Supporting Information

Synthesis and Cheminformatics-Directed Antibacterial Evaluation of Echinosulfonic Acid-Inspired Bis-Indole Alkaloids

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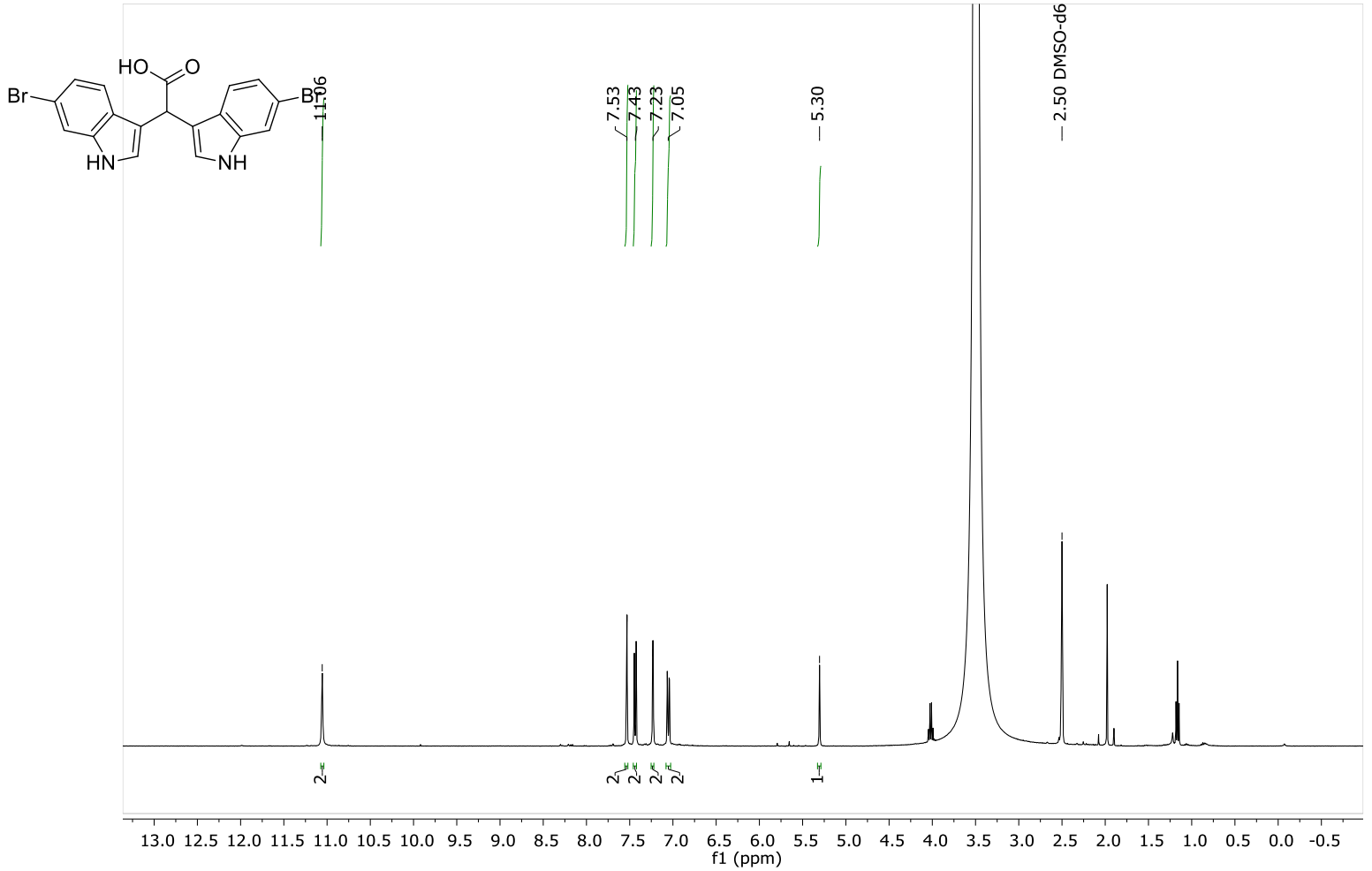


Figure S1 ^{1}H NMR spectrum (500 MHz) of 2,2-bis(6-bromo-1*H*-indol-3-yl)acetic acid (**11**) in DMSO-d_6

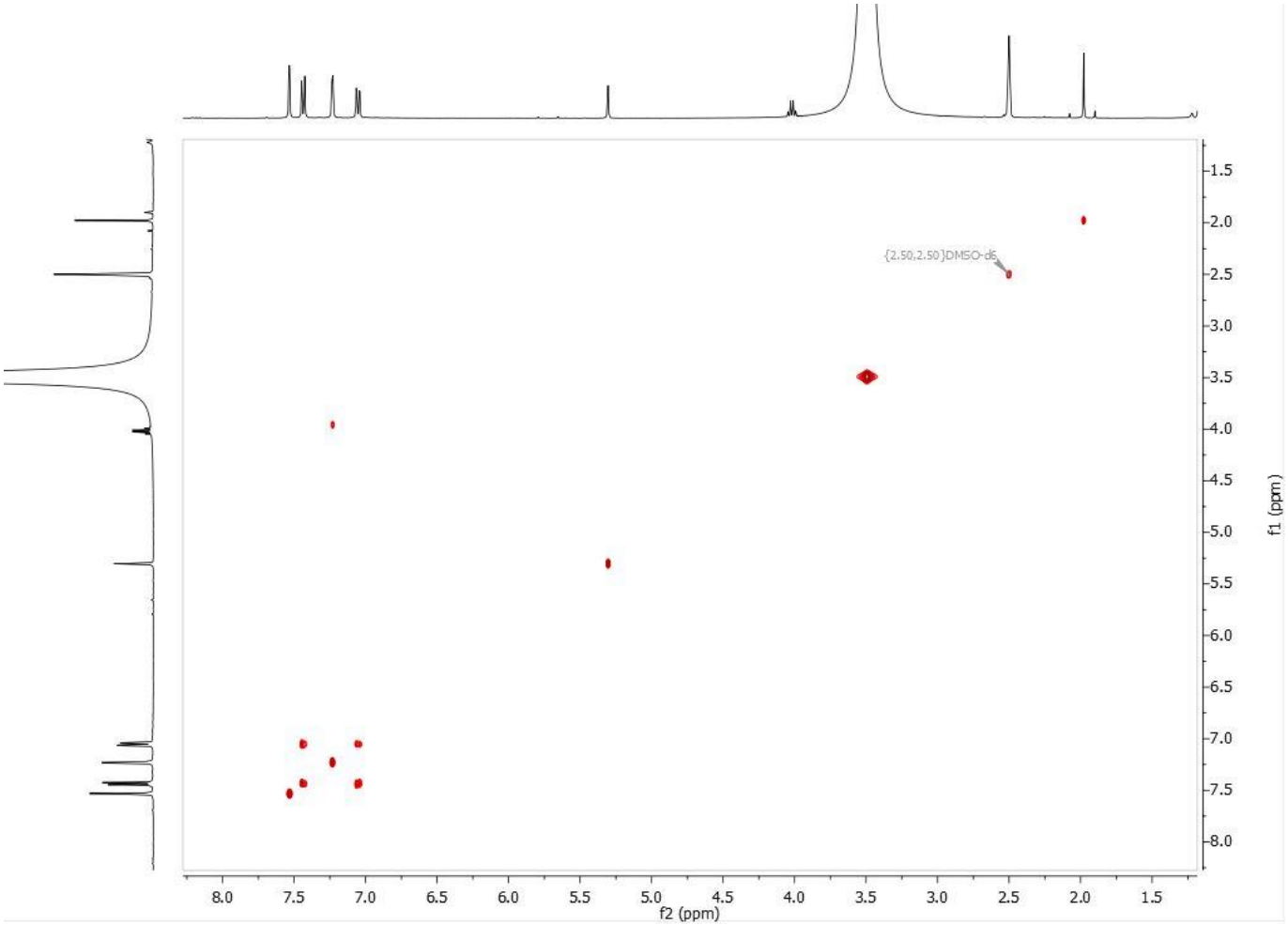


Figure S2. COSY NMR spectrum (500 MHz) of 2,2-bis(6-bromo-1*H* -indol-3-yl)acetic acid (**11**) in DMSO-d_6

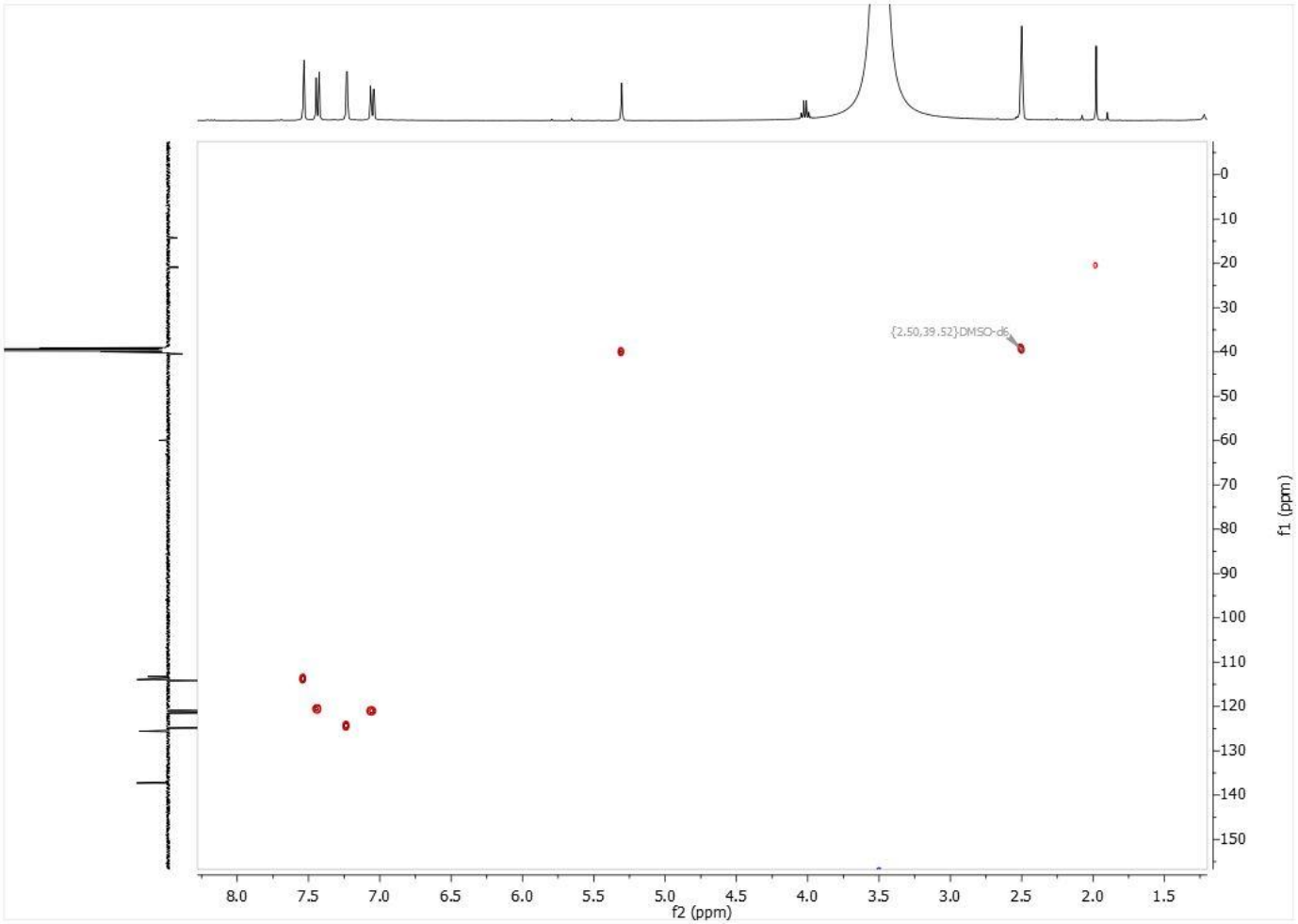


Figure S3. HSQC NMR spectrum (500 MHz) of 2,2-bis(6-bromo-1*H* -indol-3-yl)acetic acid (**11**) in DMSO-d_6

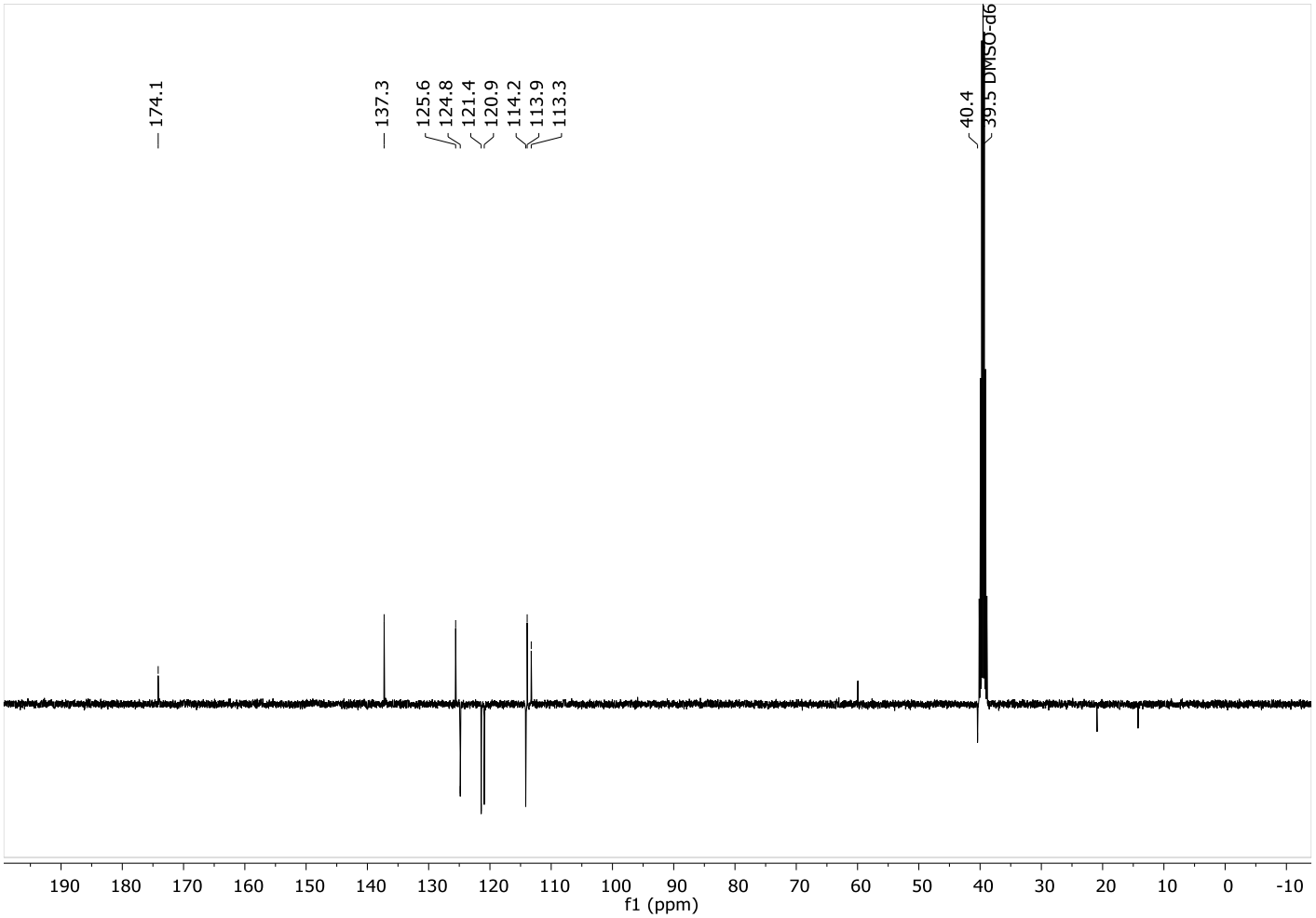


Figure S4. ^{13}C NMR spectrum (125 MHz) of 2,2-bis(6-bromo-1*H* -indol-3-yl)acetic acid (**11**) in DMSO-d_6

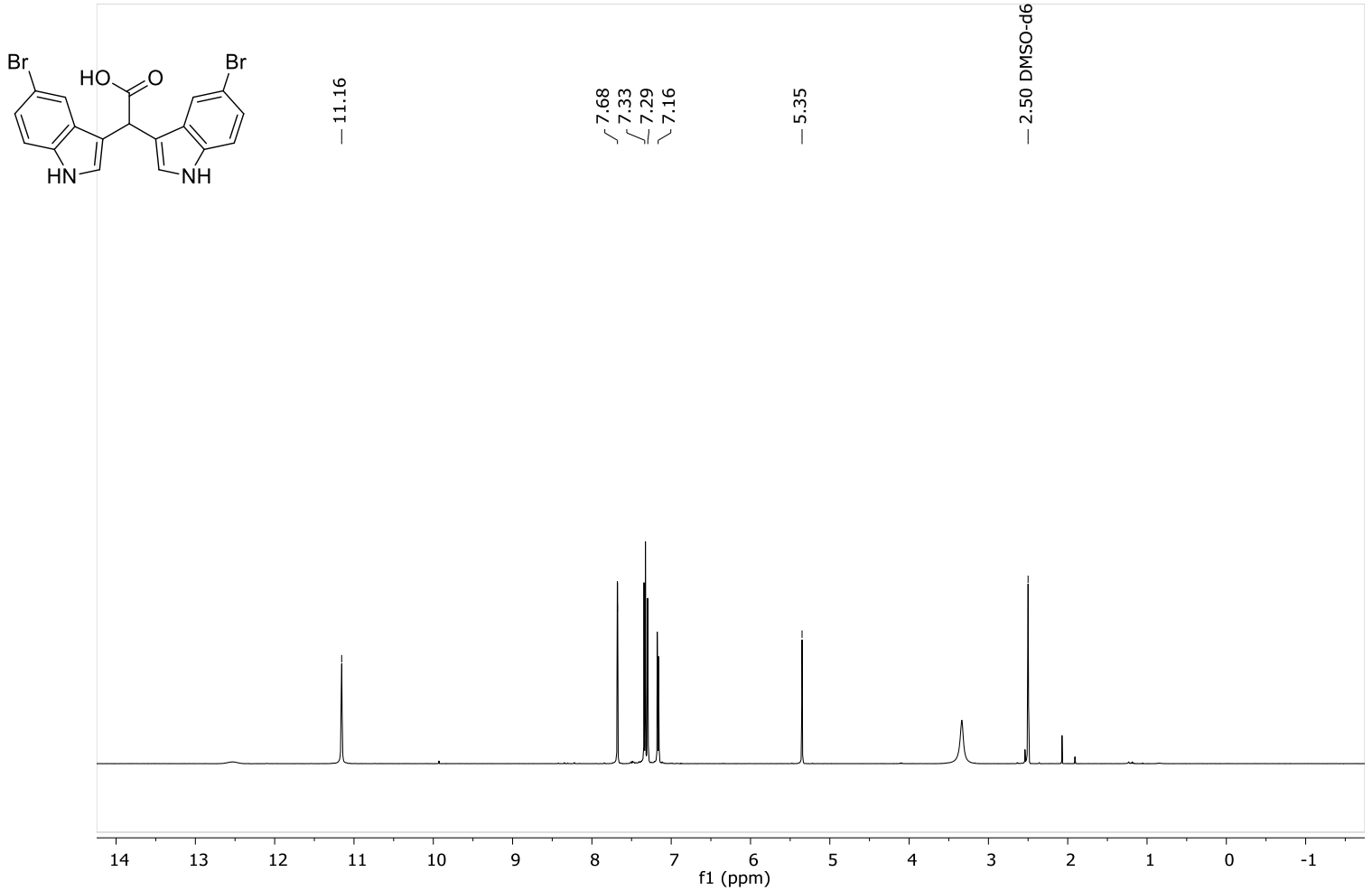


Figure S5. ^{1}H NMR spectrum (500 MHz) of 2,2-bis(5-bromo-1*H*-indol-3-yl)acetic acid (**12**) in DMSO-d_6

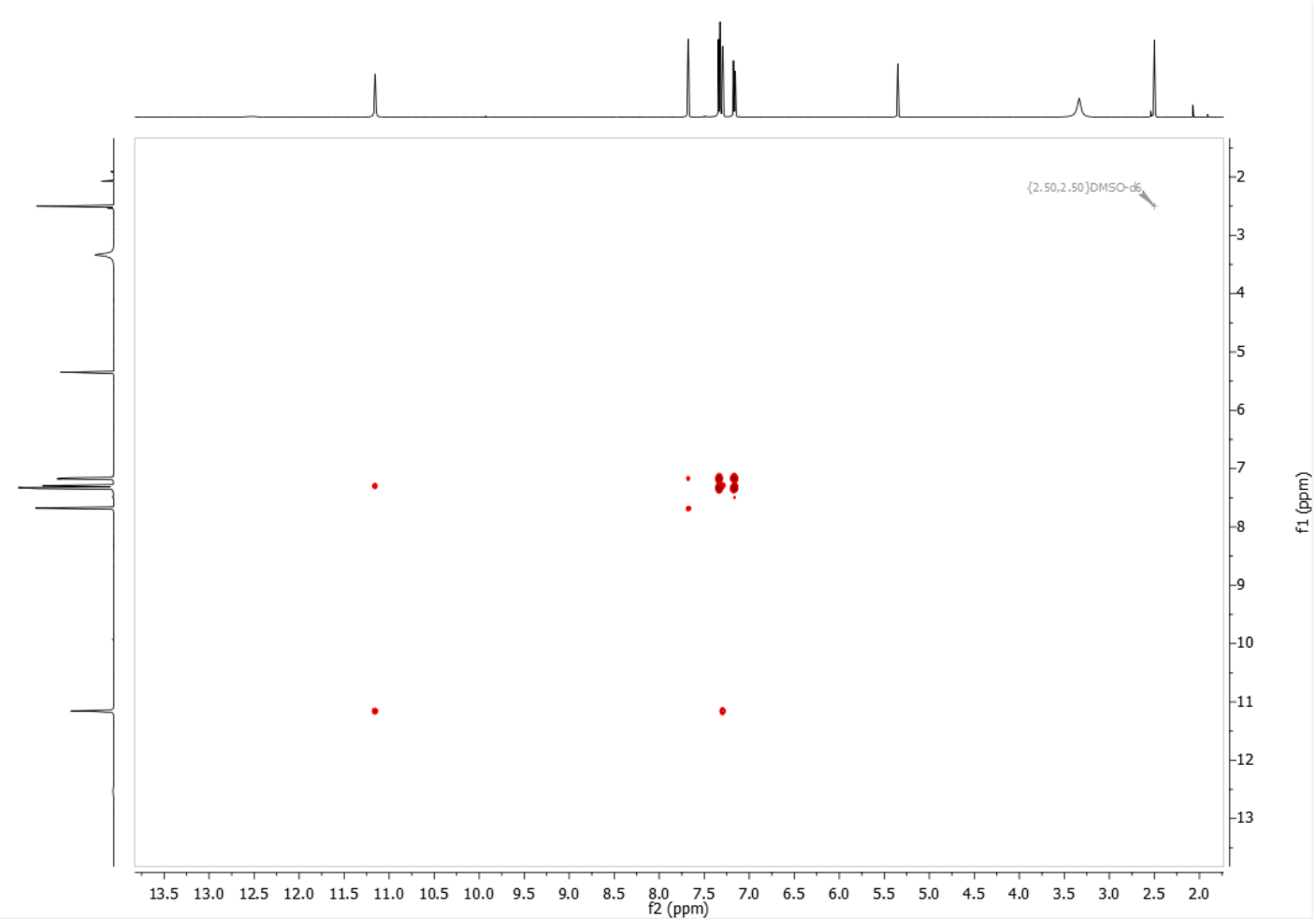


Figure S6. COSY NMR spectrum (500 MHz) of 2,2-bis(5-bromo-1*H* -indol-3-yl)acetic acid (**12**) in DMSO-d_6

