Supporting Information

Studies Directed Toward the Elucidation of the Pharmacophore of Steroid-Based Sonic Hedgehog Signaling Inhibitors

André K. Isaacs,[1] Valérie Baubet,[2] Chaomei Xiang,[2] Nadia Dahmane[*2] and Jeffrey D. Winkler[*1]

[1]Department of Chemistry, The University of Pennsylvania, Philadelphia, PA 19104 and

[2]The Wistar Institute, Molecular and Cellular Oncogenics Program, Philadelphia, PA 19104

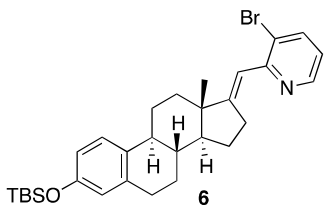
winkler@sas.upenn.edu, ndahmane@wistar.org

General Methods

Solvents used for extraction and purification were HPLC grade from Fisher Scientific. Unless otherwise indicated, all reactions were run under an inert atmosphere of Argon. Anhydrous tetrahydrofuran, ethyl ether and toluene were obtained via passage through an activated alumina column[1]. Commercial reagents were used as received. Deuterated solvents were obtained from Cambridge Isotope labs. Merck pre-coated silica gel plates (250 μm, 60 F254) were used for analytical TLC. Spots were visualized using 254 nm ultraviolet light, with either anisaldehyde or potassium permanganate stains as visualizing agents. Chromatographic purifications were performed on Sorbent Technologies silica gel (particle size 32-63 microns). ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively, in $CDCl_3$ on a Bruker AM-500 or DRX-500 spectrometer. Chemical shifts are reported relative to internal chloroform (δ 7.26 for ^{1}H, δ 77.0 for ^{13}C). Infrared spectra were recorded on a NaCl plate using a Perkin-Elmer 1600 series Fourier transform spectrometer. High-resolution mass spectra were obtained by Dr. Rakesh Kohli at the University of Pennsylvania Mass Spectrometry Service Center, on an Autospec high-resolution double-focusing electrospray ionization/chemical ionization spectrometer with either DEC 11/73 or OPUS software data system. Melting points were obtained on a Thomas Hoover capillary melting point apparatus and are uncorrected.

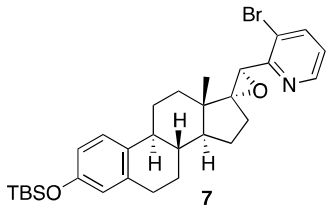
[1] Pangborn, A.B.; Giardello, M.A.; Grubbs, R.H.; Rosen, R.K.; Trimmers, F.J.; *Organometallics* **1996**, *15*, 1518.

Experimental Section



6

Alkene (6). A solution of *tert*-alcohol **5**[2] (362 mg, 0.65 mmol) in 6.5 mL acetic anhydride was heated to reflux for 24 h. The reaction mixture was cooled to room temperature and the acetic anhydride was evaporated. To the residue was added 7 mL pyridine and 7 mL ice. The mixture was allowed to stand for 0.5 h after which water (10 mL) was added. The mixture was extracted with CH_2Cl_2 (3x 30 mL). The combined organic layers were washed with 10% HCl (20 mL), sat. $NaHCO_3$ (20 mL), brine (20 mL) and dried with Na_2SO_4. The solvent was evaporated *in vacuo* and the crude product was purified by silica gel chromatography (15% ethyl acetate in hexanes) to yield **6** as a yellow solid (176 mg, 51%). mp 147–149°C. $[\alpha]^{23.8}$ D = +14.94 (*c* = 1.07, $CHCl_3$). **^{1}H NMR** ($CDCl_3$): δ = 8.50 (d, *J* = 4.5 Hz, 1H), 7.80 (dd, *J* = 8.0 and 1.1 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 1H), 6.90 (dd, *J* = 8.0 and 4.5 Hz), 6.63 (dd, *J* = 8.4 and 2.3 Hz, 1H), 6.58 (m, 2H), 3.01 (m, 1H), 2.90–2.80 (m, 3H), 2.42 (m, 1H), 2.27 (m, 1H), 2.15 (m, 1H) 1.99–1.89 (m, 2H), 1.64–1.26 (m, 6H), 0.99 (s, 9H), 0.96 (s, 3H), 0.20 (s, 6H). **^{13}C NMR** ($CDCl_3$): δ = 164.6, 155.7, 153.4, 147.3, 140.0, 137.9, 135.2, 133.2, 126.1, 121.0, 120.0, 117.2, 114.5, 52.9, 46.5, 44.2, 38.7, 36.0, 30.3, 29.8, 29.7, 26.7, 25.7, 24.5, 18.9, -4.4. **FTIR** (thin film) 2927, 2857, 1645, 1496, 1258, 839 cm^{-1}. **HRMS** (ES) Calcd. for $C_{25}H_{33}O_2N$: 537.2062 (M^+), found 538.2131 (MH^+).

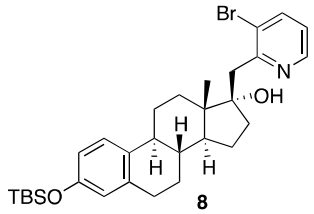


7

Epoxide (7). To a solution of alkene **6** (98 mg, 0.18 mmol) in dry $CHCl_3$ (9 ml) at 0 °C was added dropwise a solution of 77% *m*CPBA (38 mg, 0.22 mmol) in dry $CHCl_3$ (1 ml). The reaction was allowed

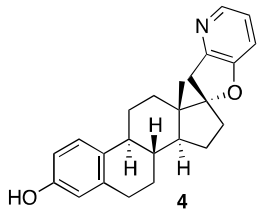
[2] Winkler, J.; Isaacs, A.; Holderbaum, L.; Tatard, V.; Dahmane, N.; *Org Lett* **2009**, *11*, 2824.

to warm to room temperature and stirred for 6 h. Another solution of 77% *m*CPBA (25 mg, 0.15 mmol) was added dropwise and the solution was allowed to stir for a further 6 h. Reaction was diluted with $CHCl_3$ (5 ml), washed with water (5 ml), saturated $NaHCO_3$ (5 ml), brine (5 ml) and dried with Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by silica gel chromatography (20% ethyl acetate in hexanes) to yield **7** as a waxy solid (43 mg, 43% yield). **^{1}H NMR** ($CDCl_3$): δ = 8.56 (d, *J* = 4.6 Hz, 1H), 7.84 (dd, *J* = 8.0 and 1.3 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.09 (dd, *J* = 8.0 and 4.7 Hz, 1H), 6.62 (dd, *J* = 8.4 and 2.5 Hz, 1H), 6.54 (d, *J* = 2.3 Hz, 1H), 4.24 (s, 1H), 2.84–2.81 (m, 2H), 2.39–2.28 (m, 2H), 1.93–1.90 (m, 2H), 1.81–1.26 (m, 9H), 1.04 (s, 3H), 0.98 (s, 9H), 0.19 (s, 6H). **^{13}C NMR** ($CDCl_3$): δ = 154.6, 153.4, 148.1, 139.8, 137.8, 133.1, 126.1, 123.4, 120.0, 119.9, 117.2, 77.2, 58.1, 51.7, 43.7, 42.7, 38.9, 30.6, 29.7, 27.5, 26.7, 25.8, 25.7, 23.3, 18.2, 16.6, -4.4. **FTIR** (thin film) 2929, 2857, 1606, 1573, 1495, 1286, 1250 cm^{-1}. **HRMS** (ES) Calcd. for $C_{30}H_{40}O_2NSiBr$: 553.2011 (M^+), found 576.1900 (MNa^+).



