Dynamic Combinatorial Chemistry

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1. Introduction

Dynamic combinatorial chemistry is defined as combinatorial chemistry under thermodynamic control; that is, in a dynamic combinatorial library (DCL), all constituents are in equilibrium. This requires the interconversion of library members into one another through a reversible chemical process, which can involve covalent bonds or noncovalent interactions including metal−ligand coordination (see section 2).

Dynamic combinatorial chemistry is unique in that the composition of the library is determined by the thermodynamic stability of each of the library members under the particular conditions of the experiment. This makes DCLs powerful tools in identifying thermodynamic minima, which is useful in a number of different contexts.

One application is the identification of the most stable structure in mixtures of structures with different conformational properties (foldamers) (Figure 1a). Those structures that have the most favorable internal noncovalent interactions will be stabilized and formed in preference over those library members that lack such stabilization (see section 4.5).

Stabilization of specific library members can also take place through *inter*molecular noncovalent interactions between library members, the library composition being biased toward those members that form stable assemblies or aggregates (Figure 1b). Although this approach has received little attention (see section 4.4), it has real potential for the discovery of self-assembling molecules including interlocked architectures and new soft materials.

Thermodynamic control in DCLs implies that changing the conditions of the experiment can induce changes in the library composition; that is, dynamic combinatorial libraries will respond to external influences. In principle, this responsiveness can be exploited in numerous fashions: for instance, for the discovery of compounds with strongly temperature- or pressure-dependent properties or compounds that respond to light or electric or magnetic fields. Giuseppone and Lehn

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Peter Corbett was born in Ascot, England, in 1978. After finishing school, he went on to read Natural Sciences at the University of Cambridge, graduating with an M.Sci. in 2001. His final year dissertation on "Dynamic Combinatorial Libraries of Disulphide-Linked Molecular Capsules" was supervised by Sijbren Otto and Jeremy Sanders. Since then he has stayed on to study the behavior of dynamic combinatorial libraries of macrocyclic disulfides for a Ph.D.: "Dynamic Combinatorial Libraries in Theory and Practice". He is currently working as a Research Associate for Prof. P. Murray-Rust, studying the automated extraction of information from the chemical literature.

Julien Leclaire graduated from the Ecole Normale Supérieure in Lyon and obtained his Ph.D. in Chemistry at the University of Toulouse in 2003 working in the field of phosphorus dendrimers under the supervision of Prof. J.-P. Majoral. He studied as a postdoctoral researcher at the University of Cambridge in 2003–2005 with Jeremy Sanders, investigating anion recognition through dynamic combinatorial chemistry and multilevel exchange in solution. In 2005, he joined the Ecole Généraliste d'Ingénieur de Marseille and the Chirotechnologies laboratory as an assistant professor.

Laurent Vial was born in 1975 and was educated at the University Claude Bernard of Lyon (France). In 2003, he received his Ph.D. degree in chemistry from the University of Geneva (Switzerland), exploring the use of chiral salts in asymmetric chemistry under the supervision of Prof. Jérôme Lacour. Since January 2004, he has been a Marie Curie Postdoctoral Research Fellow in the laboratory of Jeremy Sanders and Sijbren Otto at the University of Cambridge. His current research interests include the selection and preparation of biomimetic catalysts and receptors from dynamic combinatorial libraries.

Kevin West was born in Oxford, England, in 1980; his family returned to Edinburgh, Scotland, soon after, where he remained for his primary, secondary, and tertiary education. He attended the University of Edinburgh where he received his M.Chem. in chemistry with industrial experience in 2003. He is currently pursuing his Ph.D. at the University of Cambridge under the supervision of Sijbren Otto. The main focus of his research is on dynamic combinatorial libraries of disulfide capsules in water.

reported one of the first demonstrations that dynamic libraries can respond to selection pressures other than molecular recognition, as illustrated by the adaptive behavior of a library of imines to external stimuli in the form of a change in pH and temperature.[1] In practice, the response of the library to an added template has so far proven most valuable (sections 4.1−4.3).

In the context of this review, a template influences the overall geometry or structure of the product rather than the intrinsic chemistry. In general, after a template has directed the formation of the product(s), it can be removed.[2] When a template binds to a specific library member, this species is stabilized and the equilibrium will shift, normally resulting in an increase in the concentration of the selected library member (thermodynamic templating[3−6]). This increase in the concentration of selected species in response to the introduction of a template is commonly referred to as "amplification". Templating can be used to select for species that act as hosts or receptors (Figure 1c), as well as for the discovery of new guests or ligands (Figure 1d). These applications are reviewed in detail in sections 4.1, 4.2, and 4.3.

Ideally, amplification will be selective for the compound that binds the template most strongly. Or, more generally, the compound that is most stabilized through noncovalent interactions (either with the template, within itself, or with other library members) tends to be produced in preference over other library members. However, since DCLs strive for the lowest *overall* Gibbs energy, special cases can occur where the final equilibrium distribution favors library members other than the one that is, in isolation, the most stable species. This behavior reflects the fact that DCLs are complex molecular networks,[7] in which library members are connected through a set of equilibrium reactions. Any change to the stability of one member will be "felt" by all others. The final equilibrium distribution is determined by the sum total of the thermodynamic stabilities of all species in the mixture. Fortunately, it is in most cases possible to choose

Jean-Luc Wietor was born in Luxembourg in 1980. In 1999, he went to Strasbourg to study chemistry at the Université Louis Pasteur. In 2003, he joined Jeremy Sanders' research group as an Erasmus exchange student. After the completion of his Maîtrise in Strasbourg the same year, he started his Ph.D. work in the same group. His work is focused toward DCLs of ligands for nucleotides.

Jeremy Sanders was born in London, obtained his B.Sc. from Imperial College, London (1969), and then worked for his Ph.D. at the University of Cambridge with Dudley Williams on lanthanide shift reagents. After a postdoctoral year (1972–1973) in the U.S.A., working on protein NMR, he returned to a junior academic post in Cambridge; he has remained there ever since, being appointed to his present Chair in 1996 and becoming Head of the Department of Chemistry in 2000. His past NMR interests have covered such diverse topics as two-dimensional NMR and nOe difference spectroscopy, drug metabolism in live cells, and the biophysics of biodegradable plastics. His current major research interest is in molecular recognition and supramolecular chemistry. He is particularly well-known for templated syntheses of receptors based on metalloporphyrins, for studies of molecular recognition by metalloporphyrins, for the synthesis of catenanes and rotaxanes, and for the recent development of the concept of dynamic combinatorial chemistry.

experimental conditions such that the individually "fittest" species are indeed the ones that are preferentially produced (see section 5).

1.1. History

This brief section attempts to capture the history of dynamic combinatorial chemistry. Much of the work mentioned here is described in detail in the following sections. Dynamic combinatorial chemistry relies on the selection of the thermodynamically most stable product from an equilibrating mixture. This principle can arguably be traced back to the 19th century: the studies of Emil Fischer on carbohydrates and of Werner on coordination complexes made extensive use of this approach. A cornerstone in some of the applications of dynamic combinatorial chemistry is

Sijbren Otto received his M.Sc. (1994) and Ph.D. (1998) degrees cum laude from the University of Groningen in The Netherlands. He worked on physical organic chemistry in aqueous solutions in the group of Jan Engberts. In 1998, he moved to the United States for a year as a postdoctoral researcher with Steve Regen (Lehigh University, Bethlehem, Pennsylvania) investigating synthetic systems mediating ion transport through lipid bilayers. In 1999, he received a Marie Curie Fellowship and moved to the University of Cambridge where he worked for two years with Jeremy Sanders, introducing the disulfide chemistry. He started his independent research career in 2001 as a Royal Society University Research Fellow at the same university. His current research interests involve molecular recognition at biomembranes and dynamic combinatorial chemistry in water.

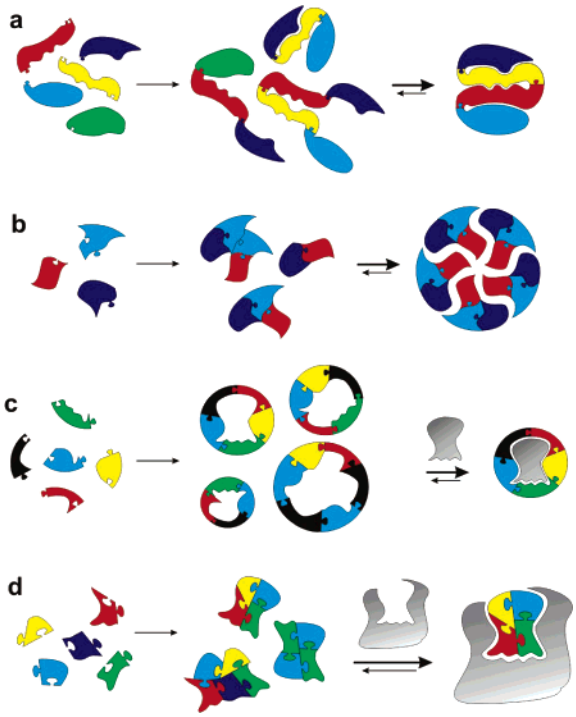
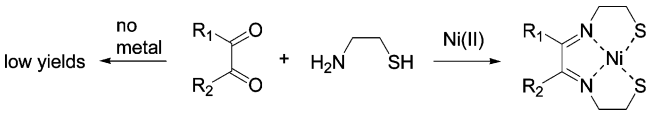


Figure 1. Different ways of selecting specific members of a dynamic combinatorial library on the basis of noncovalent interactions: (a) selection of foldamers driven by internal noncovalent interactions; (b) selection of self-assembling molecules on the basis of noncovalent interactions between different library members; (c) selection of a host by a separately introduced guest; (d) selection of a guest by a separately introduced host.

templating: Watson and Crick discovered the DNA double helix in 1953 and realized immediately that its replication involved a templated synthesis. Their work inspired Todd in Cambridge to suggest soon afterward that organic templates might one day be used to control synthetic reactions in the laboratory, and he challenged organic

chemists to explore this idea.[8] In retrospect, it is clear that metal-ion-templated syntheses of phthalocyanines had been observed as early as 1932,[9] but it is only through the pioneering work of Busch in the 1960s that templated synthesis became recognized. Busch's macrocycle synthesis shown in Scheme 1 is probably the first published example

Scheme 1. Synthesis of a Macrocycle Using Thermodynamically Controlled Templating by Ni(II)[10]



to articulate clearly the role of a template (Ni^{2+}) in stabilizing a desired product from a complex equilibrating mixture.[10] In related work, Nelson et al. stressed the role of transamination in metal-templated formation of imine macrocycles.[11] Subsequently, templated syntheses developed along parallel and largely noncommunicating organic, inorganic, and biochemical tracks. A notable contribution by Goodwin and Lynn features reversible imine-mediated synthesis on a DNA template.[12] Prior attempts to exploit template-directed synthesis in polymerization reactions sought kinetic differentiation of product distribution. DNA and RNA polymerases employ reaction reversibility and sophisticated proof-reading mechanisms to ensure high fidelity in template translations. In Goodwin and Lynn's approach, the role of the template is to shift the equilibrium position. By controlling this equilibrium, they both demonstrated unique chemistry on a DNA template and realized the first chain-length- and sequence-specific template-directed polymerizations.

Another important contribution came from Rideout who reported the covalent assembly of a cytotoxic drug at its target using hydrazone chemistry.[13,14] In 1995, Hamilton described one of the first examples of a combinatorial approach to synthetic receptors using reversible coordination around a metal ion.[15] In the same year, Harding described the guest-induced amplification of a metallo-macrocycle from a mixture.[16] Soon after, several laboratories independently evolved very similar general approaches to what is now known as dynamic combinatorial chemistry.

The Cambridge version was conceived in Dublin on 17 September 1992 and stemmed from the notion that in order to become more efficient at developing synthetic receptors, we needed a new and general approach that captured the combinatorial, selection, and amplification elements exhibited by the mammalian immune system.[17] The key idea was that guests should be able to shift an equilibrium mixture of (macrocyclic) hosts in the direction of the best binder. The first experiments were carried out by Richard Bonar-Law and involved base-catalyzed reversible transesterification. Max Gunter, on sabbatical from Australia, carried out a few experiments, but our first serious work aimed at achieving thermodynamically controlled templating was started by Paul Brady, in October 1993. Progress was slow at first, but the first paper clearly articulating the concept of dynamic combinatorial chemistry appeared in early-1996,[18] and the first modest templating results with this system soon after.[19] As explained in section 2.1.1.1, base-catalyzed transesterification has severe limitations as an exchange reaction, and it was soon supplanted in our work by hydrazone and, with the arrival in Cambridge of Sijbren Otto, disulfide exchange reactions.

During the mid-1990s, Lehn independently conceived a dynamic combinatorial approach as a result of his work on metal helicates (section 4.1.2.2, Scheme 29), observing that the major product in a dynamic mixture of helicates was determined by the nature of the counterion that binds in the center of the helicate;[20] he has reviewed his entry to the field in more detail elsewhere.[21] Huc and Lehn then extended their work to include templating of a ligand by a protein, describing the inhibition of carbonic anhydrase by a library of imines created in situ.[22] This work was preceded by a publication by Venton and co-workers who used nonspecific proteases to prepare and degrade a set of peptides reversibly, with a view to amplifying the sequences that bound most strongly to fibrinogen.[23] In 1997, Miller described the first dynamic combinatorial approach to DNA-binding compounds.[24] In the same year, Sasaki published an elegant "self-adjusting" metal-centered ligand for lectins,[25,26] and Eliseev used light-induced alkene isomerization as a reaction to drive chemical evolution in an equilibrating mixture of simple arginine receptors.[27]

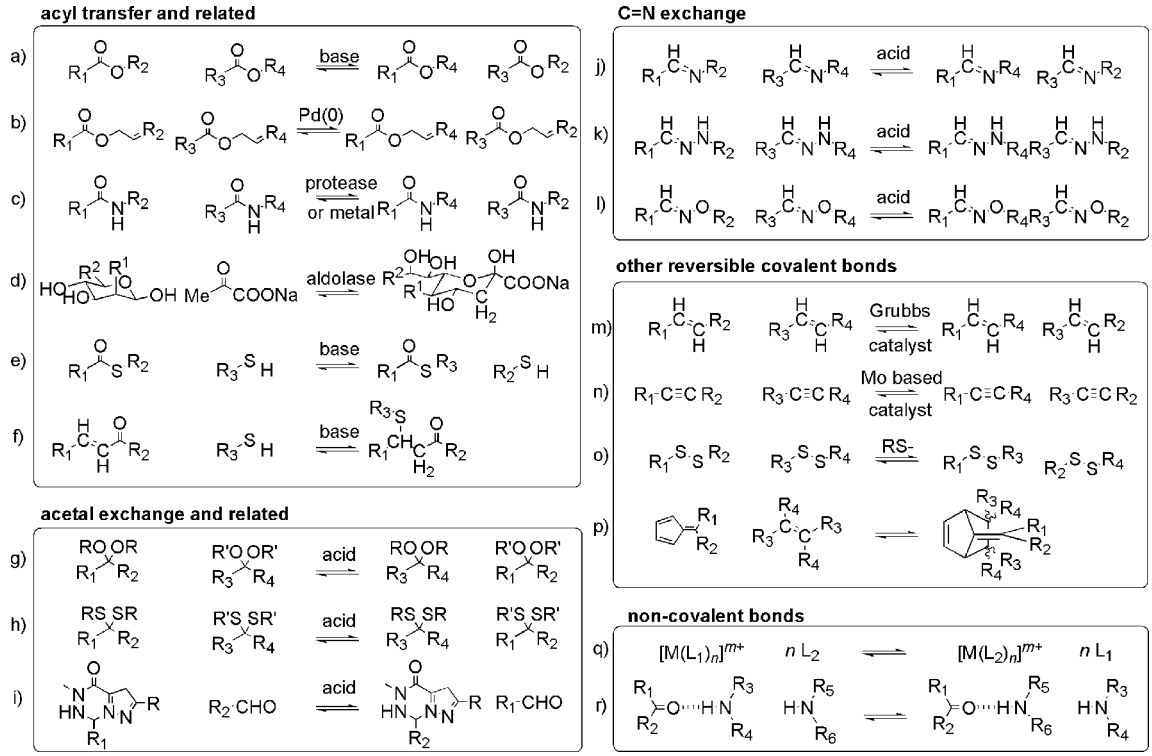
The first disulfide exchange work to articulate a version of the dynamic combinatorial idea was probably that of Hioki and Still in 1998,[28] although the reversibility of thiol−disulfide exchange has been known and exploited for many years.[29−33]

1.2. Scope and Outline of the Review

We now present a comprehensive review of all aspects of dynamic combinatorial chemistry and some related approaches. Earlier reviews on particular aspects of dynamic combinatorial chemistry can be found in refs 7, 21, and 34−50. Not included in the present review is the closely related approach of thermodynamically controlled templated synthesis, where the aim is to prepare specific predesigned molecules (an elegant example and leading references can be found in ref 51), which can sometimes be formidably complex.[52]

This review is organized as follows: In section 2, the various types of reversible chemistries will be discussed in considerable detail, focusing on the conditions and practical issues such as functional group tolerance. This overview is followed by section 3, in which we discuss some issues related to the experimental setup of a DCL, including tests for equilibration and the influence of building block concentrations and design on the composition of DCLs. Section 4 deals with applications of DCLs for the discovery of new synthetic receptors and catalysts, as well as the discovery of new ligands for proteins and nucleic acids. This is followed by a brief description of dynamic combinatorial approaches to aggregation. The final part in this section is devoted to the use of dynamic combinatorial chemistry to address the folding of biomolecules as well as synthetic polymers. While the present studies on aggregation and folding feature mixtures of only a few compounds and are therefore perhaps not truly combinatorial, we have nevertheless chosen to include this work because we feel it forms the foundation of important future work in this area. The theoretical work on DCLs is reviewed in section 5. Finally, in section 6, we discuss some approaches that resemble dynamic combinatorial chemistry, including systems in which reversible chemistry is separated from screening for affinity. We conclude the review by highlighting some exciting new approaches that combine reversible chemistry with irreversible reactions.

Scheme 2. Reversible Reactions Used for Dynamic Combinatorial Chemistry to Date[a]



[a] (a) Transesterification; (b) transallylesterification; (c) transamidation; (d) aldol exchange; (e) transthioesterification; (f) Michael/retro-Michael reactions; (g) acetal exchange; (h) thioacetal exchange; (i) pyrazolotriazone metathesis; (j) transimination; (k) hydrazone exchange; (l) oxime exchange; (m) alkene metathesis; (n) alkyne metathesis; (o) disulfide exchange; (p) Diels−Alder/retro-Diels−Alder reactions; (q) metal−ligand exchange; (r) hydrogen-bond exchange.

2. The Exchange Reactions

The key feature of dynamic combinatorial chemistry is the reversible reaction that mediates exchange of the building blocks between the different library members. This reaction needs to meet a number of requirements: (i) it needs to be reversible on a reasonable time scale; (ii) because equilibration and selection ideally occur simultaneously, the reversible reaction needs to be compatible with the experimental conditions of the selection process, including the functional groups on the building blocks and template, the solvent, and the pH (physiological for the use of biomolecules); (iii) reaction conditions need to be mild (e.g., temperature, pressure, concentration), so as not to interfere with the delicate noncovalent interactions involved in molecular recognition; (iv) it needs to guarantee the solubility of all the library members at equilibrium because any not sufficiently soluble material could act as a thermodynamic or, at slow dissolution rates, kinetic trap; (v) it should be possible to turn off the reaction so as to kinetically "freeze" the selected library member(s) enabling their isolation and handling; and finally (vi) ideally all library members should be isoenergetic in order to prevent the production of reaction mixtures that are strongly biased toward certain products, which may make it energetically costly to shift the equilibrium in any other direction. In practice, the latter requirement is rarely met; for instance, nearly all DCLs in which macrocyclic products are formed will, for entropic reasons, be biased toward smaller oligomers.

Three main types of bonds have been used to date in reversible reactions: noncovalent (hydrogen bonds), coordinative, and covalent bonds. Table 1 gives an overview of their thermodynamic stability and their kinetic lability.

Table 1. A Comparison of the Thermodynamic Stability and the Kinetic Lability for Different Bonds

interactions		energy (kJ/mol)	lability
covalent bonds		150−800[a]	low
coordinative bonds	1st row[b]	80−350	high
	2nd row[b]	80−350	medium
	3rd row[b]	80−350	low
hydrogen bonds		0−20	high

[a] Typical range for single and double bonds. [b] Localization of the metal in the d block of the periodic table (3d−5d series).

Usually, weak and labile noncovalent bonds allow equilibrium to be reached rapidly. However, the ensuing thermodynamic products are not very stable, in particular in solution, and are consequently difficult to analyze by chromatography and difficult to isolate and use in further experiments.

In contrast, covalent bond formation and breaking typically exhibit slow kinetics, which can sometimes be accelerated with the addition of a catalyst to allow equilibration on a practical time scale. This implies that by removing this catalyst it is possible to stop the exchange process, whereafter selected compounds can be isolated and handled without risk of re-equilibration.

We have compiled a comprehensive list of the covalent and noncovalent exchange reactions used to date to generate DCLs, and we have examined their conditions, yields, kinetics, and limitations (see Scheme 2 and Table 2).

2.1. Reactions Involving Covalent Bonds

A systematic classification of reversible covalent reactions used in dynamic combinatorial chemistry is difficult to establish. We have chosen to review the different types of

Table 2. References to Reversible Chemistries Used in DCLs to Date

reversible chemistry	refs
(a) transesterification	18,19,62−67,269,270
(b) transallylesterification	78
(c) transamidation	23,86,382,383
(d) aldol exchange	88,89
(e) transthioesterification	99,100,252,381
(f) Michael/retro-Michael reactions	104,105
(g) acetal exchange	173−176
(h) thioacetal exchange	177
(i) pyrazolotriazone metathesis	178
(j) transimination	1,10,12,22,24,112,113,115−118, 128,136−140,142−145,238,278, 281−283,331,339,369,380,386
(k) hydrazone exchange	13,14,151−167,169,170, 277,285,335
(l) oxime exchange	147−149
(m) alkene metathesis	197,199,202,203,296,379,387
(n) alkyne metathesis	207
(o) disulfide exchange	28,32,33,186,194,202,203,252, 253,272,273,294,295,311,327, 328,333,334,338,366,367,378
(p) Diels−Alder/ retro-Diels−Alder	181
(q) metal−ligand exchange	15,16,20,24−26,213−239, 316,370,375,376
(r) hydrogen-bond exchange	167,242−245,247,248,250, 251,253

labile bonds involved in DCLs following a classical organic chemistry classification and, in addition, focus on the symmetry of the bond, because this factor determines the design of the building blocks as well as the degree of diversity accessible within a dynamic mixture. The symmetry or directionality of a labile bond dictates whether individual building blocks can self-oligomerize or can only be involved in alternate sequences. For instance, when targeting macrocyclic species, in the case of an unsymmetrical bond, two categories of building blocks can be designed carrying either two copies of the same function and therefore being self-inert or carrying complementary functions (Figure 2a). No

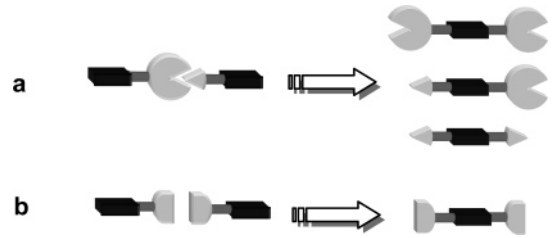


Figure 2. (a) Unsymmetrical and (b) symmetrical reversible bonds (in gray) influence the design of building blocks (right) and their possible association (left).

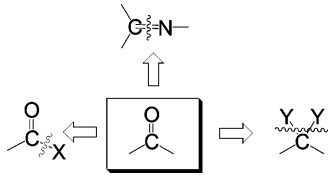
such differences occur with symmetrical linkages (Figure 2b).

The symmetrical covalent bonds are discussed in sections 2.1.5−2.1.7 and the unsymmetrical linkages in sections 2.1.1−2.1.4. Nearly all reversible unsymmetrical covalent bonds used in dynamic combinatorial chemistry involve carbonyl compounds and derivatives thereof (Scheme 3). Important classes of reactions are acyl exchange reactions, exchange reactions involving imines and derivatives thereof and reversible acetal chemistry (Scheme 2).

2.1.1. Acyl Exchange

C(O)−X bonds are relatively fragile and, as a consequence, potentially broken reversibly when X is an electro-

Scheme 3. Different Types of Unsymmetrical Covalent Exchange Reactions Involving Carbonyl Groups[a]



[a] Acyl transfer (X = NHR, OR, SR, CH_2R), C=N exchange and acetal-type exchange (Y = OR, SR, NHR).

negative fragment (O, S, or N). The mechanism of exchange usually involves a tetrahedral intermediate resulting from attack of the carbonyl group by a nucleophile X in the rate-determining step (Figure 3, inset).[53] The activation energy ($E_a(1)$ or $E_a(1')$) reflects the thermal accessibility of the equilibrium and can be approximated by considering the energy gap between the frontier orbitals of the reaction partners. In the case of acyl transfer, the HOMO holds the n_p lone pair of the nucleophile X. The LUMO of the RCO−X electrophile corresponds to the π^*_{CO} of a R−CO fragment perturbed by the interaction with the doubly occupied p orbital of the electron-donating substituent X[54,55] (this atomic orbital corresponds to the HOMO of the attacking nucleophile X).[56,57] The difference in electronegativity of X is mainly responsible for the difference in the LUMO and HOMO levels, so that transamidation (X = N) and transesterification (X = O) reactions have to surmount appreciable activation barriers. In practice, in transesterification, the reactivity of the oxygen nucleophile is enhanced by deprotonation (where the counterion can act as a Lewis-acid catalyst, coordinating to the carbonyl oxygen atom, thereby increasing its electrophilicity) rendering transesterification more accessible than transamidation.

In principle, aldol reactions (nucleophilic attack of the α-carbon of an enolate on the carbonyl group of an aldehyde) are much more energetically accessible because the reactive species is a highly nucleophilic carbanion. Under physiological conditions, reversible aldol condensations have thus far only been achieved in the presence of a biological catalyst.

Sulfur is less electronegative than nitrogen and oxygen and is much more nucleophilic and softer. Overlap of the orbitals on sulfur with the π-system of the carbonyl fragment is poor and gives a slight increase in the energy level of the π^*_{COX} orbital. Transthioesterification involves a deprotonated thiolate anion as the nucleophile (which can be generated around neutral pH due to the increased acidity of thiols as compared to alcohols), which has a higher electron density than the corresponding neutral species. The gap between both frontier orbitals is thus dramatically reduced, and thioester exchange can be performed at room temperature under neutral conditions.

2.1.1.1. Ester Exchange. Transesterification and macrolactonization under thermodynamic control have been extensively studied in polymer chemistry, focusing in particular on the conversion of macrocycles into linear polymers (see section 3.2).

Displacing the esterification/hydrolysis equilibrium toward condensation traditionally requires introducing an excess of carboxylic acid or alcohol or the removal of the condensation byproduct, that is, water. The latter is usually achieved by azeotropic distillation with a suitable cosolvent, absorption on molecular sieves, or both. If the starting material is a methyl ester, the byproduct is methanol, which can be

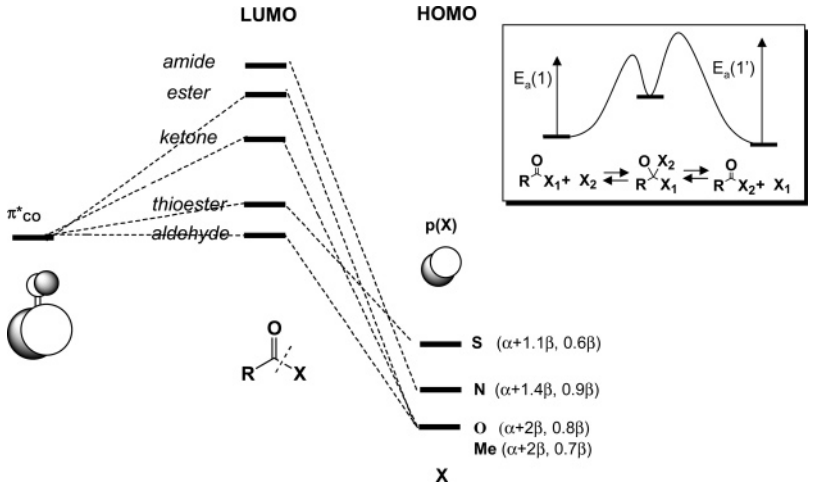
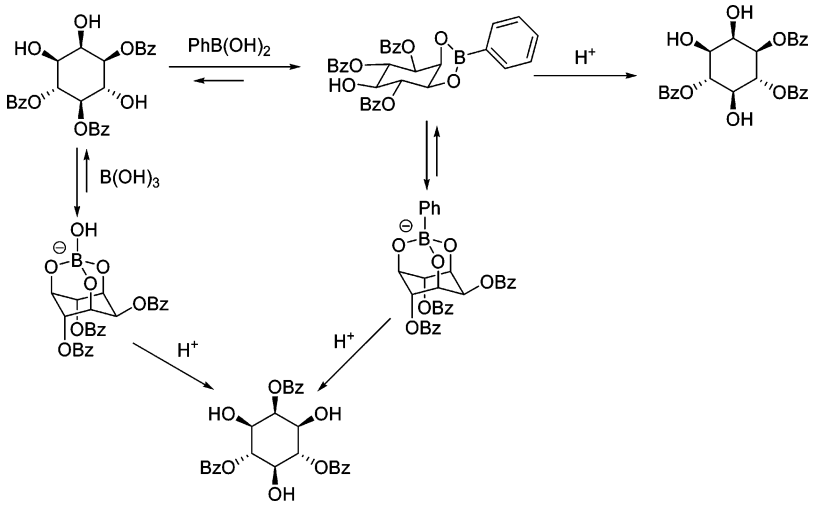


Figure 3. Orbital classification of activation energies for acyl transfer reactions.