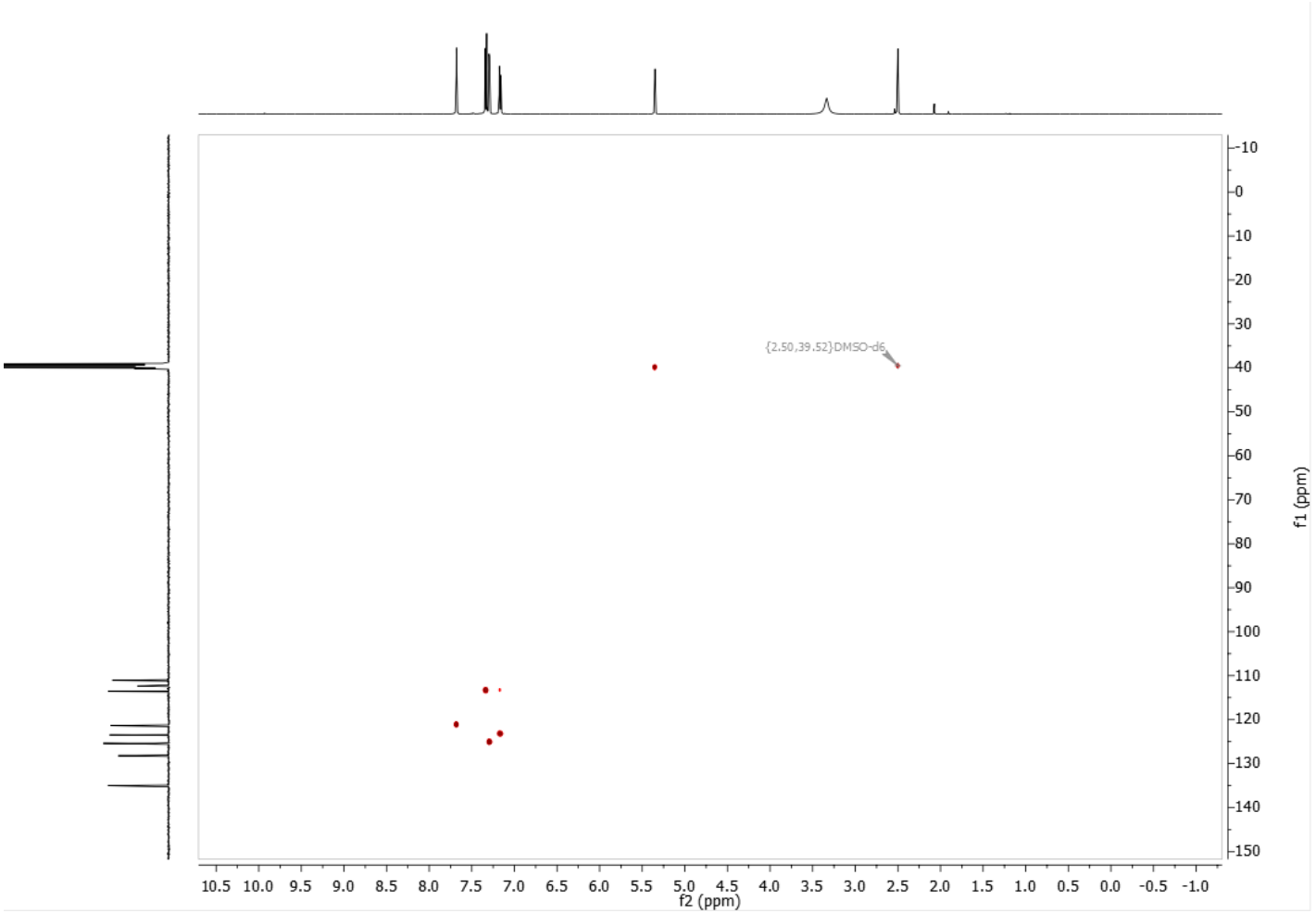


Figure S7. HSQC NMR spectrum (500 MHz) of 2,2-bis(5-bromo-1*H* -indol-3-yl)acetic acid (**12**) in DMSO-d_6

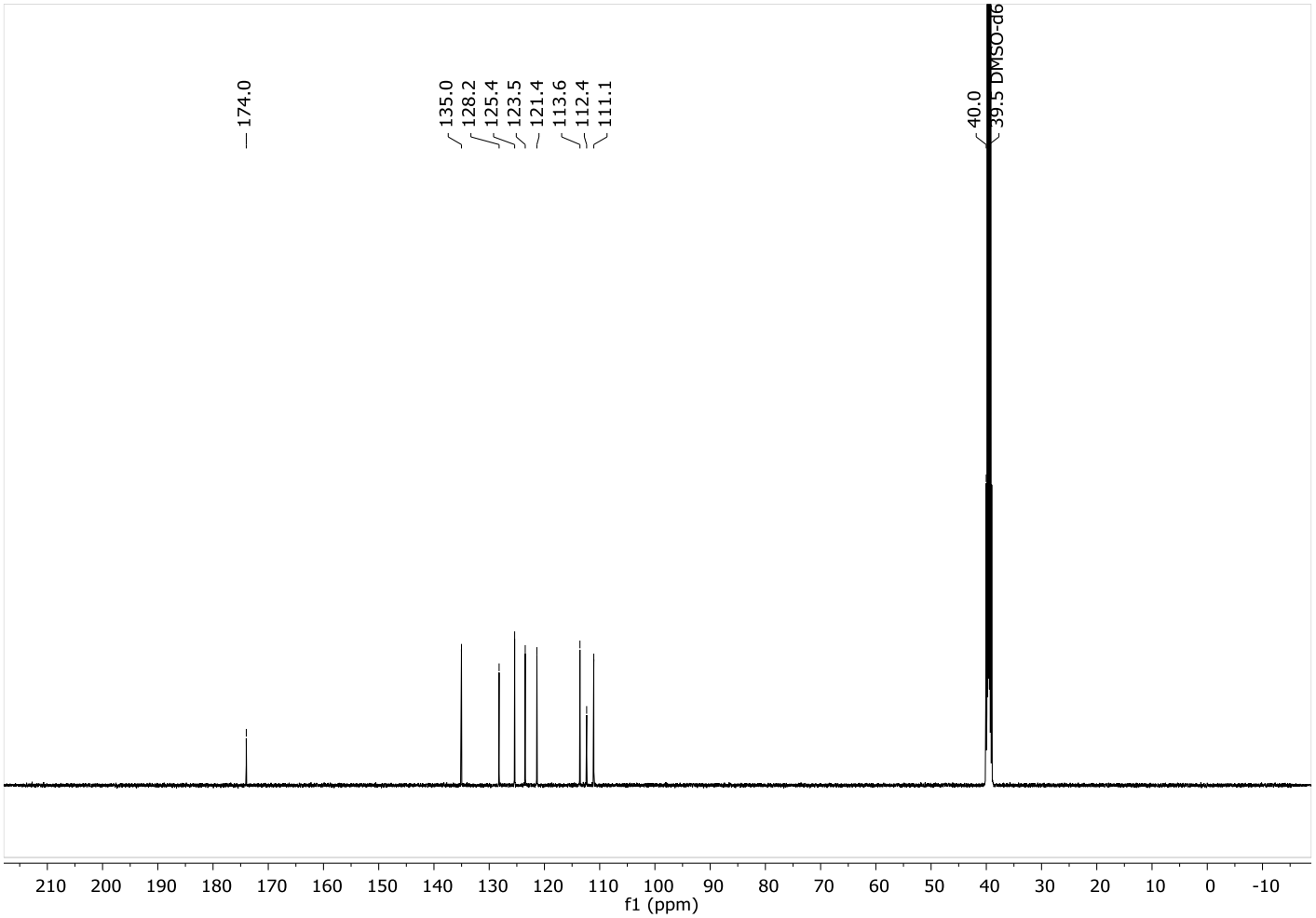


Figure S8. ^{13}C NMR spectrum (125 MHz) of 2,2-bis(5-bromo-1*H* -indol-3-yl)acetic acid (**12**) in DMSO-d_6

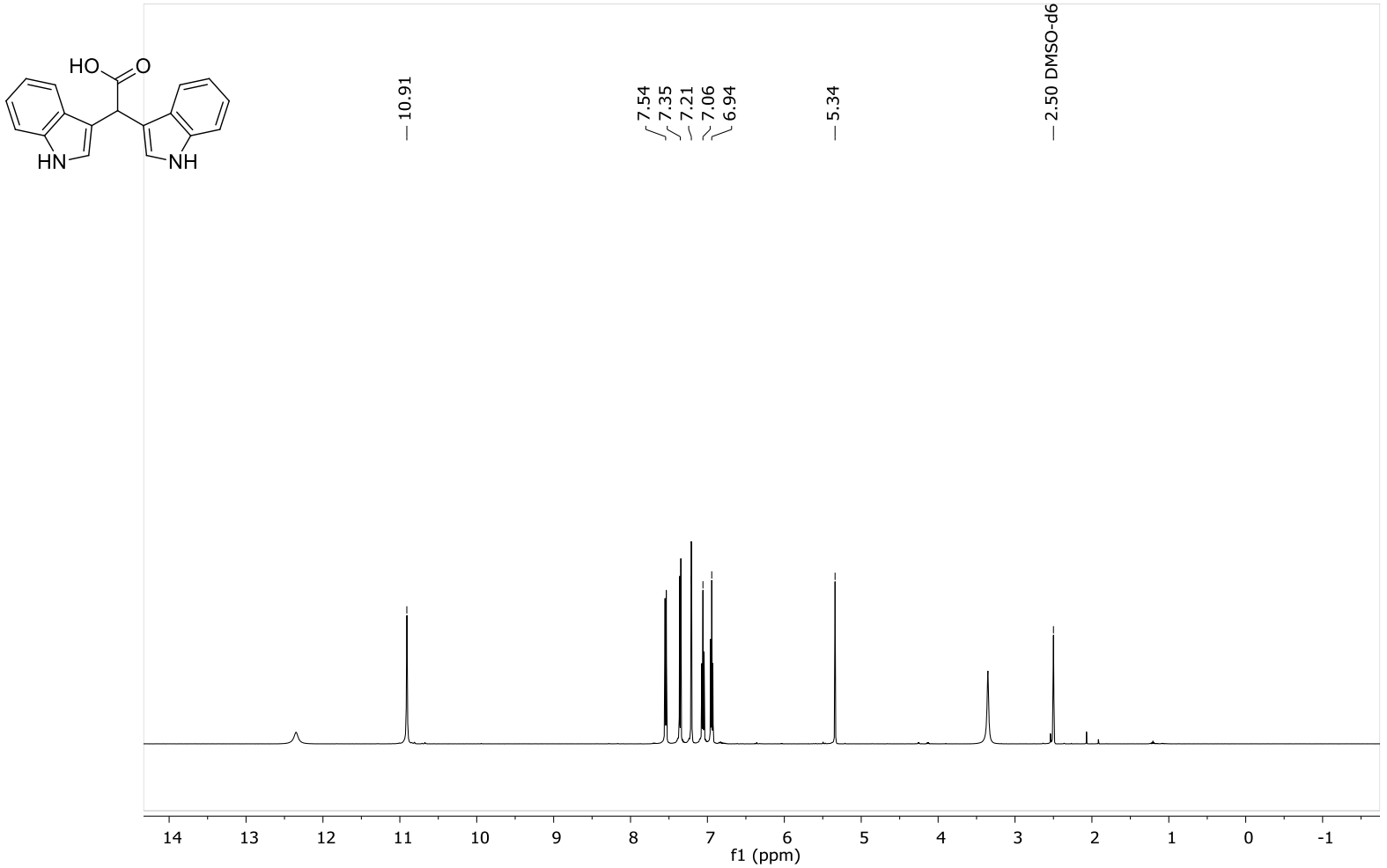


Figure S9. ^{1}H NMR spectrum (500 MHz) of 2,2-di(1*H*-indol-3-yl)acetic acid (**13**) in DMSO-d_6

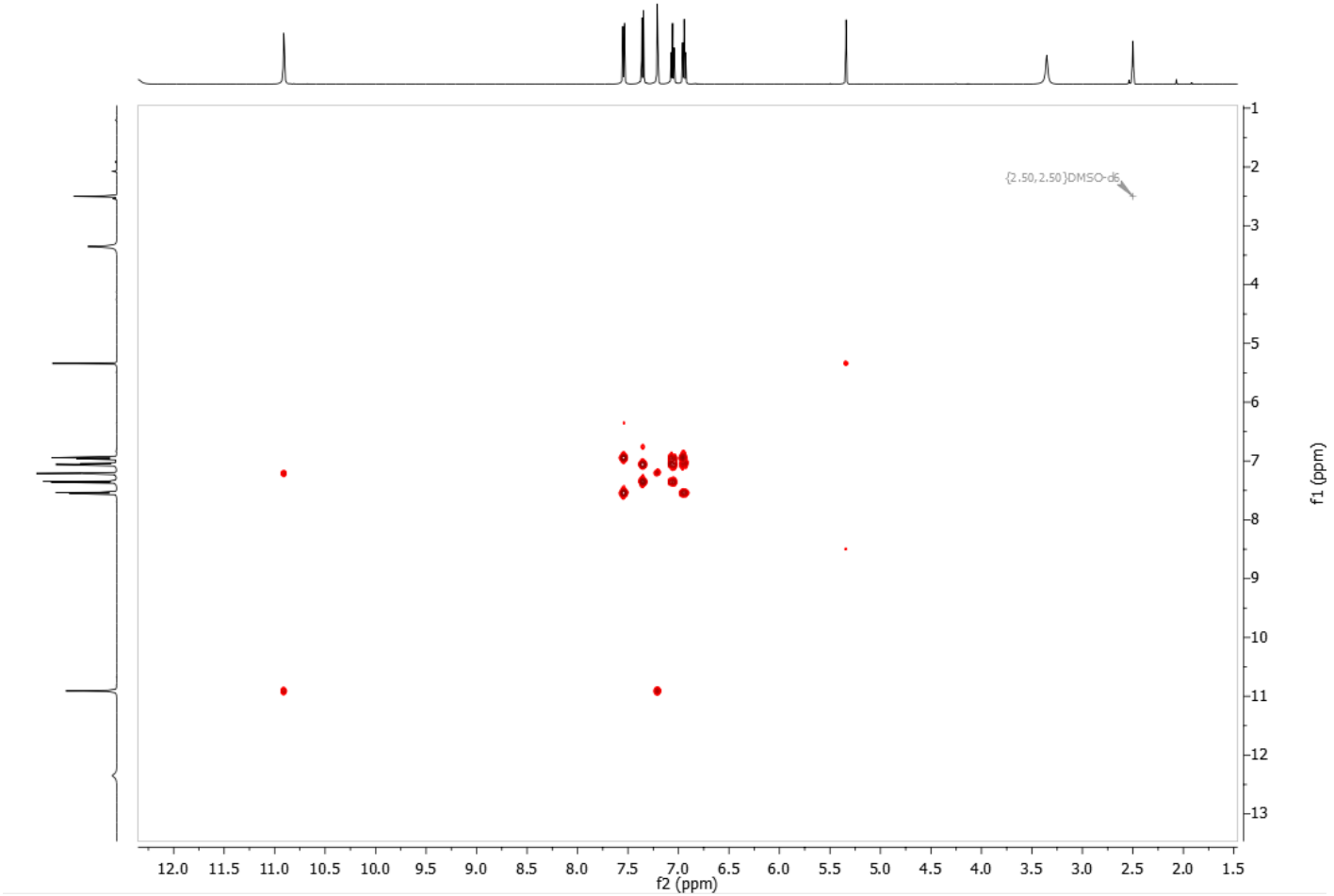


Figure S10. COSY NMR spectrum (500 MHz) of 2,2-di(1*H*-indol-3-yl)acetic acid (**13**) in DMSO-d_6

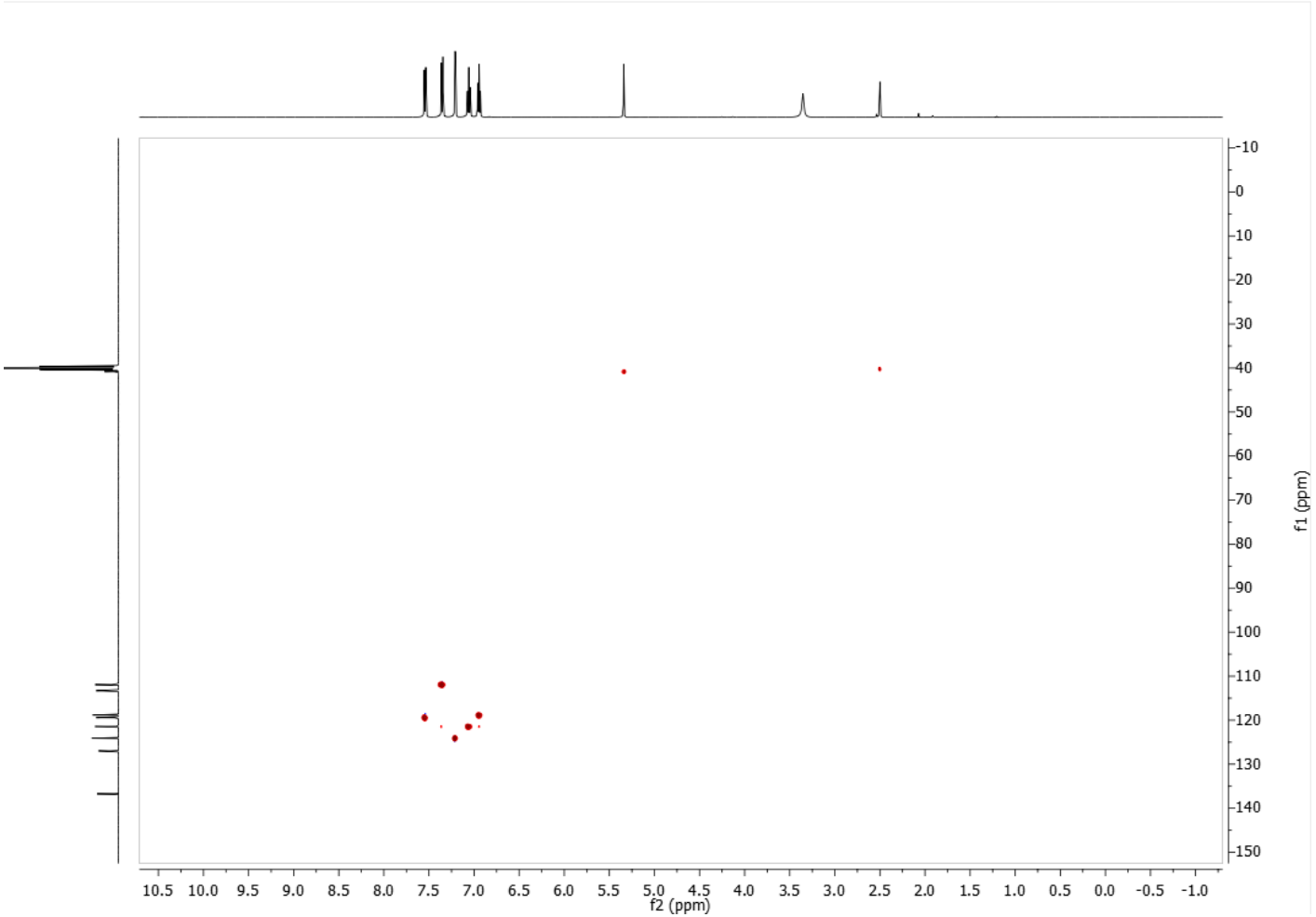


Figure S11. HSQC NMR spectrum (500 MHz) of 2,2-di(1*H*-indol-3-yl)acetic acid (**13**) in DMSO-d_6

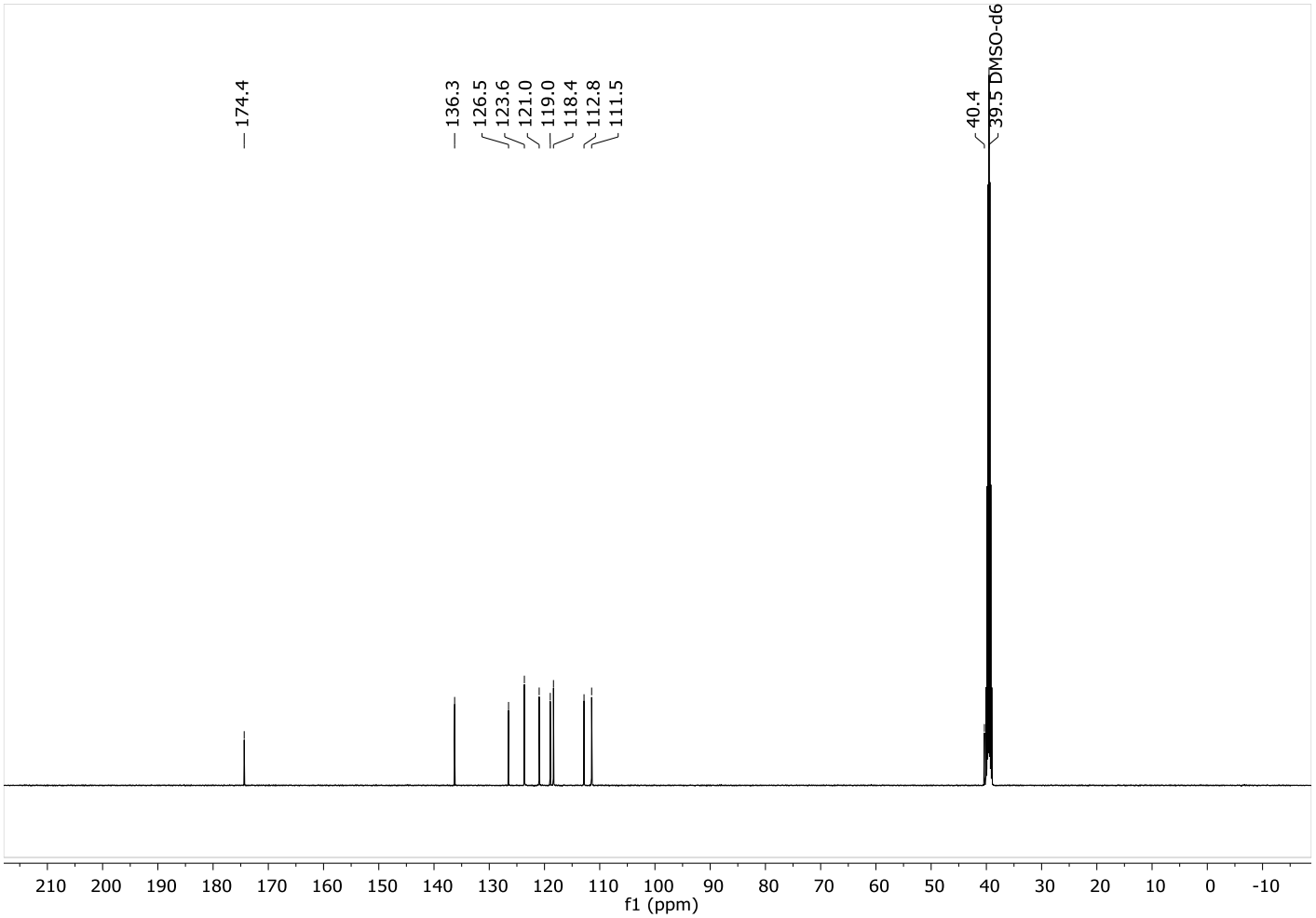


Figure S12. ^{13}C NMR spectrum (125 MHz) of 2,2-di(1*H*-indol-3-yl)acetic acid (**13**) in DMSO-d_6

