

Supporting information to

A biophysical study with carbohydrate derivatives explains the molecular basis of monosaccharide selectivity of the *Pseudomonas aeruginosa* lectin

LecB

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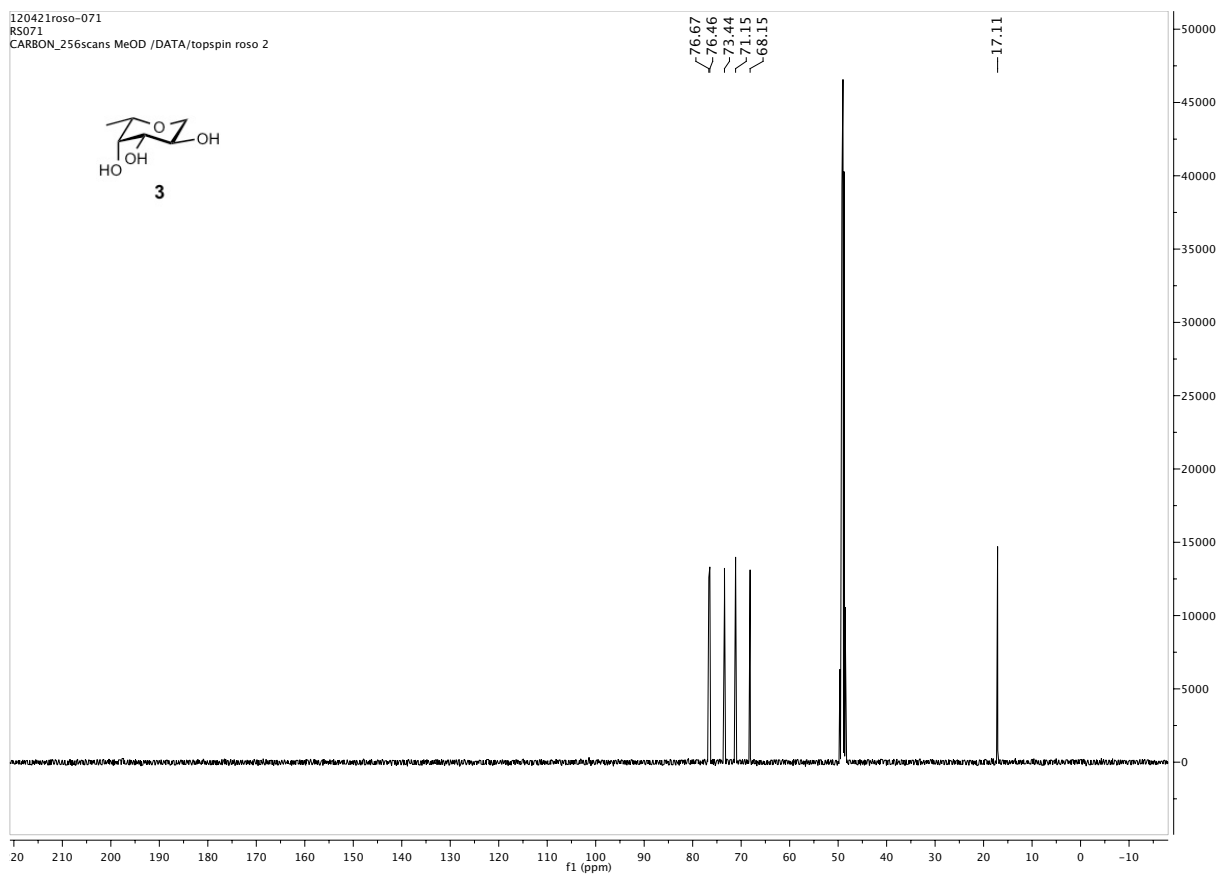
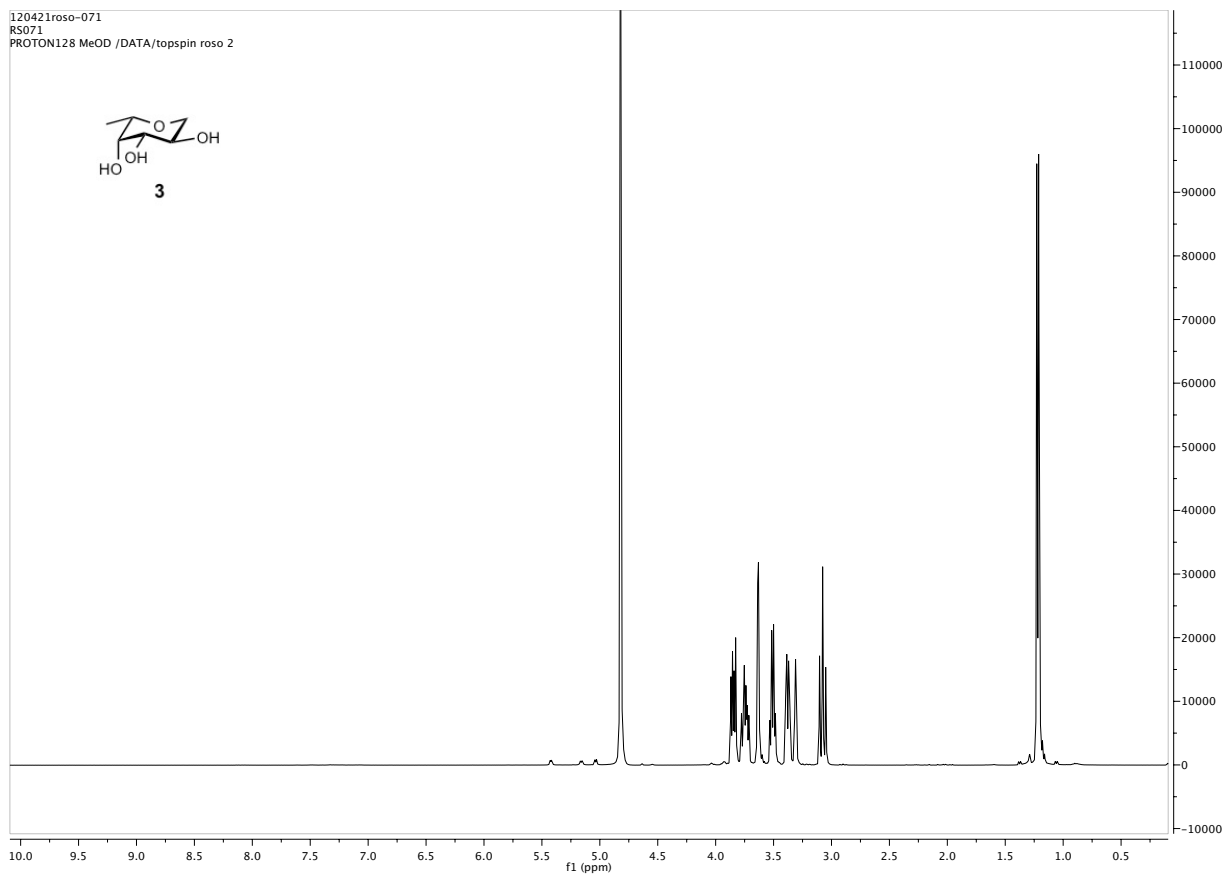
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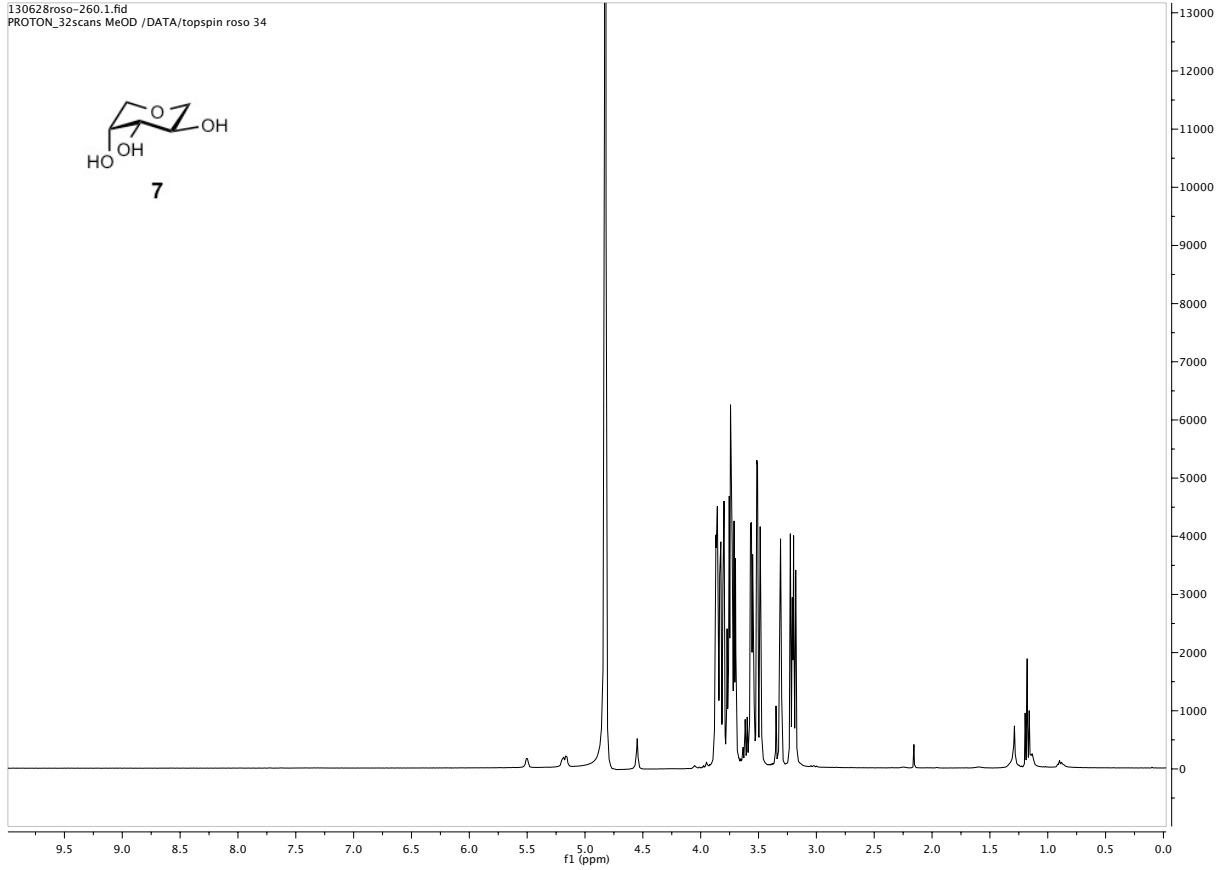
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email: alexander.titz@helmholtz-hzi.de

