Supplementary Information

for

Synthesis of Tris-Indolylmethanealkaloids by Harnessing the Nucleophilic Reactivity of Indole-BX and Studying Their Interactions with Hemoglobin

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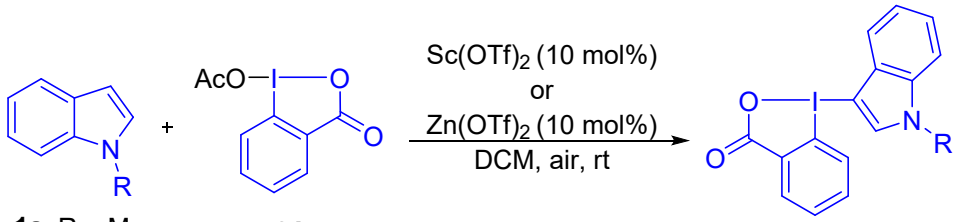
Content

1. General Information

^{1}H-NMR, ^{13}C-NMR spectrums were recorded on Bruker DPX-300 and Bruker DPX-400 using $CDCl_3$, DMSO–d^6 as solvents, respectively. ^{1}H-NMR data are reported as follows: δ, chemical shift; coupling constants (J are given in Hertz, Hz), and integration. Abbreviations to denote the multiplicity of a particular signal were s (singlet), brs (broad singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), dt (doublet of triplet) and m (multiplet). Chemical shifts were reported in ppm from the tetramethyl silane (TMS) with the solvent resonance as internal standards. Melting points were measured on a digital melting point apparatus and the temperature was uncorrected. High-resolution mass spectrometric measurements (HRMS) were performed by the waters quadruple time of flight mass spectrometer (XEVOG2 Q-TOF). All single crystal X-ray diffraction analyses study was performed using a Bruker D8 QUEST diffractometer. Reflection data were measured using graphite monochromatic MoKα radiation with a PHOTON-II detector. The collected data were reduced using the program APEX-III and an empirical absorption correction was carried out using SADABS. The structure was solved using direct methods and refined using the full-matrix least-squares method on F^2 using the WINGX software package and Olex2. The molecular graphics were created using mercury 3.8. All non-hydrogen atoms were refined with anisotropic parameters. All the CIF files of the compounds reported here have been deposited with the Cambridge Crystallographic Data Centre (CCDC). The glassware used for all the reactions was oven-dried. Unless otherwise indicated, all the chemicals and solvents were purchased from Sigma-Aldrich, Spectrochem, TCI, and BLDPharm, Rankem India pvt. Ltd. and used as such. Silica gel [(60-120, 230-400 mesh), Rankem, India] was used for chromatographic separation. Analytic grade solvents for the column chromatography and commercially available reagents were used as received.

2. Experimental procedures for the synthesis of Indole-BX reagents



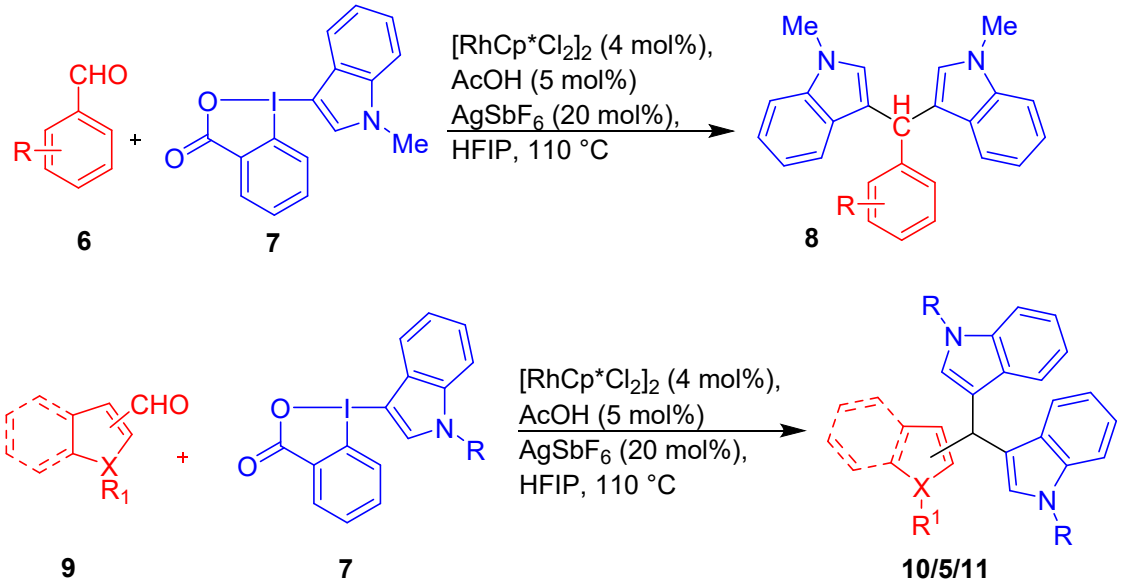
1a: R = Me
1b: R = H

2A

7a: R = Me
7b: R = H

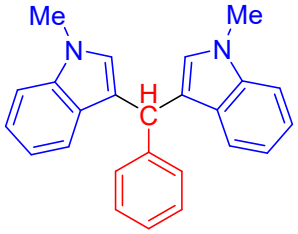
Following the reported procedure,[1] NH- and N-Methyl-Indole-BX reagents (**7a** and **7b**, 77% and 86% yield respectively) were prepared as a brown solid from Indole and its N-Methylated derivative (**1a**, **1b**) with IB-OAc (**2A**) in presence of 10 mol% $Sc(OTf)_2$ and $Zn(OTf)_2$ respectively. The values of the NMR spectra are in accordance with reported literature data.[1]

3. General procedure for the synthesis of *bis*- and *tris*-indolylmethane derivatives



To a clean oven-dried 15 mL sealed tube equipped with a magnetic stir bar were sequentially added aldehyde derivatives **6**/**9** (1.0 equiv.), $[RhCp^*Cl_2]_2$ (4 mol %), AcOH (5 mol %), $AgSbF_6$ (20 mol%). Then HFIP was added, followed by the addition of Indole-BX (3.0 equiv.) into the reaction mixture. The tube was tightly closed and placed in an oil bath at 110 °C and was stirred for 6-12 h according to the conversion estimated by TLC. After completion (monitoring by TLC), the reaction mixture was cooled to room temperature and quenched with saturated solution of $NaHCO_3$ and was diluted with EtOAc (3 x 20 mL). The combined organic extracts were dried over Na_2SO_4 and filtered. Concentration of the solution by rotary evaporation under reduced pressure gave a residue, which was purified by column chromatography using silica gel (60-120 mess) and ethyl acetate: hexanes from a range of (0.2: 9.8) to (3: 7) as eluent to afford the *bis*- and *tris*-indolylmethane product (**5**/**8**/**10**/**11**).

4. Characterization of products

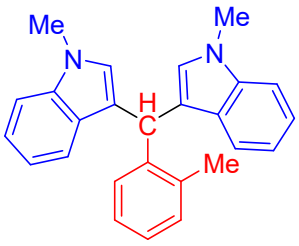


3,3'-(phenylmethylene)bis(1-methyl-1*H*-indole) (8a):

Following the general procedure, **8a** was isolated in 62% yield (41 mg, 0.12 mmol) from benzaldehyde **6a** (20 mg, 0.19 mmol) and **7a** (215 mg, 0.57 mmol); as light pink solid and the spectral data is in accordance with the literature.[2]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.34 (m, 4H), 7.31 – 7.26 (m, 4H), 7.23 – 7.19 (m, 3H), 7.00 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 2H), 6.54 (s, 2H), 5.89 (s, 1H), 3.69 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 144.4, 137.4, 128.7, 128.2, 128.2, 127.4, 125.9, 121.4, 120.0, 118.6, 118.2, 109.0, 40.1, 32.6.



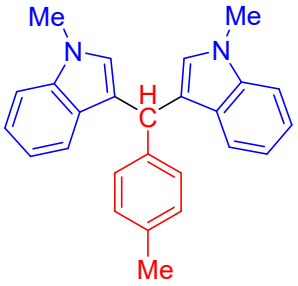
3,3'-(*o*-tolylmethylene)bis(1-methyl-1*H*-indole) (8b):

Following the general procedure, **8b** was isolated in 65% yield (40 mg, 0.11 mmol) from 2-methyl-benzaldehyde **6b** (20 mg, 0.17 mmol) and **7a** (192 mg, 0.51 mmol); as red solid and the spectral data is in accordance with the literature.[2]

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (ddt, *J* = 15.6, 8.3, 1.0 Hz, 4H), 7.23 – 7.10 (m, 5H), 7.07 – 6.97 (m, 3H), 6.44 (s, 2H), 6.02 (s, 1H), 3.67 (s, 6H), 2.38 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 142.5, 137.4, 135.9, 130.1, 128.5, 128.3, 127.5, 125.9, 125.7, 121.3, 119.8, 118.5, 117.7, 109.0, 35.9, 32.6, 19.6.

^{13}C NMR (75 MHz, $CDCl_3$) (DEPT-135) δ 130.1, 128.5, 128.4, 125.9, 125.8, 121.4, 119.9, 118.6, 109.0, 36.0, 32.7, 19.6.

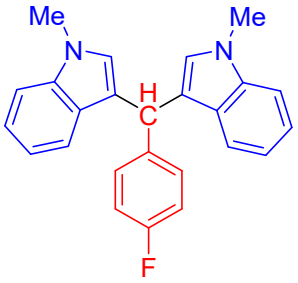


3,3'-(*p*-tolylmethylene)bis(1-methyl-1*H*-indole) (8c):

Following the general procedure, **8c** was isolated in 70% yield (43 mg, 0.12 mmol) from 4-methyl-benzaldehyde **6c** (20 mg, 0.17 mmol) and **7a** (192 mg, 0.51 mmol); as orange solid and the spectral data is in accordance with the literature.[2]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.26 – 7.19 (m, 3H), 7.10 (d, *J* = 8.0 Hz, 2H), 7.03 – 6.99 (m, 2H), 6.55 (s, 2H), 5.86 (s, 1H), 3.69 (s, 6H), 2.34 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 141.4, 137.4, 135.3, 128.9, 128.5, 128.2, 127.5, 121.3, 120.0, 118.6, 118.4, 108.9, 39.6, 32.6, 21.2.



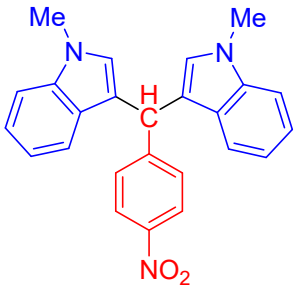
3,3'-((4-fluorophenyl)methylene)bis(1-methyl-1*H*-indole) (8d):

Following the general procedure, **8d** was isolated in 60% yield (35 mg, 0.09 mmol) from 4-fluoro-benzaldehyde **6d** (20 mg, 0.16 mmol) and **7a** (181 mg, 0.48 mmol); as light pink solid and the spectral data is in accordance with the literature.[2]

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (dt, *J* = 7.9, 1.0 Hz, 2H), 7.33 – 7.28 (m, 4H), 7.22 (ddt, *J* = 8.2, 6.9, 1.2 Hz, 2H), 7.04 – 6.94 (m, 4H), 6.52 (s, 2H), 5.88 (s, 1H), 3.69 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.5, 160.1, 140.1, 140.1, 137.4, 130.0, 129.9, 128.2, 127.3, 121.5, 119.93, 118.7, 118.1, 115.0, 114.8, 109.1, 39.3, 32.6.

