Supplementary Material

Synthesis of phenazine-2,8-dicarboxylates

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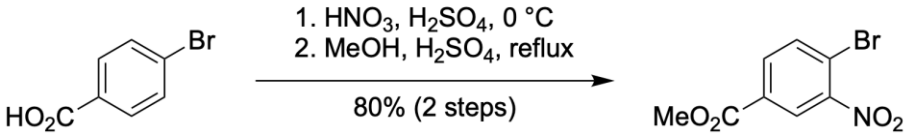
SM-1. Preparation of starting materials

Methyl 4-bromo-3-nitrobenzoate[1] (11):



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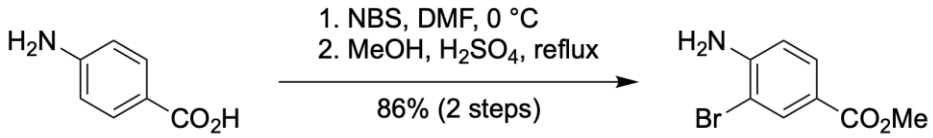
A solution of nitric acid (70%, 0.66 mL, 11 mmol) in sulfuric acid (98%, 3 mL) was added slowly to a stirring, solution of 4-bromobenzoic acid (2.00 g, 9.95 mmol) in sulfuric acid (25 mL) at 0 °C. The solution was stirred for 0.5 h then poured into water (0 °C, 250 mL). The resultant colourless precipitate was collected by filtration and redissolved in EtOAc (20 mL). Water (20 mL) was added, the phases separated, and the aqueous phase extracted with further EtOAc (2 × 20 mL). Organic extracts were combined, dried ($MgSO_4$), filtered and the filtrate concentrated under reduced pressure to afford a crude colourless solid (2.16 g). This crude material was dissolved in MeOH (20 mL), and sulfuric acid (98%, 1 mL) was slowly added. The solution was heated to reflux for 17 h, and cooled to r.t. whereupon a yellow precipitate formed. Water (10 mL) and NaOH (2 M) were added until pH7 was reached, and the solution was extracted with EtOAc (3 × 50 mL). Extracts were combined, washed with brine (30 mL), dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure and the resultant crude yellow solid was purified by flash chromatography (10–50% EtOAc in hexane). The title compound was obtained as a colourless solid (2.07 g, 80%); $\mathbf{R_f}$ 0.30 (20% EtOAc in hexane); **m.p.** 104–106 °C {lit. 102–104 °C}[1]; **IR** (neat) ν_{max} 1716 (s, C=O str.), 1538 (s), 1441 (m), 1353 (m), 1244 (s), 1033 (m) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.47 (d, *J* 2.0 Hz, 1H, H-2), 8.07 (dd, *J* 2.0, 8.3 Hz, 1H, H-6), 7.85 (d, *J* 8.3 Hz, 1H, H-5), 3.97 (s, 3H, CO_2CH_3); **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ 164.5 ($\underline{C}O_2CH_3$), 150.0 (C3), 135.6 (C5), 133.6 (C6), 130.9 (C1), 126.6 (C2), 119.7 (C4), 53.1 ($CO_2\underline{C}H_3$); **HRMS (ESI, +ve)** $C_8H_7BrNO_4^+$ $[M + H]^+$ requires *m/z* 259.9553, 261.9533, found 259.9546, 261.9525.

Methyl 4-amino-3-bromobenzoate (12):



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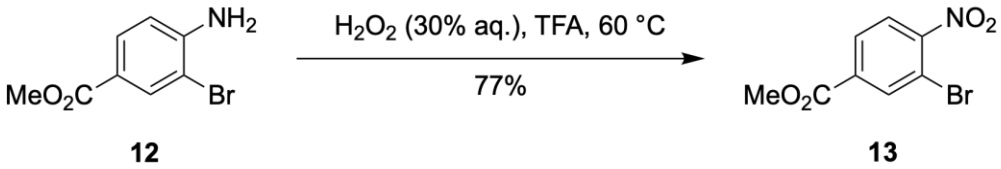
To a solution of 4-aminobenzoic acid (7.50 g, 54.7 mmol) in DMF (30 mL) at 0 °C was added *N*-bromosuccinimide (10.1 g, 56.9 mmol) in ~0.5 g portions over 1.5 h. The suspension was warmed to r.t. and stirred for a further 2.5 h, then poured into water (60 mL) at 0 °C. The resultant colourless precipitate was collected by vacuum filtration and dried under vacuum. The solid was added to a 70 °C solution of sulfuric acid (98%, 4.5 mL) in methanol (95 mL). The resulting pale brown solution was stirred for 20 h at reflux, whereupon TLC indicated the consumption of the isolated intermediate. The solution was cooled to r.t., and the volume reduced to ~3/4 under reduced pressure. Water (200 mL) and NaOH (2 M, until pH10) were added to quench the reaction. This was extracted with EtOAc (3 × 100 mL), and the combined organic extracts were washed successively with saturated $NaHCO_3$ solution (50 mL) and brine (50 mL), dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure to give an orange-brown oil, which was purified by flash

chromatography (50% EtOAc in hexane), to afford the title compound as a pale pink solid (10.8 g, 86%); **R_f** 0.30 (30% EtOAc in hexane); **m.p.** 103–106 °C (from EtOAc/hexane, pink needles); **IR** (neat) ν_{max} 3461(m, NH_2 str.), 3354 (m, NH_2 str.), 1682 (s, C=O str.), 1614 (m), 1434 (m), 1294 (s), 1244 (s), 1036 (w) cm^{-1}; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.10 (d, *J* 1.8 Hz, 1H, H-2), 7.76 (dd, *J* 8.4, 1.8 Hz, 1H, H-6), 6.71 (d, *J* 8.4 Hz, 1H, H-5), 4.43 (br s, 2H, NH_2), 3.84 (s, 3H, CO_2CH_3); **^{13}C{^{1}H} NMR** (76 MHz, $CDCl_3$) δ 166.2 ($\underline{C}O_2CH_3$), 148.3 (C4), 134.6 (C2), 130.3 (C6), 120.7 (C1), 114.3 (C5), 107.9 (C3), 52.0 ($CO_2\underline{C}H_3$); **HRMS (ESI, +ve)** $C_8H_8BrNO_2Na^+$ $[M + Na]^+$ requires *m/z* 251.9631, 253.9610, found *m/z* 251.9629, 253.9605.

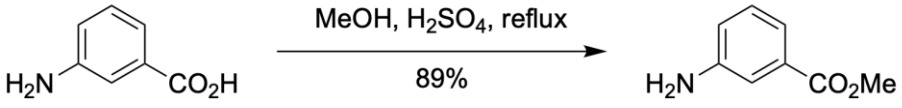
Methyl 3-bromo-4-nitrobenzoate (13):



12 **13**

To a solution of methyl 4-amino-3-bromobenzoate (**12**, 4.04 g, 17.5 mmol) in TFA (80 mL) was added H_2O_2 (30%, 11 mL) dropwise, and the solution was heated to 60 °C for 18 h. The reaction mixture was concentrated under reduced pressure, EtOAc (80 mL) added, and the solution washed with NaOH (1 M, 40 mL) and $NaHCO_3$ (40 mL). The aqueous phases were combined and extracted with EtOAc (3 × 40 mL). All organic phases were combined, washed with brine (20 mL), dried ($MgSO_4$), filtered and the filtrate concentrated under reduced pressure to afford a crude yellow-brown solid (4.36 g). This material was purified by flash chromatography (15–50% EtOAc in hexane) and crystallisation from the relevant fractions afforded the title compound as a yellow crystalline solid (3.73 g, 82%); **R_f** 0.28 (15% EtOAc in hexane); **m.p.** 73–75 °C {lit. 75–76 °C}[2]; **IR** (neat) ν_{max} 1724 (s, C=O str.), 1527 (s), 1435 (m), 1355 (m), 1258 (s) 1040 (w) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.40 (d, *J* 1.7 Hz, 1H, H-2), 8.10 (dd, *J* 1.7, 8.4 Hz, 1H, H-6), 7.85 (d, *J* 8.4 Hz, 1H, H-5), 3.98 (s, 3H, CO_2CH_3); **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ 164.2 ($\underline{C}O_2CH_3$), 152.6 (C4), 136.3 (C2), 134.4 (C1), 129.5 (C6), 125.5 (C5), 114.5 (C3), 53.2 ($CO_2\underline{C}H_3$); **HRMS (ESI, +ve)** $C_8H_7BrNO_4^+$ $[M + H]^+$ requires *m/z* 259.9553, 261.9533, found 259.9565, 261.9545.

Methyl 3-aminobenzoate[3] (14):



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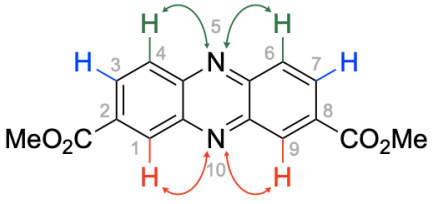
Sulfuric acid (98%, 5.5 mL) was added slowly to a solution of 3-aminobenzoic acid (6.17 g, 45.0 mmol) in methanol (110 mL) at 0 °C. The solution was subsequently heated to reflux for 18 h, then cooled to r.t. and concentrated under reduced pressure. Water (80 mL) and NaOH (1 M) was added until pH6 was reached. This solution was extracted with EtOAc (3 × 100 mL), and the combined organic extracts were washed with $NaHCO_3$ (50 mL), dried ($MgSO_4$), filtered, and the filtrate concentrated under reduced pressure. The resulting residue was purified by flash chromatography (40–60% EtOAc in hexane) affording the title compound as a yellow oil (6.05 g, 89%); **R_f** 0.33 (40% EtOAc in hexane); **IR** (neat) ν_{max} 3456 (w, NH_2 str.), 3373 (w, NH_2 str.), 1704 (s, C=O str.), 1602 (m), 1435 (m), 1293 (s), 1234 (s) cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl3_3$) δ 7.45–7.40 (m, 1H, H-6),

7.37–7.34 (m, 1H, H-2), 7.24–7.18 (m, 1H, H-5), 6.89–6.82 (m, 1H, H-4), 3.88 (s, 3H, CO_2CH_3), 3.71 (br s, 2H, NH_2); **$^{13}C\{^1H\}$ NMR** (101 MHz, $CDCl_3$) δ 167.4 ($\underline{C}O_2CH_3$), 146.5 (C3), 131.2 (C1), 129.4 (C5), 119.8 (C6), 119.5 (C4), 115.9 (C2), 52.2 ($CO_2\underline{C}H_3$); **HRMS (ESI, +ve)** $C_8H_{10}NO_2^+$ $[M + H]^+$ requires *m/z* 152.0706, found 152.0703.

SM-2. $^{1}H–^{15}N$ HMBC NMR plot of compound 5



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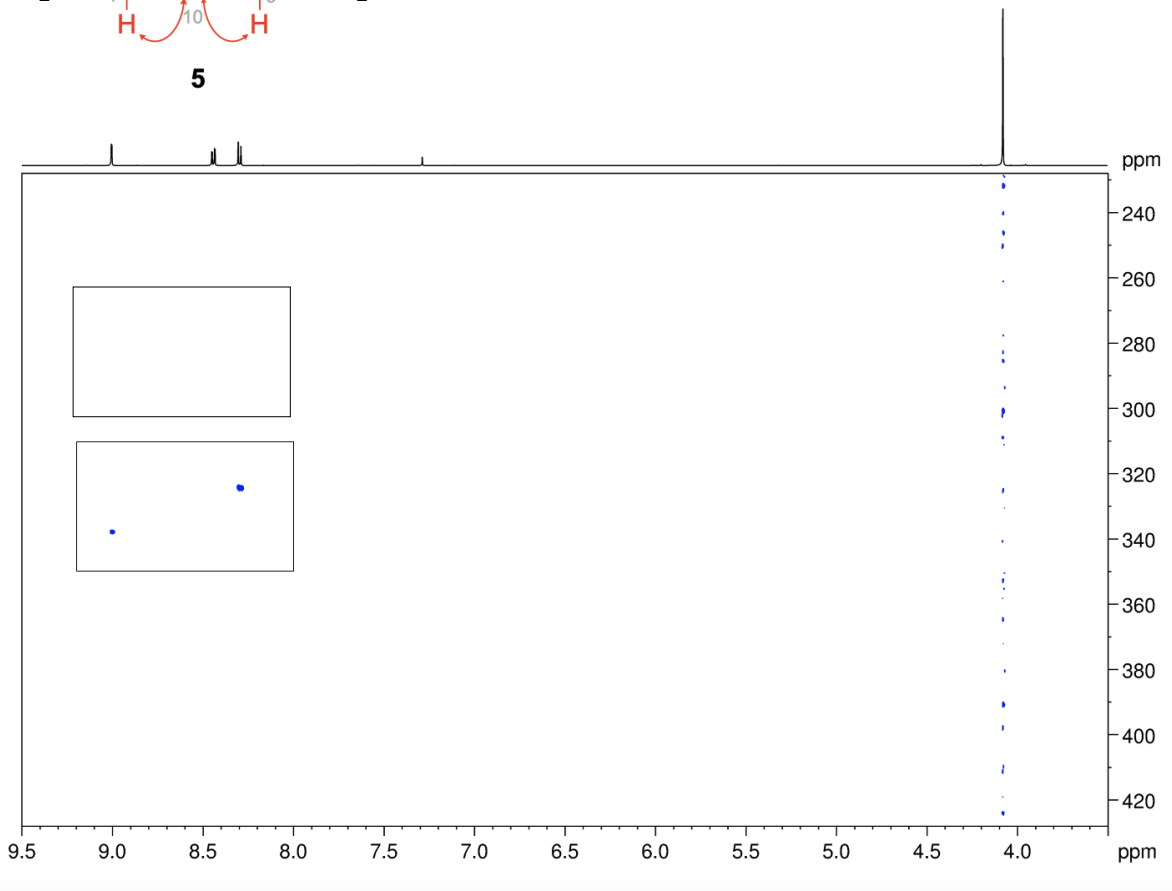


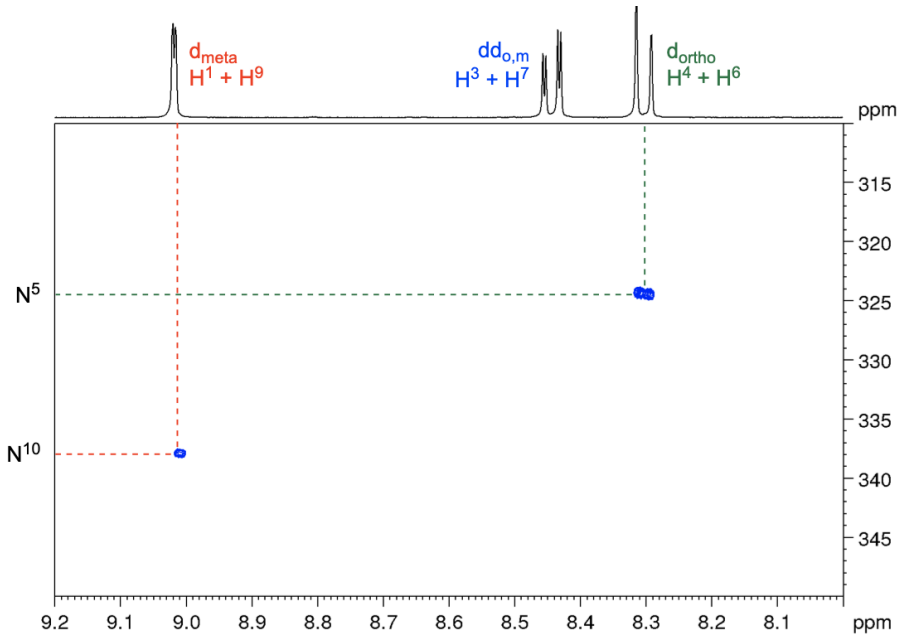


Figure S1: $^{1}H–^{15}N$ HMBC NMR plot of compound **5** (600 MHz, $CDCl_3$).

