Electronic Supplementary Material (ESI) for Chemical Communications

Tuning glycosidase inhibition through aglycone interactions: Pharmacological chaperones for Fabry disease and GM_1 gangliosidosis

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Electronic Supplementary Information

List of contents:

General Methods. Compound **2** (1-deoxygalactonojirimycin, DGJ) was obtained from 3,4-*O*-isopropylidene-1-deoxygalactonojirimycin following a reported route.[1] Reagents and solvents were purchased from commercial sources and used without further purification. Optical rotations were measured with a JASCO P-2000 polarimeter, using a sodium lamp (λ = 589 nm) at 22 ºC in 1 cm or 1 dm tubes. NMR experiments were performed at 300 (75.5) and 500 (125.7) MHz using Bruker DMX300 and DRX500. 1-D TOCSY as well as 2-D COSY and HMQC experiments were carried out to assist in signal assignment. In the FABMS spectra, the primary beam consisted of Xe atoms with maximun energy of 8 keV. The samples were dissolved in *m*-nitrobenzyl alcohol or thioglycerol as the matrices and the positive ions were separated and accelerated over a potencial of 7 keV. NaI was added as cationizing agent. Thin-layer chromatography was performed on E. Merck precoated TLC plates, silica gel 30F-245, with visualization by UV light and by carring with 10% H_2SO_4 or 0.2% w/v cerium (IV) suphate-5% ammonium molybdate in 2 M H_2SO_4 or 0.1% ninhydrin in EtOH. Column chromatography was performed on Chromagel (SDS silice 60 AC.C 70-200 μm). Elemental analyses were performed at the Servicio de Microanálisis del Instituto de Investigaciones Químicas de Sevilla, Spain.

Dulbecco's Modified Eagle's Medium (DMEM) and trypsin-EDTA were obtained from Wako (Tokyo, Japan). Fetal bovine serum (FBS) was from HyClone Lab. (Waltham, MS). 4-Methylumbelliferone (4-MU)-conjugated β-D-galactoside, α-D-galactoside, *N*-acetyl-β-D-glucosaminidase and GM_1 from bovine brain were from Sigma (St. Louis, MO).

General Procedure for the Inhibition Assay against the Commercial Enzymes. Inhibition constant (K_i) values were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *o*- (for β-galactosidase from bovine liver or *E. coli*) or *p*-nitrophenyl α- or β-D-glycopyranoside (for other glycosidases) in the presence of compounds **5**-**10**. Each assay was performed in phosphate buffer or phosphate-citrate buffer (for α- or β-mannosidase and amyloglucosidase) at the optimal pH for the enzymes. The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. The mixture was incubated for 10-30 min at 37 ºC or 55 ºC (for amyloglucosidase) and the reaction was quenched by addition of 1 M Na_2CO_3. Reaction times were appropriate to obtain 10-20% conversion of the

substrate in order to achieve linear rates. The absorbance of the resulting mixture was determined at 405 nm. Approximate values of K_i were determined using a fixed concentration of substrate (around the K_M value for the different glycosidases) and various concentrations of inhibitor. Full K_i determinations and enzyme inhibition mode were determined from the slope of Lineweaver-Burk plots and double reciprocal analysis (see Figures S1-S6 for selected examples).

Cell Culture, Transfection and Chaperone Test. Human skin fibroblasts from control subjects and patients with α-Gal or β-Gal deficiency were maintained in our laboratories.[2] These fibroblasts were cultured in DMEM supplemented with 10% FBS. Chaperone treatment for human fibroblasts was performed culturing cells in the medium with or without chaperones for 96 h.

Lysosomal Enzyme Assay. Lysosomal enzyme activities in cell lysates were measured by using 4-MU substrates as described.[2] Briefly, cells in 35-mm dishes were washed with PBS (4 °C) for 3 times and scraped into 100 μL of 0.1% Triton X-100 in H_2O. After centrifugation (6,000 rpm for 15 min at 4 °C) to remove insoluble materials, 10 μL of lysates with 20 μL of the substrate solution in 0.1 M citrate buffer (pH 4.5) was incubated at 37 °C for 30 min and the reaction was terminated by adding 0.2 M glycin-NaOH buffer (pH 10.7). The liberated 4-MU was measured with a fluorescence plate reader (excitation 340 nm; emission 460 nm; Infinite F500, TECAN Japan, Kawasaki, Japan). Protein concentrations were determined using Protein Assay Rapid Kit (WAKO, Tokyo, Japan) and enzyme activity was normalized by protein concentration. In order to determine α-Gal A activity, discarding interferences from α-Gal B (α-galactosaminidase), the method described previously by Ishii et al.[3] was followed. Briefly, 4-MU-α-D-galactopyranoside was used as a substrate with N-acetyl-D-galactosamine in 0.1 M sodium citrate buffer (pH 4.5) and the reaction was terminated with 0.2 M glycin buffer (pH 10.7). The librated 4-MU was measured with a fluorescence plate reader (Infinite F500, Tecan, Kawasaki, Japan). Protein concentration was determined by Protein Assay Rapid Kit (Wako, Tokyo, Japan).

Inhibition of α-Gal and β-Gal *in vitro*. Lysates in 0.1% Triton X-100 in H_2O of human skin fibroblasts were used for in vitro analysis. For the inhibition assay, lysates were mixed with 4-MU α-Gal or β-Gal substrate in the absence or presence of increasing concentrations of the corresponding sp^2-iminosugar.

General Procedure for the Synthesis of *N*-(*N'*-Alkylthiocarbamoyl)-1-deoxygalactonojirimycin (5-7). To a solution of 1-deoxygalactonojirimycin (**2**, 0.44 mmol) in pyridine (6.4 mL), Et_3N (74 μL, 0,53 mmol, 1.2 eq) and the corresponding isothiocyanate (0.49 mmol, 1.1 eq) were added. The mixture was further stirred for 18 h, the solvent was removed and coevaporated with toluene and the residue was purified by column chromatography with the eluent indicated in each case.

***N*-(*N'*-Phenylthiocarbamoyl)-1-deoxygalactonojirimycin (5).** Column chromatography: CH_2Cl_2-MeOH-H_2O 100:10:1 → 90:10:1. Yield: 100 mg (76%). $[\alpha]_D$ −105.1 (*c* 1.0, MeOH); R_f 0.37 (CH_2Cl_2-MeOH-H_2O 70:10:1); ^{1}H MNR (300 MHz, CD_3OD): δ 7.35-7.08 (m, 5 H, Ph), 5.05 (m, 1 H, H-5), 4.73 (da, 1 H, $J_{1a,1b}$ = 14.5 Hz, H-1a), 4.15 (m, 2 H, H-4, H-6a), 4.03 (dd, 1 H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 3.3 Hz, H-6b), 3.89 (m, 1 H, H-2), 3.79 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 3.3 Hz, H-3), 3.62 (dd, 1 H, $J_{1b,2}$ = 2.3 Hz, H-1b); ^{13}C MNR (75.5 MHz, CD_3OD): δ 186.7 (CS), 142.1, 129.4, 125.6, 125.3 (Ph), 72.9 (C-3), 71.9 (C-2), 67.6 (C-4), 64.1 (C-5), 60.2 (C-6), 46.9 (C-1); FABMS: *m/z* 321 (30, $[M + Na]^+$), 299 (10, $[M + H]^+$). Anal. Calcd for $C_{13}H_{18}N_2O_4S$: C, 52.33; H, 6.08; N, 9.39; S, 10.75. Found: C, 52.14; H, 5.86; N, 9.11; S, 10.49.

