Supporting Information for

Identification of Pyridinium with Three Indole Moieties as an Antimicrobial Agent

Masahiro Okada,†‡ Tomotoshi Sugita,†‡ Chin Piow Wong,† Toshiyuki Wakimoto,†,§ Ikuro Abe †**

† Graduate School of Pharmaceutical Sciences, The University of Tokyo,

Bunkyo-ku, Tokyo 113-0033, Japan

Table of Contents

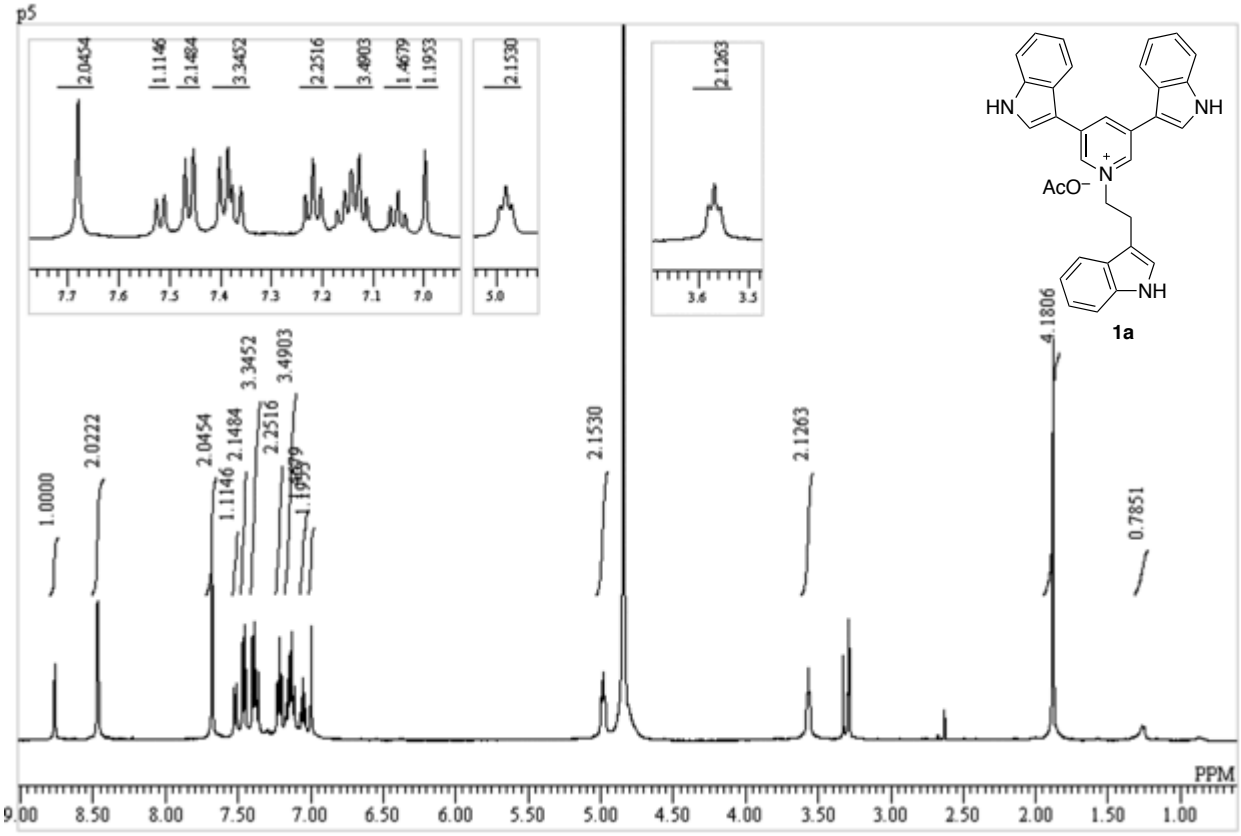


Figure S1. ^{1}H NMR (500 MHz, CD_3OD) spectrum of **1a**.

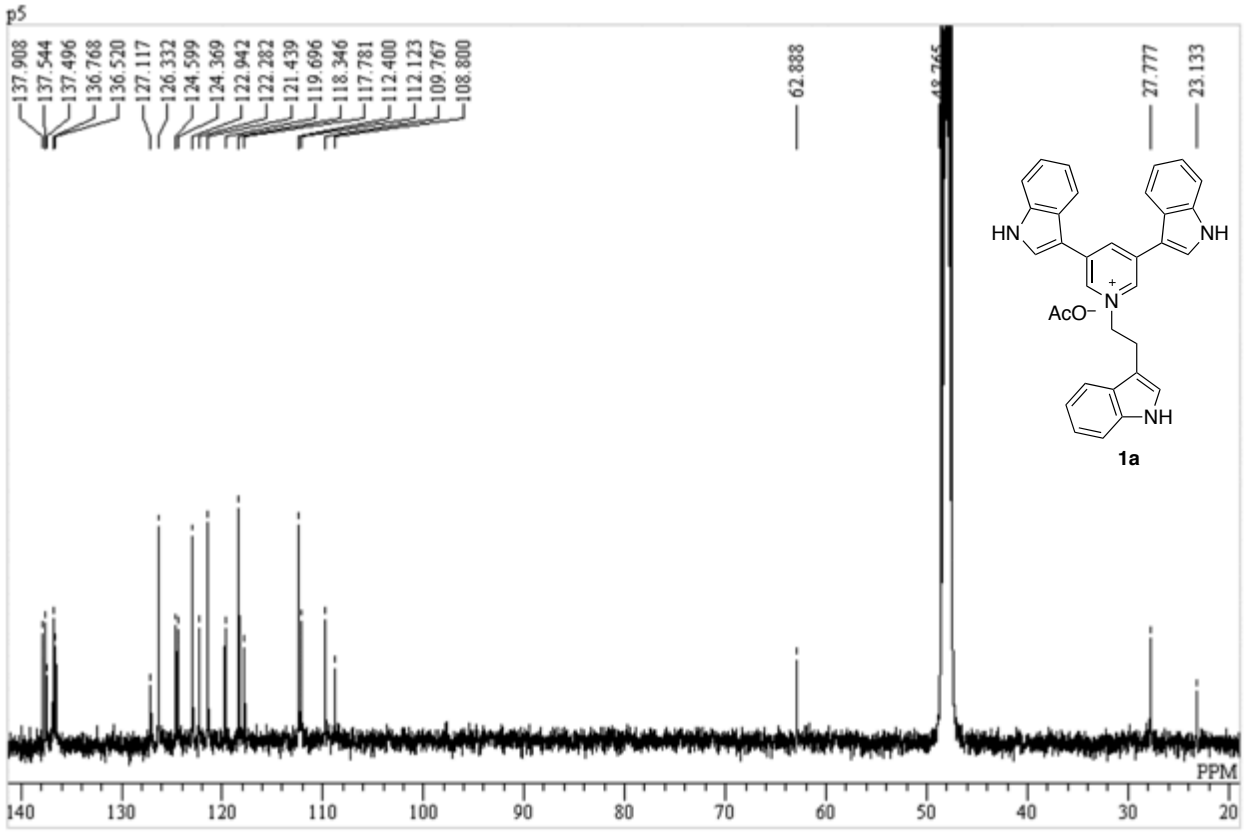


Figure S2. ^{13}C NMR (125 MHz, CD_3OD) spectrum of **1a**.

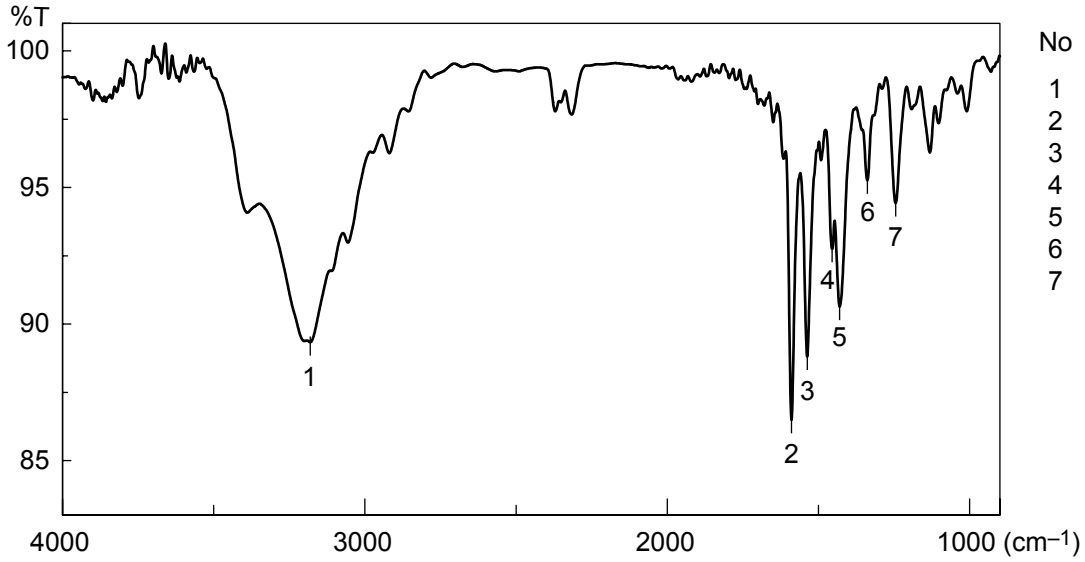


Figure S3. IR (CaF_2) spectrum of **1a**.