SM-3. X-Ray Crystallography and Discussion of Crystal Packing (Compound 5)

Experimental

Suitable single crystals of **5** were selected under the polarising microscope (Leica M165Z), mounted on a MicroMount (MiTeGen, USA) consisting of a thin polymer tip with a wicking aperture. The X-ray diffraction measurements were carried out on a Bruker D8 Quest Single Crystal diffractometer with Photon II detector at 150 K by using IμS 3.0 Microfocus Source with Mo-K*α* radiation (λ = 0.710723 Å) . The single crystal, mounted on the goniometer using cryo loops for intensity measurements, was coated with paraffin oil and then quickly transferred to the cold stream using an Oxford Cryo stream 800 attachment. Symmetry related absorption corrections using the program SADABS[4] were applied and the data were corrected for Lorentz and polarisation effects using Bruker APEX3 software.[4] The structure was solved by ShelxT (intrinsic phasing)[5] and the full-matrix least-square refinement was carried out using Shelxl[6] in Olex2.[7] The non-hydrogen atoms were refined anisotropically. The molecular graphic was generated using program Olex2.[7] Deposition Number **2058991** contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationzentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

Table S1. Experimental details

	Compound **5**
Crystal data	
Chemical formula	$C_{16}H_{12}N_2O_4$
M_r	296.28
Crystal system, space group	Orthorhombic, *Pna*2_1
Temperature (K)	150
a, *b*, *c* (Å)	7.752 (2), 29.630 (8), 5.6633 (15)
V (Å^3)	1300.8 (6)
Z	4
Radiation type	Mo *K*α
μ (mm^{-1})	0.11
Crystal size (mm)	0.09 × 0.07 × 0.02
Data collection	
Diffractometer	Bruker D8Quest
Absorption correction	Multi-scan *SADABS2016*/2[4] was used for absorption correction. wR2(int) was 0.1402 before and 0.0911 after correction. The Ratio of minimum to maximum transmission is 0.7027. The λ/2 correction factor is Not present.
T_{min}, T_{max}	0.524, 0.745
No. of measured, independent and	25880, 2663, 1534

observed [$I > 2\sigma(I)$] reflections	
R_{int}	0.218
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.627
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.088, 0.212, 1.05
No. of reflections	2663
No. of parameters	201
No. of restraints	1
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.31, −0.34
Absolute structure	Flack x determined using 440 quotients [(I+)-(I-)]/[(I+)+(I-)] [8]
Absolute structure parameter	−0.7 (10)

Computer programs: SAINT V8.40A[4], SHELXT 2014/5[5], SHELXL[6], Olex2.[7]

Discussion of Crystal Packing

The planarity of this molecule **5** (Figure S2) and 7777π–π stacking interactions with adjacent molecules (Figure S3) are of significance. Given **5**'s potential applications in DNA intercalating agents, the abilities to adopt a planar shape and form π–π interactions with DNA-bases are crucial. Of note is the offset nature of the stacked molecules (Figure S3) which nonetheless pack closely with interplanar distance ~3.5 Å.

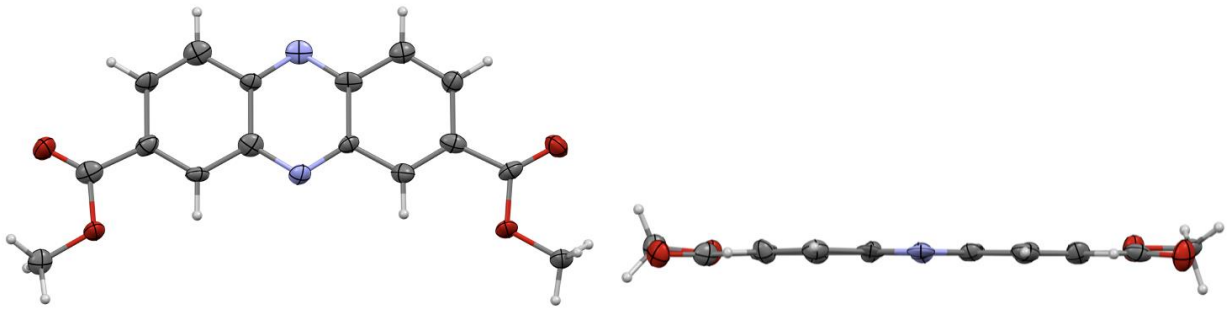


Figure S2. Single crystal structure of **5**, probability level 50%. Two orientations of a single molecule.

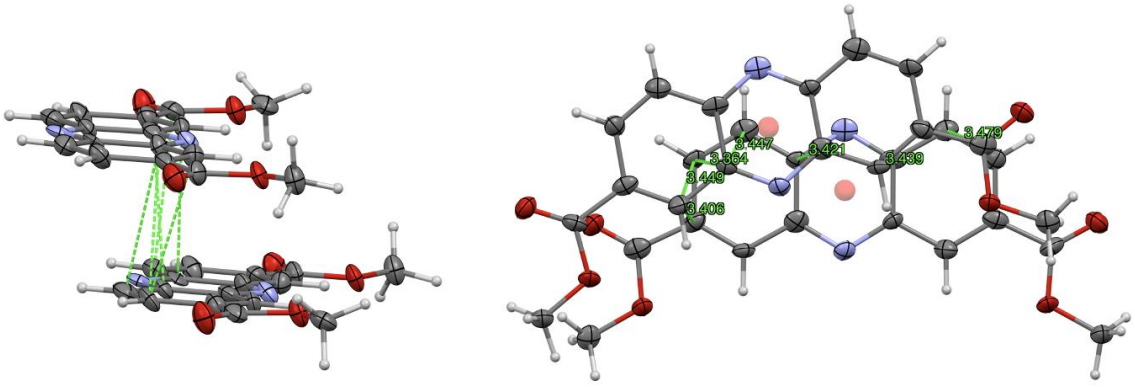


Figure S3. π–π stacking interactions between two molecules within the crystal. The centroids of the phenazine rings are visualised in red to show the offset nature of this stacking. Distances are shown in Å.

The unit cell comprises four symmetrically equivalent molecules, and has been divided into two sections, **I** and **II**, for this discussion (Figure S4). CH---O and CH---N interactions parallel to the plane of the phenazine rings hold molecules in sheet-like formations within each section (Figure S4, S5); sections **I** and **II** are linked by C–H---O contacts (C14-H14---O3, Figure S4). These sheets stack through the aforementioned π–π interactions perpendicular to the plane of the phenazine rings (Figure S6). Sections **I** and **II** both exhibit these interactions, however the orientation in space of the two stacked formations differ to result in an overall criss-cross or herringbone pattern (Figure S7).

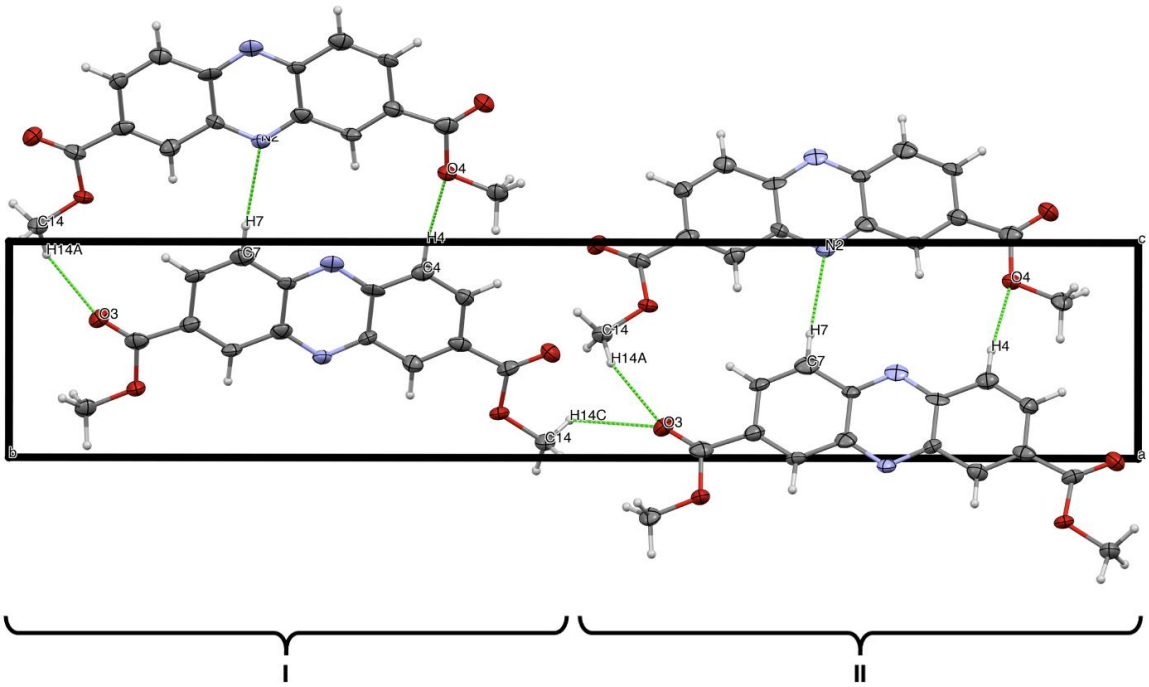


Figure S4. Four molecules of the unit cell, and Sections **I** and **II** are designated. CH---O and CH---N interactions are shown in green.

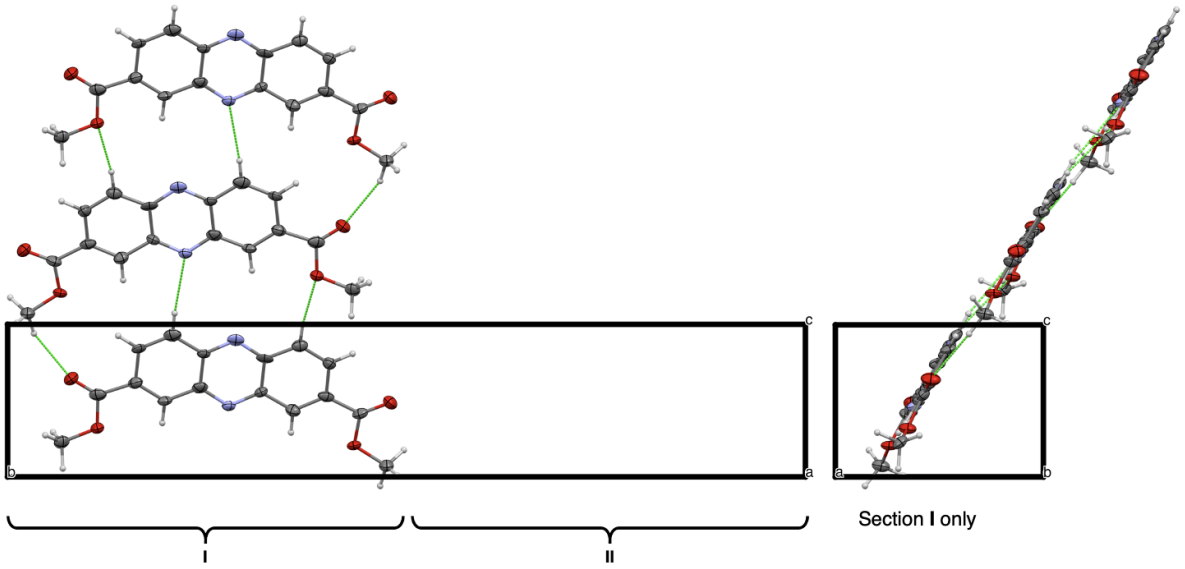


Figure S5. CH---O and CH---N interactions (within Section **I** of the unit cell only) are parallel to the plane of the phenazine rings, leading to planar sheet structures.

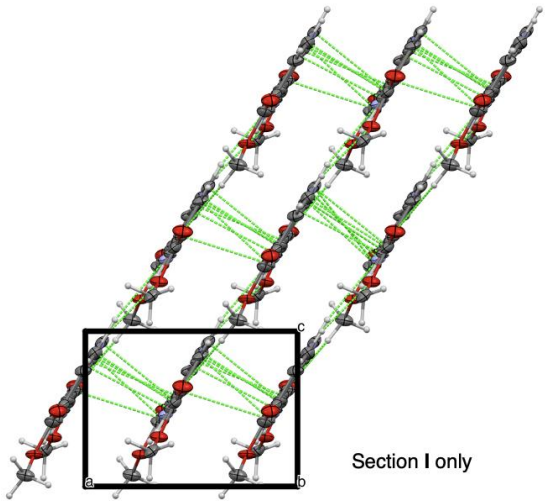


Figure S6. π–π stacking interactions (within Section **I** of the unit cell only) which are perpendicular to the plane of the phenazine rings, leading to stacks of sheets.

