Synthesis of Glycodendrimers by Modification of Poly(propylene imine) Dendrimers**

Peter R. Ashton, Sue E. Boyd, Christopher L. Brown, Sergey A. Nepogodiev, E. W. Meijer,* H. W. I. Peerlings, and J. Fraser Stoddart*

Abstract: The use of preformed poly(propylene imine) dendrimers (DAB–*dendr*–$(NH_2)_x$) for the rapid and facile construction of high molecular weight carbohydrate-coated dendrimers (glycodendrimers) is presented. An efficient attachment of spacer-armed derivatives of D-galactose and lactose to the primary amino end groups of DAB–*dendr*–$(NH_2)_x$ has been achieved by means of amide bond formation, using the *N*-hydroxysuccinimide coupling procedure. Acetate protecting groups have been employed in order to avoid side reactions at the coupling stage. Deacetylation leads to the target glycodendrimers. The reactivity of all the available DAB–*dendr*–$(NH_2)_x$ (generations 1–5) has been investigated and a series of homologous carbohydrate-coated dendrimers have been synthesized. In addition, the attachment of larger saccharide moieties has been demonstrated by the condensation of a trisgalactoside cluster with DAB–*dendr*–$(NH_2)_x$ carrying both four and eight primary amino groups. The regularity of the glycodendrimers has been proven by NMR spectroscopy, and the molecular weights of the low-generation carbohydrate-coated dendrimers have been determined by mass spectrometry. Modifications of DAB–*dendr*–$(NH_2)_x$ with biologically active carbohydrates affords a new and simple approach to high molecular weight compounds that may be considered as neoglycoconjugates with perfectly symmetrical structures and that offer much promise as multivalent ligands involved in carbohydrate–protein interactions.

Keywords
carbohydrates · dendrimers · glycosides · neoglycoconjugates · polymers

Introduction

Dendrimers are known for their well-defined, regular, highly branched architectures with a large number of reactive end groups and their guest–host properties.[1] The end groups are confined in space and are present at the periphery of the molecule. Therefore, dendrimers become unique starting materials for numerous chemical modifications. End group modification is particularly attractive for—and is mostly employed on—dendrimers made by the divergent approach.[2] From this class of compounds, the poly(amido amine) dendrimers (PAMAM dendrimers),[2c, 3] the arborols,[4] and the poly(propylene imine) dendrimers (DAB–*dendr*–$(NH_2)_x$)[5] are among those most frequently studied on account of their availability on a reasonable to large scale, even for the higher-generation dendrimers. Some of the unique properties of dendrimers, for example, dense surface packing and controled architecture, only arise in the case of dendrimers of higher generations,[6] and there is therefore a need to use dendrimers that have an almost perfect structure. The attachment of certain residues to the surface of dendrimers has attracted enormous attention. Structures obtained in this way include unimolecular (inverted) micelles,[7] catalysts,[8] metallodendrimers,[9] and dendritic boxes.[10] In addition, biomolecules such as peptides[11] and antibodies[12] have been attached to dendritic scaffolds. The modification of various functional groups on the surface of the dendrimer can also lead to changes in, or enhancement of, some of their properties as a consequence of cooperative effects.[7, 10, 13] In biological systems, we can identify numerous examples of molecular recognition processes enhanced by multiple interactions within the molecule. This kind of multivalent effect is well known for carbohydrate–protein interactions[14] for which, in many cases, binding of saccharide ligands by protein receptors can be improved significantly by the clustering of the saccharide structures.[15] It has been proposed that several sugar residues, joined to the branching point by appropriate lengths of spacer arms through covalent bonds, may be able to form much stronger complexes with protein receptors than do individual saccha-

[*] Prof. J. F. Stoddart, Dr. C. L. Brown, Dr. S. E. Boyd, Dr. S. A. Nepogodiev, P. R. Ashton
School of Chemistry, University of Birmingham
Edgbaston, B15 2TT Birmingham (UK)
Fax: Int. code +(121)414-3531
Prof. E. W. Meijer, H. W. I. Peerlings
Laboratory of Organic Chemistry, Eindhoven University of Technology
P. O. Box 513, NL-5600 MB Eindhoven (The Netherlands)
Fax: Int. code +(40)245-1036

[**] Synthetic Carbohydrate Dendrimers, Part 3. For Part 2, see: P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, J. F. Stoddart, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, issue 7. A list of abbreviations used in this paper is given in ref. [37].

© VCH Verlagsgesellschaft mbH, D-69451 Weinheim, 1997
0947-6539/97/0306-0974 $ 17.50+.50/0

rides. This belief has stimulated research on the synthesis of multivalent carbohydrate structures.[16] With these aims in mind, dendritic molecules can be considered as suitable candidates for potential carriers of oligosaccharides in the preparation of neoglycoconjugates,[17] rather than carriers based on proteins or synthetic polymers.

Recently, a series of papers on the chemical modification of dendrite-like macromolecules, aimed at the preparation of biologically active glycodendrimers, has been published by Roy and co-workers.[18] Although the concept of multivalency has been developed very elegantly in this research, the highly branched scaffoldings are not those of the spherically shaped dendrimers that are our aim. After our own research programme had started,[19] Okada et al.[20a] reported the synthesis of a so-called "sugar ball"—poly(amido amine) (PAMAM) dendrimer[3] conjugated with 48 carbohydrate residues derived from 4-*O*-(β-D-galactopyranosyl)-D-gluconic acid. Moreover, Lindhorst and Kieburg[20b] have recently described a number of cluster glycosides, also starting from PAMAM dendrimers. We now present our results on the modification of DAB-*dendr*-$(NH_2)_x$[5] involving the multiple attachment of saccharide units through a spacer arm, terminated with carboxylic acid end groups, to the peripheral primary amine groups, that is, the carbohydrate–dendrimer linkages are amide bonds. The approach combines commercially available dendrimers, simple saccharide derivatization, and standard coupling techniques to yield complex but well-defined structures.

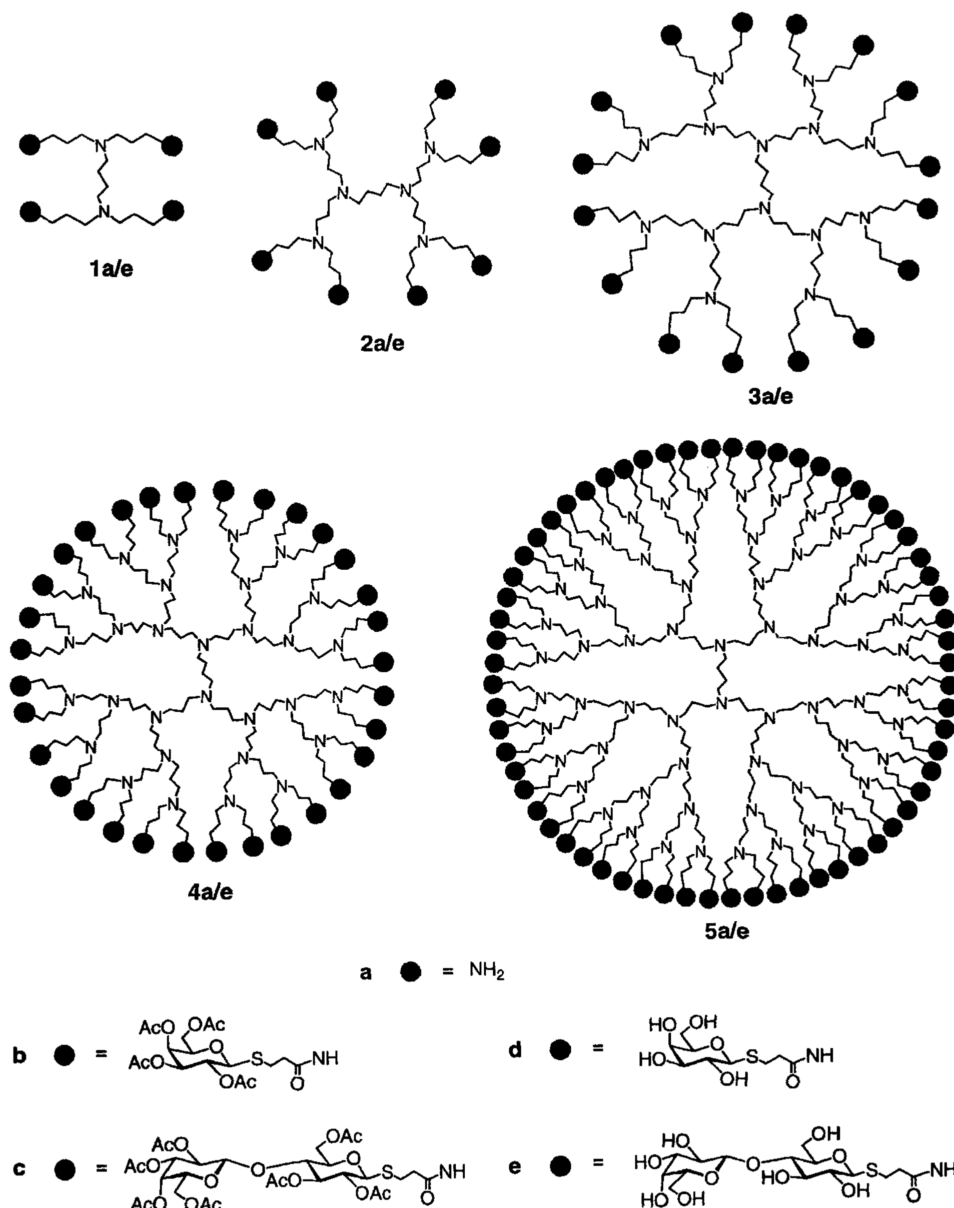


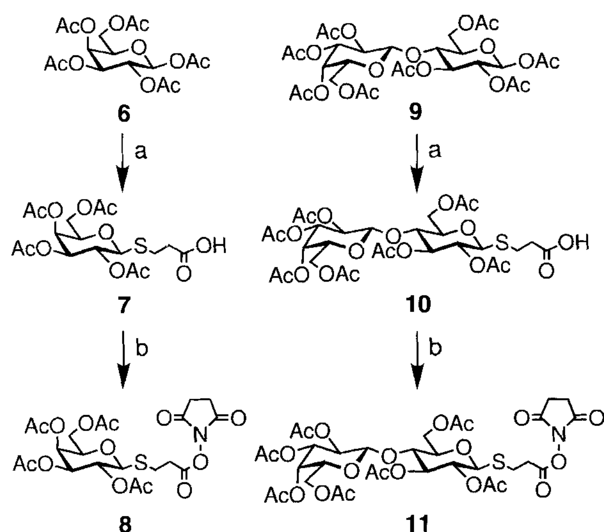
Figure 1. The poly(propylene imine) dendrimers **1a–5a** and the glycodendrimers **1b/e–6b/e** obtained according to Scheme 2.

Results and Discussion

The Synthetic Strategy: As a result of our current research to optimize the synthesis of *poly(propylene imine) dendrimers*, we have now available well-defined DAB-*dendr*-$(NH_2)_x$, **1a–5a** (Figure 1), with 4, 8, 16, 32, and 64 primary amine end groups, respectively.[5b] These dendrimers have been characterized in great detail and are among the most monodisperse dendritic compounds. Electrospray mass spectrometry has shown that the first three generations of DAB-*dendr*-$(NH_2)_x$ are defect free and that the fifth generation, with 64 terminal amine groups, possesses a polydispersity of 1.002, or a dendritic purity of 18%.[21] From our investigations, it is known that the carboxylic acid functionalized dendrimers[22] lead to complex reaction mixtures upon attempted modifications, whereas the primary amine terminated analogues DAB-*dendr*-$(NH_2)_x$ can be cleanly brought into reaction with a variety of reagents, including sulfonyl chlorides,[13a] acid anhydrides,[23] activated esters,[10, 13a] and acid chlorides.[7c] In the knowledge that the preparation of neoglycoconjugates by the covalent linking of saccharides to carriers is well established,[17b, 24] one can envisage using a range of preformed dendrimers bearing primary amino groups on the periphery as a macromolecular support for the attachment of carbohydrates. The linking of the sugars may be achieved by the conventional methods of amide bond formation, provided that the reaction proceeds quantitatively or nearly so. Since we expected that the size and complexity of the

compounds associated with the higher generation dendrimers would give problems in their characterization, we decided to synthesize a whole series of carbohydrate-coated dendrimers based on all generations of DAB-*dendr*-$(NH_2)_x$ **1a**–**5a**, starting from the chemical modification and analysis of lower generation dendrimers. We note that a very successful modification of the dendrimer **5a** has been performed[10, 13a] using activated ester derivatives of amino acids. Therefore, we decided to use the same mild coupling method for the construction of the carbohydrate-coated dendrimers.

