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Synthesis of Poly(propyl ether imine) Dendrimers and Evaluation of Their Cytotoxic Properties

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In this paper, we report the synthesis of several poly(propyl ether imine) dendrons and dendrimers. These dendrons and dendrimers were constructed by involving an ether as the linker component and an imine as the branching component. The divergent syntheses of dendrons and dendrimers were established with the aid of two alternate Michael addition reactions and two alternate reduction reactions in a four-step iterative synthetic sequence. Dendrons up to three generations were synthesized and some of the dendrons were attached to a benzenoid core so as to obtain dendrimers up to two generations containing 12 carboxylic acids at the periphery. Divergent synthesis involving ether as the core was found to be more facile, and dendrimers up to three generations having 16 carboxylic acids at the periphery were achieved in good to excellent yields in each individual step. The adopted synthetic sequence allows us to install either alcohol, an amine, or a carboxylic acid at their peripheries. The carboxylic acid-terminated dendrons and dendrimers were evaluated as to their cytotoxic properties, and while most dendrons and dendrimers did not exhibit any measurable cytotoxicity, even up to 100 μg/mL, the second-generation dendrimer with the benzenoid core exhibited a mild toxicity at concentrations above 30 μg/mL.

Introduction

The highly branched, symmetrical, and monodispersed macromolecules called "dendrimers"[1] occupy a considerable interest among synthetic chemists in the last two decades. The uniform branching, large number of peripheral functionalities, molecular weights of several kilodaltons, and a globular spherical shape with defined inner cavities are some of the features of dendrimers, upon which a large body of investigations is being carried out at present.[2] Numerous dendrimers were synthesized with an aim to explore their functional properties resulting from the unique structural features of dendrimers. In this context, both *endo* and *exo* receptor properties of dendrimers have been investigated to identify the so-called "dendritic effect" in chemical, biological, and materials related studies, establishing the fact that the principle of dendritic architecture remains the most attractive.[3] Although large varieties of dendrimers are reported, only a few represent most of the studies that have been carried out so far. Primary among them are poly(amido amine),[1b] poly(propylene imine),[1a,4] poly(benzylaryl ether),[5] polysilane,[6] and polyphosphine[7] dendrimers. Despite the synthesis of these and a number of other dendrimers,[8] identification of new monomers and syntheses of new dendrimers are a continuously evolving interest. A fundamental requirement in the synthesis of

(1) (a) Buhleier, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 155. (b) Tomalia, D. A.; Baker, H.; Dewald, J. R.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J. Tokyo* **1985**, *17*, 117. (c). Newkome, G. R.; Yao, Z.; Baker, G. R.; Gupta, V. K. *J. Org. Chem.* **1985**, *50*, 2003. (d) Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638.

(2) (a) Tomalia, D. A.; Naylor A. M.; Goddard, W. A., III. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 138. (b) Issberner, J.; Moors, R.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2413. (c) Bryce, M. R.; Devonport, W.; Moore, A. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1761. (d) Zeng, F.; Zimmerman, S. C. *Chem. Rev.* **1997**, *97*, 1681. (e) Chow, H.-F.; Mong, T. K.-K.; Nongrum, M. F.; Wan, C.-W. *Tetrahedron* **1998**, *54*, 8543. (f) Frey, H.; Lach, C.; Lorenz, K. *Adv. Mater.* **1998**, *10*, 279. (g) Majoral, J.-P.; Caminade, A.-M. *Chem. Rev.* **1999**, *99*, 845. (h) Newkome, G. R.; He, E.; Moorefield, G. N.; Vögtle, F. *Dendritic Molecules: Concepts, Synthesis, Perspectives*; VCH: Weinheim, 1996. (i) Hearshaw, M. A.; Moss, J. R. *Chem. Commun.* **1999**, 1. (j) Stoddart, F. J.; Welton, T. *Polyhedron* **1999**, *18*, 3575. (k) Fisher, M.; Vögtle, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 885.

(3) (a) Smith, D. K.; Diederich, F. *Chem. Eur. J.* **1998**, *4*, 1353. (b) Hecht, S.; Fréchet, J. M. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 74. (c) Matthews, O. A.; Shipway, A. N.; Stoddart, J. F. *Prog. Polym. Sci.* **1998**, *23*, 1. (d) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665. (e) Newkome, G. R.; He, E.; Moorefield, N. *Chem. Rev.* **1999**, *99*, 1689. (f) Inoue, K. *Prog. Polym. Sci.* **2000**, *25*, 453. (g) Gorman, C. B.; Smith, J. C. *Acc. Chem. Res.* **2001**, *34*, 60. (h) Oosterom, G. E.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. W. N. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1828.

(4) (a) Wörner, C.; Mülhaupt, R. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1306. (b) de Brabander-van den Berg, E. M. M.; Meijer, E. W. *Angew. Chem., Int. Ed.* **1993**, *32*, 1308.

(5) (a) Ref 1d. (b) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1059.

(6) (a) van der Made A. W.; van Leeuwen P. W. N. M. *J. Chem. Soc., Chem. Commun.* **1992**, 1400. (b) Knapen, J. W. J.; van der Made A. W.; de Wilde J. C.; van Leeuwen P. W. N. M.; Wijkens, P.; van Koten G. *Nature* **1994**, *72*, 659. (c) Kleij, A. W.; Gossage, R. A.; Gebbink, R. J. M. K.; Brinkmann, N.; Reijerse, E. J.; Kragl, U.; Lutz, M.; Spek, A. L.; van Koten, G. *J. Am. Chem. Soc.* **2000**, *122*, 12112. (d) Morikawa, A.; Kakimoto, M. A.; Imai, Y. *Macromolecules* **1991**, *24*, 3469. (e) Frey, H.; Schlenk, C. *Top. Curr. Chem.* **2000**, *210*, 69.

(7) (a) Rengan K.; Engel, R. *J. Chem. Soc., Chem. Commun.* **1990**, 1084. (b) Miedaner, A.; Curtis, C. J.; Barkley R. M.; DuBois, D. L. *Inorg. Chem.* **1994**, *33*, 5482. (c) Galliot, C.; Larré, C.; Caminade, A. M.; Majoral, J. P. *Science* **1997**, *277*, 1981. (d) Larré, C.; Caminade, A. M.; Majoral, J. P. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 595. (e) Majoral, J. P.; Caminade, A. M.; Maraval, V. *Chem. Commun.* **2002**, 2929.

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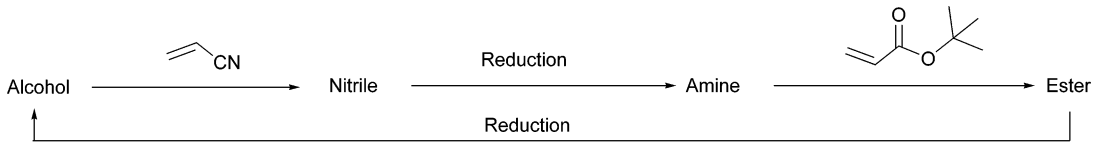


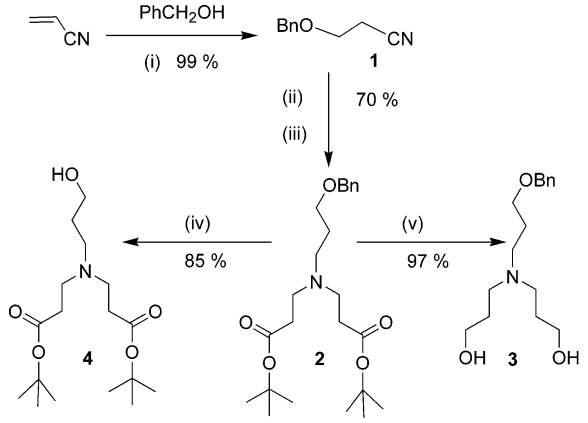
FIGURE 1. Reaction sequence adopted to synthesize poly(propyl ether imine) dendrons and dendrimers.

a dendrimer is the identification of a suitable monomer, possessing desirable functionalities that are amenable for synthetic manipulations. In this respect, a variety of functionalities have been used to construct dendrimers. The most commonly used linker functionalities are amide,[1b,c] ether,[2e,5] ester,[9] urethane,[10] phosphorus,[7] and silane,[6] and the branch junctures are those derived from amine,[1a,4] benzyl,[5] phosphorus,[7] quarternary carbon,[1c] silane,[6] and metal ions.[11] Noncovalent bonding has also been explored as branch junctures.[8d] While tertiary amine as the branch juncture and the amide as the linker component or the tertiary amine alone as the branch juncture constitute the most widely used PAMAM[1b] and poly(propylenimine)[1a,4] dendrimers, respectively, we desired to explore the synthesis of dendrimers in which the branch juncture is a tertiary amine and the linker component is an ether. We report herein the synthesis of these new types of dendrons and dendrimers in which 3-amino-1-propanol forms the constituent part of the repeating unit. This monomer unit was chosen so as to offer a tertiary amine as the branch juncture and an ether as a linking component. Apart from synthesis and characterization, we describe evaluation of cytotoxicity properties relevant to their applicability in biological studies.

Results and Discussion

The early generation dendrons and dendrimers described herein are composed of tertiary amine as the branch juncture with ether as the linker functionality. To have such a constitution of dendrimers, we had attempted initially to utilize triethanolamine as the monomer. However, difficulties encountered in protecting one or more of the hydroxyl groups and selective activation and alkylation of free hydroxyl groups, which often led to quaternization of the amine and cyclization to form three-membered aziridine derivatives and six-membered morpholine derivatives, had prevented effective use of this monomer to construct the dendrons and dendrimers. We realized that replacement of the ethyl linker with a propyl linker would overcome some of the difficulties such as the cyclization and quaternization. On the basis of the above preliminary experiments with triethanolamine, 3-aminopropan-1-ol was chosen as the monomer and a divergent synthetic methodology was found to be most appropriate to prepare the dendrons and dendrimers. The reaction sequence was divided into four major iterative reactions, namely, (i) Michael addition of acrylonitrile to alcohol; (ii) reduction of the nitrile to amine; (iii) double Michael addition of amine with *tert*-butyl acrylate; and (iv) reduction of the resulting ester to alcohol (Figure 1).

SCHEME 1[a]



[a] Reagents and conditions: (i) cat. NaOH, 6 h, rt; (ii) $CoCl_2$, $NaBH_4$, MeOH, −78 °C to room temperature; (iii) *tert*-butyl acrylate, MeOH, 6 h; (iv) 10% Pd/C, 5 days; (v) LAH, THF, 0 °C.

Synthesis of Dendrons. In the initial stages of synthesis, the hydroxyl group, in the *O*-benzyl-protected form, served as the focal point functionality. Thus, reaction of BnOH with acrylonitrile, in the presence of a catalytic amount of aqueous NaOH (40%) at room temperature, afforded *O*-benzyl-2-cyano ethanol (**1**) in nearly quantitative yield (Scheme 1). The reduction of nitrile **1** with $NaBH_4/CoCl_2$ in MeOH, followed by treatment of the resulting amine with excess *tert*-butyl acrylate, afforded the bis(*tert*-butyl acrylate) (**2**) in good yields. Reduction of **2** was performed in the presence of LAH at 0 °C to afford bis-alcohol **3** in nearly quantitative yield without necessitating column chromatographic purification.

