

Synthesis, Microcalorimetry, and Docking Analysis of Cyclic Oligosaccharide Inhibitors of Monoclonal Antibody SYA/J6

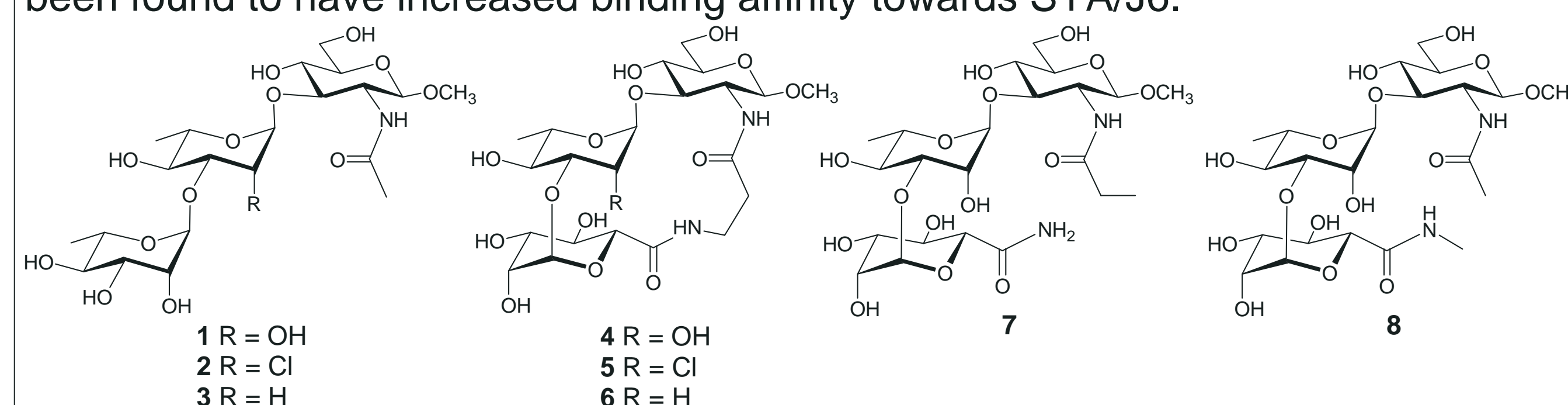


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Introduction

Investigation of the entropic penalty that accompanies binding of univalent oligosaccharide ligands suggests that the often-cited flexibility of glycosidic linkages is not the primary cause of the low affinity binding of oligosaccharides by protein receptors. We report here efforts to increase affinity by a combination of intramolecular tethering and functional group replacement. We have focused our efforts on a structurally well characterized monoclonal antibody (mAb) that binds the trisaccharide epitope, α -L-Rha-(1-3)- α -L-Rha-(1-3)- β -D-GlcNAc-OMe (Rings labelled BCD, **1**) a component of the O-polysaccharide of *Shigella flexneri* variant Y. Previous synthesis and chemical mapping of **1** has culminated in two congeners containing 2'-chloro- (**2**)¹ and 2'-deoxy (**3**)² modifications that have been demonstrated to exhibit higher affinities than **1** with mAb SYA/J6. Inspection of the co-crystallized complex^{3,4} of **1** and SYA/J6 shows the 2-acetamido and 6"-methyl groups are exposed to bulk solvent. Hence, a rationally designed cyclic trisaccharide with a β -alanine-tether spanning these two functionalities (**4**) has been found to have increased binding affinity towards SYA/J6.⁴



Here we combine molecular pre-organization with functional group replacement, each of which separately results in tighter binding when compared to binding of the native trisaccharide epitope. Presented in this work is the synthesis of β -alanine-tethered **5** and **6**, each containing a modified central rhamnose ring (Scheme 1). Acyclic control compounds **7** and **8** were synthesized in a similar fashion, and the native trisaccharide epitope was synthesized via a literature protocol. All trisaccharides were analyzed using isothermal microcalorimetry (Table 1), and these binding parameters show that only modest free energy gains are seen for pre-organized ligands. Furthermore, the net free energy gains of paired functional group modifications are not additive. It is postulated that the unnatural amide used for the conformationally restrictive tether could be responsible for possible free energy losses **4** and **5**, each with double modifications.

