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A Dynamic Combinatorial Approach for the Analysis of Weak Carbohydrate/Aromatic Complexes: Dissecting Facial Selectivity in CH/π Stacking Interactions

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Supporting Information

ABSTRACT: A dynamical combinatorial approach for the study of weak carbohydrate/aromatic interactions is presented. This methodology has been employed to dissect the subtle structure−stability relationships that govern facial selectivity in these supramolecular complexes.

Understanding how proteins and nucleic acid receptors recognize oligosaccharides represents a fundamental issue in chemical biology with far-reaching implications in fundamental biology, biotechnology, and drug design.[1] After two decades of studies, it is now widely accepted that stacking interactions involving pyranose rings and aromatic systems are pivotal to these processes.[2] While the free energy of these contacts is dominated by the dispersive component, electrostatic and solvent-dependent effects also must be considered. Despite extensive efforts in this area, a detailed comprehension of the existing relationship between these contributions and the chemical nature of the pyranose and aromatic rings is still missing. This absence can at least partially be attributed to the lack of a simple system for the study of stacking complexes that displays enough sensitivity to reveal minor stability differences. Employing concepts from dynamic combinatorial chemistry,[3] we have developed a simple and versatile model for the analysis of weak carbohydrate/aromatic complexes.

Our general strategy is outlined in Figure 1a. First, several disaccharide model systems were designed and synthesized. All of them included a common aminoaltrose ring locked in a $^4C_1$ conformation by a benzylidene group and linked to an equatorial position of a variety of potential "CH/π donor" sugar units (represented as colored ellipses). Next, equimolar amounts of the different models were dissolved in 10 mM phosphate solution [see the experimental section in the Supporting Information (SI)] and treated with 2-naphthylacetaldehyde to form a dynamic mixture of hemiaminals/imines (undetectable under the employed experimental conditions).

Previous studies have shown that these species adopt a hairpinlike major conformation stabilized by intramolecular stacking between the aromatic system and the sugar CH/π donor.[4] Since the resulting mixture is formed under thermodynamic control, the observed composition directly depends on the energy contribution of the different carbohydrate/aromatic contacts (the only distinct factor among the species). Subsequent chemical reduction of the

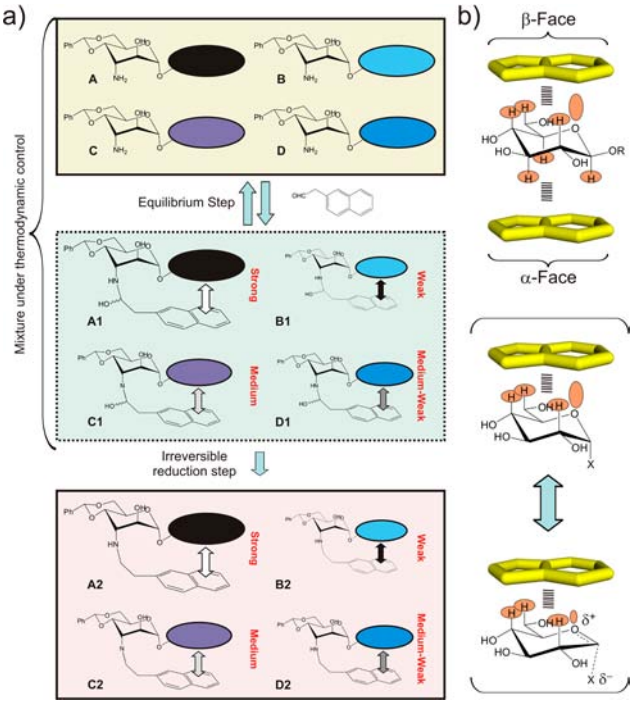

Figure 1. (a) Schematic representation of the dynamic combinatorial approach employed for the analysis of carbohydrate/aromatic interactions. (b) (top) Complexes involving the D-pyranose β/α faces for both anomers are shown (up and down). Interacting regions of the D-pyranose are highlighted. (bottom) The charge density at the ring oxygen is modulated by hyperconjugative delocalization of its lone pair (a common explanation for the anomeric effect).