***N*-(*N'*-Butylthiocarbamoyl)-1-deoxygalactonojirimycin (6).** Column chromatography: CH_2Cl_2-MeOH-H_2O 70:10:1. Yield: 96 mg (79%). $[\alpha]_D$ −186.5 (*c* 0.7, MeOH). R_f = 0.52 (CH_2Cl_2-MeOH-H_2O 40:10:1); UV (MeOH): 249 nm (ε_{mM} 10.6); ^{1}H NMR (400 MHz, CD_3OD): δ 4.82 (m, 1 H, H-5), 4.57 (d, 1 H, $J_{1a,1b}$ = 14.5 Hz, H-1a), 4.06 (dd, 1 H, $J_{4,5}$ = 6.4 Hz, $J_{3,4}$ = 3.3 Hz, H-4), 4.04 (dd, 1H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 3.4 Hz, H-6a), 3.94 (dd, 1 H, $J_{5,6b}$ = 3.4 Hz, H-6b), 3.83 (m, 1 H, H-2), 3.72 (t, 1 H, $J_{2,3}$ = 3.3 Hz, H-3), 3.57 (m, 3 H, CH_2NH, H-1b), 1.59 (m, 2 H, CH_2), 1.38 (m, 2 H, C$H_2$$CH_3$), 0.95 (t, 3 H, $^3J_{H,H}$ = 7.4 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ 186.3 (CS), 73.1 (C-3), 72.0 (C-2), 67.8 (C-4), 63.6 (C-5), 60.1 (C-6), 46.8 (CH_2N), 46.2 (C-1), 32.2 (CH_2), 21.1 (C$H_2$$CH_3$), 14.2 ($CH_3$); FABMS: *m/z* 301 (100, $[M + Na]^+$), 279 (40, $[M + H]^+$). Anal. Calcd for $C_{11}H_{22}N_2O_4S$: C, 47.46; H, 7.97; N, 10.06; S, 11.52. Found: C, 47.29; H, 7.69; N, 9.78; S, 11.26.

***N*-(*N'*-Octylthiocarbamoyl)-1-deoxygalactonojirimycin (7).** Column chromatography: CH_2Cl_2-MeOH-H_2O 90:10:1. Yield: 114 mg (78%). $[\alpha]_D$ −193.7 (*c* 1.0, MeOH). R_f 0.59 (CH_2Cl_2-MeOH-H_2O 40:10:1); UV (MeOH): 247 nm (ε_{mM} 17.3); ^{1}H NMR (500 MHz, CD_3OD): δ 4.81 (m, 1 H, H-5), 4.57 (d, 1 H, $J_{1a,1b}$ = 14.6 Hz, H-1a), 4.06 (dd, 1 H, $J_{4,5}$ = 6.3 Hz, $J_{3,4}$ = 3.1 Hz, H-4), 4.04 (dd, 1H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 8.2 Hz, H-6a), 3.94 (dd, 1H, $J_{5,6b}$ = 3.3 Hz, H-6b), 3.83 (m, 1 H, H-2), 3.72 (t, 1 H, $J_{2,3}$ = 3.3 Hz, H-3), 3.56 (m, 3 H, CH_2NH, H-1b), 1.60 (m, 2 H, CH_2), 1.32 (m, 10 H, CH_2), 0.90 (t, 3 H, $^3J_{H,H}$ = 6.7 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): δ 186.3 (CS), 73.1 (C-3), 72.0 (C-2), 67.8 (C-4), 63.6 (C-5), 60.1 (C-6), 47.1 (CH_2N), 46.2 (C-1), 33.0, 30.5, 30.4, 30.1, 28.0 (CH_2), 23.7 (C$H_2$$CH_3$), 14.4 ($CH_3$);

FABMS: *m/z* 357 (85, $[M + Na]^+$), 335 (25, $[M + H]^+$). Anal. Calcd for $C_{15}H_{30}N_2O_4S$: C, 53.86; H, 9.04; N, 8.38; S, 9.59. Found: C, 54.43; H, 8.07; N, 6.50; S, 7.32.

General procedure for the synthesis of 5-*N*,6-*S*-(*N'*- alkyl(aryl)iminomethylidene)-1-deoxy-6-thiogalactonojirimycin derivatives (8-10). To a solution of 1-deoxygalactonojirimycin (**2**, 0.63 mmol) in pyridine (9 mL), Et_3N (0.1 mL, 0.75 mmol, 1.2 eq) and the corresponding isothiocyanate (1.1 eq) were added and the mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure and the residue was coevaporated several times with toluene. The crude thioureido derivative in MeOH (10 mL), was treated with concentrated HCl was added (pH 1) and the reaction mixture was further stirred at room temperature for 12 h. The solvent was removed; the residue was coevaporated several times with MeOH (neutral pH) and purified by column chromatography using the eluent indicated in each case.

5-*N*,6-*S*-(*N'*-Phenyliminomethylidene)-1-deoxy-6-thiogalactonojirimycin (8). Column chromatography: 90:10:1 → 50:10:1 CH_2Cl_2-MeOH-H_2O. Yield: 160 mg (67%); $[\alpha]_D$ −19.2 (*c* 0.98, MeOH); R_f 0.52 (40:10:1 CH_2Cl_2-MeOH-H_2O); ^{1}H NMR (500 MHz, CD_3OD): δ 7.26-6.89 (m, 5 H, Ph), 4.22 (dd, 1 H, $J_{1a,2}$ = 5.8 Hz, $J_{1a,1b}$ = 12.6 Hz, H-1a), 3.89 (m, 2 H, H-4, H-5), 3.74 (m, 1 H, H-2), 3.46 (dd, 1 H, $J_{3,4}$ = 3.0 Hz, $J_{2,3}$ = 9.6 Hz, H-3), 3.37 (t, 1 H, $J_{5,6a}$, $J_{6a,6b}$ = 10.4 Hz, H-6a), 3.12 (dd, 1 H, $J_{5,6b}$ = 7.3 Hz, H-6b), 2.59 (dd, 1 H, $J_{1b,2}$ = 12.6 Hz, H-1b); ^{13}C NMR (75.5 MHz, CD_3OD): δ 165.0 (CN), 150.8-124.0 (Ph), 73.8 (C-3), 70.8 (C-4), 66.9 (C-5), 65.7 (C-2), 47.9 (C-1), 28.1 (C-6); FABMS: *m/z* 381 (30, $[M + H]^+$). Anal. Calcd for $C_{13}H_{16}N_2O_3S$: C, 55.70; H, 5.75; N, 9.99; S, 11.44. Found: C, 55.41; H, 5.50; N, 9.62; S, 11.12.

