

Fluorinated Carbohydrates as Lectin Ligands: Simultaneous Screening of a Monosaccharide Library and Chemical Mapping by ^{19}F NMR Spectroscopy

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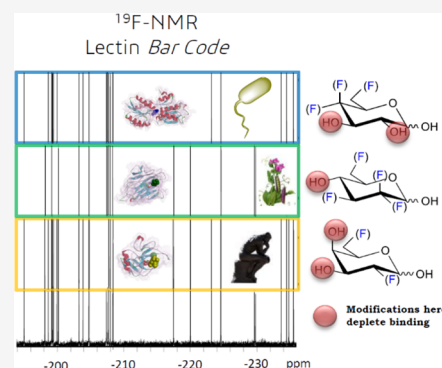
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ABSTRACT: Molecular recognition of carbohydrates is a key step in essential biological processes. Carbohydrate receptors can distinguish monosaccharides even if they only differ in a single aspect of the orientation of the hydroxyl groups or harbor subtle chemical modifications. Hydroxyl-by-fluorine substitution has proven its merits for chemically mapping the importance of hydroxyl groups in carbohydrate–receptor interactions. ^{19}F NMR spectroscopy could thus be adapted to allow contact mapping together with screening in compound mixtures. Using a library of fluorinated glucose (Glc), mannose (Man), and galactose (Gal) derived by systematically exchanging every hydroxyl group by a fluorine atom, we developed a strategy combining chemical mapping and ^{19}F NMR T_2 filtering-based screening. By testing this strategy on the proof-of-principle level with a library of 13 fluorinated monosaccharides to a set of three carbohydrate receptors of diverse origin, i.e. the human macrophage galactose-type lectin, a plant lectin, *Pisum sativum* agglutinin, and the bacterial Gal-/Glc-binding protein from *Escherichia coli*, it became possible to simultaneously define their monosaccharide selectivity and identify the essential hydroxyls for interaction.



INTRODUCTION

Molecular recognition events are at the heart of health and disease. From the chemical perspective, understanding the details of interactions for the underlying functional pairings may provide key information for innovative drug discovery and design. In this context, carbohydrate oligomers (saccharides, glycans) are ubiquitous in nature, commonly presented on cell surfaces by protein and lipid scaffolds.^{1–4} Structurally, an exceptionally large diversity can be generated by simply exploiting permutations of linkage points and anomeric position at each glycosidic linkage.⁵ As a consequence, glycans are “ideal for generating compact units with explicit informational properties”,⁶ and this information is being disclosed to be “read” and “translated” into (patho)physiological processes by lectins.^{4,5} Thus, the analysis of glycan-lectin recognition has become a topic with biomedically promising perspective^{7,8} and a fructiferous foundation to enhance the symbiosis of Chemistry and Biology as Lemieux asked for.⁹

From the molecular recognition perspective, different approaches have been tested to examine the relevance of hydroxyl groups from saccharide units in binding to receptors. One extensively applied approach rests on screening a given set of available closely related saccharides that display different stereochemistry and/or substitutions at a certain site within the

sugar ring.^{10–19} This protocol synthetically eliminates or modifies hydroxyl groups (deoxygenation, methylation, exchange by halogens).²⁰ In particular, hydroxyl-by-fluorine substitution has been used to trace key hydroxyl groups for contact with either lectins, antibodies, transporters, or enzymes.²¹

Fluorine can be considered as an isosteric mimic of the hydroxyl group, although without the capacity to act as a hydrogen-bond donor and with a diminished hydrogen-bond acceptor competence.^{22–24} Additionally, its particular physicochemical properties²⁵ introduce electronic and polar-hydrophobic effects.²⁶ Indeed, fluorine modulates the population of the conformational space^{27,28} and lipophilicity of fluorine-containing carbohydrates.²⁹ Smart use of these features has already allowed development of new molecules that efficiently act as substrates³⁰ and inhibitors of glycosidases.^{31,32} Advances for fluorine introduction into

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organic molecules have made available a large variety of mono-³³ and polyfluorinated saccharides,^{33–40} which are highly attractive as chemical probes from different point of views.²¹ It is also well-known that fluorine-containing molecules are extensively used in bioorganic and medicinal chemistry.^{23,41} Many of these studies have driven the development of ¹⁹F NMR-spectroscopy methodologies as valuable tools to study molecular recognition events or to screen compound libraries.^{42,43}

In this context, we and others have applied ¹⁹F-observed NMR strategies to study glycan–protein interactions by means of saturation transfer difference (STD NMR-spectroscopy) measurements using 1D⁴⁴ and 2D³⁴ experimental designs, by monitoring chemical shifts perturbations and exchange kinetics,^{45,46} by observing line broadening of the ¹⁹F NMR signals,^{47–49} or by employing relaxation filtering protocols.^{50,51}

Herein, we propose a robust and general method to efficiently pick up and study the interactions of a library of fluorinated sugars with a given receptor. By taking advantage of the large chemical shift range of the ¹⁹F nucleus and its sensitivity, monitoring sugar–protein interactions by a panel of 13 different monofluorinated sugars (with up to 26 well resolved ¹⁹F NMR signals considering the presence of the α and β -anomers for each sugar, Figure 1, Table S1) provides information on the selectivity of the binding event in a single setup. This methodology extends the applicability of the reported T₂-filtering strategy and overcomes the limits of ¹H NMR resolution (see spectrum in Figure 1b).

As proof-of-concept, two lectins and a sugar transporter of diverse origins and selectivities have been chosen: the human Macrophage Galactose-type Lectin (MGL, CLEC10A, CD301), a C-type lectin binding *N*-acetylgalactosamine in *O*-glycans (T_n antigen, CD175) and in *N*-glycans (LacdiNAc);^{52–54} *Pisum sativum* agglutinin (PSA), a plant lectin selective for α -mannopyranosides and -glucopyranosides;^{15,55} and the glucose/galactose-binding protein (GGBP),^{56–58} a bacterial sensor for free monosaccharides. From the analysis of data from simple 1D ¹⁹F NMR experiments by applying transversal relaxation filters, screening and chemical mapping are simultaneously achieved. In essence, information on the monosaccharide selectivity for a particular sugar receptor is obtained (screening) together with the direct identification of hydroxyls that are essential for binding and those that can be chemically substituted or modified without critically compromising the binding event (chemical mapping).

RESULTS

Three different types of sugar receptors are deliberately selected herein to illustrate broad applicability, i.e. a human lectin involving Ca²⁺ for direct ligand contact, a plant agglutinin, and a bacterial sugar transporter.

MGL. MGL belongs to the C-type lectin family characterized by containing a calcium cation at the binding site, directly involved in carbohydrate recognition by coordination bonding.^{52,53} MGL, like the hepatic asialoglycoprotein receptor, is a transmembrane protein with the carbohydrate recognition domain (CRD) on top of its extracellular stalk that oligomerizes to trimers.⁵⁹ GalNAc in α/β linkage are the preferred ligands ($K_D = 12 \mu\text{M}$ for methyl α -*N*-acetylgalactosaminide, Me α -GalNAc), galactose being a weaker binder ($K_D = 0.9 \text{ mM}$ for Me α -Gal).⁵³ To perform the recognition studies, the soluble extracellular ectodomain containing the CRD was used. It is known that the Ca²⁺ in

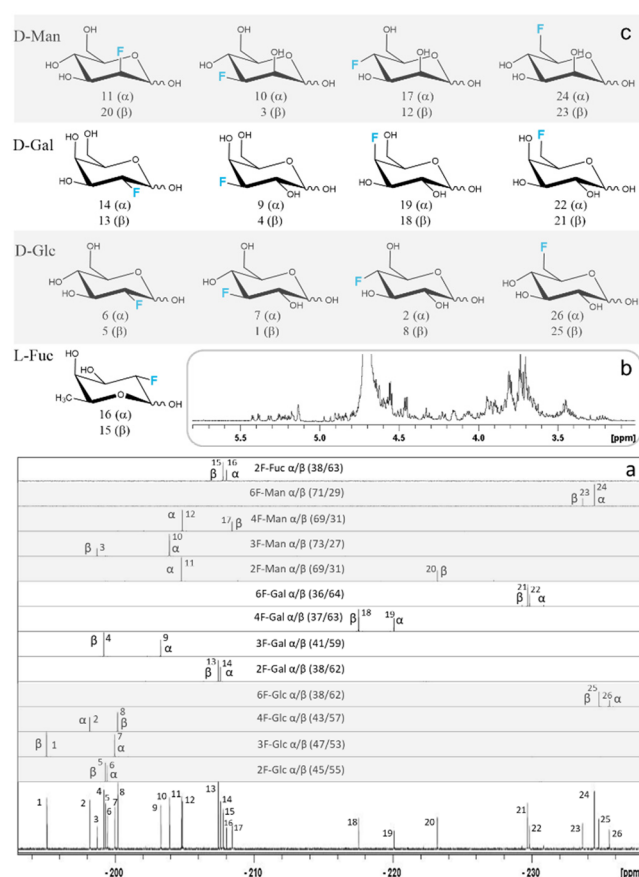


Figure 1. (a) ¹⁹F NMR (¹H-decoupled) spectra recorded for each individual monofluorinated monosaccharide as anomer mixture. The α/β anomeric ratios are given between brackets. Lower panel, ¹⁹F NMR spectrum of the full library. Each peak is numbered from lower to higher field. (b) ¹H NMR spectrum of the mixture of the 13 monosaccharides. (c) Representation of the structures of the different monodeoxy-monofluorinated monosaccharides present in the library. The corresponding peak number for each anomer in the ¹⁹F NMR spectrum is indicated.

