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Assembly of Divalent Ligands and Their Effect on Divalent Binding to *Pseudomonas aeruginosa* Lectin LecA

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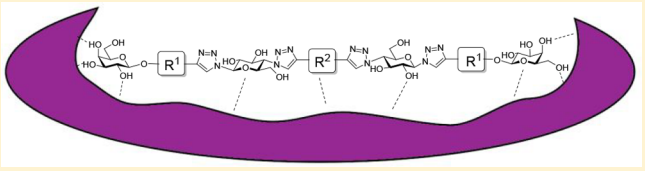
Supporting Information

ABSTRACT: Divalent ligands were prepared as inhibitors for the adhesion protein of the problematic *Pseudomonas aeruginosa* pathogen. Bridging two binding sites enables simultaneous binding of two galactose moieties, which strongly enhances binding. An alternating motif of glucose and triazole and aryl groups was shown to have the right mix of rigidity, solubility, and ease of synthesis. Spacers were varied with respect to the core unit as well as the aglycon portions in an attempt to optimize dynamics and enhance interactions with the protein. Affinities of the divalent ligands were measured by ITC, and K_d's as low as 12 nM were determined, notably for a compounds with either a rigid (phenyl) or flexible (butyl) unit at the core. Introducing a phenyl aglycon moiety next to the galactoside ligands on both termini did indeed lead to a higher enthalpy of binding, which was more than compensated by entropic costs. The results are discussed in terms of thermodynamics and theoretical calculations of the expected and observed multivalency effects.



INTRODUCTION

Protein−carbohydrate interactions are involved in many biological processes and diseases.[1−6] In this context, it is an important goal to find new specific molecular ligands for carbohydrate-binding or carbohydrate-processing proteins,[7−9] to be used as chemical probes,[10] or leads for therapeutic application.[11,12] One specific aspect of protein−carbohydrate interactions is the widespread prevalence of multivalency.[13−18] Numerous carbohydrate binding proteins of biological or medicinal interest contain more than one binding site, either identical or not. Bridging such binding sites by divalent or higher valency ligands may lead to greatly enhanced binding or inhibitory potencies.[19,20] Increasingly higher potency enhancements are being reported, and larger distances are also being covered by spacers.[21] In this context, it is likely that rigidified spacers are beneficial with potential for high potency and specificity. More flexible, often PEG-based spacers exhibit more shallow affinity optima.[22] Recent calculations involving effective molarity calculations and experiments revealed that PEG-based spacers will have no enhancing effect for bridging distant weak sites of millimolar binding affinities.[21] Nucleic acid based spacers seem to be preferred for the bridging of long distances,[19,23−27] while for shorter distances various structural types have been reported including, e.g., polyproline[28] and phenylene-ethynylene.[29] Notably, such spacers should have a persistent linear overall shape, but they should also allow for overall aqueous solubility of the multivalent construct. Previously, we described a modular spacer based on directly equatorially 1,4-linked glucose and 1,4-linked triazole moieties.[30,31] This system was used for optimization and yielded divalent ligand **1** (Figure 1) of the *Pseudomonas aeruginosa* adhesion lectin LecA with two galactose specific binding sites separated by ca. 26 Å when measured between

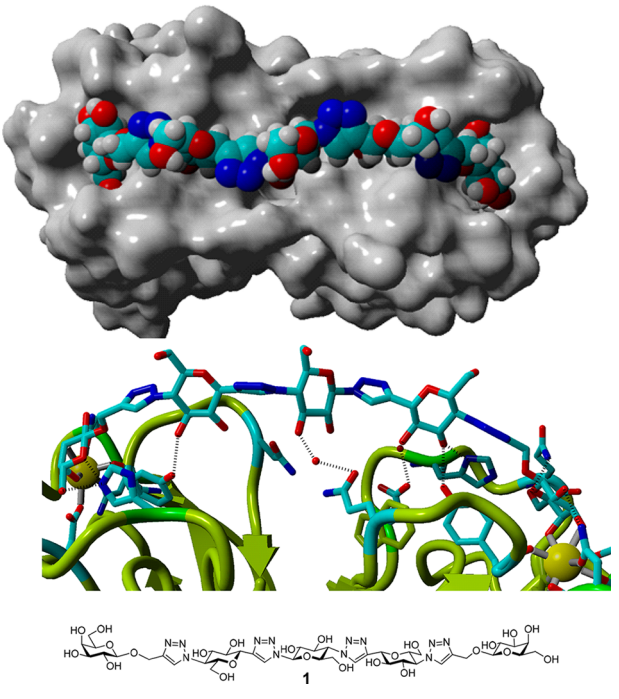


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Figure 1. X-ray structure of **1** bound to LecA (pdb 4YWA[34]).

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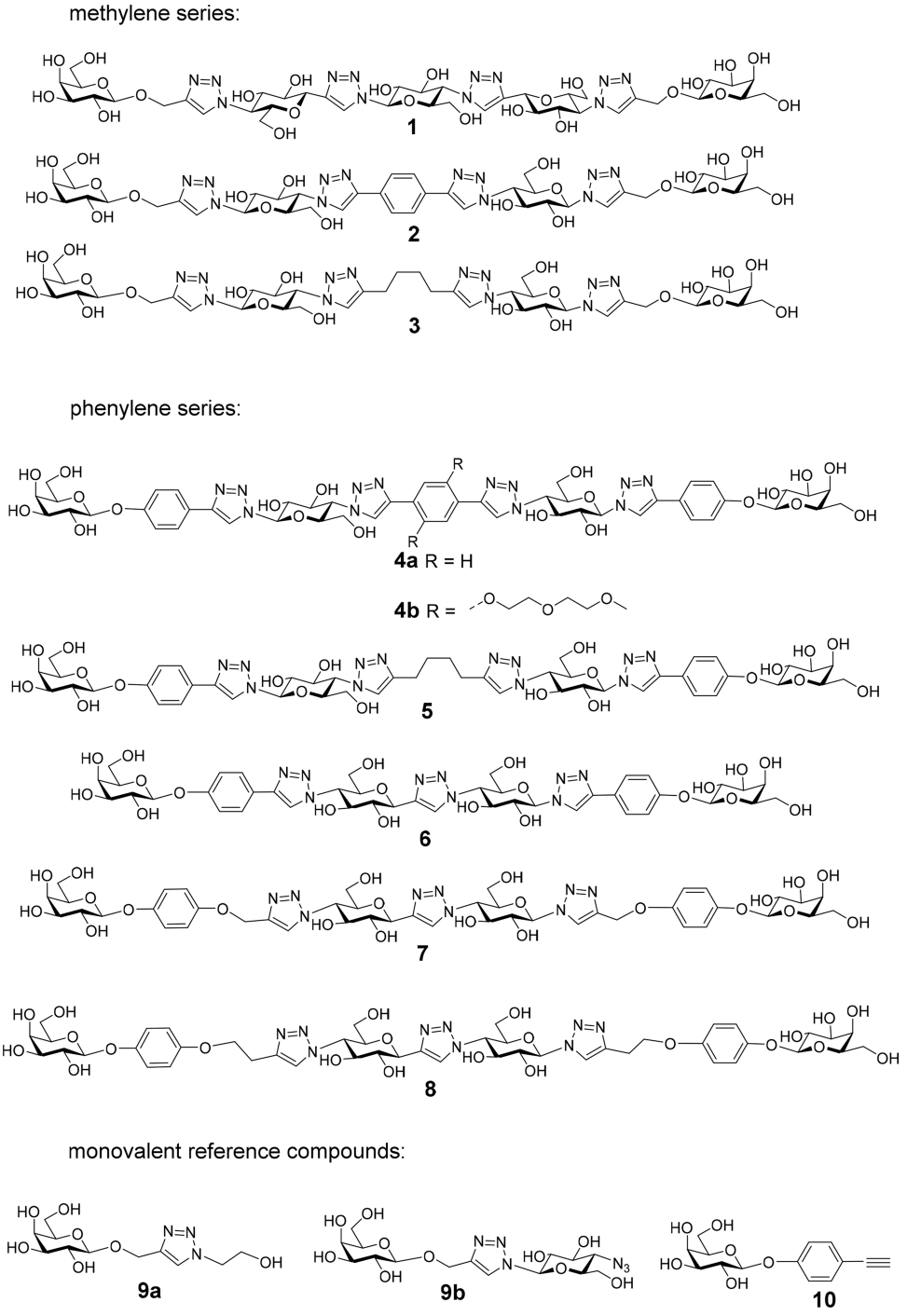

Figure 2. Structures of mono- and divalent LecA inhibitors used in this study.

the anomeric oxygens of the bound galactosides in pdb entry 1OKO.[32,33] The spacer length of **1** was optimized on the basis of inhibition and binding data (K_d = 28 nM), and it is the most potent reported divalent ligand for LecA. Its divalent binding mode was confirmed by X-ray crystallography, in which the whole spacer was visible, a feat not seen before for a synthetic spacer of this length (Figure 1).[34] The structure was also largely predicted by molecular modeling.[31] Interestingly, besides the interactions between the terminal galactoside ligands and the protein, additional interactions were observed between the protein and the spacer, possibly adding to the binding affinity. While **1** was clearly a potent LecA inhibitor, it was not clear which of its structural features were contributing significantly to its potency. We here describe the synthesis and detailed thermodynamic evaluation of a series of variants.

The aim was to first explore the synthetic possibilities of rigid spacers composed of glucose, triazole, and phenyl units and second to study the structure activity relationships between the spacer moieties and the binding to LecA. The syntheses were modular in all cases, but different strategies were explored involving building blocks based on azido-glucose derivatives and 1,4-diethynyl benzene linked together by CuAAC. ITC was used to shed more light on the effect of various components of the spacers, producing thermodynamic

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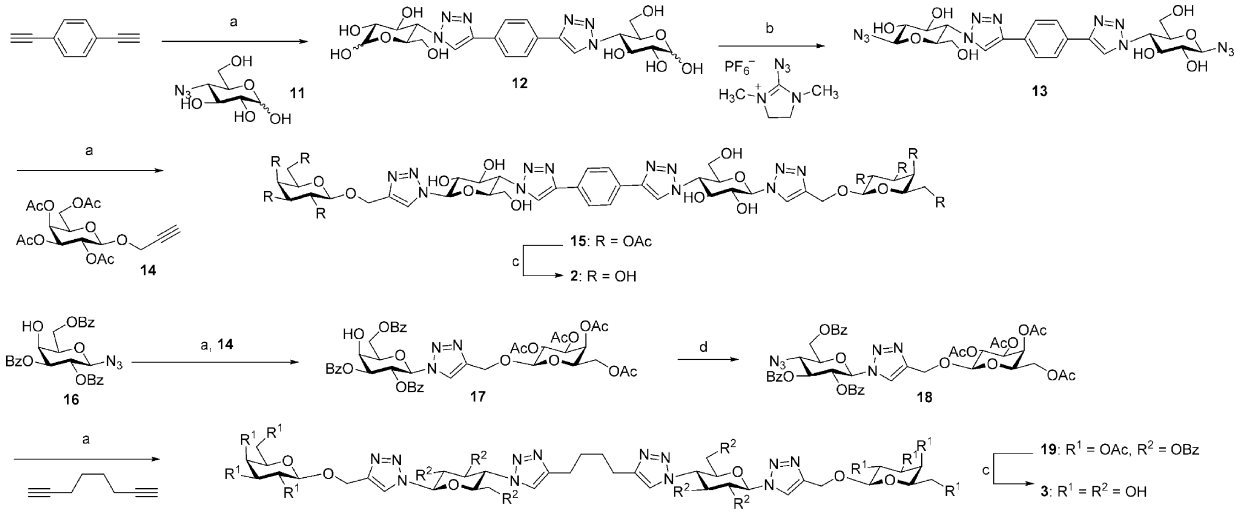
Scheme 1[a]



[a](a) $CuSO_4 \cdot 5H_2O$, Na-ascorbate, DMF/H_2O 9:1, microwave, 80 °C, 40 min, 65−85%; (b) D_2O/CH_3CN 4/1, Et_3N, 0 °C, 3 days, 50%; (c) MeONa, MeOH, 40−50% after prep HPLC; (d) (i) Tf_2O, pyridine, CH_2Cl_2, 0 °C, 1 h; (ii) NaN_3, DMF, 14 h, 80% over two steps.

parameters. Multivalent LecA inhibitors have been reported in the literature, e.g., based on fullerenes,[35] β-peptoids,[36] peptide dendrimers,[37−40] calixarenes,[41,42] cyclic carbohydrates,[43] perylene,[44] tetraphenylethylene,[45] a carbohydrate core,[46−48] and gold nanoparticles.[49] In addition, among other potent divalent ligands,[50,43] a potent divalent ligand with a K_d of 82 nM was found by library screening, and although less potent than **1**, it achieved its potency while being considerably more flexible.[51]

The lectin LecA is of medicinal interest as a virulence factor for *P. aeruginosa*, involved in the adhesion of the pathogen, biofim formation, and causing lung injury.[33,52,53] *P. aeruginosa* is a Gram-negative pathogen involved in diseases such as dermatitis, pancreatitis, urinary tract infections, keratitis, and respiratory tract infections.[54] It is regarded as a primary cause of death in immuno-compromised patients, notably those with cystic fibrosis.[55] Treating *P. aeruginosa* infection is becoming more difficult because of the increasing spread of drug-resistant strains,[56,57] which made it one of the highest priorities targets for intervention.[58] Another reason for its difficult eradication is its tendency to form biofilms.[59] In these biofilms, the bacteria are protected from the host defense system and the action of antibiotics. It was estimated that within a biofilm, bacteria are upward of 1000 times more resistant to conventional antibiotic treatment.[60−63] These issues combined make the search for *P. aeruginosa* therapeutics an urgent one. Bacterial adhesion is often a prelude to infection.[64,65] For *P. aeruginosa*, lectin LecA has been identified to play an important role in the internalization of the pathogen by binding to glycosylated targets displayed on the cell surface.[66] Therefore, inhibition of LecA is aimed at affecting adhesion of the bacteria at an early stage of the infection process and may provide an alternative to conventional antibiotics.[67] This concept[68,65] was supported by the therapeutic effect of a galactose solution against *P. aeruginosa* pneumonia in mouse models and cystic fibrosis patients through inhibiting the binding of LecA to its glycosylated targets.[53,69]

RESULTS AND DISCUSSION

From previous research, we knew that the length of the divalent ligand is a very important factor for the binding affinity.[31] For this reason, ligand **2** and **3** were designed with the same numbers of atoms in the spacer as the previously optimized **1** (Figure 2). For ligand **2**, a phenyl group replaces the central glucose moiety of **1** and maintains the number of atoms in the spacer (in terms of distance between the two galactosides). Furthermore, the two remaining glucose units in the spacer of **2** are linked in the opposite direction; i.e., the C(4) is linked to the core instead of C(1). The molecule is now also symmetrical just like its target protein. The consequences of the modification are that this synthesis does not require the use of a glucose building block with a C(1) alkyne, which is a more difficult to prepare building block. The strategy for the synthesis of **2** relied on the construction of the diazido-functionalized spacer **13** (Scheme 1). To this end, the two hemiacetals in **12** were converted to two β-azides using 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (ADMP).[70] CuAAC conjugation of **13** and **14**, followed by Zemplén deprotection, yielded **2**. Next, a totally unconstrained central unit was introduced in the design of **3** in order to evaluate the importance of the constraint in **1** and **2**. For ligand **3**, octa-1,7-diyne was used to introduce the central unit. For the synthesis, a different strategy was used than for **2**. Here, the galactoside ligand was first coupled to the spacer unit, and the resulting compound was linked to the core structure at the end. The partially benzoylated building block **16** was "clicked" with **14** to yield **17**. After activation as a triflate, the axial hydroxyl at C(4) was displaced by sodium azide leading to equatorial azide **18**. CuAAC conjugation to the central dialkyne, followed by the Zemplén deprotection afforded ligand **3**. Overall, the advantage of this strategy was to avoid the relatively low yielding ADMP step. The synthesis is now highly efficient with only nine steps from commercial peracetylated sugars and an overall yield of 13%.

The next aim was to introduce a phenyl group as the aglycon part of the terminal galactoside ligands, as this moiety is known to enhance the LecA binding by a factor of ca. 5−10 fold,[71,43,41,72,47,73] benefiting from CH−π interactions.[74] In the first approach, **13** was linked to **20a** (Scheme S1) by CuAAC to give **21** and after acetyl removal **4a** was obtained (Scheme 2).