Scheme 4. Reversible Transesterification To Effect Acyl Migration[a]



[a] Use of a boronic acid "template" induces a shift in product distribution.[66,67]

removed azeotropically using toluene and 4 Å molecular sieves.[18,19,58] However, achieving efficient exchange when none of the components is present in excess is more difficult and requires a catalyst in order to give equilibration within a reasonable time scale. While acid catalysts such as *p*-toluenesulfonic, triflic, or camphorsulfonic acid have been used extensively for transesterifications in organic synthesis, these catalysts can cause substantial decomposition of building blocks.[59] Milder alternatives such as titanium tetralkyl derivatives[60,61] or tin tributyl alkoxides do not accelerate the reactions sufficiently to be useful for dynamic combinatorial chemistry. Base catalysis appears to be the most efficient exchange method. We have used 5 mol % of the complex of potassium methoxide and dicyclohexyl-18-crown-6 in refluxing toluene, which allows the formation of alkoxide intermediates that remain soluble in the anhydrous solvent mixture at millimolar concentrations and yields the desired thermodynamic mixture of oligoester products within 10 min.[62−65] While this reaction proved useful in the early days of dynamic combinatorial chemistry to establish proof-of-principle, the conditions are harsh: naked methoxide is both a strong base and a strong reducing agent, limiting the functional groups that can be present, while the high temperature reduces the effect of templating interactions.

Nevertheless Chang and Ahn[66,67] used transesterification to effect acyl migration on *myo*-inositol tribenzoates (Scheme 4). Unsurprisingly, aromatic acyl groups appear to be more reactive (and presumably more sensitive to water) than aliphatic ones, with the exchange taking place at room temperature. The optimum conditions involve the use of a basic catalyst (DBU, 70 equiv with respect to building blocks) in anhydrous acetonitrile; increasing the amount of water accelerates benzoyl migration but also promotes ester hydrolysis. After 1.5 h at a concentration of 0.1 mM, 12 possible regioisomers were observed by LC-MS and ^{1}H NMR. A significant shift in product distribution was induced upon binding of the hydroxyl groups to a boronic acid (16 equiv with respect to building block). Due to their binding preferences for 1,2- and 1,3-cis diols, boronic acids selectively associate with some of the regioisomers of the benzoylated inositols. For example, the regioisomer carrying benzoyl groups on the positions 2, 4, and 6 is the major product in the presence of $B(OH)_3$ as a result of preferential binding of this template to the axial hydroxyl groups. Thermodynamic control is demonstrated by the convergent results obtained with pre-equilibrated libraries without template followed by the addition of the boronic acid compared with mixing all ingredients at the start of the experiment. The exchange reaction can be stopped by acidifying the reaction mixture, which also results in cleavage of the boronic ester moiety.

In the examples described above, the exchange takes place in conditions in which the ester bond is thermodynamically unstable when some water is present. In the presence of water, the equilibrium is on the side of the starting materials. This situation can have certain advantages in special cases when different library members are competing for the same building blocks (see section 5).

The tolerance to water can be improved by operating in neutral conditions (in which the ester bond is much more stable to the presence of water) and activating the exchange by using biological catalysts such as esterases. Lipases (serine hydrolases that catalyze the hydrolysis of lipids at the water−lipid interface) appear particularly effective in minimizing hydrolysis. Lipase-catalyzed reactions have been widely applied to synthesis in biphasic media (food and oil processing as well as preparation of chiral intermediates), including transesterification reactions,[68−71] in particular the lactonization of different families of hydroxyacids.[72−75]

While lipases appear promising for use in dynamic combinatorial chemistry, the extent to which they allow equilibration of the product mixture remains unclear. It appears that the product distribution differs from that obtained by conventional synthesis under kinetic control and is affected by the geometry of the substrate, the solvent, the concentration, the temperature, and the type of lipase. This last observation emphasizes a recurrent problem linked to the use of enzymes, which still remains to be solved, the kinetic specificity of the catalyst for particular substrates. Ideally, the enzyme should process the different members of the libraries at the same rate to avoid a reactivity-based (as opposed to stability-based) bias of the library composition.

2.1.1.2. Allyl Ester Exchange. π-Allyl ester palladium complexes generally undergo reversible reactions.[76,77] We have demonstrated that equilibrium can be reached within hours in the presence of mild base (40 equiv), the palladium catalyst ($Pd(PPh_3)_4$, 0.1 equiv), and moderate heating (55 °C).[78] While such conditions are compatible with some molecular recognition processes, they also promote the hydrolysis of the ester bond as soon as traces of water are present in the medium.

2.1.1.3. Amide Exchange. The prominence of amide-based molecules in living systems and the widespread use of synthetic polyamides are in part due to the stability of the amide group. Thus, achieving amide bond exchange under mild conditions is a considerable but worthwhile challenge. Work in the fields of biochemistry and polymer chemistry has led to two distinct approaches to achieve thermodynamically controlled amide exchange, which are based on biocatalysts and synthetic catalysts, respectively.

One of the most economical strategies for obtaining the synthetic dipeptide aspartame (the most used low-calorie sweetener in the world) involves a thermodynamically controlled condensation between *Z*-protected aspartic acid and phenylalanine methyl ester catalyzed by the enzyme thermolysin. Removal of an insoluble salt is used as driving force to shift the equilibrium in the synthetic direction.[79] The same enzyme was used in the first (and thus far only) transamidation-based approach to dynamic combinatorial chemistry by Venton and co-workers who used small peptides (two or three amino acid units) at 50 mM concentrations and physiological pH in aqueous medium (phosphate buffer, pH = 7.4).[23] The equilibrium is reached within several days and can be shifted toward peptide formation by employing less polar nonaqueous cosolvents and a large excess of starting material. However, water is essential for proteases; mixed aqueous media usually reduce enzyme activity considerably, and insufficiently hydrated catalyst can even be completely inactive.[80−83]

The major limitations of enzyme-mediated transamidation are the high substrate selectivity of the catalyst that may bias the reaction and the fact that the equilibrium typically lies on the side of the starting materials (acids and amines). This latter drawback can be eliminated by using an organic cosolvent in the presence of a hydrophilic solid phase. Gardossi and co-workers have used proteases (5 mg) immobilized on Celite R-640 (200 mg) in the presence of a controlled amount of water (70 μL) coabsorbed on the solid phase, which is dispersed in a nonwater miscible organic solvent (1 mL of toluene) at room temperature.[84] Such a biphasic system allows retaining enzyme activity in organic medium by tuning the water activity. Under these conditions, thermolysin and α-chymotrypsin catalyze the amide coupling and esterification of protected amino acids, respectively, giving more than 95% yield within several days. In comparison, homogeneous water−acetonitrile systems involving the same components gave only 11% of coupled products at equilibrium.[85]

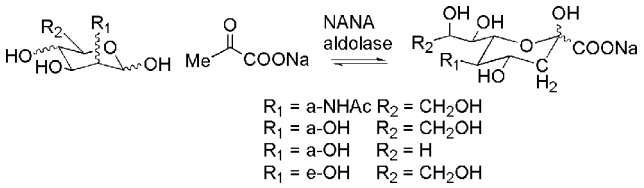
The Ulijn group has recently reported the use of thermolysin for the reversible formation of peptides in a system in which formation of a hydrogel results in the stabilization of a particular peptide.[86]

The use of synthetic catalysts for amide exchange has focused on the reaction between carboxamides (165 mM) and amines (1 equiv). These reactions were studied in organic solvent at relatively high temperature (90−120 °C) in the presence of organometallic catalysts.[87] The potential catalysts were selected from a large range of candidates (Lewis acidic metal complexes, nucleophilic alkali-metal amides, transition- and main-group amides). Aliphatic and aromatic amines and amides have been studied separately in the presence of the preselected catalysts (isoenergetic exchange). In the presence of catalyst, the reactions require around 16 h, while they normally take more than a month in the absence of catalyst. A different preference is observed between aliphatic and aromatic amines in terms of solvent and catalyst: $Al_2(NMe_2)_6$/toluene is the optimum system for aliphatic amines, whereas $Ti(NMe_2)_4$/xylene is more appropriate for anilines. The reaction is believed to proceed through a bifunctional mechanism involving both substrate activation by a Lewis acidic metal center and nucleophilic attack by a coordinated amide ligand. It may be possible to apply these catalysts for the dynamic combinatorial development of amide-based molecules and materials, although the relatively harsh conditions will limit the number of applications.

2.1.1.4 Aldol Exchange. Although a classical reversible reaction in organic chemistry, the aldol condensation has so far only been applied to dynamic combinatorial chemistry in a single case.[88,89] Aldolases are enzymes that catalyze the interconversion of hexoses and their three-carbon subunits and can be applied to a wide range of substrates. They offer an interesting water-compatible alternative to conventional aldol synthesis, which is often carried out at low temperature in organic solvents, requiring the protection of most polar groups.

N-Acetylneuraminic acid aldolase (NANA aldolase) catalyzes the addition of pyruvate to *N*-acetylmannosamine to form *N*-acetylneuraminic acid (Scheme 5). Since the equi-

Scheme 5. Enzyme-Mediated Reversible Aldol Reaction[a]



[a] a- and e- denote axial and equatorial substituents, respectively.[88,89]

librium for this reaction is close to unity, an excess of pyruvate is usually required to drive the reaction toward completion. While the specificity of the enzyme is absolute for pyruvate (the donor), it is more flexible with respect to the acceptor, and a wide range of mannosamine derivatives have been used to synthesize the corresponding aldol adducts under thermodynamic control.[90−96]

Flitsch and Turner have used NANA aldolase to create a dynamic combinatorial library of sialic acid analogues that interconvert through stereoselective aldol and retro-aldol reactions.[88,89] They used 2 equiv of pyruvate and a 5 mM concentration of aldol acceptor building block at pH 7.5 (phosphate buffer) in the presence of 0.25 equiv of aldolase per building block. The equilibrium distribution was reached within 24 h.

The prospects for use of nonenzymatic aldol exchange appear limited by complications arising from dehydration of aldol products and other side reactions.

2.1.1.5. Thioester Exchange. Thioester bonds were identified as reversible within the (bio)chemical community several decades ago[97] and applied to the reversible activation of peptide termini for subsequent coupling.[98] Thioesters have been used only relatively recently as the reversible bond in the formation of DCLs. The exchange reaction is usually performed by mixing stoichiometric amounts of thiols and thioesters in aqueous solution without the need for any activation procedure.

Ramström and co-workers have studied the catalytic screening of dynamic combinatorial libraries of thioester substrates differing in their acyl group (section 6.2).[99] Their system of aliphatic sulfur nucleophiles reaches equilibrium within 2 h at 5 mM concentration. The presence of an enzymatic template (acetylcholinesterase) in the mixture affected both the kinetics and the thermodynamics of the exchange, the binding (or more likely, the unbinding) to the biomacromolecule being much slower than the scrambling.

Woll and Gellman used peptide folding for the selection and amplification of two sequences assembled by aliphatic thioester linkages.[100] The authors used 2 mM tris-carboxyethylphosphine (TCEP) as a reducing agent to avoid disulfide formation. Interestingly, the equilibrium is reached within roughly the same time scale as in the Ramström system, even though the building block concentration that was used was 50 times lower.

We have reported an example in which disulfide exchange and thioester exchange were combined to form DCLs based on aromatic thiol and thioester building blocks (see section 2.3).

2.1.1.6. Michael Addition. Michael additions are mechanistically closely related to acyl transfers, involving a 1,4-addition of a nucleophile to an enone, proceeding through essentially the same reaction steps as the 1,2-addition to a carbonyl group described in the previous sections. In the context of dynamic combinatorial chemistry, thiol-based Michael donors are ideal reaction partners due to their softness, polarizability, and nucleophilicity even in mild aqueous conditions. The kinetics and thermodynamics of Michael/retro-Michael reactions involving thiolated[101,102] and oxygenated[103] species have been explored recently from an organic synthesis perspective. Inspired by those studies, Shi and Greaney have incorporated this labile bond into a dynamic combinatorial library involving ethacrinic acid derivatives as the Michael acceptors (100 mM) and glutathione (10 mM) as the sulfur nucleophile.[104,105] The exchange between five different adducts and their respective ethacrinic acid reactants takes place in close to neutral conditions (pH = 8) in aqueous solution (1:1 water/DMSO, no special precautions being taken to prevent glutathione dimerization). In the absence of template, the library was composed of an approximately even distribution of Michael adducts and corresponding enones.

2.1.2. C═N Exchange

The most common method for preparing imines and related C═N compounds is the acid-catalyzed reaction of aldehydes or ketones with amine derivatives. This reaction was discovered by Schiff,[106] and imines are therefore often referred to as Schiff bases and the CH═N bond as the azomethine linkage.

Because imine formation is reversible, synthesis is generally carried out by driving C═N bond formation to completion through the removal of water using azeotropic distillation or 4 Å molecular sieves.[107,108] The substitution pattern or the stereoelectronic characteristics of the reaction partners play a crucial role in the thermodynamic stability of the condensation products.[109,110] In particular, when the reaction is carried out with an amine that has an electronegative atom containing at least one lone pair of electrons adjacent to the attacking nitrogen atom, such as a hydroxylamine, a semicarbazide, or a hydrazine, the equilibrium lies far on the side of the adduct even in the presence of water. To avoid unbalanced product distributions, libraries relying on C═N exchange have thus far only been based on homologous H_2N-X families where X is the same molecular fragment ($CR_1R_2R_3$ (imines), NR_1R_2 (hydrazones), or OR (oximes)).

2.1.2.1. Imine Exchange. The substitution pattern of the imine, and in particular the carbonyl-derived part, dramatically affects the thermodynamic stability of the C═N adducts. During the imine formation, water removal is necessary with diaryl or arylalkyl ketones, but aldehydes and dialkyl ketones can usually be condensed with amines without removing the water from the reaction mixture. Aromatic aldehydes give the most stable adducts[111] and are therefore used extensively to generate imine-based libraries. In contrast, to date, there is only one example of imine-based libraries generated from ketones.[112]

The imine condensation reaction is acid catalyzed, but only aldehydes and ketones which do not aldolize easily in acidic media can be condensed efficiently with amines in the presence of strong acid catalysts. In dynamic combinatorial chemistry, imine-based libraries are often generated at mildly acidic and even basic pH (aqueous buffers between 5.0 and 8.5 or weak acids in organic media such as oxalic acid or NH_4PF_6[113]) and at room temperature, preventing aldol side reactions.

Some easily accessible carbonyl derivatives may condense with amines even more readily than the parent carbonyl compounds.[114] Acetals and ketals react with amino groups either on refluxing in a solvent or on removal of the alcohol

formed in the reaction by distillation. So far, only *gem*-diols have been used as a masked carbonyl functionality for imine exchange.[115]

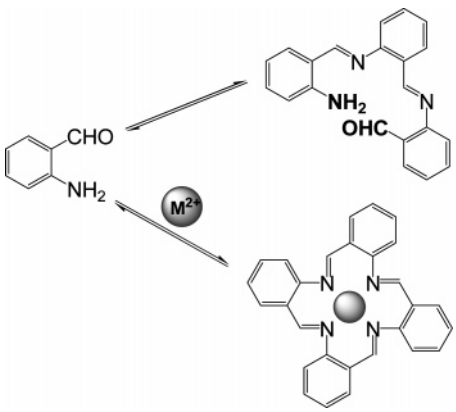
In aqueous solution, aliphatic amines are usually fully protonated in the pH domain where the reaction takes place rapidly (pH < 8.0) pulling the equilibrium toward hydrolysis. Lehn and co-workers have recently reported a systematic analysis of the equilibrium constants of formation (K) of imines in water from a structurally diverse pool of aldehydes and amines.[116] As expected, the values of K were higher for basic amines, but the correlation between log(K) and the pK_a of amines was rather poor. The authors were able to obtain much improved correlations using a three-parameter equation that includes the difference between the LUMO energies of the aldehydes and the HOMO energies of amines:

$$\log(K) = 6.668 - 1.12E_{\mathrm{LUMO}}^{\mathrm{aldehyde}} + 2.067E_{\mathrm{HOMO}}^{\mathrm{amine}} + 0.641\mathrm{p}K_{\mathrm{a}}^{\mathrm{amine}}$$

Due to the relative thermodynamic instability in the presence of water, libraries based on imine exchange tend to give poor yield of adducts and are unstable toward analysis by chromatographic methods that use water. A solution to this problem is to work in water-free conditions[117] or in biphasic systems.[118] The rate of hydrolysis of the azomethine linkage is increased by electron-withdrawing substituents, while electron donating ones decrease the reactivity.[119] The exchange reaction can be expected to follow a similar trend because the rate-determining steps of hydrolysis and transimination are similar.

Reversible imine chemistry has been used to form cyclic ligands for transition metals for several decades. The complexation of the metal ion directs the synthesis away from insoluble polymers to soluble cyclic adducts and shifts the condensation equilibrium toward completion even in pure water.[10,120−131] For example, the self-condensation of *o*-amino benzaldehyde results in a linear trimer in the absence of template (thus to an incomplete condensation reaction),[120,121] whereas closed tri- or tetradentate macrocylic ligands are formed exclusively in the presence of metal ions such as Ni(II), Zn(II), and Co(II) (Scheme 6).[128] The building blocks

Scheme 6. One of the Earliest Examples of Thermodynamically Templated Imine Synthesis[120,121,128]



that are successfully cyclized under the influence of metal ions can usually bind the metal by chelation (i.e., pyridine and pyrimidine *o*-carboxaldehydes, salicylaldehyde,[132−135] *o*-aminophenols).

Macrocyclization can even take place under conditions where not only the imine bond but also the metal ion template itself is labile. This has been elegantly demonstrated by Nitschke et al. using copper(I) (70 mM), which is kept from disproportionating in the complex.[115,136] No polymerization of the building blocks was reported despite relatively high building block concentrations (250 mM building blocks, 140 mM sodium bicarbonate).

Lehn and co-workers have recently reported the use of lanthanide cations as transimination (imine and amine, 20 mM each) catalysts in organic medium.[137] This study has provided insight into the thermodynamic and kinetic features of imines and more generally CH═N bond exchange. Sc(OTf)$_3$ is a powerful and general Lewis-acid catalyst in organic medium providing accelerations up to 10^5-fold, which is more effective than Brønsted acids at the same concentration (4 mol % in chloroform). In particular in the case of the most basic amines, catalysis by H^+ is less efficient because protonation decreases the reactivity of the amine while at the same time preventing activation of the imines. Scandium-mediated catalysis is most effective in solvents of low dielectric constant, while coordinating solvents greatly decrease the catalytic effect (the latter influence the proton-catalyzed process much less). Several lanthanides have been tested as well, and catalytic efficiency seems to decrease linearly with the ionic radii of the trivalent cations. The most reactive (i.e., most nucleophilic) amine leads to the thermodynamically most stable imine, while the exchange rates are highest for amines with comparable nucleophilicities, regardless of the direction of the reaction. Catalysis is compatible with the presence of electron-rich and chelating groups and can be extended to oxime- and hydrazone-based exchange.

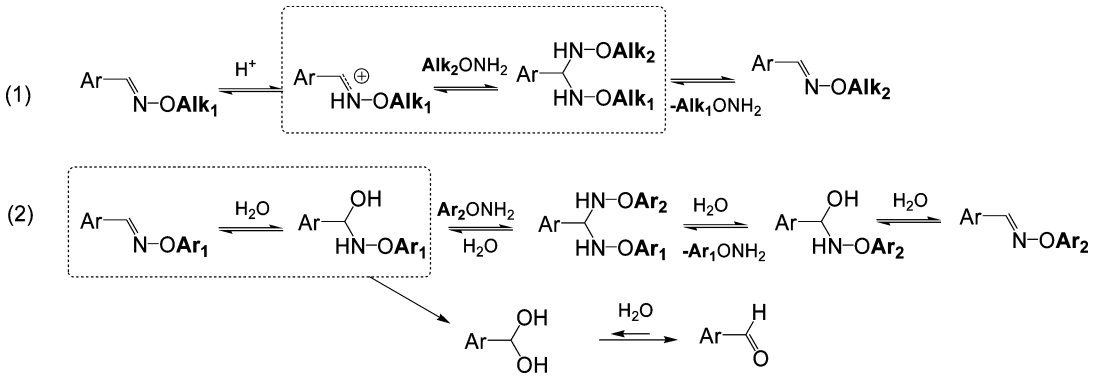
The mechanism of lanthanide catalysis involves the formation of a termolecular amine−imine−metal ion complex followed by nucleophilic attack of the amine in the rate-determining step. Note that the most strongly coordinated amine will react most efficiently, stressing the advantage of isoenergetic (or isobasic in this case) libraries.

Moore has studied imine metathesis as a reversible reaction for folding driven synthesis of oligomers in organic medium (see also section 4.5.3).[138−140] In this case, the exchange takes place directly from imine building blocks in the absence of water (5 mM in dry organic solvents such as CH_3CN and $CHCl_3$).[141] In the presence of 10 mol % oxalic acid, equilibrium is reached within several days at room temperature. A very strong influence of the solvent on the size of the oligomers is observed, indicative of the solvent sensitivity of the folding process.[138−140] The noncovalent interactions that are responsible for the folding of the oligomers also appear to accelerate the equilibration process and to strongly stabilize the adducts.

The analysis of imine libraries is hampered by the inherent lability of the imine linkage, making chromatography difficult. Analysis usually relies on NMR analysis of the complete mixture.

Alternatively, kinetic trapping of the imine-based libraries can be achieved through reduction to amines using selective reducing agents such as sodium or tetrabutylammonium[142] cyanoborohydride. The reducing agent is usually used in a 10-fold excess compared to the building blocks (between 0.05 and 25 mM), and the procedure is compatible with organic[143] as well as aqueous[142] environments. Imine reduction has two advantages: it produces derivatives of the library members that are stable to HPLC analysis and also increases,

Scheme 7. Mechanistic Pathways of Exchange Involving *O*-Alkyl and *O*-Aryl Oximes in Water[147]



by equilibrium displacement, the amount of condensation adducts, which in aqueous environments would otherwise remain very low. Providing the different library members display the same reactivity toward reduction, the amine product distribution will reflect the imine distribution.[22] However, care has to be taken to ensure that reduction of aldehydes to alcohols does not influence the outcome of the selection experiment. When proteins or peptides are used, it is important to avoid unwanted imine condensation on the amine groups of these biomolecules by using an excess of the amine building blocks.[22]

After reduction, the isolated molecule (i.e., an amine) is no longer the binder selected by the template (i.e., an imine). In a study of glycosidase inhibitors, the Vogel laboratory has observed that "imines can model the inhibitory activities of the corresponding amines".[144] In a study of stabilization of oligonucleotide complexes, Rayner and co-workers[145] also reported a correlation between the amplification of a library member and the noncovalent interactions exhibited by its reduced analogue. Nevertheless, one cannot generally expect that the binding behavior of the isolated amine is necessarily correlated to the corresponding behavior of the imine that was selected.

2.1.2.2. Oxime Exchange. The equilibrium for oxime formation lies more on the side of the adduct than in the case of imines. The pH of the reaction medium has a large influence on the position of the equilibrium, while the rate of oxime formation tends to be maximal close to neutral pH. Although this condensation reaction is acid-catalyzed, it is rarely carried out in the presence of strong acid. Only a few reactions of hydroxylamines with carbonyl compounds in strongly basic media are reported, mostly for the preparation of sterically hindered oximes.[146] Unlike imines, oximes can be isolated in syn and anti configurations, the latter being thermodynamically most stable.

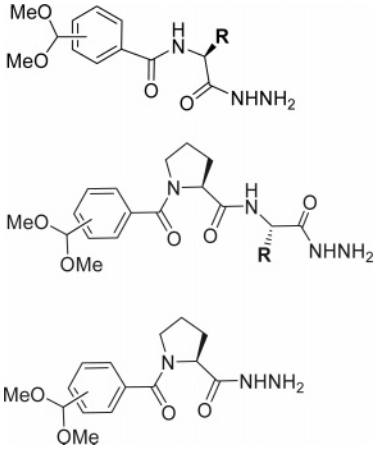
In the context of dynamic combinatorial chemistry, oxime exchange has thus far only been studied by Eliseev's laboratory.[147] Oximes obtained from aromatic aldehydes were found to be quite stable to hydrolysis in aqueous solutions, whereas oximes from aliphatic aldehydes form only incompletely upon mixing the components. The reactivity of *O*-alkyl or *O*-aryl oximes toward oxime exchange (reaction between oxime and oxyamine) in water was found to be radically different. The exchange between alkoxylamines and their oximes is first order in both reagents, the rate decreasing with increasing pH, suggesting that the rate limiting step is the formation of the tetrahedral intermediate from the preprotonated oxime (Scheme 7). Exchange reactions of aromatic analogues are first order in oxime only and are unaffected by the pH, suggesting that attack of water on the aryloxime is the rate limiting step (which can also lead to minor amounts of hydrolysis products). The rates of equilibration of the studied reactions are negligible under physiological conditions (i.e., ambient temperature in neutral aqueous media) but increase by increasing the temperature, pH, or both. Oxime chemistry was also tested successfully for the generation of diversity in acidified methanol (1% TFA, 7.5 mM).[148,149]

2.1.2.3. Hydrazone Exchange. While imines condense and hydrolyze quickly at neutral to acidic pH but are unstable to the presence of water, hydrazones can be thermodynamically stable even at low pH, while they tend to be kinetically inert under neutral conditions. The mesomeric effect decreases the electrophilicity of the hydrazone, slowing down hydrolysis and exchange.[150] Only under acidic conditions (pH 4 or below) or at high temperatures do hydrolysis and exchange become significant. Most of the applications of hydrazones in dynamic combinatorial chemistry feature acyl hydrazones in which the acyl group moderates the stabilizing influence of the amine subunit in C═N−NRR′. Without an acyl or similar electron withdrawing group hydrazones tend to be too stable for use in dynamic libraries.

The hydrazone exchange reaction is chemoselective and thus compatible with a wide range of solvents and functional groups. DCLs based on hydrazone exchange have been reported using metal ions, carbohydrates,[151] nucleic acids, or peptides as building blocks or templates without observing any interference.[152]

We have explored libraries based on pseudo-mono- and dipeptides carrying aromatic acetal-protected aldehyde and aliphatic acyl hydrazide functionalities (Chart 1). Dissolution

Chart 1. Pseudopeptide Building Blocks for DCLs of Acyl Hydrazones[153−161]



of these building blocks in organic solvent (5 mM building block in chloroform containing small amounts of methanol or DMSO) containing excess TFA (5 to 25 mM) gives an equilibrium mixture within days[153,154] or weeks.[155] Addition of crown ether to these equilibrating solutions leads to the trapping of linear hydrazides capped by the crown ether, indicative of the presence of linear species in the mixtures and indicating their likely intermediacy in the exchange process.[156] These pseudopeptides have resulted in receptors for cationic templates such as quaternary ammoniums,[155,157−159] and alkali cations[160,161] (see section 4.1.2.6).

Poulsen and co-workers recently reported a carbohydrate analogue of these amino acid building blocks.[151]

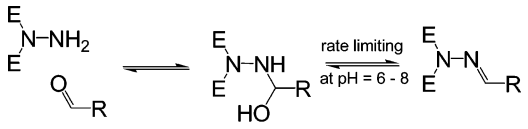
The Lehn group has used hydrazone exchange to generate DCLs in water with the aim of using biomolecules as templates.[162−164] Because the conditions for exchange (pH 4) are not compatible with most biomolecules, the equilibration reaction had to be carried out in the absence of the target, which was only introduced into the mixture after it was adjusted to physiological pH.

The same group have also used hydrazone exchange in organic solvents using TFA as a catalyst to give dynamic covalent polymers and foldamers.[165−167] Although the formation reaction takes place at room temperature within days, the exchange of monomer units usually requires heating (90−120 °C for several minutes). For hydrazones that are highly stable even at elevated temperature or in the presence of acid (e.g., hydrazones prepared from pyridine-derived difunctionalized hydrazine and carbaldehyde buiding blocks), exchange can be catalyzed using Lewis acids in $CHCl_3$.[168]

Recent studies on hydrazone-based DCLs aimed at developing new extraction agents for metal ions[169] and studies on nucleic acid replication under dilute conditions (0.01 mM)[170] demonstrate that the exchange can take place in aqueous medium close to neutrality in a reasonable time scale. However, the presence of an appropriate template is partly responsible for the relatively fast rate of equilibration.

