Investigating the promiscuity of the chloramphenicol nitroreductase from *Haemophilus influenzae* towards the reduction of 4-nitrobenzene derivatives

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Materials and instrumentation for chemistry and biological studies. 4-Chloro-2-fluorobenzylalcohol was purchased form Matrix Scientific (Columbia, SC, USA). All other chemicals for the synthesis of 4-nitrobenzyl ethers, esters, and thioethers, as well as other nitro-containing molecules were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without any further purification. Chemical reactions were monitored by thin-layer chromatography (TLC) using Merck, Silica gel 60 F_{250} plates. All ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Varian 400 MHz spectrometer. Chemical shifts (δ) and coupling constant (*J*) are provided in parts per million (ppm) and Hertz (Hz), respectively. Conventional abbreviations used for signal shape are as follows: br s = broad singlet; s = singlet; d = doublet; t = triplet; q = quartet; p = pentet; sx = sextet; sp = septet; m = multiplet. Monitoring of nitroreduction reactions was performed on a multimode SpectraMax M5 plate reader. Reversed-phase high-pressure liquid chromatography (RP-HPLC) assays were performed on an Agilent Technologies 1260 Infinity HPLC system using the following general method 1: Flow rate = 1 mL/min; λ = 254 nm; column = Vydac 201SP C18, 250 mm × 4.6 mm, 90 Å, 5 μm. Eluents: A = H_2O + 0.1% TFA; B = MeCN. Gradient profile: starting from 5% B, increasing to 100% B over 10 min, holding at 100% B from 10 to 16 min, decreasing from 100% B to 5% B from 16 to 20 min. Prior to each injection the HPLC column was equilibrated for 15 min with 5% B. Liquid chromatography-mass spectrometry (LCMS) experiments were performed on an Agilent 1200 Series Quaternary LC system and with an Eclipse XDB-C18 column (150 mm × 4.6 mm, 80 Å, 5 μm) equipped with an Agilent 6120 Quadropole MSD mass spectrometer (Agilent Technologies, Santa Clara, CA). All compounds were at least 95% pure.

CHEMISTRY

General procedure for the synthesis of 4-nitrobenzyl ethers (26a,b,d-k,o,q). 4-Nitrobenzylbromide (216 mg, 1 mmol) was added to a mixture of primary or secondary alcohol (1.1 mmol) and Ag_2O (340 mg, 1.5 mmol) in dry Et_2O (10 mL). The reaction mixture was heated at reflux temperature for 12 h. After cooling to room temperature, the solid was filtered off and washed with Et_2O (2×25 mL). The organic layer was collected, concentrated under reduced pressure, and purified by column chromatography (SiO_2, hexanes:CH_2Cl_2/1:1). 4-Nitrobenzylethyl ether (**26a**),[1] 4-nitrobenzylpropyl ether (**26b**),[2] 4-nitrobenzylbenzyl ether (**26g**),[3] 4-bromobenzyl 4-nitrobenzyl ether (**26h**),[4] di(4-nitrobenzyl) ether (**26j**),[5] and 4-nitrobenzylphenyl ether (**26q**),[3, 6]

have previously been reported. The physical appearance and spectral data of all ethers are listed below:

4-Nitrobenzylethyl ether (26a). Yellow solid (91 mg, 50%). ^{1}H NMR ($CDCl_3$, Fig. S1) δ 8.14 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 4.57 (s, 2H), 3.56 (q, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, Fig. S2) δ 146.4, 127.7, 127.6, 123.5, 71.3, 66.4, 15.1.

4-Nitrobenzylpropyl ether (26b). Off-white solid (51 mg, 26%). ^{1}H NMR ($CDCl_3$, Fig. S3) δ 8.17 (d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 4.58 (s, 2H), 3.47 (t, *J* = 6.8 Hz, 2H), 1.65 (q, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, Fig. S4) δ 146.5, 130.9, 127.6, 123.5, 72.7, 71.5, 22.9, 10.6.

1-[(Cyclobutylmethoxy)methyl]-4-nitrobenzene (26d). Colorless oil (70 mg, 31%). ^{1}H NMR ($CDCl_3$, Fig. S5) δ 8.18 (d, *J* = 8.2 Hz, 2H), 7.48 (d, *J* = 8.2 Hz, 2H), 4.58 (s, 2H), 3.46 (d, *J* = 6.8 Hz, 2H), 2.61 (p, *J* = 7.2 Hz, 1H), 2.07-2.03 (m, 2H), 1.92-1.72 (m, 4H); ^{13}C NMR ($CDCl_3$, Fig. S6) δ 147.2, 146.5, 127.6, 123.5, 75.5, 71.6, 35.0, 25.0, 18.6.

1-[(Cyclopentylmethoxy)methyl]-4-nitrobenzene (26e). Colorless oil (83 mg, 35%). ^{1}H NMR ($CDCl_3$, Fig. S7) δ 8.17 (d, *J* = 7.2 Hz, 2H), 7.49 (d, *J* = 7.2 Hz, 2H), 4.59 (s, 2H), 3.37 (d, *J* = 5.6 Hz, 2H), 2.21 (sp, *J* = 6.0 Hz, 1H), 1.76 (m, 2H), 1.52 (m, 4H), 1.26 (m, 2H); ^{13}C NMR ($CDCl_3$, Fig. S8) δ 147.2, 146.6, 127.6, 123.5, 75.6, 71.6, 39.5, 29.6, 25.4.

1-[(Cyclohexylmethoxy)methyl]-4-nitrobenzene (26f). Colorless oil (178 mg, 71%). ^{1}H NMR ($CDCl_3$, Fig. S9) δ 8.15 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 4.56 (s, 2H), 3.29 (d, *J* = 6.4 Hz, 2H), 1.78-1.60 (m, 6H), 1.21-1.11 (m, 3H), 0.95 (m, 2H); ^{13}C NMR ($CDCl_3$, Fig. S10) δ 147.2, 146.6, 127.5, 123.5, 76.9, 71.6, 38.1, 30.0, 26.5, 25.8.

4-Nitrobenzylbenzyl ether (26g). *Note*: For the preparation of compound **1f**, 4-nitrobenzyl alcohol and benzyl bromide were used instead of 4-nitrobenzyl bromide and benzyl alcohol. Pale yellow solid (122 mg, 50%). ^{1}H NMR ($CDCl_3$, Fig. S11) δ 8.19 (d, *J* = 8.8 Hz, 2H), 7.52 (d, *J* = 8.8 Hz,

2H), 7.33 (m, 5H), 4.65 (s, 2H), 4.62 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S12) δ 147.3, 146.0, 137.6, 128.6, 128.0, 127.80, 127.76, 123.6, 72.8, 70.8.

4-bromobenzyl 4-nitrobenzyl ether (26h). White solid (315 mg, 50%). ^{1}H NMR ($CDCl_3$, Fig. S13) δ 8.20 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 7.48 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), 4.62 (s, 2H), 4.55 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S14) δ 147.4, 145.6, 136.5, 131.7 (2 carbons), 129.3 (2 carbons), 127.7 (2 carbons), 123.7 (2 carbons), 121.9, 72.0, 70.9.

4-Chlorobenzyl 4-nitrobenzyl ether (26i). Yellow oil (121 mg, 44%). ^{1}H NMR ($CDCl_3$, Fig. S15) δ 8.20 (d, J = 8.8 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.8 Hz, 2H), 7.29 (d, J = 8.8 Hz, 2H), 4.62 (s, 2H), 4.56 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S16) δ 147.4, 145.6, 136.0, 133.7, 129.0 (2 carbons), 128.7 (2 carbons), 127.7 (2 carbons), 123.7 (2 carbons), 72.0, 70.9.

Di(4-nitrobenzyl) ether (26j). Off-white solid (130 mg, 45%). ^{1}H NMR ($CDCl_3$, Fig. S17) δ 8.22 (d, J = 8.4 Hz, 4H), 7.54 (d, J = 8.4 Hz, 4H), 4.72 (s, 4H); ^{13}C NMR ($CDCl_3$, Fig. S18) δ 147.5, 145.1, 127.8, 123.7, 71.5.

4-Chloro-2-fluorobenzyl 4-nitrobenzyl ether (26k). White solid (8 mg, 3%). ^{1}H NMR ($CDCl_3$, Fig. S19) δ 8.20 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.37 (m, 1H), 7.12 (m, 2H), 4.66 (s, 2H), 4.61 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S20) δ 161.73-159.24 ($^1J_{C\text{-}F}$ = 249.7 Hz), 147.5, 145.4, 134.73-134.64 ($^3J_{C\text{-}F}$ = 9.4 Hz), 130.81-130.75 ($^3J_{C\text{-}F}$ = 5.3 Hz), 127.7 (2 carbons), 124.67-124.64 ($^4J_{C\text{-}F}$ = 3.8 Hz), 123.7 (2 carbons), 123.44-123.29 ($^3J_{C\text{-}F}$ = 14.4 Hz), 116.34-116.10 ($^2J_{C\text{-}F}$ = 29.4 Hz), 71.22, 65.85-65.81 ($^3J_{C\text{-}F}$ = 3.8 Hz).

1-[(Cyclopentyloxy)methyl]-4-nitrobenzene (26o). Colorless oil (70 mg, 31%). ^{1}H NMR ($CDCl_3$, Fig. S21) δ 8.18 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.0 Hz, 2H), 4.55 (s, 2H), 4.01 (s, 1H), 1.73 (m, 6H), 1.55 (m, 2H); ^{13}C NMR ($CDCl_3$, Fig. S22) δ 147.2, 146.9, 127.5, 123.5, 81.7, 69.5, 32.3, 23.5.

4-Nitrobenzylphenyl ether (26q). White solid (141 mg, 62%). ^{1}H NMR ($CDCl_3$, Fig. S23) δ 8.22 (d, *J* = 8.4 Hz, 2H), 7.60 (d, *J* = 8.4 Hz, 2H), 7.33 (t, *J* = 7.8 Hz, 2H), 7.01 (m, 3H), 5.17 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S24) δ 158.1, 147.5, 144.7, 129.7, 127.6, 123.8, 121.5, 114.8, 68.5.

General procedure for the synthesis of 4-nitrobenzyl esters (27c-i,k-n). The carboxylic acid (1.1 mmol) and K_2CO_3 (150 mg, 1.1 mmol) were added to acetone (15 mL). The reaction mixture was stirred for 10 min, and then the 4-nitrobenzylbromide (216 mg, 1 mmol) was added portion-wise. The reaction mixture was continuously stirred at 50 °C for 6 h, and then cooled down to room temperature. The inorganic salts were removed by filtration. The organic materials were collected and concentrated under vacuum, and then partitioned between EtOAc (30 mL) and 1 M aq. K_2CO_3 (30 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, and evaporated under reduced pressure. The obtained oily materials were purified by column chromatography (SiO_2, hexanes:CH_2Cl_2/2:1). The butanoate (**27c**),[7] benzoate (**27g**),[8] 4-bromobenzoate (**27h**),[9] and 4-chlorobenzoate (**27i**) derivatives have previously been reported. The physical appearance and spectral data of all esters are listed below:

4-Nitrobenzyl butanoate (27c). White solid (162 mg, 73%). ^{1}H NMR ($CDCl_3$, Fig. S25) δ 8.14 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H), 5.16 (s, 2H), 2.33 (t, *J* = 7.6 Hz, 2H), 1.63 (sx, *J* = 7.6 Hz, 2H), 0.90 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, Fig. S26) δ 173.0, 147.5, 143.5, 128.2, 123.7, 64.4, 35.9, 18.3, 13.6.

4-Nitrobenzyl cyclobutanecarboxylate (27d). Colorless oil (208 mg, 88%). ^{1}H NMR ($CDCl_3$, Fig. S27) δ 8.20 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 8.4 Hz, 2H), 5.18 (s, 2H), 3.21 (p, *J* = 8.4 Hz, 1H), 2.32-2.18 (m, 4H), 2.02-1.88 (m, 2H); ^{13}C NMR ($CDCl_3$, Fig. S28) δ 174.9, 147.6, 143.5, 128.2, 123.8, 64.5, 37.9, 25.2, 18.4.

4-Nitrobenzyl cyclopentanecarboxylate (27e). Colorless oil (155 mg, 62%). ^{1}H NMR ($CDCl_3$, Fig. S29) δ 8.13 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 5.14 (s, 2H), 2.78 (p, *J* = 8.4 Hz, 1H), 1.85-1.52 (m, 8H); ^{13}C NMR ($CDCl_3$, Fig. S30) δ 176.2, 147.5, 143.7, 128.1, 123.6, 64.5, 44.6, 43.6, 29.9, 29.4, 25.7.

4-Nitrobenzyl cyclohexanecarboxylate (27f). Colorless oil (105 mg, 40%). ^{1}H NMR ($CDCl_3$, Fig. S31) δ 8.17 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 5.16 (s, 2H), 2.34 (tt, J_1 = 11.2 Hz, J_2 = 3.2 Hz, 1H), 1.90 (d, J = 12.0 Hz, 2H), 1.72 (m, 2H), 1.61 (m, 1H), 1.43 (q, J = 12.0 Hz, 2H), 1.30-1.16 (m, 3H); ^{13}C NMR ($CDCl_3$, Fig. S32) δ 175.5, 147.5, 143.7, 128.1, 123.7, 64.4, 43.0, 28.9, 25.6, 25.3.

4-Nitrobenzyl benzoate (27g). White solid (222 mg, 86%). ^{1}H NMR ($CDCl_3$, Fig. S33) δ 8.24 (d, J = 8.8 Hz, 2H), 8.07 (d, J = 8.4 Hz, 2H), 7.59 (m, 3H), 7.46 (t, J = 8.0 Hz, 2H), 5.45 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S34) δ 166.1, 147.7, 143.3, 133.5, 129.7, 129.4, 128.6, 128.3, 123.9, 65.2.

4-Nitrobenzyl 4-bromobenzoate (27h). White solid (226 mg, 67%). ^{1}H NMR ($CDCl_3$, Fig. S35) δ 8.24 (d, J = 8.4 Hz, 2H), 7.92 (d, J = 8.4 Hz, 2H), 7.60 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 5.43 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S36) δ 165.4, 147.8, 143.0, 131.9 (2 carbons), 131.2 (2 carbons), 128.7, 128.4 (2 carbons), 128.3, 123.9 (2 carbons), 65.4.

4-Nitrobenzyl 4-chlorobenzoate (27i). White solid (276 mg, 74%). ^{1}H NMR ($CDCl_3$, Fig. S37) δ 8.23 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 8.8 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 8.8 Hz, 2H), 5.43 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S38) δ 165.2, 147.8, 143.0, 140.0, 131.1 (2 carbons), 128.9 (2 carbons), 128.4 (2 carbons), 127.9, 123.9 (2 carbons), 65.4.

4-Nitrobenzyl 4-chloro-2-fluorobenzoate (27k). Brown solid (197 mg, 64%). ^{1}H NMR ($CDCl_3$, Fig. S39) δ 8.24 (d, J = 8.4 Hz, 2H), 7.92 (m, 1H), 7.60 (d, J = 8.0 Hz, 2H), 7.22 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 7.20 (dd, J_1 = 11.2 Hz, J_2 = 2.0 Hz, 1H), 5.43 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S40) δ 163.23-163.16 ($^{3}J_{C\text{-}F}$ = 7.6 Hz), 163.20-160.61 ($^{1}J_{C\text{-}F}$ = 258.8 Hz), 147.8, 142.7, 140.68-140.57 ($^{3}J_{C\text{-}F}$ = 10.6 Hz), 133.1, 128.3 (2 carbons), 124.83-124.80 ($^{4}J_{C\text{-}F}$ = 3.8 Hz), 123.9 (2 carbons), 118.04-117.78 ($^{2}J_{C\text{-}F}$ = 25.8 Hz), 116.61-116.51 ($^{3}J_{C\text{-}F}$ = 9.9 Hz), 65.6.

4-Nitrobenzyl 2-cyclohexylacetate (27l). Colorless oil (98 mg, 35%). ^{1}H NMR ($CDCl_3$, Fig. S41) δ 8.14 (d, J = 7.6 Hz, 2H), 7.46 (d, J = 7.6 Hz, 2H), 5.15 (s, 2H), 2.23 (d, J = 6.8 Hz, 2H), 1.74 (sp, J = 4.0 Hz, 1H), 1.60 (m, 5H), 1.21-1.06 (m, 3H), 0.95 (m, 2H); ^{13}C NMR ($CDCl_3$, Fig. S42) δ 172.5, 147.5, 143.5, 128.2, 123.7, 64.4, 41.8, 34.8, 32.9, 26.0, 25.9.

