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Triazine Dendrimers with Orthogonally Protected Amines on the Periphery. Masking Amines with Dde and BOC Groups Provides an Alternative to Carrying Protected Alcohols and Disulfides through an Iterative Synthesis

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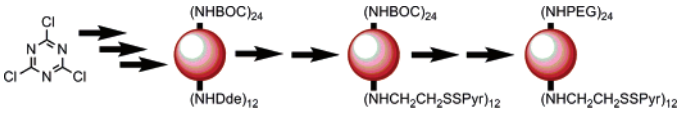

An orthogonally protected dendrimer based on melamine displaying 24 Boc-protected amines (Boc is *t*-butoxycarbonyl) and 12 Dde-protected (Dde is *N*-2-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl) amines was synthesized using a convergent route in four linear steps in 43% overall yield to provide 5 g of product. Postsynthetic manipulation of this dendrimer produced a 57 kDa macromolecule bearing poly(ethylene glycol) groups and pyridyldisulfide groups linked via carbamate and amide bonds, respectively. These orthogonally protected amines provide more reactive handles for chemical modification when compared to other groups, notably hydroxyls, that have been explored. In addition, the Dde group proves to be more stable to the synthetic methods employed than do the disulfides used previously. Monochlorotriazine and dichlorotriazine intermediates are invaluable as a route for eliminating unwanted byproducts arising from over-substitution of the triazine ring. Routes requiring three reactions on a simple, generation zero core versus six reactions on a generation one core are compared.

Introduction

The ability of dendrimers to present multiple functional groups upon a well-defined structure with very low polydispersity offers enormous advantages to these macromolecules and opportunities for multiple uses.[1] Examples of asymmetric or heterogeneously functionalized dendrimers or macromolecules are replete in the literature.[2] For instance, in the development of systems for drug delivery, the desire to covalently incorporate different species such as drug molecules, targeting moieties, radiolabels, and solubilizing groups necessitates the development of macromolecular scaffolds having surface groups amenable to covalent modifications.[3] A similar utility is seen on the other end of the application spectrum in the development of materials for organic electrooptical purposes. The creation of electronic gradients within a macromolecule is possible when the electron source and the electron sink are on the same macromolecule.[4]

While these targets can be obtained through convergent or divergent approaches, historically, heterogenously functionalized dendrimers were derived from a divergent strategy. Although the exact structure of the resulting heterogeneously functionalized dendrimers cannot be assigned due to the statistical nature of the reactions employed, utility and alteration of the physical properties of the dendrimers are observed. Early work from Newkome and co-workers is representative: amine-terminated poly(propylene)imine dendrimers were functionalized with known ratios of possible monomers (isocyanates derived from Behera's amine) leading to combinatorially generated libraries of macromolecules.[5] Convergent approaches successfully reduce

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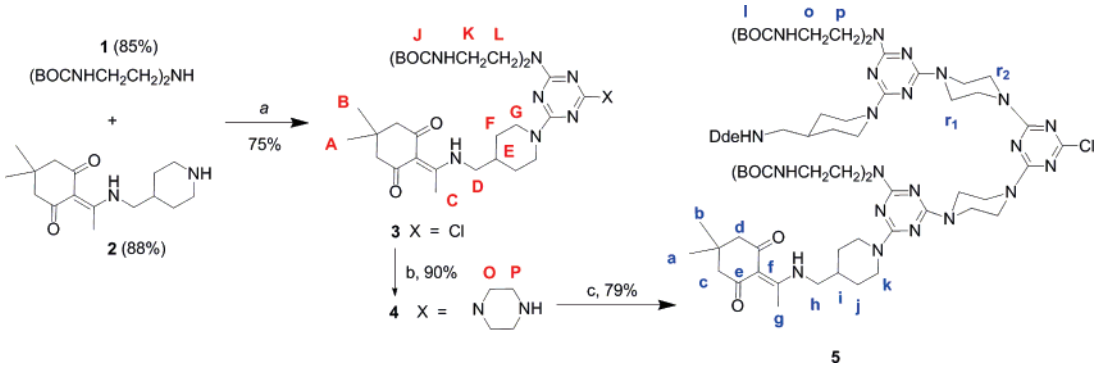
SCHEME 1[a]



[a] (a) Cyanuric chloride, DIPEA, THF, 0 °C, then **1**, then **2**; (b) 5 equiv of piperazine, THF, rt overnight; (c) 0.5 equiv of cyanuric chloride, THF, 4 equiv of DIPEA, 0 °C to rt overnight. Labels refer to assignments in the ^{1}H (red) and ^{13}C (blue) NMR spectra shown in Figure 2.

the heterogeneity. Whether the divergently derived, narrowly disperse mixtures of macromolecules are as effective as single-entity molecules will likely be dependent on their use. These comparisons, however, rest on the availability of practical routes for the latter single-entity macromolecules derived convergently.[6]

The incorporation of orthogonal protecting groups into a dendrimer provides opportunities for the systematic modification of these molecules after their convergent construction. The molecule described bears two reactive amine groups that are orthogonally protected. Our motivation for examining these groups rested in the belief that the greater nucleophilicity of the pendant amines would lead to higher conversions during postsynthetic manipulations of these materials. Previous efforts with masked alcohols suffered from low conversions attributed, in part, to the low nucleophilicity of the hydroxyl group.[7] Here, the moderate-scale synthesis of a third generation dendrimer based on melamine having two orthogonally protected amine groups is described. The Dde[8] and BOC groups are orthogonal protecting groups that can be used to differentiate amines. To establish this role in dendrimers, the Dde groups of **11** were unmasked with hydrazine and acylated with a disulfide-bearing group. Following deprotection of the BOC groups with acid, poly(ethylene glycol) (PEG) chains were installed. We pursued PEGylation over more trivial manipulations as it improves the water solubility, biocompatibility, and biodistribution of these molecules for our intended pursuit, drug delivery.[9]

Results and Discussion

Synthesis of the Core and Dendrons. An accelerated approach relying on the synthesis of an orthogonally protected dendron and a hexavalent, first generation core was adopted.[1b] The synthesis is shown in Scheme 1. Selective protection of the triamine and 4-(aminomethyl)piperidine proceeded smoothly to provide **1** and **2**, respectively. Stepwise reaction of these amines with cyanuric chloride proceeded in 75% yield after chromatography to provide dendron **3**. This step can be executed at a 20 g scale. Substitution of this monochlorotriazine with piperazine provides **4** in good yield, and iterative reaction with cyanuric chloride provides **5**. Of note: the synthesis of **4** from sequential addition of **1**, **2**, and piperazine can be achieved in one pot by manipulating the temperatures at which the addition is performed, but a side product with identical physical properties to **4** was obtained. This material, corresponding to a triazine substituted with piperazine and two Dde-bearing amines, could not be separated by chromatography. Indeed, the only evidence for this trace impurity came from mass spectrometry: the mass difference is 25 Da. This impurity persists through the synthesis and, interestingly, was most readily identified in the mass spectra of the intended product **11**. The intermediacy of **3** proves to be critical in obtaining products of high purity.

The core of the dendrimer, **10**, was prepared from triazines **6** and **8** (Scheme 2). Both building blocks can be obtained from the same reaction pot from cyanuric chloride, but each can be favorably obtained by controlling the temperature. Deprotection of surface amines of **6** and reaction with **8** at elevated temperatures in the presence of excess K_2CO_3 provided **9**. Hunig's base was found to be less efficient than K_2CO_3 for this reaction.[10] Deprotection of **9** with trifluoroacetic acid gave the hexaamine in poor yields, a situation that is remedied using methanolic HCl.

