

Rapid, Efficient Synthesis of Heterobifunctional Biodegradable Dendrimers

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A number of polymeric systems have been explored for biomedical applications such as drug delivery,¹ gene delivery,² and in vivo imaging,³ but the properties of dendrimers translate especially well into pharmacology.^{4–6} Their precise synthesis produces a polymer of low polydispersity, which increases the likelihood of reproducible behavior within a patient. Moreover, their high degree of branching provides a multivalent periphery that can be used for the conjugation of many copies of functional molecules, increasing the efficacy or spectroscopic sensitivity of each individual dendritic carrier molecule. In particular, the aliphatic polyester dendrimer derived from 2,2-bis(hydroxymethyl)propanoic acid (bis-HMPA) boasts low toxicity and immunogenicity⁷ with its non-ionic and biodegradable structure.⁸

From a single dendrimer scaffold, functional molecules may be added to provide solubility and compatibility, increased plasma residence time, targeting, imaging, therapy, or potentially any combination of these. However, controlled implementation of varied functions onto a single platform can be difficult because all peripheral groups of a symmetric dendrimer have the same reactivity. Dendrimers bearing more than one type of peripheral group can be prepared, but their synthesis, requiring numerous steps, can be challenging.^{9–11} An efficient method would be to grow a symmetric dendrimer in bulk and then tune its periphery to the desired application. Since symmetric dendrimers are easier to construct than their asymmetric counterparts, the initial part of the synthesis would be more amenable to larger-scale production. However, the latter process requires that the subsequent differentiation and coupling steps be minimal in number and efficient in reactivity. Moreover, all synthetic intermediates should be purified by simple means, preferably without chromatography.

We employed the cyclic carbonate as a symmetric substrate that can yield a bifunctional product. The reaction of a cyclic carbonate and an amine has been used previously for polyurethane synthesis¹² and has even proven efficient and selective enough to be run in water with quantitative conversions.¹³ In the reaction, the amine opens the ring, forming a carbamate linkage with concomitant liberation of an alcohol that may then be used for a subsequent ligation. Thus two different moieties may be added in immediate succession without any deprotection steps or functional group conversions. To append these groups to bis-HMPA dendrimers, synthesis of a cyclic carbonate bis-HMPA monomer was undertaken. Unfortunately, the direct addition of reactive carbonyl equivalents such as phosgene, triphosgene, and ethyl chloroformate to the free bis-HMPA led to a mixture of products. Therefore, the free acid was first protected as the benzyl ester and then treated with ethyl chloroformate and triethylamine to yield a carbonate product that could be recrystallized easily and in good yield (Scheme 1).¹⁴ The acid was then deprotected by hydrogenolysis in near quantitative yield and required no further purification. To provide a model platform for testing the reaction, dendrimer **4** with eight hydroxyl groups was synthesized in two steps from pentaerythritol.^{15,16} DCC coupling of **3** and **4** led to a pure carbonate-

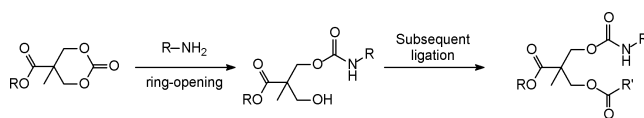
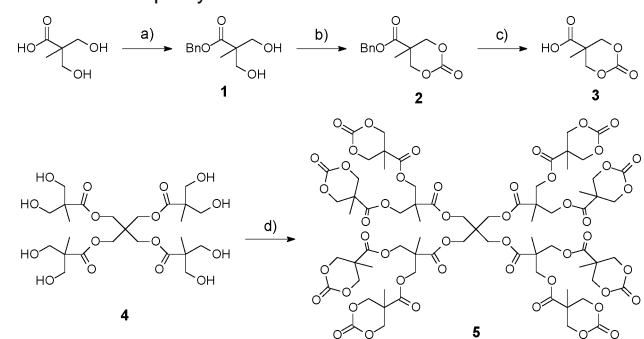


Figure 1. General two-step differentiation scheme for cyclic carbonates derived from 2,2-bis(hydroxymethyl)propanoic acid.

Scheme 1. Synthesis of [G-2] Bis-HMPA Dendrimer with a Cyclic Carbonate Periphery^a

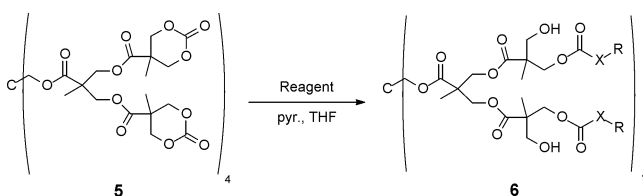


^a Reagents and conditions: (a) BnBr, Et₃N, CH₂Cl₂; (b) ethyl chloroformate, Et₃N, THF; (c) H₂, 10% Pd/C, EtOAc, 98%; (d) **3**, DCC, DMAP, DPTS, CH₂Cl₂, DMF, 81%.

bearing dendrimer after filtration, extraction, and precipitation into ether. No urea impurities could be found by NMR or IR, and MALDI-TOF MS showed only the desired product.