5-*N*,6-*S*-(*N'*-Butyliminomethylidene)-1-deoxy-6-thiogalactonojirimycin (9). Column chromatography: 90:10:1 → 50:10:1 CH_2Cl_2-MeOH-H_2O. Yield: 126 mg (77%); $[\alpha]_D$ +8.1 (*c* 0.97, MeOH); R_f 0.27 (40:10:1 CH_2Cl_2-MeOH-H_2O); ^{1}H NMR (500 MHz, CD_3OD): δ 4.35 (dt, 1 H, $J_{1,2}$ = 8.4 Hz, $J_{2,3}$ = 1.3 Hz, H-2), 4.15 (dd, 1 H, $J_{5,6a}$ = 5.8 Hz, $J_{6a,6b}$ = 13.1 Hz, H-6a), 3.93 (m, 1 H, H-3), 3.84 (m, 1 H, H-5), 3.60 (d, 2 H, H-1), 3.50 (dd, 1 H, $J_{3,4}$ = 2.8 Hz, $J_{4,5}$ = 9.5 Hz, H-4), 3.34 (t, 2 H, $^3J_{H,H}$ = 9.5 Hz C*H*$_2$N), 2.90 (dd, 1 H, $J_{5,6b}$ = 10.9 Hz, H-6b), 1.66 (m, 2 H, CH_2), 1.40 (m, 2 H, C*H*$_2$CH$_3$), 0.97 (m, 3 H, $^3J_{H,H}$ = 7.3 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): δ 171.7 (CS), 76.0 (C-4), 71.2 (C-3), 68.7 (C-2), 66.3 (C-5), 46.0 (CH_2N), 48.7 (C-6), 32.3 (CH_2), 29.0 (C-1), 20.8 (CH_2), 13.9 (CH_3); FABMS: *m/z* 261 (100, $[M + H]^+$). Anal. Calcd for $C_{11}H_{20}N_2O_3S$: C, 50.75; H, 7.74; N, 10.6. Found: C, 50.66; H, 7.67; N, 10.70.

5-*N*,6-*S*-(*N'*-Octyliminomethylidene)-1-deoxy-6-thiogalactonojirimycin (10). Column chromatography: 90:10:1 → 50:10:1 CH_2Cl_2-MeOH-H_2O. Yield: 128 mg (65%); $[\alpha]_D$ +2.3 (*c* 1.0, MeOH); R_f 0.26 (40:10:1 CH_2Cl_2-MeOH-H_2O); ^{1}H NMR (500 MHz, CD_3OD): δ 4.42 (dt, 1 H, $J_{1,2}$ = 8.4 Hz, $J_{2,3}$ = 1.6 Hz, H-2), 4.16 (dd, 1 H, $J_{5,6a}$ = 5.6 Hz, $J_{6a,6b}$ = 13.1 Hz, H-6a), 3.93 (m, 1 H, H-3), 3.85 (m, 1 H, H-5), 3.63 (dd, 2 H, $J_{1a,2}$ = 3.3 Hz, H-1), 3.51 (dd, 1 H, $J_{3,4}$ = 2.8 Hz, $J_{4,5}$ = 9.4 Hz, H-4), 3.36 (t, 2 H, $^3J_{H,H}$ = 6.7 Hz CH_2N), 2.95 (dd, 1 H, $J_{5,6b}$ = 10.9 Hz, H-6b), 1.69 (m, 2 H, CH_2), 1.34 (m, 10 H, C$H_2$$CH_3$), 0.91 (t, 3 H, $^3J_{H,H}$ = 7.1 Hz, CH_3); ^{13}C NMR (125.7 MHz, CD_3OD): δ 172.4 (CS), 75.8 (C-4), 73.5 (C-3), 69.0 (C-2), 66.2 (C-5), 49.80 (CH_2N), 48.7 (C-6), 32.9, 30.2, 30.2, 30.1, 27.6, 23.7 (CH_2), 29.0 (C-1), 14.4 (CH_3); FABMS: *m/z* 317 (100, $[M + H]^+$). Anal. Calcd for $C_{15}H_{28}N_2O_3S$: C, 56.93; H, 8.92; N, 8.85; S, 10.13. Found: C, 56.68; H, 8.70; N, 8.76; S, 9.87.

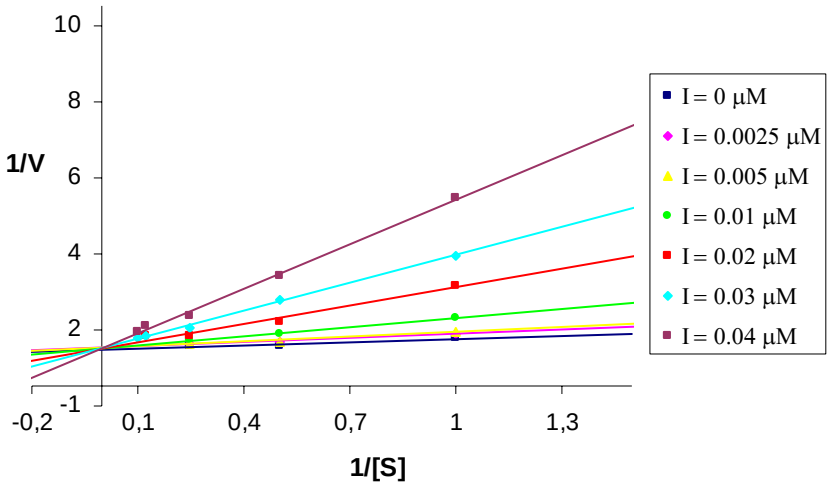

Figure S1. Lineweaver-Burk Plot for K_i determination (1.9 nM) of **5** against green coffee bean α-galactosidase (pH 6.8).

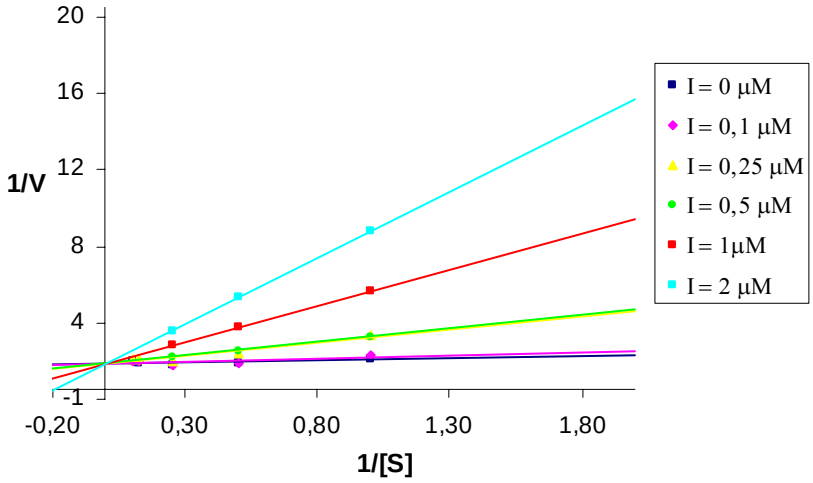

Figure S2. Lineweaver-Burk Plot for K_i determination (44 nM) of **6** against green coffee beans α-galactosidase (pH 6.8).

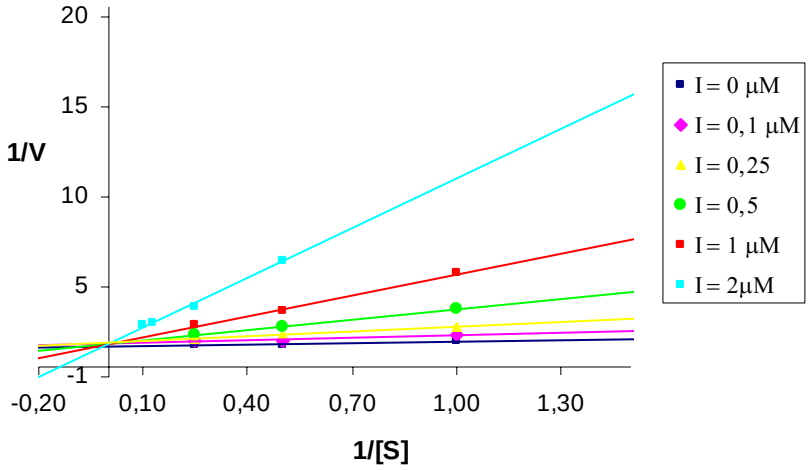

Figure S3. Lineweaver-Burk Plot for K_i determination (29 nM) of **7** against green coffee bean α-galactosidase (pH 6.8).

