JOC Article
pubs.acs.org/joc

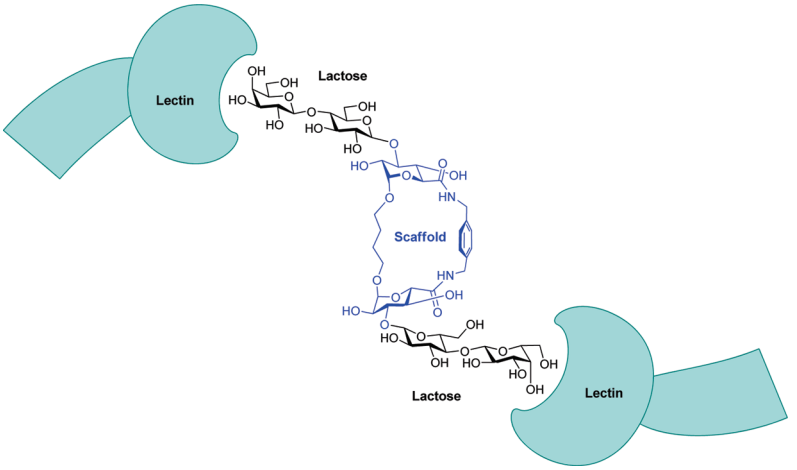
Synthesis of Bivalent Lactosides Based on Terephthalamide, *N*,*N*′-Diglucosylterephthalamide, and Glycophane Scaffolds and Assessment of Their Inhibitory Capacity on Medically Relevant Lectins

Rosaria Leyden,[†] Trinidad Velasco-Torrijos,[†] Sabine André,[‡] Sebastien Gouin,[†] Hans-Joachim Gabius,[‡] and Paul V. Murphy*[,†,§]

[†]*School of Chemistry and Chemical Biology, Centre for Synthesis and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland,* [‡]*Institute of Physiological Chemistry, Faculty of Veterinary Medicine, Ludwig-Maximilians-University Munich, Veterinärstrasse 13, 80539 Munich, Germany, and* [§]*School of Chemistry, National University of Ireland, Galway*

paul.v.murphy@nuigalway.ie

Received August 5, 2009



Glycan recognition by lectins initiates clinically relevant processes such as toxin binding or tumor spread. Thus, the development of potent inhibitors has a medical perspective. Toward this goal, we report the synthesis of both rigid and flexible bivalent lactosides on scaffolds that include secondary and tertiary terephthalamides and *N*,*N*′-diglucosylterephthalamides. Construction of these compounds involved Schmidt−Michel glycosidation, and amide coupling or Ugi reactions of relevant glycosyl amines in key steps. A glycocluster based on a rigid glycophane was also prepared from coupling of a glucuronic acid derivative and *p*-xylylenediamine with subsequent ring-closing metathesis. Finally, a more flexible bivalent lactoside was produced from lactosyl azide with use of the copper-catalyzed azide−alkyne cycloaddition. Distances between lactose residues were analyzed computationally as were their orientations for the relatively rigid subset of compounds. Distinct spacing properties were revealed by varying the structure of the scaffold or by varying the location of the lactose residue on the scaffold. To relate these features to bioactivity a plant toxin and human adhesion/growth-regulatory galectins were used as sensors in three types of assay, i.e. measuring agglutination of erythrocytes, binding to glycans of a surface-immobilized glycoprotein, or binding to human cells. Methodologically, the common hemeagglutination assay was found to be considerably less sensitive than both solid-phase and cell assays. The bivalent compounds were less effective at interfering with glycoprotein binding to the plant toxin than to human lectins. Significantly, a constrained compound was identified that displayed selectivity in its inhibitory potency between galectin-3 and its proteolytically processed form. Conversely, compounds with a high degree of flexibility showed notable ability to protect human cells from plant toxin binding. The applied conjugation chemistry thus is compatible with the long-term aim to produce potent and selective lectin inhibitors.

* To whom correspondence should be addressed. Fax: +353-91-525700

Published on Web 11/09/2009 DOI: 10.1021/jo901667r

Introduction

Carbohydrates have ideal properties to generate an unrivalled number of oligomers. They are capable of engaging in molecular recognition events in biological systems, underlying the concept of the sugar code.[1] Indeed, glycan chains presented on cell surface glycoconjugates are versatile biochemical signals by serving as direct contact sites for receptors (lectins) that bind to carbohydrates (*trans*-interactions) and as potent molecular switches regulating aspects of protein accessibility and functionality (*cis*-interactions).[1,2] Besides the structure of the headgroup of a glycan chain its presentation and local density govern the affinity in intermolecular interactions, as recently revealed in the case of physiological core substitutions in *N*-glycans which trigger significant alterations in glycan conformations and, importantly, in lectin affinity.[3] Distinct changes in glycan display including microdomain formation in natural systems can thus lead to induction of cellular responses such as anoikis/apoptosis or autoimmune suppression via lectin binding.[4] To underscore the emerging clinical relevance a tumor suppressor and responses in inflammation can apparently work on this level by remodelling cell surface glycan profiles and even lectin expression to result in host defense/antimalignancy effects.[4c,d] Clinically disadvantageous, lectin binding to glycans is also involved in disease progression. Herewith, it gives an incentive for drug design. Considering the multiple, medically relevant activities of cell surface glycans in concert with lectins in regulating, for example, adhesion, migration, proliferation, or tissue invasion or serving as docking sites for plant/bacterial toxins,[1] it clearly is an attractive goal to develop new molecules which can efficiently interfere with binding of certain effectors to cell surface glycans.

Of current interest to us toward this goal is a series of bivalent glycoclusters of varying degree of scaffold flexibility, altering ligand spacing with a view to profiling the structure−bioactivity relationships. As a first step, we recently described the synthesis and biological evaluation of a series of bivalent mannosides based on terephthalamide and glycocyclophane scaffolds, the latter being derived from phenylene-1,4-diamines.[5] Initial testing for bioactivity with leguminous lectins in cell-based assays showed that presentation of the mannose residues on a rigid macrocyclic scaffold can lead to enhanced inhibitory capacity.[5] Evidently, the mannose moieties maintained ligand-binding capacity for the tested plant lectins. Thus, this proof-of-principle result encourages further efforts with a headgroup different from that of mannose and medically relevant lectins.

Due to its positioning at branch ends of glycan chains and frequent involvement in lectin binding β-galactose satisfies the requirement for biological relevance.[1,2] Testing of bivalent scaffolds presenting β-galactosides (e.g., lactosides) in assays with a biohazardous toxin and with human lectins was therefore envisaged to provide a medical perspective. Consequently, we were interested to establish synthetic routes that would allow bivalent lactosides based on macrocyclic and terephthalamide scaffolds to be generated. As follows, the syntheses of ligands (Chart 1) with β-lactose conjugated to terephthalamide scaffolds[6] (**1**−**2**), *N*′,*N*′-diglucosylterephthalamides (**3**−**5**), and a rigid macrocyclic glycocyclophane **8** are presented. Preparations of **6** and **7** with increased flexibility compared to the conformationally arrested compound **8** are included as was the generation of the triazole derivative **9**. Geometrical aspects of presentation of the β-lactose units in the more rigid compounds were then comparatively inferred by computational methods.

Bioactivity of the sugar headgroup and the impact of structural properties were next examined in the commonly used hemeagglutination test and also in a solid-phase binding assay. The latter assay was based on evaluating the ability of synthetic compounds to inhibit binding of lectins to a natural glycoprotein (asialofetuin (ASF) with three *N*-glycosylation sites preferentially occupied by complex-type triantennary *N*-glycans). Since any clinical application would be based on impairing lectin binding to cells, we also determined the extent of lectin binding to native human cells as a function of the presence of the new compounds. Lectin selection for the assays was based on the aim to protect cells from toxicity, in addition to evaluating the potential of the test panel to interfere with progression of disease in malignancy due to activity of distinct adhesion/growth-regulatory galectins.[7] In detail, the binding of *Viscum album* agglutinin (VAA) and human galectins-3 and -4 to the surface-presented glycoprotein was monitored in the absence/presence of a test compound, using free lactose as an internal standard of inhibitory potency. Galectin-3 has two special features in this respect: it can pentamerize in the presence of a

(1) Gabius, H.-J., Ed. *The Sugar Code. Fundamentals of glycosciences*; Wiley-VCH: Weinheim, Germany, 2009.

(2) (a) Sharon, N.; Lis, H. In *Glycosciences: Status and Perspectives*; Gabius, H.-J., Gabius, S., Eds.; Chapman & Hall: London, UK, 1997; pp 133−162. (b) von der Lieth, C.-W.; Siebert, H.-C.; Kožár, T.; Burchert, M.; Frank, M.; Gilleron, M.; Kaltner, H.; Kayser, G.; Tajkhorshid, E.; Bovin, N. V.; Vliegenthart, J. F. G.; Gabius, H.-J. *Acta Anat.* (*Basel*) **1998**, *161*, 91–109. (c) Gabius, H.-J.; Siebert, H.-C.; André, S.; Jiménez-Barbero, J.; Rüdiger, H. *ChemBioChem* **2004**, *5*, 740–764. (d) Gabius, H.-J. *Crit. Rev. Immunol.* **2006**, *26*, 43–79. (e) Gabius, H.-J. *Biochem. Soc. Trans.* **2008**, *36*, 1491–1496. (f) André, S.; Kožár, T.; Kojima, S.; Unverzagt, C.; Gabius, H.-J. *Biol. Chem.* **2009**, *390*, 557–566. (g) Honke K.; Taniguchi, N. In *The Sugar Code. Fundamentals of glycosciences*; Gabius, H.-J., Ed.; Wiley-VCH: Weinheim, Germany, 2009; pp 385−401. (h) Ledeen, R. W.; Wu, G. In *The Sugar Code. Fundamentals of glycosciences*; Gabius, H.-J., Ed.; Wiley-VCH: Weinheim, Germany, 2009; pp 495−516. (i) Zuber, C.; Roth, J. In *The Sugar Code. Fundamentals of glycosciences*; Gabius, H.-J., Ed.; Wiley-VCH: Weinheim, Germany, 2009; pp 87−110.

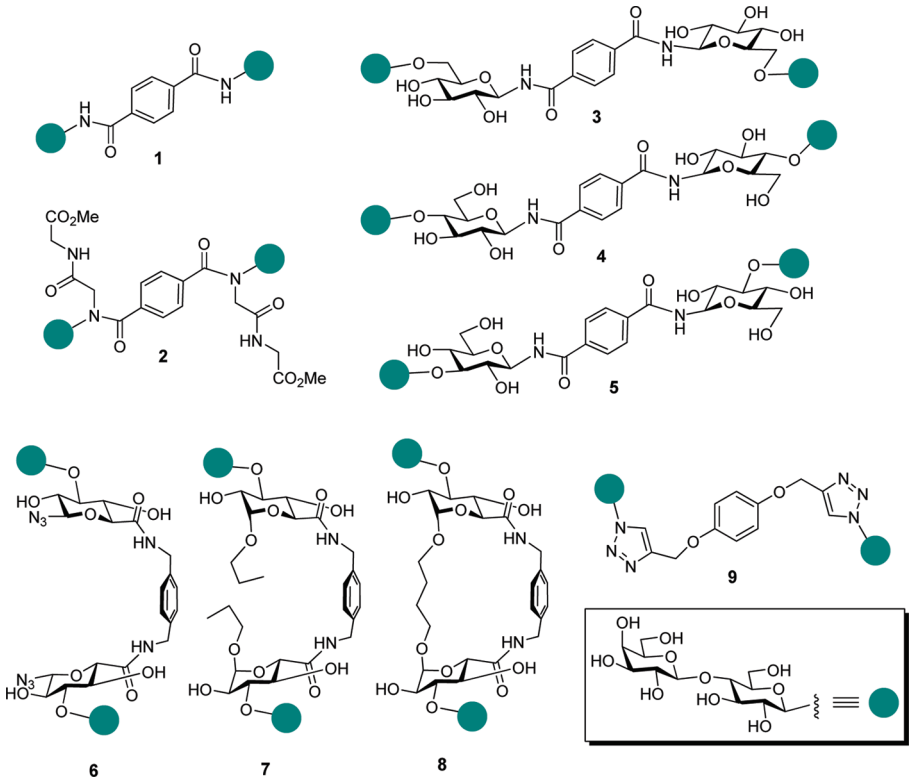
(3) (a) André, S.; Unverzagt, C.; Kojima, S.; Dong, X.; Fink, C.; Kayser, K.; Gabius, H.-J. *Bioconjugate Chem.* **1997**, *8*, 845–855. (b) Unverzagt, C.; André, S.; Seifert, J.; Kojima, S.; Fink, C.; Srikrishna, G.; Freeze, H. H.; Kayser, K.; Gabius, H.-J. *J. Med. Chem.* **2002**, *45*, 478–491. (c) André, S.; Unverzagt, C.; Kojima, S.; Frank, M.; Seifert, J.; Fink, C.; Kayser, K.; von der Lieth, C.-W.; Gabius, H.-J. *Eur. J. Biochem.* **2004**, *217*, 118–134. (d) André, S.; Kožár, T.; Schuberth, R.; Unverzagt, C.; Kojima, S.; Gabius, H.-J. *Biochemistry* **2007**, *46*, 6984–6995.

(4) (a) Kopitz, J.; von Reitzenstein, C.; André, S.; Kaltner, H.; Uhl, J.; Ehemann, V.; Cantz, M.; Gabius, H.-J. *J. Biol. Chem.* **2001**, *276*, 35917–35923. (b) Rappl, G.; Abken, H.; Muche, J. M.; Sterry, W.; Tilgen, W.; André, S.; Kaltner, H.; Ugurel, S.; Gabius, H.-J.; Reinhold, U. *Leukemia* **2002**, *16*, 840–845. (c) André, S.; Sanchez-Ruderisch, H.; Nakagawa, H.; Buchholz, M.; Kopitz, J.; Forberich, P.; Kemmner, W.; Böck, C.; Deguchi, K.; Detjen, K. M.; Wiedenmann, B.; von Knebel Doeberitz, M.; Gress, T. M.; Nishimura, S.-I.; Rosewicz, S.; Gabius, H.-J. *FEBS J.* **2007**, *274*, 3233–3256. (d) Marathe, D. D.; Chandrasekaran, E. V.; Lau, J. T. Y.; Matta, K. L.; Neelamegham, S. *FASEB J.* **2008**, *22*, 4154–4167. (e) Patsos, G.; André, S.; Roeckel, N.; Gromes, R.; Gebert, J.; Kopitz, J.; Gabius, H.-J. *Glycobiology* **2009**, *19*, 726–734. (f) Schwartz-Albiez, R. In *The Sugar Code. Fundamentals of glycosciences*; Gabius, H.-J., Ed.; Wiley-VCH: Weinheim, Germany, 2009; pp 447−467. (g) Wang, J.; Lu, Z.-H.; Gabius, H.-J.; Rohowsky-Kochan, C.; Ledeen, R. W.; Wu, G. *J. Immunol.* **2009**, *182*, 4036–4045.

(5) André, S.; Velasco-Torrijos, T.; Leyden, R.; Gouin, S.; Tosin, M.; Murphy, P. V.; Gabius, H.-J. *Org. Biomol. Chem.* **2009**, *7*, 4715–4725.

(6) (a) Avalos, M.; Babiano, R.; Carretero, M. J.; Cintas, P.; Higes, F. J.; Jiménez, J. L.; Palacios, J. C. *Tetrahedron* **1998**, *54*, 615–628. (b) Murphy, P. V.; Bradley, H.; Tosin, M.; Pitt, N.; Fitzpatrick, G. M.; Glass, W. K. *J. Org. Chem.* **2003**, *68*, 5692–5704. (c) Tosin, M.; Gouin, S. G.; Murphy, P. V. *Org. Lett.* **2005**, *7*, 211–214. (d) Bradley, H.; Fitzpatrick, G.; Glass, W. K.; Kunz, H.; Murphy, P. V. *Org. Lett.* **2001**, *3*, 2629.

CHART 1. *β*-Lactose-Based Glycoclusters



multivalent ligand and it is physiologically truncated by matrix metalloproteinases-2/-9 to yield a trimmed (truncated) version with diminished propensity for oligomer formation.[8] Galectin-3 and its truncated derivative were thus tested to reveal any difference in sensitivity to the glycocompounds and a selective inhibitor was identified. The obtained results demonstrate the bioactivity of this panel of glycoclusters as well as reveal a notable influence of assay system, compound, lectin, and matrix properties on inhibitory potency.

