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Synthesis of multivalent carbonate esters by divergent growth of branched carbamates

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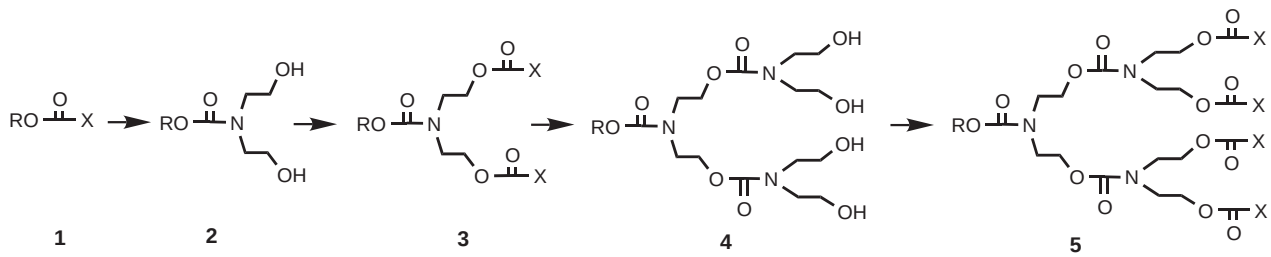
Abstract—Multivalent 4-nitrophenyl (PNP)–carbonate esters were prepared from carbonate ester-containing core groups by a new divergent-growth process. The process doubles the number of PNP–carbonate esters with each round of growth, or branching, which occurs by reacting an *N*,*N*-dihydroxylalkyl amine with a PNP–carbonate ester. The resulting *N*,*N*-dihydroxyalkyl carbamate contains two hydroxyl groups for each original PNP–carbonate ester. The hydroxyl groups are converted in situ to PNP–carbonate esters, and the process can be repeated to provide successive generations of dendrimer-like branched structures of increased valence. © 2001 Published by Elsevier Science Ltd.

A new divergent-growth process has been developed which provides convenient access to a new class of multivalent platforms comprised of carbamate linkages.† We recognized that readily available diethanolamine, or other amines containing multiple hydroxyl groups, could serve as an AB_n monomer which could react with activated carbonate derivatives to provide branched structures in relatively few synthetic steps. Scheme 1 describes the process, exemplified with diethanolamine.

A reactive carbonate derivative, **1**, is reacted with diethanolamine to provide a hydroxyl terminated structure, represented as **2**, by formation of a carbamate. The hydroxyl groups are converted to reactive carbonate derivatives to provide the first generation branched structure **3**. The process can be repeated to propagate successive generations of branching. Thus, compound **3** is reacted with diethanolamine to provide structure **4**, which is converted to compound **5**. In addition to having utility in the preparation of multivalent platforms, the chemistry described herein may be applicable to the preparation of dendrimers.[4] Multivalent platforms resemble dendrimers, because they are comprised of branching groups and linking groups. Dendrimers are highly branched molecules typically derived from



Scheme 1.

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† Multivalent platforms are branched structures which are important intermediates used to prepare structurally defined multivalent bioconjugates. Multivalent platforms are key intermediates used to prepare B cell toleragens, therapeutic agents for the treatment of autoimmune diseases. B cell toleragens are constructed by attaching multiple copies of B cell epitopes to multivalent platforms.[1–3] Multivalent platforms are metabolically-stable biocompatible, non-immunogenic molecules of defined structure with multiple reactive groups to which biologically active molecules can be attached.