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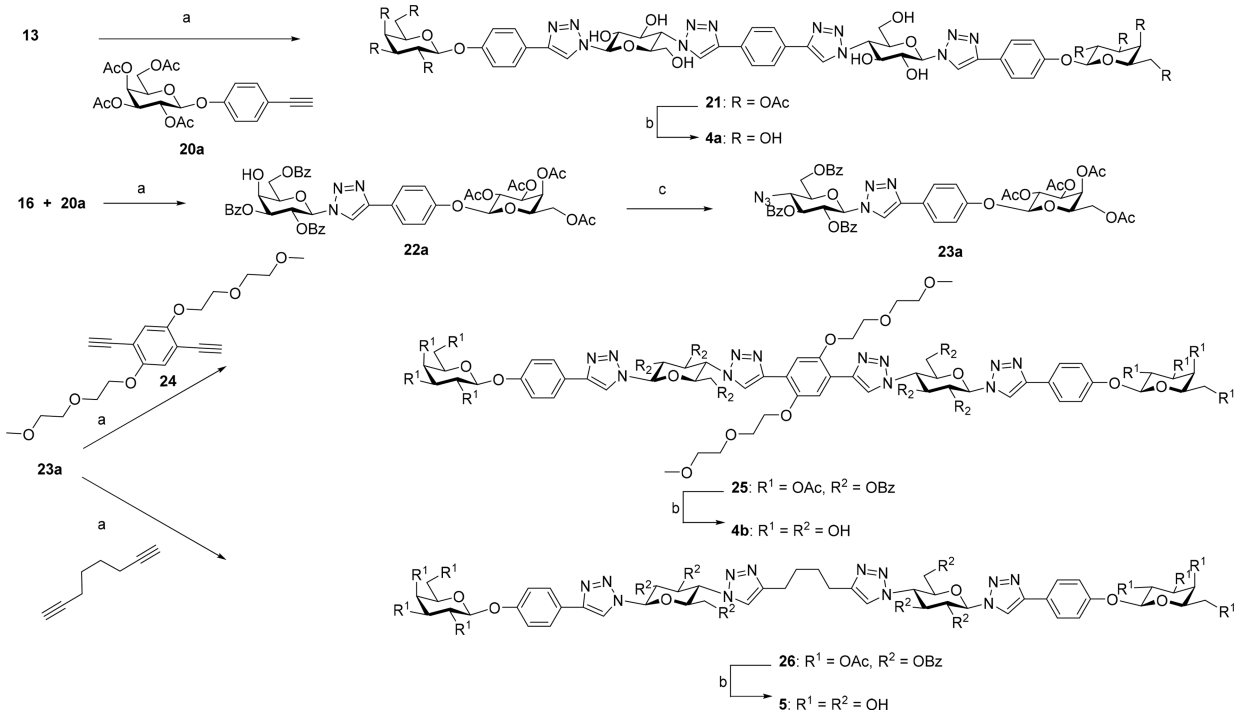
Scheme 2[a]



[a](a) $CuSO_4 \cdot 5H_2O$, Na-ascorbate, DMF/H_2O 9:1, microwave, 80 °C, 40 min, 65−85%; (b) MeONa, MeOH, 20−40% after prep HPLC; (c) (i) Tf_2O, pyridine, CH_2Cl_2, 0 °C, 1 h; (ii) NaN_3, DMF, 14 h, 73% over two steps

Table 1. LecA Binding by ITC[a]

	compd	atoms in spacer[f]	K_d	n	ΔH	$-T\Delta S$	ΔG	rel pot (per sugar)
1	D-galactose[71]		87500	1.1	−7.9	2.3	−5.5	
2	Gal-β-OMe[43]		70000	0.8	−9.3	3.6	−5.7	
3	Gal-β-OPh[71]		8800	0.9	−11.2	4.8	−6.9	
4	**9a**[31]		22000	0.92	−8.3	2.0	−6.3	0.25 (0.25)
5	**9b**		6200 ± 400	1.00 ± 0.01	−11.6 ± 0.9	4.5 ± 0.9	−7.1 ± 0.1	1 (1)
6	**10**		8400 ± 1,700	0.94 ± 0.06	−8.7 ± 1.0	−1.8 ± 0.8	−6.9 ± 0.1	1 (1)
7	**10**		7300 ± 800	0.95 ± 0.01	−9.5 ± 0.6	2.5 ± 0.6	−7.0 ± 0.1	1 (1)[d]
				Methylene Series[b]				
7	**1**	26	28[31]	0.55	−11.6	1.3	−10.3	221 (111)
8	**2**	26	12 ± 8	0.41 ± 0.09	−22.9 ± 1.9	12 ± 1.8	−10.9 ± 0.4	517 (258)
9	**3**	26	13 ± 3	0.58 ± 0.06	−14.3 ± 0.2	3.6 ± 0.3	−10.8 ± 0.14	477 (238)
				Phenylene Series[c]				
10	**4b**	32	61 ± 10	0.43 ± 0.03	−10.2 ± 0.5	0.4 ± 0.1	−9.9 ± 0.2	120 (60)[d,e]
11	**5**	32	92 ± 15	0.43 ± 0.03	−16.8 ± 0.5	7.2 ± 0.5	−9.6 ± 0.2	91 (46)
12	**6**	25	87 ± 17	0.46 ± 0.01	−16.8 ± 0.5	7.1 ± 0.6	−9.6 ± 0.1	97 (48)
13	**7**	29	94 ± 13	0.43 ± 0.01	−15.2 ± 0.2	5.6 ± 0.1	−9.6 ± 0.1	89 (45)
14	**8**	31	35 ± 15	0.50 ± 0.01	−16.1 ± 0.9	5.9 ± 1.0	−10.2 ± 0.3	240 (120)

[a]K_d in nM, ΔH, $-T\Delta S$, and ΔH in kcal/mol, Standard deviations are given over two or more experiments. [b]Relative potency determined vs **9b**. [c]Relative potency determined vs **10** in buffer. [d]Determined in buffer with 5% DMSO. [e]Relative potency determined vs **10** in buffer with 5% DMSO. [f]Number of atoms between the two anomeric oxygens of the galactosides of divalent ligands using the shortest path.

Unfortunately, **4a** proved to be insoluble in water. For this reason, the central benzene ring was outfitted with two short PEG units in the synthesis of **4b**. As before, a C4-bridged version of the molecule (**5**) was also prepared using the same synthetic strategy. The sequence started with a "click" coupling between **16** and **20a**, resulting in **22a**. Its axial C(4) hydroxyl was converted to an equatorial azide to give **23a**. CuAAC coupling of **23a** to bis-alkyne **24**[75] yielded **25** and, after deprotection **4b**, which exhibited sufficient aqueous solubility. Similarly, **23a** was coupled to octa-1,7-diyne, yielding **26**, and after acetyl removal **5** was obtained.

While the structure of compound **4b** was very close to that of compound **2**, the modification also introduces an extra six atoms, and the spacer is therefore longer. In order to keep a similar length to **2** while introducing the phenyl units adjacent to the galactose moiety, compound **6** was designed. In this compound, the shortest path between the two anomeric oxygens of the galactose ligands involves 25 atoms (Table 1), while this number is 26 for compounds **1**−**3**. To evaluate the

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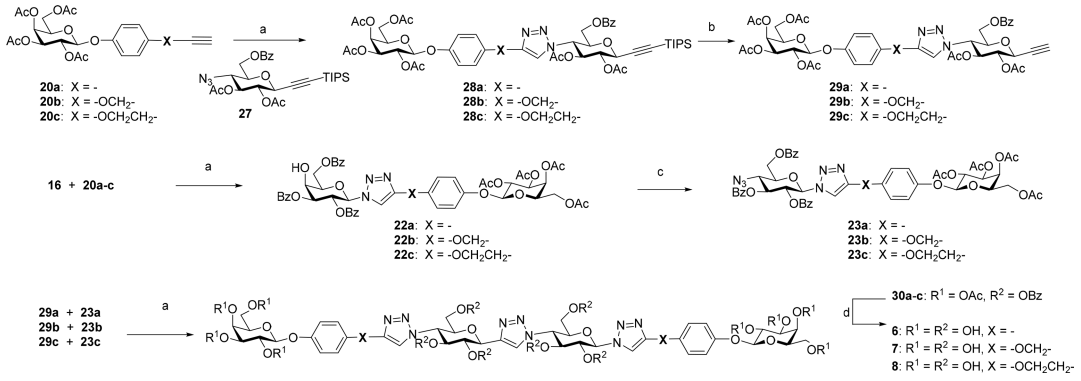
Scheme 3[a]



[a](a) $CuSO_4 \cdot 5H_2O$, Na-ascorbate, DMF/H_2O 9:1, microwave, 80 °C, 40 min, 65−85%; (b) TBAF, Et_3N, THF, 14 h, 80−90%; (c) (i) Tf_2O, pyridine, CH_2Cl_2, 0 °C, 1 h; (ii) NaN_3, DMF, 14 h, 80% over two steps; (d) MeONa, MeOH, 30−40% after prep HPLC.

sensitivity of the binding to both length and flexibility, compounds **7** and **8** were added that contain additional flexible units in the spacer.

The syntheses of **6**−**8** started with alkynes **20** (Schemes S1 and S3) that were linked to azide **27** giving **28** (Scheme 3). For **27**, a new route was developed (Schemes S2). Previously, we used a galactose moiety where the C(4) OH was inverted to the glucoside azide.[31] As the alkyne introduction chemistry typically works better on glucoside derivatives, this was used here, and a double-inversion strategy led to **27**. Removal of the TIPS groups from **28** by TBAF yielded the free alkynes **29**. The other half of the target compounds was prepared similarly, starting again with alkynes **20**, now coupled to azidosugar **16**, affording **22**. Installing the required equatorial azido groups in **23**, set the stage for the coupling with **29**. After the CuAAC coupling and removal of the acetyl and benzoyl groups, divalent ligands **6**−**8** were obtained.

The compounds were evaluated for their binding abilities of the LecA lectin using isothermal titration calorimetry (ITC) as previously reported by us and others.[31,41,76] The bivalent compounds were compared to a number of monovalent ligands reported in the literature and a few relevant reference compounds, and the numbers are shown in Table 1. Entries 1 and 2 show similar K_d's for free galactose versus its β-OMe derivative at around 70−90 μM. Entry 3 shows the effect of an aromatic aglycon moeity, which enhances the binding to a K_d of ca. 9 μM. The divalent inhibitors (**1**−**8**) were divided into two groups. In the first, the methylene series (**1**−**3**) refers to the methylene group between the galactose ligand and the spacer (triazole). In the phenylene series (**4b**−**8**) this is a phenyl group. Both series showed an n value of around 0.5, consistent with bivalent binding. In the methylene series, both compounds **2** and **3** that contain a phenyl and an n-butyl unit in the center, respectively, were more potent than the glucose-bridged **1**. The K_d's of the divalent **2** and **3** were essentially identical and reached unprecedented levels of 12−13 nM. Interestingly, the thermodynamic parameters in the methylene series varied widely. In the phenylene series, affinities varied but were lower with K_d's between 35 and 94 nM. With the exception of PEG-containing **4b** which was measured in the presence of 5% DMSO, thermodynamic parameters of the phenylene group members **5**−**8** were relatively close.

One of the notable thermodynamic features of **1** was the low entropic loss associated with its binding event. The $T\Delta S$ component was smaller than that of monovalent ligands. While it is tempting to attribute this to the rigid spacer, naturally other factors such as solvation also play important roles in the entropic component. A surprising notion was the observation that the flexible **3** was such a good ligand surpassing **1** in terms of K_d. Furthermore, this compound's entropic loss was relatively low and similar to that of the monovalent ligands. Besides this, it also has a larger favorable enthalpy than **1**, but the differences are small. Another notable aspect is the fact that turning the two glucosides around in **3** versus **1** and removing the central glucose did not have a deleterious effect. In the X-ray structure of **1** bound to the protein LecA,[31] three water-bridged hydrogen bonds and a direct hydrogen bond involving the C(3) and C(4) OH's of the central and adjacent spacer glucoside were observed.

While the flipped glucose moieties in **3** could similarly make these hydrogen bonds too, the central sugar is obviously missing in **3**. These protein−spacer interactions may have contributed some to the binding energy, but it seems their contributions were minor as the removal of the central glucose had no negative effect on binding.

Compound **2** bound with essentially the same affinity as compound **3**. Interestingly, the binding energy of compound **2** has a much higher enthalpic contribution (ca. 2-fold) and a higher entropic loss (ca. 9-fold) than **1**. The origin of this effect is not obvious but may involve the differential solvation between bound and free state. Even so, the large and opposite enthalpic and entropic components of the binding of **2** are not extreme and fall between the following two reported divalent ligands: (1) a flexible, peptide-based divalent ligand GalAG1[38] showed a ΔH of −29 kcal/mol with a K_d 83 nM, and (2) and the mentioned flexible structure discovered by library screening[51] showed a ΔH of −18 kcal/mol for a K_d 82 nM.

In the aryl-linked galactoside series (**4**−**8**), K_d differences were relatively moderate, and none of the compounds were as potent as the starting point **1**. Compound **4b** showed a remarkably low entropic loss ($-T\Delta S$ = 0.4 kcal/mol), even lower than that of **1**, and in stark contrast with **2**, but they may not be directly compared because it was measured in the presence of 5% DMSO. In the phenylene series, the enthalpic contribution is typically larger than that of the methylene-linked series (**1**−**3**), but the entropic loss is also larger leading to a weaker overall binding.

In terms of the benefit of the divalent presentation and the usefulness of building a spacer between the two ligands, a reference monovalent ligand needs to be chosen. This is a

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delicate issue, as no reference molecule is perfect in this regard. After previously using **9a**, we here use **9b** for the methylene series as the extended monovalent ligand. It is clear that it benefits somewhat from interactions to the protein with a lower K_d down from 22 μM for **9a** to 6.2 μM for **9b**. The affinity is now comparable with that of the phenyl aglycon containing **10** and related compounds. Possibly, the observed interactions between the spacer glucose moiety is the cause of this enhancement,[34] while the phenyl-linked compounds were reported to benefit from the interactions of the phenyl with the nearby CH group of a histidine.

From our results, clearly the methylene series was more potent than the phenylene series. Large relative potencies (and relative potencies per sugar) were obtained for **2** with a 542-fold (271-fold per sugar) multivalency enhancement. Note that this number is 1822 when compared to **9a**. The 271-fold K_d-based number is among the highest enhancements in the literature for LecA to the best of our knowledge. For Reymond's tetravalent peptidic system, the K_d was 2.5 nM, which calculates to a ca. 300-fold enhancement per sugar when compared to the monovalent ligand (K_d ca. 3 μM). Similarly, for a calixarene-based tetramer (K_d 90 nM) a ca. 300-fold enhancement per sugar was calculated, when compared to the reference arm.[41]

Based on recent mathematical models we calculated whether the observed enhancements were in line with expectations and possibly whether they were close to optimum or not. The recently reported models for rigid spacers indicate that rigidity is key for multivalency effects, especially when the binding sites have a weak monovalent affinity.[21] In that case, a flexible PEG spacer will not induce a measurable multivalency effect, while a rigid spacer of appropriate length does. The effective molarity is key in these discussions, which is the concentration of the second ligand around a second binding site after the first one is bound and should be higher than the monovalent K_d to have a gain in potency. For a PEG-based system, this concentration was calculated to be in the micromolar range in contrast to the rigid spacer where it was millimolar and, thus, much more likely to bind with enhanced affinity. In the present system, the binding sites have a relatively high affinity with monovalent ligands binding below 100 μM. Even a PEG-based divalent system in our hands bound with a 30-fold enhancement.[30] We calculated the effective concentration according to the paper using a 30 Å distance between the center of the two nearby binding sites in LecA and, subsequently, the predicted K_d's as a function of spacer length for rodlike ligands, with a variability of its length of 4 Å, since no spacer is perfectly rigid. The K_d's were calculated according to eq 1.[21]

$$K_{D,\mathrm{bv}} = \frac{K_{\mathrm{mono}}^2}{\exp\left[\frac{\Delta G_{\mathrm{spacer}}}{kT}\right] 2\pi c_{\mathrm{eff}}} \quad (1)$$

This equation contains the mentioned effective concentration c_{eff}. The monovalent K_d is 70 μM, i.e., K_{mono}, based on Table 1, entry 2, the affinity of the ligand without any parts of the spacer (Gal-β-OMe). The experimentally determined interaction with the spacer ($\Delta G_{\mathrm{spacer}}$) is based on entry 5, where the ligand **9b** contains a sizable part of the spacer which does contribute to the affinity (K_d = 6.2 μM).

The graph (Figure 3) shows an optimum K_d for the ideal 30 Å spacer, the spacing between the binding site centers. The K_d was predicted to be ca. 1 nM. Considering that the best

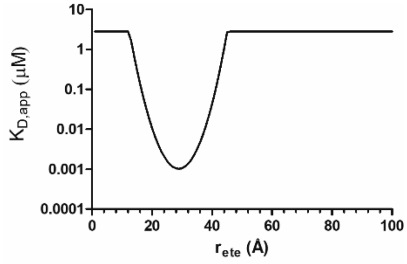

Figure 3. Calculated dissociation constants of a divalent ligands of various lengths (r_{ete} is end-to-end distance) for LecA, according to eq 1 (see the SI for details).

compound in this study has a K_d of 12 nM, our compounds are ca. 1 order of magnitude below their theoretical optimum. It should be pointed out that the experimental approach has an impact on multivalency effects, e.g., due to protein concentration. This was previously noted in the lower inhibition concentrations for **1** (IC_{50} = 2.7 nM) by ELISA,[31] and similar effects were seen for the multivalent inhibition of cholera toxin.[77]

The type of modeling described above makes it clear that major advances due to the chelation type of bivalent binding are possible and favorable for rigid spacers of the right length but would unlikely be able to distinguish between the subtle structural variations in the present series, which nevertheless show significant binding differences.

Can we look to the thermodynamic parameters for answers? Overall, the thermodynamics indicate an enthalpically driven binding that can be associated with an induced fit model.[78] If we take the profile of **2** as exceptional, the rest behaves in the following way. The addition of the phenyl aglycon does indeed help the binding enthalpy as it does for the monovalent Gal-β-OPhe (entry 3); however, unlike the case of the monovalent Gal-β-OPhe, the gains are more than balanced by increased entropic losses, resulting in overall weaker binding. Surface burial could be the source of favorable entropy, often associated with hydrophobic surfaces like the phenyl group. In the present compounds this factor does not dominate. More likely is the option that conformational rearrangements were needed in the ligand, and possibly the protein, to accommodate the galactoside ligand with its properly oriented phenyl aglycon to take advantage of the additional CH−π interactions.[71] The large difference between **2** and **3** with respect to thermodynamic parameters, while exhibiting essentially the same K_d, is intriguing. Building a CPK model and handling both compounds reveals the large difference in rigidity. Compound **2** is quite rigid, while **3** has a central hinge region that allows a range of conformations. The large entropic costs for the binding of **2** suggest major reorganization of the protein to enable all intermolecular contacts. Ironically, binding the flexible **3** costs far less entropy, as the single degree of freedom caused by the hinge is not overall very costly when compared to rearranging the protein for accomodating **2.**

CONCLUSIONS

Rigid, well-defined spacers were synthesized that were based on equatorially 1,4-linked glucose moieties or 1,4-linked phenyl rings alternated with 1,4 triazole moieties. Variations were made in the central unit and in the part linked to the galactose ligand with additional flexibility-enhancing units. Synthetic strategies varied accordingly, with the most

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successful synthesis of one the best ligands **3** being only nine steps from commercial peracetylated sugars and an overall yield of 13%. This synthesis coupled an azido-galactose moiety to the terminal propargyl galactoside ligand by CuAAC. After introducing an equatorial azido group at the galactose C(4)−OH, two of these units were linked to the central bis-alkyne.

Overall, a major affinity improvement was obtained by linking either octa-1,7-diyne or 1,4-diethynylbenzene to **19a**, which yielded divalent ligands **2** and **3** with ca. 500-fold binding enhancements.