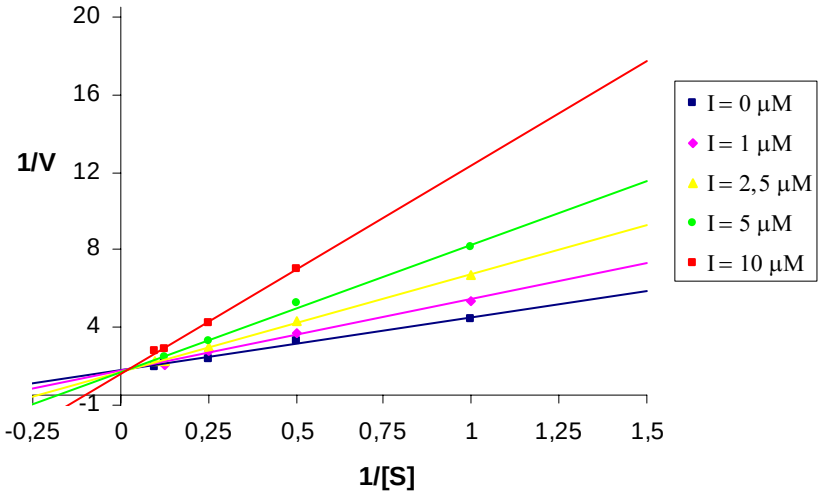

Figure S4. Lineweaver-Burk Plot for K_i determination (2.9 µM) of **9** against bovine liver β-galactosidase (pH 7.3).

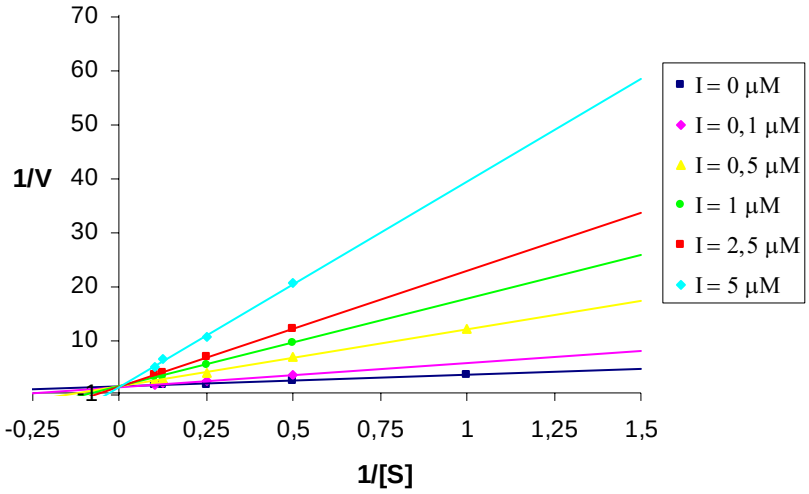

Figure S5. Lineweaver-Burk Plot for K_i determination (0.2 µM) of **10** against bovine liver β-galactosidase (pH 7.3).

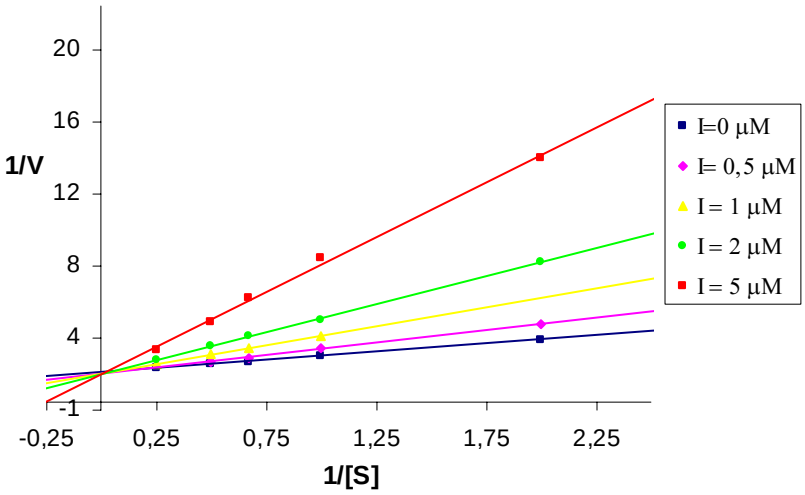

Figure S6. Lineweaver-Burk Plot for K_i determination (0.65 µM) of **10** against *E. coli* β-galactosidase (pH 7.3).

