Single Site Functional Group Replacement and Tethering of Trisaccharides Yield Non-Linear Free Energy Gains Due to Changes in Bound Ligand Orientation

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Introduction

The molecular recognition of a *Shigella flexneri* variant Y lipopolysaccharide (LPS) by a monoclonal antibody, SYA/J6, has provided several crystal structures that provide high resolution details of carbohydrate–protein interactions and have been used to design higher affinity univalent carbohydrate ligands. The biological repeating unit of the LPS is shown below and the pyranosyl residues are identified as ABCD:

-[-2)-α-L-Rha(1-2)-α-L-Rha(1-3)-α-L-Rha(1-3)-β-D-GlcNAc(1]$_n$-

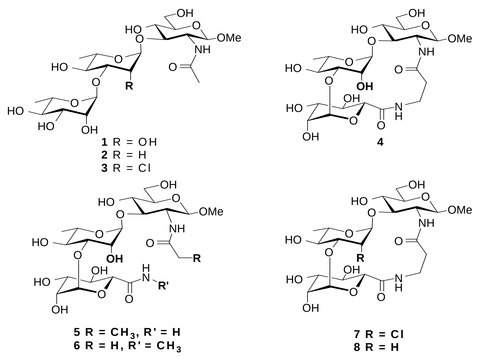

Figure 1: Native ligand **1** and derivatives **2** to **8** used for this study.

Crystal structures[1] of SYA/J6 with bound ligands (pentasaccharide ABCDA' and trisaccharide **2**) detail a deep binding groove and the position of the minimal BCD trisaccharide epitope **1**. Analysis of the contacts made to **1** and the higher affinity deoxygenated trisaccharide **2**[2] show that **2** can enter the site more deeply than **1**. Chemical mapping identified a higher affinity congener **3**, and it was assumed that chlorine was a compatible, isosteric replacement for the C-2' hydroxyl.[3] The acetamido and C-6" methyl groups pointed toward bulk solvent. Intramolecular tethering of these groups was employed to constrain the ligand in its bioactive conformation (**4**).[4]

Our efforts to design high affinity carbohydrate ligands have used the pairing of functional group modification and intramolecular pre-organization, each of which in isolation contribute favorable free energy gains (Figure 1, **7** and **8**). We found that there was little additivity of activity in one case (**7**), and a detrimental effect on binding energy in the other (**8**).[5]

We now report data from STD NMR epitope mapping studies conducted on these ligands with SYA/J6, and comment on tether construction and the additive binding activities when pairing functional group modification with intramolecular pre-organization.

