Tetrahedron 66 (2010) 3326–3331

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

# Globular carbosilane dendrimers with mannose groups at the periphery: synthesis, characterization and toxicity in dendritic cells

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ARTICLE INFO

*Article history:*
Received 24 November 2009
Received in revised form
25 February 2010
Accepted 26 February 2010
Available online 6 March 2010

*Keywords:*
Dendrimers
Mannose
Carbosilane
Dendritic cells

ABSTRACT

A synthetic strategy has been developed for the preparation of new globular carbosilane dendrimers with mannose groups at the periphery. It consists of hydrosilylation reaction of allyl tetraacetylmannose with carbosilane dendrimers containing monohydrosilane end groups and the subsequent deacetylation reaction. Evaluation of dendrimer toxicities in dendritic cells by MTT assay were carried out, and evidence a good biocompatibility.

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## 1. Introduction

Carbohydrates displayed on the surface of cells play critical roles in cell–cell recognition, adhesion, signaling between cells, and as markers for disease progression, for example, viral and bacterial infections, antibody–antigen interactions, fertilization, and other cell–cell interactions all rely on protein-carbohydrate interactions.[1] The use of dendrimers in carbohydrate chemistry, which has led to a large number of glycodendrimers, is especially attractive.[2] Dendrimers, macromolecular-branched compounds around an inner core, are gaining popularity as multivalent ligands for the study and modulation of biological events on protein surfaces. In particular, glycodendrimers, and glycopeptides dendrimers[3] have found wide use in the study of cellular events mediated by lectins and toxins, and several reviews reflect the latest progress in this field.[4] Recently, a large number of neoglycoconjugates carrying different saccharides have been synthesized by several groups and their activities have been evaluated.[5,6] Moreover, systems with multivalent presentation of mannosyl groups on an adequate scaffold and the specific targeting of mannose-capped dendrimers to mannose receptors, highly expressed in cells of the immune system, can be considered as potential antiviral drugs to inhibit the DC-SING binding of the different virus type like HIV.[7] Large number of dendrimers with mannose moiety has been synthesized.[8] However in our knowledge, there are few examples of carbosilane dendrimers functionalized with mannose groups.[5e,9]

Carbosilane dendrimers are effective scaffolds for therapeutic usefulness.[10] This type of dendrimers seems to be attractive systems for manufacturing a therapeutic reagent, due to their own characteristics, such as (a) simplicity of the synthetic process to extend the generation, (b) present a hydrophobic nature in contrast to more hydrophilic polyamine-type dendrimers, (c) chemical and biochemical stability and (d) biological inertness.[11] Carbosilanes dendrimers bearing saccharide epitopes on the surface were first introduced by Matsuoka et al.[12] When the saccharide is mannose, Matsuoka et al.[5d] prepared carbosilane dendrimers by nucleophilic displacement of a bromine leaving group on the dendritic molecule with a thio nucleophile borne by the sugaring structure. However, only one case of glycocoating carbosilane dendrimers with mannose moiety has been synthesized via a hydrosilylation reaction between di-isopropylidene protected allyl mannoside and carbosilane dendrimers with dihydrosilane surface groups and reported by Lindhorst and Boysen.[9] In this case the hydrosilylation reaction with dendrimers containing di- or trihydrosilanes end groups and different alkenes was unfavorable,[13] affording glycodendrimes in poor yield.

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doi:10.1016/j.tet.2010.02.097

In this manuscript, we described in detail the systematic synthesis and characterization of globular carbosilane dendrimers with mannose groups in the periphery via hydrosilylation of allyl tetraacetylmannose with a carbosilane dendrimers containing monohydrosilane end groups. We also present preliminary results of their biocompatibility studies.

## 2. Results and discussion

### 2.1. Synthesis and structural characterization of carbosilane dendrimers having peripheral mannose

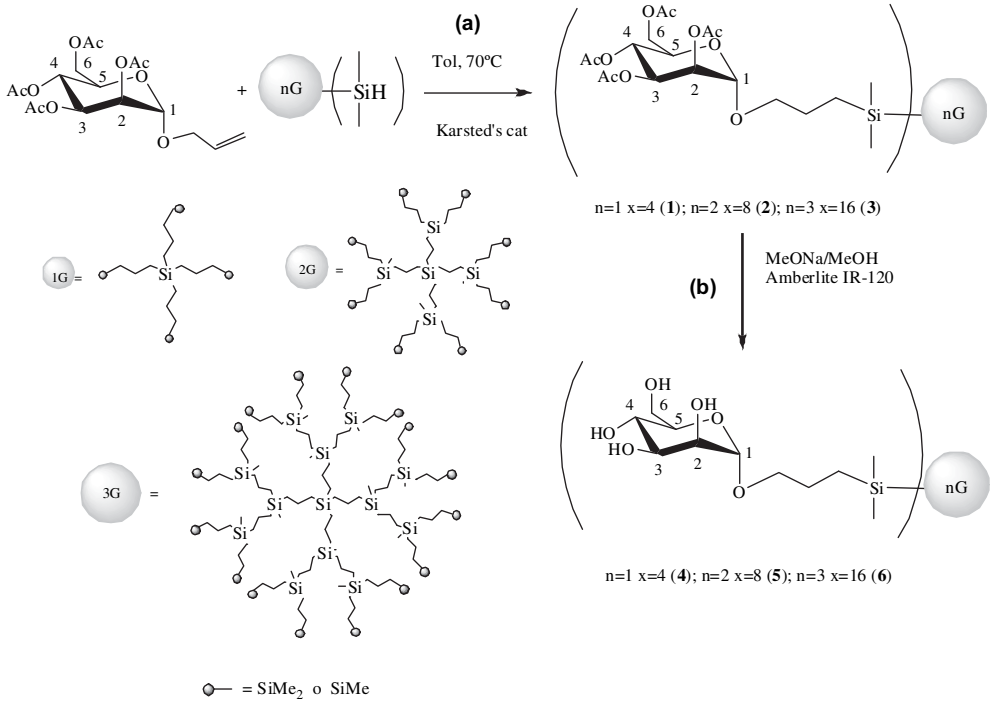
Preparative details and spectroscopic data for the new compounds are given in the Experimental Section. Only selected data will be presented for this discussion. Glycodendrimers formed by hydrosilylation with carbosilane dendrimers of type $G_n$–$H_x$ ($n$=1, 2, 3; $x$=4, 8, 16) where '$n$' means the number of generation (G) and '$x$' denotes the number of branches and hence the number of peripheral groups, were synthesized as reported previously[14] while the allyl tetraacetylmannose ligand (**I**) was obtained through the treatment of penta-*O*-acetylmannopyranose with allyl alcohol in the presence of boron trifluoride diethyl ether complex.[12a,15]

