A New Cyclic Pseudopeptide Receptor for Li^+ from a Dynamic Combinatorial Library

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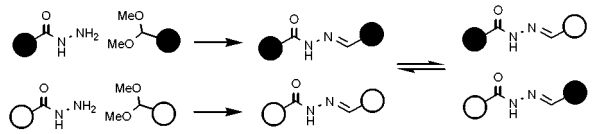
Dynamic combinatorial chemistry (DCC) has emerged as a new strategy for the discovery of novel host−guest systems.[1] In theory the dynamic combinatorial approach integrates preparation, identification, and high-yield isolation of receptors in one process.[2] To date very few examples exist where this is actually demonstrated in practice.[3,4] Herein we report one such example where Li^+ ions select an unpredictable cyclic pseudopeptide receptor from a small dynamic combinatorial library (DCL) of at least 10 different macrocycles. The cation converts this complex mixture into one that contains 98% of the Li^+ receptor. This is the first time that a new receptor has been identified and isolated from a DCL.

DCLs differ from conventional combinatorial libraries in that each library member is assembled from building blocks connected through *reversible* bonds. As a consequence, all members are interconverting to give a thermodynamically controlled product distribution. Addition of a guest molecule which binds and stabilizes one receptor in the library will increase the concentration of this receptor in the mixture at the expense of the other members.

Since our initial reports on the preparation of "living mixtures" of macrocycles,[5] research on DCLs has mainly focused on the development of chemistries that allow controlled reversible bond formation. Several reactions are now available that enable the formation of diverse DCLs under one set of conditions, whereas under other conditions the libraries become static (i.e., exchange is switched off) whereupon individual library members can be isolated.[6] Hence, research into DCLs is now entering its critical phase, where it has to be proven that the concept is actually practical.

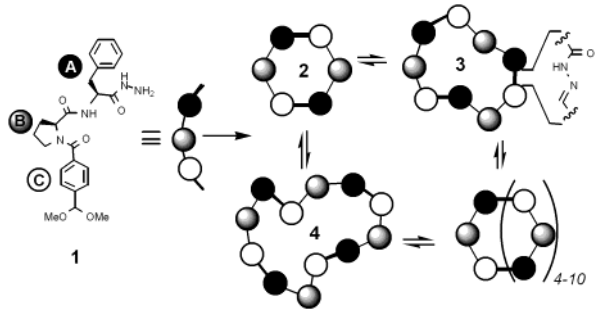
We have recently developed the use of hydrazone chemistry to prepare DCLs.[6f] By mixing hydrazides and protected aldehydes under acidic conditions, hydrazone formation occurs rapidly (Scheme 1). The presence of acid also ensures efficient exchange of the generated hydrazones. The resulting equilibrium mixture can be frozen upon neutralization of the reaction mixture, allowing for the isolation of individual library members.

Scheme 1



Using hydrazone exchange we have previously developed DCLs of macrocycles from building blocks that contain both aldehyde and hydrazide functionalities linked to a central amino acid or peptide unit.[6f] We focus here on the recognition properties of a library of macrocycles prepared from building block **1**, containing L-phenylalanine (A), L-proline (B), and an aromatic unit (C) (Scheme 2). This building block (i) has the potential to engage in hydrogen-bonding interactions, (ii) can offer Lewis-basic carbonyls to Lewis acids, and (iii) has aromatic rings for $\pi-\pi$ and cation-π interactions.

Scheme 2



Acid-catalyzed cyclization of building block **1** generated a DCL of macrocycles.[7] The mixture was analyzed by electrospray mass spectrometry (ESI-MS) and consisted of a series of macrocyclic polyhydrazones ranging from dimer to undecamer. Combination of ESI-MS and HPLC allowed the assignment of the major peaks in the chromatogram as shown in Figure 1a.

Given the range of potential recognition properties of **1** we have screened the library for its affinity for cations. When the dynamic library was exposed to different quaternary ammonium iodides,[8] no significant changes in the product distribution occurred. Likewise, addition of KI, RbI, and CsI did not affect the product distribution. However, upon addition of NaI a significant shift toward cyclic trimer was observed (Figure 1). Introduction of LiI into the reaction mixture generated the most dramatic response from the library. The amplified pseudononapeptide **3** now accounts for 98% of the peptide material in the library.[9] The selected trimer was isolated by successive filtration through a basic resin (neutralization) and silica gel.[10]

The interaction between Li^+ and the amplified trimer **3** was studied using ^1H NMR, ^{13}C NMR, ^7Li NMR, and FT-IR. Binding

(1) For reviews, see: (a) Lehn, J.-M.; Eliseev, A. V. *Science* **2001**, *291*, 2331. (b) Karan, C.; Miller, B. L. *Drug Discovery Today* **2000**, *5*, 67. (c) Cousins, G. R. L.; Poulsen, S.-A.; Sanders, J. K. M. *Curr. Opin. Chem. Biol.* **2000**, *4*, 270. (d) Klekota, B.; Miller, B. L. *Trends Biotechnol.* **1999**, *17*, 205.

