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Brief Article

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Tuning the Preference of Thiodigalactoside- and Lactosamine-Based Ligands to Galectin-3 over Galectin-1

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Supporting Information

ABSTRACT: Inhibitors for galectin-1 and -3 were synthesized from thiodigalactoside and lactosamine by derivatization of the galactose C3. Introduction of 4-phenyl-1*H*-1,2,3-triazol-1-yl substituents at the thiodigalactoside C3 by CuAAC, targeting arginine−arene interactions, increased the affinity to 13 nM but yielded little selectivity. The bulkier 4-(4-phenoxyphenyl)-1*H*-1,2,3-triazol-1-yl substituent, however, increased the preference for galectin-3 over galectin-1 to more than 200-fold. Modeling showed more arginine−arene interactions for galectin-3 than for galectin-1. Introducing 4-phenoxyaryl groups on lactosamine had a similar effect.

INTRODUCTION

The protein family of galectins is defined by their affinity for β-galactosides and their sequence homology.[1] They function intracellularly and extracellularly, and they are involved in many biological processes such as cell signaling[2] and adhesion.[3] Moreover, they play a role in various pathological pathways like metastasis,[4] tumor cell survival,[5] apoptosis,[6] and inflammatory response.[7,8] However, the exact biological role of the various galectins remains to be elucidated. Selective inhibitors for different galectins would therefore be very beneficial to deciphering the modes of action of these proteins, considering the reported opposite roles of galectins-1 and -3.[5] Furthermore, selective inhibitors are important for the possible development of therapeutics.

Previous (selective) inhibitor studies focused on peptide and carbohydrate ligands.[8−12] The peptidic structures include pentapeptides[13] and the antiangiogenic peptide anginex,[14] which was recently found to enhance the binding affinity of galectin-1 for glycoproteins.[15] The carbohydrate-based ligands have been used in a multivalent format to gain affinity.[16−18] Besides this, optimization of monovalent carbohydrates yielded nanomolar inhibitors, as shown by Nilsson et al., due to the introduction of aromatic groups on the galactose C3 position establishing favorable cation−π interactions with arginine residues.[19−24] Moreover, a triazole linkage between the carbohydrate and the aromatic moiety instead of an ether, ester, or amide linkage increased the affinity even further.[25] Previous studies with proteomics probes carrying bulky galactose C3 substituents had indicated that galectin-3 selectivity might be achievable using this strategy.[25] These foundations led to our aim to synthesize selective galectin-3 inhibitors based on thiodigalactoside (TDG) for which the protein has an affinity of 43 μM.[19] Three aromatic groups differing in size, namely, phenyl, 3-hydroxyphenyl and 4-phenoxyphenyl, were coupled to a TDG azide by copper assisted alkyne−azide cycloaddition (CuAAC).[26] First, the affinity of galectin-1 and -3 was strongly increased to the nanomolar range. Modeling studies indicated that the established arginine−arene interactions could be responsible for this phenomenon. Moreover, galectin-3 bound 230 times stronger to TDG with larger aromatic groups than galectin-1, resulting in a selective inhibitor for galectin-3. In a parallel approach, lactosamine (LacNAc), for which galectin-3 has an affinity of 69 μM,[27] was also derivatized to investigate whether a similar effect could be achieved. LacNAc is hydrolytically less stable than TDG, but the carbohydrate is easier to derivatize with fluorescent labels or radiolabels for biological studies via its reducing end. X-ray studies indicated that the two carbohydrates bind similarly to galectin-1,[28] and therefore, LacNAc was derivatized on the Gal-C3 position with 4-phenoxyaryl via an ether or a triazole linkage and an aromatic phthaloyl or benzoyl group was attached to the N2 to mimic the symmetric nature of TDG. Additionally, the LacNAc derivatives contained an azidopropyl spacer to enable their conjugation for biological studies. Several LacNAc derivatives showed similar selectivity for galectin-3 as their TDG counterparts; however, the affinities were significantly lower to around 1 μM in the best cases.