In an iterative reaction sequence, bis-alcohol **3** was subjected to Michael addition with acrylonitrile to afford **5** in nearly quantitative yields (Scheme 2).

It was noticed that vigorous stirring of **3** was required during the reaction, so as to allow quantitative *O*-

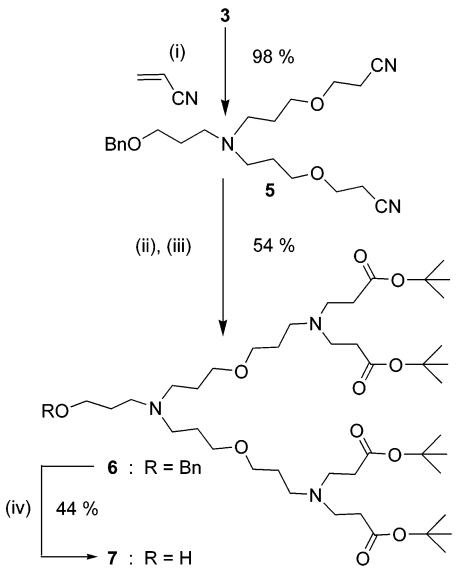
(8) Many new monomers and synthesis of dendrimers have been covered in: (a) Seebach, D.; Rheiner, P. B.; Greiveldinger, G.; Butz, T.; Sellner, H. *Top. Curr. Chem*. **1998**, *197*, 125. (b) Astruc, D., Blais, J.-C., Cloutet, L. Djakovitch, L.; Rigaut, S.; Ruiz, J.; Sartor, V.; Valério, C. *Top. Curr. Chem*. **2000**, *210*, 229. (c) Chow, H.-F.; Leung, C.-F.; Wang, G, -X.; Zhang, J. *Top. Curr. Chem*. **2002**, *217*, 1. (d) Zimmerman, S. C.; Zeng, F.; Reichert, D. E. C.; Kolotuchin, S. V. *Science* **1996**, *271*, 1095. (e) Jayaraman, M.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1998**, *120*, 12996. (f) Nithyanandhan, J.; Jayaraman, N. *J. Org. Chem*. **2002**, *67*, 6282.

(9) (a) Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1992**, *114*, 8405. (b) Twyman, L. J.; Beezer A. E.; Mitchell J. C. *J. Chem. Soc., Perkin Trans. 1* **1994**, 407.

(10) Taylor, R. T.; Puapaiboon, U. *Tetrahedron Lett*. **1998**, *39*, 8005.

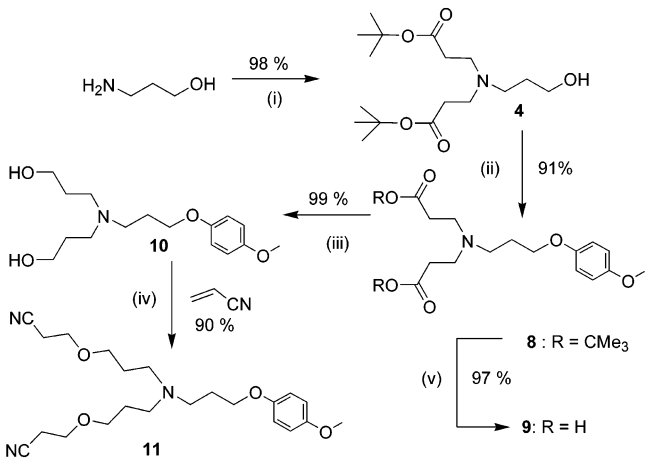
(11) (a) Newkome, G. R.; Cardullo, F.; Constable, E. C.; Moorefield, C. N.; Cargill, T. A. M. W. *J. Chem. Soc., Chem. Commun*. **1993**, 925. (b) Campagna, S.; Denti, G.; Serroni, S.; Juris, A.; Venturi, M.; Ricevuto, V.; Balzani, V. *Chem. Eur. J*. **1995**, *1*, 211. (c) Achar, S.; Puddephatt, R. J. *Organometallics* **1995**, *14*, 1681. (d) Cuadrado, I.; Casado, C. M.; Alonso, B.; Morán, M.; Losada, J.; Belsky, V. *J. Am. Chem. Soc.* **1997**, *119*, 7613. (e) Wang, R.; Zheng, Z. *J. Am. Chem. Soc.* **1999**, *121*, 3549. (f) Constable, E. C.; Eich, O.; Housecroft, C. E.; Johnston, L. A. *Chem. Commun*. **1998**, 2661. (g) Gorman, C. B.; Parkhurst, B. L. *J. Am. Chem. Soc.* **1997**, *117*, 1141. (h) Oosterom, G. E.; van Haaren, R. J.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. W. N. M. *Chem. Commun*. **1999**, 1119. (i) Cardona, C. M.; Kaifer, A. E. *J. Am. Chem. Soc.* **1998**, *120*, 4023. (j) Rama Krishna, T.; Jayaraman, N. *J. Chem. Soc., Perkin Trans. 1* **2002**, 746.

SCHEME 2[a]



[a] Reagents and conditions: (i) cat. NaOH, 6 h, rt; (ii) $CoCl_2$, $NaBH_4$, MeOH, −78 °C to room temperature; (iii) *tert*-butyl acrylate, MeOH, 6 h; (iv) 10% Pd/C, 5 days.

SCHEME 3[a]

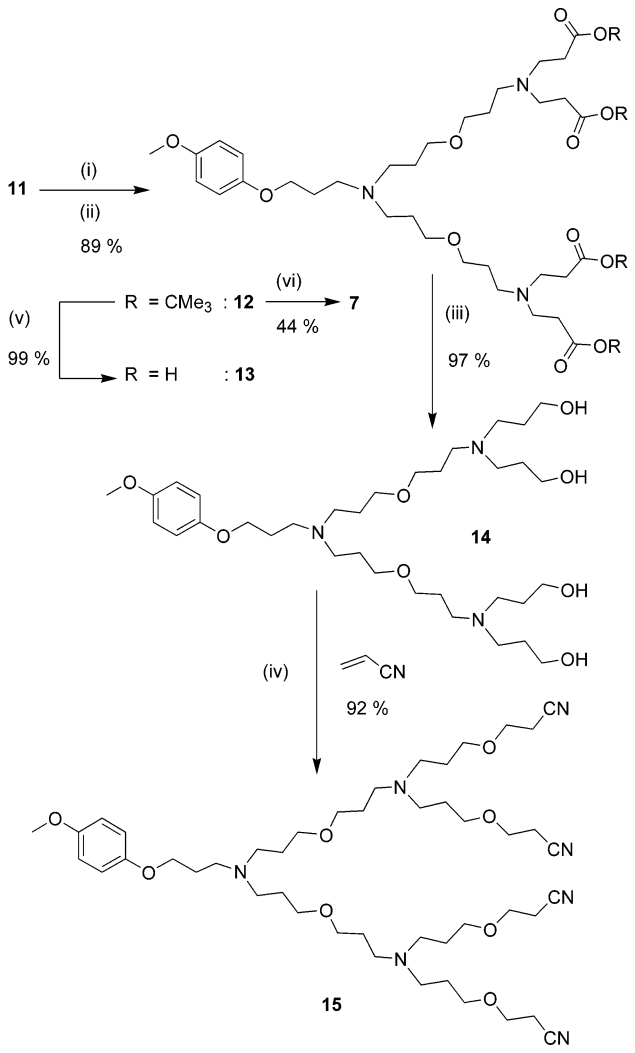


[a] Reagents and conditions: (i) *tert*-butyl acrylate, MeOH, 6 h; (ii) DIAD, PPh_3, *p*-$MeOC_6H_4OH$, THF, 15 min, 80 °C; (iii) LAH, THF, 0 °C; (iv) cat. NaOH, 15 h, rt; (v) AcCl, H_2O, CH_2Cl_2, 8 h.

alkylation. Reduction of two nitrile groups in **5** ($CoCl_2$, $NaBH_4$, −78 °C to room temperature) and subsequent double Michael reaction of the resulting diamine with *tert*-butyl acrylate afforded the desired tetrakis(*tert*-butyl ester) (**6**) in an overall moderate yield of 54% for two steps.

An alternate approach was also explored to synthesize the above dendrons, on account of lower yields encountered during reduction of multiple nitrile groups to the corresponding amines by using a $CoCl_2/NaBH_4$ reagent system, while retaining the benzyl protecting group intact at the focal point of the dendrons. Because of the requirement that efficient reactions are essential for the generation of dendrimers, we desired to use Raney Co catalyst for the reduction of nitrile groups of dendrons, as adopted by Meijer and de Brabender van den Berg in their synthesis of poly(propylenimine) dendrimers.[4b] Use of this catalyst in turn required an alternate protecting

SCHEME 4[a]



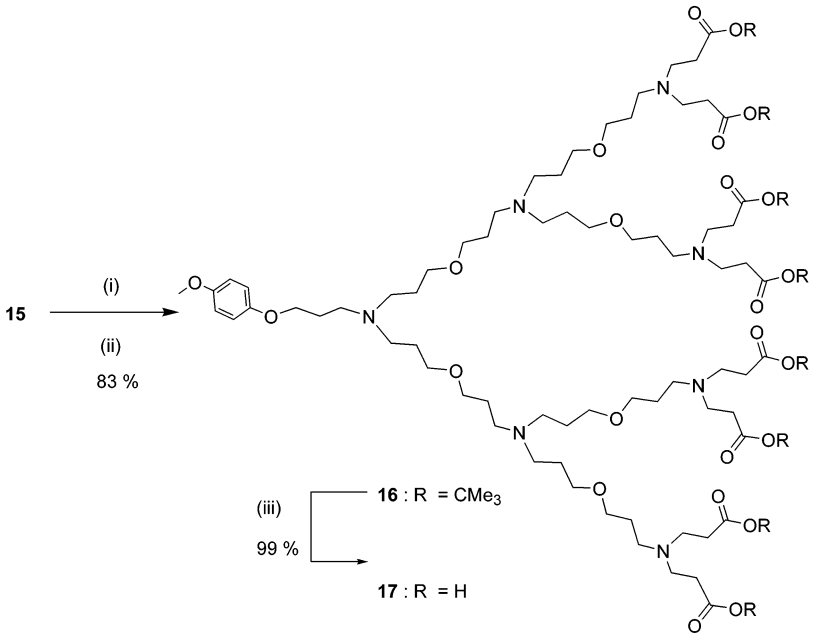
[a] Reagents and conditions: (i) Raney Co, $MeOH/H_2O$, H_2, 40 atm, 70 °C; (ii) *tert*-butyl acrylate, MeOH, 6 h; (iii) LAH, THF, 0 °C; (iv) cat. NaOH, 15 h, rt; (v) AcCl, H_2O, CH_2Cl_2, 8 h; (vi) CAN, $MeCN-H_2O$, 15 min.

group for the hydroxyl group functionality at the focal point of dendrons. *p*-Anisyl protecting was chosen for this purpose, owing to its stability under strongly acidic, basic, oxidative, and reductive conditions, yet it can be deprotected under mild conditions (CAN, $MeCN-H_2O$).[12] 3-Aminopropan-1-ol was chosen as the starting material for the construction of dendrons, which upon exhaustive Michael addition with *tert*-butyl acrylate provided bis(*tert*-butyl acrylate) (**4**) in 98% yield (Scheme 3).

