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A Convenient Route to Diversely Substituted Icosahedral Closomer Nanoscaffolds

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Supporting Information

ABSTRACT: The design and synthesis of icosahedral polyhedral borane closomer motifs based upon carbonate and carbamate anchoring groups for biomedical applications are described. Dodecacarbamate closomers containing easily accessible groups of interest at their linker termini were synthesized via activation of the B−OH vertices as aryl carbonates and their subsequent reaction with primary amines. Novel dodecacarbonate closomers were successfully synthesized for the first time by reacting $[closo\text{-}B_{12}(OH)_{12}]^{2-}$ with an excess of respective aryl chloroformates, utilizing relatively short reaction times, mild conditions and simple purification strategies, all of which had previously presented difficulties in closomer chemistry. This methodology for the 12-fold degenerate synthesis of carbonate and carbamate closomers will greatly facilitate further exploration of closomers as monodisperse nanomolecular delivery platforms.

Significant advances have been made over the past decade in the development of nanoscale pharmaceutical carriers to enhance the *in vivo* efficacy of many pharmaceuticals currently in clinical use.[1,2] Attachment of many copies of pharmaceuticals to nanocarriers such as liposomes, polymeric and metallic nanoparticles, micelles, dendrimers, etc., can be used to enhance the effectiveness of a drug's therapeutic or diagnostic functions.[3] Herein we present a novel nanocarrier based upon the B_{12}^{2-} icosahedral borane scaffold. The chemical basis of these unique nanocarriers is the discovery of the very stable and icosahedral $[closo\text{-}B_{12}(OH)_{12}]^{2-}$, **1**, and its successful 12-fold derivatization to produce general structures now known as closomers.[4−6] The syntheses of fully substituted borane closomers, in which linker groups emanate from a rigid B_{12}^{2-} core, represent a novel architecture at the interface of boron and carbon chemistries.

The 12-fold degenerate functionality of **1** is reflected in the closomer species derived from it by simple organic reactions characteristic of the hydroxyl group: carboxylate ester, ether, as well as carbonate and carbamate ester formation reported here. The resulting monodisperse closomers can accommodate closely spaced radial substituents with variable size, hydrophilicity, ionic charges, and solvation properties.[6] A schematic representation of such a system is shown in Figure 1.

One of the main objectives of this study was to develop a universal toolbox that may be used to conjugate many copies of substituents for therapeutic or diagnostic purposes as well as others. Earlier, we reported the synthesis of 12-fold degenerate

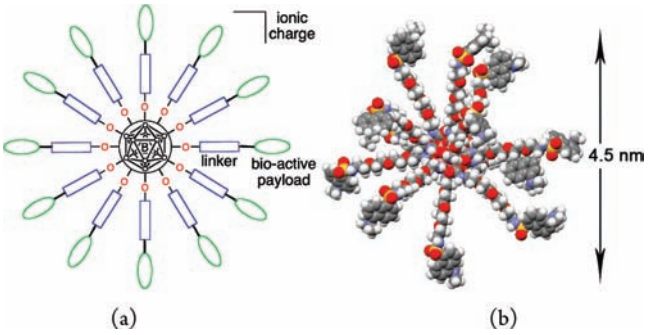

Figure 1. Monodisperse nanomolecular closomer platform based on B_{12}^{2-} core: (a) schematic representation; (b) space filling model of 12-fold degenerate carbamate closomer **3i**.

ester[6,7] and ether[8,9] derivatives of $[closo\text{-}B_{12}(OH)_{12}]^{2-}$ including a recently communicated synthesis of a 12-fold degenerate azido carboxylate ester closomer for 'click chemistry' approaches.[10] Though ester and ether derivatives have demonstrated the utility of the B_{12}^{2-} core for use as a scaffold, simple and mild reaction conditions are necessary for the successful utilization of closomers as multifunctional carriers. Consequently, we report here 12-fold degenerate carbonate and carbamate closomers as useful platforms for conjugation to bioreactive or other molecules of interest.

Carbamates are widely used as protective groups for amines in organic chemistry.[11] They are easily synthesized by reacting chloroformates with amines or more elegantly by nucleophilic attack of amines on the carbonyl group of a carbonate ester. In our earlier reports[7] we have shown that closomer carboxylate esters are relatively stable in aqueous media at moderately acidic and alkaline pH. We envisioned that carbamates, when also O-bonded to a B_{12}^{2-} cage would be stable at physiological pH. This prompted us to examine 12-fold degenerate carbonate closomers as intermediates in carbamate closomer syntheses. For the initial exploration we chose the *p*-nitrophenyl carbonate since substituted aryl carbonates having strongly electron-withdrawing substituents are preferred reactants for carbamate formation from primary amines. Thus, closomer **2j** was prepared by reacting *p*-nitrophenyl chloroformate with **1** in the presence of anhydrous pyridine and acetonitrile at room temperature for 72 h to give the corresponding 12-fold degenerate carbonate closomer

