Engineering diversity into dynamic combinatorial libraries by use of a small flexible building block

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Oligomerization of preorganized cinchona and xanthene building blocks to produce dynamic combinatorial libraries results in libraries with only a few components. Diversity is generated by the addition of semi-flexible ephedrine and cholate building blocks.

We report here a significant step forward in the generation of diverse macrocyclic dynamic combinatorial libraries (DCL); such libraries provide a potential 'selection' approach to biomimetic catalysis that is conceptually related to molecularly imprinted polymers,[1] catalytic antibodies and ribozymes.[2] A combinatorial library consists of many members ($\mathbf{M_1} \cdots \mathbf{M_n}$), each of which contains two or more building blocks (*A*, *B*, *C*, $\cdots$) arranged in a particular way. In a traditional combinatorial library[3] the covalent bonds between building blocks are fixed using irreversible chemistry during synthesis so there can be no post-synthetic interconversion between members such as *ABC* and *ACB*. In a dynamic combinatorial library the connections between building blocks are reversible and in flux, continuously being made and broken; these connections may be covalent bonds such as imine,[4] ester,[5–7] disulfide,[8] borate[9] or alkene linkages, or they may be non-covalent, utilizing metal–ligand[10] or hydrogen bonding[11] interactions. The composition of a dynamic library will be dependent on its environment (Fig. 1): addition of a template $\mathbf{T_1}$ that selectively binds one member will bias the equilibrium towards that member, while a different template $\mathbf{T_2}$ will bias the composition in a different direction.

Two complementary types of DCL have been proposed: libraries of small ligands for large receptors [Fig. 2(a)],[3] and libraries of large receptors for small ligands [Fig. 2(b)].[6–10,12] In practice, however, no dynamic libraries of large receptors have yet been reported to contain more than a very few members. We now show why attempts to create dynamic libraries with relatively rigid building blocks can easily fail, giving self-sorted homo-oligomers rather than the desired diversity,[13] and demonstrate that the use of small semi-flexible building blocks induces precisely the kind of mixing and diversity required.

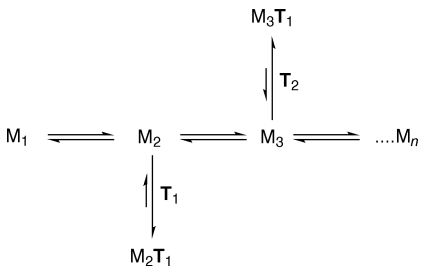

Fig. 1 Changing the equilibrium in a dynamic combinatorial library of members $\mathbf{M_1} \cdots \mathbf{M_n}$ using thermodynamic templates $\mathbf{T_m}$

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DCLs in this laboratory are currently produced using base-catalysed transesterification of the building block monomers shown in Fig. 3. Building blocks **1**–**7** possess recognition features and spectroscopic tags as part of an overall concave geometry that encourages macrocyclization; each also carries a hydroxymethylester functionality for transesterification. **1**, **2** and **6** have been described before[5,13] while the new monomers **3**–**5** were prepared by analogous methods. The new ephedrine-derived monomer **7** is discussed in more detail below. As reported earlier,[5,7] the quinine- and cinchonidine-derived monomers **1** (HO—**Cq**—OMe and HO—**Cc**—OMe, respectively) yielded cyclic trimers $\mathbf{Cq_3}$ or $\mathbf{Cc_3}$ in more than 95% yield when subjected to transesterification at 5 mM concentration. In order to explore the effect of a radically different shape within the context of otherwise identical chemistry and components, we prepared the diastereomeric quinidine-derived monomer **3** (HO—**Cd**—OMe); this yields, under the same reaction conditions, more than 95% cyclic dimer $\mathbf{Cd_2}$. Addition of an extension arm to give **2** (HO—**Ce**—OMe) and **4** (HO—**Ca**—OMe) would be expected to increase flexibility and so to expand the range of cyclic oligomers produced at equilibrium. This is indeed what is observed for **2**,[5] but to our surprise the new extended quinidine **4** still cyclized to more than 90% dimer. These results are summarized in Table 1.