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transient hemiaminals/imines (normally performed under slightly acidic conditions for an optimum reaction rate; see the SI) with an externally added reagent irreversibly converts them into a set of secondary amines, *whose relative populations must reflect free energy differences among the alternative stacking modes*. For practical reasons, we employed NMR spectroscopy to follow the chemical reactions. The populations of the different species, which were roughly invariant during the time course of the experiment, were evaluated by integration of the different sets of resolved signals. Finally, the chemical shift perturbations promoted by the naphthyl ring at the interacting pyranose in the final species were measured and taken as an indication of the subtle geometrical differences among the alternative stacking modes.

In particular, we focused our attention on the subtle factors that govern (α/β) "facial" selectivity in carbohydrate/aromatic complexes (Figure 1b). It should be noted that stacking through a D-pyranose β face implies the participation of the ring oxygen in n/π bonds with the aromatic system. This particular contact, would be expected to be modulated by the *anomeric effect*[5] and consequently should be sensitive to structural factors such as the anomeric configuration and the presence of electron-withdrawing substituents at the anomeric center.[5d,e]

The employed disaccharide library is shown in Figure 2 (also see Figures S1−S7 in the SI). Its design permitted comparison

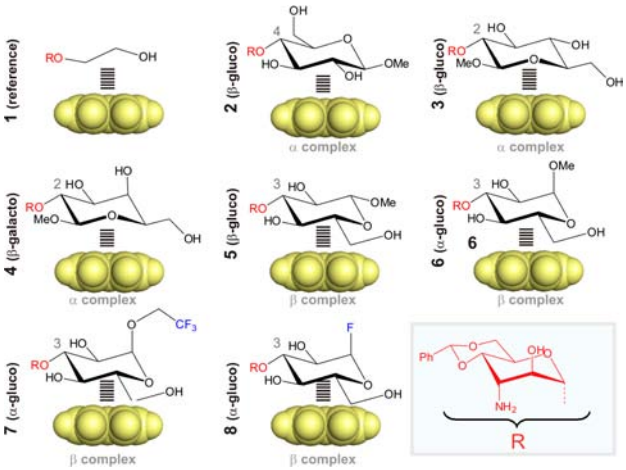

Figure 2. Representation of the synthesized disaccharide library. The configuration of the interacting pyranoses is indicated. Carbon positions involved in the glycosidic bonds are labeled in gray. Finally, carbohydrate/aromatic contacts formed upon reaction with 2-naphthylacetaldehyde are also shown.

of the stacking of the naphthyl ring against either the α (compounds **2**−**4**) or β (compounds **5**−**8**) face of the CH/π donor D-pyranose unit. These unique stacking modes are to a large extent determined by the strong conformational preferences of the $\alpha(1-4)/\alpha(1-2)$ and $\alpha(1-3)$ linkages, respectively (see Figure S8). The library also included disaccharides with different configurations for the key OH groups and punctual OH/F substitutions. Monosaccharide **1**, with no interacting additional sugar unit, was also prepared as a negative reference.

Control reductive amination assays confirmed that 2-naphthylacetaldehyde exhibits a strong preference for disaccharides **2**−**8** with respect to the reference compound **1** (3−10-fold increased reactivity). Moreover, the observed differences in reactivity were in agreement with the stabilities expected for the alternative stacking modes. In contrast, smaller aldehydes (e.g., benzaldehyde) were much less selective, in agreement with their limited ability to contact the CH/π donor unit in the disaccharide products.

As a first step in our analysis, we performed the reductive amination reactions with these disaccharides and characterized the corresponding products by NMR spectroscopy (Figure 3). Next, we carried out pairwise competition experiments with all of the library members (Figures 4, S9, and S10). Figure 4 shows the ratio of products resulting from each pair of primary amines. This value was taken as an indicator of the free energy differences among the alternative carbohydrate/aromatic stacking modes. Experiments performed with more complex mixtures (see Figure 4b) or alternative buffer conditions (Figure S11) rendered similar results. Several conclusions can be obtained from these data (Figures 4 and 5).