The free hydroxyl group was protected at this stage by reaction of **4** with *p*-methoxyphenol, under Mitsunobu conditions (DIAD, PPh_3, THF), to afford **8** (91%). Reduction of the ester in **8** was conducted adding LAH to the corresponding bis-alcohol **10** in a nearly quantitative yield. Michael addition with acrylonitrile to bis-alcohol **10** was accomplished in the presence of a catalytic amount of aqueous NaOH (40%) to obtain bis-nitrile **11** in 90% yield. Reduction of the nitrile groups in **11** was

(12) Fukuyama, T.; Laird, A. A.; Hotchkiss, L. M. *Tetrahedron Lett.* **1985**, *51*, 6291.

SCHEME 5[a]



[a] Reagents and conditions: (i) Raney Co, MeOH/H_2O, H_2, 40 atm, 70 °C; (ii) *tert*-butyl acrylate, MeOH, 6 h; (iii) AcCl, H_2O, CH_2Cl_2, 8 h.

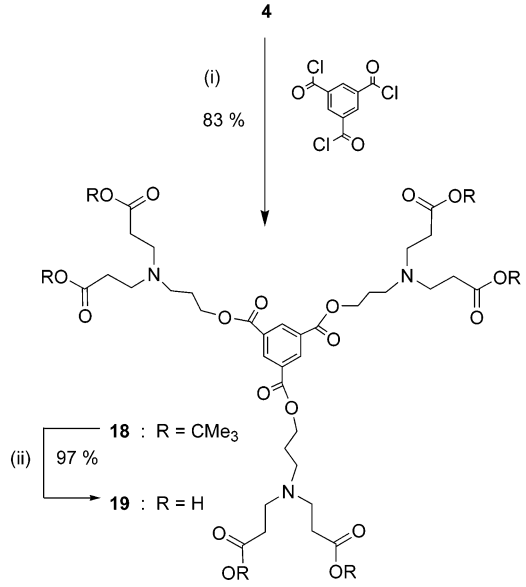
carried out effectively with the use of Raney Co and H_2 (40 atm) in water at 70 °C, and the resulting amine was subjected to exhaustive Michael addition to obtain four *tert*-butyl ester group terminated dendron **12** with an overall yield of 89% for two steps (Scheme 4).

The modified route involving the use of Raney Co as the reducing agent of nitrile groups has improved greatly the dendron synthesis with excellent yields at each individual step. Further growth of second- and third-generation dendrons was accomplished by repetition of the sequence: (i) reduction of **12** to alcohol **14** (LAH, THF, 97%); (ii) Michael addition of **14** with acrylonitrile to afford **15** (aqueous NaOH (40%), 92%); (iii) reduction of **15** to amine (Raney Co, MeOH−H_2O); followed by (iv) Michael addition of the resulting amine with *tert*-butyl acrylate (MeOH, 83% for two steps) to afford octavalent *tert*-butyl acrylate **16** (Scheme 5).

Synthesis of Dendrimers. Upon synthesis of dendrons, their attachment to a chosen core was carried out. The first- (**4**) and second- (**7**) generation dendrons were thus coupled with 1,3,5-benzenetricarbonyl chloride in the presence of DMAP to afford the first- (**18**) and second- (**20**) generation dendrimers (Schemes 6 and 7), respectively. *tert*-Butyl ester protecting groups at the peripheries of these dendrimers were deprotected (AcCl, H_2O) to afford the free carboxylic acid-containing dendrimers **19** and **21**, respectively.

With the difficulty that we were unable to remove the benzyl group at the focal point of dendrons **2** and **6** effectively, presumably due to catalyst poisoning in the presence of several tertiary amine groups, and also with the difficulty of removing the *p*-anisyl group in the anticipated manner in the higher generation dendron **16**, we focused on synthesis of 3-aminopropan-1-ol-based dendrimers by adopting a divergent growth methodology. In principle, the same synthetic sequence was required as we had followed previously for the dendron synthesis.

SCHEME 6[a]

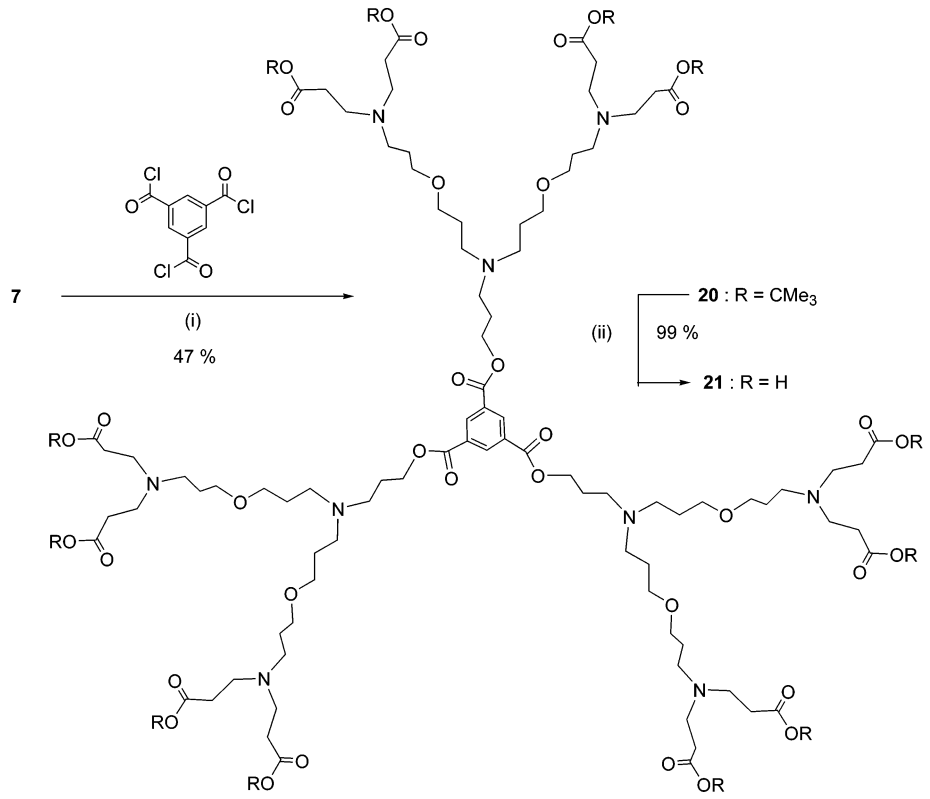


[a] Reagents and conditions: (i) DMAP, PhMe, 6 h reflux; (ii) AcCl, H_2O, CH_2Cl_2, 8 h.

The required starting material bis-nitrile **22** was obtained from acrylonitrile and aqueous NaOH (40%) (Scheme 8).

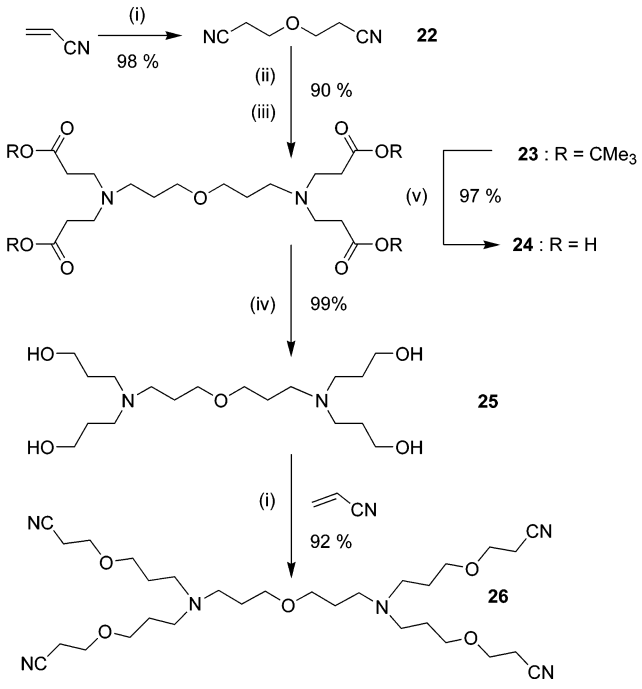
The reaction was facile and could be accomplished in multigram quantities in quantitative yields. Bis-nitrile **22** was subjected to sequential reactions: (i) reduction of nitrile in the presence of Raney Co catalyst; (ii) *tert*-butyl acrylate addition to afford first-generation tetrakis(*tert*-butyl acrylate) (**23**); (iii) reduction of peripheral *tert*-butyl acrylate with LAH to alcohol **25**; and (iv) conversion of **25** to tetrakis-nitrile **26** by reaction with acrylonitrile. This synthetic sequence was repeated with nitrile **26** to afford, respectively, the second-generation octakis(*tert*-butyl acrylate) (**27**), octakis-alcohol (**29**), and octakis-

SCHEME 7[a]



[a] Reagents and conditions: (i) DMAP, PhMe, 6 h reflux; (ii) AcCl, H_2O, CH_2Cl_2, 8 h.

