

# Synthesis of *N*-, *S*-, and *C*-Glycoside castanospermine analogues with selective neutral $\alpha$ -glucosidase inhibitory activity as antitumor agents<sup>†</sup>

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## Supporting Information

**General Methods.** (5*S*,6*S*,7*S*,8*R*,8*aR*)-5,6,7,8-Tetrahydroxy-2-oxa-3-oxoindolizidine (**4**) and its corresponding per-*O*-acetyl derivative (**8**) were prepared according to literature procedures.<sup>1</sup> Reagents and solvents were purchased from commercial sources and used without further purification. Optical rotations were measured at 22 °C in 1-dm tubes on a Perkin-Elmer 141 MC polarimeter. <sup>1</sup>H (and <sup>13</sup>C) NMR spectra were recorded at 500 (125.7) MHz with Bruker 500 DRX instrument. 2D COSY and HMQC experiments were carried out to assist in signal assignment. TLC was performed with precoated TLC plates, silica gel 30F-245, with visualization by UV light and by charring with 10% H<sub>2</sub>SO<sub>4</sub> or 0.2% w/v cerium (IV) sulphate-5% ammonium molybdate in 2 M H<sub>2</sub>SO<sub>4</sub> or 0.1% ninhydrine in EtOH. Column chromatography was carried out with Silica Gel 60 (230-400 mesh). Semi-preparative HPLC was carried out on an Agilent Series 1100 instrument. A Spherisorb column (5  $\mu$ m Silica, 10 x 250 mm), flow rate of 2.0 mL/min and 1100 Series refractive index detector -model number G1362A (Agilent Technologies)- were used. For ESI mass spectra, 0.1 pM sample concentrations were used, the mobile phase consisting of 50% aq acetonitrile at 0.1 mL min<sup>-1</sup> and were obtained with a Bruker Esquire 6000 instrument. Elemental analyses were performed at the Instituto de Investigaciones Químicas (Sevilla, Spain).

<sup>†</sup> V. M. Díaz Pérez, M. I. García Moreno, C. Ortiz Mellet, J. Fuentes, J. C. Díaz Arribas, J. Cañada, J. M. García Fernández, *J. Org. Chem.*, **2000**, 65, 136-143.

**General Procedures for Inhibition Assay.** The glycosidases  $\alpha$ -glucosidase (from yeast),  $\beta$ -glucosidase (from almonds),  $\beta$ -glucosidase (from bovine liver, cytosolic),  $\alpha$ -galactosidase (from green coffee beans), trehalase (from pig kidney), amyloglucosidase (from *Aspergillus niger*),  $\alpha$ -mannosidase (from jack bean),  $\beta$ -mannosidase (from *Helix pomatia*) and  $\beta$ -galactosidase (from *E. coli*) used in the inhibition studies, as well as the corresponding *o*- and *p*-nitrophenyl glycoside substrates, were purchased from Sigma Chemical Co. Inhibitory potencies were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *o*- (for  $\beta$ -glucosidase/ $\beta$ -galactosidase from bovine liver and  $\beta$ -galactosidase from *E. coli*) or *p*-nitrophenyl  $\alpha$ - or  $\beta$ -D-glycopyranoside or  $\alpha,\alpha'$ -trehalose (for trehalase), in the presence of the corresponding gem-diamine derivative. Each assay was performed in phosphate or phosphate-citrate (for  $\alpha$ - or  $\beta$ -mannosidase or amyloglucosidase) buffer at the optimal pH for each enzyme. The  $K_m$  values for the different glycosidases used in the tests and the corresponding working pHs are listed herein:  $\alpha$ -glucosidase (yeast),  $K_m$  = 0.35 mM (pH 6.8);  $\beta$ -glucosidase (almonds),  $K_m$  = 3.5 mM (pH 7.3);  $\beta$ -glucosidase (bovine liver),  $K_m$  = 2.0 mM (pH 7.3);  $\beta$ -galactosidase (*E. coli*),  $K_m$  = 0.12 mM (pH 7.3);  $\alpha$ -galactosidase (coffee beans),  $K_m$  = 2.0 mM (pH 6.8); trehalase (pig kidney),  $K_m$  = 4.0 mM (pH 6.2); amyloglucosidase (*Aspergillus niger*),  $K_m$  = 3.0 mM (pH 5.5);  $\beta$ -mannosidase (*Helix pomatia*),  $K_m$  = 0.6 mM (pH 5.5);  $\alpha$ -mannosidase (jack bean),  $K_m$  = 2.0 mM (pH 5.5). The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. After the mixture was incubated for 10-30 min at 37 °C or 55 °C the reaction was quenched by addition of 1 M Na<sub>2</sub>CO<sub>3</sub> or a solution of Glc-Trinder (Sigma, for trehalase). The absorbance of the resulting mixture was determined at 405 nm or 505 nm. The  $K_i$  value and enzyme inhibition mode were determined from the slope of Lineweaver-Burk plots and double reciprocal analysis using a Microsoft Office Excel 2003 program.

**Cell culture.** MCF-7 cells were grown in Eagle's minimum essential medium (EMEM; Invitrogen) containing 5% foetal calf serum (Cambrex), 2 mM L-glutamine (Invitrogen), 0.06% HEPES (Invitrogen) and penicillin (50 IU/ml)/ streptomycin (50  $\mu$ g/ml; Invitrogen) at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> in air. The growth media was renewed every 2 days.

**Cell count and viability.** Compounds **5 $\alpha$** , **6 $\alpha$**  and **7 $\alpha$**  were diluted out in DMSO. Final concentrations were obtained by appropriate dilution in an external control solution. The final DMSO concentration was < 0.1%. MCF-7 cells were grown in 60-mm Petri dishes at a density of  $1 \times 10^5$  cells/dish and allowed to attach overnight and subsequently incubated for 72 h in the same medium supplemented with different concentrations of **6**, **7** and **8**. MCF-7 growth was assessed using the standard Malassez cell method. Briefly, cells were removed by trypsinisation and diluted in Trypan blue. Cell counts were performed 6 times (in a blind manner) and the results were expressed as the percentage of viable cells measured compared to those measured under control conditions.

**Statistical analysis.** Data are presented as means  $\pm$  SD (n = number of individual experiments). Plots were produced using Origin 7.0 (Microcal Software, Inc). The ANOVA one way with Holm-sidak post hoc test was performed using Sigma Stat software to compare treatment means with control means and to test significant differences. Differences between values were considered significant (\*) when  $P < 0.05$ ; very significant (\*\*) when  $P < 0.01$  and highly significant (\*\*\*) when  $P < 0.001$ .

**Preparation of (5*S* and 5*R*,6*S*,7*S*,8*R*,8*aR*)-5-octylamino-6,7,8-trihydroxy-2-oxa-3-oxoindolizidine (5):** A solution of the 5-hydroxy-2-oxa-3-oxoindolizidine derivative **4** (152 mg, 0.74 mmol) and *n*-octylamine (124  $\mu$ L, 0.74 mmol) in MeOH (1 mL) was stirred at 65 °C for 2 h (TLC monitoring). The solvent was eliminated under reduced pressure to afford the corresponding glycosylamine **6** (68% yield) as a mixture of the corresponding  $\alpha$  and  $\beta$  anomers;  $\alpha$ : $\beta$  ratio 6:1 (H-5 integration). The  $\alpha$ -anomer (**6 $\alpha$** ) was separated in pure form by subsequent column chromatography (20:1  $\rightarrow$  10:1 CH<sub>2</sub>Cl<sub>2</sub>-MeOH).