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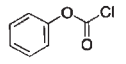
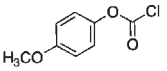
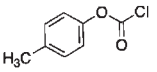
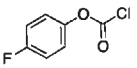
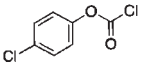
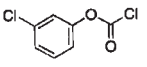
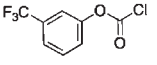
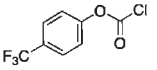
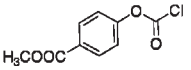
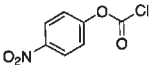
Table 1. Synthesis of 12-Fold Degenerate Carbonate Closomers

Entry	Chloroformate	12-fold carbonate product	Time / Temperature	Yield [%][a]
1		**2a**	24 h/ reflux	35
2		**2b**	24 h/ reflux	26
3		**2c**	24 h / reflux	32
4		**2d**	24 h / reflux	39
5		**2e**	24 h / reflux	68
6		**2f**	24 h / reflux	66
7		**2g**	96 h / r.t.	47
8		**2h**	96 h / r.t.	44
9		**2i**	96 h / r.t.	32
10		**2j**	72 h / r.t.	40

[a] Purified yield.

in 40% purified yield. This carbonate was highly reactive toward amines but was not suitable for use in carbamate synthesis due to its unstable nature and resulting low yield. This led us to other 12-fold degenerate carbonates for carbamate synthesis. Table 1 shows various carbonate closomers synthesized with substitutents having Hammet Sigma constants ranging from −0.27 (p-OCH_3) to 0.78 (p-NO_2).

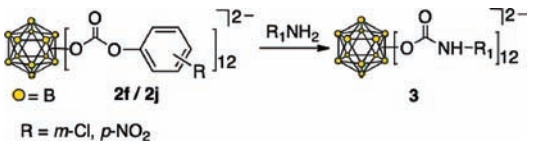
The 12-fold degenerate hydroxyl groups on the B_{12} icosahedral surface pose unique challenges in synthesis. To achieve a high yield of 12-fold degenerate carbonate closomer, the typical reaction conditions involved reacting **1** with respective chloroformate (60 mol equiv) and pyridine (60 mol equiv) in acetonitrile (excess) at the refluxing temperature for 24 h (carbonates **2a**−**2f**, method 1) or stirring at room temperature for 96 h (carbonates **2g**−**2j**, method 2). The reaction was monitored by MS analysis and ^{11}B NMR spectra of the reaction mixture. The completion of the reaction was confirmed by the appearance of a single peak around −17.4 ppm in the ^{11}B NMR. Solvent was removed under vacuum following filtration, and the residue was precipitated from dichloromethane with diethyl ether to remove the large excess of chloroformate used in the reaction. Benchtop flash column chromatography using a gradient of dichloromethane and acetonitrile over neutral alumina gave the desired 12-fold degenerate carbonate in good purity although in reduced yield along with some less than 12-substituted carbonate. The yields of 12-fold carbonate closomers presented in Table 1 are purified yields of 12-fold substituted carbonates only. Preliminary hydrolytic stability studies carried out in a DMSO/H_2O system[12−14] indicated these carbonate closomers to be stable between pH 3 and pH 11.

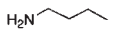
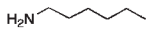
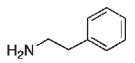
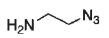
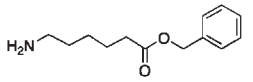
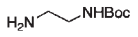
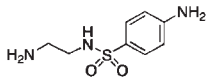
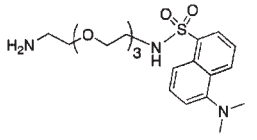
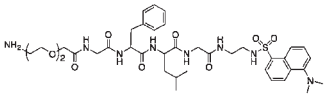
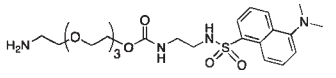
Once the carbonate closomers were obtained, their reactivities toward n-butyl amine were examined. The reaction of carbonates **2a**−**2e** with n-butyl amine (60 mol equiv in acetonitrile) required elevated temperature to obtain the corresponding 12-fold degenerate carbamates. At the same reagent concentrations, carbonates **2f**−**2j** were found to react with n-butyl amine at room temperature to give the corresponding 12-fold degenerate carbamate **3a**. This led us to conclude that carbonate closomers with substituents having Hammett σ constants of 0.37 and higher were suitable for forming carbamates at room temperature. Due to its great reactivity, described above, carbonate **2j** could not be routinely used. Alternatively, we chose carbonate **2f** as a reagent for its ease of synthesis, high reactivity toward amines, and long-term stability.

