Supporting Information

Pharmacological Chaperones for the Treatment of α-Mannosidosis

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Content list

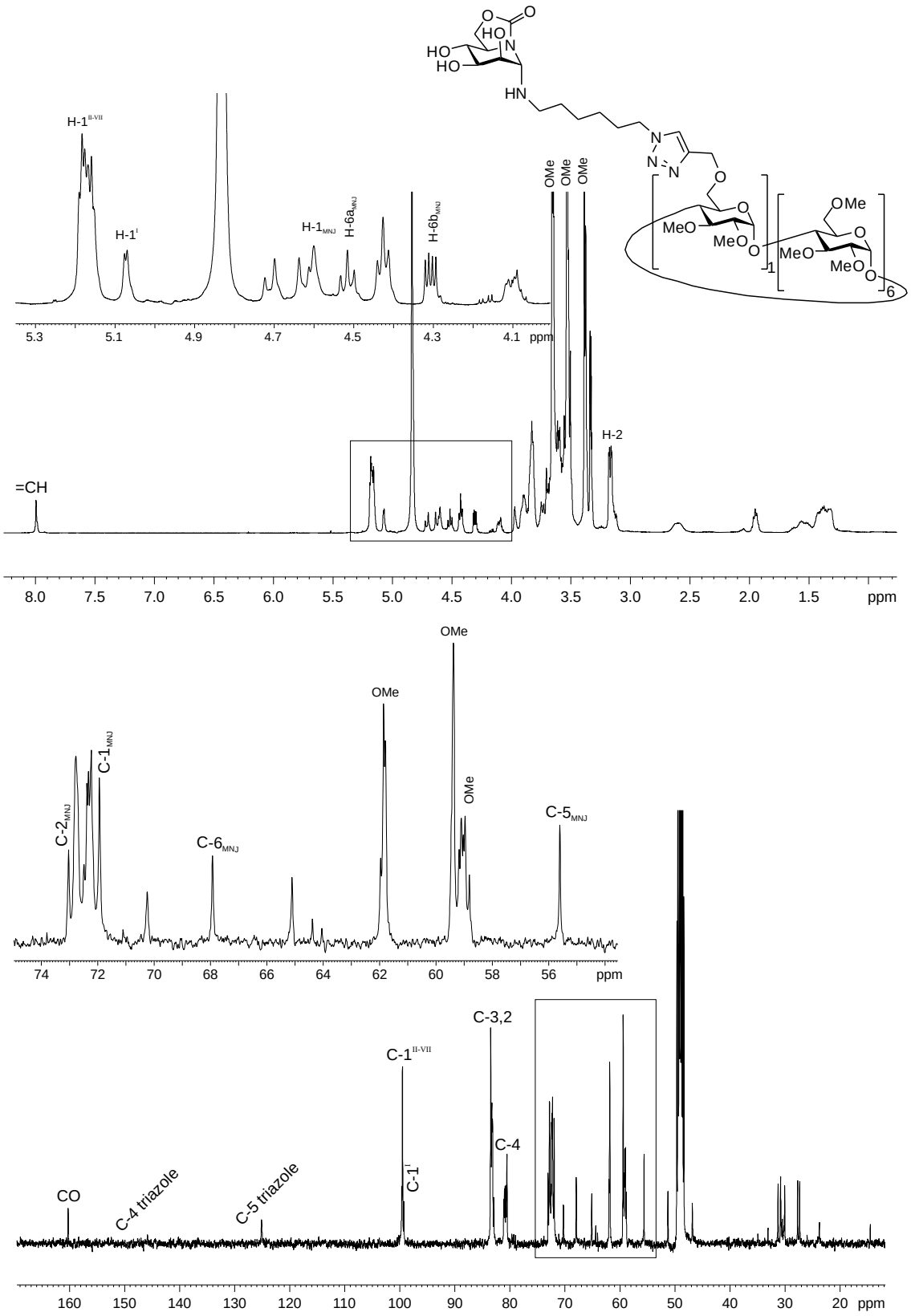

Figure S1. ^{1}H and ^{13}C NMR (500 and 125.7 MHz, CD_3OD) of **6**.

