Electronic supplementary materials *Mendeleev Commun.*, 2021, **31**, 862-864

Synthesis and antibacterial evaluation of new pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one hybrids linked to different heteroarene units

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Contents

1. Experimental

1.1. Materials

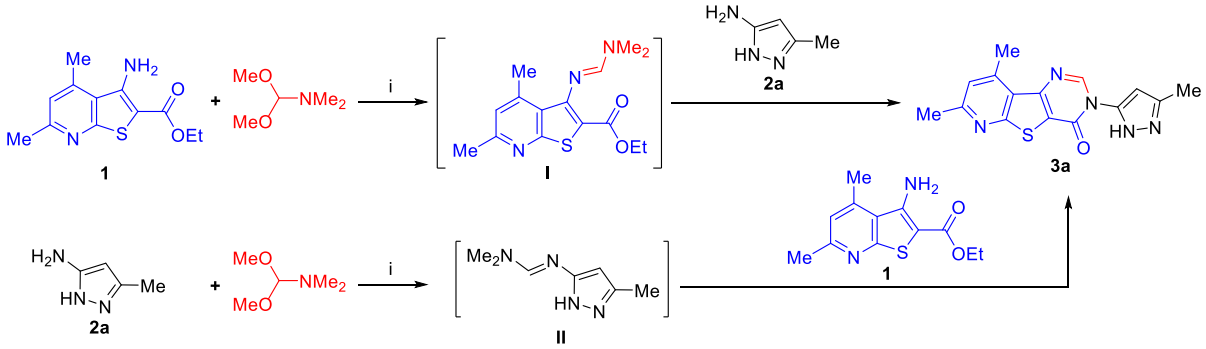
All solvents were acquired from commercial sources and used as received unless otherwise stated. All other chemicals (Merck or Aldrich) were used as purchased. Microwave experiments were performed using CEM Discover apparatus (300 W), utilizing 35 mL capped glass reaction vessels Automated power control based on temperature feedback. The melting points were measured on a Stuart melting point apparatus and are uncorrected. IR spectra were recorded on a Smart iTR, which is an ultra-high-performance, versatile Attenuated Total Reflectance (ATR) sampling accessory on the Nicolet iS10 FT-IR spectrometer. NMR spectra were recorded on Bruker Avance III 400 MHz spectrophotometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) using TMS as an internal standard and DMSO-d_6 as solvent and chemical shifts were expressed as δ ppm units. Elemental analyses were carried out on a EuroVector instrument C, H, N, S analyzer EA3000 Series.

1.2. Table S1. Optimization of the synthesis of hybrid **3a** under MW irradiation.

Entry	Solvent	Temp. (°C)	Time (min)	Yield (%)[a,b]
1	DMF	160	40	59
2	Toluene	120	45	37
3	Xylene	140	45	43
4	Pyridine	120	40	62
5	**Dioxane**	**110**	**30**	**86**
6	Dioxane	rt	45	Traces
7	Dioxane	70	45	49

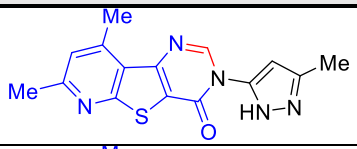
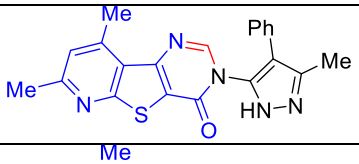
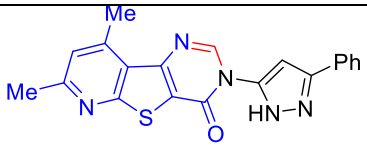
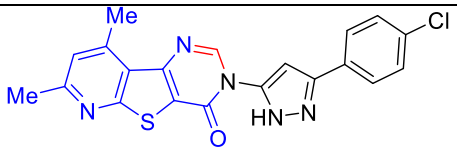
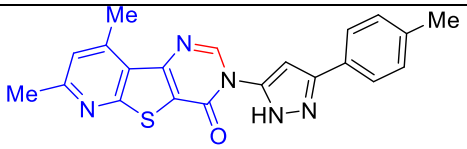
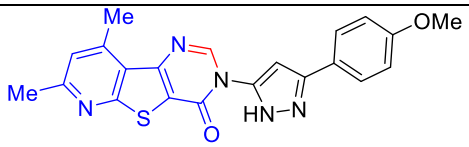
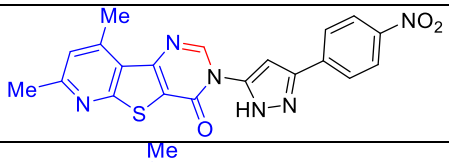
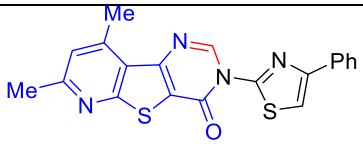
[a] All reactions were irradiated by microwaves of power 300 W at 110 °C; [b] All reactions were followed up by TLC analyses.

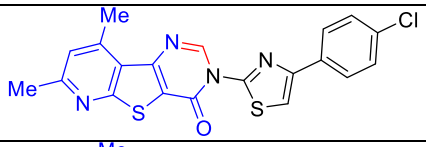
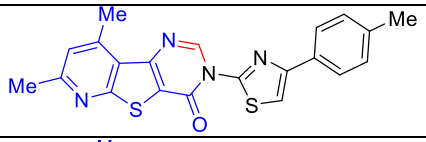
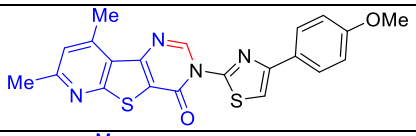
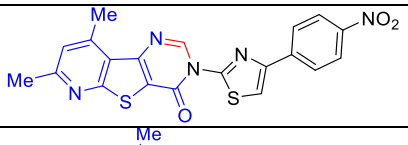
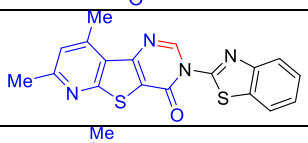
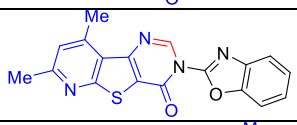
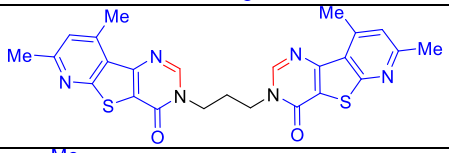
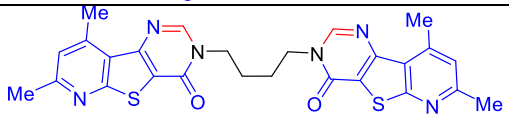
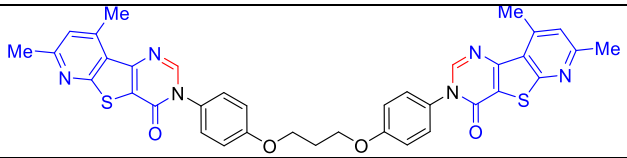
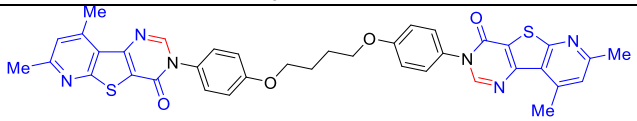
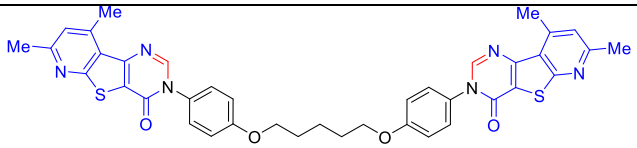
1.3. Detailed mechanism of the heterocyclization of thieno[2,3-*b*]pyridine-fused pyrimidinone hybrid **3a**.



Scheme S1 *Reagents and conditions:* i, dioxane, MW (300 W), 110 °C, 30 min.

1.4. Table S2 Synthesis of pyrimidinone hybrids **3**, **4** and **5** under microwave irradiation.

Entry	Compound	Structure	Yield%[a,b]	Time (min)
1	**3a**		86	30
2	**3b**		84	40
3	**3c**		78	40
4	**3d**		79	40
5	**3e**		80	35
6	**3f**		82	35
7	**3g**		76	40
8	**3h**		72	40

9	**3i**		74	40
10	**3j**		75	35
11	**3k**		77	35
12	**3l**		69	40
13	**3m**		75	40
14	**3n**		78	40
15	**4a**		89	20
16	**4b**		87	20
17	**5a**		84	40
18	**5b**		81	40
19	**5c**		82	40

[a] All reactions were conducted in dioxane under microwave irradiation of power 300 W at 110 °C;
[b] All reactions were followed up by TLC analyses.

1.5. The procedures and spectral data

General procedure for the synthesis of pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one hybrids 3a-n.

A mixture of ethyl 3-aminothieno[2,3-*b*]pyridine-2-carboxylate **1** (5 mmol), $Me_2NCH(OMe)_2$ (5 mmol) and the appropriate of 1*H*-pyrazol-5-amines **2a-g**, 2-aminothiazoles **2h-l**, benzo[*d*]thiazol-2-amine **2m** or benzo[*d*]oxazol-2-amine **2n** (5 mmol) in dioxane (10 mL) was irradiated by microwaves with a power of 300 W to reach a reaction temperature of 110 °C under auto-generated pressure for 30-40 min. The reaction mixture was cooled and then ethanol (2 mL) was added dropwise. The product was collected by filtration, washed with cold ethanol, dried and then recrystallized from the proper solvent.

7,9-Dimethyl-3-(3-methyl-1*H*-pyrazol-5-yl)pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidin-4(3*H*)-one (3a).

Yellow solid (dioxane / ethanol mixture); m.p. 204-206 ºC; IR (υ cm^{-1}): 1664 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 6.42 (s, 1H, pyrazole-H), 7.06 (s, 1H, pyridine-H), 8.19 (s, 1H, pyrimidine-H), 13.80 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 14.2, 20.2, 24.7, 102.5, 117.2, 118.4, 122.9, 142.8, 143.8, 145.7, 148.2, 153.5, 155.4, 156.8, 162.5; Anal. calcd. for $C_{15}H_{13}N_5OS$ (311.3): C, 57.86; H, 4.21; N, 22.49; found: C, 58.07; H, 3.97; N, 22.24%.

7,9-Dimethyl-3-(3-methyl-4-phenyl-1*H*-pyrazol-5-yl)pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidin-4(3*H*)-one (3b).

Yellow solid (dioxane / ethanol mixture); m.p. 218-220 ºC; IR (υ cm^{-1}): 1666 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 7.08 (s, 1H, pyridine-H), 7.54 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.62-7.67 (m, 4H, Ar-H's), 8.16 (s, 1H, pyrimidine-H), 12.86 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 14.3, 20.2, 24.7, 111.4, 117.8, 118.9, 122.6, 127.7, 127.9, 128.9, 132.9, 141.9, 143.4, 145.1, 147.6, 153.2, 155.7, 157.3, 163.3; Anal. calcd. for $C_{21}H_{17}N_5OS$ (387.4): C, 65.10; H, 4.42; N, 18.08; found: C, 64.89; H, 4.26; N, 18.31%.

7,9-Dimethyl-3-(3-phenyl-1*H*-pyrazol-5-yl)pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (3c).

Yellow solid (dioxane / ethanol mixture); m.p. 212-214 ºC; IR (υ cm^{-1}): 1666 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 6.56 (s, 1H, pyrazole-H), 7.06 (s, 1H, pyridine-H), 7.26 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.40 (t, *J* = 7.6 Hz, 2H, Ar-H's), 8.16 (s, 1H, pyrimidine-H), 8.21 (d, *J* = 7.6 Hz, 2H, Ar-H's), 13.22 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ

20.3, 24.7, 103.2, 116.5, 117.6, 123.2, 125.7, 128.3, 128.7, 134.4, 142.7, 144.7, 145.6, 150.4, 153.7, 155.5, 157.6, 163.9; Anal. calcd. for $C_{20}H_{15}N_5OS$ (373.4): C, 64.33; H, 4.05; N, 18.75; found: C, 64.07; H, 3.79; N, 18.99%.

3-[3-(4-Chlorophenyl)-1*H*-pyrazol-5-yl]-7,9-dimethylpyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (3d).

Yellow solid (dioxane); m.p. 222-225 ^oC; IR (υ cm^{-1}): 1668 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 6.52 (s, 1H, pyrazole-H), 7.09 (s, 1H, pyridine-H), 7.32 (d, *J* = 8.4 Hz, 2H, Ar-H's), 8.13 (s, 1H, pyrimidine-H), 8.18 (d, *J* = 8.4 Hz, 2H, Ar-H's), 13.07 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 20.1, 24.7, 103.1, 116.9, 117.8, 122.7, 126.9, 128.9, 132.7, 133.2, 142.3, 144.8, 145.2, 149.2, 153.4, 155.8, 157.2, 163.6; Anal. calcd. for $C_{20}H_{14}ClN_5OS$ (407.8): C, 58.90; H, 3.46; N, 17.17; found: C, 59.16; H, 3.27; N, 16.95%.

7,9-Dimethyl-3-[3-(*p*-tolyl)-1*H*-pyrazol-5-yl]pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidin-4(3*H*)-one (3e).

Yellow solid (dioxane); m.p. 210-213 ^oC; IR (υ cm^{-1}): 1666 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.29 (s, 3H, *p*-CH_3), 2.45 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 6.68 (s, 1H, pyrazole-H), 7.06 (s, 1H, pyridine-H), 7.24 (d, *J* = 8.4 Hz, 2H, Ar-H's), 8.14 (s, 1H, pyrimidine-H), 8.18 (d, *J* = 8.4 Hz, 2H, Ar-H's), 12.96 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 20.2, 21.0, 24.8, 103.0, 117.4, 118.5, 123.4, 125.7, 129.5, 131.7, 137.0, 141.6, 144.6, 144.9, 150.5, 154.0, 156.2, 157.7, 164.3; Anal. calcd. for $C_{21}H_{17}N_5OS$ (387.4): C, 65.10; H, 4.42; N, 18.08; found: C, 64.86; H, 4.19; N, 18.33%.

3-[3-(4-Methoxyphenyl)-1*H*-pyrazol-5-yl]-7,9-dimethylpyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (3f).

Yellow solid (dioxane); m.p. 242-245 ^oC; IR (υ cm^{-1}): 1669 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 3.87 (s, 3H, *p*-OCH_3), 6.66 (s, 1H, pyrazole-H), 7.02 (d, *J* = 8.4 Hz, 2H, Ar-H's), 7.09 (s, 1H, pyridine-H), 8.03 (d, *J* = 8.4 Hz, 2H, Ar-H's), 8.14 (s, 1H, pyrimidine-H), 13.10 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 20.0, 24.5, 55.2, 102.7, 114.3, 117.1, 118.2, 123.2, 127.0, 131.3, 141.8, 144.5, 144.6, 150.4, 153.7, 155.8, 157.3, 159.6, 163.5; Anal. calcd. for $C_{21}H_{17}N_5O_2S$ (403.4): C, 62.52; H, 4.25; N, 17.36; found: C, 62.77; H, 4.02; N, 17.14%.

7,9-Dimethyl-3-[3-(4-nitrophenyl)-1*H*-pyrazol-5-yl]pyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (3g).

Yellow solid (dioxane); m.p. 254-257 ºC; IR (υ cm^{-1}): 1664 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 6.86 (s, 1H, pyrazole-H), 7.09 (s, 1H, pyridine-H), 8.08 (d, *J* = 8.4 Hz, 2H, Ar-H's), 8.17 (s, 1H, pyrimidine-H), 8.22 (d, *J* = 8.4 Hz, 2H, Ar-H's), 13.29 (br s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ 20.4, 24.9, 104.0, 116.7, 117.9, 123.2, 124.3, 126.0, 140.4, 142.3, 144.4, 145.3, 146.8, 147.6, 153.8, 155.6, 156.4, 163.0; Anal. calcd. for $C_{20}H_{14}N_6O_3S$ (418.4): C, 57.41; H, 3.37; N, 20.09; found: C, 57.13; H, 3.60; N, 19.86%.

7,9-Dimethyl-3-(4-phenylthiazol-2-yl)pyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidin-4(3*H*)-one (3h).

Yellow solid (dioxane / ethanol mixture); m.p. 210 ºC; IR (υ cm^{-1}): 1664 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 7.10 (s, 1H, pyridine-H), 7.39 (t, *J* = 7.6 Hz, 1H, Ar-H's), 7.43 (t, *J* = 7.6 Hz, 2H, Ar-H's), 7.72 (s, 1H, thiazole-H), 7.89 (d, *J* = 7.6 Hz, 2H, Ar-H's), 8.17 (s, 1H, pyrimidine-H); ^{13}C-NMR (DMSO-d_6): δ 20.0, 24.6, 113.2, 118.4, 119.1, 124.2, 126.6, 128.2, 129.3, 133.1, 144.4, 146.0, 150.2, 152.9, 153.8, 154.9, 157.6, 164.7; Anal. calcd. for $C_{20}H_{14}N_4OS_2$ (390.4): C, 61.52; H, 3.61; N, 14.35; found: C, 61.35; H, 3.86; N, 14.11%.

3-[4-(4-Chlorophenyl)thiazol-2-yl]-7,9-dimethylpyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (3i).

Yellow solid (dioxane); m.p. 224-226 ºC; IR (υ cm^{-1}): 1667 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 7.08 (s, 1H, pyridine-H), 7.49 (d, *J* = 8 Hz, 2H, Ar-H's), 7.61 (d, *J* = 8 Hz, 2H, Ar-H's), 7.75 (s, 1H, thiazole-H), 8.14 (s, 1H, pyrimidine-H); ^{13}C-NMR (DMSO-d_6): δ 20.2, 24.5, 113.2, 118.8, 119.6, 123.7, 127.5, 128.6, 132.4, 135.9, 143.9, 146.3, 149.4, 152.2, 154.2, 155.2, 157.3, 165.2; Anal. calcd. for $C_{20}H_{13}ClN_4OS_2$ (424.9): C, 56.53; H, 3.08; N, 13.19; found: C, 56.74; H, 3.33; N, 12.97%.