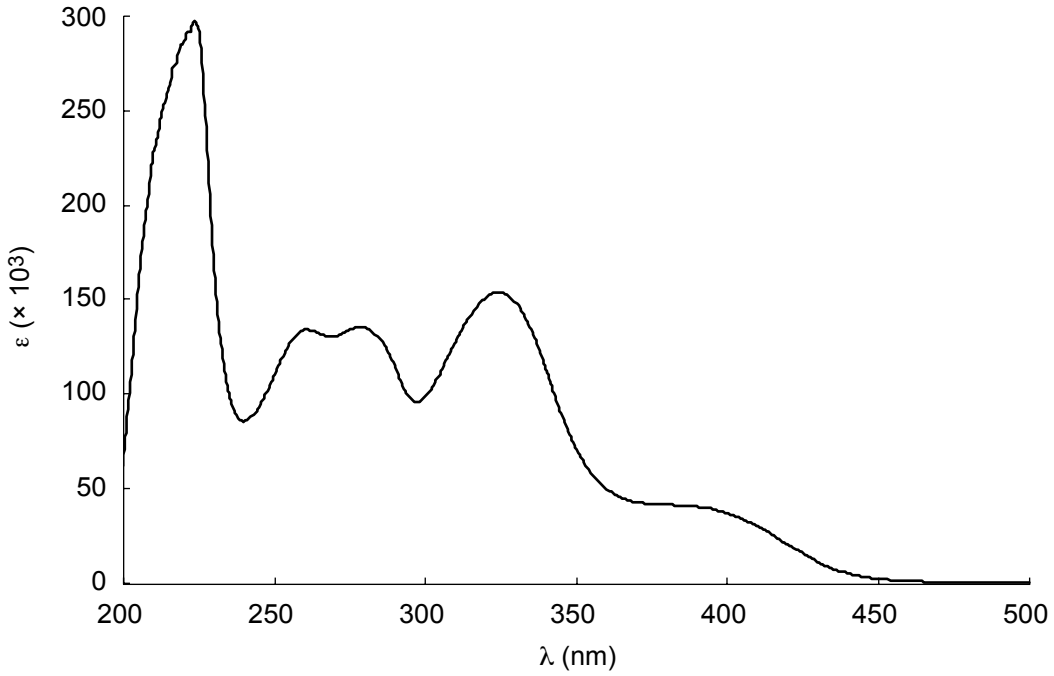
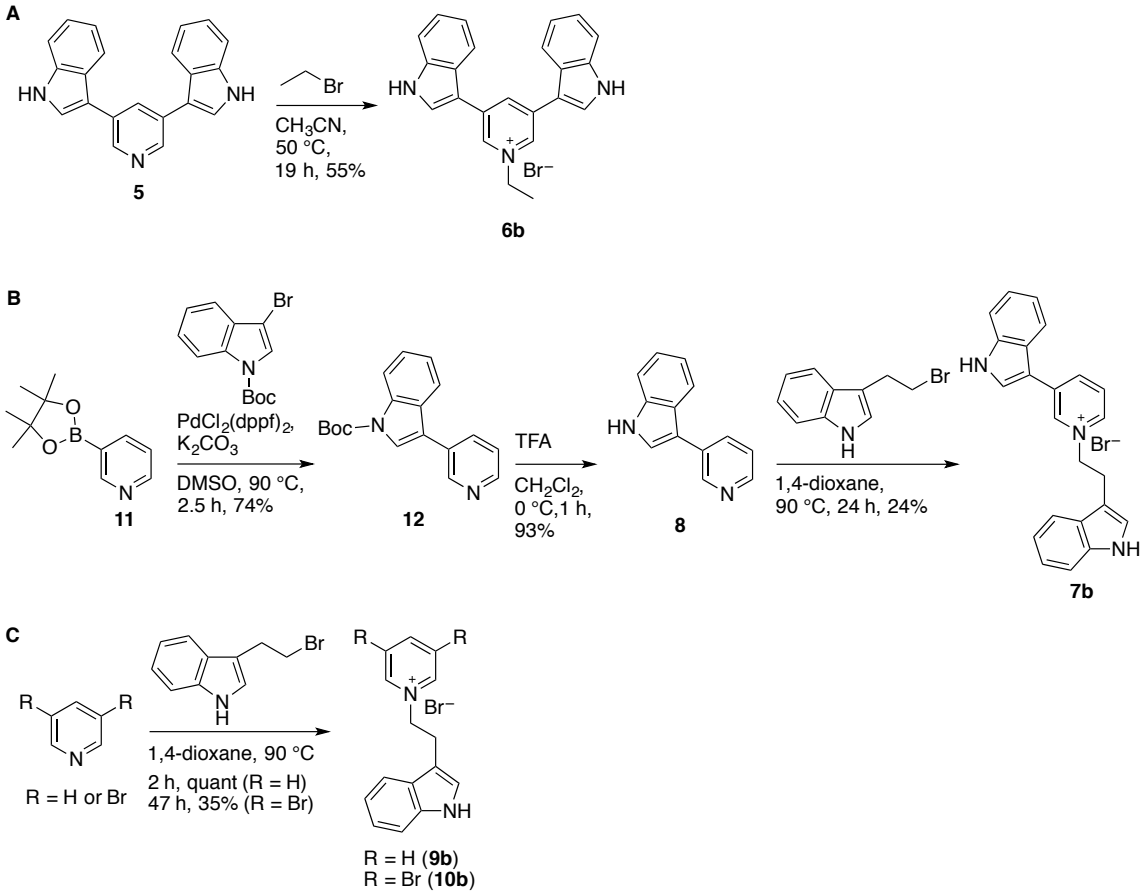


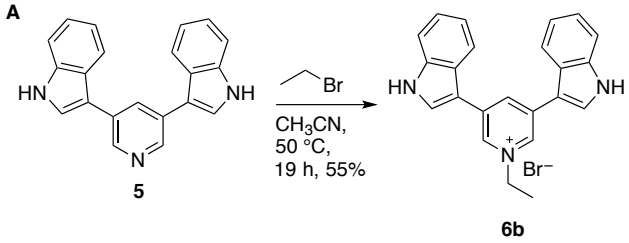
Figure S4. UV (MeOH) spectrum of **1a**.

Synthetic Protocols



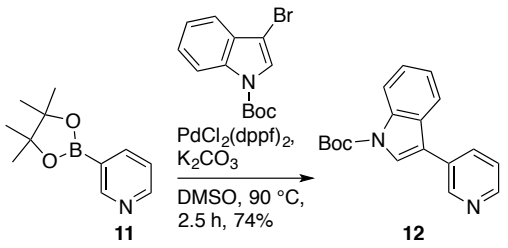
Scheme S1. Synthetic schemes of tricepyridinium analogs.

Synthesis of 1-Ethyl-3,5-di(3-indolyl)pyridinium Bromide (6b).



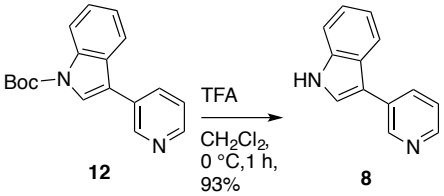
To a solution of **5** (23.9 mg, 77.3 μmol) in acetonitrile (1.0 mL), bromoethane (4.0 mL, 53.8 mmol) was added, and the mixture was stirred at 50 °C for 19 h in refluxed bromoethane. After the mixture was quenched with H_2O, EtOAc was added, and the mixture was extracted with H_2O. The aqueous layer was evaporated to give **6b** (17.7 mg, 42.3 μmol, 55%) as a yellow solid, which was used without any further purification. ^{1}H NMR (500 MHz, CD_3OD): δ_H 8.90 (2H, s), 8.78 (1H, s), 8.02-7.89 (4H, m), 7.52-7.44 (2H, m), 7.27-7.17 (4H, m), 4.75-4.65 (2H, m), 1.76-1.65 (3H, m); ^{13}C NMR (125 MHz, CD_3OD): δ_C 137.6, 136.0, 135.8, 126.5, 126.3, 124.4, 122.7, 121.1, 118.2, 112.2, 109.5, 57.3, 16.0; HRESIMS *m/z* 338.1660 $[M]^+$ (calcd for $C_{23}H_{20}N_3$, 338.1652).

Synthesis of 3-[3-(1-Boc)-indolyl]pyridine (12).



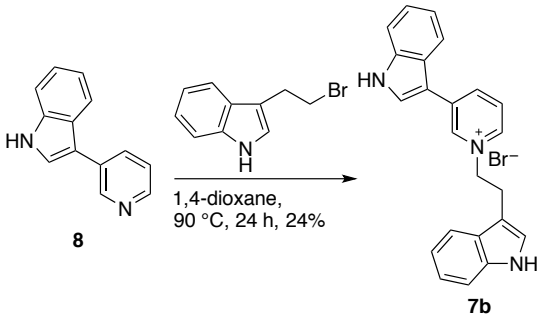
To a solution of 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine **11** (214 mg, 1.04 mmol) in DMSO (10 mL), 1-Boc-3-bromoindole (370 mg, 1.25 mmol), K_2CO_3 (725 mg, 5.25 mmol), and $PdCl_2(dppf)_2$ (58.0 mg, 71.0 μmol) were added. After the mixture was stirred at 90 °C for 2.5 h, it was quenched with water and filtered through Celite to remove the palladium catalyst. The filtrate was extracted with EtOAc, washed with saturated aqueous NaCl, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (hexane/acetone = 15/1 to 4/1) to give **12** (227 mg, 0.771 mmol, 74%) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 8.90 (1H, s), 8.59 (1H, d, J = 5.1 Hz), 8.23 (1H, brs), 7.97-7.85 (1H, m), 7.75 (2H, d, J = 8.5 Hz), 7.43-7.27 (3H, m), 1.69 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 149.6, 149.0, 148.4, 136.0, 135.1, 130.0, 128.6, 125.0, 123.7, 123.5, 123.3, 119.6, 118.7, 115.7, 84.3, 28.3; HRESIMS: *m/z* 295.1436 $[M+H]^+$ (calcd for $C_{18}H_{19}N_2O_2$, 295.1441).

Synthesis of 3-(3-Indolyl)pyridine (8).



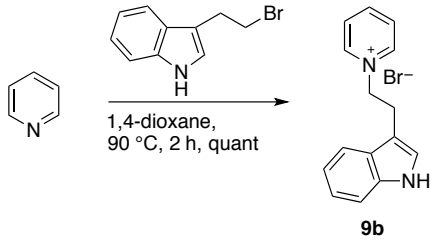
To a solution of **12** (215 mg, 0.730 mmol) in CH_2Cl_2 (2.0 mL), TFA (6.0 mL) was slowly added at 0 °C, and the mixture was stirred at 0 °C for 1 h. The reaction mixture was quenched and basified with 1 M aqueous KOH. The mixture was extracted with EtOAc, washed with saturated aqueous NaCl, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography (hexane/acetone = 6/1 to 3/1) to give **8** (132 mg, 0.680 mmol, 93%) as a yellow powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 9.56 (1H, s), 8.95 (1H, m), 8.55 (1H, d, J = 4.6 Hz), 7.96 (2H, m), 7.60-7.18 (4H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 148.3, 146.8, 137.0, 134.7, 132.1, 125.6, 124.0, 122.8, 122.8, 120.7, 119.3, 114.3, 112.0; HRESIMS *m/z* 195.0919 $[M+H]^+$ (calcd for $C_{13}H_{11}N_2$, 195.0917).

Synthesis of 1-[2-(3-Indolyl)ethyl]-3-(3-indolyl)pyridinium Bromide (7b).



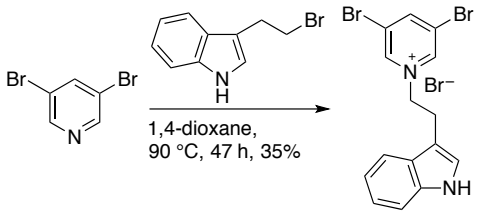
To a solution of **8** (67.0 mg, 0.345 mmol) in 1,4-dioxane (0.70 mL), 3-(2-bromoethyl)indole (114 mg, 0.509 mmol) was added, and the mixture was stirred at 90 °C for 24 h. After the mixture was quenched with H_2O, EtOAc was added, and the mixture was extracted with H_2O. The aqueous layer was evaporated to give **7b** (34.0 mg, 81.3 μmol, 24%) as a yellow solid, which was used without any further purification. ^{1}H NMR (500 MHz, CD_3OD): δ_H 8.63 (1H, d, J = 8.6 Hz), 8.50 (1H, d, J = 6.3 Hz), 8.43 (s, 1H), 7.89 (1H, dd, J = 8.0, 5.7 Hz), 7.47-7.42 (2H, m), 7.40 (1H, d, J = 8.0 Hz), 7.33 (1H, d, J = 8.6 Hz), 7.20-7.09 (3H, m), 7.07-6.99 (2H, m), 6.93 (1H, s), 4.92 (2H, t, J = 6.0 Hz), 3.49 (2H, t, J = 6.0 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ_C 140.7, 140.5, 139.2, 137.6, 137.4, 137.1, 137.0, 127.6, 126.6, 126.0, 123.9, 122.5, 121.8, 121.1, 119.2, 117.9, 117.3, 111.9, 111.6, 108.8, 108.2, 62.4, 27.2; HRESIMS *m/z* 338.1658 $[M]^+$ (calcd for $C_{23}H_{20}N_3$, 338.1652).

Synthesis of 1-[2-(3-Indolyl)ethyl]pyridinium Bromide (9b).



Pyridine (2 mL) was added to 3-(2-bromoethyl)indole (104 mg, 0.464 mmol), and the mixture was stirred at 90 °C for 2 h. After the mixture was quenched with H_2O, EtOAc was added, and the mixture was extracted with H_2O. The aqueous layer was evaporated to give **7** (145 mg, 0.478 mmol, quant.) as a yellow solid, which was used without any further purification. ^{1}H NMR (500 MHz, CD_3OD): δ_H 8.56 (2H, t, J = 5.7 Hz), 8.37 (1H, q, J = 7.2 Hz), 7.81 (2H, d, J = 6.8 Hz), 7.39-7.21 (2H, m), 7.07 (1H, q, J = 7.2 Hz), 7.00-6.84 (2H, m), 3.40 (2H, q, J = 6.4 Hz); ^{13}C-NMR (125 MHz, CD_3OD): δ_C 145.2, 144.4, 136.7, 127.6, 126.7, 123.8, 121.6, 119.1, 117.3, 111.4, 108.0, 62.5, 27.0; HRESIMS *m/z* 223.1232 $[M]^+$ (calcd for $C_{15}H_{15}N_2$, 223.1230).