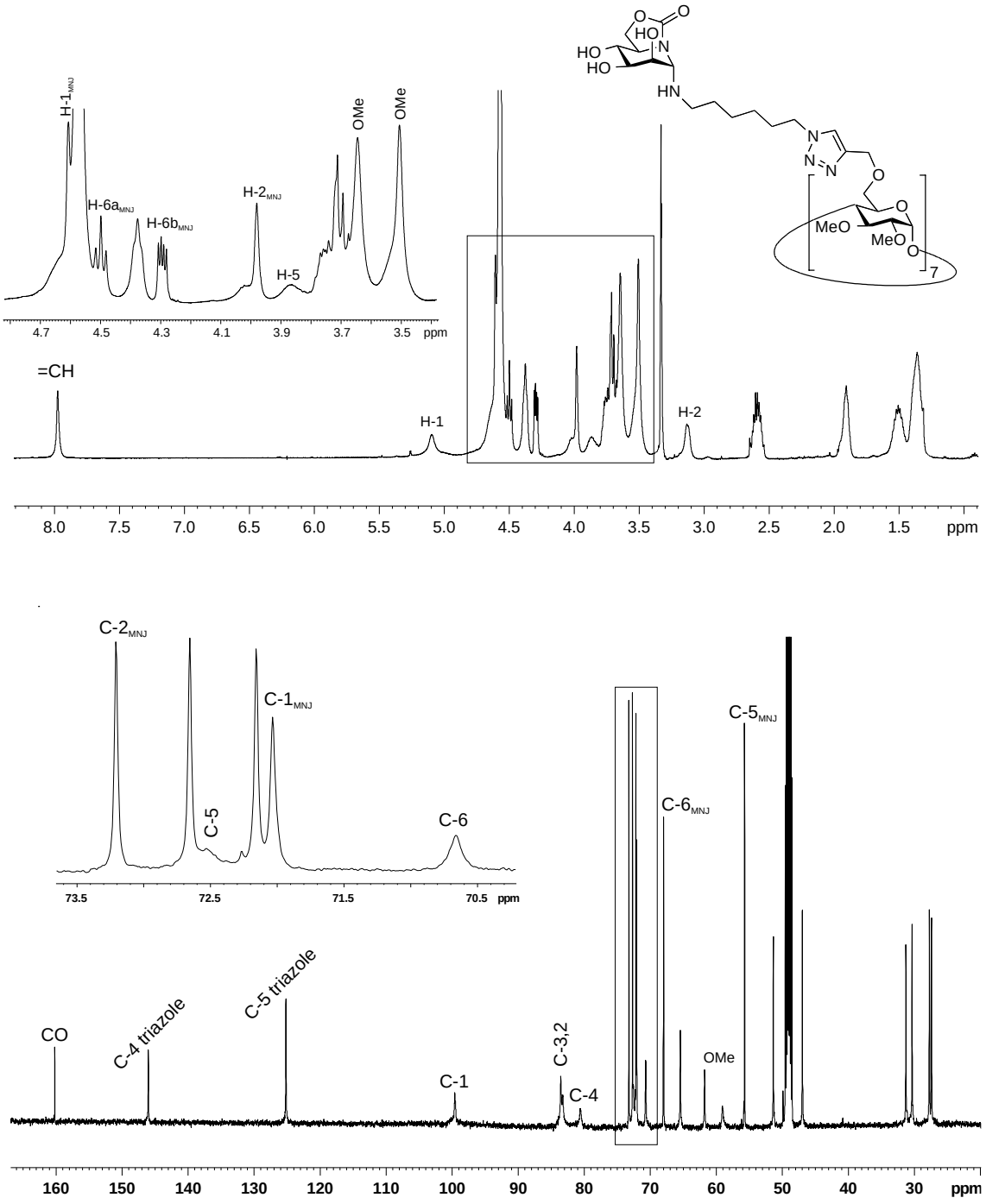

Figure S2.[1]H and [13]C NMR (500 and 125.7 MHz, CD_3OD, 323 K) of **7**.