Scheme 2: Cyclic Trisaccharides 2 and 3

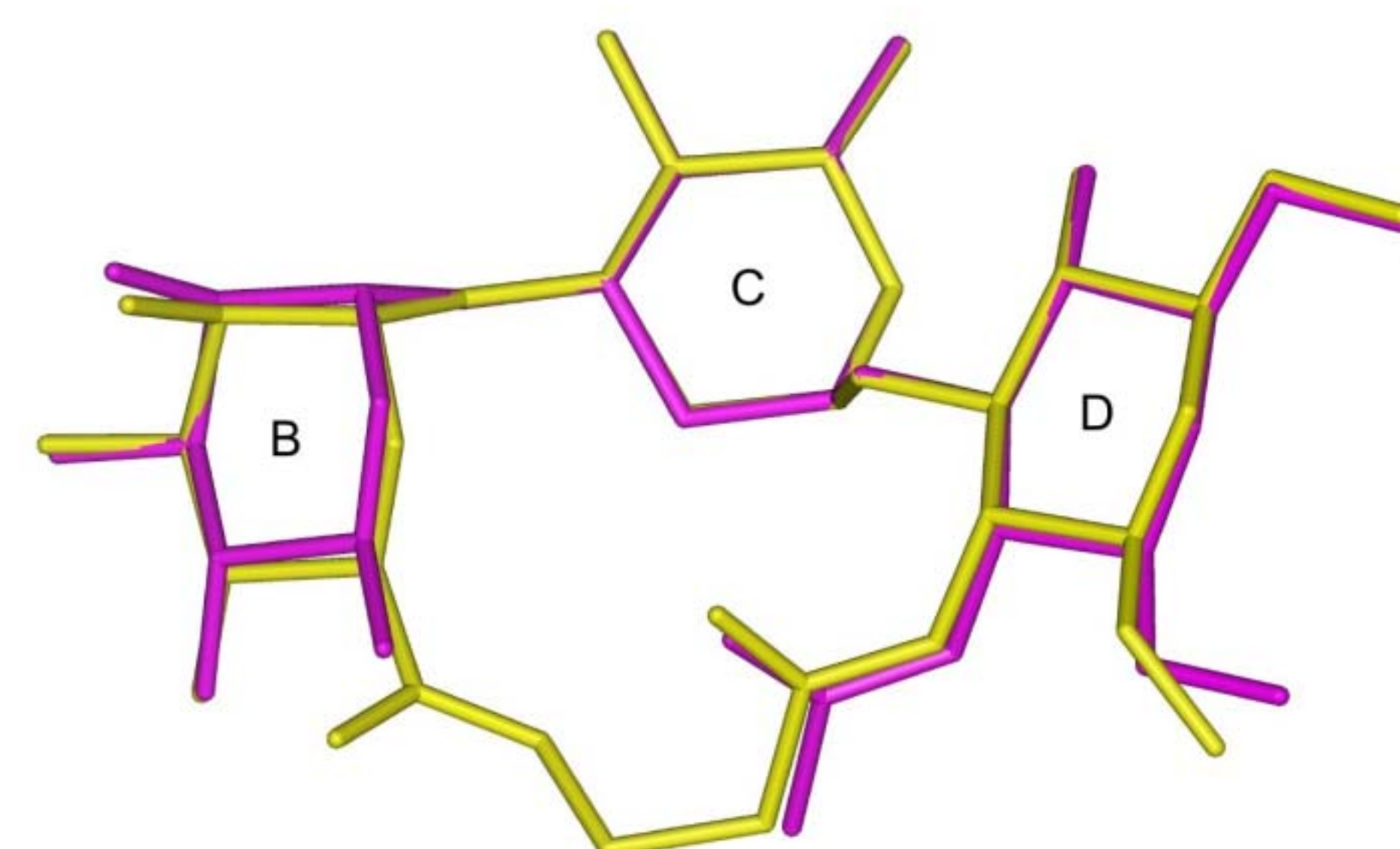