4-Nitrobenzyl 1-adamantanecarboxylate (27m). Colorless oil (112 mg, 37%). ^{1}H NMR ($CDCl_3$, Fig. S43) δ 8.19 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 5.21 (s, 2H), 2.66 (t, *J* = 5.6 Hz, 1H), 2.29 (m, 2H), 2.06 (d, *J* = 2.1 Hz, 2H), 1.81 (m, 5H), 1.63-1.54 (m, 5H); ^{13}C NMR ($CDCl_3$, Fig. S44) δ 177.0, 147.5, 143.9, 127.9, 123.8, 64.5, 53.7, 46.8, 46.4, 44.2, 43.6, 43.5, 37.4, 37.3, 34.6.

4-Nitrobenzyl lithocholate (27n). White solid (215 mg, 41%). ^{1}H NMR ($CDCl_3$, Fig. S45) δ 8.20 (d, *J* = 8.8 Hz, 2H), 7.49 (d, *J* = 8.8 Hz, 2H), 5.17 (s, 2H), 3.60 (p, *J* = 4.8 Hz, 1H), 2.43 (m, 1H), 2.28 (m, 1H), 1.92 (d, *J* = 11.6 Hz, 1H), 1.77-1.63 (m, 5H), 1.49 (m, 2H), 1.46 (m, 2H), 1.23 (m, 8H), 1.09 (m, 3H), 1.05-0.93 (m, 5H), 0.89 (s, 3H), 0.88 (s, 3H), 0.59 (s, 3H); ^{13}C NMR ($CDCl_3$, Fig. S46) δ 173.8, 147.6, 143.4, 128.4, 123.8, 71.8, 64.6, 56.4, 55.8, 42.7, 42.0, 40.4, 40.1, 36.4, 35.8, 35.3, 35.3, 34.5, 31.1, 30.9, 30.5, 28.2, 27.1, 26.4, 24.2, 23.3, 20.8, 18.2, 12.0.

General procedure for the synthesis of 4-nitrobenzyl thioethers (28g-i,k,p). The thiol derivative (1.1 mmol) and K_2CO_3 (150 mg, 1.1 mmol) were added to EtOH (15 mL). The reaction mixture was stirred for 20 min, and then 4-nitrobenzylbromide (216 mg, 1 mmol) was added portion-wise. The reaction mixture was continuously stirred at reflux temperature for 2 h, and then cooled down to room temperature. The organic materials were collected by filtration and concentrated under reduced pressure. The obtained brownish oily materials were purified by column chromatography (SiO_2, hexanes:CH_2Cl_2/1:1). 4-nitrobenzylbenzyl thioether (**28g**)[10] has previously been reported. The physical appearance and spectral data for all thioethers are listed below:

Benzyl(4-nitrobenzyl)sulfane (28g). Light yellow solid (153 mg, 59%). ^{1}H NMR ($CDCl_3$, Fig. S47) δ 8.14 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 8.8 Hz, 2H), 7.30 (d, *J* = 6.4 Hz, 2H), 7.26 (m, 3H), 3.64 (s, 2H), 3.60 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S48) δ 146.9, 146.1, 137.3, 129.8, 129.0, 128.6,127.3, 123.7, 35.8, 34.9.

4-Bromobenzyl(4-nitrobenzyl)sulfane (28h). White solid (246 mg, 73%). ^{1}H NMR ($CDCl_3$, Fig. S49) δ 8.15 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.40 (d, *J* = 8.8 Hz, 2H), 7.12 (d, *J* = 8.8

Hz, 2H), 3.62 (s, 2H), 3.53 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S50) δ 147.0, 145.7, 136.4, 131.7 (2 carbons), 130.6 (2 carbons), 129.7 (2 carbons), 123.8 (2 carbons), 121.2, 35.2, 34.9.

4-Chlorobenzyl(4-nitrobenzyl)sulfane (28i). White solid (210 mg, 71%). ^{1}H NMR ($CDCl_3$, Fig. S51) δ 8.15 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.0 Hz, 2H), 3.62 (s, 2H), 3.55 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S52) δ 147.0, 145.7, 135.8, 133.1, 130.2 (2 carbons), 129.7 (2 carbons), 128.8 (2 carbons), 123.7 (2 carbons), 35.1, 34.9.

4-Chloro-2-fluorobenzyl(4-nitrobenzyl)sulfane (28k). *Note*: For the preparation of compound **3d**, 4-nitrobenzyl bromide (108 mg, 0.50 mmol), the thiol derivative (97 mg, 0.55 mmol), and K_2CO_3 (76 mg, 0.55 mmol) were used. Yellow solid (112 mg, 72%). ^{1}H NMR ($CDCl_3$, Fig. S53) δ 8.15 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 7.21 (m, 1H), 7.07 (m, 2H), 3.70 (s, 2H), 3.60 (s, 2H); ^{13}C NMR ($CDCl_3$, Fig. S54) δ 161.72-159.24 ($^1J_{C\text{-}F}$ = 248.9 Hz), 147.1, 145.5, 133.97-133.87 ($^3J_{C\text{-}F}$ = 9.8 Hz), 131.57-131.52 ($^3J_{C\text{-}F}$ = 4.6 Hz), 129.7 (2 carbons), 124.72-124.68 ($^4J_{C\text{-}F}$ = 3.8 Hz), 123.8 (2 carbons), 123.72-123.56 ($^3J_{C\text{-}F}$ = 15.2 Hz), 116.47-116.22 ($^2J_{C\text{-}F}$ = 22.1 Hz), 35.5, 28.31-28.28 ($^3J_{C\text{-}F}$ = 3.0 Hz).

Cyclohexyl(4-nitrobenzyl)sulfane (28p). Faint yellow oil (65 mg, 26%). ^{1}H NMR ($CDCl_3$, Fig. S55) δ 8.13 (d, J = 8.8 Hz, 2H), 7.46 (d, J = 8.8 Hz, 2H), 3.78 (s, 2H), 2.51 (p, J = 6.8 Hz, 1H), 1.88 (m, 2H), 1.71 (m, 2H), 1.56 (m, 1H), 1.26 (m, 5H); ^{13}C NMR ($CDCl_3$, Fig. S56) δ 147.0, 146.8, 129.5, 123.7, 43.2, 34.0, 33.3, 25.9, 25.7.

BIOLOGICAL STUDIES

Optimization of the conditions for CNR activity. The CNR enzyme was purified as previously reported.[11] The nitroreduction activity of CNR was observed using the absorbance of NADPH (340 nm, ε = 6,220 $M^{-1}cm^{-1}$), which is used to reduce the flavin mononucleotide (FMN) of the enzyme after oxidation due to the ongoing reaction. Reactions (200 μL total volume) consisting of CAM (150 μM), Tris-HCl (50 mM, pH 8.0 adjusted at room temperature), and NADPH (0.75 mg/mL, 1.0 mM), were initiated by addition of CNR (1 μM). Reactions were monitored taking readings every 30 s for 60 min. The initial rates were determined using the first 2-5 min of the reaction.

pH: The conditions above were used with various pHs to determine the optimum pH for CNR activity. Buffers and pHs used were as follows: 100 mM citric acid buffer (pH 3, 3.5, 4, 4.5, 5, 5.5, 6), 100 mM sodium phosphate (6.0, 6.5, 7, 7.5, 8.0), and 50 mM Tris-HCl (pH 6.8, 7.5, 8.0, 8.4, 9.0). Each reaction contained one of the buffers listed above, NADPH (750 μM), CAM (500 μM), and was initiated with the addition of CNR (0.4 μM). Reactions were incubated at 25 °C and monitored as above in this section. These data are presented in Fig. S57.

Temperature: The temperature for optimum enzyme activity was tested by monitoring reactions at 20, 25, 30, 37, 42, 57, and 60 °C. Reactions contained Tris pH 8.0 (50 mM), NADPH (750 μM), CAM (500 μM), and were initiated by the addition of CNR (0.4 μM). Reactions were monitored as above in this section. These data are presented in Fig. S58.

Determination of kinetic parameters of CAM. The kinetic parameters of CAM were determined using reactions containing Tris-HCl (50 mM, pH 8.0 adjusted at room temperature), NADPH (750 μM), CAM (0, 1, 5, 10, 50, 100, 250, 500, or 2500 μM), and CNR (0.4 μM). Reactions were incubated at 37 or 50 °C and monitored for 30 min taking measurements every 30 s. The first 2-5 min of reaction were used to determine the initial rates. The resulting data were fit with a Michaelis-Menten plot and kinetic parameters were determined using SigmaPlot 13. These data are presented in Fig. 1.

Monitoring of nitroreduction by the CNR enzyme by UV-Vis assays. Reactions (200 μL total volume) consisting of nitro-containing compounds (synthesized, Scheme 2; commercially available, Fig. S59) (150 μM), Tris-HCl (50 mM, pH 8.0 adjusted at room temperature), and NADPH (0.75 mg/mL, 1.0 mM) were initiated by addition of CNR (1 μM). Reactions were monitored taking readings every 30 s for 60 min. The initial rates were determined using the first 5 min of the reaction and were normalized to the rate for chloramphenicol (CAM), a known substrate of the CNR enzyme.

Large-scale reduction reactions for LCMS. Reactions contained Tris-HCl (50 mM, pH 8.0 adjusted at room temperature), NAPDH (100 μM), glucose (40 mM), glucose dehydrogenase (4

μM, used with glucose to regenerate NADPH),[12] CNR (5 μM), and the compound of interest (1 mM). Reactions were incubated at 37 °C for 24 h. After incubation, reactions were cooled and extracted with 3×10 mL of EtOAc. The organic fractions were pooled and dried over $MgSO_4$. The remaining solvent was removed *in vacuo*. The residue was redisolved in 1 mL of MeOH and analyzed by LCMS. These data are presented in Fig. S60.

Large-scale reduction reactions for RP-HPLC time course. Reactions were identical to those used in preparation for LCMS above. After 10 min, 30 min, 60 min, 3 h, 6 h, and 24 h, a 0.5-mL aliquot of the reaction was extracted and dried as above. The resulting residues were dissolved in 100 μL of MeOH. A 10-μL portion was injected onto the RP-HPLC and run with general method 1. Results of the RP-HPLC time course are shown in Fig. S61.

References

1. Yang, M. S.; Xu, L. W.; Qiu, H. Y.; Lai, G. Q.; Jiang, J. X., Highly efficient indium-catalyzed chemoselective allylation-etherification and reductive etherification of aromatic aldehydes with functional silanes. *Tetrahedron Lett.* **2008,** *49*, 253-256.
2. Joshi, G.; Adimurthy, S., New method for the synthesis of benzyl alkyl ethers mediated by $FeSO_4$. *Synth. Commun.* **2011,** *41*, 720-728.
3. Shah, S. T. A.; Khan, K. M.; Heinrich, A. A.; Choudhary, M. I.; Voelter, W., An efficient approach towards syntheses of ethers and esters Using Csf-celite as a solid base. *Tetrahedron Lett.* **2002,** *43*, 8603-8606.
4. Wang, L.; Hashidoko, Y.; Hashimoto, M., Cosolvent-promoted *O*-benzylation with silver(I) oxide: Synthesis of 1'-benzylated sucrose derivatives, mechanistic studies, and scope investigation. *J. Org. Chem.* **2016,** *81* (11), 4464-4474.
5. Firouzabadi, H.; Iranpoor, N.; Jafari, A. A., Facile preparation of symmetrical and unsymmetrical ethers from their corresponding alcohols catalyzed by aluminumdodecatangstophosphate ($AlPW_{12}O_{40}$), as a versatile and a highly water tolerant Lewis acid. *J. Mol. Cata. A.* **2005,** *227*, 97-100.
6. Lyman, J. A.; Reid, E. E., The identification of phenols. *J. Am. Chem. Soc.* **1920,** *42*, 615-619.
7. Iwasaki, G.; Saeki, S.; Hamana, M., A novel nucleophilic substitution of the formyl group in *p*-nitrobenzaldehyde with some carbanions. *Chem. Lett.* **1986**, 173-176.

8. Mohammadpoor-Baltork, I.; Aliyan, H.; Khosropour, A. R., Bismuth(III) salts as convenient and efficient catalysts for the selective acetylation and benzoylation of alcohols and phenols. *Tetrahedron* **2001,** *57*, 5851-5854.
9. Li, Y.; Du, W.; Deng, W.-P., NHCs-mediated benzoates formation directly from aromatic aldehydes and alkyl halides. *Tetrahedron* **2012,** *68* (18), 3611-3615.
10. Riddell, N.; Tam, W., Ruthenium-catalyzed [2+2] cycloadditions of alkynyl sulfides and alkynyl sulfones. *J. Org. Chem.* **2006,** *71*, 1934-1937.
11. Berkov-Zrihen, Y.; Green, K. D.; Labby, K. J.; Feldman, M.; Garneau-Tsodikova, S.; Fridman, M., Synthesis and evaluation of hetero- and homodimers of ribosome-targeting antibiotics: antimicrobial activity, *in vitro* inhibition of translation, and drug resistance. *J. Med. Chem.* **2013,** *56* (13), 5613-5625.
12. Nguyen-Tran, H. H.; Zheng, G. W.; Qian, X. H.; Xu, J. H., Highly selective and controllable synthesis of arylhydroxylamines by the reduction of nitroarenes with an electron-withdrawing group using a new nitroreductase BaNTR1. *Chem. Commun.* **2014,** *50* (22), 2861-2864.

Figs. S1-S61:

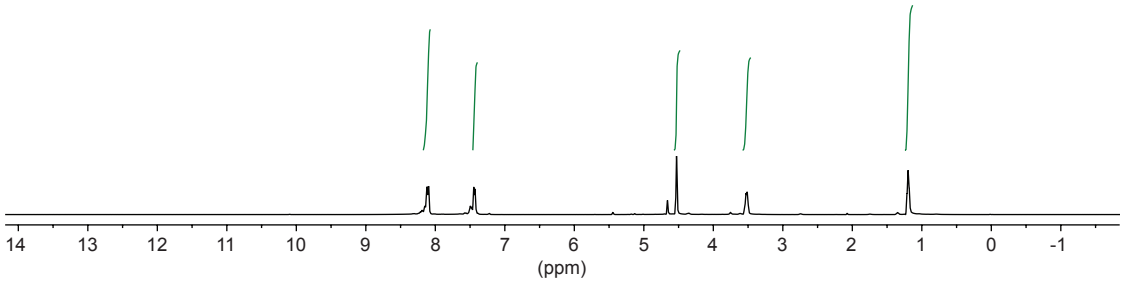

Fig. S1: ^{1}H NMR spectrum for compound **26a** in $CDCl_3$ (400 MHz).

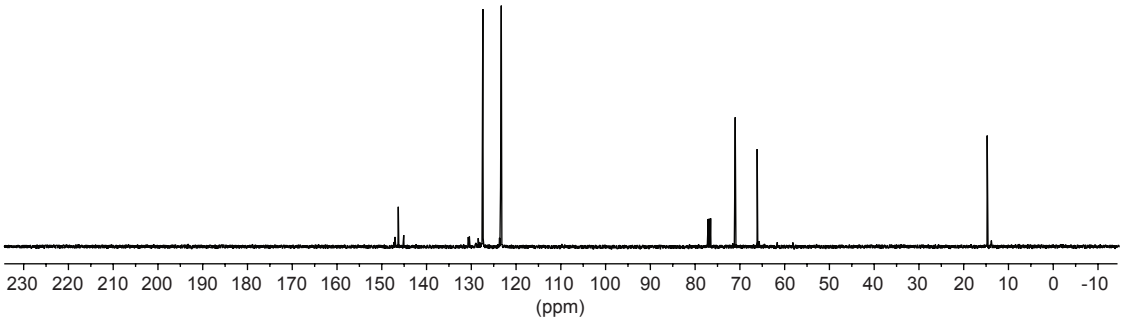

Fig. S2: ^{13}C NMR spectrum for compound **26a** in $CDCl_3$ (100 MHz).