Results and Discussion

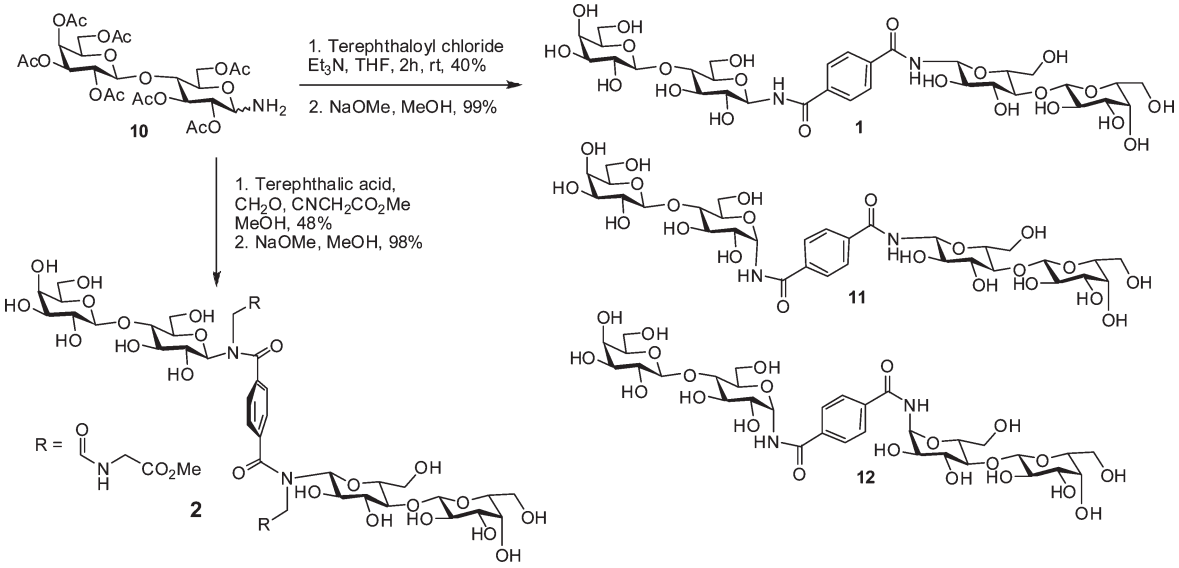
Synthesis of Glycoclusters. Synthetic routes were first established to produce **1**−**9**. It was envisaged that **1** and **2** could be prepared from lactosyl amine **10**. Fittingly, coupling of **10** with terephthaloyl chloride in the presence of triethylamine and subsequent Zemplén deacetylation gave **1**. Since the glycosyl amine **10** also contained a small amount of the α-anomer, a mixture of diamides was obtained from the coupling reaction. The bivalent compounds **11** and a lesser amount of **12** containing α-glycosyl amides were thus also obtained after HPLC-based separation of the deacetylated diamide mixture (Scheme 1). Next, the Ugi reaction of **10** in the presence of formaldehyde, methyl isocyanoacetate, and terephthalic acid and subsequent Zemplén deacetylation resulted in the bivalent compound **2**. It differs from **1** in that tertiary amides are present as opposed to secondary amides.

In the next step, approaches to bivalent lactosides based on *N*,*N*′-diglucopyranosylterephthalamide scaffolds were studied. The Schmidt−Michel glycoside coupling reaction of trichloroacetimidate **14** with **13** with TMSOTf as catalyst and subsequent reaction of the trisaccharide product with $SnCl_4$ and $TMSN_3$ generated azide **15**. Catalytic hydrogenation led to the glycosyl amine and subsequent coupling with terephthaloyl chloride followed by deacetylation as before gave **3** where the lactosyl units are attached to both 6-*O* atoms of the scaffold (Scheme 2).

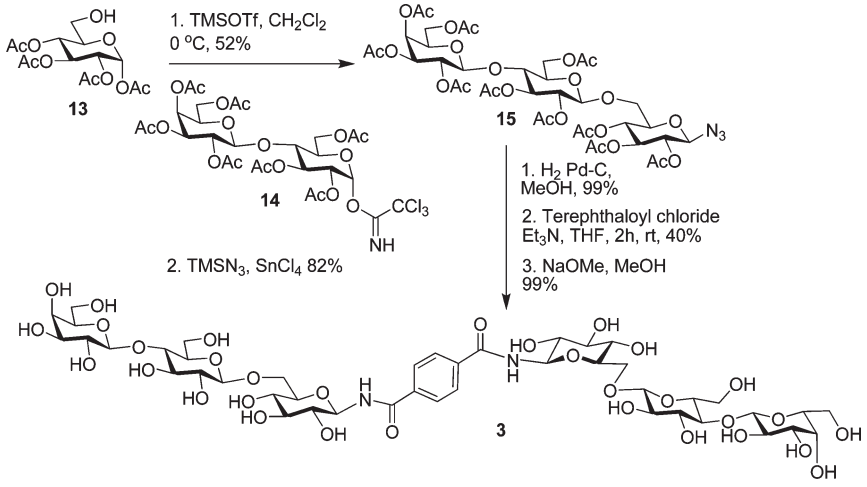
(7) (a) Gabius, H.-J. *Biochimie* **2001**, *83*, 659–666. (b) Gabius, H.-J.; Darro, F.; Remmelink, M.; André, S.; Kopitz, J.; Danguy, A.; Gabius, S.; Salmon, I.; Kiss, R. *Cancer Invest*. **2001**, *19*, 114–126. (c) Nagy, N.; Legendre, H.; Engels, O.; André, S.; Kaltner, H.; Wasano, K.; Zick, Y.; Pector, J.-C.; Decaestecker, C.; Gabius, H.-J.; Salmon, I.; Kiss, R. *Cancer* **2003**, *97*, 1849–1858. (d) Lahm, H.; André, S.; Hoeflich, A.; Kaltner, H.; Siebert, H.-C.; Sordat, B.; von der Lieth, C.-W.; Wolf, E.; Gabius, H.-J. *Glycoconj. J*. **2004**, *20*, 227–238. (e) Delacour, D.; Gouyer, V.; Zanetta, J.-P.; Drobecq, H.; Leteurtre, E.; Grard, G.; Moreau-Hannedouche, O.; Maess, E.; Pons, A.; André, S.; Le Bivic, A.; Gabius, H.-J.; Manninen, A.; Simons, K.; Huet, G. *J. Cell Biol*. **2005**, *169*, 491–501. (f) Jiménez, M.; Sáiz, J. L.; André, S.; Gabius, H.-J.; Solís, D. *Glycobiology* **2005**, *15*, 1386–1395. (g) Jiménez, M.; André, S.; Siebert, H.-C.; Gabius, H.-J.; Solís, D. *Glycobiology* **2006**, *16*, 926–937. (h) Villalobo, A.; Nogales-Gonzáles, A.; Gabius, H.-J. *Trends Glysci. Glycotechnol*. **2006**, *18*, 1–37. (i) Langbein, S.; Brade, J.; Badawi, J. K.; Hatzinger, M.; Kaltner, H.; Lensch, M.; Specht, K.; André, S.; Brinck, U.; Alken, P.; Gabius, H.-J. *Histopathology* **2007**, *51*, 681–690. (j) Moisa, A.; Fritz, P.; Eck, A.; Wehner, H.-D.; Mürdter, T.; Simon, W.; Gabius, H.-J. *Anticancer Res*. **2007**, *27*, 2131–2140. (k) André, S.; Sansone, F.; Kaltner, H.; Casnati, A.; Kopitz, J.; Gabius, H.-J.; Ungaro, R. *ChemBioChem* **2008**, *9*, 1649–1661. (l) Saussez, S.; Decaestecker, C.; Mahillon, V.; Cludts, S.; Cappouillez, A.; Chevalier, D.; Kaltner, H.; André, S.; Toubeau, G.; Leroy, X.; Gabius, H.-J. *Laryngoscope* **2008**, *118*, 1583–1590. (m) Čada, Z.; Smetana, K., Jr.; Lacina, L.; Plzáková, Z.; Štork, J.; Kaltner, H.; Russwurm, R.; Lensch, M.; André, S.; Gabius, H.-J. *Folia Biol* (*Praha*) **2009**, *55*, 145–52. (n) Stechly, L.; Morelle, W.; Dessein, A.-F.; André, S.; Grard, G.; Trinel, D.; Dejonghe, H.-J.; Leteurtre, E.; Drobecq, H.; Trugnan, G.; Gabius, H.-J.; Huet, G. *Traffic* **2009**, *10*, 438–450.

(8) (a) Herrmann, J.; Turck, C. W.; Atchison, R. E.; Huflejt, M. E.; Poulter, L.; Gitt, M. A.; Burlingame, A. L.; Barondes, S. H.; Leffler, H. *J. Biol. Chem*. **1993**, *268*, 26704–26711. (b) Ahmad, N.; Gabius, H.-J.; André, S.; Kaltner, H.; Sabesan, S.; Roy, R.; Liu, B.; Macaluso, F.; Brewer, C. F. *J. Biol. Chem*. **2004**, *279*, 10841–10847. (c) Dam, T. K.; Gabius, H.-J.; André, S.; Kaltner, H.; Lensch, M.; Brewer, C. F. *Biochemistry* **2005**, *44*, 12564–12571. (d) Kübler, D.; Hung, D.-W.; Dam, T. K.; Kopitz, J.; André, S.; Kaltner, H.; Lohr, M.; Manning, J. C.; He, L.; Wang, H.; Middelberg, A.; Brewer, C. F.; Reed, J.; Lehmann, W.-D.; Gabius, H.-J. *Biochim. Biophys. Acta* **2008**, *1780*, 716–722.

SCHEME 1. Synthesis of 1, 2, and 11



SCHEME 2. Synthesis of 3



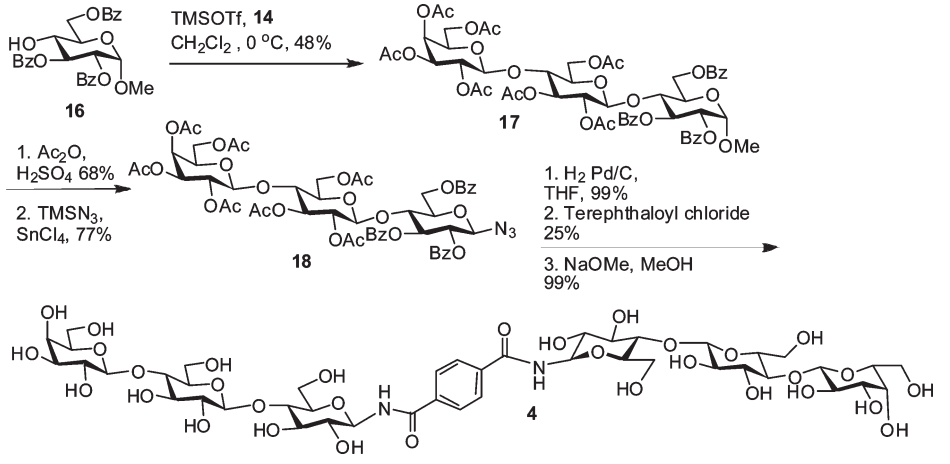
The synthesis of **4** was achieved via the glucopyranoside **16**. Glycosidation of the acetylated lactosyl trichloroacetimidate donor **14** opened the way to the desired trisaccharide **17**. Acetolysis gave the glycosyl acetate derivative and its subsequent reaction with azidotrimethylsilane in the presence of $SnCl_4$ the desired β-azide **18**. Reaction of **18** as described for **15** produced bivalent compound **4** (Scheme 3). The lactose units are covalently linked to the two 4-*O* atoms of the *N*,*N*′-diglucosylterephthalamide scaffold.

To prepare other analogues which would be available by using the 3-*O* atoms of the scaffold as an attachment site, a respective strategy was developed. Toward this aim, glycosidation reactions of the glycosyl acceptor **20** with donor **14** were not successful, which contrasted with what we observed for the other acceptors (Schemes 2 and 3). An orthoester byproduct, often detected in glycosidation reactions, was obtained instead of the desired glycosidic product. To address this problem the benzoylated lactosyl trichloroacetimidate **19** was prepared and its reaction with **20** investigated, leading to the successful formation of the desired β-glycoside **21** in good yield. Hydrolysis of the acetonide groups, subsequent acetylation followed by reaction of the product with $SnCl_4$ and $TMSN_3$, and then catalytic hydrogenation gave the amine **22**. Treatment of amine **22** with terephthaloyl chloride as before and ensuing removal of the acyl protecting groups resulted in **5** (Scheme 4).

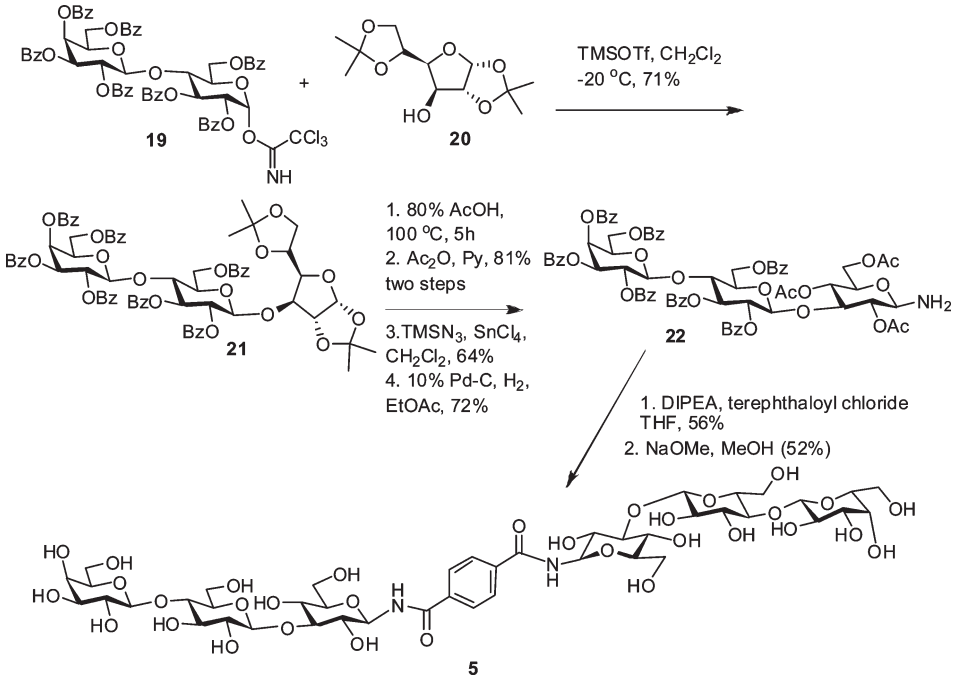
With this series of bivalent lactosides in hand we next added compounds to the panel based on scaffolds that were amides of glucuronic acid. Consequently, the acceptor **23**, which we had used previously to prepare mannosides, on reaction with acetylated lactose donor **14** gave the undesired orthoester **24**. Application of the benzoylated donor **19** in the glycosidation yielded the suitable trisaccharide intermediate, which was then converted to **25** after selective removal of the allyl ester with Pd(0) catalysis (Scheme 5).[9] In our previous work we had synthesized the bivalent mannosides via coupling of a glucuronic acid derivative with phenylene-1,4-diamine. The products of coupling of phenylene-1,4-diamine to the acid **25** were analyzed indicating that only one

(9) Kunz, H.; Waldmann, H. *Angew. Chem., Int. Ed.* **1984**, *23*, 71–72.

SCHEME 3. Synthesis of 4



SCHEME 4. Synthesis of 5

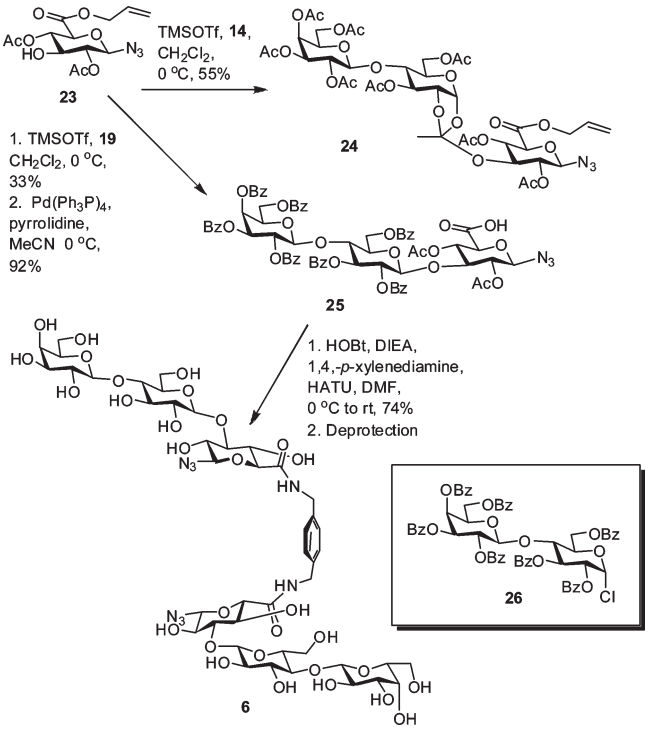


trisaccharide residue had been added to one amine group (Scheme 5). It is presumed that formation of the first amide group leads to a reduction in nucleophilicity of the second amine group of the phenylene-1,4-diamine residue precluding formation of a second amide. We thus investigated the preparation of diamides from 1,4-di(aminomethyl)benzene (*p*-xylylenediamine), where electron-withdrawing effects were expected to have been lessened. Conversion of **25** to its acid chloride with a view to using this for the amide synthesis led to the formation of the glycosyl chloride **26**, tentatively explained by the mechanistic proposal outlined in Scheme 6. The activation of *O*-glycosidic linkages by manipulation of remote carboxylic acid groups has already been delineated and favorably utilized in glycosidation reactions.[10] Herein, a glycosyl carbenium ion is proposed to be generated via the acid chloride, the former which is subsequently trapped by chloride ion (Scheme 6). Eventually, the desired coupling reaction en route to the diamide was achieved by activation of the acid **25** with HOBt, DIPEA, and HATU in the presence of the *p*-xylylenediamine. Removal of the acetate and benzoate protecting groups gave the flexible divalent compound **6** (Scheme 5).

