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Stereoselective synthesis of fluorinated galactopyranosides as potential molecular probes on galactophilic proteins: assessment of monofluorogalactosides-LecA interactions

Vincent Denavit,^[a] Danny Lainé,^[a] Chahrazed Bouzriba,^[b, c] Elena Shanina,^[d] Émilie Gillon,^[e] Sébastien Fortin,^[b, c] Christoph Rademacher,^[d] Anne Imberty,^[e] and Denis Giguère*^[a]

Abstract: The replacement of hydroxyl groups by fluorine atoms on hexopyranoside scaffolds may allow access to invaluable tools to study various biochemical processes. As part of ongoing activities toward the preparation of fluorinated carbohydrates, a systematic investigation involving the synthesis and biological evaluations of a series of mono- and polyfluorinated galactopyranosides is described. The preparation of all the monofluorogalactopyranosides, one trifluorinated galactopyranoside, and the tetrafluorinated galactopyranoside was achieved using a Chiron approach. The synthetic challenge they present combined with the scarcity of some of these compounds prompted us to evaluate their biological profile. Firstly, our fluorinated compounds were investigated as antiproliferative agents using normal human and mouse cells and compared with cancerous cells. Most of the fluorinated compounds showed no antiproliferative activity. Secondly, we used these carbohydrate probes as potential inhibitors for galactophilic lectins. We performed the first TROSY NMR, chemical shift perturbations of the backbone resonances of LecA, a virulence factor from *Pseudomonas aeruginosa*. Moreover, taking advantage of the fluorine atom, we achieved the direct detection of the ¹⁹F NMR resonance of the monofluorogalactopyranosides in presence and absence of LecA to monitor ligand binding. Lastly, these results were corroborated with the binding potency of the monofluorinated galactopyranoside derivatives by isothermal titration calorimetry experiments. Analogs with fluorine atoms at C-3 and C-4 have weaker affinities with LecA as compared to compounds with fluorine atom at C-2 and C-6. This research focused on the chemical synthesis of “drug-like” low-

molecular weight inhibitors that circumvent drawbacks typically associated with natural oligosaccharides.

Introduction

The most widespread application of fluorinated carbohydrates is as radiopharmaceuticals for cancer imaging technique.^[1] Fluorinated carbohydrates are also invaluable tools as mechanistic probes to study lectin-carbohydrate interactions and to decipher the mechanisms of glycosidases.^[2] To that end, deoxyfluoro sugars provide useful insights into the role of hydrogen bonding interactions in order to identify the key active site of enzyme-substrate interactions.

Lectins, carbohydrate binding proteins, are found in all organisms from animals and plants, to bacteria and viruses.^[3] Lectins are involved in various biological processes, and their properties are in relation with their carbohydrate binding domain.^[4] Hence, it is crucial to elucidate and understand the sugar binding properties of lectins.^[5] Among them, galactose-specific lectins are of high interest as they bind for example to β -galactose present on branches of *N*-linked glycans, or to α -galactose epitopes of some human and non-human blood groups.^[6, 7] Galactose-specific lectins of therapeutic interest include for example LecA (PA-IL lectin from the pathogenic *Pseudomonas aeruginosa*), galectins, asialoglycoprotein receptor, and macrophage galactose-binding lectin. Intense investigations have been devoted to the understanding of these lectins ability to regulate numerous biological processes. Since thorough understanding of biochemical pathways is hampered by the natural complexity of carbohydrates, chemical or chemo-enzymatic synthesis of glycomimetics ligands is likely to remain a valuable option in glycoscience. Therefore, there is a major need to develop new tools that target galactoside-specific lectins in order to decipher their binding characteristics.

The carbohydrate binding capability of numerous lectins has been demonstrated by determining their ability to bind glycan arrays and by inhibiting their interaction with glycoconjugates or glycomimetics.^[8] This allows insight into the nature of the hydrogen bonding involved between the hydroxyl groups of carbohydrates and the binding sites of the proteins. The monosaccharides used in these studies generally include fluoro-, deoxy-, and thio-hexoses, along with uronic acids and alditols.^[9] The major drawbacks to use such diverse glycomimetic libraries are their poor synthetic availability. Consequently, the synthesis of

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fluoro-, deoxy-, and thio-glycosides is of high interest. In this context, we wish to replace the carbohydrate hydroxyl groups with fluorine atoms to generate new fluorine-substituted glycoside analogs.^[10] The rationale to synthesize such compounds emerges from the likenesses between hydroxyl group and fluorine atom in regard to polarity and isosteric relationships.^[11] Also, the loss of hydrogen donating capacity for the fluorine atom and the high C–F bond energy render them resistant to rapid *in vivo* degradation.^[12] Finally, addition of a fluorine group on a hydroxypyranose core can modulate the lipophilicity, which in turn can increase cell permeability.^[13] Definitely, fluorinated carbohydrates could represent more “drug-like” tools that circumvent drawbacks typically associated with natural oligosaccharides, such as low affinity, limited metabolic stability, and high polarity leading to low bioavailability.

As part of our ongoing program related to the synthesis of fluorinated carbohydrates,^[14] our attention was turned toward the preparation of monofluorogalactopyranosides **1–4**, along with the tetrafluorinated galactopyranoside congener **6** (Figure 1). On our way to prepare the polyfluoro derivative, we were also able to access trifluorinated galactopyranoside **5**. Heavily fluorinated hexopyranosides (replacement of hydroxyl groups by fluorine atoms) are particularly interesting because of the synthetic challenge they present. We developed convenient synthetic methodologies for further biological investigations. In the expectation of increasing the molecular diversity of our library, two aglycones were installed at the anomeric position, a β -*O*-benzoic acid and a β -*S*-(2-naphthyl). This choice is no coincidence since some galactoside-specific lectins are known to bind such structural motifs.^[15]

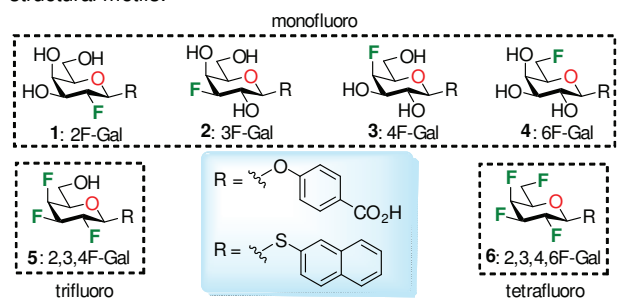


Figure 1. Mono- and polyfluorinated galactopyranosides prepared in this work.

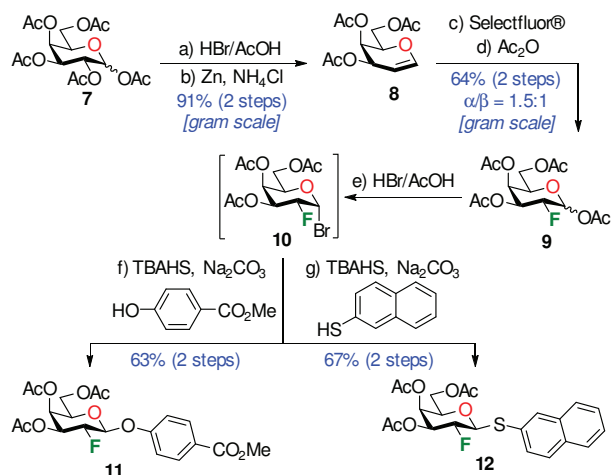
In the context of this study, it is important to point out that heavily fluorinated carbohydrates have been only used in kinetic studies,^[16] to improve protein-carbohydrate interactions,^[17] in the development of chemically modified analogs with improved antigenicity,^[18] and have been evaluated for their ability to cross erythrocyte membranes.^[19] The interesting properties of polyfluorinated carbohydrates can partly be explained by desolvation effect, together with attractive dipolar interactions mediated by polar C–F bonds.^[20] Herein, we present the first library and biological investigation of synthetic monofluorinated galactopyranosides. To the best of our knowledge, only one report described the hydrolysis of a series of fluorogalactopyranoside.^[21] However, the synthetic preparation of these compounds was not described. Here, we disclosed the first antiproliferative screening of mono- and polyfluorinated galactopyranosides on human

HaCat primary epidermal keratinocyte, human HDFn neonatal dermal fibroblast and mouse 3T3 embryonic fibroblast normal cells. These results were compared with human HT-29 colon adenocarcinoma and human M21 skin melanoma cancer cells. Also, our synthetic library could be useful as stable molecular probes with galactophilic lectins. This is exemplified by the first TROSY NMR monitoring chemical shift perturbation of LecA from the virulence factor *Pseudomonas aeruginosa*. Moreover, taking advantage of the properties of fluorine atom, the direct detection of the ¹⁹F NMR resonance of monofluorogalactopyranosides were performed on LecA to identify chemical shift changes of the ligands as well. Finally, these results were corroborated by the binding potency of the monofluorinated galactopyranoside derivatives by isothermal titration calorimetry.

