Supporting Information

Biselyngbyaside, a Macrolide Glycoside from the Marine Cyanobacterium *Lyngbya* sp.

Toshiaki Teruya, Hiroaki Sasaki, Kazuhiro Kitamura, Takeshi Nakayama, Kiyotake Suenaga*

Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kohoku-ku, Yokohama 223-8522, Japan and Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

*

Experimental Procedures and Spectral Data for All New Compounds.

General Methods. Chemicals and solvents were the best grade available and were used as received from commercial sources. Optical rotations were measured with a JASCO DIP-360 polarimeter. CD spectra were measured with a JASCO J-720 W spectropolarimeter. ^{1}H NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz) a JEOL JNM-A400 (400 MHz), or a JEOL JNM-GX400 (400 MHz) instrument. Chemical shifts are reported δ values in parts per million relative to the residual solvent signal (CHD_2OD: δ=3.31 ppm; $CHCl_3$: δ=7.26 ppm; CHD_5: δ=7.16 ppm for ^{1}H) and coupling constants are in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 (67.8 MHz) a JEOL A-GX400 (100.4 MHz), or a JEOL JNM-GX400 (100.4 MHz) instrument using CD_3OD and $CDCl_3$ as a solvent, respectively. Chemical shifts are reported in parts per million from the solvent signal ($CDCl_3$: δ=77.2 ppm; CHD_2OD: δ=49.0 ppm). The assignments of ^{1}H NMR and ^{13}C NMR spectra were determined by H-H COSY, HMQC and HMBC experiment. IR spectra were recorded on a JASCO FT/IR-410 instrument and are reported in wavenumbers (cm^{-1}). ESI mass spectra were recorded on a LCT premier EX spectrometer (Waters). Both TLC analysis and preparative TLC were conducted on E. Merck precoated silical gel 60 F254. Fuji Silysia silica gel BW-820 MH and FL-60D were used for column chromatography unless otherwise noted. Organic solvents for moisture-sensitive reactions were distilled from the following drying agents: THF (Na-benzophenone ketyl), benzene (Na), acetonitrile and triethylamine (calcium hydride), CH_2Cl_2 (P_2O_5). Anhydrous acetone, MeOH was purchased from Kanto Chemical Co., Inc., or Wako Pure Chemical Industries, Ltd., and used without further drying. All moisture-sensitive reactions were preformed under an atmosphere of nitrogen, and the starting materials were azeotropically dried with benzene before use.

Material. The marine cyanobacterium *Lyngbya* sp. was collected at the reef of Bise, Okinawa, Japan.

Extraction and isolation. Approximately 670 g (wet weight) of cyanobacterium was extracted with methanol (3 L) for one week. The extract was filtered, and the filtrate was concentrated. The residue was partitioned between ethyl acetate (3 × 0.3 L) and water (0.3 L). The material obtained from the organic layer were partitioned between 90% methanol/water and hexane. The aqueous methanol fraction (0.76 g) was first separated by column chromatography on ODS (10 g) using 40% methanol, 60% methanol, 80% methanol, and methanol. The fraction (0.29 g) eluted with 80% methanol was subjected to HPLC [Cosmosil 5C_{18}-AR-II (ϕ20 × 250 mm); flow rate 5mL/min; detection, UV 215 nm; solvent 65% MeCN] to give biselyngbyaside **1** (63.6 mg, t_R = 62.7 mim): $[\alpha]^{25}_D$ -36.0 (*c* 0.10, $CHCl_3$); IR ($CHCl_3$) 3589, 3502 (br), 1725 cm^{-1}.

^{1}H NMR Data for biselyngbyaside (1; 400 MHz, C_6D_6). δ 6.11 (dd, *J* = 16.6, 10.7 Hz, 1H), 6.05 (dd, *J* = 16.6, 10.3 Hz, 1H), 5.80 (m, 1H), 5.57 (m, 1H), 5.52 (m, 1H), 5.52 (m, 1H), 5.44 (m, 1H), 5.43 (m, 1H), 5.32 (dd, *J* = 15.1, 8.8 Hz, 1H), 5.24 (d, *J* = 8.8 Hz, 1H), 5.04 (d, *J* = 9.3 Hz, 1H), 4.67 (m, 1H), 4.41 (d, *J* = 7.8 Hz, 1H), 4.03 (dd, *J* = 11.7, 2.9 Hz, 1H), 3.84 (dd, *J* = 11.7, 5.9 Hz, 1H), 3.57 (dd, *J* = 8.8, 7.8 Hz, 1H), 3.55 (s, 3H), 3.43 (dd, *J* = 9.3, 8.8 Hz, 1H), 3.40 (m, 1H), 3.39 (m, 1H), 3.12 (dd, *J* = 8.8, 8.8 Hz, 1H), 3.07 (s, 3H), 3.06 (m, 1H), 2.79 (m, 1H), 2.63 (dd, *J* = 14.6, 8.9 Hz, 1H), 2.44 (m, 1H), 2.41 (m, 1H), 2.39 (m, 1H), 2.38 (m, 1H), 2.35 (m, 1H), 2.26 (m, 1H), 2.15 (m, 1H), 1.91 (m, 1H), 1.62 (s, 3H), 1.57 (d, *J* = 4.4 Hz, 3H), 1.54 (s, 3H), 0.90 (d, *J* = 6.3 Hz, 3H).

Methanolsis of biselyngbyaside (1). A 0.7 M solution of NaOMe in MeOH (0.5 mL) was added to bislyngbyaside (4.5 mg, 7.4 μmol), and the solution was stirred at room temperature for 30 min. The mixture was neutralized with an acidic ion-exchange resin (Amberlite IRC-50) and poured on a pad of the same resin. The resin was washed with MeOH. The filtrate and washings were combined and concentrated to give a oil,

which was subjected to preparative TLC developed with hexane-EtOAc 2:1 to afford methyl ester **2** (3.0 mg, 91%) as a colorless oil:

(*R*)-MTPA ester 3. To a stirred solution of methyl ester **2** (1.9 mg, 4.3 μmol) in pyridine (100 μL) were added 3 drops of (*S*)-MTPACl and 4-(dimethylamino)pyridine (2.2 mg). The mixture was stirred at 60 ˚C for 35 h and concentrated to give a oil, which was purified by preparative TLC (hexane-EtOAc 3:1) to afford (*R*)-MTPA ester **3** (2.1 mg, 99%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.55-7.32 (m, 5H), 7.24 (dd, *J* = 15.6, 10.7 Hz, 1H), 6.20 (dd, *J* = 15.6, 10.7 Hz, 1H), 6.03 (m, 1H), 5.96 (m, 1H), 5.93 (m, 1H), 5.79 (d, *J* = 15.6 Hz, 1H), 5.78 (m, 1H), 5.48 (m, 1H), 5.47 (m, 1H), 5.34 (m, 1H), 5.30 (m, 1H), 5.21 (d, *J* = 9.2 Hz, 1H), 5.16 (d, *J* = 9.8 Hz, 1H), 3.71 (s, 3H), 3.56 (s, 3H), 3.49 (m, 1H), 3.16 (s, 3H), 2.94 (dd, *J* = 14.7, 6.3 Hz, 1H), 2.75 (dd, *J* = 14.7, 6.3 Hz, 1H), 2.48 (m, 1H), 2.47 (m, 1H), 2.35 (m, 2H), 2.29 (m, 1H), 2.01 (m, 2H), 1.72 (s, 3H), 1.64 (d, *J* = 5.8 Hz, 3H), 1.53 (s, 3H), 0.96 (d, *J* = 6.8 Hz, 3H); HRMS (ESI) *m/z* 681.3372, calcd for $C_{38}H_{49}F_3NaO_6$ $[M+Na]^+$ 618.3379.

(*S*)-MTPA ester 4. To a stirred solution of methyl ester **2** (1.8 mg, 4.1 μmol) in pyridine (100 μL) were added 2 drops of (*R*)-MTPACl and 4-(dimethylamino)pyridine (1.3 mg). The mixture was stirred at 60 ˚C for 35 h and concentrated to give a oil, which was purified by preparative TLC (hexane-EtOAc 3:1) to afford (*S*)-MTPA ester **4** (2.0 mg, 99%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55-7.32 (m, 5H), 7.24 (dd, *J* = 15.6, 10.7 Hz, 1H), 6.20 (dd, *J* = 15.6, 10.7 Hz, 1H), 6.03 (m, 1H), 6.02 (m, 1H), 5.97 (m, 1H), 5.79 (d, *J* = 15.6 Hz, 1H), 5.74 (m, 1H), 5.50 (m, 1H), 5.47 (m, 1H), 5.46 (m, 1H), 5.31 (m, 1H), 5.16 (d, *J* = 9.8 Hz, 1H), 5.08 (d, *J* = 9.8 Hz, 1H), 3.71 (s, 3H), 3.56 (s, 3H), 3.49 (m, 1H), 3.16 (s, 3H), 2.96 (dd, *J* = 14.7, 6.3 Hz, 1H), 2.72 (dd, *J* = 14.7, 6.3 Hz, 1H), 2.48 (m, 1H), 2.47 (m, 1H), 2.45 (m, 2H), 2.29 (m, 1H), 2.02 (m, 2H), 1.68 (s, 3H), 1.64 (d, *J* = 5.8 Hz, 3H), 1.53 (s, 3H), 0.96 (d, *J* = 6.8 Hz, 3H); HRMS (ESI) *m/z* 681.3379, calcd for $C_{38}H_{49}F_3NaO_6$ $[M+Na]^+$ 618.3379.

Ozonolysis of biselyngbyaside (1). To a solution of bislyngbyaside (**1**) (3.4 mg, 5.6 μmol) in pyridine (70 μL) was added acetic anhydride (50 μL) at room temperature. The mixture was stirred at 30 ˚C for 2 h and concentrated. The residual oil was purified by preparative TLC developed with EtOAc to give biselyngbyaside triacetate (3.9 mg) as a colorless oil. Ozone was bubbled through a cooled (–78 ˚C) stirred solution of biselyngbyaside triacetate (3.9 mg) in MeOH (5 mL) for 2 min. Dimethyl sulfide (70 μL) was then added and the solution allowed to warm to room temperature. Sodium borohydride (3 mg, large excess) was added, and the solution was stirred at room temperature for 20 min, diluted with water, and extracted with ether. The organic layer was dried with Na_2SO_4 and concentrated. The residual oil was dissolved in pyridine (0.3 mL), and treated with 4-bromophenyl isocyanate (100 mg, large excess) at room temperature for 3 h. The mixture was diluted with water (1 mL), stirred at room temperature for 20 min, and concentrated. The residue was suspended with ether and filtered through a cotton plug, and the residue was washed with ether. The filtrate and the washings were combined and concentrated. The residual oil was purified by HPLC [Nacalai Tesque, Cosmosil 5C_{18}-AR-II (ϕ20 × 250 mm), 85% MeCN, flow rate 5 mL/min, detection at 215 nm] to give biscarbamate **10** (1.7 mg, 50%, t_R = 29 min) as a colorless oil: t_R = 6.5 min [Chiralpac IA (Daicel Chemical Ind., Ltd), hexane-ethanol 75:25, flow rate 1.0 mL/min, detection at 215 nm]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.36 (m, 4H), 7.31-7.24 (m, 4H), 5.20 (d, *J*=9.6 Hz, 1H), 4.17 (m, 2H), 4.13 (m, 2H), 3.55 (t, *J*=7.0 Hz, 1 H), 3.17 (s, 3H), 2.60 (m, 1H), 1.97 (m, 1H), 1.86 (m, 1H), 1.76 (m, 1H), 1.56 (m, 1H), 1.56 (s, 3H), 1.04 (d, *J*=6.8 Hz, 3H); HRMS (ESI) *m/z* 612.0393, calcd for $C_{25}H_{30}N_2NaO_5$ $[M+Na]^+$ 619.0419.

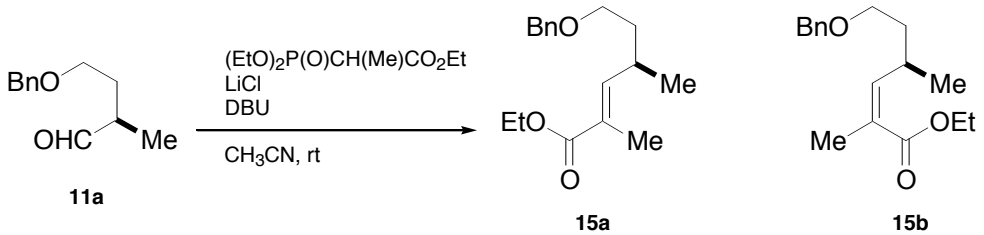
Acidic hydrolysis of biselyngbyaside (1). A mixture of biselyngbyaside (**1**) (5.1 mg, 8.4 μmol) and 5% Pd/C (2 mg) in EtOH (1 mL) was stirred under a hydrogen atmosphere at room temperature for 2 h. The mixture was filtered through a pad of Celite, and the residue was washed with MeOH. The filtrate and the

washings were combined and concentrated to give a perhydrocarboxylic acid (4.9 mg). A solution of the perhydrocarboxylic acid (4.9 mg) in 1% HCl-MeOH (1 mL) was refluxed for 2 days, and the mixture was diluted with water and extracted with ether. The organic layers were concentrated to give methyl ester **6** (0.9 mg, 25%). The aqueous layer was concentrated to afford methyl 3-*O*-methyl glucoside as a mixture of anomers (0.5 mg, 30%).

(*R*)-MTPA ester 7. To a stirred solution of methyl ester **6** (0.45 mg, 4.3 μmol) in pyridine (50 μL) were added 3 drops of (*S*)-MTPACl and 4-(dimethylamino)pyridine (1 mg). The mixture was stirred at room temperature for 1 h and concentrated to give a oil, which was purified by preparative TLC (hexane-EtOAc 5:1) to afford (*R*)-MTPA ester **3** (0.5 mg) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.37 (m, 5H), 5.48 (m, 1H), 3.66 (s, 3H), 3.54 (s, 3H), 2.70 (dd, J = 15.6, 7.8 Hz, 1H), 2.60 (dd, J = 15.6, 4.9 Hz, 1H), 1.64 (m, 2H), 1.44-1.05 (m, 33H), 0.95-0.77 (m, 12H); HRMS (ESI) *m/z* 665.4376, calcd for $C_{37}H_{61}F_3NaO_5$ $[M+Na]^+$ 665.4369.

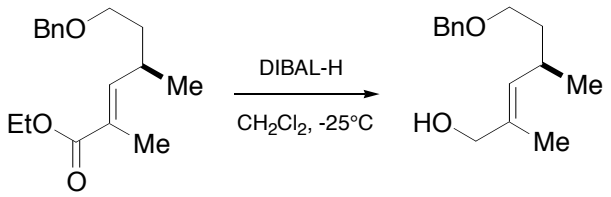
(*S*)-MTPA ester 8. To a stirred solution of methyl ester **6** (0.45 mg, 4.1 μmol) in pyridine (50 μL) were added 3 drops of (*R*)-MTPACl and 4-(dimethylamino)pyridine (1 mg). The mixture was at room temperature for 1 h and concentrated to give a oil, which was purified by preparative TLC (hexane-EtOAc 5:1) to afford (*S*)-MTPA ester **4** (0.5 mg) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.37 (m, 5H), 5.48 (m, 1H), 3.59 (s, 3H), 3.53 (s, 3H), 2.65 (dd, J = 15.6, 7.8 Hz, 1H), 2.57 (dd, J = 15.6, 4.4 Hz, 1H), 1.70 (m, 2H), 1.44-1.05 (m, 33H), 0.95-0.77 (m, 12H); HRMS (ESI) *m/z* 665.4365, calcd for $C_{37}H_{61}F_3NaO_5$ $[M+Na]^+$ 665.4369.

Methyl 2,4,6-tri-*O*-(4-bromobenzoyl)-3-*O*-methyl-β-D-glucoside 9. To a stirred solution of methyl 3-*O*-methyl glucoside (0.5 mg, 2.5 μmol) from **1** in pyridine (0.2 mL) were added 4-bromobenzoyl chloride (40 mg, large excess) and 4-(dimethylamino)pyridine (1 mg). The mixture was stirred at 60 ˚C for 14 h and concentrated to give a oil, which was purified by preparative TLC (hexane-EtOAc 1:1) and HPLC [Nacalai Tesque, Cosmosil 5C_{18}-MS-II (ϕ20 × 250 mm), 80% MeCN, flow rate 5 mL/min, detection at 215 nm] to afford Methyl 2,4,6-tri-*O*-(4-bromobenzoyl)-3-*O*-methyl-β-D-glucoside **9** (0.3 mg, t_R = 54.5 mim) and its α-anomer (0.1 mg, t_R = 59.5 mim) as a colorless oil, respectively. **9**: ^{1}H NMR (270 MHz, CD_3OD) δ 8.01-7.62 (m, 12H), 6.17 (d, J = 8.3 Hz, 1H), 5.45 (dd, J = 9.5, 8.3 Hz, 1H), 5.37 (dd, J = 9.5, 9.5 Hz, 1H), 4.15 (dd, J = 9.5, 9.5 Hz, 1H), 3.99 (m, 1H), 3.70 (dd, J = 12.7, 3.0 Hz, 1H), 3.60 (dd, J = 12.7, 5.1 Hz, 1H), 3.41 (s, 3H). Signals due to three protons were overlapped with that of CHD_2OD in CD_3OD; CD: (CH_3OH) λ_{max} 238 nm ($\Delta\varepsilon$ +7.2), 254 nm ($\Delta\varepsilon$ -17.2). **α-anomer of 9**: ^{1}H NMR (270 MHz, CD_3OD) δ 7.97-7.59 (m, 12H), 6.58 (d, J = 3.5 Hz, 1H), 5.19 (dd, J = 9.7, 3.5 Hz, 1H), 4.65 (dd, J = 12.1, 2.2 Hz, 1H), 4.52 (dd, J = 12.1, 4.9 Hz, 1H), 4.22 (m, 1H), 3.88 (dd, J = 9.7, 9.7 Hz, 1H), 3.79 (dd, J = 9.7, 9.7 Hz, 1H), 3.63 (s, 3H). Signals due to three protons were overlapped with that of CHD_2OD in CD_3OD.