Thermodynamic parameters were evaluated in detail, and surprisingly large differences were observed, while the differences in K_d's were relatively minor. The compounds in the phenylene series did generally show more favorable enthalpy as expected for additional CH−π interactions, but this advantage was more than erased by additional entropic costs possibly caused by protein rearrangement. This phenomenon made the methylene series the more effective ligands. Within this series, the large differences in thermodynamic parameters between **2** and **3** were intriguing and tentatively attributed to differences on rigidity and solvation as caused by replacing a phenyl with a butyl group.

A recent spacer modeling approach was applied and led to the conclusion that more improvements should theoretically be possible, but also that the method was too coarse grain to predict the subtle effects that were seen here. In that sense, possibly a full modeling approach may eventually become successful. Bridging ligands is a common theme in the carbohydrate recognition realm but also interfaces in general[18] or in noncarbohydrate ligands that were linked together to achieve improved properties.[79]

EXPERIMENTAL SECTION

General Methods. Unless stated otherwise, chemicals were obtain from commercial sources and were used without further purification. Compounds **11**,[80] **14**,[2] **16**,[3] and **24**[4] were synthesized following literature procedure. Solvents were purchased from Biosolve (Valkenswaard, The Netherlands). All moisture-sensitive reactions were performed under nitrogen atmosphere. Anhydrous THF was dried over Na/benzophenone and freshly distilled prior to use. All of the other solvents were dried over molecular sieves 4 or 3 Å. TLC was performed on Merck precoated silica 60 plates. Spots were visualized by UV light, 10% H_2SO_4 in MeOH, and triphenylphosphine in THF followed by ninhydrin (for azides). Microwave reactions were carried out in a Biotage microwave Initiator (Uppsala, Sweden). The microwave power was limited by temperature control once the desired temperature was reached. Sealed vessels of 2−5 and 10−20 mL were used. Analytical HPLC runs were performed on a Shimadzu automated HPLC system with a reversed-phase column (Phenomenex, C_4, 250 × 4.60 mm 5 μm 140087-2 for ligand **5**, C_{18}, 250 × 2.00 mm 5 μm 132174-4 for ligands **2**, **3**, **4a,b**, **6**−**8**, and **9b**) that was equipped with an evaporative light scattering detector (PLELS 1000, Polymer Laboratories, Amherst, MA) and a UV/vis detector operating at 220 and 254 nm. Preparative HPLC runs were performed on an Applied Biosystems workstation. Elution was effected by using a linear gradient of 5% MeCN/0.1% TFA in H_2O to 5% H_2O/0.1% TFA in MeCN. ^{1}H NMR spectra were recorded at 400, 500, and 600 MHz and ^{13}C at 101, 126, and 151 MHz. Electrospray mass experiments were performed in a Shimadzu LCMS QP-8000. High-resolution mass spectrometry (HRMS) analysis was performed using an ESI-QTOF II spectrometer (Bruker, Billerica, MA).

Isothermal Titration Microcalorimetry (ITC). The lectin LecA was obtained from Sigma-Aldrich and was dissolved in buffer (0.1 M Tris−HCl, 6 mM $CaCl_2$, pH 7.5) and degassed. Protein concentration (between 10 and 40 μM depending on the ligand affinity) was checked by measurement of optical density by using a theoretical molar extinction coefficient of 28000 units. Carbohydrate ligands were dissolved directly into the same buffer, degassed, and placed in the injection syringe. ITC was performed using a MicroCal Auto ITC200 (Malvern, Worcestershire, UK). LecA (0.01−0.04 mM) was placed into the 200 μL sample cell at 25 °C. Titration was performed with injections of carbohydrate ligands (5−20 times of LecA, 2.5 μL) every 120 s. Data were fitted using the "one-site model" using MicroCal Origin 7 software according to standard procedures. Fitted data yielded the stoichiometry (n), the association constant (K_a), the enthalpy (ΔH) and the entropy of binding. The K_d value was calculated as $1/K_a$, and T = 298 K.

Diglucoside (12). Compound **11** (63 mg, 500 μmol) and 1,4-diethynylbenzene (256 mg, 1.25 mmol) were dissolved in 0.9 mL of DMF. Then the aqueous solution of $CuSO_4{\cdot}5H_2O$ (18 mg in 25 μL of water, 75 μmol) and Na-ascorbate (30 mg in 25 μL of water, 150 μmol) was added to the resulting mixture. Finally, TBTA (40 mg, 75 μmol) was added, and the reaction system was heated by microwave irradiation at 80 °C for 40 min. TLC indicated complete conversion of the reaction. Then Cuprisorb was added, stirred for 30 min, and filtered. The filtrate was dried under vacuum, and the residue was purified by column chromatography (MeOH/DCM 1:1) to afford **12** as a colorless syrup (174 mg, 348 μmol, 65%). ^{1}H NMR (400 MHz, D_2O): δ 8.54 (s, 1H), 8.52 (s, 1H), 7.99−7.91 (m, 4H), 5.43 (d, J = 3.7 Hz, 1H), 4.92 (d, J = 8.0 Hz, 1H), 4.70 (td, J = 10.4, 3.9 Hz, 2H), 4.58 (ddd, J = 10.6, 4.2, 2.2 Hz, 1H), 4.46 (t, J = 9.9 Hz, 1H), 4.31−4.20 (m, 2H), 3.80 (dd, J = 9.6, 3.7 Hz, 1H), 3.59 (ddd, J = 12.7, 7.5, 2.2 Hz, 2H), 3.54−3.47 (m, 1H), 3.41−3.33 (m, 2H). ^{13}C{^{1}H} NMR (151 MHz, D_2O): δ 146.8, 129.1, 125.8, 122.3, 122.1, 96.1, 92.3, 74.5, 74.1, 73.4, 71.8, 70.2, 69.7, 62.3, 62.2, 59.9, 59.8. HRMS (ESI, Q-TOF): m/z calcd for $C_{22}H_{29}N_6O_{10}$ $[M + H]^+$ 537.1945, found 537.1956.

Bis-azide (13). To a D_2O/CH_3CN (4:1) solution of **12** (1.3 g, 2.4 mmol) was added triethylamine (3.4 mL, 24 mmol) dropwise and the solution cooled to 0 °C. Then 2-azido-1, 3-dimethylimidazolinium hexafluorophosphate (ADMP 4 g, 14.2 mmol) was added, and the mixture was stirred at 0 °C until most of the starting material was converted to the azide compound. Initially, two new spots formed on the TLC plate (developing eluent n-BuOH/H_2O/Acetic acid 6:3:1, **13** highest new spot, mono azide lower new spot). Within 3 days, conversion to **13** was complete. The solvent was removed under vacuum, and the residue was purified by column chromatography to give compound **13** as a white solid (703 mg, 1.2 mmol, 50%). ^{1}H NMR (400 MHz, D_2O): δ 8.44 (s, 2H, H-trizole), 7.92 (s, 4H, ArH), 4.82 (d, J = 8.7 Hz, 2H, H-1), 4.62 (t, J = 10.3 Hz, 2H, H-4), 4.26−4.17 (m, 4H, H-5, H-3), 3.62 (dd, J = 12.7 Hz, 1.8 Hz, 2H, H-6a), 3.37 (t, J = 8.8 Hz, 2H, H-2), 3.34−3.25 (m, 2H, H-6b). ^{13}C{^{1}H} NMR (101 MHz, CD_3OD): δ 147.9, 131.5, 127.2, 123.7, 92.2, 78.0, 75.4, 75.4, 63.3, 61.4. HRMS (ESI, Q-TOF): m/z calcd for $C_{22}H_{27}N_{12}O_8$ $[M + H]^+$ 587.2075, found 587.2073.

General Procedure for the "Click Reaction", Step a. Preparation of Compounds 15, 17, and 19. The alkyne compound, $CuSO_4{\cdot}5H_2O$ (0.15 equiv), and sodium ascorbate (0.3 equiv) were added to a solution of the azide compound in DMF containing 10% water. The mixture was heated under microwave irradiation at 80 °C for 40 min. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2. The organic solution was washed three times with water and brine and dried over sodium sulfate. The solvent was removed, and the residue was purified by column chromatography.

General Procedure for Removal of Acetyl and Benzoyl Protecting Groups, Preparation of Compounds 2, 3, 4a,b, and 5. The protected substrate was suspended or dissolved in methanol. Sodium methoxide was added to obtain a basic pH (pH ≈ 8). The reaction was stirred at rt, and it was monitored by HPLC. After disappearance of the substrate, the reaction was neutralized with DowexH$^+$ resin. The mixture was filtered, and the solvent was evaporated under vacuum, which was subjected to purification by Preparative-HPLC.

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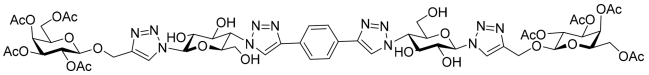
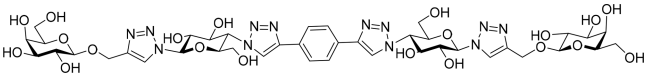
*Protected Divalent Ligand (**15**).*



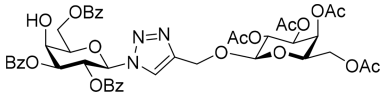
95 mg, 70 μmol, 70%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.72 (s, 2H, H-triazole), 8.37 (s, 2H, H-triazole), 7.98 (s, 4H, ArH), 5.88 (d, *J* = 9.2 Hz, 2H, H-1), 5.28 (d, *J* = 3.6 Hz, 2H, H-4′), 5.16 (dd, *J* = 10.3, 3.6 Hz, 2H, H-3′), 4.94 (t, *J* = 10.3, 7.9 Hz, 2H, H-2′), 4.89−4.78 (m, 4H, H-1′, H-6′a), 4.70 (d, *J* = 12.4 Hz, 2H, H-6′b), 4.61 (t, *J* = 10.3 Hz, 2H, H-4), 4.35−3.99 (m, 12H, H-5, H-3, H-5′, −OCH_2−, H-2), 3.29 (dd, *J* = 11.9 Hz, 2H, H-6a), 3.16−3.07 (m, 2H, H-6b), 2.00−1.85 (24H, 8 × CH_3COO−). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.0, 169.5, 169.2, 145.6, 143.1, 130.2, 125.6, 123.8, 122.1, 99.1, 87.3, 77.3, 74.1, 72.5, 70.3, 70.0, 68.6, 67.3, 61.8, 61.7, 61.3, 59.7, 20.6, 20.5, 20.4, 20.3. HRMS (ESI, Q-TOF): *m*/*z* calcd for $C_{56}H_{71}N_{12}O_{28}$ [M + H]$^+$ 1359.4500, found 1359.4471; $C_{56}H_{70}N_{12}O_{28}Na$ [M + Na]$^+$ 1381.4320, found 1381.4301.

*Divalent ligand (**2**).*



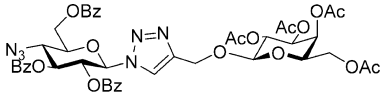
(10.2 mg, 10 μmol, 40%, white solid). ^{1}H NMR (400 MHz, D_2O): δ 8.59 (s, 2H, H-triazole), 8.43 (s, 2H, H-triazole), 7.96 (s, 4H, ArH), 6.08 (d, *J* = 9.2 Hz, 2H, H-1), 5.09−4.97 (m, 6H, 2 × −OCH_2−, H-4), 4.53 (m, 6H, H-5, H-3, H-1′), 4.31 (t, *J* = 9.2 Hz, 2H, H-2), 3.95 (d, *J* = 3.4 Hz, 2H, H-4′), 3.83−3.57 (m, 12H, 6′a, 6′b, H-2′, H-3′, H-6a, H-5′), 3.40 (dd, *J* = 12.9, 4.3 Hz, 2H, H-6b). ^{13}C{^{1}H} NMR (101 MHz, D_2O,extracted from HSQC): δ 147.3, 144.4, 129.7, 126.5, 125.0, 122.9, 102.34, 87.7, 77.3, 75.5, 73.8, 73.0, 72.9, 70.9, 68.9, 62.1, 61.8, 61.2, 59.9. HRMS (ESI, Q-TOF): *m*/*z* calcd for $C_{40}H_{55}N_{12}O_{20}$ [M + H]$^+$ 1023.3650, found 1023.3644.

*Galactoside (**17**).*



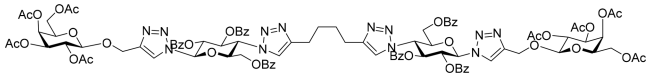
(240 mg, 266 μmol, 86%, White solid). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (s, 1H, H-triazole), 7.96 (m, 4H, ArH), 7.85−7.75 (m, 2H, ArH), 7.56−7.23 (m, 9H, ArH), 6.18−6.06 (m, 2H, H-1, H-2), 5.58 (dt, *J* = 7.9, 2.7 Hz, 1H, H-3), 5.42 (d, *J* = 3.4 Hz, 1H, H-4′), 5.18 (dd, *J* = 10.3, 7.9 Hz, 1H, H-2′), 5.05 (dd, *J* = 10.3, 3.4 Hz, 1H, H-3′), 4.90−4.80 (m, 2H, −OCH_2−), 4.71 (dd, *J* = 11.8, 5.5 Hz, 1H, H-6a), 4.59−4.49 (m, 2H, H-6b, H-4), 4.40−4.27 (m, 3H, H-1′, H-6′a, H-5), 4.08 (m, 2H, H-6′b, H-5′), 3.67 (d, *J* = 6.3 Hz, 1H, −OH), 2.15 (s, 3H, CH_3COO−), 2.13 (s, 3H, CH_3COO−), 1.96 (s, 3H, CH_3COO−). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 171.9, 170.5, 170.2, 169.7, 166.6, 165.8, 165.4, 143.3, 133.9, 133.8, 133.6, 130.0, 129.9, 129.9, 129.3, 128.8, 128.7, 128.6, 128.6, 128.1, 122.8, 97.8, 86.8, 76.2, 73.8, 71.1, 70.5, 69.0, 68.8, 67.5, 67.4, 63.0, 62.0, 60.7, 20.9, 20.8, 20.7, 20.7. HRMS (ESI, Q-TOF): *m*/*z* calcd for $C_{44}H_{46}N_3O_{18}$ [M + H]$^+$ 904.2776, found 904.2780.

*Azido-glucoside (**18**).*



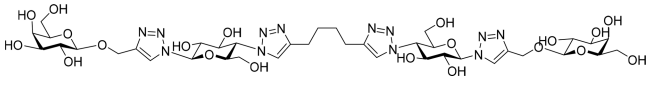
Compound **17** (240 mg, 266 μmol) was first dissolved in DCM/pyridine (10:1, 11 mL), and then triflic anhydride (0.75 g, 2.66 mmol) was added dropwise at 0 °C and reacted for 1 h at this temperature. The reaction was quenched with 1 M $KHSO_4$, and then DCM (20 mL) was added to extract the triflate intermediate. The solution was washed with water and brine and dried by sodium sulfate. The solvent was removed under vacuum to afford the crude triflate intermediate, which was used directly for the next step. To the solution of the intermediate in DMF (10 mL) was added sodium azide (87 mg, 1.33 mmol), and the mixture reacted at room temperature overnight. After removal of the solvent, DCM was added to dilute the product. The organic phase was washed with water and brine and dried with sodium sulfate. The residue was purified by column chromatography (toluene/ethyl acetate 3:1) to afford the compound as a white solid (217 mg, 234 μmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11−8.01 (m, 2H, ArH), 7.98−7.90 (m, 2H, ArH), 7.88 (s, 1H, H-triazole), 7.80−7.70 (m, 2H, ArH), 7.62−7.23 (m, 9H, ArH), 6.13 (d, *J* = 9.2 Hz, 1H, H-1), 5.90 (t, *J* = 9.6 Hz, 1H, H-3), 5.80 (t, *J* = 9.4 Hz, 1H, H-2), 5.37 (dd, *J* = 3.5, 1.1 Hz, 1H, H-4′), 5.16 (dd, *J* = 10.4, 7.9 Hz, 1H, H-2′), 4.98 (dd, *J* = 10.4, 3.4 Hz, 1H, H-3′), 4.88 (d, *J* = 12.9 Hz, 1H, −OCH_2−), 4.82−4.72 (m, 2H, −OCH_2−, H-6a), 4.65 (dd, *J* = 12.5, 4.4 Hz, 1H, H-6b), 4.44 (d, *J* = 7.9 Hz, 1H, H-1′), 4.26−4.02 (m, 4H, H-6′a, H-4, H-6′b, H-5), 3.95 (ddd, *J* = 7.2, 6.0, 1.2 Hz, 1H, H-5′), 2.12 (s, 3H, CH_3COO−), 2.04 (s, 3H, CH_3COO−), 1.94 (s, 3H, CH_3COO−), 1.76 (s, 3H, CH_3COO−). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 170.7, 170.4, 170.1, 169.6, 166.0, 165.5, 165.0, 144.0, 134.0, 134.0, 133.7, 130.0, 129.9, 129.9, 129.4, 128.7, 128.6, 128.4, 127.8, 122.1, 98.9, 86.2, 76.1, 73.7, 71.2, 71.0, 70.6, 68.7, 67.2, 63.0, 61.4, 61.4, 60.6, 20.9, 20.8, 20.7, 20.6. HRMS (ESI, Q-TOF): *m*/*z* calcd for $C_{44}H_{45}N_6O_{17}$ [M + H]$^+$ 929.2841, found 929.2859.