the binding site makes contact with its Gal/GalNAc ligands through the equatorial/axial OH-3 and OH-4 groups.⁵³ In order to study the importance of each hydroxyl group of the Gal moiety, the binding of the four possible monodeoxy-monofluorinated Gal analogues (at positions 2, 3, 4, and 6), keeping the anomeric position free, was tested to detect those hydroxyl-to-fluorine substitutions that impair binding. A similar strategy, using an extended mixture of mono- and polyfluorinated galactopyranosides and applying a diversity of techniques, has allowed identification of OH-3 and -4 as the coordinating groups in a calcium-dependent bacterial galactophilic lectin.³³

Since every monosaccharide exists as a mixture of its α and β anomers in equilibrium, eight different molecules are present in solution. The ¹⁹F NMR spectrum of the mixture is pleasingly simple, just showing eight individual ¹⁹F NMR signals (Figure 2a), one for each monofluorinated Gal anomer in the mixture. Their intensities are governed by the anomer ratio at equilibrium.⁶⁰

The transverse relaxation time (T₂) for each compound was measured in the free state in the absence of lectin, ranging between 1.2 to 1.8 s for Gal derivatives (Table S1). The monofluorinated Gal mixture was added to a solution of MGL,

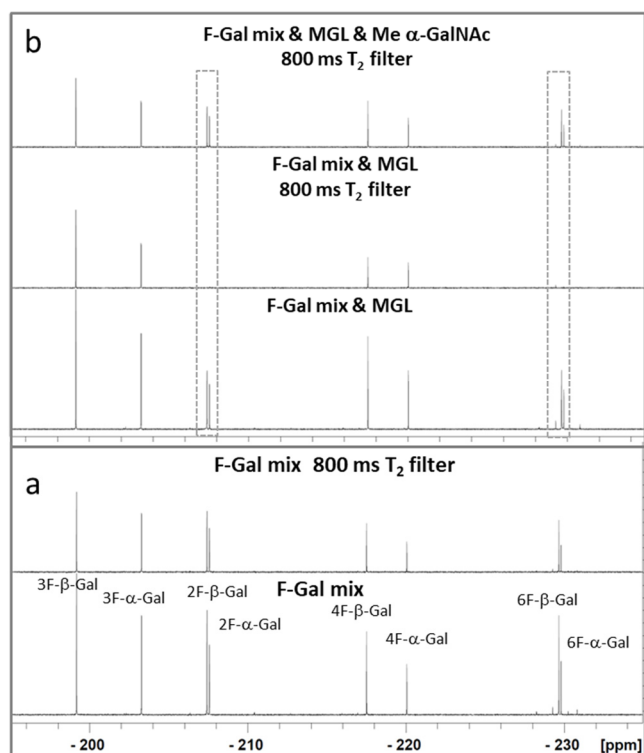


Figure 2. (a) ^{19}F NMR (^1H -decoupled) spectrum of the F-Galactose mixture (2F-Gal (0.5 mM); 3F-Gal (0.68 mM), 4F-Gal (0.37 mM), and 6F-Gal (0.45 mM) in absence of the protein, without T_2 relaxation filter (lower panel), and after applying a 800 ms T_2 relaxation filter (upper panel). (b) ^{19}F NMR (^1H -decoupled) spectrum of the same mixture in the presence of 0.015 mM MGL before (lower panel) and after (mid panel) the application of a 800 ms T_2 relaxation filter. The upper panel shows the spectrum after the addition of 0.5 mM Me α -GalNAc with the same relaxation filter.

and the T_2 filtering strategy^{50,61,62} was applied to identify the binders. Briefly, those molecules that bind to the protein drastically change their hydrodynamic behavior in the bound state, and thus their rotational motion correlation time increases toward that of the large protein, with a concomitant reduction in T_2 . Additionally, the effective transverse relaxation is also affected by the kinetics of the chemical exchange process between the free and bound states, further reducing the observed T_2 , especially if the system no longer follows the fast chemical exchange regime. This reduction in T_2 , which is in the first instance manifested in standard 1D NMR spectra as signal broadening, can be easily transformed into a signal-intensity reduction by the application of a standard Carr–Purcell–Meiboom–Gill (CPMG) spin echo pulse train sequence before acquisition. The filtered NMR spectrum displays the NMR signals of the binders significantly reduced or even suppressed, compared to those of the unbound compounds.

Figure 2b shows the comparison of the ^{19}F NMR spectra recorded for the mixture of monofluorinated galactose derivatives in the presence of MGL (lower panel) with that obtained by applying a spin–echo filter of 800 ms (central panel). The drastic reduction of the intensity of signals in the presence of protein (Figure 2b central panel) relative to the experiment in its absence (Figure 2a upper panel) corresponding to 2F-Gal ($\beta = 2\%$, $\alpha = 4\%$) and 6F-Gal ($\beta = 1\%$, $\alpha = 7\%$) is clearly observed, while the NMR signals obtained for 3F-Gal

($\beta = 57\%$, $\alpha = 73\%$) and 4F-Gal ($\beta = 98\%$, $\alpha = 80\%$) are significantly less altered.

To confirm that this selective signal reduction is due to the binding of the ^{19}F -containing Gal entities to the CRD, a competition experiment was performed by adding Me α -GalNAc to the mixture. The recovery of the 2F-Gal and 6F-Gal signals was evident, indicating that they are displaced from the binding site by the strong competitor (Figure 2b, upper panel).

Drawing a conclusion from chemical mapping^{30,49} is straightforward: the modification of either hydroxyl at 3 or 4 eliminates a coordination bond in the interaction between sugar and Ca^{2+} . Therefore, the signals corresponding to 3F-Gal and 4F-Gal are not affected by the lectin and do not show significant signal reduction. On the contrary, the hydroxyls at positions 2 and 6 can be substituted by fluorine. Their ^{19}F NMR signals are clearly reduced in the presence of the MGL due to binding.

Since the broad dispersion of ^{19}F NMR chemical shifts of the four anomeric pairs of the monofluorinated Gal analogues is more than 30 ppm (between -199 and -230 ppm), the feasibility to test a broad panel of monofluorinated monosaccharides was envisioned. Thus, the four monodeoxy-monofluorinated D-glucoses (2F-Glc, 3F-Glc, 4F-Glc, and 6F-Glc) and D-mannoses (2F-Man, 3F-Man, 4F-Man, and 6F-Man) together with 2-deoxy-2-fluoro-L-fucose (2F-Fuc) were added to provide a library with 13 different anomeric pairs of monofluorinated monosaccharide (Figure 1). All compounds were available from commercial sources except 6F-Man that was chemically synthesized (see Experimental Section).

This mixture with the 13 fluorinated monosaccharide anomeric pairs gives a very crowded ^1H NMR spectrum (Figure 1b). In contrast, its proton-decoupled ^{19}F NMR spectrum presents well-resolved individual signals for each of the 26 different molecules in the sample, which are spread over 40 ppm (Figure 1a). Thus, the extended compound library, including the monofluorinated Gal, Glc, and Man analogues, was now tested with MGL, applying again the T_2 -filtering strategy (Figure 3). For qualitative visualization of the NMR

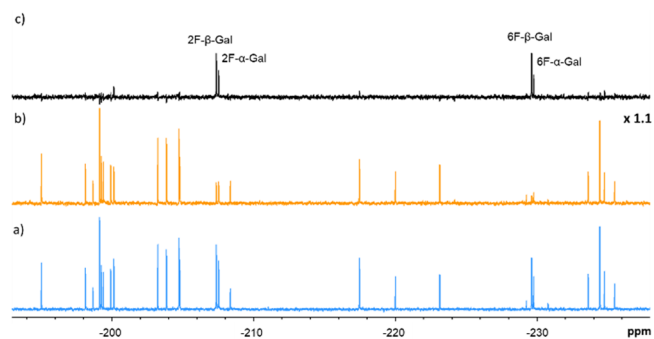


Figure 3. ^{19}F NMR (^1H -decoupled) T_2 -filtered spectra recorded for the fluorinated monosaccharide library (Man, Glc, and Gal analogues) in the presence of MGL (30 μM). (a) Spectrum acquired with a short 8 ms T_2 filter. (b) Spectrum acquired with long 160 ms T_2 filter factored 1.1 times. (c) Difference spectrum.

signals affected by the lectin, the obtained filtered ^{19}F NMR spectrum was subtracted from the nonfiltered one following the protocol described in the Experimental Section (a correction factor f was applied to the filtered spectrum to account for the signal reduction due to transversal relaxation unrelated with the presence of the protein). Only the ^{19}F NMR

signals corresponding to binders should appear in the difference spectrum. Indeed, the peaks corresponding to 2F-Gal (peaks 13 and 14) and 6F-Gal (peaks 21 and 22) are clearly displayed in the difference spectrum (Figure 3) in accordance with the results of the experiment described above for the smaller sized Gal library.