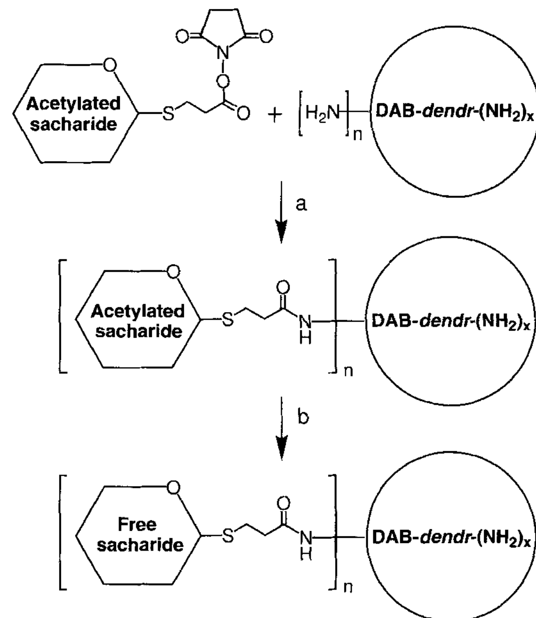
Modification of DAB-*dendr*-$(NH_2)_x$ with Saccharide Residues: Before the coupling of the saccharide residues to the dendrimers, two carbohydrates (D-galactose and D-lactose) were converted to their acetylated spacer-armed derivatives containing an activated ester as the reactive end group (Scheme 1).



Scheme 1. Synthesis of galactose and lactose derivatives for attachment to the DAB-*dendr*-$(NH_2)_x$ dendrimers. Reagents and conditions: a) $HS(CH_2)_2CO_2H/BF_3 \cdot OEt_2/CH_2Cl_2$, 25 °C, 4–5 h, 88% (**7**), 53% (**10**); b) NHS/DCC/$MeO(CH_2)_2OMe$, 0–10 °C, 20 h, 93% (**8**), 95% (**11**).

A simple and efficient procedure[25] ($HSCH_2CH_2CO_2H/BF_3 \cdot OEt_2/CH_2Cl_2$, 20 °C, 5 h) afforded the thioglycoside **7** in high yield, starting from D-galactose β-pentaacetate **6**. Employing very similar conditions, lactose octaacetate[26, 27] **9** was transformed into **10** in 53% yield. The activated esters **8** and **11** were prepared from **7** and **9**, respectively, by condensation of these carboxylic acid derivatives with *N*-hydroxysuccinimide under the influence of DCC ($MeOCH_2CH_2OMe$, 0–5 °C, 18 h).[28] The *O*-acetyl protection of the hydroxyl groups was essential for the efficient coupling of activated esters to the dendrimers: after the attachment of the saccharide to the dendrimers, they were deprotected.

Coupling of the active esters **8** and **11** with the DAB-*dendr*-$(NH_2)_x$ (Scheme 2) was carried out by using one molar equivalent of the activated ester for every primary amino end group present in the dendrimers. Reactions were performed in CH_2Cl_2 at 25 °C for 15–18 h to give carbohydrate dendrimers **1b/c**–**5b/c** (Figure 1), which were isolated after vigorous washing of the diluted (CH_2Cl_2) reaction mixtures with aqueous saturated



Scheme 2. Coupling of saccharide units with the primary amino groups in the dendrimers. Reagents and conditions: a) CH_2Cl_2, 25 °C, 15–18 h; b) 1. MeONa/$MeOH/CH_2Cl_2$, 25 °C, 10–15 min, 2. $NaOH/H_2O/MeOH$, 25 °C, 16 h.

Na_2CO_3. No starting materials or acids derived from **8** or **11** could be detected following this particular workup procedure. Deprotection of the peracetates **1b/c**–**5b/c** was achieved by subjecting them to standard Zemplen deacylation,[29] followed by treatment with an aqueous NaOH solution. The deacetylated products **1d/e**–**5d/e** (Figures 2 and 3) were completely soluble in water and their desalination was achieved successfully in all cases by means of GPC.

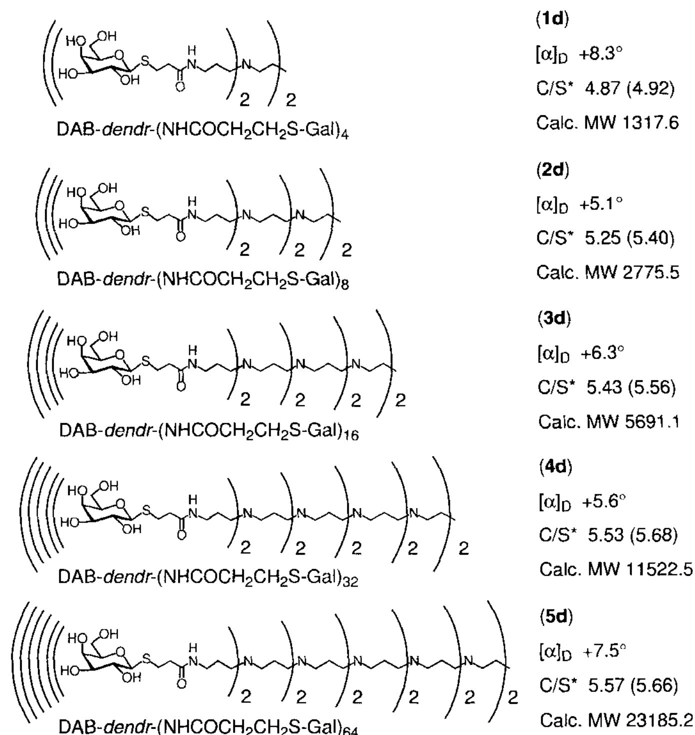


Figure 2. Galactodendrimers **1d**–**5d**. C/S* gives the calculated (and found) microanalyses.

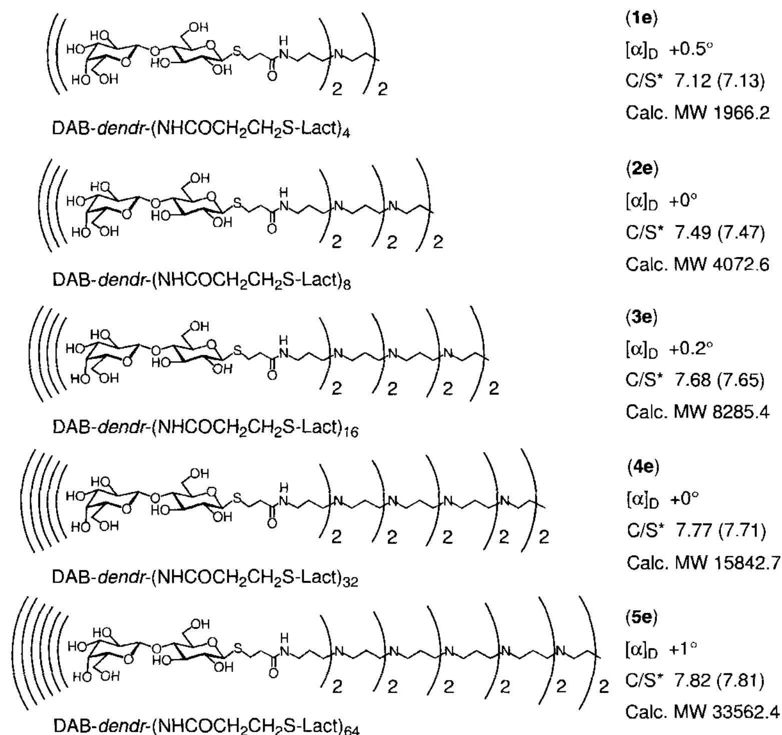


Figure 3. Lactodendrimers **1e–5e**. C/S* gives the calculated (and found) microanalyses.

Determination of the Structure of the Galacto- and Lacto-Dendrimers: Evidence for the introduction of saccharide moieties at the surface of the dendrimers **1a–5a** was forthcoming from the inspection of the NMR spectra of the coupled products. In all cases, the ^{13}C NMR spectra are more diagnostic of the products than are the ^{1}H NMR spectra. Both the galactose and lactose residues of the acetylated glycodendrimers **1b/c–5b/c** give rise to ^{13}C NMR resonances in the region $\delta = 60-101$ in the shape of well-resolved signals, namely, six and twelve for **b** and **c**, respectively (Figure 1). The chemical shifts of particular carbon atoms (C1–C6) in the saccharide residues of the five generations of dendrimers are almost coincident (Table 1). These signals were assigned on the basis of the unambiguous assignments of the resonances in the ^{13}C NMR spectra of the monosaccharide precursors **7** and **10**: the assignments were established by COSY and C-H correlation experiments. The acetyl protecting groups are very clearly evident as two sets of peaks located around $\delta = 20$ (CH_3CO) and in the region $\delta = 169-170$ (CH_3CO), where signals for the NHCO groups also appear. All the remaining signals for the inner parts of the acetylated glycodendrimers are to be found between $\delta = 26$ and 54 (see the ^{13}C NMR chemical shifts listed in Table 1).

Characteristic signals for the acetyl groups, the amide functions, and the pyranose ring protons were also identified in the ^{1}H NMR spectra of the glycodendrimers **1b/c–5b/c**. However, well-resolved spectra were obtained only for the modified low-generation dendrimers (see, for example, the ^{1}H NMR data for **1b** and **1c** recorded in the Experimental Section). In the case of the higher molecular weight dendrimers, a considerable broadening of the spectral lines was observed.

The IR spectra obtained for compounds **1b/c–5b/c** are almost identical within each generation, differing only a little in the relative intensities of selected absorption bands. A strong absorption at 1655 cm^{-1} is characteristic of the newly formed amide bond.

In order to determine the molecular weight of the carbohydrate-modified dendrimers **1b/c–5b/c**, LSI-MS techniques were applied, and the molecular ion peaks for the dendrimers **1b** and **2b**, and **1c** and **2c**, with four (**b**) and eight (**c**) saccharide end groups, were identified successfully (Table 2). A satisfactory result was also obtained using LSI-MS for dendrimer **3b** with its 16 acetylated monosaccharide units, again showing essentially the presence of a single molecular compound devoid of statistical defects. This observation is in agreement with the ES-MS of the starting materials. The molecular masses of the higher molecular weight compounds **3c**, **4b/c**, and **5b/c** cannot be determined by LSI-MS as a consequence of the limitations of the

Table 1. ^{13}C NMR spectroscopic data (selected δ values) for acetylated glycodendrimers **1b/c–5b/c** and **7** and **10** at 100.6 MHz in $CDCl_3$ at 25 °C.