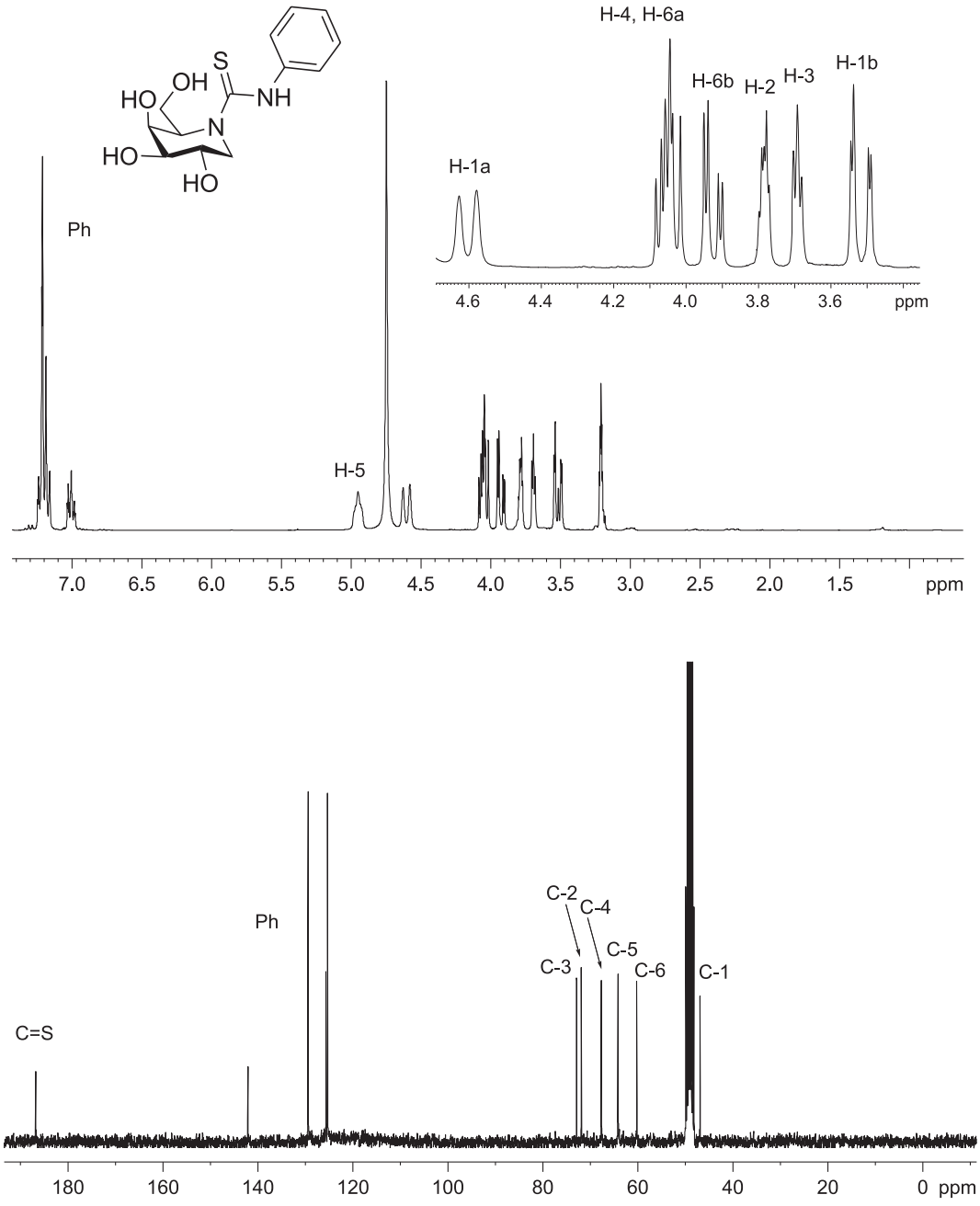

Figure S7. ^{1}H and ^{13}C NMR spectra (300 MHz, 75.5 MHz, CD_3OD) of compound **5.**

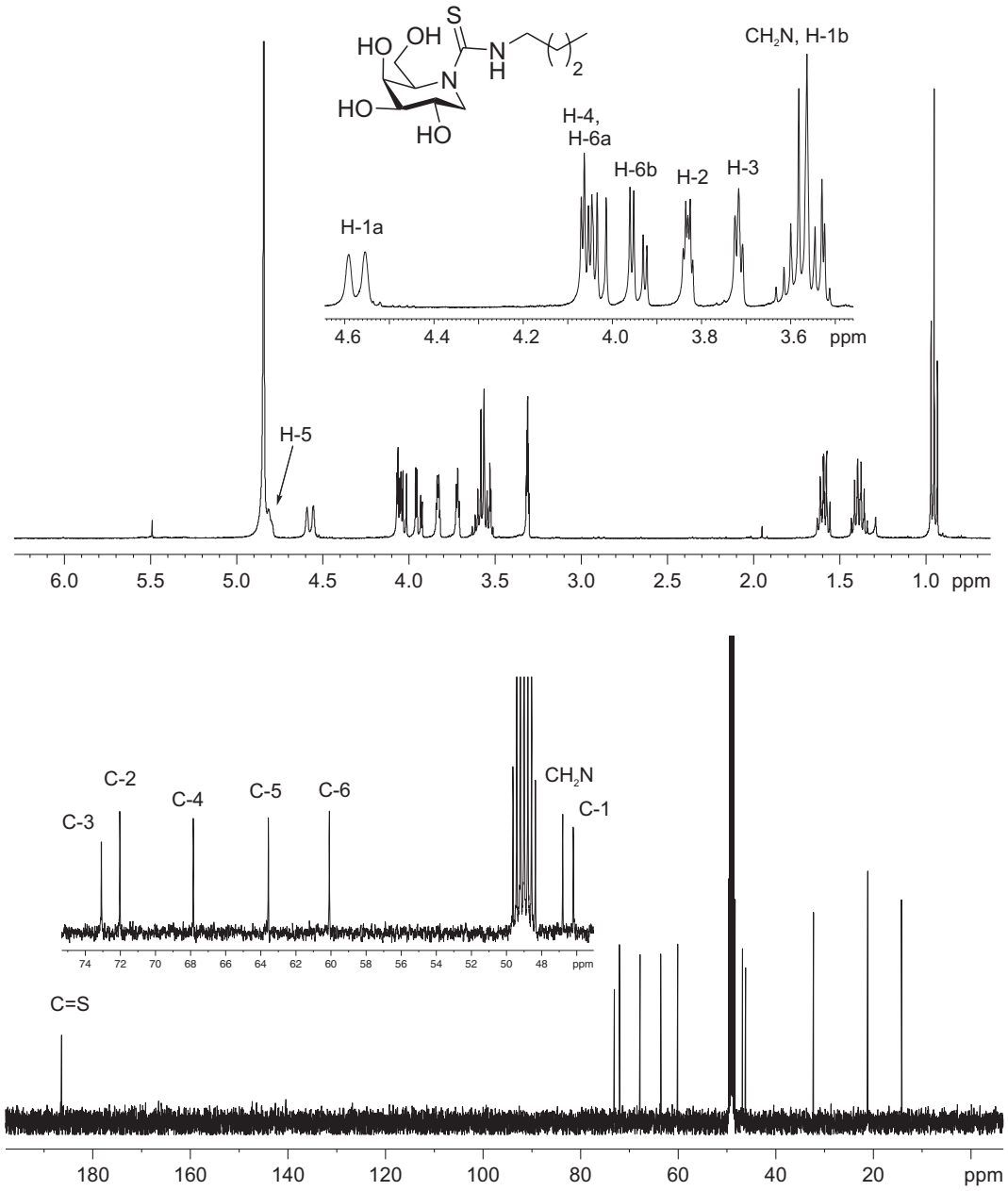

Figure S8. ^{1}H and ^{13}C NMR spectra (400 MHz, 100 MHz, CD_3OD) of compound **6.**