Given the encouraging results for the first system, the suitability of the monofluorinated monosaccharide library for simultaneous ligand screening and chemical mapping was further tested with two other types of carbohydrate receptors with different sugar selectivities.

PSA. *Pisum sativum* agglutinin (PSA), a leguminous lectin with a “jelly roll” fold,⁶³ was also tested. PSA is selective for Man/Glc-containing oligosaccharides without involvement of Ca²⁺ in contact with the sugar, but displays weak affinity for single monosaccharides: 0.53 and 1.15 mM for methyl α -mannoside (Me α -Man) and methyl α -glucoside (Me α -Glc), respectively.⁵⁵

Several ¹⁹F NMR peaks diminished (Figure 4a,b) when the T₂ filter was applied. Those present in the difference spectrum

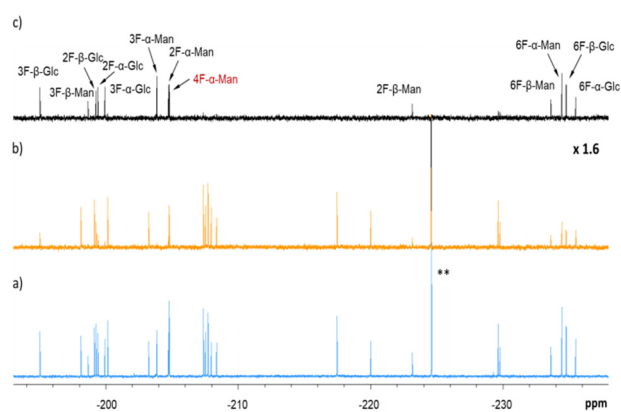


Figure 4. ¹⁹F NMR (¹H-decoupled) T₂-filtered spectra recorded for the fluorinated monosaccharide library in the presence of PSA (25 μM, ligand/protein ratio around 36:1). (a) Spectrum acquired with a short 8 ms T₂ filter. (b) Spectrum acquired with 720 ms of T₂ filter factored 1.6 times. (c) Difference spectrum. The peak corresponding to 4F- α -Man, in red, is a “false positive” (see text). **2-Fluoroethanol added as internal reference.

(Figure 4c) correspond to 2F-Glc (peaks 5 and 6) and 2F-Man (11 and 20), 3F-Glc (1 and 7), 3F-Man (3 and 10), 6F-Glc (25 and 26), and 6F-Man (23 and 24). Neither 4F-Glc nor any Gal derivatives were observed in the difference ¹⁹F NMR spectrum. However, a limitation of the method was detected. The difference ¹⁹F NMR spectrum also displays “false positives” corresponding to fast relaxing signals (see below), especially when the applied T₂ filter is long enough (720 ms in this experiment). This was the case for 4F- α -Man (peak 12, ¹⁹F-T_{2,free} = 0.7 s), whose signal relaxation was significantly faster than that of the other signals of the molecules present in the mixture (Table S1).

To confirm specific binding, the difference ¹⁹F NMR spectrum was again complemented with competition experiments (Figure 5) in the presence of a known ligand (Me α -Man). The signals corresponding to the 2F- and 3F- Man/Glc derivatives were now clearly observed, indicating that 2F-/3F-Man/Glc are indeed displaced from the binding site by Me α -Man. On the contrary, no difference in the intensities of the 4F- α -Man signal was observed in the absence and presence of

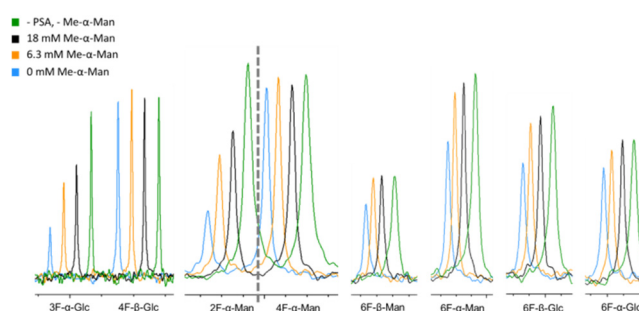


Figure 5. Close-up view of selected ¹⁹F NMR peaks recorded in the ¹⁹F NMR T₂-filtered spectra (720 ms) of the fluorinated monosaccharide library in the absence of lectin (green), in the presence of PSA (blue), and when adding different concentrations of Me α -Man.

the competitor (Figure 5), indicating that this molecule is not a binder of the lectin. Very likely, the signal observed in the difference spectrum described above is due to the intrinsic fast relaxation of 4F- α -Man (¹⁹F-T_{2,free} = 0.70 s, Table S1). Analogous results were observed in the absence of the lectin; i.e., T₂ of 4F-Man is not affected by the presence of PSA. A mixed situation took place for the 6F-Man and 6F-Glc derivatives (Figure 5). In these cases, the observed signals in the difference experiment are due to ligand binding and to fast relaxation. In fact, the intrinsic T_{2,free} for the corresponding ¹⁹F signals of the 6F-Man (¹⁹F-T_{2,free} = 1.00 s and ¹⁹F-T_{2,free} = 0.82 s for the β and α anomers, respectively) and 6F-Glc (¹⁹F-T_{2,free} = 0.92 s and ¹⁹F-T_{2,free} = 0.90 s for the β and α anomers, respectively) derivatives is also rather short. On the other side, indeed, the initial decrease in signal intensity induced by the presence of the protein was subtle, but recovery was almost complete after addition of a small concentration of competitor, thus also confirming affinity, although likely weaker.

Interestingly, it has been described that hydroxyls at positions 2 and 3 of glucose can be substituted by fluorine while retaining binding by PSA; however, when F is at the 6 position the reported binding was minimal and modifications at OH-4 abolished the binding,^{15,64} supporting the results presented here regarding PSA selectivity.

GGBP. The third receptor is the bacterial GGBP. It is involved in chemotaxis and sugar transport in bacteria and has a very high affinity for Glc (0.04 μM) and Gal (0.13 μM)^{65,66} typical for bacterial binders of free monosaccharides. Its structure consists of two globular Rossman fold domains, and differently from the tested lectins, GGBP presents a deep binding pocket at the hinge connecting and closing both domains around the monosaccharide ligand.^{56,57} When GGBP was added to the monofluorinated monosaccharide library, the signals belonging to Glc and Gal molecules with F atoms at positions 4 or 6 showed reduced peak intensities in the T₂-filtered spectrum (Figure 6). On the contrary, those signals corresponding to Glc and Gal moieties substituted at either position 2 or 3 were not affected by the presence of GGBP. This evidence indicates that the OH groups at those 2 and 3 positions are required for the binding to take place and cannot be substituted by a fluorine atom. In the difference ¹⁹F NMR spectrum, signals for 2F- α -Man, 4F- α -Man, and 6F- α -Man also appear (Figure 6c). However, when Glc was added to the library/receptor mixture as a competitor, the corresponding signals of those fluoromannoses were not recovered (Figure 7). In the cases of 4F- α -Man and 6F- α -Man, as for PSA, this

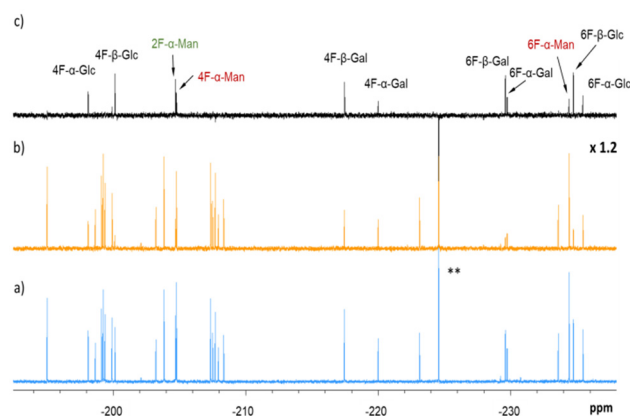


Figure 6. ^{19}F NMR (^1H -decoupled) T_2 -filtered spectra recorded for the fluorinated monosaccharide library in the presence of GGBP (25 μM , ligand/protein ratio ca. 36:1). (a) Spectrum acquired with a short 8 ms T_2 filter, (b) spectrum acquired with long 400 ms of T_2 filter factored 1.3 times, (c) difference spectrum. For peaks labeled in green and red, see text. **2-Fluoroethanol added as internal reference.

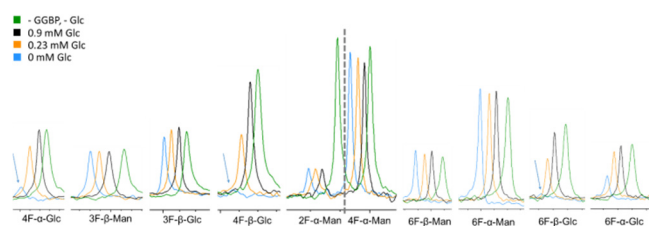


Figure 7. Close-up view of selected ^{19}F NMR peaks recorded in the ^{19}F NMR T_2 -filtered spectra of the fluorinated monosaccharide library in the absence of receptor (green), in the presence of GGBP (blue), and when adding different concentrations of the Glc competitor (0.23 mM orange and 0.9 mM black). In all cases, the NMR experiments were acquired using 720 ms of T_2 filter.

behavior is again due to the intrinsic fast relaxation of the 4F- α -Man and 6F- α -Man ^{19}F signals. Interestingly, 2F- α -Man is a special case; its signal reduction only takes place in the presence of the protein, and it is not affected by glucose (Figure 7). This result suggests 2F- α -Man interacts with GGBP but at a location different from the canonical sugar-binding site.