Compound [a]		Carbohydrate component						Dendritic component					
		C-1	C-2	C-3	C-4	C-5	C-6	SCH_2	CH_2CONH	$CONHCH_2$	CH_2NCH_2		$CH_2CH_2CH_2$
1b		84.4	67.0	71.5	67.1	74.3	61.3	26.5	36.8	37.9	51.5	53.5	26.7
2b		84.2	66.8	71.3	67.0	74.1	61.1	26.3	36.5	37.4	50.9	51.3	26.4
3b		84.4	67.0	71.5	67.0	74.2	61.3	26.5	36.7	37.7	51.1	51.7	26.8
4b		84.2	67.0	71.4	67.0	74.0	61.1	26.3	36.5	37.4	50.8	51.6	26.6
5b		84.3	67.2	71.5	67.2	74.2	61.3	26.4	36.5	37.4	50.8	51.6	26.4
7		84.6	67.1	71.8	67.3	74.5	61.6						
1c	Glc	100.7	69.9	73.4	75.7	76.6	61.6	26.7	36.9	37.8	51.3	53.5	26.7
	Gal	84.0	68.8	70.6	66.4	70.3	60.5						
2c	Glc	100.8	70.0	73.5	75.7	76.7	61.7	26.8	36.9	37.7	51.1	51.9	26.8
	Gal	84.0	68.9	70.7	66.4	70.4	60.6						
3c	Glc	100.6	69.9	73.4	75.7	76.5	61.7	26.6	36.7	37.4	50.9	51.6	26.6
	Gal	83.8	68.8	70.6	66.4	70.3	60.5						
4c	Glc	100.9	70.1	73.6	75.9	76.7	61.8	26.6	36.7	37.7	50.0	51.6	26.6
	Gal	84.1	69.0	7.08	66.5	70.5	60.6						
5c	Glc	100.6	70.0	73.5	75.3	76.5	61.7	26.6	36.7	37.3	51.1	51.1	26.6
	Gal	83.7	68.8	70.6	66.4	70.3	60.5						
10	Glc	101.8	70.0	73.5	76.0	76.5	62.1						
	Gal	83.6	69.0	70.8	66.5	70.5	60.7						

[a] For lactose units in **1c–5c** and **10**, the chemical shifts are given for glucose (Glc) and galactose (Gal) residues in separate rows.

Table 2. Mass spectrometric data for the glycodendrimers.

	Molecular formula	Calculated molec. mass	Mass spectrometric data LSI-MS	MALDI-TOF-MS
1b	$C_{84}H_{128}N_6O_{40}S_4$	1990.2	1990 $[M+H]^+$	1990 $[M+H]^+$
2b	$C_{176}H_{272}N_{14}O_{80}S_8$	4120.7	4143 $[M+Na]^+$	4124 $[M+H]^+$
3b	$C_{360}H_{560}N_{30}O_{160}S_{16}$	8381.5	8401 $[M+Na]^+$	8402 $[M+Na]^+$
1c	$C_{132}H_{192}N_6O_{72}S_4$	3143.2	3143 $[M+H]^+$	3143 $[M+H]^+$
			3165 $[M+Na]^+$	
2c	$C_{272}H_{400}N_{14}O_{144}S_8$	6426.7	6448 $[M+Na]^+$	6433 $[M+H]^+$
1d	$C_{52}H_{96}N_6O_{24}S_4$	1317.6		1319 $[M+H]^+$
				3141 $[M+Na]^+$
2d	$C_{112}H_{208}N_{14}O_{48}S_8$	2775.5		2777 $[M+H]^+$
				2799 $[M+Na]^+$
1e	$C_{76}H_{136}N_6O_{44}S_4$	1966.2		1989 $[M+Na]^+$
2e	$C_{160}H_{288}N_{14}O_{88}S_8$	4072.6		4097 $[M+Na]^+$
16a	$C_{228}H_{328}N_{14}O_{132}$	5377.2	5399 $[M+Na]^+$	
16b	$C_{132}H_{232}N_{14}O_{84}$	3359.4		3387 $[M+Na]^+$

technique. Although the use of MALDI-TOF mass spectrometry was attempted with these compounds, reliable determination of molecular weights again proved to be impossible. Clearly, statistical defects are present in these dendrimers as a result of the divergent nature of the synthesis. In contrast, the lower molecular weight compounds **1b–3b** and **1c** and **2c** exhibit good MALDI-TOF spectra, which contain peaks for both $[M+Na]^+$ and $[M+K]^+$ ions.

The structures of the unprotected carbohydrate dendrimers **1d/e–5d/e** were also analyzed by NMR spectroscopy and mass spectrometry. The ^{13}C NMR spectra of these compounds (Table 3 and Figure 4 for the galactodendrimers) obtained in D_2O show remarkable similarities in particular regions of the spectra, when they are grouped together, either according to the nature of the carbohydrate residue, or according to the generation of the DAB-*dendr*-$(NH_2)_x$ core. The resonances for the ^{13}C nuclei of the galactose or lactose residues appear as a set of six or twelve signals, respectively; the chemical shifts of the signals for comparable nuclei in different generations is absolutely identical. Therefore, the nature of the dendritic core does not influence the NMR spectroscopic behavior of the carbohydrate residues. Also, the signals for the poly(propylene imine) parts of the glycodendrimers **1d/e–5d/e** are comparable for the galactose- (**d**) and lactose-containing (**e**) compounds built up from DAB-*dendr*-$(NH_2)_x$ dendrimers of the same generation. The signals appear as sets of five to nine resonances of different intensity in the range $\delta = 21-56$ and have a specific pattern for dendrimers of a particular generation (Figure 4). The relative simplicity of these parts of the spectra for compounds **1d/e–5d/e** allows us to conclude that the dendritic cores do not contain any serious defects as a result of the chemical manipulations (e.g., basic treatment in the workup procedure or the deacetylation) performed on these compounds. Signals for the amide carbonyl groups are also observed very clearly at $\delta = 177.3-177.4$.

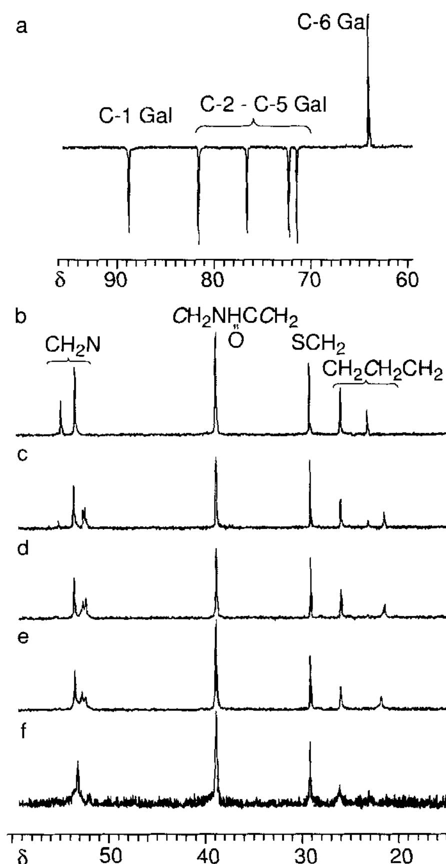


Figure 4. The ^{13}C NMR spectra of galactodendrimers **1d–5d** recorded at 75.5 MHz in D_2O at 25 °C using the PENDANT technique: a) Signals of the D-galactopyranosyl residue **1d** (this portion of the spectra is essentially the same for the whole series) and b–f) Dendritic core region of the spectra of **1d**, **2d**, **3d**, **4d**, and **5d**, respectively.

Table 3. ^{13}C NMR data (δ values) of glycodendrimers **1d/e–5d/e** and **16b** and **17b** at 75.5 MHz in D_2O at 25 °C [a].

	Generation 1			Generation 2			Generation 3		Generation 4		Generation 5	
	1d	**1e**	**16b**	**2d**	**2e**	**17b**	**3d**	**3e**	**4d**	**4e**	**5d**	**5e**
$CH_2CH_2CH_2$	23.3	23.3	23.2	21.6	21.5	21.5	21.6	22.0	21.9	22.5	24.7	22.1
				23.3	23.3	23.3						
SCH_2	26.1	26.1	26.0	26.1	26.1	26.0	26.1	26.1	26.0	26.2	26.1	26.2
$CONHCH_2CH_2$	29.3	29.0	24.3	29.2	28.9	24.3	29.2	28.9	29.1	28.9	29.3	28.9
$CH_2CONHCH_2$	38.9	38.9	38.8	38.9	38.9	38.8	38.9	38.9	38.0	39.0	39.0	39.0
CH_2NCH_2	53.4	53.3	53.1	52.4	52.4	52.3	52.4	52.7	52.3 54.0	52.4–54.0	53.4	53.4
	54.8	54.8	54.8	52.6	52.5	52.4	52.7	52.8				
				53.6	53.5	53.3	53.6	53.5				
				55.2	55.2	55.2						
$CH_2CONHCH_2$	177.4	177.3	178.9	177.4		178.8	177.4	177.2	177.3	177.2	177.3	177.2

[a] Signals of terminal saccharide units in i) galactodendrimers **1d–5d**, d 64.0 (C-6), 71.5, 72.3, 76.6, 81.6 (C-2, C-3, C-4, C-5), 88.7–88.8 (C-1), ii) lactodendrimers **1e–5e**, d 63.0–63.1 and 63.7–63.8 (C-6 Gal and C-6 Glc), 71.2, 73.6, 74.6-74.7, 75.2, 78.0–78.1, 78.5, 81.0–81.2 (C-2, C-3, C-4, C-5 of Gal and C-2, C-3, C-4, C-5 of Glc), 81.3, 87.9–88.0 (C-1 Glc), 105.5–105.6 (C-1 Gal), iii) trisgalactosidodendrimers **16b** and **17b**, d 37.2–37.3, 37.5, 45.4 ($COCH_2CH_2CH_2CO$), 62.6 (CH_2 of Tris), 63.7 (C-6), 70.3, 71.3, 73.4, 75.2, 77.8 (C-2, C-3, C-4, C-5 of Gal), 106.2 (C-1 Gal), 173.6–173.7 (NHCO of Tris).

© VCH Verlagsgesellschaft mbH, D-69451 Weinheim, 1997
0947-6539/97/0306-0978 $ 17.50+.50/0

The ^1H NMR spectra of all the glycodendrimers, recorded at 25 °C in D_2O, exhibit extensive broadening of all signals. Elevated temperature (100 °C) and the use of $(CD_3)_2SO$ as a solvent were explored in an attempt to overcome this problem. Some improvements in the quality of the spectra were achieved, particularly for the first generation in each series: interpretation of the ^1H NMR spectra of **1d** and **1e** is given in the Experimental Section. It should be noted that when ^1H NMR spectra of the original DAB-*dendr*-$(NH_2)_x$ **1a**–**5a** were recorded under the same conditions, this dramatic difference in the resolution of the spectra of lower and higher generation dendrimers was not observed. Mass spectrometric analysis using the MALDI-TOF technique was also useful for characterization of the lower molecular weight glycodendrimers **1d**, **2d**, **1e**, and **2e** (Table 2). In all these cases, the $[M+Na]^+$ ion appears as the highest intensity peak in the mass region above 1 kDa.

The chiroptical properties of the glycodendrimers were also investigated. Polarimetry revealed that the specific optical rotations of the compounds in each carbohydrate series remain nearly constant, with $[\alpha]_D$ values of about +6° for the galactose dendrimers and 0° for the lactose dendrimers (Table 4). Although these observations are not unexpected, they are in sharp contrast with the significant decline in the specific optical rotations for amino acid modified DAB-*dendr*-$(NH_2)_x$ dendrimers with increasing generations.[13a]

Table 4. Yields and specific optical rotation data for the acetylated glycodendrimers **1b/c**–**5b/c** and free glycodendrimers **1d/e**–**5d/e**.

Generation	Acetylated glycodendrimers			Unprotected glycodendrimers		
	Compd	Yield/%	$[\alpha]_D$ ($CHCl_3$)	Compd	Yield/%	$[\alpha]_D$ (H_2O)
1	**1b**	96	−4.5 (c = 1.0)	**1d**	87	+8.2 (c = 0.9)
2	**2b**	100	−4.8 (c = 1.0)	**2d**	85	+5.1 (c = 1.0)
3	**3b**	99	−4.9 (c = 1.0)	**3d**	88	+6.3 (c = 1.0)
4	**4b**	100	−5.8 (c = 1.0)	**4d**	74	+5.6 (c = 1.0)
5	**5b**	97	−5.5 (c = 1.0)	**5d**	69	+7.5 (c = 1.0)
1	**1c**	97	−5.5 (c = 1.0)	**1e**	78	+0.5 (c = 1.1)
2	**2c**	97	−7.2 (c = 1.0)	**2e**	79	0 (c = 1.2)
3	**3c**	100	−8.0 (c = 1.0)	**3e**	73	+0.2 (c = 1.1)
4	**4c**	94	−8.5 (c = 1.0)	**4e**	69	0 (c = 1.1)
5	**5c**	93	−7.7 (c = 1.1)	**5e**	64	+1 (c = 1.2)

The synthetic methodology we have employed relies upon the complete reactivity of many amino groups on the dendrimer surfaces. The full characterization by numerous methods, including mass spectrometry, of the first-, second-, and third-generation glycodendrimers allows the unambiguous assignment of completely functionalized molecules without statistical defects: they are perfect molecular compounds. For the higher generations, reliable NMR spectroscopic information, even without mass spectrometric evidence, allows us to conclude with considerable confidence that extremely efficient functionalization of the larger dendrimers—**4a** and **5a**—has been achieved. However, it is obvious that there is a small number of statistical defects, at least of the same order as those present in the starting poly(propylene imine) dendrimers.[21] The number of saccharide units incorporated in the glycodendrimers was estimated by combustion analysis,[30] which gives a reliable ratio of carbon to sulfur (Table 5); absolute values from elemental analyses were not reliable, as might be expected for such hygroscopic compounds.

