Supporting Information

trans-Stilbenoids: Potent and Selective Inhibitors for Human Cytochrome P450 1B1

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Contents

A. Experimental Methods

(a) General

All chemicals were reagent grade and were used as purchased. All reactions were performed under an inert atmosphere of dry argon or nitrogen using distilled dry solvents. Reactions were monitored by TLC analysis using silica gel 60 F-254 thin layer plates. Melting points were determined with a Büchi melting point B-540 apparatus and were not corrected. ^{1}H NMR (400 or 300 MHz) and ^{13}C NMR (100 or 75 MHz) spectra were recorded in δ units relative to the non-deuterated solvent as an internal reference. Mass spectra (MS) were recorded using electron impact (EI). High-resolution mass spectra (HRMS) were recorded using EI.

(b) General procedure for the preparation of (*E*)-stilbenes

Commercially available aromatic aldehydes **10** (1.1 mmol) were added individually to well-stirred solutions of phosphonate **9** (1.0 mmol), freshly powdered KOH (2.0 mmol), and 18-crown-6 (0.1 mmol) in 2 mL of CH_2Cl_2 at room temperature. After the mixture was stirred for an additional 3–6 h, the mixture was diluted with 15 mL of CH_2Cl_2 and washed with water (10 mL) and brine (2 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in 2 mL of CH_2Cl_2. To this solution were added Girard's reagent T ((carboxymethyl)trimethylammonium chloride hydrazide, 0.5 mmol) and AcOH (5 mmol), and the resulting mixture was stirred for 2 h at room temperature. The insoluble material was filtered off, the filtrate was concentrated in vacuo, and the residue was washed with brine (3 × 10 mL) and dried over $MgSO_4$. The solvent was then removed in vacuo to yield the desired stilbene (ca. 0.9–0.95 mmol) as a mixture of *E* and *Z* isomers. To a solution of this mixture in heptanes (5 mL) was added a catalytic amount of iodine (1 crystal), and then this mixture was heated to

reflux for 12 h. The reaction mixture was diluted with 20 mL of ether and washed with saturated aqueous sodium bisulfate (10 mL) and brine (2 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to provide the desired (*E*)-stilbene **8** (ca. 0.9–0.97 mmol).

(c) Analytical data of representative (*E*)-stilbenes

7,[1] **8a**,[2] **8d**,[3] **8g**,[4] and **8j**[5] were identified by comparison to published ^{1}H NMR and ^{13}C NMR spectra.

(*E*)-2,2',4-Trimethoxystilbene (**8b**): Yield 97%; light yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (dd, *J* = 1.5, 7.5 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.35 (d, *J* = 1.2 Hz, 2H), 7.19 (ddd, *J* = 1.8, 7.4, 8.1 Hz, 1H), 6.93 (td, *J* = 1.1, 7.5 Hz, 1H), 6.86 (dd, *J* = 0.9, 8.4 Hz, 1H), 6.50 (dd, *J* = 2.4, 8.7 Hz, 1H), 6.44 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.3, 157.9, 156.6, 128.0, 127.4, 127.2, 126.1, 123.4, 121.5, 120.7, 120.2, 110.8, 104.9, 98.4, 55.5 (2C), 55.4; MS (EI): *m/z* (%): 270 ($[M]^+$, 100), 151 (25); HRMS (EI) calcd for $C_{17}H_{18}O_3$ 270.1256 ($[M^+]$), found 270.1259.

(*E*)-2,3',4-Trimethoxystilbene (**8c**): Yield 94%; white solid; mp 48–54 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.5 Hz, 1H), 7.36 (d, *J* = 16.4 Hz, 1H), 7.22 (d, *J* = 8.1 Hz, 1H), 7.08 (d, *J* = 7.7 Hz, 1H), 6.96 (d, *J* = 16.4 Hz, 1H), 6.76 (dd, *J* = 2.0, 8.1 Hz, 1H), 6.50 (dd, *J* = 2.2, 8.5 Hz, 1H), 6.45 (d, *J* = 2.2 Hz, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.6, 159.9, 158.1, 139.8, 129.5, 127.3, 126.9, 123.7, 119.5, 119.1, 112.7, 111.5, 105.0, 98.5, 55.5 55.4, 55.3; MS (EI): *m/z* (%): 270 ($[M]^+$, 100), 239 (12); HRMS (EI) calcd for $C_{17}H_{18}O_3$ 270.1256 ($[M^+]$), found 270.1262.

