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Synthesis, Biological Evaluation, WAC and NMR Studies of *S*-Galactosides and Non-Carbohydrate Ligands of Cholera Toxin Based on Polyhydroxyalkylfuroate Moieties

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Abstract: The synthesis of several non-carbohydrate ligands of cholera toxin based on polyhydroxyalkylfuroate moieties is reported. Some of them have been linked to D-galactose through a stable and well-tolerated *S*-glycosidic bond. They represent a novel type of non-hydrolyzable bidentate ligand featuring galactose and polyhydroxyalkylfuroic esters as pharmacophoric residues, thus mimicking the GM1 ganglioside. The affinity of the new compounds towards cholera toxin was measured by weak affinity chromatography (WAC). The interaction of the best candidates with this toxin was also studied by saturation transfer difference NMR experiments, which allowed identification of the binding epitopes of the ligands interacting with the protein. Interestingly, the highest affinity was shown by non-carbohydrate mimics based on a polyhydroxyalkylfuroic ester structure.

Keywords: weak affinity chromatography (WAC) · biomimetic synthesis · carbohydrates · cholera toxin · NMR spectroscopy

Introduction

Cholera toxin (CT) from *Vibrio cholerae*, and the closely related heat-labile toxin (LT) of *Escherichia coli*, belong to the bacterial AB_5 holotoxin family, which comprises a single catalytically active component A subunit and five identical B subunits forming a regular pentamer B_5. This pentamer is responsible for binding to ganglioside GM1 (Galβ1-3GalNAcβ1-4[NeuAcα2-3]Galβ1-4Glcβ1,1-ceramide) on the surface of the membrane of intestinal cells.[1] These toxins are responsible for diarrheal diseases and several other disorders. Due to the importance of the processes that they promote, the study of the complexes between gangliosides and AB_5 toxins is very relevant. The B pentamer of CT interacts with the monovalent oligosaccharide fragment of GM1 ganglioside with a strong affinity ($K_d = 43$ nM, as measured by isothermal calorimetry). This interaction represents one of the strongest carbohydrate–protein interactions, the complex GM1:CT being an extremely well characterized pair in the field of sugar–protein interactions. In fact, the interaction between GM1 and CT has been widely studied through calorimetry,[2] X-ray diffraction,[3] and computational methods.[4] It is known that the two pharmacophoric units of GM1 are the terminal galactose and the sialic acid residues. The conformational preorganization of these pharmacophores and the distance between them is essential to interact efficiently with CT.[5] The heat-labile enterotoxin (LT) has a homology of about 80% with CT and is basically identical in binding with GM1 and in the toxic effect, although LT causes less severe symptoms than CT.[6] In consequence, the study of the interaction CT:GM1 is a valid model for the interaction with LT. Several GM1 derivatives or mimetics containing terminal galactose residues and/or sialic acid have been described to have affinity towards CT and LT. They bind reversibly to the B subunit (forming an association complex) and act as inhibitors of the recognition between toxins and host mucins, thus preventing infections.[1a,5,7]

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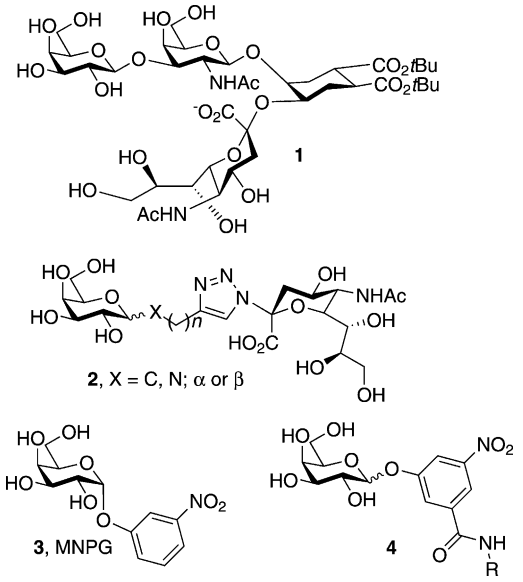


Figure 1. General structures for GM1 mimetics.

Bernardi and co-workers have reported the synthesis of a series of pseudo-tetrasaccharides bearing a cyclohexane diol moiety, such as **1** (Figure 1), as GM1 mimetics, which displayed the same affinity as the natural ligand.[8] Other pseudo-oligosaccharides obtained through replacing the sialic acid moiety by simpler hydroxyacids presented dissociation constants in the millimolar to micromolar range.[9] This group has also reported metabolically stable bifunctional compounds **2**,[10] based on *C*-galactosides having galactose and sialic acid moieties connected through a triazole moiety as linker. The affinity of the compounds towards CT was tested by weak affinity chromatography (WAC). Affinity could be enhanced by up to one or two orders of magnitude over those of the individual pharmacophoric sugar residues.

Verlinde and co-workers[11] first described that *m*-nitrophenyl-α-D-galactopyranoside (MNPG) **3** is an inhibitor of the GM1–enterotoxin interaction. On its side, Fan and co-workers[7b,12] have described that **3** exhibits an IC_{50} of 600 μM. Galactose itself displays a high specificity but low affinity relative to CT (IC_{50} = 45 mM). Ohlson and co-workers[13] have determined for MNPG **3** an affinity constant K_d = 1.1 mM, by means of the WAC method based on immobilized cholera toxin subunit B (CTB). Derivatives such as **4** are also CTB_5 antagonists in the low millimolar range (Figure 1).[14]

Our group has reported the preparation and biological studies of novel thiofucosides incorporating hydroxylated non-proteinogenic amino acids ending in carboxylate groups as aglycons (Figure 2). These compounds have shown affinity towards E- and P-selectins in the millimolar range.[15] Selectins are glycoproteins that present heterophilic binding with their receptors,[16] thereby mediating cellular interactions through the lectin domain and the cell surface carbohydrate ligands.[17] Another group of proteins that, like selectins, presents heterophilic binding with their receptors are pathogen-secreted enterotoxins.[18]

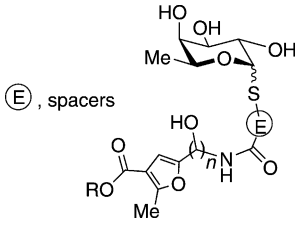


Figure 2. General structure for E- and P-selectin ligands.

Based on our previous results on polyhydroxyalkylfuryl thiofucosides, which show affinity towards selectins,[15c] we hypothesized that *S*-galactosides bearing a polyhydroxyalkylfuran aminoester moiety as a non-proteinogenic polyhydroxylated amino acid could interact with the enterotoxins CT and LT, thus acting as GM1 glycomimetics. We report herein the preparation and biological study by WAC of new *S*-galactosides of Generations I and II (Figure 3). Both generations of compounds are *S*-galactosides containing a polyhydroxyalkylfuran moiety in the aglycon, which is linked through a spacer to the galactose moiety. For compounds of Generation I, the polyhydroxyalkylfuran moiety is attached to the spacer by the furan unit (Generation I: "head connection"). In the case of compounds of Generation II, a polyhydroxyalkylfuran moiety is attached to the spacer by a polyol chain (Generation II: "tail connection").[22]

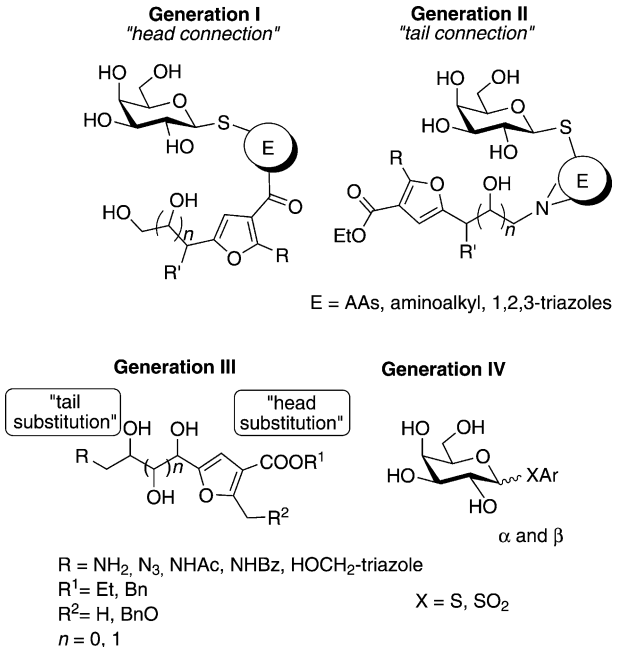


Figure 3. General structures of new *S*-linked galactosides and fragments. AA = amino acid, Bz = benzoyl, Bn = benzyl.

These compounds are structurally simpler and hydrolytically more stable than the natural ganglioside GM1 and have appropriate groups to generate structural diversity and to further assemble them into multivalent structures. We demonstrate that the polyhydroxyalkylfuran moiety contrib-

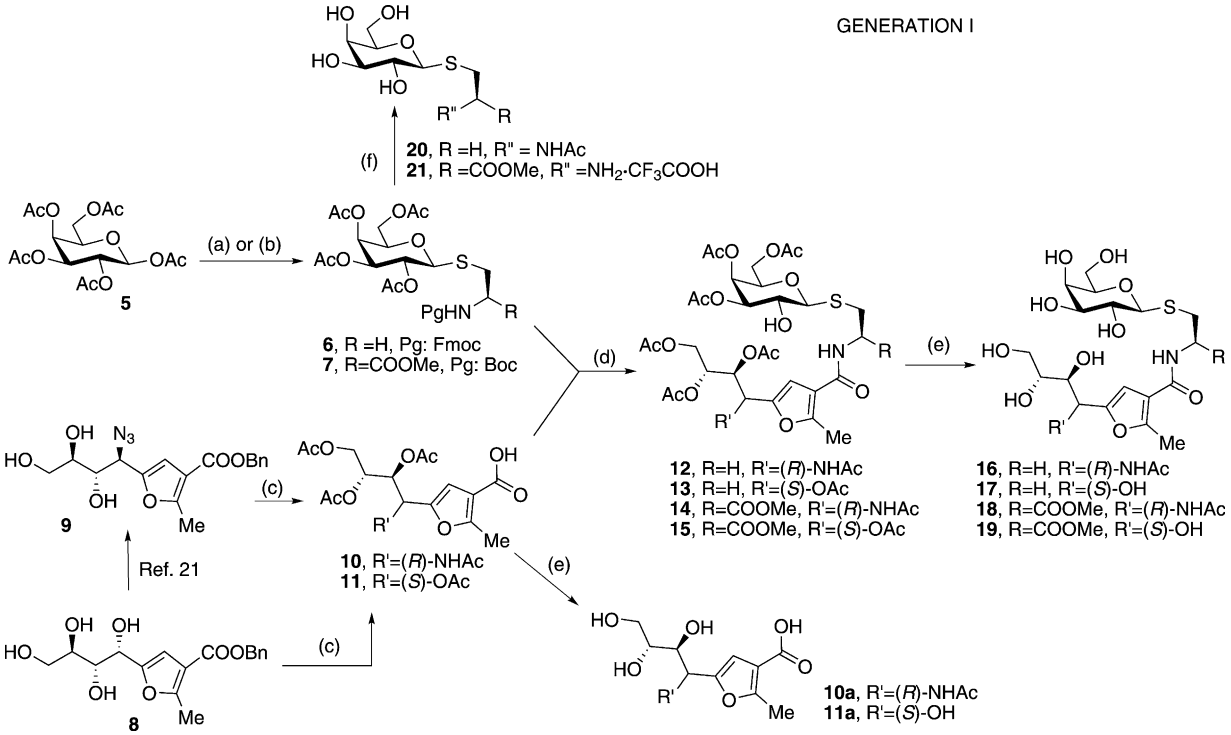
utes effectively to the affinity, and that the type of connection to galactose, head or tail connection, is crucial for the binding affinity. To establish which part of the molecule is more important for binding affinity, fragment compounds have also been prepared and evaluated by WAC. Thus, Generation III is constituted by differently configured and substituted polyhydroxyalkylfurans.

We show herein that some of these compounds present binding affinities in the same order as that of known sugar-derived MNPG **3**. Their binding affinity depends on the type and site of substitution, that is, in the furan ring ("head substitution") or in the polyolic side chain ("tail substitution"). Some of the prepared compounds represent the first examples of non-sugar-based antagonists of enterotoxins. With the aim of accurately obtaining structural information on the interactions of this family of ligands with the CTB, saturation transfer difference (STD) NMR experiments are also presented.

