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Synthesis and Affinity Evaluation of a Small Library of Bidentate Cholera Toxin Ligands: Towards Nonhydrolyzable Ganglioside Mimics

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Abstract: A small library of nonhydrolyzable mimics of GM1 ganglioside, featuring galactose and sialic acid as pharmacophoric carbohydrate residues, was synthesized and tested. All compounds were synthesized from readily available precursors using high-performance reactions, including click chemistry protocols, and avoiding *O*-glycosidic bonds. Some of the most active molecules also feature a point of further derivatization that can be used for conjugation with polyvalent aglycons. Their affinity towards cholera toxin was assessed by weak affinity chromatography, which allowed a systematic evaluation and selection of the best candidates. Affinity could be enhanced up to one or two orders of magnitude over the affinity of the individual pharmacophoric sugar residues.

Keywords: carbohydrates · cholera toxin · combinatorial chemistry · glycosides · weak affinity chromatography

Introduction

Cholera toxin (CT) belongs to the AB_5 bacterial toxins family, named after the characteristic architecture comprising a single catalytically active component, A, and a nontoxic receptor-binding pentamer of B subunits.[1] The B_5 pentamer is responsible for binding to GM1 ganglioside at the surface of host cells; the complete AB_5 holotoxin is required for the toxic effects. The CT family includes enterotoxins that are responsible for several disorders, from the relatively mild traveller's diarrhea (from *E. coli* heat-labile toxin, LT) to the much more serious cholera. Complexes formed between gangliosides and AB_5 toxins offer a paradigmatic model for studying the structural and thermodynamic basis of protein–carbohydrate interactions. For medicinal chemistry, they may also provide key insights for the structure-based design of glycomimetic drug leads.[2] A rational design of galactose-based ligands for CT and LT has been reported and recently reviewed.[3] A series of CT ligands designed to mimic the three-dimensional structure of GM1 ganglioside has been reported[4] and C-galactosides have been used as scaffolds to prepare a small library of nonhydrolyzable inhibitors of binding.[5]

The B pentamer of CT (CTB) interacts with the soluble, monovalent oligosaccharide portion of GM1 (Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc, GM1os, Scheme 1) with a strong affinity (43 nM at room temperature, as measured by isothermal calorimetry (ITC)).[6] The X-ray structure of the CTB-GM1os complex[7] shows a "two-fingered grip" of the sugar on the toxin, created by a sialic acid "thumb" and a Galβ1-3GalNAc "forefinger". The terminal galactose residue in the forefinger reaches a well-defined galactose binding pocket, which is lined by the indole side chain of Trp-88 and shielded from the solvent. The rest of the toxin binding site is shallow and exposed to solvent. The sialic acid (NeuAc) thumb interacts with a carboxylate binding region, which includes one highly conserved crystallographic water molecule. It has been shown by ITC that the individual "fingers" interact very weakly with the toxin (with dissociation constant in the high mM range),[6] so the high binding affinity of the CTB–GM1os pair has been credited to structural preorganization of GM1os,[8] which allows a near lock-and-key interaction with CTB.

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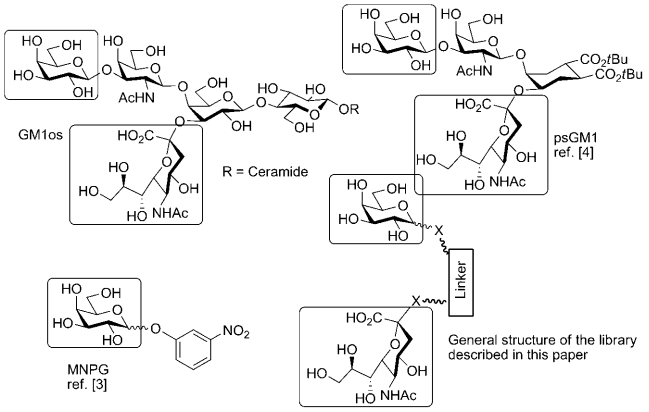
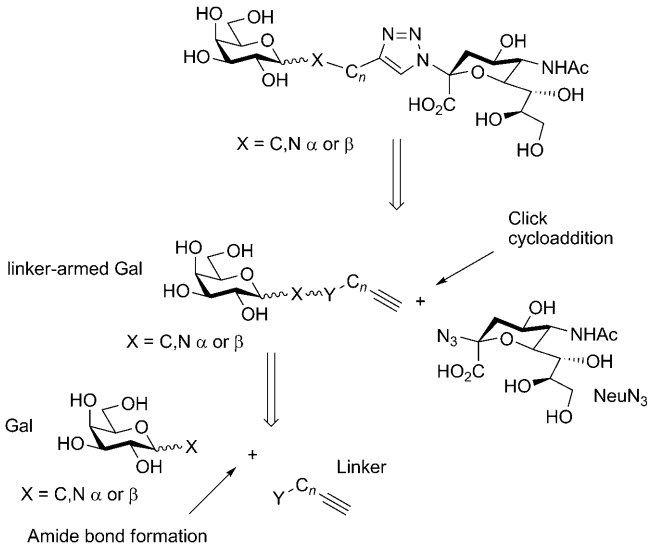


Scheme 1. GM1os, some known CT ligands (ps-GM1, MNPG), and general structure of the proposed library. The pharmacophoric sugar fragments are highlighted in the boxes.



Scheme 2. General strategy for library assembly.

This analysis suggests that functional mimics of GM1 may be designed by stringing together the two pharmacophoric sugar fragments, galactose and sialic acid, while trying to preserve the relative orientation adopted in GM1os. This concept was implemented in our initial work by generating pseudo-GM1 (psGM1, Scheme 1) structures that are close mimics of GM1os.[4] Like other known inhibitors of CT binding, such as *meta*-nitrophenylgalactoside (MNPG, Scheme 1),[3] this molecule contains enzymatically labile *O*-glycosidic linkages that limit their potential application in vivo. Furthermore, the synthetic methods used to connect the pharmacophoric sugar moeties in psGM1 are those of traditional carbohydrate chemistry, which are often laborious and low-yielding procedures. To develop metabolically stable and synthetically more accessible mimics of GM1os, we set our efforts towards the synthesis of a library of bifunctional compounds of the general formula shown in Scheme 1. The target molecules contain a galactose and a sialic acid moiety connected through a linker. By taking advantage of the terminal alkyne of the linker and the ready availablility of the sialic acid azide **26**,[9] a triazole was used to connect the NeuAc residue to the linker (Scheme 2) through a Cu-catalyzed (click) triazole formation reaction.[10] The library design (Scheme 2) relied on combinatorial selection of the alkyne linkers and the galactose-mimicking fragments, with the following two constraints: 1) *O*-glycosides were excluded to stabilize the constructs against the activity of hydrolytic enzymes; 2) the functionalization of the galactose ring (X in Schemes 1 and 2) was chosen to allow a facile conjugation to the putative linkers. Selection of the linker and identification of the best bidentate ligand was achieved by synthesizing a small library of compounds and testing their CTB affinity by weak affinity chromatography (WAC). Herein we report our initial results.

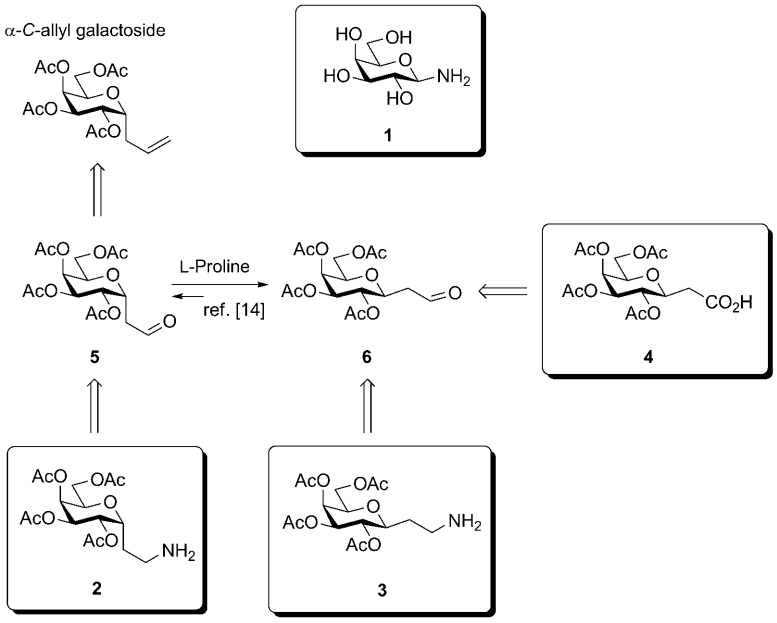
Results and Discussion

Synthesis of the library: A library of bidentate ligands was synthesized using linear alkyne linkers by following the strategy outlined in Scheme 2. Four functionalized Gal fragments were conjugated through an amide bond to linear alkyne linkers of variable length to obtain linker-armed galactose mimics. Incorporation of the sialic acid residue using click chemistry was planned as the final step of the synthesis, since Cu-catalyzed cycloaddition is known to tolerate a wide range of substrates, solvents, and reaction conditions.

The four functionalized Gal fragments (Scheme 3) used were: β-galactosyl amine **1**, α- or β-*C*-aminoethyl galactosides **2** and **3**, respectively, and β-carboxylic acid **4**. All of these fragments can be connected by amide bonds to the alkyne linkers to set the stage for the final "click" sialylation step. β-Galactosyl amine **1** is a well known and readily available compound.[11] The remaining three *C*-galactose derivatives were obtained from known, easily accessible α-*C*-allyl galactoside[12] via α-*C*-galactosyl aldehyde **5**[13] (Scheme 3).

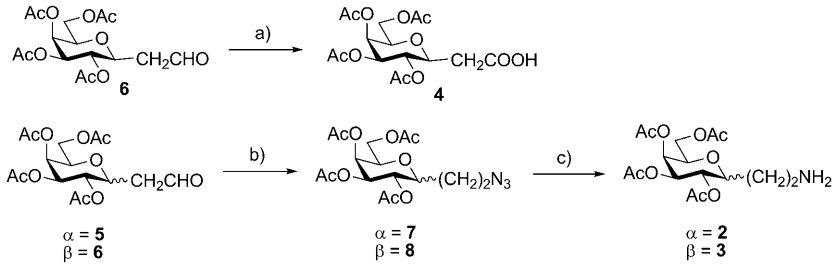
The crucial step of the synthesis of β-*C*-galactosides **3** and **4** was the inversion of configuration of **5** to the β-*C*-galactosyl aldehyde **6**, which was achieved by using L-proline catalysis, as recently described.[14] However, scale up of this step was complicated by proline-catalyzed autocondensation of **6** during work up. The problem was solved by using polyethylene glycol (PEG)-supported proline[15] as the catalyst, which can be precipitated from the reaction mixture before work up. With this modification of the reported procedure, yields improved from moderate to excellent and purification issues reported in the original paper were resolved (we have performed the reaction on up to 1.5 g of aldehyde **5** with 99% yield of **6**).

Oxidation of **6** with 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) and bis[acetoxy(iodo)]benzene (BAIB) in a 1:1 mixture of acetonitrile and water,[16] afforded acid **4** in good



Scheme 3. The functionalized Gal fragments used in this work.

yields (Scheme 4). Since direct reductive amination of α- and β-*C*-galactosyl aldehydes **5** and **6** to the corresponding amines **2** and **3** was complicated by an N-bisalkylation process and proceeded with poor yield, a two-step procedure via intermediate formation of azides **7** and **8** was used instead (Scheme 4).



Scheme 4. Synthesis of **2**–**4**. Reagents and conditions: a) TEMPO/BAIB, 1:1 CH_3CN/H_2O; b) i) diphenylphosphoryl azide (DPPA), 120 °C, microwave (MW); ii) $NaBH_3(OAc)$; c) H_2, Pd/C.

For the synthesis of the linker-armed Gal fragments from amines **1**–**3**, three commercially available, linear, terminal alkynoic acids **9**–**11** (from C_5 to C_7) were converted into the corresponding pentafluorophenyl esters **12**–**14** and coupled with either freshly prepared crude galactosylamine **1**, or aminoethyl galactosides **2** and **3** (Scheme 5). Attempts to apply a direct Staudinger acylation procedure by treating pentafluorophenyl esters **12**–**14** with azides **7** or **8**[17] did not lead to any significant improvement in overall yields.

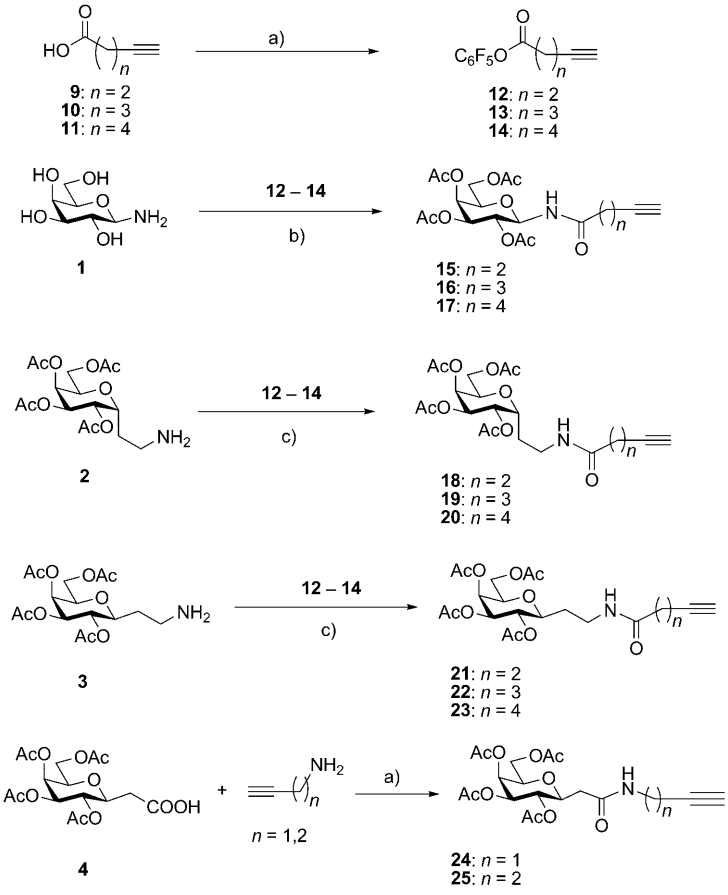
Ligands with shorter linker chains were prepared by coupling the β-galactosyl acid **4**, with either propargylamine or butynylamine to afford alkynes **24** and **25**, respectively (Scheme 5).

Finally, Cu-catalyzed (click) cycloaddition of alkynes **15**–**25** with fully acetylated sialyl azide **26**[9] under Sharpless reaction conditions ($CuSO_4$, sodium ascorbate) was performed[10] (Scheme 6). Complete deprotection of peracetylated coupling products **27**–**37** gave the target bidentate adducts **38**–**48**. These compounds can be grouped into three general families of ligands according to the anomeric composition and configuration of the galactose moiety. For clarity, in the rest of the text these families will be identified as: β-Gal-N (compounds **38**–**40**), α-Gal-C (**41**–**43**), and β-Gal-C (**44**–**48**).

As will be shown, compounds of the β-Gal-C family and, in particular, the pentynoic acid derivative **44**, turned out to be the most active members of this library. Thus, the library was expanded to include molecules featuring an additional branching point on the pentynoic acid backbone. Such a functional group on the linker could also act as a point of conjugation with a multivalent aglycon to allow the synthesis of multivalent ligands in a strategy that has previously been successfully employed against CT.[18]

To introduce a branching point, we used commercially available propargyl glycine **49** (Scheme 7) as the linker precursor. Initial studies were performed with racemic **49** to obtain both possible isomers at the same time; this approach capitalized on the ability of weak affinity chromatography (WAC) to analyze mixtures of compounds (see below). Starting from **49**, the *N*-acetyl pentafluorophenyl ester **50** was prepared and allowed to react with β-aminoethyl galactoside **3** to give alkyne **51**. The latter underwent click cycloaddition with sialyl azide to give, after deprotection, the target divalent ligand **52** as a 1:1 mixture of two inseparable epimers.

Similarly, another group of eight compounds **56**–**59** (four pairs of epimers at the linker's stereocenter) was prepared according to Scheme 8 to evaluate the effect of lipophilic substituents on the linker. Thus, the *N*-Boc-protected ligand **55** was prepared as previously described for **52**, deprotected



Scheme 5. Synthesis of linker-armed Gal fragments **15**–**25**. Reagents and conditions: a) C_6F_5OH, dicyclohexylcarbodiimide (DCC), THF; b) i) Et_3N, DMF; ii) Ac_2O/Py; c) Et_3N, THF.