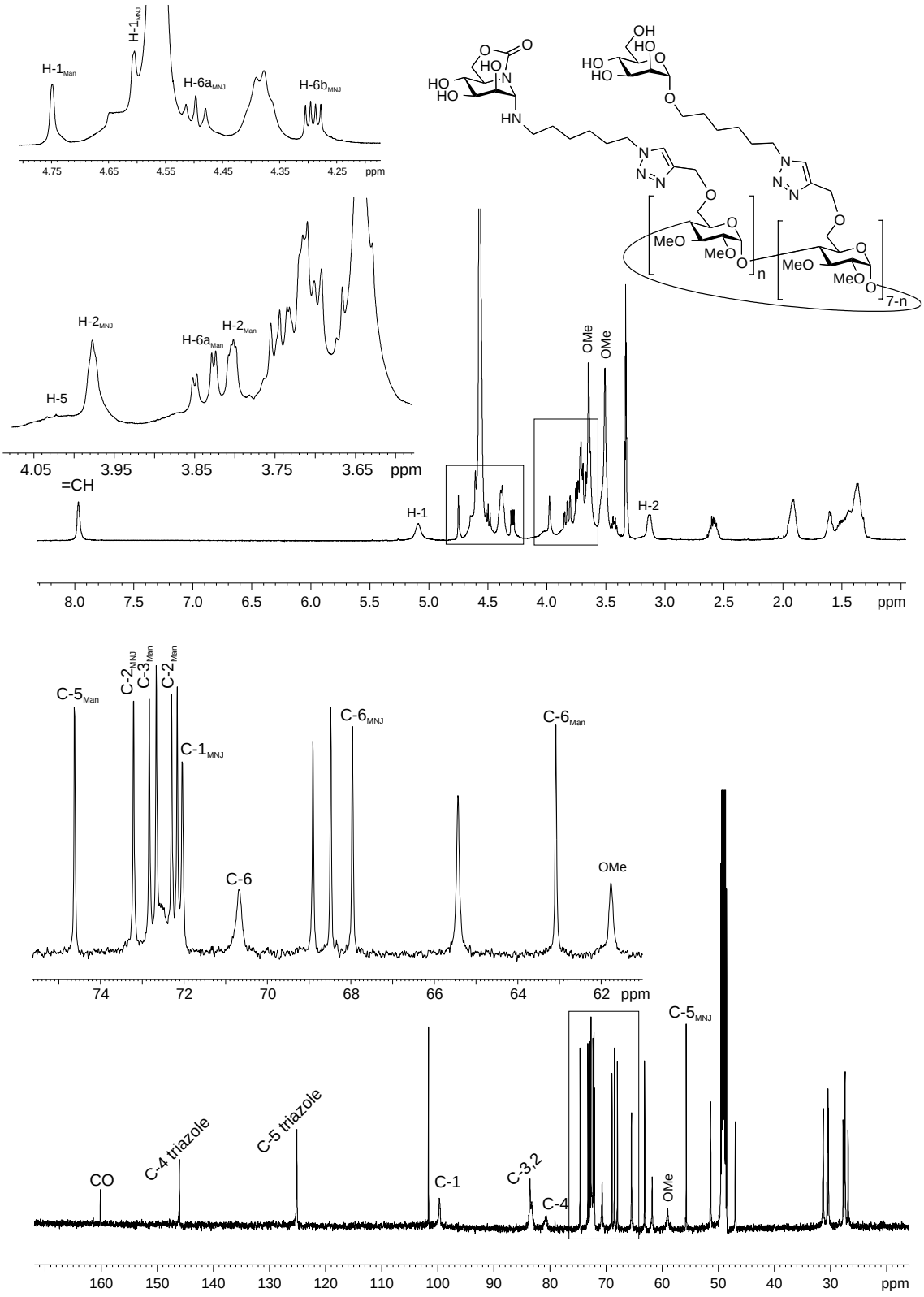

Figure S3.[1]H and [13]C NMR (500 and 125.7 MHz, CD_3OD, 323 K and 313 K, respectively) of **8**.