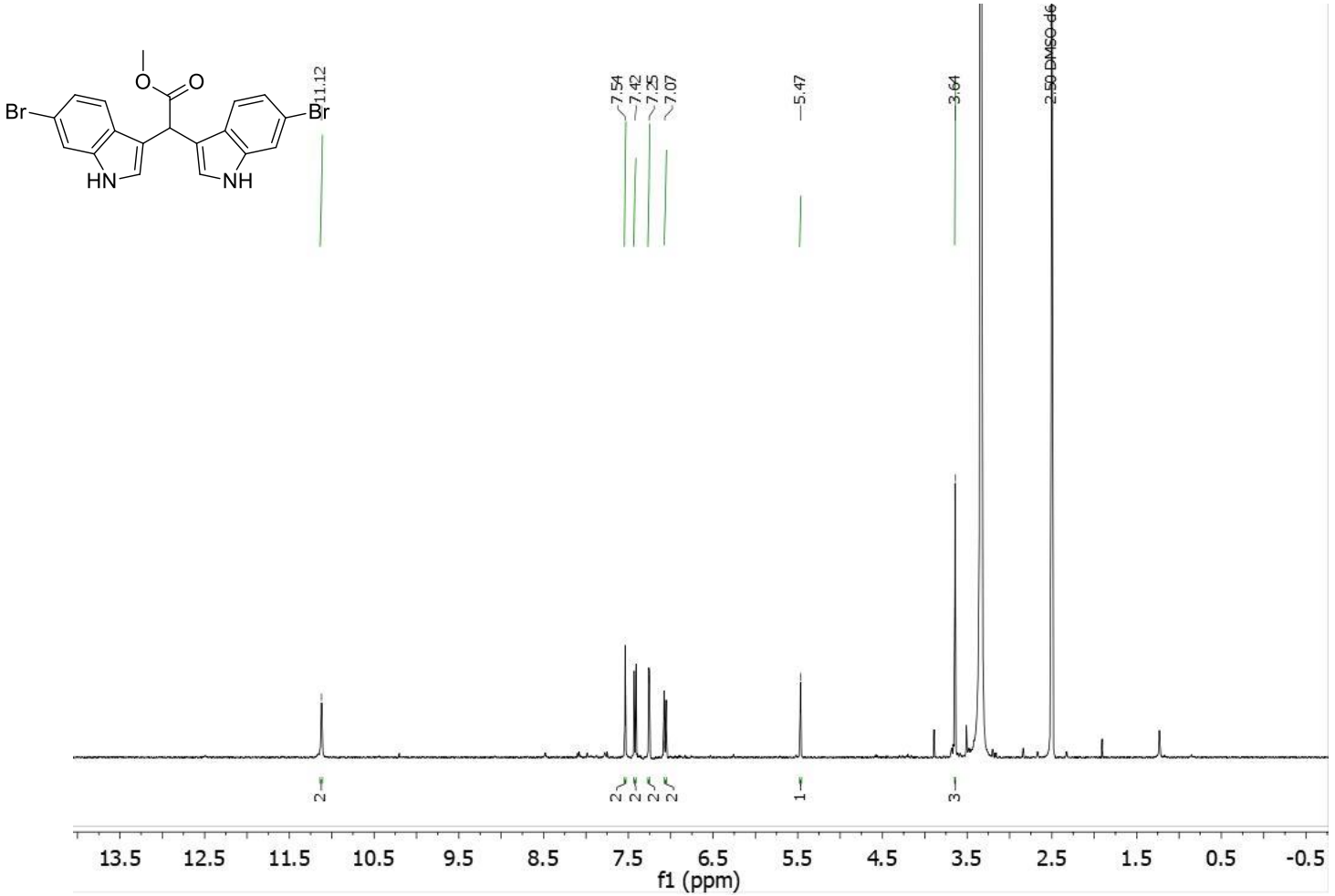


Figure S13. ^{1}H NMR spectrum (500 MHz) of methyl 2,2-bis(6-bromo-1*H* -indol-3-yl)acetate (**14**) in DMSO-d_6

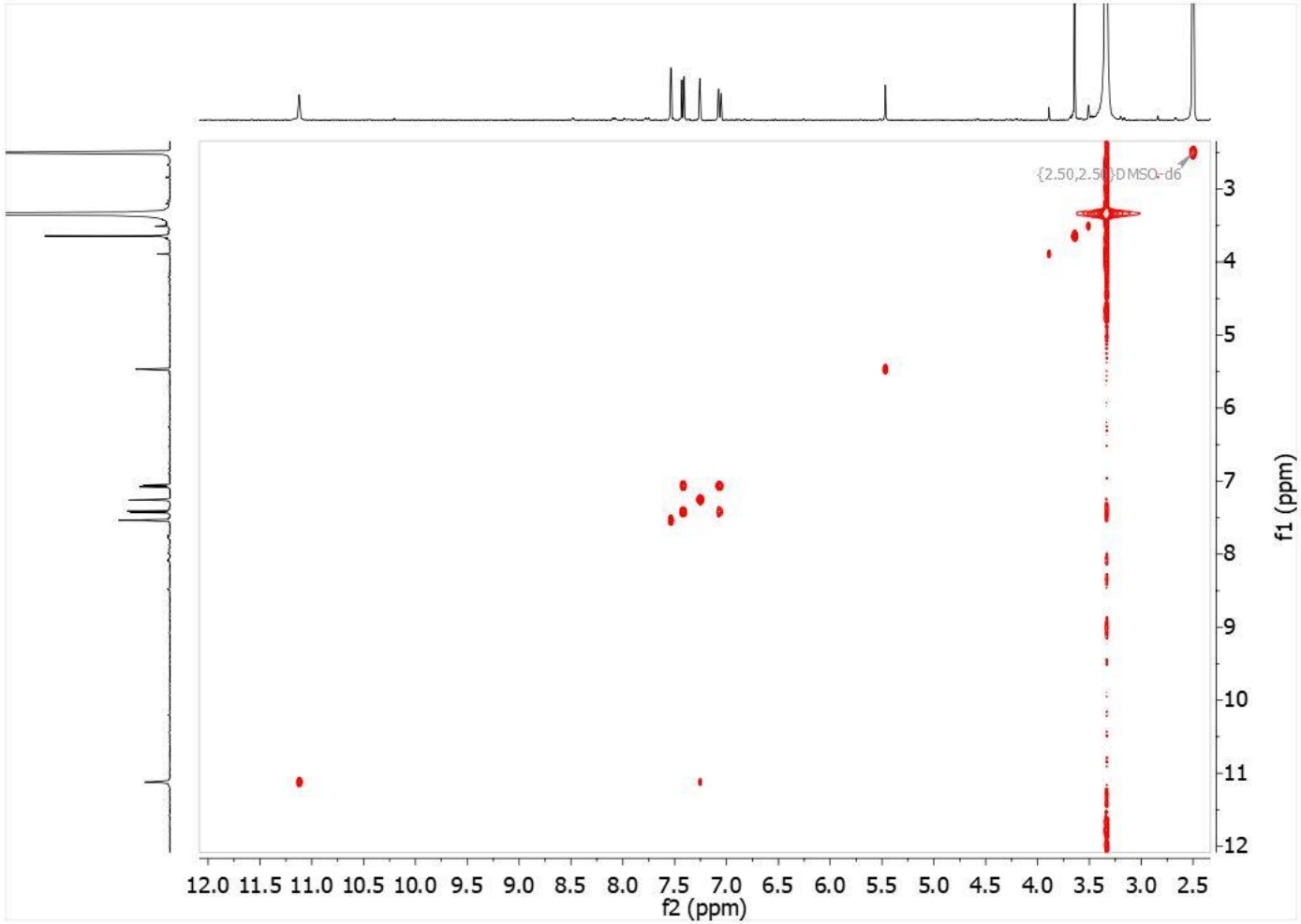


Figure S14. COSY spectrum (500 MHz) of methyl 2,2-bis(6-bromo-1*H* -indol-3-yl)acetate (**14**) in DMSO-d_6

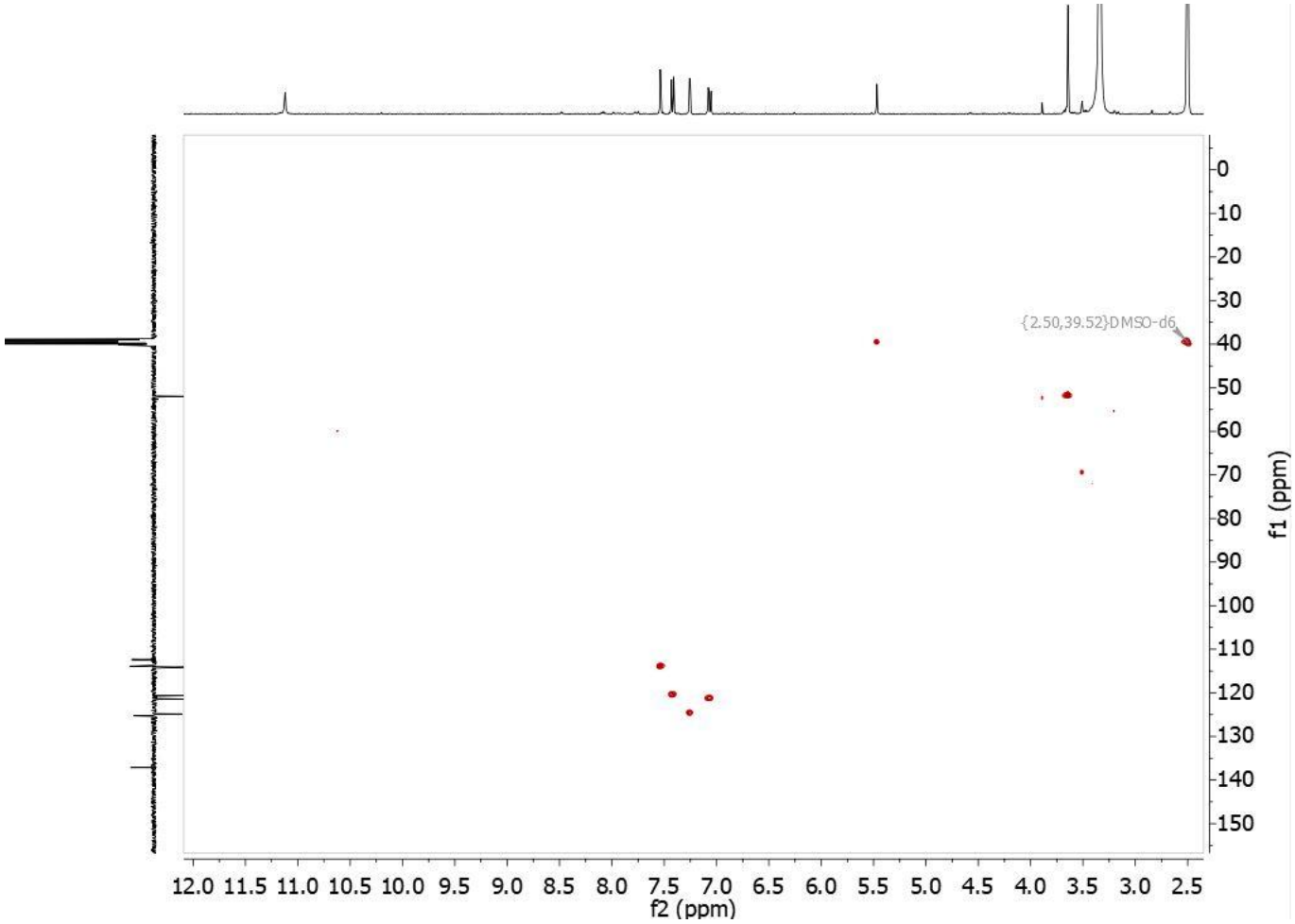


Figure S15. HSQC spectrum (500 MHz) of methyl 2,2-bis(6-bromo-1*H* -indol-3-yl)acetate (**14**) in DMSO-d_6

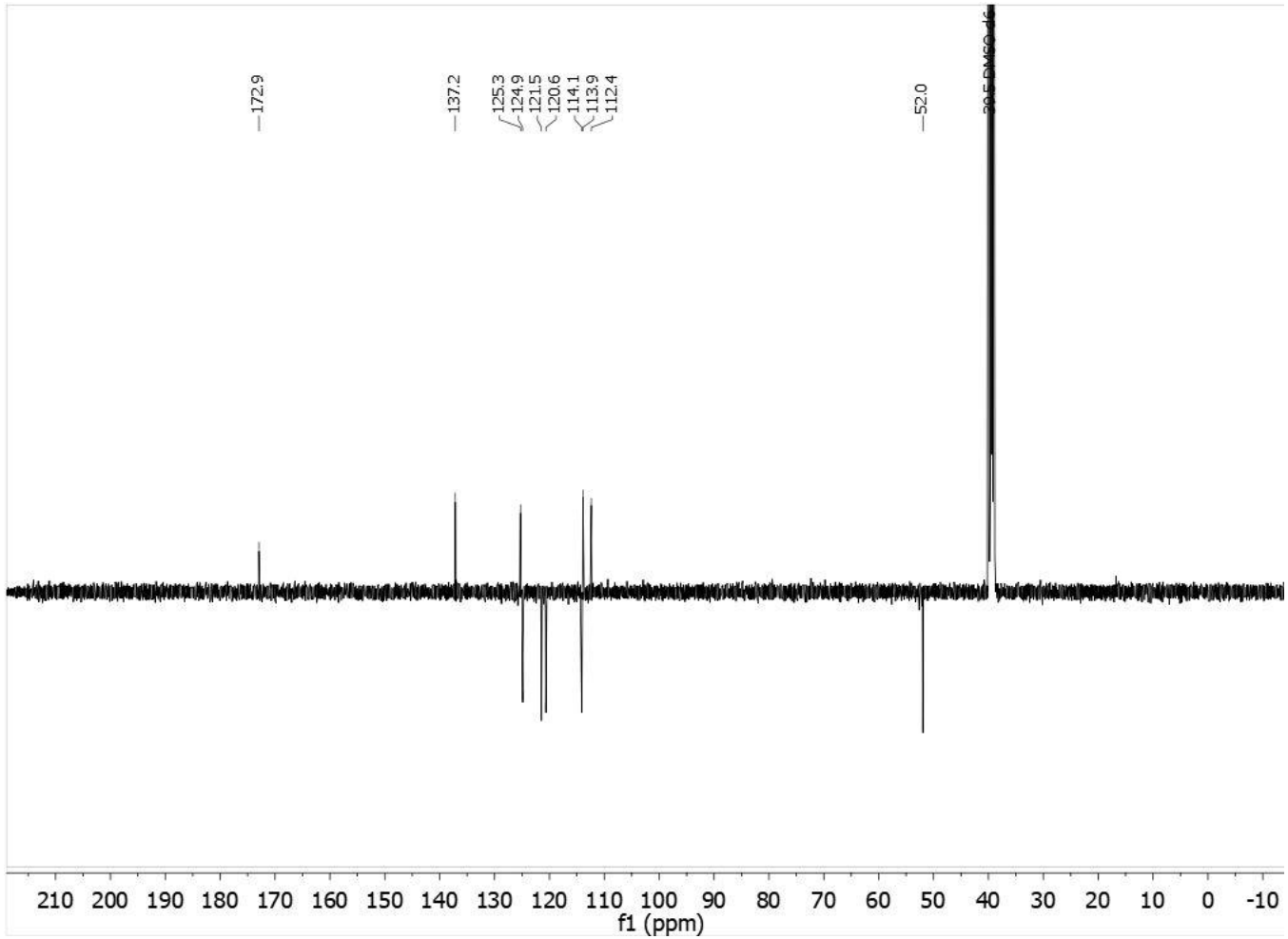


Figure S16. ^{13}C NMR spectrum (125 MHz) of methyl 2,2-bis(6-bromo-1*H* -indol-3-yl)acetate (**14**) in DMSO-d_6

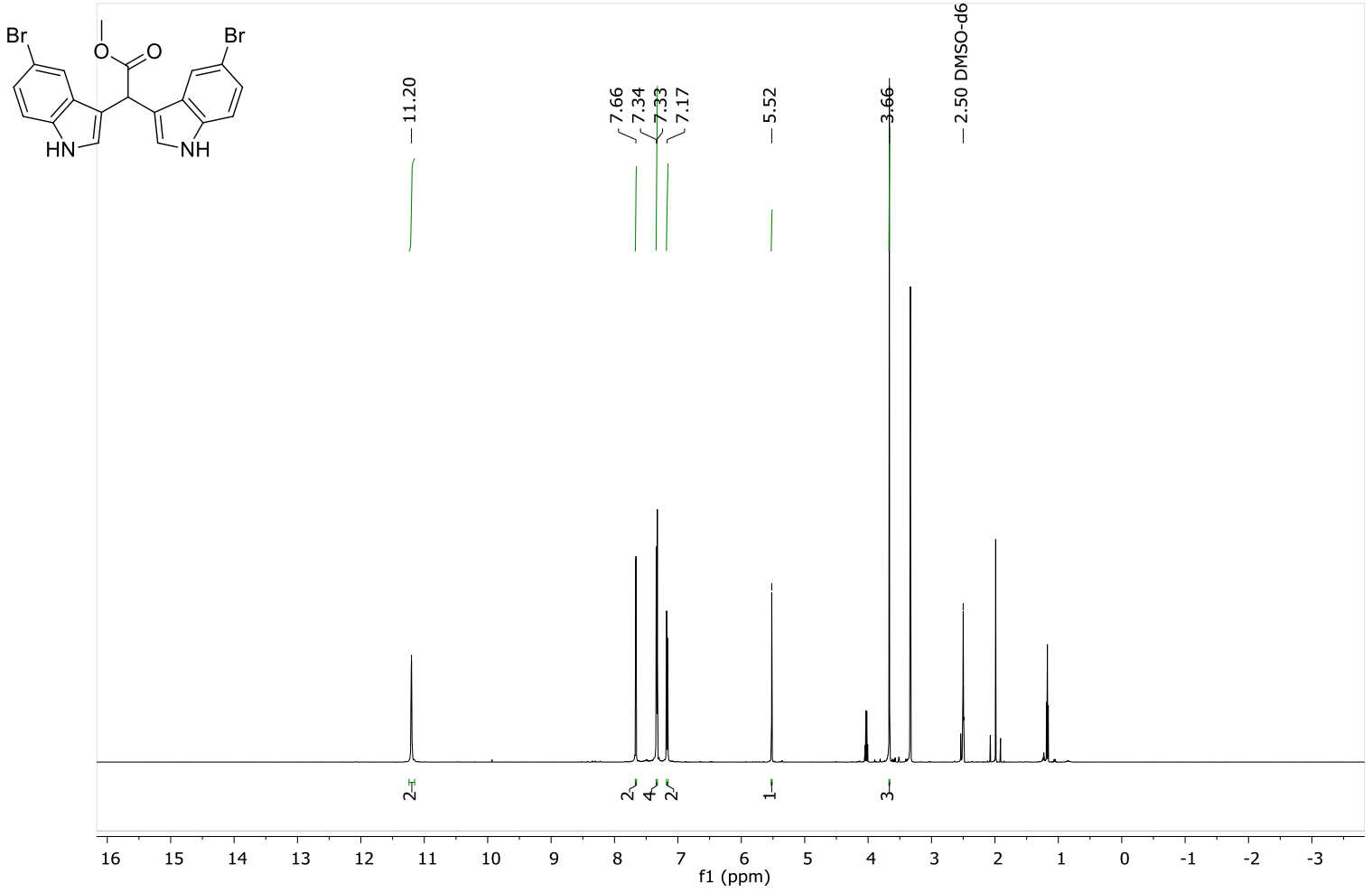


Figure S17. ^{1}H NMR spectrum (500 MHz) of methyl 2,2-bis(5-bromo-1*H* -indol-3-yl)acetate (**15**) in DMSO-d_6