7,9-Dimethyl-3-[4-(*p*-tolyl)thiazol-2-yl]pyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidin-4(3*H*)-one (3j).

Yellow solid (dioxane / ethanol mixture); m.p. 214 ºC; IR (υ cm^{-1}): 1665 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.38 (s, 3H, *p*-CH_3), 2.46 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 7.06 (s, 1H, pyridine-H), 7.30 (d, *J* = 8 Hz, 2H, Ar-H's), 7.69 (s, 1H, thiazole-H), 7.86 (d, *J* = 8 Hz, 2H, Ar-H's), 8.18 (s, 1H, pyrimidine-H); ^{13}C-NMR (DMSO-d_6): δ 20.1, 20.6, 24.7, 112.6, 118.9, 120.2, 124.7, 126.1, 129.0, 131.2, 139.2, 143.1, 145.6, 149.7, 152.4, 154.3, 155.2, 156.9, 163.5; Anal. calcd. for $C_{21}H_{16}N_4OS_2$ (404.5): C, 62.36; H, 3.99; N, 13.85; found: C, 62.10; H, 4.24; N, 14.06%.

3-[4-(4-Methoxyphenyl)thiazol-2-yl]-7,9-dimethylpyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (3k).

Yellow solid (dioxane); m.p. 226-229 ºC; IR (υ cm^{-1}): 1668 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.40 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 3.83 (s, 3H, *p*-OCH_3), 7.02 (d, *J* = 8 Hz, 2H, Ar-H's), 7.11 (s, 1H, pyridine-H), 7.58 (d, *J* = 8 Hz, 2H, Ar-H's), 7.74 (s, 1H, thiazole-H), 8.18 (s, 1H, pyrimidine-H); ^{13}C-NMR (DMSO-d_6): δ 20.3, 24.8, 55.5, 113.8, 114.2, 117.7, 118.5, 123.6, 127.2, 128.1, 143.8, 144.9, 151.1, 153.0, 153.9, 154.4, 156.8, 159.4, 163.6; Anal. calcd. for $C_{21}H_{16}N_4O_2S_2$ (420.5): C, 59.98; H, 3.84; N, 13.32; found: C, 6.21; H, 4.05; N, 13.17%.

7,9-Dimethyl-3-[4-(4-nitrophenyl)thiazol-2-yl]pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidin-4(3*H*)-one (3l).

Yellow solid (dioxane); m.p. 236-238 ºC; IR (υ cm^{-1}): 1670 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 7.07 (s, 1H, pyridine-H), 7.74 (s, 1H, thiazole-H), 8.05 (d, *J* = 8 Hz, 2H, Ar-H's), 8.20 (s, 1H, pyrimidine-H), 8.31 (d, *J* = 8 Hz, 2H, Ar-H's); ^{13}C-NMR (DMSO-d_6): δ 20.3, 24.8, 112.4, 117.6, 118.4, 122.9, 123.8, 126.2, 135.6, 141.8, 144.7, 148.5, 149.4, 151.6, 153.8, 155.7, 156.5, 164.4; Anal. calcd. for $C_{20}H_{13}N_5O_3S_2$ (435.4): C, 55.16; H, 3.01; N, 16.08; found: C, 54.92; H, 2.75; N, 15.91%.

3-(Benzo[*d*]thiazol-2-yl)-7,9-dimethylpyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidin-4(3*H*)-one (3m).

Yellow solid (dioxane); m.p. 240 ºC; IR (υ cm^{-1}): 1671 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 7.05 (s, 1H, pyridine-H), 7.36 (t, *J* = 7.6 Hz, 1H, H6′), 7.42 (t, *J* = 7.6 Hz, 1H, H5′), 7.79 (d, *J* = 7.6 Hz, 1H, H7′), 7.88 (d, *J* = 7.6 Hz, 1H, H4′), 8.17 (s, 1H, pyrimidine-H); ^{13}C-NMR (DMSO-d_6): δ 20.2, 24.6, 118.2, 118.9, 120.4, 120.5, 122.5, 123.3, 125.7, 132.3, 142.4, 144.8, 151.7, 153.4, 153.6, 155.2, 156.8, 163.8; Anal. calcd. for $C_{18}H_{12}N_4OS_2$ (364.4): C, 59.32; H, 3.32; N, 15.37; found: C, 59.08; H, 3.54; N, 15.13%.

3-(Benzo[*d*]oxazol-2-yl)-7,9-dimethylpyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidin-4(3*H*)-one (3n).

Yellow solid (dioxane); m.p. 254 ºC; IR (υ cm^{-1}): 1667 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 7.09 (s, 1H, pyridine-H), 7.27-7.35 (m, 2H, H5´ and H6´), 7.59 (d, *J* = 7.6 Hz, 1H, H7´), 7.65 (d, *J* = 7.6 Hz, 1H, H4´), 8.14 (s, 1H, pyrimidine-H); ^{13}C-NMR (DMSO-d_6): δ 20.4, 24.9, 110.7, 117.5, 117.9, 119.5, 122.8, 123.2, 125.0, 141.4, 143.4, 145.6, 148.9, 150.9, 154.7, 155.7, 157.4, 165.2; Anal. calcd. for $C_{18}H_{12}N_4O_2S$ (348.3): C, 62.06; H, 3.47; N, 16.08; found: C, 61.81; H, 3.72; N, 15.93%.

General procedure for the synthesis of bis(pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one) hybrids 4 and 5.

A mixture of ethyl 3-aminothieno[2,3-*b*]pyridine-2-carboxylate **1** (10 mmol), $Me_2NCH(OMe)_2$ (10 mmol) and the appropriate bis(amine) (5 mmol) in dioxane (10 mL) was irradiated by microwaves with a power of 300 W to reach a reaction temperature of 110 °C under autogenerated pressure for 20-40 min. The reaction mixture was cooled, and then ethanol (2 mL) was added dropwise. The product was collected by filtration, washed with cold ethanol, dried and then recrystallized from the proper solvent.

3,3'-(Propane-1,3-diyl)bis(7,9-dimethylpyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidin-4(3*H*)-one) (4a).

Yellow solid (dioxane / ethanol mixture); m.p. 200-203 ºC; IR (υ cm^{-1}): 1664 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.92 (quint, *J* = 6.8 Hz, 2H, NCH_2CH_2), 2.42 (s, 6H, 2 CH_3), 2.54 (s, 6H, 2 CH_3), 4.19 (t, *J* = 6.8 Hz, 4H, 2 NCH_2), 7.13 (s, 2H, 2 pyridine-H's), 8.15 (s, 2H, 2 pyrimidine-H's); ^{13}C-NMR (DMSO-d_6): δ 20.1, 24.8, 27.2, 43.6, 118.6, 119.9, 123.7, 142.5, 146.2, 154.8, 157.0, 158.6, 163.8; Anal. calcd. for $C_{25}H_{22}N_6O_2S_2$ (502.6): C, 59.74; H, 4.41; N, 16.72; found: C, 59.99; H, 4.15; N, 16.49%.

3,3'-(Butane-1,4-diyl)bis(7,9-dimethylpyrido[3',2':4,5]thieno[3,2-*d*]-pyrimidin-4(3*H*)-one) (4b).

Yellow solid (dioxane / ethanol mixture); m.p. 214-216 ºC; IR (υ cm^{-1}): 1670 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.70 (t, *J* = 6.8 Hz, 4H, 2 NCH_2CH_2), 2.46 (s, 6H, 2 CH_3), 2.58 (s, 6H, 2 CH_3), 4.17 (t, *J* = 6.8 Hz, 4H, 2 NCH_2), 7.11 (s, 2H, 2 pyridine-H's), 8.14 (s, 2H, 2 pyrimidine-H2's), ^{13}C-NMR (DMSO-d_6): δ 20.2, 24.8, 25.9, 45.2, 118.9, 119.7, 123.3, 141.9, 146.3, 154.5, 156.8, 157.9, 163.4; Anal. calcd. for $C_{26}H_{24}N_6O_2S_2$ (516.6): C, 60.45; H, 4.68; N, 16.27; found: C, 60.71; H, 4.40; N, 16.02%.

3,3'-{[Propane-1,3-diylbis(oxy)]bis(4,1-phenylene)}bis(7,9-dimethylpyrido-[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one) (5a).

Yellow solid (dioxane); m.p. 274-276 ºC; IR (υ cm^{-1}): 1667 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.13 (quint, *J* = 6.8 Hz, 2H, OCH_2CH_2), 2.47 (s, 6H, 2 CH_3), 2.62 (s, 6H, 2 CH_3), 4.15 (t, *J* = 6.8 Hz, 4H, 2 OCH_2CH_2), 6.83 (d, *J* = 7.6 Hz, 4H, Ar-H's), 7.09 (s, 2H, 2 pyridine-H's), 7.18 (d, *J* = 7.6 Hz, 4H, Ar-H's), 8.11 (s, 2H, 2 pyrimidine-H's); ^{13}C-NMR (DMSO-d_6): δ 20.4, 24.9, 29.5, 66.7, 115.2, 117.8, 119.4, 122.2, 123.3, 141.9, 144.7, 146.0, 153.2, 155.6, 156.9, 158.6, 164.2; Anal. calcd. for $C_{37}H_{30}N_6O_4S_2$ (686.8): C, 64.71; H, 4.40; N, 12.24; found: C, 64.96; H, 4.17; N, 11.98%.

3,3'-{[Butane-1,4-diylbis(oxy)]bis(4,1-phenylene)}bis(7,9-dimethylpyrido-[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one) (5b).

Yellow solid (dioxane); m.p. 268-270 ºC; IR (υ cm^{-1}): 1667 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.83 (t, *J* = 6.8 Hz, 4H, 2 OCH_2CH_2), 2.44 (s, 6H, 2 CH_3), 2.59 (s, 6H, 2 CH_3), 4.09 (t, *J* = 6.8 Hz, 4H, 2 OCH_2CH_2), 6.84 (d, *J* = 7.6 Hz, 4H, Ar-H's), 7.07 (s, 2H, pyridine-H's), 7.19 (d, *J* = 7.6 Hz, 4H, Ar-H's), 8.13 (s, 2H, 2 pyrimidine-H's); ^{13}C-NMR (DMSO-d_6): δ 20.4, 24.9, 26.9, 69.3, 115.6, 118.2, 119.1, 121.8, 122.9, 142.3, 144.7, 145.1, 153.7, 155.9, 157.3, 158.7, 163.6; Anal. calcd. for $C_{38}H_{32}N_6O_4S_2$ (700.8): C, 65.13; H, 4.60; N, 11.99; found: C, 64.90; H, 4.87; N, 12.24%.

3,3'-{[Pentane-1,5-diylbis(oxy)]bis(4,1-phenylene)}bis(7,9-dimethylpyrido-[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one) (5c).

Yellow solid (dioxane); m.p. 254-257 ºC; IR (υ cm^{-1}): 1666 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.58 (br s, 2H, $OCH_2CH_2CH_2$), 1.69 (br s, 4H, 2 OCH_2CH_2), 2.46 (s, 6H, 2 CH_3), 2.61 (s, 6H, 2 CH_3), 4.07 (br s, 4H, 2 OCH_2CH_2), 6.83 (d, *J* = 7.6 Hz, 4H, Ar-H's), 7.05 (s, 2H, pyridine-H's), 7.17 (d, *J* = 7.6 Hz, 4H, Ar-H's), 8.12 (s, 2H, 2 pyrimidine-H's); ^{13}C-NMR (DMSO-d_6): δ 20.2, 22.2, 24.8, 29.1, 69.6, 114.9, 118.4, 118.8, 122.4, 123.5, 140.9, 142.9, 145.3, 151.5, 153.5, 155.8, 158.4, 163.2; Anal. calcd. for $C_{39}H_{34}N_6O_4S_2$ (714.8): C, 65.53; H, 4.79; N, 11.76; found: C, 65.25; H, 5.02; N, 11.64%.

1.6. Minimum inhibitory concentration (MIC) determination.

The inhibitory activities against three different Gram-positive bacterial strains [*Staphylococcus aureus* (ATCC:6538), *Streptococcus mutans*, (ATCC:25175) and *Enterococcus faecalis* (ATCC:29212)] were selected as well as three different Gram-negative strains [*Escherichia coli* (ATCC:9637), *Pseudomonas aeruginosa* (ATCC:27953) and *Klebsiella pneumonia* (ATCC:10031)] were estimated. MIC values were determined using microbroth serial dilution method [S1,S2] in a sterile 96-well microtiter plate after overnight incubation of tested bacteria at 37°C. This assay was performed in triplicates for consistency in accordance with guidelines provided by CLSI (2012) [S3]. Ciprofloxacin (100 μg susceptibility disc) was used as a standard drug. The concentration of the tested hybrids as well as Ciprofloxacin used in the study ranged from 250 to 0.9 μg/mL. The sterile Muller-Hinton broth (MHB) was enriched with 2% NaCl before the tested antimicrobial agents were inserted into the well at concentration gradient in a serial dilution. Then the diluted bacterial suspension at final inoculum of 10^6 CFU/mL was added. The tested compound in MHB was used as negative control to ensure medium sterility, while the inoculum in MHB served as positive control to ensure the adequacy of the broth for bacterial growth. To facilitate the observation of the growth of bacteria in each well, 20 μL of 2,3,5-triphenyltetrazolium chloride (TTC) at 2 mg/mL was added into each well.

2. References

[S1] A. E. M. Mekky, S. M. H. Sanad, *Bioorg. Chem.***,** 2020, 102, 104094.

[S2] H. Mohammad, P. N. Reddy, D. Monteleone, A. S. Mayhoub, M. Cushman, M. N. Seleem, *Eur. J. Med. Chem.*, 2015, **94**, 306.

[S3] CLSI. 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically-7th edition. Approved standard M07-A9, Clinical and Laboratory Standards Institute Wayne, PA.

3. ^{1}H- and ^{13}C-NMR copies of all new hybrids

Figure S1. ^{1}H-NMR spectrum of compound **3a**.

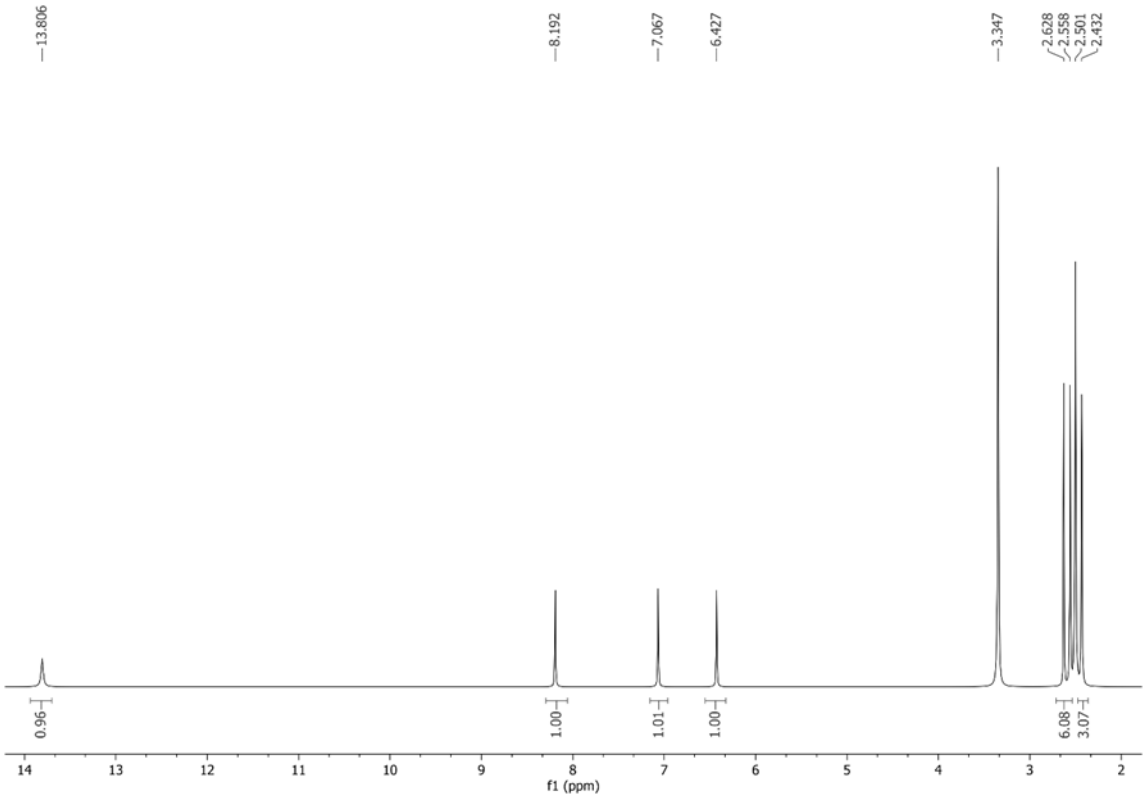


Figure S2. ^{13}C-NMR spectrum of compound **3a**.