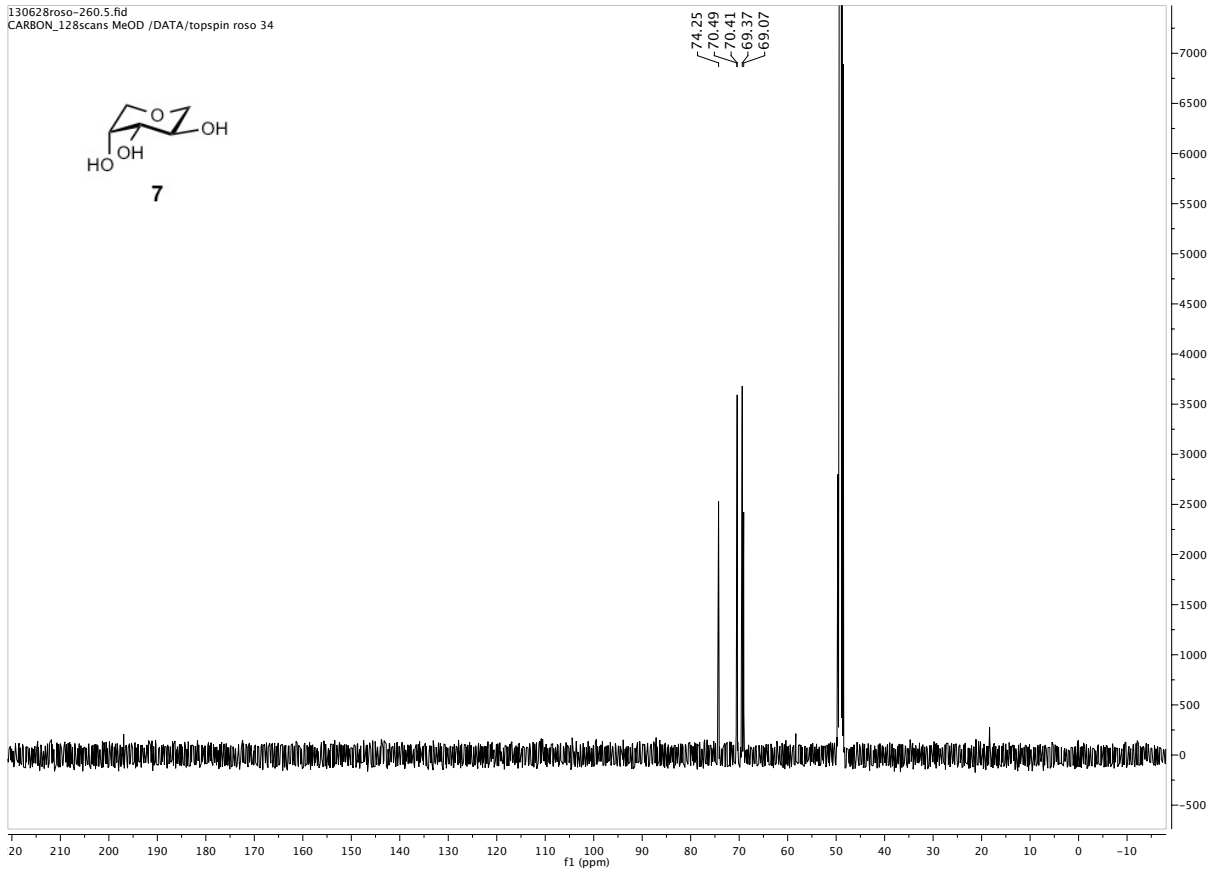
Data S1: ^1H - and ^{13}C -NMR spectra of new compounds 3, 4, 6, 7, 18, 19