When mixtures of two of the alkaloid building blocks **1**–**4** were subjected to transesterification, the outcome ranged from effective mixing (*e.g.*, **1** and **2**) to almost perfect 'self-sorting' (Table 1). The two most striking examples of self-sorting are **3** and **4**, which yielded around 90% of homo-dimers when transesterified together, and **1b** and **3**, which gave almost exclusively a mixture of $\mathbf{Cc_3}$ and $\mathbf{Cd_2}$. Such self-sorting—which occurs despite the fact that many mixed intermediates must be

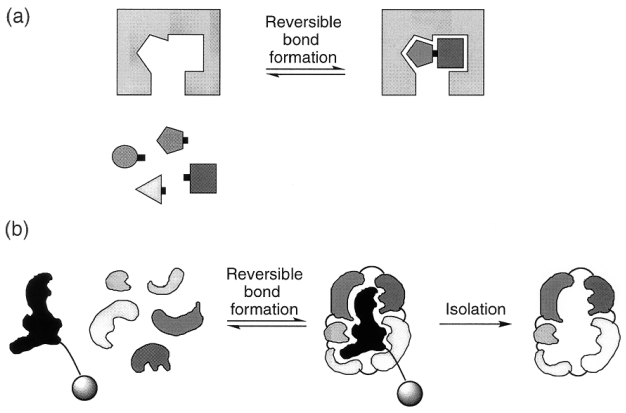

Fig. 2 The use of dynamic combinatorial libraries to generate (a) the optimum ligand for a large biological receptor and (b) the optimum receptor for a tethered ligand

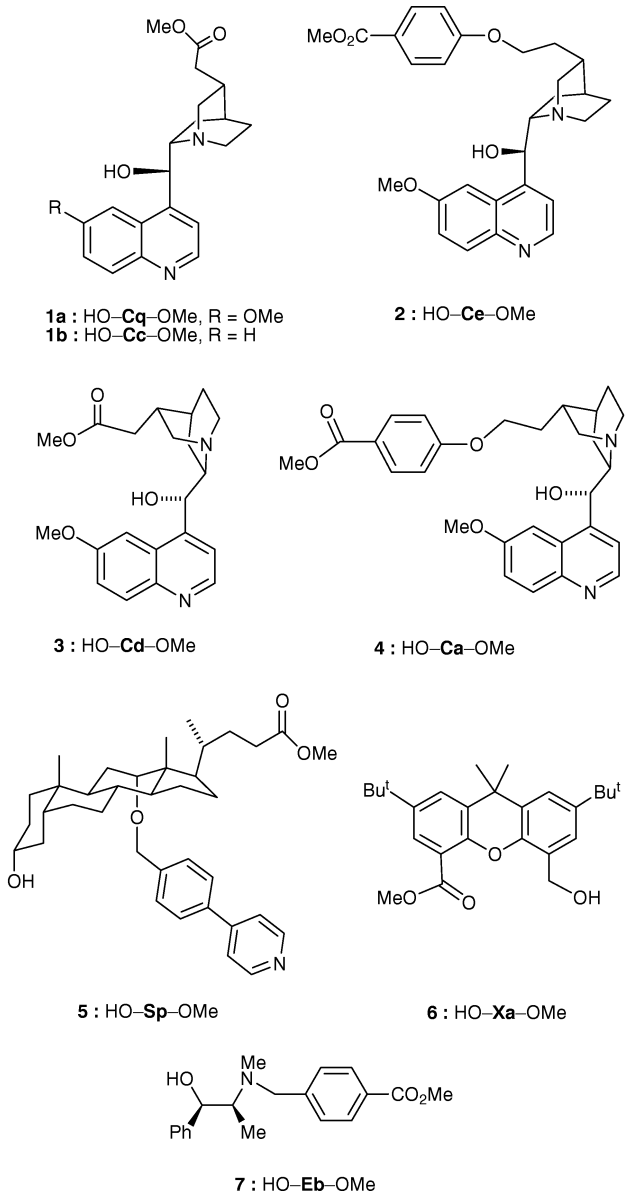

Fig. 3 Building block monomers explored for potential production of dynamic combinatorial libraries. Each monomer is identified by a unique two-letter code and with HO/OMe labels to indicate that this is the hydroxymethylester series

formed in the course of the reaction—is a unique property of thermodynamically controlled chemistry.[14] Some of its origin can be understood by considering the reaction between two monomers of the same shape but different 'bite size' [Fig. 4(a)]: the heterodimer will exhibit some bond or angle strain [Fig. 4(b)] and so will be destabilized relative to the self-sorted homo-dimers [Fig. 4(c)]. Because all the reactions are