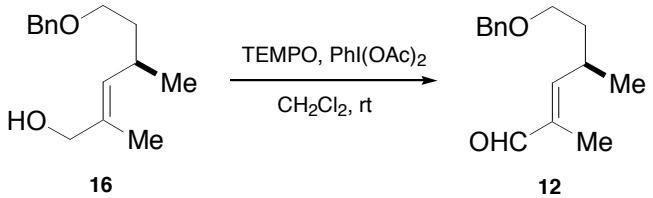
Ester 15a and 15b. To a stirred solution of triethyl 2-phosphonopropionate (0.14 mL, 0.64 mmol), DBU (0.098 mL, 0.66 mmol) and LiCl (27.6 mg, 0.65 mmol) in CH_3CN (0.2 mL) was added a solution of aldehyde **11a** (50 mg, 0.26 mmol) in CH_3CN (2×0.15 mL). The mixture was stirred at room temperature for 5 min, diluted with H_2O (5 mL) and AcOEt (5 mL), extracted with EtOAc (3×20 mL). The combined extracts

were washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by flash column chromatography on silical gel (13 g, hexane-EtOAc 45:1→20:1) to give ester **15a** (51.6 mg, 72%) and ester **15b** (14.6 mg, 20%) as a colorless oil, respectively: **15a:** $[\alpha]^{25}_{D}$ -28.2 (*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 1708, 1454, 1366, 1268, 1210, 1095, 1027, 749, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.28-7.20 (m, 5H), 6.46 (d, *J*=9.6 Hz, 1H), 4.41 (s, 2H), 4.13 (q, *J*=7.2 Hz, 2H), 3.39 (m, 1H), 3.32 (m, 1H), 2.67 (m, 1H), 1.78 (s, 3H), 1.70 (m, 1H), 1.53 (m, 1H), 1.24 (t, 3H), 0.95 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 168.2, 146.9, 138.2, 128.2, 127.5, 127.4, 126.8, 72.9, 68.2, 60.5, 36.6, 30.0, 20.1, 14.3, 12.5; HRMS (ESI) *m/z* 299.1630, calcd for $C_{17}H_{24}NaO_3$ $[M+Na]^+$ 229.1623; TLC, R_f 0.24 (hexane-EtOAc 20:1). **15b:** $[\alpha]^{25}_{D}$ -0.4 (*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 1713, 1454, 1369, 1239, 1204, 1161, 1096, 1027, 735, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.28-7.20 (m, 5H), 5.60 (d, *J*=10.4 Hz, 1H), 4.43 (s, 2H), 4.12 (q, *J*=7.2 Hz, 2H), 3.40 (m, 2H), 3.20 (m, 1H), 1.83 (s, 3H), 1.58 (m, 2H), 1.23 (t, 3H), 0.95 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 168.0, 147.1, 138.5, 128.2, 127.5, 127.4, 126.1, 72.8, 68.7, 60.0, 37.1, 30.7, 20.7, 20.7, 14.2; HRMS (ESI) *m/z* 299.1613, calcd for $C_{17}H_{24}NaO_3$ $[M+Na]^+$ 229.1623; TLC, R_f 0.26 (hexane-EtOAc 20:1).



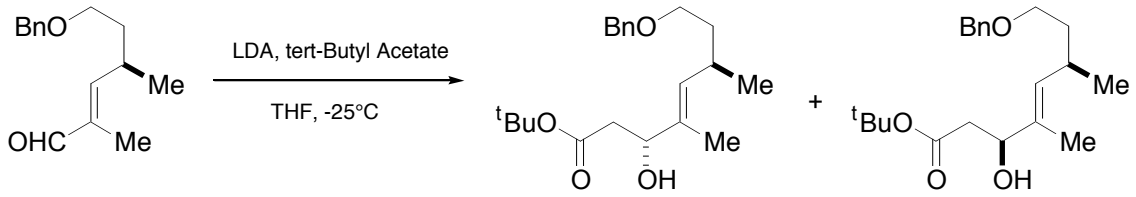
15a **16**

Alcohol 16. To a stirred solution of ester **15a** (94 mg, 0.341 mmol) in CH_2Cl_2 (2 mL) cooled at -25 ^oC was added a 1.0 M solution of diisobutylaluminum hydride (0.85 mL, 0.85 mmol). The mixture was stirred at -25 ^oC for 5 min, and the reaction was quenched by addition of methanol (2 mL). The mixture was warmed to room temperature, diluted with 0.5 M aqueous Na/K tartrate (8 mL), and stirred for 10 min. The organic layer was separated, and the aqueous layer was extracted with EtOAc (3×50 mL). The combined extracts were washed with brine (25 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by flash column chromatography on silical gel (10 g, hexane-EtOAc 6:1→3:1) to give alcohol **16** (65 mg, 81%) as a colorless oil: $[\alpha]^{25}_{D}$ -31.7(*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 3398, 1454, 1364, 1310, 1206, 1101, 1011, 865, 736, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30-7.19 (m, 5H), 5.10 (d, *J*=10.0 Hz, 1H), 4.40 (s, 2H), 3.91 (s, 2H), 3.36 (m, 2H), 2.53 (m, 1H), 1.62 (m, 1H), 1.59 (s, 3H), 1.43 (m, 1H), 0.89 (d, *J*=6.4 Hz, 3H); HRMS (ESI) *m/z* 235.1691, calcd for $C_{15}H_{23}O_2$ $[M+H]^+$ 235.1698; TLC, R_f 0.20 (hexane-EtOAc 5:1).



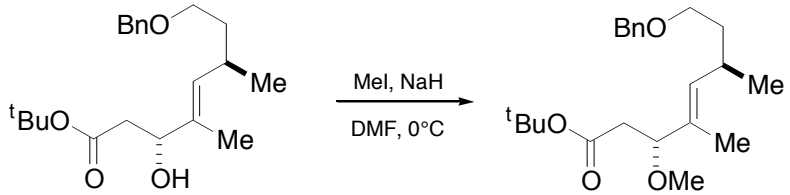
16 **12**

Aldehyde 12. To a solution of alcohol **16** (50 mg, 0.214 mmol) in CH_2Cl_2 (0.5 mL) were added bis(acetoxy)iodobenzene (89.4 mg, 0.278 mmol) and TEMPO (21.3 mg, 0.137 mmol). The mixture was stirred at room temperature for 30 min, diluted with EtOAc (10 mL) and saturated aqueous $Na_2S_2O_3$ (10 mL), and extracted with EtOAc (3×20 mL). The combined extracts were washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (8 g, hexane-EtOAc 30:1) to give aldehyde **12** (46.5 mg, 93%) as a colorless oil: $[\alpha]^{25}_{D}$ -38.0 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 1706, 1455, 1364, 1205, 1100, 1027, 737, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.35 (s, 1H), 7.31-7.20 (m, 5H), 6.21 (d, *J*=9.2 Hz, 1H), 4.41 (s, 2H), 3.42 (m, 1H), 3.34 (m, 1H), 2.91 (m, 1H), 1.75 (m, 1H), 1.71 (s, 3H), 1.59 (m, 1H), 1.04 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 195.1, 159.1, 137.9, 137.7, 127.9, 127.2, 127.2, 72.7, 67.5, 36.1, 30.0, 19.4, 8.8; TLC, R_f 0.16 (hexane-EtOAc 20:1).



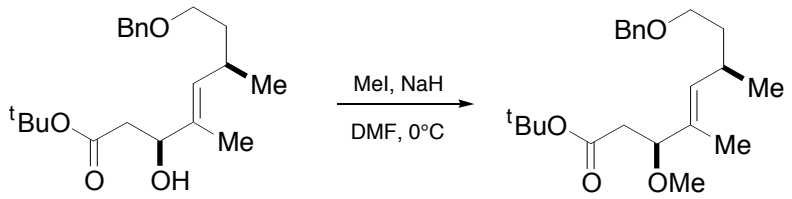
12 **13a** **13b**

Hydroxy ester 13a and 13b. To a stirred solution of diisopropylamine (39 μL, 0.276 mmol) in THF (0.3 mL) cooled at -78 °C was added a solution of 1.6 M *n*-BuLi (0.166 mL, 0.266 mmol), and the mixture was stirred at -78 °C for 15 min. To the mixture tert-butyl acetate (41 μL, 0.306 mmol) was added, and the reaction mixture was stirred for 30 min. A solution of aldehyde **12** (31.8 mg, 0.137 mmol) in THF (0.2 mL, 0.1 mL) was added, and the mixture was stirred at -78 °C for 15 min. The mixture was diluted with saturated aqueous NH_4Cl (1 mL), extracted with EtOAc (3×15 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (5 g, hexane-EtOAc 10:1) followed by reversed-phase HPLC [Nacalai Tesque, Cosmosil $5C_{18}$-AR-II (20×250 nm), 55% aqueous CH_3CN, 6.0 mL/min, detection at UV 215 nm] to give hydroxyl ester **13a** (t_R = 100 min, 22.6 mg, 47%) and **13b** (t_R = 106 min, 14.3 mg, 30%) as a colorless oil, respectively; **13a:** $[\alpha]^{25}_D$ -20.2 (*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 3446, 1728, 1455, 1367, 1149, 1102, 1027, 953, 736, 698 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.31-7.19 (m, 5H), 5.16 (d, *J*=9.5 Hz, 1H), 4.40 (s, 2H), 4.30 (dd, *J*=3.5, 8.9 Hz 1H), 3.33 (m, 2H), 2.53 (m, 1H), 2.38 (m, 1H), 2.38 (m, 1H), 1.63 (m, 2H), 1.56 (s, 3H), 1.40 (s, 9H), 0.90 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.9, 138.4, 134.5, 132.0, 128.2, 127.5, 127.3, 81.2, 73.3, 72.9, 68.4, 41.2, 37.2, 28.8, 28.1, 21.0, 12.1; HRMS (ESI) *m/z* 371.2170, calcd for $C_{21}H_{32}NaO_4$ $[M+Na]^+$ 371.2198; TLC, R_f 0.14 (hexane-EtOAc 10:1); **13b:** $[\alpha]^{25}_D$ -35.6 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 3446, 1728, 1455, 1367, 1149, 1102, 1027, 954, 736, 698 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.31-7.19 (m, 5H), 5.16 (d, *J*=9.5 Hz, 1H), 4.40 (s, 2H), 4.29 (t, *J*=6.0 Hz, 1 H), 3.36 (m, 2H), 2.53 (m, 1H), 2.37 (d, *J*=6.0 Hz, 2H), 1.63 (m, 2H), 1.57 (s, 3H), 1.44 (s, 9H), 0.88 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.9, 138.4, 134.5, 131.9, 128.2, 127.5, 127.3, 81.1, 73.2, 72.9, 68.5, 41.3, 37.2, 28.8, 28.1, 21.0, 12.2; HRMS (ESI) *m/z* 371.2188, calcd for $C_{21}H_{32}NaO_4$ $[M+Na]^+$ 371.2198; TLC, R_f 0.14 (hexane-EtOAc 10:1).



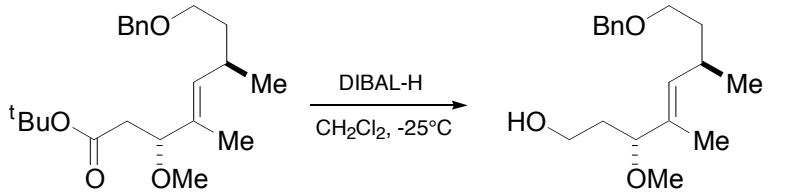
13a **17a**

Methyl ether 17a. To a stirred solution of hydroxy ester **13a** (22.6 mg, 64.9 μmol) in DMF (0.15 mL) at 0 °C were added methyl iodide (40.7 μL, 0.65 mmol) and NaH (60% oil suspension 15.2 mg, 0.38 mmol). The mixture was stirred at room temperature for 5 min, diluted with cooled H_2O (5 mL) and AcOEt (2 mL), then extracted with EtOAc (3×20 mL). The combined extracts were washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (6 g, hexane-EtOAc 15:1) to give methyl ether **17a** (19.8 mg, 84%) as a colorless oil: $[\alpha]^{25}_D$ -29.7 (*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 1731, 1455, 1366, 1288, 1148, 1098, 735, 698 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 7.29-7.16 (m, 5H), 5.13 (d, *J*=9.5 Hz, 1H), 4.38 (s, 2H), 3.80 (dd, *J*=5.1, 8.6 Hz, 1 H), 3.31 (m, 2H), 3.09 (s, 3H), 2.57 (m, 1H), 2.43 (dd, *J*=8.6, 15.0 Hz, 1H), 2.20 (dd, *J*=5.1, 15.0 Hz, 1H), 1.62 (m, 2H), 1.47 (s, 3H), 1.38 (s, 9H), 0.91 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.3, 138.3, 135.0, 132.0, 128.2, 127.5, 127.3, 83.5, 80.3, 73.0, 68.4, 55.8, 41.1, 37.1, 29.0, 28.1, 21.2, 10.9; HRMS (ESI) *m/z* 385.2335, calcd for $C_{22}H_{34}NaO_4$ $[M+Na]^+$ 385.2355; TLC, R_f 0.16 (hexane-EtOAc 20:1).



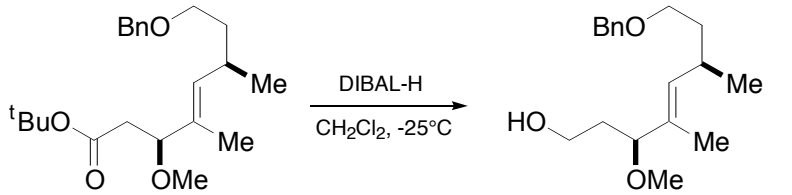
13b **17b**

Methyl ether 17b. To a stirred solution of hydroxy ester **13b** (21.4 mg, 61.5 μmol) in DMF (0.15 mL) at 0 °C were added methyl iodide (38.5 μL, 0.617 mmol) and NaH (60% oil suspension 14.4 mg, 0.36 mmol). The mixture was stirred at room temperature for 30 min, diluted with cooled H_2O (5 mL) and AcOEt (5 mL), then extracted with EtOAc (3×15 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (3.5 g, hexane-EtOAc 15:1) to give methyl ether **17b** (19.4 mg, 87%) as a colorless oil: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.31-7.19 (m, 5H), 5.13 (d, *J*=9.7 Hz, 1H), 4.42 (s, 2H), 3.81 (dd, *J*=5.8, 8.2 Hz, 1 H), 3.37 (m, 2H), 3.07 (s, 3H), 2.59 (m, 1H), 2.46 (dd, *J*=8.2, 14.7 Hz, 1H), 2.28 (dd, *J*=5.8, 14.6 Hz, 1H), 1.67 (m, 2H), 1.54 (s, 3H), 1.45 (s, 9H), 0.89 (d, *J*=6.8 Hz, 3H).



17a **18a**

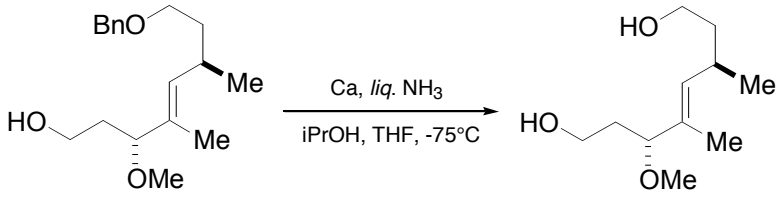
Alcohol 18a. To a stirred solution of methyl ether **17a** (18.4 mg, 50.8 μmol) in CH_2Cl_2 (0.40 mL) cooled at -25 °C was added a 1.0 M solution of diisobutylaluminum hydride (0.25 mL, 0.25 mmol). The mixture was stirred at -25 °C for 30 min, and the reaction was quenched by addition of methanol (0.5 mL). The mixture was warmed to room temperature, diluted with saturated aqueous Na/K tartrate (3 mL), and stirred for 5 min. The organic layer was separated, and the aqueous layer was extracted with EtOAc (3×20 mL). The combined extracts were washed with brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (3.5 g, hexane-EtOAc 4:1→2:1) to give alcohol **18a** (11.3 mg, 76%) as a colorless oil: $[\alpha]^{25}_D$ -8.2 (*c* 0.50, $CHCl_3$); IR ($CHCl_3$) 3420, 1454, 1363, 1100, 736, 698 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.30-7.19 (m, 5H), 5.08 (d, *J*=9.5 Hz, 1H), 4.39 (s, 2H), 3.60 (m, 2H), 3.60 (m, 1H), 3.32 (m, 2H), 3.10 (s, 3H), 2.56 (m, 1H), 1.83 (m, 1H), 1.61 (m, 1H), 1.50 (m, 2H), 1.47 (s, 3H), 0.92 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 138.3, 134.6, 132.8, 128.2, 127.6, 127.4, 87.2, 73.0, 68.5, 61.4, 55.6, 37.2, 36.3, 29.0, 21.3, 10.9; HRMS (ESI) *m*/*z* 315.1936, calcd for $C_{18}H_{28}NaO_3$ $[M+Na]^+$ 315.1936; TLC, R_f 0.42 (hexane-EtOAc 1:1).



17b **18b**

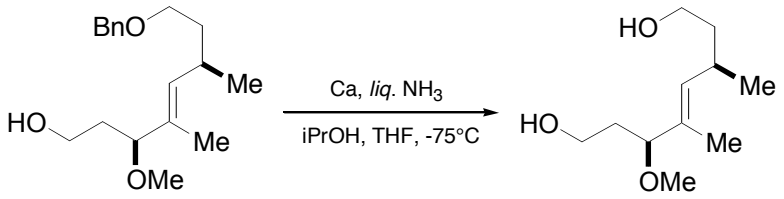
Alcohol 18b. To a stirred solution of methyl ether **17b** (3.0 mg, 8.3 μmol) in CH_2Cl_2 (50 μL) cooled at -25 °C was added a 1.0 M solution of diisobutylaluminum hydride (47 μL, 47 μmol). The mixture was stirred at -25 °C for 15 min, and the reaction was quenched by addition of methanol (0.1 mL). The mixture

was warmed to room temperature, diluted with saturated aqueous Na/K tartrate (1 mL), and stirred for 5 min. The organic layer was separated, and the aqueous layer was extracted with EtOAc (3×5 mL). The combined extracts were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (1 g, hexane-EtOAc 5:1→2:1) to give alcohol **18b** (1.9 mg, 78%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.27 (m, 5H), 5.14 (d, *J*=9.6 Hz, 1H), 4.47 (s, 2H), 3.73 (t, *J*=5.4 Hz, 2 H), 3.65 (dd, *J*=4.4, 8.8 Hz, 1 H), 3.43 (m, 2H), 3.13 (s, 3H), 2.63 (m, 1H), 1.90 (m, 1H), 1.72 (m, 1H), 1.56 (m, 2H), 1.55 (s, 3H), 0.95 (d, *J*=6.4 Hz, 3H).