In a recent fundamental study, Nguyen and Huc have demonstrated that hydrazine derivatives bearing electron-withdrawing substituents can form hydrazones that are sufficiently activated to be hydrolyzed and exchanged even at neutral pH.[152] The rate-determining step in the hydrolysis reaction is the acid-catalyzed attack of water on the hydrazone, while for the reverse reaction (hydrazone formation), the dehydration step is rate limiting (Scheme 8). In agreement

Scheme 8. Reversible Hydrazone Formation[a]



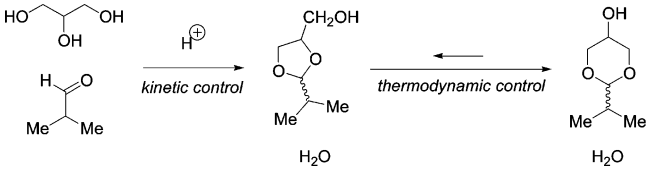
[a] E represents an electron-withdrawing group.[152]

with the above mechanism, the rate increases with the electron-withdrawing strength of nitrogen substituents E, presumably because of enhanced electrophilicity of the hydrazone. In the presence of strongly electron-withdrawing substituents equilibrium can be reached within minutes at pH 6−8 (building block concentrations 0.5−10 mM). Above pH 8, the exchange becomes base catalyzed and also here strongly electron-withdrawing groups on the hydrazine accelerate the process. The authors have varied the nature of the aldehyde and have observed that the equilibrium constant of hydrazone formation of a given hydrazine is several times higher with an aromatic aldehyde such as 4-carboxybenzaldehyde than with an aliphatic one such as isobutyraldehyde.

2.1.3. Acetal Exchange

The well-known acid-catalyzed formation of cyclic acetals from diols and aldehydes provides a good illustration of a thermodynamically controlled reaction.[171] A classical example is the condensation, under acidic conditions, of glycerol with isobutyraldehyde (Scheme 9).[172]

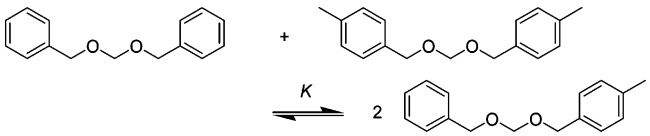
Scheme 9. Thermodynamic Control in the Formation of Five- and Six-Membered Cyclic Acetals[172]



The five-membered ring adducts (dioxolane, cis and trans isomers) are formed faster. However, the six-membered ring compounds (dioxane, cis and trans isomers) are thermodynamically more stable and, as the reaction is reversible, they become the major products in the course of time. The acid-catalyzed equilibration of the dioxolane/dioxane mixture does not necessarily need water because it can proceed via an oxycarbenium ion.

The Mandolini group have shown that the transacetalation between formaldehyde acetals (Scheme 10) proceeds within

Scheme 10. Transacetalation between Formaldehyde Acetals[173]



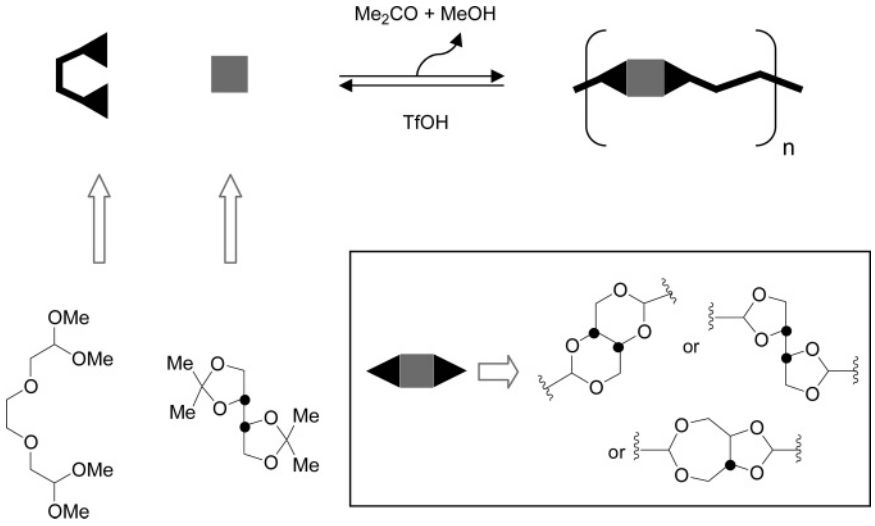
a few days at millimolar concentrations after addition of a Brønsted acid catalyst (triflic acid) in organic solvents (CD_3CN or $CDCl_3$).[173] The authors have used a simple but clever procedure to demonstrate that the process is under thermodynamic control. Two libraries were prepared at two different concentrations, leading to two different library compositions. After equilibration, the most concentrated library was diluted, so as to reach the concentration of the least concentrated library. At the end of this procedure, analyses by ^1H NMR revealed two identical library compositions.

The Fuchs lab used the transacetalation between a D-threitol derivative and a diacetal to generate a very complex mixture of linear and cyclic compounds (Scheme 11).[174]

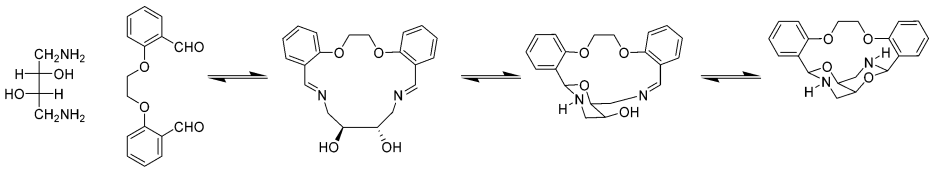
The same authors have used diaminobutanediols instead of threitol to generate equilibrium mixtures of tautomeric macrocycles in DMSO (Scheme 12).[175,176]

The reversible formation of dithioacetals, well-known in synthetic chemistry as carbonyl protecting groups, has been exploited by Hirsch and co-workers to generate equilibrium mixtures of tris(thioacetals).[177] They demonstrated that condensation of a benzene hexathiol unit with *p*-tolualdehyde in refluxing $CHCl_3$ in the presence of a Lewis acid ($Zn(OTf)_2$, 10 mol %) went to completion in 24−72 h (Scheme 13). ^1H NMR and mass spectrometry analyses showed that isomeric α,α,α and α,α,β tris(thioacetals) had been produced in a statistical ratio (1:3 respectively). The products could be isolated, confirming the inertness of the compounds in

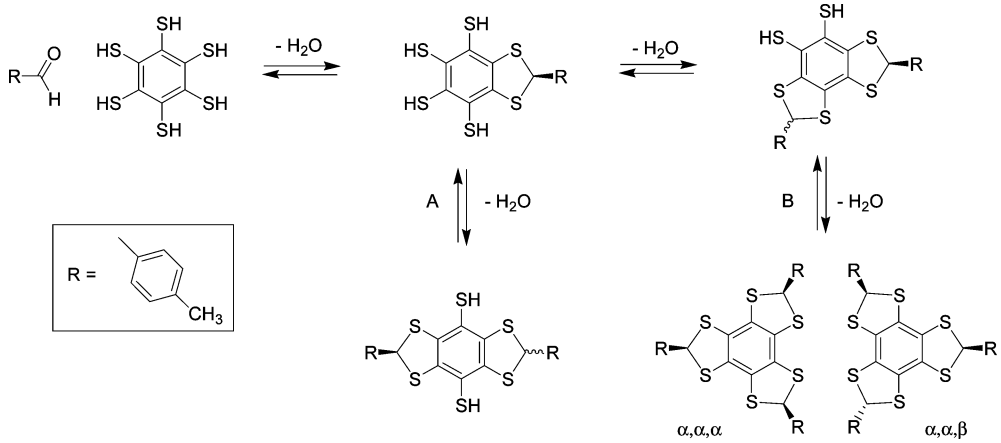
Scheme 11. Thermodynamic Control over the Formation of Oligomeric Acetals[174]



Scheme 12. A Thermodynamically Controlled Tautomeric Mixture of Macrocycles[175,176]



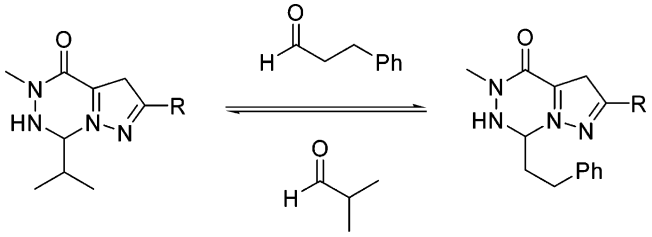
Scheme 13. Thermodynamically Controlled Formation of Isomeric α,α,α and α,α,*β* Tris(thioacetals)[177]



the absence of catalyst. The Lewis-acid catalyst induced reversibility in pathway A, leading to the bis(thioacetals), but not in pathway B, giving the tris(thioacetals).

Wipf et al. recently reported a new reversible cyclocondensation reaction between a pyrazolotriazone and one or more aldehydes (Scheme 14).[178] The exchange reaction

Scheme 14. Metathesis of Pyrazolotriazones under Thermodynamic Control[178]



proceeds in water at pH 4, and equilibrium was reached in 3 days at 40 °C (1 mM concentration for each starting material). Exchange could be stopped by raising the pH to 7, allowing for the isolation of selected library members.

2.1.4. Reversible Diels–Alder Chemistry

It is well established that some Diels−Alder reactions can be reversible.[179,180] The [4 + 2] process is exothermic since two π-bonds are converted into two σ-bonds. Therefore the cycloreversion is anticipated to involve a relatively high enthalpy of activation, which would make it less suitable for use in dynamic combinatorial chemistry. However, cycloadditions are facilitated if one of the reactants is electron-rich (normally the diene) and the other is electron-poor (normally the dienophile), the result being a relatively small energy gap between the reaction orbitals ($HOMO_{diene}$ and $LUMO_{dienophile}$).

Following this principle, the Lehn group have recently described a Diels−Alder reaction that is designed to be reversible under particularly mild conditions.[181] For example, mixing functionalized fulvenes with diethyl dicyanofumarate in chloroform (100 mM) leads to an equilibrium mixture of

starting material and cycloadduct within a minute at 25 °C (Scheme 15). The equilibrium position depends on the nature

Scheme 15. Diels−Alder Chemistry under Thermodynamic Control[181]

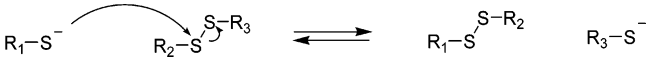
of the substituents on the reactants and shifts toward the adduct when the temperature is reduced.

Diene exchange experiments confirmed that the reaction is indeed under thermodynamic control. For instance, addition of an excess (5 equiv) of a different fulvene to an equilibrated mixture leads to the displacement of the equilibrium toward the adduct incorporating the new fulvene. Reversible Diels−Alder chemistry is an attractive new tool in dynamic combinatorial chemistry that may well lead to practical applications since, for instance, cycloadducts of polycyanoalkenes are well-known for their biological activity. However, the questions of the scope of the substrates able to give such behavior in mild conditions and of finding a way to freeze the exchange process still need to be addressed.

2.1.5. Disulfide Exchange

The disulfide exchange reaction plays an important role in biology because it is involved in the folding of proteins,[182] in the maintenance of the redox state of cells,[31] and in, for instance, the cleavage of DNA by calicheamicin and esperamicin.[183] The mechanism involves nucleophilic displacement of a thiolate anion from the disulfide through attack by another thiolate anion (Scheme 16).[184] Exchange requires

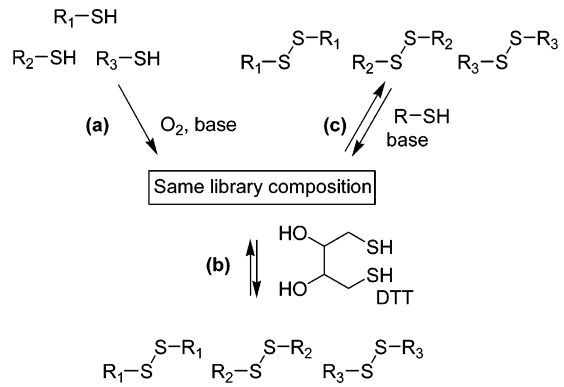
Scheme 16. Disulfide Exchange



deprotonated thiol and is therefore highly pH-dependent, allowing the exchange process to be halted by lowering the pH. Near neutral conditions (pH 7−9) are usually adequate to generate a sufficient amount of thiolate anion to mediate disulfide exchange.

Two starting positions are possible for generating DCLs of disulfides: (i) from thiols, which can be submitted to irreversible air oxidation and simultaneous disulfide exchange mediated by residual thiolate (Scheme 17a), or (ii) from

Scheme 17. Different Approaches to DCLs of Disulfides



"preoxidized" disulfides, reactivated by the addition of a catalytic amount of reducing agent such as dithiothreitol[185] (DTT; Scheme 17b) or "free thiol" (Scheme 17c).

We have described the first example of large dynamic libraries of macrocyclic disulfides formed from a range of aliphatic dithiol building blocks, including carbohydrate and α-amino acid derivatives, in water.[186] When four different dithiols (10 mM overall) were submitted to (irreversible) air oxidation and simultaneous (reversible) disulfide exchange (in water under atmospheric oxygen at pH 7.5 for 24 h), a mixture of macrocyclic disulfides was obtained. To ascertain that the mixture was under thermodynamic control, two different pre-equilibrated libraries were made that contained only two of the four building blocks. After oxidation of these sublibraries was complete, they were mixed, and the exchange reaction was reactivated by addition of a substoichiometric amount of DTT (15 mol %). The final composition obtained after equilibration of this mixture was identical to the one obtained from the library in which all four building blocks were mixed together from the start. Under acidic conditions (pH ≤ 2.5), no exchange was detected.

Disulfide exchange is one of the few reversible covalent reactions that are compatible with biomolecules (as long as surface-exposed thiol or disulfide groups are not present).[187−189] Under highly dilute conditions (usually as a result of limited availability or solubility of the biomolecule template), equilibration through disulfide exchange is inevitably slow. The labs of Kumar[190] and Balasubramanian[191−193] have tried to solve this problem by performing the experiments in the presence of excess glutathione. The glutathione (GSH)/oxidized glutathione (GSSG) pair acts as a redox buffer and allows the use of relatively low concentrations of building blocks (40−200 μM at pH = 7.40−7.50 in the presence of GSH/GSSG: 500 μM/125 μM), while reaching equilibrium within 12−48 h at room temperature. Equilibration was confirmed by starting from dimeric species generated under kinetic conditions (for instance, with $K_3[Fe(CN)_6]$ as oxidant) and re-equilibrating in the presence of glutathione redox buffer to give identical distributions compared to the libraries set up from the thiol building blocks. Because the GSH/GSSG pair is used in large excess compared to the building blocks, a distinct disadvantage of this approach is the resulting bias toward the formation of adducts between glutathione and the building blocks.

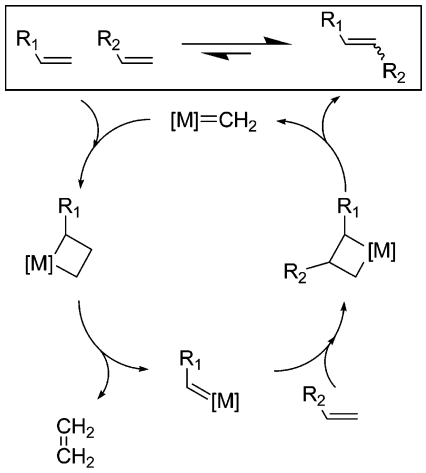
Disulfide exchange can also take place in organic solvents. For instance, when a solution of two aromatic disulfides in chloroform (8.6 mM) was treated with a catalytic amount of a monomeric thiol and triethylamine (2−10 mol % each) a nearly statistical distribution of the three expected disulfides was obtained in the absence of template after 36 h at room temperature.[28] We have used aliphatic bisthiols as building blocks to generate DCLs of macrocyclic disulfides in chloroform; the less acidic aliphatic thiols required use of a stronger base (DBU) to give oxidation and equilibration in 3−4 days.[194] Because the use of a strong base may not be compatible with all types of building blocks, catalysis of the exchange process by rhodium salts in acetone is an attractive and milder alternative. This procedure has been used successfully to perform disulfide exchange on peptides without any trace of racemization[195] and might well become a useful tool in dynamic combinatorial chemistry.

2.1.6. Alkene Metathesis

Alkene metathesis has emerged as an indispensable tool for chemists for the formation of C−C bonds through an

essentially reversible process using organometallic catalysts under mild conditions. The reaction proceeds through a series of [2 + 2] cycloaddition/cycloreversion steps, which involve alkenes, metallacyclobutanes, and carbene complexes (Scheme 18).[196] Since all individual steps are in principle reversible, an equilibrium mixture should be obtained.

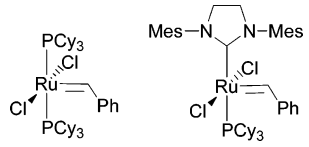
Scheme 18. Basic Catalytic Cycle for Cross-Metathesis[a]



[a] Since all steps are in principle reversible, the overall transformation should proceed under thermodynamic control.

Grubbs' catalyst [$(PCy_3)_2Ru(CHPh)Cl_2$] (Chart 2) is probably the most used transition-metal complex for cross-metathesis reactions due to its reasonable stability toward oxygen, water, and minor impurities that can be present in solvents. It is, in addition, largely compatible with a wide range of aliphatic and aromatic reactants, for instance, alkenes carrying epoxide, ester, sulfone, or aromatic aldehyde functions, as demonstrated in the preparation of large equilibrium libraries in the presence of Grubbs' catalyst (2 mol %) in CH_2Cl_2 at 45−50 °C for 16 h.[197] However, the catalyst may be deactivated by functional groups that can coordinate to the catalyst (e.g., phosphines, amines, phenols, nitriles).

Chart 2. First- (Left) and Second-Generation (Right) Grubbs' Catalysts (Mes = 2,4,6-trimethylphenyl)



The functional group tolerance of the first generation Grubbs' catalyst, as well as its activity, can be enhanced by the substitution of one of the phosphine ligands by a N-heterocyclic carbene to produce the second-generation Grubbs' catalyst (Chart 2).[198] This catalyst has been shown to be compatible with a pyridine-based template in a library prepared from alkene functionalized building blocks.[199] Another example involves α,β-unsaturated esters, which are "metathesis-inactive" substrates when the first generation catalyst is used yet produce a mixture of dimeric, trimeric, and tetrameric macrocycles in the presence of the second-generation catalyst in CH_2Cl_2 under reflux for 11 h.[200]

Alkene cross-metathesis can also be used with internal alkenes as starting materials. Ward and Brändli performed pairwise reactions from a pool of different internal alkenes (ca. 2 mM in CH_2Cl_2 with 0.01 equiv of first-generation Grubbs' catalyst, 36 h stirring under argon).[201] Statistical mixtures of all 10 expected alkenes, 20 including (*E*)/(*Z*) isomers, were observed. To prove that the reaction is under thermodynamic control, the authors introduced a third alkene to an equilibrated mixture of two compounds. After 48 h, analyses revealed the anticipated statistical distribution of products.

Alkene metathesis can also be performed in water. Nicolaou et al.[202,203] have made libraries using up to eight alkene building blocks (110 μM each), which were dissolved in degassed water in the presence of a phase-transfer agent ($C_{12}H_{25}NMe_3Br$, 2.75 mM) and Grubbs' catalyst (first generation, 176 μM) in CH_2Cl_2. The solvent was then removed until only a trace remained (<5%), and after 24 h of stirring at room temperature, the mixtures were analyzed. The libraries consisted of a statistical mixture of the various adducts in the absence of target, whereas exposure to a template introduced some bias in the library distribution, probably as the result of a kinetic template effect.

Attempts to generate a water-soluble metathesis catalyst have thus far met with only moderate success,[204] but further developments in this direction are eagerly awaited.

One practical difficulty in the use of alkene metathesis in dynamic combinatorial chemistry is the separation of the catalyst to stop the exchange process. A possible solution would be to immobilize the catalyst on a solid support, allowing removal through a simple filtration. However, thus far, immobilized versions of Grubbs' catalyst are at least 2 orders of magnitude less active than their solution-phase analogues.[205]

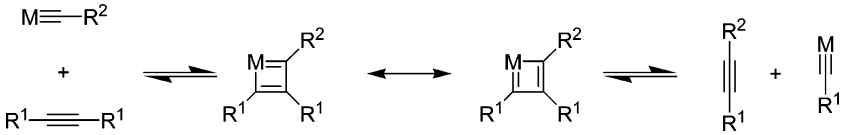
Equilibration usually requires a large number of individual exchange reactions, and in our experience, the current generations of metathesis catalysts tend not to survive a sufficient number of turnovers to achieve complete equilibration.

2.1.7. Alkyne Metathesis

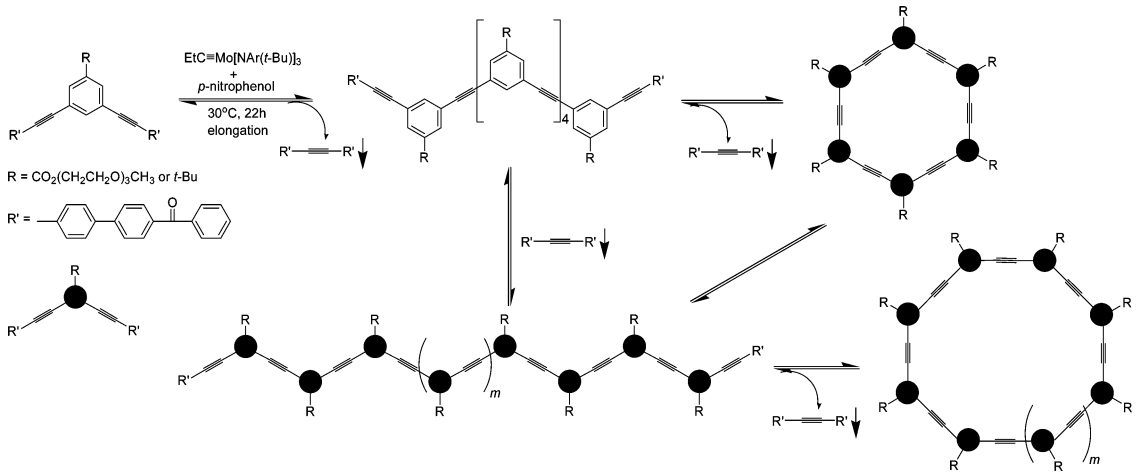
So far, alkyne metathesis has remained in the shadow of alkene metathesis. But the recent development of new alkylidyne complexes that allow alkyne metathesis of highly functionalized substrates under mild conditions should stimulate a more widespread use of this reaction. The accepted mechanism of the process is outlined in Scheme 19.[206]

In an elegant study, Zhang and Moore have used a molybdenum-based metathesis catalyst to generate an equilibrium mixture of arylene ethynylene macrocycles (conditions, 22 h at 30 °C in CCl_4) (Scheme 20).[207] Analysis of the reaction mixture during the early stages of the reaction revealed the presence of linear oligomers and larger macrocycles. As the reaction progressed, these were transformed into the thermodynamically more stable hexameric product.

Scheme 19. Mechanism of Alkyne Metathesis



Scheme 20. Alkyne Metathesis under Thermodynamic Control Leading to the Preferential Formation of Cyclic Hexamer[207]



The same product was formed when starting from kinetically synthesized phenylene ethynylene polymer. The authors observed that alkyl-substituted alkynes can be problematic in cross-metathesis experiments involving arylene ethynylene chemistry. Alkyl alkynes are more reactive than aryl alkynes, so the catalyst spends most of its time turning over the alkyl-substituted species with the result that the cross-metathesis of arylene ethynylenes is severely impeded.

2.2. Reactions Involving Noncovalent Bonds

Kinetically labile hydrogen and coordinative bonds have been exploited with success by chemists for the self-assembly of various supramolecular architectures.[208−211] The relatively predictable directionality offered by both metal−ligand and hydrogen bonds has traditionally been exploited to direct self-assembly processes to well-defined target structures. More recently, efforts are being made to combine different building blocks to form dynamic combinatorial libraries of noncovalent assemblies.

2.2.1. Metal−Ligand Coordination

The use of metal−ligand interactions in dynamic combinatorial chemistry requires ligand substitution reactions to be fast. The rates of these reactions depend on a large number of factors such as the nature of the metal, charge, steric and electronic effects of the ligands, and additives. The kinetic lability of a metal−ligand bond depends mainly on crystal-field effects, such as ligand-field stabilization energy (LFSE) and Jahn−Teller distortion, and the characteristics of the ligands. For instance, the LFSE may constitute a barrier to the geometrical change required in the transition state of both dissociative and associative ligand exchange mechanisms leading to high Gibbs energies of activation (e.g., Cr^{3+} and Co^{3+} ions tend to form kinetically inert complexes). In contrast, Jahn−Teller distortion usually leads to a complex with two elongated metal−ligand bonds and rapid ligand exchange (e.g., complexes based on Cu^{2+} and Cr^{2+} are kinetically very labile).

A rough idea of the influence of the nature of the metal ion on ligand exchange rates can be obtained from the rate of substitution of water on metal ions (Figure 4).[212]

So far, complexes based on Co(II),[213−215] Cu(I),[216] Cu(II),[217−219] Fe(II),[20,25,26,215,220−222] Ga(III),[223] In(III),[224] Ir(II),[225] Ni(II),[226] Pd(II),[216,227−230] Rh(II),[231,232] Ru(II),[116,225,232] Ti(IV),[224,233−235] and Zn(II)[16,24,231,236−239] ions have been used to generate DCLs via metal−ligand exchange. The exact

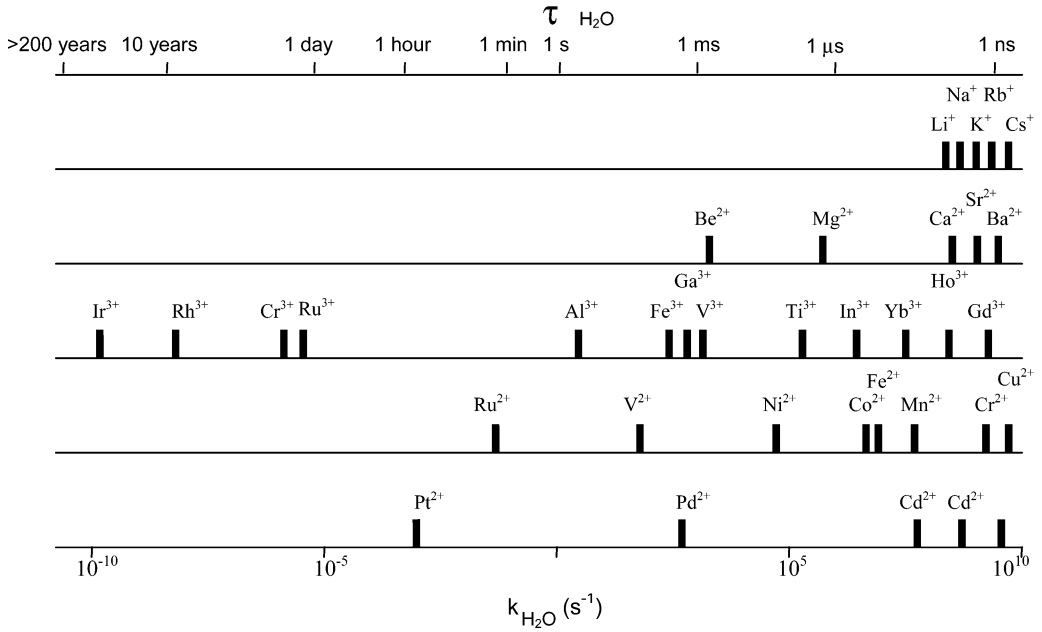
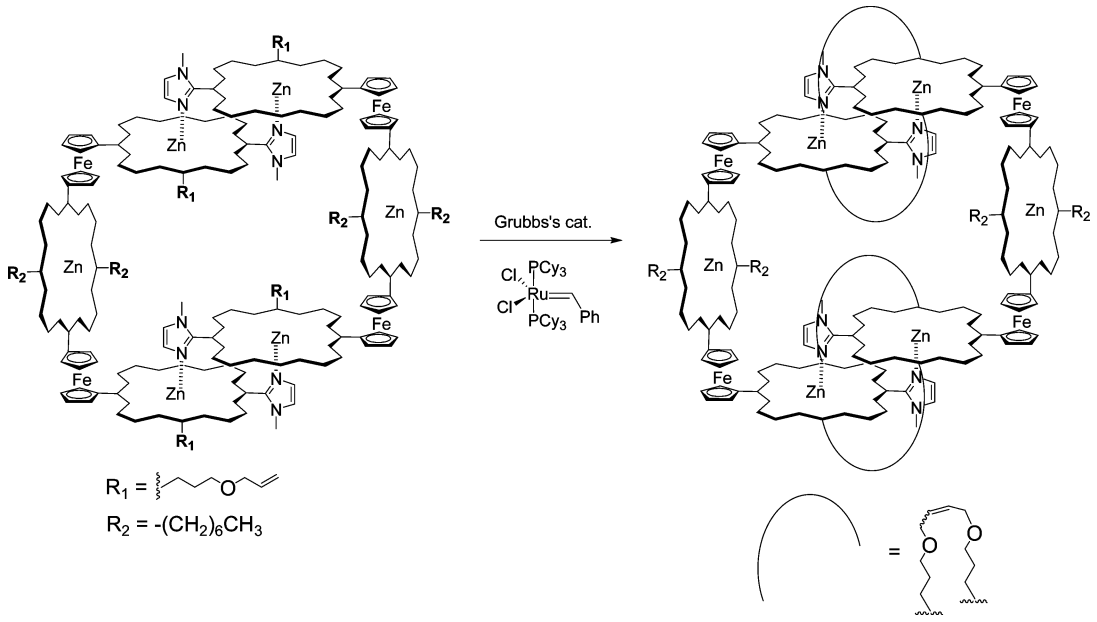


Figure 4. Water exchange rate constants (k) and mean lifetime (τ) in the first coordination sphere of metal ions at 25 °C.