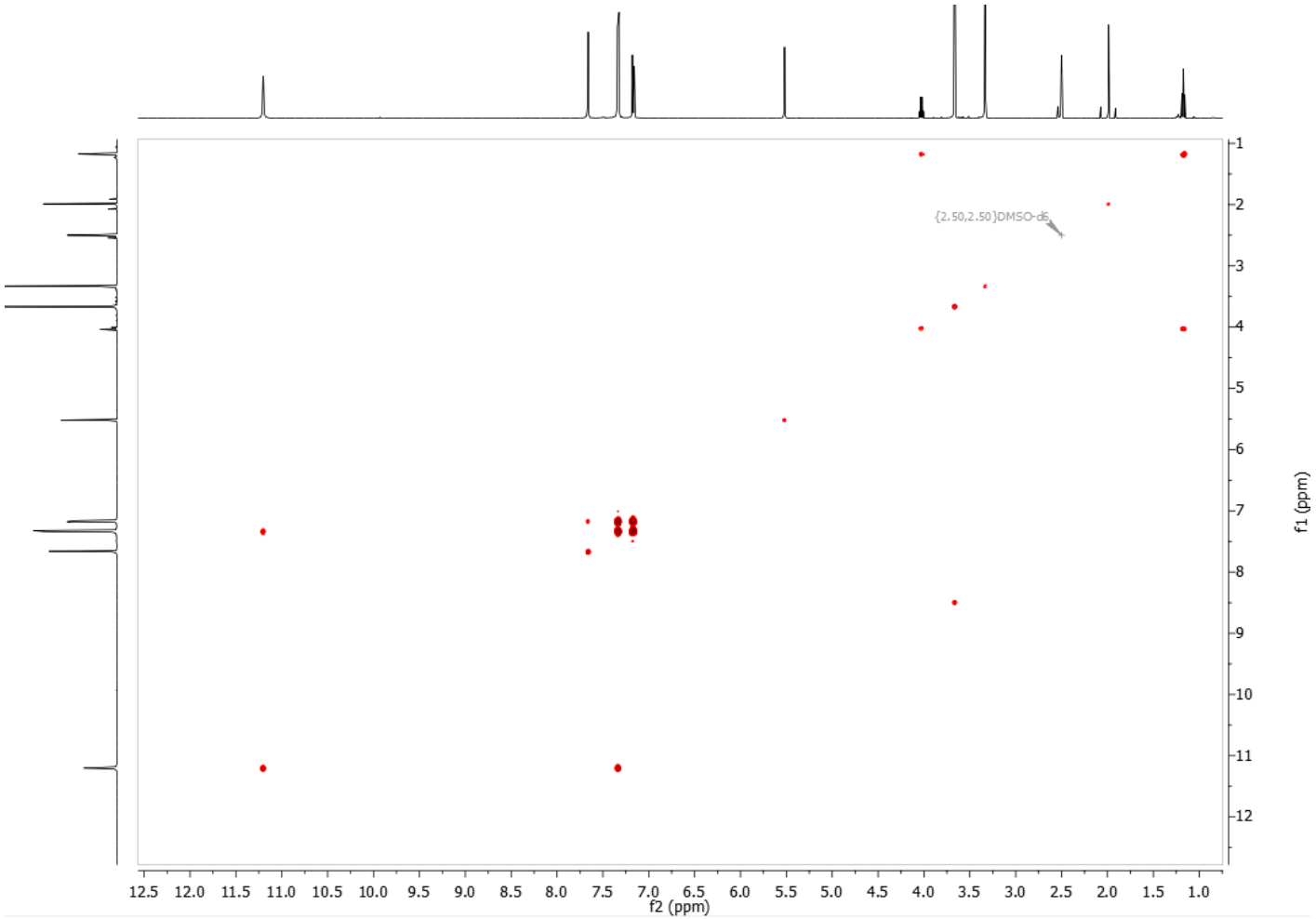


Figure S18. COSY NMR spectrum (500 MHz) of methyl 2,2-bis(5-bromo-1*H* -indol-3-yl)acetate (**15**) in DMSO-d_6

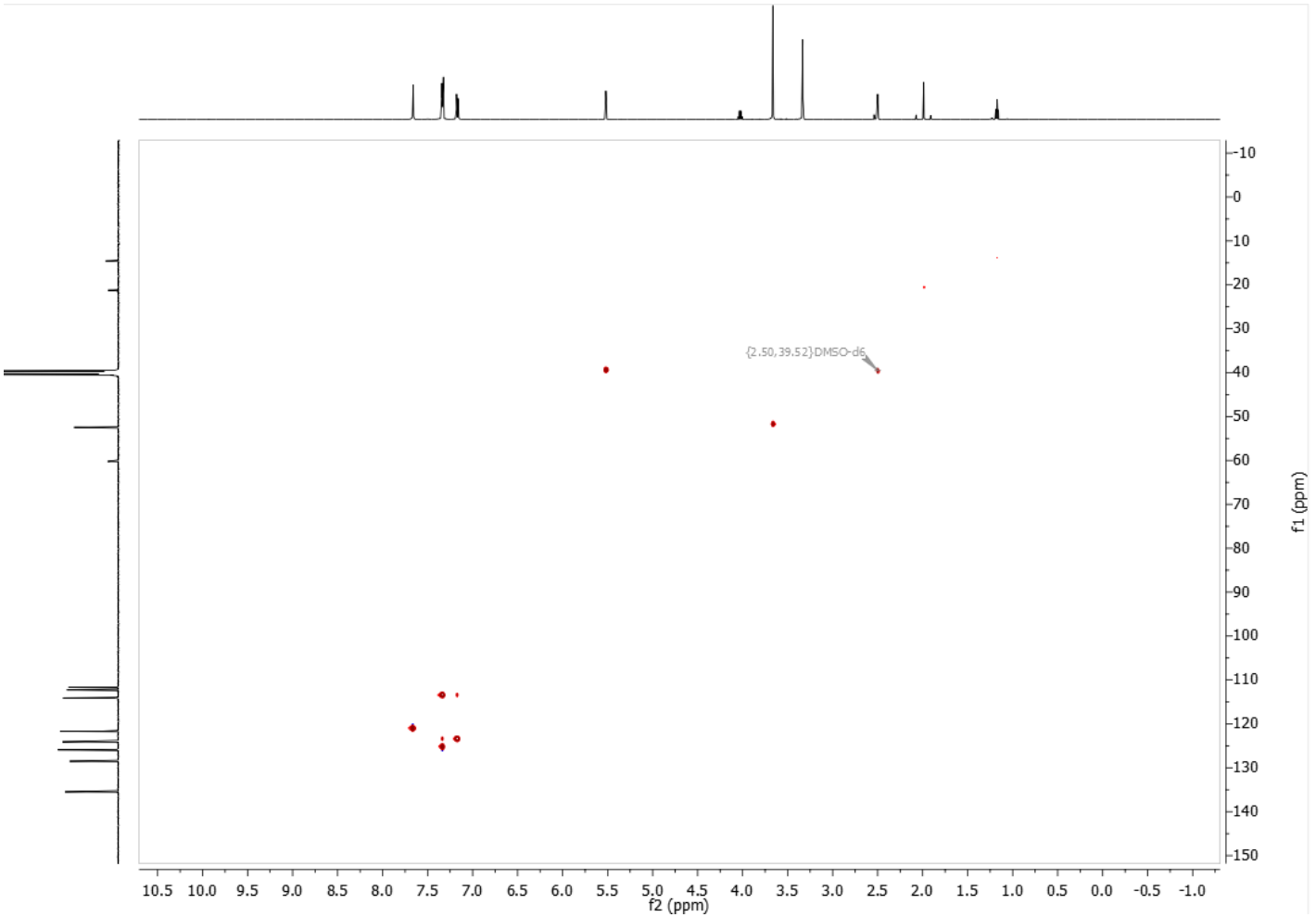


Figure S19. HSQC NMR spectrum (500 MHz) of methyl 2,2-bis(5-bromo-1*H* -indol-3-yl)acetate (**15**) in DMSO-d_6

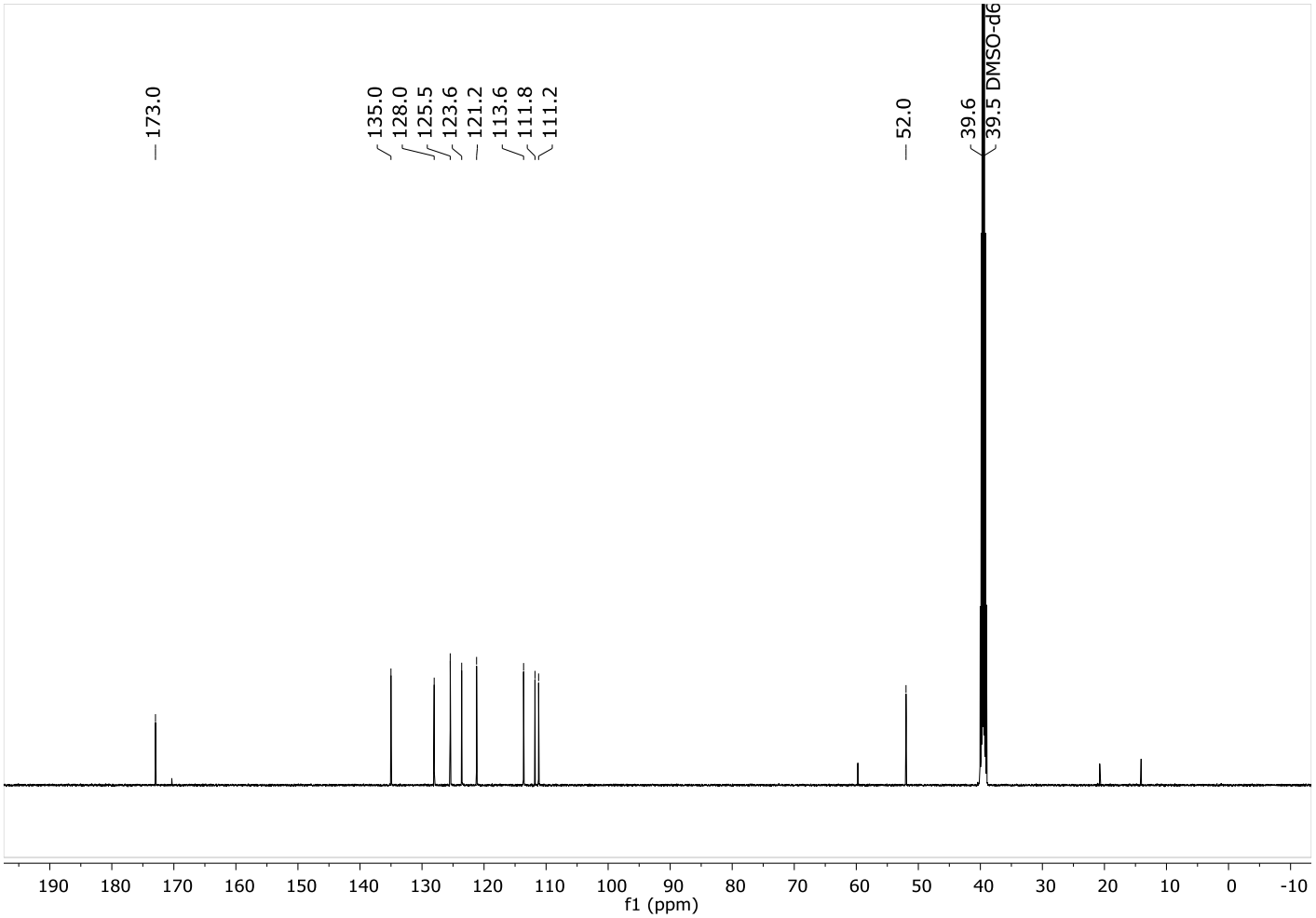


Figure S20. ^{13}C NMR spectrum (125 MHz) of methyl 2,2-bis(5-bromo-1*H* -indol-3-yl)acetate (**15**) in DMSO-d_6

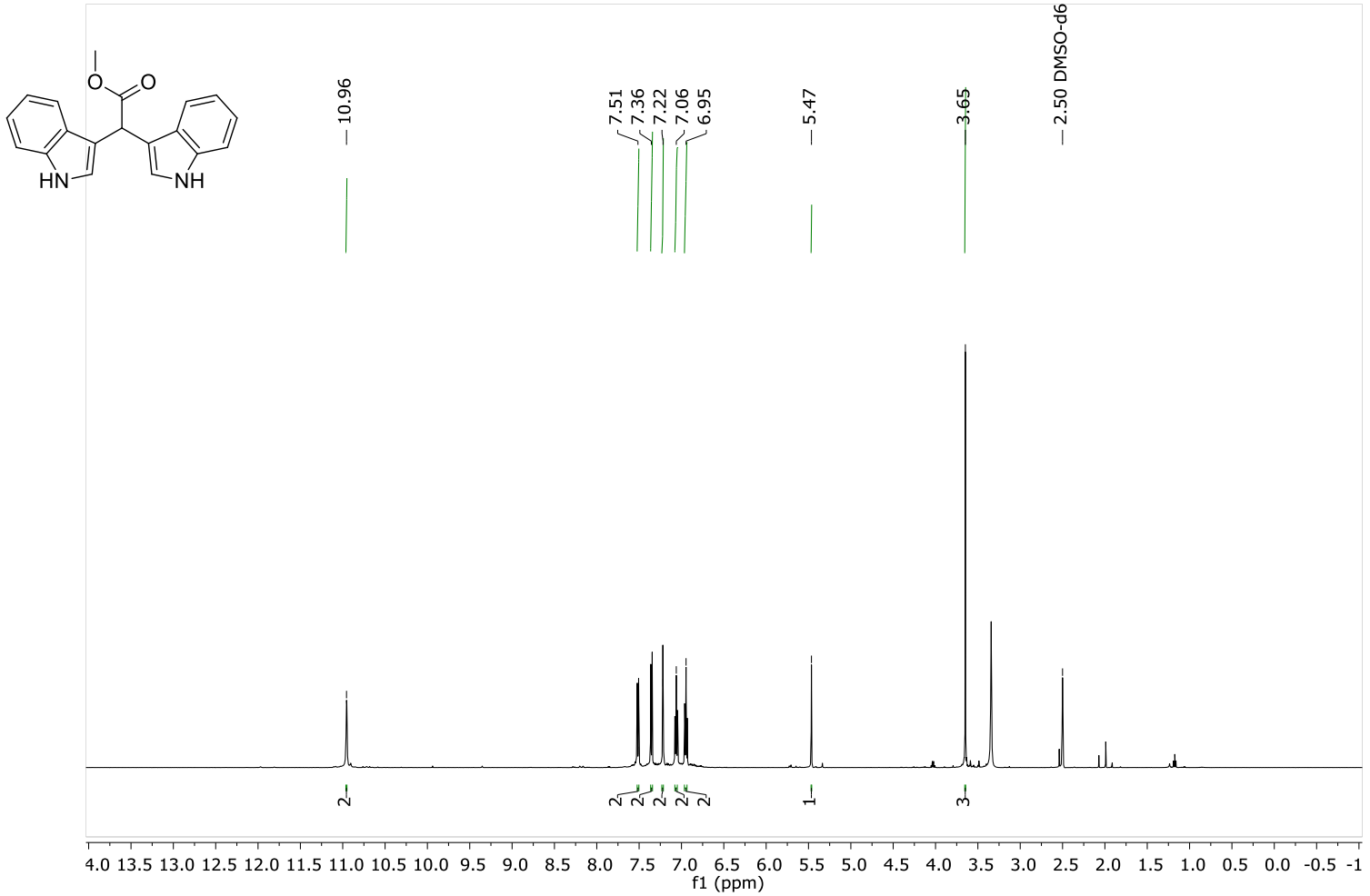


Figure S21. ^{1}H NMR spectrum (500 MHz) of methyl 2,2-di(1*H*-indol-3-yl)acetate (**16**) in DMSO-d_6

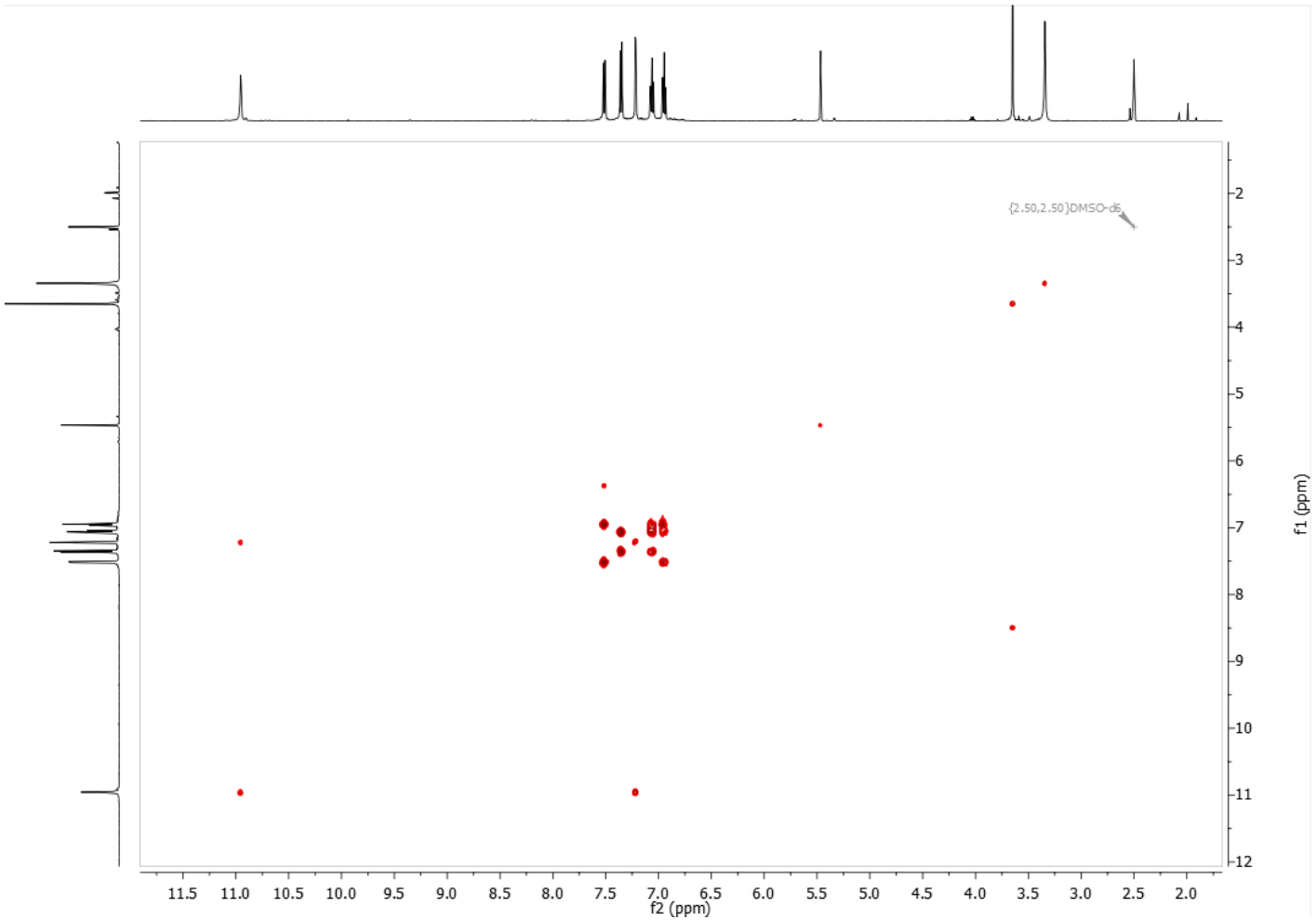


Figure S22. COSY NMR spectrum (500 MHz) of methyl 2,2-di(1*H*-indol-3-yl)acetate (**16**) in DMSO-d_6

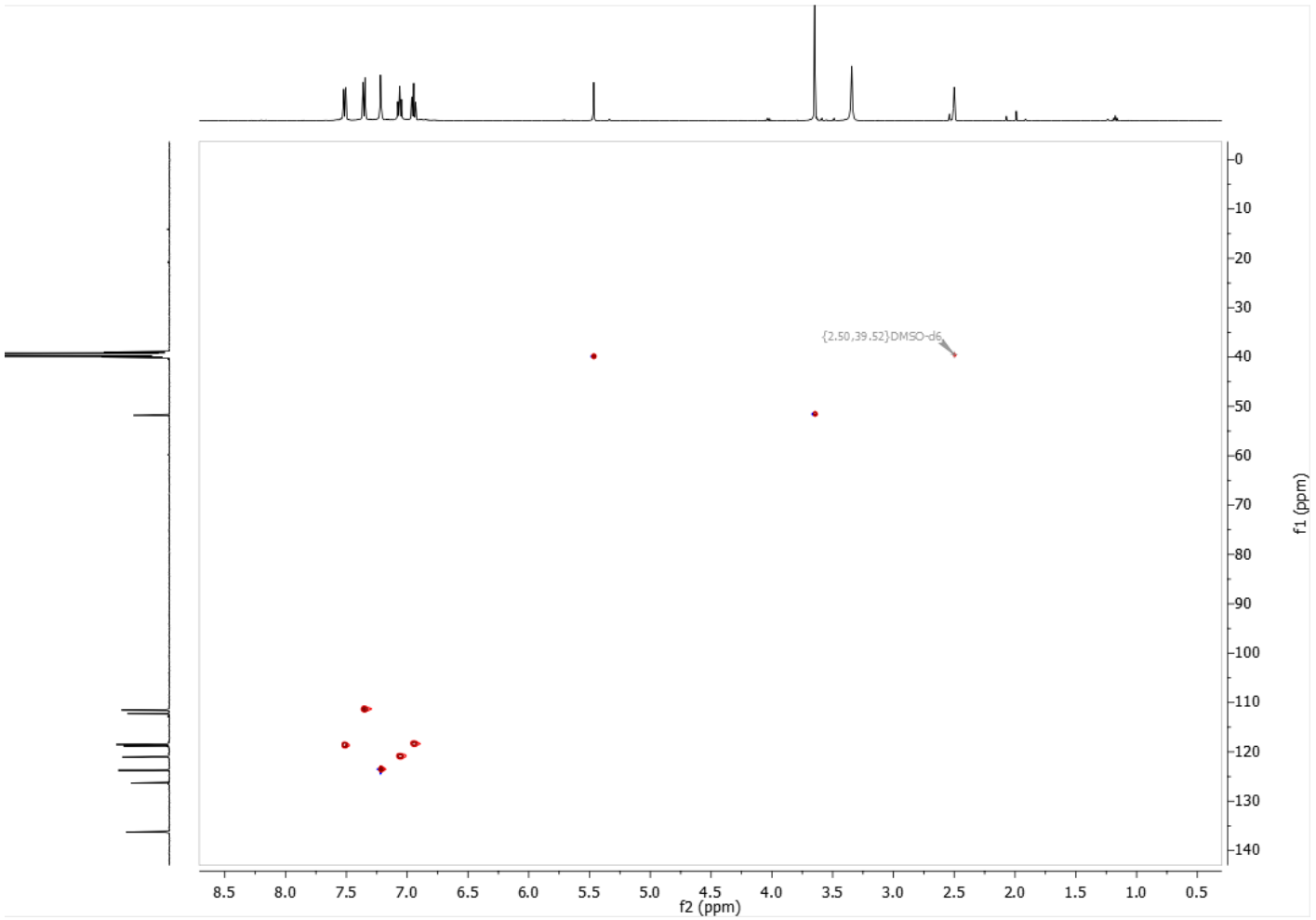


Figure S23. HSQC NMR spectrum (500 MHz) of methyl 2,2-di(1*H*-indol-3-yl)acetate (**16**) in DMSO-d_6

