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2,6-Disubstituted Benzoates As Neighboring Groups for Enhanced Diastereoselectivity in β-Galactosylation Reactions: Synthesis of β-1,3-Linked Oligogalactosides Related to Arabinogalactan Proteins

Nathan W. McGill and Spencer J. Williams*

School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria 3010, Australia

sjwill@unimelb.edu.au

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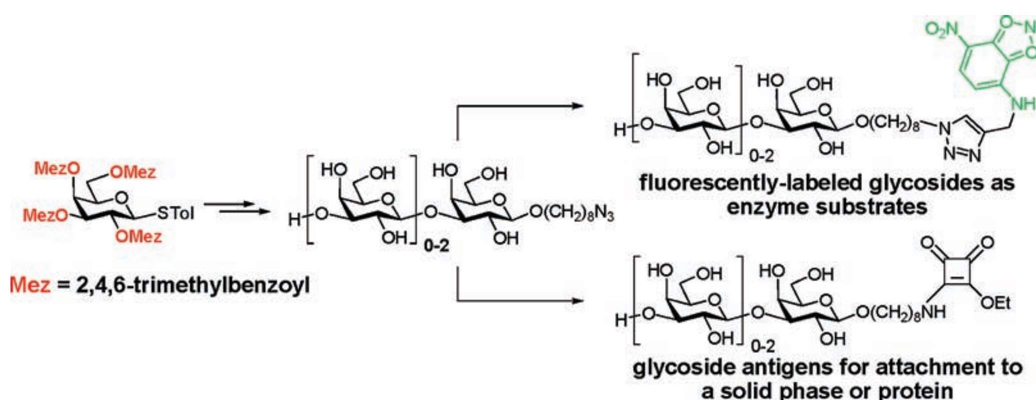

Arabinogalactan proteins (AGPs) are plant glycoproteins which contain a β-1,3-linked galactan core. The synthesis of the β-galactopyranose-1,3-β-galactopyranose linkage using various 2-*O*-acyl-protected glycosyl donors has been plagued with poor stereoselectivity and side reactions including orthoester formation and transesterification of the 2-*O*-acyl group from the donor to the acceptor. We have investigated the use of 2,6-disubstituted benzoyl groups as bulky neighboring groups on the glycosyl donor. A 2,4,6-trimethylbenzoyl group was found to be optimal and enabled the formation of the β-galactopyranose-1,3-β-galactopyranose linkage to disarmed ester-protected acceptors, suppressing transesterification and reducing orthoester formation while enhancing the β-selectivity of galactosylation reactions. A series of β-1,3-linked oligogalactosides were prepared and elaborated to neoglycoconjugates for the study of AGP biosynthesis and AGP binding proteins.

Introduction

Arabinogalactan proteins (AGPs) are plant glycoproteins with emerging roles in embryogenesis, cellular homeostasis, and developmental processes.[1,2] They are significant articles of commerce, being the major constituent of gum arabic, which possesses a wide range of applications in the food and pharmaceutical industries owing to its activities as an emulsion stabilizer, excipient, and demulcent (soothing agent).[3] Gum arabic is exulted as the premier emulsifier for oil-in-water emulsions such as citrus- and cola-based beverages. AGPs are characterized by a hydroxyproline-rich protein core, which is often modified at the C-terminus through the installation of a glycosylphosphatidyl anchor.[4] The hydroxyproline residues are O-glycosylated with complex glycans rich in D-arabinofuranose and D-galactopyranose.[2] The core regions of these glycans contain a linear galactan chain, comprising β-1,3-linked oligogalactosides interrupted with β-1,6-linkages, as demonstrated by sensitivity to periodate cleavage.[5]

*Corresponding author. Tel: +61 3 8344 2422. Fax: +61 3 9347 8124.

(1) Seifert, G. J.; Roberts, K. *Annu. Rev. Plant Biol.* **2007**, *58*, 137–161.
(2) Showalter, A. M. *Cell. Mol. Life Sci.* **2001**, *58*, 1399–1417.
(3) Islam, A. M.; Phillips, G. O.; Sljivo, A.; Snowden, M. J.; Williams, P. A. *Food Hydrocolloids* **1997**, *11*, 493–505.
(4) Gaspar, Y.; Johnson, K. L.; McKenna, J. A.; Bacic, A.; Schultz, C. J. *Plant Mol. Biol.* **2001**, *47*, 161–176.
(5) Tan, L.; Qiu, F.; Lamport, D. T.; Kieliszewski, M. J. *J. Biol. Chem.* **2004**, *279*, 13156–13165.

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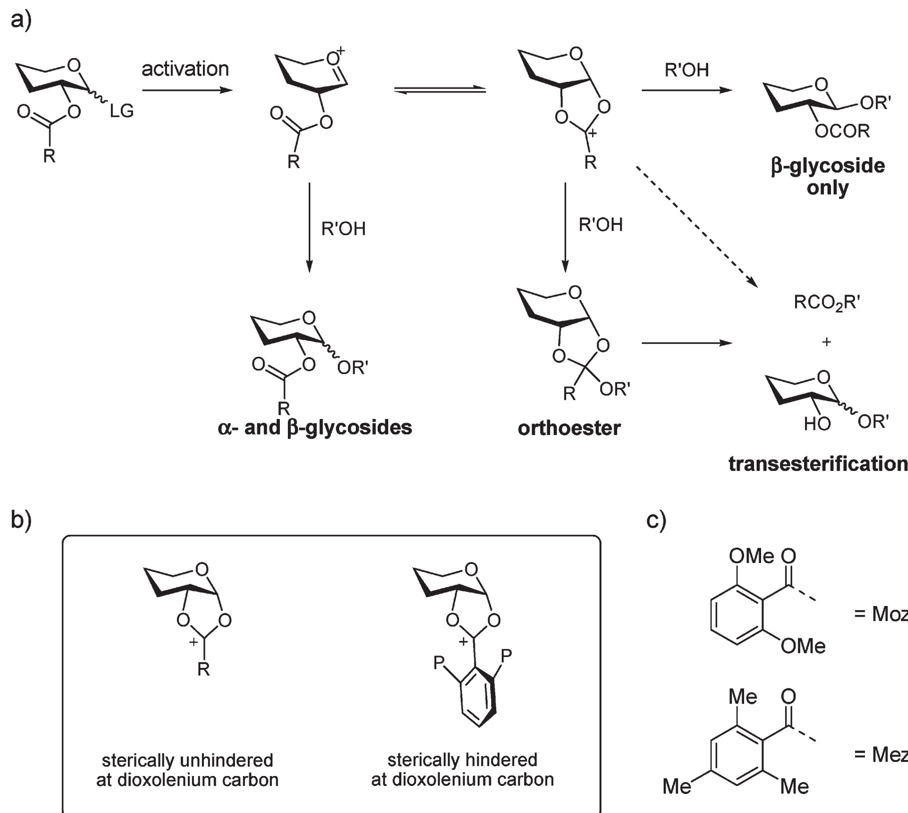
SCHEME 1. **(a) Mechanism of Glycosylation with Neighboring Group Participation, and Side Reactions Leading to Poor Stereoselectivity, Orthoester Formation, And Transesterification; (b) Comparison of Dioxolenium Ion Intermediates of Standard and 2,6-Disubstituted Benzoyl Groups; (c) Protecting Groups Investigated in This Work: Moz = 2,6-Dimethoxybenzoyl; Mez = 2,4,6-Trimethylbenzoyl**



The synthesis of the β-D-galactopyranosyl-1,3-β-D-galactopyranose linkage has attracted some attention owing to its occurrence in the tetrasaccharide core of glycosaminoglycan proteoglycans,[6] the type V core of the ABO blood group antigens,[7] protozoan parasites such as leishmania,[8,9] and various plant-based materials including arabinogalactan[10] and AGPs.[11−13] However, poor stereoselectivity is commonly observed in many galactosylation reactions.[14−20] In a landmark study, Kovác and co-workers reported the synthesis of a series of β-1,3-linked oligogalactosides, but this work was plagued by the frequent and unexpected occurrence of α-galactosides even with a 2-*O*-benzoyl group on the donor that should be capable of neighboring group participation.[17] Kong and co-workers have reported the development of galactosyl donors that give stereoselective α-galactosylation; these glycosyl donors possess an electron-rich alkyl substituent at O-3 and a 2-*O*-benzoyl group.[15] Other complicating processes have been seen with 2-*O*-acyl galactosyl donors, including the formation of orthoesters[21−23] and transesterification of the 2-*O*-acyl group to the acceptor alcohol.[24−26] These processes most likely occur through nucleophilic attack of the acceptor alcohol on the dioxolenium ion carbon of the intermediate formed upon activation of the glycosyl donor.[27] Whitfield and co-workers have presented computational evidence that orthoester formation and transesterification are separate processes but which proceed through a common dioxolenium ion (Scheme 1a).[25,28]

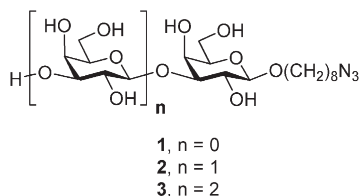
(6) Allen, J. G.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1999**, *121*, 468–469.
(7) Meloncelli, P. J.; Lowary, T. L. *Aust. J. Chem.* **2009**, *62*, 558–574.
(8) McConville, M. J.; Thomas-Oates, J. E.; Ferguson, M. A.; Homans, S. W. *J. Biol. Chem.* **1990**, *265*, 19611–19623.
(9) McConville, M. J.; Turco, S. J.; Ferguson, M. A.; Sacks, D. L. *EMBO J.* **1992**, *11*, 3593–3600.
(10) Bürgermeister, J.; Paper, D. H.; Vogl, H.; Linhardt, R. J.; Franz, G. *Carbohydr. Res.* **2002**, *337*, 1459–1466.
(11) Chowdhary, M. S.; Navia, J. L.; Anderson, L. *Carbohydr. Res.* **1986**, *150*, 173–185.
(12) Ziegler, T.; Adams, B.; Kovac, P.; Glaudemans, C. P. J. *J. Carbohydr. Chem.* **1990**, *9*, 135–158.
(13) Wang, L. X.; Sakairi, N.; Kuzohara, H. *J. Carbohydr. Chem.* **1993**, *10*, 349–361.
(14) Yang, F.; Du, Y. *Carbohydr. Res.* **2002**, *337*, 485–491.
(15) Chen, L.; Kong, F. *Tetrahedron Lett.* **2003**, *44*, 3691–3695.
(16) Jacquinet, J.-C. *Carbohydr. Res.* **2004**, *339*, 349–359.
(17) Kovác, P.; Taylor, R. B.; Glaudemans, C. P. J. *J. Org. Chem.* **1985**, *50*, 5323–5333.
(18) Li, A.; Kong, F. *Carbohydr. Res.* **2005**, *340*, 1949–1962.
(19) Yang, F.; He, H.; Du, Y.; Lü, M. *Carbohydr. Res.* **2002**, *337*, 1165–1169.
(20) Chen, L.; Kong, F. *Carbohydr. Res.* **2002**, *337*, 1373–1380.
(21) Yang, Z.; Lin, W.; Yu, B. *Carbohydr. Res.* **2000**, *329*, 879–884.
(22) Wilstermann, M.; Magnusson, G. *Carbohydr. Res.* **1995**, *272*, 1–7.
(23) Seeberger, P. H.; Eckhardt, M.; Gutteridge, C. E.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10064–10072.
(24) Ziegler, T.; Kovác, P.; Glaudemans, C. P. J. *Liebigs Ann. Chem.* **1990**, 613–615.
(25) Bérces, A.; Whitfield, D. M.; Nukada, T.; do Santos, Z., I.; Obuchowska, A.; Krepinsky, J. J. *Can. J. Chem.* **2004**, *82*, 1157–1171.
(26) Murakami, T.; Hirono, R.; Sato, Y.; Furusawa, K. *Carbohydr. Res.* **2007**, *342*, 1009–1020.
(27) Crich, D.; Dai, Z.; Gastaldi, S. *J. Org. Chem.* **1999**, *64*, 5224–5229.
(28) Nukada, T.; Bérces, A.; Zgierski, M. Z.; Whitfield, D. M. *J. Am. Chem. Soc.* **1998**, *120*, 13291–13295.