Compounds of Generation IV (Figure 3) present the sugar fragments of compounds of Generations I and II, which additionally are the thio analogues of the reported MNPG series.[12,13] Notably, the introduction of a sulfur group at the anomeric position provides good acid and enzyme-mediated hydrolytic stability.[19] In addition, the higher water solubility of sulfur derivatives relative to their oxygen counterparts is an important advantage for our compounds with regard to tolerance by most biological systems.[20]

Results and Discussion

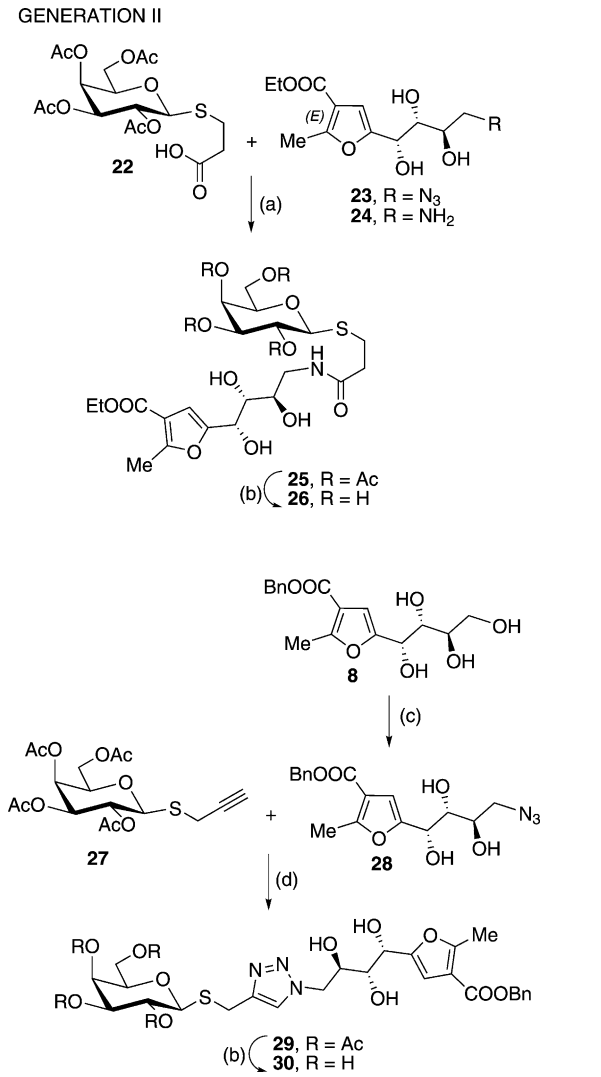
Synthesis of compounds from Generations I and II: The syntheses of compounds of Generations I and II were carried out by coupling different thioglycosides with conveniently protected polyhydroxyalkylfuroates. Thus, the preparation of compounds **16**–**19** (Generation I) was carried out starting from penta-*O*-acetyl-β-D-galactopyranoside (**5**) by applying two main strategies. Direct glycosylation of a thiol-containing compound such as Fmoc-cysteamine in the presence of TMSOTf (method A) gave aminoalkyl β-D-thiogalactopyranoside **6** in 72% yield. Transformation of **5** into acetobromogalactose and glycosylation with Boc-cysteine methyl ester (method B) afforded β-D-thiogalactosyl aminoester **7** in 57% yield (Scheme 1). 1′-Acetamido-2′,3′,4′-tri-*O*-acetyl-1-yl-furancarboxylic acid **10** and per-*O*-acetyl-1-yl-furancarboxylic acid **11** were obtained by hydrogenation of compounds **9** and **8**, respectively, followed by acetylation. The introduction of the azido moiety at C-1′ in compound **8** was carried out as described previously by our group,[21] through



Scheme 1. Reagents and conditions: a) Method A: TMSOTf, CH_2Cl_2, $FmocNHCH_2CH_2SH$, 72% for **6**; b) Method B: 1. HBr/AcOH 33%, 2. Na_2CO_3, TBAHS, AcOEt/H_2O, Boc-Cys-OMe, 57% for **7**; c) 1. H_2, Pd-C, 2. Ac_2O, Py, DMAP, 80% for **10**, 60% for **11**; d) 1. For Pg:Fmoc, PIP/DMF (20%) and for Pg:Boc, TFA/CH_2Cl_2 (20%), 2. PyBOP, DIPEA, DMF, 56% for **12**, 42% for **13**, 89% for **14**, 89% for **15**; e) NaOMe, MeOH, 100% for **16**–**19**, **10a**, and **11a**; f) 1. PIP/DMF (20%), 2. Ac_2O/Py, 3. NaMeO/MeOH, 63% (three steps) for **20**; 1. NaMeO/MeOH, 2. TFA/CH_2Cl_2 (20%), 94% (two steps) for **21**. TMSOTf = trimethylsilyl triflate, Fmoc = 9-fluorenylmethoxycarbonyl, TBAHS = tetrabutyl ammonium hydrogenosulfate, Boc = *tert*-butoxycarbonyl, Cys = cysteine, Py = pyridine, DMAP = 4-dimethylaminopyridine, Pg = protecting group, PIP = piperidine, TFA = trifluoroacetic acid, PyBOP = (benzotriazol-1-yloxy)trispyrolidinophosphonium hexafluorophosphate, DIPEA = *N*,*N*-diisopropylethylamine.

the formation of a cyclic sulfite as intermediate. Removal of the Fmoc protecting group in **6** with piperidine/DMF (20%) followed by coupling with furancarboxylic acids **10** and **11** by using PyBOP and DIPEA as condensing agents furnished adducts **12** and **13** in 56 and 42% yield, respectively. On the other hand, acid treatment of **7** with TFA/CH_2Cl_2 (20%) followed by coupling with **10** and **11**, under the same reaction conditions, afforded **14** and **15** in 89% yield in both cases. Final deprotection under Zemplén conditions gave compounds **16**–**19** in quantitative yield. With the purpose of comparing the biological activities of the compounds of Generation I with their corresponding fragments, deprotection of **6**, **7**, **10**, and **11** under standard conditions gave **20**, **21**, **10a**, and **11a**, respectively, in good overall yields (Scheme 1).

Compounds from Generation II (**26**, **30**) bearing an alkyl polyhydroxyalkylfuroate moiety were prepared through two synthetic sequences. The synthesis of compound **26** was carried out from known ethyl 4′-aminotriol-1-yl furoate **24**, which was obtained from the corresponding azido derivative **23** as described previously by our group[22] (Scheme 2). Similarly, compound **30** was prepared from benzyl 4′-azidotriol-1-yl furoate **28**, obtained from compound **8**[21] in good overall yield, after chemoselective tosylation of the hydroxyl primary position and conventional azido displacement. Reaction of **24** with carboxyalkyl-β-D-thiogalactoside **22**,[23] under peptide coupling conditions, with PyBOP and DIPEA gave **25** in 49% yield, which was deprotected under Zemplén conditions furnishing **26** in quantitative yield. "Click chemistry" coupling between azido derivative **28** and β-galactosyl alkyne **27** furnished triazole **29** in 82% yield, which was finally deprotected to give **30** in quantitative yield. β-Galactosyl alkyne **27** was obtained from per-*O*-acetylated β-galactopyranoside through Lewis acid catalyzed (BF_3•Et_2O) glycosylation with thiourea, according to the procedure reported by Ibatullin and co-workers,[24] followed by treatment with Et_3N[25] and reaction with propargyl bromide (Scheme 2).



Scheme 2. Reagents and conditions: a) DIPEA, PyBOP, DMF, 49%; b) NaOMe, MeOH, 100%; c) 1. tosyl chloride (TsCl), Py, −15°C, 69%, 2. NaN_3, DMF, 80°C, 85%; d) $CuSO_4$, sodium ascorbate, MeOH/H_2O, 82%.

Ligand evaluation of compounds from Generations I and II by weak affinity chromatography: To evaluate the binding affinity of these ligands and their corresponding fragments to CT, the method of WAC, based on immobilized CTB, was used. The method is mostly appropriate for quantifying transient binding events, such as sugar–protein interactions. In our case, it was found to be the method of choice, because for our ligands the binding affinity was suspected to be rather weak, in the millimolar range. This method has previously been used for the evaluation of CTB binders, both in terms of small compounds and galactosyl derivatives as bidentate ligands.[10,13] A HPLC column with immobilized recombinant CTB (rCTB) was used and small amounts of the synthesized ligands were injected. An elution solution was subsequently applied to the column to make each ligand run through the column to be released from the entrapment. The retardation (expressed as the retention factor k') is directly related to the affinity of the interaction.[26] A compound of known affinity was used as a reference, and the dissociation constant of the ligand, K_d, was calculated from k' by calibrating the retention with the K_d of the reference compound. In our case, MNPG, a well-known CT ligand, was used as reference to calibrate our experiments. The results of the WAC analysis of our ligand library and the corresponding fragments compared with MNPG are summarized in Table 1.

Compounds **16**–**19** bearing a "head connection" (Generation I) did not show binding affinity to CTB (Table 1, entries 2–5), and neither did *N*,*O*-unprotected β-D-thiogalactopyranoside fragments **20** and **21** and polyhydroxyalkylfuran carboxylic acids **10a** and **11a** (Table 1, entries 8–11). In contrast, compounds bearing a "tail connection" (Generation II) and their corresponding fragments showed significant activities, and some interesting candidates were identified. Thus, the best result for Generation II was obtained for compound **30** with $K_d = 1.05$ mM. Compound **26** showed

Table 1. Retention factor (k') and dissociation constant (K_d) of CT ligands and their fragments at 22 °C determined with WAC at pH 7.

	Entry	Compound	Generation	k'	K_d [mM]	K_{MNPG}/K_d
Ligands	1	MNPG	–	1.58	1.1[a]	1
	2	**16**	I	0.19	NB	–
	3	**17**	I	0.18	NB	–
	4	**18**	I	0.14	NB	–
	5	**19**	I	0.12	NB	–
	6	**26**	II	0.36	4.7	0.23
	7	**30**	II	1.63	1.05	1.05
Fragments	8	**20**	I	0.13	NB	–
	9	**21**	I	0.15	NB	–
	10	**10a**	I	0.03	NB	–
	11	**11a**	I	0.20	NB	–
	12	**24**	II	0.52	3.3	0.33
	13	**28**	II	2.63	0.6	1.83

[a] Ref. [13]. NB = no binding.

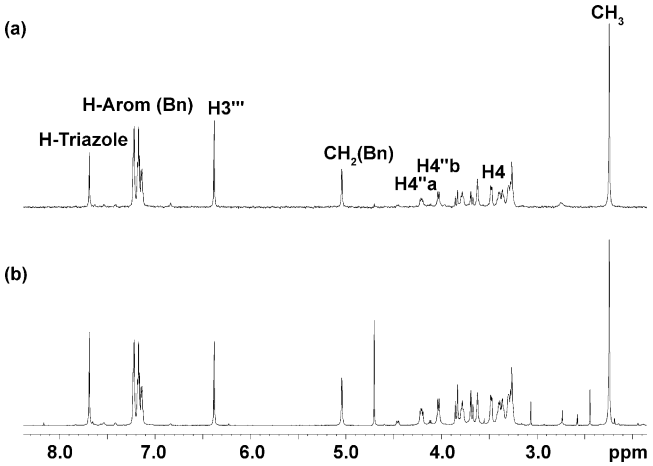


Figure 4. STD NMR (a) and reference (b) spectra of a sample containing 1 mM ligand **30** in the presence of 5 μM subunit B of cholera toxin (CTB; 25 μM in binding sites). The spectra were obtained at 700 MHz and 278 K (saturation time, $t_{sat} = 2$ s). Key signals are labeled on the difference spectrum (a) .

$K_d = 4.7$ mM. Interestingly, the aminopolyhydroxyalkylfuran ethyl ester **24** and the azidopolyhydroxyalkylfuran benzyl ester **28** exhibited remarkable binding affinities of $K_d =$ 3.3 mM and 0.6 mM, respectively. The latter is almost twice as active as MNPG under the same conditions.[13]

Interaction studies by NMR spectroscopy: binding epitopes: To obtain structural information on the interactions of this family of ligands with the CTB, we carried out STD NMR experiments.[27] With these experiments we identified the main contact points of the ligands with the protein in the binding pocket (the so-called ligand binding epitope).[28] This is possible because the binding process transfers ^{1}H magnetization, created selectively on the protein, onto the ligand molecule due to intermolecular ^{1}H–^{1}H NOE processes in the bound state and the fast exchange with the free state.[27–29] The presence of signals in the resulting STD NMR spectrum of the ligand reveals the existence of binding in solution and, in addition, the intensities of the STD signals of the ligand are related to ligand–protein spatial proximity; that is, the stronger the signal, the closer the contact of that part of the ligand to the protein surface.

The STD NMR spectrum of **30** in the presence of rCTB is shown in Figure 4. This compound was selected for a detailed structural study by NMR spectroscopy as the best representative of the diverse set of synthesized molecules, as it contains different chemical elements sought to afford affinity for CTB. The experiments were carried out at 700 MHz on a sample containing 1 mM ligand and 5 μM rCTB, which, due to its pentameric nature, corresponds to a total concentration of 25 μM in binding sites (i.e., ligand-to-receptor ratio of 40:1). The spectra at different saturation times (see Experimental Section) showed clear signals (in Figure 4, $t_{sat} = 2$ s), thus confirming that we were able to detect the binding of **30** to rCTB in solution.

The evolution of the STD signals with saturation time (buildup curves) is shown in Figure 5. It shows that **30** received saturation all along the molecule, so that the binding is not simply through one end of the molecule, which is frequently the case in lectin–carbohydrate interactions. On the other hand, Figure 6 shows the relative saturation values for each ligand proton, obtained from normalization of the STD initial growth rates (see Experimental Section), which gives an accurate picture of the binding epitope of **30** for its interaction with CTB. Onto this quantitative map of ligand binding, it is clear that the entire molecule makes contacts with the surface of CTB in the bound state. Interestingly, the saturation level decreases from both terminal ends of **30** towards the triazole ring, indicative of a bidentate-like binding to CTB. What is more, the furan and carboxybenzyl-containing end of the molecule received the largest amount of saturation (Figure 5, bottom right, and Figure 6). This is a key result, as it explains the previously observed contribution of the polyhydroxyalkylfuran moiety to the binding affinity to CTB, by WAC experiments. The protein indeed closely recognizes this moiety, most likely enhancing the enthalpy of the interaction due to the increase in the number of contacts of the ligand with the protein surface.

In addition, we also carried out transferred NOESY experiments, to elucidate whether the binding process involves conformational selection on the ligand, which would contribute negatively to the free energy of the interaction process by an entropic penalty. Yet, the similarity of the NOESY experiments in the presence and in the absence of CTB (see Supporting Information) indicated that the protein does not select any particular conformer of **30**, or that it simply recognizes the most populated one in the free state.