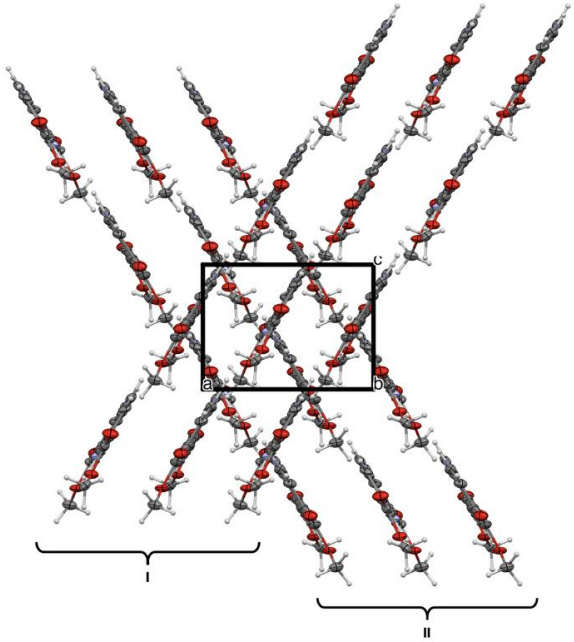
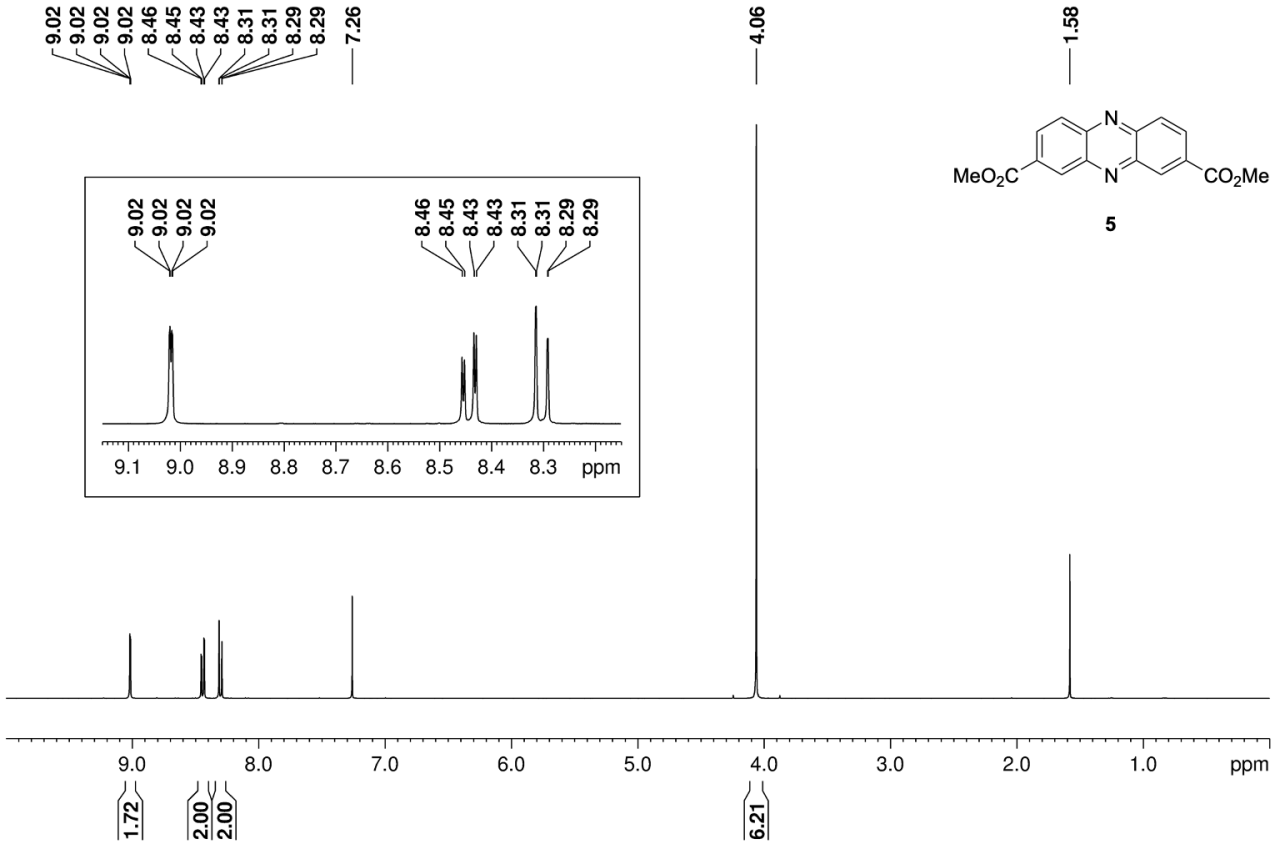


Figure S7. Herringbone-like pattern exhibited by the crystal packing.

SM-4. Relevant Spectra

The following pages contain ^{1}H and ^{13}C NMR spectra are provided for isolated compounds, and IR and HRMS spectra for key compounds **5** and **6**.

^{1}H NMR (400 MHz, $CDCl_3$) of 5



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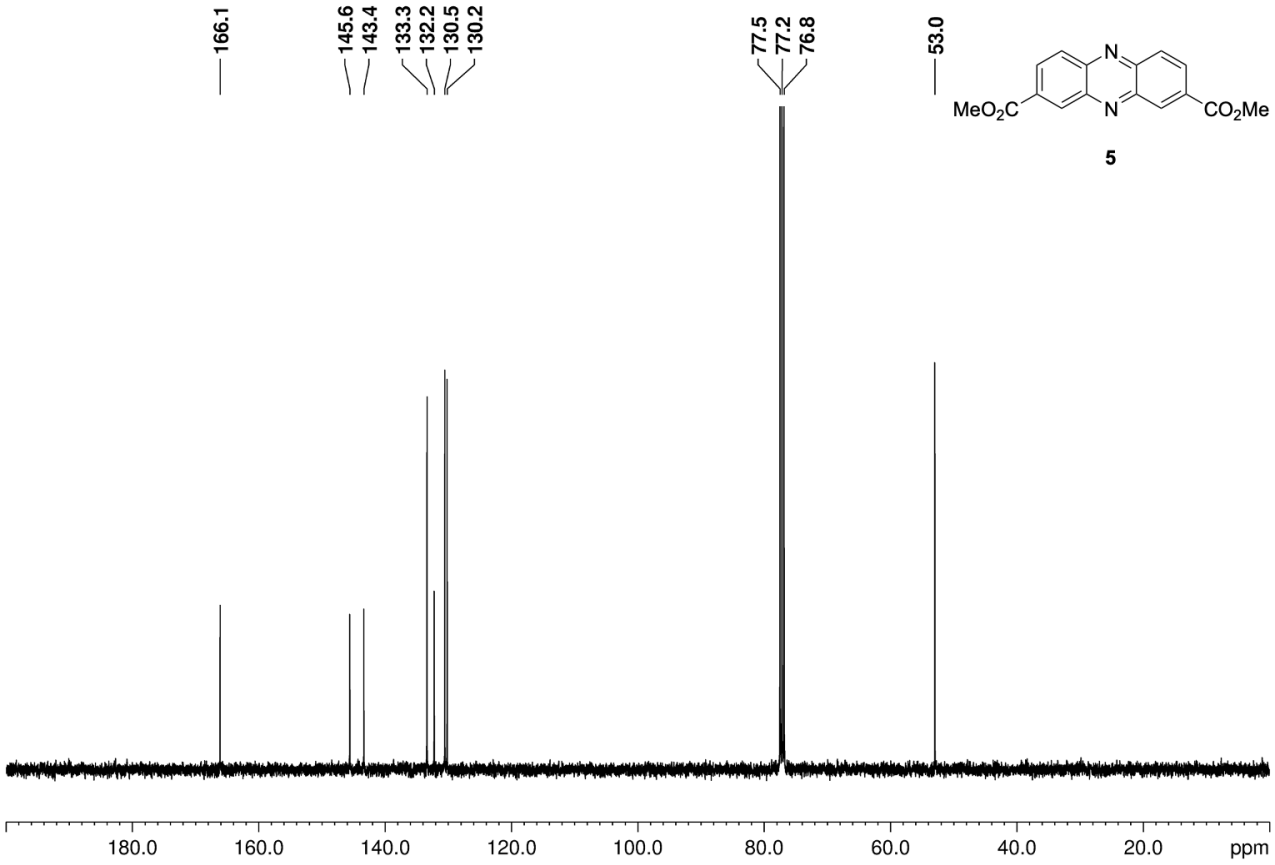
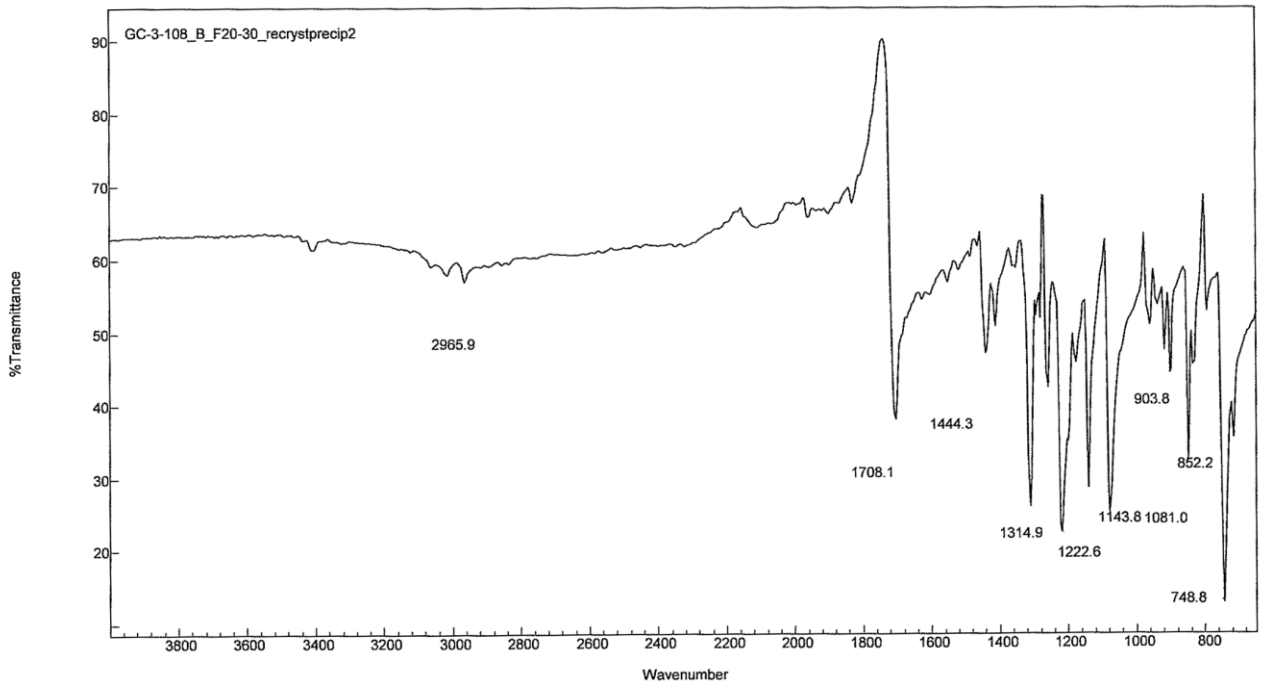
^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) of 5