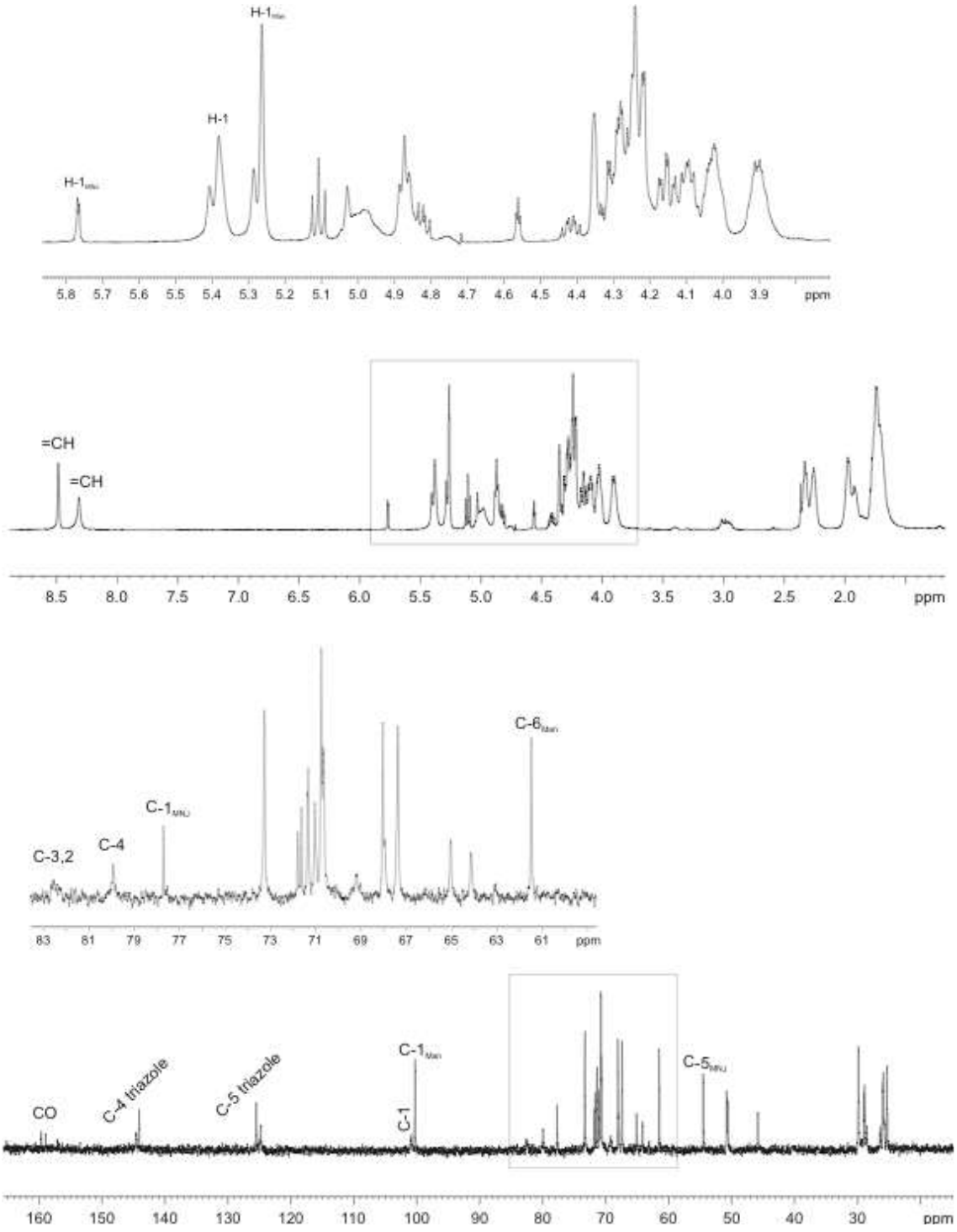

Figure S4.[1]H and [13]C NMR (500 and 100.6 MHz, D_2O, 343 K and 333 K, respectively) of **9**.