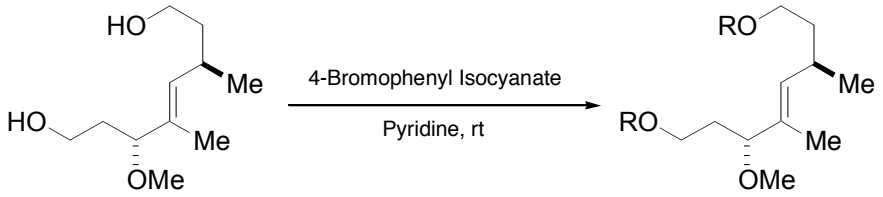
18a **19a**

Diol 19a. Calcium (108 mg, 2.7 mmol) was added to a stirred solution of alcohol **18a** (10.2 mg, 34.9 μmol) in THF (0.60 mL), isopropyl alcohol (0.20 mL), and liquid NH_3 (0.5 mL) cooled at -78 ^oC. After the mixture was stirred at -78 ^oC for 30 min, NH_4Cl (100 mg) and $Fe(NO_3)_3{\cdot}9H_2O$ (100 mg) were added. The mixture was stirred at -78 ^oC for 10 min and allowed to warm to room temperature. The residue was diluted with H_2O (5 mL), and the mixture was stirred at room temperature for 10 min and extracted with EtOAc (3×50 mL). The combined extracts were washed with brine (25 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (3 g, hexane-EtOAc 1:2→0:1) to give diol **19a** (5.1 mg, 72%) as a colorless oil: $[\alpha]^{25}_D$ -0.5 (*c* 0.20, $CHCl_3$); IR ($CHCl_3$) 3374, 1455, 1374, 1215, 1052, 755, 667 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.21 (d, *J*=9.7 Hz, 1H), 3.70 (m, 2 H), 3.70 (m, 1 H), 3.59 (m, 2H), 3.19 (s, 3H), 2.61 (m, 1H), 1.93 (m, 1H), 1.66 (m, 2H), 1.60 (s, 3H), 1.50 (m, 1H), 1.01 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 134.6, 132.9, 87.1, 61.4, 55.6, 40.1, 36.2, 29.1, 21.3, 19.7, 10.9; HRMS (ESI) *m/z* 225.1462, calcd for $C_{11}H_{22}NaO_3$ $[M+Na]^+$ 225.1467; TLC, R_f 0.17 (hexane-EtOAc 1:2).



18b **19b**

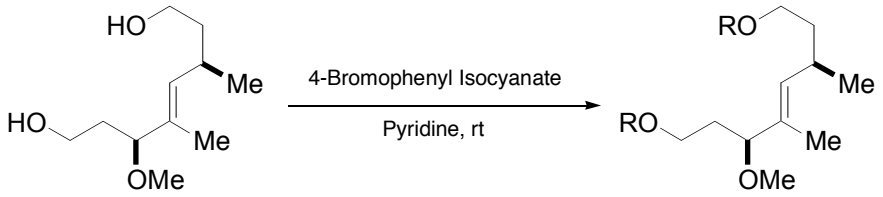
Diol 19b. Calcium(5.2 mg, 0.13 mmol) was added to a stirred solution of alcohol **18b** (1.9 mg, 6.5 μmol) in THF (0.20 mL), isopropyl alcohol (0.07 mL), and liquid NH_3 (0.5 mL) cooled at -78 ^oC . After the mixture was stirred at -78 ^oC for 40 min, NH_4Cl (10 mg) and $Fe(NO_3)_3{\cdot}9H_2O$ (10 mg) were added. The mixture was stirred at -78 ^oC for 10 min and allowed to warm to room temperature. The residue was diluted with H_2O (1mL), and the mixture was stirred at room temperature for 10 min and extracted with EtOAc (3×15 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (0.7 g, hexane-EtOAc 1:1→0:1) to give diol **19b** (1.2 mg, 91%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.19 (d, *J*=9.6 Hz, 1H), 3.74 (m, 2 H), 3.67 (m, 1 H), 3.66 (m, 2H), 3.19 (s, 3H), 2.61 (m, 1H), 1.91 (m, 1H), 1.57 (m, 1H), 1.57 (m, 2H), 1.55 (s, 3H), 0.97 (d, *J*=7.2 Hz, 3H).



19a **14a**

R=*p*-BrC_6H_4NHCO

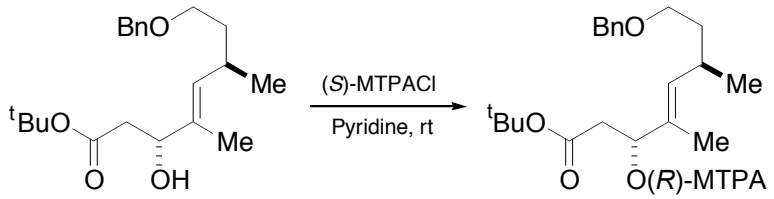
Fragment 14a. To a stirred solution of diol **19a** (2.6 mg, 12.9 μmol) in pyridine (0.20 mL) was added 4-bromophenyl isocyanate (25.3 mg, 0.128 mmol). The mixture was stirred at room temperature for 10 min, diluted with H_2O (0.1 mL) and ether (3 mL). The mixture was concentrated in *vacuo* and purified by column chromatography on silical gel (1.0 g, hexane-EtOAc 5:1→3:1→2:1) and (1.0 g, hexane-EtOAc 5:1→3:1) to give **14a** (7.6 mg, 99%) as a colorless oil: $[\alpha]^{25}_{D}$ -21.6 (*c* 0.33, $CHCl_3$); IR ($CHCl_3$) 1704, 1594, 1536, 1489, 1400, 1307, 1223, 1075, 824, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40-7.36 (m, 4H), 7.31-7.24 (m, 4H), 5.20 (d, *J*=9.6 Hz, 1H), 4.17 (m, 2H), 4.13 (m, 2H), 3.55 (t, *J*=7.0 Hz, 1 H), 3.17 (s, 3H), 2.60 (m, 1H), 1.97 (m, 1H), 1.86 (m, 1H), 1.76 (m, 1H), 1.56 (m, 1H), 1.56 (s, 3H), 1.04 (d, *J*=6.8 Hz, 3H); HRMS (ESI) *m*/*z* 619.0430, calcd for $C_{21}H_{32}NaO_4$ $[M+Na]^+$ 619.0419; TLC, R_f 0.16 (hexane-EtOAc 5:1)



19b **14b**

R=*p*-BrC_6H_4NHCO

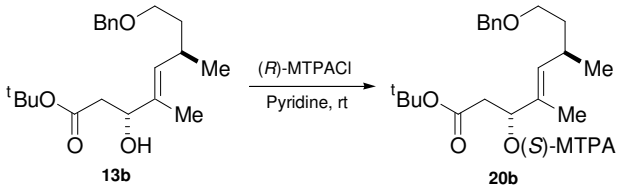
Fragment 14b. To a stirred solution of diol **19b** (1.2 mg, 5.9 μmol) in pyridine (0.10 mL) was added 4-bromophenyl isocyanate (11.7 mg, 59 μmol). The mixture was stirred at room temperature for 10 min, diluted with H_2O (0.1 mL) and ether (3 mL). The mixture was concentrated in *vacuo* and purified by column chromatography on silical gel (1.0 g, hexane-EtOAc 10:1→5:1→3:1) to give **14b** (2.5 mg, 75%) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 7.42-7.39 (m, 4H), 7.30-7.27 (m, 4H), 5.18 (d, *J*=9.2 Hz, 1H), 4.19 (m, 2H), 4.15 (m, 2H), 3.57 (t, *J*=6.8 Hz, 1 H), 3.19 (s, 3H), 2.61 (m, 1H), 1.97 (m, 1H), 1.79 (m, 2H), 1.57 (m, 1H), 1.57 (s, 3H), 0.99 (d, *J*=6.8 Hz, 3H).



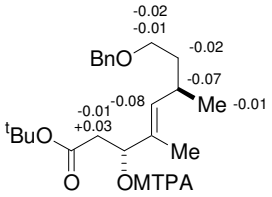
13a **20a**

MTPA ester 20a. To a stirred solution of hydroxyl ester **13a** (2.0 mg, 5.8 μmol) in pyridine (15 μL) was added DMAP (1.0 mg, 8.2 μmol) and (*S*)-MTPACl (2 drops). The mixture was stirred at room temperature for 45 min, diluted with saturated aqueous NH_4Cl (2 mL) and AcOEt (2 ml), then extracted with EtOAc (3×15 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (1.5 g, hexane-EtOAc 20:1) to give (*R*)-MTPA ester **20a** (3.3 mg, 100%) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 7.49-7.47 (m, 2H), 7.38-7.27 (m, 8H), 5.80 (dd, *J*=4.4, 9.4 Hz, 1H), 5.42 (d, *J*=9.6 Hz, 1H), 4.44 (s, 2H), 3.50 (s, 3H), 3.41 (m, 1H), 3.34 (m, 1H), 2.69 (dd, *J*=9.4, 16.0 Hz, 1H), 2.60 (m, 1H), 2.44 (dd, *J*=4.4, 16.0 Hz, 1H), 1.65

(m, 2H), 1.57 (s, 3H), 1.40 (s, 9H), 0.95 (d, J=6.8 Hz, 3H); HRMS (ESI) m/z 587.2605, calcd for $C_{31}H_{39}F_3NaO_6$ $[M+Na]^+$ 587.2596.

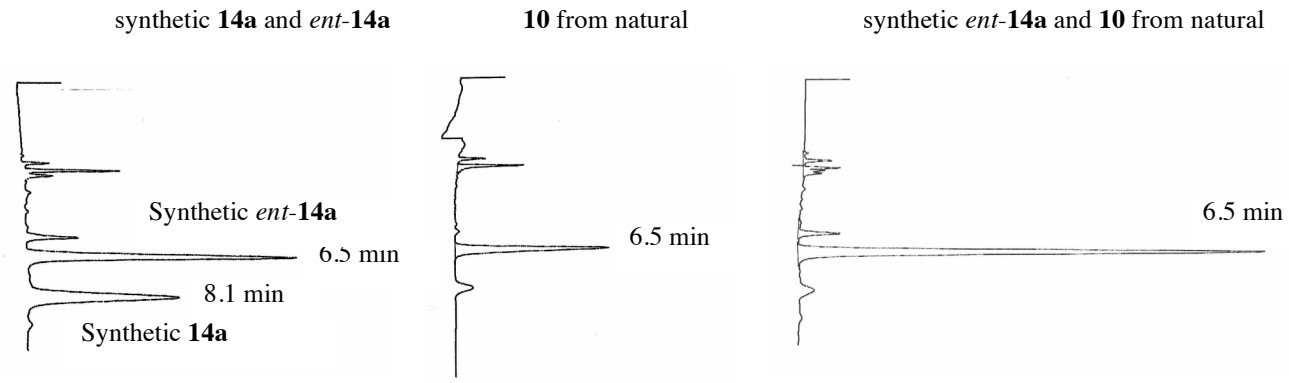


MTPA ester 20b. To a stirred solution of hydroxyl ester **13a** (2.0 mg, 5.8 μmol) in pyridine (15 μL) was added DMAP (1.0 mg, 8.2 μmol) and (R)-MTPACl (4 drops). The mixture was stirred at room temperature for 5 min, diluted with saturated aqueous NH_4Cl (2 mL) and AcOEt (2 ml), then extracted with EtOAc (3×15 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silical gel (1.5 g, hexane-EtOAc 20:1) to give (S)-MTPA ester **20b** (3.4 mg, 100%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50-7.48 (m, 2H), 7.38-7.27 (m, 8H), 5.76 (dd, J=4.0, 9.6 Hz, 1H), 5.34 (d, J=9.6 Hz, 1H), 4.44 (s, 2H), 3.55 (s, 3H), 3.39 (m, 1H), 3.33 (m, 1H), 2.72 (dd, J=9.6, 16.0 Hz, 1H), 2.53 (m, 1H), 2.43 (dd, J=4.0, 16.0 Hz, 1H), 1.63 (m, 2H), 1.56 (s, 3H), 1.42 (s, 9H), 0.95 (d, J=6.8 Hz, 3H); HRMS (ESI) m/z 587.2575, calcd for $C_{31}H_{39}F_3NaO_6$ $[M+Na]^+$ 587.2596.

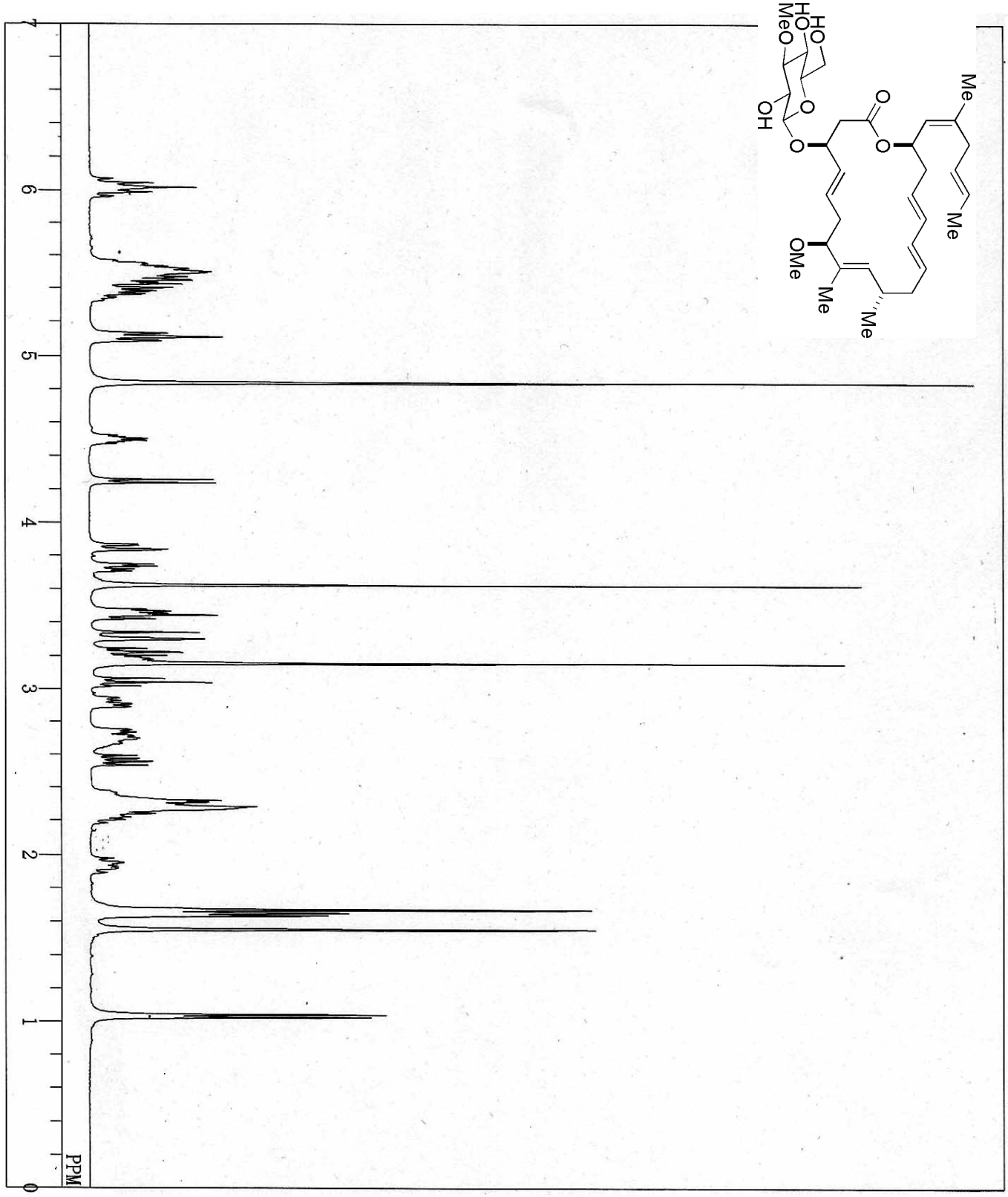


$\Delta\delta$ values ($\Delta\delta_{S-R}$) in ppm for bis-S- and bis-R-MTPA esters (**20a** and **20b**)

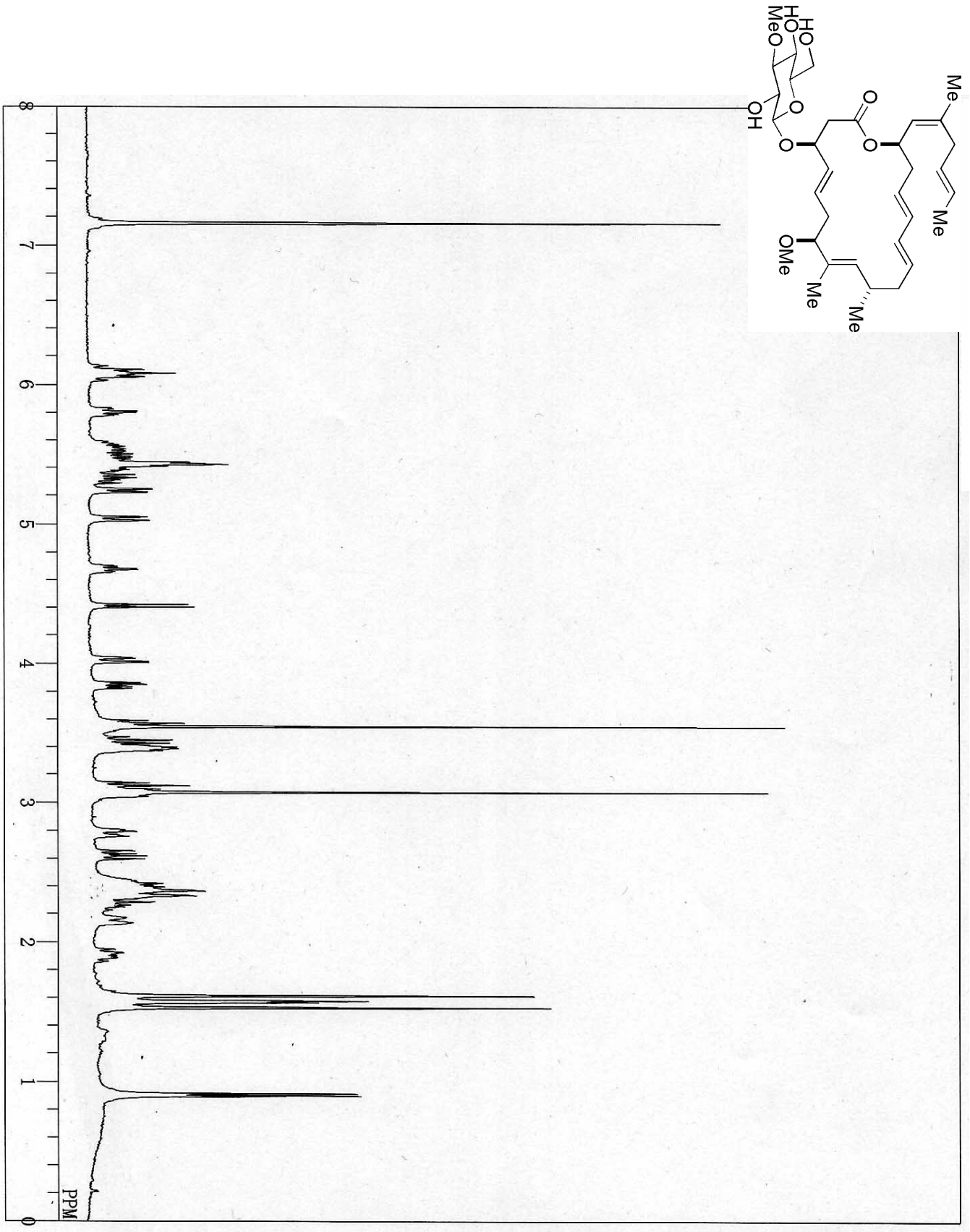
Chiral HPLC analysis of 10, 14a, and *ent*-14a. To determine the absolute stereochemistry of **10**, **14a** and the enantiomeric (3*R*, 6*R*)-biscarbamate *ent*-**14a** was prepared from (2*R*)-aldehyde **11b** and (2*S*)-aldehyde **11b,** respectively, and both enantiomers were analyzed by chiral HPLC. Of the two synthetic enantiomers **14a** and *ent*-**14a**, the retention time of *ent*-**14a** was identical to that for **10** from natural.