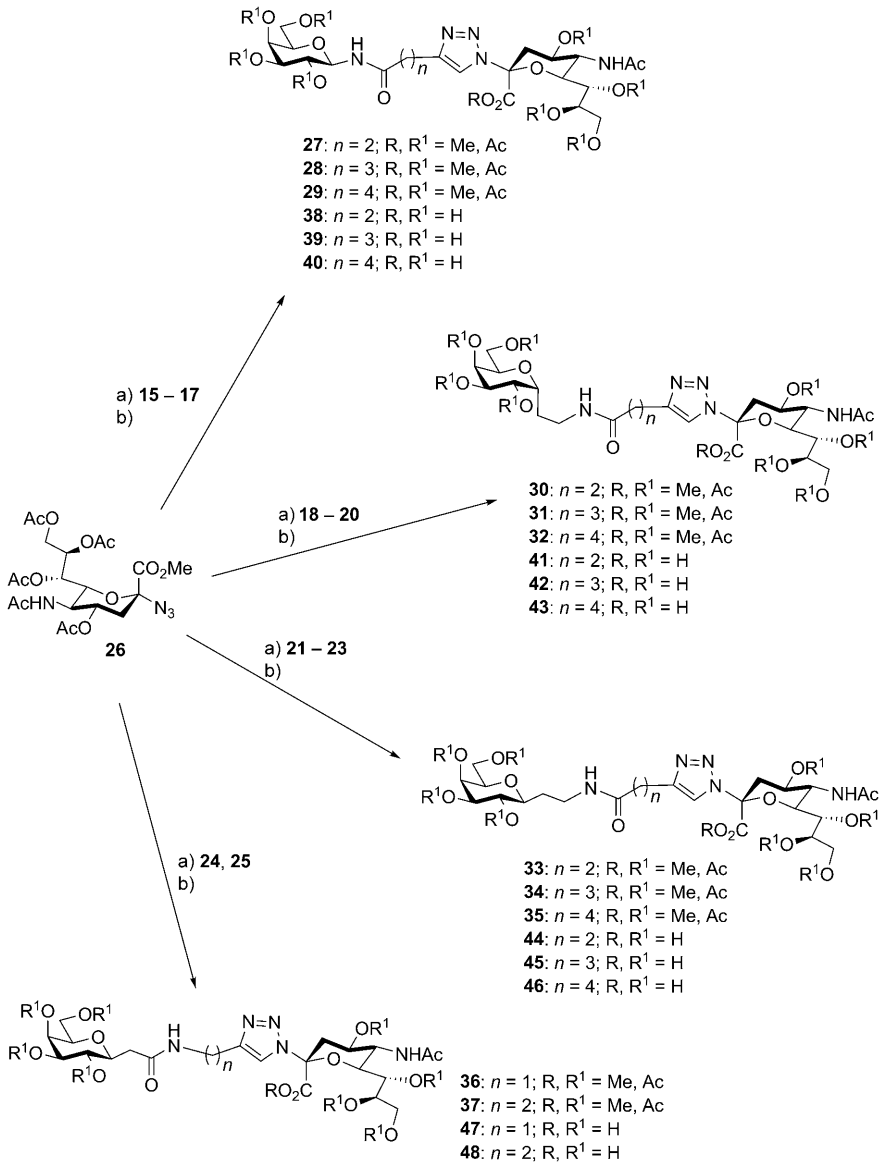
(trifluoroacetic acid (TFA)), and treated with either benzoyl chloride or the pentafluorophenyl ester of phenylacetic acid to afford, after deprotection, amides **56** and **57** as a pair of inseparable isomers. Alternatively, acylation of the free amine with either phenylisocyanate or benzylisocyanate, followed by deacetylation, yielded the two ureas **58** and **59**.

Ligand ranking by weak affinity chromatography (WAC): The affinity of the ligands for CT is often determined by fluorescence titrations using the intrinsic fluorescence of Trp-88 in the protein binding site.[19] This method could not be used in this case because of interference of the triazole moiety in the tested structures. WAC has previously been shown to be an interesting alternative for the study of CT ligands[20] and it was found to be the method of choice in our case. This approach is especially suitable for quantifying transient binding events such as sugar–protein interactions.[21] A HPLC column with immobilized recombinant CTB was used and small amounts (0.1 μg) of the synthesized ligands were injected. Because the concentrations of the injected compounds were low, a linear binding isotherm might be assumed and, under these conditions, the retardation (expressed as the retention factor, k') should be directly related to the affinity of the interaction.[22] The k' value can be transformed into the dissociation constant, K_d, of the ligand by calibrating the retardation with a compound of known affinity. The K_d values of galactose (51 mM)[20] and GalβOMe (15 mM)[6] have been determined by inhibition chromatography and ITC, respectively. However, these values are too low to be used as a reference in WAC because they fall below the revelation threshold. Instead, *meta*-nitropenyl-α-D-galactopyranoside (MNPG), a well-known CT ligand,[3] was used to calibrate our experiments. Although MNPG does not contain the critical NeuAc residue, X-ray data[23] have revealed that the oxygen atoms of the nitro group displaces a conserved water molecule from the binding site to make a hydrogen bond with a backbone N–H in the toxin. The increased entropy associated with the displacement of this water into bulk solvent accounts for a large part of the affinity gain of MNPG relative to galactose, which is estimated to be one or two orders of magnitude greater, depending on the methods adopted. The reported dissociation constants for the MNPG–CTB complex vary in the range from 1 to 0.2 mM.[3,20,24] For consistency, comparison with the WAC-determined value (Table 1, entry 1) will be made throughout this paper. The results of the WAC analysis of the ligand library compared with MNPG are shown in Table 1 (entries 1–12).

The galactosylamine derivatives **38**–**40** (Table 1, entries 2–4) and the two amides **47** and **48** obtained from acid **4** (Table 1, entries 11 and 12) bound below the measurable threshold at pH 5.5 and pH 7.0. In contrast, the affinity of ligands **41**–**46** (Table 1, entries 5–10) could be measured and some interesting candidates were identified. The most active ligand of this set, compound **44** (Table 1, entry 8), gave a k' of 1.25 at pH 7, which corresponds to a K_d value of 1.3 mM, thus matching the affinity of the reference MNPG. By varying the mobile phase in the WAC analysis, the pH dependence of the interaction could be studied (Figure 1). Interestingly, the interaction with ligands **41**–**46** was found to be strongly pH dependent, with a maximum retardation at pH 6. MNPG binding was less pH dependent and displayed maximum affinity at pH 7. The retention factor (k') and dissociation constant (K_d) of all ligands at pH 6 and 7 are collected in Table 1; the pH affinity dependence of **44** and of MNPG is shown in Figure 1.

The K_d value of **44** at pH 6 was found to be 0.8 mM, whereas MNPG had a K_d of 1.3 mM (Table 1, entries 8 and 1, respectively). The pH-dependent affinity of compounds **41**–**46** may be related to the interaction of their sialic acid moiety with the His13 residue in the CTB binding site, which should be optimal when the His side chain is protonated. The X-ray structure of the CTB–MNPG complex is known[23] and, as expected for this small ligand, no contact between MNPG and His13 was observed.

From the structural point of view, WAC analysis of **38**–**48** clearly shows some interesting trends. In particular, a group of three compounds in the β-Gal-C family (**44**–**46**) and compound **42** in the α-Gal-C family, displayed affinities one

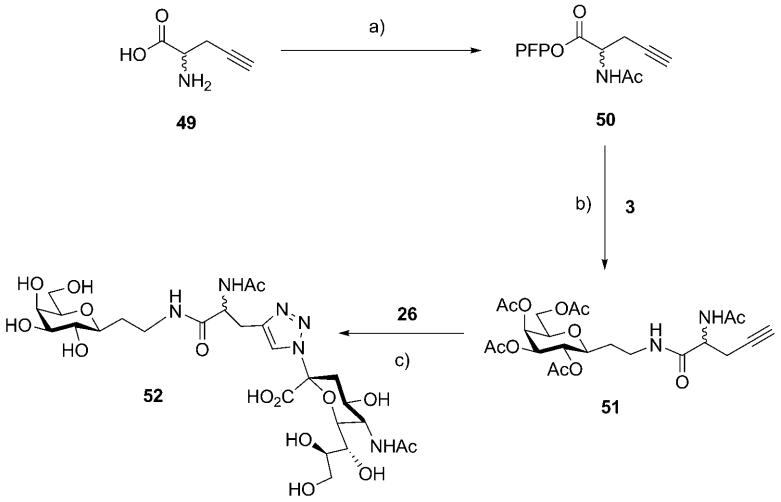


Scheme 6. Synthesis of the bidentate adducts **38**–**48**. Reagents and conditions: a) $CuSO_4$/Na ascorbate; b) NaOH, MeOH/H_2O.

order of magnitude higher than the revelation threshold. These values may be assumed to result from simultaneous interaction of the two sugar fragments with the toxin, which apparently is optimally allowed in this series by the framework of ligand **44** (C_5 linker chain from β-Gal-C framework).

As mentioned above, further characterization of the interaction of **44** with CTB by fluorescence spectroscopy was precluded by the interference of the triazole moiety, which absorbs in the same range as CTB Trp-88, and, therefore, filters the excitant radiation. However, saturation transfer difference (STD) NMR spectroscopy[25] experiments allowed the observation of binding events between CT and ligand **44**. In STD experiments, irradiation of the protein is followed by transfer of magnetization to the ligand protons, which, in turn, causes a signal enhancement that can be best appreciated in the difference spectrum. STD experiments were carried out in the presence of CTB (96 μM) in D_2O (80 mM phosphate buffer, pH 7.8) at several ligand to protein ratios (from 6 to 245) and different saturation times (from 0.5 to 3 s). Quantitative data analysis and epitope mapping were complicated by severe signal overlap, nonetheless the experiments showed clear signals corresponding to the galactose (Gal-H2 at 3.33 ppm) and sialic acid (NeuAc-H5 at $\delta =$ 3.89 ppm) fragments, thus confirming that **44** operates as a bidentate ligand. The binding affinity was also estimated by STD experiments at different ligand to protein ratios (from 6 to 250) at constant concentration of the protein (96 μM) in D_2O (80 mM phosphate buffer, pH 7.8) with a saturation time of 2 s. Plotting the STD amplification factors[26] against the concentration of the added ligand for the proton with the largest STD amplification factor (Gal-H2) allowed us to estimate an EC50 of 2.7 mM for the CTB–**44** complex (see Figure SI-1 in the Supporting Information), which is consistent with the WAC results at similar pH.

On the basis of the above findings, the propargyl glycine derivatives **52**–**59** were synthesized to obtain compounds with the same framework as **44**, but capable of further derivatization and conjugation to multivalent aglycons. Starting from racemic propargyl glycine, epimer pairs could be synthesized and their CTB affinity simultaneously evaluated by WAC. As an example, WAC analysis of the epimeric mixture of **52** is shown in Figure 2. The analysis clearly revealed two peaks, corresponding to the two epimers of **52**. One of the two isomers demonstrated some inhibition activity (was retarded on the column), although it was lower than that of the simple linear ligand **44**, while the second eluted close to the nonretarded void fraction. The corresponding evaluated retention factors and dissociation constants are reported in Table 1 (Entries 13 and 14. For the configurational assignment in the Table, see below.)

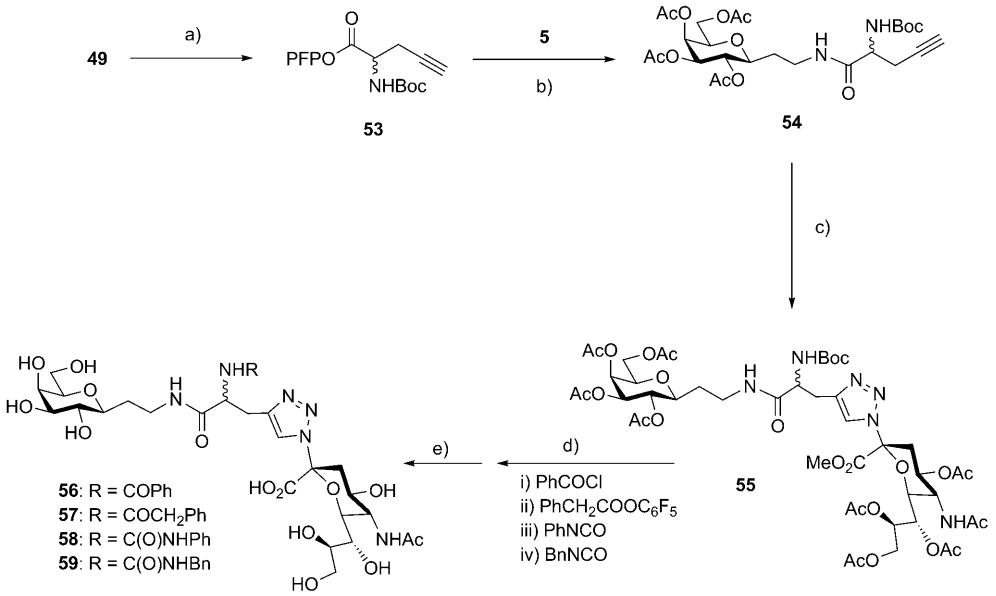


Scheme 7. Reagents and conditions: a) i) Ac_2O; ii) C_6F_5OH, DCC, THF; b) Et_3N, THF; c) i) $CuSO_4$/sodium ascorbate; ii) NaOH, MeOH/H_2O.

The loss of affinity upon branching of the linker suggests that the presence of the NHAc substituent disfavors the conformation required to allow simultaneous contact of the two pharmacophoric sugars with the toxin. However, this unfavorable factor could be offset by additional interactions between the linker side chain and the toxin binding site. Since the CTB binding site is known to possess a lipophilic patch near the sialic acid side-chain binding region,[27] the contribution of more lipophilic side chains was explored by testing ligands **56**–**59** (Table 1, entries 15–21), which showed similar pH dependence to the parent compound **44**. As can be seen from Table 1, the affinity of most structures improved significantly compared with the *N*-acetyl derivative **52**, to approach the potency of the underivatized ligand **44**. Among the compounds tested, one of the epimers of phenylacetamide (**57**) and one of the phenylureas (**58**) were found to be the most active ligands, with K_d values of 1.2 and 0.8 mM, respectively. When the two *R* and *S* isomers of **58** were synthesized separately, starting from enantiomerically pure D- or L-propargyl glycine, WAC analysis clearly showed that the *R* isomer of **58** had higher affinity than the corresponding *S* isomer (Table 1). This, in fact, had been suggested by docking models obtained by using Glide,[28] which showed that the *R* epimer of the side-chain stereocenter should direct the N-substituent towards the lipophilic region of the binding site (Figure 3).

Conclusion

A modular approach to a library of new glycomimetic CT ligands has been developed. The cornerstone of this approach consists of tethering carbohydrate epitopes that are known to interact with the CTB binding site, galactose, and sialic acid, to a properly designed linker through nonhydrolyzable, non-*O*-glycosidic bonds. The Gal epitope was introduced as one of four simple *C*- or *N*-galactosides. The NeuAc residue



Scheme 8. Synthesis of functionalized ligands **56**–**59**. Reagents and conditions: a) i) Boc_2O (Boc = *tert*-butyloxycarbonyl); ii) C_6F_5OH, DCC, THF; b) Et_3N, THF; c) i) $CuSO_4$/sodium ascorbate, **26**; d) CF_3COOH/CH_2Cl_2, followed by the appropriate reagent i–iv; e) NaOH, MeOH/H_2O. Bn = benzyl.

Table 1. Retention factor (k') and dissociation constant (K_d) of CT ligands at 23 °C determined with WAC at pH 6 and 7.

		pH 6		pH 7	
Entry	Ligand	k'	K_d [mM]	k'	K_d [mM]
1	MNPG[a]	1.28	1.3	1.58	1.1[b]
2	**38**	<0.05	>30	<0.05	>30
3	**39**	<0.05	>30	<0.05	>30
4	**40**	<0.05	>30	<0.05	>30
5	**41**	0.17	9.9	0.09	19
6	**42**	0.38	4.4	0.19	9.1
7	**43**	0.09	20	0.05	31
8	**44**	2.17	0.8	1.25	1.3
9	**45**	0.76	2.2	0.37	4.6
10	**46**	0.25	6.6	0.14	12
11	**47**	<0.05	>30	<0.05	>30
12	**48**	<0.05	>30	<0.05	>30
13	(*R*)-**52**[c]	0.70	2.4	0.47	3.6
14	(*S*)-**52**[c]	0.07	26	<0.05	>30
15	(*R*/*S*)-**56**[c]	1.10	1.5	0.71	2.4
16	(*R*)-**57**[c]	1.35	1.2	0.86	2.0
17	(*S*)-**57**[c]	1.19	1.4	0.86	2.0
18	(*R*)-**58**[c]	2.20	0.8	1.43	1.2
19	(*S*)-**58**[c]	1.39	1.2	0.93	1.8
20	(*R*)-**59**[c]	1.86	0.9	1.23	1.4
21	(*S*)-**59**[c]	1.18	1.4	0.80	2.1

[a] *meta*-Nitrophenyl-α-D-galactopyranoside. [b] From ref. [20]. [c] Configuration of the linker's stereocenter.