Synthesis of 3,5-Dibromo-1-[2-(3-indolyl)ethyl]pyridinium Bromide (10b).



10b

To a solution 3,5-dibromopyridine (290 mg, 1.22 mmol) in 1,4-dioxane (1.50 mL), 3-(2-bromoethyl)indole (168 mg, 0.750 mmol) was added, and the mixture was stirred at 90 °C for 47 h. After the mixture was quenched with H_2O, EtOAc was added, and the mixture was extracted with H_2O. The aqueous layer was evaporated to give **10b** (121 mg, 0.262 mmol, 35%) as an yellow solid, which was used without any further purification. ^{1}H NMR (500 MHz, D_2O): δ_H 8.68 (1H, s), 8.53 (2H, s), 7.37 (1H, d, J = 8.5 Hz), 7.18 (1H, d, J = 7.4 Hz), 7.10 (1H, m), 7.01-6.91 (2H, m), 3.32 (2H, m); ^{13}C NMR (125 MHz, CD_3OD): δ_C 137.6, 136.9, 133.9, 126.6, 124.0, 121.9, 121.8, 119.2, 119.0, 116.8, 111.4, 69.7, 26.8; HRESIMS *m/z* 378.9444 $[M]^+$ (calcd for $C_{15}H_{13}Br_2N_2$, 378.9440).

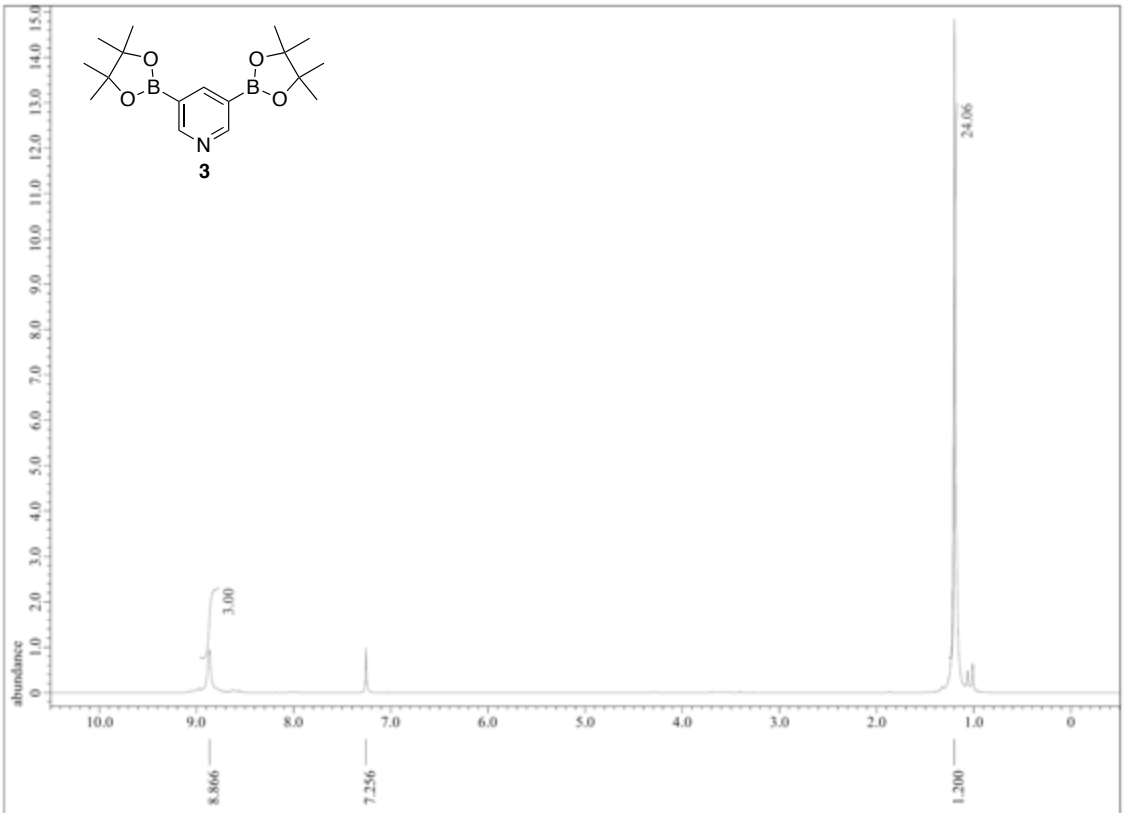


Figure S5. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **3**.

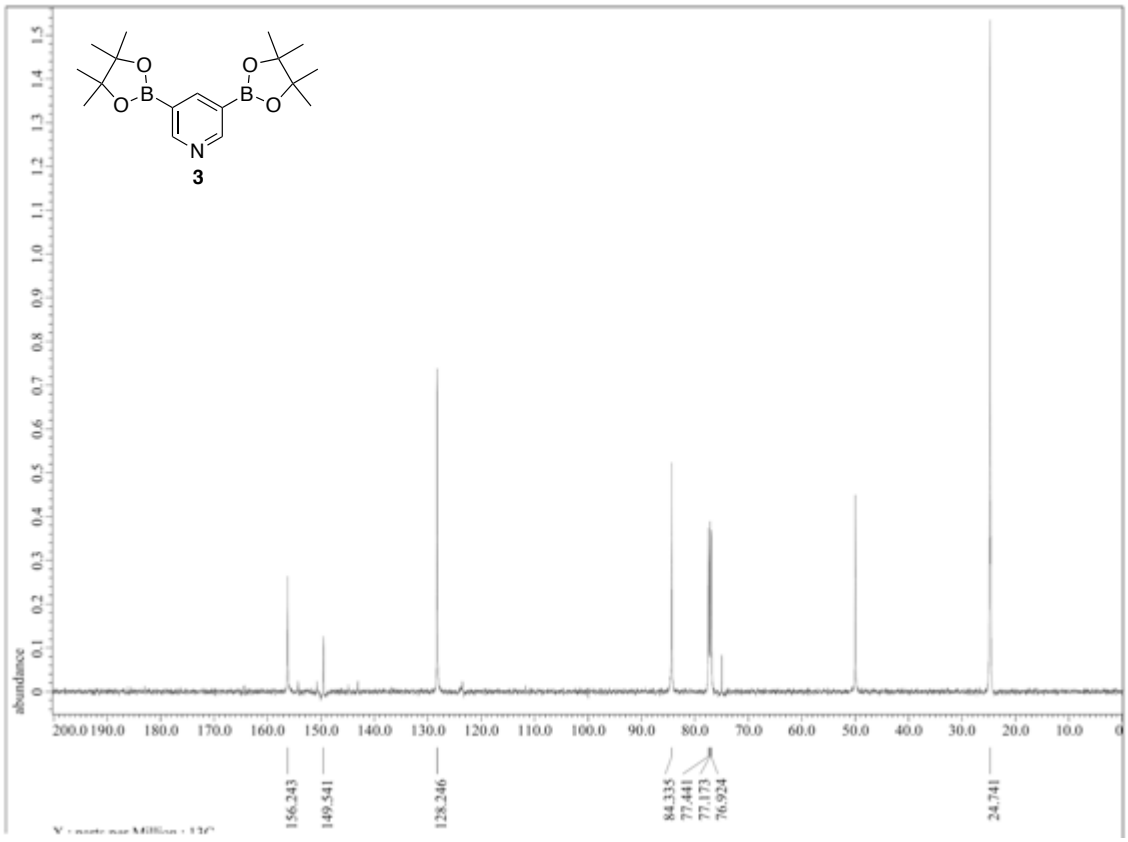


Figure S6. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **3**.

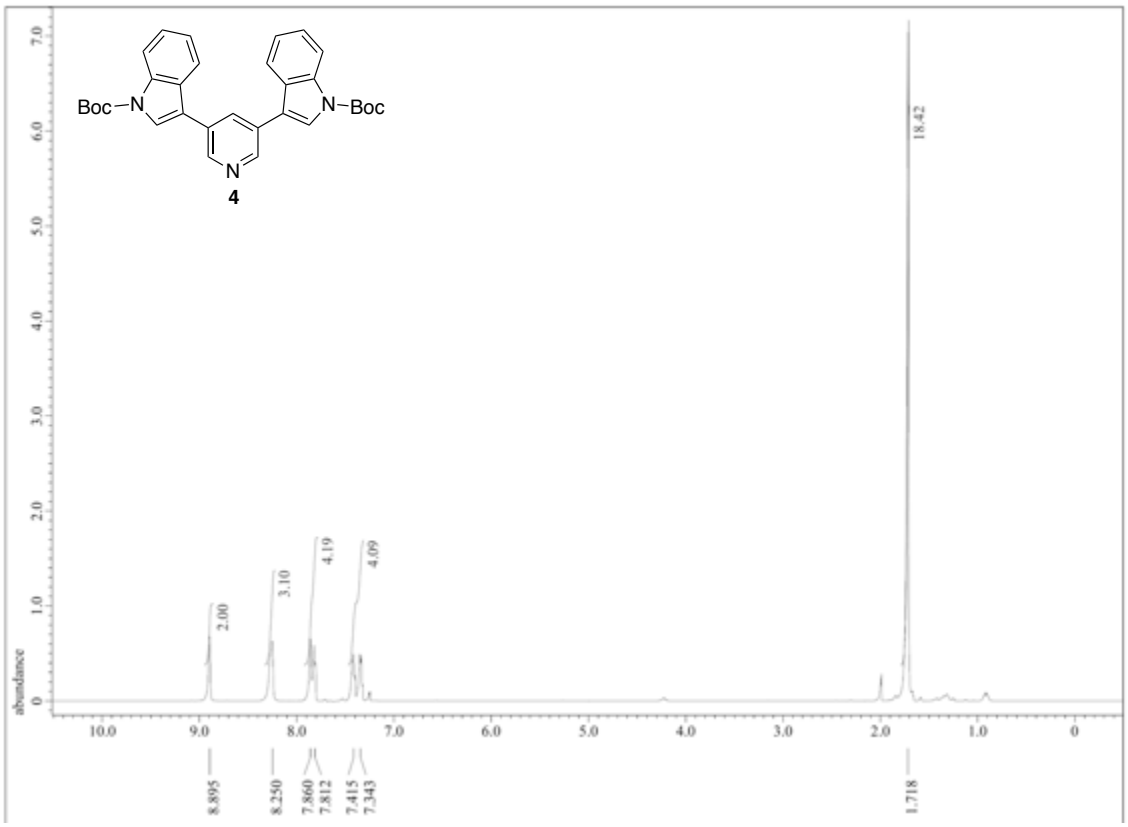


Figure S7. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **4**.

