# "Clickable" PEG−Dendritic Block Copolymers

Eduardo Fernandez-Megia,† Juan Correa, and Ricardo Riguera*

*Departamento de Química Orgánica, Facultad de Química, and Unidad de RMN de Biomoléculas Asociada al CSIC, Universidad de Santiago de Compostela, Avenida de las Ciencias S.N. 15782 Santiago de Compostela, Spain*

*Received June 16, 2006; Revised Manuscript Received July 14, 2006*

Three generations of azido-terminated PEG−dendritic block copolymers have been synthesized and completely characterized by NMR and MALDI-TOF. A radial decrease of density, leading to more mobile protons at the outermost periphery, and an increasingly higher compactness of the core with generation have been determined by $T_1$ and $T_2$ relaxation time studies. The efficient surface decoration of these dendritic polymers by means of click chemistry has been demonstrated by the incorporation of unprotected carbohydrate units in very good to excellent yields. The reaction proceeds at room temperature, under aqueous conditions, and requires just catalytic amounts of Cu. The modified block copolymers are conveniently purified by ultrafiltration. The glycodendrimers functionalized with α-mannose form aggregates with concanavalin A as determined by absorbance experiments at 400 nm. This aggregation ability increases with generation.

## Introduction

Since the early reports on dendrimers, these highly branched and monodisperse type of polymers have attracted a great deal of attention on different fields related to nanotechnology.[1] Especially interesting have been their applications to drug delivery[2] and biomedicine.[3] At the same time, poly(ethylene glycol) (PEG) has consolidated itself as the hydrophilic polymer of choice for many of these applications, due to its ability to confer lower toxicity and immunogenicity, as well as a better biodistribution, to any molecule, polymer, or surface to which it is covalently bonded.[4]

With the aim of conjugating the favorable properties of both polymeric systems, several PEG−dendritic block copolymers have been recently described and found various applications.[5−7] Unfortunately, when functionalization of these block copolymers at the dendrimer periphery was desired, additional deprotection/activation steps were mandatory, very much limiting their usefulness and scope.

We have recently reported on the decoration of dendrimers by means of click chemistry.[8] More specifically, the Cu(I)-catalyzed azide−alkyne [3 + 2] cycloaddition[9,10] proved to be an ideal reaction for the complete surface functionalization of azide-terminated dendrimers in reproducible high yields. The reaction proceeds at room temperature, under aqueous conditions, and requires just catalytic amounts of Cu.[11]

Herein we describe that application of this strategy to PEG−dendritic block copolymers allows the ready and efficient decoration of the dendritic block when functionalized with terminal azides, avoiding lengthening deprotection/activation steps. In this paper we report the synthesis of three generations of a new family of block copolymers PEG-[Gn]-$N_3$ (Scheme 1), as well as the procedure and conditions for their complete surface functionalization with up to 27 unprotected alkyne-derived carbohydrate units in very good to excellent yields. An NMR relaxation study of these block copolymers revealed a radial decrease of density from the core to the periphery, more intense on increasing the generation of the dendritic block.

## Results and Discussion

To build a PEG−dendritic block copolymer with terminal azides, a dendritic core comprised of gallic acid and triethylene glycolazide (**1**) was chosen based on its resemblance to PEG and its good solubility in both organic and aqueous solvents (Scheme 1).[8,12] Three generations of hydrolytically stable PEG−dendritic block copolymers were synthesized divergently from monomer **1** and a narrow polydispersity monomethyl ether PEG amino (PEG−$NH_2$, $M_n$ = 5610). Due to the properties of PEG as a soluble polymeric support,[13] the resulting block copolymers were purified by precipitation, rendering solvent extractions and tedious chromatographic purifications unnecessary.

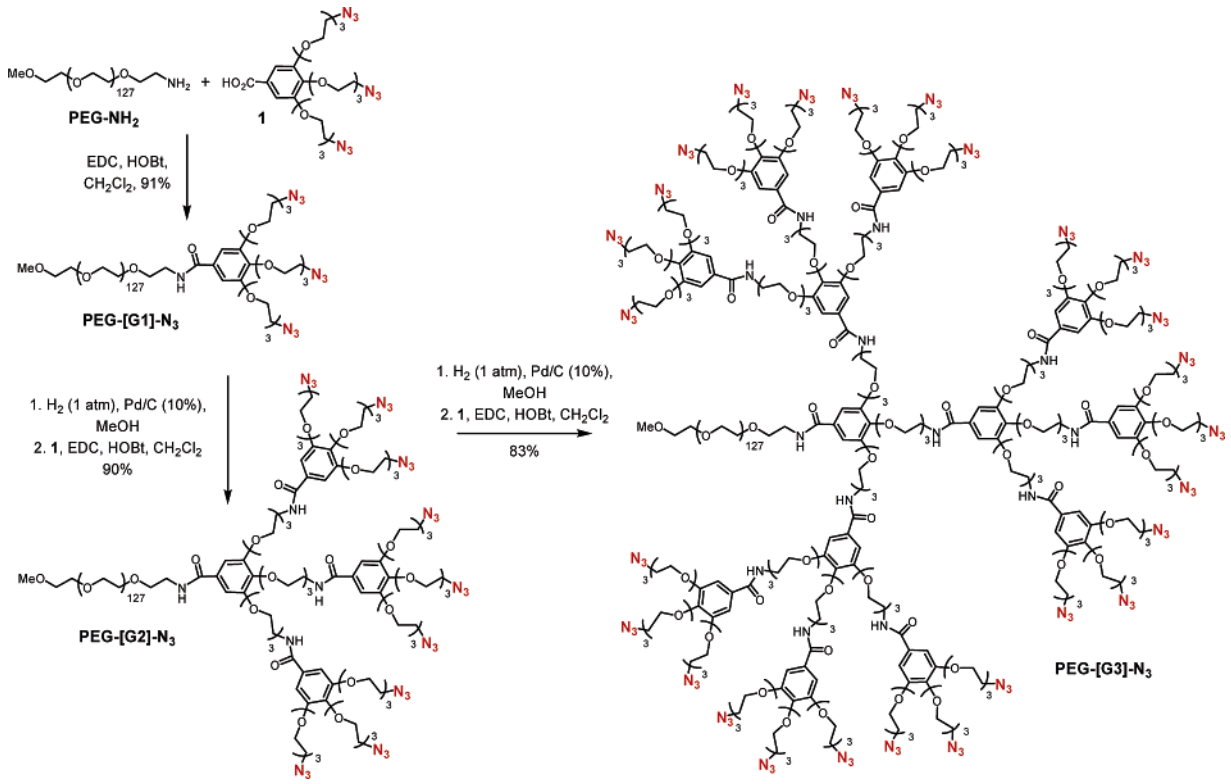
Thus, block copolymer of generation one (PEG-[G1]-$N_3$) was easily obtained in an excellent 91% yield (Scheme 1) from PEG−$NH_2$ and 3 equiv of monomer **1** (EDC, HOBt, DCM). After the reaction was completed, the solvent was evaporated, and excess reagents and urea were removed by straightforward precipitation with MeOH/$^i$PrOH, leading to pure PEG-[G1]-$N_3$. Catalytic hydrogenation of the terminal azides of PEG-[G1]-$N_3$ [$H_2$ (1 atm), Pd/C (10%), MeOH], followed by treatment of the resulting triamine with monomer **1** (6 equiv) as above (EDC, HOBt, DCM), led to generation two PEG-[G2]-$N_3$ in an excellent 90% yield. Again, the pure compound was isolated by precipitation of the reaction mixture with MeOH/$^i$PrOH. Application of the same hydrogenation−amide formation−precipitation sequence to PEG-[G2]-$N_3$ (18 equiv of monomer **1**) led to the third generation, PEG-[G3]-$N_3$, in a very good 83% yield (Scheme 1).

These block copolymers were completely characterized by $^1$H and $^{13}$C NMR. The dendritic generation growth was easily monitored by NMR spectroscopy ($CDCl_3$), following the disappearance of the signals of the methylene protons adjacent to the azide groups (3.35−3.40 ppm in $^1$H NMR, 50.4 ppm in $^{13}$C NMR) and the advent of those adjacent to the amino groups (2.84−2.91 ppm in $^1$H NMR, 40.0 ppm in $^{13}$C NMR).