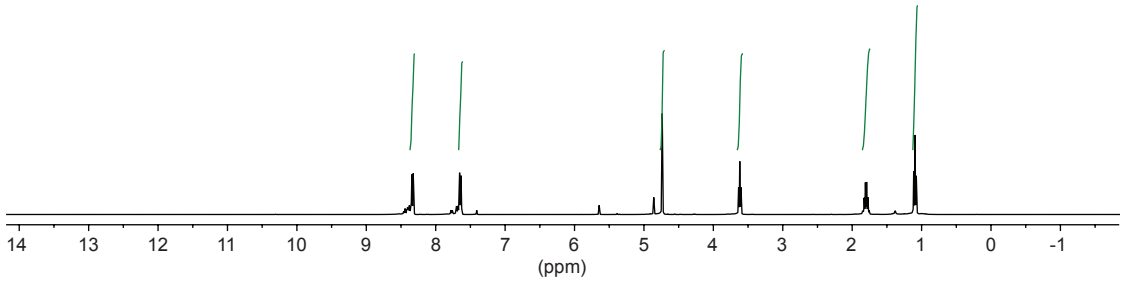

Fig. S3: ^{1}H NMR spectrum for compound **26b** in $CDCl_3$ (400 MHz).

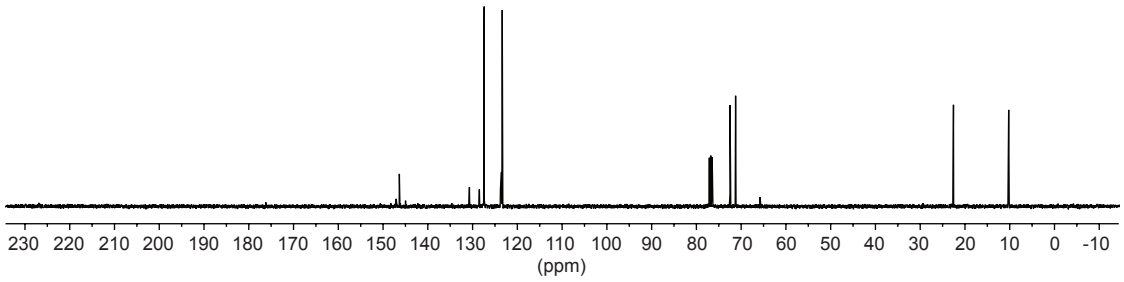

Fig. S4: ^{13}C NMR spectrum for compound **26b** in $CDCl_3$ (100 MHz).

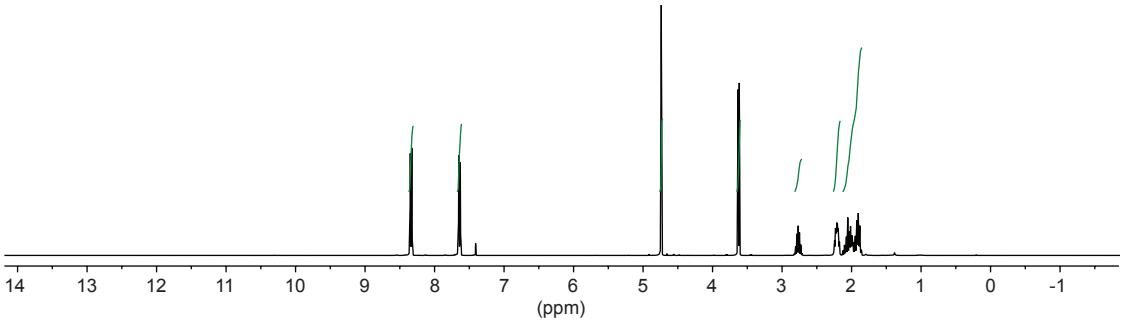

Fig. S5: ^{1}H NMR spectrum for compound **26d** in $CDCl_3$ (400 MHz).

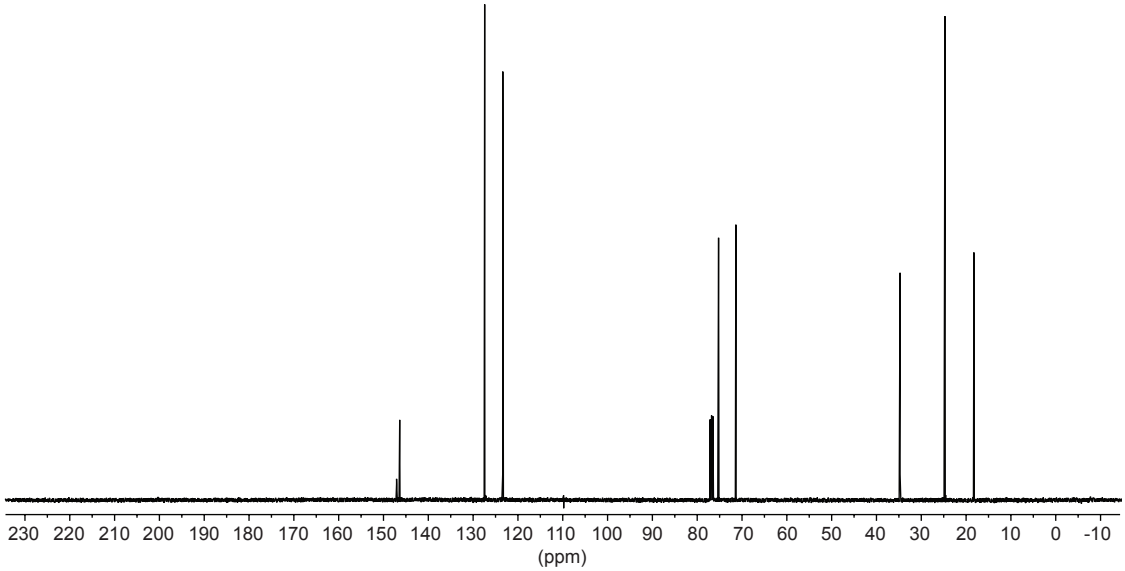

Fig. S6: ^{13}C NMR spectrum for compound **26d** in $CDCl_3$ (100 MHz).

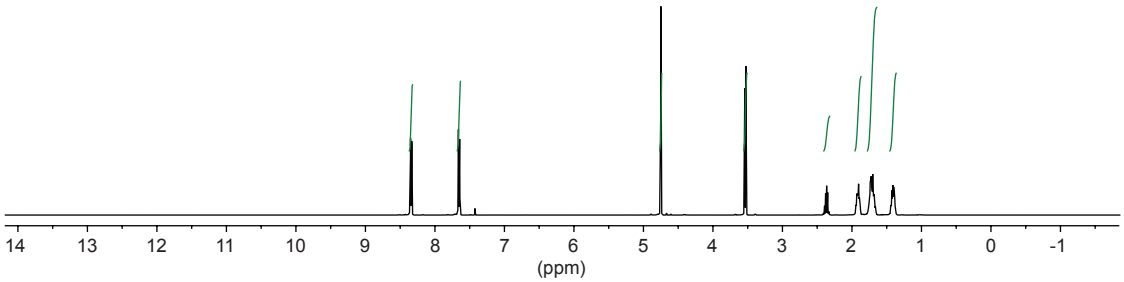

Fig. S7: ^{1}H NMR spectrum for compound **26e** in $CDCl_3$ (400 MHz).

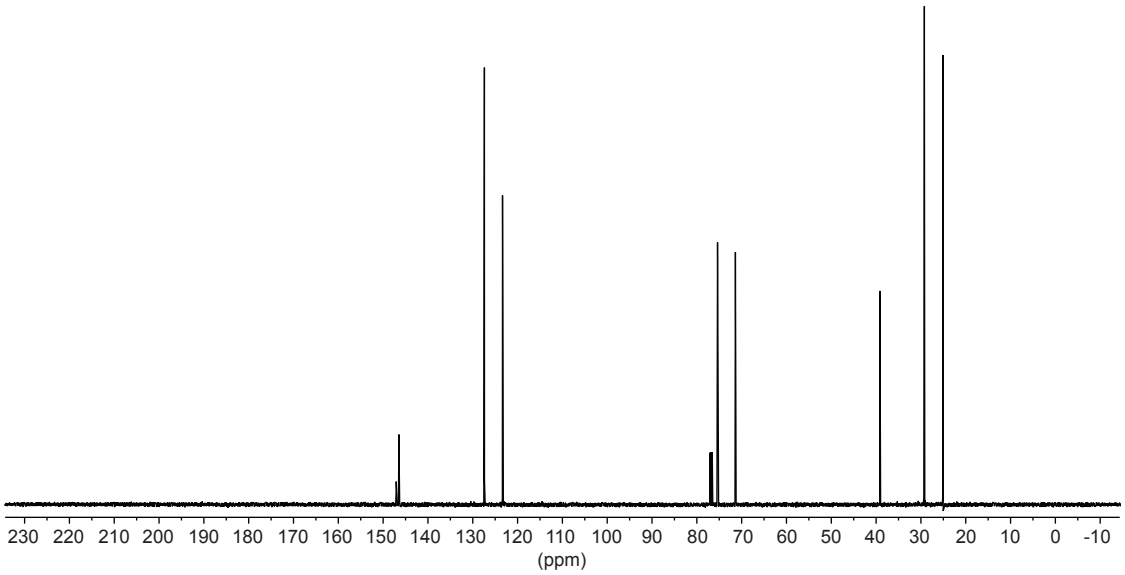

Fig. S8: ^{13}C NMR spectrum for compound **26e** in $CDCl_3$ (100 MHz).

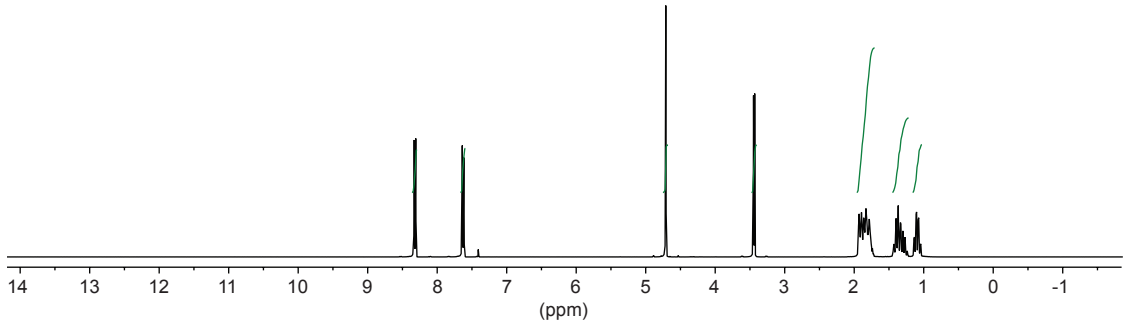

Fig. S9: ^{1}H NMR spectrum for compound **26f** in $CDCl_3$ (400 MHz).

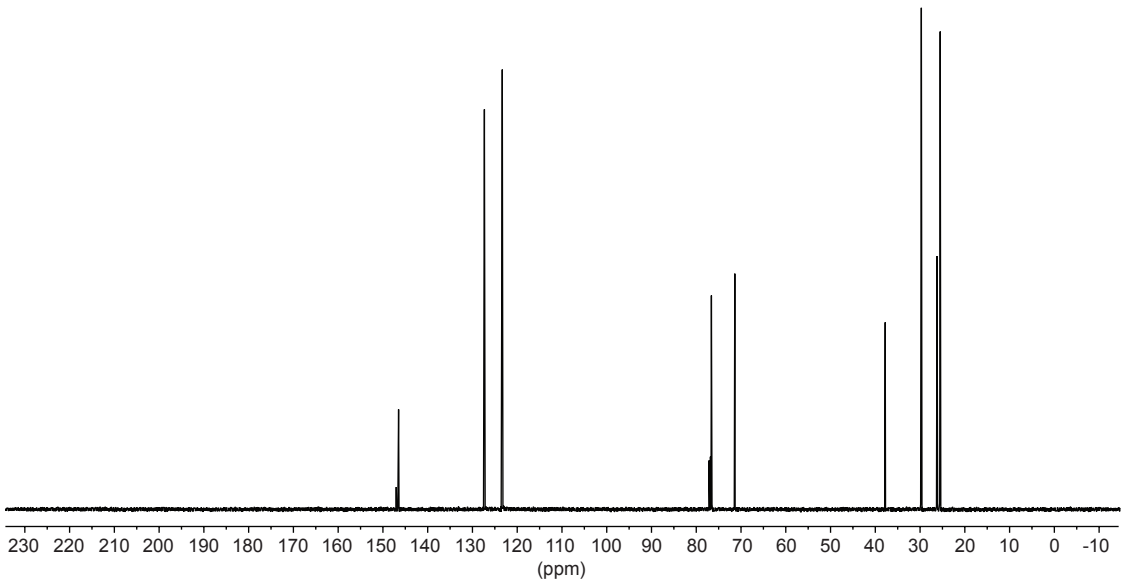

Fig. S10: ^{13}C NMR spectrum for compound **26f** in $CDCl_3$ (100 MHz).

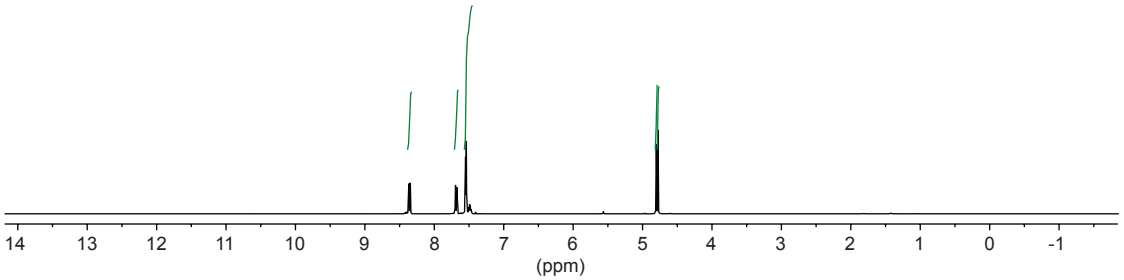

Fig. S11: ^{1}H NMR spectrum for compound **26g** in $CDCl_3$ (400 MHz).

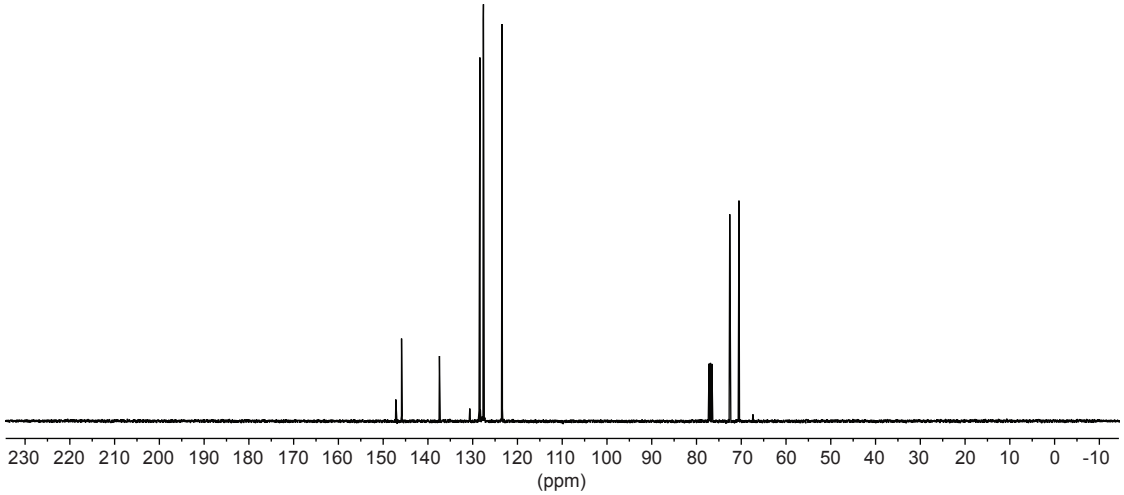

Fig. S12: ^{13}C NMR spectrum for compound **26g** in $CDCl_3$ (100 MHz).

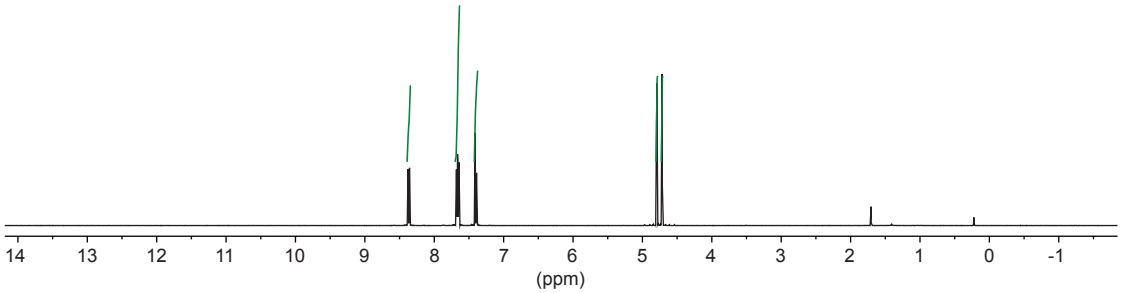

Fig. S13: ^{1}H NMR spectrum for compound **26h** in $CDCl_3$ (400 MHz).

