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Dynamic Combinatorial Libraries of Macrocyclic Disulfides in Water

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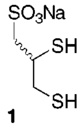
Since our initial studies in 1996[1] the interest in dynamic combinatorial libraries (DCLs) has been growing rapidly,[2] but the number of reversible chemical reactions which have been explored for the creation of DCLs is still limited. In this paper we report the first example of the successful use of the disufide exchange reaction for the generation of DCLs of structurally diverse macrocyclic disulfides.[3] FTICR ESI-MS analysis of a DCL made from four different dithiol building blocks revealed the presence of over 100 macrocycles which were in some cases separated in mass by only 0.17 amu.

DCLs differ from conventional combinatorial libraries in that the individual members of the library are constantly interconverting, that is the composition of the library is under thermodynamic control. As a consequence a DCL has the unique ability to respond to influences that affect the stability of its constituents. More specifically, introduction of a template molecule will stabilize those members of the DCL that happen to bind to the template. Consequently, *the concentration of those members will increase* which facilitates the identification of the desired molecules. In theory, isolation of these molecules directly from the DCLs in high yield is possible by repeated isolation−equilibration cycles.

At present, research on DCLs is still in the proof-of-principle stage. Considerable success has been achieved in templating DCLs built up through noncovalent linking of building blocks,[4] encouraging the development of libraries based on reversible covalent bond formation. As yet, there are only a small number of reversible reactions available. After our initial studies on DCLs based on transesterification[1] Lehn et al. have reported a library based on the exchange of Schiff bases.[5] Unfortunately, transesterification requires harsh conditions, while exchange of Schiff bases can only be turned off by hydrogenation. DCLs based on the hydrazone exchange reaction have recently been proven to be highly practical.[6] Reversible C═N bond formation can also be used to create libraries of oximes.[7] Finally, two reports exist of DCLs generated through olefin metathesis[8] and enzymatic transamination.[9]

In an attempt to expand the number of reversible reactions that can be used to generate DCLs we have been exploring the potential of the disulfide exchange reaction. Extensive mechanistic studies on thiols and disulfides by Whitesides and others[10] indicate that (i) disulfides tend to form readily from thiols in the presence of oxygen and small amounts of base, (ii) disulfide exchange takes place efficiently under mild conditions in the presence of a catalytic amount of thiol, (iii) disulfide exchange is negligible under acidic conditions, and (iv) disulfides are stable toward many different functional groups. Equilibrium mixtures of disulfides have been used by Regen et al. to study lipid mixing in bilayer systems,[11] while work by Still et al. has demonstrated that the composition of an equilibrium system of three different disulfides can indeed be influenced by host−guest interactions.[12]

Aiming at the formation of DCLs of macrocyclic disulfides, we selected a number of structurally diverse dithiol building blocks (**1**−**6**) including a carbohydrate (**5**) and an α-amino acid derivative (**6**)[13] as well as a number of fully synthetic dithiols.[14]



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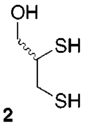

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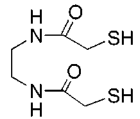

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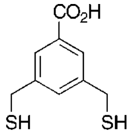

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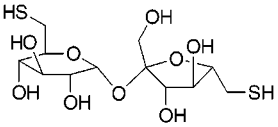

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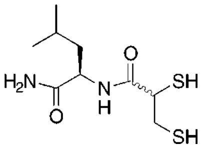

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Stirring the dithiols in an open vial at a concentration of 10 mM at pH 7.5 resulted in the expected formation of a mixture of macrocyclic disulfides, as illustrated schematically in Scheme 1. Oxidation was monitored by quantifying the remaining amount of thiol using Ellman's reagent[15] and was in most cases essentially complete within 24 h.[16]

The composition of the libraries was studied using triple quadrupole electro spray ionization mass spectroscopy (QQQ ESI-MS) and Fourier transform ion cyclotron resonance (FTICR) ESI-MS.[17] Analysis of reaction mixtures obtained upon oxidation of **1**, **4**, and **5** individually[18] revealed the presence of all expected cyclic species up to octamers, pentamers, and heptamers, respectively.