CHEMISTRY

Synthesis of Thiodigalactoside Derivatives. The thiodigalactoside-based inhibitors were synthesized from a common thiodigalactoside intermediate with azide moieties on the C3 and C3′ positions to allow the introduction of alkyne containing aromatic groups through CuAAC (Scheme 1). To this end, 3-3′-azidothiodigalactoside **1** was synthesized as

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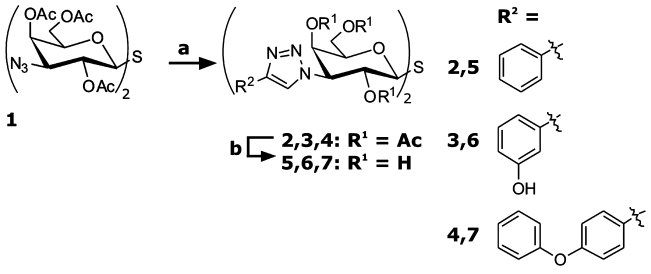
Scheme 1[a]



[a]Reaction conditions: (a) phenylacetylene, 3-hydroxyphenylacetylene, or 1-ethynyl-4-phenoxybenzene, $CuSO_4$, sodium ascorbate, DMF/H_2O, 80 °C, microwave (86−100%); (b) NaOMe, MeOH (86−100%).

previously described[25] in two steps by bromination of the anomeric center of 3-azidogalactoside[29] followed by glycosylation with the thiourea treated 3-azidogalactoside acceptor. Several aromatic groups varying in size and hydrophilicity were subsequently coupled to **1** using CuAAC under microwave irradiation to give the phenyl-, 3-hydroxylphenyl-, and 4-phenoxyphenyl derivatives **2**, **3**, and **4** in good to excellent yields. Finally, deacetylation of the hydroxyl groups yielded the free thiodigalactosides **5**, **6**, and **7**.

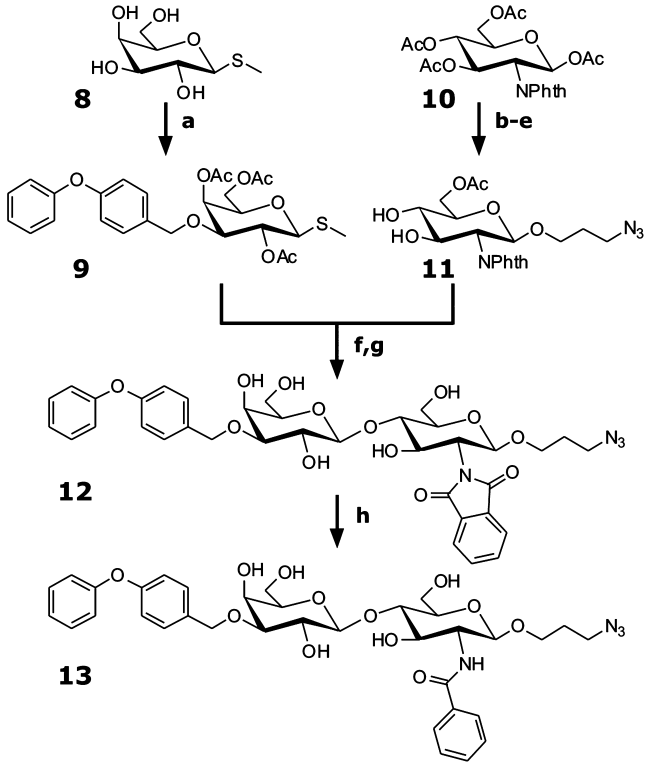
Synthesis of 4-(4-Phenoxybenzyl) Ether Linked Lactosamine. Larger aromatic groups on TDG resulted in higher selectivity of galectin-3 vs galectin-1. Therefore, phenoxyaryl groups were introduced on the lactosamine derivatives. The LacNAc derivatives with an ether-linked aromatic moiety were obtained by the glycosylation of derivatized galactoside and glucosamine (Scheme 2). First, the known thiomethylgalactoside donor **8**[30] was treated with dibutyltin oxide to form the C3 to C4 stannylideneacetal,[31] which was then functionalized with 4-phenoxybenzene bromide on C3 and acetylated to give compound **9**. Acceptor **11** was synthesized from the fully protected glucosamine **10**,[32] which was glycosylated with 3-bromopropanol using $BF_3{\cdot}OEt_2$ followed by bromide displacement with sodium azide, deacetylation, and selective low temperature acetylation of C6.

