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Selectively Functionalized Glycerol/Diacid Dendrimers via Click Chemistry of Azido Fatty Acids

Jonathan A. Zerkowski · Alberto Nuñez · Daniel K. Y. Solaiman

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Abstract Dendrimers consisting of glycerol and dicarboxylic acid units were synthesized using a convergent or "outside-in" strategy. The multimeric arms, or dendrons, are joined to a central core in the final step using the azide/alkyne click reaction. The combination of these approaches allows the preparation of dendrimers with variable and controlled degrees of substitution at their periphery. For example, a single protected amino acid has been situated at the periphery, with all other substituents being hydrophobic. In another example, all the peripheral substituents are protected amino acids. The identity of the dendrimers is unambiguous due to the purification and characterization of all the synthetic intermediates. Functional groups have also been selectively incorporated along the arms (dicarboxylic acids) and at the cores.

Keywords Dendrimers · Nanochemistry · Functionalized oligoesters · Click reaction

Introduction

Glycerol, with its three reactive functional groups, is an obvious choice as a building block for highly branched oligomers or polymers. The practical advantages that a highly branched structure, as opposed to a linear one, can possess have been thoroughly reviewed elsewhere [1]; two that deserve mention are the increased density of terminal or surface or capping groups, which can be chosen to impart a property of choice, and the possibility of entanglement or hosting of a guest molecule at the interior. This combination of features has led to the use of hyperbranched materials as drug delivery agents and matrices for tissue engineering [2, 3]. As the partner reactant for glycerol, dicarboxylic acids are also logical choices, considering the ease of making ester linkages, and the likely biocompatibility of these building blocks. (Polyglycerol, which is also highly branched, will not be considered here since as a homopolyether it is chemically quite distinct, and it is not necessarily prepared from glycerol itself but from other three-carbon units [4]). There have been two general styles of routes to glycerol/diacid hyperbranched materials. First is the "all-at-once" route, where the two reactants are combined in a selected ratio and esterified using a catalyst (either inorganic or enzymatic) [3, 5–7]. Second is a stepwise route where protected monomer units are added one layer or shell at a time [8]. Both routes have their strengths and drawbacks. The first is simple and suited to larger scale, but there is little control over the extent of reaction, and many glycerol OH groups may remain unreacted. The second offers better control over the thoroughness of reaction, although once again at later stages the increased number of reacting groups may make 100% completion difficult to achieve, and there is the complementary problem that every single protecting group needs to be removed cleanly. Furthermore, the completed macromolecule still can only be derivatized globally and not at selected sites. These two routes are illustrative of the distinction between hyperbranched polymers (the former route) where an incomplete reaction leads to incomplete branching and "dangling" or free OH groups, and a true

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J. A. Zerkowski (✉) · A. Nuñez · D. K. Y. Solaiman
USDA, ARS, Eastern Regional Research Center,
600 E. Mermaid Lane, Wyndmoor, PA 19038, USA
e-mail: jonathan.zerkowski@ars.usda.gov

"dendrimer" structure (the latter) where the degree of branching is exactly 1 and all OH groups have been esterified (except for any that are intentionally left at the final surface). Another important distinction between the two routes is that molecular characterization of the product of the one-pot route, e.g., by NMR or mass spectrometry, can be extremely challenging, since there are many alternative incomplete branching patterns, while the stepwise nature of the other route allows characterization along the way. In fact, the broad product distribution of the former route makes clear-cut structure/function relationships murky at best.

To demonstrate the versatility of structural and functional control that the basic pattern of glycerol/diacid oligomers can offer, but that has yet to be taken full advantage of, we report here the modular synthesis of glycerol/dicarboxylic oligomers that are tailored at their periphery, arms, and core. Our method hinges on two key strategies. First is a convergent approach that builds the dendrimer from the outside in. Second is use of the azide/alkyne "click" reaction for linking the bulky branched arms to a central core, a reaction that has proven to be reliable in a number of sterically demanding situations, e.g., at cell surfaces [9] as well as in the construction of dendrimers [10–12]. We recently prepared some azido fatty acids and used the click reaction to ligate them to biomolecules [13], so we were prompted to consider their use in the construction of larger aggregates such as dendrimers. One specific aim was to be able to choose the extent of labeling at the exterior of the dendrimer, that is, to vary the coverage from single-point derivatization to full coating with a functional group of choice, which is an area of active research in dendrimer chemistry [14]. A further aim was to construct nanoscale molecules of a defined (monodisperse) size and shape from bioderived, renewable building blocks.

Materials and Methods

17-Azido stearic acid was synthesized as previously described [13]. Chemicals and reagents were obtained commercially from Sigma-Aldrich (St. Louis, MO, USA) and Lancaster Synthesis (Alfa Aesar, Ward Hill, MA, USA). All solvents and reagents were used as received. Silica gel (Grade 60 Å, Mesh 230–400, particle size 40–63 μm), used for all column chromatography, was obtained from Fisher Scientific (Fairlawn, NJ, USA). Solvents were removed on a Yamato rotary evaporator. NMR spectra were recorded in $CDCl_3$ on either a Varian Associates (Walnut Creek, CA, USA) Gemini 200 MHz or Inova 400 MHz instrument and were referenced to $Si(CH_3)_4$. Atmospheric pressure chemical ionization (APCI) MS data were recorded on a Waters/Micromass (Milford, MA, USA) ZMD instrument, using direct injection without an LC column. Matrix-Assisted Laser Desorption/Ionization mass spectra with automated tandem time of flight fragmentation of selected ions (MALDI-TOF/TOF) were acquired with an ABI 4700 Proteomics Analyzer mass spectrometer (Applied Biosystems, Framingham, MA, USA) in the positive reflectron mode. Masses were determined as sodiated adducts of the products, $[M + Na]^+$, using 2,5-dihydroxybenzoic acid as matrix in a concentration of 10 mg/mL in acetonitrile/water (1:1) with 0.1% TFA. Approximately 0.7 μL of matrix was spotted on the MALDI plate and allowed to dry. The sample (0.5–1 μL) dissolved in chloroform or acetone at a concentration of 1–2 mg/mL was spotted on the top of the matrix crystals. Averages of 1,000–2,000 spectra were acquired for optimal signal to noise ratio.

Synthesis of Benzyl Glyceryl Adipate, **1b**

To a solution of adipic acid (5.0 g, 34 mmol), benzyl alcohol (3.7 g, 34 mmol), solketal (4.5 g, 34 mmol), and triethylamine (20 mL, 0.14 mol) in 50 mL THF, cooled in an ice bath and stirred magnetically, is added a solution of benzoyl chloride (9.5 g, 68 mmol) in 10 mL THF dropwise over the course of 5 min [15]. After about half of the benzoyl chloride solution has been added, 4-dimethylamino pyridine (DMAP, 200 mg, 1.6 mmol) is added, and when the dropwise addition is complete (a thick precipitate forms), another 200 mg of DMAP is added. The solution is allowed to warm to room temperature overnight. Solvent is removed, and the crude is taken up in 100 mL ethyl acetate and washed with 50 mL each saturated citric acid, 1 M NaOH, and water. Solvent is again removed, and the crude is chromatographed on silica gel ($R_f = 0.3$ in 3:1 hexane/ethyl acetate) to yield **1a** (4.8 g, 81% based on an ideal 1:2:1 ratio of the mixed diester to the two symmetrical diesters).

