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Dynamic Combinatorial Libraries of Disulfide Cages in Water

Kevin R. West,[†] Kyle D. Bake,[‡] and Sijbren Otto*,[†]

Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK

so230@cam.ac.uk

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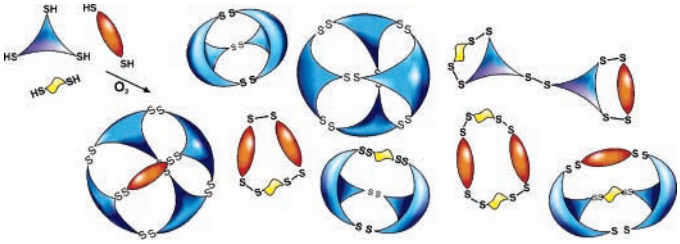
ABSTRACT



Dynamic combinatorial libraries (DCLs) containing water-soluble disulfide-linked cages (alongside macrocyclic structures) have been generated and characterized. Unlike most other strategies for generating molecular cages, the structures are held together by covalent bonds, which are formed under thermodynamic control. The diversity of the cages generated opens new possibilities for a generalized combinatorial strategy toward molecular encapsulation.

As synthetic molecular capsules have reached their twentieth birthday, two main approaches for capsule formation dominate the literature: (i) formation through covalent bonds under kinetic control[1] and (ii) self-assembly through noncovalent interactions or metal−ligand coordination under thermodynamic control.[2] Covalent molecular capsules, as pioneered by Cram and co-workers in the 1980s, have been demonstrated to be robust and stable with the remarkable ability to shield a guest from the outside environment. However, the synthesis of such capsules is often accompanied by the formation of oligomeric side products and release of trapped guests under mild conditions is usually not possible. During the synthesis of capsules held together by noncovalent interactions the formation of oligomeric side products is less frequent although often the ensuing complexes are relatively labile.

Dynamic covalent chemistry is an attractive alternative approach to molecular encapsulation, providing robust capsules that are connected through covalent bonds while benefiting from thermodynamically controlled synthesis. Moreover, many reversible covalent bonds can be cleaved under mild conditions, allowing for controlled release of the contents of the capsules.

Whereas only a few examples exist of molecular capsules formed under thermodynamic control through dynamic covalent chemistry (all in organic solvents),[3] this approach

[†] University of Cambridge. Fax: (+44) 1223-336017. Tel: (+44) 1223-336509.

[‡] Current address: Department of Chemistry, Tufts University, 62 Talbot Avenue, Medford, MA 02155.

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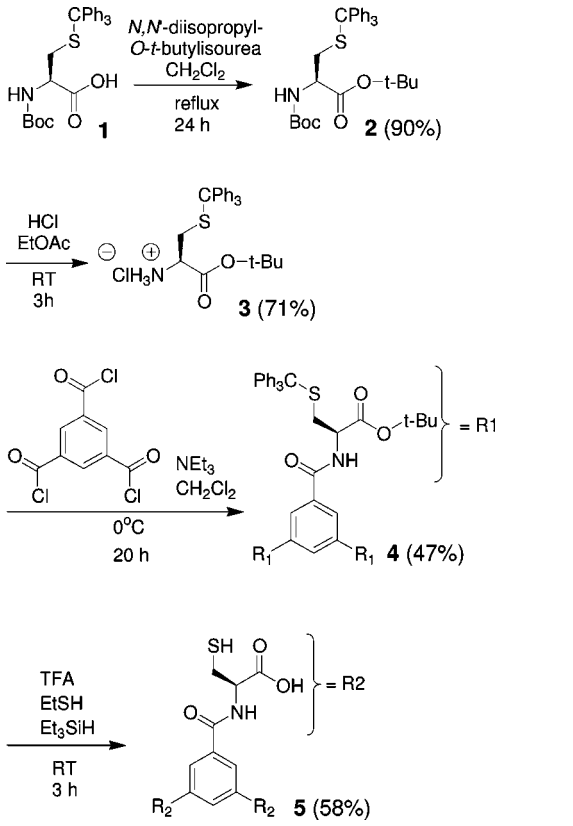
holds great potential, particularly when combined with combinatorial chemistry. Dynamic combinatorial libraries (DCLs) of noncovalent capsules or cages[4] have been previously realized by others.[5] We now report the first example of a DCL containing covalent cages.