8

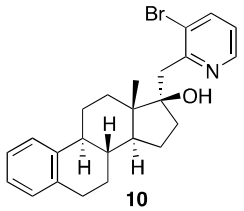
Alcohol (8). To a solution of epoxide **7** (17 mg, 0.035 mmol) in HMPA (0.33 mL) at room temperature was added 0.09M SmI_2 in THF (1mL, 0.09 mmol). A 0.17M solution of pivalic acid in THF was added (0.3 mL, 0.05 mmol) and the solution was allowed to stir for 18 h. The reaction was quenched with a solution of sodium potassium tartrate (1 mL). The mixture was extracted with diethyl ether (3x 2 mL) and the organic layer was washed with H_2O (2x 2 mL) and dried with Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by silica gel chromatography (20% ethyl acetate in hexanes) to yield **8** as a yellow oil (11mg, 65% yield). **^{1}H NMR** ($CDCl_3$): δ = 8.42 (d, *J* = 4.6 Hz, 1H), 7.88 (d, *J* = 8.1 Hz, 1H), 7.07 (m, 2H), 6.58 (dd, *J* = 8.4 and 2.4 Hz, 1H), 6.54 (d, *J* = 1.9 Hz, 1H), 6.29 (s, 1H), 3.24 (s, 2H), 2.79 (m, 2H), 2.22 (m, 2H), 2.01 (m, 2H), 1.71–1.92 (m, 4H), 1.38–1.44 (m, 3H), 1.24–1.31 (m, 3H), 0.97 (s, 9H), 0.83 (s, 3H), 0.12 (s, 6H). ^{13}C **NMR** ($CDCl_3$): δ = 159.2, 153.2, 146.4, 140.9, 138.0, 133.5, 126.0, 122.7, 122.0, 119.9, 117.0, 84.1, 48.8, 47.7, 43.5, 39.8, 39.2, 37.8, 31.4, 29.8, 27.9, 26.4, 25.7, 23.6, 18.2, 16.0, -4.4. **FTIR** (thin film) 3382, 2925, 2853, 1604, 1495, 1251 cm^{-1}. **HRMS** (ES) Calcd. for $C_{30}H_{42}O_2NSiBr$: 555.2168 (M^+), found 556.2269 (MH^+).



4

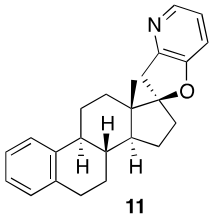
C-17 Epi Analog (4). A resealable Schlenk tube was charged with alcohol **8** (11 mg, 0.02 mmol), $Pd(OAc)_2$ (1 mg, 0.004 mmol), BINAP (3 mg, 0.004 mmol) and Cs_2CO_3 (10 mg, 0.03 mmol). Dry toluene (0.2 mL) was added and the tube was capped under argon. The resulting mixture was allowed to stir at 80 ^oC for 12 h. The mixture was allowed to cool to room temperature, filtered through Celite, concentrated and purified by silica gel chromatography (15% ethyl acetate in hexanes) to yield a spirocycle as a waxy solid (6 mg, 64%): $[\alpha]^{24.4}$ D = +13.31 (*c* = 0.57, $CHCl_3$). **^{1}H NMR** ($CDCl_3$): δ = 8.0 (d, *J* = 4.7 Hz, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 7.00–6.94 (m, 2H), 6.61 (dd, *J* = 8.4 and 2.5 Hz, 1H), 6.56 (d, *J* = 2.4 Hz, 1H), 3.41 (d, *J* = 16.9 Hz, 1H), 3.08 (d, *J* = 16.9 Hz, 1H), 2.83 (m, 2H), 2.33–2.17 (m, 3H), 1.91–2.07 (m, 4H), 1.70 (m, 1H), 1.50–1.37 (m, 5H), 0.99 (s, 9H), 0.85 (s, 3H), 0.19 (s, 6H). **^{13}C NMR** ($CDCl_3$): δ = 153.4, 152.7, 151.0, 140.9, 137.9, 133.0, 126.1, 122.4, 120.0, 117.2, 115.0, 100.1, 49.5, 47.3, 43.6, 39.3, 38.4, 36.0, 30.0, 29.7, 27.8, 25.9, 25.7, 23.6, 18.2, 15.5, -4.4. **FTIR** (thin film) 2927, 2856, 1606, 1495, 1256 cm^{-1}. **HRMS** (ES) Calcd. for $C_{30}H_{41}O_2NSi$: 475.2906 (M^+), found 476.2973 (MH^+).

To a solution of the spirocycle (8 mg, 0.02 mmol) in dry THF (1 mL) at 0^oC was added TBAF (1M in THF, 34 μL, 0.03 mmol) dropwise. The reaction was allowed to warm to room temperature and was complete after 2 h of stirring. The reaction was quenched with 1 mL of sat. NH_4Cl. The mixture was extracted with Et_2O (2x 3 mL) and dried with Na_2SO_4. The solvent was removed *in vacuo*, and the product was purified by silica gel chromatography (50% ethyl acetate in hexanes) to yield **4** as a yellow solid (5 mg, 96%). mp 200–202^oC. $[\alpha]^{27.4}$ D = +14.1 (*c* = 0.36, $CHCl_3$). **^{1}H NMR** ($CDCl_3$): δ = 8.0 (d, *J* = 4.2 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 1H), 7.02–6.96 (m, 2H), 6.62 (dd, *J* = 8.4 and 2.5 Hz, 1H), 6.58 (d, *J* = 2.2 Hz), 5.00 (s, 1H), 3.41 (d, *J* = 16.9 Hz, 1H), 3.08 (*J* = 16.9 Hz, 1H), 2.85 (m, 2H), 2.32–2.17 (m, 3H), 2.07–1.91 (m, 3H), 1.70 (m, 1H), 1.50–1.37 (m, 3H), 0.84 (s, 3H). **^{13}C NMR** ($CDCl_3$): δ = 153.5, 152.6, 151.0, 140.8, 138.3, 132.6, 126.5, 122.5, 115.3, 112.7, 100.1, 49.5, 47.3, 43.5, 39.4, 38.3, 35.9, 30.0, 29.7, 27.7, 26.0, 23.6, 15.5. **FTIR** (thin film) 2927, 2856, 1606, 1495, 1256 cm^{-1}. **HRMS** (ES) Calcd. for $C_{24}H_{27}O_2N$: 361.2042 (M^+), found 362.2109 (MH^+).



10

Alcohol (10). To a solution of diisopropylamine (368 μL, 2.62 mmol) in dry Et_2O (13 mL) stirred at –20 °C under argon was added dropwise a solution on 2.5 M *n*-BuLi in hexanes (1.05 mL, 2.62 mmol). The mixture was stirred at –20 °C for 30 minutes. 2-methyl-3-bromopyridine (451 mg, 2.62 mmol) was added dropwise. The resulting red mixture was stirred at –20 °C for 2 h under argon. The known 3-deoxyestrone[3] **9** (334 mg, 1.31 mmol) in Et_2O (20 mL) was added dropwise and stirred at –20 °C for 1 h. The reaction flask was allowed to warm up to room temperature and was quenched slowly with H_2O (10 mL). The mixture was extracted with Et_2O (3x 40 mL), washed with 10% HCl (30 mL), saturated $NaHCO_3$ (30 mL), brine (30 mL), and dried with Na_2SO_4. The solvent was removed *in vacuo*, and the crude product was purified by silica gel chromatography (20% ethyl acetate in hexanes) to yield **10** as a pale yellow waxy solid (530 mg, 95 %). $[\alpha]^{26.7}$ D = +10.2 (*c* = 0.98, $CHCl_3$). **^{1}H NMR** ($CDCl_3$): δ = 8.43 (dd, *J* = 4.7 and 1.5 Hz, 1H), 7.90 (dd, *J* = 8.1 and Hz, 1H), 7.29 (d, *J* = 7.2 Hz, 1H), 7.15–7.06 (m, 4H), 6.25 (s, 1H), 3.34 (d, *J* = 15.3 Hz, 1H), 3.15 (d, *J* = 15.3 Hz, 1H), 2.92–2.87 (m, 2H), 2.36–2.28 (m, 2H), 1.96–1.93 (m, 1H), 1.80–1.56 (m, 7H), 1.45–1.42 (m, 3H), 1.00 (s, 3H). **^{13}C NMR** ($CDCl_3$): δ = 159.5, 146.6, 140.8, 140.5, 136.8, 129.0, 125.6, 125.5, 125.3, 122.8, 122.6, 83.6, 50.0, 47.1, 44.4, 41.7, 39.3, 36.6, 32.6, 29.6, 27.6, 26.2, 23.6, 14.2. **FTIR** (thin film) 3365, 2931, 2870, 1429 cm^{-1}. **HRMS** (ES) Calcd. for $C_{24}H_{28}NOBr$: 425.1354 (M^+), found 426.1438 (MH^+).