TABLE 1. Effect of Protecting Group on the Glycosylation of 8-Azidooctan-1-ol Using Donors 5, 7, 9, and 10

entry	donor	yield[a] (%)
1	**5**, R = Bz	25[b]
2	**7**, R = Piv	45[b]
3	**9**, R = Moz	44[c]
4	**10**, R = Mez	75

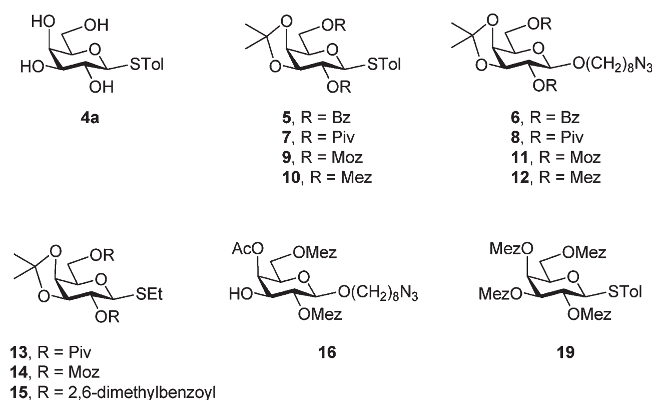
[a]Reactions were performed using donor (1 equiv), acceptor (1.2 equiv), NIS (1.4 equiv), and TfOH (0.25 equiv) in CH_2Cl_2 at 0 °C. [b]Transesterification product was isolated in 10% yield. [c]α-Glycoside was isolated in 6% yield; transesterification product was isolated in 15% yield.

We are interested in synthesizing a series of azidooctyl *β*-1,3-oligogalactosides **1−3** as central intermediates in the preparation of neoglycoconjugates for use as artificial substrates for AGP biosynthetic enzymes and as antigens to investigate AGP binding proteins. In this work, we have investigated 2,6-disubstituted benzoates as bulky protecting and neighboring groups to attempt to overcome poor stereoselectivity and transesterification seen in difficult galactosylations (Scheme 1b). Such 2,6-disubstituted benzoates have been previously investigated by others as neighboring groups for glycosylation of aliphatic alcohols.[25,29,30] This work represents the first to investigate their use for the formation of more complex oligosaccharides in a form suitable for applications as neoglycoconjugates for the study of AGP biosynthesis and corresponding binding proteins.



Results and Discussion

Owing to their stability to many of the conditions required for protecting group manipulation, we employed thioglycosides as glycosyl donors. The 3,4-di-*O*-isopropylidene galactoside **5** was selected as a glycosyl donor as it was anticipated that the isopropylidene group could be readily removed and the resultant diol converted to a glycosyl acceptor suitable for sequential nonreducing-end galactosylations to deliver the oligogalactosides **1−3**. Compound **5** was prepared in two steps from tetraol **4a**[31] (see the Supporting Information). Glycosylation of 8-azidooctan-1-ol using dibenzoate donor **5** afforded the *β*-galactoside **6** in only 25% yield, accompanied by transesterification product (10%) (Table 1). An attempt to remedy this poorly performing glycosylation through the use of a pivalyl-protected donor **7** provided little improvement; a 45% yield of the *β*-galactoside **8** was obtained. The observation of low yields of the desired *β*-galactosides, along with amounts of α-anomer and transesterification products, is unusual for a 2-*O*-pivalyl donor, which is widely acknowledged for its insensitivity to transesterification.[23,32] These results may be readily rationalized in terms of activation of the glycosyl donor leading first to an oxocarbenium ion, which is captured by the neighboring group to give a dioxolenium ion (Scheme 1a).[27] In the normal course of events, this dioxolenium ion should undergo S_N2-like reaction with a nucleophilic alcohol. However, a reactivity mismatch with an acceptor alcohol may lead to either nucleophilic attack on the intermediate at the dioxolenium carbon, leading to transesterification, or reversion to the oxocarbenium ion, allowing bottom-face attack and formation of the α-anomer.

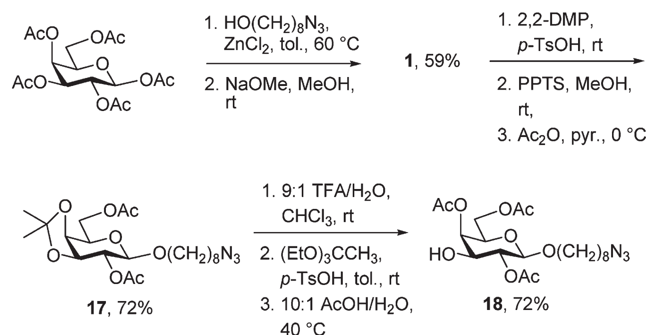


These unexpected, but not unprecedented,[32] results prompted us to investigate the use of a 2,6-disubstituted benzoate as a neighboring group, with the expectation that the *ortho* substituents on the benzoyl group should sterically shield the intermediate dioxolenium ion and prevent transesterification through preventing nucleophilic attack at this site (Scheme 1b). In addition, the steric bulk of this group might prevent 1,2-*cis*-substitution on an intermediate oxonium ion in the event of a reactivity mismatch between an acceptor alcohol and the derived dioxolenium ion (Scheme 1a). We chose to investigate 2,6-dimethoxybenzoyl chloride (MozCl), and owing to its lower cost, 2,4,6-trimethylbenzoyl chloride (MezCl) was identified as a pragmatic alternative to 2,6-dimethylbenzoyl chloride (Scheme 1c). In a glycosylation reaction with 8-azidooctan-1-ol, 2,6-dimethoxybenzoate **9** gave **11** with poor stereoselectivity and also suffered from transesterification (Table 1). On the other hand, the mesitoate **10** was an effective glycosyl donor and afforded the *β*-galactoside **12** in a yield of 75% with no α-anomer or transesterification products observed. These results are especially interesting in the context of work by Whitfield and co-workers, who examined the glycosylation of a PEG-based primary alcohol acceptor using the closely related glycosyl donors: pivalate **13**, 2,6-dimethoxybenzoate **14**, and 2,6-dimethylbenzoate **15**.[25,32] While the pivalate donor **13** was susceptible to transesterification, the 2,6-dimethylbenzoate **15** was an adequate glycosyl donor, although it was prone to orthoester formation. Conversely, the 2,6-dimethoxybenzoate **14** was pronounced as the optimal donor for glycosylation, although it underwent transesterification to the acceptor to a limited extent.

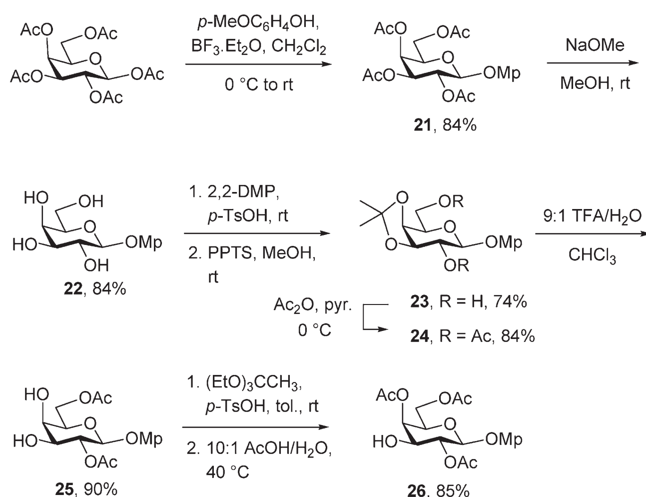
The next phase in our synthesis required the elaboration of the azidooctyl galactoside **12** to glycosyl acceptor **16**. This

(29) Nunomura, S.; Mori, M.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1989**, *30*, 6713–6716.
(30) Nunomura, S.; Mori, M.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1989**, *30*, 5619–5622.
(31) Yde, M.; De Bruyne, K. *Carbohydr. Res.* **1973**, *26*, 227–229.
(32) Nukada, T.; Bérces, A.; Whitfield, D. M. *J. Org. Chem.* **1999**, *64*, 9030–9045.

SCHEME 2



SCHEME 3



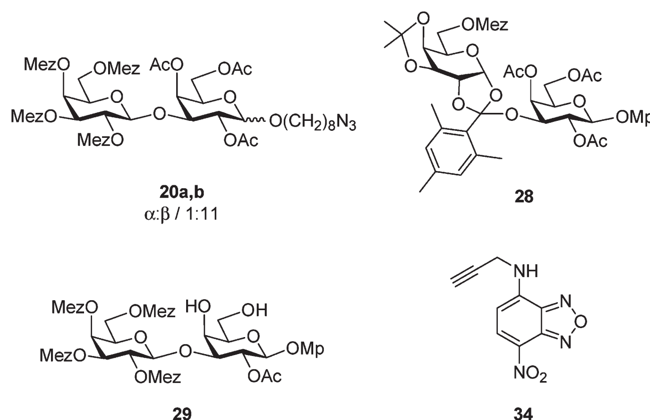
was readily achieved by hydrolysis of the isopropylidene acetal and regioselective 4-*O*-acetylation via the intermediate orthoacetate according to the methodology of King and Allbutt.[33,34] However, under a range of conditions, coupling of mesitoate donor **10** and acceptor **16** was completely unsuccessful, with no glycoside product being isolated.

Together these results raised some important questions. Was the failure to glycosylate related to poor reactivity of the glycosyl donor **10**, owing to the presence of the bulky 2-*O*-mesitoyl group, or the poor reactivity of the acceptor **16**, which also bears a 2-*O*-mesitoyl group adjacent to the hydroxy group?[35] More generally, can conditions be found that 2-*O*-mesitoyl-protected glycosyl donors can be used to glycosylate carbohydrate alcohols? Finally, can mesitoyl groups be utilized as stereodirecting groups to complete the synthesis of our target β-1,3-linked oligogalactosides **1**−**3**?

The failure of the condensation between **10** and **16** was attributed to the severe steric congestion arising from the presence of a 2-*O*-mesitoyl group on both the donor and acceptor adjacent to the sites of reaction. As a 2-*O*-mesitoyl group was required on the donor as a neighboring group, we elected to modify the acceptor. Compound **18** was obtained by $ZnCl_2$-catalyzed[26] coupling of galactose pentaacetate and 8-azidooctan-1-ol, followed by deacetylation to give **1**. Isopropylidenation of **1**, followed by acetylation afforded **17**; sequential isopropylidene hydrolysis, orthoacetate formation, and regioselective hydrolysis furnished **18** (Scheme 2). We also elected to prepare a simpler glycosyl donor, the tetramesitoate **19** (see the Supporting Information). When treated with alcohol **18**, **19** proved a competent glycosyl donor, allowing the synthesis of the disaccharides **20a**,**b** in 47% (1:11 α:β). Compound **20b** was deprotected using 1 M LiOH in MeOH/THF/H_2O to afford **2**.