^{19}F NMR (377 MHz, $CDCl_3$) δ -117.54.

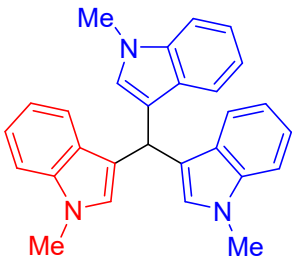


3,3'-((4-nitrophenyl)methylene)bis(1-methyl-1*H*-indole) (8e):

Following the general procedure, **8e** was isolated in 58% yield (30 mg, 0.07 mmol) from 4-nitro-benzaldehyde **6e** (20 mg, 0.13 mmol) and **7a** (150 mg, 0.40 mmol); as yellow solid and the spectral data is in accordance with the literature.[2]

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.8 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 2H), 7.34 (ddt, *J* = 8.5, 3.9, 1.0 Hz, 4H), 7.26 – 7.21 (m, 2H), 7.03 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 2H), 6.55 (s, 2H), 5.99 (s, 1H), 3.71 (s, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 152.3, 146.5, 137.4, 129.4, 128.3, 127.0, 123.6, 121.8, 119.6, 119.0, 116.6, 109.3, 40.1, 32.7.

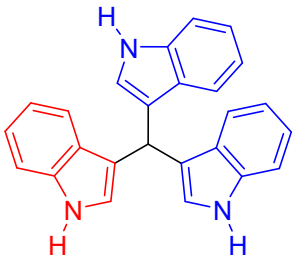


tris(1-methyl-1*H*-indol-3-yl)methane (10):

Following the general procedure, **10** was isolated in 56% yield (29 mg, 0.07 mmol) from 1-methyl-1*H*-indole-3-carbaldehyde **9a** (20 mg, 0.12 mmol) and **7a** (136 mg, 0.36 mmol); as orange solid and the spectral data is in accordance with the literature.[3]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, *J* = 7.6 Hz, 3H), 7.29 (d, *J* = 8.4 Hz, 3H), 7.19 (t, *J* = 7.6 Hz, 3H), 6.99 (t, *J* = 7.4 Hz, 3H), 6.64 (s, 3H), 6.16 (s, 1H), 3.66 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 137.4, 127.9, 127.5, 121.1, 120.1, 118.4, 118.2, 108.94, 32.6, 29.7.



tri(1*H*-indol-3-yl)methane (5):

Following the general procedure, **5** was isolated in 44% yield (22 mg, 0.06 mmol) from 1H-indole-3-carbaldehyde **9b** (20 mg, 0.14 mmol) and **7b** (152 mg, 0.42 mmol); as orange solid and the spectral data is in accordance with the literature.[3]

^{1}H NMR (300 MHz, *d*^{6}-DMSO) δ 10.70 (s, 3H), 7.39 (d, *J* = 7.8 Hz, 3H), 7.33 (dt, *J* = 8.1, 1.0 Hz, 3H), 7.01 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 3H), 6.94 – 6.93 (m, 3H), 6.84 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 3H), 6.04 (s, 1H).

^{13}C NMR (101 MHz, *d*^{6}-DMSO) δ 137.0, 127.2, 123.7, 121.1, 119.7, 118.7, 118.4, 111.8, 31.4.

3,3'-((1-methyl-1*H*-pyrrol-2-yl)methylene)bis(1-methyl-1*H*-indole) (11):

Following the general procedure, **11** was isolated in 18% yield (12 mg, 0.03 mmol) from 1-methyl-1*H*-pyrrole-2-carbaldehyde **9c** (20 mg, 0.18 mmol) and **7a** (207 mg, 0.55 mmol); as off-white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dt, *J* = 8.0, 1.0 Hz, 3H), 7.30 – 7.28 (m, 3H), 7.21 – 7.17 (m, 3H), 7.01 – 6.97 (m, 2H), 6.64 (s, 2H), 6.16 (s, 1H), 3.66 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 137.4, 127.9, 127.5, 121.2, 120.2, 118.4, 118.2, 108.9, 32.6, 30.8. (3 additional peak at aliphatic due to trace amount of Ethyl acetate solvent; total number of carbon less due to merge at aromatic region)

HRMS (ESI) *m/z*: $[M+H]^+$ calculated for $C_{24}H_{23}N_3$: 354.1970, found: 354.1965.

5. Biology study details

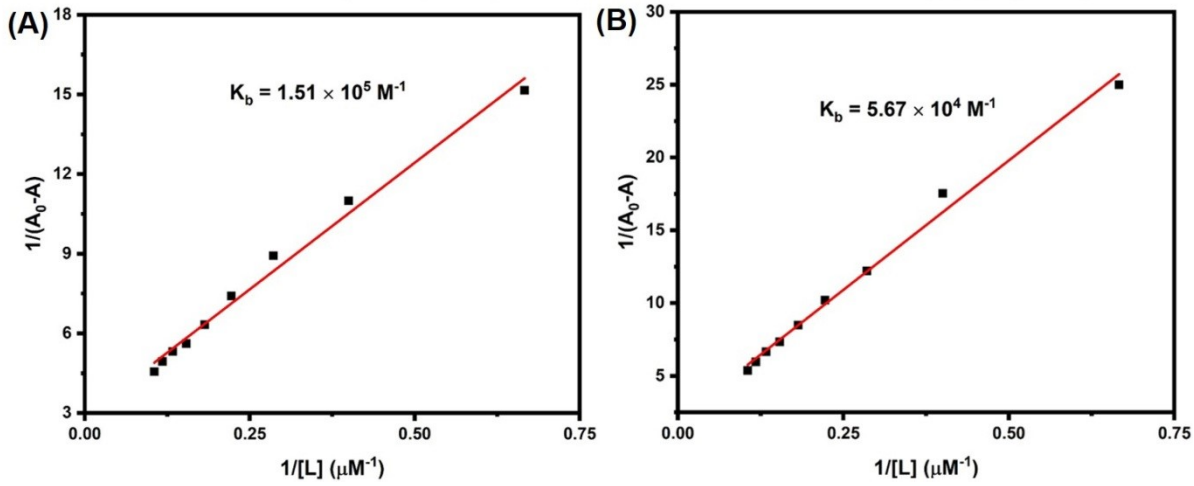


Figure S1. Modified Benesi–Hildebrand equation for determining the binding constant between (A) Hb and TIM 10 and (B) Hb and TIM 5.

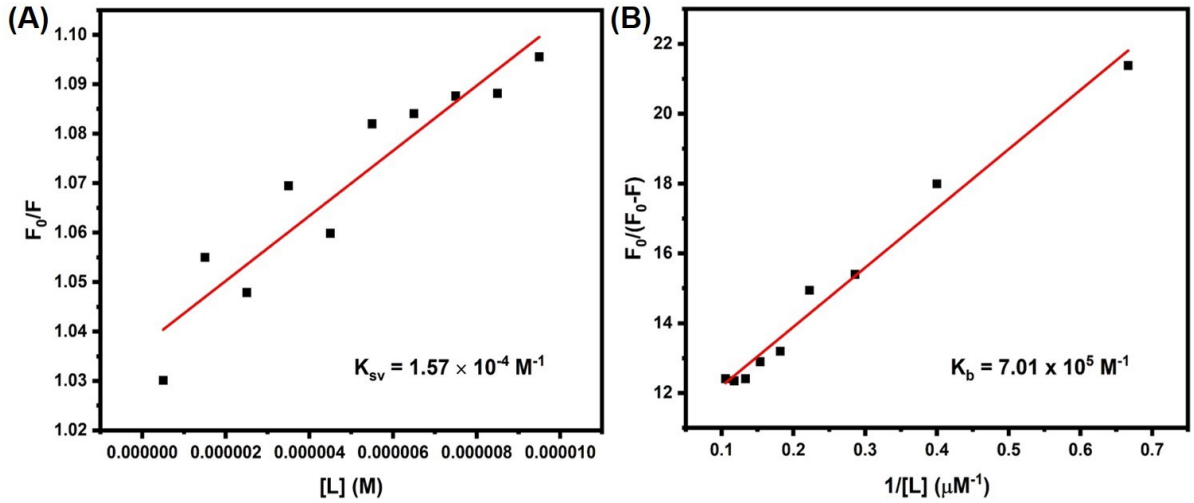


Figure S2. (A) Stern-Volmer plot and (C) modified Stern-Volmer plot of interaction between Hb and **TIM 10** at 25℃.

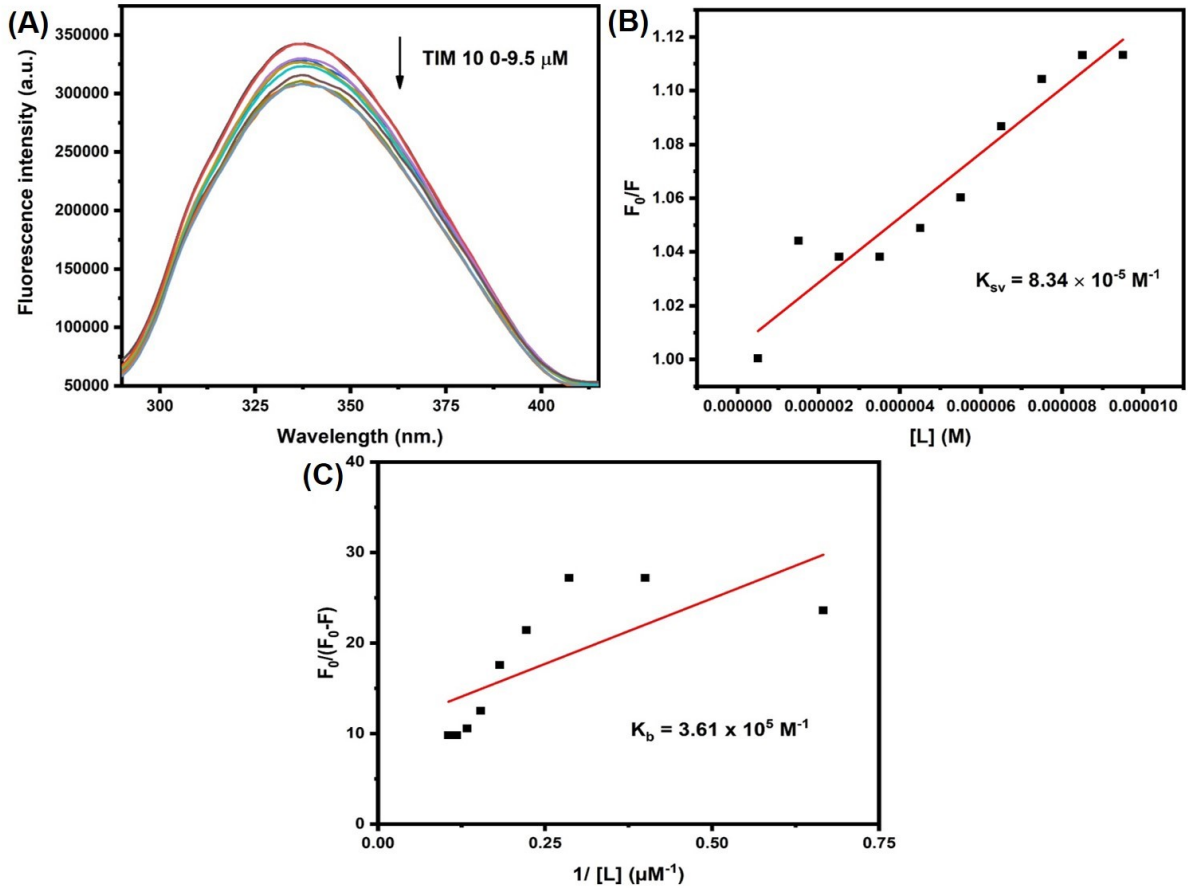


Figure S3. Fluorescence spectroscopic study of interaction between Hb and **TIM 10** at 30℃. (A) Fluorescence spectrum of Hb in the absence and presence of **TIM 10**, (B) Stern-Volmer plot and (C) modified Stern-Volmer plot of interaction between Hb and **TIM 10**.