* Corresponding author. E-mail: ricardo@usc.es.
† Dr. Eduardo Fernandez-Megia is a Ramón y Cajal researcher.

10.1021/bm060580d CCC: $33.50 
Published on Web 10/11/2006

**Scheme 1**




Especially interesting was the effect of the growing generation on the shift and shape of the aromatic signals (Figure 1). Thus, while a sharp singlet at 7.08 ppm appeared in the $^1$H NMR spectrum of PEG-[G1]-$N_3$, broad signals centered at 7.10 ppm were found in the spectra of PEG-[G2]-$N_3$ and PEG-[G3]-$N_3$. This trend is similar to that already observed in the non-PEGylated series of these dendrimers (Figure 2) and obeys the inverse proportionality between the line width and the spin−spin relaxation time ($T_2$).[8] This suggests that at the core of the dendrimers of higher generations $T_2$ is reduced. In fact, an analysis of the variation of $T_2$ for the aromatic signals in [Gn]-$N_3$ and PEG-[Gn]-$N_3$ revealed a sharp decrease on growing from G1 to G3 for both PEGylated and non-PEGylated dendrimers, with the PEGylated copolymers always showing the lower $T_2$ values (Figure 2).

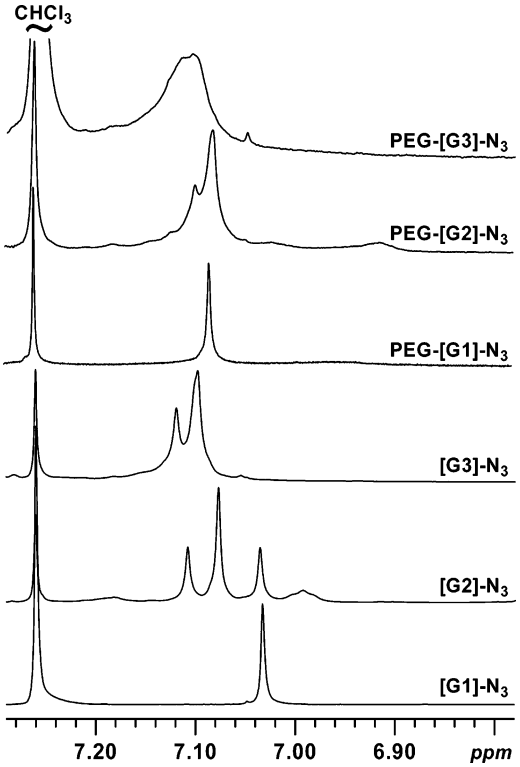



**Figure 1.** Partial $^1$H NMR spectra of [Gn]-$N_3$ and PEG-[Gn]-$N_3$ (500 MHz, $CDCl_3$).

From a structural point of view, this variation of $T_2$ reflects the different mobility and relative density distribution within the dendrimers, and this could be better defined if a more complete study, covering several signals, and not only $T_2$, but also spin−lattice relaxation times ($T_1$), were carried out (Figure 2).[14]

Thus, a complete relaxation time study of several signals of [Gn]-$N_3$ and PEG-[Gn]-$N_3$ was performed. The $T_2$ analysis revealed the following facts: (a) $T_2$ values for the internal and core protons (a, b, c, and d) are lower than for the external protons (e) in both types of dendrimers, independently of the generation, suggesting the presence of congested protons at the interior and core, surrounded by external more flexible protons (e). (b) $T_2$ values of all the protons show a continuous decrease on increasing the generation from G1 to G3, but the degree of reduction is always less pronounced for the external protons (e) than for those at the interior and core.

Overall, these data suggest that as generation increases, a more dense packing appears and this effect is more severe at the interior and core than at the surface. Also, there is a radial decrease in density on going from the core to the periphery that fits the model of Lescanec and Muthukumar,[15] as opposed to that formulated by Gennes and Hervert.[16]

When the $T_2$ values obtained for the PEG-[Gn]-$N_3$ block copolymers are compared to those displayed by the same generation of the non-PEGylated series, a reduction of about 30% in the $T_2$ values of all the resonances is observed. This homogeneous decrease of $T_2$ due to the presence of PEG reflects

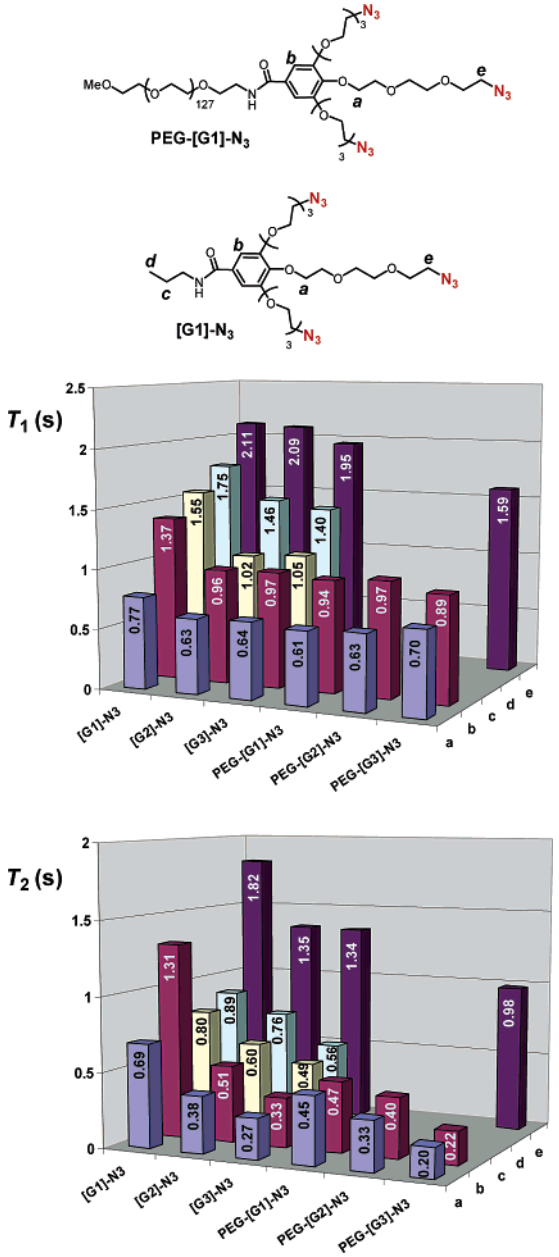



**Figure 2.** $^1$H NMR relaxation times $T_1$ and $T_2$ of [Gn]-$N_3$ and PEG-[Gn]-$N_3$ (500 MHz, $CDCl_3$).

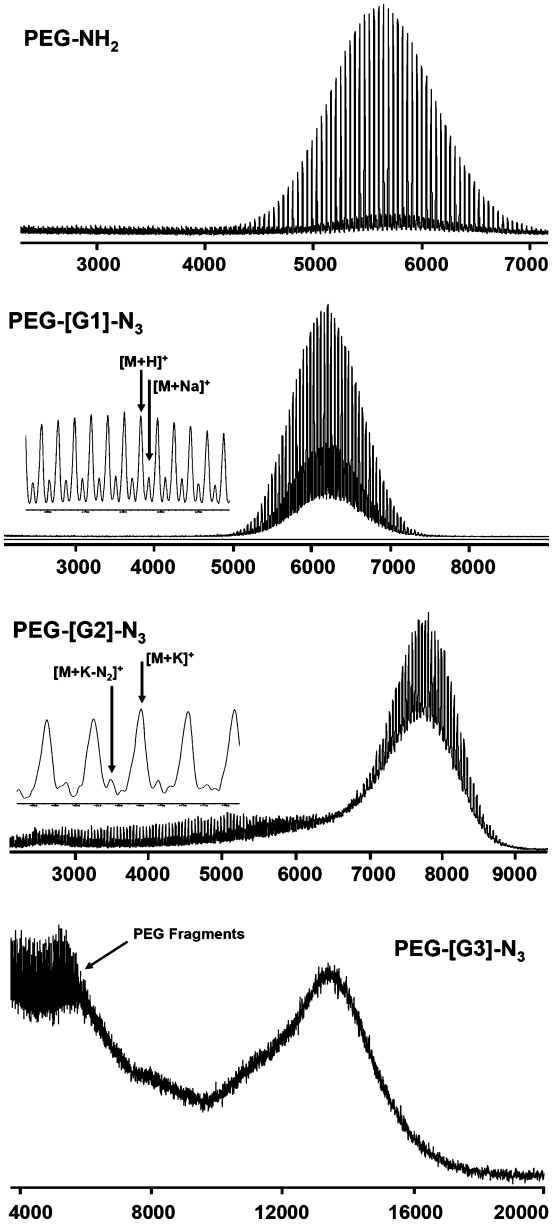



**Figure 3.** MALDI-TOF MS of PEG−$NH_2$, PEG-[G1]-$N_3$, PEG-[G2]-$N_3$, and PEG-[G3]-$N_3$.

a reduction in the overall motion of the block copolymers by the presence of the heavy PEG chain, apparently equally affecting all the protons in the block copolymer.