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(1) (a) Brady, P. A.; Bonar-Law, R. P.; Rowan, S. J.; Suckling, C. J.; Sanders, J. K. M. *Chem. Commun.* **1996**, 319. (b) Rowan, S. J.; Brady, P. A.; Sanders, J. K. M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2143. (c) Rowan, S. J.; Brady, P. A.; Sanders, J. K. M. *Tetrahedron Lett.* **1996**, *37*, 6013.

(2) For reviews, see: (a) Ganesan, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2828. (b) Klekota, B.; Miller, B. L. *Trends Biotechnol.* **1999**, *17*, 205. (c) Lehn, J.-M. *Chem. Eur. J.* **1999**, *5*, 2455. (d) Cousins, G. R. L.; Poulsen, S.-A.; Sanders, J. K. M. *Curr. Opin. Chem. Biol.* **2000**, *4*, 270. (e) Karan, C.; Miller, B. L. *Drug Discovery Today* **2000**, *5*, 67.

(3) During the process of submission of this paper a DCL of carbohydrate dimers linked though a disulfide bond has been reported. Ramstrom, O.; Lehn, J.-M. *ChemBioChem* **2000**, *1*, 41.

(4) (a) Klekota, B.; Hammond, M. H.; Miller, B. L. *Tetrahedron Lett.* **1997**, *38*, 8639. (b) Sakai, S.; Shigemasa, Y.; Sasaki, T. *Tetrahedron Lett.* **1997**, *38*, 8145. (c) Hiraoka, S.; Fujita, M. *J. Am. Chem. Soc.* **1999**, *121*, 10239. (d) Crego Calama, M.; Timmerman, P.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **2000**, *39*, 755. (e) Hof, F.; Nuckolls, C.; Rebek, J., Jr. *J. Am. Chem. Soc.* **2000**, *122*, 4251.

(5) Huc, I.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2106.

(6) Cousins, G. R. L.; Poulsen, S.-A.; Sanders, J. K. M. *Chem. Commun.* **1999**, 1575.

(7) Polyakov, V. A.; Nelen, M. I.; Nazarpack-Kandlousy, N.; Ryabov, A. D.; Eliseev, A. V. *J. Phys. Org. Chem.* **1999**, *12*, 357.

(8) Giger, T.; Wigger, M.; Audetat, S.; Benner, S. A. *Synlett* **1998**, 688.

(9) Swann, P. G.; Casanova, R. A.; Desai, A.; Frauenhoff, M. M.; Urbancic, M.; Slomczynska, U.; Hopfinger, A. J.; Le Breton, G. C.; Venton, D. L. *Biopolymers* **1996**, *40*, 617.

(10) (a) Lees, W. J.; Whitesides, G. M. *J. Org. Chem.* **1993**, *58*, 642 and references therein. (b) Gilbert, H. F. *Methods Enzymol.* **1995**, *251*, 8. (c) Wallace, T. J.; Schriesheim, A.; Bartok, W. *J. Org. Chem.* **1963**, *28*, 1311.

(11) Davidson, S. M. K.; Regen, S. L. *Chem. Rev.* **1997**, *97*, 1269.

(12) Hioki, H.; Still, W. C. *J. Org. Chem.* **1998**, *63*, 904.

(13) Disulfides containing **6** appear to be losing one sulfur atom per unit of **6** in the mass spectrometer. Conversion of the disulfides back into dithiols using tricarboxyethylphosphine (Burns, J. A.; Butler, J. C.; Moran, J.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 2648) regenerated **6**, indicating that loss of sulfur takes place only in the mass spectrometer.

(14) **1** and **2** are commercially available; procedures have been reported for the synthesis of **4** (Staab, H. A.; Kirrstetter, R. G. H. *Liebigs Ann. Chem.* **1979**, 886) and **5** (Lees, W. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 1860). The synthesis of **3** and **6** is described in the Supporting Information.

(15) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70.

(16) Oxidation of **4** required 2 weeks, most likely a result of the rigid character of this building block inhibiting ring closure. When mixed with other building blocks, oxidation was essentially complete within 24 h.