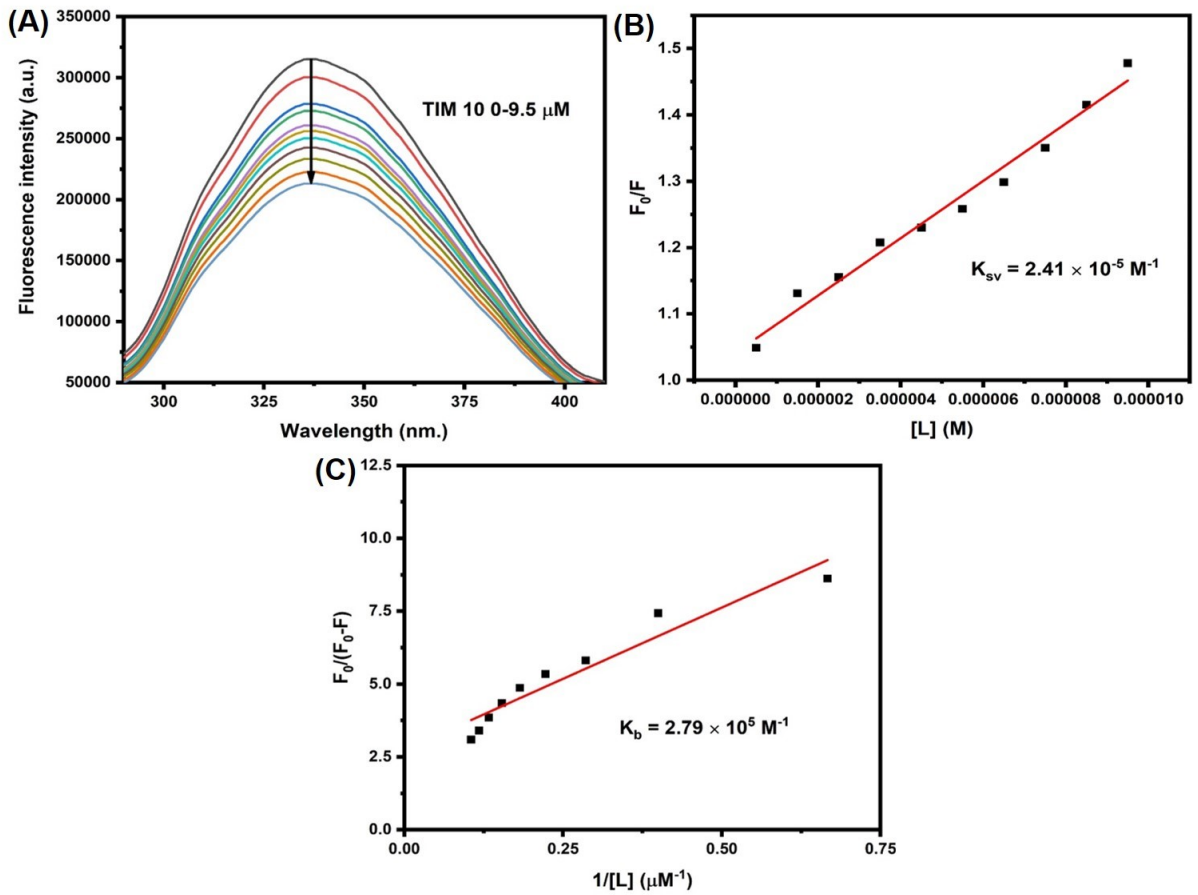


Figure S4. Fluorescence spectroscopic study of interaction between Hb and **TIM 10** at 37℃. (A) Fluorescence spectrum of Hb in the absence and presence of **TIM 10**, (B) Stern-Volmer plot and (C) modified Stern-Volmer plot of interaction between Hb and **TIM 10**.

Table S1. Thermodynamic parameters of the interaction between Hb and **TIM 10**.

Temperature (K)	K_{sv} (M^{-1})	K_b (M^{-1})	ΔG^0 (KJ M^{-1})	ΔH^0 (KJ M^{-1})	ΔS^0 (J M-1)
298	1.57×10^{-4}	7.01×10^5	-14.48	-2.99	-4.23
303	8.34×10^{-5}	3.61×10^5	-14.00		
310	2.41×10^{-5}	2.79×10^5	-14.04		

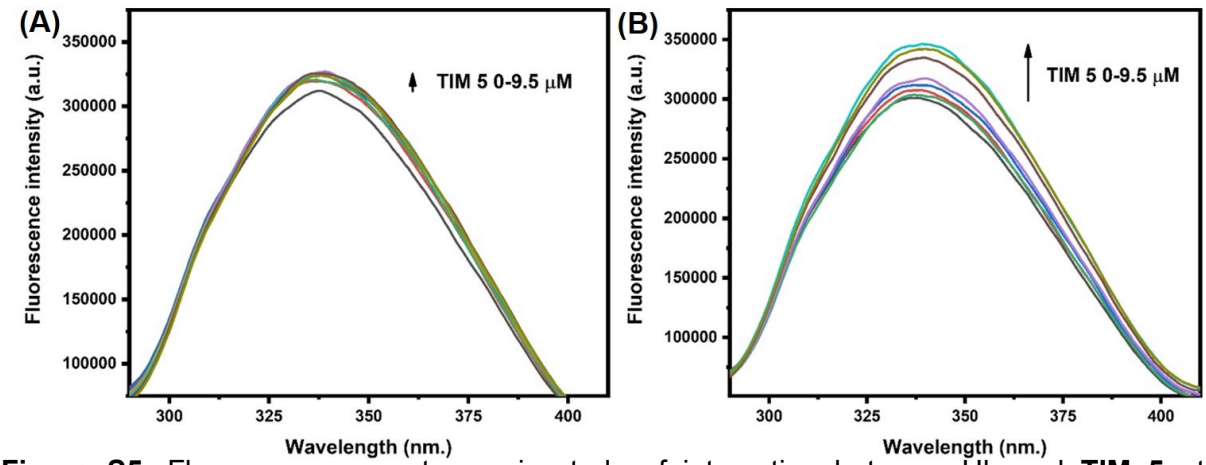


Figure S5. Fluorescence spectroscopic study of interaction between Hb and **TIM 5** at different temperatures; (A) 30℃, and (B) 37℃.

6. References

[1] Caramenti, P.; Nicolai, S.; Waser, J. Indole-and Pyrrole-BX: Bench-Stable Hypervalent Iodine Reagents for Heterocycle Umpolung. *Chem. Eur. J.* **2017**, *23*, 14702-14706.

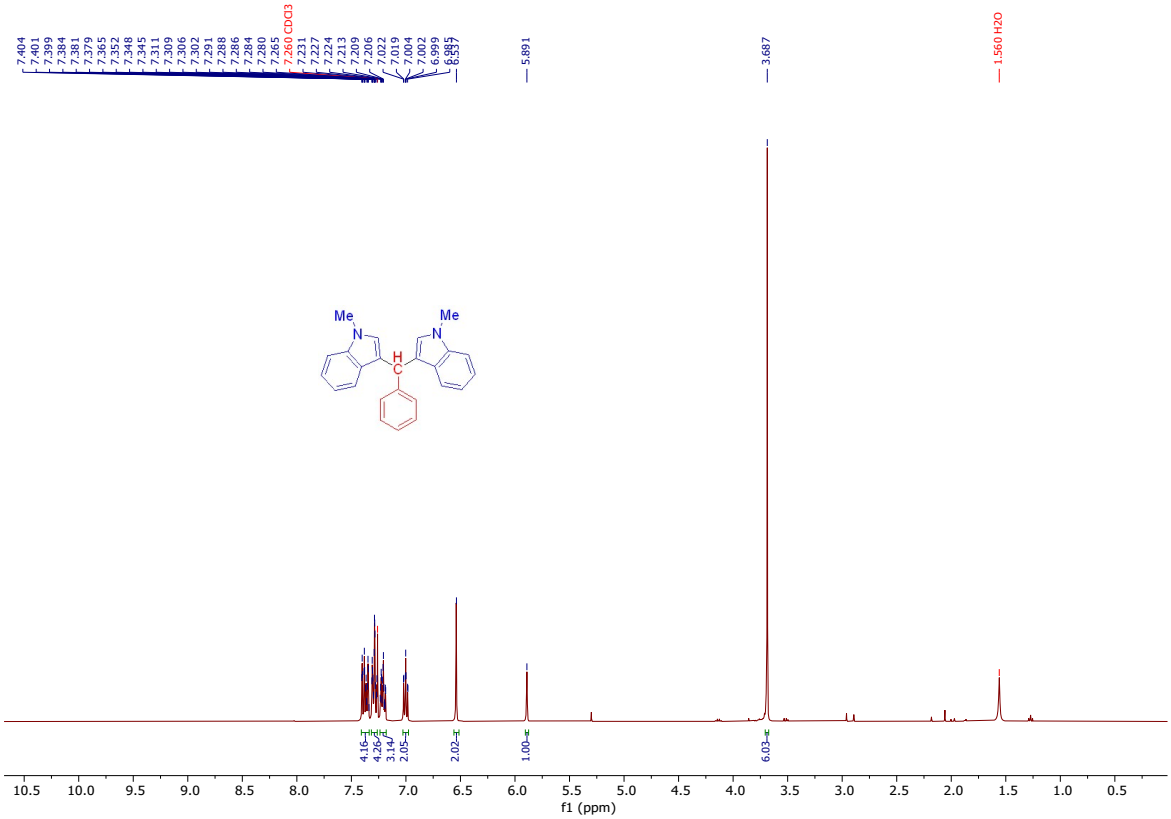
[2] Qi, X.; Ai, H.-J.; Zhang, N.; Peng, J.-B.; Ying, J.; Wu, X.-F. Palladium-catalyzed carbonylative bis (indolyl) methanes synthesis with TFBen as the CO source. *J. Catal.* **2018**, *362*, 74-77.

[3] Naskar, S.; Hazra, A.; Paira, P.; Sahu, K. B.; Banerjee, S.; Mondal, N. B. NH4Cl-promoted synthesis of symmetrical and unsymmetrical triindolylmethanes under solvent-free conditions. *J. Chem. Res.* **2008**, *2008*, 568-571.