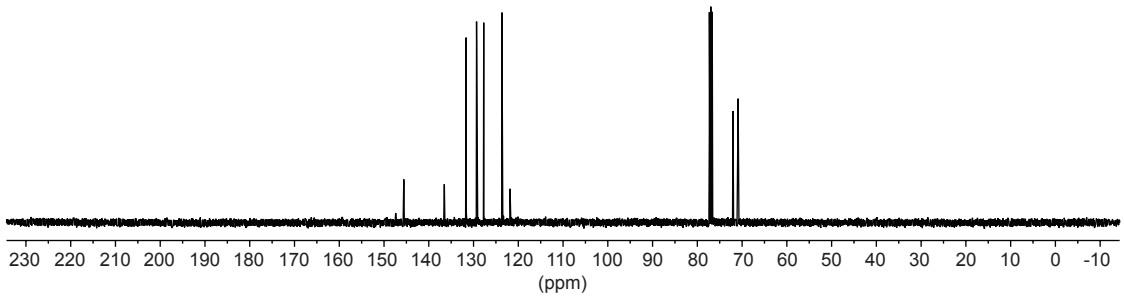

Fig. S14: ^{13}C NMR spectrum for compound **26h** in $CDCl_3$ (100 MHz).

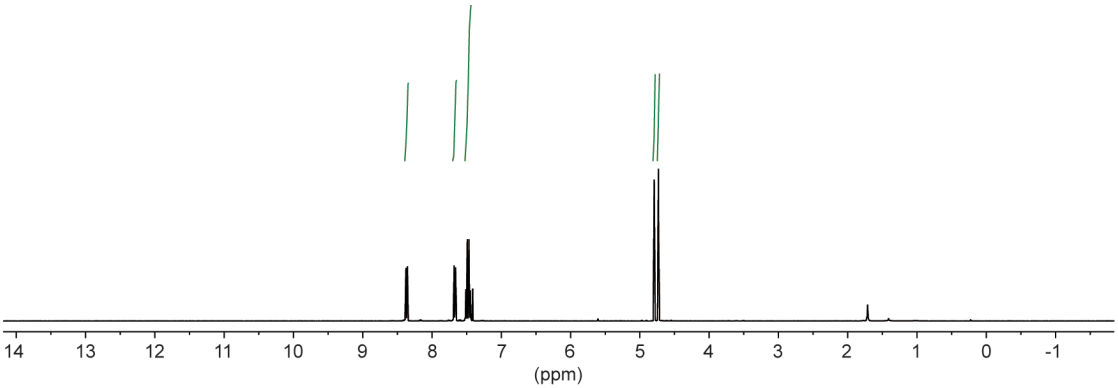

Fig. S15: ^{1}H NMR spectrum for compound **26i** in $CDCl_3$ (400 MHz).

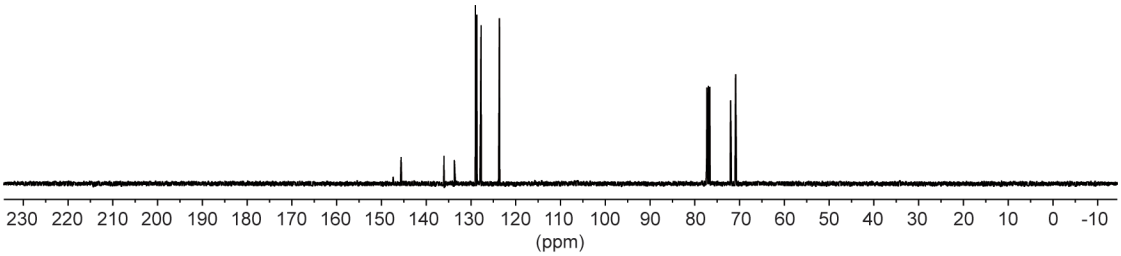

Fig. S16: ^{13}C NMR spectrum for compound **26i** in $CDCl_3$ (100 MHz).

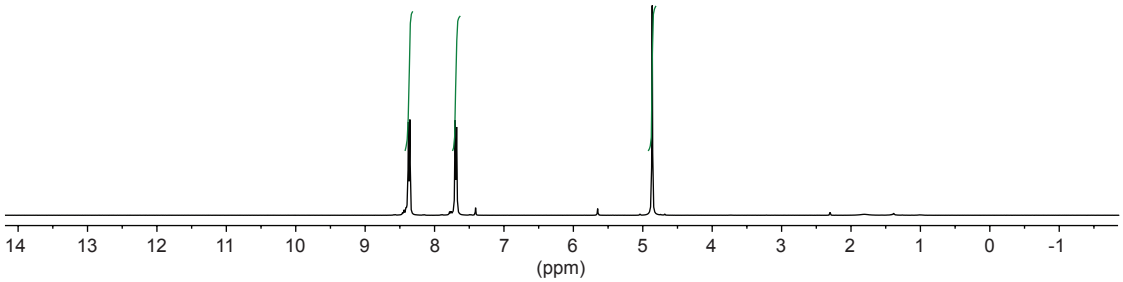

Fig. S17: ^{1}H NMR spectrum for compound **26j** in $CDCl_3$ (400 MHz).

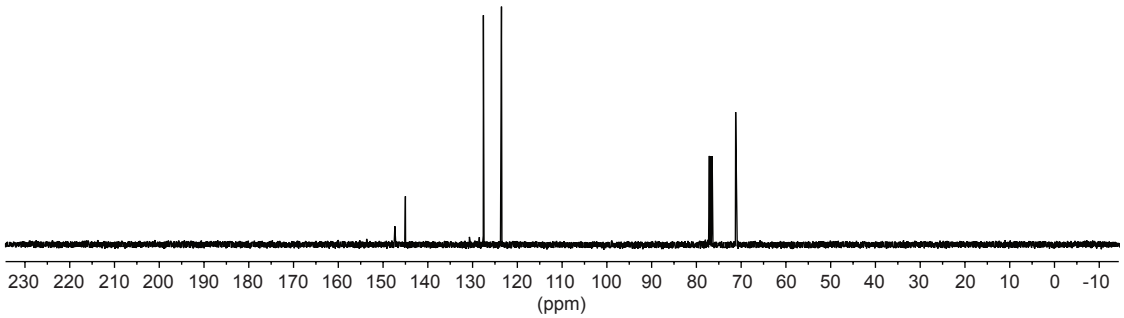

Fig. S18: ^{13}C NMR spectrum for compound **26j** in $CDCl_3$ (100 MHz).

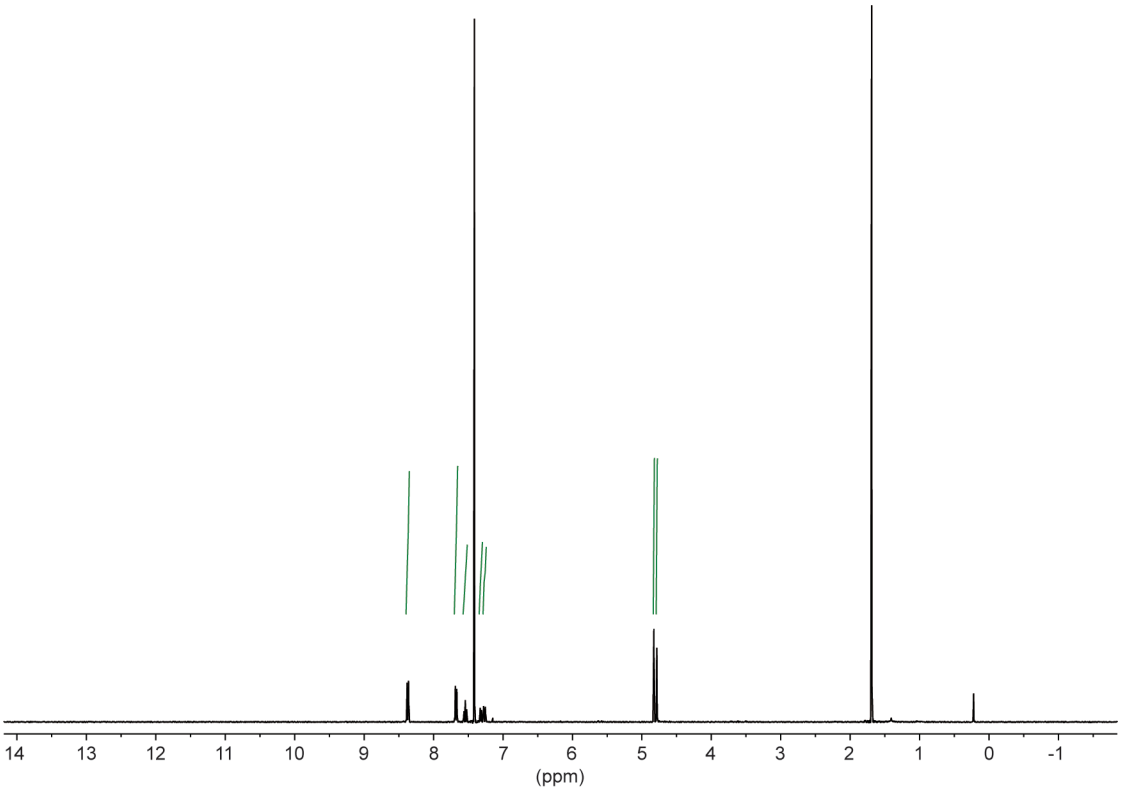

Fig. S19: ^{1}H NMR spectrum for compound **26k** in $CDCl_3$ (400 MHz).

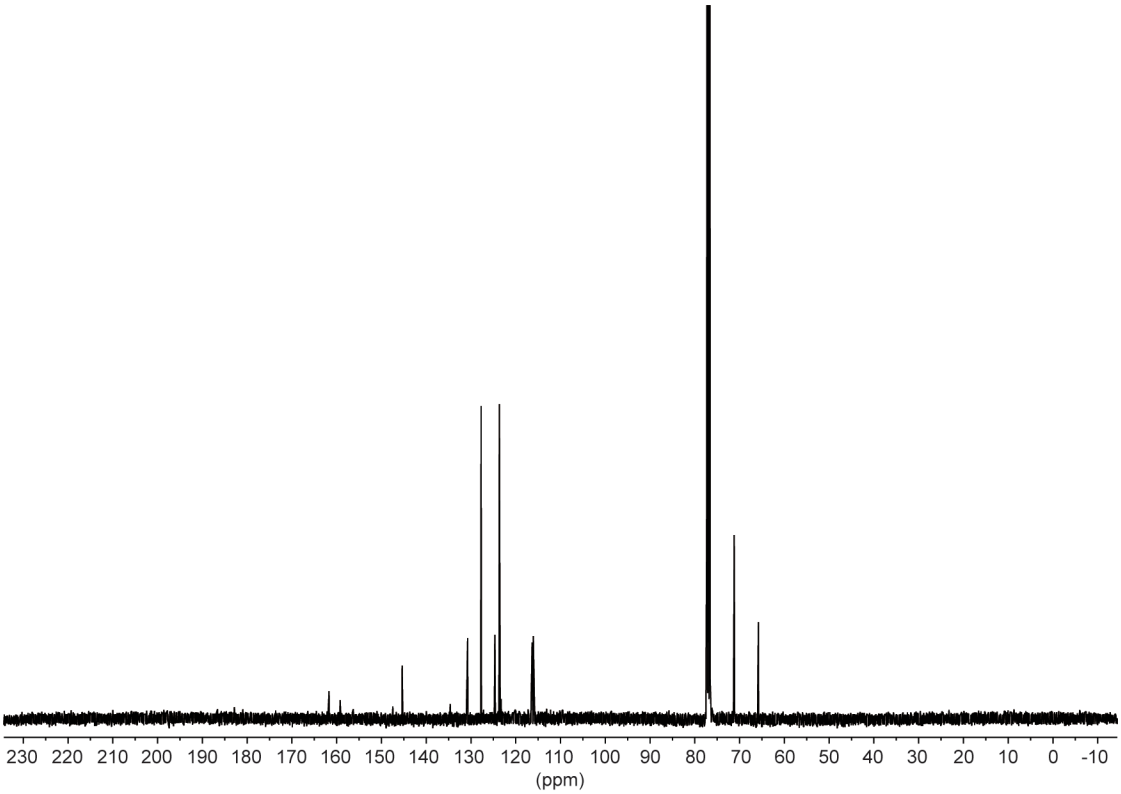

Fig. S20: ^{13}C NMR spectrum for compound **26k** in $CDCl_3$ (100 MHz).

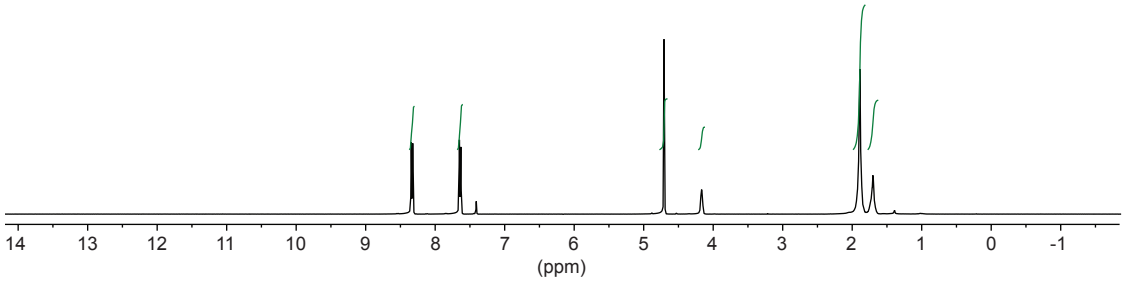

Fig. S21: ^{1}H NMR spectrum for compound **26o** in $CDCl_3$ (400 MHz).

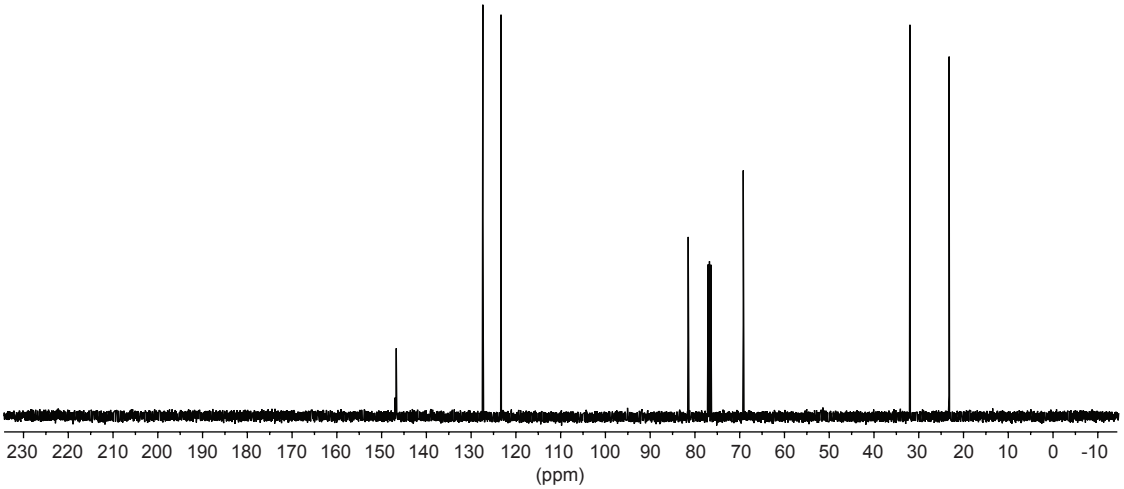

Fig. S22: ^{13}C NMR spectrum for compound **26o** in $CDCl_3$ (100 MHz).