Results and Discussion

Our synthetic endeavours started with the preparation of 2-fluorogalactopyranosides. Compound such as 2F-glycosides are important tools used as specific mechanism-based glycosidase inhibitors.^[16a, 22] The synthesis of 2-deoxy-2-fluorogalactopyranoside derivatives is summarized in **Scheme 1**. Thus, acetylated galactoside **7** was transformed in two steps into known 2,3,6-tri-*O*-*D*-galactal **8** in 91% yield. Treatment of compound **8** with Selectfluor® on large scale exclusively led to 2-deoxy-2-fluoro-*D*-galactopyranoside **9** after *O*-acetyl protection of the anomeric position ($\alpha/\beta = 1.5:1$).^[23] Stereoselective installation of the anomeric aglycone proceeded through the α -galactosyl bromide **10**. The crude bromide product underwent a phase transfer catalyzed nucleophilic displacement with methyl 4-hydroxybenzoate or 2-naphthalenethiol, leading to derivative **11** (63% yield over 2 steps) and **12** (55% yield over 2 steps) respectively. The β configurations were determined by direct NMR coupling of the anomeric proton with the H-2 proton, and the fluorine at C-2 (¹H NMR (500 MHz) δ 5.23 (dd, ³*J*_{H1-H2} = 7.4 Hz, ³*J*_{H1-F2} = 3.8 Hz) for **11** and 4.82 (dd, ³*J*_{H1-H2} = 9.6 Hz, ³*J*_{H1-F2} = 2.8 Hz, 1H, H1) for **12**). This methodology will be applied for the determination of the anomeric configuration of all the galactopyranoside derivatives prepared in this study.^[24]

Our next challenge involved the preparation of 3-deoxy-3-fluorogalactopyranoside derivatives, which was based on our previously described method and summarized in **Scheme 2**.^[14b] Briefly, Cerny’s epoxide **13**^[25] was treated with potassium fluoride in ethylene glycol at 200 °C for 5 hours and yielded the desired 3-deoxy-3-fluorogalactopyranoside **14** in 65% yield as the sole isomer. With the required 3-deoxy-3-fluoro derivative in hand, the next task involves inversion of configuration at C-4. Thus, benzylation of the free hydroxyl group was followed by deprotection of the 4-*O*-benzyl group using TiCl₄. Then, compound **15** was subjected to a Lattrell-Dax epimerization on gram scale through formation of triflate **16**.^[26] The crude mixture was treated with KNO₂ in DMF and generated the desired 1,6-anhydro- β -*D*-galactopyranoside derivative **17** in 72% yield over 2 steps. Acetolysis using a mixture of H₂SO₄ and Ac₂O allowed

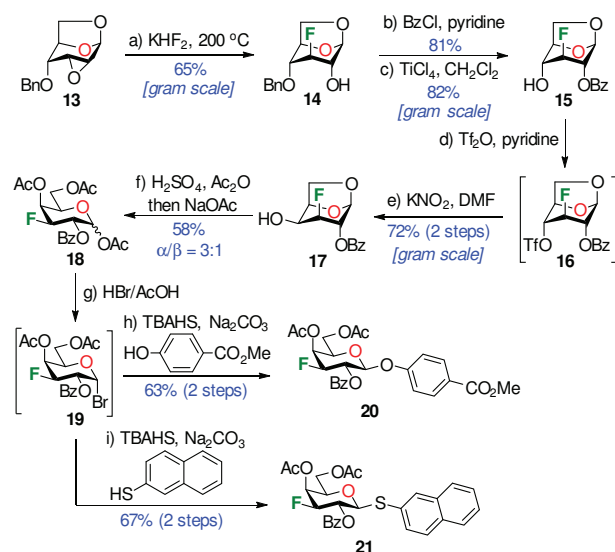


the generation of protected 3-deoxy-3-fluoro-D-galactopyranose **18** in 58% yield ($\alpha/\beta = 3:1$). The stereoselective functionalization of the anomeric position was easily achieved as described above. Thus, the mixture HBr/AcOH generated bromide **19**, which was subsequently subjected to the phase transfer catalyzed reactions. Methyl 4-hydroxybenzoate **20** and 2-naphthalenethiol **21** were isolated in 63% and 67% yields, respectively over 2 steps.

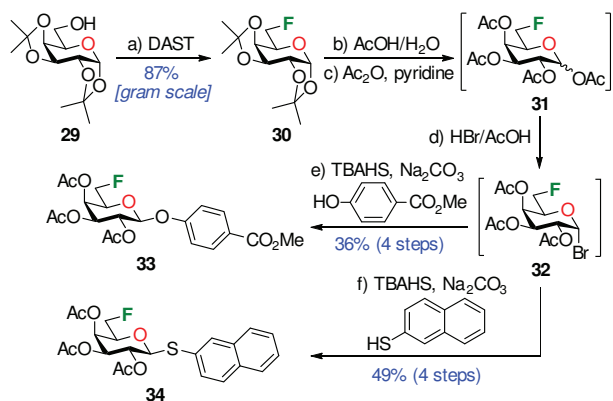
The syntheses of 4-deoxy-4-fluoro-galactopyranoside derivatives were initiated by the use of known 4-*O*-*p*-toluenesulfonyl **22** (Scheme 3).^[27] Protection of the residual hydroxyl group allowed the preparation of compound **23** in high yield and the latter was subjected to nucleophilic fluorination using TBAF in boiling THF. Compound **24** was isolated together with an unidentifiable impurity, consequently it was subjected to the next reaction. The mixture was directly subjected to acetolysis under acidic condition and the concomitant removal of the MOM protecting group proceeded as expected, generating intermediate **25** in 52% yield over 2 steps ($\alpha/\beta = 3:1$).^[16c] Finally, the anomeric groups were installed using the strategy mentioned above, allowing the isolation of products **27** and **28** via bromide intermediate **26**.

The easiest fluorinated analog to prepare was undoubtedly the 6-deoxy-6-fluoro-galactopyranose. The synthetic route was straightforward and initiated with 1,2,3,4-di-*O*-isopropylidene- α -D-galactopyranose **29** as inexpensive starting material (Scheme 4). Fluoro derivative **30** was isolated in 87% yield using Me-DAST with microwave irradiation (80 °C) for 1 hour. This method provided higher yield as compared to conventional heating.^[14b] Next, isopropylidene hydrolysis was followed by acetyl protection allowing the preparation of anomeric mixture of 6-deoxy-6-fluoro-galactopyranose **31**. The latter was subjected to acidic conditions followed by phase transfer catalyzed reactions. Product **33** was

isolated in 36% yield over 4 steps (78% per step) and product **34** was isolated in 49% yield over 4 steps (83% per step).

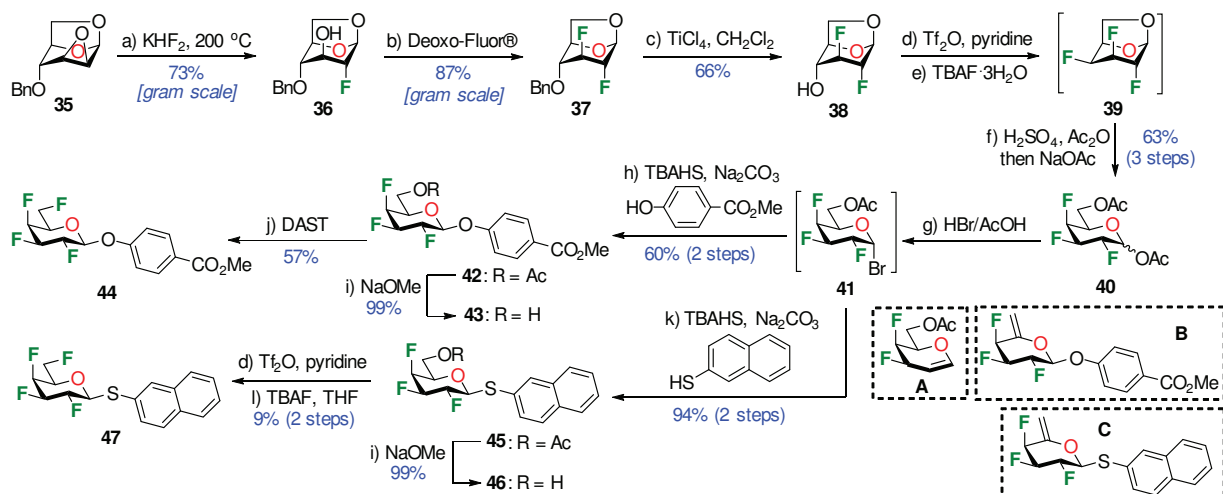


Scheme 3. Synthesis of 4-deoxy-4-fluoro-galactopyranoside **27** and **28**. a) MOMCl (10.0 equiv), DIPEA (11.0 equiv), CH₂Cl₂, 40 °C, 18 h, 97%; b) TBAF (10.0 equiv), THF, 66 °C, 72 h, 31%, based on 76% purity; c) H₂SO₄ (10.0 equiv), Ac₂O (30.0 equiv), rt, 18 h; then NaOAc (20.0 equiv), rt, 0.3 h, 52% over 2 steps, $\alpha/\beta = 3:1$; d) HBr/AcOH, CH₂Cl₂, rt, 1 h; e) methyl 4-hydroxybenzoate (3.0 equiv), TBAHS (1.0 equiv), AcOEt/1M Na₂CO₃ (1:1), rt, 18 h, 40% over 2 steps; f) 2-thionaphthyl (3.0 equiv), TBAHS (1.0 equiv), AcOEt/1M Na₂CO₃ (1:1), rt, 18 h, 69% over 2 steps. Ac₂O = acetic anhydride, DIPEA = *N,N*-diisopropylethylamine, MOMCl = chloromethyl methyl ether, NaOAc = sodium acetate, TBAF = tetrabutylammonium fluoride, TBAHS = tetrabutylammonium hydrogen sulfate.