Table 5. C/S ratios, theoretical and calculated from microanalytical data, for the free glycodendrimers **1d**–**5d** and **1e**–**5e**.

Compd	Molecular formula	C/S (calcd)	C/S (found)
1d	$C_{52}H_{96}N_6O_{24}S_4$	4.87	4.92
2d	$C_{112}H_{208}N_{14}O_{48}S_8$	5.25	5.40
3d	$C_{232}H_{432}N_{30}O_{96}S_{16}$	5.43	5.56
4d	$C_{472}H_{880}N_{62}O_{192}S_{32}$	5.53	5.68
5d	$C_{952}H_{1776}N_{126}O_{384}S_{64}$	5.57	5.66
1e	$C_{76}H_{136}N_6O_{44}S_4$	4.87	4.92
2e	$C_{160}H_{288}N_{14}O_{88}S_8$	7.49	7.67
3e	$C_{328}H_{592}N_{30}O_{176}S_{16}$	7.68	7.65
4e	$C_{664}H_{1200}N_{62}O_{352}S_{32}$	7.77	7.71
5e	$C_{1336}H_{2416}N_{162}O_{704}S_{64}$	7.82	7.81

In general, the degree of functionalization of the amino groups in the DAB-*dendr*-$(NH_2)_x$ dendrimers by saccharides will probably depend to a large extent on the structure of the saccharides. We might reasonably expect very large oligosaccharides to reveal a tendency toward random and incomplete attachment to the amino groups on the surface of the dendrimers, particularly those of higher generations.

Analysis of the galactodendrimer **5d**, which contains 64 galactose units, by the use of simulated annealing stochastic molecular dynamics (as implemented in the program Macromodel 5.0,[31] at a simulated bath temperature of 300 K using the GB/SA solvent model[32] for water), revealed the highly globular nature of the dendritic macromolecule (Figure 5). It

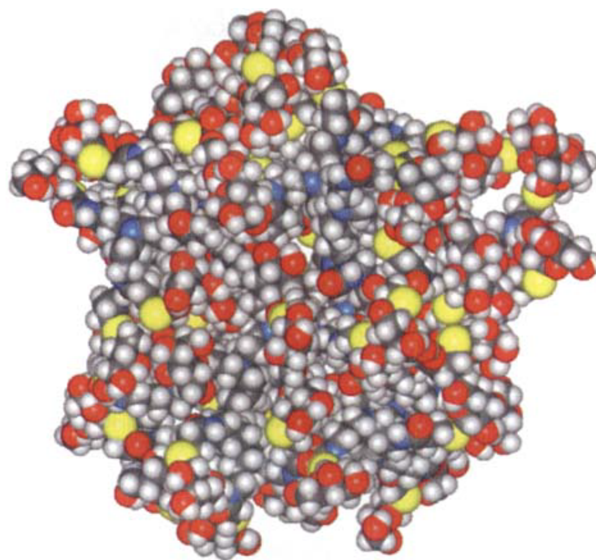


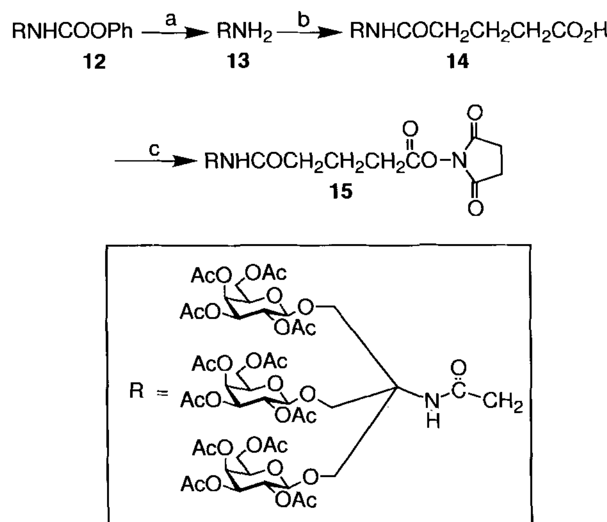
Figure 5. The molecular visualization of **5d** in the form of a sample taken at 300 K from the simulated annealing molecular dynamics simulation. Atoms are colored as follows: carbon, grey; hydrogen, white; nitrogen, blue; oxygen, red; and sulfur, yellow.

occupies a molecular volume of about 17400 Å^3 and has an average molecular radius of 28 Å (taken as the average of 15 distances described by a point located at the center of the central bond of the core unit to various points located at the outermost periphery on the surface). This visualization has given us some insight into the dimensions and transient dynamic motions of molecules of this type. Investigation of the "movie" file obtained from the output of the molecular dynamics simula-

 © VCH Verlagsgesellschaft mbH, D-69451 Weinheim, 1997 0947-6539/97/0306-0979 $ 17.50+.50/0

tion allowed us to observe transient voids and channels on the surface of the sphere, as well as the formation of deeper "holes" permitting access to the central region of the dendrimer. As expected, most of the galactose residues are evident on the surface of the macromolecule with only a few residues being located within the dendrimer interior. Although computational studies of this type can only give an impression of the dynamic behavior of these types of molecules in solution, they can afford some insight into a molecular "world" that is not easy to appreciate in any other way.

Construction of the Trisgalactoside-Modified DAB-*dendr*-$(NH_2)_x$ Dendrimers: Recently, we demonstrated[19] the efficiency of a convergent approach[33] in the synthesis of carbohydrate-based dendrimers. In this particular approach, the saccharides are connected first to a small branching unit to form a cluster that then serves as a building block in a further step. In this investigation, we used one of the known cluster glycosides in the form of compound **12**[16a, 34] to modify the first two generations (**1a** and **2a**) of the DAB-*dendr*-$(NH_2)_x$ dendrimers. In order to introduce a spacer arm with a carboxylic acid end group onto the trisgalactoside **12** (Scheme 3), the latter



Scheme 3. Synthesis of the active ester **15** from the trisgalactoside **12**. Reagents and conditions: a) H_2/Pd-C/EtOAc, 30 °C, 10 h, 61%; b) glutaric anhydryde/CH_2Cl_2, 20 °C, 4 h, 75%; c) NHS/DCC/$MeO(CH_2)_2OMe$, 0-10 °C, 20 h, 95%.

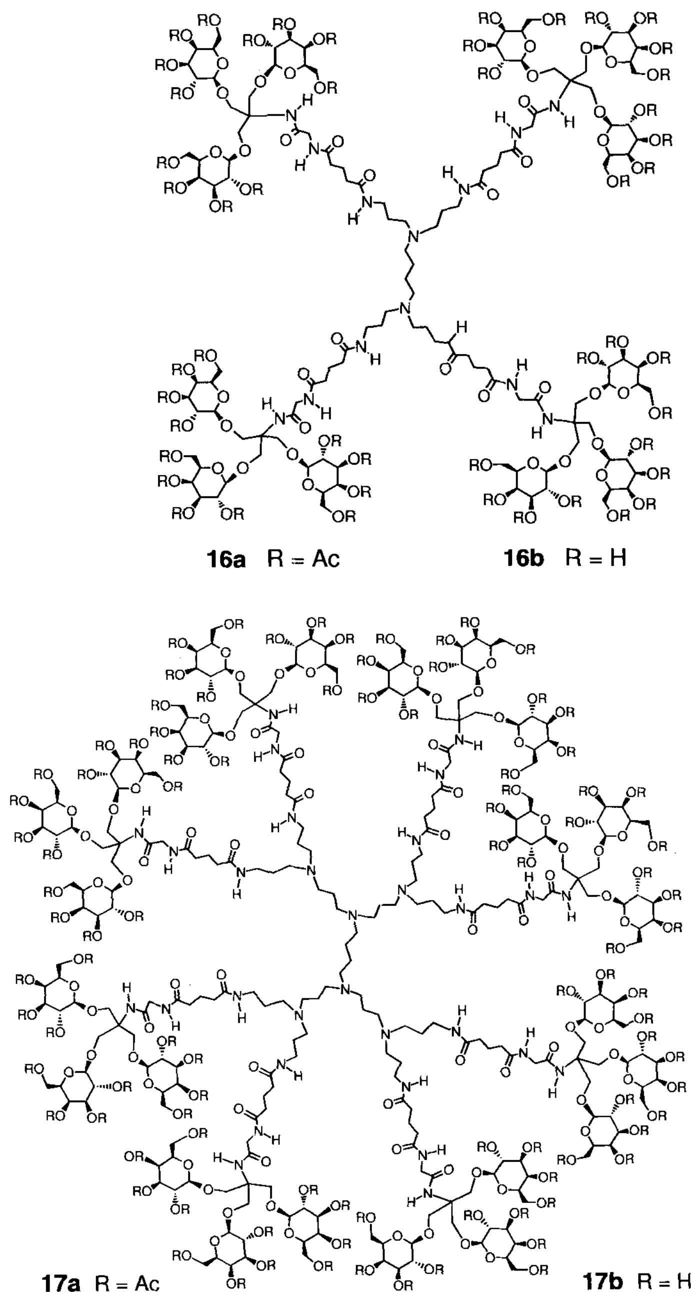


Figure 6. The structure of acetylated trisgalactosidodendrimers **16a** and **17a** synthesized by attachment (Scheme 3) of active ester **15** to the (DAB)-*dendr*-$(NH_2)_x$ dendrimers **1a** (top) and **2a** (bottom) followed by deacetylation according to Scheme 2.

was *N*-deprotected (H_2, 10% Pt/C, 40 °C, 8 h) to afford the amine **13**, which was then acylated with glutaric anhydride to give compound **14** in an overall yield of 46%. Condensation of **14** with *N*-hydroxysuccinimide using the standard procedure[28] yielded the activated ester **15** in near quantitative yield. Reaction of the dendrimers **1a** and **2a** with 4.0 and 8.0 equiv of **15**, respectively, was carried out under the same conditions as described earlier in this paper, affording compounds **16a** and **17a** (Figure 6) in 90 and 84% yield, respectively.

Deacetylation of **16a** and **17a** (MeONa/MeOH, followed by $NaOH/H_2O/MeOH$ at 22 °C for 8 h) produced the carbohydrate dendrimers **16b** and **17b** (Figure 6) bearing 12 and 24 monosaccharide residues, respectively. On account of the symmetry present in **16a/b**-**17a/b**, simple NMR spectra were obtained. The internal non-carbohydrate part of the glycodendrimers was revealed most distinctly in the ^{13}C NMR spectra of the compounds recorded in D_2O. With the exception of the difference brought about by the "new" spacer arm, there was a close correspondence between the ^{13}C and ^1H chemical shifts for the methylene protons in the NMR spectra of **16b** and **17b** and for the signals of the analogous galacto- and lactodendrimers (Tables 1 and 3). LSI-MS and MALDI-TOF-MS helped to confirm the structure of the low-generation dendrimers **16a** and **16b** (Table 2). It is interesting to note that the specific optical rotations of both compounds turned out to be zero, that is, **16a** and **16b**, exhibit no special chiroptical behavior.