(a) First, in regard to the α complexes (formed by **2**−**4**), derivative **2** leads to a favored interaction relative to that established by **3.** The observed preference probably reflects the contribution of the carbohydrate/aromatic contacts mediated by the hydroxymethyl moiety in **2**, which are hardly feasible in **3** and **4**. This hypothesis was backed up by inspection of the geometries of the corresponding complexes and by the $\Delta\delta$ values observed in the reaction products (Figure 5).

(b) It is known that axial OH groups exposed on the interacting face of the pyranose are highly disruptive for carbohydrate/aromatic stacking.[1,2] Our results highlight the unfavorable influence that equatorial OH functions can also have on these complexes. In particular, inversion of position 4 from the D-gluco (in **3**) to the D-galacto configuration (in **4**) leads to preferred CH/π contacts, together with a pronounced adjustment of the stacking geometry (as revealed by the $\Delta\delta$ values). The destabilizing role of this particular OH in **3** most likely results from a combination of repulsive electrostatic interactions and nonfavorable desolvation effects.[4,6]

(c) In the case of an identical β-OMe glucose unit, complexation through the D-pyranose α face (compound **3**) was preferred over the β-face (compound **5**). *In conclusion, n/π interactions mediated by the ring oxygen are less stable than the CH/π analogues.* The observed effect amounts to 0.2−0.4 kcal/mol.

(d) This nonfavorable influence would be expected to be partially relieved upon inversion of the anomeric position (from β in **5** to α in **6**; see Figures 4 and 5) as a result of hyperconjugative delocalization of the ring oxygen lone pair (a common explanation for the anomeric effect). In fact, our experiments indicate that derivative **6** allows the formation of a tighter carbohydrate/aromatic complex with a slightly different geometry (see the $\Delta\delta$ values in Figure 5). However, the stabilization promoted by inversion of the anomeric position in **5** (to give **6**) was identical to that promoted by inversion of the equivalent position 4 in **3** (to give **4**) within the experimental error (Figure 5). In conclusion, the observed effect *is not specific for the anomeric position* and seems simply to reflect the unfavorable influence of particular equatorial OH moieties of the D-pyranose. According to our data, the modulation *of* the β-type carbohydrate/aromatic stacking by the anomeric effect is below the 0.2 kcal/mol limit.

(e) Further enhancements of the hyperconjugative interactions promoted by the incorporation of electron-withdrawing substituents at the anomeric center (in **7** and **8**) were barely reflected in either the stability or the geometry of the resulting complexes. The obtained results are consistent with a minor

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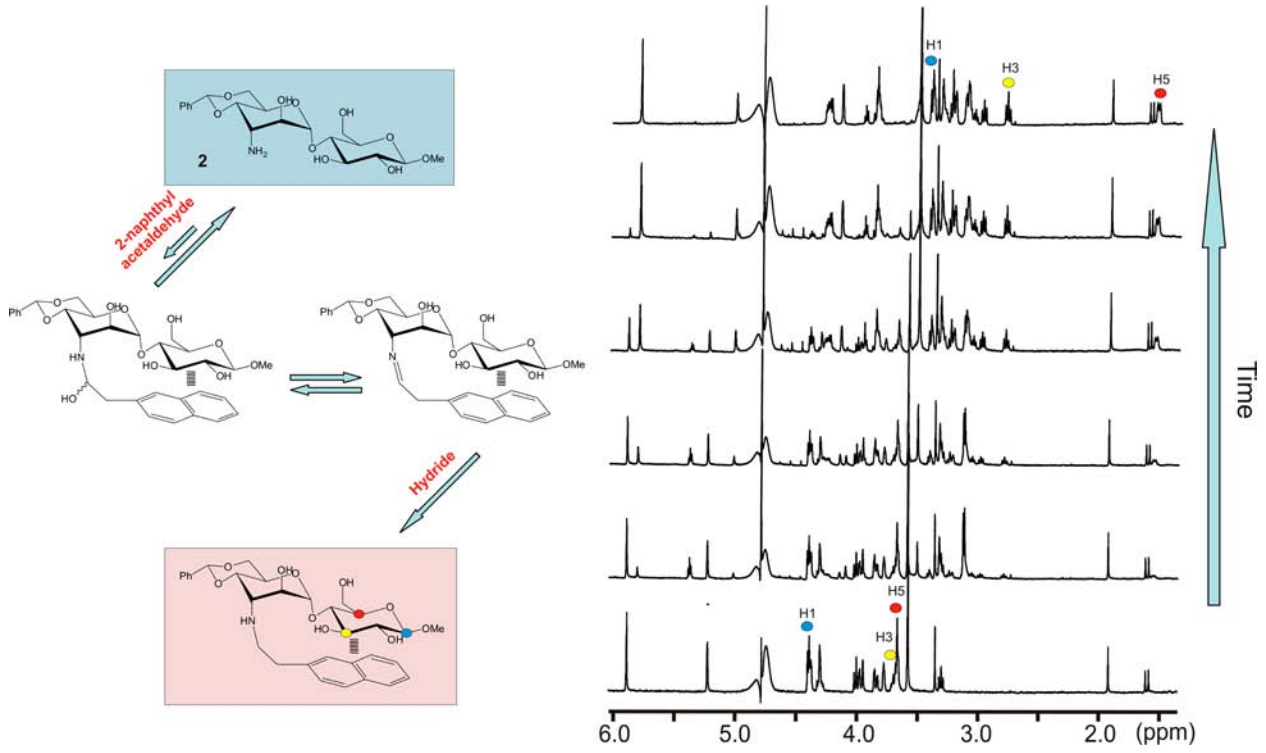