Scheme 4. Synthesis of 6-deoxy-6-fluoro-galactopyranoside **33** and **34**. a) Me-DAST (1.2 equiv), 2,4,6-collidine (2.4 equiv), CH_2Cl_2 , 80 °C, microwave irradiation, 1 h, 87 %; b) AcOH/ H_2O (4:1), reflux, 18 h; c) Ac_2O , pyridine, rt, 72 h; d) HBr/AcOH, CH_2Cl_2 , rt, 2 h; e) methyl 4-hydroxybenzoate (3.0 equiv), TBAHS (1.0 equiv), AcOEt/1M Na_2CO_3 (1:1), rt, 18 h, 36% over 4 steps; f) 2-thionaphthyl (3.0 equiv), TBAHS (1.0 equiv), AcOEt/1M Na_2CO_3 (1:1), rt, 18 h, 49% over 4 steps. AcOH = acetic acid, Ac_2O = acetic anhydride, Me-DAST = dimethylaminosulfur trifluoride, TBAHS = tetrabutylammonium hydrogen sulfate.

In order to increase the molecular diversity of our library, our next task focused on the preparation of tetrafluorinated galactopyranosides.^[14a] Along the way, it became obvious that we could also access one trifluorinated galactopyranoside. Hence, **Scheme 5** described our synthetic endeavours toward this end and starts with easily accessible Cerny's epoxide **35** obtained in 4 steps from levoglucosan.^[28] Nucleophilic fluorination of the 2,3-anhydro derivative **35** was achieved upon exposure to potassium hydrogen fluoride in 73% yield. Treatment of **36** with Deoxo-Fluor® furnished 2,3-dideoxy-difluoro-glucose **37** in high yield with complete retention of configuration. This result can be



Scheme 5. Synthesis of trifluorinated **43** and **46** and tetrafluorinated **44** and **47** galactopyranoside derivatives. a) KHF_2 (7.0 equiv), ethylene glycol, 200 °C, 2.5 h, 73%; b) Deoxo-Fluor® (2.0 equiv), THF, microwave irradiation, 100 °C, 1.5 h, 87%; c) TiCl_4 (1.1 equiv), CH_2Cl_2 , 0 °C, 0.5 h, 66%; d) Tf_2O (2.0 equiv), pyridine (3.0 equiv), 0 °C, 0.2 h; e) TBAF· $3\text{H}_2\text{O}$ (1.5 equiv), CH_2Cl_2 , rt, 15 h; f) Ac_2O (30.0 equiv), H_2SO_4 (10.0 equiv), 0 °C to rt, 18 h, then NaOAc (20.0 equiv), rt, 0.3 h, 63% over 3 steps, $\alpha/\beta = 5:1$; g) HBr/AcOH, CH_2Cl_2 , rt, 66 h; h) methyl *p*-hydroxybenzoate (3.0 equiv), TBAHS (1.0 equiv), EtOAc/1M Na_2CO_3 (1:1), rt, 18 h; 60% over 2 steps; i) 1M NaOMe, MeOH, rt, 1 h, 99% for **43**, 99% for **46**; j) DAST (3.0 equiv), CH_2Cl_2 , microwave irradiation, 100 °C, 1 h, 57%; k) 2-thionaphthyl (3.0 equiv), TBAHS (1.0 equiv), EtOAc/1M Na_2CO_3 (1:1), rt, 18 h; 94% over 2 steps; l) Tf_2O (2.0 equiv), pyridine (10.0 equiv), 0 °C, 0.5 h; m) 1M TBAF in THF (15 equiv), -78 °C to rt, 15 h, 9% over 2 steps. Ac $_2$ O = acetic anhydride, DAST = diethylaminosulfur trifluoride, Deoxo-fluor® = bis(2-methoxyethyl)aminosulfur trifluoride, TBAF = tetrabutylammonium fluoride, TBAHS = tetrabutylammonium hydrogen sulfate, Tf_2O = trifluoromethanesulfonic anhydride.

explained by a trans-diaxially positioned benzyloxy group at C-4 capable of participation through an oxiranium intermediate species.^[29] A TiCl_4 -mediated benzyl deprotection allowed the generation of compound **38** in 66% yield, which was the ideal precursor to generate the 1,6-anhydro-2,3,4-trideoxy-trifluoro- β -D-galactopyranose **39**. Thus, the free O-4 hydroxyl group on compound **38** was activated as a triflate and subjected to a nucleophilic fluorination using TBAF allowing the generation of compound **39** with complete inversion of configuration. The isolation and purification of trifluoro **39** proved to be difficult due to its high volatility. Consequently, the crude mixture was treated under acidic conditions (H_2SO_4 , Ac_2O) generating the acetylsis product **40** in 63% yield over 3 steps.^[30] The configurations of the fluorine atoms were ascertained based on ^{19}F NMR spectroscopy (^{19}F NMR (470MHz): $^3J_{\text{F}2-\text{H}3} = 12.8$ Hz, $^3J_{\text{F}3-\text{F}4} = 13.4$ Hz, $^3J_{\text{F}3-\text{H}4} = 6.4$ Hz, $^3J_{\text{F}4-\text{H}5} = ^3J_{\text{F}4-\text{H}6} = 27.0$ Hz). The aglycones were installed using the same strategy as before, allowing the preparation of compounds **42** and **45** via bromide **41**. In both reactions, side product **A** was isolated from the mixture originating from elimination of the anomeric bromine (**Scheme 5**). The last task was the deoxofluorination at C-6 and represented a more challenging task than expected.^[14a] Upon extensive experimentation, a DAST-mediated deoxofluorination on **43** allowed the generation of polyfluorohexopyranose **44** in 57% yield [together with 32% of the *L-arabino*-hex-5-enopyranoside derivative **B**]. For compound **45**, due to the instability of the thionaphthyl moiety under the deoxofluorination conditions,^[31] a different approach was followed. After de-O-acetylation, the free hydroxyl group of **46** was activated as a triflate and a nucleophilic fluorination using TBAF gave a disappointing 9% yield. The major side product of this transformation was the elimination of the C-6

leaving group leading to derivative **C** in 83% yield. This example clearly shows the limitation of deoxyfluorination with aryl thiohexopyranoside analogs.

The complete deprotection of our target products was achieved according to two distinct protocols, depending on the substrates (Table 1). In the case of the methyl *p*-(*O*-galactosyl)benzoate analogs, lithium hydroxide was used for concomitant de-*O*-acetylation and generation of the acid moiety, allowing preparation of compounds **48**, **50**, **52**, **54**, **56**, and **57**. For thiogalactoside analogs, a classical Zemplén de-*O*-acetylation (NaOMe, MeOH) provided the desired analogs **49**, **51**, **53**, and **55**. Both methods furnished clean products in high yields.

In order to complement this study, we investigated key physical properties of some of our fluorinated carbohydrates. Firstly, in order to establish unambiguously the configuration of the fluorine atoms of the polyfluorinated galactopyranoside derivatives, an X-ray crystallographic analysis of **57** was achieved (Figure 2).^[32] We obtained a crystalline polymorph with different space group than what was previously reported.^[14a, 33] In the solid state, compound **57** is a dimer and the pyran ring adopts a ⁴C₁ conformation. Secondly, we compared the ¹⁹F NMR spectra of compound **48**, **50**, **52**, **54**, **56**, and **57** (Figure 3). The assessment of coupling constants in ¹⁹F and ¹H NMR represents a reliable tool for determination of the absolute configuration and conformation of fluorinated carbohydrates. Figure 3 shows that the multiplicities are very consistent, and depend on the relative spatial relationships between neighbouring atoms. All fluorinated carbohydrates adopts the ⁴C₁ conformation. For polyfluorinated analogs **56** and **57**, a comparison of fluorine signals with their monofluorinated counterparts reveals that the F-H coupling constants are similar with a slight change in chemical shift. Finally, the C-6 fluorine atom in compound **54** adopt either a TG or GT conformation. This was confirmed after analysis of the ¹H NMR spectrum (500 MHz). The proton at C-5 has a chemical shift of 4.02 ppm with, amongst others, a coupling constant ³J_{H5-F6} of 15.2 Hz, corresponding to a gauche conformation with F-6. A similar result is obtained for compound **57** (proton at H-5 = 4.52 ppm with ³J_{H5-F6} = 12.9 Hz). This result is in opposition with the conformation of the fluorine atom at C-6 of compound **57** in the solid state (Figure 2). The GG conformer, corresponding to the one from the crystal structure, adopts one of the highest energetic conformation.^[14a, 34] This demonstrates that fluorine NMR are an invaluable tool to analyse structural conformation of organofluorine compounds.

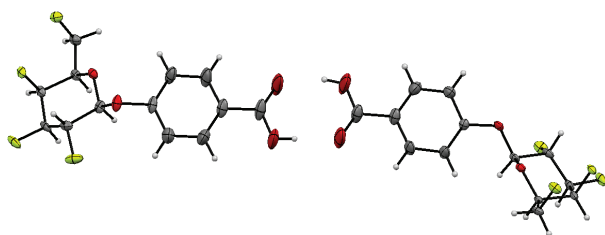


Figure 2. X-ray crystallographic analysis derived ORTEP of **57** showing 50% thermal ellipsoid probability, carbon (gray), oxygen (red), fluorine (light green), hydrogen (white).

Table 1. Deprotection of acetylated fluorogalactopyranosides generating analogs **48–57**.