(*E*)-2,3',4,4'-Tetramethoxystilbene (**8e**): Yield 92%; white solid; mp 114–117 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.1 Hz, 1H), 7.23 (d, *J* = 16.5 Hz, 1H), 7.06 (d, *J* = 1.8 Hz, 1H), 7.02 (dd, *J* = 2.1, 8.4 Hz, 1H), 6.94 (d, *J* = 16.5 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.50 (dd,

J = 2.4, 8.7 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.2, 157.8, 149.0, 148.4, 131.4, 126.94, 126.85, 121.4, 119.7, 119.5, 111.1, 108.6, 104.9, 98.4, 55.9, 55.8, 55.4, 55.3; MS (EI): *m/z* (%): 300 ($[M]^+$, 100), 285 (53), 150 (14); HRMS (EI) calcd for $C_{18}H_{20}O_4$ 300.1361 ($[M^+]$), found 300.1360.

(*E*)-2,2',4,5'-Tetramethoxystilbene (**8f**): Yield 93%; light yellow solid; mp 68–70 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.4 Hz, 1H), 7.36 (d, *J* = 3.6 Hz, 2H), 7.20 (d, *J* = 3.0 Hz, 1H), 6.81 (d, *J* = 8.7 Hz, 1H), 6.75 (dd, *J* = 2.9, 8.9 Hz, 1H), 6.51 (dd, *J* = 2.6, 8.6 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.815 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.4, 157.9, 153.7, 151.2, 128.3, 127.2, 123.6, 121.2, 119.9, 112.9, 112.2, 111.4, 104.9, 98.3, 56.3, 55.7, 55.4, 55.3; MS (EI): *m/z* (%): 300 ($[M]^+$, 100), 285 (15), 151 (17), 121 (31); HRMS (EI) calcd for $C_{18}H_{20}O_4$ 300.1361 ($[M^+]$), found 300.1358.

(*E*)-2,2',3',4-Tetramethoxystilbene (**8h**): Yield 92%; white solid; mp 54–57 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.7 Hz, 1H), 7.44-7.25 (m, 3H), 7.02 (t, *J* = 8.1 Hz, 1H), 6.78 (dd, *J* = 1.4, 8.3 Hz, 1H), 6.52 (dd, *J* = 2.4, 8.4 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.823 (s, 3H), 3.819 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.2, 157.8, 149.0, 148.4, 131.4, 126.94, 126.85, 121.4, 119.7, 119.5, 111.1, 108.6, 104.9, 98.4, 55.9, 55.8, 55.4, 55.3; MS (EI): *m/z* (%): 300 ($[M]^+$, 100), 285 (11), 151 (36); HRMS (EI) calcd for $C_{18}H_{20}O_4$ 300.1361 ($[M^+]$), found 300.1356.

(*E*)-2,2',4,6'-Tetramethoxystilbene (**8i**): Yield 92%; light yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (d, *J* = 16.8 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 1H)), 7.28 (d, *J* = 16.8 Hz, 1H), 7.12 (t, *J* = 8.3 Hz, 1H), 6.57 (d, *J* = 8.4 Hz, 2H), 6.51 (dd, *J* = 2.6, 8.6 Hz, 1H), 6.45 (d, *J* = 2.1 Hz, 1H), 3.87 (s, 6H), 3.84 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.0, 158.4, 157.8, 127.4, 127.0, 126.9, 121.6, 118.3 (2C), 115.6, 104.8, 104.0 (2C), 98.3, 55.8 (2C), 55.5, 55.3; MS

(EI): *m/z* (%): 300 ([M]$^+$, 100), 164 (23), 151 (38), 121 (21); HRMS (EI) calcd for $C_{18}H_{20}O_4$ 300.1361 ([M$^+$]), found 300.1362.