The reactions were performed in ampoules with J Young valves using toluene as solvent and the Karstedt's catalyst[16] and heating the mixture at 70 °C for 8 h to afford the corresponding dendrimers $G_n$–[Man(OAc)]$_x$ ($n$=1, $x$=4 (**1**); $n$=2, $x$=8 (**2**); $n$=3, $x$=16 (**3**)) in good yields as pale yellow oils (see Scheme 1 (a)). All these derivatives are soluble in common organic solvents. $^1$H NMR spectroscopy was used to follow the progress of the reactions by monitoring the loss of the Si–H and the characteristic resonance of the allyl group of compound **I**. The processes occurred exclusively by β-hydrosilylation to give the Man–$OCH_2CH_2CH_2Si$ group, and not α-hydrosilylation was observed.

In order to obtain the deacetylated dendrimers, the $G_n$-[Man-(OAc)]$_x$ compounds were treated under argon atmosphere with a solution of NaOMe in MeOH. The resulting solution was stirred at room temperature during 2 h. After the reaction, Amberlite IR-120 was added to neutralize the reaction solution for 12 h. The suspension was filtrated and evaporated, affording a residue that was purified by column size chromatography (see Scheme 1 (b)). Dendrimers $G_n$-[Man]$_x$ ($n$=1, $x$=4 (**4**); $n$=2, $x$=8 (**5**); $n$=3, $x$=16 (**6**)) were obtained as a pale yellow solid in good yields. This family of dendrimers is soluble in THF, MeOH, and DMSO and insoluble in water. In this case, the reaction was followed by $^{13}$C NMR spectroscopy, through the extinction of the carbonyl resonances of acetate groups.

Structural characterization of dendrimers **1**–**6** has been carried out using elemental analysis, $^1$H, $^{13}$C, and $^{29}$Si NMR spectroscopy and mass spectrometry. The NMR spectroscopic and analytical data for derivatives **1**–**6** are consistent with their proposed structures (for some examples see Fig. 1).

$^1$H NMR spectra in $CDCl_3$ showed for the carbosilane framework of dendrimers **1**–**3** almost identical chemical shifts for analogous nuclei in different generations. The tetraacetylmannose unit presents five sets of signals. For H-3, H-4, and H-2 a multiplet around $\delta$=5.25 ppm is observed while for H-5 another multiplet is located at $\delta$=3.98 ppm. A broad singlet is observed for H-1 at 4.78 ppm and the signals centered at $\delta$=4.24 ppm are assigned to H6a and H6b. The acetylated groups show four resonances between $\delta$=2.12 and 1.99 ppm. Respecting to the methylene groups of the carbosilane skeleton five sets of resonances are observed with the expected integration ratio. The hydrogen atoms of the methylene group bonded to oxygen, Man–$OCH_2CH_2CH_2Si$, exhibit two multiplets at 3.58 and 3.38 ppm as a result of their different chemical environment. The central methylenes of $SiCH_2CH_2CH_2Si$ and Man–$OCH_2CH_2CH_2Si$ branches are located at $\delta$=1.22 and 1.57 ppm, whilst the methylene groups bond directly to silicon atoms are centered at $\delta$=0.55 ($SiCH_2CH_2CH_2Si$) and 0.42 ppm (Man–$OCH_2CH_2CH_2Si$). The latter assignment is based on the enlargement of the signal intensity of the resonance at $\delta$=0.55 ppm on increasing dendrimer generation. $^{13}$C{$^1$H} NMR spectra show four resonances between 170.0 and 168.0 ppm attributed to the carbonyl group of the acetylated units, the C-1 resonance appear at 97.5 ppm and the carbon atom of the methylene group bonded to –OMan give a resonance at




