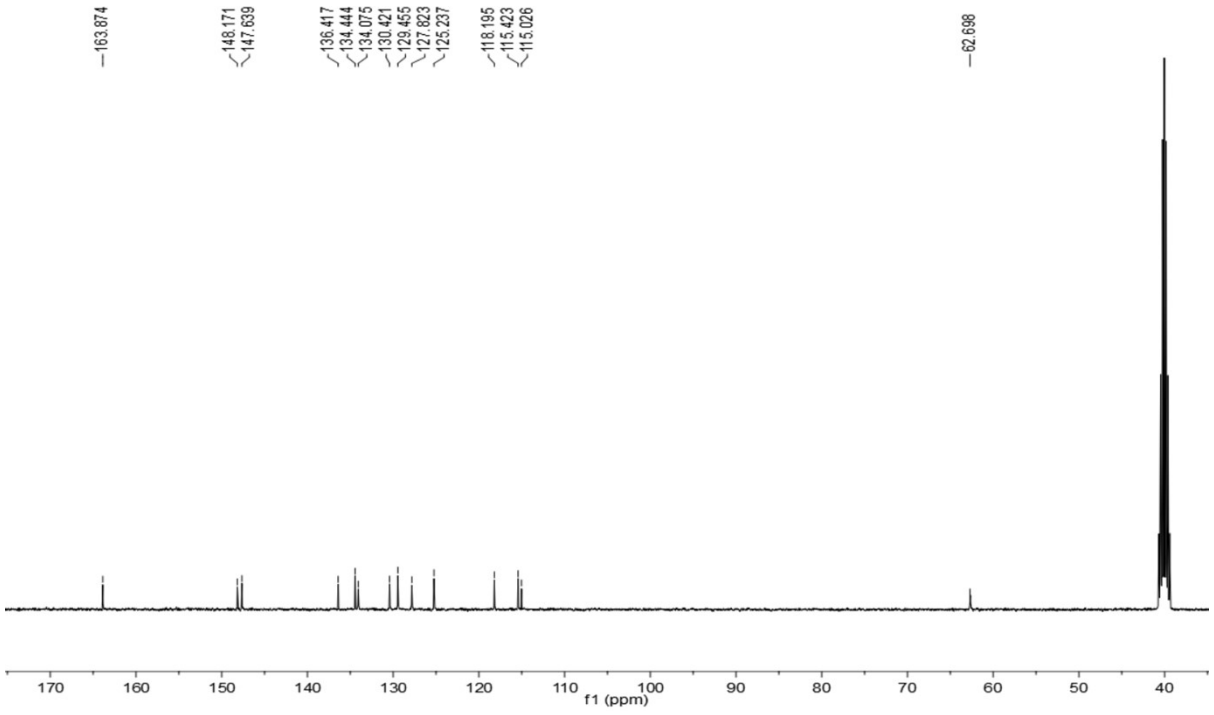

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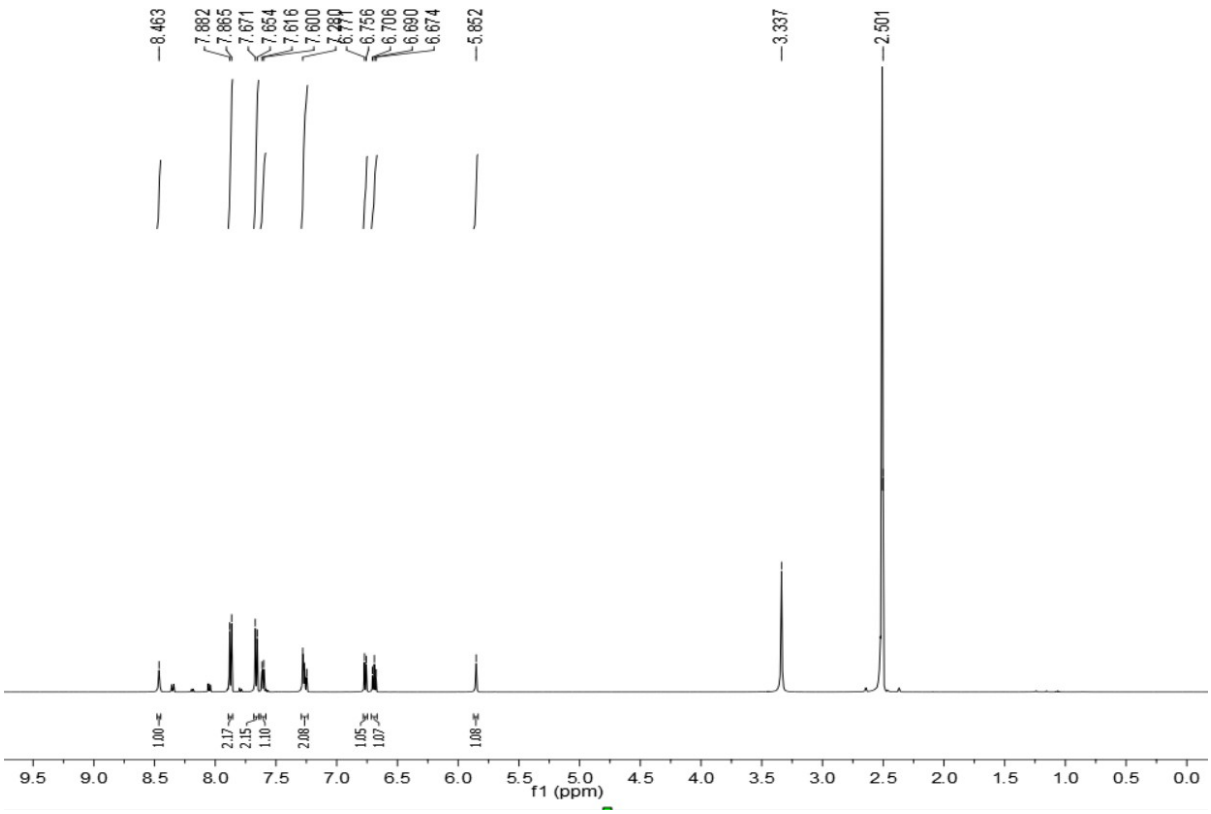