(*E*)-2,2',4,4',5'-Pentamethoxystilbene (**8k**): Yield 90%; white solid; mp 87–91 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.4 Hz, 1H), 7.32-7.13 (m, 3H), 6.50-6.44 (m, 3H), 3.893 (s, 3H), 3.886 (s, 3H), 3.85 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 157.7, 151.4, 149.1, 143.4, 126.9, 121.2 (2C), 120.3, 119.4, 109.3, 104.9, 98.4, 97.9, 56.9, 56.6, 56.0, 55.5, 55.4; MS (EI): *m/z* (%): 330 ([M]$^+$, 100), 315 (23), 293 (11), 165 (17), 151 (17); HRMS (EI) calcd for $C_{19}H_{22}O_5$ 330.1467 ([M$^+$]), found 330.1462.

(*E*)-2,2',4,4',6'-Pentamethoxystilbene (**8l**): Yield 91%; light pink solid; mp 110–113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, *J* = 6.8 Hz, 1H), 7.52 (d, *J* = 8.7 Hz, 1H), 7.20 (d, *J* = 16.8 Hz, 1H), 6.49 (dd, *J* = 2.4, 8.7 Hz, 1H), 6.43 (d, *J* = 2.7 Hz, 1H), 6.15 (s, 2H), 3.85 (s, 6H), 3.83 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7, 159.6, 159.2 (2C), 157.6, 126.6, 124.6, 122.0, 118.3, 108.9, 104.8, 98.3, 90.7 (2C), 55.7 (2C), 55.5, 55.3, 55.2; MS (EI): *m/z* (%): 330 ([M]$^+$, 100), 315 (13), 165 (18), 151 (14); HRMS (EI) calcd for $C_{19}H_{22}O_5$ 330.1467 ([M$^+$]), found 330.1464.

(*E*)-2,2',3',4,6'-Pentamethoxystilbene (**8m**): Yield 92%; white solid; mp 89–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 16.8 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 1H), 7.19 (d, *J* = 16.8 Hz, 1H), 6.72 (d, *J* = 9.0 Hz, 1H), 6.58 (d, *J* = 9.0 Hz, 1H), 6.51 (dd, *J* = 2.2, 8.5 Hz, 1H), 6.45 (d, *J* = 2.2 Hz, 1H), 3.83 (s, 3H), 3.82 (s, 6H), 3.81 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.4, 158.1, 152.5, 148.2, 147.4, 128.2, 127.1, 121.8, 121.3, 118.3, 110.8, 106.2, 104.9, 98.5, 60.4, 56.5, 56.1, 55.6, 55.4; MS (EI): *m/z* (%): 330 ([M]$^+$, 100), 151 (48); HRMS (EI) calcd for $C_{19}H_{22}O_5$ 330.1467 ([M$^+$]), found 330.1466.

(d) Biological assay

Bacterial coexpression plasmids for human CYP1A1, 1A2, 1B1, and NADPH-P450 reductase were kindly provided by Dr. F. Peter Guengerich (Vanderbilt University, Nashville, TN). Bicistronic bacterial membranes were prepared in accordance with Guengerich et al.[6] Protein concentrations were determined using the bicinchoninic acid method according to the supplier's recommendations (Pierce Chemical Co., Rockford, IL). To measure CYP1A1, 1A2, or 1B1 enzyme activities, EROD was determined. Bicistronic membranes containing 5 nM of CYP1A1, 1A2, or 1B1 were added to 0.1 M potassium phosphate buffer (pH 7.4) containing 2 M ethoxyresorufin and varying concentrations of inhibitors.[7] The reaction mixtures were preincubated at 37 °C for 3 min. The reactions were initiated by the addition of an NADPH-generating system consisting of 5 mM glucose 6-phosphate, 0.5 units mL-1 glucose 6-phosphate dehydrogenase, and 0.5 mM $NADP^+$. After 10 min of incubation at 37 °C, the reactions were terminated by the addition of 1 mL of MeOH. The formation of resorufin was determined fluorometrically using a Perkin-Elmer LS 5 spectrofluorometer at excitation and emissions wavelengths of 550 and 585 nm, respectively.

