ORGANIC LETTERS

2012
Vol. 14, No. 23
5816–5819

Two-Stage Amplification of Receptors Using a Multilevel Orthogonal/Simultaneous Dynamic Combinatorial Library

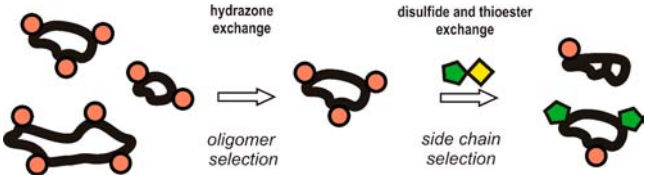
Andrea M. Escalante, A. Gastón Orrillo, Ignacio Cabezudo, and Ricardo L. E. Furlan*

Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina

rfurlan@fbioyf.unr.edu.ar

Received August 6, 2012

ABSTRACT



Sequential activation of different reversible exchange reactions in a dynamic combinatorial library allows directed exploration of the chemical space: initially a macrocyclic scaffold is selected by the template and finally side chain and conformational constrains are introduced into such a scaffold.

In the past decade, Dynamic Combinatorial Chemistry[1] has proven invaluable for the identification of novel binders for a variety of targets, impacting several fields such us materials science,[2] drug and fragrance delivery,[3] two phase transport,[4] biosensing,[5] and systems chemistry.[6]

Dynamic combinatorial libraries (DCLs) have become a powerful source of high affinity receptors with complex and, sometimes unexpected, structures.[7]

Most of the DCLs prepared to date have involved only one reversible exchange process; however, there are some examples where two exchange reactions are combined in the same system. Depending on the relative functional state of the exchange processes (activated/inactivated), the systems can be simultaneous or orthogonal. In simultaneous DCLs, both exchange reactions occur at the same time, as observed in the dynamic system based in thioesters and disulfides reported by Otto et al.[8] In orthogonal DCLs, each reversible reaction used can be activated under different conditions allowing their manipulation in a sequential order.[9] In previous work the groups of Otto,[10] Miller,[11] and ourselves[12] have described the combination of hydrazones and disulfides to prepare orthogonal DCLs.

(1) (a) Cougnon, F. B. L.; Sanders, J. K. M. *Acc. Chem. Res.* **2012**, ASAP article DOI 10.1021/ar200240m. (b) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J.-L.; Sanders, J. K. M.; Otto, S. *Chem. Rev.* **2006**, *106*, 3652.

(2) (a) aMoulin, E.; Cormos, G.; Giuseppone, N. *Chem. Soc. Rev.* **2012**, *41*, 1031. (b) Hirsch, A. K. H; Buhler, E.; Lehn, J.-M. *J. Am. Chem. Soc.* **2012**, *134*, 4177. (c) Folmer-Andersen, J. F.; Lehn, J.-M. *J. Am. Chem. Soc.* **2011**, *133*, 10966. (d) Ruff, Y.; Buhler, E.; Candau, S.-J.; Kesselman, E.; Talmon, Y.; Lehn, J.-M. *J. Am. Chem. Soc.* **2010**, *132*, 2573.

(3) (a) Buchs (née Levrand), B.; Fieber, W.; Vigouroux-Elie, F.; Sreenivasachary, N.; Lehn, J.-M.; Herrmann, A. *Org. Biomol. Chem.* **2011**, *9*, 2906.(b) Buchs (née Levrand), B.; Godin, G.; Trachsel, A.; de Saint Laumer, J.-Y.; Lehn, J.-M.; Herrmann, A. *Eur. J. Org. Chem.* **2011**, 681. (c) Herrmann, A. *Org. Biomol. Chem.* **2009**, *7*, 3195. (d) Herrmann, A.; Giuseppone, N.; Lehn, J.-M. *Chem.—Eur. J.* **2009**, *15*, 117. (e) West, K.; Otto, S. *Curr. Drug Discovery Technol.* **2005**, *2*, 123.

(4) (a) Pérez-Fernández, R.; Pittelkow, M.; Belenger, A. M.; Lane, L. A.; Robinson, C. V.; Sanders, J. K. M. *Chem. Commun.* **2009**, 3708. (b) Saggiomo, V.; Lüning, U. *Chem. Commun.* **2009**, 3711.