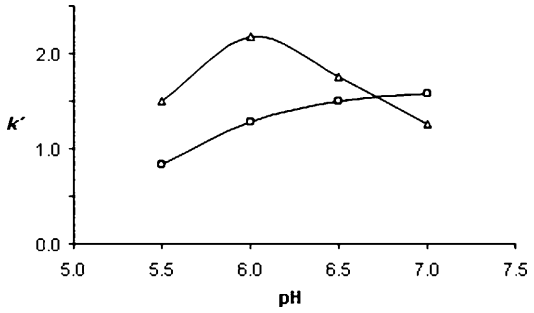


Figure 1. Affinity (k') of **44** and MNPG versus pH. △: **44**; □: MNPG.

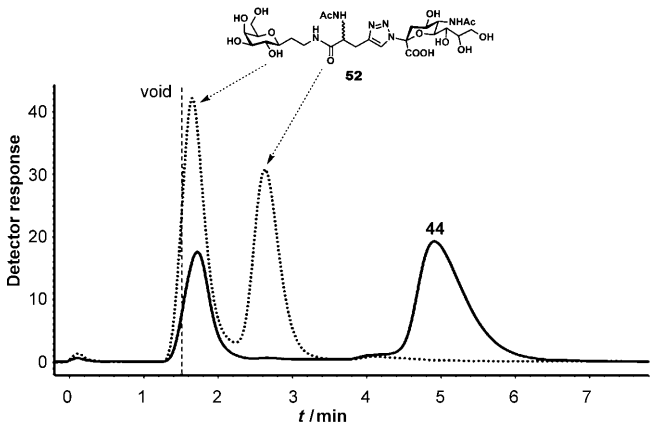


Figure 2. WAC diagram for compounds **52** (1:1 isomers, dotted line) and **44** (solid line) at pH 6.

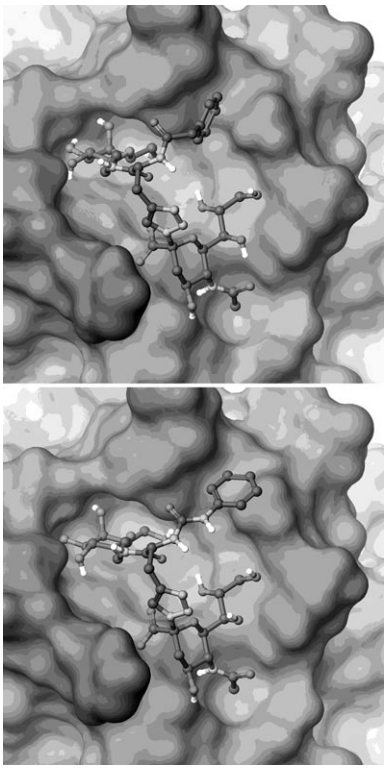


Figure 3. Docked conformations of ligands (*R*)-**57** (top) and (*R*)-**58** (bottom) in the CTB binding site (the stereochemical descriptor refers to the linker stereocenter).

was connected through a triazole spacer, starting from the known sialyl azide. Simple linear linkers were used to connect the sugar fragments. The affinity of the bidentate ligands was evaluated by WAC[20] with immobilized CTB. The results showed that only some appropriate combinations of fragments led to a measurable improvement of affinity over the individual epitopes. In particular, a group of molecules was identified that displayed affinities at least one order of magnitude higher than galactose. Such values may be assumed to result from simultaneous interaction of the two sugar fragments with the toxin.

Although docking studies could, in principle, have been used to select favorable conformational properties of the linkers and to identify ligands capable of engaging both binding areas in the toxin, in practice, the nature of CTB binding site, which is large and shallow in the area that should be covered by the linker (see Figure 3), does not lend itself to reliable predictive use of computational models. Indeed, initial docking experiments conducted by using Glide[28] software did not reveal significant differences in docking scores between similar structures containing the Gal residue in the form of *N*- or *C*-glycoside in either α- or β-anomeric configuration, albeit with some preference for the latter. The linear linker developed in this study can now be used as a platform for rational ligand design, and be directed towards the stabilization of the bound conformation

of the ligand, thus increasing its preorganization and, ultimately, its affinity for CT.

All compounds reported herein could be synthesized from precursors that are readily available on a large scale by using high-performance reactions, including click chemistry protocols. Previously known artificial CT ligands basically fall into two classes: the MNPG derivatives introduced by the group of Fan[3] and the structural mimics of the GM1os reported by our group (Scheme 1).[4] Both types contain enzymatically labile *O*-glycosidic linkages. Several of the non-hydrolyzable ligands described herein demonstrated reasonably high CT affinities that were similar to or higher than the affinity of MNPG.[20,24] They are therefore among the most active monovalent CT ligands that do not closely reproduce the GM1 structure. Some of the most active molecules identified herein also feature a point of further derivatization that can be used for conjugation with polyvalent aglycons. Even though there was either no, or negligible, affinity gained by linker derivatization, this approach opens the way to the creation of polyvalent constructs that may be used to block the toxin in a therapeutically relevant context.[18]

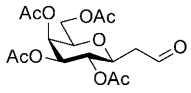
Interesting findings, such as pH dependence of the binding affinity of the ligands and the structural preference of the CTB binding site towards one of the two diastereomeric forms of the ligand, were assessed with WAC, which will be useful for further development of inhibitors of CT binding.

Experimental Section

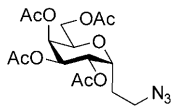
General: WAC was performed essentially as described previously.[20] In short, recombinant CTB (SBL vaccines, Stockholm, Sweden) was immobilized with reductive amination to aldehyde-derivatized Nucleosil silica (10 μm, 300 Å; Macherey–Nagel, Düren, Germany) and packed into a 50×2.1 mm column. The number of binding sites on the column was determined with frontal chromatography to be 261 nmol at pH 7. All chromatography experiments were performed on an Agilent 1100 series HPLC system (Agilent Technologies, Santa Clara, CA, USA); the mobile phase was 0.15 M sodium chloride with 10 mM sodium phosphate (adjusted to pH 5.5, 6.0, 6.5, or 7.0 as indicated in the text); the flow rate was 0.1 mL min^{-1} and the temperature was 23 °C; the injection volume was 5 μL and the concentration of each sample was 20 μg mL^{-1}. Detection was performed at 220 nm and the detector signal was collected with an Agilent ChemStation chromatography system. The retention factor (k') was calculated from the retention time (T_r) and the mobile phase hold up time, also called the void time (T_m), according to the equation: $k'=(T_r-T_m)/T_m$. The void time of the column was determined to be 1.55 min. Dissociation constants (K_d) of the ligands were calculated from the following equation: $K_d=B_{tot}/(k'*V_m)$ in which B_{tot} is the number of binding sites in the column and V_m is the void volume.[21,22] It seems reasonable to assume that B_{tot} and V_m are constant under the conditions studied and the equation can be simplified to $K_d=\text{const}/k'$. By using this relationship, the affinity (K_d) of any compound can easily be calculated from the k' by comparing the retardation with a compound of known affinity.

Solvents were dried by standard procedures: dichloromethane, methanol and triethylamine (TEA) were dried over calcium hydride; THF was dried on sodium; anhydrous DMF was purchased from Fluka. Reactions requiring anhydrous conditions were performed under nitrogen. Microwave-assisted reactions were performed on a CEM Discover instrument. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz on a Bruker AVANCE-400 instrument; samples were measured in $CDCl_3$ at room temperature, unless otherwise specified. Chemical shifts (δ) for ^{1}H and ^{13}C NMR spectra are expressed in ppm relative to internal tetramethylsilane as standard. Assignments were aided by homo- and heteronuclear two-dimensional experiments. Common mass spectra were obtained with a Bruker ion-trap Esquire 3000 plus apparatus (ESI ionization), exact mass spectra were registered with a Bruker Daltonics ICR-FTMS APEX II spectrometer (ESI ionization). TLC was carried out with precoated Merck F_{254} silica gel plates. TLC detection was performed by treatment with phosphomolybdic acid, ninhydrin, potassium permanganate, or vanillin. Automated flash direct and reverse-phase chromatography were carried out on a Biotage SP1 system equipped with Biotage SNAP cartridges.

Compound 6: L-Proline PEG conjugate (2.27 g, ≈0.1 equiv)[15] was added to a stirred solution of the corresponding α-aldehyde **5** (1.58 g, 4.22 mmol)[13] in MeOH (11 mL) at 0 °C. The mixture was sonicated and then subjected to microwave irradiation for 4 h at a controlled temperature of 50 °C (constant power 13 W, cooling by compressed air). The reaction was monitored by ^{1}H NMR ($H_{1Gal\alpha}$: m, $\delta=4.86$ ppm; $H_{1Gal\beta}$: m, $\delta=4.00$ ppm). The reaction mixture was allowed to cool to RT then the solvent was evaporated and solid residue was purified by automated flash chromatography to afford pure β-anomer **6** (1.57 g, 99 %); analytical data corresponded to those reported in the literature.[14] ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=9.72$ (t, $J=3$ Hz, 1H; -COH), 5.41 (t, $J=3$ Hz, 1H; H-4), 5.28 (dd, $J_1=5$, $J_2=9$ Hz, 1H; H-2), 5.17 (dd, $J_1=3$, $J_2=9$ Hz, 1H; H-3), 4.03–4.14 (m, 2H; H-5, H-6a), 4.03 (ddd, $J_1=9.6$, $J_2=8$, $J_3=4$ Hz, 1H; H-1), 3.95 (t, $J=6.5$ Hz, 1H; H-6b), 2.78 (ddd, $J_1=19$, $J_2=9.6$, $J_3=2$ Hz, 1H; $GalCH_{2a}$), 2.60 (ddd, $J_1=19$, $J_2=4$, $J_3=2$ Hz, 1H; $GalCH_{2b}$), 2.01–2.20 ppm (m, 12H; $4\times CH_3CO$); MS (ESI): 414.9 [M^++K].

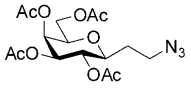


Compound 7: Acetic acid (100 μL, 1.76 mmol) and $NaBH_4$ (63 mg, 1.76 mmol) were added to a stirred solution of aldehyde **5** (332 mg, 0.88 mmol)[13] in anhydrous THF (10 mL). The reaction mixture was vigorously stirred at RT for ≈2 h until reduction was complete. The reaction was quenched with acetic acid (200 μL) and the solvent was evaporated. The excess acetic acid was removed by co-evaporation with toluene and the solid residue was filtered through silica gel (hexane/EtOAc 1:3) to give the alcohol intermediate, which was subsequently dissolved in anhydrous DMF (2.5 mL). Diphenyl phosphoryl azide (266 μL, 1.23 mmol, 1.4 equiv) and 1,8-diazabicyclo[5.4.0]undec-7-ene (184 μL, 1.23 mmol, 1.4 equiv) were added while stirring. The mixture was then subjected to microwave irradiation for 20 min at 120 °C (dynamic control) at 200 W power. The solvent was evaporated and the mixture was purified by flash chromatography (hexane/EtOAc) to afford the azide **7** (247 mg, 70 %). $[\alpha]_D=+36.45$ ($c=0.92$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=5.45$ (t, $J_1=J_2=3$ Hz, 1H; H-4), 5.29 (dd, $J_1=5$, $J_2=9$ Hz, 1H; H-2), 5.21 (dd, $J_1=3$, $J_2=9$ Hz, 1H; H-3), 4.31–4.40 (m, 2H; H-1, H-6a), 4.07–4.16 (m, 2H; H-5, H-6a), 3.35–3.50 (m, 2H; CH_2N_3), 2.09–2.15 (m, 12H; $4\times CH_3CO$), 1.87–2.00 (m, 1H; Gal-CH_{2a}), 1.63–1.78 ppm (m, 1H; Gal-CH_{2b}); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta=170.6$, 170.0, 169.8, 169.7 ($4\times CH_3CO$), 69.0 (C-5), 68.8 (C-1), 68.3 (C-2), 67.9 (C-3), 67.2 (C-4), 61.2 (C-6), 47.7 (CH_2N_3), 26.0 (Gal-CH_2), 20.74, 20.66 ppm ($4\times CH_3CO$); MS (ESI): m/z: 423.9 [M^++Na].

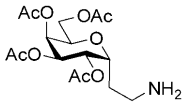


Compound 8: Acetic acid (206 μL, 3.606 mmol) and $NaBH_4$ (137 mg, 3.606 mmol) were added to a stirred solution of the aldehyde **6** (672 mg, 1.8 mmol) in anhydrous THF (10 mL). The reaction mixture was stirred for ≈2 h until reduction was complete, then quenched with acetic acid (200 μL). The same protocol described above yielded the intermediate alcohol, which was subsequently dissolved in anhydrous DMF (5 mL). Diphenyl phosphoryl azide (544 μL, 2.52 mmol, 1.4 equiv) and 1,8-diazabicyclo[5.4.0]undec-7-ene (376 μL, 2.52 mmol, 1.4 equiv) were added

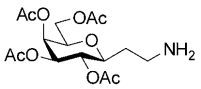
while stirring. The mixture was then subjected to microwave irradiation for 20 min at 120 °C (dynamic control) at 200 W power. The solvent was evaporated and the mixture was purified by flash chromatography (hexane/EtOAc) to afford the azide **8** (560 mg, 77.3 %). $[\alpha]_D = +1.8$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.41$ (dd, $J_1 = 1$, $J_2 = 3$ Hz, 1H; H-4), 5.07 (t, $J = 10$ Hz, 1H; H-2), 5.01 (dd, $J_1 = 3$, $J_2 = 10$ Hz, 1H; H-3), 4.16–4.46 (m, 2H; H-6), 3.86 (br t, $J = 7$ Hz, 1H; H-5), 3.69 (td, $J_1 = 4$, $J_2 = 9$ Hz, 1H; H-1), 3.41 (dt, $J = 6$ Hz, 2H; CH_2N_3), 1.92–2.15 (m, 12H; $4 \times CH_3CO$), 1.74–1.82 ppm (m, 2H; $CH_2CH_2N_3$); ^{13}C NMR: $\delta =$ 170.5, 170.2, 170.1, 169.9 ($4 \times CH_3CO$), 75.0 (C-1) , 74.3 (C-5), 72.0 (C-3), 69.2 (C-2), 67.7 (C-4), 61.7 (C-6), 47.1 (CH_2N_3), 31.0 ($CH_2CH_2N_3$), 20.8, 20.7, 20.6 ppm ($4 \times CH_3CO$); MS (ESI): m/z: 423.9 [M^++Na].



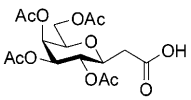
Compound 2: Acetic acid (500 μL) and a catalytic amount of Pd/C was added to a stirred solution of the azide **7** (636 mg, 1.6 mmol) in a mixture of MeOH/H_2O (5:1, 30 mL). The reaction vessel was filled with hydrogen and the reaction mixture was vigorously stirred at RT for ≈2 h until the reduction was complete. The reaction mixture was filtered through Celite and the resulting solution was evaporated and dried in vacuo to afford amine **2** as the acetate salt (834 mg, 100 %). $[\alpha]_D = +25.4$ ($c = 1$ in MeOH); ^{1}H NMR (400 MHz, CD_3Cl_3): $\delta = 5.39$ (t, $J_1 = J_2 = 3$ Hz, 1H; H-4), 5.25 (dd, $J_1 = 3$, $J_2 = 8$ Hz, 1H; H-3), 5.19 (dd, $J_1 = 5$, $J_2 = 8$ Hz, 1H; H-2), 4.42–4.52 (m, 1H; H-6a), 4.30–4.40 (m, 1H; H-1), 4.20 (dt, $J_1 = 3$, $J_2 =$ 8 Hz, 1H; H-5), 4.08 (dd, $J_1 = 4$, $J_2 = 8$ Hz, 1H; H-6b), 2.98–3.10 (m, 2H; CH_2NH_2), 1.97–2.19 (m, 13H; Gal-CH_{2a}, $4 \times CH_3CO$), 1.93 (s, 3H; CH_3COO^-), 1.78–1.88 ppm (m, 1H; Gal-CH_{2b}); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 172.4$, 171.6, 171.3, 171.2 ($4 \times CH_3CO$), 70.8 (C-5), 70.5 (C-1), 69.6 (C-2), 68.93 (C-3), 68.94 (C-4), 62.2 (C-6), 38.0 (CH_2NH_2), 25.8 (Gal-CH_2), 23.4 (CH_3COO^-), 20.7, 20.6, 20.6, 20.5 ppm ($4 \times CH_3CO$); HRMS (FT-ICR, ESI): m/z calcd for $C_{16}H_{26}NO_9$: 376.16021 [M^++H]: found: 376.16054.