(2) Ganesan, A.; *Angew. Chem., Int. Ed.* **1998**, *37*, 2828.

(3) Cousins, G. R. L.; Furlan, R. L. E.; Ng, Y.-F.; Redman, J. E.; Sanders, J. K. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 423.

(4) Amplification of several noncovalently assembled receptors has been reported. (a) Crego Calama, M.; Timmerman, P.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **2000**, *39*, 755. (b) Hof, F.; Nuckolls, C.; Rebek, J. *J. Am. Chem. Soc*. **2000**, *122*, 4251. (c) Albrecht, M.; Blau, O.; Fröhlich, R. *Chem. Eur. J*. **1999**, *5*, 48. (d) Hiraoka, S.; Fujita, M. *J. Am. Chem. Soc*. **1999**, *121*, 10239. (e) Huc, I.; Krische, M. J.; Funeriu, D. P.; Lehn, J.-M. *Eur. J. Inorg. Chem.* **1999**, 1415. Since the building blocks of these receptors are connected through weak noncovalent interactions, they have limited stability in absence of the guest.

(5) (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.

(6) Some suitable covalent reactions for the preparation of DCLs are the following: Olefin metathesis: (a) Giger, T.; Wigger, M.; Audètat, S.; Benner, S. A. *Synlett* **1998**, 688. Transimination: (b) Huc, I.; Lehn, J.-M. *Proc. Natl. Acad. Sci. U.S.A.*, **1997**, *94*, 2106. Oxime: (c) Nazarpack-Kandlousy, N.; Zweigenbaum, J.; Henion, J.; Eliseev, A. V. *J. Comb. Chem*. **1999**, *1*, 199. Disufide: (d) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *J. Am. Chem. Soc*. **2000**, *122*, 12063. (e) Ramström, O.; Lehn, J.-M. *ChemBioChem* **2000**, *1*, 41. Hydrazone exchange: (f) Cousins, G. R. L.; Poulsen, S.-A.; Sanders, J. K. M. *Chem. Commun.* **1999**, 1575.

(7) The general procedure for cyclization experiments entailed dissolution of monomer **1** (5 mM) in $CHCl_3$/MeOH (98:2) containing 1.5% of TFA at room temperature. The equilibrium is reached within 3 days. The amplification observed is achieved by addition of 0.4 equiv of the salt (with respect to **1**) either when the reaction is started or after it has reached equilibrium.

(8) The quaternary ammonium salts used were: Me_4NI, Et_4NI, Bu_4NI, $BnMe_3NI$, $BnEt_3NI$, and $BnBu_3NI$.

(9) The same experiment carried out in 100% chloroform, adding excess of either LiCl, LiBr, or LiI (with respect to **1**) leads to quantitative formation of the amplified trimer **3** allowing for its isolation on a 20 mg scale.

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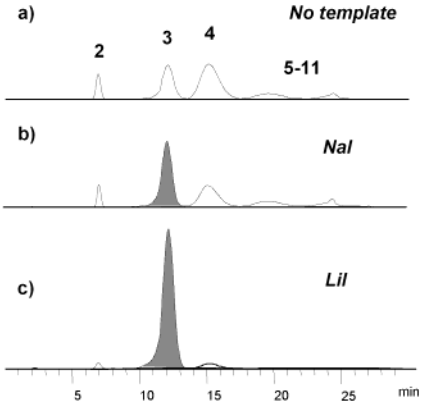

Figure 1. HPLC traces of the library (a) control without template, (b) in the presence of NaI, (c) in the presence of LiI.

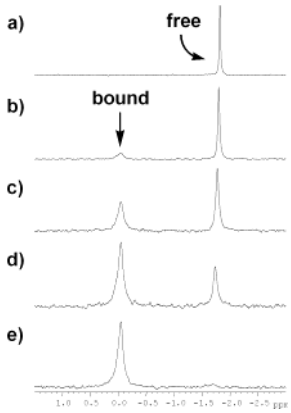

Figure 2. 155.5 MHz ^{7}Li NMR spectra of a 5 mM solution of LiI in $CDCl_3$/MeOD (98:2): (a) in absence; (b) in the presence of 0.25 equiv; (c) 0.50 equiv; (d) 0.75 equiv; and (e) 1.00 equiv of **3**.

of lithium to the trimer was found to be slow on the NMR time scale, giving rise to separate signals for bound and free Li^+ (Figure 2). A titration experiment established that lithium binds to the trimer in a 1:1 stoichiometry.