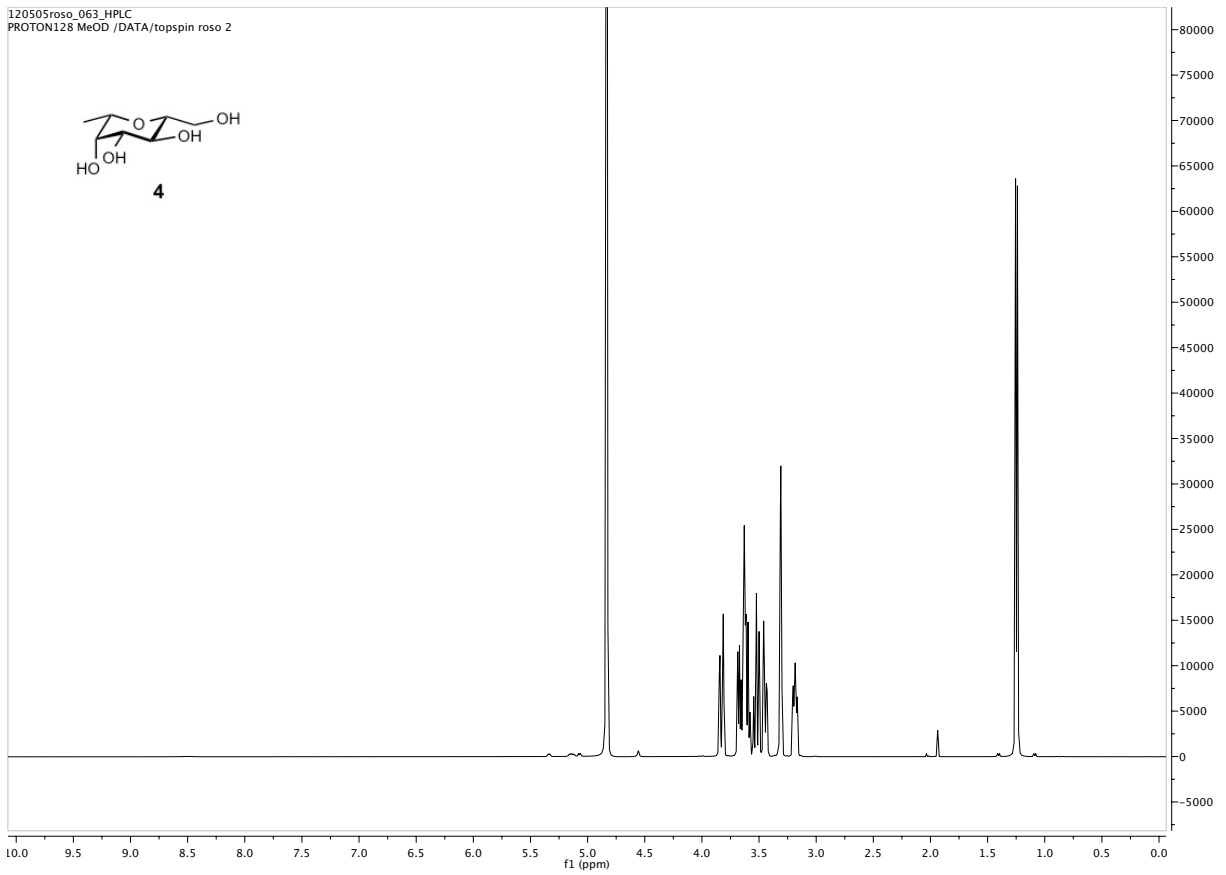
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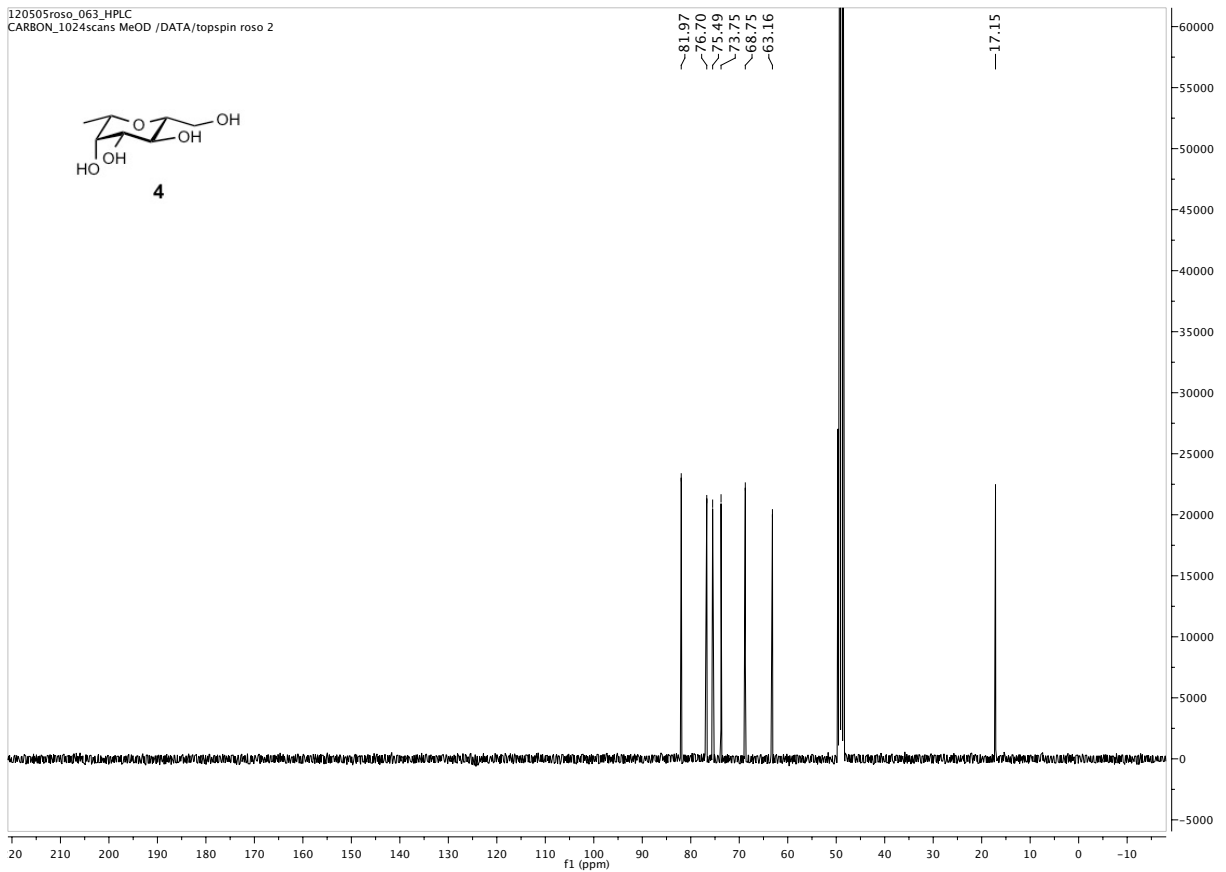
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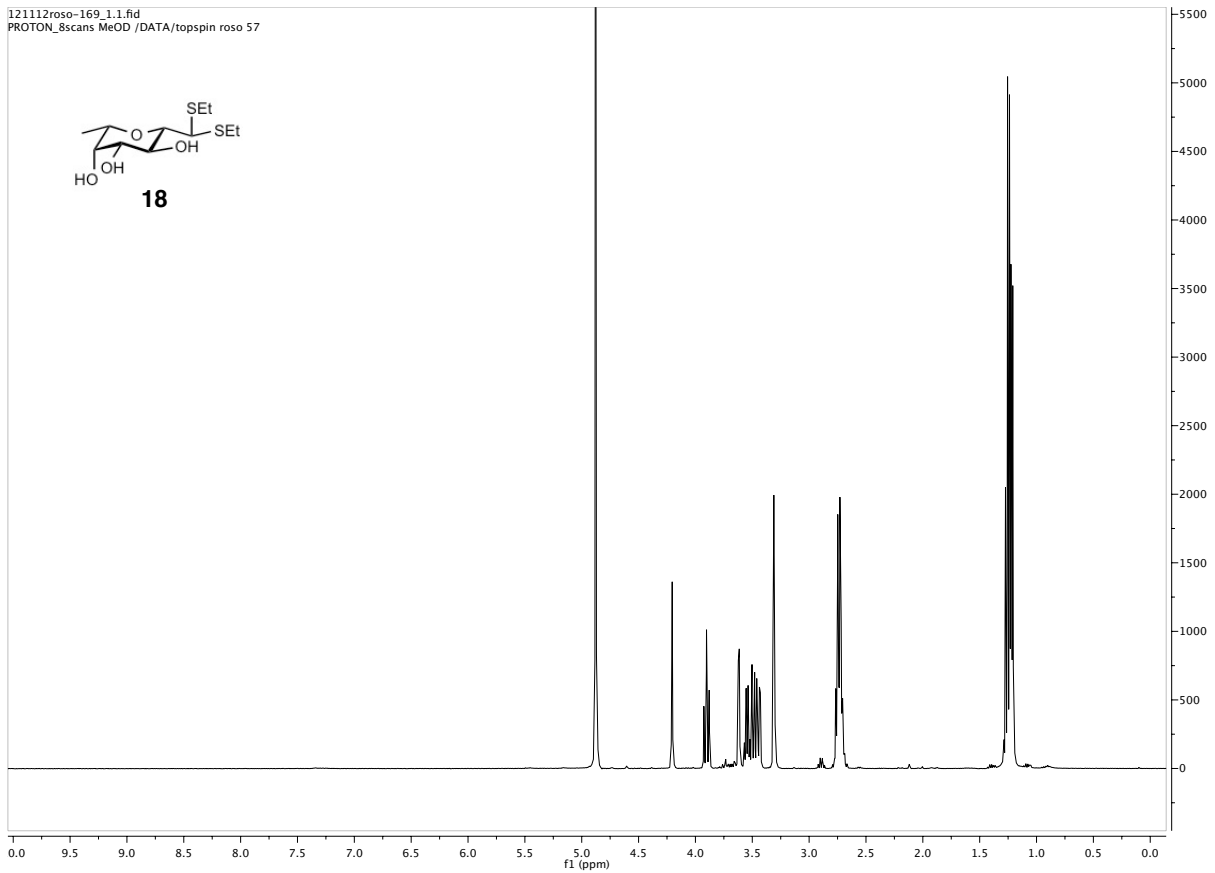
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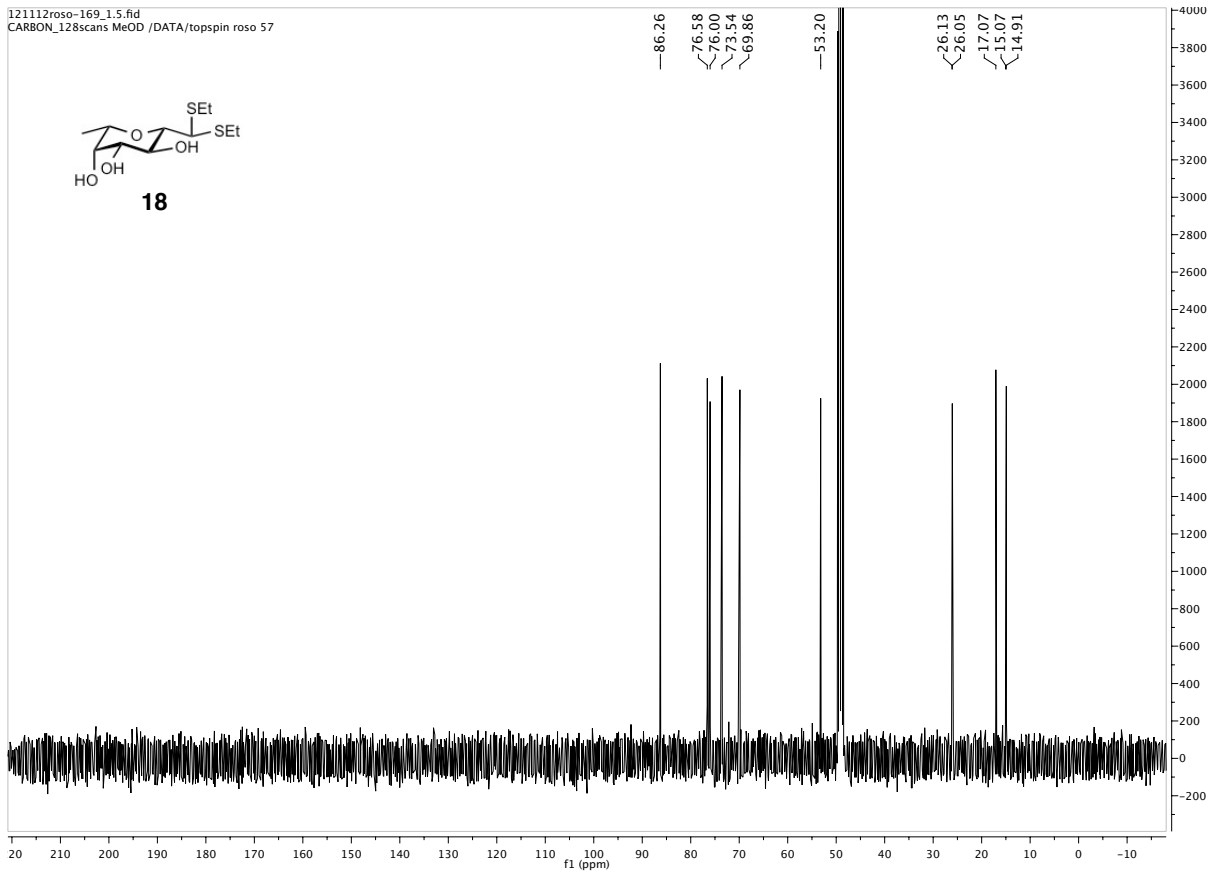
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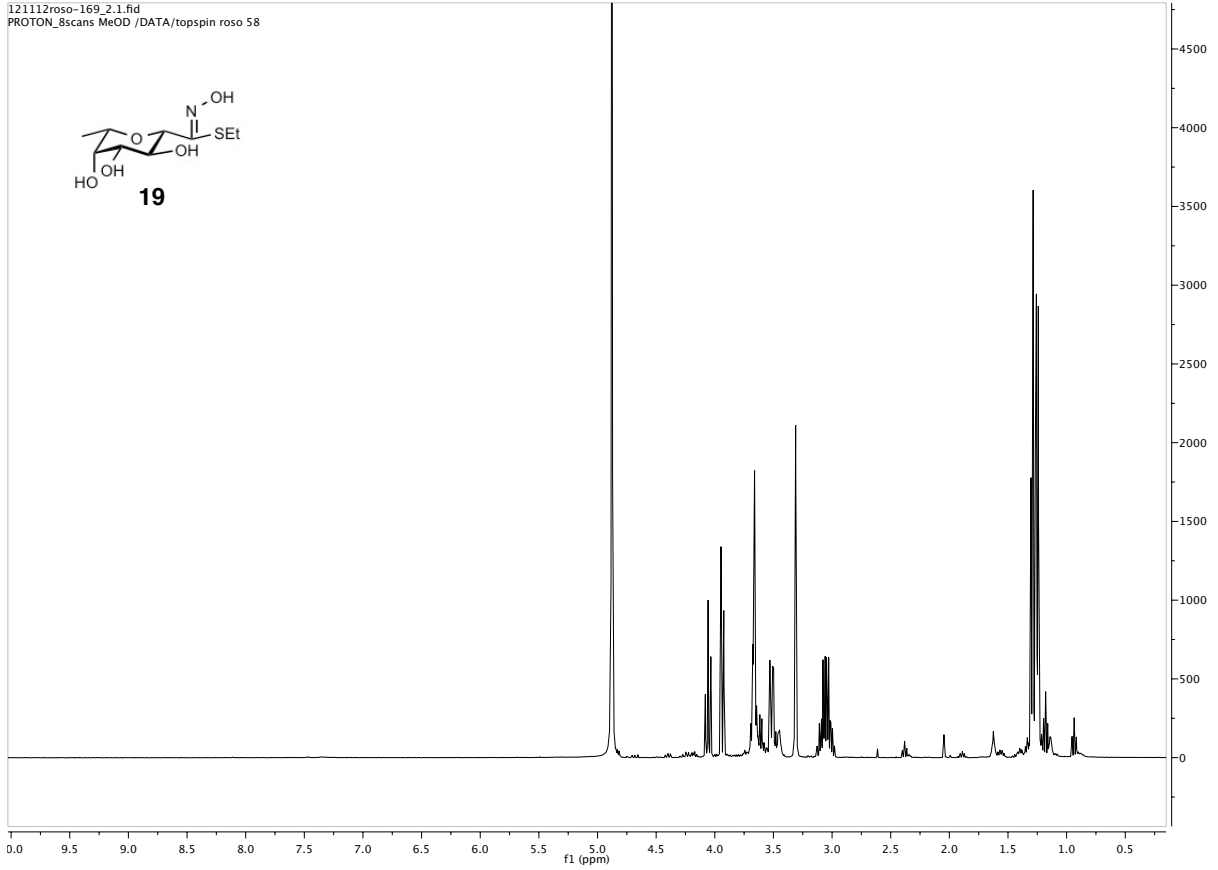
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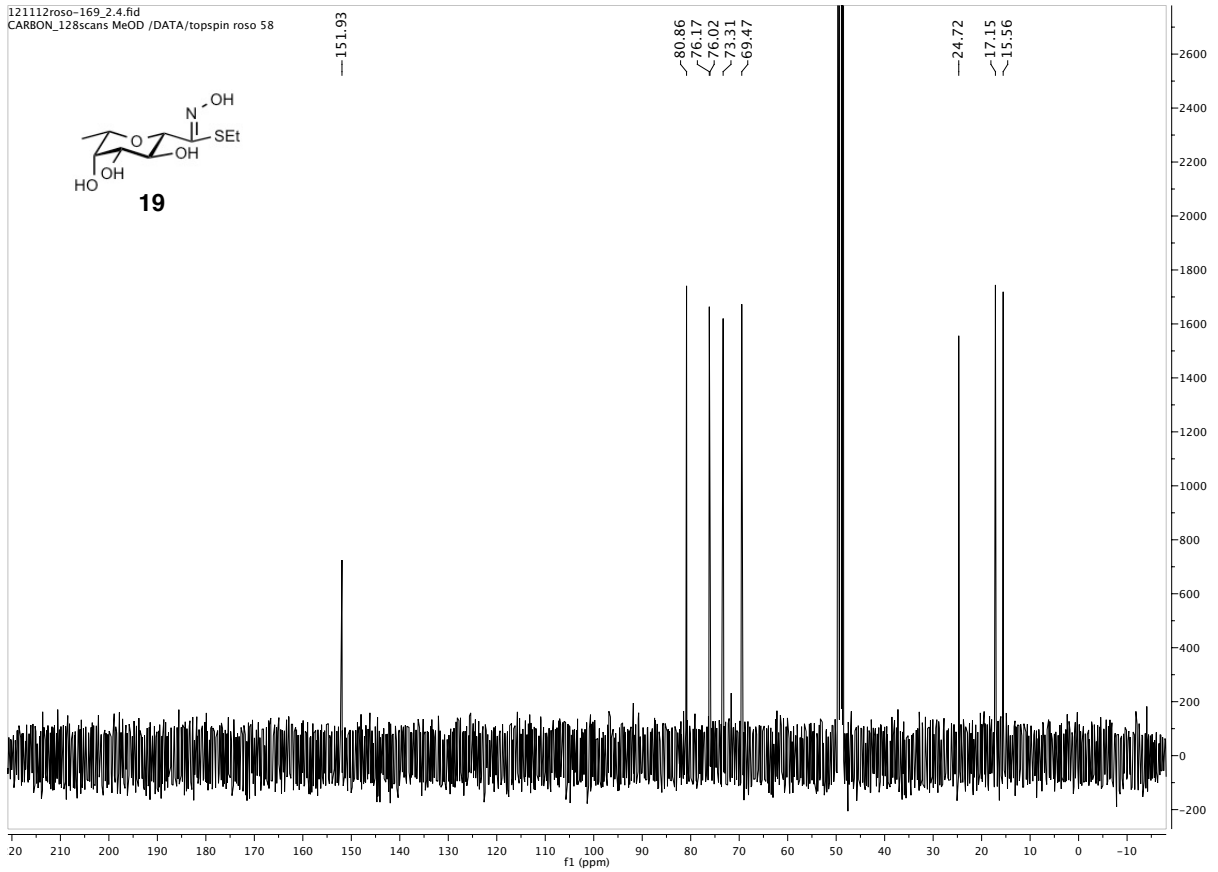
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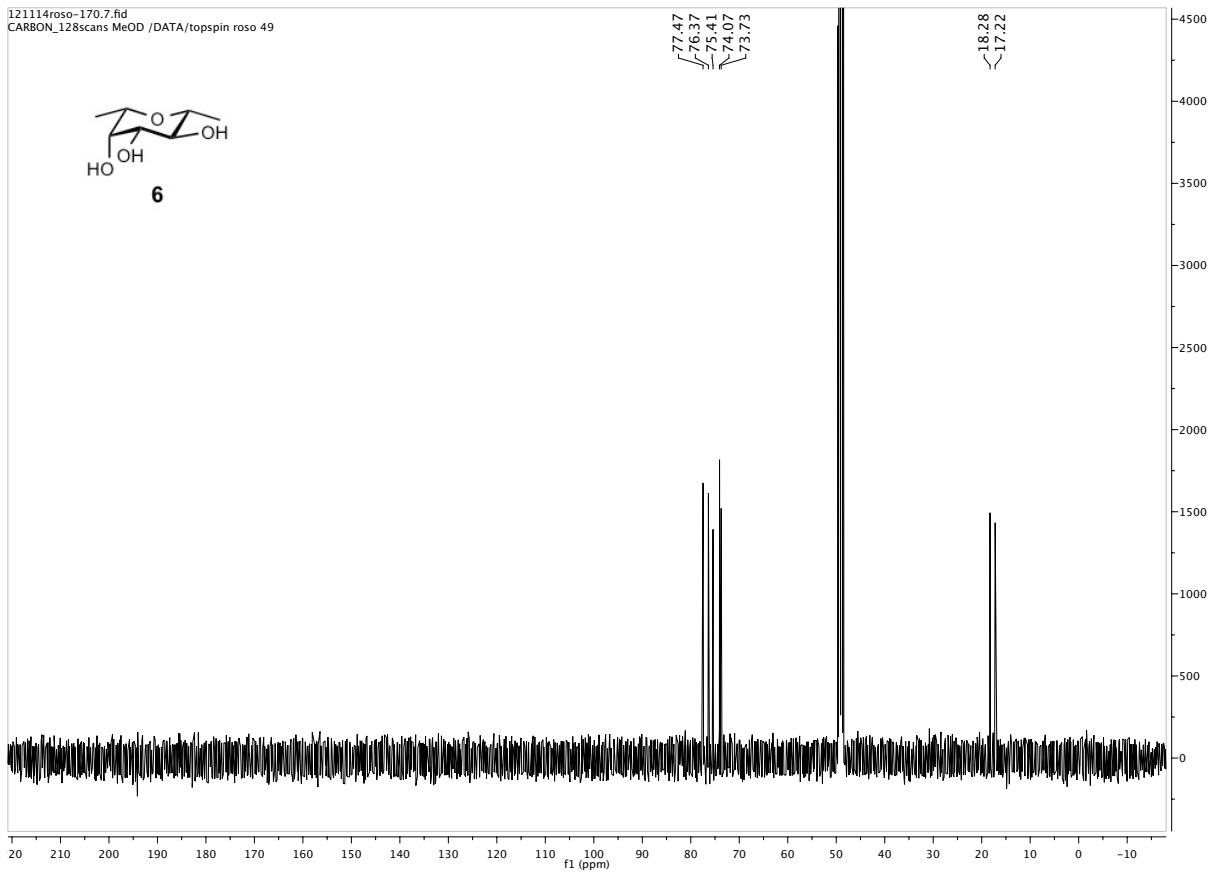
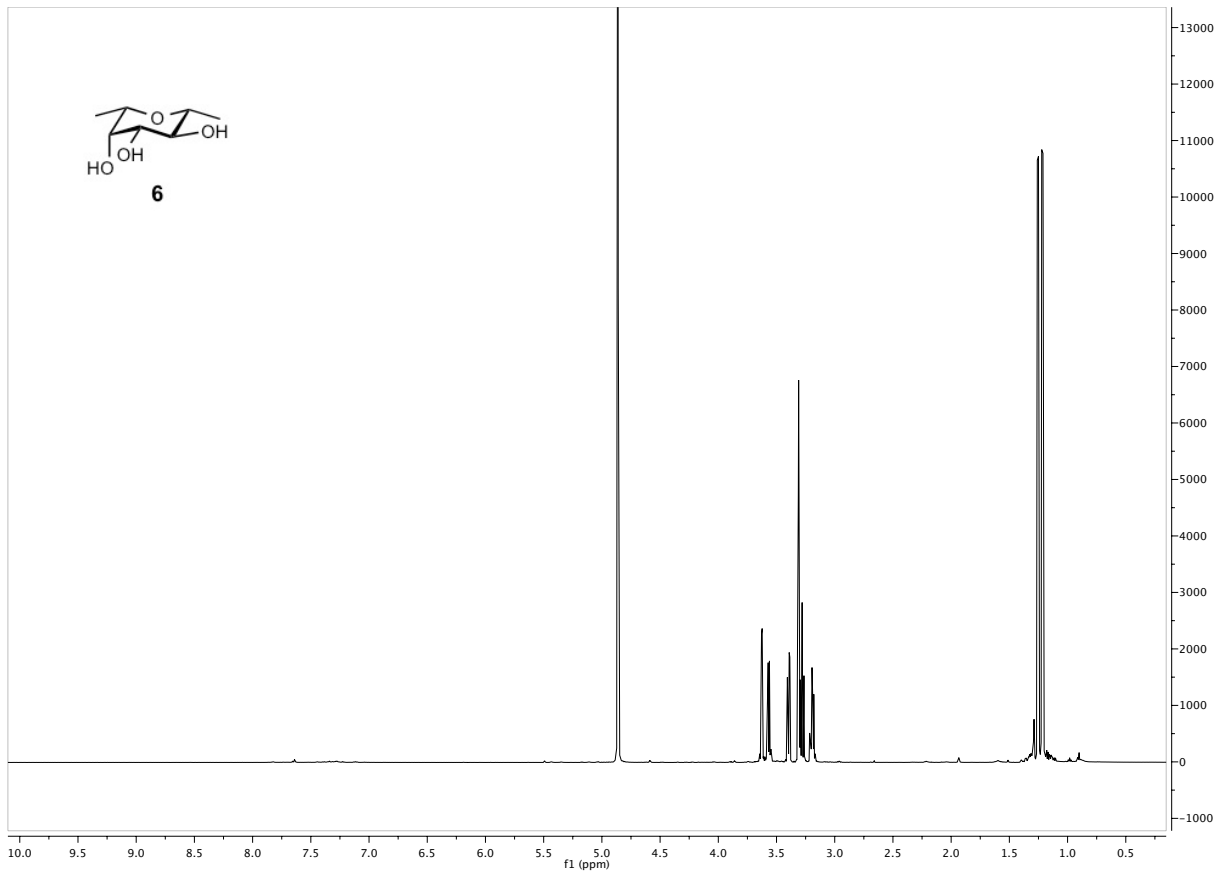


