Cyclodextrin-mediated crystallization of acid β–glucosidase in complex with amphiphilic bicyclic nojirimycin analogues

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Supporting Information

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General procedure for the inhibition assay against recombinant β-glucocerebrosidase (prGCD). Inhibition constant (K_i) values were determined by spectrophotometrically measuring the residual hydrolytic activities of prGCD against *p*-nitrophenyl β-D-glucopyranoside in the presence of NOI-NJ or 6S-NOI-NJ. Each assay was performed in phosphate buffer (pH 7.3) or phosphate-citrate buffer (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. The mixture was incubated for 10-30 min at 37 ºC 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. Representative examples of the Lineweaver-Burk plots, with typical profile for competitive inhibition mode, are shown in Figures S1 to S4.

Tensiometry. The surface tension of NOI-NJ and 6S-NOI-NJ was measured in phosphate-citrate buffer (pH 5.5) and phosphate buffer (pH 7.3) with a homemade tensiometer based on a Mettler Toledo AL 204 balance.

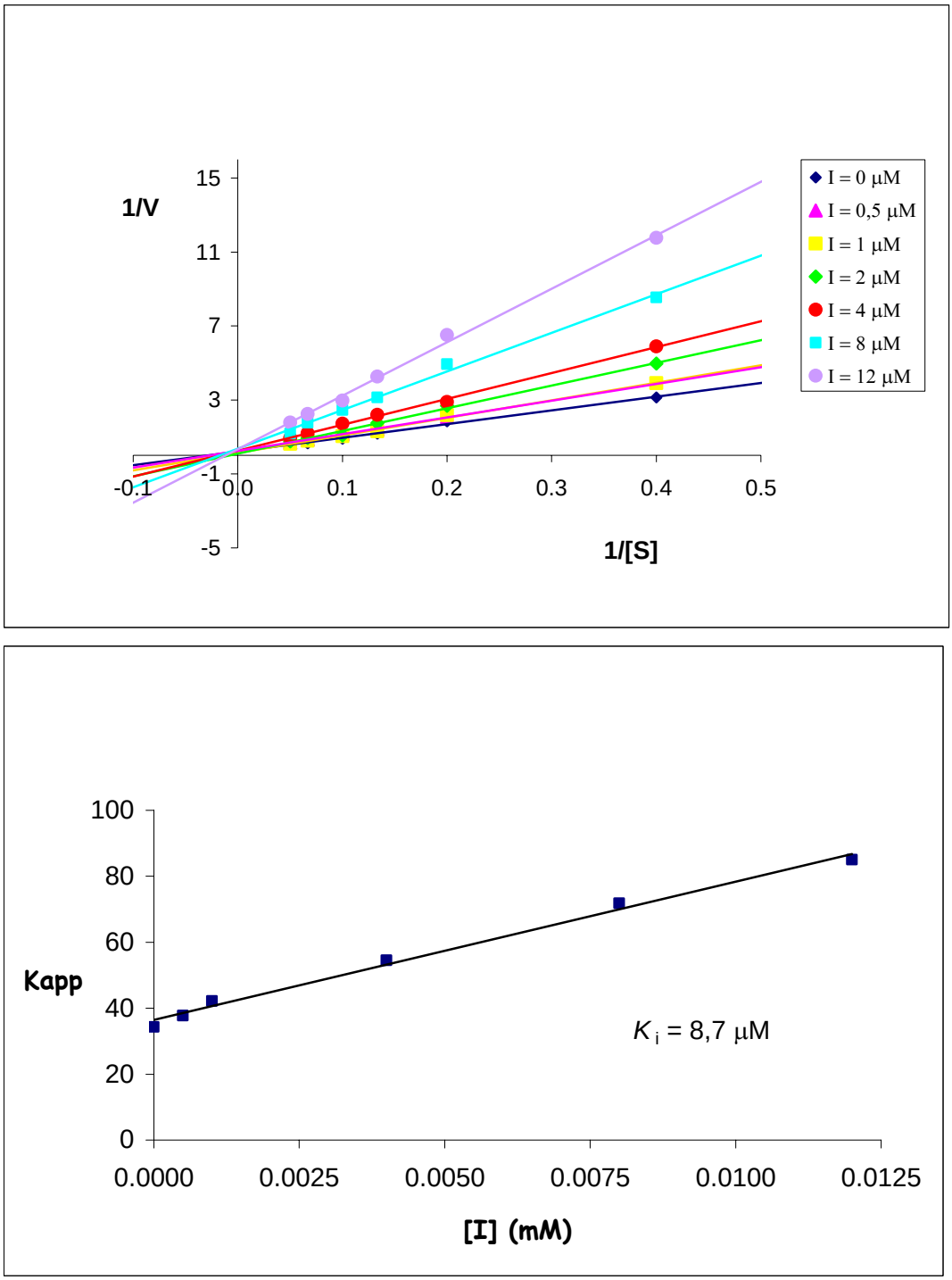


Figure S1. Lineweaver-Burk Plot for K_i determination (8.7 µM) of NOI-NJ against prGCD (pH 5.5).

