Quantifying the role of N-acetylation in sialic acid recognition

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Introduction

Multiple receptor proteins recognize sialic acid as their target ligand molecule, including influenza hemagglutinin (HA), siglecs, etc [1] as illustrated in Fig.1. The CH-π interaction makes a significant contribution but is frequently overlooked by theoreticians.

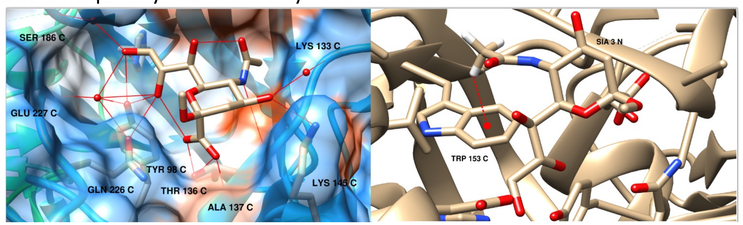


Figure 1. Influenza HA (PDB 3UBQ). Left, a hydrophobicity surface of the sialic acid binding site. Hydrogen bonding interactions are shown with red solid lines. Right: a zoomed-in depiction of the CH-π interaction between TRP 153 side chain and the N-acetyl moiety of sialic acid.

Research Question

We seek to quantify the role of the N-Acetyl moiety in the recognition of sialic acid, with a specific focus on CH-π interactions. This will enhance understanding on how proteins evolve to recognize carbohydrates and will help improve the accuracy of theoretical modeling methods. Therefore, we synthesized α-Methyl sialoside **1** [2,3] and its free amine analog **2**, (Scheme 1). Based on our recently developed CH-π model, removal of the N-acetyl moiety was predicted to result in a loss of 1.8 kcal/mol in binding ΔG [4]. We will attempt to compare our predictions to experimentally measured IC_{50} and *Ki* values using a BLI-based competition assay, Fig. 2 [5].

Methods

The free amine of the methyl ester of methyl glycoside **8** was prepared in a simple, high-yield (75%) approach by hydrolysis of per-acetylated glycoside analog **6** using methane-sulfonic acid. The 1D proton NMR spectrum for **8** is shown in Fig. 3.

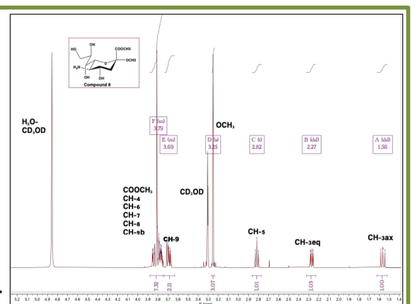
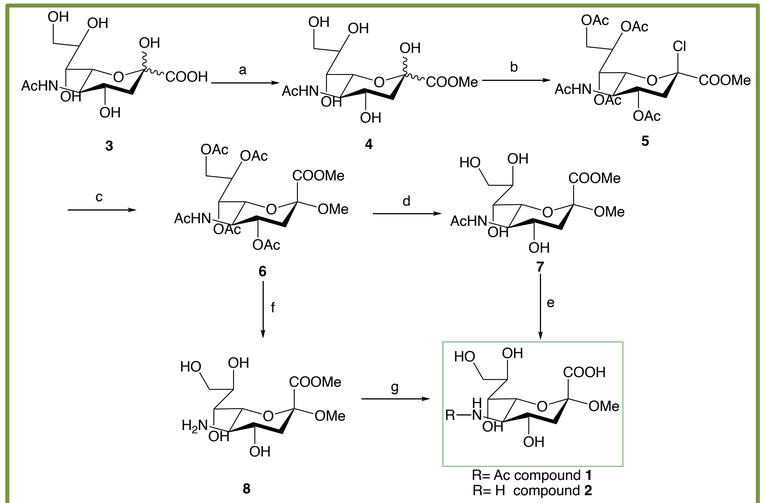


Figure 3. ^{1}H-NMR for compound **8**.

Synthesis Results



Scheme 1. *Reagents and conditions:* ***(a)*** Anh. MeOH, Amberlite IR-120 (H), RT, 3 d; ***(b)*** CH_3COOCl, RT, 16 h; ***(c)*** Anh. MeOH, RT, 3-5 h; ***(d)*** Methanolic CH_3ONa, Anh. MeOH, 0 °C, 16 h; ***(e)*** 1M NaOH, 0°C, 0.5 h. ***(f)*** CH_3SO_3H, Anh MeOH, Reflux, 20-24 h; ***(g)*** LiOH.H_2O, Ethanol: H_2O, 0°C, 0.5 h.

Conclusions

- We successfully designed a simple, high-yielding pathway for the preparation of free amine methyl ester methyl glycoside **8** by acidic deacetylation using the methanesulfonuc acid starting from commercially available sialic acid **3** in 4 steps only.
- We have optimized a BLI-based competition assay to measure IC_{50} values of sialoside analogs **1 & 2.**
- The IC_{50} and Ki for **1** has been determined to be 1.6 mM and 0.65 mM
- The IC_{50} and Ki for **2** has been determined to be 4.9 mM and 2.02 mM
- Using: $\Delta\Delta G = RT\ln(Ki_1/Ki_2)$, we computed the binding energy difference between **1** and **2** to be 0.7 kcal/mol (favoring **1**).

Acknowledgements

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IC_{50} Assay Results

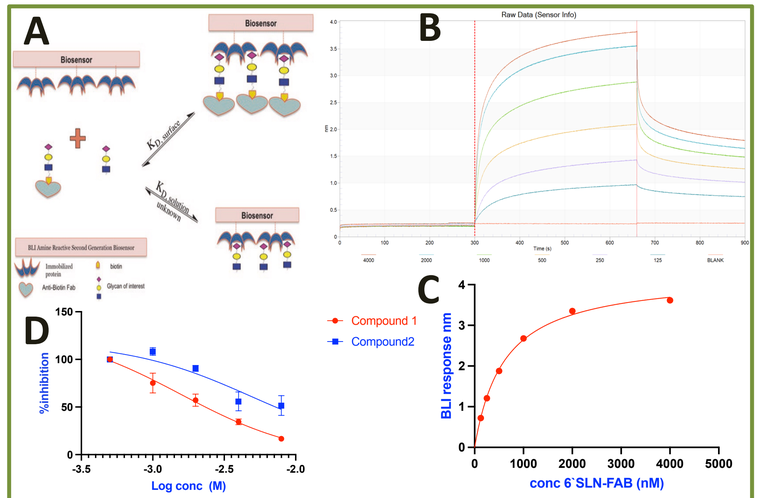

Figure 2. A. Schematic illustration of the BLI-based competition assay. **B.** BLI sensorgram of 6'SLN-Fab binding to H1N1 AC04 on AR2G biosensor. **C.** Steady-state derivation of $K_{D,surf}$ for 6'SLN-Fab binding to H1N1 AC04 **D.** IC_{50} curves for Compound **1 & 2** inhibiting the binding of 6`SLN-FAB. i) preliminary the $K_{D,surf}$ for the 6`SLN-FAB with immobilized H1N1 AC04 on AR2G biosensors was measured as 0.7uM . ii) IC_{50} curves for Compound **1 & 2** inhibiting the binding of 6`SLN-FAB with immobilized H1N1 ACO4.
Using the Cheng-Prussof relationship [6], the inhibitory constants (*Ki*) for compounds **1** and **2**, will be determined: $K_i = IC_{50} / (1+ [\text{Fab-glycan}]/K_{D,surf})$

References

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