11

[3] Nicolaou, K. C.; Barnette, W.E., et al.; *J. Org. Chem.* **1980**, *45*, 1463-1470.

C3-Deoxy Analog (11). A resealable Schlenk tube was charged with spirocycle **10** (156 mg, 0.37 mmol), $Pd(OAc)_2$ (17 mg, 0.07 mmol), BINAP (46 mg, 0.07 mmol) and Cs_2CO_3 (181 mg, 0.56 mmol). Dry toluene (7.5 mL) was added and the tube was capped under argon and the resulting mixture was allowed to stir at 80 ^oC for 1 h. The mixture was allowed to cool to room temperature, filtered through Celite, concentrated and purified by silica gel chromatography (15% ethyl acetate in hexanes) to yield **11** as a waxy solid (100 mg, 79%): $[\alpha]^{26.7}$ D = -38.91 (*c* = 0.97, $CHCl_3$). **^{1}H NMR** ($CDCl_3$): δ = 8.01 (d, *J* = 4.6 and 1.5 Hz, 1H), 7.27 (d, *J* = 1.8 Hz, 1H), 7.15–7.08 (m, 3H), 7.00–6.95 (m, 2H), 3.55 (d, *J* = 16.7 Hz, 1H), 3.01 (d, *J* = 16.7 Hz, 1H), 2.91–2.88 (m, 2H), 2.38–2.26 (m, 3H), 2.04–1.92 (m, 2H), 1.87–1.81 (m, 1H), 1.62–1.39 (m, 7H), 1.05 (s, 3H). **^{13}C NMR** ($CDCl_3$): δ = 153.5, 151.2, 141.0, 139.9, 136.6, 129.0, 125.68, 125.65, 125.3, 122.2, 114.7, 98.2, 48.9, 46.3, 44.1, 40.3, 39.0, 36.9, 31.6, 29.5, 27.2, 25.9, 22.4, 14.3. **FTIR** (thin film) 2932, 2871, 1430, 737 cm^{-1}. **HRMS** (ES) Calcd. for $C_{24}H_{27}NO$: 345.2093 (M^+), found 346.2154 (MH^+).

NMR Spectra

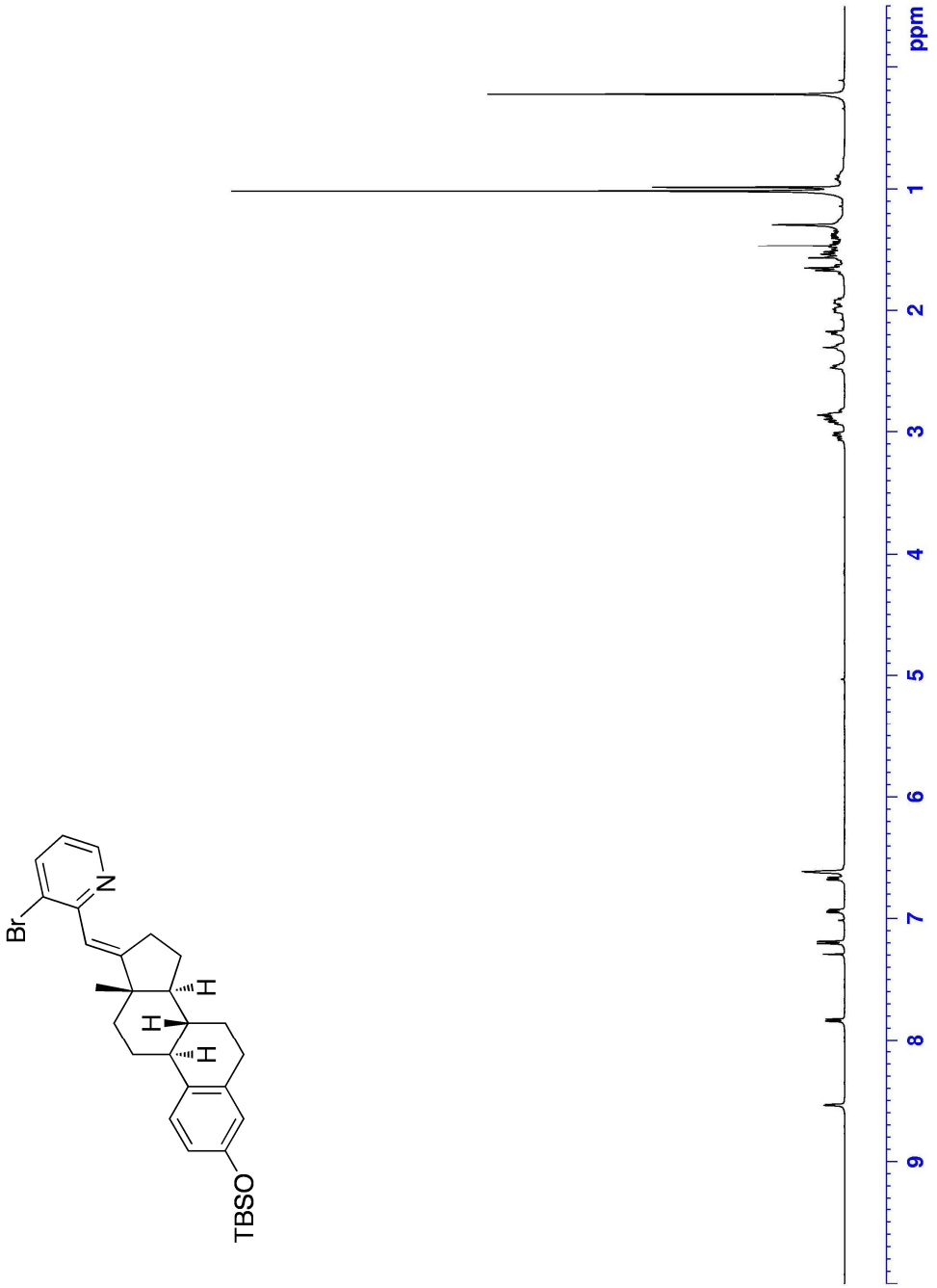


Figure 1: ^{1}H NMR ($CDCl_3$, 500 MHz) of **6**.