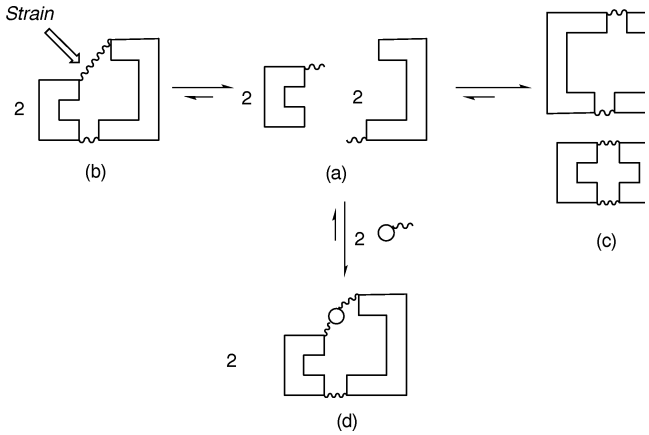

Fig. 4 How incompatible 'bite size' can lead to self-sorting of building blocks in thermodynamically controlled chemistry: (a) a mixture of large and small building blocks, (b) mixed dimer exhibiting bond and/or angle strain, (c) self-sorted mixture of strain-free dimers and (d) the use of a small diversifier to facilitate formation of mixed oligomers

reversible, the product distribution will move to minimize the concentration of strained (*i.e.*, mixed) products.[15] An argument based on bite angle or relative conformational stability of the monomer once incorporated in a cyclic oligomer, which could lead to trimers or tetramers, would be just as effective, and it seems likely that the self-sorting observed here is due to a combination of size, angle and conformational effects. Of course, two complementary monomers might lead efficiently to a single hetero-dimer but that does not improve diversity in any general way.

One way to increase diversity and avoid self-sorting would be to use only small and very flexible building blocks but the likely cavity collapse that would result might minimize substrate binding and selectivity. We chose, therefore, to explore the effect of adding a small, semi-flexible building block to mixtures of the larger monomers: we reasoned that the smaller unit should act *in situ* as a diversifier to facilitate mixing of monomers with incompatible bite sizes, [Fig. 4(d)]. This approach should allow fine-tuning of macrocyclic cavity size while at the same time giving access to relatively small oligomers containing large monomers in low-energy, unstrained conformations. (1*R*, 2*S*)-(–)-Ephedrine adopts a similar conformation to quinine,[16] but is more flexible; in order to impose some rigidity and give the required hydroxymethylester functionality, ephedrine was refluxed with methyl bromomethyl benzoate in acetonitrile to produce the new monomer **7** (HO—**Eb**—OMe).[17] When cyclized alone at 5 mM under thermodynamic conditions, **7** produced a distribution of macrocycles, from the cyclic dimer $\mathbf{Eb_2}$ through to the cyclic heptamer $\mathbf{Eb_7}$, confirming that the monomer is relaxed in the desired way.

When the quinine monomer **1a** and ephedrine monomer **7** were subjected to transesterification together, we obtained a library of macrocycles that included all possible compositions

Table 1 Outcome of transesterification reactions of the alkaloids **1**–**4**

	HO—**Cc**—OMe	HO—**Ce**—OMe	HO—**Cd**—OMe	HO—**Ca**—OMe
HO—**Cc**—OMe Quinine	>95% trimer	Good mixing	Self-sorting	Good mixing
HO—**Ce**—OMe Extended quinine		Dimer < trimer >tetramer	Mainly dimers, including mixed	—
HO—**Cd**—OMe Quinidine			>95% dimer	Self-sorting, <5% mixing
HO—**Ca**—OMe Extended quinidine				>90% dimer