DISCUSSION

The tested screening method is based on the dramatic differences in transverse relaxation observed for binders within a library of fluorinated monosaccharides, when acquiring NMR spectra in the absence or presence of a carbohydrate-binding protein. The transversal relaxation time is related to the rotational motion correlation time of the molecule, and it sharply decreases as the correlation time increases. When monosaccharides interact with a large receptor, they adopt the correlation time of the macromolecule during the time the complex is associated and, thus, undergo a critical decrease of their T_2 . This change in T_2 may be followed in a straightforward manner under fast exchange conditions between bound and free states. Thus, only a single ^{19}F NMR signal appears in the spectrum at the averaged chemical shift of the exchanging states weighted by their corresponding molar fractions. In fact, the observed effective T_2 also depends on the kinetics of the exchange between the free and bound forms.

Both rotational motion and exchange effects add together in the T_2 filtering strategy and allow the efficient detection of medium- to low-affinity binders (from low micromolar to millimolar K_D), even using high ligand/protein ratios.⁶² The application of the CPMG-based T_2 filtering scheme is fairly straightforward, and usually a reasonable number of spin-echo loops before acquisition is sufficient to obtain highly sensitive NMR spectra with the required information discriminating binders from nonbinders. From the practical perspective, the current library renders very well resolved ^{19}F NMR spectra with separated signals for all different monosaccharide moieties in the mixture. Obviously, other fluorinated saccharides could well be added to the mixture increasing the screening power of the concept. As an added value for the ^{19}F observation, the experiments do not require any deuterated buffer, thus simplifying the experimental setup.

Regarding the screening process, in the first instance, and assuming that all ^{19}F nuclei in the library have similar T_2 relaxation times when free in solution, it should be possible to qualitatively visualize those signals that are affected by the protein. To do so, a difference NMR spectrum is obtained by subtracting the spectrum recorded using a short spin-echo delay from a second one measured employing a longer delay. However, the ^{19}F NMR signals of some molecules, such as 4F- α -Man, 6F- α/β -Man, and 6F- α/β -Glc (Table 1), relax significantly faster ($T_2 < 1$ s) than the others ($T_2 > 1.2$ s) and their peaks consistently appear in the difference spectrum when long spin-echo relaxation delays are used. Therefore, to unambiguously assess the existence of specific binders at the carbohydrate-binding site, the difference ^{19}F spectrum should be complemented with the information provided by additional competition experiments carried out by adding a known ligand of the lectin. The comparison of the recovered ^{19}F NMR signals in the presence of an excess of the competitor in the lectin/library sample can be expressed as the ratio of signal intensities, $I_{t(+C/-C)}$, measured in spectra acquired with a relaxation filter t in presence (+C) and absence (-C) of competitor (C), thus highlighting the specific binders (Figure 8).

At least qualitatively, these signal recovery data allow the specificities of the three sugar receptors to be distinguished, correlating them with their known monosaccharide selectivity: MGL only recognizes Gal moieties and PSA binds Glc and Gal analogues, while GGBP interacts with Glc and Gal monosaccharides. Additionally, information on the selectivity for the anomeric configuration can be gleaned from the signal recovery data in the T_2 -filtered competition experiments. For instance, for PSA, the α -anomers show a higher recovery ratio than their corresponding β -anomers in accordance with previous reports. On the contrary, based on X-ray and NMR structural data, GGBP has been described to display specificity for β -anomers.⁵⁷ The recovery ratio data here presented not only are in agreement with that selectivity but also show that the α -anomers are binders, as previously suggested by means of ligand-binding kinetic experiments.^{65,66}

As mentioned above, the OH by F substitution has been extensively used in carbohydrate chemistry to map the key hydroxyl groups of a given sugar that are involved in their recognition by lectins, antibodies, transporters, or enzymes.^{10–20} The methodology presented herein, which employs a rationally assembled collection of monofluorinated monosaccharides for which their hydroxyl groups have been systematically substituted by fluorine atoms, allows dissecting

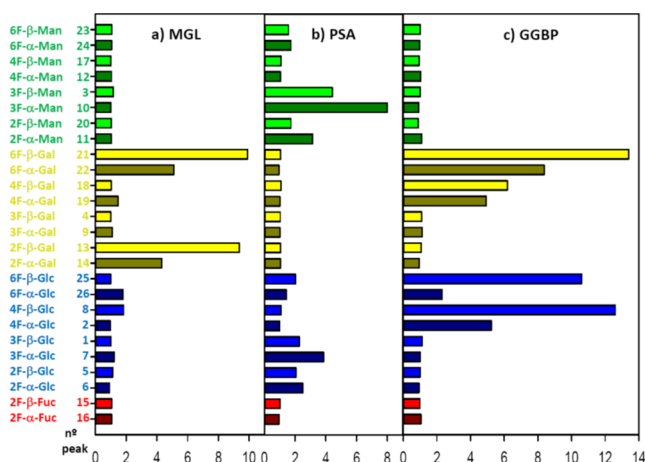


Figure 8. Competition experiments and ^{19}F NMR T_2 -filtered spectra for the analysis of the interaction of the fluorinated monosaccharides library with the different lectins. The x axis corresponds to the signal intensity recovery, $I_{t(+C/-C)}$, expressed as the ratio between the relative decay at time t in the presence (+) and absence (-) of competitor (C). From left to right: (a) MGL, T_2 filter $t = 720$ ms, MGL (30 μM) and competitor Me α -GalNAc (0.9 mM); (b) PSA, T_2 filter $t = 720$ ms, PSA (25 μM) and competitor Me α -Man (18 mM); and (c) GGBP, T_2 filter $t = 400$ ms, GGBP (25 μM) and competitor Glc (0.9 mM). In all cases the mixture with 0.9 ± 0.3 mM of each monosaccharide was used.

chemical mapping information regarding the importance of each individual hydroxyl group in the interaction with its receptor. For MGL, the same experiment allows identifying its selectivity for Gal moieties and simultaneously shows that hydroxyls at positions 3 and 4 are essential to keep the interaction ability of the Gal analogue, while hydroxyls 2 and 6 can be modified while still maintaining binding to MGL. PSA can recognize Man and Glc, epimers at position 2. Thus, the orientation of OH-2, axial in Man, equatorial in Glc, is not essential for binding, and consequently, both fluorinated epimers 2F-Man and 2F-Glc are recognized. Moreover, it can be inferred that modifications at OH-3 are tolerated, as F to OH substitution at this position does not block binding to the lectin. On the contrary, OH-4 is essential for binding while the modification at position 6 still sustains a weak interaction. Finally, for GGBP, which also recognizes two monosaccharides that share the equatorial configuration at C2, i.e. Glc and Gal, OH-2 and OH-3 are necessary for binding, while OH-4 (either axial in Gal or equatorial in Glc) and OH-6 can be modified. Thus, the binding pattern is completely opposite to that observed for PSA. Additionally, for GGBP, the possibility of a secondary binding site has been deduced, given the existence of binding to 2F- α -Man (see Figure 6; 2F- α -Man is marked in green) and the fact that this interaction is not abolished by Glc (see Figure 7, the signal intensity of 2F- α -Man is not recovered after addition of Glc), the canonical ligand of GGBP. The implications of this result remain to be explored.

Although T_2 filtering has been merely applied herein from a qualitative perspective, the obtained data clearly pave the way to perform further quantitative affinity studies. In fact, such values could be in principle deduced for each isolated monosaccharide from competition experiments, using a competitor with a known affinity constant.⁶⁷

In summary, using this ^{19}F NMR-based T_2 -filtering strategy using a library of fluorinated monosaccharides generated through systematic OH-to-F substitutions allows (i) defining

sugar selectivity of the tested receptor, (ii) detecting its anomer preference, and (iii) identifying the key hydroxyls for binding, distinguishing them from those that can be chemically modified in the quest to find new binders. Extending this approach to other saccharides (aminosugars and sialosides) and to synthetic libraries of disaccharides will especially be attractive to screen a variety of carbohydrate–receptor families, on the way “from biology to drug target”.⁶⁸ In this sense, Siglecs, sialoside receptors proposed to act as “immune cell checkpoints in disease”,⁶⁹ or the multifunctional galectins,^{70,71} look like exciting targets to start with. The versatility of the described strategy is evident: it shows applicability to lectins and sensor/transport proteins and proved to be suitable to cover diverse selectivities and wide-ranging affinities, from sub-micromolar (40 nM for the Glc-GGBP complex) to over millimolar (1.15 mM for the Me β -Glc/PSA complex) dissociation constants. Thus, the method is robust and envisioned to find wide application.