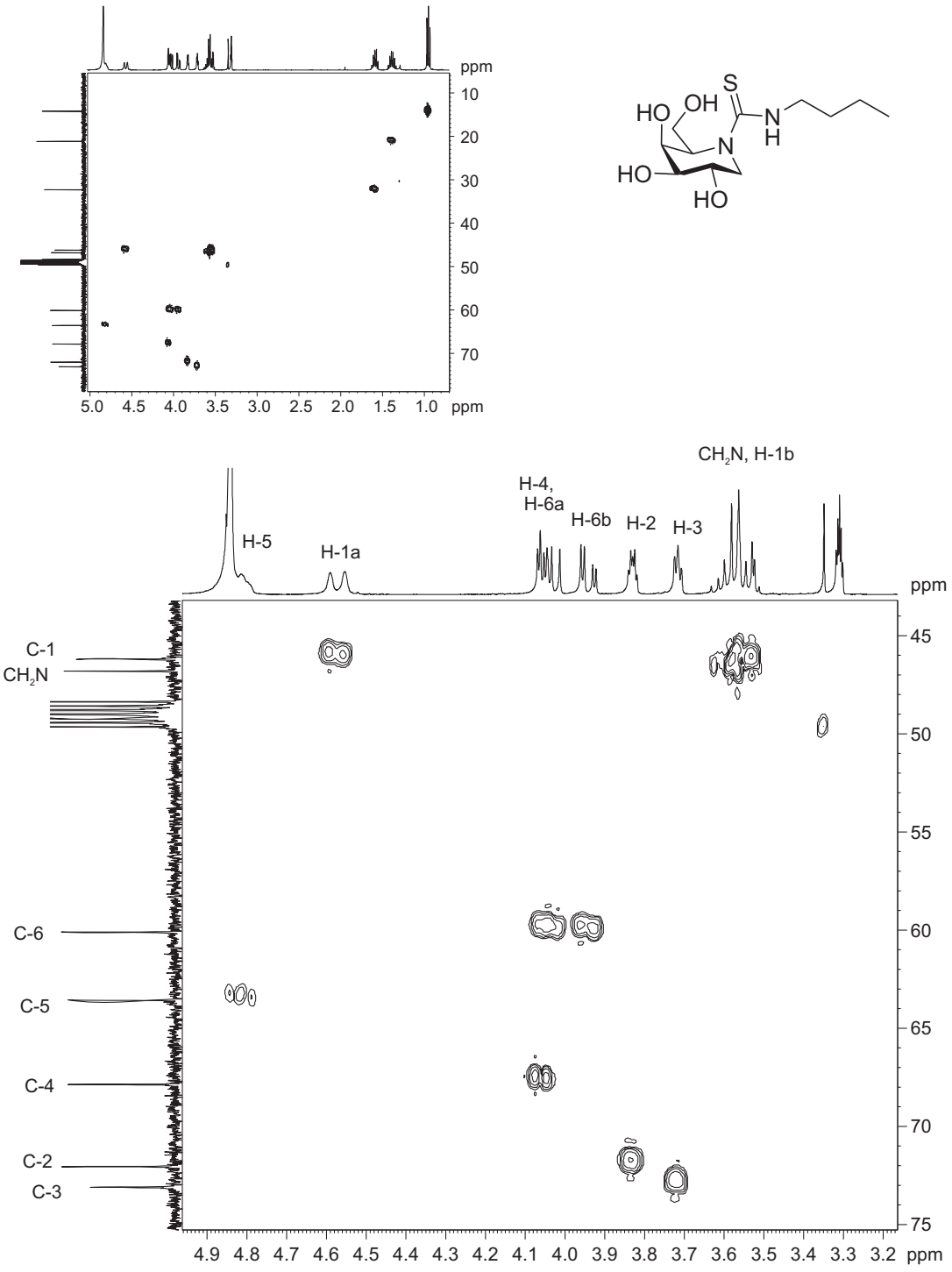

Figure S9. HMQC spectrum (400 MHz, CD_3OD) of compound **6.**

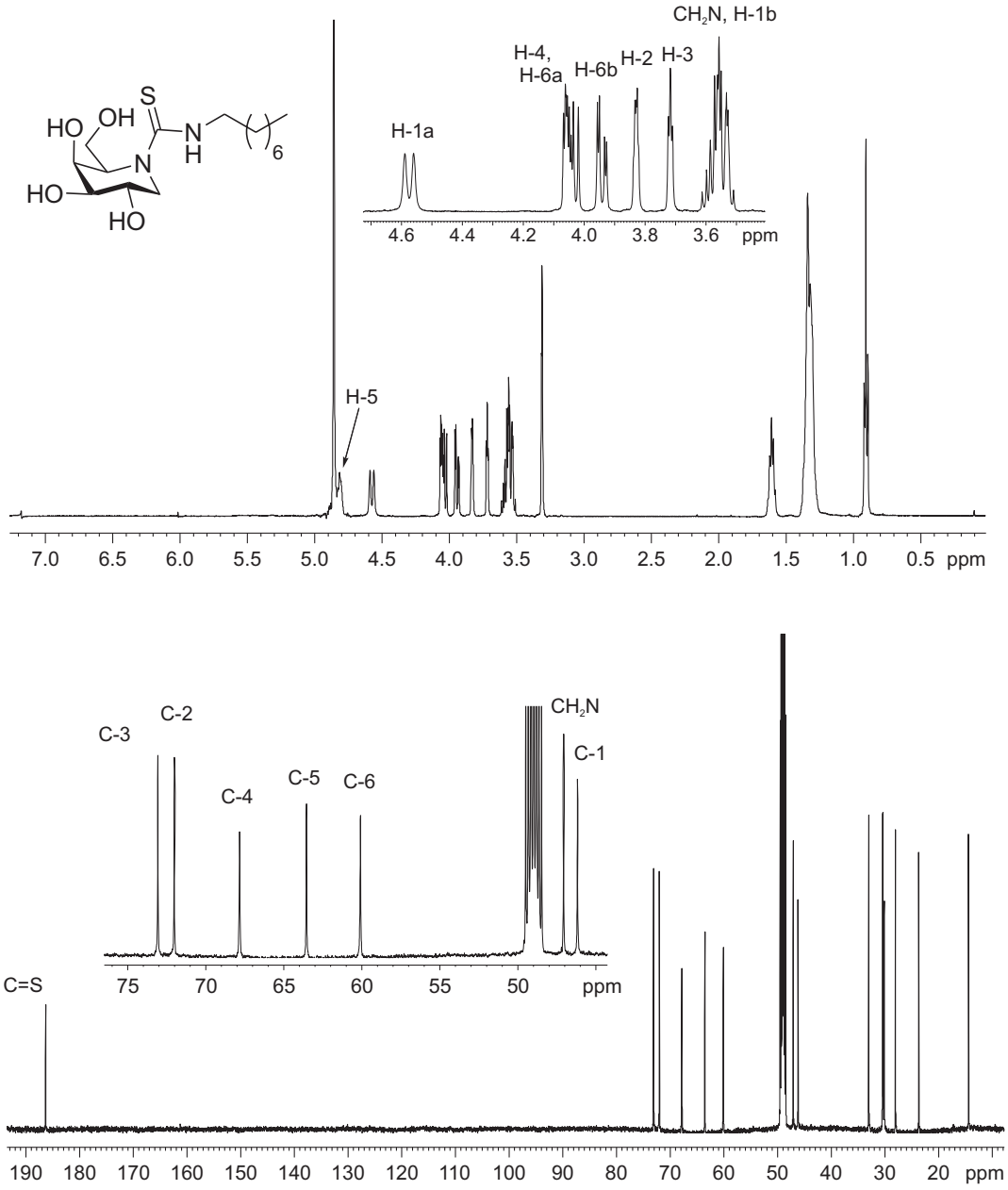

Figure S10. ^{1}H and ^{13}C NMR spectra (400 MHz, 100 MHz, CD_3OD) of compound **7.**