SCHEME 8[a]

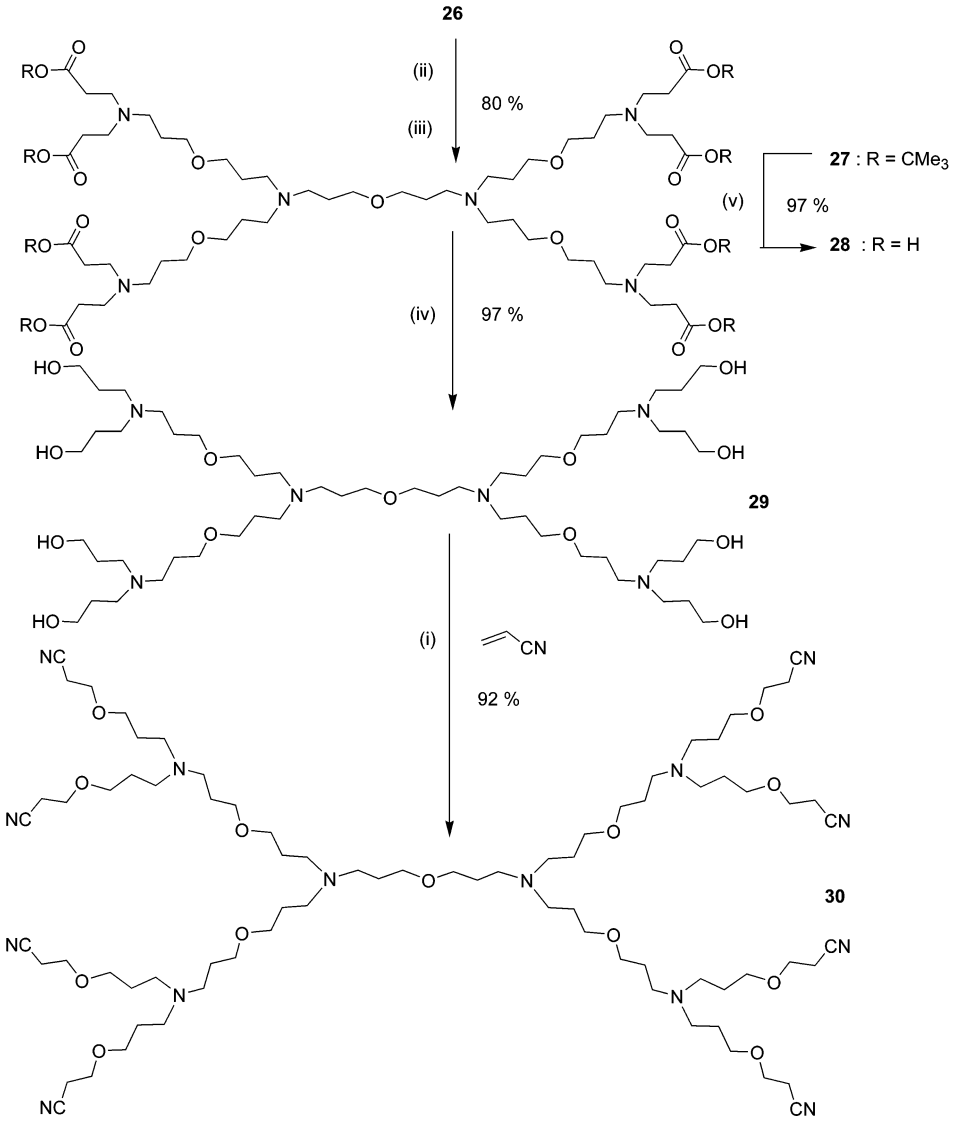


[a] Reagents and conditions: (i) cat. NaOH, 15 h, rt; (ii) Raney Co, MeOH/H_2O, H_2, 40 atm, 70 °C; (iii) *tert*-butyl acrylate, MeOH, 6 h; (iv) LAH, THF, 0 °C; (v) AcCl, H_2O, CH_2Cl_2, 6 h.

nitrile (**30**) (Scheme 9). Higher generation dendrimer **31** was synthesized, starting from nitrile **30**, by performing reduction of the nitrile groups using Raney Co catalyst, followed by addition with *tert*-butyl acrylate to the resulting octakis-amine to afford the third-generation dendrimer **31** with 16 peripheral *tert*-butyl ester groups in excellent yield (Scheme 10). In summary, this sequence of reactions could be conducted in excellent yields, characterizing the facile nature of the reactions involved in the syntheses of dendrimers.

Characterization. All the dendrons and dendrimers were characterized by routine physical methods. Since the synthetic sequence involved changes in the functional group at their peripheries, monitoring the progress of reaction was easy by using IR spectroscopy. Thus, formation of nitrile (ν 2251), reduction of nitrile to amine (ν 3460), formation of *tert*-butyl ester (ν 1727), and conversion of the ester to the corresponding alcohol (ν 3390) could be followed rather comprehensively by IR spectroscopy for all the dendrons and dendrimers. Characteristics of ^{1}H NMR spectra were the changes of chemical shifts of the $-CH_2-$ groups at the peripheries, depending on the functional group present. Thus, disappearance of the triplet at ~2.60 ppm of the CH_2CN group and appearance of resonances at ~1.70 and ~2.50 ppm, corresponding to CH_2CH_2N, were distinct for this nitrile reduction. Likewise, disappearance of the resonance at 2.30 ppm of $CH_2-CO_2{}^tBu$ and appearance of resonances at ~1.70 and ~3.70 ppm of CH_2CH_2OH could be used comfortably to monitor the progress of the extent of functional group conversion and the growth of the dendrons and dendrimers. While the resonances were sharp and distinct for modifications at the peripheries, the internal CH_2 protons became broader progressively as the generations advanced, indicating the changing morphologies of the dendritic architecture.

SCHEME 9[a]



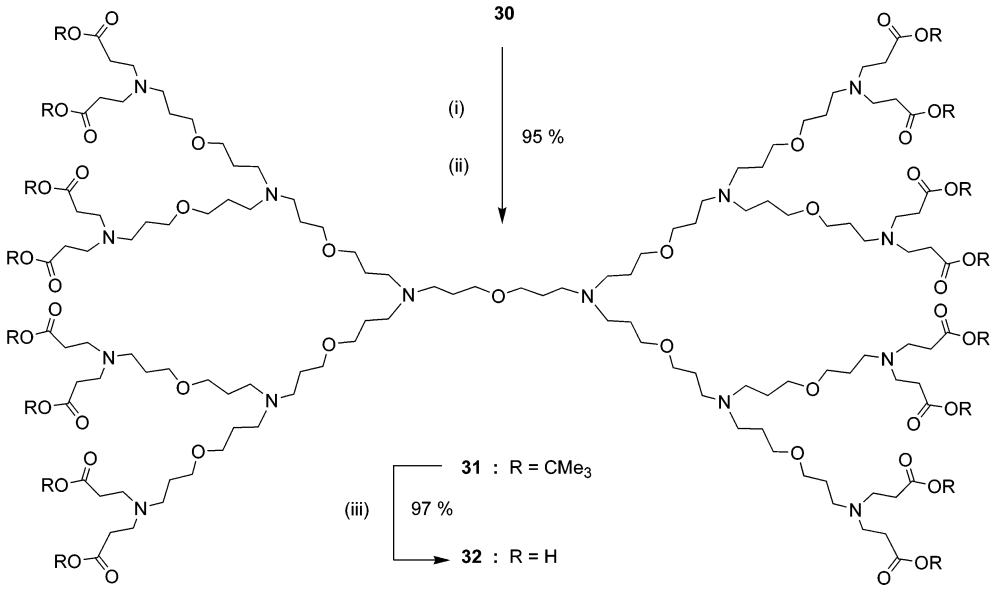
[a] Reagents and conditions: (i) cat. NaOH, 15 h, rt; (ii) Raney Co, MeOH, H_2O, 70 °C, 40 atm; (iii) *tert*-butyl acrylate, MeOH, 6 h; (iv) LAH, THF, 0 °C; (v) AcCl, H_2O, CH_2Cl_2, 8 h.

Similar changes in ^{13}C NMR chemical shifts could also be used to monitor the conversions of functional groups at the peripheries of dendrons and dendrimers. Thus, disappearance of the resonance due to the nitrile group at ~117 ppm and appearances of the carbonyl resonance of the ester group at ~172 ppm and of the CH_2OH group resonance at 62 ppm resulting from the reduction of the ester group could be observed distinctly, thereby confirming the required changes in the functionalities as the generations of dendrons and dendrimers grew. The constitution of the dendrons and dendrimers was further ascertained by mass spectrometric analysis. The calculated molecular ion peak, including in the form of Na and K adducts, could be obtained as the most intense peak in almost all the compounds (Figure 2). For smaller molecular weight dendrons and dendrimers (molecular weight < 1000 g/mol), high-resolution ES-MS analysis was performed, and the error between the observed mass position and the calculated mass position was often less than 5 ppm. The ES-MS behavior of carboxylic acid-terminated dendrons and dendrimers was different in each case, except in those containing a phenyl group, wherein molecular ion peaks were either Na or K adducts. In the case of dendrimer **24**, the mass spectrum showed the molecular ion peak along with a few more peaks corresponding to the addition of 14 molecular mass units. Whereas in the case of dendrimers **28** and **32**, with free carboxylic acids, it was difficult to analyze by ES-MS analysis; only a cluster of peaks of much lesser molecular weights could be seen. Also, elemental analysis could not be obtained reliably due to the hygroscopic nature of these dendrons and dendrimers.

Evaluation of the Cytotoxic Properties of Dendrons and Dendrimers. Dendrons and dendrimers synthesized herein have molecular features corresponding to polyimines and polyethers, both of which have been studied in detail for their biological applications.[13,14] On the basis of the properties of the constituent imine and ether linkages, we tested the cytotoxic properties of some

(13) (a) Ferruti, P.; Knobloch, S.; Ranucci, E.; Duncan, R.; Gianasi. *Macromol. Chem. Phys.* **1998**, *199*, 2565. (b) Wroblewski, S.; Kopeckova, J. *Macromol. Chem. Phys.* **1998**, *199*, 2601.

SCHEME 10[a]



[a] Reagents and conditions: (i) Raney Co, MeOH, H_2O, 70 °C, 700 psi; (ii) *tert*-butyl acrylate, MeOH, 6 h; (iii) AcCl, H_2O, CH_2Cl_2, 6 h.

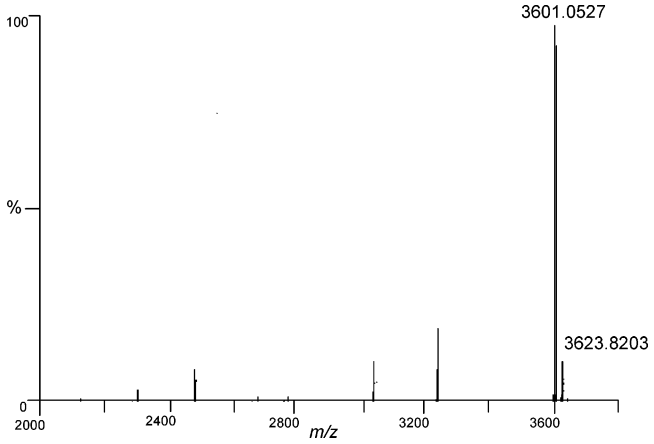


FIGURE 2. Low-resolution ES-MS of the third-generation dendrimer **31**.

