Synthetic Carbohydrate-Containing Dendrimers**

Narayanaswamy Jayaraman, Sergey A. Nepogodiev, and J. Fraser Stoddart*

Abstract: Dendrimers coated with carbohydrates on their exterior surfaces have been constructed by using both convergent and divergent synthetic routes. Alternatively, cluster glycosides in the form of highly branched oligosaccharides can serve as dendritic wedges in the subsequent elaboration of fully carbohydrate dendrimers. It is anticipated that these novel saccharide-containing polymers, which are highly branched and water-soluble, will find applications of a biological nature as well as in the context of new materials.

Keywords
carbohydrates · cluster glycosides · convergent synthesis · dendrimers · divergent synthesis

Introduction

Constructing large molecules with precise (macro)molecular structures in a controlled manner is an extremely attractive proposition because of the possibility of creating new materials and biologically active compounds. Since the early 1980s, one of the ways of addressing this objective has been the iterative assembly of monodisperse, highly branched macromolecules[1] in the form of so-called *dendrimers*. Many organic, organometallic, and polymer chemists have made their contributions to the remarkable advances in dendrimer chemistry that have emerged in this now highly interdisciplinary field of research as new conceptual approaches to the syntheses of dendrimers have evolved. As a result, a wide range of building blocks have been incorporated as components into dendrimers. However, until fairly recently, no saccharides had been employed for this purpose. The desire to understand the biological functions of carbohydrates[2] has established this class of natural products as one of the major focuses of current biological research[3] and has prompted the remarkable progress in the synthesis of oligosaccharides[4] and glycoconjugates[5] of the last decade or so. Needless to say, there is an infinite array of possibilities and opportunities in relation to the preparation of synthetic glycoconjugates. The unique features of carbohydrates, which are supremely adaptable to aqueous environments and widely used by Nature, are, as yet, under-explored and hardly exploited as a relatively inexpensive means of creating unnatural macromolecules with potentially useful properties for new materials and biologically active compounds. Recently, several groups—including our own—have turned their attention to the synthesis of carbohydrate-containing dendrimers. In this short article, we will discuss some of the early achievements and try to assess the outlook for a rapidly expanding area of multidisciplinary research that seems to be full of promise.

Observations

There are two limiting types of carbohydrate-containing dendritic molecules[6] which may be distinguished by the way in which the saccharide residues are incorporated into them. The first type, the carbohydrate-coated dendrimer, is characterized by the presence of saccharides attached to the termini of a non-carbohydrate interior skeleton (Figure 1 a). Alternatively, the saccharides could be used as multifunctional building blocks, giving rise to dendrimers that are totally carbohydrate (Figure 1 b). Both these types of carbohydrate-containing dendrimers may be considered as analogues of polysaccharides, retaining some of their features—for example, water solubility and biodegradability.

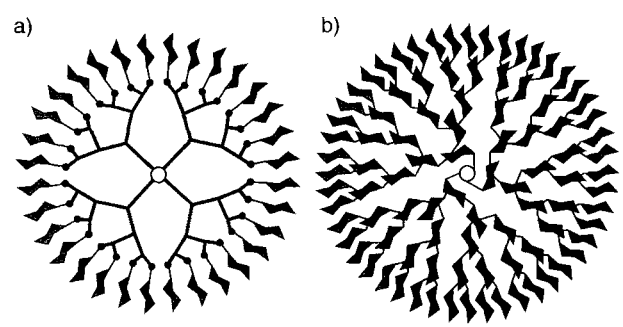

Figure 1. Cartoon representations of the two possible types of carbohydrate-containing dendrimers. a) Carbohydrate-coated dendrimers, where the saccharide residues are located on the outer surface of the dendrimer. b) Fully carbohydrate dendrimers, where the inner saccharide residues play a role as branching units and are linked to three other saccharides.

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[**] This paper should be regarded as Part 4 of a series on Synthetic Carbohydrate Dendrimers: For Part 3, ref. [16]. A list of abbreviations used in this article is given in ref. [39].