**(5*S*,6*S*,7*S*,8*R*,8*aR*)-5-Octylamino-6,7,8-trihydroxy-2-oxa-3-oxoindolizidine (5 $\alpha$ ):** Yield: 142 mg (60%).  $R_f$  0.45 (9:1 CH<sub>2</sub>Cl<sub>2</sub>-MeOH).  $[\alpha]_D^{+69.2}$  ( $c$  1.0 in MeOH). <sup>1</sup>H NMR (500 MHz, MeOD)  $\delta$  4.64 (d, 1 H,  $J_{5,6} = 5.0$  Hz, H-5), 4.50 (t, 1 H,  $J_{1a,8a} = J_{1a,1b} = 8.5$  Hz, H-1a), 4.29 (dd, 1 H,  $J_{1b,8a} = 5.0$  Hz, H-1b), 3.85 (ddd, 1 H,  $J_{8a,8} = 9.5$  Hz, H-8a), 3.63 (t, 1 H,  $J_{6,7} = J_{7,8} = 9.5$  Hz, H-7), 3.49 (dd, 1 H, H-6), 3.28 (t, 1 H, H-8), 2.60 (m, 2 H, CH<sub>2</sub>NH), 1.53 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>NH), 1.35 (m, 10 H, CH<sub>2</sub>), 0.92 (t, 3 H,  $^3J_{H,H} = 7.0$  Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (125.7 MHz, MeOD)  $\delta$  160.5 (CO), 76.9 (C-8), 75.8 (C-7), 73.6 (C-6), 70.8 (C-5), 69.2 (C-1), 55.9 (C-8a), 48.8 (CH<sub>2</sub>NH), 34.3-25.0 (CH<sub>2</sub>),

15.7 (CH<sub>3</sub>). ESIMS:  $m/z$  339.2 [M + Na]<sup>+</sup>. Anal. Calcd for C<sub>15</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>: C, 56.94; H, 8.92; N, 8.85. Found: C, 56.76; H, 8.83; N, 8.71.

**(5*R*,6*R*,7*S*,8*R*,8*aR*)-5-Fluoro-6,7,8-tri-*O*-acetyl-2-oxa-3-oxoindolizidine (10):**

Compound **9** (560 mg, 1.50 mmol) was placed in a polyethylene vessel cooled at -40 °C and treated with poly(hydrogen fluoride)pyridinium complex (70% HF; 2.8 m). The reaction mixture was stirred at this temperature for 80 min (TLC monitoring), diluted with Et<sub>2</sub>O (30 mL), washed with saturated aq KF (15 mL) and extracted with Et<sub>2</sub>O (3 x 30 mL). The organic layer was washed with saturated aq NaHCO<sub>3</sub> (10 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. The resulting residue was purified by column chromatography (1:2 EtOAc-petroleum ether) to afford **10** (300 mg, 60% yield). Unreacted **9** (150 mg, 27%) was also recovered. Compound **10** had  $R_f$  0.77 (2:1 EtOAc-petroleum ether). [ $\alpha$ ]<sub>D</sub> +21.6 ( $c$  0.9 in CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  6.17 (dd, 1 H,  $J_{5,F}$  = 52.5 Hz,  $J_{5,6}$  = 3.5 Hz, H-5), 5.60 (t, 1 H,  $J_{6,7}$  =  $J_{7,8}$  = 10.0 Hz, H-7), 5.00 (ddd, 1 H,  $J_{6,F}$  = 14.0 Hz, H-6), 4.99 (t, 1 H,  $J_{8,8a}$  = 10.0 Hz, H-8), 4.51 (dd, 1 H,  $J_{1a,1b}$  = 9.0 Hz,  $J_{1a,8a}$  = 8.0 Hz, H-1a), 4.32 (t, 1 H,  $J_{1b,8a}$  = 9.0 Hz, H-1b), 4.17-4.09 (m, 1 H, H-8a), 2.15-2.09 (3 s, 9 H, MeCO). <sup>13</sup>C NMR (125.7 MHz, CDCl<sub>3</sub>)  $\delta$  170.0-169.5 (MeCO), 154.2 (CO), 87.5 (C-5, d,  $J_{C5,F}$  = 211.6 Hz), 72.0 (C-8), 69.8 (C-6, d,  $J_{C6,F}$  = 24.8 Hz), 68.6 (C-7), 67.2 (C-1), 52.0 (C-8a), 20.4 (MeCO). ESIMS:  $m/z$  356.1 [M + Na]<sup>+</sup>. Anal. Calcd for C<sub>13</sub>H<sub>16</sub>NO<sub>8</sub>F: C 46.85, H 4.84, N 4.20. Found: C 46.77, H 4.71, N 4.02.

**Preparation of (5*R* and 5*S*,6*R*,7*S*,8*R*,8*aR*)-5-Octylthio-6,7,8-trihydroxy-2-oxa-3-oxoindolizidine (9):** To a stirred solution of **9** (59 mg, 0.16 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (3 mL) at 0 °C, BF<sub>3</sub>·Et<sub>2</sub>O (70  $\mu$ L, 0.57 mmol and 1-octanethiol (58  $\mu$ L, 0.33 mmol) were dropwise added under N<sub>2</sub> atmosphere. The mixture was stirred for 15 min (TLC monitoring), diluted with CH<sub>2</sub>Cl<sub>2</sub> (25 mL) and washed with water (5 mL), aq NaHCO<sub>3</sub> (5 mL) and water (5 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to afford the corresponding octyl thioglycoside **9** ( $\alpha$ : $\beta$  ratio 20:1; H-5 integration). The pure anomers **9 $\alpha$**  and **9 $\beta$**  were obtained in pure form after subsequent column chromatography (2:3 EtOAc-petroleum ether).

**(5*R*,6*R*,7*S*,8*R*,8*aR*)-5-Octylthio-6,7,8-tri-*O*-acetyl-2-oxa-3-oxoindolizidine**

**(9 $\alpha$ ):** Yield: 24 mg (73%).  $R_f$  0.75 (1:1 EtOAc-petroleum ether). [ $\alpha$ ]<sub>D</sub> +70.8 ( $c$  0.7 in CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  5.69 (d, 1 H,  $J_{5,6}$  = 6.0 Hz, H-5), 5.44 (t, 1 H,  $J_{6,7}$  =  $J_{7,8}$  = 10.0 Hz, H-7), 4.97 (dd, 1 H, H-6), 4.94 (t, 1 H,  $J_{8a,8}$  = 9.5 Hz, H-8), 4.47

(dd, 1 H,  $J_{1a,1b} = 9.5$  Hz,  $J_{1a,8a} = 8.5$  Hz, H-1a), 4.30 (dd, 1 H,  $J_{1b,8a} = 6.5$  Hz, H-1b), 4.18 (ddd, 1 H, H-8a), 2.63 (ddd, 1 H,  $^2J_{H,H} = 12.5$  Hz,  $^3J_{H,H} = 8.5$  Hz,  $^3J_{H,H} = 7.0$  Hz, SCH<sub>2</sub>), 2.49 (ddd, 1 H, SCH<sub>2</sub>), 2.11-2.05 (3 s, 9 H, MeCO), 1.68-1.50 (m, 2 H, SCH<sub>2</sub>CH<sub>2</sub>), 1.42-1.24 (m, 10 H, CH<sub>2</sub>), 0.89 (t, 3 H,  $^3J_{H,H} = 7.0$  Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (125.7 MHz, CDCl<sub>3</sub>) δ 170.0-169.5 (MeCO), 155.3 (CO), 72.6 (C-8), 70.2 (C-6), 69.9 (C-7), 66.2 (C-1), 57.7 (C-5), 51.2 (C-8a), 31.8-22.6 (CH<sub>2</sub>), 20.6-20.5 (MeCO), 14.1 (CH<sub>3</sub>). ESIMS:  $m/z$  481.8 [M + Na]<sup>+</sup>. Anal. Calcd for C<sub>21</sub>H<sub>33</sub>NO<sub>8</sub>S: C 54.88, H 7.24, N 3.05, S 6.98. Found: C 54.75, H 7.12, N 2.89, S 6.67.

