Synthesis and Binding Analysis of Cyclic Oligosaccharide Inhibitors of Monoclonal Antibody SYA/J6

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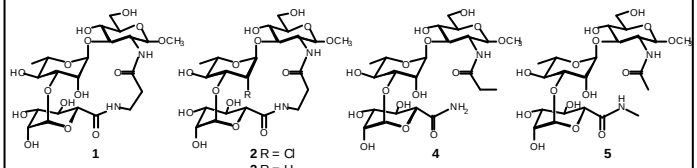
Introduction

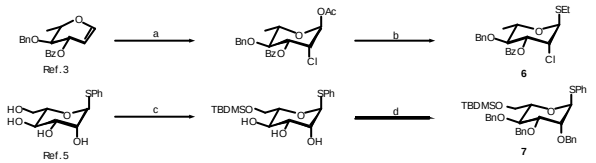
Investigation of the entropic penalty that accompanies binding of univalent oligosaccharide ligands suggests that the often-cited flexibility of glycosidic linkages is not the primary cause of the low affinity binding of oligosaccharides by protein receptors. We report here efforts to increase affinity 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 (*a*-L-Rha-(1-3)-*a*-L-Rha-(1-3)-*b*-D-GlcNAc-OMe), a component of the O-polysaccharide of *Shigella flexneri* variant Y. Inspection of the complex of the trisaccharide and SYA/J6 shows the 2acetamido and 6′-methyl groups are exposed to bulk water, and a cyclic trisaccharide with a *b*-alanine-tether spanning these two functionalities (**1**) has been found to have increased binding affinity towards SYA/J6. [1] 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 tethered **2** and **3**, containing previously reported beneficial modifications to the central rhamnose ring (2′-chloro- and 2-chloro-2′-deoxy), [2,3,4] and the synthesis of acyclic derivatives **4** and **5**. The native trisaccharide epitope was synthesized, using known methodologies, for comparative measurement of isothermal mircocalorimetric titration and ELISA. These data 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.



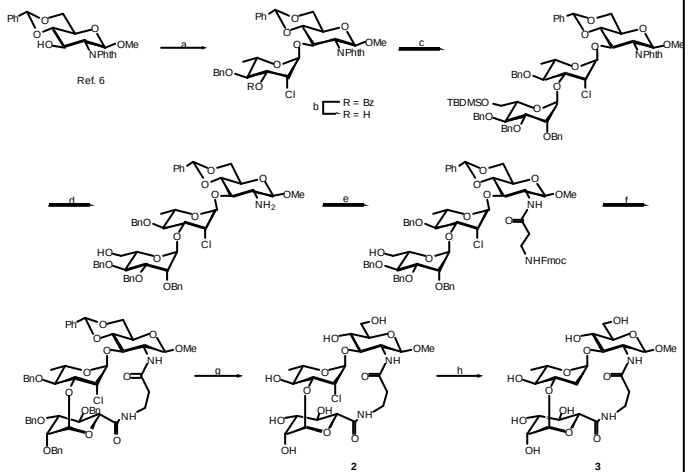
1 **2** R = Cl, **3** R = H, **4**, **5**

Scheme 1: Monosaccharide Donors **6** and **7**



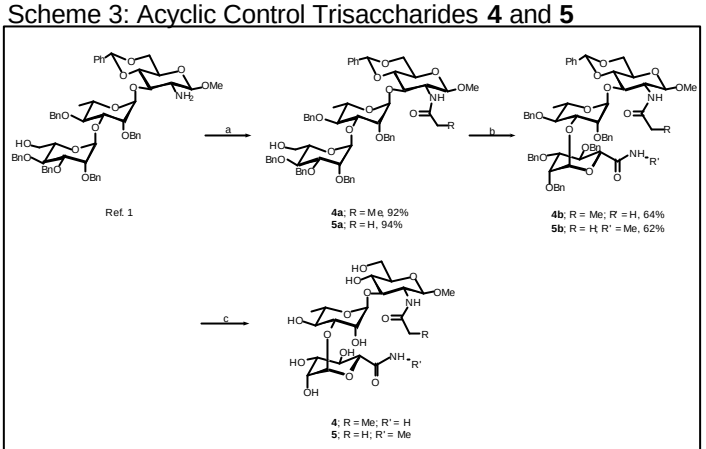
Reagents and Conditions: a) (*i*) Cl_2, CCl_4, -10°C (*ii*) AgOAc, AcOH, 70% (b) EtSH, BF_3-OEt_2, CH_2Cl_2, 92% (c) TBDMS-Cl, Pyr, Et_3N,91% (d) NaH, BnBr, DMF, 92%.