*Protected Divalent Ligand (**19**).*



81 mg, 41 μmol, 87%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02−7.92 (m, 6H, 4 × H-triazole, 2 × ArH), 7.72 (m, 8H, ArH), 7.59−7.53 (m, 2H, ArH), 7.48−7.34 (m, 10H, ArH), 7.22 (m, 8H, ArH), 6.54−6.44 (m, 4H, H-1, H-3), 6.05 (t, *J* = 9.5 Hz, 2H, H-2), 5.37 (dd, *J* = 3.5, 1.1 Hz, 2H, H-4′), 5.27−5.11 (m, 6H, H-4, H-2′, H-5), 4.99 (dd, *J* = 10.4, 3.4 Hz, 2H, H-3′), 4.90 (d, *J* = 12.9 Hz, 2H, −OCH_2−), 4.79 (d, *J* = 12.9 Hz, 2H, −OCH_2−), 4.50−4.44 (m, 4H, H-1′, H-6a), 4.28−4.20 (m, 4H, H-6b, H-6′a), 4.07 (dd, *J* = 11.2, 7.1 Hz, 2H, H-6′b), 3.96 (m, 2H, H-5′), 2.46 (m, 4H, 2 × −CH_2−), 2.12 (s, 6H, 2 × CH_3COO−), 2.05 (s, 6H, 2 × CH_3COO−), 1.94 (s, 6H, 2 × CH_3COO−), 1.75 (s, 6H, 2 × CH_3COO−), 1.36 (m, 4H, 2 × −CH_2−). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 170.7, 170.4, 170.1, 169.6, 166.0, 165.0, 164.8, 148.4, 144.1, 133.9, 133.8, 133.7, 130.0, 129.9, 129.7, 129.2, 128.7, 128.6, 128.6, 128.1, 127.8, 122.4, 121.7, 99.0, 86.1, 75.4, 72.9, 71.4, 71.0, 70.6, 68.7, 67.2, 62.7, 61.5, 61.4, 60.4, 28.0, 25.0, 20.9, 20.8, 20.7, 20.6. HRMS (ESI, Q-TOF): *m*/*z* calcd for $C_{96}H_{99}N_{12}O_{34}$ [M + H]$^+$ 1963.6386, found 1963.6364; $C_{96}H_{98}N_{12}O_{34}Na$ [M + Na]$^+$ 1985.6201, found 1985.6166.

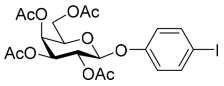
*Divalent Ligand (**3**).*



DOI: 10.1021/acs.joc.8b02727
J. Org. Chem. 2019, 84, 2470−2488

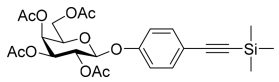
13 mg, 13 μmol, 42%, white solid. ^{1}H NMR (400 MHz, D_2O): δ 8.39 (s, 2H, H-triazole), 7.95 (s, 2H, H-triazole), 6.01 (d, *J* = 9.2 Hz, 2H, H-1), 5.06 (d, *J* = 12.7 Hz, 2H, −OCH$_2$−), 4.95 (d, *J* = 12.7 Hz, 2H, −OCH$_2$−), 4.84 (m, 2H, H-4), 4.52 (d, *J* = 7.8 Hz, 2H, H-1′), 4.46−4.33 (m, 4H, H-3, H-5), 4.23 (t, *J* = 9.2 Hz, 2H, H-2), 3.93 (d, *J* = 3.4 Hz, 2H, H-4′), 3.85−3.49 (m, 12H, H-5′, H-6′a, H-6′b, H-3′, H-2′, H-6a), 3.27 (dd, *J* = 13.0, 4.3 Hz, 2H, H-6b), 2.79 (m, 4H, 2 × −CH$_2$−), 1.71 (m, 4H, 2 × −CH$_2$−). ^{13}C{^{1}H} NMR (101 MHz, D_2O): δ 148.5, 143.9, 124.6, 123.4, 101.9, 87.3, 76.9, 75.1, 73.4, 72.6, 72.5, 70.6, 68.5, 61.7, 61.2, 60.8, 59.5, 27.5, 23.9. HRMS (ESI, Q-TOF): *m*/*z* calcd for $C_{38}H_{59}N_{12}O_{20}$ [M + H]$^+$ 1003.3968, found 1003.3983; $C_{38}H_{58}N_{12}O_{20}Na$ [M + Na]$^+$ 1025.3788, found 1025.3802.

*Galactoside (**20a.1**).*



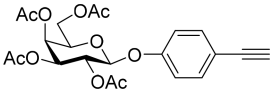
20a.1. To the solution of pentaacetyl β-D-galactopyranoside (200 mg, 513 μmol) in CH_2Cl_2 (3 mL) with 4 Å molecular sieves were added 4-iodophenol (147 mg, 667 μmol) and BF_3·Et_2O (124 mg, 872 μmol) slowly at 0 °C. Then the mixture was allowed to warm to room temperature and reacted for further 32 h. The reaction was quenched with water (0.5 mL) and diluted with ethyl acetate. The organic layer was washed with 1 M HCl, saturated $NaHCO_3$, water, and brine and dried with sodium sulfate. The solvent was removed, and the residue was purified by column chromatography (PE/EtOAc 4:1) to obtain the pure β isomer as a white solid (169 mg, 308 μmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62−7.53 (m, 2H, ArH), 6.81−6.72 (m, 2H, ArH), 5.50−5.41 (m, 2H, H-2, H-4), 5.09 (dd, *J* = 10.4, 3.4 Hz, 1H, H-3), 4.99 (d, *J* = 7.9 Hz, 1H, H-1), 4.25−4.07 (m, 2H, H-6a, H-6b), 4.04 (m, 1H, H-5), 2.17 (s, 3H, CH_3COO−), 2.05 (s, 6H, 2 × CH_3COO−), 1.98 (s, 3H, CH_3COO−). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.4, 156.8, 138.6, 119.3, 99.6, 86.2, 71.2, 70.8, 68.6, 66.9, 61.5, 20.8, 20.8, 20.7. The spectral data are in accordance with literature data.[5]

*Galactoside (**20a.2**).*



Compound **20a.1** (150 mg, 273 μmol), $Pd(PPh_3)_2Cl_2$ (5.74 mg, 8.2 μmol), and CuI (1.56 mg, 8.2 μmol) were added to a round-bottomed flask and degassed for 30 min. Then the previously degassed triethylamine (3 mL) was added to the flask, and finally ethynyltrimethylsilane (40.3 mg, 410 μmol) was added via syringe. The resulting system reacted at rt overnight. Triethylamine was removed under vacuum, and CH_2Cl_2 was added to extract the product. The organic layer was washed with water and brine and dried with sodium sulfate. After removal of the solvent, the residue was purified by column chromatography (PE/EtOAc 4:1) to obtain **20a.2** as a white solid (114 mg, 218 μmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41−7.33 (m, 2H, ArH), 6.93−6.85 (m, 2H, ArH), 5.50−5.39 (m, 2H, H-2, H-4), 5.09 (dd, *J* = 10.4 Hz, 3.4 Hz, 1H, H-3), 5.02 (d, *J* = 8.0 Hz, 1H, H-1), 4.21−4.11 (m, 2H, H-6a, H-6b), 4.06−4.03 (m, 1H, H-5), 2.15 (s, 3H, CH_3COO−), 2.03 (s, 6H, 2 × CH_3COO−), 1.98 (s, 3H, CH_3COO−), 0.21 (s, 9H, Si(CH_3)$_3$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.4, 156.9, 133.6, 118.2, 116.7, 104.5, 99.3, 93.7, 71.3, 70.9, 68.7, 67.0, 61.5, 20.8, 20.8, 20.7. HRMS (ESI, Q-TOF): *m*/*z* calcd for $C_{25}H_{32}O_{10}SiNa$ [M + Na]$^+$ 543.1663, found 543.1659.

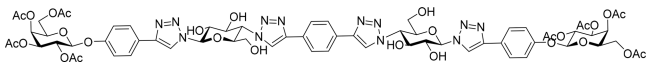
*Galactoside (**20a**).*



Compound **20a.2** (108 mg, 219 μmol) was dissolved in THF (10 mL). TBAF·3H_2O (83 mg, 263 μmol) was added, and the mixture was stirred at room temperature for 1 h. The solvent was removed under vacuum, and the residue was purified by column chromatography (PE/EtOAc 2:1) to afford **20a** as a brown solid (74 mg, 164 μmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46−7.38 (m, 2H, ArH), 6.98−6.89 (m, 2H, ArH), 5.52−5.42 (m, 2H, H-2, H-4), 5.10 (dd, *J* = 10.4, 3.4 Hz, 1H, H-3), 5.05 (d, *J* = 8.0 Hz, 1H, H-1), 4.24−4.12 (m, 2H, H-6a, H-6b), 4.08−4.05 (m, 1H, H-5), 3.03 (s, 1H, CH ≡ C−), 2.17 (s, 3H, CH_3COO−), 2.05 (s, 6H, 2 × CH_3COO−), 2.00 (s, 3H, CH_3COO−). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.4, 157.1, 133.7, 117.1, 116.8, 99.3, 83.1, 77.4, 71.3, 70.9, 68.6, 66.9, 61.5, 20.8, 20.8, 20.7. HRMS (ESI, Q-TOF): *m*/*z* calcd for $C_{22}H_{24}O_{10}Na$ [M + Na]$^+$ 471.1267, found 471.1267.

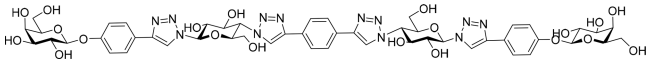
General procedure for the "Click Reaction", Preparation of Compounds 21, 22a, 25, and 26. The compounds were prepared following the procedure previously described for the synthesis of compound **12**.

*Protected Divalent Ligand (**21**).*



96 mg, 65 μmol, 65%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.85 (s, 2H, H-trizole), 8.75 (s, 2H, H-trizole), 7.99 (s, 4H, ArH), 7.87 (d, *J* = 8.4 Hz, 4H, ArH), 7.10 (d, *J* = 8.5 Hz, 4H, ArH), 5.90 (d, *J* = 9.2 Hz, 2H, H-1), 5.53 (d, *J* = 7.6 Hz, 2H, H-1′), 5.36 (m, 2H, H-3′), 5.33−5.19 (m, 4H, H-2′, H-4′), 4.64 (t, *J* = 10.2 Hz, 2H, H-4), 4.46 (t, *J* = 6.5 Hz, 2H, H-5′), 4.41−4.33 (m, 2H, H-5), 4.24 (t, *J* = 9.5 Hz, 2H, H-3), 4.18−3.97 (m, 6H, H-2, H-6′a, H-6′b), 3.32 (m, 2H, H-6a), 3.14 (m, 2H, H-6b), 2.16 (s, 6H, 2 × CH_3COO−), 2.07 (s, 6H, 2 × CH_3COO−), 2.04 (s, 6H, 2 × CH_3COO−), 1.96 (s, 6H, 2 × CH_3COO−). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.0, 169.9, 169.6, 169.3, 156.2, 146.1, 145.7, 130.2, 128.7, 126.6, 125.7, 125.4, 122.1, 120.2, 116.9, 97.6, 87.5, 77.3, 74.1, 72.6, 70.4, 70.2, 68.4, 67.3, 61.9, 61.4, 59.7, 20.5, 20.5, 20.4, 20.4. HRMS (ESI, Q-TOF): *m*/*z* calcd for $C_{66}H_{75}N_{12}O_{28}$ [M + H]$^+$ 1483.4813, found 1483.4819.

*Divalent Ligand (**4a**).*



11 mg, 9.5 μmol, 20%, white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.57 (s, 2H, H-triazole), 8.51 (s, 2H, H-triazole), 7.76 (s, 4H, ArH), 7.60 (d, *J* = 8.6 Hz, 4H, ArH), 6.91 (d, *J* = 8.3 Hz, 4H, ArH), 5.67 (d, *J* = 9.2 Hz, 2H, H-1), 4.66 (d, *J* = 7.7 Hz, 2H, H-1′), 4.42 (t, *J* = 10.2 Hz, 2H, H-4), 4.13 (dt, *J* = 10.7, 3.4 Hz, 2H, H-5), 4.01 (t, *J* = 9.5 Hz, 2H, H-3), 3.84 (t, *J* = 9.0 Hz, 2H, H-2), 3.49−3.21 (12H, H-5′, H-2′, H-6′a, H-6′b, H-3′, H-4′), 3.10 (m, 2H, H-6a), 2.92 (m, 2H, H-6b). ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6): δ 157.6, 146.6, 146.0, 130.3, 126.7, 126.0, 124.3, 122.2, 120.2, 116.9, 101.1, 87.7, 77.4, 75.7, 74.1, 73.3, 72.7, 70.4, 68.3, 62.1, 60.6, 59.9. HRMS

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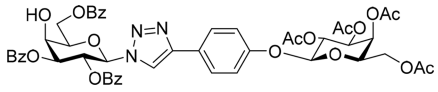
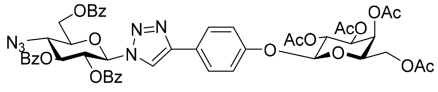
(ESI, Q-TOF): m/z calcd for $C_{50}H_{59}N_{12}O_{20}$ $[M + H]^+$ 1147.3968, found 1147.3954.

*Galactoside (**22a**).*



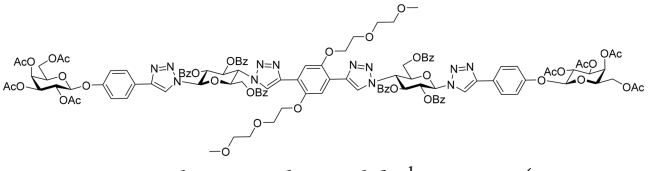
365 mg, 378 μmol, 87%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 1H, H-trizole), 7.93 (d, J = 8.0 Hz, 2H, ArH), 7.86 (d, J = 8.0 Hz, 2H, ArH), 7.67 (m, 4H, ArH), 7.50−7.16 (m, 9H, ArH), 6.98−6.90 (m, 2H, ArH), 6.22 (t, J = 9.7 Hz, 1H, H-2), 6.08 (d, J = 9.4 Hz, 1H, H-1), 5.51 (dd, J = 10.0, 2.7 Hz, 1H, H-3), 5.48−5.34 (m, 2H, H-2′, H-4′), 5.07−4.94 (m, 2H, H-3′, H-1′), 4.71 (dd, J = 11.7, 6.0 Hz, 1H, H-6a), 4.53−4.40 (m, 2H, H-6b, H-4), 4.30 (t, J = 6.2 Hz, 1H, H-5), 4.17−3.95 (m, 3H, H-5′, H-6′a, H-6′b), 3.18 (d, J = 4.0 Hz, 1H, −OH), 2.09 (s, 3H, CH_3COO−), 2.02−1.90 (s, 9H, 3 × CH_3COO−). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 170.0, 169.9, 169.6, 169.3, 165.6, 165.1, 164.4, 156.4, 146.5, 133.8, 133.7, 133.6, 129.3, 129.3, 129.2, 129.1, 129.0, 128.8, 128.8, 128.7, 128.3, 126.8, 124.9, 119.8, 116.8, 97.6, 84.9, 75.2, 74.3, 70.4, 70.2, 69.2, 68.3, 67.3, 66.2, 63.8, 61.40, 20.5, 20.5, 20.4, 20.4. HRMS (ESI, Q-TOF): m/z calcd for $C_{49}H_{48}N_3O_{18}$ $[M + H]^+$ 966.2933, found 966.2928; $C_{49}H_{47}N_3O_{18}Na$ $[M + Na]^+$ 988.2753, found 988.2742; $C_{49}H_{47}N_3O_{18}K$ $[M + K]^+$ 1004.2492, found 1004.2476.

*Azido-galactoside (**23a**).*



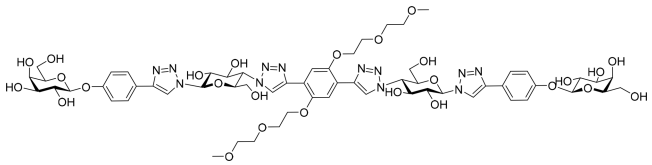
The compound was prepared following the procedure previously described for the synthesis of compound **18**. 225 mg, 227 μmol, 73%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (m, 2H, ArH), 8.00 (s, 1H, H-trizole), 7.97−7.94 (m, 2H, ArH), 7.76−7.27 (m, 13H, ArH), 7.04−7.01 (m, 2H, ArH), 6.23−6.13 (m, 1H, H-1), 5.97−5.86 (m, 2H, H-2, H-4), 5.54−5.42 (m, 2H, H-2′, H-4′), 5.16−5.02 (m, 2H, H-1′, H-3′), 4.79 (d, J = 12.1 Hz, 1H, H-6a), 4.67 (dd, J = 12.4, 3.6 Hz, 1H, H-6b), 4.26−4.03 (m, 5H, H-3, H-5, H-6′a, H-6′b, H-5′), 2.18 (s, 3H, CH_3COO−), 2.11−1.99 (s, 9H, 3 × CH_3COO−). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 169.5, 166.1, 165.5, 165.1, 157.2, 147.9, 134.0, 133.9, 133.7, 130.0, 129.9, 129.4, 128.8, 128.7, 128.6, 128.4, 127.9, 127.4, 125.4, 117.5, 117.4, 99.7, 86.1, 76.0, 74.0, 71.3, 70.9, 70.8, 68.8, 67.0, 63.1, 61.6, 60.7, 20.9, 20.8, 20.7. HRMS (ESI, Q-TOF): m/z calcd for $C_{49}H_{47}N_6O_{17}$ $[M + H]^+$ 991.2997, found 991.2992.

*Protected Divalent Ligand (**25**).*



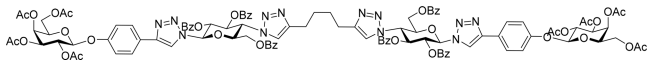
80 mg, 34 μmol, 85%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.97 (s, 2H, H-triazole), 8.64 (s, 2H, H-triazole), 7.96 (d, J = 7.6 Hz, 4H, ArH), 7.90−7.34 (m, 32H, ArH), 7.07 (d, J = 8.4 Hz, 4H, ArH), 6.95 (d, J = 9.1 Hz, 2H, H-1), 6.59 (t, J = 9.8 Hz, 2H, H-3), 6.36 (t, J = 9.2 Hz, 2H, H-2), 5.70 (t, J = 10.2 Hz, 2H, H-4), 5.52 (d, J = 7.7 Hz, 2H, H-1′), 5.36−5.20 (m, 8H, H-5, H-3′, H-2′, H-4′), 4.45 (t, J = 7.0 Hz, 4H, $-CH_2-$), 4.26−4.06 (m, 10H, H-5′, H-6b, H-6′b, $-CH_2-$), 3.71 (m, 4H, H-6a, H-6′a), 3.60 (t, J = 4.6 Hz, 4H, $-CH_2-$), 3.48 (t, J = 4.3 Hz, 4H, $-CH_2-$), 3.25 (s, 6H, 2 × $-OCH_3$), 2.20−1.90 (24H, 8 × CH_3COO−). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 170.0, 169.8, 169.6, 169.2, 165.2, 164.5, 164.1, 156.5, 149.0, 146.5, 141.8, 134.1, 134.0, 133.6, 129.4, 129.1, 129.1, 128.9, 128.8, 128.8, 128.7, 127.9, 127.8, 126.8, 124.7, 124.4, 120.3, 119.4, 116.9, 111.4, 97.6, 84.3, 73.8, 72.8, 71.4, 70.4, 70.1, 69.5, 68.8, 68.3, 68.2, 67.2, 62.7, 61.4, 59.5, 58.1, 58.1, 20.5, 20.5, 20.4, 20.4. HRMS (ESI, Q-TOF): m/z calcd for $C_{118}H_{119}N_{12}O_{40}$ $[M + H]^+$ 2343.7646, found 2343.7637.

