Keywords: alkynes · cyclophanes · fullerenes · mass spectrometry

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Strategies for the Dynamic Integration of Combinatorial Synthesis and Screening[**]

Arasu Ganesan[*]

The generation and screening of large populations is a familiar concept in biology. It is central to recombinant DNA techniques and occurs in nature during evolution. Such approaches are only now being truly appreciated by chemists. Combinatorial organic synthesis, linked to high-throughput screening, has become an important part of the drug discovery process.[1] Areas such as materials science, catalysis, and engineered biosynthesis are also increasingly influenced by combinatorial ways of thinking.[2] Traditionally, library synthesis and screening are two separate activities which are performed sequentially. Here, recent examples are highlighted where the composition of a library undergoes fluid and dynamic change during selection for a desired property.[3]

This idea originated in the field of molecular recognition. When a metal cation is mixed with a set of ligands, a large number of complexes is possible. However, under equilibrating conditions, the actual composition is dictated by thermodynamic stability.[4] The best binder can be identified simply by determination of the predominant component without the need to test each ligand individually. Lehn et al.[5] have described this approach as a virtual combinatorial library.[6]

The Sanders group has used transesterification as a route to combinatorial libraries. For example, the steroidal hydroxy ester **1** (Scheme 1) was mainly converted into the macrocyclic trimer, tetramer, and pentamer (83:12:5 respectively, by mass percentage) upon heating.[7] When the experiment was repeated with an equimolar amount of NaI, the equilibrium

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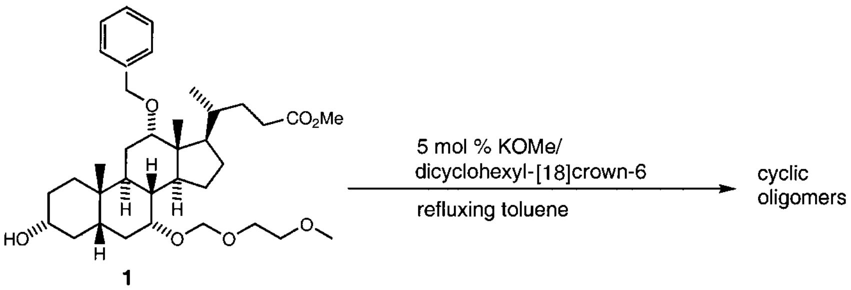
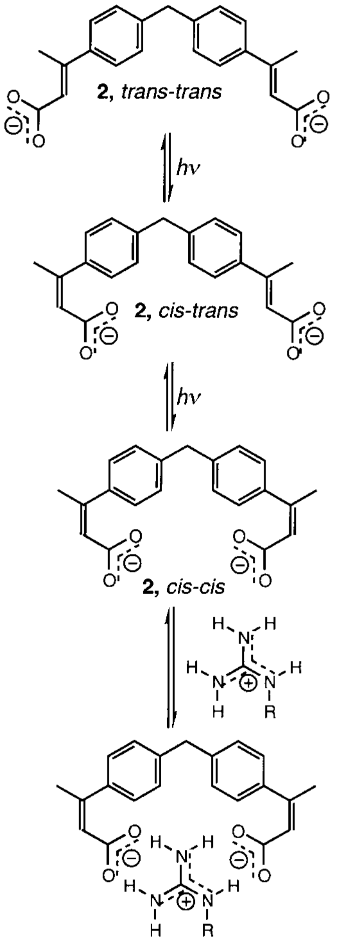


Scheme 1. Thermodynamically controlled transesterification.

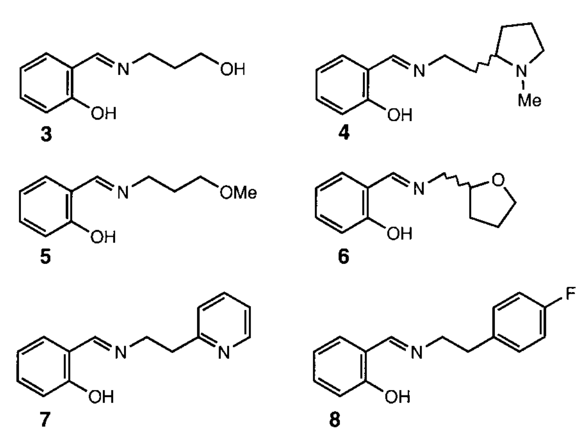
shifted towards the higher oligomers (61:24:14), probably due to a template effect by the polyether substituent. Mixed macrocycles have also been obtained by the combination of two hydroxyester monomers.[8]

Photochemically induced *cis–trans* isomerization of the dicarboxylate **2** (Scheme 2) was used by Eilseev and Nelen to drive the equilibration reaction.[9] Of the three geometrical isomers, the *cis,cis* form has an optimal structure for guanidine binding through salt bridges. Approximately 30 cycles of irradiation and passage over an immobilized arginine column altered the proportion of *cis,cis*, *cis,trans*, and *trans,trans* isomers in solution from 3:28:69 to 48:29:23, while **2** retained by the column had the ratio 85:13:2.