The binding epitope of **30** from STD NMR spectroscopy (Figure 6), and the affinity studies, were both supportive of a bidentate binding of the ligand in which both terminal ends, the galactose and the furan-carboxybenzyl residues, are strongly involved in establishing contacts with the protein. Nevertheless, to confirm this issue and to rule out nonspecific interactions, we formally "dissected" the ligand into the two constituent moieties. We then carried out STD

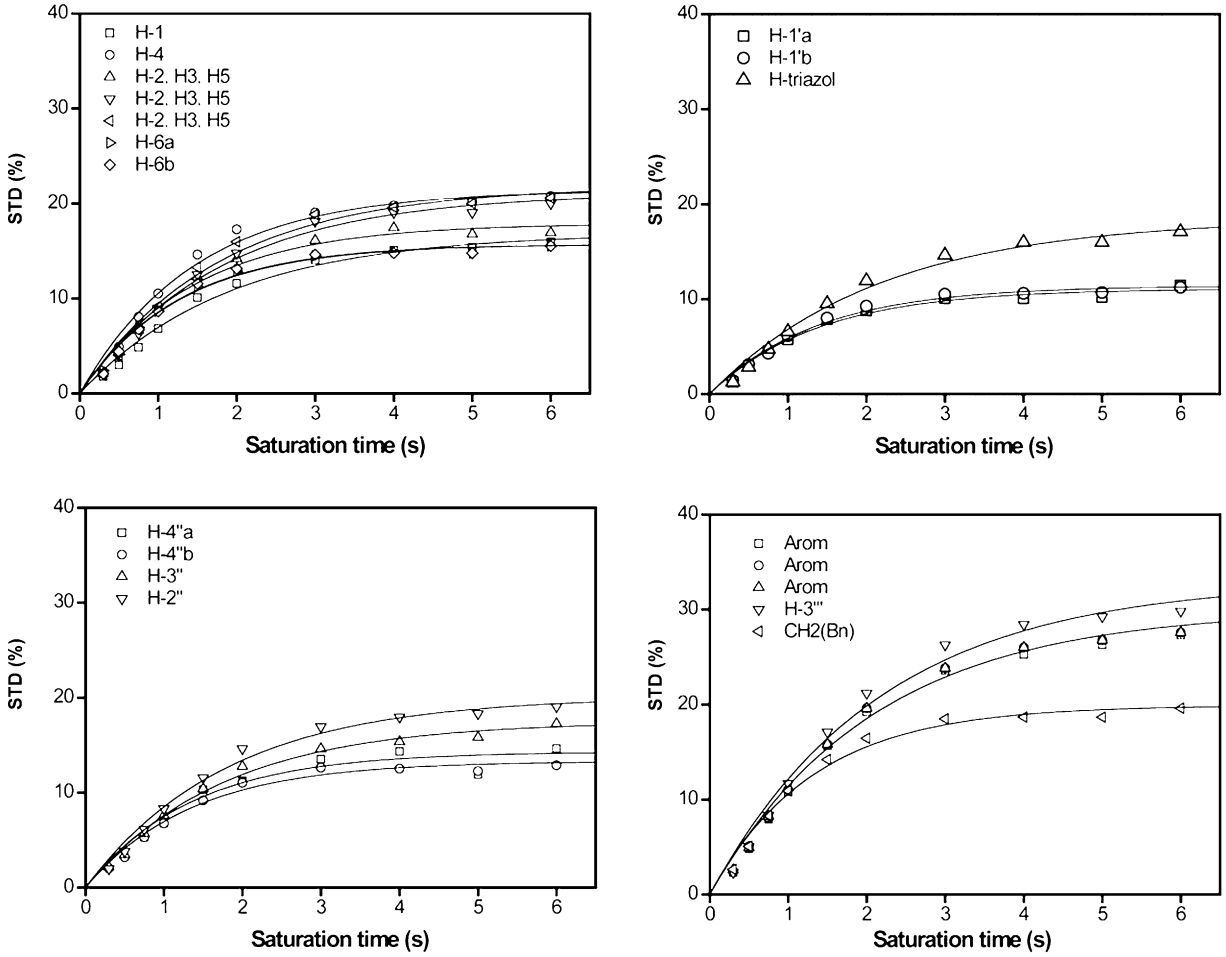


Figure 5. STD buildup curves of **30** interacting with the CTB. The signals have been split for simplicity into four graphs (four different regions of the ligand **30**). The most intense STD responses came from the galactose and aromatic ends.

NMR experiments on a ligand constituted by the galactose terminal end **20** (see Scheme 1) and another one constituted by the polyhydroxyalkylfuran moiety **33** (see Scheme 3, Generation III). Their STD NMR spectra in the presence of CTB are shown in Figure 7. Both molecules showed significant STD NMR signals in their spectra, which indicates that both are binding to CTB in solution.

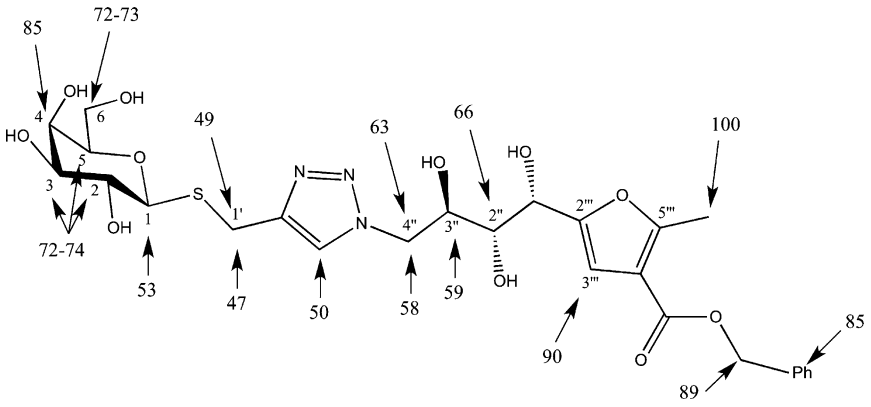
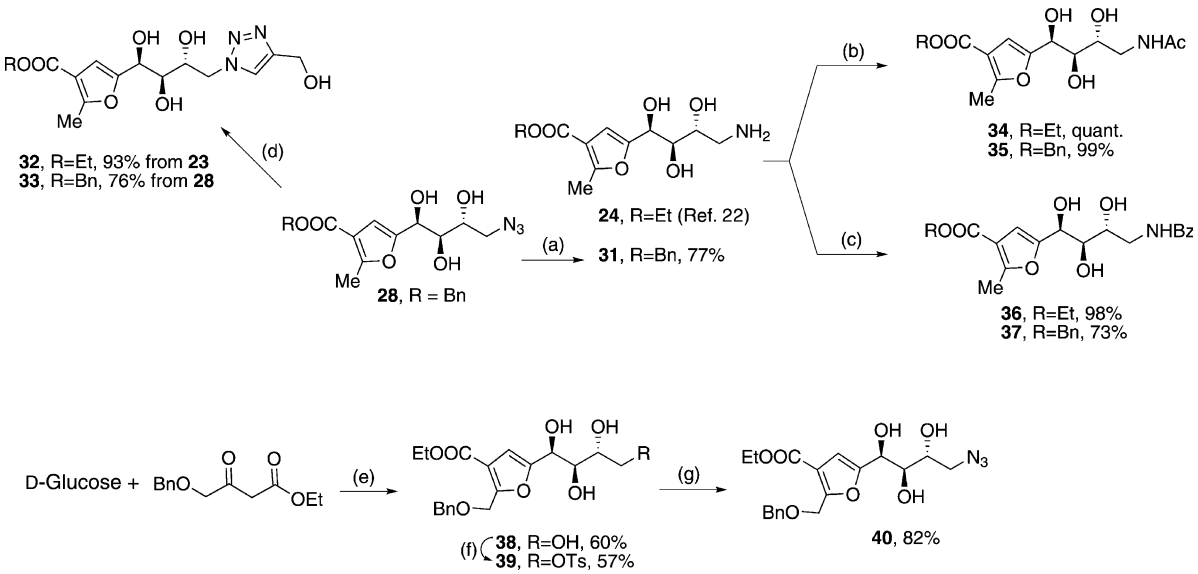


Figure 6. Binding epitope of **30** for its interaction with the CTB. The numbers represent relative values of saturation after their normalization related to the most intense one (assigned 100%), obtained from STD initial slopes (see Experimental Section). Both molecular ends receive the largest amounts of saturation from the protein, supportive of a bidentate binding.

It is worth mentioning the observation of STD signals from **20**, which highlights the outstanding sensitivity of STD NMR spectroscopy for weak protein–ligand interactions, as **20** binding was not detectable by the WAC experiments. The comparison of global STD intensities of **30**, **20**, and **33** (see Supporting Information) clearly indicated that **20** is the weakest binder, in agreement with the WAC data (Tables 1 and 2). The binding epitopes from the analysis of the corresponding STD buildup curves are shown in Figure 8.

The resulting binding epitopes confirm that ligands **20**



Scheme 3. Synthesis of compounds of Generation III (1). Reagents and conditions: a) H_2S(g), Py/H_2O (1:1), 0 °C→RT; b) acetic anhydride, MeOH, RT; c) BzCl, Et_3N, dry DMF, 5 °C→RT; d) propargyl alcohol, $CuSO_4$ (aq), sodium ascorbate (aq), MeOH, RT; e) $CeCl_3{\cdot}7H_2O$, NaI, SiO_2, MeCN; f) TsCl, Py, −15 °C; g) NaN_3, DMF, 80 °C.

and **33** bind to CTB in solution with binding modes (Figure 8) that involve very close contacts with the protein through the galactose residue and the furan-carboxybenzyl end, respectively, similar to those observed for the binding of **30**. Therefore, with these two extra binding experiments we were able to demonstrate that both terminal ends of **30** are indeed specific binders of CTB, as independent molecules. The protein recognizes both structures on ligands **20** and **33** and when linked on the same molecule, **30**, demonstrates that both the sugar and the non-sugar moieties are contributing to the binding affinity of the ligand **30** for the CTB.

It is well known that a terminal galactose residue constitutes a pharmacophoric unit of the natural ligand GM1 ganglioside. Our NMR data on **30** and **20** are in very good agreement with this, showing that the region of the sugar ring outlined by carbon atoms C4, C5, and C6 is involved in close contacts with the protein (Figure 6 and Figure 8a), thus strongly supporting the participation of this part of the hexopyranose ring in the stacking interactions with the side chain of the amino acid Trp-88, as reported in the case of GM1.[3b] The NMR results also highlight the relevance of the furan moiety of **33** in the binding process (Figure 6 and Figure 8b), and the importance of the distance between the two pharmacophoric moieties is demonstrated by the dramatic reduction in binding affinity to CTB in the case of ligands of Generation I (head-connected).

Table 2. Retention factor (k') and dissociation constant (K_d) of CT ligands from Generation III at 22 °C determined with WAC at pH 7.

Entry	Compound	k'	K_d [mM])	K_{MNPG}/K_d
1	MNPG	1.58	1.1[a]	1
2	**23**	0.43	3.6	0.3
3	**24**	0.52	3.3	0.33
4	**34**	0.15	NB	–
5	**32**	0.14	NB	–
6	**36**	1.20	1.3	0.85
7	**28**	2.63	0.6	1.83
8	**31**	3.32	0.47	2.34
9	**48**	0.16	NB	–
10	**33**	1.16	1.35	0.8
11	**35**	1.16	1.35	0.8
12	**37**	0.31	5.1	0.2
13	**40**	7.6	0.2	5.5
14	$\mathbf{45}_{\text{D-arab}}$	0.34	4.6	0.24
15	**47**	0	NB	–
16	$\mathbf{49}_{\text{D-arab}}$	0.36	4.4	0.25
17	$\mathbf{49}_{\text{L-arab}}$	0.38	4.1	0.27
18	$\mathbf{49}_{\text{D-xyl}}$	0.38	4.1	0.23
19	$\mathbf{49}_{\text{L-xyl}}$	0.38	4.1	0.23
20	$\mathbf{50}_{\text{D-arab}}$	3.1	0.5	2.2
21	$\mathbf{50}_{\text{L-arab}}$	3.2	0.5	2.2
22	$\mathbf{50}_{\text{D-xyl}}$	3.2	0.5	2.2
23	$\mathbf{50}_{\text{L-xyl}}$	3.1	0.5	2.2
24	$\mathbf{51}_{\text{D-arab}}$	3.06	0.51	2.16
25	$\mathbf{51}_{\text{L-arab}}$	2.5	0.6	1.83
26	$\mathbf{51}_{\text{D-xyl}}$	2.1	0.7	1.57
27	$\mathbf{51}_{\text{L-xyl}}$	1.9	0.8	1.37
28	**52**	0.16	NB	–
29	**32**	0.14	NB	–

[a] Ref. [13]. NB = no binding.

Synthesis of compounds from Generation III: towards novel, easily available, and better alternatives to MNPG: This intriguing result obtained with both subunits prompted us to synthesize libraries of a panel of differently configured and substituted polyhydroxyalkylfurans (Generation III) and thio analogues of MNPG (Generation IV; see Supporting Information). The polyhydroxyalkylfuran skeleton is easily obtained from sugars by condensation with β-ketoesters.[30] The synthesis of compounds of Generation III is outlined in Schemes 3 and 4. From azido derivatives **23** and **28**, different functionalities were attached at the end of the

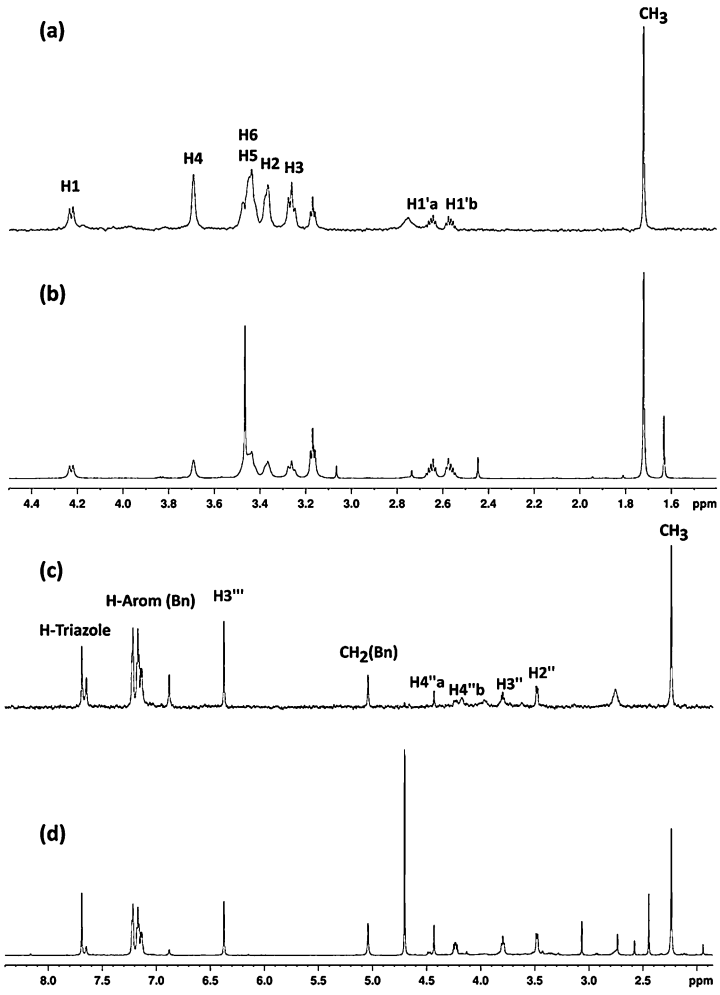


Figure 7. STD NMR (a,c) and reference (b,d) spectra of the interactions of **20** and **33**, respectively, with the CTB. Each sample contained 1 mM ligand in the presence of 5 μM CTB (25 μM in binding sites). The spectra were obtained at 700 MHz and 278 K (saturation time, 2 s). Key signals are labeled on the difference spectra (a and c).

side chain on ethyl and benzyl tetritol-1-yl furoates. Thus, transformation of the 4′-amino or 4′-azido groups into acetamido, benzamido, or triazole moieties under standard conditions gave rise to derivatives **32**–**37** (Scheme 3). Reaction of D-glucose with ethyl benzyloxymethylcarbonyl acetate[31] following Bartoli's modification of the García González reaction,[32] furnished ethyl tetritol-1-yl furoate **38** bearing a benzyloxy appendage in 60% yield. Conventional tosylation and azido displacement gave azido derivative **40** in moderate to good overall yield.