(5) (a) Otto, S.; Severin, K. *Top. Curr. Chem.* **2007**, *277*, 267. (b) Buryak, A.; Severin, K. *Angew. Chem., Int. Ed.* **2005**, *44*, 7935. (c) Rochat, S.; Gao, J.; Qian, X.; Zaubitzer, F.; Severin, K. *Chem.—Eur. J.* **2010**, *16*, 104. (d) Zaubitzer, F.; Riis-Johannessen, T.; Severin, K. *Org. Biomol. Chem.* **2009**, *7*, 4598. (e) Müller-Graff, P.-K.; Szelke, H.; Severin, K.; Krämer, R. *Org. Biomol. Chem.* **2010**, *8*, 2327.

(6) (a) Hunt, R. A. R.; Otto, S. *Chem. Commun.* **2011**, 847. (b) Ludlow, R. F.; Otto, S. *Chem. Soc. Rev.* **2008**, *37*, 101.

(7) Otto, S. *Acc. Chem. Res.* **2012**, ASAP article DOI 10.1021/ar200246j.

(8) Leclaire, J.; Vial, L.; Otto, S.; Sanders, J. K. M. *Chem. Commun.* **2005**, 1959.

(9) Goral, V.; Nelen, M. I.; Eliseev, A. V.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1347.

(10) Rodriguez-Docampo, Z.; Otto, S. *Chem. Commun.* **2008**, 5301.

(11) Gromova, A. V.; Ciszewski, J. M.; Miller, B. L. *Chem. Commun.* **2012**, *48*, 2131.

(12) Orrillo, A. G.; Escalante, A. M.; Furlan, R. L. E. *Chem. Commun.* **2008**, 5298.

10.1021/ol3027335
Published on Web 11/15/2012

Later on, we described the combination of hydrazones, disulfides, and thioesters to prepare multilevel DCLs wherein orthogonal and simultaneous levels of diversity coexist.[13] In such libraries each orthogonal reversible reaction can be used to explore independently its own dimension in structural space, opening the possibility for evolutionary approaches. In addition, incorporation of simultaneous exchange processes in a given orthogonal level increases the size of the diversity space explored by that level.

In this work we use such a multilevel DCL setup to build up diversity in a directed stepwise fashion.

Building block **1** is equipped with an acyl-hydrazide and a protected aldehyde. Both groups can react to give rise to a hydrazone-based DCL of macrocycles (Figure 1). In addition, the cysteine residue in **1** is connected via a disulfide bond to an adamantane group. Reduction of the disulfide produces thiols which, in turn, can engage in exchange reactions with thioesters and disulfides.

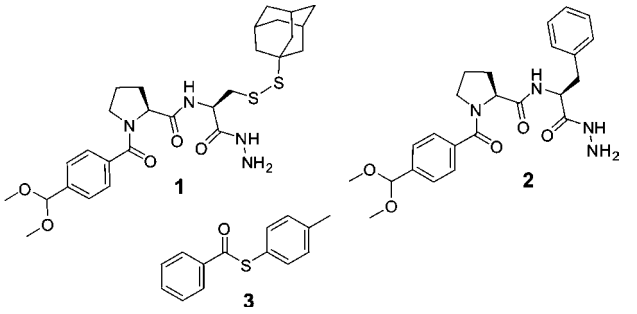


Figure 1. Building blocks **1**–**3**.

Building block **1** is inspired by a family of dipeptide building blocks developed by the Sanders group.[14] One of those building blocks, compound **2**, has been used in the preparation of dynamic systems that respond to the presence of several templates,[15] including alkaline metal salts.[16]

Acid-catalyzed cyclization of building block **1** generated a hydrazone-based DCL of macrocycles. The procedure for cyclization entailed stirring a solution of monomer **1** (0.75 mM) in $CHCl_3$/MeOH (98:2) containing TFA (30 mM) at room temperature. The mixture was analyzed by liquid chromatography coupled to electrospray mass spectrometry (LC-ESI-MS); we observed the presence of macrocyclic hydrazones formed by two and three units of **A** (Figure 2a). The equilibrium is reached within 6 days; at that time the area for the cyclic trimer **AAA** peak represented 59% of the total area for the library whereas the cyclic dimer **AA** peak area represented 41% of the total area.

Given the recognition properties of **2**, we have screened the library for its affinity for LiBr. Cyclization of **1** was carried out in the presence of LiBr (3.75 mM), and after 3 days, most of the DCL peptide material had converged to form **AAA** which then represents 99% of the total area of the DCL chromatogram (Figure 2b).

