Recognition of Septanose Carbohydrates by Concanavalin A

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Total Pages: 18

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Details of STD Experiments 500MHz Brüker

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scaling in F2           [Hz/cm]
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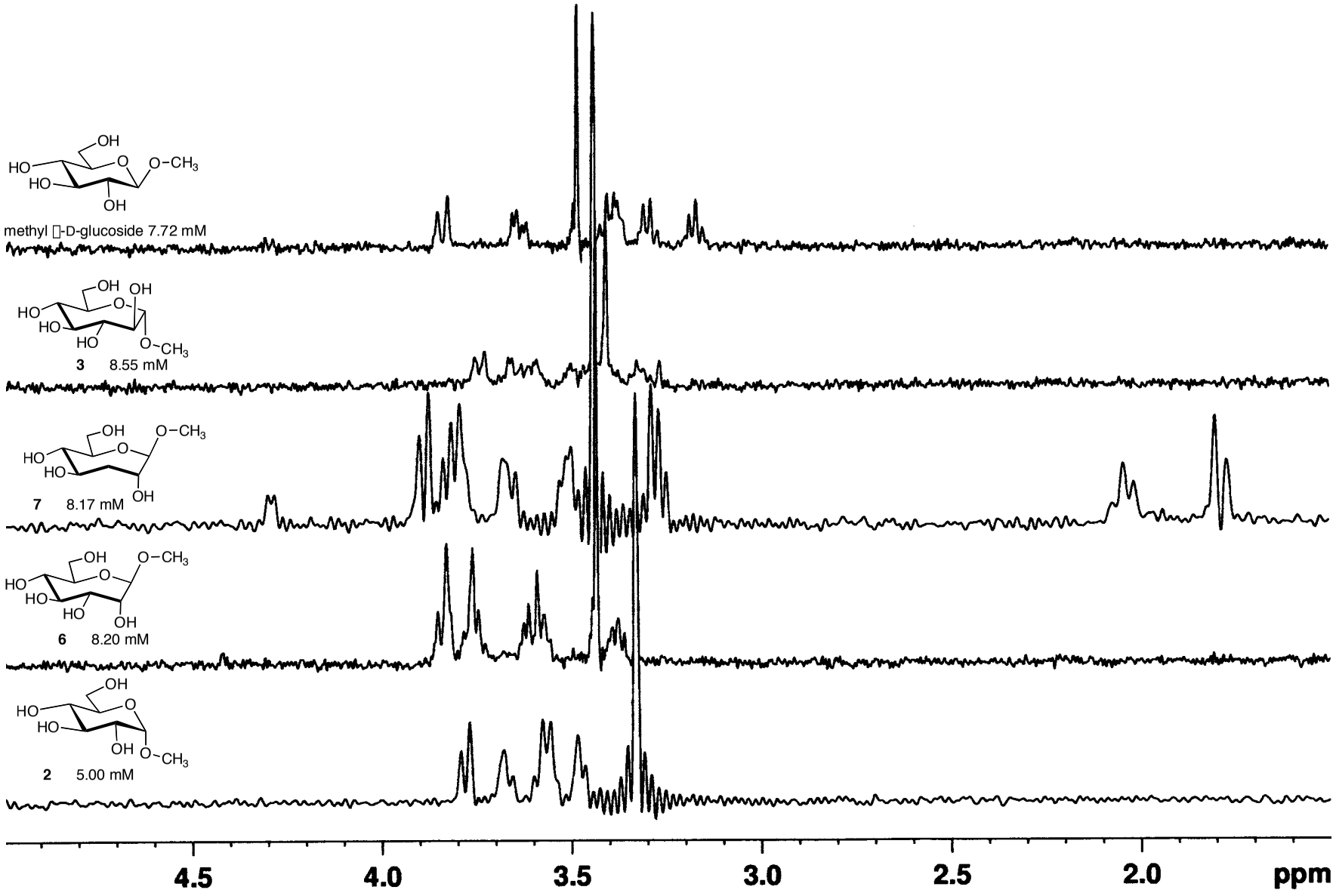
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3 8.55 mM
7 8.17 mM
6 8.20 mM
2 5.00 mM
4.5
4.0
3.5
3.0
2.5
2.0
ppm

References and details for the preparation of **3-7**

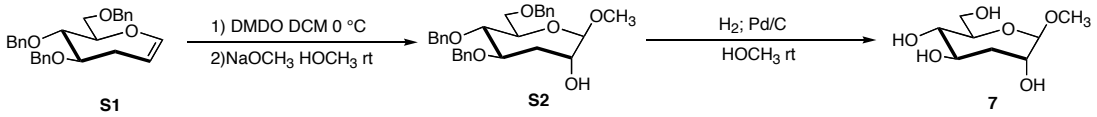
The preparation of **3**-**6** have been reported in the literature.

3, **6**: DeMatteo, M.; Snyder, N. L..; Morton, M.; Baldisseri, D. M.; Hadad, C. M.; Peczuh, M. W.; Septanose Carbohydrates: Synthesis and Conformational Studies of Methyl-α-D-*Glycero*-D-idoseptanoside and Methyl β-D-*Glycero*-D-guloseptanoside. *J. Org. Chem.* **2005***, 70*, 24-38.

4: Castro. S.; Peczuh, M. W.; Sequential Cyclization-Elimination Route to Carbohydrate Based Oxepines *J. Org. Chem.* **2005**, *70*, 3312-3315.

5: Peczuh, M. W.; Snyder, N. L.; Fyvie, W. S. Synthesis, crystal structure and reactivity of a D-xylose based oxepine. *Carbohydr. Res.* **2004**, *339*, 1163-1171.