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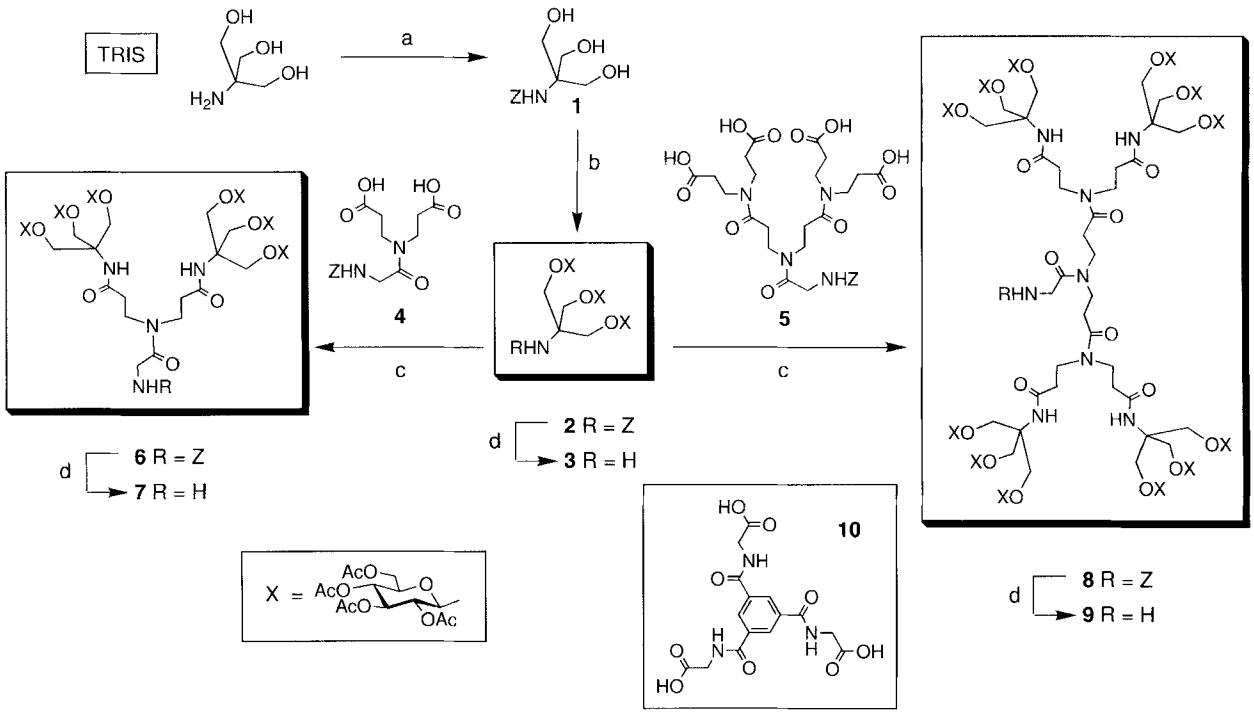

Scheme 1. The construction of the dendritic building blocks **3**, **7**, and **9**, bearing trisglucoside clusters and containing a focal NH_2 group for the attachment to the central core component **10**. Reagents: a) Z-Cl/Na_2CO_3/H_2O; b) 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide/AgOTf/collidine/CH_2Cl_2/$MeNO_2$; c) DCC/HOBT/CH_2Cl_2/DMF; d) H_2/Pd/C/EtOAc/MeOH.

1. Carbohydrate-Coated Dendrimers: The synthesis of this type of glycodendrimer can be approached by a convergent method or in a divergent fashion through end-group modifications of a pre-existing non-carbohydrate dendritic core. Since the chemical manipulation with free saccharides is still a somewhat delicate exercise, the use of *O*-protected saccharides is advantageous when employing either of these two synthetic strategies, provided that the dendritic core is stable under the conditions required for deprotection.

Convergent Synthetic Routes: A convergent strategy for the synthesis of glycodendrimers implies that branched building blocks should carry saccharides from the very beginning of the synthesis. In other words, the first step in such a strategy involves the attachment of two or more saccharide residues to a polyfunctional branching unit. This objective may be achieved by a variety of methods involving the formation of *O*-, *S*-, or *N*-glycosides, with or without the insertion of an additional spacer arm between the sugars and the branching unit. Quite a number of small molecules can be considered as feasible branching components[7] for the attachment of saccharides. In our initial investigations,[8, 9] we chose the compound TRIS (Scheme 1), because of the possibility of elaborating it further through its NH_2 group, following the attachment of glycosyl residues to the CH_2OH groups. Hence, the trisglucosylated derivative **2**, prepared in excellent yield by a well-established glycosylation technique from the *N*-protected TRIS derivative **1**, was converted into the amine **3**, which was used for the construction of the larger dendritic wedges **6** and **8** by forming amide bonds. These compounds were obtained in good yields, by coupling of two or four molecules of **3** with the dicarboxylic acid **4** or tetracarboxylic acid **5**. Removal of the *N*-protecting group from **6** and **8** afforded, respectively, the hexa- and dodecavalent cluster glycosides **7**[8] and **9**.[9] In summary, dendrons **7** and **9** are accessible in good overall yields and in high purities by using repetitive sequences of reactions and without having to resort to employing exotic reagents. We anticipate that much higher generation dendritic wedges could also be prepared in this manner.

