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Toward the Next-Generation Drug Delivery Vehicle: Synthesis of a Dendrimer with Four Orthogonally Reactive Groups

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Abstract: The synthesis of a dendrimer based on melamine that displays multiple copies of four orthogonally reactive groups, three on the surface and one on the interior, is described. The three groups on the surface are nucleophilic and include four free hydroxyl groups, four hydroxyl groups masked as *tert*-butyldiphenylsilyl (TBDPS) ethers, and 16 amines masked as *tert*-butoxycarbonyl (BOC) groups. The core of the dendrimer displays two electrophilic monochlorotriazines. The dendrimer is available in seven linear steps (eight total steps) at 55% overall yield for the longest linear sequence.

Keywords: Dendrimer; drug delivery

Introduction

We, and the drug-delivery community, have been intrigued by dendrimers for use as drug delivery vehicles.[1−5] These highly branched, globular macromolecules present well-defined (often uniquely monodisperse) structures and a large number of reactive surface groups. They may afford passive selectivity for tumors based on the enhanced permeability and retention time (EPR) effect.[6] Many of the molecular details for optimizing these vehicles to function in the complex environment presented by an organism are poorly understood, but advances are being made by numerous investigators, each favoring a specific subclass of dendrimers including polyesters,[7] poly(amidoamines),[8] poly(propylene imines),[9] and poly(amides).[10] We favor triazines linked by diamines.[11−17] The systematic replacement of chlorine atoms around the triazine ring of cyanuric chloride offers tremen-

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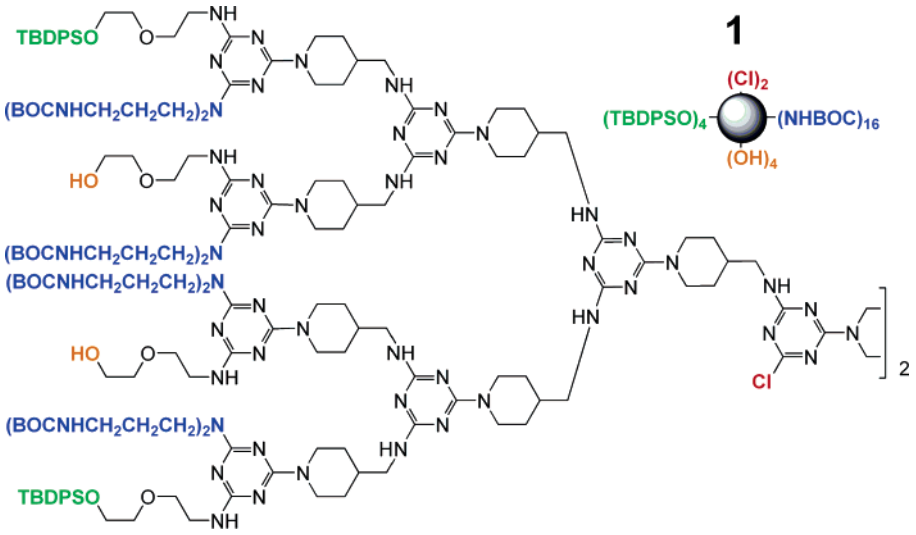
Chart 1. Dendrimer **1** in Atomic and Schematic Detail



dous potential for compositional diversity and a hydrophobic interior for sequestering hydrophobic drugs. Preliminary studies suggest that dendrimers based on triazine show low toxicity in vivo upon PEGylation: Acute dosing of one PEGylated dendrimer reached in mice 1.3 g/kg (intravenous administration) and 2.6 g/kg (intraperitoneal administration) without detectable changes to blood urea nitrogen or alanine transaminase levels.[12]

Here, we employ control over composition to synthesize a dendrimer with four different groups of reactive sites. We envision that these sites will be used for (i) conjugating drugs, (ii) enhancing solubility and biocompatibility (PEGylation), (iii) presenting biodistribution tags, and (iv) displaying targeting ligands. Accordingly, the number of sites necessary for each function varies. We envision that drug conjugation sites and PEGylation sites should be in abundance, compared with targeting ligands, which, too, outnumber a small number of sites for the incorporation of tags for probing biodistribution.