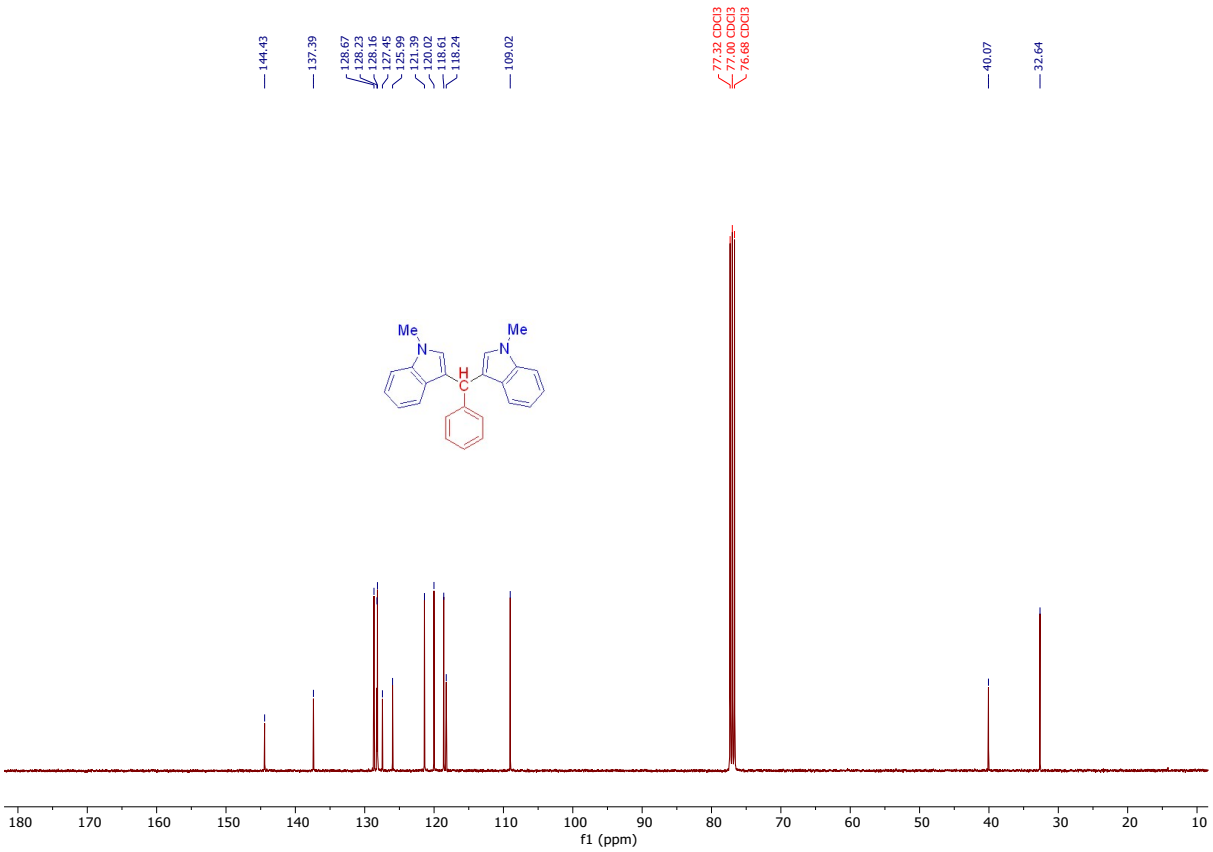
7. Copies of NMR Spectra

3,3'-(phenylmethylene)bis(1-methyl-1*H*-indole) (8a):

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

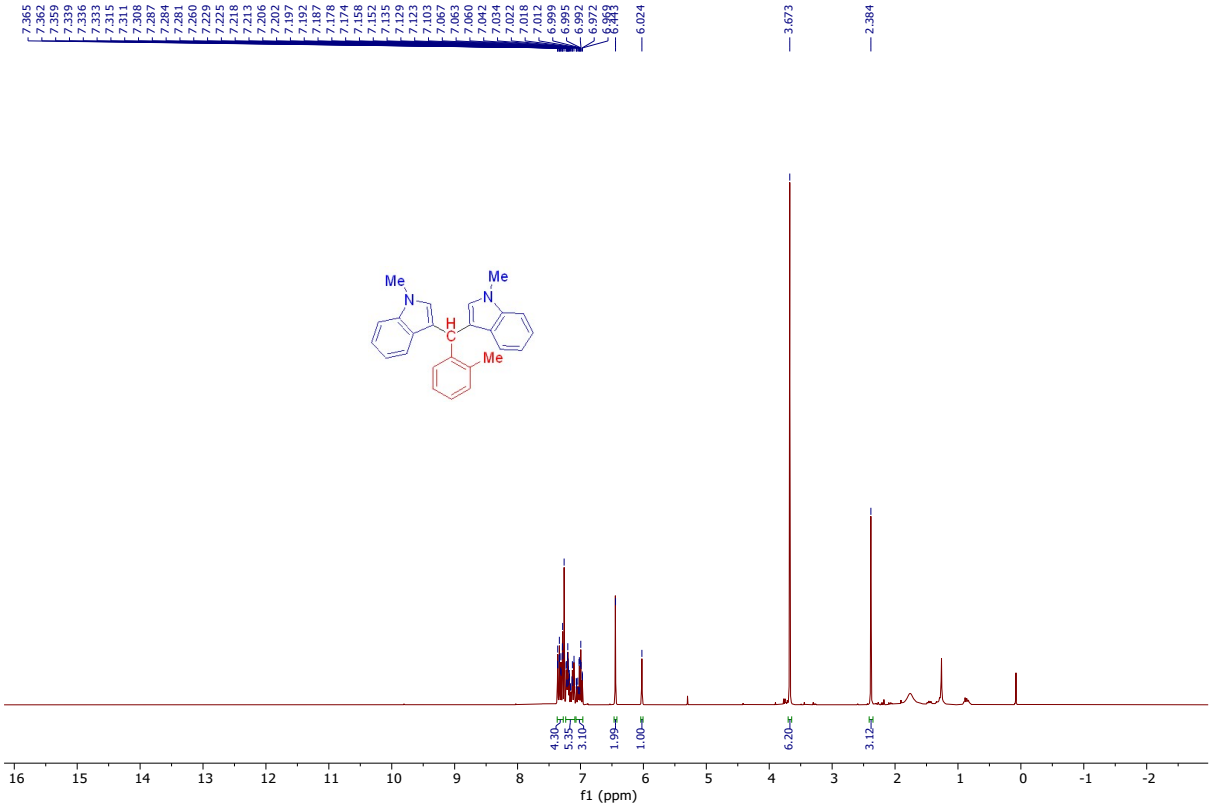


^{13}C NMR (101 MHz, $CDCl_3$)

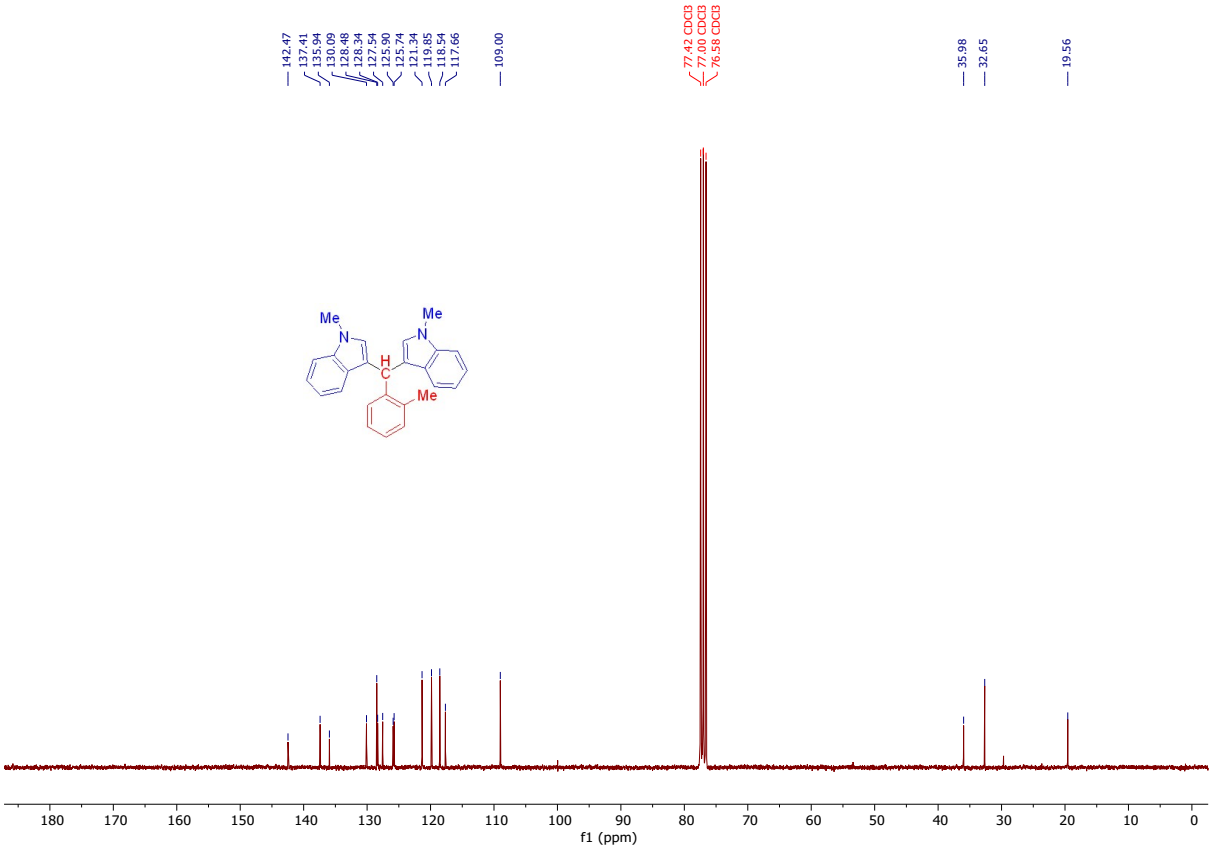


3,3'-(*o*-tolylmethylene)bis(1-methyl-1*H*-indole) (8b):

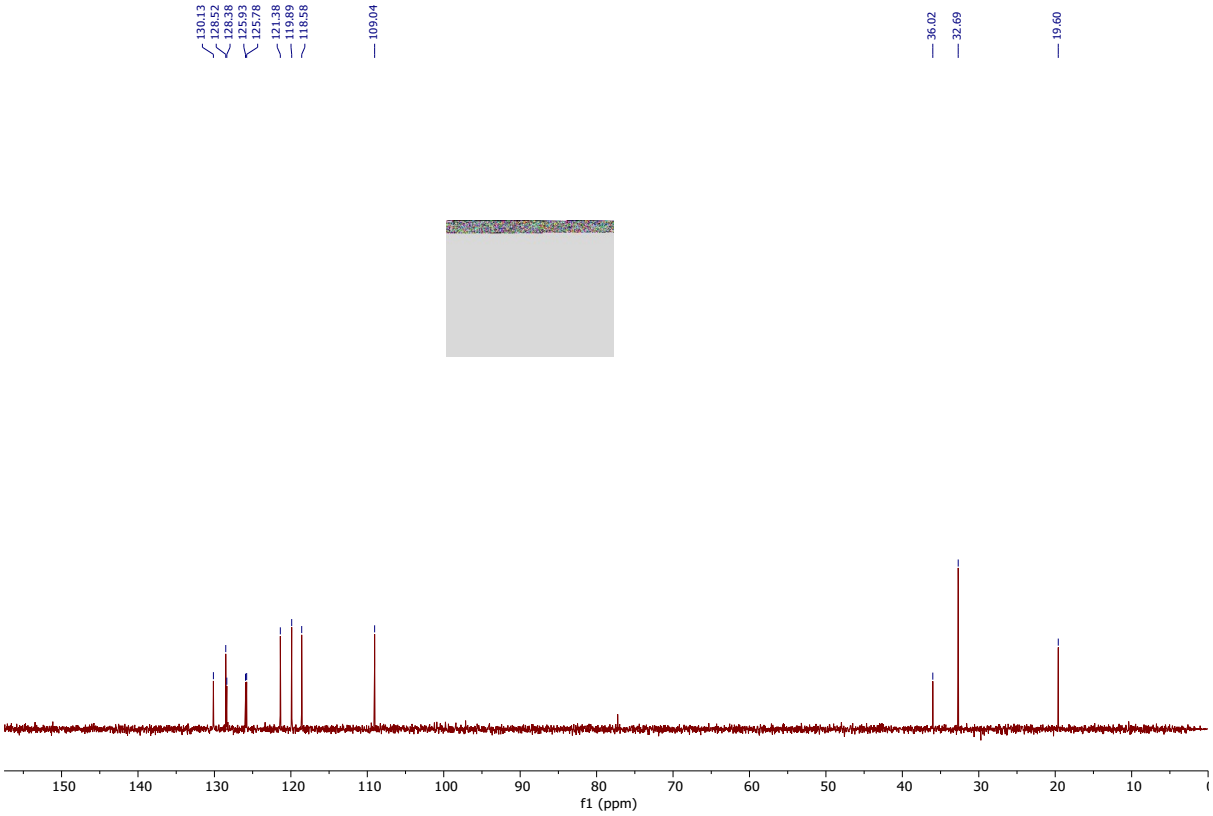
^{1}H NMR (300 MHz, $CDCl_3$)