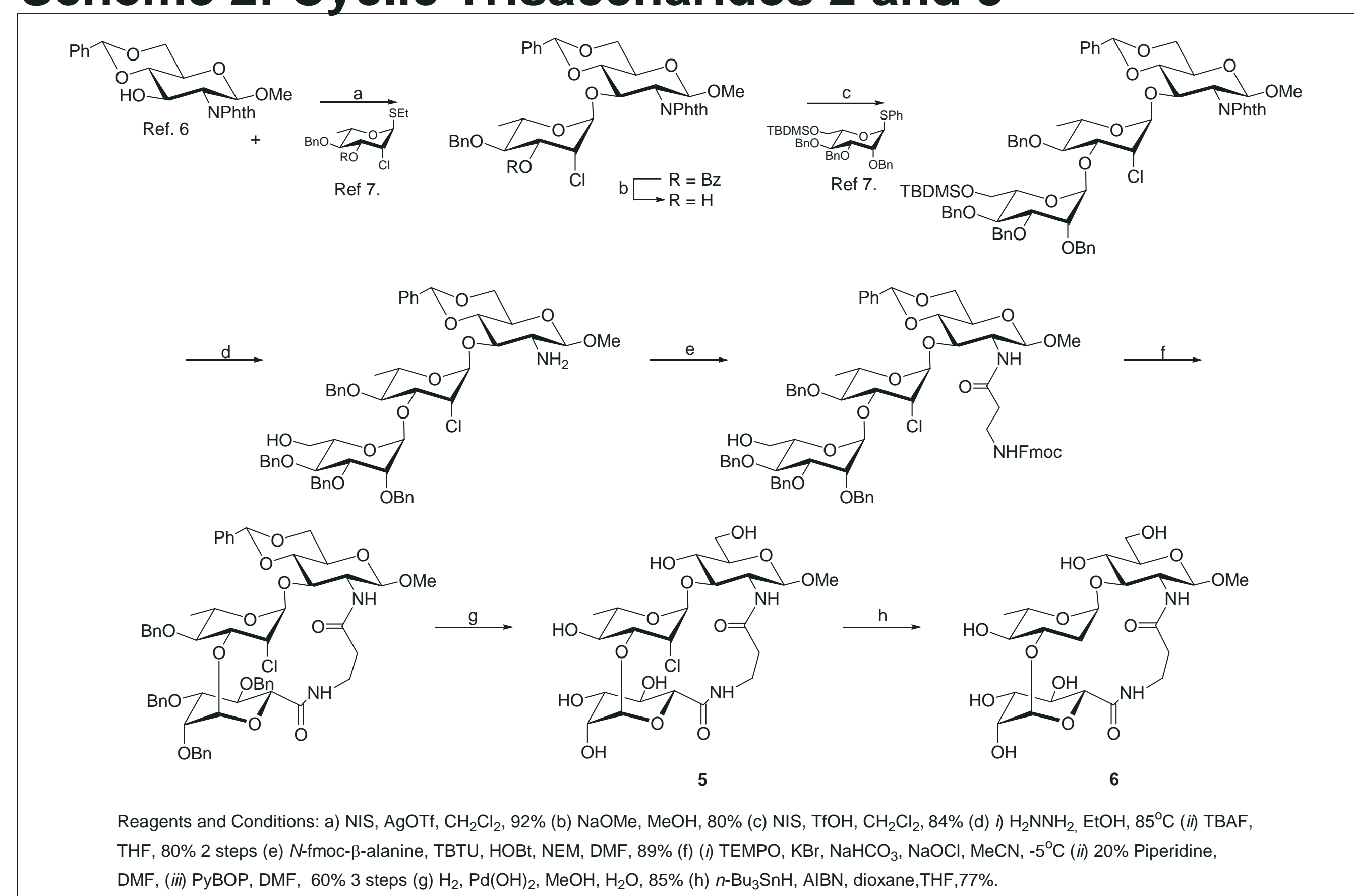