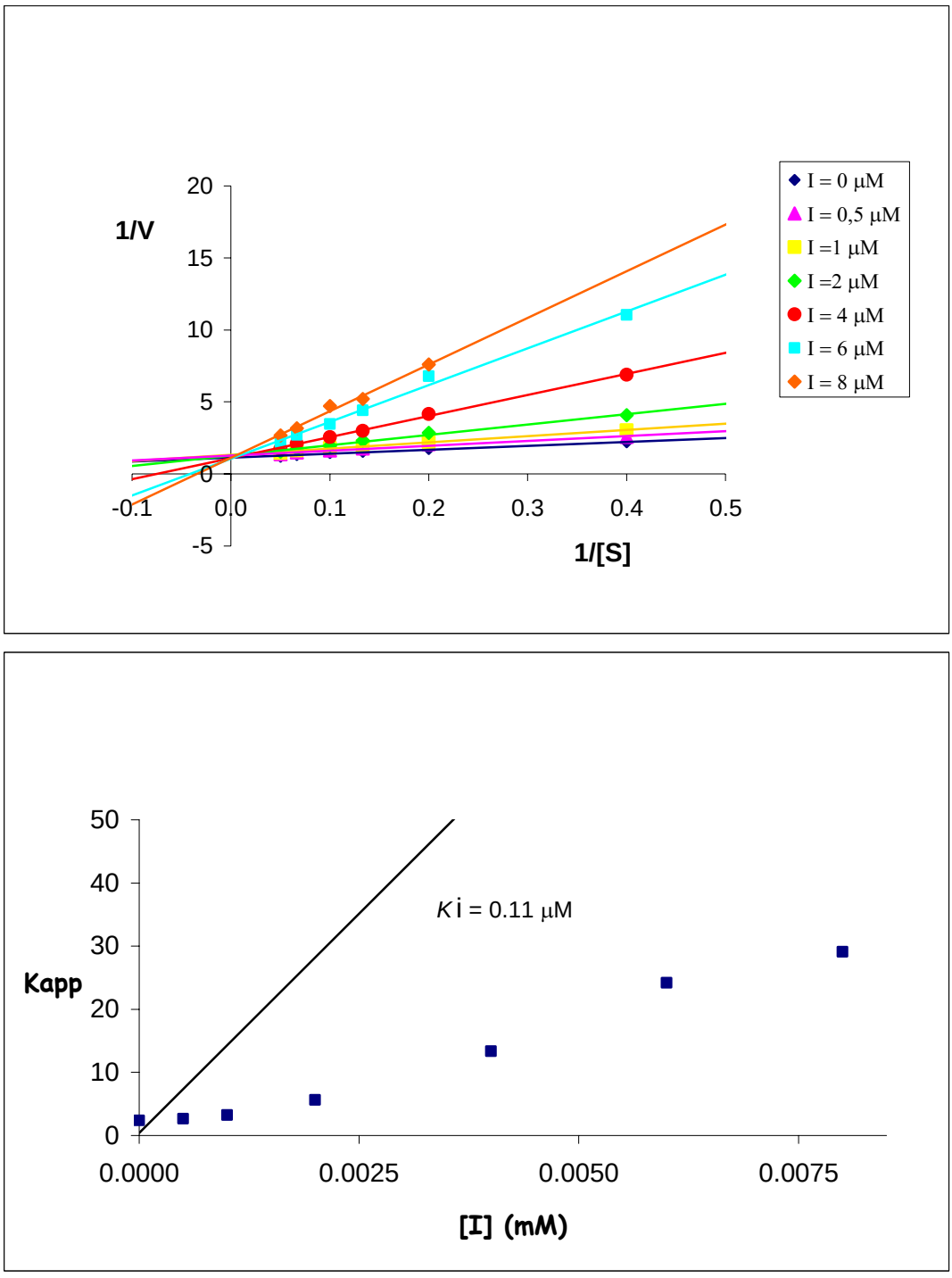
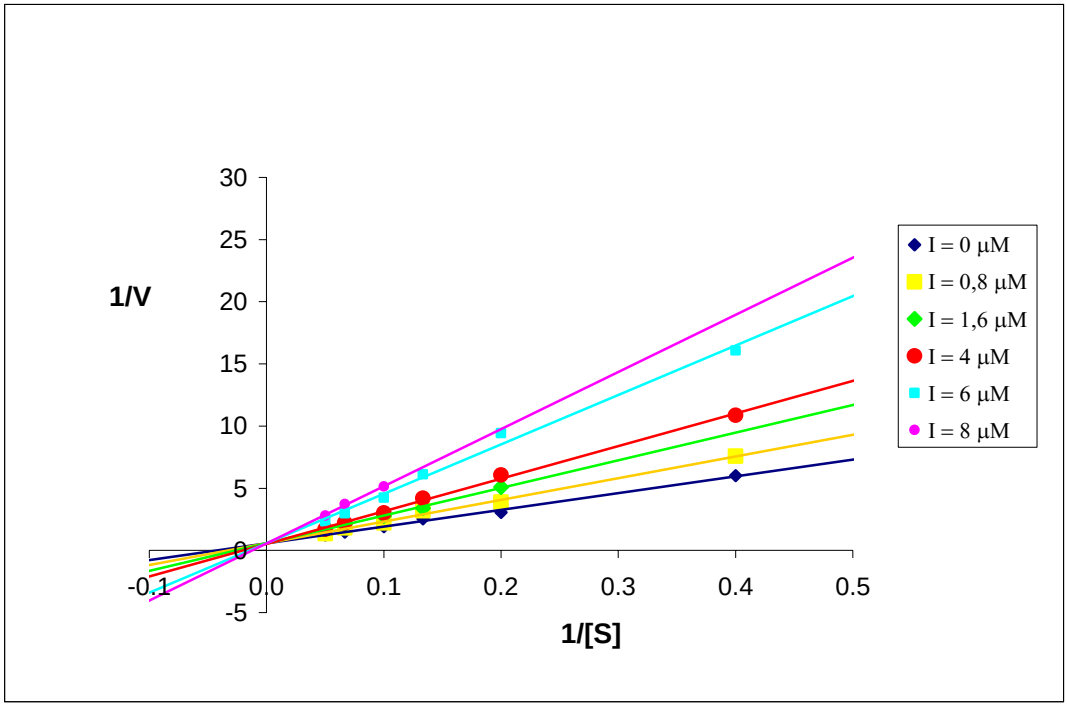


