Macromolecules

Article

pubs.acs.org/Macromolecules

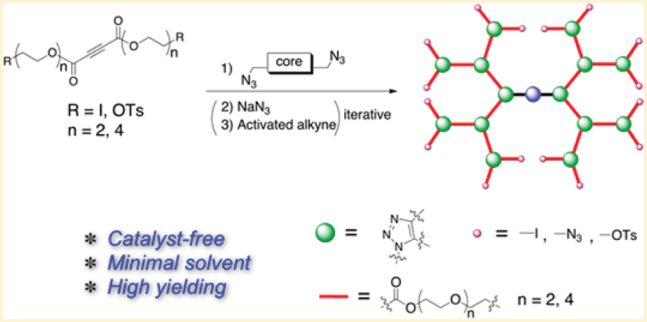
Efficient and Rapid Divergent Synthesis of Ethylene Oxide-Containing Dendrimers through Catalyst-Free Click Chemistry

Mathieu Arseneault, Isabelle Levesque, and Jean-François Morin*

Département de chimie and Centre de Recherche sur les Matériaux Avancés (CERMA), 1045 Ave de la Médecine, Université Laval, Québec, Canada G1V 0A6

Supporting Information

ABSTRACT: Third and fourth generation ethylene oxide-containing dendrimers containing triazole units were prepared divergently through metal-free Huisgen 1,3-dipolar cycloaddition ("click") reaction between activated disubstituted alkyne and terminal azide groups. The growth of the dendrimers was achieved in high yield with minimal purification effort and without the participation of toxic metal or excess of reagents. Preliminary *in vitro* testing on different cell lines indicates that this series of dendrimers is very promising for bio-related applications.



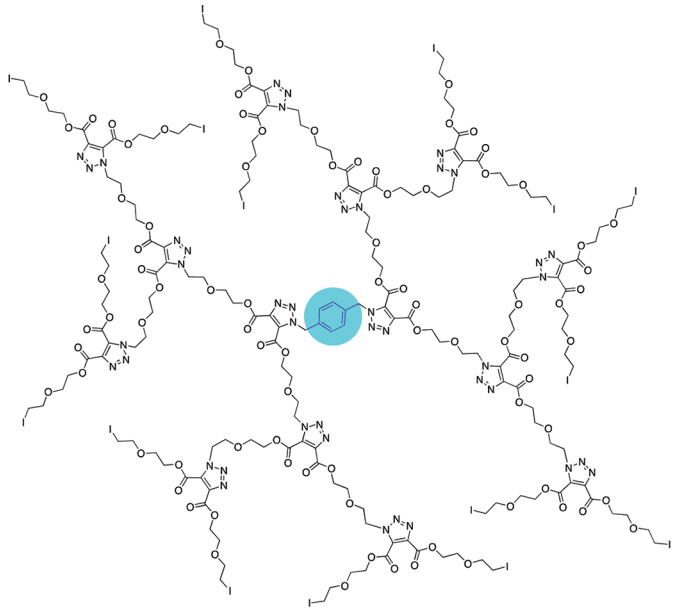
INTRODUCTION

Dendrimer materials are now considered to be one of the most important and promising classes of macromolecules for applications ranging from electronics to nanomedicine.[1,2] For bio-related applications, many water-soluble and biocompatible dendrimers were developed over the past few decades including poly(amidoamine) (PAMAM),[3] glycerol,[4] and ethylene oxide (EO)-based dendrimers[5] to name but a few. When properly decorated with active molecules, those particular dendrimeric structures were found to be very useful in various biological appplications such as drug delivery,[6] boron neutron capture therapy (BNCT),[7] and magnetic resonance imaging (MRI).[8,9] However, even the most promising classes of dendrimers suffer from serious drawbacks that could limit their penetration in real-world applications. For example, poly(amidoamine) (PAMAM) dendrimers, one of the most studied families of dendrimers, have significant disadvantages, the most important being their thermal and chemical instability. In fact, PAMAM dendrimers can undergo retro-Michael reactions, resulting in loss of branches and the creation of secondary amines that can further undergo an intramolecular transamidation reaction.[10,11] Moreover, the hydrolysis (or solvolysis) of terminal ester functionalities into carboxylic acids makes the dendrimer termini unreactive toward ethylenediamine, thus impeding the dendrimer growth to higher generations.[3] Finally, a large excess of reagent has to be used at each step in order to avoid the occurrence of side reactions, including intramolecular bridging and intermolecular cross-linking.[3] Since most of these drawbacks are inherent to the PAMAM chemical structure, the preparation of a new family of readily accessible dendrimers circumventing all these negative aspects is desired.

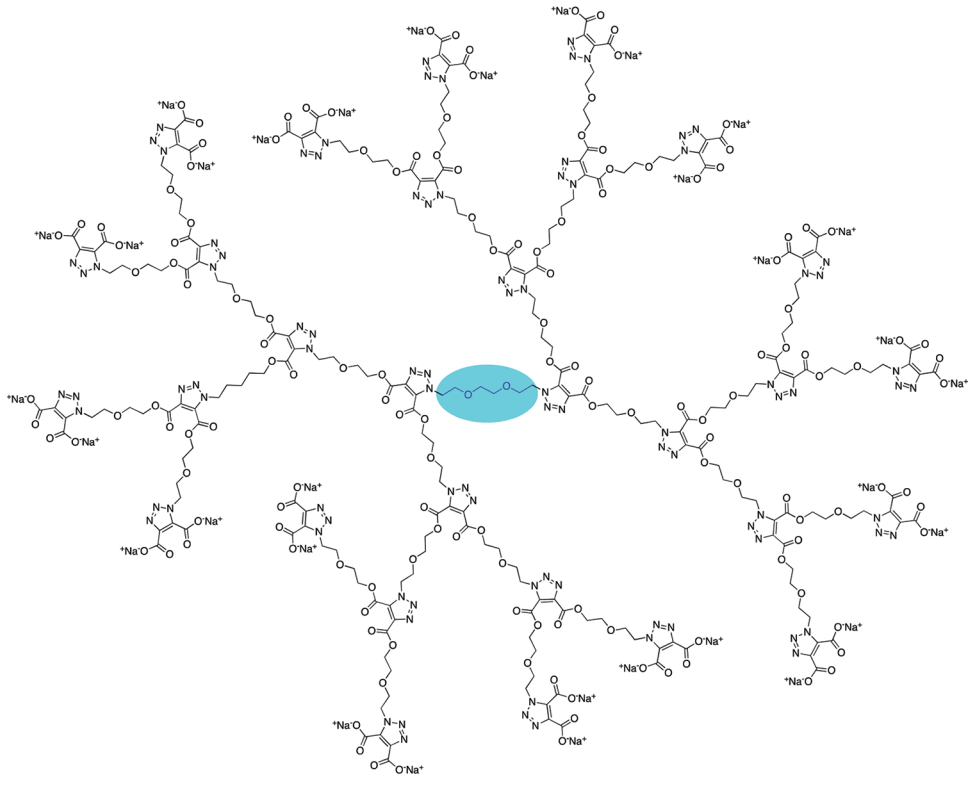
In this context, the development of more stable and water-soluble dendrimers made from readily accessible biocompatible building blocks such as glycerol and ethylene oxide (EO) becomes very attractive. The extensive use of EO in bio-related areas comes from, among other things, its very good solubility in aqueous media, chemical stability in physiological environments, low toxicity, and a prolonged circulation period in the human body due to its nonimmunogenicity.[12] However, EO does not offer good chemical versatility because only a few EO-based building blocks with two or more reactive or functional ends are available. To circumvent this lack of chemical versatility, chemists often used EO moieties to decorate the surface of nanoparticles,[13] quantum dots,[14] dendrimers,[15] and biomolecules[16] since these strategies require EO with only one reactive end. With the exception of linear EO polymers and oligomers, more complex molecular architectures such as dendrons and dendrimers in which the EO moiety is part of the molecular scaffold are thus rather limited.