Entry	Starting material	Method ^[a]	Product	Yield (%) ^[b]
	$\begin{array}{ccc} \begin{array}{c} \text{R} \quad \text{R} \\ \diagdown \quad / \\ \text{O} \\ / \quad \backslash \\ \text{R} \quad \text{X-Ar} \end{array} & \xrightarrow[\text{or}]{\begin{array}{c} \text{Method A:} \\ \text{LiOH} \end{array}} & \begin{array}{c} \text{R} \quad \text{R} \\ \diagdown \quad / \\ \text{O} \\ / \quad \backslash \\ \text{R} \quad \text{X-Ar} \end{array} \\ \text{R} = \text{OAc or F} & & \text{R} = \text{OH or F} \\ \text{X} = \text{O or S} & & \text{X} = \text{O or S} \end{array}$			
1	11	A		89
2	12	B		97
3	20	A		92
4	21	B		98
5	27	A		86
6	28	B		94
7	33	A		93
8	34	B		98
9	43	A		94
10	44	B		97

[a] Method A: 1M LiOH (10.0 equiv), H₂O/MeOH/THF (2:3:5); Method B: NaOMe in MeOH.

[b] Yields refer to isolated pure products.

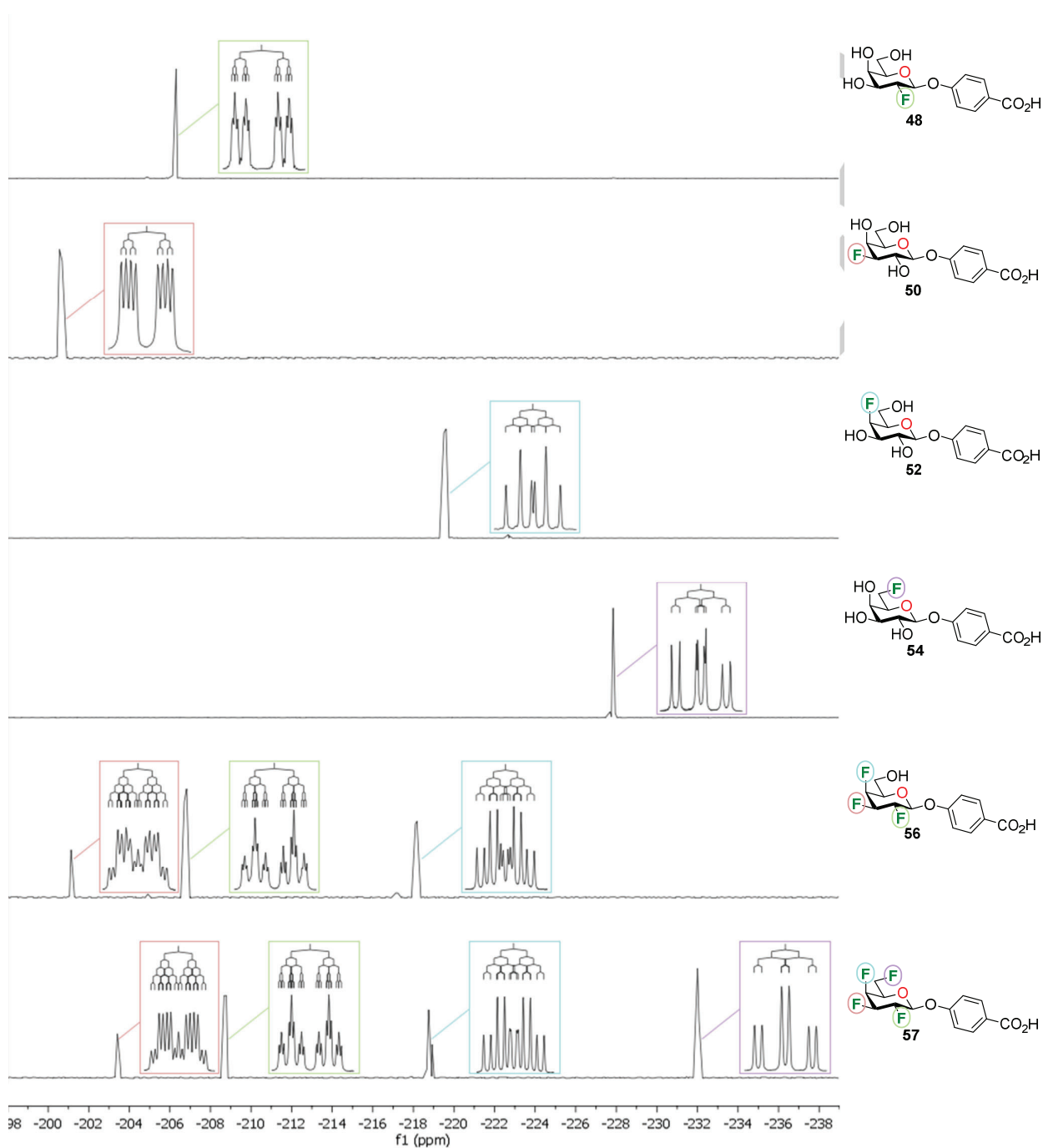


Figure 3. Direct comparison of ^{19}F resonances of compounds 48, 50, 52, 54, 56, and 57 (^{19}F NMR, 470 MHz). Expansions from the spectrum display coupling constant of each signals.

Antiproliferative activity

We wished to use our library in various biological systems. Consequently, we evaluated the antiproliferative profile of fluorinated galactopyranosides. Compounds 43, 46, 48–56 were

tested for their antiproliferative activity on human HaCaT primary epidermal keratinocyte, human HDFn neonatal dermal fibroblast and mouse 3T3 embryonic fibroblast normal cells as compared with human HT-29 colon adenocarcinoma and human M21 skin melanoma cancer cells (Table 2). Most of the fluorinated compounds (43 and 48–56) showed no antiproliferative activity

against normal or cancer cell lines and could therefore be used in various cells assays. In contrast, trifluorinated galactose derivative **46** with a thionaphthyl moiety presented some activity with no selectivity towards normal cell lines ($IC_{50} = 45\text{--}69\ \mu\text{M}$) and cancer cell lines ($IC_{50} = 34\text{--}38\ \mu\text{M}$). This is a weak antiproliferative agent when compared to Topotecan, a known chemotherapeutic active compound.

Table 2. Antiproliferative activity (IC_{50}) of molecular probe (**48–56**) derivatives on human HaCaT primary epidermal keratinocyte, human HDFn neonatal dermal fibroblast and mouse 3T3 embryonic fibroblast normal cells as compared with human HT-29 colon adenocarcinoma and human M21 skin melanoma cancer cells.

Compound	IC_{50} (μM) ^[a]				
	Normal cell lines			Cancer cell lines	
	HaCaT	HDFn	3T3	HT-29	M21
43	> 100	> 100	> 100	> 100	> 100
46	69	45	56	38	34
48–56	> 100	> 100	> 100	> 100	> 100
Topotecan	0.18	0.24	0.48	0.34	2.0

[a] IC_{50} is expressed as the concentration of drug inhibiting cell proliferation by 50% after 48 h of treatment.

Biophysical measurements

Pseudomonas aeruginosa is a Gram-negative bacterium and a leading pathogen for infections of immune-compromised patients and patients suffering from cystic fibrosis.^[35] LecA is a virulence factor crucial for biofilm formation by *P. aeruginosa* and has been demonstrated to be involved in adhesion to lung tissues.^[36] This lectin has a strong specificity for α -galactopyranose terminating oligosaccharides, however β -galactopyranoside possessing an aromatic aglycon were reported to be efficient binders.^[15b, 37] Moreover, 2-naphthyl-1-thio- β -D-galactopyranoside were reported to have high affinity with LecA (K_D of $6.3\ \mu\text{M}$), an affinity increase nicely supported by the available X-ray crystal structure with the lectin (PDB code 3ZYF).^[15b] In the context of this study, we proposed that our library of fluorinated galactosides could represent efficient synthetic glycomimetics of natural α -linked oligosaccharides with improved properties. To unveil such interactions, we first looked at the chemical shift perturbations in $^1\text{H},^{15}\text{N}$ -TROSY (transverse relaxation-optimized spectroscopy) spectra of the backbone amide resonances of ^{15}N -labelled LecA introduced by the interaction with our fluorinated probes. It is important to note, that we performed the first TROSY NMR experiments of LecA and this milestone could be useful for future related experiments. Complementary to these studies, we followed the perturbation of the isolated and sensitive ^{19}F resonances of the compounds. Finally, these NMR experiments were corroborated using isothermal titration calorimetry experiments.

The $^1\text{H},^{15}\text{N}$ -TROSY- NMR spectrum of ^{15}N -labelled LecA clearly demonstrated that the position of the fluorine atom on the pyranose ring strongly influence the binding of galactose to the protein. This is exemplified in **Figure 4a** for the spectrum of 2-deoxy-2-fluoro-galactopyranoside **48** in the presence (orange) and absence (blue) of ^{15}N -labelled LecA. Multiple perturbations of backbone chemical shifts and line broadening of these resonances were visible particularly related to amino acid constituting the carbohydrate recognition domain as inferred from experiments with unsubstituted galactose. In contrast, 4-deoxy-4-fluoro-galactopyranoside **52** induces significantly less perturbations in the protein backbone indicative for a reduced interaction (**Figure 4b**). Clearly, the fluorine atom at C-4 abrogates binding to LecA.