Target **12** was obtained by glycosylation of the galactoside donor **9** and glucosamine acceptor **11** and careful deacetylation without removal of the phthaloyl protection group. Lactosamine derivative **13** was obtained by removal of the phthaloyl group of **12** with ethylenediamine followed by benzoylation.

Synthesis of 4-(4-Phenoxyphenyl)triazole-Linked Lactosamine. For the triazole-linked lactosamine derivatives the known 3-azidogalactoside **14**[29] and the bromide **16** were the starting point (Scheme 3). Compound **14** was reacted with (methylthio)trimethylsilane to give the desired methylthio donor **15**. The acceptor **17** was synthesized from **16** by low-temperature acetylation.

In this route, the bromide was not displaced by an azide immediately to avoid the formation of a diazidolactosamine after glycosylation. Glycosylation of **15** and **17** was performed as before and yielded the desired 1,4-linked disaccharide as the major product and the 1,3-isomer as a side product. This mixture was coupled with 1-ethynyl-4-phenoxybenzene by CuAAC followed by azide introduction and careful deacetylation without removal of the phthaloyl protection group. Preparative HPLC yielded isomerically pure **18**. The benzoyl analogue **19** was prepared by removal of the phthaloyl group and subsequent benzoylation of the nitrogen.

Scheme 2[a]



[a]Reaction conditions: (a) (i) Bu_2SnO, MeOH; (ii) 4-phenoxybenzene bromide, NBu_4Br, benzene; (iii) Ac_2O, py, 22%; (b) 3-bromo-1-propanol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 81%; (c) NaN_3, DMF, 91%; (d) NaOMe, MeOH, quant; (e) AcCl, 2,4,6-trimethylpyridine, CH_2Cl_2, quant, −60 °C → −20 °C; (f) TfOH, NIS, CH_2Cl_2, 80%; (g) NaOMe, MeOH, 73%; (h) (i) ethylene diamine, *n*-BuOH; (ii) Bz_2O, DiPEA, MeCN, 95%.

AFFINITY STUDIES

The binding affinities of galectin-1 and -3 for **5**−**7**, **12**, **13**, **18**, and **19** and reference compounds thiodigalactoside and LacNAc-β-OMe were evaluated by a fluorescent polarization assay (Table 1).[33] The symmetrical thiodigalactoside derivatives **5**−**7** strongly benefited from the introduction of aromatic substituents in comparison to the parent compound. The K_d of galectin-3 for TDG was 43 μM and decreased 1000−2000 times to 44 and 22 nM, respectively, for **5** and **6**. For galectin-1 the enhancement was between 500 and 1850 times for the same compounds. Interestingly, the bulkier **7** showed different behavior. Its K_d for galectin-3 was still submicromolar with 0.36 μM, but for galectin-1 the affinity was even worse than the free TDG with a K_d of 84 μM. The result was that **7** has a 230-fold preference for galectin-3. For the LacNAc derivatives (**12**, **13**, **18**, **19**) that all contained the bulky phenoxyaryl group, several effects are apparent. First, three of the four compounds showed similar galectin-3 affinities of around 1−2 μM. The ether-linked phenoxybenzyl group of **12** and **13** strongly reduced galectin-1 binding with K_d of 280 and 180 μM, respectively. This resulted in a selectivity factor of 230 for **12**. The triazole-linked phenoxyphenyl group (**18** and **19**) reduced the galectin-1 affinity less than in the TDG series. Additional observations include the affinity of galectin-1 benefiting more from a triazole-linked than an ether-linked phenoxyaryl and at least in one case (**12**) galectin-3 binding benefiting from a phthaloyl on the N2 over the benzoyl group.