Recently, Gnanou et al. have developed practical approaches to prepare functional high-generation dendrimer-like molecules based on EOs with narrow size distribution (PDI $\leq$1.5) through the divergent anionic ring-opening polymerization of ethylene oxide.[17−19] They have demonstrated that those structures show interesting properties in terms of water solubility, possibility of functionalization, and pH responsiveness.[20] Using a different approach, Veronese et al. have prepared low-generation EO-based dendrons through an amidation reaction for targeted drug delivery applications.[21] Almost at the same time, Hildgen et al. reported on the synthesis of monodisperse EO-based dendrimers (up to the third generation) through a combination of convergent and divergent synthetic steps, also for drug delivery purposes.[22,23]

Received: March 29, 2012
Revised: April 13, 2012
Published: April 18, 2012



X-EO2-G3-I



EO2-EO2-G4-COO⁻ Na⁺

Figure 1. Examples of third and fourth EO-containing dendrimers grown by metal-free click chemistry.

Although the properties of those EO-based dendrimers are very promising, their preparation involves either toxic metal and chemicals, harsh conditions, long reaction times, or excess of chemical reagents resulting in chemical wastes. Thus, an efficient and clean preparative method for monodisperse dendrimers for bio-related applications still represents an important cornerstone that has not been totally reached yet. The search for the "perfect" conditions to grow dendrimers represents a demanding challenge because the synthetic strategy must be very efficient in order to avoid structural defects, free of side reactions and byproducts to ensure dendrimer monodispersity and conducted without the

dx.doi.org/10.1021/ma300648r | *Macromolecules* 2012, 45, 3687−3694

participation of toxic metal. The latter requirement is of utmost importance since polar dendrimers such as PAMAM are known to trap metal ions very efficiently, as shown by us[24] and others.[25] Herein we report the divergent synthesis of third and fourth generation EO-containing dendrimers (Figure 1), using sequential azidation/metal-free 1,3-dipolar cycloaddition (click) reactions.[26−28] These dendrimers and the reactions used to prepare them have carefully been designed for biorelated applications. First, the dendritic structures include 1,2,3-triazole heterocycles, which are known for their promising pharmacological properties.[29] Second, the ester groups directly attached to the triazole units should allow the dendrimers to be decomposed *in vivo* through enzymatic hydrolysis reactions to create low molecular weight units that could be easily assimilated by the human body.[11] Third, the presence of either azide or iodide termini provides us with versatile dendrimers in terms of surface decoration. Finally, the synthetic strategy we developed is relatively simple, high yielding, and free of toxic metal and reagent.

EXPERIMENTAL SECTION

Materials. All solvents (ACS grade) were distilled and put through a Vac Atmosphere (Hawthorne, CA) solvent purification system. All the reagents were purchased from either Sigma-Aldrich Co., TCI America, or Oakwood Products and used as received. All equivalents are molar. 2-(2-Iodoethoxy)ethanol (compound **2**)[30] and tetraethylene glycol mono(*p*-toluenesulfonate) (compound **7**)[31] were synthesized according published procedures.

Characterization. SEC analysis was performed using a Jordi DBV 500A in THF. ^{1}H and ^{13}C NMR spectra were recorded on a Varian AS400 apparatus in appropriate deuterated solvent solution at 298 K. Chemical shifts were reported as values (ppm) relative to internal tetramethylsilane. High resolution mass spectrometry (HRMS) was performed on an Agilent model 62-10 MS-TOF.

General Procedure for Iodo- or Tosylate-Terminated Generations. In a round-bottom flask, either 2, 4, or 8 equiv of compound **3** or **8** were added to 1 equiv of the azido compound moieties. A minimal amount of chloroform was added (typically 2 mL/g of substrate). The reaction was started in an ice bath at 0 °C for 2 h and stirred for 1 h, allowing the mixture to reach room temperature. The mixture was then stirred at 60 °C overnight. The reaction was allowed to cool down at 25 °C, and the chloroform was then evaporated. The compound was used without any further purification.

General Procedure for Azido-Terminated Generations. The iodo- or tosylate-terminated dendrimer was dissolved in DMF (0.1 M), and 2 equival of NaN_3 per iodine terminals was added to the solution. The reaction was then stirred and heated at 70 °C for 24 h. The mixture was then allowed to cool down to 25 °C and extracted with AcOEt/"half-brine". "Half-brine" is brine that has been diluted by an equal volume of distilled water. We found that this was a good compromise to obtain good phase separation while eliminating DMF. This was done because standard ether/water extractions failed to dissolve the dendrimer at any given generation. The aqueous layer was then washed with AcOEt, and the organic layers were combined. They were then washed 10 times with "half-brine". The organic layer was then dried and concentrated.

Synthesis of Compound 3. In a round-bottom flask fitted with a Dean−Stark apparatus, acetylenedicarboxylic acid (3.83 g, 33.6 mmol, 1 equiv) was dissolved in benzene (66 mL). To the solution, *p*-toluenesulfonic acid (0.638 g, 3.36 mmol, 0.1 equiv) and 2-(2-iodoethoxy)ethanol (16.0 g, 73.8 mmol, 2.2 equiv) were added. The mixture was refluxed for 24 h and was then allowed to cool down at room temperature. Diethyl ether and $NaHCO_3$ (1.41 g, 16.8 mmol, 0.5 equiv) were then added and stirred for 1 min. The purification was achieved through column chromatography (silica gel, DCM and hexanes 4:1 as the eluent). Yield is 75% (12.7 g, 24.9 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.40 (t, 4H, J = 4.6 Hz), 3.75 (m, 8H), 3.26 (t, 4H, J = 6.7 Hz). ^{13}C NMR: 151.75, 75.08, 72.00, 68.07, 65.74, 2.36. IR (ATR): 1037, 1109, 1232, 1714, 2869, 2958 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{12}H_{16}I_2O_6$: 509.9036; found: 532.8963 (M + Na^+).

Synthesis of X-EO2-G1-I. Following the above procedure for iodo-terminated generations, α,α'-diazido-*p*-xylene (0.184 g, 0.980 mmol, 1 equiv) was used with compound **3** (1.00 g, 1.96 mmol, 2 equiv). No purification was needed. The reaction gave a viscous dark yellow oil. Yield is over 98% (1.184 g, 0.980 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.29 (s, 4H), 5.81 (s, 4H), 4.51 (t, 4H, J = 4.7 Hz), 4.46 (t, 4H, J = 4.6 Hz), 3.83 (t, 4H, J = 4.7 Hz), 3.75 (m, 12H), 3.23 (dt, 8H, J = 5.2 Hz). ^{13}C NMR: 160.10, 158.17, 140.71, 134.96, 129.89, 128.96, 72.12, 71.94, 68.54, 68.12, 65.73, 64.97, 53.72. IR (ATR): 1056, 1117, 1195, 1264, 1358, 1551, 1724, 2869, 2955 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{32}H_{40}I_4N_6O_{12}$: 1207.8883; found: 1208.8925 (M + H^+).