Scheme S1 Synthesis of **7**

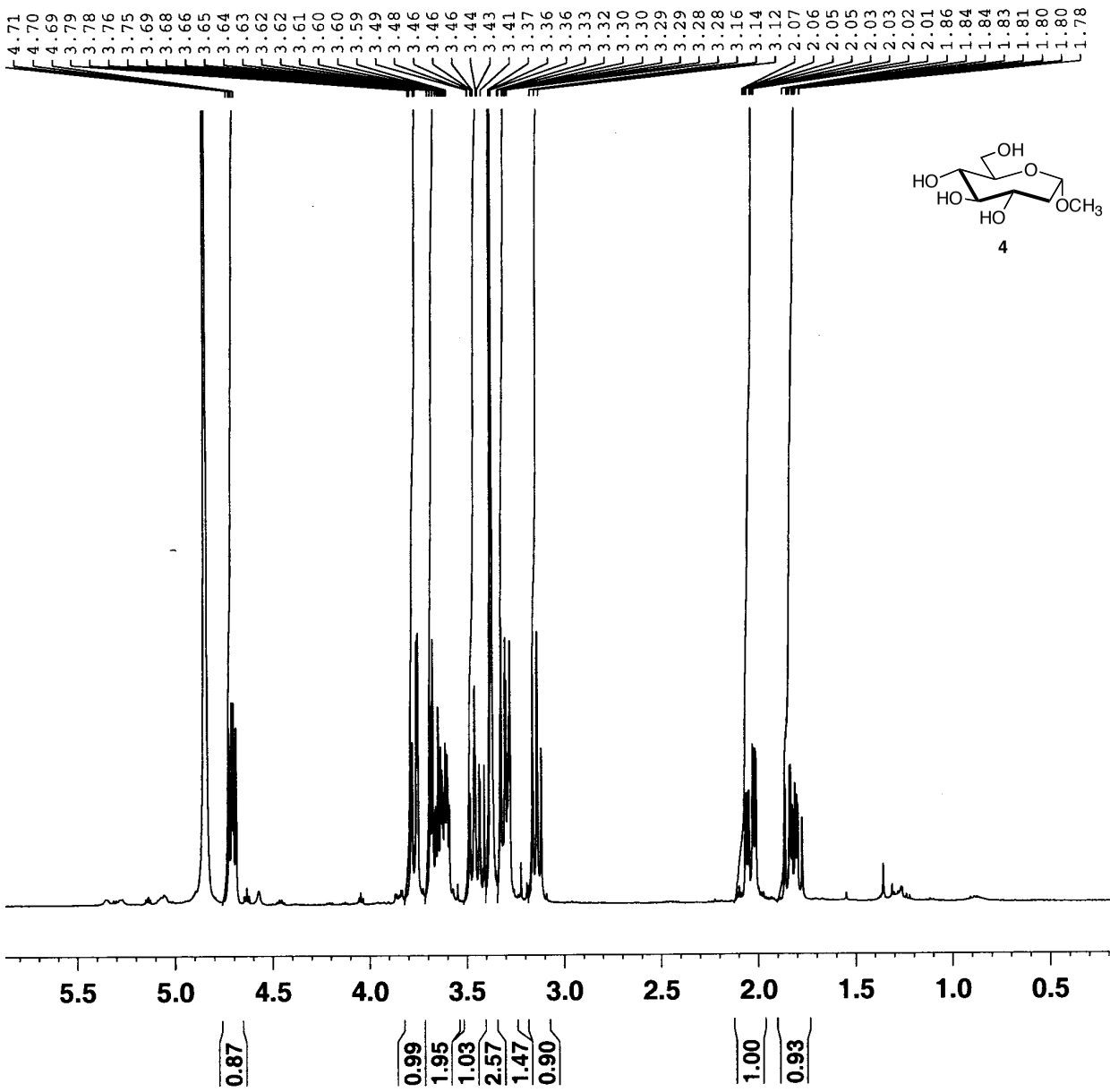


S1 S2 7

Methyl 4,5,7-tri-O-benzyl-3-deoxy-α-D-*glycero*-D-guloseptanoside (S2) Oxepine **S1**[1] (0.050 g, 0.116 mmol) was azeotroped from toluente (3 x 5 mL), dissolved in dry CH_2Cl_2 (5 mL), and cooled to 0 °C. DMDO (0.278 mL of a 0.2 M solution in CH_2Cl_2) was added and the mixture was stirred at 0 °C for 30 min. The solvent was removed under reduced pressure and a solution of $NaOCH_3$ (0.010 g) in CH_3OH (5 mL) was added to the residue. The mixture was stirred overnight (12 h) at rt. The reaction was quenched with water (2 mL) and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (15 mL) and washed with water (2 x 15 mL), dried (Na_2SO_4) and the solvent was removed under reduced pressure. The residue was purified by column chromatography (1:3 EtOAc-Hexanes) to give a clear and colorless oil (0.034 g, 61%). R_f=0.65 (1:1 EtOAc-Hexanes); $[\alpha]_D$ -6.79° (*c* 2.40, $CHCl_3$); ^{1}H NMR 400 MHz (CDCl) δ 7.34-7.17 (m, 15H), 4.65 (d, 1H, *J* = 12.1 Hz), 4.55 (m, 3H), 4.51 (d, 1H, *J* = 2.9 Hz), 4.31 (d, 1H, *J* = 11.4 Hz), 4.17 (d, 1H, *J* = 7.5 Hz), 3.89 (ddd, 1H, J = 13.0, 8.9, 4.2 Hz), 3.83 (dd, 1H, *J* = 7.2, 4.4 Hz), 3.70-3.68 (m, 1H), 3.63-3.61 (m, 2H), 3.53-3.50 (m, 4H) 2.20 (ddd, 1H, *J* = 10.5, 6.9, 2.7 Hz), 1.89 (dd, 1H *J* = 14.7, 9.1 Hz); ^{13}C NMR 100 MHz (CDCl) δ 138.5, 138.0, 128.6 (2), 128.5, 128.1, 128.0 (2), 127.9, 127.8 (2), 110.3, 80.7, 79.9, 76.2, 73.5, 73.0, 71.7, 71.3, 70.0, 56.2, 31.6; FAB-MS *m/z* $[M+H]^+$ calcd 479.2434, found 479.2431.