Having established conditions for the formation of **6**, we next worked toward the synthesis of bivalent lactosides based on a glycocyclophane scaffold derived from the *p*-xylylenediamine. The acceptor **28** was therefore prepared from **27** as described previously[5] and its reaction with **19** and subsequent chemoselective deallylation with Pd(0) gave **29**. Reaction of **29** with *p*-xylylenediamine in the presence of HOBt, HATU, and DIPEA generated the protected divalent compound **30** (Scheme 7), a suitable precursor for ring closure metathesis (RCM).

(10) Kim, K. S.; Kim, J. H.; Yong, Lee, J.; Lee, Y. J.; Park, J. *J. Am. Chem. Soc.* **2001**, *123*, 8477–8481.

SCHEME 5. Synthesis of 6

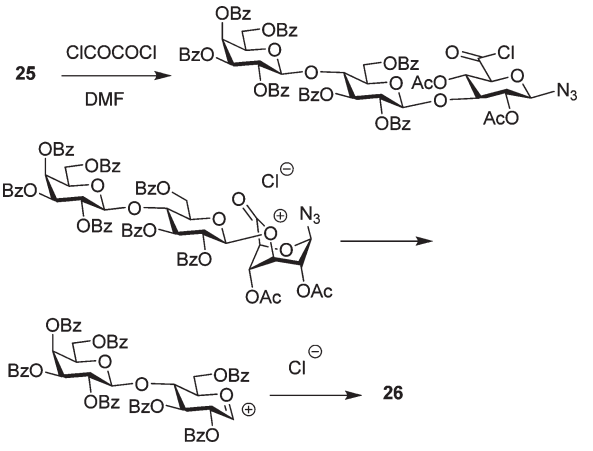


The subsequent RCM of **30** with the Grubbs-I catalyst gave a mixture of macrocyclic isomers containing *E*- and *Z*-alkene groups, and unreacted **30** was also recovered. Hydrogenation of the mixture produced **31** and **32**. These compounds were readily separated by chromatography. The removal of the protecting groups from **31** and **32** was the last step of the route providing the acyclic derivative **7** and the rigid macrocyclic derivative **8** (Scheme 8).

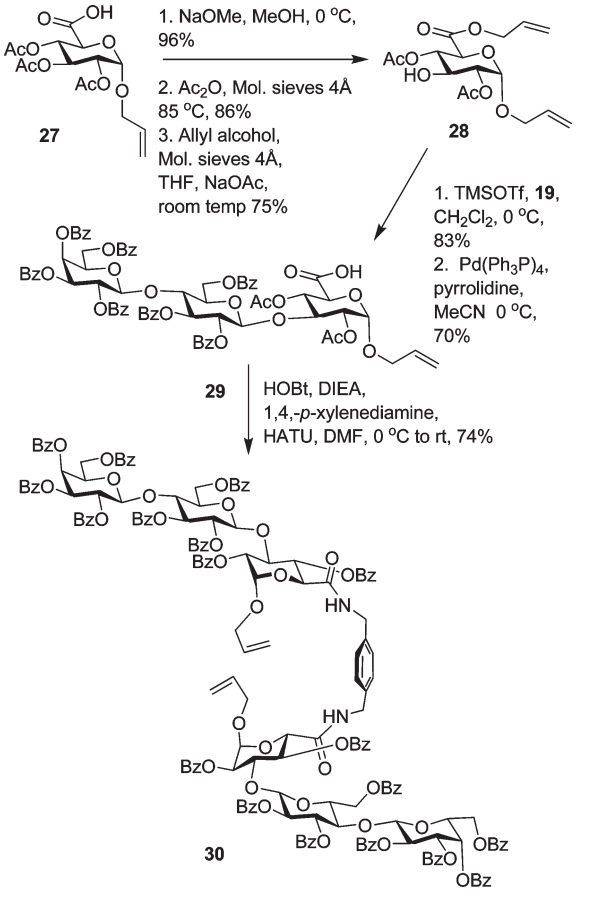
Finally, the bivalent lactoside **9** was prepared from **34** and the lactosyl azide **33** with the copper-catalyzed azide−alkyne cycloaddition reaction.[11] To facilitate purification of **9**, the product from the click reaction was acetylated, purified by chromatography, and subsequently deacetylated (Scheme 9). Having herewith completed the synthetic part, we next proceeded to calculate geometrical aspects of ligand positioning in the bivalent systems.

Analysis of Ligand Spacing. The use of rigid scaffolds presenting ligands can avoid entropic penalties that occur upon binding to a receptor, particularly if they are preorganized into a constellation favorable for binding. If the two sugar headgroups react with different lectin molecules, then orientation and spacing may also have a bearing on bioactivity. The aim to relate structure to function explains our interest to calculate interligand spatial relationships for the bivalent lactosides. At the outset, the panel of compounds was separated into two categories based on their relative flexibility. Two parameters were chosen to define the interligand spacing: the distances between the two Glc C-1 atoms of the lactose residues was defined as the interlactose distance whereas the torsion Gal C4−Glc C1−Glc C1−Gal C4 corresponding to the lactose residues was defined as the

SCHEME 6. Proposed Route of Formation of 26



SCHEME 7. Synthesis of 30

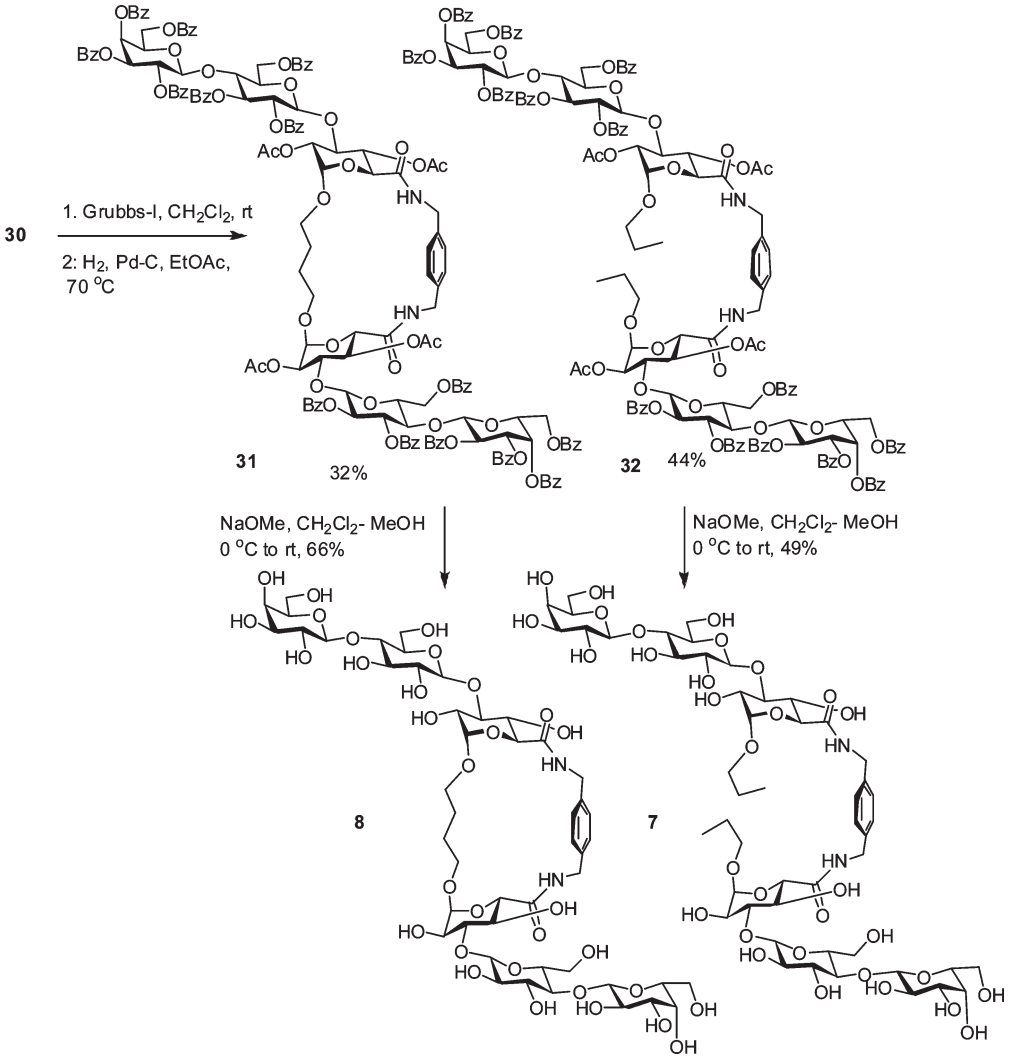


lactose orientation (see Figure 2D). Since the β-lactosides **1**−**5** and **8** were judged to have fewer degrees of freedom when compared to **6**, **7**, and **9**, conformational analysis was focused on the former structures. When calculating the spatial relationships between the two lactose residues in compounds **1**−**5** and **8**, we used Macromodel for model building and all subsequent computations.[12] Experimentally observed

(11) (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021. (b) Meldal, M.; Tornoe, C. W. *Chem. Rev.* **2008**, *108*, 2952–3015. (c) Leyden, R.; Murphy, P. V. *Synlett* **2009**, 1949–1950.

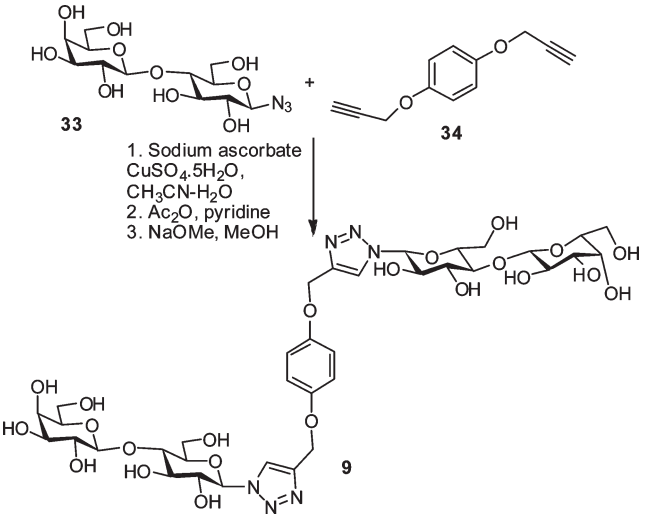
(12) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.

SCHEME 8. Synthesis of 7 and 8



glycosidic torsion angles (ϕ = O5−C1−O1−Cx; ψ = C1−O1−Cx−C(x+1); ω = O5−C5−C6−O1) were used to guide the building[13] of model structures establishing the structure from which conformational searching commenced. In detail, the glycosidic torsions from a published crystal structure of a β-lactoside[14] ($\phi = -88$; $\psi = -161$) defined the corresponding torsions in the lactose residues of the model structures. To complete the models of **3**−**5** additional torsions for the glycosidic linkage between the glucose residue of the scaffold and the glucose residue of the lactose needed to be defined; the ϕ, ψ, and ω values were again obtained from X-ray crystal structural data. For the β-D-Glc(1−6)-β-D-Glc linkage in **3** the ϕ, ψ, and ω were −59°, −155°, and −61°, respectively;[15] for the β-D-Glc(1−4)-β-D-Glc linkage in **4**, the ϕ and ψ used were −76° and 132°;[16] for the β-D-Glc(1−3)-β-D-Glc linkage in **4**, the ϕ and ψ used were −94° and 78°.[17]

SCHEME 9. Synthesis of 9



Equally relevant studies on the structural orientations of *N*-glycosyl benzamides had been carried out previously, providing a basis to define the preferred torsional parameters

(14) Qingfeng, P.; Noll, B. C.; Serianni, A. S. *Acta Cryst. C* **2005**, *61*, 674–677.
(15) Arene, F.; Newman, A.; Longchambon, F. *C. R. Acad. Sci.* **1979**, *288*, 331–334.
(16) Chu, S. S.; Jeffrey, G. A. *Acta Cryst. B* **1968**, *24*, 830–8.
(17) Noguchi, K.; Okyama, K.; Kitamura, S.; Takeo, K. *Carbohydr. Res.* **1992**, *237*, 33–42.

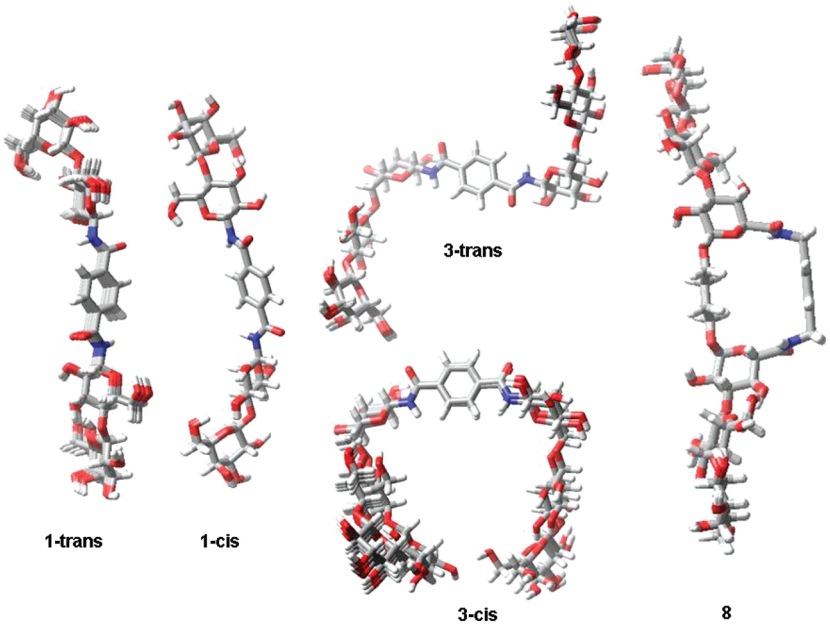


FIGURE 1. The ten lowest energy structures for **1**, **3**, and **8** generated by conformational search with Macromodel are superimposed. For **1** and **3** structures were obtained where the terephthalamide torsion was *cis* or *trans* and these are shown separately.