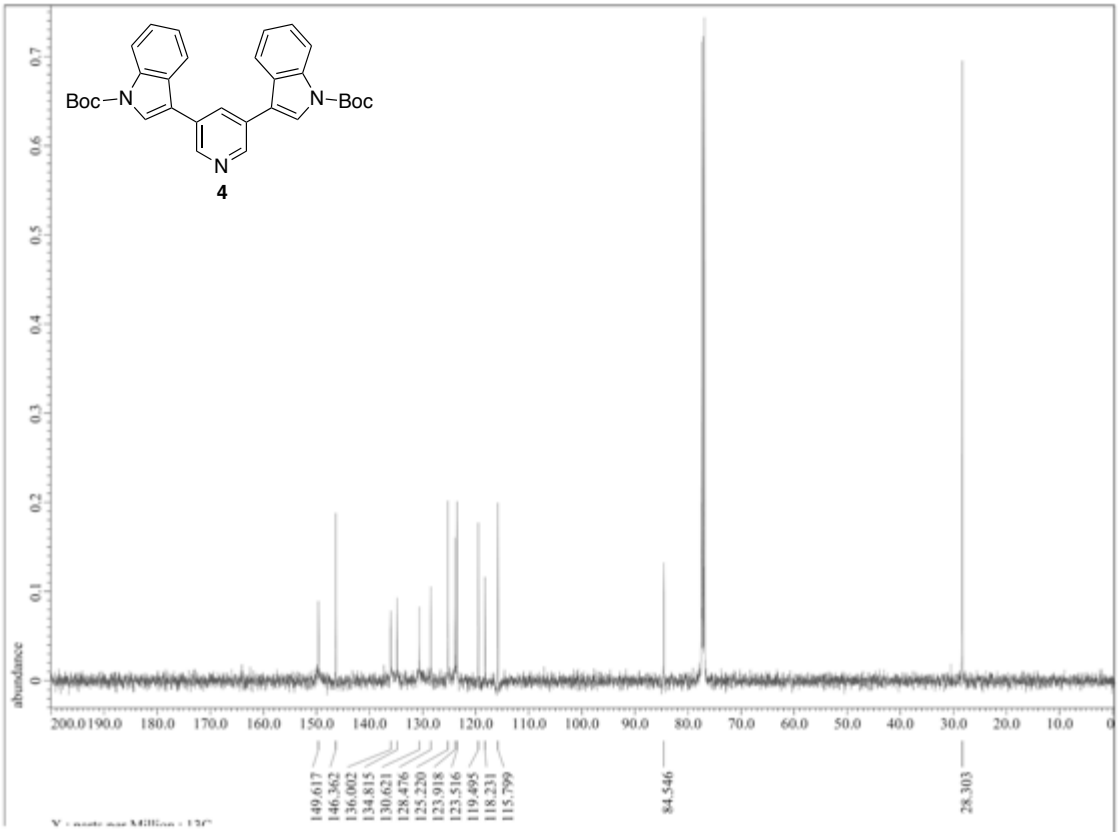


Figure S8. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **4**.

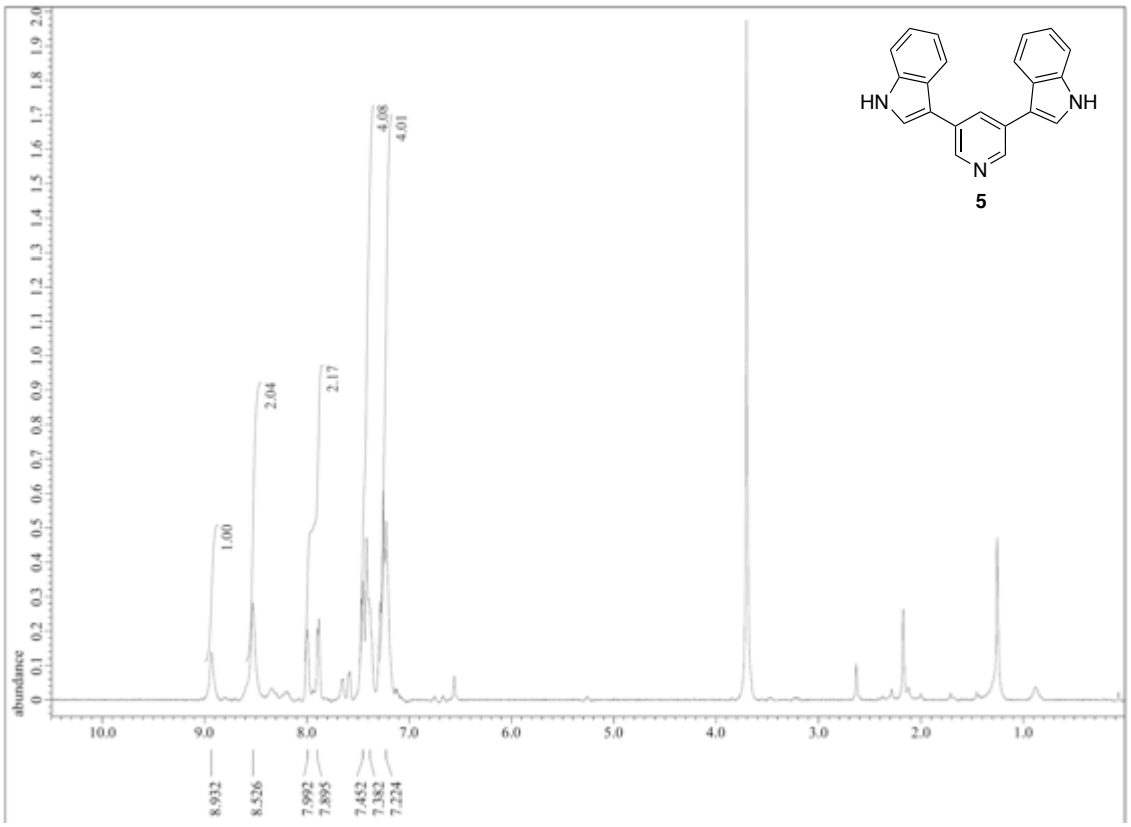


Figure S9. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **5**.

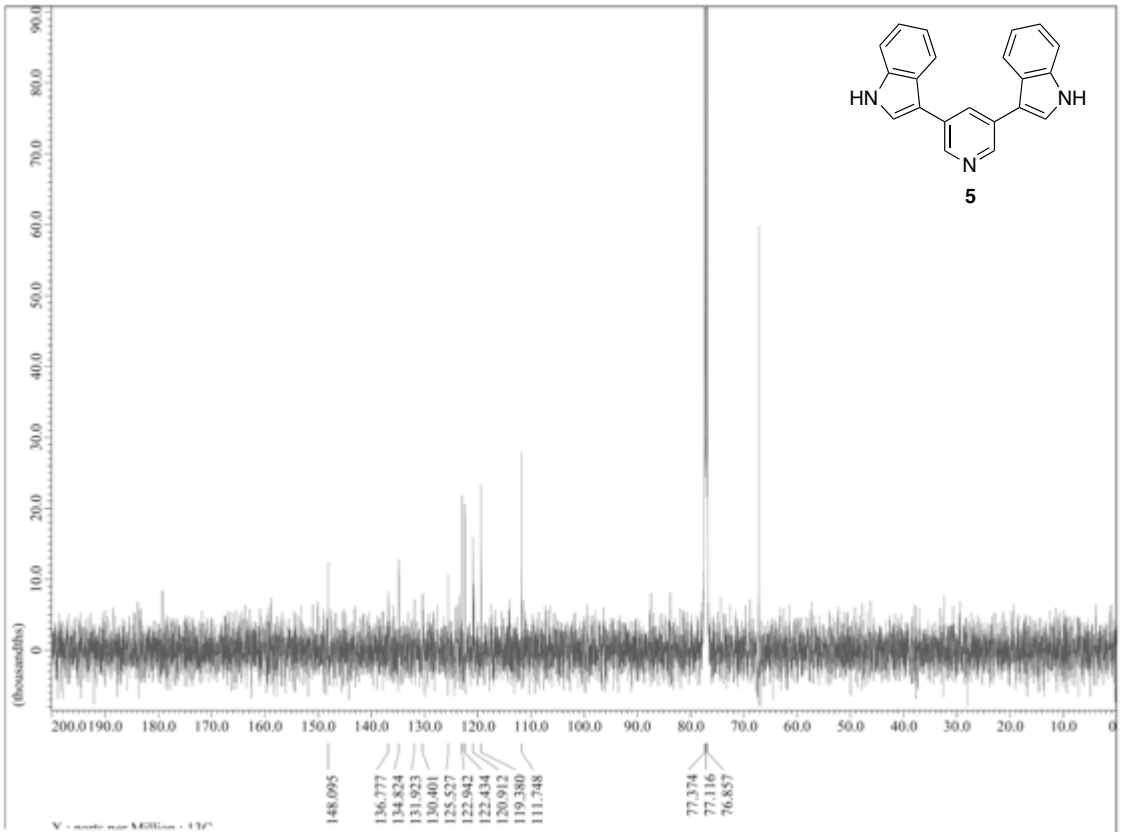


Figure S10. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **5**.

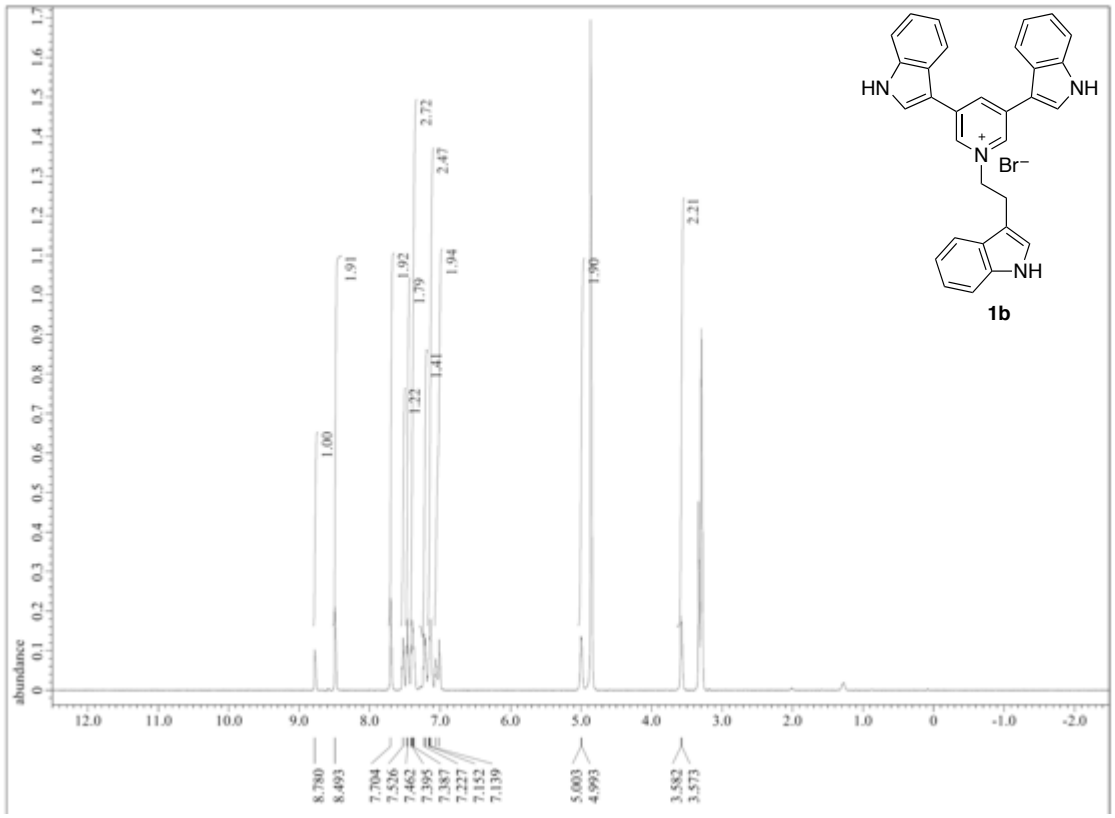


Figure S11. ^{1}H NMR (500 MHz, CD_3OD) spectrum of **1b**.