The ^{1}H NMR spectrum of the trimer[11] was strongly affected by the presence of Li^+. Addition of 1 equiv of LiI to a 5 mM solution of **3** in $CDCl_3$/MeOD (98:2) produces significant shifts in every resonance of the receptor (Figure 3). This indicates that there is a substantial change in the geometry of the trimer upon binding to Li^+; hence, the receptor is *not* pre-organized. Apparently the guest selects one conformer out of the many possible. From the change in the chemical shifts in the spectrum an equilibrium constant for binding of Li^+ to the trimer of 4×10^4 M^{-1} was estimated.

It has been reported that lithium salts can alter the conformational properties of peptides by coordination to carbonyls.[12] A similar interaction is suggested in our system by a shift in the

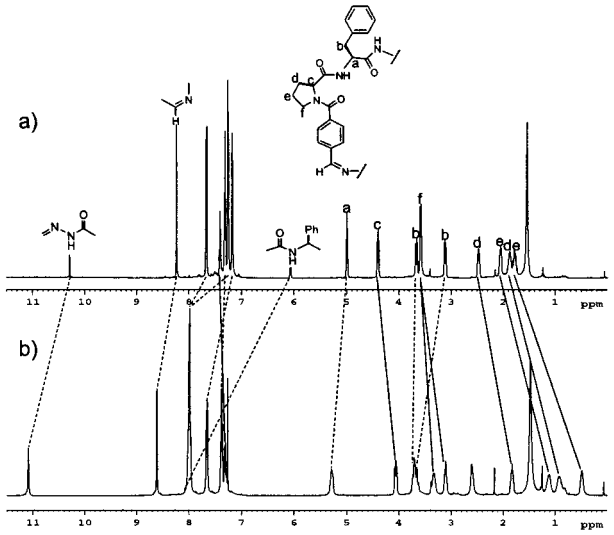

Figure 3. 500 MHz ^{1}H NMR spectra of **3** in $CDCl_3$/MeOD (98:2). (a) In absence of guest, (b) in the presence of 1 equiv of LiI.

carbonyl-stretching frequency in the FT-IR spectrum from 1684 to 1666 cm^{-1} after complexation with LiI. In addition, the ^{13}C NMR spectrum of **3** shows downfield shifts of 1.0, 4.5, and 4.5 ppm for the signals corresponding to the carbonyl carbons of the proline and phenylalanine units,[13] and to the hydrazone carbon (C═N), respectively.

In summary, we have described a one-pot synthesis, identification, and isolation of a new receptor for Li^+ from a dynamic combinatorial library. The receptor is rather flexible and changes its conformation upon binding. Such flexible receptors, although widespread in nature, are still extremely difficult to create by design. In fact, despite a large amount of data on the **3**·Li^+ complex, we still do not know its exact conformation. Hence, **3** would not have been discovered through design. This illustrates the potential of the dynamic combinatorial approach for the discovery of novel conformationally flexible host (or guest) molecules. We are currently studying the recognition properties of DCLs prepared from different building blocks related to **1** in order to identify receptors for other cations.

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Supporting Information Available: QQQ ESI-MS procedures and data for the DCL before and after addition of LiI; HMBC and FT-IR spectra of **3** and the **3**·Li complex showing the shift in the carbonyl signals; HPLC traces of the Li^+ templated library in chloroform at different times (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) To eliminate the possibility that the amplification observed is only the consequence of an acceleration in the formation of a thermodynamically more favorable macrocycle, the reaction was reinitiated by addition of TFA, and **3** was recycled to regenerate all the other macrocycles in their original relative concentrations.

(11) The ^{1}H NMR spectrum of template-free receptor **3** in $CDCl_3$:MeOD (98:2) is consistent with averaged C_3-symmetric conformations. Since the spectrum is not significantly affected by varying the concentration in the range 5.0−0.5 mM, intermolecular association of the receptor under these conditions is improbable.

(12) (a) Boger, D. L.; Patane, M. A.; Zhou, J. *J. Am. Chem. Soc*. **1995**, *117*, 7357. (b) Seebach, D.; Bossler, H. G.; Flowers, R.; Arnett, E. M.; *Helv. Chim. Acta* **1994**, *77*, 291. (c) Seebach, D.; Beck, A. K.; Bossler, H. G.; Gerber, C.; Ko, S. Y.; Murtiashaw, C. W.; Naef, R.; Shoda, S.; Thaler, A.; Krieger, M.; Wenger, R. *Helv. Chim. Acta* **1993**, *76*, 1564. (d) Köck, M.; Kessler, H.; Seebach, D.; Thaler, A. *J. Am. Chem. Soc*. **1992**, *114*, 2676. (e) Kessler, H.; Hehlein, W.; Schuck, R. *J. Am. Chem. Soc*. **1982**, *104*, 4534.

(13) The signals corresponding to these two carbonyl carbons are also split, which could be the result of either a lack of C_3-symmetry detected only by those carbonyls or the formation of two diastereomers as a consequence of the chiral ligand coordinating to Li^+ in an octahedral way.