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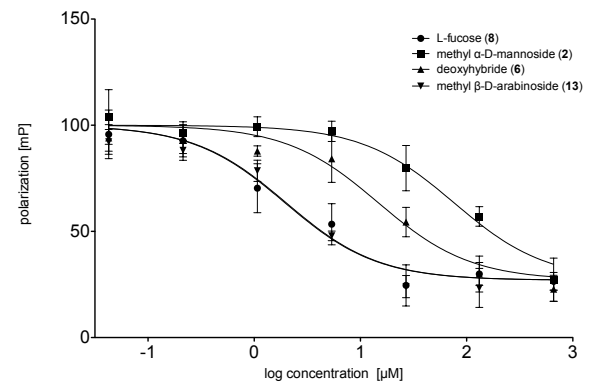
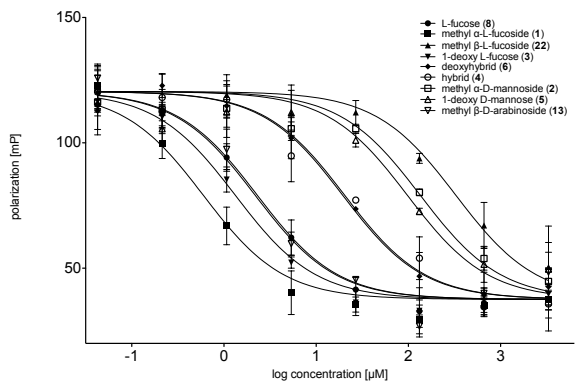
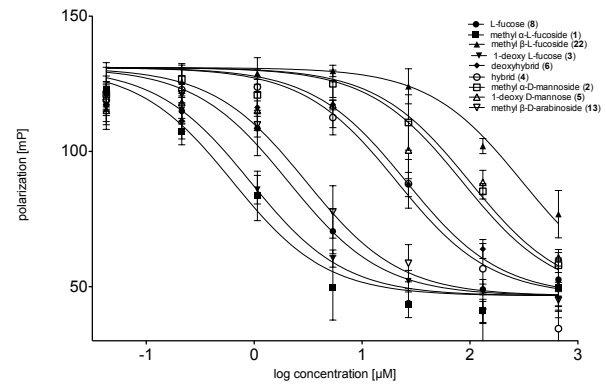
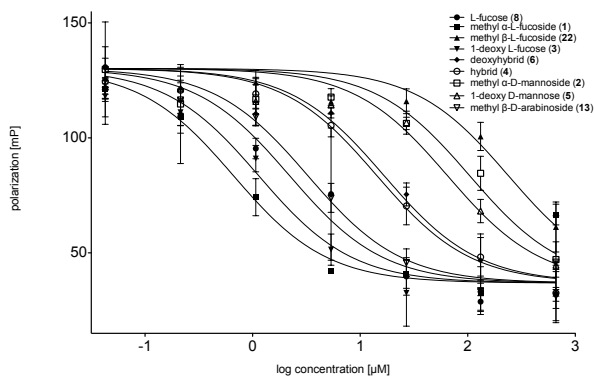
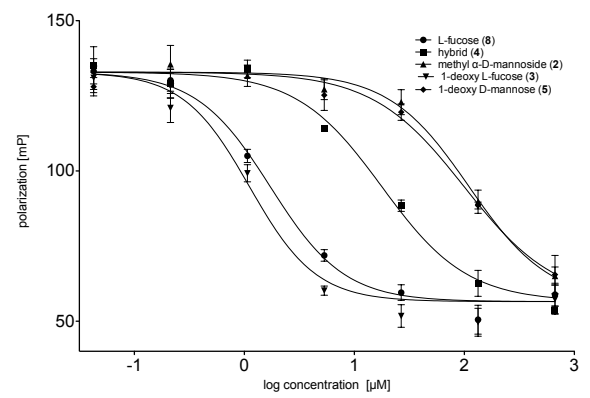
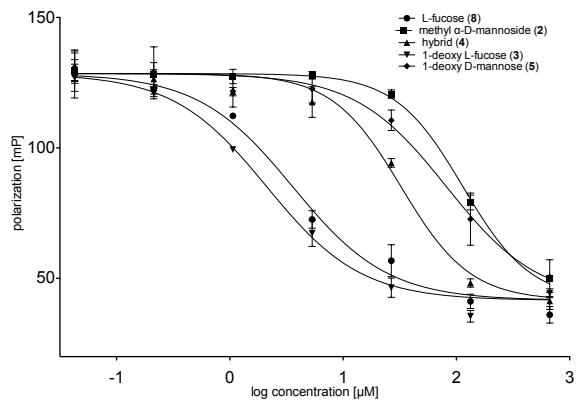
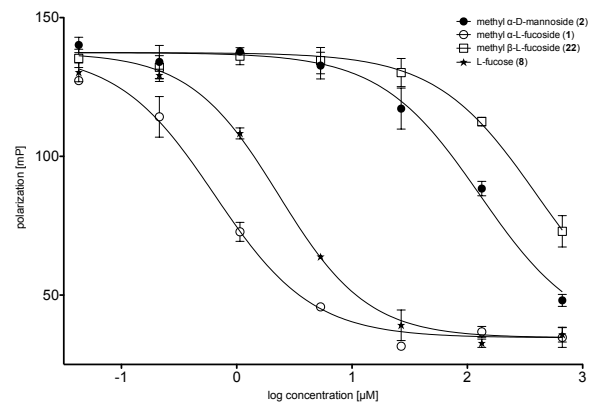
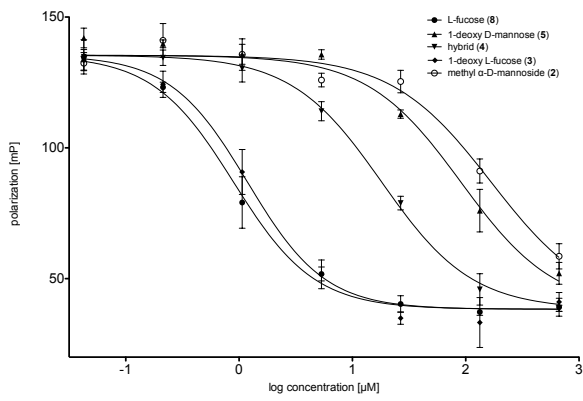