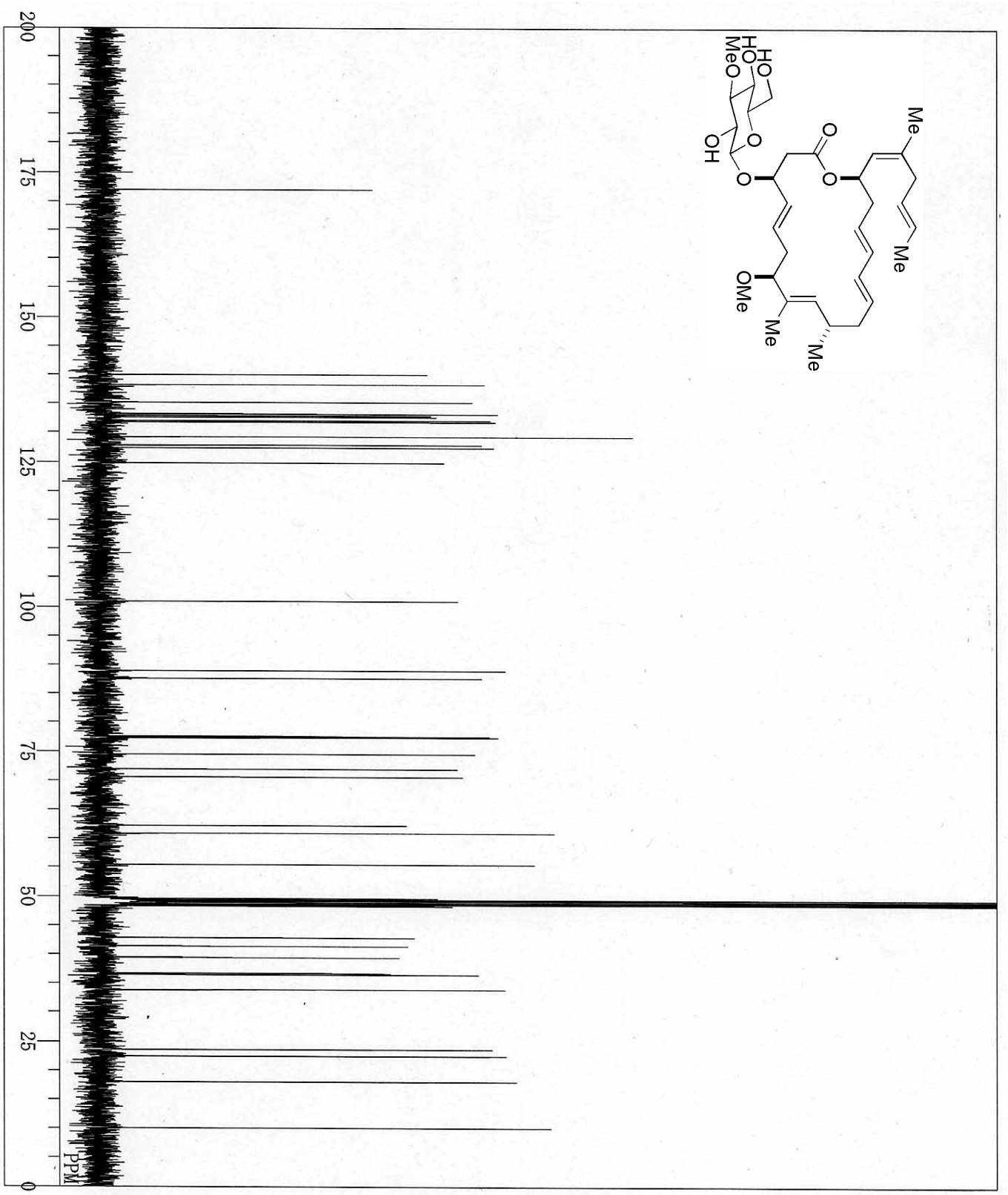
Condition	Column	Chiralpac IA (Daicel Chemical Ind., Ltd)
	Eluent	hexane-ethanol 75:25
	Flow rate	1.0 mL/min
	Detection	UV (215 nm)



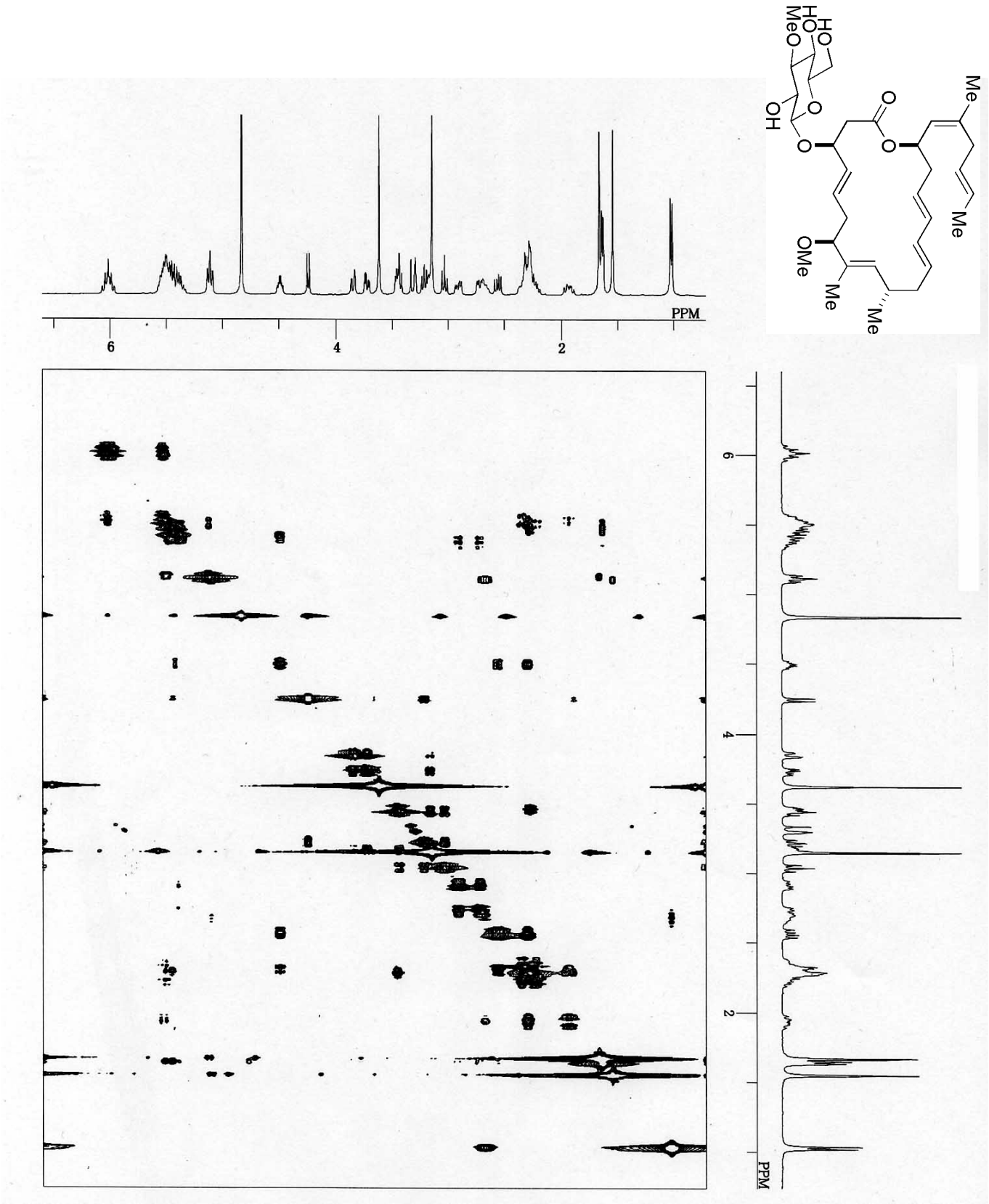
^{1}H NMR spectrum of **1** [400 MHz, CD_3OD].



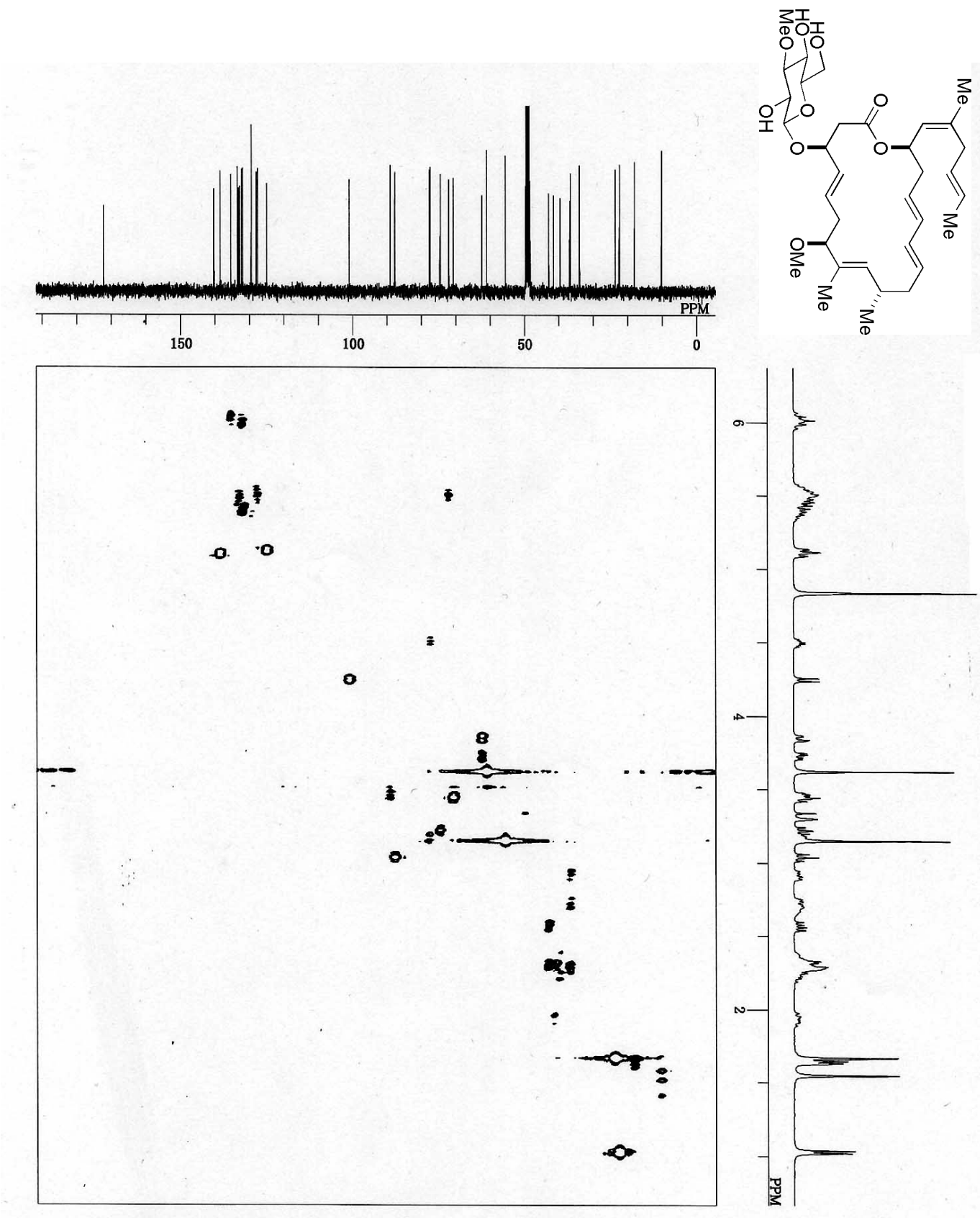
^{1}H NMR spectrum of **1** [400 MHz, C_6D_6].



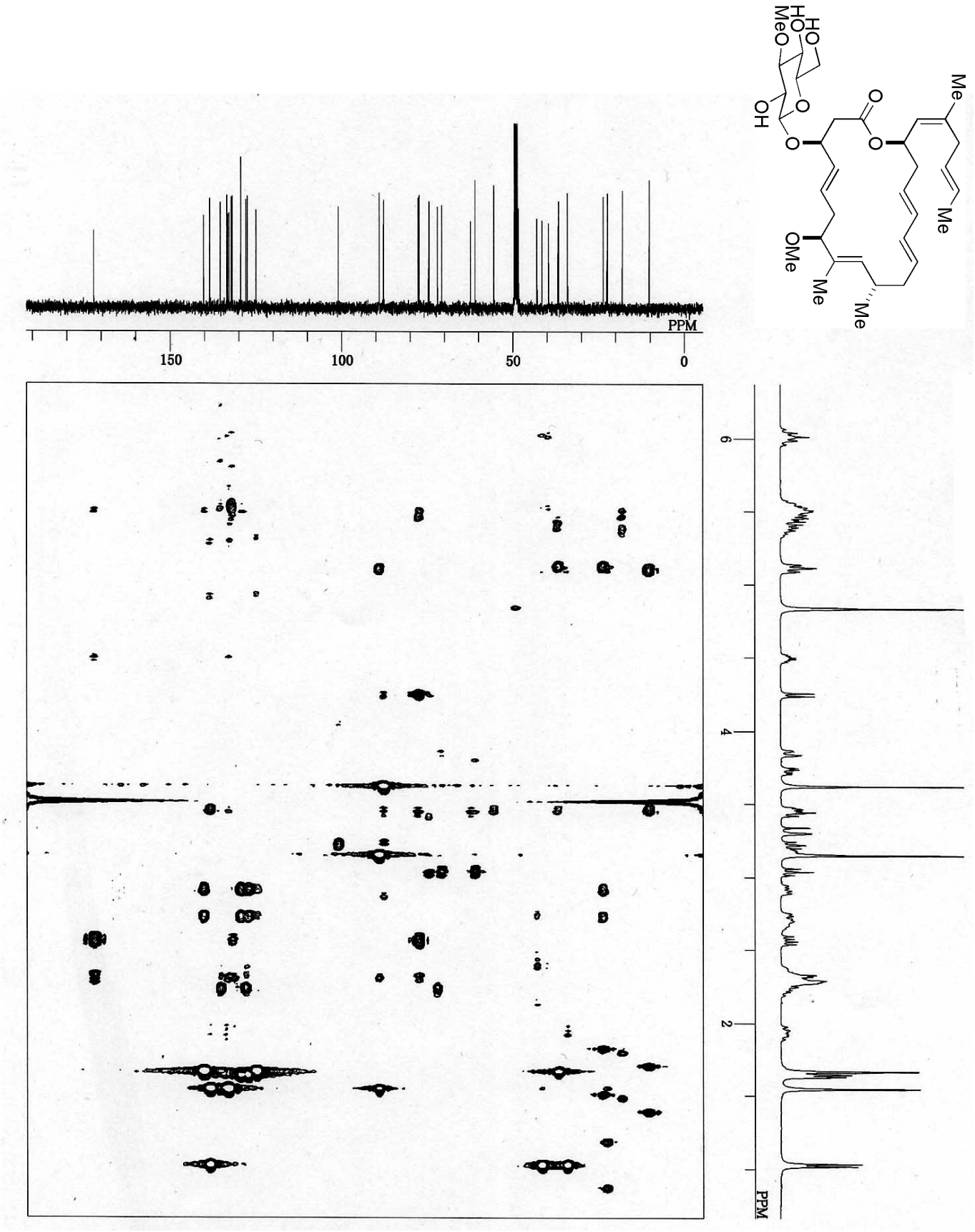
^{13}C NMR spectrum of **1** [100 MHz, CD_3OD].



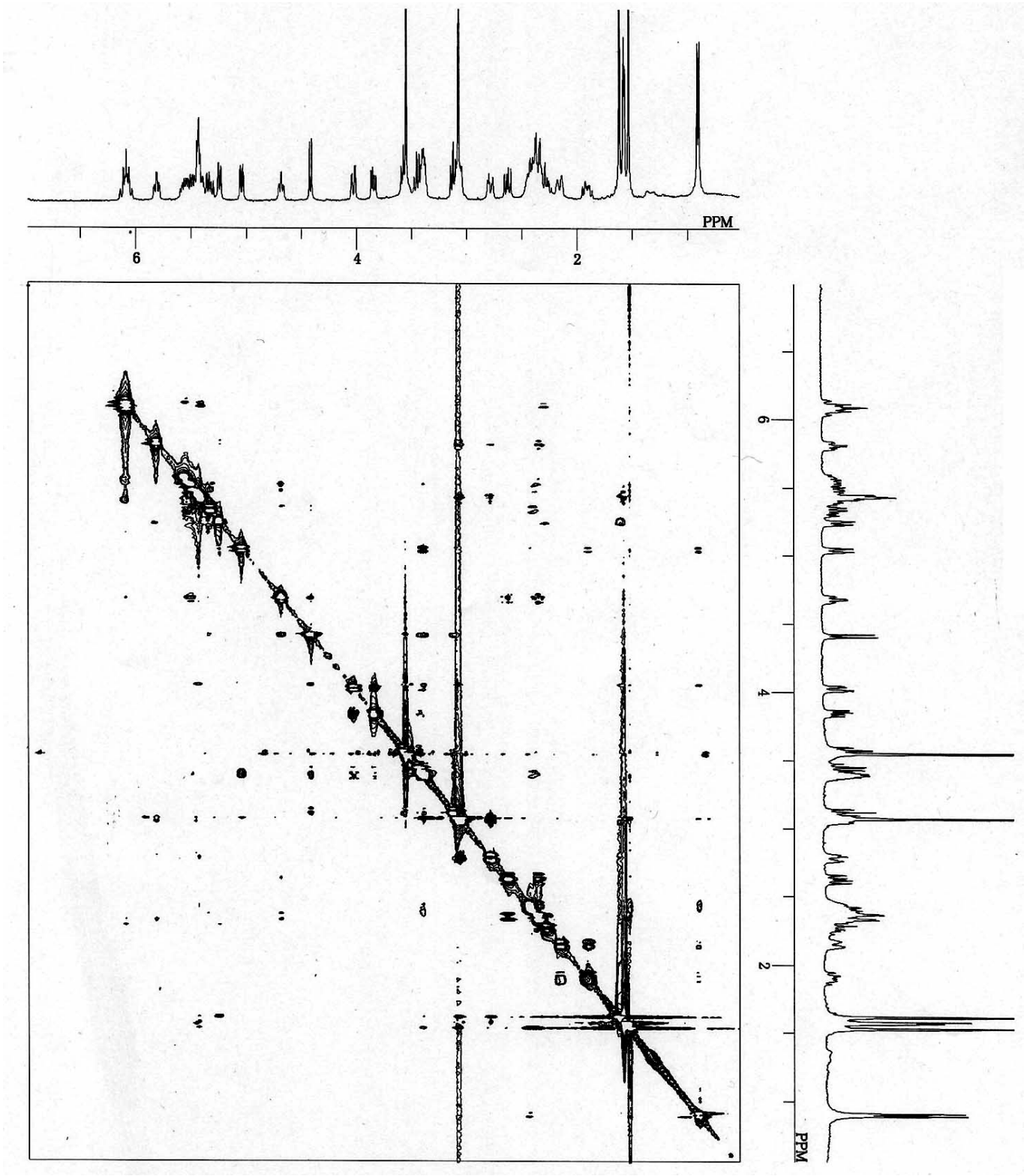
^{1}H-^{1}H spectrum of **1** [400 MHz, CD_3OD].