**(5S,6R,7S,8R,8aR)-5-Octylthio-6,7,8-tri-*O*-acetyl-2-oxa-3-oxoindolizidine**

**(9β):** Yield: 4 mg (12%). R<sub>f</sub> 0.53 (1:1 EtOAc-petroleum ether). [α]<sub>D</sub> -9.0 (*c* 0.3 in CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 5.29 (dd, 1 H,  $J_{8a,8} = 10.5$  Hz,  $J_{7,8} = 7.0$  Hz, H-8), 5.23 (t, 1 H,  $J_{5,6} = J_{6,7} = 4.0$  Hz, H-6), 5.12 (dd, 1 H, H-7), 4.72 (d, 1 H, H-5), 4.42 (t, 1 H,  $J_{1a,1b} = J_{1a,8a} = 8.0$  Hz, H-1a), 4.17 (t, 1 H,  $J_{1b,8a} = 9.0$  Hz, H-1b), 4.00 (ddd, 1 H, H-8a), 2.96-2.84 (m, 2 H, SCH<sub>2</sub>), 2.15-2.10 (3 s, 9 H, MeCO), 1.70-1.20 (m, 12 H, CH<sub>2</sub>), 0.90 (t, 3 H,  $^3J_{H,H} = 7.0$  Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (125.7 MHz, CDCl<sub>3</sub>) δ 169.9-168.7 (MeCO), 156.1 (CO), 73.6 (C-7), 73.3 (C-6), 72.7 (C-8), 67.2 (C-1), 59.1 (C-5), 53.9 (C-8a), 34.3-22.6 (CH<sub>2</sub>), 20.9-20.6 (MeCO), 14.1 (CH<sub>3</sub>). ESIMS:  $m/z$  482.2 [M + Na]<sup>+</sup>. HRFABMS Calcd for C<sub>21</sub>H<sub>33</sub>NO<sub>8</sub>SNa [M + Na]<sup>+</sup> 482.1825, found 482.1830.

**(5R,6R,7S,8R,8aR)-5-Octylthio-6,7,8-trihydroxy-2-oxa-3-oxoindolizidine**

**(7α):** Compound **7α** was obtained by conventional de-*O*-acetylation of **10α** (54 mg, 0.12 mmol) with NaOMe in MeOH and purified by column chromatography (EtOAc). Yield: 36 mg (92%). R<sub>f</sub> 0.33 (EtOAc). [α]<sub>D</sub> +104.2 (*c* 0.8 in MeOH). <sup>1</sup>H NMR (500 MHz, MeOD) δ 5.28 (d, 1 H,  $J_{5,6} = 5.5$  Hz, H-5), 4.58 (t, 1 H,  $J_{1a,8a} = J_{1a,1b} = 8.5$  Hz, H-1a), 4.30 (dd, 1 H,  $J_{1b,8a} = 5.5$  Hz, H-1b), 3.95 (td, 1 H,  $J_{8a,8} = 8.5$  Hz, H-8a), 3.68 (dd, 1 H,  $J_{6,7} = 9.5$  Hz, H-6), 3.55 (t, 1 H,  $J_{7,8} = 9.5$  Hz, H-7), 3.37-3.31 (m, 1 H, H-8), 2.60 (ddd, 1 H,  $^2J_{H,H} = 13.0$  Hz,  $^3J_{H,H} = 8.0$  Hz,  $^3J_{H,H} = 6.0$  Hz, SCH<sub>2</sub>), 2.53 (ddd, 1 H, SCH<sub>2</sub>), 1.72-1.56 (m, 2 H, SCH<sub>2</sub>CH<sub>2</sub>), 1.48-1.28 (m, 10 H, CH<sub>2</sub>), 0.92 (t, 3 H,  $^3J_{H,H} = 7.0$  Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (125.7 MHz, MeOD) δ 157.0 (CO), 74.3 (C-8), 73.8 (C-7), 71.1 (C-6), 66.9 (C-1), 61.0 (C-5), 53.1 (C-8a), 31.6-22.3 (CH<sub>2</sub>), 13.0 (CH<sub>3</sub>). ESIMS:  $m/z$  355.8 [M + Na]<sup>+</sup>. Anal. Calcd for C<sub>15</sub>H<sub>27</sub>NO<sub>5</sub>S: C 54.03, H 8.16, N 4.20, S 9.62. Found: C 53.72, H 7.90, N 4.13, S 9.37.

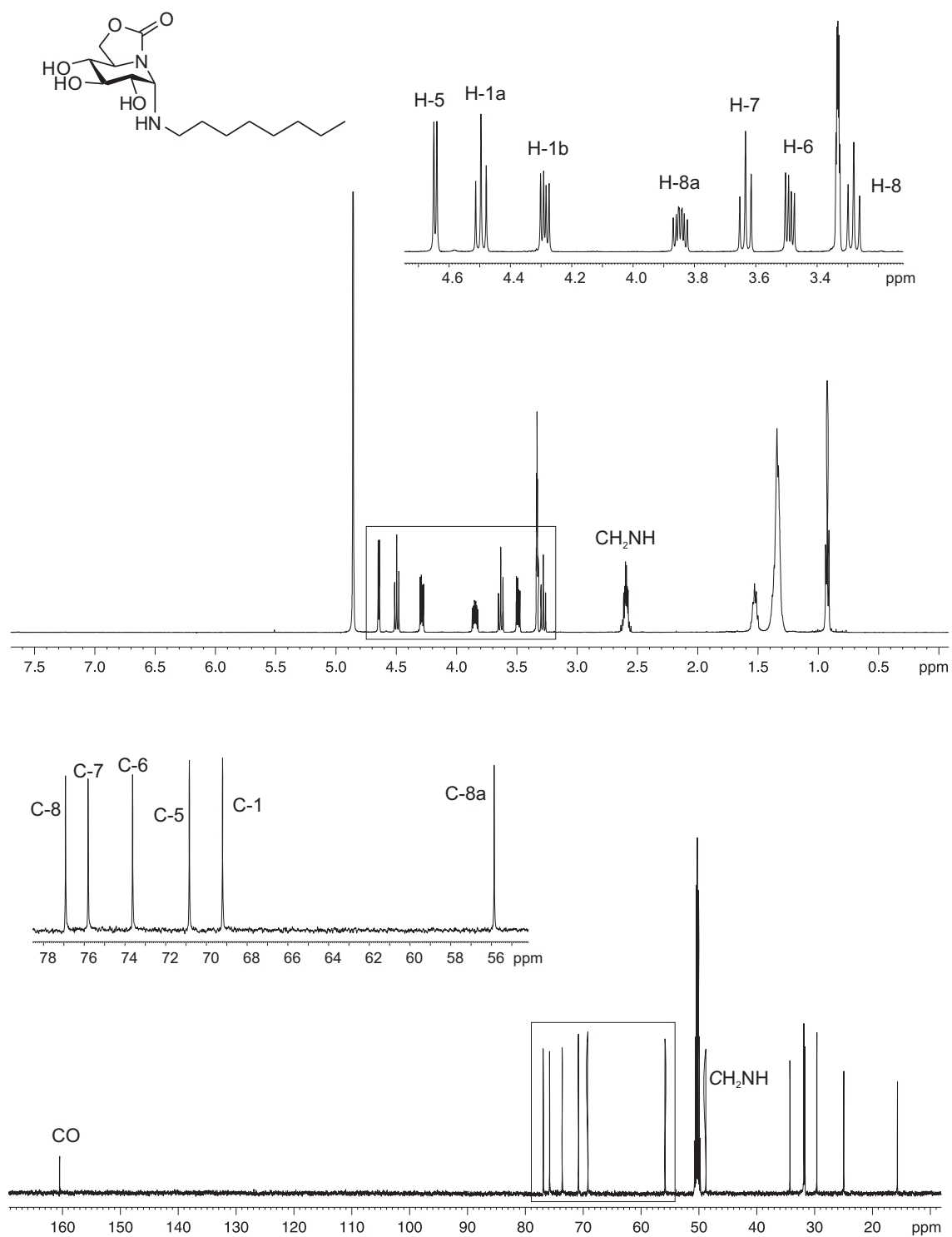
**Preparation of (5*R* and 5*S*,6*R*,7*S*,8*R*,8*aR*)-5-octyl-6,7,8-trihydroxy-2-oxa-3-oxoindolizidine (12):** A solution of the fluoro derivative (**11**, 300 mg, 0.90 mmol, 1.0 equiv.) in dry toluene (10 mL) under nitrogen atmosphere was cooled to 0 °C. Then, trioctylaluminum (3.8 mL, 1.80 mmol, 2.0 equiv.) was added and the reaction mixture was stirred for 3 h (TLC monitoring). A saturated solution of NH<sub>4</sub>Cl (10 mL) was added and the mixture was extracted with EtOAc (3 x 30 mL). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. The resulting residue was purified by column chromatography (1:5 → 1:3.5 EtOAc-petroleum ether) to afford **12** (260 mg, 68%) as a mixture of the corresponding α and β anomers (α:β ratio 4:1; H-7 integration). Compounds **12α** and **12β** were separated by HPLC (mobile phase EtOAc:hexane, 33:67).