Methyl 3-deoxy-α-D-glycero-D-guloseptanoside (7) 10% Pd/C (0.006 g) was added to a solution of **S2** (0.034 g, 0.071 mmol) in CH_3OH (5 mL). The reaction was placed under an H_2 atmosphere via a balloon and the mixture was stirred for 4 h at rt. The balloon was removed from the flask and the mixture was filtered through a short pad of celite. The celite was washed with additional CH_3OH (4 x 5 mL). The solvent was removed from the combined filtrates by rotary evaporation under reduced pressure to give a clear, colorless oil (0.015 g, 98%). $[\alpha]_D$ -6.2° (*c* 1.54, CH_3OH); ^{1}H NMR 400 MHz (CD_3OD) δ 4.2 (d, 1H, *J* = 6.7 Hz), 3.84 (dd, 1H, *J* = 11.8, 2.6 Hz), 3.80-3.74 (m, 2H), 3.65 (dd, 1H, *J* = 11.8, 6.7 Hz), 3.5 (s, 3H), 3.41-3.35 (m, 1H), 3.20 (dd, 1H, *J* = 8.2, 8.2 Hz), 2.02 (ddd, 1H, *J* = 14.7, 10.5, 4.8 Hz), 1.81 (ddd, 1H, *J* = 14.5, 3.6, 1.4 Hz); ^{13}C NMR 100 MHz (CD_3OD) δ 112.3, 84.2, 76.6, 71.7, 70.7, 64.3, 56.4, 35.7; FAB-MS *m/z* $[M-H]^+$ calcd 207.0869, found 207.0886.

(1) Peczuh, M. W.; Snyder, N. L. Carbohydrate Based Oxepines: Ring Expanded Glycals for the Synthesis of Septanose Saccharides. *Tetrahedron Lett.* **2003***, 44,* 4057-4061.

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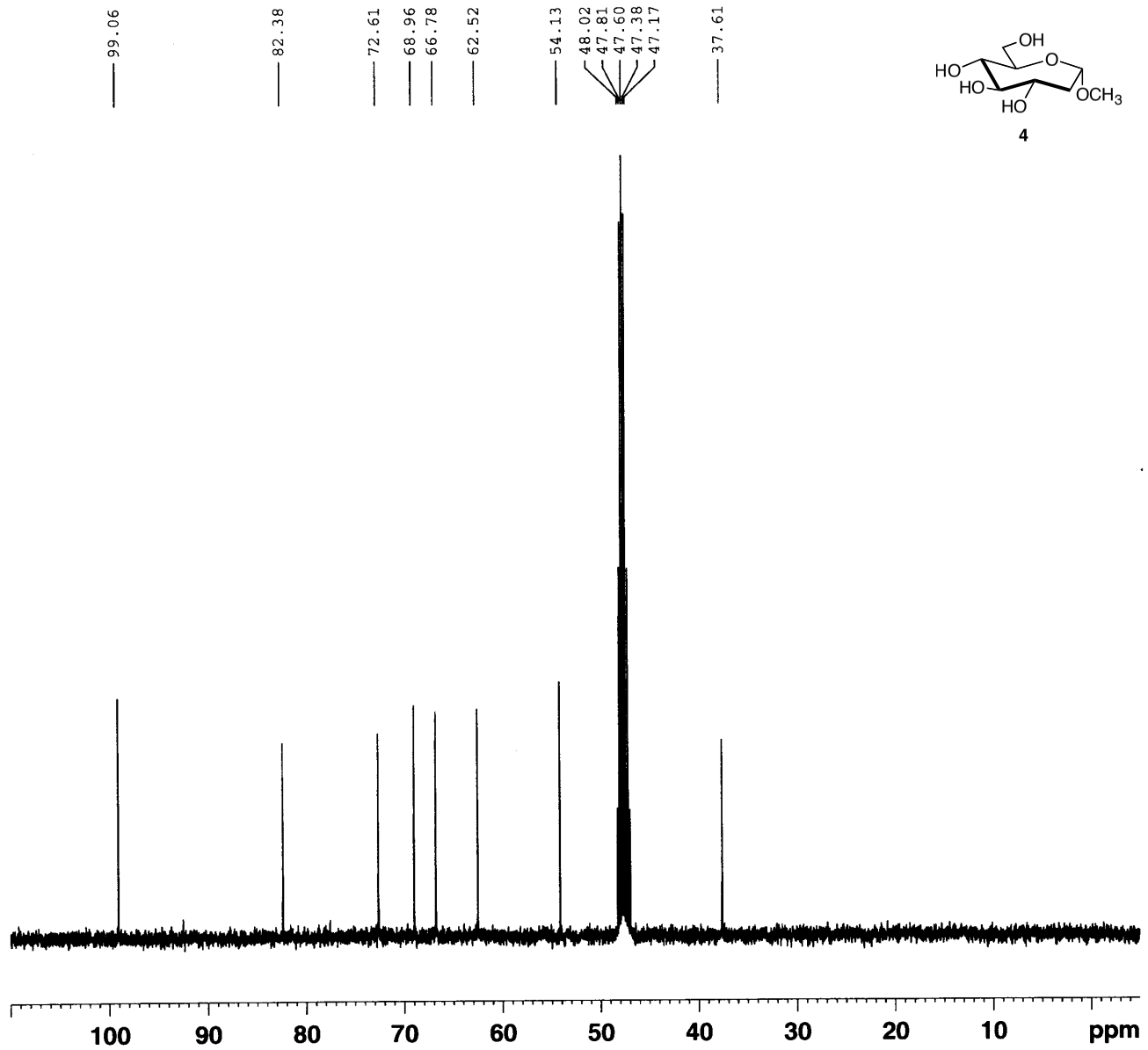


