Supporting Information

Synthesis of Multibranched Australine Derivatives from Reducing Castanospermine Analogues through the Amadori Rearrangement of *gem*-Diamine Intermediates: Selective inhibitors of β-Glucosidase

Elena M. Sánchez Fernández,[†] Eleuterio Álvarez,[‡] Carmen Ortiz Mellet,[†,*] and José M. García Fernández[‡,*]

[†]*Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, c/ Profesor García González nº 1, E-41012 Sevilla, Spain*

[‡]*Instituto de Investigaciones Químicas, CSIC – Universidad de Sevilla, c/ Américo Vespucio nº 49, Isla de la Cartuja, E-41092 Sevilla, Spain*

mellet@us.es, jogarcia@iiq.csic.es

List of Contents

1. General Procedure for the Glycosidase Inhibition Assay

Inhibitory potencies were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *o*- (for β-glucosidase/β-galactosidase from bovine liver and β-galactosidase from *E. coli*) or *p*-nitrophenyl α- or β-D-glycopyranoside, in the presence of the corresponding 3-*epi*-australine derivative. Each assay was performed in phosphate 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: α-glucosidase (yeast), K_m = 0.35 mM (pH 6.8); isomaltase (yeast) K_m = 1.0 mM (pH 6.8), β-glucosidase (almonds), K_m = 3.5 mM (pH 7.3); β-glucosidase/β-galactosidase (bovine liver), K_m = 2.0 mM (pH 7.3); β- galactosidase (*E. coli*), K_m = 0.12 mM (pH 7.3); α-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); β-mannosidase (*Helix pomatia*), K_m = 0.6 mM (pH 5.5); α-mannosidase (jack bean), K_m = 2.0 mM (pH 5.5); naringinase (*Penicillium decumbens*, β-glucosidase/β-rhamnosidase activity). 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 ºC or 55 ºC the reaction was quenched by addition of 1 M Na_2CO_3. The absorbance of the resulting mixture was determined at 405 nm or 505 nm. Each experiment was performed in duplicate using [I] = 2, 0.4, 0.08, 0.04 y 0.02 μM and [S] nearly K_m value. In those cases were K_i values lower that 10 μM were obtained by this procedure (**12b** and **12c** against bovine liver β-glucosidase), refined K_i values and the enzyme inhibition mode were determined from the slope of Lineweaver-Burk plots and double reciprocal analysis (Figures S1-S2).

2. Lineweaver-Burk and Double Reciprocal Analysis Plots

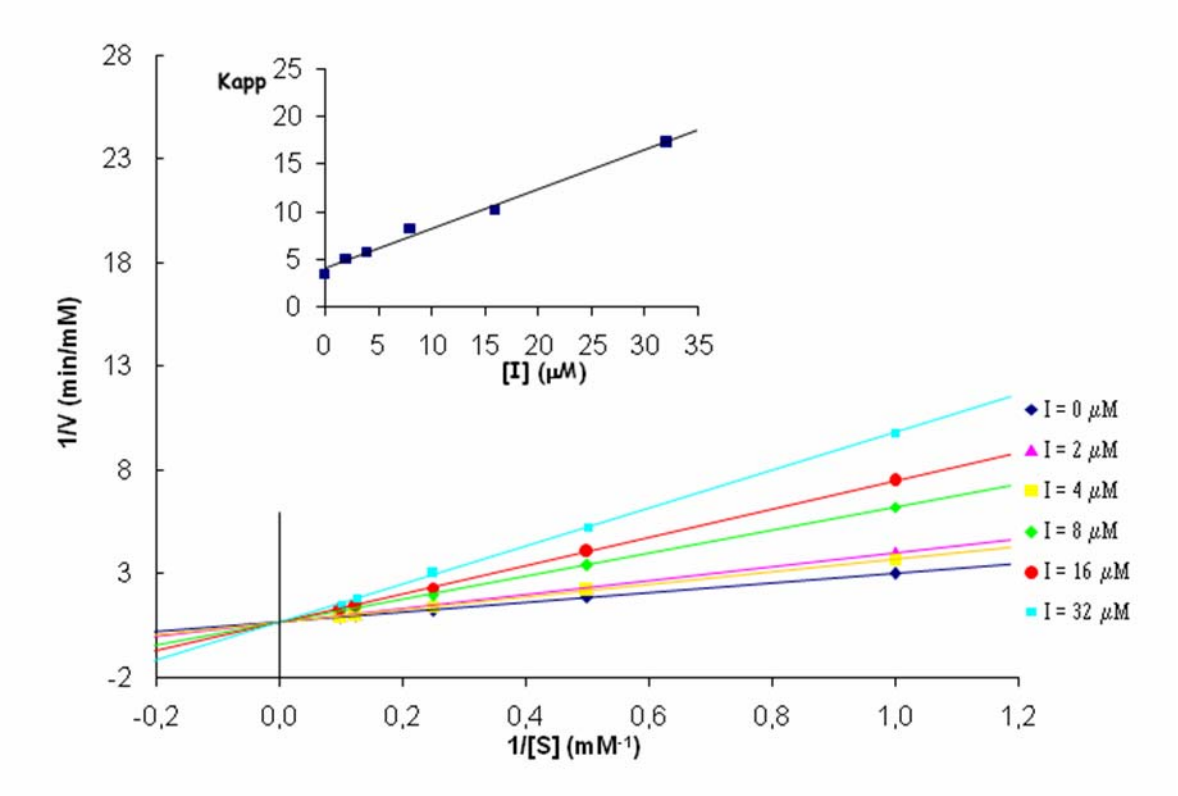

Figure S1. Lineweaver-Burk Plot for K_i determination (9.6 μM) of **12b** against bovine liver β-glucosidase.

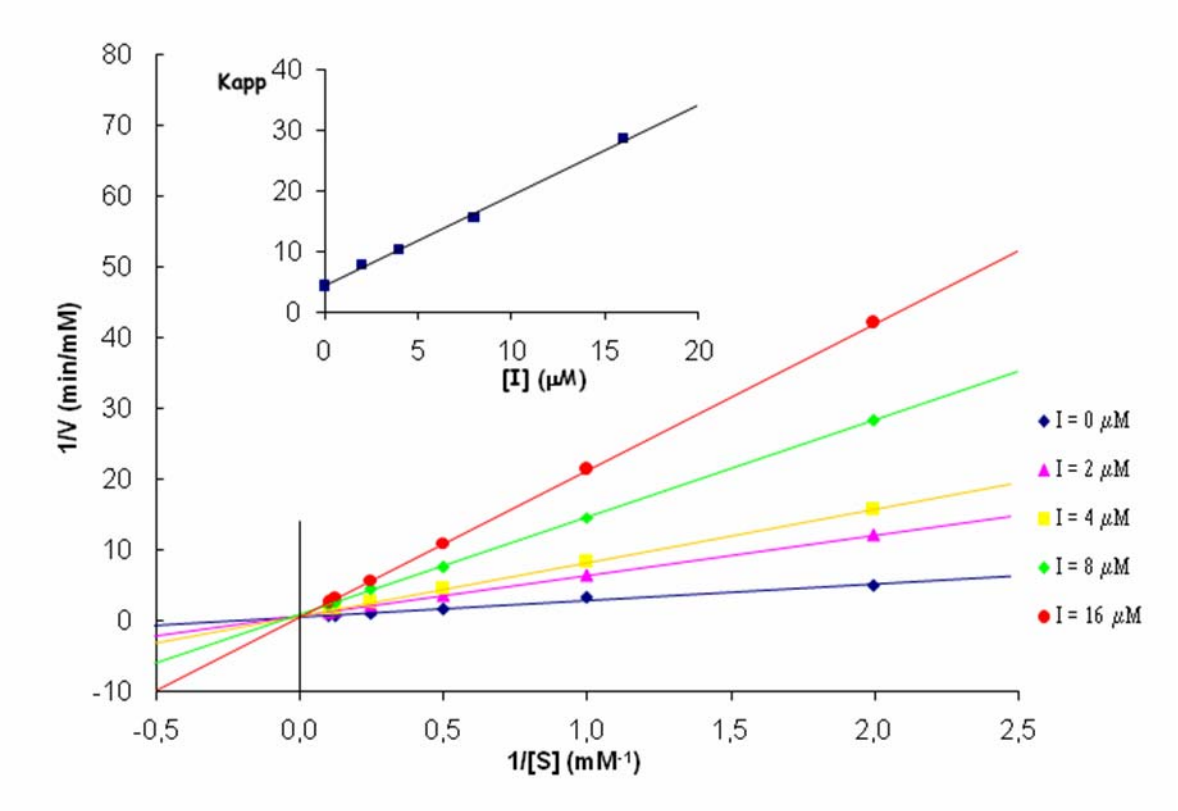

Figure S2. Lineweaver-Burk Plot for K_i determination (2.9 μM) of **12c** against bovine liver β-glucosidase.