Synthesis of the Target Dendrimer. To prepare the target dendrimer **11**, a slight excess of **5** and **10** were reacted in the presence of the polymer-supported base, 1,5,7-triazabicyclo-[4.4.0]dec-5-ene (p-TBD). With 6.6 equiv of **5** and 12 equiv of the base, a complete substitution of all the amines of the triazine core was observed. This reaction was monitored by thin layer chromatography (TLC) as well as MALDI-TOF MS of the crude reaction mixture (Figure 1). The top trace shows a predominant line for the starting material, **5**, at the left side of the trace and intermediates along the route to complete the desired product (right-most line, *m*/*z* 10476) that include substitution of three, four, and five amines of the core with dendron **5**.

Although the difference in retention factors of **11** and the excess reactant **5** as measured in TLC by preferred solvent systems (i.e., 9:1 CH_2Cl_2/CH_3OH) is not substantial to effect an efficient separation by flash column chromatography, purification of the product was possible using lower concentrations of methanol in dichloromethane (99:1). However, for large-scale reactions, the observed behavior of **11** in flash column

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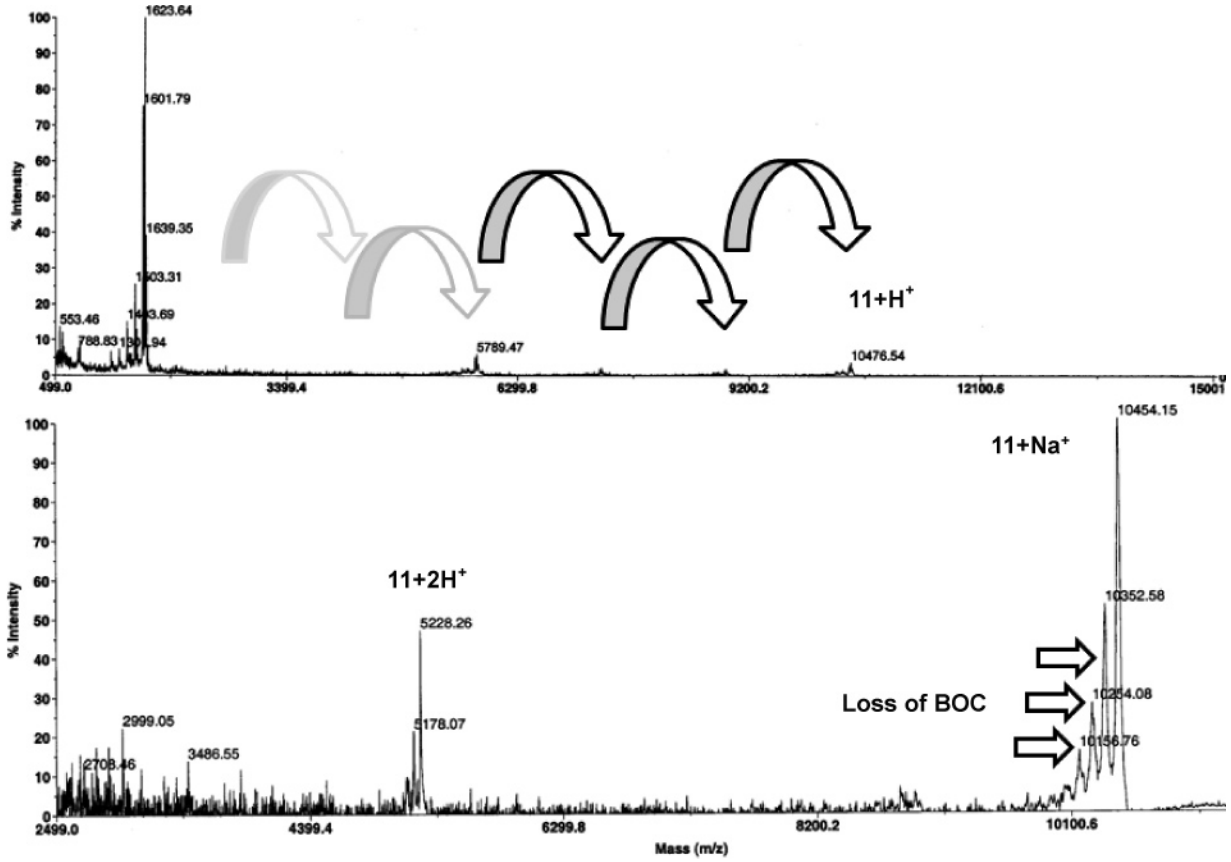
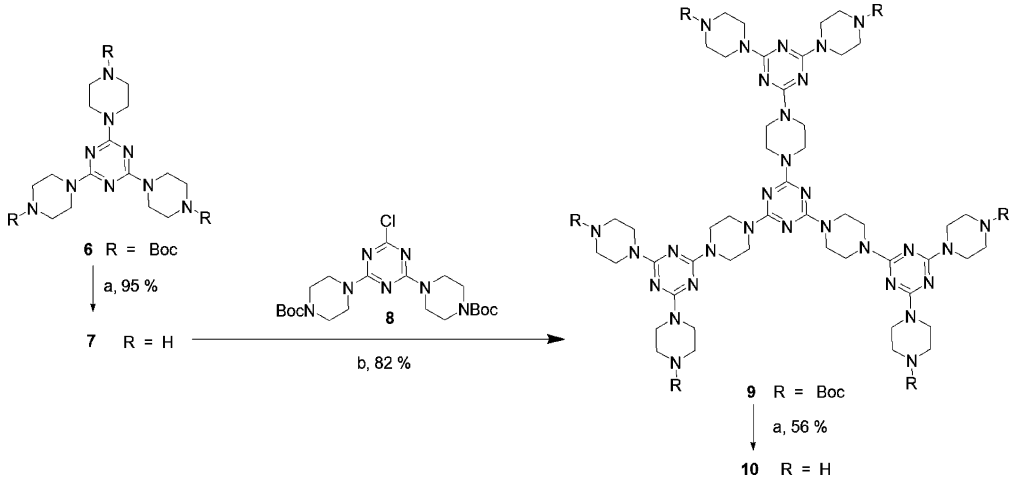


FIGURE 1. MALDI-TOF MS of the reaction mixture at 24 h (top) and 48 h (bottom). In addition to starting material (major species), the sequential addition of dendrons can be inferred (light arrows) and observed (dark arrows) in the top spectrum. The bottom spectra reveal complete conversion to the desired product. Loss of BOC groups (arrows) occurs during ionization in the MALDI-TOF experiment.

SCHEME 2[a]

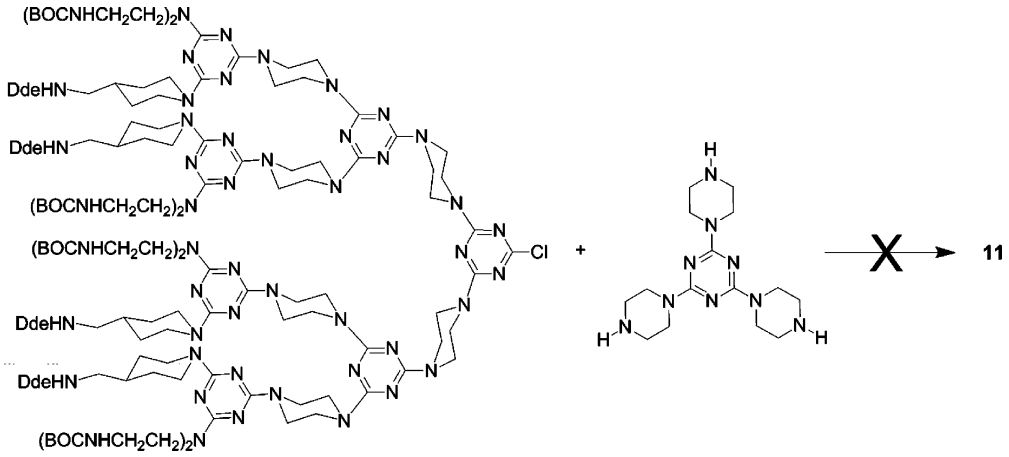


[a] (a) 1:1 TFA/CH_2Cl_2, rt overnight and (b) 6 equiv of **8**, K_2CO_3, $CHCl_3$, THF, rt overnight.

chromatography was different. The majority of the dendrimer eluted before excess **5**, although some of it was retained and eluted in the manner predicted by TLC. Calculated yields refer to the early eluting fraction.