^{13}C NMR (75 MHz, $CDCl_3$)

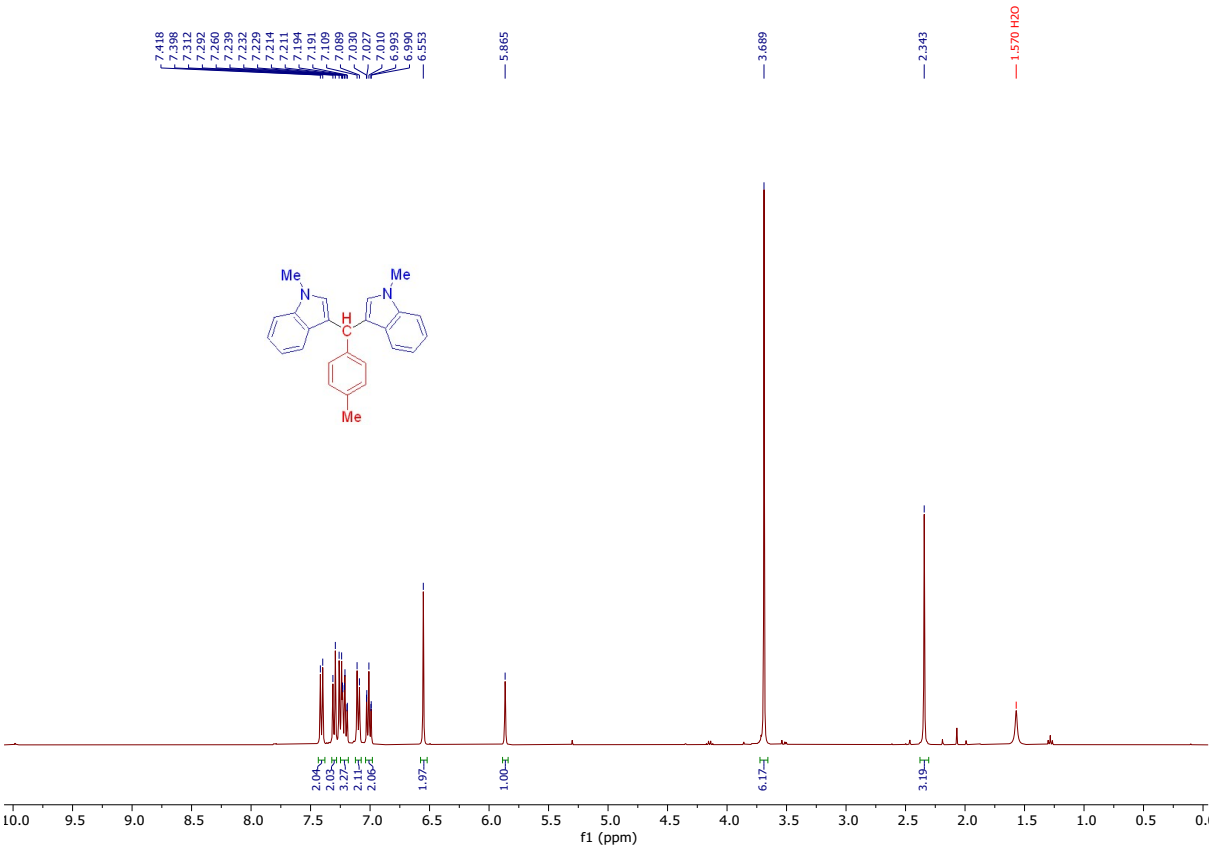


^{13}C NMR (75 MHz, $CDCl_3$) (DEPT-135)

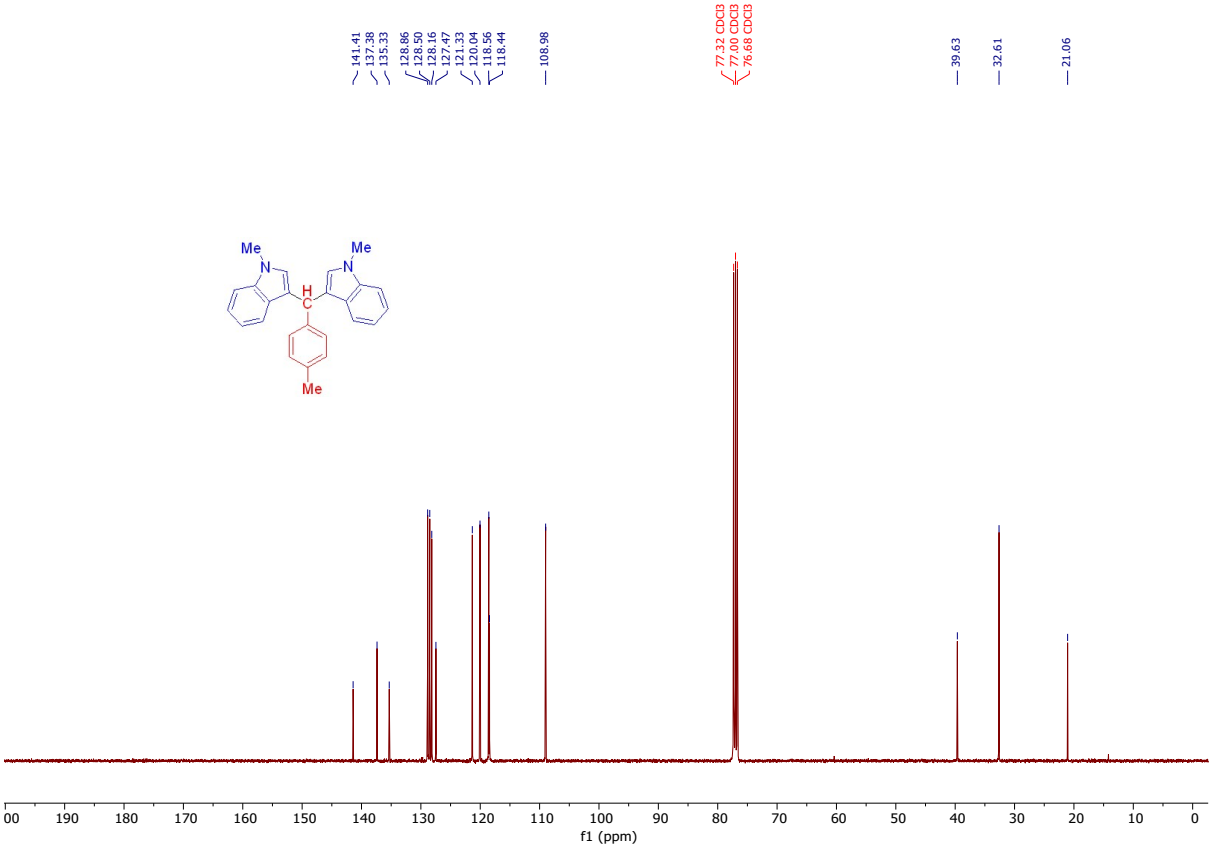


3,3'-(*p*-tolylmethylene)bis(1-methyl-1*H*-indole) (8c):

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

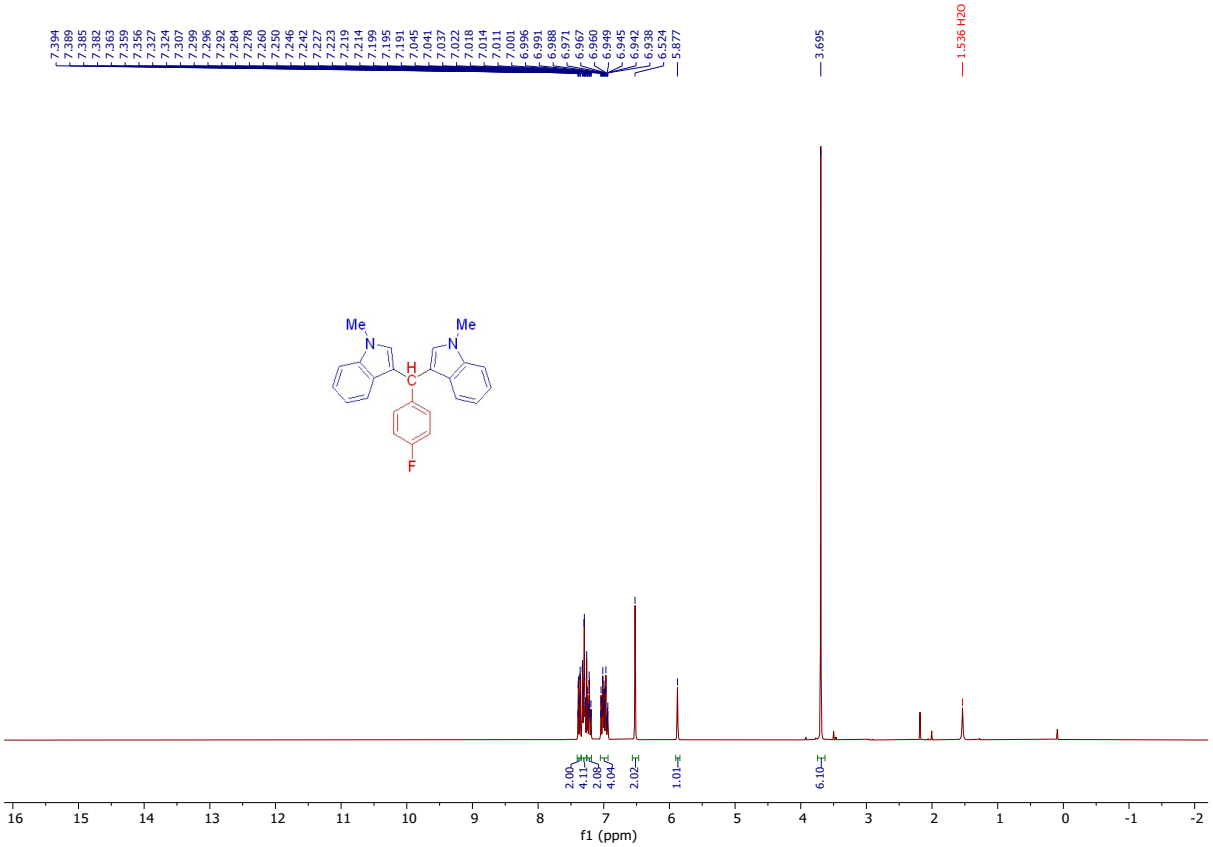


^{13}C NMR (101 MHz, $CDCl_3$)