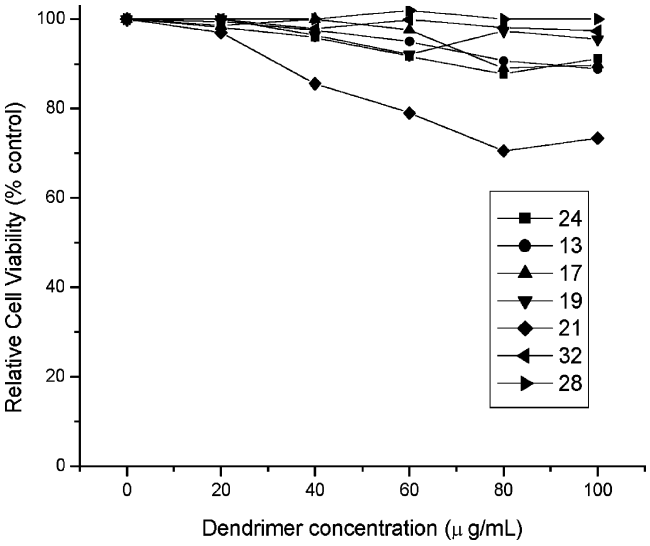


FIGURE 3. In vitro cytotoxicity studies on the mammalian CHO cell line (**13**, **17**, **19**, **21**, and **24**) and breast cancer T47D cell line (**28** and **32**), at various concentrations of dendrons and dendrimers.

of the dendrons and dendrimers as a means to evaluate their acceptance in the biological studies.[15] The cytotoxicity studies were performed using a tetrazolium-based MTT colorimetric assay. This assay relies on quantitation of cells surviving after treatment of the live cells by testing their enzymatic dehydrogenase activity.[16] The assessment of enzymatic activity is based on changes of the water-soluble tetrazolium salt to a purple, insoluble formazan derivative, and this change can be monitored by UV−vis spectroscopy. Either a Chinese hamster ovarian cell line or breast cancer T47D cell line was used for testing the cytotoxic properties of a few carboxylic acid-terminated dendrons and dendrimers. Cells were incubated for 24 h with increasing concentration of dendrons and dendrimers in the presence of bovine serum. The relative cell viability in the presence of dendrons and dendrimers at different concentrations is presented in Figure 3. The cell survival rate was more than 90% in the case of **13**, **17**, **19**, **24**, **28**, and **32**, and the rate had reduced slightly to 80% in the case of the second-generation dendrimer **21**, for concentrations up to 100 μg/mL of the solutions. It is clear from this assay that while no measurable cytotoxicity is observed for **13**, **17**, **19**, **24**, **28**, and **32**, a mild toxicity at concentrations above 30 μg/mL is shown by **21**. The observation that the dendrons and dendrimers tested herein allow cell sur-

(14) (a) Boussif, O.; Lezoualch, F.; Zanta, M. A.; Mergny, M. D.; Scherman, D.; Demeneix, B.; Behr, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7297. (b) Elizabeth R. G.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2002**, *124*, 14137. (c) Choi, J. S.; Joo, D. K.; Kim, C. H.; Kim, K.; Park, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 474.

(15) For toxicity studies of amine and carboxylic acid terminated dendrimers, see: Malik, R.; Wiwattanapatapee, R.; Klopsch, R.; Lorentz, K.; Frey, H.; Weener, J. W.; Meijer, E. W.; Paulus, W.; Duncan, R. *J. Controlled Release* **2000**, *65*, 133.

(16) Mosmann, T. *J. Immun. Methods* **1983**, *65*, 55.

(17) For an alternate synthesis of **22**, see: Masasane, I.; Kanemitsu, M.; Akira, I.; Yamauchi, A. *Jpn. Kokai Tokkyo Koho* **1989**, 4 (*Chem. Abstr.* **1989**, *111*, 133654y).

vival ability of more than 80% indicates that these compounds are significantly less toxic, are biocompatible, and thus are suitable for in vitro biological studies.

Conclusion

We have established a four-step iterative protocol to synthesize poly(propyl ether imine) dendrons and dendrimers. The yields of individual reactions are excellent often, indicating the facile *N*- and *O*-alkylations, as well as the reduction reactions. The facile conversions also allow us to have either a carboxylic acid, an alcohol, or an amine easily at their peripheries. In addition, their molecular features, having the ether and imine functionalities, are common, as in the case of several polyethers and polyimines, and thus are likely to have similar properties for further chemical and other interfacial studies. Evaluation of the extent of cytotoxicities demonstrates that the toxicity levels of the dendrons and dendrimers are either nearly none or very mild. These experiments point to the possibility of incorporation of these poly(propyl ether imine) dendrons and dendrimers in biological and related studies as well.

Experimental Section

General Procedure for Michael Addition of Acrylonitrile (I). Acrylonitrile was added to a mixture of alcohol and aqueous NaOH (40%) while maintaining the temperature below 30 °C. The reaction mixture was stirred for 6 h at room temperature, neutralized with aqueous HCL (1 N), and diluted with $CHCl_3$. The organic layer was washed with 5% aqueous NaOH followed by brine, evaporated in vacuo, and dried thoroughly to afford the desired compound.

General Procedure for the Reduction of Nitrile Using $NaBH_4/CoCl_2$ and Michael Addition of Resulting Amines (II). To a solution of the nitrile compound in MeOH was added $CoCl_2 \cdot 6H_2O$ followed by addition of $NaBH_4$ in portions at −78 °C (violent gas evolution occurred during addition). The reaction mixture was gradually brought to room temperature, stirred for an additional 2 h, and acidified cautiously with concentrated HCl, and the solvents were removed in vacuo. The resulting residue was treated with aqueous NH_3 solution (30%) to give a deep-blue solid residue, and the residue was extracted with $CHCl_3$. The organic extracts were dried and concentrated to give a crude yellow-colored oil product. A solution of this crude product in MeOH was added with *tert*-butyl acrylate, and the reaction mixture was stirred for 6 h at room temperature. Excess of *tert*-butyl acrylate was then removed in vacuo, and the resulting product was purified by column chromatography (SiO_2).

General Procedure for Reduction of *tert*-Butyl Esters (III). To a suspension of LAH in THF at 0 °C was added dropwise the ester in THF for ∼30 min. The suspension was stirred at 0 °C for 30 min and brought to room temperature, and stirring continued for an additional 1 h. The reaction mixture was cooled to 0 °C and quenched with ice (∼1 g), solid Na_2SO_4 was added, and the mixture was left until LAH was quenched and the gray residue became a free white suspension and settled. The suspension was passed through a plug of Celite, and the filtrate was dried and concentrated to obtain alcohol as a colorless liquid.

General Procedure for Hydrolysis of *tert*-Butyl Esters (IV). To a solution of the ester in CH_2Cl_2 were added slowly AcCl and H_2O, and the solution was stirred at room temperature for 8 h. Solvents were removed in vacuo, and the resulting residue was triturated several times with hexane and CH_2Cl_2 to afford the desired acid as a white, foamy solid.

General Procedure for Reduction of Nitrile Using Raney Cobalt Catalyst and Subsequent Michael Addition of Resulting Amines (V). The nitrile derivative in MeOH was transferred to a hydrogenation reactor vessel and was admixed with Raney Co catalyst in H_2O (40 mL). The mixture was hydrogenated (H_2, 40 atm) at 70 °C for 1 h, cooled, and decanted, and the solvents were evaporated off to afford amine as a colorless oil. A solution of the resulting crude amine in MeOH (5 mL) was treated with *tert*-butyl acrylate and stirred for 6 h. Excess *tert*-butyl acrylate and solvents were removed in vacuo, and the resulting product was purified (alumina).

General Procedure for the Attachment of Dendron to the Trimesityl Chloride Core (VI). A two-necked flask, fitted with a Dean Stark trap and a condenser, was charged with dendron and DMAP in PhMe (20 mL), refluxed for 2 h, then cooled to room temperature. 1,3,5-Benzenetricarbonyl trichloride was added to the reaction mixture and refluxed for 4 h, and the solvents were removed in vacuo. The product was purified to afford dendrimer as a colorless liquid.

Compound 1. Acrylonitrile (5.83 g, 0.11 mol) was added to a mixture of BnOH (10.8 g, 0.1 mol) and aqueous NaOH (40%) (1 mL), and the mixture was stirred for 6 h and worked up as described in the general procedure I to obtain **1** as a colorless liquid (15.9 g, 99%). FT-IR (neat) ν: 2251, 1455, 1363, 1104. EI-MS *m/z*: 161 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 2.62 (t, 2 H, J = 6.9 Hz), 3.68 (t, 2 H, J = 6.9 Hz), 4.58 (s, 2 H), 7.28−7.39 (m, 5 H). ^{13}C NMR ($CDCl_3$) δ: 18.8, 64.5, 73.3, 117.8, 127.7, 128.0, 128.6, 137.2.

Compound 2. A solution of **1** (1.61 g, 10 mmol) in MeOH (80 mL) was treated with $CoCl_2 \cdot 6H_2O$ (2.38 g, 10 mmol) and $NaBH_4$ (7.56 g, 0.2 mol), and the reaction continued as described in the general procedure II. The amine (1.5 g), resulting from this reaction, was then treated with *tert*-butyl acrylate (3.83 g, 30 mmol) and processed further as described in the general procedure II to afford, after purification (hexane/EtOAc, 90:10), **2** as a colorless liquid (2.95 g, 70%). FT-IR (neat) ν: 1726, 1455, 1367, 1151. EI-MS *m/z*: 422 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 18 H), 1.74 (m, 2 H), 2.35 (t, 4 H, J = 7.2 Hz), 2.50 (t, 2 H, J = 6.3 Hz), 2.71 (t, 4 H, J = 7.2 Hz), 3.50 (t, 2 H, J = 6.3 Hz), 4.49 (s, 2 H), 7.29−7.34 (m, 5 H). ^{13}C NMR ($CDCl_3$) δ: 27.7, 28.1, 33.8, 49.4, 50.4, 68.5, 72.9, 80.2, 127.5, 127.6, 128.3, 138.6, 172.1. HRMS *m/z:* calcd for $C_{24}H_{39}O_5N$ 422.2906, found 422.2912.

Compound 3. To a suspension of LAH (0.10 g, 2.3 mmol) in THF at 0 °C was added dropwise **2** (0.4 g, 0.95 mmol) in THF (10 mL), and the reaction continued further as described in the general procedure III to obtain **3** as a colorless liquid (0.26 g, 97%). FT-IR (neat) ν: 3390, 1455, 1365, 1069. EI-MS *m/z*: 282 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.72 (q, 4 H, J = 6.6, 6.9 Hz), 1.81 (q, 2 H, J = 6.6, 6.9 Hz), 2.53 (t, 2 H, J = 6.9 Hz), 2.62 (t, 4 H, J = 6.3 Hz), 3.51 (t, 2 H, J = 6.6 Hz), 3.72 (t, 4 H, J = 6.0 Hz) 4.50 (s, 2 H), 7.27−7.37 (m, 5 H). ^{13}C NMR ($CDCl_3$) δ: 26.9, 28.5, 51.0, 52.7, 62.5, 68.4, 73.0, 127.6, 127.7, 128.4, 138.3. HRMS *m/z:* calcd for $C_{16}H_{27}O_3N$ 282.2089, found 282.2074.

Compound 4. A mixture of 3-aminopropan-1-ol (4.91 g, 65.4 mmol) and *tert*-butyl acrylate (21.87 g, 171.0 mmol) in MeOH (25 mL) was stirred at room temperature for 6 h. Excess *tert*-butyl acrylate and solvents were removed in vacuo, the crude product was diluted with $CHCl_3$ and washed with brine, and the organic portion was dried and concentrated to afford **4** as a colorless viscous liquid (21.3 g, 98%). Alternatively, a suspension of **2** (1.0 g, 2.37 mmol) and Pd/C (10%, 0.1 g) in EtOAc (25 mL) was attached with a H_2 gas-filled balloon and stirred for 5 days at room temperature. The suspension was filtered and washed, and the filtrate was concentrated and purified to afford **4** (0.2 g, 84.8% after recovery of 0.7 g of starting material). FT-IR (neat) ν: 3429, 1729, 1459, 1368, 1158. EI-MS *m/z*: 331 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 18 H), 1.66 (q, 2 H, J = 6.0 Hz), 2.37 (t, 4 H, J = 7.2 Hz), 2.60 (t, 2 H, J = 6.0 Hz), 2.72 (t, 4 H, J = 7.2 Hz), 3.71 (t, 2 H, J = 6.0 Hz). ^{13}C NMR ($CDCl_3$) δ: 28.1, 28.4, 33.3, 49.4, 53.0, 63.2, 80.7, 171.7. HRMS *m/z:* calcd for $C_{17}H_{33}O_5N$ 332.2437, found 332.2440.