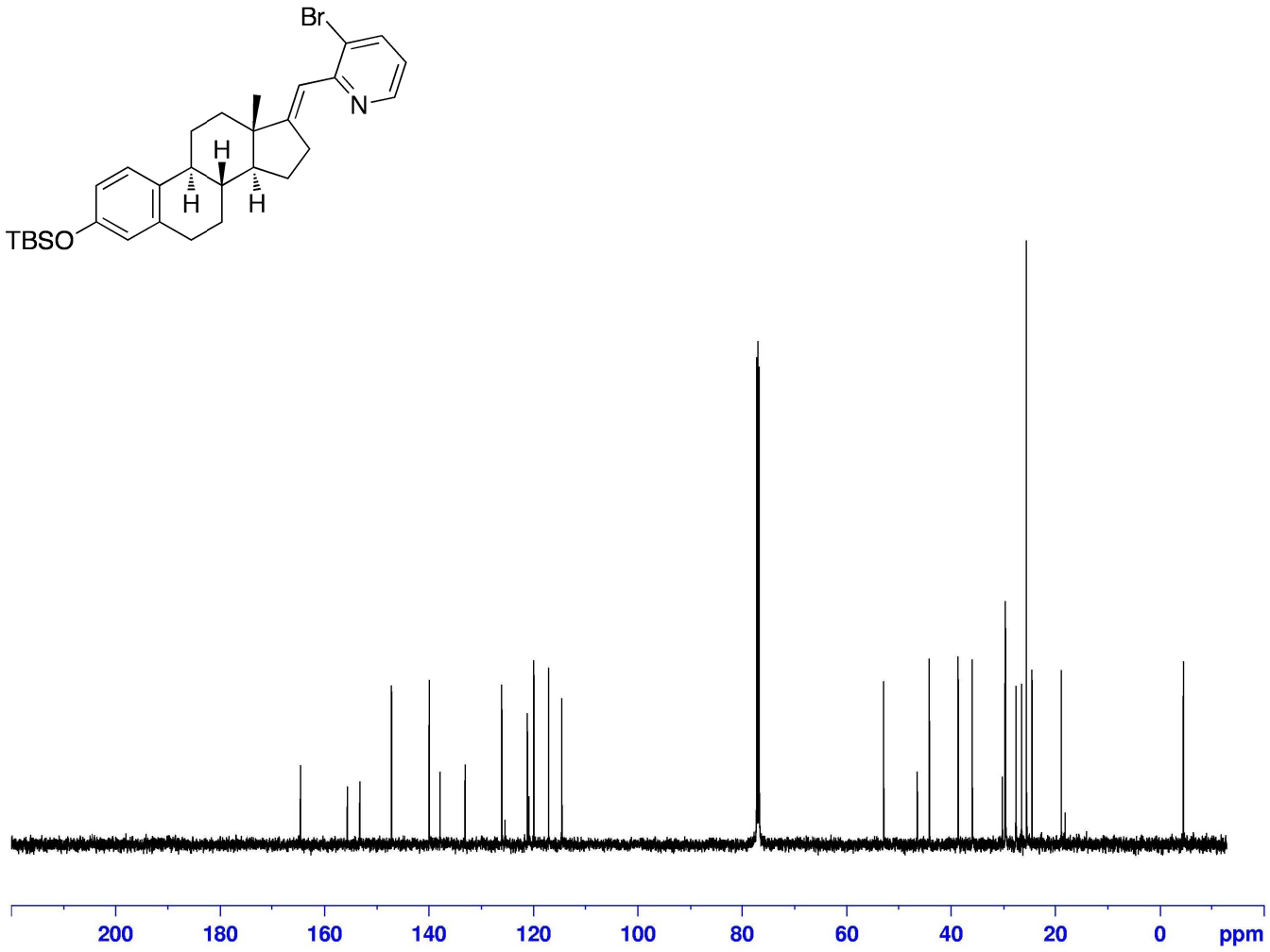


Figure 2: ^{13}C NMR ($CDCl_3$, 125 MHz) of **6**.

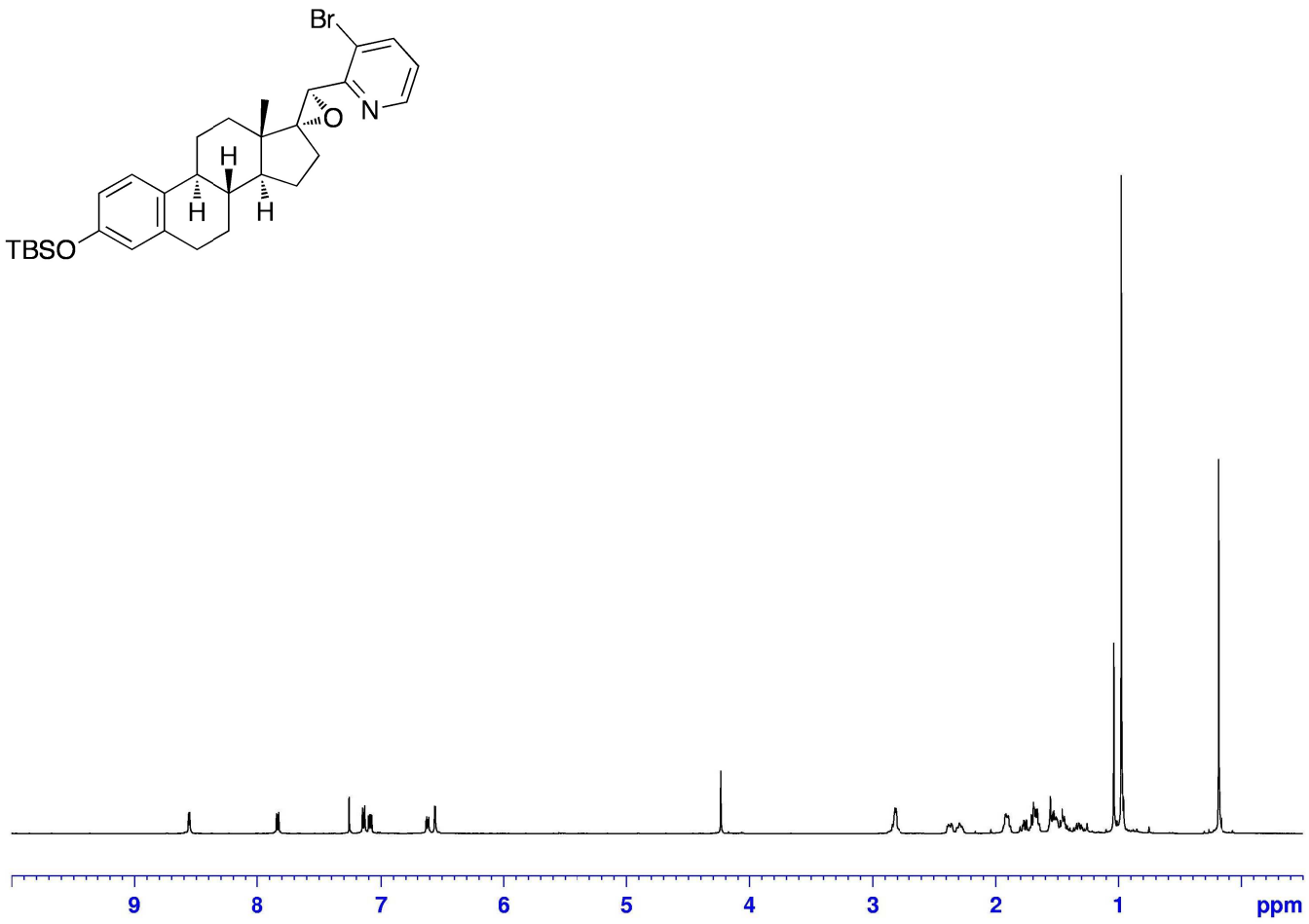


Figure 3: ^{1}H NMR ($CDCl_3$, 500 MHz) of **7**.