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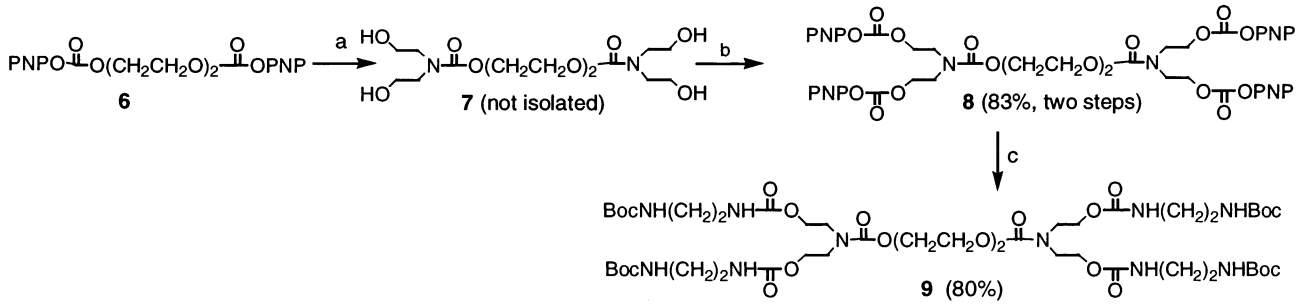
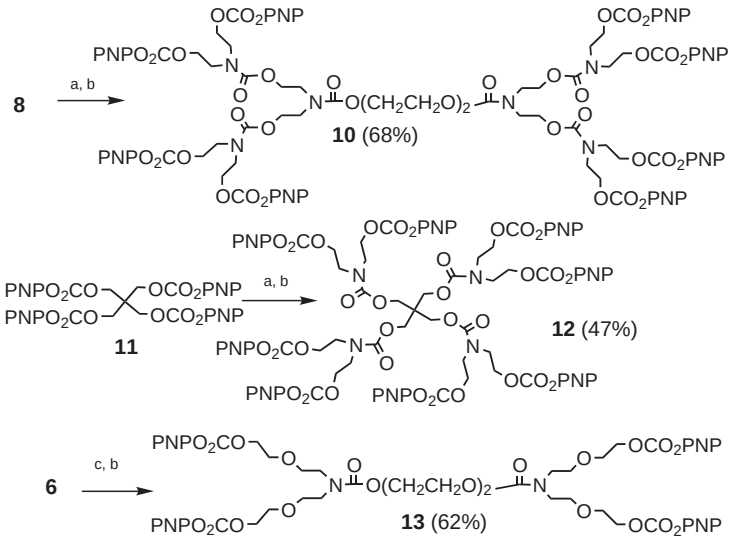


Scheme 2. (a) Diethanolamine, pyridine; (b) PNP–chloroformate, pyridine; (c) $BocNH(CH_2)_2NH_2$, Et_3N, CH_2Cl_2.



Scheme 3. (a) Diethanolamine, pyridine; (b) PNP–chloroformate, pyridine/CH_2Cl_2; (c) di-(2-(2-hydroxyethoxy)ethyl)amine, pyridine.

AB_n monomers where n generally equals 2 or 3. For more information on dendrimers, the reader is referred to key review articles.[5–9]

Divergent growth involving the formation of carbamates appeared to be an attractive entry into multivalent platforms, because carbamates are metabolically stable and otherwise biocompatible as evidenced by lack of reported toxicity or immunogenicity. Carbamates are more resistant to proteases than are amides,[10,11] and tertiary carbamates are more resistant than secondary carbamates.[12] Surprisingly, carbamate formation has seldom been used to prepare dendrimer-like compounds. Dendrimeric polymers containing carbamates with a reversed orientation were prepared by polymerization of 3,5-bis-((benzyloxycarbonyl)imino)-benzyl alcohols.[13] These were polydisperse compounds with secondary carbamate linkages. We are not aware of any dendrimer-like structures comprised of carbamates of the orientation described below.

Attempts to react diethanolamine with tri(ethylene glycol) bis-chloroformate led to uncharacterizable mixtures, presumably due to acylation of both the hydroxyl groups and the secondary amine. Less activated carbonate derivatives, such as 4-nitrophenyl (PNP)–carbonate esters, are reactive enough to acylate amines but not significantly reactive with hydroxyl groups. Reaction of diethanolamine with compound **6**, prepared in 78% yield by treating di(ethylene glycol) with PNP–chloroformate in pyridine, provided the desired tetrahydroxy compound, **7**, as described in Scheme 2.