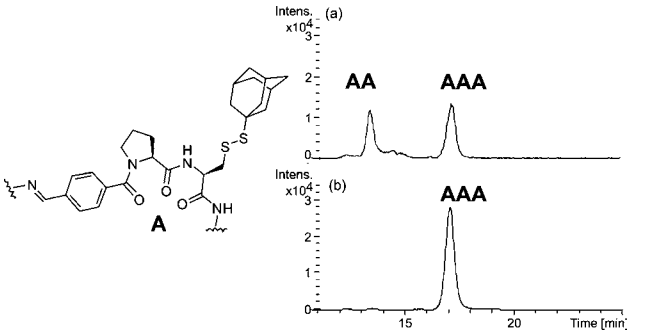


Figure 2. Chromatograms obtained by LC-MS of the DCL generated from **1** (0.75 mM) in $CHCl_3$/MeOH (98:2) and TFA (30 mM) in the absence (a) or in the presence (b) of LiBr (3.75 mM).

The hydrazone-based DCL in the presence of LiBr was scaled up and the trimer **AAA** was isolated by filtration of TFA through a basic resin[16a] (neutralization), followed by chromatography on silica gel. **AAA** was characterized by NMR, ESI-MS, and MS/MS experiments (Figure 3; see NMR data and Figure S3 in the Supporting Information).[17]

Once the macrocycle size was selected using hydrazone exchange, an orthogonal diversity level was used to explore side chains. This second level of diversity is orthogonal to the first level and includes two simultaneous exchange processes: disulfide and thioester exchange.

In principle, the locations of the disulfide bonds in **AAA** allow the exploration of a diversity of side chains without affecting the size of the central scaffold of **AAA**. With the aim of activating the disulfide–thioester exchange level, the disulfide bonds contained in **AAA** were reduced with DTT. The procedure entailed stirring in an open vial, a solution of **AAA** (0.25 mM), DTT (1.125 mM), and TEA (5 mM) in $CHCl_3$/MeOH (98:2). Thioester **3** (1.125 mM) was added (Figure 1), and the reaction mixture was split into two fractions. One control fraction was stirred without addition of further reagents, and the other fraction was treated with the template LiBr (1.25 mM). The assignment

(13) Escalante, A. M.; Orrillo, A. G.; Furlan, R. L. E. *J. Comb. Chem.* **2010**, *12*, 410.

(14) (a) Cousins, G. R. L.; Poulsen, S.-A.; Sanders, J. K. M. *Chem. Commun.* **1999**, 1575. (b) Furlan, R. L. E.; Cousins, G. R. L.; Sanders, J. K. M. *Chem. Commun.* **2000**, 1761. (c) Liu, J.; West, K. R.; Bondy, C. R.; Sanders, J. K. M. *Org. Biomol. Chem.* **2007**, *5*, 778. (d) Chung, M.-K.; Severin, K.; Lee, S. J.; Waters, M. L.; Gagné, M. R. *Chem. Sci.* **2011**, *2*, 744. (e) Chung, M.-K.; Hebling, C. M.; Jorgenson, J. W.; Severin, K.; Lee, S. J.; Gagné, M. R. *J. Am. Chem. Soc.* **2008**, *130*, 11819.

(15) (a) Lam, R. T. S.; Belenguer, A.; Roberts, S. L.; Naumann, C.; Jarrosson, T.; Otto, S.; Sanders, J. K. M. *Science* **2005**, *308*, 667. (b) Bulos, F.; Roberts, S. L.; Furlan, R. L. E.; Sanders, J. K. M. *Chem. Commun.* **2007**, 3092.

(16) (a) Furlan, R. L. E.; Ng, Y.-F.; Otto, S.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2001**, *123*, 8876. (b) Roberts, S. L.; Furlan, R. L. E.; Otto, S.; Sanders, J. K. M. *Org. Biomol. Chem.* **2003**, *1*, 1625.

(17) The isolation of trimer **AAA** was carried out in order to remove the template and to carry out its complete characterization; however it is not strictly necessary for the activation of the second diversity level that can be activated without removal of reagents as described in ref 13.