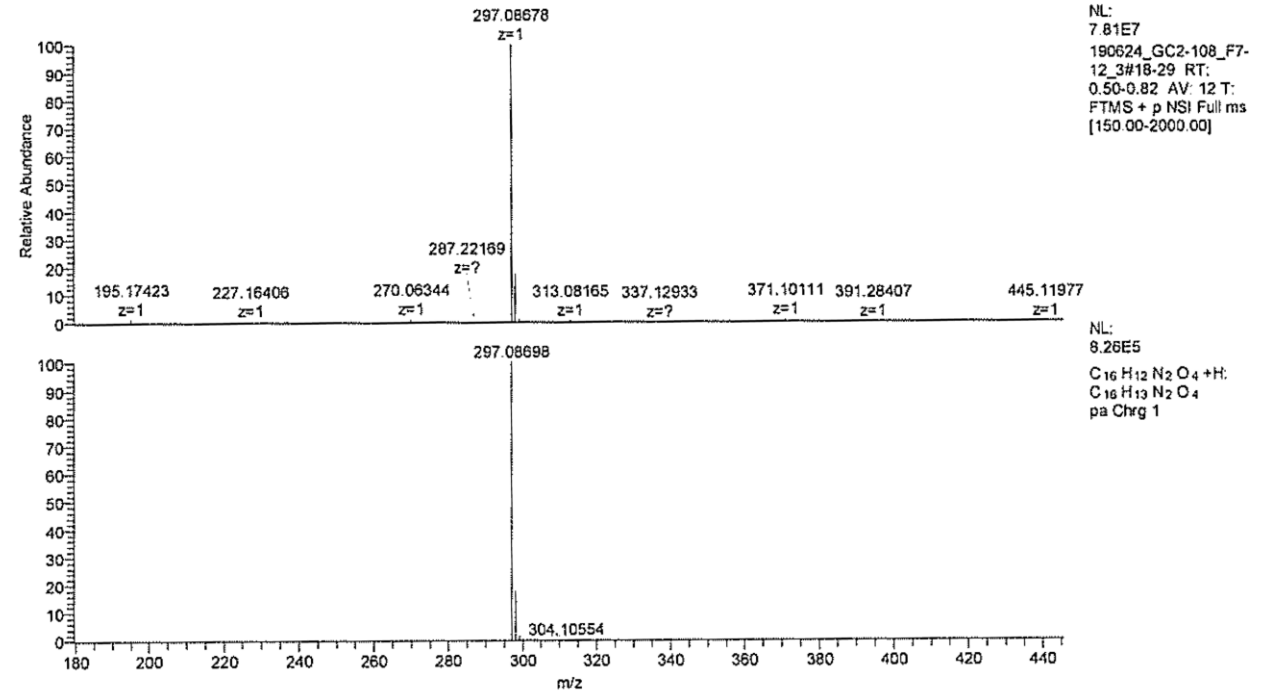


5

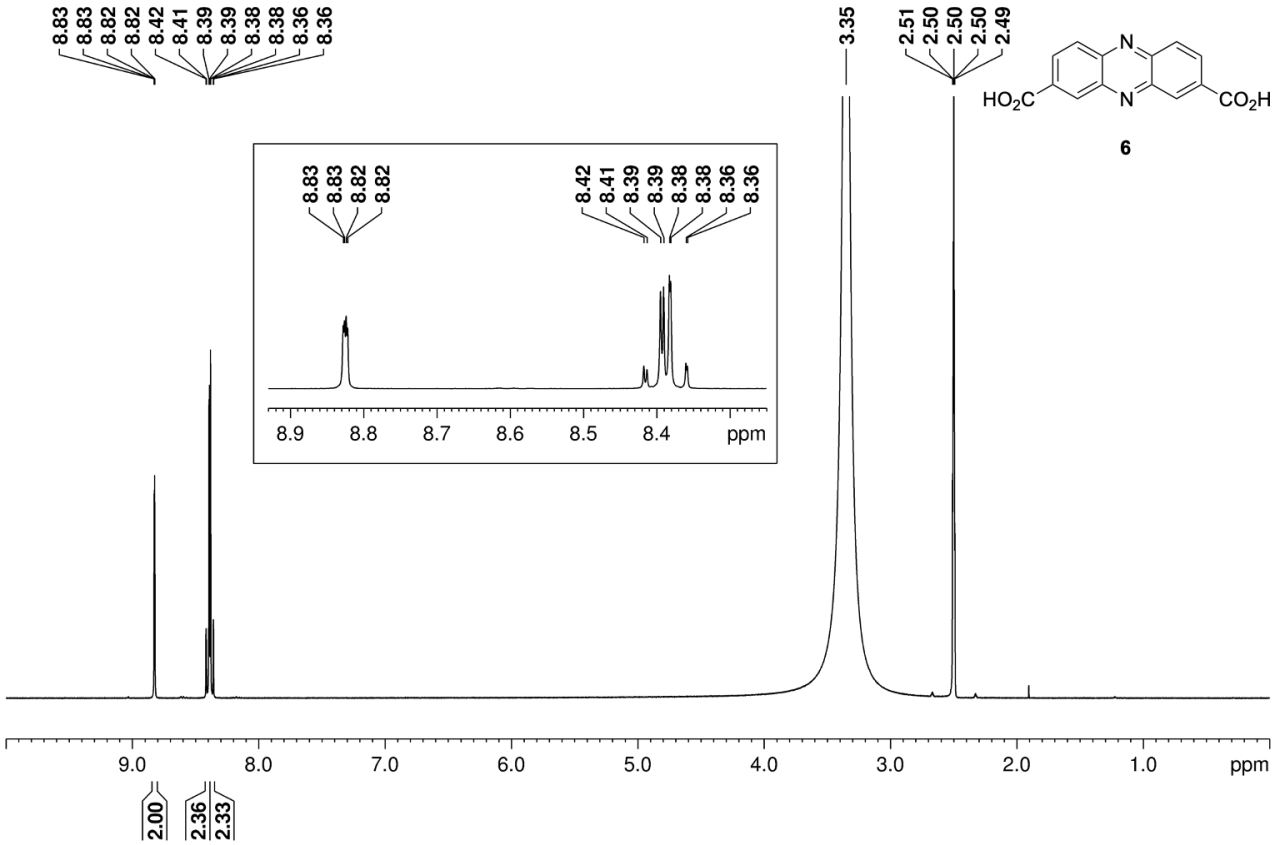
IR (neat) of 5



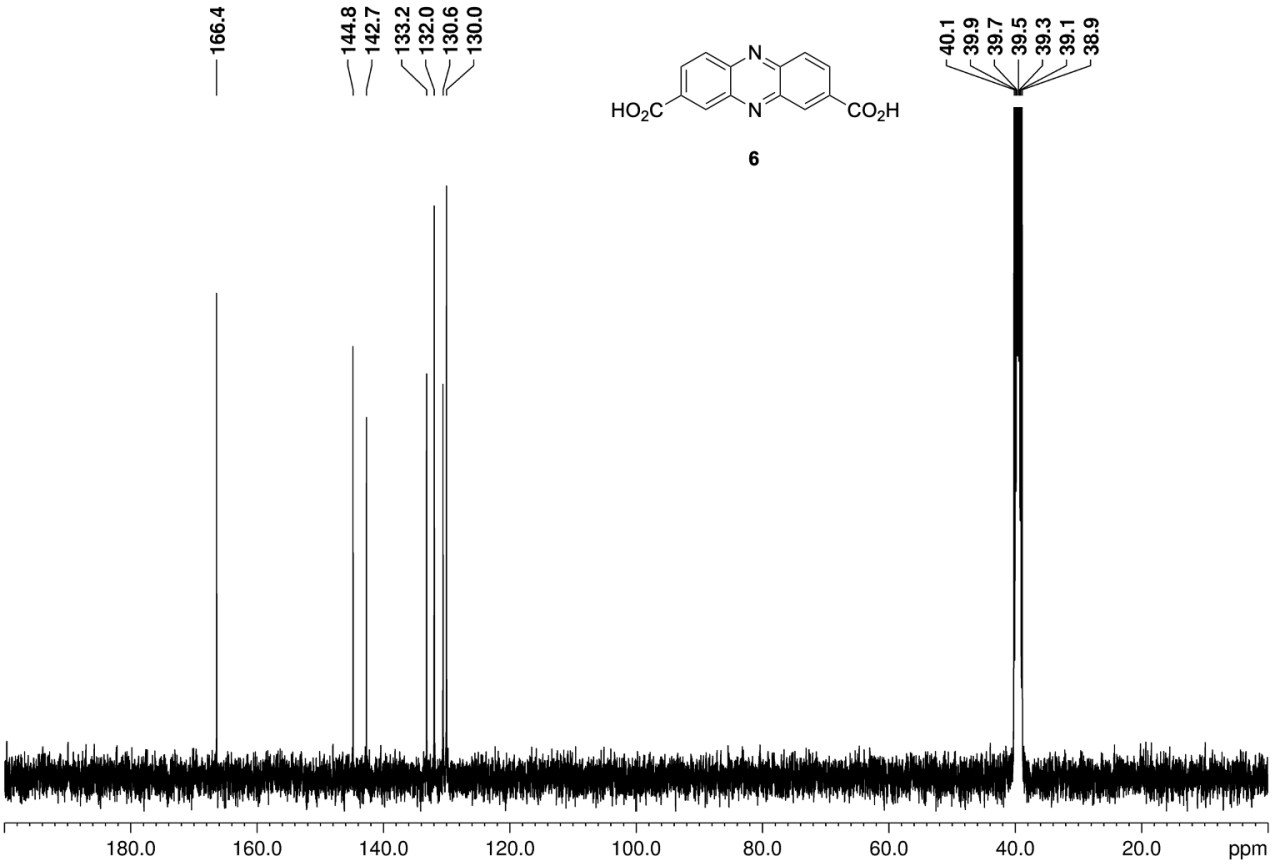
HRMS (ESI, +ve) of 5



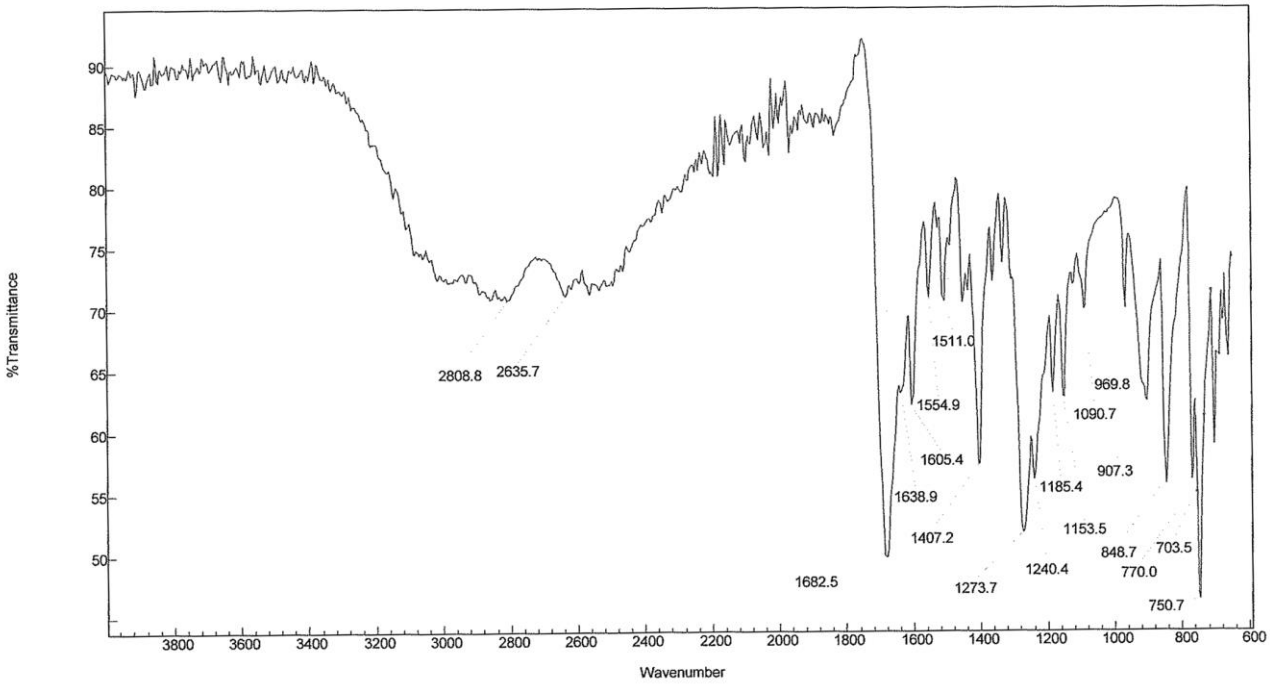
^{1}H NMR (400 MHz, DMSO-d6) of 6



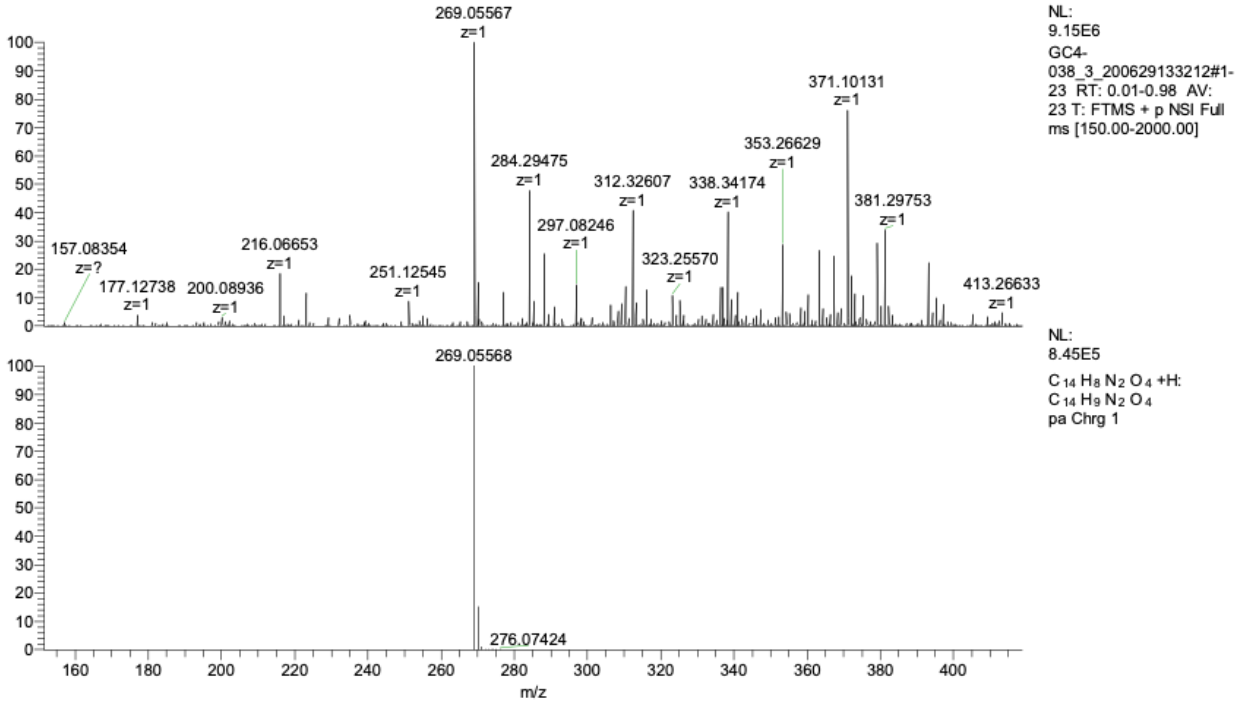
^{13}C{^{1}H} NMR (101 MHz, DMSO-d6) of 6