Figure S2. Lineweaver-Burk Plot for K_i determination (0.11 µM) of NOI-NJ against prGCD (pH 7.3).



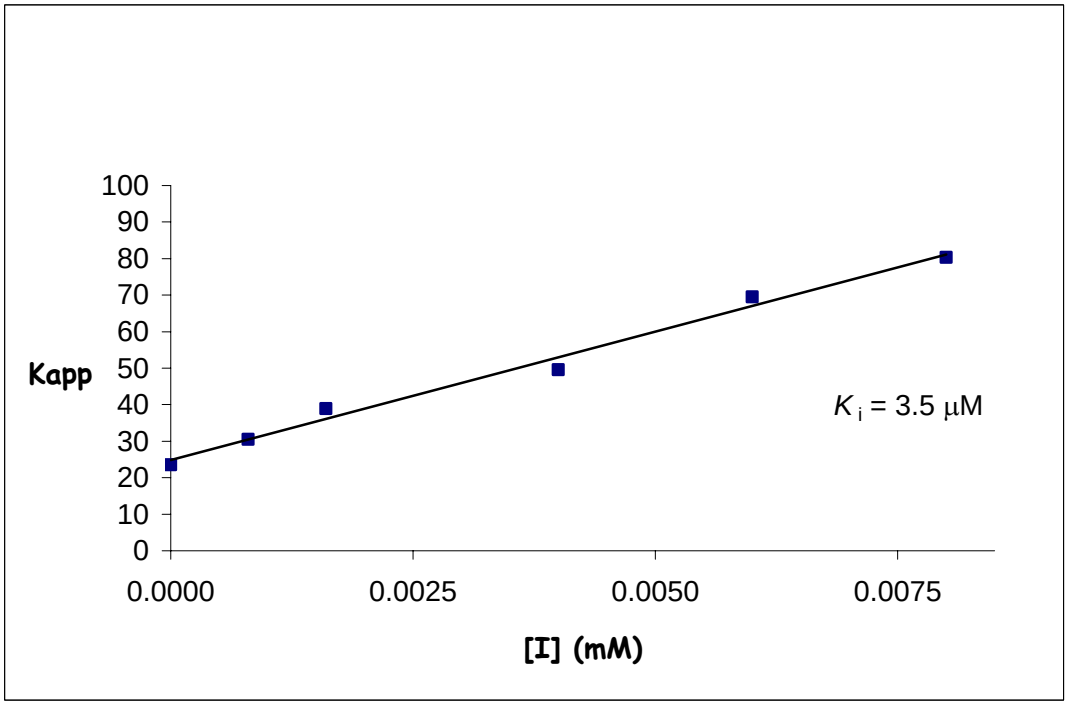


Figure S3. Lineweaver-Burk Plot for K_i determination (3.5 µM) of 6-S-NOI-NJ against prGCD (pH 5.5).