Compound 5. Acrylonitrile (0.60 mg, 11.0 mmol) was added to a mixture of **3** (1.30 g, 4.70 mmol) and aqueous NaOH (40%) (0.50 mL), and the mixture was stirred for 6 h and worked up as described in the general procedure I to afford **5** as a colorless liquid (1.8 g, 98%). FT-IR (neat) ν: 2251, 1456, 1365, 1114. EI-MS *m/z*: 387 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.72 (m, 6 H), 2.48 (t, 2 H, J = 6.3 Hz), 2.57 (t, 4 H, J = 6.6 Hz), 2.65 (t, 4 H, J = 6.0 Hz), 3.50 (t, 4 H, J = 6.0 Hz), 3.61 (t, 2 H, J = 6.3 Hz), 3.57 (t, 4 H, J = 6.6 Hz) 4.49 (s, 2 H), 7.27−7.39 (m, 5 H). ^{13}C NMR ($CDCl_3$) δ: 18.9, 27.4, 27.5, 50.5, 50.8, 65.3, 65.9, 68.6, 72.9, 117.4, 127.5, 127.6, 128.4, 138.5. HRMS *m/z*: calcd for $C_{22}H_{33}O_3N_3$ 388.2600, found 388.2589.

Compound 6. A solution of **5** (0.25 g, 0.65 mmol) in MeOH (25 mL) was treated with $CoCl_2 \cdot 6H_2O$ (0.31 g, 1.29 mmol) and $NaBH_4$ (0.98 g, 25.8 mmol), and the reaction continued as described in the general procedure II. The amine (0.25 g), resulting from this reaction, was then treated with *tert*-butyl acrylate (0.87 g, 5.34 mmol) and processed further as described in the general procedure II to afford, after putifications (alumina) (hexane/EtOAc, 85:15), **6** as a colorless liquid (0.25 g, 54% combined yield for nitrile group reduction and Michael addition reaction). FT-IR (neat) ν: 1728, 1455, 1367, 1151. MALDI-TOF-MS *m/z*: 908 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 36 H), 1.68 (b, 10 H), 2.34 (t, 8 H, J = 7.2 Hz), 2.46 (t, 10 H, J = 6.9 Hz), 2.71 (t, 8 H, J = 7.2 Hz), 3.39 (t, 8 H, J = 6.3 Hz), 3.50 (t, 2 H, J = 6.3 Hz), 4.49 (s, 2 H), 7.29−7.36 (m, 5 H). ^{13}C NMR ($CDCl_3$) δ: 27.5, 27.7, 28.1, 33.8, 49.4, 50.6, 50.9, 68.5, 68.9, 69.1, 72.9, 80.1, 127.5, 128.3, 129.2, 138.6, 172.1. HRMS *m/z*: calcd for $C_{50}H_{89}O_{11}N_3$ 908.6575, found 908.6580.

Compound 7. A solution of **12** (0.5 g, 0.55 mmol) in MeCN/H_2O (5:1) (25 mL) was admixed with CAN (0.45 g, 0.83 mmol), stirred at room temperature for 15 min, filtered through a plug of Celite, and washed with EtOAc. The filtrate was concentrated and purified (alumina) (hexane/EtOAc, 70:30) to afford **7** as a colorless liquid (0.14 g, 30%). Alternatively, **6** (50 mg, 0.055 mmol) in EtOAc (25 mL) was added with Pd/C (10%) (24 mg), attached with a H_2-filled balloon, stirred at room temperature for 5 days, filtered, and washed, and the filtrate was concentrated and purified to afford **7** as a colorless liquid (4 mg, 44% after recovery of 40 mg of starting material). FT-IR (neat) ν: 3440, 1729, 1461, 1367, 1157. MALDI-TOF-MS *m/z*: 818 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 36 H), 1.68 (b, 10 H), 2.34 (t, 8 H, J = 7.2 Hz), 2.47 (m, 8H), 2.64 (t, 2 H, J = 5.7 Hz), 2.68 (t, 8 H, J = 7.2 Hz), 3.38 (b, 8 H), 3.77 (t, 2 H, J = 5.4 Hz). ^{13}C NMR ($CDCl_3$) δ: 27.1, 27.6, 28.1, 29.7, 33.8, 49.4, 50.5, 51.0, 54.9, 64.4, 69.0, 80.2, 172.1. HRMS *m/z*: calcd for $C_{43}H_{83}O_{11}N_3$ 818.6106, found 818.6103.

Compound 8. Diisopropyl azidodicarboxylate (DIAD) (6.36 g, 31.5 mmol) was added to a mixture of **4** (8.0 g, 24.2 mmol), *p*-methoxyphenol (9.0 g, 73 mmol), and PPh_3 (8.5 g, 32 mmol) in THF (75 mL), and the mixture was refluxed for 15 min. Solvents were then removed in vacuo, and the crude product was diluted with EtOAc (300 mL), washed with saturated aqueous NaOH (6 × 150 mL) solution and brine (2 × 150 mL), dried, and concentrated. The resulting crude product was purified (SiO_2) (hexane/EtOAc, 90:10) to afford **8** as a colorless liquid (9.63 g, 91%). FT-IR (neat) ν: 1729, 1509, 1367, 1232, 1155. EI-MS *m/z*: 437 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 18 H), 1.89 (q, 2 H, J = 6.6 Hz), 2.36 (t, 4 H, J = 7.2 Hz), 2.58 (t, 2 H, J = 6.6 Hz), 2.74 (t, 4 H, J = 7.2 Hz), 3.76 (s, 3 H), 3.93 (t, 2 H, J = 6.3 Hz), 6.82 (s, 4 H). ^{13}C NMR ($CDCl_3$) δ: 27.3, 28.1, 33.8, 49.4, 50.2, 55.7, 66.5, 80.2, 114.6, 115.4, 153.2, 153.6, 172.0. HRMS *m/z*: calcd for $C_{24}H_{39}O_6N$ 438.2855, found 438.2854.

Compound 9. To a solution of **8** (1.57 g, 3.5 mmol) in CH_2Cl_2 (50 mL) were added AcCl (1.4 mL) and H_2O (0.36 mL), and the deprotection reaction was continued and worked up as given in the general procedure IV for hydrolysis of *tert*-butyl esters to afford **9** as white foamy solid (1.2 g, 97%). FT-IR (KBr) ν: 3414, 1717, 1509, 1229, 1025. ES-MS *m/z*: 325 $[M+]^+$. ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ: 2.32 (b, 2 H), 2.98 (t, 4 H, J = 6.6 Hz), 3.31 (t, 2 H, J = 6.6 Hz), 3.42 (t, 4 H, J = 6.9 Hz), 3.78 (s, 3 H), 4.03 (b, 2 H), 6.83 (s, 4 H). ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ: 28.5, 48.5, 49.6, 50.6, 55.4, 65.1, 114.5, 115.3, 152.1,153.9, 171.3.

Compound 10. A solution of **8** (0.96 g, 2.2 mmol) in THF (25 mL) was added dropwise to a suspension of LAH (0.20 g, 5.28 mmol) in THF (10 mL), and the reaction was continued further as described in the general procedure III to obtain **10** as a colorless liquid (0.65 g, 99%). FT-IR (neat) ν: 3383, 1508, 1467, 1231, 1039. ES-MS *m/z*: 298 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.73 (q, 4 H, J = 6.0 Hz), 1.96 (q, 2 H, J = 6.0 Hz), 2.60−2.67 (m, 6 H), 3.73 (t, 4 H, J = 6.0 Hz), 3.76 (s, 3 H), 3.95 (t, 2 H, J = 6.0 Hz), 6.83 (s, 4 H). ^{13}C NMR ($CDCl_3$) δ: 26.6, 28.5, 50.8, 52.8, 55.7, 62.4, 66.5, 114.6, 115.4, 152.9, 153.8. HRMS *m/z*: calcd for $C_{16}H_{27}O_4N$ 298.2018, found 298.2045.

Compound 11. Acrylonitrile (0.25 g, 4.84 mmol) was added to a mixture of **10** (0.65 g, 2.2 mmol) and aqueous NaOH (40%) (22 μL), and the mixture was stirred 15 h and worked up as described in the general procedure I to afford **11** as a colorless liquid (0.80 g, 90%). FT-IR (neat) ν: 2251, 1508, 1232, 1116. ES-MS *m/z*: 404 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.70 (q, 4 H, J = 6.3 Hz), 1.87 (q, 2 H, J = 6.6 Hz), 2.49 (t, 4 H, J = 6.9 Hz), 2.56 (t, 6 H, J = 6.3 Hz), 3.49 (t, 4 H, J = 6.3 Hz), 3.58 (t, 4 H, J = 6.3 Hz), 3.76 (s, 3 H), 3.95 (t, 2 H, J = 6.3 Hz), 6.83 (s, 4 H). ^{13}C NMR ($CDCl_3$) δ: 18.8, 27.1, 27.3, 50.2, 50.4, 55.7, 65.2, 69.3, 66.3, 114.5, 115.2, 117.9, 153.1, 153.6. HRMS *m/z*: calcd for $C_{22}H_{33}O_4N_3$ 404.2549, found 404.2560.

Compound 12. Bis-nitrile derivative **11** (0.80 g, 1.98 mmol) in MeOH (0.5 mL) was added with Raney Co catalyst (40 mL), and the reaction was continued further as given in the general procedure V to afford the amine intermediate. A solution of amine (0.81 g, 1.96 mmol) in MeOH (5 mL) was treated with *tert*-butyl acrylate (4.37 g, 34.1 mmol), and the reaction was followed as given in general procedure V to afford, after purifications (hexane/EtOAc, 85:15), **12** as a colorless liquid (1.62 g, 89% combined yield for nitrile reduction and Michael addition). FT-IR (neat) ν: 1728, 1508, 1367, 1232, 1156. ES-MS *m/z*: 924 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.40 (s, 36 H), 1.60−1.68 (m, 8 H), 1.84 (q, 2 H, J = 6.6 Hz), 2.31 (t, 8 H, J = 7.2 Hz), 2.40−2.48 (m, 8 H), 2.52 (m, 2 H), 2.68 (t, 8 H, J = 7.2 Hz), 3.32−3.38 (app. q, 8 H, J = 6.3 Hz), 3.73 (s, 3 H), 3.91 (t, 2 H, J = 6.0 Hz), 6.82 (s, 4 H). ^{13}C NMR ($CDCl_3$) δ: 27.6, 28.0, 33.7, 49.4, 50.5, 50.8, 55.7, 66.7, 68.9, 69.1, 80.2, 114.5, 115.3, 153.2, 153.4, 172.0. HRMS *m/z*: calcd for $C_{50}H_{89}O_{12}N_3$ 924.6524, found 924.6549.