The final step in the completion of a convergent dendrimer synthesis is the attachment of the dendritic wedges to a central core component. In our research so far, we have condensed compounds **3**, **7**, and **9**, all containing free amino groups at their focal points, with the tricarboxylic acid **10** shown in Scheme 1. In this manner, the dendrimers **9-mer**, **18-mer**, and **36-mer** (Figure 2) bearing 9, 18, and 36 β-D-glucopyranosyl residues, respectively, on their peripheries have been obtained in good yields. Once the dendrimer assemblies are complete, all the carbohydrate protecting groups can be removed to uncover the free saccharide dendrimers.

The primary advantage of the convergent synthetic methodology is that it allows the creation of dendrimers with perfect chemical constitutions, since the number of reactions at any particular stage is kept to the sheer minimum. In this regard, the logic of glycodendrimer synthesis is very similar to the principles behind assembling glycopeptides.[10] Additional possibilities associated with the convergent approach include: 1) the controllable introduction of different wedges (which may be carbohydrate or non-carbohydrate in nature) for the preparation of unsymmetrical and mixed dendrimers, and 2) ease of variation of the central core component.

Divergent Modifications of Pre-existing Dendritic Cores: One of the most straightforward ways to reach carbohydrate-coated dendrimers is through the grafting of saccharide residues on to

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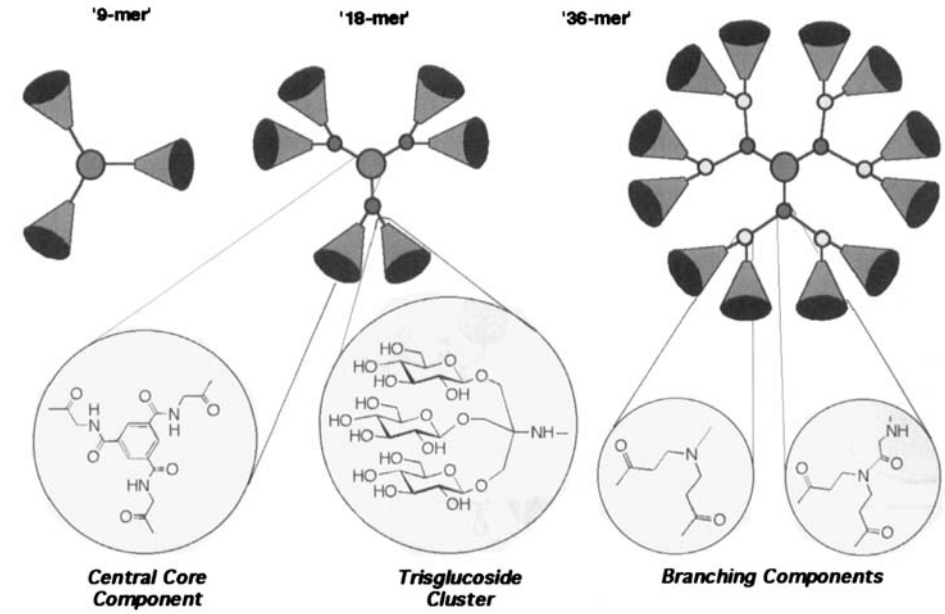
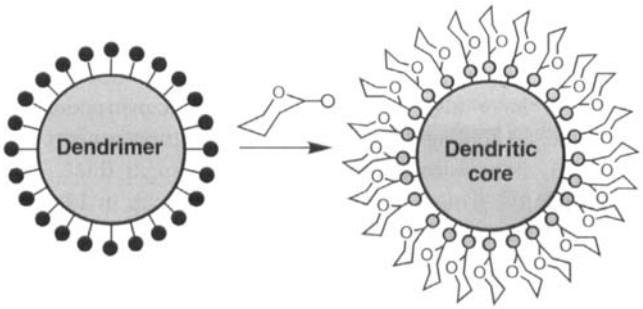


Figure 2. A schematic representation of the carbohydrate dendrimers—the **9-mer**, the **18-mer**, and the **36-mer**—bearing 9, 18, and 36 terminal D-glucopyranose residues, respectively, assembled by the convergent approach [8,9].

A range of other techniques, which had been developed for the synthesis of neoglycoconjugates,[13] could be extended to the preparation of carbohydrate-coated dendrimers. Thus, Lindhorst and Kieburg[14] have shown that acetylated glycosyl isothiocyanates can be linked with the PAMAM dendrimers: after removal of the acetyl groups under standard Zémplen conditions, water-soluble glycodendrimers can be isolated. The efficiency of this approach was demonstrated by the synthesis of tetra-, hexa-, and octa-antennary thiourea-bridged cluster α-D-glycopyranosides. A similar technique, involving the formation of thiourethane linkages at the conjugation step, was employed by Roy et al.[15] in the preparation of a hexavalent dendrimer, also terminated by α-D-mannopyranosyl residues.