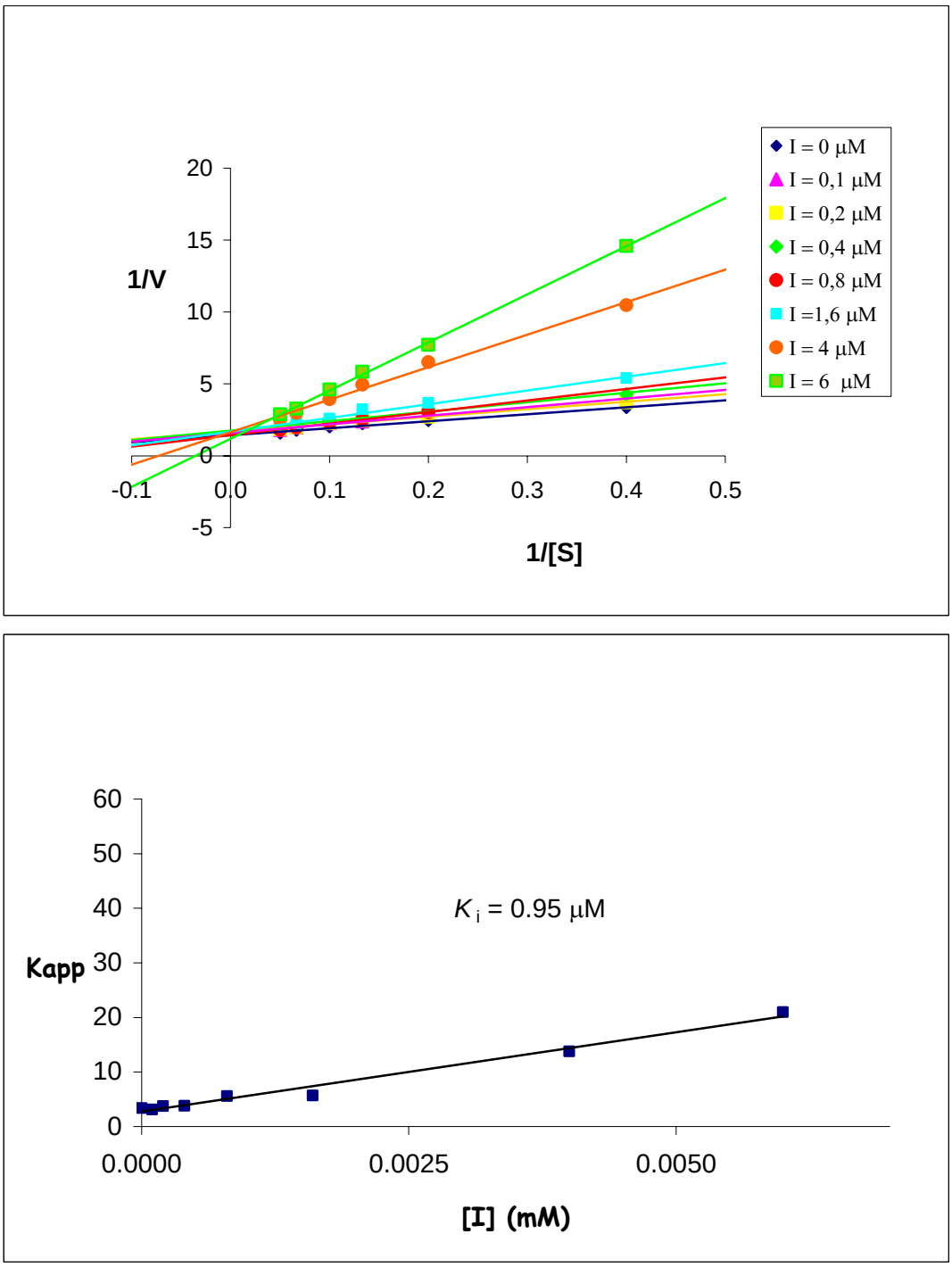


Figure S4. Lineweaver-Burk Plot for K_i determination (0.95 µM) of 6-S-NOI-NJ against prGCD (pH 7.3).

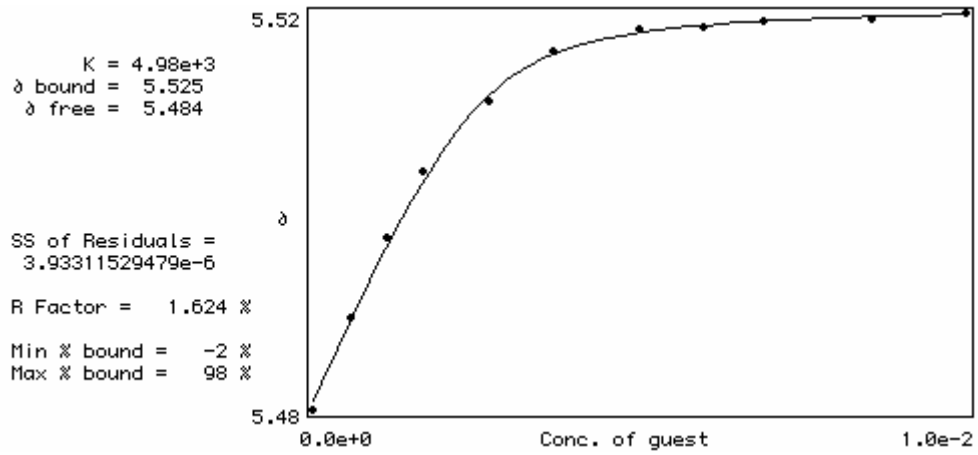


Figure S5. Titration plot obtained from the changes in the chemical shift of the H-1 resonance of NOI-NJ (2.64 mM) in the presence of increasing concentrations of βCD.