3. Copies of ^{1}H and ^{13}C NMR Spectra of 8a, 12a-c, 13a-c, 16-19

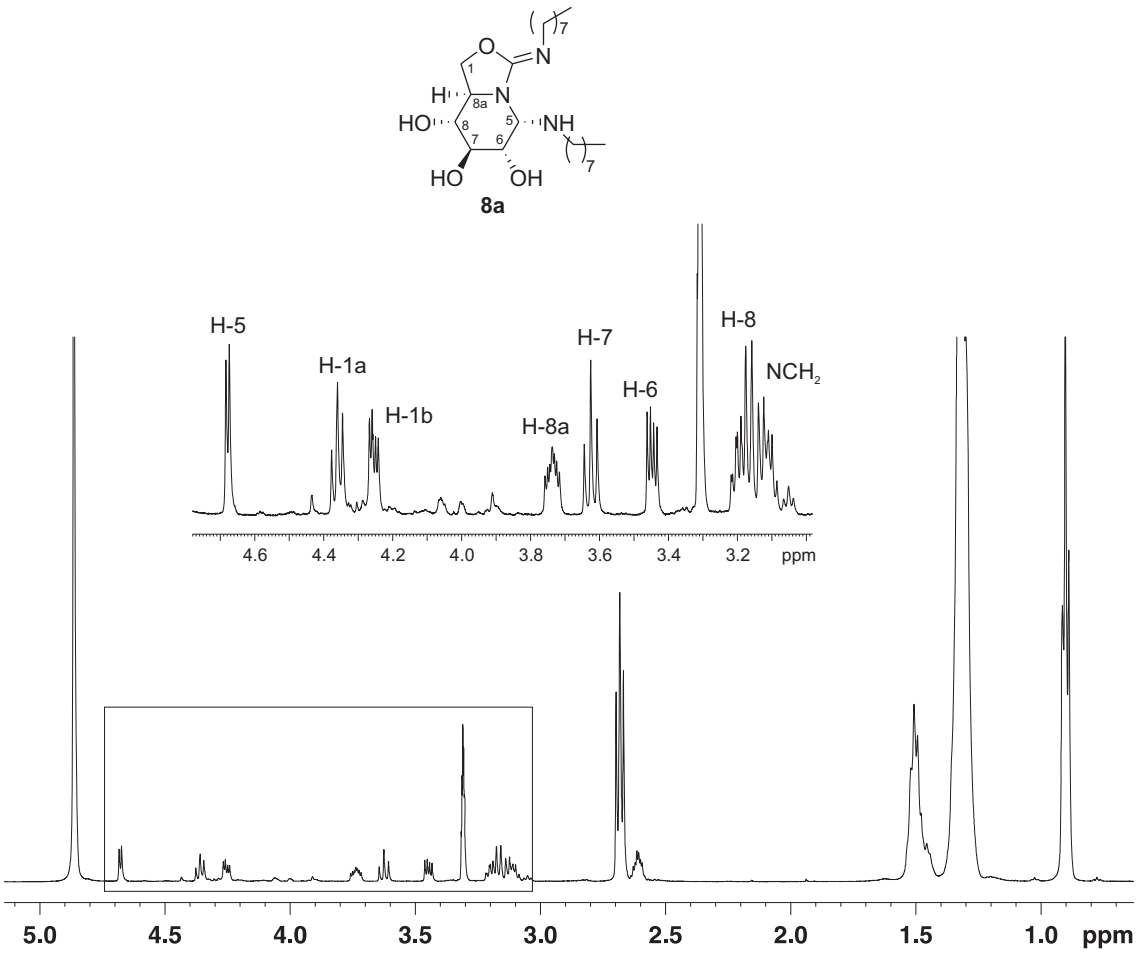

Figure S3. ^{1}H NMR spectrum (500 MHz, CD_3OD) of **8a**, n = 7.

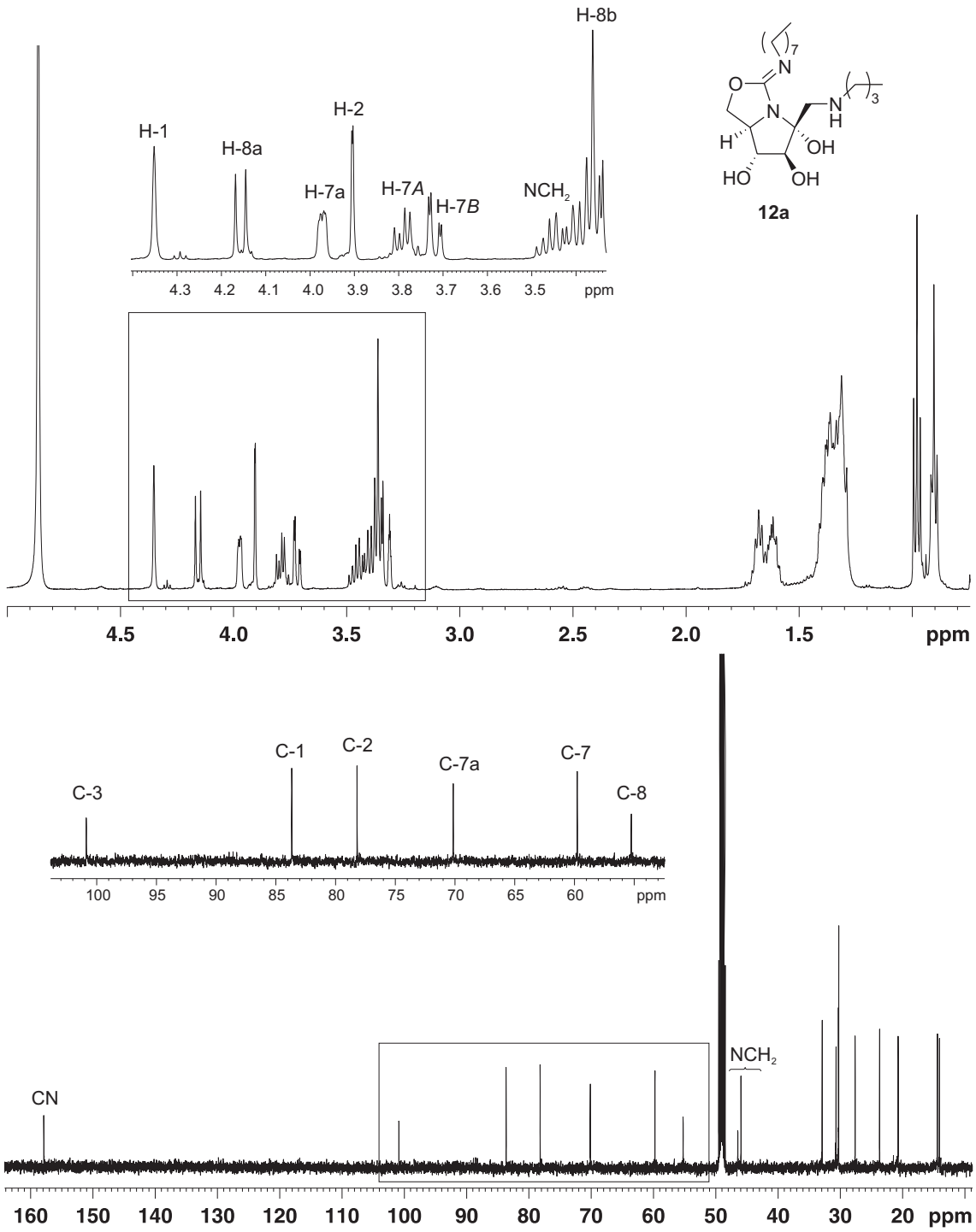

Figure S4. ^{1}H and ^{13}C NMR spectra (500 MHz and 125.7 MHz, respectively, CD_3OD) of **12a.**