Figure 1 shows the global minimum conformation of cyclic-deoxygenated **6** (gold) superimposed over the conformation of **3** (cyan, taken from its co-crystallized complex with SYA/J6). Dynamics calculations were conducted using Insight II with the AMBER Plus force field. It can be seen that the calculated minimum energy conformation agrees almost perfectly with that of the bound trisaccharide. This indicates that the pre-organization imparted by the tether ought to give rise to entropic gains in binding affinities.

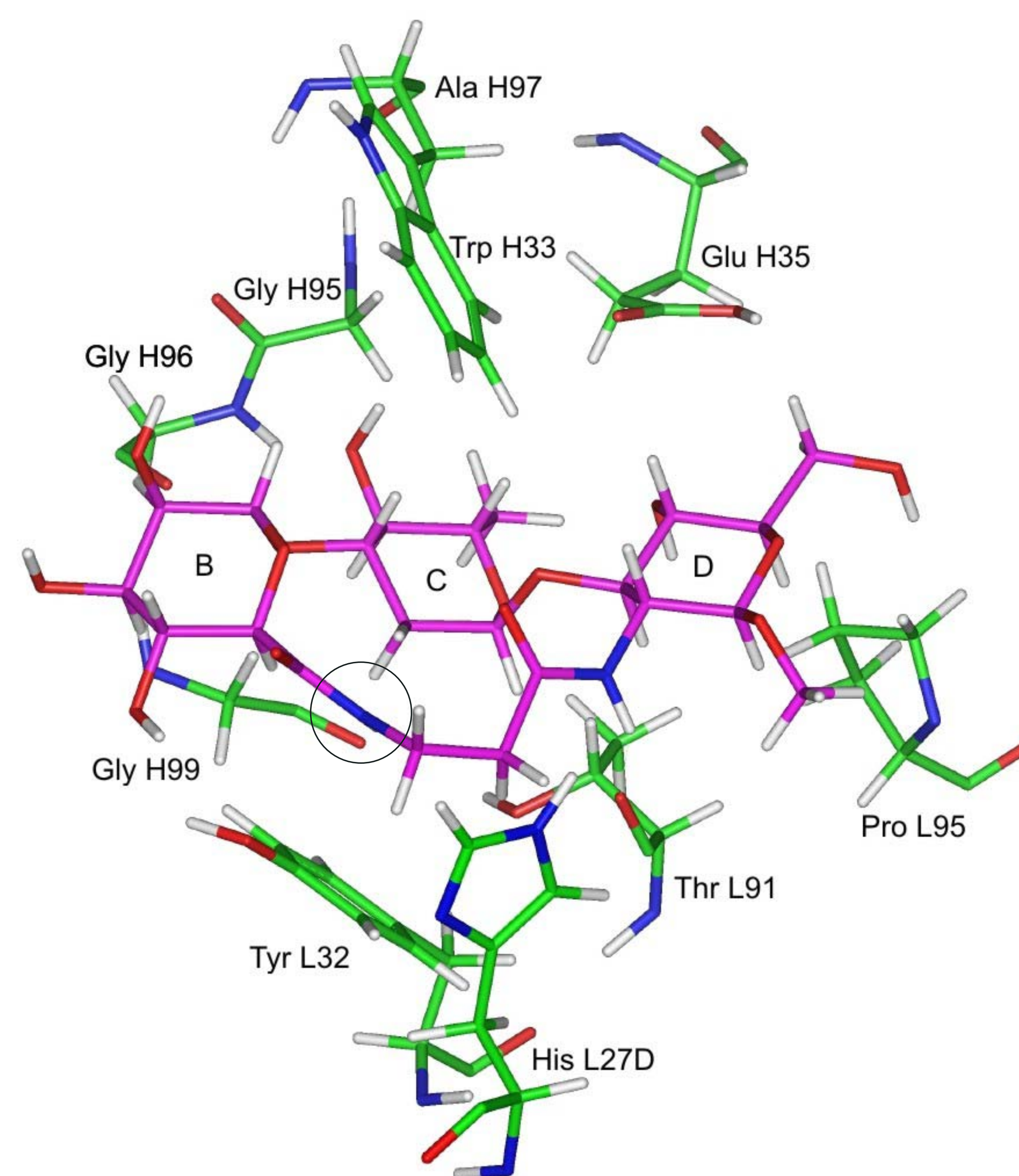
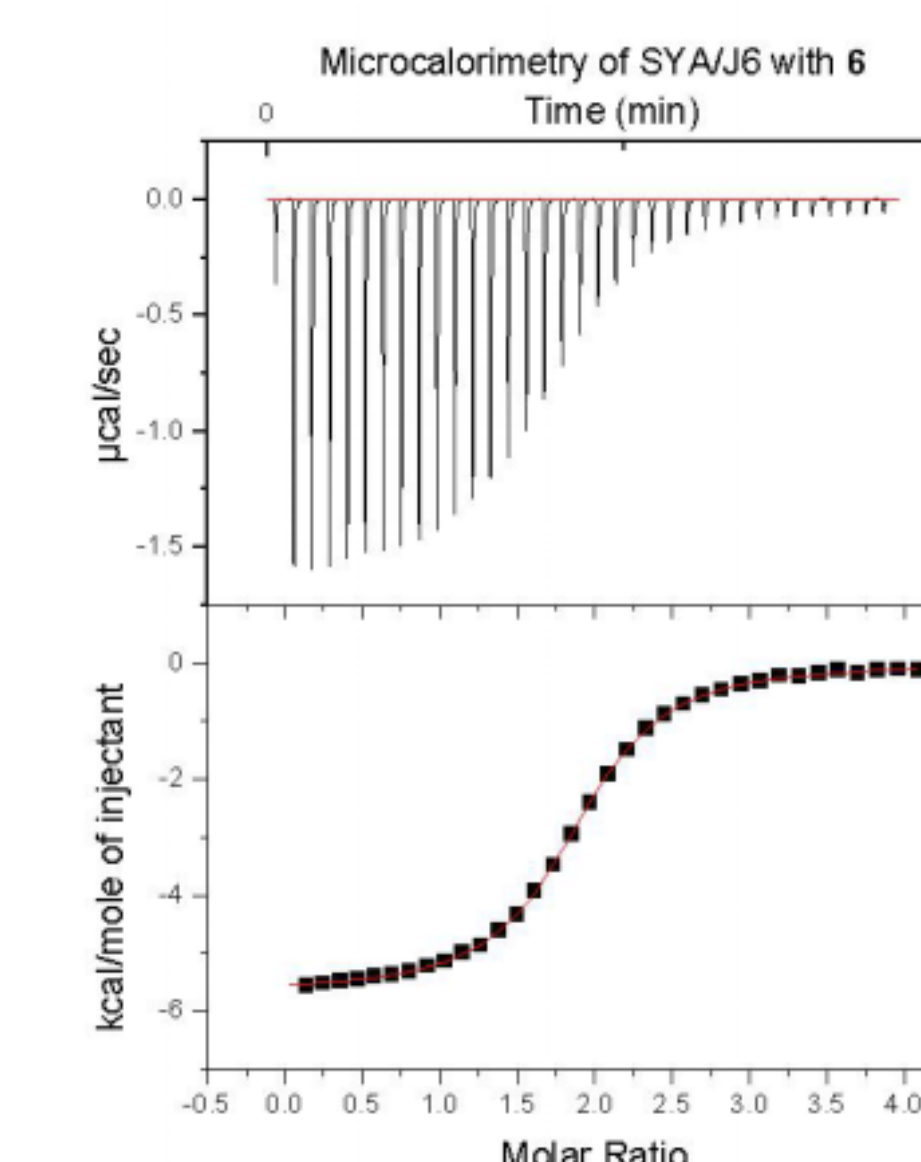


