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Evaluation of Amino Acids as Chiral Ligands for the Enantiodifferentiation of Carbohydrates by TOCSY NMR

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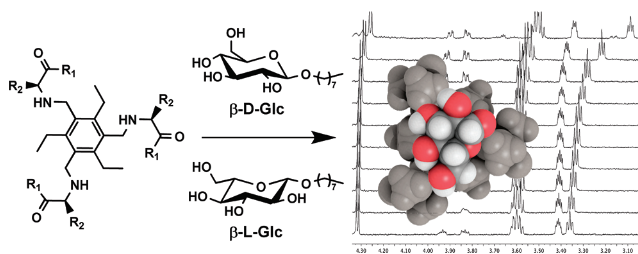

The use of natural amino acids as chiral ligands on a triethylbenzene scaffold for the binding and enantiodifferentiation of carbohydrates has resulted in moderate affinity and selectivity values for glucose. Selective 1D TOCSY experiments revealed this as a powerful titration technology surpassing the severe overlapping of receptor and carbohydrate signals in ^{1}H NMR spectra.

Carbohydrate recognition events mediate a myriad of biological processes such as cell–cell interactions, infection by pathogens, tumor metastasis, and certain pathways of the immune response.[1] As this recognition takes place in Nature under aqueous conditions, it represents an important challenge for supramolecular chemistry. Thus, water molecules compete with the carbohydrate hydroxyl groups for the receptor binding site, and only the precise arrangement of polar and apolar domains (hydrogen bonds and van der Waals forces) enables the saccharide recognition to occur. Consequently, a great effort has been devoted to the understanding of these recognition mechanisms and to the development of synthetic carbohydrate receptors.[2]

Two main families of synthetic receptors can be considered attending to their recognition mechanisms: (i) boronic acid derivatives with the ability to bind diols covalently have proven effective carbohydrate receptors under aqueous conditions, albeit the null resemblance of their recognition mechanism to that of lectins (natural carbohydrate recognition proteins),[2,3] and (ii) receptors interacting with carbohydrates in a biomimetic way through noncovalent interactions. In this case, the strong hydrogen bonds between carbohydrates/synthetic receptors and water molecules render recognition under aqueous conditions more elusive and of limited efficiency.[2,4] Actually, most of these receptors work exclusively in organic solvents (e.g., $CHCl_3$ and MeCN),[2,5,6] which limits very much their potential bioapplications, but conversely offers a good playground for the screening of candidate scaffolds and ligands.

In spite of the stereoselective carbohydrate recognition displayed by lectins, it is surprising that the enantiodifferentiation capacity of synthetic carbohydrate receptors has been barely investigated.[7−9] Furthermore, the ability of amino acids, the constituting building blocks of lectins, to bind carbohydrates[10] and to induce a chiral recognition[9] has been narrowly exploited.

In this Note we describe our results on the noncovalent recognition and enantiodifferentiation of carbohydrates in solution ($CHCl_3$) by means of a new family of amino acid-decorated

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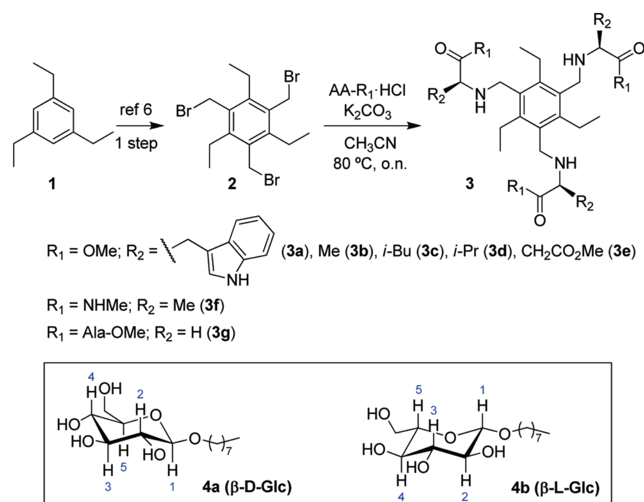
SCHEME 1



receptors based on 1,3,5-triethylbenzene (**1**) (Scheme 1). Compound **1** is a common scaffold in the construction of artificial receptors due to its marked preference for an alternate conformation when fully substituted.[11]

With this aim, the bromomethylated derivative **2**[6] was treated with various methyl L-α-amino esters (AA-OMe) derived from tryptophan (Trp), alaline (Ala), leucine (Leu), valine (Val), and aspartate (Asp), leading to the desired receptors **3a**–**e** in very good to excellent yields (86–96%). In a similar fashion, receptors **3f** and **3g**, incorporating L-Ala-NHMe and the dipeptide Gly-Ala-OMe, were also synthesized (Scheme 1 and Supporting Information).[12]