In our previous studies, we reported the synthesis of versatile dendrimer with five different functional groups, including free hydroxyls, levulinic acid esters, *tert*-butyldiphenylsilyl ethers, pyridyl disulfide (PyrSSR) groups, and *tert*-butyloxycarbonate (BOC) protected amines.[11] The choice of these groups derived from Wong's orthogonally protected sugars.[18] This original, orthogonally reactive dendrimer, however, suffered from three issues that limited its potential role as a building block for manipulation. First, the levulinic acid ester groups and the pyridyl disulfides were not as chemically robust as the other protecting groups during the dendrimer synthesis. Accordingly, yields for reactions involving these groups were lower (~80%) compared with manipulations of the intermediate containing the other groups (>95%). Second, the dendrimer was more complex than necessary; only four unique groups of sites are believed to be required. Third, too many of the orthogonal groups were hydroxyl groups which are less reactive and less selective than the electrophilic triazines incorporated here. Here we describe a dendrimer, **1**, with four groups that are amenable for postsynthetic manipulation (Chart 1) that incorporates both nucleophilic and electrophilic sites. We envision that this dendrimer will serve as the basis for future studies of biodistribution and efficacy in tumor-bearing animals.

Results and Discussion

Dendrimer **1** was synthesized using a strategy similar to that of the original dendrimer described by our group.[11] This new dendrimer has a total of 26 reactive sites for manipulation. This architecture represents the current generation of

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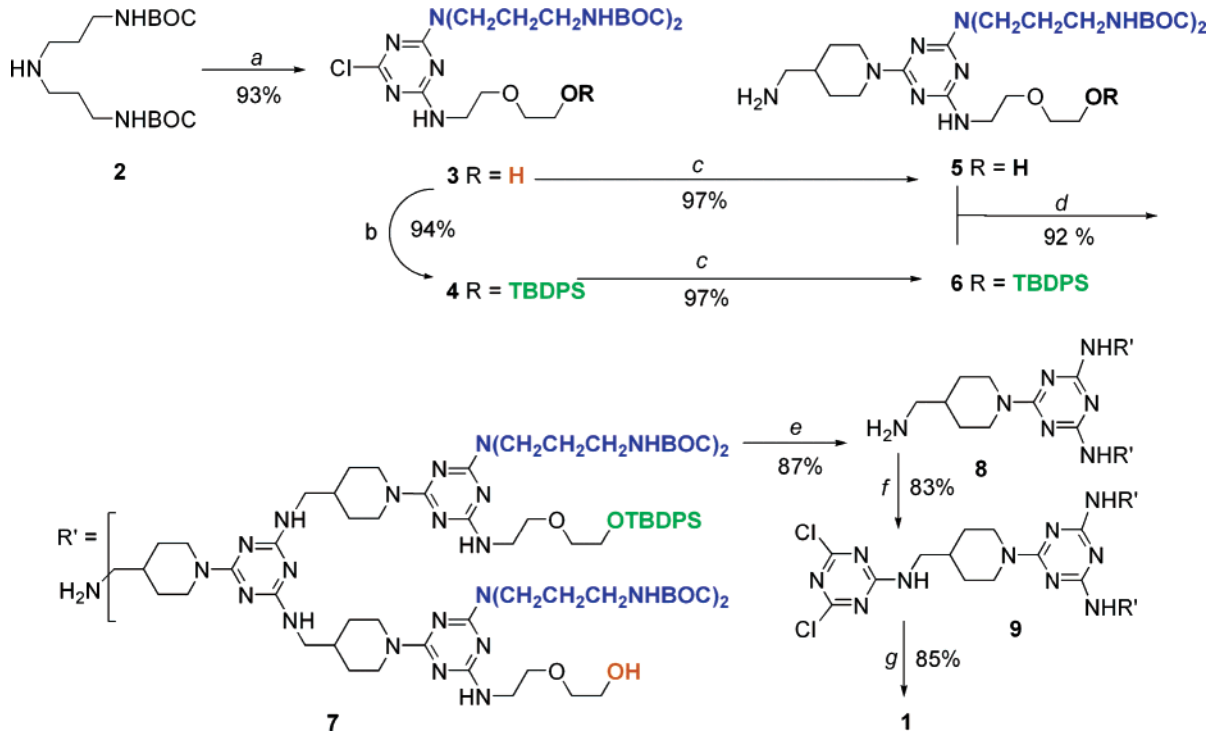
Scheme 1 [a]