**(5*R*,6*S*,7*R*,8*R*,8*aR*)-5-Octyl-6,7,8-tri-*O*-acetyl-2-oxa-3-oxoindolizidine (12α):** Colourless oil. Retention time HPLC: 23.6 min. *R<sub>f</sub>* 0.51 (1:1 EtOAc-petroleum ether). [α]<sub>D</sub> +36.1 (*c* 1.0 in CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 5.34 (t, 1 H, *J*<sub>6,7</sub> = *J*<sub>7,8</sub> = 9.5 Hz, H-7), 5.04 (dd, 1 H, *J*<sub>5,6</sub> = 6.0 Hz, H-6), 4.96 (t, 1 H, *J*<sub>8,8a</sub> = 9.5 Hz, H-8), 4.41 (dd, 1 H, *J*<sub>1a,1b</sub> = 9.0 Hz, *J*<sub>1a,8a</sub> = 8.0 Hz, H-1a), 4.27 (dd, 1 H, *J*<sub>1b,8a</sub> = 4.5 Hz, H-1b), 4.31-4.24 (m, 1 H, H-5), 3.82 (ddd, 1 H, H-8a), 2.08-2.04 (3 s, 9 H, *MeCO*), 1.76-1.53 (m, 2 H, CH<sub>2</sub>), 1.43-1.22 (m, 12 H, CH<sub>2</sub>), 0.90 (t, 3 H, <sup>3</sup>*J*<sub>H,H</sub> = 7.0 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (125.7 MHz, CDCl<sub>3</sub>) δ 170.0-169.1 (*MeCO*), 156.3 (CO), 72.5 (C-8), 69.9 (C-7), 69.7 (C-6), 65.5 (C-1), 52.1 (C-8a), 51.0 (C-5), 31.8-22.6 (CH<sub>2</sub>), 20.7-20.6 (*MeCO*), 14.1 (CH<sub>3</sub>). ESIMS: *m/z* 450.3 [M + Na]<sup>+</sup>. Anal. Calcd for C<sub>21</sub>H<sub>33</sub>NO<sub>8</sub>: C 59.00, H 7.78, N 3.28. Found: C 59.11, H 7.81, N 3.21.

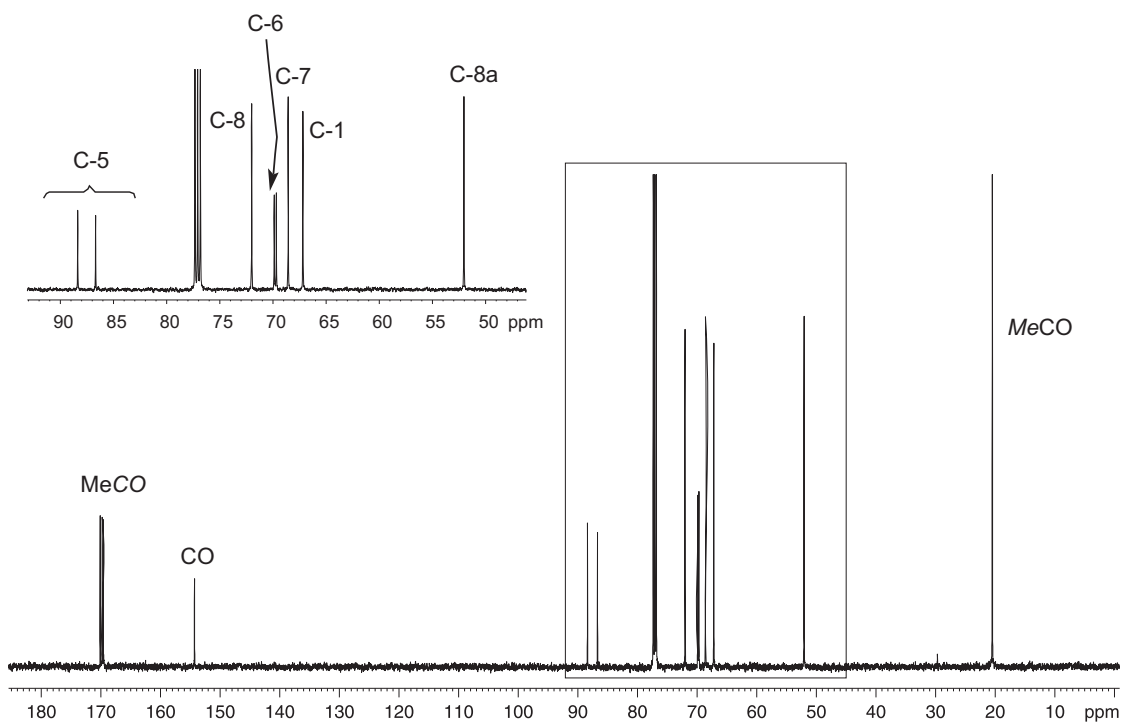
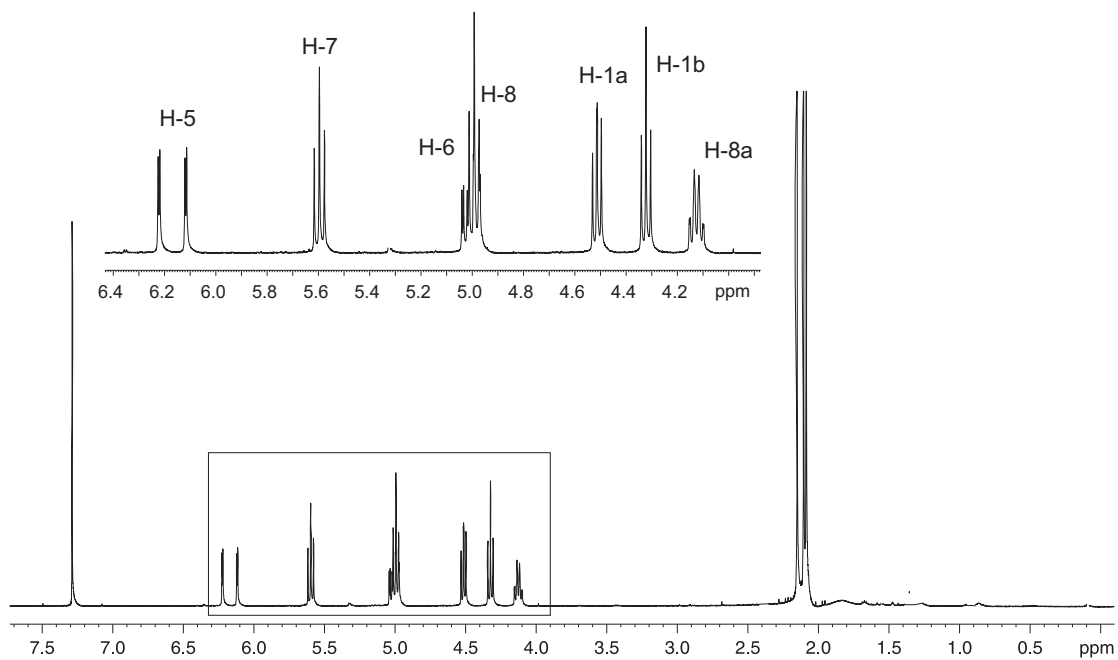
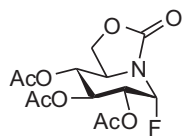
**(5*S*,6*S*,7*R*,8*R*,8*aR*)-5-Octyl-6,7,8-tri-*O*-acetyl-2-oxa-3-oxoindolizidine (12β):** White foam. Retention time HPLC: 26.2 min. *R<sub>f</sub>* 0.50 (1:1 EtOAc-petroleum ether). [α]<sub>D</sub> -14.7 (*c* 1.0 in CHCl<sub>3</sub>). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 5.16 (t, 1 H, *J*<sub>6,7</sub> = *J*<sub>7,8</sub> = 9.5 Hz, H-7), 5.08 (t, 1 H, *J*<sub>8,8a</sub> = 9.5 Hz, H-8), 5.04 (t, 1 H, *J*<sub>5,6</sub> = 9.5 Hz, H-6), 4.33 (dd, 1 H, *J*<sub>1a,1b</sub> = 9.0 Hz, *J*<sub>1a,8a</sub> = 7.0 Hz, H-1a), 4.12 (dd, 1 H, *J*<sub>1b,8a</sub> = 4.0 Hz, H-1b), 3.70 (ddd, 1 H, H-8a), 3.31 (td, 1 H, <sup>3</sup>*J*<sub>H,H</sub> = 4.5 Hz, H-5), 2.40-2.30 (m, 1 H, CH<sub>2</sub>), 2.08-2.04 (3 s, 9 H, *MeCO*), 1.80-1.50 (m, 2 H, CH<sub>2</sub>), 1.40-1.20 (m, 11 H, CH<sub>2</sub>), 0.90 (t, 3 H, <sup>3</sup>*J*<sub>H,H</sub> = 7.0 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (125.7 MHz, CDCl<sub>3</sub>) δ 170.0-169.2 (*MeCO*), 155.0 (CO), 74.1 (C-7), 71.1-70.9 (C-6, C-8), 69.4 (C-1), 57.7 (C-8a), 57.4 (C-5), 31.8-22.6 (CH<sub>2</sub>), 20.6 (*MeCO*), 14.1 (CH<sub>3</sub>). ESIMS: *m/z* 450.2 [M + Na]<sup>+</sup>. Anal. Calcd for C<sub>21</sub>H<sub>33</sub>NO<sub>8</sub>: C 59.00, H 7.78, N 3.28. Found: C 59.08, H 7.69, N 3.20.