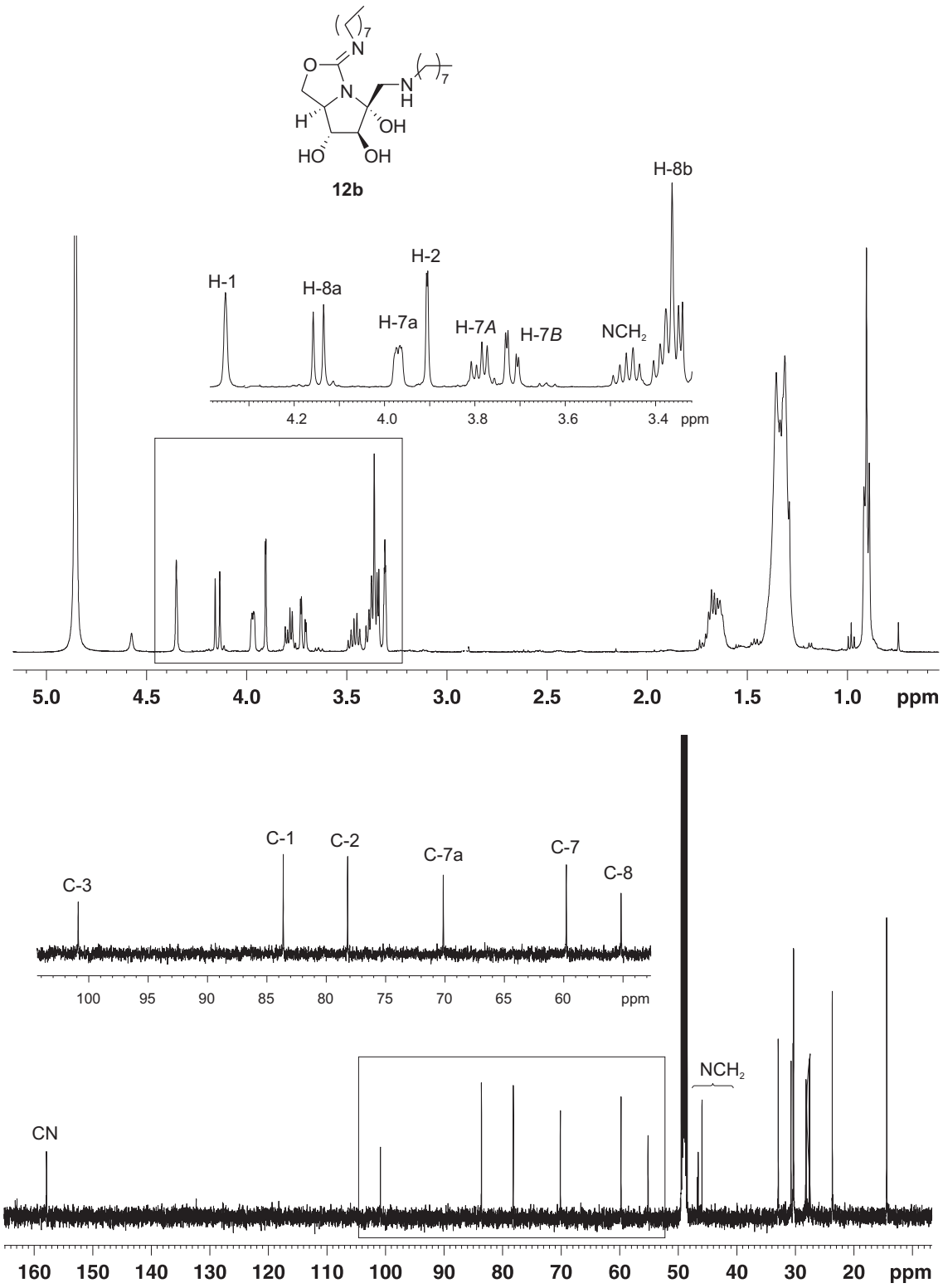

Figure S5. ^{1}H and ^{13}C NMR spectra (500 MHz and 125.7 MHz, respectively, CD_3OD) of **12b.**

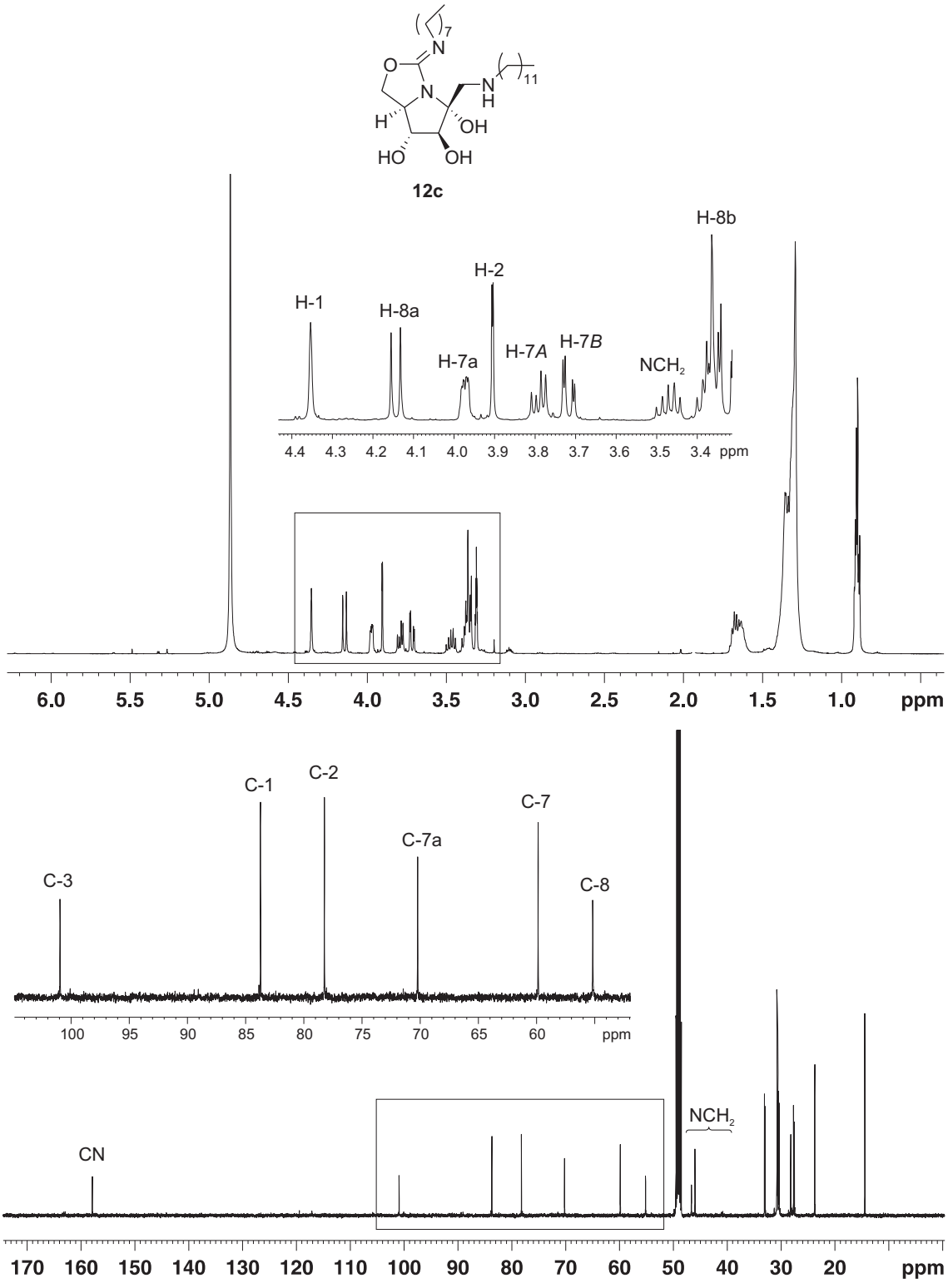

Figure S6. ^{1}H and ^{13}C NMR spectra (500 MHz and 125.7 MHz, respectively, CD_3OD) of **12c.**