Other ethyl and benzyl polyhydroxyalkylfuroates (**41**–**52**) with shorter side chains, presumably with least conformational freedom, were also prepared starting from L- and D-arabinose and L- and D-xylose (Scheme 4). Compounds $\mathbf{42}_{\text{D-arab}}$ and $\mathbf{42}_{\text{D-xyl}}$ have been obtained previously[33] in our group by reaction of the corresponding aldose with benzyl acetoacetate catalyzed by $CeCl_3/NaI/SiO_2$ under Bartoli's conditions.[32] We have applied the same methodology for the preparation of compounds **41** and **42** in moderate to good yields. Chemoselective tosylation of the primary hydroxy group followed by azido displacement afforded compounds **45** and **46** in good overall yields. Ester hydrolysis on $\mathbf{45}_{\text{D-arab}}$ furnished **47** in good yield. Additionally, click reaction of azido derivative $\mathbf{45}_{\text{D-arab}}$ with propargyl alcohol gave furantriazole **48** in good yield. Hydrogenation of compounds **45** or reduction with H_2S of derivatives **46** afforded aminoesters **49** and **50**. Chemoselective benzoylation of compounds **49** furnished derivatives **51**. Similarly, chemoselective acetylation of $\mathbf{49}_{\text{D-arab}}$ gave **52**.

Ligand evaluation by weak affinity chromatography of compounds from Generation III: All these compounds were evaluated by WAC for their affinity to rCTB, and the results are summarized in Table 2. Several structure–activity relationships could be drawn. Thus, D-glucose-derived polyhydroxyalkylfuran derivatives bearing a COOEt moiety (alkyl head substitution; compounds **23** and **24**, Table 2, entries 2 and 3) present worse activity than MNPG. An acetamido or a hydroxymethyltriazole moiety at the end of the side chain (compounds **34** and **32**, Table 2, entries 4 and 5) abolishes the binding affinity. On the contrary, the presence of a benzamido moiety at the same position (compound **36**, Table 2, entry 6) slightly improves the activity, being in the same order as MNPG. An aromatic moiety contributes to the binding affinity with slighter efficacy than an alkyl moiety, especially when it is linked to the furan ring (aryl head substitution). Thus, benzyl ester analogues of the above compounds show better affinity properties: **23** (K_d = 3.6)/**28** (K_d = 0.6), Table 2, entries 2/7; **24** (K_d = 3.3)/**31** (K_d = 0.47), Table 2, entries 3/8; **34** (NB)/**35** (K_d = 1.35), Table 2, entries 4/11. However, two aromatic moieties at both ends in this type of compound are not beneficial for binding (**37**, Table 2, entry 12). An acetamido or a hydroxymethyltriazole moiety, instead of an amino group, also diminishes the affinity in this series: **31** (K_d = 0.47)/**33** (K_d = 1.35)/**35** (K_d = 1.35), Table 2, entries 8/10/11). The best compound in this series bears a benzyloxymethyl group at the furan ring (**40** (K_d = 0.2), Table 2, entry 13), being 5.5 times more active than MNPG itself (see Supporting Information for a typical weak affinity chromatogram of compound **33**).

Shortening the side chain slightly diminishes the binding affinity: **23** (K_d = 3.6)/$\mathbf{45}_{\text{D-arab}}$ (K_d = 4.6), Table 2, entries 2/14). For ethyl(3-amino-1,2-dihydroxypropyl)furoates (diastereoisomeric compounds **49**, Table 2, entries 16–19) the same K_d was observed, thus indicating no influence of the stereochemistry in the affinity. On changing to benzyl esters (aryl head substitution, compounds **50**, Table 2, entries 20–23) the affinity improved considerably, but no influence of the stereochemistry was observed.

On the contrary, for aryl-tail-substituted compounds (**51**, Table 2, entries 24–27), appreciable slight differences in the binding affinities could be observed among the four diastereoisomers, which diminished the affinity in the order: $\mathbf{51}_{\text{D-arab}} > \mathbf{51}_{\text{L-arab}} > \mathbf{51}_{\text{D-xyl}} > \mathbf{51}_{\text{L-xyl}}$. It could be speculated that for compounds **50**, the aminodihydroxypropyl side chain has

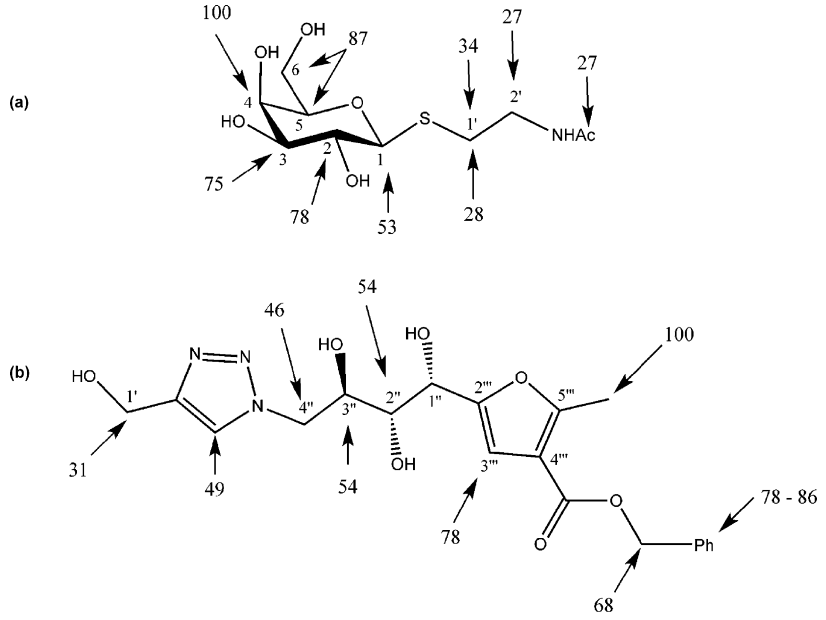
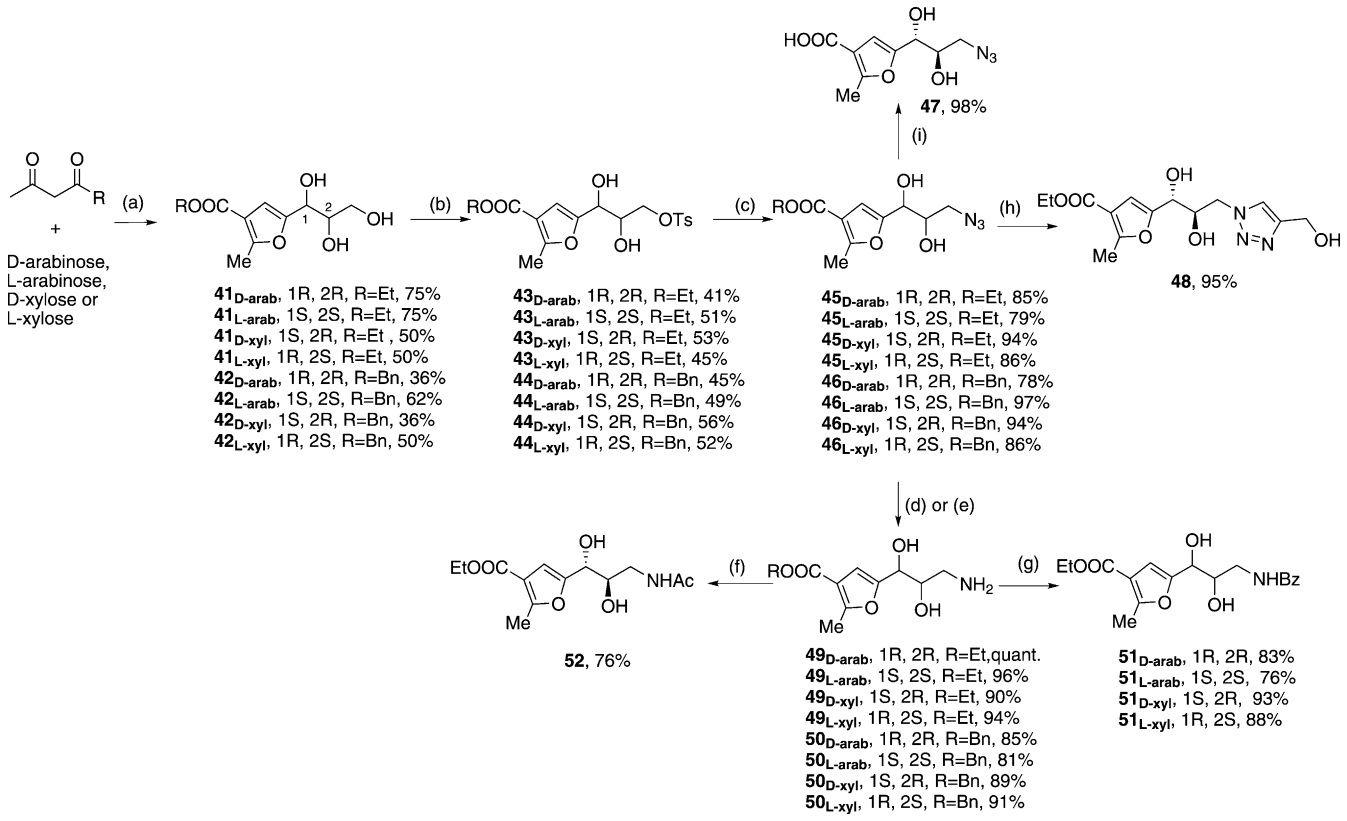


Figure 8. Binding epitopes of a) **20** and b) **33** for their interactions with the CTB. The values represent relative values of saturation after their normalization related to the most intense one (assigned 100%), obtained from STD initial slopes (see Experimental Section).

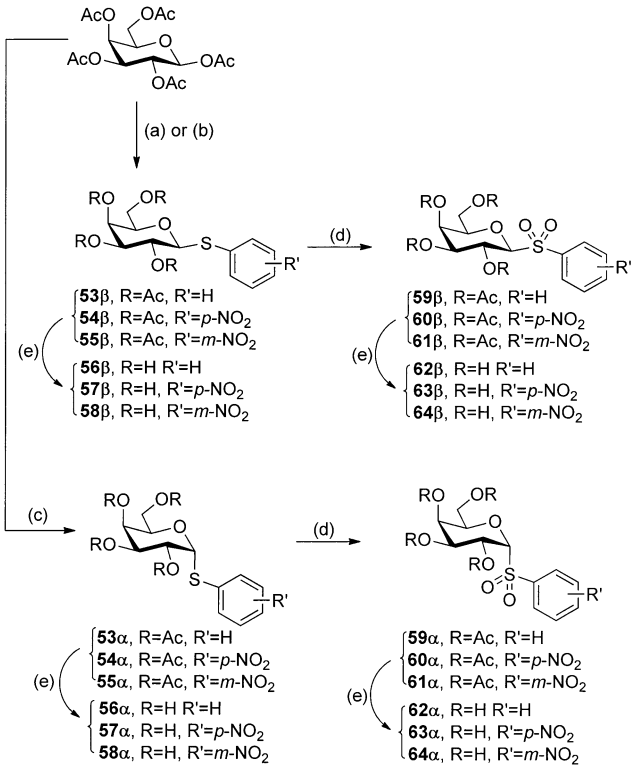
a lot of conformational freedom, once the benzyl moiety ("head-substituted", see Figure 3) interacts with the CTB binding pocket as was observed in the NMR study of polyhydroxyalkylfuran **33** (see Figure 8b). This is not the case for compounds **51** ("tail-substituted", see Figure 3), in which the interaction with the binding pocket presumably takes place through the benzoyl moiety. The four diastereoisomers present different retention factors, thus showing differences in binding affinities (see Supporting Information for a typical weak affinity chromatogram of compounds $\mathbf{51}_{\text{D-arab}}$, $\mathbf{51}_{\text{L-arab}}$, $\mathbf{51}_{\text{D-xyl}}$, and $\mathbf{51}_{\text{L-xyl}}$).

Finally, a carboxylic acid moiety at the furan ring abolishes the affinity (**47**/$\mathbf{45}_{\text{D-arab}}$, Table 2, entries 15/14; **10a**/**11a**, Table 1, entries 10/11). As suspected, amide **52** and triazole derivative **32** did not show binding affinity (Table 2, entries 28 and 29).