The study of $T_1$ led to similar conclusions to those just obtained from the analysis of $T_2$. Thus, within each generation, the highest $T_1$ values were observed again for the external protons (e) in both dendrimers and PEG-containing block copolymers. Also, the lowest molecular weight dendrimer [G1]-$N_3$ displayed the highest $T_1$ values for all types of protons. However, in contrast to the behavior of $T_2$, $T_1$ values remain nearly unchanged with generation, and even a small increase is observed in some cases on going from G2 to G3.

This apparently surprising behavior of $T_1$ could support a situation of motional freedom being independent of generation. Nevertheless, a careful examination of the curve describing the variation of $T_1$ as a function of the correlation time ($\tau_c$) reveals the full coherence of the $T_1$ behavior.[17] Thus, protons in [G1]-$N_3$, and external e protons in all the dendrimers and block copolymers are mobile and in the plot of $T_1$ versus $\tau_c$ are located in the left side of the $T_1$ minimum (fast motion regime). On the contrary, core and internal protons (a, b, c, and d) in [G2]-$N_3$, [G3]-$N_3$, and in all the block copolymers, are less mobile, present longer correlation times, and in the curve lie close to the $T_1$ minimum or at its right side (slow motion regime).

An important practical observation that can be derived from these results is that $T_1$ relaxation data alone should not be used for molecular motion studies, because it can induce misinterpretations about the relative density and motional freedom, due to the particular shape of its variation with $\tau_c$. Thus, to get reliable molecular motion data, $T_2$ relaxation times must be better considered.

Block copolymers were also characterized by MALDI-TOF spectrometry (Figure 3). The spectrum of PEG-[G1]-$N_3$ revealed a series of 44 Da spaced peaks corresponding to the protonated oligomers in the sample, accompanied by lower intensity peaks from the sodium adducts. No signals resulting from unreacted PEG−$NH_2$ or side products were seen, confirming the purity of the product. Also, experimental $M_n$ and $M_p$ were in agreement with calculated values (Table 1). Similarly, the spectrum of

**Table 1.** Molecular Weight of PEG-[Gn]-$N_3$ and Clicked Conjugates Determined by MALDI-TOF MS

| copolymer | $M_n$[a] | | $M_w$ | $M_p$[a] | | PDI |
|---|---|---|---|---|---|---|
| PEG−$NH_2$ | 5610 | | 5655 | 5649.0[b] | | 1.008 |
| PEG-[G1]-$N_3$ | 6197 | [6234] | 6219 | 6203.1 | [6250.4][c] | 1.004 |
| PEG-[G2]-$N_3$ | 7702 | [8027] | 7720 | 7812.4 | [8081.3][d] | 1.002 |
| PEG-[G3]-$N_3$ | 1322 2 | [13405] | 1364 5 | 13411 | [13422][c] | 1.032 |
| PEG-[G1]-Man | 6867 | [6888] | 6881 | 6900.1 | [6905.0][c] | 1.002 |
| PEG-[G2]-Man | 9887 | [9990] | 1006 0 | 9935 | [10007][c] | 1.017 |
| PEG-[G3]-Man | 1909 9 | [19296] | 2059 3 | 18459 | [19313][c] | 1.078 |

[a] Numbers in brackets are calculated molecular weights. [b] $[M + Na]^+$. [c] $[M + H]^+$. [d] $[M + K]^+$.

PEG-[G2]-$N_3$ showed pure G2 block copolymer with well-resolved potassium adducts as mayor peaks. Some low-intensity signals derived from decomposition of azide groups by loss of $N_2$ were present.[18]

As expected, on increasing the generation of the dendritic block, copolymers flew with more difficulty, and higher laser power had to be applied in the MALDI experiment.[6,19] Consequently, MALDI-TOF of PEG-[G3]-$N_3$ resulted in a broad peak with no individual oligomer resolution, accompanied by a series of 44 Da spaced peaks in the low *m*/*z* region, attributed to PEG oligomers produced by fragmentation at the PEG−dendrimer amide bond during the MALDI experiment.

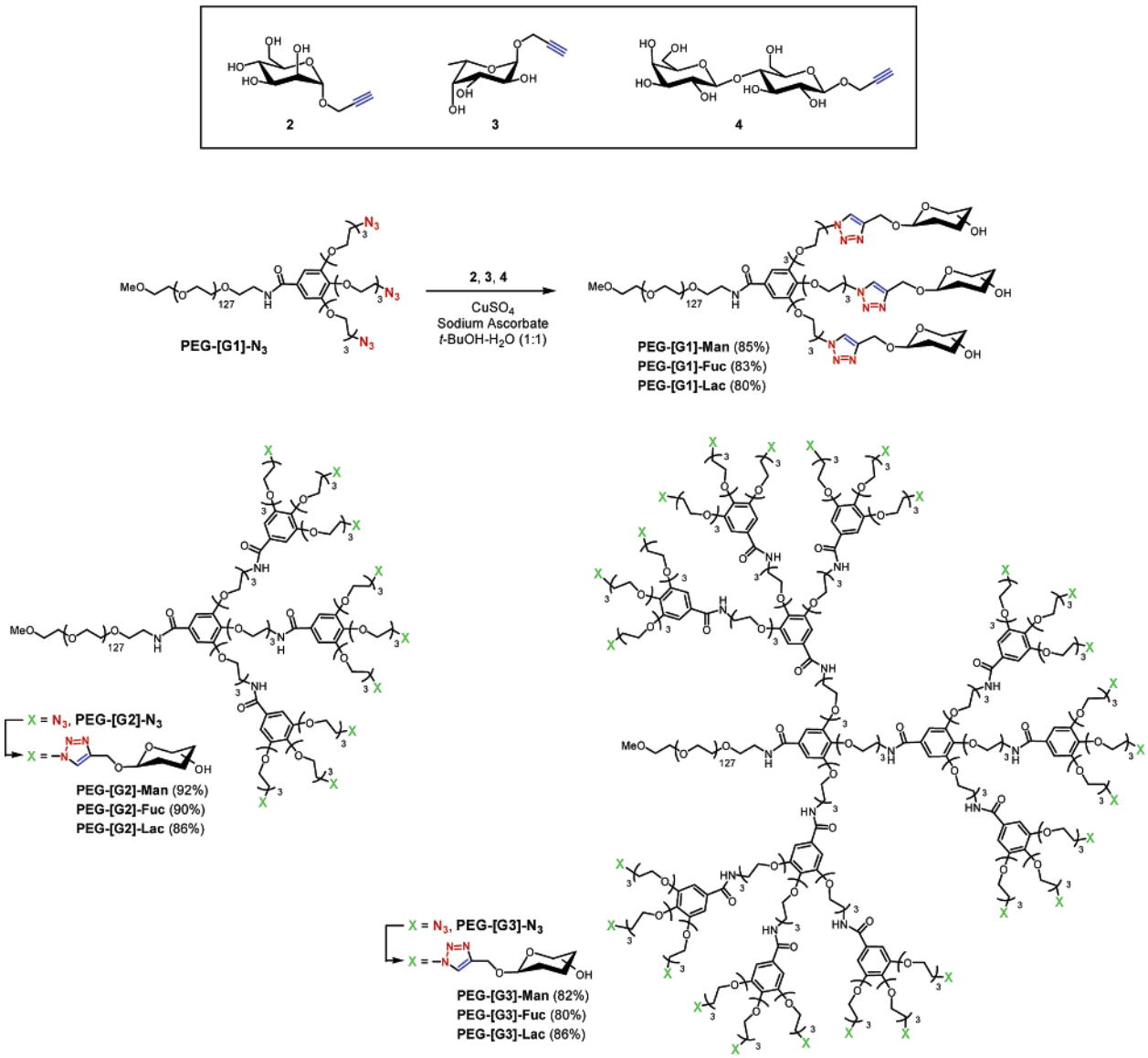
Demonstration of the usefulness and potential of these azide-containing block copolymers for their surface functionalization by means of click chemistry was carried out by reaction with three alkyne-functionalized unprotected carbohydrates derived from α-D-mannose (**2**), α-L-fucose (**3**), and β-D-lactose (**4**) (Scheme 2) that were selected based on the significance of the multivalent carbohydrate−receptor interaction in nature.[20,21]