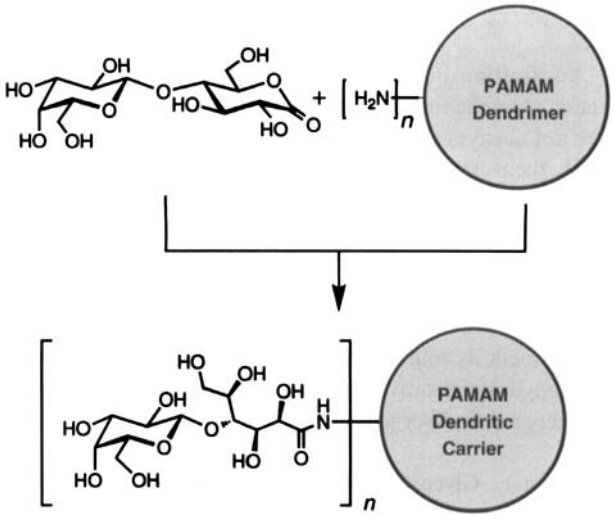
the pre-existing dendritic molecules (Scheme 2). Thus, preformed non-carbohydrate dendrimers can be employed as well-defined matrices for the attachment of sugar residues in an analogous fashion to the use of proteins as carriers of saccharides in the preparation of neoglycoproteins.[5a] This approach requires the presence of numerous reactive groups on the outer surface of the dendrimers and a highly efficient means of functionalizing them.



Scheme 2. A cartoon representation of the construction of carbohydrate-coated dendrimers by modification of non-carbohydrate dendritic matrices. Black dots and open circles represent reactive functionalities located at the dendrimer outer-surface and at the saccharide aglycone, respectively. These functionalities allow the saccharides to be attached to the dendrimer.

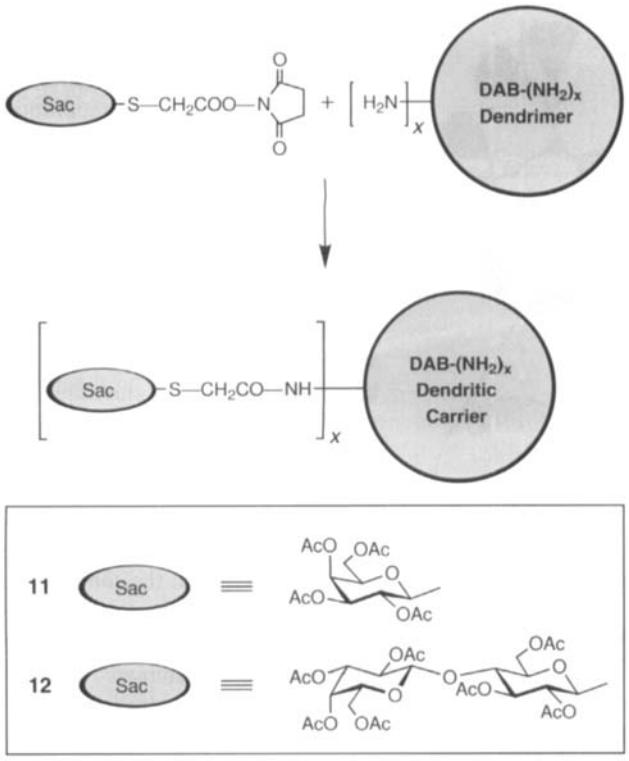
There are many examples of dendritic macromolecules[1] that bear reactive terminal functional groups (e.g. NH_2 and CO_2H) suitable for further chemical modifications. In 1995, Okada et al.[11] reported the functionalization of Tomalia's poly(amido amine) dendrimers[12] (PAMAM dendrimers) with both maltose and lactose derivatives. This modification was achieved by reaction of the terminal amino groups of the PAMAM dendrimers with the corresponding aldonolactones (Scheme 3). On the basis of ^{13}C NMR spectroscopic and GPC data, the authors claim that the substitution of the primary NH_2 groups by the carbohydrates was complete in their "sugar balls".

In an attempt to establish a simple and general way of making carbohydrate-coated dendrimers, we have performed[16] a series



Scheme 3. The attachment of saccharides to a PAMAM dendrimer by amidation of its terminal NH_2 groups with aldonolactones [11]. Free lactonolactone and maltonolactone (not shown) were used in the modification of several PAMAM dendrimers of generations two, three, and four (n = 12, 24, and 48, respectively).

of successful modifications of the poly(propylene imine) dendrimers (DAB-*dendri*-$(NH_2)_x$)[17] with sugar residues. The readily available thiogalactoside and thiolactoside derivatives **11** and **12**, respectively—each endowed with an activated propionic acid aglycone unit—have been attached (Scheme 4) through amide bonds to the terminal NH_2 groups of DAB-*dendri*-$(NH_2)_x$. Application of the well-known *N*-hydroxysuccinimide-mediated coupling technique has allowed an extremely high degree of substitution of the surface NH_2 groups with spacer-armed saccharide residues to be accomplished.