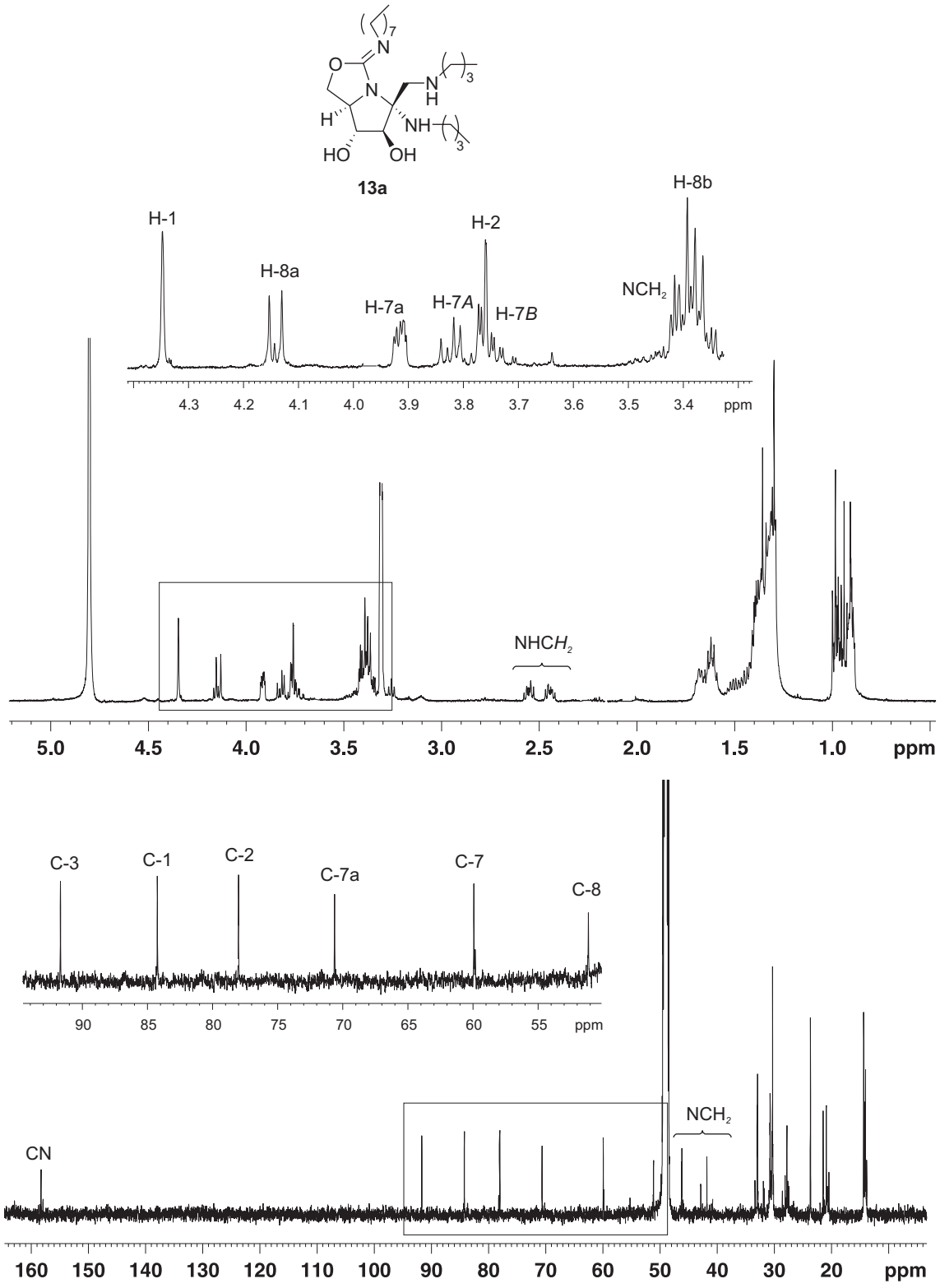

Figure S7. ^{1}H and ^{13}C NMR spectra (500 MHz and 125.7 MHz, respectively, CD_3OD) of **13a.**

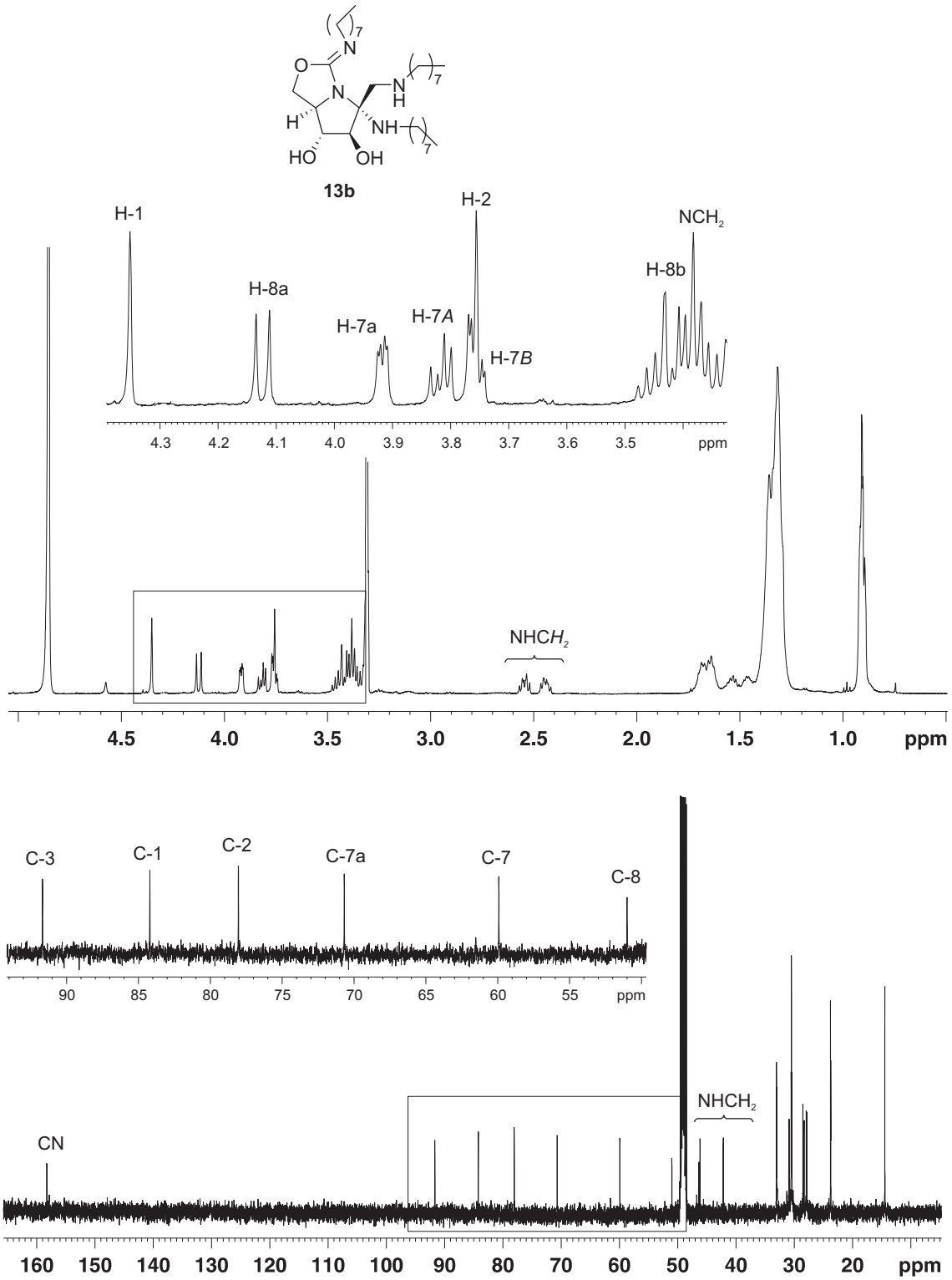

Figure S8. ^{1}H and ^{13}C NMR spectra (500 MHz and 125.7 MHz, respectively, CD_3OD) of **13b.**