EXPERIMENTAL SECTION

Materials. PSA was from a commercial source (Sigma-Aldrich-Merck) and dissolved in phosphate-buffered saline at pH 7.5. MGL ectodomain was recombinantly produced in *E. coli* and routinely checked for purity and activity as previously described, including ascertaining GalNAc-inhibitable histochemical staining.^{53,72} The samples for NMR were prepared in deuterated Tris buffer (10 mM), containing CaCl_2 (1 mM) and NaCl (75 mM) at pH 7.5 by means of five ultrafiltration–dilution buffer exchange steps with a 10 kDa cutoff membrane. GGBP was expressed in *E. coli* and purified as previously described,⁵⁶ and the samples were prepared in 20 mM Tris, containing 150 mM NaCl and 10 mM CaCl_2 at pH 7.0. Protein concentrations were measured by UV spectrometry.

The monofluorinated monosaccharide mixtures were prepared from concentrated stock solutions of each individual monosaccharide depending on their availability, either commercial or from synthesis. Final concentrations in the mixtures were centered around 0.5 mM or 0.9 mM, depending on the experiment, with variations in $\pm 35\%$ range. Given the intrinsic different equilibrium populations of the different anomers for a given monosaccharide, it is impossible to use the same concentration for each individual species.

Fluorinated Monosaccharides. 2-Deoxy-2-fluoro-glucose, 3-deoxy-3-fluoro-glucose, 4-deoxy-4-fluoro-glucose, 6-deoxy-6-fluoro-glucose, 2-deoxy-2-fluoro-galactose, 3-deoxy-3-fluoro-galactose, 4-deoxy-4-fluoro-galactose, 6-deoxy-6-fluoro-galactose, 2-deoxy-2-fluoro-mannose, 3-deoxy-3-fluoro-mannose, 4-deoxy-4-fluoro-mannose, 2-deoxy-2-fluoro-fucose, 2-fluoroethanol, Me α -N-acetylgalactosamine, and Me α -mannopyranoside were from commercial sources (Sigma-Aldrich Merck, Spain; Carbosynth, UK). 6-Deoxy-6-fluoro-mannose was synthesized as described in the Supporting Information, and its analytical data were consistent with literature values.⁷³ Characterizations of the intermediates in reaction steps in the synthesis are described below. NMR peak assignments were made using correlation spectroscopy (COSY) and heteronuclear single-quantum coherence (HSQC).

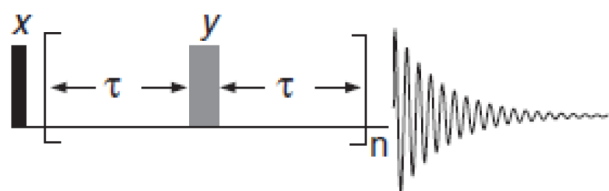
Methyl 2,3,4-Tri-O-benzoyl-6-deoxy-6-fluoro- α -D-mannopyranoside (2). Compound **1**⁷⁴ (50 mg, 0.098 mmol) was dissolved in dry CH_2Cl_2 (1.2 mL), then the solution was cooled to -78°C , and DAST (98 μL , 0.74 mmol) was slowly added dropwise. The reaction mixture was kept at -78°C for 30 min, then warmed to rt, and left stirring overnight. The solution was then cooled to -20°C , and the reaction was quenched with MeOH. The solvents were evaporated, and the residue was purified by silica gel column chromatography (toluene/EtOAc, 98:2 \rightarrow 8:2, v/v) to give **2** as a yellowish solid (37 mg, 0.07 mmol, 74%). $R_f = 0.8$, toluene/EtOAc 8:2; ^1H NMR (400 MHz, CDCl_3): δ 8.14–8.05 (m, 2H, H_{Bz}), 8.02–7.94 (m, 2H, H_{Bz}), 7.86–7.78 (m, 2H, H_{Bz}), 7.64–7.59 (m, 1H, H_{Bz}), 7.56–7.46 (m, 3H, H_{Bz}), 7.46–7.36 (m, 3H, H_{Bz}), 7.29–7.23 (m, 2H, H_{Bz}), 5.94–5.85 (m, 2H, H-3, H-4), 5.68 (dd, $J = 3.0, 1.8$ Hz, 1H, H-2), 5.02 (d, $J =$

1.8 Hz, 1H, H-1), 4.64 (dt, $J = 46.9, 3.7$ Hz, 2H, H-6ab), 4.34–4.21 (m, 1H, H-5), 3.55 (s, 3H, OCH₃). ¹⁹F NMR (376 MHz, CDCl₃): δ -231.70 (td, $J = 47.2, 22.9$ Hz). All analytical data were consistent with literature values.⁷⁴

1-O-Acetyl-2,3,4-tri-O-benzoyl-6-deoxy-6-fluoro- α -D-mannopyranoside (3). Compound 2 (180 mg, 0.35 mmol) was dissolved in Ac₂O/AcOH (2:1, 3.5 mL). H₂SO₄ (4 μ L, 0.07 mmol) was slowly added dropwise at 0 °C, and the mixture was stirred for 5 h. The reaction was then diluted with AcOEt and washed with sat. NaHCO₃(aqueous). The organic layer was dried over MgSO₄, filtered, and concentrated. The residue was purified by silica gel flash column chromatography (Toluene/EtOAc, 98:2 \rightarrow 8:2, v/v) to give 3 as a white powder (150 mg, 0.28 mmol, 80%). $R_f = 0.62$, Tol/AcOEt 9:1; $[\alpha]_D^{20} = -64.1$ ($c = 0.6$, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 8.13–8.06 (m, 2H, H_{Bz}), 8.02–7.94 (m, 2H, H_{Bz}), 7.86–7.77 (m, 2H, H_{Bz}), 7.66–7.60 (m, 1H, H_{Bz}), 7.56–7.52 (m, 1H, H_{Bz}), 7.50 (t, $J = 7.8$ Hz, 2H, H_{Bz}), 7.47–7.43 (m, 1H, H_{Bz}), 7.40 (t, $J = 7.8$ Hz, 2H, H_{Bz}), 7.28 (t, $J = 7.9$ Hz, 2H, H_{Bz}), 6.39 (d, $J = 2.0$ Hz, 1H, H-1), 5.99 (t, $J = 10.1$ Hz, 1H, H-4), 5.92 (dd, $J = 10.1, 3.3$ Hz, 1H, H-3), 5.72 (dd, $J = 3.4, 2.0$ Hz, 1H, H-2), 4.69–4.56 (m, 2H, H-6ab), 4.34 (ddt, $J = 23.3, 10.1, 3.2$ Hz, 1H, H-5), 2.28 (s, 3H, OCOCH₃). ¹³C{¹H} NMR (126 MHz, CDCl₃): δ 168.25 (O₂COCH₃), 165.70 (CO_{Bz}), 165.40 (CO_{Bz}), 165.35 (CO_{Bz}), 133.86 (C_{Bz}), 133.78 (C_{Bz}), 133.53 (C_{Bz}), 130.13 (2C_{Bz}), 129.94 (2C_{Bz}), 129.89 (2C_{Bz}), 129.02 (C_{Bz}), 128.89 (C_{Bz}), 128.85 (C_{Bz}), 128.81 (2C_{Bz}), 128.67 (2C_{Bz}), 128.51 (2C_{Bz}), 90.81 (C-1), 81.34 (d, $J = 176.4$ Hz, C-6), 71.92 (d, $J = 19.3$ Hz, C-5), 69.72 (C-3), 69.30 (C-2), 65.78 (d, $J = 7.0$ Hz, C-4), 21.11 (OCOCH₃). ¹⁹F NMR (376 MHz, CDCl₃): δ -232.65 (td, $J = 47.0, 23.3$ Hz). HRMS (ESI-TOF) m/z [M + Na]⁺ calcd for C₂₉H₂₅FO₉Na 559.1380; found 559.1396.

6-Deoxy-6-fluoro-D-mannose (4). Compound 3 (150 mg, 0.28 mmol) was dissolved in dry MeOH (2 mL), and then solid sodium methoxide was added until pH = 10–11. The reaction was stirred for 3 h, then quenched with Dowex 50WX8 H⁺ form, filtered, and concentrated. The residue was purified by silica gel flash column chromatography (CH₂Cl₂/MeOH, 98:5, v/v) to give 4 as a white solid (40 mg, 0.22 mmol, 78%, α : β 9:1). $R_f = 0.2$, CH₂Cl₂/MeOH 9:1; ¹H NMR (500 MHz, CD₃OD): δ 5.11 (d, $J = 1.7$ Hz, 1H, H-1 α), 4.73–4.54 (m, 2H, H-6a, H-6b), 3.91 (dddd, $J = 26.0, 10.0, 4.7, 2.0$ Hz, 1H, H-5), 3.83 (dd, $J = 3.4, 1.7$ Hz, 1H, H-2), 3.80 (dd, $J = 9.2, 3.4$ Hz, 1H, H-3), 3.69 (t, $J = 9.6$ Hz, 1H, H-4). ¹⁹F NMR (470 MHz, CD₃OD) δ -233.99 (td, $J = 47.9, 23.8$ Hz), -234.81 (td, $J = 47.8, 25.8$ Hz). All analytical data were consistent with literature values.⁷³

NMR Experiments. All NMR spectra were recorded on a 500 MHz Bruker spectrometer (470.56 MHz for fluorine) equipped with a ¹⁹F probe (¹⁹F, ¹H SEF from Bruker) at 298 K in D₂O unless otherwise is indicated. Standard pulse sequences 1D ¹H with and without decoupling ¹⁹F and 1D-¹⁹F with and without decoupling 1H included in Topspin acquisition software were used. For measuring transversal relaxation times, T_2 , the CPMG pulse sequence was used.^{75,76} It was as follows: [D-90_x-(τ -180_y- τ)_n-acquire], with a prescan delay of 4 s and a pre and post 180° pulse echo delay τ of 2 ms. The number n of echo loops varies from 2 to 2000, depending on the experiment. The 90_x and 180_y pulse durations were calculated for each sample. The total time used for the relaxation filter corresponds to n times the spin echo pulse was applied: $n(2\tau+180_y)$ (typically between 8 ms to 8 s).