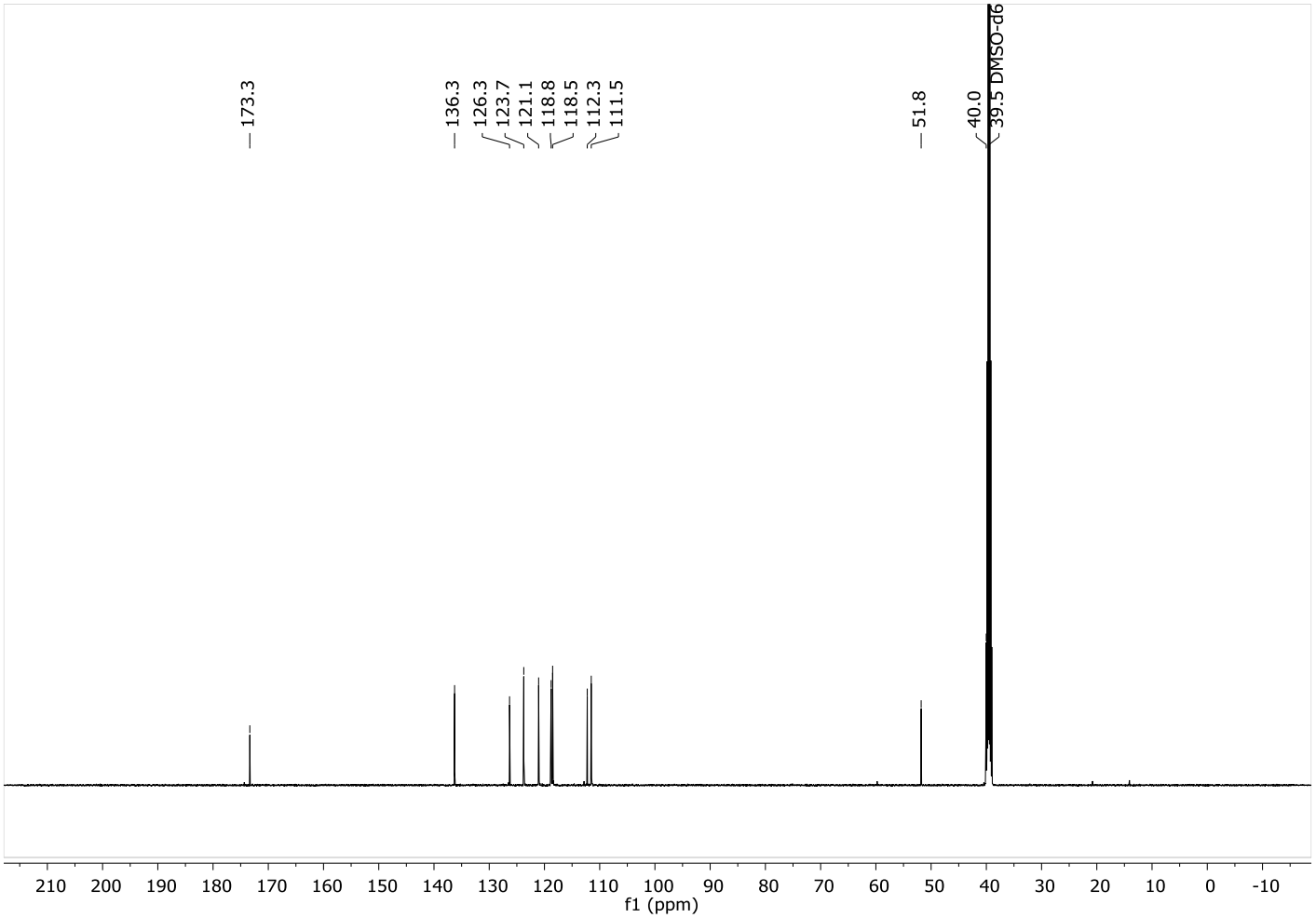


Figure S24. ^{13}C NMR spectrum (125 MHz) of methyl 2,2-di(1*H*-indol-3-yl)acetate (**16**) in DMSO-d_6

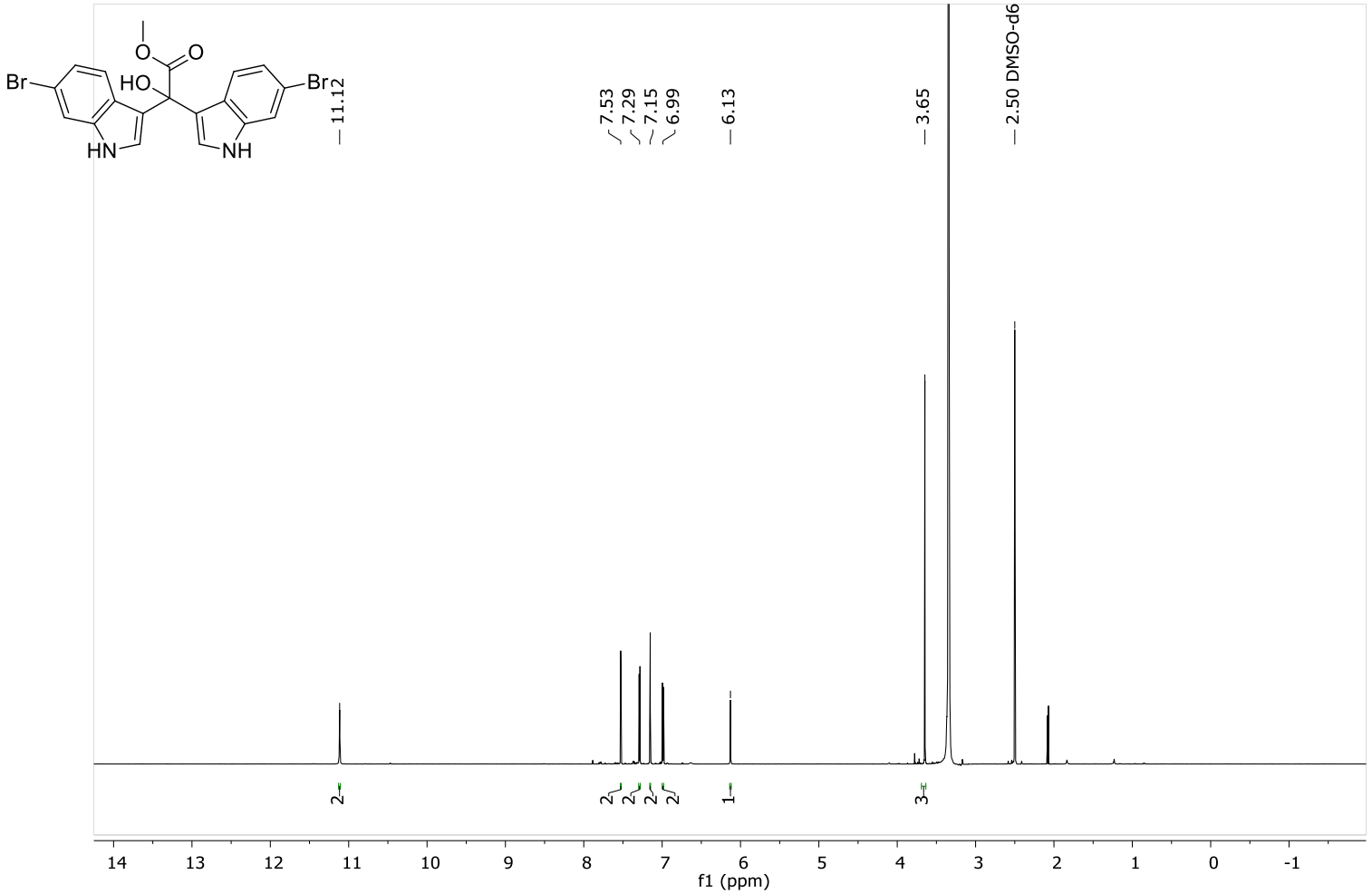


Figure S25. ^{1}H NMR spectrum (800 MHz) of methyl 2,2-bis(6-bromo-1*H* -indol-3-yl)-2-hydroxyacetate (**17**) in DMSO-d_6

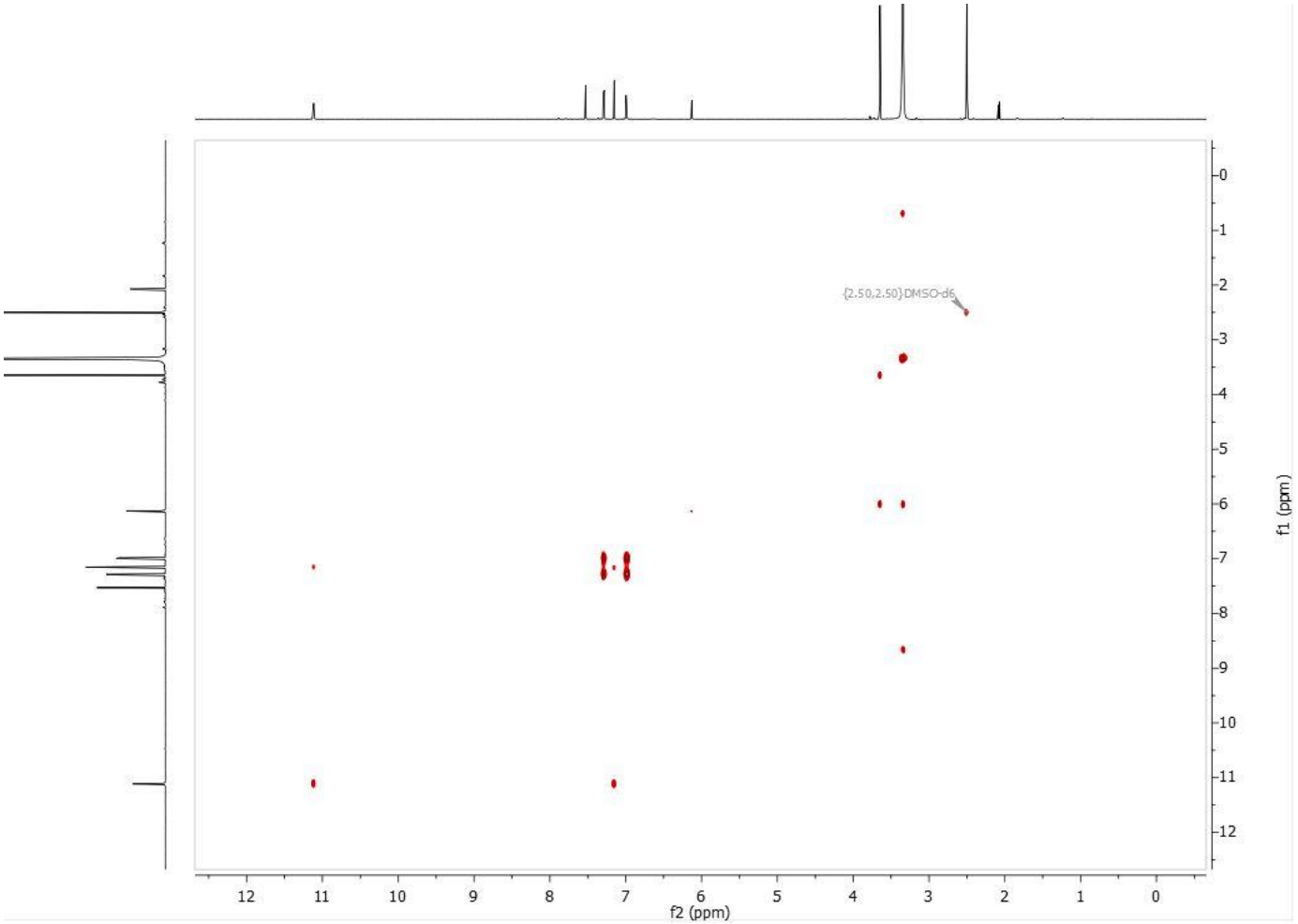


Figure S26. COSY NMR spectrum (800 MHz) of methyl 2,2-bis(6-bromo-1*H* -indol-3-yl)-2-hydroxyacetate (**17**) in DMSO-d_6

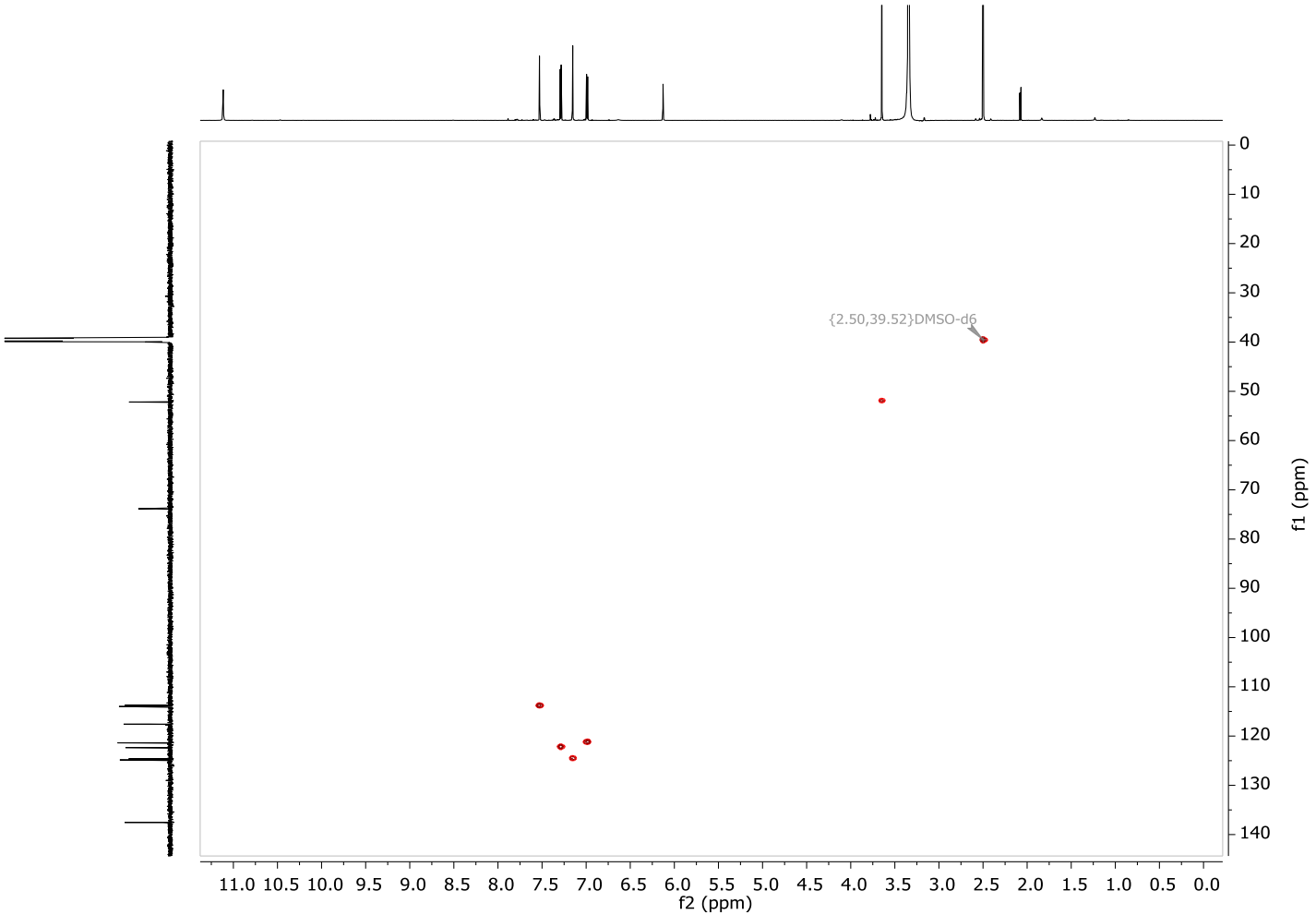


Figure S27. HSQC spectrum (500 MHz) of methyl 2,2-bis(6-bromo-1*H* -indol-3-yl)-2-hydroxyacetate (**17**) in DMSO-d_6

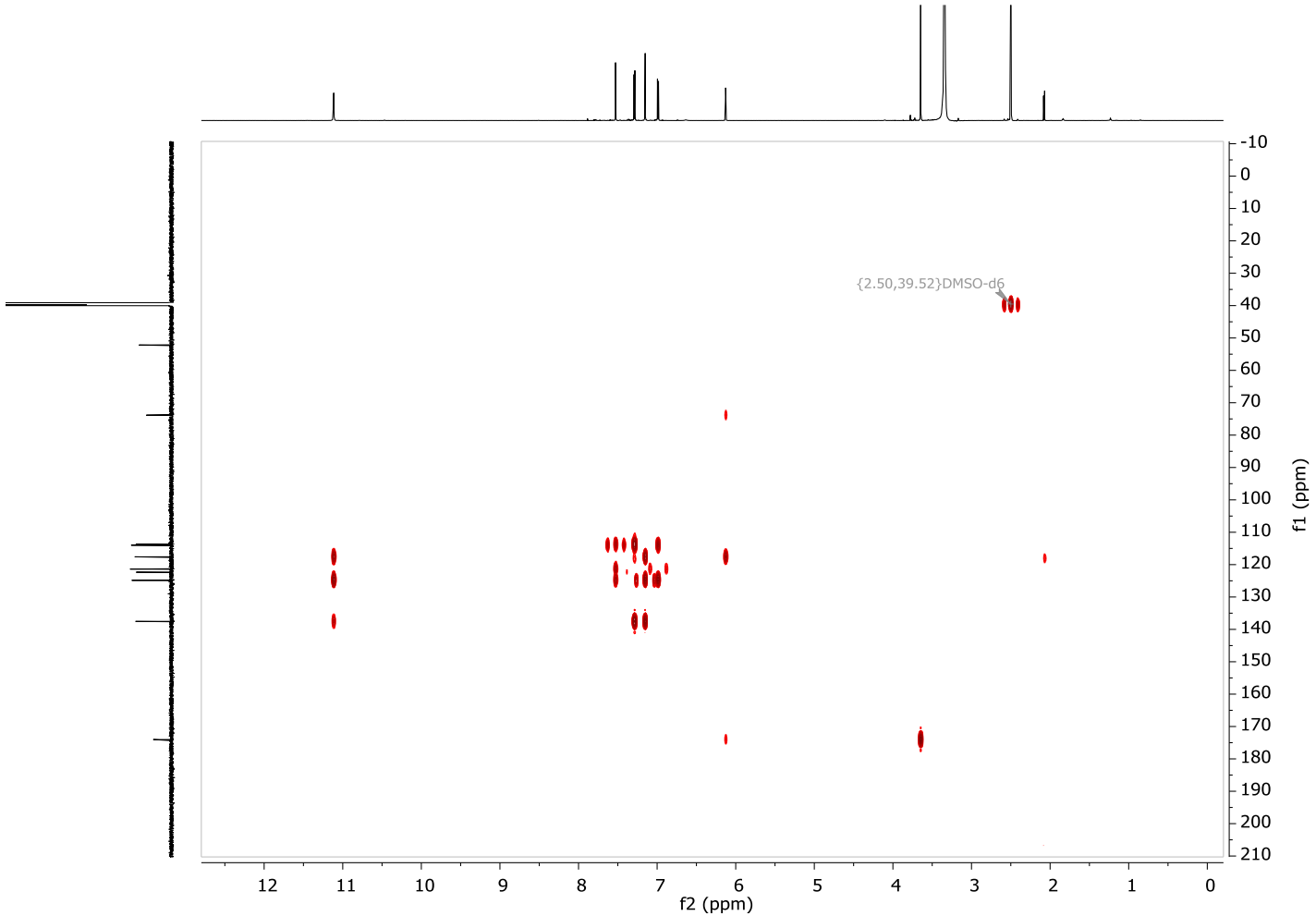


Figure S28. HMBC spectrum (500 MHz) of methyl 2,2-bis(6-bromo-1*H* -indol-3-yl)-2-hydroxyacetate (**17**) in DMSO-d_6

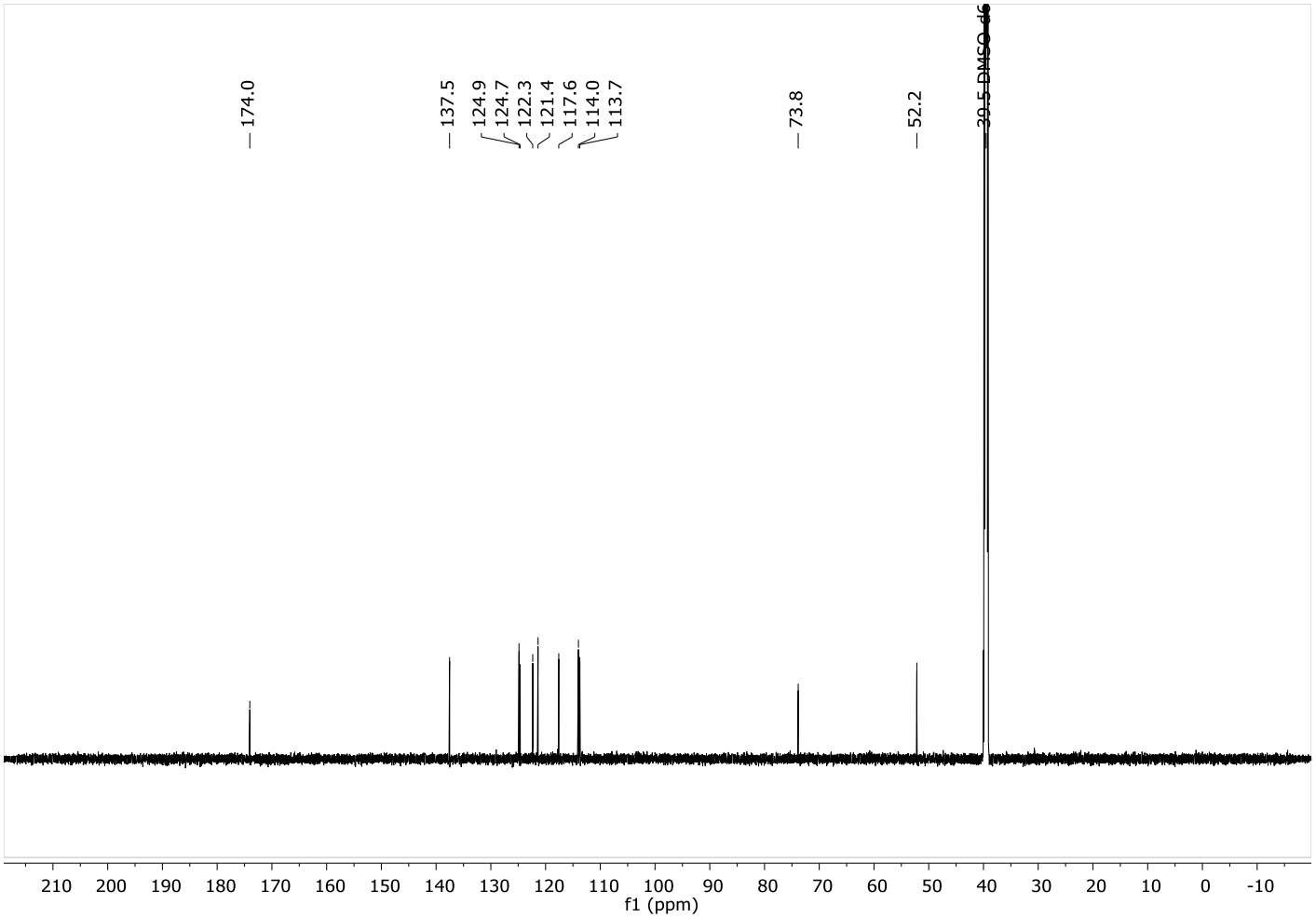


Figure S29. ^{13}C NMR spectrum (200 MHz) of methyl 2,2-bis(6-bromo-1*H* -indol-3-yl)-2-hydroxyacetate (**17**) in DMSO-d_6