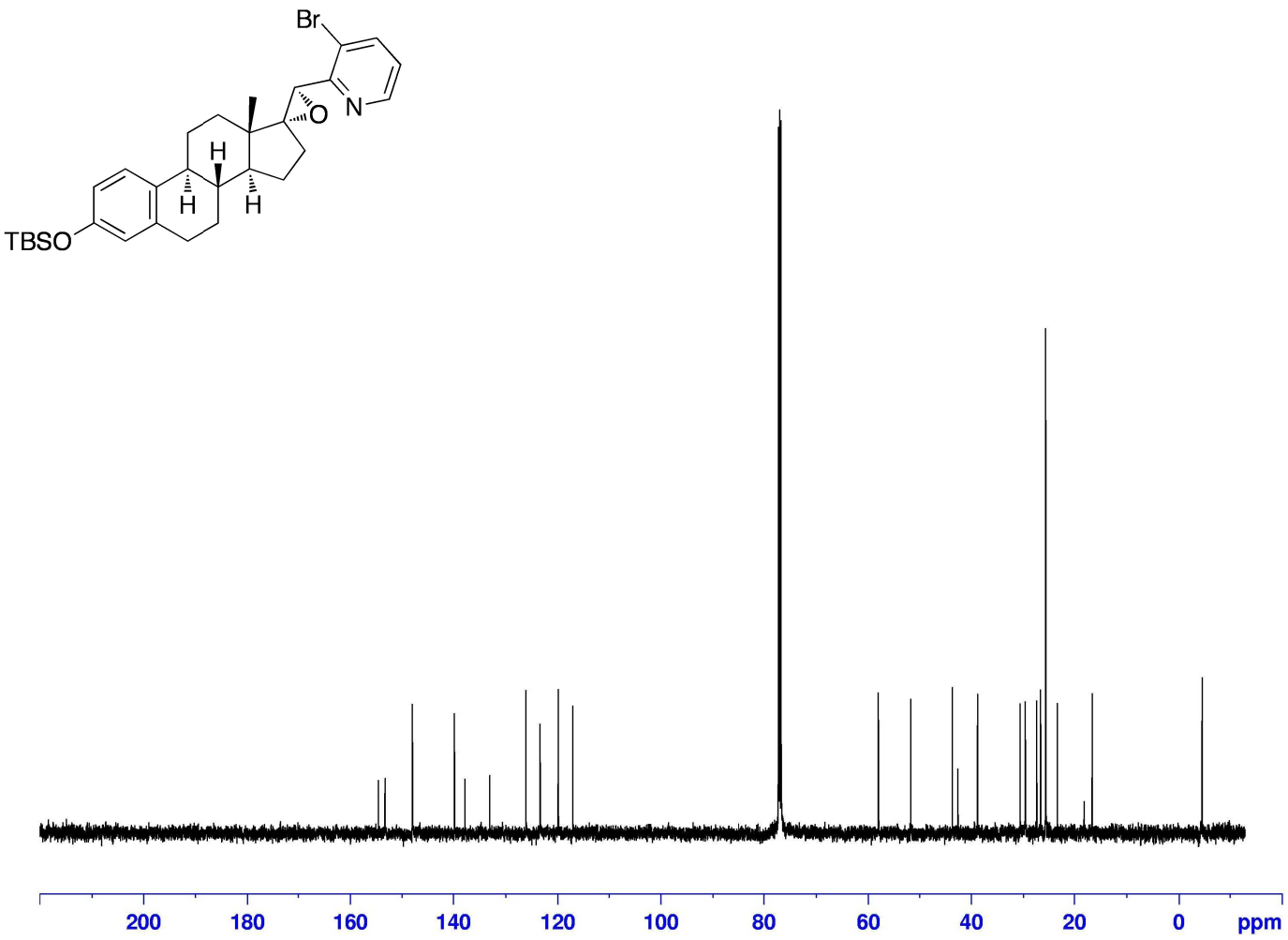


Figure 4: ^{13}C NMR ($CDCl_3$, 125 MHz) of **7**.

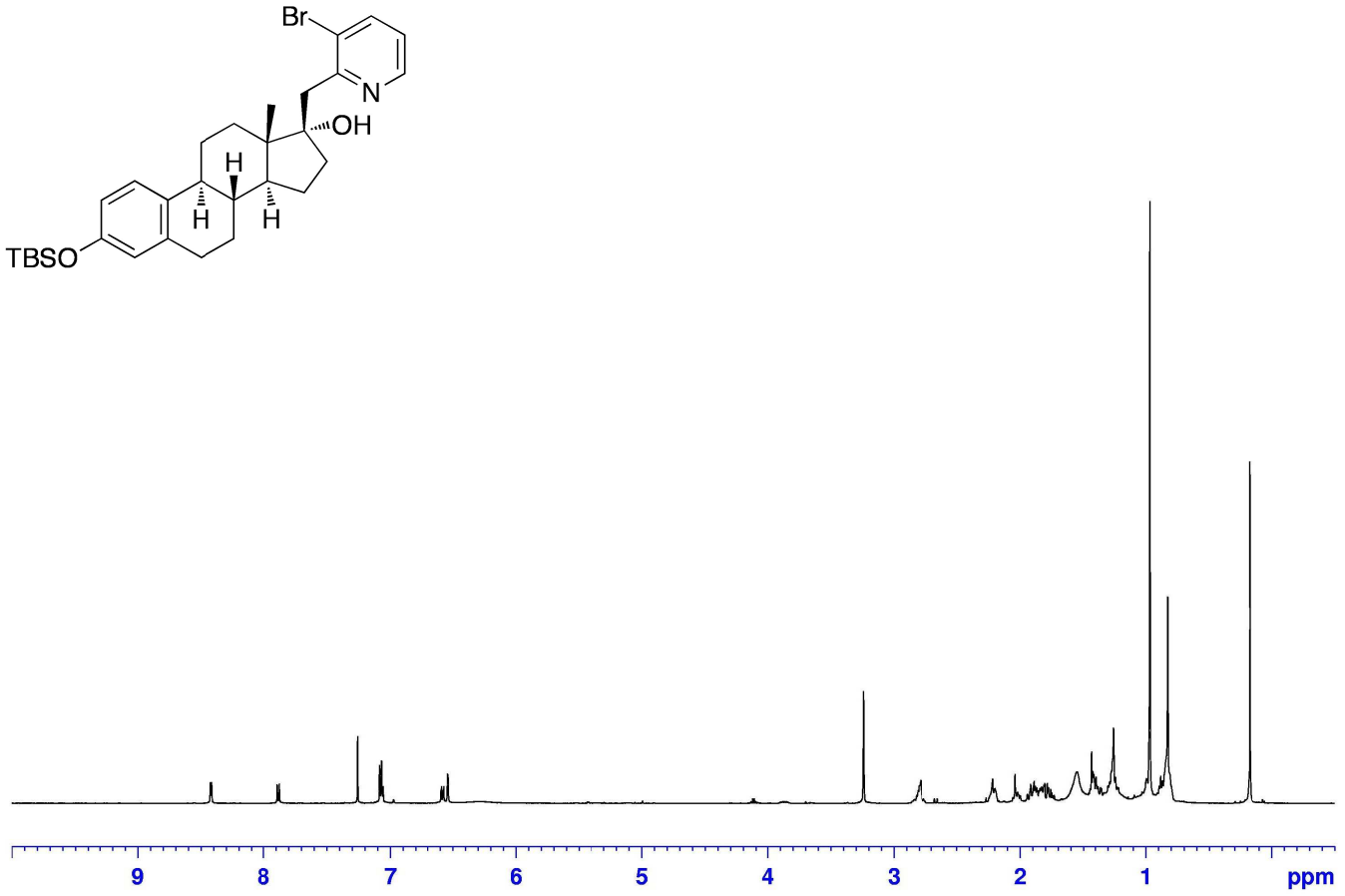


Figure 5: ^{1}H NMR ($CDCl_3$, 500 MHz) of **8**.

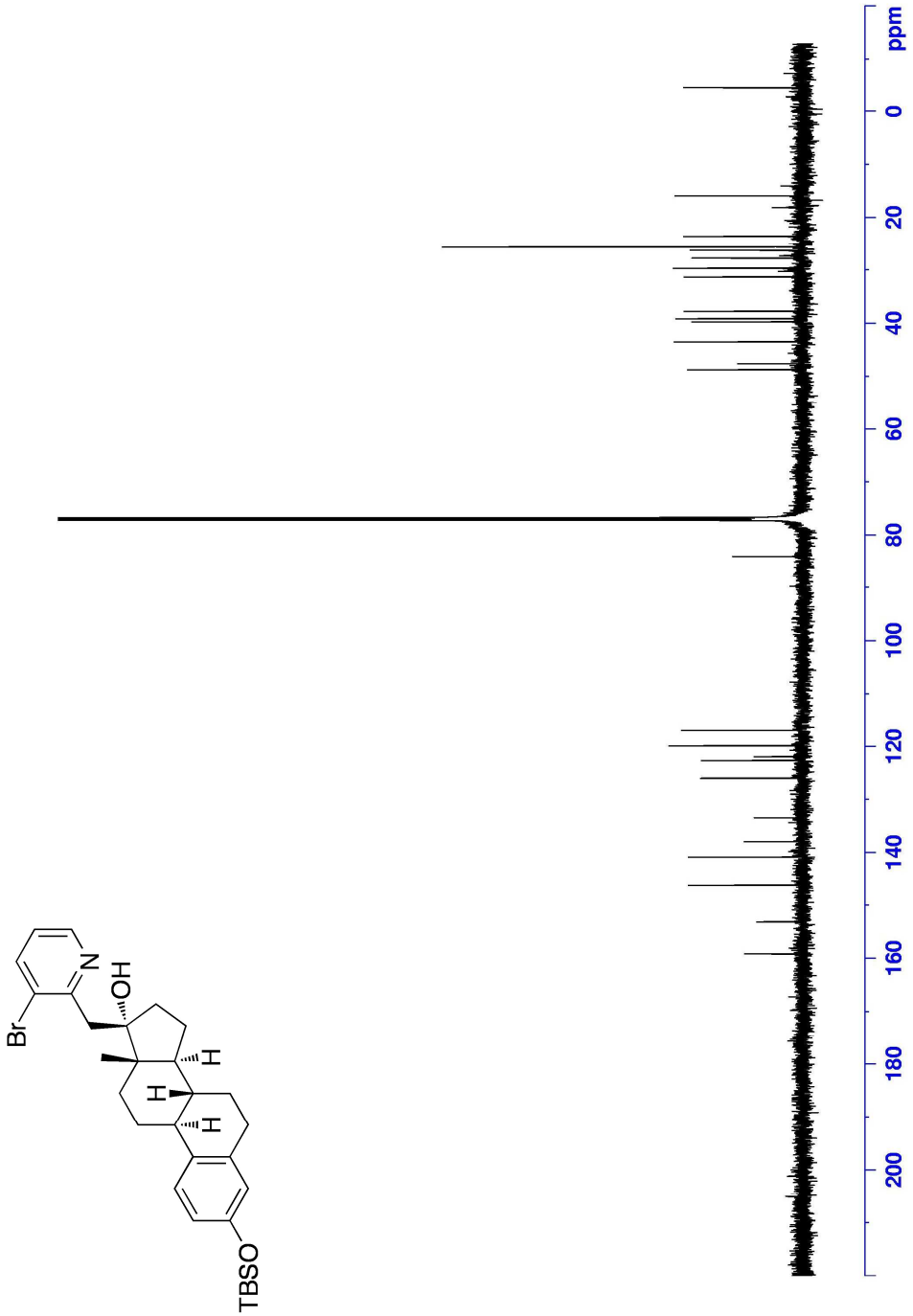


Figure 6: ^{13}C NMR ($CDCl_3$, 125 MHz) of **8**.