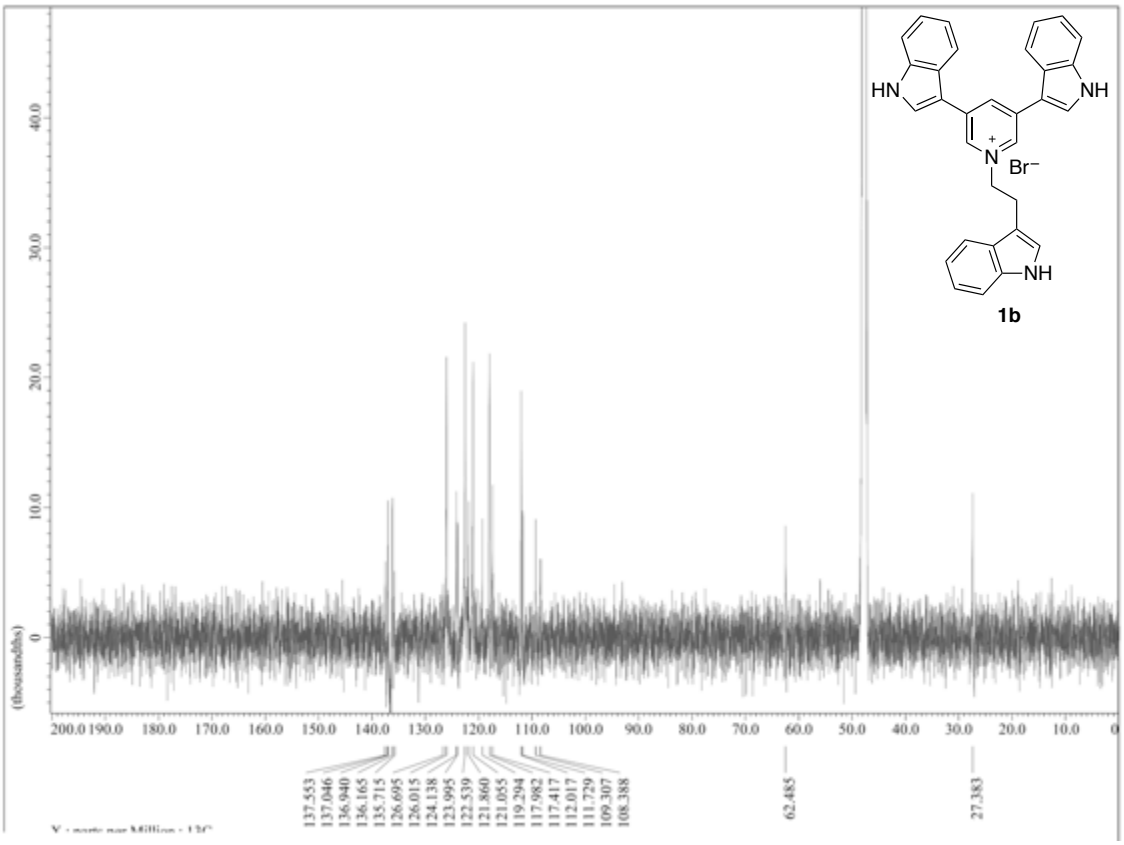


Figure S12. ^{13}C NMR (125 MHz, CD_3OD) spectrum of **1b**.

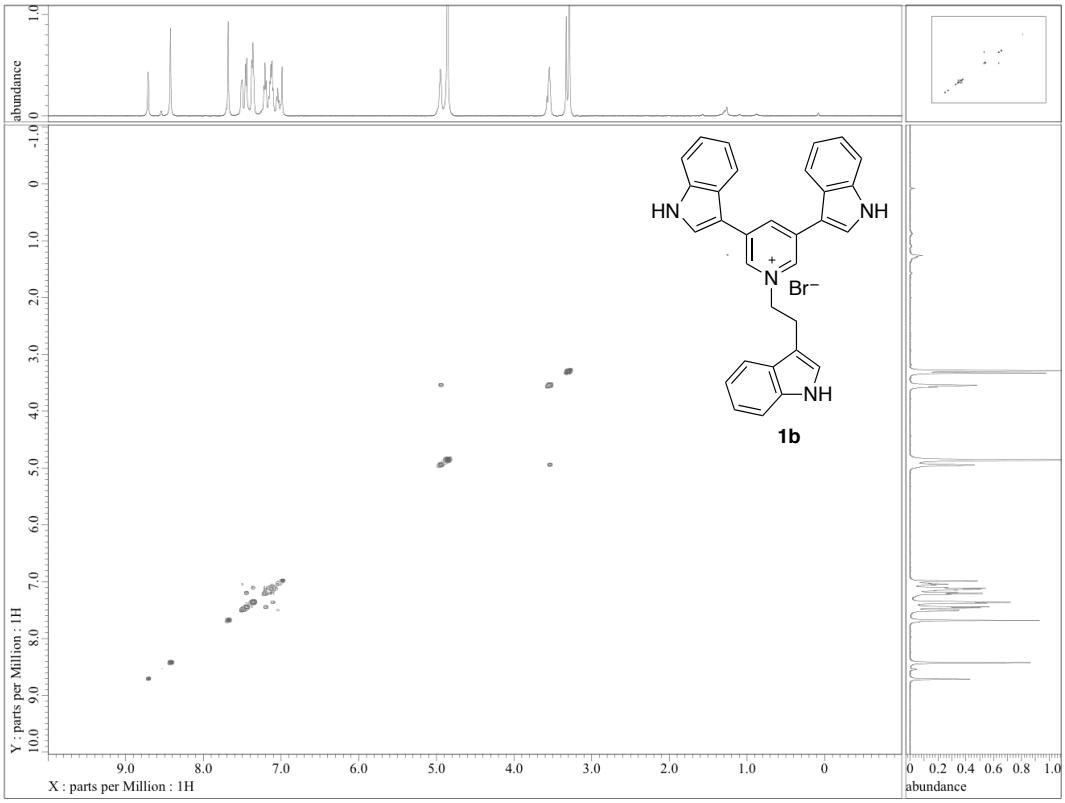


Figure S13. COSY (500 MHz, CD_3OD) spectrum of **1b**.

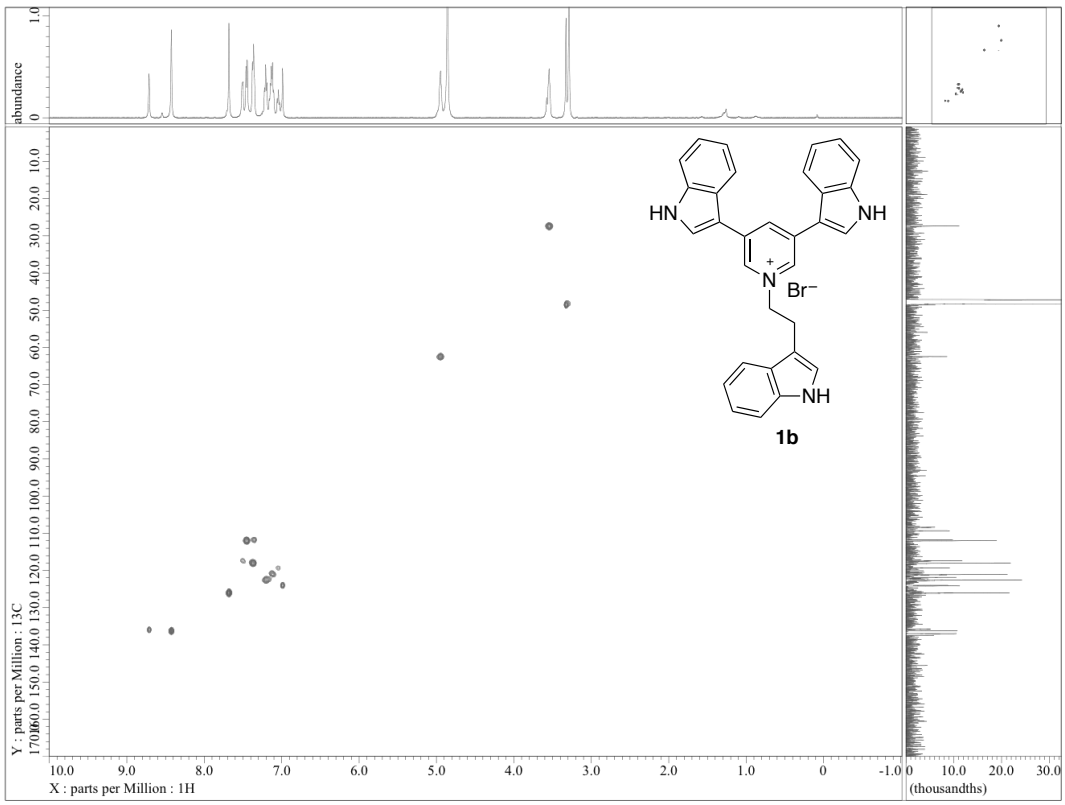


Figure S14. HMQC (500 MHz, CD_3OD) spectrum of **1b**.

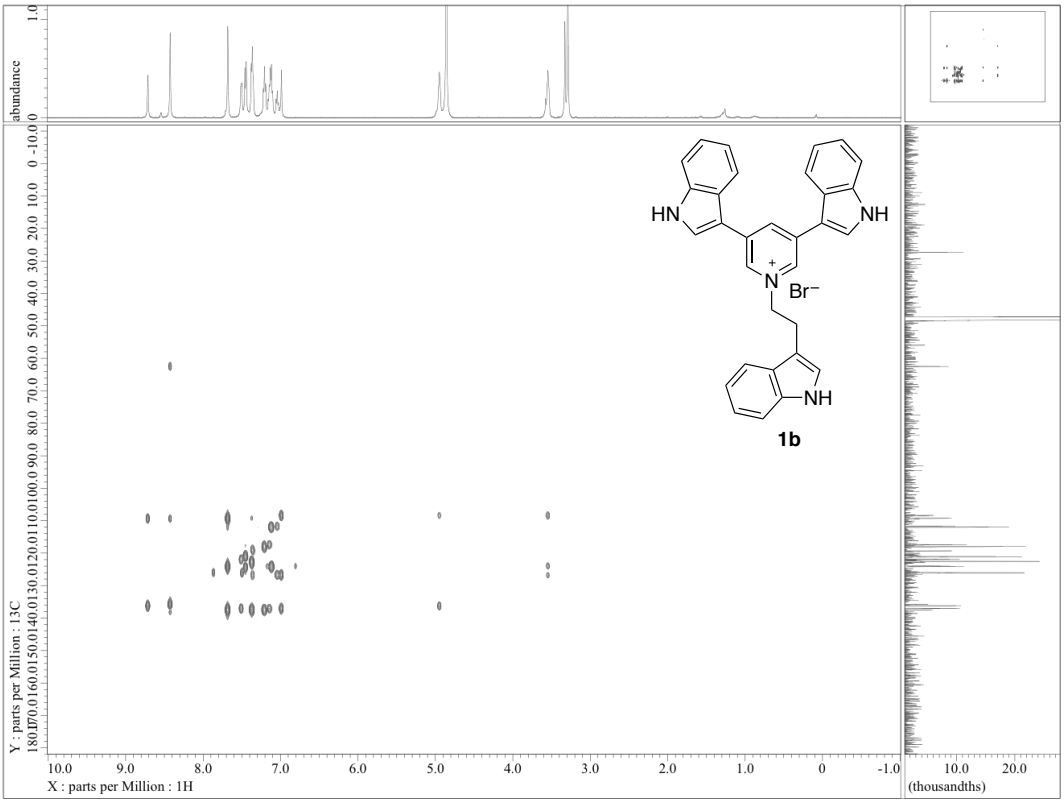


Figure S15. HMBC (500 MHz, CD_3OD) spectrum of **1b**.