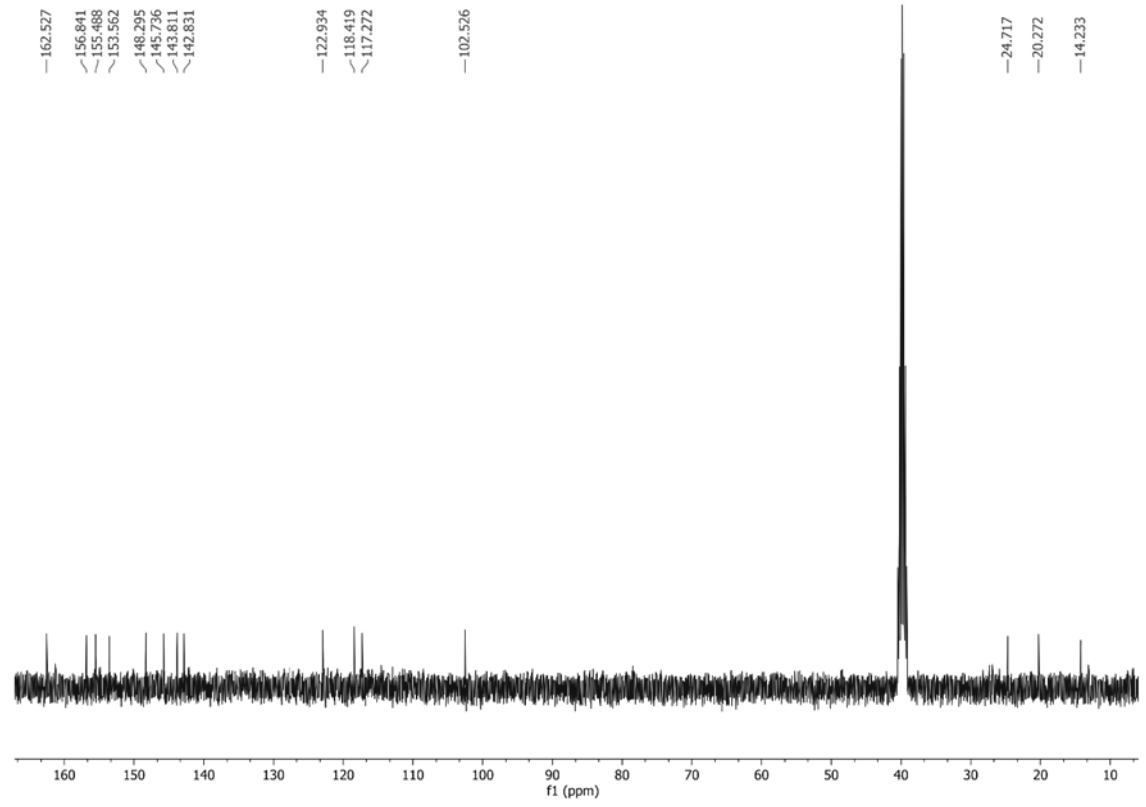


Figure S3. ^{1}H-NMR spectrum of compound **3b**.

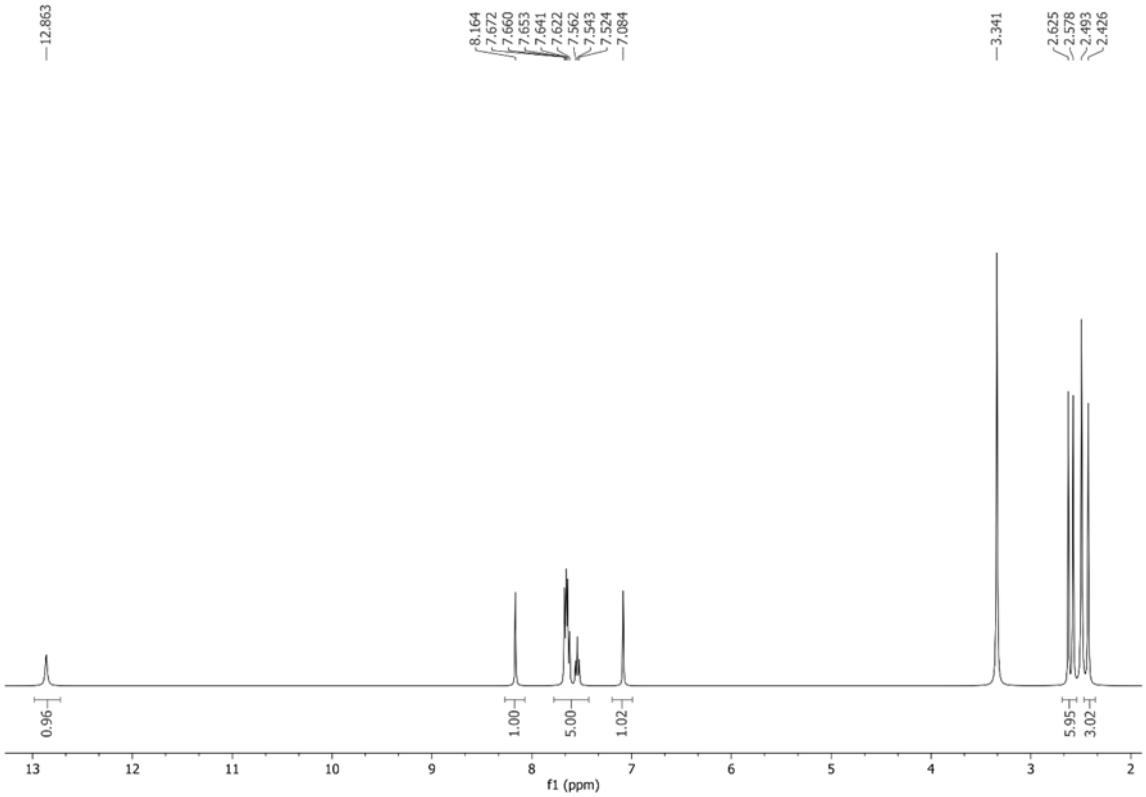


Figure S4. ^{13}C-NMR spectrum of compound **3b**.

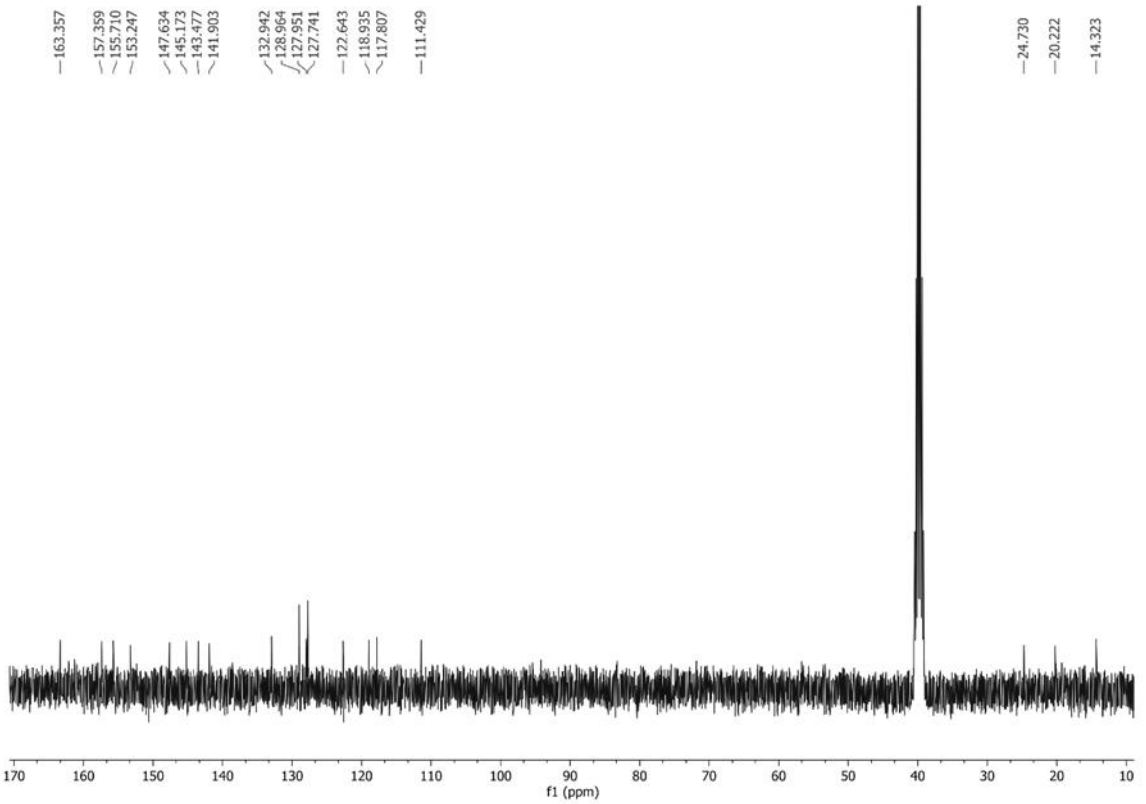


Figure S5. ^{1}H-NMR spectrum of compound **3c**.

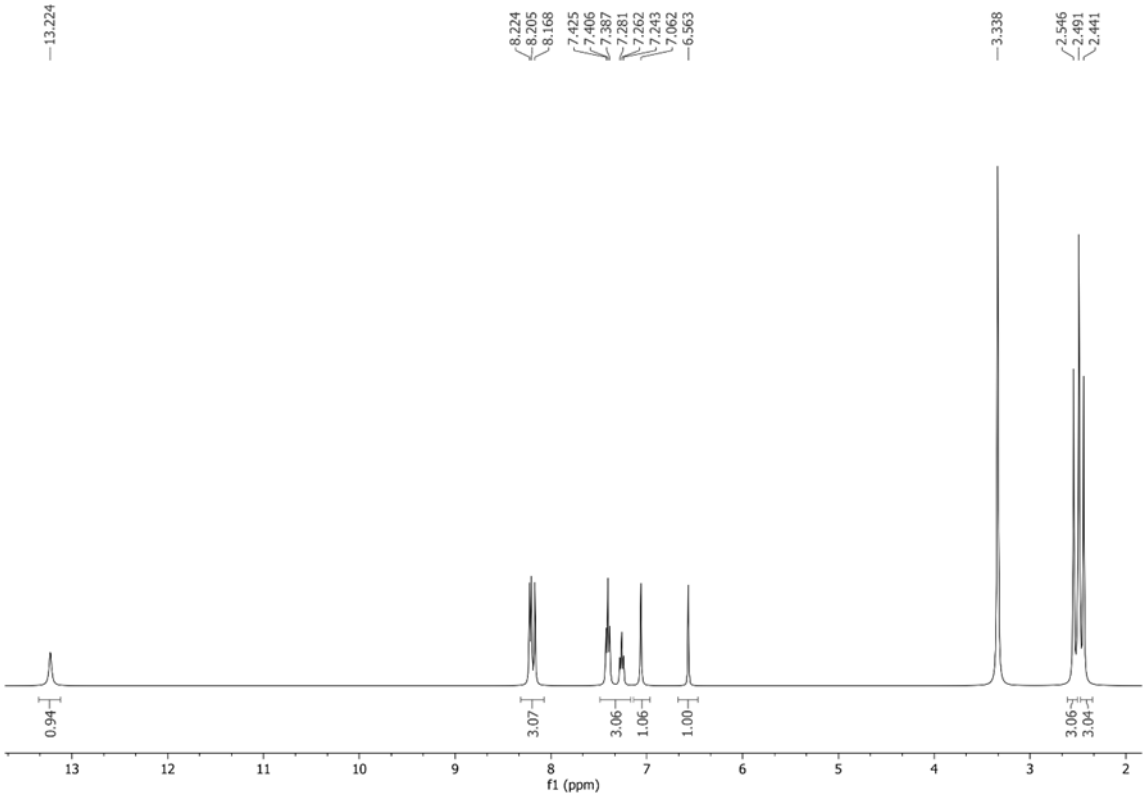


Figure S6. ^{13}C-NMR spectrum of compound **3c**.

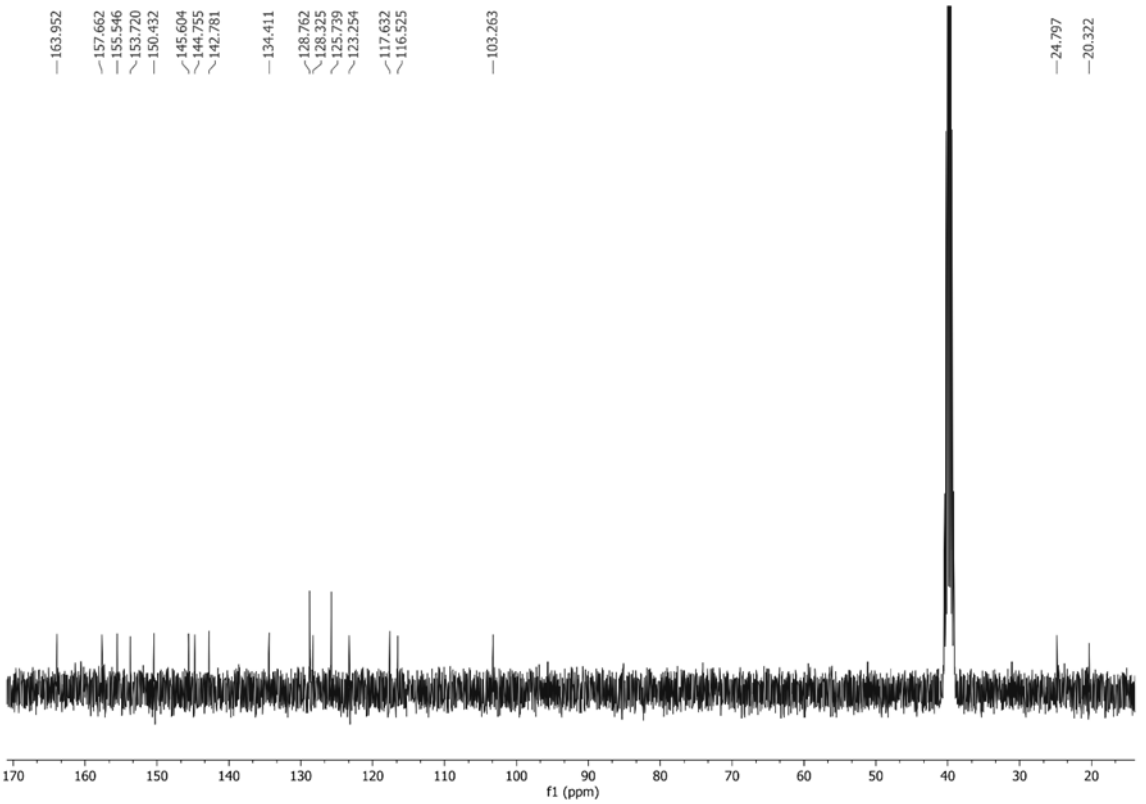


Figure S7. ^{1}H-NMR spectrum of compound **3d**.

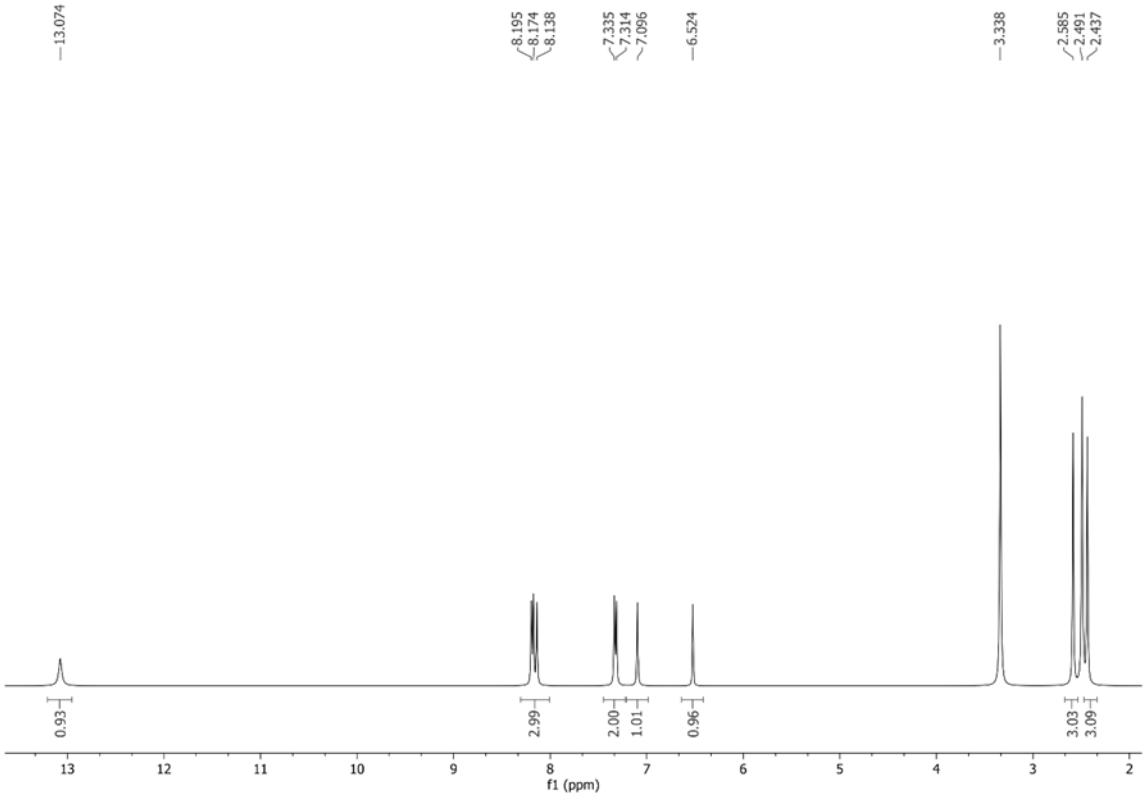


Figure S8. ^{13}C-NMR spectrum of compound **3d**.