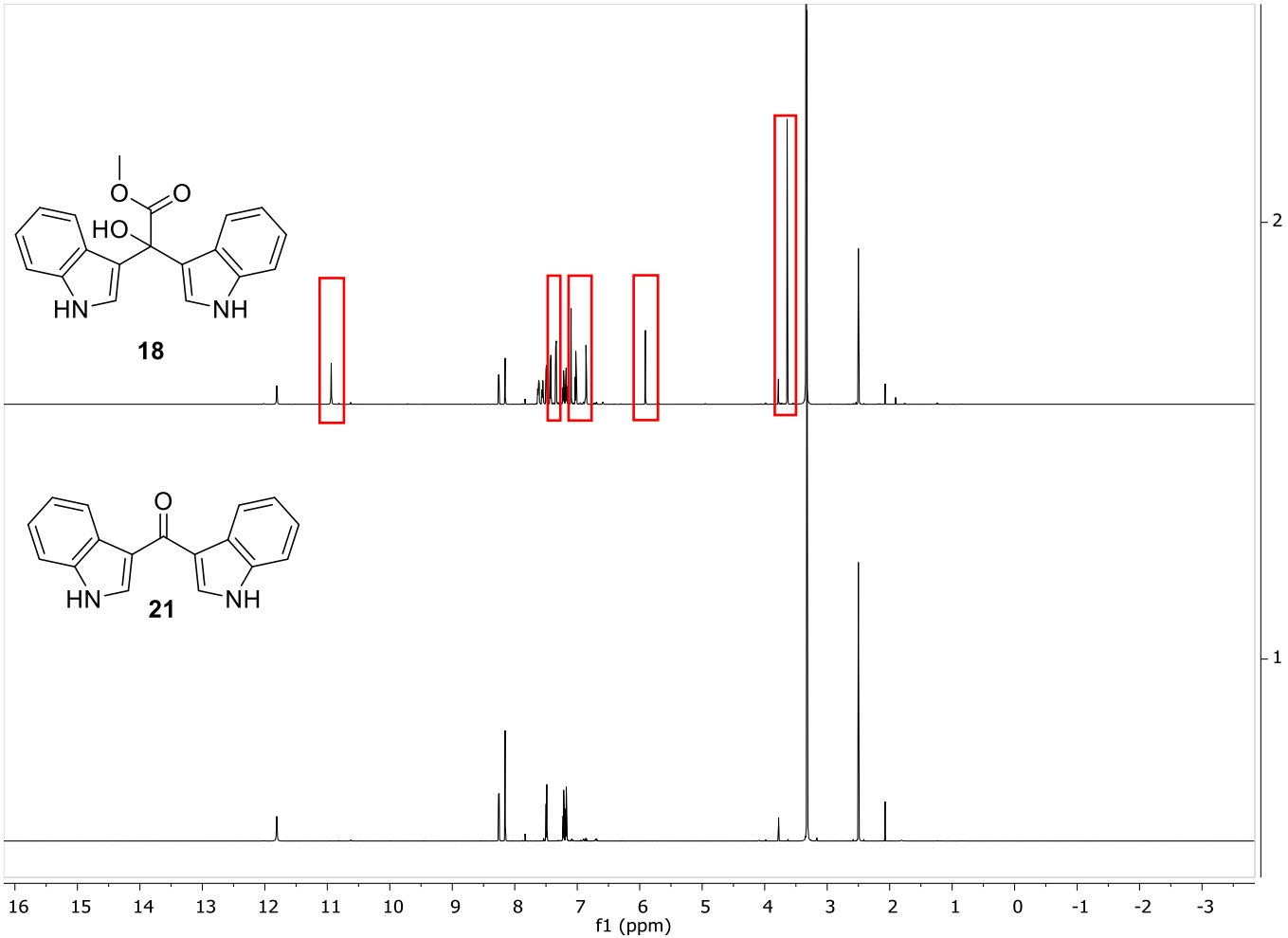


Figure S30. ^{1}H NMR spectrum (500 MHz) of fraction containing 2:1 methyl 2-hydroxy-2,2-di(1*H* -indol-3-yl)acetate (**18** - red highlighted) and di(1*H* -indol-3-yl)methanone (**21** - bottom spectrum) DMSO-d_6

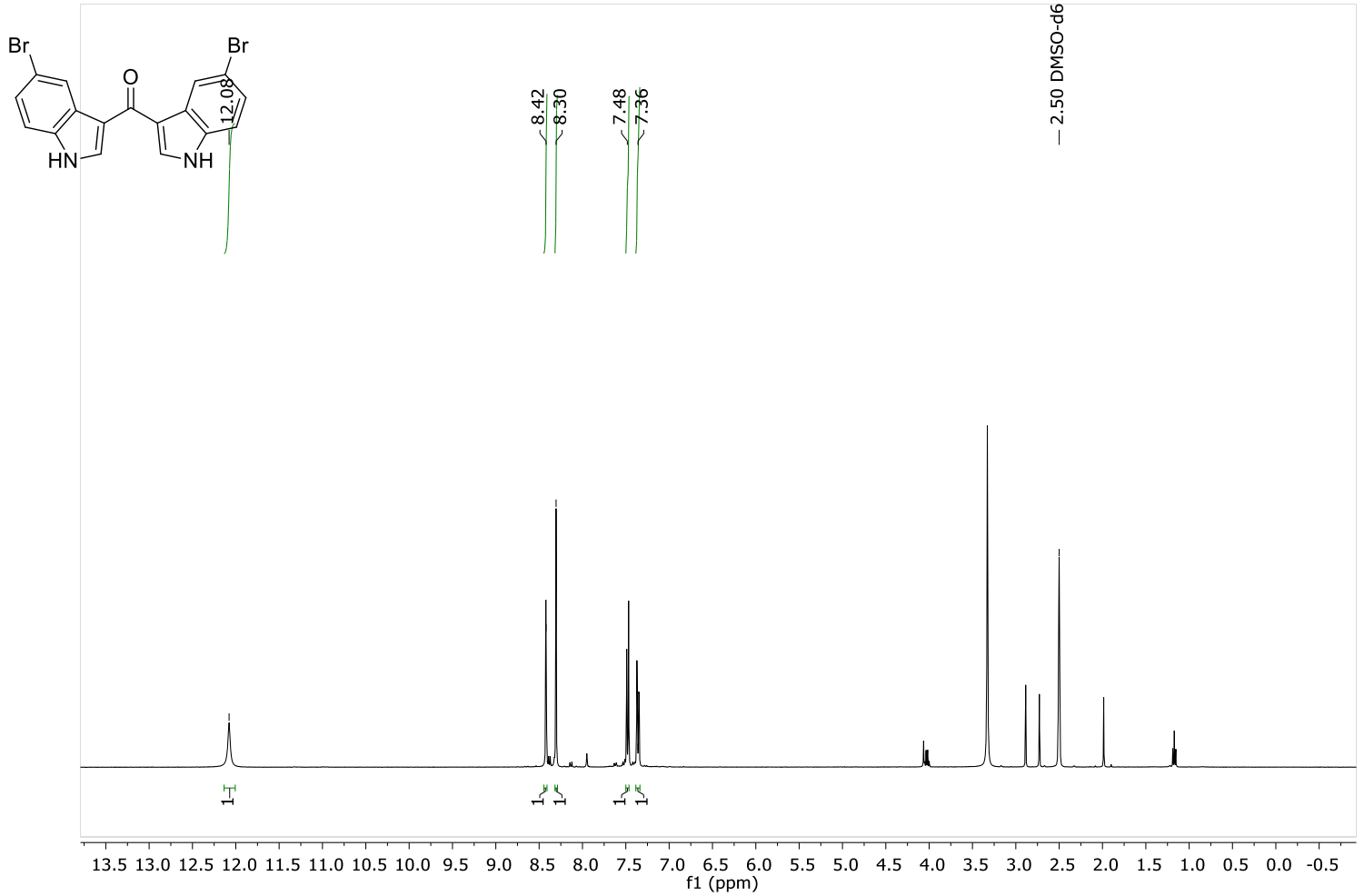


Figure S31. ^{1}H NMR spectrum (400 MHz) of bis(5-bromo-1*H* -indol-3-yl)methanone (**19**) in DMSO-d_6

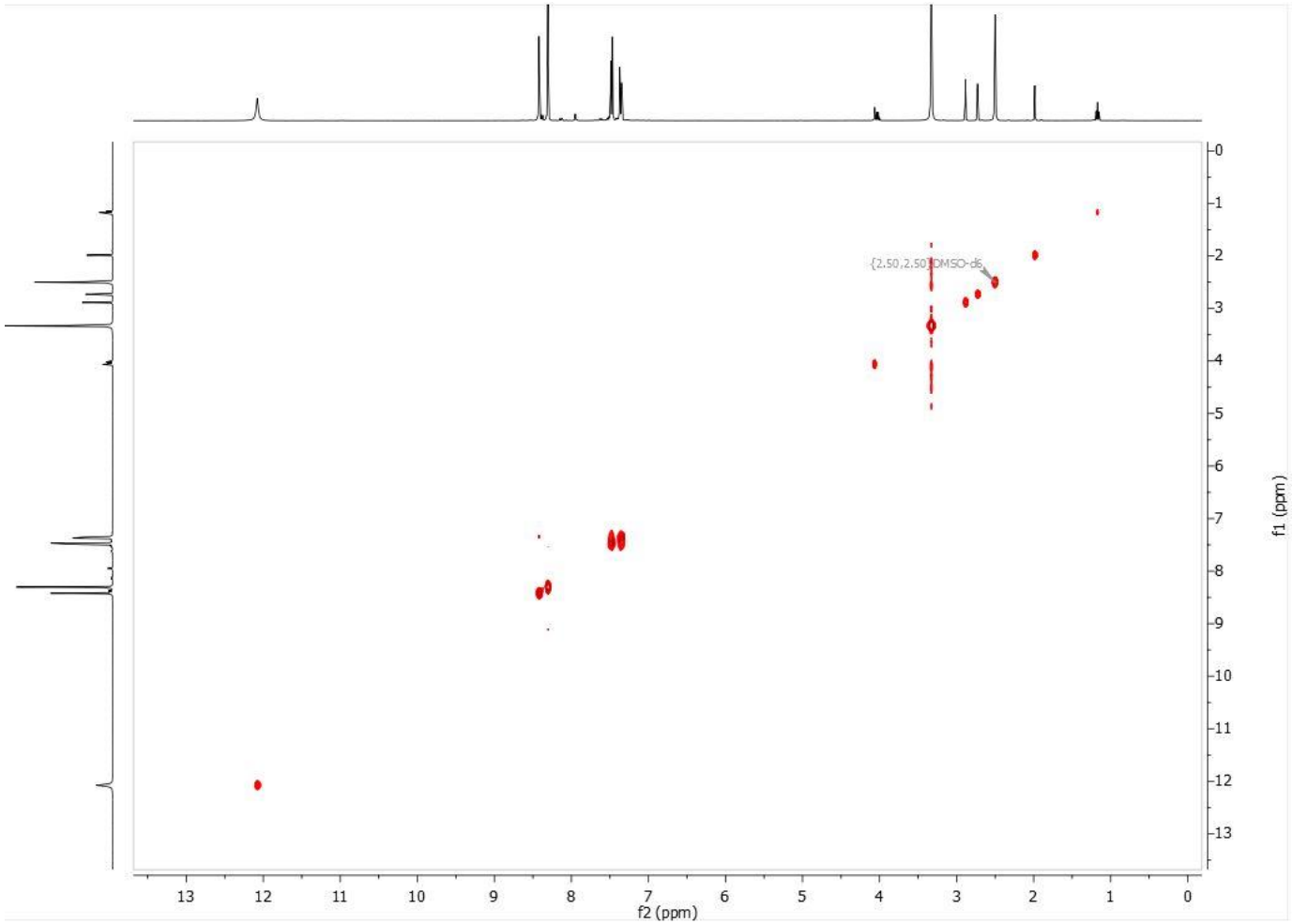


Figure S32. COSY NMR spectrum (400 MHz) of bis(5-bromo-1*H* -indol-3-yl)methanone (**19**) in DMSO-d_6

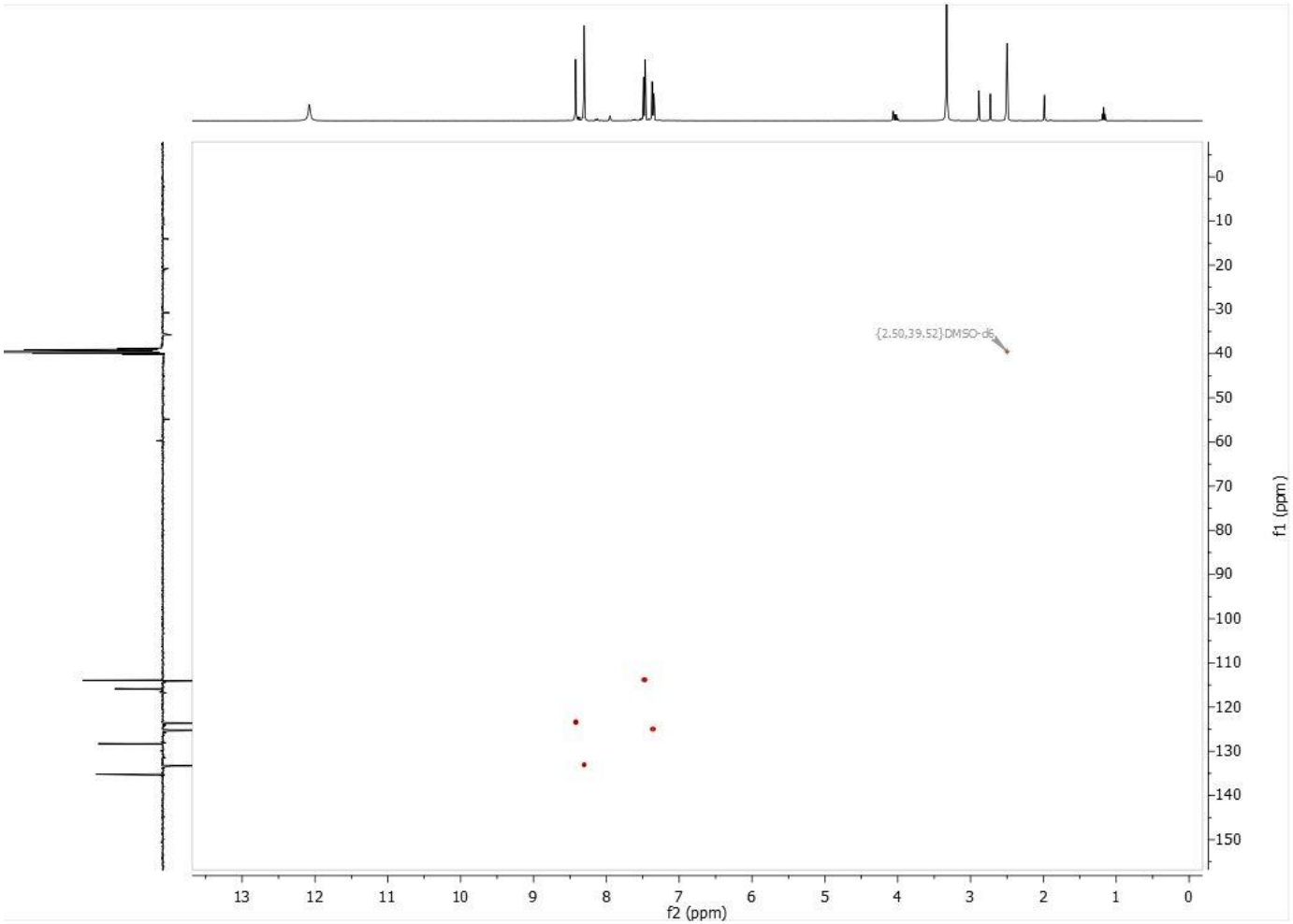


Figure S33. HSQC NMR spectrum (400 MHz) of bis(5-bromo-1*H* -indol-3-yl)methanone (**19**) in DMSO-d_6

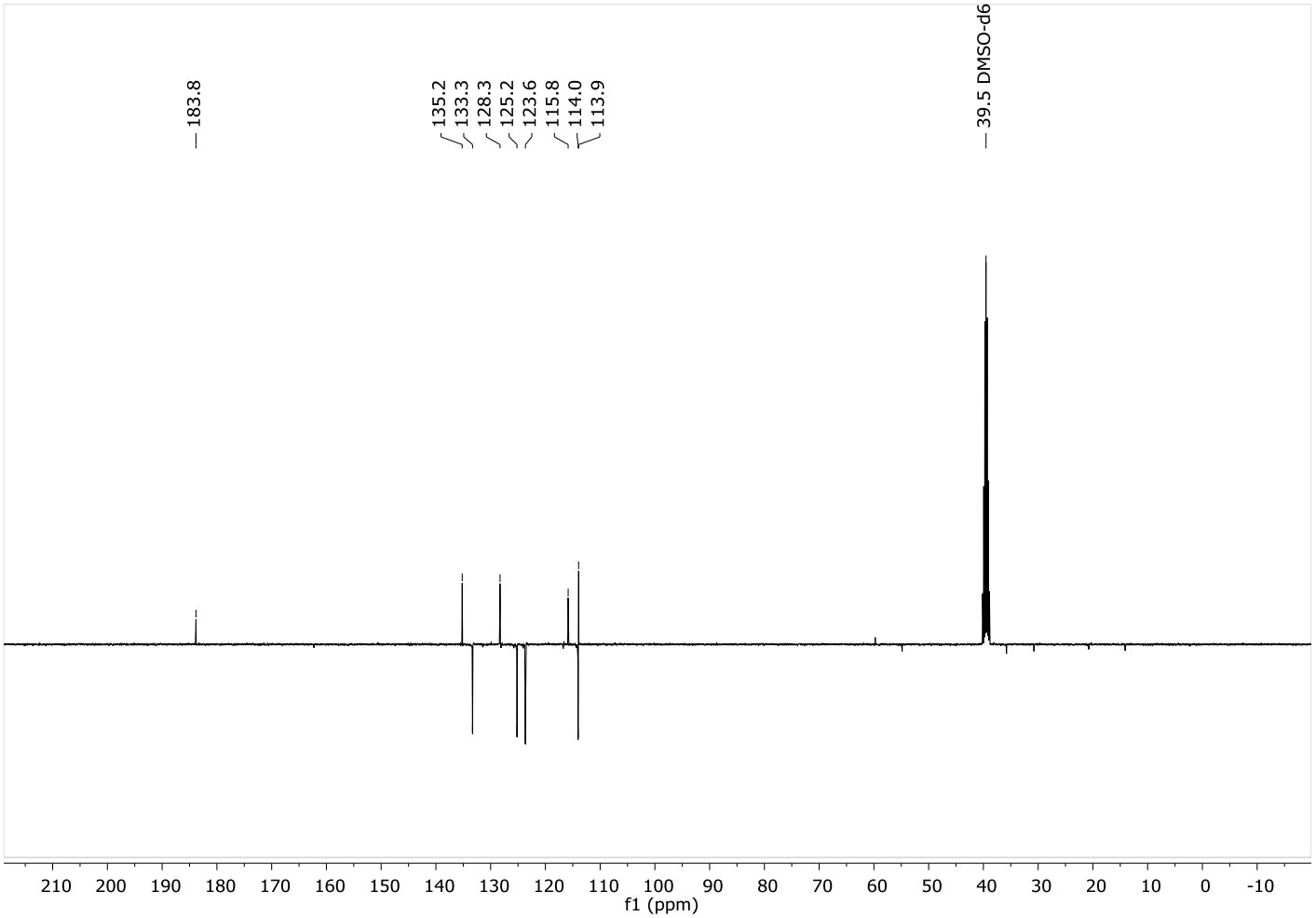


Figure S34. ^{13}C DEPT NMR spectrum (101 MHz) of bis(5-bromo-1*H* -indol-3-yl)methanone (**19**) in DMSO-d_6

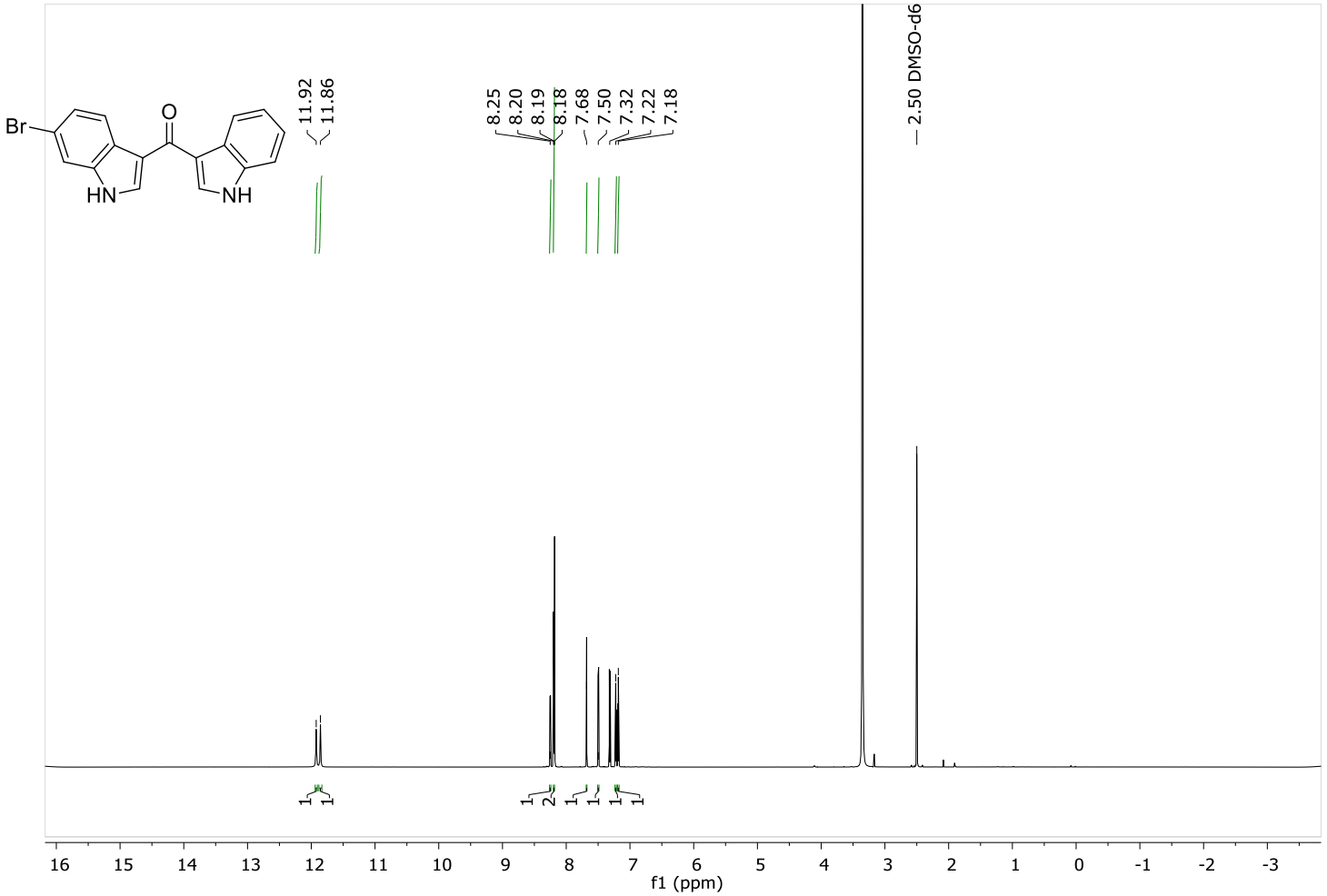


Figure S35. ^{1}H NMR spectrum (500 MHz) of (6-bromo-1*H* -indol-3-yl)(1*H* -indol-3-yl)methanone (**20**) in DMSO-d_6