Thus, following our previously described conditions,[8] the Cu(I)-catalyzed azide−alkyne [3 + 2] cycloaddition between the PEG−dendritic block copolymers and the unprotected carbohydrates **2**, **3**, and **4** was performed. Typical reaction times ranged from 24 h (G1) to 72 h (G3) at room temperature under aqueous conditions (*t*-BuOH/$H_2O$, 1:1), in the presence of $CuSO_4$ (1 mol % per terminal azide) as the source of copper and sodium ascorbate (5 mol % per terminal azide) as the reducing agent.

The resulting glycosylated block copolymers, incorporating 3, 9, and 27 mannoses, fucoses, and lactoses were purified by ultrafiltration and were obtained in excellent yields (80−92%), (Scheme 2).

The completion of the glycoconjugation was monitored by $^1H$ NMR ($D_2O$) thanks to the disappearance of the signal for the methylene protons adjacent to the azide groups. Also, the

**Scheme 2**



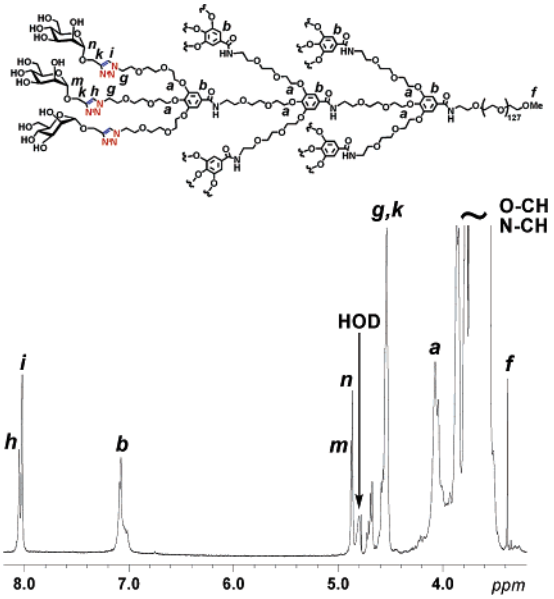



**Figure 4.** $^1$H NMR spectra of PEG-[G3]-Man (750 MHz, $D_2O$).

incorporation of the carbohydrate units, and the success of the click reaction were confirmed by the appearance in the $^1$H NMR spectra of two singlets (ratio 1:2) at around 8.05 ppm, corresponding to the triazol protons (h, i) and of two singlets (ratio 1:2) at around 4.90 ppm due to the anomeric protons (m, n) (Figure 4). These sets of signals in a 1:2 ratio have been ascribed to the protons of the triazol and carbohydrate units linked to the para and the meta aromatic positions, respectively.

Another interesting characteristic of the NMR spectra of the glycosylated block copolymers concerns the signals of the aromatic protons (b). While PEG-[G1]-Man gives only a sharp singlet at 7.18 ppm, three broad peaks at 7.10, 7.09, and 7.05 ppm are seen in the spectrum of PEG-[G2]-Man, and a broad multiplet (7.14−6.98 ppm) is found for PEG-[G3]-Man. This line width dependence on generation, similar to the one described above for PEG-[Gn]-$N_3$, reveals again a radial decrease of density within the dendrimer block and an increasingly higher compactness of the core with generation.

The glycodendritic copolymers derived from mannose were also characterized by MALDI-TOF MS, allowing us to ascertain their purity and the completion of the Cu (I)-catalyzed conjugation process (Figure 5). In all cases, $M_p$ and $M_n$ matched well the expected values, and no signals from unreacted PEG-[Gn]-$N_3$ or partially glycosylated products were seen. In the case of G2 and G3, a series of 44 Da spaced peaks in the *m*/*z* range of the starting PEG−$NH_2$ appeared again, as a result of a MALDI fragmentation due to the high laser power required on increasing generation.

PEGylated glycoconjugates derived from fucose and lactose were less prone to fly in MALDI experiments than those derived from mannose. Although experimental $M_p$ molecular weights were coincident with expected values in PEG-[G1]-Fuc and PEG-[G1]-Lac, much broader peaks, lower oligomer resolution, and more extensive fragmentation than in the mannose counterparts were observed.[22]

Clearly, the combination of PEG and the gallic acid−triethylene glycol-based dendrimers, along with the hydrophilicity of the terminal carbohydrates, hampers the ability of these entities to fly when compared with homopolymers of comparable molecular weight. The differences observed between the mannose and the fucose and lactose counterparts indicate that minute modifications at the periphery of these block copolymers

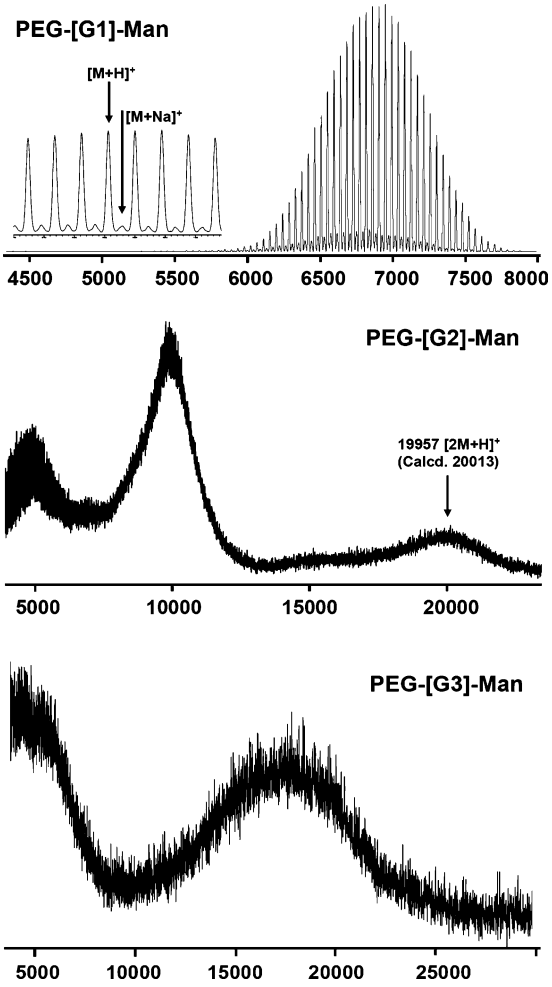



**Figure 5.** MALDI-TOF MS of PEG-[G1]-Man, PEG-[G2]-Man, and PEG-[G3]-Man.

critically determine their ability to fly in MALDI-TOF and render the characterization of these glycosylated block copolymers by MALDI-TOF MS still a challenge at the present time.

To demonstrate the existence of effective interaction between these multivalent glycoconjugates and their natural receptors, solutions of the PEGylated and non-PEGylated glycodendrimers functionalized with mannose were incubated with increasing concentrations of concanavalin A (ConA), and the absorbance at 400 nm measured (Figure 6). ConA is a lectin with a high affinity for α-mannose,[23] that exists as a tetramer at the pH of the experiment. If large aggregates between ConA and the mannosylated dendrimers [Gn]-Man and PEG-[Gn]-Man were produced, turbidity, and even precipitation, should be observed.[20]

Thus, while [G1]-Man and PEG-[G1]-Man did not show sings of aggregation (not even after the addition of 6 mL of the ConA stock solution), [G2]-Man and PEG-[G2]-Man showed a steady increase of absorbance after the addition of 600 $\mu$L of the ConA solution. At this point, the concentration of mannose ligand and ConA were, respectively, 1.19 mM and 9.81 $\mu$M. Higher amounts of ConA markedly increased the turbidity that reaches its maximum after the addition of 1.5 mL of ConA. In accordance with the reversible nature of the interaction, the subsequent addition of 150 $\mu$L of a saturated solution of α-methyl-D-mannopyranoside completely removed turbidity and precipitation. With G3, the absorbance started at lower ConA concentrations compared to that of G2, revealing the higher capacity of the larger dendrimer generations to aggregate with the lectin.