**Scheme 1.** Synthesis of globular carbosilane dendrimers with mannose groups at the periphery.

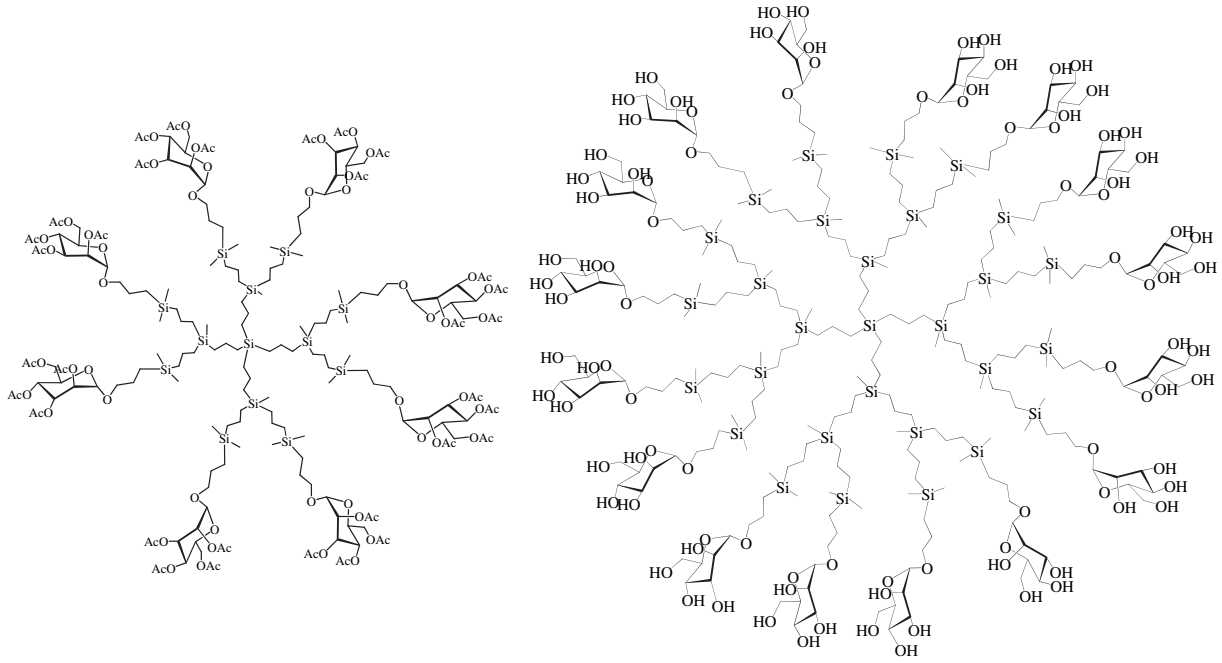


**Figure 1.** Molecular representation of $G_2$-[Man(OAc)]$_8$ (**2**) and $G_3$-[Man]$_{16}$ (**6**).

68.5 ppm while the chemical shift of the carbon atom bonded to Si in the fragment Man–$OCH_2CH_2CH_2Si$ appears around 11.2 ppm. The $^{29}$Si chemical shifts of these dendrimers have been determined by a 2D {$^1$H–$^{29}$Si}-HMBC experiment, where the outermost Si atom appears at 2.1 ppm and for the subsequent inner silicon atom around 1.0 ppm (Fig. 2).

In the deacetylated derivatives, the $^1$H NMR spectra of the dendrimers **4**–**6** were recorded in DMSO-$d_6$ at room temperature, although in this solvent the line widths of these spectra tended to be broader than those of derivatives soluble in common organic solvents. The spectra show that the carbosilane framework is insignificantly affected by the deacetylation reaction. A broad multiplet for all the protons of mannose group between $\delta$=4.00 and 3.20 ppm and a broad singlet centered at $\delta$=4.56 ppm for H-1 are observed. In $^{13}$C{$^1$H} NMR spectra, the most important fact is the disappearance of the signals attributed to the carbonyl groups of acetylated units.

Dendrimers were also analyzed by mass spectroscopy (MALDI-TOF MS) using 1,8,9-trihydroxyanthracene (dithranol) as a matrix. Very clean MALDI-TOF spectra were obtained for the $G_1$ glycodendrimers, with fine peaks centered at $m/z$ values matching those for the calculated sodium adducts (see Experimental section). However, for the second and third generations the molecular peaks were not observed because the dendrimers fly with more difficulty in MALDI-TOF MS and the spectra obtained showed a broader peak, as well as some fragmentation.

### 2.2. Dendrimer toxicity

The dendrimers **4**–**6** were tested on dendritic cells (from healthy donors) as an initial screen for biocompatibility. Dendritic cells (DC) are the first line of defense against HIV-1 infections and undergo maturation during virus infection and thereby become potent stimulators of cell-mediated immunity.[17] The toxicity evaluation for **4**–**6** has been carried out by means of MTT assay. Due to the low solubility of all these systems in water, small quantities of DMSO (less than 2.5% in water, e.g., 0.5% for $G_1$, 1.0% for $G_2$, and 1.5% for $G_3$) were added, where no visual aggregation was observed, resulting in clear solutions for the cytotoxicity studies. At this DMSO concentration the cell bioavailability is not affected. Figure 3A shows that up to 10 μM concentration mitochondrial activity at 24 h was above 80–90% suggesting that $G_1$-[Man]$_4$, $G_2$-[Man]$_8$, and $G_3$-[Man]$_{16}$ were not toxic at that concentration in both immature and mature DC (Fig. 3A), while at 20 μM the mitochondrial activity decrease 30–35%. The MTT assay at 48 h shows the same results (Fig. 3B).

## 3. Conclusion

A synthetic strategy has been developed for introducing mannose groups on the periphery of spherical carbosilane dendrimers in good yields via hydrosilylation of allyl tetraacetylmannose with carbosilane dendrimers containing monohydrosilane end groups and the subsequent deacetylation reaction.

The lack of toxicity obtained, both in immature and mature dendritic cells by the different dendrimer generations with mannose groups at the periphery, shows that the new glycodendrimers can be good candidates as antiviral drugs to interact with lectin receptors like DC-SIGN.

## 4. Experimental section

### 4.1. General remarks

All manipulations of oxygen- or water-sensitive compounds were carried out under an atmosphere of argon using standard Schlenk techniques. Toluene solvent was dried and freshly distilled under argon prior to use sodium, unless otherwise stated, reagents were obtained from commercial sources and used as received. The carbosilane dendrimers of different generations nG–$H_x$ were prepared according to reported methods.[14] $^1$H, $^{13}$C, and $^{29}$Si NMR spectra were recorded on Varian Unity VXR-300 and Varian 500 Plus Instruments. Chemical shifts ($\delta$, ppm) were measured relative to residual $^1$H, $^{13}$C, and $^{29}$Si NMR resonances for $CDCl_3$ and DMSO-$d_6$, used as solvents. C, H analyses were carried out with a Perkin–Elmer 240 C microanalyzer. MALDI-TOF MS samples were prepared in a 1,8,9-trihydroxyanthracene (dithranol) matrix, and spectra were recorded on a Bruker Reflex II spectrometer equipped with