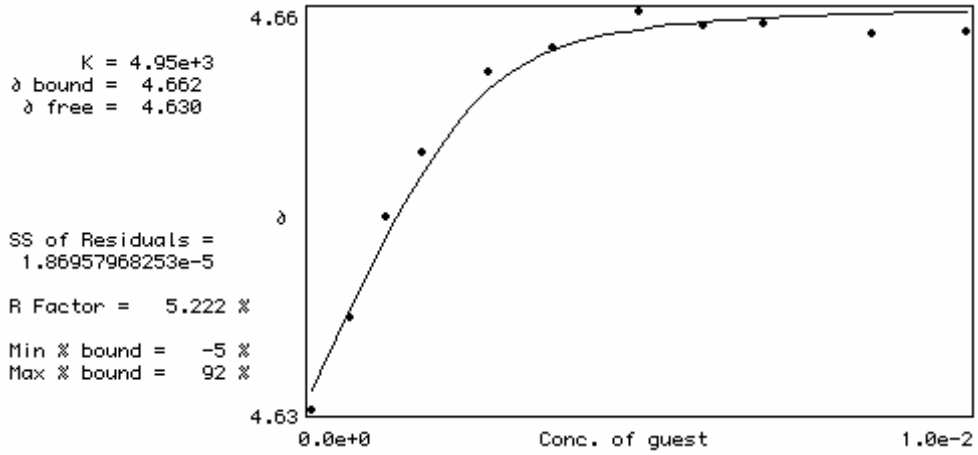


Figure S6. Titration plot obtained from the changes in the chemical shift of the H-6a resonance of NOI-NJ (2.64 mM) in the presence of increasing concentrations of βCD.

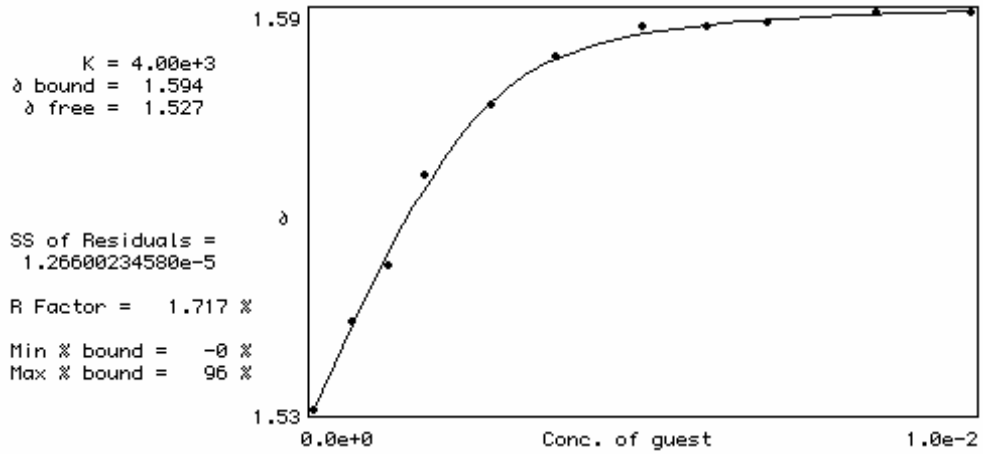


Figure S7. Titration plot obtained from the changes in the chemical shift of the H-6a resonance of NOI-NJ (2.64 mM) in the presence of increasing concentrations of βCD.

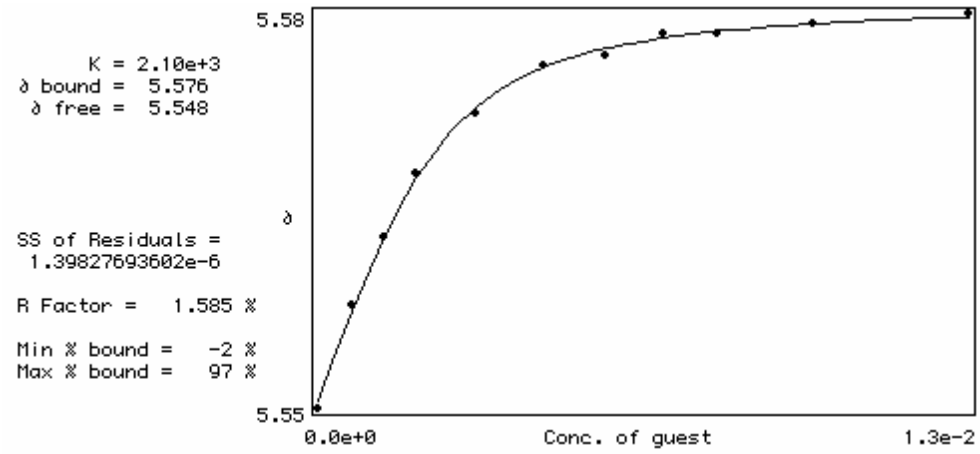


Figure S8. Titration plot obtained from the changes in the chemical shift of the H-1 resonance of 6S-NOI-NJ (2.41 mM) in the presence of increasing concentrations of βCD