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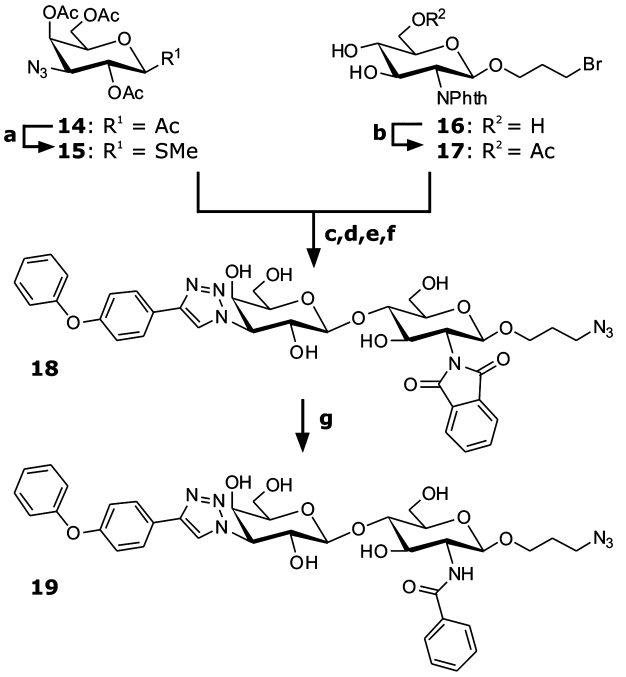
Scheme 3[a]



[a]Reaction conditions: (a) $MeSSiMe_3$, TMSOTf, DCE, 71%; (b) AcCl, 2,4,6-trimethylpyridine, CH_2Cl_2, 79%; (c) TfOH, NIS, CH_2Cl_2, 64%; (d) 1-ethynyl-4-phenoxybenzene, $CuSO_4$, sodium ascorbate, DMF/H_2O, 70%; (e) NaN_3, DMF, 83%; (f) NaOMe, MeOH, 84%; (g) (i) ethylenediamine, *n*-BuOH; (ii) Bz_2O, DiPEA, MeCN, 73%.

Table 1. K_d Values of Galectin-1 and -3 for the Thiodigalactoside and Lactosamine Derivatives As Measured by a Fluorescence Polarization Assay

	galectin-1		galectin-3		
compd	K_d (μM)	rel aff	K_d (μM)	rel aff	ratio[a]
TDG	24	1	43	1	0.6
5	0.049 ± 0.012	490	0.044 ± 0.005	980	1
6	0.013 ± 0.004	1850	0.022 ± 0.007	1950	0.6
7	84 ± 46	0.3	0.36 ± 0.09	120	230
LacNAc-β-OMe	70	1	69	1	1
12	280 ± 8	0.3	1.2 ± 0.13	58	230
13	180 ± 25	0.4	8.1 ± 0.92	8.5	22
18	28 ± 7.6	2.5	2.2 ± 0.29	31	13
19	6.7 ± 1.0	10	2.8 ± 0.69	25	2.4

[a]Galectin-3 preference, K_d(gal-1)/K_d(gal-3).

To better understand the experimental observations, computer simulations of the ligands were undertaken following a rigid docking protocol (see Supporting Information). The lowest energy structures obtained for reference compounds TDG and LacNAc in galectin-1 and -3 by rigid docking mimicked the known X-ray structures of Gal-X-TDH and Gal-Y-LacNAc-OX with rmsd of ~1.1 Å (Gal-1 PDB code 3t2t, Gal-3 PDB code 1kjr), and the calculated affinities accurately resembled the experimentally measured data.[27,34] The calculated free energy of binding for the newly synthesized compounds showed a similar trend as the experimental K_d; however, the trends were less pronounced. Modeling of **5** showed similar interactions with the two galectins. Arg73 for galectin-1 and Arg186 and Arg144 for galectin-3 showed cation−π interactions with the phenyl and triazole groups that could explain its enhanced affinities for both galectins in comparison to TDG. Interestingly, galectin-3 was modeled to have three arginine−arene interactions with **7** while for galectin-1 there was only one, which might account for the large observed differences in affinity (Figure 1). Additionally, the larger structures could cause steric hindrance and hence lower the affinity of galectin-1; however, this is not apparent from the computational data.