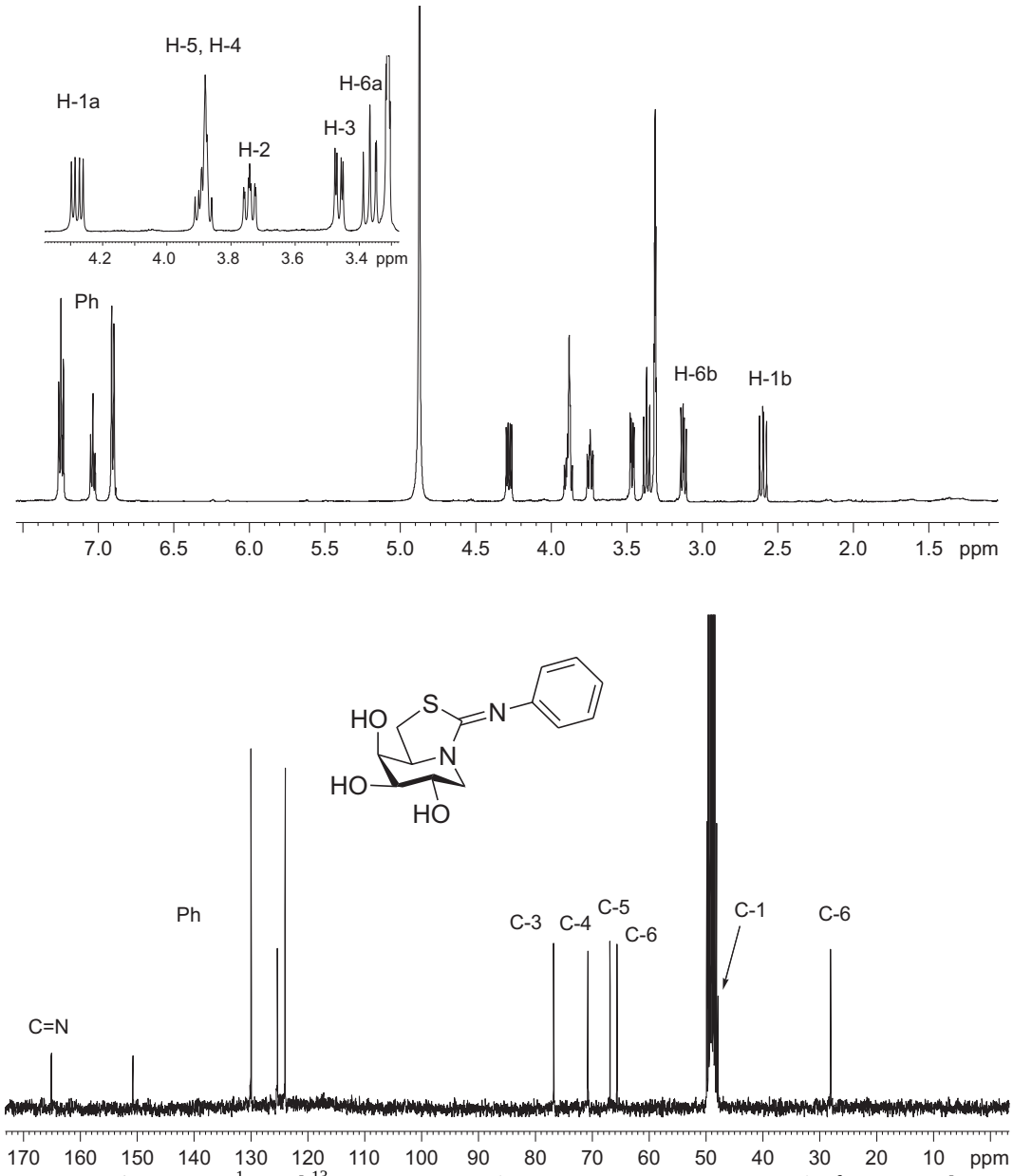

Figure S11. ^{1}H and ^{13}C NMR spectra (500 MHz, 75.5 MHz, CD_3OD) of compound **8.**

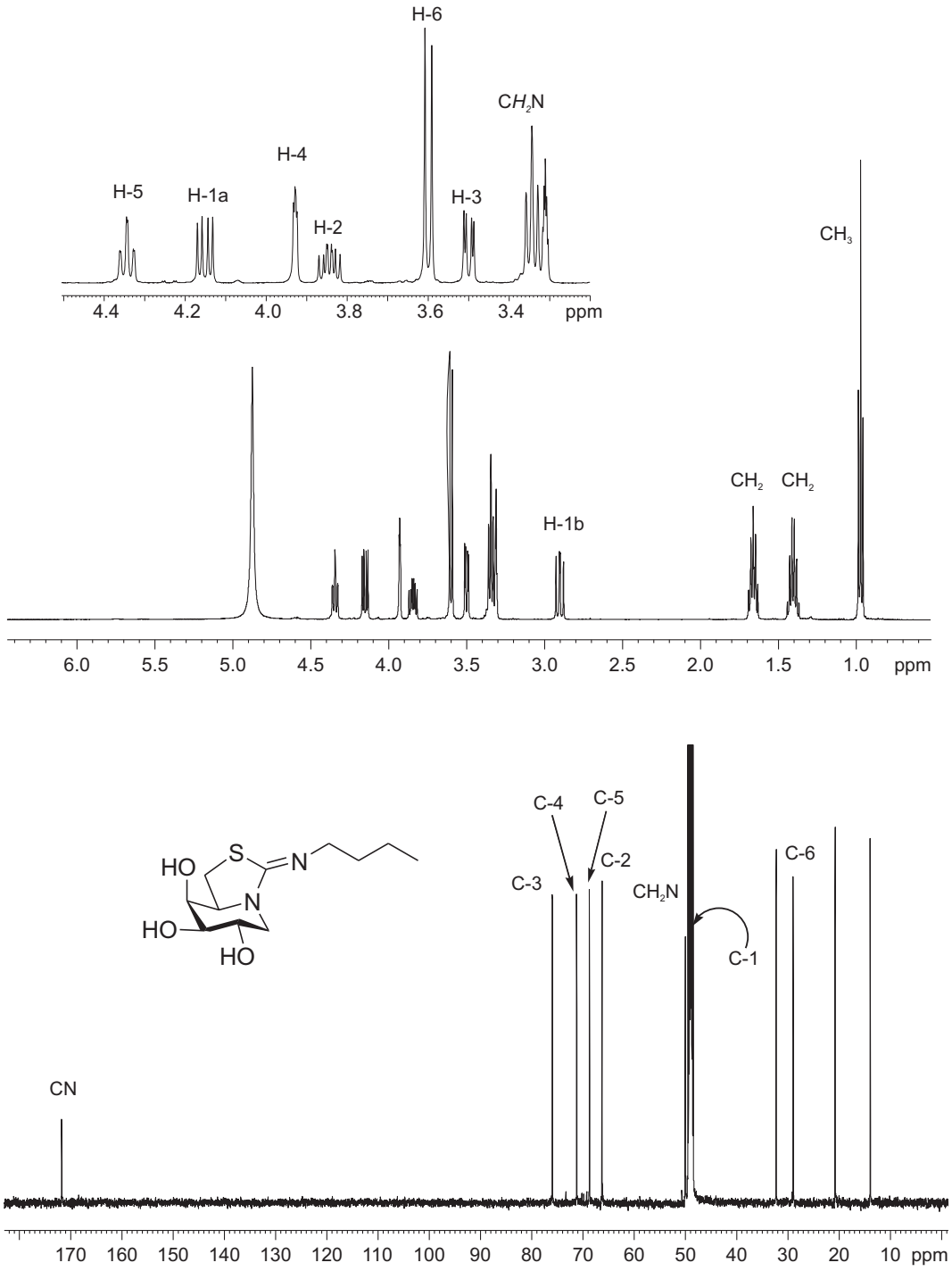

Figure S12. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD) of compound **9.**