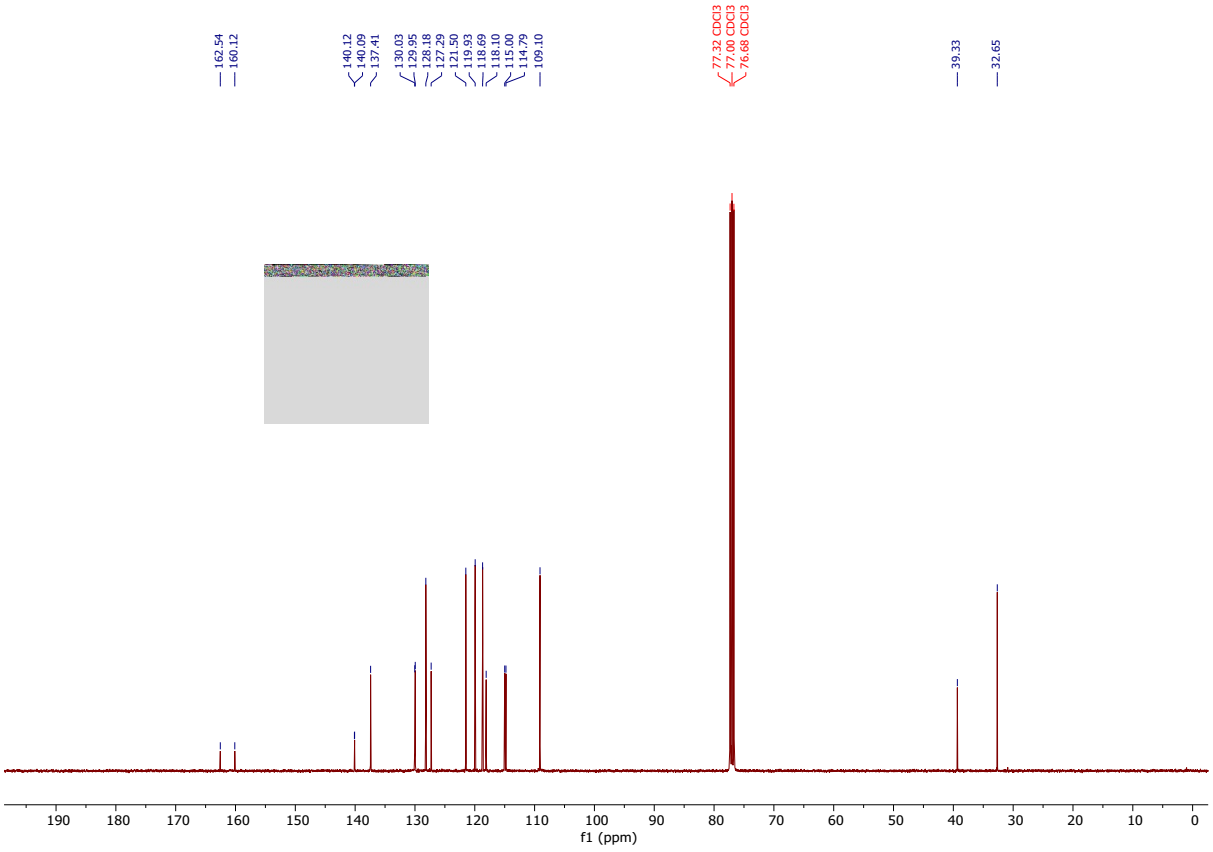


3,3'-((4-fluorophenyl)methylene)bis(1-methyl-1*H*-indole) (8d):

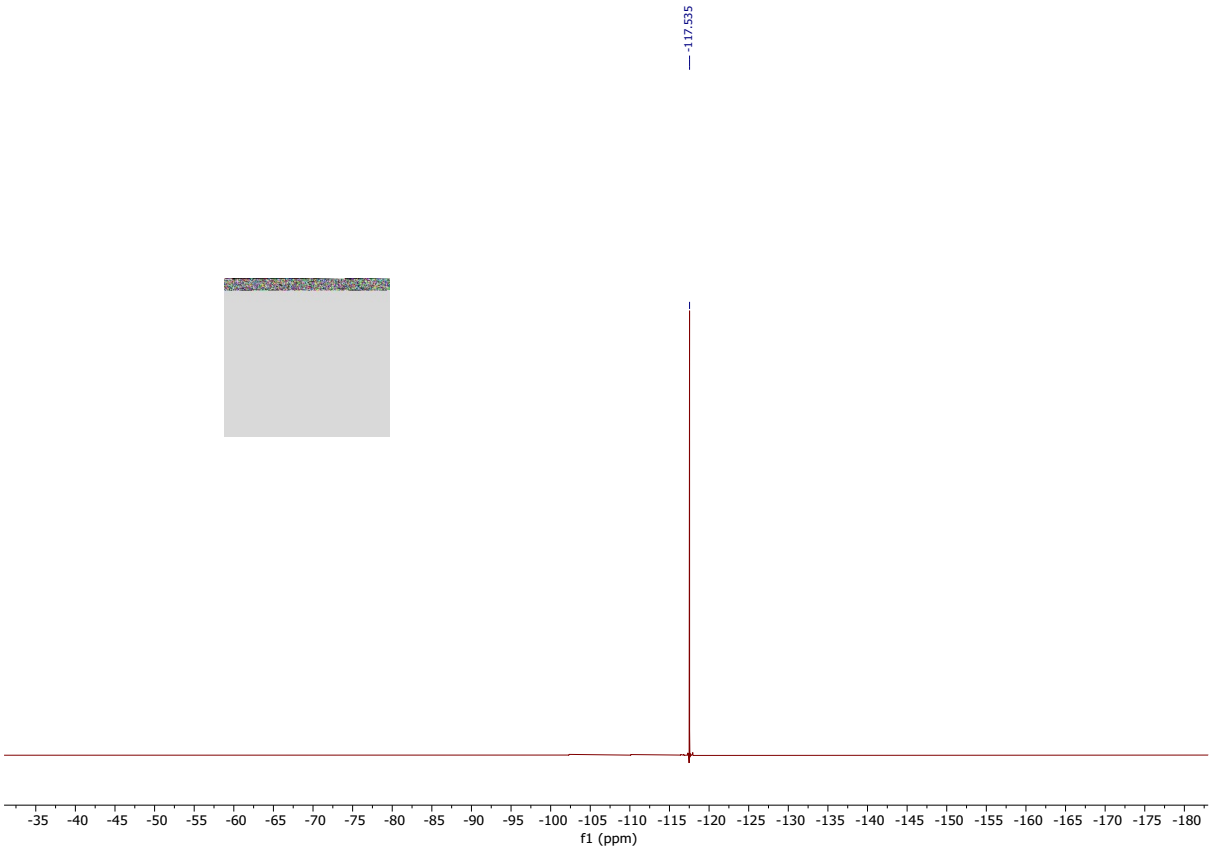
^{1}H NMR (300 MHz, $CDCl_3$)



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

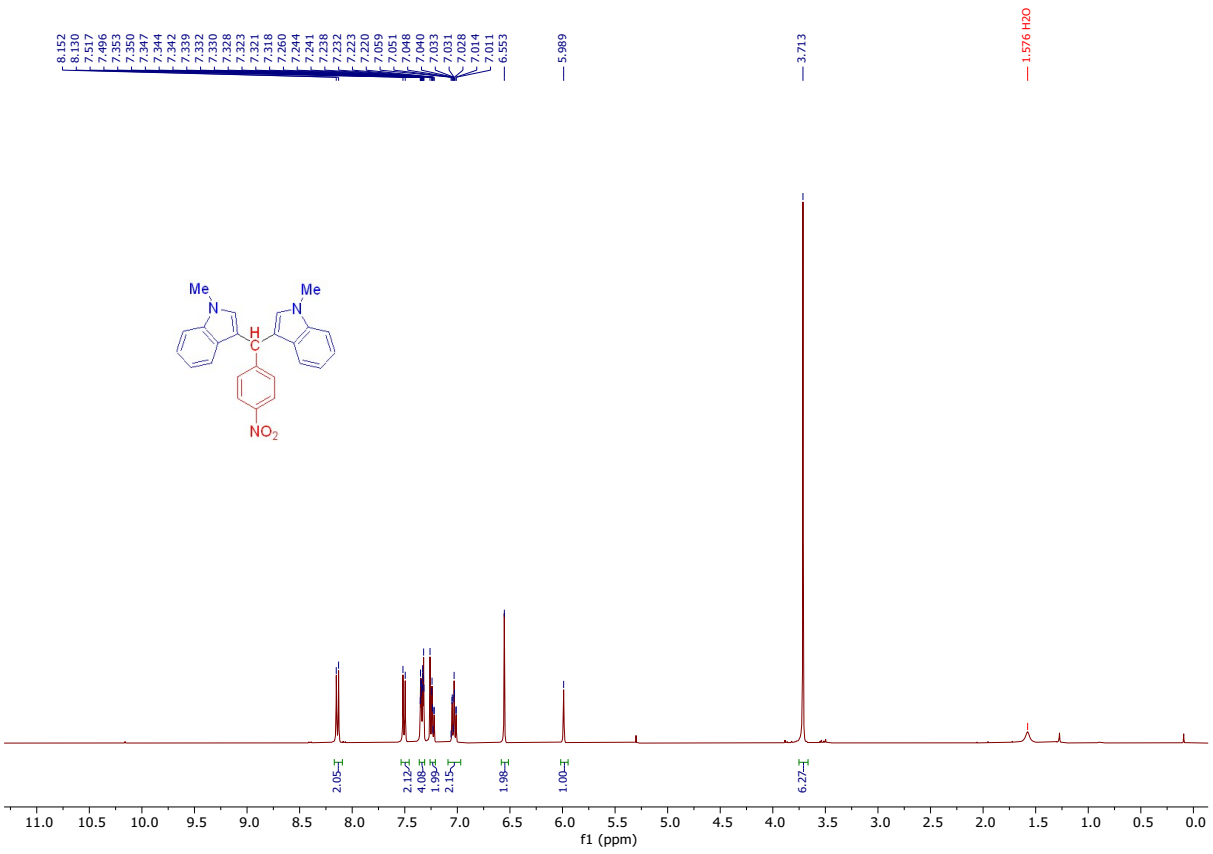


^{19}F NMR (377 MHz, $CDCl_3$)