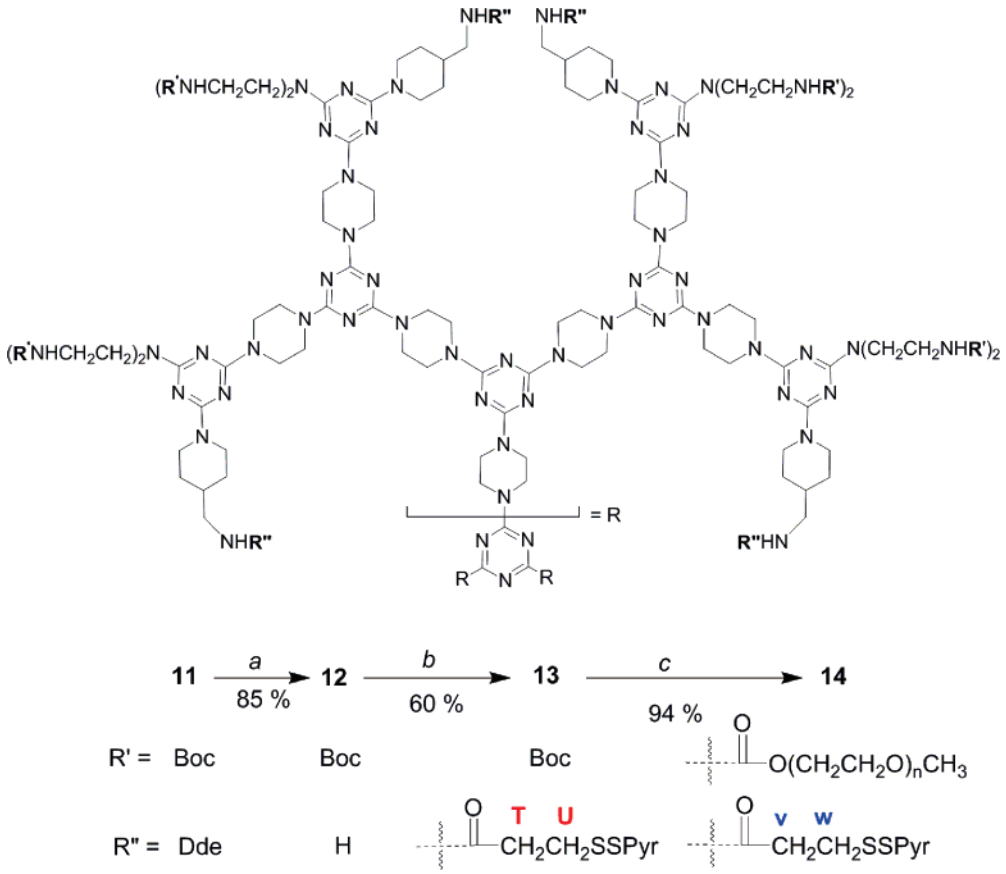
Attempts to synthesize **11** using a tris(piperazine) triazine core and a third generation dendron bearing four Dde-protected and eight Boc-protected amines using Hunig's base were unsuccessful. We attribute this failure to steric crowding and the lower basicity of Hunig's base relative to the piperazine amines in the small triazine core (Scheme 3).[10] Using K_2CO_3 as well as the unsupported form of TBD were equally unsuccessful. Instead, a product or products with very high polarity formed, as shown by the thin layer chromatogram of the reaction mixture, which we attribute to the loss of the Dde group. Unlike most of the chemistries we have pursued, the incorporation of Dde groups into this material led us to be more rigorous in the exclusion of water: ordinarily, these reactions are carried out in wet solvents in air. These considerations and the expense of

SCHEME 3. Preliminary Route toward Synthesis of 11[a]



[a] Generation three dendron is available in 87% overall yield from **4** in the two-step sequence commencing with piperazine, followed by treatment with cyanuric chloride. Reaction to form **11** was plagued with impurities.

SCHEME 4[a]



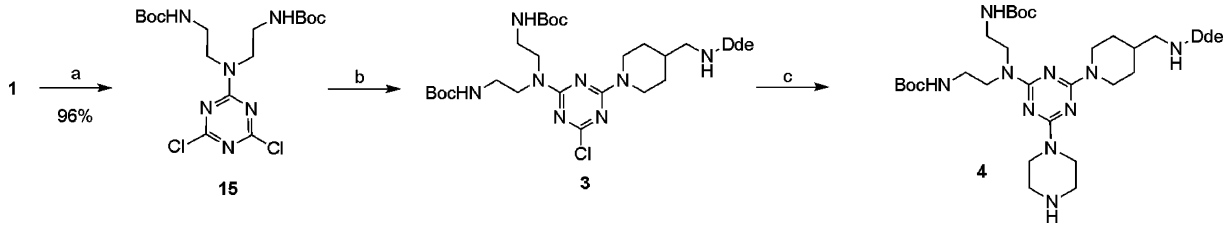
[a] (a) 30% NH_2NH_2 in MeOH; (b) SPDP, CH_2Cl_2; (c) 1:1 TFA/CH_2Cl_2, then dialysis, then 4-nitrophenylPEGOMe.

polymer-supported TBD represent practical limitations of scale with regard to this approach.

Postsynthetic Modification. The orthogonally protected amines of **11** were unmasked and reacted sequentially to afford target **14**, a macromolecule containing 12 thiopyridyl groups and 24 methoxy-terminated PEG groups (Scheme 4). To avoid the known N → N intramolecular migration of Dde groups,[8] Dde-protected amines were first unmasked using hydrazine in methanol. Surprisingly, purification of the resulting polyamine, **12**, can be accomplished by flash column chromatography using silica gel.

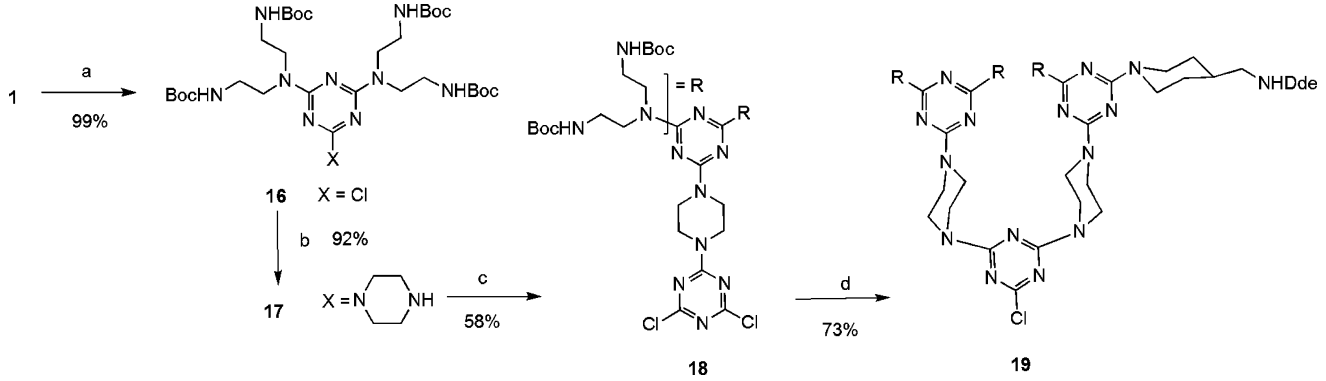
The disulfide groups were installed using 24 equiv of the NHS ester of thiopyridyl-protected 3-thiolpropanoic acid (SPDP) in dichloromethane at room temperature. While the reaction can be monitored using TLC, MALDI-TOF MS was more useful in ensuring complete substitution. Mass spectra of the crude

SCHEME 5[a]



[a] (a) 1.0 equiv of cyanuric chloride, 1.5 equiv of DIPEA, THF, 0 °C, 1 h; (b) 1.0 equiv of Dde-AMP, 3.0 equiv of DIPEA, THF, rt overnight; (c) 5.0 equiv of piperazine, 2.0 equiv of DIPEA, THF, rt overnight. Overall yield for steps b and c was 92%.