Scheme 21. Freezing the Composition of a Dynamic Library Based on Metal−Ligand Interactions by Covalent Capture through Alkene Metathesis[239]



experimental conditions required for efficient exchange vary considerably depending on the metal and the type of ligand used.

Not only the metal ion but also the ligand has a large influence on the exchange kinetics of coordination complexes. Where most Ru(II)−bipyridine complexes are kinetically inert, we have generated equilibrium mixtures of Ru(II) porphyrin−phosphine complexes by mixing a bis-diphenylphosphine (2.5 mM) with a stoichiometric amount of a Ru(II) porphyrin in chloroform at ambient temperature.[231,232]

Metal−ligand exchange can be compatible with biomolecules (e.g., peptides, oligonucleotides) as templates, building blocks, or both. For example, libraries of bis(salicylaldiminato)zinc(II),[24] mono- and bis(salicylamide)copper(II),[218] and bis(pyridine)iron(II)[25,26,221] complexes have been generated successfully in physiological buffers within 0.5−32 h at room temperature.

The Achilles' heel of using metal−ligand interactions is the fact that they are usually labile, which severely hampers the isolation and handling of individual library members. Exceptions are cases where the number of noncovalent connections within the complex is very large such as in Lehn's Fe(II)−bipyridine helicates, which are held together by 30−36 coordinative bonds.[20] In most other cases, a means of "freezing" the exchange process is desirable.

Since a number of metal ions have comparable geometries yet vastly different exchange kinetics, synthesis of stable analogues of labile complexes identified in dynamic combinatorial experiments is possible. For instance, McLendon and co-workers have used kinetically labile Fe(II)−bipyridine complexes in screening and subsequently synthesized kinetically inert Ru(II)−bipyridine analogues of library members of interest for further studies.[221]

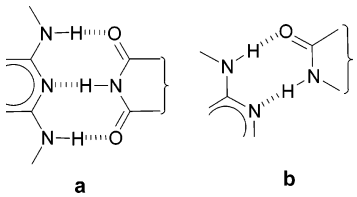
For some complexes, the exchange can be stopped in situ by changing the oxidation state of the metal center. For example, the oxidation of cobalt(II) to cobalt(III) in self-assembled superstructures results in kinetically inert complexes.[213,214] Another method for freezing the library involves covalent capture of the library members. For example, trisporphyrins carrying allyl ether substituents at their *meso*-positions have been used to generate a DCL of macrocyclic structures through nitrogen−zinc exchange.[239] After equilibration, addition of Grubbs' catalyst covalently linked the building blocks together (Scheme 21). A final but less general solution is the selective crystallization of specific library members as reported by Duan, Meng, and co-workers.[219]

2.2.2. Hydrogen Bonding

Hydrogen bonds between neutral partners in solution typically have energies between 0 and 20 kJ/mol[240] and have a preference for a linear arrangement of the three atoms involved (X−H···A angle ∼180°). The orientation of the donor with respect to the acceptor is usually determined by the position of the lone pair of electrons on this atom.

Allen et al. have conducted a statistical analysis of the hydrogen-bond arrangements that occur in the >160 000 crystal structures in the Cambridge Structural Database (CSD), revealing that motifs **a** and **b** (Chart 3), which have

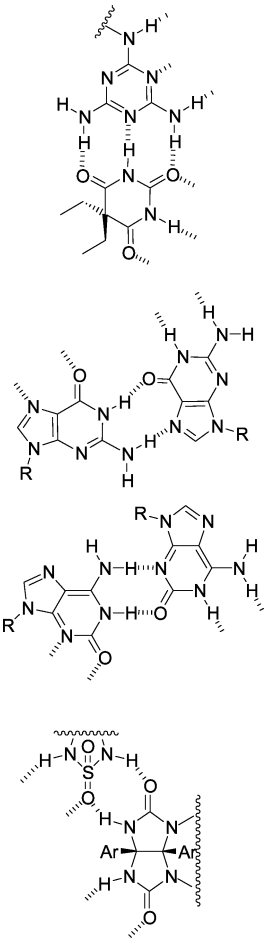
Chart 3. Prevalent Hydrogen-Bond Motifs



strong analogies with nucleotide recognition in DNA, have the highest probability of formation (these H-bonding arrangements formed in 97% and 91% of CSD entries that contained both subunits, respectively).[241]

Inspired by these complementary hydrogen motifs from nature, Reinhoudt, Crego Calama, Timmerman, and co-workers described the first example of a DCL based on hydrogen-bonded assemblies in 1998.[242] Using combinations of donors and acceptors (Chart 4), they have built highly complex superstructures held together by an impressive number of up to 36 hydrogen bonds.[243] The libraries were generated by mixing the building blocks (5 mM each) in

Chart 4. H-Bonding Motifs Exploited to Build DCLs by the Groups (from Top to Bottom) of Reinhoudt, Crego Calama, and Timmerman,[242−244,250,251] Davis,[245] and Rebek.[248]



apolar solvents at 0 °C in order to maximize the strength of the hydrogen bonds. To initiate the exchange process, the temperature had to be raised to 15 °C, reflecting the relatively high stability of the linkages in apolar solvent. Equilibration occurs within seconds in more polar solvent, such as $CDCl_3$, in which the library is kinetically inert only below −50 °C. To "freeze" the exchange process permanently, covalent linkages between the building blocks have been introduced.[244] The authors used modified building blocks carrying oct-7-enyl side chains that can undergo ring-closing metathesis under standard conditions in toluene, without affecting the hydrogen-bonding network.

Hydrogen-bonding patterns inspired by natural products have also been studied by the Davis lab.[245] Upon dissolution of guanosine or isoguanosine in organic solvents (CD_2Cl_2, 5.5 mM) at room temperature in the presence of a cation template, homotetrameric (for guanosine) and homopentameric (for isoguanosine) macrocycles were obtained. Each of these arrangements display their own unique hydrogen-bonding pattern as shown in Chart 4. When guanosine and isoguanosine were mixed in the same library, the result was the self-association of each isomer. This phenomenon of self-sorting has been extensively investigated in hydrogen-bonded assemblies by Wu, Isaacs, and co-workers using a large range of classical building blocks, including the molecules reported in the previous paragraph, which, among them, can form a potentially very large number of partnerships.[246,247] However, from analysis of a mixture of nine building blocks in $CDCl_3$, the authors have demonstrated that the association takes place preferentially between the established host−guest pairs.

Thus, while the structural information encoded within the H-bond linkage tends to dictate the size and the shape of hydrogen-bonded assemblies to give a unique final structure, diversity can be achieved through the use of different substituents on the skeleton of the building blocks. Rebek and co-workers have used this strategy to form a dynamic library of capsules through the self-assembly of a cyclic sulfamide donor and a glycoluril acceptor carrying different substituents on the spacer (Chart 4; monomer concentration of 0.1−1 mM in CH_2Cl_2).[248]

A major limitation of H-bonded assemblies is that the resulting aggregates usually do not survive in polar solvents and do not tolerate the presence of water. Wu et al. have recently reported that the combined use of $\pi-\pi$ interactions and hydrogen bonds provides an improvement in the stability of aggregates, which remain stable in solvents ranging from C_6D_6 to D_2O.[249]

2.3. Dynamic Combinatorial Libraries Involving Multiple Exchange Processes

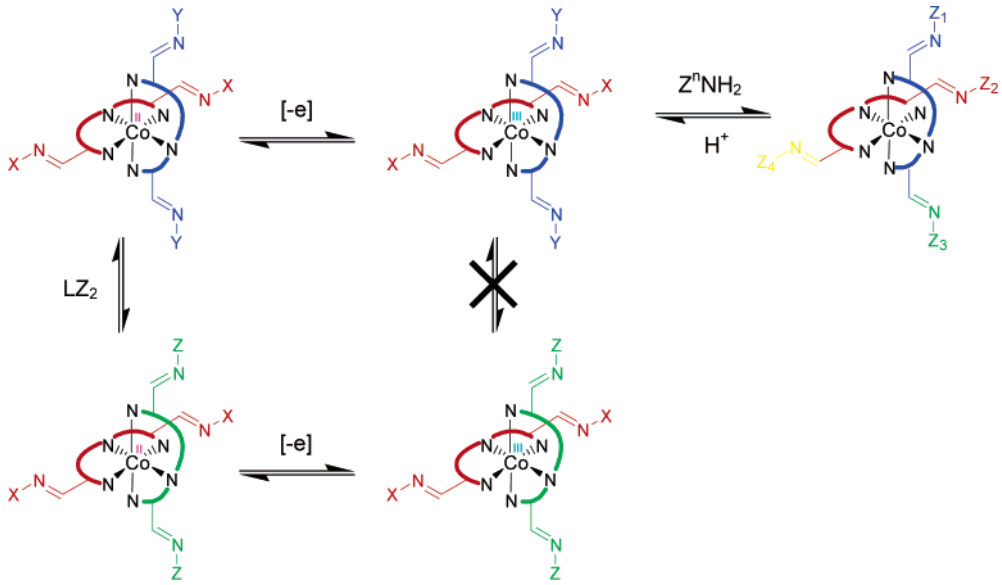
Having more than one exchange reaction in a DCL has some intriguing benefits but also brings with it additional complications in addressing the chemistries independently. When multiple exchange processes are used that are orthogonal, the possibility arises for an evolutionary approach: each reversible chemistry can carry its own dimension in structural space, and these dimensions can be explored alternatingly, keeping one dimension constant (exchange switched off) while exploring the other (exchange switched on) and vice versa.

Another advantage of such "multilevel" libraries is the extension of the diversity within the libraries since the building blocks are held together by a variety of linkages. Thus far, only a few examples of libraries involving more than one exchange process have been reported.

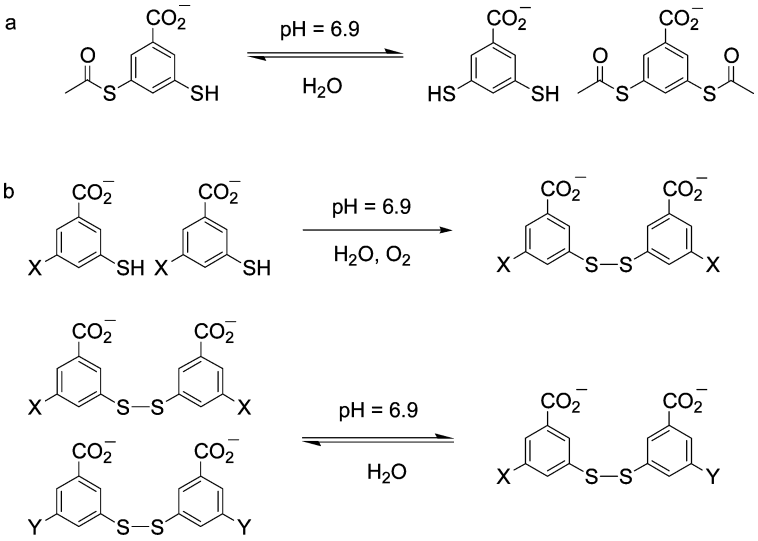
Eliseev, Lehn, and co-workers have described the only example to date of an *orthogonal* library, where the two exchange reactions can be addressed independently.[213] The first process is the formation of cobalt complexes with terpyridine-based ligands, and the second is imine formation with aldehyde substituents on the terpyridine moieties (Scheme 22). When they started from cobalt(II) complexes (3×10^{-4} to 3×10^{-6} M in 30% acetonitrile/water at pH 7.0 and 25 °C), ^{1}H NMR and ESI-MS analysis indicated a rapid exchange with a second competing ligand (Scheme 22, LZ_2). Metal−ligand exchange can be stopped by oxidation of the metal center to form kinetically inert cobalt(III) complexes. The second exchange process (imine exchange) is activated by lowering the pH to values below 3.0 and heating to 60 °C in the presence of various amines (Scheme 22, Z^nNH_2).

An example where two exchange reactions (thioester exchange and disulfide exchange) can be performed simultaneously and communicate with each other, has been recently described by us.[252] When a building block carrying a thiol and a thioester functionality was dissolved (5 mM) in the absence of oxygen in 10% methanol/water at pH 6.9 and 25 °C, only thioester exchange took place (Scheme 23a). Exposing the mixture to atmospheric oxygen causes disulfide oxidation and subsequently disulfide exchange. (Scheme 23b). Thus, the two exchange processes can be activated consecutively but then proceed simultaneously. The exchange

Scheme 22. First Example of Orthogonal Exchange Processes in Dynamic Combinatorial Chemistry[213]



Scheme 23. Simultaneous Thioester (a) and Disulfide (b) Exchange[252]



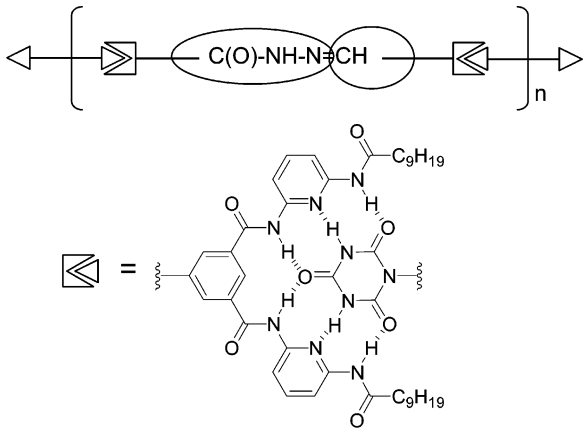
processes communicate with each other because they share the same functional groups to generate a dynamic library of linear and cyclic oligomers from a single building block.

Two examples of libraries involving covalent and hydrogen bonds have been reported. Kolomiets and Lehn used reversible acylhydrazone bonds and hydrogen bonds to generate polymeric materials from bis-functionalized building blocks (5 mM in [D_2]tetrachloroethane; see Chart 5).[167]

The double dynamic nature of the process has been demonstrated by covalent and noncovalent end-capping experiments with mono-functionalized species (e.g., hexanal), which led to a shortening of the polymers.

Sijbesma, Meijer, and co-workers have used simultaneous disulfide and hydrogen-bond exchange (Chart 6).[253] Starting from preoxidized disulfides in $CDCl_3$ in the presence of triethylamine (1 equiv) and DTT (0.05 equiv), an equilibrium mixture of cyclic and linear oligomers was obtained after 20 or 50 days depending on the concentration (150 or 53 mM respectively).

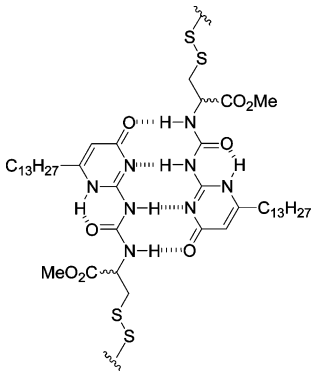
Chart 5. Double Dynamic Polymers Based on Reversible Hydrazone and Hydrogen-Bond Linkages[167]



3. Experimental Considerations

Apart from the chemistry related to the specific reversible reactions that can be used to generate a DCL, there are a number of general experimental considerations that need to be addressed before the DCLs can be used for screening for molecular recognition. We will discuss three important issues below. Section 3.1 reviews some methods that can be used to check that experiments are run under thermodynamic

Chart 6. Dynamic Polymers Based on Reversible Disulfide Exchange and Hydrogen-Bonding Interactions[253]



control; sections 3.2 and 3.3 address issues related to the interplay between building block concentration and polymerization and between building block design and self-sorting, respectively, that can arise when building blocks carry two reversible linkages.

3.1. Methods for Confirming That Equilibrium Has Been Reached

Molecular recognition induced shifts in product distribution are the key to the identification of hits in dynamic libraries. These shifts rely on the library being under thermodynamic control, and it is therefore important to check that the experimental conditions used allow the reaction mixture to indeed reach equilibrium. The most common method is to demonstrate that the same product distribution is obtained from two (or more) different starting points. For example, the product distribution in a library made by mixing a series of building blocks together at the start of the experiment should be the same as that obtained upon mixing two sublibraries that contain a subset of the building blocks (see section 2.1.5).

Alternatively, it is possible to monitor a change in the library composition in response to a change in an experimental variable such as temperature, concentration, pressure, or solvent composition and ascertain that the library composition returns to the starting composition after reverting to the original experimental conditions (see section 2.1.3 and ref 173 for an example). It is also possible to add a template to shift the equilibrium in one direction and then to remove the template by complexation to a further component, thereby reverting to the original composition.[156]

Finally, one can isolate one member from a library and demonstrate that upon subjecting it to the exchange conditions, it produces a product mixture identical to that from which it was isolated. This method is only applicable when the isolated species and the original library have the same building block composition.

3.2. Building Block Concentrations and Ring–Chain Equilibria

The building block concentration in a dynamic combinatorial library is a key parameter because it influences the rates of the exchange reactions and, in the case of building blocks having multiple connections, dramatically influences the oligomer-to-polymer ratio. Separate concentration ranges exist in which such building blocks form predominantly macrocycles and where the major products are polymeric. In fact, macrocycles are commonly used as starting materials in equilibrium polymerization[44] to produce a large range of different types of organic,[44] inorganic,[254,255] and noncovalent polymers.[256−259] The physics behind this process was described in a pioneering theoretical study by Jacobsen and Stockmayer (referred to as the JS theory).[260] One of the predictions of the JS theory is the existence of a critical concentration below which the equilibrium composition of the system consists entirely of small macrocycles. This is a result of the fact that chain extension is a second-order reaction whereas cyclization is a first-order process, so that at low concentrations cyclization is faster than chain extension. This difference in reactivity is captured in the effective molarity, which is defined as the ratio of the first-order rate constant for *intramolecular* ring closure and the second-order rate constant of the *intramolecular* chain extension reaction.[261] Above the critical concentration, the concentration of smaller macrocycles is constant, and all additional material is incorporated into the polymer.

More recently, this theoretical description has been revisited in theoretical and experimental studies by Mandolini and others.[261−263] The occurrence of a critical monomer concentration has been confirmed for covalent[259,264] and noncovalent[228,253,264] reversible linkages. The transition from macrocycles to polymers can be detected by different techniques including size exclusion chromatography, NMR,[265] mass spectrometry,[259,264] and specific viscosity measurements.[266,267]

For reactions carried out below the critical monomer concentration, the distribution of cyclic oligomers depends on the building block concentration modulated by ring strain, which may destabilize individual macrocycles,[263] and intramolecular recognition, which may stabilize selected macrocycles.[253]

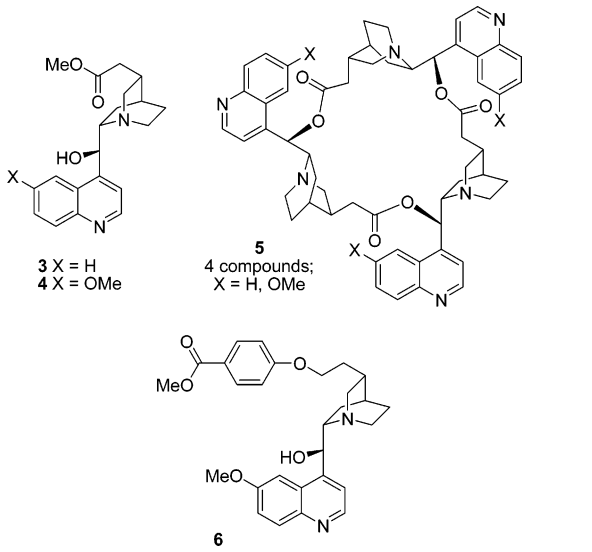
Interesting possibilities for the development of responsive "smart" materials arise when linear chains coexist with cyclic structures. Such systems will respond to external stimuli such as light[268] or heat[264] by a dramatic change in physical properties.

Building blocks can be designed to be predisposed for macrocyclization or polymerization. For instance, using rigid building blocks in which the number of available conformations is reduced can decrease the entropic penalty for cyclization. On the other hand, steric constraints may prevent cyclization and therefore drive the assembly toward linear or helical species (cf. section 4.5.3).

3.3. Building Block Design and Library Diversity

Our early work on DCLs generated through transesterification revealed an interesting relationship between the structure of the building blocks and the diversity of the resulting library. Starting from building blocks **3** and **4** (Chart 7; derived from cinchona alkaloids), all four possible trimeric compounds **5** were obtained in a statistical 1:3:3:1 ratio, together accounting for 95% of the small DCL.[62] Under thermodynamic control, hardly any dimers or tetramers or higher oligomers were formed, whereas cyclizations under kinetic control produced a mixture of trimers, tetramers, and higher oligomers. The pronounced preference for trimer production under thermodynamic conditions was believed to be linked to the rigidity of the backbone. In a later study,[269] one of the building blocks was replaced by the extended building block **6**. The extra flexibility provided by this species now allowed the formation of cyclic dimers, trimers, and tetramers, giving a total of 11 detectable species as a

Chart 7. Building Blocks and Trimers in DCLs of Alkaloid Derivatives



mixture of hetero-oligomers and homo-oligomers.[65] Diversity was further increased by incorporating additional building blocks in which the configuration of the asymmetric centers or the length of the group carrying the ester functionality was varied.[270]

Some new units differing radically from the parent cinchonidine backbone were also investigated for their increased flexibility, reduced dimensions, or both.[269,270] The main conclusion from these studies is that rigid concave building blocks have a tendency to self-sort when they differ from each other in terms of size and "bite angle" (Figure 5).

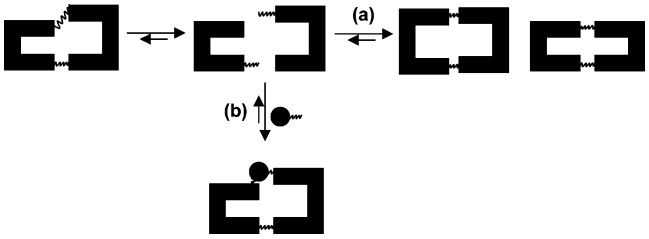


Figure 5. (a) Rigid building blocks have a tendency to self-sort; (b) introduction of a small or flexible building block allows access to mixed species.[269,270]

Any mixed species are destabilized through ring strain and therefore represent only minor species within the mixture. Diversity can be increased by the use of small and flexible units, which can bridge between rigid and disparate building blocks.

4. Applications

The unique advantage of dynamic combinatorial chemistry over traditional combinatorial chemistry is the fact that library members that engage in noncovalent interactions are favored (present in higher concentration) over their less strongly interacting counterparts. This makes DCLs attractive tools to screen for compounds that play a role in molecular recognition of some kind. At present, the main applications are in (i) selection of foldamers on the basis of internal noncovalent interactions (see section 4.5), (ii) selection of aggregates on the basis of noncovalent interactions between separate library members (section 4.4), (iii) selection of a guest by a host (section 4.3), and (iv) selection of a host by a guest (section 4.1). In the latter case, catalytically active hosts can be obtained by using guests that resemble the transition state of a chemical reaction (section 4.2).

4.1. Synthetic Receptors

Supramolecular chemists have spent decades developing synthetic receptors for a wide variety of guests. Many elegantly designed molecules have been prepared and studied, and our understanding of noncovalent interactions has undeniably improved as a result. Nevertheless, developing new synthetic receptors remains hard work and all too often involves a lengthy process of design, synthesis, evaluation, and improving the initial design followed by re-evaluation. Even then comparing the final product with the hosts produced by nature is often a humbling exercise.[271] So it is worthwhile pursuing alternatives to the design approach, and dynamic combinatorial chemistry is a particularly attractive one. Instead of having to design and synthesize complete receptors, one simply designs and synthesizes potential fragments of receptors and lets the molecules decide which of these to assemble and in which way. Considerable progress has been made over the past decade, and several synthetic receptors with impressive submicromolar affinity have been discovered from DCLs.

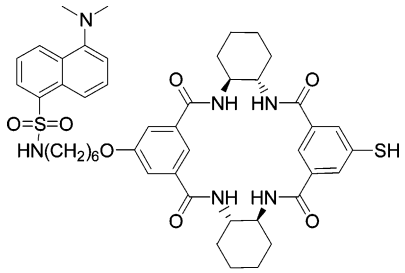
In the next sections, we review the various receptors grouped by the architecture of the building blocks starting with linear receptors made from monofunctionalized (or mixtures of mono- and bisfunctionalized) building blocks, followed by macrocyclic receptors, which are accessed through the assembly of bisfunctionalized building blocks, and finally capsule-like receptors in which individual building blocks carry more than two groups that participate in the formation of exchangeable bonds. Where applicable, subdivisions are made according to the reversible chemistry used.

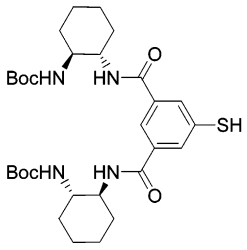
4.1.1. Linear Receptors

One of the first "linear" receptors based on dimerization of monofunctionalized building blocks was reported by Hioki and Still in 1998. The building blocks used were a thiol-functionalized macrocycle and a noncyclic analogue or benzenethiol as a bystander. The disulfide-linked bis-macrocycle was previously identified as an artificial receptor for the tripeptide D-Pro-L-Val-D-Val in chloroform.[28] The library is made from two building blocks: macrocycle A−SH and open B−SH (or benzenethiol, Chart 8). In presence of the tripeptide template, immobilized on a resin, the essentially statistical mixture shifted away from the mixed dimers and toward the homodimers, as a result of the preferential binding of the homodimer A−S−S−A to the tripeptide. The cyclic peptide dimer was obtained in high purity by a two-stage washing procedure: first the nonbinding library members were removed from the template-functionalized beads by washing with chloroform; subsequent washing with DMF liberated the receptor A−S−S−A.

In collaboration with Kubik, we have reported a receptor for inorganic anions that is constituted of a linear array of three building blocks.[272,273] Dynamic combinatorial chemistry was used here to optimize the spacer between two cyclic peptide rings. Kubik and co-workers had previously observed that the cyclic peptide **7** (Chart 9) is an efficient receptor for anions such as sulfate and iodide even in competitive mixed aqueous organic solvents.[274] Anions were found to be sandwiched between two peptide rings forming a 2:1 complex. We reasoned that linking the two peptide rings

Chart 8. Building Blocks for a Small DCL Targeting the Tripeptide D-Pro-L-Val-D-Val in Chloroform Solution[28]

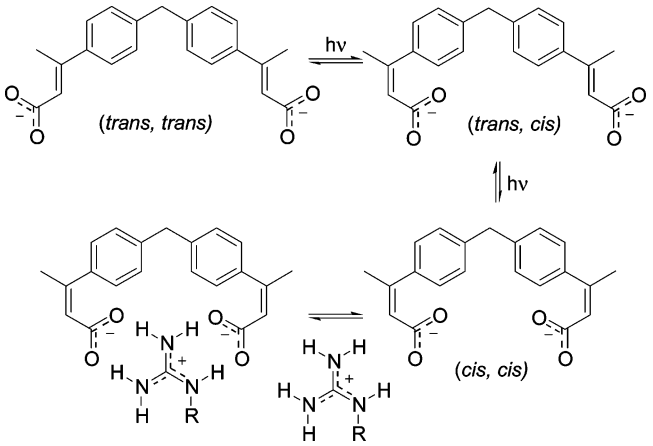




covalently would make the binding process entropically more favorable, and two parallel approaches to the problem of finding the best linker were explored. One was based on computer modeling and analysis of the X-ray crystal structure of the 2:1 complex of **7** with iodide, while the other was based on dynamic combinatorial chemistry. The modeling approach suggested adipic acid as the optimum spacer to give the oyster-like receptor **8**, which did indeed show an improved affinity for anions as compared to the original cyclic peptide **7**.[275] In the dynamic combinatorial approach, we prepared a small DCL by mixing disulfide dimer **9** and dithiol building blocks **a**−**f**. Exposure of the library to K_2SO_4 induced the amplification of receptors **9b** and **9c**, which exhibited anion affinities that were an order of magnitude larger than those of the designed receptor **8**.[272,273] The new disulfides are the best neutral synthetic anion receptors described to date, with micromolar binding affinities in 2:1 acetonitrile−water.