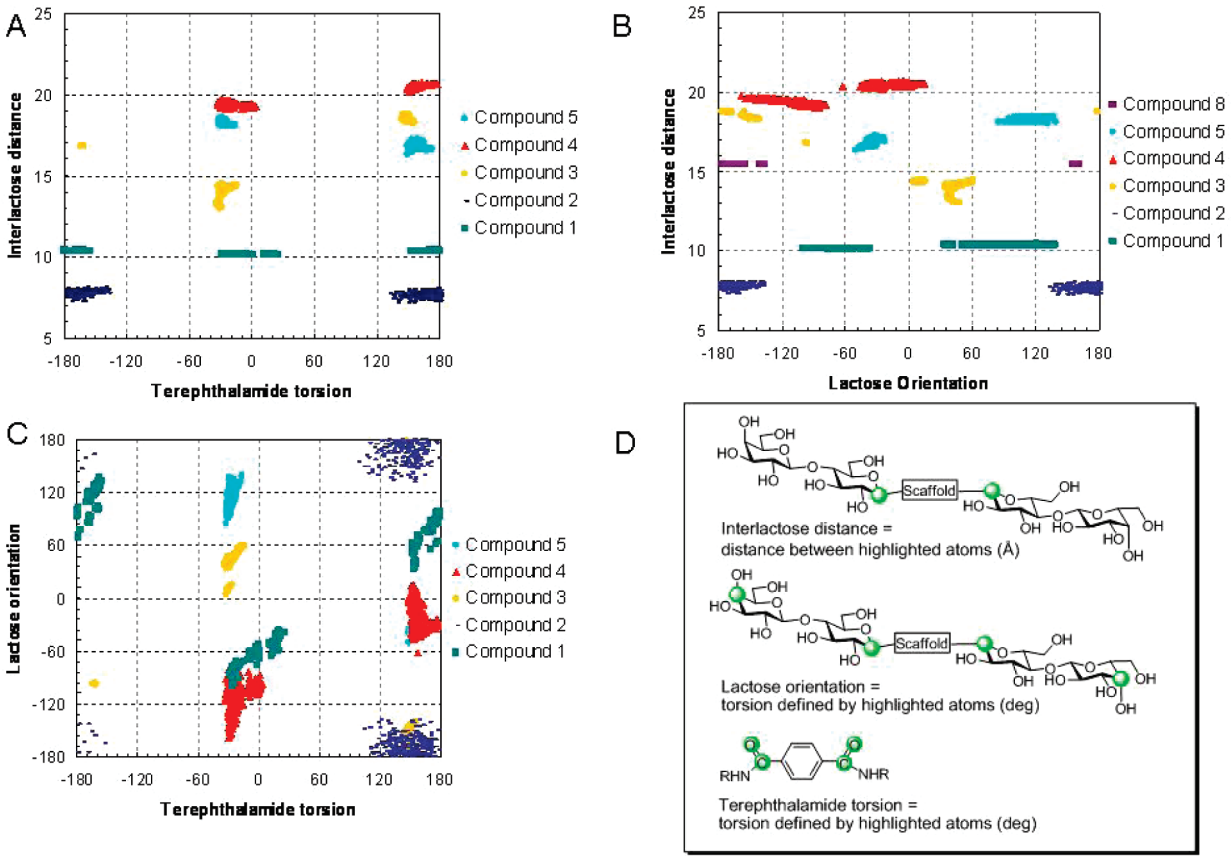


FIGURE 2. Structural profiles of low-energy conformational isomers of **1**−**5** and **8** (A−C) and definition of parameters (D). Data for **2** were obtained from stochastic molecular dynamics simulations for the isomer where the two carbonyl groups of the terephthalamide residue were *trans*. Data for other compounds was obtained from low-energy structures within 12 kJ/mol of minimum obtained by systematic conformational searches.

for glycosyl amide residues of **1**−**5**. Thus *N*-glycosyl benzamides that contain secondary amides adopt the *Z*-configuration (or *trans* amide), whereas those that are tertiary amides prefer the *E*-configuration (*cis* amide). The glycosyl amide torsion defined by H1−C1−N−H was constrained at a value of 171° (*anti*-conformation); this is close to the average of

the angles observed (168.2° to 175.0°) in a number of X-ray crystal structures of related secondary glycosyl amides and supported by ^{1}H NMR ($J_{H1-NH} \approx 9.0$ Hz) in solution.[18] The two carbonyl groups of the terephthalamide residues were constructed with a *trans* coplanar arrangement (dihedral defined by O═C—C═O 180 ± 60°) for **1** and **3**−**5**. During modeling of **2**, the aromatic groups were not coplanar with the carbonyl groups for reasons discussed previously.[6] All these parameters combined facilitated the establishment of the initial models of **1**−**5**. A starting structure of the glycocyclophane derivative **8** was also established and together with the models of **1**, **2**, and **3**−**5** was used to start systematic conformational searches; for **1** and **2** stochastic molecular dynamics was also explored. The AMBER (assisted model building with energy refinement) force field and the GB/SA continuum for water in Macromodel were generally applied. For the conformational searches all isomers within 12 kJ/mol of the global minimum were retained and used in the subsequent analysis. The terephthalamides containing secondary amides (**1**, **3**−**5**) consistently gave low-energy structures where the carbonyl groups of the terephthalamides were either *cis* or trans (shown for low-energy structures of **1** and **3** in Figure 1). This torsion, defined by O═C—C═O atoms of the terephthalamide residue, is referred to as the terephthalamide torsion subsequently. Our analysis (Figure 2A,C) for all the low-energy structures generated showed how the terephtalamide torsion affected interlactose distance and lactose orientation. For **1** the interlactose distance was constrained between 10.1 and 10.4 Å for all low-energy structures. Increased flexibility was observed for **3** where interlactose distance ranged from 13.0 to 18.8 Å, whereas intermediate flexibility was observed for **4** (19.1−20.7 Å) and **5** (16.3−17.2 and 18.0−18.4 Å). In all low-energy conformers of **8** (Figure 1), which was the most rigid structure, the glucuronic acid residues inherent in the macrocycle adopted a *cis* relationship (or a U-shape) with respect to the benzene residue, as was also found in related macrocycles derived from phenylene-1,4-diamine. The interlactose distance was firmly constrained between 15.4 and 15.5 Å in **8**. Conformational searches on **2** led to highly folded low-energy structures which were deemed unlikely to be accessed by lectins and which possibly were artifacts. Thus, a stochastic molecular dynamics simulation instead explored the conformational space for **2** and commenced from a structure where the carbonyl groups of the terephthalamide residue were *trans* (O═C—C═O torsion ~90°). In the molecular dynamics runs a temperature of 300 K, time step of 1.5 fs, equilibration time of 1.0 ps, and simulation time of 2 ns were set and 500 structures were sampled over the duration of the simulation. The result derived from the sampled structures is also included in Figure 2. The interlactose distance was found to be constrained between 7.2 and 8.1 Å for **2**. Similar stochastic molecular dynamics simulations were also performed for **1** (not shown), coming up with reasonable correlations between the interlactose distances and lactose orientations of structures generated in the dynamics simulations and in conformational searching.

In addition, plots were generated for each set of conformers showing the relationship between interlactose distance and lactose orientation (Figure 2B), which further demonstrated the unique spatial profile for each molecule. This analysis was also useful to depict the constraints imposed on each of the molecules. For example, for **5** there are two low-energy clusters: the first corresponds to a set of conformers with a lactose orientation that varies from −50° to −22° and a corresponding interlactose distance of 16.3−17.2 Å; the second cluster is a set of conformers where the lactose orientation varies between 84° and 139° and a corresponding interlactose distance of 18.0−18.4 Å. In general, the analysis shows that, while the subset of glycoclusters can adopt a range of interlactose distances (~7−21 Å) as well as a very large range of interlactose orientations from −180° to +180°, not all regions of space definable by both these parameters are occupied. Running a similar conformational search for the triazole derivative **9** indicated that it should be more flexible than the selected rigid subset. For **9** the interlactose distance was shown to vary from 9 to 17 Å and the lactose orientation varied between −165° and + 165°, suggesting it can have spacing arrangements not accessible to **1**−**5** and **8**. This would be the case also for **6** and **7**. If bioactive, it will thus be a key question whether flexible compounds such as **6** and **9** or less flexible substances will exhibit relatively higher potency for interfering with lectin activity. Having prepared the panel of glycoclusters and examined their conformational properties, the next step was therefore to ascertain the implied bioactivity of the carbohydrate headgroups and, if possible, to establish structure−activity profiles for inhibitory lectin binding.

Inhibition Studies. At the outset, we performed hemeagglutination tests classical to lectin research.[1] Using trypsin-treated, glutaraldehyde-fixed rabbit erythrocytes, the activity of lactose or the glycoclusters was assessed for their ability to inhibit lectin-mediated hemeagglutination in a total volume of 50 μL. Lactose (but not controls such as mannose) was effective at blocking lectin activity at concentrations of 2 and 0.8 mM, respectively, when using the plant toxin (25 ng/50 μL) and galectin-3 (2 μg/50 μL). The sugar units in compound **9** matched the activity of free lactose but no inhibitory activity was seen for any other test substance up to a concentration of 25 mM (given as sugar concentration). To infer whether the setting of this assay, where cell agglutination was the measured parameter, may have a bearing on sensitivity to pick up compound activity, we next employed an assay that measured the ability of a glycocluster to inhibit binding, in this case to a surface-immobilized glycoprotein. In the solid-phase setting, the extent of the lectin−glycoprotein interaction and inhibition thereof are determined spectrophotometrically.

To set up optimal conditions for this assay the coating density and lectin concentration were systematically tested until the dependence of signal intensity on lectin concentration was in the linear range. The signal was shown to be consistently dependent on the protein−carbohydrate interaction, as revealed by haptenic inhibition with lactose. Under these subsaturating conditions, the sensitivity to determine inhibitory potency of the synthetic compounds was optimal. As seen in Figure 3, glycoclusters from the synthetic panel blocked lectin binding to the glycans of the glycoprotein with higher efficiency than free lactose. Obviously, the conjugation chemistry did not impair the bioactivity, and attachment to a scaffold could enhance the

(18) Rawe, S. L.; Doyle, D.; Zaric, V.; Rozas, I.; McMahon, K.; Tosin, M.; Mueller Bunz, H.; Murphy, E. P.; O' Boyle, K. M.; Murphy, P. V. *Carbohydr. Res.* **2006**, *341*, 1370–1390.

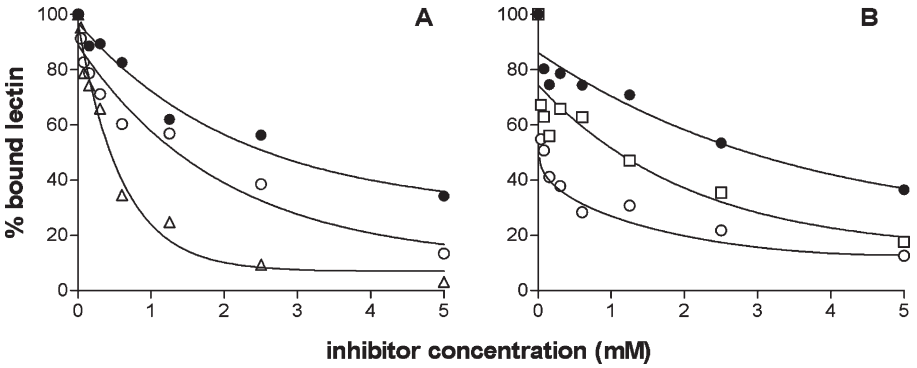


FIGURE 3. Inhibition of binding of biotinylated human galectin-3 (A) and human galectin-4 (B) to the glycans of surface-immobilized asialofetuin by lactose (●) and the lactose-presenting compounds **2** (○), **6** (Δ), and **8** (□) (see Table 1 for IC_{50} values). The inhibitor concentration given is normalized to lactose for direct comparison. Assays were routinely done in triplicates for up to five independent series with standard deviations not exceeding 17%.

inhibitory potency of lactose. Titrations were used to determine the concentration required to reach 50% inhibition (IC_{50}; Table 1). The inhibitory effects were most pronounced for the proteolytically truncated form of human galectin-3 and for galectin-4, when using ASF as matrix. The minimal interlactose distance observed for compound **2** translated into potent inhibition in these two cases. In comparison, the plant toxin (VAA), where the two readily accessible lectin sites of the 2γ subdomains are separated by 87 Å,[7g] showed no notable response to cluster formation or scaffold rigidification. Even more important, the unprocessed form of galectin-3 was much less sensitive to the presence of **2**. Thus, inhibitory capacity depended on the type of lectin and scaffold used to present the lactose as well as lectin-site presentation (e.g., galectin-3 vs. truncated galectin-3). It might also depend on matrix properties. To address this issue the solid-phase assay was carried out with a glycoprotein with only one *N*-glycosylation site, this strictly bearing bi- instead of triantennary *N*-glycans, i.e. serum amyloid P component (SAP). The effect of using SAP as the matrix was thus to reduce the glycan microdensity. In this case, enhancements of inhibitory activity were measured for galectin-3, with all compounds being at least 5-fold more active than free lactose and substances **4**−**9** being the most potent reaching up to a 50-fold activity increase. In contrast, toxin binding remained less susceptible. Considering this aspect and attaining increased physiological relevance, we next probed binding of the plant toxin and galectin-3 to the surface of native cells, in this case human B-lymphoblastoid Croco II cells. Interference by the glycoclusters was quantified.

All measurements were routinely performed with aliquots of the same cell batch and passage, prolonged culture periods with inevitable impact on cell features were hereby avoided. The extent of carbohydrate-dependent cell binding was experimentally determined by fluorescent staining in FACScan analysis, obtaining the percentage of positive cells and signal intensity on a logarithmic scale as read-out, as presented in Figure 4. The binding of the labeled lectin to the cell surface shifted the curve representing the staining profile of each lectin-exposed cell population from the position of the control (given as the shaded area in each figure), and the presence of an effective inhibitor will move the curve back into the direction of the control. Optimal conditions with a subsaturating lectin concentration and a lactose concentration in the linear range of signal intensity were identified first by systematic testing (Figure 4A,B). Routinely, measurements were done in triplicates with up to three independent series. As exemplarily illustrated in Figure 4C−F, compounds **6** and **9** were considerably more active than free lactose to block plant toxin binding to the cells, which correlated reasonably well with the binding data obtained from the solid-phase assay. In the case of galectin-3, the measurements (scans not shown) came up with a broadly similar trend as observed in the solid-phase assay, and **8** was less potent than **6** and **9** in this setting. In detail, five compounds superseded the activity of free lactose. With standard deviations of less than 11%, the difference between the flexible and rigid compounds **6** and **8** seen in solid-phase assays remained with respective values of 34.8 vs. 39.8 relative to 49.9 for lactose in mean intensity (at 0.5 mM sugar concentration), whereas compounds **2** and **3** were less potent than lactose with intensity values of 51.8 and 61.6. Compound **9** also resulted in marked inhibition to 35.1 in intensity. Thus, the substances **6** and **9** are particularly effective to reduce lectin association to this type of native human cells. Flexibility of bivalent compounds thus appears to be a favorable factor when aiming to inhibit the toxin and galectin-3.

TABLE 1. IC_{50} Values (mM per lactose residue) for Blocking Binding of Lectins to Surface-Immobilized ASF

compd	lactose units per molecule	VAA[a] [0.5 μg mL^{-1}]	galectin-3 [5 μg mL^{-1}]	galectin-3 (truncated) [30 μg mL^{-1}]	galectin-4 [5 μg mL^{-1}]
1	2	0.7	1.85	0.15	0.3
2	2	1.4	1.6	0.06	0.08
3	2	1.3	2.1	0.15	1.1
4	2	0.9	0.7	0.6	0.12
5	2	0.8	1.8	0.15	0.3
6	2	0.3	0.6	0.15	0.12
7	2	0.9	1.4	5	0.3
8	2	1.2	1.6	0.3	1.1
9	2	0.4	1.2	0.15	0.15
11	2	1.0	1.8	0.08	0.15
lactose	1	0.7	2.7	0.6	2.5

[a] *Viscum album* agglutinin/toxin.

Summary and Conclusions

We have reported the synthesis of different types of bivalent lactosides based on terephthalamides and glycocyclophanes as well as other analogues. Rather rigid

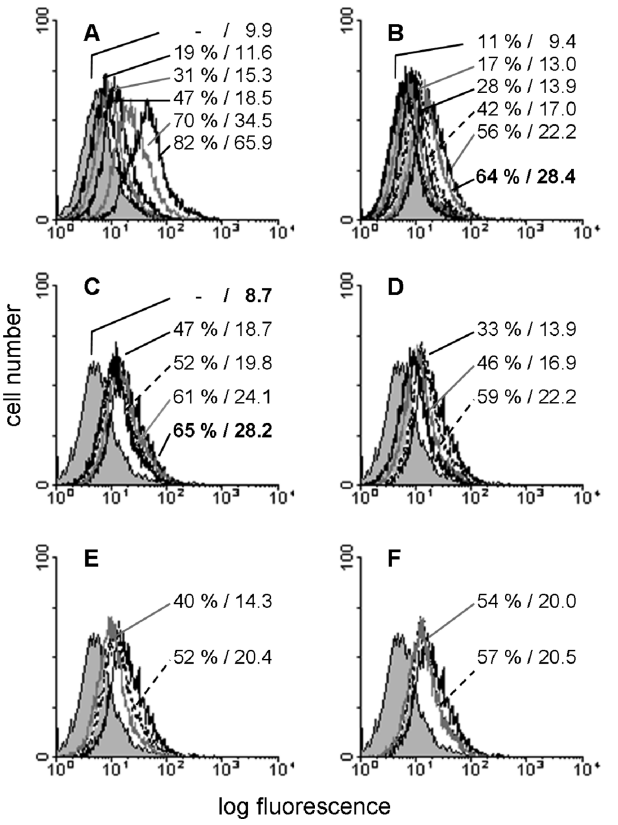


FIGURE 4. Illustration of binding of labeled plant toxin to cells of the human B-lymphoblastoid line Croco II as a function of lectin concentration (A), of the presence of lactose as haptenic inhibitor (B), and of the compounds at a fixed concentration of 2 mM lactose per assay (C−F). Controls in the absence of lectin and in the absence of inhibitor are given by the shaded area and the solid black line. Quantitative data on the percentage of positive cells and mean fluorescence intensity are listed in each panel. Lectin concentrations at 0.05, 0.1, 0.2, 0.5, and 1 μg/mL resulting in signals of increasing level (A); assays at 0.4 μg/mL lectin with increasing concentrations of lactose at 1, 2, 4, 20, and 40 mM document corresponding decreases of level of signal (B). Using a constant concentration of 2 mM normalized to the sugar content the panel of test substances was assayed: compounds **2** (thin gray line), **1** (dashed black line), and **4** (black line) in panel C where data for positive and negative controls are listed as bold numbers, compounds **3** (dashed black line), **5** (thin gray line), and **6** (black line) in panel D, compounds **8** (dashed black line) and **9** (thin gray line) in panel E, and compounds **7** (dashed black line) and **11** (thin gray line) in panel F.

compounds were characterized computationally with respect to geometry of presentation of the sugar headgroups. Bioactivity of the glycocompounds was systematically tested in hemeagglutination as well as solid-phase and cell assays. Haemagglutination as a test system proved rather insensitive, where only one compound was found to be active as inhibitor of lectin-dependent erythrocyte agglutination. In contrast, measuring the extent of lectin binding to glycoproteins on a solid phase or on cell surfaces as the experimental end point disclosed remarkable bioactivity. These experiments added substantial support to the concept that both terephthalamide and glycocyclophanes[19] can be considered as suitable scaffolds for presentation of carbohydrate ligands.[20,21] Either introducing structural changes to a scaffold (e.g., compare **1**, **2**, and **8**) or strategically grafting a lactose residue to different locations of a common scaffold (compare **3**−**5**) led to diverse interlactose spacing. These changes in geometry can apparently factor into the observed alterations for inhibitory capacity, especially for the truncated form of galectin-3 and the tandem-repeat-type galectin (galectin-4). In addition, increasing rigidity of a scaffold by macrocyclisation (**6** and **7** < **8**) also affects lectin reactivity to bivalent scaffolds, with a trend toward activity loss in assays with the plant toxin as compounds become more rigid. Protection of cells from association with the plant toxin was best achieved with the flexible compound **6**, in accord with the data from solid-phase assays. The relatively increased activity for the flexible derivatives could be due to their ability to access favorable ligand spacings not possible for the more rigid compounds. In addition, the chemical characteristics of the spacer and its environment may also play a role in enhancing binding properties, as does the choice of glycoprotein used as matrix and the cell surface, rendering it difficult to draw unambiguous a priori conclusions.