Scheme 4. The synthesis of glycodendrimers by modification [16] of a series of DAB-*dendri*-$(NH_2)_x$ dendrimers. Five different generations of dendrimers were employed with the number terminal primary NH_2 groups x equal to 4, 8, 16, 32, and 64.

Purification and characterization of the glycodendrimers synthesized by the functionalization of high-generation dendrimers are not always easy tasks. However, the efforts spent to accomplish them are compensated by the relative simplicity of the methodology, which allows the synthetic chemist to prepare different carbohydrate-coated dendrimers in a rather limited number of steps, starting from the "standard" dendritic precursors, such as PAMAM or DAB-*dendri*-$(NH_2)_x$, or other readily accessible dendrimers. Moreover, we can anticipate the use of dendrimers as multivalent cores and attaching to them large carbohydrate dendrons, which may be constructed either by convergent or divergent approaches.

2. Cluster Glycosides and Carbohydrate-Coated Dendritic Wedges: Concurrent with developments in the synthesis of carbohydrate-containing dendrimers, attention has also become focused on the elaboration of approaches to multivalent cluster glycosides, based on dendritic wedgelike carriers. The main driving force for this research has been the well-established principle[18] of the enhancement of the binding affinity of some natural carbohydrates toward carbohydrate-binding proteins, such as lectins, resulting from the clustering of carbohydrate units. In order to achieve very strong binding between the saccharide ligands and the protein receptors (e.g. leading to irreversible blocking of such receptors and thus inhibiting their interactions with natural ligands), a number of small carbohydrate clusters[18, 19] have been synthesized, in addition to the development of well-known neoglycoconjugates, constructed through the attachment of carbohydrates to carriers such as proteins[20] and synthetic polymers.[21] Thus, it seems logical to extend the principle of carbohydrate multivalency to dendritic systems. Indeed, since 1993, Roy and his co-workers[22] have published a series of seminal papers describing the synthesis of dendritic glycosides and evaluating their biological activities. The first compounds in this class[23] (e.g. the octamer illustrated in **14** in Figure 3) incorporated *N*-acetylneuraminic acid (sialic acid)[24] as terminal residues. A series of similar polylysine-

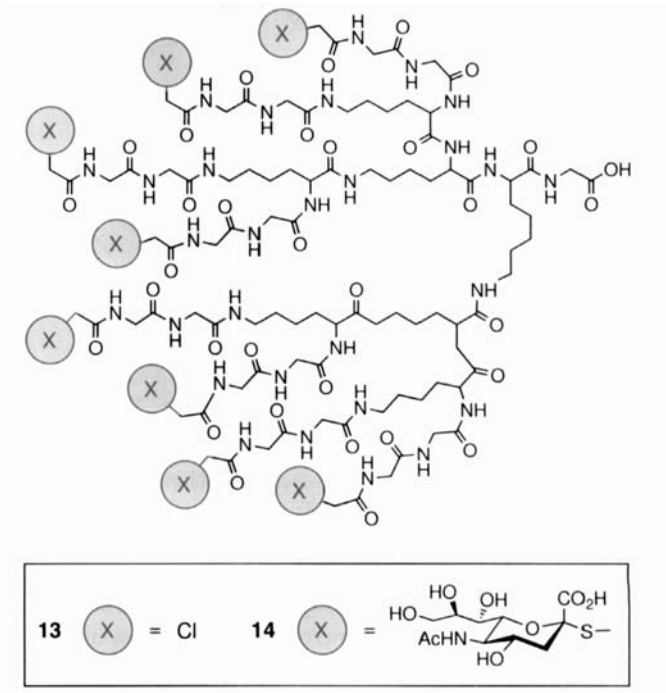
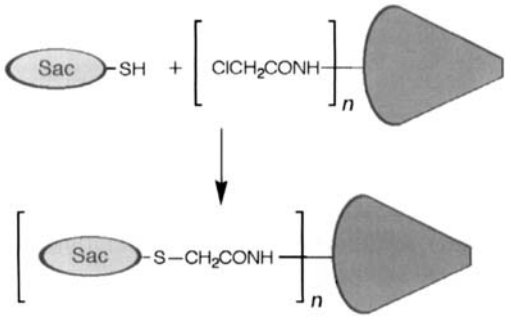


Figure 3. The dendritic polysialoside **14** based on the highly branched oligopeptide core **13** [23].

based dendritic glycosides bearing nonreducing β-glucosaminyl, β-lactosyl, β-lactosaminyl,[25] and α-mannosyl[26] residues, respectively, have also been prepared. The construction of polylysine scaffoldings,[27] such as in the multibranched compound **13**, along with the key couplings of sugar thiols with *N*-chloroacetyl groups located at all eight termini in **13**, were performed on a solid support (Scheme 5). It should be noted that saccharide hydroxyl groups were acetyl-protected during the couplings and that deprotection was performed after releasing the dendrites from the polymer support.