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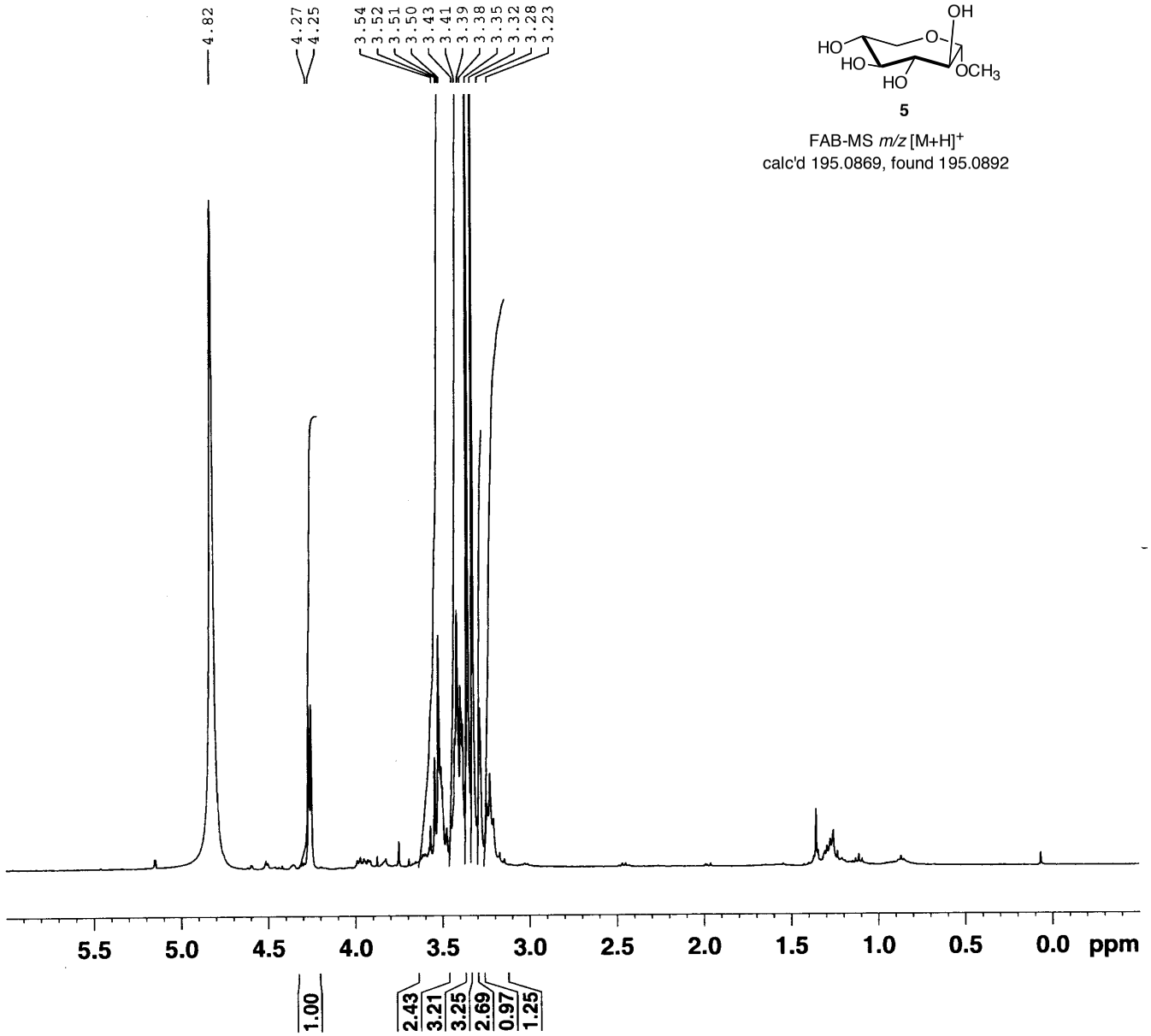

5

FAB-MS *m/z* $[M+H]^+$
calc'd 195.0869, found 195.0892

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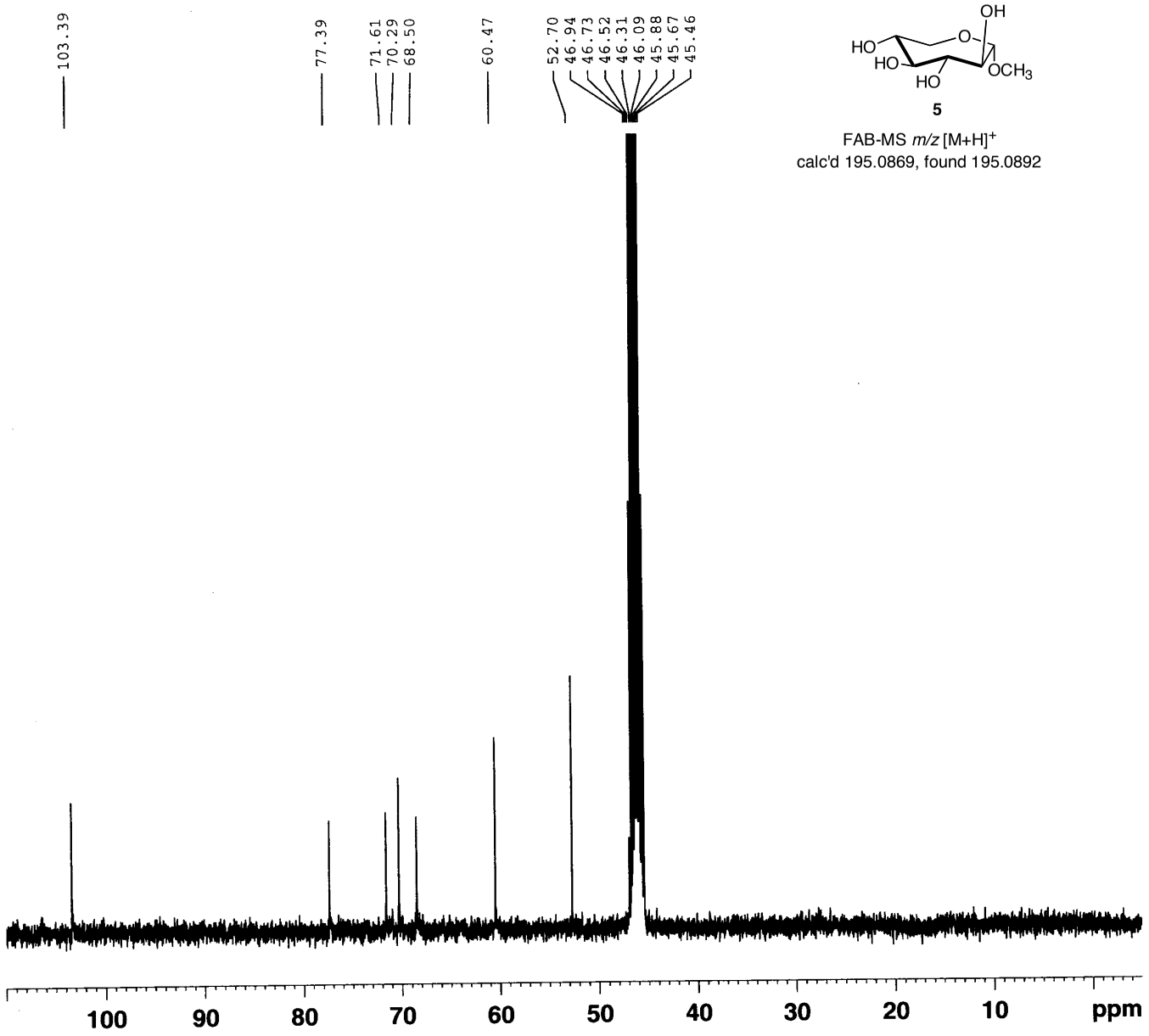