The capacity of **3** to establish hydrogen bonds (amino ester side arms with donor–acceptor ability) and hydrophobic interactions (benzene core) with carbohydrates was assessed in the binding and enantiodifferentiation of the octyl-β-glucopyranosides **4a** and **4b**,[13] convenient organosoluble derivatives of D- and L-glucose (selected as a model monosaccharide based on the presence of the D enantiomer as terminal sugar in glycans that participate in saccharide recognition processes, Scheme 1). As Trp is present at the binding site of many lectins, and the indol group resembles the pyridine/pyrrol present in some of the most successful carbohydrate receptors based on **1**,[14] we decided to analyze first the behavior of the Trp-based receptor **3a**. The interaction of **3a** with both enantiomers of **4** was studied by ^{1}H NMR in $CDCl_3$ (Figure 1). Titrations were performed with a solution of **4a** at a constant concentration (0.5 mM)[15] and increasing concentrations of **3a**. The chemical shifts of the time-averaged signals for the free and complexed **4a** were monitored. Unfortunately, as seen in Figure 1a, severe overlapping of carbohydrate and receptor resonances was overwhelming even at 750 MHz, with the anomeric proton being the only clearly distinguishable signal of the carbohydrate in the whole set of spectra.

Since the analysis of a single carbohydrate signal noticeably limits the quality of the pursued affinity data, 1D DPFGSE-TOCSY (Total Correlation Spectroscopy)[16] NMR experiments were attempted with the aim of filtering off the receptor signals. Indeed, by selective irradiation at the anomeric proton at 4.3 ppm, the transfer of magnetization through the whole spin system of **4a** (via vicinal-coupled protons, mixing time 120 ms) resulted in the selective and clean visualization of the carbohydrate ring protons with no significant increase in titration time (Figure 1b). In this way, it was possible to analyze the chemical shift variations of all the carbohydrate resonances independently on the carbohydrate/receptor ratio, and so a more precise measurement of the binding affinity was performed. It was revealed that no saturation of the sugar signals occurred even after the addition of 11 equiv of **3a**, in agreement with a moderate affinity (Figure 1c). Attemps to determine the binding stoichiometry by using mole ratio and job plots led to inconsistent results, suggesting a complex binding mode. Actually, an accurate fitting resulted when a 1:1 and 1:2 mix model (sugar to receptor ratio) was used for the calculation of the binding affinity (EQNMR software).[17,18] Average values for the cumulative association constants β_{11} (119.7 ± 4.12 M^{-1}) and β_{12} ($9.10 \times 10^3 \pm 263\ M^{-2}$) were obtained for the binding of **3a** and **4a**, with averaged standard deviations below 10% (Table 1).

At this point the enantiomeric differentiation capacity of receptor **3a** was evaluated by titration with octyl-β-L-glucopyranoside **4b**.[13] Association constants β_{11} and β_{12} in the range of those shown for the enantiomer **4a** evidenced an almost negligible chiral differentiation (Table 1). To elucidate the reasons behind the moderate affinity and null selectivity values for **3a**, semiempirical (AM1) energy calculations of the binding pocket geometry were performed with Gaussian 03.[19] An interesting feature emerged: the most stable geometry for **3a** places the indol residues slightly below the binding cavity for glucose, imparting a rather rigid conformation with limited adaptability, and hence reduced recognition capacity (Figure 2). In this situation, the role of the indol group as a binding motif is limited.

In light of these considerations, we decided to investigate the steric factors at the amino acid side chain influencing the recognition of **4a** and **4b** by this family of receptors. The binding affinities of a series of Ala-, Leu-, and Val-decorated receptors **3b**–**d** (Scheme 1), characterized by an increasing degree of substitution, were determined by TOCSY NMR titration (1:1 and 1:2 mix model, Table 1 and Supporting Information).[18] In addition to β, median binding concentrations (BC_{50}), an affinity descriptor independent of the binding nature of the association, were also determined.[20] As shown in Table 1, there seems to be an influence of the bulkiness of the amino acid side chain in the