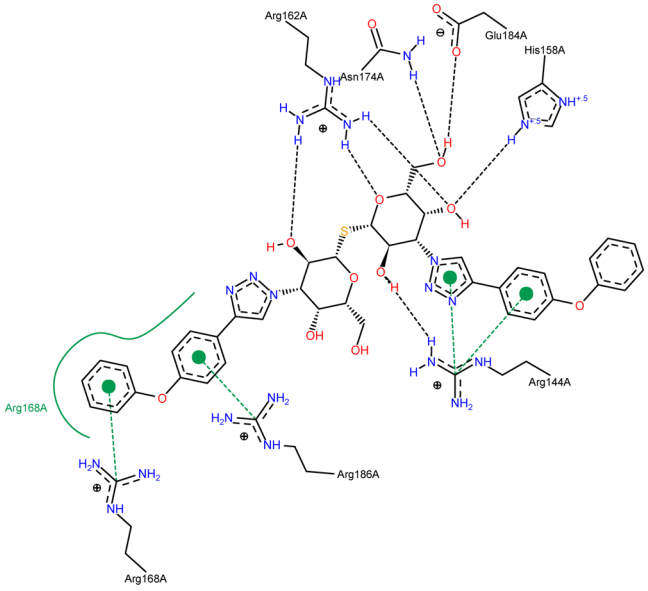


Figure 1. PoseView of **7** positioned in galectin-3 via molecular modeling in which Arg168, Arg186, and Arg144 interact with the aromatic substituents.

CONCLUSIONS

Aromatic substitution at C3 of TDG with 4-aryltriazol groups results in potent galectin-3 inhibitors. Moreover, larger aromatic groups provide selective inhibitors for galectin-3 vs -1, presumably because of a larger number of favorable aryl−arginine interactions for galectin-3 and possible steric impediments for galectin-1. Furthermore, substitution of LacNAc on the C3 position and the N2 similarly resulted in selectivity especially for **12**. These results indicate that the newly synthesized TDG and LacNAc inhibitors can be very useful for the biological investigations of galectin-3 or possibly for therapeutics development. Attempts in the former direction have already been made with the conjugation of TDG and LacNAc derivatives to DOTA chelators for radiolabeling. These results will be presented in due course.

EXPERIMENTAL SECTION

General 1,3-Cycloaddition of 3-Azidothiodigalactoside. 3-Azidothiodigalactoside **1** (75 mg, 114 μmol), alkyne (58 mg pf phenylacetylene, 67 mg of 3-hydroxyphenylacetylene, or 111 mg of 1-ethynyl-4-phenoxybenzene, 570 μmol), $CuSO_4{\cdot}5H_2O$ (27 mg, 108 μmol), and sodium ascorbate (23 mg, 116 μmol) were dissolved in DMF/H_2O (5 mL, 4:1). The 1,3-cycloaddition reaction was performed under microwave irradiation at 80 °C for 40 min. Subsequently, the solvent was evaporated and the residue was redissolved in CH_2Cl_2 (100 mL), washed with H_2O (50 mL) and saturated aqueous NaCl (50 mL), dried over Na_2SO_4, and filtered. The residue was purified by silica chromatography (CH_2Cl_2/MeOH 49:1),

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and products **2**–**4** were obtained as white solids. Bis-{2,4,6-tri-*O*-acetyl-3-deoxy-3-[4-(phenyl)-1*H*-1,2,3-triazol-1-yl]-*β*-D-galactopyranosyl}sulfane **2**: yield 100 mg, quant, R_f = 0.6 (CH_2Cl_2/MeOH 19:1). Bis-{2,4,6-tri-*O*-acetyl-3-deoxy-3-[4-(3-hydroxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*β*-D-galactopyranosyl}sulfane **3**: yield 97 mg, quant, R_f = 0.29 (CH_2Cl_2/MeOH 19:1). Bis-{2,4,6-tri-*O*-acetyl-3-deoxy-3-[4-(4-phenoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*β*-D-galactopyranosyl}sulfane **4**: yield 102 mg, 86%, R_f = 0.29 (CH_2Cl_2/MeOH 19:1).