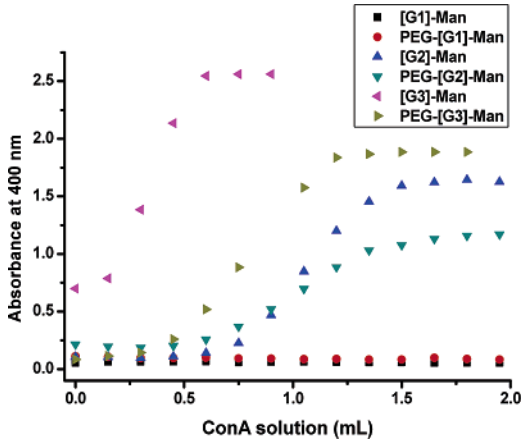



**Figure 6.** Aggregation of [Gn]-Man and PEG-[Gn]-Man (1.5 mL, 1.67 mM of terminal mannose) with ConA (0.9 mg/mL) in 20 mM Tris−HCl, 250 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, determined at 400 nm.

## Conclusions

Three generations of azido-terminated PEG−dendritic block copolymers have been synthesized and completely characterized by NMR and MALDI-TOF. A radial decrease of density within the dendrimer, leading to more mobile protons at the outermost periphery, and an increasingly higher compactness of the core with generation have been determined by $T_1$ and $T_2$ relaxation times. The efficient surface decoration of these dendritic polymers by means of click chemistry has been demonstrated by the incorporation of up to 27 unprotected carbohydrate units in excellent yields. The reaction proceeds at room temperature, under aqueous conditions, and requires only catalytic amounts of Cu. The resulting PEGylated glycodendrimers have demonstrated an increased capacity to aggregate lectins with generation. They represent a novel glycoconjugate architecture benefiting from the advantageous properties of PEG, that are envisioned as potential tools for the study of carbohydrate−receptor interactions and as attractive building blocks for the construction of active targeted drug delivery systems. Further quantitative analyses of the interaction of these glycoconjugates with lectins are being performed and will be published in due time.

## Experimental Section

**Materials.** Methylene chloride was distilled from $CaH_2$. Ultrafiltration was performed on stirred cells with Amicon YM1 membranes. NMR spectra were recorded on 500 and 750 MHz spectrometers in $D_2O$ and $CDCl_3$. Chemical shifts are reported in ppm ($\delta$ units) downfield from internal tetramethylsilane ($CDCl_3$) or the HOD signal ($D_2O$). Alkyne-functionalized unprotected carbohydrates **2**, **3**, and **4** were prepared following reported procedures.[8] ConA was purchased from Sigma. Ultrafiltration was performed on Amicon stirred cells with Amicon YM1 membranes. NMR spectra were recorded at Varian Inova 400 MHz, Bruker DPX 250 MHz, and Varian Inova 750 MHz spectrometers in $D_2O$ or $CDCl_3$. Chemical shifts are reported in ppm ($\delta$ units) downfield from internal tetramethylsilane ($CDCl_3$) or the HOD signal ($D_2O$). (+)-Sodium l-ascorbate (≥98%) was purchased from Sigma, $CuSO_4 \cdot 5H_2O$ (98.5%) was obtained from Prolabo, and 10% Pd on charcoal was from Fluka. PEG−$NH_2$ ($M_n$ = 5610) was purchased from Nektar.

**Methods.** *Relaxation Time Measurements.* $T_1$ and $T_2$ values were measured using standard inversion recovery and Carr−Purcell−Meiboom−Gill pulse sequences, respectively, on a 500 MHz spectrometer at 25 °C. Freshly distilled $CDCl_3$ was used as solvent. Samples were prepared under an Ar atmosphere, in 5 mm Young's tap NMR tubes. The concentration was set at 1.19 mM. The resulting solutions were degassed by several freeze−pump−thaw cycles.

The $T_1$ and $T_2$ relaxation times of protons a (and b) in [G3]-$N_3$, PEG-[G2]-$N_3$, and PEG-[G3]-$N_3$ (Figure 2) are averaged values due to the impossibility of distinguishing between resonances for the different layers of a (and b) protons. The same applies for the a protons in [G2]-$N_3$.

The relaxation times shown in Figure 2 for the protons b in [G2]-$N_3$ are the averaged values of three aromatic signals at 7.04, 7.09, and 7.12 ppm and are reported with the only purpose of comparison with those of other dendrimers. The individual $T_1$ and $T_2$ values for these three signals are as follows ($\delta$, $T_1$, $T_2$): 7.04 ppm, 0.93 s, 0.50 s; 7.09 ppm, 0.97 s, 0.55 s; and 7.12 ppm, 0.94 s, 0.36 s. The signal at 7.12 ppm corresponds to the most internal aromatic proton due to its low $T_2$ value.

Relaxation times for the external e protons in PEG-[G1]-$N_3$ and PEG-[G2]-$N_3$ are not reported due to overlap with the methoxy signal of PEG. In the case of PEG-[G3]-$N_3$, the ratio between the e and methoxy protons (54:3) minimizes the effect of the overlap over the relaxation times, and therefore, these values were included in Figure 2.

The resonance of the external e protons in the three generations of the [Gn]-$N_3$ series overlaps with that of the methylene protons at the C1 position in the *N*-propyl group. The large difference of relaxation times between both types of protons, and the higher proportion of e protons in the overlapped signal, led us to conclude that no significant variation in the relaxation times of the e protons should arise from considering them as those resulting from the overlapped signal.

*Interaction with ConA.* A solution of ConA (0.9 mg/mL in 20 mM Tris−HCl, 250 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, pH = 5.86) was added in 150 $\mu$L portions to a solution of glycodendrimer [Gn]-Man or PEG-[Gn]-Man (1.5 mL, 1.67 mM of terminal mannose in 20 mM Tris−HCl, 250 mM NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, pH = 6.17). After each addition, the resulting mixture was allowed to stand for 5 min before the absorbance being measured at 400 nm and the next ConA addition was realized.

*MALDI-TOF MS.* MALDI-TOF MS experiments were carried out operating in linear mode for masses higher than 8500 Da and in reflected mode for lower masses.

When 2-(4-hydroxyphenylazo)benzoic acid (HABA) was used as the matrix, NaCl was employed as the cationizing agent. Samples were dissolved in MeOH/$H_2O$ (1:1) at a concentration of $5 \times 10^{-4}$ M. HABA was dissolved in dioxane at a concentration of 0.05 M. Sample (20 $\mu$L) and matrix (80 $\mu$L) solutions were mixed, and then 80 $\mu$L of 0.02 M NaCl was added. Finally, 1 $\mu$L of the resulting mixture was placed on the MALDI plate.

In the case of PEG-[G3]-$N_3$, the sample was dissolved in MeOH (1 mg/mL) and the matrix (dithranol) in $CH_2Cl_2$. When 2,5-dihydroxybenzoic acid (DHB) was used as the matrix, samples were dissolved in $H_2O$ or mixtures $H_2O$/MeOH (1 mg/mL) and DHB in mixtures MeOH/$H_2O$.

**PEG-[G1]-$N_3$.** HOBt·*n*$H_2O$ (15 mg, 0.105 mmol) and EDC·HCl (20 mg, 0.105 mmol) were added to a solution of PEG−$NH_2$ (198 mg, 0.035 mmol) and acid **1**[8,12] (67 mg, 0.105 mmol) in $CH_2Cl_2$ (3.5 mL). The resulting solution was stirred at room temperature for 20 h under Ar, and then it was concentrated and precipitated from MeOH/$^i$PrOH to give pure PEG-[G1]-$N_3$ as a white powder (200 mg, 91%). IR (KBr): 2945, 2888, 2107, 1655, 1113 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, $Me_4Si$) $\delta$: 7.08 (s, 2H), 6.81 (br s, 1H), 4.24−4.19 (m, 6H), 3.88−3.46 (m, 534H), 3.40−3.37 (m, 9H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 166.8, 152.2, 141.0, 129.6, 104.6, 71.7, 70.3, 69.9, 69.6, 68.8, 58.8, 50.5, 39.8. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): $M_p$ 6203.1, $M_n$ 6197. Calcd: $M_p$ 6250.4 ([M + H]$^+$), $M_n$ 6234.