(17) (a) FTICR-MS is a powerful technique for the analysis of combinatorial libraries. See, for example: Walk, T. B.; Trautwein, A. W.; Richter, H.; Jung, G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1763. (b) Previous work in our group has demonstrated the potential of FTICR-MS for sequencing of the oligomers in DCLs. See: Poulsen, S.-A.; Gates, P. J.; Cousins, G. R. L.; Sanders, J. K. M. *Rapid Comm. Mass Spectrosc.* **2000**, *14*, 44.

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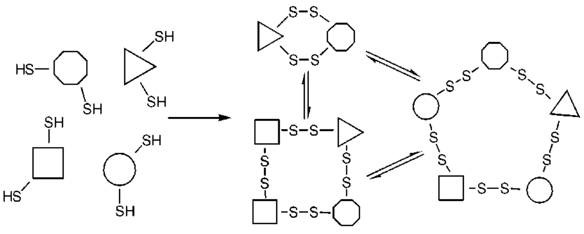
Scheme 1. Formation of a DCL of Macrocyclic Disulfides from Dithiol Building Blocks



We have also analyzed several DCLs formed by oxidizing mixtures of building blocks. High-resolution FTICR analysis was required for the analysis of DCLs in which building blocks **1** as well as **5** were incorporated since cyclic oligomers that contain **5** differ only by 0.17 amu from analogues containing 2 equiv of **1**.[19] The resolution of the FTICR mass spectrometer allowed for peaks up to *m*/*z* = 1167 to be analyzed for the presence of the two different oligomers. Considering all macrocycles up to pentamers within this range, peaks due to both of the two expected compounds were observed in 17 out of 26 cases. Figure 1 shows an example. Also shown is a comparison of the observed isotopic substitution patterns (Figure 1b) with the calculated ones (Figure 1a).

Detailed analysis of a DCL formed upon oxidation of **1**, **2**, **4**, and **5** revealed the presence of 119 different macrocyclic compounds. Considering all macrocycles up to tetramers, we were able to confirm the presence of 56 of the theoretical number of 66 species with unique masses. The failure to detect the remaining species might be due to the very low concentrations of these species in the mixture but could also be caused by inefficient ionization of some of the macrocycles. We have made no attempt to identify sequence isomers,[17b] regioisomers, and stereoisomers.

To ascertain that the disulfide libraries are under thermodynamic control we have performed a set of control experiments in which the same library was generated via two different routes. Figure 2a shows part of the ESI-MS spectrum of the target library formed directly by oxidation of a mixture of **1**, **2**, **3**, and **5**. In addition, two separate libraries were generated by oxidizing mixtures of (**1** and **2**) and (**3** and **5**), respectively. The latter two libraries were mixed and analyzed immediately after mixing (Figure 2b). After partial reduction by addition of 15 mol % of dithiothreitol and subsequent oxidation, the mass spectrum of the mixture (Figure 2c) is nearly identical to that of Figure 2a. These results clearly indicate that, under the conditions used, the oxidation process is sufficiently slow to allow for equilibration of the different macrocyclic disulfides.

Practical use of DCLs requires control over the exchange process. To be able to isolate molecules from a DCL *conditions should exist where exchange does not occur*. In line with literature observations we found that disulfide exchange becomes exceedingly slow with increasing acidity of the medium. For instance, when two libraries that were obtained by oxidizing mixtures of (**1** and **2**) and (**3** and **5**), respectively, were mixed at pH 2.5 no new mixed disulfides could be detected after a period of 12 days.

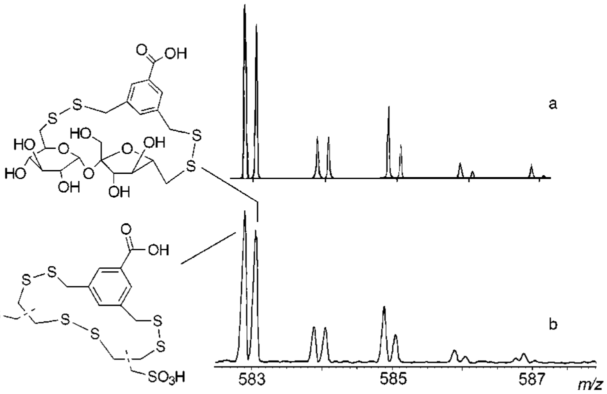


Figure 1. Part of the negative ion FTICR ESI-mass spectrum of a DCL formed after oxidation of a mixture of **1**, **2**, **4**, and **5**, showing peaks due to two different macrocyclic disulfides differing by 0.17 amu, comparing calculated (a) and observed (b) isotopic substitution patterns. Observed peaks correspond to $[M - 1]^-$.