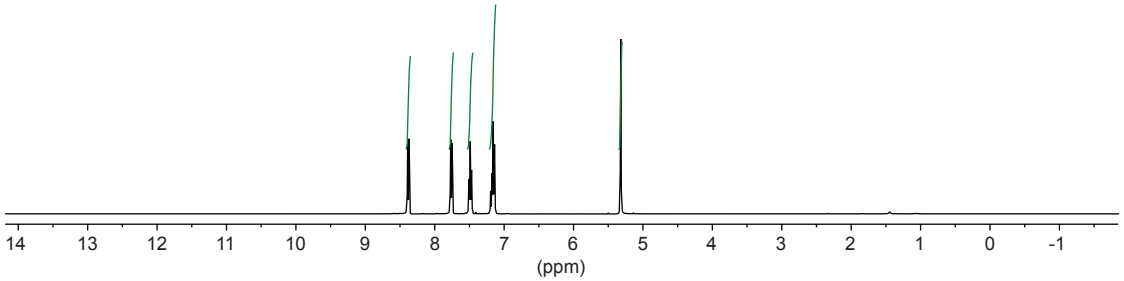

Fig. S23: ^{1}H NMR spectrum for compound **26q** in $CDCl_3$ (400 MHz).

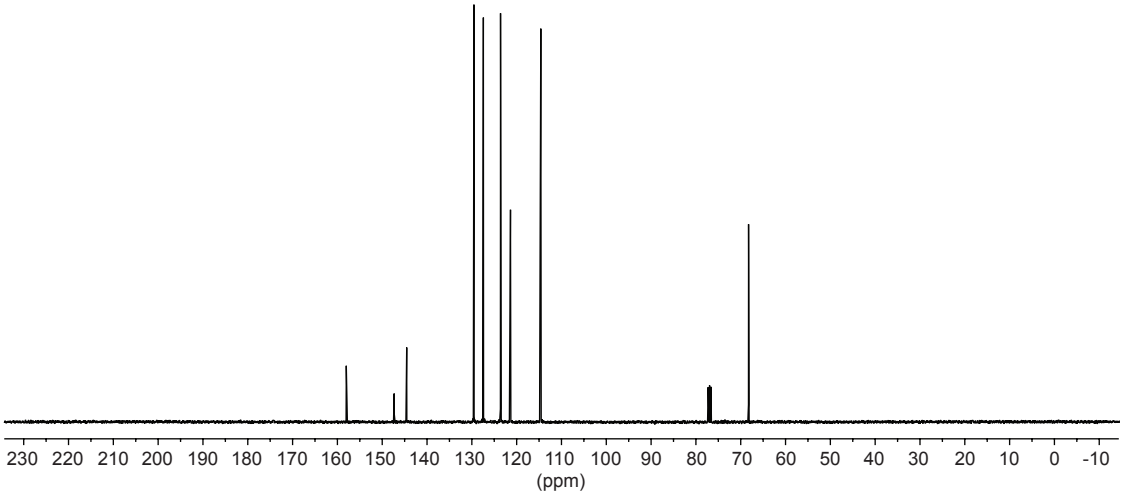

Fig. S24: ^{13}C NMR spectrum for compound **26q**in $CDCl_3$ (100 MHz).

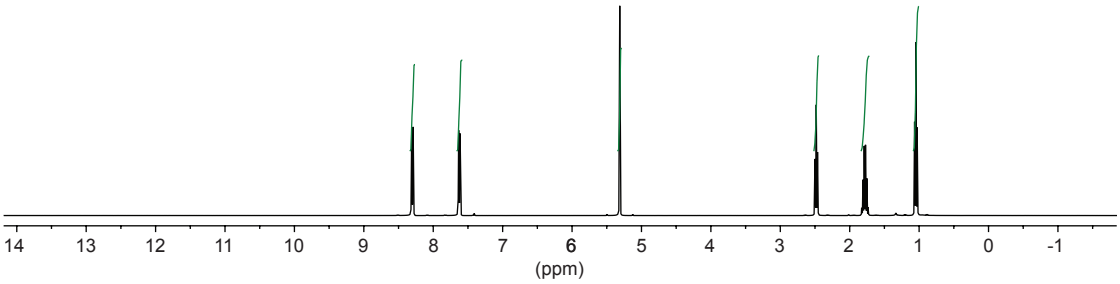

Fig. S25: ^{1}H NMR spectrum for compound **27c** in $CDCl_3$ (400 MHz).

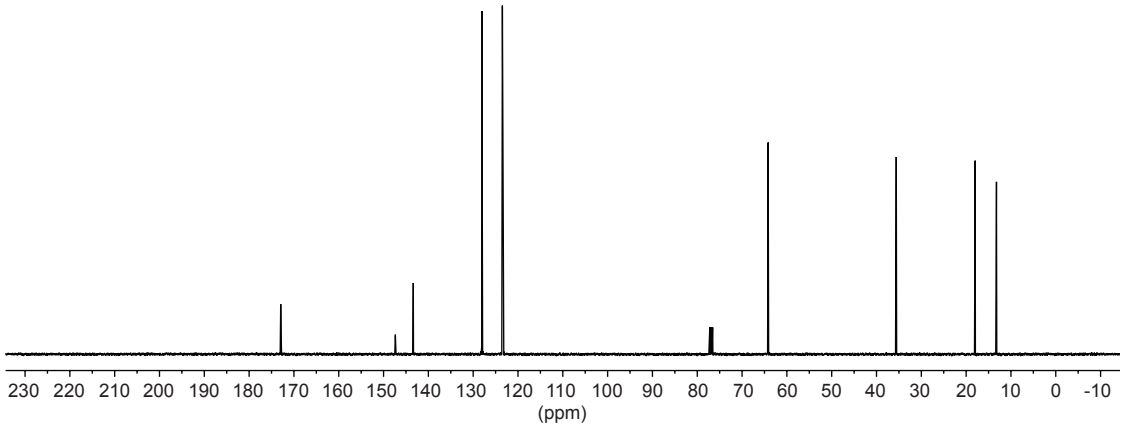

Fig. S26: ^{13}C NMR spectrum for compound **27c** in $CDCl_3$ (100 MHz).

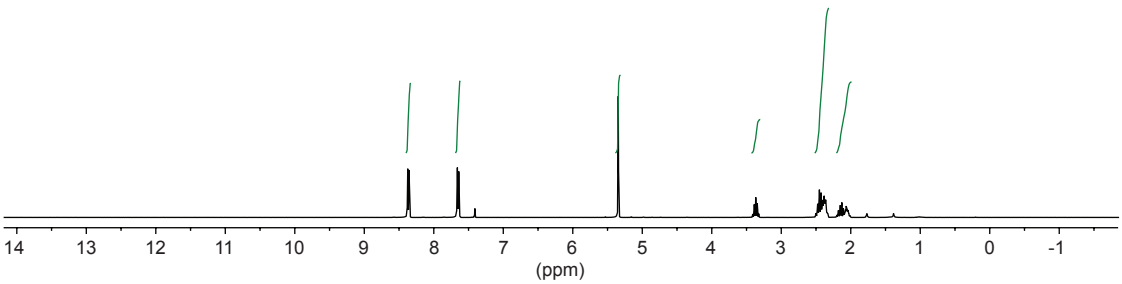

Fig. S27: ^{1}H NMR spectrum for compound **27d** in $CDCl_3$ (400 MHz).

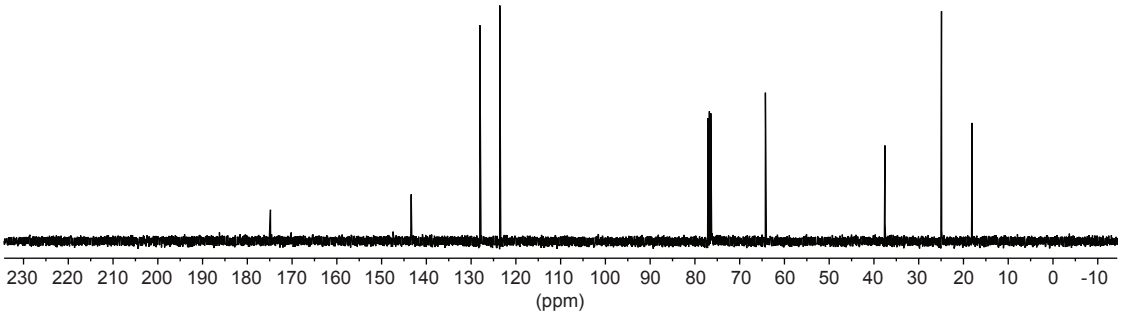

Fig. S28: ^{13}C NMR spectrum for compound **27d** in $CDCl_3$ (100 MHz).

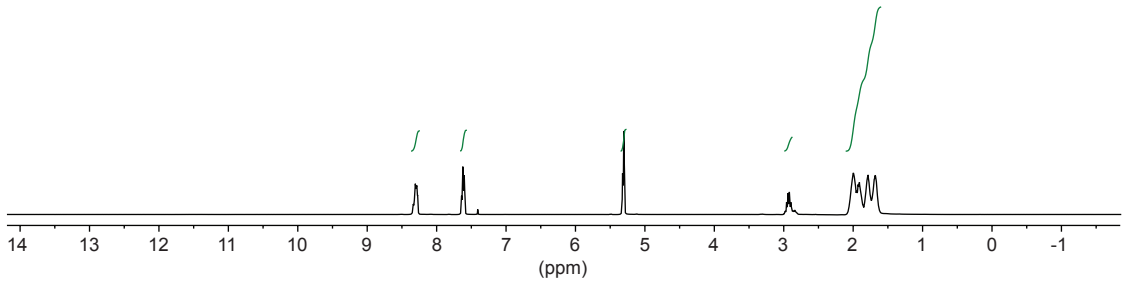

Fig. S29: ^{1}H NMR spectrum for compound **27e** in $CDCl_3$ (400 MHz).

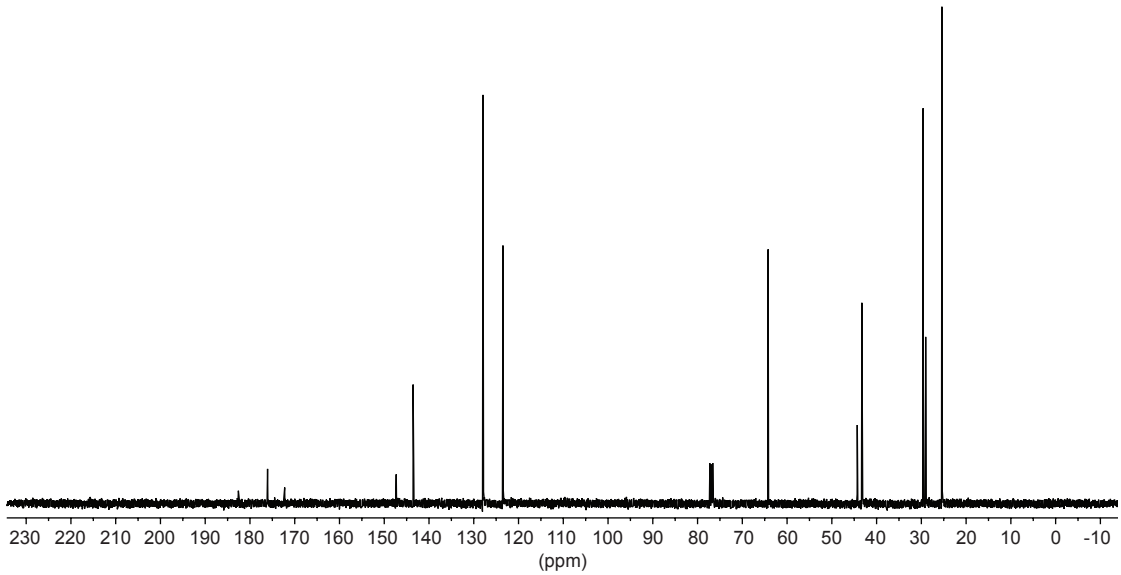

Fig. S30: ^{13}C NMR spectrum for compound **27e** in $CDCl_3$ (100 MHz).

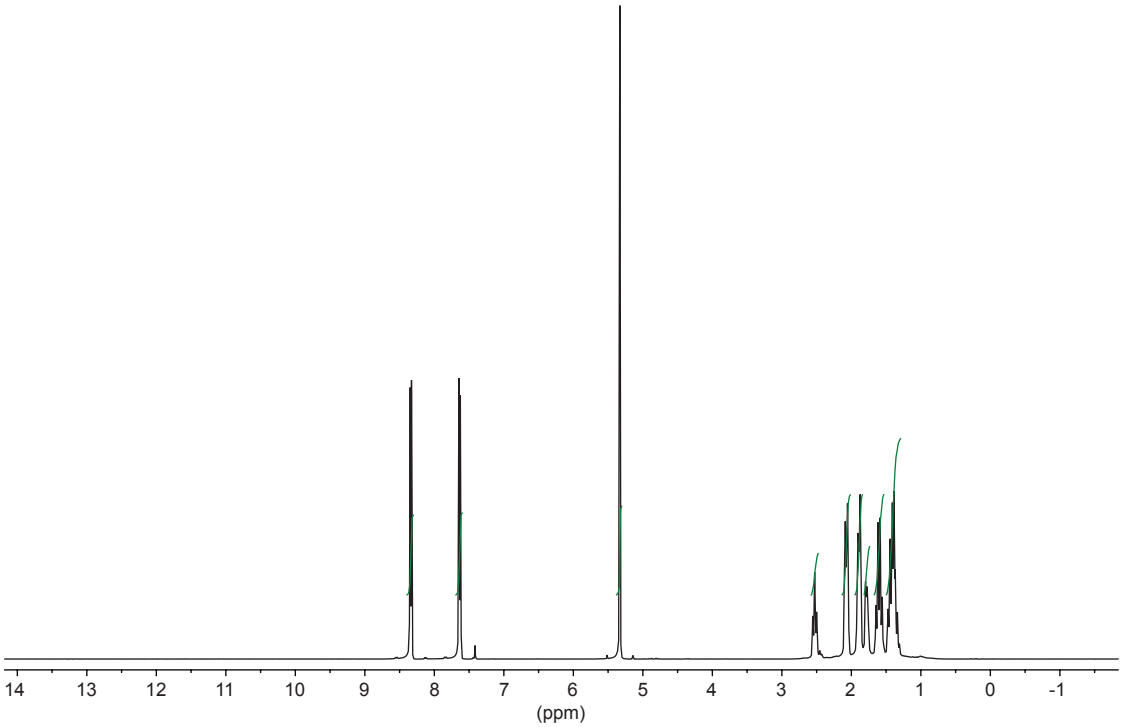

Fig. S31: ^{1}H NMR spectrum for compound **27f** in $CDCl_3$ (400 MHz).

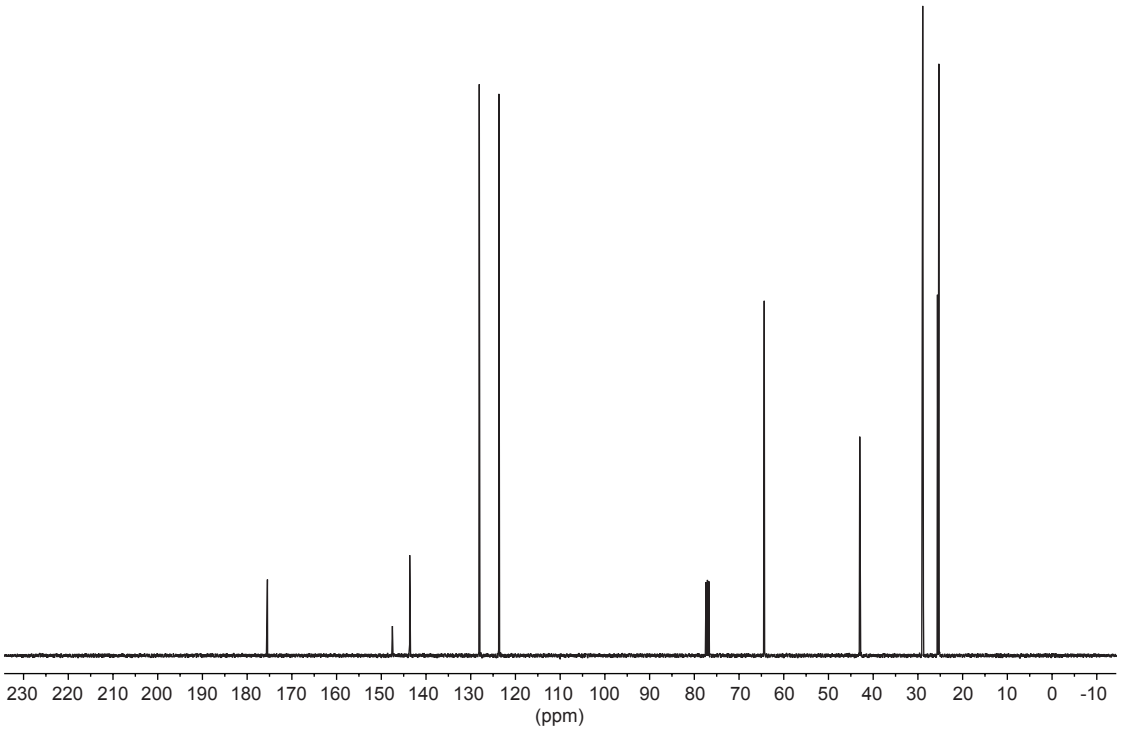

Fig. S32: ^{13}C NMR spectrum for compound **27f** in $CDCl_3$ (100 MHz).