**PEG-[G2]-$N_3$.** Pd/C (10 mg, 10%) was added to a solution of PEG-[G1]-$N_3$ (50 mg, 8 $\mu$mol) in MeOH (0.8 mL). The resulting mixture was stirred under $H_2$ (1 atm) for 24 h. Then, the catalyst was removed by filtration and the filtrate was concentrated. HOBt·*n*$H_2O$ (6 mg, 0.043

mmol) and EDC·HCl (8 mg, 0.043 mmol) were added to a solution of the above residue and acid **1** (27 mg, 0.043 mmol) in $CH_2Cl_2$ (0.7 mL). The resulting solution was stirred at room temperature for 48 h under Ar, and then it was concentrated and precipitated from MeOH/$^i$PrOH to give pure PEG-[G2]-$N_3$ as a white powder (50 mg, 90%). IR (KBr): 2926, 2887, 2106, 1654, 1114 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, $Me_4Si$) $\delta$: 7.12−7.10 (m, 8H), 4.20−4.17 (m, 24H), 3.89−3.45 (m, 612H), 3.38−3.37 (m, 21H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 166.9, 166.8, 152.4, 152.3, 152.0, 140.9, 129.6, 106.9, 106.7, 72.1, 70.5, 70.4, 70.0, 69.9, 69.8, 69.6, 69.5, 68.7, 58.8, 50.4, 39.7. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): $M_p$ 7812.4, $M_n$ 7702. Calcd: $M_p$ 8081.3 ($[M + K]^+$), $M_n$ 8027.

**PEG-[G3]-$N_3$.** Pd/C (6 mg, 10%) was added to a solution of PEG-[G2]-$N_3$ (30 mg, 3.7 $\mu$mol) in MeOH (0.8 mL). The resulting mixture was stirred under $H_2$ (1 atm) for 24 h. Then, the catalyst was removed by filtration and the filtrate was concentrated. HOBt·$n$$H_2O$ (8.9 mg, 0.067 mmol) and EDC·HCl (12 mg, 0.067 mmol) were added to a solution of the above residue and acid **1** (42 mg, 0.067 mmol) in $CH_2Cl_2$ (0.7 mL). The resulting solution was stirred at room temperature for 48 h under Ar, and then it was concentrated and precipitated from MeOH/$^i$PrOH to give pure PEG-[G3]-$N_3$ as a white powder (41 mg, 83%). IR (KBr): 2927, 2871, 2106, 1652, 1111 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$, $Me_4Si$) $\delta$: 7.07−7.06 (m, 26H), 4.16−4.11 (m, 78H), 3.86−3.41 (m, ∼958H), 3.35−3.32 (m, 57H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: 167.1, 167.0, 152.7, 152.5, 152.3, 141.1, 129.6, 107.1, 106.9, 72.3, 70.7, 70.6, 70.5, 69.9, 69.8, 69.7, 68.9, 59.9, 50.9, 50.8, 50.7, 50.6, 50.4, 39.8, 39.7. MALDI-TOF MS (dithranol, linear mode, *m*/*z*): $M_p$ 13411, $M_n$ 13222. Calcd: $M_p$ 13422 ($[M + H]^+$), $M_n$ 13405.

**General Procedure for the Multivalent Glycosylation of PEG-[Gn]-$N_3$.** PEG−dendritic block copolymers PEG-[Gn]-$N_3$ were dissolved in *t*-BuOH/$H_2O$ (1:1) to give a 0.1 M final concentration of terminal azides. Then, pyranosides **2**, **3**, or **4**[8] (200 mol % per terminal $N_3$) and aq solutions of $CuSO_4$ (1 mol % per $N_3$) and sodium ascorbate (5 mol % per $N_3$) were added. The resulting solution was stirred at room temperature for 24−72 h, and the product was isolated by ultrafiltration of the concentrated reaction mixture (Amicon YM1, acetone/$H_2O$ 1:1, 5 × 30 mL).

**PEG-[G1]-Man.** From PEG-[G1]-$N_3$ (50 mg, 8.06 $\mu$mol), **2** (11 mg, 0.048 mmol), 0.05 M sodium ascorbate (24 $\mu$L), and 0.05 M $CuSO_4$ (5 $\mu$L) dissolved in *t*-BuOH (0.135 mL)/$H_2O$ (0.102 mL), and after 48 h of reaction time according to the general glycosylation procedure, PEG-[G1]-Man (45 mg, 85%) was obtained as a white foam. $^1H$ NMR (750 MHz, $D_2O$) $\delta$: 8.07 (s, 1H), 8.05 (s, 2H), 7.17 (s, 2H), 4.87 (d, $J$ = 1.1 Hz, 1H), 4.86 (d, $J$ = 1.0 Hz, 2H), 4.73−4.55 (m, 12H), 4.21−4.20 (m, 4H), 4.14−4.13 (m, 2H), 3.93−3.55 (m, ∼548H), 3.39 (s, 3H). $^{13}C$ NMR (63 MHz, $D_2O$) $\delta$: 170.2, 153.0, 144.4, 130.6, 126.5, 122.2, 107.4, 100.3, 78.8, 73.9, 72.0, 71.5, 70.6, 69.7, 67.6, 61.8, 60.6, 51.0, 40.6. MALDI-TOF MS (HABA, reflected mode, *m*/*z*): $M_p$ 6900.1, $M_n$ 6867. Calcd: $M_p$ 6905.0 ($[M + H]^+$), $M_n$ 6888.

**PEG-[G2]-Man.** From PEG-[G2]-$N_3$ (56 mg, 6.96 $\mu$mol), **2** (27 mg, 0.125 mmol), 0.05 M sodium ascorbate (62 $\mu$L), and 0.05 M $CuSO_4$ (13 $\mu$L) dissolved in *t*-BuOH (0.350 mL)/$H_2O$ (0.270 mL), and after 60 h of reaction time according to the general glycosylation procedure, PEG-[G2]-Man (63 mg, 92%) was obtained as a white foam. $^1H$ NMR (750 MHz, $D_2O$) $\delta$: 8.08 (s, 3H), 8.05 (s, 6H), 7.12 (s, 2H), 7.11 (s, 4H), 7.07 (s, 2H), 4.90−4.88 (m, 9H), 4.75−4.56 (m, 42H), 4.27−3.42 (m, ∼686H), 3.40 (s, 3H). $^{13}C$ NMR (63 MHz, $D_2O$) $\delta$: 169.1, 152.2, 143.8, 140.1, 130.7, 129.6, 129.5, 125.9, 125.8, 106.7, 106.6, 99.8, 73.3, 72.6, 72.5, 72.4, 72.2, 72.1, 71.3, 70.3, 70.1, 70.0, 69.9, 69.8, 69.5, 69.4, 69.0, 68.7, 67.0, 62.8, 61.2, 59.9, 50.3, 40.1. MALDI-TOF MS (DHB, linear mode, *m*/*z*): $M_p$ 9935, $M_n$ 9887. Calcd: $M_p$ 10007 ($[M + H]^+$), $M_n$ 9990.

**PEG-[G3]-Man.** From PEG-[G3]-$N_3$ (21 mg, 1.57 $\mu$mol), **2** (18 mg, 0.085 mmol), 0.05 M sodium ascorbate (42 $\mu$L), and 0.05 M $CuSO_4$ (9 $\mu$L) dissolved in *t*-BuOH (0.212 mL)/$H_2O$ (0.161 mL), and after 72 h of reaction time according to the general glycosylation procedure, PEG-[G3]-Man (24 mg, 82%) was obtained as a white foam. $^1H$ NMR (750 MHz, $D_2O$) $\delta$: 8.05−8.02 (m, 27H), 7.09−701 (m, 26H), 4.88−4.86 (m, 27H), 4.73−4.54 (m, 132H), 4.07−3.44 (m, ∼1064H), 3.38 (s, 3H). $^{13}C$ NMR (63 MHz, $D_2O$) $\delta$: 170.1, 169.9, 169.1, 152.9, 144.5, 140.8, 130.3, 126.5, 107.3, 100.5, 74.0, 73.8, 73.2, 72.8, 71.6, 71.0, 70.7, 70.3, 70.2, 69.8, 69.5, 67.7, 63.6, 61.9, 60.7, 51.1, 40.8. MALDI-TOF MS (DHB, linear mode, *m*/*z*): $M_p$ 18459, $M_n$ 19099. Calcd: $M_p$ 19313 ($[M + H]^+$), $M_n$ 19296.