¹⁹F was set as the observed nucleus, and proton decoupling was carried out during acquisition using the WALTZ-16 scheme.

Transverse Relaxation Time of F-Monosaccharides. To carry out the relaxation filtered experiments, individual stock solutions

around 50 mM in deuterated water of each fluorinated monosaccharide (glucose, galactose, mannose, and L-fucose) were prepared. These stock solutions were appropriately mixed and diluted to the final concentration used in each experiment. The concentrations were estimated by integrating the corresponding signals in the ¹⁹F-spectrum. 2-Fluoroethanol was added to the mixture as the internal reference. To prepare the samples of the monosaccharide library in the presence of proteins, 0.2 or 0.5 mL (for using 2 mm and 5 mm NMR tubes, respectively) aliquots of the mixture with 0.9 mM of each fluorinated monosaccharide were dried in a speed-vac, and the resulting powder reconstituted with the same volume of the corresponding buffer with and without protein. T_2 values were obtained from a series of CPMG experiments recorded with increasing number n (spin echo loops). Experiments with up to 16 different spin echo total relaxation times ranging from 8 to 8000 ms were determined.

Detection of Ligand Binding by T_2 -Filtered Experiments. A general protocol was followed using a protein-containing solution with a concentration between 10 μ M and 30 μ M. The mixtures of monofluorinated monosaccharides were prepared by mixing aliquots of each monosaccharide from highly concentrated. The final concentration of each monosaccharide in the mixture was around 0.9 mM ($[\alpha]+[\beta]$) ranging between 0.6 and 1.2 mM depending their availability. The ligand to protein ratio (L/P) was maintained between a 20- to 150-fold excess, optimized in each case to yield comparable T_2 decay responses between the three systems (PSA, GGBP, and MGL). CPMG experiments were carried out as previously described, but recording an initial reference experiment with 2 CPMG loops with $\tau = 2$ ms (8 ms total relaxation time) and one to five additional experiments with CPMG filters between 16 and 400 CPMG echo loops (64 to 1600 ms, respectively; the exact values of the spectra selected are indicated in each experiment).

In order to obtain the difference spectrum, the T_2 filtered spectra were multiplied by a factor f to correct the signal decay in the absence of protein. f is defined as the mean value of the ratio of ¹⁹F signal intensities after the first (I_1) and the last (I_n) CPMG experiments for all the fluorinated monosaccharides in the mixture: $f = I_1/I_n$. The first CPMG spectrum is acquired with $t_1 = 8$ ms, and the last one at $t = 160, 720, \text{ and } 400$ ms in each case, yielding a factor f of 1.1, 1.6, and 1.2 as shown in Figures 3, 4, and 6, respectively.

Detection of Ligand Binding by Competition Experiments. Competition (displacement) experiments were performed by adding an excess of a cognate sugar to the lectin/monofluorinated monosaccharide mixtures. In particular, Me α -N-acetylgalactosamine (12 μ M K_d) up to 1 mM for MGL,⁵³ Me α -mannopyranoside (530 μ M)⁵⁵ up to 18 mM for PSA, and glucose (0.04 μ M)⁶⁵ up to 1 mM for GGBP. Equivalent experiments, with the same CPMG relaxation filter parameters to those used for detection of ligand binding, were carried out to observe recovery in signals that had previously diminished as a consequence of binding. Each experiment was repeated upon sequential addition of the competing ligand.

The signal recovery ratio represented in Figure 8 for each fluorinated monosaccharide in the presence of the lectin after relaxation time t_i , $I_{ti}(+C/-C)$, with (+C) or without (-C) competitor was calculated from the ratio of relative signal decays in the presence (I_{ti}/I_{t1})_{+C} and in the absence (I_{ti}/I_{t1})_{-C} of competitor for $t_1 = 8$ ms and $t_i = 720, 720, \text{ and } 400$ ms for MGL, PSA, and GGBP, respectively.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.joc.0c01830>.

Includes the scheme of synthesis for 6F-Man and characterization spectra of intermediates and final product, the individual ¹⁹F spectra for each monofluorinated monosaccharide, and a table with their ¹⁹F transversal relaxation times. (PDF)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

NMR, nuclear magnetic resonance; CPMG, Carr–Purcell–Meiboom–Gill; STD, saturation transfer difference; 1D, one-dimensional; MGL, Macrophage Galactose-type Lectin; PSA, *Pisum sativum* agglutinin; GGBP, glucose/galactose-binding protein; CRD, carbohydrate recognition domain; CLEC, C-Type lectin; CD, cluster of differentiation

REFERENCES

- (1) Ginsburg, V.; Neufeld, E. F. Complex Heterosaccharides of Animals. *Annu. Rev. Biochem.* **1969**, *38*, 371.
- (2) Varki, A. Biological roles of glycans. *Glycobiology* **2017**, *27*, 3.
- (3) Gagneux, P.; Aebi, M.; Varki, A. In *Essentials of Glycobiology*, 3rd ed.; Varki A, C. R., Esko, J. D., et al., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor (NY), 2017; p 253.
- (4) Kaltner, H.; Abad-Rodríguez, J.; Corfield, A. P.; Kopitz, J.; Gabius, H.-J. The sugar code: letters and vocabulary, writers, editors and readers and biosignificance of functional glycan-lectin pairing. *Biochem. J.* **2019**, *476*, 2623.
- (5) Böhm, M.; Bohne-Lang, A.; Frank, M.; Loss, A.; Rojas-Macias, M. A.; Lütke, T. Glycosciences.DB: an annotated data collection linking glycomics and proteomics data (2018 update). *Nucleic Acids Res.* **2019**, *47*, D1195.
- (6) Winterburn, P. J.; Phelps, C. F. The Significance of Glycosylated Proteins. *Nature* **1972**, *236*, 147.
- (7) Solís, D.; Bovin, N. V.; Davis, A. P.; Jiménez-Barbero, J.; Romero, A.; Roy, R.; Smetana, K., Jr.; Gabius, H.-J. A guide into glycosciences: How chemistry, biochemistry and biology cooperate to crack the sugar code. *Biochim. Biophys. Acta, Gen. Subj.* **2015**, *1850*, 186.
- (8) Agre, P.; Bertozzi, C.; Bissell, M.; Campbell, K. P.; Cummings, R. D.; Desai, U. R.; Estes, M.; Flotte, T.; Fogleman, G.; Gage, F.; Ginsburg, D.; Gordon, J. I.; Hart, G.; Hascall, V.; Kiessling, L.; Kornfeld, S.; Lowe, J.; Magnani, J.; Mahal, L. K.; Medzhitov, R.; Roberts, R. J.; Sackstein, R.; Sarkar, R.; Schnaar, R.; Schwartz, N.; Varki, A.; Walt, D.; Weissman, I. Training the next generation of biomedical investigators in glycosciences. *J. Clin. Invest.* **2016**, *126*, 405.
- (9) Lemieux, R. U. Toward Enhanced symbiosis of Chemistry and Biology. *FASEB J.* **1994**, *8*, 273.
- (10) Bessell, E. M.; Foster, A. B.; Westwood, J. H. The use of deoxyfluoro-D-glucopyranoses and related compounds in a study of yeast hexokinase specificity. *Biochem. J.* **1972**, *128*, 199.
- (11) Glaudemans, C. P. J. Mapping of subsites of monoclonal anti-carbohydrate antibodies using deoxy and deoxyfluorosugars. *Chem. Rev.* **1991**, *91*, 25.
- (12) Fernández, P.; Jiménez-Barbero, J.; Martín-Lomas, M. Synthesis of all the possible monomethyl ethers and several deoxyhaloanalogs of methyl n-lactoside as ligands for the *Ricinus communis* lectins. *Carbohydr. Res.* **1994**, *254*, 61.
- (13) Hevey, R. Bioisosteres of carbohydrate functional groups in glycomimetic design. *Biomimetics* **2019**, *4*, 53.
- (14) Barnett, J. E. G.; Holman, G. D.; Munday, K. A. Structural requirements for binding to sugar-transport system of human erythrocyte. *Biochem. J.* **1973**, *131*, 211.
- (15) Vanwauwe, J. P.; Loontjens, F. G.; Debruyne, C. K. Carbohydrate binding specificity of lectin from pea (*Pisum sativum*). *Biochim. Biophys. Acta, Protein Struct.* **1975**, *379*, 456.
- (16) Bhattacharyya, L.; Brewer, C. F. Lectin-carbohydrate interactions. Studies of the nature of hydrogen-bonding between D-galactose and certain D-galactose-specific lectins, and between D-mannose and concanavalin-A. *Eur. J. Biochem.* **1988**, *176*, 207.
- (17) Withers, S. G.; Street, I. P.; Percival, M. D. Fluorinated carbohydrates as probes of enzyme specificity and mechanism. *ACS Symp. Ser.* **1988**, *374*, 59.