Compound 13. To a solution of **12** (0.60 g, 0.6 mmol) in CH_2Cl_2 (15 mL) were added AcCl (1.1 mL) and H_2O (0.24 mL), and the deprotection reaction was continued and worked up as given in the general procedure IV for hydrolysis of *tert*-butyl esters to afford **13** as a white, foamy solid (0.45 g, 99%). FT-IR (KBr) ν: 3414, 1717, 1509, 1229, 1025. ES-MS *m/z*: 700 $[M + 1]^+$. ^{1}H NMR (D_2O) δ: 1.88 (b, 8 H), 2.00 (b, 2 H), 2.70 (b, 8 H), 3.10 (b, 10 H), 3.25 (b, 8 H), 3.40 (b, 8 H), 3.61 (s, 3 H), 3.98 (b, 2 H), 6.81 (s, 4 H). ^{13}C NMR (D_2O) δ: 29.1, 50.2, 51.8, 52.7, 53.5, 56.6, 66.7, 68.4, 68.7, 116.0, 116.9, 152.8, 154.4, 175.1. HRMS *m/z*: calcd for $C_{34}H_{57}O_{12}N_3$ 700.4020, found 700.3992.

Compound 14. To a suspension of LAH (84 mg, 2.28 mmol) in THF (10 mL) was added dropwise **12** (0.43 g, 0.463 mmol) in THF (5 mL), and the reaction continued further as described in the general procedure III to afford **14** as a colorless liquid (0.29 g, 97%). FT-IR (neat) ν: 3383, 1508, 1467, 1231, 1111, 1060. ES-MS *m/z*: 644 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.71 (b, 16 H), 1.85 (b, 2 H), 2.51 (b, 10 H), 2.61 (t, 8 H, J = 6.0 Hz), 3.41 (m, 8 H), 3.71 (t, 8 H, J = 5.4 Hz), 3.76 (s, 3 H), 3.94 (t, 2 H, J = 6.0 Hz), 6.82 (s, 4 H). ^{13}C NMR ($CDCl_3$) δ: 25.5, 26.9, 27.1, 28.5, 50.4, 50.7, 50.8, 52.6, 55.7, 62.2, 66.5, 68.7, 69.0, 114.5, 115.3, 153.0, 153.6. HRMS *m/z*: calcd for $C_{34}H_{65}O_8N_3$ 644.4850, found 644.4830.

Compound 15. Acrylonitrile (0.20 g, 3.84 mmol) was added to a mixture of **14** (0.54 g, 0.82 mmol) and aqueous NaOH (40%) (36 μL), and the reaction mixture was worked up as described in the general procedure I. The resulting product

was purified (alumina) (hexane/EtOAc, 1:1) to afford **15** as a colorless liquid (0.66 g, 92%). FT-IR (neat) ν: 2251, 1508, 1231, 1118. ES-MS *m/z*: 856 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.70 (b, 16 H), 1.87 (q, 2 H, J = 6.6 Hz), 2.46 (b, 18 H), 2.59 (t, 8 H, J = 6.3 Hz), 3.39 (b, 8 H), 3.53 (t, 8 H, J = 6.3 Hz), 3.63 (t, 8 H, J = 6.3 Hz), 3.75 (s, 3 H), 3.94 (t, 2 H, J = 6.0 Hz), 6.82 (s, 4 H). ^{13}C NMR ($CDCl_3$) δ: 18.8, 27.1, 27.2, 27.3, 50.4, 50.7, 55.6, 65.2, 65.8, 69.0, 69.3, 114.5, 115.2, 117.9, 153.1, 153.6. HRMS *m/z*: calcd for $C_{46}H_{77}O_8N_7$ 856.5912, found 856.5908.

Compound 16. Tetrakis-nitrile **15** (0.60 g, 0.7 mmol) in MeOH (0.5 mL) was added with Raney Co catalyst in H_2O (40 mL), and the reaction was continued further as given in the general procedure V to afford the amine intermediate. A solution of the amine (0.50 g, 0.28 mmol) in MeOH (5 mL) was treated with *tert*-butyl acrylate (4.37 g, 34.1 mmol), and the reaction was followed as given in general procedure V to afford, after purifications (hexane/EtOAc, 85:15), **16** as a colorless liquid (1.08 g, 83% combined yield for nitrile reduction and Michael addition reaction). FT-IR (neat) ν: 1728, 1508, 1367, 1232, 1156. MALDI-TOF *m/z*: 1897 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.41 (s, 72 H), 1.67 (b, 24 H), 1.85 (b, 2 H), 2.31 (t, 16 H, J = 7.2 Hz), 2.44 (b, 24 H), 2.55 (b, 2 H), 2.68 (t, 16 H, J = 7.2 Hz), 3.37 (b, 24 H), 3.74 (s, 3 H), 3.91 (t, 2 H, J = 6.3 Hz), 6.79 (s, 4 H). ^{13}C NMR ($CDCl_3$) δ: 27.6, 28.1, 33.7, 49.4, 50.5, 50.8, 55.7, 69.0, 80.2, 114.6, 115.4, 153.2, 153.4, 172.0.

Compound 17. To a solution of **16** (0.60 g, 0.31 mmol) in CH_2Cl_2 (15 mL) were added AcCl (1.0 mL) and H_2O (0.22 mL), and the deprotection reaction was continued and worked up as given in the general procedure IV to afford **17** as a white, foamy solid (0.45 g, 99%). FT-IR (KBr) ν: 3414, 1717, 1509, 1229, 1025. ES-MS *m/z*: 1450 $[M]^+$. ^{1}H NMR (D_2O) δ: 1.88 (m, 26 H), 2.71 (t, 16 H, J = 6 Hz), 3.05 (t, 16 H, J = 6.9 Hz), 3.15 (t, 26 H, J = 6.6 Hz), 3.30−3.40 (b, 24 H), 3.62 (s, 3 H), 4.00 (b, 2 H), 6.83 (s, 4 H). ^{13}C NMR (D_2O) δ: 27.5, 29.2, 50.3, 51.4, 51.7, 53.0, 56.6, 68.3, 68.8, 116.0, 116.9, 152.9, 159.9, 175.1.

Compound 18. A mixture of **4** (1.0 g, 3.0 mmol) and DMAP (1.1 g, 0.9 mmol) in PhMe (20 mL) was added with 1,3,5-benzenetricarbonyl trichloride (0.2 g 0.8 mmol), and the reaction was continued and worked up as described in the general procedure VI to afford, after purification (SiO_2) (hexane/EtOAc, 85:15), **18** as a colorless oil (0.8 g, 83%). FT-IR (neat) ν: 1728, 1457, 1367, 1241, 1157. MALDI-TOF *m/z*: 1151 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 54 H), 1.95 (q, 6 H, J = 6.3 Hz), 2.36 (t, 12 H, J = 6.9 Hz), 2.57 (t, 6 H, J = 6.3 Hz), 2.74 (t, 12 H, J = 6.9 Hz), 4.40 (t, 6 H, J = 6.3 Hz), 8.82 (s, 3 H). ^{13}C NMR ($CDCl_3$) δ: 26.8, 28.1, 33.8, 49.4, 50.1, 64.1, 80.3, 131.5, 134.4, 165.0, 171.7.

Compound 19. To a solution of **18** (0.73 g, 0.63 mmol) in CH_2Cl_2 (15 mL) were added AcCl (2.5 g, 31.7 mmol) and H_2O (0.5 g, 28.5 mmol), and the deprotection reaction was continued and worked up as given in the general procedure IV for hydrolysis of *tert*-butyl esters to afford **19** as a white, foamy solid (0.5 g, 97%). FT-IR (neat) ν: 2568, 1731, 1440, 1244, 1107. MALDI-TOF *m/z*: 897 $[M + 2Na + K]^+$. ^{1}H NMR (D_2O) δ: 2.20 (b, 6 H), 2.76 (t, 12 H, J = 6.3 Hz), 3.32 (t, 6 H, J = 7.2 Hz), 3.39 (t, 12 H, J = 6.3 Hz), 4.38 (t, 6 H, J = 5.4 Hz), 8.67 (s, 3 H). ^{13}C NMR (D_2O) δ: 23.6, 29.1, 51.6, 53.6, 63.6, 131.5, 135.4, 167.0, 173.4.

Compound 20. A mixture of **7** (0.6 g 0.13 mmol) and DMAP (0.25 mg, 2 mmol) in PhMe (10 mL) was added with 1,3,5-benzenetricarbonyl chloride (54 mg, 0.2 mmol), and the reaction was continued and worked up as described in the general procedure VI to afford, after purification (SiO_2) (hexane/EtOAc, 70:30), to afford **20** as a colorless liquid (0.25 g, 47%). FT-IR (neat) ν: 1731, 1463, 1366, 1244, 1157. ES-MS *m/z*: 2610 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 108 H), 1.62−1.68 (m, 30 H), 2.34 (t, 24 H, J = 7.2 Hz), 2.46 (b, 30 H), 2.71 (t, 24 H, J = 7.2 Hz), 3.40 (m, 24 H), 4.42 (t, 6 H, J = 6.3 Hz), 8.81 (s, 3 H). ^{13}C NMR ($CDCl_3$) δ: 26.8, 27.4, 27.7, 33.8, 49.4, 50.5, 50.8, 64.3, 69.0, 80.2, 131.5, 135.4, 165.0, 172.0.

Compound 21. To a solution of **20** (0.8 g, 0.3 mmol) in CH_2Cl_2 (15 mL) were added AcCl (1.6 mL) and H_2O (0.36 mL), and the deprotection reaction was continued and worked up as given in the general procedure IV for hydrolysis of *tert*-butyl esters to afford **21** a white, foamy solid (0.6 g, 99%). FT-IR (neat) ν: 2618, 1729, 1462, 1244, 1106. ES-MS *m/z*: 1938 $[M]^+$. ^{1}H NMR (D_2O) δ: 1.90 (b, 24 H), 2.14 (b, 6 H), 2.71 (t, 24 H, J = 6.3 Hz), 3.16 (t, 30 H, J = 6.3 Hz), 3.29 (t, 24 H, J = 6.3 Hz), 3.44 (m, 24 H), 4.35 (t, 6 H, J = 5.7 Hz), 8.66 (s, 3 H). ^{13}C NMR (D_2O) δ: 23.6, 24.0, 24.2, 29.3, 49.9, 50.4, 51.7, 52.9, 53.6, 64.1, 68.4, 68.9, 131.5, 135.4, 167.0, 175.3.