Synthesis of X-EO2-G1-N_3. Following the above procedure for azido-terminated generations, **X-EO2-G1-I** (0.800 g, 0.660 mmol, 1 equiv) was used with NaN_3 (0.34 g, 5.29 mmol, 8 equiv) and DMF. No further purification was needed. The reaction gave a viscous bright yellow oil. Yield is 97% (0.560 g, 0.64 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.28 (s, 4H), 5.78 (s, 4H), 4.51 (t, 4H, J = 4.6 Hz), 4.46 (t, 4H, J = 4.6 Hz), 3.83 (t, 4H, J = 4.8 Hz), 3.74 (t, 4H, J = 4.8 Hz), 3.70 (t, 4H, J = 4.9 Hz), 3.64 (t, 4H, J = 4.9 Hz), 3.36 (dt, 8H, J = 5.2 Hz). ^{13}C NMR: 160.10, 158.20, 140.60, 134.95, 129.93, 128.84, 70.28, 70.28, 68.95, 68.56, 65.69, 64.93, 53.58, 50.79, 50.36. IR (ATR): 1058, 1122, 1197, 1464, 1727, 2095, 2872, 2923, 2952 (broad band) ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{32}H_{40}N_{18}O_{12}$: 868.3073; found: 869.3238 (M + H^+).

Synthesis of X-EO2-G2-I. Following the above procedure for iodo-terminated generations, **X-EO2-G1-N_3** (0.535 g, 0.616 mmol, 1 equiv) was used with compound **3** (1.26 g, 2.46 mmol, 4 equiv). No purification was needed. The reaction gave a viscous yellow oil. Yield is over 98% (1.795, 0.616 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.28 (s, 4H), 5.78 (s, 4H), 4.81 (dt, 8H, J = 5 Hz), 4.51 (m, 16H,), 4.38 (m, 8H), 3.78 (m, 44H), 3.65 (t, 4H), 3.25 (m, 16H). ^{13}C NMR: 160.06, 160.04, 159.91, 158.50, 158.46, 158.10, 140.34, 139.92, 139.72, 135.01, 131.96, 131.43, 130.06, 128.89, 72.11, 72.09, 72.07, 71.87, 71.84, 69.61, 69.35, 69.09, 68.59, 68.54, 68.20, 68.13, 65.77, 65.68, 65.51, 64.87, 64.80, 64.74, 52.69, 50.39, 50.34, 3.17, 3.09, 3.07, 3.05. IR (ATR): 1059, 1116, 1198, 1269, 1462, 1552, 1724, 2872, 2956 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{80}H_{104}N_{18}I_8O_{36}$: 2907.9218; found: 2926.9660 (M + NH_4^+).

Synthesis of X-EO2-G2-N_3. Following the above procedure for azido-terminated generations, **X-EO2-G2-I** (0.50 g, 0.172 mmol, 1 equiv) was used with NaN_3 (0.179 g, 2.75 mmol, 16 equiv) and DMF. No further purification was needed. The reaction gave a viscous bright yellow oil. Yield is 72% (0.278 g, 0.125 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.28 (s, 4H), 5.78 (s, 4H), 4.81 (dt, 8H, J = 5 Hz), 4.51 (m, 16H,), 4.38 (m, 8H), 3.83 (m, 44H), 3.68 (m, 4H), 3.39 (m, 16H). ^{13}C NMR: 159.99, 159.98, 159.86, 158.38, 158.34, 157.99, 140.17, 139.75, 139.56, 134.97, 131.84, 131.35, 130.06, 128.70, 70.12 70.02, 69.95, 69.45, 69.20, 68.90, 68.83, 68.51, 68.46, 68,41, 65.63, 65.57, 65.44, 64.75, 64.69, 64.58, 53.52, 50.73, 50.71, 50.19. IR (ATR): 960, 1062, 1125, 1203, 1273, 1462, 1554, 1730, 2102, 2874, 2923, 2955 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{80}H_{104}N_{42}O_{36}$: 2228.7598; found: 2247.7653 (M + NH_4^+).

Synthesis of X-EO2-G3-I. Following the above procedure for iodo-terminated generations, **X-EO2-G2-N_3** (0.278 g, 0.125 mmol, 1 equiv) was used with compound **3** (0.509 g, 0.998 mmol, 8 equiv). No purification was needed. The reaction gave a viscous dark yellow oil. Yield is over 98% (0.787 g, 0.125 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.29 (s, 4H), 5.78 (s, 4H), 4.81 (m, 22H), 4.51 (m, 30H,), 4.40 (m, 26H), 3.79 (m, 114H), 3.25 (m, 32H). ^{13}C NMR: image available below. IR (ATR): 1060, 1102, 1269, 1461, 1552, 1723, 2872, 2956 (broad band) ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{176}H_{232}I_{16}N_{42}O_{84}$: 6307.9888; found: 3172.9986 (m/z = M^{2+} + NH_4^+).

Synthesis of EO3-EO2-G1-I. Following the above procedure for iodo-terminated generations, 1,2-bis(2-azidoethoxy)ethane (**5**) (0.587 g, 2.94 mmol, 1 equiv) was used with compound **3** (3.00 g, 5.88 mmol, 2 equiv). No purification was needed. The reaction gave a viscous amber oil. Yield is over 98% (3.59 g, 2.94 mmol). ^{1}H NMR ($CDCl_3$,

400 MHz): 4.81 (t, 4H, J = 5.3 Hz), 4.23 (m, 8H), 3.79 (m, 20H), 3.44 (s, 4H), 3.26 (t, 8H, J = 6.5 Hz). ^{13}C NMR: 160.15, 158.50, 139.84, 131.74, 72.12, 71.93, 70.66, 69.49, 68.58, 68.21, 65.68, 64.83, 50.43. IR (ATR): 1061, 1102, 1198, 1270, 1462, 1553, 1725, 2870, 2954 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{30}H_{44}I_4N_6O_{14}$: 1219.9094; found: 1220.9173 (M + H$^+$).

Synthesis of EO3-EO2-G1-N$_3$. Following the above procedure for azido-terminated generations, **EO3-EO2-G1-I** (0.705 g, 0.577 mmol, 1 equiv) was used with NaN_3 (0.300 g, 4.62 mmol, 8 equiv) and DMF. No further purification was needed. The reaction gave a viscous bright yellow oil. Yield is 98% (0.500 g, 0.566 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.79 (t, 4H, J = 5.1 Hz), 4.53 (t, 8H, J = 4.5 Hz), 3.84 (qt, 8H, J = 4.9 Hz), 3.77 (t, 4H, J = 5.1, 3.70 (qt, 8H, J = 4.9 Hz), 3.43 (s, 4H), 3.50 (t, 8H, J = 4.8 Hz). ^{13}C NMR: 160.16, 158.49, 139.82, 131.73, 70.60, 70.31, 70.20, 69.46, 69.01, 68.68, 65.61, 64.83, 50.89, 50.35. IR (ATR): 1062, 1112, 1200, 1273, 1463, 1728, 2096, 2870, 2922, (broad band) ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{32}H_{40}N_{18}O_{12}$: 880.3284; found: 881.3745 (M + H$^+$).

Synthesis of EO3-EO2-G2-I. Following the above procedure for iodo-terminated generations, **EO3-EO2-G1-N$_3$** (0.300 g, 0.341 mmol, 1 equiv) was used with compound **3** (0.695 g, 1.36 mmol, 4 equiv). No purification was needed. The reaction gave a viscous amber oil. Yield is over 98% (0.965, 0.341 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.84 (q, 8H, J = 4.7 Hz), 4.74 (t, 4H, J = 4.9 Hz), 4.51 (m, 16H), 4.42 (q, 8H, J = 4.3 Hz), 3.90 (q, 8H, J = 4.3 Hz), 3.80 (m, 20H), 3.60 (m, 24H), 3.42 (s, 4H), 3.396 (m, 16H). ^{13}C NMR: 72.11, 72.08, 71.89, 71.87, 68.55, 68.22, 68.14, 65.79, 65.71, 64.80, 64.60, 64.57, 64.74, 50.41, 3.04. IR (ATR): 1061, 1103, 1198, 1272, 1462, 1553, 1726, 2872, 2956 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{78}H_{108}I_8N_{18}O_{38}$: 2919.9429; found: 1461.4744 (m/z = M^{2+} + 2H$^+$).