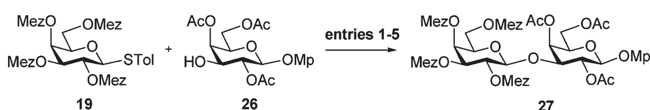
It was now apparent that the nonreducing-end strategy for the construction of the target oligosaccharides **1**−**3**, involving the sequential addition of individual monosaccharide residues to the nonreducing-end of the growing chain, was incompatible with the use of a 2-*O*-mesitoyl group as a neighboring group, as each subsequent glycosylation would require the union of a glycosyl donor and acceptor, each bearing a 2-*O*-mesitoyl group. We therefore required a disaccharide donor that could be used to assemble a trisaccharide in a [2 + 1] fashion. For this purpose, we required a galactose acceptor that could be readily converted to a glycosyl donor after conversion to a disaccharide. 4-Methoxyphenyl alcohol **26** was prepared from galactose pentaacetate by an eight step route (Scheme 3). This reliable synthetic procedure requires only two recrystallizations and no chromatography, and can provide 10 g of alcohol **26** in just 3 days from galactose pentaacetate.

We next sought to compare the performance of the isopropylidene-protected donor **10** and the tetramesitoate **19**. We were mindful of the precedent of Whitfield and coworkers that the reactivity of related glycosyl donors toward simple primary alcohol acceptors was optimal when using relatively high amounts of NIS and TfOH, which also served to suppress acyl transfer.[25] Reaction of **19** and **26** in the presence of 2.5 equiv of NIS and 1.4 equiv of TfOH provided the glycoside **27** in only 33% yield (Table 2). Reducing the amount of TfOH (0.25 equiv) resulted in a sluggish reaction, which suggested that the reactivities of the donor and acceptor were mismatched. The reaction conditions were modified to enhance the reactivity of the donor, while the amount of NIS was reduced to prevent side reactions. Reducing the amount of NIS (1.5 equiv) did not affect the outcome of the reaction, whereas increasing the amount of TfOH (0.7 equiv) resulted in acceleration of the reaction and an improvement in yield, presumably owing to an increased concentration of iodonium triflate in the reaction mixture. Switching to acid-washed molecular sieves[22] resulted in a



(33) King, J. F.; Allbutt, A. D. *Tetrahedron Lett.* **1967**, *8*, 49–54.
(34) King, J. F.; Allbutt, A. D. *Can. J. Chem.* **1970**, *48*, 1754–1769.
(35) An attempted glycosylation of donor **19** and acceptor **16** was also unsuccessful.

TABLE 2. Effects of Promotion Conditions, Molecular Sieves, and Temperature on the Glycosylation of Galactoside 26 with Donor 19



entry	NIS (equiv)	TfOH (equiv)	mol sieves (Å)	temp (°C)	yield[a] (%)
1	2.5	1.4	4	0	33[b]
2	2.5	0.25	4	0	44
3	1.5	0.25	4	0	47
4	1.5	0.7	4	0	67
5	1.5	0.7	5 (AW)	0	69
6	1.5	0.7	5 (AW)	0 to rt	80

[a]Reactions were performed in CH_2Cl_2. [b]12% of the α-anomer was isolated.

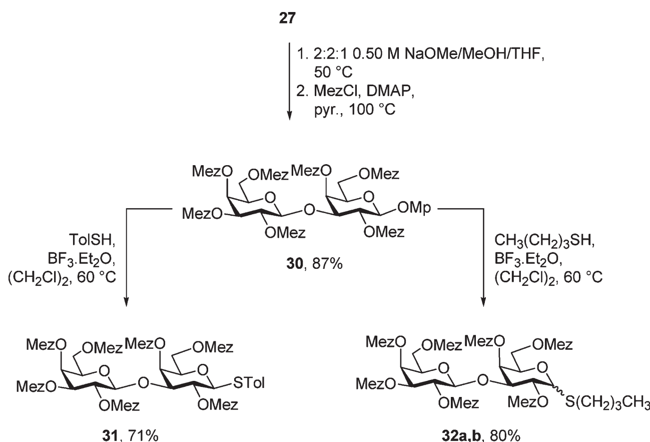
minor improvement in yield. Finally, running the reaction at room temperature afforded the disaccharide **27** in 80% yield. Attempted glycosylation of the alcohol **26** with the donor **10** was completely unsuccessful. Orthoester **28** was the major product, resulting from nucleophilic attack of the acceptor on the carbon of the dioxolenium ion intermediate. This result is not without precedent; Bérces et al. observed the formation of an orthoester in attempted glycosylations of a primary alcohol with the donor **15**.[25] Nonetheless, this result is striking, as it demonstrates that the *ortho*-substituents of the 2-*O*-mesitoyl group do not completely sterically block reaction at the dioxolenium ion carbon, even for a sterically hindered secondary alcohol. Our attempts to rearrange this orthoester to the glycoside were fruitless and led only to recovery of the acceptor **26**.

In order to complete the synthesis of the trisaccharide **3**, the 4-methoxyphenyl disaccharide **27** needed to be converted into a glycosyl donor. Deacetylation of **27** proved to be nontrivial. Under a range of mild conditions (cat. NaOMe/MeOH, DBU/MeOH, or guanidine/MeOH) the 2-acetate remained refractory, affording **29**. This result is reminiscent of the difficulty seen in cleaving a hindered acetate in the N3 antigen.[36] Application of more forcing conditions (0.5 M NaOMe in MeOH/THF at 50 °C for 24 h) resulted in cleavage of the three acetates and incomplete cleavage of the mesitoyl groups (Scheme 4). The heptamesitoate **30** was obtained by treatment of the crude product with mesitoyl chloride in pyridine at 100 °C. Treatment of **30** with thiocresol afforded the thioglycoside **31** as solely the β-anomer. However, the yield was somewhat better using a more nucleophilic alkyl thiol, n-butylthiol, affording the corresponding thioglycosides **32a**,**b** as an anomeric mixture. In this thioglycosylation reaction, a prolonged reaction time is necessary to rearrange the intermediate thioorthoesters[37,38] to the thioglycosides.

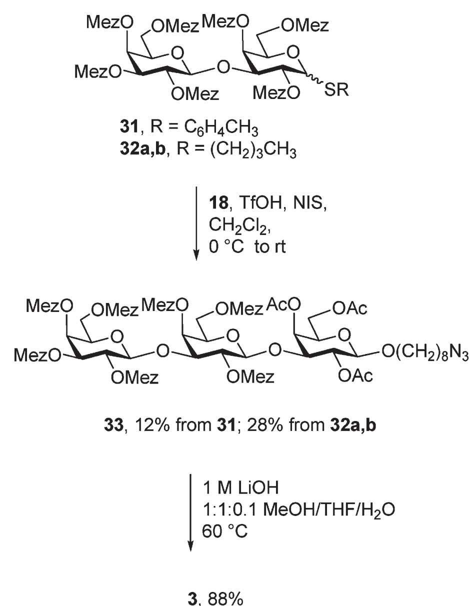
Glycosylation of the acceptor **18** was attempted using the previously optimized conditions (Scheme 5). In this case, the thiobutyl glycosides **32** performed significantly better than the corresponding thiotolyl glycoside **31**. Removal of the protecting groups from **33** was achieved by saponification using LiOH in a MeOH/THF/H_2O mixture. Compound **33**

(36) Kim, H. M.; Kim, I. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 35–48.
(37) Magnusson, G. *J. Org. Chem.* **1976**, *42*, 913–914.
(38) Zhang, Z.; Magnusson, G. *Carbohydr. Res.* **1996**, *295*, 41–55.

SCHEME 4



SCHEME 5

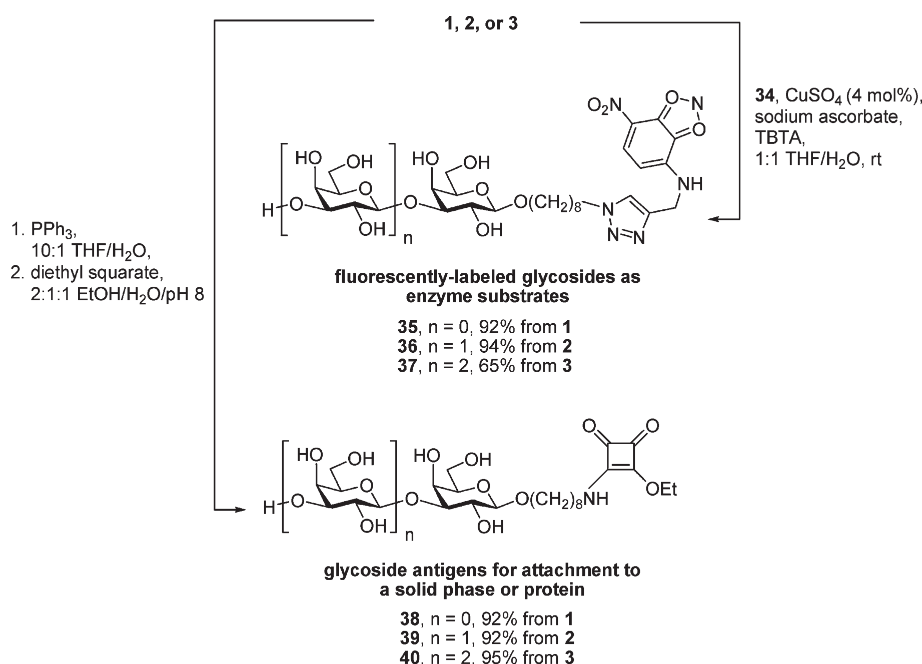


bears seven mesitoyl and three acetyl groups, and complete deprotection required 5 days. The products were purified by reversed phase chromatography, which was facilitated by the lipophilic nature of the azidooctyl aglycon.

Synthesis of Neoglycoconjugates. Neoglycoconjugates are carbohydrates linked to a noncarbohydrate group such as a protein, fluorescent label, or insoluble polymer support. Such adducts have become indispensible biochemical tools owing to the combination of the biological activity of the carbohydrate with the biological, chemical, or physical properties of the noncarbohydrate group. Like others interested in neoglycoconjugates, we have recognized that the azidooctyl chain provides significant advantages in terms of (a) its lipophilicity, allowing biphasic partitioning between water/butanol or purification by reversed-phase chromatography, (b) its potential for reduction to a aminooctyl chain for conjugation chemistry, and (c) its potential for conjugation with terminal alkynes using the Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction.[39] We were

(39) Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952–3015.

SCHEME 6



interested in the application of the mono-, di-, and trigalactosides **1**−**3** as substrates for AGP-modifying enzymes such as glycoside hydrolases[40−44] and glycosyltransferases,[45,46] and as antigens for the study of AGP-recognizing proteins.[2,41] For the former case, the ability to introduce a dye using the CuAAC reaction would allow the monitoring of reaction products by HPLC using sensitive fluorescence detection; in the latter case, a method for the conjugation of the synthetic galactosides to proteins was required.

Nitrobenzodiazoles (NBDs) are a useful fluorescent label owing to their small size and superior water solubility relative to many commonly used alternatives. Hindsgaul and co-workers have used neoglycoconjugates with NBD dyes to streamline the detection of carbohydrate−lectin interactions.[47] The alkynyl NBD-dye **34** was prepared in one step from commercially available 4-chloro-7-nitrobenzofurazan by nucleophilic aromatic substitution with propargylamine. This alkyne was coupled with the azidooctyl galactosides **1**−**3** upon exposure to $CuSO_4$, sodium ascorbate, and the Cu(I)-stabilizing ligand tris(benzyltriazolylmethyl)amine[48,49] to afford the corresponding conjugates **35**−**37** (Scheme 6). The conjugates displayed excellent fluorescent properties with λ_{ex} = 470 nm, λ_{em} = 540 nm (see the Supporting Information).