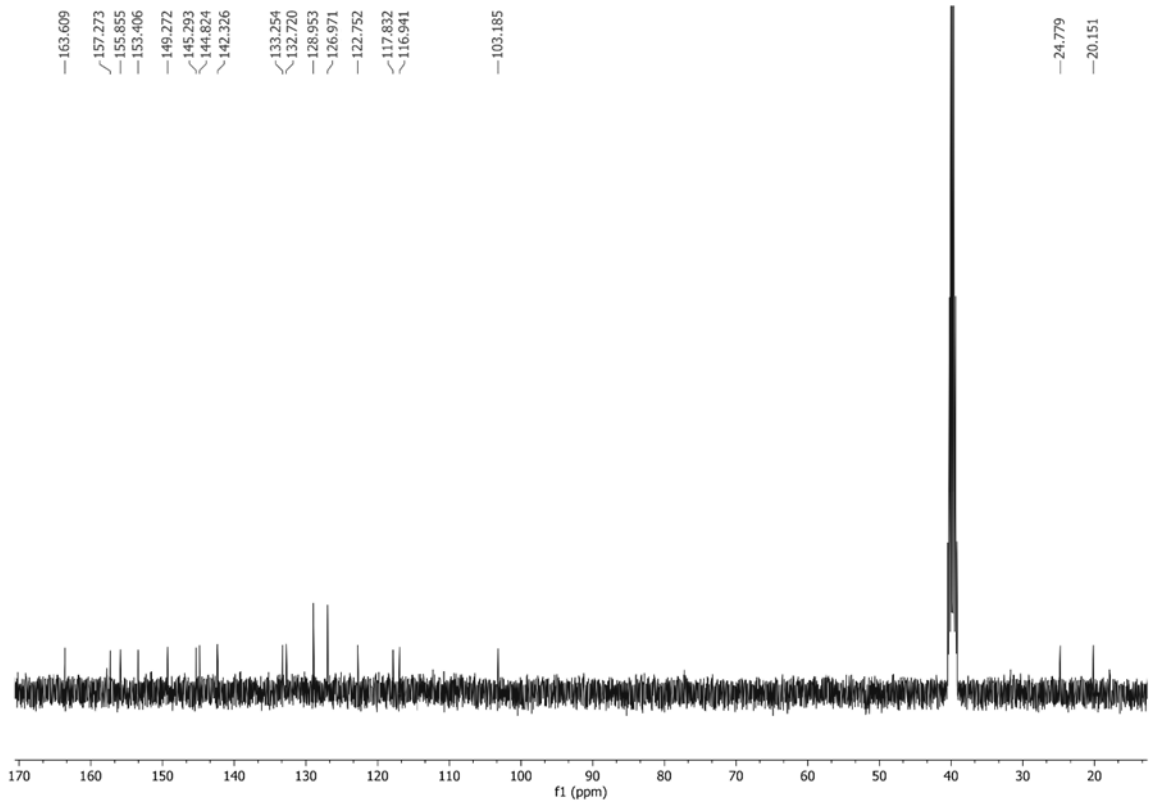


Figure S9. ^{1}H-NMR spectrum of compound **3e**.

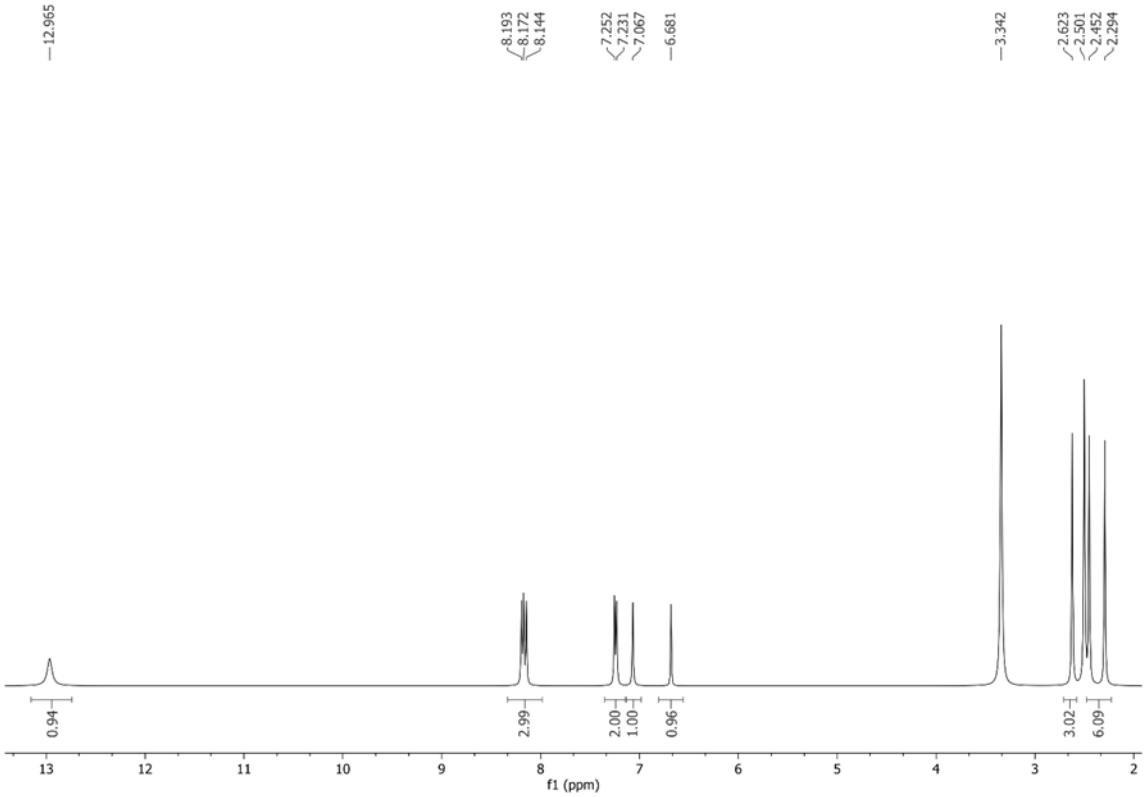


Figure S10. ^{13}C-NMR spectrum of compound **3e**.

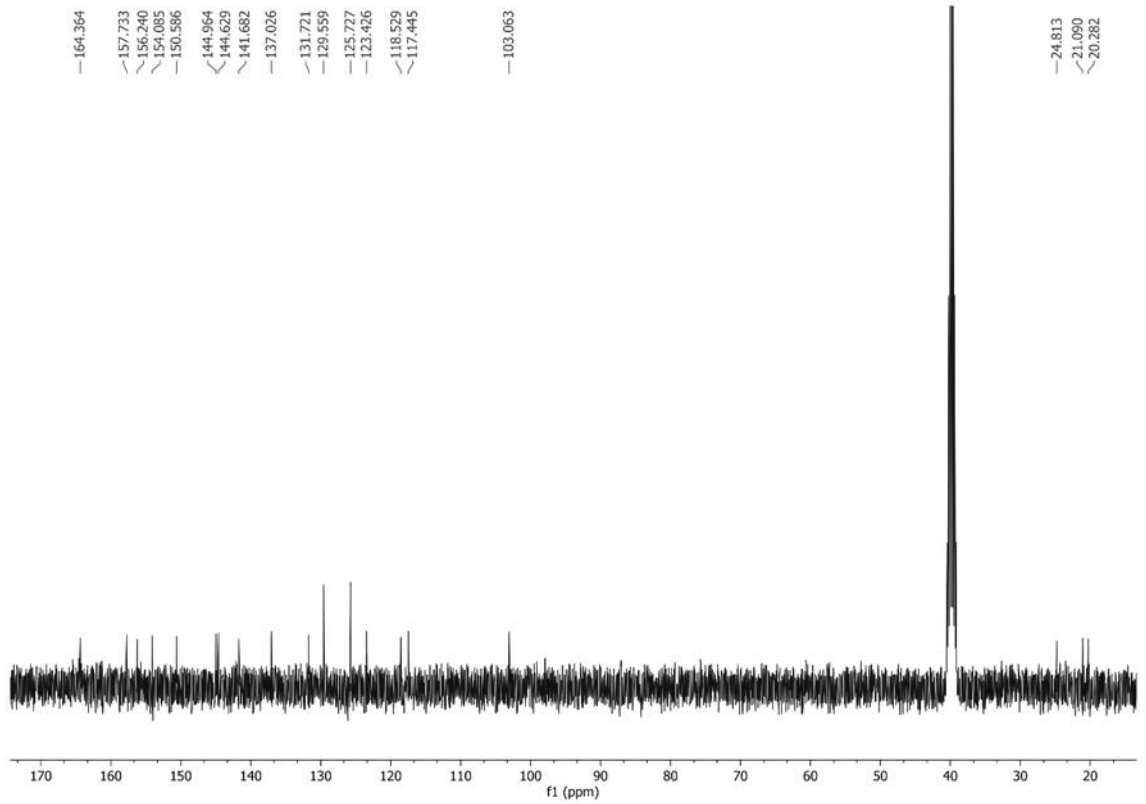


Figure S11. ^{1}H-NMR spectrum of compound **3f**.

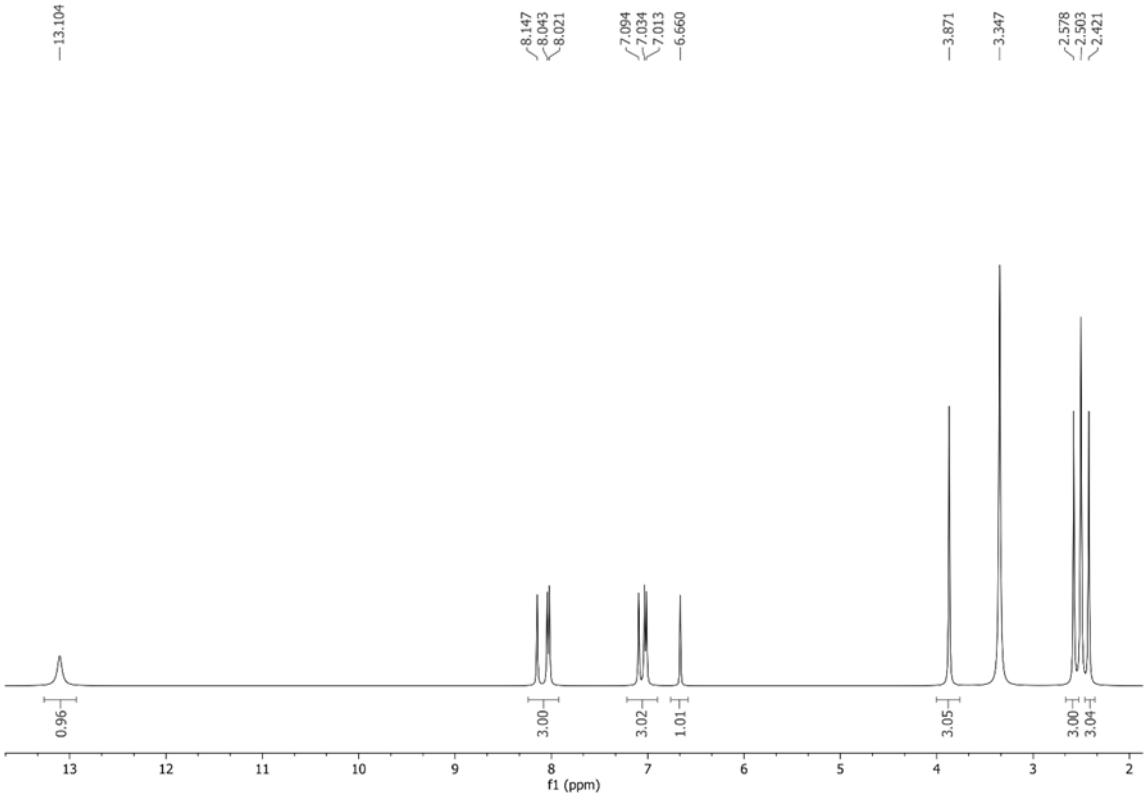


Figure S12. ^{13}C-NMR spectrum of compound **3f**.

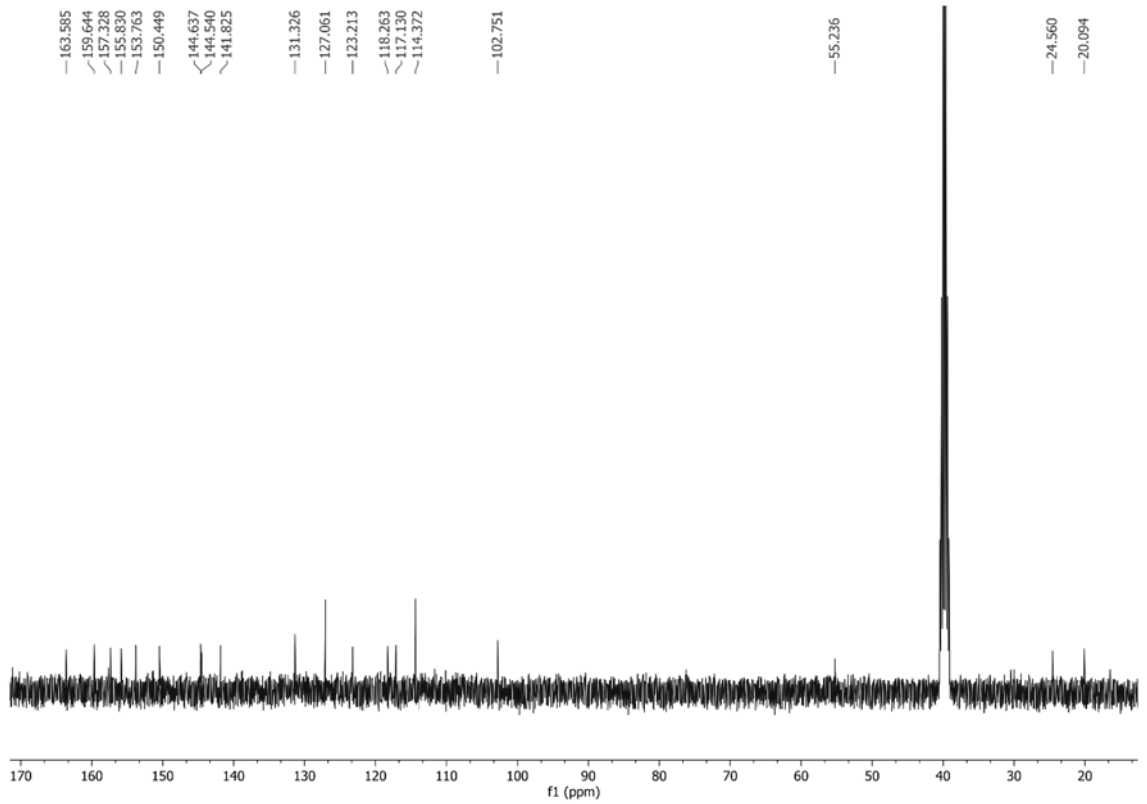


Figure S13. ^{1}H-NMR spectrum of compound **3g**.

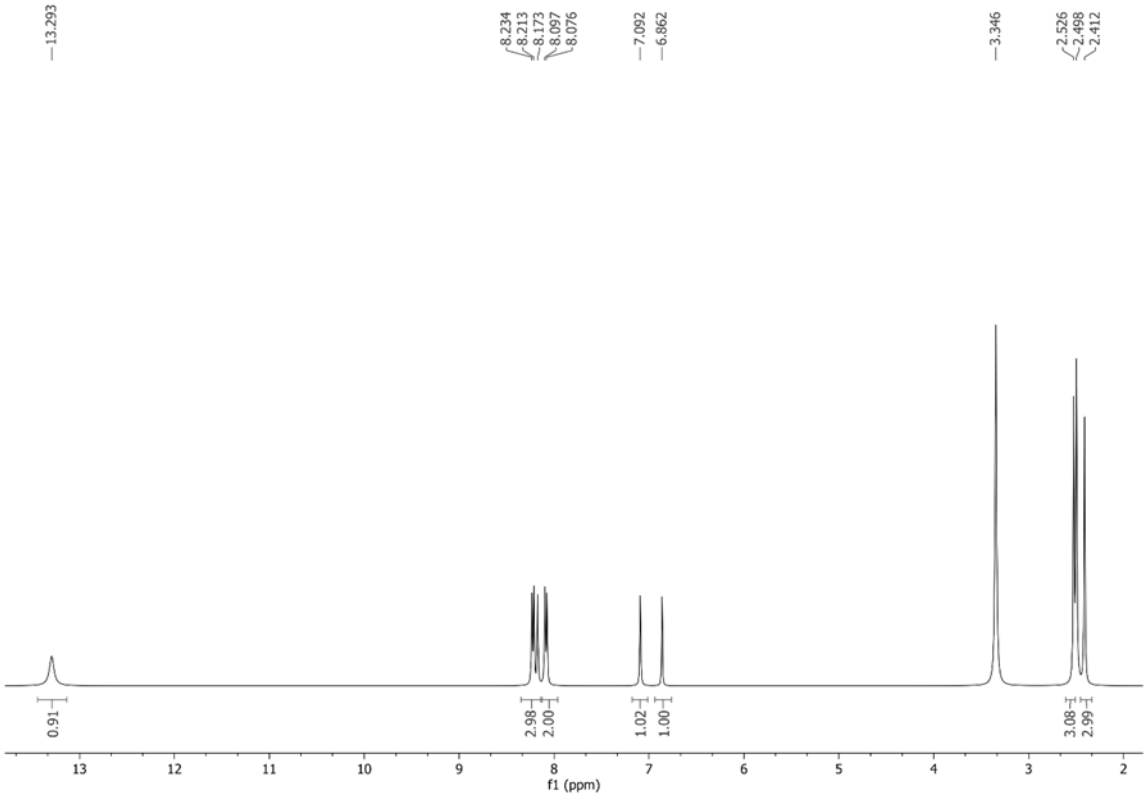


Figure S14. ^{13}C-NMR spectrum of compound **3g**.