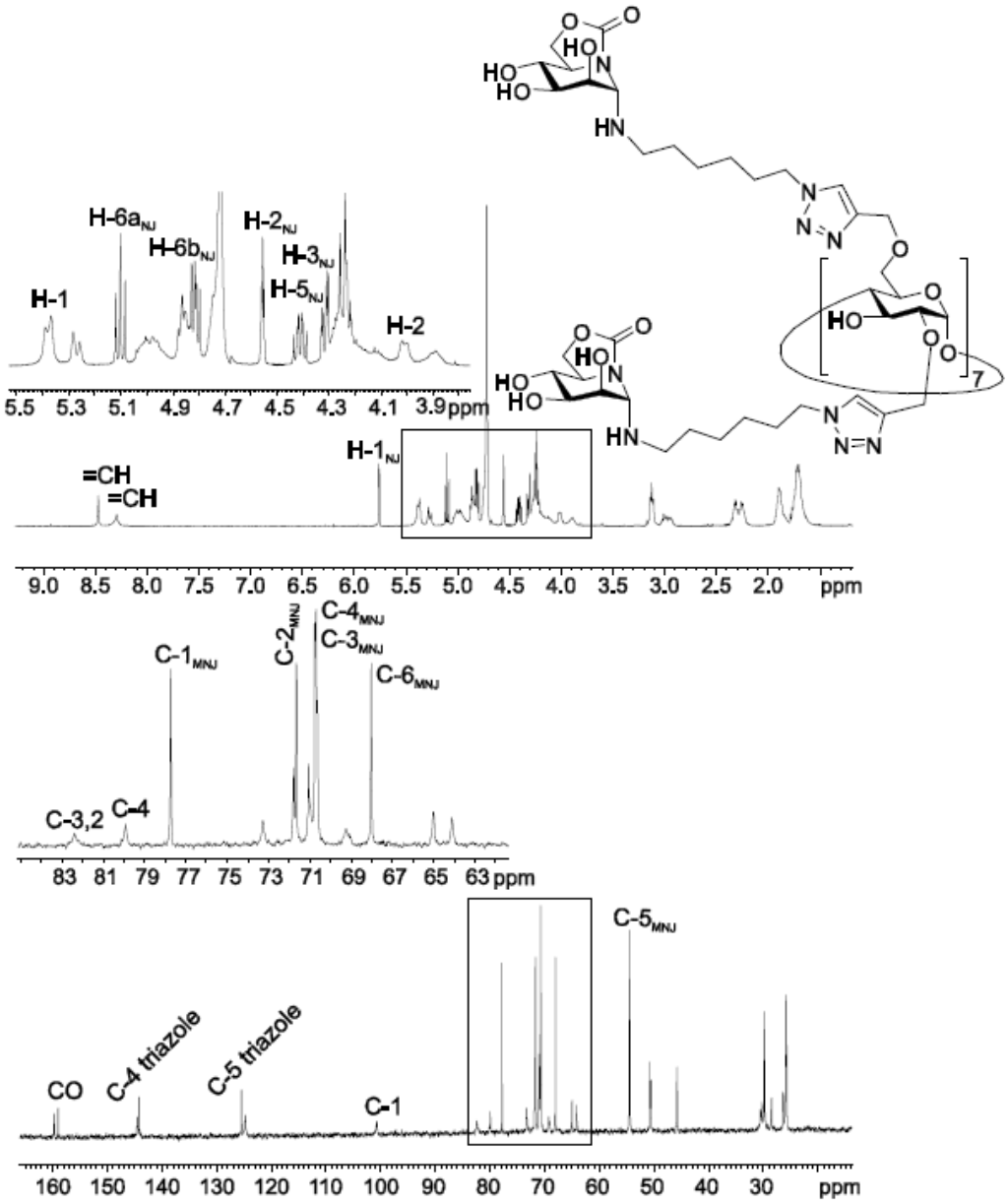

Figure S5.^{1}H and ^{13}C NMR (500 and 100.6 MHz, D_2O, 343 K and 333 K, respectively) of **10**.