Compound 3: A catalytic amount of Pd/C was added to a stirred solution of the azide **8** (136.4 mg, 0.340 mmol) in a mixture of MeOH/H_2O/AcOH (25:5:3, 10 mL). The reaction vessel was filled with hydrogen and the reaction mixture was vigorously stirred at RT for ≈2 h until reduction was complete. The reaction mixture was filtered through Celite and the resulting solution was evaporated and dried in vacuo to afford amine **3** as the acetate salt (148 mg, 100 %). $[\alpha]_D = +4.1$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 5.40$ (dd, $J_1 = 1$, $J_2 = 3$ Hz, 1H; H-4), 5.09 (dd, $J_1 = 3$, $J_2 = 10$ Hz, 1H; H-3), 5.00 (t, $J = 10$ Hz, 1H; H-2), 4.03–4.13 (m, 3H; H-5, H-6), 3.69 (td, $J_1 = 3$, $J_2 = 10$ Hz, 1H; H-1), 3.07 (td, $J_1 = 3$, $J_2 =$ 7 Hz, 2H; CH_2NH_2), 1.88–2.11 (m, 13H; $4 \times CH_3CO$, $CH_{2b}CH_2N_3$), 1.75–1.85 ppm (m, 1H; $CH_{2a}CH_2N_3$); ^{13}C NMR: $\delta = 172.3$, 172.0, 171.8, 171.6 ($4 \times CH_3CO$), 77.0 (C-1), 74.9 (C-5), 72.7 (C-3), 69.5 (C-4), 69.4 (C-2), 62.3 (C-6), 37.6 (CH_2N_3), 22.9 ($CH_2CH_2N_3$), 20.8, 20.7, 20.6 ppm ($4 \times CH_3CO$); HRMS (FT-ICR, ESI): m/z calcd for $C_{16}H_{26}NO_9$: 376.16021 [M^++H]; found: 376.16059.

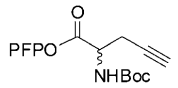


Compound 4: TEMPO (25 mg, 0.160 mmol) and BAIB (565 mg, 1.75 mmol) were added to a solution of β-aldheyde **6** (300 mg, 0.797 mmol) in MeCN/H_2O (1:1, 3 mL). The reaction mixture was stirred for ≈3 h until oxidation was complete. The reaction was quenched with an aqueous solution of $Na_2S_2O_3$ (2 mL, 1 M) then diluted with CH_2Cl_2 (15 mL) and filtered through Celite. The solvents were evaporated and the resulting solid was purified by automated flash chromatography ($MeOH/CH_2Cl_2$, 0–25 %) to afford pure acid **4** (311 mg, 100 %), the analytical data for which corresponded to those reported.[29] ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.45$ (dd, $J_1 = 1$, $J_2 = 3$ Hz, 1H; H-4), 5.16 (t, $J_1 = 10$ Hz, 1H; H-2), 5.07 (dd, $J_1 = 3$, $J_2 = 10$ Hz, 1H; H-3), 4.05–4.17 (m, 2H; H-6), 3.85–3.95 (m, 2H; H-5, H-1), 2.63 (dd, $J_1 = 6.1$, $J_2 = 16$ Hz, 2H; CH_2COOH), 1.98–2.21 ppm (m, 12H; $4 \times CH_3CO$).



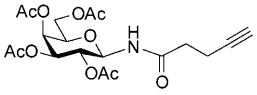
General procedure for the synthesis of 12–14 and 50: 2,3,4,5,6-Pentafluorophenol (1.5 equiv) was added to a solution of alkynoic acid (0.5 M) in anhydrous THF at RT followed, after 15 min, by dicyclohexylcarbodiimide (1.5 equiv). The resulting suspension was vigorously stirred overnight at RT, diluted with ethyl acetate, and filtered through Celite. The filtrate was concentrated and purified by flash chromatography (hexane/EtOAc gradient) to afford pure pentafluorophenyl ester.

Compound 53: Anhydrous Et_3N (500 μL) and Boc_2O (308 mg, 1.41 mmol) were added to a stirred suspension of *C*-propargylglycine (133 mg, 1.18 mmol) in MeOH (3.2 mL). During the course of reaction (≈30 min) gradual dissolution of the starting material was observed, indicating completion of carbamate formation. The solvent was evaporated, the resulting solid was dried in vacuo and the crude carbamate was converted into its pentafluorophenyl ester as described in the general procedure to afford **53** (440 mg, 98 %). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.37$ (d, $J = 8$ Hz, 1H; NH), 4.75–4.90 (m, 1H; C*H*NH), 2.97 (br d, $J = 17$ Hz, 1H; H-3a), 2.85 (ddd, $J_1 = 3$, $J_2 = 5$, $J_3 = 17$ Hz, 1H; H-3b), 2.15 (br s, 1H; C≡CH), 1.48 ppm (s, 9H; *tBu*).

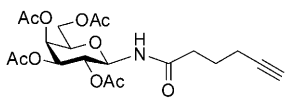


General procedure for the synthesis of 15–17: 2,3,4,5,6-Pentafluorophenyl akynoate (1.05 mmol) was added to a stirred solution of galactosylamine **1** (180 mg, 1.0 mmol) in DMF (3 mL). The reaction mixture was stirred overnight at RT then pyridine (3 mL, 37.1 mmol) and acetic anhydride (1 mL, 10.60 mmol) were added to the reaction mixture and stirring was continued at RT overnight. The resulting solution was diluted with EtOAc (30 mL), washed with 1 M HCl (15 mL), water (15 mL), and a saturated aqueous solution of $NaHCO_3$ (15 mL). Organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography (hexane/EtOAc).

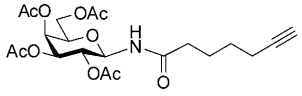
Compound ***15***: Yield: 162 mg (0.379 mmol, 38 %); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.49$ (d, $J = 1$ Hz, 1H; NH), 5.44 (d, $J = 2$ Hz, 1H; H-4), 5.26 (t, $J = 9$ Hz, 1H; H-1), 5.07–5.15 (m, 2H; H-2, H-3), 4.00–4.15 (m, 3H; H-5, H-6a, H-6b), 2.35–2.60 (m, 4H; $(CH_2)_2$), 1.95–2.20 ppm (m, 13H; C≡CH, $4 \times CH_3CO$).



Compound ***16***: Yield: 156 mg (0.353 mmol, 35 %); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.49$ (d, $J = 1$ Hz, 1H; NH), 5.43 (d, $J = 2$ Hz, 1H; H-4), 5.24 (t, $J = 9$ Hz, 1H; H-1), 5.07–5.15 (m, 2H; H-2, H-3), 4.00–4.15 (m, 3H; H-5, H-6a, H-6b), 1.70–2.40 ppm (m, 17H; $(CH_2)_3$, C≡CH, $4 \times CH_3CO$).



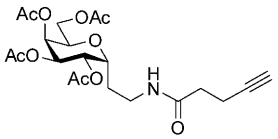
Compound ***17***: Yield: 126 mg (0.277 mmol, 28 %); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.25$ (d, $J = 1$ Hz, 1H; NH), 5.43 (d, $J = 2$ Hz, 1H; H-4), 5.25 (t, $J = 9$ Hz, 1H; H-1), 5.05–5.15 (m, 2H; H-2, H-3), 4.00–4.17 (m, 3H; H-5, H-6a, H-6b), 1.95–2.35 (m, 15H; NHC(O)CH_2, C≡CH, $4 \times CH_3CO$), 1.50–1.90 ppm (m, 6H; $(CH_2)_3$).



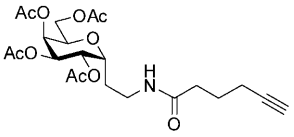
General procedure for the synthesis of 18–23, 51, and 54: Alkynoic acid 2,3,4,5,6-pentafluorophenyl ester (1.2–1.5 equiv) and triethylamine (3 equiv) were added to a stirred solution of aminoethylgalactoside **2** or **3**

(acetate salt; 0.5 M) in THF. The reaction mixture was stirred at RT overnight, then the solvent was evaporated, and resulting solid was purified by flash chromatography (hexane/EtOAc).

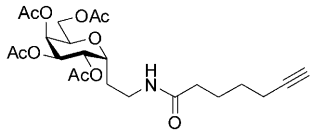
Compound ***18***: The reaction of α-aminoethylgalactoside **2** (30 mg, 0.069 mmol, *N*-acetate form) with pentafluorophenyl ester **12** (22 mg, 0.083 mmol) afforded alkyne **18** (25 mg, 0.055 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.18 (br t, J = 6.5 Hz, 1H; NH), 5.38 (t, J = 3 Hz, 1H; H-4), 5.05–5.15 (m, 2H; H-2, H-3), 4.50 (dd, J_1 = 8.3, J_2 = 11 Hz, 1H; H-6b), 4.20 (dt, J_1 = 3, J_2 = 11 Hz, 1H; H-5), 4.05–4.10 (m, 1H; H-1), 3.95 (dd, J_1 = 11, J_2 = 5 Hz, 1H; H-6a), 3.38–3.50 (m, 1H; $CH_{2b}NC(O)$), 3.08–3.18 (m, 1H; $CH_{2a}NC(O)$), 2.45–2.55 (m, 2H; $HNC(O)CH_2CH_2CCH$), 2.40–2.50 (m, 2H; $HNC(O)CH_2CH_2CCH$), 1.95–2.07 (m, 13H; C≡C*H*, 4×CH_3CO), 1.75–1.95 ppm (m, 2H; $CH_2CH_2NC(O)$).



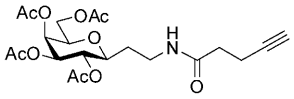
Compound ***19***: The reaction of α-aminoethylgalactoside **2** (32 mg, 0.073 mmol, *N*-acetate form) with pentafluorophenyl ester **13** (31 mg, 0.111 mmol) afforded alkyne **19** (27 mg, 0.058 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.10 (br t, J = 6.5 Hz, 1H; N*H*), 5.38 (t, J = 3 Hz, 1H; H-4), 5.10–5.20 (m, 2H; H-2, H-3), 4.57 (dd, J_1 = 8.3, J_2 = 11 Hz, 1H; H-6b), 4.23 (dt, J_1 = 3, J_2 = 11 Hz, 1H; H-5), 4.10–4.15 (m, 1H; H-1), 4.00 (dd, J_1 = 11, J_2 = 5 Hz, 1H; H-6a), 3.40–3.50 (m, 1H; $CH_{2b}NC(O)$), 3.15–3.22 (m, 1H; $CH_{2a}NC(O)$), 2.18–2.40 (m, 4H; $HNC(O)CH_2CH_2CCH$), 1.95–2.15 (m, 13H; C≡CH, 4×CH_3CO), 1.65–1.95 ppm (m, 4H; $CH_2CH_2CH_2$, $CH_2CH_2NC(O)$).



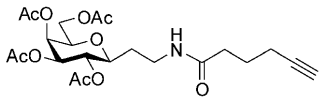
Compound ***20***: The reaction of α-aminoethylgalactoside **2** (47 mg, 0.108 mmol, *N*-acetate form) with pentafluorophenyl ester **14** (38 mg, 0.130 mmol) afforded alkyne **20** (41 mg, 0.085 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.08 (br t, J = 6.5 Hz, 1H; NH), 5.40 (t, J = 3.2 Hz, 1H; H-4), 5.20 (dd, J_1 = 3.2, J_2 = 7.9 Hz, 1H; H-3), 5.14 (dd, J_1 = 7.9, J_2 = 4.0 Hz, 1H; H-2), 4.66–4.60 (m, 1H; H-6b), 4.24 (dt, J_1 = 3.6, J_2 = 11.1 Hz, 1H; H-5), 4.13 (dt, J_1 = 4.0, J_2 = 8.1 Hz, 1H; H-1), 4.00 (dd, J_1 = 4.4, J_2 = 11.1 Hz, 1H; H-6a), 3.40–3.50 (m, 1H; $CH_{2b}NC(O)$), 3.14–3.21 (m, 1H; $CH_{2a}NC(O)$), 2.16–2.30 (m, 4H; $HNC(O)CH_2(CH_2)_2CH_2CCH$), 2.02–2.14 (m, 12H; 4×$CH_3CO$), 1.95 (t, J = 2.6 Hz, 1H; C≡CH), 1.50–1.90 ppm (m, 6H; $CH_2(CH_2)_2CH_2$, $CH_2CH_2NC(O)$).



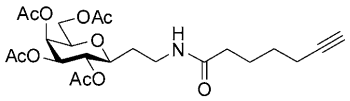
Compound ***21***: The reaction of β-aminoethylgalactoside **3** (30 mg, 0.069 mmol, *N*-acetate form) with pentafluorophenyl ester **12** (22 mg, 0.083 mmol) afforded alkyne **21** (29 mg, 0.064 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.0 (br t, J = 6.5 Hz, 1H; NH), 5.40 (d, J = 3.3 Hz, 1H; H-4), 5.02 (t, J = 10.4 Hz, 1H; H-2), 4.93 (dd, J_1 = 10.4, J_2 = 3.3 Hz, 1H; H-3), 3.98–4.14 (m, 2H; H-6), 3.82 (t, J = 6.2 Hz, 1H; H-5), 3.50–3.60 (m, 1H;$CH_{2b}NC(O)$), 3.43 (dt, J_1 = 10.4, J_2 = 3.0 Hz, 1H; H-1), 3.15–3.25 (m, 1H; $CH_{2a}NC(O)$), 2.42–2.52 (m, 2H; $HNC(O)CH_2CH_2CCH$), 2.20–2.30 (m, 2H; $HNC(O)CH_2CH_2CCH$), 1.93–2.13 (m, 13H; C≡CH, 4×CH_3CO), 1.57–1.85 ppm (m, 2H; $CH_2CH_2NC(O)$).



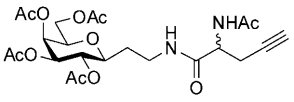
Compound ***22***: The reaction of β-aminoethylgalactoside **3** (30 mg, 0.069 mmol, *N*-acetate form) with pentafluorophenyl ester **13** (23 mg, 0.083 mmol) afforded alkyne **22** (29 mg, 0.062 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.90 (br t, J = 6.5 Hz, 1H; NH), 5.38 (d, J = 3.3 Hz, 1H; H-4), 5.02 (t, J = 10.4 Hz, 1H; H-2), 4.94 (dd, J_1 = 10.4, J_2 = 3.3 Hz, 1H; H-3), 4.00–4.15 (m, 2H; H-6), 3.81 (t, J = 6.2 Hz, 1H; H-5), 3. 50–3.58 (m, 1H; $CH_{2b}NC(O)$), 3.43 (dt, J_1 = 10.4, J_2 = 3.0 Hz, 1H; H-1), 3.15–3.25 (m, 1H; $CH_{2a}NC(O)$), 2.17–2.30 (m, 4H; $HNC(O)CH_2CH_2CH_2CCH$), 1.90–2.15 (m, 12H; 4×CH_3CO), 1.80 (t, J = 6.5 Hz, 1H; C≡C*H*), 1.52–1.82 ppm (m, 4H; $CH_2CH_2CH_2$, $CH_2CH_2NC(O)$).



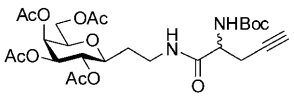
Compound ***23***: The reaction of β-aminoethylgalactoside **3** (23 mg, 0.053 mmol, *N*-acetate form) with pentafluorophenyl ester **14** (15 mg, 0.051 mmol) afforded alkyne **23** (12 mg, 0.025 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.90 (br t, J = 6.5 Hz, 1H; NH), 5.45 (d, J = 3.3 Hz, 1H; H-4), 5.13 (t, J = 10.0 Hz, 1H; H-2), 5.03 (dd, J_1 = 3.3, J_2 = 10.0 Hz, 1H; H-3), 4.15 (ddd, J_1 = 6.9, J_2 = 11.0, J_3 = 17.0 Hz, 2H; H-6), 3.90 (t, J = 6.9 Hz, 1H; H-5), 3.57–3.65 (m, 1H; $CH_{2b}NC(O)$), 3.51 (dt, J_1 = 10.0, J_2 = 6.6 Hz, 1H; H-1), 3.23–3.33 (m, 1H; $CH_{2a}NC(O)$), 2.20–2.29 (m, 4H; $HNC(O)CH_2(CH_2)_2CH_2CCH$), 2.0–2.19 (m, 12H; 4×$CH_3CO$), 1.98 (t, J = 2.7 Hz, 1H; C≡CH), 1.53–1.90 ppm (m, 4H; $CH_2(CH_2)_2CH_2$, $CH_2CH_2NC(O)$).