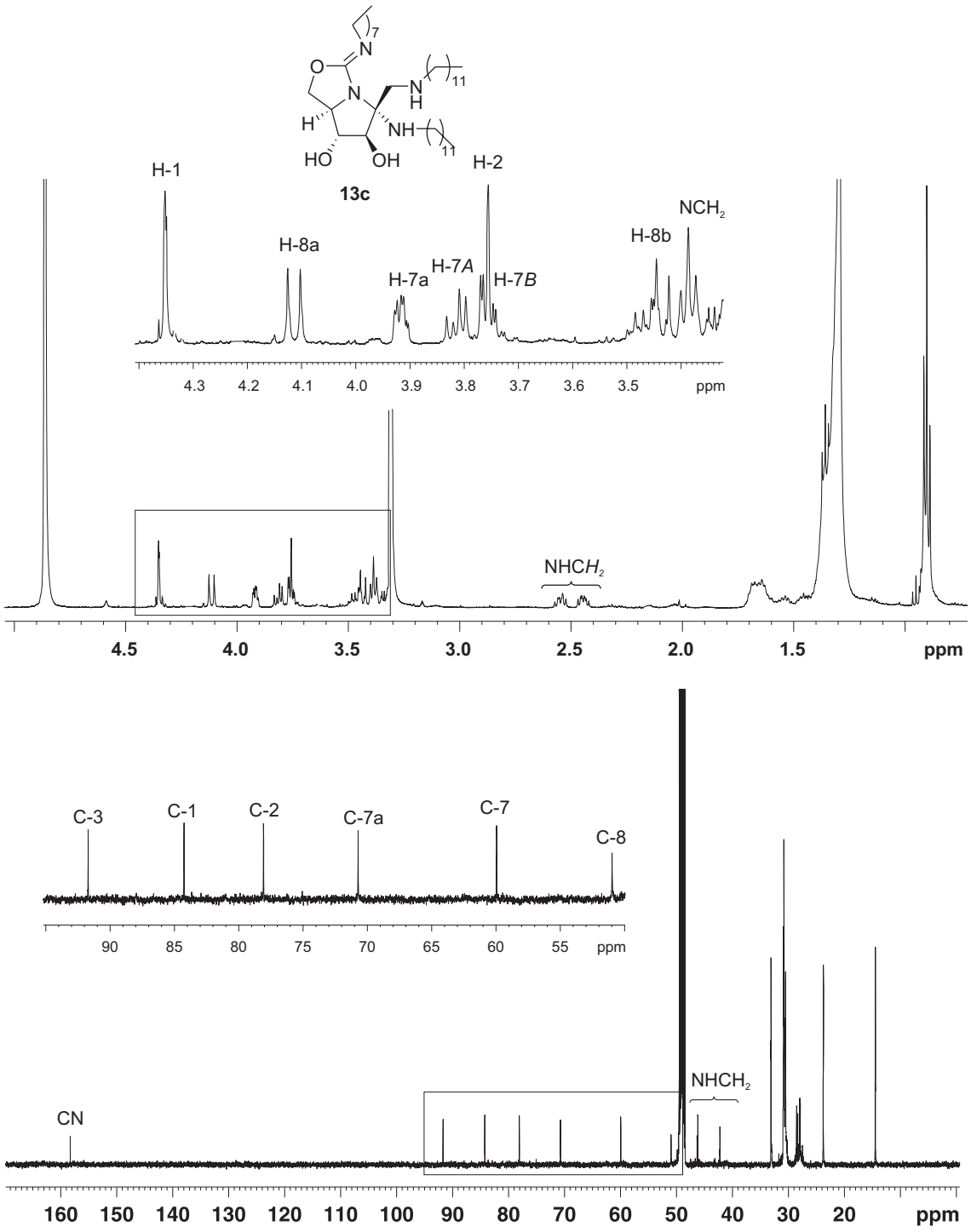

Figure S9. ^{1}H and ^{13}C NMR spectra (500 MHz and 125.7 MHz, respectively, CD_3OD) of **13c.**

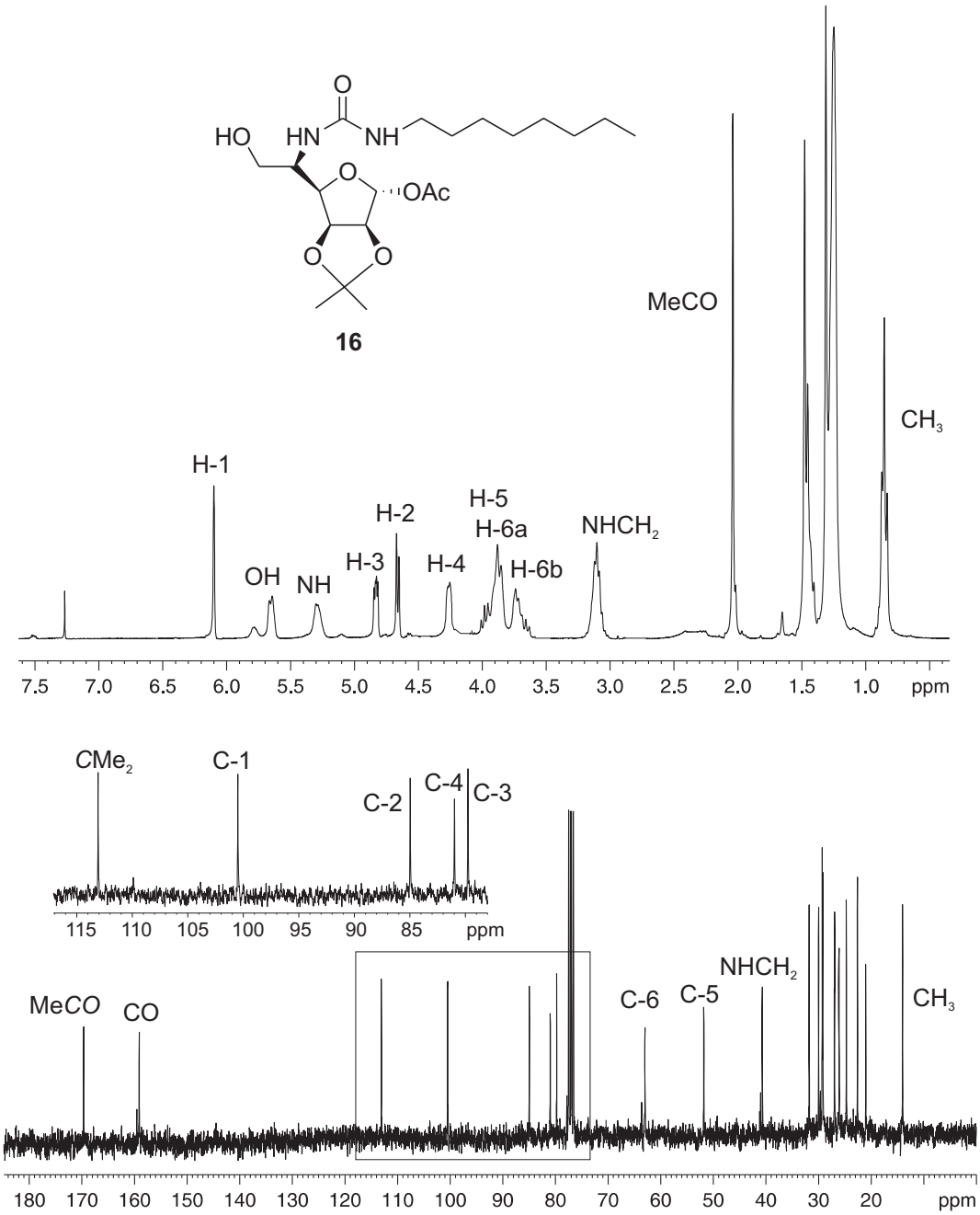

Figure S10. ^{1}H and ^{13}C NMR spectra (300 MHz and 75.5 MHz, respectively, $CDCl_3$) of **16.**