Tietze and co-workers introduced the use of squaric acids as linkers for the conjugation of amine-derivatized carbohydrate haptens to proteins.[50] Conjugation is realized through the stepwise reaction of the carbohydrate amine with diethyl squarate followed by the reaction of the intermediate ethyl squaramate adduct with the amino residues of a carrier protein. While a range of other linker methodologies are available, the squaric acid diester protocol uses commercially available dialkyl squarates and exhibits good selectivity for each coupling step, and the chemical stability of the intermediate squaramate ester allows its purification and storage. One significant limitation for the use of this methodology is the potent immunogenicity of the squaramide linker,[51] which means that squaramide-linked antigens should not be used for raising antibodies, but rather for their characterization.

Reduction of the azido groups of **1**−**3** was achieved by exposure to triphenylphosphine in 10:1 THF/water (Scheme 6). The crude products were extracted into water and coupled with diethyl squarate in pH 8.0 phosphate buffer, according to the excellent procedure of Kovác and co-workers.[52] The products were purified by reversed-phase chromatography to afford the ethyl squaramate adducts **38**−**40** in 92−98% yield.

Conclusions

The β-galactopyranose-1,3-β-galactopyranose linkage presents a special challenge for chemical synthesis owing to the reactivity mismatch commonly found between requisite

(40) Pellerin, P.; Brillouet, J.-M. *Carbohydr. Res.* **1994**, *264*, 281–291.
(41) Ichinose, H.; Yoshida, M.; Kotake, T.; Kuno, A.; Igarashi, K.; Tsumuraya, Y.; Samejima, M.; Hirabayashi, J.; Kobayashi, H.; Kaneko, S. *J. Biol. Chem.* **2005**, *280*, 25820–25829.
(42) Ichinose, H.; Kotake, T.; Tsumuraya, Y.; Kaneko, S. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 2745–2750.
(43) Ichinose, H.; Kuno, A.; Kotake, T.; Yoshida, M.; Sakka, K.; Hirabayashi, J.; Tsumuraya, Y.; Kaneko, S. *Appl. Environ. Microbiol.* **2006**, *72*, 3515–3523.
(44) Tsumuraya, Y.; Mochizuki, N.; Hashimoto, Y.; Kovác, P. *J. Biol. Chem.* **1990**, *265*, 7207–7215.
(45) Qu, Y.; Egelund, J.; Gilson, P. R.; Houghton, F.; Gleeson, P. A.; Schultz, C. J.; Bacic, A. *Plant Mol. Biol.* **2008**, *68*, 43–59.
(46) Kato, H.; Takeuchi, Y.; Tsumuraya, Y.; Hashimoto, Y.; Nakano, H.; Kovác, P. *Planta* **2003**, *217*, 271–282.
(47) Dowlut, M.; Hall, D. G.; Hindsgaul, O. *J. Org. Chem.* **2005**, *70*, 9809–9813.
(48) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853–2855.
(49) Donnelly, P. S.; Zanatta, S. D.; Zammit, S. C.; White, J. M.; Williams, S. J. *Chem. Commun.* **2008**, 2459–2461.
(50) Tietze, L. F.; Schröter, C.; Gabius, S.; Brinck, U.; Goerlach-Graw, A.; Gabius, H.-J. *Bioconjugate Chem.* **1991**, *2*, 148–153.
(51) Wu, X.; Ling, C. C.; Bundle, D. R. *Org. Lett.* **2004**, *6*, 4407–4410.
(52) Hou, S.-J.; Saksena, R.; Kovác, P. *Carbohydr. Res.* **2008**, *343*, 196–210.

donor and acceptor molecules, resulting in side reactions such as transesterification and orthoester formation. In the present work, we demonstrate the utility of 2-*O*-mesitoate esters as neighboring groups for synthesis of this challenging linkage. The 2-*O*-mesitoyl group suppressed transesterification seen for benzoyl- and pivalyl-protected glycosyl donors and afforded excellent β-stereoselectivity in most cases. Interestingly, the 2-*O*-mesitoyl group did not prevent orthoester formation, indicating that steric hindrance of the central carbon of the intermediate dioxolenium ion does not fully suppress reactivity at this site. The mesitoyl group requires relatively robust conditions for its removal (LiOH in MeOH/THF/H_2O at 80 °C), which may prove a limitation for its use in sensitive systems. In this regard, the investigation of 2,6-dimethyl-4-X-benzoyl groups, where "X" is an electron-withdrawing group, may prove fruitful in allowing the development of milder deprotection conditions.[25] Using the 2-*O*-mesitoyl group we have reported a direct synthesis of a series of β-1,3-linked oligogalactosides useful for the study of arabinogalactan proteins and demonstrated their elaboration into fluorescently labeled neoglycoconjugates and activated forms suitable for synthesis of artificial antigens. On the basis of these results, the 2-*O*-mesitoyl group is a convenient participating group for the synthesis of 1,2-*trans*-glycosidic linkages in systems sensitive to transesterification and/or poor diastereoselectivity and which are sufficiently robust to withstand the basic conditions required for their removal.

Experimental Section

General experimental details have been provided previously.[53]

8-Azidooctyl 3,4-*O*-isopropylidene-2,6-di-*O*-mesitoyl-β-D-galactopyranoside (12). TfOH (13 μL, 0.16 mmol) was added to a mixture of donor **10** (0.49 g, 0.62 mmol), 8-azidooctan-1-ol (0.13 g, 0.75 mmol), NIS (0.19 g, 0.87 mmol), and 4 Å mol sieves in CH_2Cl_2 at 0 °C and stirred for 5 min. The reaction was quenched with 0.50 M sodium thiosulfate (10 mL), and the organic phase was washed with satd aq $NaHCO_3$ (2 × 25 mL) and brine (2 × 25 mL) and dried ($MgSO_4$). The solvent was evaporated to give an oil, which was subjected to flash chromatography (5% EtOAc/toluene) to give **12** (0.31 g, 75%) as a colorless oil: $[\alpha]^{23}{}_D$ +16 (*c* 0.8 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.26−1.62 (12 H, m), 1.37−1.65 (6 H, 2 × s), 2.29−2.33 (18 H, 4 × s), 3.25 (2 H, t, *J* 7.5 Hz), 3.41 (1 H, ddd, *J* 10.0, *J* 7.0, *J* 7.0 Hz), 3.87 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 4.14 (1 H, ddd, *J* 2.0, 5.0, 7.0 Hz, H5), 4.23−4.27 (2 H, m), 4.40 (1 H, d, *J* 8.0 Hz, H1), 4.60 (1 H, dd, *J* 7.0, 11.5 Hz), 4.65 (1 H, dd, *J* 5.0, 11.5 Hz), 5.29 (1 H, dd, *J* 8.0, 7.0 Hz), 6.86−6.87 (4 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.9−29.8 (14 C), 51.7 (1 C), 64.3 (1 C), 69.8, 71.2, 73.3, 74.1, 77.1 (5 C), 100.5 (1 C), 111.1 (1 C), 128.5−139.8 (12 C), 168.8, 170.0 (2 C); HRMS (ESI$^+$) *m*/*z* 688.3569 ($C_{37}H_{51}N_3NaO_8$ $[M + Na]^+$ requires 688.3568).

8-Azidooctyl 2,6-Di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (17). A solution of **12** (60 mg, 0.090 mmol) and methanolic LiOH (1 mL, 1 M) in 90% aqueous THF (2 mL) was stirred at 60 °C for 5 d. Amberlite IR-120 resin (H^+ form) was added to neutralize the solution. Filtration and evaporation of the solvent from the filtrate gave a residue that was dissolved in CH_2Cl_2 (10 mL) and chilled to 0 °C. Acetic anhydride (34 μL, 0.36 mmol) and pyridine (72 μL, 0.90 mmol) were added to the solution, which was stirred for 90 min. The reaction was quenched with aqueous HCl (10 mL, 1 M) and the resultant mixture extracted with CH_2Cl_2 (2 × 10 mL). The organic extract was washed with 1 M HCl (3 × 10 mL), satd $NaHCO_3$ (2 × 10 mL), and brine (10 mL) then dried ($MgSO_4$). The solvent was removed by evaporation to afford an oil, which was purified by flash chromatography (EtOAc/petroleum spirits 40%) to yield **17** (40 mg, 51%) as a colorless oil: $[\alpha]^{22}{}_D$ +19 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30−1.60 (12 H, m), 1.33−1.56 (6 H, 2 × s), 2.08−2.09 (6 H, 2 × s), 3.52 (2 H, t, *J* 7.0 Hz), 3.63 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 3.85 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 3.97 (1 H, ddd, *J* 2.0, 6.0, 6.0 Hz), 4.15 (1 H, dd, *J* 7.0, 6.0 Hz), 4.16 (1 H, dd, *J* 6.0, 2.0 Hz), 4.31 (1 H, d, *J* 8.0 Hz), 4.33 (2 H, m), 4.95 (1 H, dd, *J* 8.0, 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.8, 20.9 (2 C), 25.8−29.4 (8 C), 51.4 (1 C), 63.4 (1 C), 69.6, 70.8, 72.9, 73.6, 77.2 (5 C), 100.4 (1 C), 110.7 (1 C) 169.5, 170.8 (2 C); HRMS (ESI$^+$) *m*/*z* 480.2320 ($C_{21}H_{35}N_3NaO_8$ $[M + Na]^+$ requires 480.2316).

8-Azidooctyl 2,4,6-Tri-*O*-acetyl-β-D-galactopyranoside (18). Compound **17** (180 mg, 0.393 mmol) was dissolved in chloroform (10 mL) containing 90% aqueous TFA (2 mL) and was stirred at rt for 1 h. Evaporation of the solvent gave a residue which was dissolved in toluene (10 mL) containing *p*-TsOH (17 mg, 0.090 mmol). Triethyl orthoacetate (270 μL, 1.49 mmol) was added to the solution, which was stirred at rt for 2 h. The reaction was neutralized (Et_3N) and evaporated to give a solid residue, which was dissolved in 80% aqueous acetic acid (10 mL) and stirred at 40 °C for 15 min. Evaporation of the solvent gave a white residue, which was recrystallized from EtOAc/petroleum spirits to afford **18** (120 mg, 72% three steps) as fine white needles: mp 89 °C; $[\alpha]^{20}{}_D$ −10 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32−1.62 (12 H, m), 2.07−2.19 (9 H, 3 × s), 3.27 (2 H, t, *J* 7.0 Hz), 3.48 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 3.84 (1 H, ddd, *J* 1.0, 7.0, 7.0 Hz), 3.85 (1 H, dd, *J* 10.0, 3.5 Hz), 3.89 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 4.15 (1 H, dd, *J* 7.0, 11.5 Hz), 4.18 (1 H, dd, *J* 7.0, 11.5 Hz), 4.42 (1 H, d, *J* 8.0 Hz), 4.96 (1 H, dd, *J* 8.0, 10.0 Hz), 5.33 (1 H, dd, *J* 3.5, 1.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.7−20.9 (3 C), 25.7−29.4 (6 C), 51.4 (1 C), 61.8 (1 C), 69.7, 70.1, 70.8, 71.5, 72.8 (5 C), 101.0 (1 C), 170.5−171.1 (3 C); HRMS (ESI$^+$) *m*/*z* 482.2114 ($C_{20}H_{33}N_3NaO_9$ $[M + Na]^+$ requires 482.2120). Anal. Calcd for $C_{20}H_{33}N_3O_9$: C, 52.28; H, 7.24. Found: C, 52.31; H, 7.21.