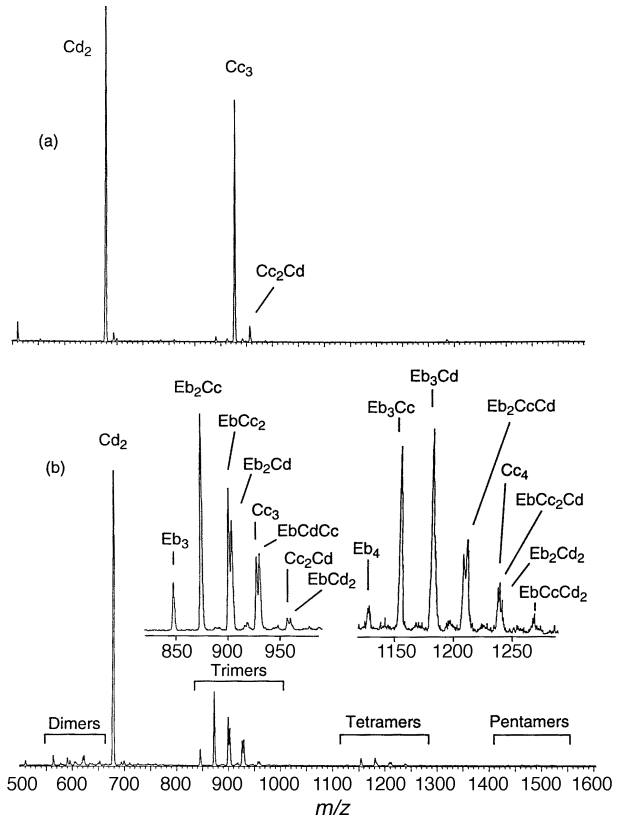

Fig. 5 Part of the ESI-MS spectra of the reaction products resulting from two transesterification reactions. In both cases the dominant $\mathbf{Cd_2}$ peak has been truncated. (a) A mixture of cinchonidine **1b** and quinidine **3** monomers at initial concentrations of 5 mM each: the only visible mixed product is a trace of $\mathbf{Cc_2Cd}$. (b) The same monomers together with 5 mM ephedrine monomer **7**. Insets show the trimer and tetramer region of the spectrum, expanded for clarity

of mixed cyclic dimers, trimers, tetramers and even some mixed pentamers.[18] A more severe test was the effect of **7** on the transesterification of a mixture of the cinchonidine[19] **1b** and quinidine **3** monomers, which self-sort as described above and as illustrated in Fig. 5(a). The effect of **7**, as observed by

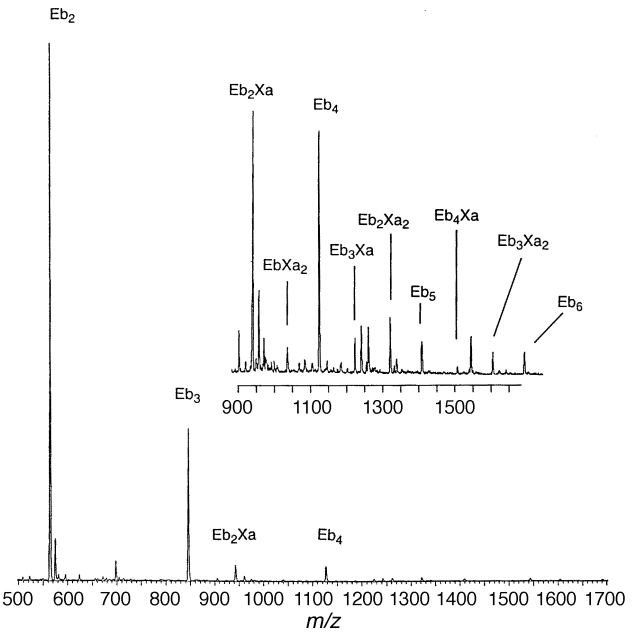

Fig. 6 Part of the ESI-MS spectrum of the reaction products resulting from transesterification of 2.5 mM xanthene monomer **6** with 2.5 mM ephedrine monomer **7**. The high-mass region is expanded to allow labelling of key peaks

ESI-MS [electrospray ionization mass spectrometry, Fig. 5(b)], is a library of cyclic oligomers that ranges up to pentamers. Both ESI-MS and high pressure liquid chromatography indicate that $\mathbf{Cd_2}$ is still the most favoured product, but quinidine–ephedrine and cinchonidine–ephedrine conjugates and, most importantly, mixed macrocycles containing all three monomers (*e.g.*, **EbCcCd**, $\mathbf{Eb_2CcCd}$, $\mathbf{EbCc_2Cd}$, $\mathbf{Eb_2CcCd}$) are obtained; not surprisingly perhaps, the most abundant pentamers (not shown) contain relatively large proportions of the ephedrine monomer.