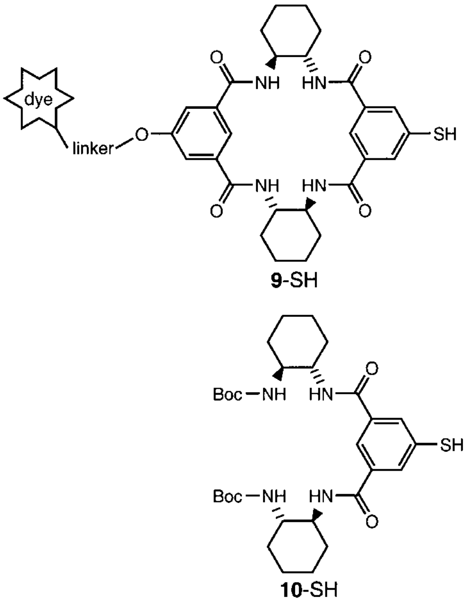
Scheme 2. Amplification of a guanidine receptor by *cis–trans* isomerization.

In the above examples, the number of components that undergo selection is very small. A system with more practical potential was reported by Klekota et al.[10] The aldimines **3**–**8** (Scheme 3) can form a total of 36 unique bis(salicylaldiminato) complexes with divalent cations. In the presence of excess zinc, the amount of **4** eluted from an affinity column of double-stranded poly-d(AT) DNA was significantly decreased. The complex $Zn^{2+}(\mathbf{4})_2$ was independently shown to bind DNA with an apparent equilibrium constant K_d of 1.1 μM.



Scheme 3. Salicylaldimines tested for DNA binding in the presence of zinc cations.

Equilibration by reversible disulfide bond formation was demonstrated by Hioki and Still.[11] Screening of a tripeptide library immobilized on beads identified a number of ligands for the fluorescent disulfide **9**-SS-**9**, including Ac-D-Pro-L-Val-D-Val-polystyrene (Scheme 4). Incubation of the disulfide

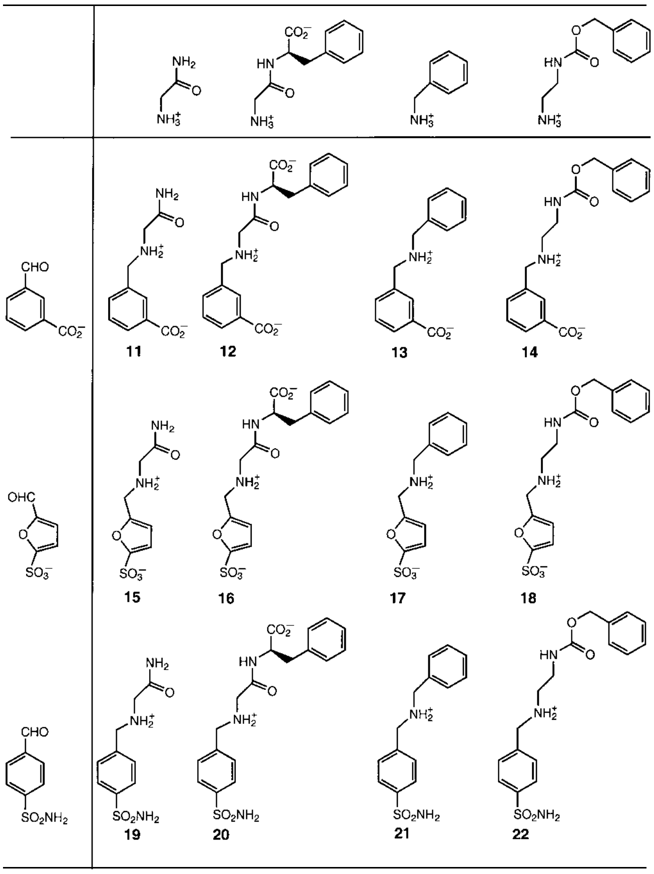


Scheme 4. Tripeptide receptors used in disulfide equilibration reactions; Boc = butoxycarbonyl.