To remove the acetonide, the benzyl/solketal adipic diester **1a** is dissolved in approximately 10 mL of 80% $AcOH/H_2O$. The solution is heated at 45 °C for 3–4 h, then stirred at RT overnight. The syrup is poured into a crystallizing dish and evaporated under a stream of nitrogen. It is then dissolved in 1:1 EtOAc/hexane and passed through a short silica column to remove all traces of acetic acid. The yield is quantitative. ^{1}H NMR: 1.64–1.78 (m, 4H, internal CH_2), 2.33–2.47 (m, 4H, $C(O)CH_2$), 3.55–3.76 (m, 2H, CH_2OH), 3.83–4.01 (m, 1H, $CH(OH)$), 4.11–4.28 (m, 2H, $C(O)OCH_2$), 5.14 (s, 2H, CH_2Ar), 7.36 (br s, 5H, Ar). ^{13}C NMR: 24.3, 24.4, 33.9 $C(O)CH_2$, 63.4 CH_2OH, 65.4 CH_2Ar, 66.4 CHOH, 70.3 esterified gly CH_2, (128.3, 128.4, 128.7, and 136.0) all Ar, 173.5 and 173.7 C=O.

General Procedure for Pivalic Anhydride Couplings [16]: Synthesis of Core **3**

To a solution of 17-azido stearic acid [13] (0.65 g, 2.0 mmol) and glycerol (52 mg, 0.56 mmol) in 10 mL dioxane was added a solution of pivalic anhydride (0.39 g, 0.43 mL, 2.1 mmol) in 2 mL THF, followed by 4-dimethylamino pyridine (DMAP, 10 mg, 0.08 mmol). The mixture was stirred vigorously with a magnetic stir bar at RT overnight, then solvent was removed on the rotary evaporator and the crude was purified by flash chromatography on silica gel to afford **3** (0.53 g, 93%). In the synthetic steps that follow, the "standard procedure" is to use the coupling agent, pivalic anhydride, in slight excess (10–20%) relative to the carboxylic acid component, along with 10–20 mg DMAP as catalyst. This method routinely gave yields better than 80% and frequently better than 90%. ^{1}H NMR: 1.18–1.41 (m, 81H), 1.41–1.52 (m, 6H), 1.55–1.70 (m, 6H),

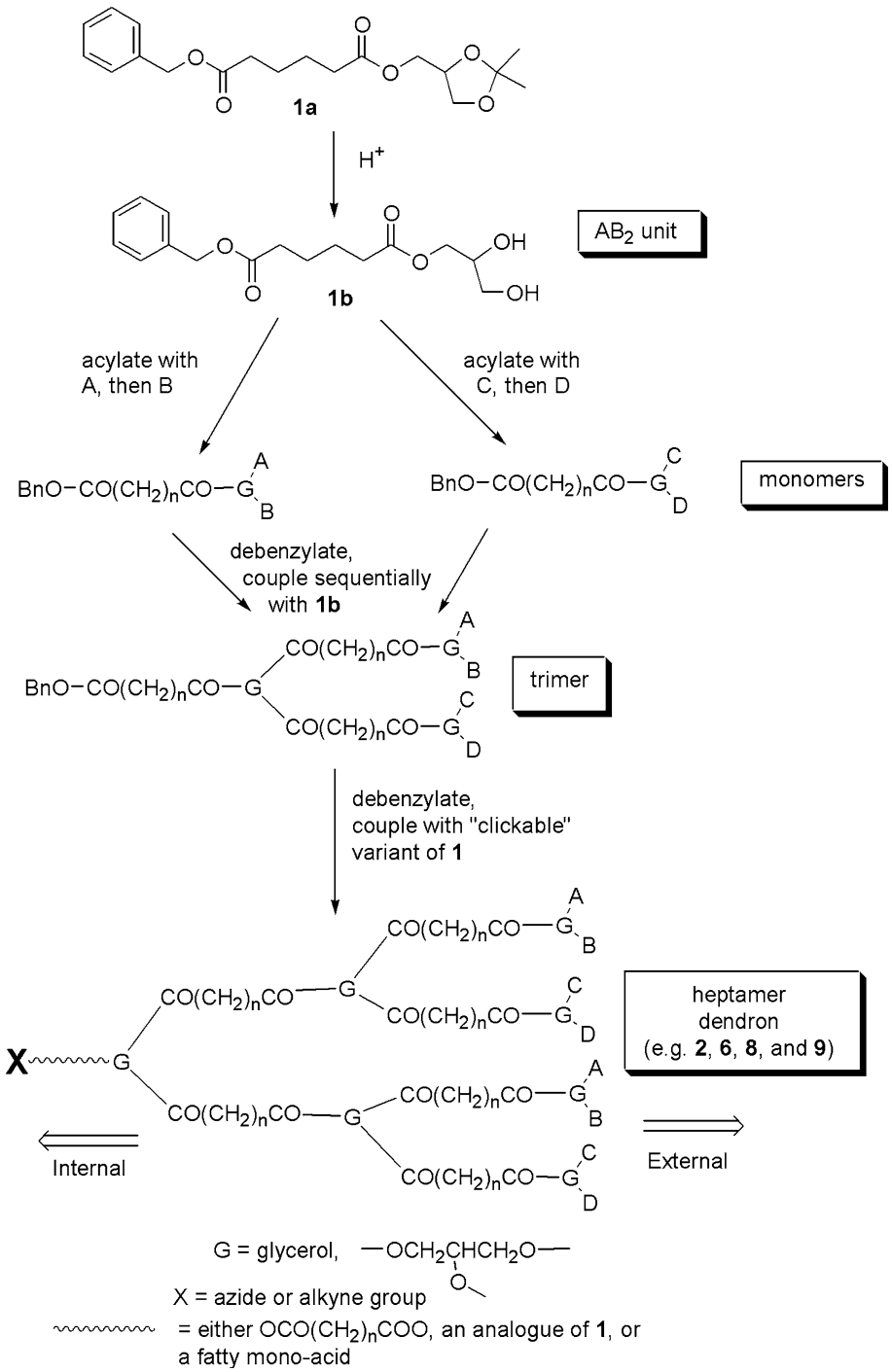
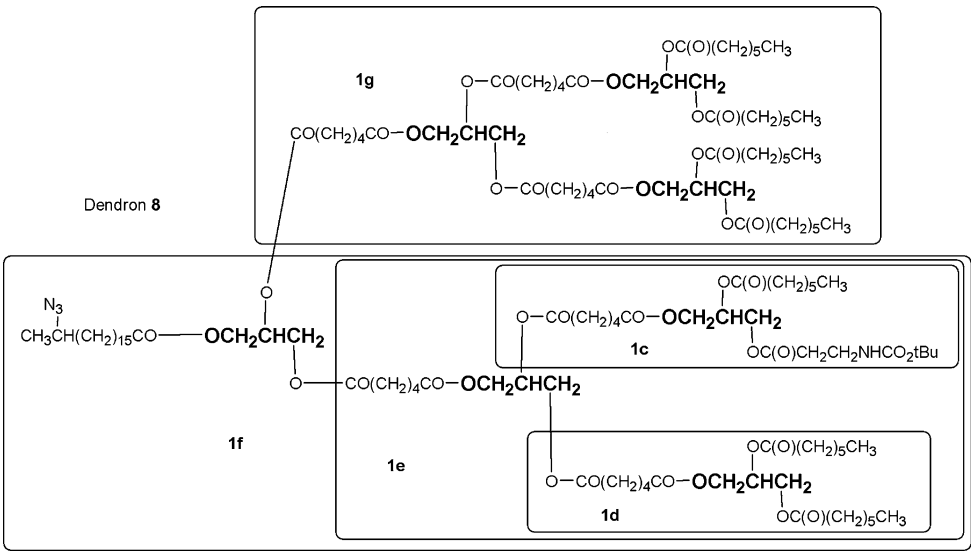