**(5*R*,6*S*,7*R*,8*R*,8*aR*)-5-Octyl-6,7,8-trihydroxy-2-oxa-3-oxoindolizidine (8α):**

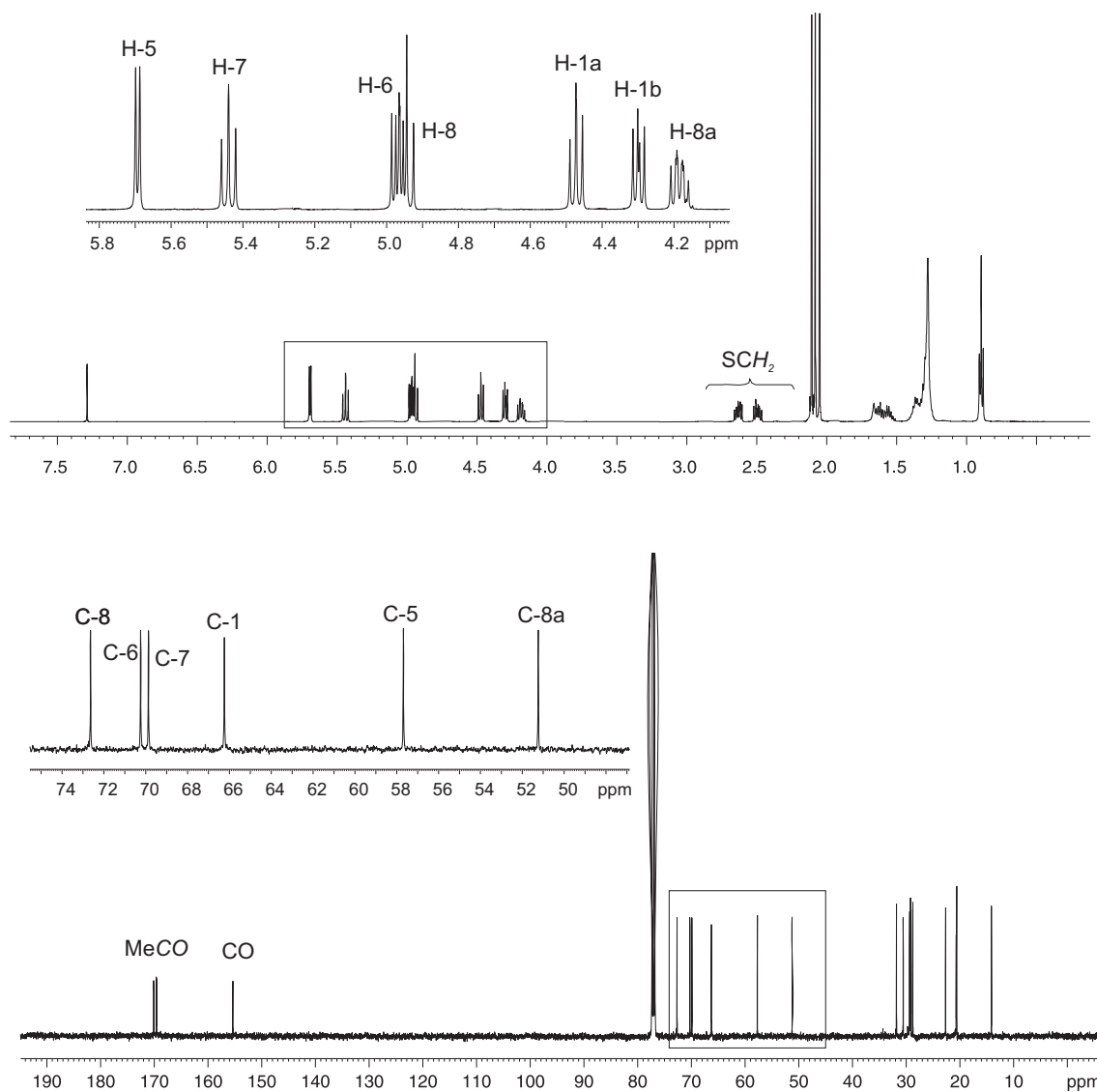
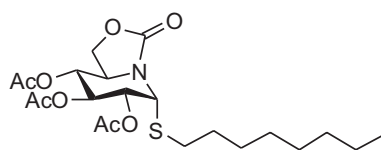
Compound **8α** was obtained by conventional de-*O*-acetylation of **12α** (91 mg, 0.21 mmol) with NaOMe in MeOH. Yield: 61 mg (95%).  $R_f$  0.75 (9:1 EtOAc-MeOH).  $[\alpha]_D^{+42.3}$  ( $c$  1.0 in MeOH).  $^1\text{H}$  NMR (500 MHz, MeOD)  $\delta$  4.47 (dd, 1 H,  $J_{1a,1b} = 9.0$  Hz,  $J_{1a,8a} = 8.5$  Hz, H-1a), 4.31 (dd, 1 H,  $J_{1b,8a} = 4.0$  Hz, H-1b), 3.92 (ddd, 1 H,  $^3J_{H,H} = 8.5$  Hz,  $^3J_{H,H} = 3.0$  Hz,  $J_{5,6} = 5.5$  Hz, H-5), 3.64 (ddd, 1 H,  $J_{8,8a} = 9.5$  Hz, H-8a), 3.52-3.44 (m, 2 H, H-6, H-7), 3.25 (bt, 1 H,  $J_{7,8} = 9.5$  Hz, H-8), 1.94-1.85 (m, 1 H, CH<sub>2</sub>), 1.51-1.24 (m, 13 H, CH<sub>2</sub>), 0.92 (t, 3 H,  $^3J_{H,H} = 7.0$  Hz, CH<sub>3</sub>).  $^{13}\text{C}$  NMR (125.7 MHz, MeOD)  $\delta$  158.3 (CO), 74.1 (C-8), 73.3 (C-7), 70.9 (C-6), 66.1 (C-1), 54.2 (C-5), 53.9 (C-8a), 31.6-22.3 (CH<sub>2</sub>), 13.1 (CH<sub>3</sub>). ESIMS:  $m/z$  324.2  $[\text{M} + \text{Na}]^+$ . Anal. Calcd for C<sub>15</sub>H<sub>27</sub>NO<sub>5</sub>: C 59.78, H 9.03, N 4.65. Found: C 59.70, H 8.95, N 4.49.