Table 1. Inhibition constants values (K_i, μM) for the OMJ derivatives **1**-**10** against commercial glycosidases.

Compound	Enzyme	
	β-glucosidase (bovine liver)	α-mannosidase (Jack beans)
1	260±26	3.2±0.4
5	728±67	4.5±0.5
6	378±30	47±5
7	149±15	0.51±0.05
8	145±14	3.1±0.3
9	n.i.	2.3±0.2
10	147±15	0.026±0.003

[a]Inhibition was competitive in all cases; no inhibition was observed for any of the compounds at 2 mM on yeast α-glucosidase (except **10**, K_i 59±6 μM), yeast Isomaltase (except **10**, K_i 239±24 μM), A*spergillus niger* amyloglucosidase, green coffee α-galactosidase, *E. coli* β-galactosidase, or *Helix pomatia* β-mannosidase (except **10**, K_i 9±0.4 μM). [b]n.i.: no inhibition observed at 2 mM.

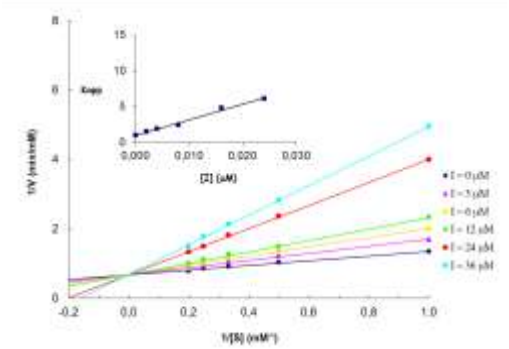

Figure S6. Lineweaver-Burk Plot for K_i determination (4.5 μM) of **5** against Jack bean α-mannosidase.

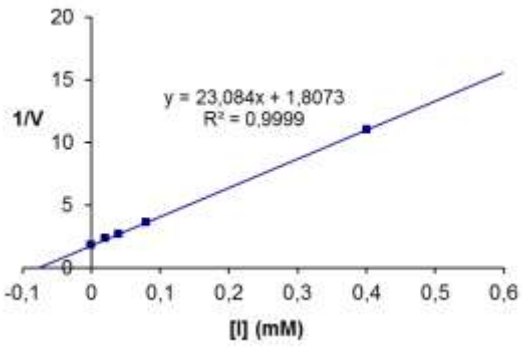

Figure S7. Dixon Plot for K_i determination (47 μM) of **6** against Jack bean α-mannosidase.

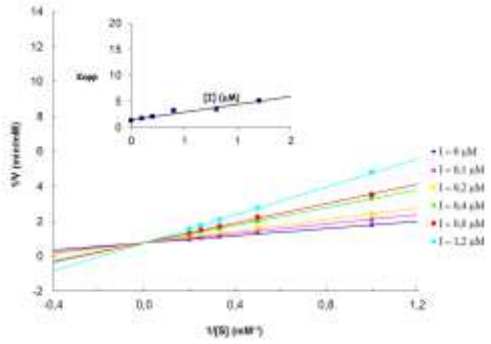

Figure S8. Lineweaver-Burk Plot for K_i determination (0.51 μM) of **7** against Jack bean *α-mannosidase*

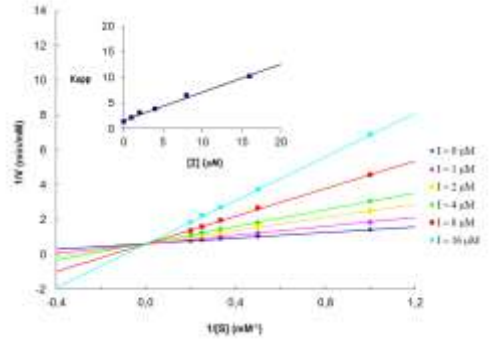

Figure S9. Lineweaver-Burk Plot for K_i determination (3.1 μM) of **8** against Jack bean *α-mannosidase*.

