DOI: 10.1002/ejoc.200((will be filled in by the editorial staff))

Differential recognition of mannose-based polysaccharides by tripodal receptors based on a triethylbenzene scaffold substituted with trihydroxybenzoyl moieties

Paula Carrero,[a] Ana Ardá,[b] Mónica Alvarez,[c] Elisa G. Doyagüez,[a] Eva Rivero-Buceta, [a] Ernesto Quesada,[a] Alicia Prieto,[d] Dolores Solís,[c,e] María José Camarasa,[a] María Jesús Peréz-Pérez,[a] Jesús Jiménez-Barbero*[b] and Ana San-Félix*[a]

Keywords: Molecular recognition / Carbohydrates / Conformational analysis / NMR spectroscopy / Receptors

Two novel tripodal receptors (**1** and **2**) based on a triethylbenzene scaffold substituted with trihydroxybenzoyl groups have been synthesized. The conformational preferences and carbohydrate-binding ability of **1** and **2** have been examined by NMR spectroscopy and modeling procedures. The results reveal that the particular structural pre-organization of **2** facilitates the recognition, in highly competitive media (DMSO), of a mannose-based polysaccharide showing a linear saccharide chain continuously decorated by α(1→2) mannose branched moieties. By contrast, other α(1→2) substituted polysaccharides or different monosaccharides are not bound, revealing the selectivity of the interaction. Due to the importance of α(1→2) mannosides, which are abundantly present on the glycan shield of several pathogens, the results here reported open attractive perspectives for the potential application of **2** or compounds derived thereof in future anti-infective strategies.

[a] Instituto de Química Médica (IQM-CSIC), Juan de la Cierva 3, 28006 Madrid (Spain); Fax: +34 915644853; Tel: +34 915622900; E-mail: *anarosa@iqm.csic.es*
[b] Chemical and Physical Biology, Centro de Investigaciones Biológicas (CIB-CSIC), Ramiro de Maeztu 9, 28040 Madrid (Spain); Fax: +34 915360432, Tel: +34 918373112; E-mail: jjbarbero@cib.csic.es
[c] Instituto de Química Física Rocasolano (IQFR-CSIC), Serrano 119, 28006 Madrid (Spain); Fax: +34 915642431; Tel: +34 915619400; E-mail: d.solis@iqfr.csic.es
[d] Enviromental Biology, Centro de Investigaciones Biológicas (CIB-CSIC), Ramiro de Maeztu 9, 28040 Madrid (Spain)
[e] Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Bunyola, Mallorca, Illes Balears (Spain),
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.xxxxxxxxx.

Introduction

Molecular recognition of carbohydrates is crucial in many relevant physiological processes, starting from fertilization, embryogenesis, tissue maturation and immune response and extending to different pathologies such as microbial infections and tumor metastasis.[1] Different synthetic carbohydrate receptors as model systems have been developed over the past years to investigate the basic molecular features that govern carbohydrate recognition in natural systems. These synthetic receptors present interesting opportunities for different practical applications, including biomedicine and analytical chemistry.[2] With respect to the biomedical applications, the synthetic receptors could be potentially used in diagnostic methods to detect carbohydrate biomarkers of disease[3] and to develop future anti-infective therapies aimed to avoid infection by pathogens (viruses, bacteria, parasites and fungi).[4]

Two main classes of artificial carbohydrate receptors have been developed to date. The first one involves the exploitation of non-natural bonding interactions and relies on boron-based systems that operate through covalent bond formation. These systems have been relatively succesful in recognizing carbohydrates even in aqueous solution.[5] The second class of carbohydrate receptors include compounds that act through noncovalent interactions.[6] Such systems are of special interest because they better reproduce the non-covalent carbohydrate-receptor interactions observed in nature. Whereas several carbohydrate receptors among this second group showed good degrees of affinity and selectivity in organic solvents, only a few tolerate significant quantities of protic cosolvents. One exception is a particular powerful family of receptors developed by Davis *et al.* that exhibits outstanding recognition properties for carbohydrates with all-equatorial stereochemistry (β-glucose, β-glucosides, cellobiose, etc..) even in water.[2,7]

Among the numerous artificial receptors designed to interact with carbohydrates through noncovalent interactions, tripodal receptors based on the 2,4,6-triethylbenzene scaffold occupy a prominent position. It has been demonstrated that this scaffold is able to preorganize the binding elements improving the binding affinity of supramolecular hosts.[8] In this context, the research groups of Roelens,[9] Mazik,[6e,f,10] Abe,[11] and Schmuck,[12] demonstrated that compounds with a tripodal 1,3,5-substituted 2,4,6-triethylbenzene scaffold, bearing on the side arms three carbohydrate recognition units, are able to bind different monosaccharides and even short oligosaccharides in organic media with substantial affinities and selectivities.

The design of this second group of artificial receptors has been at least partially inspired by lectins, carbohydrate-binding proteins that are the most common class of natural carbohydrate receptors. The X-ray crystallography structure of lectin-carbohydrate complexes has revealed that carbohydrate recognition is generally established through multiple non-covalent forces, mainly hydrogen bonding through the sugar hydroxy groups and polar residues of the protein but also hydrophobic interactions between sugar CHs and non-polar residues of the protein.[13] Interestingly, these non-polar residues are frequently aromatic, suggesting the presence of CH-π interactions between sugar CHs and aromatic amino acids.[14-17] In particular, the importance of the aromatic amino acids has been confirmed using a variety of experimental techniques, including site-directed mutagenesis.[14b,15] Depending on the particular system, either hydrogen bonds or CH-π interactions may drive the molecular recognition process, although typically both are simultaneously operative.

It should be said that although some noncovalent receptors showing di- *vs* monosaccharide preferences[18] or even capacity to discriminate tetrasaccharides from their lower (mono-, di- and trisaccharides) homologues have been described,[19] reports on the selective recognition of oligosaccharides by this type of receptors are still scarce.

On the other hand, it is known that natural polyphenols are amphipathic molecules having both hydrophobic aromatic rings and hydrophilic hydroxyl groups, allowing them to bind simultaneously at several sites on the surface of different types of macromolecules, including polysaccharides.[20,21] Their complexation with these macromolecules involves both hydrophobic effects, considered as the main driving force in the association, and hydrogen-bonding.[22] Factors such as molecular size, conformation, number and position of free phenolic hydroxyl groups affect the binding capacity and selectivity of polyphenols.[20]

Based on all these precedents, two novel tripodal receptors **1** and **2** (Figure 1) based on a triethylbenzene scaffold substituted with trihydroxybenzoyl groups have been synthesized. We hypothesized that the central and phenolic aromatic rings of these compounds might establish stabilizing CH-π interactions with the sugar CHs of polysaccharides, while the acidic phenolic OHs might give additional hydrogen-bonding interactions with the polar groups (OHs and oxygen of the glycosidic linkage) of carbohydrates. Phenolic entities have been connected to the central scaffold by amide bonds. The choice of amido groups as spacers was made in account of the double H-bonding donor-acceptor nature of this functionality. Consequently, these groups could provide "extra" H-bonding interactions with the polar groups of carbohydrates.

In addition to the synthesis, the carbohydrate binding ability of **1** and **2** has been examined by UV-vis and NMR spectroscopy. As a non-demanding technique (regarding sample concentration), UV-vis spectroscopy appeared as an appropriate methodology to screen a set of carbohydrates in aqueous media. Such pre-evaluation of the interaction of **1** and **2** indicated that both

compounds showed a preference for a certain mannose-based polysaccharide. Further analysis by NMR in a highly competitive media (DMSO) revealed that the tripodal receptor **2** showed a unique selectivity for a certain type of α(1→2) mannose polysaccharide that resembles those abundantly present on the glycan shield of several pathogens.[4a,23] The interaction with this type of polysaccharide has been recently envisaged as an original therapeutic concept for the treatment of various pathogen infections.[4]

The selectivity found for **2**, with three 2,3,4-trihydroxybenzoyl moieties on a triethylbenzene scaffold, contrasts with **1**, with three gallic acid (3,4,5-trihydroxybenzoyl) moieties as substituents (Figure 1). To explore the origin of this selectivity, additional ^{1}H NMR and molecular modeling studies have been performed.

Results and Discussion

Synthesis

The target compounds **1** and **2** were prepared following the synthetic pathway shown in Scheme 1.

The central scaffold, 1,3,5-trisamine-2,4,6-triethylbenzene (**3**), was prepared in three steps from the commercially available 1,3,5-triethylbenzene following previously reported procedures.[24] Subsequently, **3** was reacted with the respective benzyl-protected trihydroxybenzoic acids (**4**)[25] and (**6**)[26] in the presence of BOP and triethylamine to afford **5** and **7** in 70% and 63% yields, respectively. Attempts to conduct the reaction with the unprotected trihydroxybenzoic acids failed under several conditions, giving untractable mixtures. On the other hand, the alternative protection of the phenolic hydroxyl groups as methyl ethers proved not to be suitable, as subsequent deprotection required harsh conditions.

Deprotection of the benzyl groups of **5** and **7** by catalytic hydrogenation in the presence of 10% Pd/C afforded the corresponding phenol deprotected derivatives **1** (99%) and **2** (76%) in excellent yields (Scheme 1).

UV spectroscopy evaluation of the carbohydrate binding ability of 1 and 2

The carbohydrate binding ability of the synthesised compounds was initially evaluated by scrutinising possible changes in their UV spectra in the absence and presence of different mono- and polysaccharides in aqueous media. As expected, **1** and **2** exhibited similar spectra characterized by a band centered at 265 nm and a trough at 243/247 nm (for **1/2**, respectively). No changes in the spectra were visible in the presence of mannose, galactose or glucose at 12 mg.mL$^{-1,}$ or even at a higher 18 mg.mL^{-1} (0.1 M) monosaccharide concentration. However, isosbestic points were observed in the presence of 12 mg.mL^{-1} of mannan (Figure 2), a mannose-based polysaccharide, together with some significant changes. In particular, the absorbance intensity of the band at 265 nm decreased noticeably while the trough minimum was slightly red-shifted. There were no perceptible changes in the presence of 12 mg.mL^{-1} dextran (a glucose-based polysaccharide). Altogether, the results were taken as a hint of the existence of a selective interaction of **1** and **2** with mannan in aqueous solvent. Taking into account that mannan from *Saccharomyces cerevisae* is composed of a linear α(1→6) mannose chain with α(1→3) mannose branching and variable concentrations of α(1→2) mannose linked to the α(1→3) units, three different DMSO-soluble mannose-based polysaccharides with similar connectivities were selected for NMR analysis.

^{1}H NMR analysis of the carbohydrate binding ability of 1 and 2

The interaction of different monosaccharides with simple aromatic moieties has been previously investigated by NMR spectroscopy.[27] Changes in the relaxation properties or in chemical shifts of the different protons of the interacting partners were monitored to provide non-ambiguous evidence of the existence of interaction. Similar methodologies have been employed to study the interaction of simple glycosides with synthetic receptors.[9,10] With this precedent, and prompted by the UV spectroscopy indications of selective compound-sugar interactions, we also used NMR experiments to evaluate the binding of the novel tripodal receptors, **1** and **2,** to different mannose-containing polysaccharides of different chemical architecture. In addition, the interaction with different monosaccharides was scrutinized in polar solvents.

Most studies to determine the carbohydrate binding properties of synthetic receptors have employed NMR spectroscopy measurements using non-competitive organic solvents such as $CDCl_3$ where H-bonding interactions are enhanced, often dramatically, compared to water and other competitive polar media.[10d,e] In our case, ^{1}H NMR titrations were performed in DMSO-d_6, a competitive solvent.