In dynamic combinatorial chemistry,[6] a mixture of compounds is generated by linking building blocks together through a reversible reaction. Reversibility ensures that the DCL is in thermodynamic equilibrium and responsive to external influences. Upon exposure of a DCL to a molecular target, those library members that bind to the target are stabilized; the equilibrium shifts and strong binders are amplified at the expense of poor binders in the library.[7] After the exchange of building blocks has been turned off, the amplified compound(s) can be isolated directly from the frozen library. Thus, dynamic combinatorial chemistry not only is a method of selection but also provides a synthetic route for the selected compounds.

Following our successful work on DCLs of linear and macrocyclic receptors starting from mono and dithiols,[8] we have started to explore the use of trithiols to generate DCLs of cage-like structures. We now report the first results of these studies, based on cysteine-derived trithiol building block **5**.

The synthesis of trithiol **5** is shown in Scheme 1. We have chosen to protect the thiol and carboxylate groups of the cysteine subunits with acid labile protecting groups (trityl and *tert*-butyl, respectively). The protected cysteine **3** should allow straightforward coupling to any suitable carboxylic-acid derived scaffold after which the thiol moiety (for disulfide formation and exchange) and carboxylic acid (for water solubility) can be liberated in a single clean deprotection step.

Scheme 1. Synthesis of Trithiol Building Block **5**[9−12]



In this case, the protected cysteine **3** was coupled to 1,3,5-benzenetricarbonyl chloride to produce the protected trithiol **4**. Finally, removal of the *tert*-butyl and trityl protecting groups to yield **5** was achieved using trifluoroacetic acid and triethylsilane. In addition, ethanethiol had to be added to prevent migration of the *tert*-butyl group to the cysteine thiol.

We have prepared and analyzed DCLs made from trithiol building block **5** and two dithiol building blocks **6** and **7**, which should allow access to mixed cages (Figure 1).

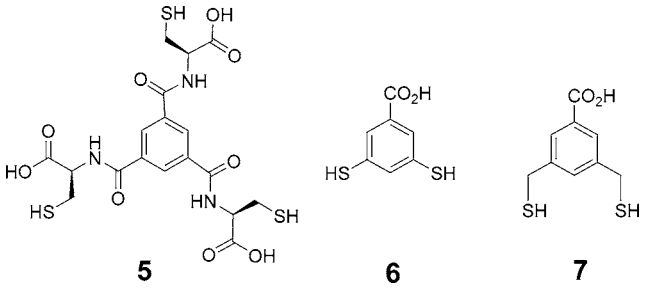


Figure 1. Trithiol and dithiol building blocks used in DCLs.

Disulfide formation occurs readily by oxidation of an aqueous solution of the thiols upon exposure to air. Exchange

(4) As the term "capsules" is associated with structures that have receptor characteristics, we use the term "cages" to describe the macrobicyclic structures in our libraries. Others have used this term before to describe structures that do not necessarily have receptor characteristics (see ref 2d).

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takes place under mild conditions (pH 7−9) in the presence of thiolate and is switched off under acidic conditions or after removal of the thiolate.[8a]

Oxidation of a 5mM solution of trithiol building block **5** produced the dimeric cage $(\mathbf{5})_2$[13] essentially quantitatively, judging by LC−MS, although trace amounts of a tetrameric cage could be detected using ESI−MS.