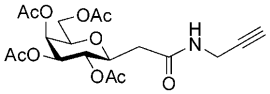
Compound ***51***: The reaction of β-aminoethylgalactoside **3** (40 mg, 0.092 mmol, *N*-acetate form) with pentafluorophenyl ester **50** (30 mg, 0.093 mmol) afforded alkyne **51** (20 mg, 0.039 mmol, 43%) together with the byproduct from *O*- to *N*-acetyl transfer in **3** (19 mg, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.52, 6.37 (2H; 2×NH), 5.36 (d, J = 3.3 Hz, 1H; H-4), 5.03 (t, J = 9.8 Hz, 1H; H-2), 4.94 (dd, J_1 = 3.4, J_2 = 10.0 Hz, 1H; H-3), 4.40–4.50 (m, 1H; C*H*NHAc), 3.97–4.17 (m, 2H; H-6), 3.75–3.85 (m, 1H; H-5), 3.49–3.58 (m, 1H; $CH_{2b}NC(O)$), 3.43 (dt, J_1 = 10.0, J_2 = 3.4 Hz, 1H; H-1), 3.21–3.31 (m, 1H; $CH_{2a}NC(O)$), 2.47–2.73 (m, 2H; $CH_2C{\equiv}CH$), 1.86–2.13 (m, 13H; 4×CH_3CO, C≡C*H*), 1.61–1.82 ppm (m, 2H; $CH_2CH_2NC(O)$).



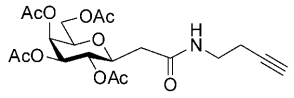
*Compound **54***: The reaction of the pentafluorophenyl ester **53** (234 mg, 0.619 mmol) with β-aminoethylgalactoside **3** (*N*-acetate salt, 224.5 mg, 0.516 mmol), afforded alkyne **54** (178 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.59$ (s, 0.5H; CH_2NH), 6.53 (t, $J = 6$ Hz, 0.5H; CH_2NH), 5.42 (brs, 1H; H-4), 5.28 (s, 1H; NHBoc), 5.08 (t, $J_1 = 10$ Hz, 1H; H-2), 4.99 (dd, $J_1 = 3$, $J_2 = 10$ Hz, 1H; H-3), 4.20–4.31 (m, 1H; *CH*NH), 4.06–4.18 (m, 2H; H-6), 3.86 (qd, $J_1 = 1$, $J_2 = 6$ Hz, 1H; H-5), 3.44–3.64 (m, 2H; H-1, $CH_2CH_{2a}NH$), 3.25–3.40 (m, 1H; $CH_2CH_{2b}NH$), 2.71–2.83 (m, 1H; $CH_{2a}C{\equiv}CH$), 2.55–2.68 (m, 1H; $CH_{2b}C{\equiv}CH$), 1.94–2.18 (m, 13H; 4× CH_3CO, C≡C*H*), 1.61–1.92 ppm (m, 2H; CH_2CH_2NH); ^{13}C NMR: $\delta =$ 170.6–170.0 (4× CH_3CO, CH_2NHCO), 77.4 (C-1), 74.7 (C-5), 71.7 (C-3), 69.1 (C-2), 67.9 (C-4), 62.0 (C-6), 53.2 (*C*HNH), 36.6 (CH_2NH), 31.0 (CH_2CH_2NH), 28.4 (*tBu*), 22.8 ($CH_2C{\equiv}CH$), 20.7–20.9 ppm (4× CH_3CO).



Compound 24: The pentafluorophenyl ester of β-caboxymethylgalactoside **4** (127 mg, 0.229 mmol) prepared as described above, was treated with propargylamine hydrochloride (21 mg, 0.229 mmol) in THF in the presence of triethylamine (3 equiv). The reaction mixture was stirred at RT overnight, then the solvent was evaporated, and the resulting solid was purified by flash chromatography (hexane/EtOAc) to afford alkyne **24** (83 mg, 0.194 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.26$ (t, $J = 4$ Hz, 1H; $NHCH_2$), 5.53 (brd, $J =$ 3 Hz, 1H; H-4), 5.09 (t, $J_1 = 10$ Hz, 1H; H-2), 5.02 (dd, $J_1 = 3$, $J_2 = 10$ Hz, 1H; H-3), 4.00–4.15 (m, 4H; H-6, $CH_2C{\equiv}CH$), 3.83–3.96 (m, 2H; H-1, H-5), 2.40–2.50 (m, 2H; $CH_2C(O)$), 2.22 (t, $J = 2$ Hz, 1H; $CH_2C{\equiv}CH$), 1.93–2.18 ppm (m, 12H; 4× CH_3CO).

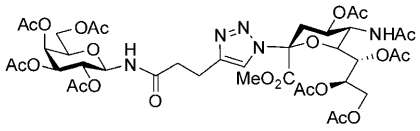


Compound 25: 1,5-Diazabicyclo[5.4.0]undec-5-ene (DBU; 125 μL, 0.890 mmol) and DPPA (192 μL, 0.890 mmol) were added to a solution of 4-butynol (69 mg, 0.99 mmol) in anhydrous DMF (300 μL). The reaction mixture was stirred at RT until consumption of the alcohol was complete. The mixture was then heated to 120 °C and stirred for ≈2 h, until disappearance of the intermediate phosphate was observed. The crude azide solution was cooled to RT, PMe_3 (0.988 mmol, 1 M in toluene) was added, and the reaction was stirred until no further formation of N_2 was observed. The crude amine solution was added by means of a cannula into a second vessel containing the pentafluorophenyl ester derivative of β-caboxymethylgalactoside **4** (55 mg, 0.0988 mmol). The reaction was stirred overnight under a nitrogen atmosphere, then solvent was evaporated, and the crude material was purified by flash chromatography (hexane/EtOAc) to afford alkyne **25** (15.4 mg, 0.0349 mmol, 35% yield overall from 4-butynol). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.37$ (t, $J =$ 6 Hz, 1H; $NHCH_2$), 5.45 (dd, $J = 1$, $J = 3$ Hz, 1H; H-4Gal), 5.11 (t, $J =$ 10 Hz, 1H; H-2Gal), 5.04 (dd, $J_1 = 3$, $J_2 = 10$ Hz, 1H; H-3Gal), 4.00–4.20 (m, 2H; H-6Gal), 3.84–3.96 (m, 2H; H-1Gal, H-5Gal), 3.33–3.48 (m, 3H; H-9, H-12), 2.40–2.50 (m, 2H; H-7, H-10), 1.94–2.19 ppm (m, 12H; 4× CH_3CO).

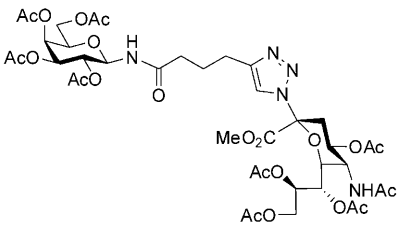


General procedure for the synthesis of 27–37, and 55 (click cycloaddition): Aqueous sodium ascorbate (1.1 equiv, 1 M) and an aqueous solution of $CuSO_4$ (0.11 equiv, 0.3 M) were added to a solution of alkyne (0.1 M) and sialic acid azide **26**[8] (1.1 equiv) in methanol. The reaction vessel was protected from light and the reaction mixture was vigorously stirred for 2–3 h. The solvent was then evaporated and the solid residue was extracted with CH_2Cl_2 (15 mL). The combined organic layer was concentrated and purified by flash chromatography.

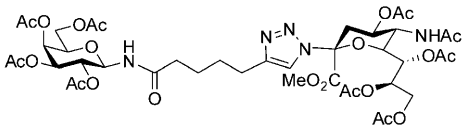
*Compound **27***: The reaction of alkyne **15** (56 mg, 0.131 mmol) with **26** following the general procedure described above afforded compound **27** (100 mg, 0.106 mmol, 81%). $[\alpha]_D = +9.9$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 8.00$ (s, 1H; H-Triaz), 5.39–5.54 (m, 3H; H-4Gal, H-7Neu, H-8Neu), 5.33 (d, $J = 9$ Hz, 1H; H-1Gal), 5.23 (dd, $J_1 = 10$, $J_2 = 4$ Hz, 1H; H-3Gal), 5.17 (t, $J = 9$ Hz, 1H; H-2Gal), 5.09 (dt, $J_1 = 4.5$, $J_2 = 11$ Hz, 1H; H-4Neu), 4.45–4.00 (m, 7H; H-9Neu, H-5Neu, H-6Neu, H-5Gal, H-6Gal), 3.85 (s, 3H; $COOCH_3$), 3.34 (dd, $J_1 = 13.0$, $J_2 = 4.2$ Hz, 1H; H-3_{eq}Neu), 3.01 (brs, 2H; $CH_2CH_2C(O)NH$), 2.52–2.67 (m, 3H; H-3_{ax}Neu, $CH_2CH_2C(O)NH$), 1.90–2.20 ppm (m, 27H; 9× CH_3CO); ^{13}C NMR (400 MHz, CD_3OD): $\delta = 168.0$–172.0 (11× *C*=*O*), 137.5 (C-Triaz), 124.9 (CH-Triaz), 79.2, 74.0, 73.0, 71.6, 69.1, 69.0, 68.5, 67.8, 67.6 (C-1Gal, C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 63.3, 62.0 (C-6Gal, C-9Neu), 53.9 ($COOCH_3$), 49.0 (C-5Neu), 35.5 (C-3Neu, $CH_2CH_2C(O)NH$), 21.6–21.9 ppm (9× CH_3CO, $CH_2CH_2C(O)NH$); HRMS (FT-ICR, ESI): *m*/*z* calcd for $C_{39}H_{53}N_5O_{22}$: 966.307995[*M*+Na^+]; found: 966.30482.



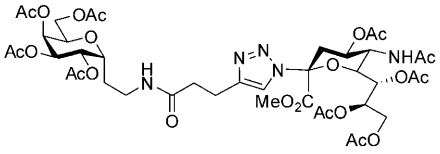
*Compound **28***: The reaction of alkyne **16** (90 mg, 0.204 mmol) with **26** following the general procedure described above afforded **28** (152 mg, 0.159 mmol, 78%). $[\alpha]_D = +12.4$ ($c = 1$ in $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.95$ (s, 1H; H-Triaz), 5.37–5.50 (m, 3H; H-4Gal, H-7Neu, H-8Neu), 5.33 (d, $J = 9$ Hz, 1H; H-1Gal), 5.23 (dd, $J_1 = 10$, $J_2 = 4$ Hz, 1H; H-3Gal), 5.18 (t, $J = 9$ Hz, 1H; H-2Gal), 5.11 (dt, $J_1 = 4.5$, $J_2 = 11$ Hz, 1H; H-4Neu), 4.00–4.45 (m, 7H; H-9Neu, H-5Neu, H-6Neu, H-5Gal, H-6Gal), 3.85 (s, 3H; $COOCH_3$), 3.37 (dd, $J_1 = 13.0$, $J_2 = 4.5$ Hz, 1H; H-3_{eq}Neu), 2.75 (t, $J = 7.5$ Hz, 2H; $CH_2CH_2C(O)NH$), 2.58 (t, $J = 12.5$ Hz, 1H; H-3_{ax}Neu), 2.30 (t, $J = 7.5$ Hz, 1H; $CH_2CH_2CH_2C(O)NH$), 1.80–2.25 ppm (m, 29H; 9× CH_3CO, $CH_2CH_2CH_2C(O)NH$); ^{13}C NMR (400 MHz, CD_3OD): $\delta = 168.0$–174.0 (11× *C*=*O*), 147.5 (C-Triaz), 120.2 (CH-Triaz), 77.8, 73.8, 72.0, 71.5, 68.9, 68.3, 68.0, 67.6, 66.8 (C-1Gal, C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 61.9, 61.2 (C-6Gal, C-9Neu), 53.2 ($COOCH_3$), 48.5 (C-5Neu), 35.5 (C-3Neu), 37.4 ($CH_2CH_2CH_2C(O)NH$), 25.0 ($CH_2C(O)NH$), 24.2 ($CH_2CH_2CH_2$), 19.0–21.3 ppm (9× CH_3CO, $CH_2CH_2C(O)NH$); HRMS (FT-ICR, ESI): *m*/*z* calcd for $C_{40}H_{55}N_5O_{22}$: 980.323645 [*M*+Na^+]; found: 980.32300.



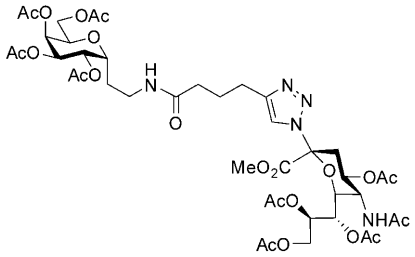
*Compound **29***: The reaction of alkyne **17** (10 mg, 0.022 mmol) with **26** following the general procedure described above afforded crude **29** (16 mg, 0.016 mmol), which was not further purified but subsequently deprotected to ligand **40**.



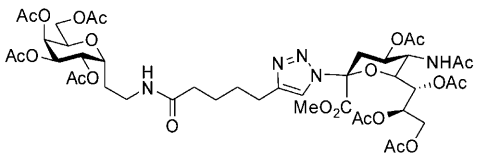
*Compound **30***: The reaction of alkyne **18** (23 mg, 0.050 mmol) with **26** following the general procedure described above afforded **30** (31 mg, 0.032 mmol, 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.71 (s, 1H; H-Triaz), 6.45 (br s, 1H; NH), 5.28–5.40 (m, 3H; H-4Gal, H-7Neu, H-8Neu), 5.25 (d, J=9.8 Hz, 1H; AcNH), 5.00–5.17 (m, 3H; H-4Neu, H-2Gal, H-3Gal), 4.46–4.50 (m, 1H; H-6bGal), 3.90–4.30 (m, 7H; H-9Neu, H-5Neu, H-6Neu, H-1Gal, H-5Gal, H-6aGal), 3.71 (s, 3H; $COOCH_3$), 3.28–3.46 (m, 2H; $CH_{2b}NC(O)$, H-3_{eq}Neu), 3.24–3.06 (m, 1H; $CH_{2a}NC(O)$), 2.49–2.71 (m, 5H; H-3_{ax}Neu, $CH_2CH_2C(O)NH$), 1.80–2.15 (m, 27H; 9×CH_3CO), 1.60–1.75 (m, 1H; $CH_{2b}CH_2NC(O)$), 1.50–1.60 ppm (m, 1H; $CH_{2a}CH_2NC(O)$).



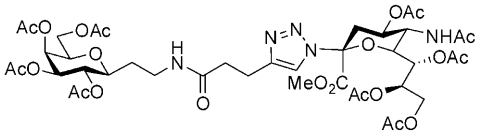
*Compound **31***: The reaction of alkyne **19** (27 mg, 0.058 mmol) with **26** following the general procedure described above afforded **31** (51 mg, 0.052 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.70 (s, 1H; H-Triaz), 6.30 (br s, 1H; NH), 5.36–5.48 (m, 3H; H-4Gal, H-7Neu, H-8Neu), 5.33 (d, J=9.0 Hz, 1H; AcNH), 5.11–5.23 (m, 3H; H-4Neu, H-2Gal, H-3Gal), 4.45 (t, J=7.5 Hz, 1H; H-6bGal), 4.23–4.37 (m, 3H; H-9Neu, H-5Gal), 4.00–4.19 (m, 4H; H-5Neu, H-6Neu, H-1Gal, H-6aGal), 3.70 (s, 3H; $COOCH_3$), 3.39–3.53 (m, 2H; $CH_{2b}NC(O)$, H-3_{eq}Neu), 3.12–3.30 (m, 1H; $CH_{2a}NC(O)$), 2.68–2.90 (m, 2H; $CH_2CH_2CH_2C(O)NH$), 2.68 (t, J=12 Hz, 1H; H-3_{ax}Neu), 2.28 (t, J=7 Hz, 2H; $CH_2CH_2C(O)NH$), 1.60–2.25 ppm (m, 31H; 9×CH_3CO, $CH_2CH_2NC(O)$, $CH_2CH_2CH_2$).