First, polysaccharides of general structures **A** and **C** (Figure 3) consisting of a main linear α(1→6) mannose chain continuously decorated by α(1→2) glucose (polysaccharide **A**) or α(1→2) mannose (polysaccharide **C**) units were used. In addition, polysaccharide **B**, in which α(1→2) mannose is attached every three residues of the main chain, was also investigated. The addition of polysaccharide **C** (23 mg/mL) to a DMSO-d_6 solution (0.7 mM) of **2** caused significant broadening of its aromatic H-5 and H-6 signals**,** thus indicating the participation of these groups in interaction with the sugar (see Figures 4 and 5a). Broadening of the signals of NH and OH groups of **2** was also observed. When the reverse titration was performed,[28] i.e., polysaccharide **C** was titrated with **2**, no unambiguous confirmation of the interaction could be obtained. This can be explained by the heterogeneity of the polysaccharide, whose molecular weight range encompasses several dozens of units following a Gaussian distribution. Thus, upon titration of **C** with the "small" receptor **2**, the effects are diluted among the different units of the polysaccharide with very tiny chemical shift variations, below the experimental error. This also accounts for the success of the direct experiment: a single polysaccharide molecule can accommodate several receptor molecules, thereby allowing the detection of the effect. In contrast, in the presence of polysaccharides **A** and **B,** broadening of the proton signals of **2** was not observed in analogous ^{1}H NMR experiments, underscoring the selectivitiy of the interaction between

polysaccharide **C** and receptor **2**.

A rough estimation of the binding affinity between **2** and polysaccharide **C** was made by measuring the variations in intensity and linewidth (which is related to the transverse relaxation rate, $1/T_2$*) of protons H-5 and H-6 of **2** upon stepwise additions (5 µL) of polysaccharide **C** (Figure 4). As described by Shortridge *et al*,[29] an estimated K_d was calculated from these values, using Equation 1

$$\frac{I_{obs}}{I_0} = \frac{1}{1+\frac{c\cdot[P]_T}{[P]_T + K_D}} \quad (1)$$

where $[P]_T$ is the total polysaccharide concentration, I_{obs} and I_0 are the intensities of **2** in the presence and absence of polysaccharide **C**, and the non-dimensional parameter c is defined as $c = (\upsilon_B / \upsilon_F - 1)$, where υ_F and υ_B are the linewidhts of free and bound **2**, respectively. An estimated K_d (averaged between the values for H-5 and H-6) of 6 ± 4 mM was obtained.

On the other hand, in the case of **1,** no changes were observed in the aromatic region of the ^{1}H NMR spectrum upon addition of any of the three polysaccharides **A**, **B** and **C.** Furthermore, the NMR spectrum of *N*-benzyl-2,3,4-trihydroxybenzamide, used as model compound,[26] did not show significant changes either in chemical shifts or in line broadening in the presence of polysaccharide **C** (20-25 mg/mL) suggesting the absence of interaction under these experimental conditions (Figure 5b). Therefore, NMR experimental evidence of the interaction in solution was selectively achieved for a unique receptor-ligand pair. Only receptor **2** was able to recognize polysaccharide **C**, which shows a linear saccharide chain continuously decorated by α(1→2) mannose branched moieties. A smaller degree of branching or the substitution of mannose by glucose did not provide interaction.

In parallel, the binding abilities of **1** and **2** toward a set of monosaccharides were also investigated in DMSO-d_6 at T = 298 K. As glycosides, methyl α-D glucopyranoside, methyl β-D glucopyranoside, methyl α-D galactopyranoside, methyl β-D galactopyranoside, methyl α-D mannopyranoside, *N*-acetylglucosamine, and disaccharides as α,α-trehalose were used at 20-25 mg/mL. The NMR spectra of **1** and **2** did not show significant changes either in chemical shifts or in line broadening in the presence of these sugars, suggesting the absence of interaction in DMSO solution under these experimental conditions, as similarly inferred from the UV spectroscopy analysis in aqueous conditions.

Overall, NMR data indicate that the interaction in DMSO-d_6 of the polyphenolic compounds with the mannose-containing polysaccharides here studied is fairly selective and depends on the chemical nature of the substitution of the central benzene unit (only **2,** and not **1,** provides interaction) and, additionally, on the presentation of the mannose units of the polysaccharide. The differential recognition by compounds **1** and **2** of mannose-based polysaccharides justified the analysis of the conformational preferences of the two compounds.

Conformational studies of 1 and 2 using NMR and Molecular Modeling

The conformational preferences of compounds **1** and **2** were studied by NMR and molecular modeling procedures. Previous work has shown that preorganization of *ortho*-hydroxyl (*o*-OH) substituted benzamides on triethyl benzene scaffolds exists in the solid state and is maintained in polar solutions, through the establishment of intramolecular hydrogen bonding between the *o*-OH proton of the benzamide group and the CO moiety.[30]

The ^{1}H NMR spectra of compounds **1** and **2** in DMSO-d_6 allowed to detect all the exchangeable OH and NH as sharp singlet peaks. A first hint of the possible participation of the *o*-OH proton of **2** in intra-molecular hydrogen bonding was its remarkable downfield shift (δ = 12.5 ppm at 294 K) with respect to the *meta*- and *para*-hydroxyl protons (Figure S9, Supporting Information). In contrast, for **1**, such a large difference between the chemical shifts of the different OH protons was not observed. The temperature coefficients[31] ($\Delta\delta/\Delta T$) of each exchangeable OH and NH protons for both **1** and **2** were calculated by acquiring ^{1}H NMR spectra at different temperatures (T = 294 to 328 K) (Table 1). The lower temperature coefficient observed for the *o*-OH proton in **2**, in comparison with those for the other hydroxyl protons, supports its participation in hydrogen bonding.

In addition, 2D NOE (NOESY) studies were performed in DMSO-d_6 at 294 K. For **2**, strong NOE cross-peaks between the amide NH/aromatic H-6 and aromatic H-6/H-5 protons were observed (Figure 6 and Figure S9 Supporting Information). In addition weak, but noticeable, NOEs between the amide NH and the aromatic H-5 proton, amide NH/ethyl CH_3 and H-6/ethyl CH_3 were observed. It should be mentioned that the observed NOE between the amide NH and the aromatic H-6 proton is even stronger than those observed between the two adjacent H-6 and H-5 aromatic protons. This indicates that the distance between the amide NH and the aromatic H-6 is very short and, therefore, that the H-6 proton should be oriented towards the amide NH. Consequently, the *o*-OH proton faces the amide CO moiety. In conclusion, the obtained ^{1}H NMR data for compound **2** support that the phenol ring and the amide bond are in the same plane, and at the adequate distance for the formation of an intramolecular hydrogen bond between the *o*-OH proton and the amide carbonyl oxygen.

Molecular Modeling calculations were performed through Monte Carlo conformational search using the MMFFs force field to provide three-dimensional structures of these molecules in solution. The applied protocol (see experimental section) for **2** yielded a structure adopting a so-called *ababab* disposition as the lowest energy conformation (Figure 7I). In this geometry, the three polyphenolic rings (arms) and the three ethyl groups (legs) alternately orient above (*a*) and below (*b*) the benzene ring. This alternating geometrical pattern agrees with that reported for other tripodal receptors[8-10] and orients all the polyphenolic rings to the same upper side of the central benzene core.

Another key feature of this geometry was the formation of an intramolecular hydrogen bond between the *o*-OH proton and the amide carbonyl oxygen, thus resulting in a nearly planar disposition of the phenolic ring. In this structure, the distance between the amide NH and H-6 proton is 2.23 Å, while the H-5/H-6 distance is 2.45 Å, in agreement with the corresponding

observed relative NOE intensities. It should be noticed that, in this conformation, the polyphenolic rings are oriented displaying the three phenolic OH groups out of the molecule, while the two aromatic protons (H-5 and H-6) point inwards.

However, some observed weak NOEs (amide NH/ethyl CH_3 and H-6/ethyl CH_3) can not be satisfactorily explained by considering this unique structure. Thus, it was necessary to consider the participation of a second conformational family (Figure 7II). In this second family, one of the polyphenolic arms is oriented with respect to the central benzene ring in an alternative manner to the other two arms. Then, distance values of 2.71 and 2.51 Å were measured between the corresponding NH/ethyl CH_3 and H-6/ethyl CH_3 proton pairs, explaining the observed NOEs. Thus, the existence of a minor population of conformers of this type must be assumed.

With respect to compound **1,** the NMR data suggested that no phenolic OHs were involved in hydrogen bonds. The observed NOEs are consistent with the minimum energy structure obtained from the conformational search using the MMFFs force field shown in Figure 8I. For this structure, the alternating *ababab* pattern is observed. The distances between NH/ethyl CH_3 (4.62 Å), NH/$(CH_2)_{NH}$ (2.77Å) and NH/$CH_{aromatic}$ (2.16 Å) protons agree with the observed NOEs.

Nevertheless, the molecular modeling protocol suggested that different torsion angle values may exist between the phenolic moieties and the carbonyl oxygen (Figure 8II). These results are in contrast with those mentioned above for **2**. In this molecule, the presence of the intramolecular hydrogen bond already described significantly restricts the motion around this linkage. The corresponding hydrogen bond does not exist in **1** and, therefore, more conformations are possible.

Compound **1** also showed a set of weak NOEs between the aromatic CHs protons of the phenolic moieties and the ethyl groups attached to the central benzene unit that can not take place in the *ababab* conformation (these protons are at a distance of 6.50 Å). However, they can be explained by considering additional flexibility around the C(benzene)-CH_2CH_3 and C(benzene)-CH_2NHCO bonds, giving rise to conformations different from the *ababab* arrangement. This is the case of the local minimum (destabilized in 1.5 kcal/mol) shown in figure 9, where the distance between the aromatic CHs and the ethyl CH_3 is 2.69 Á.

Therefore, from the conformation search protocol, it can be concluded that compound **2** has a clear preference to adopt a global conformation in which a control of the orientation of the substituents (polyphenol rings) attached to the central skeleton (triethylbenzene) does exist. Therefore, in compound **2**, a well-defined structure is energetically favored. In contrast, for **1**, a much larger degree of conformational mobility takes place.

With the aim to explain the interaction observed by ^{1}H NMR between **2** and polysaccharide **C**, additional molecular modeling studies were performed. A Manα(1→2)Man disaccharide was taken as a model of the branch of polysaccharide **C**. The obtained global minimum (Figure 10) revealed that the cavity provided by **2** offers the correct shape and size for the encapsulation of the side mannose chain of polysaccharide **C**. In the obtained structure, the branching α(1→2) linked mannose is located within the cleft formed by the three phenolic arms of the receptor, whereas the backbone mannose residue is oriented in such a way that the O1 anomeric and O6 atoms point outside the cleft. Thus, the linear Manα(1→6)Man polysaccharide backbone should not interfere with the interaction, although it provides the proper orientation of the branching residue to interact with the receptor. Also, the two aromatic protons H-5/H-6 of the trihydroxybenzoyl moieties are oriented inwards this cavity, allowing the interaction with the bound carbohydrate.

In any case, the monovalent interaction is expected to be very weak, as commonly observed for protein-carbohydrate interactions in nature.[32] Indeed, no interaction could be detected for the single Man monosaccharide, either as α- or β-methyl glycoside. Only the presence of many branched Man units in the polysaccharide generated detectable interaction signals.