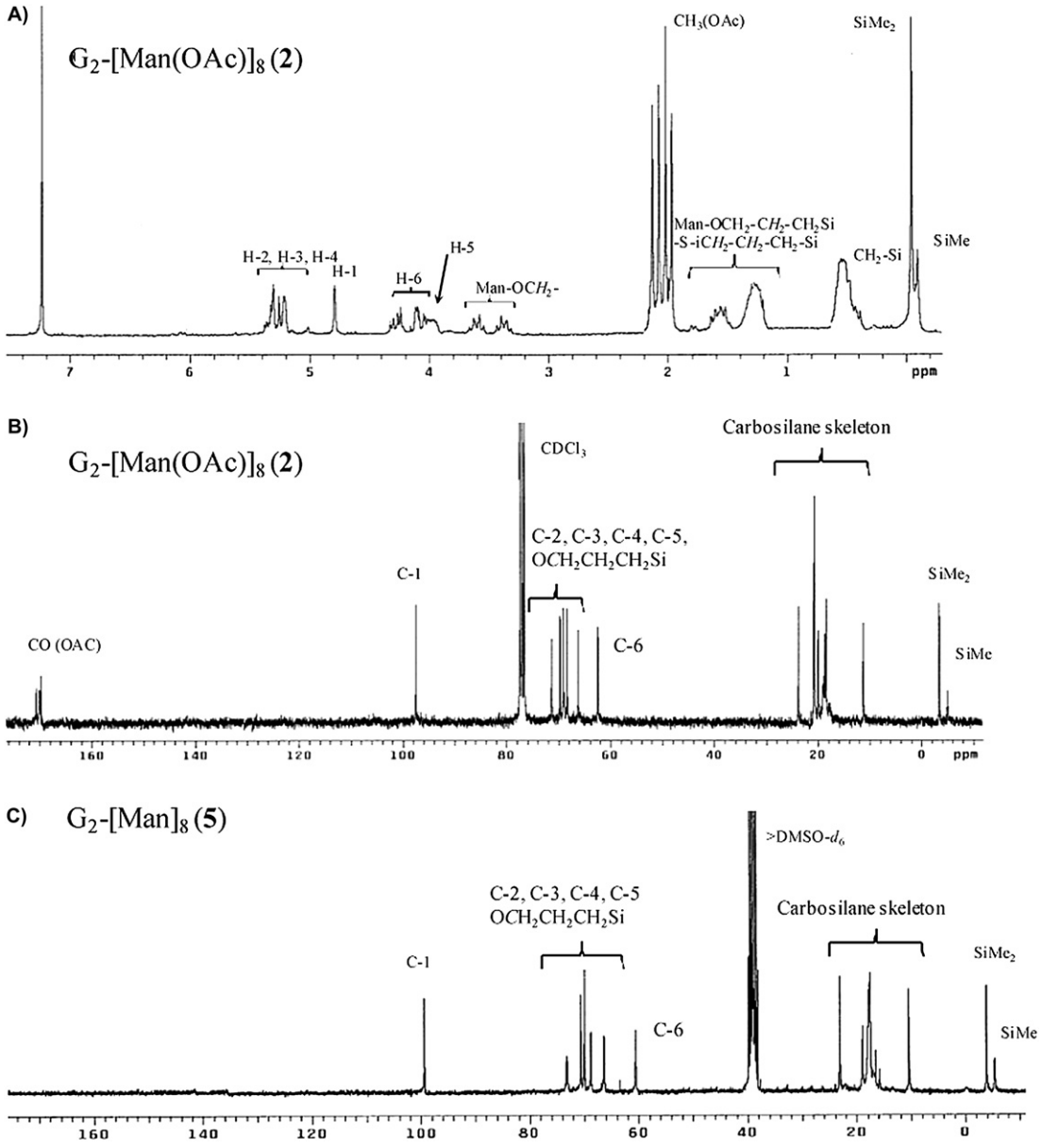



**Figure 2.** $^{1}H$ NMR (A) and $^{13}C$ NMR (B) of acetylated dendrimer **2** in $CDCl_3$ as a solvent. $^{13}C$ NMR (C) of deacetylated dendrimer **5** in DMSO-$d_6$ as a solvent.

a nitrogen laser emitting at 337 nm and operated in the reflection mode at an accelerating voltage in the range 23–25 kV.

*4.1.1. Synthesis of $G_1$-[Man(OAc)]$_4$ (**1**).* The compound **I** (0.45 g, 1.17 mmol) and two drops of Karsted's catalyst were added to a solution of first-generation hydrogen-terminated dendrimer $G_1$–$H_4$ (0.12 g, 0.29 mmol) in toluene (10 mL). The reaction mixture was heated at 70 °C for one night and then evaporated to dryness to remove the solvent to give **1** as a pale yellow oil (0.50 g, 87%). $^{1}H$ NMR ($CDCl_3$): $\delta$=5.31–5.21 (m, 3H, H-3, H-4, and H-2); 4.78 (br s, 1H, H-1); 4.24 (m, 2H, H-6a, and H-6b); 3.98 (m, 1H, H-5); 3.58 (m, 1H, Man–OC*H*$_2$CH$_2$CH$_2$Si); 3.38 (m, 1H, Man–OC*H*$_2$CH$_2$CH$_2$Si); 2.13, 2.08, 2.02, and 1.97 (s, 12H, $CH_3$(OAc)); 1.54 (m, 2H, Man–OCH$_2$C*H*$_2$CH$_2$Si); 1.28 (m, 2H, SiCH$_2$C*H*$_2$CH$_2$Si); 0.55 (m, 4H, SiC*H*$_2$CH$_2$C*H*$_2$Si); 0.45 (m, 2H, Man–OCH$_2$CH$_2$C*H*$_2$Si); −0.04 (s, 6H, $SiMe_2$). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$): 170.6, 170.0, 169.9, 169.7 (CO (OAc)); 97.5 (C-1); 71.3 (C-2); 69.7 (C-3); 69.1 (C-5); 68.3 (Man–OCH$_2$CH$_2$CH$_2$Si); 66.2 (C-4); 62.4 (C-6); 23.7 (Man–OCH$_2$*C*H$_2$CH$_2$Si); 20.9–20.7 ($CH_3$ (OAc)); 20.0, 18.5, 17.5 (SiCH$_2$CH$_2$*C*H$_2$Si); 11.2 (Man–OCH$_2$CH$_2$*C*H$_2$Si); −3.4 ($SiMe_2$). $^{29}Si\{^{1}H\}$ NMR ($CDCl_3$): 0.9 ($G_0$–Si); 2.2 ($G_1$–Si). Elemental analysis calcd (%) for $C_{88}H_{148}O_{40}Si_5$: C 53.21, H 7.51; found C 53.01, H 7.44. MALDI-TOF: [M+$Na^+$]=2008.9 uma (calcd=2008.8 uma).