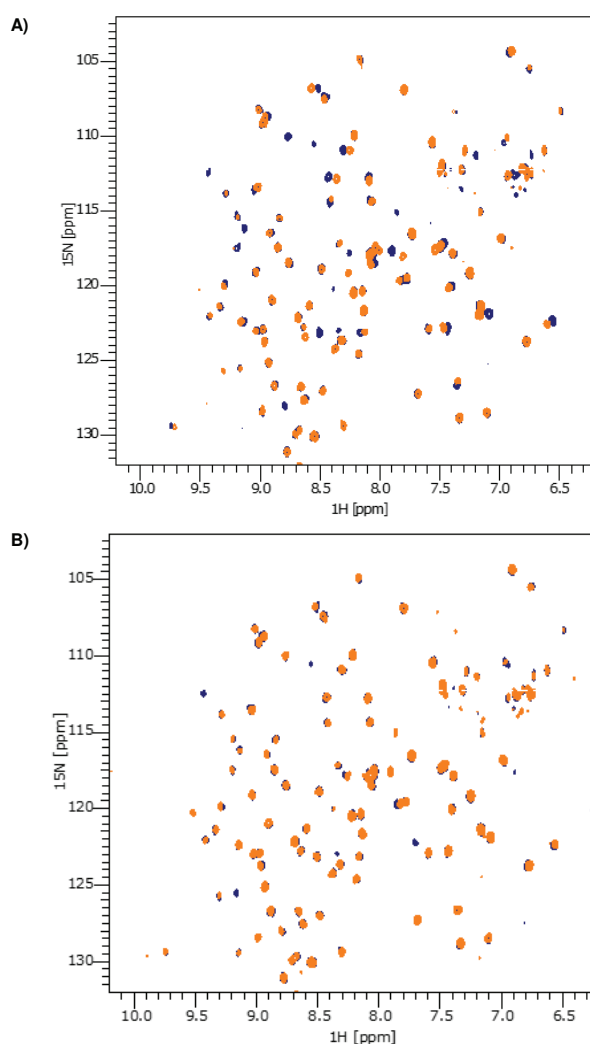


Figure 4. Chemical shift perturbations of backbone resonances of LecA upon binding to galactose derivatives **48** and **52** using $^1\text{H},^{15}\text{N}$ -TROSY NMR experiments. Spectra of $350\ \mu\text{M}$ ^{15}N -labelled LecA in the absence (blue) and presence (orange) of monofluorinated galactoside are shown for **A)** $0.2\ \text{mM}$ **48** and **B)** $1.0\ \text{mM}$ **52**. Fluorination in C2 (**48**) induces multiple chemical shift perturbation and line broadening while fluorine atom at C-4 (**52**) abrogates binding to LecA.

Following-up on these results, we thus performed the ^1H , ^{15}N -TROSY experiments over all the monofluorinated galactosides. The results are shown in **Figure 5** and summarize the perturbed backbone resonances upon addition of monofluorinated galactopyranosides **48–55**.^[38] Since no assignment backbone resonance is available, labeling of the peaks is arbitrary and we utilized these perturbations for fingerprinting the interaction patterns. Similar perturbation patterns would indicate similar binding patterns. First of all, analogs with fluorine atoms at C-3 (**50** and **51**) and C-4 (**52** and **53**) showed limited chemical shift perturbations or changes in peak intensities, thus suggesting no or weak affinities with LecA. Hydroxyl group at C-3 and C-4 of galactose are involved in a coordination to the calcium ion bound to LecA.^[39] Consequently, fluorine atoms at these positions abrogate efficient binding to LecA. Furthermore, as for compounds with fluorine atom at C-2 (**48** and **49**) and C-6 (**54** and **55**), strong change in peak intensity or chemical shift perturbation were noticed. In fact, the largest changes were observed for residues involved in the carbohydrate recognition domain as inferred from galactose binding (**Figure 5**, top).^[39] Overall, a consensus of perturbed resonances can be deduced from all compounds suggesting that a common binding site is shared (**Figure 5**, bottom).^[40]

Thus far, perturbations of the protein resonances were used to monitor the interaction. Since, the incorporation of a fluorine offers exquisite possibilities in NMR we chose to complement our studies and to take advantage of this and perform ^{19}F NMR of monofluorinated galactoside derivatives in presence and absence of LecA (**Figure 6**). Changes in peak intensity in the ^{19}F NMR spectra were followed as exemplified with 2-deoxy-2-fluoro-

galactopyranoside **48** (**Figure 6a**). Upon addition of LecA to **48**, there is a broadening of the peak associated with a reduction of the peak intensity by about 40% indicating binding to the lectin. All monofluorinated galactopyranosides **48–55** were analysed analogously (**Figure 6b**). Taken together, derivatives with fluorine atoms at C-2 (**48** and **49**) and C-6 (**54** and **55**) showed large changes in peak intensity (35–80%). Also, for these derivatives, compounds with the β -S-(2-naphthyl) aglycone (**49** and **55**) experienced largest changes in peak intensity of up to 80%. Finally, while no changes in peak intensity could be observed for compound **50**, significant changes in chemical shift were recorded, indicative of a faster exchange rate on the NMR chemical shift time scale.

To evaluate the affinity of the best compounds, we performed isothermal titration calorimetry experiments on our monofluorinated galactopyranoside analogues (**48–49**). A typical thermogram of LecA interacting with compound **49** is given in **Figure 7a**. First of all, no binding was observed for compounds with fluorine atom at position 3 and 4, which are involved in coordination of calcium ion. Fluorination at position 2 resulted in a slight decrease in affinity. Fluorination at position 6 has a stronger effect with decrease of one or two order of magnitude. This is in agreement with the role of each hydroxyl group in the complex between LecA and galactose (1OKO) (**Figure 7b**). Oxygens at position 3 and 4 are crucial for the interaction, being involved in direct coordination of the bridging calcium ion and in hydrogen bonds with amino acid side chains. Oxygen O-6 is involved in two direct hydrogen bonds with the protein, and oxygen O-2 in only one.

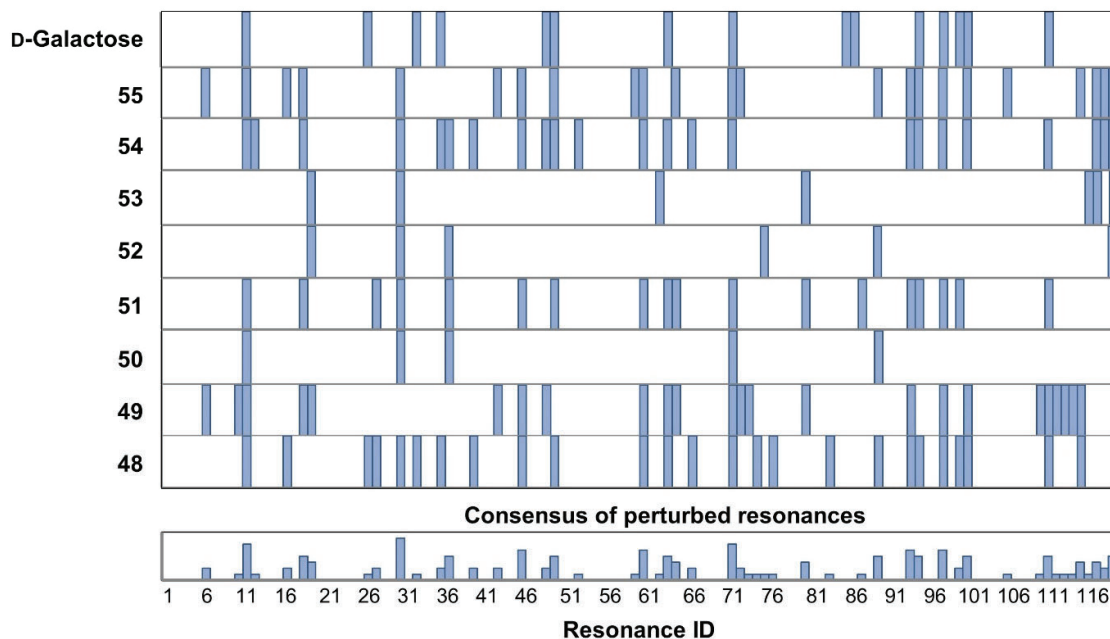


Figure 5. Summary of backbone resonances of LecA perturbed by the presence of monofluorinated galactosides (**48–55**) and galactose using ^1H , ^{15}N -TROSY NMR. Resonance IDs are arbitrary and cannot be associated with amino acid residues in absence of an assignment, but serve as fingerprints to visualize interaction patterns. If the change in peak intensity is more than 20% or if there is a chemical shift perturbation exceeding 0.025 ppm, the residue was attributed a blue color.

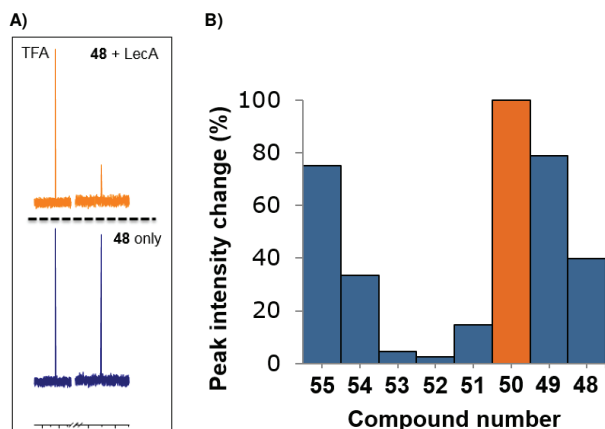


Figure 6. Direct observation of ¹⁹F resonances of monofluorinated galactosides upon binding to LecA. **A)** ¹⁹F NMR spectrum of compound **48** (blue) and in presence of LecA (orange): Reduction of the signal intensity of the galactoside indicates binding to LecA. Both spectra were normalized to reference trifluoroacetic acid (TFA: -75.6 ppm). **B)** Percent peak intensity changes of ¹⁹F monofluorinated galactosides **48–55** in presence of LecA (blue) compared to spectra recorded in absence of LecA. Compound **50** was arbitrarily assigned with 100% to indicate binding inferred from chemical shift perturbation (orange) in absence of intensity reduction.