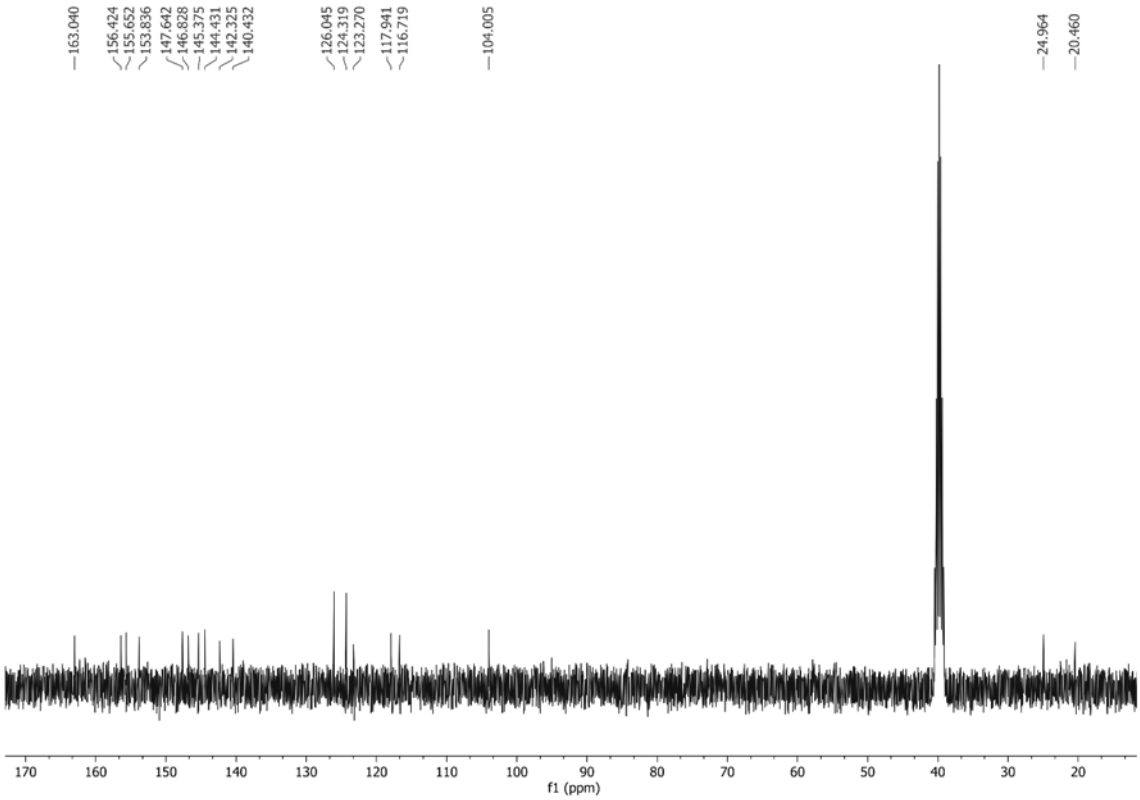


Figure S15. ^{1}H-NMR spectrum of compound **3h**.

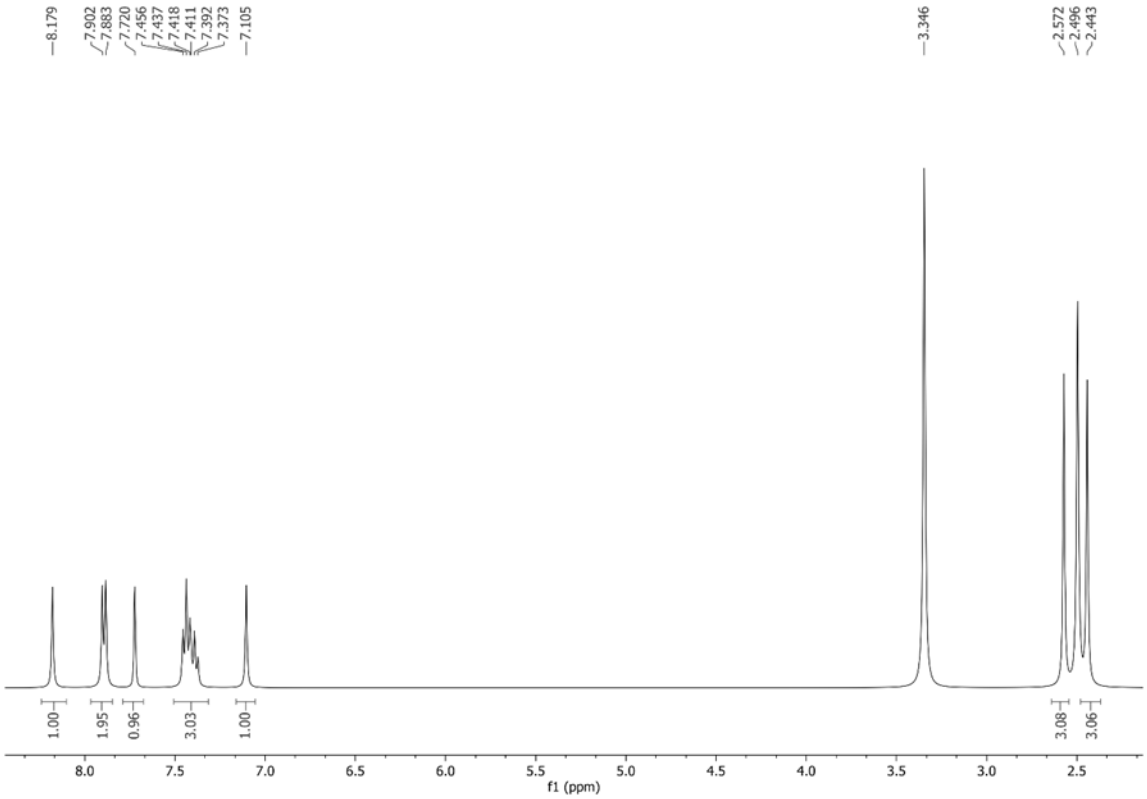


Figure S16. ^{13}C-NMR spectrum of compound **3h**.

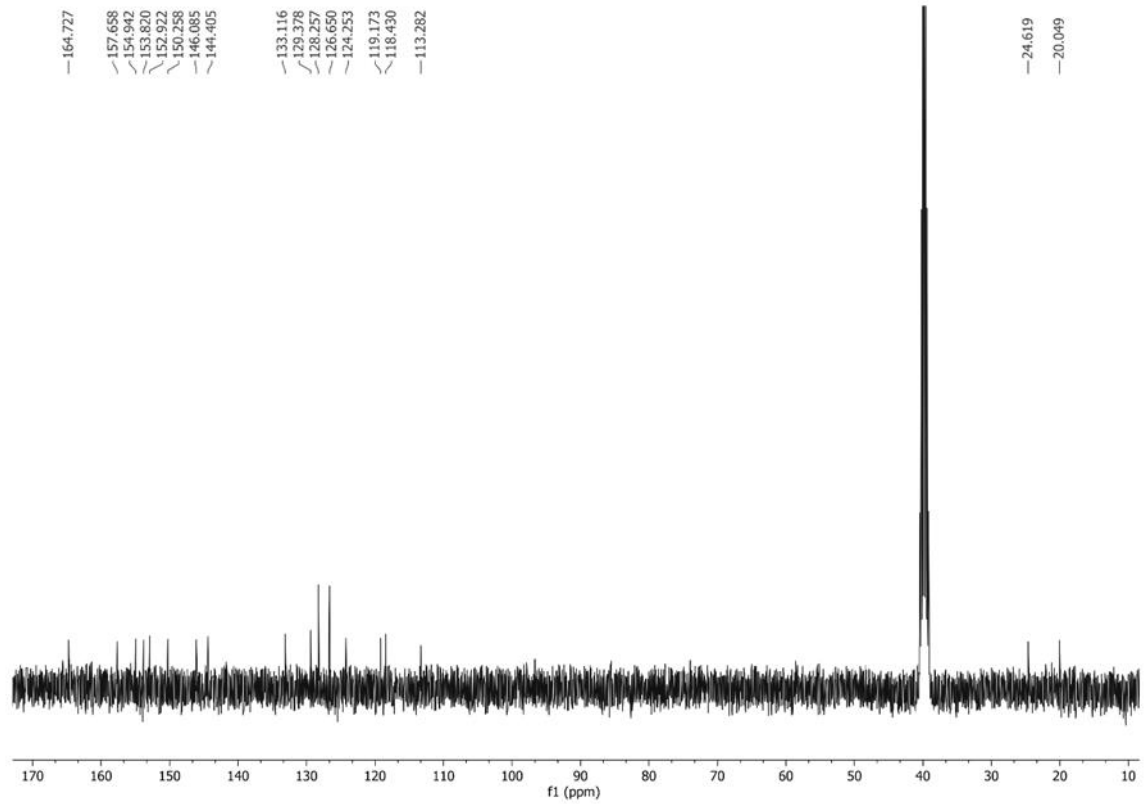


Figure S17. ^{1}H-NMR spectrum of compound **3i**.

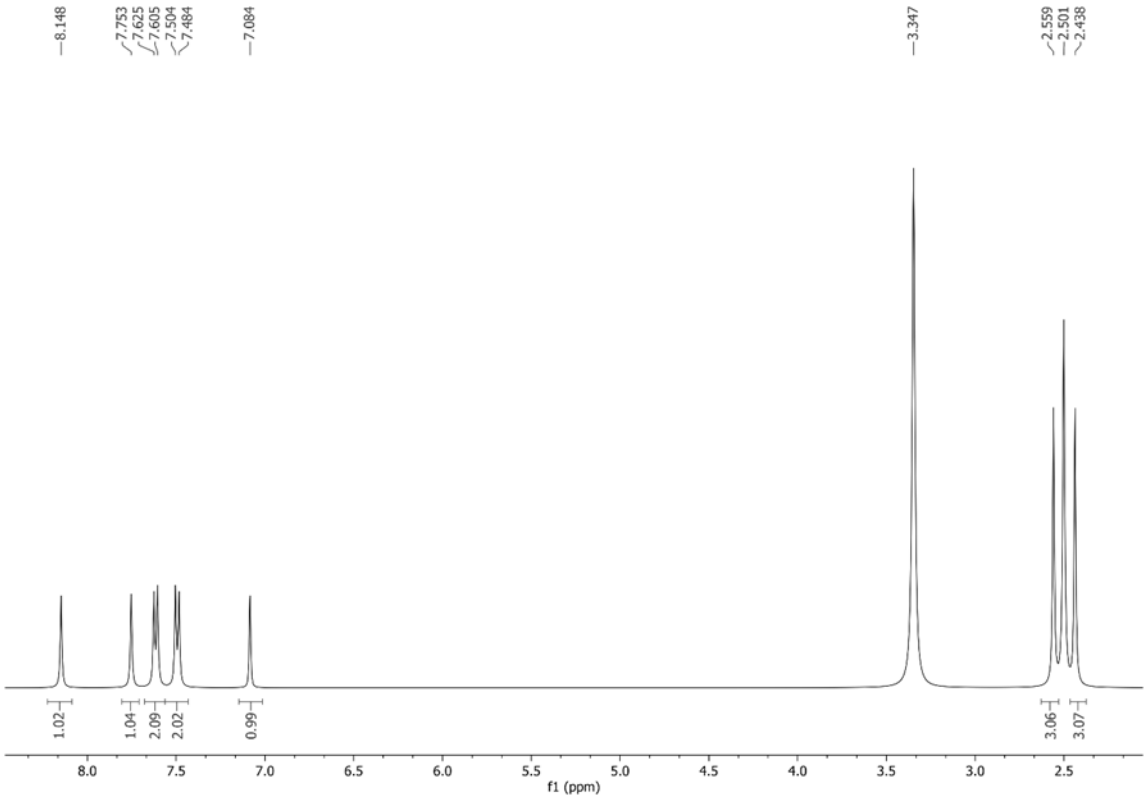


Figure S18. ^{13}C-NMR spectrum of compound **3i**.

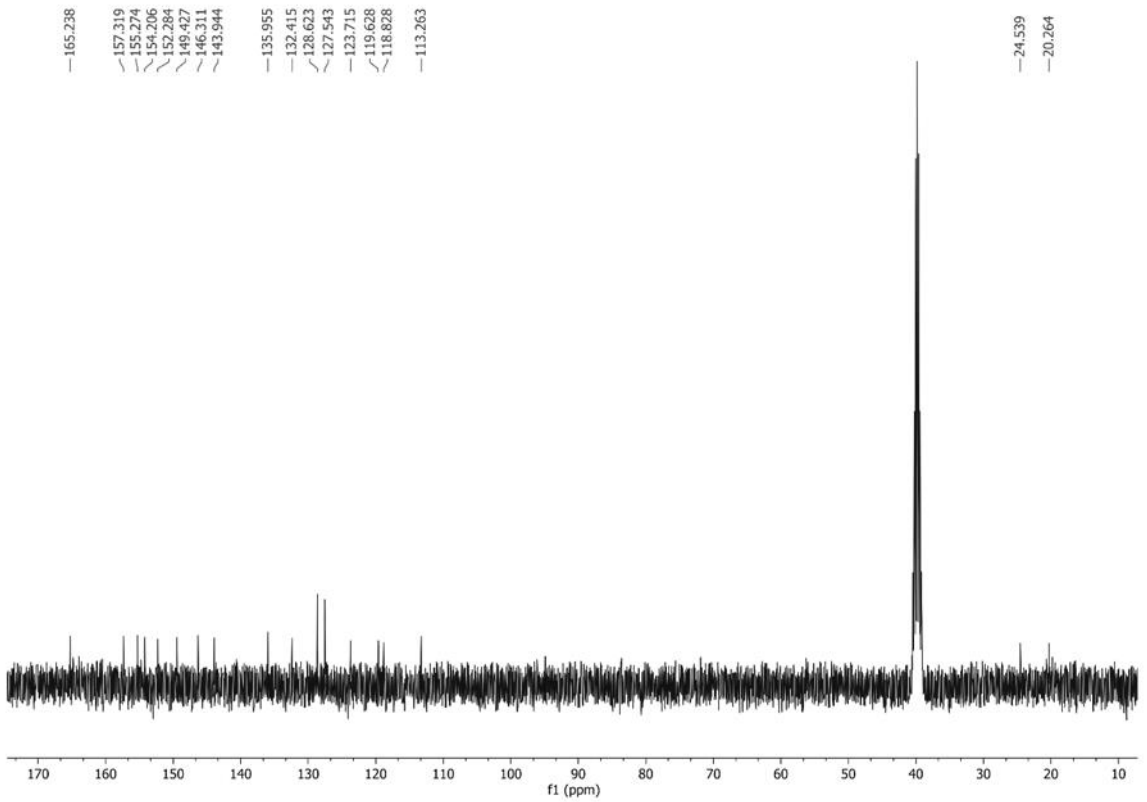


Figure S19. ^{1}H-NMR spectrum of compound **3j**.

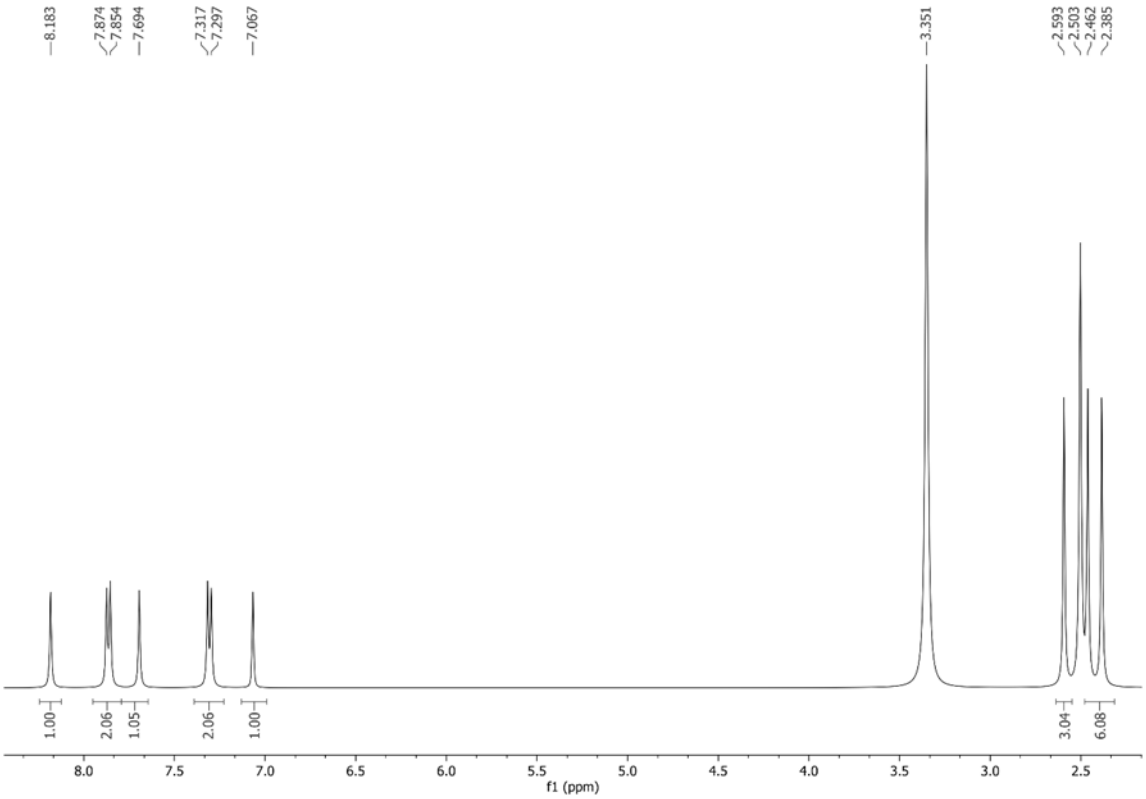


Figure S20. ^{13}C-NMR spectrum of compound **3j**.