© VCH Verlagsgesellschaft mbH, D-69451 Weinheim, 1997
0947-6539/97/0306-0980 $ 17.50+.50/0 Chem. Eur. J. 1997, 3, No. 6

Conclusion

In the current investigation, we have demonstrated that modification of the readily available DAB-*dendr*-$(NH_2)_x$ dendrimer series ($x = 4, 8, 16, 32, 64$) by mono- and disaccharides, as well as by a cluster trisgalactoside, can be achieved using a common amide bond forming procedure. Notwithstanding the fact that the exhaustive characterization of high molecular weight, carbohydrate-clothed dendrimers still represents a considerable challenge on account of their complexity and polymeric nature, there is no indication of the presence of major structural defects in these compounds. This conjecture is strongly supported by our results on the modifications of the lower-generation DAB-*dendr*-$(NH_2)_x$ dendrimers **1a** ($x = 4$) and **2a** ($x = 8$), which afforded large, multibranched molecules possessing uniform structure and distinct molecular weight, as determined by mass spectrometry. The use of acetylated saccharides for attachment to the dendritic cores does not seem to suffer from steric hindrance to the extent that there is any interference between the many condensation reactions. Obviously, after the removal of the protecting groups, the space between the carbohydrate residues will increase, allowing their motion to be freer on account of the flexible linker connecting the carbohydrate residues to the dendrimers. In this investigation, we have successfully used a rather long spacer arm in the synthesis of both the galacto- and lactodendrimers. It is reasonable to propose that the efficiency of the attachment would not be affected adversely by using a longer spacer arm. From the biological standpoint, elongation of a spacer arm may have a highly beneficial effect on the ability of a carbohydrate dendrimer to form a strong complex with, for example, a protein receptor. It is conceivable that such an elongation could provide just the right distance between the carbohydrate ligands that is needed for optimal binding to the protein receptor. A further improvement of the properties of dendrimer-based neoglycoconjugates might be achieved by combining the divergent and convergent approaches to their synthesis—and even applying the relatively new double exponential growth technique.[18b, 35] The divergent approach, described here for the construction of the galacto- and lactodendrimers, has made it possible for us to obtain high molecular weight compounds in one step by using a simple reaction many times over. The convergent approach, which has been used in the synthesis of trisgalactosido-dendrimers **16b** and **17b**, has the advantage of using a precisely designed saccharide wedge for the final attachment to a dendrimer core.

Experimental Section

General: Chemicals, including lactose and 1,2,3,4,6-penta-*O*-acetyl-*β*-D-galactose, were purchased from Aldrich. Poly(propylene imine) dendrimers **1a–5a** were supplied by DSM Research (The Netherlands). For deacetylations, anhydrous MeOH was prepared by reflux over Mg and distillation. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with Kieselgel 60 F_{254} (Merck). The plates were inspected under UV light and developed with 5% H_2SO_4 in EtOH at 120 °C. Column chromatography was carried out using silica gel 60 F (Merck 40–63 mm). Gel permeation chromatography (GPC) was performed on a column (80 × 1.6 cm with $V_0 \approx 60$ mL) packed with Fractogel TSK HW-40(S) (Merck) in H_2O. Fractions were monitored using a Differential Refractometer 141 supplied by Waters. Optical rotations were measured at 22 ± 2 °C on a Perkin-Elmer 457 polarimeter. ^{1}H NMR Spectra were recorded on either a Bruker AC300 (300 MHz) spectrometer or a Bruker AMX400 (400 MHz) spectrometer with either the solvent reference or TMS as internal standard. ^{13}C NMR Spectra were recorded on a Bruker AC300 (75.5 MHz) or a Bruker AMX400 (100.6 MHz) spectrometer using the PENDANT pulse train. Liquid secondary ion mass spectra (LSI-MS) were recorded on a VG Zabspec mass spectrometer equipped with a cesium gun operating at ≈30 keV. Matrix-assisted laser-desorption ionization/time-of-flight mass spectra (MALDI-TOF-MS) were recorded on a Kratos Kompact MALDI III instrument using a 2,5-dihydroxybenzoic acid matrix. Microanalyses were performed by the University of North London Microanalytical Services.

3-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosylthio)propionic Acid (7): The title compound was prepared from the *β*-acetate **6** according to the literature[25] to afford a colorless oil in a yield of 88%, R_F 0.67 (PhMe/EtOAc/AcOH, 80:19:1); $[\alpha]_D = -9$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.97, 2.04, 2.05, 2.14 (4 × s, 4 × 3H; CH_3CO), 2.74 (m, 2H; SCH_2CH_2), 2.93 (m, 2H; SCH_2CH_2), 3.91 (m, 1H; H-5), 4.08 (dd, $J_{5,6a}$ = 6.5 Hz; $J_{6a,6b}$ = 11.2 Hz, 1H; H-6a), 4.14 (dd, $J_{5,6b}$ = 7.0 Hz, 1H; H-6b), 4.53 (d, $J_{1,2}$ = 9.8 Hz, 1H; H-1), 5.03 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.5 Hz, 1H; H-3), 5.21 (pt, $J_{1,2} \approx J_{2,3}$ = 10 Hz, 1H; H-2), 5.41 (dd, $J_{4,5}$ = 1.0 Hz, 1H; H-4); ^{13}C NMR (100.6 MHz, $CDCl_3$, 25 °C): δ = 20.7, 20.8, 21.45 ($COCH_3$), 25.35 (SCH_2), 35.4 (CH_2CO_2H), 61.6 (C-6), 67.1 (C-2), 67.3 (C-4), 71.8 (C-3), 74.5 (C-5), 84.6 (C-1), 169.7, 170.1, 170.3, 170.6 (OCH_3), 176.8 (CO_2H). A small portion of **7** was deacetylated using 0.1 M MeONa in MeOH, followed by deionization with Amberlite IR-120 (H^+), to give crude 3-(*β*-D-galactopyranosylthio)propionic acid; ^{1}H NMR (400 MHz, D_2O, 25 °C) d 2.67 (m, 2H; SCH_2CH_2), 2.87 (m, 2H; SCH_2CH_2), 3.44 (pt, $J_{1,2} \approx J_{2,3}$ = 9.7 Hz, 1H; H-2), 3.53 (d, $J_{3,4}$ = 3.4 Hz, 1H; H-3), 3.56–3.61 (m, 2H, H-5, H-6a), 3.65 (dd, $J_{5,6b}$ = 8.6 Hz; $J_{6a,6b}$ = 12.0 Hz, 1H; H-6b), 3.86 (dd, 1H; H-4), 4.38 (d, 1H; H-1); ^{13}C NMR (100.6 MHz, D_2O, 5 °C): δ = 27.7 (CH_2CO_2H), 37.5 (SCH_2CH_2), 63.6 (C-6), 71.3 (C-4), 72.1 (C-2), 76.45 (C-3), 81.4 (C-5), 88.6(C-1), 179.0 (C=O); MALDI-TOF-MS *m*/*z* 290 $[M+Na]^+$: $C_9H_{16}O_7S$ (268.3).

***N*-Hydroxysuccinimidoyl-3-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galacto-pyranosylthio)propionate (8)**: DCC (3.5 g, 17 mmol) and NHS (1.9 g, 16.5 mmol) were added to a solution of compound **7** (7.2 g, 16.5 mmol) in $MeO(CH_2)_2OMe$ (50 mL) and the mixture was stirred at 0–10 °C for 20 h. The precipitated product was filtered off and the solvent was removed by evaporation, before the residue was dissolved in $CHCl_3$ (150 mL). The solution was filtered again, the filtrate was concentrated to ca. 50 mL and then poured into cyclohexane (250 mL). The solvent was decanted, the remaining viscous oil was dissolved in a minimum amount of $CHCl_3$, and the product precipitated again with cyclohexane. The solvent was decanted and the remaining oil was dried to give a white foam, **8** (7.1 g, 93%); R_f 0.64 (PhMe/EtOAc, 7:3); $[\alpha]_D = -8.3$ ($c = 1.08$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 2.00, 2.06, 2.08, 2.17 (4 × s, 4 × 3H; CH_3CO), 2.86 (s, 4H; CH_2 Suc), 2.96–3.17 (m, 4H; SCH_2CH_2), 3.97 (m, 1H; H-5), 4.14 (m, 2H; H-6a, H-6b), 4.59 (d, $J_{1,2}$ = 9.9 Hz, 1H; H-1), 5.07 (dd, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 3.3 Hz, 1H; H-3), 5.23 (pt, 1H; H-2), 5.44 (d, 1H; H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 20.5, 20.6 (×2), 20,7 ($COCH_3$), 24.7 (SCH_2), 25.5 (CH_2 Suc), 32.7 (CH_2CO_2-Suc), 61.6 (C-6), 66.9 (C-2), 67.2 (C-4), 71.7 (C-3), 74.5 (C-5), 84.1 (C-1), 167.0 (CH_2CO_2-Suc), 168.9 (C=O Suc), 169.7, 170.0, 170.2, 170.5 (CH_3CO); MALDI-TOF-MS: *m*/*z* 555 $[M+Na]^+$, 571 $[M+K]^+$: $C_{21}H_{27}O_{13}NS$ (533.5).

3-[2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galacto-pyranosyl)-*β*-D-glucopyranosylthio]propionic Acid (10): A solution of lactose heptaacetate **9** (13.0 g, 19.2 mmol, a mixture of *α*/*β* isomers, 1.0/3.7, prepared by acetylation of lactose with Ac_2O/AcONa by the standard procedure[26]) and 3-thiopropionic acid (6.7 mL, 77 mmol) in CH_2Cl_2 (100 mL) was treated with $BF_3 \cdot OEt_2$ (3.5 mL, 28 mmol) and the mixture was left to stand for 5 h at room temperature, before being diluted with CH_2Cl_2 (100 mL) and washed with 1 M HCl (3 × 40 mL). The product was isolated by column chromatography (SiO_2:PhMe/EtOAc/AcOH, 80:18:2) to afford **10** (7.4 g, 53%); R_f 0.34 (PhMe/EtOAc/AcOH, 80:19:1); $[\alpha]_D = -11$ ($c = 1.32$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.97, 2.05, 2.12, 2.15 (4 ×, 7 × 3H; CH_3CO), 2.70 (m, 2H; CH_2CO_2H), 2.84 (m, 1H; SCH_aH_b), 2.94 (m, 1H; SCH_aH_b), 3.66 (m, 1H; H-5), 3.83 (pt, $J_{3,4} = J_{4,5}$ = 9.2 Hz, 1H; H-4′), 3.91 (pt, $J_{5',6a'} \approx J_{5',6b'}$ = 7.5 Hz, 1H; H-5′), 4.10 (m, 3H; H-6a, H-6a′, H-6b′), 4.49 (m, 1H, H-6b), 4.51 (d, $J_{1',2'}$ = 7.6 Hz, 1H; H-1′), 4.53 (d, $J_{1,2}$ = 9.2 Hz, 1H;

H-1), 4.92 (pt, $J_{1,2} \approx J_{2,3}$ = 9.2 Hz, 1H; H-2), 4.99 (dd, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ = 3.4 Hz, 1H; H-3'), 5.09 (dd, 1H; H-2'), 5.19 (pt, 1H; H-3), 5.32 (d, 1H; H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$, 25 °C): δ = 20.3, 20.4, 20.5 ($COCH_3$), 25.1 (SCH_2), 35.0 (CH_2CO_2H), 60.7 (C-6'), 62.1 (C-6), 66.6 (C-4'), 69.0 (C-2'), 70.0 (C-2), 70.5 (C-5'), 70.8 (C-3'), 73.5 (C-3), 76.0 (C-4), 76.5 (C-5), 83.6 (C-1), 100.8 (C-1'), 169.0-170.4 (7 C; CH_3CO), 175.9 (CO_2H); MALDI-TOF-MS: *m/z* 747 $[M+Na]^+$, 763 $[M+K]^+$: $C_{29}H_{40}O_{19}S$ (724.7).