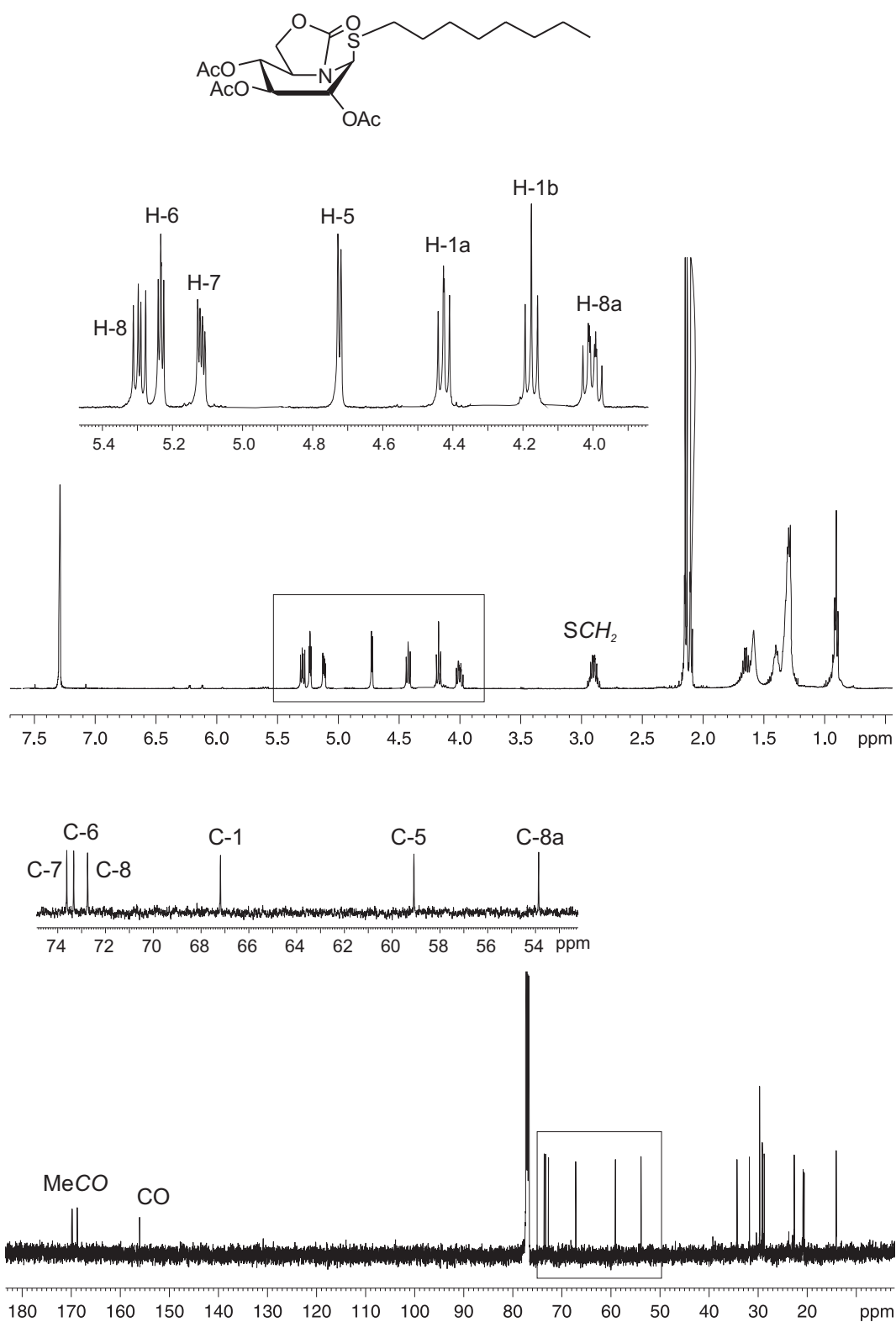
$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (500 MHz, 125.7 MHz, MeOD) of **6a**