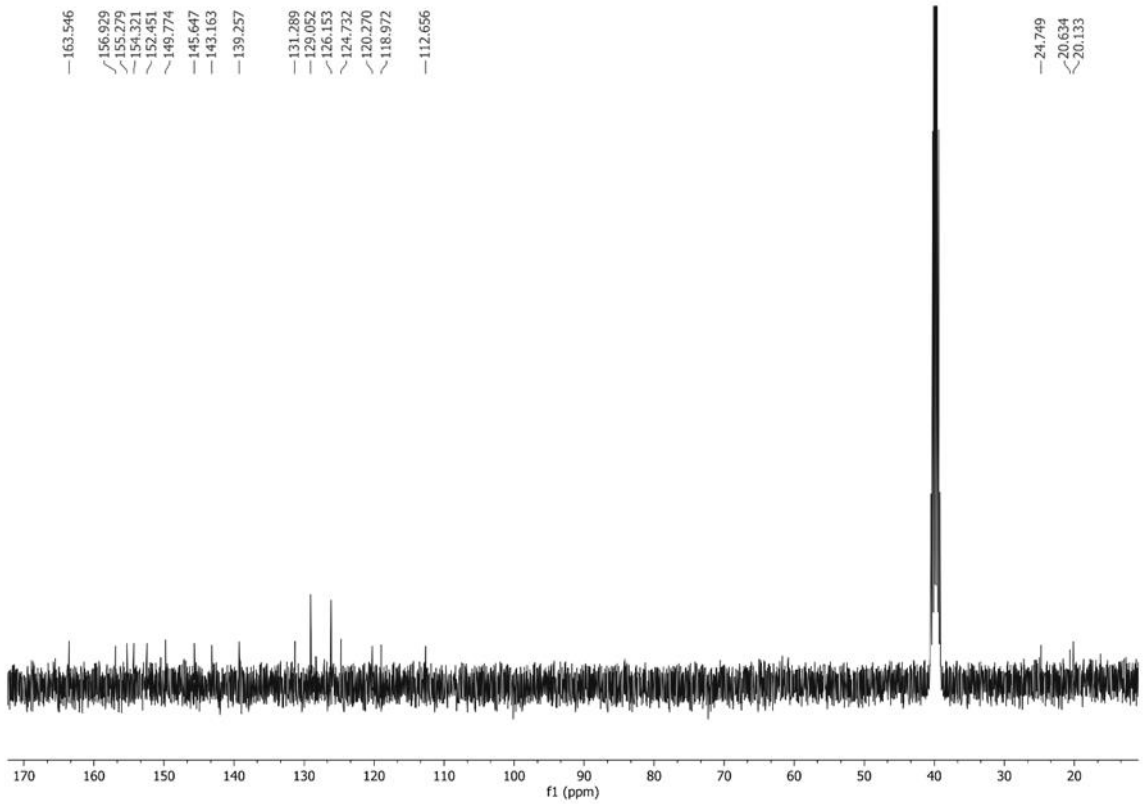


Figure S21. ^{1}H-NMR spectrum of compound **3k**.

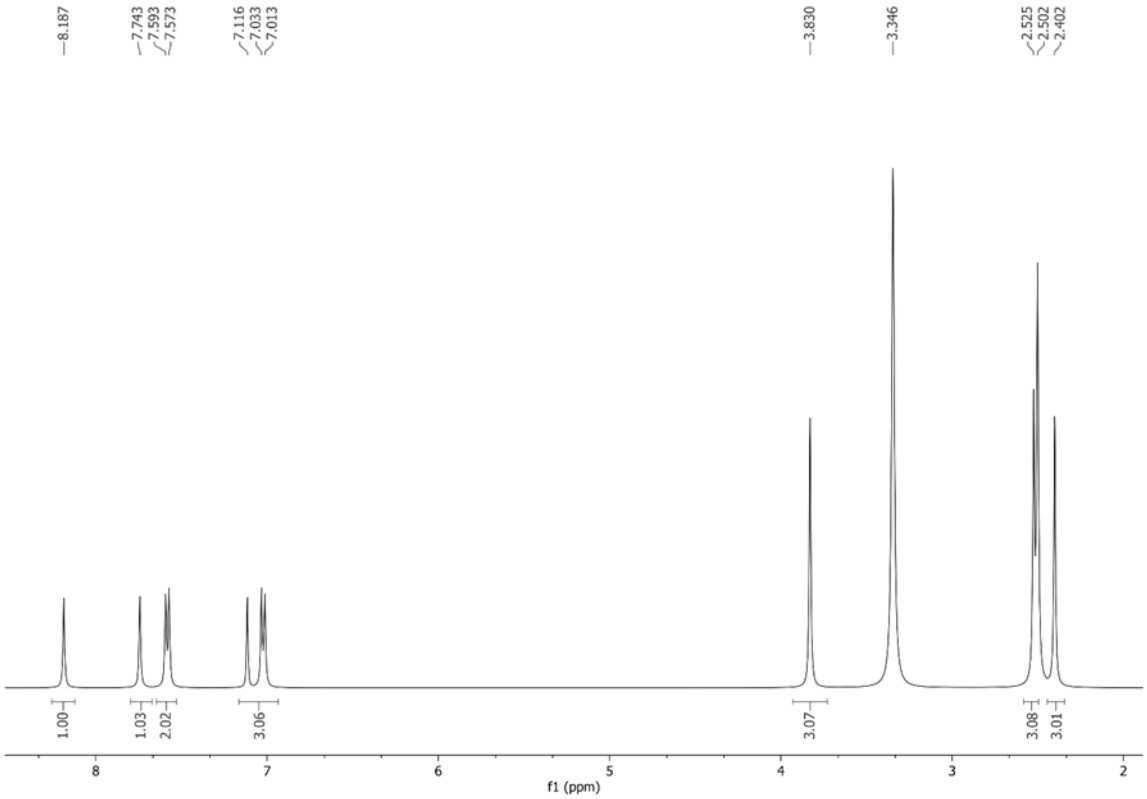


Figure S22. ^{13}C-NMR spectrum of compound **3k**.

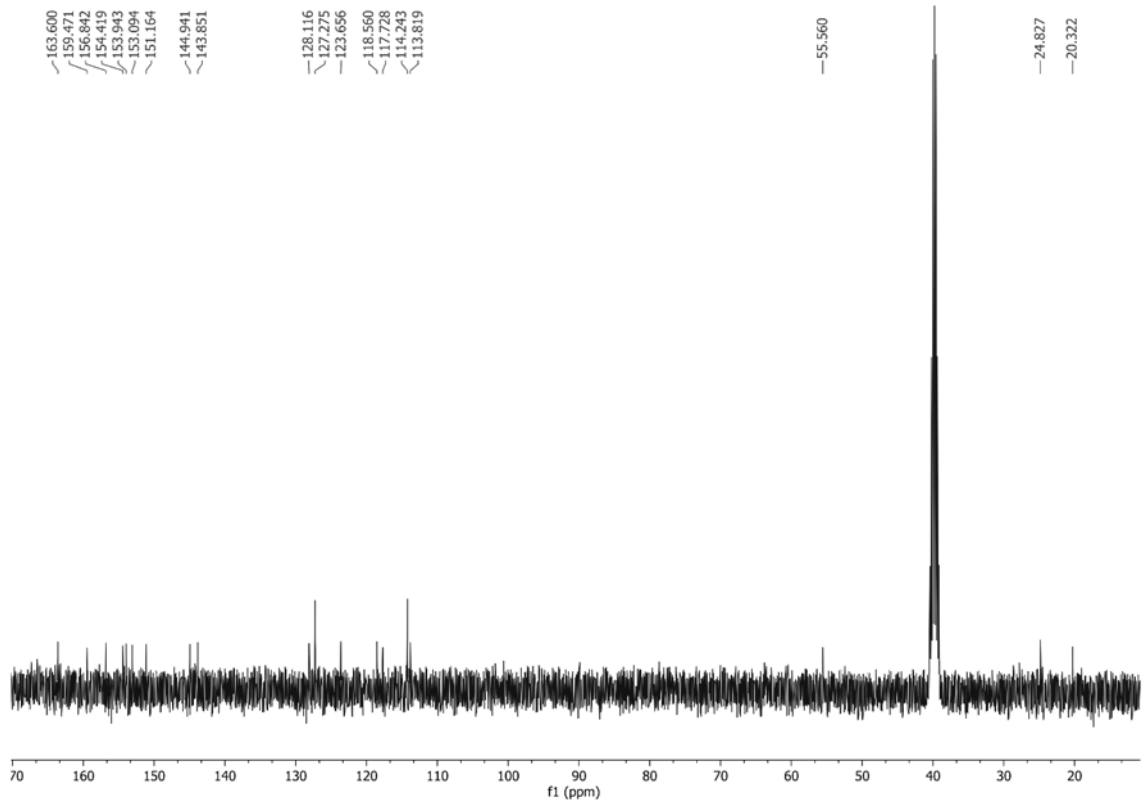


Figure S23. ^{1}H-NMR spectrum of compound **3l**.

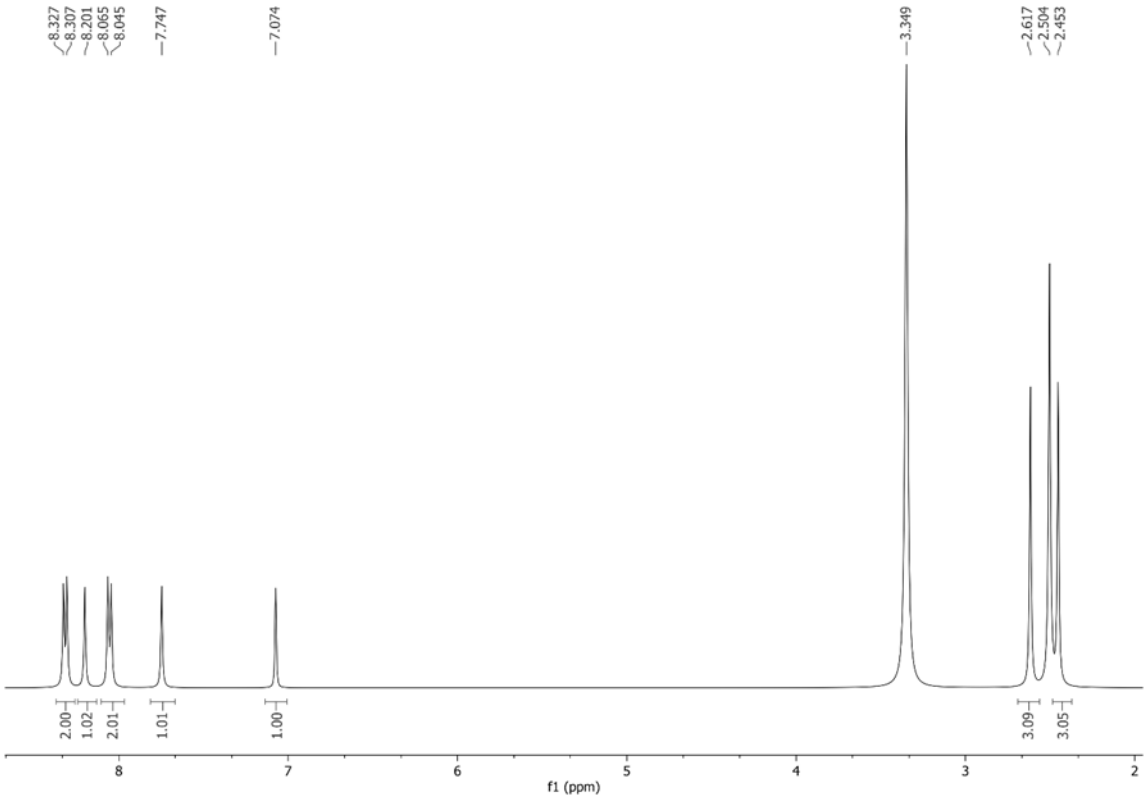


Figure S24. ^{13}C-NMR spectrum of compound **3l**.

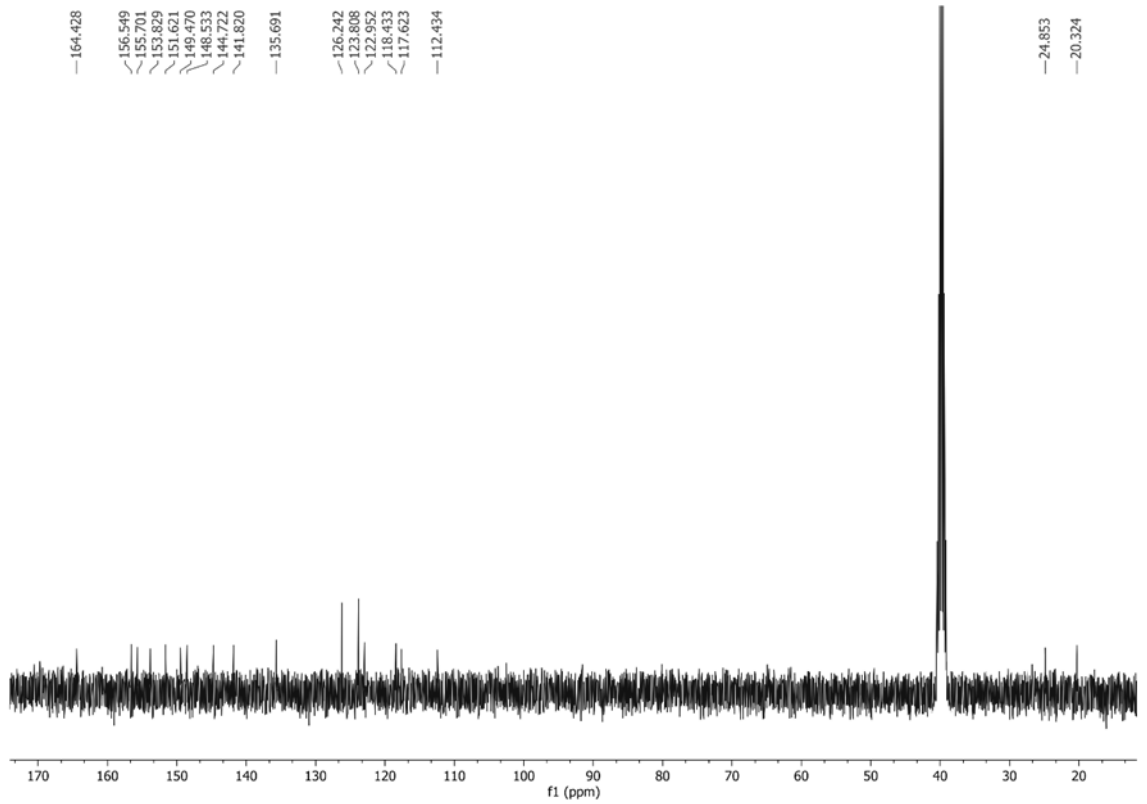


Figure S25. ^{1}H-NMR spectrum of compound **3m**.

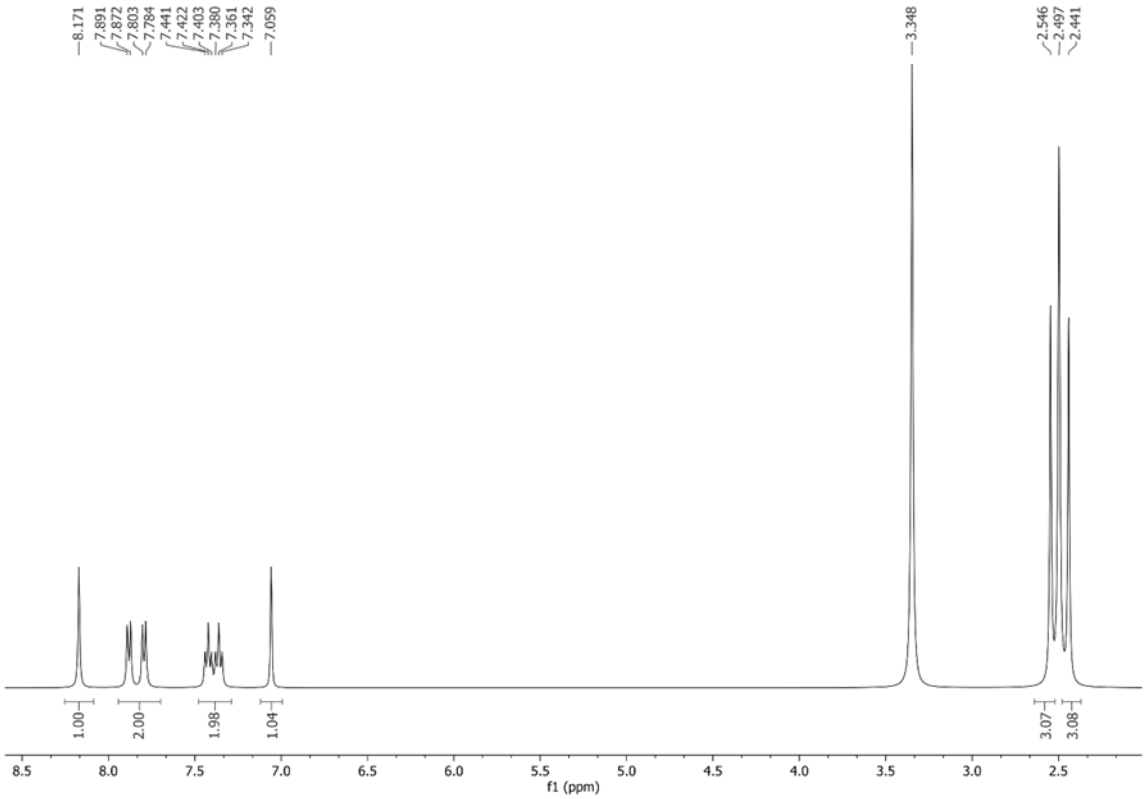


Figure S26. ^{13}C-NMR spectrum of compound **3m**.