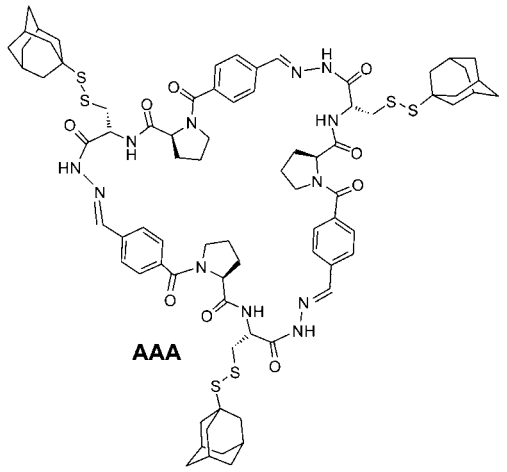


Figure 3. Molecular structure of **AAA** selected in the hydrazone exchange level.

of library members was carried out using LC-MS and HRMS experiments.

After 15 days, the control DCL showed **AAA** as the main component, together with at least five new macrocycles including thioesters, disulfide, and thiol groups (Figure 4a). In compounds **AAB** and **ABB** one or two adamantyl groups from parent homotrimer **AAA** have been exchanged with benzoyl moieties. Their peaks account for 30% of the total area of the DCL chromatogram. The peaks for three other library members account for 7% of the total area and include the fully reduced trithiol macrocycle **HHH**, one macrocycle equipped with a new disulfide moiety **AAT**, and one macrobicycle with an intramolecular disulfide bond **AS-S** (Figure 4a).

The DCL treated with LiBr showed a different composition (Figure 4b). The clear amplification of two macrocycles containing a benzoyl ester side chain was observed: a 3.3-fold increase in the area of the peak for **ABB**, that in the presence of template represents 44% of the total area of the DCL chromatogram, and a 19-fold increase in the area the peak of **BBB** that now represents 12%. The preference of LiBr for members containing benzoyl chains such as **ABB** and **BBB** suggests its coordination with carbonyls.

Additionally, one previously undetected compound was generated in the presence of the template (**HS-S**). HRMS experiments indicated that this compound contains one free thiol group and one intramolecular disulfide bond (Figure 5). Incorporation of an intramolecular disulfide bond in **HS-S** probably decreases the flexibility and introduces conformational changes through a strategy that is very commonly used in nature to control function.

Electrospray ionization mass spectrometry is an important analytical technique for identifying and characterizing

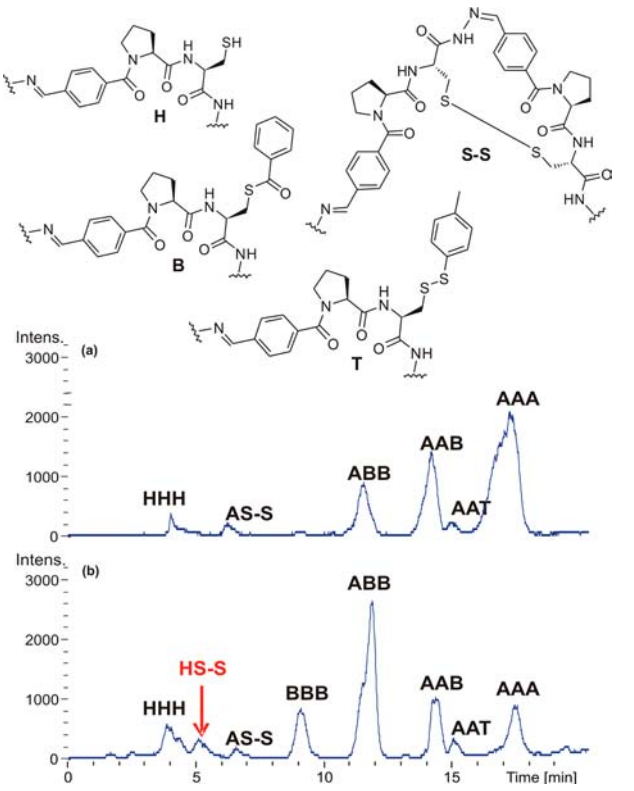


Figure 4. Chromatograms obtained by LC-MS of the exchange between **AAA** and **3** in the absence (a) or in the presence (b) of LiBr.