Synthesis of EO3-EO2-G2-N$_3$. Following the above procedure for azido-terminated generations, **EO3-EO2-G2-I** (4.00 g, 1.369 mmol, 1 equiv) was used with NaN_3 (1.424 g, 21.91 mmol, 16 equiv) and DMF. No further purification was needed. The reaction gave a viscous amber yellow oil. Yield is 92% (2.82 g, 1.26 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.84 (q, 8H, J = 4.7 Hz), 4.74 (t, 4H, J = 4.9 Hz), 4.51 (m, 16H), 4.42 (q, 8H, J = 4.3 Hz), 3.90 (q, 8H, J = 4.3 Hz), 3.80 (m, 20H), 3.60 (m, 24H), 3.42 (s, 4H), 3.39 (m, 16H). ^{13}C NMR: 160.11, 160,08, 159.99, 158.52, 158.49, 158.29, 139.98, 139.72, 139.65, 131.94, 131.75, 131.32, 70.49 70.30, 70.21, 70.13, 69.69, 69.39, 69.27, 69.07, 68.99, 68.69, 68.63, 65.72, 65.65, 65.36, 65.03, 64.87, 64.80, 64.59, 50.89, 50.87, 50.85, 50.45, 50.33, 50.31. IR (ATR): 1061, 1115, 1199, 1273, 1461, 1553, 1727, 2098, 2874, 2905, 2953 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{78}H_{108}N_{42}O_{38}$: 2240.7810; found: 1121.8963 (m/z = M^{2+} + H$^+$).

Synthesis of EO3-EO2-G3-I. Following the above procedure for iodo-terminated generations, **EO3-EO2-G2-N$_3$** (0.598 g, 0.267 mmol, 1 equiv) was used with compound **3** (1.088 g, 2.13 mmol, 8 equiv). No purification was needed. The reaction gave a viscous amber oil. Yield is over 98% (1.686 g, 0.267 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.80 (m, 26H), 4.50 (m, 30H), 4.40 (m, 28H), 3.90 (m, 22H,), 3.78 (m, 98H), 3.26 (m, 32H). ^{13}C NMR: 160.09, 159.93, 158.54, 158.45, 158.31, 139.56, 70.12 72.04, 71.85, 69.60, 69.26, 69.06, 68.55, 68.20, 68.14, 67.97, 67.68, 65.85, 65.80, 65.71, 65.47, 65.40, 65.38, 64.88, 64.80, 64.55, 50.36, 3.26. IR (ATR): 1062, 1107, 1200, 1268, 1462, 1553, 1725, 2873, 2918, 2957 (broad band) ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{174}H_{236}I_{16}N_{42}O_{86}$: 6320.0100' found: 2125.3672 (m/z = M^{3+} + NH$_4^+$ + H$^+$).

Synthesis of EO3-EO2-G3-N$_3$. Following the above procedure for azido-terminated generations, **EO3-EO2-G3-I** (4.00 g, 0.63 mmol, 1 equiv) was used with NaN_3 (1.424 g, 21.91 mmol, 16 equiv) and DMF. No further purification was needed. The reaction gave a viscous amber yellow oil. Yield is 86% (2.70 g, 0.54 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.80 (m, 26H), 4.52 (m, 34H), 4.39 (m, 22H) 3.89 (d, 24H, J = 4.4 Hz), 3.82 (m, 38H), 3.70 (m, 56H), 3.39 (m, 36H).). ^{13}C NMR: 160.10, 160.08, 160.07, 160.00, 159.93, 159.92, 158.51, 158.50, 158.44, 158.28, 139.97, 139.76, 139.70, 139.67, 139.63, 139.57, 131.99, 131.96, 131.85, 131.74, 131.39, 131.37, 131.23, 70.46, 70.28, 70.18, 70.12, 69.60, 69.56, 69.35, 69.23, 69.04, 68.97, 68.66, 68.60, 68.72, 65.65, 65.43, 64.99, 64.86, 64.79, 64.63, 64.59, 50.87, 50.41, 50.32. IR (ATR): 1061, 1114, 1273, 1461, 1553, 1726, 2098, 2974, 2915, 2954 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{174}H_{236}N_{90}O_{86}$: 4961.6860; found: 1672.5885 (m/z = M^{3+} + NH$_4^+$ + H$^+$).

Synthesis of EO3-EO2-G4-COOH. Following the above procedure for iodo-terminated generations, **EO3-EO2-G3-N$_3$** (1.000 g, 0.201 mmol, 1 equiv) was used with acetylenedicarboxylic acid (0.735 g, 6.45 mmol, 32 equiv). No purification was needed. The reaction gave a viscous dark amber oil insoluble in most solvent. Yield is over 98% (1.735 g, 0.201 mmol). ^{1}H NMR (D_2O, 400 MHz): 4.91−5.22 (m), 4.41−4.15 (m), 3.99−3.59 (m). Image available below. IR (ATR): 1063, 1117, 1210, 1278, 1370, 1455 1551, 1627, 1724, 2878, 2957, 3400 ν cm^{-1}. MS data could not be obtained due to the limitations of our apparatus.

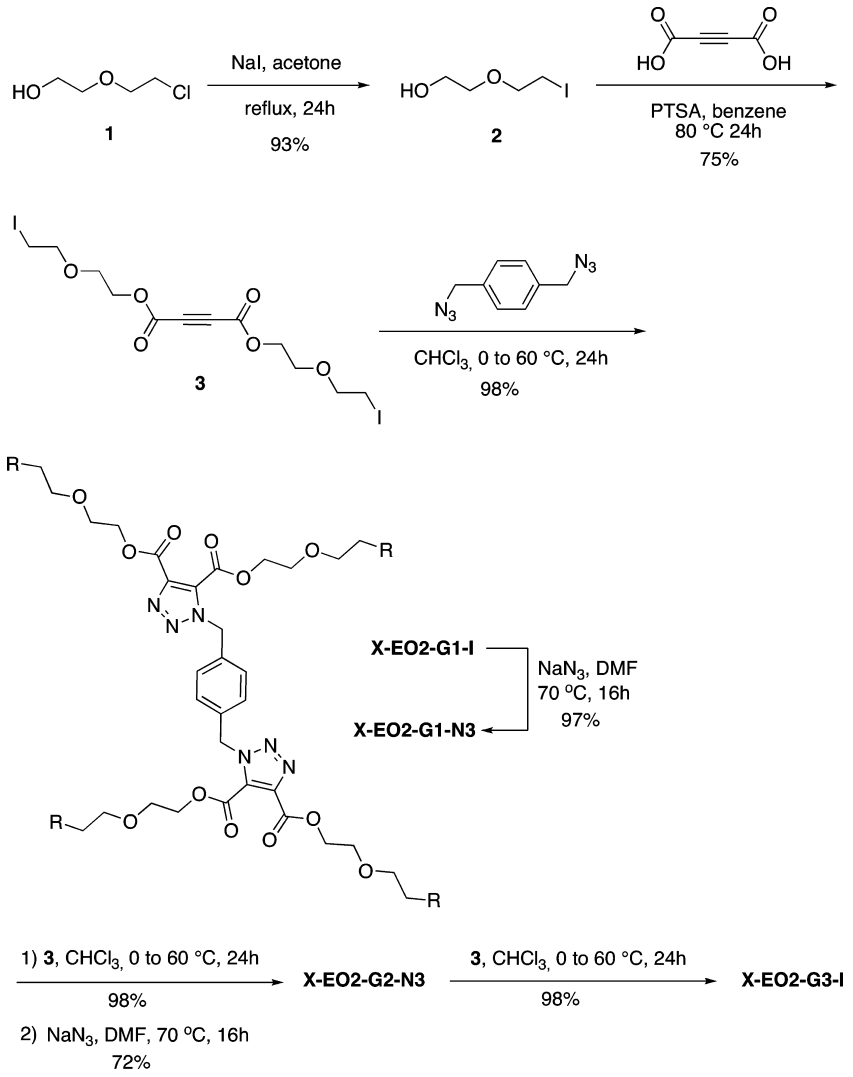
Preparation of EO3-EO2-G4-COO$^-$Na$^+$ Salt for Cytotoxicity Assays. **EO3-EO2-G4-COOH** was mixed with deionized water (1.735 g in 25 mL) and $NaHCO_3$ (1 equiv per −COOH group) was added. The mixture was vigorously stirred until all dendrimer was dissolved. To remove any smaller species, dialysis was performed in nanopure water. Dialysis tubing was left to soak in nanopure water for 30 min to remove most of its preservative. The tube was then clamped at the bottom, and five small glass beads (prewashed with nanopure water) were added. Typically, 10 mL of the dendrimer solution mentioned above was used. The tube was clamped at its top and put in a 1 L beaker filled with nanopure water with gentle magnetic stirring for 24 h. Nanopure water was replaced two more times, each at 24 h intervals. The tube was then emptied, and the water was evaporated under reduced pressure. A pale yellow powder was obtained.