SCHEME 6[a]



[a] (a) 0.5 equiv of cyanuric chloride, 4 equiv of DIPEA, THF, rt; (b) 5.0 equiv of piperazine, 2.0 equiv of DIPEA, THF, rt overnight; (c) 1.0 equiv of cyanuric chloride, 2.0 equiv of DIPEA, THF, 0 °C, 4 h; (d) 1.0 equiv of dendron **4**, THF, rt overnight.

reaction mixture on the third day of the reaction indicated a complete functionalization. Dendrimer **13** was purified by flash chromatography. Deprotection of the Boc groups and PEGylation using a known procedure[11] afforded **14** after 10 days. Complete PEGylation required 52 mol of activated methoxy-PEG per mole of the dendrimer. MALDI-TOF of this product showed a molecular mass centered at 57 kDa.

Alternate Route to 4. Success at reducing levels of impurities through the intermediacy of monochlorotriazine **3** led us to examine alternative routes that furthered these aims. Specifically, we pursued isolating dichlorotriazine **15** (Scheme 5) before installing the Dde-protected amine **2**. Compound **15** formed rapidly and precipitated cleanly, with no need for column chromatography, thus eliminating the possibility of multiple Dde-amine substitutions on the triazine ring. Dendron **3** was still isolated before reaction with piperazine, but no column chromatography was necessary. The overall yield for this scheme is comparable to that shown in Scheme 1, but fortuitously, one chromatographic step is eliminated.

This facile isolation strategy can be readily extended. Dendron **16** with four Boc-protected amines was made from bis-Boc-protected diethylenetriamine and cyanuric chloride in 99% yield without chromatography (Scheme 6). Piperazine can be added to form compound **17**, which was used to synthesize the dichlorotriazine **18** in 58% yield after precipitation. We attribute the low yield to the precipitation protocol and byproduct that result when piperazine substitutes two chlorine atoms. Dendron **19** was formed by reaction of dichlorotriazine **18** with dendron **4** in a process that employed only two chromatographic purifications.

Characterization. All intermediates and products gave satisfactory ^{1}H and ^{13}C NMR spectra and traces by ESI and MALDI-TOF MS. Throughout the discussion of the NMR traces, uppercase letters refer to proton NMR assignments. Lowercase letters refer to carbon NMR assignments. To underscore this difference, these assignments are shown in red and blue, respectively, throughout the schemes and figures.

The three singlets at ~δ 1.0, 2.3, and 2.5 in the ^{1}H NMR spectra of **3**−**5** and **11** (**A**, **B**, and **C** in Figure 2) correspond to the three sets of unique and independent methyl groups of the Dde group (Scheme 1). Derivatization of **3** with piperazine led to new triplet peaks, **O** and **P**, corresponding to the methylene protons of piperazine, at δ 2.9 and 3.8 for **4**, which overlapped at δ 3.8 in the monochloride **5**. Protons of 4-(aminomethyl)-piperidine were assigned using 2-D NMR (not shown). The axial and equatorial protons of **F** and **G** (Scheme 1) had different chemical shifts that did not change throughout the synthesis. In the ^{13}C NMR spectra of these intermediates, characteristic peaks due to carbonyl, vinyl, allyl, and quaternary carbons of the Dde group (**d** and **e** and **f** and **g**, and **b**; Scheme 1) and Boc group (**l** shown) were diagnostic for assignment. Appearance of the piperazine carbons in the spectra of **4** was observed ($\mathbf{r_1}$ and $\mathbf{r_2}$, Figure 2). Installation of the triazine ring to yield **5** led to peak overlap. The expected doubling of lines for the methylene carbons **c** and the carbonyl carbons **d** of the cyclic Dde group was established with an HMQC spectra of dendron **4** (Supporting Information). The ^{1}H and ^{13}C NMR spectra of **11** were very similar to those of **5**. Most noteworthy, the line at δ 169.6 corresponding to the triazine carbon bearing chlorine was replaced with one at δ 165 as a result of substitution with the amine of the core in forming **11** (Supporting Information).

The intermediates in the postsynthetic modification of **11** were also characterized by NMR spectroscopy. Loss of the charac-

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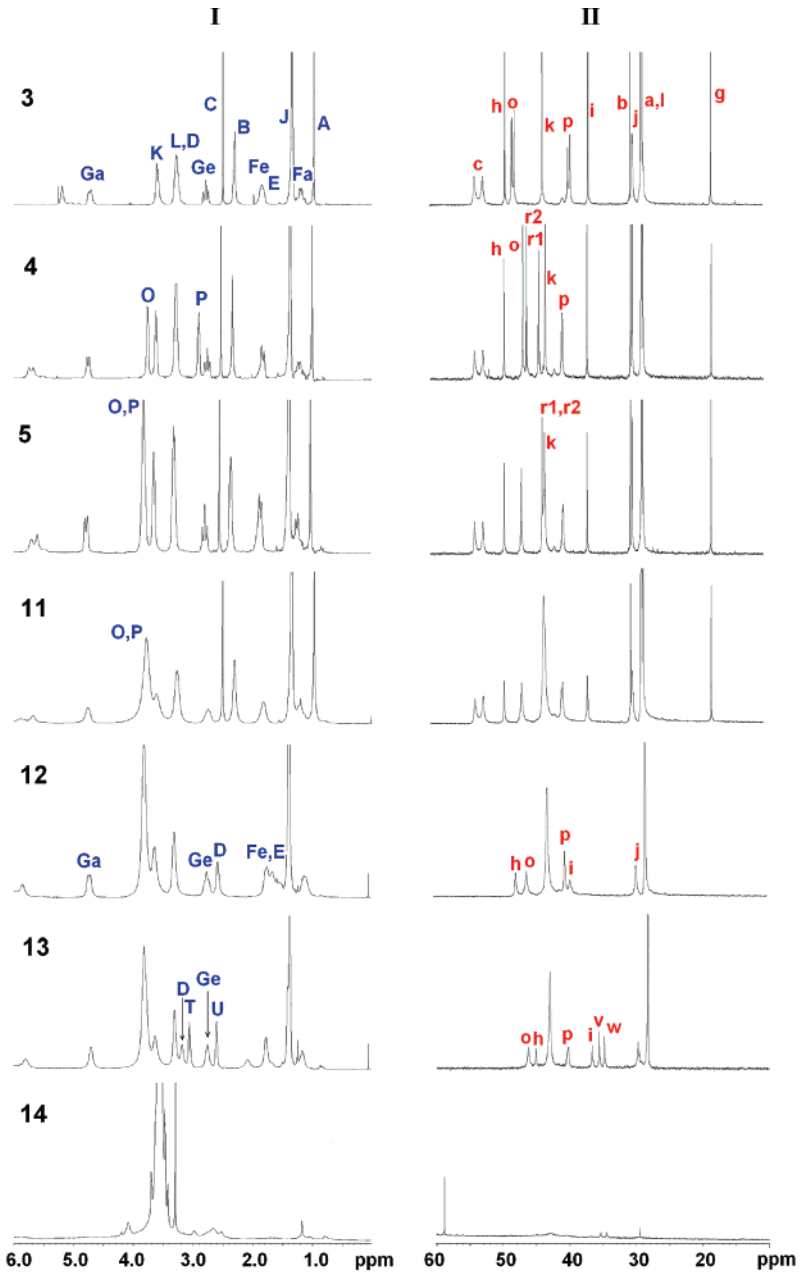