Another system contemporary with the earliest dynamic combinatorial chemistry experiments was developed by Eliseev and Nelen.[27] A receptor was designed and synthesized to incorporate two key features: a pair of photoisomerizable double bonds, and a pair of carboxylate residues for guanidinium recognition (Scheme 24). The cis−cis isomer

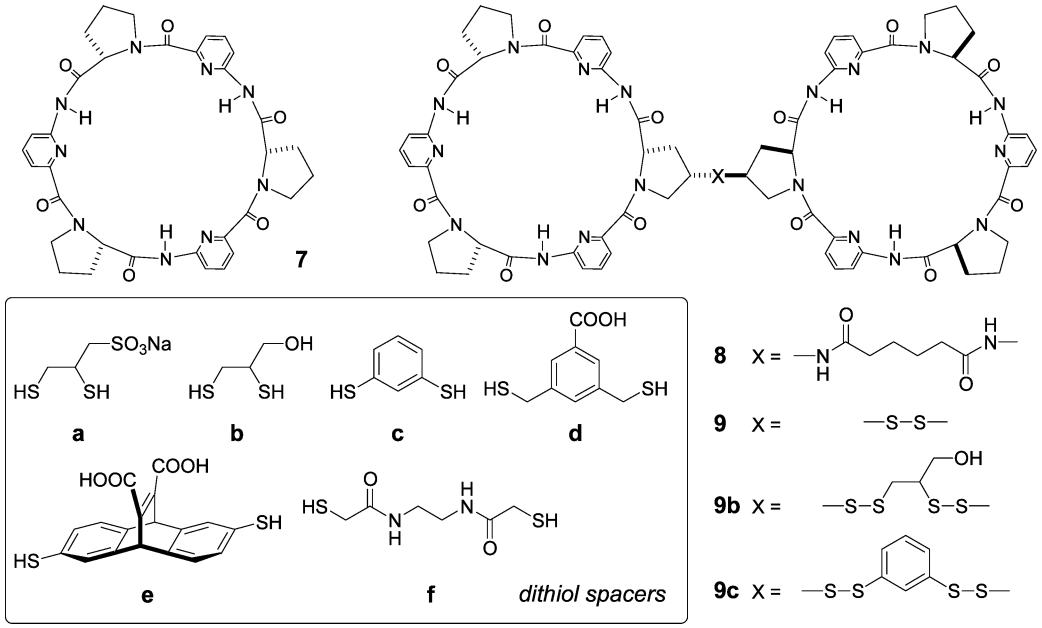
Scheme 24. A Small DCL of Photoisomerizable Guanidine Receptors[27]



was predicted by molecular modeling to be well-suited for guanidinium recognition, whereas the other isomers were expected to show a lower affinity. Binding studies with methylguanidinium chloride confirmed this.

Because the conditions for photoisomerization were found to be incompatible with those of molecular recognition, a circulation setup was constructed (Figure 6). In one chamber, the building block solution was irradiated to cause photoisomerization. The solution was then pumped into the second chamber, an affinity column bearing immobilized arginine. Most of the cis−cis isomer was retained on the column, whereas the rest of the isomer mixture was pumped back into the irradiation chamber to repeat the cycle. After 30

Chart 9. Development of Synthetic Receptors for Inorganic Anions Based on Cyclic Peptides Using Disulfide-Based DCLs To Identify the Optimum Spacer between Two Peptide Rings[272,273]



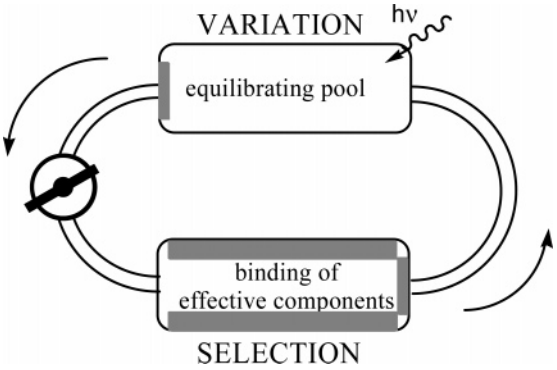


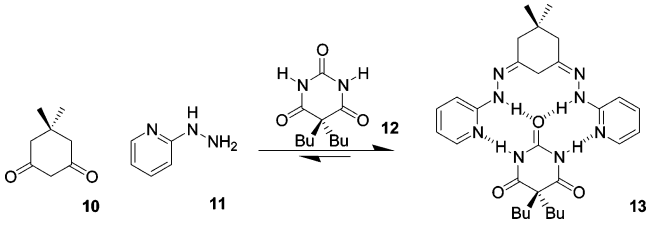
Figure 6. Schematic of the apparatus used for separating selection and equilibration of DCLs while still benefiting from template-induced equilibrium displacements.[27]

cycles, the mixture of isomers on the column was found to be 85:13:2 cis−cis/cis−trans/trans−trans, whereas the photostationary distribution in the absence of guanidinium template was 52:31:17. A mathematical model of the process,[276] as described in more detail in section 5, demonstrated that the outcome of the technique was equivalent to direct equilibration in the presence of the template.

In our work on DCLs of predominantly macrocyclic hydrazones, we have observed the amplification of a linear hydrazone by addition of [18]crown-6.[156] When this crown ether is added to a DCL of the **PFm** building block, the normally observed cyclic dimer, trimer, and higher species are consumed, and the library becomes dominated by a previously undetectable species, which was identified by ESI mass spectrometry as the deprotected aldehyde **PFm**, protonated and complexed to [18]crown-6 (Scheme 25). A small amount of linear hydrazone dimer complexed to [18]crown-6 was also detected.

The Lehn group has used a hydrazone DCL to produce a linear barbiturate receptor that binds with induced fit.[277] They used 5,5-dimethyl-1,3-cyclohexanedione **10** and 2-hydrazinopyridine **11** in chloroform to produce a dynamic mixture of conformational and configurational isomers of mono- and bis-condensed products (Scheme 26). On addition of the barbiturate guest **12**, the (*Z*/*Z*) dihydrazone isomer is strongly favored because it binds to **12** to produce the resultant complex **13** as the major library component.

Scheme 26. Amplification of a Linear Barbiturate Receptor from a DCL Based on Hydrazone Exchange[277]



Folded, linear receptors that bind to rod-like guests have been developed by the Moore lab using DCLs based on imine metathesis.[278] In this approach, the imine building blocks are based on *m*-phenylene ethyne oligomers that can fold to form a tubular cavity. To allow variation in the length of the foldamers produced, three building blocks were used: **14**, which has two *N*-terminal imines, and **15a** and **15b**, which have one C-terminal imine but are of different chain length (Chart 10). When equilibrated in an apolar solvent (chloroform), the resulting oligomers do not fold, and all six possible

Scheme 25. The Amplification of a Linear Species from a Hydrazone DCL Using [18]Crown-6[156]

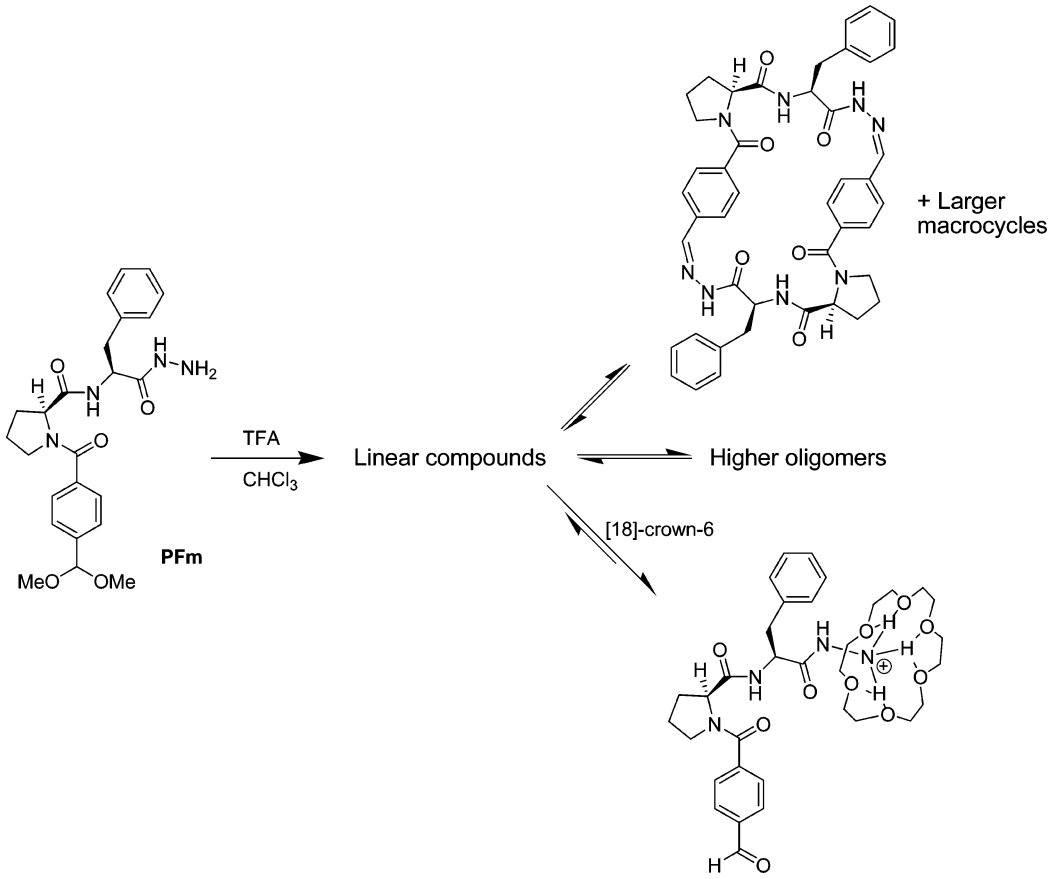
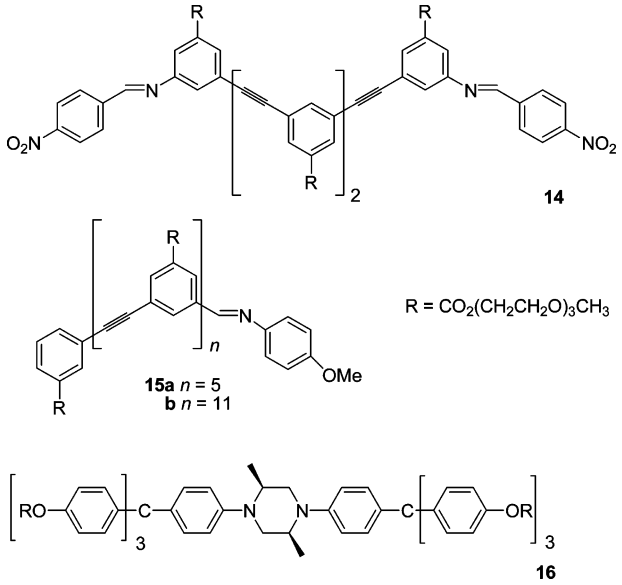


Chart 10. Imine Building Blocks 14 and 15 That Are Used To Form a Small DCL of Linear Oligomers, One of Which Is Capable of Folding around Guest 16[278]



products are formed in roughly the same proportion. However, when equilibrated in acetonitrile, folding is favorable and becomes a driving force to generate the oligomers capable of folding; thus the three oligomers corresponding to **14(15)**$_2$ were amplified in a statistical distribution. On addition of the rod-like guest **16**, one of these oligomers, the best binder **14(15a)(15b)**, was strongly favored. Other work by the same group to investigate folding in closely related systems is discussed in section 4.5.3.

4.1.2. Macrocyclic Receptors

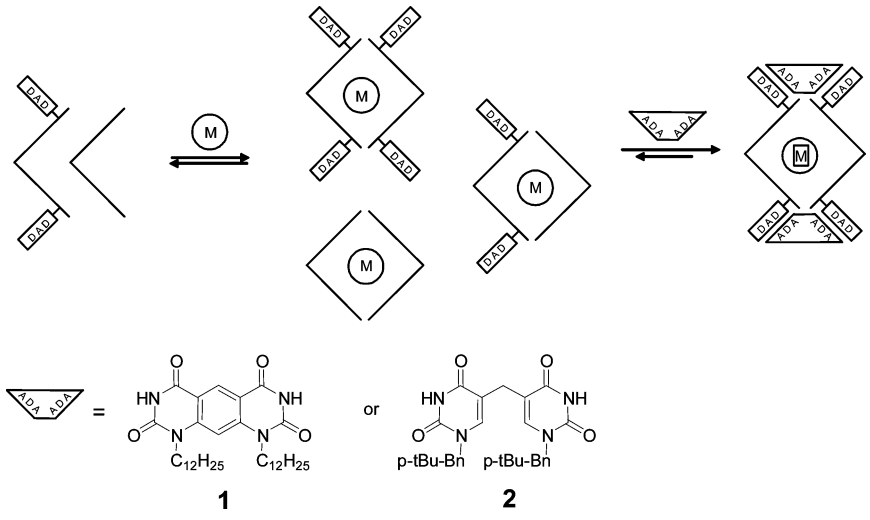
Since the discovery of crown ethers by Pedersen,[279,280] macrocycles have become the most widely studied topology of synthetic receptors. Dynamic combinatorial chemistry has proven to be no exception, and most of the examples of receptors generated from DCLs have been macrocycles.

4.1.2.1. Macrocyclic Receptors Using Hydrogen Bonds. Due to the labile nature of noncovalent forces, macrocycles held together by these interactions tend to be relatively fragile, and hence few examples of noncovalent macrocycles generated from DCLs exist. However, DCLs that use hydrogen bonds or metal−ligand interactions as the linkages that hold the macrocycles together have met with considerable success.

Lehn and co-workers developed a system where macrocycle formation is responsive to the addition of a transition-metal ion such as Cu(I) or Pd(II).[216] In this study, 2,2′-bipyridines were functionalized at the 4-positions with a H-bond donor−acceptor−donor (DAD) pattern (Scheme 27). This ligand and bipyridines without H-bond functionalization were mixed with complementary ADA bisimides of varying flexibility. Addition of a transition metal ion template directs two bipyridines into a planar [in the case of Pd(II)] or tetrahedral [for Cu(I)] geometry, thus aligning the ligands and DAD arrays either parallel or at a 90° angle. In the absence of a bisimide molecule, ions are complexed indiscriminately by the bipyridines. On addition of a suitable bisimide, the complexes are sorted into those with and without functionalized bipyridine ligands in order to form macrocycles with bridging bisimides. Depending on the dihedral angle at the metal center, a particular bisimide will "fit" into the array best, bridging the ligands to complete the hydrogen-bonded macrocycle.

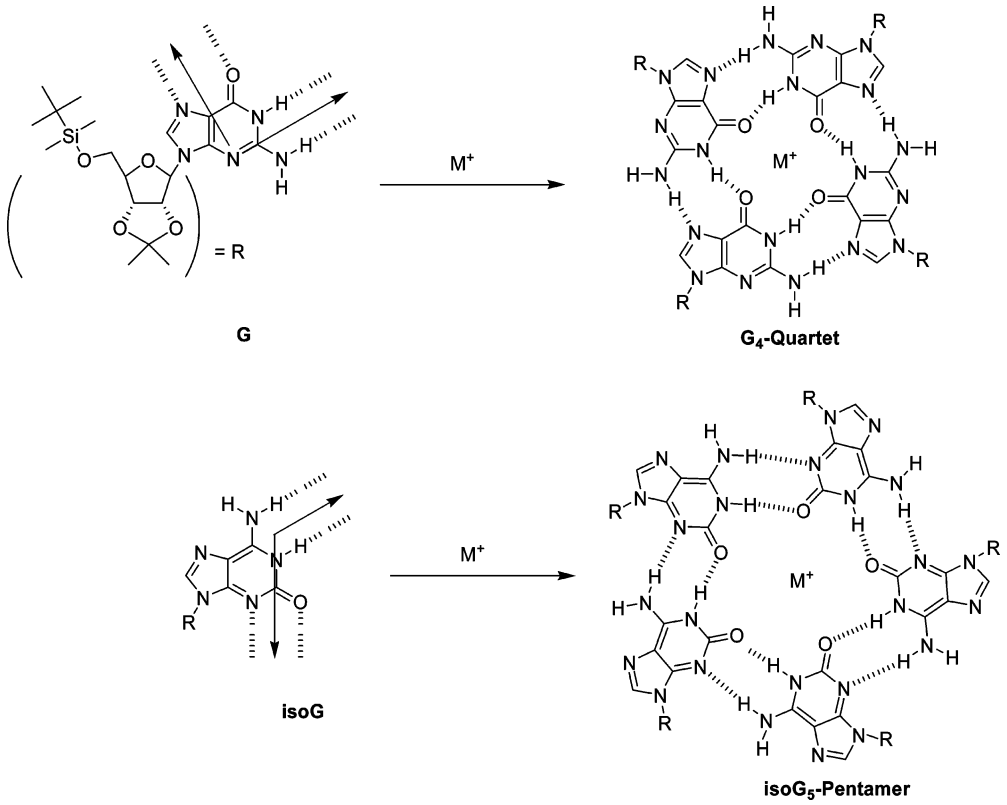
Davis and co-workers have used lipophilic nucleosides that spontaneously form macrocycles that act as receptors for alkali and Ba^{2+} cations in organic solvents.[245] In this study, two nucleotides, **G** and **isoG**, were used as building blocks (Scheme 28). In the absence of an alkali cation, the two nucleotides cross-associate to form a base pair dimer. Upon addition of a suitable cation, the nucleotides self-sort into cyclic **G** tetramers and **isoG** pentamers. Some selectivity toward specific alkali cations was observed, the **G** tetramer being moderately selective for binding K^+ over Na^+ and Rb^+, while the **isoG** pentamer was strongly selective for binding Cs^+. In addition to existing as distinct macrocycles, the species generated were also shown to stack into sandwich complexes. For example, on addition of barium picrate to a mixture of the two nucleosides, discrete complexes of four **G** tetramers stacking around two Ba^{2+} ions, $(\mathbf{G})_{16}\cdot 2Ba^{2+}$, and a sandwich complex of two **isoG** pentamers around a Ba^{2+} ion, $(\mathbf{isoG})_{10}\cdot Ba^{2+}$, were formed.

Scheme 27. A Small DCL Based on Metal−Ligand Interactions in Which the Metal Dictates the Preference for Flat Guest 1 or Flexible Guest 2[a]



[a] M = Cu(I) or Pd(II); A = H-bond acceptor; D = H-bond donor.[216]

Scheme 28. DCLs of Macrocycles Using Hydrogen-Bonding and Metal−Ligand Interactions[245]

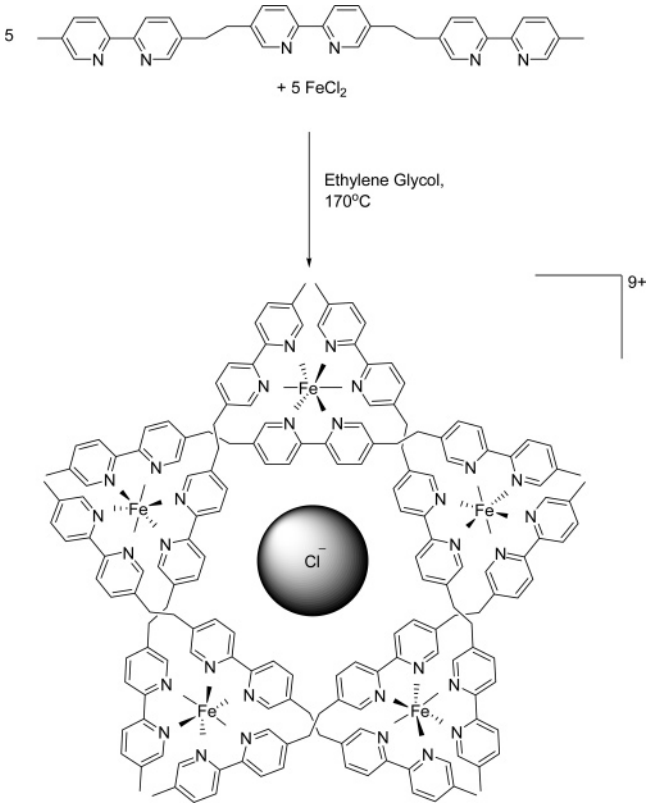


4.1.2.2. Macrocyclic Receptors Using Metal−Ligand Interactions. Lehn and co-workers' first foray into dynamic combinatorial chemistry came from their work on transition metal helicates, using chains of bipyridine and terpyridine ligands. Normally, each bipyridine moiety in the molecule would chelate a transition metal ion, allowing two or three ligand molecules to join together to form a double or triple helix. When iron(II) chloride was used to generate helices, a cyclic double helicate, consisting of five ligand chains and five metal ions, was formed.[220] An X-ray crystal structure of the complex revealed the presence of a tightly bound chloride ion in the center (Scheme 29). The structure generated was found to be dependent on the nature of the transition metal salt used: $NiCl_2$ resulted in the production of a linear triple helicate, whereas $Fe(BF_4)_2$ gave rise to a hexameric species. A later study revealed that hexamers were also formed using other counterions and that a tetramer could be formed using a more flexible ligand chain.[20]

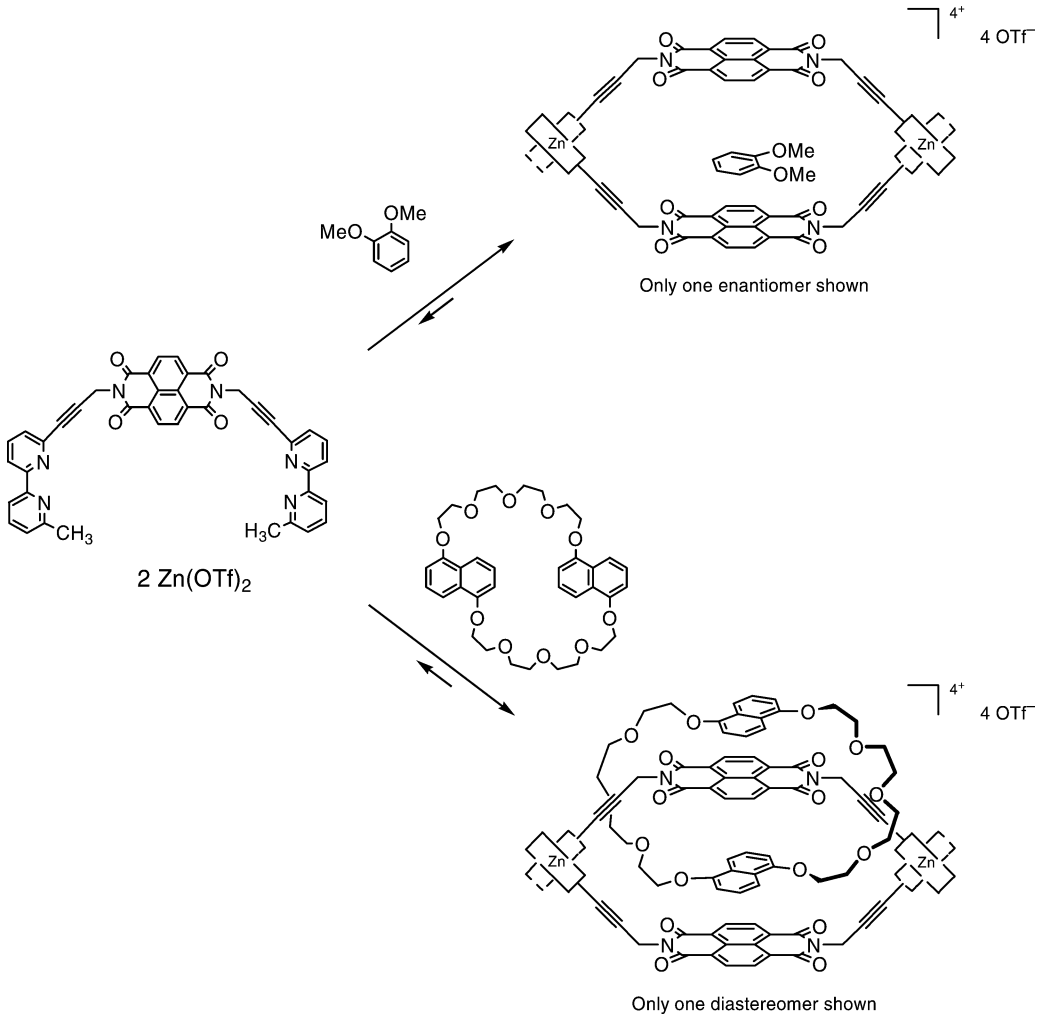
In a pioneering study, Harding showed that the bis-bipyridyl ligand in Scheme 30, which contains an electron-poor naphthodiimide spacer, forms a complex mixture of cyclic and linear species with 2 equiv of Zn^{2+} ions. Addition of an electron-rich aromatic guest such as *o*-dimethoxybenzene rapidly leads to selection and amplification of a single chiral metallomacrocycle, templated by host−guest donor−acceptor interactions.[16,236] Replacement of the guest by the electron-rich macrocycle illustrated leads to virtually quantitative synthesis of the corresponding catenane.[237]

Our use of metal−ligand coordination in dynamic combinatorial chemistry has focused on metallo-porphyrins to generate cage-like macrocycles templated by 4,4′-bipyridines and other related compounds.[231] An initial study used Rh(III) and bisphosphine-substituted Zn(II) porphyrin units as building blocks. Because phosphines coordinate reversibly to Rh(III), an equilibrium mixture of oligomers and macrocycles is established when the building blocks are mixed (Scheme 31). In the absence of template, multiple resonances for the various species in solution were observed by ^1H and ^{31}P NMR. Addition of 0.5 equiv of 4,4′-bipyridine amplifies the cyclic porphyrin tetramer to essentially 100% abundance

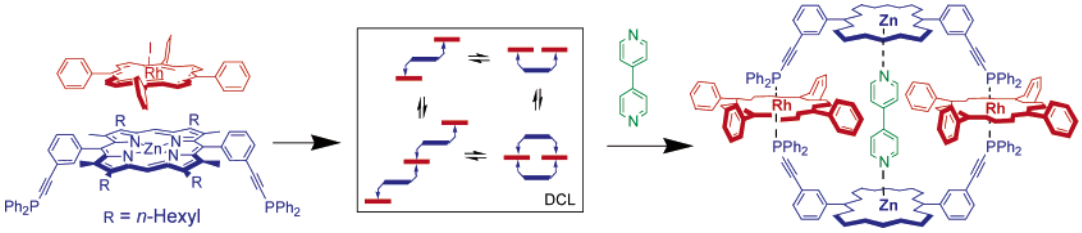
Scheme 29. Chloride Anion Bound by a Cyclic Helicate Formed under Thermodynamic Control[20,220]



Scheme 30. Template-Directed Amplification of a Macrocycle and a Catenane from a Small DCL of Zn Complexes[16,236,237]



Scheme 31. A DCL Formed by Rh(III) and Bisphosphine-Substituted Zn(II) Porphyrin Units Is Templated by Bipyridine To Give a Single Macrocyclic Product[231]

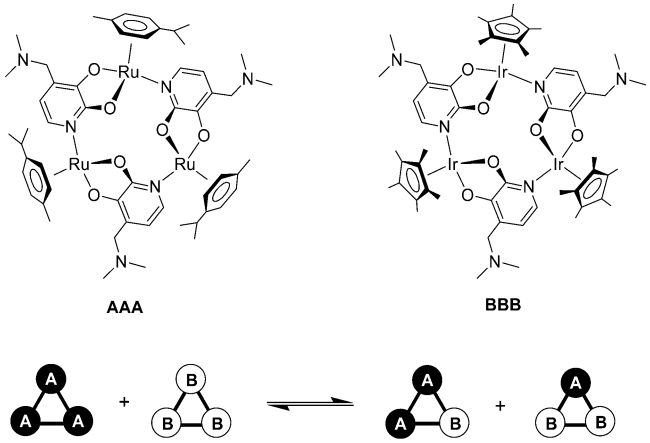


because it coordinates to the two Zn(II) porphyrins, stabilizing the cyclic structure. The template could be removed from the macrocycle by adding an excess of competing Zn(II) porphyrin, leading to the disassembly of much of the tetramer and the reappearance of other species in the re-equilibrated library.

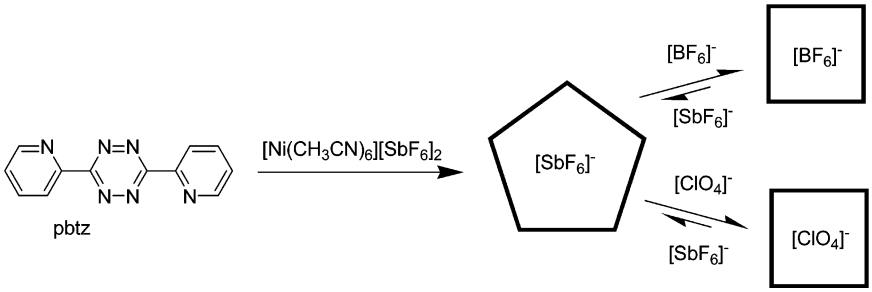
The system was expanded by adding sterically demanding templates and acceptor Rh(III) and Ru(II) porphyrins. Highly sterically hindered porphyrins were found to be unable to accommodate a 4,4′-bipyridine template. More moderately hindered porphyrins showed limited selectivity for the less hindered templates, but it was found that the cyclic cage structure could be significantly distorted to accommodate a guest.[232]

Saur and Severin have made small DCLs of trinuclear metallamacrocyclic complexes **AAA** and **BBB** (Scheme 32) and observed that at high template concentrations the most

Scheme 32. A Small Dynamic Library of Trinuclear Organometallic Macrocycles[225]



Scheme 33. The Formation of the pbtz [Ni]$^{10+}$ Pentagon and Anion-Mediated Interconversion to Tetrameric Squares[226]



amplified species in this DCL is not the most effective template binder.[225] The macrocycles are similar in structure, both being trimers of the pyridone ligand and a transition metal carrying an aromatic ligand. Both of these macrocycles are receptors for Li^+ in water, but **AAA** has a significantly higher affinity than **BBB** ($K_{AAA} = 4.4 \times 10^3\ M^{-1}$, $K_{BBB} = 1.1 \times 10^{-1}\ M^{-1}$). When mixed, the macrocycles interconvert to form mixed species **AAB** and **ABB**. In the absence of Li^+, the product distribution is a statistical 1:3:3:1 mixture. Upon addition of a small amount (0.2 equiv) of Li^+, macrocycle **AAA** is amplified to become the major library member. However, as the concentration of Li^+ is increased to excess concentrations, the library composition shifts back to favor the formation of **AAB**. In section 5, we will treat these and similar effects in greater detail.

