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Molecular amplification in a dynamic combinatorial library using non-covalent interactions

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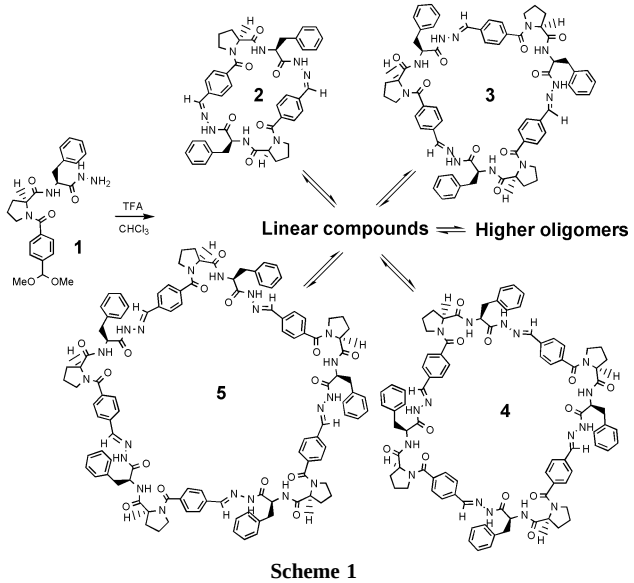
A trace component within a dynamic combinatorial library of pseudo-peptide hydrazones has been dramatically amplified in a reversible manner through recognition by [18]crown-6.

Dynamic combinatorial libraries (DCLs) have attracted great interest because of their potential for the identification of new catalysts, enzyme inhibitors and host–guest systems.[1] Their successful implementation will hinge on our ability to effect control over the library composition using non-covalent interactions with a template: because of the dynamic equilibria established in a DCL, the stabilisation of any given member by molecular recognition will lead to its amplification by virtue of the Le Chatelier principle. In principle, even species which are present in only trace quantities should be able to be amplified, but since our original proposal of thermodynamic templating of 'living' mixtures in 1996,[2] only small changes in the product distribution of covalent DCLs induced by templates have been reported.[3,4] We now report that recognition by [18]crown-6 leads to dramatic amplification of a trace component within a pseudo-peptide DCL; we also show that this amplification is controllable and reversible.

Our group initially showed that DCLs generated by methoxide-catalysed transesterification of steroidal macrocycles can be influenced by the presence of NaI,[3b] but more recently we have sought to generate libraries using chemistry which offers exchange under milder conditions more suitable for supramolecular recognition such as transimination[5,6] and palladium-catalysed allyl transesterification.[7] A DCL of pseudo-peptide hydrazones can be prepared in chloroform from monomer **1** (5 mM) using TFA as catalyst (Scheme 1).[5a] Analysis of the library using ESI-MS† revealed the presence of 10 macrocyclic compounds ranging from dimer to undecamer. These major macrocyclic products must interconvert *via* at least 10 linear hydrazide species which are present in the DCL but at concentrations too low for detection by HPLC or ESI-MS. HPLC analysis of the library after 24 h showed as major products the dimer **2**, trimer **3**, tetramer **4**, pentamer **5** and higher cyclic oligomers (Fig. 1a). The products were identified using LC-MS.‡



Scheme 1

Unlike the cyclic members, the linear species present in the DCL contain a free hydrazide functionality, which under the acidic conditions used is likely to be protonated. As the affinity of [18]crown-6 for primary alkylammonium[8] and hydrazinium[9] ions is well known, similar behaviour could be expected for the closely related protonated hydrazides present in our system. Introduction of 4 eq. of [18]crown-6 to the reaction mixture shifted the equilibrium by formation of a hydrazide–crown ether complex. HPLC analysis showed a decrease in the concentration of the larger macrocycles and the presence of a new peak corresponding to **6** with a retention time of 3 min (Fig. 1b). At equilibrium, **6**, previously present in an undetectable amount, was the major component of the library (67%).§ As a control experiment, the library was prepared in the presence of [18]crown-6 by treating a solution of **1** and the crown ether with TFA, and the same distribution of products was achieved.