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FAB-MS *m/z* $[M+H]^+$
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PL13              19.00 dB
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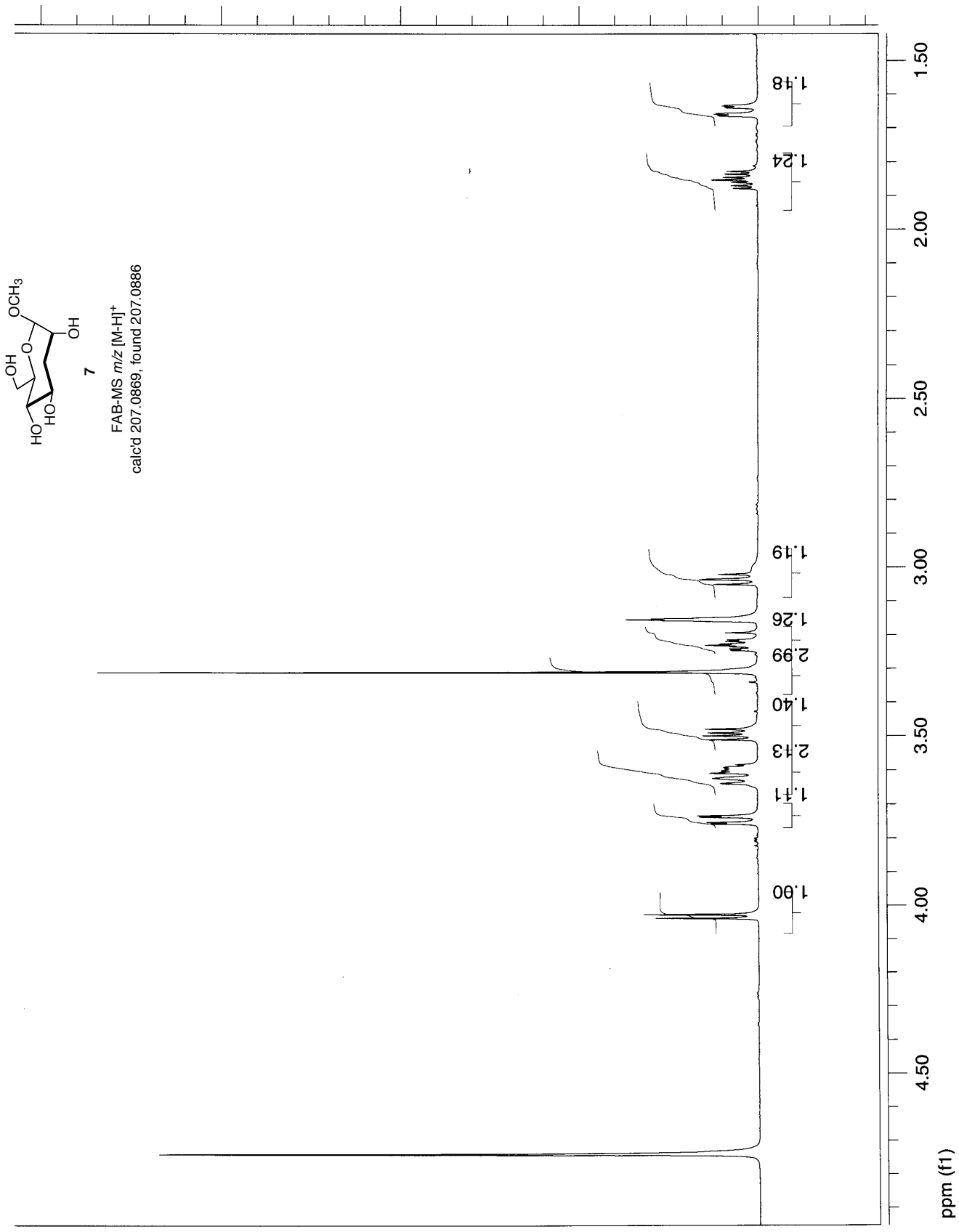
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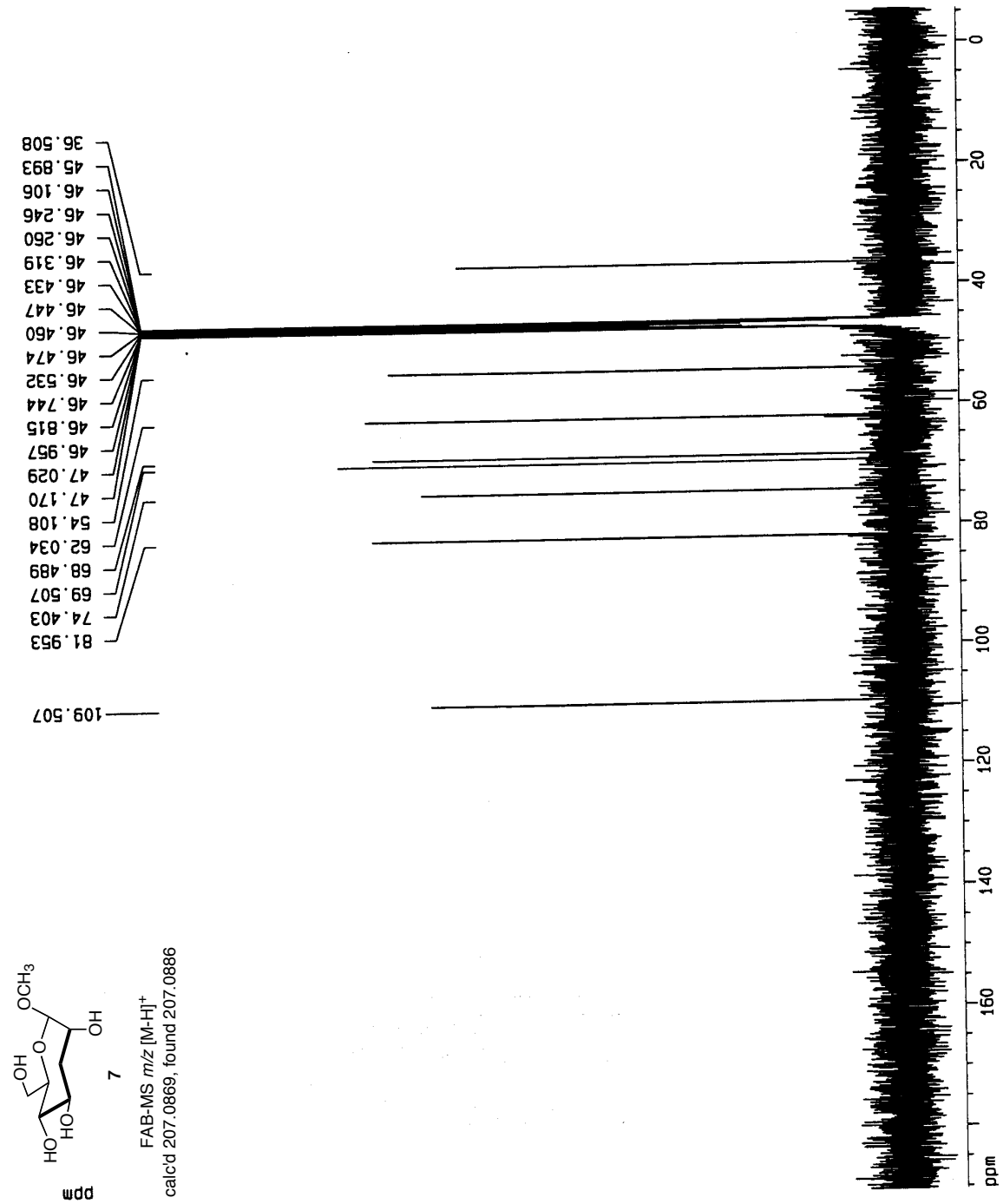
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FAB-MS m/z [M-H]+
calc'd 207.0869, found 207.0886