FIGURE 2. NMR spectra of **12**−**14** and intermediates **3**−**11** monitor the iterative reaction. Column I, ^{1}H NMR spectra (δ 0−6 ppm) and column II, ^{13}C NMR spectra (δ 10−60 ppm).

teristic peaks due to Dde was evident in the spectra of **12** (Figure 2). Furthermore, the ^{1}H NMR spectra of this product showed an upfield shift of proton **D**, the exocyclic α proton of (4-aminomethyl)piperidine, in the TOCSY spectra of **12** (Supporting Information). The line for the corresponding carbon, **h**, also shifted upfield. Substitution of the free amines of **12** to give the target **13** derivatized with pyridyl disulfide groups led to a downfield shift of the same protons. The corresponding carbon, however, shifted upfield. Peaks due to aromatic protons and carbons appeared in all the spectra. The ^{1}H and ^{13}C NMR spectra of **14** showed the loss of the peaks due to the loss of Boc, but peaks due to PEG overwhelmed a majority of the signals associated with the dendrimer. The aromatic peaks of pyridyl groups were, however, discernible. The mass spectra of these compounds can be found in the Supporting Information. Table 1 summarizes the calculated and found masses for compounds during the postsynthetic modification. The discrepancies observed reflect the analytical limits of the instrument when used in linear mode and are not necessarily a failure of the chemistries described. Gas phase molecular simulations of target **14** were performed to evaluate the extent of steric hindrance that the PEG chains might communicate (Figure 3). The low energy structure shown illustrates that PEGylation does not necessarily shield all of the dendrimer core nor the disulfide groups from solvent.

TABLE 1. Mass of 11−14 as Obtained by MALDI-TOF MS

compound	M^+ calculated	M^+ found
11	10435	10454
12	8466	8468
13	10830	10847
14	56909	56709

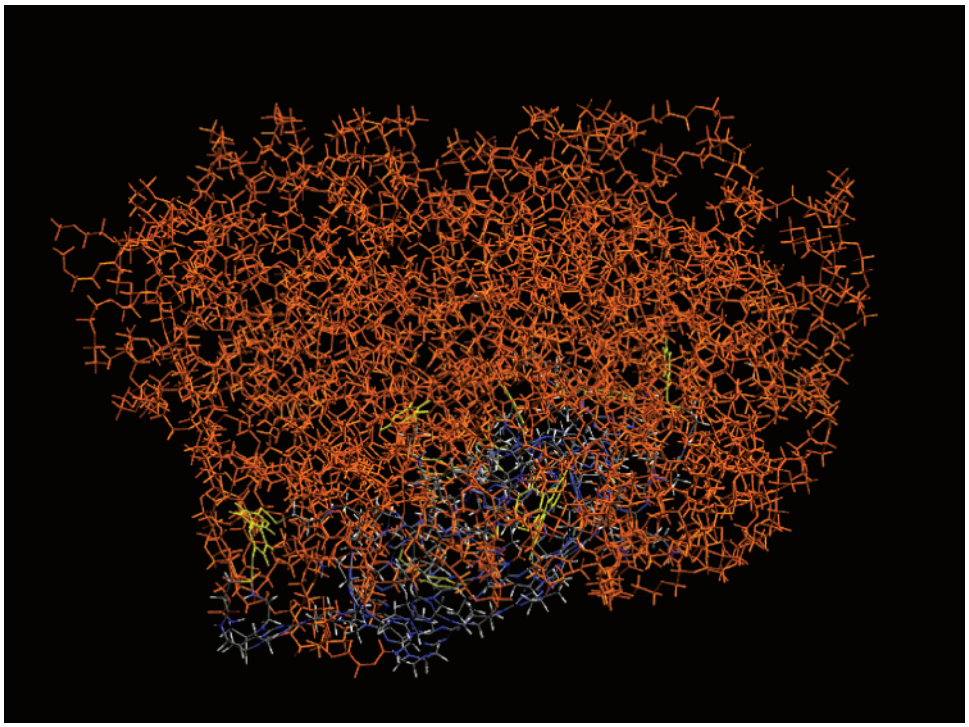

FIGURE 3. Molecular model of **14**. Yellow: pyridyl disulfide groups; blue: dendrimer scaffold; and orange: PEG groups.

Conclusion

A dendrimer based on melamine having orthogonally protected terminal amine groups was synthesized in moderate scale. Postsynthetic manipulation can lead to dendrimers with structurally diversified surface groups in a well-defined manner. PEGylation and loading with thiopyridyl groups were illustrated in this study. The resulting dendrimer had a mass of about 57 kDa while having 12 thiopyridyl groups reactive toward thiol-containing molecules. We have shown previously that small molecules and peptides can be appended to these architectures through thiol-disulfide exchange.[12]

Experimental Section

General. Triazine trichloride, piperazine, 4-(aminomethyl)-piperidine (TCI), diisopropylethylamine or DIPEA, and BOC−ON were used as-is. 1,5,7-Triazabicyclo[4.4.0]dec-5-ene bound to polystyrene cross-linked with 2% DVB (2.6 mmol/g resin) was purchased from a chemical supplier. MALDI-TOF mass spectra (in the positive mode) were acquired on a Voyager-DE STR mass spectrometer equipped with a pulsed nitrogen laser emitting at 337 nm. Samples were analyzed in linear mode using an extraction delay time set at 350 ns and an accelerating voltage operating at 25 kV utilizing trihydroxyacetophenone as the matrix. To improve the signal-to-noise ratio, 100 single shots were averaged for each mass spectrum, and typically, four individual spectra were accumulated to generate a summed spectrum. An external mass spectrum calibration was performed using the calibration mixture 3 of the Sequazyme Protein Mass Standards Kit, including known protein standards in a mass range from 5 to 25 kDa. Electrospray mass spectra were acquired in the positive ion mode using a MDS Sciex API Qstar Pulsar using an Ionwerks time-to-digital converter, TDCx4, for data recording at 625 ps time resolution. Samples were electrosprayed from acetonitrile at 50 mM under the conditions listed next. The ion spray (needle) voltage was held constant at 4.5 kV. The nozzle skimmer potential was set to +10 V to minimize fragmentation in that region. TOF voltages were tuned to optimize the resolving power over the mass range observed, but usually the following parameters were used: grid −338 V, plate +360 V, mirror +960 V, and liner +4000 V. Acquisition and data analysis were performed with the Analyst QS software.

Intermediate 1.[16] Diethylenetriamine (1.00 g, 9.70 mmol, 1.0 equiv) and triethylamine (2.94 g, 29.1 mmol, 3.0 equiv) were dissolved in 50 mL of THF and cooled in an ice bath. 2-(Boc-oxyimino)-2-phenylacetonitrile (BOC−-ON) (4.78 g, 19.40 mmol, 2 equiv), dissolved in 20 mL of THF, was added dropwise to this mixture, which was stirred for an hour in an ice bath and then at room temperature for another hour. The solvent was evaporated, and the residue was dissolved in 100 mL of dichloromethane. The mixture was washed with 5% NaOH. The product was purified by flash column chromatography (silica, 9:1 DCM/MeOH, 1% NH_4-OH), to give 2.47 g (85%). $R_f = 0.63$ (4:1 DCM/MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.19 (q, $J = 5.7$ Hz, 4H), 2.70 (t, $J = 5.7$ Hz, 4H), 1.85 (br, 2H) 1.30 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.4, 79.4, 49.0, 40.4, 28.6. MS: Calcd, 304.0 (M^+); Found, 304.1 (M^+).