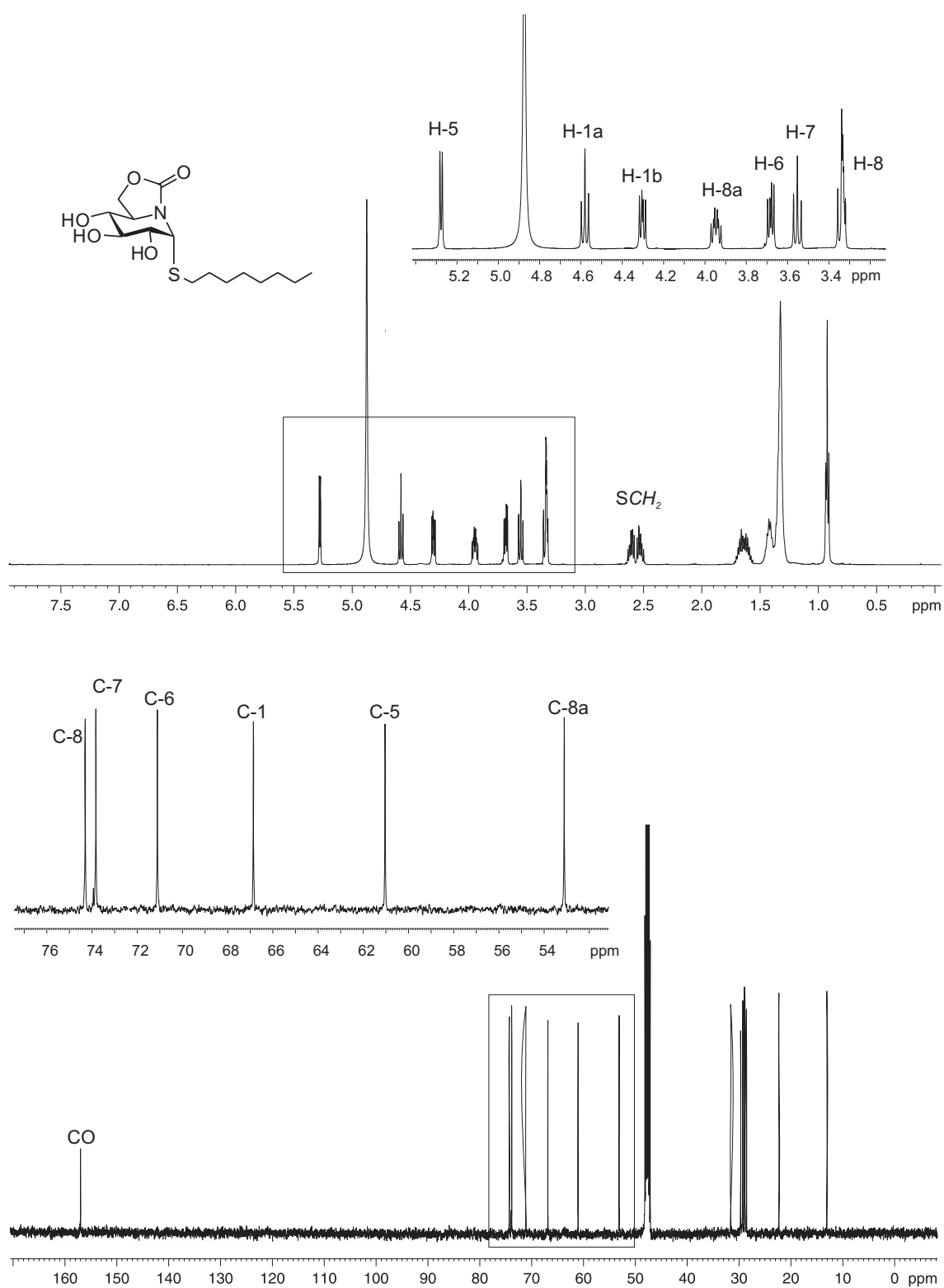
$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (500 MHz and 125.7 MHz,  $\text{CDCl}_3$ ) of **11**



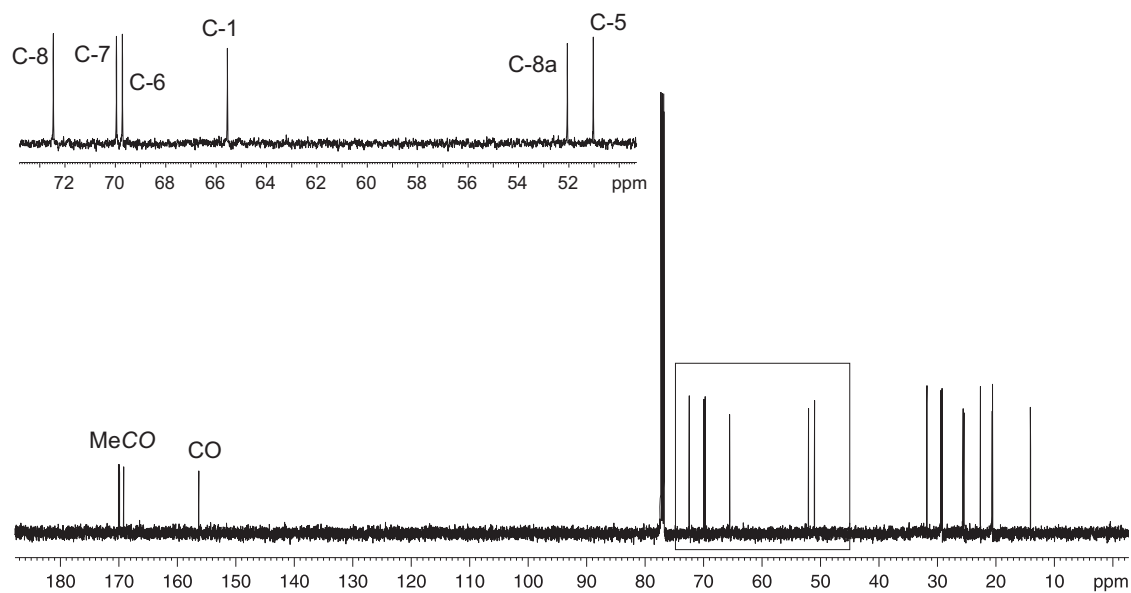
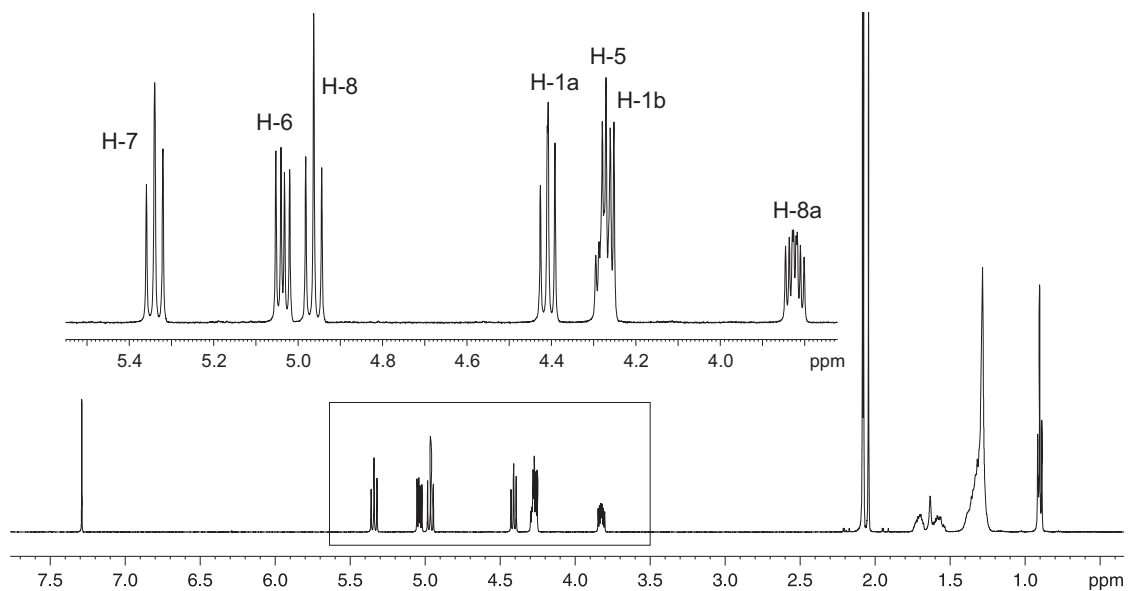
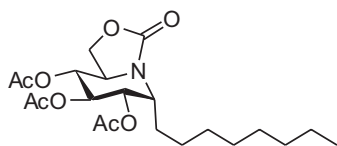
<sup>1</sup>H and <sup>13</sup>C NMR spectra (500 MHz and 125.7 MHz, CDCl<sub>3</sub>) of **10α**