Synthesis of Compound 8. In a round-bottom flask fitted with a Dean−Stark apparatus, acetylenedicarboxylic acid (0.708 g, 6.22 mmol, 1 equiv) was dissolved in benzene (22 mL). To the solution, p-toluenesulfonic acid (0.118 g, 0.622, 0.1 equiv) and Ts-tetraglycol (4.77 g, 13.691 mmol, 2.2 equiv) were added. The mixture was refluxed for 24 h and was then allowed to cool down at room temperature. Diethyl ether and $NaHCO_3$ (0.261 g, 3.112 mmol, 0.5 equiv) were then added and stirred for 1 min before being concentrated. The purification was achieved through column chromatography (silica gel, AcOEt and hexanes 4:1 as the eluent). Yield is 47% (2.27 g, 2.93 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.79 (t, 4H, J = 4.1 Hz), 7.34 (t, 4H, J = 4.1 Hz), 4.37 (t, 4H, J = 4.7 Hz), 4.16 (t, 4H, J = 4.8 Hz), 3.73 (t, 4H, J = 4.6 Hz), 3.69 (t, 4H, J = 4.8 Hz), 3.63 (bs, 8H), 3.59 (s, 8H), 2.45 (s, 6H). ^{13}C NMR: 151.90, 145.09, 133.13, 130.08, 128.20, 75.11, 70.94, 70.87, 70.83, 70.77, 69.52, 68.89, 68.63, 66.09, 21.89. IR (ATR): 916, 1095, 1174, 1251, 1352, 1451, 1598, 1720, 2872, 2949 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{34}H_{46}O_{16}S_2$: 774.2227; found: 792.2985 (M + NH$_4^+$).

Synthesis of EO3-EO4-G1-Ts. Following the above procedure for iodo-terminated generations, 1,2-bis(2-azidoethoxy)ethane (**5**) (0.08 g, 0.400 mmol, 1 equiv) was used with compound **8** (0.619 g, 0.800 mmol, 2 equiv). No purification was needed. The reaction gave a viscous yellow oil. Yield is over 98% (0.699 g, 0.400 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.79 (d, 8H, J = 7.8 Hz), 7.34 (d, 8H, J = 7.8 Hz), 4.37 (t, 8H, J = 4.6 Hz), 4.16 (t, 8H, J = 4.8 Hz), 3.63 (m, 60H), 2.44 (d, 12H, J = 3.0 Hz). ^{13}C NMR: 160.24, 158.50, 145.06, 139.84, 133.14, 130.08, 128.21, 70.97, 70.96, 70.87, 70.82, 70.77, 70.70, 70.57, 69.53, 69.52, 69.47, 68.96, 68.91, 68.90, 68.87 68.64, 65.79, 64.95, 50.39, 21.90 IR (ATR): 919, 1096, 1175, 1352,1453, 1731, 2872, 2919, 2952, 3010 (broad band) ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{74}H_{104}N_6O_{34}S_4$: 1748.5476; found: 1766.585 (M + NH$_4^+$).

Synthesis of EO3-EO4-G1-N$_3$. Following the above procedure for azido-terminated generations, **EO3-EO4-G1-Ts** (0.7423 g, 0.424 mmol, 1 equiv) was used with NaN_3 (0.179 g, 3.395 mmol, 8 equiv) and DMF. No further purification was needed. The reaction gave a viscous bright yellow oil. Yield is 63% (0.330 g, 0.267 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.79 (t, 4H, J = 4.9 Hz), 4.50 (t, 8H, J = 4.6 Hz), 3.80 (m, 12H), 3.66 (m, 40H,), 3.42 (s, 4H), 3.38 (m, 8H). ^{13}C NMR: 160.25, 158.52, 139.87, 131.77, 70.90, 70.84, 70.74, 70.61, 70.29, 70.27, 69.50, 68.97, 68.64, 65.78, 64.97, 50.90, 50.88, 32.10. IR (ATR): 1063, 1104, 1201, 1274, 1462, 1552, 1729, 2098, 2869 νcm^{-1}. HRMS (ESI$^+$): calcd for $C_{46}H_{76}N_{18}O_{22}$: 1232.5382; found: 1250.5932 (M + NH$_4^+$).

Scheme 1. Synthesis of X-EO2-G3-I



Synthesis of EO3-EO4-G2-Ts. Following the above procedure for iodo-terminated generations, **EO3-EO4-G1-N_3** (0.313 g, 0.254 mmol, 1 equiv) was used with compound **8** (0.787 g, 1.015 mmol, 4 equiv). No purification was needed. The reaction gave a viscous yellow oil. Yield is over 98% (1.1 g, 0.254 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.77 (d, 12H, J = 8.1 Hz), 7.33 (d, 12H, J = 8.1 Hz), 4.78 (m, 12H), 4.48 (m, 24H,), 4.14 (m, 16H), 3.78 (m, 34H), 3.58 (m, 122H), 3.41 (s, 4H), 2.42 (m, 24H). ^{13}C NMR: 160.24, 159.49, 145.07, 133.18, 130.09, 128.21, 70.99, 70.96, 70.95, 70.91, 70.87, 70.86, 70.83, 70.81, 70.79, 70.76, 70.71, 70.65, 70.61, 70.57, 69.54, 69.52, 69.48, 69.46, 68.96, 68.93, 68.91, 68.88, 68.66, 68,61, 65.76, 65.74, 65.72, 64.94, 64.92, 50.41, 50.39, 50.38, 21.89. IR (ATR): 917, 1012, 1064, 1096, 1174, 1274, 1351, 1456, 1552, 1598 1729, 2871, 2920, 2954 ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{182}H_{260}N_{18}O_{86}S_8$: 4329.4291; found: 1084.1100 (m/z = 4 + 2H$^+$).