Intermediate 2. 4-Aminomethylpiperidine (1.66 g, 14.5 mmol) and 2-acetyldimedone (2.64 g, 14.5 mmol) were dissolved in 100 mL of absolute ethanol and were refluxed for 3 h. The solvent was evaporated, and the product was purified by flash column chromatography (silica, 4:1 DCM/MeOH, 1% NH_4OH), to give a yield of 3.50 g (87.5%). $R_f = 0.075$ (9:1 DCM/MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.27 (t, $J = 6.0$ Hz, 2H), 2.62 (m, 2H), 2.55 (s, 3H), 2.36 (br, 4H), 1.77 (m, 3H), 1.24 (m, 2H), 1.02 (s, 6H). ^{13}C NMR (125 MHz, CD_3OD) 199.9 (br), 175.8, 108.9, 53.6, 50.5, 46.7, 37.5, 32.0, 31.2, 28.5, 18.0. MS: Calcd, 279.2 (M^+); Found, 279.2 (M^+).

Intermediate 3. Cyanuric chloride (1.73 g, 9.38 mmol) and 3 mL of DIPEA was dissolved in 100 mL of THF and cooled to 0 °C. Intermediate **1** (2.85 g, 9.38 mmol) was dissolved in 50 mL of THF and added dropwise to the cold solution of cyanuric chloride and DIPEA. After all of the amine had reacted, as monitored by TLC, another 3 mL of DIPEA and intermediate **2** (2.61 g, 9.38

(12) Umali, A. P.; Simanek, E. E. *Org. Lett.* **2003**, *5*, 1245−1247.

mmol) were added, and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was dissolved in about 50 mL of CH_2Cl_2. The resulting solution was washed with water in three 100 mL portions. The CH_2Cl_2 layer was dried with $MgSO_4$, and the solvent was evaporated. The product was isolated by flash column chromatography (silica, 3:1 DCM/EtOAc) (4.87 g, 75%). R_f = 0.30 (3:1 DCM/EtOAc). ^{1}H NMR (500 MHz, CD_3OD) δ 4.77 (br, 2H), 3.65 (m, 4H), 3.46 (m, 2H), 3.31 (m, 4H), 2.93 (t, J = 12.5 Hz, 2H), 2.59 (s, 3H), 2.39 (s, 4H), 2.01 (br, 1H), 1.89 (d, J = 10.5 Hz, 2H), 1.42 (s, 18H), 1.30 (m, 2H), 1.05 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.2, 196.8, 173.7, 169.4, 165.8, 163.9, 156.3, 156.0, 108.0, 79.3, 79.2, 53.5, 52.3, 48.9, 47.9, 47.5, 43.3, 39.5, 39.1, 36.4, 30.2, 29.9, 29.7, 28.6, 28.4, 28.4, 28.3,18.0. MS: Calcd, 693.39 (M^+); Found, 693.39 (M^+), 699.40 ($M + Li^+$), 715.37 ($M + Na^+$).

Intermediate 4. Intermediate **3** (3.49 g, 5.04 mmol) and piperazine (2.17 g, 25.2 mmol) were dissolved in about 150 mL of THF. The mixture was stirred at room temperature overnight. The solvent was evaporated, and the product was obtained by flash column chromatography (19:1 DCM/MeOH) (3.20 g, 90%). R_f = 0.33 (9:1 DCM/MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.79 (br, 1H), 5.69 (br, 1H), 4.78 (m, 2H), 3.74 (m, 4H), 3.64 (br, 4H), 3.30 (m, 5H), 2.89 (m, 4H), 2.75 (m, 2H), 2.55 (s, 3H), 2.36 (br, 5H), 1.88 (m, 3H), 1.39 (s, 18H), 1.22 (m, 2H), 1.02 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.0, 196.8, 173.5, 166.5, 165.4, 165.1, 164.8, 164.5, 156.1, 107.9, 78.9, 68.1, 61.5, 53.3, 52.2, 49.0, 46.3, 45.5, 43.6, 40.0, 36.5, 29.8, 28.2, 17.9. MS: Calcd, 743.49 (M^+); Found (ESI), 743.46 (M^+).

Intermediate 5. Intermediate **4** (2.82 g, 3.79 mmol) and 3 mL of DIPEA were dissolved in 150 mL of THF. The resulting solution was placed in an ice bath. Cyanuric chloride (0.35 g, 1.90 mmol) was added, and the reaction mixture was stirred at room temperature overnight. The solvent was removed, and the residue was dissolved in DCM. The resulting mixture was washed with three 50 mL portions of distilled water. The DCM layer was dried with $MgSO_4$, and DCM was removed in vacuo. Flash column chromatography (silica, 9:1 DCM/MeOH) gave 2.18 g of the product (79%). R_f = 0.63 (9:1 DCM/MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.65 (br, 2H), 5.58 (br, 2H), 4.76 (d, J = 12.9 Hz, 4H), 3.80 (m, 16H), 3.63 (br, 8H), 3.29 (m, 12H), 2.76 (t, J = 12.3 Hz, 4H), 2.54 (s, 6H), 2.34 (m, 8H), 1.84 (m, 6H), 1.34 (s, 36H), 1.23 (m, 4H), 1.01 (s, 12H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.4, 196.9, 173.6, 169.8, 166.6, 165.0, 164.7, 164.6, 156.2, 108.1, 79.1, 68.0, 53.6, 52.4, 49.1, 46.7, 43.5, 43.0, 40.4, 36.7, 30.2, 29.9, 28.5, 25.7, 18.0. MS: Calcd, 1596.94 (M^+); Found (ESI), 798.96 ($M + 2H^+$).

Intermediate 6. Cyanuric chloride (0.85 g, 4.59 mmol) and mono(*t*-butoxycarbonyl)piperazine (2.65 g, 14.2 mmol) were dissolved in 100 mL of THF in a round-bottomed flask and stirred at 80 °C in the presence of K_2CO_3 (5.7 g, 41.3 mmol) overnight. THF was evaporated off, and the resulting residue was dissolved in dichloromethane. The mixture was washed with three 50 mL portions of water. The dichloromethane layer was dried with $MgSO_4$ and concentrated in vacuo. Flash column chromatography (silica, 4:1 DCM/EtOAc) of the residue gave 2.59 g of product (86%). R_f = 0.64 (9:1 DCM/MeOH). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.72 (t, J = 5 Hz, 12H), 3.42 (t, J = 5 Hz, 12H), 1.46 (s, 27H). ^{13}C NMR (125 MHz, $CDCl_3$) 165.5, 155.0, 80.0, 76.2, 43.2, 28.6. MS: Calcd, 634.40 (M^+); Found (ESI), 634.40 (M^+).

Intermediate 7. Intermediate **6** (2.01 g, 3.18 mmol) was dissolved in 25 mL of 1:1 TFA/DCM solution and was stirred at room temperature for 3 h. The solvent was evaporated, and the residue was treated with 5 M NaOH until highly basic (pH 14 using pH paper). The product was extracted with three 50 mL portions of chloroform. The organic layer was dried with $MgSO_4$, and chloroform was evaporated off. The residue was dried in vacuo, giving 1.01 g of the amine (95%). ^{1}H NMR (300 MHz, CD_3OD) δ 4.04 (t, J = 5.3 Hz, 12H), 3.23 (t, J = 5.1 Hz, 12H). ^{13}C NMR (75 MHz, CD_3OD) 166.6, 44.4, 41.2. MS: Calcd, 334.25 (M^+); Found (ESI), 335.25 (M^+).