*Divalent Ligand (**4b**).*



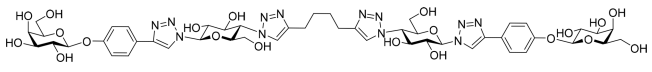
8.7 mg, 6.3 μmol, 21%, white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.74 (s, 2H, H-triazole), 8.51 (s, 2H, H-triazole), 7.91 (s, 2H, ArH), 7.82 (d, J = 8.2 Hz, 4H, ArH), 7.14 (d, J = 8.2 Hz, 4H, ArH), 5.91 (d, J = 9.0 Hz, 2H, H-1), 4.88 (d, J = 7.7 Hz, 2H, H-1′), 4.67 (t, J = 10.3 Hz, 2H, H-4), 4.36 (m, 2H, H-5), 4.29 (m, 4H, $-OCH_2-$), 4.22 (t, J = 9.5 Hz, 2H, H-3), 4.07 (t, J = 9.0 Hz, 2H, H-2), 3.94−3.87 (m, 4H, $-OCH_2-$), 3.73−3.67 (m, 6H, H-5′, $-OCH_2-$), 3.63−3.51 (m, 12H, $-OCH_2-$, H-2′, H-3′, H-6′a, H-6′b), 3.44 (dd, J = 9.9, 3.2 Hz, 2H, H-4′), 3.29 (m, 8H, $-OCH_3$, H-6a), 3.14 (m, 2H, H-6b). $^{13}C\{^1H\}$ NMR (126 MHz, DMSO-$d6$): δ 157.4, 148.9, 146.3, 141.2, 126.4, 124.9, 124.2, 120.0, 119.5, 116.6, 110.6, 100.9, 87.4, 77.3, 75.6, 74.1, 73.3, 72.7, 71.4, 70.3, 69.7, 69.0, 68.2, 68.0, 61.7, 60.4, 59.8, 58.2. HRMS (ESI, Q-TOF): m/z calcd for $C_{60}H_{79}N_{12}O_{26}$ $[M + H]^+$ 1383.5228, found 1383.5230.

*Protected Divalent Ligand (**26**).*



94 mg, 45 μmol, 90%, white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.96 (s, 2H, H-triazole), 8.04 (s, 2H, H-triazole), 7.99−7.91 (m, 4H, ArH), 7.82−7.24 (m, 30H, ArH), 7.03 (d, J = 8.6 Hz, 4H, ArH), 6.80 (d, J = 9.1 Hz, 2H, H-1), 6.48 (t, J = 9.8 Hz, 2H, H-3), 6.29 (t, J = 9.2 Hz, 2H, H-2), 5.57−5.46 (m, 4H, H-4, H-1′), 5.34−5.16 (m, 8H, H-4′, H2′, H-5, H-3′), 4.43−4.30 (m, 4H, H-5′, H-6a), 4.09 (m, 6H, H-6b, H-6′a, H-6′b), 2.32 (m, 4H, 2 × $-CH_2-$), 2.16−2.07 (6H, 2 × CH_3COO−), 2.06−1.80 (18H, 6 × CH_3COO−), 1.20 (d, J = 6.4 Hz, 4H, $-CH_2-$). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 170.0, 169.8, 169.6, 169.2, 165.2, 164.4, 164.1, 156.4, 147.0, 146.5, 134.1, 133.8, 133.6, 129.5, 129.1, 129.1, 128.9, 128.9, 128.8, 128.7, 127.9, 127.8, 126.7, 124.7, 121.9, 120.2, 116.9, 97.6, 84.4, 73.7, 72.8, 71.4, 70.4, 70.1, 68.3, 67.2, 62.5, 61.4, 59.1, 27.6, 24.3, 20.5, 20.5, 20.4, 20.4. HRMS (ESI, Q-TOF): m/z calcd for $C_{106}H_{103}N_{12}O_{34}$ $[M + H]^+$ 2087.6699, found 2087.6693.

*Divalent Ligand (**5**).*



10.5 mg, 9.3 μmol, 39%, white solid. ^{1}H NMR (400 MHz, D_2O): δ 8.54 (s, 2H, H-triazole), 7.91 (s, 2H, H-triazole), 7.77 (d, J = 8.7 Hz, 4H, ArH), 7.22 (d, J = 8.7 Hz, 4H, ArH), 6.04 (d, J = 9.2 Hz, 2H, H-1), 5.11 (d, J = 7.3 Hz, 2H, H-1′), 4.86 (t, J = 10.6 Hz, 2H, H-4), 4.43 (m, 4H, H-3, H-5), 4.26 (t, J =

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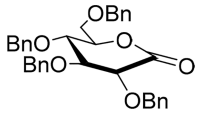
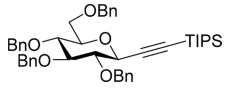
9.2 Hz, 2H, H-2), 4.02 (d, J = 3.2 Hz, 2H, H-4′), 3.91−3.77 (m, 10H, H-5′, H-6′a, H-6′b, H-3′, H-2′), 3.57 (m, 2H, H-6a), 3.31 (dd, J = 13.0, 4.4 Hz, 2H, H-6b), 2.78 (s, 4H, 2 × $-CH_2-$), 1.69 (q, J = 7.6 Hz, 4H, 2 × $-CH_2-$). $^{13}C\{^1H\}$ NMR (101 MHz, D_2O, deduced from HSQC): δ 127.6, 117.3, 100.9, 87.8, 77.3, 75.8, 73.8, 72.9, 72.8, 70.7, 68.7, 61.6, 61.0, 60.0, 59.9, 59.9, 27.9, 24.4. HRMS (ESI, Q-TOF): m/z calcd for $C_{48}H_{63}N_{12}O_{20}$ $[M + H]^+$ 1127.4281, found 1127.4267.

*(3R,4R,5R,6R)-3,4,5-Tris(benzyloxy)-6-((benzyloxy)methyl)-tetrahydro-2H-pyran-2-one (**27.1**).*



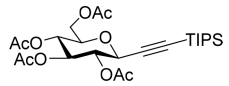
In a two-neck round-bottom flask under nitrogen atmosphere, a solution of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (7.007 g, 12.96 mmol) in dry CH_2Cl_2 (65 mL) was treated with Dess−Martin periodinane (DMP) (6.596 g, 15.55 mmol) at rt and the reaction mixture was stirred at the same temperature until complete conversion of the starting material (monitored by TLC, PE/EtOAc 2:1). After 1 h and 45 min, the reaction mixture was filtered through a silica pad and rinsed with 1.4 L of CH_2Cl_2 to afford the lactone **27.1** as a pale yellow oil (6.388 g, 11.86 mmol, 92%) which was used in the next step without any further purification. ^1H NMR (400 MHz, $CDCl_3$): δ 7.41−7.15 (m, 20H, ArH), 4.99 (d, J = 11.4 Hz, 1H, PhCH), 4.76−4.43 (m, 8H, PhCH, H-5), 4.12 (d, J = 6.5 Hz, 1H, H-2), 3.88−3.98 (m, 2H, H-3, H-4), 3.73 (dd, J = 11.0, 2.4 Hz, 1H, H-6a), 3.67 (dd, J = 11.0, 3.3 Hz, 1H, H-6b). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 169.5, 137.7, 137.7, 137.6, 137.1, 128.6, 128.6, 128.5, 128.3, 128.2, 128.1, 128.1, 128.0, 81.1, 78.3, 77.6, 76.2, 74.1, 73.9, 73.7, 68.4. MS (ESI): m/z calcd for $C_{34}H_{34}O_6Na$ $[M + Na]^+$ 561.23, found 561.55, m/z calcd for $C_{34}H_{38}NO_6$ $[M+NH_4]^+$ 556.27 found 556.55. Spectroscopic data were in accordance with literature data.[6]

*Triisopropyl(((2S,3S,4R,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)ethynyl)silane (**27.2**).*



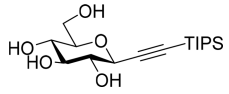
In a round-bottom flask under argon atmosphere, a solution of triisopropylsilyl acetylene (6.5 mL, 29.0 mmol) in dry THF (35 mL) was treated dropwise at −78 °C with a 2.5 M solution of n-BuLi in hexane (7.0 mL, 17.4 mmol) and stirred for 15 min. Subsequently, a solution of compound **27.1** (6.256 g, 11.61 mmol) in dry THF (12 mL) was added dropwise within 2 min at −78 °C and stirred for an hour at −78 °C (monitored by TLC, PE/EtOAc 2:1). When the conversion of **27.1** was complete, the reaction was neutralized by the addition of Amberlite IR120 H^+ form resin (checked using pH-paper), allowing at the same time the temperature to increase slowly from −78 °C to rt. The resin was filtered, washed with CH_2Cl_2, and the solvent was removed *in vacuo*. The residue, a yellow oil, was dissolved in dry CH_3CN/CH_2Cl_2 1:1 (130 mL in total) and transferred to a three-necks round-bottom flask. The solution was cooled to −15 °C by means of an ice and salt bath, and Et_3SiH (11.0 mL, 68.9 mmol) was added at once, followed by dropwise addition of $BF_3{\cdot}OEt_2$ (8.5 mL, 68.9 mmol), while the temperature inside the flask was monitored to avoid it exceeding −10 °C. The reaction mixture was stirred for 1h at −15 °C, and then, as the reaction was not complete, overnight at −20 °C. The reaction was quenched by pouring the mixture into $Et_2O/NaHCO_3$ (satd) 1:1 (200 mL in total). Et_2O (150 mL) and H_2O (100 mL) were added and the layers were separated. The aqueous phase was extracted with Et_2O (3 × 150 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (PE 95%, EtOAc 3%, $CHCl_3$ 2%) gave 6.45 g (9.15 mmol, 79% in 2 steps) of **27.2** as white needles. ^1H NMR (400 MHz, $CDCl_3$): δ 7.39−7.16 (m, 20H, ArH), 5.11 (d, J = 10.7 Hz, 1H, PhCH), 4.90 (d, J = 11.1 Hz, 1H, PhCH), 4.85−4.81 (m, 3H, PhCH), 4.66−4.52 (m, 3H, PhCH), 4.07−4.01 (m, 1H, H-2), 3.77 (dd, J = 11.2 Hz, 2.1 Hz, 1H, H-6a), 3.70 (dd, J = 11.2, 4.5 Hz, 1H, H-6b), 3.68−3.59 (m, 3H, H-1, H-3, H-4), 3.47−3.41 (m, 1H, H-5), 1.10 (s, 21H, $SiCH(CH_3)_2$ and $SiCH(CH_3)_2$). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 138.7, 138.4, 138.3, 138.2, 128.5, 128.5, 128.5, 128.4, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 104.8, 87.5, 86.3, 82.7, 79.4, 77.9, 75.8, 75.4, 75.2, 73.6, 70.4, 68.8, 18.8, 11.4. HRMS (ESI, Q-TOF): m/z calcd for $C_{45}H_{60}NO_5Si$ $[M+NH_4]^+$ 722.4235, found 722.4245.

*(2R,3R,4R,5S,6S)-2-(acetoxymethyl)-6-((triisopropylsilyl)ethynyl)-tetrahydro-2H-pyran-3,4,5-triyl triacetate (**27.3**).*



To a solution of **27.2** (6.230 g, 8.84 mmol) in acetic anhydride (45 mL) was added slowly $BF_3{\cdot}OEt_2$ (9 mL, 74 mmol) at 0 °C. The solution was then warmed to rt and stirred for 3 days at rt. After the solution was cooled to 0 °C, the reaction was neutralized by addition of $NaHCO_3$ (satd) (100 mL). The mixture was diluted with EtOAc (200 mL) and H_2O (200 mL), and the layers were separated. The aqueous phase was extracted with EtOAc (200 mL), and then the combined organic layers were washed with H_2O (3 × 200 mL), $NaHCO_3$ (satd) (3 × 200 mL), and brine (200 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (PE/EtOAc 5:1) to obtain compound **27.3** as a thick yellow syrup (3.527 g, 6.88 mmol, 78%). ^1H NMR (400 MHz, $CDCl_3$): δ 5.19−5.04 (m, 3H), 4.30−4.19 (m, 2H), 4.10 (dd, 12.4 Hz, 2.3 Hz, 1H, H-6a) 3.64 (m, 1H, H-5), 2.09 (s, 3H, CH_3COO-), 2.01 (s, 3H, CH_3COO-), 2.01 (s, 3H, CH_3COO-), 1.99 (s, 3H, CH_3COO-), 1.04 (br s, 21H, $SiCH(CH_3)_2$ and $SiCH(CH_3)_2$). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.9, 170.5, 169.5, 169.1, 100.7, 89.3, 76.0, 74.0, 71.5, 69.2, 68.3, 62.2, 20.9, 20.8, 20.8, 20.7, 18.6, 11.2. HRMS (ESI, Q-TOF): m/z calcd for $C_{25}H_{41}O_9Si$ $[M + H]^+$ 513.2520, found 513.2544; $C_{25}H_{40}O_9SiNa$ $[M + Na]^+$ 535.2340, found 535.2351.

*(2R,3S,4R,5R,6S)-2-(Hydroxymethyl)-6-((triisopropylsilyl)ethynyl)-tetrahydro-2H-pyran-3,4,5-triol (**27.4**).*



Compound **27.3** (3.515 g, 6.86 mmol) was dissolved in MeOH (40 mL). The minimum amount of dioxane necessary to obtain a clear solution was added, and the reaction mixture was treated with an aqueous solution of NaOH (1 M, 500 μL) to obtain a basic pH (pH ≈ 8). The reaction mixture was stirred at rt until complete conversion of the starting material (checked by TLC PE/EtOAc 3:1), and then it was neutralized with Amberlite IR120 H^+ form resin (monitored using pH

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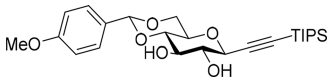
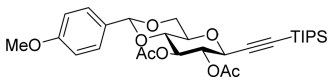
paper). After filtration, the resin was washed with MeOH, and removal of the solvent under reduced pressure gave 2.36 g of **27.4** as a white foam (quantitative yield). The crude compound was used in the next step without any further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.03 (d, J = 9.1 Hz, 1H, H-1), 3.92 (ddd, 12.2 Hz, 5.6 Hz, 3.5 Hz, 1H, H-6a), 3.80 (dd, J = 12.0, 5.2 Hz, 1H, H-6b), 3.61 (t, J = 9.0 Hz, 1H, H-4), 3.54 (t, J = 8.7 Hz, 1H, H-3), 3.47 (t, J = 9.1 Hz, H-2), 3.36 (m, 1H, H-5), 1.08 (s, 21H, SiC*H*$(CH_3)_2$ and SiCH$(CH_3)_2$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 103.3, 88.5, 79.5, 77.6, 74.2, 71.4, 69.8, 62.0, 18.8, 18.8, 11.2. HRMS (ESI, Q-TOF): m/z calcd for $[M + H]^+$ 345.2097, found 345.2093.

*(2R,4aR,6S,7R,8R,8aS)-2-(4-Methoxyphenyl)-6-((triisopropylsilyl)-ethynyl)hexahydropyrano[3,2-d][1,3]dioxine-7,8-diol (**27.5**).*



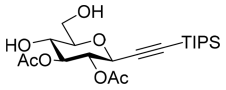
Compound **27.4** (2.356 g, 6.84 mmol) and CSA (450 mg, 1.94 mmol) were dissolved in DMF (15 mL) in a 50 mL round-bottom flask and reacted with anisalaldehyde dimethyl acetal (2.4 mL, 13.68 mmol) at 60 °C under reduced pressure on a rotary evaporator. After 1 h, the reaction was completed (monitored by TLC, PE/EtOAc 1:3). The reaction mixture was cooled to rt and neutralized with triethyl amine (5 mL), which changed the color of the solution from red to bright yellow. The mixture was concentrated under reduced pressure and the crude product was purified by column chromatography (PE/EtOAc 3:1) to afford **27.5** as a white foam (2.56 g, 5.54 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44−7.38 (m, 2H, ArH), 6.92−6.85 (m, 2H, ArH), 5.49 (s, 1H, *p*-OMe-C_6H_4-C*H*), 4.34 (dd, J = 10.5, 4.9 Hz, 1H, H-6a), 4.12 (d, J = 9.4 Hz, 1H, H-1), 3.82−3.70 (m, 5H, OCH_3, H-6b and H-3), 3.64 (t, J = 9.3 Hz, 1H, H-2), 3.55 (t, J = 9.2 Hz, 1H, H-4), 3.45 (m, 1H, H-5), 2.87(s, 1H, OH), 2.50 (s, 1H, OH), 1.09 (s, 21H, SiC*H*$(CH_3)_2$ and SiCH$(CH_3)_2$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 160.4, 129.5, 127.7, 113.8, 102.7, 102.0, 89.2, 80.6, 75.0, 74.3, 71.9, 70.8, 68.7, 55.5, 18.7, 11.2. HRMS (ESI, Q-TOF): m/z calcd for $C_{25}H_{39}O_6Si$ $[M + H]^+$ 463.2510, found 463.2515.