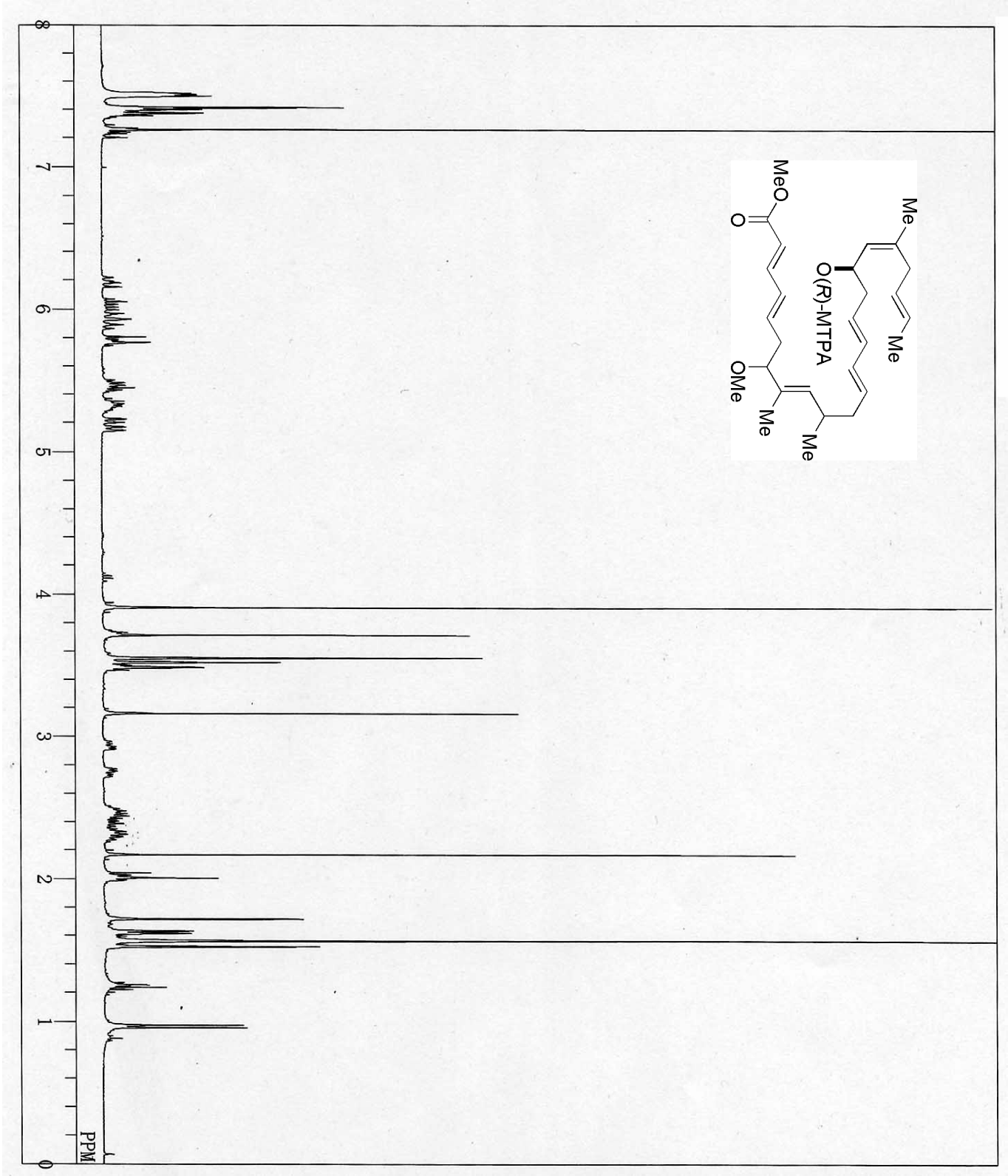
HMQC spectrum of **1** [400 MHz, CD_3OD].



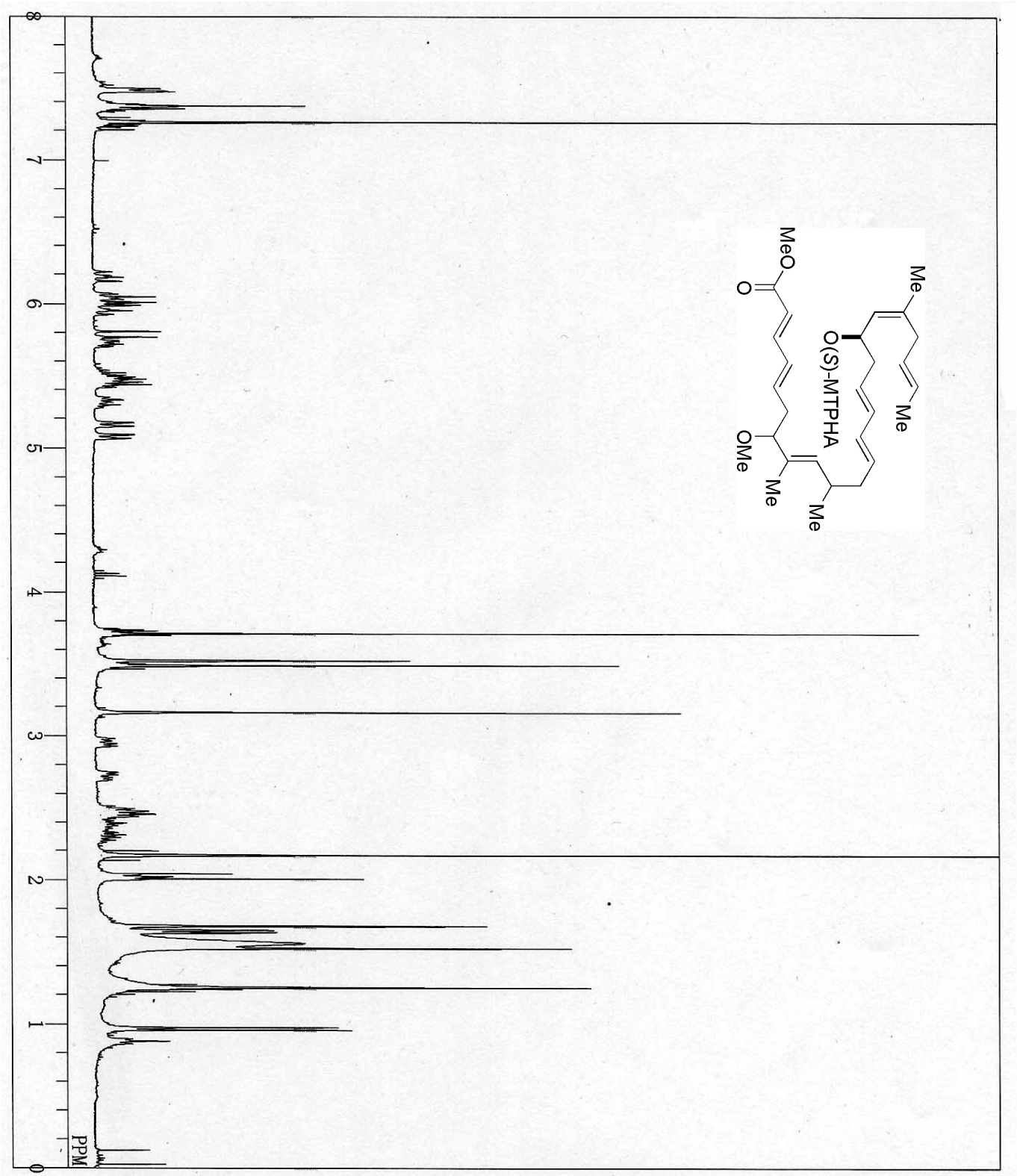
HMBC spectrum of **1** [400 MHz, CD_3OD].



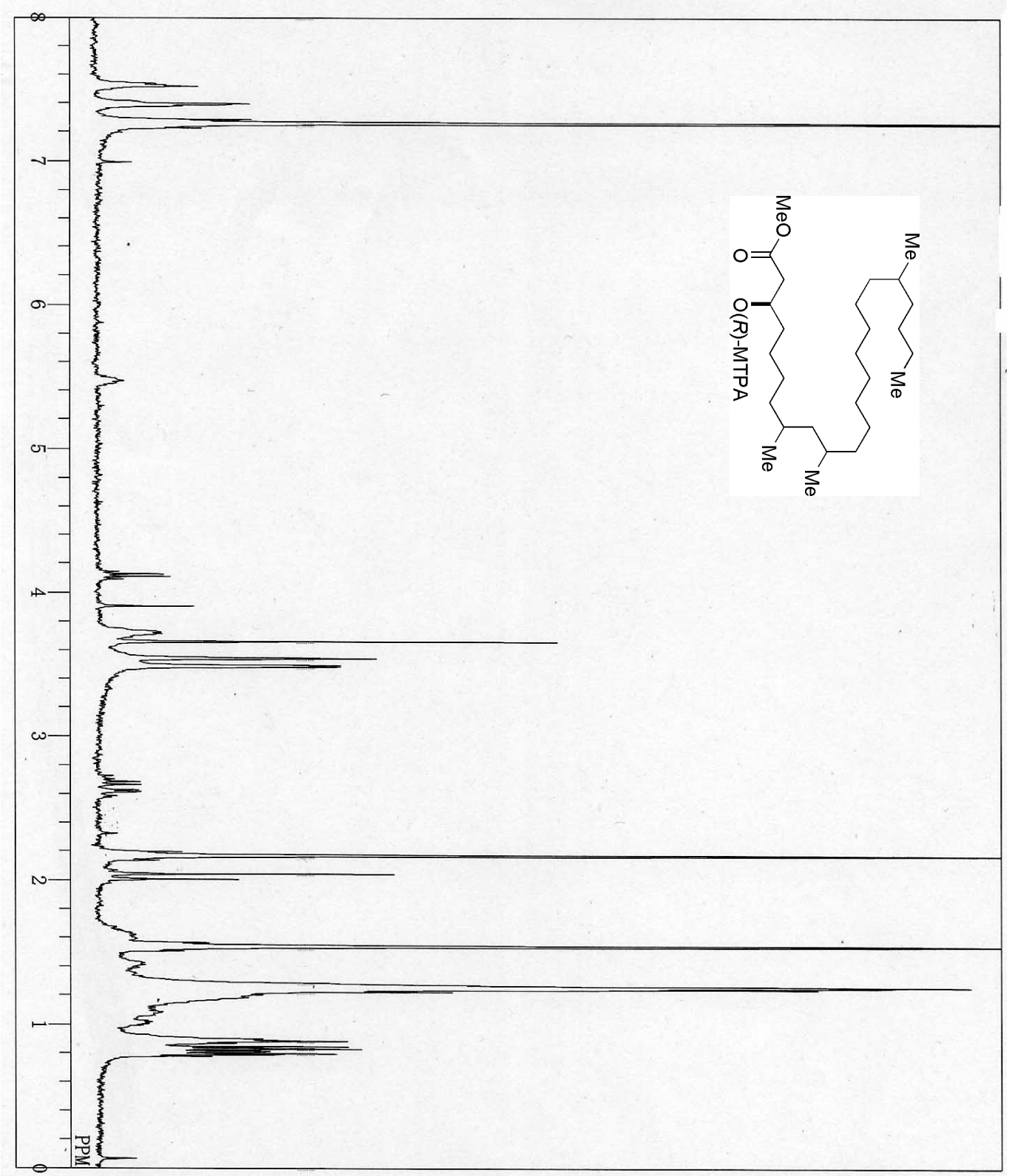
NOESY spectrum of **1** [400 MHz, C_6D_6].



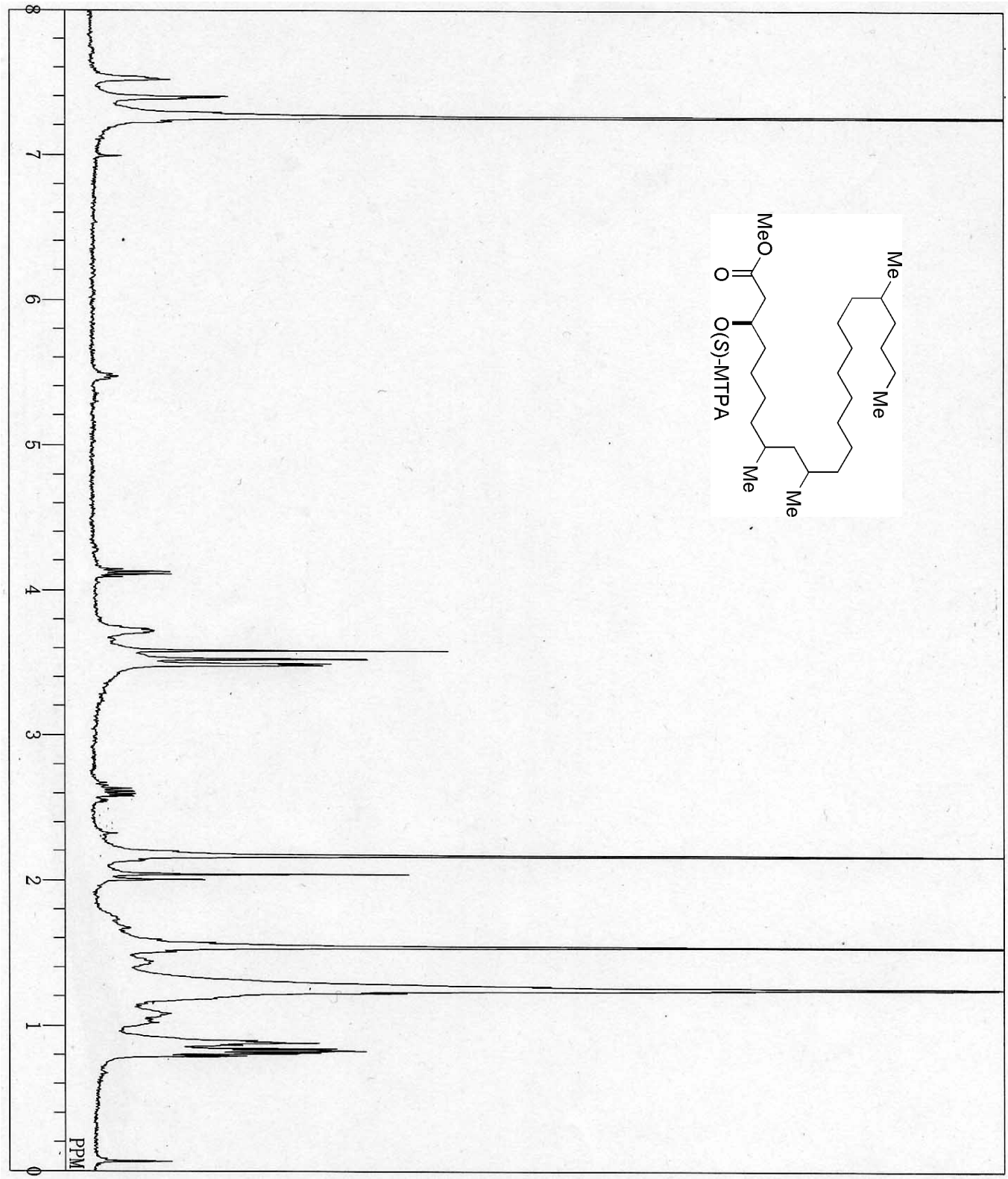
^{1}H NMR spectrum of (*R*)-MTPA ester **3**. [400 MHz, $CDCl_3$].



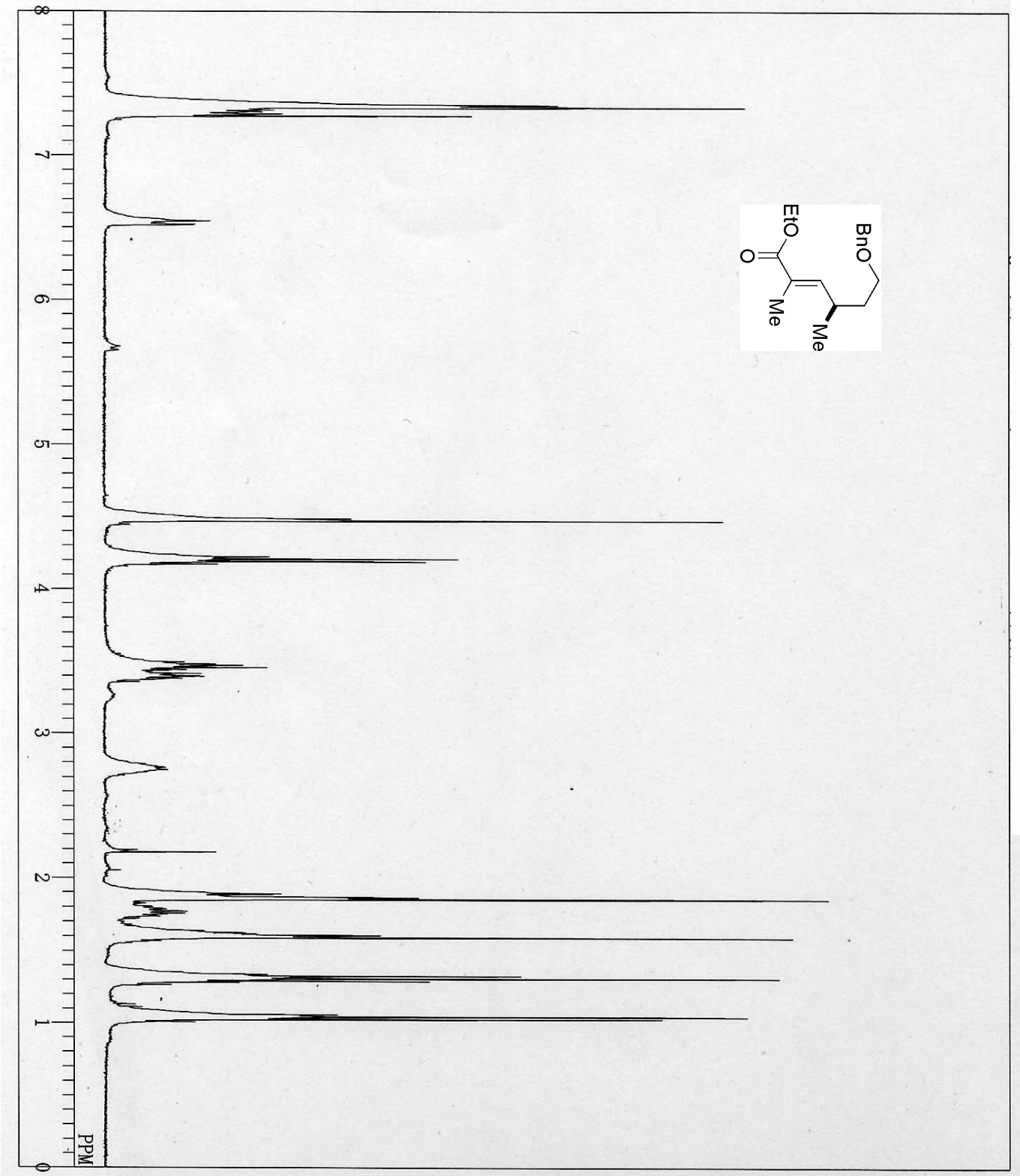
^{1}H NMR spectrum of (*S*)-MTPA ester **4**. [400 MHz, $CDCl_3$]