It is also of interest to evaluate the effect of fluorination on the thermodynamics, by analysing the enthalpy and entropy contribution of the 2F-derivative compared to native compounds. Fluorination at position 2 decreases strongly the enthalpy contribution (loss of 20%) but the entropy barrier is also significantly decreased (**Table 3**). As a result, the decrease in affinity is limited, corresponding to a loss of about 10% in free energy. The decrease in binding enthalpy can be correlated to the loss of the hydrogen bond to Asn₁₀₇, and the gain in entropy contribution to a modification of the water network. Moreover, as previously observed, the β-S-(2-naphthyl) aglycone (**49** and **55**)

Table 3. *K_d* values and thermodynamics for the binding LecA to selected fluorinated galactopyranoside derivatives and reference compounds in ITC assays. Experiments were performed twice and standard deviations was lower than 10%.

Ligand	-Δ <i>H</i> ^o [KJ mol ⁻¹]	-TΔ <i>S</i> ^o [KJ mol ⁻¹]	-Δ <i>G</i> ^o [KJ mol ⁻¹]	<i>K_d</i> [μM]
α-Gal-O-Me	40.9	16.3	24.6	50.0
β-Gal-O-Me	19.0	5.3	24.3	55.7
β-Gal-O- <i>p</i> -benzoic acid	38.9	11.1	27.8	13.0
β-Gal-S-2-thionaphthyl	45.1	14.8	30.3	4.8
48	31.9	6.9	25.0	41
49	35.3	8.1	27.2	17
54 ^[a]	-	-	20.1	303
55 ^[a]	-	-	22.3	124.2

[a] Due to low affinity, a sigmoid curve could not be obtained for compounds **54** and **55**, which precluded the determination of reliable value for Δ*H*.

provided analogues with higher affinity as compared to their β-*O*-benzoic acid counterpart (**48** and **54**). Furthermore, it is important to point out that compound **49** constitute a privilege class of LecA inhibitors (3 times more potent than methyl β-D-galactopyranose). For one thing, the aromatic thioglycoside represent a stable functional group in biological medium by the glycosidically stable thio linkage. Also, not only the high C–F bond energy renders them resistant to *in vivo* degradation, fluorine group can increase lipophilicity, which in turn can increase cell permeability.^[41] Finally, in the context of this study, the limitation of our library are the low water solubility of polyfluorinated analogues (especially for compounds with the β-*S*-(2-naphthyl aglycone) and the low affinity between fluorine atoms with the calcium cation. One can assume that our library of fluorinated analogues would be more suitable as ligand for other galactophilic proteins that does not bear a cationic metal in the carbohydrate recognition domain.

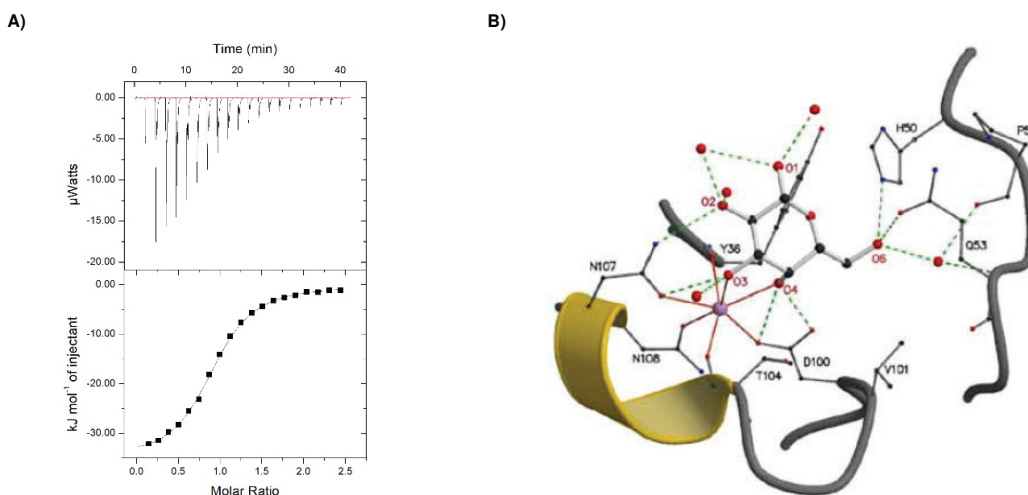


Figure 7. **A)** Thermogram of LecA interacting with compound **49**. The ITC plot (measure by VP-ITC Microcal) in the lower panel shows the total heat released as a function of total ligand concentration for the titration shown in the upper panel. The solid line denotes the best least square fit to experimental data using a one site model. **B)** *D*-Galactose in the carbohydrate recognition domain of LecA (1OKO).^[39]

The preparation and characterization of the first library of fluorinated galactopyranosides was achieved with two aglycones at the anomeric position: β -*O*-benzoic acid and β -*S*-(2-naphthyl). Most of the fluorinated compounds showed no antiproliferative activity against normal or cancer cell lines. Also, our library of stable glycomimetics can be used as molecular probes with galactophilic lectins. The first TROSY NMR following chemical shift perturbation of LecA and ^{19}F NMR in presence and absence of LecA suggested that analogs with fluorine atoms at C-3 and C-4 seems to have weak affinities with LecA. Furthermore, compounds with fluorine atom at C-2 and C-6 showed strong changes in peak intensity or chemical shift perturbations. This results was corroborated with isothermal titration calorimetry experiments. Compound **49** was a high affinity ligand for LecA with dissociation constants of 17 μM . The present investigation clearly shows the importance of systematic investigations in the search of stable and potent glycomimetics as lectin ligand. By blending organic synthesis and biological studies endeavors, we strongly believe that the resulting molecules could serve as useful tools to deepen investigations on the use of stable glycomimetics and to underscore their relevance and their yet underestimated potential to medicine.

Experimental Section

Chemical synthesis

All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF), toluene, benzene, diethyl ether (Et_2O), *N,N'*-dimethylformamide (DMF), and methylene chloride (CH_2Cl_2) were obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. Yields refer to chromatographically and spectroscopically (NMR) homogeneous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 200 μm SiliaPlate™ Aluminium backed TLC (indicator F-254) using UV light as visualizing agent and an ethanolic solution of phenol and sulphuric acid, and heat as developing agents. SiliaFlash® P60 (particle size 40 – 63 μm , 230 – 400 mesh) was used for flash column chromatography. NMR spectra were recorded on Agilent DD2 (at 500 MHz for ^1H , 470 MHz for ^{19}F and 126 MHz for ^{13}C) instruments and calibrated using residual undeuterated solvent (Chloroform-*d*: $\delta\text{H} = 7.26$ ppm, $\delta\text{C} = 77.16$ ppm; DMSO-*d*₆: $\delta\text{H} = 2.50$ ppm, $\delta\text{C} = 39.52$ ppm; Acetone-*d*₆: $\delta\text{H} = 2.05$ ppm, $\delta\text{C} = 29.84$ ppm; Methanol-*d*₄: $\delta\text{H} = 3.31$ ppm, $\delta\text{C} = 49.0$ ppm) as an internal reference. Calibration of ^{19}F NMR were done using hexafluorobenzene, which have been measured at -162.29 ppm compared to the chemical shift of the reference compound CFCl_3 . The following abbreviations were used to designate multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, h = sextet, m = multiplet, br = broad. Infrared (IR) spectra were recorded on a Thermo Nicolet 380 FT-IR spectrometer, with ZnSe crystal plate. High-resolution mass spectra (HRMS) were recorded on an Agilent serial 1200 TOF (time of flight) 6210 mass spectrometer using ESI (electrospray ionization). Melting points are uncorrected and were recorded on a Stanford Research Systems OptiMelt MPA100 automated melting point system. Optical rotations were recorded

on a JASCO DIP-360 digital polarimeter at 589 nm, and are reported in units of 10^{-1} (deg $\text{cm}^2 \text{g}^{-1}$).

Cell lines culture

HaCaT primary epidermal keratinocyte and human HDFn neonatal dermal fibroblast human cells were purchased from Thermo Fisher Scientific, while mouse 3T3 embryonic fibroblast and human HT-29 colon adenocarcinoma cells were purchased from the American Type Culture Collection (Manassas, VA). M21 human skin melanoma cells were kindly provided by Dr. David Cheresch (University of California, San Diego School of Medicine). HaCaT and 3T3 cells were cultured in high-glucose Dulbecco's minimal essential medium (DMEM, Gibco, Thermo Fisher Scientific) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific) and 1% antibiotic penicillin-streptomycin (5,000 U/mL). HDFn cells were cultured in DMEM supplemented with 2% (v/v) FBS, 1% penicillin-streptomycin, fibroblast growth factor (3 ng/mL), epidermal growth factor (10 ng/mL), hydrocortisone (1 ng/mL) and heparin (10 ng/mL). HT-29 and M21 cells were cultured in DMEM supplemented with 5% of FBS. Cells were maintained at 37 °C in a moisture-saturated atmosphere containing 5% CO_2 .