Fig. 1 General outline for the iterative construction of clickable dendrons. In this paper, $n = 4$ (adipic acid). Compare to Scheme 1, where the "nested" character of specific intermediates is diagrammed explicitly in the building up of dendron **8**

Scheme 1



2.33 (t, 7.2 Hz, 6H, $C(O)CH_2$), 3.42 (hex, 6.5 Hz, 3H, C(17)H-N_3), 4.08–4.21 and 4.25–4.38 (m, 4H, gly CH_2), 5.21–5.37 (m, 1H, gly CH). ^{13}C NMR: 19.6 CH_3, 25.0, 26.2, 29.2–29.8, 34.2, 36.3 C(O)*C*H_2, 58.1 C(17)-N_3, 62.2 gly CH_2, 69.0 gly CH, 173.0 and 173.4 C=O. APCI: calculated for $C_{57}H_{107}N_9O_6{\cdot}Na^+$: 1,036.8 Da, found 1,037.0 Da.

Representative Synthesis of a Dendron (Molecule **8**) (Refer to Figs. 1, 5; Scheme 1)

To a solution of diol **1b** (1.15 g, 3.70 mmol) and Boc-*β*-alanine (0.53 g, 2.8 mmol) in 10 mL THF is added a solution of pivalic anhydride (0.52 g, 2.8 mmol) in 2 mL THF dropwise, followed by DMAP (15 mg, 0.1 mmol). A substoichiometric amount of the alanine is used to minimize formation of its di-adduct onto **1b**. The solution is stirred magnetically overnight. Solvent is removed and the diacylglycerol product—$C_6H_5CH_2OC(O)(CH_2)_4C(O)OCH_2CH(OH)CH_2OC(O)CH_2CH_2NHCO_2tBu$—is isolated by column chromatography, which is also used to purify all subsequent synthetic intermediates (0.99 g, 74%). This diacylglycerol (0.6 g, 1.3 mmol) is then reacted with caproic acid (0.2 g, 1.7 mmol) using the standard pivalic anhydride/DMAP protocol (260 mg, 1.4 mmol pivalic anhydride, 10 mg DMAP) to yield adipoyl triacylglycerol **1c**, schematically represented by the AB monomer in Fig. 1 (0.65 g, 86%). After it is purified, it is dissolved in THF and the benzyl ester is removed hydrogenolytically over 5% Pd/C to yield 0.49 g (1.0 mmol). A second monomer **1d**, having two identical acyl units attached to the glycerol unit, (i.e. C=D in Fig. 1) was prepared similarly from **1b** (1.2 g, 3.9 mmol) and two equivalents of caproic acid (1.0 g, 8.6 mmol) and likewise debenzylated. The next step is constructing a trimer (see Fig. 1), which is done by coupling these two monomers sequentially to a new unit of diol **1b**. First the dicaproic monomer **1d** (0.42 g, 1.0 mmol) was coupled to **1b** (0.65 g, 2 mmol). The resulting mono-hydroxy compound (0.60 g, 0.85 mmol) was then reacted with the Boc-*β*-Ala/caproic monomer **1c** (0.49 g, 1.0 mmol). This trimer is shown in condensed fashion in the open rectangle at the top of Fig. 5 and explicitly as **1e** in Scheme 1. The benzyl ester is removed hydrogenolytically, and the resulting free acid (0.82 g, 0.75 mmol) is coupled to the monoacylglycerol of 17-azido stearic acid, $H_3C–CH(N_3)(CH_2)_{15}CO–OCH_2$-$CH(OH)CH_2(OH)$ (1.0 g, 2.5 mmol) to afford a tetramer unit **1f** that still possesses one free OH. An excess of the mono-17-azido stearoyl glycerol is used so that di-acylation is minimized; unreacted mono-17-azido stearoyl glycerol is readily recovered with column chromatography. After purification, this molecule **1f** (0.85 g, 0.58 mmol) is coupled with a second trimer **1g** (0.68 g, 0.67 mmol) possessing four caproic units (Scheme 1 and shaded rectangle at the top of Fig. 5), to afford the heptameric dendron **8** (see Fig. 5 and Scheme 1), 1.2 g (0.49 mmol, 84% for this final acylation). ^1H NMR: 0.88 (t, 6.6 Hz, 21H, caproic CH_3), 1.20–1.41 m, 1.44 (s, 9H, Boc tBu), 1.53–1.76 m, 2.22–2.45 (m, 40H, $C(O)CH_2$), 2.56 (t, 6.0 Hz, 2H, *β*-Ala $C(O)CH_2$), 3.41 (m, 3H, *β*-Ala C*H*$_2$NH and C(17)H-N_3), 4.09–4.23 and 4.25–4.50 (m, 28H, gly CH_2's), 5.04–5.16 (m, 1H, Boc-NH), 5.21–5.36 (m, 14H, gly CH). ^{13}C: 14.0, 21.1, 22.4, 24.2–24.9, 27.2, 28.5, 29.2–29.9, 31.2, 33.6–34.6, 36.1, 41.1, 58.2 C(17)–N_3, 62.1–62.6, 68.8–69.1, 79.5, 155.6, 172.3–173.3. MALDI: calculated for $C_{125}H_{208}N_4O_{44}{\cdot}Na^+$: 2,492.41 Da, found 2,492.46 Da.

Dendrons **2**, **6**, and **9** were built up using similar iterative steps. Other Dendron MALDI data:

Dendron **2**: calculated for $C_{154}H_{254}N_8O_{60}{\cdot}Na^+$ 3,198.70 Da, found 3,198.75 Da.

Dendron **6**: calculated for $C_{137}H_{229}N_3O_{46}{\cdot}Na^+$ 2,675.57 Da, found 2,675.61 Da.
Dendron **9**: calculated for $C_{117}H_{189}NO_{44}{\cdot}Na^+$ 2,335.25 Da, found 2,335.26 Da.

Synthesis of the Triazine Core (**5**, Fig. 4)