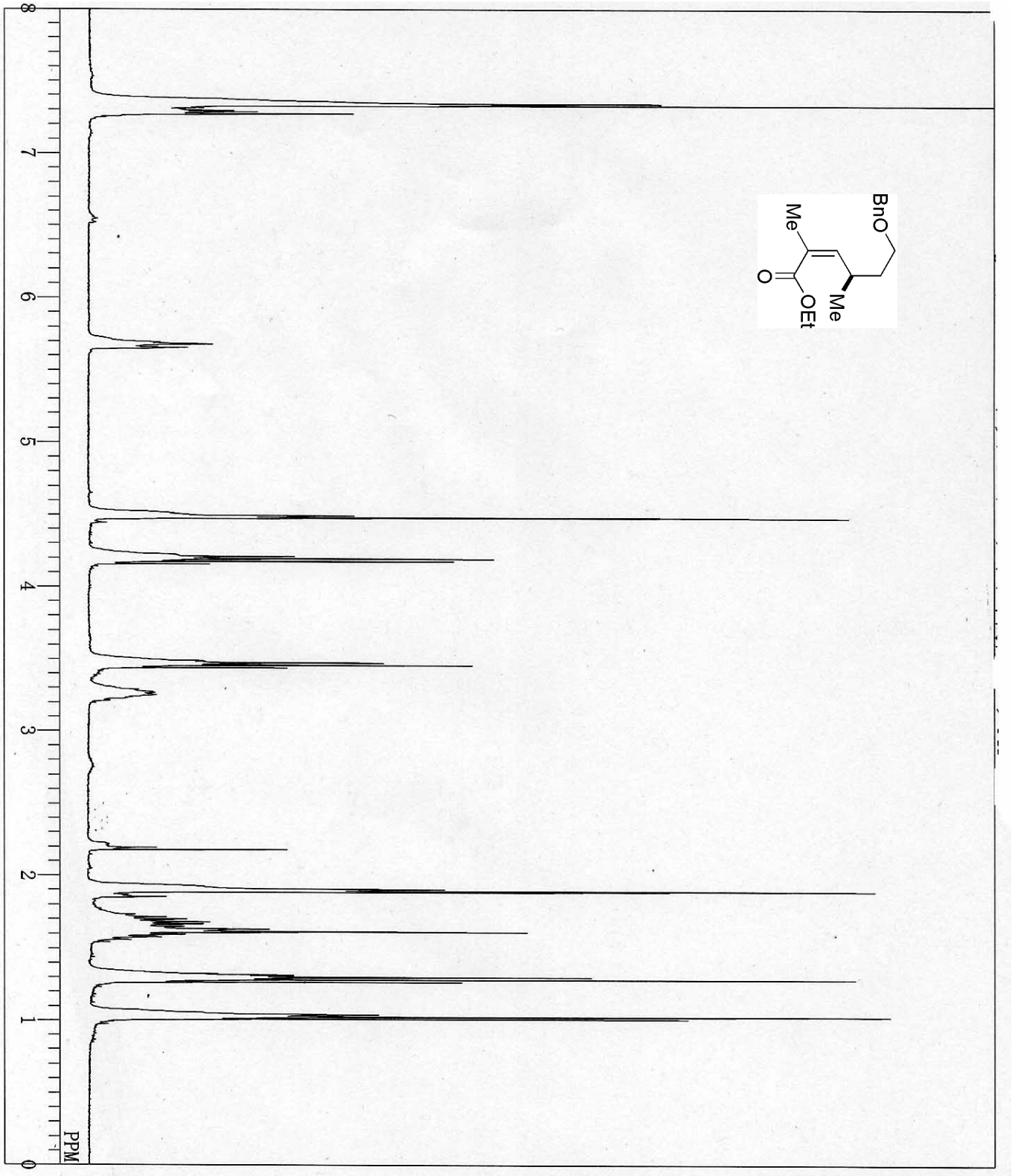
^{1}H NMR spectrum of (*R*)-MTPA ester **7**. [400 MHz, $CDCl_3$]



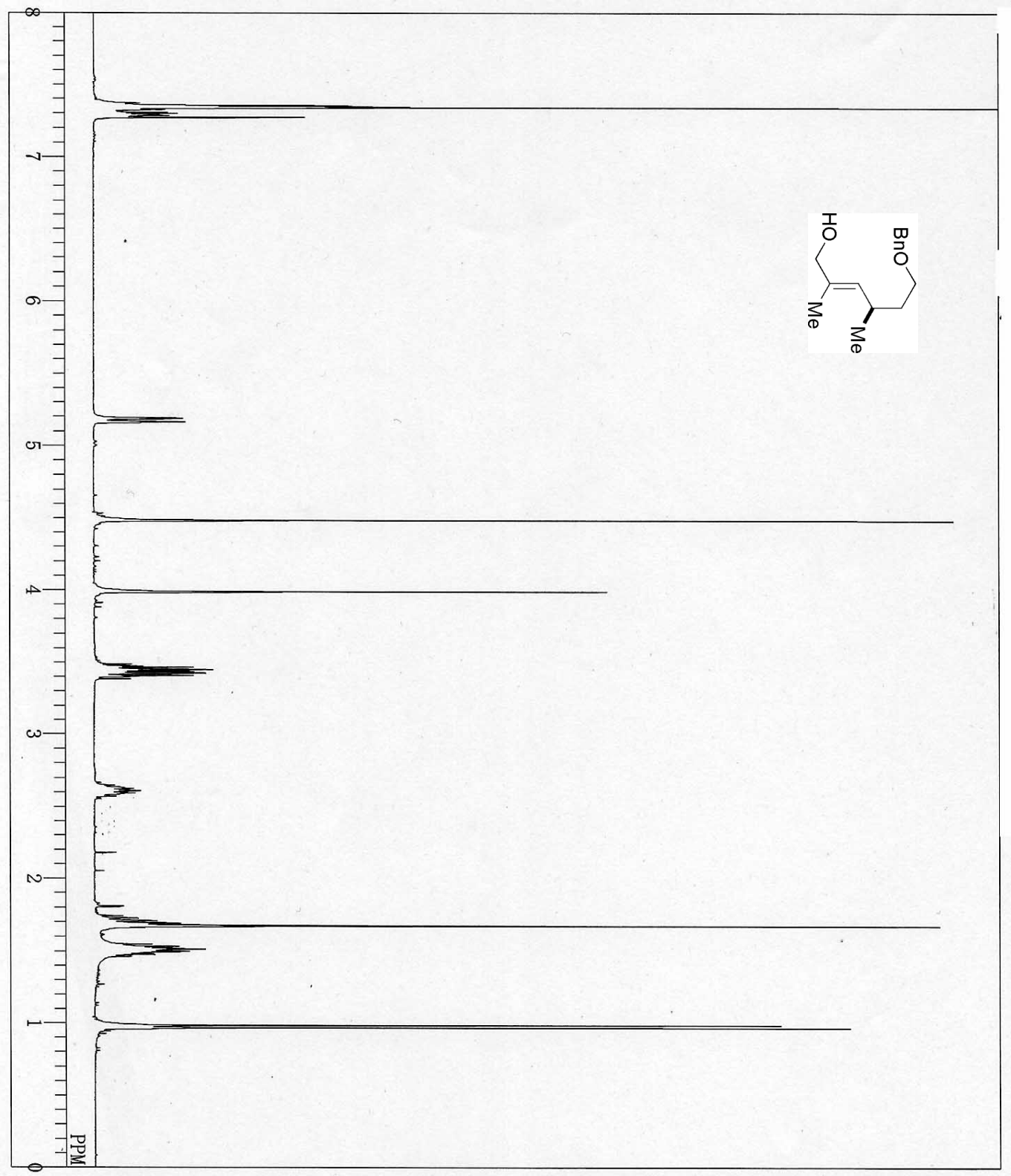
^{1}H NMR spectrum of (*S*)-MTPA ester **8**. [400 MHz, $CDCl_3$]



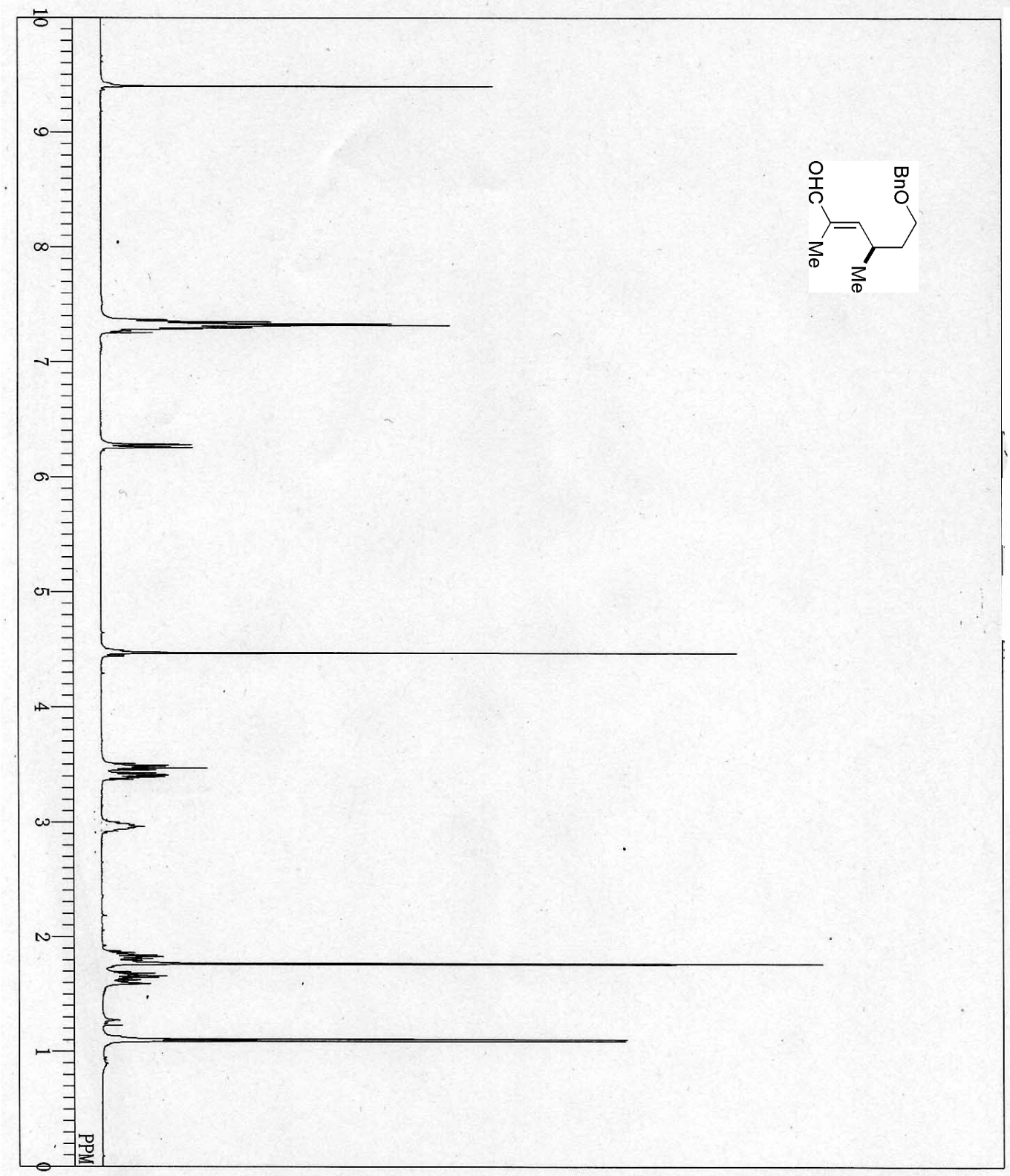
^{1}H NMR spectrum of **15a**. [400 MHz, $CDCl_3$]



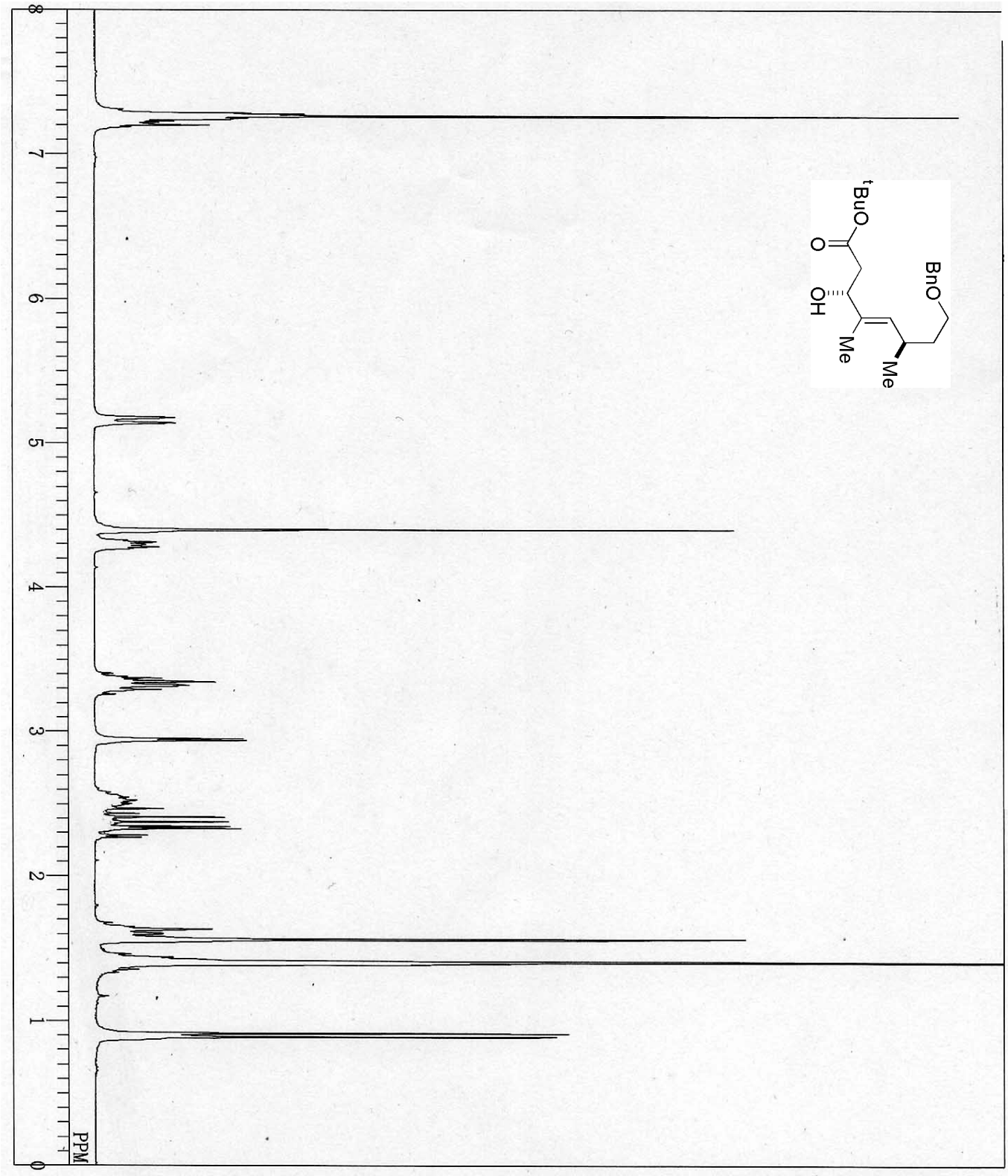
^{1}H NMR spectrum of **15b**. [400 MHz, $CDCl_3$]



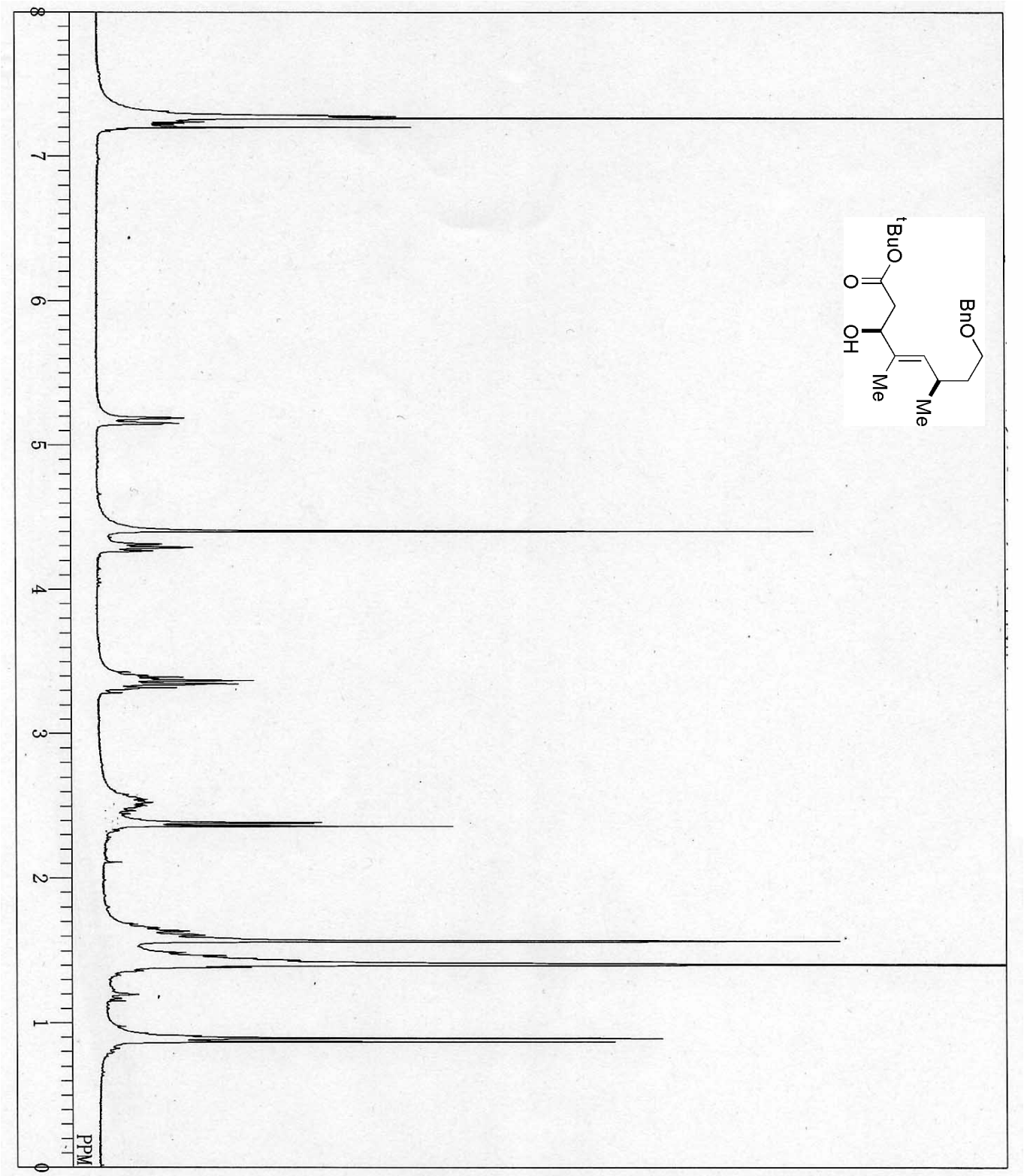
^{1}H NMR spectrum of **16**. [400 MHz, $CDCl_3$]