8-Azidooctyl β-D-Galactopyranoside (1). $ZnCl_2$ (83.9 mg, 6.14 mmol) was added to a mixture of β-D-galactose pentaacetate (2.00 g, 5.12 mmol) and 8-azidooctan-1-ol (1.05 g, 6.14 mmol) in anhydrous toluene (70 mL) at rt. The suspension was warmed to 60 °C and stirred for 3 days. The reaction was quenched with satd aq $NaHCO_3$ (5 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 20 mL) and brine (10 mL) and dried ($MgSO_4$). The solvent was evaporated to afford a residue, which was dissolved in MeOH (20 mL) containing catalytic NaOMe. The solution was stirred for 15 min, neutralized with Amberlite IR-120 resin (H^+ form), and concentrated to give a pale yellow oil, which was purified by flash chromatography (50% EtOAc/petroleum spirits) to yield the tetraol **1** (1.01 g, 59%) over two steps: $[\alpha]^{22}{}_D$ −12 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, MeOH-d_4) δ 1.36−1.65 (12 H, m), 3.92 (2 H, t, *J* 6.5 Hz), 3.46 (1 H, dd, *J* 9.5, 3.5 Hz), 3.50 (1 H, ddd, *J* 1.0, 6.5, 6.5 Hz), 3.51 (1 H, dd, *J* 7.5, 9.5 Hz), 3.55 (1 H, ddd, *J* 9.5, 7.0, 7.0 Hz), 3.72 (1 H, dd, *J* 6.5, 11.0 Hz), 3.75 (1 H, dd, *J* 6.5, 11.0 Hz), 3.84 (1 H, dd, *J* 3.5, 1.0 Hz), 3.90 (1 H, ddd, *J* 9.5, 6.5, 6.5 Hz), 4.21 (1 H, d, *J* 7.5 Hz); ^{13}C NMR (125 MHz, MeOH-d_4) δ 27.0−30.8 (6 C), 52.5 (1 C), 62.5 (1 C), 70.4, 70.8, 72.6, 75.1, 76.6 (5 C), 105.0 (1 C); HRMS (ESI$^+$) *m*/*z* 356.1795 ($C_{14}H_{27}N_3NaO_6$ $[M + Na]^+$ requires 356.1792). Anal. Calcd for $C_{14}H_{27}N_3O_6$: C, 50.44; H, 8.16; N, 12.60. Found: C, 50.50; H, 8.25; N, 12.52.

(53) Dinev, Z.; Gannon, C. T.; Egan, C.; Watt, J. A.; McConville, M. J.; Williams, S. J. *Org. Biomol. Chem.* **2007**, *5*, 952–959.

8-Azidooctyl 2,3,4,6-Tetra-*O*-mesitoyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (20a) and 8-Azidooctyl 2,3,4,6-tetra-*O*-mesitoyl-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (20b). TfOH (121 μL, 1.37 mmol) was added to a mixture of donor **19** (2.05 g, 2.35 mmol), acceptor **18** (0.901 g, 1.96 mmol), NIS (0.658 g, 2.94 mmol), and freshly activated 5 Å AW molecular sieves in CH_2Cl_2 (15 mL) at 0 °C. The mixture was allowed to warm to rt and was stirred for 4 h. The reaction was quenched by the addition of 0.50 M sodium thiosulfate (10 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 25 mL) and brine (2 × 25 mL) and dried ($MgSO_4$). The solvent was evaporated to give an oil, which was subjected to flash chromatography (20% EtOAc/petroleum spirits). First to elute was the α-linked galactoside **20a** (105 mg, 4%) as a colorless oil: $[\alpha]^{20}_D$ +84 (*c* 1.0 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.27−1.60 (12 H), 2.03−2.37 (45 H), 3.28 (2 H, t, *J* 7.0 Hz), 3.33−3.36 (2 H, m), 3.80 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 3.91 (1 H, dd, *J* 10.0, 3.0 Hz), 3.92 (1 H, d, *J* 8.0 Hz), 3.98 (1 H, dd, *J* 6.0, 12.0 Hz), 4.07 (1 H, dd, *J* 6.0, 12.0 Hz), 4.44−4.51 (1 H, m), 4.58 (1 H, dd, *J* 7.0, 11.0 Hz), 5.23 (1 H, dd, *J* 8.0, 10.0 Hz), 5.31 (1 H, dd, *J* 3.5, 10.0 Hz), 5.43 (1 H, d, *J* 3.0 Hz), 5.67 (1 H, dd, *J* 10.0, 3.5 Hz), 5.77 (1 H, d, *J* 3.5 Hz), 5.98 (1 H, d, *J* 3.5 Hz), 6.69−6.89 (8 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.0−29.7 (21 C), 51.5 (1 C), 61.5 (1 C), 63.4−71.0 (10 C), 91.2, 101.6 (2 C), 127.6−140.5 (24 C), 167.7−170.3 (7 C); HRMS (ESI$^+$) *m*/*z* 1228.5564 ($C_{66}H_{83}N_3NaO_{18}$ [M + Na]$^+$ requires 1228.5564). Next to elute was the β-linked galactoside **20b** (1.01 g, 43%) as a colorless oil: $[\alpha]^{19}_D$ +32 (*c* 0.50 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.27−1.60 (12 H), 2.05−2.35 (45 H), 3.25 (2 H, t, *J* 7.0 Hz), 3.37 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 3.74 (1 H, dd, *J* 6.5, 6.5 Hz), 3.83 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 3.99 (1 H, dd, *J* 10.0, 3.5 Hz), 4.04 (1 H, dd, *J* 6.5, 12.0 Hz), 4.12 (1 H, dd, *J* 6.5, 12.0 Hz), 4.22 (1 H, dd, *J* 6.0, 6.0 Hz), 4.27 (1 H, d, *J* 8.0 Hz), 4.47 (1 H, dd, *J* 6.0, 12.0 Hz), 4.56 (1 H, dd, *J* 6.0, 12.0 Hz), 5.00 (1 H, d, *J* 8.0 Hz), 5.17 (1 H, dd, *J* 8.0, 10.0 Hz), 5.42 (1 H, d, *J* 3.5 Hz), 5.44 (1 H, dd, *J* 8.0, 10.0 Hz), 5.63 (1 H, dd, *J* 10.0, 3.0 Hz), 5.96 (1 H, d, *J* 3.0 Hz), 6.74−6.89 (8 H, m), 6.77−6.89 (4 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.0−29.3 (21 C), 51.4 (1 C), 62.1−75.5 (11 C), 101.4, 101.6 (2 C), 128.0−140.3 (24 C), 167.1−170.4 (7 C); HRMS (ESI$^+$) *m*/*z* 1228.5564 ($C_{66}H_{83}N_3NaO_{18}$ [M + Na]$^+$ requires 1228.5595).

4-Methoxyphenyl 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranoside (21). $BF_3 \cdot Et_2O$ (1.95 mL, 15.4 mmol) was added to a stirred solution of *p*-methoxyphenol (1.91 g, 15.4 mmol) and D-galactose pentaacetate (5.00 g, 12.8 mmol) in CH_2Cl_2 (100 mL) at 0 °C. The reaction was warmed to rt and stirred for 2 h. The mixture was quenched with HCl (1 M, 50 mL), and the organic phase was washed with satd aq $NaHCO_3$ (2 × 50 mL) and brine (20 mL). The organic phase was dried ($MgSO_4$) and evaporated to give a pale yellow residue. The residue was recrystallized from MeOH to afford tetraacetate **21** (4.87 g, 84%) as colorless crystals: mp 106−108 °C (lit.[54] mp 104 °C); $[\alpha]^{23}_D$ +4 (*c* 1.0 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 2.00−2.17 (12 H, 4 × s), 3.76 (3 H, s), 4.00 (1 H, m), 4.15 (1 H, dd, *J* 6.4, 11.2 Hz), 4.22 (1 H, dd, *J* 7.2, 11.2 Hz), 4.91 (1 H, d, *J* 8.0 Hz), 5.08 (1 H, dd, *J* 10.4, 3.6 Hz), 5.42−5.46 (2 H, m), 6.79−6.95 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5−20.7 (4 C), 55.6 (1 C), 61.2, 66.9, 68.7, 70.8, 70.9 (5 C), 100.8 (1 C), 114.5−155.7 (6 C), 169.3−170.3 (4 C); HRMS (ESI$^+$) *m*/*z* 477.1373 ($C_{21}H_{26}NaO_{11}$ [M + Na]$^+$ requires 477.1367).

4-Methoxyphenyl β-D-Galactopyranoside (22). A small portion of sodium metal was added to a solution of tetraacetate **21** (3.00 g, 6.60 mmol) in MeOH (100 mL), and the mixture was stirred at rt for 1 h. Amberlite IR-120 resin (H^+ form) was added to neutralize the solution. Filtration and evaporation of the solvent from the filtrate gave a pale yellow residue, which was recrystallized from *n*-propanol/petroleum spirits to afford tetraol **22** (1.58 g, 84%) as a white powder: mp 162 °C (lit.[55] mp 160−161 °C); $[\alpha]^{23}_D$ −39 (*c* 1 in $CHCl_3$) [lit.[55] $[\alpha]^{23}_D$ −41.8 (*c* 0.28 in $CHCl_3$)]; ^1H NMR (500 MHz, CD_3OD) δ 3.59 (1 H, dd, *J* 9.5, 3.0 Hz), 3.66 (1 H, dd, *J* 6.0, 6.0 Hz), 3.76 (3 H, s), 3.77−3.83 (3 H, m), 3.92 (1 H, d, *J* 3.0 Hz), 4.75 (1 H, d, *J* 8.0 Hz), 6.84−7.09 (4 H, m); ^{13}C NMR (125 MHz, CD_3OD) δ 56.2 (1 C), 62.6, 70.3, 72.5, 75.0, 77.0 (5 C), 104.2 (1 C), 115.6−156.7 (6 C); HRMS (ESI$^+$) *m*/*z* 309.0948 ($C_{13}H_{18}NaO_7$ [M + Na]$^+$ requires 309.0945).

4-Methoxyphenyl 3,4-*O*-Isopropylidene-β-D-galactopyranoside (23). A solution of tetraol **22** (2.0 g, 7.0 mmol) and *p*-TsOH (0.13 g, 0.70 mmol) in 2,2-DMP (22 mL) was stirred at rt for 3 h. The mixture was quenched with Et_3N, and the solvent was evaporated to give an off-white foam. The foam was dissolved in MeOH (25 mL) containing PPTS (0.18 g, 0.70 mmol) and was stirred at rt for 10 min. The reaction was quenched (Et_3N) and the solvent evaporated to give white crystals, which were recrystallized from EtOAc/petroleum spirits to yield **23** (1.69 g, 74%) as white needles: mp 120−121 °C; $[\alpha]^{27}_D$ −24 (*c* 1.2 in $CHCl_3$); ^1H NMR (500 MHz, CD_3OD) δ 1.35,1.52 (6 H, 2 × s), 3.65 (1 H, dd, *J* 8.0, 8.0 Hz), 3.75 (3 H, s), 3.78 (1 H, dd, *J* 4.5, 11.5 Hz), 3.81 (1 H, dd, *J* 5.5, 11.5 Hz), 3.98 (1 H, ddd, *J* 2.0, 4.5, 5.5 Hz), 4.11 (1 H, dd, *J* 8.0, 6.0 Hz), 4.25 (1 H, dd, *J* 6.0, 2.0 Hz), 4.72 (1 H, d, *J* 8.0 Hz), 6.83−7.05 (4 H, m); ^{13}C NMR (125 MHz, CD_3OD) δ 26.8, 28.6 (2 C), 55.2 (1 C), 62.6, 74.4, 75.2, 75.4, 81.0 (5 C), 103.2 (1 C), 111.3 (1 C), 115.7−156.9 (6 C); HRMS (ESI$^+$) *m*/*z* 349.1257 ($C_{16}H_{22}NaO_7$ [M + Na]$^+$ requires 349.1258). Anal. Calcd for $C_{16}H_{22}O_7$: C, 58.89; H, 6.79. Found: C, 58.85; H, 6.69.