Synthesis of EO3-EO4-G2-N_3. Following the above procedure for azido-terminated generations, **EO3-EO4-G2-Ts** (0.625 g, 0.144 mmol, 1 equiv) was used with NaN_3 (0.150 g, 2.31 mmol, 16 equiv) and DMF. No further purification was needed. The reaction gave a viscous bright yellow oil. Yield is 92% (0.437 g, 0.132 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 4.67 (m, 8H), 4.36 (m, 16H), 3.67 (m, 40H), 3.51 (m, 96H), 3.39 (m, 22H), 3.29 (s, 4H), 3.24 (t, 16H, J = 4.8 Hz). ^{13}C NMR: 160.10, 158.37, 158.36, 139.69, 139.67, 139.60, 131.66, 131.64, 70.75, 70.73, 70.69, 70.67, 70.60, 70.55, 70.51, 70.47, 70.44, 70.13, 70.11, 69.48, 69.42, 69.34, 69.20, 68.84, 68.81, 68.53, 68.49, 65.64, 64.84, 64.81, 63.71, 50.75, 53.73, 53.31, 50.29, 21.09. IR (ATR): 940, 1063, 1102, 1201, 1274, 1462, 1552, 1729, 2100, 2869, 2915 (broad band) ν cm^{-1}. HRMS (ESI$^+$): calcd for $C_{126}H_{204}N_{42}O_{62}$: 3297.4101; found: 1666.8335 (m/z = 2 + NH_4^+).

Synthesis of EO3-EO4-G3-Ts. Following the above procedure for iodo-terminated generations, **EO3-EO4-G2-N_3** (0.350 g, 0.106 mmol, 1 equiv) was used with compound **8** (0.657 g, 0.848 mmol, 8 equiv). No purification was needed. The reaction gave a viscous yellow oil. Yield is over 98% (1.007 g, 0.106 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): 7,77 (d, 32H, J = 7,6 Hz); 7.33 (d, 4H, J = 7.9 Hz), 4.80 (m, 24H), 4.48 (m, 24H), 4.14 (m, 36H), 3.83 (m), 3.79 (m), 3.61 (m), 3.56 (br s), 3.51 (br s), 3.41 (br s), 2.42 (s, 48H) all peaks from 3.83 to 3.41 integrated for 348H combined. ^{13}C NMR: 160.23, 158.48, 145.08, 133.17, 130.09, 128.20, 70.94 70.86, 70.82 70.80, 70.76, 70.70, 70.63, 69.54, 68.94, 68.89, 68.87, 68.65, 65.72, 65.44, 64.75, 64.94, 64.92, 64.88, 50.40, 21.87. IR (ATR): 916, 1096, 1175, 1275, 1351, 1453, 1522, 1729, 2096, 2871 ν cm^{-1}.

RESULTS AND DISCUSSION

The synthetic strategy for the synthesis of EO-containing dendrimers is depicted in Scheme 1. For our initial attempt, very short EO chains (−[CH_2CH_2O]−, named **EO2** thereafter) were used. A phenyl-containing core obtained from a xylene derivative (named **X** thereafter) was chosen to enable the monitoring of the dendrimer growth using thin layer chromatography and size-exclusion chromatography (SEC,

dx.doi.org/10.1021/ma300648r | Macromolecules 2012, 45, 3687−3694

UV/vis detector). In order to conduct metal-free click chemistry without using harsh heating conditions, an activated alkyne bearing at least one electron-deficient unit must be used.[32,33] Since internal alkyne is needed for the preparation of dendrimers using a divergent approach, we chose to start with the commercially available acetylenedicarboxylic acid. Dehaen et al. have shown that this building block is reactive enough to attach multiple dendrons on a highly functionalized core without the participation of copper or other metal catalysts.[34] Thus, acetylenedicarboxylic acid was coupled with 2-(2-iodoethoxy)ethanol (compound **2**) using *p*-toluenesulfonic acid in benzene using a Dean−Stark apparatus as developed by Finn et al. to provide the compound **3** in 75% yield.[35] It is noteworthy that the commercially available 2-(2-chloroethoxy)-ethanol was transformed into its iodo analogue in order to enable further azidation reaction. In fact, the reaction conditions needed to quantitatively displace the chlorine atom by the azide on our substrates were too harsh and led to the partial decomposition of our dendrimers as observed by ^{1}H NMR. The replacement of the chlorine atom by a better leaving group toward S_N2 reactions allowed us to conduct the azidation reactions at a lower temperature for a shorter period of time without any trace of decomposition.

The key step and major feature of our dendrimer lie in the convenient character of the branching click reaction. Inspired by Brook et al.,[26] we mixed neat compounds **3** and 1,4-bis(azidomethyl)benzene[36] in the appropriate 1:2 molar ratio and stirred for 24 h at room temperature with no other precaution. Initially, no change was observed, but on a larger scale, we noticed the mixture turned yellow and thickened. The ^{1}H NMR spectrum showed all the expected shifts with faint traces of both starting materials (less than 2%), but the HRMS showed a very clean spectrum with nothing but the desired product. In large quantities (>2 g scale), an intense exothermic reaction lasting a few seconds was observed, bringing the reactants to their boiling point and, therefore, to the decomposition of the starting materials and the desired product. This exothermic reaction can be considered as empirical proof of the high efficiency of the click reaction with these specific moieties. After a series of optimizations that were monitored by ^{1}H NMR and ESI MS, we chose to start the reaction with a minimum amount of chloroform at 0 °C under stirring for about an hour before the mixture was heated to 60 °C for 16 h. The addition of a small amount of chloroform or dichloromethane was necessary to decrease the viscosity and increase the homogeneity of the reaction mixture. Also, the gentle heating was necessary to bring the reaction to completion. Indeed, small amounts (up to 10%) of unreacted starting materials were still present when the reaction was started in an ice bath instead of at room temperature when no further heat was applied. Following these optimized conditions, we obtained the desired **X-EO2-G1-I** in 98% yield using this simple protocol. This reaction can also be achieved on multigrams scale with the same result.

The second step in the iterative process to build the dendrimer involved the replacement of all the terminal iodo atoms into azides. This reaction was performed at 70 °C in DMF as a solvent for 16 h to provide **X-EO2-G1-N$_3$** in 97% yield. ^{1}H NMR (Figure 2) easily assessed the complete conversion of iodo into azides since both EO chains on any given generation are not exactly symmetrical toward the triazole branching point, meaning that each methylene unit is well resolved. Furthermore, the chemical shifts of the protons of the terminal methylene groups are very dependent on the nature of the heteroatom attached. Thus, the signals coming from iodomethylene (3.25 ppm) and azidomethylene (3.37 ppm) never overlap and it is very clear whether or not the click or the S_N2 reaction (I→ N_3) has been brought to completion, even at higher generations. This analysis can also be applied to detect if there is any unreacted diester synthon (compound **3**) remaining in the mixture. Thus, careful examination of the 3.10−3.50 ppm region suggests that all the starting materials were consumed after each click reaction on any generation, meaning that potential impurities are present at a concentration below 2% (*vide infra*). These clean conversions are quite surprising considering that no purification except usual workup (to remove the DMF after the iodo conversion to azide) was performed all along the divergent growth. This is a tremendous advantage compared to other divergently grown dendrimers.[1] With the aforementioned optimized reactions, **X-EO2-G2-I**, **X-EO2-G2-N3**, and **X-EO2-G3-I** were readily obtained in similar yields and excellent purity with great ease.