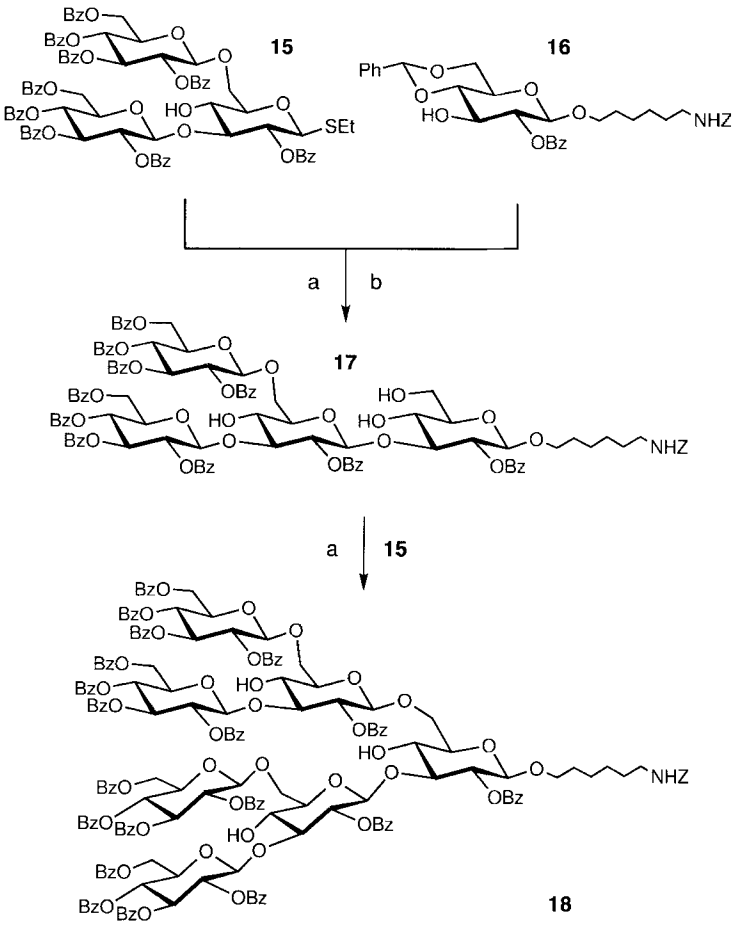
Scheme 5. Coupling of thioylated saccharides with *N*-chloroacetyl termini of a dendritic wedge. The SH group could be positioned either directly at the anomeric center or on the end of the saccharide aglycone. The number of chloroacetylated NH_2 groups (n) is typically 4–16. The method has been utilized for the construction of dendritic glycosides by Roy et al [23, 25–31].

In addition to lysine-based dendritic wedges, the Roy group have also studied dendrons using gallic acid,[28] the phosphotriester group,[22, 29] and, more recently, 3,3′-iminobis(propylamine)[30] as the branching components. Modifications of these dendrons with glucosamine, lactose, and sialic acid residues, respectively, were carried out according to previously described methodologies.[23]

Despite the fact that most of the high-valency glycosides designed and synthesized by Roy's group[22] are not strictly speaking dendrimers,[15] the principles employed in their construction could readily be extended to the synthesis of symmetrical carbohydrate-coated dendrimers. Their synthetic protocols could rather easily be adapted 1) to use solid supports for assembling dendritic peptide carriers, 2) to develop efficient coupling methodologies for attaching carbohydrates to dendritic carriers, and 3) to exploit the recently suggested[31] application of enzymatic glycosylation for the proliferation of dendritic glycosides. It should be possible to create real dendrimers by uniting some of the carbohydrate dendritic wedges already described in the literature by Roy's group. Toward this end, reactions of the focal functionalities of the dendritic wedges with appropriately functionalized multidirectional core molecules might prove to be worthwhile.

3. Toward Fully Carbohydrate Dendrimers: Synthetic fully carbohydrate dendrimers are polysaccharide analogues, which could obviously mimic a broad spectrum of polysaccharide structures and properties. Also, the three-dimensional closely packed architecture of highly branched dendritic oligosaccharides could be responsible for the inclusion properties associated with the inner parts of the molecules. Here, the analogy with the remarkable ability of cyclodextrins[32] to form inclusion complexes with a wide variety of guest molecules comes to mind. It might be anticipated that water-soluble fully carbohydrate dendrimers could possess—like the cyclodextrins—internal voids, capable of encapsulating and solubilizing hydrophobic organic molecules.