Compound **6** cannot be isolated as it reacts with itself to form hydrazones, but its UV spectrum was recorded using the diode array facility of the HPLC; it does not show the characteristic 288 nm absorption of the cyclic aromatic hydrazones but a band at 257 nm is observed which corresponds well with the spectrum of the model aldehyde **7**.¶ The ESI mass spectrum of the reaction mixture (Fig. 2) was dominated by a major peak at 673.4 corresponding to the **6**·[18]crown-6·H^+ complex, accompanied by small peaks for **6**H^+(409.4), cyclic dimer **2**H^+(781.6) and trimer **3**H^+(1171.7), as well as the complex of the protonated linear dimer **8** with [18]crown-6 (1063.5).

As the DCL contains a complex network of equilibria, it is important to show that kinetic traps have not been introduced

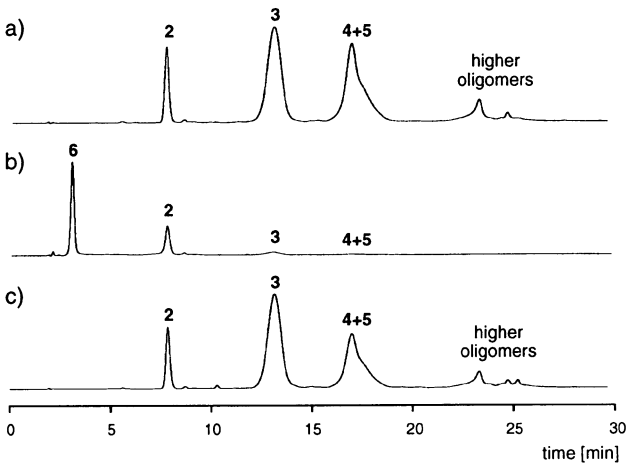


Fig. 1 (a) Chromatogram of the DCL at 24 h, (b) chromatogram of the DCL after addition of 18-crown-6, (c) chromatogram of the DCL showing the regeneration of the cyclic products by addition of 3 eq. of KBr with respect to the amount of [18]crown-6.

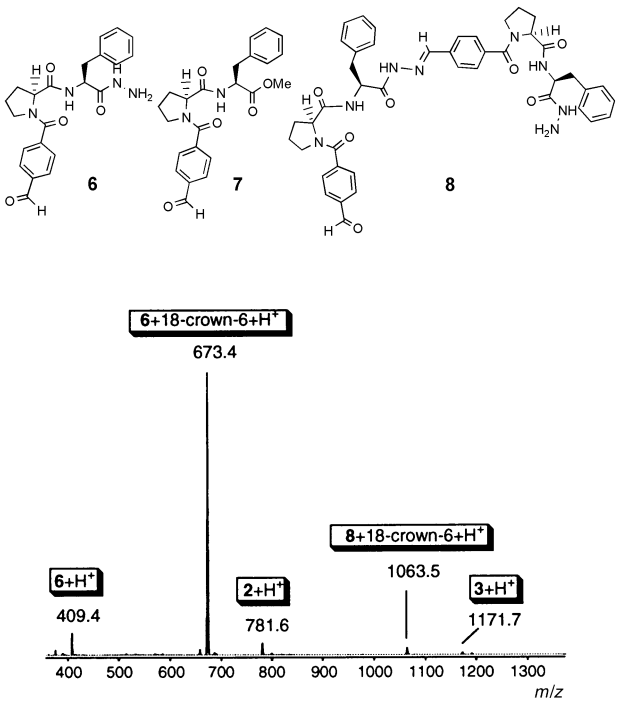


Fig. 2 ESI-MS of the library in presence of 4 eq. of [18]crown-6.

into the system. When KBr was added as a competitive guest that strongly interacts specifically with the crown ether, the original cyclic products were regenerated (Fig. 1c). In a control experiment, the same amount of KBr was added to the library in the absence of crown ether and no change in the composition was observed over 2 days.