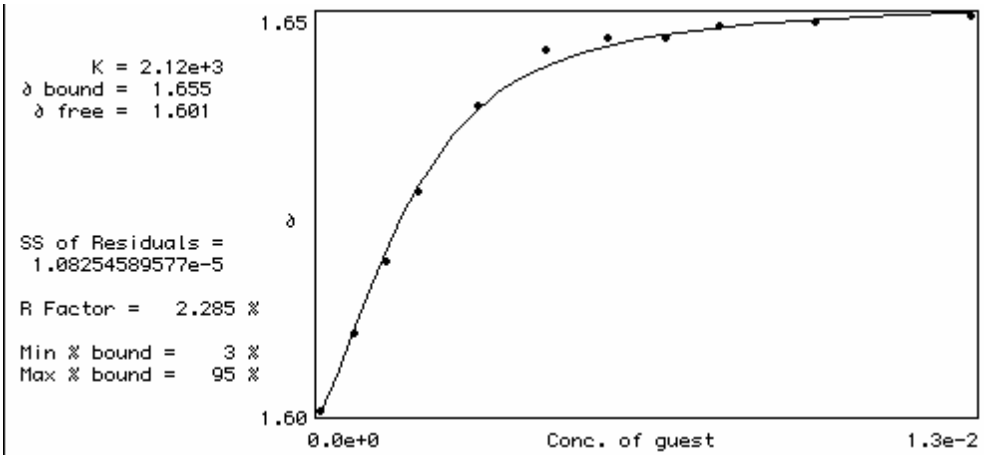


Figure S9. Titration plot obtained from the changes in the chemical shift of the H-6a resonance of 6S-NOI-NJ (2.41 mM) in the presence of increasing concentrations of βCD.

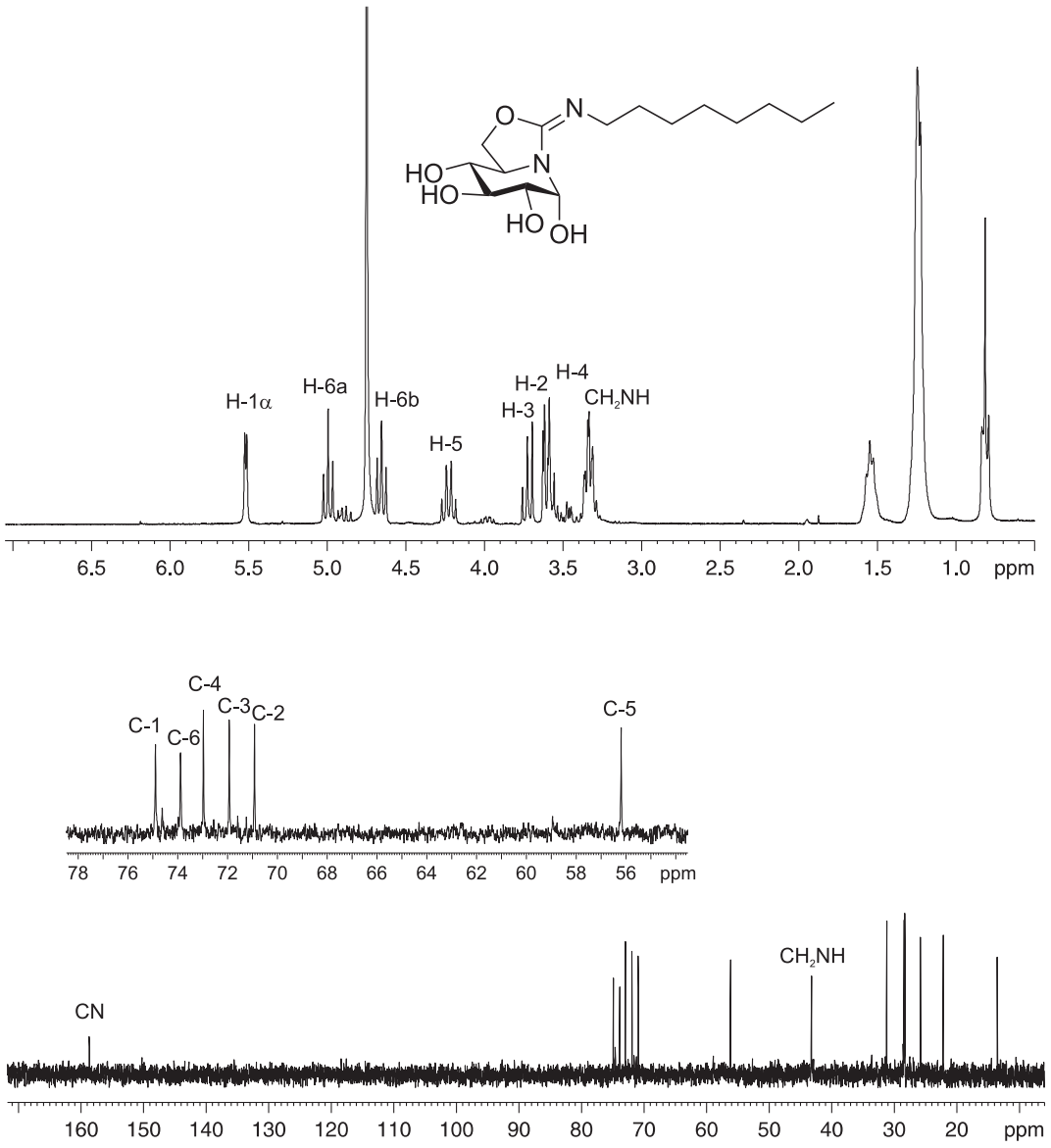


Figure S10. ^{1}H and ^{13}C NMR spectra (300 MHz, 75.5 MHz, D_2O) of NOI-NJ.