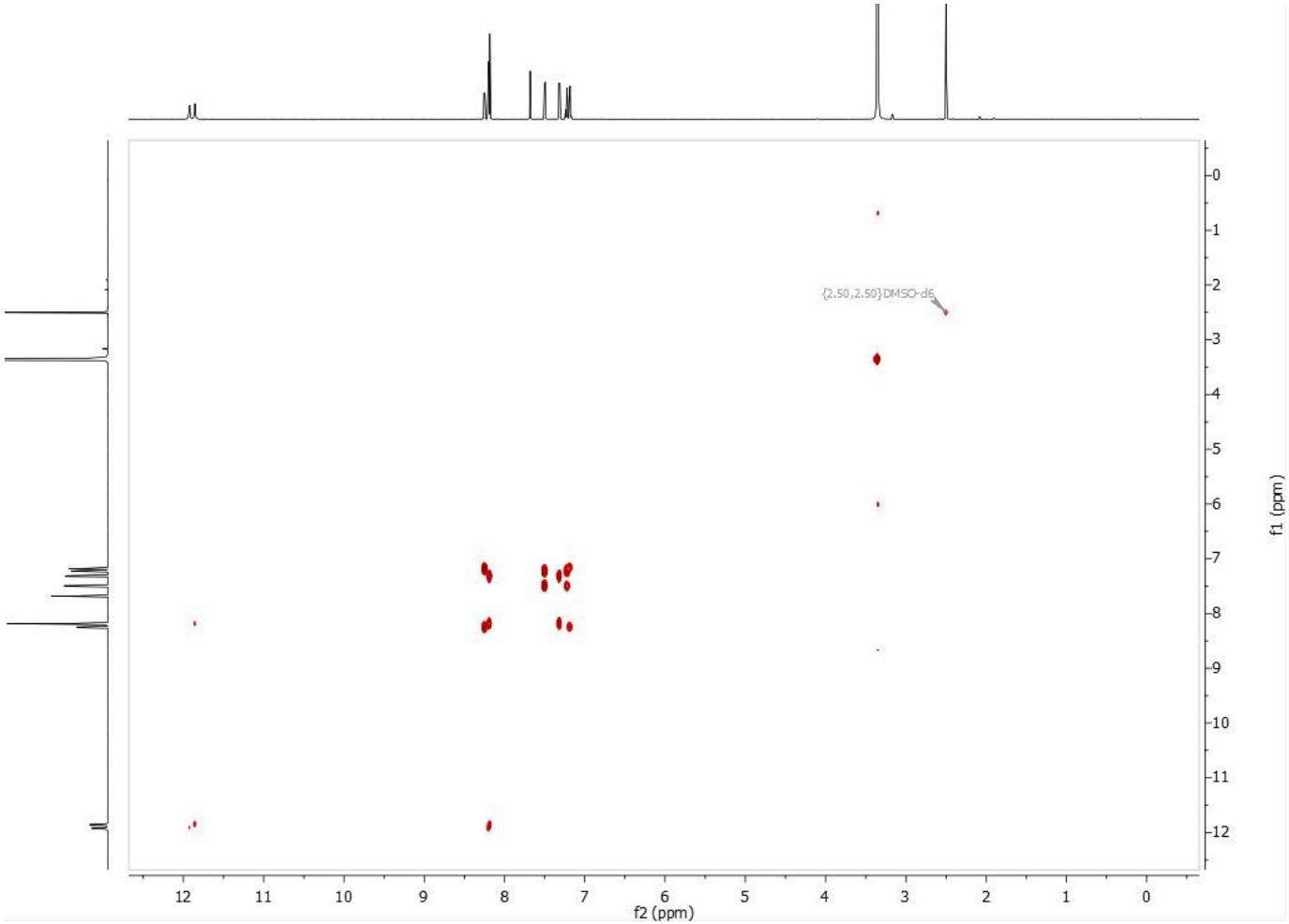


Figure S36. COSY NMR spectrum (500 MHz) of (6-bromo-1*H* -indol-3-yl)(1*H* -indol-3-yl)methanone (**20**) in DMSO-d_6

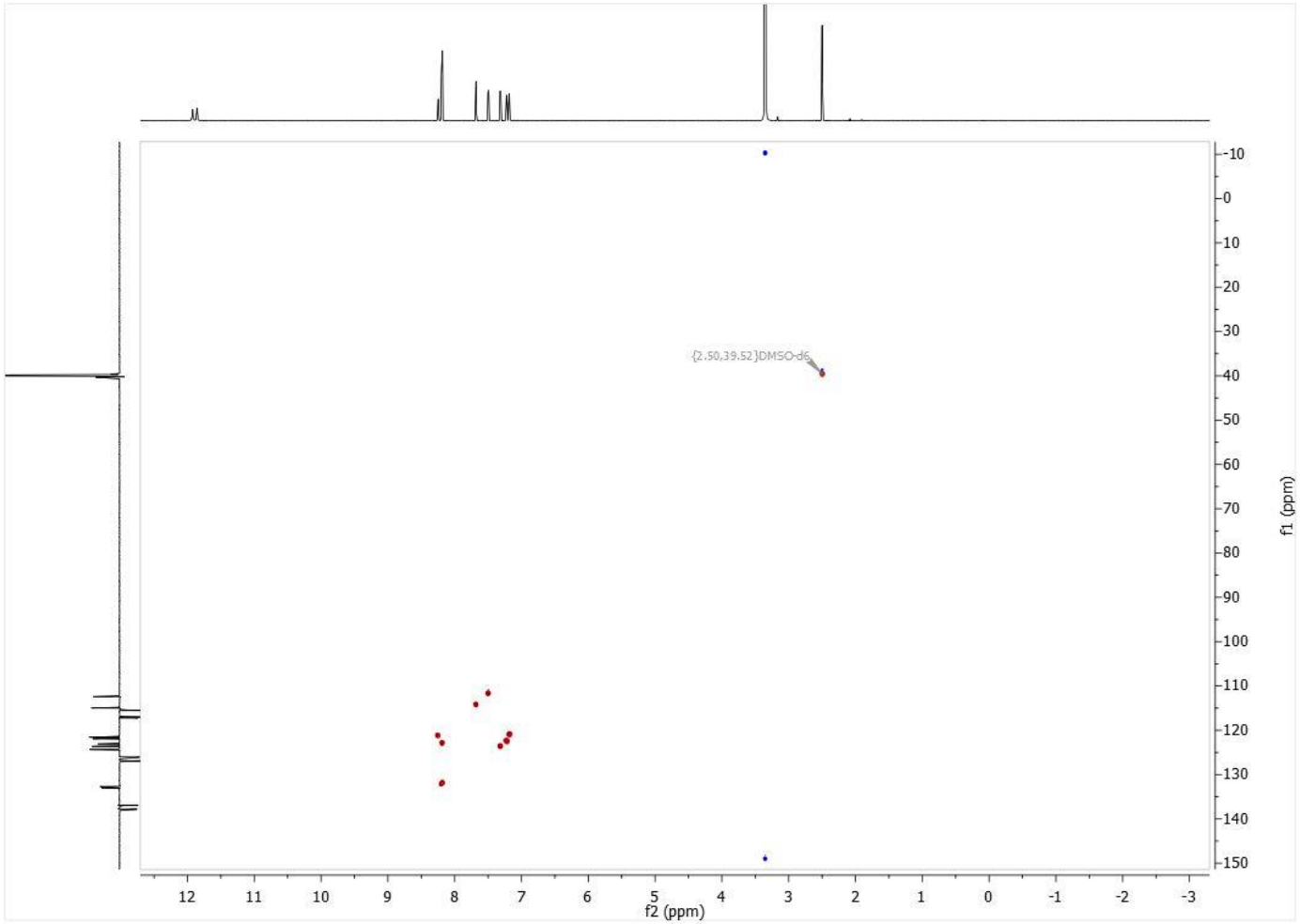


Figure S37. HSQC NMR spectrum (500 MHz) of (6-bromo-1*H* -indol-3-yl)(1*H* -indol-3-yl)methanone (**20**) in DMSO-d_6

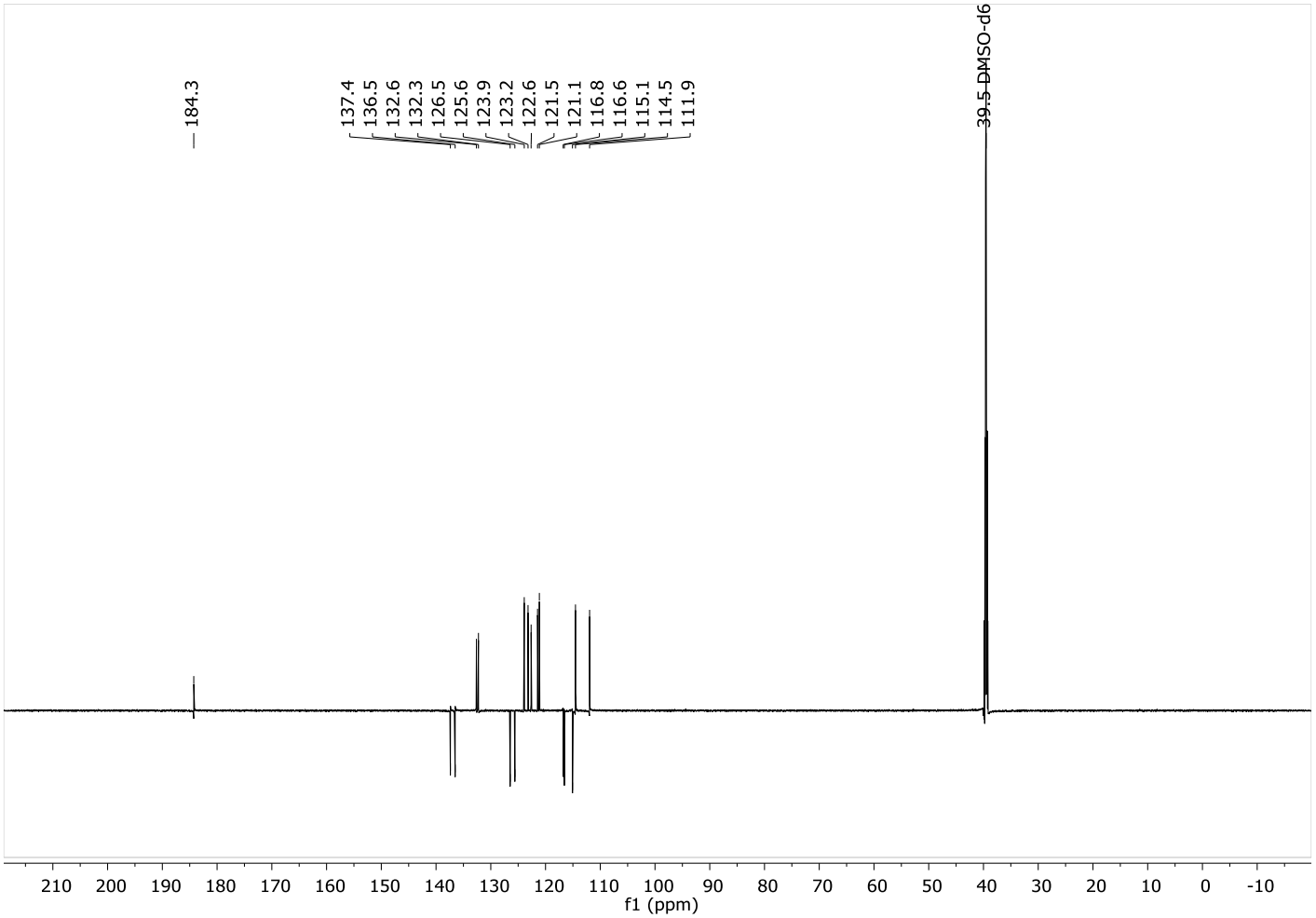


Figure S38. ^{13}C DEPT NMR spectrum (125 MHz) of (6-bromo-1*H* -indol-3-yl)(1*H* -indol-3-yl)methanone (**20**) in DMSO-d_6

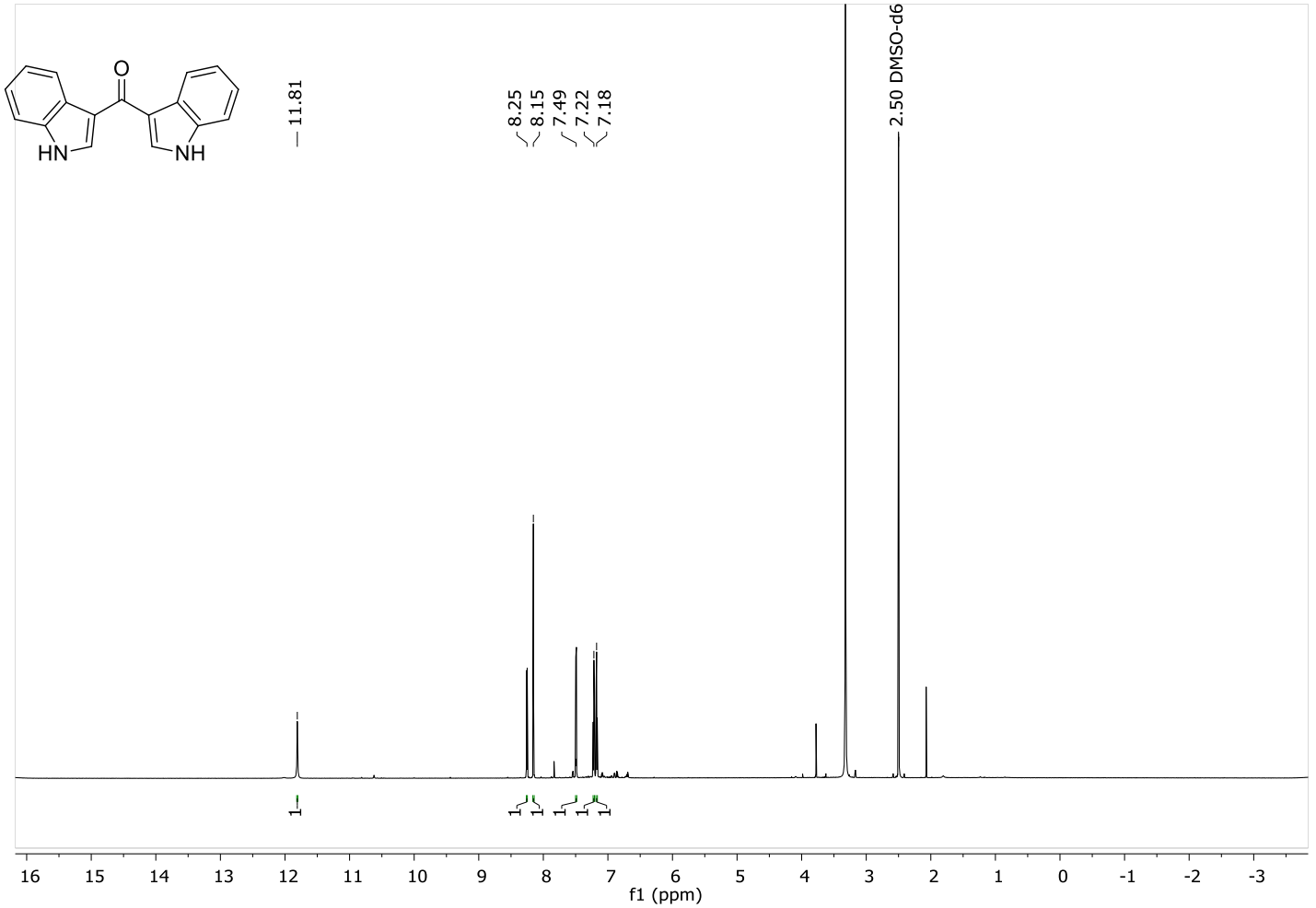


Figure S39. ^{1}H NMR spectrum (500 MHz) of di(1*H* -indol-3-yl)methanone (**21**) in DMSO-d_6

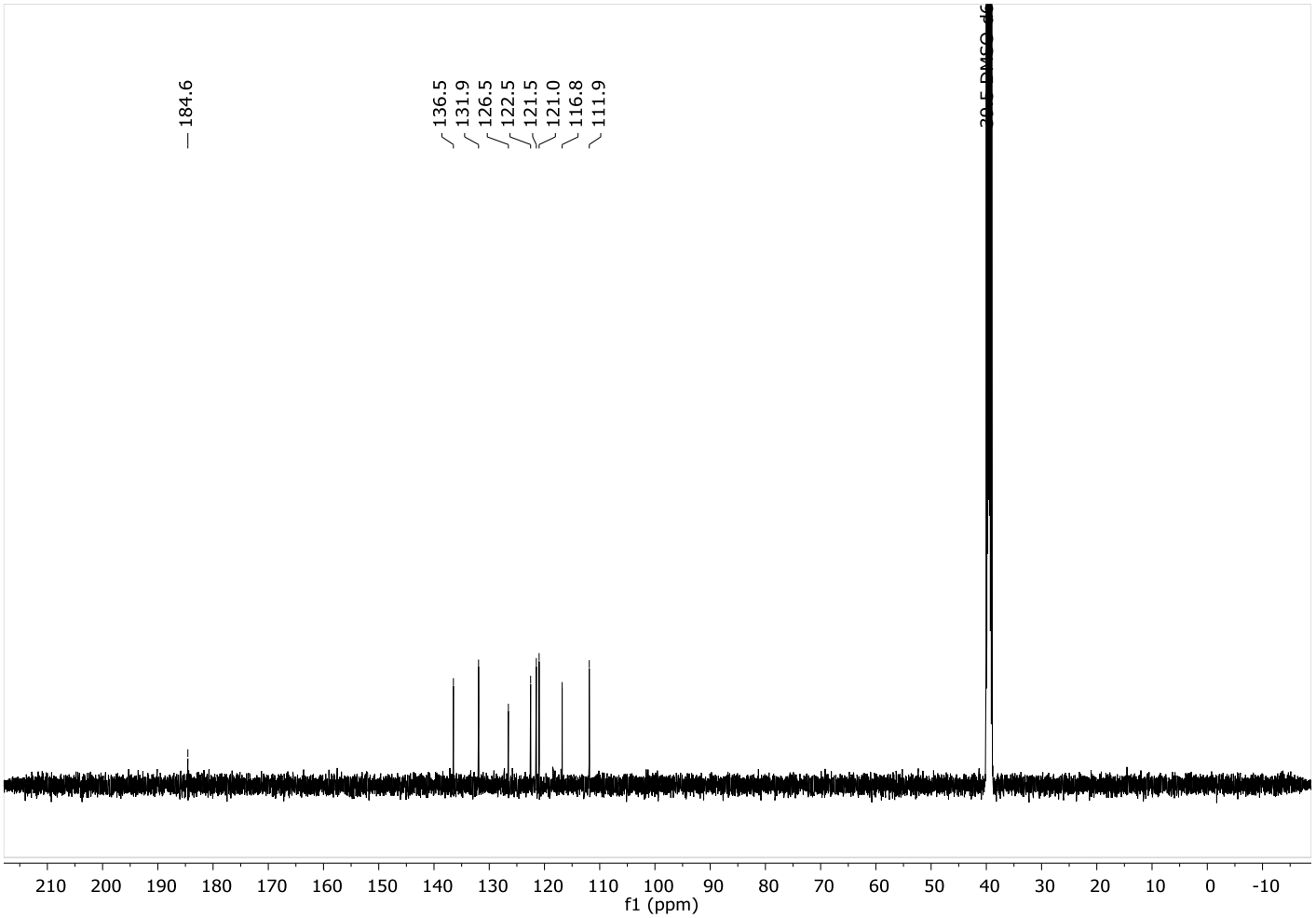


Figure S40. ^{13}C NMR spectrum (125 MHz) of di(1*H* -indol-3-yl)methanone (**21**) in DMSO-d_6

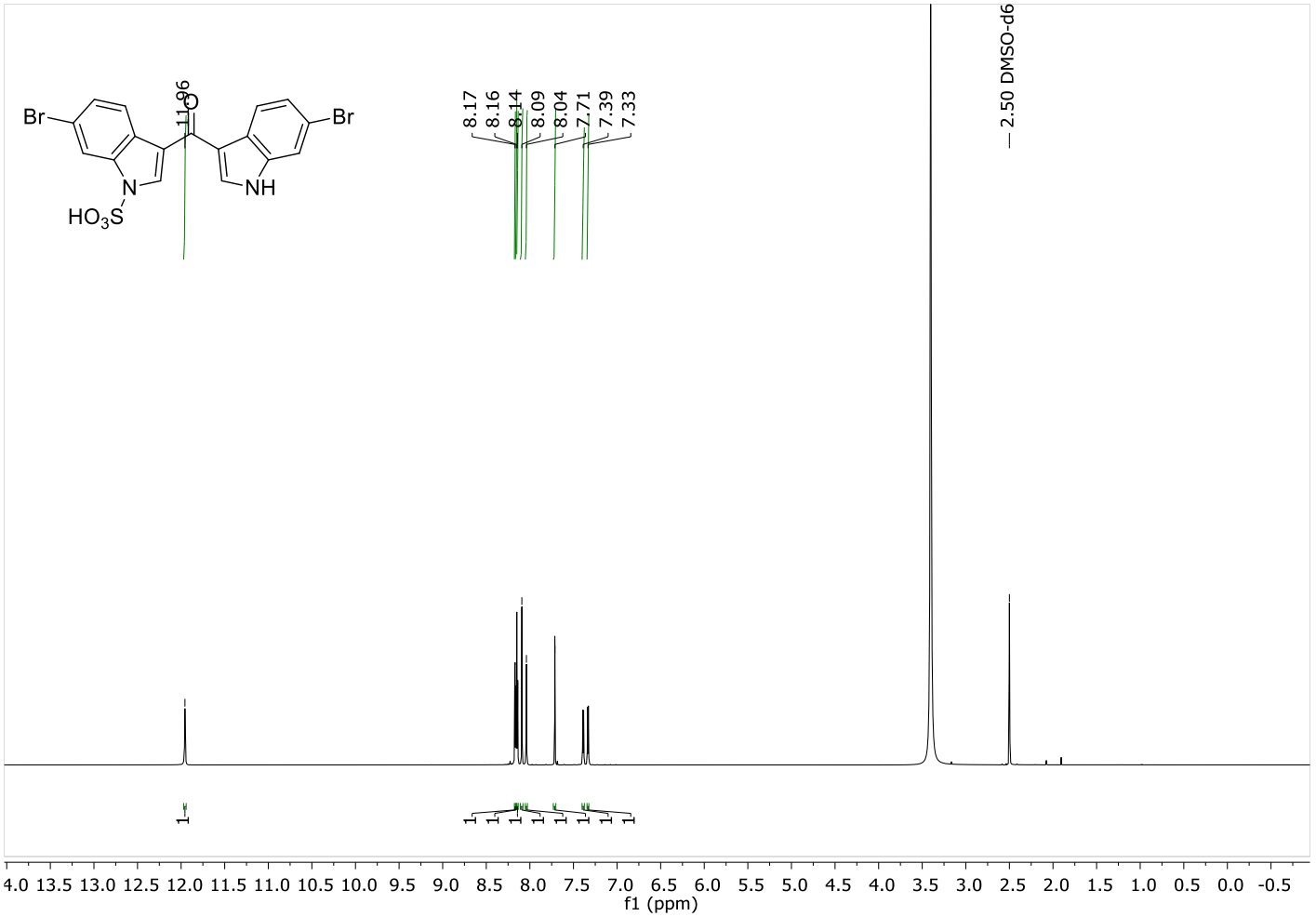


Figure S41. ^{1}H NMR spectrum (800 MHz) of synthetic echinosulfone A (**1**) in DMSO-d_6