(**A**) Propylamino-dichloro-triazine was prepared by reacting propylamine (0.16 g, 2.7 mmol) with cyanuric chloride (0.5 g, 2.7 mmol) at RT in 15 mL THF for 3 h. Removal of solvent and column chromatography afforded the product (0.24 g, 86%), $CH_3CH_2CH_2NH(C_3N_3)Cl_2$. (**B**) *N*-phenoxyacetamidoyl *γ-tert*-butyl glutamic piperazine amide: Z-piperazine (Z = $C_6H_5CH_2OCO$, 0.77 g, 3.5 mmol) was coupled to Fmoc-Glu(OtBu)-OH (1.5 g, 3.5 mmol), with diisopropylcarbodiimide (DIC, 0.44 g, 3.5 mmol) in 20 mL CH_2Cl_2 for 2 h. Solvent was removed, then 10 mL *N*,*N*-diisopropylethylamine and 1,8-diazabicycloundecene (0.76 g, 5 mmol) were added to remove the Fmoc group. Reaction proceeded for 18 h, then solvent was removed, 15 mL dioxane was added, and phenoxy acetic acid (0.61 g, 4 mmol) and DIC (0.44 g, 3.5 mmol) were added. The mixture was stirred magnetically overnight. Solvent was removed, and column chromatography gave 1.15 g, 61 % from Fmoc-Glu(OtBu)OH. Finally the Z group was removed from the piperazine with H_2 on 5% Pd/C in 25 mL EtOH to give *N*-phenoxyacetamidoyl *γ-tert*-butyl glutamic piperazine amide, $C_6H_5OCH_2C(O)NHCH(CH_2CH_2COOtBu)C(O)(NC_4H_8NH)$ (0.80 g, 95%). (**C**) To a solution of propylamino-dichloro-triazine (0.17 g, 0.80 mmol) in 25 mL dioxane was added *N*-phenoxyacetamidoyl *γ-tert*-butyl glutamic piperazine amide (0.73 g, 1.8 mmol) and the solution was refluxed under N_2 for 6 h. Over the course of this time, *N*,*N*-diisopropylethylamine was added periodically (0.23 g, 1.8 mmol) to neutralize the HCl that is formed. After removal of solvent, the product was purified by column chromatography to give 636 mg product (84%). The *t*-Bu protecting groups were removed with CF_3CO_2H, which was then removed under a stream of N_2. To prepare the di-propargyl ester core **5**, the free diacid (0.44 g, 0.53 mmol) and propargyl alcohol (280 mg, 5 mmol) were reacted using the pivalic anhydride/DMAP protocol (0.37 g, 2.0 mmol, and 20 mg, respectively). After purification (R_f = 0.4 in ethyl acetate), 415 mg (87%) of **5** was obtained. Calculated for $C_{46}H_{56}N_{10}O_{10}{\cdot}H^+$ 909.4, found 909.8 Da by APCI. The NMR spectrum is incorporated within that for dendrimers **7a** and **7b** below.

Synthesis of the Azelaic/Glycerol Carbamate Unit (Fig. 5)

The monobenzyl ester of azelaic acid (2.6 g, 9.3 mmol) and solketal (20 mL, 0.16 mol) were dissolved in 20 mL THF, K_2CO_3 (1.4 g, 10 mmol) was added, and the solution was warmed to approximately 45 °C under N_2. A solution of diphenylphosphoryl azide (2.6 g, 9.5 mmol) in THF was added and the heat was increased to reflux the solution for 7 h, after which time it was stirred at RT overnight. Solvent was removed and the crude was taken up in EtOAc and washed with water. Column chromatography yielded $C_6H_5CH_2OCO(CH_2)_7NHCO$-solketal, which was then deprotected using H_2 over Pd/C to give the free acid, 1.67 g, 57% from benzyl azelate. This material was coupled with propargyl alcohol using the pivalic anhydride/DMAP method to give the clickable ester, which was subsequently deprotected at the diol end with aq. HOAc as described for **1b** above to give $HCCCH_2OC(O)(CH_2)_7NHCO_2CH_2CH(OH)CH_2(OH)$, 1.41 g, 79%. This diol is coupled with 2 equiv of trimer **1 g** for the preparation of dendron **9**. ^{1}H NMR: 1.24–1.42 (m, 6H), 1.42–1.56 (m, 4H), 1.56–1.75 (m, 4H), 2.37 (t, 7.4 Hz, 2H, $CH_2C(O)$), 2.49 (t, 2.6 Hz, alkyne CH), 3.15 (q, 6.7 Hz, 2H, CH_2NH), 3.54–3.74 (m, 2H, CH_2OH), 3.82–3.96 (m, 1H, $CH(OH)$), 4.11–4.27 (m, 2H, $C(O)OCH_2$), 4.69 (d, 2.4 Hz, alkyne–CH_2–O), 5.05–5.20 (m, 1H, NH). ^{13}C NMR: 24.7, 26.5, 28.9, 29.0, 29.8, 34.0 $C(O)CH_2$, 41.2 CH_2NH, 51.9 OCH_2–alkyne, 63.3 gly CH_2OH, 65.9 gly CHOH, 70.8 esterified gly OCH_2, 74.9 HC (alkyne), 77.9 quaternary alkyne, 157.2 carbamate C=O, 173.0 ester C=O. APCI: calculated for $C_{15}H_{25}NO_6{\cdot}Na^+$ 338.2 Da, found 338.3 Da.

Representative Conditions for the Click Reaction

Triacylglycerol core **3** (Fig. 2) (0.11 g, 0.11 mmol) and dendron **2** (1.25 g, 0.39 mmol) were dissolved in 10 mL 2:1 H_2O/t-BuOH and 3 mL THF. The solution was stirred vigorously with a magnetic stir bar. An aqueous solution of $CuSO_4{\cdot}5\ H_2O$ (10 mol%, 10 mg in 0.5 mL) was added, followed immediately by sodium ascorbate (20 mol%, 15 mg in 0.5 mL) and reaction was continued overnight [17]. TLC of the crude showed no remaining **3**. Solvent was removed and the crude was purified by column chromatography to afford dendrimer **4** (1.0 g, 90%). ^{1}H NMR: 0.94 (d, 6.5 Hz, 144 H, Leu CH_3), 1.09–1.35, 1.45 (s, 216 H, Boc tBu), 1.50–1.78, 2.22–2.40 (m, 90H, (C(O)–CH_2), 4.08–4.41 (m, 112H, gly CH_2's and Leu C(α)H), 4.63 (h, 6.8 Hz, 3H, C(17)H–N), 4.92–5.19 (m, 24H, Boc NH), 5.21 (s, 6H, OCH_2–triazole), 5.20–5.38 (m, 22H, gly CH), 7.58 (s, 3H, triazole). ^{13}C NMR (When a range of ppm values is listed, there are several overlapping peaks).: 21.4 $C(18)H_3$, 21.8 Leu CH_3, 22.9, 24.2, 24.9, 26.1, 28.4, 29.2–29.7, 33.6, 33.7, 34.1, 37.3, 41.4–41.6 Leu β-CH_2, 52.2 Leu α-CH, 57.6 C(17)–triazole, 62.0–62.7 gly CH_2 and OCH_2–triazole, 68.9–69.8 gly CH, 79.9 Boc quaternary, 121.6 triazole C, 142.6 triazole CH, 155.5 Boc C=O, 172.4–173.4 carbonyls. MS:

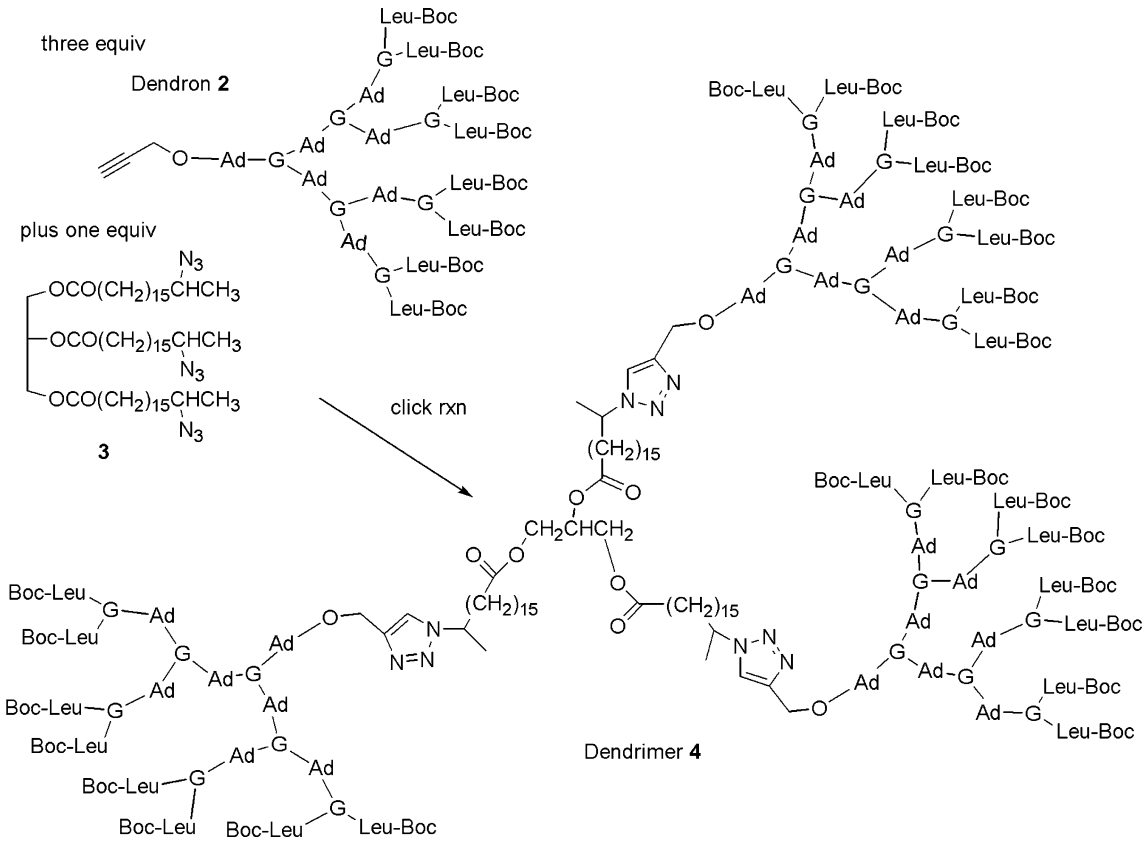


Fig. 2 Construction of a dendrimer with 24 external units. *Ad* adipic, *G* glycerol, *Leu-Boc* C(O)CH(CH$_2$CH(CH$_3$)$_2$)NHC(O)OC(CH$_3$)$_3$

calculated for $C_{519}H_{869}N_{33}O_{186}{\cdot}Na^+$, 10,563.9 Da, found 10,566.7 Da.

Dendrimer **7a**: 0.85 (t, 6.5 Hz, 36H, caproic CH_3), 0.93 (t, 7.3 Hz, 3H, propyl CH_3), 1.04–1.37 m, 1.39 (s, 36H, tBu), 1.46–1.73 m, 1.75–1.91 m, 2.18 (t, 7.2 Hz, 8H, tBuOC(O)CH_2), 2.21–2.47 (m, 84H, other C(O)CH_2), 3.32 (q, 6.9 Hz, 2H, propyl NHCH_2), 3.50–3.70 (m, 8H, piperazine), 3.70–3.91 (m, 8H, piperazine), 4.04–4.19 (m, 28H, gly CH_2), 4.19–4.36 (m, 28H, gly CH_2), 4.47 (m, 4H, CH_2OAr), 4.62 (h, 6.6 Hz, 2H, C(17)H–triazole), 4.86 (t, 6.5 Hz, 1H, propyl NH), 5.05–5.14 (m, 2H, Glu C(α)H), 5.14–5.34 (m, 18H, gly CH and OCH_2–triazole), 6.86–7.03 (m, 6H, Ar), 7.20–7.31 (m, 4H, Ar), 7.57 (d, 8.7 Hz, 2H, NH Glu), 7.64 (s, 2H, triazole). ^{13}C NMR: 11.6 propyl CH_3, 14.0 caproic CH_3, 21.4 C(18)H_3, 22.3, 23.1, 24.2, 24.6–25.0, 26.0, 27.1, 28.2, 28.9–29.7, 31.2, 33.6, 34.1, 35.5, 37.2, (42.2–43.3, 45.4, and 47.6 piperazine and propyl NHCH_2), 57.6 C(17)-triazole, 58.2 Glu C(α)H, 62.1–62.3 gly CH_2 and OCH_2–triazole, 67.2 CH_2OAr, 68.9 gly CH, 79.9 tBu quaternary, 114.8 Ar, 121.5 triazole CH, 122.2 Ar, 129.8 Ar, 142.6 triazole C, 157.1 O–C(Ar) quaternary, 165.3 and 166.4 triazine C, 168.2 and 169.5 amide C=O, 172.3–173.3 carbonyls. MALDI: calculated for $C_{320}H_{514}N_{16}O_{102}{\cdot}H^+$ 6,218.63 Da, found 6,220.51 Da.

Dendrimer **7b** (see text and Fig. 3): 0.88 (t, 6.6 Hz, 36H, caproic CH_3), 0.93 (t, 7.3 Hz, 3H, propyl CH_3), 1.07–1.44 m, 1.49–1.77 m, 1.77–1.93 m, 2.22–2.45 (m, 92H, C(O)CH_2), 2.49 (t, 2.6 Hz, 4H, alkyne CH), 3.36 (q, 6.9 Hz, 2H, propyl NHCH_2), 3.55–3.72 (m, 8H, piperazine CH_2), 3.72–3.94 (m, 8H, piperazine CH_2), 4.05–4.22 (m, 28H, gly CH_2), 4.22–4.38 (m, 28H, gly CH_2), 4.49 (m, 4H, CH_2OAr), 4.63 (q, 6.6 Hz, 2H, C(17)H–triazole), 4.66 (d, 2.5 Hz, 8H, OCH_2–alkyne), 4.94 (t, 6.5 Hz, 1H, propyl NH), 5.08–5.19 (m, 2H, Glu C(α)H), 5.19–5.36 (m, 18H, gly CH and OCH_2–triazole), 6.90–7.07 (m, 6H, Ar), 7.23–7.39 (m, 4H, Ar), 7.60 (d, 8.5 Hz, 2H, NH Glu), 7.67 (s, 2H, triazole). ^{13}C NMR: 11.6 propyl CH_3, 14.0 caproic CH_3, 21.4 C(18)H_3, 22.4, 23.1, 24.2, 24.6–24.9, 26.1, 27.2, 28.3, 28.9–29.8, 31.2, 33.6, 34.2, 37.3, (42.3–43.3, 45.4, and 47.6 piperazine and propyl NHCH_2), 51.8 OCH_2–alkyne, 57.6 C(17)–triazole, 58.2 glu C(α), 62.1–62.4 gly CH_2 and OCH_2–triazole, 67.2 CH_2O–Ar, 68.9 and 69.1 gly CH, 74.9 alkyne CH, 77.9 quaternary alkynyl C, 114.8 Ar, 121.5 triazole CH, 122.2 Ar, 129.8 Ar, 142.6 triazole C, 157.2 O–C(Ar) quaternary, 165.3 and 166.4 triazine C, 168.2 and 169.5 amide C=O, 172.3–173.3 carbonyl. MALDI: calculated for $C_{316}H_{490}N_{16}O_{102}{\cdot}H^+$ 6,142.37 Da, found 6,142.54 Da.