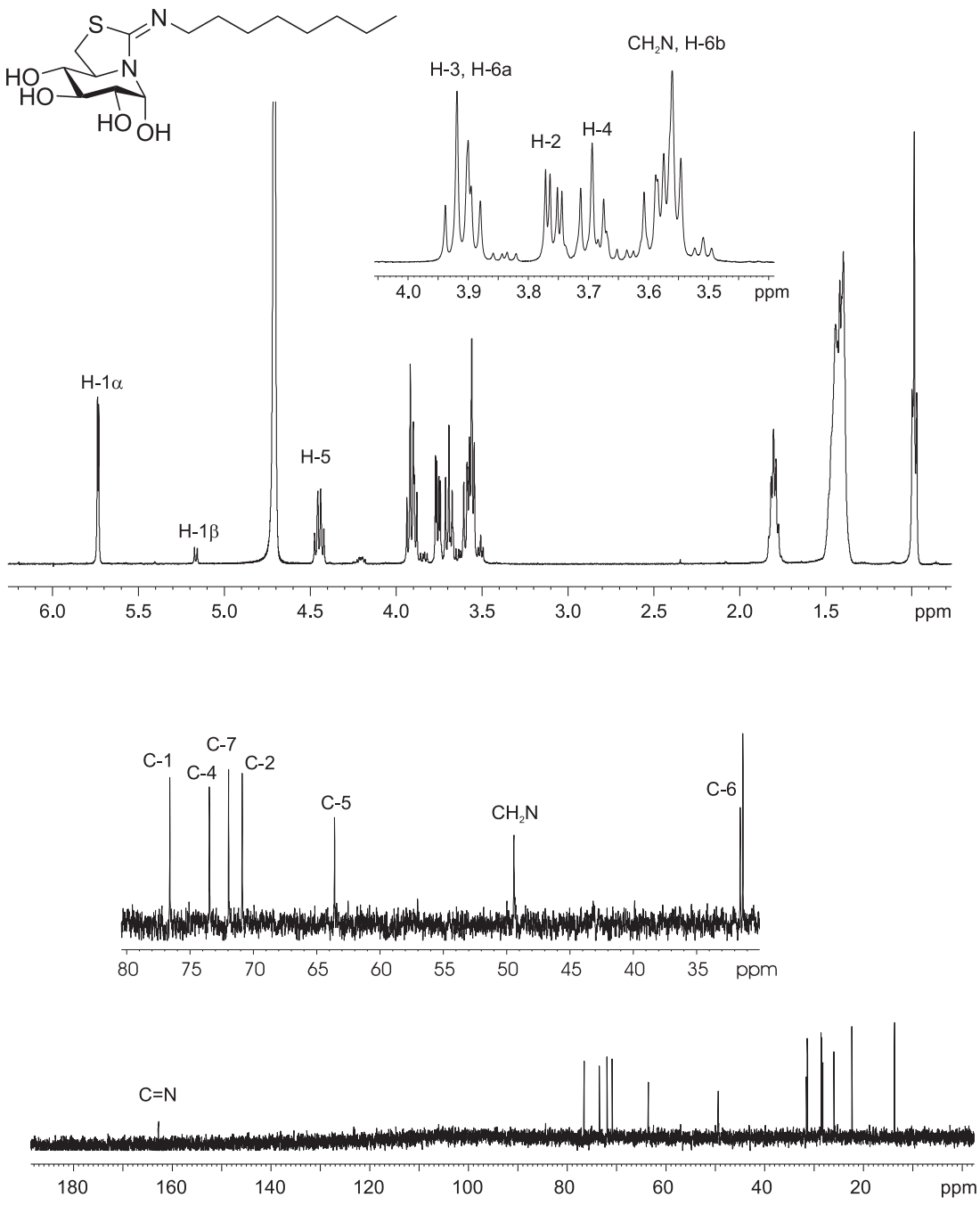


Figure S11. ^{1}H and ^{13}C NMR spectra (500 MHz, 75.5 MHz, D_2O) of 6S-NOI-NJ.