Scheme 2: Cyclic Trisaccharides **2** and **3**



Reagents and Conditions: a) **6**, NIS, AgOTf, CH_2Cl_2, 92% (b) NaOMe, MeOH, 80% (c) **7**, NIS, TfOH, CH_2Cl_2, 84% (d) *i*) H_2NNH_2, EtOH, 85°C (*ii*) TBAF, THF, 80% 2 steps (e) *N*-fmoc-β-alanine, TBTU, HOBt, NEM, DMF, 89% (f) (*i*) TEMPO, KBr, $NaHCO_3$, NaOCl, MeCN, -5°C (*ii*) 20% Piperidine, DMF, (*ii*) PyBOP, DMF, 60% 3 steps (g) H_2, $Pd(OH)_2$, MeOH, H_2O, 85% (h) *n*-Bu_3SnH, AIBN, dioxane, THF,77%.

Scheme 3: Acyclic Control Trisaccharides **4** and **5**



Reagents and Conditions: a) RC(O)OC(O)R, MeOH, (b) (*i*) TEMPO, KBr, $NaHCO_3$, NaOCl, MeCN, -5°C (*ii*) CDI, CH_2Cl_2, then NH_3 or $MeNH_2$ in THF (c) H_2, $Pd(OH)_2$, MeOH, H_2O, 82% for **4**; 85% for **5**

Results

Table 1 summarizes the binding affinities of the modified trisaccharide derivatives. Competitive ELISA was performed on the native trisaccharide epitope and acyclic derivatives **4** and **5**. Isothermal titration calorimetry was used to measure the binding parameters of the native epitope and cyclic ligands **2** and **3**. Both techniques were performed as previously described. [7,8] A representative binding isotherm (SYA/J6 with **3**) is also displayed.

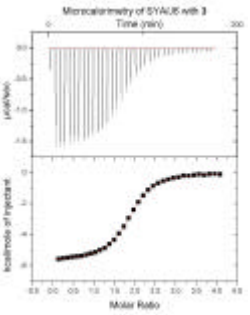


Table 1

Compound	IC50 (μM)	K_A	ΔG (kcal/mol)	ΔH (kcal/mol)	-TΔS (kcal/mol)
Native Epitope	15	1.1×10^5	-6.8	-3.8	-3.0
1 (Lit.)	-	2.0×10^6	-8.7	-5.7	-3.0
2	-	4.0×10^6	-8.8	-4.8	-4.0
3	-	4.3×10^5	-7.4	-5.6	-1.8
4	94	-	-	-	-
5	92	-	-	-	-

Summary and Conclusions

An efficient synthetic route to tethered cyclic oligosaccharide inhibitors and acyclic reference compounds is described. ITC data shows that the bulk of the free energy gain observed for the modified cyclic derivatives is enthalpic. The pairing of site specific modifications and intramolecular pre-organization result in higher affinity ligands but the free energy gains of single site variants are not additive. This suggests that the contacts with protein for each ligand, modified at single sites, differs so that it is difficult to productively pair tethering and functional group replacement. It is envisioned that the use of STD-NMR techniques can identify the contact residues responsible for this difficulty. Investigations toward modifying the tether linkage at the 6′′-position is underway and focused on removing all hydrogen bond donor and acceptor elements from this position.

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