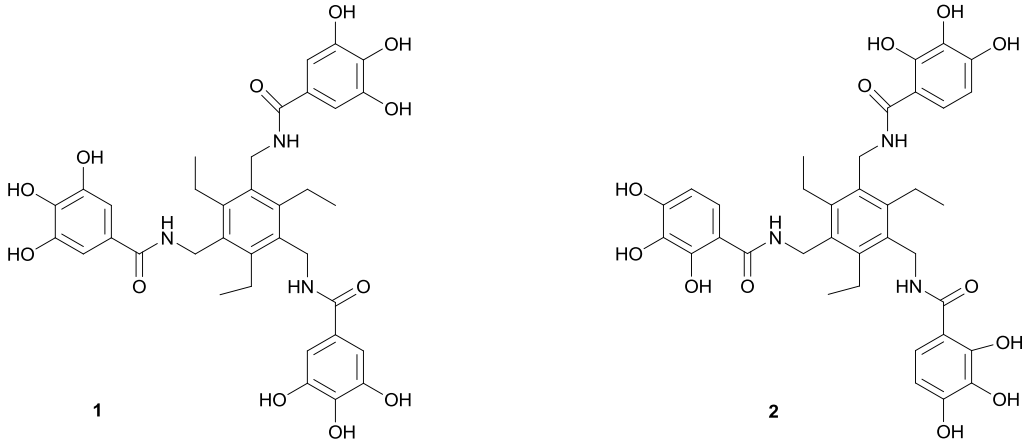
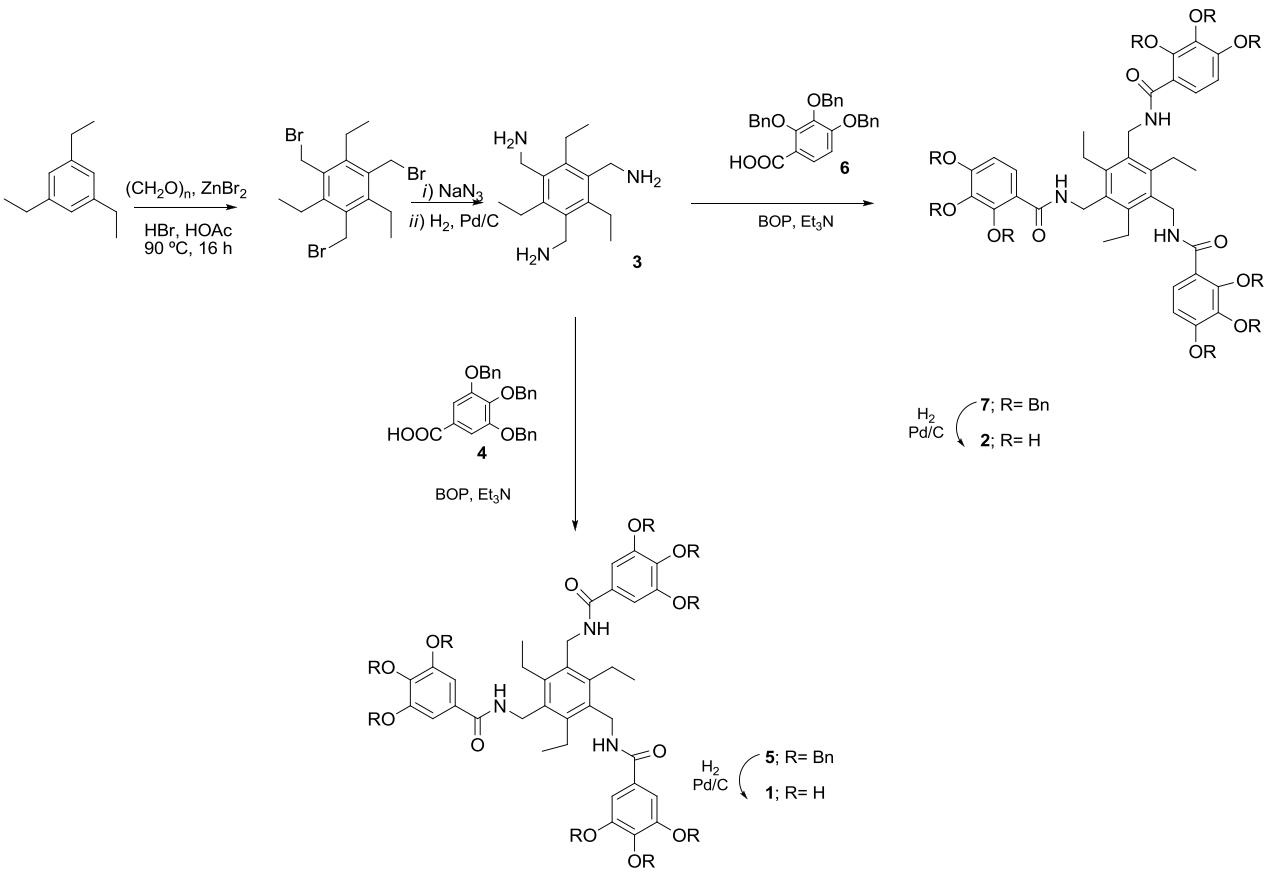


Figure 1. Structures of **1** and **2**



Scheme 1. Synthesis of **1** and **2**

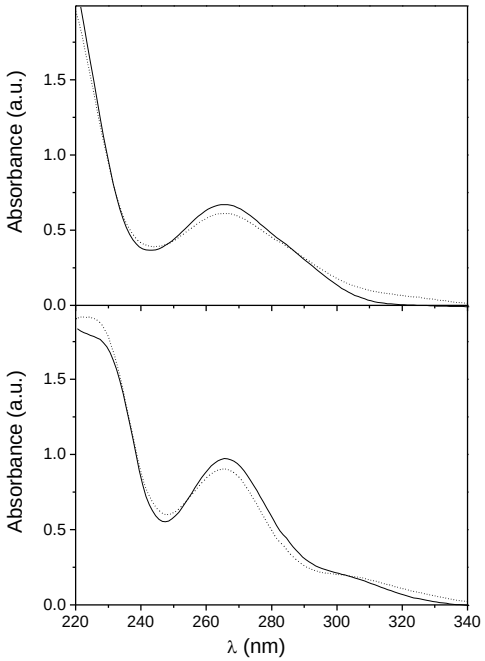


Figure 2. Mannan-induced changes in the UV spectra of compounds **1** and **2**. The spectra of $2.42 \cdot 10^{-4}$ M solutions of **1** (upper panel) and $2.46 \cdot 10^{-4}$ M of **2** (lower panel) in water containing 30% acetonitrile and 10 mM DTT, were registered in the absence (solid lines) and presence (dotted lines) of 12 $mg.mL^{-1}$ mannan

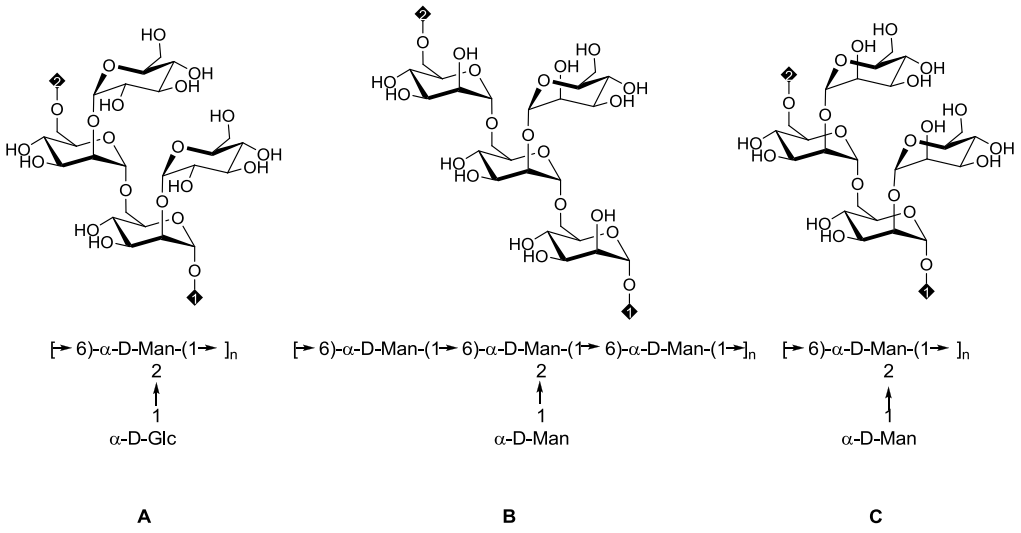


Figure 3. Structure of polysaccharides **A**, **B** and **C**

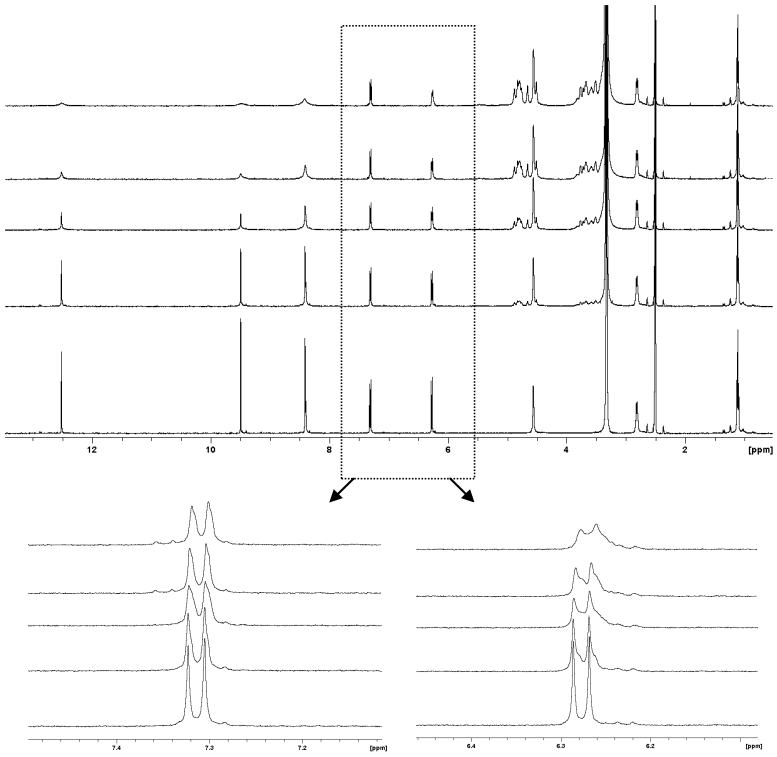


Figure 4. From bottom to top: ^{1}H NMR spectra of **2** in DMSO-d_6 alone and after stepwise additions (5µL) of polysaccharide **C** solution (23 mg/mL). The lower panel shows an enlargement of the aromatic region, highlighting the broadening of the signals corresponding to H-5 and H-6 protons

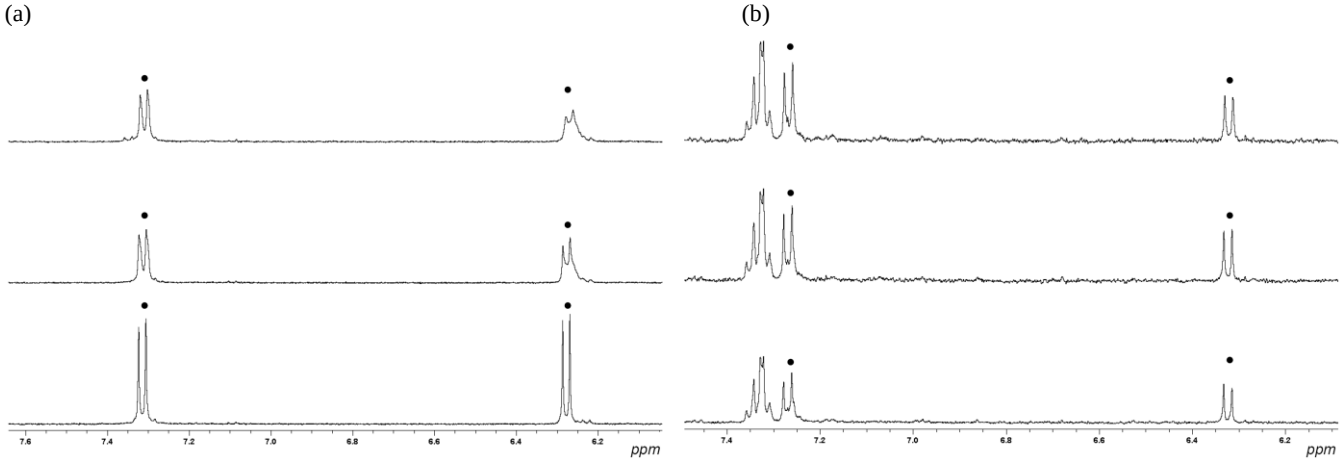


Figure 5. (a) Aromatic region of **2** in DMSO-d_6 after stepwise additions (5µL) of polysaccharide **C** solution (23 mg/mL), showing the broadening of the signals corresponding to H-5 and H-6 protons (•); (b) broadening of the signals corresponding to H-5 and H-6 protons (•) was not observed in an analogous ^{1}H NMR experiment using *N*-benzyl-2,3,4-trihydroxybenzamide