Deacetylation of 3-Triazolylthiodigalactoside. Compounds **2**–**4** (15 μmol) were separately dissolved in MeOH (5 mL) and treated with NaOMe (100 μL, 30% w/v in MeOH) for 2.5 h. The mixture was neutralized with DOWEX H^+ resin, filtered, and concentrated in vacuo. Products **5**–**7** were obtained after preparative HPLC (elution gradient of 5% MeCN and 0.1% TFA in H_2O to 5% H_2O and 0.1% TFA in MeCN) as white solids. Bis-{3-deoxy-3-[4-(phenyl)-1*H*-1,2,3-triazol-1-yl]-*β*-D-galactopyranosyl}sulfane **5**: Yield 9 mg, 98%. HRMS (*m*/*z*): calcd for $C_{28}H_{32}N_6O_8S$ $[M + H]^+$ 613.2080; found 613.2045. Bis-{3-deoxy-3-[4-(3-hydoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*β*-D-galactopyranosyl}sulfane **6**: yield 7.5 mg, 86%. HRMS (*m*/*z*): calcd $C_{28}H_{32}N_6O_{10}S$ $[M + H]^+$ 645.1979; found 645.1877. Bis-{3-deoxy-3-[4-(4-phenoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-*β*-D-galactopyranosyl}sulfane **7**: yield 12 mg, quant. HRMS (*m*/*z*): calcd $C_{40}H_{40}N_6O_{10}S$ $[M + H]^+$ 797.2605; found 797.2514.

[3-*O*-(4-Phenoxybenzyl)-*β*-D-galactopyranosyl]-*β*-(1,4)-2-deoxy-2-*N*-phthalimido-3-azidopropyl-*β*-D-glucopyranoside 12. Donor **9** (295 mg, 0.57 mmol) and acceptor **11** (206 mg, 0.47 mmol) were azeotropically dried by triple coevaporation with toluene. The reagents and NIS (160 mg, 1.5 mmol) were dissolved in dry CH_2Cl_2 (15 mL), and the mixture was stirred under N_2 flow in the presence of molecular sieves (4 Å, 2.5 g) at −50 °C. After 15 min triflic acid (8.3 μL, 94 μmol) was added and the mixture was stirred for 1 h. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated aqueous $Na_2S_2O_5$ (50 mL) and the organic layer dried over Na_2SO_4, filtered, and evaporated. Silica chromatography (Hex/EtOAc 2:1 to 1:2) yielded the disaccharide **12I** as white foam (80%, 342 mg). R_f = 0.41 (Hex/EtOAc 1:1). Subsequently, NaOMe (17 μL, 30% w/v in MeOH) was added to a solution of the dissaccharide (35 mg, 39 μmol) in MeOH (14 mL) and the mixture was stirred for 6 h at rt. The solution was neutralized with DOWEX-H^+ resin, filtered, and evaporated. **12** was obtained as a white solid after preparative HPLC (73%, 21 mg). HRMS (*m*/*z*): calcd $C_{36}H_{40}N_4O_{13}$ $[M + Na]^+$ 759.2490; found 759.2418.

[3-*O*-(4-Phenoxybenzyl)-*β*-D-galactopyranosyl]-*β*-(1,4)-2-deoxy-2-*N*-benzamido-3-azidopropyl-*β*-D-glucopyranoside 13. A solution of **12** (4 mg, 5 μmol) in *n*-butanol (2.5 mL) and ethylenediamine (0.5 mL) was heated to 80 °C for 20 h. Subsequently, the solvent was evaporated and the compound was purified by column chromatography (CH_2Cl_2/MeOH 4:1). The nitrogen was immediately protected with benzoic anhydride (9.4 mg, 41 μmol) in acetonitrile (2 mL) and DiPEA (12 μL) for 1 h. Column chromatography (CH_2Cl_2/MeOH 9:1) yielded **13** as a white solid (95%, 3.4 mg). HRMS (*m*/*z*): calcd $C_{35}H_{42}N_4O_{12}$ $[M + H]^+$ 711.2877; found 711.2869.