with thiophenol resulted in an equilibrium mixture of 65% of **9**-SS-**9** and Ph-SS-Ph as well as 35% of **9**-SS-Ph. When the reaction was carried out in the presence of immobilized tripeptide ligand (3.7 equiv), the composition shifted to 95% of **9**-SS-**9** and Ph-SS-Ph. Virtually all the Ph-SS-Ph was in solution, while the beads to which **9**-SS-**9** was bound could be readily identified by the fluorescent tag. The disulfide was subsequently released in high purity. In a separate experiment, the mixed disulfide **9**-SS-**10** was equilibrated with **10**-SH. Once again, the peptide ligand biased the population towards **9**-SS-**9** and **10**-SS-**10**, with the equilibrium constant changing from 1.8 to 32 in favor of the symmetrical disulfides.

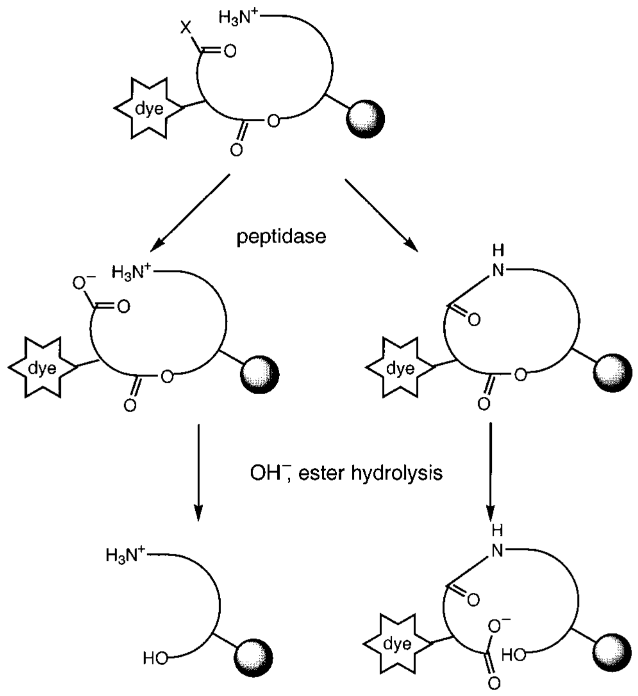
Dynamic equilibration as a route to the identification of enzyme inhibitors was explored by Huc and Lehn.[12] When a set of four amines and three aldehydes are mixed, twelve imines are generated reversibly, which can be trapped by reduction to give the mixture **11**–**22** (Table 1). When the reaction was carried out in the presence of carbonic anhydrase (one equivalent based on the aldehyde; a 15-fold excess of amines was used to compensate for reactions with the lysine side chains of the enzyme), HPLC analysis revealed that the enzyme almost doubled the relative proportion of **21.** *para*-Substituted sulfonamides are known to be carbonic anyhdrase inhibitors, and the selection of **21** over **19**, **20**, and **22** is consistent with the steric requirements of the enzyme.

Table 1. A set of three aldehydes and four amines used to generate potential carbonic anhydrase inhibitors.