*4.1.2. Synthesis of $G_2$-[Man(OAc)]$_8$ (**2**).* This dendrimer was prepared using a similar method to that described for **1**, starting from $G_2$–$H_8$ (0.17 g, 0.14 mmol), compound **I** (0.45 g, 1.16 mmol) and two drops of Karsted's catalyst to obtain compound **2** as a pale yellow oil (0.48 g, 78%). $^{1}H$ NMR ($CDCl_3$): $\delta$=5.31–5.22 (m, 6H, H-3, H-4, and H-2); 4.79 (br s, 2H, H-1); 4.25 (m, 4H, H-6a, and H-6b); 3.98 (m, 2H, H-5); 3.59 (m, 2H, Man–OC*H*$_2$CH$_2$CH$_2$Si); 3.39 (m, 2H, Man–OC*H*$_2$CH$_2$CH$_2$Si); 2.13, 2.08, 2.02, and 1.97 (s, 24H, $CH_3$(OAc)); 1.55 (m, 4H, Man–OCH$_2$C*H*$_2$CH$_2$Si); 1.28 (m, 6H, SiCH$_2$C*H*$_2$CH$_2$Si); 0.54 (m, 12H, SiC*H*$_2$CH$_2$C*H*$_2$Si); 0.46 (m, 4H, Man–OCH$_2$CH$_2$C*H*$_2$Si); −0.04 (s, 12H, $SiMe_2$); −0.10 (s, 3H, SiMe). $^{13}C\{^{1}H\}$ NMR($CDCl_3$): 170.6, 170.1, 169.9, 169.7 (CO (OAc)); 97.5 (C-1); 71.3 (C-2); 69.7 (C-3); 69.1

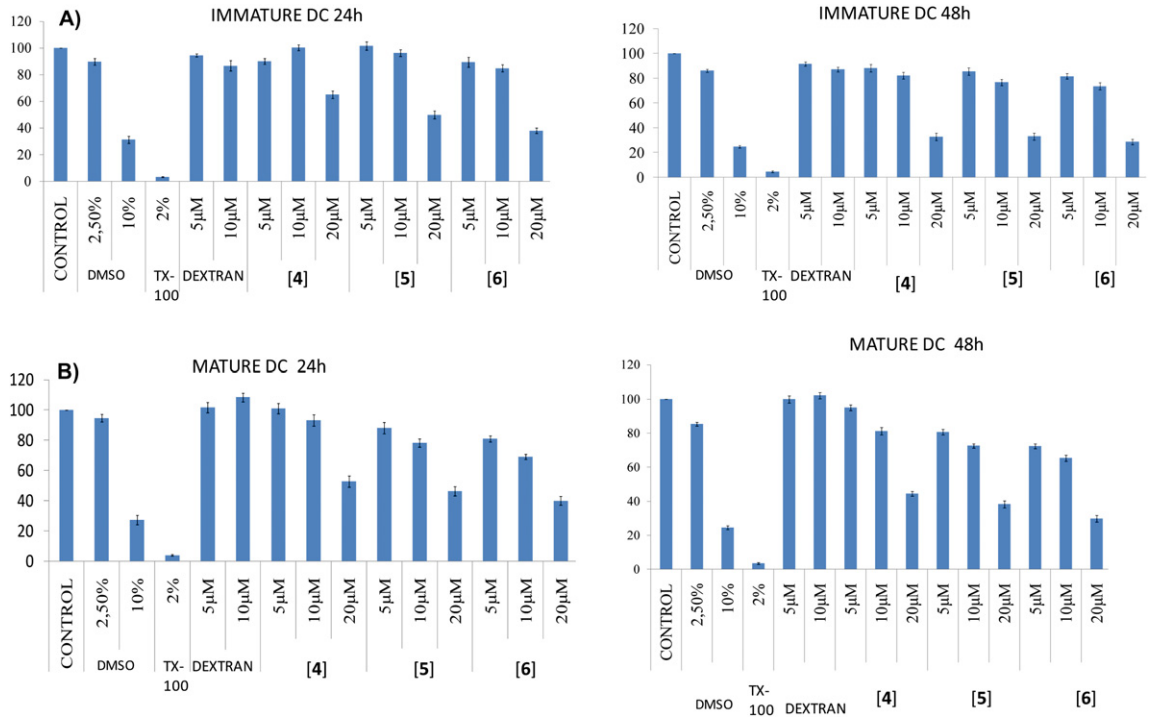



**Figure 3.** MTT assay of dendrimers **4**–**6** in immature DC and mature DC (A) after 24 h (B) after 48 h. The graphic represents the media of three independent experiments.

(C-5); 68.3 (Man–O$CH_2CH_2CH_2$Si); 66.2 (C-4); 62.4 (C-6); 23.8 (Man–O$CH_2CH_2CH_2$Si); 20.9–20.7 ($CH_3$ (OAc)); 20.0–17.9 (Si$CH_2CH_2CH_2$Si); 11.2 (Man–O$CH_2CH_2CH_2$Si); −3.4 ($SiMe_2$); −5.0 (SiMe). $^{29}Si\{^1H\}$ NMR($CDCl_3$): 0.9 ($G_0$–Si); 1.1 ($G_1$–Si); 2.2 ($G_2$–Si). Elemental analysis calcd (%) for $C_{192}H_{332}O_{80}Si_{13}$: C 53.81, H 7.31; found C 52.67, H 7.15.

*4.1.3. Synthesis of $G_3$-[Man(OAc)]$_{16}$ (**3**).* This dendrimer was prepared using a similar method to that described for **1**, starting from $G_3$–$H_{16}$ (0.14 g, 0.05 mmol), compound **I** (0.32 g, 8.32 mmol) and two drops of Karsted's catalyst to obtain compound **3** as a pale yellow oil (0.26 g, 56%). $^1H$ NMR ($CDCl_3$): $\delta$=5.33–5.20 (m, 12H, H-3, H-4, and H-2); 4.79 (br s, 4H, H-1); 4.26 (m, 8H, H-6a, and H-6b); 3.98 (m, 4H, H-5); 3.58 (m, 4H, Man–O$CH_2$$CH_2CH_2$Si); 3.38 (m, 4H, Man–O$CH_2$$CH_2CH_2$Si); 2.13, 2.07, 2.02, and 1.97 (s, 48H, $CH_3$(OAc)); 1.58 (m, 8H, Man–O$CH_2CH_2$$CH_2$Si); 1.28 (m, 14H, Si$CH_2CH_2$$CH_2$Si); 0.54 (m, 28H, Si$CH_2CH_2$$CH_2$Si); 0.45 (m, 8H, Man–O$CH_2CH_2$$CH_2$Si); −0.04 (s, 24H, $SiMe_2$); −0.10 (s, 9H, SiMe). $^{13}C\{^1H\}$ NMR($CDCl_3$): 170.6, 170.0, 169.9, 169.7 (CO (OAc)); 97.5 (C-1); 71.3 (C-2); 69.7 (C-3); 69.1 (C-5); 68.3 (Man–O$CH_2CH_2CH_2$Si); 66.2 (C-4); 62.4 (C-6); 23.7 (Man–O$CH_2CH_2CH_2$Si); 20.9–20.7 ($CH_3$ (OAc)); 20.0–17.9 (Si$CH_2CH_2CH_2$Si); 11.2 (Man–O$CH_2CH_2CH_2$Si); −3.4 ($SiMe_2$); −4.9 (SiMe). $^{29}Si\{^1H\}$-NMR ($CDCl_3$): 0.9 ($G_0$–Si); 1.1 (overlapped signals of $G_1$–Si and $G_2$–Si); 2.2 ($G_3$–Si). Elemental analysis calcd (%) for $C_{400}H_{700}O_{160}Si_{29}$: C 54.08, H 7.94; found C 53.25, H 7.56.