***N*-Hydroxysucinimidoyl-3-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*β*-D-glucopyranosylthio]propionate (11)**: Compound **10** (5.9 g, 8.1 mmol) was treated with NHS (0.95 g, 8.1 mmol) and DCC (1.83 g, 8.9 mmol) in $MeO(CH_2)_2OMe$ (25 mL) for 7 h at 0–5 °C and the product was isolated, as described for the preparation of **8**, to give the ester **11** (6.3 g, 95%); R_f 0.30 (PhMe/EtOAc/AcOH, 80:19:1); $[\alpha]_D$ = −11.7 (c = 1.04 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.97, 2.05, 2.12, 2.15 (4 × s, 7 × 3H; CH_3CO), 2.75–3.01 (m, 8H; $SCH_2CH_2CO_2Suc$), 3.61 (m, 1H, H-5), 3.72 (pt, $J_{3,4} = J_{4,5}$ = 9.0 Hz, 1H; H-4'), 3.85 (pt, $J_{5',6a'} = J_{5',6b'}$ = 7.5 Hz, 1H; H-5'), 4.00–4.10 (m, 4H; H-6a, H-6b, H-6a', H-6b'), 4.45 (d, $J_{1,2}$ = 7.6 Hz, 1H; H-1'), 4.51 (d, $J_{1,2}$ = 9.0 Hz, 1H; H-1), 4.88 (pt, $J_{1,2} \approx J_{2,3}$ = 9.0 Hz, 1H; H-2), 4.90 (dd, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 3.4 Hz, 1H; H-3'), 5.02 (dd, 1H; H-2'), 5.16 (pt, 1H; H-3), 5.28 (d, 1H; H-4); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 20.3–20.6 (7 C, $COCH_3$), 24.5 (SCH_2), 25.4 (CH_2 Suc), 32.6 (CH_2CO_2-Suc), 60.65 (C-6'), 61.9 (C-6), 66.9 (C-3), 68.8 (C-2'), 70.0 (C-2), 70.4 (C-5'), 70.8 (C-3'), 73.3 (C-3), 75.9 (C-4'), 76.5 (C-5), 83.3 (C-1), 100.6 (C-1'), 167.0 (CH_2CO_2), 168.9 (C=O Suc), 169.5-170.3 (CH_3CO); MALDI-TOFMS: *m/z* calcd for $C_{93}H_{43}NO_{21}S$ = 821.8, found 844 $[M+Na]^+$, 860 $[M+K]^+$.

General Procedure for Coupling of Activated Esters with DAB-*dendr*-$(NH_2)_x$: To a solution of the DAB-*dendr*-$(NH_2)_x$ dendrimer (about 50 mg, which corresponds to approximately 0.16, 0.065, 0.03, 0.014, and 0.007 mmol of dendrimers **1a**, **2a**, **3a**, **4a**, and **5a**, respectively) in CH_2Cl_2 (10 mL), the activated ester **8** or **11** (0.65, 0.53, 0.48, 0.45, and 0.45 mmol for reactions with **1a**, **2a**, **3a**, **4a**, and **5a**, respectively) was added and the mixture was stirred for 18 h at room temperature. After dilution with CH_2Cl_2 (40 mL), the solution was washed with saturated aqueous Na_2CO_3 (5 × 30 mL, usually 1–3 h was required for the complete separation of layers), dried (Na_2SO_4), filtered, and concentrated to give a white solid foam, which was dried in vacuo. Yields and $[\alpha]_D$ values are given in Table 4. ^{13}C NMR and mass spectroscopic data are listed in Tables 1 and 2, respectively. ^{1}H NMR spectra of compounds **1b** and **1c** are described below.

Galactodendrimer 1b: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.35 (brm, 4H; $NCH_2(CH_2)_2CH_2N$), 1.58 (brm, 8H; $NCH_2CH_2CH_2N$), 1.92, 1.99, 2.00, 2.10 (4s, 48H; Ac) 2.25–2.55 (m, 20H; CH_2N and CH_2NHCO), 2.82–3.02 (m, 8H, SCH_2), 3.21 (brm, 8H; CH_2CONH), 3.90 (pt, $J_{5,6a} \approx J_{5,6b}$ = 6 Hz, 4H; H-5 Gal), 4.03 (dd, $J_{5,6a}$ = 6.0 Hz, $J_{6a,6b}$ = 11 Hz, 4H; H-6a Gal), 4.11 (dd, $J_{5,6b}$ = 6.0 Hz, 4H; H-6b Gal), 4.52 (d, $J_{1,2}$ = 9.5 Hz, 4H; H-1 Gal), 5.00 (dd, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.0 Hz, 4H; H-3 Gal), 5.13 (pt, $J_{1,2} \approx J_{2,3}$ = 9.5 Hz, 4H; H-2 Gal), 5.37 (d, 4H; H-4 Gal), 6.80 (t, J = 5.0 Hz, 4H; NH).

Lactodendrimer 1c: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.39 (brm, 4H; $NCH_2(CH_2)_2CH_2N$), 1.63 (brm, 8H; $NCH_2CH_2CH_2N$), 1.97, 2.05, 2.06, 2.07, 2.10, 2.14 (6s, 84H; Ac) 2.33–2.50 (m, 20H; CH_2N and CH_2NHCO), 2.86 (m, 4H; SCH_aH_b), 2.99 (m, 4H; SCH_aH_b), 3.28 (brm, 8H; CH_2CONH), 3.66 (m, 4H; H-5 Glc), 3.82 (pt, $J_{3,4} \approx J_{4,5}$ = 9.5 Hz, 4H; H-4 Glc), 3.93 (pt, $J_{5,6a} \approx J_{5,6b}$ = 6.8 Hz, 4H; H-5 Gal), 4.06–4.17 (m, 12H; H-6a Glc, H-6a, H-6b Gal), 4.56 (d, $J_{1,2}$ = 8 Hz, 4H; H-1 Gal), 4.57 (d, $J_{1,2}$ = 10.0 Hz, 4H; H-1 Glc), 4.60 (brd, $J_{6a,6b}$ = 11 Hz, 4H; H-6b Glc), 4.91 (pt, $J_{1,2} \approx J_{2,3}$ = 9.5 Hz, 4H; H-2 Gal), 4.99 (dd, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 3.4 Hz 4H; H-3 Gal), 5.11 (dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.2 Hz, 4H; H-2 Glc), 5.21 (dd, $J_{2,3} \approx J_{3,4}$ = 9.5 Hz, 4H; H-3 Glc), 5.36 (d, $J_{3,4}$ = 3.4 Hz, 4H; H-4 Gal), 6.93 (brt, J = 5.3 Hz, 1H, NHCO).

General Procedure for Deacetylation of Protected Carbohydrate Dendrimers 1b/c–5b/c: A solution of acetylated glycodendrimer (200–400 mg) in a mixture of dry CH_2Cl_2 (2 mL) and dry MeOH (3 mL) was treated with 1 M MeONa in MeOH (0.5 mL) and stirred for about 15 min at room temperature. A white precipitate was formed and the mixture was concentrated in vacuo and the residue was dissolved in H_2O (5–8 mL) and MeOH (1–2 mL). The solution was stirred overnight at room temperature, neutralized with 1 M HCl to pH 6, concentrated to 1 mL, and subjected to GPC in H_2O. All fractions, which were detected using a differential refractometer and eluted before salt, were collected, combined, concentrated, and freeze-dried from H_2O to give white powders. The yields and values of $[\alpha]_D$ are listed in Table 4, ^{13}C NMR spectra of **1d/e–5d/e** are given in Table 3, mass spectroscopic data are listed in Table 2, and the C/S ratios from elemental analysis are listed in Table 5.

1d and **1e**: In order to give an indication of the resonances present in ^{1}H NMR spectra, the data for compounds **1d** and **1e** are described below.

Galactodendrimer 1d: ^{1}H NMR (400 MHz, 100 °C, $(CD_3)_2SO$): δ 1.82 (m, 4H; $NCH_2CH_2CH_2CH_2N$), 1.92 (m, 8H; $NCH_2CH_2CH_2NHCO$), 2.46 (m, 8H; CH_2CONH), 2.79–2.92 (m, 8H; SCH_2), 3.05–3.23 (2m, 12H and 8H; CH_2NCH_2 and CH_2NHCO), 3.35 (dd, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 3.4 Hz, 4H; H-3 Gal), 3.42 (pt, $J_{1,2} \approx J_{2,3}$ = 9.3 Hz, 4H; H-2 Gal), 3.44 (ddd, $J_{4,5}$ = 1.1 Hz, $J_{5,6a} = J_{5,6b}$ = 6.0 Hz, 4H; H-5 Gal), 3.55 (dd, $J_{6a,6b}$ = 13.2 Hz, 8H; H-6a, H-6b Gal), 3.77 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.1 Hz, 4H; H-4 Gal), 4.27 (d, $J_{1,2}$ = 9.3 Hz, 4H; H-1 Gal), 7.78 (bs, 4H; NHCO); IR spectrum (KBr): $\tilde{\nu}$ (cm^{-1}) = 919, 951, 1054, 1083, 1152, 1225, 1372, 1437, 1546, 1654, 1752, 2962, 3395.

Lactodendrimer 1e: ^{1}H NMR (400 MHz, 100 °C, $(CD_3)_2SO$): δ 1.82 (m, 4H; $NCH_2CH_2CH_2CH_2N$), 1.92 (m, 8H; $NCH_2CH_2CH_2NHCO$), 2.46 (m, 8H; CH_2CONH), 2.79–2.92 (m, 8H; SCH_2), 3.05–3.23 (2m, 12H and 8H; CH_2NCH_2 and CH_2NHCO), 3.34–3.43 (m, 20H; H-24 and H-3 Gal, H-3, H-4, H-5 Glc), 3.48 (m, 4H; H-5 Gal), 3.55–3.63 (m, 8H; H-6a and H-6b Gal), 3.67 (dd, $J_{5,6a}$ = 4.8 Hz, $J_{6a,6b}$ = 12.4 Hz, 4H; H-6a Glc), 3.71 (dd, $J_{3,4}$ = 2.8 Hz, $J_{4,5}$ = 1.4 Hz, 4H; H-4 Gal), 3.82 (dd, $J_{5,6b}$ = 2.5 Hz, 4H; H-6b Glc), 4.23 (d, $J_{1,2}$ = 7.3 Hz, 4H; H-1 Gal), 4.23 (d, $J_{1,2}$ = 9.6 Hz, 4H; H-1 Glc), 7.80 (bs, 4H; NHCO); IR spectrum (KBr): $\tilde{\nu}$ (cm^{-1}) = 913, 954, 1048, 1232, 1371, 1435, 1544, 1659, 1756, 2943, 3396.

Tris(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyloxymethyl)methylamido-glycine (13): A solution of **12** (3.08 g, 2.36 mmol) in EtOAc (30 mL) was hydrogenated over Pd/C (10%, 0.5 g) at 30 °C for 10 h, filtered through Celite, and concentrated. The residue was subjected to column chromatography (SiO_2, EtOAc:EtOH 99:1 to 94:6) to give the amine **13** (1.57 g, 61%), R_f 0.20 ($CHCl_3$:Me_2CO 5:1); $[\alpha]_D$ = −14.3 (c = 1.05 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 1.97, 2.05, 2.06, 2.15 (4 × s, 21H; $COCH_3$), 3.27 (s, 2H; NH_2), 3.82 (d, J = 10.0 Hz, 3H; C(quat)CH_aH_b), 3.91 (m, 4H; H_a Gly, H-5 Gal), 4.08–4.21 (m, 8H; H_b Gly, C(quat)CH_aH_b, H-6a and H-6b Gal), 4.42 (d, $J_{1,2}$ = 7.9 Hz, 3H; H-1 Gal), 5.01 (dd, $J_{2,3}$ = 10.2 Hz; $J_{3,4}$ = 3.2 Hz, 3H; H-3 Gal), 5.13 (dd, 3H; H-2 Gal), 5.37 (d, 3H; H-4 Gal), 7.20 (s, 1H; C(quat)NH); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 20.5, 20.6 (×2), 20.8 ($COCH_3$), 45.3 (CH_2NH_2), 58.72 (C(quat)), 61.0 (C-6), 68.1 (C(quat)CH_2), 66.9 (C-4), 69.0 (C-2), 70.6 (C-3), 70.7 (C-5), 101.4 (C-1), 169.4, 170.0, 170.2, 170.4 (CH_3CO), 173.0 (CONH); MALDI-TOF-MS *m/z*: 1191 $[M+Na]^+$, 1208 $[M+K]^+$: $C_{48}H_{68}N_2O_{31}$ (1169.07).