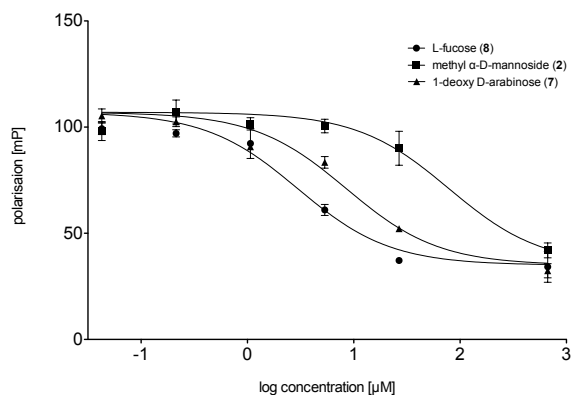
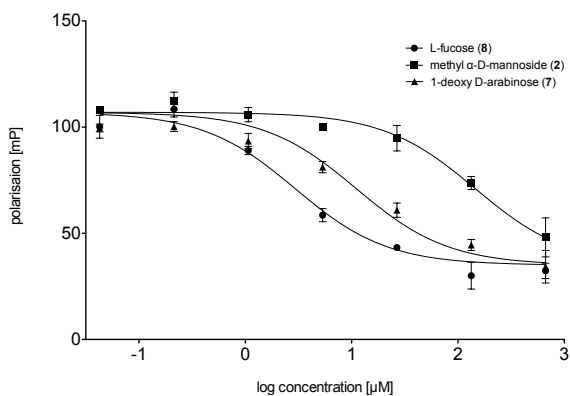
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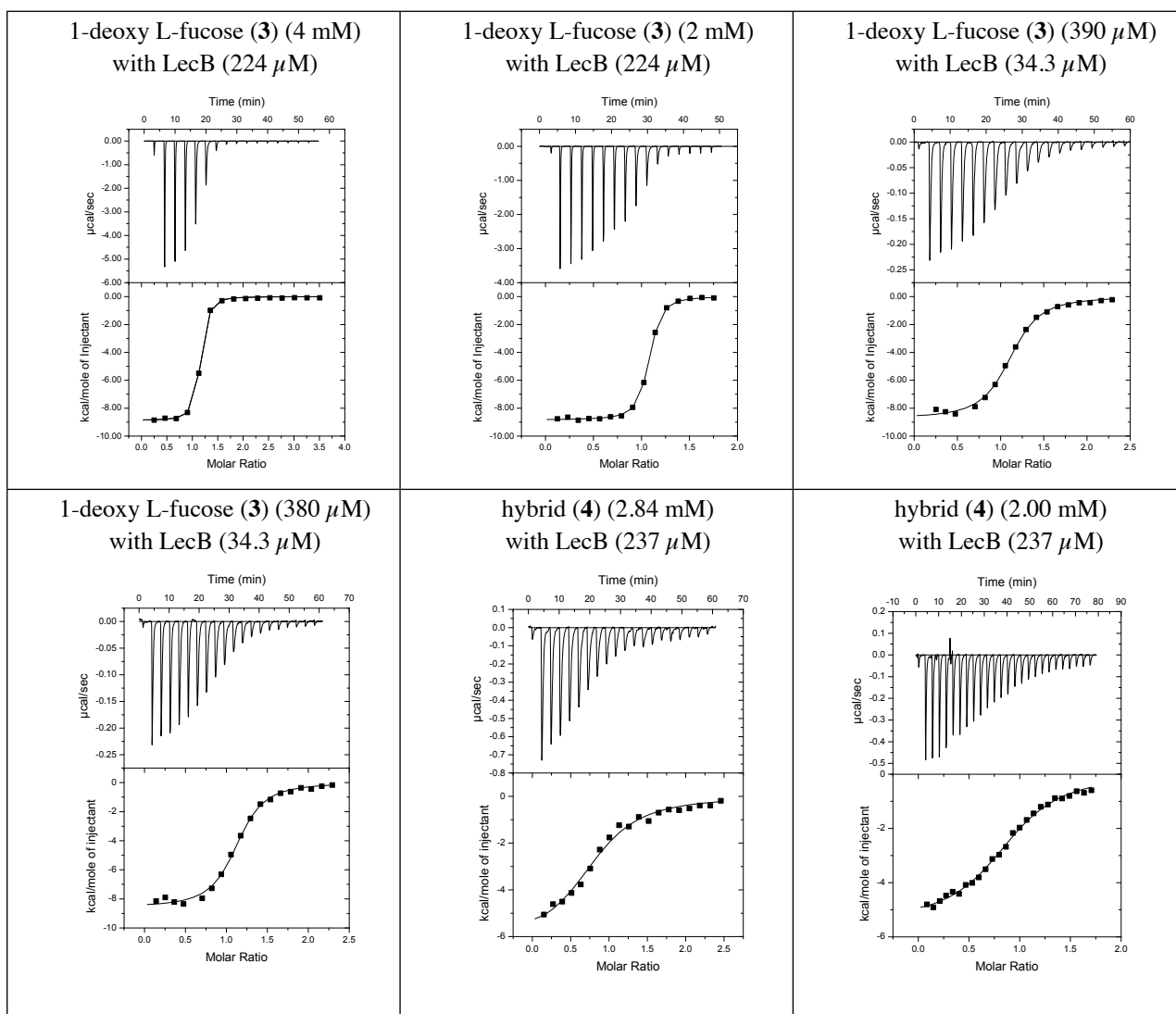