Any variation in the strength or extent of hydrazide–crown ether binding should result in a corresponding change in the library composition. When the amount of added [18]crown-6 was varied in the range 0.4–5.0 eq., the proportion of peptide material present as **6** similarly ranged from 20–74%. When different crown ethers were introduced to the library the observed response from the system was related to the size of the ring. Within the series of unsubstituted crown ethers the amount of **6** increases with the size of the crown ether up to [18]crown-6; and in the series of the dibenzo crown ethers, the yield of **6** decreased with crown ethers larger than dibenzo [18]crown-6 (Fig. 3). These results are likely to be related to the affinity of the protonated hydrazide for the crown ethers. It is well known that [18]crown-6 possess the right size and shape to bind primary ammonium cations through three-point hydrogen bonds.[10]

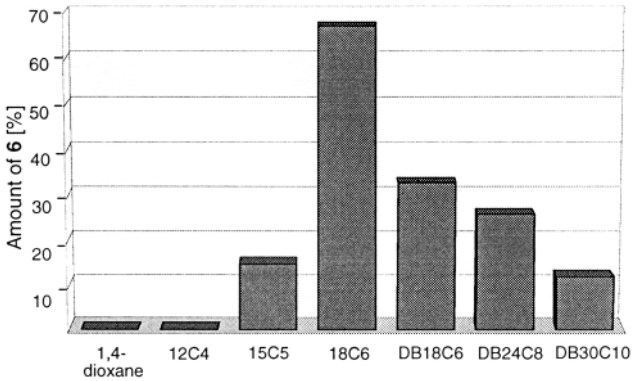


Fig. 3 Amount of **6** in the library when 4 eq. of differents crown ethers were added. 12C4 = [12]crown-4; 15C5 = [15]crown-5; 18C6 = [18]crown-6; DB18C6 = dibenzo [18]crown-6; DB24C8 = dibenzo [24]crown-8; DB30C10 = dibenzo [30]crown-10.

In summary, we have demonstrated that non-covalent interactions between a protonated hydrazide and [18]crown-6 can be used to generate a strong response from a hydrazone-based DCL. The response consists in the amplification of a member previously present in trace amounts to become the major constituent. That increase in concentration is achieved through the preferential consumption of all the unselected cyclic products. The amplification can be reversed by adding a competitive guest that specifically interacts with the crown ether, leading to the regeneration of the original macrocyclic products. To the best of our knowledge, this is the first example of a very substantial amplification in a covalent DCL.

The interaction between protonated hydrazide and crown ether can also be regarded as a *non-covalent* protection of the hydrazide, competing efficiently with the reaction between the hydrazide and an aldehyde to form new *covalent* bonds. Further exploration of the concept of non-covalent protection is under investigation in our laboratory.

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Notes and references

† Electrospray mass spectra were recorded on a Micromass Quattro-LC triple quadrupole apparatus fitted with a z-spray electrospray source. The electrospray source was heated to 100 °C and the sampling cone voltage was 65 V. Samples were introduced into the mass spectrometer source with an LC pump (Shimadzu LC-9A LC pump) at a rate of 4 μL min^{-1} of MeCN–H_2O (1:1).

‡ HPLC analysis was carried out using a Hewlett-Packard 1050 instrument, coupled to a HP 1050 DAD; data were analysed using HP ChemStation. LC-MS was carried out using an identical HPLC system containing a solvent splitter which diverted 1% of the eluant to a Micromass Platform MS operating in simultaneous positive and negative electrospray modes with a cone voltage of 30 V. The UV and MS data sets were analysed using a MassLynx software suite. Reverse phase HPLC separations were carried out using a 15 cm × 4.6 mm i.d. 3 μM particle size, Supelco ABZ+ C16 alkylamide column using acetonitrile and water gradients.

§ As the extinction coefficient of the new compound is different from that of a hydrazone, its concentration was indirectly determined by the absolute loss of signal intensity belonging to the macrocycles. Loss of hydrazone intensity was directly related to the increase in the signal due to the peak at 3 min.

¶ Aldehyde **7** was prepared from carboxybenzaldehyde dimethoxy acetal, Cbz-proline and phenylalanine methyl ester using standard peptide chemistry. Removal of the dimethoxy acetal was achieved with TFA.

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