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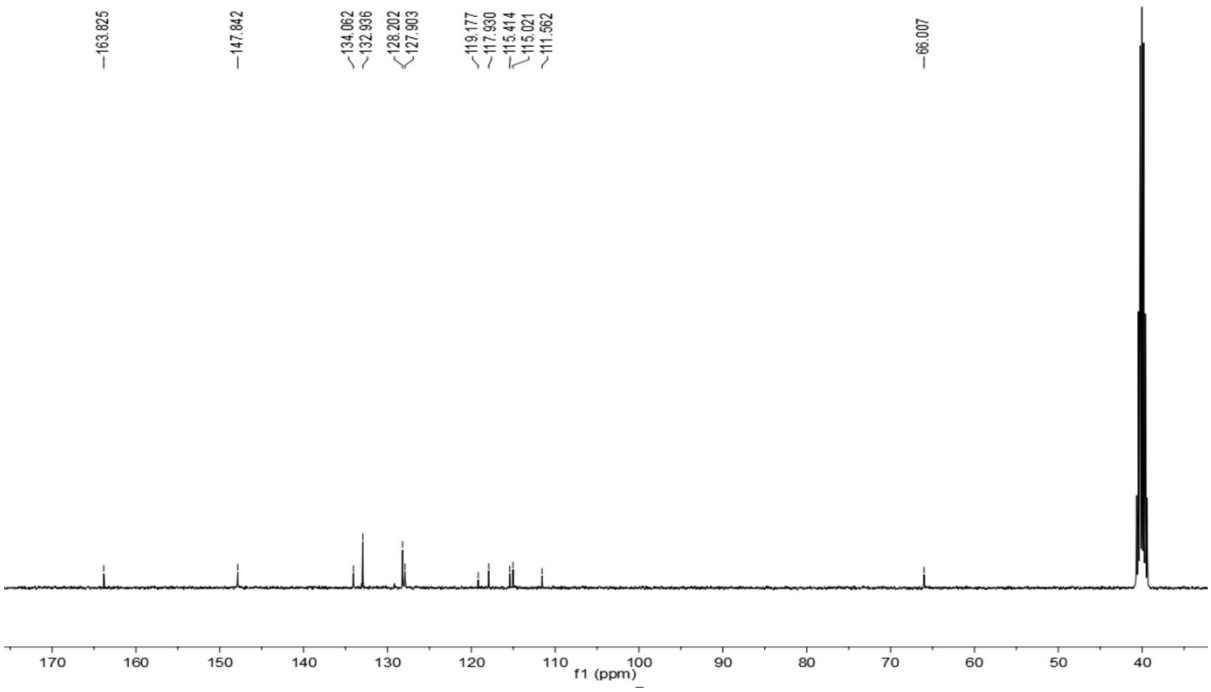

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