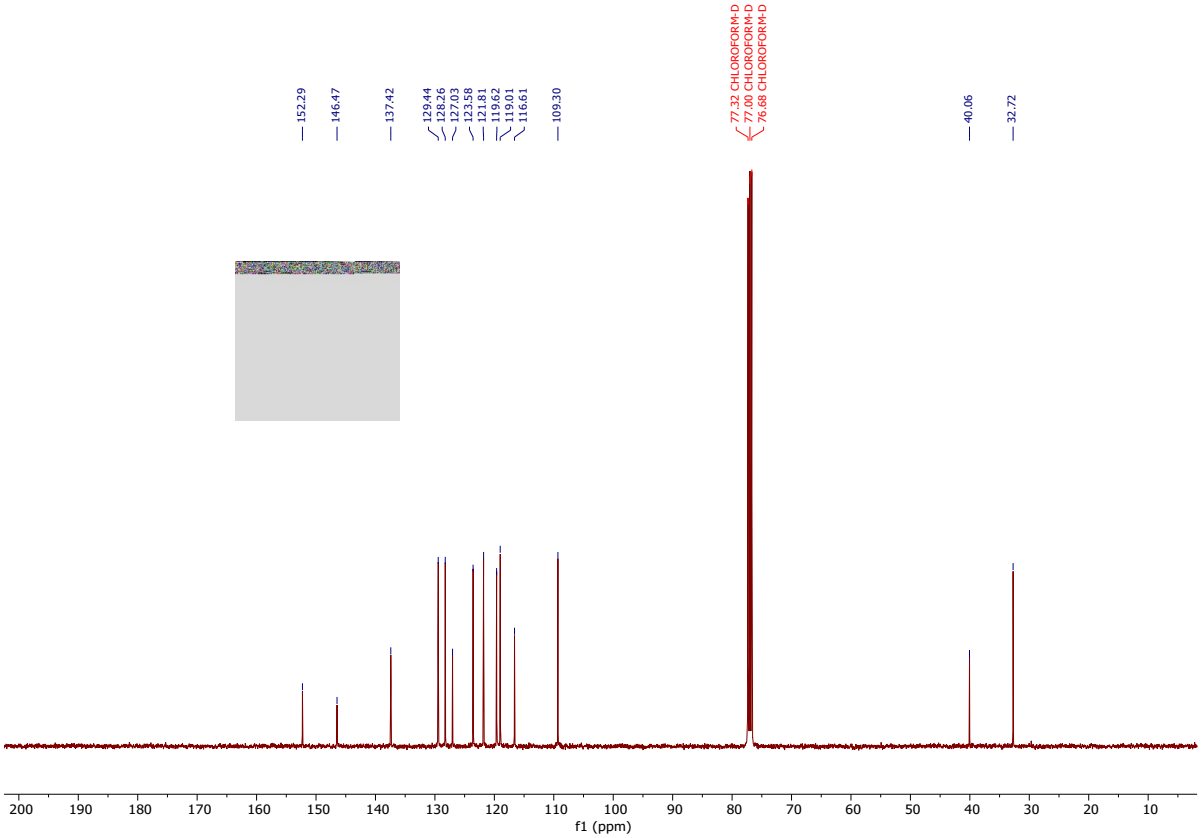


3,3'-((4-nitrophenyl)methylene)bis(1-methyl-1*H*-indole) (8e):

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

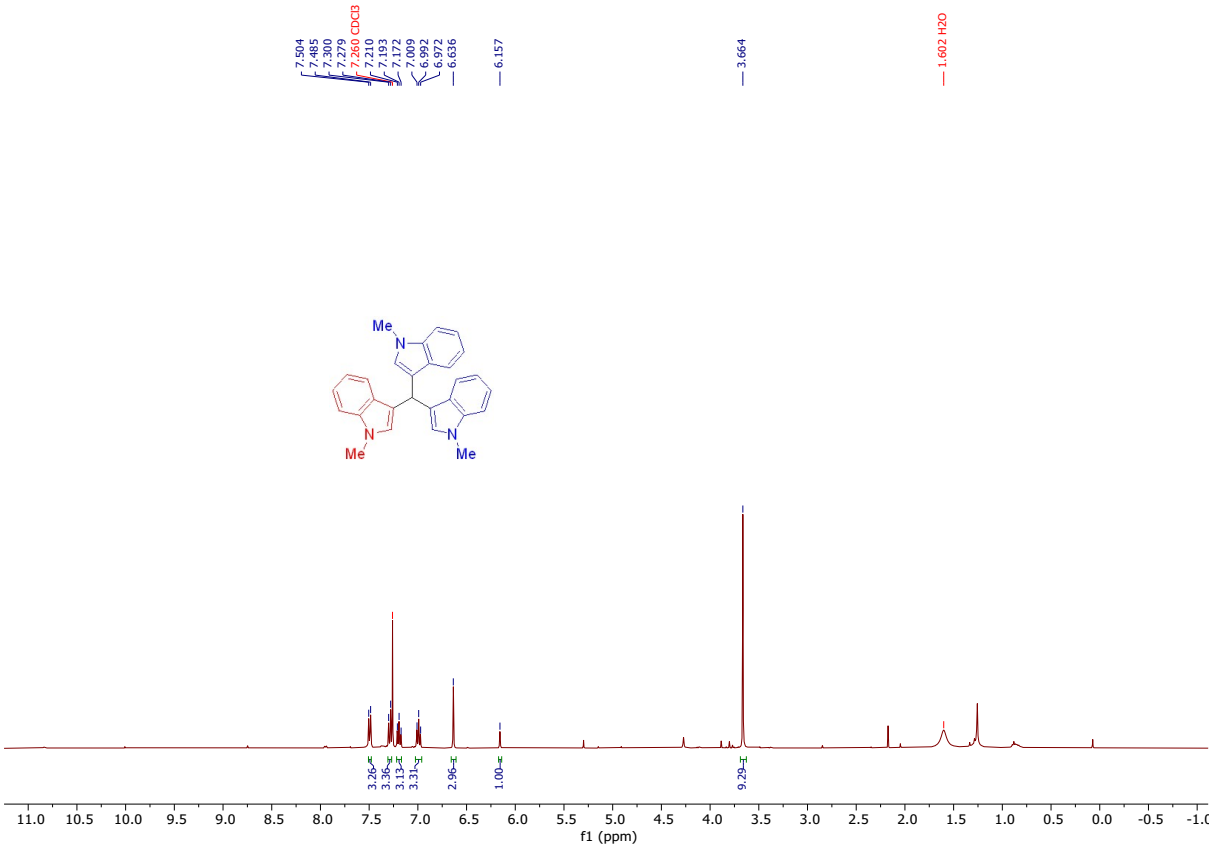


^{13}C NMR (101 MHz, $CDCl_3$)

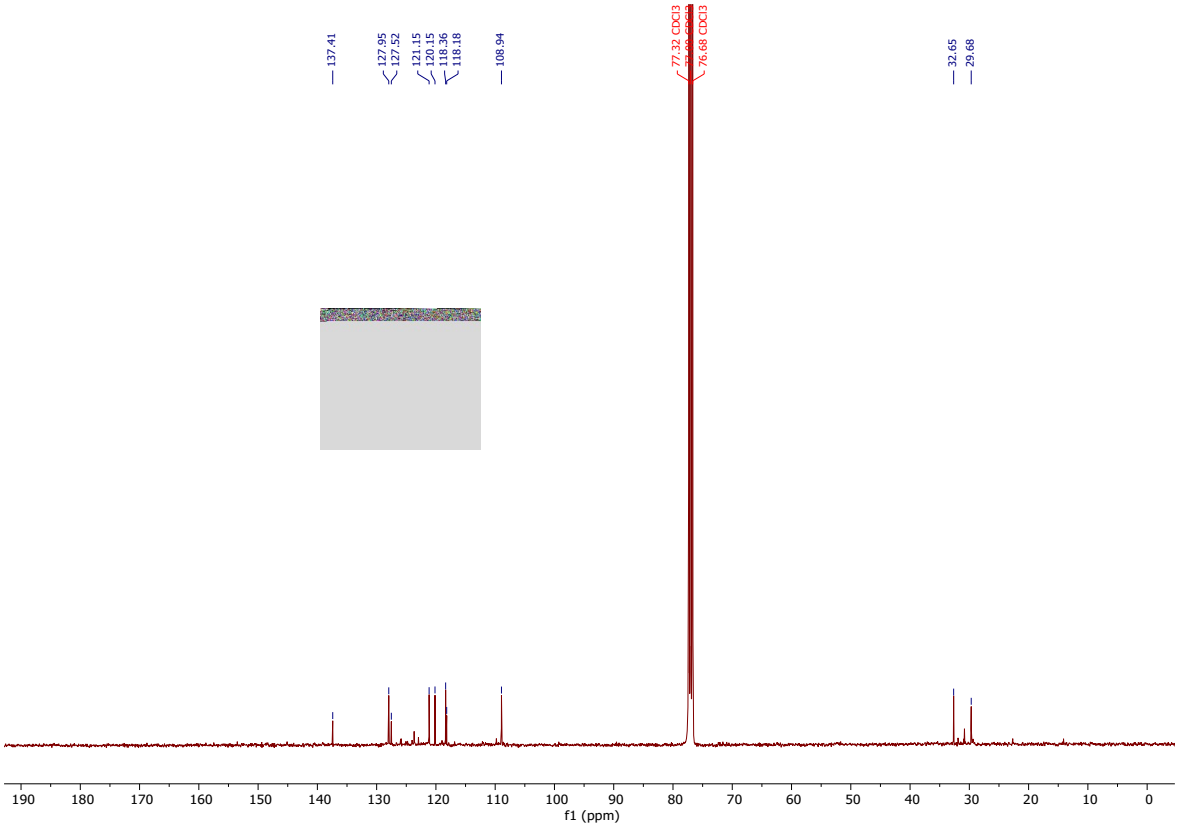


tris(1-methyl-1*H*-indol-3-yl)methane (10):

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

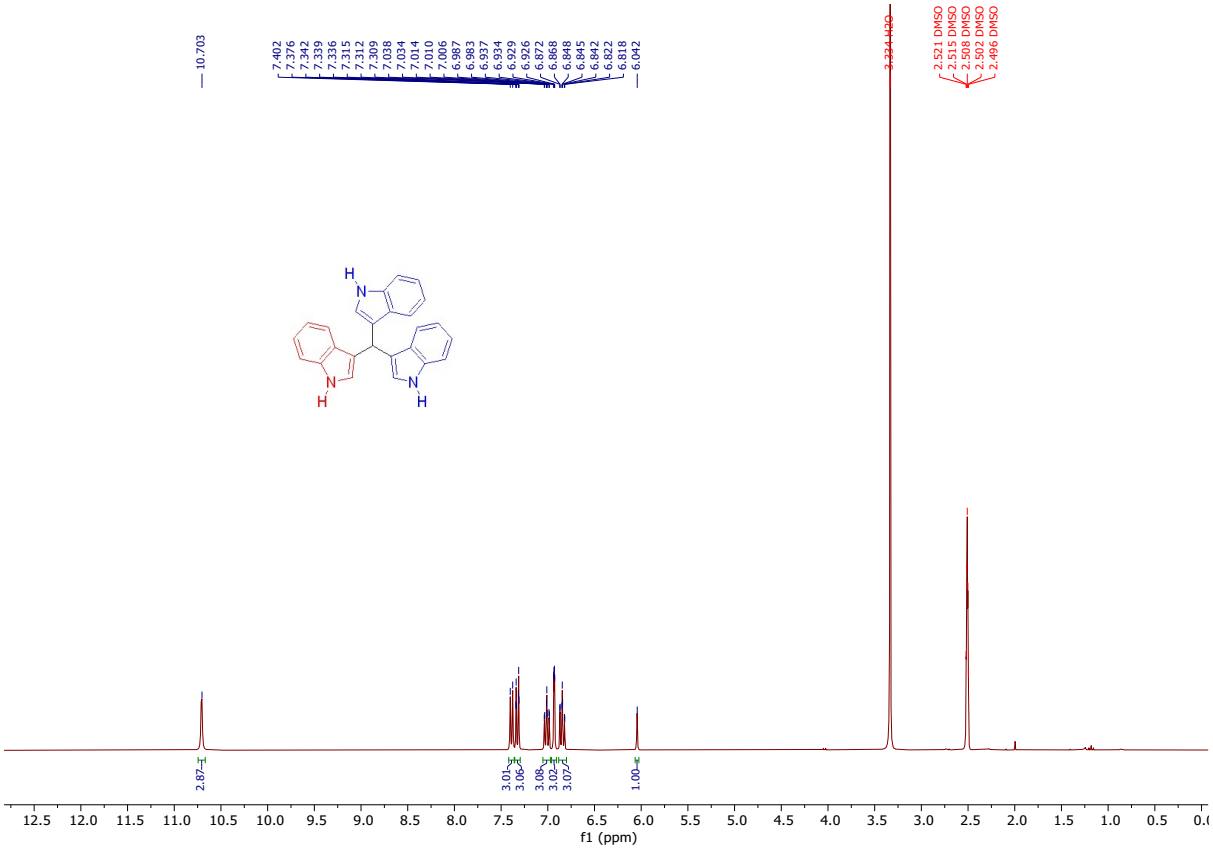


^{13}C NMR (101 MHz, $CDCl_3$)