The structural analysis indicates that it is unlikely that compounds exert their inhibition by binding to multiple carbohydrate recognition sites on one lectin as would be the case, for example, for the glycoside cluster effect of triantennary *N*-glycans homing in on the hepatic asialoglycoprotein receptor; it seems more likely that the effects would be due to bivalent compounds binding to more than one lectin molecule, possibly promoting lectin clustering. At any rate, the sugar headgroups maintained bioactivity after conjugation and attained differential reactivity to the tested human lectins, as seen especially for compound **2** and the truncated form of galectin-3 and galectin-4 with a more than 10-fold and even higher increase of sugar activity relative to free lactose. This gain in activity seen in solid-phase assays could be due to constraint of the lactose residues into a favorable presentation at minimal interlactose distance, a benefit of using a scaffold based on the tertiary terephthalamide. The differential activity of **2** between galectin-3 and its naturally derived truncated form illustrates the potential to exploit physiological changes in binding-site presentation of a lectin for selective inhibition. Synthesis of a bivalent structure **11** incorporating an α-lactosyl amide linkage led to notable activity against the two galectins noted above but

(19) For applications of both cyclic peptides and cyclophanes as scaffolds for multivalent ligand display see: (a) Hayashida, O.; Hamachi, I. *J. Org. Chem.* **2004**, *69*, 3509–3516. (b) Wittmann, V.; Seeberger, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 4348–4352. (c) Ohta, T.; Miura, N.; Funitani, N.; Nakajima, F.; Niikura, K.; Sadamoto, R.; Guo, C.-T.; Suzuki, T.; Suzuki, Y.; Yasuo, M.; Monde, K.; Nishimura, S.-I. *Angew. Chem., Int. Ed.* **2003**, *42*, 5186–5189.

(20) Hirschmann, R. *Angew. Chem., Int. Ed.* **1991**, *30*, 1278.

(21) The application of carbohydrates and carbohydrate-derived structures as scaffolds for presentation of recognition groups in chemical biology and medicinal chemistry has recently been reviewed. (a) Yamazaki, N.; Kojima, S.; Bovin, N. V.; André, S.; Gabius, S.; Gabius, H.-J. *Adv. Drug Delivery Rev.* **2000**, *43*, 225–244. (b) Murphy, P. V.; O' Brien, J. L.; Gorey-Feret, L. J.; Smith, A. B., III *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1763–66. (c) Meutermans, W.; Le, G. T.; Becker, B. *ChemMedChem* **2006**, *1*, 1164–1194. (d) Murphy, P. V.; Dunne, J. L. *Curr. Org. Synth.* **2006**, *3*, 403–437. (e) Velter, I.; La Ferla, B.; Nicotra, F. *J. Carbohydr. Chem.* **2006**, *25*, 97–138. (f) Murphy, P. V. *Eur. J. Org. Chem.* **2007**, 4177–4187. (g) Murphy P. V.; Velasco-Torrijos, T. In *Glycoscience-Chemistry and Chemical Biology*, 2nd ed.;. Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds.; Springer-Verlag: Berlin-Heidelberg, Germany, 2008; p 997. (h) Chabre, Y. M.; Roy, R. In *The Sugar Code. Fundamentals of glycosciences*; Gabius, H.-J.,Ed.; Wiley-VCH: Weinheim, Germany, 2009; pp 53−70.

not the plant toxin. In sum, the conjugation chemistry is compatible with the long-term aim to develop inhibitors with selectivity to certain members among a family of medically relevant human lectins. Inhibitory efficiency was obtained with bivalent compounds, offering the perspective to increase ligand density. Also of note for further work, fine-specificity differences between homologous lectins could be exploited by respective tailoring of the structure of the headgroup, hereby reducing cross-reactivity.

Experimental Section

***N,N′*-Di(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl)terephthalamide 1 and *N*-(β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl)-*N′*-(β-D-galactopyranosyl-(1→4)-α-D-glucopyranosyl)terephthalamide 11.** The lactosyl amine **10**[22] (0.473 g, 0.744 mmol) and DIPEA (0.16 mL, 0.967 mmol) in dry THF (10 mL) were added dropwise at rt into freshly recrystallized terephthaloyl chloride (68 mg, 0.335 mmol) in dry THF. After 2 h the solvent was removed and chromatography of the residue (EtOAc−cyclohexane, gradient elution, 1:1 to 3:2 to 2:1) gave a mixture of diamides as a white amorphous solid (0.432 g, 92%). Deacetylation of the diamide mixture (0.187 g, 0.133 mmol) gave a 79:20:1 mixture of **1**, **11**, and **12** (0.108 g, quantitative yield). Semipreparative HPLC (isocratic elution with water−CH_3CN, 99:1, flow rate 10 mL/min) was used to separate **1**, **11**, and **12**. Analytical data for **1**: $[\alpha]_D$ +58.7 (*c* 0.7, H_2O); ^{1}H NMR (500 MHz, D_2O, 30 °C) δ 7.94 (s, 4H), 5.24 (d, J = 9.2 Hz, 2H), 4.48 (d, J = 7.8 Hz, 2H), 3.96 (d, J = 12.0 Hz, 2H), 3.93 (d, J = 3.3 Hz, 2H), 3.85 (dd, J = 12.3 Hz, J = 2.3 Hz, 2H), 3.83−3.71 (m, 12H), 3.67 (dd, J = 10.0 Hz, J = 3.4 Hz, 2H), 3.62 (t, J = 9.0 Hz, 2H), 3.57 (dd, J = 9.9 Hz, J = 7.9 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 171.2 (*C*ONH), 136.8 (C), 128.2 (CH), 103.1, 80.0, 78.0, 76.8, 75.6, 75.4, 72.8, 71.7, 71.2, 68.8 (each CH), 61.3, 60.1 (each CH_2); IR (KBr) 3481−3259 (br signal), 1657, 1545, 1383, 1298, 1082, 1041 cm^{-1}; HRMS (ESI) found 811.2609 $[M - H]^-$, $C_{32}H_{47}N_2O_{22}$ requires 811.2620, and found 835.2626 $[M + Na]^+$, $C_{32}H_{48}N_2NaO_{22}$ requires 835.2596. Analytical data for **11**: $[\alpha]_D$ +42.6 (*c* 0.96, H_2O); ^{1}H NMR (500 MHz, D_2O, 25 °C) δ 7.93 (2 d, J = 8.4 Hz, 2H), 5.83 (d, J = 4.6 Hz, 1H), 5.27 (d, J = 9.2 Hz, 1H), 4.51 (dd, J = 7.8 Hz, J = 4.1 Hz, 1H), 4.00 (br s, 1H), 3.98 (br s, 1H), 3.96 (s, 1H), 3.89 (s, 2H), 3.86−3.73 (m, 6H), 3.72−3.68 (m, 1H), 3.66 (t, J = 9.0 Hz, 1H), 3.59 (dt, J = 10.4 Hz, J = 7.9 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 172.0, 170.9 (each CO), 136.9, 136.2 (each C), 128.0, 127.8, 102.8, 79.7, 77.1, 78.0, 77.7, 76.5, 75.3, 75.1, 72.5, 71.7, 71.6, 71.4, 70.9, 69.2, 68.5 (each CH), 61.0, 59.8 (each CH_2); HRMS (ESI) found 835.2635 $[M + Na]^+$, $C_{32}H_{48}N_2NaO_{22}$ requires 835.2596.

***N,N′*-Di(β-D-galactopyranosyl-β-(1→4)-β-D-glucopyranose)-*N,N′*-di[(1-methoxycarbonyl)methylamino-2-oxoethyl]terephthalamide 2.** Terephthalic acid (73 mg, 0.44 mmol), amine **10** (0.558 g, 0.878 mmol), and formaldehyde (80 μL of a 37% soln, 1.023 mmol) were suspended in MeOH (13 mL) and the mixture was stirred at rt for 1 h. Methyl isocyanoacetate (80 μL, 0.878 mmol) was then added and the mixture was stirred at rt for 18 h. The reaction was then heated to 45 °C for 6 h and then cooled to rt and left to stir for a further 18 h and then solvent was removed under diminished pressure. Chromatography of the residue (gradient elution, EtOAc to EtOAc−MeOH, 98:2), obtained after removal of solvent, gave the protected intermediate as a white amorphous solid (0.352 g, 48%). Zemplén deacetylation of this intermediate (0.352 g, 0.212 mmol) gave a yellow powder (0.222 g, 98%) that was purified further by semipreparative HPLC to give **2** as an interconverting mixture (83:38) of *EE* and *EZ* isomers;[6] $[\alpha]_D$ +18 (*c* 1.22, H_2O); ^{1}H NMR (600 MHz, D_2O, 30 °C) data for *EE* isomer: δ 7.70 (s, 4H), 4.85 (d, J = 8.9 Hz, 2H), 4.42 (d, J = 7.7 Hz, 2H), 4.37 (d, J = 14.5 Hz, 4H), 4.12 (d, 4H), 3.97 (dd, J = 12.5 Hz, J = 1.5 Hz, 2H), 3.90 (d, J = 3.1 Hz, 2H), 3.83−3.72 (m, 10H), 3.73−3.62 (m, 10H), 3.57−3.47 (m, 6H); selected ^{1}H NMR data for the EZ isomer: δ 7.67 (d, J = 6.0 Hz, 2H), 7.61 (d, J = 5.9 Hz, 2H), 5.77 (d, J = 9.2 Hz, 2H, H-1), 4.48 (d, J = 7.7 Hz, 2H, H-1′); ^{13}C NMR (125 MHz, D_2O) data for EE isomer: δ 174.4, 172.2 (*C*O_2Me, *C*ONH), 136.1 (C), 127.9, 103.1, 87.5, 77.8, 73.0, 75.6, 74.3, 72.7, 71.1, 70.1, 68.8 (each CH), 61.3, 60.3 (each CH_2), 53.1 (CH_3), 45.0, 41.6 (each CH_2); IR (KBr) 3456−3305 (br s), 1743, 1655, 1444, 1383, 1225, 1078 cm^{-1}; LRMS (ESI) 1069.5 $[M - H]^-$, 1093.1 $[M + Na]^+$; HRMS (ESI) found 1069.3503 $[M - H]^-$, $C_{42}H_{61}N_4O_{28}$ requires 1069.3472.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)- 2,3,4-tri-*O*-acetyl-1-azido-1-deoxy-β-D-glucopyranose 15. Trichloroacetamide **14**[23] (65 mg, 84 μmol), acceptor **13** (29.2 mg, 84 μmol),[24] and molecular sieves 4 Å (166 mg) were dried at high vacuum for 2 h. Dry dichloromethane was then added (2 mL) and the solution was stirred for 2 h at rt. TMSOTf (1.54 μL, 8.4 μmol) was added and the mixture was stirred for 30 min at rt. Satd $NaHCO_3$ was then added and the stirring continued for a further 30 min. The mixture was then filtered through Celite, washing with dichloromethane (5 mL). The solvent was removed and chromatography of the residue (EtOAc−cyclohexane, 3:2) gave a protected trisaccharide intermediate (42 mg, 52%) as a white powder. This protected trisaccharide (0.966 g, 1 mmol) was dissolved in dry CH_2Cl_2 (20 mL) under N_2. Azidotrimethylsilane (340 μL, 2.5 mmol,) and tin(IV) chloride (60 μL, 0.5 mmol) were added and the mixture was stirred for 15 min. Solid $NaHCO_3$ (0.5 g) was then added and stirring continued for an additional 30 min. The reaction mixture was then diluted with dichloromethane (20 mL), washed with satd $NaHCO_3$ (2 mL) and water (30 mL), dried ($MgSO_4$), and filtered, then the solvent was removed under vacuum. Chromatography of the residue (cyclohexane−EtOAc, 4:6) afforded **15** as a white solid (0.78 g, 82%); mp 104 °C; $[\alpha]^{20}_D$ −12 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 5.35 (d, J = 3.9 Hz, 1H), 5.19 (t, J = 9.3 Hz, 1H), 5.19 (t, J = 9.0 Hz, 1H), 5.11 (dd, J = 7.8 Hz, J = 10.2 Hz, 1H), 4.96 (dd, J = 10.2 Hz, J = 3.3 Hz, 1H), 4.94−4.86 (m, 4H), 4.63 (d, J = 9.0 Hz, 1H), 4.55−4.48 (m, 3H), 4.15−4.05 (m, 3H), 3.90−3.73 (m, 4H), 3.80 (t, J = 9.3 Hz, 1H), 3.63−3.56 (m, 2H), 3.52 (dd, J = 10.8 Hz, J = 6.0 Hz, 1H), 2.15, 2.13, 2.10, 2.07, 2.06, 2.05 (2s), 2.03, 2.00, 1.97 (each s); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.4, 170.3, 170.2, 170.0, 169.5, 169.3, 169.2 (each CO), 101.2, 100.6 (each CH), 87.8 (CH), 76.2, 75.4, 72.9, 72.7, 71.5, 71.1, 71.0, 69.2, 68.6 (each CH), 68.1 (CH_2), 66.7 (CH), 61.9, 60.9 (each CH_2), 20.9, 20.7, 20.6 (each CH_3); HRMS (ESI) found 972.2709 $[M + Na^+]$, $C_{38}H_{51}N_3NaO_{25}$ requires 972.2709.

***N,N′*-Di(β-D-galactopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl)terephtalamide 3.** Catalytic hydrogenation of the azide **15** (0.102 g, 0.105 mmol) gave the desired glycosyl amine intermediate as a white solid (96 mg, 99%). Reaction of this amine (0.132 g, 0.143 mmol) with terephthaloyl chloride (11 mg, 0.0715 mmol) as described for **10** gave, after chromatography (cyclohexane−EtOAc, 1:4 to 1:3), the protected diamide (0.114 g, 40%). Deacetylation (0.1 g, 0.57 mmol) gave **3** as a white powder (quantitative yield); $[\alpha]^{20}_D$ +2 (*c* 0.2, H_2O); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.96 (s, 4H), 5.25 (d, J = 8.4 Hz, 2H), 4.55 (d, J = 8.1 Hz, 2H), 4.45

(22) (a) Peto, C.; Batta, G.; Györgydeák, Z.; Sztaricskai, F., *Liebigs Ann. Chem.* **1991**, *5*, 505−507. (b) Aravind, S.; Park, W. K. C.; Brochu, S.; Roy, R. *Tetrahedron Lett*. **1994**, *35*, 7739–7742.

(23) Li, H.; Cai, M.-S.; Li, Z.-J. *Carbohydr. Res*. **2000**, *328*, 611–615.

(24) Lee, G. S.; Lee, Y.-J.; Choi, S. Y.; Park, Y. S.; Yoon, K. B. *J. Am. Chem. Soc*. **2000**, *122*, 12151–12157.