Intermediate 8. Cyanuric chloride (0.44 g, 2.41 mmol) and mono(*t*-butoxycarbonyl)piperazine (0.90 g, 4.82 mmol) were dissolved in about 75 mL of THF. The mixture was stirred overnight at room temperature. The solvent was removed in vacuo. The residue was then dissolved in DCM and washed with three 100 mL portions of water. DCM was removed after drying the mixture with $MgSO_4$. Flash column chromatography (50:1 DCM/MeOH) gave 2.17 g of product (88%). R_f = 0.34 (50:1 DCM/MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.77 (m, 8H), 3.46 (m, 8H), 1.48 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 164.7, 154.9, 80.5, 43.5, 28.6. MS: Calcd, 484.24 (M^+); Found (ESI), 484.24 (M^+).

Intermediate 9. Intermediate **7** (1.00 g, 3.00 mmol) and intermediate **8** (4.50 g, 9.30 mmol) were dissolved in about 50 mL of $CHCl_3$. K_2CO_3 (3.73 g, 27.0 mmol) was added to the mixture, which was then stirred at 80 °C for 2 days or until the triazine tris(piperazine) was exhausted as indicated by TLC. The reaction mixture was washed with three 50 mL portions of water, and the chloroform layer was dried with $MgSO_4$. The solvent was evaporated, and the product was purified by flash column chromatography (silica, 24:1 DCM/MeOH, R_f = 0.16) to give 4.12 g (82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.81 (s, 24H), 3.76 (t, J = 5.0 Hz, 24H), 3.45 (t, J = 5.1 Hz, 24H), 1.49 (s, 56H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.7, 165.6, 155.1, 80.1, 43.3, 43.3, 28.7. MS: Calcd, 1676.02 (M^+), 838.52 $(M + 2H)^{2+}$; Found (ESI), 838.53 $(M + 2H)^{2+}$.

Intermediate 10. To a 15 mL solution of protected core **9** (1.02 g, 0.609 mmol) in DCM was added 15 mL of TFA. This was stirred at room temperature for 3 h. The solvent was evaporated, and the residue was made basic with 5 M NaOH (about pH 14). The product was extracted with five 50 mL portions of chloroform. The organic layer was dried with $MgSO_4$, chloroform was evaporated off, and the residue was dried in vacuo to give 0.365 g of product (56%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.76 (br, 24H), 3.72 (t, J = 5.0 Hz, 24H), 2.84 (t, J = 5.0 Hz, 24H). ^{13}C NMR (75 MHz, $CDCl_3$) 165.6, 46.3, 44.6, 43.3. MS: Calcd, 334.25 (M^+); Found (ESI), 335.25 (M^+).

Dendrimer 11. Intermediate **10** (35.3 mg, 0.033 mmol) and intermediate **5** (0.35 g, 0.22 mmol) were completely dissolved in 4:1 $CHCl_3$/MeOH. Polystyrene-supported 1,5,7-triazabicyclo[4.4.0]-dec-5-ene (12 equiv, 0.20 mmol) was added to the mixture which was refluxed at 80 °C. Loss of the amine core was detected by TLC (9:1 DCM:MeOH) after 48 h. The polymer-supported base was filtered off, and the product was obtained by flash column chromatography (silica, 40:1 and then 19:1 DCM/MeOH) to give 0.23 g, 66% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.89, 5.69 (br, 36H), 4.78 (br, 24H), 3.82 (br, 168H), 3.62 (br, 48H), 3.28 (br, 72H), 2.76 (m, 24H), 2.51 (s, 36H), 2.31 (m, 48H), 1.83 (m, 36H), 1.35 (s, 216H), 1.21 (m, 24H), 0.98 (s, 72H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.8, 166.8, 165.7, 165.6, 165.2, 164.8, 156.4, 108.2, 79.2, 68.2, 53.7, 52.5, 49.3, 46.7, 43.4, 43.2, 40.7, 40.5, 36.8, 30.3, 30.1, 28.7, 28.7, 28.5, 25.8, 18.2. MS: Calcd, 10,435.45 (M^+); Found (MALDI-TOF), 10,454.15 (M^+), 5228.26 $(M + 2H)^{2+}$.

Dendrimer 12. Dendrimer **11** (2.54 g, 0.243 mmol) was dissolved in 50 mL of 1:3 DCM/MeOH. Anhydrous hydrazine (25 mL) was added and stirred at room temperature for 7 h or until all of **11** had reacted and was monitored by TLC. The solvent was evaporated, and the residue was dissolved in 100 mL of chloroform. This mixture was then washed with 50 mL portions of distilled water 3 times. The chloroform layer was dried with $MgSO_4$ and evaporated. Flash chromatography using DCM from silica gave 1.74 g of product (84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.96, 5.86 (br, 24H) 4.74 (m, 24H), 3.82, 3.65 (br, 216H), 3.32 (br, 48H), 2.77 (m, 24H), 2.58 (br, 24H), 1.76 (br, 24H), 1.60 (m, 36H), 1.39 (s, 216H), 1.14 (m, 24H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6, 165.4, 165.3, 164.8, 164.5, 156.0, 78.8, 47.8, 46.2, 43.1, 40.4, 39.6, 29.7, 28.4. MS: Calcd, 8466.44 (M^+); Found (MALDI-TOF), 8468.68 (M^+), 8491.08 ($M + Na^+$).

Dendrimer 13. Dendrimer **12** (0.397 g, 0.0468 mmol) and *N*-succinimidyl 3-(2-pyridyldithio)propionate (0.427 g, 1.37 mmol)

were dissolved in 1.0 mL of 1:1 dichloromethane/chloroform and stirred at room temperature for 3 days. The reaction was monitored by TLC and MALDI-TOF MS. The product was isolated by flash column chromatography using dichloromethane as solvent. R_f = 0.28 (9:1 DCM/MeOH), 0.304 g (60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (m, 12H), 7.62 (m, 24H), 7.11 (m, 12H), 6.85 (br, 12H), 5.81 (br, 24H), 4.71 (br, 24H), 3.82, 3.64 (br, 216H), 3.31 (br, 48H), 3.19 (br, 24H), 3.06 (m, 24H), 2.76 (m, 24H), 2.61 (m, 24H), 1.78 (br, 24H), 1.38 (br, 216H), 1.18 (br, 24H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 166.5, 165.3, 165.2, 164.8, 164.4, 159.4, 156.1, 149.4, 121.0, 120.2, 78.9, 46.3, 45.2, 43.1, 40.3, 36.7, 35.7, 34.9, 29.6, 28.4. MS: Calcd, 10830.42 (M^+); Found (MALDI-TOF), 10847.93 (M^+).