*(2R,4aR,6S,7S,8S,8aR)-2-(4-Methoxyphenyl)-6-((triisopropylsilyl)-ethynyl)hexahydropyrano[3,2-d][1,3]dioxine-7,8-diyl Diacetate (**27.6**).*



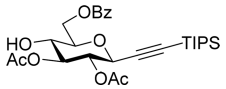
To the solution of **27.5** (2.544 g, 5.50 mmol) in acetic anhydride (25 mL) was added DABCO (617 mg, 5.50 mmol). The reaction was stirred at rt overnight. After disappearance of the starting material, the reaction mixture was poured onto crushed ice. The resulting precipitate was collected by vacuum filtration, washed with ice-cold water, and dried on a Buchner funnel to afford pure **27.6** as a pale yellow solid (3.01 g, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38−7.33 (m, 2H, ArH), 6.90−6.84 (m, 2H, ArH), 5.45 (s, 1H, *p*-OMe-C_6H_4-C*H*), 5.25 (t, J = 9.3 Hz, 1H, H-3), 5.17 (t, J = 9.5 Hz, 1H, H-2), 4.36 (dd, J = 10.5, 4.8 Hz, 1H, H-6a), 4.32 (d, J = 9.7 Hz, 1H, H-1), 3.81−3.73 (m, 4H, OCH_3 and H-6b), 3.70 (t, J = 9.5 Hz, 1H, H-4), 3.50 (dt, J = 9.8 Hz, 5.0 Hz, 1H, H-5), 2.04 (s, 3H, CH_3COO−), 2.03 (s, 3H, CH_3COO−), 1.05 (s, 21H, SiC*H*$(CH_3)_2$ and SiCH$(CH_3)_2$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 170.4, 169.4, 160.3, 129.4, 127.6, 113.7, 101.6, 101.1, 89.0, 78.4, 72.8, 72.4, 71.0, 69.7, 68.6, 55.4, 21.0, 20.8, 18.6, 11.2. HRMS (ESI, Q-TOF): m/z calcd for $C_{29}H_{43}O_8Si$ $[M + H]^+$ 547.2727, found 547.2748; $C_{29}H_{46}NO_8Si$ $[M + NH_4]^+$ 564.2993, found 564.3034.

*(2S,3S,4S,5R,6R)-5-Hydroxy-6-(hydroxymethyl)-2-((triisopropylsilyl)ethynyl)tetrahydro-2H-pyran-3,4-diyl Diacetate (**27.7**).*



To the solution of **26.7** (2.887 g, 5.28 mmol) in MeOH/THF (2:1, 30 mL) was added pyridinium paratoluensulfonate (PPTS, 132.7 mg, 0.528 mmol). After being stirred for 3 days at rt, the mixture was diluted with Et_2O (100 mL) and neutralized with $NaHCO_3$ (satd, 75 mL). The reaction was slightly exothermic and led to the precipitation of a white solid, which remained in the aqueous phase. The phases were separated and the aqueous layer was extracted three times with Et_2O (100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give a yellow oil, which was purified by column chromatography (PE/EtOAc 3:2). Pure **27.7** was recovered as a colorless oil (2.053 g, 4.79 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.04 (t, J = 9.5 Hz, 1H, H-2), 4.97 (t, J = 9.2 Hz, 1H, H-3), 4.23 (d, J = 9.6 Hz, 1H, H-1), 3.91 (dd, J = 12.1, 3.1 Hz, 1H, H-6a), 3.80 (dd, J = 12.2 Hz, 4.5 Hz, 1H, H-6b), 3.72 (t, J = 9.3 Hz, 1H, H-4), 3.37 (dt, J = 9.7, 3.4, 3.4 Hz, 1H, H-5), 3.02 (br s, 2H, OH), 2.07 (s, 3H, CH_3COO−), 2.01 (s, 3H, CH_3COO−), 1.03 (br s, 21H, SiC*H*$(CH_3)_2$ and SiCH-$(CH_3)_2$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 171.8, 169.3, 101.3, 88.8, 79.7, 77.0, 71.6, 69.3, 69.0, 62.2, 21.0, 20.8, 18.6, 11.2. HRMS (ESI, Q-TOF): m/z calcd for $C_{21}H_{37}O_7Si$ $[M + H]^+$ 429.2303, found 429.2318.

*(2S,3S,4S,5R,6R)-6-((Benzoyloxy)methyl)-5-hydroxy-2-((triisopropylsilyl)ethynyl)tetrahydro-2H-pyran-3,4-diyl Diacetate (**27.8**).*



Benzoyl chloride (834 μL, 7.18 mmol) was added slowly to a solution of **27.7** (2.05 g, 4.79 mmol) in dry pyridine (45 mL) at 0 °C, and the reaction mixture was stirred for another 30 min at 0 °C. The reaction was quenched by the addition of methanol, and after dilution with EtOAc, it was washed successively with water (2 × 150 mL), HCl (2M) (2 × 150 mL), $NaHCO_{3(satd)}$ (2 × 150 mL), and brine (150 mL). Drying over Na_2SO_4 and evaporation of the solvent in vacuo gave a pale yellow oil which was purified by column chromatography (PE/EtOAc 3:1) to afford pure **27.8** as a white foam (1.966 g, 3.69 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11−8.05 (m, 2H, ArH), 7.63−7.56 (m, 1H, ArH), 7.50−7.43 (m, 2H, ArH), 5.09−5.03 (m, 2H, H-2, H-3), 4.75 (dd, J = 12.3, 3.8 Hz, 1H, H-6a), 4.55 (dd, J = 12.3, 2.2 Hz, 1H, H-6b), 4.24 (d, J = 9.6 Hz, 1H, H-1), 3.67 (t, J = 9.2 Hz, 1H, H-4), 3.59 (m, 1H, H-5), 2.07 (s, 3H, CH_3COO−), 2.03 (s, 3H, CH_3COO−), 1.04 (br s, 21H, SiC*H*$(CH_3)_2$ and SiCH$(CH_3)_2$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 171.5, 169.3, 167.5, 133.6, 130.1, 129.6, 128.6, 101.3, 88.8, 78.5, 76.4, 71.5, 69.3, 68.9, 63.5, 21.0, 20.8, 18.6, 11.2. HRMS (ESI, Q-TOF): m/z calcd for $C_{28}H_{41}O_8Si$ $[M + H]^+$ 533.2565, found 533.2578.

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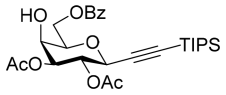
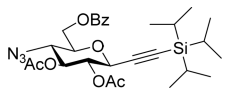
*(2S,3S,4S,5S,6R)-6-((Benzoyloxy)methyl)-5-hydroxy-2-((triisopropylsilyl)ethynyl)tetrahydro-2H-pyran-3,4-diyl Diacetate (**27.9**).*



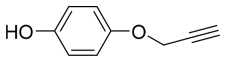
A solution of **27.8** (1. 960 g, 3.68 mmol) in dry CH_2Cl_2 (25 mL) was cooled to −15 °C, and dry pyridine (2.5 mL, 10% v/v) was added at once, followed by neat triflic anhydride (2 mL, 11.09 mmol), which was added dropwise at −15 °C. The solution was stirred at −15 °C for 30 min and then quenched by addition of $KHSO_4$ (1 M). The reaction mixture was allowed to reach rt and then diluted with CH_2Cl_2 and water. The phases were separated, and the organic layer was washed with water (2 × 40 mL) and brine (40 mL) and dried over Na_2SO_4. Removal of solvent under reduced pressure gave a dark yellow oil, which was dissolved in a minimum amount of DMF (10 mL) and reacted with $NaNO_2$ (890 mg, 12.9 mmol) at rt for 19 h. Brine (20 mL) was added, and the mixture was stirred for another 30 min to hydrolyze the nitro ester intermediate. After dilution with CH_2Cl_2 (50 mL) and separation of the phases, the organic layer was washed two more times with brine (2 × 40 mL), dried over Na_2SO_4, and concentrate in vacuo. The crude product was purified by column chromatography (PE/EtOAc 4:1) to afford **27.9** as a white foam. 1.38 g, 4.15 mmol, 70% in two steps. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06−7.96 (m, 2H, ArH), 7.58−7.50 (m, 1H, ArH), 7.46−7.37 (m, 2H, ArH), 5.44 (t, J = 10.0 Hz, 1H, H-2), 4.96 (dd, J = 10.0, 3.2 Hz, 1H, H-3), 4.62 (dd, J = 11.0, 5.5 Hz, 1H, H-6a), 4.49 (dd, J = 11.4, 6.0 Hz, 1H, H-6b), 4.22 (d, J = 10.0 Hz, 1H, H-1), 4.15−4.11 (m, 1H, H-4), 3.86 (t, J = 6.5 Hz, 1H, H-5), 2.53 (br s, 1H, OH), 2.10 (s, 3H, CH_3COO−), 2.04 (s, 3H, CH_3COO−), 1.06 (s, 21H, SiCH(CH_3)$_2$ and SiCH(CH_3)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.3, 169.2, 166.6, 133.5, 129.9, 129.7, 128.6, 101.2, 88.7, 76.0, 74.0, 69.6, 69.1, 67.6, 62.7, 21.0, 20.9, 18.6, 11.2. HRMS (ESI, Q-TOF): m/z calcd for $C_{28}H_{44}NO_8Si$ [M +NH_4]$^+$ 550.2836, found 550.2849.

*(2S,3S,4S,5R,6S)-5-Azido-6-((benzoyloxy)methyl)-2-((triisopropylsilyl)ethynyl)tetrahydro-2H-pyran-3,4-diyl Diacetate (**27**).*



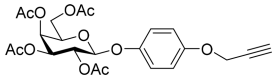
A solution of **27.9** (871 mg, 1.64 mmol) in dry CH_2Cl_2 (10 mL) was cooled to −15 °C, and dry pyridine (1 mL, 10% v/v) was added at once, followed by neat triflic anhydride (800 μL, 4.67 mmol) which was added dropwise at the same temperature. The solution was stirred at −15 °C for 20 min and then quenched by the addition of $KHSO_4$ (1M, 10 mL). The reaction mixture was allowed to reach rt and then was diluted with CH_2Cl_2 and water. The phases were separated, and the organic layer was washed with water (2 × 30 mL) and brine (30 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure gave a yellow oil, which was dissolved in acetone (8 mL), treated with an aqueous solution of NaN_3 (533.0 mg, 8.2 mmol, in 2 mL of water), and stirred at rt for 24 h. The acetone was subsequently removed under reduced pressure, and the residue was dissolved in EtOAc (40 mL), washed twice with $NaHCO_{3\ (satd)}$ (30 mL), once with water (30 mL) and once with brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. Purification by column chromatography (PE/EtOAc 92:8) gave 616.5 mg of **27** as a white solid (1.11 mmol, 67% in two steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09−8.04 (m, 2H, ArH), 7.62−7.55 (m, 1H, ArH), 7.50−7.43 (m, 2H, ArH), 5.18−5.10 (m, 2H, H-2, H-3), 4.64 (dd, J = 12.3, 2.3 Hz, 1H, H-6a), 4.50 (dd, J = 12.3, 4.4 Hz, 1H, H-6b), 4.24 (d, J = 10.0 Hz, 1H, H-1), 3.74 (t, J = 9.9 Hz, 1H, H-4), 3.54 (m, 1H, H-5), 2.09 (s, 3H, CH_3COO−), 2.03 (s, 3H, CH_3COO−), 1.03 (s, 21H, SiCH(CH_3)$_2$ and SiCH(CH_3)$_2$). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 170.1, 169.5, 166.2, 133.4, 129.9, 129.8, 128.6, 100.8, 89.3, 76.3, 74.9, 71.7, 69.2, 63.6, 60.4, 20.8, 20.7, 18.6, 11.1. HRMS (ESI, Q-TOF): m/z calcd for $C_{28}H_{40}N_3O_7Si$ [M + H]$^+$ 558.2635, found 558.2662; $C_{28}H_{39}N_3O_7SiNa$ [M + Na]$^+$ 580.2455, found 580.2473.

*4-(Prop-2-yn-1-yloxy)phenol (**20b.1**).*



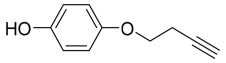
To the solution of hydroquinone (0.220 g, 2.0 mmol) in DMF (10 mL) were added sequentially potassium carbonate (0.138 g, 1.0 mmol) and propargyl bromide (0.117 g, 1.0 mmol). The resulting system reacted at 60 °C. After 4 h, dichloromethane (50 mL) was added. The organic layer was washed with 10% HCl and water and dried with sodium sulfate. After removal of the solvent, the compound was purified by column to afford the product as a yellowish syrup (121 mg, 820 μmol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.91−6.82 (m, 2H, ArH), 6.82−6.73 (m, 2H, ArH), 5.41 (br s, 1H, −OH), 4.66−4.60 (d, J = 2.3 Hz, 2H, −CH_2−), 2.51 (t, J = 2.3 Hz, 1H, −C≡CH). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 151.8, 150.4, 116.5, 116.2, 78.9, 75.6, 56.8. The spectrum was in accordance with a published paper.[7]

*Galactoside (**20b**).*



The compound was prepared following the procedure previously described for the synthesis of compound **20a.1**. 101 mg, 211 μmol, 75%, colorless syrup. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.99−6.84 (m, 4H, ArH), 5.51−5.39 (m, 2H, H-2, H-4), 5.07 (dd, J = 10.5, 3.4 Hz, 1H, H-3), 4.92 (d, J = 7.9 Hz, 1H, H-1), 4.63 (d, J = 2.4 Hz, 2H, −CH_2−), 4.26−3.96 (m, 2H, H-6a, H-6b), 4.00 (t, J = 6.7 Hz, 1H, H-5), 2.53−2.47 (t, J = 2.4 Hz, 1H, −C≡CH), 2.12 (s, 3H, CH_3COO−), 2.06 (s, 3H, CH_3COO−), 2.03 (s, 3H, CH_3COO−), 1.99 (s, 3H, CH_3COO−). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.5, 153.7, 151.7, 118.6, 116.0, 100.7, 78.7, 75.7, 71.0, 70.9, 68.8, 67.0, 61.4, 56.4, 20.8, 20.7, 20.7. HRMS (ESI, Q-TOF): m/z calcd for $C_{23}H_{30}O_{11}N$ [M + NH_4]$^+$ 496.1819, found 496.1814; $C_{23}H_{26}O_{11}K$ [M + K]$^+$ 517.1112, found 517.1101.

*4-(But-3-yn-1-yloxy)phenol (**20c.1**).*



Diethyl azodicarbonate (348 mg, 2.0 mmol) was added dropwise to a magnetically stirred solution of hydroquinone (220 mg, 2.0 mmol), 3-butyn-1-ol (140 mg, 2.0 mmol), and triphenylphosphine (525 mg, 2.0 mmol) in dry THF (15 mL) under nitrogen. The resulting system reacted overnight at room temperature. After removal of the solvent, the compound was purified by column to afford the product as yellowish syrup (81 mg, 500 μmol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.91−6.66 (m, 4H, ArH), 4.50 (br, s, 1H, −OH), 4.04 (t, J =

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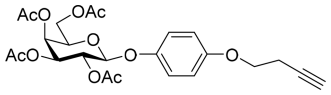
7.0 Hz, 2H, $-OCH_2-$), 2.65 (tdd, J = 7.0, 2.6, 0.6 Hz, 2H, $-CH_2C \equiv$), 2.03 (td, J = 2.6, 0.7 Hz, 1H, CH ≡ C−). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 152.6, 150.0, 116.2, 116.2, 80.7, 70.0, 67.0, 19.7. HRMS (ESI, Q-TOF): m/z calcd for $C_{10}H_{11}O_2$ $[M + H]^+$ 163.0759, found 163.0769.

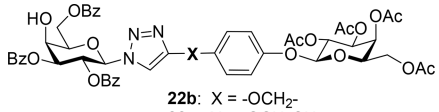
*Galactoside (**20c**).*



20c

The compound was prepared following the procedure previously described for the synthesis of compound **20b**. 129 mg, 263 μmol, 80%, yellowish syrup. ^1H NMR (400 MHz, $CDCl_3$): δ 7.00−6.89 (m, 2H, ArH), 6.89−6.77 (m, 2H, ArH), 5.49−5.40 (m, 2H, H-2, H-4), 5.08 (dd, J = 10.5, 3.4 Hz, 1H, H-3), 4.92 (d, J = 8.0 Hz, 1H, H-1), 4.25−4.12 (m, 2H, H-6a, H-6b), 4.09−3.97 (m, 3H, H-5, $-OCH_2$), 2.66 (td, J = 7.0, 2.7 Hz, 2H, $-CH_2C \equiv$), 2.18 (s, 3H, CH_3COO-), 2.08−1.99 (m, 10H, 3 × CH_3COO-, CH ≡ C−). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 169.5, 154.7, 151.5, 118.8, 115.7, 100.9, 80.5, 71.1, 71.0, 70.0, 68.9, 67.0, 66.7, 61.5, 20.9, 20.8, 20.7, 19.7. HRMS (ESI, Q-TOF): m/z calcd for $C_{24}H_{32}O_{11}N$ $[M+NH_4]^+$ 510.1976, found 510.1969; $C_{24}H_{28}O_{11}Na$ $[M + Na]^+$ for 515.1530, found 515.1522.

Compounds **22b,c**, **28a,b,c**, and **30a−c** were prepared following the procedure previously described for the synthesis of compound **22a**

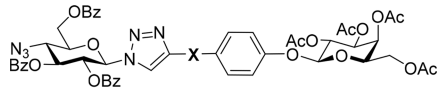


22b: X = -OCH_2-
22c: X = -OCH_2CH_2-

*Galactoside (**22b**).* 64 mg, 65 μmol, 77%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.82 (s, 1H, H-trizole), 7.77−7.71 (m, 2H, ArH), 7.71−7.64 (m, 2H, ArH), 7.54−7.48 (m, 2H, ArH), 7.35−7.27 (m, 1H, ArH), 7.26−7.12 (m, 4H, ArH), 7.09−6.99 (m, 4H, ArH), 6.69−6.63 (m, 2H, ArH), 6.62−6.56 (m, 2H, ArH), 5.98 (t, J = 9.6 Hz, 1H, H-2), 5.90 (d, J = 9.4 Hz, 1H, H-1), 5.32 (dd, J = 9.9, 3.0 Hz, 1H, H-3), 5.23−5.16 (m, 2H, H-2′, H-4′), 4.83 (m, 3H, H-3′, $-CH_2-$), 4.69 (d, J = 8.0 Hz, 1H, H-1′), 4.50 (dd, J = 11.7, 5.7 Hz, 1H, H-6a), 4.34−4.26 (m, 2H, H-6b, H-4), 4.12 (t, J = 6.2 Hz, 1H, H-5), 3.98−3.87 (m, 2H, H-6a′, H-6b′), 3.77 (td, J = 6.6, 1.2 Hz, 1H, H-5′), 3.34 (d, J = 4.3 Hz, 1H, −OH), 1.92 (s, 3H, CH_3COO-), 1.82 (s, 3H, CH_3COO-), 1.75 (6H, 2 × CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.6, 170.4, 170.3, 169.5, 166.7, 165.7, 165.2, 154.4, 151.5, 144.9, 133.8, 133.7, 133.6, 130.0, 129.9, 129.8, 129.3, 128.8, 128.7, 128.6, 128.5, 128.3, 121.4, 118.7, 116.0, 100.8, 86.6, 75.8, 74.2, 71.0, 68.9, 68.7, 67.2, 67.1, 63.0, 62.6, 61.5, 20.9, 20.8, 20.7, 20.7. HRMS (ESI, Q-TOF): m/z calcd for $C_{50}H_{50}N_3O_{19}$ $[M + H]^+$ 996.3038, found 996.3024; $C_{50}H_{49}N_3O_{19}Na$ $[M + Na]^+$ 1018.2858, found 1018.2839.