*4.1.4. Synthesis of $G_1$-[Man]$_4$ (**4**).* Over a methanolic solution of glycodendrimer **1** (0.19 g, 0.09 mmol) was added 0.01 g of NaOMe dissolved in 2 mL of MeOH. The resulting solution was stirred at room temperature for 1 h 30 min and then was neutralized with Amberlite IR-120 one night; filtered, and evaporated, affording a residue that was purified by column size chromatography. The dendrimer **4** was obtained as a pale yellow solid (0.07 g; 56%). $^1H$ NMR (DMSO-$d_6$): $\delta$=4.56 (br s, 1H, H-1); 3.99–3.21 (m, 8H, H-3, H-4, H-2, H-5; H-6, and Man–O$CH_2$$CH_2CH_2$Si); 1.43 (m, 2H, Man–O$CH_2CH_2$$CH_2$Si); 1.29 (m, 2H, Si$CH_2CH_2$$CH_2$Si); 0.52 (m, 4H, Si$CH_2CH_2$$CH_2$Si); 0.43 (m, 2H, Man–O$CH_2CH_2$$CH_2$Si); −0.06 (s, 6H, $SiMe_2$). $^{13}C\{^1H\}$-NMR (DMSO-$d_6$): 99.2 (C-1); 73.3 (C-2); 70.5 (C-3); 69.9 (C-5); 68.6 (Man–O$CH_2CH_2CH_2$Si); 66.4 (C-4); 60.6 (C-6); 23.1 (Man–O$CH_2CH_2CH_2$Si); 18.9, 17.7, 16.4 (Si$CH_2CH_2CH_2$Si); 10.5 (Man–O$CH_2CH_2CH_2$Si); −3.9 ($SiMe_2$). $^{29}Si\{^1H\}$ NMR (DMSO-$d_6$): 1.0 ($G_0$–Si); 2.4 ($G_1$–Si). Elemental analysis calcd (%) for $C_{56}H_{116}O_{24}Si_5$: C 51.19, H 8.90; found C 49.92, H 8.36. MALDI-TOF: [M+$Na^+$]=1335.7 uma (calcd=1335.7 uma).

*4.1.5. Synthesis of $G_2$-[Man]$_8$ (**5**).* This second-generation dendrimer was prepared using a similar method to that described for **4**, starting from **2** (0.21 g; 0.05 mmol) and 0.01 g of NaOMe dissolved in 2 mL of MeOH. Compound **5** was isolated as a pale yellow solid (0.09 g, 60%). $^1H$ NMR (DMSO-$d_6$): $\delta$=4.56 (br s, 2H, H-1); 4.08–3.21 (m, 24H, H-3, H-4, H-2, H-5; H-6, and Man–O$CH_2$$CH_2CH_2$Si); 1.45 (m, 4H, Man–O$CH_2CH_2$$CH_2$Si); 1.29 (m, 6H, Si$CH_2CH_2$$CH_2$Si); 0.52 (m, 16H, Si$CH_2CH_2CH_2$Si, and Man–O$CH_2CH_2CH_2$Si); −0.07 (s, 15H, $SiMe_2$, and SiMe). $^{13}C\{^1H\}$ NMR(DMSO-$d_6$): 99.2 (C-1); 73.2 (C-2); 70.5 (C-3); 69.8 (C-5); 68.6 (Man–O$CH_2CH_2CH_2$Si); 66.4 (C-4); 60.9 (C-6); 23.1 (Man–O$CH_2CH_2CH_2$Si); 18.8–16.4 (Si$CH_2CH_2CH_2$Si); 10.4 (Man–O$CH_2CH_2CH_2$Si); −3.9 ($SiMe_2$); −5.4 (SiMe). $^{29}Si\{^1H\}$ NMR(DMSO-$d_6$): $G_0$ not observed, 0.9 ($G_1$–Si); 2.3 ($G_2$–Si). Elemental analysis calcd (%) for $C_{128}H_{268}O_{48}Si_{13}$: C 52.28, H 9.19; found C 51.59, H 8.63.

*4.1.6. Synthesis of $G_3$-[Man]$_4$ (**6**).* This dendrimer was prepared using a similar method to that described for **4**, starting from **3** (0.18 g; 0.02 mmol) and 0.01 g of NaOMe dissolved in 2 mL of MeOH. Compound **6** was isolated as a pale yellow solid (0.07 g, 55%). $^1H$ NMR (DMSO-$d_6$): $\delta$=4.56 (br s, 4H, H-1); 4.08–3.25 (m, 32H, H-3, H-4, H-2, H-5; H-6, Man–O$CH_2CH_2CH_2$Si,); 1.45 (m, 8H, Man–O$CH_2CH_2$$CH_2$Si); 1.29 (m, 14H, Si$CH_2CH_2$$CH_2$Si); 0.52 (m, 30H, Si$CH_2CH_2CH_2$Si, and Man–O$CH_2CH_2CH_2$Si); −0.07 (s, 33H, $SiMe_2$ and SiMe). $^{13}C\{^1H\}$ NMR(DMSO-$d_6$): 99.3 (C-1); 73.1 (C-2); 70.5 (C-3); 69.9 (C-5); 68.7 (Man–O$CH_2CH_2CH_2$Si); 66.3 (C-4); 60.6 (C-6); 23.1 (Man–O$CH_2CH_2CH_2$Si); 18.9–16.4 (Si$CH_2CH_2CH_2$Si); 10.5 (Man–O$CH_2CH_2CH_2$Si); −3.9 ($SiMe_2$); −5.5 (SiMe). $^{29}Si\{^1H\}$ NMR (DMSO-$d_6$): $G_0$–Si and $G_1$–Si not observed, 0.9 ($G_2$–Si); 2.2

($G_3$–Si). Elemental analysis calcd (%) for $C_{272}H_{572}O_{96}Si_{29}$: C 52.74, H 9.31; found C 51.99, H 9.01.