Synthesis of thio analogues of MNPG Generation IV: The synthesis of thio analogues of MNPG **5** (Generation IV) is



Scheme 4. Synthesis of compounds of Generation III (2). Reagents and Conditions: a) $CeCl_3 \cdot 7H_2O$, NaI, silica gel, MeCN, 50°C; b) TsCl, dry Py, −15°C; c) NaN_3, DMF, 80°C; d) H_2, Pd-C, EtOH, RT for **45** or e) H_2S(g), Py/H_2O (1:1) 0°C→RT for **46**; f) Ac_2O, MeOH, RT; g) BzCl, Et_3N, dry DMF, 5°C→RT; h) propargyl alcohol, $CuSO_4$ (aq), sodium ascorbate (aq), MeOH, RT; i) 1 M NaOH, EtOH, 60°C.



Scheme 5. Reagents and conditions: a) ArSH, TMSOTf, 1,2-dichloroethane, 0°C→RT, 86% for **53β**; b) 1. HBr/AcOH 33%, 2. ArSH, Bu_4NHSO_4, NaOH(aq)/CH_2Cl_2, 83% for **54β**, 71% for **55β**; c) 1. PCl_5, $BF_3(Et_2O)$, CH_2Cl_2, 2. ArSNa, DMF, 50°C, 82% for **53α**, 66% for **54α**, 73% for **55α**; d) *m*-chloroperoxybenzoic acid, anhydrous CH_2Cl_2, 0°C→RT, 100% for **59β** and **61β**, 76% for **60β**, 100% for **59α**, 83% for **60α**, 95% for **61α**; e) NaOMe/MeOH, 100% for **56β**, 74% for **57β**, 84% for **58β**, 100% for **56α**, **57α**, **58α**, 94% for **62β**, 77% for **63β**, 88% for **64β**, 98% for **62α** and **63α**, 82% for **64α**.

outlined in Scheme 5. The preparation of the aryl β-thiogalactopyranosides was accomplished by following two methodologies. Direct glycosylation of penta-*O*-acetyl-β-D-galactopyranose with thiophenol, in the presence of TMSOTf, afforded **53β** in 86% yield. *Para*- and *meta*-NO_2-aryl thiogalactopyranosides **54β** and **55β** were obtained in 83 and 71% yield, respectively, by phase-transfer catalysis from acetobromogalactose and the corresponding aryl thiols by using a mixture of $NaOH_{aq}/CH_2Cl_2$ and tetrabutylammonium hydrogenosulfate as catalyst. The α series were obtained by reaction of penta-*O*-acetyl-β-D-galactopyranose with PCl_5 in the presence of $BF_3{\cdot}Et_2O$ to give highly reactive tetra-*O*-acetyl-β-D-galactopyranosyl chloride,[34] which reacts with the appropriate aryl thiols furnishing **53α**, **54α**, and **55α** in 82, 66, and 73% yield, respectively.

Oxidation of the aryl β- and α-tetra-*O*-acetyl-D-thiogalactosides gave the corresponding sulfones **59–61** (α and β) in good yields. Deprotection under Zemplén conditions of both series of compounds gave **56–58** (α and β) and **62–64** (α and β) in excellent yields.

Ligand evaluation by weak affinity chromatography of compounds from Generation IV: The biological evaluation of these compounds was carried out by the WAC method as indicated above. Compounds of Generation IV are *S* analogues of the reported MNPG series. They all showed lower affinity than MNPG itself, although it can be concluded that the presence of a *m*-NO_2 group is necessary for the affinity. *m*-Nitrophenyl α-D-thiogalactoside **58α** (Table 3, entry 10) showed better affinity than its corresponding β-D-thiogalactoside **58β** (Table 3, entry 4). Oxidation of the sulfur to sulfone in these compounds did not improve their binding affinity. Although aryl *O*-galactoside MNPG[12] is a slightly better CTB binder than its *S* analogue, the introduction of a sulfur group at the anomeric position provides better acid and enzyme-mediated hydrolytic stability. In addition, the higher water solubility of sulfur derivatives relative to their oxygen counterparts is an important advantage for our compounds with regard to tolerance by most biological systems.[20]

Table 3. Retention factor (k') and dissociation constant (K_d) of CT ligands at 22°C determined with WAC at pH 7 for Generation IV.

Entry	Compound	k'	K_d [mM]	K_{MNPG}/K_d
1	**MNPG**	1.58	1.1[a]	1
2	**56β**	0.16	NB	–
3	**57β**	0.19	NB	–
4	**58β**	0.33	4.7	0.21
5	**62β**	0.07	NB	–
6	**63β**	0.11	NB	–
7	**64β**	0.08	NB	–
8	**56α**	0.19	NB	–
9	**57α**	<0.2	NB	–
10	**58α**	0.63	2.5	0.44
11	**62α**	0.07	NB	–
12	**63α**	0.06	NB	–
13	**64α**	0.28	5.5	0.20

[a] Ref. [13]. NB = no binding.

Conclusion

The synthesis of a library of novel nonhydrolyzable compounds that act as rCTB ligands has been developed. An important point to note in this approach is that polyhydroxyalkylfuroate derivatives presented the highest affinities, being the first examples of non-carbohydrate ligands of CT. They constitute a new type of ligand apart from the well-known MNPG derivatives and the structural mimics of GM1 reported previously.[8–10] These compounds are better ligands themselves than when attached to D-galactose.

Biological evaluation has been carried out by WAC. NMR experiments in the presence of rCTB indicated that the furan moiety and the spacer to thiogalactose are crucial for the ligand–CT interaction. Tail-connected benzyl polyhydroxyalkylfuroates (Generation II) interact along the whole molecule but less tightly in the middle of it, thus indicating a bidentate ligand. However, with head-connected compounds (Generation I), the affinity is dramatically reduced probably because the furan moiety is placed under the thio-

galactose ring by means of stabilizing carbohydrate–π interactions.[35]

For polyhydroxyalkylfuroates (Generation III), besides the furan, the presence of another aromatic moiety is beneficial for the binding affinity, that is, ethyl benzyloxymethyl furoate (**40**) is 5.5 times more active than MNPG itself. Shortening the side chain slightly diminishes the binding affinity. For this type of aryl-tail-substituted compound, with less conformational freedom, a relationship between binding affinity and stereogenic centers was observed.

The presence of S at the anomeric position of D-galactose in the thio analogues of MNPG, although increasing the hydrolytic stability of the compounds, is not beneficial for the binding affinity. Finally, the compounds presented herein represent an important step towards low-cost potent ligands for CT with applications in therapy and detection. The good hydrolytic stability and their easy preparation from carbohydrates that are readily available on a large scale, by using high-performance reactions, will provide new possibilities for the discovery of low-molecular-weight blockers of rCTB. Progress in this area, to find more efficient CTB antagonists, is currently under way in our laboratory.

Experimental Section

General procedures: Optical rotations were measured in a 1.0 cm or 1.0 dm tube with a Jasco P-2000 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were obtained for solutions in CD_3OD, D_2O, $CDCl_3$, and [D_6]DMSO. All the assignments were confirmed by two-dimensional NMR experiments. The fast atom bombardment (FAB)–liquid secondary ion mass spectra were obtained by using glycerol or 3-nitrobenzyl alcohol as the matrix. TLC was performed on silica gel HF_{254} (Merck), with detection by UV light and charring with H_2SO_4, ninhidrin, or Pancaldi reagent [$(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O]. Silica gel 60 (Merck, 230 mesh) was used for preparative chromatography.

STD NMR and transferred NOESY experiments: The NMR binding experiments were carried out at 278 K on a Bruker Avance III 700 MHz spectrometer equipped with a four-channel TCI cryoprobe. For STD NMR experiments, a pulse sequence that included two trim pulses (2.5 and 5 ms) and 3 ms spoil gradient before the first 90° pulse was used, to remove unwanted magnetization in the *x*,y plane between consecutive scans. Water suppression was carried out by excitation sculpting, and the broad signals from the protein were removed by using a 20 ms spin-lock (T1ρ) filter at a field strength of 6.9 kHz. Selective saturation was achieved by using a train of 50 ms Gaussian pulses at 0 ppm (on-resonance experiment), whereas 40 ppm was the frequency of low-power irradiation for the control (off-resonance) experiment. NOESY experiments were carried out at a mixing time of 300 ms. Samples contained 5 μM pentameric subunit B of cholera toxin (rCTB) and 1 mM ligand, on a D_2O pH 7 buffer solution of 20 mM phosphate and 150 mM NaCl, which led to a ligand-to-receptor ratio of 40:1 (five binding sites per protein subunit). To obtain the binding epitopes of the ligands, STD NMR experiments were carried out at different saturation times (0.3, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 s) and the resulting building curves were fitted mathematically to a monoexponential equation, from which the initial slopes were obtained. From these values, the binding epitope was obtained by dividing all by the largest value, to which an arbitrary value of 100% was assigned.

Weak affinity chromatography: Recombinant CTB (Crucell Company, Sweden) was immobilized into Nucleosil silica (10 μm, 300 Å) and packed into a 50×2.1 mm column. The number of active groups of CTB was estimated to be 261 nmol. All chromatographic experiments were performed on an Agilent-1100/Agilent-1200 HPLC system. The mobile phase was 10 mM sodium phosphate, 0.15 mM sodium chloride, pH 7. The flow rate was 0.1 mL min^{-1} and the temperature 22 °C. The sample volume was 5 μL and the sample concentration 35 μM. Detection was performed at 220 nm. The retention factor (k') and the affinity (K_d) of the derivatives were calculated as described previously[13] by using MNPG as reference compound.

Compounds from Generation I

2-(Fluoren-9-yl-methoxycarbonylamino)ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (6): Molecular sieve (4 Å) was added to a solution of *N*-Fmoc-aminoethanothiol (430 mg, 1.44 mmol) and penta-*O*-acetyl-β-D-galactopyranose **5** (375 mg, 0.96 mmol) in dry dichloromethane (10 mL), and the mixture was stirred for 15 min. Then, trimethylsilyl trifluoromethanesulfonate (265 μL, 1.44 mmol) was added, and the solution was stirred at RT overnight. After this period, the mixture was washed with a saturated aqueous solution of $NaHCO_3$, water, and brine, dried with Na_2SO_4, filtered, and concentrated. The resulting residue was purified by column chromatography (AcOEt/petroleum ether, 1:2→1:1) to give **6** (432 mg, 1.22 mmol, 72%) as a white solid. $[\alpha]_D^{27} = -17$ (c = 0.58 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 7.80–7.29 (m, 8H; H-Ar), 5.62 (br t, 1H; N*H*Fmoc), 5.25 (d, $J_{4,3}$ = 3 Hz, 1H; H-4), 5.12 (t, $J_{2,1} = J_{2,3}$ = 9.9 Hz, 1H; H-2), 4.94 (dd, 1H; H-3), 4.51 (d, 1H; H-1), 4.75 (dd, $^2J_{H-H}$ = 10.8, $^3J_{H-H}$ = 5.4 Hz, 1H; C*H*H of Fmoc), 4.55 (dd, $^3J_{H-H}$ = 5.1 Hz, 1H; CH*H* of Fmoc), 4.28–4.14 (m, 2H; H-1, CH of Fmoc), 3.90 (dd, $J_{6a,5}$ = 8.1 Hz, 1H; H-6a), 3.80 (dd, $J_{6b,6a}$ = 11.4, $J_{6b,5}$ = 4.2 Hz, 1H; H-6b), 3.69–3.53 (m, 1H; H-2a′), 3.28–3.10 (m, 2H; H-2b′, H-5), 3.80–3.67 (m, 2H; H-1a′, H-1b′), 2.13, 2.07, 2.01, 1.69 ppm (4s, 12H; Me of OAc); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C, mixture of rotamers): δ = 170.1–169.5 (4 C=O of OAc), 156.6 (C=O of Fmoc), 146.8, 141.3, 127.8, 127.2, 124.7, 120.0 (C-Ar), 85.1 (C-1), 74.1 (C-5), 71.5 (C-3), 67.3 (C-4, C-2), 65.4 (*C*H$_2$ of Fmoc), 62.4 (C-6), 47.3 (*C*H of Fmoc), 42.3 (C-2′), 32.5 (C-1′), 20.8–20.6 ppm (4 Me of OAc); IR: $\tilde{\nu}$ = 1743 (C=O), 1216, 1037 cm^{-1}; MS (FAB): *m/z* (%): 652 (20) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{31}H_{35}NO_{11}NaS$ (*M*+Na)$^+$: 652.1829; found: 652.1832.

(2*S*)-2-[2-(*tert*-Butoxycarbonylamino)-2-methoxycarbonyl]ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (7): A solution of HBr/AcOH (33%, 3.5 mL) was added to a solution of penta-*O*-acetyl-β-D-galactopyranose **5** (1.20 g, 3.08 mmol) in dry dichloromethane (20 mL) cooled to 0 °C. The reaction mixture was stirred for 1 h at 0 °C, and then at RT for 3.5 h. Then, the mixture was washed sequentially with cooled aqueous solutions of 1% $NaHCO_3$, saturated $NaHCO_3$, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude was dissolved in AcOEt (25 mL) and added to a solution of *N*-Boc-L-Cys-OMe (723 mg, 3.08 mmol) in a 10% aqueous solution of Na_2CO_3 (25 mL). Then, TBAHS (4.20 g, 12.32 mmol) was added and the reaction mixture was stirred vigorously at RT overnight. After this period, the reaction mixture was diluted with AcOEt and washed with a saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The resulting crude was purified by column chromatography (AcOEt/petroleum ether, 1:2) to give **7** (986 mg, 1.74 mmol, 57%) as a white solid. $[\alpha]_D^{27} = -26$ (c = 1.01 in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 5.63 (br d, $J_{NH,2'}$ = 7.5 Hz, 1H; N-H), 5.43 (dd, $J_{4,3}$ = 3.3, $J_{4,5}$ = 0.9 Hz, 1H; H-4), 5.22 (t, $J_{2,1} = J_{2,3}$ = 9.9 Hz, 1H; H-2), 5.04 (dd, 1H; H-3), 4.52 (m, 1H; H-2′), 4.50 (d, 1H; H-1), 4.16 (d, $J_{6,5}$ = 6.6 Hz, 2H; H-6), 3.95 (td, 1H; H-5), 3.75 (s, 3H; COOC*H*$_3$), 3.22 (dd, $^2J_{1a',1b'}$ = 14.4, $J_{1a',2'}$ = 4.2 Hz, 1H; H-1a′), 3.03 (dd, $J_{1b',2'}$ = 6.3 Hz, 1H; H-1b′), 2.16, 2.08, 2.06, 1.98 (4s, 12H; Me of OAc), 1.45 ppm (s, 9H; C(C*H*$_3$)$_3$); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25 °C): δ = 171.4 (*C*OOMe), 170.5, 170.3, 170.1, 169.8 (4C, C=O of OAc), 155.6 (C=O of Boc), 83.7 (*C*(CH$_3$)$_3$), 80.3 (C-1), 75.0 (C-5), 71.8 (C-3), 67.2, 66.8 (C-4, C-2), 61.4 (C-6), 53.5 (C-2′), 52.7 (COO*C*H$_3$), 32.1 (C-1′), 28.5 (C(*C*H$_3$)$_3$), 20.9, 20.8, 20.7 ppm (4C, Me of OAc); IR: $\tilde{\nu}$ = 1754, 1715 (C=O), 1216, 1032 cm^{-1}; MS (FAB): *m/z* (%): 588 (100) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{23}H_{35}NO_{13}SNa$ (*M*+Na)$^+$: 588.1727; found: 588.1737.