In attempts directed toward the generation of fully carbohydrate dendrimers, we have designed and identified a branched oligosaccharide representing a wedge component of a dendrimer. Taking advantage of the recent developments[33] in the synthesis of the phytoalexin elicitor analogues and employing a highly convergent scheme, we have completed the multistep synthesis of the gluco-heptasaccharide **18** possessing β-(1 → 3) and β-(1 → 6) interglycosidic bonds (Scheme 6).[34] The reaction sequence involved the initial preparation of the trisaccharide thioglycoside building block **15**, which was then used as a glycosyl donor in two sequential glycosylations of 3-positions in the glucosyl acceptor **16** and the 6-position in the tetrasaccharide intermediate **17**. The attachment of this heptasaccharide wedge **18** to a central trifunctional core should afford a C_3-symmetrical glucodendrimer of the type anticipated by the cartoon drawn in Figure 1 b.



Scheme 6. Synthesis of the branched glucoheptaoside **18**, which can be used as a fully carbohydrate dendritic wedge for further construction of glycodendrimers. The focal NH_2 group, which can be generated by removal of the Z protecting group, is available to react with a central core component. Reagents and conditions: a) CF_3SO_3H/*N*-iodosuccinimide/CH_2Cl_2, 20 °C; b) 90% CF_3CO_2H/CH_2Cl_2.

Reflections

The wide range of different modes of synthesis of dendritic saccharides demonstrates that the basis for the construction of carbohydrate-containing dendrimers is now established. Small and medium-sized dendritic carbohydrates can be prepared at present by using one of many different approaches. The synthesis of high molecular weight carbohydrate dendrimers matching the high molecular weights of some commonly known neoglycoconjugates is the next challenging task, which demands progress beyond previously elaborated synthetic methodologies. It seems that one of the optimal strategies for glycodendrimer construction might lie in a combination of the convergent approach with the divergent methodology of terminal group modification. Thus, multifunctional dendrimers could be regarded as core components themselves to be used for attachment of large saccharide dendritic wedges in a convergent manner. The carbohydrate-containing dendritic wedges themselves could be constructed, either 1) by the modification of pre-existing dendrons or 2) by using convergent schemes. The second approach would be much more versatile for constructing dendrimers coated with complex oligosaccharides. It is also worth drawing attention to the potential use of the chemoenzymatic syntheses for the construction of both carbohydrate-coated and fully carbohydrate dendrimers. Some well-recognized advantages of this approach—such as, for example, there being no need for the involvment of protecting groups—may mean that it has a unique role to play in glycodendrimer synthesis.

For creating dendritic cores, the use of the solid support technology seems to offer a promising prospect. It might be expected

that such dendrites would be based on highly branched peptide structures, like the polylysine dendron **13**. Such molecular architectures suffer from the disadvantage associated with their low symmetries, which makes their characterization difficuilt. However, replacement of natural amino acids with a symmetrical component, such as compound **4**[8] (Scheme 1) or *N*,*N*-bis-(3-aminopropyl)glycine[30] could provide a solution to this problem. Many attempts have been made to synthesize oligosaccharides by application of the solid support technique.[35] However, spectacular as recent advances have been, we are still somewhat distant from a general methodology that would allow it to be applied forthwith to the synthesis of fully carbohydrate dendrimers. There is, however, another way to employ solid phase synthesis for the preparation of fully carbohydrate dendrimers, which is associated with creating intersaccharide linkages by means of amide bond formation. The syntheses of these types of oligosaccharide mimetics by using solution chemistry were reported recently.[35] The application of this approach to the construction of fully carbohydrate dendrimers seems to be ready for investigation.

The incentive to create carbohydrate-containing dendrimers has emerged in part because of the potential interest which these macromolecules, exhibiting multivalency, as they do, could arouse in the fascinating area of glycoscience. Extensive biochemical studies of dendrimers covered by specific carbohydrate epitopes are currently in progress.[11, 14, 15, 23, 24] With improvements in the quality and availability of dendritic carriers, as well as advances in the development of techniques for constructing carbohydrate dendrimers employing a convergent approach, the attractive concept of anti-adhesive drugs[37] may become a reality. We might also envisage that, in the case of fully carbohydrate dendrimers, the combination of the carbohydrate cluster effect with the ability of the dendrimers to bind and deliver drugs, could be the basis for a potent new therapeutic regime. Further, synthetic carbohydrate based vaccines are being increasingly realized for the development of new therapeutics to induce immunological responses against cancer cells.[38] Application of dendrimer chemistry may also find an avenue in the production of such carbohydrate-based vaccines.[38a]

The physicochemical properties of synthetic dendritic carbohydrates need to be explored in much more detail. So many questions need to be answered. Do glycodendrimers have globular shapes? Do they aggregate in aqueous solution? How do the constitutions of their interior skeletons influence their overall shapes? Is it possible to use the unique properties of glycodendrimers to grow crystalline materials suitable for X-ray diffraction studies? These and many other questions have yet to be answered before the potential of these new dendritic systems can reveal their full scientific and technological significance.[40]

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[2] A. Varki, *Glycobiology* **1993**, *3*, 97–130.