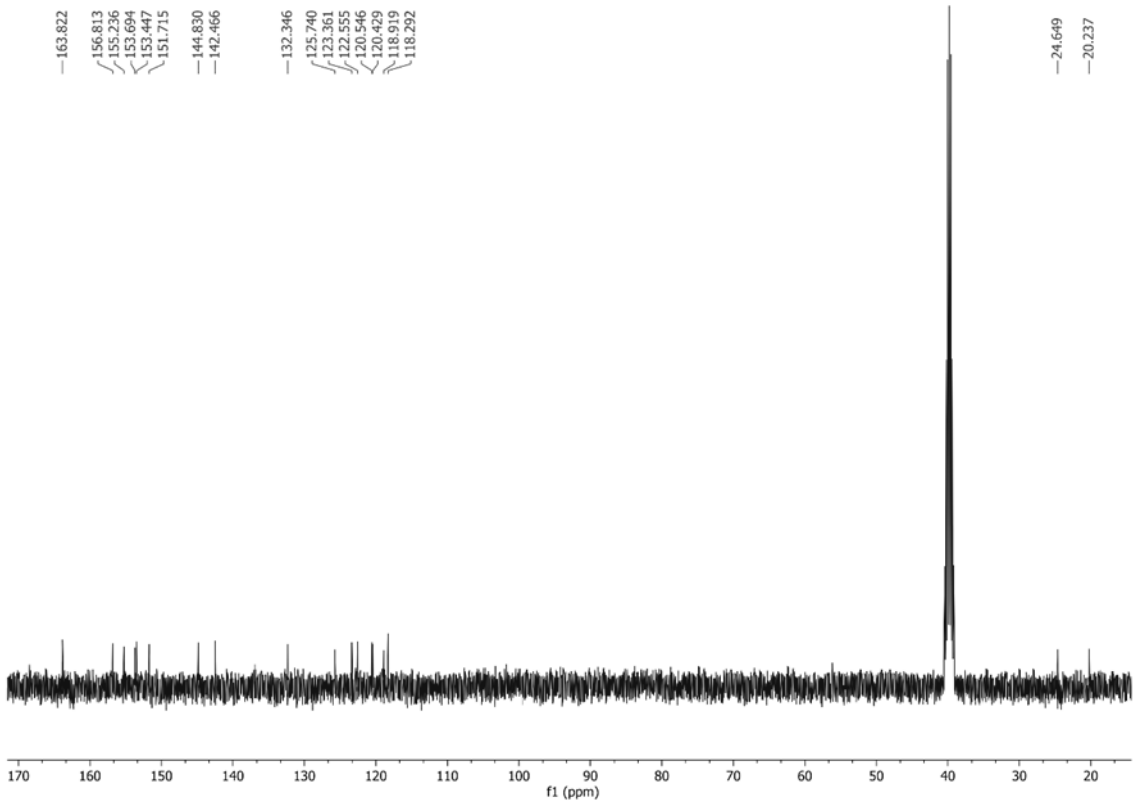


Figure S27. ^{1}H-NMR spectrum of compound **3n**.

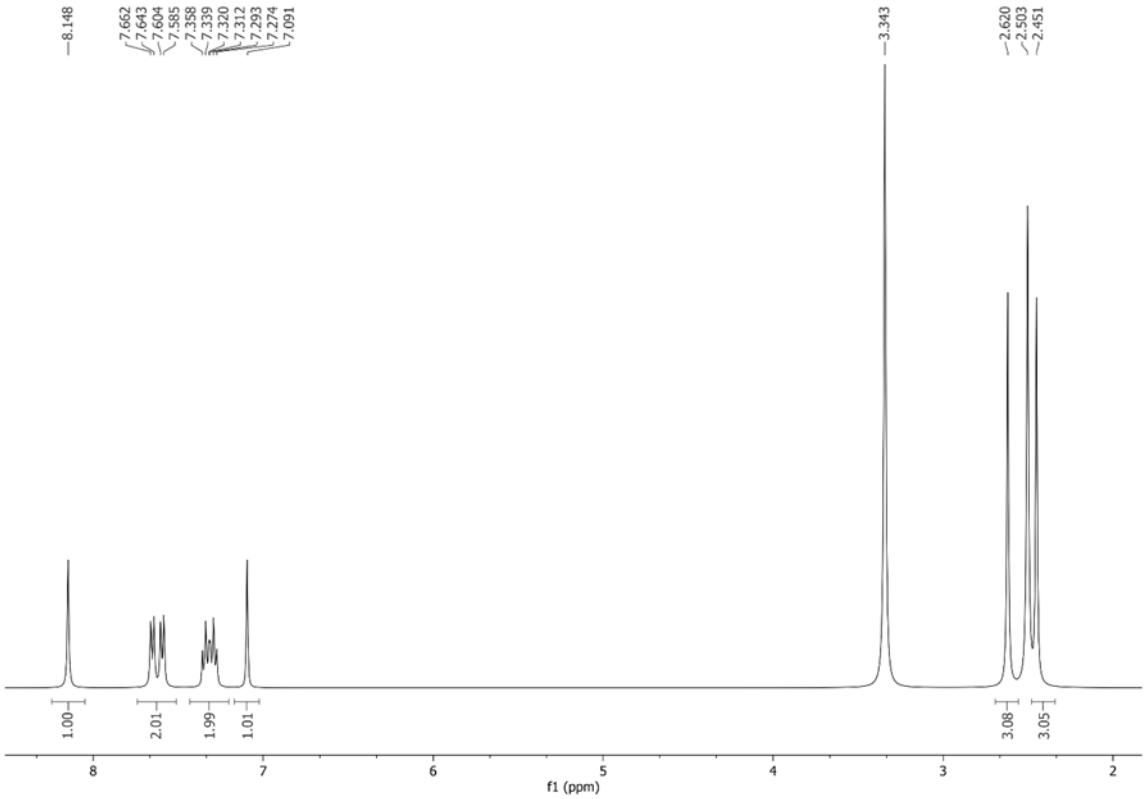


Figure S28. ^{13}C-NMR spectrum of compound **3n**.

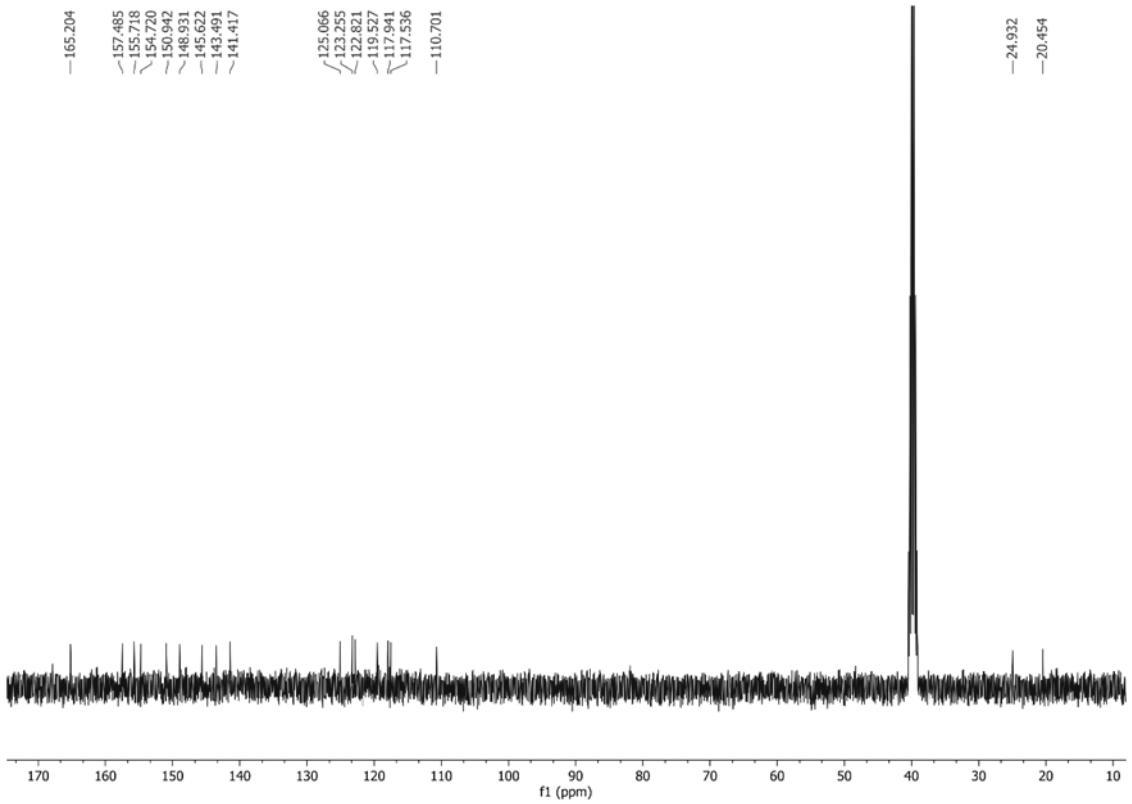


Figure S29. ^{1}H-NMR spectrum of compound **4a**.

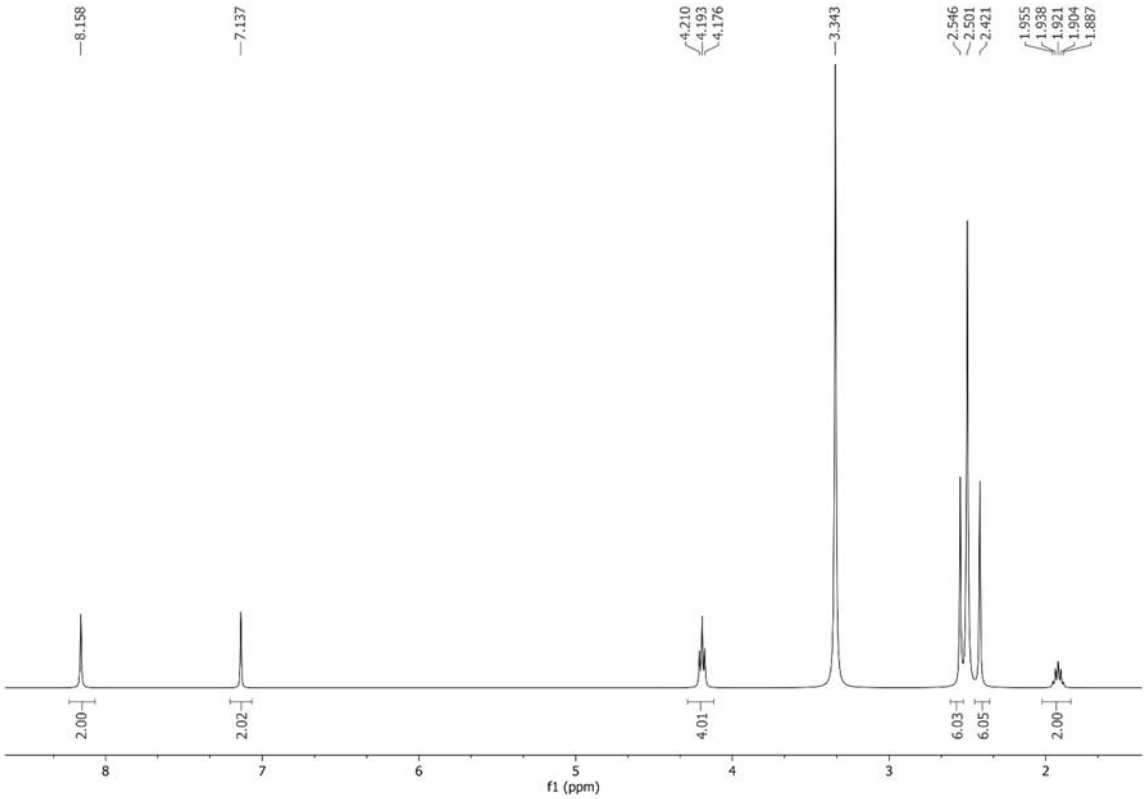


Figure S30. ^{13}C-NMR spectrum of compound **4a**.

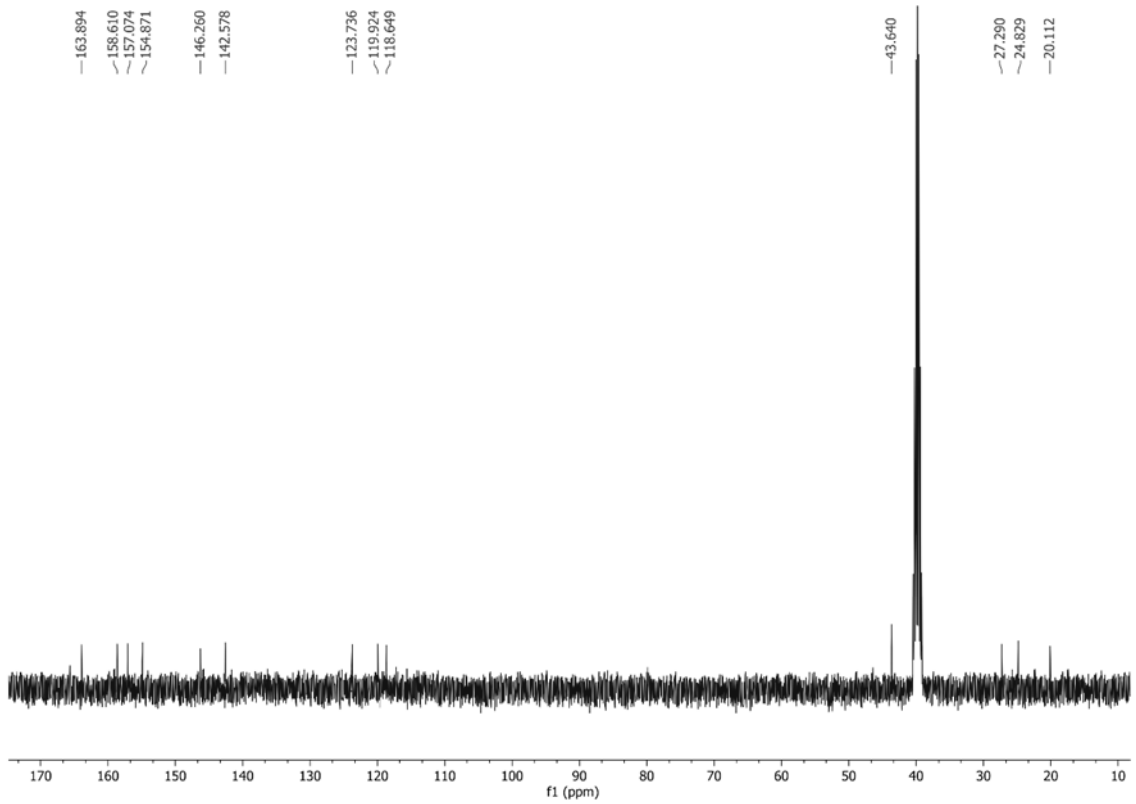


Figure S31. ^{1}H-NMR spectrum of compound **4b**.

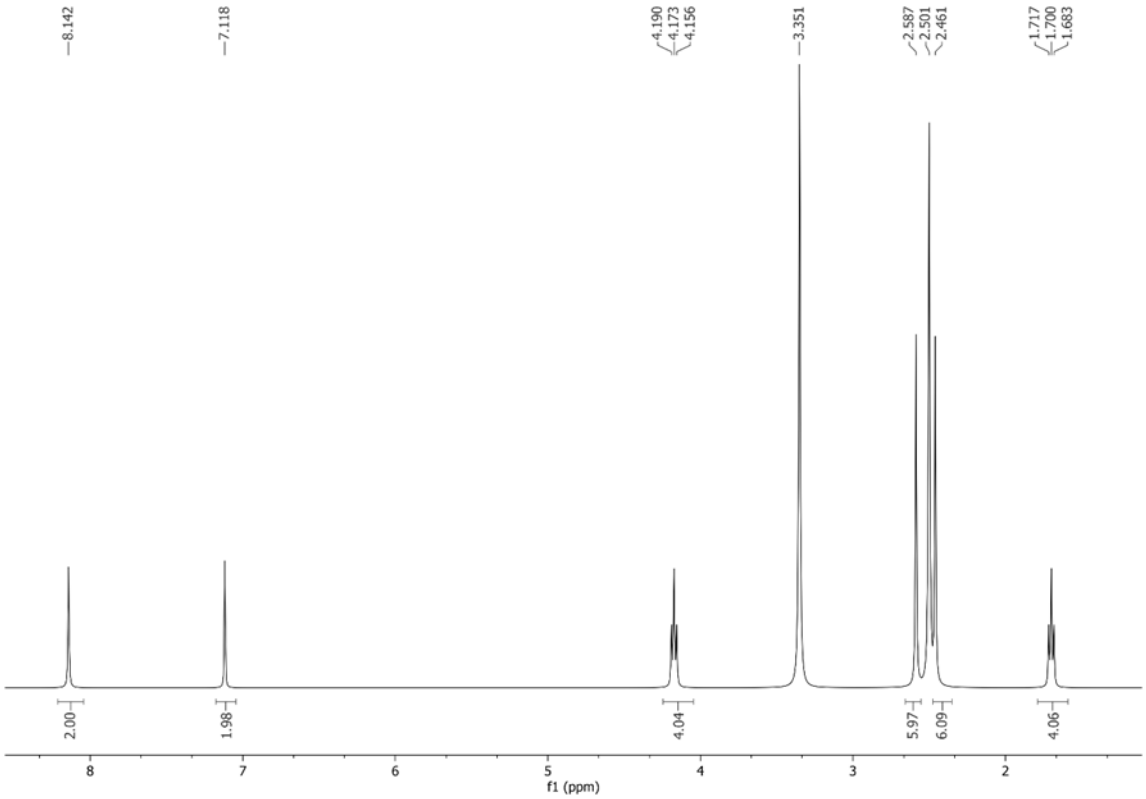


Figure S32. ^{13}C-NMR spectrum of compound **4b**.