5-(1-Acetamido-2,3,4-tetra-*O*-acetyl-1-deoxy-D-ribotetritol-1-yl)-2-methyl-3-furoic acid (10): A solution of **9**[21] (318 mg, 0.88 mmol) in anhy-

drous MeOH (10 mL) was hydrogenated under atmospheric pressure for 3 h using Pd-C (10%) as catalyst. Then, the solution was filtered through Celite and the catalyst was washed with MeOH. The filtered solution was concentrated, and conventionally acetylated. Column chromatography (CH_2Cl_2/MeOH, 25:1) gave pure **10** (287 mg, 0.70 mmol, 80%) as a white solid. $[\alpha]_D^{27} = -66$ ($c = 0.62$ in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C): $\delta = 6.50$ (s, 1H; H-4), 6.49 (br d, $J_{NH,1'} = 9.3$ Hz, 1H; N*H*Ac), 5.43 (dd, $J_{1',2'} = 5.1$ Hz, 1H; H-1′), 3.34 (dd, $J_{2',3'} = 6.0$ Hz, 1H; H-2′), 3.17 (td, $J_{3',4b'} = 6.0$, $J_{3',4a'} = 2.7$ Hz, 1H; H-3′), 4.36 (dd, $^2J_{4a',4b'} = 12.6$ Hz, 1H; H-4a′), 4.20 (dd, 1H; H-4b′), 2.56 (s, 3H; Me), 2.07, 2.06, 2.05, 2.04 ppm (4s, 12H; Me of Ac); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25°C): $\delta = 170.9$, 170.6, 170.3, 169.8 (C=O of Ac), 168.1 (*C*OOH), 160.8 (C-2), 148.5 (C-5), 113.7 (C-3), 108.9 (C-4), 72.1 (C-2′), 69.8 (C-3′), 62.1 (C-4′), 47.5 (C-1′), 23.4, 21.0, 20.8 (Me of Ac), 14.0 ppm (Me); IR: $\tilde{\nu} = 1743$ (C=O), 1211, 1047 cm^{-1}; MS (CI): *m/z* (%): 414 (30) [*M*+H$^+$]; HRMS (CI): *m/z* calcd for $C_{18}H_{24}NO_{10}$ (*M*+H)$^+$: 414.1400; found: 414.1385.

5-(1-Acetamido-1-deoxy-D-ribotetritol-1-yl)-2-methyl-3-furoic acid (10a): NaOMe in methanol (1M, 0.1 equiv per *O*-acetyl group) was added to a solution of compound **10** (50 mg, 0.12 mmol) in anhydrous MeOH (1 mL), and the reaction mixture was stirred for 1 h at RT. Then the mixture was neutralized with Amberlite IR-120H$^+$, filtered, and washed with MeOH. The filtered solution was concentrated to give pure **10a** (quant.) as a white solid. $[\alpha]_D^{27} = +48$ ($c = 0.73$ in MeOH); ^{1}H NMR (300 MHz, CD_3OD, 25°C): $\delta = 6.58$ (s, 1H; H-4), 5.35 (d, $J_{1',2'} = 3.9$ Hz, 1H; H-1′), 3.81–3.69 (m, 2H; H-2′, H-4a′), 3.59 (dd, $^2J_{4b',4a'} = 11.4$, $J_{4b',3'} = 5.9$ Hz, 1H; H-4b′), 3.43 (m, 1H; H-3′), 2.54 (s, 3H; Me), 1.99 ppm (s, 3H; Me of NHAc); ^{13}C NMR (75.4 MHz, CD_3OD, 25°C): $\delta = 172.4$ (2C, C=O of NHAc, *C*OOH), 159.4 (C-2), 151.3 (C-5), 114.6 (C-3), 110.4 (C-4), 74.4 (C-2′), 73.3 (C-3′), 64.7 (C-4′), 50.4 (C-1′), 22.6 (Me of NHAc), 13.8 ppm (Me); IR: $\tilde{\nu} = 3306$ (COOH, OH), 2927, 1649 (C=O), 1226, 1068 cm^{-1}; MS (FAB): *m/z* (%): 310 (27) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{12}H_{17}NO_7Na$ (*M*+Na)$^+$: 310.0903; found: 310.0901.

2-[5-(1-Acetamido-2,3,4-tri-*O*-acetyl-1-deoxy-D-ribotetritol-1-yl)-2-methyl-3-furancarboxamido]ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (12): Compound **6** (200 mg, 0.32 mmol) was dissolved in piperidine/DMF (20%; 8.4 mL) and the mixture was stirred at RT for 15 min. Then, the solvent was removed and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 8:1) affording the unprotected amine (83 mg, 0.20 mmol, 63%) as a yellow oil. The resulting amine (72 mg, 0.18 mmol) was dissolved in DMF (4 mL), and acid derivative **10** (80 mg, 0.20 mmol), DIPEA (132 μL, 0.78 mmol), and PyBOP (105 mg, 0.20 mmol) were added sequentially. The reaction mixture was stirred vigorously at RT overnight. Then the solvent was removed, and the residue was diluted with AcOEt and washed with 1M HCl, a saturated aqueous solution of $NaHCO_3$, and water. The organic phase was dried (Na_2SO_4), filtered, and concentrated. Chromatographic purification on silica gel (ether/acetone, 7:1→5:1) afforded the protected ligand **12** (76 mg, 0.10 mmol, 56%) as a yellow solid. $[\alpha]_D^{27} = +29$ ($c = 0.80$ in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, 25°C): $\delta = 6.56$ (br d, $J_{NH,1''} = 8.9$ Hz, 1H; N*H*Ac), 6.34 (s, 1H; H-4‴), 6.29 (br t, 1H; N-H), 5.44 (dd, $J_{4,3} = 3.0$ Hz, 1H; H-4), 5.40 (dd, $J_{1'',2''} = 5.2$ Hz, 1H; H-1″), 5.34 (br t, $J_{2'',3''} = 5.8$ Hz, 1H; H-2″), 5.30 (t, $J_{2,1} = J_{2,3} = 9.9$ Hz, 1H; H-2), 5.19 (ddd, $J_{3'',4b''} = 6.1$, $J_{3'',4b''} = 2.6$ Hz, 1H; H-3″), 5.06 (dd, 1H; H-3), 4.52 (d, 1H; H-1), 4.36 (dd, $^2J_{4a'',4b''} = 12.1$ Hz, 1H; H-4a″), 4.20 (dd, 1H; H-4b″), 4.14 (dd, $^2J_{6a,6b} = 11.2$, $J_{6a,5} = 6.9$ Hz, 1H; H-6a) 4.05 (dd, $J_{6b,5} = 6.1$ Hz, 1H; H-6b), 3.96 (br td, 1H; H-5), 3.68 (m, 1H; H-2a′), 3.53 (m, 1H; H-2b′), 3.03 (m, 1H; H-1a′), 2.84 (m, 1H; H-1b′), 2.55 (s, 3H; Me), 2.19–1.96 ppm (8s, 24H; Me of Ac); ^{13}C NMR (125.7 MHz, $CDCl_3$, 25°C): $\delta = 170.4$–169.5 (8C, C=O of OAc), 163.4 (*C*ONH), 156.8 (C-2‴), 148.1 (C-5‴), 116.2 (C-3‴), 106.5 (C-4‴), 84.0 (C-1), 74.8 (C-5), 72.0 (C-2″), 71.7 (C-3), 69.8 (C-3″), 67.3 (C-4), 67.1 (C-2), 61.9 (C-4″), 61.7 (C-6), 47.3 (C-1″), 38.9 (C-2′), 30.5 (C-1′), 23.2 (Me of NHAc), 20.9–20.5 (8C, Me of OAc), 13.7 ppm (Me); IR: $\tilde{\nu} = 1739$ (C=O), 1211, 1046 cm^{-1}; MS (FAB): *m/z* (%): 825 (100) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{34}H_{46}N_2O_{18}NaS$ (*M*+Na)$^+$: 825.2364; found: 825.2377.

2-[5-(1,2,3,4-Tetra-*O*-acetyl-D-arabinotetritol-1-yl)-2-methyl-3-furancarboxamido]ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (13): This compound was prepared following the procedure described for **12** except that acid derivative **11**[22] was used as starting material. Purification on silica gel (ether/acetone, 15:1) afforded **13** in 42% yield as a yellow oil. $[\alpha]_D^{26} = -16$ ($c = 0.90$ in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C): $\delta = 6.53$ (s, 1H; H-4‴), 6.23 (br t, 1H; N-H), 6.01 (d, $J_{1'',2''} = 5.1$ Hz, 1H; H-1″), 5.61 (dd, $J_{2'',3''} = 7.2$ Hz, 1H; H-2″), 5.44 (dd, $J_{4,3} = 3.3$, $J_{4,5} = 0.9$ Hz, 1H; H-4), 5.26 (t, $J_{2,1} = J_{2,3} = 9.9$ Hz, 1H; H-2), 5.16 (ddd, $J_{3'',4b''} = 8.7$, $J_{3'',4b''} = 3.3$ Hz, 1H; H-3″), 5.06 (dd, 1H; H-3), 4.51 (d, 1H; H-1), 4.25 (dd, $^2J_{4a'',4b''} = 12.6$ Hz, 1H; H-4a″), 4.18–4.06 (m, 3H; H-4b″, H-6a, H-6b), 3.96 (br td, $J_{5,6} = 5.8$ Hz, 1H; H-5), 3.71 (m, 1H; H-2a′), 3.52 (m, 1H; H-2b′), 3.01 (m, 1H; H-1a′), 2.84 (m, 1H; H-1b′), 2.57 (s, 3H; Me), 2.17, 2.10, 2.08, 2.07, 2.05, 2.04, 2.03, 1.99 ppm (8s, 24H; Me of OAc); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25°C): $\delta = 170.1$–169.5 (8C, C=O de OAc), 163.4 (*C*ONH), 157.8 (C-2‴), 146.7 (C-5‴), 116.5 (C-3‴), 109.0 (C-4‴), 84.1 (C-1), 75.0 (C-5), 71.9 (C-3), 70.1 (C-2″), 68.9 (C-3″), 67.5 (C-4), 67.3 C-2), 66.0 (C-1″), 61.7 (C-4″, C-6), 38.9 (C-2′), 30.7 (C-1′), 20.9–20.7 (8C, Me of OAc), 13.7 ppm (Me); IR: $\tilde{\nu} = 1739$ (C=O), 1206, 1041 cm^{-1}; MS (FAB): *m/z* (%): 826 (35) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{34}H_{45}NO_{19}NaS$ (*M*+Na)$^+$: 826.2206; found: 826.2204.

(2*S*)-2-{5-[(1-Acetamido-2,3,4-tri-*O*-acetyl-1-deoxy-D-ribotetritol-1-yl)-2-methyl-3-furancarboxamido]-2-methoxycarbonyl}ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (14): Compound **7** (100 mg, 0.18 mmol) was dissolved in TFA/CH_2Cl_2 (20%, 3 mL) and the mixture was stirred at RT for 2 h. The resulting amine was dissolved in DMF (4 mL) and acid derivative **10** (81 mg, 0.20 mmol), DIPEA (146 μL, 0.86 mmol), and PyBOP (105 mg, 0.20 mmol) were added sequentially. The reaction mixture was stirred vigorously at RT overnight. Then the solvent was removed, and the residue was diluted with AcOEt and washed with 1M HCl, a saturated aqueous solution of $NaHCO_3$, and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated. Chromatographic purification on silica gel (ether/acetone, 6:1) afforded the protected ligand **14** (134 mg, 0.16 mmol, 89%) as a white solid. $[\alpha]_D^{26} = +24$ ($c = 1.01$ in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C): $\delta = 6.97$ (br d, $J_{NH,1''} = 9.0$ Hz, 1H; N*H*Ac), 6.80 (br d, $J_{NH,2'} = 7.2$ Hz, 1H; N-H), 6.41 (s, 1H; H-4‴), 5.44 (dd, $J_{1'',2''} = 6.0$ Hz, 1H; H-1″), 5.40–5.36 (m, 2H; H-4, H-2″), 5.21 (t, $J_{2,1} = 10.0$ Hz, 1H; H-2), 5.17 (m, 1H; H-3″), 5.02 (dd, $J_{3,2} = 10.0$, $J_{3,4} = 3.3$ Hz, 1H; H-3), 4.95 (m, 1H; H-2′), 4.55 (d, 1H; H-1), 4.34 (dd, $^2J_{4a'',4b''} = 12.4$, $J_{4a'',3''} = 2.7$ Hz, 1H; H-4a″), 4.22 (dd, $J_{4b'',3''} = 6.6$ Hz, 1H; H-4b″), 4.03 (dd, $J_{6a,5} = 7.8$, $J_{6b,5} = 1.8$ Hz, 2H; H-6a, H-6b), 3.88 (m, 1H; H-5), 3.79 (s, 3H; COOC*H*$_3$), 3.34 (dd, $^2J_{1a',1b'} = 14.4$, $J_{1a',2'} = 4.5$ Hz, 1H; H-1a′), 3.12 (dd, $J_{1b',2'} = 6.0$ Hz, 1H; H-1b′), 2.56 (s, 3H; Me), 2.15, 2.11, 2.06, 2.05, 2.04, 2.04, 2.03, 2.00 ppm (8s, 24H; Me of Ac); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25°C): $\delta = 171.0$, 170.9, 170.7, 170.6, 170.4, 170.3, 170.2, 170.0, 169.8 (8C, C=O of ester), 163.2 (*C*ONH), 157.6 (C-2‴), 149.1 (C-5‴), 115.8 (C-3‴), 106.2 (C-4‴), 84.2 (C-1), 74.9 (C-5), 71.9, 71.8 (C-3, C-2″), 70.1 (C-3″), 67.4, 67.3 (C-4, C-2), 62.0 (C-4″), 61.5 (C-6), 53.0 (COO*C*H$_3$), 52.2 (C-2′), 47.2 (C-1″), 31.8 (C-1′), 23.3 (Me of NHAc), 21.0–20.7 (7C, Me of Ac), 13.8 ppm (Me); IR: $\tilde{\nu} = 1739$ (C=O), 1211, 1046 cm^{-1}; MS (FAB): *m/z* (%): 883 (100) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{36}H_{48}N_2O_{20}NaS$ (*M*+Na)$^+$: 883.2419; found: 883.2399.