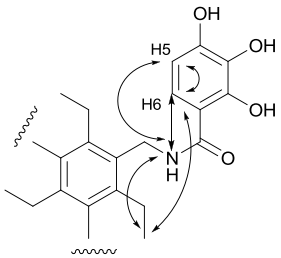


Figure 6. Observed NOEs for compound **2**. NOEs between H-6/H-5, NH/H-6 and NH/H-5 define the preferred conformation in which the distance NH/H-5 is shorter than the distance H-5/H-6

I) II)

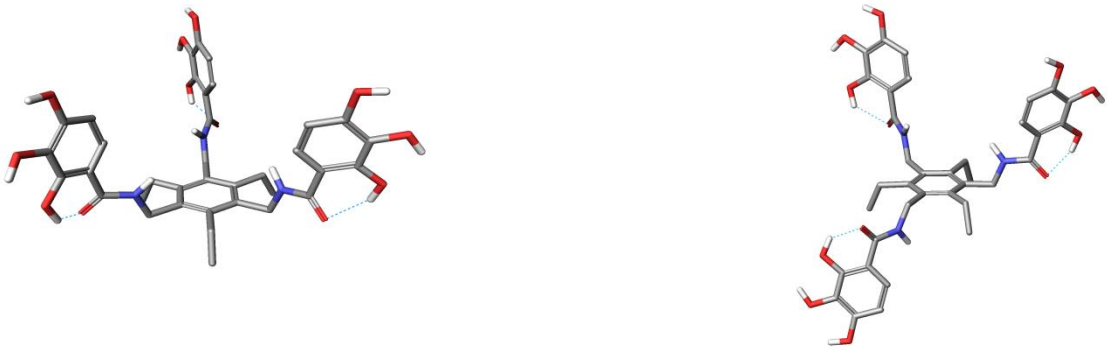


Figure 7. Compound **2**: I) Structure of the global minimum geometry and II) local minimum conformation as deduced by the MMFFs force field. The dotted lines represent the intramolecular hydrogen bond between the *o*-OH proton and the amide carbonyl oxygen

I) II)

Figure 8. Compound **1**: I) Structure of the global minimum geometry. II) Superimposition of different energy minima structures as obtained from the molecular modeling protocol. The polyphenolic benzene rings may adopt different orientations

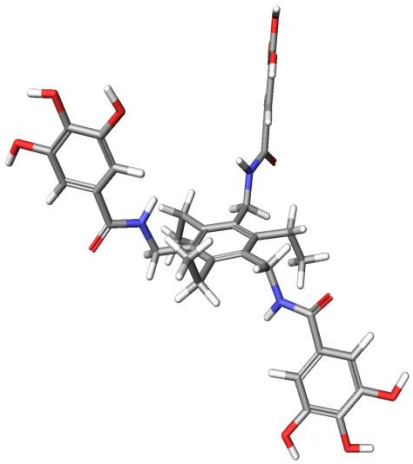


Figure 9. Structure of **1** adopting an alternative conformation to the *ababab* arrangement

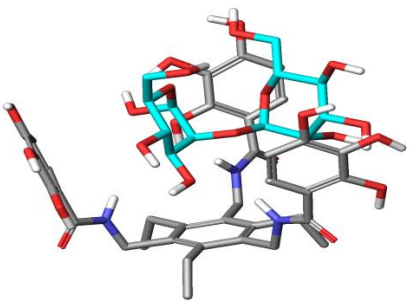


Figure 10. Minimum energy conformer for the complex of **2** with Manα(1→2)Man from the conformational search with MMFFs force field. In the obtained structure, the branching α(1→2) linked mannose is located within the cleft formed by the receptor, whereas the backbone mannose residue is oriented in such a way that the O1 anomeric and O6 atoms point outside the cleft

Table 1. Chemical shifts and temperature coefficients [Δδ/ΔT] of the exchangeable OH and NH protons of 1 and 2

T [K]	*δ* [ppm]						
	Compound **2**				Compound **1**		
	o-OH	*m*-OH	*p*-OH	NH	*p*-OH	2 *m*-OH	NH
294	12.541	8.442	9.523	8.422	8.648	8.999	7.715
298	12.521	8.414	9.497	8.403	8.619	8.973	7.691
303	12.496	8.379	9.465	8.397	8.583	8.943	7.662
308	12.471	8.344	9.432	8.355	8.547	8.911	7.632
313	12.447	8.309	9.400	8.331	8.511	8.879	7.602
318	12.421	8.273	9.368	8.306	8.476	8.848	7.572
323	12.395	8.238	9.335	8.282	8.440	8.816	7.541
328	12.368	8.202	9.302	8.256	8.405	8.785	7.511
[Δ*δ*/Δ*T*] [ppb/º]	5.100	6.500	7.100	5.000	7.200	6.300	6.000

Conclusions

In the present work two novel tripodal receptors (**1** and **2**) based on a triethylbenzene scaffold substituted with trihydroxybenzoyl groups have been prepared.

Compound **2** mainly adopts a well-defined conformation in polar (DMSO-d_6) solution, in which the phenol rings and the amide bonds are in the same plane, thus allowing the adequate distance for the formation of an intramolecular hydrogen bond between the *ortho*-hydroxyl proton and the amide carbonyl oxygen. This is not the case in compound **1**, which is much more flexible and does not show the corresponding intramolecular hydrogen bonds. On this basis, molecular modeling studies showed, for compound **2**, a well defined major structure in which the *ortho*-hydroxyl protons are strongly hydrogen-bonded to the amide carbonyl oxygen, resulting in the outward projection of the phenolic OH groups and the inward presentation of the aromatic protons. In contrast, **1** may adopt a large variety of conformations, thus resulting in the lack of a well-defined geometry.

UV spectroscopic indications pointed out a selective recognition of mannan by **1** and **2** in water containing 30% acetonitrile, over dextran (a glucose-based polysaccharide) and different monosaccharides. Mannan from *Saccharomyces cerevisae* consists of a linear mannose chain with variable concentrations of α(1→2) mannose linked to α(1→3) units. Furthermore, NMR evidence revealed that **2** discriminated between different mannose-based polysaccharides and recognized polysaccharide **C**, a polymannan which shows a linear saccharide chain continuously decorated by α(1→2) mannose branched moieties. Significant selectivity for the presence of branched mannose residues was deduced from these studies. From the receptor's perspective, pre-organization seems to be required for the existence of the recognition process with polysaccharide **C**, since there are no major changes in the orientation of the phenolic aromatic rings between the free and bound states of **2**.

Thus, **2** displays the basic features in terms of chemical groups and structural pre-organization requirements to be able to recognize, in highly competitive media (DMSO), a certain α(1→2) mannose polysaccharide that mimics the mannose composition of the high-mannose oligosaccharide chains found in several pathogen glycoproteins. This property could be of interest for the potential application of compound **2** in future anti-infective strategies.

Experimental Section

Chemical Synthesis

General

Commercial reagents and solvents were used as received from the suppliers without further purification unless otherwise stated. Dichloromethane was dried prior to use by distillation from CaH_2 and stored over Linde type activated 4Å molecular sieves.

Analytical thin-layer chromatography (TLC) was performed on aluminium plates pre-coated with silica gel 60 (F_{254}, 0.25 mm). Products were visualised from the TLC by exposure to ultraviolet light (254 nm) or by heating on a hot plate (approx. 200 ºC), directly or after treatment with a 5% solution of phosphomolybdic acid or vanillin in ethanol. Separations were performed by preparative Centrifugal Circular Thin-Layer Chromatography (CCTLC) (Kieselgel 60 PF_{254} gipshaltig, layer thickness of 1 mm, flow rate 4 mL·min^{-1}). For HPLC analysis was used an Alliance 2695 (Waters) equipped with a PDA (Photo Diode Array) detector Waters 2996. Acetonitrile was used as mobile phase A with 0.08% of formic acid, and water was used as mobile phase B with 0.1% of formic acid at a flow rate of 1 mL·min^{-1}. Two different methods were used, one on a XBridge C_{18} (2.1 x 100 mm, 3.5 μm) column with 5-80% of A, that will be noted as $t_{R(X)}$, and the other on a SunFire C_{18} (4.6 x 50 mm, 3.5 μm) column with 0-100% of A, that will be noted as $t_{R(S)}$. All retention times are quoted in minutes. Melting points were measured on a Reichert-Jung Kofler. Standard NMR (^{1}H, ^{13}C NMR) spectra were recorded on a 300 MHz (Inova 300) and 400 MHz (Inova 400) spectrometers, using $CDCl_3$ or CD_3OD as solvents at room temperature. Chemical shift values are reported in parts per million (*δ*) relative to tetramethylsilane (TMS) in ^{1}H and $CDCl_3$ (*δ* =77.0) in ^{13}C NMR. Coupling constant (*J* values) are reported in hertz (Hz), and spin multiplicities are indicated by the following symbol: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were registered in a quadrupole mass spectrometer 1100 equipped with an electrospray source. Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument.

General procedure for the reaction of trisamine 3 with benzoic acids. Benzotriazol-1-yloxytris(dimethylamino)phosphoniumhexafluorophosphate (BOP) (3.3 eq) was added to a solution of the corresponding benzoic acid (3.3 eq) in dry dichloromethane (5 mL). After 5 min, trisamine **3**[24] (0.05 g, 1 eq) and triethylamine (3.3 eq) were added. The mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was treated with ethyl acetate (10 mL) and washed successively with saturated solutions of citric acid (3x 30 mL), $NaHCO_3$ (3 x 30 mL) and brine (1 x 30 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated to dryness. The residue was purified by CCTLC using dichlorometane/methanol, (9:1) as eluent.

1,3,5-Tris(3,4,5-tribenzyloxybenzamidomethyl)-2,4,6-triethylbenzene (5): Trisamine derivative **3**[24] (0.05 g, 0.20 mmol) and **4**[25] (0.32 g, 0.72 mmol) were reacted according to the general procedure to give 0.21 g (70%) of **5**, as a white solid: mp 178-180 ºC. HPLC: $t_{R(X)}$ = 20.44. ^{1}H NMR ($CDCl_3$, 300 MHz): *δ* [ppm] = 1.18 (t, *J* = 6.9 Hz, 9H, CH_3-CH_2), 2.73 (q, *J* = 6.7 Hz, 6H, CH_3-CH_2), 4.61 (s, 6H, CH_2-NH), 4.99 (s, 18H, CH_2-Ph), 5.66 (s, 3H, NH), 6.93 (s, 6H, H-Ar), 7.18-7.25 (m, 45H, H-Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): *δ* [ppm] = 17.15 (CH_3), 23.78 (CH_2), 39.18 (CH_2), 72.09 (CH_2), 75.63 (CH_2), 107.76 (CH), 127.96-129.78 (CH), 132.78 (C), 136.96 (C), 137.68 (C), 142.11 (C), 145.22 (C),

153.25 (C), 167.20 (CO). MS (ES+): *m/z* = 1517 $(M+H)^+$, 1539 $(M+Na)^+$.