[3] The rapidly growing interest in carbohydrate–protein interactions has led to the evolution of glycobiology as a field of intense scientific inquiry. For an excellent review, see: R. Dwek, *Chem. Rev.* **1996**, *96*, 683–720.

[4] a) K. Toshima, K. Tatsuta, *Chem. Rev.* **1993**, *93*, 1503–1531; b) G.-J. Boons, *Tetrahedron*, **1996**, *52*, 1095–1121, c) S. J. Danishefsky, M. T. Bilodeau, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380–1419.

[5] The majority of the research performed in this area is directed toward creating synthetic analogues of natural glycoconjugates—the so-called neoglycoconjugates. These compounds provide an insight into the biological functions of saccharides and are an indispensable aid to understanding many aspects of glycobiology. For leading references, see: a) C. P. Stowell, Y. C. Lee, *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225–281; b) *Neoglycoconjugates: Preparation and Applications* (Eds.: Y. C. Lee, R. T. Lee), Academic Press, San Diego, **1994**; c) *Methods in Enzymology, Vol. 242, Neoglycoconjugates, Part A, Synthesis* (Eds.: Y. C. Lee and R. T. Lee), Academic Press, San Diego, **1994**; d) *Methods in Enzymology, Vol. 247, Neoglycoconjugates, Part B, Biomedical Applications* (Eds.: Y. C. Lee and R. T. Lee), Academic Press, San Diego, **1994**.

[6] The term *glycodendrimers* has also been suggested by Roy for this class of compounds: in practice, it is used for dendrimers, dendrons, and even compounds consisting of relatively small multi-antennary molecules. An article entitled *Glycodendrimere* has recently been published in German; see: T. K. Lindhorst, *Nachr. Chem. Tech. Lab.* **1996**, *44*, 1073–1079.

[7] For a discussion on the wide range of building blocks available for constructing dendrimers, see: G. R. Newkome, C. N. Moorefield, G. R. Baker, *Aldrichim. Acta.* **1992**, *25*, 31–38.

[8] P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, S. A. Nepogodiev, J. F. Stoddart, *Chem. Eur. J.* **1996**, *2*, 1115–1128.

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[11] K. Aoi, K. Itoh, M. Okada, *Macromolecules* **1995**, *28*, 5391–5393.

[12] D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, P. Smith, *Polym. J.* **1985**, *17*, 117–132. A range of Tomalia's PAMAM dendrimers from generations one to five is now available from the Aldrich Chemical Company.

[13] Most of the synthetic techniques rely upon covalent bond formation between spacer-armed saccharides and functional groups on the polymer carriers. For more details, consult ref. [5].

[14] K. Lindhorst, C. Kieburg, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1953–1956.

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[40] **Note added in proof:** The feasibility of using unprotected carbohydrates in dendrimer syntheses has been established very recently. Kieburg and Lindhorst have demonstrated that unprotected carbohydrates, tethered with isothiocyanate functional groups, can readily form thiourea bridges with amine-terminated PAMAM dendrimers (C. Kieburg, T. K. Lindhorst, *Tetrahedron Lett.* **1997**, *38*, 3885–3888). In a convergent synthetic approach, we have synthesized some lower-generation carbohydrate-containing dendrimers using unprotected carbohydrates (N. Jayaraman, J. F. Stoddart, *Tetrahedron Lett.*, submitted for publication). The key reaction, which proceeds in DMF/Pyridine (9:1) at 60°C, is one between a three-directional core, carrying *N*-hydroxysuccinimide ester functions, and free glucoside and mannoside-containing dendritic wedges with amino groups at their focal points. The development of synthetic routes utilizing unprotected carbohydrates to prepare dendrimers has the advantages that i) it circumvents steric inhibition caused by the presence of protecting groups on the saccharide residues in a growing dendrimer and ii) it avoids the consequent reduction in the surface densities of the final free saccharide-containing dendrimers upon removal of the protecting groups. The absence of any protecting groups on the peripheral glycoside units should now make it possible to prepare large densely-packed carbohydrate-containing dendrimers without the need to resort to protecting group manipulations on the saccharide residues.

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