3-[4-(4-Phenoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-3-deoxy-*β*-D-galactospyranosyl)-*β*(1,4)-2-deoxy-2-*N*-phthalimido-3-azidopropyl-*β*-D-glucopyranoside 18. Donor **15** (620 mg, 1.7 mmol) and acceptor **17** (675 mg, 1.43 mmol) were azeotropically dried by coevaporation with toluene. Then the reagents were dissolved in dry CH_2Cl_2 (30 mL) and combined with NIS (482 mg, 2.1 mmol). The mixture was stirred under N_2 flow with activated molecular sieves (4 Å, 6 g) at −50 °C. After 15 min triflic acid (32 μL, 0.36 mmol) was added and the mixture was stirred for 1 h. The mixture was diluted with CH_2Cl_2 (100 mL) and filtered to remove the molecular sieves. The mixture was washed with saturated aqueous $Na_2S_2O_5$ (50 mL) and the organic layer dried over Na_2SO_4, filtered, and evaporated. Silica chromatography (Hex/EtOAc 1:1) yielded the disaccharide **18I** as a white foam (1 g, 64%) with galactospyranosyl-*β*(1,3)-glucopyranoside as side product. R_f = 0.15 (Hex/EtOAc 1:1). Disaccharide **18I** (75 mg, 90 μmol), 1-ethynyl-4-phenoxybenzene (36 mg, 180 mmol), $CuSO_4 \cdot 5H_2O$ (12 mg, 45 μmol), and sodium ascorbate (9 mg, 45 μmol) were dissolved in DMF/H_2O (19:1) and reacted at 80 °C under microwave irradiation for 40 min. The solvents were evaporated. The residue was redissolved in CH_2Cl_2 (50 mL) and washed with H_2O (25 mL) and aqueous NaCl (25 mL, saturated). The organic layer was dried over Na_2SO_4, filtered, and concentrated. Compound **18II** was obtained after silica chromatography (Hex/EtOAc 1:1) as a solid (62 mg, 70%). R_f = 0.63 (Hex/EtOAc 1:3). Compound **18II** (50 mg, 51 μmol) and NaN_3 (16 mg, 73 μmol) were dissolved in dry DMF (1.4 mL) and stirred at 100 °C for 18 h. The organic solvent was evaporated, and the residue was redissolved in EtOAc (50 mL) and washed with H_2O (50 mL) and saturated aqueous NaCl (50 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated. The crude product was subjected to silica chromatography (Hex/EtOAc 1:1) to give **18III** as a clear oil (83%, 40 mg). R_f = 0.51 (Hex/EtOAc 1:3). NaOMe (12.5 μL, 30% w/v in MeOH) was added to a solution of **18III** (43 mg, 39 μmol) in MeOH (17 mL). The mixture was stirred for 1.5 h at rt. The solution was neutralized with DOWEX-H^+ resin, filtered, and evaporated. Compound **18** was obtained as a white solid after preparative HPLC (84%, 29 mg). HRMS (*m*/*z*): calcd for $C_{37}H_{39}N_7O_{12}$ $[M + H]^+$ 774.2735; found 774.2654.

3-[4-(4-Phenoxyphenyl)-1*H*-1,2,3-triazol-1-yl]-3-deoxy-*β*-D-galactopyranosyl)-*β*(1,4)-2-deoxy-2-*N*-benzamido-3-azidopropyl-*β*-D-glucopyranoside 19. A solution of **18** (10 mg, 13 μmol) in *n*-butanol (2.5 mL) and ethylenediamine (0.5 mL) was heated to 80 °C for 20 h. Then the solvent was evaporated and the compound was purified by column chromatography (CH_2Cl_2/MeOH 4:1). The free N_2 was immediately protected with benzoic anhydride (12.8 mg, 54 μmol) in acetonitrile (2 mL) and DiPEA (17 μL) for 1 h. After preparative HPLC **19** was obtained as a white solid (73%, 7.3 mg). HRMS (*m*/*z*): calcd for $C_{36}H_{41}N_7O_{11}$ $[M + H]^+$ 748.2942; found 748.2892.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, characterization data, and modeling data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare the following competing financial interest(s): U.J.N. and H.L. own shares in Galacto Biotech AB.

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