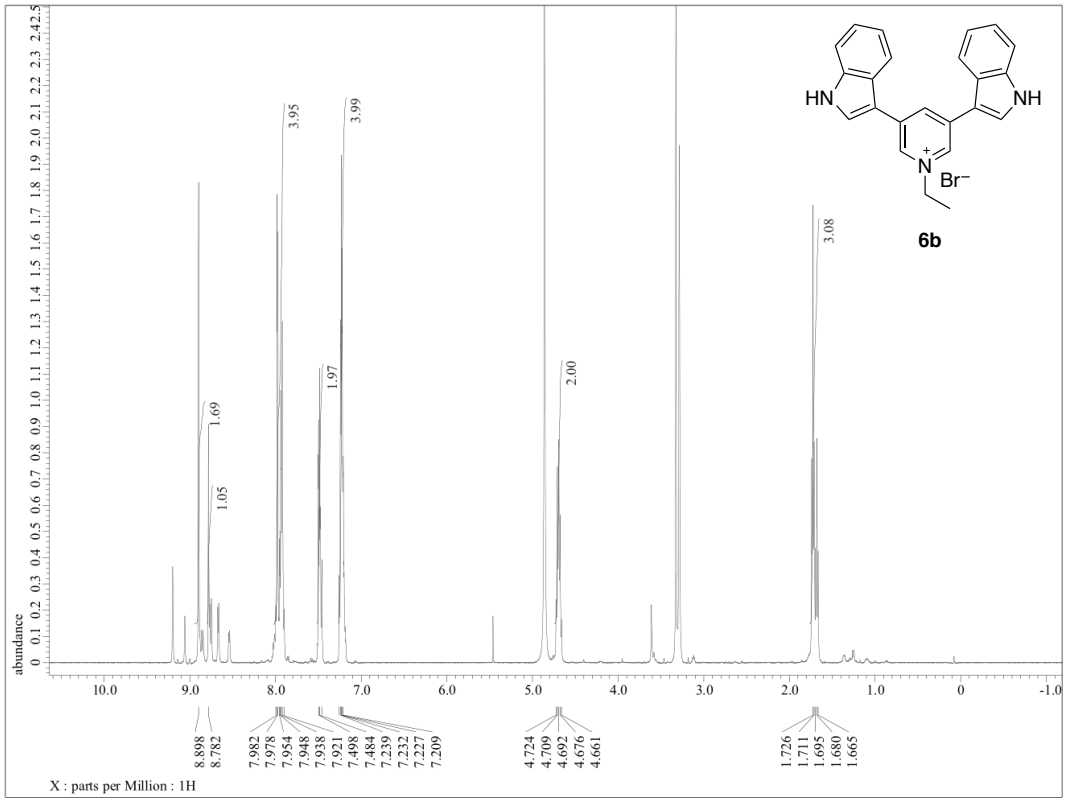


Figure S16. ^{1}H NMR (500 MHz, CD_3OD) spectrum of **6b**.

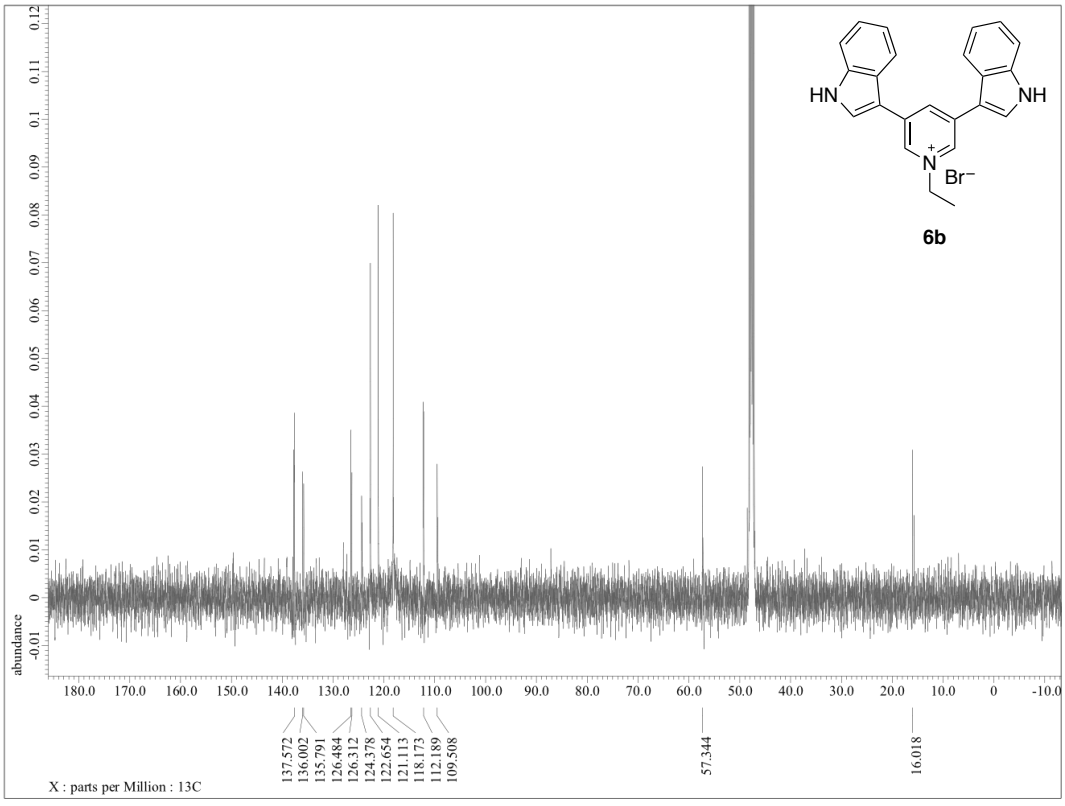


Figure S17. ^{13}C NMR (125 MHz, CD_3OD) spectrum of **6b**.

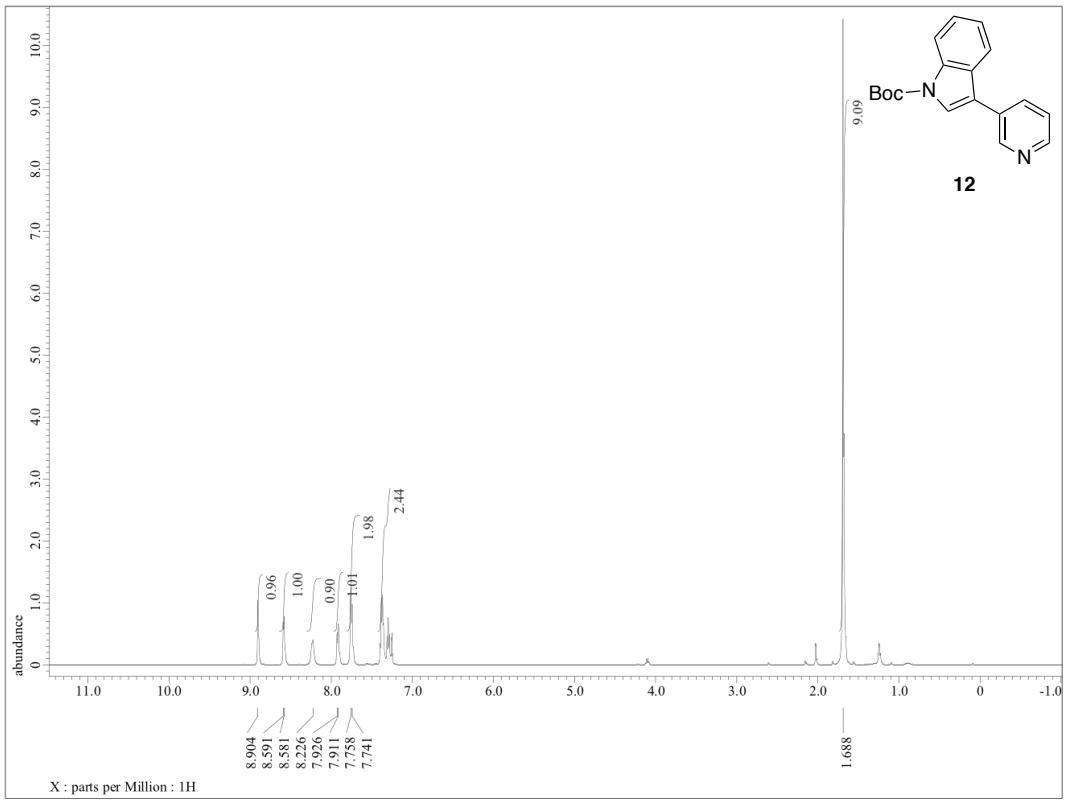


Figure S18. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **12**.

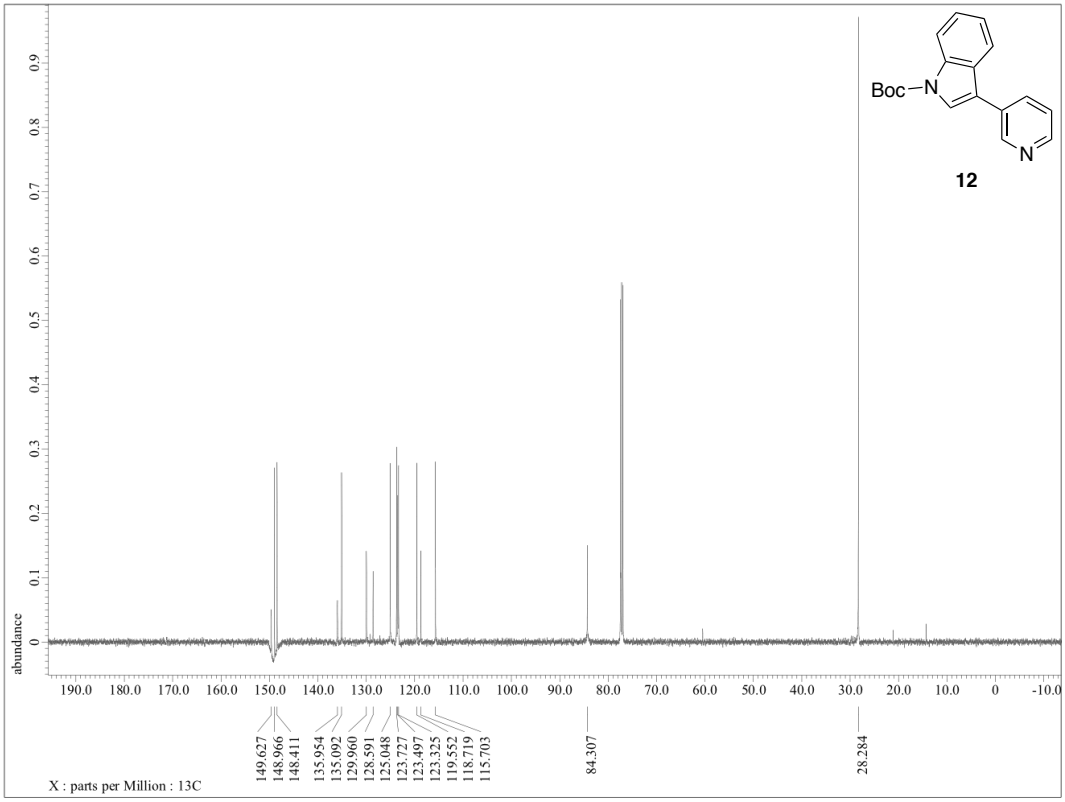


Figure S19. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **12**.

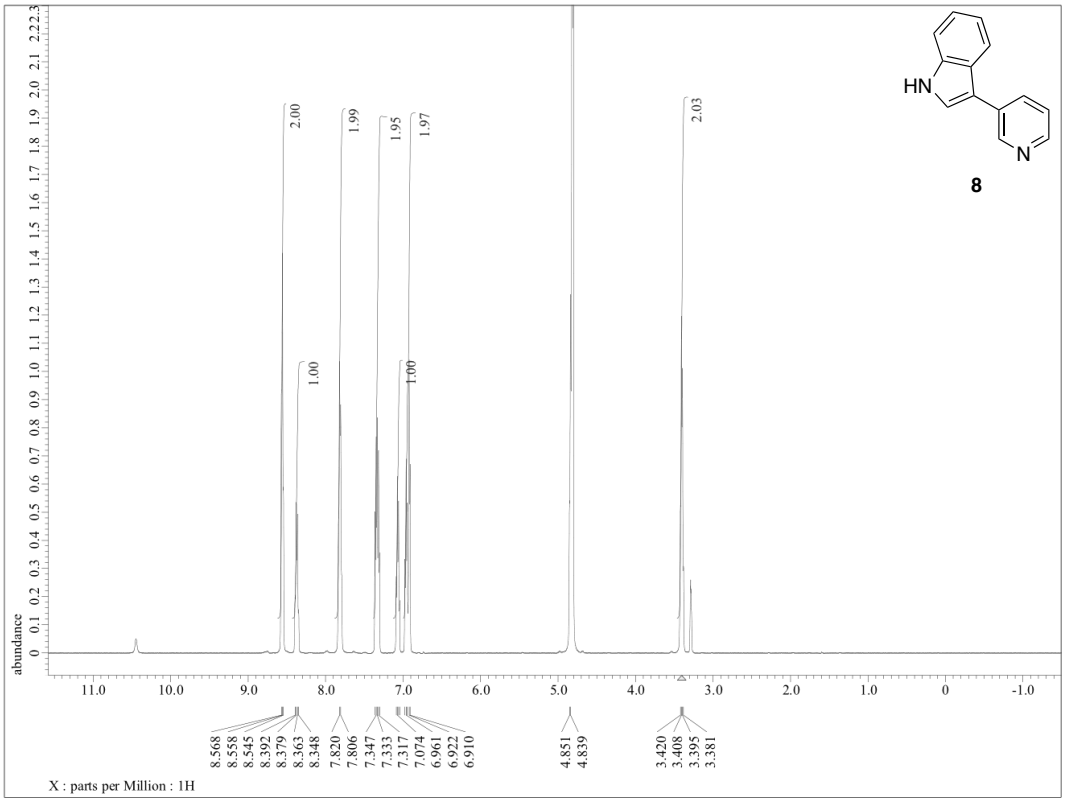


Figure S20. ^{1}H NMR (500 MHz, CD_3OD) spectrum of **8**.