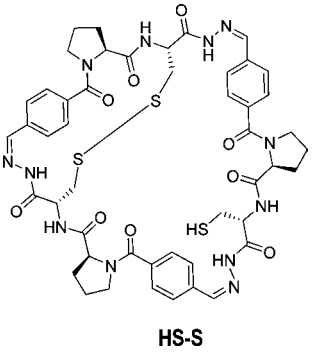


Figure 5. Molecular structure of the **HS-S** generated in the thiol-thioester level.

noncovalent interactions.[18] The mass spectrum of the templated library, acquired by direct infusion in the presence of LiBr, shows the singly charged adducts of each of the macrocycles **AAA**, **BBB**, **ABB**, and **HS-S**, with H^+, Na^+, K^+, or Li^+.[19] The relative proportion of the complexes indicated that they have different cation preferences

(18) (a) Adaligil, E.; Davis, B. D.; Hilmey, D. G.; Shen, Y.; Spruell, J. M.; Brodbelt, J. S.; Houk, K. N.; Paquette, L. A. *J. Org. Chem.* **2007**, *72*, 6215. (b) Paquette, L. A.; Selvaraj, P. R.; Keller, K. M.; Brodbelt, J. S. *Tetrahedron* **2005**, *61*, 231. (c) Williams, S. M.; Brodbelt, J. S.; Bartsch, R. A. *J. Am. Soc. Mass Spectrom.* **2003**, *14*, 1215.

(19) Although Na^+ and K^+ have not been added to the mixture, its presence is due to contamination of solvents, glass, and the spectrometer itself.

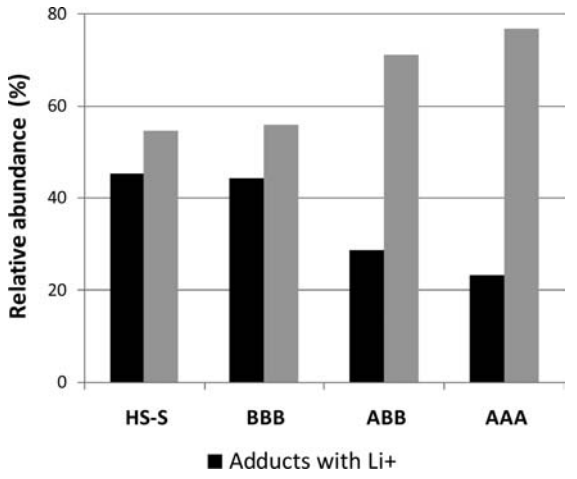


Figure 6. Relative abundance of the different macrocycle/Li^+ complexes for macrocycles **HS-S**, **BBB**, **ABB**, and **AAA** compared with the other adducts that include each of these macrocycles.

(Figure 6). The peak for complex **HS-S**:Li^+ represents 45% of the abundance of the peaks for the species that include the macrobicycle **HS-S**. Similarly, the peak for **BBB**:Li^+ represents 44% of the species that include **BBB**. In the case of macrocycle **ABB**, amplified to a lesser extent, the peak for its complex with Li^+ represents 29% of the total species that include this macrocycle. Finally, the peak for the complex **AAA**:Li^+ represents 23% of the total species that include the macrocycle **AAA**. These results suggest that the amplified receptors, particularly **HS-S** and **BBB**, possess a stronger preference for LiBr than its precursor **AAA**.

In summary, we have demonstrated how the diversity space can be explored sequentially using three different reversible exchange processes that can be activated orthogonally or simultaneously. Using this approach, hydrazone chemistry was initially used to select an appropriate macrocycle size for the template LiBr.[20] Finally, the simultaneous exchange of disulfides and thioesters was applied to build up diversity on the previously selected macrocycle. Diversity was increased by exchanging side chains through disulfide and thioester bonds and also changing the rigidity and conformation through the formation of an intramolecular disulfide. This multistep approach to dynamic combinatorial chemistry work could shed light in studying evolutionary processes.

Acknowledgment. This work was supported by CONICET (PIP 0796) and ANPCYT (PICT 0918). A.M.E. and R.L.E.F. are CONICET Researchers. We thank Prof. Jeremy K. M. Sanders from the University of Cambridge for his helpful comments on the manuscript.

Supporting Information Available. Materials and experimental procedures; LC data for preparation of libraries. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(20) Although coordination of Li^+ with carbnoyls has been proposed as in the case of related macrocycles (ref 16), LiBr forms a strong ion pair in $CHCl_3$. Therefore, it could be the ion pair that acts as the template.