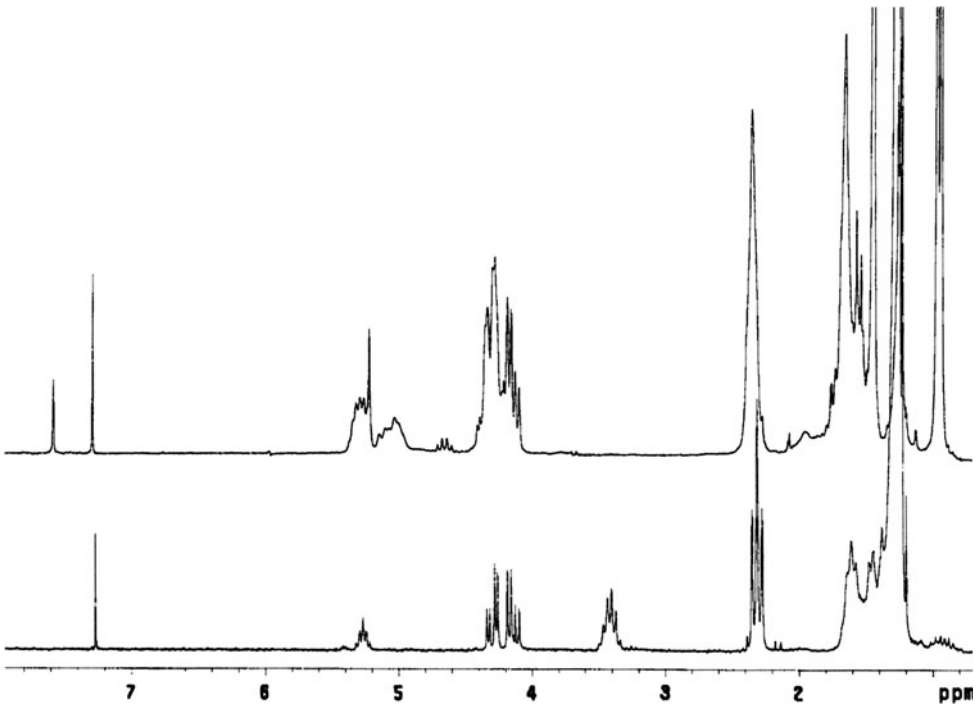


Fig. 3 Comparison of the ^{1}H NMR traces for core **3** (*bottom*) and dendrimer **4** (*top*). Note the complete conversion of the C(17)H–N_3 protons around 3.4 ppm in core **3** to the "clicked" triazole product around 4.6 ppm

Dendrimer **10**: 0.85 (t, 6.0 Hz, 69H, caproic CH_3), 1.07–1.40 m, 1.42 (s, 9H, Boc tBu), 1.48–1.78 m, 1.78–2.02 m, 2.20–2.45 (m, 128H, C(O)CH_2), 2.54 (t, 6.8 Hz, 2H, β-Ala C(O)CH_2), 3.13 (q, 6.6 Hz, 4H, carbamate CH_2NH), 3.38 (q, 6.5 Hz, 2H, β-Ala CH_2NH), 4.03–4.21 (m, 44H, gly CH_2), 4.21–4.39 (m, 44H, gly CH_2), 4.64 (h, 7.1 Hz, 3H, C(17)H–triazole), 4.96–5.11 (m, 1 Boc NH and 2H azelaic carbamate NH), 5.18 (s, 6H, OCH_2–triazole), 5.21–5.35 (m, 22H, gly CH), 7.58 (s, 3H, triazole). ^{13}C NMR: 13.9 caproic CH_3, 21.4 C(18)H_3, 22.3, 24.2, 24.6–24.8, 26.0, 26.5, 27.1, 28.4, 28.8–29.8, 31.2, 33.5, 34.1, 37.2 carbamate CH_2NH, 41.1 β-Ala CH_2NH, 57.6 C(17)–triazole, 62.1–62.3 gly CH_2 and OCH_2–triazole, 68.7–69.5 gly CH, 79.3 Boc quaternary C, 121.5 triazole CH, 142.7 triazole C, 155.8 Boc C=O, 172.3–173.6 carbonyl. MALDI: calculated for $C_{410}H_{676}N_{12}O_{140}{\cdot}Na^{+}$ 8,036.74 Da, found 8,035.92 Da.

Results and Discussion

Synthetic Strategies

There are two routes that can be followed in the construction of dendrimers. The more obvious, at first glance, is by starting at a core and adding successive layers, moving "inside-out". But this divergent procedure has the drawback that the number of reacting groups at each stage increases, for example from four to eight to sixteen hydroxy groups. Since the critical aspect of dendrimer synthesis is obtaining 100% reaction at each site, with no sites "skipped", the odds of attaining a complete reaction decreases at each stage. Even if acylation goes in 99% yield at each site, an octa-hydroxy compound would afford $(0.99)^8$ or 92% yield. Purifying the intermediates, at least using silica gel chromatography, is also difficult, since a highly polar multiple-hydroxy compound can be hard to elute, and there would be practically no chance of separating out incompletely reacted side products that have slightly different degrees of acylation. There is also the matter of requiring 100% removal of the protecting groups, as well as any reagents used for deprotection that could interfere with future reactions. As an aside, these problems are reminiscent of those faced by peptide chemistry that were essentially solved by the introduction of solid-phase protocols.

A better strategy is the "outside-in" or convergent one, which facilitates unambiguous characterization and purification at every stage of reaction. The external units are constructed first, and protecting groups are removed when needed. Using AB_2 monomers, each reaction stage occurs at two sites at most, for example between a diol-acid protected at its carboxy end and two equivalents of free carboxylic acids (see Fig. 1). Differences in polarity are such that separation of unreacted starting materials from product, including partially reacted variants where one OH of the diol-acid is left unreacted on purpose, is straightforward. In an iterative way, the multimeric arms, referred to as dendrons, are built up after removal of the carboxy protecting group and subsequent rounds of coupling

(Fig. 1). The dendrons we employed are heptamers, since they contain seven glycerol and seven dicarboxylic or fatty acid units, not counting the external capping groups. While we speculate that our iterative methodology would have been successful in preparing dendrons of a larger size as well (the next generation would be a pentadecamer), since the ester-forming reactions did not appear to get slower as the dendrons got larger—and if they did, the temperature could be raised—we chose to stop at the heptamer stage due to anticipated analytical limitations, that is, we were concerned that dendrimers larger than about 10 kDa might be difficult to ionize and characterize by mass spectrometry. The final step, then, is linkage of dendrons to the core, and again only a small number of reactions occur at this stage, three at most in our examples. Depending on the size of the dendrons, steric interference when bringing together several units of kilodalton size toward the central core could be severe. Since we need these reactions to go with a high degree of confidence in virtually 100% yield, we chose the azide/alkyne "click" reaction for the final step, which has been shown to meet this criterion [9–12, 17]. An additional benefit is that it is compatible with a wide range of functional groups.

A brief comment on the isomeric nature of these dendrons and dendrimers is in order. When differential substitution is used on the external units—that is, when A ≠ B in the monomer molecules shown in Fig. 1—the acylations can occur at either the free primary or the secondary OH of the glycerol. We have found that the 1,2 and 1,3 diacylglycerol isomers, e.g., **1b** acylated with one equivalent of Boc-β-alanine as described in the synthesis of dendron **8** in the methods section, can be separated using silica gel chromatography, but we have chosen not to separate them. Our hypothesis is that any local effects imposed on an acyl unit by attachment at a primary versus a secondary position of glycerol will be minuscule compared to the overall sterics and flexibility of the dendrimer.