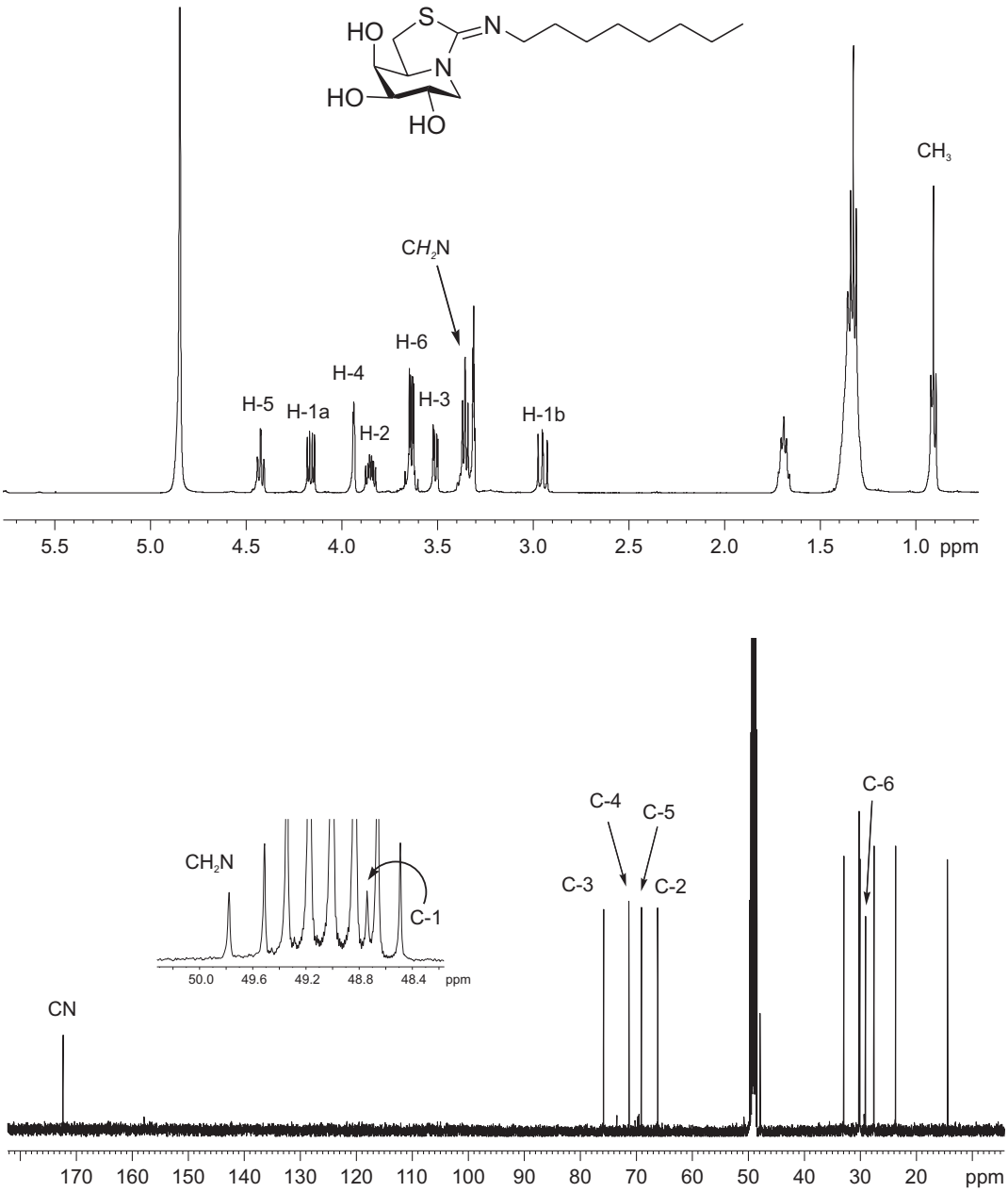

Figure S13. ^{1}H and ^{13}C NMR spectra (500 MHz, 125.7 MHz, CD_3OD) of compound **10.**

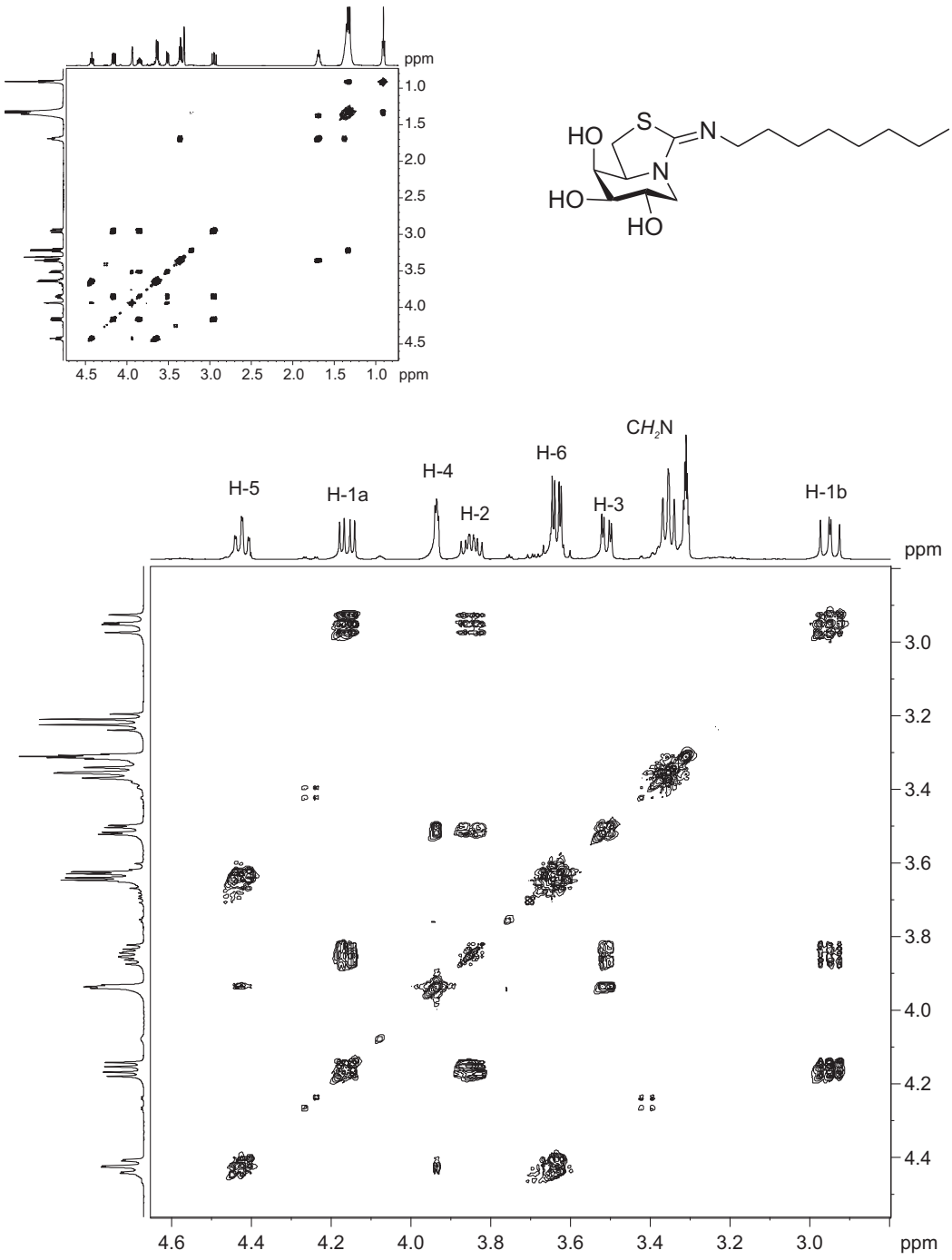

Figure S14. COSY spectrum (500 MHz, MeOD) of compound **10.**

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[2] Iwasaki, H.; Watanabe, H.; Iida, M.; Ogawa, S.; Tabe, M.; Higaki, K.; Nanba, E.; Suzuki, Y. *Brain Dev.* **2010**, *28*, 482.
[3] Ishii, S.; Chang, H. H.; Kawasaki, K.; Yasuda, K.; Wu, H. L.; Garman, S. C.; Fan, J. Q. *Biochem. J.* **2007**, *406*, 285.