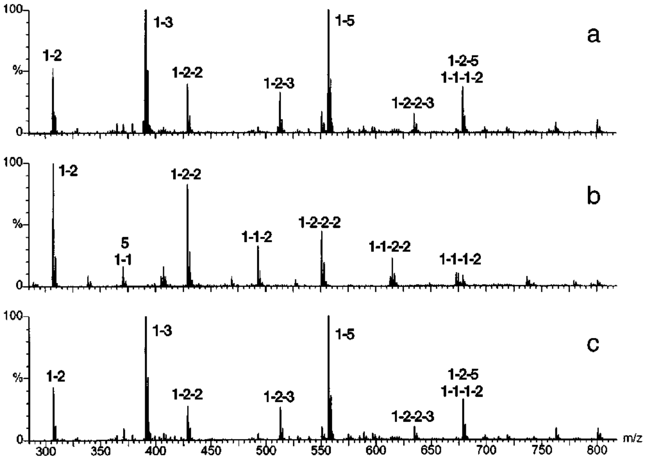


Figure 2. Part of the negative ion QQQ ESI-mass spectrum of disulfide libraries formed after (a) oxidation of a mixture of **1**, **2**, **3**, and **5**; (b) mixing of two preformed libraries made from building blocks (**1** and **2**) and (**3** and **5**), respectively; (c) addition of 15 mol % of DTT to the mixture described under b followed by 12 h of stirring in an open vial.

In summary, we have demonstrated that considerable structural diversity can be generated in a single step using a variety of simple dithiol building blocks under extremely mild conditions. Even building blocks such as **3** and **5**, which have been reported to form relatively stable intramolecular disulfides[14] are readily incorporated into the DCLs. The disulfide chemistry forms an important addition to the as yet limited number of reactions that is available for the generation of DCLs. It complements existing libraries in that exchange takes place under neutral or mildly basic conditions, whereas C═N bond-based DCLs require acidic conditions for reversibility. It surpasses existing DCLs in the fact that no external catalyst is required for the exchange process.

Inspired by the reported use of macrocyclic disulfides as receptors and as synthetic ionophores[20] we are currently screening disulfide-based DCLs for molecules with these properties.[21]

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Supporting Information Available: FTICR and QQQ ESI-MS procedures and data for the DCL formed from **1**, **2**, **4**, and **5** and synthesis of **3** and **6** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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[18] Only **1**, **4**, and **5** are sufficiently hydrophilic to allow the formation of libraries from one building block without any precipitation occurring. Libraries made from **2**, **3**, and **6** require the presence of **1**, **4**, or **5** to prevent precipitation.

[19] When creating DCLs of macrocyclic compounds it is difficult to avoid generating compounds with similar masses since this would require all building blocks and also *all possible combinations of building blocks* to have unique masses.

(20) (a) Ranganathan, D.; Haridas, V.; Karle, I. L. *J. Am. Chem. Soc.* **1998**, *120*, 2695. (b) Ranganathan, D.; Haridas, V.; Madhusudanan, K. P.; Roy, R.; Nagaraj, R.; John, G. B.; Sukhaswami, M. B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1105. (c) Ranganathan, D.; Haridas, V.; Madhusudanan, K. P.; Roy, R.; Nagaraj, R.; John, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 11578. (d) Ranganathan, D.; Haridas, V.; Kurur, S.; Nagaraj, R.; Bikshapathy, E.; Kunwar, A. C.; Sarma, A. V. S.; Vairamani, M. *J. Org. Chem.* **2000**, *65*, 365.

(21) We have recently demonstrated efficient templating of equilibrium mixures based on hydrazone and Pd-allyl chemistry. See: (a) Furlan, R. L. E.; Cousins, G. R. L.; Sanders. J. K. M. *Chem. Commun.* **2000**, 1761 and (b) Kaiser, G.; Sanders, J. K. M. *Chem. Commun.* **2000**, 1763, respectively.