Considering the structural features of a quasi-spherical dendrimer, there are three elements that can be varied to "tune" the properties of the completed oligomer, for example in terms of size, polarity, or functional groups. These elements are the peripheral units (A–D in Fig. 1), the arms or dicarboxylic acids, and finally the core. As our basic AB_2 arm unit we chose the mono-glycerol adipic ester **1b**. We have no reason to believe that longer diacids would not work (see for example reference [3], where sebacic acid was used), but the adipate NMR pattern is simple, and the two carboxylic units should be far enough apart to not interfere with each other, either sterically or reactively. Linear, non-hyperbranched glycerol/adipic polyesters have also found use in drug delivery [18]. Succinic acid, by contrast, could conceivably participate in side-reactions where one free carboxylate cyclizes with an OH at the other end. The glycerol side is "outward" and the benzyl group is the inward end. It is true that this convergent strategy requires the use of protecting groups, but the benefits of specificity, that is, being able to perform a reaction at only selected sites when desired, far outweigh any drawbacks or tedium arising from a few extra steps. Furthermore, the particular protecting groups we used are easy to remove mildly, and just as with the bond-forming reactions in the synthetic direction, only a small number of groups need to be removed at each step, which is again a major contrast with the divergent route described above. The acetonide on solketal only needs to be removed in an initial batch step, not repeatedly during preparation of dendrons. It is removed with 80% acetic acid/water at room temperature or slightly above (40–50 °C). These mild conditions do not destroy the ester linkages. All traces of acetic acid do, however, need to be removed, otherwise they would compete with the incoming carboxylic component as a capping/acylating agent. The benzyl ester protecting group is removed by hydrogenation over palladium on carbon; again, ester bonds in the rest of the molecule are undisturbed. The only limitation of course is that olefins cannot be used elsewhere in the molecule.

Synthetically, ester bonds are the connections used to build up the dendrons, and for these we have employed two variants of mixed anhydride routes that are very mild and go in high yields. For construction of all the dendron ester bonds, we use pivalic anhydride [16], but for construction of the diester starting materials, e.g., **1**, we found that the benzoyl chloride route works better [15]. As would be expected, three different diester products are obtained—the dibenzyl, di-solketal, and desired mixed ester **1a**—but they are easily separated on silica gel, having ΔR_f's of at least 0.1. It may be that the polarity of the solketal acetonide unit contributes to the ease of separation, however, a similar effect was noted with the three products obtained in preparing the benzyl/*tert*-butyl diester of azelaic acid, used for the synthesis of dendron **6**. We propose that the benzoyl chloride mixed anhydride method may be a general way for preparing non-symmetrical diesters of dicarboxylic acids. Interestingly, this route does not work for adding a *tert*-butyl ester on azelaic acid, giving only dark decomposition products, presumably due to acidity of benzoyl chloride and accompanying HCl, but the pivalic method does work, although the reaction is sluggish and needs to be refluxed in THF. The benzyl/*tert*-butyl azelaic diester had to be prepared by first making the mono benzyl ester through the benzoyl chloride route, then attaching the *tert*-butyl group with the pivalic route. The benzoyl chloride route is also less satisfactory for preparation of the dendron units, since a minor side product (approximately 5–10%) is benzoylation of free hydroxy groups in **1b** or its congeners. We have found that the pivalic route does not afford a similar side product (the trimethylacetylated derivative).

Design and Preparation of Targets

The three dendrimers we have prepared using this methodology were designed to exhibit varying degrees of structural complexity. The first and most straightforward combines dendrons that are homogeneously capped with a symmetrical core (molecule **4**, Fig. 2). The 24 external units of the dendrons are protected amino acids, the arms are all adipic acid, and the connection point to the core is a propargyl ester. The core is the triacylglycerol prepared using 17-azido stearic acid. As expected, the threefold click reaction went in high yield. There was no "partial-click" product observed, where only one or two of the azide units of core **3** reacted, as shown by the complete disappearance of the C(17)*H*–N_3 signal at 3.4 ppm (Fig. 3). One potential application of this macromolecule is that it would serve as a polycation after deprotection of the amino groups. Structures of this sort have been referred to as "unimolecular micelles" and are actively being studied for their self-assembling properties [19].

The second target is composed of a polyfunctional core, namely the triazine shown in Fig. 4. This core was designed to demonstrate one way in which functional groups and/or hydrogen-bonding moieties could be "hidden" at the center of the dendrimer. To accomplish this, a multifunctional linker, glutamic acid, is used to build out the core. The phenoxyacetic amide groups, attached to the N-terminus of glutamic acid, were simply chosen for convenience; in a dendrimer geared toward some specific application they could have been some other aromatic or acyl group, perhaps a fluorescent one, or a binding point for a specific guest. The attachment to the triazine nucleus is via the piperazine amide at the C-terminus, and the side chain COOH of Glu provides the attachment point for the clickable dendrons, a propargyl ester. The third substituent of the triazine ring, the propylamino group, was again chosen for convenience and its clear NMR signal; as with the phenoxy groups above, a more "useful" functionalized unit could have been in its place. One interesting advantage of the triazine nucleus is that it can be substituted in a sequential fashion, that is, three different Glu units, each with its own unique substituent at the N-terminus, could have been attached. The dendrons used in this target are substituted with functional groups to a medium extent, namely two reactive groups each (the *tert*-butyl protected carboxyl groups of azelaic acid) with the other caps being hydrophobic caproic acid units. The *t*-Bu groups are an excellent choice for pursuing further derivatization once the dendrimer is constructed: we removed them using trifluoroacetic acid, then installed new propargyl esters in their place (molecule **7b**). In this way, the dendrimer is "reprimed" for further rounds of click chemistry, although other amide or ester derivatives of the azelaic units could have been added. The final dendrimer has a bola shape.

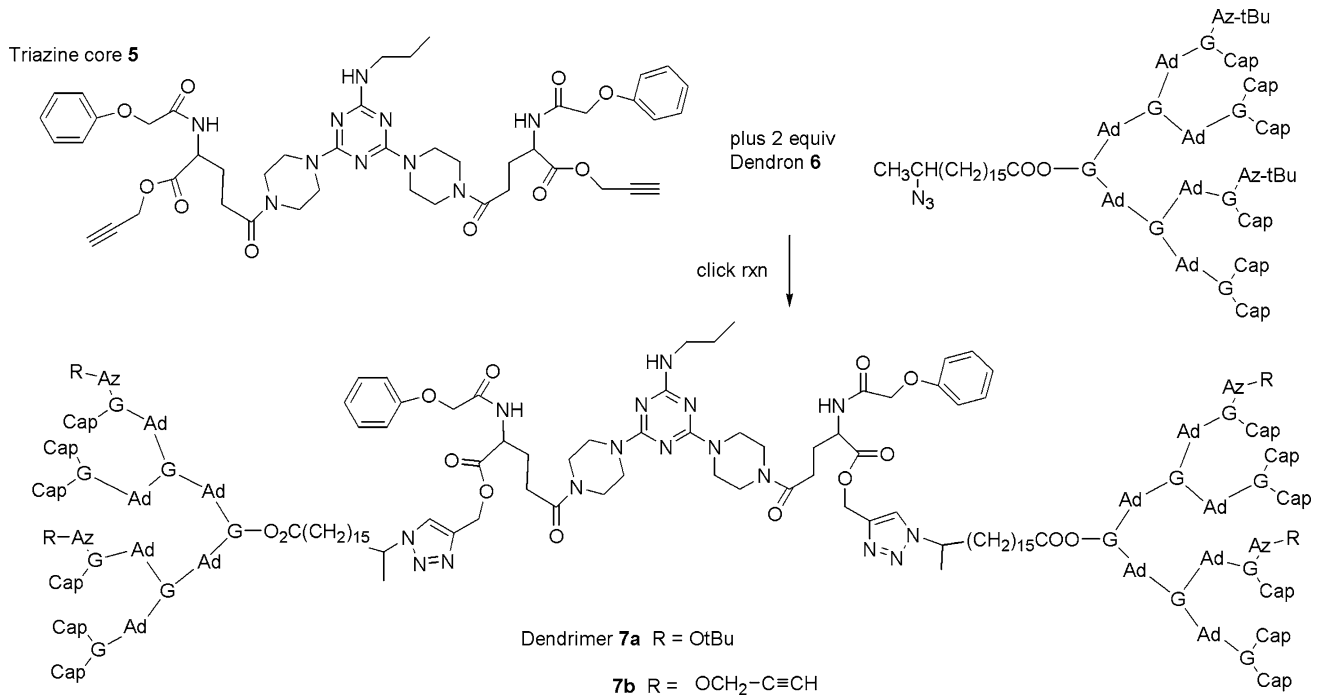


Fig. 4 Construction of a *bola-shaped* dendrimer with a functionalized triazine core. *G* glycerol, *Ad* adipic, *Cap* caproic, *Az* azelaic. Removal of the *t*-Bu esters with CF_3CO_2H, then coupling with propargyl alcohol, affords the alkynyl dendrimer **7b** that could undergo further extension via click chemistry