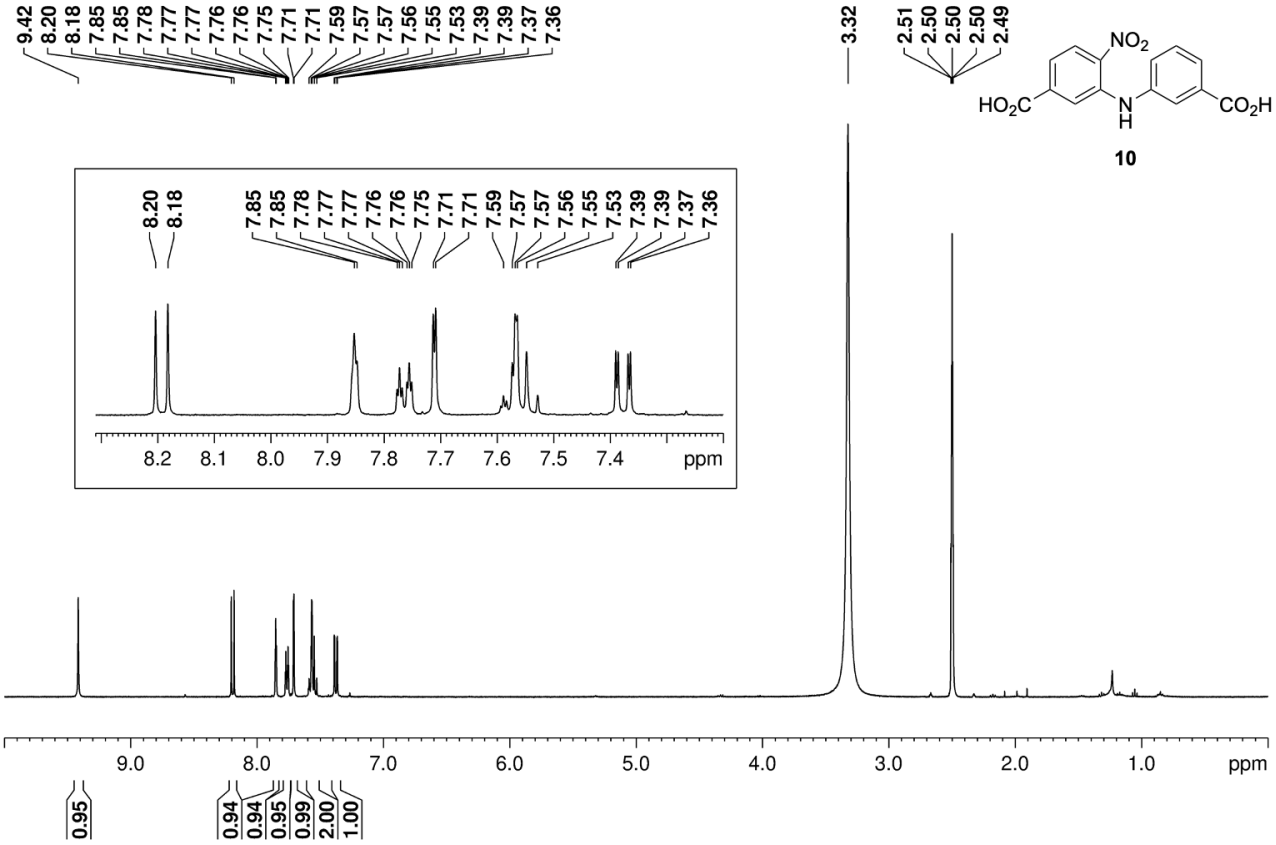
IR (neat) of 6



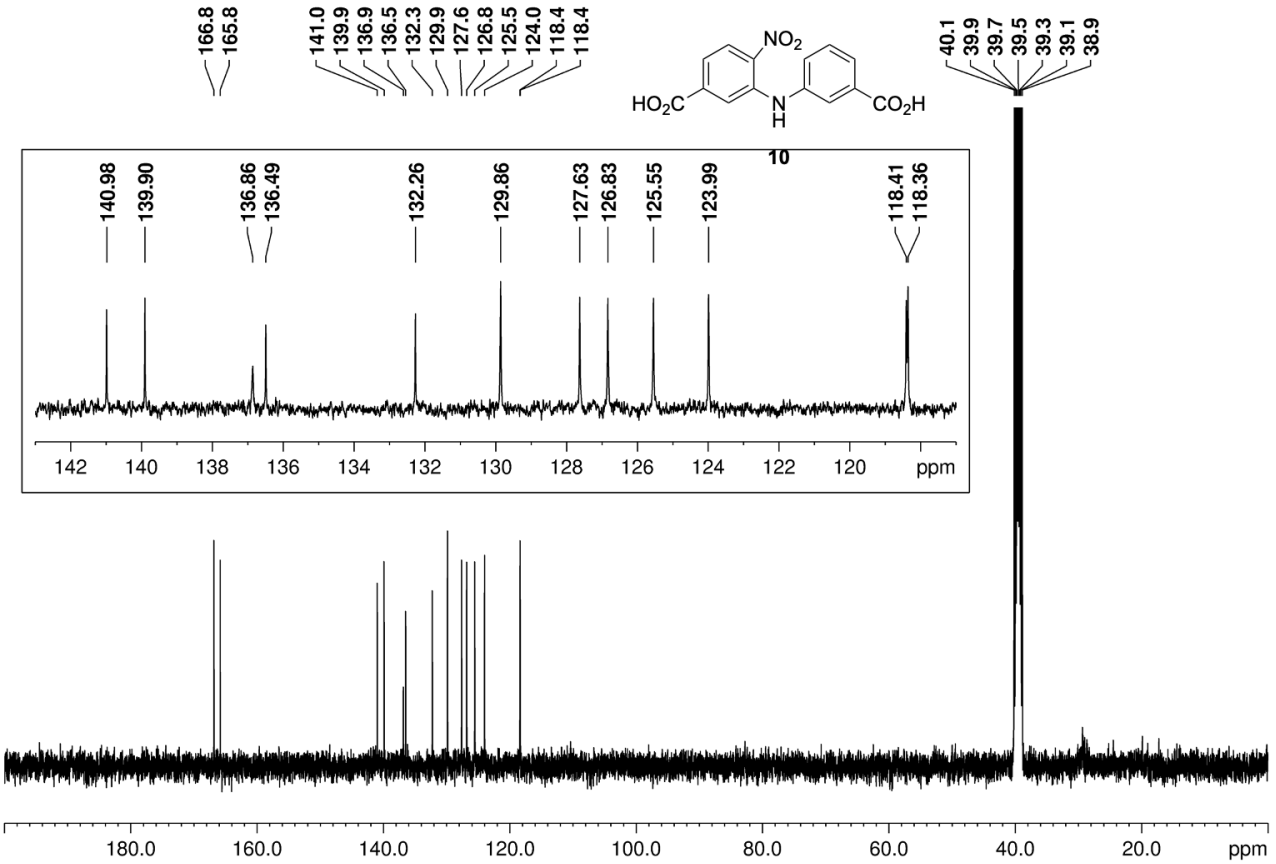
HRMS (ESI, +ve) of 6



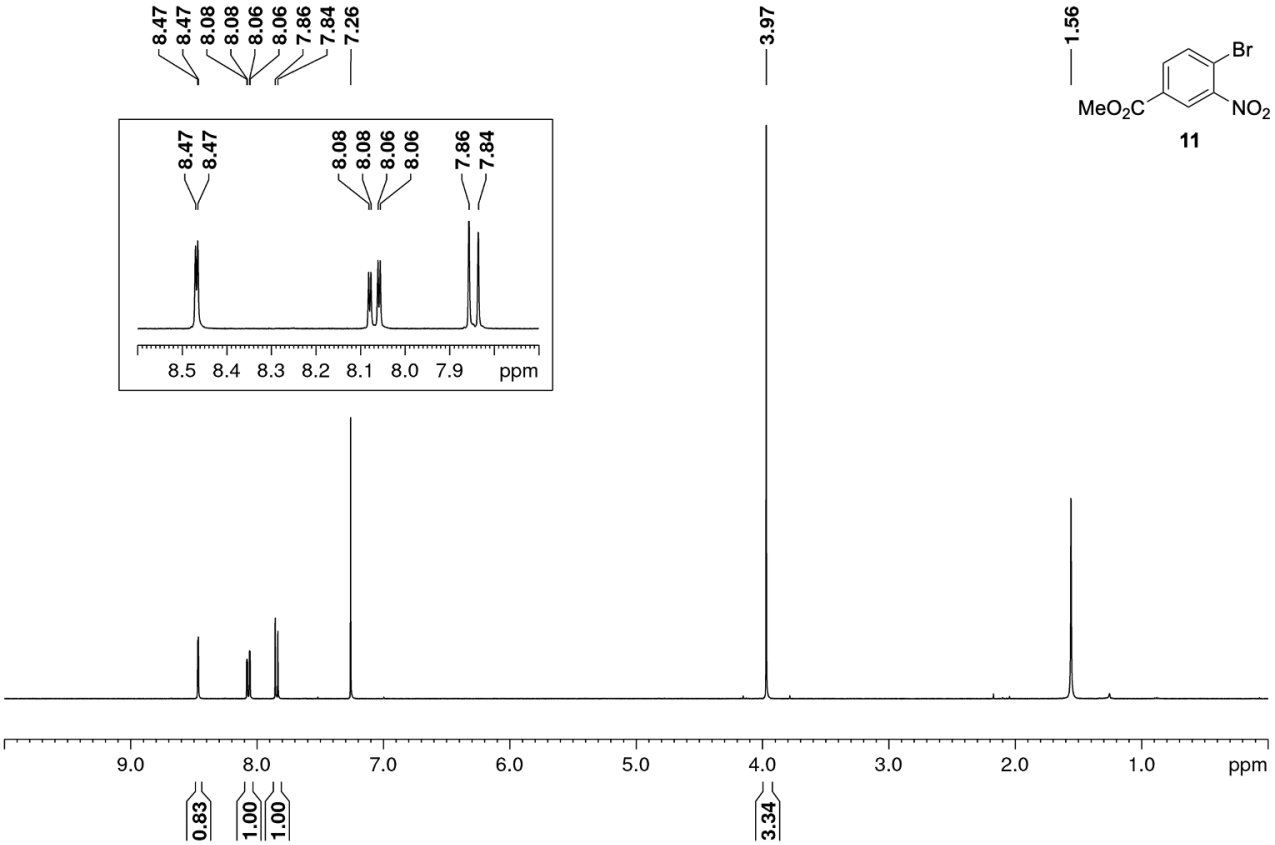
^{1}H NMR (400 MHz, DMSO-d6) of 10



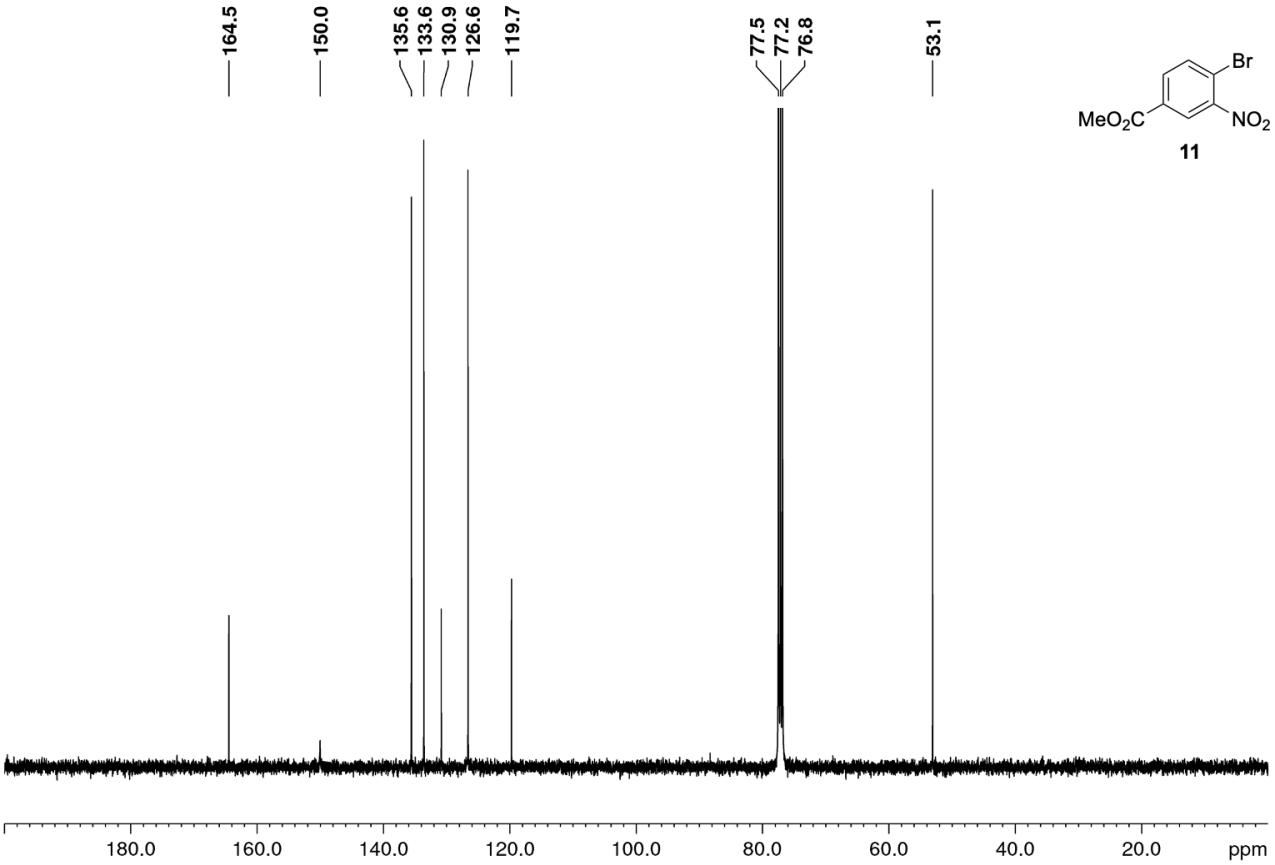
^{13}C{^{1}H} NMR (100 MHz, DMOS-d6) of 10



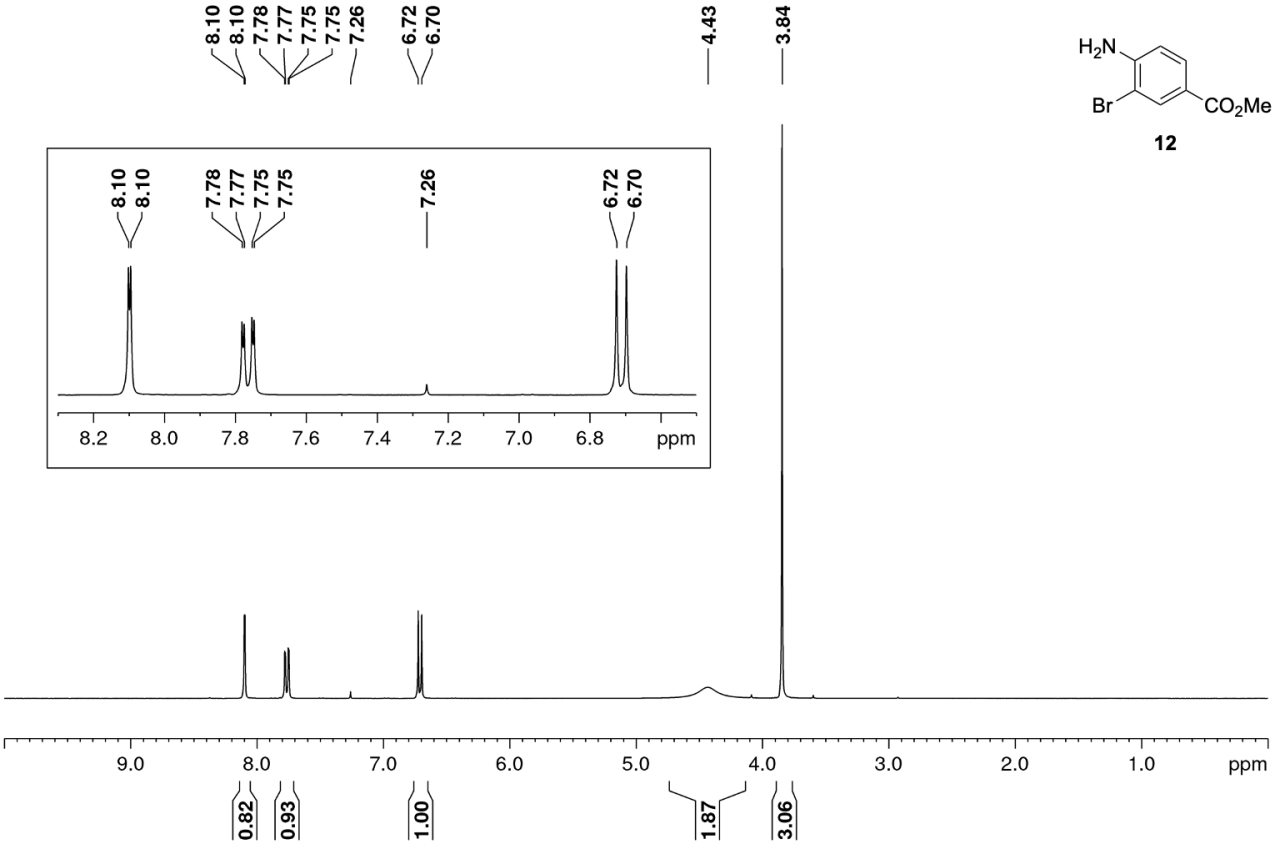
^{1}H NMR (400 MHz, $CDCl_3$) of 11



^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) of 11



^{1}H NMR (300 MHz, $CDCl_3$) of 12



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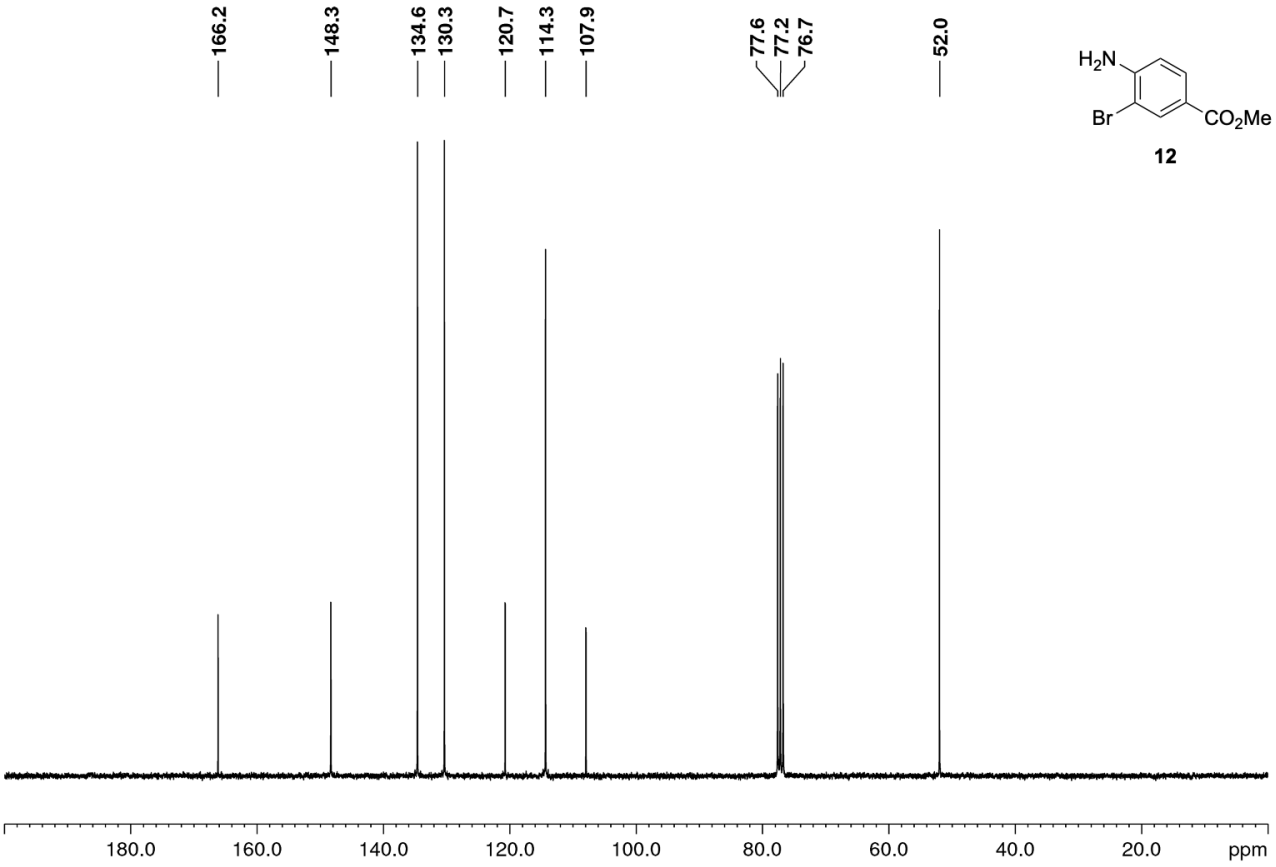
^{13}C{^{1}H} NMR (76 MHz, $CDCl_3$) of 12