**PEG-[G1]-Fuc.** From PEG-[G1]-$N_3$ (26 mg, 4.17 $\mu$mol), **3** (6 mg, 0.025 mmol), 0.005 M sodium ascorbate (125 $\mu$L), and 0.005 M $CuSO_4$ (25 $\mu$L) dissolved in *t*-BuOH (0.70 mL)/$H_2O$ (0.55 mL), and after 48 h of reaction time according to the general glycosylation procedure, PEG-[G1]-Fuc (23 mg, 83%) was obtained as a white foam. $^1H$ NMR (750 MHz, $D_2O$) $\delta$: 8.04 (s, 1H), 8.03 (s, 2H), 7.17 (s, 2H), 4.92 (d, $J$ = 3.4 Hz, 1H), 4.91 (d, $J$ = 3.1 Hz, 2H), 4.68−4.56 (m, 12H), 4.23−4.18 (m, 4H), 4.15−4.11 (m, 2H), 3.96−3.55 (m, ∼548H), 3.37 (s, 3H), 1.08−1.03 (m, 9H). MALDI-TOF MS (DHB, linear mode, *m*/*z*): $M_p$ 6868.5. Calcd: $M_p$ 6879.0 ($[M + Na]^+$).

**PEG-[G2]-Fuc.** From PEG-[G2]-$N_3$ (30 mg, 3.7 $\mu$mol), **3** (14 mg, 0.066 mmol), 0.05 M sodium ascorbate (33 $\mu$L), and 0.05 M $CuSO_4$ (7 $\mu$L) dissolved in *t*-BuOH (0.195 mL)/$H_2O$ (0.153 mL), and after 60 h of reaction time according to the general glycosylation procedure, PEG-[G2]-Fuc (33 mg, 90%) was obtained as a white foam. $^1H$ NMR (750 MHz, $D_2O$) $\delta$: 8.03 (s, 3H), 8.01 (s, 6H), 7.09 (s, 2H), 7.08 (s, 4H), 7.03 (s, 2H), 4.92−4.90 (m, 9H), 4.68−4.51 (m, 42H), 4.15−3.52 (m, 668H), 3.37 (s, 3H), 1.08−1.01 (m, 27H).

**PEG-[G3]-Fuc.** From PEG-[G3]-$N_3$ (16 mg, 1.19 $\mu$mol), **3** (13 mg, 0.064 mmol), 0.05 M sodium ascorbate (32 $\mu$L), and 0.05 M $CuSO_4$ (6 $\mu$L) dissolved in *t*-BuOH (0.200 mL)/$H_2O$ (0.160 mL), and after 72 h of reaction time according to the general glycosylation procedure, PEG-[G3]-Fuc (18 mg, 80%) was obtained as a white foam. $^1H$ NMR (750 MHz, $D_2O$) $\delta$: 8.05−8.00 (m, 27H), 7.14−6.98 (m, 26H), 4.94−4.89 (m, 27H), 4.70−4.52 (m, 96H), 4.24−3.46 (m, ∼1010H), 3.28 (s, 3H), 1.11−1.03 (m, 81H).

**PEG-[G1]-Lac.** From PEG-[G1]-$N_3$ (26 mg, 4.17 $\mu$mol), **4** (10 mg, 0.025 mmol), 0.005 M sodium ascorbate (125 $\mu$L), and 0.005 M $CuSO_4$ (25 $\mu$L) dissolved in *t*-BuOH (0.70 mL)/$H_2O$ (0.55 mL), and after 48 h of reaction time according to the general glycosylation procedure, PEG-[G1]-Lac (24 mg, 80%) was obtained as a white foam. $^1H$ NMR (750 MHz, $D_2O$) $\delta$: 8.07 (s, 1H), 8.06 (s, 2H), 7.17 (s, 2H), 4.90 (d, $J$ = 12.5 Hz, 1H), 4.87 (d, $J$ = 12.6 Hz, 2H), 4.76 (d, $J$ = 12.2 Hz, 1H), 4.72 (d, $J$ = 12.2 Hz, 2H), 4.61−4.56 (m, 6H), 4.49−4.41 (m, 6H), 4.22−4.20 (m, 4H), 4.14−4.12 (m, 2H), 4.00−3.50 (m, ∼570H), 3.37 (s, 3H), 3.29 (t, $J$ = 8.3 Hz, 3H). MALDI-TOF MS (DHB, linear mode, *m*/*z*): $M_p$ 7403.2. Calcd: $M_p$ 7413.4 ($[M + Na]^+$).

**PEG-[G2]-Lac.** From PEG-[G2]-$N_3$ (30 mg, 3.7 $\mu$mol), **4** (25 mg, 0.066 mmol), 0.05 M sodium ascorbate (33 $\mu$L), and 0.05 M $CuSO_4$ (7 $\mu$L) dissolved in *t*-BuOH (0.195 mL)/$H_2O$ (0.153 mL), and after 60 h of reaction time according to the general glycosylation procedure, PEG-[G2]-Lac (37 mg, 86%) was obtained as a white foam. $^1H$ NMR (750 MHz, $D_2O$) $\delta$: 8.04 (s, 3H), 8.03 (s, 6H), 7.09 (s, 2H), 7.08 (s, 4H), 7.02 (s, 2H), 4.91−4.84 (m, 9H), 4.76−4.69 (m, 9H), 4.58−4.38 (m, 42H), 4.15−3.49 (m, 731H), 3.37 (s, 3H), 3.33−3.31 (m, 9H).

**PEG-[G3]-Lac.** From PEG-[G3]-$N_3$ (16 mg, 1.19 $\mu$mol), **4** (16 mg, 0.064 mmol), 0.05 M sodium ascorbate (32 $\mu$L), and 0.05 M $CuSO_4$ (6 $\mu$L) dissolved in *t*-BuOH (0.200 mL)/$H_2O$ (0.160 mL), and after 72 h of reaction time according to the general glycosylation procedure, PEG-[G3]-Lac (24 mg, 86%) was obtained as a white foam. $^1H$ NMR (750 MHz, $D_2O$) $\delta$: 8.09−8.00 (m, 27H), 7.13−6.96 (m, 26H), 4.93−4.81 (m, 27H), 4.75−4.65 (m, 27H), 4.61−4.37 (m, 132H), 4.24−3.42 (m, ∼1199H), 3.38 (s, 3H), 3.31−3.24 (m, 27H).

**Acknowledgment.** This work was financially supported by the Spanish Government and the XUGA (Grants CTQ2005-00874/BQU, NAN2004-09230-C04-03/, CTQ2004-08106, and SAF2003-08765-C03-01).

CDV

## References and Notes


(1) (a) Tomalia, D. A.; Fréchet, J. M. J. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 2719. (b) Tomalia, D. A. *Aldrichimica Acta* **2004**, *37*, 39. (c) Gitsov, I.; Lin, C. *Curr. Org. Chem.* **2005**, *9*, 1025. (d) Tomalia, D. A. *Prog. Polym. Sci.* **2005**, *30*, 294.

(2) (a) Kukowska-Latallo, J. F.; Candido, K. A.; Cao, Z.; Nigavekar, S. S.; Majoros, I. J.; Thomas, T. P.; Balogh, L. P.; Khan, M. K.; Baker, J. R., Jr. *Cancer Res.* **2005**, *65*, 5317. (b) Fuchs, S.; Otto, H.; Jehle, S.; Henklein, P.; Schlüter, A. D. *Chem. Commun.* **2005**, 1830. (c) Majoros, I. J.; Myc, A.; Thomas, T.; Mehta, C. B.; Baker, J. R., Jr. *Biomacromolecules* **2006**, *7*, 572.