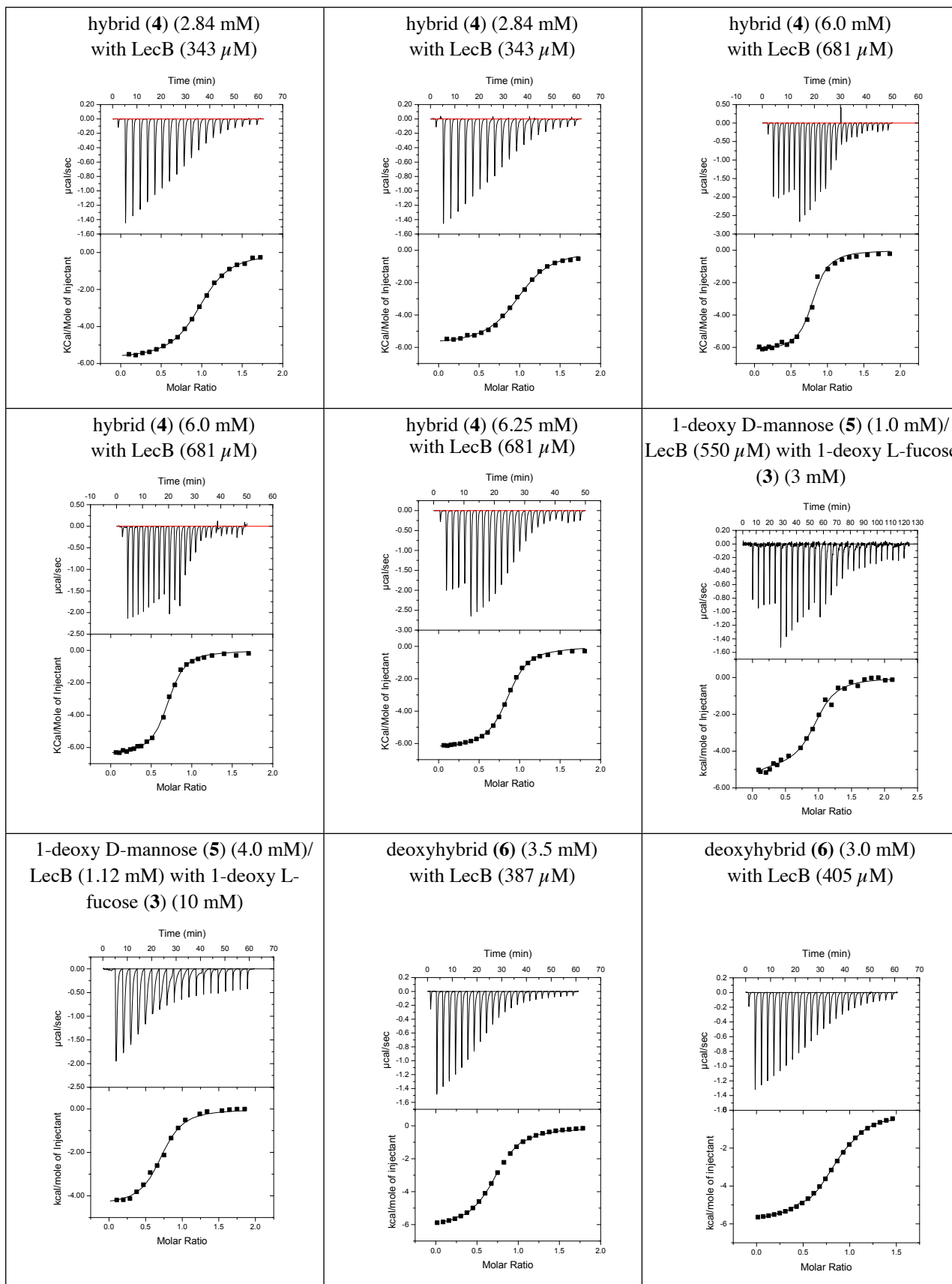
Data S2: Individual titration curves from competitive binding assay

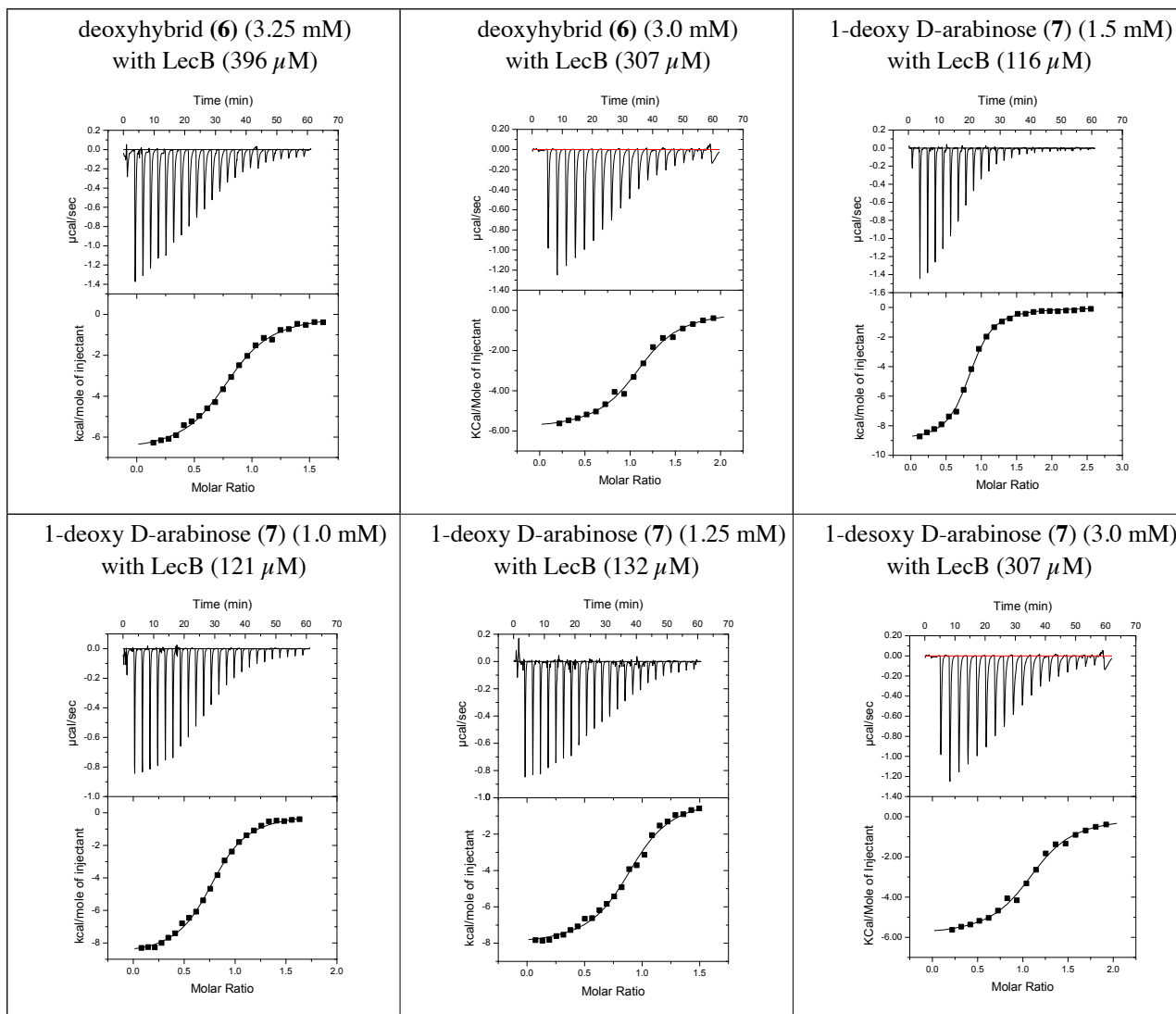




Data S3: Individual titration curves from isothermal calorimetry experiments

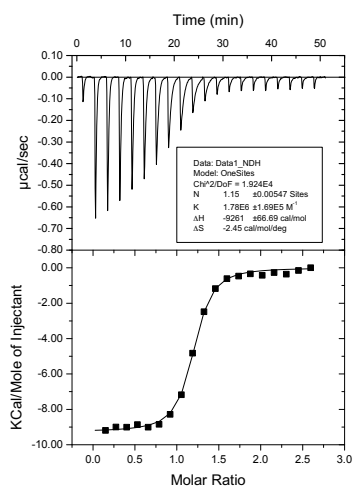






Experimental ITC data on the same systems may vary between different laboratories. For validation of the literature ITC data and to guarantee the validity of our comparisons between our data and data from the lab of Anne Imberty (Grenoble, France), we performed a titration of **1** with LecB. The data obtained are in very good agreement with the literature data by Sabin *et al.*[1]

Titration of LecB (56.1 μM) with **1** (700 μM):



Thermodynamic parameters obtained:

ΔG (kJ/mol): -35.67, ΔH (kJ/mol): -38.73, $T\Delta S$ (kJ*K/mol): -3.05, N: 1.15

Molecular Modeling

Flexibility of the Ligands in 15 ns MD Simulations of LecB Complexes:

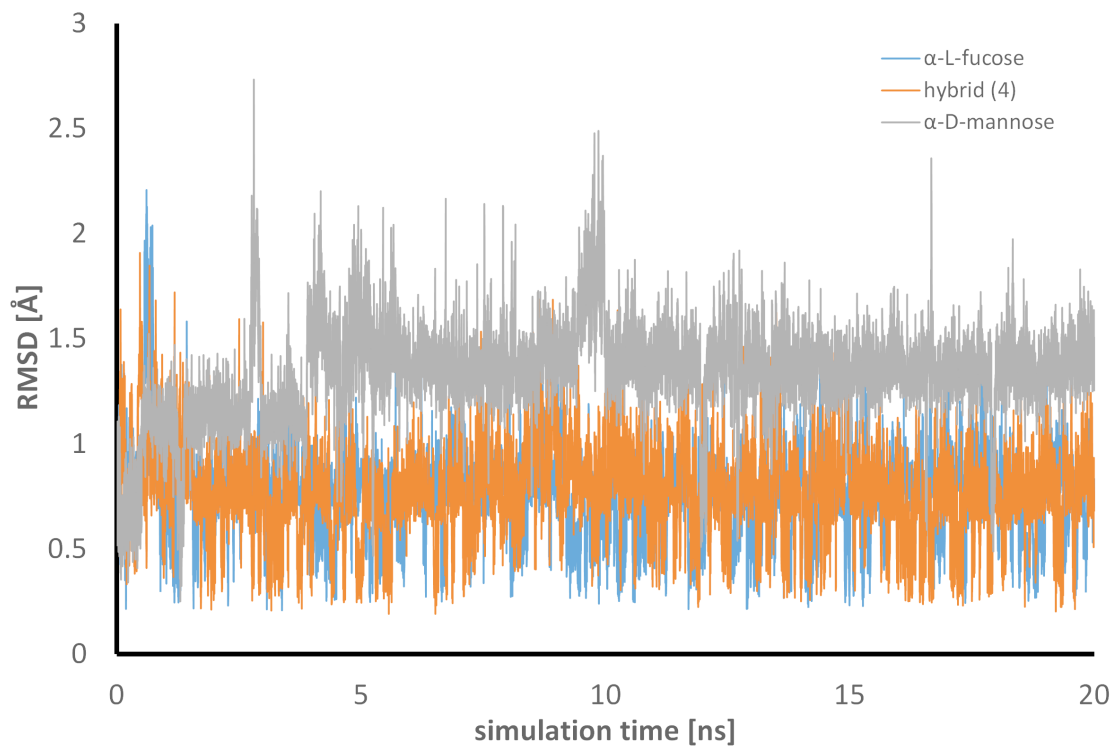


Figure S1: Time series of the root mean square deviation (RMSD) of all ligand atoms in the simulation of three complexes of LecB. Before the RMSD calculations the snapshots were aligned based on the binding site residues.