Dendrimer 14. Dendrimer **13** (0.234 g, 0.0216 mmol) was dissolved in 10 mL of a 2:1 TFA/CH_2Cl_2 mixture and stirred overnight. The solvent was removed, and the residue was dissolved in a minimum amount of methanol. The salt product was precipitated out and washed with diethylether. After drying, the deprotection product was dissolved in DMSO and stirred with MeO-PEG 4-nitrophenylcarbonate[13] (3.07 g, 72 equiv) and 7 mL of triethylamine for 10 days. The reaction mixture was dissolved in 10 mL of water and dialyzed using a dialysis bag with a molecular weight cutoff of 12−14 kDa in 1:1 water/methanol for 24 h, in 2:1 water/methanol for 48 h, and then in water alone for another 48 h, changing the solution as often as possible. Water was removed, and the product was vacuum-dried (1.14 g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (br), 7.56 (br), 7.03 (br), 4.08 (br), 3.70 (m), 3.57 (m), 3.48 (m), 3.42 (m), 3.30 (m), 3.16 (m), 2.98 (br), 2.66 (br), 2.53 (br), 1.68 (br), 1.17 (m), 1.05 (m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 165.0, 159.4, 156.3, 149.3, 137.0, 120.8, 119.8, 80.5, 73.3, 72.3, 71.6, 71.3, 70.3, 70.0, 69.3, 67.2, 64.1, 63.3, 61.4, 58.8, 45.0, 42.8, 40.8, 35.3, 34.4, 29.5. MS: Calcd, 56909 (M_{av}^+); Found (MALDI-TOF), 56798 (M_{av}^+).

Intermediate 15. Cyanuric chloride (2.91 g, 15.8 mmol) was dissolved in 100 mL of THF and cooled to 0 °C in an ice bath. DIPEA (4.25 mL, 23.7 mmol) was added to the cold solution. Intermediate **1** (4.8 g, 15.8 mmol) was dissolved in 100 mL of THF and added dropwise to the cold cyanuric chloride solution over 1 h. The mixture was stirred at 0 °C for an additional 45 min. The solvent was removed in vacuo, and the resulting solids were dissolved in 50 mL of dichloromethane. The solution was washed with 50 mL of water (2 times) and with 50 mL of brine (1 time). The organic layer was dried with Na_2SO_4 and filtered, and the solvent was removed in vacuo. The resulting residue was dissolved in a minimal amount of dichloromethane, and 100 mL of hexanes was added slowly. The resulting precipitate was filtered and dried under vacuum overnight (6.814 g, 96%). R_f = 0.51 (3:2 DCM/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.273 (s, 0.5H), 5.015 (br, 1.5H), 3.711 (t, $^3J_{H-H}$ = 6 Hz, 4H) 3.365 (m, 4H), 1.361 (s, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 165.9, 156.3, 79.9, 48.9, 38.8, 28.5. MS: Calcd, 450.15 (M^+); Found (ESI), 457.16 ($M + Li)^+$.

Intermediate 16. Cyanuric chloride (0.260 g, 1.41 mmol) and intermediate **1** (0.86 g, 2.83 mmol) were dissolved in 20 mL of THF. DIPEA (1.01 mL, 5.65 mmol) was added, and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo, and the residue was dissolved in DCM. The organic layer was washed with 20 mL of water (2 times) and with 20 mL of brine (1 time). The organic layer was dried with Na_2SO_4 and filtered, and the solvent was removed in vacuo to yield a white solid (0.9996 g, 99%). R_f = 0.44 (3:2 DCM/EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.607 (br, 1.5 H), 5.195 (br, 1.5 H), 3.602 (m, 8H), 3.291 (m, 8H), 1.384 (s, 36H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.1, 165.5, 156.4, 79.5, 48.1, 47.4, 39.7, 37.9, 28.6. MS: Calcd, 717.40 (M^+); Found (ESI), 718.40 ($M + H)^+$.

Intermediate 17. Intermediate **16** (4.5 g, 6.26 mmol) and piperazine (2.698 g, 31.32 mmol) were dissolved in 180 mL of THF, and the solution was stirred at room temperature for 2 days. The solvent was removed in vacuo, and the residue was purified by silica column chromatography with 4:1 DCM/MeOH. R_f = 0.65, 4.450 g, 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.715 (br, 3H), 5.258 (s, 1H), 3.686 (br, 4H), 3.611 (br, 4H), 3.536 (br, 4H), 3.271 (br, 8H), 2.840 (br, 4H). NMR (75 MHz, $CDCl_3$) δ 166.1, 164.7, 156.4, 79.4, 47.5, 46.8, 46.0, 43.4, 40.8, 38.6, 28.6. MS: Calcd, 767.5018 (M^+); Found (ESI), 768.5382 ($M + H)^+$.

Intermediate 18. Cyanuric chloride (1.07 g, 5.782 mmol) was dissolved in 50 mL of THF and cooled to 0 °C in an ice bath. Intermediate **17** (3.7 g, 4.82 mmol) was dissolved in 50 mL of THF and added dropwise to the cold cyanuric chloride solution. The mixture was stirred at 0 °C for 4 h, and the solvent was removed in vacuo. The residue was dissolved in dichloromethane and washed with three 50 mL portions of water. The organic layer was dried with Na_2SO_4 and filtered, and the solvent was removed in vacuo. The solids were dissolved in a minimal amount of DCM, and hexanes were added to precipitate the product (2.566 g, 58% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.580 (br, 3H), 5.287 (s, 1H), 3.907 (m, 4H), 3.844 (m, 4H), 3.652 (br, 4H), 3.579 (br, 4H), 3.313 (m, 8H), 1.400 (s, 36H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 166.0, 164.4, 156.5, 156.3, 79.5, 47.7, 47.1, 44.3, 40.7, 38.5, 28.7, 28.6. MS: Calcd, 914.44 (M^+); Found (MALDI-TOF), 915.25 ($M + H)^+$, 937.24 ($M + Na)^+$.

Intermediate 19. Intermediate **18** (0.122 g, 0.1331 mmol) and intermediate **4** (0.099 g, 0.1331 mmol) were dissolved in 20 mL of THF. DIPEA was added (0.07 mL, 0.3993 mmol), and the mixture was stirred for 16 h at room temperature. The solvent was removed in vacuo. The resulting residue was dissolved in 15 mL of DCM and was washed with 10 mL of water (2 times) and with 10 mL of brine (1 time). The organic layer was dried with Na_2SO_4 and filtered, and the solvent was removed in vacuo. The resulting residue was dissolved in a minimal amount of dichloromethane, and hexanes were added to form a precipitate (0.1575 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.632 (br, 5H), 4.766 (d, $^3J_{H-H}$ = 12 Hz, 2H), 3.806 (br, 16H), 3.592 (br, 12H), 3.305 (br, 14H), 2.782 (t, $^3J_{H-H}$ = 12 Hz, 2H), 2.539 (s, 3H), 2.349 (s, 4H), 2.018 (br, 1H), 1.849 (d, $^3J_{H-H}$ = 12 Hz, 2 H), 1.398 (br, 54H), 1.237 (br, 2H), 1.015 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) 199.2, 196.9, 173.8, 169.9, 166.0, 164.7, 156.4, 156.3, 108.2, 79.3, 53.7, 49.3, 47.7, 46.9, 46.7, 43.6, 43.2, 40.8, 40.5, 38.5, 36.8, 31.8, 30.3, 30.1, 28.6, 22.9, 18.2. MS: Calcd, 1620.95 (M^+); Found (MALDI-TOF), 1621.68 ($M + H)^+$, 1643.67 ($M + Na)^+$, 1659.63 ($M + K)^+$.

Molecular Modeling. The energy-minimized structure of **18** (gas phase) was obtained using the software package Cerius2 4.6. The pcff second-generation force field in the open force field (OFF) program was used in minimizations and dynamics. The structure was initially minimized in the fully extended conformation. Constant volume and temperature (NVT) molecular dynamics (MD) calculations were then performed on the minimized extended structure via simulated annealing. The simulated annealing was carried out for 840.0 ps, over a temperature range of 300−1000 K, with ΔT = 50 K, using the Nosé temperature thermostat, a relaxation time of 0.1 ps, and a time step of 0.001 ps. The dendrimer was minimized after each annealing cycle, resulting in 300 minimized structures of **14**.

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Supporting Information Available: ^{1}H and ^{13}C NMR and mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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