(d, J = 7.8 Hz, 2H), 4.25 (d, J = 10.5 Hz, 2H), 3.37−4.00 (m, 32H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 173.7 (CO), 139.3 (C), 130.8, 105.8, 105.2, 82.8, 81.3, 79.5, 79.3, 78.2, 77.6, 77.1, 75.6, 75.4, 74.5, 73.8 (each CH), 72.1 (CH_2), 71.4 (CH), 63.8, 62.9 (CH_2); HRMS (ESI) found 1159.3596 [M + H^+], $C_{44}H_{68}N_2NaO_{32}$ requires 1159.3653.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-1-*O*-methyl-α-D-glucopyranose 17. Glycosidation coupling of **16** with **14** as described for **13** gave, after chromatography (EtOAc−cyclohexane, 35:75 to 40:60), **17** (48%); mp 121 °C; $[\alpha]^{20}_D$ +51 (c 0.5, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.08 (d, J = 7.2 Hz, 2H), 8.00 (d, J = 7.5 Hz, 2H), 7.93 (d, J = 7.2 Hz, 2H), 7.62 (m, 1H), 7.50 (t, J = 7.2 Hz, 4H), 7.35 (m, 4H), 6.03 (t, J = 9.6 Hz, 1H), 5.29 (d, J = 3.3 Hz, 1H), 5.09 (m, 2H), 5.06 (t, 1H, J = 9.3 Hz, 1H), 4.99 (dd, 1H, J = 7.8 Hz, J = 10.5 Hz, 1H), 4.89−4.77 (m, 2H), 4.75 (d, 1H, J = 12.0 Hz), 4.68 (d, 1H, J = 7.8 Hz), 4.44 (dd, 1H, J = 12.0 Hz, J = 5.1 Hz, 1H), 4.27 (d, 1H, J = 7.8 Hz, 1H), 4.23−4.19 (m, 1H), 4.84 (dd, J = 10.2 Hz, J = 3.6 Hz, 1H), 4.50−4.46 (m, 2H), 4.11−3.97 (m, 4H), 3.79 (t, J = 6.9 Hz, 1H), 3.67−3.59 (m, 2H), 3.28 (m, 1H), 3.42 (s, 3H), 2.11, 2.04, 2.02, 1.92 (each s, each CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.4, 170.2, 170.1 (2s), 169.8, 169.7, 169.0, 166.1, 166.0, 165.0, 133.4 (each C), 130−128.4 (15 × CH), 101.1, 100.1, 96.9, 75.6, 73.0, 72.7, 72.4, 72.2, 71.1, 70.7, 70.4, 69.0 (each CH), 62.8, 61.8, 60.9 (each CH_2), 55.6 (CH), 20.9, 20.8, 20.7, 20.6 (each CH_3); HRMS (ESI) found 1147.3267 [M + Na^+], $C_{54}H_{60}NaO_{26}$ requires 1147.3271.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl Azide 18. Trisaccharide **17** (1.17 g, 1.00 mmol) was dissolved in acetic anhydride (10 mL) and an acetic anhydride−sulfuric acid mixture (1:50, 20 mL) was added and the mixture was stirred for 2 h at rt. Dichloromethane was then added (100 mL) and the organic layer washed with water (100 mL), satd $NaHCO_3$ (100 mL), and water (100 mL), dried ($MgSO_4$), and filtered. The solvent was removed under diminished pressure. Chromatography of the residue (cyclohexane−EtOAc, 7:3) gave the glycosyl acetate intermediate as a white solid (0.81 g, 70%). Reaction of this glycosyl acetate (0.8 g, 0.69 mmol) with azidotrimethylsilane (0.4 g, 3.45 mmol) and $SnCl_4$ as described for the preparation of **15** gave the title compound (0.6 g, 77%); mp 102 °C; $[\alpha]^{20}_D$ +7 (c 0.3, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.09 (d, J = 7.2 Hz, 2H), 8.00 (d, d, J = 7.2 Hz, 2H), 7.91 (d, J = 7.2 Hz, 2H), 7.63 (m, 1H), 7.53−7.48 (m, 4H), 7.37−7.27 (m, 4H), 5.75 (t, J = 9.3 Hz, 1H), 5.33 (t, J = 9.0 Hz, 1H), 5.29 (s, 1H), 5.05 (t, 1H, J = 9.0 Hz, 1H), 4.98 (dd, J = 9.0 Hz, J = 7.5 Hz, 1H), 4.90−4.80 (m, 4H), 4.65 (d, J = 7.8 Hz, 1H), 4.44 (dd, J = 12.0 Hz, J = 4.8 Hz, 1H), 4.26 (d, J = 7.8 Hz, 1H), 4.15 (dd, J = 9.0 Hz, 1H), 4.04 (m, 4H), 3.95 (d, J = 10.5 Hz, 1H), 3.77 (t, J = 6.9 Hz, 1H), 3.59 (t, J = 6.9 Hz, 1H), 3.50 (dd, 1H, J = 10.5 Hz, J = 5.1 Hz), 3.28 (m, 1H), 2.11, 2.00, 1.97, 1.93 (each s, 7 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 170.4, 170.2, 170.1, 170.0, 169.8, 169.6, 169.0, 166.0, 165.2, 165.0, 133.6 (each C), 130−128.5 (15 × CH), 101.0, 100.3, 88.0, 76.1, 75.6, 75.3, 72.9 (2s), 72.8, 72.0, 71.5, 71.0, 70.7, 69.0, 66.6 (each CH), 62.4, 61.8, 60.8 (each CH_2), 20.9, 20.8, 20.7, 20.6 (each CH_3); HRMS (ESI) found 1158.3213 [M + Na^+], $C_{53}H_{57}N_3NaO_{25}$ requires 1158.3179.

***N,N′*-Di(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl)terephtalamide 4.** Catalytic hydrogenation of azide **18** (0.21 g, 0.176 mmol) as described for **15** gave an amine intermediate. Reaction of the amine (0.25 g, 0.22 mmol) with terephthaloyl chloride (44 mg, 0.22 mmol) as described for the preparation of **15** and following chromatography (EtOAc−cyclohexane, 60:40 to 55:45) yielded the protected divalent compound as a white powder (63 mg, 25%). This intermediate (23.5 mg, 10 μmol) was dissolved in dry MeOH (1 mL). Potassium carbonate was then added (15 mg), and the resulting solution was stirred for 20 h at rt. The solvent was removed under reduced pressure and water was added (2 mL). Amberlite IR-120(plus) was added, until the pH reached 6. Water was then removed by lyophilization to obtain **4** (white powder) in quantitative yield; mp 187 °C; $[\alpha]^{20}_D$ −4 (c 0.1, CH_2Cl_2); ^{1}H NMR (300 MHz, D_2O) δ (ppm) 7.94 (s, 4H), 5.27 (d, J = 9.0 Hz, 2H), 4.54 (d, J = 8.1 Hz, 2H), 4.48 (d, J = 7.8 Hz, 2H), 4.00−3.54 (m, 36H), 3.41 (t, J = 8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 173.8, 139.4 (each C), 130.8, 105.8, 105.2, 82.0, 81.0, 80.8, 79.4, 78.2, 77.9, 77.7, 77.0, 75.7, 75.4, 74.4, 73.8, 71.4 (each CH), 63.9, 62.8, 62.7 (each CH_2); HRMS (ESI) found 1159.3639 [M + Na^+], $C_{44}H_{68}N_2NaO_{32}$ requires 1159.3653.

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-1,2:5,6-di-*O*-isopropylidene-β-D-glucofuranoside 21. Glycosidation coupling of trichloroacetimidate **19** (1.08 g, 0.89 mmol) with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **20** (0.193 g, 0.742 mmol) as described for **13** gave, after chromatography (cyclohexane−EtOAc, gradient elution, 4:1 to 3:1), **21** as a white foam (0.69 g, 71%); R_f 0.36 (cyclohexane−EtOAc, 2:1); $[\alpha]^{20}_D$ +33.2 (c 2.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.02−7.94 (m, 10 H), 7.90 (d, J = 7.3 Hz, 2H), 7.72 (dd, J = 8.4, J = 1.1 Hz, 2H), 7.65−7.45 (m, 8H), 7.44−7.28 (m, 9H), 7.23−7.14 (m, 4H), 5.79 (t, J = 9.4 Hz, 1H), 5.75−5.70 (m, 2H), 5.46 (d, J = 3.7 Hz, 1H), 5.41 (dd, J = 6.7, 3.0 Hz, 1H), 5.40 (dd, J = 6.4, 2.9 Hz, 1H), 4.89 (d, J = 7.9 Hz, 1H), 4.82 (d, J = 7.9 Hz, 1H), 4.59 (dd, J = 12.1 Hz, J = 1.5 Hz, 1H), 4.49 (dd, J = −12.2 Hz, J = 4.2 Hz, 1H), 4.32−4.17 (m, 5H), 3.97 (dd, J = 8.6 Hz, J = 6.5 Hz, 1H), 3.92 (dd, J = 8.5 Hz, J = 6.0 Hz, 1H), 3.91 (t, J = 6.4, 1H), 3.82 (ddd, J = 9.9 Hz, J = 3.9 Hz, J = 1.8 Hz, 1H), 3.75 (d, J = 6.7 Hz, 2H), 1.39, 1.32, 1.13, 1.09 (each s, each 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.8, 165.5, 165.4, 165.3, 165.2, 164.8 (2s) (each C), 133.5, 133.4 (2s), 133.3, 133.2, 129.7 (4s), 129.6 (2s), 129.5, 129.4 (2s), 129.1, 128.9, 128.7, 128.6 (3s), 128.5, 128.3, 128.2 (each CH), 111.9, 108.5 (each C), 104.9, 101.1, 99.8, 82.7, 81.4, 80.4, 75.9, 73.4, 73.0, 72.7, 71.7 (2s), 71.4, 69.9, 67.5 (each CH), 67.0, 66.1, 62.0 (each CH_2), 26.6, 26.5, 25.9, 25.0 (each CH_3); IR (KBr) 3064, 2985, 1730, 1603, 1453, 1269, 1093, 1070 cm^{-1}; HRMS (ESI) found 1335.3987 [M + $Na]^+$, $C_{73}H_{68}NaO_{23}$ requires 1335.4049.

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl Amine 22. A solution of **21** (0.59 g, 0.45 mmol) in 80% acetic acid (100 mL) was heated at 100 °C for 16 h. The solvents were then removed under diminished pressure. Methanol (2 × 10 mL) and CH_2Cl_2 (2 × 10 mL) were evaporated from the residue to give the desired trisaccharide intermediate as a white foam (quantitative yield, HRMS (ESI): 1255.3361 [M + $Na]^+$). This intermediate was dissolved in pyridine (5 mL) and acetic anhydride (5 mL) and the mixture was stirred for 15 h. The volatile reagents were removed and toluene (2 × 10 mL) and CH_2Cl_2 (2 × 10 mL) were evaporated from the residue. Chromatography (cyclohexane−EtOAc, gradient elution, 3.5:1 to 1:1) gave the protected glycosyl acetate intermediate as a white amorphous solid (0.51 g, 81% over two steps, mixture of anomers, α:β = 3:2). Reaction of this glycosyl acetate (0.492 g, 0.351 mmol) with azidotrimethylsilane (0.155 mL, 0.878 mmol) as described for preparation of **15** gave, after chromatography (cycohexane−EtOAc, gradient elution, 3:1 to 1:1), the glycosyl azide precursor to **22** as a white foam (0.294 g, 61%). Catalytic hydrogenation of this glycosyl azide (0.252 g, 0.182 mmol) as described for **15** gave, after chromatography (cyclohexane−EtOAc, 1:1 to EtOAc−CH_2Cl_2, 9:1), **22** as a white foam (0.179 g, 72%); R_f 0.44 (EtOAc−CH_2Cl_2, 9:1); $[\alpha]^{20}_D$ +12.6 (c 1.04, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04−7.95 (m, 8H), 7.90−7.82 (m, 4H), 7.71 (dd, J = 8.2 Hz, J = 1.0 Hz, 2H),

7.64−7.56 (m, 3H), 7.55−7.28 (m, 14H), 7.20 (t, J = 7.9 Hz, 2H), 7.13 (t, J = 7.8 Hz, 2H), 5.79 (t, J = 9.5 Hz, 1H), 5.71−5.67 (m, 2H), 5.37 (dd, J = 9.8 Hz, J = 8.0 Hz, 1H), 5.32 (dd, J = 10.3 Hz, J = 3.4 Hz, 1H), 4.93 (t, J = 9.6 Hz, 1H), 4.85 (d, J = 7.9 Hz, 1H), 4.80 (d, J = 7.9 Hz, 1H), 4.68 (t, J = 9.4 Hz, 1H), 4.58 (dd, J = 12.2 Hz, J = 1.4 Hz, 1H), 4.47 (dd, J = 12.3 Hz, J = 4.1 Hz, 1H), 4.29 (t, J = 9.5 Hz, 1H), 4.07−4.04 (m, 2H), 3.95 (d, J = 9.0 Hz, 1H), 3.87−3.76 (m, 4H), 3.66 (tt, J = 10.1 Hz, J = 5.2 Hz, 1H), 3.47 (td, J = 10.1 Hz, J = 3.6 Hz, 1H), 2.05, 1.92, 1.72 (each s, each CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 169.7, 168.4, 165.9, 165.8, 165.7, 165.6, 165.5, 165.4, 165.0 (each C), 133.9, 133.8 (2s), 133.7, 133.5 (2s), 13.4, 130.2, 130.1, 130.0, 129.9 (3s) (each CH), 129.7 (CH), 129.7 (2s), 129.5, 129.1 (each C), 128.9 (each CH), 128.9 (C), 128.8 (CH), 128.8 (C), 128.5 (2s), 128.4 (each CH), 101.4, 101.2, 85.4, 79.5, 75.9, 73.9, 73.3, 73.1, 73.0, 72.2, 72.0, 71.7, 70.2, 68.7, 67.8 (each CH), 62.8, 61.3 (each CH_2), 21.2, 21.0, 20.6 (each CH_3); IR (film on NaCl) 2958, 1730, 1375, 1267, 1092, 1068, 1028 cm^{-1}; HRMS (ESI) found 1358.4141 $[M + H]^+$, $C_{73}H_{68}NO_{25}$ requires 1358.4080.

***N,N′*-Di(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl)terephthalamide 5.** Reaction of the amine **22** (48.5 mg, 0.036 mmol) with terephthaloyl chloride as described for **10** gave, after chromatography (EtOAc−cyclohexane, gradient elution, 1:1 to 3:2), the protected diamide as a white amorphous solid (28.6 mg, 56%). Deprotection of the diamide (27 mg, 0.01 mmol) with the Zemplén procedure gave **5** (5.6 mg, 52%) as a white solid after semipreparative HPLC; $[\alpha]^{20}_D$ +512 (*c* 0.16); ^{1}H NMR (500 MHz, D_2O, 30 °C) δ 7.93 (s, 4H), 5.24 (d, J = 9.3 Hz, 2H), 4.83 (d, J = 8.0 Hz, 2H), 4.45 (d, J = 7.8 Hz, 2H), 3.99 (dd, J = 12.2 Hz, J = 1.8 Hz, 2H), 3.92 (d, J = 2.9 Hz, 2H), 3.91−3.86 (m, 2H), 3.84−3.74 (m, 10H), 3.74−3.61 (m, 14H), 3.61−3.57 (m, 2H), 3.54 (dd, J = 9.9 Hz, J = 7.8 Hz, 2H), 3.42 (dd, J = 9.3 Hz, J = 8.0 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 171.2, 136.7 (each C), 128.1, 103.2, 102.8, 80.0, 85.2, 78.6, 77.7, 75.6, 75.1, 74.5, 73.4, 72.8, 71.7, 71.2, 68.8, 68.0 (each CH), 61.3, 60.8, 60.3 (each CH_2); IR (KBr) 3473−3313 (br s), 1657, 1552, 1385, 1071, 1043 cm^{-1}; HRMS (ESI) found 1135.3638 $[M - H]^-$, $C_{44}H_{67}N_2O_{32}$ requires 1135.367.

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→3)-2,4-di-*O*-acetyl-1-azido-1-deoxy-β-D-glucopyranuronic Acid 25. Reaction of the trichloroacetimidate **19** (1.64 g, 1.35 mmol) with acceptor **23**[5,19c] (0.745 g, 2.18 mmol) as described for **13** gave, after chromatography (cyclohexane−toluene−EtOAc, 3:2:1), the glycosidic product as a white foam (0.62 g, 33%). To this intermediate (0.264 g, 0.189 mmol) in dry CH_3CN (2 mL) at 0 °C were added $Pd(Ph_3)_4$ (0.022 g, 0.019 mmol) and pyrrolidine (0.016 mL, 0.189 mmol), respectively, with stirring for 1 h. The mixture was then filtered through Celite and the solvent was removed. The residue was dissolved in EtOAc and the organic layer was washed with H_2O (2 × 15 mL). The water layer was then washed with EtOAc (3 × 15 mL) and the organic layers combined, dried ($MgSO_4$), and filtered, then solvent was removed under diminished pressure to give **25** (0.238 g, 92%); $[\alpha]^{20}_D$ +6.6 (*c* 2.55, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.02−7.92 (m, 7H), 7.86 (dd, J = 13.4 Hz, J = 7.6 Hz, 4H), 7.71 (d, J = 7.4 Hz, 2H), 7.67 (dd, J = 12.0 Hz, J = 7.2 Hz, 2H), 7.64−7.28 (m, 16H), 7.20 (t, J = 7.8 Hz, 2H), 7.12 (t, J = 7.8 Hz, 2H), 5.76 (t, J = 9.4 Hz, 1H), 5.70−5.66 (m, 2H), 5.37 (d, J = 8.1 Hz, 1H), 5.31 (dd, J = 10.3 Hz, J = 3.4 Hz, 1H), 5.14−5.02 (br s, 1H), 4.84−4.81 (m, 1H), 4.79 (overlapping d, J = 7.9 Hz, 2H), 4.54 (d, J = 11.0 Hz, 1H), 4.46 (dd, J = 12.2 Hz, J = 3.9 Hz, 1H), 4.31 (broad d, J = 8.1 Hz, 1H), 4.27 (t, J = 9.4 Hz, 1H), 3.94−3.71 (m, 5H), 3.66 (dd, J = 11.1 Hz, J = 6.5 Hz, 1H), 1.85, 1.70 (each s, each CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.4, 169.2, 168.4, 165.7, 165.5, 165.4 (2s), 165.2 (2s), 164.8, 133.6 (each C), 133.5 (CH), 133.2 (C), 132.1 (2s), 131.9 (2s), 129.9, 129.8, 129.7 (2s), 129.6 (2s), 129.5 (each CH), 129.5, 129.4, 129.3, 128.9 (each C), 128.7 (CH), 128.7 (C), 128.6, 128.5 (2s), 128.4, 128.2, 79.0, 76.0, 75.5, 73.0, 72.8, 72.0, 71.9, 71.7, 71.4, 69.9, 69.5, 67.5 (each CH), 62.5, 61.0 (CH_2), 20.6, 20.5 (each CH_3); IR (film on NaCl) 3483−3305 (br s), 2121, 1746, 1619, 1429, 1376, 1232, 1046, 901 cm^{-1}; LRMS (ES) 1355.2 $[M - H]^-$; HRMS (ESI) found 1378.3523 $[M + Na]^+$, $C_{71}H_{61}NaN_3O_{25}$ requires 1378.3492.