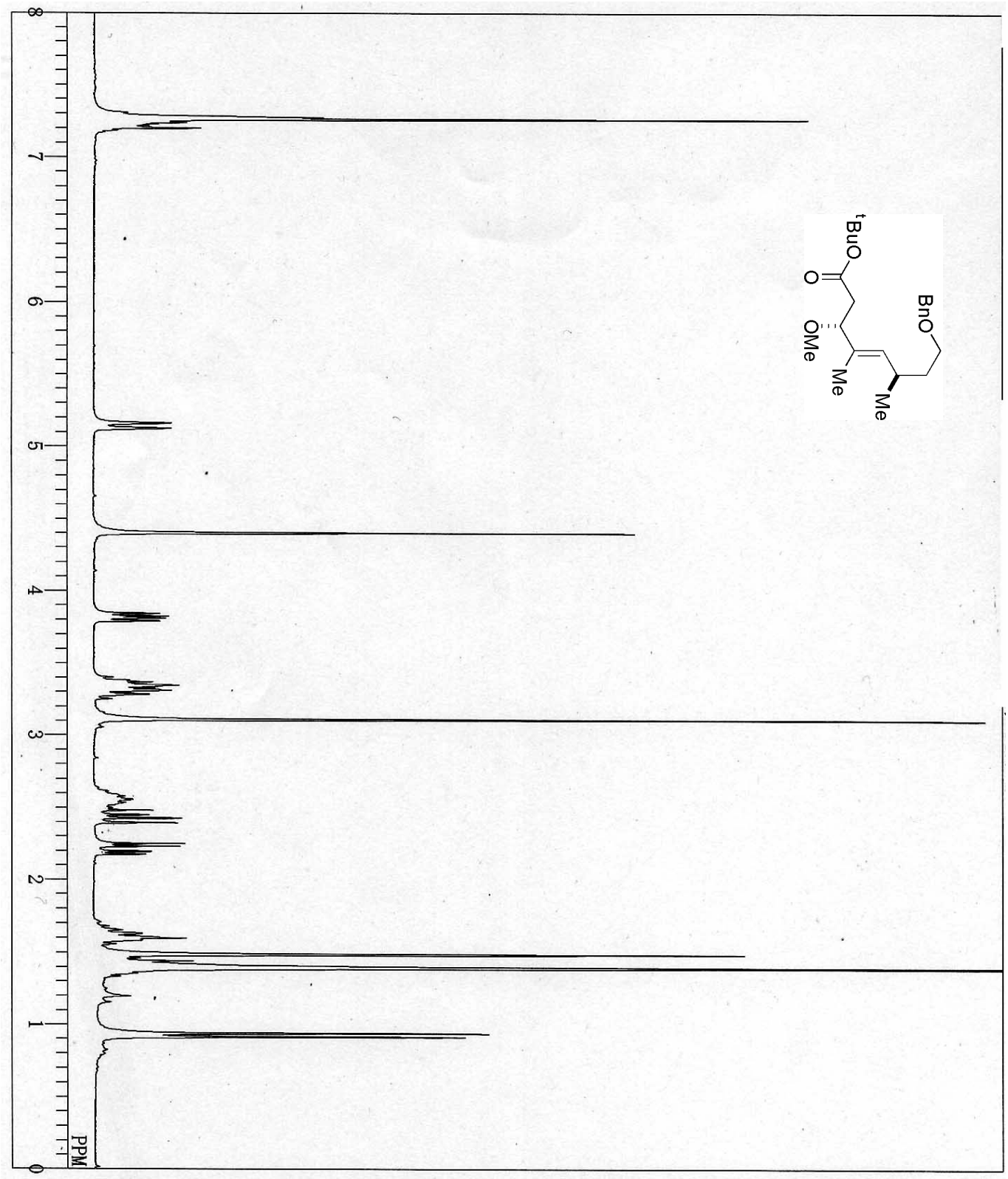
^{1}H NMR spectrum of **12**. [400 MHz, $CDCl_3$]



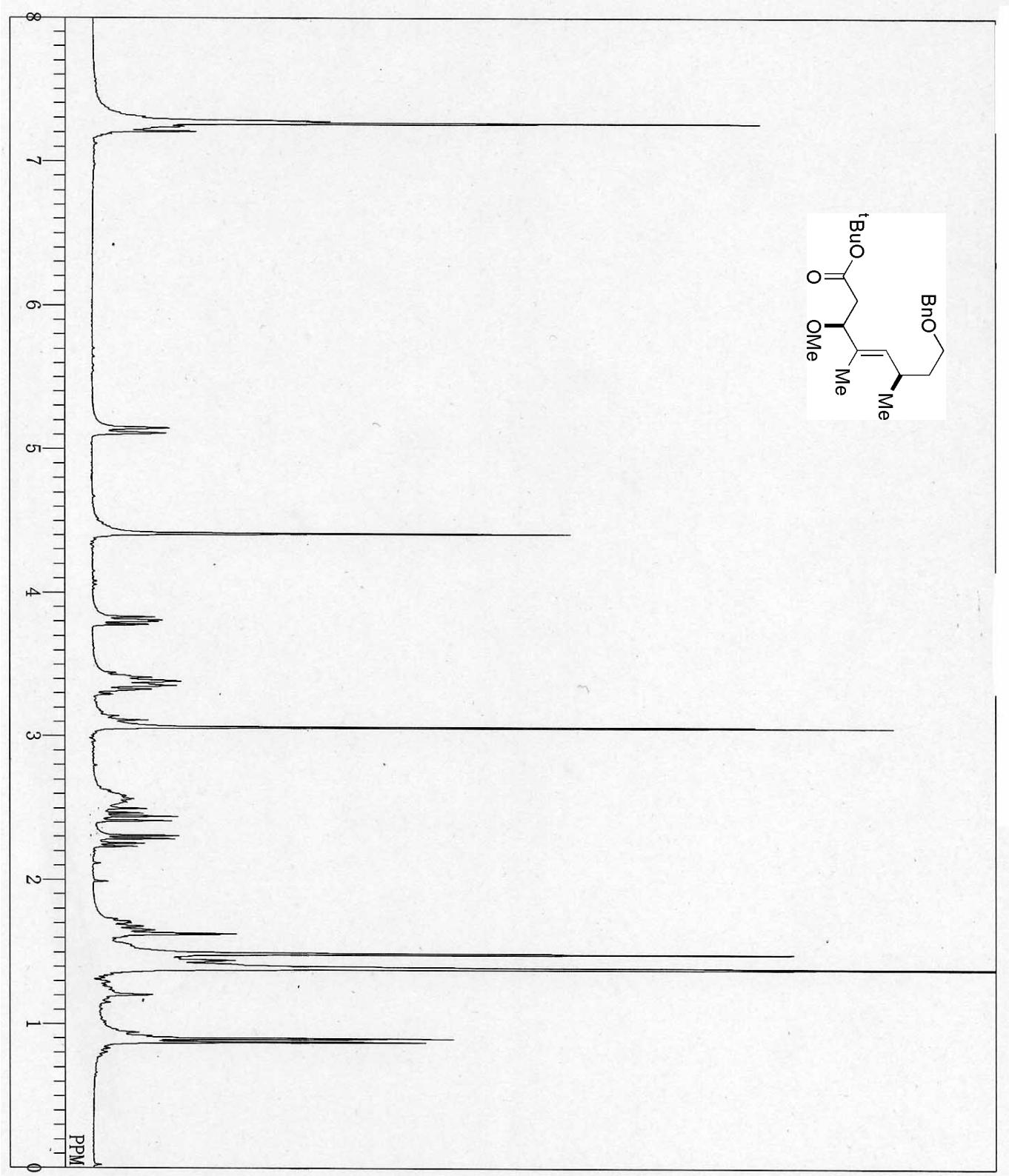
^{1}H NMR spectrum of **13a**. [270 MHz, $CDCl_3$]



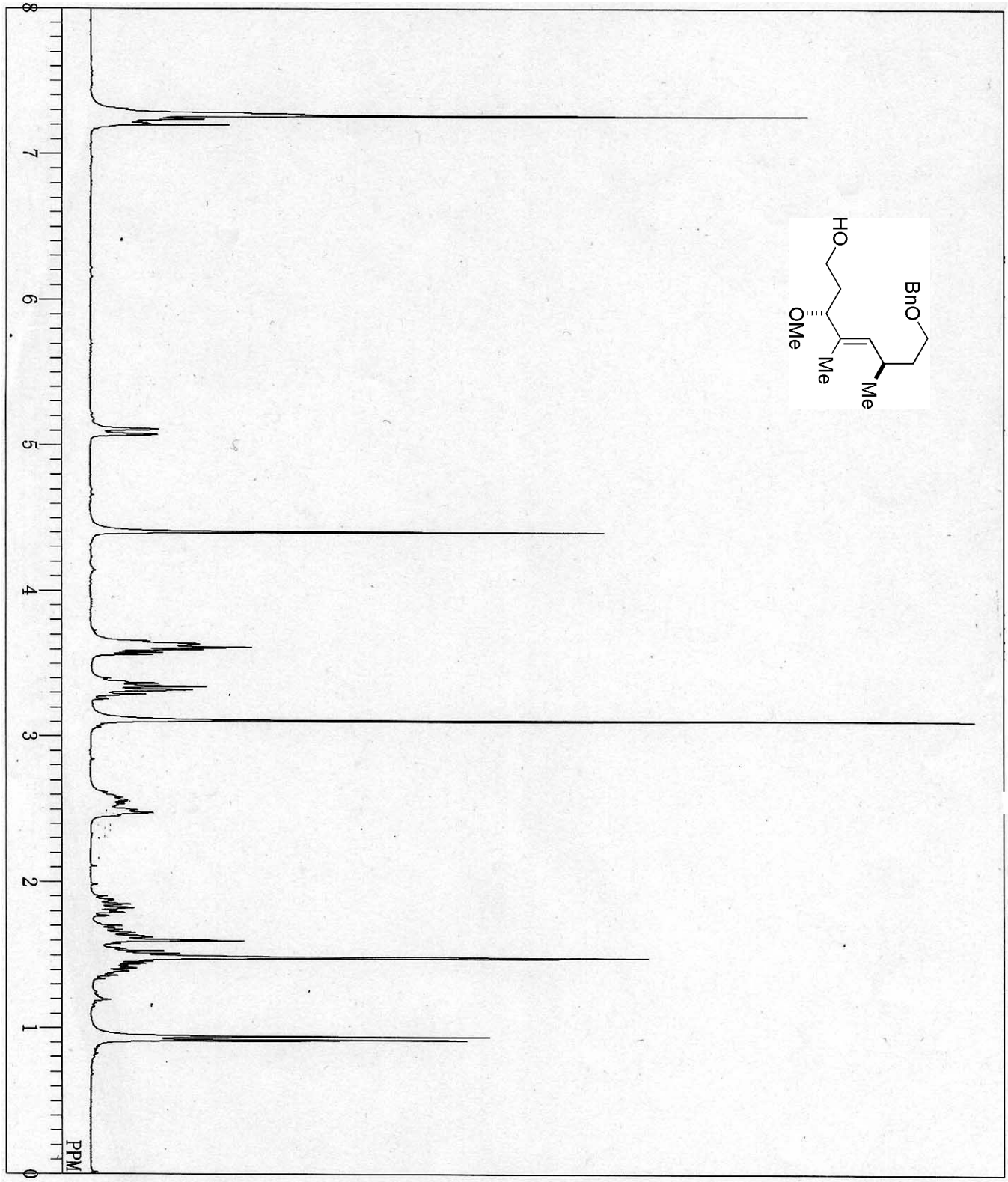
^{1}H NMR spectrum of **13b**. [270 MHz, $CDCl_3$]



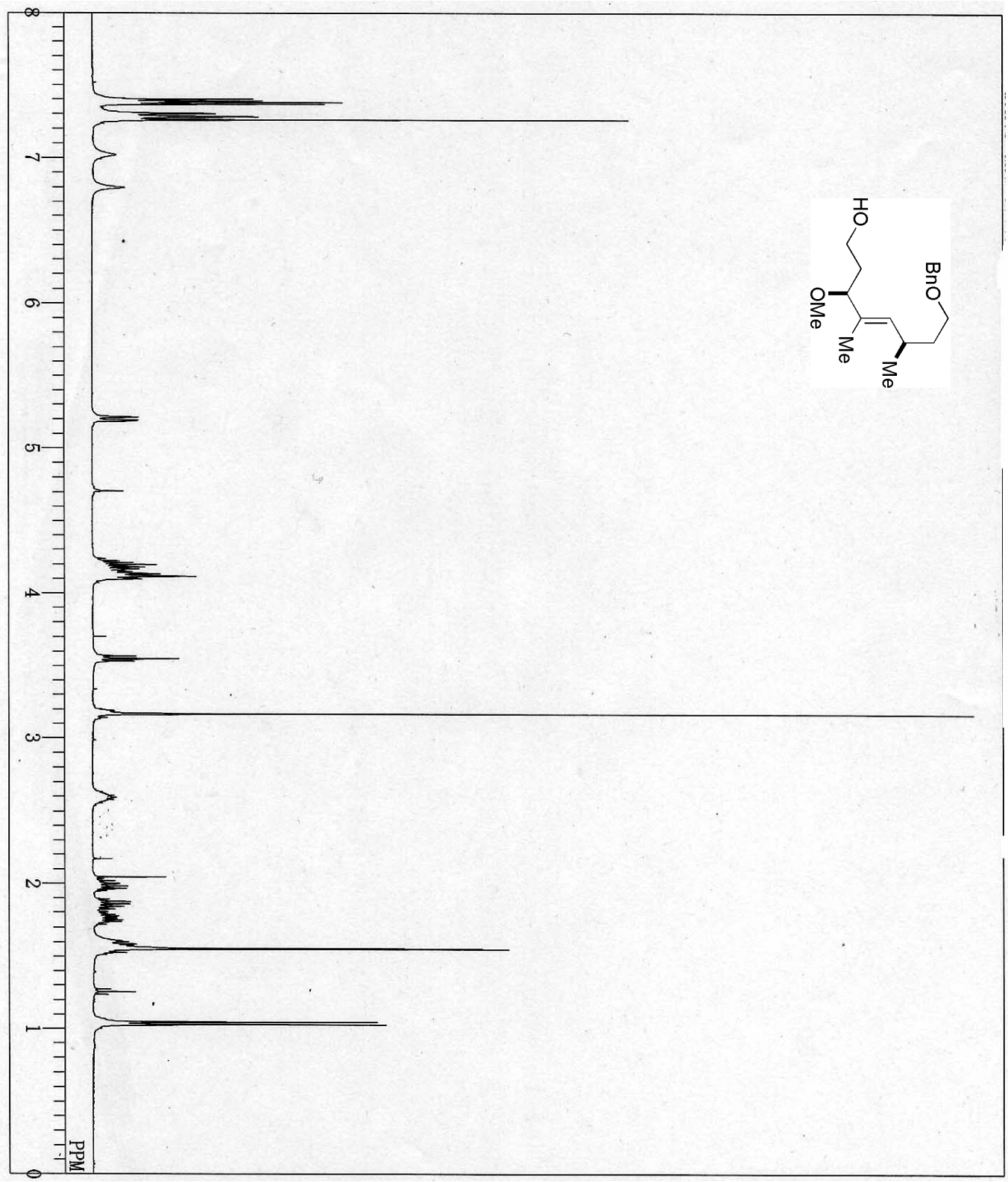
^{1}H NMR spectrum of **17a**. [270 MHz, $CDCl_3$]



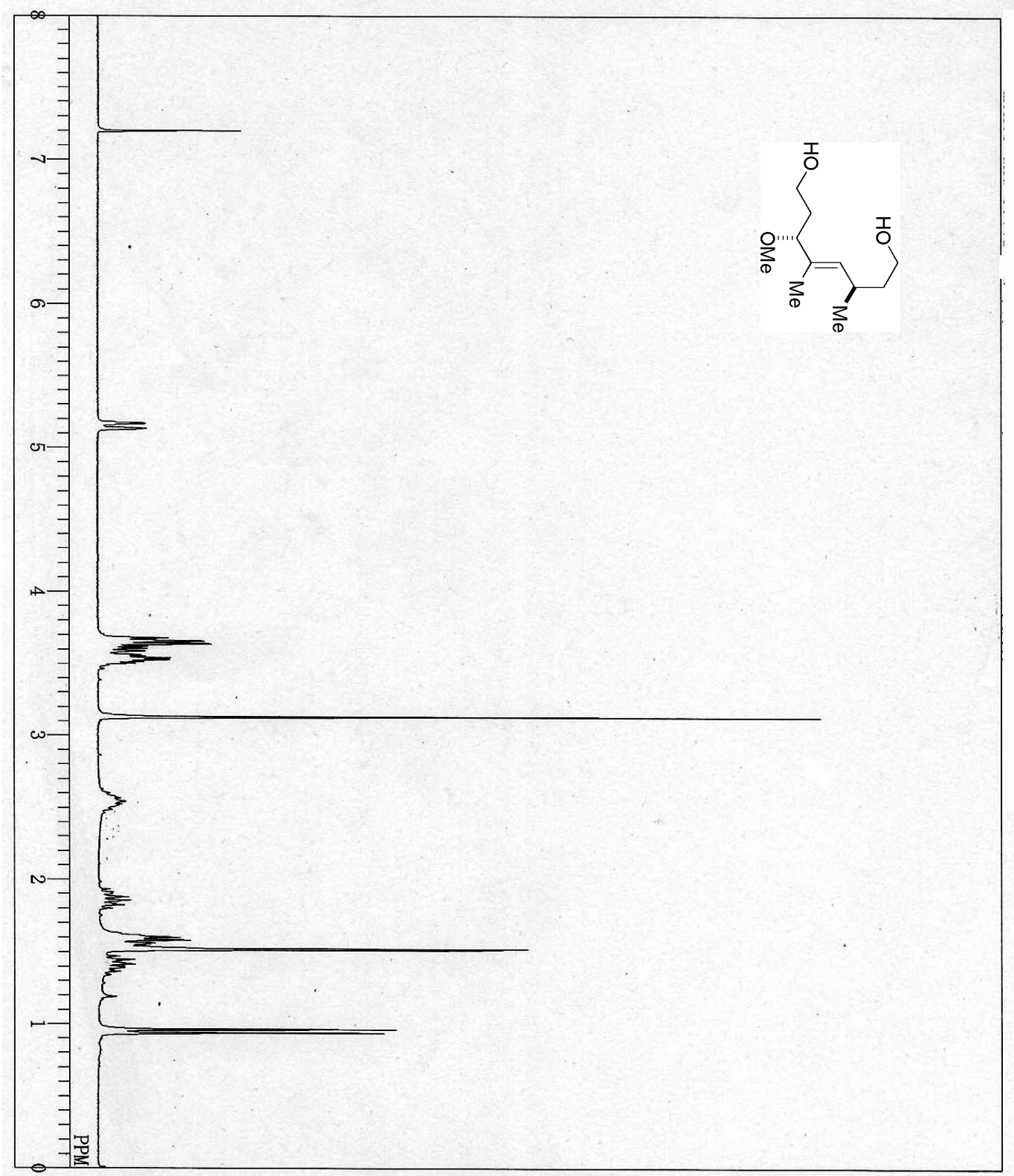
^{1}H NMR spectrum of **17b**. [270 MHz, $CDCl_3$]