Isothermal Titration Calorimetry

Measurements were made with MicroCal VP-ITC. The buffer used in all titrations was 50 mM 3,3-dimethylglutarate pH 5.2, 250 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$. For a given experiment, a solution of ConA ranging in concentration from 200 mM to 300 mM was placed in the cell and titrated with a solution of the saccharide ligand (**1**-**7**) over a course of 35 7 □L injections. A delay time of 5 min between injections was utilized. The cell volume was 1.4167 mL. Heat was generated upon each injection which resulted from protein-ligand association. Heats of dilution, collected from a blank titration, were then subtracted from the titration data before analysis. Titration data was then integrated to give a binding curve. Nonlinear least squares fits of the data, using Origin software from the calorimeter manufacturer, provided K_a (binding constant), □H (enthalpy of binding), and N (stoichiometry) values.

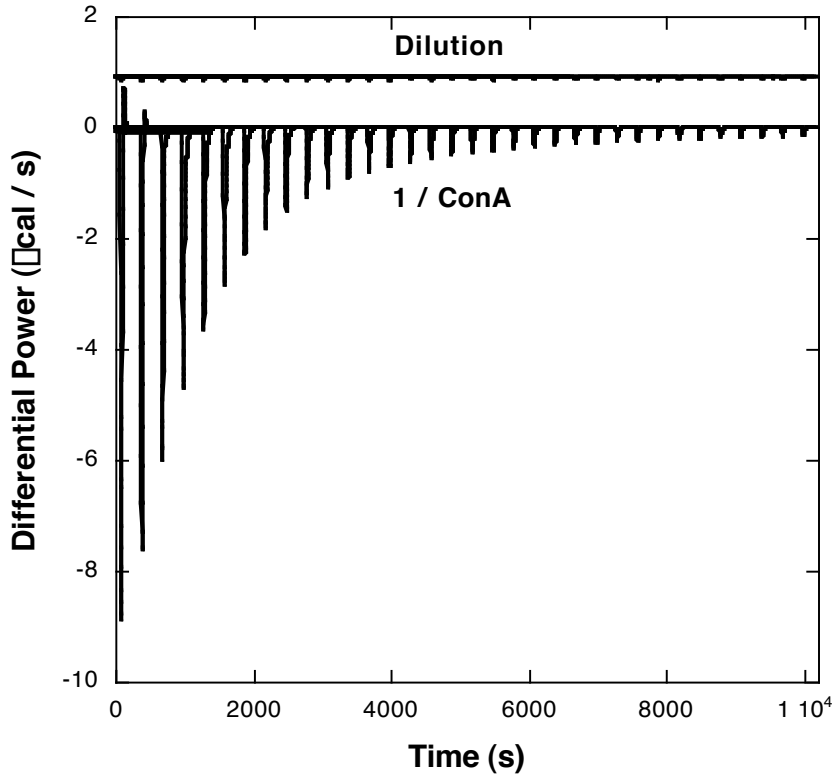
Note on the value of *c*:

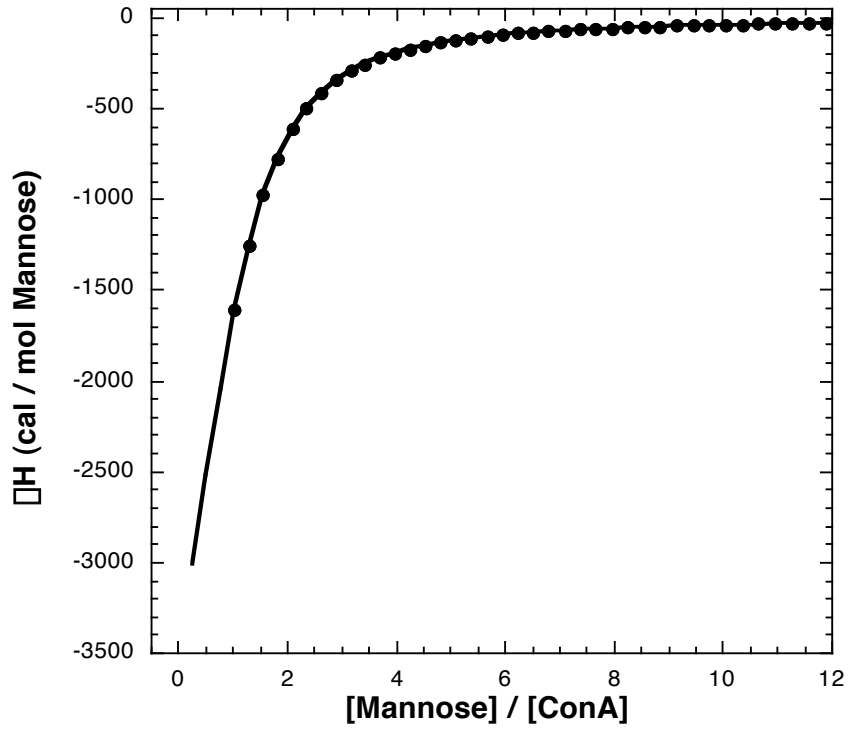
The binding isotherm of an ITC experiment is characterized by the value of c, which is defined as:

$$c = K_a[\mathrm{M}]n$$

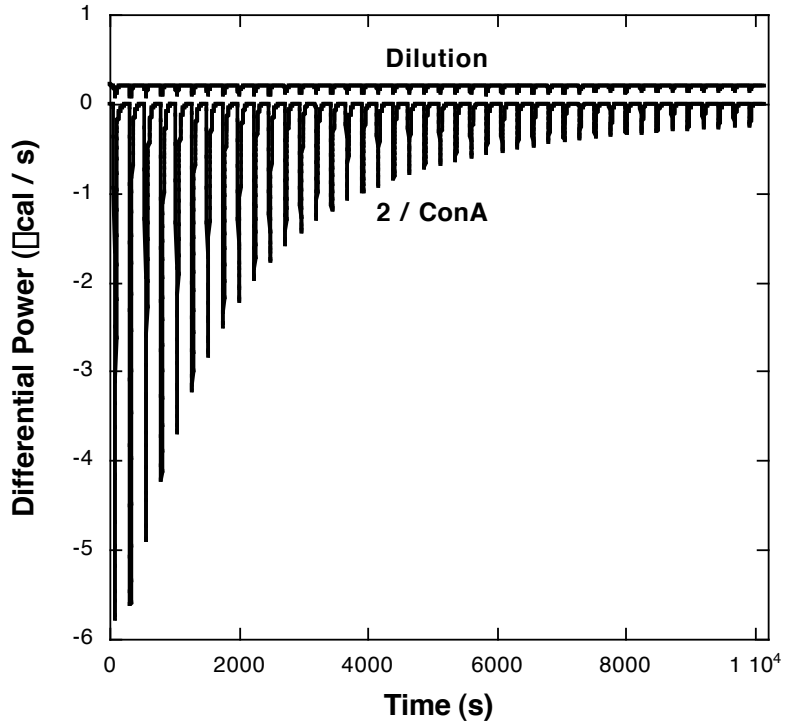
where K_a is the association constant of the interaction, [M] is the concentration of the macromolecule, and n is the stoichiometry of the interaction. The concentration of the macromolecule (ConA) in the present system is expressed in the terms of the monomer binding sites making the n value in the above expression 1. The c value has no units and is a way to describe the shape of the binding isotherm in ITC. This shape is used in determining the inflection point of a titration. Optimal c values range from between 1 and 1000; values below this range generate isotherms that are too linear, making the equivalence point hard to determine. Conversely, values above this range of c have too few points near inflection and make determination of thermodynamic parameters similarly difficult.