[a] Reagents and conditions: (a) cyanuric chloride, DIPEA, THF, 0 °C, 2 h; then 2-(2-aminoethoxy)ethanol with CH_3OH, rt, 24 h; (b) TBDPS-Cl, imidazole, DIPEA, THF, rt, 4 h; (c) 4-(aminomethyl)piperidine, THF, rt, 8 h; (d) cyanuric chloride, DIPEA, THF, 0 °C, 2 h; then **5**, rt, 24 h; then 4-(aminomethyl)piperidine, 24 h; (e) cyanuric chloride, DIPEA, THF, rt, 48 h; then 4-(aminomethyl)piperidine, rt, 24 h; (f) cyanuric chloride, DIPEA, THF, 0 °C, 5 h; (g) piperazine, DIPEA, THF:CH_3OH = 7:1, 0 °C to rt, 48 h.

dendrimers that we are exploring for drug delivery. Such a vehicle will carry a cargo of drugs linked by biolabile, covalent bonds, ligands for targeting, poly(ethylene glycol) (or PEG) for biocompatibility and tuning size which in turn affects circulation time and excretion route, and a beacon that indicates location. The hydroxyl groups are subject to acylation directly, or upon cleavage of the silyl ethers with tetrabutylammonium fluoride (TBAF) for further manipulation. The BOC groups can be unmasked to provide amines amenable to multiple manipulation strategies. All three of these groups were present in the previous generation vehicle. However, here the levulinic esters and pyridyl disulfide groups have been replaced with monochlorotriazines. These groups react efficiently with amine nucleophiles: we have invested significant energies in the study of such groups and find that cyclic secondary amines are well suited to this reaction.[14] In addition to detailing the synthesis of **1**, we show that a piperazine-containing derivative of the Bolton−Hunter reagent, useful for the incorporation of radioactive iodine,[19] can be installed quantitatively.

The synthesis of dendrimer **1** is outlined in Scheme 1 and employs the convergent strategy in which the surface groups are elaborated iteratively toward the core group. Intermediate **2** is prepared by protecting the primary amines of 3,3′-diaminodipropylamine with BOC-ON. Subsequent reactions with cyanuric chloride and 2-(2-aminoethoxy)ethanol respectively will produce monochlorotriazine **3**, which can selectively react with the secondary amine of 4-(aminomethyl)piperidine (4-AMP) to yield **5**. Using TBDPSCl, the free hydroxyl group of **3** is protected to afford **4**, which is then reacted with 4-AMP to give **6**. The synthesis of **7** is conducted in a single-pot reaction by first reacting **6** with cyanuric chloride then followed by the addition of **5** and then completed with the addition of 4-AMP. In a similar way, intermediate **7** is sequentially reacted with cyanuric chloride and then 4-AMP to give **8**. Dendrimer **1** was prepared in two steps from **8** through the intermediate dichlorotriazine **9** followed by dimerization with piperazine. The synthesis is executed in seven linear (8 total) steps from **2** to give the target dendrimer in 55% overall yield from the protected triamine. The route as described can be used to prepare moderate quantities of material. For example, intermediate **8** is useful for this dendrimer and related structures: it has been prepared on 20 g scale. This effort takes between 7 and 8 days at this scale. The final dendrimer has been prepared on 6 g scale.

The synthesis is followed by mass spectrometry and NMR spectroscopy. Mass spectrometry is particularly valuable (Figure 1). With rare exception, the reaction proceeds spot-to-spot as judged by thin layer chromatography and yields products that appear to be single compounds after chromatography. The mass spectrum shows lines corresponding to the protonated material and sodium and potassium ion