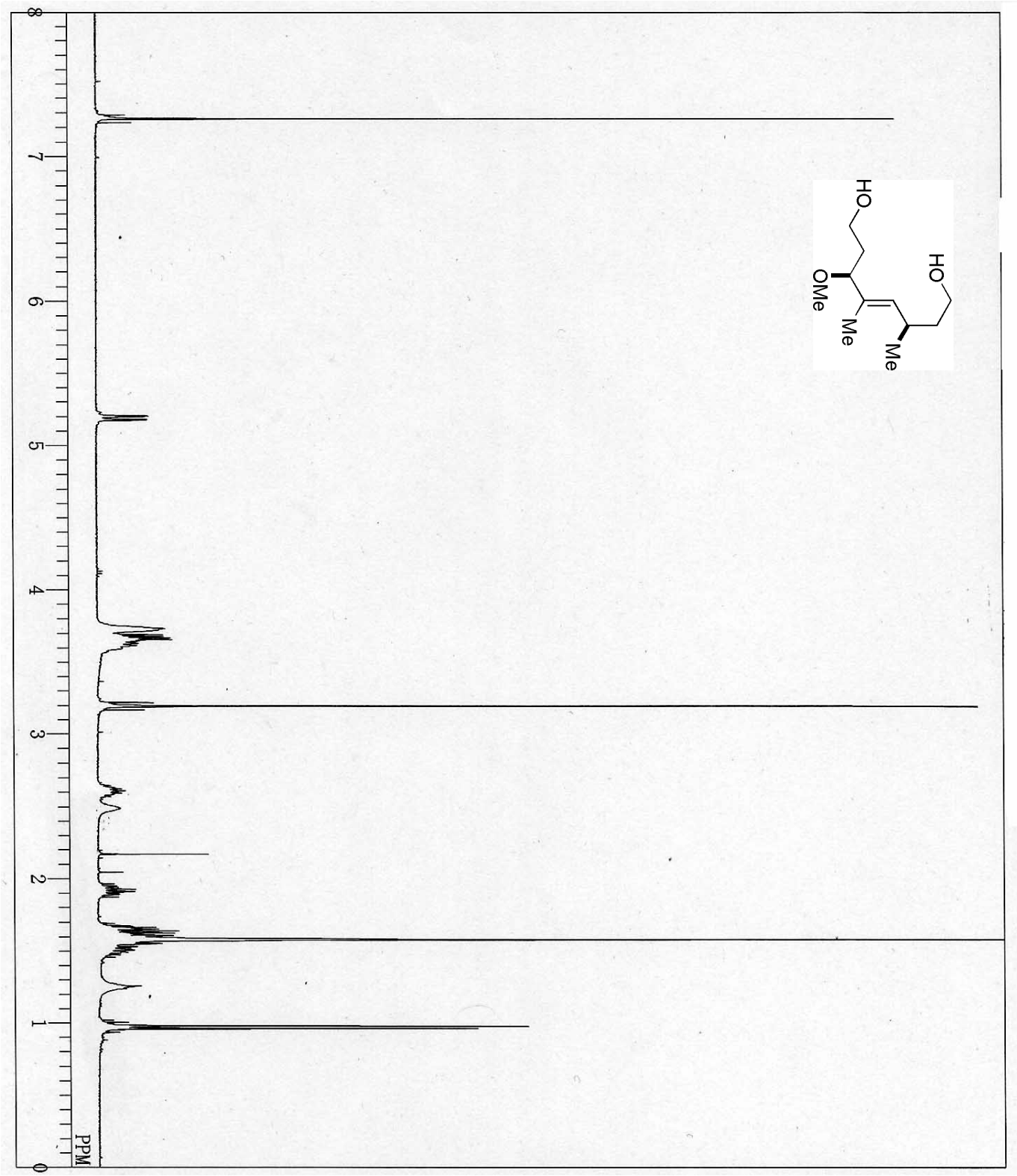
^{1}H NMR spectrum of **18a**. [270 MHz, $CDCl_3$]



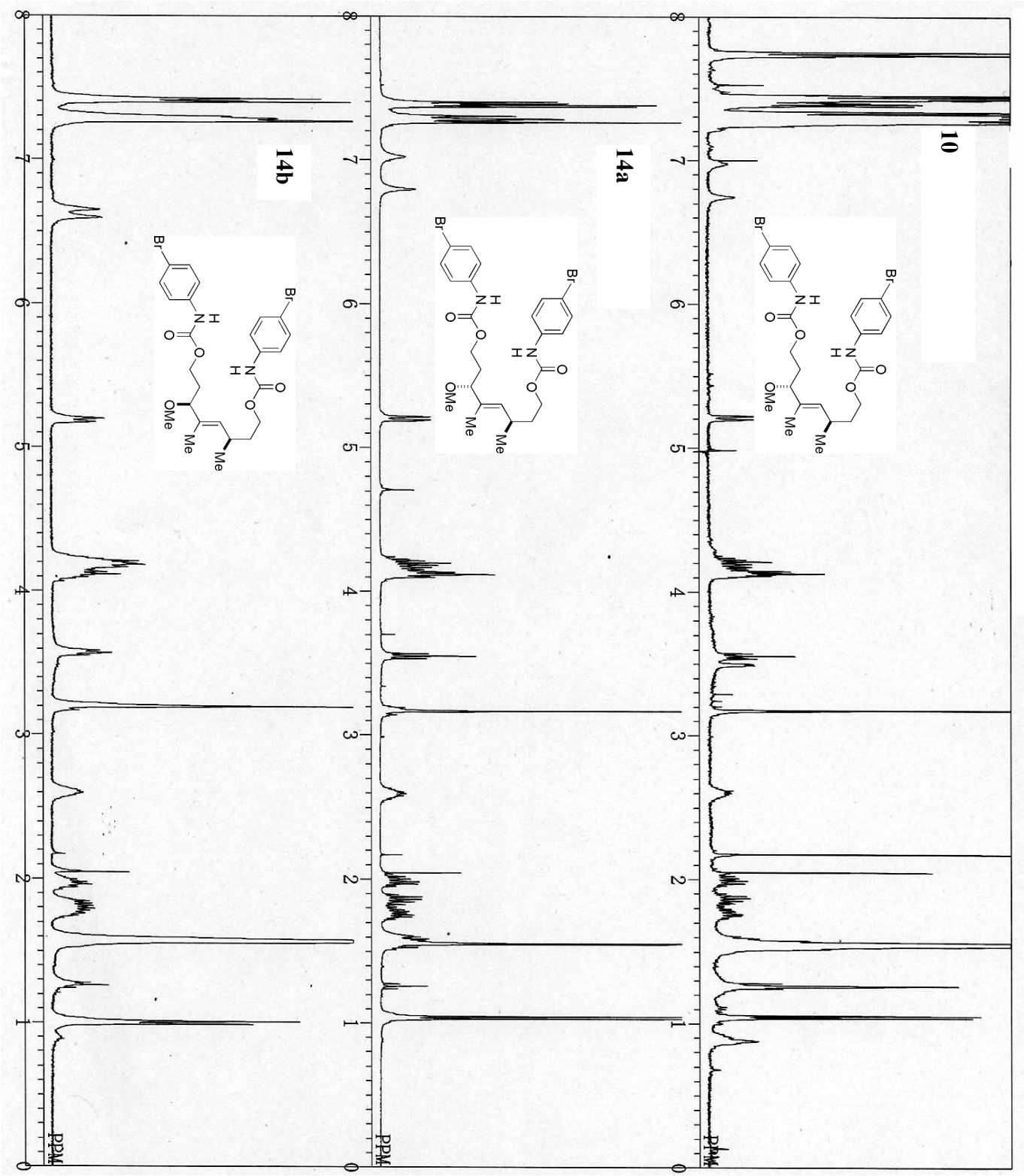
^{1}H NMR spectrum of **18b**. [400 MHz, $CDCl_3$]



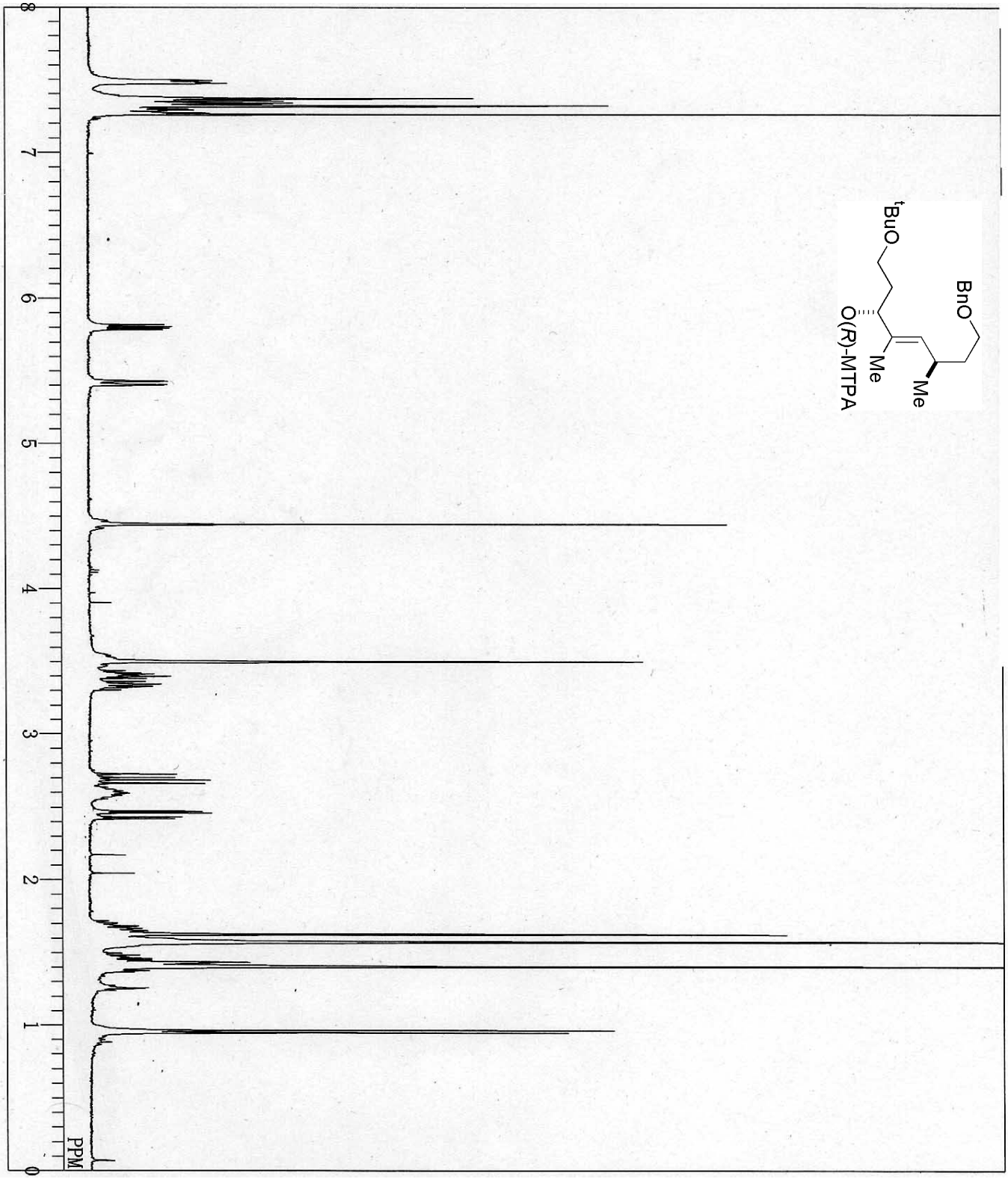
^{1}H NMR spectrum of **19a**. [270 MHz, $CDCl_3$]



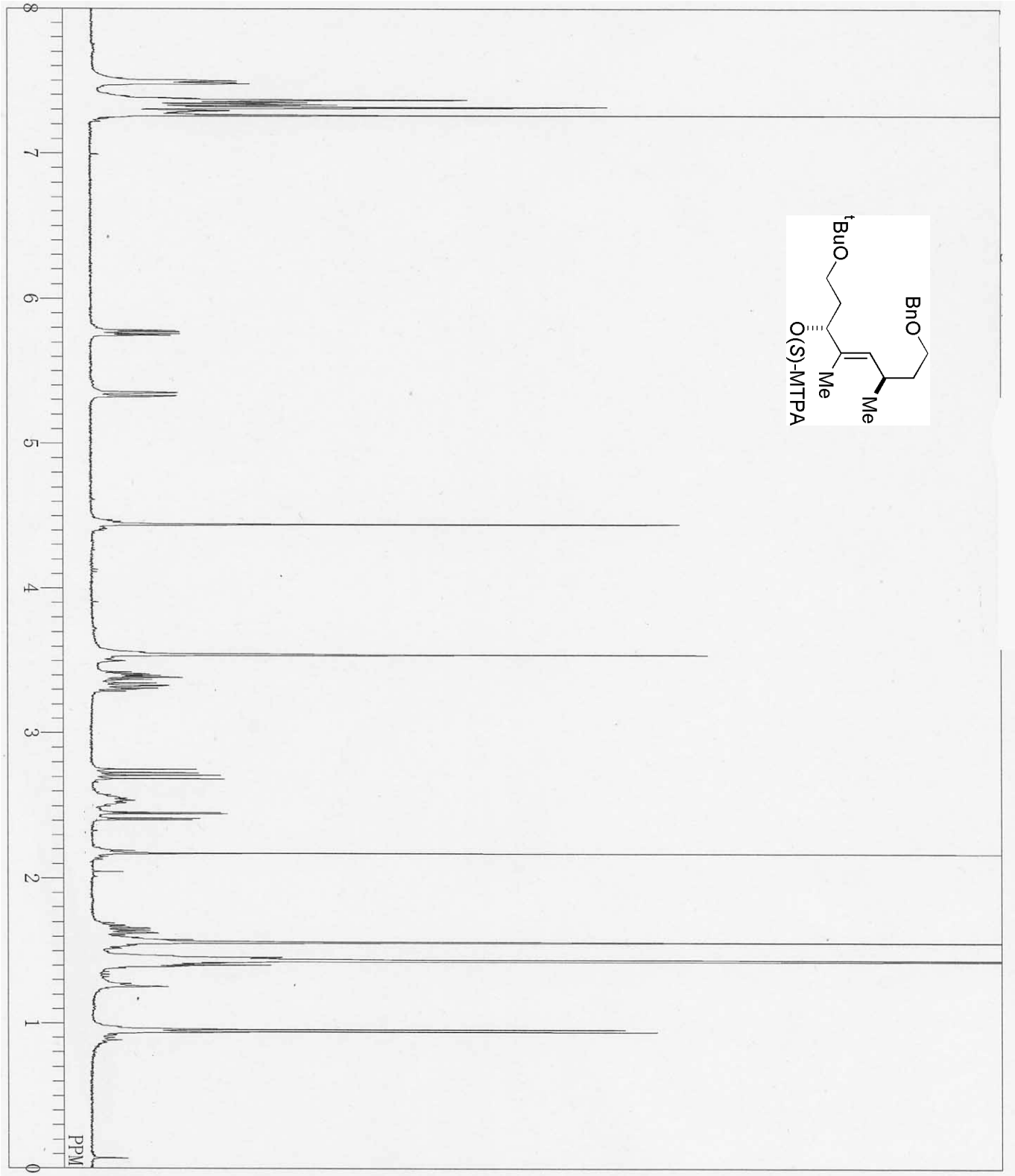
^{1}H NMR spectrum of **19b**. [400 MHz, $CDCl_3$]



^{1}H NMR spectra of **10**, **14a**, and **14b**. [400 MHz, $CDCl_3$]



^{1}H NMR spectrum of **20a**. [400 MHz, $CDCl_3$]



^{1}H NMR spectrum of **20b**. [400 MHz, $CDCl_3$]

GI_{50} values of biselyngbyaside (**1**) against 39 human cancer cell lines

Type of cancer	Cell line	GI_{50} [a,b](mM)
Breast	HBC-4	1.9
	BSY-1	0.31
	HBC-5	0.29
	MCF-7	0.16
	MDA-MB-23	0.35
Central nervous system	U251	0.41
	SF-268	5.6
	SF-295	0.58
	SF-539	0.56
	SNB-75	0.16
	SNB-78	0.038
Colon	HCC2998	0.72
	KM-12	0.66
	HT-29	0.37
	HCT-15	5.3
	HCT-116	0.39
Lung	NCI-H23	0.87
	NCI-H226	0.40
	NCI-H522	0.067
	NCI-H460	0.71
	A549	0.50
	DMS273	0.81
	DMS273	0.24
Melanoma	LOX-IMVI	0.22
Ovary	OVCAR-3	0.35
	OVCAR-4	0.67
	OVCAR-5	0.50
	OVCAR-8	4.9
	SK-OV-3	2.4
Kidney	RXF-631L	6.1
	ACHN	1.7
Stomach	St-4	3.0
	MKN1	5.9
	MKN7	0.63
	MKN28	0.90
	MKN45	0.29
	MKN74	0.17
Prostate	DU-145	2.6
	PC-3	0.21
MG-MID[c]		0.60
Delta[d]		1.22
Range[e]		2.21

[a] Concentrations for the inhibition of cell growth at 50% relative to control.
[b] Cell growth was determined according to the sulforhodamine B assay.
[c] Mean GI_{50} value in all of the cell lines tested.
[d] Difference in the GI_{50} value between the most-sensitive cells and the MG-MID value.
[e] Difference in the log GI_{50} value between the most- and least-sensitive cells.