1,3,5-Tris(2,3,4-tribenzyloxybenzamidomethyl)-2,4,6-triethylbenzene (7): Trisamine derivative **3**[24] (0.05 g, 0.20 mmol) and **6**[26] (0.29 g, 0.66 mmol) were reacted according to the general procedure to give 0.19 g (63 %) of **7** as a yellow oil. HPLC: $t_{R(S)}$ = 5.74. ^{1}H NMR ($CDCl_3$, 300 MHz): δ [ppm] = 1.03 (t, *J* = 7.3 Hz, 9H, CH_3-CH_2), 2.47 (q, *J* = 7.3 Hz, 6H, CH_3-CH_2), 4.53 (s, 6H, CH_2-NH), 4.83 (s, 6H, CH_2-Ph), 4.84 (s, 6H, CH_2-Ph), 5.12 (s, 6H, CH_2-Ph), 6.8-7.4 (m, 48H, H-Ar), 7.73 (t, *J* = 4.4 Hz, 3H, NH), 7.91 (d, *J* = 8.9 Hz, 3H, H-Ar). ^{13}C NMR ($CDCl_3$, 75 MHz): δ [ppm] = 16.27 (CH_3), 22.84 (CH_2), 37.88 (CH_2), 70.88 (CH_2), 75.52 (CH_2), 76.49 (CH_2), 109.25 (C), 119.68 (C), 126.78 (CH), 127.54-128.06 (CH), 132.34 (C), 135.73 (C), 136.14 (C), 136.88 (C), 141.05 (C), 143.90 (C), 151.42 (C), 155.72 (C), 164.42 (C=O). MS (ES+): *m/z* = 1517 $(M+H)^+$, 1539 $(M+Na)^+$.

General procedure for benzyl group deprotection. A solution of the corresponding benzyl-protected derivative (1 mmol) in THF/methanol (1:1) (100 mL) containing 30 wt-% of Pd/C (10%) was hydrogenated at 2.85 atm (42 psi) at 30 ºC overnight. The Pd/C was filtered through Whatman® filter paper 42 and the solvent was removed under reduced pressure to give the corresponding deprotected derivatives as unique products.

1,3,5-Tris(3,4,5-trihydroxybenzamidomethyl)-2,4,6-triethylbenzene (1): Following the deprotection procedure, benzyl derivative **5** (0.10 g, 0.07 mmol) gave 0.05 g (99 %) of **1** as a white solid: mp 178-180 ºC. HPLC: $t_{R(X)}$ = 3.04. ^{1}H NMR (CD_3OD, 300 MHz): δ [ppm] = 1.21 (t, *J* = 7.3 Hz, 9H, CH_3-CH_2), 2.90 (q, *J* = 7.1 Hz, 6H, CH_3-CH_2), 4.61 (s, 6H, CH_2-NH), 6.80 (s, 6H, H-Ar), 7.82 (wide s, 3H, NH). ^{13}C NMR (CD_3OD, 100 MHz): δ [ppm] = 15.56 (CH_3), 22.90 (CH_2), 38.64 (CH_2), 106.75 (CH), 124.83 (C), 131.74 (C), 136.93 (C), 144.52 (C), 145.43 (C), 169.32 (C=O). MS (ES+): *m/z* = 706 $(M+H)^+$, 728 $(M+Na)^+$. Anal. Calcd. for $C_{36}H_{39}N_3O_{12}$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.00; H, 5.60; N, 5.98.

1,3,5-Tris(2,3,4-trihydroxybenzamidomethyl)-2,4,6-triethylbenzene (2): Following the deprotection procedure, benzyl derivative **7** (0.19 g, 0.12 mmol) gave 0.07 g (76 %) of **2** as a white solid: mp 154-156 ºC. HPLC: $t_{R(S)}$ = 3.62. ^{1}H NMR (CD_3OD, 300 MHz): δ [ppm] = 1.21 (t, *J* = 7.3 Hz, 9H, CH_3-CH_2), 2.88 (q, *J* = 7.0 Hz, 6H, CH_3-CH_2), 4.66 (s, 6H, CH_2-NH), 6.33 (d, *J* = 8.8 Hz, 3H, H-Ar), 7.17 (d, *J* = 8.8 Hz, 3H, H-Ar). ^{13}C NMR (CD_3OD, 75 MHz): δ [ppm] = 16.64 (CH_3), 24.04 (CH_2), 39.16 (CH_2), 108.09 (C), 109.47 (CH), 120.36 (CH), 132.87 (C), 133.75 (C), 145.83 (C), 150.44 (C), 150.66 (C), 170.73 (C=O). MS (ES+): m/z = 706 $(M+H)^+$, 728 $(M+Na)^+$. Anal. Calcd. for $C_{36}H_{39}N_3O_{12}$: C, 61.27; H, 5.57; N, 5.95. Found: C, 60.98; H, 5.63; N, 6.12.

UV-vis spectroscopy

UV-vis spectra were measured in a CARY/1E/UV-Visible Spectrophotometer (Varian, Cary winUV software) at room temperature and 600 nm/min, with a 2 nm slit. 0.1 cm path-length quartz cells were used. Stock solutions of **1** and **2** were prepared in a concentration around 10^{-3} M in water containing 30% acetonitrile. DTT (10 mM) was used in order to prevent a potential oxidation. Measured solutions had concentrations in the range of 10^{-4} M. To test the effect of the monosaccharides and polysaccharides, solutions of **1** and **2** in the appropriate concentration were added to the undissolved sugars and the spectrum of the resulting mixture compared to the one obtained for the pure tripodal receptor.

NMR procedures

NMR titrations of **1** and **2** with polysaccharides **A**, **B** and **C** were performed at 500 MHz in a Bruker AVANCE spectrometer, at 298 K, unless otherwise stated. The experiments were performed in DMSO-d_6. Experiments for the free species were recorded at 2.1 mM for **1**, and at 1.2 mM for **2**. Besides regular 1D ^{1}H NMR spectra, TOCSY (35 ms mixing time) and NOESY (500 ms mixing time) experiments, using standard BRUKER sequences, were also acquired.

For the tritrations, stepwise additions (5 μL) of a concentrated polysaccharide solution (ca. 23 mg/mL) were added to solutions of **1** or **2** (0.7 mM) and the corresponding NMR spectra were recorded and monitored, looking for chemical shift changes or line broadening on the signals of **1** or **2**.

Molecular Modeling procedures

Compounds **1** and **2** were built using Maestro[33] and minimized using conjugate gradients with the AMBER* force field,[34] and a dielectric constant of 37.5 Debyes with extended cutoff to treat remote interactions. A maximum number of 5000 iterations were employed with the PRCG scheme, until the convergence energy threshold was 0.05. Once the optimum geometries had been achieved, a conformational search protocol was adopted for both compounds, using a Monte Carlo torsional sampling method (MCMM) with automatic setup during the calculation, energy window of 50 kJ/mol, 1000 maximum number of steps and 100 steps per torsion to be rotated bond. The best structures obtained from this calculation in terms of energy were chosen and then, models for a mannose monosaccharide and a Manα(1→2)ManαOMe disaccharide were manually docked within the cavity and further minimized. Different complexes were found to be stable. From those which showed the sugar inside the receptor cavity, the most stable one was submitted to additional Conformational Search calculations.

Polysaccharides

Polysaccharides **A**, **B** and **C** were obtained as previously described.[35,36] Mannan from *Saccharomyces cerevisiae* (Sigma) and dextran T40 (Pharmacia) were used without further purification.

Supporting Information (see footnote on the first page of this article): Copies of the ^{1}H NMR and ^{13}C NMR spectra for all key intermediates and final products are included, as well as NOESY spectrum of **2** and the ^{1}H NMR control experiment with *N*-benzyl-2,3,4-trihydroxybenzamide.

Acknowledgments

The Spanish MEC/MCINN (project SAF 2009-13914-C02-01), the Spanish Consejo Superior de Investigaciones Científicas (project PIF08-022), the Spanish CAM (project BIPEDD 2-CM-S2010/BMD-2457) and the CIBER of Respiratory Diseases (CIBERES), an initiative from the Spanish Institute of Health Carlos III (ISCIII), are acknowledged for their financial support.

[1] a) A. Varki, *Glycobiology*, 1993, **3**, 97; b) H.-J. Gabius, H.-C. Siebert, S. André, J. Jimenez-Barbero and H. Rüdiger, *ChemBioChem*. **2004**, *5*, 740-764; c) *The sugar code: Fundamentals of Glycosciences* (Ed.: H.-

J. Gabius), Wiley-VCH, Weinheim, **2009**; d) B. Ernst, W. Hart and P. Sinay in *Carbohydrates in Chemistry and Biology*, Wiley-VCH, Weinheim, **2000**, Part I, vol. 2 and Part II, vol. 4; e) T. K. Lindhorst, in *Essentials of Carbohydrate Chemistry and Biochemistry*, Wiley-VCH, Weinheim, **2000**; f) C. R. Bertozzi and L. L. Kiessling, *Science* **2001**, *291*, 2357-2364; g) R. A. Dwek and T. D. Butters, *Chem. Rev*. **2002**, *102*, 283-284; h) H-J. Gabius, S. André, J. Jiménez-Barbero A. Romero, D. Solís, , *Trends Biochem. Sci*. **2011**, *36*, 298-313.

[2] A. P. Davis and R. S. Wareham, *Angew. Chem. Int. Ed*. **1999**, *38*, 2978-2996.

[3] a) A. Pal, M. Bérubé and D.G. Hall, *Angew. Chem. Int. Ed*. **2010**, *49*, 1492-1495; b) A. P. Davis, *Nature* **2010**, *464*, 169-170.

[4] a) J. Balzarini, *Nature Reviews* **2007**, *5*, 583- 597; b) J. Balzarini, *Antiviral Chem. Chemother*. **2007**, *18*, 1-11; c) J. Balzarini, *Lancet Infect. Dis.* **2005**, *5*, 726-731.

[5] a) T. A. Houston, *ChemBioChem*. **2010**, *11*, 954-957; b) *Boronic Acids; Preparation and Applications in Organic Synthesis and Medicine*. (Ed.: D.G. Hall.) 1. Edition, Wiley-VCH,Weinheim, **2005**; c) T.D. James, M.D.Phillips, S. Shinkai. In *Boronic Acids in Saccharide Recognition*, 1 ed. The Royal Society of Chemistry, Cambridge, **2006**; d) M. Dowlut and D. G. Hall, *J. Am. Chem. Soc*. **2006**, *128*, 4226-4227; e) S. Jin, Y. Cheng, S. Reid, M. Li and B. Wang, *Med. Research Rev*. **2010**, *30*, 171-257; f) T.D. James and S. Shinkai, *Top. Curr. Chem.* **2002**, *218*, 159-200; g) S. Striegler, *Curr. Org. Chem*. **2003**, *7*, 81-102.

[6] a) A. P. Davis and T.D. James, in *Functional Synthetic Receptors*; (Eds.: T. Schrader and A.D. Hamilton), Wiley-VCH; Weinheim, **2005**, pp 45-109; b) D.B. Walker, G. Joshi and A.P. Davis, *Cell. Mol. Life Sci*. **2009**, *66*, 3177-3191; c) D.B.Walker, G. Joshi and A.P. Davis, *Cell Mol. Life Sci*. **2009**, *66*, 3177-3191; d) A.P. Davis, *Org. Biomol. Chem.* **2009**, *7*, 3629-3638; e) M. Mazik, *ChemBioChem*, **2008**, 9, 1015-1017; f) M. Mazik, *Chem. Soc, Rev*. **2009**, 935-956; g) *Host-Guest Chemistry-Mimetic approaches to Study Carbohydrate Recognition.* (Ed.: S. Penadés) *Top. Curr. Chem.*, Springer-Verlag:, Berlin, **2002,** vol. 218.