4-Methoxyphenyl 2,6-Di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (24). Acetic anhydride (0.87 mL, 9.2 mmol) was added to a solution of **23** (1.00 g, 3.10 mmol) and DMAP (112 mg, 0.917 mmol) in pyridine (5 mL) at 0 °C. The mixture was stirred under N_2 for 1 h. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with aq HCl (1 M, 25 mL), satd aq $NaHCO_3$ (2 × 50 mL), and brine (50 mL). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure to afford an off-white amorphous solid, which was recrystallized from EtOH/H_2O to yield **24** (1.61 g, 84%) as colorless needles: mp 87−88 °C; $[\alpha]^{23}_D$ +27 (*c* 1.3 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.36, 1.60 (6 H, 2 × s), 2.09−2.13 (6 H, 2 × s), 3.77 (3 H, s), 4.09 (1 H, ddd, *J* 2.0, 5.0, 5.5 Hz), 4.22 (1 H, dd, *J* 6.0, 2.0 Hz), 4.26 (1 H, dd, *J* 7.0, 6.0 Hz), 4.37−4.44 (2 H, m), 4.78 (1 H, d, *J* 8.0 Hz), 5.21 (1 H, dd, *J* 8.0, 7.0), 6.78−6.97 (4 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.8−20.9 (2 C), 26.3, 27.5 (2 C), 55.6 (1 C), 63.3, 71.0, 72.5, 73.3, 76.9 (5 C), 99.9 (1 C), 111.0 (1 C), 114.4−155.5 (6 C), 169.5, 170.7 (2 C); HRMS (ESI$^+$) *m*/*z* 433.1468 ($C_{20}H_{26}NaO_9$ [M + Na]$^+$ requires 433.1469). Anal. Calcd for $C_{20}H_{26}O_9$: C, 58.53; H, 6.39. Found: C, 58.54; H, 6.32.

4-Methoxyphenyl 2,6-Di-*O*-acetyl-β-D-galactopyranoside (25). Compound **24** (2.10 g, 5.08 mmol) was dissolved in chloroform (40 mL) containing 90% aqueous TFA (3 mL) and was stirred ar rt for 1 h. Evaporation of the solvent gave a white residue, which was recrystallized from EtOAc/petroleum spirits to yield diol **25** (1.71 g, 90%) as white needles: mp 112−113 °C; $[\alpha]^{27}_D$ +8 (*c* 1.1 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 2.08−2.16 (6 H, 2 × s), 3.73 (1 H, dd, *J* 9.6, 3.2 Hz), 3.75 (1 H, m), 3.77 (3 H, s), 3.97 (1 H, d, *J* 3.2 Hz), 4.34 (1 H, dd, *J* 6.8, 11.6 Hz), 4.39 (1 H, dd, *J* 6.0, 11.6 Hz), 4.81 (1 H, d, *J* 8.0 Hz), 5.19 (1 H, dd, *J* 8.0, 9.6 Hz), 6.78−6.98 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 21.0 (2 C), 55.6 (1 C), 62.6, 68.6, 72.3, 72.5, 73.0 (5 C), 100.4 (1 C), 114.5−155.6 (6 C), 171.1, 171.5 (2 C); HRMS (ESI$^+$) *m*/*z* 393.1152 ($C_{17}H_{22}NaO_9$ [M + Na]$^+$ requires 393.1156). Anal. Calcd for $C_{17}H_{22}O_9$: C, 55.13; H, 5.99. Found: C, 55.09; H, 5.94.

(54) Robertson, A. *J. Chem. Soc.* **1929**, 1820–1823.

(55) Murakata, C.; Ogawa, T. *Carbohydr. Res.* **1992**, *235*, 95–114.

4-Methoxyphenyl 2,4,6-Tri-*O*-acetyl-*β*-D-galactopyranoside (26). Diol **25** (2.00 g, 5.40 mmol) was dissolved in toluene (20 mL) containing *p*-TsOH (300 mg, 1.62 μmol). Triethyl orthoacetate (4.9 mL, 27 mmol) was added, and the mixture was stirred at rt for 1 h. The reaction was quenched (Et_3N), and the solvent evaporated to give a yellow oil, which was dissolved in 80% aqueous acetic acid (20 mL) and stirred at 40 °C for 30 min. Evaporation of the solvent gave a residue, which was recrystallized from EtOAc/petroleum spirits to yield **26** (1.89 g, 85%) as fine colorless needles: mp 134−135 °C; $[\alpha]^{22}_D$ +2 (*c* 0.9 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.06−2.20 (9 H, 3 × s), 2.70 (1 H, d, *J* 6.5 Hz), 3.77 (3 H, s), 3.91 (1 H, ddd, *J* 6.5, 10.0, 3.5 Hz), 3.94 (1 H, ddd, *J* 1.0, 6.5, 6.5 Hz), 4.18−4.19 (2 H, m, H6,6), 4.87 (1 H, d, *J* 8.0 Hz), 5.22 (1 H, dd, *J* 8.0, 10.0 Hz), 5.37 (1 H, dd, *J* 1.0, 3.5 Hz), 6.80−6.98 (4 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.7−20.9 (3 C), 55.6 (1 C), 61.9, 69.5, 71.2, 71.4, 72.6 (5 C), 100.5 (1 C), 114.5−155.7 (6 C), 170.4−171.1 (3 C); HRMS (ESI$^+$) *m*/*z* 435.1260 ($C_{19}H_{24}NaO_{10}$ [M + Na]$^+$ requires 435.1262). Anal. Calcd for $C_{19}H_{24}O_{10}$: C, 55.34; H, 5.87. Found: C, 55.64; H, 5.88.

4-Methoxyphenyl 2,3,4,6-Tetra-*O*-mesitoyl-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-*β*-D-galactopyranoside (27). TfOH was added to a mixture of donor **19** (1.20 g, 1.40 mmol), acceptor **26** (0.470 g, 1.10 mmol), NIS (0.370 g, 1.65 mmol), and freshly activated 5 Å AW molecular sieves in CH_2Cl_2 (15 mL) at 0 °C. The mixture was allowed to warm to rt and was stirred for 4 h. The reaction was quenched by the addition of 0.50 M sodium thiosulfate (10 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 25 mL) and brine (2 × 25 mL) and dried ($MgSO_4$). The solvent was evaporated to give a foam which was recrystallized from EtOH to afford disaccharide **27** (1.30 g, 80%) as white crystals: mp 126−127 °C; $[\alpha]^{23}_D$ +47 (*c* 1.0 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.05−2.34 (45 H), 3.76 (3 H), 3.83 (1 H, dd, *J* 6.0, 7.0 Hz), 4.04 (1 H, dd, *J* 10.0, 3.5 Hz), 4.07 (1 H, dd, *J* 6.0, 11.5 Hz), 4.14 (1 H, dd, *J* 7.0, 11.5 Hz), 4.23 (1 H, dd, *J* 6.0, 6.0 Hz), 4.60 (1 H, dd, *J* 6.0, 11.5 Hz), 4.64 (1 H, dd, *J* 6.0, 11.5 Hz), 4.70 (1 H, d, *J* 8.0 Hz), 5.00 (1 H, d, *J* 8.0 Hz), 5.42 (1 H, dd, *J* 8.0, 10.0 Hz), 5.46 (1 H, d, *J* 3.5 Hz), 5.48 (1 H, dd, *J* 8.0, 10.0 Hz), 5.63 (1 H, dd, *J* 10.0, 3.0 Hz), 5.95 (1 H, d, *J* 3.0 Hz), 6.74−6.80 (8 H, m), 6.77−6.89 (4 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.0−21.3 (15 C), 55.6 (1 C), 62.2−75.7 (10 C), 101.0, 101.6 (2 C), 114.4−140.4 (30 C), 167.1−170.4 (7 C); HRMS (ESI$^+$) *m*/*z* 1181.4729 ($C_{65}H_{74}NaO_{19}$ [M + Na]$^+$ requires 1181.4717). Anal. Calcd for $C_{65}H_{74}O_{19}$: C, 67.34; H, 6.34. Found: C, 67.21; H, 6.35.

4-Methoxyphenyl 2,3,4,6-Tetra-*O*-mesitoyl-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-mesitoyl-*β*-D-galactopyranoside (30). NaOMe in MeOH (0.50 M, 10 mL) was added to a solution of **27** (1.0 g, 0.87 mmol) in MeOH/THF (2:1, 18 mL), and the mixture was stirred overnight at 50 °C. The solution was neutralized with Amberlite IR-120 resin (H^+ form), and the solvent was removed by evaporation to afford an oil. Mesitoyl chloride (3.20 g, 17.5 mmol), DMAP (107 mg, 0.870 mmol), and pyridine (10 mL) were added to the oil, and the mixture was warmed to 100 °C and stirred for 2 d. The mixture was diluted with H_2O (25 mL) and extracted into CH_2Cl_2 (50 mL). The organic phase was washed with aq HCl (1 M, 4 × 50 mL), satd aq $NaHCO_3$ (2 × 50 mL), and brine (25 mL) and dried ($MgSO_4$). Evaporation of the solvent gave a residue, which was purified by flash chromatography (10−15% EtOAc/petroleum spirits) to afford the protected disaccharide **30** (1.10 g, 86% two steps) as an off-white foam. An analytical sample was crystallized from EtOH affording white cubic crystals: mp 176−177 °C; $[\alpha]^{20}_D$ +32 (*c* 1.0 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.04−2.35 (63 H), 3.69 (3 H, s), 4.00 (1 H, dd, *J* 3.5, 9.0 Hz), 4.21 (1 H, dd, *J* 6.0, 6.0 Hz), 4.26 (1 H, dd, *J* 10.0, 3.5 Hz), 4.44−4.53 (1 H, dd, *J* 3.5, 12.0 Hz), 4.44−4.53 (1 H, dd, *J* 9.0, 12.0 Hz), 4.48−4.58 (1 H, dd, *J* 6.0, 12.0 Hz), 4.48−4.58 (1 H, dd, *J* 6.0, 12.0 Hz), 4.95 (1 H, d, *J* 8.0 Hz), 4.97 (1 H, d, *J* 8.0 Hz), 5.46 (1 H, dd, *J* 10.5, 3.5 Hz), 5.60−5.69 (2 H, m), 5.92 (1 H, d, *J* 3.5 Hz), 5.94 (1 H, d, *J* 3.5 Hz), 6.56−6.79 (4 H, m), 6.51−6.84 (14 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.8−21.2 (21 C), 55.5 (1 C), 62.8−77.4 (10 C), 99.9−102.4 (2 C), 114.3−140.3 (48 C), 166.8−169.4 (7 C); HRMS (ESI$^+$) *m*/*z* 1493.6604 ($C_{89}H_{98}NaO_{19}$ [M + Na]$^+$ requires 1493.6595).