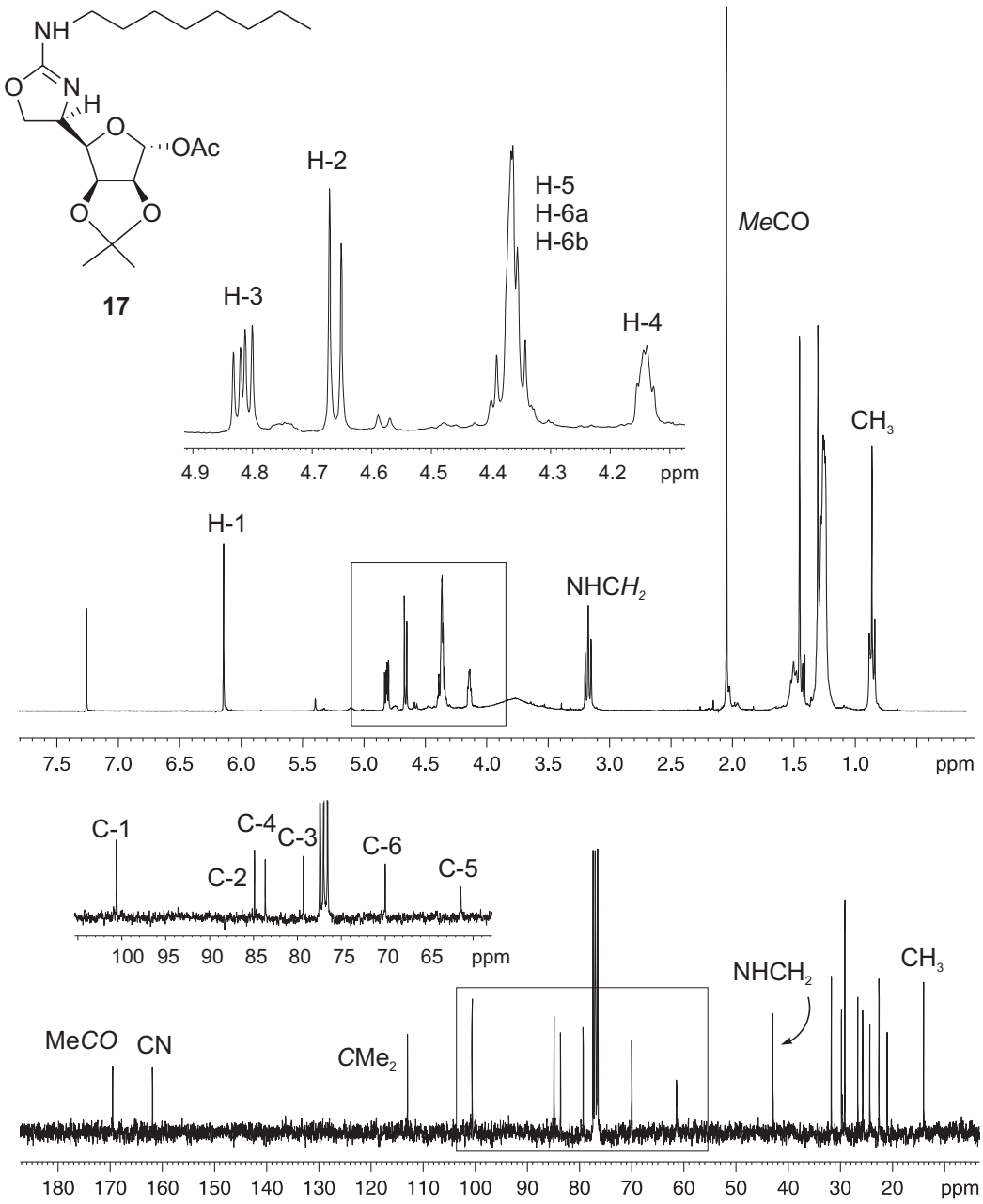

Figure S11. ^{1}H and ^{13}C NMR spectra (300 MHz and 75.5 MHz, respectively, $CDCl_3$) of **17.**

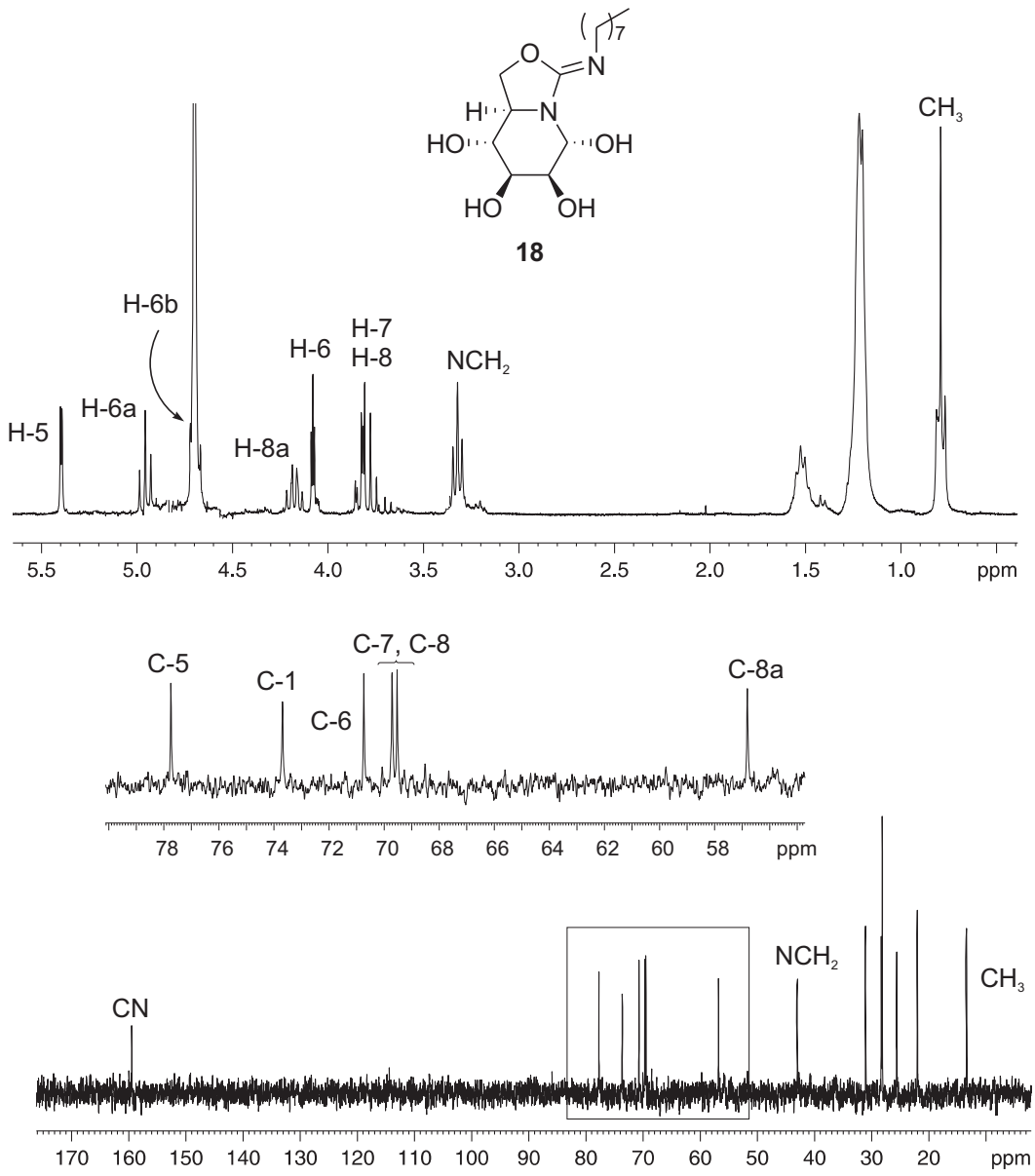

Figure S12. ^{1}H and ^{13}C NMR spectra (300 MHz and 75.5 MHz, respectively, D_2O) of **18.**