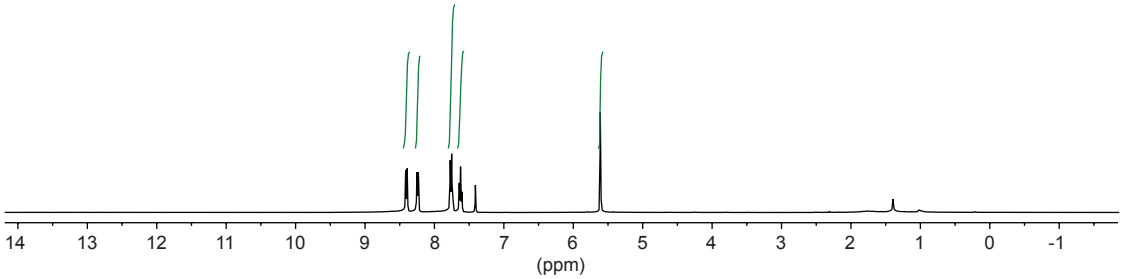

Fig. S33: ^{1}H NMR spectrum for compound **27g** in $CDCl_3$ (400 MHz).

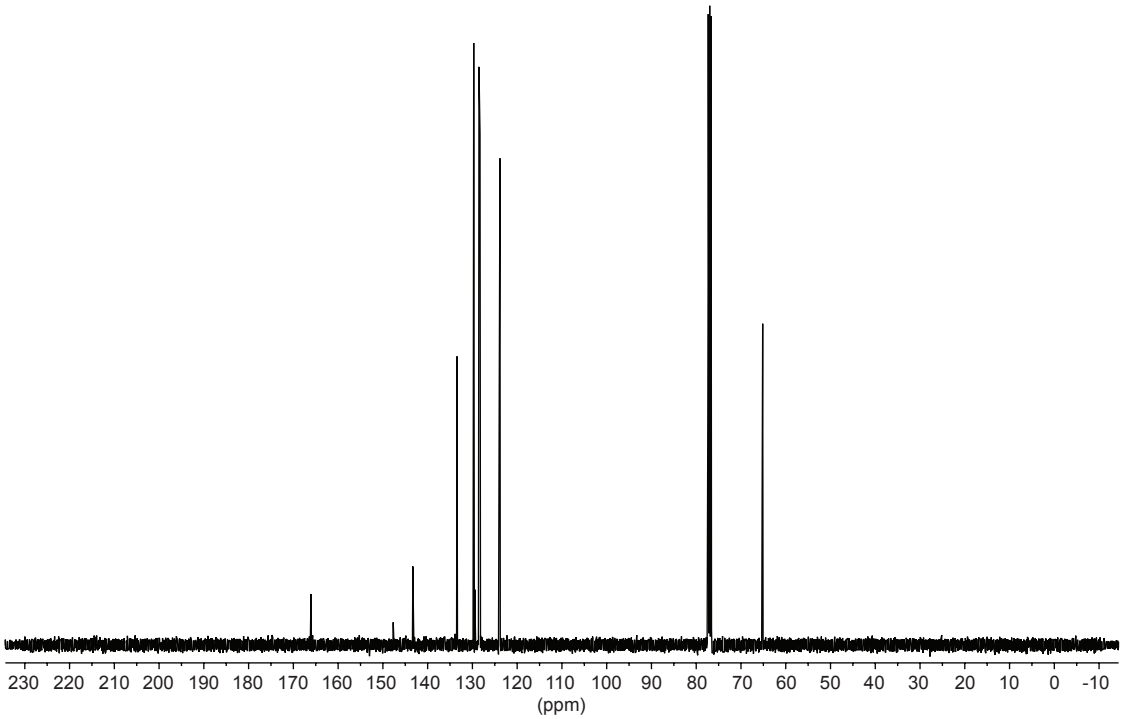

Fig. S34: ^{13}C NMR spectrum for compound **27g** in $CDCl_3$ (100 MHz).

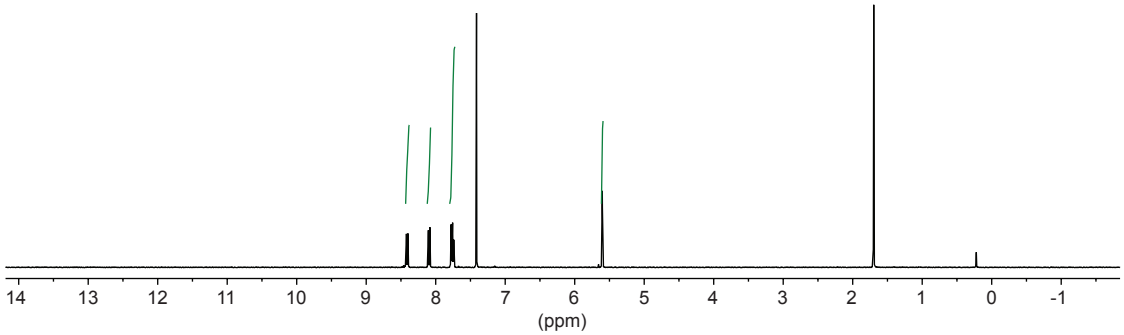

Fig. S35: ^{1}H NMR spectrum for compound **27h** in $CDCl_3$ (400 MHz).

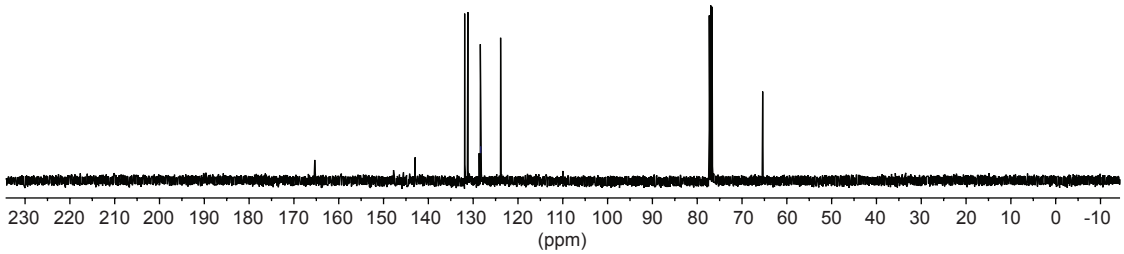

Fig. S36: ^{13}C NMR spectrum for compound **27h** in $CDCl_3$ (100 MHz).

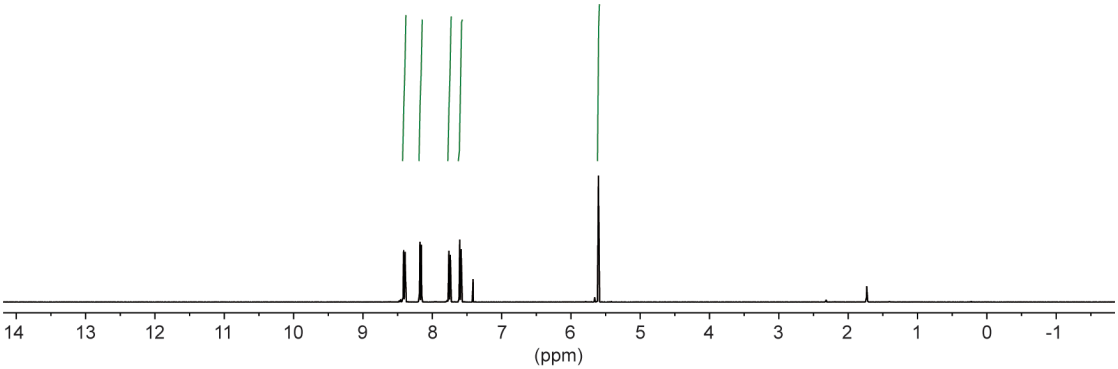

Fig. S37: ^{1}H NMR spectrum for compound **27i** in $CDCl_3$ (400 MHz).

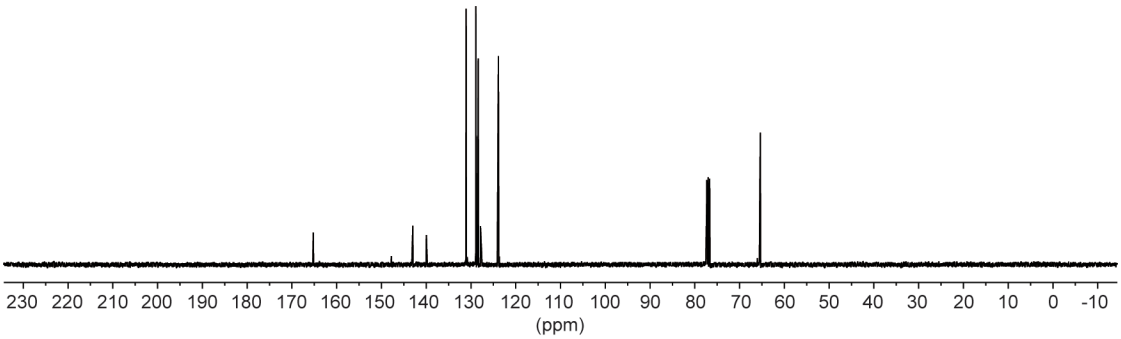

Fig. S38: ^{13}C NMR spectrum for compound **27i** in $CDCl_3$ (100 MHz).

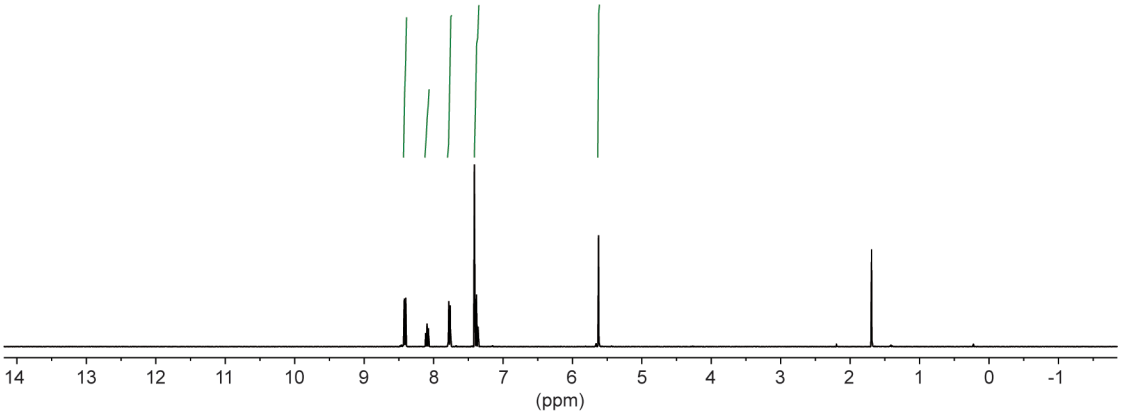

Fig. S39: ^{1}H NMR spectrum for compound **27k** in $CDCl_3$ (400 MHz).

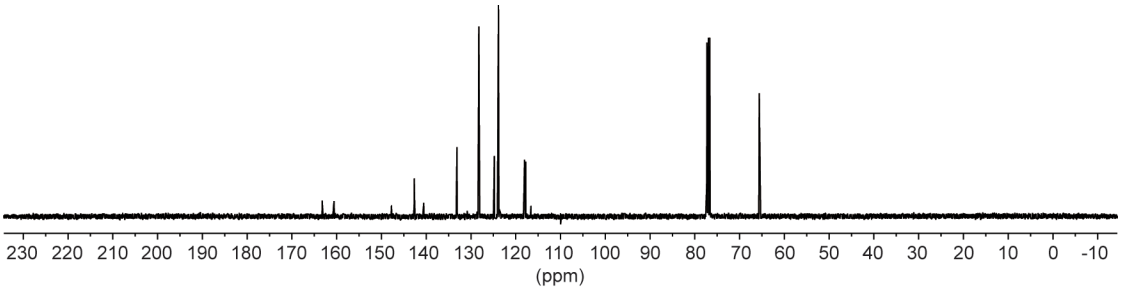

Fig. S40: ^{13}C NMR spectrum for compound **27k** in $CDCl_3$ (100 MHz).

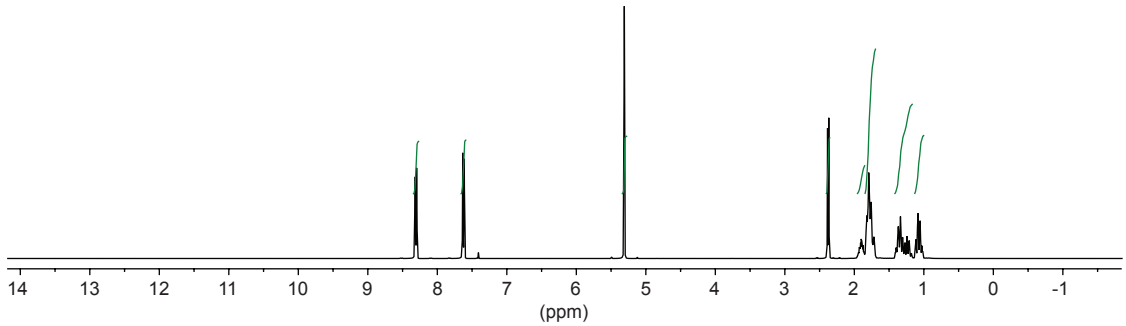

Fig. S41: ^{1}H NMR spectrum for compound **27l** in $CDCl_3$ (400 MHz).

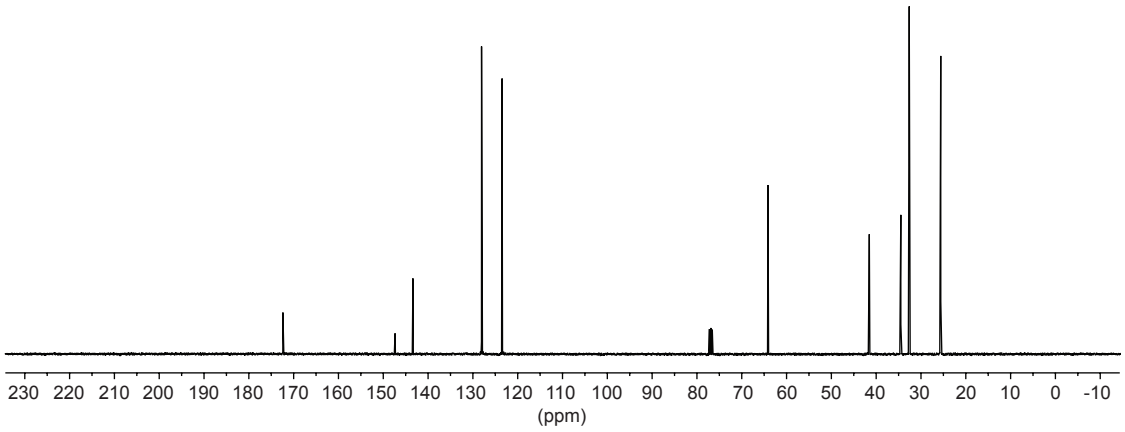

Fig. S42: ^{13}C NMR spectrum for compound **27l** in $CDCl_3$ (100 MHz).

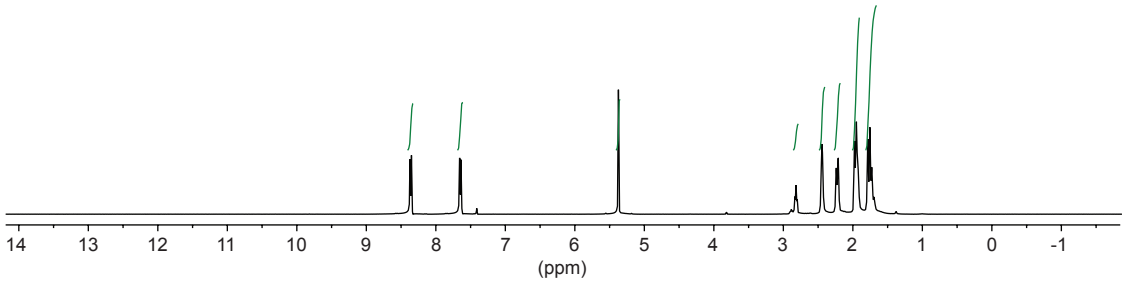

Fig. S43: ^{1}H NMR spectrum for compound **27m** in $CDCl_3$ (400 MHz).