1,4-Di[(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl)-(1→3)-1-azido-1-deoxy-β-D-glucopyranuronamidomethyl]benzene 6. Acid **25** (117 mg, 0.88 mmol), HOBt (27 mg, 0.201 mmol), *p*-xylylenediamine (5 mg, 0.04 mmol), and DIPEA (0.018 mL, 0.1 mmol) were dissolved in dry DMF (0.8 mL) at 0 °C under N_2. HATU (43 mg, 0.11 mmol) was added and the reaction was stirred for 1 h at 0 °C and then overnight at rt. Ethyl acetate was added and the mixture was washed with H_2O (2 × 10 mL), the organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was removed. Chromatography of the residue (toluene−EtOAc, 2:1) gave the intermediate protected diamide as a pale yellow solid (72 mg, 65%). To this protected diamide (33 mg, 0.012 mmol) in MeOH−CH_2Cl_2 (2.5 mL, 4:1) was slowly added a solution of NaOMe (0.1 mL, 25 M in MeOH) and the resulting mixture was stirred for 3 h at 0 °C. Solid CO_2 was added, the mixture was stirred for 20 min, and the solvent was then removed under diminished pressure. The residue was dissolved in water and then lyophilized to give **6** as a white powder. ^{1}H NMR (600 MHz, D_2O) δ 7.32 (s, 4H), 4.85 (d, J = 8.9 Hz, 2H), 4.80 (d, J = 8.0 Hz, 2H), 4.46 (s, 4H), 4.44 (d, J = 7.9 Hz, 2H), 4.05 (d, J = 9.9 Hz, 2H), 3.99 (dd, J = 12.2 Hz, J = 1.7 Hz, 2H), 3.93 (d, J = 3.2 Hz, 2H), 3.85 (t, J = 9.1 Hz, 2H), 3.83−3.70 (m, 10H), 3.69−3.64 (m, 6H), 3.63−3.59 (m, 2H), 3.55 (dd, J = 9.0 Hz, J = 8.9 Hz, 4H), 3.40 (dd, J = 8.3 Hz, J = 8.8 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 169.9, 136.9 (each C), 127.7, 103.2, 102.7, 90.2, 83.4, 78.7, 76.9, 75.6, 75.1, 74.4, 73.4, 72.8, 72.5, 71.2, 69.8, 68.8 (each CH), 61.3, 60.4, 42.8 (each CH_2); IR (KBr) 3483−3305 (br s), 2121, 1660, 1385, 1277, 1070 cm^{-1}; LRMS (ES) 1185.4 $[M - H]^-$; HRMS (ESI) found 1209.3837 $[M + Na]^+$, $C_{44}H_{66}N_8NaO_{30}$ requires 1209.3783, and found 1185.3833 $[M - H]^-$, $C_{44}H_{65}N_8O_{30}$ requires 1185.3807.

Allyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4-di-*O*-acetyl-1-*O*-allyl-α-D-glucopyranuronate 28. Deacetylation of **27**[19b] (940 mg, 2.61 mmol) gave allyl α-D-glucopyranosiduronic acid as a yellow foam (589 mg, 96%). This intermediate (1.56 g, 6.30 mmol) was suspended in acetic anhydride (23 mL) in the presence of molecular sieves and stirred in a preheated bath at 85 °C for 2 h. The solvent was coevaporated with toluene to give a brown oil that was dried under high vacuum. The oil was dissolved in cyclohexane−EtOAc (1:1) then filtered, and the filtrate was passed through a short column of silica gel eluting with cyclohexane−EtOAc (1:1). This gave a 6,3-lactone intermediate as a yellow oil (1.63 g, 86%), which was used in the next reaction without further purification. Allyl alcohol (2.5 mL, 36.7 mmol) was added to a solution of the 6,3 lactone intermediate (1.63 g, 5.33 mmol) in dry THF (8 mL) in the presence of NaOAc (217 mg, 2.65 mmol) and molecular sieves at rt and under N_2. The mixture was stirred at rt for 24 h then filtered, and the filtrate was evaporated under reduced pressure to give a brown oil. This oil was dissolved in dichloromethane (30 mL) and washed with brine. The aq phase was further extracted with dichloromethane (2 × 15 mL) and the combined organic extracts were dried ($MgSO_4$) and filtered, then the solvent was removed under reduced pressure. Chromatography of the residue (cyclohexane−EtOAc, 2:1) gave **28** as a white solid (1.03 g, 75%); R_f 0.45 (cyclohexane−EtOAc, 1:1); $[\alpha]_D$ +20.8 (*c* 0.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.98−5.78 (m, 2H), 5.39−5.20 (m, 4H), 5.17−5.15 (d, J = 3.6 Hz, 1H), 5.11−5.04 (t, J = 9.6 Hz, 1H), 4.83−4.79 (dd, J = 3.9 Hz, J = 10.2 Hz, 1H),

4.69−4.55 (m, 2H), 4.31−4.28 (d, J = 10.2, 1H), 4.25−4.09 (m and overlapping t, J = 9.6 Hz, 2H), 4.06−3.99 (m, 1H,), 2.45 (d, J = 5.4 Hz, 1H), 2.13, 2.08 (each s, 3H, each CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.7, 170.4, 167.8 (each C), 133.0, 131.2 (each CH), 118.5, 118.2 (each CH_2), 95.4, 72.9, 72.0, 69.4 (each CH), 69.1 (CH_2), 68.7 (CH), 66.6 (CH_2), 20.9, 20.8 (each CH_3); IR (film from dichloromethane) 3480, 2996, 1748, 1374, 1230, 1050 cm^{-1}; HRMS (ESI) found 359.1349 $[M + H]^+$, $C_{16}H_{23}O_9$ requires 359.1342.

2,3,4,6-Tetra-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranosyl-(1→3)-2,4-di-*O*-acetyl-1-*O*-allyl-α-D-glucopyranuronic Acid 29. Glycoside coupling reaction of acceptor **28** (1.31 g, 3.65 mmol) and trichloroacetimidate **19** (3.22 g, 2.65 mmol) as described for **13** gave, after chromatography (eluant cyclohexane−toluene−EtOAc 3:2:1 to 5:4:2), the protected trisaccharide intermediate as a white solid (3.11 g, 83%). To this protected trisaccharide (1.45 g, 1.027 mmol) in dry acetonitrile (8 mL), cooled on an ice bath and under N_2, tetrakis(triphenylphosphine)palladium (117 mg, 0.10 mmol) was added followed by pyrrolidine (85 μL, 1.032 mmol). The reaction mixture was stirred on an ice bath for 1 h and then the solvent was removed under diminished pressure. The residue was dissolved in dichloromethane and washed with 0.1 M HCl and brine and then dried ($MgSO_4$) and filtered, then the solvent was removed under diminished pressure. The residue was filtered through a short column of silica gel (eluant: dichloromethane then MeOH) to give **29** as a pale yellow solid (980 mg, 70%); R_f 0.15 (dichloromethane−MeOH, 95:5); $[\alpha]^{20}_D$ +4.26 (c 0.26, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 8.00−7.96 (m, 8H), 7.88 (d, J = 7.2 Hz, 2H), 7.79 (d, J = 6 Hz, 2H), 7.72 (d, J = 7.2 Hz, 2H), 7.62−7.56 (m, 2H), 7.50−7.31 (m, 15H), 7.21−7.19 (t, J = 7.8 Hz, 2H), 7.14−7.12 (m, 2H), 5.77−5.67 (m, 4H), 5.36−5.32 (m, 2H), 5.18−5.15 (d, J = 17 Hz, 1H), 5.11−4.94 (m, 3H), 4.86 (d, J = 6.6 Hz, 1H), 4.81 (d, J = 7.8 Hz, 1H), 4.60−4.53 (m, 2H), 4.47−4.45 (m, 1H), 4.27−4.24 (t, J = 8.4 Hz, 1H), 4.11−4.01 (m, 3H), 3.90−3.69 (m, 5H), 1.76, 1.67 (each s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.4, 165.7, 165.6, 165.5, 165.4, 165.2, 164.8 (each C), 133.5, 133.2, 129.9, 129.8, 129.7, 129.6 (2s) (each CH), 129.6 (C), 129.5, 129.4, 129.2, 128.9, 128.7, 128.6, 128.5, 128.2 (each CH), 118.0 (CH_2), 101.1, 100.9, 95.0, 75.6, 73.1, 72.7, 72.1, 71.7, 71.4, 69.8 (each CH), 68.9 (CH_2), 67.5 (CH), 62.4, 61.0 (each CH_2), 20.5, 20.4 (each CH_3); IR (film from dichloromethane) 3598, 3374, 1729, 1602, 1423, 1371, 1267, 1103, 1050 cm^{-1}; HRMS (ESI) found 1393.3729 $[M + Na]^+$, $C_{74}H_{66}O_{26}$ requires 1393.3740.

1,4-Di[2,3,4,6-tetra-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranosyl-(1→3)-2,4-di-*O*-acetyl-1-*O*-allyl-α-D-glucopyranuronamidomethyl]benzene 30. To acid **29** (980 mg, 0.71 mmol) in dry DMF (7.5 mL) and under N_2 were added HOBt monohydrate (134 mg, 0.98 mmol) followed by *p*-xylylenediamine (41 mg, 0.3 mmol). Diisopropylethylamine (180 μL, 1.07 mmol) was added and the mixture became cloudy, then it was cooled on an ice bath and HATU (350 mg, 0.92 mmol) was added. The reaction mixture was allowed to attain rt and was then stirred for 15 h. Dichloromethane was added and the mixture was washed with water. The aq phase was extracted with dichloromethane and the combined organic extracts were dried ($MgSO_4$) and filtered and the solvent was removed under diminished pressure. Chromatography (toluene−EtOAc, 2.5:1 and EtOAc) gave **30** as a white solid (629 mg, 74%); R_f 0.55 (toluene−EtOAc, 1:1); $[\alpha]^{20}_D$ +2.41 (c 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 8.02−7.97 (m, 14H), 7.87 (d, J = 7.2 Hz, 4H), 7.81 (d, J = 7.2 Hz, 4H), 7.71 (d, J = 7.2 Hz, 4H), 7.62−7.59 (m, 4H), 7.57−7.55 (m, 2H), 7.51−7.30 (m, 24H), 7.26−7.23 (t, J = 6 Hz, 4H), 7.20 (s, 4H) overlapping 7.20−7.11 (m, 10H), 6.52−6.50 (t, J = 6 Hz, 2H), 5.80−5.77 (t, J = 9.6 Hz, 2H), 5.77−5.68 (m, 6H), 5.36−5.34 (dd, J = 8.4 Hz, J = 9.3 Hz, 2H), 5.34−5.32 (dd, J = 10.2 Hz, J = 3.0 Hz, 2H), 5.21−5.14 (m, 4H), 5.03−5.00 (t, J = 9.6, 2H), 5.00 (d, J = 3.6 Hz, 2H), 4.87 (d, J = 7.8 Hz, 2H), 4.81 (d, J = 8.4 Hz, 2H), 4.58−4.54 (m, 4H), 4.48−4.46 (dd, J = 4.5 Hz, J = 13.2 Hz, 2H), 4.38−4.30 (m, 4H), 4.30−4.27 (t, J = 9.6 Hz, 2H), 4.18−4.15 (t, J = 9.6 Hz, 2H), 4.13 (d, J = 10 Hz, 2H), 4.09−4.05 (m, 2H), 3.89−386 (m, 2H), 3.82−3.77 (m, 6H), 3.68−3.66 (m, 2H), 1.82, 1.78 (each s, 3H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 169.5, 169.4, 167.3, 165.7, 165.6, 165.5, 165.4, 165.2, 165.1, 164.8, 137.0 (each C), 133.6, 133.5 (2s), 133.4, 133.2 (each CH), 133.2 (C), 133.1, 132.7, 129.9, 129.8, 129.7 (2s) (each CH), 129.6 (2s) (C), 129.6, 129.5 (2s) (each CH), 129.4, 129.3, 128.9 (each C), 128.7 (2s) (each CH), 128.6 (C), 128.5, 128.3 (2s), 128.2 (each CH), 118.5 (CH_2), 101.2, 100.9, 94.5, 75.7, 75.6, 73.0, 72.9, 72.8, 72.1, 71.7, 71.5, 69.9, 69.5 (each CH), 69.2 (CH_2), 68.7, 67.6 (each CH), 62.4, 61.1, 43.7 (each CH_2), 20.5, 20.4 (each CH_3); IR (film from dichloromethane) 3432, 3343, 3064, 2944, 1729, 1687, 1585, 1527, 1452, 1371, 1263, 1099, 1051 cm^{-1}; HRMS (ESI) found 1421.4327 $[M + 2H]^{2+}$, $C_{156}H_{140}N_2O_{50}$ requires $m/2z$ 1421.4315.

Macrocyclic Compound 31 and Acyclic Compound 32. A degassed solution of the diamide **30** (629 mg, 0.22 mmol) in dry dichloromethane (190 mL, 1.15 mM) and under N_2 was treated with Grubbs-I catalyst (53 mg, ∼9%) for 60 h. The solvent was evaporated, then the resulting black solid was dissolved and subjected to fractionation through a short column of silica gel (toluene−EtOAc, 2:1) yielding a mixture of products that was used in the next reaction without further purification. Three cycles of catalytic hydrogenation of the mixture (530 mg) using the H-Cube (70 °C) hydrogenation apparatus with Pd as catalyst and EtOAc as solvent and subsequent chromatography (toluene−cyclohexane-EtOAc, 3:1:1 to 1:1:1) gave both **31** (265 mg, 43%) and **32** (233 mg, 44%). Analytical data for **32**: R_f 0.75 (toluene−EtOAc 1:1); $[\alpha]_D$ +2.86 (c 0.24, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.02−7.96 (m, 14H), 7.86 (d, J = 7.2 Hz, 4H), 7.82 (d, J = 7.2 Hz, 4H), 7.71 (d, J = 7.2 Hz, 4H), 7.63−7.60 (t, J = 7.2 Hz, 4H), 7.57−7.55 (t, J = 7.8 Hz, 2H), 7.52−7.30 (m, 28H), 7.21−7.19 (t, J = 7.8 Hz, 4H) overlapping with 7.20 (s, 4H), 7.16−7.12 (m, 6H), 6.52−6.49 (t, J = 5.4 Hz, 2H, NH), 5.80−5.77 (t, J = 9.6 Hz, 2H), 5.72−5.68 (m, 4H), 5.37−5.34 (dd, J = 8.4 Hz, J = 10.2 Hz, 2H), 5.34−5.31 (dd, J = 5.4 Hz, J = 10.2 Hz, 2H), 5.01−4.98 (t, J = 9 Hz, 2H), 4.96 (d, J = 3.0 Hz, 2H), 4.88 (d, J = 7.8 Hz, 2H), 4.81 (d, J = 7.8 Hz, 2H), 4.59−4.57 (m, 2H), 4.52−4.50 (dd, J = 3.6 Hz, J = 9.8 Hz), 4.49−4.46 (dd, J = 3.6 Hz, J = 12 Hz, 2H), 4.37−4.27 (m, 6H), 4.14−4.09 (m, 4H), 3.82−376 (m, 6H), 3.68−3.65 (m, 2H), 3.54−3.50 (m, 2H), 3.26−3.22 (m, 2H), 1.82, 1.78 (each s, 3H), 1.53−1.47 (m, 4H), 0.85−0.83 (t, J = 6.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.5 (2 s), 167.4, 165.7, 165.6, 165.5, 165.4, 165.2, 165.1, 164.8, 137.0, 133.6 (each C), 133.5 (2s), 133.2 (each CH), 133.2, 133.1 (each C), 129.9, 129.8, 129.7 (3s), 129.5 (CH), 129.4, 129.3, 128.9 (C), 128.7(2s), 128.6, 128.3 (3s) (each CH), 101.2, 100.9, 95.2, 75.8, 75.6, 73.2, 73.0, 72.8, 72.1, 71.8, 71.5 (each CH), 70.5 (CH_2), 69.9, 69.6, 68.5, 67.6 (each CH), 62.5, 61.1, 42.7, 22.4 (each CH_2), 20.5, 20.4, 10.4 (each CH_3); IR (film from dichloromethane) 3394, 3264, 2967, 2856, 1731, 1681, 1585, 1521, 1452, 1369, 1265, 1176, 1085, 1070, 1025 cm^{-1}; HRMS (ESI) found 1423.4458 $[M + 2H]^{2+}$, $C_{156}H_{144}N_2O_{50}$ requires 1423.4472. Anal. Calcd for $C_{156}H_{144}N_2O_{50}$: C 65.82, H 5.10, N 0.98. Found: C 65.51, H 5.26, N 0.83. Analytical data for **31**: R_f = 0.7 (toluene−EtOAc 1:1); $[\alpha]_D$ +0.61 (c 0.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.02−7.96 (m, 14H), 7.87 (d, J = 7.2 Hz, 4H), 7.83 (d, J = 7.2 Hz, 4H), 7.71 (d, J = 7.2 Hz, 4H), 7.62−7.60 (t, J = 6.6 Hz, 4H), 7.58−7.54 (m, 2H), 7.52−7.31 (m, 28H), 7.27−7.24 (t, J = 7.8 Hz, 4H), 7.26 (s, 4H), 7.21−7.11 (m, 6H), 6.74−6.72 (dd, J = 3.6 Hz, J = 9.6 Hz, 2H), 5.79−5.76 (t, J = 9.0 Hz, 2H), 5.69−5.65 (m, 4H), 5.38−5.36 (dd, J = 7.9 Hz, J = 10.0 Hz, 2H), 5.35−5.32 (dd, J = 3.3 Hz, J = 8.6 Hz,