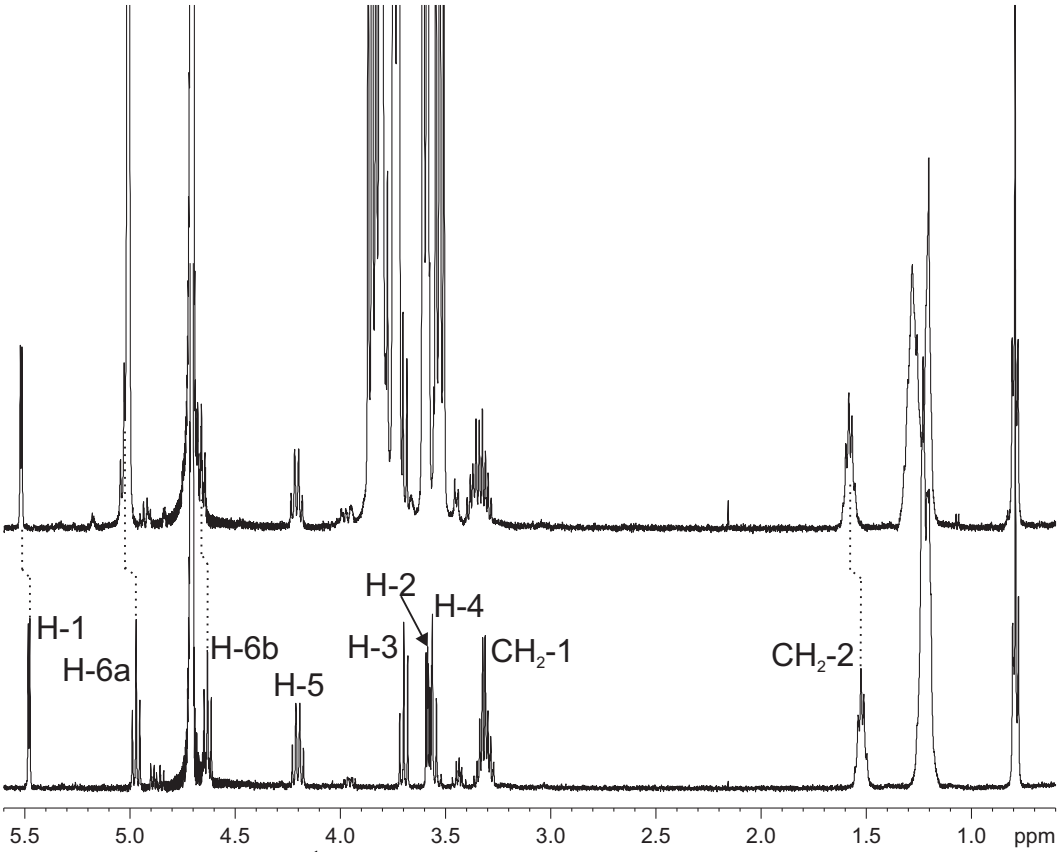


Figure S12. Comparative ^{1}H NMR Spectra of NOI-NJ (2.64 mM, 500 MHz, 298 K, D_2O) in the absence (bottom) and in the presence of βCD (1:1.4 NOI-NJ:βCD ratio; top).

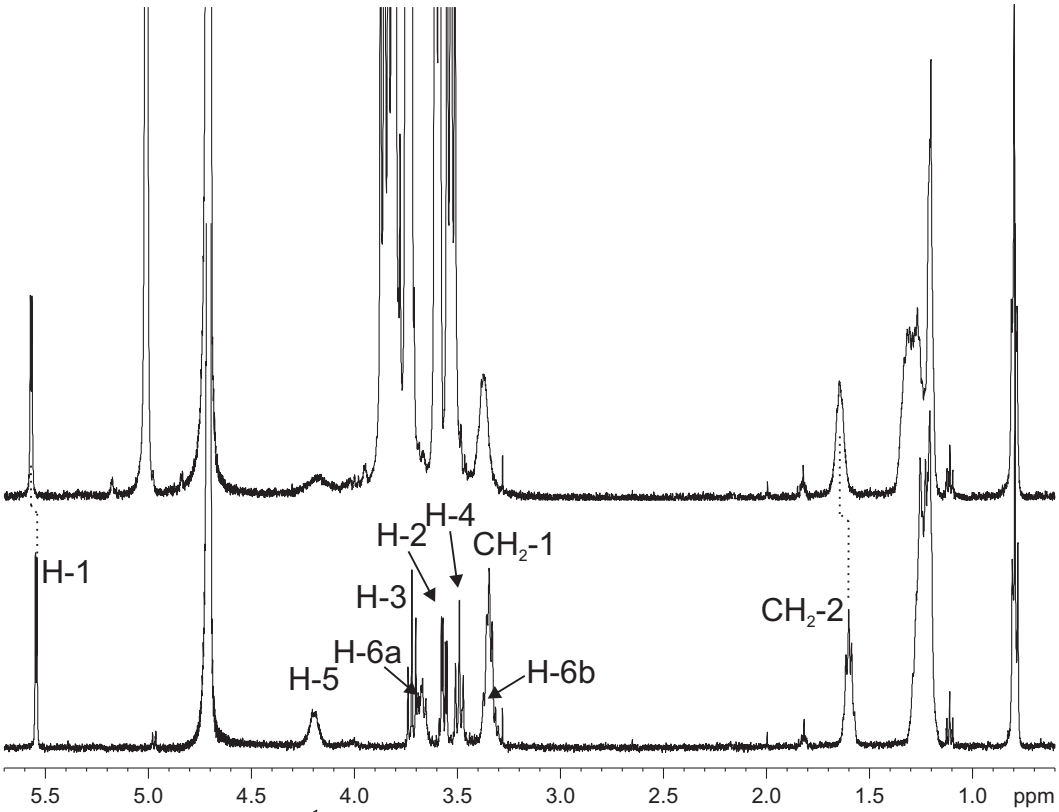


Figure S13. Comparative ^{1}H NMR Spectra of 6S-NOI-NJ (2.41 mM, 500 MHz, 298 K, D_2O) in the absence (bottom) and in the presence of βCD (1:1.7 **6S-NOI-NJ**:βCD ratio; top).