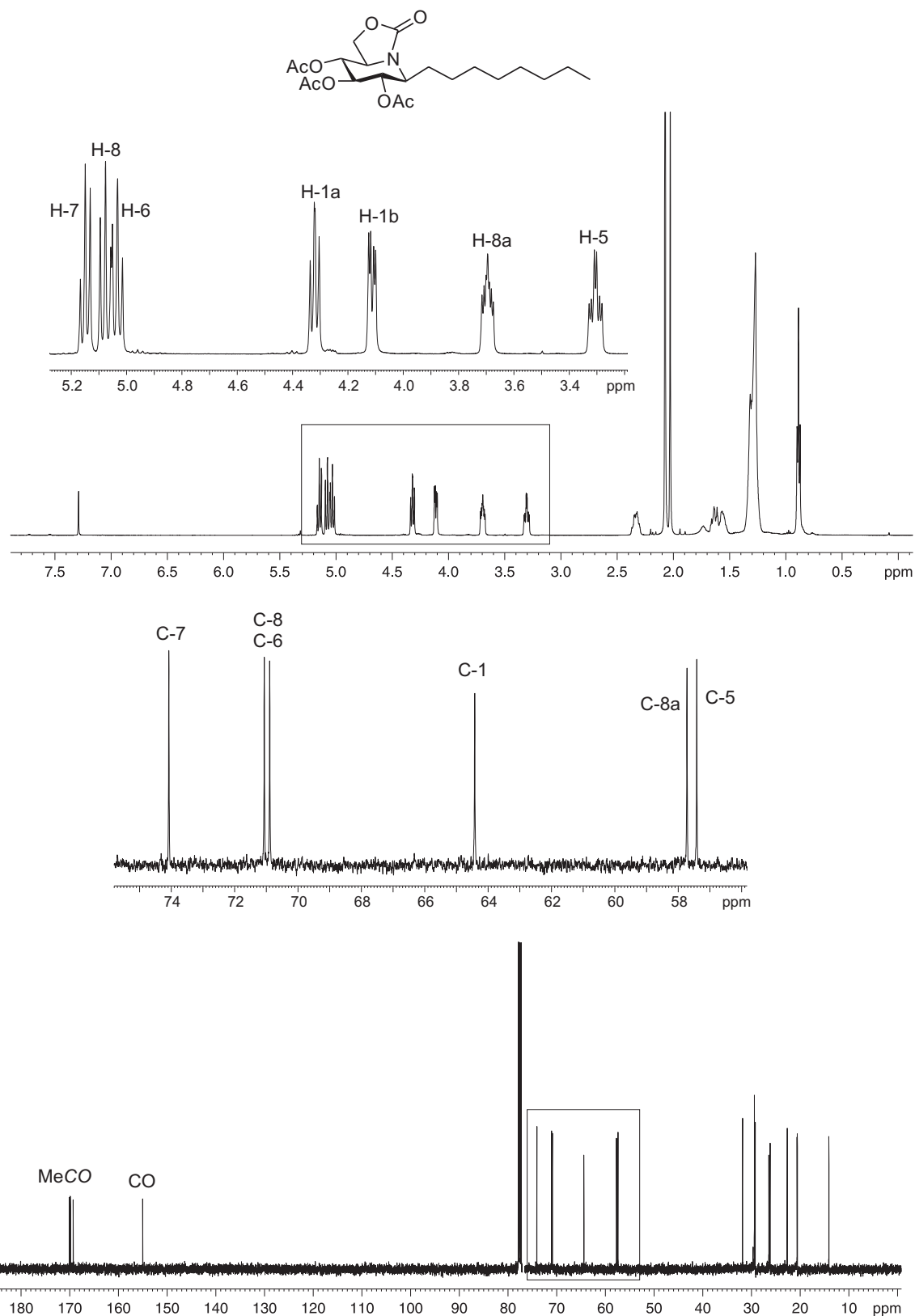
$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (500 MHz and 125.7 MHz,  $\text{CDCl}_3$ ) of **10β**



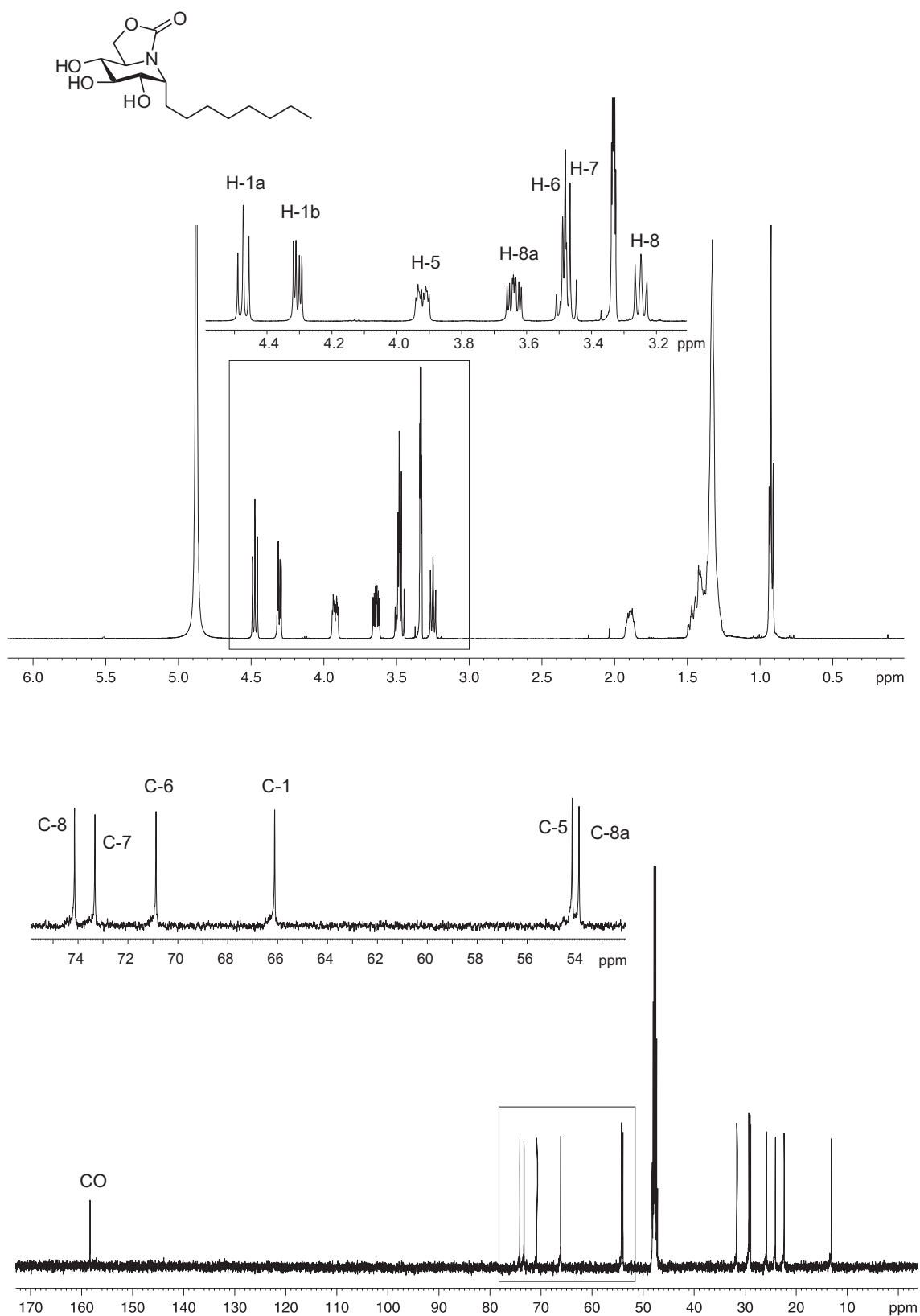
$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (500 MHz and 125.7 MHz, MeOD) of **7α**



$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (500 MHz and 125.7 MHz,  $\text{CDCl}_3$ ) of **12α**



$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (500 MHz and 125.7 MHz,  $\text{CDCl}_3$ ) of **12 $\beta$**



$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (500 MHz and 125.7 MHz, MeOD) of **8α**