The ring-opening reaction with amines proved to be quite facile. As **5** was soluble in highly polar solvents such as DMF or pyridine, solvent screening reactions with benzylamine as a model nucleophile revealed that a 1:1 mixture of THF and pyridine worked best, although pyridine alone could be used as well if desired. Next, the generality of this reaction was explored. To examine the effect of steric hindrance of the amine, test reactions were performed with benzylamine, *sec*-butylamine, and piperidine. Piperidine and benzylamine proved to be most reactive, with ¹H NMR analysis suggesting full conversion after 1 and 3 h, respectively. MALDI-TOF analysis of the reaction product showed the presence of a smaller peak corresponding to a product resulting from seven rather than eight reactions, suggesting an overall conversion of 95–99% (see Supporting Information). *sec*-Butylamine reacted more slowly, with some starting material still apparent after overnight stirring. Other nucleophiles were not as reactive. After 16 h stirring, phenol, aniline, and benzyl alcohol showed only 13, 7, and 12% conversion, respectively; thus the reaction appeared to favor amines. It should be noted that no side products could be observed by ¹H NMR during these experiments.

We explored the elaboration of dendrimer **5** to produce dendrimers with reactive sites amenable to future conjugation. Encouraged by the difference in reactivity between amines and phenols, tyramine was employed to build a dendrimer containing phenolic substituents for either PET radiolabeling¹⁷ or subsequent ligations^{18,19} (**6i**). The NMR spectrum of **6i** showed no peaks corre-

Table 1. Reactions of Cyclic Carbonate Dendrimer **5**^a


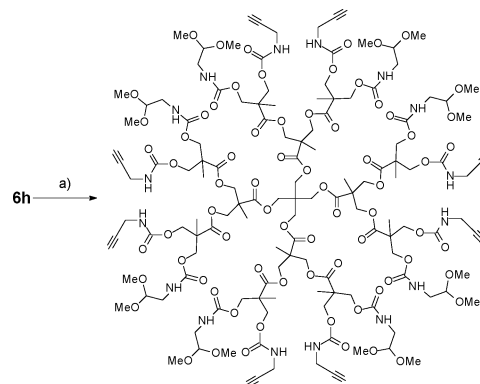
entry	reagent	time (h)	conversion ^b (%)	yield ^c (%)
6a	benzylamine	1	63	—
6a	benzylamine	3	95–99	92
6b	<i>sec</i> -butylamine	3	26	—
6b	<i>sec</i> -butylamine	16	90	74
6c	piperidine	1	95–99	96
6d	aniline	16	13	—
6e	phenol	16	7	—
6f	benzyl alcohol	16	12	—
6g	propargylamine	16	95–99	89
6h	(MeO) ₂ CHCH ₂ NH ₂	16	95–99	91
6i	tyramine	16	95–99	94

^a Performed in 1:1 pyridine/THF. ^b Conversion per carbonate measured by ¹H NMR. ^c Dendrimer yield after workup.

sponding to acyclic carbonates, as would be expected if the phenol had reacted with the cyclic carbonates, and peak integration data for the carbamate peaks were consistent with those of the inner dendrimer framework. Reaction of **5** with propargylamine, intended to provide a dendrimer with alkynes for click chemistry,¹⁹ also met with similar success, affording **6g**. Finally, reaction with aminoacetaldehyde dimethyl acetal gave a 90% yield of dendrimer **6h** with eight acetals. Deprotection of these acetals would provide free aldehydes, which could be used for subsequent oxime, hydrazone, or thiazolidene formation.^{18c} In each reaction, the dendrimer products were purified simply by washing the reaction mixture with aqueous acid to remove both the pyridine and any unreacted amine.

The free alcohols were then employed to introduce other functional substituents. Since oxime formation and click chemistry are orthogonal to one another, a dendrimer containing both protected aldehydes and alkynes was synthesized. The free alcohols of **6h** were activated with 1,1'-carbonyldiimidazole, then reacted in situ with propargylamine (Scheme 2). Excess propargylamine and propargylurea byproduct were washed away with water, affording bifunctional dendrimer **7** as a white powder in 80% yield.

This work has demonstrated the rapid and scalable synthesis of heterobifunctional bis-HMPA dendrimers derived from a cyclic carbonate periphery. The symmetric carbonate periphery reacted cleanly, efficiently, and selectively with primary amines to produce bifunctional dendrimers. Use of this methodology yielded a dendrimer bearing both alkynes and protected aldehydes for orthogonal ligation reactions. This was accomplished in nine steps from commercially available materials, requiring only extraction, precipitation, or recrystallization to purify the synthetic intermediates. We are exploring the use of this methodology to construct future platforms for the delivery of therapeutic agents.

Scheme 2. Synthesis of Bifunctional Dendrimer **7**^a

^a Reagents and conditions: (a) 1,1'-carbonyldiimidazole in CH₂Cl₂, 1 h, then propargylamine, 16 h, 80%.

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Supporting Information Available: Experimental procedures and characterization data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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