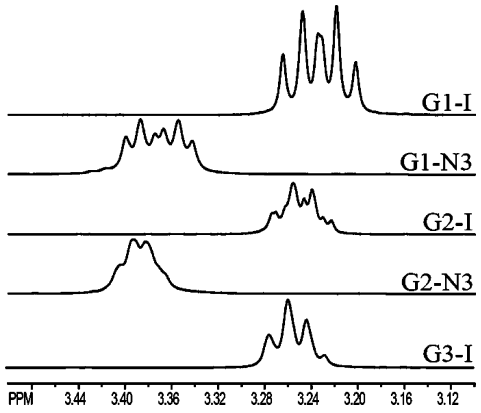


Figure 2. ^{1}H NMR spectra in the terminal methylene region during the growth of **X-EO2-G3-I**.

Size-exclusion chromatography (SEC) analysis was performed in THF for **X-EO2-G1-I**, **X-EO2-G2-I**, and **X-EO2-G3-I**, and the results are shown in Figure 3. As expected, all the dendrimers show a very sharp and well-resolved peak. However, unlike what was observed by ^{1}H NMR analysis, very small amounts of the previous generation dendrimer can

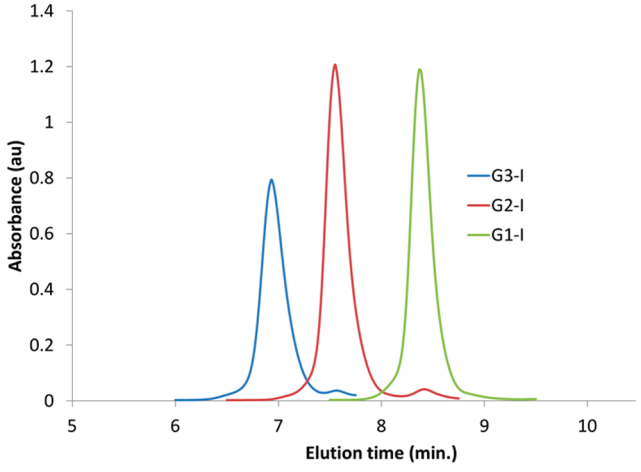
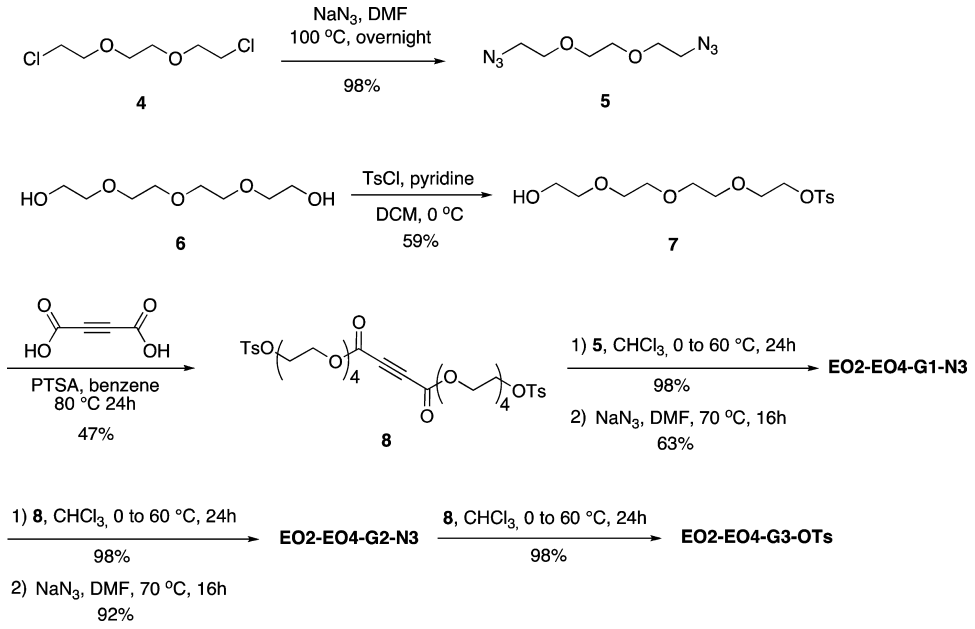


Figure 3. SEC traces for **X-EO2-G1-I**, **X-EO2-G2-I**, and **X-EO2-G3-I** (UV/vis detector, THF as solvent).

dx.doi.org/10.1021/ma300648r | *Macromolecules* 2012, 45, 3687−3694

Scheme 2. Synthesis of X-EO2-G3-I



be found in **X-EO2-G2-I** and **X-EO2-G3-I** (Figure 3). A quantitative analysis of those peaks confirms that those lower generation dendrimers are present in very low concentrations (<2%), which is still very low for unpurified materials. One can assume that the preparation of such dendrimers on larger scale would be beneficial regarding dendrimers purity since the amount of reagents can be better controlled. Studies in this regard are currently underway since biological applications required very pure materials. Nevertheless, analytically pure samples can be obtained by purification using preparative SEC.

In order to extend the scope of our strategy, we prepared analogous dendrimers with structural differences. Initially, we replaced the xylene core by a small ethylene oxide moiety (see Supporting Information). As expected, the reaction yields and the purity of the dendrimers were also very good. A more significant change was to extend the length of the ethylene oxide moieties in the dendrimers. As with any dendritic architecture, branch length dictates, in part, how densely a dendrimer will pack on itself.[37] Denser packing may lead to steric hindrance, which in turn results in a restricted growth passed a certain generation. Furthermore, we hypothesized that longer EO branches could enhance the hydrophobicity of the dendrimers. Thus, tetraethylene glycol was selected to investigate these issues. Since unsymmetrical tetraethylene glycol derivatives are not commercially available, a dissymmetrization reaction was performed directly on tetraethylene glycol. Following Bauer et al.,[31] we introduced a tosyl group to one end of the tetraglycol (Scheme 2) to yield compound **7** in a 59% yield. The above-mentioned esterification method using Dean–Stark apparatus was used to couple compound **7** to acetylenedicarboxylic acid in a 47% yield. The click reactions and the conversions of tosylate to azido groups proceeded very efficiently until the third generation was reached. At that point, IR analysis showed some unreacted azide groups and SEC analysis (see Supporting Information) showed remaining monomer **8** and dendrimer precursors in an estimated 5% ratio. Trying to push the reaction toward completion, 2% molar excess monomer was added and the reaction was heated again for 72 h. Unfortunately, no change was observed. Simple geometric optimization indicated that dendrimer termini lie relatively closer to each other, thus harming the azide reactivity toward disubstituted alkynes. This new series of dendrimers is probably packed much more densely than its shorter-chain counterpart. In light of this result, we turned back to the **EO2-EO2-G*n*** series to optimize biocompatibility. A fourth generation dendrimer bearing 32 carboxylate groups (**EO2-EO2-G4-COO$^-$Na$^+$**, Figure 1) was synthesized following the iterative strategy we developed. As expected, **EO2-EO2-G4-COO$^-$Na$^+$** is very soluble in water. NMR and MS analysis failed to acertain its completion, but IR showed a clean spectrum, free of any azide group, even at the fourth generation (see Supporting Information). The choice of carboxylate as a terminal decoration was motivated by previous evidence with a PAMAM dendrimer that anionic dendrimers are far less toxic than their cationic versions.[38]

A proliferation assessment on two cell lines (PANC-1 and Ovcar-3) with concentrations ranging from 0.1 to 100 μM was performed alongside a positive control of doxorubicine. In all cases, compound **EO2-EO2-G4-COO$^-$Na$^+$** showed no toxicity, as cell proliferation was never impeded upon treatment with this dendrimer (see Supporting Information). These preliminary results confirm that the EO-containing dendrimers presented here did not present any toxicological activity as expected and should be suitable for biomedical applications. Detailed biological evaluation of this new class of dendrimers is now underway.