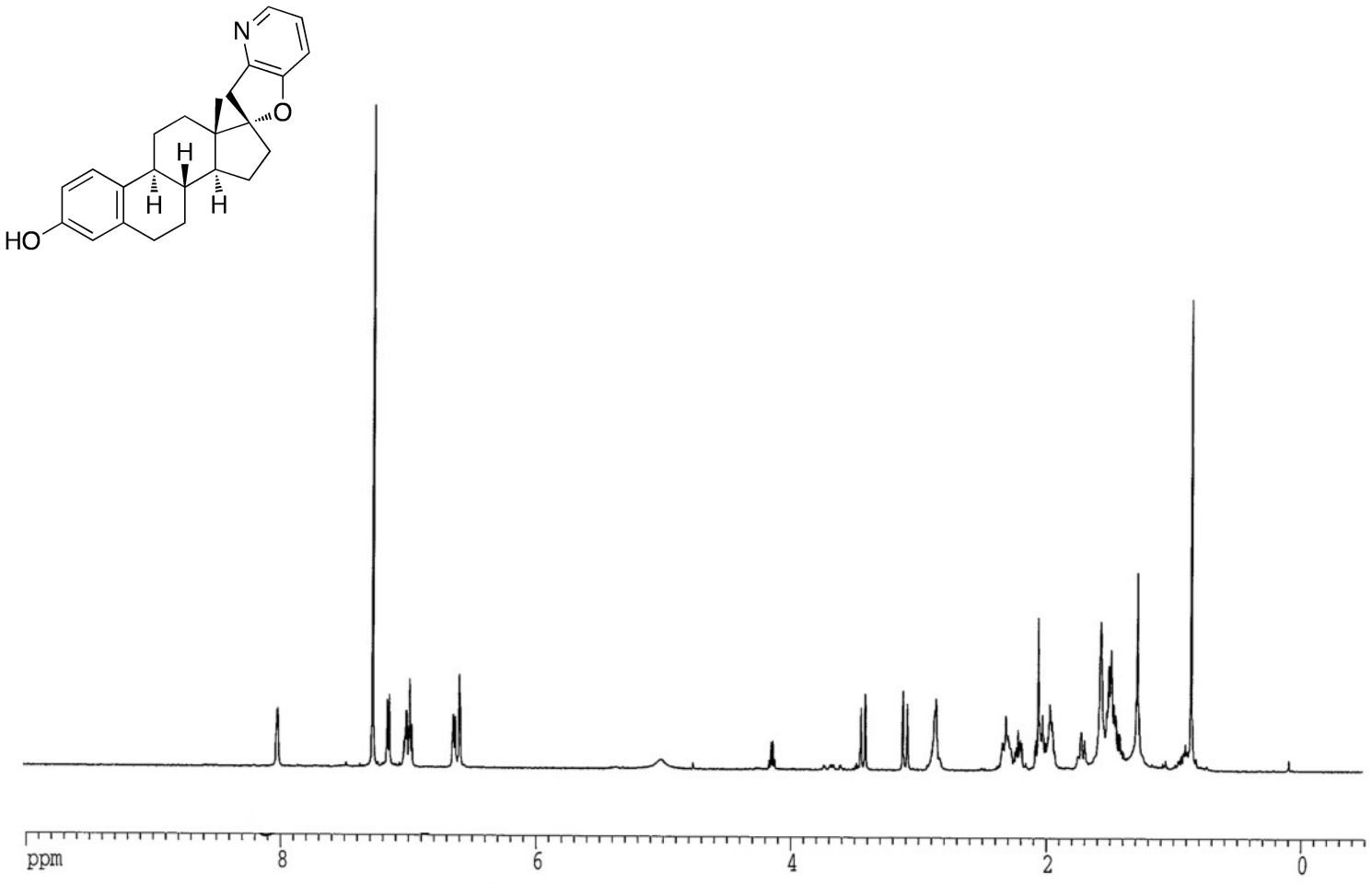


Figure 7: ^{1}H NMR ($CDCl_3$, 500 MHz) of **4**.

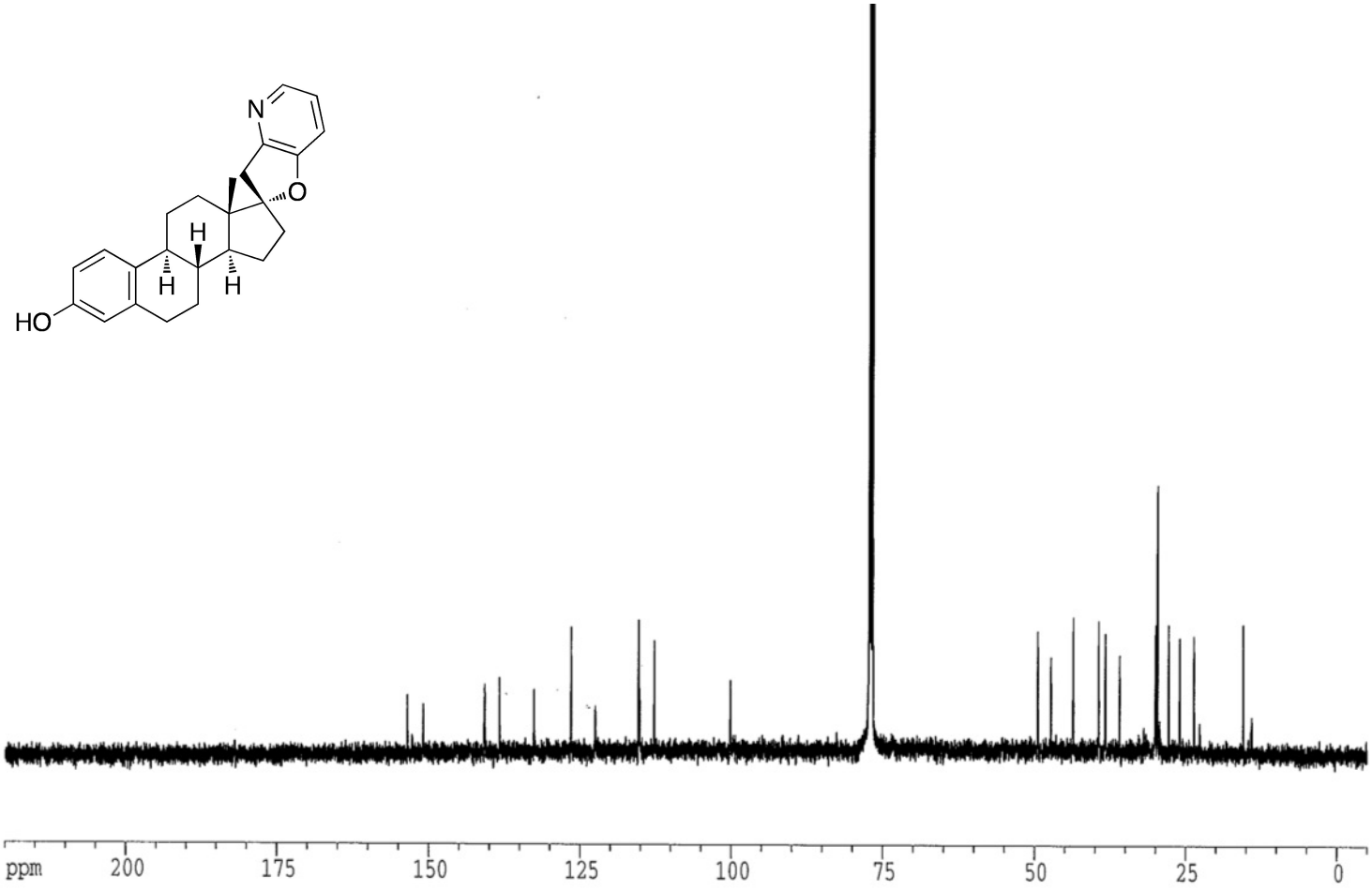


Figure 8: ^{13}C NMR ($CDCl_3$, 125 MHz) of **4**.