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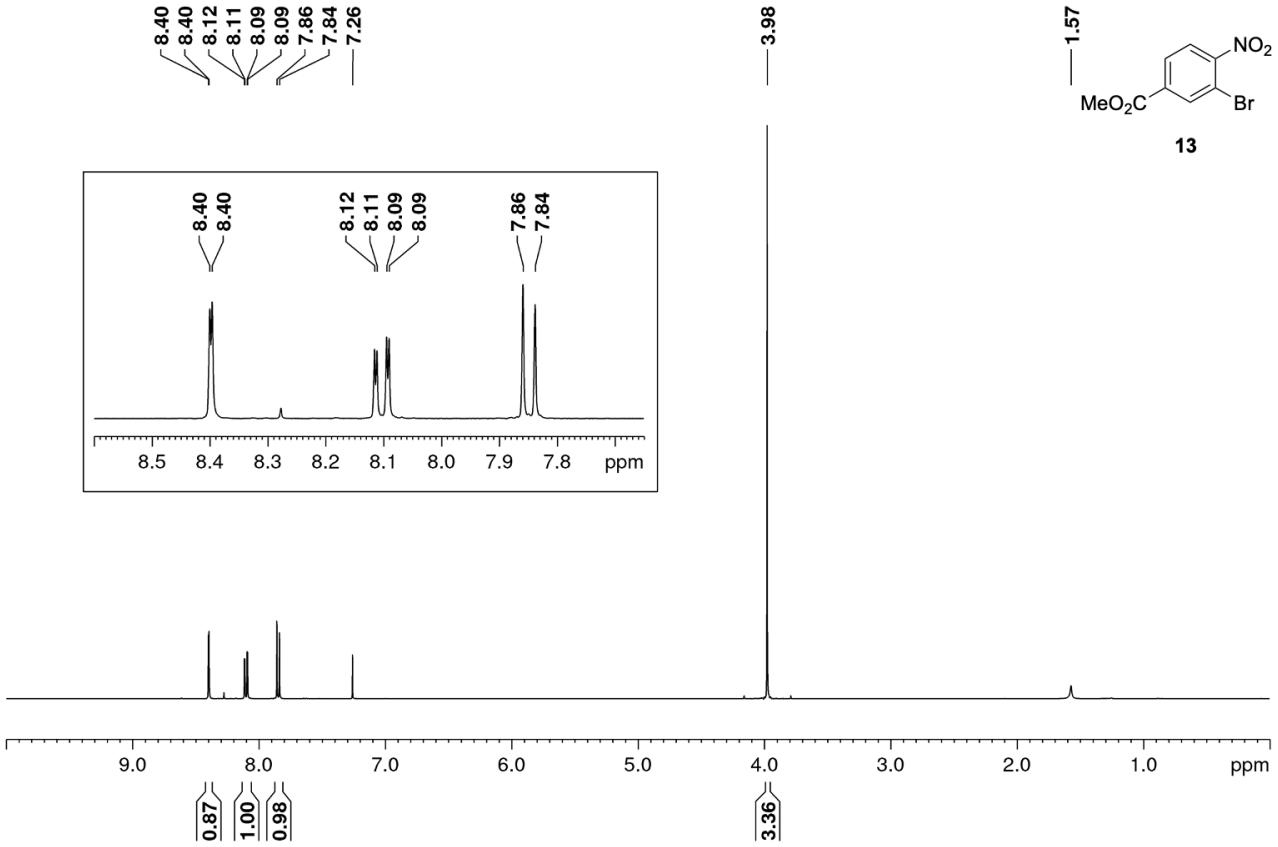
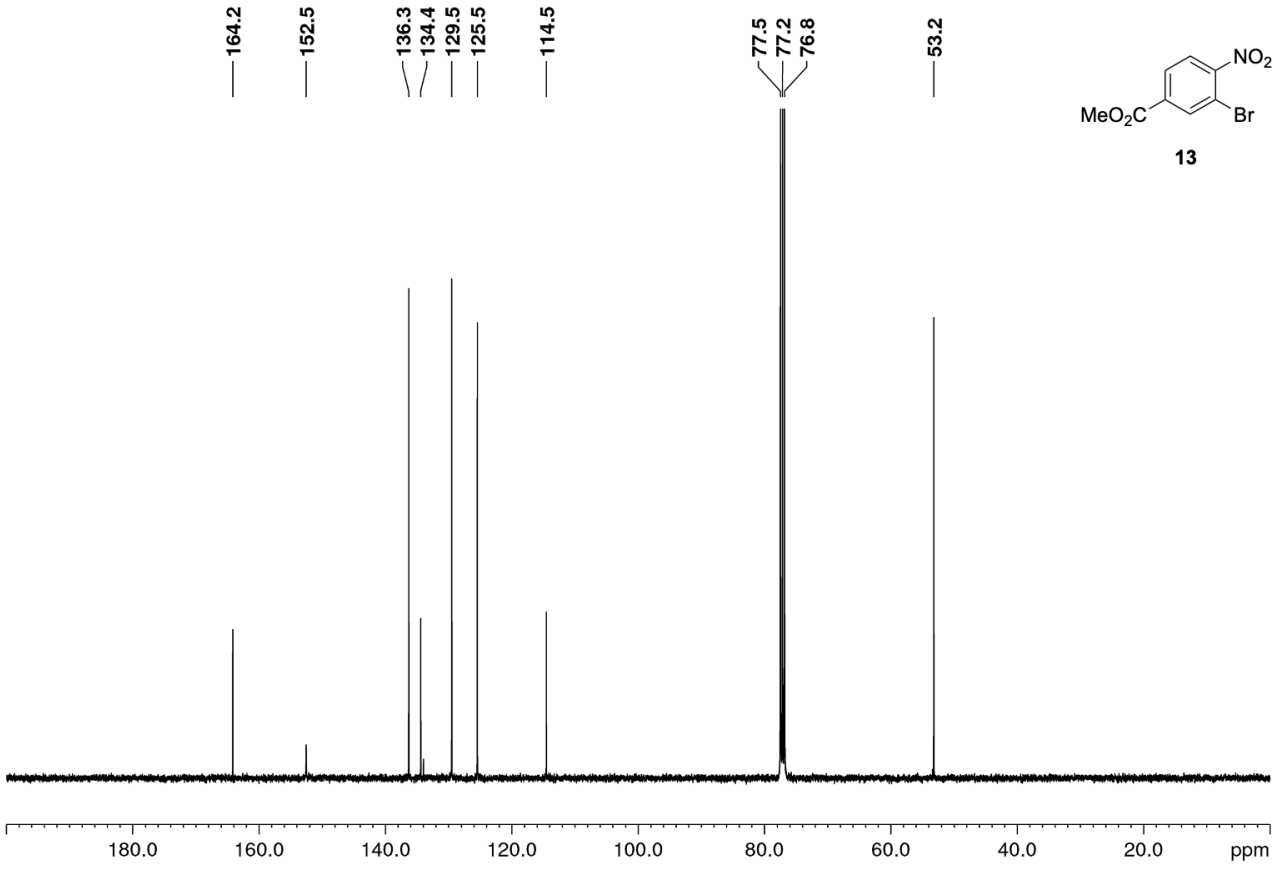
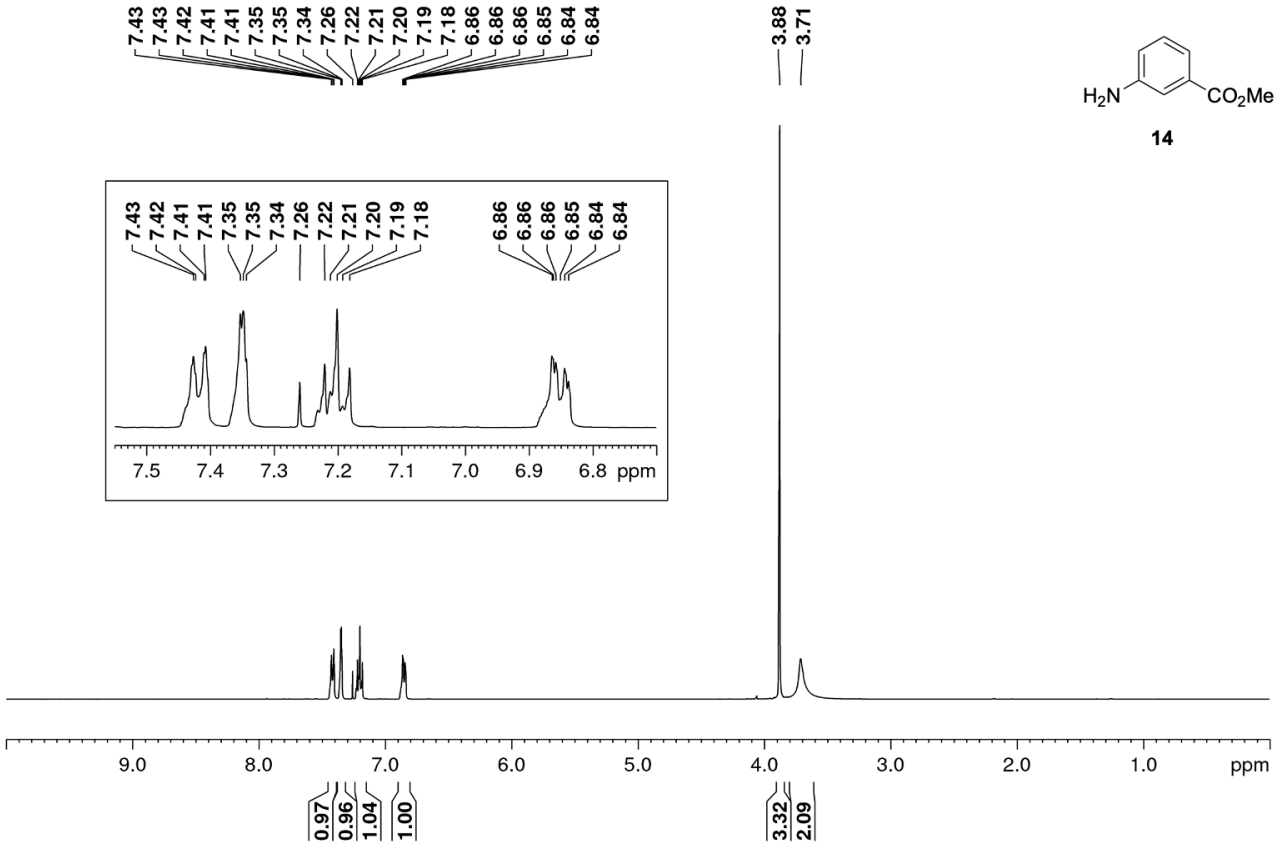
^{1}H NMR (400 MHz, $CDCl_3$) of 13



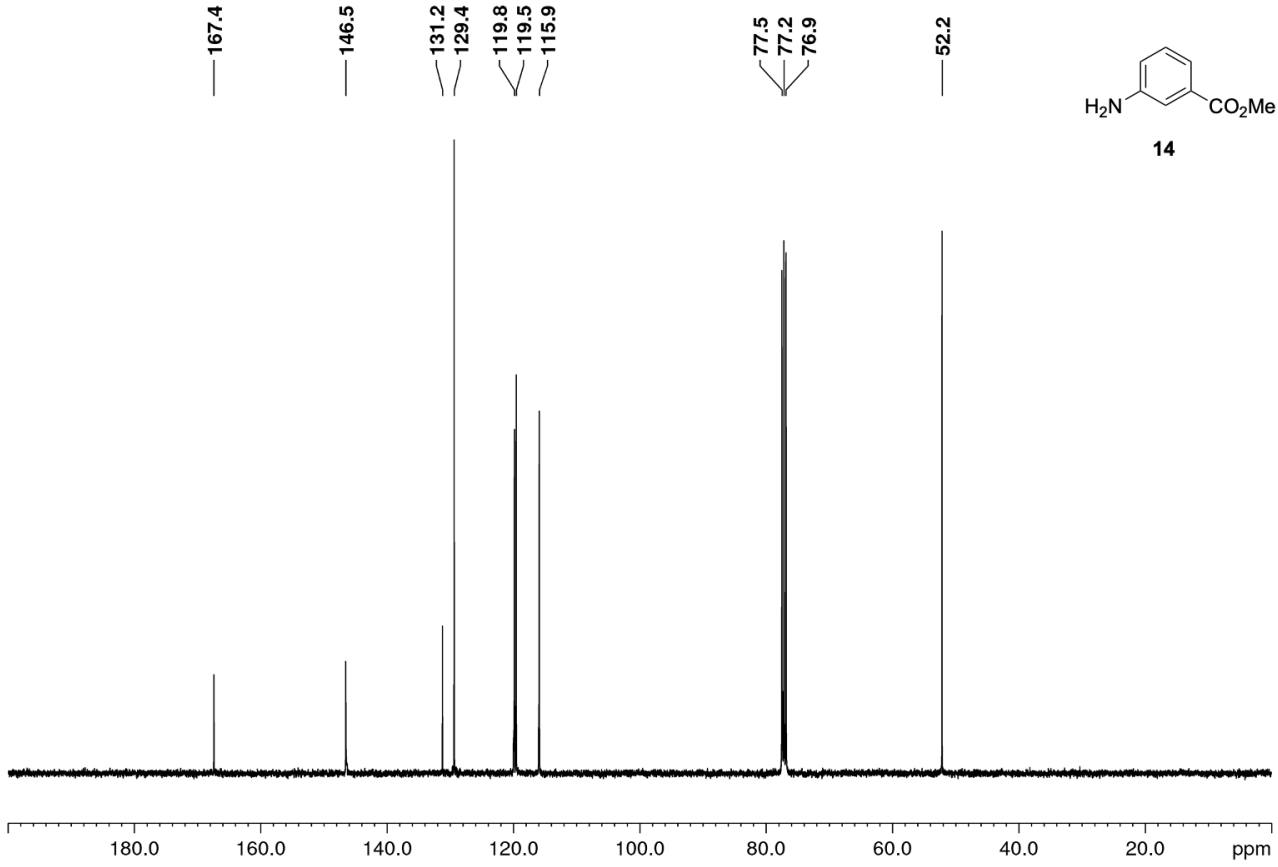
^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) of 13