4-Methylphenyl 2,3,4,6-Tetra-*O*-mesitoyl-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-mesitoyl-1-thio-*β*-D-galactopyranoside (31). $BF_3 \cdot Et_2O$ (8.40 μL, 0.068 mmol) was added dropwise to a solution of **30** (100 mg, 0.068 mmol) and thiocresol (17 mg, 0.14 mmol) in dry $(CH_2Cl)_2$ (2 mL) at rt. The reaction was heated to 60 °C and stirred overnight. The mixture was cooled and the reaction quenched by addition of satd aq $NaHCO_3$ (5 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 5 mL) and brine (1 × 5 mL) and dried ($MgSO_4$). Evaporation of the solvent gave an oil, which was purified by flash chromatography (15% EtOAc/pet. spirits) to yield **31** (71 mg, 71%) as a colorless foam: $[\alpha]^{20}_D$ +36 (*c* 0.85 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.07−2.34 (66 H), 3.92 (1 H, dd, *J* 3.0, 9.0 Hz), 4.15 (1 H, dd, *J* 10.0, 3.5 Hz), 4.18 (1 H, dd, *J* 6.0, 6.0 Hz), 4.39−4.55 (4 H, m), 4.62 (1 H, d, *J* 10.0 Hz), 4.92 (1 H, d, *J* 8.0 Hz), 5.42 (1 H, dd, *J* 10.0, 10.0 Hz), 5.44 (1 H, dd, *J* 10.5, 3.0 Hz), 5.64 (1 H, dd, *J* 8.0, 10.5 Hz), 5.91 (2 H, m), 6.50−7.27 (18 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.1−21.6 (22 C), 63.0−79.3 (10 C), 87.1 (1 C), 102.6 (1 C), 127.7−140.5 (48 C), 166.8−169.7 (7 C); HRMS (ESI$^+$) *m*/*z* 1493.6429 ($C_{89}H_{98}NaO_{17}S_1$) [M + Na]$^+$ requires 1493.6417).

***n*-Butyl 2,3,4,6-Tetra-*O*-mesitoyl-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-mesitoyl-1-thio-α-D-galactopyranoside (32a) and *n*-Butyl 2,3,4,6-Tetra-*O*-mesitoyl-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-mesitoyl-1-thio-*β*-D-galactopyranoside (32b).** $BF_3 \cdot Et_2O$ (84.0 μL, 0.679 mmol) was added dropwise to a solution of **30** (1.00 g, 0.679 mmol) and *n*-butylmercaptan (180 mg, 2.00 mmol) in dry $(CH_2Cl)_2$ (20 mL) at rt. The reaction was heated to 60 °C and stirred overnight. The mixture was cooled and the reaction quenched by addition of satd aq $NaHCO_3$ (20 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 10 mL) and brine (1 × 10 mL) and dried ($MgSO_4$). Evaporation of the solvent gave an oil, which was purified by flash chromatography (50% EtOAc/peroleum spirits), a 1:1 α/β mixture of **32a** and **32b** (781 mg, 80%) as a colorless foam. The anomers may be separated by flash chromatography (15% EtOAc/petroleum spirits). The α-linked disaccharide **32a** eluted as a colorless foam: $[\alpha]^{19}_D$ +93 (*c* 1.0 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.13−1.46 (7 H, $(CH_2)_2CH_3$), 2.01−2.37 (63 H), 2.37−2.49 (2 H, m), 4.23 (1 H, dd, *J* 6.5, 6.5 Hz), 4.37 (1 H, dd, *J* 10.0, 3.0 Hz), 4.45 (4 H, m), 4.73 (1 H, dd, *J* 4.0, 8.0 Hz), 5.03 (1 H, d, *J* 8.0 Hz), 5.43 (1 H, dd, *J* 5.5, 10.0 Hz), 5.46 (1 H, dd, *J* 11.0, 3.0 Hz), 5.55 (1 H, dd, *J* 8.0, 11.0 Hz), 5.92−5.95 (2 H, m), 5.92 (1 H, d, *J* 5.5 Hz), 6.50−6.85 (14 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.6-31.5 (3 C), 20.1−22.1 (22 C), 63.0−74.8 (10 C), 82.3 (1 C), 102.5 (1 C), 127.9−140.3 (42 C), 166.7−169.7 (7 C); HRMS (ESI$^+$) *m*/*z* 1459.6575 ($C_{86}H_{100}$-$NaO_{17}S_1$ [M + Na]$^+$ requires 1459.6573). The *β*-linked disaccharide **32b** eluted as a colorless foam: $[\alpha]^{19}_D$ +36 (*c* 1.0 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.83−1.20 (7 H), 2.08−2.39 (63 H), 2.68 (2 H, m), 3.99 (1 H, dd, *J* 5.0, 8.0 Hz), 4.16 (1 H, dd, *J* 10.0, 3.5 Hz), 4.23 (1 H, dd, *J* 6.5, 6.5 Hz), 4.42 (1 H, d, *J* 10.0 Hz), 4.49−4.60 (4 H, m), 4.97 (1 H, d, *J* 8.0 Hz), 5.49 (1 H, dd, *J* 11.0, 3.5 Hz), 5.53 (1 H, dd, *J* 10.0, 10.0 Hz), 5.70 (1 H, dd, *J* 8.0, 11.0 Hz), 5.96−5.98 (2 H, 2 × d, *J* 3.5, 3.5 Hz), 6.53−6.89 (14 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.4−31.4 (3 C), 19.8−21.8 (22 C), 62.7 (1 C), 64.2−79.0 (10 C), 83.9 (1 C), 102.3 (1 C), 127.3−140.2 (42 C), 166.4−169.4 (7 C); HRMS (ESI$^+$) *m*/*z* 1459.6577 ($C_{86}H_{100}NaO_{17}S_1$) [M + Na]$^+$ requires 1459.6573).

8-Azidooctyl 2,3,4,6-Tetra-*O*-mesitoyl-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-mesitoyl-*β*-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-1-*β*-D-galactopyranoside (33). (i) TfOH (8.1 μL, 0.091 mmol) was added to a mixture of **31** (190 mg, 0.130 mmol), acceptor **18** (75 mg, 0.16 mmol), NIS (43.9 mg, 0.195 mmol), and freshly activated 5 Å AW molecular sieves in CH_2Cl_2 (5 mL) at 0 °C. The mixture was allowed to warm to rt and was stirred for 2 h. The reaction was quenched by the addition of 0.50 M sodium thiosulfate (10 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 25 mL) and brine (2 × 25 mL) and dried ($MgSO_4$). The solvent was evaporated to give an oil, which was subjected to flash chromatography (20% EtOAc/petroleum spirits) to yield trisaccharide **33** (28 mg, 12%) as an amorphous solid: $[\alpha]^{20}_D$ +32 (*c* 1.0 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.30−1.60 (12 H, m), 1.98−2.36 (72 H), 3.20 (2 H, t, *J* 7.0), 3.31 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 3.60 (1 H, dd, *J* 6.5, 6.5 Hz), 3.78 (1 H, ddd, *J* 10.0, 7.0, 7.0 Hz), 3.82 (1 H, dd, *J* 10.0, 3.5 Hz), 3.95 (1 H, dd, *J* 3.0, 6.5 Hz), 3.99 (1 H, dd, *J* 6.5, 11.5 Hz), 4.03 (1 H, dd, *J* 6.5, 11.5 Hz), 4.16 (1 H, d, *J* 8.0 Hz), 4.18 (1 H, dd, *J* 10.0, 3.5 Hz), 4.23 (1 H, dd, *J* 6.5, 6.5 Hz), 4.38−4.59 (4 H, m), 4.80−4.95 (2 H, 2 × d, *J* 8.0, 8.0 Hz), 5.02−5.06 (2 H, 2 × d, *J* 10.0, 10.0 Hz), 5.33 (1 H, d, *J* 3.5 Hz), 5.52 (1 H, dd, *J* 11.0, 3.5 Hz), 5.62 (1 H, dd, *J* 8.0, 11.0 Hz), 5.89−5.92 (2 H, 2 × d, *J* 3.5, 3.5 Hz), 6.43−6.86 (14 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.8−29.7 (30 C), 51.4 (1 C), 62.2 (1 C), 62.7−78.2 (15 C), 101.1−102.8 (3 C), 127.0−140.3 (42 C), 166.4−170.4 (10 C); HRMS (ESI^+) *m*/*z* 1828.8318 ($C_{102}H_{123}N_3NaO_{26}$ $[M + Na]^+$ requires 1828.8287). (ii) According to the procedure outlined above, a 1:1 anomeric mixture of **32a**,**b** (950 mg, 0.658 mmol) was condensed with acceptor **18** (280 mg, 0.601 mmol). After purification by flash chromatography (15% EtOAc/petroleum spirits), trisaccharide **33** was isolated as an amorphous solid (308 mg, 28%).

8-Azidooctyl *β*-D-Galactopyranosyl-(1→3)-*β*-D-galactopyranoside (2). A solution of disaccharide **20b** (32 mg, 0.027 mmol) in a 1:1:0.1 mixture of 1 M LiOH in MeOH, THF, and H_2O (2 mL) was heated at 80 °C for 5 d. The reaction was neutralized by addition of Amberlite IR-120 resin (H^+ form), and the solvent was evaporated to afford an oil, which was dissolved in H_2O (2 mL) and purified by reversed-phase chromatography (0−40% MeOH/H_2O) to yield disaccharide **2** (11 mg, 86%) as an amorphous solid: $[\alpha]^{21}_D$ +2 (*c* 0.8 in H_2O); ^1H NMR (500 MHz, MeOH-d_4, internal acetone) δ 1.36−1.66 (12 H, m), 3.33 (2 H, t, *J* 7.0 Hz), 3.61−3.98 (13 H, ddd), 4.21 (1 H, m), 4.46 (1 H, d, *J* 8.0 Hz), 4.62 (1 H, d, *J* 8.0 Hz); ^{13}C NMR (100 MHz, MeOH-d_4, acetone) δ 25.5−29.3 (6 C), 51.8 (1 C), 61.4 (1 C), 61.5−83.1 (10 C), 103.0−104.9 (2 C); HRMS (ESI^+) *m*/*z* 518.2320 ($C_{20}H_{37}N_3NaO_{11}$ $[M + Na]^+$ requires 518.2320). Anal. Calcd for $C_{20}H_{37}N_3O_{11}$: C, 48.48; H, 7.53; N, 8.48. Found: C, 48.34; H, 7.67; N, 8.59.

8-Azidooctyl *β*-D-Galactopyranosyl-(1→3)-*β*-D-galactopyranosyl-(1→3)-*β*-D-galactopyranoside (3). Trisaccharide **33** (100 mg, 0.0553 mmol) was dissolved in 1:1:0.1 mixture of 1 M LiOH in MeOH, THF, and H_2O (10.5 mL), and the solution was heated at 80 °C for 5 d. The reaction was neutralized with Amberlite IR-120 resin (H^+ form), and the solvent was evaporated to afford an oil, which was dissolved in H_2O (2 mL) and purified by reversed-phase chromatography (0−40% MeOH/H_2O) to yield trisaccharide **3** (26 mg, 72%) as a colorless glass: $[\alpha]^{21}_D$ 22 (*c* 1.0 in H_2O); ^1H NMR (500 MHz, D_2O) δ 1.22−1.52 (12 H, m), 3.19 (2 H, t, *J* 7.0 Hz), 3.61−3.98 (16 H, m), 4.07 (2 H, m), 4.33−4.55 (3 H, 3 × d, $J_{1,2}$ 8.0 Hz); ^{13}C NMR (100 MHz, MeOH-d_4, acetone) δ 25.4−30.8 (6 C), 51.8 (1 C), 61.4−82.9 (16 C), 102.9, 104.4, 104.8 (3 C); HRMS (ESI^+) *m*/*z* 680.2842 ($C_{26}H_{47}N_3NaO_{16}$ $[M + Na]^+$ requires 680.2849).