*Galactoside (**22c**).* 78 mg, 77 μmol, 77%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.95−7.87 (m, 2H, ArH, H-triazole), 7.87−7.79 (m, 2H, ArH), 7.77 (s, 1H, ArH), 7.67−7.59 (m, 2H, ArH), 7.47 (ddt, J = 8.7, 7.0, 1.3 Hz, 1H, ArH), 7.42−7.30 (m, 4H, ArH), 7.27−7.21 (m, 2H, ArH), 7.19−7.14 (m, 2H, ArH), 6.85−6.78 (m, 2H, ArH), 6.71−6.64 (m, 2H, ArH), 6.15 (t, J = 9.7 Hz, 1H, H-2), 6.02 (d, J = 9.4 Hz, 1H, H-1), 5.46 (dd, J = 10.1, 3.0 Hz, 1H, H-3), 5.39−5.32 (m, 2H, H-2′, H-4′), 4.99 (dd, J = 10.5, 3.4 Hz, 1H, H-3′), 4.82 (d, J = 8.0 Hz, 1H, H-1′), 4.66 (dd, J = 11.7, 6.1 Hz, 1H, H-6a), 4.47−4.39 (m, 2H, H-6b, H-4), 4.28−4.23 (m, 1H, H-5), 4.13−4.00 (m, 4H, H-6′a, H-6′b, $-OCH_2-$), 3.91 (td, J = 6.6, 1.2 Hz, 1H, H-5′), 3.14 (d, J = 4.0 Hz, 1H, −OH), 3.03 (t, J = 6.6 Hz, 2H, $-CH_2CH_2O-$), 2.07 (s, 3H, CH_3COO-), 2.02−1.82 (9H, CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 169.6, 166.8, 165.7, 165.2, 154.9, 151.3, 145.3, 133.9, 133.7, 130.0, 129.8, 129.3, 128.8, 128.7, 128.6, 128.5, 128.4, 120.4, 118.7, 115.6, 100.9, 86.5, 75.7, 74.2, 71.1, 71.0, 68.9, 68.5, 67.3, 67.2, 67.1, 62.8, 61.5, 26.2, 20.9, 20.8, 20.8, 20.7. HRMS (ESI, Q-TOF): m/z calcd for $C_{51}H_{52}N_3O_{19}$ $[M + H]^+$ 1010.3195, found 1010.3199; $C_{51}H_{51}N_3O_{19}Na$ $[M + Na]^+$ 1032.3015, found 1032.3022.

Compounds **23b,c** were prepared following the procedure previously described for the synthesis of compound **23a**

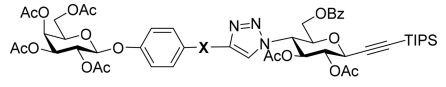


23b: X = -OCH_2-
23c: X = -OCH_2CH_2-

*Azidogalactoside (**23b**).* 46 mg, 45 μmol, 90%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.09−8.01 (m, 2H, ArH), 7.96−7.87 (m, 3H, 1 H-trizole, 2 × ArH), 7.69 (dd, J = 8.1, 1.3 Hz, 2H, ArH), 7.64−7.22 (m, 9H, ArH), 6.93−6.78 (m, 4H, ArH), 6.15 (d, J = 8.8 Hz, 1H, H-1), 5.94−5.79 (m, 2H, H-2, H-4), 5.48−5.38 (m, 2H, H-2′, H-4′), 5.10−5.05 (m, 3H, H-3′, $-CH_2-$), 4.90 (d, J = 7.9 Hz, 1H, H-1′), 4.74 (dd, J = 12.5, 1.5 Hz, 1H, H-6a), 4.63 (dd, J = 12.5, 3.8 Hz, 1H, H-6b), 4.26−4.02 (m, 4H, H-6a′, H-6b′, H-3, H-5), 3.98 (t, J = 6.8 Hz, 1H, H-5′), 2.15 (s, 3H, CH_3COO-), 2.03−1.99 (9H, 3 × CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.5, 166.1, 165.5, 164.9, 154.3, 151.5, 145.0, 133.9, 133.9, 133.7, 129.9, 129.9, 129.9, 129.4, 128.7, 128.7, 128.5, 128.4, 127.9, 121.3, 118.7, 115.8, 100.7, 86.0, 76.0, 73.9, 71.1, 71.0, 70.9, 68.9, 67.0, 63.0, 62.5, 61.4, 60.6, 20.9, 20.8, 20.8, 20.7. HRMS (ESI, Q-TOF): m/z calcd for $C_{50}H_{49}N_6O_{18}$ $[M + H]^+$ 1021.3103, found 1021.3087; $C_{50}H_{48}N_6O_{18}Na$ $[M + Na]^+$ 1043.2923, found 1043.2899; $C_{50}H_{48}N_6O_{18}K$ $[M + K]^+$ 1059.2662, found 1059.2647.

*Azidogalactoside (**23c**).* 49 mg, 48 μmol, 85%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.09−8.06 (m, 2H, ArH), 7.97−7.94 (m, 2H, ArH), 7.74 (s, 1H, H-triazole), 7.71−7.68 (m, 2H, ArH), 7.65−7.61 (m, 1H, ArH), 7.52−7.39 (m, 6H, ArH), 7.28−7.25 (m, 2H, ArH), 6.89 (d, J = 2.3 Hz, 2H, ArH), 6.78−6.75 (m, 2H, ArH), 6.14−6.11 (m, 1H, H-1), 5.89−5.86 (m, 2H, H-2, H-4), 5.47−5.43 (m, 2H, H-2′, H-4′), 5.11−5.08 (m, 1H, H-3′), 4.91 (d, J = 8.0 Hz, 1H, H-1′), 4.77 (dd, J = 12.5, 1.8 Hz, 1H, H-6a), 4.68−4.64 (m, 1H, H-6b), 4.19−4.08 (m, 6H, H-6′a, H-6′b, H-3, H-5, $-OCH_2-$), 4.02−3.99 (m, 1H, H-5′), 3.13 (d, J = 6.4 Hz, 2H, $-CH_2CH_2O-$), 2.18 (s, 3H, CH_3COO-), 2.10−2.00 (m, 12H, 4 × CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 169.5, 166.1, 165.5, 165.0, 154.8, 151.4, 145.4, 134.0, 133.8, 133.7, 130.0, 129.9, 129.4, 128.8, 128.7, 128.5, 128.5, 128.0, 120.4, 118.7, 115.6, 100.9, 86.0, 76.0, 74.0, 71.1, 71.0, 70.8, 68.9, 67.1, 67.0, 63.1, 61.5, 60.7, 26.2, 20.9, 20.8, 20.8, 20.7. HRMS (ESI, Q-TOF): m/z calcd for $C_{51}H_{51}N_6O_{18}$ $[M + H]^+$ 1035.3260, found 1035.3260; $C_{51}H_{50}N_6O_{18}Na$ $[M + Na]^+$ 1057.3080, found 1057.3079.

*Galactoside (**28a**).*



28a: X = -
28b: X = -OCH_2-
28c: X = -OCH_2CH_2-

60 mg, 60 μmol, 85%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 7.99−7.91 (m, 2H, ArH), 7.70−7.63 (m, 3H, 1 × H-trizole and 2 × ArH), 7.57−7.50 (m, 1H, ArH), 7.40 (t, J = 7.8 Hz, 2H, ArH), 7.04−6.98 (m, 2H, ArH), 5.71 (t, J = 10.2 Hz, 1H, H-3), 5.52−5.43 (m, 2H, H-2′, H-4′), 5.27 (t, J = 9.8, 1H, H-2), 5.13 (dd, J = 10.5 Hz, 3.4 Hz, 1H, H-3′), 5.08 (d, J = 8.0 Hz, 1H, H-1′), 4.81 (t, J = 10.3 Hz, 1H, H-4), 4.52 (d, J = 10.0 Hz, 1H, H-1), 4.44 (m, 2H, H-5, H-6a), 4.22−4.07 (m, 4H, H-5′, H-6′a, H-6′b, H-6b), 2.17 (s, 3H, CH_3COO-), 2.09−1.93 (12H, 4 × CH_3COO-), 1.83 (s, 3H, CH_3COO-), 1.05 (s, 21H, Tips). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.4, 170.3, 170.2, 169.5, 169.4, 169.3, 165.9, 157.1, 147.3, 133.4, 129.8, 129.3, 128.6, 127.2, 125.2, 119.5, 117.3, 100.5,

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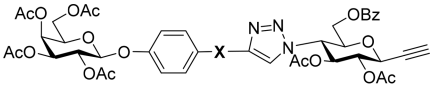
99.6, 89.7, 75.9, 73.3, 72.0, 71.2, 70.9, 69.4, 68.7, 70.0, 63.0, 61.5, 60.8, 20.8, 20.8, 20.8, 20.7, 20.4, 18.6, 11.1. HRMS (ESI, Q-TOF): *m/z* calcd for $C_{50}H_{64}N_3O_{17}Si$ $[M + H]^+$ 1006.4005, found 1006.4001; $C_{50}H_{63}N_3O_{17}SiNa$ $[M + Na]^+$ 1028.3825 found 1028.3812.

*Galactoside (**28b**)*. 74 mg, 71 μmol, 85%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.00−7.92 (m, 2H, ArH), 7.60−7.53 (m, 2H, H-trizole and ArH), 7.45 (t, *J* = 7.7 Hz, 2H, ArH), 6.93−6.87 (m, 2H, ArH), 6.83−6.77 (m, 2H, ArH), 5.62 (dd, *J* = 10.4, 9.3 Hz, 1H, H-3), 5.46−5.40 (m, 2H, H-2′, H-4′), 5.23 (t, *J* = 9.68 Hz, 1H, H-2) 5.11−5.04 (m, 3H, H-3′, $-CH_2-$), 4.90 (d, *J* = 7.9 Hz, 1H, H-1′), 4.75 (t, *J* = 10.3 Hz, 1H, H-4), 4.50−4.38 (m, 3H, H-1, H-5, H-6a), 4.21−4.07 (m, 3H, H-6′a, H-6′b, H-6b), 4.00 (td, *J* = 6.6, 1.2 Hz, 1H, H-5′), 2.16 (s, 3H, CH_3COO-), 2.09−1.96 (12H, 4 × CH_3COO-), 1.74 (s, 3H, CH_3COO-), 1.04 (s, 21H, Tips). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.4, 170.4, 170.2, 169.5, 169.4, 169.1, 165.9, 154.2, 151.6, 144.6, 133.5, 129.8, 129.4, 128.6, 123.2, 118.7, 115.8, 100.8, 100.4, 89.8, 75.8, 73.4, 71.9, 71.1, 71.0, 69.5, 68.8, 67.0, 62.8, 62.6, 61.4, 60.7, 60.5, 20.9, 20.8, 20.7, 20.2, 18.6, 11.1. HRMS (ESI, Q-TOF): *m/z* calcd for $C_{51}H_{66}N_3O_{18}Si$ $[M + H]^+$ 1036.4110, found 1036.4104; $C_{51}H_{65}N_3O_{18}SiNa$ $[M + Na]^+$ for 1058.3930, found 1058.3918; $C_{51}H_{65}N_3O_{18}SiK$ $[M + K]^+$ for 1074.3669, found 1074.3664.

*Galactoside (**28c**)*. 79 mg, 75 μmol, 75%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.02−7.93 (m, 2H, ArH), 7.62−7.56 (m, 1H, ArH), 7.50−7.42 (m, 2H, ArH), 7.38 (s, 1H, H-trizole), 6.94−6.86 (m, 2H, ArH), 6.77−6.70 (m, 2H, ArH), 5.64 (dd, *J* = 10.4, 9.3 Hz, 1H, H-3), 5.47−5.42 (m, 2H, H-2′, H-4′), 5.25 (t, *J* = 9.8, 9.6 Hz, 1H, H-2), 5.08 (dd, *J* = 10.5, 3.4 Hz, 1H, H-3′), 4.90 (d, *J* = 8.0 Hz, 1H, H-1′), 4.73 (t, *J* = 10.3 Hz, 1H, H-4), 4.50−4.38 (m, 3H, H-1, H-5, H-6a), 4.22−4.08 (m, 5H, H-6′a, H-6′b, H-6b, $-OCH_2-$), 4.00 (td, *J* = 6.7, 1.1 Hz, 1H, H-5′), 3.10 (t, *J* = 6.3 Hz, 2H, $-OCH_2CH_2-$_, 2.18 (s, 3H, CH_3COO-), 2.08−2.01 (12H, 4 × CH_3COO-), 1.77 (s, 3H, CH_3COO-), 1.05 (s, 21H, Tips). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 169.5, 169.4, 169.2, 165.9, 154.8, 151.4, 144.9, 133.5, 129.9, 129.5, 128.7, 122.6, 118.7, 115.5, 100.9, 100.5, 89.7, 75.9, 73.4, 72.0, 71.1, 71.0, 69.5, 68.9, 67.2, 67.0, 62.9, 61.4, 60.5, 26.2, 20.9, 20.8, 20.7, 20.3, 18.6, 11.1. HRMS (ESI, Q-TOF): *m/z* calcd for $C_{52}H_{68}N_3O_{18}Si$ $[M + H]^+$ 1050.4267, found 1050.4268; $C_{52}H_{67}N$ $_3O_{18}SiNa$ $[M + Na]^+$ 1072.4087, found 1072.4091.

Compounds **29a−c** were prepared following the procedure previously described for the synthesis of compound **20a**.



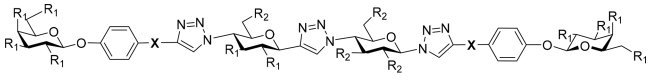
29a: X = -
29b: X = -OCH_2-
29c: X = -OCH_2CH_2-

*Galactoside (**29a**)*. 34 mg, 40 μmol, 80%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.01−7.93 (m, 2H, ArH), 7.73−7.65 (m, 3H, H-trizole and ArH), 7.59−7.53 (m, 1H, ArH), 7.42 (t, *J* = 7.8 Hz, 2H, ArH), 7.06−7.00 (m, 2H, ArH), 5.73 (t, *J* = 9.56 Hz, 1H, H-3), 5.53−5.43 (m, 2H, H-2′, H-4′), 5.30 (t, *J* = 9.7 Hz, 1H, H-4), 5.16−5.06 (m, 2H, H-3′, H-1′), 4.81 (t, *J* = 10.4 Hz, 1H, H-2), 4.54−4.47 (m, 2H, H-1, H-5), 4.41 (dd, *J* = 12.6, 2.8 Hz, 1H, H-6a), 4.25−4.13 (m, 3H, H-6′a, H-6′b, H-6b), 4.11−4.06 (m, 1H, H-5′), 2.57 (d, *J* = 2.1 Hz, 1H, −C≡CH), 2.18 (s, 3H, CH_3COO-), 2.13−1.93 (12H, 4 × CH_3COO-), 1.86 (s, 3H, CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 170.2, 169.7, 169.5, 169.2, 165.9, 157.2, 147.4, 133.6, 129.8, 129.3, 128.6, 127.3, 125.2, 119.5, 117.4, 99.6, 77.5, 76.2, 76.0, 73.0, 71.6, 71.3, 70.9, 68.9, 68.7, 67.0, 62.9, 61.5, 60.6, 20.9, 20.8, 20.8, 20.7, 20.7, 20.4. HRMS (ESI, Q-TOF): *m/z* calcd for $C_{41}H_{44}N_3O_{17}$ $[M + H]^+$ 850.2670, found 850.2659; $C_{41}H_{43}N_3O_{17}Na$ $[M + Na]^+$ 872.2490, found 872.2471; $C_{41}H_{43}N_3O_{17}K$ $[M + K]^+$ 888.2229, found 888.2217.

*Galactoside (**29b**)*. 36 mg, 41 μmol, 61%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.00−7.94 (m, 2H, ArH), 7.63−7.56 (m, 1H, ArH), 7.55 (s, 1H, H-trizole), 7.49−7.42 (m, 2H, ArH), 6.94−6.88 (m, 2H, ArH), 6.85−6.78 (m, 2H, ArH), 5.65 (t, *J* = 10.2, 9.5 Hz, 1H, H-3), 5.47−5.39 (m, 2H, H-2′, H-4′), 5.27 (t, *J* = 10.0, 9.6, 9.8 Hz, 1H, H-2), 5.12−5.04 (m, 3H, H-3′, $-CH_2-$), 4.91 (d, *J* = 8.0 Hz, 1H, H-1′), 4.75 (t, *J* = 10.3 Hz, 1H, H-4), 4.53−4.44 (m, 2H, H-5, H-1), 4.40 (dd, *J* = 12.6, 2.6 Hz, 1H, H-6a), 4.22−4.08 (m, 3H, H-6′a, H-6′b, H-6b), 4.00 (td, *J* = 6.7, 1.1 Hz, 1H, H-5′), 2.56 (s, 1H, CH ≡ C−), 2.17 (s, 3H, CH_3COO-), 2.12−1.92 (12H, 4 × CH_3COO-), 1.76 (s, 3H, CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 170.2, 169.6, 169.5, 169.1, 165.9, 154.2, 151.6, 144.7, 133.6, 129.9, 129.3, 128.7, 123.3, 118.7, 115.8, 100.8, 76.1, 76.0, 73.1, 71.5, 71.1, 71.0, 68.9, 68.9, 67.0, 62.7, 62.6, 61.4, 60.5, 20.9, 20.8, 20.7, 20.2. HRMS (ESI, Q-TOF): *m/z* calcd for $C_{42}H_{46}N_3O_{18}$ $[M + H]^+$ 880.2776, found 880.2775; $C_{42}H_{45}N_3O_{18}Na$ $[M + Na]^+$ 902.2596, found 902.2586; $C_{42}H_{45}N_3O_{18}K$ $[M + K]^+$ 918.2335, found 918.2330.