Antiproliferative activity assay

The growth inhibition potency of all compounds was assessed using the procedure recommended by the National Cancer Institute (NCI) Developmental Therapeutics Program for its drug screening program with slight modifications.^[42] Briefly, 96-well Costar microtiter clear plates were seeded with 75 μL of a suspension of either HaCaT (4.5×10^3), 3T3 (3×10^3), HDFn (3×10^3), HT-29 (5×10^3), or M21 (3×10^3) cells per well in the appropriate medium. Plates were incubated for 24 h. Freshly solubilised drugs in DMSO (40 mM) were diluted in fresh medium and 75 μL aliquots containing serially diluted concentrations of the drug were added. Final drug concentrations ranged from 100 μM to 78 nM. DMSO concentration was kept constant at < 0.5% (v/v) to prevent any related toxicity. Plates were incubated for 48 h, after which growth was stopped by the addition of cold trichloroacetic acid to the wells (10% w/v, final concentration). Afterward, plates were incubated at 4 °C for 1 h. Then, plates were washed 5-times with distilled water and a sulforhodamine B solution (0.1% w/v) in 1% acetic acid was added to each well. After 15 min at room temperature, the exceeding dye was removed and was washed 5-times with a solution of 1% acetic acid. Bound dye was solubilized in 20 mM Tris base and the absorbance was read using an optimal wavelength (530-580 nm) with a SpectraMax® i3x (Molecular Devices). Data obtained from treated cells were compared to the control cell plates fixed on the treatment day and the percentage of cell growth was thus calculated for each drug. The experiments were done at least twice in triplicate. The assays were considered valid when the coefficient of variation was < 10% for a given set of conditions within the same experiment.

^1H , ^{15}N TROSY NMR

Fluorinated galactopyranosides binding to LecA were validated using protein-observed ^1H , ^{15}N TROSY NMR. All ^1H , ^{15}N TROSY measurements were performed at 310 K in Norell S-3-800-7 3mm tubes (Norell) on a Bruker Ascend 700 MHz spectrometer (Bruker, Billerica, MA, USA) equipped with a 5mm TCI700 CryoProbe™.

Briefly, ^1H , ^{15}N TROSY spectra of 350 μM (U: ^{15}N) LecA were recorded in 20 mM HEPES, 150 mM NaCl, pH 7.4 buffer containing 10 mM CaCl_2 , 100 μM DSS as internal reference and 10% D_2O . 0.2-1.5 mM of fluorinated D-galactose compound dissolved in DMSO-*d*₆ was added and the respective spectrum was compared to a spectrum with the same amount (v/v) of DMSO-*d*₆ to factor out changes caused by the solvent. A ^1H , ^{15}N TROSY pulse sequence with WATERGATE solvent suppression, 128 increments, and 16 scans per increment was applied. Data were processed in NMRpipe^[43] and further analysed in CCPN.^[44]

¹H,¹⁵N TROSY resonances were indexed with IDs from 1 to 118 due to lack of protein backbone resonance assignment. Based on this reference spectrum, resonance IDs were transferred to the spectrum with compound in order to compare changes in presence of a compound. In case of ambiguities caused by strongly overlapping or disappearing peaks among reference spectra, resonance IDs were not transferred. Chemical shift perturbation (CSP) were calculated according to:

$$\Delta\delta = \sqrt{\Delta\delta_H^2 + (\alpha\Delta\delta_N)^2}$$

with $\Delta\delta$, as the difference in chemical shift (in ppm) and α an empirical weighing factor of 0.14 for all amino acid backbone resonances.^[45] The threshold value was set to 0.015 ppm based on four independent measurements of reference spectra. In addition to CSPs, peaks that reduced at least 20% in normalized signal intensity compared to reference spectrum were used as indicators for carbohydrate binding.

¹⁹F NMR measurements

The measurement using ligand-observed ¹⁹F NMR was performed to validate binding of fluorinated galactopyranosides to LecA. Briefly, a spectrum of 50 μ M compound alone and with 100 μ M LecA in TBS buffer (25 mM Tris, 150 mM NaCl, pH 7.8, 10 mM CaCl₂, 100 μ M TFA, 10% D₂O) was recorded at 310 K in Norell S-3-800-7 3mm tubes (Norell) on a Bruker Ascend 700 MHz spectrometer (Bruker, Billerica, MA, USA) equipped with a 5mm TCI700 CryoProbe™. All spectra were normalized to internal reference TFA at -75.6 ppm and analysed for changes in peak intensity or chemical shift. Compounds were defined to bind LecA in ¹⁹F NMR experiment when changes in peak intensity above 20% or a chemical shift of 0.025 ppm occurred in presence of LecA.

Isothermal titration calorimetry

Recombinant lyophilized LecA was dissolved in buffer (20 mM TRIS-HCl, 100 μ M CaCl₂, 100 mM NaCl, pH 7.5) and degassed. Protein concentration (50 to 300 μ M) was checked by measurement of optical density by using a theoretical molar extinction coefficient of 28000. Galactose derivatives were dissolved directly into the same buffer, degassed, and placed in the injection syringe (concentrations varying from 1.3 to 1.5 mM). ITC was performed using a ITC-200 microcalorimeter (MicroCal Inc). LecA was placed into the 200 μ M sample cell, at 25 °C. Titration was performed with 2 μ L injections of carbohydrate ligands every 120 s. Data were fitted using the "one-site model" using MicroCal Origin 7 software according to standard procedures. Fitted data yielded the stoichiometry (n), the association constant (K_a), and the enthalpy of binding (Δ H). Other thermodynamic parameters (i.e., changes in free energy Δ G and entropy Δ S) were calculated from the equation Δ G = Δ H - T Δ S = -RTlnK_a in which T is the absolute temperature and R = 8.314 J.mol⁻¹.K⁻¹. Two or three independent titrations were performed for each ligand tested.

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Keywords: organic synthesis • fluorinated glycoside • isothermal titration calorimetry • LecA • TROSY NMR

- [1] a) S. M. Ametamey, M. Honer, P. A. Schubiger, *Chem. Rev.* **2008**, *108*, 1501–1516; b) S. Preshlock, M. Tredwell, G. Gouverneur *Chem. Rev.* **2016**, *116*, 719–766; c) H. H. Coenen, P. H. Elsinga, R. Iwata, M. R. Kilbourn, M. R. A. Pillai, M. G. R. Rajan, H. N. Wagner, J. J. Zaknum, *Nucl. Med. Biol.* **2010**, *37*, 727–740; d) D. A. Mankoff, F. Dehdashti, A. F. Shields, *Neoplasia* **2000**, *2*, 71–88.
- [2] a) M. Namchuk, C. Braun, J. D. McCarter, S. G. Withers, Fluorinated sugars as probes of glycosidase mechanism. In *ACS Symposium Series*, Ed.: Ojima, I.; McCarthy, J.; Welch, J. T. **1996**, *639*, 279–293; b) S. J. Williams, S. G. Withers, *Carbohydr. Res.* **2000**, *327*, 27–46; c) S. A. Allam, H. H. Jensen, B. Vijayakrishnan, J. A. Garnett, E. Leon, Y. Liu, D. C. Anthony, N. R. Sibson, T. Feizi, S. Matthews, B. G. Davis, *ChemBioChem* **2009**, *10*, 2522–2529.
- [3] H. Lis, N. Sharon, *Chem. Rev.* **1998**, *98*, 637–674.
- [4] A. Varki, R. Cummings, J. Esko, H. Freeze, G. Hart, J. Marth, *Essentials of Glycobiology*, Eds.; Cold Spring Harbor Laboratory Press, **1999**.
- [5] T. K. Dam, F. C. Brewer, *Chem. Rev.* **2002**, *102*, 387–429.
- [6] Subcellular Biochemistry, Volume 32: α -Gal and anti-Gal: α -1,3-galactosyltransferase, α -Gal epitopes, and the natural anti-Gal antibody, Eds. U. Galili, J. L. Avila. Kluwer Academic, New York. **1999**, 394pp.
- [7] a) R. Lotan, β -Galactoside-binding vertebrate lectins: synthesis, molecular biology, function. In *Glycoconjugates* Eds.: H. J. Allen, E. C. Kisailus. **1992**, 635–671; b) J. T. Powell, F. L. Harrison, *Am. J. Physiol.* **1991**, *261*, L236–L239; c) K. Kasai, *Adv. Lectin Res.* **1990**, *3*, 10–35; d) M. Caron, D. Bladier, R. Joubert, *Int. J. Biochem.* **1990**, *22*, 1379–1385; e) A. M. Wu, S. Sugii, *Adv. Exp. Med. Biol.* **1988**, *228*, 505–263.
- [8] a) A. Imberty, Y. M. Chabre, R. Roy, *Chem. Eur. J.* **2008**, *14*, 7490–7499; b) L. Bhattacharyya, F. C. Brewer, *Eur. J. Biochem.* **1988**, *176*, 207–212.
- [9] O. Hindsgaul, T. Norberg, J. Le Pendu, R. U. Lemieux, *Carbohydr. Res.* **1982**, *109*, 109–142.
- [10] T. Diercks, A. S. Infantino, L. Unione, J. Jiménez-Barbero, S. Oscarson, H.-J. Gubius, *Chem. Eur. J.* **2018**, *24*, 15761–15765.
- [11] M. Hoffmann, J. Rychlewski, *Int. J. Quant. Chem.* **2002**, *89*, 419–427.
- [12] M. Reindl, A. Hoffmann-Röder, *Curr. Top. Chem.* **2014**, *14*, 840–854.
- [13] a) H. J. Bohm, D. Banner, S. Bendels, M. Kansy, B. Kuhn, K. Muller, U. Obst-Sander, M. Stahl, *ChemBioChem*, **2004**, *5*, 637–643; b) S. Purser, P. R. Moore, S. Swallow, V. Gouverneur, *Chem. Soc. Rev.* **2008**, *37*, 320–330; c) E. P. Gillis, K. J. Eastman, M. D. Hill, D. J. Donnelly, N. A. Meanwell, *J. Med. Chem.* **2015**, *58*, 8315–8359.
- [14] a) V. Denavit, D. Lainé, J. St-Gelais, P. A. Johnson, D. Giguère, *Nat. Commun.* **2018**, *9*, 4721; b) D. Lainé, V. Denavit, D. Giguère, *J. Org. Chem.* **2017**, *82*, 4986–4992; c) V. Denavit, D. Lainé, G. Le Heiget, D. Giguère, Fluorine-containing carbohydrates: synthesis of 6-deoxy-6-fluoro-1,2,3,4-di-O-isopropylidene- α -D-galactopyranose. In *Carbohydrate chemistry: Proven synthetic methods, volume 4*, Eds.: C. Vogel, P. V. Murphy, Ed., WILEY-VCH, **2017**, Chapter 30, pp. 241–246.
- [15] a) D. Giguère, S. Sato, C. St-Pierre, S. Sirois, R. Roy, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1668–1672; b) J. Rodrigue, G. Ganne, B. Blanchard, C. Saucier, D. Giguère, T. C. Shiao, A. Varrot, A. Imberty, R. Roy, *Org. Biomol. Chem.* **2013**, *11*, 6906–6918; c) J. C. Diaz Arribas, A. G. Herrero, M. Martin-Lomas, F. J. Canada, S. He, S. G. Withers, *Eur. J. Biochem.* **2000**, *267*, 6996–7005.
- [16] a) S. G. Withers, M. D. Percival, I. P. Street, *Carbohydr. Res.* **1989**, *187*, 43–66; b) B. P. Rempel, S. G. Withers, *Aust. J. Chem.* **2009**, *62*, 590–599; c) J.-S. Zhu, N. E. McCormick, S. C. Timmons, D. L. Jakeman, *J. Org. Chem.* **2016**, *81*, 8816–8825.