- (18) Nycholat, C. M.; Bundle, D. R. Synthesis of monodeoxy and mono-O-methyl congeners of methyl beta-D-mannopyranosyl-(1 → 2)-beta-D-mannopyranoside for epitope mapping of anti-*Candida albicans* antibodies. *Carbohydr. Res.* **2009**, *344*, 555.
- (19) McCarter, J. D.; Adam, M. J.; Withers, S. G. Binding energy and catalysis. Fluorinated and deoxygenated glycosides as mechanistic probes of *Escherichia-coli* (LacZ) β -galactosidase. *Biochem. J.* **1992**, *286*, 721.
- (20) Lemieux, R. U. The origin of the specificity in the recognition of oligosaccharides by proteins. *Chem. Soc. Rev.* **1989**, *18*, 347.
- (21) Linclau, B.; Ardá, A.; Reichardt, N.-C.; Sollogoub, M.; Unione, L.; Vincent, S. P.; Jiménez-Barbero, J. Fluorinated carbohydrates as chemical probes for molecular recognition studies. Current status and perspectives. *Chem. Soc. Rev.* **2020**, *49*, 3863.
- (22) Bégue, J.-P.; Bonnet-Delpon, D. I. *Bioorganic and medicinal chemistry of fluorine*; John Wiley & Sons: Hoboken, NJ, 2008.
- (23) Gillis, E. P.; Eastman, K. J.; Hill, M. D.; Donnelly, D. J.; Meanwell, N. A. Applications of Fluorine in Medicinal Chemistry. *J. Med. Chem.* **2015**, *58*, 8315.
- (24) Dalvit, C.; Invernizzi, C.; Vulpetti, A. Fluorine as a hydrogen-bond acceptor: experimental evidence and computational calculations. *Chem. Eur. J.* **2014**, *20*, 11058.
- (25) Dunitz, J. D. Organic fluorine: odd man out. *ChemBioChem* **2004**, *5*, 614.
- (26) Biffinger, J. C.; Kim, H. W.; DiMugno, S. G. The polar hydrophobicity of fluorinated compounds. *ChemBioChem* **2004**, *5*, 622.
- (27) Unione, L.; Xu, B.; Díaz, D.; Martín-Santamaría, S.; Poveda, A.; Sardinha, J.; Rauter, A. P.; Bleriot, Y.; Zhang, Y.; Cañada, F. J.; Sollogoub, M.; Jiménez-Barbero, J. Conformational Plasticity in Glycomimetics: Fluorocarbamethyl-L-idopyranosides Mimic the Intrinsic Dynamic Behaviour of Natural Idose Rings. *Chem. - Eur. J.* **2015**, *21*, 10513.
- (28) St-Gelais, J.; Côté, É.; Lainé, D.; Johnson, P. A.; Giguère, D. Addressing the Structural Complexity of Fluorinated Glucose Analogues: Insight into Lipophilicities and Solvation Effects. *Chem. - Eur. J.* **2020**, DOI: 10.1002/chem.202002825.
- (29) Linclau, B.; Wang, Z.; Compain, G.; Paumelle, V.; Fontenelle, C. Q.; Wells, N.; Weymouth-Wilson, A. Investigating the Influence of (Deoxy) fluorination on the Lipophilicity of Non-UV-Active Fluorinated Alkanols and Carbohydrates by a New log P Determination Method. *Angew. Chem., Int. Ed.* **2016**, *55*, 674.
- (30) Williams, S. J.; Withers, S. G. Glycosyl fluorides in enzymatic reactions. *Carbohydr. Res.* **2000**, *327*, 27.
- (31) Withers, S. G.; Street, I. P.; Bird, P.; Dolphin, D. H. 2-Deoxy-2-Fluoroglycosides. A Novel Class of Mechanism-Based Glucosidase Inhibitors. *J. Am. Chem. Soc.* **1987**, *109*, 7530.
- (32) Tysoe, C.; Withers, S. G. Fluorinated Mechanism-Based Inhibitors: Common Themes and Recent Developments. *Curr. Top. Med. Chem.* **2014**, *14*, 865.
- (33) Denavit, V.; Laine, D.; Bouzriba, C.; Shanina, E.; Gillon, E.; Fortin, S.; Rademacher, C.; Imbert, A.; Giguere, D. Stereoselective Synthesis of Fluorinated Galactopyranosides as Potential Molecular Probes for Galactophilic Proteins: Assessment of Monofluorogalactoside-LecA Interactions. *Chem. - Eur. J.* **2019**, *25*, 4478.
- (34) Diercks, T.; Infantino, A. S.; Unione, L.; Jiménez-Barbero, J.; Oscarson, S.; Gabius, H.-J. Fluorinated Carbohydrates as Lectin Ligands: Synthesis of OH/F-Substituted N-Glycan Core Trimannoside and Epitope Mapping by 2D STD-TOCSYreF NMR spectroscopy. *Chem. - Eur. J.* **2018**, *24*, 15761.
- (35) N'Go, L.; Golten, S.; Arda, A.; Cañada, F. J.; Jiménez-Barbero, J.; Linclau, B.; Vincent, S. P. Tetrafluorination of Sugars as Strategy for Enhancing Protein-Carbohydrate Affinity: Application to UDP-Galp Mutase Inhibition. *Chem. Eur. J.* **2014**, *20*, 106.
- (36) Cheng, Y.; Guo, A.-L.; Guo, D.-S. Recent Progress in Synthesis and Applications of Fluorinated Carbohydrates. *Curr. Org. Chem.* **2010**, *14*, 977.
- (37) Uhrig, M. L.; Lantano, B.; Postigo, A. Synthetic strategies for fluorination of carbohydrates. *Org. Biomol. Chem.* **2019**, *17*, 5173.
- (38) Card, P. J. Synthesis of fluorinated carbohydrates. *J. Carbohydr. Chem.* **1985**, *4*, 451.
- (39) Dax, K.; Albert, M.; Ortner, J.; Paul, B. J. Synthesis of deoxyfluoro sugars from carbohydrate precursors. *Carbohydr. Res.* **2000**, *327*, 47.
- (40) Denavit, V.; Laine, D.; St-Gelais, J.; Johnson, P. A.; Giguere, D. A Chiron approach towards the stereoselective synthesis of polyfluorinated carbohydrates. *Nat. Commun.* **2018**, *9*, 11.
- (41) Wang, J.; Sanchez-Rosello, M.; Acena, J. L.; del Pozo, C.; Sorochinsky, A. E.; Fustero, S.; Soloshonok, V. A.; Liu, H. Fluorine in pharmaceutical industry: fluorine-containing drugs introduced to the market in the last decade (2001–2011). *Chem. Rev.* **2014**, *114*, 2432.
- (42) Dalvit, C. Ligand- and substrate-based ^{19}F NMR screening: Principles and applications to drug discovery. *Prog. Nucl. Magn. Reson. Spectrosc.* **2007**, *51*, 243.
- (43) Dalvit, C.; Vulpetti, A. Ligand-Based Fluorine NMR Screening: Principles and Applications in Drug Discovery Projects. *J. Med. Chem.* **2019**, *62*, 2218.
- (44) Diercks, T.; Ribeiro, J. P.; Cañada, F. J.; André, S.; Jiménez-Barbero, J.; Gabius, H.-J. Fluorinated Carbohydrates as Lectin Ligands: Versatile Sensors in ^{19}F -Detected Saturation Transfer Difference NMR Spectroscopy. *Chem. Eur. J.* **2009**, *15*, 5666.
- (45) Matei, E.; Andre, S.; Glinschert, A.; Infantino, A. S.; Oscarson, S.; Gabius, H.-J.; Gronenborn, A. M. Fluorinated Carbohydrates as Lectin Ligands: Dissecting Glycan-Cyanovirin Interactions by Using F-19 NMR Spectroscopy. *Chem. Eur. J.* **2013**, *19*, 5364.
- (46) Shishmarev, D.; Fontenelle, C. Q.; Kuprov, I.; Linclau, B.; Kuchel, P. W. Transmembrane Exchange of Fluorosugars: Characterization of Red Cell GLUT1 Kinetics Using $(^{19}\text{F})\text{NMR}$. *Biophys. J.* **2018**, *115*, 1906.
- (47) Unione, L.; Alcalá, M.; Echeverría, B.; Serna, S.; Arda, A.; Franconetti, A.; Cañada, F. J.; Diercks, T.; Reichardt, N.; Jiménez-Barbero, J. Fluoroacetamide Moieties as NMR Spectroscopy Probes for the Molecular Recognition of GlcNAc-Containing Sugars: Modulation of the CH-p Stacking Interactions by Different Fluorination Patterns. *Chem. Eur. J.* **2017**, *23*, 3957.
- (48) Katiyar, S.; Singh, A.; Surolia, A. The interaction of N-trifluoroacetylgalactosamine and its derivatives with winged bean (*Psophocarpus tetragonolobus*) basic agglutinin reveals differential mechanism of their recognition: a fluorine-19 nuclear magnetic resonance study. *Glycoconjugate J.* **2014**, *31*, 537.
- (49) Krishna Sastry, M. V.; Swamy, M. J.; Surolia, A. Analysis of dynamics and mechanism of ligand binding to Artocarpus integrifolia agglutinin. A ^{13}C and ^{19}F NMR study. *J. Biol. Chem.* **1988**, *263*, 14826.
- (50) Martínez, J. D.; Valverde, P.; Delgado, S.; Romano, C.; Linclau, B.; Reichardt, N. C.; Oscarson, S.; Arda, A.; Jiménez-Barbero, J.; Cañada, F. J. Unraveling Sugar Binding Modes to DC-SIGN by Employing Fluorinated Carbohydrates. *Molecules* **2019**, *24*, 2337.
- (51) Wamhoff, E. C.; Hanske, J.; Schnirch, L.; Aretz, J.; Grube, M.; Silva, D. V.; Rademacher, C. F-19 NMR-Guided Design of Glycomimetic Langerin Ligands. *ACS Chem. Biol.* **2016**, *11*, 2407.
- (52) van Vliet, S. J.; Saeland, E.; van Kooyk, Y. Sweet preferences of MGL: carbohydrate specificity and function. *Trends Immunol.* **2008**, *29*, 83.
- (53) Marcelo, F.; García-Martín, F.; Matsushita, T.; Sardinha, J.; Coelho, H.; Oude-Vrielink, A.; Koller, C.; Andre, S.; Cabrita, E. J.; Gabius, H.-J.; Nishimura, S.-I.; Jiménez-Barbero, J.; Cañada, F. J. Delineating Binding Modes of Gal/GalNAc and Structural Elements of the Molecular Recognition of Tumor-Associated Mucin Glycopeptides by the Human Macrophage Galactose-Type Lectin. *Chem. - Eur. J.* **2014**, *20*, 16147.
- (54) Pirro, M.; Rombouts, Y.; Stella, A.; Neyrolles, O.; Bulet-Schiltz, O.; van Vliet, S. J.; de Ru, A. H.; Mohammed, Y.; Wuhler, M.; van Veelen, P. A.; Hensbergen, P. J. Characterization of Macrophage Galactose-type Lectin (MGL) ligands in colorectal cancer cell lines. *Biochim. Biophys. Acta, Gen. Subj.* **2020**, *1864*, 129513.
- (55) Schwarz, F. P.; Puri, K. D.; Bhat, R. G.; Surolia, A. Thermodynamics of monosaccharide binding to concanavalin A,