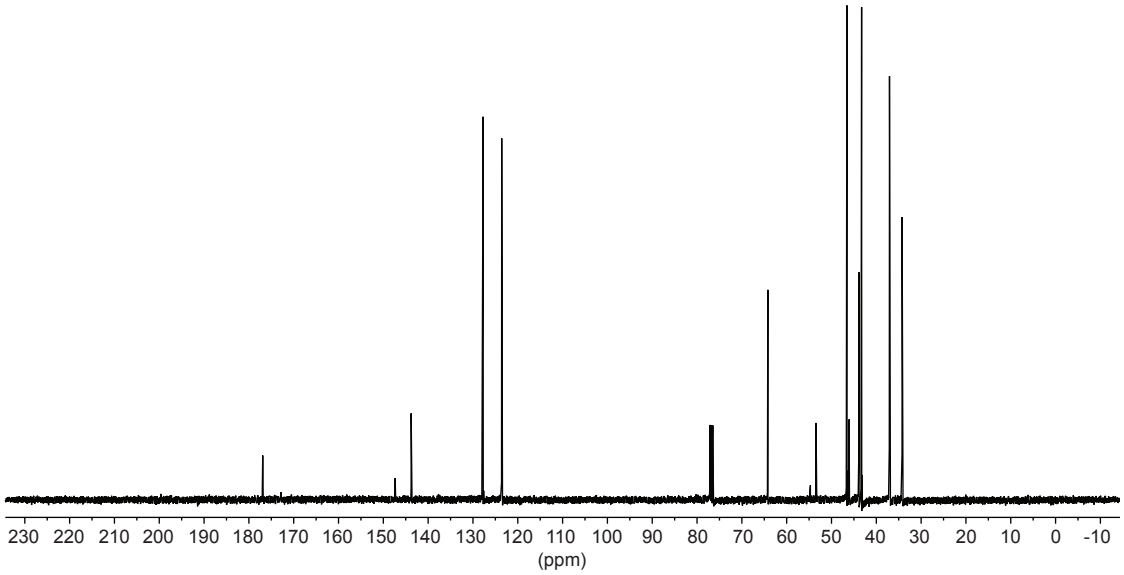

Fig. S44: ^{13}C NMR spectrum for compound **27m** in $CDCl_3$ (100 MHz).

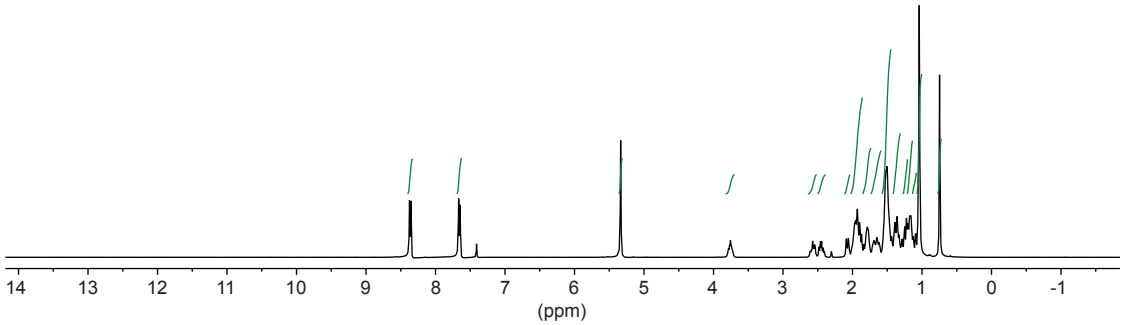

Fig. S45: ^{1}H NMR spectrum for compound **27n** in $CDCl_3$ (400 MHz).

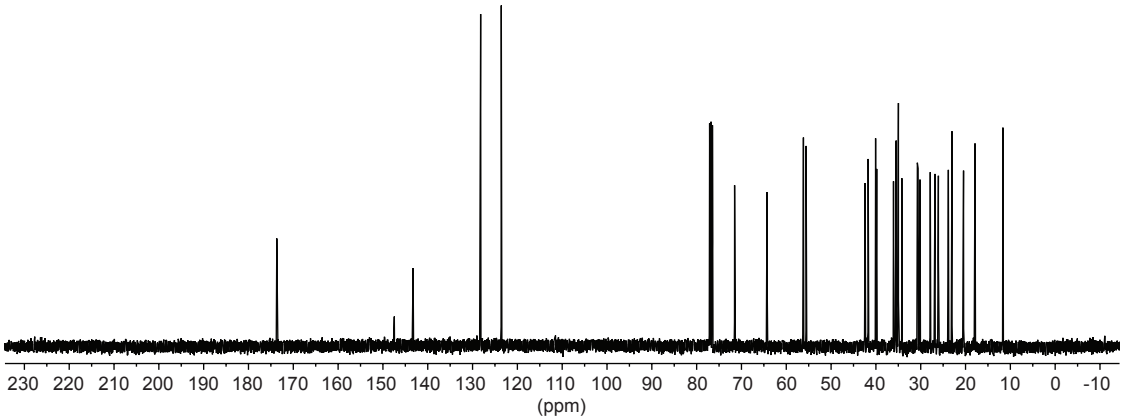

Fig. S46: ^{13}C NMR spectrum for compound **27n** in $CDCl_3$ (100 MHz).

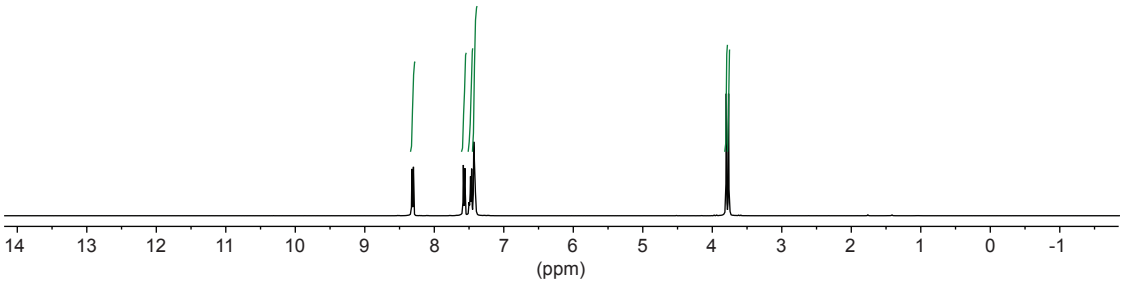

Fig. S47: ^{1}H NMR spectrum for compound **28g** in $CDCl_3$ (400 MHz).

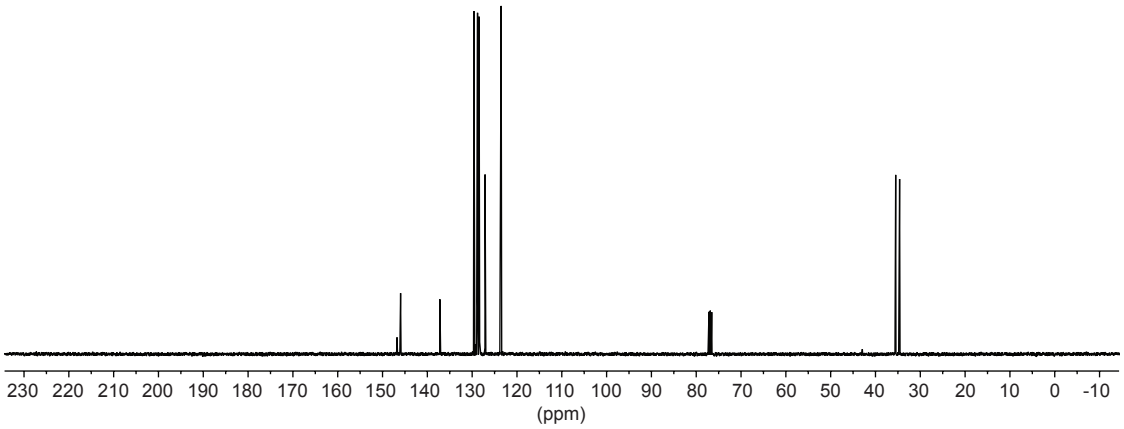

Fig. S48: ^{13}C NMR spectrum for compound **28g** in $CDCl_3$ (100 MHz).

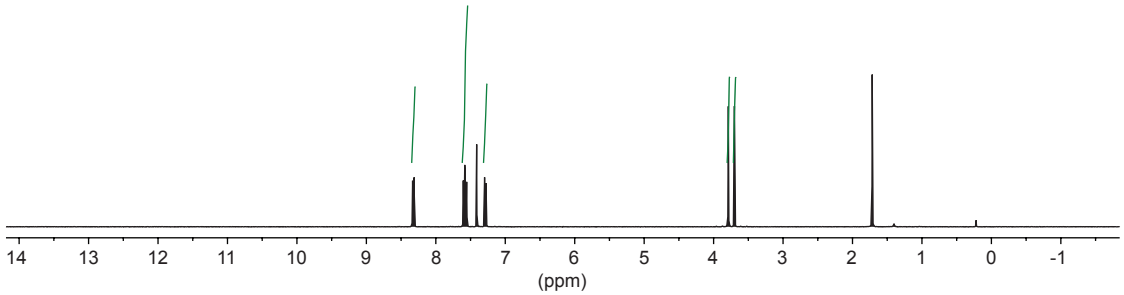

Fig. S49: ^{1}H NMR spectrum for compound **28h** in $CDCl_3$ (400 MHz).

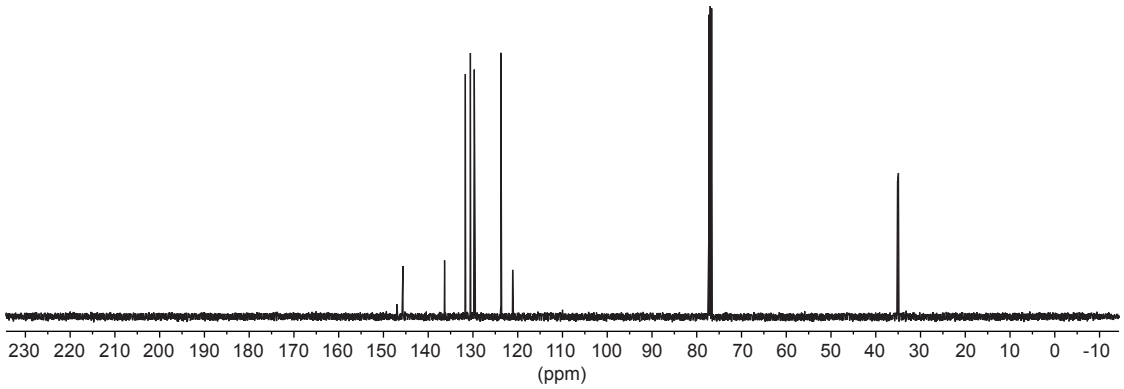

Fig. S50: ^{13}C NMR spectrum for compound **28h** in $CDCl_3$ (100 MHz).

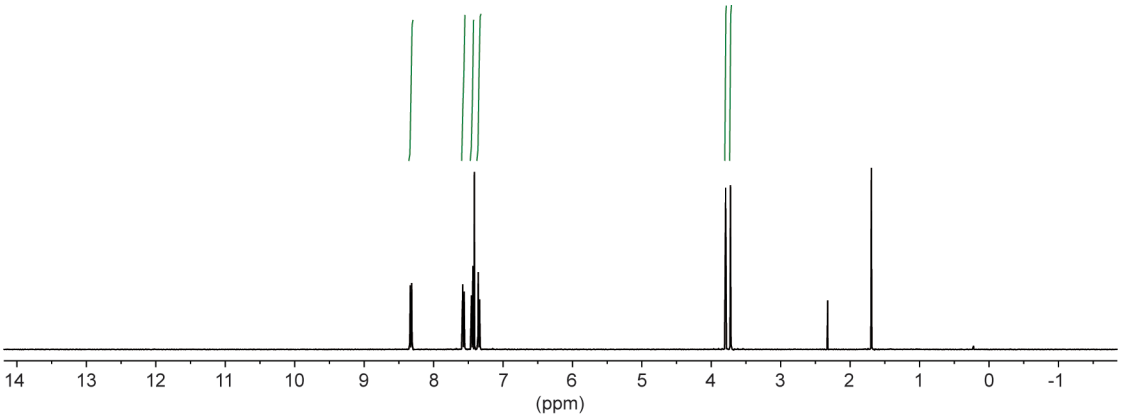

Fig. S51: ^{1}H NMR spectrum for compound **28i** in $CDCl_3$ (400 MHz).

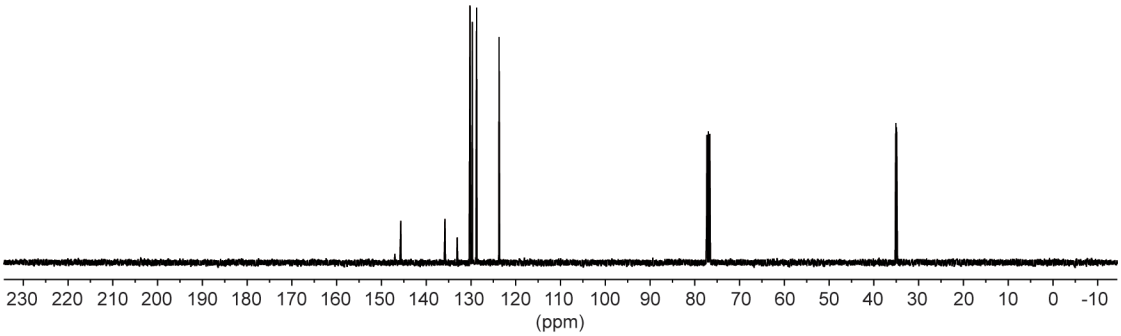

Fig. S52: ^{13}C NMR spectrum for compound **28i** in $CDCl_3$ (100 MHz).

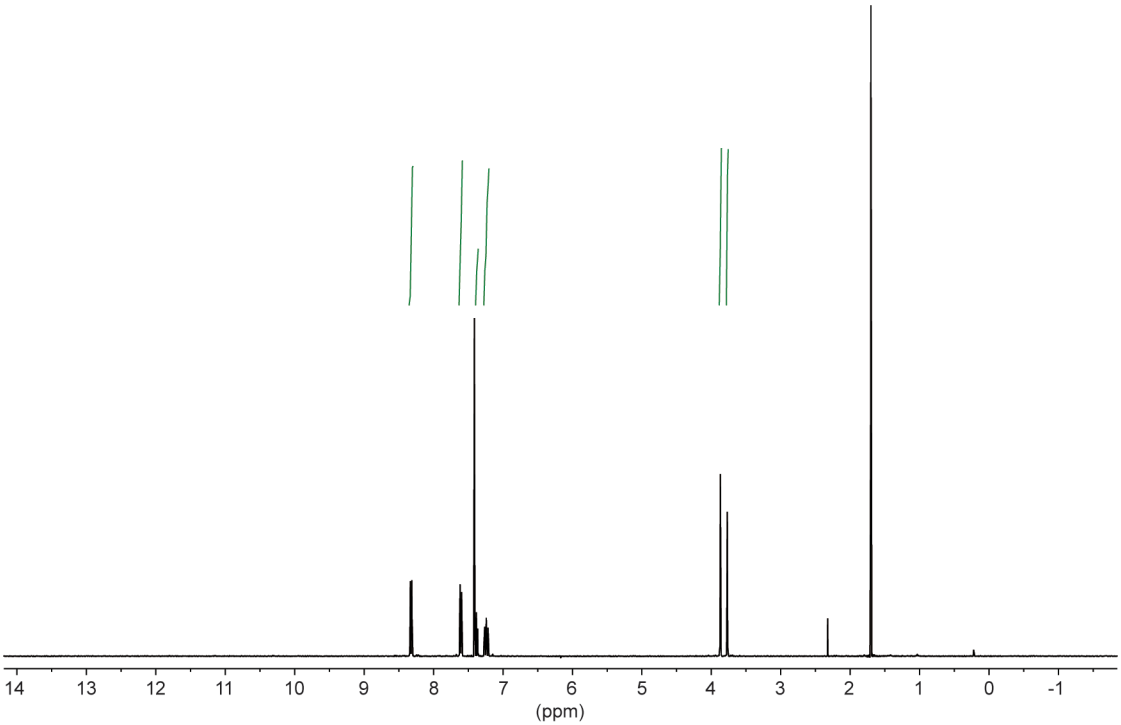

Fig. S53: ^{1}H NMR spectrum for compound **28k** in $CDCl_3$ (400 MHz).