### 4.2. Biocompatibility studies

*Dendritic cells* (DC) were obtained using the following procedure. Monocytes were separated from the leukophoresed blood with *anti*-human CD14 monoclonal antibody (mAb)-coated MicroBeads using MACS single-use separation columns (Miltenyi Biotec, Germany). Purified monocytes were suspended in RPMI 1640 medium (Sigma, US) containing 5% heat-inactivated fetal calf serum, 100 U/ml penicillin G, and 100 μg/ml streptomycin (Sigma). Monocytes obtained from one donor were plated in a flat-bottomed 6-well tissue culture plate (2×106/well) and cultured with 50 ng/ml rh GM-CSF or 20 ng/ml rh IL-4 (ImmunoTools, Germany) at conditions stated above. Cells, fresh culture medium containing rh GM-CSF and rh IL-4 is added to the immature DC (iDC) culture every two days. iDC were matured by 20 ng/ml LPS (Sigma, Z) for the last two days. iDC and mDC were tested for immature or mature markers by FACS (EPICS MX-MCL, Beckman Coulter, US) and analyzed by CXP Software (Beckman Coulter).

### 4.3. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay

This method was selected to analyze detrimental intracellular effects on mitochondria and metabolic activity. MT-2 cells were seeded in 96 well plates in OPTIMEM® I medium containing 5% FBS (100×105 cells in 200 μl/well) and submitted to 5, 10, and 20 μM treatment with $G_1$-$[Man]_4$, $G_2$-$[Man]_8$, and $G_3$-$[Man]_{16}$. After 20 h, 20 μl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium-bromide) substrate solution (5 mg/ml) was added to the cells to measure mitochondrial activity. After 4 h, the supernatant was removed and the formed crystals were dissolved in 200 μl DMSO and absorbance was measured at 550 nm with a reference of 690 nm. All points were performed in triplicate.

### Acknowledgements

This work has been supported by grants from Fondo de Investigación Sanitaria (FIS) of Ministerio de Ciencia e Innovación FIS (PI052476, PI061479); Fundación para la Investigación y la Prevención del SIDA en España (FIPSE 24632/07); MNT-ERA NET 2007 (ref. NAN2007-31198-E), Red RIS RD06-0006-0035; Fundación Caja Navarra and Comunidad de Madrid (S-SAL-0159-2006) to MAMF and MNT-ERA NET 2007 (ref. NAN2007-31135-E) and Fondo de Investigación Sanitaria (PI080222) to UA.