- [17] a) I. P. Street, C. R. Armstrong, S. G. Withers, *Biochemistry*, **1986**, *25*, 6021–6027; b) H. W. Kim, P. Rossi, R. K. Shoemaker, S. G. DiMugno, *J. Am. Chem. Soc.* **1998**, *120*, 9082–9083; c) I. N'Go, S. Goltén, A. Arda, J. Canada, J. Jimenez-Barbero, B. Linclau, S. P. Vincent, *Chem. Eur. J.* **2014**, *20*, 106–112; d) K. E. van Straaten, J. R. A. Kuttiyatveetil, C. M. Sevrain, S. A. Villaume, J. Jimenez-Barbero, B. Linclau, S. P. Vincent, D. A. R. Sanders, *J. Am. Chem. Soc.* **2015**, *137*, 1230–1244.
- [18] a) T. Oberbillig, C. Mersch, S. Wagner, A. Hoffmann-Röder, *Chem. Commun.* **2012**, *48*, 1487–1489; b) A. Baumann, S. Marchner, M. Daum, A. Hoffmann-Röder, *Eur. J. Org. Chem.* **2018**, 3803–3815.
- [19] a) S. Bresciani, T. Lebl, A. M. Z. Slawin, D. O'Hagan, *Chem. Commun.* **2010**, *46*, 5434–5436; b) J. C. Biffinger, H. W. Kim, S. G. DiMugno, *ChemBioChem* **2004**, *5*, 622–627.
- [20] a) J. A. Olsen, D. W. Banner, P. Seiler, U. Obst-Sander, A. D'Arcy, M. Stihle, K. Müller, F. Diederich, *Angew. Chem. Int. Ed.* **2003**, *42*, 2507–2511; b) R. Paulini, K. Müller, F. Diederich, *Angew. Chem. Int. Ed.* **2005**, *44*, 1788–1805; c) K. Müller, C. Faeh, F. Diederich, *Science* **2007**, *317*, 1881–1886.
- [21] J. D. McCarter, M. J. Adam, S. G. Withers, *Biochem. J.* **1992**, *286*, 721–727.
- [22] a) A. G. Watts, P. Oppezzo, S. G. Withers, P. M. Alzari, A. Buschiazzo, *J. Biol. Chem.* **2006**, *281*, 4149–4155; b) M. N. Namchuk, J. D. McCarter, A. Becalski, T. Andrews, S. G. Withers, *J. Am. Chem. Soc.* **2000**, *122*, 1270–1277; c) S. G. Withers, K. Rupitz, I. P. Street, *J. Biol. Chem.* **1988**, *263*, 7929–7932; d) S. G. Withers, I. P. Street, D. H. Dolphin, *J. Am. Chem. Soc.* **1987**, *109*, 7530–7531.
- [23] a) M. Carmelita, K. Ayaka, K. Hatanaka, *J. Fluorine Chem.* **2007**, *128*, 562–565; b) J. Ortner, M. Albert, H. Weber, K. Dax, *J. Carbohydr. Chem.* **1999**, *18*, 297–316.
- [24] See supporting information for more details.
- [25] a) M. Cerny, J. Stanek, *Adv. Carbohydr. Chem. Biochem.* **1977**, *34*, 23–178; b) M. Cerny, O. Julakova, J. Pacak, *Collect. Czech. Chem. Commun.* **1974**, *39*, 1391–1396.
- [26] a) R. Lattrell, G. Lohaus, *Justus Liebig's Ann. Chem.* **1974**, 901–920; b) R. Albert, K. Dax, R. W. Link, A. E. Stutz, *Carbohydr. Res.* **1983**, *118*, C5–C6.
- [27] T. B. Grindley, R. Thangarasa, *Carbohydr. Res.* **1988**, *172*, 311–318.
- [28] T. Trnka, M. Cerny, *Collect. Czech. Chem. Commun.* **1971**, *36*, 2216–2225.
- [29] S. Hornik, L. C. Stastna, P. Curinova, J. Sykora, K. Kanova, R. Hrstka, M. Dracinsky, J. Karban, *Beilstein J. Org. Chem.* **2016**, *12*, 750–759.
- [30] P. Sarda, F. C. Escibano, R. J. Alves, A. Olesker, G. Lukacs, *J. Carbohydr. Chem.* **1989**, *8*, 115–123.
- [31] P.-O. Lin, A. K. Adak, S.-H. Ueng, L.-D. Huang, K.-T. Huang, J.-a. A. Ho, C.-C. Lin, *J. Org. Chem.* **2009**, *74*, 4041–4048.
- [32] CCDC 1824899 contains the supplementary crystallographic data for compound **57**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.
- [33] CCDC 1848261 contains the previously described supplementary crystallographic data for **57**.
- [34] K. Bock, J. O. Duus, *J. Carbohydr. Chem.* **1994**, *13*, 513–543.
- [35] L. B. Rice, *J. Infect. Dis.* **2008**, *197*, 1079–10881.
- [36] C. Chemani, A. Imberty, S. de Bentzman, M. Pierre, M. Wimmerová, B. P. Guery, K. Faure, *Infect. Immun.* **2009**, *77*, 2065–2075.
- [37] a) N. Garber, U. Guempel, A. Belz, N. Gilboa-Garber, R. J. Doyle, *Biochim. Biophys. Acta*, **1992**, *1116*, 331–333; b) R. U. Kadam, M. Bergmann, M. Hurley, D. Garg, M. Cacciarini, M. A. Swiderska, C. Nativi, M. Sattler, A. R. Smyth, P. Williams, M. Cámara, A. Stocker, T. Darbre J.-L. Reymond, *Angew. Chem., Int. Ed.* **2011**, *50*, 10631–10635; c) I. Otsuka, B. Blanchard, R. Borsali, A. Imberty, T. Kakuchi, *ChemBioChem*, **2010**, *11*, 2399–2408.
- [38] For a more detailed account of the chemical shift perturbation, see supplementary information.
- [39] a) G. Cioci, E. P. Mitchell, C. Gautier, M. Wimmerova, D. Sudakevitz, S. Pérez, N. Gilboa-Garber, A. Imberty, *FEBS Lett.* **2003**, *555*, 297–301; b) R. U. Kadam, M. Bergmann, M. Hurley, D. Garg, M. Cacciarini, M. A. Swiderska, C. Nativi, M. Sattler, A. R. Smyth, P. Williams, M. Camara, A. Stocker, T. Darbre, J.-L. Reymond, *Angew. Chem. Int. Ed.* **2011**, *50*, 10631–10635.
- [40] S. Wagner, D. Hauck, M. Hoffmann, R. Sommer, I. Joachim, R. Müller, A. Imberty, A. Varrot, A. Titz, *Angew. Chem. Int. Ed.* **2017**, *56*, 16559–16564.
- [41] B. Linclau, Z.; Wang, G. Compain, V. Paumelle, C. Q. Tontenelle, N. Wells, A. Weymouth-Wilson, *Angew. Chem. Int. Ed.* **2016**, *55*, 674–678.
- [42] Developmental therapeutics program human tumor cell line screen, National Cancer Institute (NCI/NIH), https://ntp.cancer.gov/discovery_development/nci-60/default.htm, accessed February 21, 2018].
- [43] F. Delaglio, S. Grzesiek, G. Vuister, G. Zhu, J. Pfeifer, A. Bax, *J. Biomol. NMR* **1995**, *6*, 277–293.
- [44] W. F. Vranken, W. Boucher, T. J. Stevens, R. H. Fogh, A. Pajon, M. Llinas, E. L. Ulrich, J. L. Markley, J. Ionides, E. D. Laue, *Proteins* **2005**, *59*, 687–696.
- [45] M. P. Williamson, *Prog. Nucl. Magn. Reson. Spectrosc.* **2013**, *73*, 1–16.