The effect of ephedrine monomer **7** on a pair of components that already mix well, for example, the quinine and extended quinine monomers **1a** and **2**, was to produce a large library that contains all possible trimers and some of the possible tetramers. The almost complete absence of pentamers suggests that the lower mixed oligomers are more stable than they are in the **CcCdEb** mixed reaction. This should not be surprising: in the absence of any strain, entropy arguments tend to favour formation of small oligomers because that leads to the largest number of independent molecules,[20] and the use of flexible building blocks inevitably reduces strain. When four different monomers (**1a**, **1b**, **5** and **7**) were subjected to transesterification together the result was a library containing over 30 different compositions of species, many of which undoubtedly disguise the presence of several different isomers.[21] All mixed cyclic trimer compositions are observed along with some tetramers; the most abundant cyclic tetramer observed by ESI-MS is the statistically most favourable composition containing all four monomers, **EbCcCqSp**.

The most severe test of **7** as a diversifier was the xanthene monomer **6** (HO—**Xa**—OMe): this monomer is so strongly pre-organized to give cyclic dimers that it self-sorts almost exclusively, even in the presence of the extended quinine **2** and cholate **5**. However, when **6** and **7** were transesterified together, small but significant quantities of mixed trimers and tetramers were obtained (Fig. 6). It is important to note that for a dynamic combinatorial library to be effective in the sense implied by Figs. 1 and 2 it is not necessary for all members to be formed in equal, or even comparable, quantities: we need only to know that a receptor is thermodynamically accessible, that is, formed in trace quantities. If it is selected by thermodynamic templating, then its concentration will increase and it will be possible to isolate and identify it.

Absolute quantititative conclusions cannot be drawn from the mass spectra shown in Figs. 5 and 6 because the intrinsic detectability of each species depends on its component building blocks, on its degree of oligomerization and on the presence of multiply charged ions; however, relative intensities within different spectra containing the same components do correlate directly with relative concentrations.[22] The larger proportion of $\mathbf{Eb_3}$ relative to $\mathbf{Eb_2}$ visible in Fig. 5(b) by comparison with Fig. 6 is consistent with the higher total monomer concentration used in the three-component experiment: larger oligomers are favoured by higher concentrations.

In summary, we have shown (*i*) why the counter-intuitive phenomenon of covalent self-sorting is common when pre-organized building blocks are subjected to thermodynamically equilibrating conditions[23] and (*ii*) that a small flexible building block can facilitate mixing and produce diversity in dynamic combinatorial libraries. We now have to turn to the more challenging task of selecting interesting members of such libraries through the use of tethered templates.

Experimental

Deoxycholate **7** was prepared similarly to the *p*-phenylbenzyl derivative[6] except that *p*-bromobenzyl-2,2,2-trichloroacetimidate was used instead of *p*-phenylbenzyl-2,2,2-trichloroacetimidate. The *p*-bromobenzyl cholate ether was then subjected to a Stille coupling using 4-trimethylstannyl pyri-

dine and deprotected as before.[6] All new monomers were characterized by ^{1}H and ^{13}C NMR and accurate FAB-MS (fast atom bombardment). Transesterification conditions and preparation of samples for ESI-MS were as described before.[7]

Monitoring of combinatorial libraries was mainly carried out using positive-ion ESI mass spectrometry. Spectra were obtained on a VG BioQ triple quadrupole apparatus with a m/z range up to 4000 (VG Bio Tech Ltd, Altrincham, UK). The electrospray source was heated to 70 °C, with 80 V sampling cone voltage (V_c). The samples were introduced into the mass spectrometer source with an LC pump (Shimadzu LC-9A LC pump) at a flow rate of 4 μL min^{-1} of acetonitrile–water (1 : 1). The data system was operated as a multichannel analyzer and several scans were summed to obtain the final spectrum.

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