■ CONCLUSION

In summary, we have prepared EO-containing dendrimers containing triazole units using a relatively simple and efficient divergent method. No toxic metal and excess of reagents were required to obtain nearly defect free dendrimers in high yield. The hydrophilic nature of these dendrimers and the absence of metal during the synthesis make these dendrimers very promising candidates for bio-related applications. Preliminary *in vitro* proliferation testing on different cell lines shows that the anionic version of this series of dendrimers is not toxic for ovarian and pancreatic cells. This opens the way to the use of

dx.doi.org/10.1021/ma300648r | *Macromolecules* 2012, 45, 3687–3694

this readily prepared dendrimeric scaffold for bio-related applications. Dendrimers with functional and bioactive termini are under preparation.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and characterization data for all the new compounds; SEC traces and toxicological assays results for **EO3-EO2-G4-COO$^-$Na$^+$**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jean-francois.morin@chm.ulaval.ca.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the National Science and Engineering Council of Canada (NSERC) for financial support and Dr. Donald Poirier and Lucie Carolle Kenmogne from CRCHUQ for toxicological assays. Mathieu Arseneault thanks the NSERC for a PhD scholarship. We are also grateful for professional support from CQMF and CERMA.

■ REFERENCES

(1) Grayson, S. M.; Fréchet, J. M. J. *Chem. Rev.* **2001**, *101*, 3819−3867.

(2) Fréchet, J. M. J., Tomalia, D. A., Eds.; *Dendrimers and other Dendritic Polymers*; John Wiley & Sons: West Sussex, UK, 2001.

(3) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117−132.

(4) For a recent example, see: Zill, A.; Rutz, A. L.; Kohman, R. E.; Alkilany, A. M.; Murphy, C. J.; Kongb, H.; Zimmerman, S. C. *Chem. Commun.* **2011**, *47*, 1279−1281.

(5) Dhanikula, R. S.; Hildgen, P. *Bioconjugate Chem.* **2006**, *17*, 29−41.

(6) Liu, M.; Fréchet, J. M. J. *Pharm. Sci. Technol. Today* **1999**, *2*, 393−401.

(7) Barth, R. F.; Adams, D. M.; Soloway, A. H.; Alam, F.; Darby, M. V. *Bioconjugate Chem.* **1994**, *5*, 58−66.

(8) Venditto, V. J.; Regino, C. A. S.; Brechbiel, M. W. *Mol. Pharmaceutics* **2005**, *2*, 302−311.

(9) New, K.; Bryant, L. H.; Brechbiel, M. W. *Bioconjugate Chem.* **2010**, *21*, 1014−1017.

(10) Peterson, J.; Allikmaa, V.; Pehk, T.; Lopp, M. *Proc. Estonian Acad. Sci. Chem.* **2001**, *50*, 167−172.

(11) Medina, S. H.; El-Sayed, M. E. H. *Chem. Rev.* **2009**, *109*, 3141−3157.

(12) Veronese, F. M.; Harris, J. M. *Adv. Drug Delivery Rev.* **2002**, *54* (4).

(13) Rucareanu, S.; Maccarini, M.; Shepherd, J. L.; Lennox, R. B. *J. Mater. Chem.* **2008**, *18*, 5830−5834.

(14) Ballou, B.; Lagerholm, B. C.; Ernst, L. A.; Bruchez, M. P.; Waggoner, A. S. *Bioconjugate Chem.* **2004**, *15*, 79−86.

(15) Kojima, C.; Kono, K.; Maruyama, K.; Takagishi, T. *Bioconjugate Chem* **2000**, *11*, 910−917.

(16) Haag, R.; Kratz, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 1198−1215 and references therein.

(17) Feng, X.-S.; Taton, D.; Chaikof, E. L.; Gnanou, Y. *J. Am. Chem. Soc.* **2005**, *127*, 10956−10966.

(18) Feng, X.; Taton, D.; Ibarboure, E.; Chaikof, E. L.; Gnanou, Y. *J. Am. Chem. Soc.* **2008**, *130*, 11662−11676.

(19) Feng, X.; Taton, D.; Gnanou, Y. *Macromolecules* **2009**, *42*, 7292−7298.

(20) Feng, X.; Taton, D.; Borsali, R.; Chaikof, E. L.; Gnanou, Y. *J. Am. Chem. Soc.* **2006**, *128*, 11551−11562.

(21) Berna, M.; Dalzoppo, D.; Pasut, G.; Manunta, M.; Izzo, L.; Jones, A. T.; Duncan, R.; Veronese, F. M. *Biomacromolecules* **2006**, *7*, 146−153.

(22) Dhanikula, R. S.; Hildgen, P. *Bioconjugate Chem.* **2006**, *17*, 29−41.

(23) Dhanikula, R. S.; Hildgen, P. *Biomaterials* **2007**, *28*, 3140−3152.

(24) Arseneault, M.; Dufour, P.; Levesque, I.; Morin, J.-F. *Polym. Chem.* **2011**, *2*, 2293−2298.

(25) Zhang, Q.; Sha, Y.; Wang, J.-H. *Molecules* **2010**, *15*, 2962−2971.

(26) Metal-free click chemistry was used by Prof. Mike Brook (McMaster University, Canada) to prepare a completely different dendrimer with other building blocks. No report on this from the Brook group has been published yet.

(27) Qin, A.; Jim, C. K. W.; Lu, W.; Lam, J. W. Y.; Haüssler, M.; Dong, Y.; Sung, H. H. Y.; Williams, I. D.; Wong, G. K. L.; Tang, B. Z. *Macromolecules* **2007**, *40*, 2308−2317.

(28) Li, H.; Wang, J.; Sun, J. Z.; Hu, R.; Qin, A.; Tang, B. Z. *Polym. Chem.* **2012**, *3*, 1075−1083.

(29) Kharb, R.; Sharma, P. C.; Yar, M. S. *J. Enzyme Inhib. Med. Chem.* **2011**, *26*, 1−21.

(30) Saha, S.; Johansson, E.; Flood, A. H.; Tseng, H. R.; Zink, J. I.; Stoddart, J. F. *Chem.—Eur. J.* **2005**, *11*, 6846−6858.

(31) Bauer, H.; Stier, F.; Petry, C.; Knorr, A.; Stadler, C.; Staab, H. A. *Eur. J. Org. Chem.* **2001**, 3255−3278.

(32) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021.

(33) Abu-Orabi, S. T.; Al-Hamdany, R.; Atfah, A.; Ali, A. A. S.; Abu-Shandi, K. *Asian J. Chem.* **1999**, *11*, 774−783.

(34) Wuytswinkel, G. V.; Verheyde, B.; Compernolle, F.; Toppet, S.; Dehaen, W. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1337−1340.

(35) Kislukhin, A. A.; Higginson, C. J.; Finn, M. G. *Org. Lett.* **2011**, *13*, 1832−1835.

(36) Abu-Orabi, S. T.; Harmon, R. E. *J. Chem. Eng. Data* **1986**, *31*, 379−380.

(37) Ruiz, J.; Lafuente, G.; Marcen, S.; Ornelas, C.; Lazare, S.; Cloutet, E.; Blais, J.-C.; Astruc, D. *J. Am. Chem. Soc.* **2003**, *125*, 7250−7257.

(38) Wiwattanapatapee, R.; Carreno-Gomez, B.; Malik, N.; Duncan, R. *Pharm. Res.* **2000**, *17*, 991−998.