The third dendrimer (Fig. 5) shows the kind of structure that can be assembled by performing sequential click reactions with different dendrons. The core begins as a monoacylglycerol built from azelaic acid, monoesterified at the other end of the dicarboxylic acid with a clickable propargyl group. The first dendron added is singly substituted, that is, it possesses at its periphery only a single group (the NH-Boc) that can be further reacted with, for example,

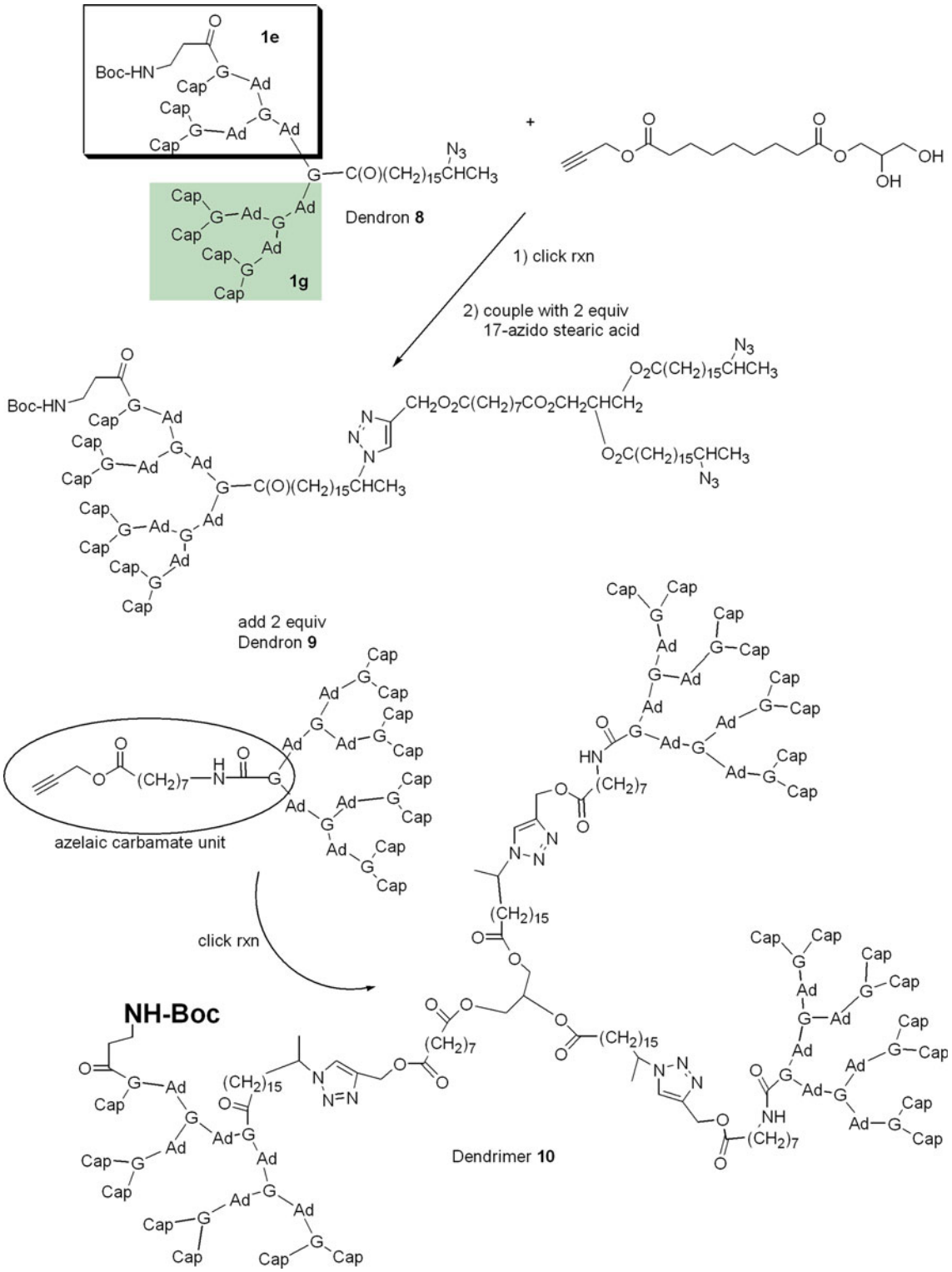


Fig. 5 Construction of a dendrimer with a single reactive group (NH-Boc) at its periphery. Abbreviations as in Fig. 3

a peptide or a fluorescent group. It was gratifying that the click reaction could be performed with the free dihydroxy glycerol group. The next step was to introduce two new clickable units, namely 17-azido stearates, converting the monoacylglycerol to a triacylglycerol. The final two click reactions are then performed using dendrons that are homogeneously substituted with hydrophobic (caproic) exteriors. In addition, we explored the use of modified dicarboxylic arms by preparing the monocarbamate derivative of azelaic acid (see Dendron **9** in Fig. 5). This group is interesting in its own right as a way to increase hydrogen bonding along the arms (relative to ester units) but it also presents a way to introduce "side-chain" functionality to the otherwise featureless dicarboxylic acids, e.g. by alkylating the amine. In general, the diphenyl phosphoryl azide-modified Curtius rearrangement is a convenient way to prepare a carbamate AB_2 monomer that should be more resistant to hydrolysis than the corresponding ester **1b**.

The three dendrimers described here show how the extent of functional coverage at the periphery of a quasi-spherical polyester can be monotonically varied, from single-point substitution to tetra-substituted to 24 substituents. Functionalized triacylglycerols and fatty acids obviously have a contribution to make to this important area of nanotechnology. It may be possible to engineer the selective targeting or delivery of an encapsulated drug to particular biological sites by surface modification of the dendrimer, either by attaching a signaling peptide or by controlling the pattern of charges. Some fundamental issues in macromolecular science that this methodology opens for study is how "sticky" these oligomers could be toward each other. To what extent for example would dendrimer X, coated with 12 positively charged groups, associate with its congener Y, coated with 12 negatively charged groups, as a function of solvent polarity? Alternatively, dendrimers with positive charges on one face, negative charges on another, and hydrophobic groups on a third might self-assemble to novel structures, such as fibrils that lead to gelation [20] or "artificial lipid clusters" [21]. Finally, there is the issue of their size. Crude molecular modeling (Chem3D and CPK models) suggests that these molecules would be approximately 10 nm in diameter. Since they contain flexible alkyl chains, that number can only be an average subject to molecular oscillations. The variability of the dicarboxylic arms would allow construction of a family of dendrimers of increasing size, e.g., from adipic to octadecanedioic acid, and a comparison of physical properties across such a family.

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