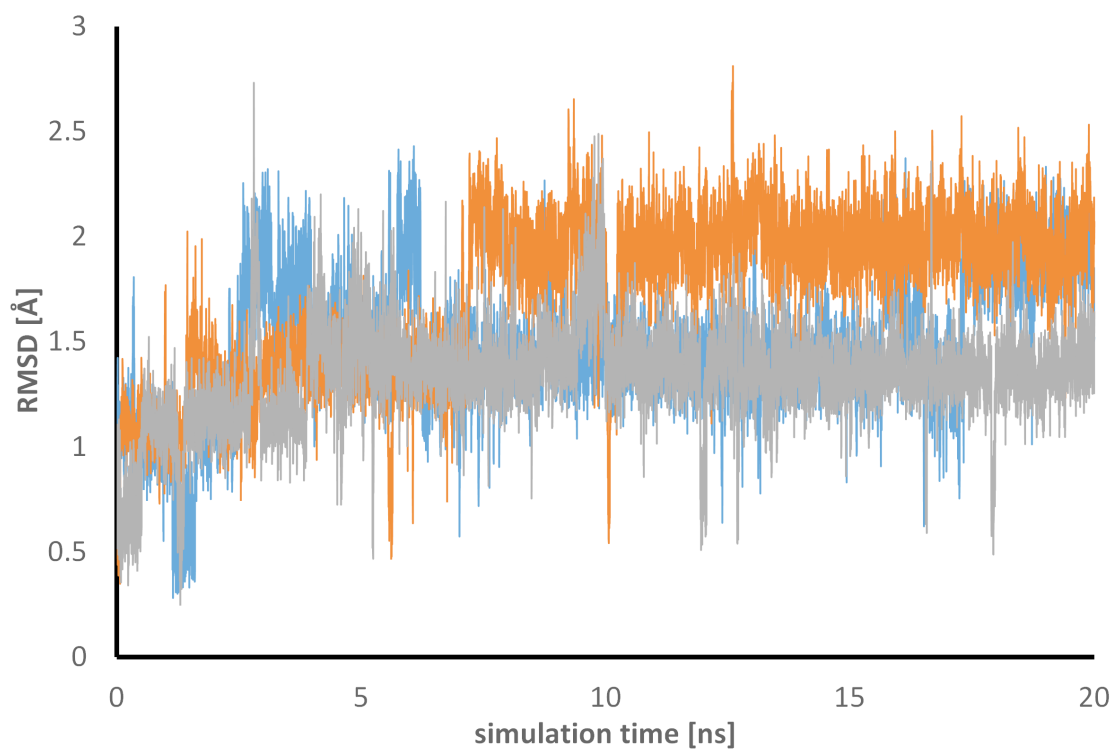


Figure S2: Time series of the root mean square deviation (RMSD) of all ligand atoms in the three independent simulation of three complexes of LecB with α -D-mannose. Before the RMSD calculations the snapshots were aligned based on the binding site residues.

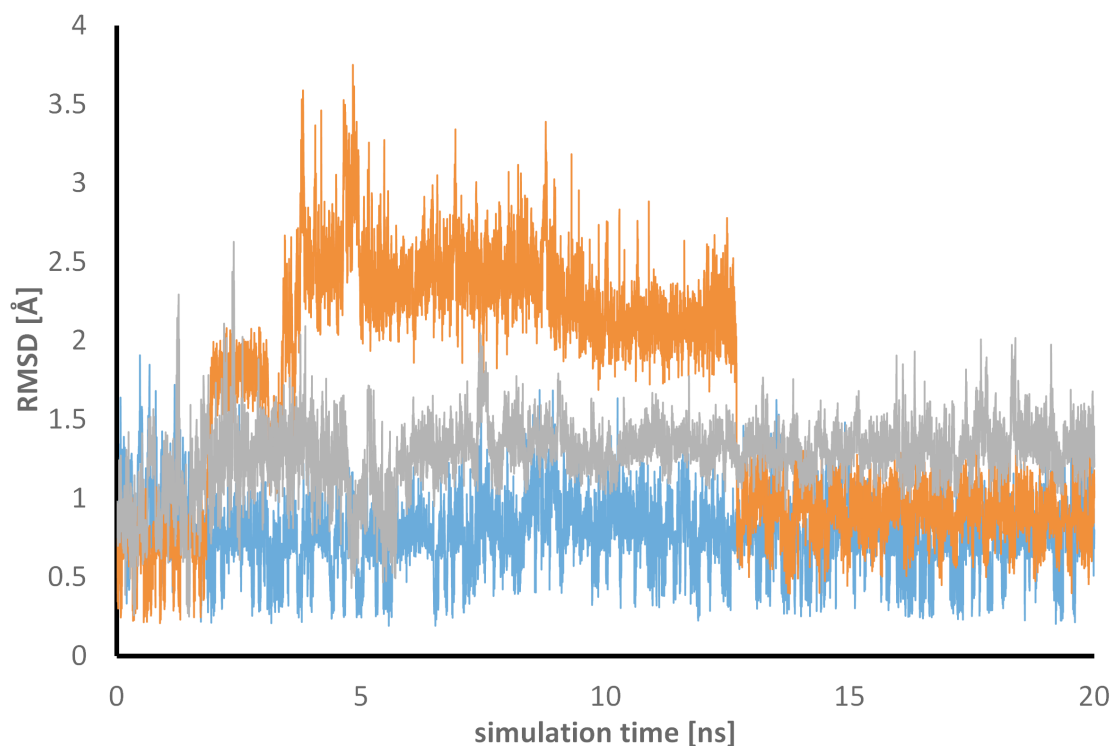


Figure S3: Time series of the root mean square deviation (RMSD) of all ligand atoms in the three independent simulation of three complexes of LecB with hybrid 4. Before the RMSD calculations the snapshots were aligned based on the binding site residues.

Hydrogen Bond Between Asp96 and Ser22 in 15 ns Long MD Simulations of LecB Complexes:

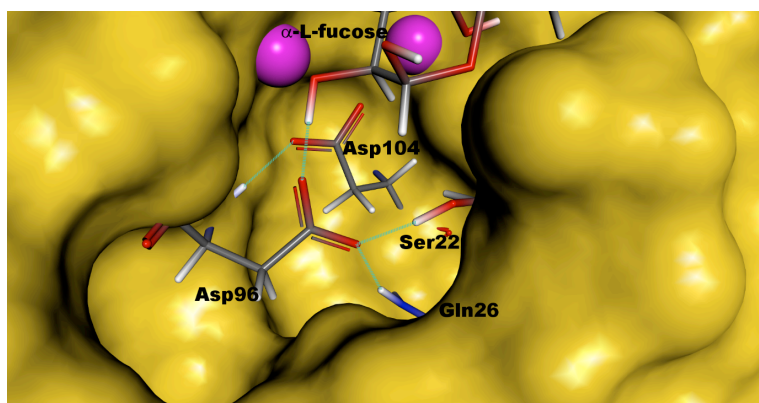


Figure S4: Full hydrogen-bond network of Asp96 with Ser22, Gln26, Asp104, and α -L-fucose. The calcium ions are shown as purple spheres.

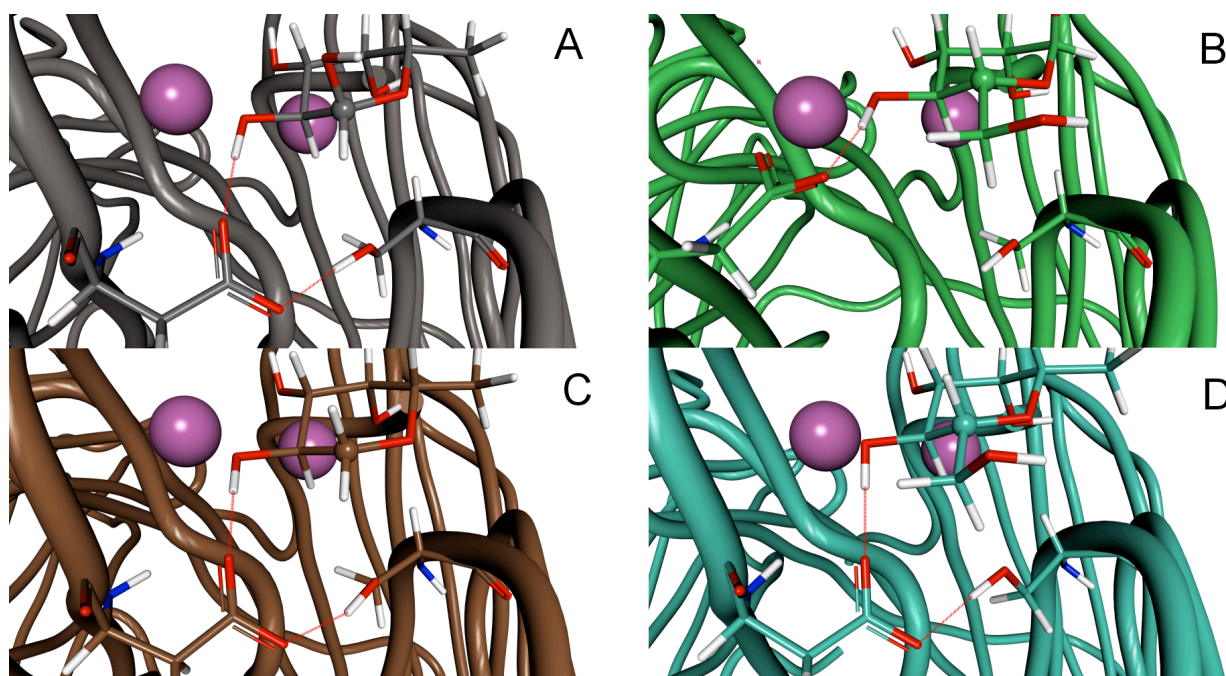


Figure S5: Orientation of Asp96 in the representative structure of the simulations of four complexes of LecB: (A) α -L-fucose (closed), (B) α -D-mannose (open), (C) 1-deoxy L-fucose (3, closed), (D) hybrid 4 (closed); The hydrogen bonds between Asp96 and Ser22 as well as Asp96 and the ligand are indicated by red dashed lines. The calcium ions are shown as purple spheres.