(3) (a) Cloninger, M. J. *Curr. Opin. Chem. Biol.* **2002**, *6*, 742. (b) Boas, U.; Heegaard, P. M. H. *Chem. Soc. Rev.* **2004**, *33*, 43. (c) Shaunak, S.; Thomas, S.; Gianasi, E.; Godwin, A.; Jones, E.; Teo, I.; Mireskandari, K.; Luthert, P.; Duncan, R.; Patterson, S.; Khaw, P.; Brocchini, S. *Nat. Biotechnol.* **2004**, *22*, 977. (d) McCarthy, T. D.; Karellas, P.; Henderson, S. A.; Giannis, M.; O'Keefe, D. F.; Heery, G.; Paull, J. R. A.; Matthews, B. R.; Holan, G. *Mol. Pharm.* **2005**, *2*, 312. (e) Lee, C. C.; MacKay, J. A.; Fréchet, J. M. J.; Szoka, F. C. *Nat. Biotechnol.* **2005**, *23*, 1517.

(4) (a) *Poly(ethylene glycol). Chemistry and Biological Applications*; Harris, J. M., Zalipsky, S., Eds.; ACS Symposium Series 680; American Chemical Society: Washington, DC, 1997. (b) Monfardini, C.; Veronese, F. M. *Bioconjugate Chem.* **1998**, *9*, 418. (c) Greenwald, R. B.; Choe, Y. H.; McGuire, J.; Conover, C. D. *Adv. Drug Delivery Rev.* **2003**, *55*, 217.

(5) (a) Gitsov, I. Linear-Dendritic Block Copolymers. Synthesis and Characterization. In *Advances in Dendritic Macromolecules*; Newkome, G. R., Ed.; Elsevier Science: Amsterdam, 2002; Vol. 5, pp 45−87. (b) Choi, J. S.; Joo, D. K.; Kim, C. H.; Kim, K.; Park, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 474. (c) Luman, N. R.; Smeds, K. A.; Grinstaff, M. W. *Chem. Eur. J.* **2003**, *9*, 5618. (d) Lambrych, K. R.; Gitsov, I. *Macromolecules* **2003**, *36*, 1068. (e) Jones, D. S.; Branks, M. J.; Campbell, M.-A.; Cockerill, K, A.; Hammaker, J. R.; Kessler, C. A.; Smith, E. M.; Tao, A.; Ton-Nu, H.-T.; Xu, T. *Bioconjugate Chem.* **2003**, *14*, 1067. (f) Gillies, E. R.; Fréchet, J. M. J. *Bioconjugate Chem.* **2005**, *16*, 361.

(6) (a) Ihre, H.; Padilla De Jesús, O. L.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2001**, *123*, 5908. (b) Gillies, E. R.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2002**, *124*, 14137.

(7) (a) Fréchet, J. M. J.; Gitsov, I.; Monteil, Th.; Rochat, S.; Sassi, J. F.; Vergelati, C.; Yu, D. *Chem. Mater.* **1999**, *11*, 1267. (b) Gitsov, I.; Lambrych, K.; Lu, P.; Nakas, J.; Ryan, J.; Tanenbaum, S. W. Nondestructive Regioselective Modification of Laccase by Linear-Dendritic Copolymers. Enhanced Oxidation of Benzo-α-Pyrene in Water. In *Polymer Bicatalysis and Biomaterials*; Cheng, H. N., Gross, R. A., Eds.; ACS Symposium Series Vol. 900; American Chemical Society: Washington, DC, 2005; pp 80−94.

(8) Fernandez-Megia, E.; Correa, J.; Rodríguez-Meizoso, I.; Riguera, R. *Macromolecules* **2006**, *39*, 2113.

(9) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. (b) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686. (c) Link, A. J.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 11164. (d) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192. (e) Helms, B.; Mynar, J. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15020. (f) Punna, S.; Kuzelka, J.; Wang, Q.; Finn, M. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 2215. (g) Parrish, B.; Breitenkamp, R. B.; Emrick, T. *J. Am. Chem. Soc.* **2005**, *127*, 7404. (h) Rodionov, V. O.; Fokin, V. V.; Finn, M. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 2210. (i) Ossipov, D. A.; Hilborn, J. *Macromolecules* **2006**, *39*, 1709. (j) Johnson, J. A.; Lewis, D. R.; Díaz, D. D.; Finn, M. G.; Koberstein, J. T.; Turro, N. J. *J. Am. Chem. Soc.* **2006**, *128*, 6564.

(10) For the use of click chemistry in the context of dendrimer synthesis, see: (a) Wu, P.; Feldman, A. K.; Nugent, A. K.; Hawker, C. J.; Scheel, A.; Voit, B.; Pyun, J.; Fréchet, J. M. J.; Sharpless, K. B.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2004**, *43*, 3928. (b) Joralemon, M. J.; O'Reilly, R. K.; Matson, J. B.; Nugent, A. K.; Hawker, C. J.; Wooley, K. L. *Macromolecules* **2005**, *38*, 5436.

(11) For the decoration of dendrimers via click chemistry, requiring the incorporation of click functionality on each generation of previously synthesized dendrimers, see: (a) Malkoch, M.; Schleicher, K.; Drockenmuller, E.; Hawker, C. J.; Russell, T. P.; Wu, P.; Fokin, V. V. *Macromolecules* **2005**, *38*, 3663. (b) Joosten, J. A. F.; Tholen, N. T. H.; Maate, F. A. L.; Brouwer, A. J.; van Esse, G. W.; Rijkers, D. T. S.; Liskamp, R. M. J.; Pieters, R. J. *Eur. J. Org. Chem.* **2005**, 3182.

(12) Meunier, S. J.; Wu, Q.; Wang, S.-N.; Roy, R. *Can. J. Chem.* **1997**, *75*, 1472.

(13) (a) Gravert, D. J.; Janda, K. D. *Chem. Rev.* **1997**, *97*, 489. (b) Zhao, X.-Y.; Metz, W. A.; Sieber, F.; Janda, K. D. *Tetrahedron Lett.* **1998**, *39*, 8433.

(14) (a) Chai, M.; Niu, Y.; Youngs, W. J.; Rinaldi, P. L. *J. Am. Chem. Soc.* **2001**, *123*, 4670. (b) Baille, W. E.; Malveau, C.; Zhu, X. X.; Kim, Y. H.; Ford, W. T. *Macromolecules* **2003**, *36*, 839. (c) Welch, K. T.; Arévalo, S.; Turner, J. F. C.; Gómez, R. *Chem. Eur. J.* **2005**, *11*, 1217.

(15) Lescanec, R. L.; Muthukumar, M. *Macromolecules* **1990**, *23*, 2280.

(16) De Gennes, P. G.; Hervet, H. *J. Phys., Lett.* **1983**, *44*, 351.

(17) Bakhmutov, V. I. *Practical NMR Relaxation for Chemists*; John Wiley & Sons Ltd.: Chichester, U.K., 2004; Chapter 4.

(18) *March's Advanced Organic Chemistry. Reactions, Mechanisms, and Structure*, 5th ed.; Smith, M. B., March, J., Eds.; John Wiley & Sons: New York, 2001; p 1412.

(19) Yu, D.; Vladimirov, N.; Fréchet, J. M. J. *Macromolecules* **1999**, *32*, 5186.

(20) (a) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754. (b) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555. (c) Lindhorst, T. K. *Top. Curr. Chem.* **2002**, *218*, 201. (d) *Carbohydrate-Based Drug Discovery*; Wong, C.-H., Ed.; Wiley-VCH: Weinheim, Germany, 2003.

(21) (a) Zanini, D.; Roy, R. *J. Org. Chem.* **1996**, *61*, 7348. (b) Röckendorf, N.; Lindhorst, T. K. *Top. Curr. Chem.* **2001**, *217*, 201. (c) Bezoušska, K. *Rev. Mol. Biotechnol.* **2002**, *90*, 269. (d) Turnbull, W. B.; Stoddart, J. F. *Rev. Mol. Biotechnol.* **2002**, *90*, 231. (e) Roy, R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 291.

(22) Several matrixes, solvent mixtures, laser power intensities, and MALDI-TOF spectrometers were tested.

(23) (a) Dam, T. K.; Brewer, C. F. *Chem. Rev.* **2002**, *102*, 387. (b) Ambrosi, M.; Cameron, N. R.; Davis, B. G. *Org. Biomol. Chem.* **2005**, *3*, 1593. (c) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637.


BM060580D