The Dunbar group have produced anion-templated metallacyclophane DCLs where the size of the metallacyclophane amplified can be dictated by the size of the anion template.[226] In their system, a bis-bipyridine ligand was used with first row transition metal ions M(II) (particularly Ni^{2+}) to produce the metallacyclophanes in acetonitrile. The $[Ni]^{10+}$ pentagon was prepared using the relatively bulky $[SbF_6]^-$ as the counterion for Ni^{2+}. This complex was found to be significantly less stable than the $[Ni]^{8+}$ tetrameric square and could be easily converted to the tetramer by adding an excess of $[BF_4]^-$ or $[ClO_4]^-$ (Scheme 33). Transformation back to the pentagon is more difficult and requires a large excess of $[SbF_6]^-$ and refluxing for 2 days.

4.1.2.3. Macrocyclic Receptors Using Ester Exchange. The first macrocycles generated using dynamic combinatorial chemistry were prepared using transesterification. Initial studies used building blocks based upon cholates functionalized by a methyl ester and a hydroxyl group (Scheme 34).[18] Transesterification was catalyzed by potassium methoxide/dicyclohexyl[18]crown-6 in refluxing toluene. Linear cholate oligomers are produced as kinetic products (not shown) but rapidly give way, forming an equilibrium mixture of cyclocholate macrocycles. Several cyclic products were observed including trimers, tetramers, and pentamers. Dimers were only observed when the building blocks lacked bulky C7 substituents. Proof of the thermodynamic nature of the system was obtained by purifying the trimers and tetramers and allowing them to re-equilibrate, regenerating the original product distribution.

Adding alkali metal iodide salts as templates induced modest changes in product distribution.[19] The most significant shift in library composition was induced by sodium iodide, which changed the trimer/tetramer/pentamer ratio from 83:12:5 to 61:24:14.

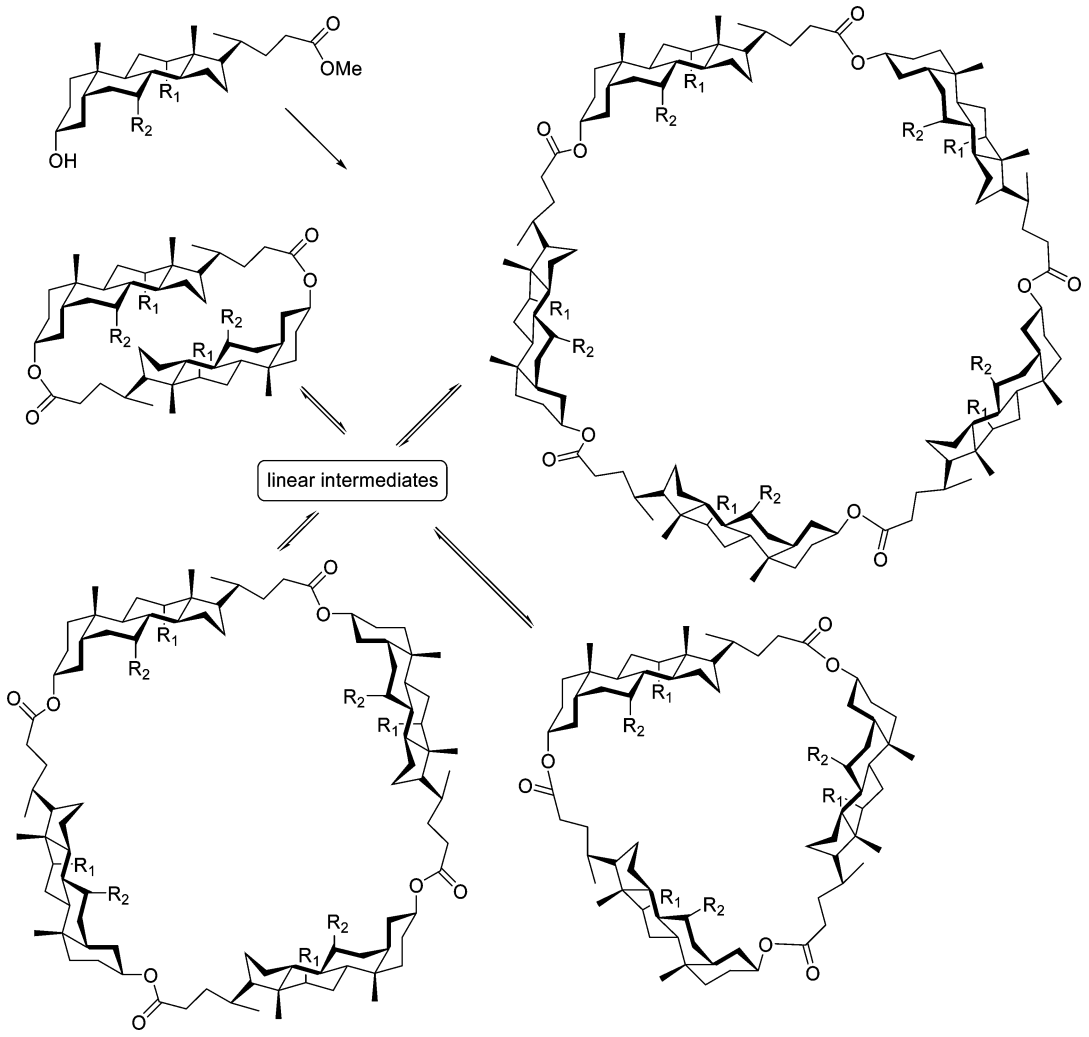
4.1.2.4. Macrocyclic Receptors Using Allyl Ester Exchange. We have explored reversible allyl ester chemistry to select the appropriate spacer for the closure of a zinc porphyrin dimer designed to recognize polypyridine templates (Scheme 35).[78] In kinetic conditions, the same molecular ingredients fail to produce the desired receptor. The porphyrin building block (10 mM) was mixed with the potential linker (8 equiv) in the presence of base (40 equiv) and the palladium catalyst [$Pd(PPh_3)_4$, 0.1 equiv] with or without the dipyridine template in chloroform at 55 °C. After 6 h, the product mixture was analyzed. In the absence of template, less than 2% of product could be detected, whereas the dipyridine guest induced a 6-fold amplification, resulting in a still modest 10% yield regardless of the nature of the linkers selected. Low yields in this system are attributed to decomposition of the building blocks rather than failure of the exchange chemistry.

4.1.2.5. Macrocyclic Receptors Using Imine Exchange. Storm and Lüning have used imine exchange to produce DCLs containing a variety of macrocyclic receptors for metal-ion templates.[143,281] Pyridinedicarboxaldehyde **17** (Scheme 36) was mixed with diamines of varying chain length in methanol (**18a**−**c**) to produce a DCL containing an assortment of macrocyclic and other species in equilibrium. Libraries had to be frozen by addition of excess sodium borohydride, reducing the imines to amines before analysis by ^1H NMR spectroscopy.

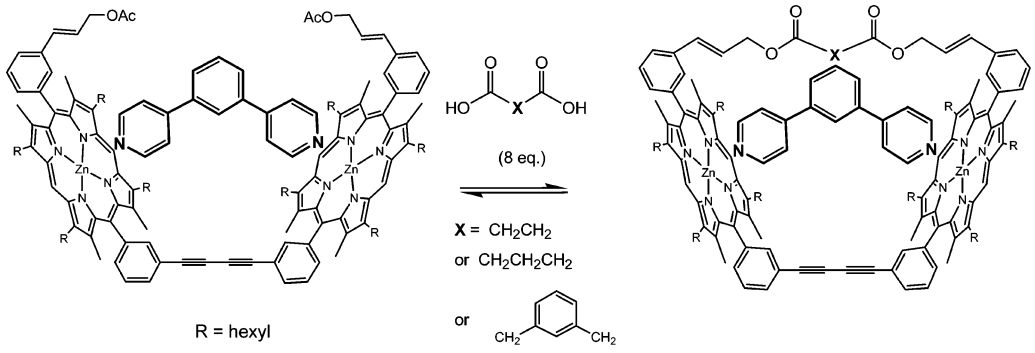
In untemplated libraries containing **17** and all three diamines, the only macrocycle that was detectable from the reduced library was **19b** (in 9% yield). Addition of template ions to the system generates more interesting results. For example, addition of the small Mg^{2+} ion to a DCL of **17** and **18a** results in the diimine macrocycle **19a** being amplified and its reduced amine counterpart **20a** is detected in an 86% yield. When larger template ions such as Ca^{2+} and Sr^{2+} are used in a DCL made from the same components, the yields of **20a** are poorer (60% and 45% respectively). However, a larger [2 + 2] macrocycle **22a**, which is not detected in Mg^{2+}-templated libraries, is now generated in 22% and 34% yields. As might be expected Ca^{2+}, Sr^{2+}, and Ba^{2+} ions selectively amplify [1 + 1] macrocycles with longer diamines **18b** and **18c**. In a DCL containing **17**, **18a**−**c**, and three ions, Mg^{2+}, Ca^{2+}, and Ba^{2+}, good yields for each [1 + 1] macrocycle **20a**−**c** were obtained.

Gotor and co-workers have used imine exchange to produce macrocycles from (*R*,*R*)-cyclohexane-1,2-diamine **23** and pyridine-1,2-dicarboxaldehyde **17**.[282] Macrocycles containing between two and six of each subunit (Scheme 37) were observed by ESI-MS and ^1H NMR. From this DCL, members were amplified upon the addition of metal ions. Addition of Ba^{2+} led to the amplification of the [2 + 2] macrocycle **24** to almost quantitative levels, while addition of Cd^{2+} resulted in the essentially quantitative formation of the [3 + 3] macrocycle **25**. Interconversion between these macrocycles was shown to be possible by adding an excess

Scheme 34. DCL of Cholate Esters[18]



Scheme 35. Template-Directed Capping of a Porphyrin Dimer under Thermodynamic Control[78]



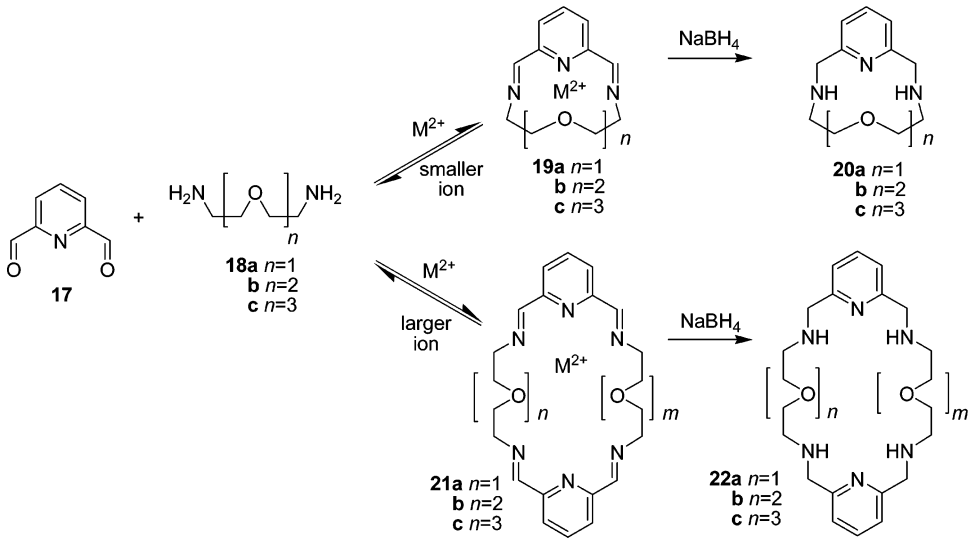
of Cd^{2+} salt to the equilibrated Ba^{2+} [2 + 2] macrocycle complex. Upon reduction, the resulting [3 + 3] amine macrocycle was the only product observed. Other cations were also used as templates, but these resulted in more complex mixtures with modest amplification of individual components.

Following this initial study on macrocyclic imines the Gotor lab recently reported the highly diastereoselective amplification induced by cadmium(II) of the heterochiral form of macrocycle **25** starting from a mixture of (*R*,*R*) and (*S*,*S*) *trans*-cyclohexane-1,2-diamine **23**.[283]

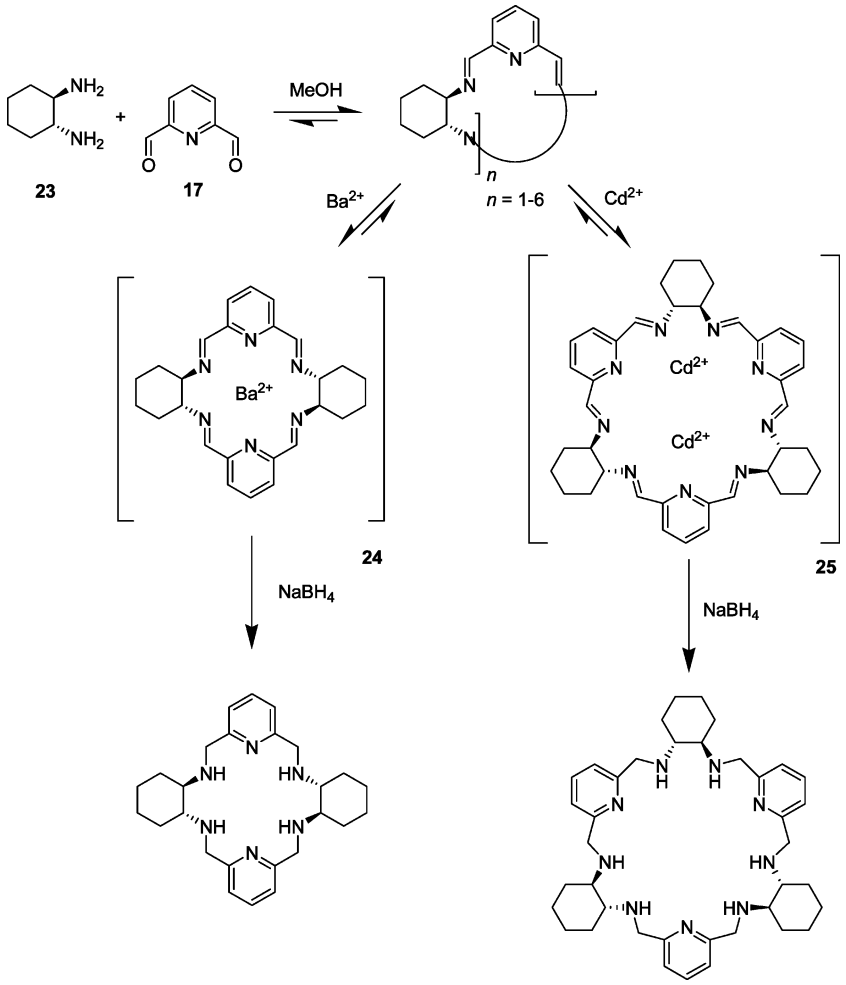
Note that in the examples of Schemes 36 and 37, the reduced diamine macrocycles are significantly worse ligands for the metal ions than their parent imines, underlining an inherent drawback associated with postsynthetic reduction of imine DCLs.

4.1.2.6. Macrocyclic Receptors Using Hydrazone Exchange. To date, one of the most successful reactions used in the formation of macrocyclic receptors from DCLs has been hydrazone exchange. We have developed a series of pseudo-peptide building blocks containing proline units (frequently found in protein β-turns) in order to predispose the pseudo-peptide to macrocyclization. Each monomer is equipped with a hydrazide and an aldehyde functionality (the latter protected as an acid-labile dimethoxy acetal) allowing macrocycles of any size to be formed.

Scheme 36. The Reversible Formation of [1 + 1] and [2 + 2] Imine Macrocycles and Subsequent Reduction to Static Amine Products[143,281]

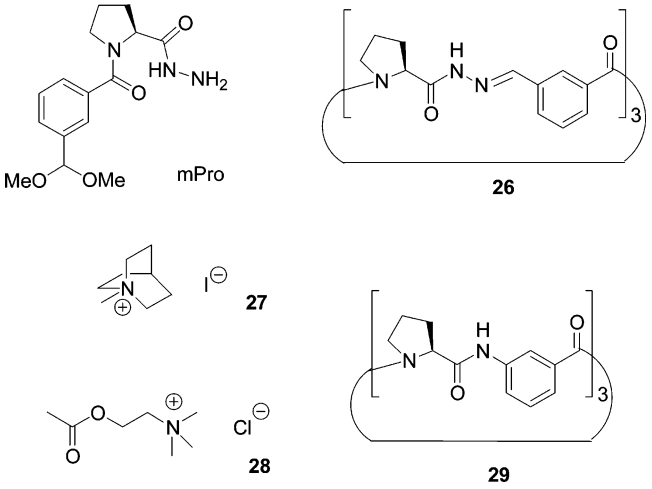


Scheme 37. Formation of a DCL of Cyclic Oligoimines Followed by Templating by Metal Ions and Subsequent Reduction to Stable Amine Macrocycles[282]



The first macrocyclic hydrazone receptor that we discovered from these libraries was amplified by ammonium cations.[157] The hydrazide building block ***m*Pro** (Chart 11) was used. This particular building block resembles the repeating unit in Kubik's cyclic peptide **29**, which binds ammonium cations such as *N*-methylquinuclidinium iodide **27** and acetylcholine iodide **28**.[284] In the absence of template, ***m*Pro** equilibrated to form predominantly cyclic dimer (88%) together with a small amount of trimer (11%). Addition of 2 equiv of **27** to the library dramatically changed this distribution, and trimer **26** became the favored library member. The amount of dimer falls to 41%, and the amount of trimer rises to 56%. Template **28** has an even greater effect resulting in 86% trimer and 13% dimer under the same

Chart 11. Mixing a Proline-Based Building Block with Ammonium Ion Templates Results in the Amplification of a Trimeric Receptor 26, Which Resembles the Cyclic Peptide Receptor 29 Previously Described by Kubik[157,284]



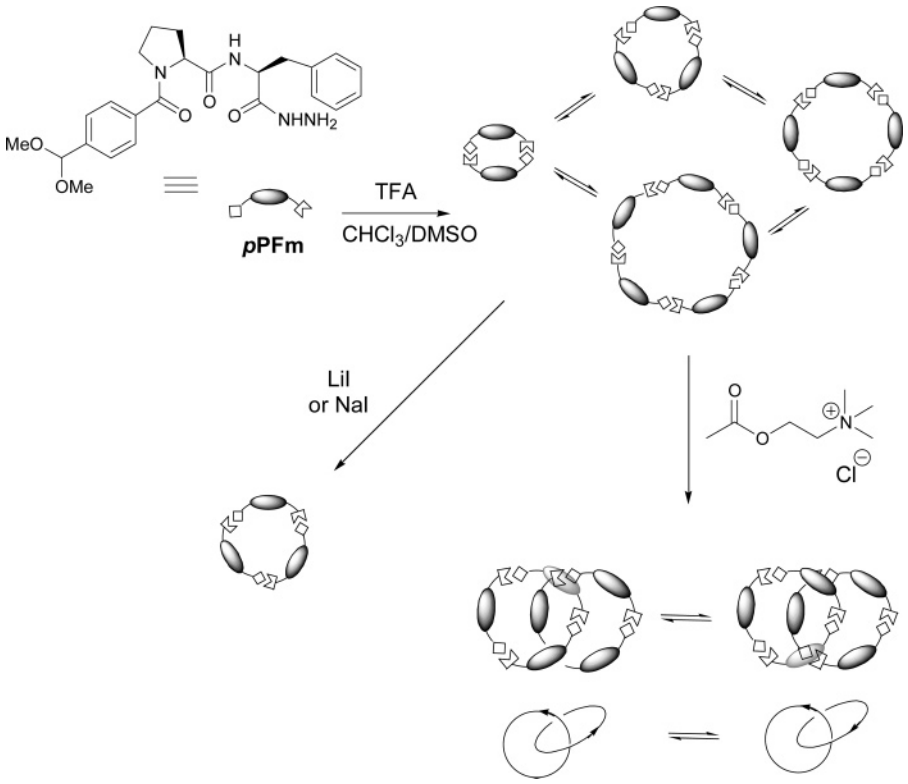
conditions. Tributyl- or triethylammonium salts produced weak nonspecific amplification of higher oligomers.[158] Binding constants between the cyclic trimer and the two templates were estimated to be relatively small: 150 and 230 M^{-1} for **27** and **28**, respectively.[158] In comparison, Kubik's cyclic peptides bind **27** and **28** with binding constants of 4.22×10^4 M^{-1} and 1.10×10^4 M^{-1} in pure chloroform.[284] ^{1}H NMR spectroscopy showed that **26** exists as multiple exchanging conformers but that acetylcholine selected a single conformer.[157,158]

Notably, using trimethylammonium salts attached to a solid support (Amberlyst-27 resin, which contains benzyltrimethylammonium chloride groups) we were able to simultaneously select, amplify, and isolate receptors from an ***m*Pro** DCL.[159] The resin turned out to amplify the formation of ***m*Pro** cyclic trimer in much the same way as trimethylammonium salts in solution. After equilibration, the resin was filtered from the library and washed with chloroform to remove dimer and other species that were nonspecifically absorbed to the resin. Subsequent washing of the resin with methanol, which breaks up the noncovalent interactions between template and receptor, yielded essentially pure trimer in 40% yield. This approach is particularly attractive because it allows the selection, amplification, and isolation of receptors to be carried out in a single step.

We have also investigated DCLs based on the pseudo-dipeptide building block ***p*PFm**, containing a phenylalanine unit in addition to the usual proline residue (Scheme 38).[160] Untemplated DCLs of this building block contained a series of macrocycles of different ring sizes (in 98:2 $CHCl_3$/MeOH as the solvent). Addition of lithium and, to a lesser extent, sodium salts induced the amplification of the cyclic trimer. Indeed, the trimer was found to be a good receptor for Li^+, with a binding constant of 4×10^4 M^{-1} (in 98:2 $CHCl_3$/MeOH as estimated by ^{1}H NMR titration). ^{1}H NMR revealed significant shifts in every resonance of the receptor indicating that the trimer is not pre-organized but that there is a significant conformational change upon Li^+ binding. Very similar results were obtained using an analogous building block in which the phenyl ring of the phenylalanine unit was replaced by a cyclohexyl ring, which indicates that the phenyl rings are not directly involved in interactions with the cation.[161]

Perhaps the single most impressive yet surprising result to have come out of pseudopeptide-based hydrazone DCLs is the generation of a [2]-catenane that strongly binds to acetylcholine.[155] The same pseudo-dipeptide building block

Scheme 38. A Dynamic Combinatorial Library Based on the Pseudo-Dipeptide Building Block *p*PFm[a]



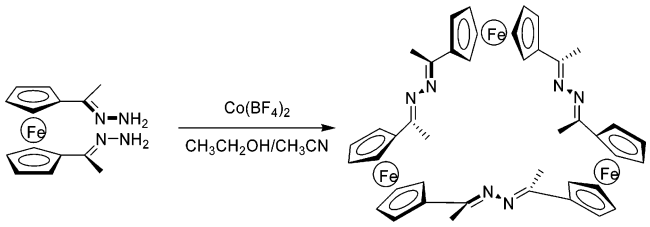
[a] Addition of LiI or NaI induces the amplification of the cyclic trimer,[160,161] whereas addition of acetylcholine chloride leads to an entirely unexpected catenane receptor.[155]

***p*PFm** that produced the receptors for Li^+ and Na^+ responded dramatically to the addition of acetylcholine chloride (Scheme 38). Initially, the cyclic dimer became the major component of the library. However, this proved to be a kinetic product, and within an hour, a new product isomeric to the cyclic hexamer appeared. Analysis by LC-MS indicated that, while the new library member had a different retention time from the cyclic hexamer by HPLC, its *m*/*z* is identical to the hexamer. When the two library members were compared by their MS/MS behavior, the distinction between the two isomers was revealed. The simple hexamer fragmented into a pentamer and successively smaller oligomers, while the new product fragmented directly to the trimer before being fragmented further. This behavior is typical of a [2]-catenane and indicates that the new component generated in the acetylcholine-templated DCL consisted of two interlocked cyclic trimers. NMR confirmed that the topology of the product is indeed that of a catenane and that only one of two possible diastereomers is found.

The catenane is an exceptionally good receptor for acetylcholine ($K = 1.4 \times 10^7$ M^{-1} in 95:5 $CHCl_3$/DMSO determined by ITC) with binding being strongly enthalpy-driven. Control experiments confirmed that acetylcholine binds the catenane much more strongly than any of the other macrocycles present in the library. The DCL takes an unusually long time to equilibrate, and only after 44 days does the catenane reach its maximum concentration, accounting for 70% of the total library material. We speculate that the reason behind the prolonged equilibration time is that the catenane formation pathway is inherently improbable[155] but that acetylcholine effectively captures and stabilizes the catenane as it is formed.

Meng and co-workers have also used metal-ion-templated hydrazone DCLs to produce a macrocyclic receptor for Co(II).[285] In their case, Co(II) acts as both the guest and catalyst for hydrazone exchange. As a building block 1,1′-diacetylferrocene dihydrazone was used (Scheme 39). The building

Scheme 39. A Hydrazine-Derived Hydrazone Macrocyclic Ligand for Co^{2+} [285]



block design is fundamentally different from the hydrazide monomers described above, because both linking ends are hydrazones as opposed to a single hydrazide and aldehyde. The hydrazones can, in effect, act as either aldehydes (by losing hydrazine upon attack) or hydrazides (by attacking another hydrazone) when condensing to form oligomers. In the absence of an acid catalyst, the monomer does not cyclize. However, in the presence of Ba^{2+}, Mo^{2+}, or hydrochloric acid, the hydrazone building block dimerizes to form a cyclic diazone. If the reaction is carried out in the presence of $Co(BF_4)_2$ in an ethanol/acetonitrile mixture, a cyclic trimer is produced. This cyclic trimer acts as a ligand for Co(II), and an X-ray crystal structure showed the Co(II) in the center of the macrocyclic ligand. No experiments were performed to demonstrate the reversibility of the hydrazone chemistry used here.

4.1.2.7. Macrocyclic Receptors Using Disulfide Exchange. After we had established that disulfide exchange can be used to make diverse DCLs of macrocyclic disulfides (see section 2.1.5),[186] the next challenge was to prove that molecular-recognition-induced changes in library composition can be large enough to allow identification of new receptors from larger libraries. At the time, the only theoretical study was rather skeptical about this prospect,[286] while all experimental work at the time was confined to model libraries that contained only a few species. We approached the problem using a set of building blocks that were inspired by the family of cyclophane receptors of general architecture **30** (Scheme 40), which were developed by the Dougherty lab.[287−293] We prepared dithiol building blocks **31**−**33** and analyzed how DCLs made from these building blocks responded to the addition of a variety of ammonium guests (**34**−**36**).[294,295] Our naive expectation was that we should obtain direct disulfide analogues of the Dougherty cyclophane by introducing guests into the library that have a high affinity (on the order of 10^5 M^{-1} in water) for this receptor. Surprisingly, guest **34**, which is the preferred guest for the original cyclophane host, induced the amplification of a different host, heterotrimer **37**. Binding studies revealed that the affinity for this host is of the same order as the Dougherty host. Remarkably, exposure of the library to guests that are either larger (quaternized morphine **35**)[294] or smaller (tetramethylammonium iodide **36**)[295] induced the amplification of new receptors **38** and **39** with, particularly in the case of **39**, unexpected architectures. The resulting receptors were found to bind their guest with impressive affinities (2.5×10^5 M^{-1} for **34·37**, 7.1×10^5 M^{-1} for **35·38**, and 4×10^6 M^{-1} for **36·39**).