Figure 3. Reductive amination reaction performed with disaccharide **2** and 2-naphthylacetaldehyde in D_2O (10 mM phosphate, pH 6.2). The time evolution of the reaction mixture is represented at the right. Key signals of the glucose unit that were shifted upfield (by up to 2.12 ppm) because of the proximity of the naphthyl ring are highlighted.

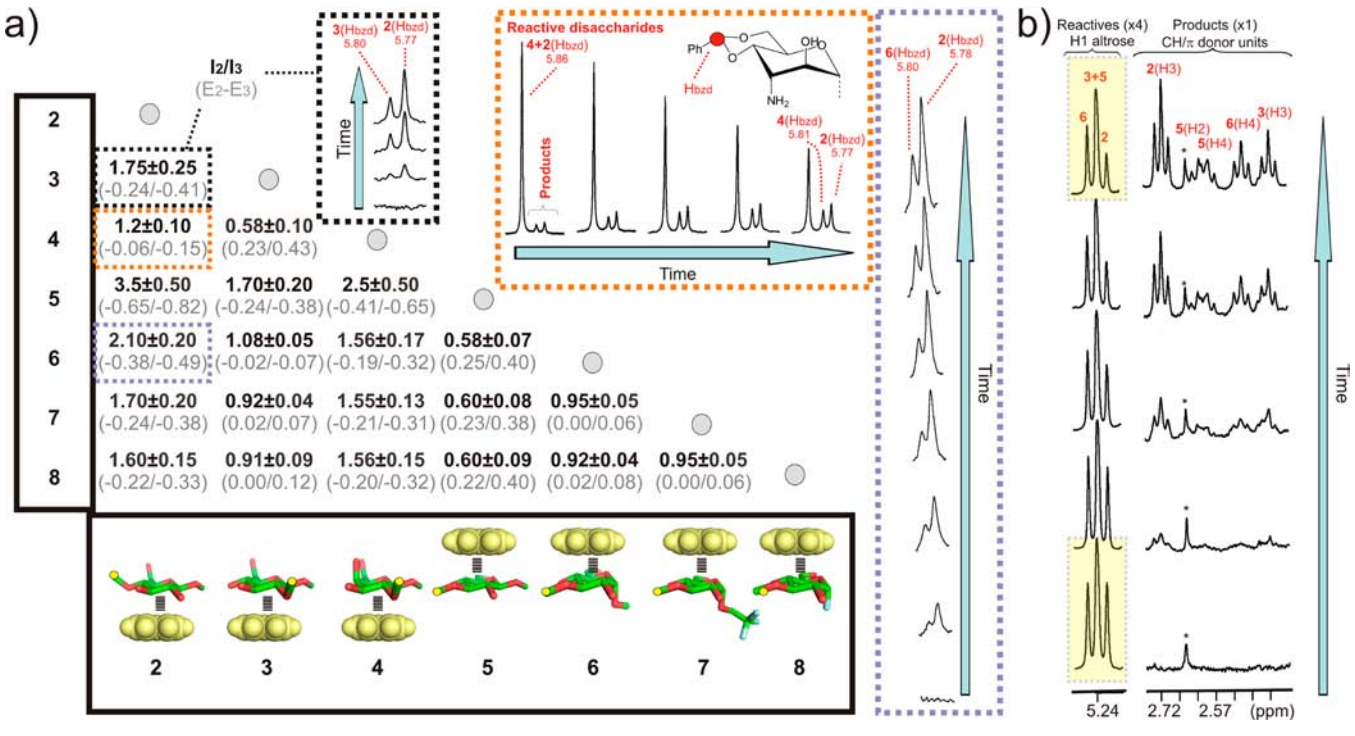

Figure 4. (a) Pairwise competition experiments performed with the library described herein. Equimolecular mixtures of two disaccharides in D_2O (10 mM phosphate, pH 6.2) were treated with substoichiometric amounts of 2-naphthylacetaldehyde. After a short equilibration period, the reductive amination reactions were carried out by adding sodium cyanoborohydride. The ratio of secondary amine products obtained for each disaccharide pair (I_x/I_y) is indicated. These values were taken as indicators of the free energy differences between the alternative stacking modes ($E_x - E_y$, in kcal/mol). Dotted boxes highlight the formation of the reaction products for pairs **2**/**3**, **2**/**4**, and **2**/**6** as revealed by NMR spectroscopy. For each signal, the compound number (in bold), proton assignment (in brackets), and chemical shifts in ppm are indicated. (b) Competition experiment performed with a 1:1:1:1 mixture of **2**, **3**, **5**, and **6**. Representative NMR signals for the disaccharide models and reaction products are shown. The compound numbers (in bold) and proton assignments (in brackets) are indicated.

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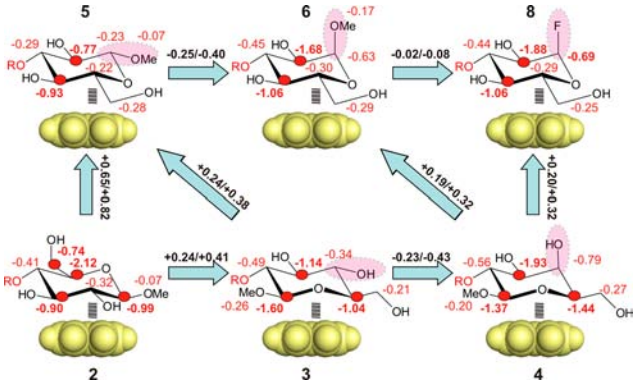