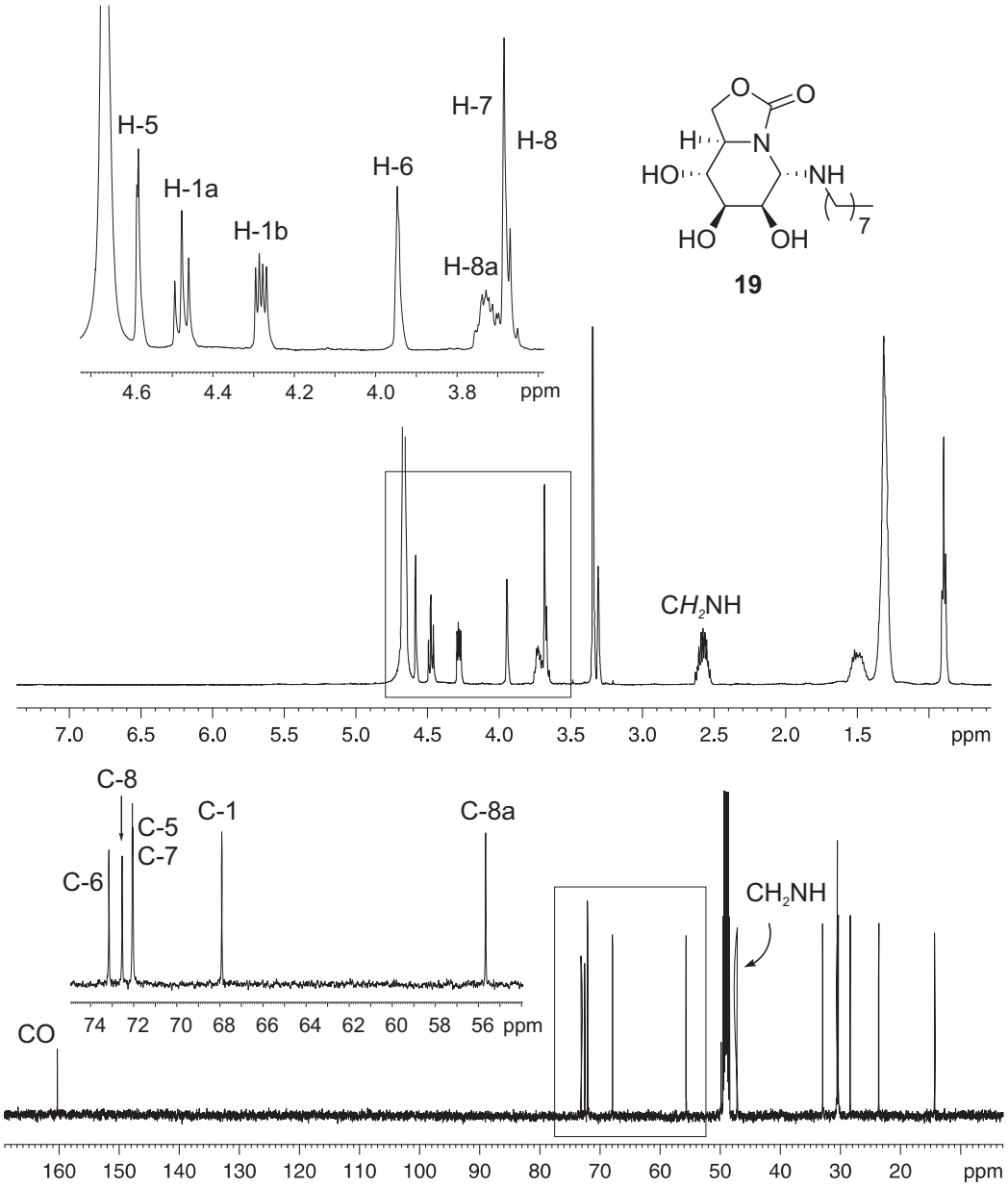

Figure S13. ^{1}H and ^{13}C NMR spectra (500 MHz and 125.7 MHz, respectively, CD_3OD) of **19.**

4. NOESY Spectra of 12b and 13b.

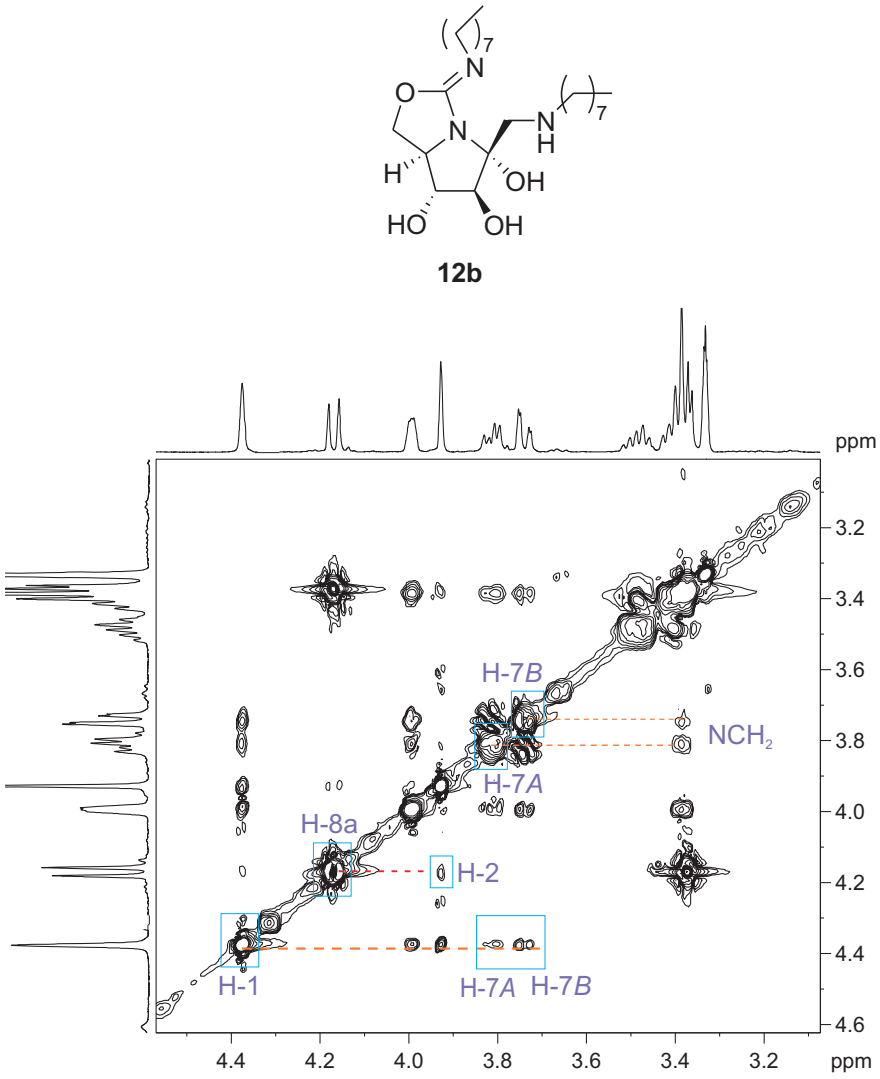

Figure S14. NOESY spectrum of **12b** (500 MHz).