(e) Molecular modeling

A 3-D structure of **8m** was generated by Concord and was energy minimized using MMFF94s force field and MMFF94 charge until the rms of the conjugated gradient was 0.05 kcal $mol^{-1}A^{-1}$ in SYBYL 8.1.1 (Tripos International, St. Louis, MO, USA). The X-ray crystal structure of human CYP1A2 (PDB code: 2HI4)[8] and the homology model of human CYP1B1[9] were prepared using Biopolymer Structure Preparation Tool in SYBYL. The docking study was performed using the Surflex-Dock program implemented in SYBYL, and the protomols were generated based on the ligand mode. The ligand was docked using default settings, except

additional starting conformations per molecule of 5 and 30 poses for each ligand. The Fast Connolly surfaces of the proteins and the Van der Waals surfaces of the ligand were generated by MOLCAD in SYBYL. All computation calculations were undertaken on an Intel® Xeon™ Quad-core workstation with Linux Cent OS release 4.6.

B. References of Supporting Information

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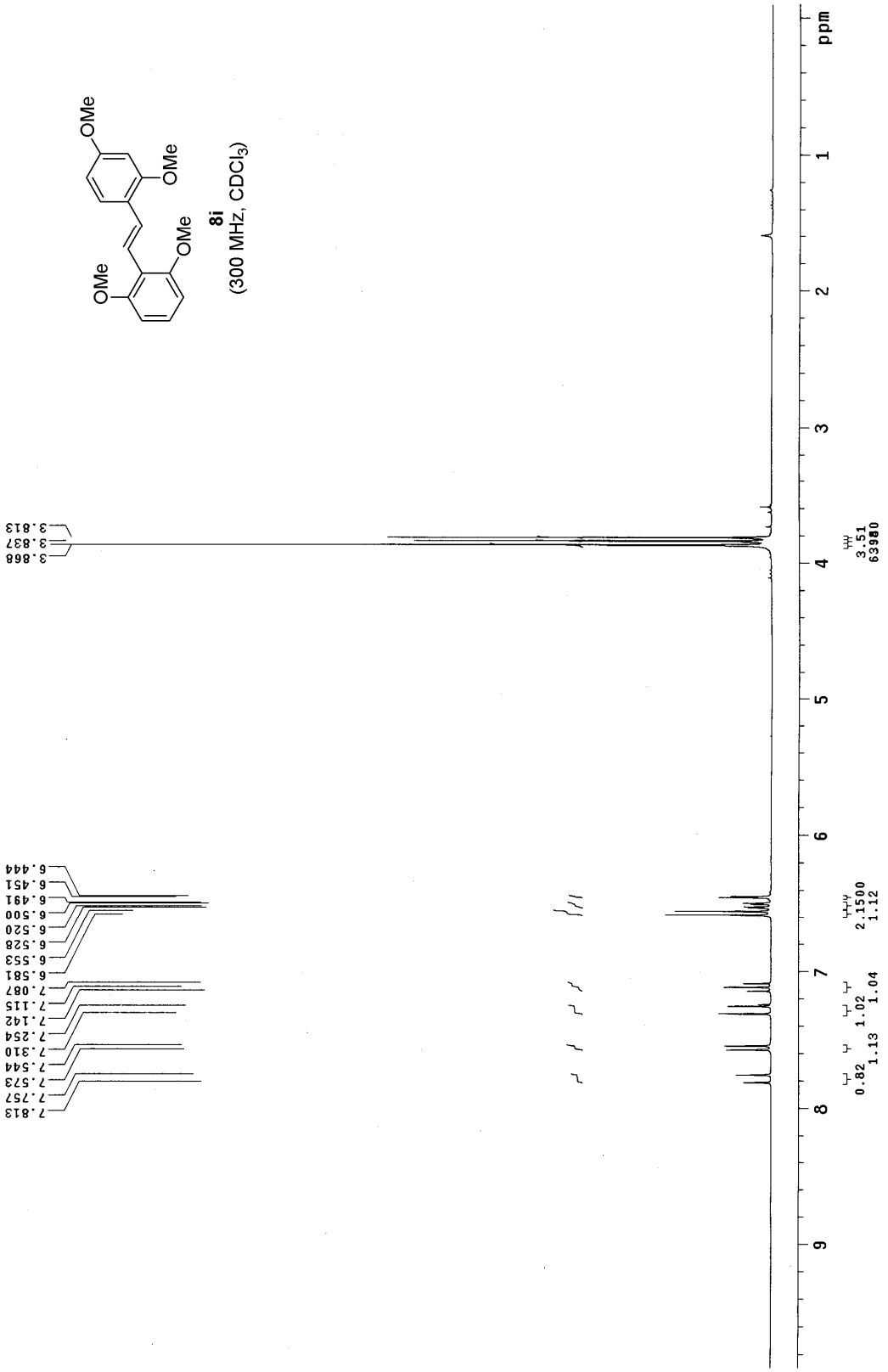
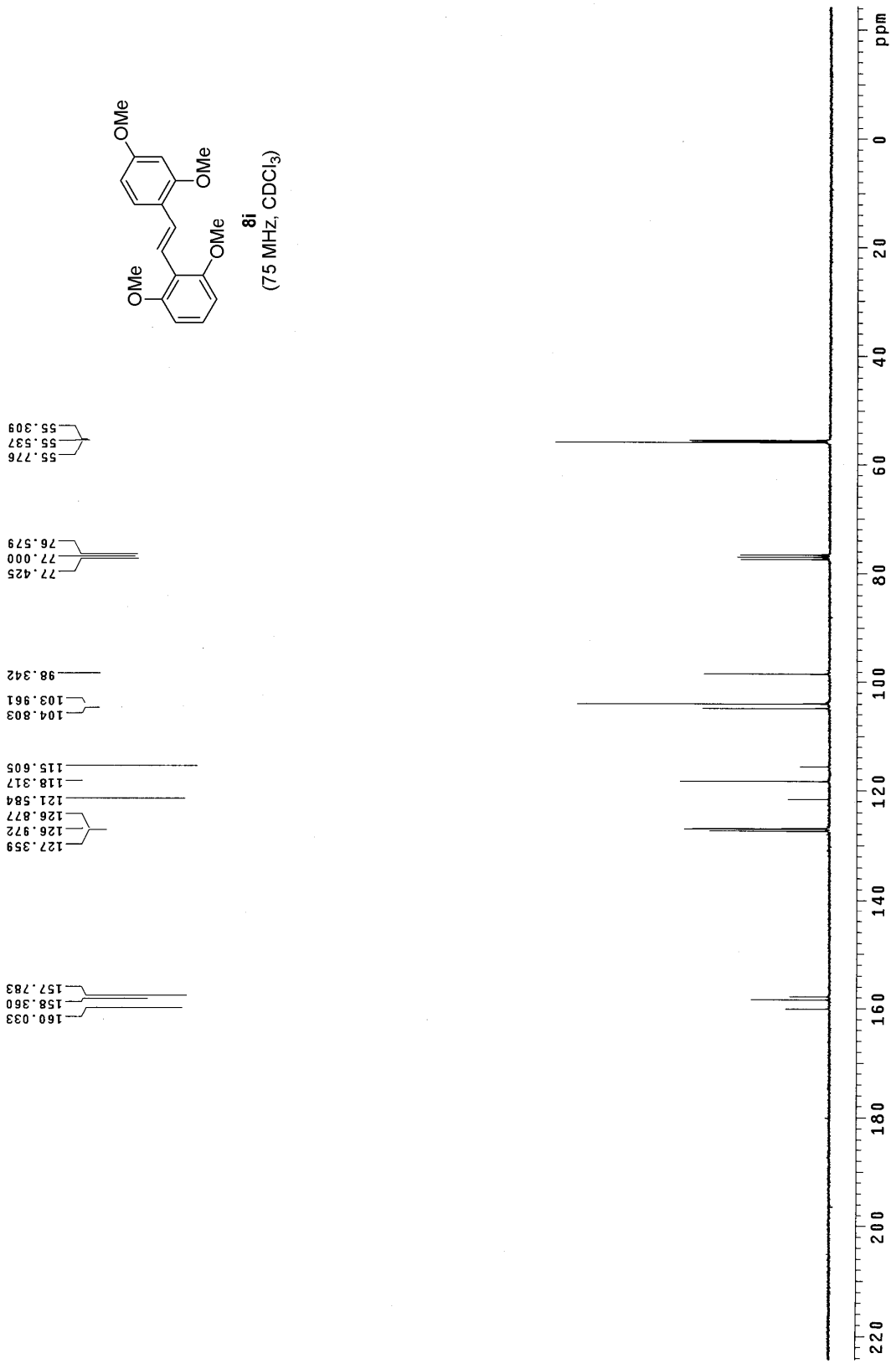
C. ^{1}H and ^{13}C NMR spectra for selected (*E*)-stilbenes