Figure 5. Stability differences for stacking complexes. Chemical shift perturbations promoted by the aromatic ring at the interacting D-pyranose unit in the reaction products are shown. For derivatives **3**–**8**, the key positions 1 and 4 in the β and α complexes, respectively, are highlighted in purple.

strengthening of the carbohydrate/aromatic forces in **7** and **8** (as also reflected in the $\Delta\delta$ values). However, according to our data, this effect amounts to less than 0.1 kcal/mol (Figures 4, 5, and S10). This conclusion is in agreement with a recent theoretical study showing that the hyperconjugative electron delocalization at the anomeric center in pyranose rings is fairly weak.[7]

In summary, the dynamic combinatorial approach herein presented provides a simple and versatile method for the study of weak carbohydrate/aromatic complexes. It can be observed that the maximum energy differences revealed by our data amount to 0.7 kcal/mol, which represents a very significant fraction of the whole carbohydrate/aromatic interaction free energy (estimated to be 1–2 kcal/mol).[1] On the other hand, the employed strategy allows the detection of minor stability differences as small as 0.1 kcal/mol, a limit hardly detectable by other means. Efforts to extend these studies to alternative carbohydrate models including a variety of chemical modifications and configurations and different aromatic systems are currently underway.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis, NMR competition experiments, and Figures S1–S11. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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