Tris(2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyloxymethyl)methylamido-glycinamidoglutaric Acid (14): Glutaric anhydride (170 mg, 1.5 mmol) was added to a solution of the amine **13** (1.57 g, 1.34 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 4 h at 20 °C and concentrated, and the product was purified by column chromatography (SiO_2, EtOAc:EtOH 99:1 to 94:6) to afford the corresponding acid **14** (1.28 g, 75%); R_f 0.39 ($CHCl_3$:Me_2CO 5:1); $[\alpha]_D$ = −14.0 (c = 1.04 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 31 °C): δ = 1.96–2.02 (m, 11H; $COCH_3$ and $CH_2CH_2CH_2$), 2.09, 2.18 (2 × s, 27H; 2 × $COCH_3$), 2.37–2.46 (m, 4H; $CH_2CH_2CH_2$), 3.78 (d, J = 10.3 Hz, 3H; C(quat)CH_aH_b), 3.87 (dd, J = 4.8 Hz, J = 6.8 Hz, 1H; H_a Gly), 3.97 (pt, $J_{5,6a} \approx J_{5,6b}$ = 7 Hz, 3H; H-5 Gal), 3.98 (m, 1H; H_b Gly overlapping with H-5 Gal); 4.14 (dd, $J_{5,6a}$ = 6.1 Hz, $J_{6a,6b}$ = 11.2 Hz, 3H; H-6a Gal overlapping with C(quat)CH_aH_b), 4.14 (d, 3H; C(quat)CH_aH_b overlapping with H-6a Gal), 4.20 (dd, $J_{5,6b}$ = 6.7 Hz, 3H; H-6b Gal), 4.45 (d, $J_{1,2}$ = 7.7 Hz, 3H; H-1 Gal), 5.05 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.3 Hz, 3H; H-3 Gal), 5.13 (dd, 3H; H-2 Gal), 5.40 (d, 3H; H-4 Gal), 6.39 (s, 1H; C(quat)NH), 6.94 (t, J = 5.2 Hz, 1H; NH Gly); ^{13}C NMR (100.6 MHz, $CDCl_3$, 31 °C): δ = 20.3, 20.4, 20.5, 20.6 (CH_3CO and $CH_2CH_2CH_2$), 32.7, 34.6 ($CH_2CH_2CH_2$), 42.6 (CH_2 Gly), 59.3 (quat. C), 60.9 (C-6), 66.8 (C-4), 67.9 (C(quat)CH_2), 68.9 (C-2), 70.4 (C-3), 70.7 (C-5), 101.2 (C-1), 169.5 (NHCO Gly), 169.4, 169.8, 170.0, 170.2 (CH_3CO), 172.7 (C(quat)NHCO), 175.9 (CO_2H); MALDI-TOF-MS *m/z*: 1306 $[M+Na]^+$, 1321 $[M+K]^+$; $C_{53}H_{74}N_2O_{34}$ (1283.17): calcd C 49.61, H 5.81, N 2.18; found C 49.68, H 5.71, N 2.08.

© VCH Verlagsgesellschaft mbH, D-69451 Weinheim, 1997
0947-6539/97/0306-0982 $ 17.50+.50/0 Chem. Eur. J. 1997, 3, No. 6

N-Hydroxysuccinimidoyl-tris(2,3,4,6-tetra-O-acetyl-β-D-galacto-pyranosyl-oxymetyl)methylamidoglycinamidoglutarate (15): A solution of the acid **14** (1.11 g, 0.87 mmol), DCC (206 mg, 1.0 mmol), and NHS (103 mg, 0.9 mmol) in $MeO(CH_2)_2OMe$ (10 mL) was stirred for 17 h at 4–10 °C. The workup procedure, which was analogous to that described for **8**, afforded a syrupy mass of the activated ester **15** (1.14 g, 95%), $[\alpha]_D = -13.4°$ ($c = 1.0$, $CHCl_3$), ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.99$ (s, 11H; $COCH_3$, $CH_2CH_2CH_2$), 2.08 (s, 18H; $COCH_3$), 2.17 (s, 9H; $COCH_3$), 2.42 (t, $J = 7.2$ Hz, 2H; CH_2CH_2CONH), 2.73 (t, $J = 7.2$ Hz, 3H; CH_2CO_2-Suc), 3.77–3.82 (m, 4H; H_a Gly, C(quat)CH_aH_b), 3.96–4.01 (m, 4H; H_b Gly, H-5 Gal), 4.11–4.22 (m, 9H; C(quat)CH_aH_b, H-6a and H-6b Gal), 4.44 (d, $J_{1,2} = 7.8$ Hz, 3H; H-1 Gal), 5.03 (dd, $J_{2,3} = 10.4$ Hz; $J_{3,4} = 3.4$ Hz, 3H; H-3 Gal), 5.13 (dd, 3H; H-2), 5.39 (d, 3H; H-4), 6.23 (s, 1H; C(quat)NH), 6.56 (m, 1H; NH Gly); ^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 20.2$, 20.3, 20.4, 20.5 (CH_3CO, $CH_2CH_2CH_2$), 25.3 (CH_2 Suc), 29.7, 33.8 ($CH_2CH_2CH_2$), 42.8 (CH_2 Gly), 58.9 (C(quat)), 60.9 (C-6), 67.8 (C(quat)CH_2), 66.7 (C-4), 68.7 (C-2), 70.3, 70.5 (C-3, C-5), 101. (C-1), 168–171.8 (C=O).

Glycodendrimer 16a: This compound was prepared from **1a** (22 mg, 0.07 mmol) and **15** (398 mg, 0.228 mmol) according to the general procedure for coupling of activated esters with PA-dendrimers. Yield 340 mg (90%), $[\alpha]_D = -11.6$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta = 1.40$ (brm, 4H; $NCH_2(CH_2)_2CH_2N$), 1.64 (m, 8H, $NCH_2CH_2CH_2N$), 1.98 (brs, 20H; $COCH_3$ and $COCH_2CH_2CH_2CO$), 2.08, 2.17 (2×s, 36H; $COCH_3$), 2.22–2.45 (m, 28H; $COCH_2CH_2CH_2CONH(CH_2)_2CH_2NCH_2$), 3.26 (m, 8H; CH_2CH_2NHCO), 3.78 (d, 4H, $J = 10.2$ Hz; C(quat)CH_aH_b), 3.86 (m, 4H; H_a Gly), 3.98 (pt, 3H; $J_{5,6a} \approx J_{5,6b} \approx 7$ Hz, 12H; H-5 Gal), 4.11–4.21 (m, 28H; H-6a and H-6b Gal, C(quat)CH_aH_b), 4.46 (d, $J_{1,2} = 7.7$ Hz, 12H; H-1 Gal), 5.05 (dd, $J_{2,3} = 10.5$ Hz, $J_{3,4} = 3.3$ Hz, 12H; H-3 Gal), 5.13 (dd, 12H; H-2 Gal), 5.39 (d, 12H; H-4 Gal), 6.28 (s, 4H; C(quat)NH), 6.84 (t, $J = 5$ Hz, 4H; NHCO Gly), 7.09 (t, $J = 5$ Hz, 4H; NHCO dendrimer); ^{13}C NMR (100.6 MHz, $CDCl_3$, 31 °C): $\delta = 20.4$, 20.5, 20.6, 20.7 (CH_3CO and $COCH_2CH_2CH_2CO$), 25.8, 27.0 ($CH_2CH_2CH_2$), 33.8, 34.9, 35.3, ($COCH_2CH_2CH_2CONHCH_2$), 42.7 (CH_2 Gly), 49.3, 52.0 (CH_2NCH_2), 59.2 (C (quat)), 67.0 (C-6), 66.8 (C-4), 68.0 (C(quat)CH_2), 68.9 (C-2), 70.4 (C-3), 70.6 (C-5), 101.2 (C-1), 168.8 (NHCO dendrimer), 169.3, 169.9, 170.0, 170.3 (CH_3CO), 172.6, 172.8, (C(quat)NHCO, NHCO Gly); for the MALDI-TOF mass spectrum see Table 2.

Glycodendrimer 17a: This compound was prepared as described previously for **16a** starting from **2a** and **15** in 84% yield, $[\alpha]_D = -11.6$ ($c = 1.0$ in $CHCl_3$).

Glycodendrimer 16b: This compound was prepared in 74% yield by deacetylation of **16a** according to the description given in the general procedure, $[\alpha]_D = +0.3$ ($c = 1.2$ in H_2O); ^1H NMR (400 MHz, $(CD_3)_2SO$, 100 °C): $\delta = 1.74$–1.93 (m, 20H; $COCH_2CH_2CH_2CONCH_2CH_2$, $NCH_2(CH_2)_2CH_2N$), 2.12–2.24 (m, 16H; $COCH_2CH_2CH_2CO$), 3.06–3.20 (m, 20H; $CONHCH_2CH_2CH_2NCH_2$), 3.30–3.40 and 3.52–3.65 (2×m, 48H; H-2, H-3, H-5, H-6 Gal), 3.69 (m, 20H; CH_2 Gly and H-4 Gal), 3.83 (d, $J = 10$ Hz, 12H; C(quat)CH_aH_b), 4.06 (d, 12H; C(quat)CH_aH_b), 4.17 (d, $J = 8$ Hz, 12H; H-1 Gal), 6.85 (s, 4H, NHCO Gly), 7.65 (brs, 8H, $NHCO(CH_2)_3CONH$). The ^{13}C NMR spectrum and MS data are listed in Tables 3 and 2, respectively.

Glycodendrimer 17b: This compound was prepared as described for **16b** in 67% yield, $[\alpha]_D = 0$ ($c = 1.0$ in H_2O). The ^{13}C NMR spectroscopic data are listed in Table 3.

Molecular Simulation: Simulations were carried out using the AMBER forcefield as implemented in Macromodel[31] (version 5.0) running on a Silicon Graphics Indigo2 Workstation. The dendrons were assembled within the Macromodel INPUT submode and then fully minimized (final gradient < 0.5 kJ Å^{-1}) using the Polak Ribiere Conjugate Gradient (PRCG) algorithm with extended cut-off. Solvation was included in the form of the GB/SA solvation model for H_2O.[32] The individual dendron units were then attached to the central core unit. The whole assembly was then fully minimized using the above method (final gradient < 0.5 kJ Å^{-1}). Simulated annealing using stochastic molecular dynamics (SD), was performed within Macromodel. The system was allowed 20 ps of equilibration at 300 K (time step 1.5 fs) then "cooled" to 50 K over 80 ps to afford a structure removed from the starting geometry. This structure was then fully minimized (AMBER*, PRCG, GB/SA solvation for H_2O) until the RMS deviation was < 0.5 kJ Å^{-1}. Molecular volume calculations were performed using Biosym's Insight and Discover[36] software, running on a Silicon Graphics Indi workstation.

Acknowledgement: This research was supported by The Netherlands Foundation for Chemical Research (SON) and by The Netherlands Organization for Scientific Research (NWO) in the Netherlands and by Biotechnology and Biological Sciences Research Council, the Engineering and Physical Sciences Research Council, and the British Council in the UK. DSM is gratefully acknowledged for an unrestricted grant and for providing us with samples of poly(propylene imine) dendrimers.

Received: November 26, 1996 [F 528]

[1] a) G. R. Newkome, C. N. Moorefield, F. Vögtle, *Dendritic Molecules: Concepts, Syntheses, Perspectives*, VCH, **1996**; b) D. A. Tomalia, A. M. Naylor, W. A. Goddard III, *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 138–175; c) G. R. Newkome, C. N. Moorefield, G. R. Baker, A. L. Johnson, R. K. Behera, *J. Org. Chem.* **1992**, *57*, 358–362; d) Z. Xu, J. S. Moore, *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1354–1357; e) C. Wörner, R. Mülhaupt, *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1306–1308; f) K. L. Wooley, C. J. Hawker, J. M. J. Fréchet, *J. Am. Chem. Soc.* **1991**, *113*, 4252–4261; g) K. L. Wooley, C. J. Hawker, J. M. J. Fréchet, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 82–85; h) T. M. Miller, T. X. Neenan, E. W. Kwock, S. M. Stein, *J. Am. Chem. Soc.* **1993**, *115*, 356–357; i) J. Issberner, R. Moors, F. Vögtle, *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2413–2420.