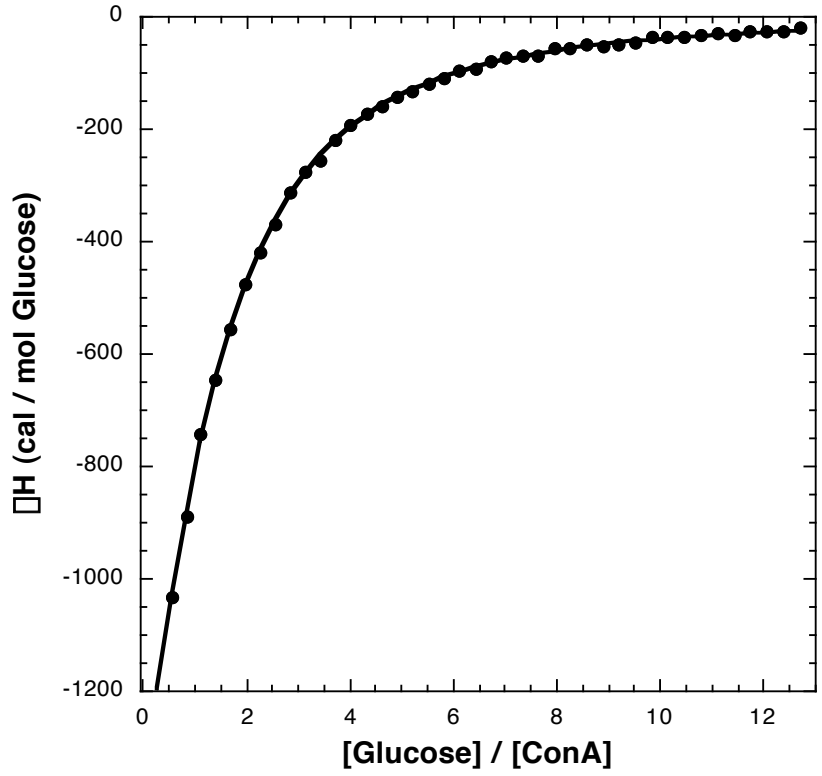
ITC 15 mM **1** / 289 μM ConA at 298 K



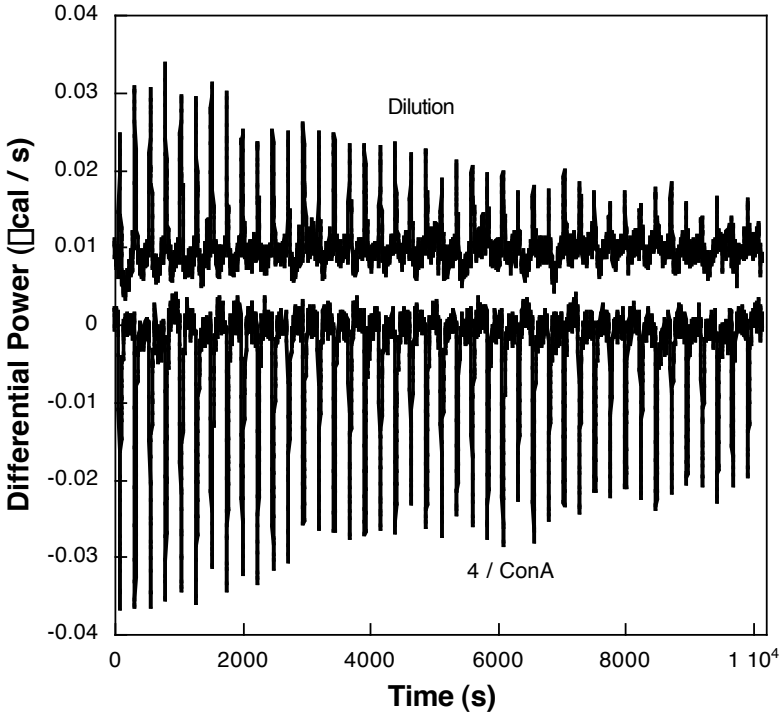


ITC 18 mM ***2*** / 250 μM ConA at 298 K

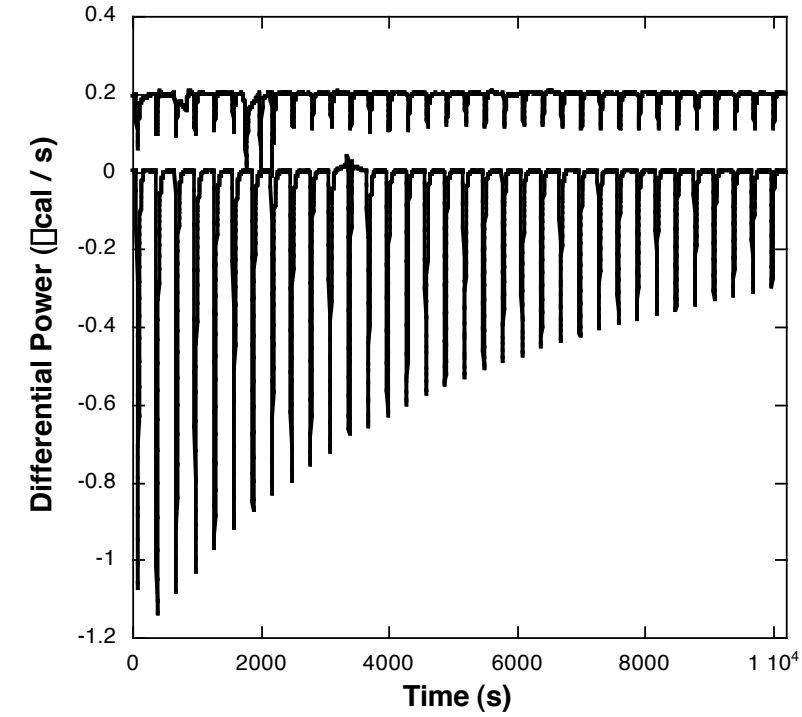


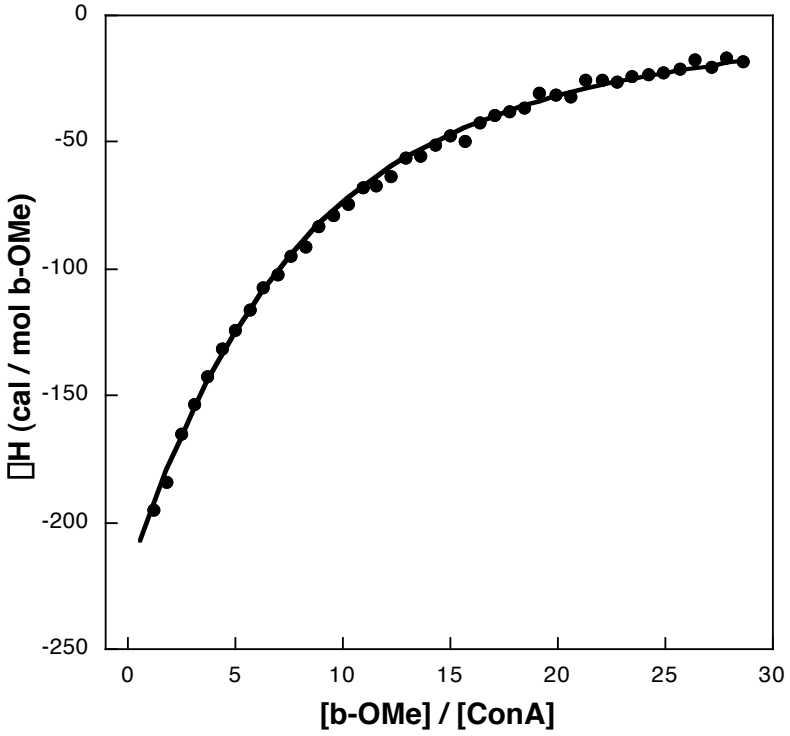


ITC 12.3 mM ***4*** / 146 mM ConA at 298 K
This plot is representative of ITC data acquired for 4, 5, and 6



ITC 25 mM ***6*** / 200 μM ConA at 298 K
(same data as Figure 2.)





ITC 25 mM *7* / 250 μM ConA at 298 K