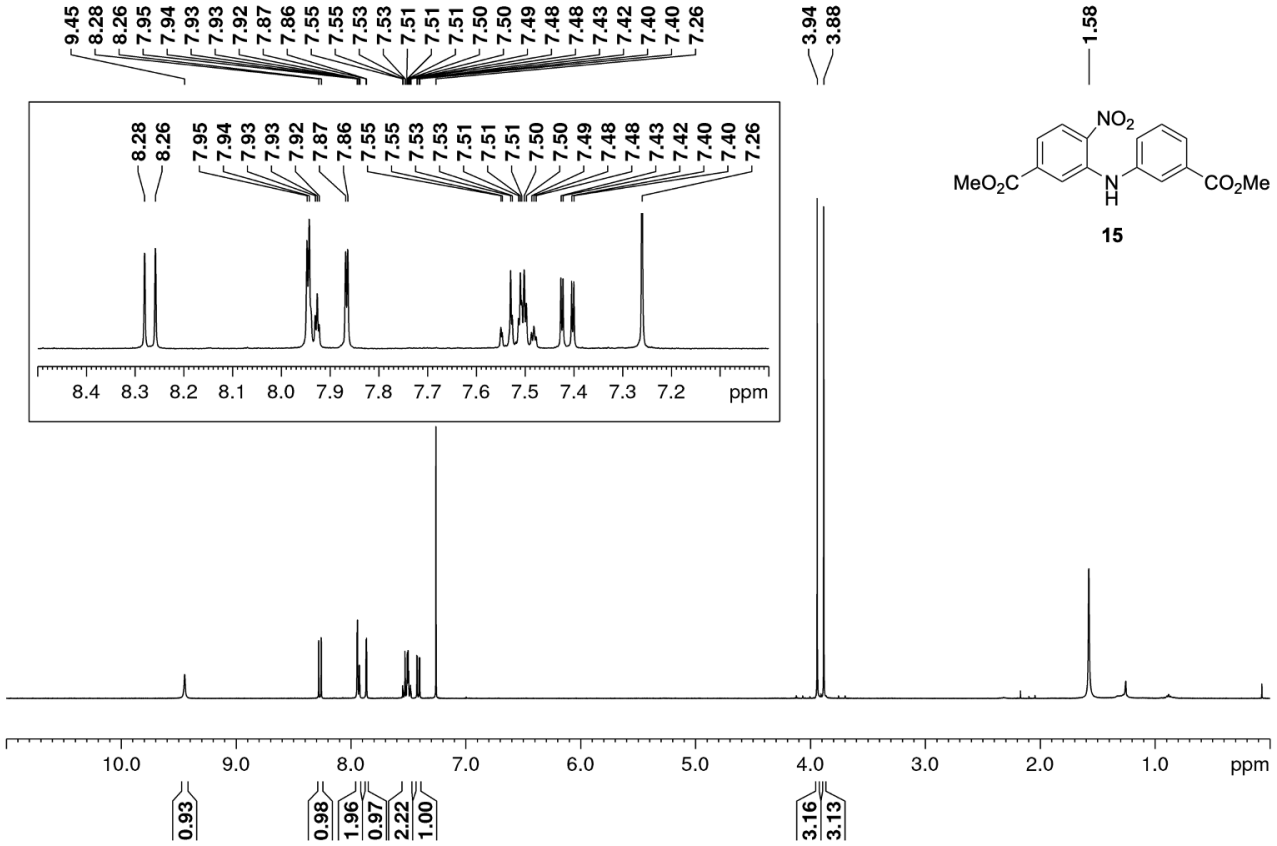
^{1}H NMR (400 MHz, $CDCl_3$) of 14



^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) of 14

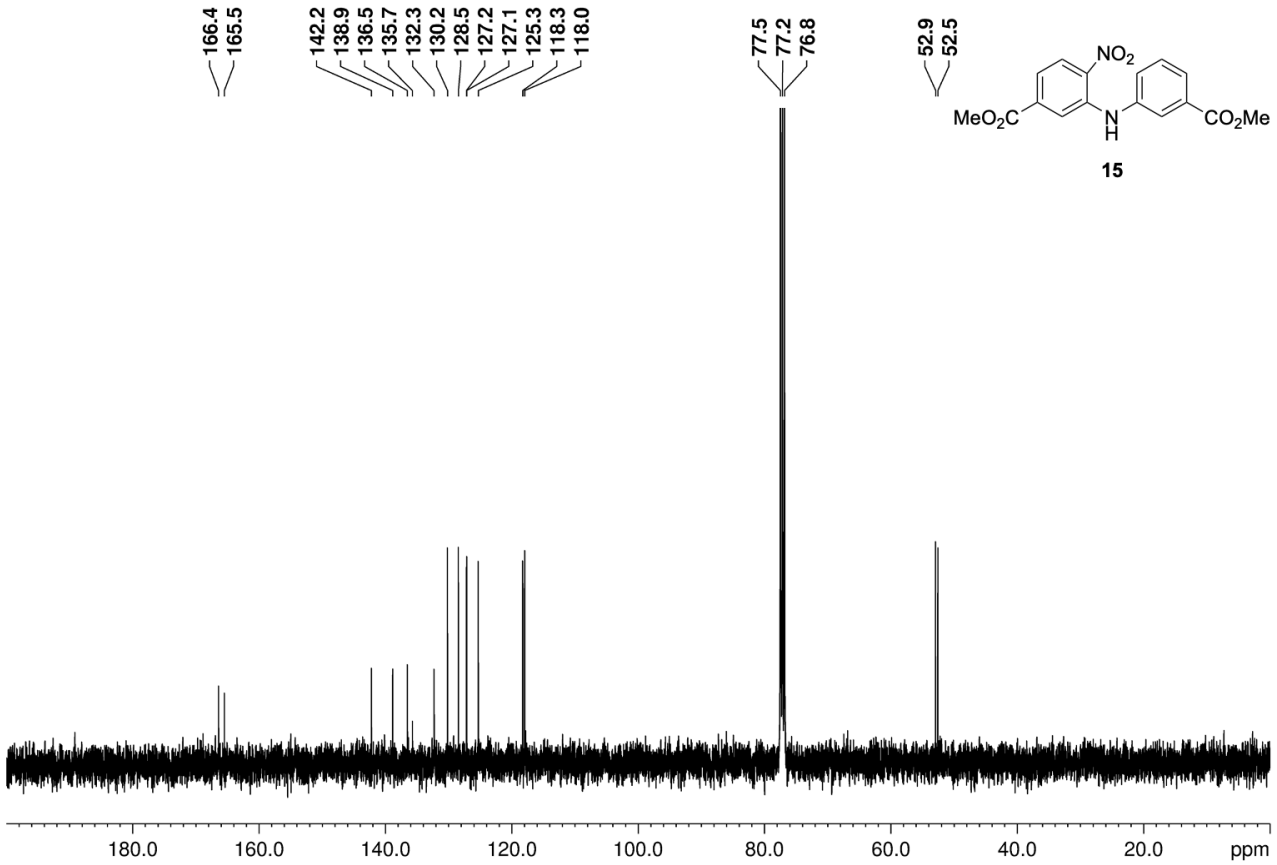


^{1}H NMR (400 MHz, $CDCl_3$) of 15



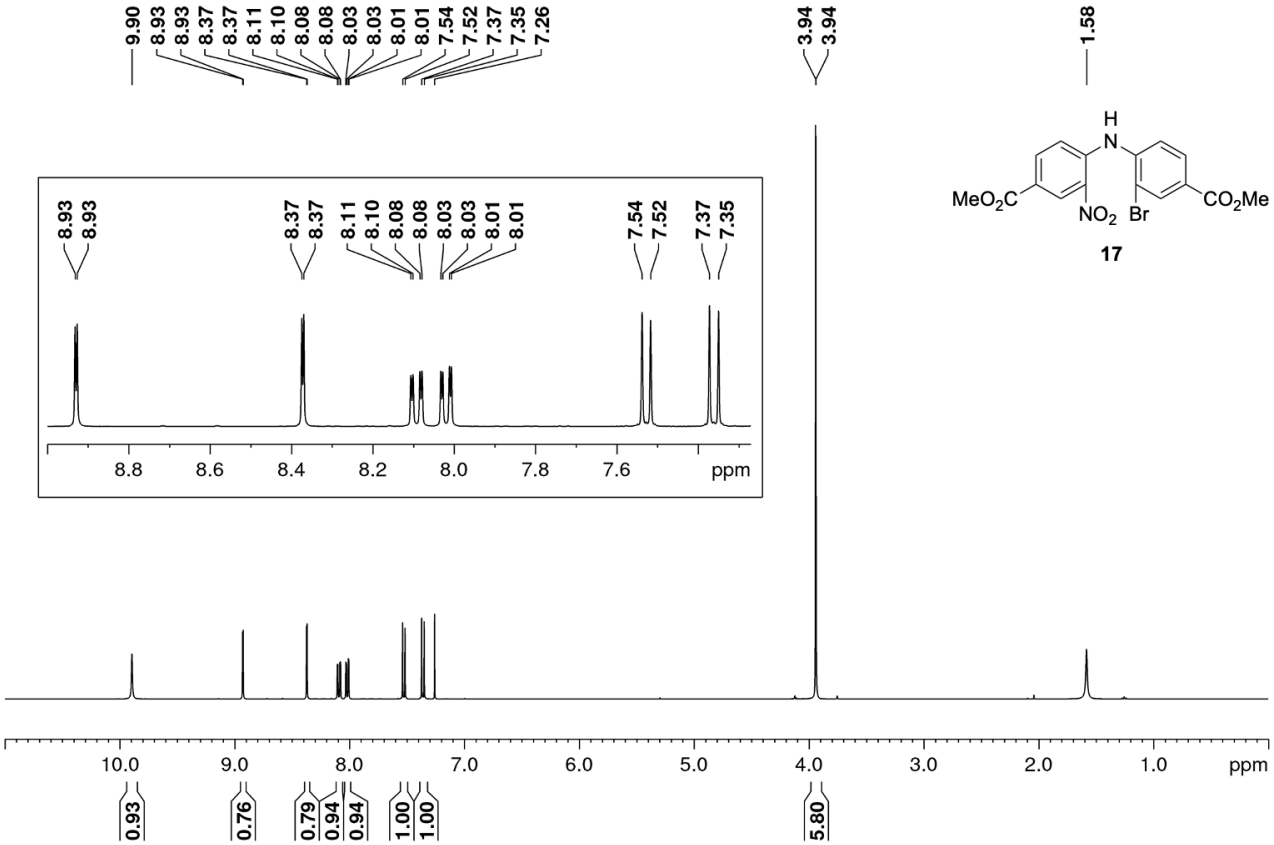
15

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) of 15

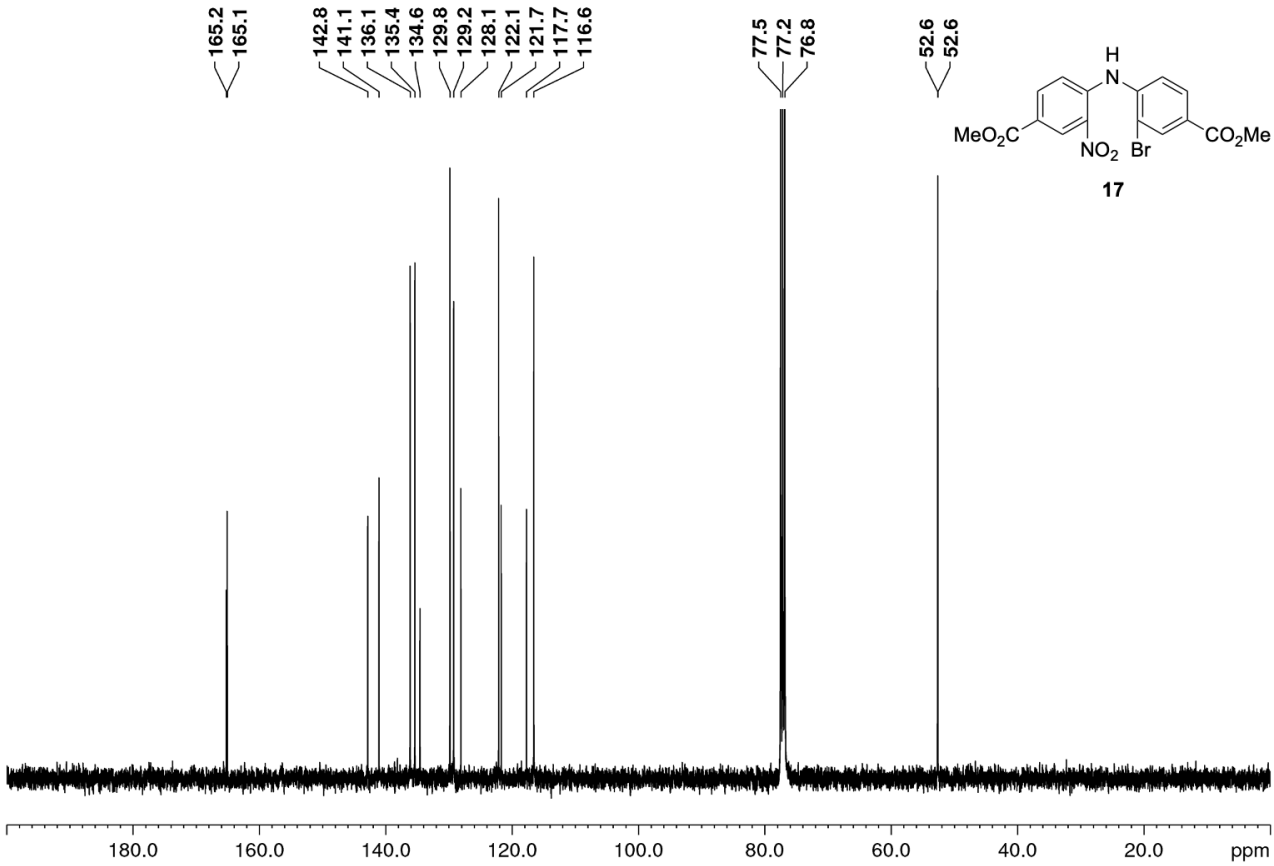


15

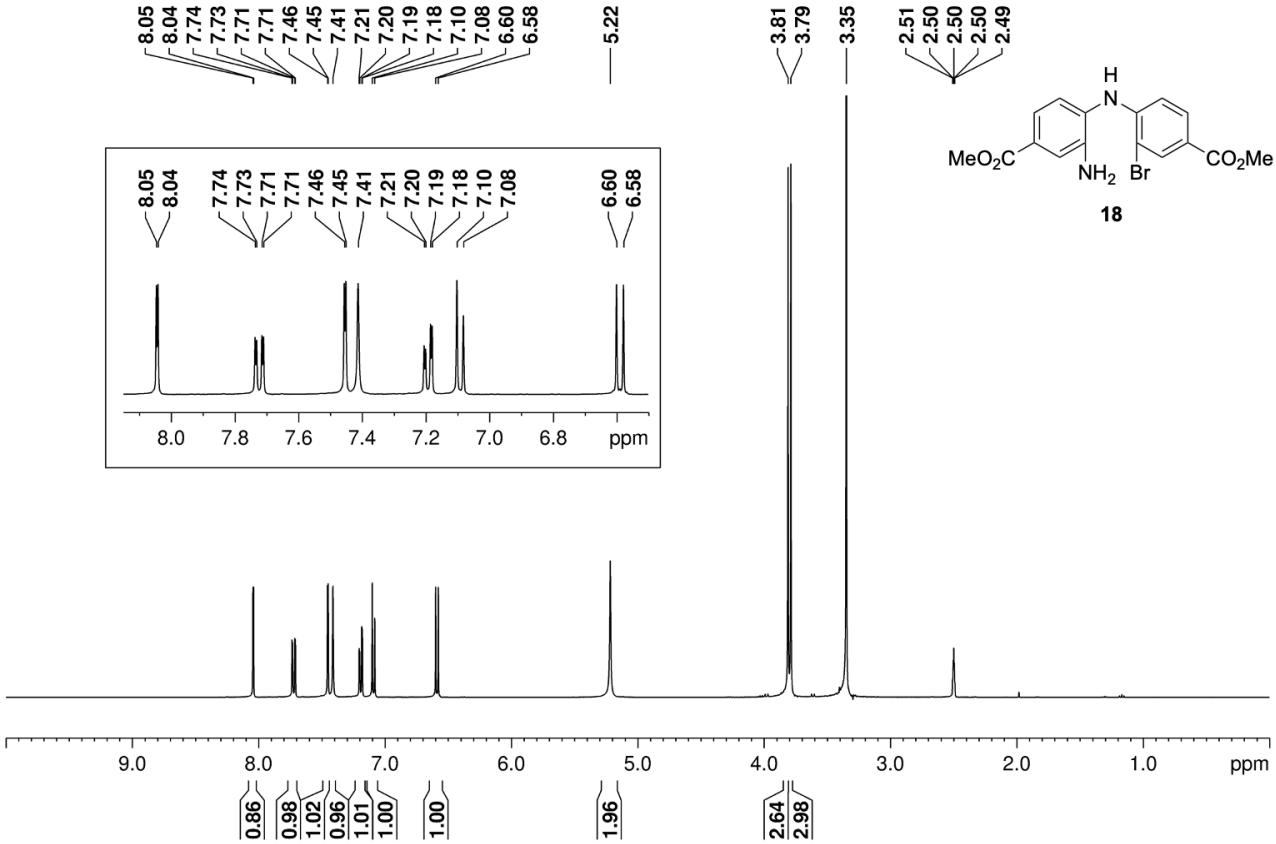
^{1}H NMR (400 MHz, $CDCl_3$) of 17



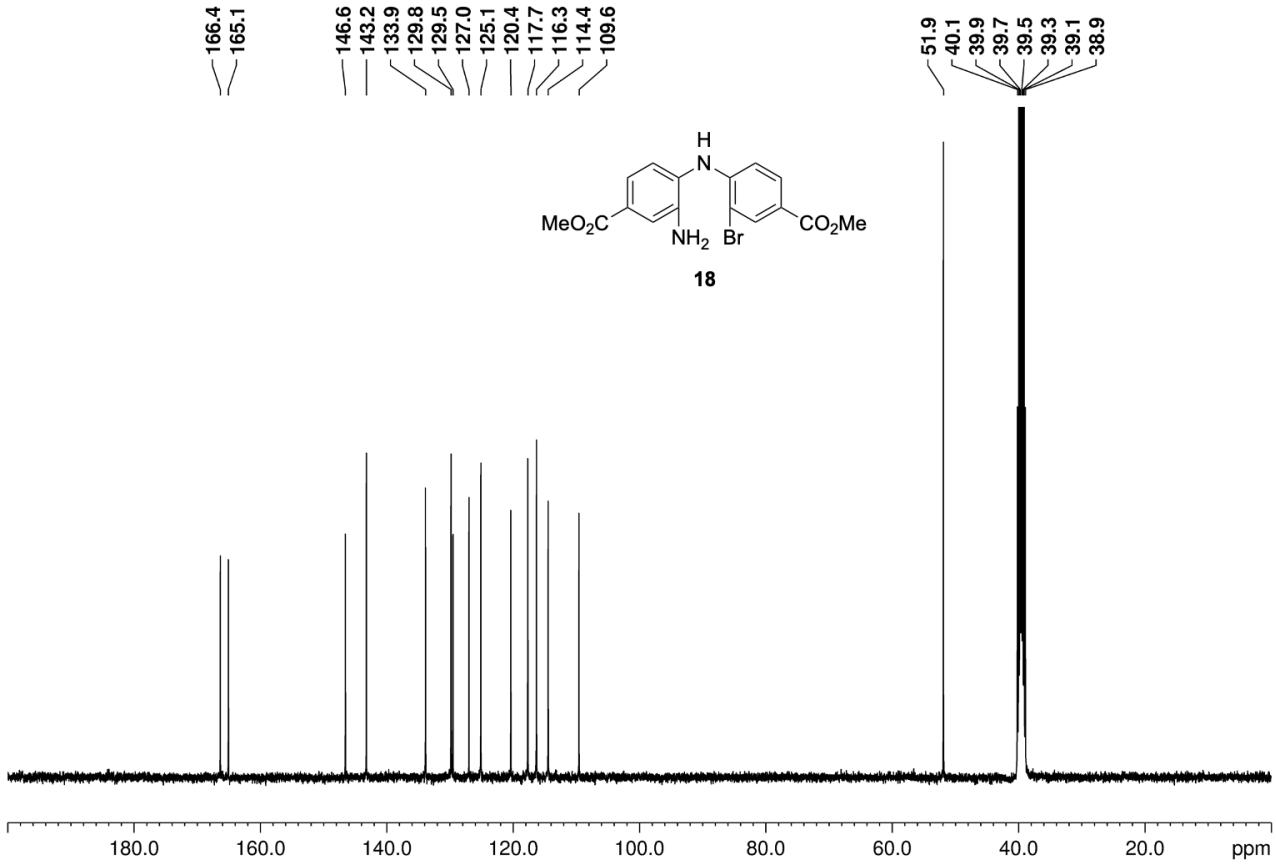
^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) of 17



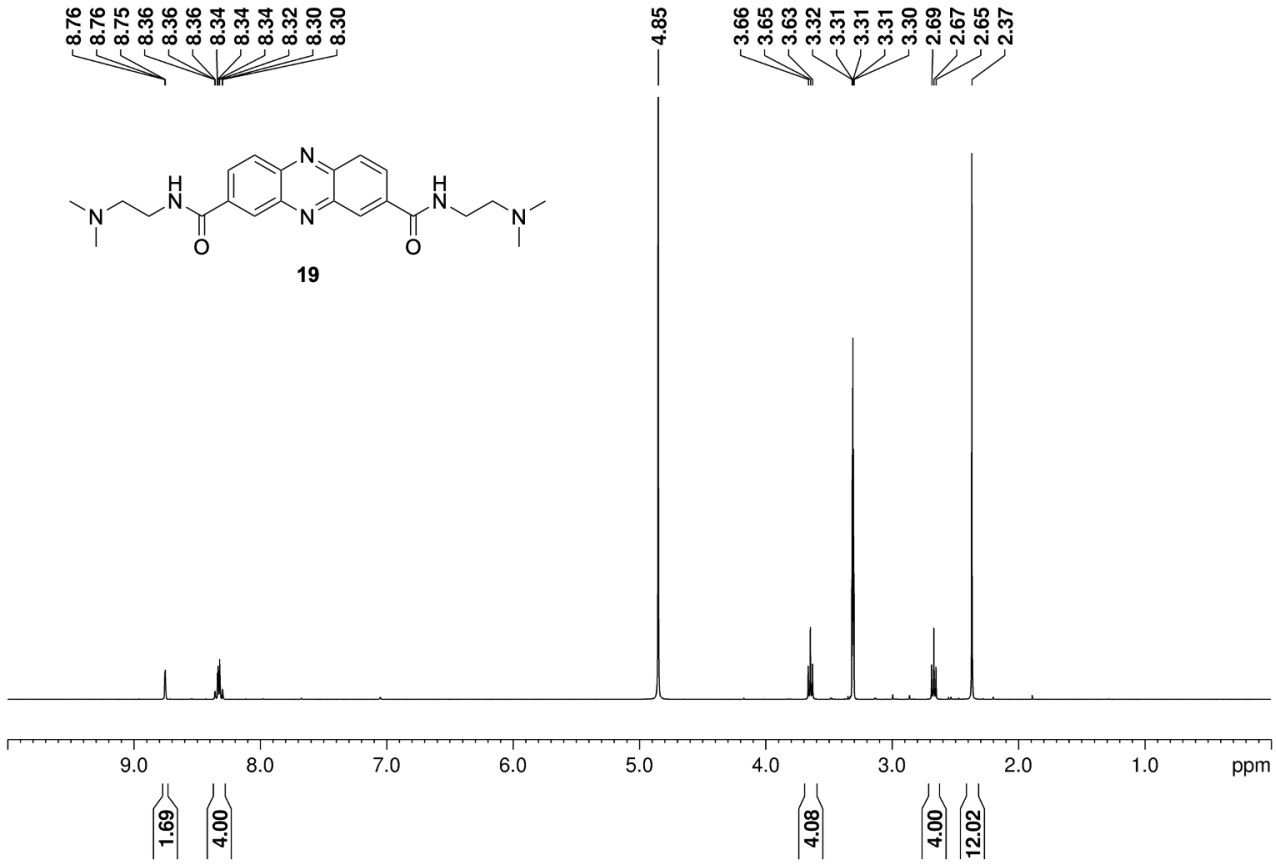
^{1}H NMR (400 MHz, DMSO-d6) of 18



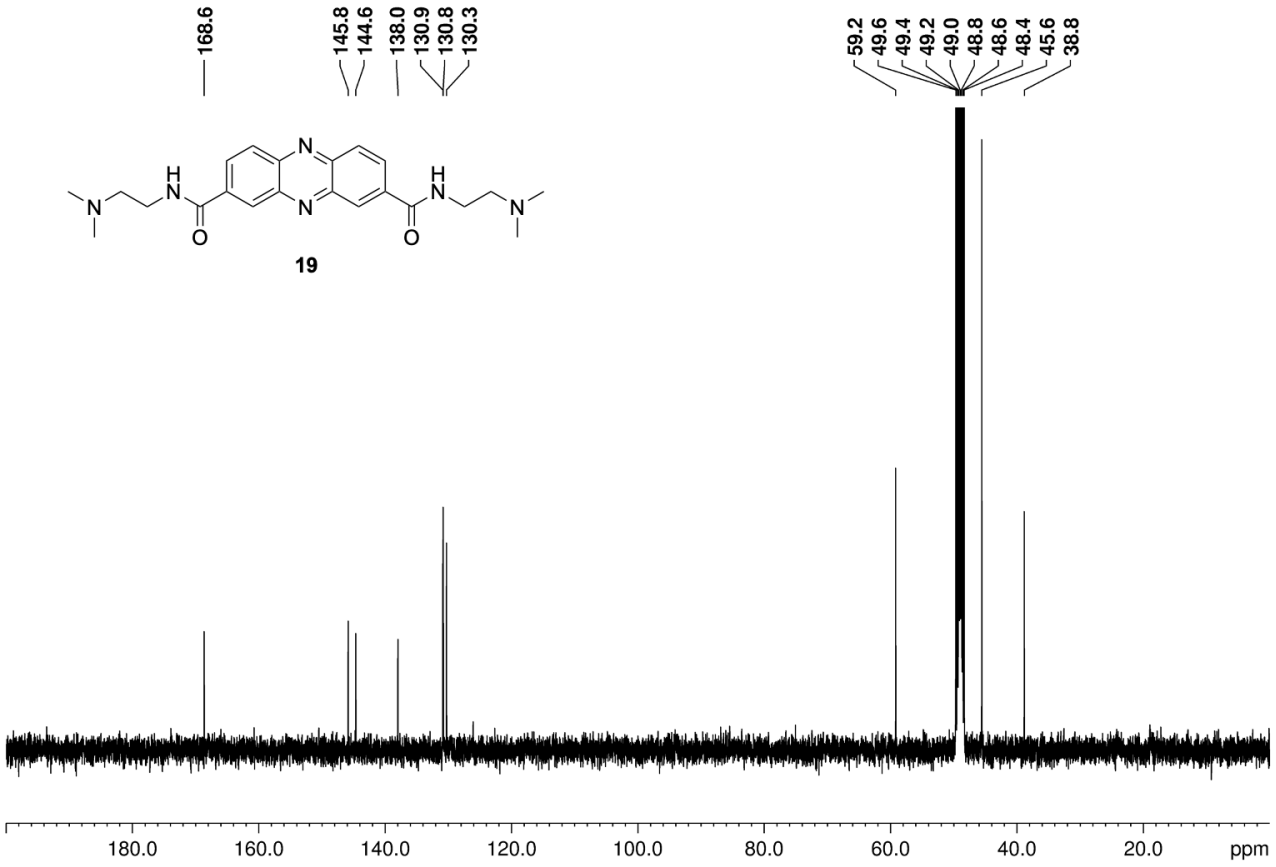
^{13}C{^{1}H} NMR (101 MHz, DMSO-d6) of 18



^{1}H NMR (400 MHz, CD_3OD) of 19



^{13}C{^{1}H} NMR (101 MHz, CD_3OD) of 19



SM-5. References

1 Luis Díaz, J.; Dobarro, A.; Villacampa, B.; Velasco, D. *Chem. Mater.* **2001**, *13*, 2528–2536.
2 Rieger, M.; Westheimer, F. H. *J. Am. Chem. Soc.* **1950**, *72*, 28–32.
3 Nicolaou, K. C.; Stepan, A. F.; Lister, T.; Li, A.; Montero, A.; Tria, G. S.; Turner, C. I.; Tang, Y.; Wang, J.; Denton, R. M.; Edmonds, D. J. *J. Am. Chem. Soc.* **2008**, *130*, 13110–13119.
4 Bruker, *APEX3, SAINT and SADABS*, Bruker AXS Inc., Madison, Wisconsin, USA, **2016**.
5 Sheldrick, G. M. *Acta Crystallogr.* **2015**, *A71*, 3–8.
6 Sheldrick, G. M. *Acta Crystallogr.* **2015**, *C71*, 3–8.
7 Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H. *J. Appl. Crystallogr.* **2009**, *42*, 339–341.
8 Parsons, S.; Flack, H. D.; Wagner, T. Acta Crystallogr. **2013**, *B69*, 249–259.