The utility of the carbonate closomer, a precursor for 12-fold degenerate carbamate closomers attached by suitable linkers, depends upon the steric environment near the site of the reaction. It was prudent to employ a flexible linker next to the terminal amine group for reaction with the carbonate closomer **2f**. Typical reaction conditions involved reaction of carbonate closomer **2f** with an excess of the primary amine (60 mol equiv corresponding to 5 mol equiv per vertex) in an appropriate solvent (acetonitrile or DMF) at room temperature for 24−120 h (Table 2). The progress of the reaction was monitored by MS analysis and shifting of the ^{11}B NMR singlet from −17.4 to −18.3 ppm. To explore the versatility of carbonate closomers as intermediates, we prepared a variety of 12-fold degenerate carbamate closomers shown in Table 2. These include simple alkyl and aralkyl carbamates **3a**−**3c** as well as peripherally functionalized carbamates **3d**−**3k**. Carbamates **3d** and **3e** have alkyne and azido groups, respectively, on their periphery for click chemistry applications. Carbamates **3f** with a benzyl-protected carboxylic acid function and **3g** with a Boc protected primary amino group are available for further conjugation to peptides or bioactive molecules of interest through amide linkages. Carbamate closomer **3f** and carbamate **3h** having 12 sulfanilamide groups on the periphery were synthesized using carbonate **2j** instead of carbonate **2f**.

To further explore carbamate closomer chemistry, we synthesized a series of 12-fold degenerate carbamates **3i**−**3k** having a fluorescent dansyl group on the closomer as a surrogate for a bioactive molecule. The dansyl group (5-(dimethylamino)-1-naphthalenesulfamido) absorbs strongly in the near UV region and fluoresces in the visible region. The dansyl group is robust, easily derivatized, and commonly used as a fluorescent marker and sensor.[15−18] In this series of 12-fold degenerate carbamate closomers, the linkers with variable length and functions attached to dansyl were preassembled and then conjugated with the B_{12}^{2-} core in the final step. For example, the reactive amine for carbamate **3i** was synthesized in five steps starting from commercially available tetraethylene glycol,

Table 2. Synthesis of 12-Fold Carbamate Closomers



Entry	Amine	12-fold carbamate product / Time	Yield [%][a]
1		**3a** / 48 h	89
2		**3b** / 48 h	84
3		**3c** / 72 h	76
4		**3d** / 96 h	72
5		**3e** / 72 h	82
6		**3f** / 24 h	37[b]
7		**3g** / 72 h	73
8		**3h** / 24 h	48[b]
9		**3i** / 120 h	84
10		**3j** / 120 h	78
11		**3k** / 120 h	82

[a] Purified yield. [b] Carbonate **2j** was used for the synthesis.

which was converted to a dimesylate derivative.[19] Both of the mesylate groups were then converted to azide using sodium azide.[20] This diazide was selectively reduced to give corresponding monoamine azide in 65% yield. This mono amine was reacted with dansyl chloride to give the corresponding dansyl conjugated azide. The terminal azide was reduced to the corresponding primary amine, and this amine was reacted (12-fold excess per vertex) with carbonate **2f** in dimethylformamide (DMF) at room temperature for 72 h. The crude product was purified by benchtop size exclusion column chromatography over lypophilic resin LH20 in methanol and subsequent dialysis through 1000 MW membrane in DMSO/water mixture to give the carbamate closomer **3i** in 84% yield.

Absorbance spectrum of the 12-fold degenerate dansyl closomer **3i** in acetonitrile exhibits an intense absorbance band in the near-UV region (λ_{max} = 221, 250, and 335 nm, $\in_{max}$ = 5.24 × 10^5, 1.93 × 10^5, and 5.07 × 10^4 M^{-1} cm^{-1}, respectively) and a strong fluorescence band in the visible region (λ_{max} = 516 nm, excitation at 335 nm). When compared to dansylamide, a monodansyl model compound (absorbance λ_{max} = 220, 250, and 335 nm, $\in_{max}$ = 4.84 × 10^4, 1.82 × 10^4, and 4.07 × 10^3 M^{-1} cm^{-1}, respectively), closomer **3i** shows approximately 12-fold increase in the absorbance. Similarly, on excitation at 335 nm in acetonitrile, the intensity of a fluorescence band at 516 nm was 12 times that of dansylamide, corresponding to 12 dansyl units on the closomer (please see Supporting Information for details). Protonation of dansyl groups with triflic acid causes the disappearance of this fluorescence band and the appearance of a new fluorescence band with λ_{max}= 338 nm for the protonated dansyl group.[21]

In addition to closomer **3i**, other 12-fold carbamate closomers **3j** and **3k** having cleavable linkers were synthesized. Carbamate **3j** has a tetrapeptide *Gly-Phe-Leu-Gly* sequence as a part of the linker. This tetrapeptide is very specific to the cathepsin-B enzyme, which is overexpressed in breast and other cancers.[22−25] The carbamate **3k** has a tetraethylene glycol linker with an acid-labile carbamate function attached to the terminal dansyl ethylene diamine moiety. These carbamate closomers were synthesized analogously to closomer **3i**, made by building the respective linker with a peripherally attached dansyl group and reacting it with carbonate closomer **2f** to give the corresponding 12-fold carbamates in good yields.

In summary, we have shown the versatility of 12-fold degenerate carbonate and carbamate closomers for the attachment of a wide variety of linkers. Such an approach can be utilized to construct a delivery system carrying 12 copies of an active pharmaceutical.

■ ASSOCIATED CONTENT

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

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