### References and notes

1. (a) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674; (b) Bovin, N. V.; Gabius, H. J. *Chem. Soc. Rev.* **1995**, *24*, 413–421; (c) Sharon, N.; Lis, H. *Sci. Am.* **1993**, 82–89; (d) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321–327; (e) Dwek, R. A. *Chem. Rew* **1996**, *96*, 683–720; (f) Gabius, H. J. *Eur. J. Biochem.* **1997**, *243*, 543–576; (g) Karlsson, K. A. *Biochem. Soc. Trans.* **1999**, *27*, 471–474; (h) Smith, A. E.; Helenius, A. *Science* **2004**, *304*, 237–242; (i) Angata, T.; Varki, A. *Chem. Rev.* **2002**, *102*, 439–470.
2. (a) Cloninger, M. J. *Mol. Recognit. Polym.* **2008**, 335–358; (b) Hadad, C.; Majoral, J.-P.; Muzart, J.; Caminade, M.; Bouquillon, S. *Tetrahedron lett.* **2009**, *50*, 1902–1905; (c) Kantchev, E. A. B.; Bader, S. J.; Parquette, J. R. *Tethahedron* **2005**, *61*, 8329–8338; (d) Han, S.; Baigude, H.; Hattori, K.; Yoshida, T.; Uryu, T. *Carbohydr. Polym.* **2007**, *68*, 26–34; (e) Turnbull, W. B.; Stoddart, J. F. *Rev. Mol. Biotechnol.* **2002**, *90*, 231–255; (f) Elsner, K.; Boysen, M. M. K.; Lindhorst, T. K. *Carbohydr. Res.* **2007**, *342*, 1715–1725.
3. Specker, D.; Wittman, V. *Top. Curr. Chem.* **2007**, *267*, 65–107.
4. (a) Chabre, Y. M.; Roy, R. *Curr. Top. Med. Chem.* **2008**, *8*, 1237–1285; (b) Heegaard, P. M. H.; Boas, U. *Recent Pat. AntiInfect. Drug Discov.* **2006**, *1*, 331–351; (c) Li, Y.; Cheng, Y.; Xu, T. *Curr. Drug Discov. Technol.* **2007**, *4*, 246–254; (d) Niederhafner, P.; Sebestík, J.; Jezek, J. *Glycopeptide dendrimers. Part I. J. Pept. Sci.* **2008**, *14*, 2–43; (e) Niederhafner, P.; Sebestík, J.; Jezek, J. *Glycopeptide dendrimers. Part II. J. Pept. Sci.* **2008**, *14*, 44–65; (f) Niederhafner, P.; Reinis, M. J.; Jezek, J. *Glycopeptide dendrimers, Part III J. Pept. Sci.* **2008**, *14*, 556–587.
5. (a) Oka, H.; Onaga, T.; Koyama, T.; Guo, C. T.; Suzuki, Y.; Esumi, Y.; Hatano, K.; Terunuma, D.; Matsuoka, K. *Bioorg. Med. Chem.* **2009**, *17*, 5465–5475; (b) Miyagawa, A.; Kurosawa, H.; Watanabe, T.; Koyama, T.; Terunuma, D.; Matsuoka, K. *Carbohydr. Polym.* **2004**, *57*, 441–450; (c) Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R. *Nature* **2000**, *403*, 669–672; (d) Nishida, Y.; Dohi, H.; Uzawa, H.; Kobayashi, K. *Tetrahedron Lett.* **1998**, *39*, 8681–8684; (e) Mori, T.; Hatano, K.; Matsuoka, Y. E.; Toone, E. J.; Terunuma, D. *Tetrahedron* **2005**, *61*, 2751–2760; (f) Matsuoka, Y. E.; Kurosawa, H.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Carbohydr. Res.* **2000**, *329*, 725–772.
6. (a) Yamada, A.; Hatano, K.; Koyama, T.; Matsuoka, K.; Takahashi, N.; Hidari, K. I. P. J.; Suzuki, T.; Suzuki, Y.; Terunuma, D. *Bioorg. Med. Chem.* **2007**, *15*, 1606–1614; (b) Yamada, A.; Hatano, K. K.; Koyama, T.; Matsuoka, K.; Esumi, Y.; Koshino, H.; Hino, K.; Nishikawa, K.; Natori, Y.; Terunuma, D. *Tetrahedron* **2006**, *62*, 5074–5083; (c) Nishikawa, K.; Matsuoka, K.; Kita, E.; Okabe, N.; Mizuguchi, M.; Hino, K.; Miyazawa, S.; Yamasaki, C.; Aoki, J.; Takashima, S.; Yamakawa, Y.; Nishijima, M.; Terunuma, D.; Kuzuhara, H.; Natori, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 7669–7674; (d) Watanabe, M.; Matsuoka, K.; Kita, E.; Igai, K.; Higashi, N.; Miyagawa, A.; Watanabe, T.; Yanoshita, R.; Samejima, Y.; Terunuma, D.; Natori, Y.; Nishikawa, K. *J. Infect. Dis.* **2004**, *189*, 360–368; (e) Mulvey, G. L.; Marcato, P.; Kitov, P. I.; Sadowska, J.; Bundle, D. R.; Armstrong, G. D. *J. Infect. Dis.* **2003**, *187*, 640–649; (f) Fukudaa, T.; Onogia, S.; Miura, Y. *Thin Solid Films* **2009**, *518*, 880–888.
7. (a) Tabarani, G.; Reina, J. J.; Ebel, C.; Vives, C.; Lortat-Jacb, H.; Rojo, J.; Fieschi, F. *FEBS Lett.* **2006**, *580*, 2402–2408; (b) Baigude, H.; katsuraya, K.; Okuyama, K.; Uryu, T. *Macromol. Chem. Phys.* **2004**, *205*, 684–691; (c) Geijtenbeeka, T. B. H.; van Vlieta, S. J.; van Duijnhovena, G. C. F.; Figdora, C. G.; van Kooyk, Y. *Placenta* **2001**, *22*, S19–S23.
8. (a) Chabre, Y. M.; Contino-Pépin, C.; Placide, V.; Shiao, T. C.; Roy, R. *J Org Chem.* **2008**, *73*, 5602–5605; (b) Greatrex, B. W.; Brodie, S. J.; Furneaux, R. H.; Hook, S. M.; McBurney, W. T.; Painter, G. F.; Rades, T.; Rendle, P. M. *Tetrahedron* **2009**, *65*, 2939–2950; (c) Hayes, W.; Osborn, H. M. I.; Osborne, S. D.; Rastall, R. A.; Romagnoli, B. *Tetrahedron* **2003**, *59*, 7983–7996; (d) Page, D.; Roy, R. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1765–1770; (e) Touaibia, M.; Roy, R. *J. Org. Chem.* **2008**, *73*, 9292–9302; (f) Woller, E. K.; Walter, E. D.; Morgan, J. R.; Singel, D. J.; Cloninger, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 8820–8826; (g) Fernandez-Megia, E.; Correa, J.; Riguera, R. *Biomacromol.* **2006**, *7*, 3104–3111.
9. Boysen, M. M. K.; Lindhorst, T. K. *Tetrahedron* **2003**, *59*, 3895–3898.
10. (a) Ortega, P.; Bermejo, J. F.; Chonco, L.; de Jesús, E.; de la Mata, F. J.; Fernández, G.; Flores, J. C.; Gómez, R.; Serramía, M. J.; Muñoz-Fernández, M. A. *Eur. J. Inorg. Chem.* **2006**, 1388; (b) Bermejo, J. F.; Ortega, P.; Chonco, L.; Eritja, R.; Samaniego, R.; Müllner, M.; Fernández, G.; Flores, J. C.; Gómez, R.; Serramía, M. J.; Muñoz-Fernández, M. A. *Chem.—Eur. J.* **2007**, *13*, 483–495; (c) Weber, N.; Ortega, P.; Clemente, M. I.; Shcharbin, D.; Bryszewska, M.; de la Mata, F. J.; Gómez, R.; Muñoz-Fernández, M. A. *J. Controlled Release* **2008**, *132*, 55–64.
11. van der Made, A. W.; Leeuwen, P. W. N. M. *J. Chem. Soc., Chem. Commun.* **1992**, 1400–1401.
12. (a) Matsuoka, K.; Terabatake, M.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 7839–7842; (b) Matsuoka, K.; Kurosawa, H.; Esumi, Y.; Terunuma, D.; Kuzuhara, H. *Carbohydr. Res.* **2000**, *329*, 765–772; (c) Matsuoka, K.; Oka, H.; Koyma, T.; Esumi, Y.; Terunuma, D. *Tetrahedron Lett.* **2001**, *42*, 3327–3330.
13. Omotowa, B. A.; Keefer, K. D.; Kirchmeier, R. L.; Shreeve, J. M. *J. Am. Soc.* **1999**, *121*, 11130–11138.
14. (a) Seyferth, D.; Son, D. Y.; Rheingold, A. L.; Ostrander, R. L. *Organometallics* **1994**, *13*, 2682–2690; (b) Cuadrado, I.; MorGn, M.; Losada, J.; Casado, C. M.; Pascual, C.; Alonso, B.; Lobete, F. In *Advances in Dendritic Macromolecules*; Newkome, G. R., Ed.; JAI: Greenwich, CT, 1996; Vol. 3, pp 151–195.
15. Takano, T.; Nakatsubo, F.; Murakami, K. *Carbohydr. Res.* **1990**, *203*, 341–342.
16. Karstedt, B.D.U.S. Patent 3,775,452, 1973.
17. Rinaldo, C. R. *J. Intern. Med.* **2009**, *265*, 138–158.