7-Nitro-4-(prop-2-ynylamino)benzofurazan (34). A solution of 4-chloro-7-nitro-benzofurazan (200 mg, 0.992 mmol), propargylamine (71 μL, 1.1 mmol), and pyridine (8 μL, 0.1 mmol) in EtOH (5 mL) was stirred at rt for 30 min. The solvent was evaporated, and the residue was purified by flash chromatography (30% EtOAc/pet. spirits) to afford **34** (108 mg, 50%) as an orange residue: mp 145−147 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 2.37 (1 H, t, *J* 2.0 Hz), 3.08 (1 H, s), 4.21 (2 H, d, *J* 2.5 Hz), 6.28−8.48 (2 H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 33.5 (1 C), 74.3 (1 C), 78.8 (1 C); 101.1−145.6 (6 C); HRMS (ESI^+) *m*/*z* 241.0332 ($C_9H_6N_4NaO_3$ $[M + Na]^+$ requires 241.0332). Anal. Calcd for $C_9H_4N_4O_3$: C, 49.55; H, 2.77; N, 25.68. Found: C, 49.47; H, 2.91; N, 25.70.

4-[(7-Nitrobenzofurazan-4-yl)aminomethyl]-triazol-1-yloctyl *β*-D-Galactopyranoside (35). A solution of azide **1** (19.7 mg, 59.1 μmol), alkyne **34** (15.4 mg, 69.9 μmol), $CuSO_4$ (0.37 mg, 2.3 μmol), sodium ascorbate (2.3 mg, 18 μmol), and TBTA (1.6 mg, 2.3 μmol) in THF/H_2O (1:1, 5 mL) was stirred at rt for 1 h. The mixture was evaporated to dryness to give a residue, which was purified by flash chromatography (17:2:1 EtOAc/MeOH/H_2O) to afford **35** (30 mg, 92%) as an orange residue: $[\alpha]^{20}_D$ −13 (*c* 0.50 in DMSO); ^1H NMR (500 MHz, DMSO-d_6) δ 1.16−1.80 (12 H, m), 3.29−3.40 (5 H, m), 3.42−3.54 (2 H, m), 3.62 (1 H, m), 3.87 (1 H, ddd, *J* 9.5, 6.5, 6.5 Hz), 4.29 (1 H, d, *J* 8.0 Hz), 4.32 (2 H, m), 6.49−8.51 (2 H, m), 8.10 (1 H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.8−30.1 (6 C), 49.8 (2 C), 60.8 (1 C), 68.6−75.5 (5 C), 100.3−145.0 (6 C), 103.4 (1 C), 123.7, 143.0 (2 C); HRMS (ESI^+) *m*/*z* 574.2232 ($C_{23}H_{33}N_7NaO_9$ $[M + Na]^+$ requires 574.2232).

4-[(7-Nitrobenzofurazan-4-yl)aminomethyl]triazol-1-yloctyl *β*-D-Galactopyranosyl-(1→3)-*β*-D-galactopyranoside (36). A solution of azide **2** (84.0 mg, 170 μmol), alkyne **34** (44.0 mg, 202 μmol), $CuSO_4$ (1.1 mg, 6.7 μmol), sodium ascorbate (6.7 mg, 34 μmol), and TBTA (4.3 mg, 34 μmol) in THF/H_2O (1:1, 10 mL) was stirred at rt for 1 h. The mixture was evaporated, and the residue was purified by reversed-phase chromatography (0−40% MeOH/H_2O) to afford **36** (113 mg, 94%) as an orange residue: $[\alpha]^{20}_D$ −7 (*c* 0.50 in DMSO); ^1H NMR (500 MHz, MeOH-d_4) δ 1.31−1.89 (12 H, m), 3.46−3.77 (13 H, m), 3.82 (1 H, d, *J* 3.0 Hz), 3.87 (1 H, ddd, *J* 9.5, 6.5, 6.5 Hz), 4.12 (1 H, dd, *J* 3.0, 0.5 Hz), 4.26 (1 H, d, *J* 8.0 Hz), 4.39 (2 H, t, *J* 7.0 Hz), 4.49 (1 H, d, *J* 8.0 Hz), 6.46−8.53 (2H, m), 8.02 (1 H, s); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.8−30.1 (6 C), 49.8 (2 C), 60.8 (1 C), 67.9−84.1 (10 C), 100.4−145.5 (6 C), 103.2, 105.7 (2 C), 123.8 (1 C), 143.0 (1 C); HRMS (ESI^+) *m*/*z* 736.2754 ($C_{29}H_{43}N_7NaO_{14}$ $[M + Na]^+$ requires 736.2760).

4-[(7-Nitrobenzofurazan-4-yl)aminomethyl]triazol-1-yloctyl *β*-D-Galactopyranosyl-(1→3)-(*β*-D-galactopyranosyl)-(1→3)-*β*-D-galactopyranoside (37). A solution of azide **3** (5.0 mg, 7.6 μmol), alkyne **34** (1.8 mg, 8.1 μmol), $CuSO_4$ (49 μg, 0.31 μmol), sodium ascorbate (31 μg, 1.5 μmol), and TBTA (160 μg, 0.31 μmol) in THF/H_2O (1:1, 2 mL) was stirred at rt for 1 h. The mixture was evaporated to dryness to give a residue, which was purified by reversed-phase chromatography (0−40% MeOH/H_2O) to afford **37** (4.4 mg, 65%) as an orange residue: $[\alpha]^{19}_D$ −15 (*c* 0.1 in MeOH); ^1H NMR (500 MHz, D_2O) δ 1.13−1.85 (12 H, m), 3.51−3.84 (19 H), 3.90, 4.16, 4.19 (3 H, 3 × d, *J* 3.2 Hz), 4.37, 4.59, 4.65 (3 H, 3 × d, *J* 8.0 Hz), 4.40 (2 H, m), 6.41−8.58 (2H, m), 8.04 (1 H, s); ^{13}C NMR (200 MHz, D_2O) δ 25.1−29.0 (6 C), 50.5 (2 C), 61.1 (1 C), 68.1−82.1 (15 C), 102.3, 103.9, 104.1 (3 C), 123.8 (1 C), 124.1, 138.6 (2 C); HRMS (ESI^+) *m*/*z* 898.3290 ($C_{35}H_{53}N_7NaO_{19}$ $[M + Na]^+$ requires 898.3288). ^{13}C NMR data were acquired by HSQC spectroscopy.

8-(2-Ethoxycyclobutene-3,4-dione-1-ylamino)octyl *β*-D-Galactopyranoside (38). PPh_3 (94 mg, 0.36 mmol) was added to a stirred solution of azide **1** (30 mg, 0.090 mmol) in THF/H_2O (10:1, 5 mL) at 0 °C. The mixture was warmed to rt and stirred overnight. The mixture was concentrated under reduced pressure to give a residue, which was dissolved in EtOAc (5 mL). The organic phase was extracted with H_2O (5 × 3 mL), and the

aqueous phase was concentrated to dryness to afford the crude amine. The amine was dissolved in a mixture of EtOH/H_2O/0.1 M phosphate buffer (2:1:1, 4 mL, pH 8), and diethyl squarate (613 μL, 90 μmol) was added. The mixture was stirred for 3 h and then concentrated to dryness. The residue was purified by reversed-phase chromatography (0−50% MeOH/H_2O) to afford **38** (36 mg, 92%) as a colorless oil: ^{1}H NMR (500 MHz, D_2O) δ 1.36−1.65 (15 H, m), 3.40−3.62 (6 H, m), 3.73 (1 H, dd, *J* 6.0, 11.5 Hz), 3.75 (1 H, dd, *J* 6.5, 11.0 Hz), 3.83 (1 H, dd, *J* 3.5, 1.0 Hz), 3.89 (1 H, ddd, *J* 9.5, 6.5, 6.5 Hz), 4.21 (1 H, d, *J* 7.0 Hz), 4.74 (2 H, m); HRMS (ESI$^+$) *m*/*z* 454.2048 ($C_{20}H_{33}NNaO_9$ [M + Na]$^+$ requires 454.2048). The ^{13}C NMR spectrum for this compound was poorly resolved. We attribute this to the presence of tautomeric isomers.

8-(2-Ethoxycyclobutene-3,4-dion-1-ylamino)octyl *β*-D-galactopyranosyl-(1→3)-*β*-D-galactopyranoside (39). PPh_3 (36 mg, 137 μmol) was added to a stirred solution of azide **2** (17 mg, 34 μmol) in THF/H_2O (5:1, 3 mL) at 0 °C. The mixture was warmed to rt and stirred overnight. The mixture was concentrated under reduced pressure to give a residue, which was dissolved in EtOAc (4 mL). The organic phase was extracted with H_2O (5 × 3 mL), and the aqueous phase was concentrated to dryness to afford the crude amine. The amine was dissolved in a mixture of EtOH/H_2O/0.1 M phosphate buffer (2:1:1, 3 mL, pH 8), and diethyl squarate (6.5 μL, 45 μmol) was added. The mixture was stirred for 3 h and then concentrated to dryness. The residue was purified by reversed-phase chromatography (0−20% MeOH/H_2O) to afford **39** (18 mg, 92%) as a colorless oil: ^{1}H NMR (500 MHz, D_2O) δ 1.37−1.65 (15 H, m), 3.41−3.91 (13 H, m), 3.83 (1 H, dd, *J* 3.0, 1.0 Hz), 3.91 (1 H, ddd, *J* 9.5, 6.5, 6.5 Hz), 4.12 (1 H, dd, *J* 3.0, 1.0 Hz), 4.27 (1 H, d, *J* 7.5 Hz), 4.50 (1 H, d, 7.5 Hz), 4.76 (2 H, m); HRMS (ESI$^+$) *m*/*z* 616.2578 ($C_{26}H_{43}NNaO_{14}$ [M + Na]$^+$ requires 616.2576). The ^{13}C NMR spectrum for this compound was poorly resolved. We attribute this to the presence of tautomeric isomers.

8-(2-Ethoxycyclobutene-3,4-dion-1-ylamino)octyl *β*-D-galactopyranosyl-(1→3)-(*β*-D-galactopyranosyl)-(1→3)-*β*-D-galactopyranoside (40). PPh_3 (30 mg, 115 μmol) was added to a stirred solution of azide **3** (19 mg, 29 μmol) in THF/H_2O (5:1, 2.2 mL) at 0 °C. The mixture was warmed to rt, stirred overnight, and then concentrated under reduced pressure to give a residue, which was dissolved in EtOAc (3 mL). The organic phase was extracted with H_2O (5 × 3 mL), and the aqueous phase was concentrated to dryness to afford the crude amine. The amine was dissolved in a mixture of EtOH/H_2O/0.1 M phosphate buffer (2:1:1, 2 mL, pH 8), and diethyl squarate (5.5 μL, 37 μmol) was added. The mixture was stirred for 3 h and then concentrated to dryness. The residue was purified by reversed-phase chromatography (0−20% MeOH/H_2O) to afford **40** (22 mg, 95%) as a colorless oil: ^{1}H NMR (500 MHz, D_2O) δ 1.37−1.65 (15 H, m), 3.37−3.80 (20 H, m), 4.06−4.07 (2 H, 2 × d, *J* 3.0 Hz), 4.31, 4.49, 4.55 (3 H, 3 × d, *J* 7.5 Hz), 4.65 (2 H, m); HRMS (ESI$^+$) *m*/*z* 778.3109 ($C_{32}H_{53}NNaO_{19}$ [M + Na]$^+$ requires 778.3104). The ^{13}C NMR spectrum for this compound was poorly resolved. We attribute this to the presence of tautomeric isomers.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for **1**−**3**, **4b**, **5**−**19**, **20a**,**b**, and **23**−**40**, fluorescence spectra for **35**−**37**, HPLC chromatograms for **37** and **40**, and experimental procedures for compounds **4b**, **5**−**11**, **16**, **19**, **28**, and **29**. This material is available free of charge via the Internet at http://pubs.acs.org.