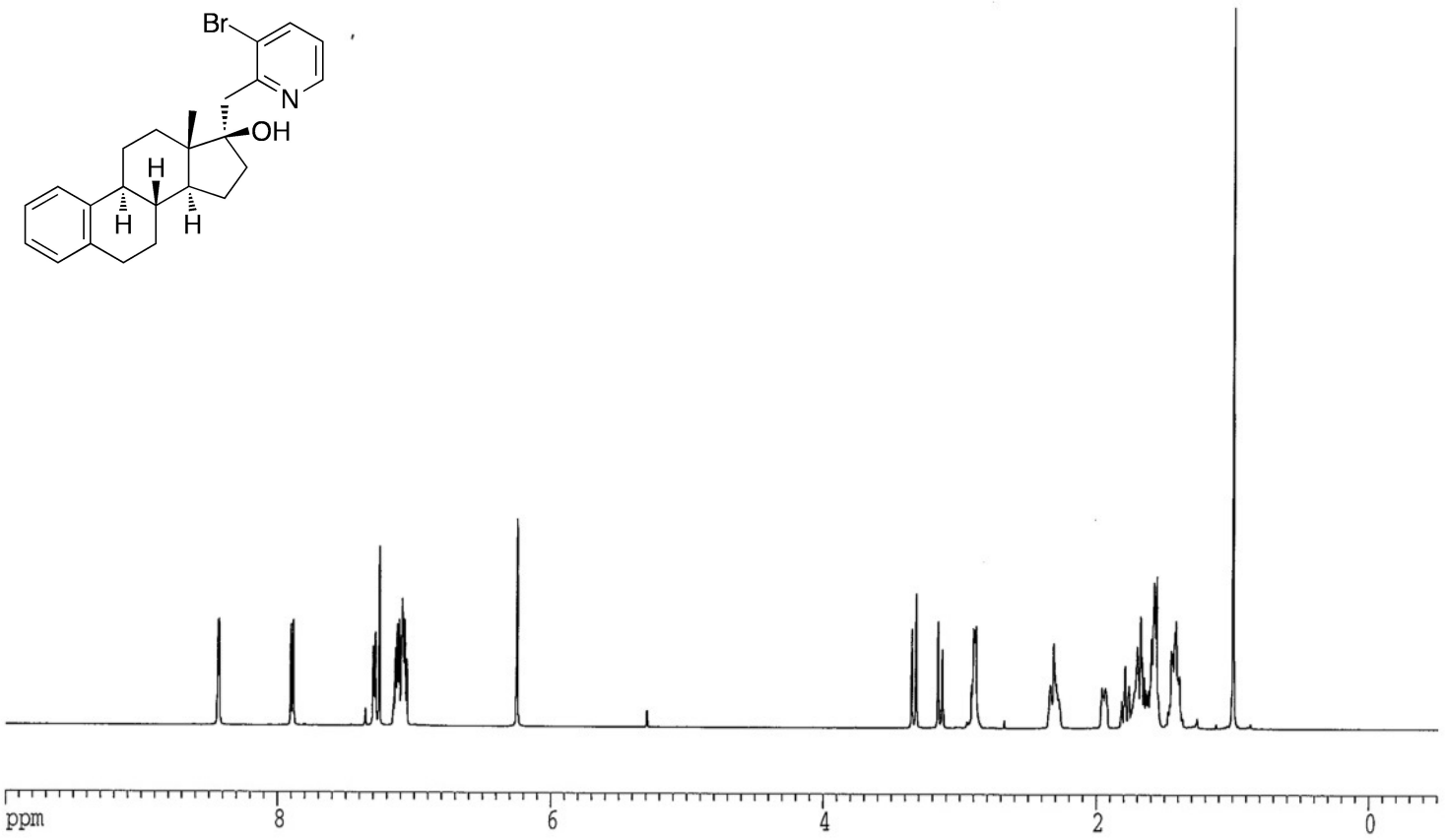


Figure 9: ^{1}H NMR ($CDCl_3$, 500 MHz) of **10**.

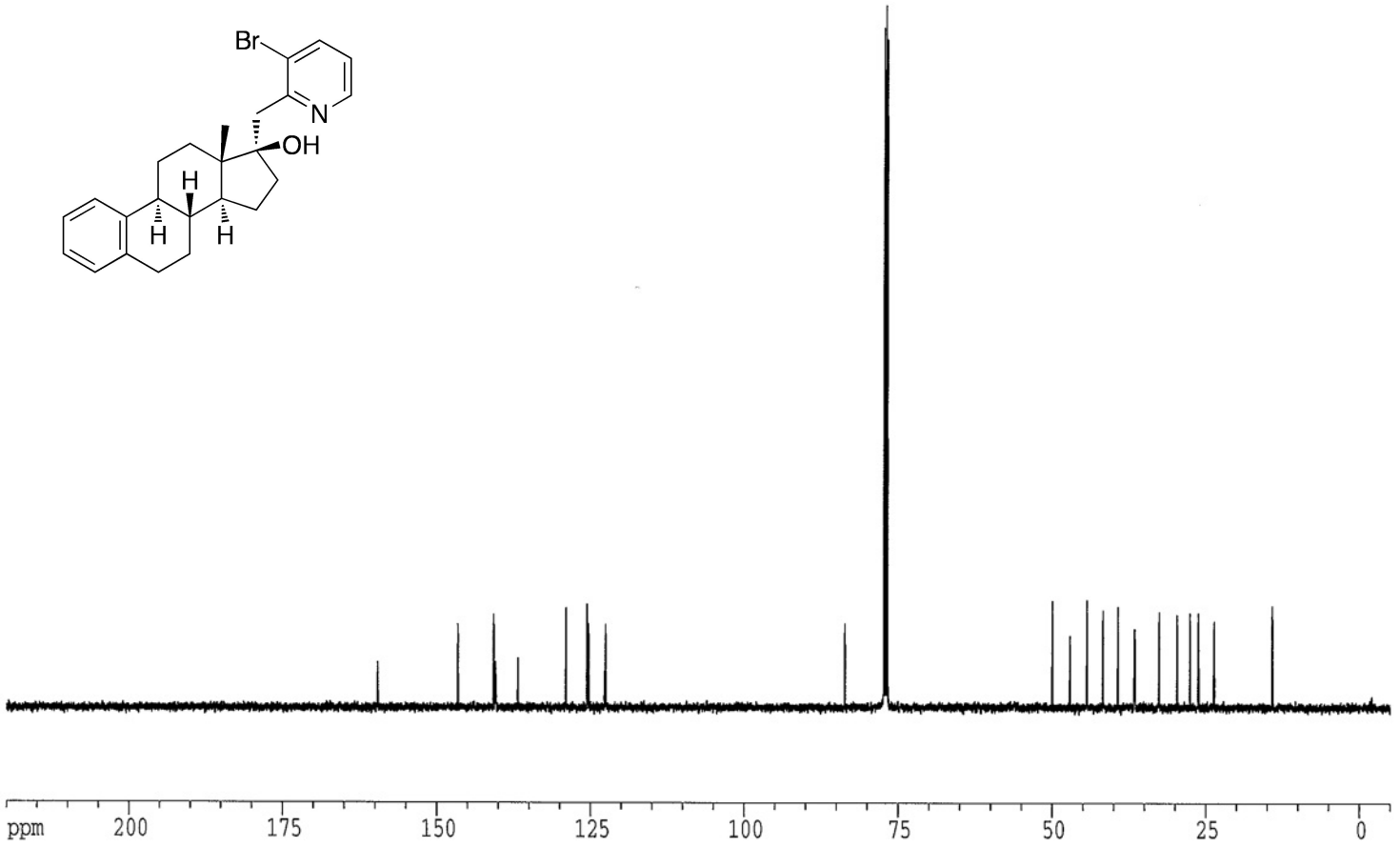


Figure 10: ^{13}C NMR ($CDCl_3$, 125 MHz) of **10**.