Isolation and purification of compound **7** was problematic due to its high polarity. It is water-soluble and tends to remain in the aqueous layer when extractive workups are attempted. Attempts to purify compound **7** by silica gel chromatography were unsuccessful. Due to the difficulty of obtaining compound **7** in purified form, and because an activated carbonate ester such as compound **8** was ultimately desired, a more convenient process was developed which avoids the isolation of **7** altogether. Compound **7** was converted in situ to compound **8** by addition of an excess of PNP–chloroformate to the reaction mixture, thus effecting the one-pot two-step conversion of **6** to **8** in 83% yield. Compound **8** can be reacted with an amine to provide desired functionality at the termini as exemplified by the conversion of **8** to **9** which was accomplished in 80% yield by reaction with mono-Boc-ethylenediamine.‡

‡ Mono-Boc-ethylenediamine was prepared as described in Ref. 3.

Scheme 3 diagrams additional examples of divergent growth which provide PNP–carbonate esters of increased valence. Branched structures of higher valence can be prepared by repetition of the process, subjecting the purified multivalent PNP–carbonate ester products to additional rounds of growth. Compound **8**, was converted to an octavalent carbonate ester, compound **10** in 68% yield. Similarly, the tetravalent carbonate ester derived from pentaerythritol, compound **11**, was converted to the octavalent structure **12** in 47% yield. As expected, the process is not restricted to the use of diethanolamine as the AB_n monomer. Other dihydroxy amines can be used as demonstrated by the conversion of compound **6** to compound **13** in 62% yield using di-(2-(2-hydroxyethoxy)ethyl)amine.[14]

A typical procedure is described as follows for the preparation of compound **8**. A solution of 2.5 g (5.7 mmol) of compound **6** in 17 mL of pyridine was added to a 0°C solution of 1.8 g (17.2 mmol) of diethanolamine in 3 mL of pyridine. The cooling bath was removed, and the mixture was stirred for 5 h at room temperature. The mixture was cooled to 0°C, and 40 mL of CH_2Cl_2 was added. To the resulting mixture was added a solution of 11.55 g (57.3 mmol) of 4-nitrophenylchloroformate in 60 mL of CH_2Cl_2, and the mixture was stirred for 20 h at room temperature. The mixture was cooled to 0°C, acidified with 1N HCl, and partitioned between 300 mL of 1N HCl and 2×200 mL of CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give 13.6 g of yellow solid. Purification by silica gel chromatography (CH_2Cl_2/MeOH and EtOAc/hexanes) provided 4.91 g (83%) of compound **8** as a sticky amorphous solid. All new compounds gave satisfactory analytical data.[15]

The multivalent carbonate esters, which we have described, can be readily reacted with primary and secondary amine containing compounds. Work is in progress to use the multivalent active carbonate esters which have been described to prepare multivalent conjugates of biologically active molecules. In addition, we plan to explore the preparation of dendrimers of higher valence.