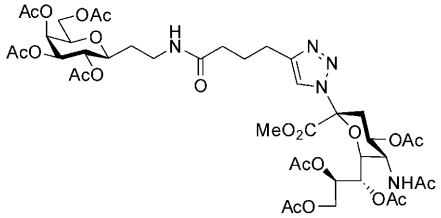
*Compound **32***: The reaction of alkyne **20** (31 mg, 0.064 mmol) with **26** following the general procedure described above afforded **32** (56 mg, 0.056 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.61 (s, 1H; H-Triaz), 6.13 (br s, 1H; NH), 5.25–5.42 (m, 4H; AcNH, H-4Gal, H-7Neu, H-8Neu), 5.04–5.16 (m, 3H; H-4Neu, H-2Gal, H-3Gal), 4.45 (t, J=8 Hz, 1H; H-6bGal), 4.14–4.29 (m, 3H; H-9Neu, H-5Gal), 3.90–4.12 (m, 4H; H-5Neu, H-6Neu, H-1Gal, H-6aGal), 3.71 (s, 3H; $COOCH_3$), 3.31–3.44 (m, 2H; $CH_{2b}NC(O)$, H-3_{eq}Neu), 3.12 (m, 1H; $CH_{2a}NC(O)$), 2.68 (t, J=7.5 Hz, 2H; $CH_2(CH_2)_2CH_2C(O)NH$), 2.60 (t, J=12 Hz, 1H; H-3_{ax}Neu), 2.17 (t, J=7.5 Hz, 2H; $(CH_2)_3CH_2C(O)NH$), 1.60–2.13 ppm (m, 33H; 9×CH_3CO, $CH_2CH_2NC(O)$, $CH_2(CH_2)_2CH_2$).



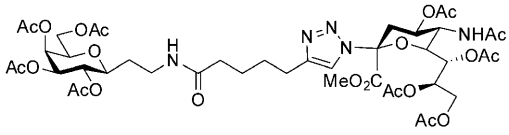
*Compound **33***: The reaction of alkyne **21** (25 mg, 0.055 mmol) with **26** following the general procedure described above afforded **33** (36 mg, 0.037 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.80 (s, 1H; H-Triaz), 6.28 (br s, 1H; NH), 5.26–5.41 (m, 3H; H-4Gal, H-7Neu, H-8Neu), 5.08–5.12 (m, 1H; H-4Neu), 5.03 (t, J=9.6 Hz, H-2Gal), 4.95 (dd, J_1=3.4, J_2=10 Hz, 1H; H-3Gal), 4.17–4.28 (m, 2H; H-9bNeu, H-6Neu), 3.95–4.10 (m, 4H; H-5Neu, H-9aNeu, H-6Gal), 3.84 (t, J=6.5 Hz, 1H; H-5Gal), 3.71 (s, 3H; $COOCH_3$), 3.31–3.55 (m, 3H; H-1Gal, $CH_{2b}NC(O)$, H3$_{eq}$Neu), 3.20–3.30 (m, 1H; $CH_{2a}NC(O)$), 2.49–2.80 (m, 3H; H-3_{ax}Neu, $CH_2CH_2C(O)NH$), 1.80–2.25 (m, 29H; 9×CH_3CO, $CH_2CH_2C(O)NH$), 1.50–1.80 ppm (m, 2H; $CH_2CH_2NC(O)$).



*Compound **34***: The reaction of alkyne **22** (25 mg, 0.053 mmol) with **26** following the general procedure described above afforded **34** (34 mg, 0.034 mmol, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.30 (br s, 1H; NH), 5.25–5.42 (m, 3H; H-4Gal, H-7Neu, H-8Neu), 5.08–5.12 (m, 1H; H-4Neu), 5.03 (t, J=9.6 Hz, H-2Gal), 4.93 (dd, J_1=3.4, J_2=10 Hz, 1H; H-3Gal), 4.17–4.28 (m, 2H; H-9bNeu, H-6Neu), 3.95–4.11 (m, 4H; H-5Neu, H-9aNeu, H-6Gal), 3.81 (t, J=6.7 Hz, 1H; H-5Gal), 3.72 (s, 3H; $COOCH_3$), 3.30–3.52 (m, 3H; H-1Gal, $CH_{2b}NC(O)$, H-3_{eq}Neu), 3.20–3.30 (m, 1H; $CH_{2a}NC(O)$), 2.49–2.80 (m, 3H; H-3_{ax}Neu, $CH_2CH_2CH_2C(O)NH$), 1.50–2.27 ppm (m, 33H; 9×CH_3CO, $CH_2CH_2CH_2C(O)NH$, $CH_2CH_2NC(O)$).

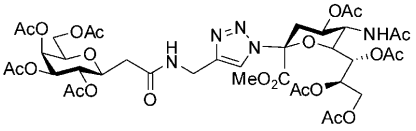


*Compound **35***: The reaction of alkyne **23** (9 mg, 0.019 mmol) with **26** following the general procedure described above afforded **35** (13 mg, 0.013 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.45 (br s, 1H; NH), 5.23–5.42 (m, 3H; H-4Gal, H-7Neu, H-8Neu), 5.08–5.12 (m, 1H; H-4Neu), 5.03 (t, J=9.5 Hz, H-2Gal), 4.91 (dd, J_1=3.4, J_2=10 Hz, 1H; H-3Gal), 4.20–4.32 (m, 2H; H-9bNeu, H-6Neu), 3.95–4.13 (m, 4H; H-5Neu, H-9aNeu, H-6Gal), 3.81 (t, J=6.7 Hz, 1H; H-5Gal), 3.70 (s, 3H; $COOCH_3$), 3.30–3.52 (m, 3H; H-1Gal, $CH_{2b}NC(O)$, H-3_{eq}Neu), 3.20–3.30 (m, 1H; $CH_{2a}NC(O)$), 2.47–2.80 (m, 3H; H-3_{ax}Neu, $CH_2(CH_2)_2C(O)NH$), 1.50–2.25 ppm (m, 35H; 9×CH_3CO, $(CH_2)_3CH_2C(O)NH$, $CH_2CH_2NC(O)$).

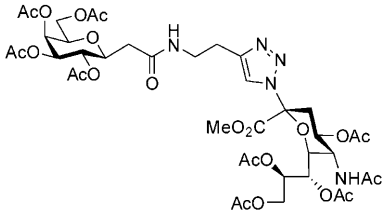


*Compound **36***: The reaction of alkyne **24** (84.2 mg, 0.197 mmol) with **26** following the general procedure described above afforded **36** (136.1 mg, 0.147 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.85 (s, 1H; CH-

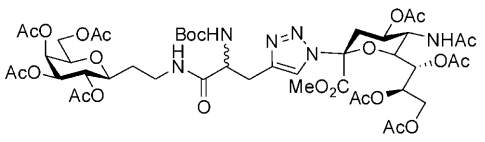
Triaz), 6.80 (br s, 1H; N*H*CH$_2$), 5.31–5.51 (m, 3H; H-4Gal, H-8Neu, H-7Neu), 4.96–5.20 (m, 3H; H-4Neu, H-2Gal, H-3Gal), 4.47–4.67 (m, 2H; H-6Neu, H-9aNeu), 4.21–4.36 (m, 2H; H-5Neu, H-9bNeu), 3.90–4.17 (m, 6H; H-1Gal, H-5Gal, H-6Gal, NHCH_2-Triaz), 3.78 (s, 3H; CH_3OCONeu), 3.40 (dd, J_1=4, J_2=13 Hz, H-3$_{eq}$Neu), 2.65 (t, J=12 Hz, 1H; H-3$_{ax}$Neu), 2.48 (br s, 2H; Gal-CH_2-), 1.80–2.20 ppm (m, 27H; 8× CH_3CO, CH_3CONH).



*Compound **37***: The reaction of alkyne **25** (13.1 mg, 0.0297 mmol) with **26** following the general procedure described above afforded **37** (8.0 mg, 0.0132 mmol, 45%). ^{1}H NMR (400 MHz, CDCl$_3$): δ=7.77 (s, 1H; CH-Triaz), 6.79 (t, J=6 Hz, 1H; N*H*CH$_2$), 5.40–5.48 (m, 2H; H-4Gal, H-8Neu), 5.37 (dd, J_1=2, J_2=9 Hz, 1H, H-7Neu), 5.18 (dt, J_1=4, J_2= 12 Hz, 1H; H-4Neu), 5.10 (t, J=10 Hz, 1H; H-2Gal), 5.05 (dd, J_1=3, J_2=10 Hz, 1H; H-3Gal), 4.28–4.35 (m, 2H; H-6Neu, H-9aNeu), 4.03–4.15 (m, 4H; H-6Gal, H-5Neu, H-9bNeu), 3.91–3.99 (m, 2H; H-1Gal, H-5Gal), 3.80 (s, 3H; CH_3OCONeu), 3.55–3.72 (m, 2H; C*H*NH), 3.43 (dd, J_1=4, J_2=13 Hz, H-3$_{eq}$Neu), 2.93 (t, J=6 Hz, 2H; CHCH_2-Triaz), 2.66 (dd, J_1=12, J_2=13 Hz, 1H; H-3$_{ax}$Neu), 2.43 (br s, 2H; Gal-CH_2), 1.95–2.20 (m, 24H; 8×CH_3CO), 1.91 ppm (s, 3H; CH_3CONH).

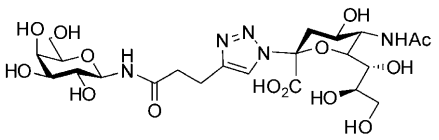


*Compound **55***: The reaction of alkyne **54** (100.0 mg, 0.175 mmol) with **26** following the general procedure described above afforded **55** (190.0 mg, 0.175 mmol, 99.7%). ^{1}H NMR (400 MHz, CDCl$_3$): δ=7.81 (s, 1H; CH-Triaz), 6.97 (br s, 1H; N*H*CH$_2$), 5.89 (s, 1H; NHBoc), 5.32–5.46 (m, 2H; H-4Gal, H-7Neu, H-8Neu), 5.16 (dt, J_1=4, J_2=12 Hz, 1H; H-4Neu), 5.07 (t, J=10 Hz, 1H; H-2Gal), 5.00 (dd, J_1=3, J_2=10 Hz, 1H; H-3Gal), 4.45–4.52 (m, 1H; H-10), 4.25–4.29 (m, 2H; H-6Neu, H-9aNeu), 4.01–4.17 (m, 4H; H-6Gal, H-5Neu, H-9bNeu), 3.90 (t, J=6 Hz, 1H; H-5Gal), 3.78 (s, 3H; CH_3OCONeu), 3.35–3.55 (m, 3H; H-1Gal, H-3$_{eq}$Neu, CH$_2$CH_{2a}NH), 3.25–3.35 (m, 2H; CH$_2$CH_{2b}NH, CHCH_2-Triaz), 2.60 (dd, J_1=12, J_2=13 Hz, 1H; H-3$_{ax}$Neu), 1.93–2.20 (m, 24H; 8×CH_3CO), 1.90 (s, 3H; CH_3CONH), 1.60–1.80 ppm (m, 2H; Gal-CH_2); ^{13}C NMR (400 MHz, CDCl$_3$): δ=167.5–168.7 (8×CH$_3$*C*O, CH$_2$CH$_2$NH*C*O, C-1Neu, CH$_3$*C*ONHNeu), 164.0 (NH*C*OO-*t*Bu), 72.0 (C-5Gal), 71.5 (C-6Neu), 69.7 (C-3Gal), 66.8 (C-2Gal), 66.1 (C-4Neu), 65.9 (C-4Gal), 65.4 (C-8Neu), 64.6 (C-7Neu), 60.0 (C-9Neu), 59.3 (C-6Gal), 51.8 (*C*H$_3$OCONeu), 51.5 (*C*(CH$_3$)$_3$), 51.4 (CHNH), 47.0 (C-5Neu), 33.8 (2C, C-3Neu, CH$_2$NH), 28.5 (Gal-*C*H$_2$), 26.0 (*t*Bu), 25.8 (CH*C*H$_2$Triaz), 20.9 (*C*H$_3$CONHNeu), 18.7–18.9 ppm (8×*C*H$_3$CO).

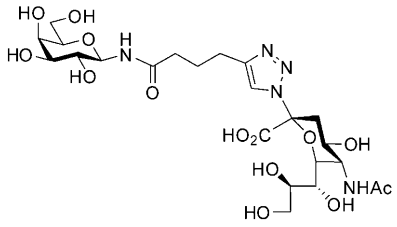


Typical deprotection procedure for synthesis of 38–48, 52 and 56–59: Sodium methoxide in methanol (1 M, 4 equiv) was added to a solution of acetylated ligand in anhydrous MeOH (≈0.05 M), and the reaction mixture was stirred for 30 min at RT. Water (≈6 equiv) was added and the reaction was stirred for a further 20 min at RT, then neutralized with Amberlyte [H$^+$], filtered and purified by reverse-phase (C-18) automatic chromatography using a water/methanol gradient.

*Ligand **38***: Deprotection of **27** (30 mg, 0.032 mmol) following the general procedure described above afforded ligand **38** (18 mg, 0.030 mmol, 95%). $[\alpha]_D$=+3.2 (c=0.8 in MeOH); ^{1}H NMR (D$_2$O, 400 MHz): δ=7.90 (s, 1H; H-triaz), 4.77 (d, J=9 Hz, 1H; H-1Gal), 3.40–3.95 (m, 13H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-2Gal, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.11 (dd, J_1=12.6, J_2=4.0 Hz, 1H; H-3$_{eq}$Neu), 2.94 (t, J=7.00 Hz, 2H; CH_2CH$_2$C(O)NH), 2.60 (t, J=7.00 Hz, 2H; CH$_2$CH_2C(O)NH), 2.11 (t, J=11 Hz, 1H; H-3$_{ax}$Neu), 1.90 ppm (s, 3H; CH_3C(O)NH); ^{13}C NMR (D$_2$O, 400 MHz): δ=176.4, 175.0 (*C*=*O*), 146.2 (C-Triaz), 120.9 (CH-triaz), 90.8 (C-2Neu), 79.7 (C-1Gal), 76.8, 74.0, 73.4, 71.2 , 69.3, 68.3, 68.2, 68.1, 68.0 (C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 62.5, 60.9 (C-6Gal, C-9Neu), 51.5 (C-5Neu), 39.6 (C-3Neu), 34.9 (CH$_2$*C*H$_2$C(O)NH), 22.1 (NHCO*C*H$_3$,), 20.7 ppm (CH$_2$*C*H$_2$C(O)NH); HRMS (FT-ICR, ESI): m/z calcd for $C_{22}H_{35}N_5O_{14}$: 593.218055 [M^+]; found: 593.21307.

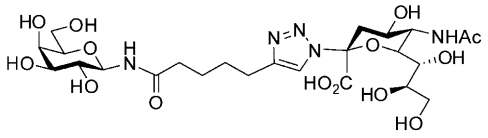


*Ligand **39***: Deprotection of **28** (68 mg, 0.071 mmol) following the general procedure described above afforded ligand **39** (42 mg, 0.069 mmol, 97%). $[\alpha]_D$=+6.8 (c=0.7 in MeOH); ^{1}H NMR (D$_2$O, 400 MHz): δ=7.95 (s, 1H; H-triaz), 4.77 (d, J=9 Hz, 1H; H-1Gal), 3.40–3.95 (m, 13H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-2Gal, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.11 (d, J=9 Hz, 1H; H-3$_{eq}$Neu), 2.61–2.68 (m, 2H; CH_2(CH$_2$)$_2$C(O)NH), 1.98–2.24 (m, 2H; (CH$_2$)$_2$CH_2C(O)NH), 2.11 (t, J=11.5 Hz, 1H; H-3$_{ax}$Neu), 1.80–1.95 ppm (m, 5H; NHC(O)CH_3, CH$_2$CH_2CH$_2$); ^{13}C NMR (D$_2$O, 400 MHz): δ=177.3, 175.0 (C=O), 120.0 (CH-triaz), 79.8 (C-1Gal), 76.7, 74.1, 73.4, 71.2, 70.4, 69.3, 68.9, 68.1, 67.9, 66.7 (C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-5Neu, C-6Neu, C-7Neu, C-8Neu), 62.8, 60.9 (C-6Gal, C-9Neu), 51.5 (C-5Neu), 39.5 (C-3Neu), 34.4 ((CH$_2$)$_2$CH$_2$C(O)NH), 24.4 (CH$_2$CH$_2$CH$_2$C(O)NH), 24.0 (*C*H$_2$(CH$_2$)$_2$C(O)NH), 21.0 ppm (NHCO*C*H$_3$); HRMS (FT-ICR, ESI): m/z calcd for $C_{23}H_{37}N_5O_{14}$: 607.233705 [M^+]; found: 607.22861.