*Galactoside (**29c**)*. 40 mg, 45 μmol, 83%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.00−7.91 (m, 2H, ArH), 7.62−7.55 (m, 1H, ArH), 7.45 (t, *J* = 7.7 Hz, 2H, ArH), 7.40 (s, 1H, H-triazole), 6.93−6.86 (m, 2H, ArH), 6.76−6.71 (m, 2H, ArH), 5.68−5.62 (t, *J* = 8.0 Hz, 12.0 Hz, 1H, H-3), 5.47−5.41 (m, 2H, H-2′, H-4′), 5.27 (t, *J* = 8.0 Hz, 12.0 Hz, 1H, H-2), 5.08 (dd, *J* = 10.5, 3.4 Hz, 1H, H-3′), 4.90 (d, *J* = 8.0 Hz, 1H, H-1′), 4.72 (t, *J* = 8.0 Hz, 12.0 Hz, 1H, H-4), 4.51−4.44 (m, 2H, H-1, H-5), 4.38 (dd, *J* = 12.6, 2.6 Hz, 1H, H-6a), 4.23−4.07 (m, 5H, H-6′a, H-6′b, $-OCH_2-$, H-6b), 4.00 (td, *J* = 6.6, 1.2 Hz, 1H, H-5′), 3.11 (t, *J* = 6.3 Hz, 2H, $-OCH_2CH_2-$, 2.51 (s, 1H, CH≡C−), 2.17 (s, 3H, CH_3COO-), 2.12−1.91 (12H, 4 × CH_3COO-), 1.78 (s, 3H, CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 170.2, 169.6, 169.5, 169.1, 165.9, 154.8, 151.4, 144.9, 133.6, 129.8, 129.3, 128.7, 122.6, 118.7, 115.5, 100.9, 77.6, 76.1, 76.0, 73.1, 71.6, 71.1, 71.0, 68.9, 68.9, 67.2, 67.0, 62.8, 61.4, 60.3, 26.1, 20.9, 20.8, 20.7, 20.3. HRMS (ESI, Q-TOF): *m/z* calcd for $C_{43}H_{48}N_3O_{18}$ $[M + H]^+$ for 894.2933, found 894.2938; $C_{43}H_{47}N_3O_{18}Na$ $[M + Na]^+$ 916.2753, found 916.2752.

*Protected Divalent Ligand (**30a**)*.



30a: R^1 = OAc, R^2 = OBz, X = -
30b: R^1 = OAc, R^2 = OBz, X = -OCH_2-
30c: R^1 = OAc, R^2 = OBz, X = -OCH_2CH_2-

46 mg, 25 μmol, 63%, white solid. ^1H NMR (400 MHz, DMSO-d_6): δ 9.01 (s, 1H, H-trizole), 8.72 (s, 1H, H-trizole), 8.61 (s, 1H, H-trizole), 8.08−7.97 (m, 2H, ArH), 7.95−7.49 (m, 16H, ArH), 7.49−7.31 (m, 6H, ArH), 7.07 (dd, *J* = 8.8, 3.2 Hz, 4H, ArH), 6.79 (d, *J* = 9.2 Hz, 1H, H-7), 6.64 (t, *J* = 9.8 Hz, 1H, H-9), 6.35 (t, *J* = 9.2 Hz, 1H, H-8), 5.92 (t, *J* = 9.8 Hz, 1H, H-3), 5.70 (t, *J* = 10.4 Hz, 1H, H-10), 5.51 (dd, *J* = 7.7, 1.4 Hz, 2H, H-1′, H-7′), 5.40−5.19 (m, 10H, H-1, H-2′, H-2, H-3′, H-4, H-4′, H-8′, H-9′, H-10′, H-11), 4.81 (dt, *J* = 9.6, 3.6 Hz, 1H, H-5), 4.45 (m, 2H, H-5′, H-11′), 4.29−4.06 (m, 8H, H-6a, H-6b, H-6′a, H-6′b, H-12a, H-12b, H-12′a, H-12′b), 2.15−1.94 (24H, 8 × CH_3COO-), 1.78 (s, 3H, CH_3COO-), 1.59 (s, 3H, CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, DMSO-d_6): δ 170.0, 169.9, 169.6, 169.3, 169.1, 168.6, 165.2, 164.6, 164.1, 156.5, 156.3, 146.6, 146.1, 144.0, 134.1, 133.9, 133.7, 133.5, 129.5, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.7, 128.0, 127.8, 126.8, 126.6, 125.2, 124.7, 120.5, 120.2, 116.9, 97.7, 97.6, 84.4, 75.0, 73.9, 72.8, 72.7, 71.6, 71.5, 71.4, 70.4, 70.1, 68.4, 68.3, 67.2, 61.4, 60.2, 59.2, 20.5, 20.5, 20.4, 20.4, 20.0, 19.8. HRMS (ESI, Q-TOF): *m/z* calcd for $C_{90}H_{90}N_9O_{34}$ $[M + H]^+$ 1840.5590, found 1840.5620. Yield: 63%.

*Protected Divalent Ligand (**30b**)*. 65 mg, 34 μmol, 85%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.01−7.93 (m, 3H, H-triazole, ArH), 7.92−7.85 (m, 2H, ArH), 7.79−7.70 (m, 3H, H-triazole, ArH), 7.69−7.63 (m, 2H, ArH), 7.60−7.50 (m, 3H, ArH), 7.48−7.34 (m, 6H, ArH), 7.26−7.22 (m, 4H, ArH), 6.92−6.74 (m, 8H, ArH), 6.45−

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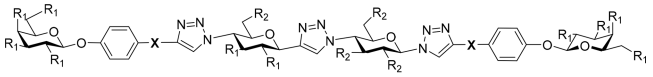
6.36 (m, 2H, H-7, H-9), 6.05 (t, J = 9.4 Hz, 1H, H-8), 5.75 (t, J = 9.96, 9.84 Hz, 1H, H-3), 5.47−5.36 (m, 4H, H-2′, H-8′, H-4′, H-10′), 5.28 (t, J = 9.76 Hz, 1H, H-2), 5.19 (t, J = 10.4 Hz, 1H, H-10), 5.13−5.02 (m, 6H, H-9′, H-3′, 2 × $-OCH_2-$), 4.98−4.88 (m, 4H, H-11, H-7′, H-1, H-1′), 4.80 (t, J = 10.3 Hz, 1H, H-4), 4.56 (dt, J = 10.3, 3.4 Hz, 1H, H-5), 4.44 (dd, J = 12.9, 2.3 Hz, 1H, H-6a), 4.28 (dd, J = 12.5, 2.8 Hz, 1H, H-6b), 4.22−4.04 (m, 6H, H-6′a, H-6′b, H-12a, H-12b, H-12′a, H-12′b), 3.99 (m, 2H, H-5′, H-11′), 2.32−1.84 (24H, 3 × 8 CH_3COO-), 1.68 (6H, 2 × CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.5, 170.4, 170.3, 169.6, 169.5, 169.2, 165.8, 164.9, 164.7, 154.4, 154.2, 151.6, 145.2, 144.8, 144.5, 134.0, 133.9, 133.8, 133.5, 129.9, 129.8, 129.3, 129.2, 128.8, 128.7, 128.6, 128.0, 127.7, 123.2, 123.0, 121.5, 118.7, 115.8, 100.8, 100.8, 86.1, 76.3, 75.7, 73.3, 73.3, 72.9, 71.6, 71.1, 71.0, 68.9, 67.0, 63.0, 62.6, 62.2, 61.4, 61.0, 60.6, 20.9, 20.8, 20.7, 20.2. HRMS (ESI, Q-TOF): m/z calcd for $C_{92}H_{94}N_9O_{36}$ $[M + H]^+$ 1900.5801, found 1900.5816.

*Protected Divalent Ligand (**30c**).* 58 mg, 30 μmol, 76%, white solid. ^1H NMR (400 MHz, $CDCl_3$): δ 8.00−7.92 (m, 2H, ArH), 7.90−7.84 (m, 2H, ArH), 7.80 (s, 2H, H-triazole), 7.75−7.69 (m, 2H, ArH), 7.63−7.50 (m, 4H, ArH), 7.47−7.33 (m, 7H, H-triazole, ArH), 7.26−7.17 (m, 4H, ArH), 6.91−6.83 (m, 4H, ArH), 6.76−6.68 (m, 4H, ArH), 6.47−6.37 (m, 2H, H-7, H-9), 6.07 (t, J = 9.48 Hz, 1H, H-8), 5.77 (t, J = 10.0, 9.76 Hz, 1H, H-3)5.45−5.37 (m, 4H, H-2′, H-8′, H-4′, H-10′), 5.30−5.20 (m, 2H, H-2, H-10), 5.10−5.04 (dd, J = on10.4, 3.3 Hz, 2H, H-3′, H-9′), 4.98−4.87 (m, 4H, H-11, H-1, H-1′, H-7′), 4.78 (t, J = 10.4 Hz, 1H, H-4), 4.53 (dt, J = 10.3, 3.6 Hz, 1H, H-5), 4.44 (dd, J = 12.9, 2.3 Hz, 1H, H-6a), 4.24−3.98 (m, 13H, H-6′a, H-6′b, H-12′a, H-12′b, H-6b, H-12a, H-12b, 2 × $-OCH_2-$, H-5′, H-11′), 3.10 (t, J = 6.4 Hz, 4H, 2 × $-CH_2CH_2O-$), 2.20−2.10 (6H, 2 × CH_3COO-), 2.09−1.85 (18H, 6 × CH_3COO-), 1.71 (s, 3H, CH_3COO-), 1.63 (s, 3H, CH_3COO-). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$): δ 170.4, 170.4, 170.2, 169.6, 169.5, 169.2, 165.8, 165.8, 164.8, 164.7, 154.8, 151.3, 145.5, 145.0, 144.5, 133.9, 133.8, 133.7, 133.5, 129.9, 129.8, 129.8, 129.3, 129.2, 128.7, 128.6, 128.5, 128.5, 128.0, 127.8, 123.2, 122.2, 120.6, 118.7, 118.6, 115.6, 115.5, 100.8, 85.9, 76.3, 75.6, 73.3, 73.2, 72.9, 71.7, 71.0, 71.0, 68.9, 67.2, 67.1, 67.0, 63.1, 62.2, 61.4, 60.8, 60.6, 26.2, 26.1, 20.9, 20.8, 20.7, 20.3, 20.2. HRMS (ESI, Q-TOF): m/z calcd for $C_{94}H_{98}N_9O_{36}$ $[M + H]^+$ 1928.6114, found 1928.6093.

Compounds **6**−**8** were prepared following the procedure previously described for the synthesis of compound **2**.



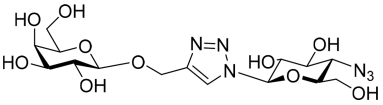
6: $R^1 = R^2$ = OH, X = -
7: $R^1 = R^2$ = OH, X = $-OCH_2-$
8: $R^1 = R^2$ = OH, X = $-OCH_2CH_2-$

*Divalent ligand (**6**).* 10 mg, 9.8 μmol, 39%, white solid. ^1H NMR (500 MHz, D_2O): δ 8.47 (s, 1H, H-triazole), 8.34 (s, 1H, H-triazole), 8.29 (s, 1H, H-triazole), 7.70 (dd, J = 8.8, 2.9 Hz, 4H, ArH), 7.19−7.12 (m, 4H, ArH), 5.96 (d, J = 9.2 Hz, 1H, H-7′), 5.02 (d, J = 7.6 Hz, 2H, H-1, H-1′), 4.90−4.70 (m, 3H, H-10, H-7, H-10′), 4.46−4.37 (m, 2H, H-11, H-9′), 4.32−4.18 (m, 3H, H-9, H-11′, H-8′), 3.95−3.89 (m, 3H, H-3, H-3′, H-8), 3.81−3.68 (m, 10H, H-2, H-2′, H-6′a, H-6′b, H-6a, H-6b, H-4, H-4′, H-5, H-5′), 3.52 (m, 2H, H-12a, H-12′a), 3.26 (m, 2H, H-12b, H-12′b). ^{13}C NMR (126 MHz, D_2O): δ 157.1, 157.0, 147.4, 147.2, 144.7, 127.4, 127.3, 125.5, 124.1, 124.0, 121.9, 120.9, 117.5, 117.0, 115.1, 100.5, 87.5, 78.3, 77.0, 75.4, 74.6, 73.8, 73.6, 73.2, 72.5, 70.5, 68.5, 62.2, 61.6, 60.7, 60.0, 59.6. HRMS (ESI, Q-TOF): m/z calcd for $C_{42}H_{54}N_9O_{20}$ $[M + H]^+$ 1004.3485, found 1004.3473.

*Divalent Ligand (**7**).* 13 mg, 12 μmol, 40%, white solid. ^1H NMR (400 MHz, D_2O): δ 8.39 (s, 1H, H-triazole), 8.37 (s, 1H, H-triazole), 8.23 (s, 1H, H-triazole), 7.21−6.99 (m, 8H, ArH), 6.04 (d, J = 9.2 Hz, 1H, H-7), 5.29 (d, J = 6.1 Hz, 4H, 2 × $-OCH_2-$), 5.00−4.82 (m, 5H, H-1′, H-7′, H-4, H-1, H-10), 4.53−4.44 (m, 2H, H-5, H-9), 4.36−4.23 (m, 3H, H-3, H-11, H-8), 4.02−3.94 (m, 3H, H-3′, H-9′, H-2), 3.90−3.69 (m, 10H, H-2′, H-8′, H-6′a, H-6′b, H-12′a, H-12′b, H-4′, H-10′, H-5′, H-11′), 3.65−3.59 (m, 1H, H-6a), 3.50 (dd, J = 13.0, 2.2 Hz, 1H, H-12a), 3.35 (dd, J = 13.1, 4.3 Hz, 1H, H-6b), 3.21 (dd, J = 12.9, 4.5 Hz, 1H, H-12b). $^{13}C\{^1H\}$ NMR (101 MHz, D_2O): δ 152.9, 152.8, 151.7, 151.6, 144.6, 143.7, 143.5, 125.6, 125.5, 124.5, 118.0, 117.9, 117.1, 116.7, 101.5, 87.3, 78.2, 76.9, 75.3, 74.5, 73.7, 73.5, 73.1, 72.5, 72.4, 70.5, 68.4, 62.1, 62.0, 61.8, 61.5, 60.7, 59.9, 59.5. HRMS (ESI, Q-TOF): m/z calcd for $C_{44}H_{58}N_9O_{22}$ $[M + H]^+$ 1064.3696, found 1064.3682.

*Divalent Ligand (**8**).* 13.4 mg, 12.3 μmol, 41%, white solid. ^1H NMR (400 MHz, D_2O): δ 8.37 (s, 1H, H-triazole), 8.19 (s, 1H, H-triazole), 8.03 (s, 1H, H-triazole), 7.16−7.04 (m, 4H, ArH), 7.03−6.90 (m, 4H, ArH), 5.99 (d, J = 9.2 Hz, 1H, H-7), 4.97−4.84 (m, 4H, H-1′, H-7′, H-4, H-1), 4.73 (t, J = 10.3 Hz, 1H, H-10), 4.52−4.42 (m, 2H, H-5, H-9), 4.41−4.21 (m, 7H, 2 × $-OCH_2-$, H-3, H-11, H-8), 4.02−3.93 (m, 3H, H-3′, H-9′, H-2), 3.90−3.70 (m, 10H, H-6′a, H-6′b, H-12′a, H-12′b, H-2′, H-8′, H-4′, H-10′, H-5′, H-11′), 3.62 (dd, J = 13.0, 2.1 Hz, 1H, H-6a), 3.52−3.46 (m, 1H, H-12a), 3.38−3.32 (dd, J = 13.0, 4.1 Hz, 1H, H-6b), 3.22 (m, 5H, H-12b, 2 × $-CH_2CH_2O-$). $^{13}C\{^1H\}$ NMR (101 MHz, D_2O): δ 153.5, 151.3, 145.3, 145.1, 144.6, 125.5, 124.1, 123.2, 118.0, 116.2, 101.6, 87.2, 78.2, 76.8, 75.3, 74.5, 73.7, 73.6, 73.1, 72.5, 72.4, 70.5, 68.4, 67.7, 67.6, 61.9, 61.5, 60.7, 59.9, 59.5, 25.1, 25.0. HRMS (ESI, Q-TOF): m/z calcd for $C_{46}H_{62}N_9O_{22}$ $[M + H]^+$ 1092.4009, found 1092.4008. Yield: 41%.

*Galactoside (**9b**).*



14 mg, 31 μmol, 61%, white solid. ^1H NMR (600 MHz, CD_3OD): δ 8.27 (s, 1H, H-triazole), 5.63 (d, J = 9.2 Hz, 1H), 5.00 (d, J = 12.6 Hz, 1H), 4.85 (d, J = 12.6 Hz, 1H), 4.38 (d, J = 7.6 Hz, 1H), 3.95 (t, J = 9.1 Hz, 1H), 3.87−3.78 (m, 3H), 3.78−3.72 (m, 3H), 3.64 (t, J = 9.9 Hz, 1H), 3.59−3.53 (m, 3H), 3.49 (dd, J = 9.7, 3.4 Hz, 1H). 13C NMR (151 MHz, CD_3OD): δ 144.6, 123.2, 103.0, 88.0, 77.7, 76.3, 75.4, 73.5, 72.7, 71.1, 69.0, 61.7, 61.3, 61.2, 60.8. HRMS (MALDI-TOF): m/z calcd for $C_{15}H_{25}N_6O_{10}$ $[M + H]^+$ 449.1627, found 449.1634.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.8b02727.

Synthetic schemes, NMR spectra, HPLC, and ITC binding experiments (PDF)

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Notes

The authors declare no competing financial interest.

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