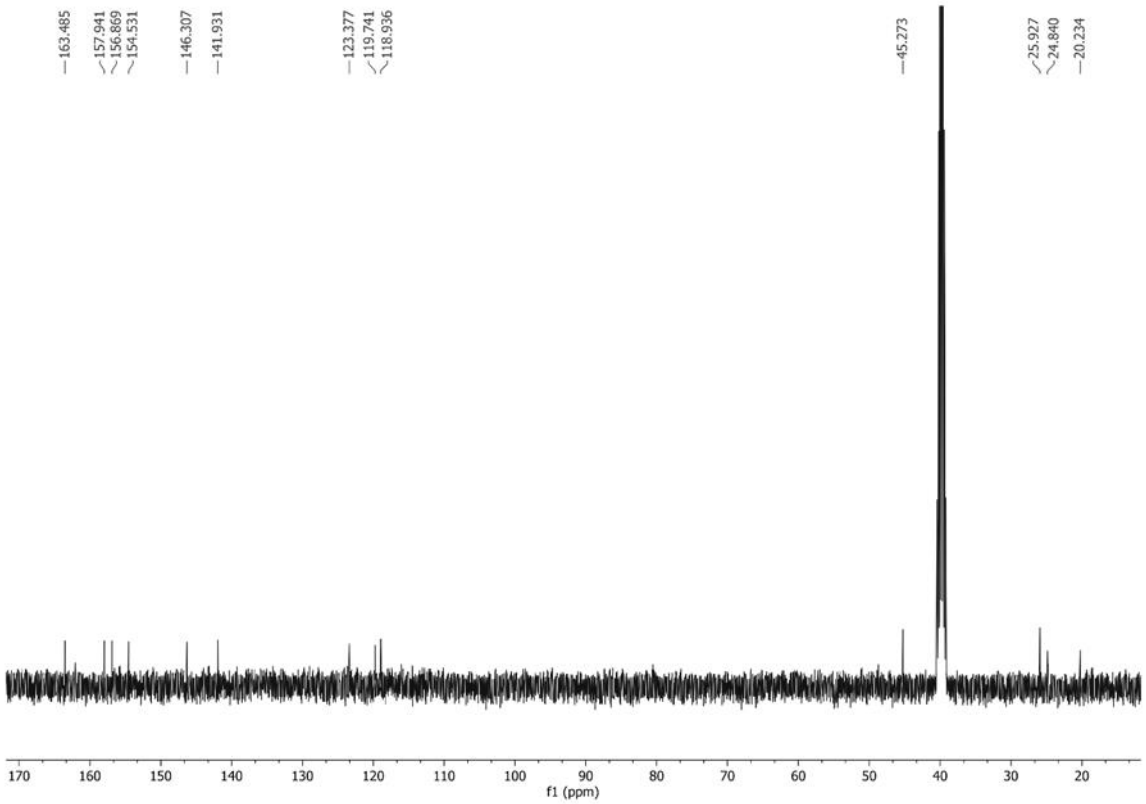


Figure S33. ^{1}H-NMR spectrum of compound **5a**.

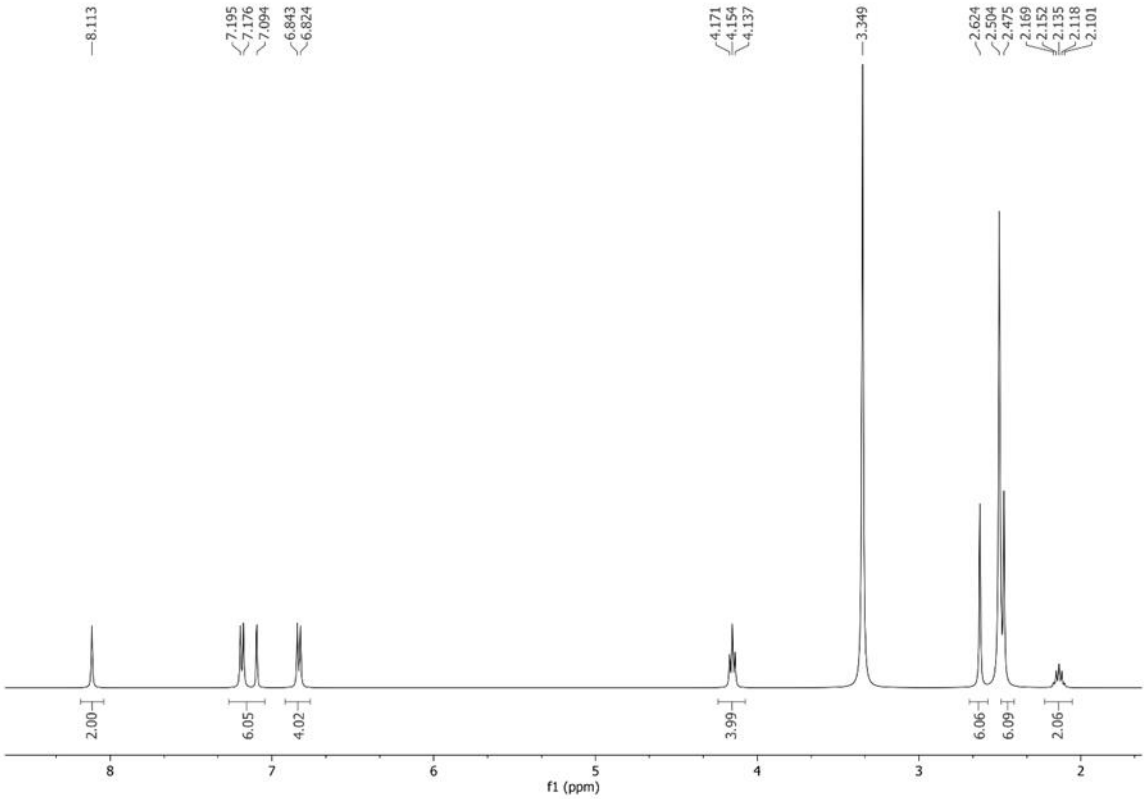


Figure S34. ^{13}C-NMR spectrum of compound **5a**.

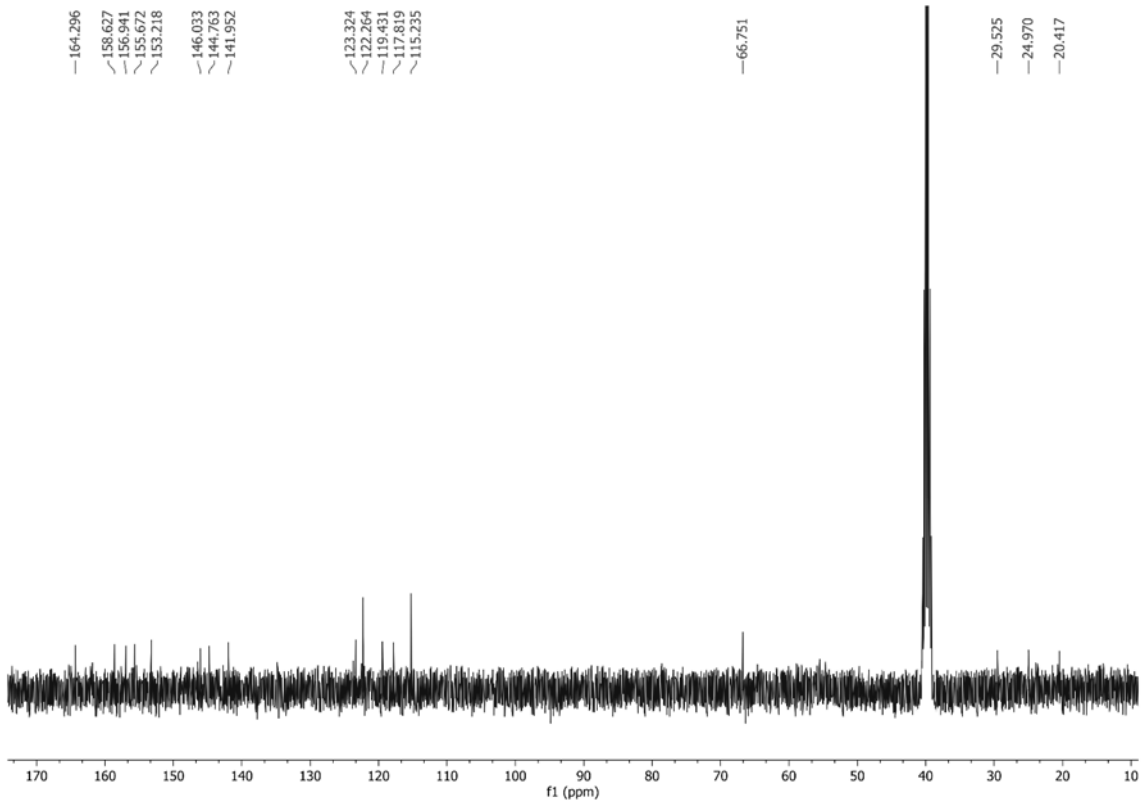


Figure S35. ^{1}H-NMR spectrum of compound **5b**.

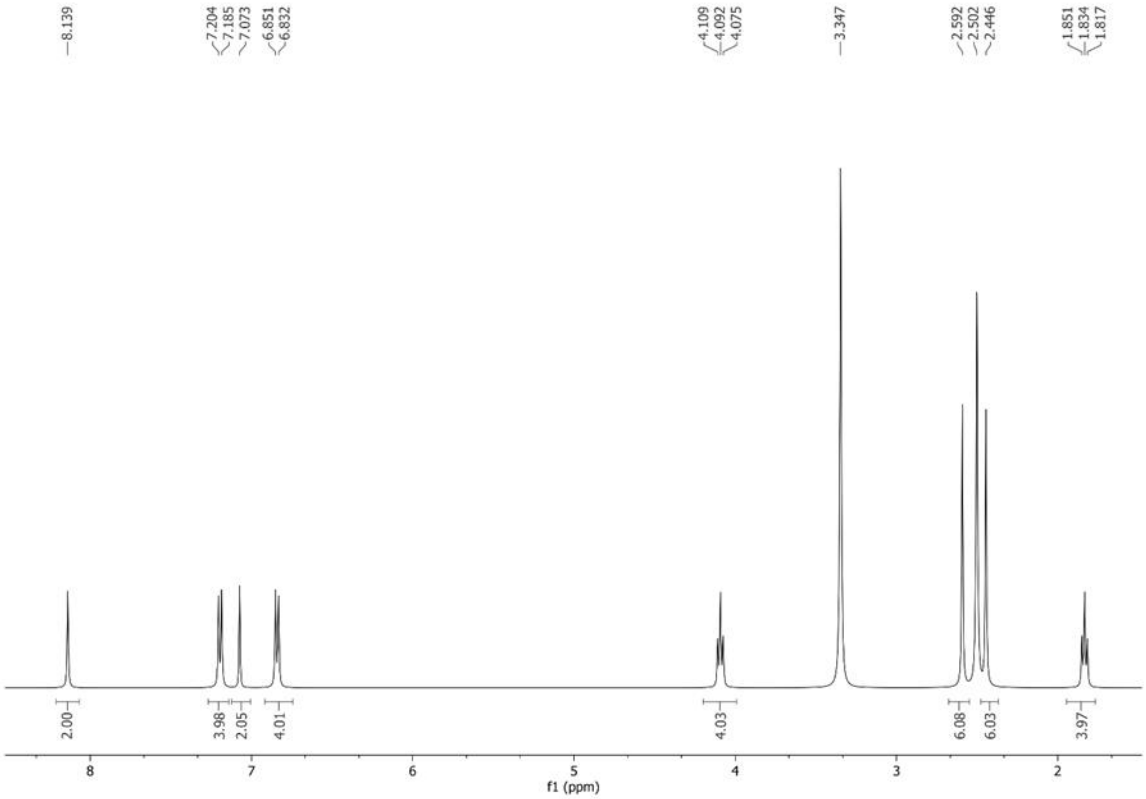


Figure S36. ^{13}C-NMR spectrum of compound **5b**.

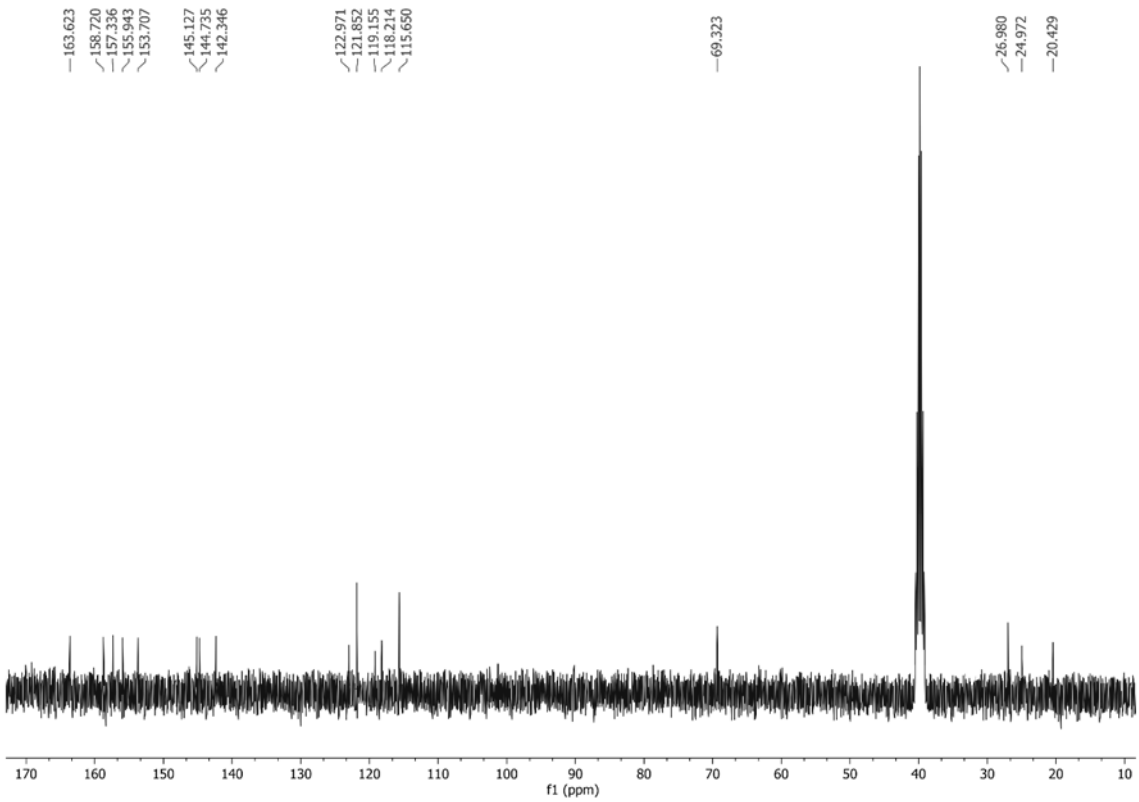


Figure S37. ^{1}H-NMR spectrum of compound **5c**.

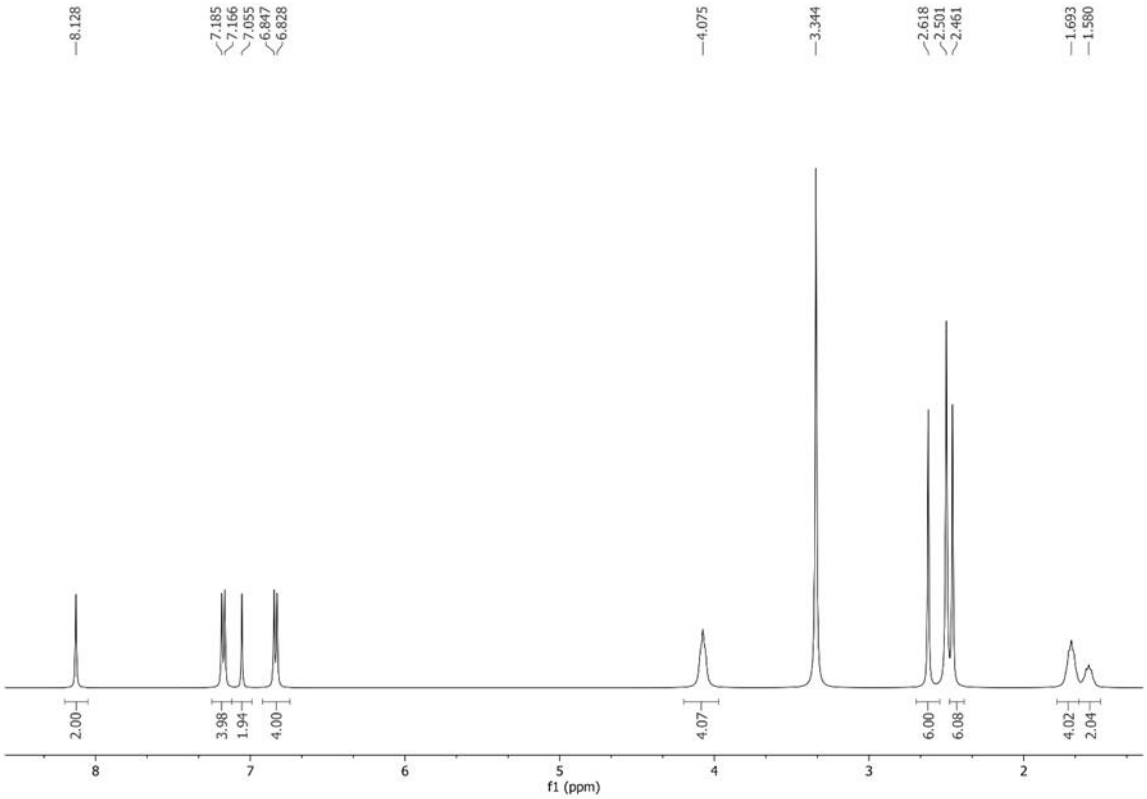


Figure S38. ^{13}C-NMR spectrum of compound **5c**.