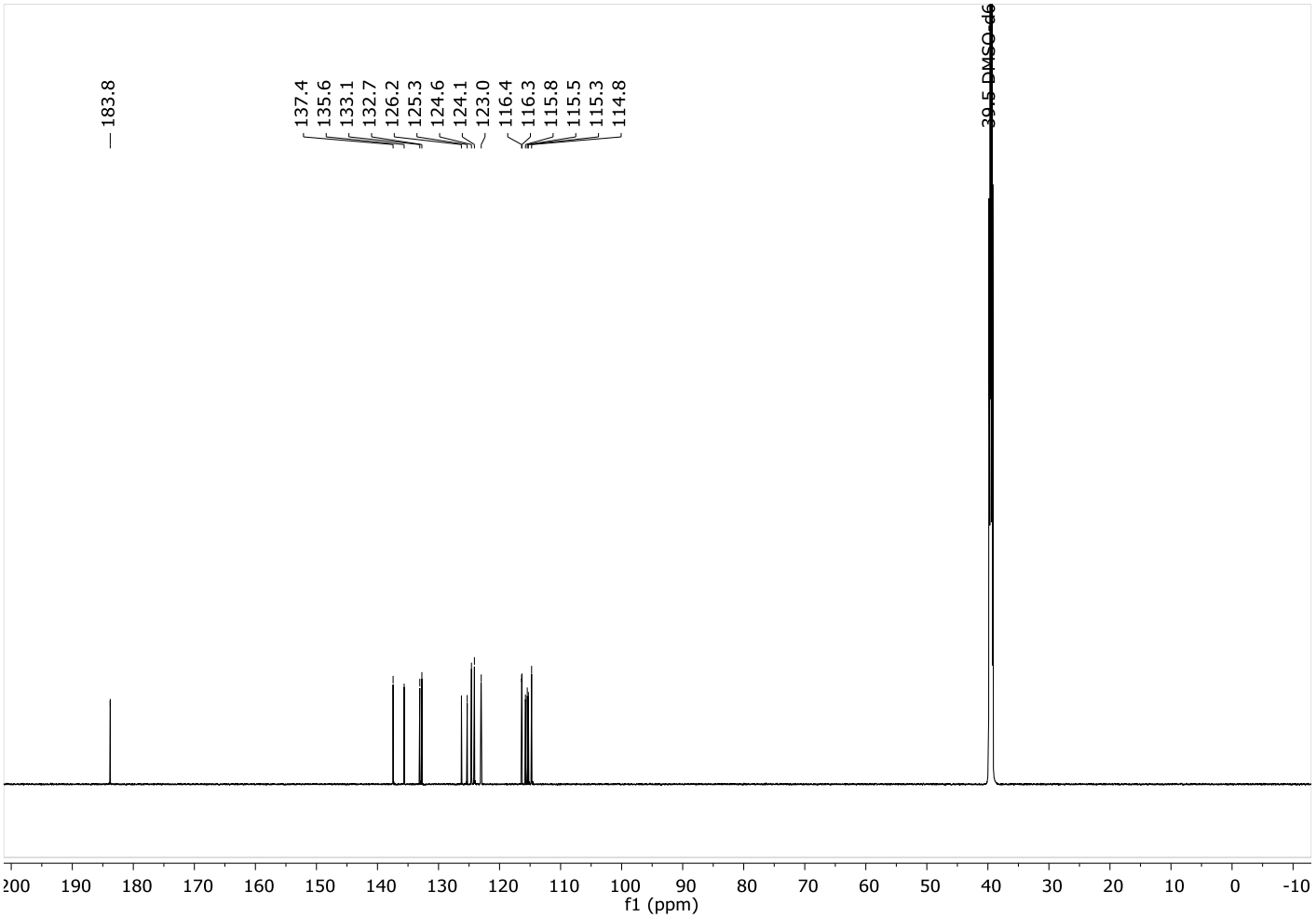


Figure S42. ^{13}C NMR spectrum (200 MHz) of echinosulfone A (**1**) in DMSO-d_6

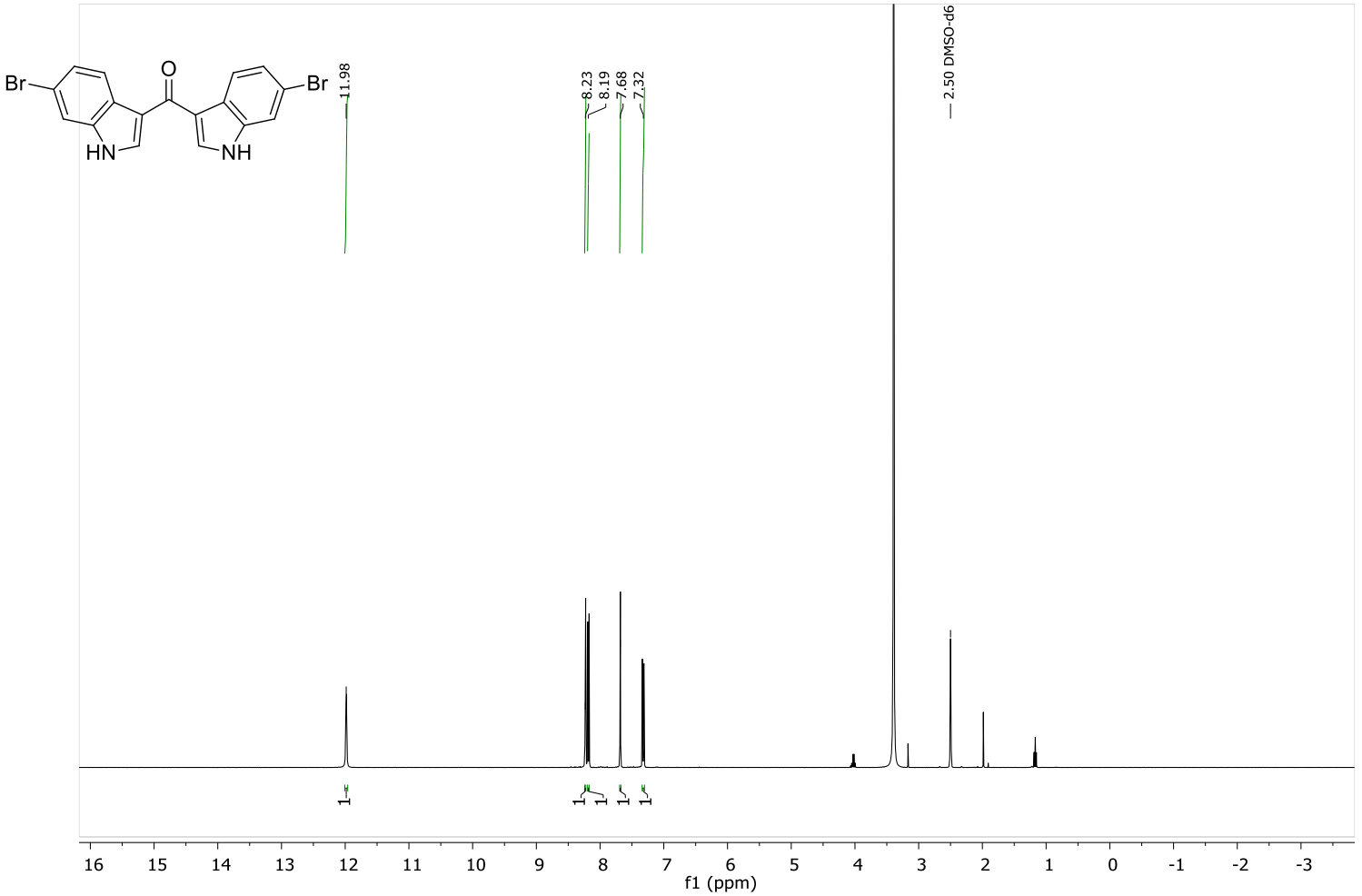


Figure S43. ^{1}H NMR spectrum (500 MHz) of bis(6-bromo-1*H* -indol-3-yl)methanone (**22**) in DMSO-d_6

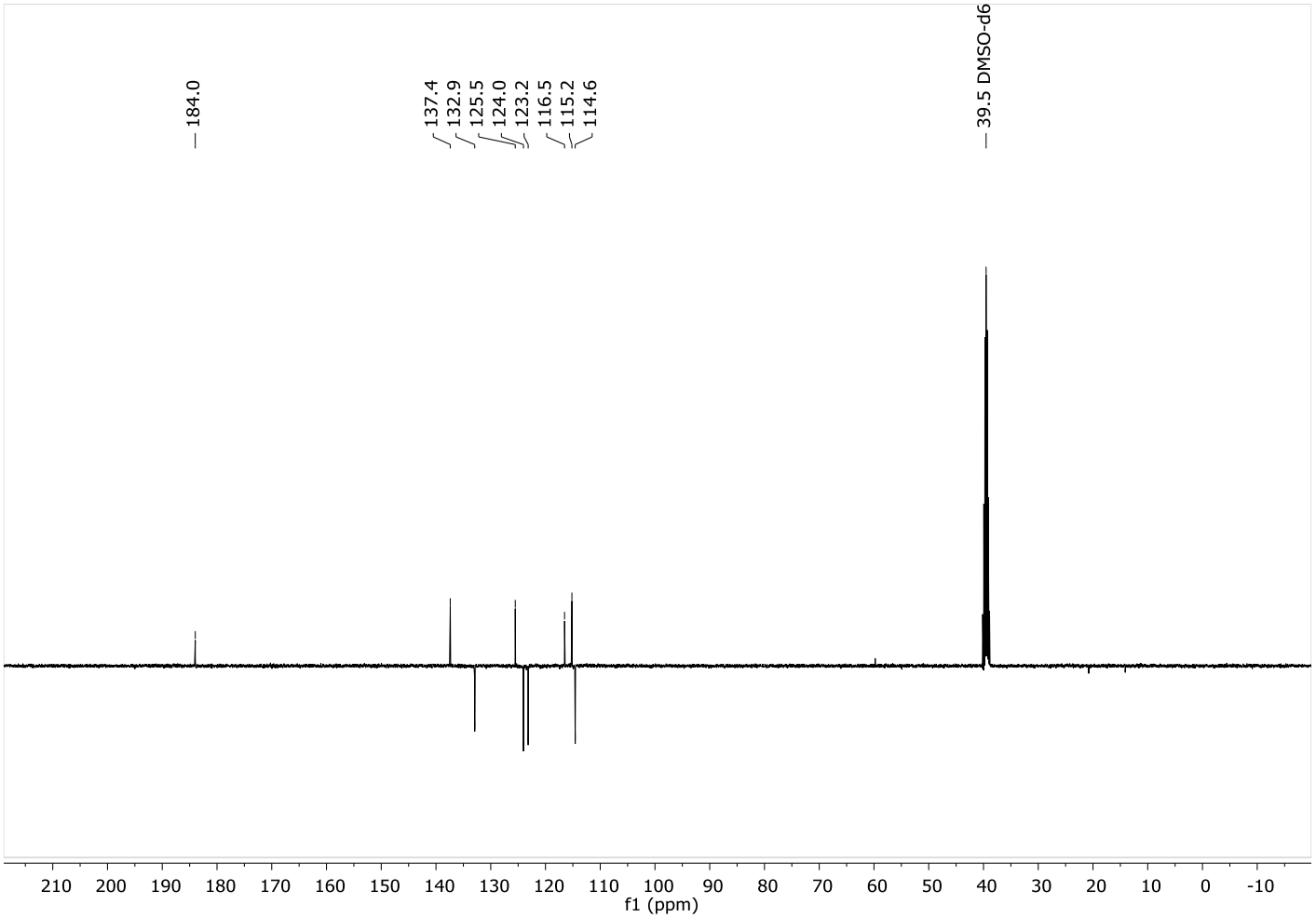


Figure S44. ^{13}C NMR spectrum (125 MHz) of echinosulfone A (**1**) bis(6-bromo-1*H* -indol-3-yl)methanone (**22**) in DMSO-d_6

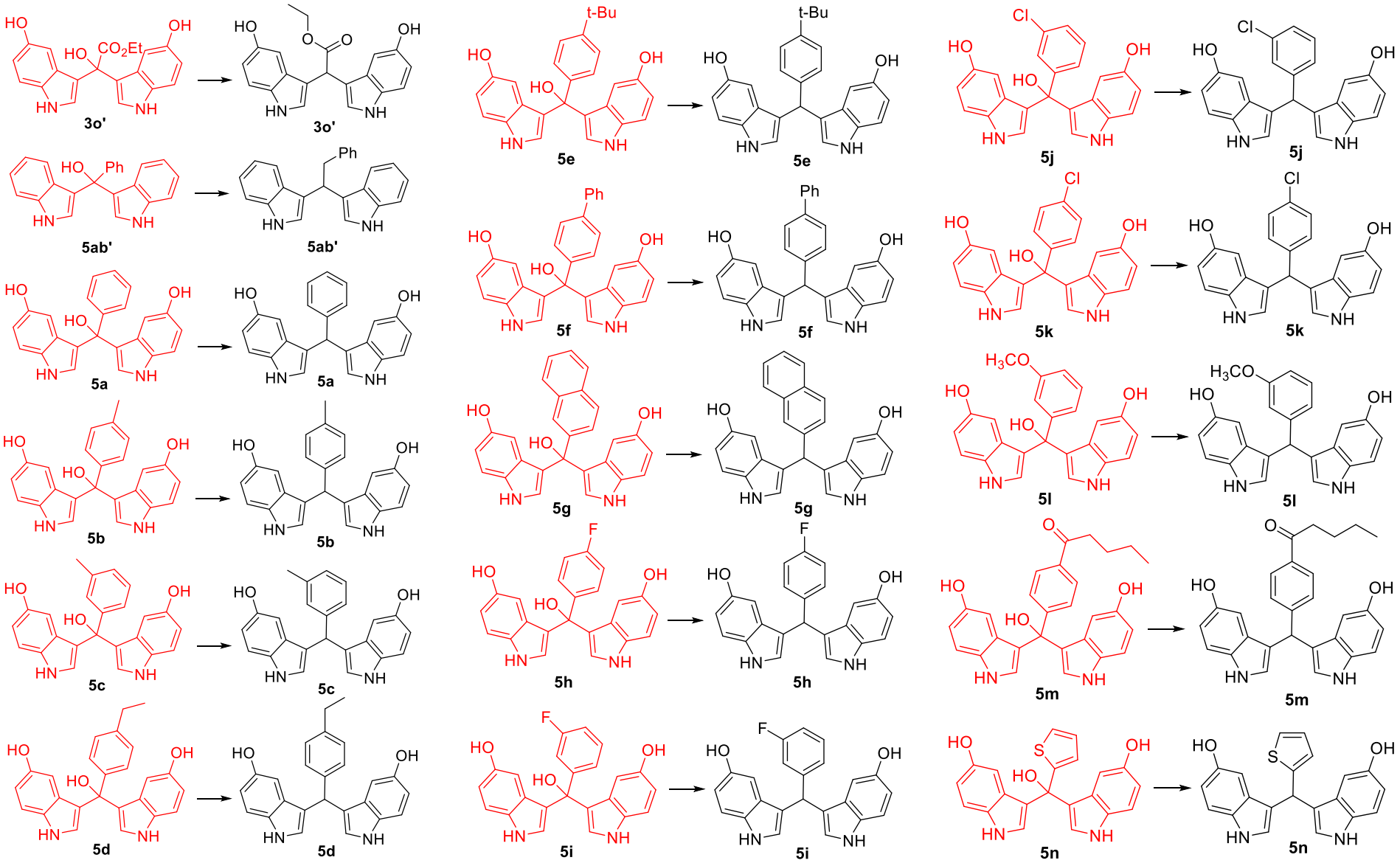


Figure S45. Incorrectly assigned synthetic α-hydroxy bis-indoles (red) and revised structures (black) for **3o'**, **5ab'**, and **5a-n**.[1]

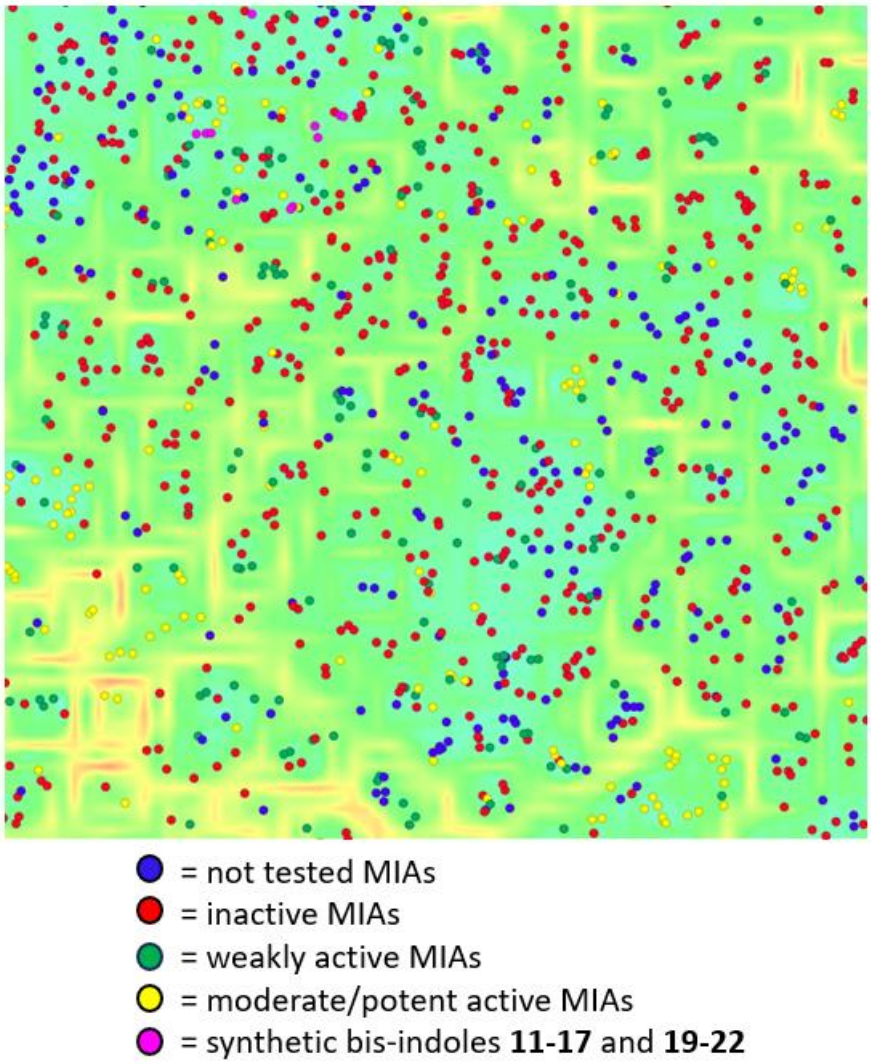
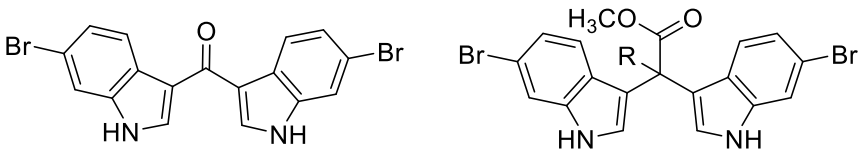


Figure S46. Chemical diversity of marine indole alkaloid (*n* = 2048) integrated with synthetic bis-indoles **11-17** and **19-22** visualized as 50 x 50 self-organizing map (SOM) using the Skelspheres 1024-bit chemical fingerprint descriptor. The reported biological activities for marine indole alkaloids (Holland and Carroll)[2] are coloured according to potency outlined in Table S1.[2,3]



22 **3a** R = OCH_3

Figure S47. Desulfonation products of echinosulfone A (**22**) and echinosulfonic acid B (**3a**) from Sala et al.[4]

Table S1. Bioactivity classifications used for cheminformatic analysis of marine indole alkaloids (adapted from Holland and Carroll,[2] and Carroll et al.)[3]

Bioactivity criteria	IC_{50}
Cytotoxic/antiparasitic/antioxidant/anti-inflammatory/antiviral/enzyme inhibitory*	
Potent	<0.1 μM
Moderate	0.1–1.0 μM
Weak	1.0–10 μM
Inactive	>10 μM
Antibacterial/Antifungal Activity	
	MIC
Potent	<1.0 μg/mL
Moderate	1.0–8.0 μg/mL
Weak	8.0–32.0 μg/mL
Inactive	>32.0 μg/mL

References.

(1) Li, Q.; Liang, X. X.; Zhang, W.; Han, M. Y. Friedel–Crafts Reaction of Acylsilanes: Highly Chemoselective Synthesis of 1-Hydroxy-Bis(Indolyl)Methanes and 1-Silyl-Bis(Indolyl)Methanes Derivatives. *Molecules* **2023**, *28* (15). https://doi.org/10.3390/molecules28155685.

(2) Holland, D. C.; Carroll, A. R. Marine Indole Alkaloid Diversity and Bioactivity. What Do We Know and What Are We Missing? *Natural Product Reports*. Royal Society of Chemistry February 15, 2023. https://doi.org/10.1039/d2np00085g.

(3) Carroll, A. R.; Copp, B. R.; Davis, R. A.; Keyzers, R. A.; Prinsep, M. R. Marine Natural Products. *Nat Prod Rep* **2022**, *39* (6), 1111–1368. https://doi.org/10.1039/d1np00076d.

(4) Sala, S.; Nealon, G. L.; Sobolev, A. N.; Fromont, J.; Gomez, O.; Flematti, G. R. Structure Reassignment of Echinosulfone A and the Echinosulfonic Acids A − D Supported by Single-Crystal X - Ray Di Ff Raction and Density Functional Theory Analysis. *J Nat Prod* **2020**. https://doi.org/10.1021/acs.jnatprod.9b00902.