Compound 22.[17] Acrylonitrile (5.83 g, 0.11 mol) was added with aqueous NaOH (40%) (1 mL), and the reaction was conducted as given in the general procedure I to afford **22** as a colorless liquid (12.0 g, 98%). FT-IR (neat) ν: 2251, 1455, 1363, 1104. ^{1}H NMR ($CDCl_3$) δ: 2.62 (t, 4 H, J = 6.3 Hz), 3.68 (t, 4 H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ: 18.8, 65.7, 117.5.

Compound 23. Bis-nitrile **22** (0.80 g, 6.56 mmol) in MeOH (0.5 mL) was added with Raney cobalt catalyst in H_2O (40 mL), and the reaction was continued further as given in the general procedure V to afford the amine intermediate. A solution of the amine (0.82 g, 6.55 mmol) in MeOH (5 mL) was treated with *tert*-butyl acrylate (4.37 g, 34.1 mmol), and the reaction was followed as given in general procedure V to afford, after purifications (hexane/EtOAc, 85:15), **23** as a colorless liquid (3.78 g, 90% combined yield for nitrile reduction and Michael addition reaction). FT-IR (neat) ν: 1729, 1457, 1367, 1153. EI-MS *m/z*: 644 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 36 H), 1.69 (q, 4 H, J = 6.3, 6.9 Hz), 2.35 (t, 8 H, J = 7.2 Hz), 2.47 (t, 4 H, J = 6.9 Hz), 2.72 (t, 8 H, J = 7.2 Hz), 3.40 (t, 4 H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ: 27.6, 28.1, 33.7, 49.4, 50.5, 68.9, 80.2, 172.0. HRMS *m/z*: calcd for $C_{34}H_{64}O_9N_2$ 645.4690, found 645.4666.

Compound 24. To a solution of **23** (0.64 g, 1 mmol) in CH_2Cl_2 (15 mL) were added AcCl (1.7 mL) and H_2O (0.36 mL), and the deprotection reaction was continued and worked up as given in the general procedure IV for hydrolysis of *tert*-butyl esters to afford **24** as a white, foamy solid (0.41 g, 97%). FT-IR (neat) ν: 2568, 1729, 1416, 1186. ES-MS *m/z*: 421 $[M]^+$. ^{1}H NMR (D_2O) δ: 1.89 (b, 4 H), 2.74 (t, 8 H, J = 6.3 Hz), 3.18 (b, 4 H), 3.32 (t, 8 H, J = 6.3 Hz), 3.44 (b, 4 H). ^{13}C NMR (D_2O) δ: 23.9, 29.1, 50.3, 52.7, 68.8, 175.4. HRMS *m/z*: calcd for $C_{18}H_{32}O_9N_2$ 421.2186, found 421.2227.

Compound 25. A solution of **23** (3.78 g, 5.87 mmol) in THF (10 mL) was added dropwise to a suspension of LAH (1.06 g, 28 mmol) in THF (15 mL), and the reaction was continued further as described in the general procedure III to afford **25** as a colorless liquid (2.14 g, 99%). FT-IR (neat) ν: 3378, 1465, 1243, 1061. ES-MS *m/z*: 365 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.74 (m, 12 H), 2.53 (t, 4 H, J = 6.9 Hz), 2.60 (t, 8 H, J = 6.3 Hz), 3.45 (t, 4 H, J = 6.3 Hz), 3.71 (t, 8 H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ: 26.8, 28.6, 50.9, 52.8, 62.4, 68.8. HRMS *m/z*: calcd for $C_{18}H_{40}O_5N_2$ 365.3015, found 365.3016.

Compound 26. Acrylonitrile (1.06 g, 20 mmol) was added to a mixture of **25** (1.53 g, 4.2 mmol) and aqueous NaOH (40%) (0.17 mL), and the reaction was continued and worked up as given in the general procedure I to afford, after purification (alumina) ($CHCl_3$/MeOH, 99:1), **26** as a colorless liquid (2.2 g, 92%). FT-IR (neat) ν: 2251, 1508, 1232, 1116. ES-MS *m/z*: 578 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.66 (b, 12 H), 2.44 (b-m, 12 H), 2.56 (t, 8 H, J = 6.3 Hz), 3.36 (t, 4 H, J = 6.3 Hz), 3.47 (t, 8 H, J = 6.3 Hz), 3.59 (t, 8 H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ: 18.8, 27.3, 50.4, 50.7, 65.7, 69.3, 117.5. HRMS *m/z*: calcd for $C_{30}H_{53}O_5N_6$ 577.4077, found 577.4054.

Compound 27. Tetrakis-nitrile **26** (0.8 g, 1.38 mmol) in MeOH (0.5 mL) was added with Raney cobalt catalyst in H_2O (40 mL), and the reaction was continued further as given in the general procedure V to afford the amine intermediate. A solution of the amine (0.81 g, 1.38 mmol) in MeOH (5 mL) was treated with *tert*-butyl acrylate (4.37 g, 34.1 mmol), and the reaction was followed as given in general procedure V to afford, after purifications (hexane/EtOAc, 85:15), **27** as a colorless liquid (1.81 g, 80% combined yield for nitrile reduction and Michael addition reaction). FT-IR (neat) ν: 1728, 1458, 1367,

1255, 1156. MALDI-TOF *m/z*: 1618 $[M]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.45 (s, 72 H), 1.69 (b-m, 20 H), 2.34 (t, 16 H, J = 7.2 Hz), 2.40−2.46 (m, 20 H), 2.71 (t, 16 H, J = 7.2 Hz), 3.40 (m, 20 H). ^{13}C NMR ($CDCl_3$) δ: 27.6, 28.1, 33.8, 49.4, 50.5, 50.8, 68.9, 69.1, 80.2, 172.0.

Compound 28. To a solution of **27** (0.34 g, 0.21 mmol) in CH_2Cl_2 (15 mL) were added AcCl (0.70 mL) and H_2O (0.16 mL), and the deprotection reaction was continued and worked up as given in the general procedure IV to afford **28** as a white, foamy solid (0.23 g, 97%). FT-IR (neat) ν: 2568, 1729, 1416, 1186. ^{1}H NMR (D_2O) δ: 1.88 (b, 20 H), 2.78 (b, 16 H), 3.15 (b, 20 H), 3.34 (b, 16 H), 3.45 (b, 20 H). ^{13}C NMR (D_2O) δ: 24.8, 24.9, 27.4, 29.0, 29.9, 50.4, 50.7, 51.0, 52.1, 53.4, 68.9, 69.2, 69.4, 175.1.

Compound 29. A solution of **27** (0.36 g, 0.22 mmol) in THF (5 mL) was added dropwise to a suspension of LAH (84 mg, 2.28 mmol) in THF (5 mL), and the reaction was continued further as described in the general procedure III to afford **29** as a colorless liquid (0.23 g, 97%). FT-IR (neat) ν: 3383, 1508, 1467, 1231, 1111, 1060. MALDI-TOF *m/z*: 1087 $[M + Na]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.75 (m, 20 H), 1.86 (m, 16 H), 2.45 (m, 20 H), 2.60 (t, 16 H, J = 6.3 Hz), 3.43 (m, 16 H), 3.75 (t, 20 H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ: 25.6, 26.9, 27.1, 50.7, 50.9, 52.7, 62.3, 67.9, 68.8, 69.1.

Compound 30. Acrylonitrile (0.12 g, 2.26 mmol) was added to a mixture of **29** (0.27 g, 0.26 mmol) and aqueous NaOH (40%) (23 μL), and the reaction was continued and worked up as given in the general procedure I to afford, after purification (alumina) ($CHCl_3$/MeOH, 98:2), **30** as a colorless liquid (0.35 g, 92%). FT-IR (neat) ν: 2251, 1508, 1231, 1118. ES-MS *m/z*: 1484 $[M + 1]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.63−1.75 (m, 36 H), 2.36−2.47 (m, 20 H), 2.58 (t, 32 H, J = 6.3 Hz), 3.40 (t, 20 H, J = 7.2 Hz), 3.51 (t, 16 H, J = 6.6 Hz), 3.63 (t, 16 H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$) δ: 18.8, 27.2, 27.3, 50.4, 50.7, 65.8, 69.0, 69.4, 117.4.

Compound 31. Octakis-nitrile **30** (0.35 g, 0.24 mmol) was added with Raney cobalt catalyst in H_2O (40 mL), and the reaction was continued further as given in the general procedure V to afford the amine intermediate. A solution of the amine (0.25 g, 0.16 mmol) in MeOH (5 mL) was treated with *tert*-butyl acrylate (4.37 g, 34.1 mmol), and the reaction was followed as given in general procedure V to afford, after purifications (alumina) (hexane/EtOAc, 60:40), **31** as a colorless liquid (0.8 g, 95% combined yield for nitrile reduction and Michael addition reaction). FT-IR (neat) ν: 1729, 1458, 1367, 1255, 1157. ES-MS *m/z*: 3601 $[M + K]^+$. ^{1}H NMR ($CDCl_3$) δ: 1.39 (s, 144 H), 1.64 (q, 52 H, J = 6.9 Hz), 2.34 (t, 32 H, J = 6.9 Hz), 2.46 (b-t, 52 H, J = 7.2 Hz), 2.71 (t, 32 H, J = 7.2 Hz), 3.39 (app. t, 52 H). ^{13}C NMR ($CDCl_3$) δ: 28.3, 28.5, 29.0, 34.6, 50.3, 51.4, 51.7, 69.8, 70.1, 70.3, 81.0, 172.9.

Compound 32. To a solution of **31** (0.52 g, 1 mmol) in CH_2Cl_2 (15 mL) were added AcCl (1.0 mL) and H_2O (0.36 mL), and the deprotection reaction was continued and worked up as given in the general procedure IV to afford **32** as a white, foamy solid (0.38 g, 97%). FT-IR (neat) ν: 2568, 1729, 1416, 1186. ^{1}H NMR (D_2O) δ: 1.90 (b, 52 H), 2.74 (b, 32 H), 3.15 (b, 52 H), 3.34 (b, 32 H), 3.45 (b, 52 H). ^{13}C NMR (D_2O) δ: 24.0, 24.3, 28.0, 29.3, 30.3, 49.7, 50.1, 50.3, 50.4, 51.3, 51.4, 68.3, 68.4, 68.8, 175.1.

Cytotoxicity Studies (MTT Assay). Chinese hamster ovarian cells or breast cancer T47D cells were seeded at a density of 10 000 cells/well in a 96-well plate and grown in 95 μL of culture medium supplemented with 10% fetal bovine serum for 24 h. Following exposure of the cells with 5 μL of aqueous solutions of dendrons or dendrimers for 24 h, 25 μL of 5 mg/mL stock solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was added to each well. After 2 h of incubation at 37 °C, 100 μL of extraction buffer (containing 20% w/v SDS in a solution of 50% of DMF, pH 4.7) was added. Absorbance was measured at 570 nm after an overnight incubation at 37 °C.

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Supporting Information Available: General methods of experimental details, ^{1}H NMR spectrum of **31**, ^{13}C NMR spectra of dendrons and dendrimers **1**−**21** and **23**−**32**. This material is available free of charge via the Internet at http://pubs.acs.org.

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