[2] a) E. Buhleier, W. Wehner, F. Vögtle, *Synthesis* **1978**, 155–158; b) R. G. Denkewalter, J. Kolc, W. J. Lukasavage, *U. S. Pat. 4,289,872* (Sept. 15, **1981**) (*Chem. Abst.* **1985**, *102*, 79324q); c) D. A. Tomalia, H. Baker, J. R. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, P. Smith, *Polym. J.* **1985**, *17*, 117–132; d) G. R. Newkome, C. N. Moorefield, G. R. Baker, A. L. Johnson, R. K. Behera, *J. Org. Chem.* **1992**, *57*, 358–362.

[3] D. A. Tomalia, H. Baker, J. R. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, P. Smith, *Macromolecules* **1986**, *19*, 2466–2468.

[4] G. R. Newkome, Z.-Q. Yao, G. R. Baker, V. K. Gupta, *J. Org. Chem.* **1985**, *50*, 2003–2004.

[5] a) E. M. M. de Brabander-van der Berg, E. W. Meijer, *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1308–1311; b) E. M. M. de Brabander-van den Berg, A. Nijenhuis, M. Muré, J. Keulen, R. Reintjens, F. van den Booren, F. Bosman, R. de Raat, T. Frijns, S. van der Wal, M. Castelijns, J. Put, E. W. Meijer, *Macromol. Symp.* **1994**, *77*, 51–62.

[6] G. R. Newkome, G. R. Baker, J. K. Young, J. G. Traynham, *J. Polym. Sci. A: Polym. Chem.*, **1993**, *31*, 641–651.

[7] a) G. R. Newkome, C. N. Moorefield, G. R. Baker, A. L. Johnson, R. K. Behera, *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1176–1180; b) G. R. Newkome, C. N. Moorefield, *U. S. Pat. 5,154,853* (Oct. 13, **1992**); c) S. Stevelmans, J. C. M. van Hest, J. F. G. A. Jansen, D. A. F. J. van Boxtel, E. M. M. de Brabander-van den Berg, E. W. Meijer, *J. Am. Chem. Soc.* **1996**, *118*, 7398–7399.

[8] a) J. W. J. Knapen, A. W. van der Made, J. C. de Wilde, P. W. N. M. van Leeuwen, P. Wijkens, D. M. Grove, G. van Koten, *Nature (London)* **1994**, *372*, 659–663; b) C. Bolm, N. Derrien, A. Seger, *Synlett* **1996**, 387–388.

[9] a) M. P. Andrews, G. A. Ozin, *Chem. Mater.* **1989**, 174–178; b) A. W. Bosman, A. P. H. J. Schenning, R. A. J. Janssen, E. W. Meijer, *Chem. Ber.* **1997**, *130*, issue 6.

[10] J. F. G. A. Jansen, E. M. M. de Brabander-van den Berg, E. W. Meijer, *Science* **1994**, *266*, 1226–1229.

[11] a) C. Rao, J. P. Tam, *J. Am. Chem. Soc.* **1994**, *116*, 6975; b) J. Haensler, F. C. Szoka, Jr., *Bioconj. Chem.* **1993**, *4*, 372–379.

[12] P. Singh, F. Moll, III, S. H. Lin, C. Ferzli, K. S. Yu, R. K. Koski, R. G. Saul, P. Gronin, *Clin. Chem.* **1994**, *40*, 1845–1849.

[13] a) J. F. G. A. Jansen, H. W. I. Peerlings, E. M. M. de Brabander-van den Berg, E. W. Meijer, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1206–1209; b) P. Murer, D. Seebach, *ibid.* **1995**, *34*, 2116–2119.

[14] a) Y. C. Lee, R. T. Lee, K. Rice, Y. Ichikawa, T.-C. Wong, *Pure Appl. Chem.* **1991**, *63*, 499–506; b) R. Lee; Y. C. Lee, in *Neoglycoconjugates* (Eds.: Y. C. Lee and R. Lee), Academic Press, San Diego, **1994**, pp. 23–50.

[15] a) Y. C. Lee, R. R. Townsend, M. R. Hardy, J. Arnarp, M. Haraldson, J. Lönn, *J. Biol. Chem.* **1983**, *258*, 199–202; b) S. Sabesan, J. O. Duus, S. Neira, P. Domaille, S. Kelm, J. C. Paulson, K. Bock, *J. Am. Chem. Soc.* **1992**, *114*, 8363–8375; c) K. H. Mortell, R. V. Weatherman, L. L. Liessling, *ibid.* **1996**, *118*, 2297–2298; d) G. B. Sigal, M. Mammen, G. Dahmann, G. M. Whitesides, *ibid.* **1996**, *118*, 3789–3800.

[16] a) H. J. M. Kempen, C. Hoes, J. H. van Boom, H. H. Spanjer, J. de Lange, A. Langedoen, T. J. C. van Berkel, *J. Med. Chem.* **1984**, *27*, 1306–1312; b) G. Kretzschmar, U. Sprengard, H. Kunz, E. Bartnik, W. Schmidt, A. Toepfer, B. Hörsch, M. Krauze, D. Seiffge, *Tetrahedron* **1995**, *51*, 13015–13030; c) S. A. DeFrees, W. Kosch, W. Way, J. C. Paulson, S. Sabesan, R. L. Halcomb, D.-H. Huang, Y. Ichikawa, C.-H. Wong, *J. Am. Chem. Soc.* **1995**, *117*, 66–79;

 © VCH Verlagsgesellschaft mbH, D-69451 Weinheim, 1997 0947-6539/97/0306-0983 $ 17.50+.50/0

d) E. R. Wijsman, D. Filippov, A. R. P. M. Valentijn, G. A. van der Marel, J. H. van Boom, *Rec. Trav. Chim. Pays-Bas* **1996**, *115*, 397–401; e) A. Kichler, F. Schuber, *Glycoconj. J.* **1995**, *12*, 275–281.
[17] a) *Neoglycoconjugates* (Eds.: Y. C. Lee and R. Lee), Academic Press, San Diego, **1994**; b) *Methods in Enzymology vol. 242, Neoglycoconjugates, part A, Synthesis* (Eds. Y. C. Lee and R. Lee), Academic Press, San Diego, **1994**; c) C. P. Stowell, Y. C. Lee, *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225–281.
[18] a) R. Roy, *Polymer News*, **1996**, *21*, 226–232; b) D. Zanini, R. Poy, *J. Org. Chem.* **1996**, *61*, 7348–7354; c) D. Page, S. Aravind, R. Roy, *Chem. Commun.* **1996**, 1913–1914; d) D. Page, R. Roy, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1765–1770; e) R. Roy, U. K. Saha, *Chem. Commun.* **1996**, 201–202; f) R. Roy, W. K. C. Park, Q. Q. Wu, S. N. Wang, *Tetrahedron Lett.* **1995**, *36*, 4377–4380; g) D. Zanini, W. K. C. Park, R. Roy, *Tetrahedron Lett.* **1995**, *36*, 7383–7386; h) W. K. C. Park, S. Aravind, A. Romanowska, J. Renaud, R. Roy, *Methods in Enzymology*, **1994**, *242*, 294–304; i) R. Roy, D. Zanini, S. J. Meunier, A. Romanowska, *ACS Symposium Series*, **1994**, *560*, 104–119; j) W. K. C. Park, S. Brochu, R. Roy, *Tetrahedron Lett.* **1994**, *35*, 7739–7742; k) R. Roy, D. Zanini, S. J. Meunier, A. Romanowska, *J. Chem. Soc. Chem. Commun.* **1993**, 1869–1872.
[19] P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, S. A. Nepogodiev, J. F. Stoddart, *Chem. Eur. J.* **1996**, *2*, 1115–1128.
[20] a) K. Aoi, K. Itoh, M. Okada, *Macromolecules* **1995**, *28*, 5391–5393; b) T. K. Lindhorst, C. Kieburg, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1953–1956.
[21] The purity in the case of this DAB-dendr-$(NH_2)_{64}$ is 18%, which means that the real amount of dendrimer with 64 terminal amine end groups is 18%. The rest consists of defects with one or more branches missing. However, this mixture is very well defined in terms of polydisperisity, which is equal to 1.002. Details will be published soon: J. C. Hummelen, J. L. J. van Dongen, E. W. Meijer, *Chem. Eur. J.* in press.
[22] DAB-*dendr*-$(CO_2H)_{64}$ as well as DAB-*dendr*-$(NH_2)_{64}$ can be prepared from the same precursor, DAB-*dendr*-$(CN)_{64}$, by acid hydrolysis or catalytic reduction, respectively. See J. C. M. van Hest, M. W. P. L. Baars, C. Elissen-Román, M. H. P van Genderen, E. W. Meijer, *Macromolecules* **1995**, *28*, 6689–6691.
[23] E. W. Meijer, unpublished results.
[24] C. Stowell, Y. C. Lee, *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225–281.
[25] M. Elofsson, B. Walse, J. Kihlberg, *Tetrahedron Lett.* **1991**, *32*, 7613–7616.
[26] C. S. Hudson, J. M. Johnson, *J. Am. Chem. Soc.* **1915**, *37*, 1270–1275.
[27] The β-heptaacetate of **9** was used as a main component of the mixture containing both anomers. The α-heptacetate of **9** remained unchanged during the treatment with $HSCH_2CH_2CO_2H/BF_3 \cdot OEt_2$ and did not interfere with the separation of the product.
[28] G. W. Anderson, J. E. Zimmerman, F. M. Callahan, *J. Am. Chem. Soc.* **1964**, *86*, 1839–1842.
[29] G. Zemplen, E. Pacsu, *Ber.* **1929**, *62*, 1613–1614.
[30] Sulfur combustion analysis was used for the same purpose in the case of protein-based neoglycoconjugates: J. Dahmén, T. Frejd, G. Magnusson, G. Noori, A.-S. Carlström, *Carbohydr. Res.* **1984**, *127*, 15–25.
[31] Macromodel V5.0: F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comp. Chem.* **1990**, *11*, 440–467.
[32] W. C. Still, A. Tempczyk, R. C. Hawley, T. Hendrickson, *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.
[33] a) C. J. Hawker, J. M. J. Fréchet, *J. Am. Chem. Soc.* **1990**, *112*, 7638–7647; b) M. M. Miller, T. X. Neenan, R. Zayas, H. E. Bair, *J. Am. Chem. Soc.* **1992**, *114*, 1018–1025; c) For a comprehensive review of convergent procedures for constructing dendrimers, see ref. [1a], pp. 107–163.
[34] T. V. Dudukina, S. A. Nepogodiev, D. M. Belen'ky, *Biokhimia* **1990**, 55, 1474–1480.
[35] a) T. Kawaguchi, K. L. Walker, C. L. Wilkins, J. S. Moore, *J. Am. Chem. Soc.* **1995**, *117*, 2159–2165; b) H.-T. Cheang, C.-T. Chen, T. Kondo, G. Siuzdak, K. B. Sharpless, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 182–186; c) See also T. Nagasaki, O. Kimura, M. Ukon, S. Arimori, I. Hamachi, S. Shinkai, *J. Chem. Soc. Perkin Trans. 1*, **1994**, 75–81.
[36] Biosym Technologies, 9685 Scranton Road, San Diego, CA 92121–2777, USA.
[37] Ac = acetyl; Suc = succinimidoyl; Gal = galactosyl residue; Glc = glucosyl residue; Gly = glycine; DCC = 1,3-dicyclohexylcarbodiimide; NHS = *N*-hydroxysuccinimide; DAB-*dendr*-$(NH_2)_x$ = poly(propylene imine) dendrimers; GPC = gel permeation chromatography; br = broad; pt = pseudotriplet.