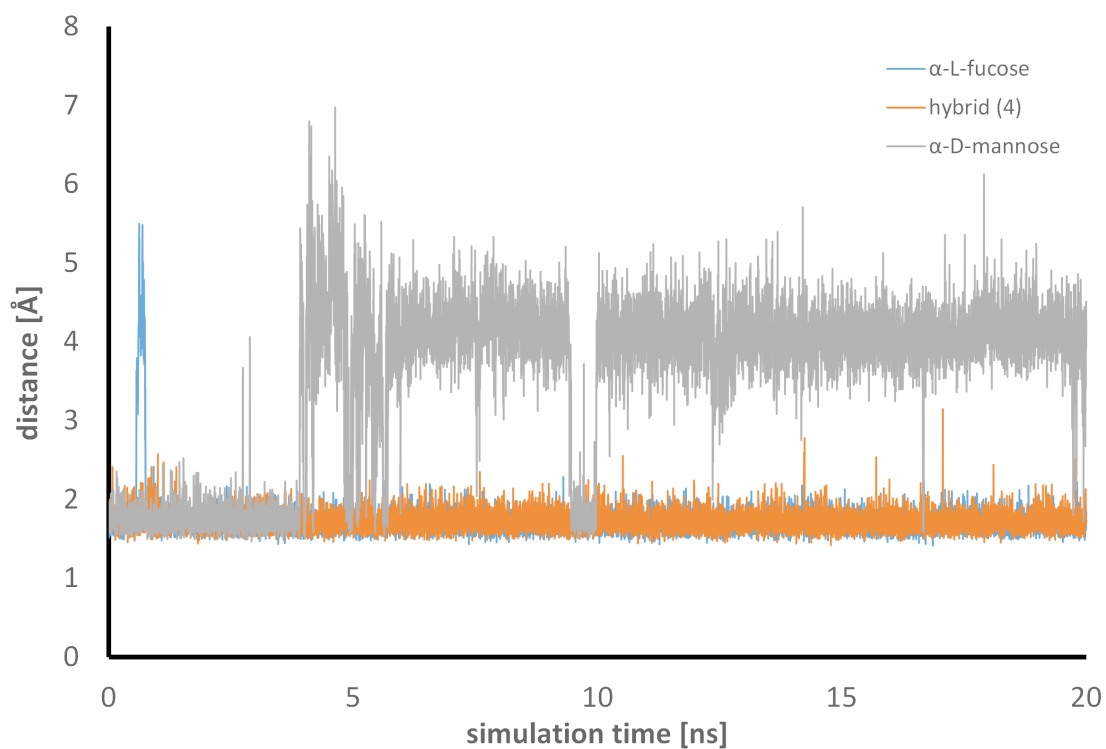


Figure S6: Time series of the shortest distance between HG of Ser22 and OD1/OD2 of Asp96 of simulations of three complex of LecB

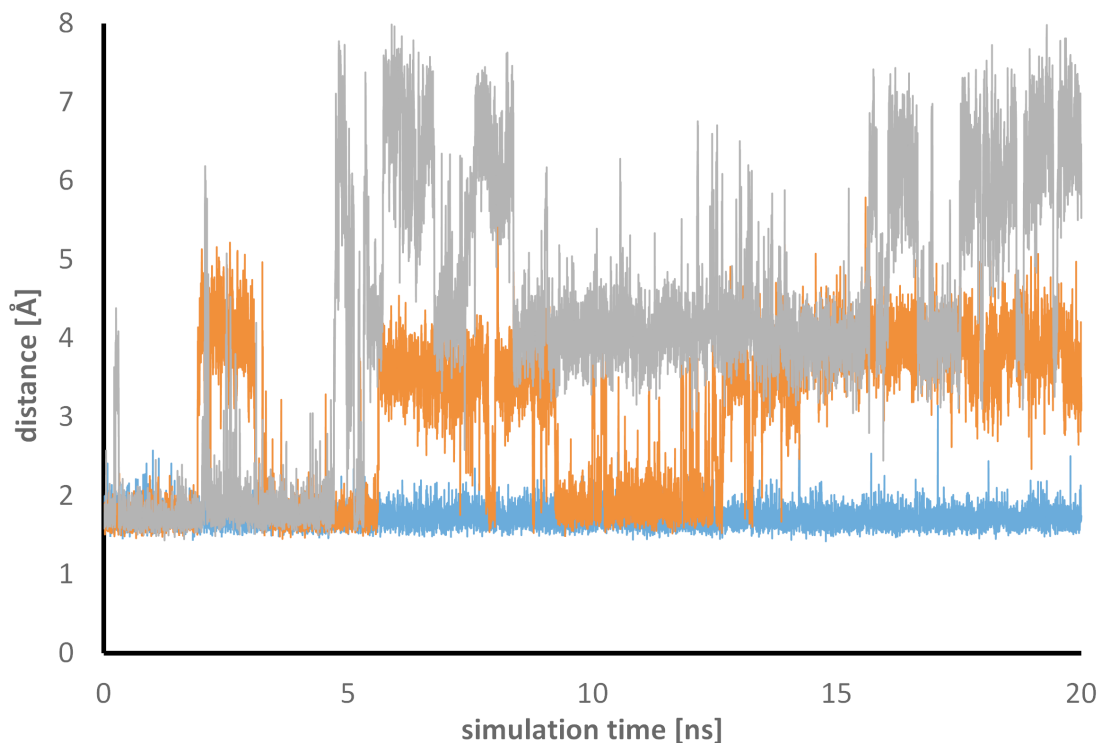


Figure S7: Time series of the shortest distance between HG of Ser22 and OD1/OD2 of Asp96 of three independent simulations of the complex of LecB with hybrid 4

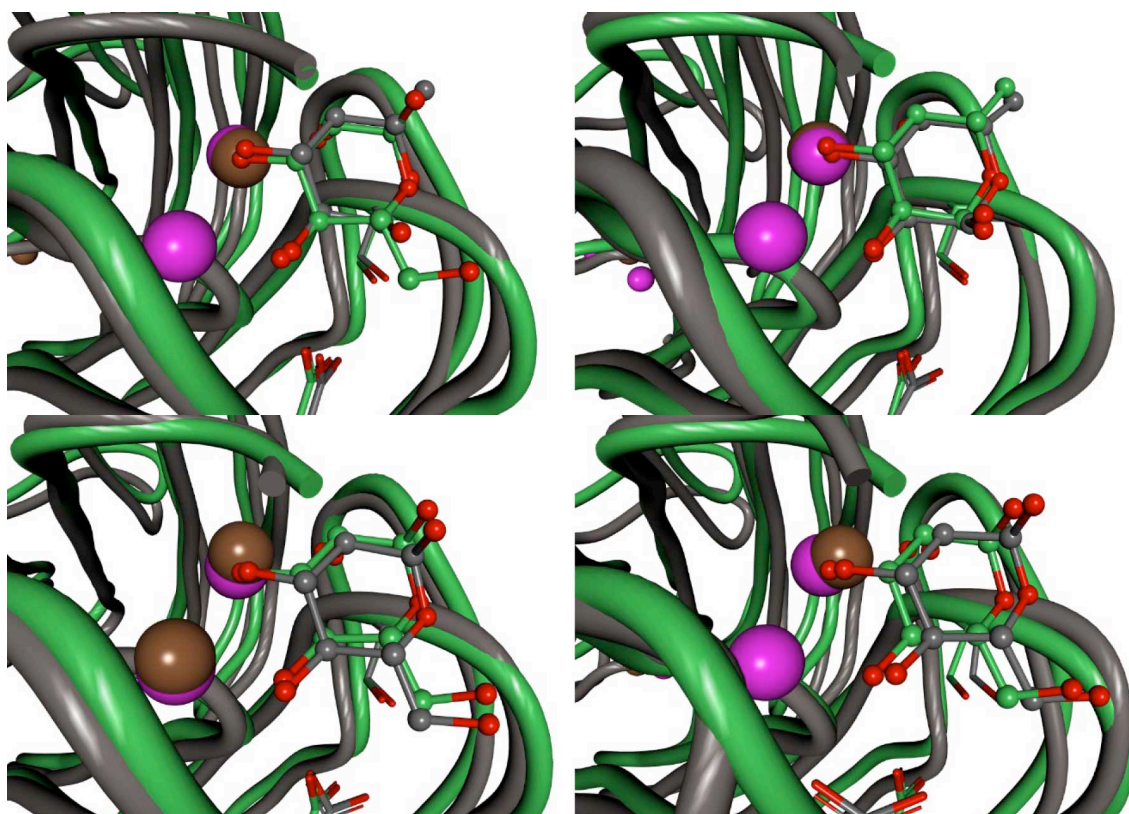


Figure S8: Overlays of experimental structures and representative structures from the MD simulations: The first structure is shown as a green ribbon, with green carbon atoms and with Ca(II) ions in magenta. The second structure is color coded in grey with brown Ca(II) ions. Upper left: X-ray structure of the fucose (1oxc) and mannose (1our) complex; Upper right: X-ray (1oxc) and MD structure of the fucose complex; Lower left: X-ray structure (1our) and closed form of the mannose complex; Lower right: X-ray structure (1our) and open form of the mannose complex

Contributions of Individual Steps of TI Simulation for Transition from 1-Deoxy L-Fucose (3) to Hybrid 4:

Table S1: Relative binding free energy for the transition from 1-deoxy L-fucose (3) to hybrid 4 calculated using thermodynamic integration and its partitioning into the free individual steps. The values for two independent calculations of 5 ns length as well as the averages are given.

Step	Starting Structure 1 [kJ/mol]	Starting Structure 2 [kJ/mol]	Average [kJ/mol]
Destruction of partial charges of vanishing group	1.32	1.39	1.35
Mutation of vanishing group into appearing group	13.10	14.78	13.94
Adding partial charges to appearing group	-3.48	-6.35	-4.92
Sum	10.93	9.81	10.37

Supplementary References

[1] Sabin C, Mitchell EP, Pokorná M, Gautier C, Utile J, Wimmerová M, Imberty A (2006) Binding of different monosaccharides by lectin PA-III from *Pseudomonas aeruginosa*: thermodynamics data correlated with X-ray structures. FEBS Lett 580: 982-7.