More diverse mixtures were obtained by mixing in dithiol building blocks **6** and **7**. Figure 2 shows the composition of

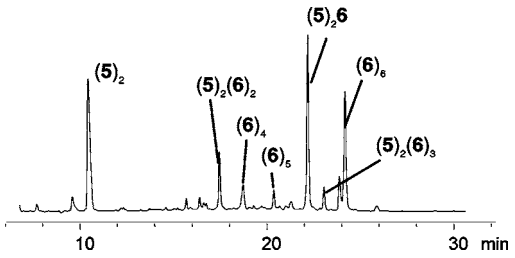

Figure 2. LC−MS analysis (negative ion mode; gradient elution using acetonitrile and water containing 0.1% formic acid) of a DCL made from **5** and **6** (5mM each).

a DCL made from **5** and **6** containing the mixed disulfides $(\mathbf{5})_2\mathbf{6}$, $(\mathbf{5})_2(\mathbf{6})_2$, and $(\mathbf{5})_2(\mathbf{6})_3$ alongside the dimeric cage $(\mathbf{5})_2$ and various macrocyclic oligomers of **6**.

Upon mixing **5** and **7** we were also able to detect a number of mixed species. Figure 3a shows the product distribution

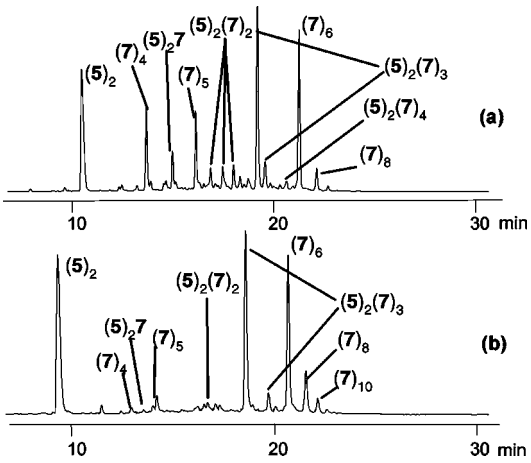

Figure 3. LC−MS analysis of the DCL made from **5** and **7** (a) after 7 days and (b) after equilibrium had been reached following the addition of dithiothreitol.

after 7 days. This time mixed disulfides containing two units of **5** and up to four units of **7** were detected. However, unlike the DCL made from **5** and **6**, this library turned out to equilibrate very slowly and equilibrium was only reached[14] after adding 15 mol % dithiothreitol to the solution to generate some more thiol to revive the disulfide exchange.[8a] The final equilibrium composition is shown in Figure 3b.

Libraries made from mixtures of dithiols and trithiols can give rise to structural isomers, depending on how the dithiols insert into the structural framework formed by the trithiols. For example, Figure 4 shows the three possible structural