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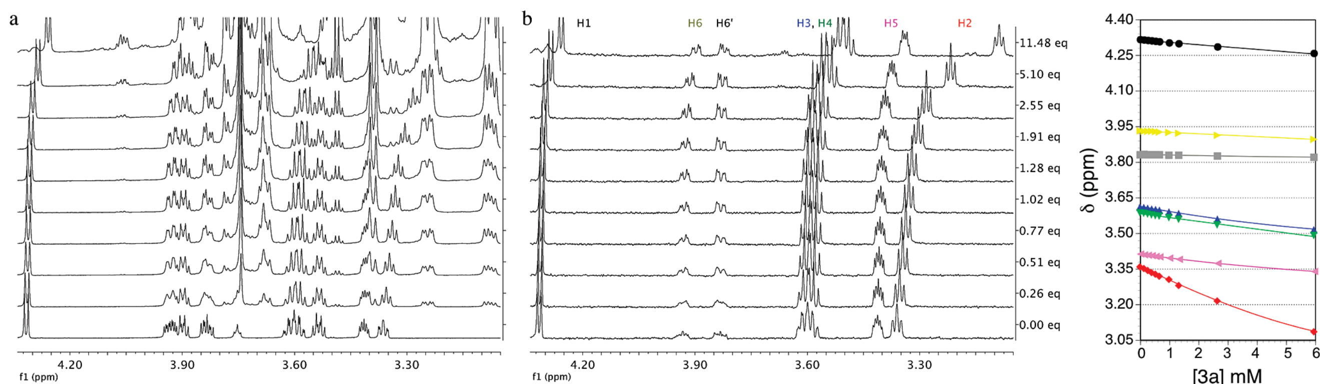

FIGURE 1. Titration of **4a** (0.52 mM in $CDCl_3$, 25 °C, 750 MHz) with Trp-based receptor **3a**: (a) ^{1}H NMR and (b) TOCSY NMR spectra. (c) Plot of the observed (symbol) and calculated (line) upfield chemical shifts of **4a** as a function of the concentration of added **3a**.

TABLE 1. Cumulative Association Constants β_{11} (M^{-1}) and β_{12} (M^{-2}), Standard Free Energies of Binding $-\Delta G°$ (kJ mol^{-1}), Median Binding Concentration BC_{50} (mM), and Relative Affinity (RA) for 1:1 and 2:1 Complexes of Receptors 3 and Glucopyranosides 4 (0.5 mM)

	β_{11}	$-\Delta G°_{11}$	$\beta_{12}/10^3$	$-\Delta G°_{12}$	BC_{50}	RA[a]
3a·4a	119.7	11.9	9.1	22.6	6.14	1.01
3a·4b	113.7	11.8	9.6	22.8	6.23	
3b·4a	269.0	13.9	18.4	24.4	3.37	1.58
3b·4b[b]	465.0	15.3	44.4	26.6	2.12	
3c·4a	84.3	11.1	5.2	21.3	8.35	1.37
3c·4b	58.5	10.1	3.4	20.2	11.4	
3e·4a	100.2	11.4	14.3	23.8	5.96	1.13
3e·4b	133.4	12.2	14.5	23.8	5.27	
3f·4a	110.2	11.7	10.9	23.1	6.12	1.28
3f·4b	80.2	10.9	7.2	22.1	7.83	
3g·4a	93.4	11.3	4.8	21.1	7.99	1.58
3g·4b	132.6	12.1	12.8	23.5	5.07	

[a] Defined as $(BC_{50})_{max}/BC_{50}$. [b] A receptor with D-Ala was prepared [(D)-**3b**] and its binding affinity to **4a** evaluated, showing identical binding constants.

binding strength of these receptors. Thus, receptor **3b**, having a methyl group at the side chain, offers the lowest steric hindrance and the highest binding affinity. As steric hindrance increases, affinity is reduced (Me in **3b** > *i*-Bu in **3c** > *i*-Pr in **3d**), with **3d** being a limiting example where the affinity constants could not be determined as a result of its poor binding ability.

In addition, a correlation between binding affinity and chiral discrimination was observed within this family of receptors, as **3b** also showed the highest relative affinity (RA 1.58, based on BC_{50}) for the L-glucopyranoside **4b** relative to the natural saccharide configuration **4a** (Table 1).

Having explored the steric effects over the binding constants and RA, we introduced variations at the number and nature of binding motifs at the amino acid side chain (Scheme 1). Comparison of receptor **3c** with the Asp derivative **3e** (similar steric hindrance) revealed the ester group in **3e** as positive for the binding, with reductions up to 50% in BC_{50} values (Table 1 and Supporting Information). Conversely, the increase in number of binding sites led to reduced RA, probably as a result of the higher adaptability of the receptor cavity to both carbohydrate enantiomers. The nature of the terminal carboxylic acid was also analyzed. Substitution of the methyl ester in the Ala derivative **3b** for a methyl amide (receptor **3f**) led to reduced binding strength and chiral discrimination (Table 1 and Supporting Information).[18]

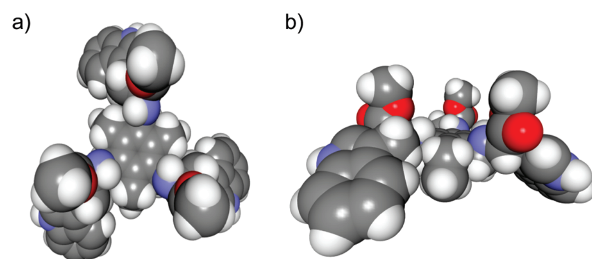

FIGURE 2. Energy minimized geometry (AM1) of receptor **3a**: (a) axial and (b) equatorial views.