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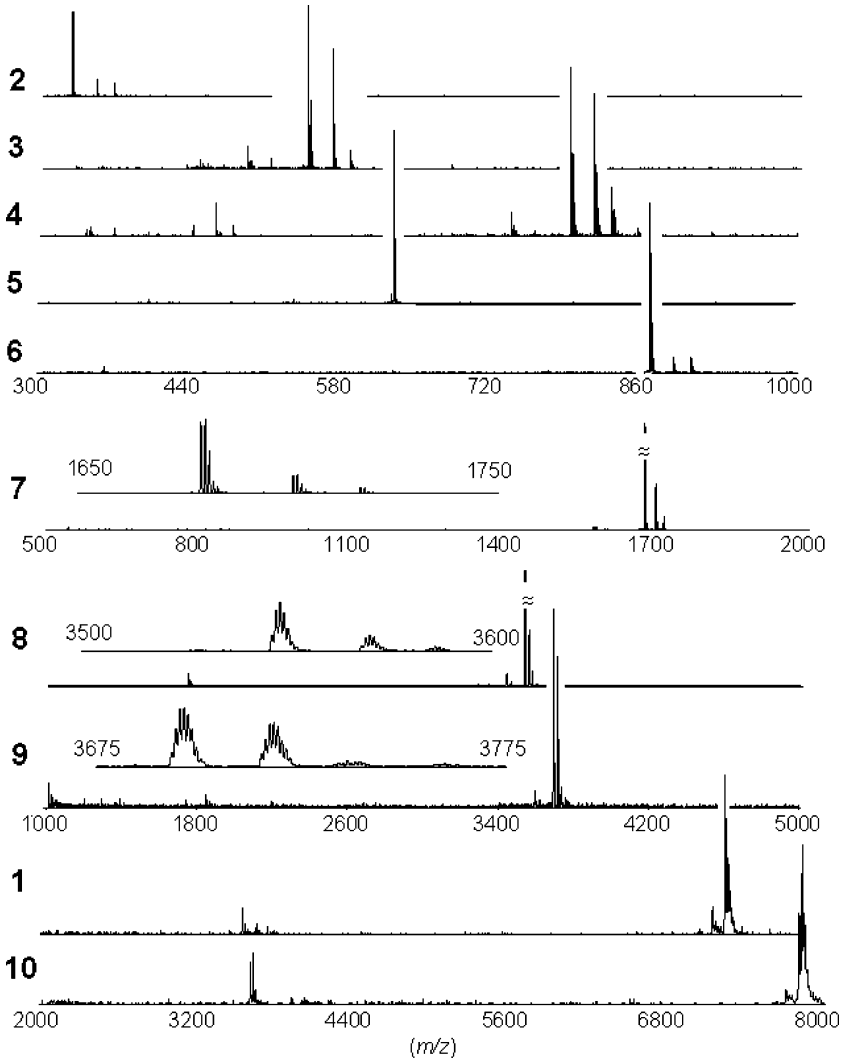

Figure 1. Mass spectrograms of **2**−**9**, **1**, and **10** acquired by ESI-TOF or MALDI-TOF MS.

Scheme 2. Preparation of **10** Using DIPEA in CH_2Cl_2:CH_3OH (1:2) at Room Temperature over 7 Days Is Quantitative

11 **1** **10**

adducts. Ladders of lines corresponding to loss of BOC groups that occurs presumably during the ionization process are often observed for larger species. Doubly charged species are observed in the spectrograms of **1** and **10** (a derivative of **1**), which are both isotopically resolved adducts with H^+, Na^+, and K^+ as well as fragment peaks corresponding to loss of one and two BOC groups.

NMR spectroscopy also is diagnostic for the reactions, but interpretation is hampered by opportunities for the existence of slowly interconverting rotamers derived from hindered rotation about the triazine−NHR bond. These rotamers manifest themselves as broad lines in the proton NMR traces and often as double lines in the ^{13}C NMR traces. Replacement of the chlorine atoms of monochlorotriazines with 4-(aminomethyl)piperidine typically simplifies the spectrum due presumably to either the reduction of rotamer number or the dissipation in the magnitude of the C−Cl dipole.

To evaluate the reactivity of the monochlorotriazine groups, a modified Bolton−Hunter reagent, **11**, was prepared in two steps by quantitatively condensing BOC-piperazine with the free acid using PyBOP in dimethylformamide.[20] Treatment of this material for 4 h with trifluoroacetic acid yielded the nucleophilic reagent. Reaction with **1** proceeded almost quantitatively over 1 week at room temperature in

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methylene chloride:methanol (1:2) (Scheme 2). No efforts were made to optimize this reaction.

Conclusions

We have prepared a versatile dendrimer with four orthogonally reactive groups: monochlorotriazine, free hydroxyl, TBDPS, and BOC groups. Each of the functional surface groups can be readily utilized to improve solubility in physiological condition, load drugs, link ligands or antibodies for targeting specific diseased cells, and attaching imaging agents for biodistribution assay. All of the synthetic steps are well established to afford the moderate scale preparation of the target. Complete details of the peripheral manipulation and biological applications will be pursued in due course.

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Supporting Information Available: Details of synthesis and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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