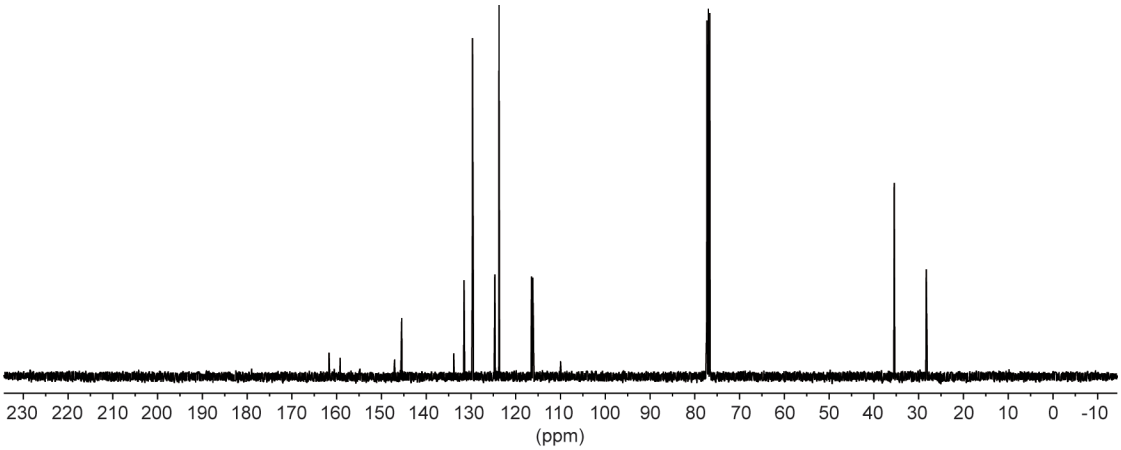

Fig. S54: ^{13}C NMR spectrum for compound **28k** in $CDCl_3$ (100 MHz).

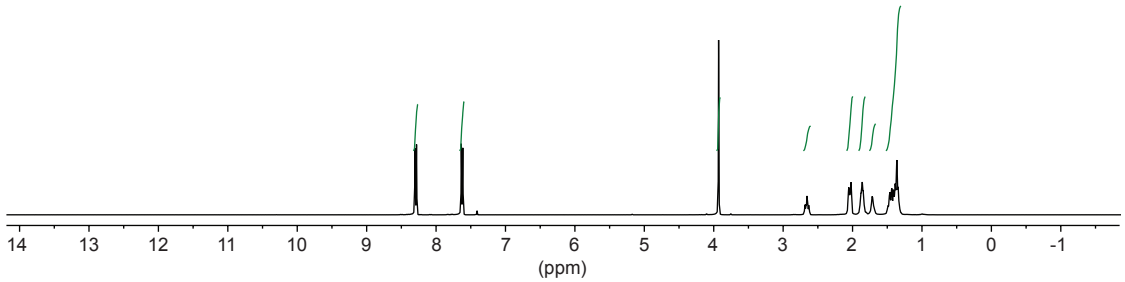

Fig. S55: ^{1}H NMR spectrum for compound **28p** in $CDCl_3$ (400 MHz).

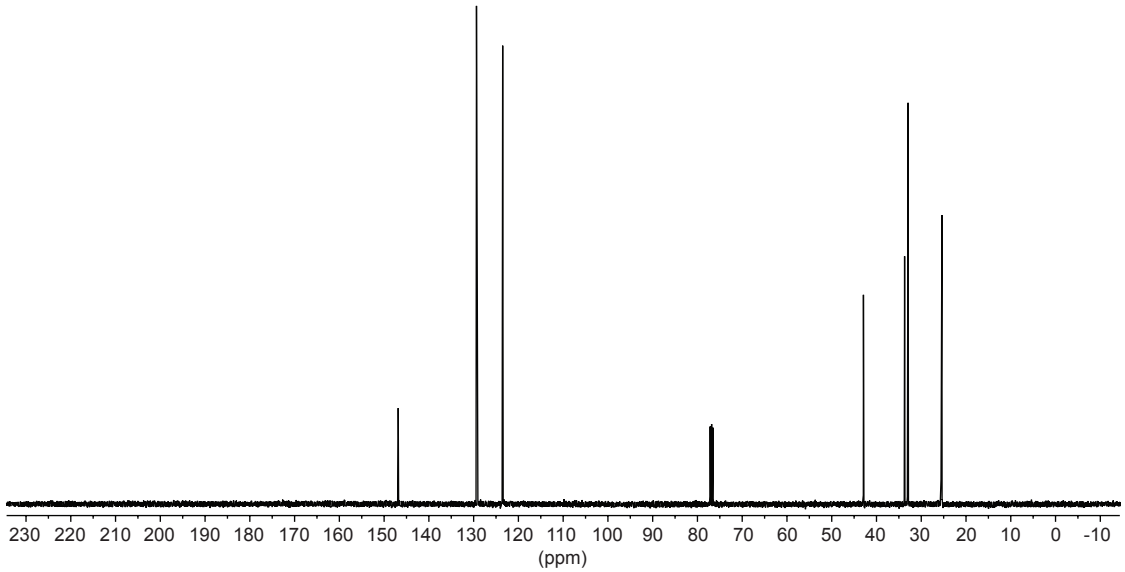

Fig. S56: ^{13}C NMR spectrum for compound **28p** in $CDCl_3$ (100 MHz).

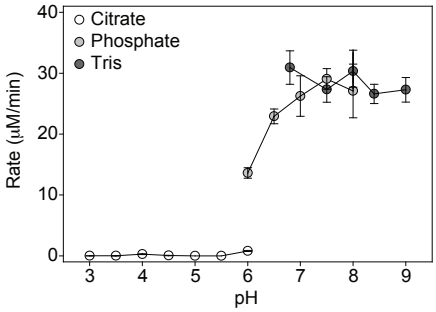

Fig. S57: Rate of reaction of CNR with CAM at different pHs in increments of 0.5 (pH 3-6 using citrate buffer; pH 6-8 using sodium phosphate buffer; pH 7-9 using Tris-HCl buffer).

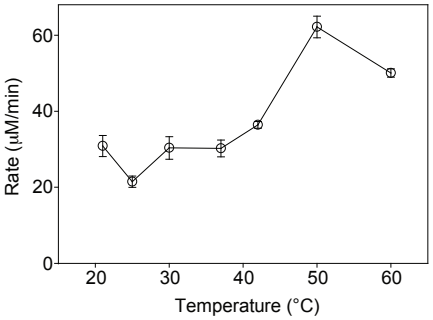

Fig. S58: Rate of reaction of CNR with CAM at different temperatures.

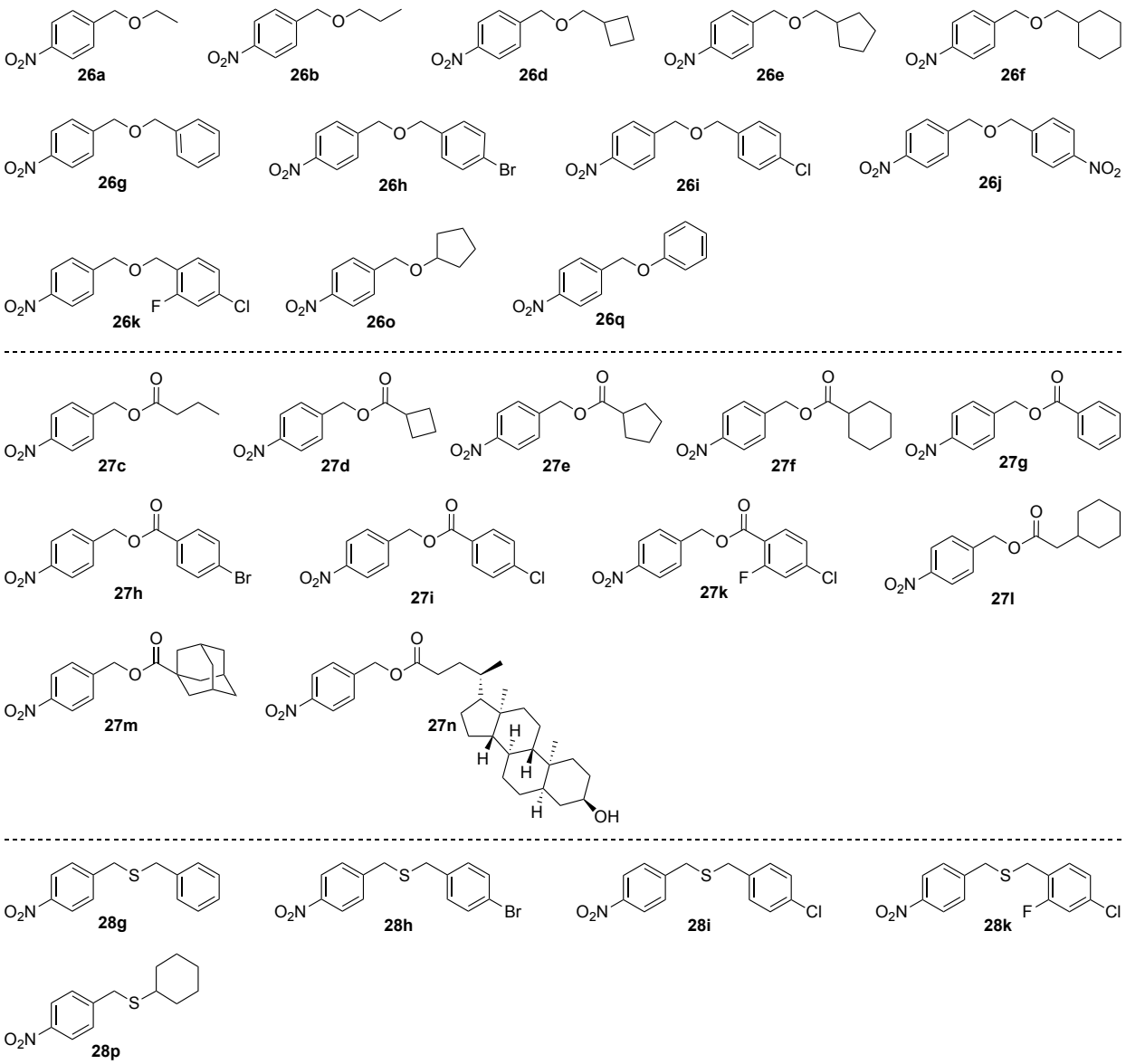

Fig. S59: Structures of synthesized nitro-containing molecules tested with CNR. *Note*: The synthetic schemes along with yields are presented in Scheme 2.

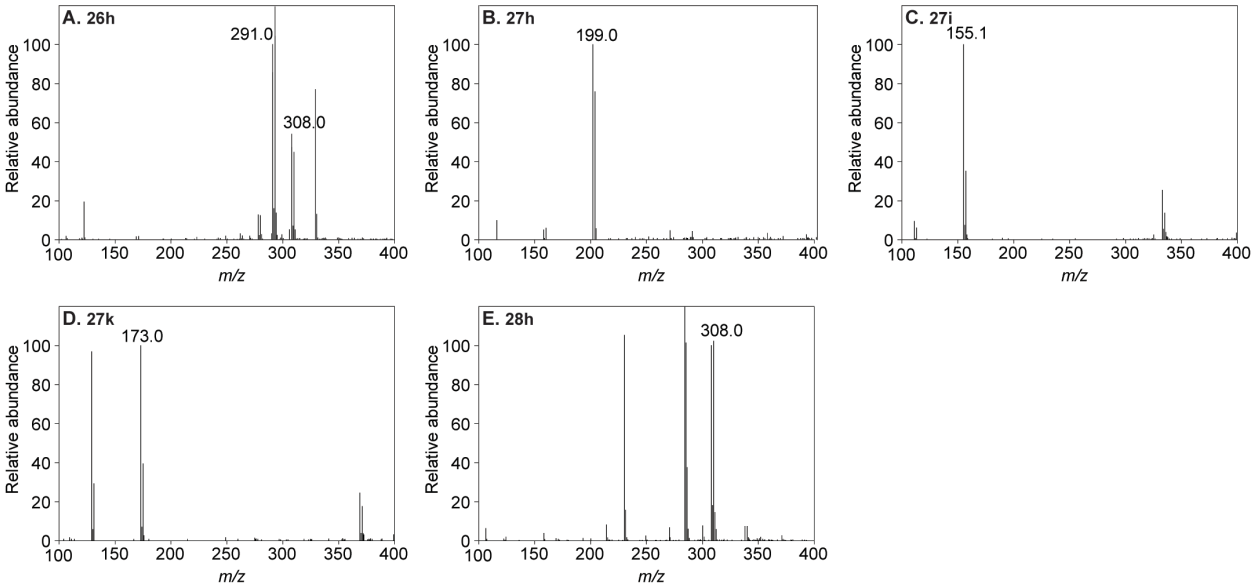

Fig. S60: MS traces of CNR-catalyzed large-scale reduction reactions of **A.** compound **26h** showing the masses of the reduced products 291.0 (M^+, amine) and 308.0 (hydroxylamine, $[M+H]^+$), **B.** compound **27h** with the deprotection mass of 199.0 ($[M-H]^-$), **C.** compound **27i** having the deprotected mass of 173.0 (($[M-H]^-$), **D.** compound **27k** with a mass of 173.0 (($[M-H]^-$) indicating deprotection, and **E.** compound **28h** showing the reduction to the amine with a mass of 308.0 ($[M+H]^+$).

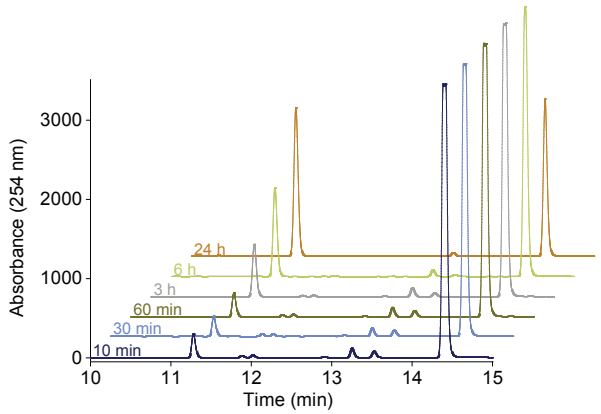

Fig. S61: RP-HPLC traces of a reaction of compound **27h** with CNR and NADPH. Samples were taken at 10 min, 30 min, 60 min, 3 h, 6 h, and 24 h.

Table S1: CNR activity (in %) against 4-nitrobenzyl derivatives.[a]

Compound	Activity (%)	Compound	Activity (%)
CAM	100 ± 0	**26a**	56 ± 8
1	0 ± 0	**26b**	0 ± 0
2	0 ± 0	**26d**	7.5 ± 1.7
3	23 ± 3	**26e**	21 ± 2
4	0 ± 0	**26f**	7.0 ± 1.8
5	0 ± 0	**26g**	29 ± 4
6	88 ± 5	**26h**	116 ± 4
7	21 ± 3	**26i**	90 ± 11
8	3.5 ± 0.6	**26j**	21 ± 4
9	157 ± 4	**26k**	88 ± 7
10	77 ± 4	**26o**	67 ± 12
11	122 ± 7	**26q**	45 ± 5
12	0 ± 0	**27c**	11 ± 5
13	16 ± 1	**27d**	1.8 ± 0.8
14	40 ± 8	**27e**	0 ± 0
15	132 ± 2	**27f**	9 ± 0
16	125 ± 5	**27g**	0 ± 0
17	18 ± 7	**27h**	0 ± 0
18	0 ± 0	**27i**	2.2 ± 0.2
19	0 ± 0	**27k**	0 ± 0
20	44 ± 4	**27l**	0 ± 0
21	82 ± 6	**27m**	120 ± 7
22	11 ± 4	**27n**	1.1 ± 0.1
23	32 ± 6	**28g**	136 ± 16
24	19 ± 4	**28h**	92 ± 7
25	0 ± 0	**28i**	133 ± 14
		28k	124 ± 3
		28p	62 ± 5

[a] These values were used to generate Fig. 3.