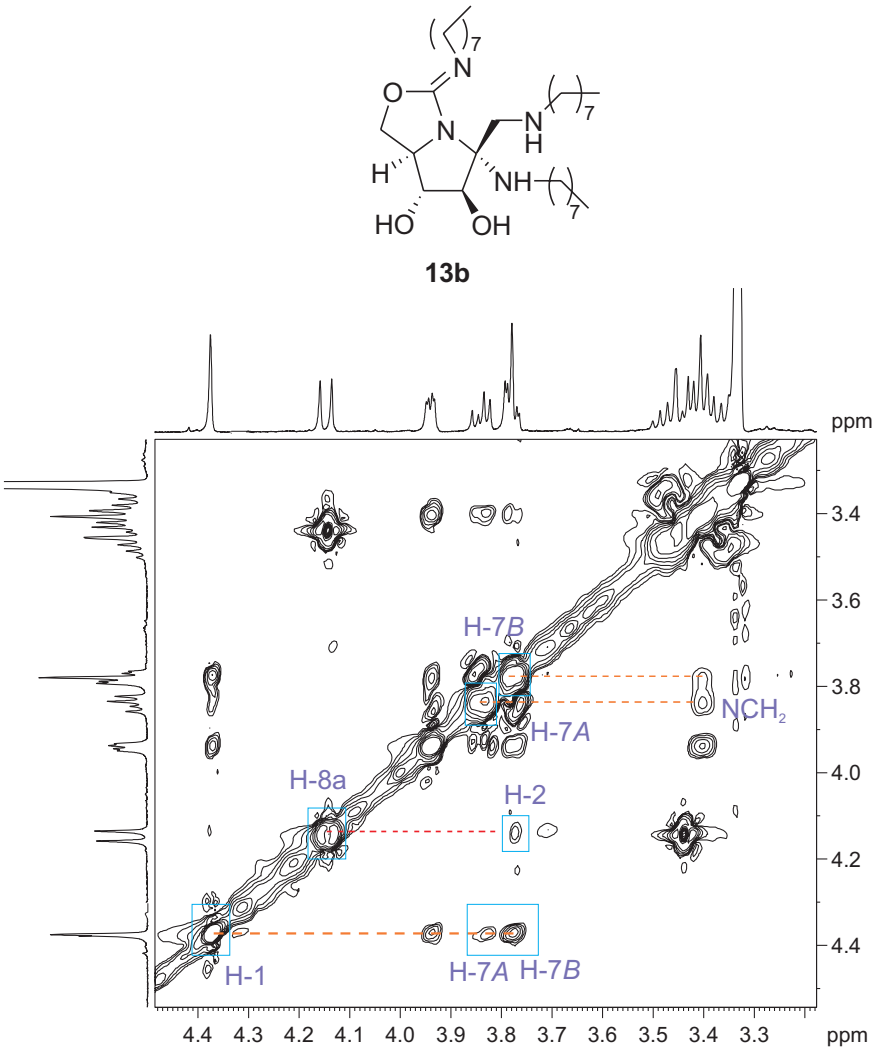

Figure S15. NOESY spectrum of **13b** (500 MHz).

5. X-Ray Data for 19.

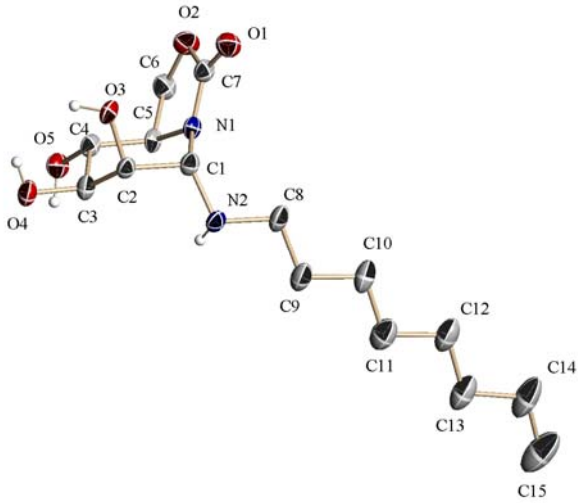

Figure S16. Molecular structure of **19** showing the atom labeling scheme. Thermal ellipsoids are draw at the 50% probability level; all hydrogen atoms from carbon atoms are omitted for clarity.

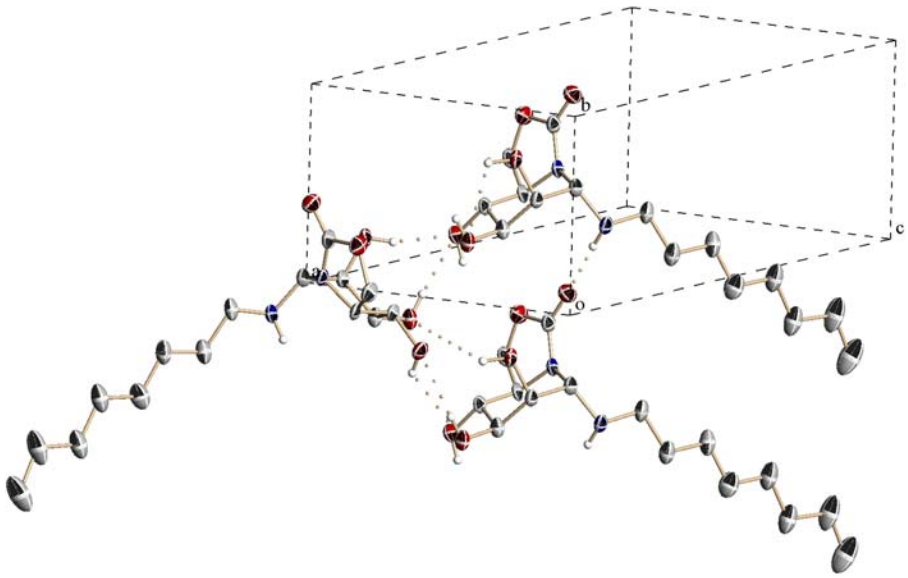

Figure S17. Packing diagram of compound **19** showing the hydrogen-bond interactions as dotted lines.

Table S1. Crystal Data and Structure Refinement for **19**.

Empirical formula	$C_{15}H_{28}N_2O_5$	
Formula weight	316.39	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 8.836(2) Å	α= 90°.
	b = 6.0632(19) Å	β= 100.167(7)°.
	c = 16.037(5) Å	γ = 90°.
Volume	845.7(4) $Å^3$	
Z	2	
Density (calculated)	1.242 Mg/m^3	
Absorption coefficient	0.093 mm^{-1}	
F(000)	344	
Crystal size	0.48 x 0.08 x 0.06 mm^3	
Theta range for data collection	1.29 to 26.33°.	
Index ranges	-10<=h<=10, -7<=k<=6, -20<=l<=19	
Reflections collected	13814	
Independent reflections	1861 [R(int) = 0.0669]	
Completeness to theta = 26.33°	98.2 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9945 and 0.9926	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	1861 / 0 / 203	
Goodness-of-fit on F^2	1.096	
Final R indices [I>2sigma(I)]	R1 = 0.0513, wR2 = 0.1319	
R indices (all data)	R1 = 0.0689, wR2 = 0.1718	
Largest diff. peak and hole	0.458 and -0.305 $e.Å^{-3}$	

Table S2. Intermolecular Hydrogen Bonds for **19** in the Crystal (distance in Å; angles in °).

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(5)-H(5)...O(4)#1	0.84	2.51	2.975(6)	116.0
O(4)-H(4)...O(5)#2	0.84	2.19	2.975(6)	154.9
O(3)-H(3)...O(4)	0.84	2.46	2.882(6)	111.8
O(3)-H(3)...O(5)#2	0.84	1.91	2.722(5)	163.1
N(2)-H(2N)...O(1)#3	0.86	2.10	2.951(6)	171.2

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,y-1/2,-z #2 -x+1,y+1/2,-z #3 x,y-1,z