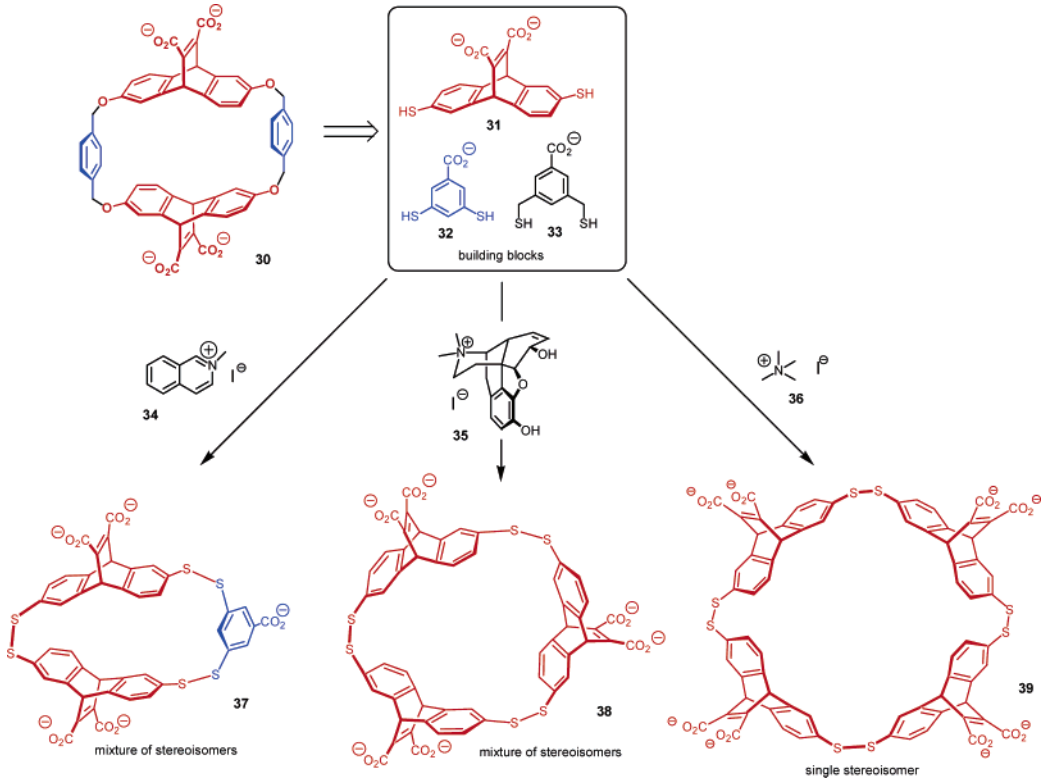
The discovery of receptor **39**, containing four units of the chiral building block **31** (prepared as a racemate), was particularly intriguing because amplification was selective for one out of four possible diastereomers.[295] It turned out that the selected receptor, in which the four building blocks have alternating chiralities (*RR*, *SS*, *RR*, *SS*), is the only one that is able to fold into a conformation in which it can completely surround the small guest **36**. Capturing this level of subtlety in the design of a new synthetic receptor would have been a huge challenge, yet dynamic combinatorial chemistry leads directly to the optimum structure. Use of a single enantiomer of **31** would have led to failure; racemic mixtures add to the structural diversity in a DCL and improve the chance of success.

Receptors **37**−**39** were all isolated from biased DCLs that contained only those building blocks that are part of the receptors. For instance, while receptor **37** constituted only 5−10% of the library material at the screening stage, in a biased library made from building blocks **31** and **32** in a 2:1 ratio, the desired host is formed in 60−65% yield (Figure 7). This strategy worked even better for receptors **38** and **39** (not shown). These results established dynamic combinatorial chemistry as a practical method, not only for screening but also for receptor synthesis.

DCLs based on building blocks **31**−**33** were also used in studies aimed at the discovery of receptors with catalytic activity (described in more detail in section 4.2) and to investigate the effects of competition between different receptors for the same building blocks (see section 5).

While the above examples were performed using water as the solvent, we have also developed macrocyclic receptors using disulfide exchange in organic solvent. We have

Scheme 40. Dithiol Building Blocks Inspired by the Cyclophane Receptor Family Developed by Dougherty and Co-Workers[a]



[a] DCLs made from these building blocks produce different receptors when exposed to different guests.[294,295]

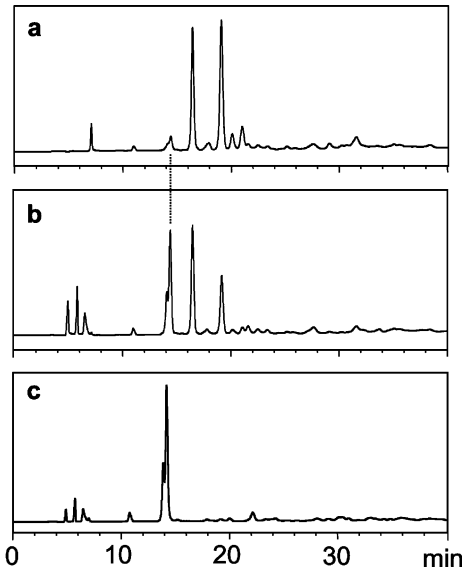


Figure 7. HPLC analysis showing the amplification of receptor **37** induced by guest **34** in a library made from (a) building blocks **31**−**33** (3.33 mM each) in the absence of the guest, (b) the same library in the presence of guest **34**, and (c) a library made from only building blocks **31** (6.67 mM) and **32** (3.33 mM) in the presence of guest **34**.[294]

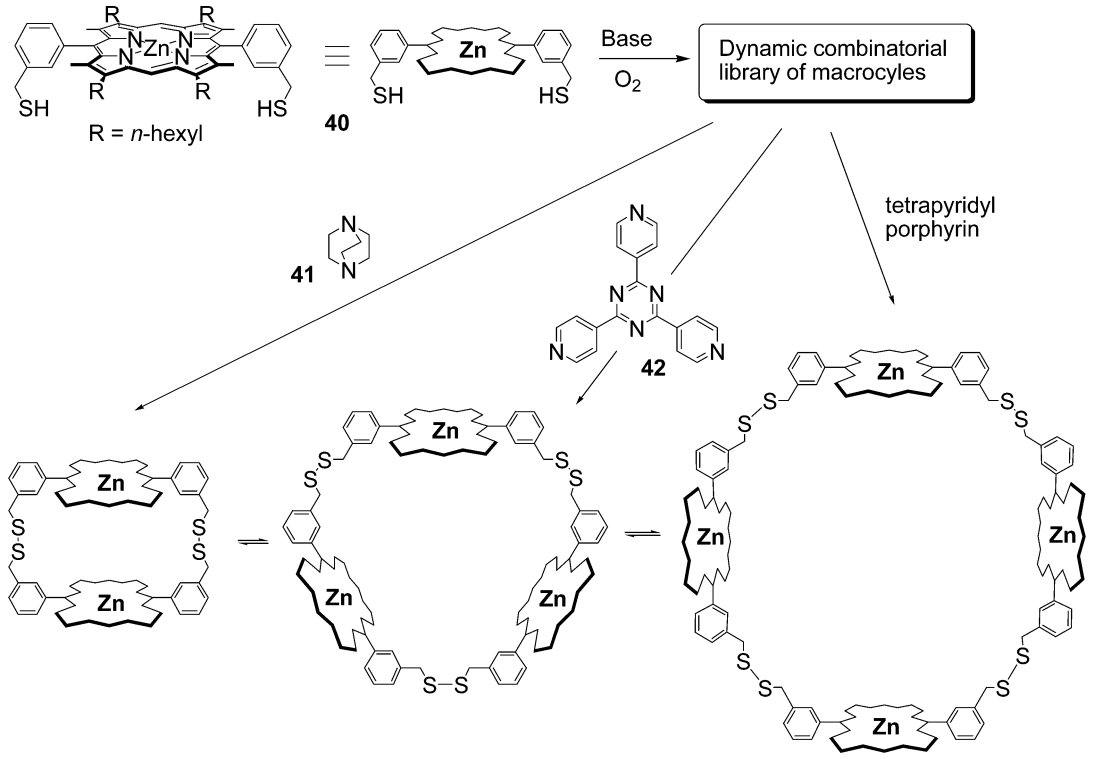
produced a DCL starting from dithioporphyrin **40** in chloroform solution using DBU as a base (Scheme 41).[194] Exposing this library to DABCO (**41**) led to the amplification of the cyclic dimer, whereas tripyridyl triazine **42** induced the formation of cyclic trimer. Similarly, tetrapyridyl porphyrin amplified the cyclic tetramer. In all cases, amplification was essentially quantitative, making templated macrocyclization under thermodynamic control a better synthetic method than our previous template-directed kinetically controlled cyclizations.

4.1.2.8. Macrocyclic Receptors Using Alkene Metathesis. Recently Nolte, Rowan, and co-workers reported a porphyrin tetramer very similar to that in Scheme 41 that is amplified from a dynamic mixture using tetrapyridylporphyrin as a template and alkene metathesis as the reversible chemistry.[199] In the same year, Langford and co-workers published an essentially identical approach to a very similar porphyrin tetramer.[296]

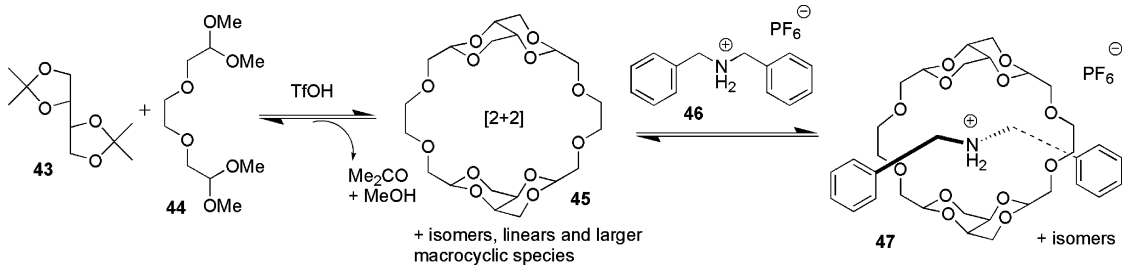
4.1.2.9. Macrocyclic Receptors Using Acetal Exchange. Stoddart, Fuchs, and co-workers used acetal exchange to produce chiral crown ethers based on tetraoxadecalin cores, which can form pseudo-rotaxanes upon complexation with dialkylammonium ions.[174] The diacetonide of D-threitol **43** underwent transacetalation with diacetal **44** in the presence of a triflic acid catalyst in $CDCl_3$ to form a DCL of cyclic and linear products of which the [2 + 2] macrocycle **45** was a member (Scheme 42). When dibenzylammonium hexafluorophosphate **46** was added to the DCL, the formation of [2 + 2] macrocycles was strongly favored. The majority of these macrocycles (64%) contained two fused six-membered diacetal rings ("6/6") forming the pseudo-rotaxane complex **47**, although diacetals of two linked five membered rings ("5/5") and fused five and seven membered rings ("5/7") were also observed (see section 2.1.3). When the template was changed to $CsPF_6$, it was found that the "6/6" diacetal was strongly favored (>95% in $CDCl_3$).

Acetal exchange has also been used by the Mandolini lab to produce cyclophane receptors for Ag^+ ions.[173] In their study, a series of cyclophane oligomers and polymers was produced under kinetic control, starting from 1,4-benzenedimethanol and bromochloromethane in the presence of NaH in boiling THF. Isolated cyclic oligomers were then equilibrated for 5 h under acidic conditions (0.5 mM TfOH) in chloroform at 25 °C to give a series of cyclophane oligomers

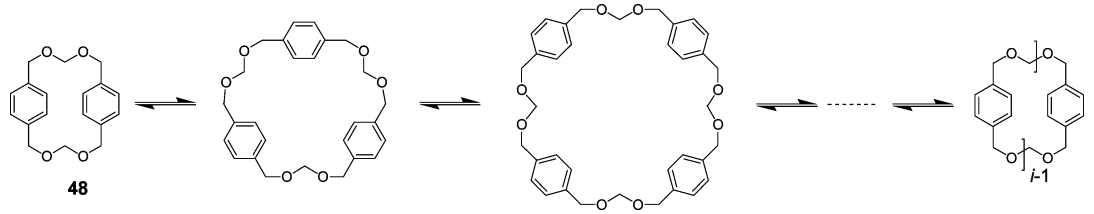
Scheme 41. Small DCL of Porphyrin Macrocycles Made from a Porphyrin Dithiol Building Block, Showing How Three Different Amine Guests Induce the Amplification of Three Different Porphyrin Hosts[194]



Scheme 42. The Dynamic Formation of Crown Ether Acetals and Complexation to Dibenzylammonium[174]



Scheme 43. A DCL of Cyclophane Formals Based on Acetal Exchange[173]



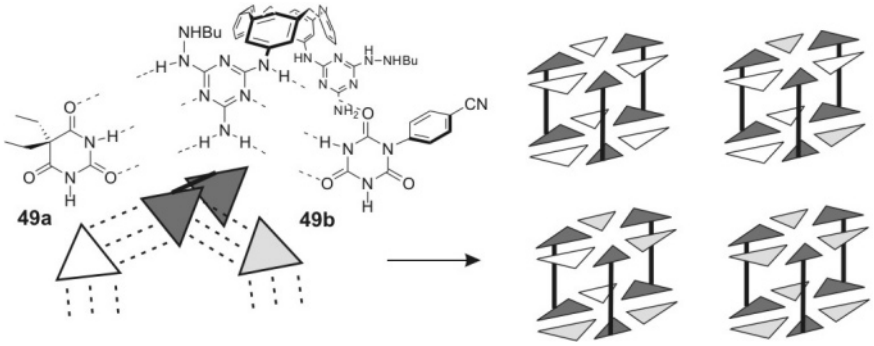
(Scheme 43). While the equilibrium mixture was independent of the nature of the starting oligomer, it was found to be dependent on the concentration of cyclophane; unsurprisingly, lower concentrations favored lower molecular weight oligomers, while higher concentrations (up to 82 mM monomer units) favored the larger oligomers. Addition of Ag^+ to the DCL amplified the formation of cyclophane **48**, and it was shown by ^1H NMR that Ag^+ and **48** form a stable complex. Using TfOAg in chloroform caused the yield of **48** to be increased from 2.2% to 11%, while using the more soluble silver salt $(CF_3SO_2)_2NAg$ increased the yield to 60%.

4.1.3. Molecular Capsules and Related Structures

Synthetic molecular capsules were pioneered by Cram in the 1980s, when he and co-workers succeeded in encapsulating guest molecules inside two hemispherical cavitands covalently joined at their upper rim.[297,298] Inspired by this early work, many others have put considerable efforts into devising new molecular capsules and related three-dimensional receptor cages. Most strategies to this end rely on using either irreversible covalent bonds[299,300] or noncovalent interactions[301−306] to facilitate the formation of a capsule structure from smaller components.

The inherent stability of the covalent bond allows covalent capsules to be exceptionally robust and stable, capable of shielding their contents from the outer environment. Capsules that are entirely covalent in connectivity have been used to permanently imprison their "guests" resulting in so-called *carceplexes*. The remarkable stability of such systems has been demonstrated in cases where guests are retained at high

Scheme 44. A Small DCL of Capsular Structures Is Formed through Hydrogen-Bonding Interactions between Calix[4]arene Bismelamines and Barbiturate Building Blocks[242]



temperatures; even transient reactive intermediates can be isolated at near ambient temperatures.[307] This approach to capsule formation does, however, have one key drawback: because several covalent bonds must be made in the formation of a capsule, there are many opportunities for "incorrect" side products to be formed, which cannot be corrected under kinetic control.

This predisposition toward forming unwanted oligomeric side products has led to the rise in popularity of *self-assembling capsules*, chiefly through noncovalent interactions such as hydrogen-bonding and metal−ligand interactions. Self-assembly offers the possibility of proof-reading during capsule formation, allowing the unwanted side products to be recycled to form capsules. This approach is, however, limited by the relative instability of the resulting capsule complexes. Labile capsule assemblies held together by noncovalent interactions are constantly exchanging in solution, a serious problem if the intention is to shield guests from the outer environment. Despite this restriction, many impressive examples of self-assembling capsules have been recorded.[301−306]

The vast majority of molecular capsule DCLs feature noncovalent interactions. It is perhaps surprising that dynamic covalent chemistry has not made a greater impact on molecular encapsulation in the same way it has impacted macrocyclic and linear receptors. In principle, dynamic covalent chemistry allows the self-assembly of capsules while retaining the benefits in stability that the covalent connectivity offers. Yet, there have been very few examples in the literature that employ dynamic covalent chemistry for capsule formation,[308−311] and only one of these introduces diversity in such a manner as to be considered dynamic combinatorial chemistry.[311]

4.1.3.1. DCLs of Molecular Capsules Using Hydrogen Bonds. One of the earliest examples of a molecular capsule DCL was by Crego Calama, Timmerman, Reinhoudt, and co-workers.[242] Previous work from the same group had already demonstrated that calix[4]arene bismelamines and barbiturate building blocks could self-assemble into capsular structures in apolar solvents.[243,312] Some degree of diversity was introduced by variation of the substituents on the barbiturate building blocks (**49a** and **49b** in Scheme 44). Building block exchange was found to be temperature dependent. Mixing two homocapsules in deuterated toluene at 0 °C in a 2:1 ratio gave no exchange. However at temperatures above 25 °C, equilibration was achieved after 2.5 h. It was found that equilibration times could be reduced to seconds by using the more polar solvent $CDCl_3$. Individual library members were also identified by Ag^+-assisted MALDI-TOF MS.

The same research group modified this system to produce a capsule DCL responsive to a template.[251] A DCL was produced containing a 1:1 mixture of unmodified and zinc-porphyrin-tethered calix[4]arene bismelamine units (**50a** and **50b**, respectively, in Scheme 45) and the complementary barbiturate unit **49a** (Scheme 45). In the absence of a template, a statistical mixture of capsules was obtained, but upon introduction of a 1,3,5-benzenetripyridine template **51**, the library self-sorts into homomeric capsules that contain either only unmodified or only modified calixarene units. Self-sorting arises from the strong metal−ligand interactions between the porphyrin-tethered homomeric capsule and the trispyridine template. The remaining unmodified calixarenes have no choice but to form the other homomeric capsule.

A templated hydrogen-bonded capsule DCL with greater diversity has been reported by Hof, Nuckolls, and Rebek.[248] The building blocks were based on glycoluril and sulfamide units that were connected to either end of an aromatic spacer and designed to self-assemble into tetrameric capsules by head-to-tail hydrogen bonds (Chart 12). Five different variations of aromatic substituents provided the diversity in the library. In the absence of a template, no capsular structures were formed, but on addition of ammonium cation templates (ethyltrimethylammonium and methylquinuclidinium tetrafluoroborate), tetrameric capsules containing the ammonium guest were formed and identified by ESI-MS. The distribution of capsule masses did not match the statistically predicted distribution and was significantly different for each template, indicating that the template selects its favored capsule host.

A rather different approach to dynamic combinatorial chemistry using hydrogen-bonded capsules was used by Wu and Isaacs.[247] The authors tested the preference of nine well-known hydrogen-bonded aggregates including three capsules (Reinhoudt's calixarene bis(rosette) discussed earlier,[243,312] Rebek's tennis ball,[313] and calixarene tetraurea capsules[314]) to either self-sort or form hetero-aggregates. This was achieved by simply mixing the aggregates in $CDCl_3$, allowing the system to equilibrate, and looking for the appearance of new resonances in the ^1H NMR not seen in the spectra of the individual aggregates. Reassuringly, diversity was selected against; heteromeric assemblies were scarcely detectable, and the components self-sorted into the homomeric assemblies that they were designed to form.

4.1.3.2. DCLs of Molecular Capsules Using Metal−Ligand Interactions. Metal−ligand interactions have been used by Albrecht and co-workers to create a DCL of ethylene-bridged di(8-hydroxyquinoline) ligands **52a−c** and Ga(III) ions, which self-assemble into metalla-cryptands and other capsule-like polycyclic structures (Scheme 46).[223] The

Scheme 45. Template-Induced Amplification of a Capsular Structure Carrying Six Zn Porphyrin Units from a Model DCL[251]

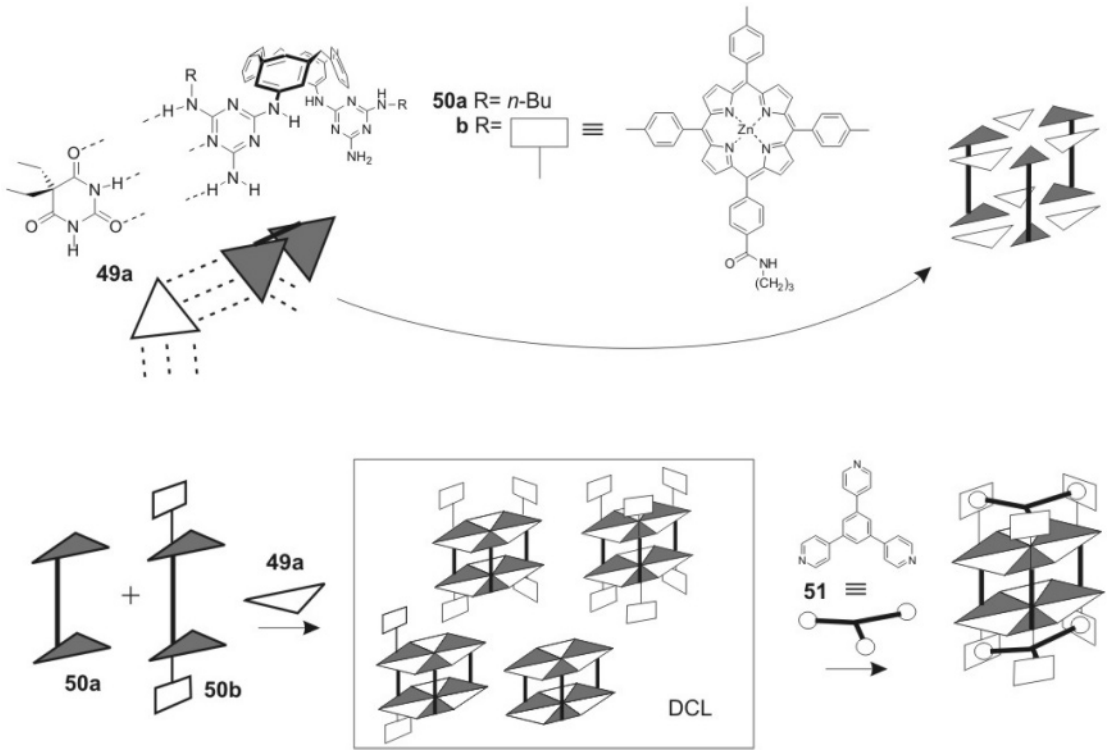
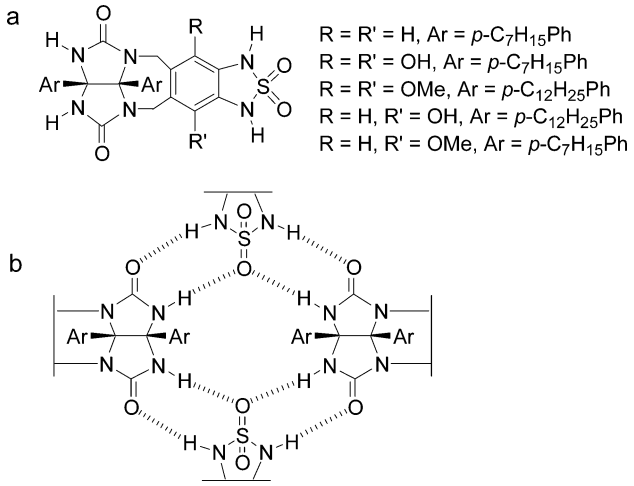


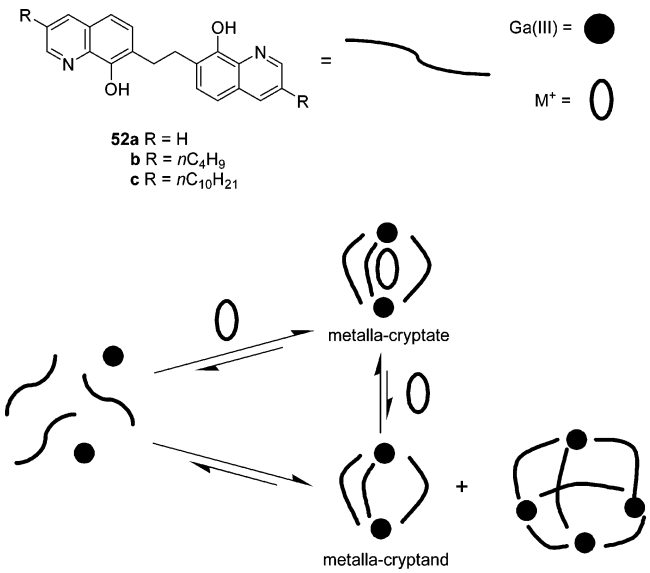
Chart 12. (a) Self-Complementary Building Blocks Carrying a Variety of Substituents; (b) Hydrogen-Bonding Pattern Directing the Self-Assembly Process[248]



formation of the metalla-cryptands was amplified by addition of suitable cations (Na^+, K^+, NH_4^+, Rb^+) that bind to the oxygen donors of the dihydroxyquinoline ligand, forming the cryptand as the thermodynamic product. Cryptand formation can be templated by cations with differing ionic radii because the gallium−gallium distance can be varied by twisting the cryptand. The cation-dependent shortening or lengthening was confirmed by X-ray crystallography and ^1H NMR.

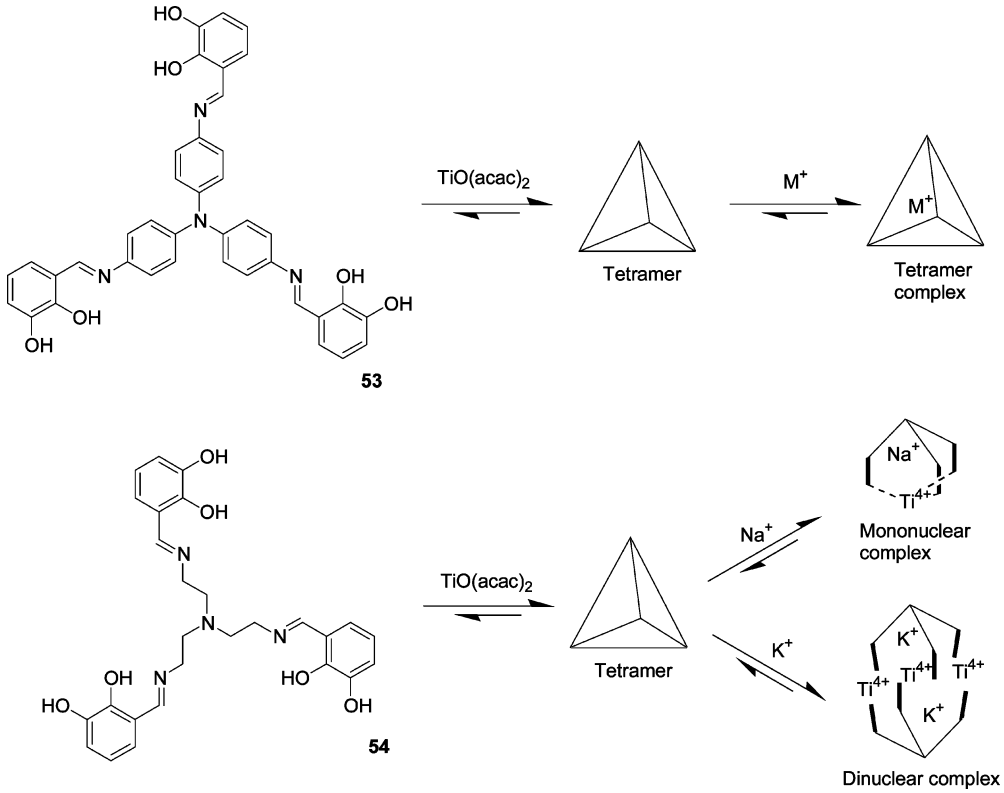
The same group has also used C_3-symmetric tris-catechol imines complexed to titanium(IV) to produce tetrameric (Ti_4L_4) capsules with large internal cavities (Scheme 47) and other metalla-cryptand complexes.[233−235] While the tris-catechol ligand **53** was found to strongly favor the formation of a tetrameric capsule, the more flexible ligand **54** was able to produce three different complexes with libraries containing

Scheme 46. Self-Assembly of Di(8-hydroxyquinoline) Ligands and Ga(III) Ions into a Small DCL of Metalla-cryptands and Other Capsule-Like Polycyclic Structures[223]



sodium or potassium ion templates or both. In the absence of such templates, oxygen and nitrogen lone pairs repel one another leading to a conformation where the imine points away from the nearby catechol oxygen. This more open conformation promotes the formation of the tetrameric capsule [(**54**)$_4$(Ti)$_4$]. Upon addition of K_2CO_3 or Na_2CO_3 to a DCL of **54** and [TiO(acac)$_2$] the conformation of the imine changes to point toward the nearby catechol oxygen atom in order to complex the metal ion through nitrogen and oxygen coordination. When a sodium ion is complexed, a mononuclear metalla-cryptate complex is formed, whereas in the presence of a potassium ion, a dinuclear complex is obtained (Scheme 47). Of all the species formed in DCLs

Scheme 47. Tris-catechol Imine Building Blocks and Their Templated Capsule Structures[233−235]



of **54**, the mononuclear complex was found to be the most thermodynamically stable.