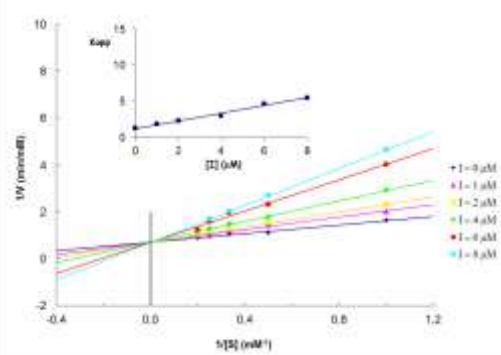

Figure S10. Lineweaver-Burk Plot for K_i determination (2.3 μM) of **9** against Jack bean α-mannosidase.

Procedure to Monitor the Stability of the Heteromultivalent PCs 8 and 9 towards GH38 α-Mannosidase. The susceptibility of towards α-mannosidase hydrolysis of **8** and **9** was examined by incubating each conjugate with the enzyme at 37 ºC under identical conditions to those used for determination of the inhibition constants during 4 h and monitoring the formation of free mannose by gas chromatography-flash ionization detection (GC-FID). For GC analysis, the samples were subjected to an oximation-trimethylsilylation protocol. Briefly, immediately after quenching, the samples were freezedried. To 15-20 mg of each sample, deionized water (1 mL) was added. To 100 µL of the resulting solution, 100 µL of internal standard (I.S.; 4 mg mL^{-1} phenyl β-D-glucopyranoside in acetone-water 1:9, v/v) was then added and the final solution was evaporated to dryness at 60 ºC (drying oven). The residue was treated with 1 mL of a solution of hydroxylamine in pyridine (20 mg mL^{-1}) at 60 ºC over 50 min. with mixing at intervals. Hexamethyldisilazane (200 µL) and trimethylchlorosilane (100 µL) were then added, and the reaction mixtures were kept at 60 ºC over a further 40 min period. Formation of a white precipitate was observed during this operation, which was separated by centrifugation (13.000 rpm, 5 min) before injection in the GC apparatus. It is worth noting that following oximation-trimethylsilylation derivatization, reducing monosaccharides provides two peaks in the GC chromatograms, corresponding to the syn- and anti-TMS-oximes, whereas the I.S. provides a single peak.

GC-FID Analysis. GC-FID was carried out using an Agilent 7820A chromatograph with an EPC injector fitted with a cross-linked 5% phenyl-dimethylsiloxane column (HP-5; 30 m x 320 µm x 0.25 µm). Operating conditions were: injection port temperature 310 ºC; splitting ratio 25:1; injection volume 1 µL of derivatized samples; column oven temperature programmed from180 to 310 ºC at 5 ºC min^{-1}, with a 25 min hold at 310 ºC; carrier gas helium (constant flow at 1.2 mL min-1); detector port temperature 310 ºC. Total acquisition time was 45 min. The identity of D-mannose (elution time 4.8/5.1 min) was confirmed by comparison with the GC chromatrograms of authentic samples. A calibration curve for quantitative determination was built by using a range of concentrations, from which the response factor relative to the I.S. (elution time 8.1 min) was determined.

No formation of free monosaccharides was observed in the above experiments for any of the heterovalent conjugates **8** or **9** against α-mannosidase, meaning that they are resistant to

enzymatic hydrolysis. In contrast, control experiments showed that methyl α-D-mannopyranoside and high mannos oligosaccharide (Man_5 and Man_9) were hydrolyzed by α-mannosidase under these conditions.