2H), 4.99−4.96 (m, 4H), 4.86 (d, J = 7.7 Hz, 2H), 4.85−4.77 (m, 4H), 4.55−4.51 (m, 4H), 4.49−4.45 (m, 2H), 4.30−4.27 (t, J = 9.6 Hz, 2H), 4.09−4.06 (t, J = 9.0 Hz, 2H), 3.95 (d, J = 10.8 Hz, 2H), 3.82−374 (m, 8H), 3.69−3.65 (m, 2H), 3.27−3.23 (m, 2H), 3.19−3.14 (m, 2H), 1.85, 1.76 (each s, each 3H), 1.48−1.42 (m, 2H), 1.31−1.22 (m, 2H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 169.5 (2s, 167.3, 166.0, 165.7, 165.6, 165.3, 165.2, 165.0, 164.8 (each CO), 138.2, 133.6 (C), 133.5 (2s, CH), 133.4 (C), 133.3 (CH), 133.2, (C), 129.9, 129.8, 129.7 (2s), 129.6 (2s), 129.5 (2s), 129.4 (each CH), 129.2 (C), 128.9, 128.7, 128.6 (3s), 128.3 (2s), 128.2 (each CH), 127.9 (C), 127.8 (CH), 127.8, 127.7 (each C), 101.0, 100.9, 96.1, 75.8, 75.5, 73.0, 72.9, 72.8, 72.1, 71.7, 71.5, 69.9 (each CH), 69.4 (CH_2), 69.3, 67.9, 67.6 (each CH), 62.4, 61.1, 42.2, 25.56 (each CH_2), 20.4, 20.3 (each CH_3); IR (film from dichloromethane) 3598, 3374, 1729, 1602, 1423, 1371, 1267, 1103, 1050, 3405, 3315, 3062, 2980, 2921, 2856, 1731, 1683, 1600, 1523, 1452, 1399, 1315, 1263, 1067, 1027 cm^{-1}; HRMS (ESI) found $m/2z$ 1408.4218 $[M + 2H]^{2+}$, $C_{154}H_{138}N_2O_{50}$ requires 1408.4237.

1−4-Di[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-1-*O*-*n*-propyl-α-D-glucopyranuronamido]benzene 7. Removal of the protecting groups from **32** (48 mg, 0.017 mmol) as described for **6** gave, after semipreparative HPLC (gradient elution: H_2O-CH_3CN, 99:1 to 96:4) and lyophilization, **7** (10 mg, 49%); $[\alpha]_D$ +2.19 (*c* 0.10, H_2O); ^{1}H NMR (D_2O, 35 °C, 600 MHz) δ 7.30 (s, 4H), 4.98 (d, J = 3.6 Hz, 2H), 4.74−4.73 (d, J = 7.8 Hz, 2H), 4.47−4.40 (m, 6H), 4.15−4.13 (d, J = 10.2 Hz, 2H), 3.99−3.97 (dd, J = 2.4 Hz, J = 12.6 Hz, 2H), 3.95−3.93 (m, 4H), 3.82−3.75 (m, 8H), 3.74−3.71 (m, 2H), 3.69−3.63 (m, 10H), 3.62−3.61 (m, 2H), 3.55−3.52 (m and t overlapping, J = 9.6 Hz, 4H), 3.41−3.38 (m, 2H), 1.66−1.60 (m, 4H), 0.92−0.90 (t, J = 7.8 Hz, 6H); ^{13}C NMR (D_2O, 150 MHz) δ 171.1, 137.0 (each C), 127.7, 103.2, 102.9, 98.7, 81.9, 78.7, 75.6, 75.1, 74.5, 73.5, 72.8, 71.8, 71.2, 70.9 (each CH), 70.9 (CH_2), 70.3 (CH_2), 68.9 (CH), 61.3, 60.4, 42.8, 22.4 (each CH_2), 10.2 (CH_3); HRMS (ESI) found 1220.4763 $[M + H]^+$, $C_{50}H_{80}N_2O_{32}$ requires 1220.4694.

Glycophane 8. Removal of the protecing groups from **31** (62 mg, 0.021 mmol) as described for formation of **6** gave **8**, after semipreparative HPLC (gradient elution: H_2O-CH_3CN, 99:1 to 96:4) and lyophilization, as a white solid (17 mg, 66%); $[\alpha]_D$ +2.0 (*c* 0.17, H_2O); ^{1}H NMR (D_2O, 35 °C, 500 MHz) δ 7.30 (s, 4H), 4.93 (d, J = 2.0 Hz, 2H), 4.72−4.70 (d, J = 7 Hz, 2H), 4.51−4.48 (d, J = −14.0 Hz, 2H), 4.43−4.42 (d, J = 8.0 Hz, 2H), 4.28−4.25 (d, J = −14.0 Hz, 2H), 3.98−3.95 (m, 4H), 3.90 (d, J = 2.0 Hz, 2H), 3.85−3.83 (d, J = 9.0 Hz, 2H), 3.80−3.74 (m, 6H), 3.72−3.64 (m, 12H), 3.59−3.56 (m, 2H), 3.54−3.51 (m, 4H), 3.47−3.42 (m, 2H), 3.40−3.35 (m, 2H), 1.60−1.53 (m, 2H), 1.48−1.39 (m, 2H); ^{13}C NMR (D_2O, 125 MHz) δ 170.5, 138.2 (each C), 128.4, 103.2, 102.8, 99.2, 82.0, 78.6, 75.6, 75.1, 74.5, 73.4, 72.8, 71.7, 71.2, 70.9, 69.9 (each CH), 69.7 (CH_2), 68.9 (CH), 61.3, 60.4, 42.7, 25.5 (each CH_2); LRMS (ESI) 1189.2 $[M - H]^-$, 593.6 $[M - 2H]^{2-}$; HRMS (ESI) found 1190.4271 $[M + H]^+$, $C_{48}H_{74}N_2O_{32}$ requires 1190.4225;

1,4-Di[1-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl)-1,2,3-triazol-4-ylmethyloxy]benzene 9. Lactosyl azide **33**23a (331 mg, 0.901 mmol) was dissolved in CH_3CN-H_2O (1:1, 4 mL), then *p*-bispropargyloxybenzene[11c] **34** (84 mg, 0.451 mmol) and sodium ascorbate (0.22 mL of 0.4 M) followed by $CuSO_4 \cdot 5H_2O$ (0.22 mL of 0.2 M) were subsequently added and the mixture was stirred for 12 h, after which it was diluted with H_2O (15 mL) and extractd with EtOAc (2 × 5 mL). After lyophilization of the aq solution the pale green powder (0.397 g) that was obtained was added to pyridine−Ac_2O (1:1, 10 mL) and the mixture was stirred overnight. The volatile reagents were then removed under diminished pressure and toluene (3 × 10 mL) was evaporated from the residue. Chromatography (EtOAc−cyclohexane, gradient elution 1:1 to 2:1) gave the peracetate as an off-white foam (0.517 g, 76% over two steps). Deacetylation of this peracetate (0.314 g, 0.208 mmol) in MeOH−CH_2Cl_2 (10 mL, 4:1) as described for **6** gave, after lyophilization, **9** as a brown solid (0.191 g, quantitative); $[\alpha]_D$ +38.5 (*c* 1.0, H_2O); ^{1}H NMR (500 MHz, D_2O) δ 8.24 (s, 2H), 6.97 (s, 4H), 5.75 (d, J = 9.2 Hz, 2H, H-1), 5.16 (s, 4H), 4.49 (d, J = 7.8 Hz, 2H), 4.03 (t, J = 9.1 Hz, 2H), 3.95−3.91 (m, 4H), 3.89−3.81 (m, 8H), 3.81−3.70 (m, 6H), 3.67 (dd, J =10.0 Hz, J = 3.4 Hz, 2H), 3.57 (dd, J = 9.9 Hz, J = 7.8 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 152.5, 144.0 (each CH), 124.7 (C), 117.1, 103.2, 87.5, 78.0, 77.7, 75.6, 74.8, 72.8, 72.3, 71.2, 68.8 (each CH), 62.2, 61.3, 60.0 (each CH_2); IR (KBr) 3502−3197 (br s), 1643, 1508, 1460, 1385, 1225, 1126, 1070 cm^{-1}; LRMS (ESI) 943.1 $[M + Na]^+$; HRMS (ESI) found 921.3257 $[M + H]^+$, $C_{36}H_{53}N_6O_{22}$ requires 921.3213, and found 919.3021 $[M - H]^-$, $C_{36}H_{53}N_6O_{22}$ requires 919.3056.

Molecular Modeling. The AMBER force field in Macromodel and the GB/SA continuum[25] for water were generally applied in all calculations. Systematic conformational searches with the SUMM method in Macromodel were carried out with 10 000 structures being generated starting from the initial model structures. Both glycosyl amide torsions (H_1-C_1-N-H) in the divalent molecules **1**, **3**−**5** were constrained to 171° with a force constant of 2000 applied throughout the calculations. Each conformer generated was minimized to convergence and all structures within 12 kJ/mol of the global minimum for each compound were retained and included in the analysis shown in Figure 2. Conformational searches with **2** led to highly folded low-energy structures which seemed unlikely to bind to lectins. Thus stochastic molecular dynamics were also carried out for **2** beginning from an initial structure where the carbonyl group of the terephthalamide residue was *trans* (O═C—C═O torsion ~90°). During the dynamics simulations a temperature of 300 K, time step of 1.5 fs, equilibration time of 1.0 ps, and simulation time 2 ns were applied and 500 structures were selected randomly over the duration of the simulation. The structures obtained from the conformational searches and dynamics simulations were analyzed in the same manner. In general the lactose glycosidic torsions were consistent in all structures generated. For example, the average observed values for **1** from the conformational search were −72.0° ± 5.3° for ϕ and 132.1° ± 1.6° for ψ.

Lectins and Activity Assays. Extracts of dried mistletoe leaves and of pellets of bacteria after recombinant production were the sources for lectin purification by affinity chromatography on lactosylated Sepharose 4B as crucial step.[7g,k,26] Proteolytic truncation by collagenase-dependent cleavage at the Tyr106/Gly107 and Glu229/Ile230 peptide bonds in human galectin-3 was performed by a standard protocol.[4a,8d] Purity control by one- and two-dimensional gel electrophoresis preceded biotinylation under activity-preserving conditions and the following activity controls by hemeagglutination with trypsin-treated and glutaraldehyde-fixed rabbit erythrocytes as well as binding to glycoproteins and human tumor cells.[26a,27] Haemagglutination

(25) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc*. **1990**, *112*, 6127–6129.

(26) (a) Gabius, H.-J.; Engelhardt, R.; Rehm, S.; Cramer, F. J. *Natl. Cancer Inst*. **1984**, *73*, 1349–1357. (b) Gabius, H.-J.; Engelhardt, R.; Cramer, F.; Bätge, R.; Nagel, G. A. *Cancer Res*. **1985**, *45*, 253–257. (c) Gabius, H.-J. *Anal. Biochem*. **1990**, *189*, 91–94. (d) Gabius, H.-J.; Walzel, H.; Joshi, S. S.; Kruip, J.; Kojima, S.; Gerke, V.; Kratzin, H.; Gabius, S. *Anticancer Res*. **1992**, *12*, 669–676. (e) André, S.; Kaltner, H.; Furuike, T.; Nishimura, S.-I.; Gabius, H.-J. *Bioconjugate Chem*. **2004**, *15*, 87–98.

(27) (a) Gabius, H.-J.; Wosgien, B.; Hendrys, M.; Bardosi, A. *Histochemistry* **1991**, *95*, 269–277. (b) Purkrábková, T.; Smetana, K., Jr.; Dvořánková, B.; Holíková, Z.; Böck, C.; Lensch, M.; André, S.; Pytlik, R.; Liu, F.-T.; Klíma, J.; Smetana, K.; Motlík, J.; Gabius, H.-J. *Biol. Cell* **2003**, *95*, 535–545. (c) André, S.; Pei, Z.; Siebert, H.-C.; Ramström, O.; Gabius, H.-J. *Bioorg. Med. Chem*. **2006**, *14*, 6314–6326. (d) André, S.; Maljaars, C. E. P.; Halkes, K. M.; Gabius, H.-J.; Kamerling, J. P. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 793–798.

assays were performed with 2-fold serial dilutions of sugar in triplicates with at least five independent test series. For studies in vitro, cells of the human B lymphoblastoid line Croco II were processed in aliquots of the same batch in triplicates and at least three independent series with incubation of lectin-containing solution at 4 °C for 30 min as labeling step and with streptavidin-*R*-phycoerythrin as reporter conjugate.[27c,d] Controls included omission of the labeling step to determine the level of lectin-independent background staining and application of noncognate sugar to infer osmolarity effects. The solid-phase assay with spectrophotometric signal detection at 490 nm was performed under optimized conditions as described previously.[28] In detail, the plastic surface of the microtiter plate wells was coated with ligand by incubation of 50 μL of phosphate-buffered saline (pH 7.2) containing the lectin-reactive glycoprotein (serum amyloid P component: 0.5 μg; asialofetuin; 0.5 μg) at 4 °C overnight. Following blocking residual sites for protein adsorption by an incubation with 100 μL of buffer containing 1% (w/v) carbohydrate-free bovine serum albumin for 1 h at 37 °C and washing, 50 μL of solution with biotinylated lectin was applied at a concentration resulting in signal intensity within the linear range (please see top part of Table 1 for details), and bound lectin was detected by its biotin label using streptavidin-peroxidase conjugate (0.5 μg/mL for 1 h at 37 °C; Sigma, Munich, Germany) and the chromogenic substrates *o*-phenylenediamine (1 mg/mL) and H_2O_2 (1 μL/mL). Up to five series of independent experiments were run in triplicates with standard deviations not exceeding 17%.

(28) (a) André, S.; Ortega, P. C.; Perez, M. A.; Roy, R.; Gabius, H.-J. *Glycobiology* **1999**, *9*, 1253–1261. (b) André, S.; Kojima, S.; Prahl, I.; Lensch, M.; Unverzagt, C.; Gabius, H.-J. *FEBS J.* **2005**, *272*, 1986–1998.

Acknowledgment. Generous funding was provided by Science Foundation Ireland, the European Commission through Marie Curie IntraEuropean Fellowships ((MEIF-CT-2003-500748, 514958, to S.G.G. and T.V.T.), a Marie Curie Research Training Network grant (contract no. CT-2005-19561), the Programme for Research in Third-Level Institutions (PRTLI), administered by the HEA (of Ireland), the research initiative LMU*excellent*, and the Verein zur Förderung des biologisch-technologischen Fortschritts in der Medizin e. V. (Heidelberg, Germany). Inspiring discussions with Drs. B. Friday and S. Namirha are gratefully acknowledged.

Supporting Information Available: Experimental details, analytical data for intermediates, and ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.