Finally, we analyzed receptor **3g** incorporating the dipeptide Gly-Ala-OMe. The Gly spacer introduces an extra binding motif (amide) and places the Ala residue further away from the triethylbenzene scaffold, when compared to receptor **3b** (Scheme 1). Although binding affinity was not improved,[21] probably again as a result of the enhanced substitution around the benzene ring, **3g** was able to induce a RA similar to **3b**.

In addition to the quantitative affinity data offered by β and BC_{50}, structural information on the interaction can be obtained from the ^{1}H chemical shift differences ($\Delta\delta = \delta_{max} - \delta_0$) for the carbohydrates during titration thanks to the TOCSY filtering (Figure 1, as well as Figures S9−S16 in the Supporting Information). Thus, significant $\Delta\delta$ resulted when comparing the NMR spectra for the free and bound glucopyranosides, with the largest shifts (up to 1.8 ppm) observed for H2 and H4 protons (Figure S16, Supporting Information). This feature is in agreement with the β face of **4** being in close proximity to the benzene ring of the receptors in the complex, as recently reported by the groups of Roelens and Jiménez-Barbero for alternative receptors based on **1** (NMR and modeling calculations).[8]

In conclusion, natural amino acids, in spite of being the constituting building blocks of lectins, have demonstrated moderate affinity and selectivity for the binding and enantiodifferentiation of carbohydrates, when combined with a triethylbenzene scaffold. We have also proven TOCSY experiments to be a useful filtering strategy widening the scope of NMR titrations to receptors with complex structures

(21) Fitting the data for **3g** proved to be more difficult than for the other receptors, suggesting a different binding mode. Nevertheless, the 1:1 and 1:2 mix model afforded again the most accurate correlation.

where severe overlapping hampers the direct analysis of the carbohydrate resonances.

Experimental Section

General Procedure for the Preparation of Receptors 3a−g. A solution of 1,3,5-tris(bromomethyl)-2,4,6-triethylbenzene (**2**) (1 equiv) in CH_3CN was added to a suspension of the corresponding aminoacid hydrochloride (4 equiv) and K_2CO_3 (6 equiv) in CH_3CN. Reaction was stirred at 80 °C. Then, it was filtered (Celite) and evaporated. The resulting crude product was dissolved in CH_2Cl_2 and 4-benzyloxybenzaldehyde resin was added. The mixture was gently shaken for 5 h, after which the resin was filtered off, and pure **3** was isolated after evaporation. In those cases where it was needed, flash chromatography was employed to further purify the receptors.

Receptor 3a. From 51 mg (0.12 mmol) of starting **2**, 94 mg (95%) of receptor **3a** were obtained after purification by flash chromatography (silica, CH_2Cl_2/MeOH 90:10). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (br s, 3H), 7.62 (d, $J = 8.2$ Hz, 3H), 7.30 (d, $J = 7.6$ Hz, 3H), 7.18 (dd, $J = 8.2$, 7.6 Hz, 3H), 7.12 (dd, $J = 8.2$, 7.6 Hz, 3H), 6.92 (d, $J = 2.1$ Hz, 3H), 3.73 (s, 9H), 3.71−3.67 (m, 3H), 3.67 (d, $J = 11.3$ Hz, 3H), 3.38 (d, $J = 11.3$ Hz, 3H), 3.23 (dd, $J = 14.5$, 6.7 Hz, 3H), 3.06 (dd, $J = 14.5$, 6.7 Hz, 3H), 2.54−2.42 (m, 3H), 2.36−2.26 (m, 3H), 1.52 (br s, 3H), 0.89 (t, $J = 7.4$ Hz, 9H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 175.4, 142.6, 136.3, 133.5, 127.1, 123.5, 121.8, 119.1, 118.5, 111.4, 110.6, 62.2, 51.8, 46.4, 29.1, 21.6, 16.4; HR-MS calcd for $C_{51}H_{61}N_6O_6$ (MH^+) 852.4574, found 852.4598.

NMR Titrations and Data Analysis. Titration experiments were monitored by ^{1}H NMR spectroscopy (750 MHz) in $CDCl_3$ at 298 K. Five millimeter NMR tubes and Gilson micropipets were used. To avoid the interference of traces of acid in solution, $CDCl_3$ was filtered over basic alumina, and stored over 4 Å molecular sieves. Mathematical analysis of data was done with EQNMR software,[17] using a mix model of 1:1 and 1:2 sugar:receptor binding. BC_{50} values were calculated as reported by Roelens and co-workers (Supporting Information).[6]

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Supporting Information Available: Experimental details for the synthesis and characterization of receptors **3a−g**, NMR titration and data analysis, and complete refs 3, 5, and 19. This material is available free of charge via the Internet at http://pubs.acs.org.