Figure 2 shows the global minimum of cyclic-2'-deoxy **6** (cyan) docked to the co-complex of **3** and SYA/J6. Included are the residues in key van der Waals contact (<4Å) to the central rhamnose ring. Also evident is the nitrogen atom from the unnatural amide contained within the tether (circled) pointing into the hydrophobic pocket surrounding the central rhamnose-C residue.

Table 1: Microcalorimetry Data

Compound	K _A	ΔG (kcal/mol)	ΔH (kcal/mol)	-T ΔS (kcal/mol)
1	1.1 x 10 ⁵	-6.8	-3.8	-3.0
2	2.7 x 10 ⁶	-7.4	-5.7	-1.7
3	3.1 x 10 ⁶	-8.8	-8.5	-0.3
4	2.0 x 10 ⁶	-8.7	-5.7	-3.0
5	4.0 x 10 ⁶	-8.8	-4.8	-4.0
6	4.3 x 10 ⁵	-7.4	-5.6	-1.8
7	2.0 x 10 ⁴	-5.8	-3.5	-2.3
8	2.9 x 10 ⁴	-6.0	-3.1	-2.9



Results and Conclusions

Table 1 summarizes the binding affinities of the modified trisaccharide derivatives. Isothermal titration calorimetry^{7,8} was used to measure the binding parameters of all compounds. A representative binding isotherm (SYA/J6 with **6**) is also displayed. The binding parameters show that cyclic **4** (2'-OH) and cyclic **5** (2'-Cl) show increased affinity over their acyclic counterparts, but despite modification, cyclic **6** (2'-H) is a weaker binder than acyclic **3**. A possible explanation can be visualized via docking studies with the global minimum structure of **6** and SYA/J6. It is evident that the central rhamnosyl ring is heavily involved in van der Waals interactions (Figure 2), and it is likely the deoxygenation in **3** that extends this hydrophobic region, accounting for its increased affinity. What is also clear is that the nitrogen of the unnatural amide used for the tethering methodology points directly into this region, and is likely the cause for drastically reducing binding affinity.

Free energy gains for paired modifications are not additive and certainly not predominantly entropic. It is envisioned that further dynamics and docking analysis coupled with STD-NMR techniques could lead to increased understanding of this system, allowing the rational design of inhibitors of SYA/J6 at nanomolar concentration

Acknowledgements

Financial support for this work was provided by research grants from the Natural Sciences and Engineering Research Council of Canada (NSERC), the University of Alberta, and an NSERC postgraduate scholarship to R.S. McGavin. Special thanks go to Ms. J. Sadowska and Ms. B. Chen for antibody preparation and purification.

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