Epitope Mapping Using STD-NMR

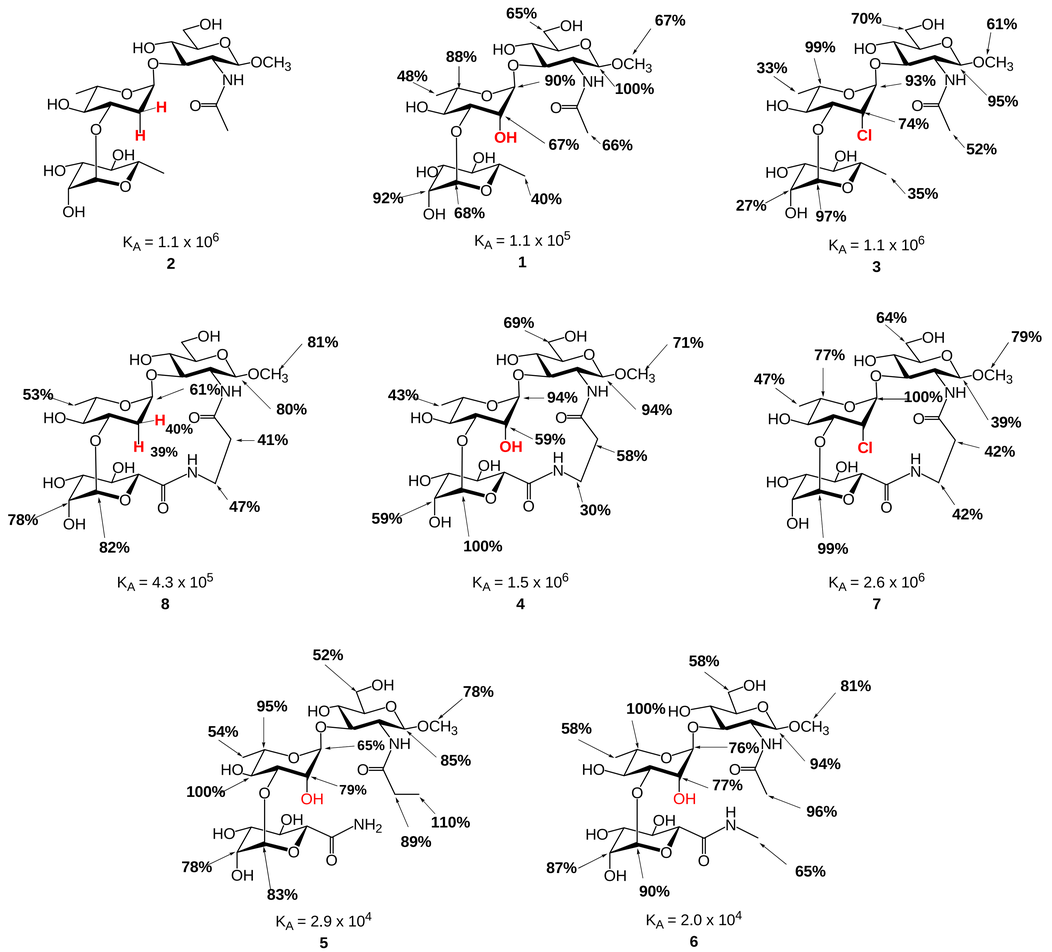

Figure 2: STD Percentage enhancements expressed relative to the proton with the greatest intensity (100%).

Several compounds show a similar semi-quantitative pattern of STD enhancements for the two residues Rha C and GlcNAc D. Compounds **1**, **3**, **4**, **6** and **8** show substantially different STD effects for the protons of residue Rha B. This implies that the position of Rha B, the most solvent exposed of the three "buried" residues varies with respect to the protein surface.

The two modified cyclic compounds **7** and **8** experience STD effects that differ from each other and from the group above. However, there are some similarities between the tethered trisaccharides **4** and **7**. Analysis of the data for the 2,6-dideoxy trisaccharide **2** suggests that this compound binds quite differently from its tethered counter part **5**.

Results and Conclusions

The deep binding site of mAb SYA/J6 appears to accommodate multiple binding modes depending on the modifications made to the ligand. It can not be ruled out that the relatively slow NMR time-scale allows a ligand to occupy multiple bound configurations, giving averaged epitope maps. Each bound ligand enters the groove to a substantial degree since every resolvable ligand resonance exhibited transferred magnetization from the protein.

Although similar epitope maps are observed for some ligands (**5**, **6** and **8**) the STD data are not consistent with single binding mode for all ligands. The most exposed residue Rha B appears to be adopting different orientations in compounds **1**, **3**, **4**, **6** and **8**.

The data for **5** indicate that a portion of the tether prevents the central 2,6-dideoxy residue of **8** from entering the binding site as deeply as **2**. This is also the likely origin of the inability to achieve additive free energy gains by pairing functional group modification with tethering. A similar conclusion may be reached for the pairs **1** and **4**, and **3** and **7** (Figure 2). This evidence is also indicates that the tether assembly prevents the pre-organized ligands from achieving their optimal position in the site.

The semi-quantitative interpretation of the STD-NMR experiment conducted here is clearly inadequate for development of a complete picture of the binding mode for each ligand. Laborious modeling and iterative re-calculation of expected versus observed STD effects would provide a more definitive picture. However, it is doubtful whether these calculations are warranted since there is sufficient evidence to suggest that segmental positioning of the three primary contact residues is the underlying cause of the diverse, affinity enhancements for the various tethered ligands.

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