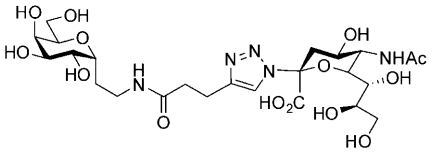


*Ligand **40***: Deprotection of crude **29** following the general procedure described above afforded ligand **40** (9 mg, 0.014 mmol, 66% from **17** (two steps)). $[\alpha]_D$=+22.3 (c=0.3 in H$_2$O); ^{1}H NMR (D$_2$O, 400 MHz): δ=7.95 (s, 1H; H-triaz), 5.11 (d, J=7 Hz, 1H; H-1Gal), 3.37–4.14 (m, 13H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-2Gal, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.08 (d, J=8.7 Hz, 1H; H-3$_{eq}$Neu), 2.56–2.63 (m, 2H; CH_2(CH$_2$)$_3$C(O)NH), 2.15–2.26 (m, 2H; (CH$_2$)$_3$CH_2C(O)NH), 2.08 (t, J=12 Hz, 1H; H-3$_{ax}$Neu), 1.90 (s, 1H; NHC(O)CH_3), 1.38–1.62 ppm (m, 4H; (CH_2)$_2$); ^{13}C NMR (D$_2$O,

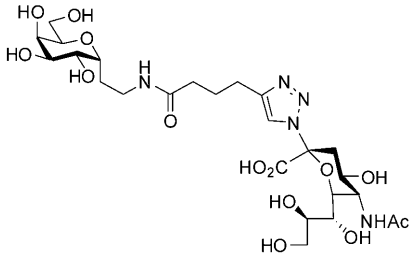
400 MHz): δ = 177.1, 175.2 (C=O), 121.4 (CH-triaz), 79.0 (C-1Gal), 76.7, 74.3, 73.1, 71.1, 70.7, 68.9, 68.7, 67.8, 67.6, 66.8 (C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-5Neu, C-6Neu, C-7Neu, C-8Neu), 62.7, 61.1 (C-6Gal, C-9Neu), 51.6 (C-5Neu), 39.4 (C-3Neu), 34.6 ($(CH_2)_3CH_2C(O)NH$), 25.0 ($CH_2(CH_2)_2CH_2C(O)NH$), 24.2 ($CH_2(CH_2)_3C(O)NH$), 21.1 ppm ($NHCOCH_3$); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{24}H_{39}N_5O_{14}$ $[M-H]^-$: 620.241530 found: 620.24059.



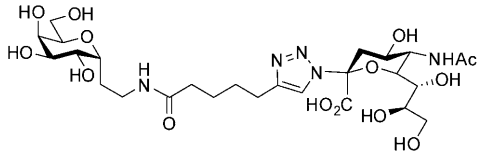
Ligand ***41***: Deprotection of **30** (23 mg, 0.024 mmol) following the general procedure described above afforded ligand **41** (14 mg, 0.023 mmol, 95%). $[\alpha]_D$ = +40.9 (*c* = 0.2 in MeOH); ^{1}H NMR (D_2O, 400 MHz): δ = 7.87 (s, 1H; H-triaz), 3.95–4.00 (m, 14H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-1Gal, H-2Gal, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.03–3.15 (m, 3H; H-3_{eq}Neu, CH_2NHC(O)), 2.87 (t, *J* = 7.1 Hz, 2H; $CH_2CH_2C(O)NH$), 2.44 (t, *J* = 7.1 Hz, 2H; $CH_2CH_2C(O)NH$), 2.08 (m, 1H; H-3_{ax}Neu), 1.90 (s,1H; NHC(O)CH_3), 1.50–1.75 ppm (m, 2H; $CH_2CH_2NHC(O)$); ^{13}C NMR (D_2O, 400 MHz): δ = 177.4, 174.8 (*C*=*O*), 120.0 (CH-triaz), 72.9, 72.1, 70.8, 70.2, 70.7, 68.5, 67.8, 66.9 (C-1Gal, C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-5Neu, C-6Neu, C-7Neu, C-8Neu), 61.6, 59.9 (C-6Gal, C-9Neu), 50.4 (C-5Neu), 38.4 (C-3Neu), 35.0 (CH_2NHC(O)), 34.3 ($CH_2CH_2C(O)NH$), 23.0 ($CH_2CH_2C(O)NH$), 20.9 ppm ($NHCOCH_3$); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{24}H_{39}N_5O_{14}$: 620.241530 $[M-H]^-$; found: 620.24069.



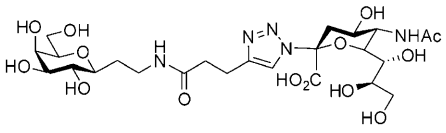
Ligand ***42***: Deprotection of **31** (16 mg, 0.016 mmol) following the general procedure described above afforded ligand **42** (10 mg, 0.016 mmol, 97%). $[\alpha]_D$ = +80.8 (*c* = 0.06 in MeOH); ^{1}H NMR (D_2O, 400 MHz): δ = 8.07 (s, 1H; H-triaz), 4.10–4.14 (m, 1H; H-1Gal), 3.60–4.06 (m, 13H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-2Gal, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.22–3.35 (m, 3H; H-3_{eq}Neu, CH_2NHC(O)), 2.79 (t, *J* = 7.2 Hz, 2H; $CH_2(CH_2)_2C(O)NH$), 2.22–2.37 (m, 3H; $CH_2CH_2C(O)NH$, H-3_{ax}Neu), 2.08 (s,1H; NHC(O)CH_3), 1.75–2.05 ppm (m, 4H; $CH_2CH_2CH_2$, $CH_2CH_2NHC(O)$); ^{13}C NMR (D_2O, 400 MHz): δ = 177.3, 175.0 (C=O), 119.9 (CH-triaz), 72.1 (C-1Gal), 72.9, 71.0, 70.4, 70.7, 68.8, 68.5, 67.1 (C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-5Neu, C-6Neu, C-7Neu, C-8Neu), 61.6, 60.03 (C-6Gal, C-9Neu), 51.2 (C-5Neu), 38.4 (C-3Neu), 35.0 (CH_2NHC(O)), 33.8 ($CH_2CH_2C(O)NH$), 23.6 ($CH_2(CH_2)_2C(O)NH$), 20.9 ($NHCOCH_3$), 21.0 ($CH_2CH_2C(O)NH$), 20.7 ppm ($CH_2CH_2CH_2$); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{25}H_{41}N_5O_{14}$: 634.257180 $[M-H]^-$; found: 634.25653.



Ligand ***43***: Deprotection of **32** (37 mg, 0.037 mmol) following the general procedure described above afforded ligand **43** (17 mg, 0.026 mmol, 71%). $[\alpha]_D$ = +41.9 (*c* = 0.49 in MeOH); ^{1}H NMR (D_2O, 400 MHz): δ = 7.92 (s, 1H; H-triaz), 4.00–4.05 (m, 1H; H-1Gal), 3.54–3.99 (m, 13H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-2Gal, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.15–3.28 (m, 3H; H-3_{eq}Neu, CH_2NHC(O)), 2.69 (t, *J* = 7.1 Hz, 2H; $CH_2(CH_2)_2C(O)NH$), 2.15–2.25 (m, 3H; $CH_2CH_2C(O)NH$, H-3_{ax}Neu), 2.01 (s,1H; NHC(O)CH_3), 1.50–1.94 ppm (m, 6H; $CH_2(CH_2)_2CH_2$, $CH_2CH_2NHC(O)$); ^{13}C NMR (D_2O, 400 MHz): δ = 176.0, 174.3 (C=O), 120.0 (CH-triaz), 72.0 (C-1Gal), 72.9, 70.8, 70.2, 68.5, 67.8, 67.0, 66.9 (C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-5Neu, C-6Neu, C-7Neu, C-8Neu), 61.5, 60.0 (C-6Gal, C-9Neu), 50.4 (C-5Neu), 38.5 (C-3Neu), 35.0 (CH_2NHC(O)), 34.3 ($CH_2CH_2C(O)NH$), 26.8, 23.5 ($CH_2(CH_2)_2CH_2$), 23.1 ($CH_2(CH_2)_3C(O)NH$), 22.3 ($CH_2CH_2C(O)NH$), 20.9 ppm ($NHCOCH_3$); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{26}H_{43}N_5O_{14}$: 648.272830 $[M-H]^-$; found: 648.27190.

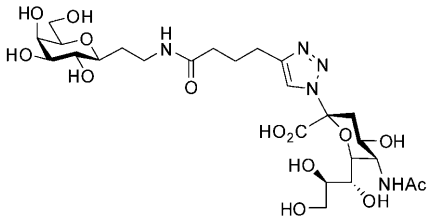


Ligand ***44***: Deprotection of **33** (25 mg, 0.026 mmol) following the general procedure described above afforded ligand **44** (16 mg, 0.026 mmol, 100%). $[\alpha]_D$ = +16.1 (*c* = 0.25 in MeOH); ^{1}H NMR (D_2O, 400 MHz): δ = 7.73 (s, 1H; H-triaz), 3.26–3.80 (m, 12H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.17 (t, *J* = 7.5 Hz, 1H; H-2Gal), 2.97–3.10 (m, 3H; CH_2NHC(O), H-3_{eq}Neu), 2.93 (dt, J_1 = 7.5, J_2 = 2.3 Hz, 1H; H-1Gal), 2.78 (t, *J* = 7.2 Hz,1H; $CH_2CH_2C(O)NH$), 2.78 (t, *J* = 7.2 Hz, 1H; $CH_2CH_2C(O)NH$), 1.99 (t, *J* = 11.2 Hz, 1H; H-3_{ax}Neu), 1.82 (s, 3H; NHC(O)CH_3), 1.72–1.76 (m, 1H; $CH_{2b}CH_2NH$), 1.30 ppm (m, 1H; $CH_{2a}CH_2NH$); ^{13}C NMR (D_2O, 400 MHz): δ = 173.7, 169.5 (C=O), 145.0 (C-triaz), 119.7 (CH-triaz), 89.6 (C-2Neu), 77.3, 73.0, 72.8, 69.5, 68.0, 66.9 (C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 76.6 (C-1Gal), 69.9 (C-2Gal), 61.5, 60.2 (C-6Gal, C-9Neu), 50.4 (C-5Neu), 38.4 (C-3Neu), 34.8 (CH_2NHC(O)), 34.2 ($CH_2CH_2C(O)NH$), 29.3 ($CH_2CH_2NHC(O)$), 20.9 ($NHCOCH_3$), 20.2 ppm ($CH_2CH_2C(O)NH$); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{24}H_{39}N_5O_{14}$: 620.241530 $[M-H]^-$; found: 620.24065.

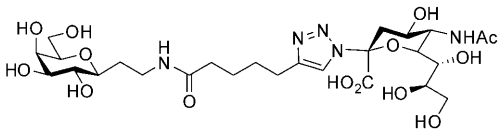


Ligand ***45***: Deprotection of **34** (20 mg, 0.020 mmol) following the general procedure described above afforded ligand **45** (12 mg, 0.019 mmol, 93%). $[\alpha]_D$ = +8.5 (*c* = 0.64 in MeOH); ^{1}H NMR (D_2O, 400 MHz): δ = 7.89 (s, 1H; H-triaz), 3.37–3.90 (m, 12H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.28 (t, *J* = 9.5 Hz, 1H; H-2Gal), 2.99–3.23 (m, 4H; CH_2NHC(O), H-3_{eq}Neu, H-1Gal), 2.60 (t, *J* = 9.2 Hz,1H; $CH_2CH_2C(O)NH$), 2.01–2.17 (m, 3H; $CH_2CH_2C(O)NH$, H-3_{ax}Neu), 1.75–19.5 (m, 6H; $CH_2CH_2CH_2$, NHC(O)CH_3, $CH_{2b}CH_2NH$), 1.42–1.52 ppm (m, 1H; $CH_{2a}CH_2NH$); ^{13}C NMR (D_2O, 400 MHz): δ = 173.7 (C=O), 120.27 (CH-triaz), 89.6 (C-2Neu), 77.3, 72.9, 72.8, 70.1, 68.4, 68.0, 66.9, 66.8 (C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 76.6 (C-1Gal), 69.6 (C-2Gal), 61.6, 60.2 (C-6Gal, C-9Neu), 50.3 (C-5Neu), 38.4 (C-3Neu), 34.8

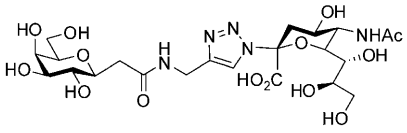
(CH_2NHC(O)), 33.7 (CH_2CH_2C(O)NH), 29.3 (CH_2CH_2NHC(O)), 23.8 ($CH_2CH_2CH_2$), 22.7 (CH_2CH_2C(O)NH), 20.9 ppm (NHCOCH_3); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{25}H_{41}N_5O_{14}$: 634.257180 $[M-H]^-$; found: 634.25636.



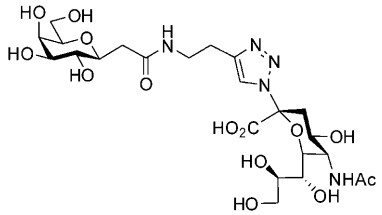
Ligand ***46***: Deprotection of **35** (13 mg, 0.013 mmol) following the general procedure described above afforded ligand **46** (8 mg, 0.012 mmol, 95%). $[\alpha]_D = +27.4$ ($c = 0.11$ in MeOH); ^{1}H NMR (D_2O, 400 MHz): $\delta = 7.81$ (s, 1H; H-triaz), 3.31–3.90 (m, 12H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.27 (t, J = 9.4 Hz, 1H; H-2Gal), 3.00–3.23 (m, 4H; CH_2NHC(O), H-3$_{eq}$Neu, H-1Gal), 2.57 (t, J = 9.4 Hz,1H; CH_2CH$_2$C(O)NH), 2.03–2.15 (m, 3H; CH$_2$CH_2C(O)NH, H-3$_{ax}$Neu), 1.83–1.96 (m, 4H; NHC(O)CH_3, CH_{2b}CH$_2$NH), 1.37–1.57 ppm (m, 5H; (CH_2)$_2$, CH_{2a}CH$_2$NH); ^{13}C NMR (D_2O, 400 MHz): δ = 175.5, 173.8, 169.7 (C=O), 146.8 (C-triaz), 119.5 (CH-triaz), 89.5 (C-2Neu), 77.4, 72.9, 72.8, 70.2, 68.0, 66.9, 66.8 (C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 76.2 (C-1Gal), 69.6 (C-2Gal), 61.6, 60.2 (C-6Gal, C-9Neu), 50.4 (C-5Neu), 38.4 (C-3Neu), 34.7 (CH_2NHC(O)), 34.4 (CH_2CH_2C(O)NH), 29.3, 26.7, 23.6 (CH_2CH_2NHC(O), (CH_2)$_2$), 23.0 (CH_2CH_2C(O)NH), 20.9 ppm (NHCOCH_3); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{26}H_{43}N_5O_{14}$: 648.272830 $[M-H]^-$; found: 648.27212.



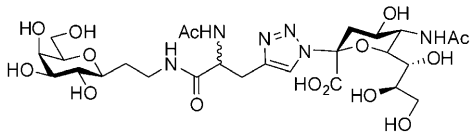
Ligand ***47***: Deprotection of **36** (136.1 mg, 0.144 mmol) following the general procedure described above afforded ligand **47** (26.9 mg, 0.0453 mmol, 31.4%). ^{1}H NMR (400 MHz, D_2O): δ = 8.14 (s, 1H; CH-Triaz$_1$), 3.54(s, 2H; CH_2-Triaz), 3.86–4.08 (m, 6H; H-2Gal, H-3Gal, H-4Neu, H-7Neu, H-8Neu, H-9aNeu), 3.59–3.74 (m, 7H; H-1Gal, H-4Gal, H-6Gal, H-5Neu, H-6Neu, H-9aNeu), 3.50 (t, J = 10 Hz, 1H; H-5Gal), 3.28 (dd, J_1 = 4, J_2 = 13 Hz, 1H; H-3$_{eq}$Neu), 2.86 (dd, J_1 = 3, J_2 = 15 Hz, 1H; CH-2aGal), 2.50 (dd, J_1 = 9, J_2 = 15 Hz, 1H; CH-2bGal), 2.28 (dd, J_1 = 11, J_2 = 13 Hz, 1H; H-3$_{ax}$Neu), 2.10 ppm (s, 3H; CH_3CONH); ^{13}C NMR (400 MHz, D_2O): δ = 173.83, 172.44, 169.43 (CH$_2$NH*C*O, C-1Neu, CH$_3$*C*ONHNeu), 143.2 (C$_q$Triaz$_1$), 120.4 (CH-Triaz$_1$), 89.7 (C-2Neu), 77.3, 75.5, 72.9, 72.6, 70.1, 69.4, 67.9, 66.9, 66.8 (C-1Gal, C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 61.6 (C-9Neu), 60.0 (C-6Gal), 50.4 (C-5Neu), 38.5 (C-3Neu), 37.5 (GalCH$_2$), 33.3 (NHCH$_2$Triaz), 20.9 ppm (NHCOCH_3); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{22}H_{34}N_5O_{14}$: 592.21077 $[M-H]^-$; found: 592.21028.