[7] a) E. Klein, Y. Ferrand, N. P. Barwell and A. P. Davis, *Angew. Chem. Int. Ed.* **2008**, *47*, 2693-2696; b) Y. Ferrand, M. P. Crump and A. P. Davis, *Science* **2007**, *318*, 619-622; c) Y. Ferrand, E. Klein, N. P. Barwell, M. P. Crump, J. Jiménez-Barbero, C. Vicent, G-J. Boons, S. Ingale and A. P. Davis, *Angew. Chem. Int. Ed.* **2009**, *48***,** 1775-1779.

[8] X. Wang and F. Hof, *Belstein J. Org. Chem.*, **2012**, *8*, 1-10.

[9] a) M. Cacciarini, C. Nativi, M. Norcini, S. Staderini, O. Francesconi and S. Roelens, *Org. Biomol. Chem.* **2011**, *9*, 1085-1091; b) A. Ardá; C. Venturi, C. Nativi, O. Francesconi, G. Gabrielli, F. J. Cañada, J. Jiménez-Barbero and S. Roelens, *Chem. Eur. J.* **2010**, *16*, 414-418; c) A. Vacca, C. Nativi, M. Cacciarini, R. Pergoli and S. Roelens, *J. Am. Chem. Soc.* **2004**, *126*, 16456-16465; d) M. Cacciarini, E. Cordiano, C. Nativi and S. Roelens, *J. Org. Chem*. **2007**, *72***,** 3933-3936; e) C. Nativi, M. Cacciarini, O. Francesconi, A. Vacca, G. Moneti, A. Lenco and S. Roelens, *J. Am. Chem. Soc.* **2007**, *129*, 4377-4385; f) C. Nativi, O. Francesconi, G. Gabrielli, I. De Simone, B. Turchetti, T. Mello, L. D. C. Mannelli, C. Ghelardini, P. Buzzini, S. Roelens, *Chem. Eur. J.* **2012**, *18*, 5064-5072.

[10] a) M. Mazik, *RSC Advances*, **2012**, *2*, 2630-2642; b) M. Mazik, A. Hartmann and P.G. Jones, *Chem. Eur. J.* **2009**, *15*, 9147-9159; c) M. Mazik, H. Bandmann and W. Sicking, *Angew. Chem. Int. Ed*. **2000**, *39*, 551-554; d) M. Mazik, M. Kuschel and W. Sicking, *Org. Lett*. **2006**, *8*, 855; e) M. Mazik, H. Cavga and P. G. Jones, *J. Am. Chem. Soc*. **2005**, *127*, 9045-9052; e) M. Mazik and A. Hartmann, *J. Org. Chem.* **2008**, *73*, 7444-7450; (f) M. Mazik and A.C. Buthe, *J. Org. Chem.* **2007**, *72*, 8319-8326.

[11] H. Abe, Y. Aoyagi and M. Inouye, *Org. Lett.* **2005**, *7*, 59-61.

[12] C. Schmuck and M. Schwegmann, *Org. Lett.* **2005**, *7*, 3517-3520.

[13] a) F. A. Quiocho, *Pure Appl. Chem*. **1989**, *61*, 1293-1306; b) R. U. Lemieux, *Chem. Soc. Rev*. **1989**, *18*, 347-374; c) H. Lis and N. Sharon, *Chem. Rev*. **1998**, *98*, 637-674; d) W. I. Weiss and K. Drickamer, *Annu. Rev. Biochem.* **1996**, *65*, 441-473; e) H. Lis and N. Sharon in *Lectins*, Kluwer Academic Publishers, Dordrecht, The Netherlands, **2003**. f) H.-J. Gabius, S. André, J. Jiménez-Barbero, A. Romero and D. Solís, *Trends Biochem. Sci.* **2011**, ***36,*** 298-313.

[14] a) M. Nishio, Y. Umezawa, M. Hirota, Y. Takeuchi, *Tetrahedron*, **1995**, *51*, 8665-8701; b) M. Nishio, M. Hirota and Y. Umezawa in *The CH/π interaction: evidence, nature and consequences*, Wiley-VCH, New York, **1998**.

[15] M. Muraki, K. Harata, N. Sugita and K. Sato, *Biochemistry* **2000**, *39*, 292.

[16] H. Kogelberg, D. Solís and J. Jiménez-Barbero, *Curr. Opin. Struct. Biol*. **2003**, *13*, 646-653.

[17] a) K.K.S. Ng, A.R. Kolatkar, S. Park-Snyder, H. Feinberg, D. A. Clark, K. Drickamer and W. I. Weis, *J. Biol. Chem*. **2002**, *277*, 16088-16095; b) G. Colombo, M. Meli, J. Cañada, J. L. Asensio and J. Jiménez-Barbero, *Carbohydr. Res.* **2004**, *339*, 985-994.

[18] a) Y. Ferrand, M.P. Crump and A.P Davis, *Science*, **2007**, *318*, 619-622; b) U. Neidlein and F. Diederich, *Chem. Commun*. **1996**, 1493-1494; c) M. Mazik and A.C. Buthe, *Org. Biomol. Chem*. **2009**, *7*, 2063-2071; d) G. Lecollinet, A.P. Dominey, T. Velasco and A.P. Davis, *Angew. Chem. Int. Ed*. **2002**, *41*, 4093-4096; e) M. Mazik and A. C. Buthe, *J. Org. Chem.* **2007**, *72*, 8319-8326; f) M. Mazik and H. Cavga, *J. Org. Chem*. **2006**, *71*, 2957-2963; g) M. Mazik and A. C. Buthe, *Org. Biomol. Chem*. **2008**, *6*, 1558-1568.

[19] G. Fukuhara and M. Inouye, *Chem. Commun.* **2010**, *46*, 9128-9130.

[20] C. le Bourvellec and C.M.G.C. Renard, *Crit. Rev. Food Sci. and Nutr.* **2012**, *52*, 213-248.

[21] a) R.L. Hanlin, M. Hrmova, J.F. Harbertson and M.O. Downey. *Aust. J.Grape wine Res.* **2010**, *16*, 173-188; b) R. Ficarra, S. Tommasini, D. Raneri, M.L Calabro, M.R. Di Bella, C Rustichelli, M.C. Gamberini and P. Ficarra, *J. Pharm. Biomed. Anal.* **2002**, *29*, 1005-1014.

[22] E. Haslam, in *Practical polyphenolics: from structure to molecular recognition and physiological action*. Cambridge University Press, Cambridge **1998**.

[23] a) *Carbohydrate Recognition: Biological problems, Methods and Applications*, (Eds.: B. Wang and G-J. Boons), John Wiley and sons Inc. Hoboken, New Jersey, **2011**; b) I. Botos and A. Wlodawer, *Prog. Biophys. and Mol. Biol*. **2005,** *88*, 233-282; c) C.A. Bewley, *Structure* **2001**, *9*, 931-940; d) A.J. Bolmstedt; B.R. O'Keefe; S.R. Shenoy; McMahon and M.R. Boyd, *Mol. Pharmacol.* **2001**, *59*, 949-954.

[24] a) C. Walsdorff, W. Saak and S. Pohl, *J. Chem. Res. Synop*. **1996**, *27*, 282-283; b) A. Metzger, V. M. Lynch and E.V. Anslyn, *Angew. Chem.* **1997**, *109*, 911-914; c) A. Metzger, V. M. Lynch and E.V. Anslyn, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 862-865; d) K. J. Wallace, R. Hanes, E. Anslyn, J. Morey, K. Kilway and J. Siegel, *Synthesis* **2005**, *12*, 2080-2083.

[25] Belin, P. Barthélémy, K. Ruiz, J. M. Lacombe and B. Pucci, *Helv. Chim. Acta* **2003**, *86*, 247-265.

[26] N. Takashi, K. Kumiko, A. Akiko, M. Kenji and T. Koichiro, JP8143525 (A), 1996-06-04.

[27] S. Vandenbussche, D. Díaz, M. C. Fernández-Alonso, W. Pan, S. Vincent, G. Cuevas, J. Cañada, J. Jiménez-Barbero and K. Bartik, *Chem. Eur. J.* **2008**, *14*, 7570-7578.

[28] H-K Cho, H-J Kim, K. H. Lee and J-I Hong, *Bull. Korean. Chem. Soc.* **2004**, *25*, 1714-1716.

[29] M. D. Shortridge, D. S. Hage, G. S. Harbison, and R. Powers, *J. Comb. Chem.* **2008**, *10*, 948-958.

[30] T. D. P. Stack, Z. Hou, K. N. Raymond *J. Am. Chem. Soc.* **1993**, *115*, 6466-6467.

[31] B. Hakkarainen, L. Kenne, M. Lahmann, S. Oscarson and C. Sandström, *Magn. Reson. Chem.* **2007**, *45*, 1076-1080.

[32] a) M. Mammen, S-K. Choi and G. M. Whitesides, *Angew. Chem. Int. Ed.* **1998**, *37*, 2754-2794; b) D. R. Bundle and N. M. Young, *Curr. Opin. Struct. Biol*. **1992**, *2*, 666-673.

[33] *Maestro*, A powerful, all-purpose molecular modeling environment, version 8.5, Schrödinger, LLC, New York, NY **2008.**

[34] a) Weiner, S. J.; Kollman, P. A.; Case, D.; Singh, U.C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc*. **1984**, *106*, 765-784; b) S. J. Weiner, P. A. Kollman, D. T. Nguyen, and D. A. Case *J. Comput. Chem*. **1986**, *7*, 230-252.

[35] J. Jiménez-Barbero, M. Bernabé, J. A. Leal, A. Prieto, B. Gómez-Miranda, *Carbohydr. Res.* **1993**, *250*, 289-299.

[36] J. Jiménez-Barbero, A. Prieto, B. Gómez-Miranda, J. A. Leal, M. Bernabé, *Carbohydr. Res.* **1995**, *272*, 121-128.

Received: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

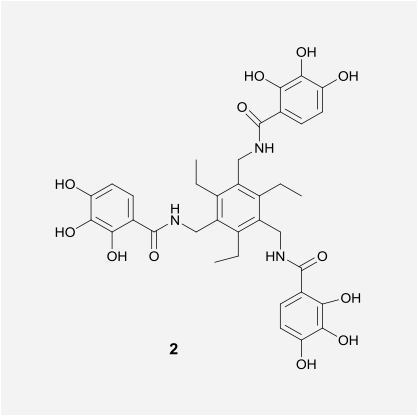
Entry for the Table of Contents

Layout 1:

Molecular recognition of Carbohydrates

P. Carrero, A. Ardá, M. Alvarez, E. G. Doyagüez, E. Rivero-Buceta, E. Quesada, A. Prieto, D. Solís, M-J. Camarasa, M-J. Peréz-Pérez, J. Jiménez-Barbero* and A. San-Félix*

Two novel tripodal receptors based on a trietylbenzene scaffold substituted with trihydroxybenzoyl groups have been synthesized and their conformational preferences and carbohydrate binding ability have been examined by NMR spectroscopy and molecular modeling. One of these receptors has a particular pre-organization that facilitates the recognition, in DMSO, of mannose-containing polysaccharides that mimic the mannose composition of those found in the envelope or cell walls of certain pathogens. These results open attractive perspectives for the development and potential application of **2** or compounds derived thereof in future anti-infective strategies.



2

Differential recognition of mannose-based polysaccharides by tripodal receptors based on a triethylbenzene scaffold substituted with trihydroxybenzoyl moities

Keywords: Molecular recognition/ Carbohydrates/Conformational analysis /NMR spectroscopy/Receptors

Supporting Information

Table of contents

^{1}H NMR and ^{13}C NMR spectra of compounds **5, 7, 1** and **2.** NOESY spectrum of **2** and the ^{1}H NMR control experiment with *N*-benzyl-2,3,4-trihydroxybenzamide.