Acknowledgements

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15. Compound **6** was isolated as a white powder: mp 110°C; ^{1}H NMR ($CDCl_3$): δ 3.89 (t, 4H), 4.50 (t, 4H), 7.40 (d, 4H), 8.26 (d, 4H); ^{13}C NMR ($CDCl_3$): δ 68.1, 68.8, 121.9, 125.4, 145.5, 152.6, 155.6; anal. calcd for $C_{18}H_{16}N_2O_{11}$: C, 49.55; H, 3.70; N, 6.42. Found: C, 49.38; H, 3.76; N, 6.38. Compound **8** was isolated as a sticky amorphous solid: ^{1}H NMR ($CDCl_3$): δ 3.72 (m, 12H), 4.31 (t, 4H), 4.48 (m, 8H), 7.40 (m, 8H), 8.29 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 47.1, 47.6, 64.9, 67.1, 67.3, 69.2, 121.8, 121.9, 125.4, 145.5, 152.4, 155.4, 155.5, 156.0; mass spectrum (ESI): m/z $(M+Na)^+$ 1051; HRMS (MALDI) calculated for $C_{44}H_{42}N_4NaO_{25}$ (M+Na): 1051.1941. Found: 1051.1632.Compound **9** was isolated as a white crystalline solid: mp 55–64°C; ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 36H), 3.26 (s, 16H), 3.50 (m, 8H), 3.71 (t, 4H), 4.20 (t, 8H), 4.26 (m, 4H), 5.35 (brd s, 4H), 5.81 (brd s, 2H), 5.98 (brd s, 2H); ^{13}C NMR ($CDCl_3$): δ 28.4, 40.4, 41.2, 47.8, 48.3, 62.7, 64.6, 69.3, 79.4, 156.1, 156.8; HRMS (MALDI) calculated for $C_{46}H_{84}NaN_{10}O_{21}$ (M+Na): 1135.5705. Found: 1135.5729. Compound **10** was isolated as a crystalline solid: mp 67–69°C; ^{1}H NMR ($CDCl_3$): δ 3.50–3.80 (m, 28H), 4.22 (m, 12H), 4.43 (m, 16H), 7.40 (m, 16H), 8.30 (m, 16H); ^{13}C NMR ($CDCl_3$): δ 46.7, 47.0, 47.4, 47.6, 63.3, 63.9, 64.5, 66.9, 67.3, 69.2, 121.8, 125.3, 145.5, 152.4, 155.3, 155.7, 155.9; mass spectrum (ESI): m/z $(M+Na)^+$ 2235; anal. calcd for $C_{90}H_{88}N_{14}O_{53}$: C, 48.83; H, 4.01; N, 8.86. Found: C, 49.07; H, 4.21; N, 8.45; HRMS (ESI) calculated for $C_{90}H_{88}NaN_{14}O_{53}$ (M+Na): 2235.4519. Found: 2234.4494. Compound **11** was isolated as a white crystalline solid: mp 175°C; ^{1}H NMR ($CDCl_3$): δ 4.61 (s, 8H), 7.40, (m, 8H), 8.30 (m, 8H); ^{13}C NMR ($CDCl_3$): δ 42.4, 66.8, 122.5, 125.4, 145.2, 151.7, 155.1; anal. calcd for $C_{33}H_{24}N_4O_{20}$: C, 49.76; H, 3.04; N,

7.03. Found: C, 49.55; H, 3.10; N, 7.06. Compound **12** was isolated as a sticky viscous oil: ^{1}H NMR ($CDCl_3$): δ 3.69 (m, 16H), 4.31 (s, 8H), 4.41 (m, 16H), 7.39 (m, 16H), 8.25 (m, 16H);); ^{13}C NMR ($CDCl_3$): δ 43.8, 47.3, 48.0, 63.4, 66.9, 121.9, 125.5, 145.8, 152.6, 155.3, 155.4, 155.5; mass spectrum (ESI): m/z $(M+Na)^+$ 2003; HRMS (ESI) calculated for $C_{81}H_{72}NaN_{12}O_{48}$ (M+Na): 2003.3498. Found: 2003.3460. Compound **13** was isolated as a viscous oil: ^{1}H NMR ($CDCl_3$): δ 3.55 (m, 8H), 3.64 (m, 12H), 3.73 (m, 8H), 4.22 (t, 4H), 4.40 (t, 8H), 7.40 (d, 8H), 8.30 (d, 8H); ^{13}C NMR ($CDCl_3$): δ 47.7, 48.2, 64.3, 68.2, 68.3, 68.4, 69.3, 69.6, 69.9, 121.7, 125.3, 145.4, 152.4, 155.4, 156.0; HRMS (FAB) $C_{50}H_{56}CsN_6O_{29}$ (M+Cs): 1337.2146. Found: 1337.2079.