pea (*Pisum-sativum*) lectin, and lentil (*Lens-culinaris*) lectin. *J. Biol. Chem.* **1993**, *268*, 7668.

(56) Ortega, G.; Castaño, D.; Diercks, T.; Millet, O. Carbohydrate Affinity for the Glucose-Galactose Binding Protein Is Regulated by Allosteric Domain Motions. *J. Am. Chem. Soc.* **2012**, *134*, 19869.

(57) Borrok, M. J.; Kiessling, L. L.; Forest, K. T. Conformational changes of glucose/galactose-binding protein illuminated by open, unliganded, and ultra-high-resolution ligand-bound structures. *Protein Sci.* **2007**, *16*, 1032.

(58) Johan; Mowbray, S. L. Sugar Recognition by a Glucose/Galactose Receptor. Evaluation of Binding Energetics from Molecular Dynamics Simulations. *J. Biol. Chem.* **1995**, *270*, 9978.

(59) Jégouzo, S. A. F.; Quintero-Martínez, A.; Ouyang, X.; dos Santos, A.; Taylor, M. E.; Drickamer, K. Organization of the extracellular portion of the macrophage galactose receptor: a trimeric cluster of simple binding sites for N-acetylgalactosamine. *Glycobiology* **2013**, *23*, 853.

(60) Kerins, L.; Byrne, S.; Gabba, A.; Murphy, P. V. Anomer Preferences for Glucuronic and Galacturonic Acid and Derivatives and Influence of Electron-Withdrawing Substituents. *J. Org. Chem.* **2018**, *83*, 7714.

(61) Hajduk, P. J.; Olejniczak, E. T.; Fesik, S. W. One-dimensional relaxation- and diffusion-edited NMR methods for screening compounds that bind to macromolecules. *J. Am. Chem. Soc.* **1997**, *119*, 12257.

(62) Dalvit, C.; Piotta, M. F-19 NMR transverse and longitudinal relaxation filter experiments for screening: a theoretical and experimental analysis. *Magn. Reson. Chem.* **2017**, *55*, 106.

(63) Srinivas, V. R.; Reddy, G. B.; Ahmad, N.; Swaminathan, C. P.; Mitra, N.; Surolia, A. Legume lectin family, the 'natural mutants of the quaternary state', provide insights into the relationship between protein stability and oligomerization. *Biochim. Biophys. Acta, Gen. Subj.* **2001**, *1527*, 102.

(64) Schwarz, F. P.; Misquith, S.; Surolia, A. Effect of substituent on the thermodynamics of D-glucopyranoside binding to concanavalin A, pea (*Pisum sativum*) lectin and lentil (*Lens culinaris*) lectin. *Biochem. J.* **1996**, *316* (Pt 1), 123.

(65) Miller, D. M.; Olson, J. S.; Quijcho, F. A. The Mechanism of Sugar binding to the Periplasmic Receptor for Galactose Chemotaxis and Transport in *Escherichia coli*. *J. Biol. Chem.* **1980**, *255*, 2465.

(66) Vyas, M. N.; Vyas, N. K.; Quijcho, F. A. Crystallographic analysis of the epimeric and anomeric specificity of the periplasmic transport/chemosensory protein receptor for D-glucose and D-galactose. *Biochemistry* **1994**, *33*, 4762.

(67) Dalvit, C.; Parent, A.; Vallée, F.; Mathieu, M.; Rak, A. Fast NMR Methods for Measuring in the Direct and/or Competition Mode the Dissociation Constants of Chemical Fragments Interacting with a Receptor. *ChemMedChem* **2019**, *14*, 1115.

(68) O'Sullivan, J. A.; Chang, A. T.; Youngblood, B. A.; Bochner, B. S. Eosinophil and mast cell Siglecs: From biology to drug target. *J. Leukocyte Biol.* **2020**, *108*, 73.

(69) Duan, S.; Paulson, J. C. Siglecs as Immune Cell Checkpoints in Disease. *Annu. Rev. Immunol.* **2020**, *38*, 365.

(70) Garcia Caballero, G.; Kaltner, H.; Kutzner, T. J.; Ludwig, A.-K.; Manning, J. C.; Schmidt, S.; Sinowatz, F.; Gabius, H.-J. How galectins have become multifunctional proteins. *Histol. Histopathol.* **2020**, *35*, 509.

(71) Ludwig, A.-K.; Michalak, M.; Xiao, Q.; Gilles, U.; Medrano, F. J.; Ma, H.; FitzGerald, F. G.; Hasley, W. D.; Melendez-Davila, A.; Liu, M.; Rahimi, K.; Kostina, N. Y.; Rodriguez-Emmenegger, C.; Moeller, M.; Lindner, I.; Kaltner, H.; Cudic, M.; Reusch, D.; Kopitz, J.; Romero, A.; Oscarson, S.; Klein, M. L.; Gabius, H.-J.; Percec, V. Design-functionality relationships for adhesion/growth-regulatory galectins. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 2837.

(72) Kaltner, H.; Manning, J. C.; Caballero, G. G.; Di Salvo, C.; Gabba, A.; Romero-Hernandez, L. L.; Knospe, C.; Wu, D.; Daly, H. C.; O'Shea, D. F.; Gabius, H.-J.; Murphy, P. V. Revealing biomedically relevant cell and lectin type-dependent structure-activity profiles for

glycoclusters by using tissue sections as an assay platform. *RSC Adv.* **2018**, *8*, 28716.

(73) Khedri, Z.; Muthana, M. M.; Li, Y.; Muthana, S. M.; Yu, H.; Cao, H.; Chen, X. Probe sialidase substrate specificity using chemoenzymatically synthesized sialosides containing C9-modified sialic acid. *Chem. Commun.* **2012**, *48*, 3357.

(74) Esmurziev, A. M.; Simic, N.; Hoff, B. H.; Sundby, E. Synthesis and Structure Elucidation of Benzoylated Deoxyfluoropyranosides. *J. Carbohydr. Chem.* **2010**, *29*, 348.

(75) Carr, H. Y.; Purcell, E. M. Effects of Diffusion on Free Precession in Nuclear Magnetic Resonance Experiments. *Phys. Rev.* **1954**, *94*, 630.

(76) Meiboom, S.; Gill, D. Modified Spin-Echo Method for Measuring Nuclear Relaxation Times. *Rev. Sci. Instrum.* **1958**, *29*, 688.