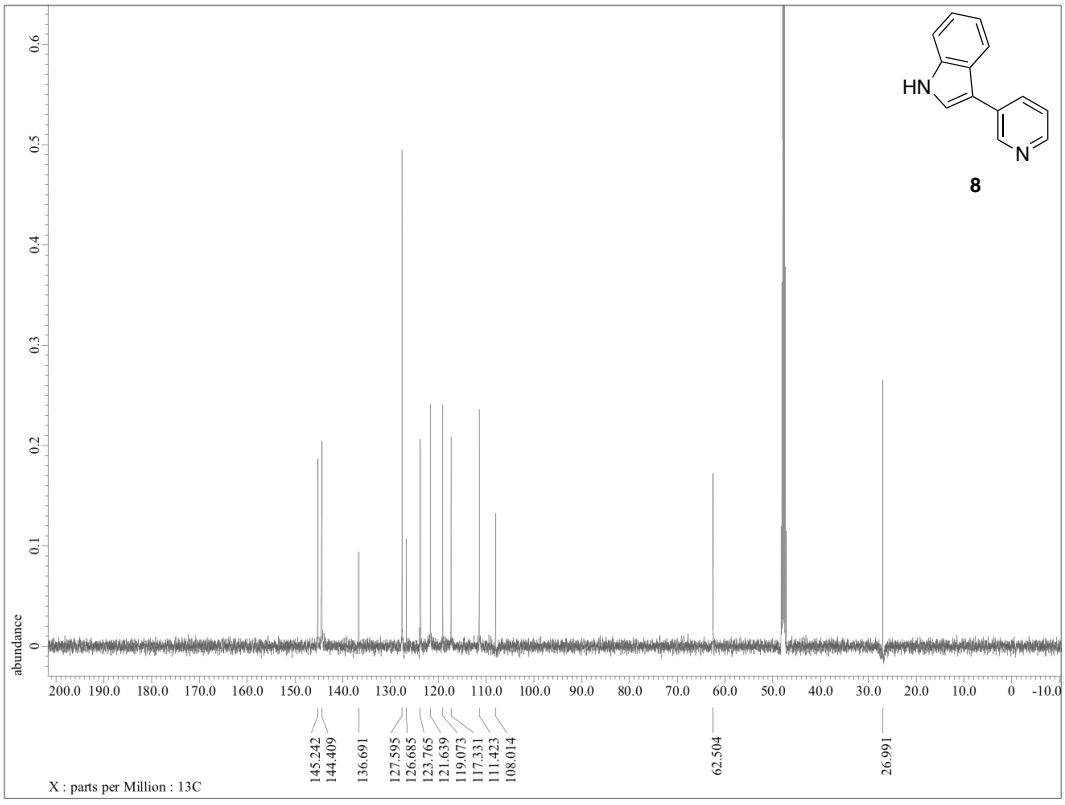


Figure S21. ^{13}C NMR (125 MHz, CD_3OD) spectrum of **8**.

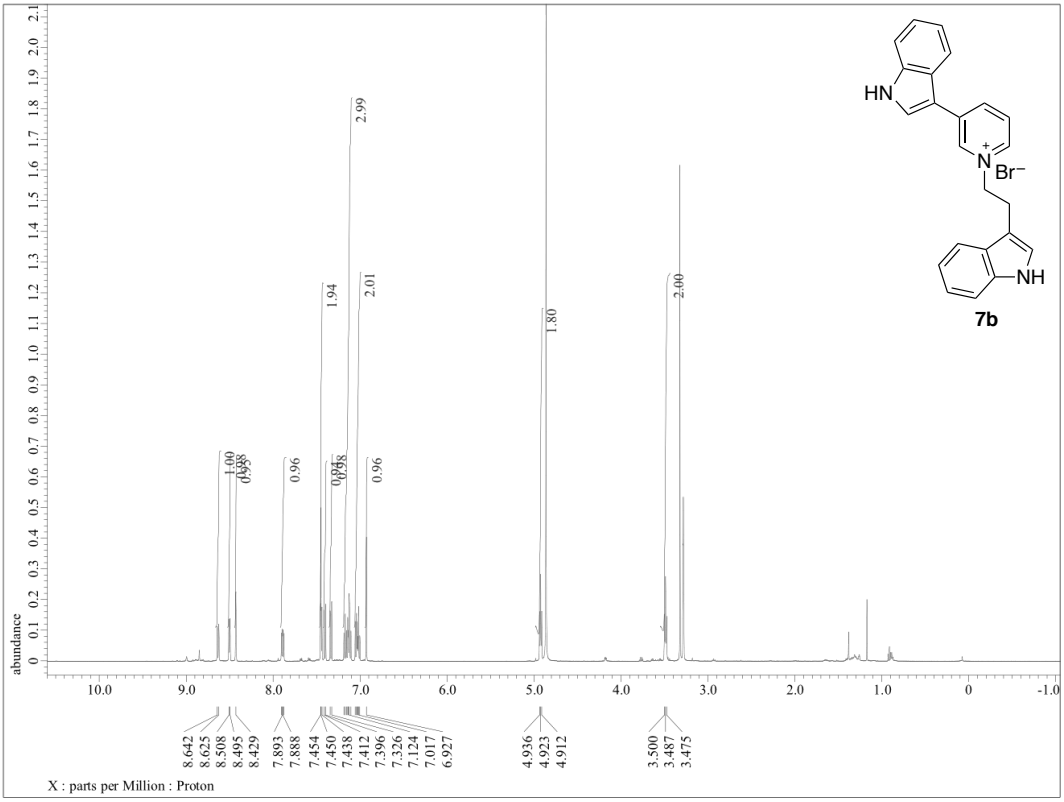


Figure S22. ^{1}H NMR (500 MHz, CD_3OD) spectrum of **7b**.

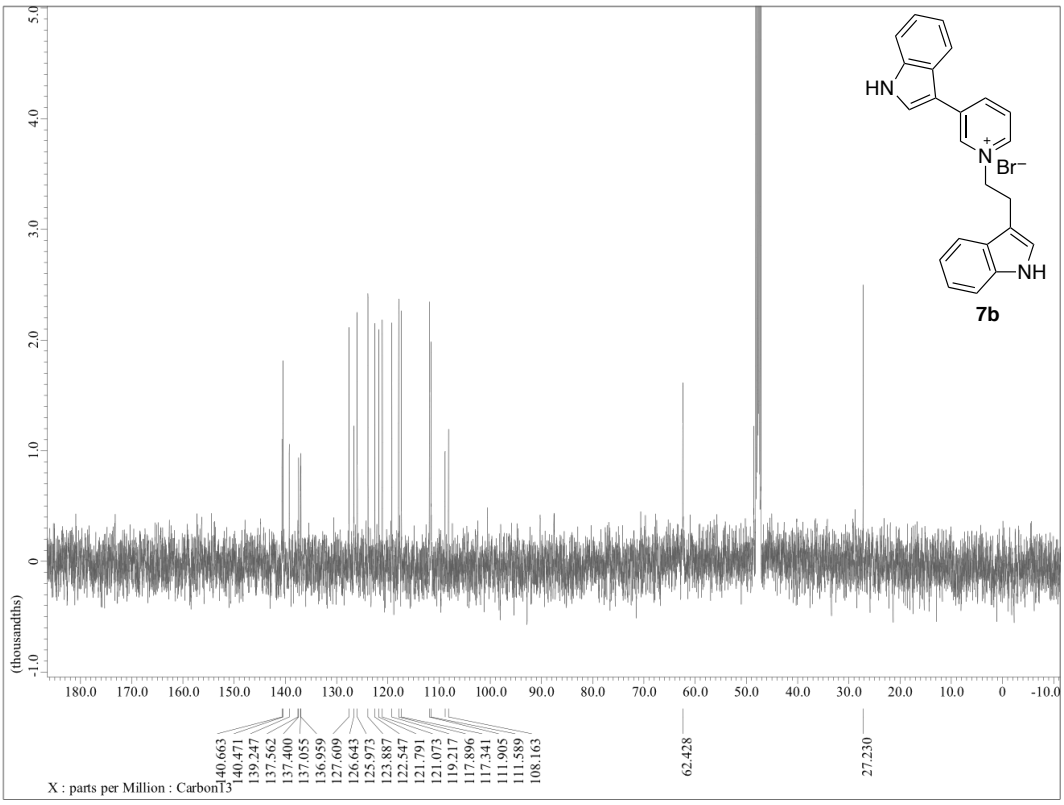


Figure S23. ^{13}C NMR (125 MHz, CD_3OD) spectrum of **7b**.

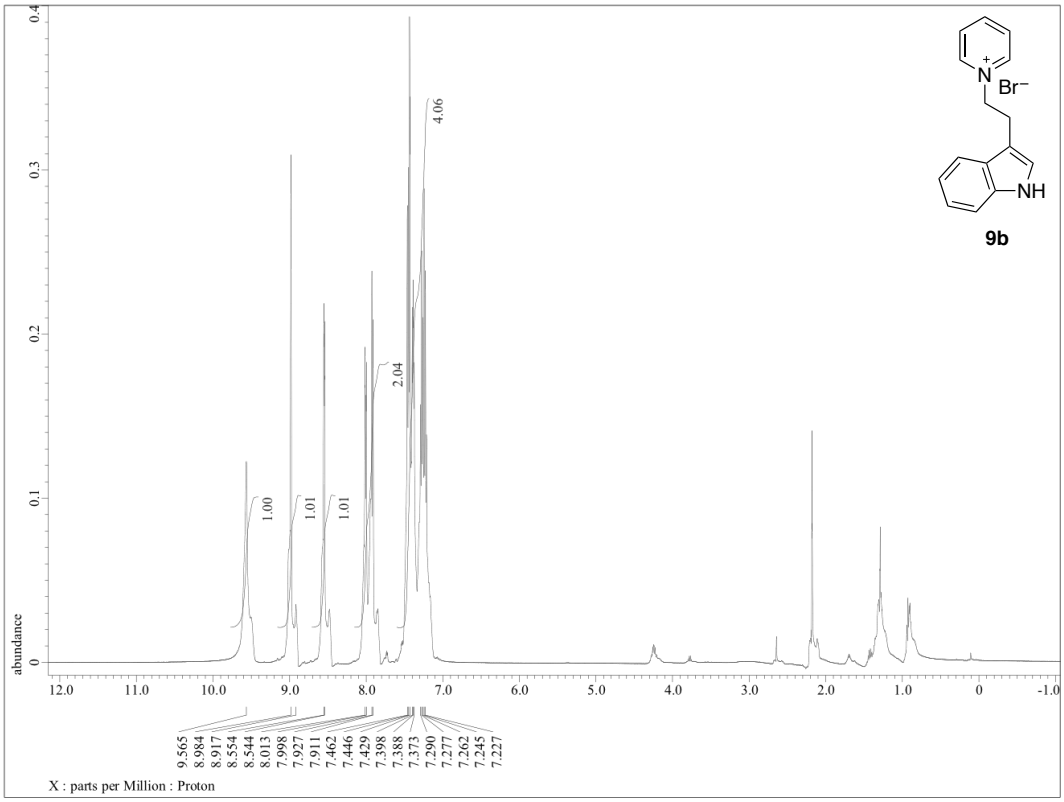


Figure S24. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **9b**.