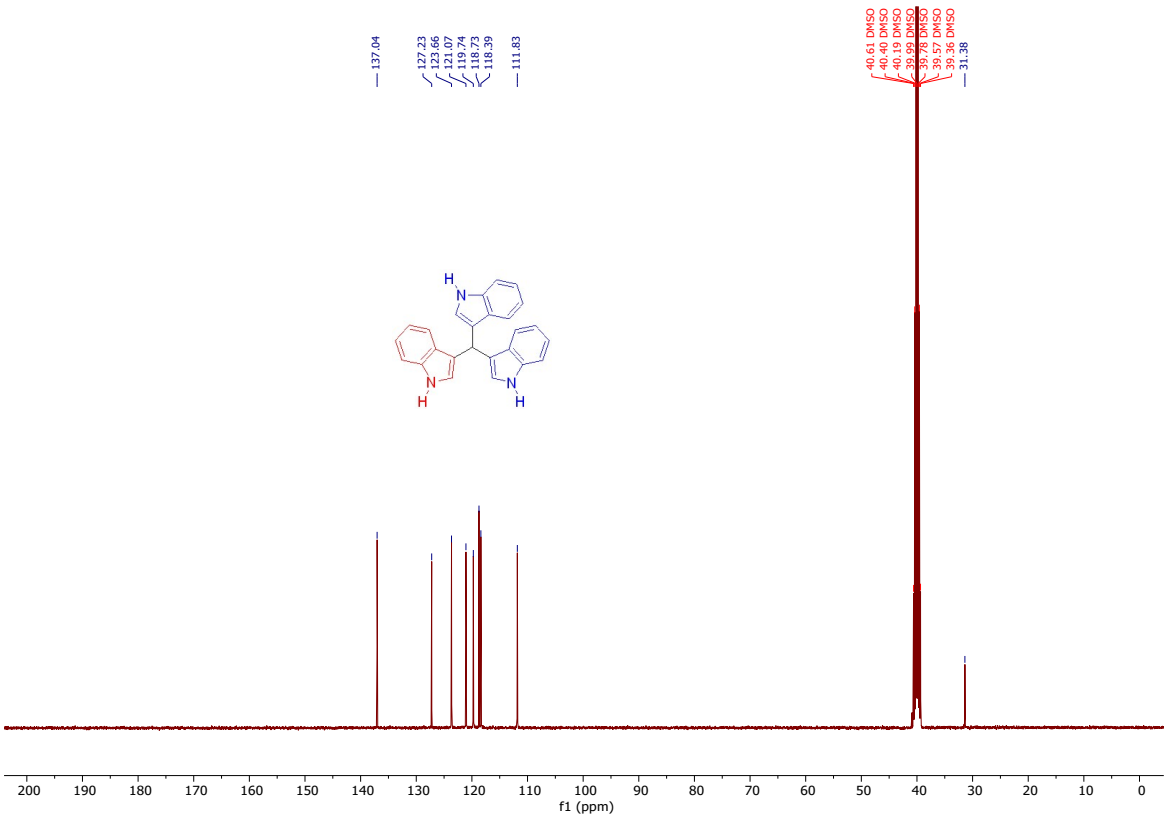


tri(1*H*-indol-3-yl)methane (5):

^{1}H NMR (300 MHz, *d*^{6}-DMSO)

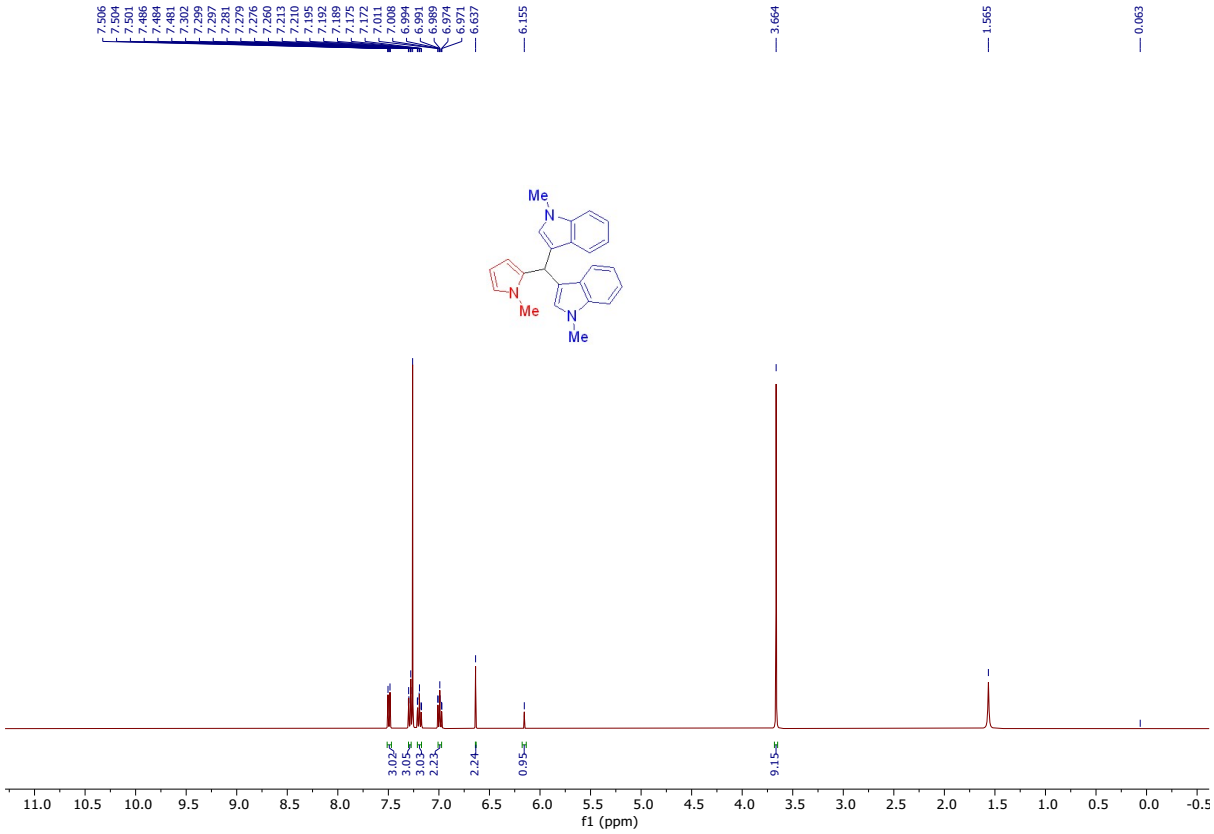


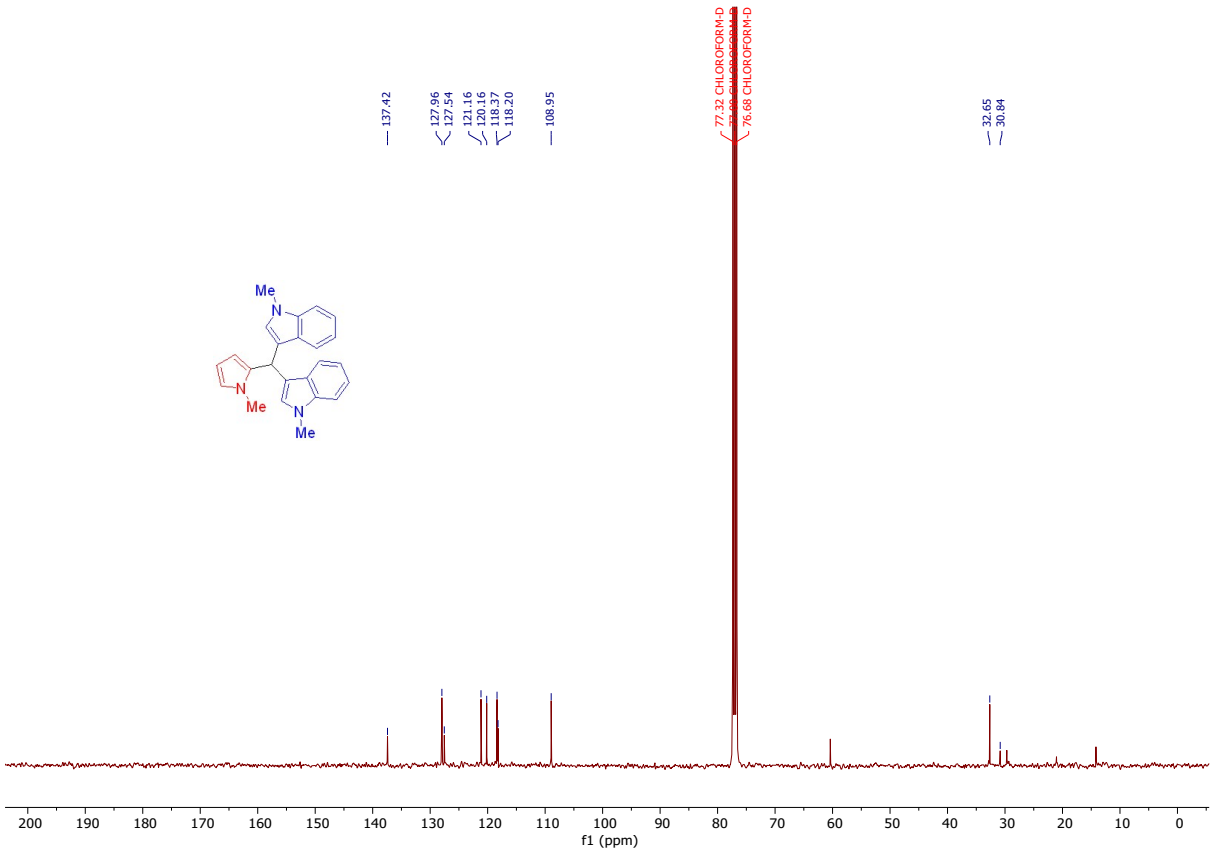
^{13}C NMR (101 MHz, *d*^{6}-DMSO)



3,3'-((1-methyl-1*H*-pyrrol-2-yl)methylene)bis(1-methyl-1*H*-indole) (11):

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





HRMS (ESI) *m/z*: $[M+H]^+$