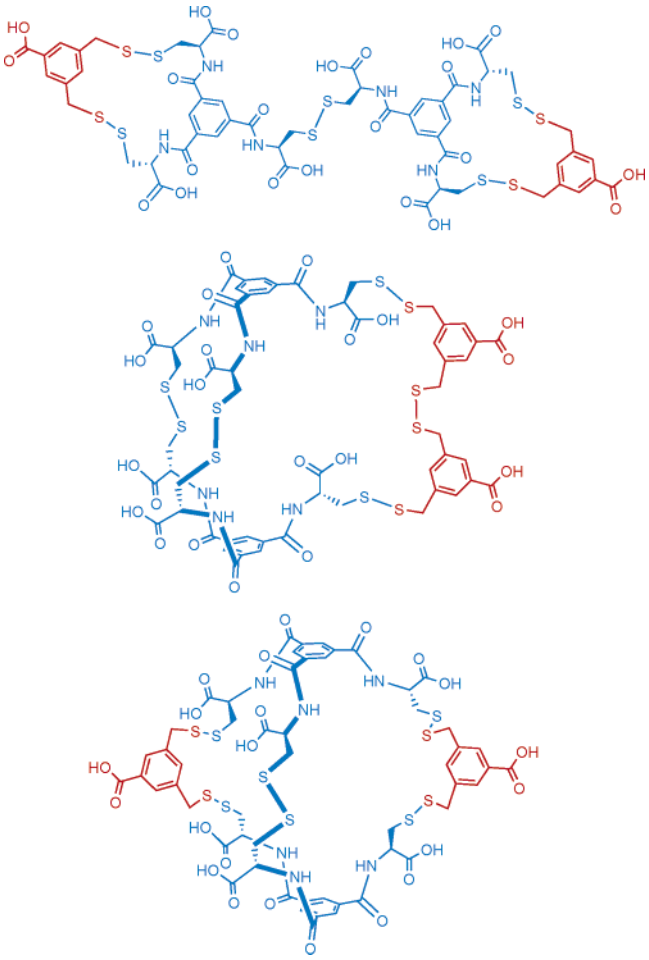

Figure 4. Three structural isomers corresponding to $(\mathbf{5})_2(\mathbf{7})_2$.

isomers that can be formed from two units of **5** and two units of **7**. LC−MS analysis of the library of Figure 3a confirms the presence of all three expected products, as

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(13) In theory, **5** could dimerize to form a noncage structure in which the two building blocks are linked though a single disulfide bond, whereas the remaining two disulfide bonds are formed within each building block subunit. However, this scenario appears unlikely: we have prepared an analogue of **5** containing only two cysteine subunits. Upon oxidation of this building block no intramolecular disulfide formation could be detected, suggesting that intra-building-block cyclization is unfavourable. Inspection of the ^{1}H NMR of $(\mathbf{5})_2$ leads to the same conclusion: all cysteine subunits in the spectrum are equivalent, which is consistent only with a cage structure.

illustrated by the chromatogram of $m/z = 1455$ shown in Figure 5. No efforts were made to assign individual peaks to individual isomers.

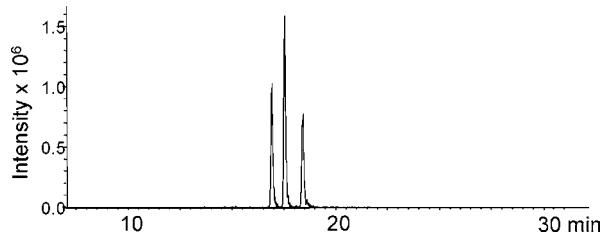

Figure 5. Extracted ion chromatogram for $m/z = 1455$ of the DCL made from **5** and **7** after 7 days.

Finally, a library was made containing all three building blocks **5**−**7**. In addition to previously observed cages and macrocyclic structures, library members of composition $(\mathbf{5})_2\mathbf{6},\mathbf{7}$ and $(\mathbf{5})_2\mathbf{6}(\mathbf{7})_2$ were detected. Control experiments[14] confirmed that in this case equilibrium had been reached without the need to use dithiothreitol.

These results indicate, for the first time, that dynamic combinatorial chemistry can be used to produce a diverse array of *covalent* cages under thermodynamic control from simple building blocks. The structures described here are relatively open and flexible making them likely to only incarcerate particularly bulky guests. We are currently developing more compact and less flexible trithiol building blocks that should be able to encapsulate a variety of small molecules. The resulting water-soluble disulfide-linked carceplexes should offer the exciting possibility of redox-controlled guest release. We envisage application of such systems in drug targeting as disulfide linkages have already been successfully employed in redox-triggered polymeric drug and gene delivery agents in vitro and in vivo.[15,16]

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Supporting Information Available: Synthetic procedures and characterization for **1**−**5**; LC−MS conditions and extracted ion chromatograms for cagelike library members and details for the experiments outlined in ref 13. This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) To check that equilibrium had been reached the separate building blocks were oxidized independently and subsequently mixed together, followed by addition of 15 mol % dithiothreitol to initiate disulfide exchange.[8a] Product distributions for DCLs formed through this procedure were similar to those obtained starting directly from the thiol building blocks. Only for the library made by mixing **5** and **7**, 15 mol % dithiothreitol had to be added to aid the exchange process.

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