(2*S*)-2-{5-[(1,2,3,4-Tetra-*O*-acetyl-D-arabinotetritol-1-yl)-2-methyl-3-furancarboxamido]-2-methoxycarbonyl}ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (15): This compound was prepared following the procedure described for **14** except that acid derivative **11** was used as starting material. Purification by column chromatography on silica gel (ether/acetone, 15:1) afforded **15** in 89% yield as a white solid. $[\alpha]_D^{26} = -33$ ($c = 1.04$ in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$, 25°C): $\delta = 6.61$ (s, 1H; H-4‴), 6.54 (br d, $J_{NH,2'} = 7.2$ Hz, 1H; N-H), 6.01 (d, $J_{1'',2''} = 5.0$ Hz, 1H; H-1″), 5.59 (dd, $J_{2'',3''} = 7.2$ Hz, 1H; H-2″), 5.41 (dd, $J_{4,3} = 3.0$, $J_{4,5} = 0.6$ Hz, 1H; H-4), 5.21 (t, $J_{2,1} = J_{2,3} = 9.9$ Hz, 1H; H-2), 5.17 (m, 1H; H-3″), 5.05 (dd, 1H; H-3), 4.91 (td, 1H; H-2′), 4.52 (d, 1H; H-1), 4.25 (dd, $^2J_{4a'',4b''} = 12.3$, $J_{4a'',3''} = 3$ Hz, 1H; H-4a″), 4.12 (dd, $J_{4b'',3''} = 5.4$ Hz, 1H; H-4b″), 4.06 (dd, $J_{6a,5} = 7.2$, $J_{6b,5} = 0.9$ Hz, 2H; H-6a, H-6b), 3.91 (m, 1H; H-5), 3.79 (s, 3H; COOC*H*$_3$), 3.33 (dd, $^2J_{1a',1b'} = 13.8$, $J_{1a',2'} = 4.8$ Hz, 1H; H-1a′), 3.08 (dd, $J_{1b',2'} = 6.6$ Hz, 1H; H-1b′), 2.58 (s, 3H; Me), 2.16, 2.11, 2.07, 2.06, 2.05, 2.05, 2.02, 1.98 ppm (8s, 24H; Me of OAc); ^{13}C NMR (75.4 MHz, $CDCl_3$, 25°C): $\delta = 170.1$–169.5 (8C, C=O of ester), 163.0 (*C*ONH), 158.4 (C-2‴), 146.9 (C-5‴), 115.9 (C-3‴), 109.0 (C-4‴), 83.7 (C-1), 74.8 (C-5), 71.7 (C-3), 70.1 (C-2″), 68.8 (C-3″), 67.3, 67.2 (C-4, C-2), 66.0 (C-1″), 1.8 (C-4″), 61.4 (C-6), 53.0 (COO*C*H$_3$), 51.8 (C-2′), 31.6

(C-1′), 20.9–20.7 (8C, Me of OAc), 13.8 ppm (Me); IR: $\tilde{\nu}$ = 1739 (C=O), 1211, 1046 cm^{-1}; MS (FAB): *m/z* (%): 884 (100) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{36}H_{47}NO_{21}NaS$ (*M*+Na)$^+$: 884.2259; found: 884.2268.

General deacetylation procedures for the synthesis of 16–19: Sodium methoxide in methanol (0.1 M, 10% molar) was added to a solution of acetylated ligand in anhydrous MeOH (≈0.05 M), and the reaction mixture was stirred for 1–6 h at RT. Then, the mixture was neutralized with Amberlite IR-120H$^+$, filtered, and concentrated.

2-[5-(1-Acetamido-1-deoxy-D-ribotetritol-1-yl)-2-methyl-3-furancarboxamido]ethyl 1-thio-β-D-galactopyranoside (16): Deprotection of **12** (61 mg, 0.08 mmol), by following the general procedure described above, afforded compound **16** (quant.) as a white solid. $[\alpha]_D^{26}$ = +25 (*c* = 0.44 in MeOH); ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 6.55 (s, 1H; H-4‴), 5.21 (d, $J_{1'',2''}$ = 4.4 Hz, 1H; H-1″), 4.49 (d, $J_{1,2}$ = 9.4 Hz, 1H; H-1), 3.95 (d, $J_{4,3}$ = 4.1 Hz, 1H; H-4), 3.85 (dd, $J_{2'',3''}$ = 8.1 Hz, 1H; H-2″), 3.73 (dd, $^2J_{4a'',4b''}$ = 11.9, $J_{4a'',3''}$ = 2.7 Hz, 1H; H-4a″), 3.70–3.46 (m, 9H; H-4b″, H-6a, H-6b, H-2, H-5, H-3, H-2a′, H-2b′, H-3″), 3.05–2.80 (m, 2H; H-1a′, H-1b′), 2.47 (s, 3H; Me), 2.02 ppm (s, 3H; Me of NHAc); ^{13}C NMR (75.4 MHz, D_2O, 25 °C): δ = 173.3 (C=O of NHAc), 166.6 (*C*ONH), 156.8 (C-2‴), 148.8 (C-5‴), 115.6 (C-3‴), 107.5 (C-4‴), 86.0 (C-1), 78.9 (C-5), 73.9 (C-3), 72.1 (C-2″), 71.5 (C-3″), 69.7 (C-2), 68.7 (C-4), 62.5 (C-4″), 61.0 (C-6), 48.9 (C-1″), 39.5 (C-2′), 29.5 (C-1′), 21.8 (Me of NHAc), 12.8 ppm (Me); IR: $\tilde{\nu}$ = 3313 (OH), 1626 (C=O), 1036 cm^{-1}; MS (FAB): *m/z* (%): 531 (25) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{20}H_{32}N_2O_{11}NaS$ (*M*+Na)$^+$: 531.1625; found: 531.1616.

2-[5-(D-*Arabino*tetritol-1-yl)-2-methyl-3-furancarboxamido]ethyl 1-thio-β-D-galactopyranoside (17): Deprotection of **13** (56 mg, 0.07 mmol), by following the general procedure described above, afforded compound **17** (quant.) as a white solid. $[\alpha]_D^{26}$ = −12 (*c* = 0.80 in H_2O); ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 6.60 (s, 1H; H-4‴), 4.89 (d, $J_{1'',2''}$ = 4.0 Hz, 1H; H-1″), 4.52 (d, $J_{1,2}$ = 9.3 Hz, 1H; H-1), 3.98 (d, $J_{4,3}$ = 3.3 Hz, 1H; H-4), 3.90 (dd, $J_{2'',3''}$ = 7.2 Hz, 1H; H-2″), 3.79 (dd, $^2J_{4a'',4b''}$ = 11.4, $J_{4a'',3''}$ = 3.0 Hz, 1H; H-4a″), 3.77–3.55 (m, 8H; H-4b″, H-6a, H-6b, H-5, H-3, H-2a′, H-2b′, H-3″), 3.57 (t, 1H; H-2), 3.09–2.87 (m, 2H; H-1a′, H-1b′), 2.50 ppm (s, 3H; Me); ^{13}C NMR (75.4 MHz, D_2O, 25 °C): δ = 167.4 (*C*ONH), 157.3 (C-2‴), 152.8 (C-5‴), 116.3 (C-3‴), 107.2 (C-4‴), 86.7 (C-1), 79.6 (C-5), 74.6 (C-3), 73.3 (C-2″), 71.8 (C-3″), 70.3 (C-2), 69.4 (C-4), 67.2 (C-1″), 63.2 (C-4″), 61.7 (C-6), 40.1 (C-2′), 30.2 (C-1′), 12.4 ppm (Me); IR: $\tilde{\nu}$ = 3243 (OH), 1081, 1051, 838 cm^{-1}; MS (FAB): *m/z* (%): 490 (5) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{18}H_{29}NO_{11}NaS$ (*M*+Na)$^+$: 490.1359; found: 490.1340.

(2*S*)-2-{5-[(1-Acetamido-1-deoxy-D-*ribo*tetritol-1-yl)-2-methyl-3-furancarboxamido]-2-methoxycarbonyl}ethyl 1-thio-β-D-galactopyranoside (18): Deprotection of **14** (111 mg, 0.13 mmol), by following the general procedure described above, afforded compound **18** (quant.) as a white solid. $[\alpha]_D^{31}$ = −2 (*c* = 1.38 in H_2O); ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 6.74 (s, 1H; H-4‴), 5.36 (d, $J_{1'',2''}$ = 3.9 Hz, 1H; H-1″), 4.84 (dd under CD_3OD, 1H; H-2′), 4.37 (d, $J_{1,2}$ = 9.6 Hz, 1H; H-1), 3.88 (br d, $J_{4,3}$ = 2.7 Hz, 1H; H-4), 3.80 (dd, $J_{2'',3''}$ = 8.4 Hz, 1H; H-2″), 3.75 (s, 3H; COOC*H*$_3$), 3.76–3.33 (m, 9H; H-4a″, H-4b″, H-3″, H-1a′, H-6a, H-6b, H-5, H-3, H-2), 3.08 (dd, $^2J_{1b',1a'}$ = 14.1, $J_{1b',2'}$ = 7.8 Hz, 1H; H-1b′), 2.53 (s, 3H; Me), 2.00 ppm (s, 3H; Me of NHAc); ^{13}C NMR (75.4 MHz, CD_3OD, 25 °C): δ = 172.9 (*C*OOCH$_3$), 172.5 (C=O of NHAc), 166.3 (*C*ONH), 157.9 (C-2‴), 151.3 (C-5‴), 116.9 (C-3‴), 108.5 (C-4‴), 87.7 (C-1), 80.9 (C-5), 76.2 (C-3), 74.3 (C-2″), 73.3 (C-3″), 71.6 (C-2), 70.5 (C-4), 64.7 (C-6), 62.7 (C-4″), 54.2 (C-2′), 53.0 (COO*C*H$_3$), 49.9 (C-1″), 32.9 (C-1′), 22.7 (Me of NHAc), 13.7 ppm (Me); IR: $\tilde{\nu}$ = 3315 (OH), 1737, 1638 (C=O), 1524, 1227, 1036 cm^{-1}; MS (FAB): *m/z* (%): 589 (31) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{22}H_{34}N_2O_{13}NaS$ (*M*+Na)$^+$: 589.1679; found: 589.1700.

(2*S*)-2-{5-[(D-*arabino*tetritol-1-yl)-2-methyl-3-furancarboxamido]-2-methoxy-carbonyl}ethyl 1-thio-β-D-galactopyranoside (19): Deprotection of **15** (108 mg, 0.13 mmol), by following the general procedure described above, afforded compound **19** (quant.) as a white solid. $[\alpha]_D^{31}$ = −65 (*c* = 0.82 in H_2O); ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 6.66 (s, 1H; H-4‴), 4.89 (d, $J_{1'',2''}$ = 3.9 Hz, 1H; H-1″), 4.82 (dd, 1H; H-2′), 4.50 (d, $J_{1,2}$ = 9.3 Hz, 1H; H-1), 3.96 (br d, $J_{4,3}$ = 3.0 Hz, 1H; H-4), 3.89 (dd, $J_{2'',3''}$ = 6.9 Hz, 1H; H-2″), 3.79 (s, 3H; COOC*H*$_3$), 3.78–3.58 (m, 7H; H-4a″, H-4b″, H-3″, H-6a, H-6b, H-5, H-3), 3.56 (t, $J_{2,3}$ = 9.3 Hz, 1H; H-2), 3.44 (dd, $^2J_{1a',1b'}$ = 14.4, $J_{1a',2'}$ = 5.1 Hz, 1H; H-1a′), 3.12 (dd, $J_{1b',2'}$ = 9 Hz, 1H; H-1b′), 2.50 ppm (s, 3H; Me); ^{13}C NMR (75.4 MHz, D_2O, 25 °C): δ = 173.3 (*C*OOCH$_3$), 167.3 (*C*ONH), 158.1 (C-2‴), 152.9 (C-5‴), 115.7 (C-3‴), 107.1 (C-4‴), 86.1 (C-1), 79.7 (C-5), 74.5 (C-3), 73.3 (C-2″), 71.8 (C-3″), 70.3 (C-2), 69.3 (C-4), 67.2 (C-1″), 63.2 (C-6), 61.6 (C-4″), 53.8 (COO*C*H$_3$), 53.5 (C-2′), 31.2 (C-1′), 13.5 ppm (Me); IR: $\tilde{\nu}$ = 3284 (OH), 1735 (C=O), 1636, 1223, 1037 cm^{-1}; MS (FAB): *m/z* (%): 548 (15) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{20}H_{31}NO_{13}NaS$ (*M*+Na)$^+$: 548.1414; found: 548.1400.