Figure S1. Compound **5**, ^{1}H NMR, $CDCl_3$, 300MHz

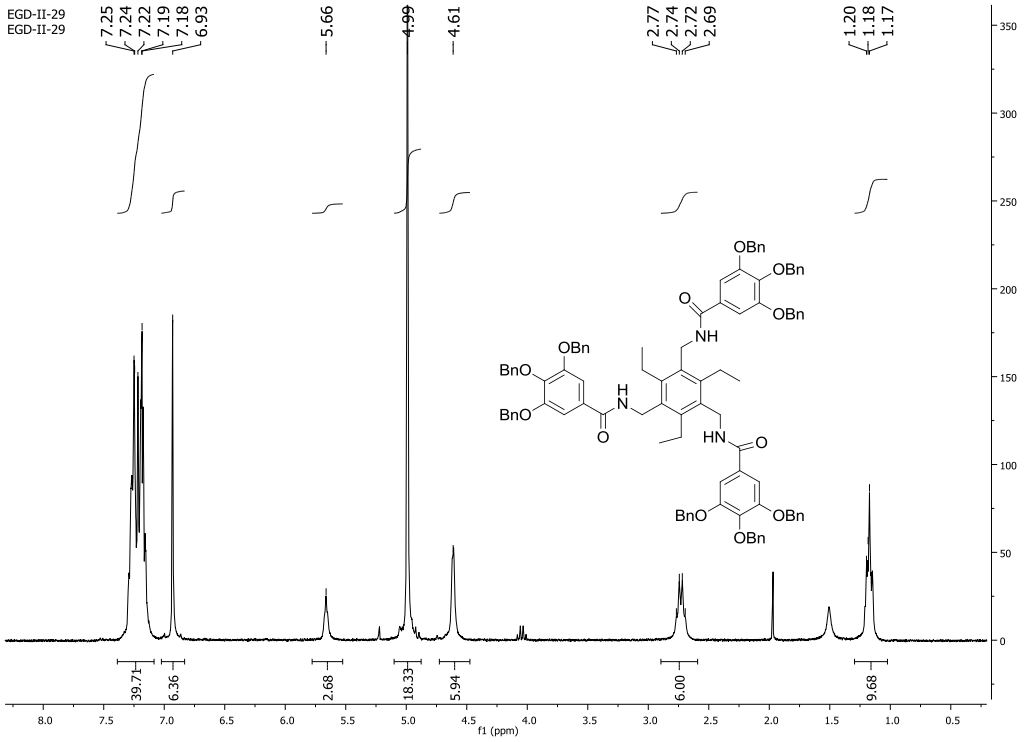


Figure S2. Compound **5**, ^{13}C NMR, $CDCl_3$, 75 MHz

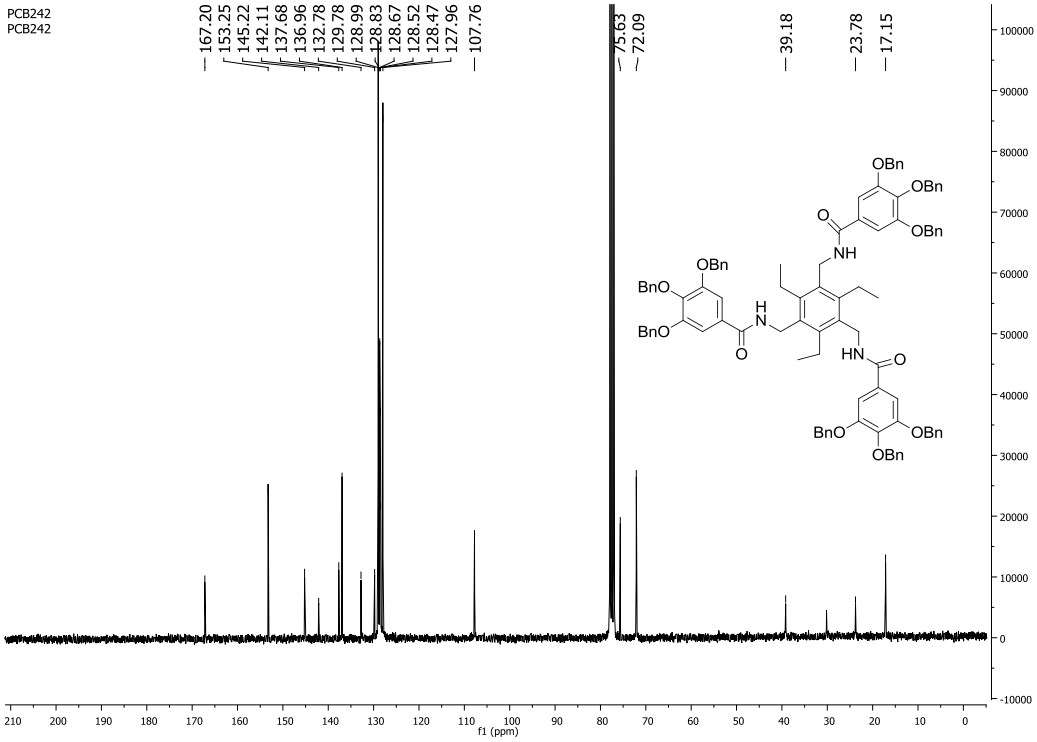


Figure S3. Compound **7**, ^{1}H NMR, $CDCl_3$, 300 MHz

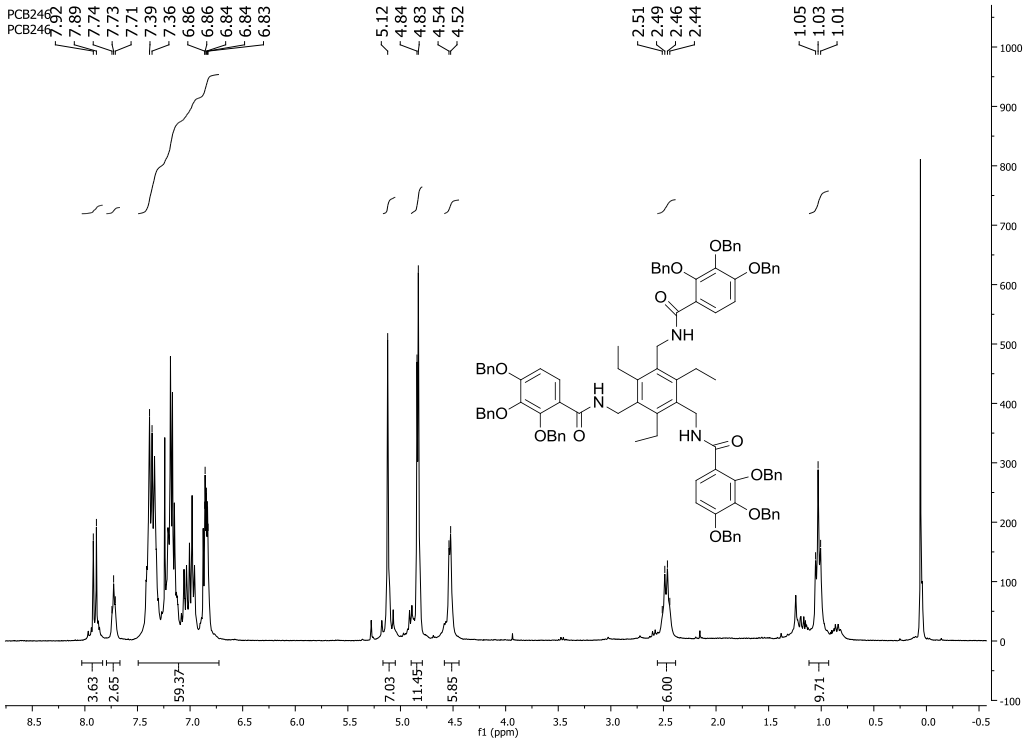


Figure S4. Compound **7**, ^{13}C NMR, $CDCl_3$, 75 MHz

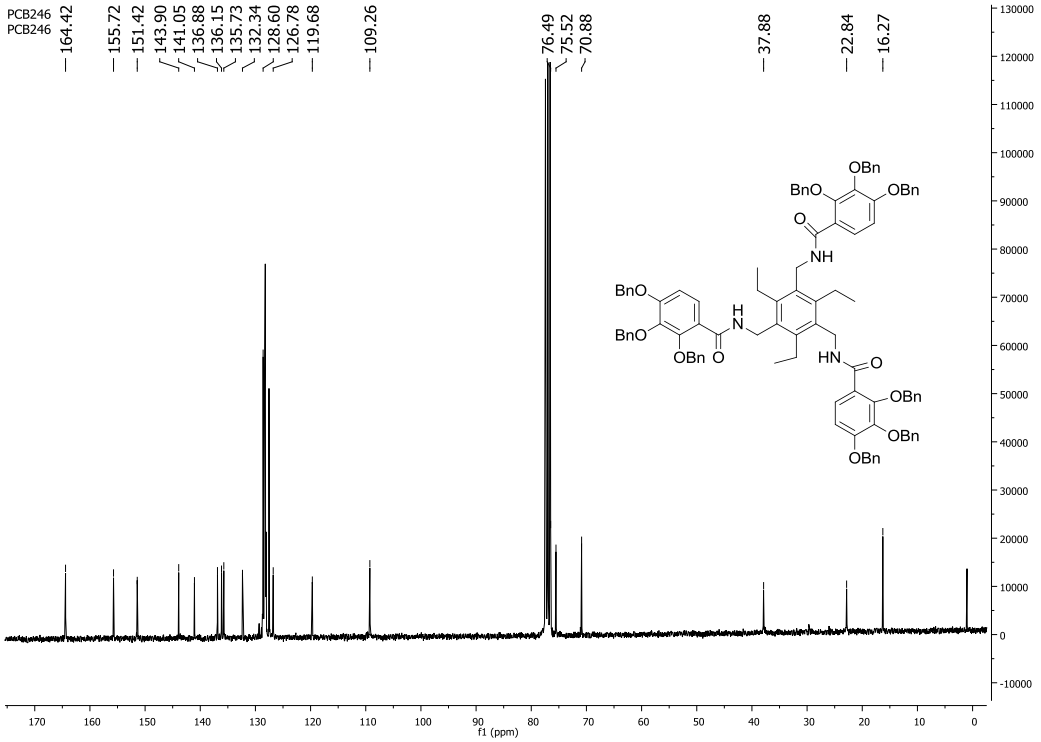


Figure S5. Compound **1**, ^{1}H NMR, CD_3OD, 300 MHz

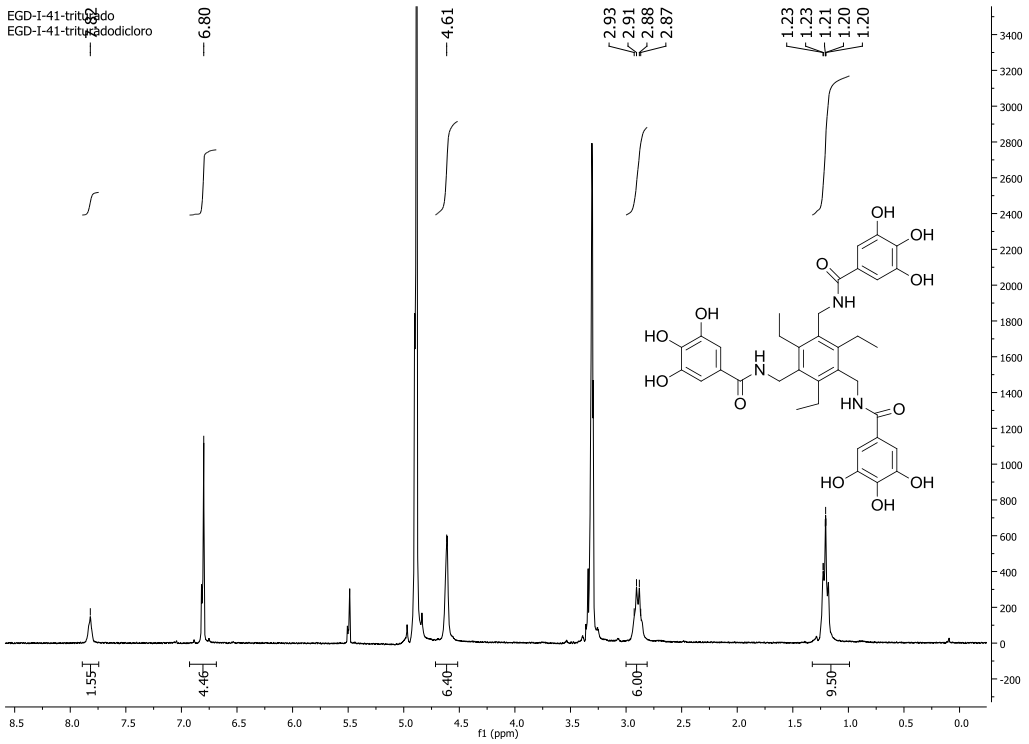


Figure S6. Compound **1**, ^{13}C NMR, CD_3OD, 100 MHz