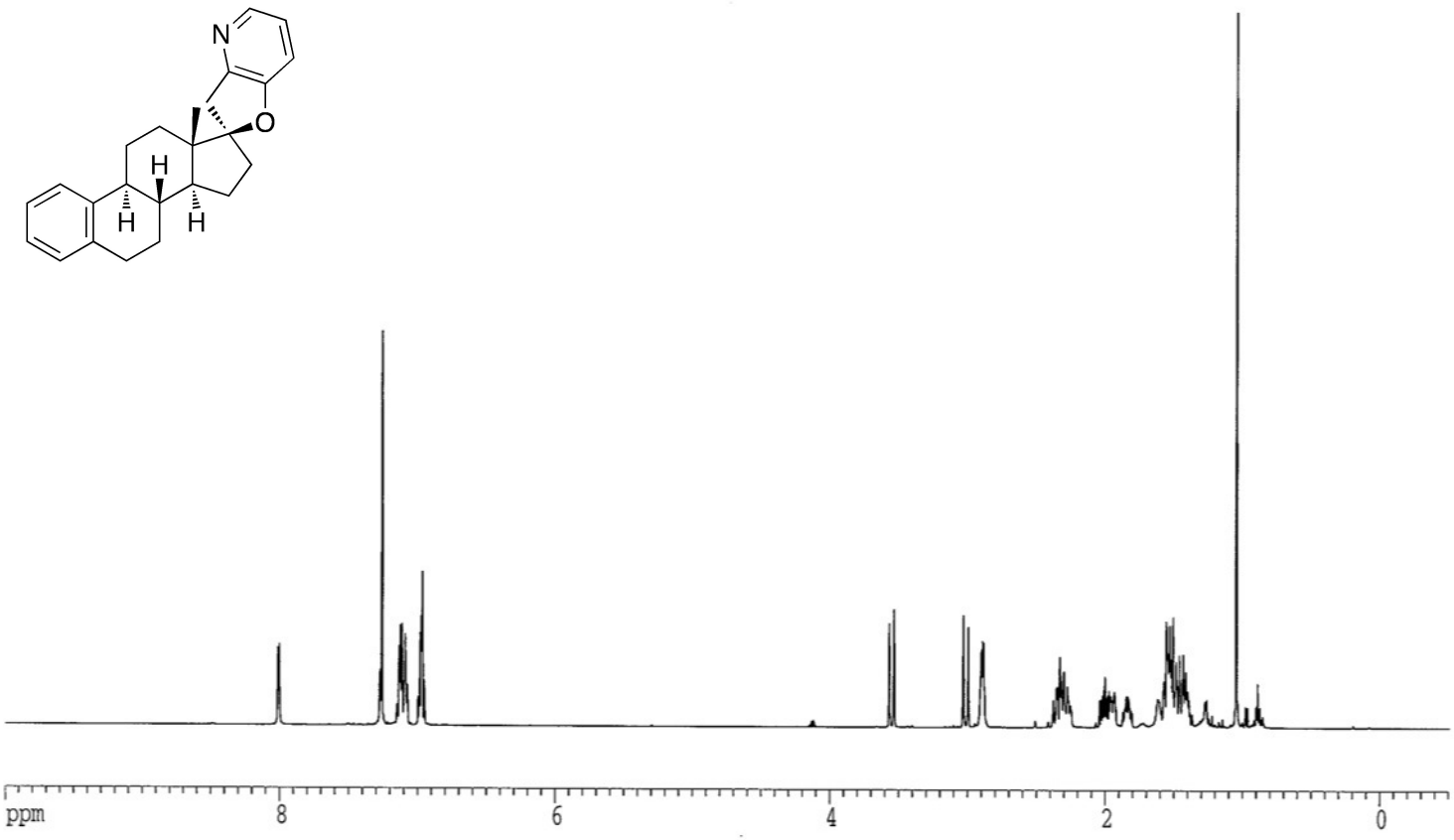


Figure 11: ^{1}H NMR ($CDCl_3$, 500 MHz) of **11.**

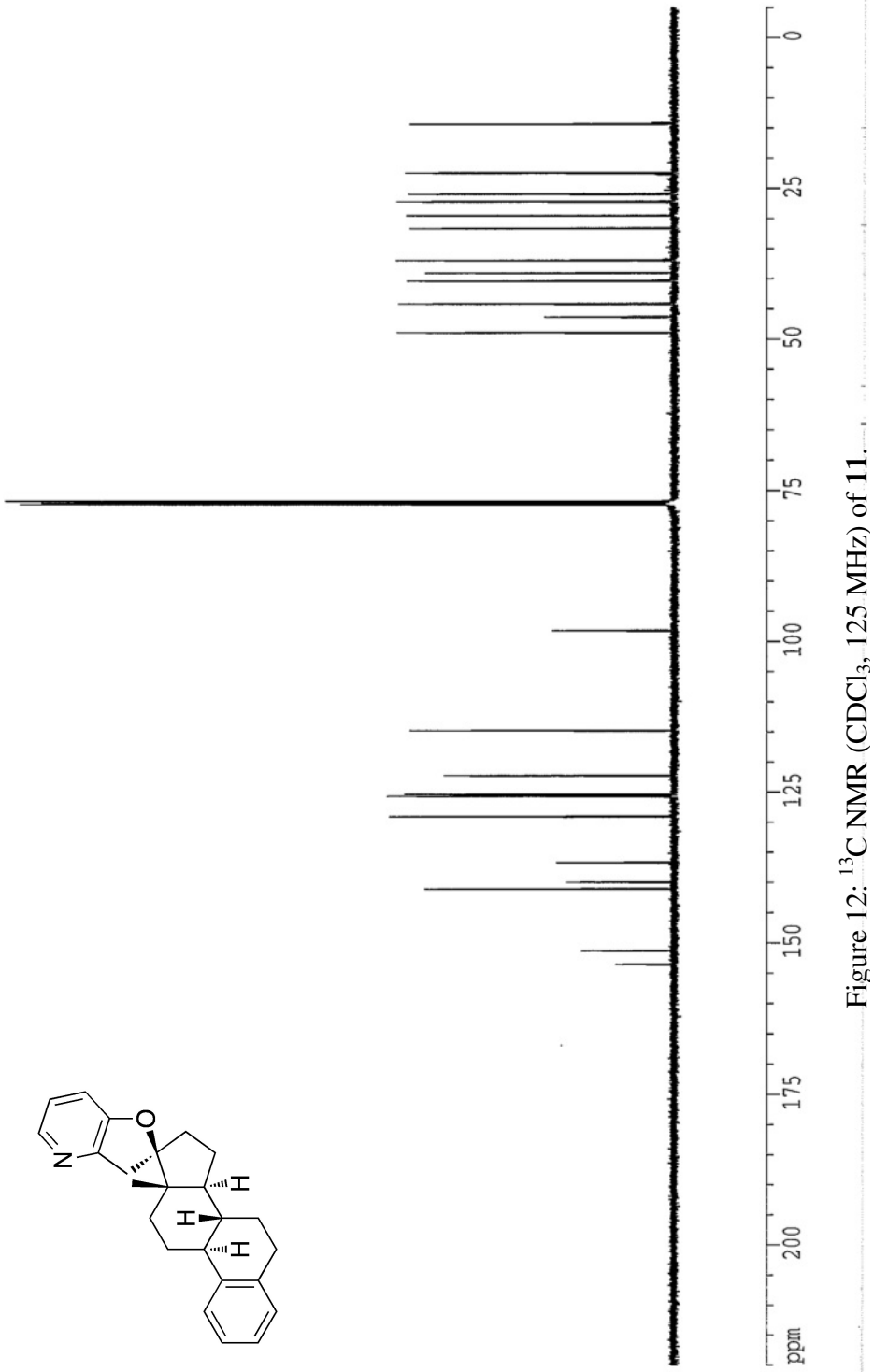


Figure 12: ^{13}C NMR ($CDCl_3$, 125 MHz) of **11**.