2-(Acetamido)ethyl 1-thio-β-D-galactopyranoside (20): Compound **6** (200 mg, 0.32 mmol) was dissolved in piperidine/DMF (20%, 8.4 mL), and the mixture was stirred at RT overnight. Evaporation of the solvent followed by conventional acetylation and purification by column chromatography (CH_2Cl_2/MeOH, 8:1) afforded the deprotected amine (83 mg, 0.20 mmol, 63%) as a yellow oil. Deacetylation of the resulting amine (108 mg, 0.13 mmol) by following the general procedure described above and purification of the residue by column chromatography (CH_2Cl_2/MeOH/NH_4OH, 2:1:0.35) afforded **20** (quant.) as a yellow solid. $[\alpha]_D^{26}$ = −17 (*c* = 0.50 in MeOH); ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 4.34 (d, $J_{1,2}$ = 9.4 Hz, 1H; H-1), 3.89 (dd, $J_{4,3}$ = 3.2, $J_{4,5}$ = 0.8 Hz, 1H; H-4), 3.77 (dd, $^2J_{6a,6b}$ = 11.5, $J_{6a,5}$ = 7.1 Hz, 1H; H-6a), 3.69 (dd, $J_{6b,5}$ = 5.0 Hz, 1H; H-6b), 3.56 (t, 1H; H-2), 3.56 (m, 1H; H-5), 3.51–3.39 (m, 3H; H-3, H-2a′, H-2b′), 2.88 (m, 1H; H-1a′), 2.76 (m, 1H; H-1b′), 1.95 ppm (s, 3H; Me); ^{13}C NMR (75.5 MHz, CD_3OD, 25 °C): δ = 173.4 (C=O of NHAc), 87.8 (C-1), 80.8 (C-5), 76.2 (C-3), 71.4 (C-2), 70.6 (C-2), 62.8 (C-6), 41.2 (C-2′), 30.7 (C-1′), 22.6 ppm (Me); IR: $\tilde{\nu}$ = 3320 (OH), 1630 (C=O), 1225, 1004 cm^{-1}; MS (CI): *m/z* (%): 282 (4) [*M*+H$^+$]; HRMS (CI): *m/z* calcd for $C_{10}H_{20}NO_6S$ (*M*+Na)$^+$: 282.1011; found: 282.1012.

(2*S*)-2-(2-Amino-2-methoxycarbonyl)ethyl 1-thio-β-D-galactopyranoside (21): Deacetylation of compound **7** (100 mg, 0.17 mmol), by following the general procedure described above, and purification by column chromatography (CH_2Cl_2/MeOH, 10:1) afforded the corresponding *O*-deprotected derivative (65 mg, 0.16 mmol, 94%). This compound was dissolved in TFA/CH_2Cl_2 (20%, 4 mL) and the mixture was stirred at RT for 1.5 h. The reaction mixture was concentrated and purified by column chromatography (CH_2Cl_2/MeOH, 4:1) to give pure **21** (quant.) as its trifluoroacetate salt (yellow oil). $[\alpha]_D^{26}$ = −15 (*c* = 0.63 in MeOH); ^{1}H NMR (300 MHz, CD_3OD, 25 °C): δ = 4.32 (d, $J_{1,2}$ = 9.4 Hz, 1H; H-1), 3.89 (m, 1H; H-4), 3.82–3.72 (m, 2H; H-6a, H-2′), 3.75 (s, 3H; COOMe), 3.69 (dd, $^2J_{6b,6a}$ = 11.4, $J_{6b,5}$ = 4.9 Hz, 1H; H-6b), 3.62–3.52 (m, 2H; H-2, H-5), 3.48 (dd, $J_{3,2}$ = 9.4, $J_{3,4}$ = 3.3 Hz, 1H; H-3), 3.22 (dd, $^2J_{1a',1b'}$ = 14.2, $J_{1a',2'}$ = 4.5 Hz, 1H; H-1a′), 2.88 (dd, $J_{1b',2'}$ = 7.6 Hz, 1H; H-1b′); ^{13}C NMR (125.7 MHz, CD_3OD, 25 °C, acid–base equilibrium): δ = 175.0 (*C*OOMe), 163.2 (c, $^3J_{C,F}$ = 34.8 Hz, CF_3COO^-), 116.8 (c, $^1J_{C,F}$ = 292.7 Hz, CF_3COO^-), 92.2 (C-2′), 88.5, 87.6 (C-1), 81.2, 80.8 (C-5), 76.3, 76.1 (C-3), 71.4, 71.0, 70.7, 70.6, 70.5, 70.3 (C-2, C-4, Me of COOMe), 62.8, 62.7 (C-6), 36.0, 35.0 ppm (C-1′); IR: $\tilde{\nu}$ = 3368 (OH, NH), 1673 (C=O), 1196, 1132 cm^{-1}; MS (ESI): *m/z* calcd for $C_{12}H_{19}F_3NO_9S$ (M^+): 411.1; found: 412.1.

Compounds from Generation II

2-[4-Carbamoyl-1-(4-ethoxycarbonyl-5-methyl-furan-2-yl)-D-*arabino*tetritol]ethyl 1-thio-β-D-galactopyranoside (26): Compound **22**[23] (760 mg, 1.74 mmol) was dissolved in DMF (7 mL), and amine **24**[22] (500 mg, 1.91 mmol) followed by DIPEA (299 μL, 1.74 mmol) and PyBOP (934 mg, 1.74 mmol) were added. The reaction mixture was stirred vigorously at RT overnight. Then the solvent was removed, and the residue was diluted with AcOEt and washed with 1 M HCl, a saturated aqueous solution of $NaHCO_3$, and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 15:1) to give derivative **25** (586 mg, 0.85 mmol, 49%) as a yellow oil. Deacetylation of this compound by following the general procedure afforded **26** (quant.) as a white solid. $[\alpha]_D^{27}$ = +3 (*c* = 0.81 in MeOH); ^{1}H NMR (500 MHz, CD_3OD, 298 K): δ = 6.57 (s, 1H; H-4‴), 4.87 (d, $J_{1'',2''}$ = 2.4 Hz, 1H; H-1″), 4.35 (d, $J_{1,2}$ = 9.6 Hz, 1H; H-1), 4.27 (q, $^3J_{H,H}$ = 7.1 Hz, 2H; C*H*$_2$CH$_3$), 3.86 (br d, 1H; H-4), 3.82–3.74 (m, 2H; H-6a, H-3″), 3.72–3.66 (m, 2H; H-6b, H-2″), 3.58–3.52 (m, 3H; H-2, H-5, H-4a″), 3.46 (dd, $J_{3,4}$ = 9.6, $J_{3,4}$ = 3.3 Hz,

1H; H-3), 3.37 (dd, $^{2}J_{4b'',4a''}=14.0$, $J_{4b'',3''}=6.5$ Hz, 1H; H-4b″), 3.03 (dt, $^{2}J_{1a',1b'}=13.7$, $J_{1a',2'}=7.3$ Hz, 1H; H-1a′), 2.92 (dt, $J_{1b',2'}=6.5$ Hz, 1H; H-1b′), 2.61 (m, 2H; H-2a′, H-2b′), 2.54 (s, 3H; Me), 1.33 ppm (t, 2H; CH_2CH_3); ^{13}C NMR (75.4 MHz, CD_3OD, 25 °C): $\delta=175.3$ (*C*ONH), 165.8 (*C*OOEt), 159.6 (C-2‴), 155.4 (C-5‴), 115.1 (C-3‴), 108.5 (C-4‴), 88.2 (C-1), 80.8 (C-5), 76.2 (C-3), 74.7 (C-2″), 71.2 (C-2, C-3″), 70.7 (C-4), 67.6 (C-1″), 62.9 (C-6), 61.3 (CH_2CH_3), 44.0 (C-4″), 38.0 (C-2′), 27.9 (C-1′), 14.7 (CH_2CH_3), 13.8 ppm (Me); IR: $\tilde{\nu}=3325$ (OH), 2924, 1761, 1630, 1558 (C=O), 1227, 1081 cm^{-1}; MS (FAB): *m/z* (%): 546 (100) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{21}H_{33}NO_{12}NaS$ (*M*+Na)$^+$: 546.1621; found: 546.1605.

Benzyl 5-(4-azido-4-deoxy-D-*arabino*tetritol-1-yl)-2-methyl-3-furoate (28): A cooled solution of tosyl chloride (2.84 g, 14.89 mmol) in anhydrous pyridine (5 mL) was added to a solution of **8**[21] (2 g, 5.95 mmol) in anhydrous pyridine (16 mL) cooled to −15 °C. The reaction mixture was stirred at −15 °C for 1.5 h and then water (1.5 mL) was added and the solvent removed. The resulting residue was diluted with CH_2Cl_2 and sequentially washed with 1 M HCl, saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated. The resulting residue was purified by column chromatography (AcOEt/petroleum ether, 1:1) to give the corresponding tosyl derivative (2.02 g, 4.12 mmol, 69%) as a yellow oil. NaN_3 (160 mg, 2.46 mmol) was added to a solution of this compound (604 mg, 1.23 mmol) in DMF (10 mL), and the mixture was stirred at 80 °C for 1 h. After evaporation of the solvent, the residue was diluted with AcOEt and washed with water. The organic layer was then dried (Na_2SO_4), filtered, and concentrated. Chromatographic purification on silica gel (AcOEt/petroleum ether, 1:1) afforded **28** (336 mg, 0.93 mmol, 76%) as a white solid. $[\alpha]_D^{27}=-11$ (*c*=0.99 in CH_2Cl_2); ^{1}H NMR (300 MHz, CD_3OD, 25 °C): $\delta=7.45$–7.38 (m, 5H; H-Ar), 6.61 (s, 1H; H-4), 5.26 (s, 2H; CH_2Ph), 4.88 (d, $J_{1',2'}=2.1$ Hz, 1H; H-1′), 3.84 (ddd, $J_{3',2'}=8.6$, $J_{3',4b'}=6.3$, $J_{3',4a'}=2.7$ Hz, 1H; H-3′), 3.70 (dd, 1H; H-2′), 3.52 (dd, $^{2}J_{4a',4b'}=12.8$ Hz, 1H; H-4a′), 3.38 (dd, 1H; H-4b′), 2.54 ppm (s, 3H; CH_3); ^{13}C NMR (75.4 MHz, CD_3OD, 25 °C): $\delta=165.4$ (*C*OOBn), 159.9 (C-2), 155.6 (C-5), 137.8 (C_q-Ar), 129.6, 129.2, 129.1 (C-Ar), 114.9 (C-3), 108.5 (C-4), 74.2 (C-2′), 71.6 (C-3′), 67.6 (C-1′), 66.9 (*C*H$_2$Ph), 55.5 (C-4′), 13.8 ppm (CH_3); IR: $\tilde{\nu}=$ 3298 (OH), 2084 (N_3), 1696 (C=O), 1162 cm^{-1}; MS (FAB): *m/z* (%): 384 (36) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{17}H_{19}N_3O_6Na$ (*M*+Na)$^+$: 384.1172; found: 384.1163.

Triazole derivative 30: Aqueous sodium ascorbate (1.1 equiv, 1 M) and an aqueous solution of $CuSO_4$ (0.11 equiv, 0.3 M) were added to a solution of alkyne **27**[25] (110 mg, 0.27 mmol) and azide **28** (109 mg, 0.30 mmol) in MeOH (2.75 mL). The reaction vessel was protected from light and the reaction mixture was stirred vigorously at RT overnight. The mixture was then diluted with MeOH and filtered. The solution was evaporated to dryness, and the resulting crude was purified by column chromatography (CH_2Cl_2/MeOH, 25:1) to give **29** (164 mg, 0.22 mmol, 82%) as a white solid. This compound (114 mg, 0.15 mmol) was deacetylated by following the general procedure to give **30** (quant.) as a white solid. $[\alpha]_D^{27}=-38$ (*c*=0.60 in H_2O/DMSO); ^{1}H NMR (300 MHz, CD_3OD, 25 °C): $\delta=7.96$ (s, 1H; H-triazole), 7.45–7.28 (m, 5H; H-Ar), 6.61 (s, 1H; H-3‴), 5.27 (s, 2H; CH_2Ph), 4.88 (under water, 1H; H-1″), 4.76 (dd, $^{2}J_{4a'',4b''}=14.0$, $J_{4a'',3''}=2.7$ Hz, 1H; H-4a″), 4.39 (dd, $J_{4b'',3''}=8.1$ Hz, 1H; H-4b″), 4.27 (d, $J_{1,2}=9.6$ Hz, 1H; H-1), 4.11 (d, $^{2}J_{1a',1b'}=14.6$ Hz, 1H; H-1a′), 4.04 (td, $J_{3'',2''}=8.1$ Hz, 1H; H-3″), 3.91 (d, 1H; H-1b′), 3.85 (br d, $J_{4,3}=3.4$ Hz, 1H; H-4), 3.78 (dd, $^{2}J_{6a,6b}=11.5$, $J_{6a,5}=7.3$ Hz, 1H; H-6a), 3.71–3.61 (m, 2H; H-6b, H-2″), 3.60 (t, 1H; $J_{2,3}=9.6$ Hz, H-2), 3.52 (m, 1H; H-5), 3.41 (dd, 1H; H-3), 2.54 ppm (s, 3H; Me); ^{13}C NMR (75.4 MHz, CD_3OD, 298 K): $\delta=165.4$ (*C*OOBn), 160.0 (C-5‴), 155.3 (C-2‴), 146.2 (C-triazole), 137.8 (C_q-Ar), 129.6, 129.2, 129.1 (C-Ar), 126.0 (CH-triazole), 115.0 (C-4‴), 108.6 (C-3‴), 86.4 (C-1), 80.8 (C-5), 76.2 (C-3), 75.0 (C-2″), 71.4 (C-2), 71.3 (C-3″), 70.7 (C-4), 67.6 (C-1″), 67.0 (CH_2Ph), 62.9 (C-6), 54.7 (C-4″), 24.2 (C-1′), 13.8 ppm (Me); MS (FAB): *m/z* (%): 618 (32) [*M*+Na$^+$]; HRMS (FAB): *m/z* calcd for $C_{26}H_{33}N_3O_{11}NaS$ (*M*+Na)$^+$: 618.1734; found: 618.1763.

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