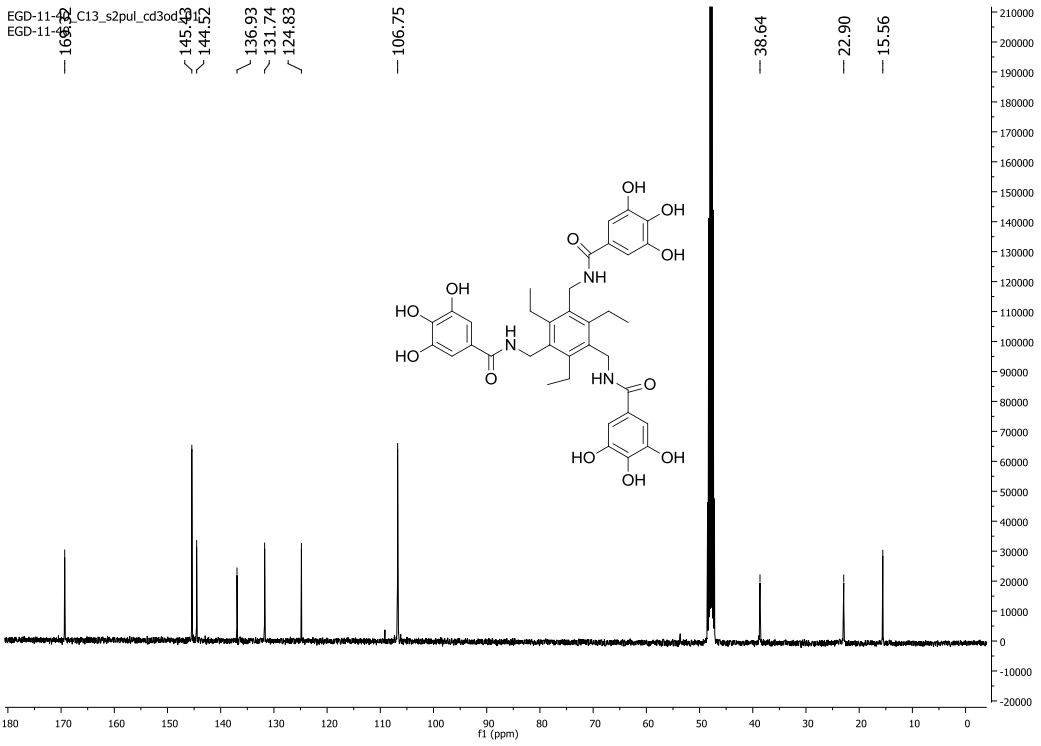


Figure S7. Compound **2**, ^{1}H NMR, CD_3OD, 300 MHz

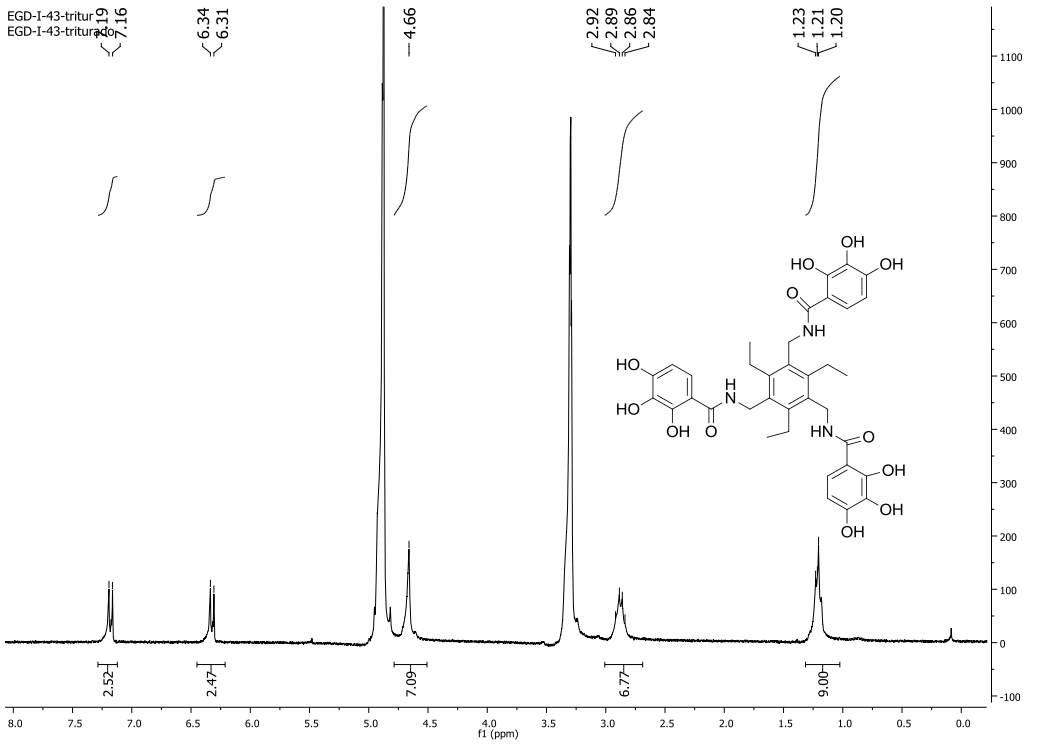


Figure S8. Compound **2**, ^{13}C NMR, CD_3OD, 75 MHz

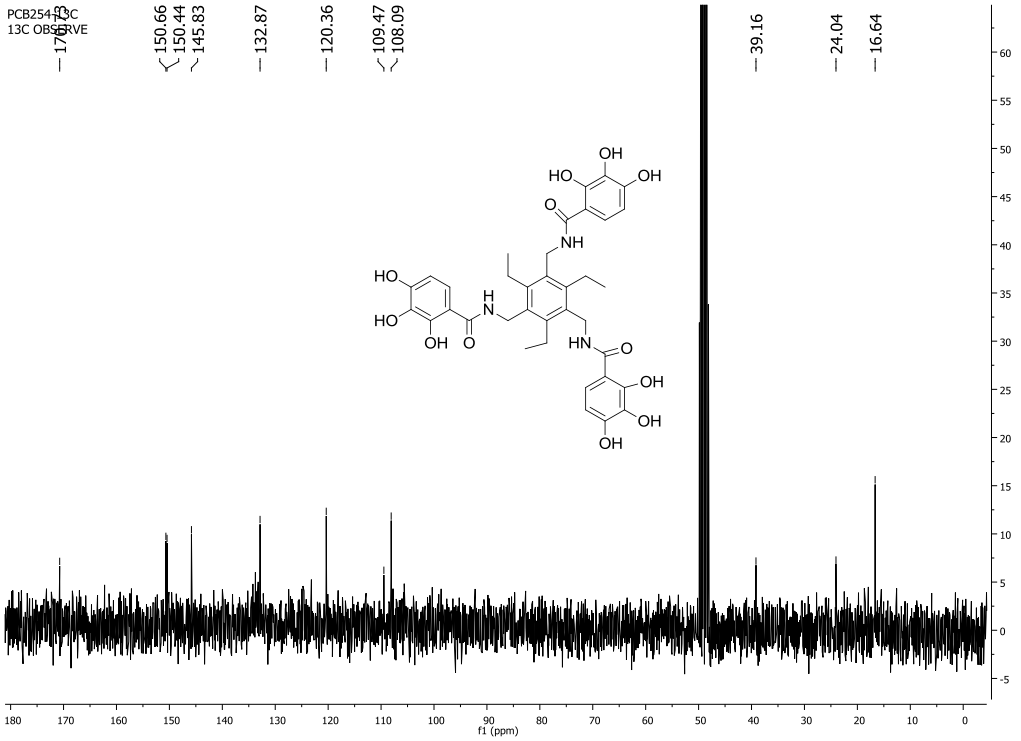


Figure S9. NOESY spectrum of **2** in DMSO-d_6 at 294 K with 500 ms mixing time

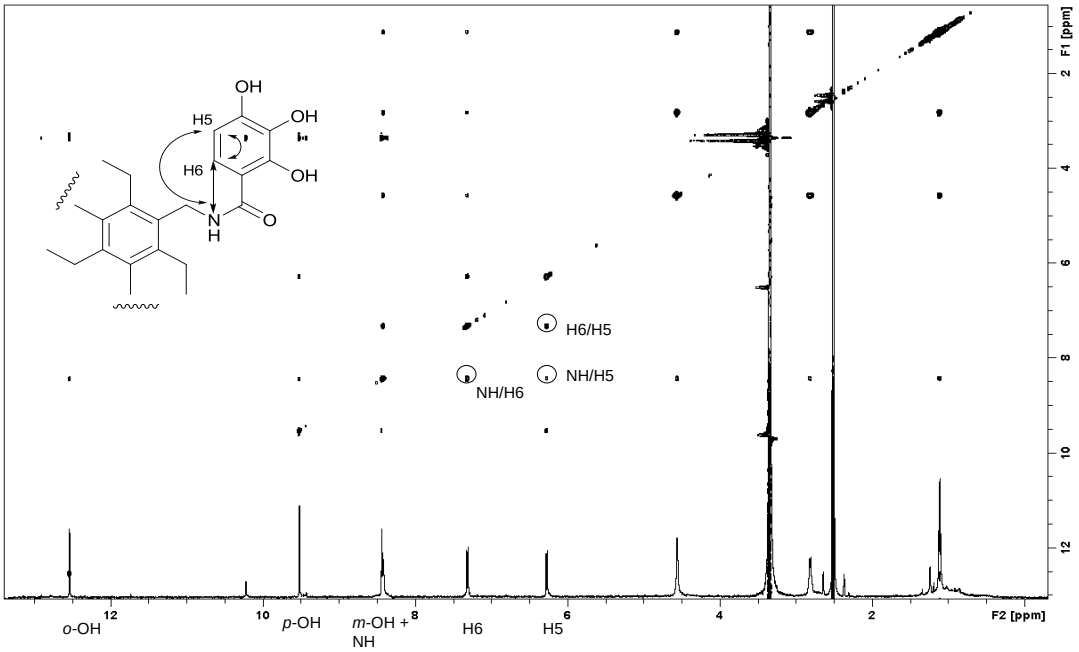


Figure S10. ^{1}H NMR spectra of **2** (left) and *N*-benzyl-2,3,4-trihydroxybenzamide (right) in DMSO-d_6 after stepwise additions (5µL) of polysaccharide **C** solution (23 mg/mL). Only the signals between 1 and 8 ppm are shown