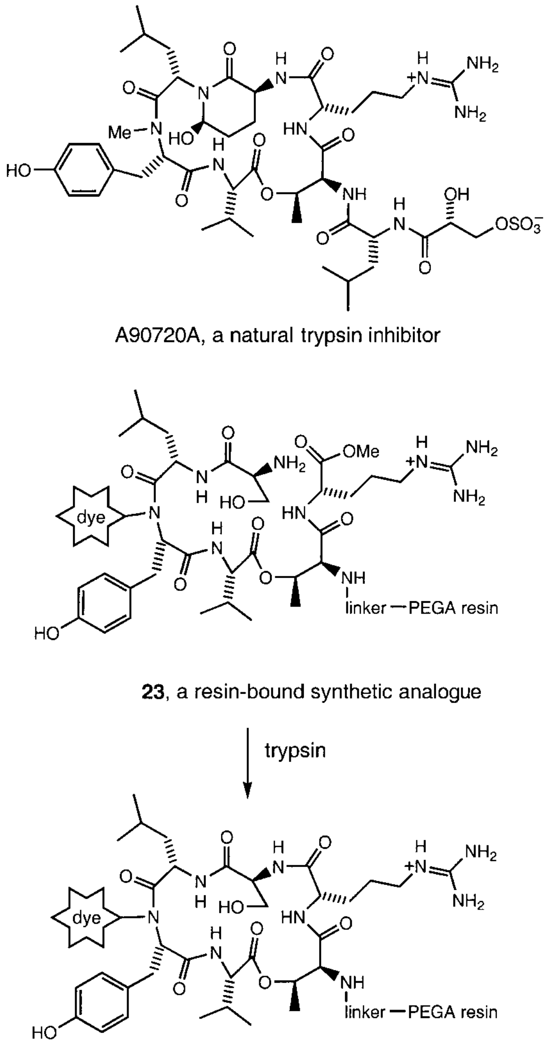


Swann et al.[13] used a nonspecific protease to catalyze both peptide bond formation and hydrolysis. Incubation of the peptides Tyr-Gly-Gly and Phe-Leu with thermolysin resulted in at least fifteen products, as observed by HPLC. The reaction was then repeated by placing thermolysin and the peptides in one half of a dialyzer, while the other half contained a monoclonal antibody ($c = 1\,\mu g\,mL^{-1}$) able to recognize the N terminus of β-endorphin (Tyr-Gly-Gly-Phe-Leu, $K_d = 7.1$ nM). Amplification of this pentapeptide could not be proven directly, but the mixture modestly inhibited radiolabeled β-endorphin binding to the antibody. Similarly, trypsin hydrolysates of bovine serum albumin and the peptide Gly-Pro-Arg (a known fibrinogen binder) were incubated with thermolysin in the presence of fibrinogen. Two tetrapeptides, Gly-Pro-Arg-Leu and Gly-Pro-Arg-Phe, were identified that were weakly bound ($K_d = 100\,\mu M$) to fibrinogen. If the considerable practical problems related to efficient peptide–peptide equilibration are overcome, this method could become an alternative to phage peptide display.

Although equilibration is not involved, Burger and Bartlett[14] have recently reported a creative example of the preselection of suitable library members by the target. Since both peptide bond formation and hydrolysis proceed via the same transition state, linear peptides that are cyclized by a peptidase would also be good templates for inhibitors. A strategy was devised (Scheme 5) which enabled bead-bound substrates that undergo cyclization to be picked out of a library. The synthetic peptide **23** (Scheme 6) is based on the blue-green algal trypsin inhibitor A90720A (equilibrium constant $K_i = 10$ nM against bovine trypsin). The peptide



Scheme 5. A strategy for the identification of macrocyclic substrates and inhibitors of proteases: The unfavorable substrate (left) does not cyclize in the presence of the peptidase, but the favorable substrate (right) does. The product obtained after ester hydrolysis (right) is fluorescent in contrast to the dye-free product on the left (the circles represent the resin beads).



A90720A, a natural trypsin inhibitor

23, a resin-bound synthetic analogue

24

Scheme 6. Design of a trypsin inhibitor. PEGA = poly(ethylene glycolamide).

was cyclized by trypsin in a mixed aqueous-organic solvent to form the macrocycle **24**. As expected, **24** is a substrate for trypsin in aqueous solution, and slow turnover at low enzyme concentrations enabled an apparent K_i of 230 nM to be calculated. Presumably, replacement of the scissile bond by a transition-state analogue would result in a true inhibitor. As in the example from Still et al., selection of components immobilized on beads should enable the rapid screening of large libraries.

The concept of dynamically tailoring library composition is still in its infancy. The examples so far have concentrated on proof of principle in situations where the selected component could be predicted beforehand (with the exception of DNA-binding $Zn^{2+}(\mathbf{4})_2$). A number of issues have to be addressed before the full potential of this approach can be realized:

1. The need for reversibility severely limits the types of chemical reaction suitable for equilibration. Even with fully reversible reactions, kinetic barriers are likely to arise with complex systems, which prevent complete exploration of all possible components within a reasonable timeframe.
2. Selection of a biological target, such as a protein, would impose further restrictions (e.g. aqueous media, physiologically compatible temperatures, and pH value). The target would also have to be present in equimolar quantities or in excess. For these reasons, applications related to materials science rather than drug discovery may be easier to achieve.
3. Unless the amplification is dramatic, careful analysis of library composition with and without selection is necessary. This places practical limits on the library size.

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