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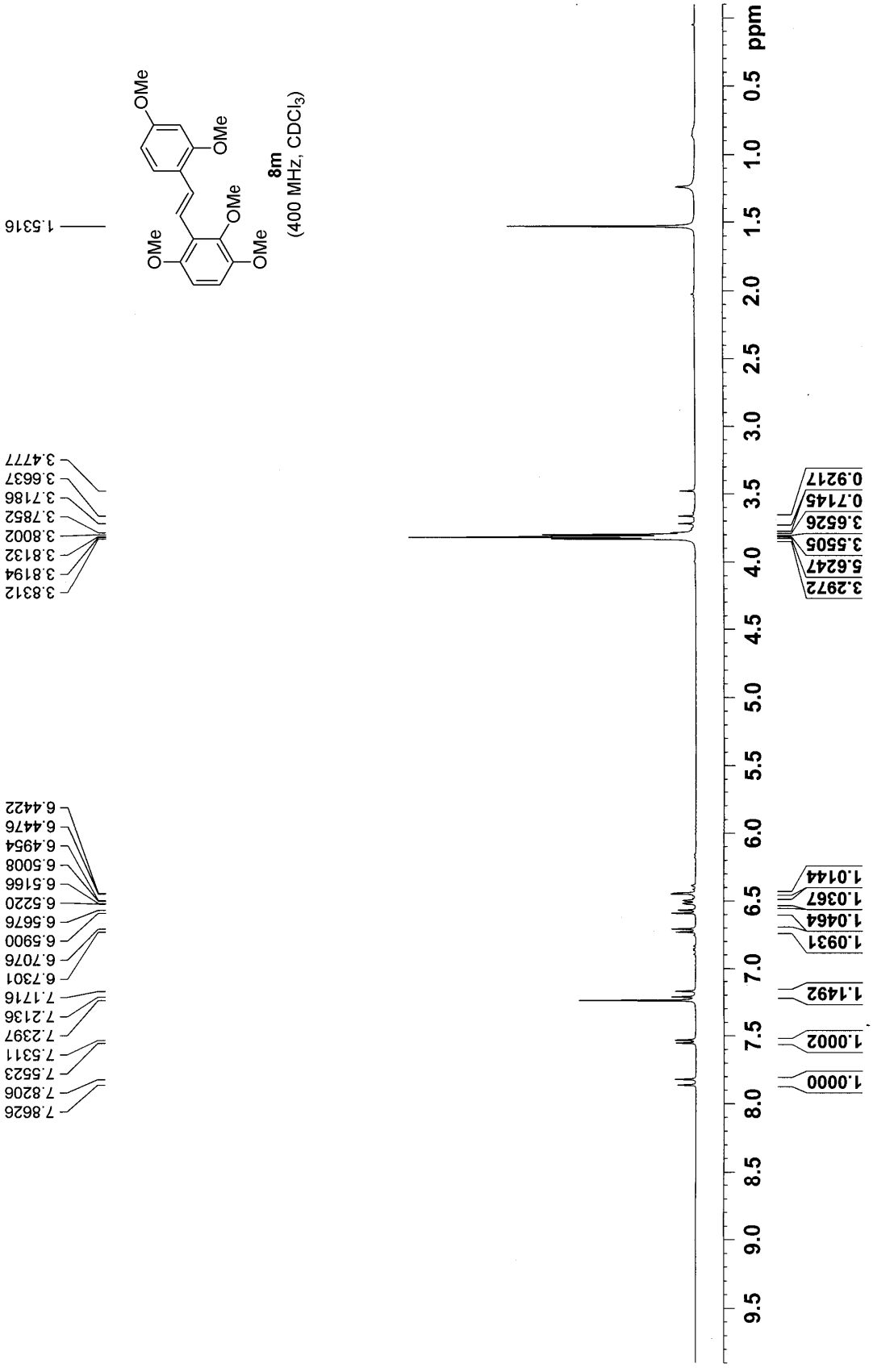