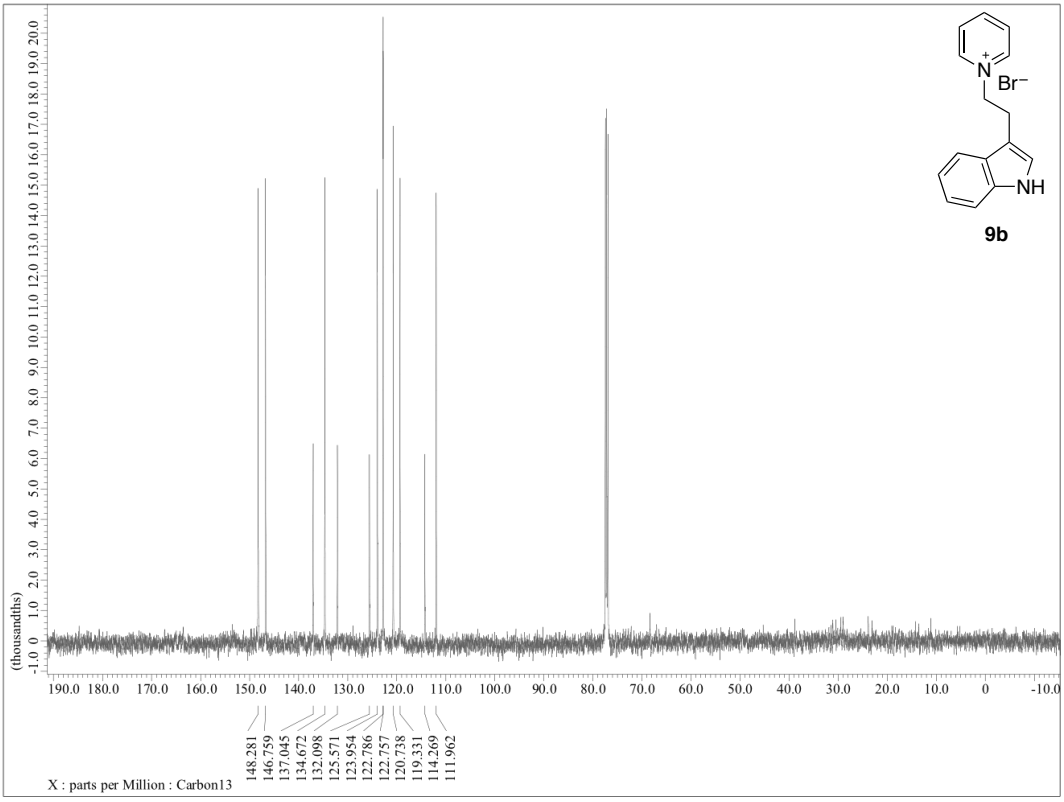


Figure S25. ^{13}C NMR (125 MHz, $CDCl_3$) spectrum of **9b**.

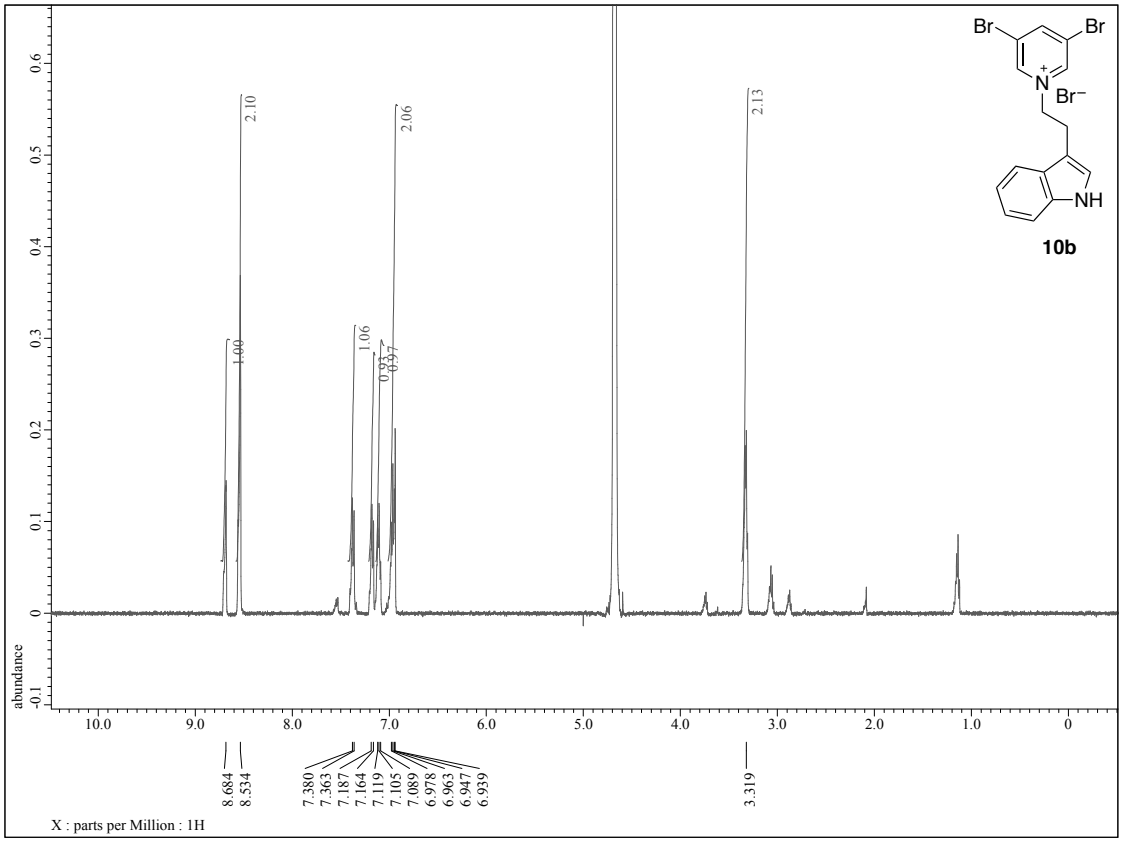


Figure S26. ^{1}H NMR (500 MHz, D_2O) spectrum of **10b**.

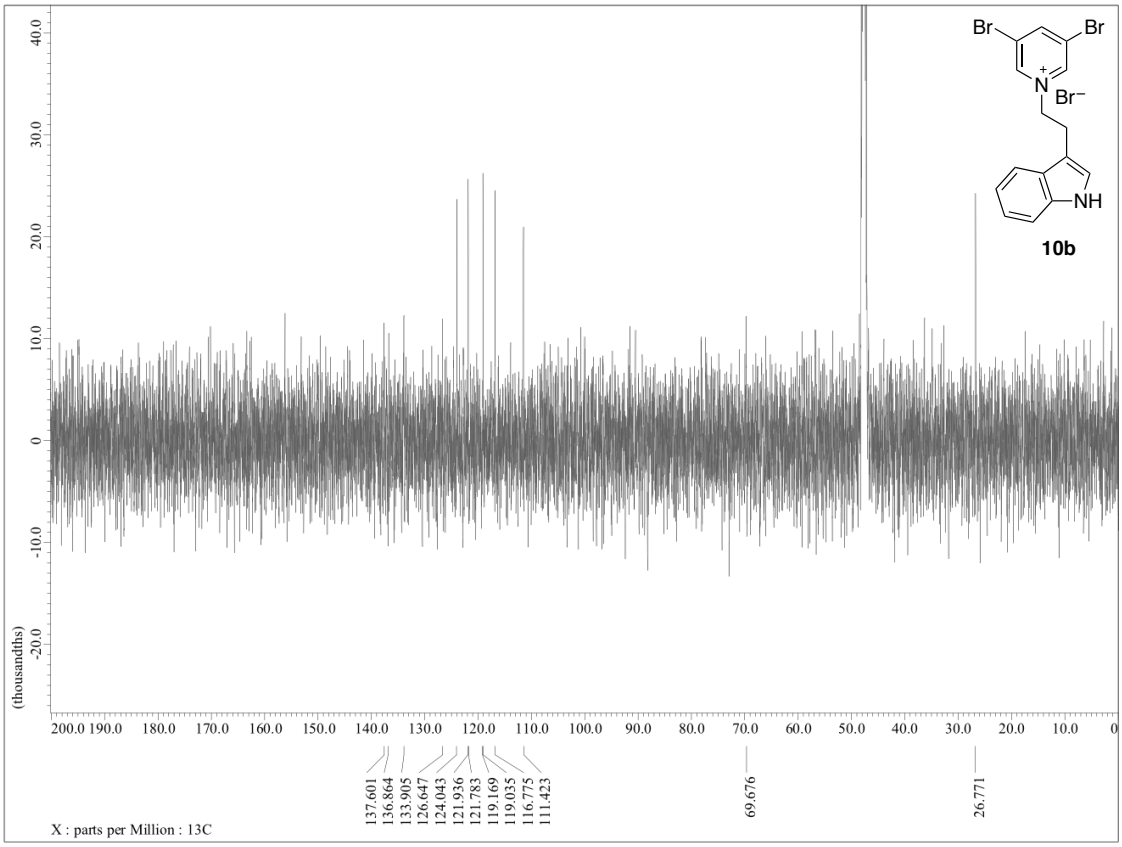
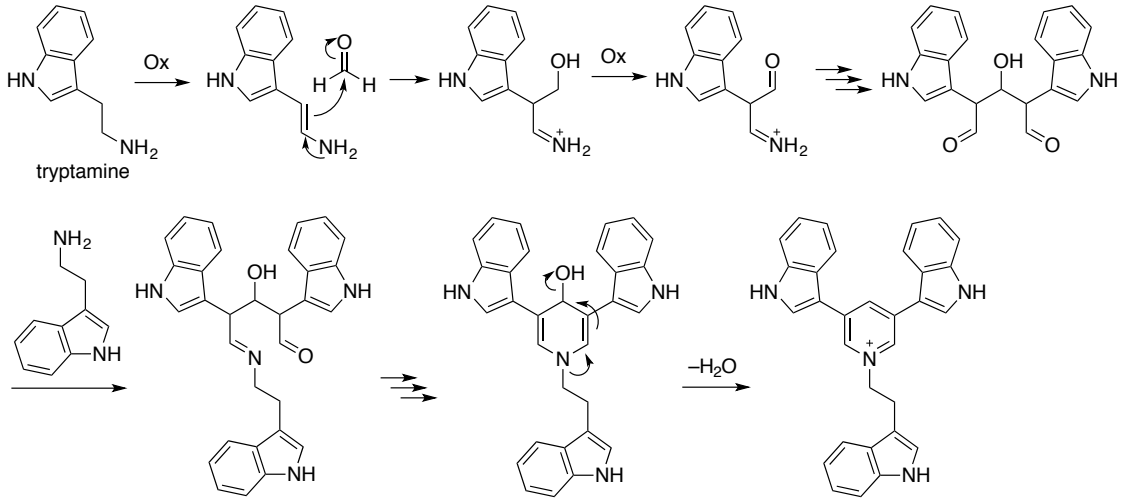


Figure S27. ^{13}C NMR (125 MHz, CD_3OD) spectrum of **10b**.



Scheme S2. Plausible biosynthetic pathway of tricepyridinium.

Table S1. Cytotoxic Activities against P388 Cells of Tricepyridinium Bromide and Its Analogs.

	IC_{50} [μg/mL] (μM)
compound	P388 cells
1b	0.53 ± 0.01 (1.0 ± 0.1)
5	14 ± 1 (45 ± 3)
6b	0.093 ± 0.029 (0.22 ± 0.07)
7b	21 ± 1 (50 ± 3)
8	1.9 ± 0.2 (10 ± 1)
9b	16 ± 1 (53 ± 3)
10b	35 ± 5 (76 ± 10)
Doxorubicin	0.19 ± 0.10 (0.35 ± 0.02)
Cisplatin	14 ± 1 (46 ± 4)