Ligand ***48***: Deprotection of **37** (9.2 mg, 0.0096 mmol) following the general procedure described above afforded ligand **48** (4.0 mg, 0.0453 mmol, 69%). ^{1}H NMR (400 MHz, D_2O): δ = 7.87 (s, 1H; CH-Triaz), 3.65–3.89 (m, 6H; H-2Gal, H-3Gal, H-4Neu, H-7Neu, H-8Neu, H-9aNeu), 3.40–3.54 (m, 6H; H-4Gal, H-6Gal, H-5Neu, H-6Neu, H-9aNeu), 3.31–3.39 (m, 3H; H-1Gal, NHCH_2CH$_2$Triaz), 3.27 (t, J = 10 Hz, 1H; H-5Gal), 3.09 (dd, J_1 = 4, J_2 = 12 Hz, 1H; H-3$_{eq}$Neu), 2.78 (t, J = 6 Hz, 2H; NHCH$_2$CH_2Triaz), 2.58 (dd, J_1 = 3, J_2 = 15 Hz, 1H; CH-2aGal), 2.21 (dd, J_1 = 9, J_2 = 15 Hz, 1H; CH-2bGal), 2.10 (dd, J_1 = 11, J_2 = 12 Hz, 1H; H-3$_{ax}$Neu), 1.90 ppm (s, 3H; CH_3CONH); ^{13}C NMR (400 MHz, D_2O): δ = 173.9, 172.5, 169.5 (CH$_2$NH*C*O, C-1Neu, CH$_3$*C*ONHNeu), 77.3 75.62, 7.95, 72.6, 70.2, 69.5, 67.9, 66.93, 66.87 (C-1Gal, C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 61.6 (C-9Neu), 60.0 (C-6Gal), 50.4 (C-5Neu), 38.4 (C-3Neu), 37.6 (NHCH_2CH_2Triaz), 37.5 (GalCH$_2$), 23.5 (NHCH$_2$CH_2Triaz), 20.9 ppm (NHCOCH_3); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{23}H_{36}N_5O_{14}$: 606.22642 $[M-H]^-$; found: 606.22665.

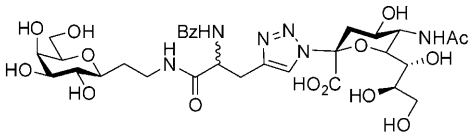


Ligand ***52***: Aqueous sodium ascorbate (0.1 mL, 1 M) and an aqueous solution of $CuSO_4$ (0.1 mL, 0.3 M) were added to a solution of alkyne **51** (20 mg, 0.039 mmol) and **26**[9] (24 mg, 0.047 mmol) in methanol (1 mL). The reaction vessel was protected from light and the reaction mixture was vigorously stirred overnight. The solvent then was evaporated and the solid residue was extracted with CH_2Cl_2 (15 mL). The extracts were concentrated and purified by flash chromatography to give the crude acetylated coupling product (27 mg, 0.026 mmol), which was not further purified, but subsequently deprotected, as described above for compounds **38**–**48**, to provide ligand **52** (17 mg, 0.025 mmol) as a mixture of stereoisomers, in 64% overall yield. ^{1}H NMR (400 MHz, D_2O): δ = 7.96 (s, 1H; H-triaz), 4.42–4.50 (m, 1H; CH(NHAc)CH$_2$), 3.41–4.05 (m, 12H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.34 (t, J = 10 Hz, 1H; H-2Gal), 2.95–3.28 (m, 5H; CH(NHAc)CH_2, CH$_2$CH_2NHC(O), H-3$_{ax}$Neu), 2.15 (m, 1H; H-3$_{eq}$Neu), 1.84–2.00 (m, 4H; CH_3CO, CH_{2b}CH$_2$NHC(O)), 1.42–1.52 ppm (m, 1H; CH_{2a}CH$_2$NHC(O)). ^{13}C NMR (400 MHz, D_2O): δ = 122.0 (C-triaz), 79.0, 77.3, 74.0, 71.2, 70.0, 68.9, 67.6 (C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 77.6 (C-1Gal), 70.3 (C-2Gal), 62.2, 61.0 (C-6Gal, C-9Neu), 54.3 (*C*H(NHAc)CH$_2$), 52.1 (C-5Neu), 39.4 (C-3Neu), 30.7 (CH_2CH_2NHC(O)), 26.8 (CH_2CH_2NHC(O)), 21.9 ppm (*C*H$_3$CO); HRMS (FT-ICR, ESI): *m/z* calcd for $C_{26}H_{42}N_6O_{15}$: 677.262994 $[M-H]^-$; found: 677.26189.

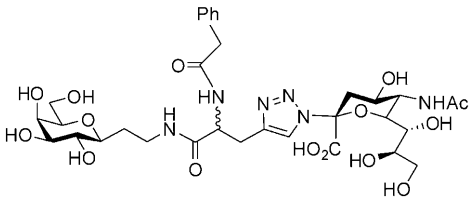


Ligand ***56***: CF_3COOH (900 μL) was added to a solution of **55** (15.2 mg, 0.014 mmol) in MeOH (2 mL). The mixture was stirred for ≈2 h until deprotection of the amino group was complete. Then the solvent was evaporated and the acid was eliminated by co-evaporation with toluene. The resulting amine was dissolved in anhydrous THF (1.5 mL), and triethylamine (21 μL, 0.15 mmol) and benzoyl chloride (7 μL, 0.06 mmol) were added. The mixture was stirred at RT for 4 h then the solvent was

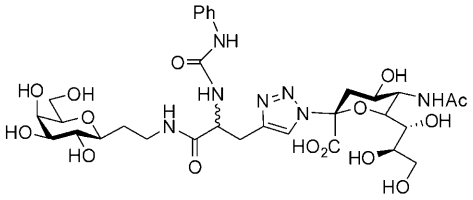
evaporated, and the crude product was purified by flash chromatography (CH_2Cl_2/acetone gradient). Deprotection of the product (9 mg, 0.0082 mmol) afforded ligand **56** as a 1:1 mixture of diastereomers (6.0 mg, 0.0081 mmol, 58%). ^{1}H NMR (400 MHz, D_2O): $\delta = 8.13$ (s, 1H; H-triaz), 7.77 (dd, $J = 4.3$, 5.9 Hz, 2H; Ph), 7.67–7.73 (m, 1H; Ph), 7.60 (t, $J = 7.5$ Hz, 2H; Ph), 4.85> (br t, $J = 6.5$ Hz, 1H; C*H*(NHBz)CH_2), 3.48–4.00 (m, 12H; H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, 2×H-9Neu, H-3Gal, H-4Gal, H-5Gal, 2×H-6Gal), 3.43 (m, 1H; H-2Gal), 3.17–3.38 (m, 5H; CH(NHBz)C*H*$_2$, CH_2CH_2NHC(O), H-3$_{ax}$Neu), 2.20–2.30 (m, 1H; H-3$_{eq}$Neu), 1.85–2.07 (m, 4H; *CH*$_3$CO, C*H*$_{2b}CH_2$NHC(O)), 1.50–1.64 ppm (m, 1H; C*H*$_{2a}CH_2$NHC(O)); ^{13}C NMR (400 MHz, D_2O): $\delta =$ 132.6, 128.8, 127.8 (Ph), 122.1 (C-triaz), 78.7, 77.5, 74.1, 71.2, 69.7, 68.7, 67.7 (C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 77.5 (C-1Gal), 70.7 (C-2Gal), 62.4, 61.2 (C-6Gal, C-9Neu), 54.1 (*C*H-(NHAc)CH_2), 51.3 (C-5Neu), 39.5 (C-3Neu), 30.1 (*C*H_2CH_2NHC(O)), 27.0 (CH_2*C*H_2NHC(O)), 21.8 ppm (*C*H_3CO); HRMS (FT-ICR, ESI): *m*/*z* calcd for $C_{31}H_{43}N_6O_{15}$: 739.27919 $[M-H]^-$; found: 739.27851.



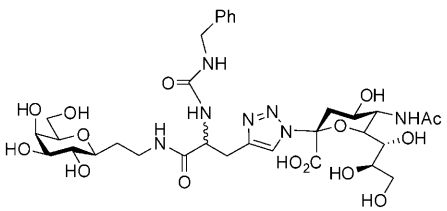
*Ligand **57***: CF_3COOH (250 μL) was added to a solution of **55** (20.2 mg, 0.0186 mmol) in CH_2Cl_2 (1 mL). The mixture was stirred for ≈2 h until deprotection of the amino group was complete. Then the solvent was evaporated and the acid was removed by co-evaporation with toluene. The amine was dissolved in anhydrous THF (0.5 mL) and treated with the pentafluorophenyl ester of phenylacetic acid (8.4 mg, 0.0279 mmol) and triethylamine (13 μL, 0.0929 mmol) for 4 h. The solvent was evaporated in vacuum and the product was purified by flash chromatography (CH_2Cl_2/acetone gradient). The product was deprotected as described above to afford ligand **57** (15.9 mg, 0.0144 mmol, 71%). ^{1}H NMR (400 MHz, D_2O): $\delta = 8.04$ (d, 1H; CH-Triaz(*R*/*S*)), 7.34–7.47 (m, 3H; H-Ph$_{meta}$, H-Ph$_{para}$), 7.30–7.25 (m, 2H; H-Ph$_{ortho}$), 4.45–4.65 (m, 1H; C*H*NH), 3.11–4.09 (m, 21H; H-1Gal, H-2Gal, H-3Gal, H-4Gal, H-5Gal, H-6Gal, H-1Neu, H-2Neu, H-3$_{eq}$Neu, H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, H-9Neu, GalCH$_2$*CH*$_2$NH, CH*CH*$_2$-Triaz, CO*CH*$_2$Ph), 2.19 (dd, $J_1 = 11$, $J_2 = 18$ Hz, 1H; H-3$_{ax}$Neu), 2.00–2.10 (m, 1H; GalCH$_{2a}$), 2.10 (s, 3H; C*H*$_3$CONH), 1.50–1.64 ppm (m, 1H; GalC*H*$_{2b}$); ^{13}C NMR (400 MHz, D_2O): $\delta = 173.9$, 173.2, 171.0, 169.3 (CH_2NH*C*O, C-1Neu, CH_3*C*ONHNeu, NH*C*OCH_2Ph), 133.5 (C^{q}Triaz), 127.7–127.9 (C-Ph$_{ortho}$, C-Ph$_{meta}$), 126.2 (C-Ph$_{para}$), 77.3, 76.3, 76.0, 72.9, 72.8, 70.2, 69.6, 68.0, 66.9 (C-1Gal, C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 61.5 (C-9Neu), 60.3 (C-6Gal), 52.6 (*C*HNH), 50.4 (C-5Neu), 40.8 (CH_2Ph), 38.5 (C-3Neu), 34.9, 34.6 (GalCH$_2$*C*H$_2$NH), 29.2 (GalCH$_2$), 26.0 (CH_2Triaz), 20.9 ppm (NHCO*C*H$_3$); HRMS (FT-ICR, ESI): *m*/*z* calcd for $C_{32}H_{45}N_6O_{15}$: 753.29484 $[M-H]^-$; found: 753.29366.



*Ligand **58***: CF_3COOH (750 μL) was added to a solution of Boc-protected acetylated ligand **55** (61.0 mg, 0.0561 mmol) in CH_2Cl_2 (3 mL). The mixture was stirred for ≈2 h until deprotection of the amino group was complete, then the solvent was evaporated and CF_3COOH was removed by co-evaporation with toluene. The amine was dissolved in anhydrous pyridine (1.5 mL), then anhydrous Et_3N (79 μL, 0.561 mmol) and PhNCO (20 μL, 0.168 mmol) were added. The mixture was stirred overnight at RT, then the solvent was evaporated in vacuum and the mixture was purified by flash chromatography (CH_2Cl_2/acetone gradient). The product was deprotected as described above to afford **58** (31.8 mg, 0.0421 mmol, 75%). ^{1}H NMR (400 MHz, D_2O): $\delta = 8.08$ (s, 1H; CH-Triaz), 7.39 (t, $J = 8$ Hz, 2H; H-Ph$_{meta}$), 7.30–7.35 (m, 2H; H-Ph$_{ortho}$), 7.17 (t, $J = 7$ Hz, 1H; H-Ph$_{para}$), 4.52 (t, $J = 6$ Hz, 1H; C*H*NH), 3.13–4.04 (m, 19H; H-1Gal, H-2Gal, H-3Gal, H-4Gal, H-5Gal, H-6Gal, H-1Neu, H-2Neu, H-3$_{eq}$Neu, H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, H-9Neu, GalCH$_2$*CH*$_2$NH, CH*CH*$_2$-Triaz), 2.20–2.30 (m, 1H; H-3$_{ax}$Neu), 2.02–2.12 (m, 1H; GalCH$_{2a}$), 1.94 (s, 3H; C*H*$_3$CONH), 1.55–1.65 ppm (m, 1H; GalCH$_{2b}$); ^{13}C NMR (400 MHz, D_2O): $\delta = 173.9$, 172.1, 168.9, 155.9 (CH_2NH*C*O, C-1Neu, CH_3*C*ONHNeu, NH*C*ONH), 136.54 (C^{q}Triaz), 128.1 (C-Ph$_{meta}$), 123.0 (C-Ph$_{para}$), 120.0 (CH-Triaz), 119.2 (C-Ph$_{ortho}$), 77.3, 76.5, 76.1, 73.0, 72.8, 70.2, 69.6, 68.0, 66.9 (C-1Gal, C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 61.5 (C-9Neu), 60.3 (C-6Gal), 52.9 (*C*HNH), 50.4 (C-5Neu), 38.6 (C-3Neu), 35.1 (GalCH$_2$*C*H$_2$NH), 29.1 (GalCH$_2$), 26.3 (CH_2Triaz), 20.9 ppm (NHCO*C*H$_3$); HRMS (FT-ICR, ESI): *m*/*z* calcd for $C_{31}H_{44}N_7O_{15}$: 754.29009 $[M-H]^-$; found: 754.29121.



*Ligand **59***: CF_3COOH (300 μL) was added to a solution of **55** (24.8 mg, 0.0228 mmol) in CH_2Cl_2 (1.2 mL). The mixture was stirred for ≈2 h until deprotection of the amino group was complete, then the solvent was evaporated, and CF_3COOH was removed by co-evaporation with toluene. The amine was dissolved in anhydrous pyridine (0.6 mL), then anhydrous Et_3N (32 μL, 0.228 mmol) and BnNCO (6 μL, 0.0456 mmol) were added. The mixture was stirred overnight at RT then the solvent was evaporated in vacuum and the mixture was purified by flash chromatography (CH_2Cl_2/acetone gradient). The product was deprotected as described above to afford **59** (0.013 mmol, 56%). ^{1}H NMR (400 MHz, D_2O): $\delta = 8.07$ (s, 1H; CH-Triaz), 7.00–7.43 (m, 5H; Ph), 4.43–4.53 (m, 1H; C*H*NH), 4.29 (qd, $J_1 = 5$, $J_2 = 16$ Hz, NHC*H*$_2$Ph), 3.07–4.16 (m, 19H; H-1Gal, H-2Gal, H-3Gal, H-4Gal, H-5Gal, H-6Gal, H-1Neu, H-2Neu, H-3$_{eq}$Neu, H-4Neu, H-5Neu, H-6Neu, H-7Neu, H-8Neu, H-9Neu, GalCH$_2$*CH*$_2$NH, CH*CH*$_2$-Triaz), 2.00–2.25 (m, 1H; H-3$_{ax}$Neu), 2.08 (s, 3H; C*H*$_3$CONH), 1.98–2.07 (m, 1H; GalCH$_{2a}$), 1.55–1.65 ppm (m, 1H; GalCH$_{2b}$); ^{13}C NMR (400 MHz, D_2O): $\delta = 173.9$, 172.3, 169.1 (CH_2NH*C*O, C-1Neu, CH_3*C*ONHNeu), 158.1 (NH*C*ONH), 138.2 (C^{q}Triaz), 127.6 (C-Ph$_{meta}$), 125.4 (C-Ph$_{ortho}$), 125.5 (C-Ph$_{ortho}$), 77.4, 76.5, 76.2, 73.0, 72.8, 70.1, 69.2, 68.0, 67.1, 66.9 (C-1Gal, C-2Gal, C-3Gal, C-4Gal, C-5Gal, C-4Neu, C-6Neu, C-7Neu, C-8Neu), 61.5 (C-9Neu), 60.2 (C-6Gal), 53.0 (CHNH), 50.4 (C-5Neu), 42.1 (CH_2Ph), 38.5 (C-3Neu), 34.7 (GalCH$_2$*C*H$_2$NH), 29.2 (GalCH$_2$), 25.9 (CH_2Triaz), 20.9 ppm



(NHCOCH_3); HRMS (FT-ICR, ESI): m/z calcd for $C_{32}H_{46}N_7O_{15}$: 768.30574 $[M-H]^-$; found: 768.30668.

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