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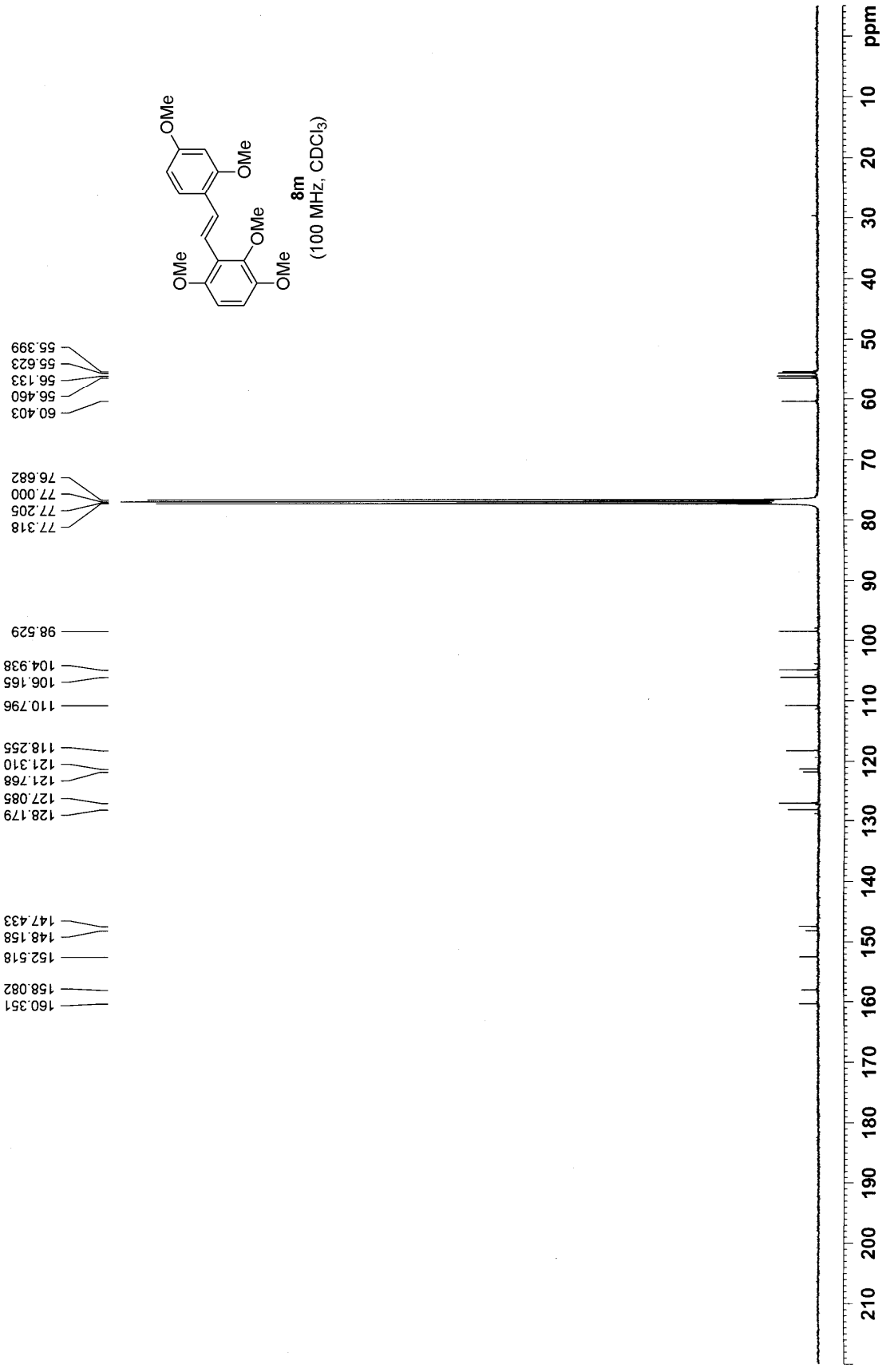
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