Raymond and co-workers have used metal−ligand interactions to produce diverse DCLs of tetrahedral capsule structures from biscatecholate ligands (Chart 13a) and Ga(III) ions.[224] Previously, the same group had prepared an M_4L_6 tetrahedral capsule based on Ga(III) and **56** that encapsulated tetraalkylammonium ions, in particular tetraethylammonium chloride in water (Chart 13b).[315] When the synthesis of this capsule was repeated using two biscatecholate ligands (**56** and **57**), a small DCL of 12 different capsule stereoisomers was formed, each of which encapsulated a tetraethylammonium guest. The different isomers could be distinguished because each capsule causes a slightly different upfield shift in the ^{1}H NMR resonances of the guest. Capsules were also identified by ESI-FTICR-MS. DCLs with further biscatecholate ligands **55**−**59** produced greater diversity in the capsules formed, although some capsules were found to be disfavored. For example, tetrahedral capsules formed from ligands **56** and **59** were kinetically inert at room temperature but thermodynamically unstable. When heated, the heteromeric capsules self-sorted into homomeric $[Ga_4(\mathbf{56})_6(NEt_4)]^{11-}$ and $[Ga_4(\mathbf{59})_6(NEt_4)]^{11-}$ capsules.

Fujita and co-workers have used Pd−pyridyl interactions to produce some of the most elegant examples of molecular capsules based on metal−ligand interactions. DCLs containing tri- and dipyridyl ligands complexed to Pd(II) were used to produce capsules responsive to their guests. Their first example of such an approach used an asymmetric tripyridyl ligand **60**, which, when linked by three bridging Pd(II) centers **61**, dimerized into a molecular capsule.[227] An equilibrium mixture consisting of two isomers of this capsule (**62** and **63**, Scheme 48) was formed. The isomer **62** has a relatively flat cavity compared to the more spherical cavity of **63**. Upon addition of a globular guest such as $CBrCl_3$ or CBr_4, the equilibrium is shifted to favor the formation of **63**, whereas on addition of a flatter guest such as xylene or trimesic acid, the formation of **62** is favored.

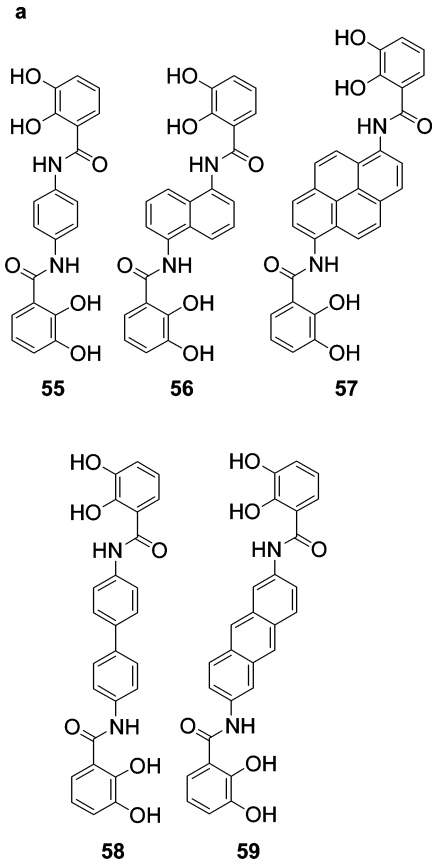
This system was further diversified in a later study where another, more flexible tripyridyl ligand **64** was added.[228] In this case, four Pd(II)-linked capsules were generated by mixing the two ligands and Pd(II) (Scheme 49). Due to the greater diversity of capsules available to the guests, templating effects were more noteworthy; guests such as CCl_4 and $CBrCl_3$ were found to selectively amplify the formation of the heteroligand capsule **66**, while other bulkier guests such as adamantane-1-carboxylic acid and adamantane-1-ol selectively amplified the homoligand capsules **65**. The flat trimesic acid guest also selectively amplified the homoligand capsules, but in this case it was owing to a strong affinity for the homoligand capsule **62** rather than **65**.

The most diverse DCLs prepared by Fujita and co-workers involve the use of two tripyridyl ligands (**67** and **64**) and three dipyridyl ligands (**68**−**70**) to generate Pd(II)-linked capsules and other receptors.[229] From this library, a new receptor for trichloroacetate **71** (Scheme 50) was identified using difference NMR.

Kobayashi and co-workers have recently used tetracyano and tetrapyridyl cavitands to produce Pd(II)- and Pt(II)-linked capsules.[316] Depending on the steric demands and the coordinative stability of the cavitand ligands, small thermodynamically controlled libraries where cavitands selectively self-assembled into either homo- or heterocapsules were made.

4.1.3.3. DCLs of Molecular Capsules Using Reversible Covalent Chemistry. We have begun to explore DCLs of molecular cages or capsules using disulfide exchange.[311] This approach is much the same as that used with the water-

Chart 13. (a) Biscatecholate Ligands Used by Raymond and Co-Workers To Form (b) Self-Assembling Capsules around a Tetraethylammonium Ion Guest[224,315]



soluble dithiols in macrocycle-producing DCLs (section 4.1.2.7), except that a trithiol building block is introduced to allow the formation of macrobicyclic structures. Our initial experiments used trithiol building block **72** based on three cysteine residues coupled to a trimesoyl scaffold (Chart 14). When **72** is oxidized by itself, a dimeric macrobicyclic structure is produced essentially quantitatively. However, when **72** is allowed to oxidize in the presence of dithiols **32** and **33**, a variety of mixed cage structures is generated as evident from LC-MS analysis. In some cases, all possible isomers for a particular building block composition were detected; for example, three different species corresponding to $(\mathbf{72})_2(\mathbf{33})_2$ were detected in a DCL containing **72** and **33**.

While we have so far not observed encarceration of guests by these capsules, such structures would have great potential in drug delivery, allowing release of the capsules' contents by disulfide reduction under physiologically relevant conditions.[317]

4.2. Catalysts from Dynamic Combinatorial Libraries

The remarkable efficiency and selectivity of enzymes has filled many scientists with envy. Designing synthetic equivalents that share at least some of the characteristics of Nature's catalysts remains a huge challenge, largely because we do not yet fully understand what makes enzymes as efficient as they are. While this situation persists, rather than designing catalysts from scratch, several approaches to the discovery of new catalytic systems are being developed that rely on selection of active species from a large collection of potential catalysts.[318−320] For in vitro evolutionary screening of ribozymes, selection is based on catalytic activity.[321,322] Alternatively, selection can be based on affinity for the transition state of a given reaction. Transition-state binding should be accompanied by stabilization and, provided that this effect exceeds any initial-state stabilization, lead to a reduced Gibbs energy of activation. Since it is extremely difficult to directly screen for transition-state affinity, in practice screening is based on affinity for a stable transition-state analogue (TSA). This strategy has been used with some success in molecular imprinting and in catalytic antibodies. The former approach involves forming a polymer around a TSA that is subsequently removed to generate catalytically active molecular-sized cavities.[323] The latter mobilizes the mammalian immune system to produce antibodies against a TSA.[324−326]

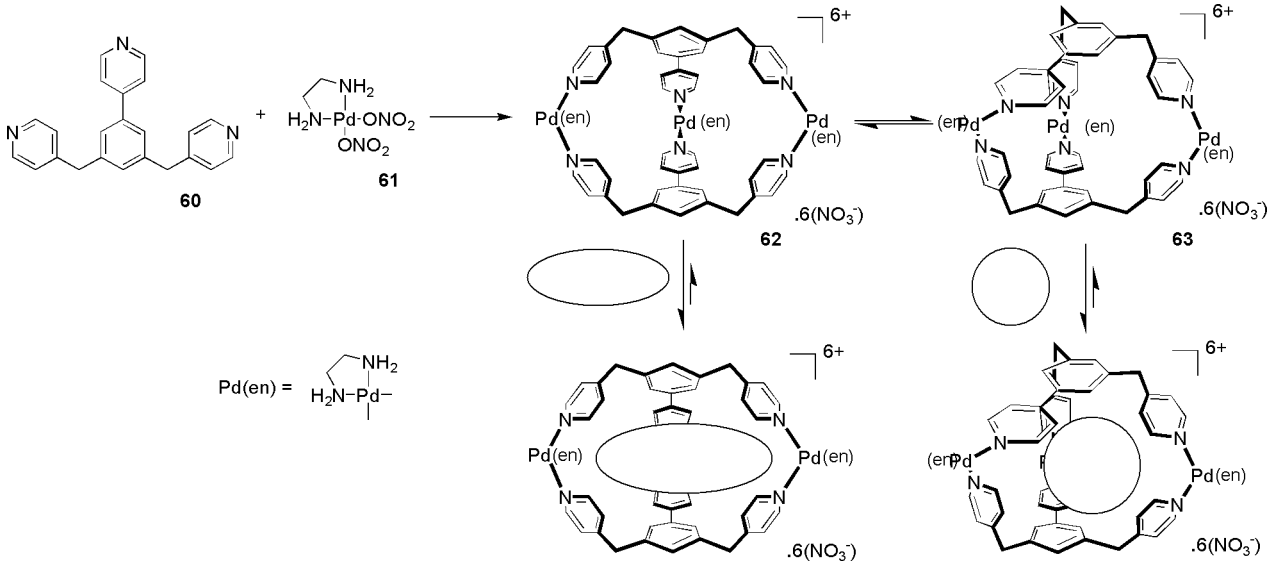
Dynamic combinatorial chemistry has considerable potential to develop into an attractive alternative to the imprinting and antibody approaches. We have reported two systems in which screening a DCL for affinity for a TSA has resulted in the discovery of new catalysts.

In the first example, a library made from building blocks **31**−**33** (Scheme 40, section 4.1.2.7) was screened for a TSA for the Diels−Alder reaction shown in Scheme 51a.[327] Given that the transition state of this reaction closely resembles the product **75**, the latter was used as the TSA. In the presence of this compound, macrocycles **37** and **38** (Scheme 40) were amplified simultaneously. Analysis of the affinities of both macrocycles for starting material **73** and product/TSA **75** revealed some interesting differences: the larger macrocycle **38** was found to bind product **75** more strongly than the starting material **73**, whereas the smaller macrocycle **37** showed exactly the opposite behavior. These results suggested that only the larger macrocycle is big enough to simultaneously bind both starting materials **73** and **74**; therefore, **38** but not **37** should be catalytically active. Kinetic experiments confirmed this prediction: **38** was able to induce an approximately 10-fold increase in the rate of the reaction, whereas **37** exhibited no catalytic activity.

Note that when the product of a reaction is used as a TSA, any catalyst that is identified will be product-inhibited. Control experiments revealed that this was indeed the case, but because the affinities of starting material and product were comparable, turnover was still possible.

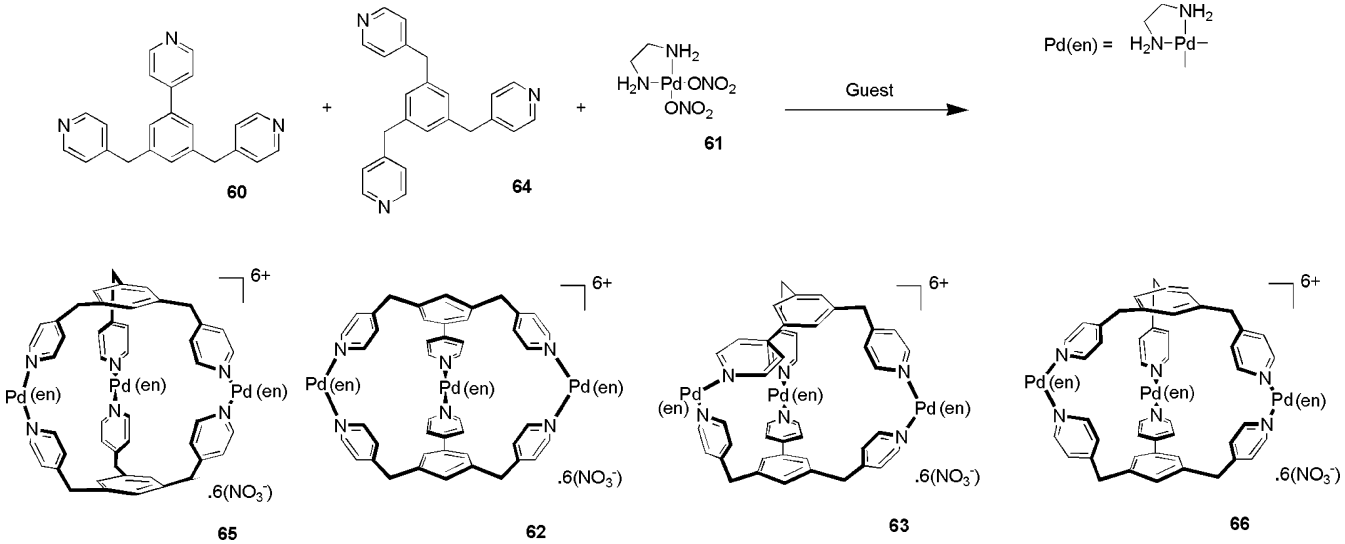
In the second example, a library was screened against a transition state analogue for an acetal hydrolysis reaction (Scheme 51b).[328] Acetal hydrolysis proceeds through a protonation pre-equilibrium followed by a rate-determining unimolecular dissociation of the acylium cation intermediate to give the hydrolysis products. Quaternary ammonium ion **76** was used as the TSA. Exposing a DCL made from a set of dithiol building blocks to **76** induced the amplification

Scheme 48. The Formation of Two Dynamic Pd-Linked Capsule Isomers Where Isomer 62 Has a Cavity Significantly Flatter than 63[a]

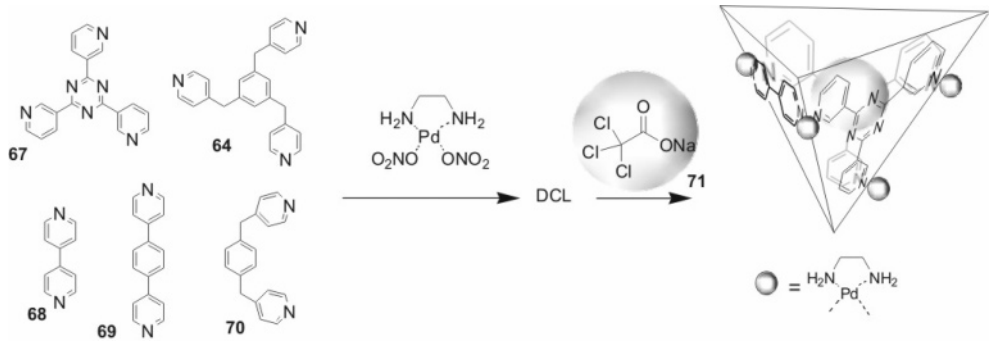


[a] The equilibrium can be biased to favor either capsule by the addition of suitable "flat" or "globular" guest.[227]

Scheme 49. A Small DCL Containing Four Pd-Linked Capsules from Two Tripyridyl Ligands[228]



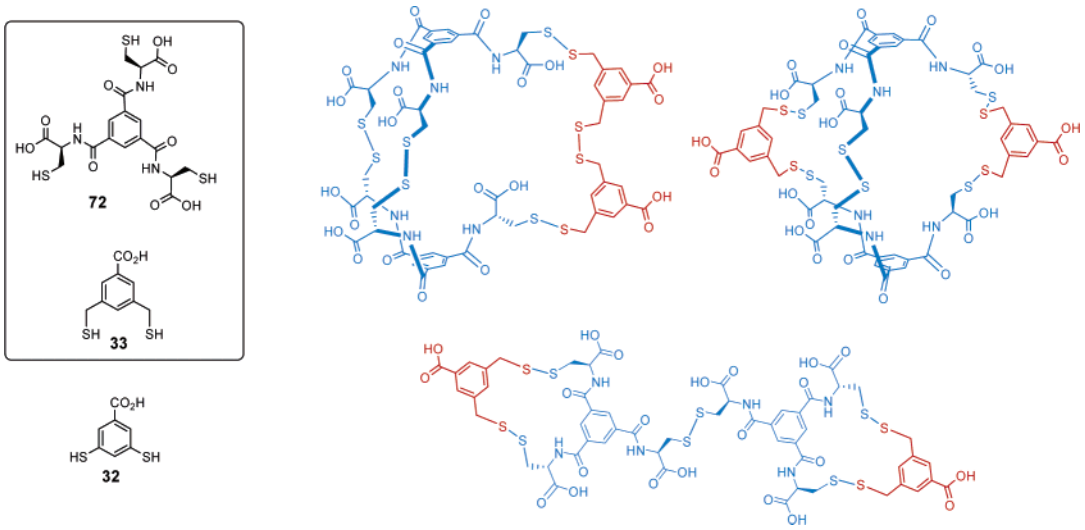
Scheme 50. A DCL of Pd-Linked Capsules and Cages Composed of Dipyridyl and Tripyridyl Ligands from Which a Sodium Trichloroacetate Receptor Is Generated[229]



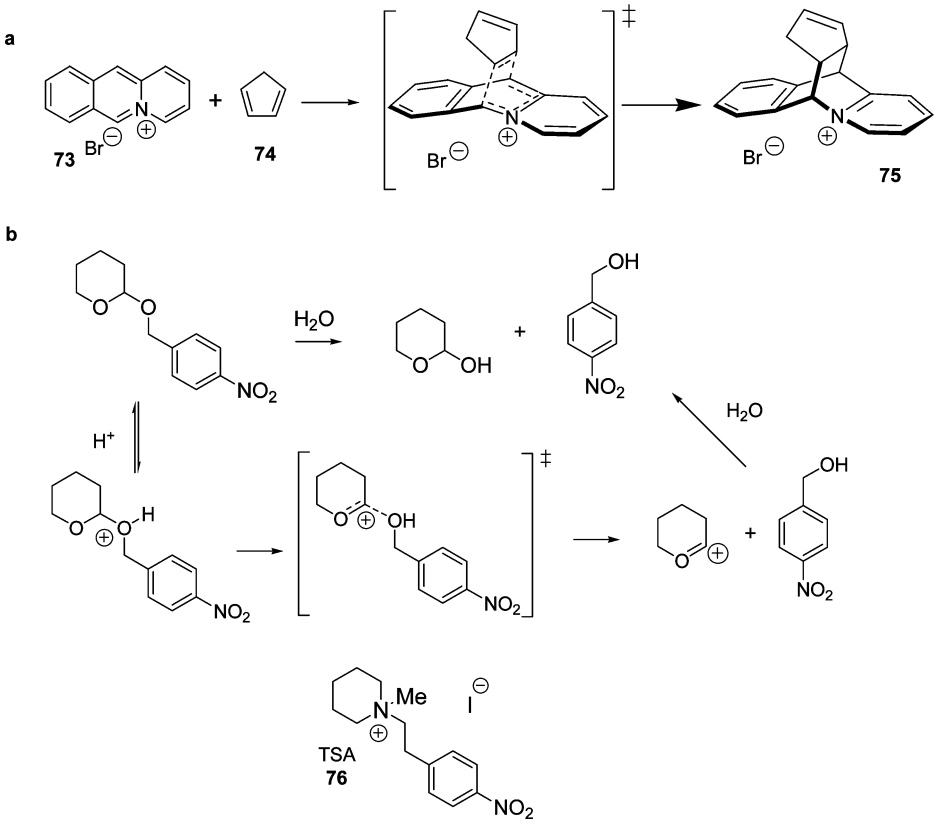
of, once again, macrocycle **38** (Scheme 40). This pluripotent catalyst was found to induce a modest 2-fold acceleration of the acetal hydrolysis reaction.

While these systems provide proof-of-principle, the catalytic activity leaves much to be desired, and clearly more work is required to establish the practicality of the method. However, there are some intrinsic advantages using dynamic combinatorial chemistry compared with the more established methods of catalyst discovery. The dynamic combinatorial method is more controllable and experimentally less demanding than the catalytic antibody approach and generates better-defined systems than imprinted polymers.

Chart 14. Some of the Isomeric Macrobicyclic Structures Produced in a DCL Made from 72 and 33[311]



Scheme 51. Reactions for Which Catalysts Have Been Developed Using Dynamic Combinatorial Chemistry[327,328]



4.3. Ligands for Biomolecules

Dynamic combinatorial chemistry has considerable potential as a tool for the discovery of new ligands for biomolecules in general and for drug discovery in particular. The ability of a DCL to shift its product distribution toward library members that are stabilized through noncovalent interactions can be extremely useful with systems such as proteins or nucleic acids, where the exact three-dimensional structure is often unknown, difficult to model, or even strongly dependent on the ligand bound.

Of the range of exchange reactions available to date (section 2), only relatively few are suited for use with biomolecules. At present, disulfide exchange (section 2.1.5) is one of the reactions that have been used most widely in this context.[187,188,190−192,202,203,329,330] Also imine exchange (section 2.1.2.1) is extensively used in the presence of biomolecules,[12,22,24,112,142,144,145,238,331] although normally a reduction step is required to convert the hydrolytically labile imines into amines. In contrast to imines, hydrazones are stable at physiological pH, but their formation and exchange typically require acidic conditions (for an exception, see ref 152), which are incompatible with most biological targets. Some special approaches that nevertheless combine hydrazone chemistry and biological targets will be discussed in section 6. Finally, thioester exchange[99,100] and enzymatic reactions such as the exchange of peptidic bonds[23] and aldol reactions[88,89] are also compatible with biological targets, although thus far only two enzymes have actually been used

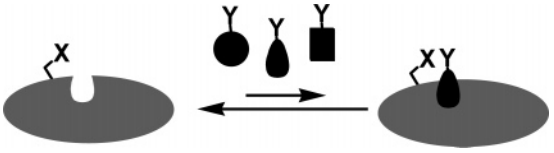


Figure 8. Schematic representation of a tethering DCL.[330]

for generating DCLs.

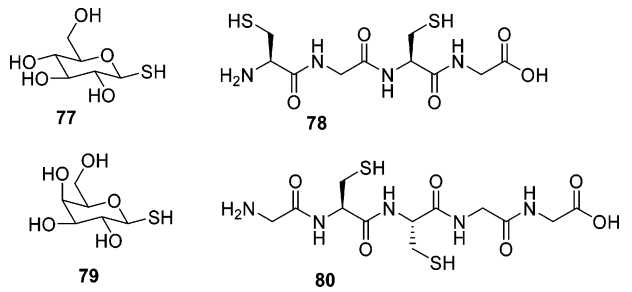
In the following sections, we review successful examples where biomolecules act as templates for the selection and formation of their own ligands from DCLs (Figure 1d). In these examples, the association between ligand and biomolecule is strictly noncovalent. Recently a related tethering approach has been developed in which a reversible covalent linkage is used that tethers the ligand to the biomolecule. Because this approach has been reviewed in detail by Erlanson and Hansen,[330] we only give a brief description of this elegant concept here. The tethering technique is used to develop new ligands for specific binding locations on proteins. In general, a cysteine residue (either wild-type or introduced by mutation) near the region that is screened is used as a covalent anchor for the ligand. In the presence of various small thiol molecules a variety of different disulfides can be formed under thermodynamic control. Those adducts where the thiol building block interacts most favorably with the protein will be formed preferentially (Figure 8). The main advantage of tethering is that it facilitates the binding of the ligands so that also low-affinity binders can be detected that would otherwise not bind efficiently enough to the protein.

4.3.1. Targeting Peptides and Proteins

The very subtle mechanisms of protein folding and flexibility, the difficulty in predicting the strength of interactions between ligands and proteins, and the fact that the structure of many proteins is not known precisely makes the development of good ligands a tedious and often unrewarding task. Dynamic combinatorial chemistry is one possible answer to the question of how to speed up and optimize drug discovery processes. DCLs are able to respond to very subtle interactions, the precise understanding of which is not indispensable.

4.3.1.1. Untemplated Libraries. Because both peptides and glycosides offer high potential for recognition of proteins, glycopeptide DCLs are of considerable interest. Sando et al. recently published a DCL made from peptide and glycoside building blocks using disulfide chemistry.[188] The peptides **78** and **80**, each containing two cysteine residues, were mixed with thiosugars **77** and **79** resulting in libraries of cyclic peptides, thiosugar dimers, and mixed linear species (Chart 15). While the authors intended to use these libraries to

Chart 15. Building Blocks for a Glycopeptide DCL[188]

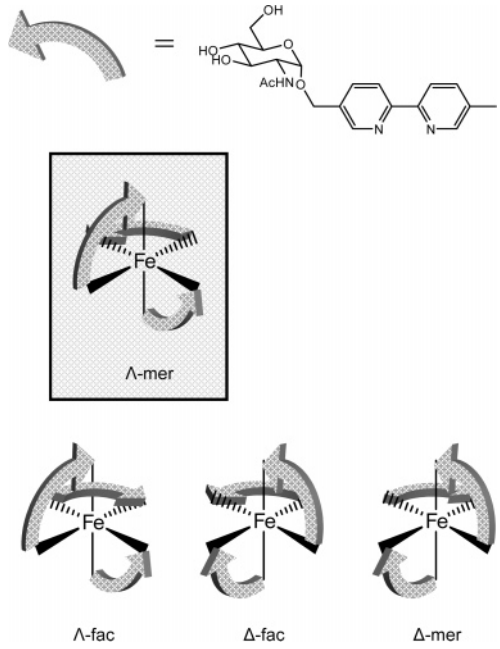


screen for affinity for sugar-binding proteins such as lectins, no templating studies have yet appeared.

4.3.1.2. Proofs-of-Principle. One of the first studies that aimed to use a protein as a template to drive an equilibrium mixture toward the strongest binding members was published in 1996. Venton and co-workers have prepared a DCL of peptides using the proteins fibrinogen and the monoclonal antibody 3E7 as targets.[23] The exchange process was based on cleavage and formation of peptide bonds mediated by the peptidase thermolysin. The system was divided in two compartments separated by a dialysis membrane permeable to library members but not to the protease or to the protein templates. In one compartment, peptide fragments were equilibrated with each other, and the DCL was formed. In the second compartment, the "frozen" library members were allowed to interact with the template protein. The presence of the targets induced some weak but detectable changes in product distribution.

Another early study used a DCL obtained from diastereoisomeric Fe(II) complexes in the presence of *Vicia villosa* B_4 lectin, a protein that specifically binds to *N*-acetyl-D-galactosamine (Chart 16).[25,26] A single ligand, an asymmetric

Chart 16. A DCL of Bipyridine−Iron Complexes, Which upon Screening against a Lectin, Gave Predominantly the Λ-mer Isomer[25,26]

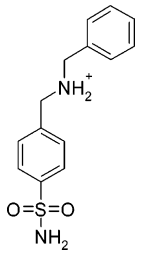


bipyridine functionalized with an *N*-acetyl-D-galactosamine moiety, yielded a DCL of four isomeric tris-bipyridine Fe(II) complexes. Due to the different binding affinities of the different diastereomers to the lectin, the composition of the DCL was shifted from an essentially statistical mixture of the library members in the absence of template toward a significant amplification of the Λ-mer isomer when the lectin was present.

Huc and Lehn's seminal study on carbonic anhydrase inhibitors was probably the first paper that clearly described the characteristics and potential of DCLs with biomolecules.[22] A library of 12 imine-linked members generated from three aldehydes and four amines was used; the imines generated were slowly reduced into the corresponding amines before HPLC analysis. The comparison of the HPLC traces of an untemplated control library with the carbonic anhydrase-templated DCL showed amplification of several species.

Amplification of a sulfonamide related to a known inhibitor was shown to be strongest (Chart 17).

Chart 17. Carbonic Anhydrase Inhibitor Amplified by the Enzyme from a DCL of Imines[22]



A slightly different approach was used in a disulfide library screened against the lectin concanavalin A, a protein that generally binds a trimannoside carbohydrate.[187] A library of different sugars linked to an ω-thiol-functionalized tether was used to mimic the natural binder. Modest amplification of the dimer made of two mannose units was observed in the presence of the lectin template. Note that the good binders were identified on the basis of their depletion from the solution rather than their amplification. However, the best binders could also be observed by eluting them from an immobilized version of the template.

The early work on enzyme-mediated exchange[23] encouraged the design of other libraries in which an enzyme mediates the exchange reaction.[88,89] Flitsch, Turner, and coworkers have used *N*-acetylneuraminic acid aldolase, which catalyses the aldol reaction between a ketone and the formal aldehyde of an aldose, thus forming and cleaving a C−C bond (section 2.1.1.4, Scheme 5). Using up to four different aldoses in the presence of an excess of ketone, they generated a DCL in which up to four aldol products were formed. These model DCLs were screened against the lectin wheat germ agglutinin and amplification of the library member with affinity for the lectin was observed.

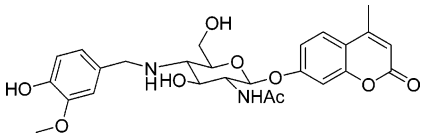
The Hamilton group has reported an elegant strategy in which they functionalized short sequences of single-stranded DNA with small organic fragments with potential affinity for protein targets.[332] The idea is to select for the duplex that carries the preferred recognition elements through a sequence of heating (to promote duplex dissociation) and binding steps. Initial proof-of-principle studies targeted streptavidin.

Danieli et al. used two potential antitumor compounds that were tethered with thiol-containing linkers of various lengths.[333] The nonpolar nature of the building blocks necessitated the use of organic solvents in order to form soluble DCLs. The mixtures were then exposed to two proteins that served as models for the desired target proteins, tubulins, which are themselves not compatible with organic solvents. Target-induced shifts in the equilibrium distribution were observed.

4.3.1.3. Discovery of New Ligands. While the examples in the previous section demonstrate proof-of-principle, recent work has resulted in the discovery of some exciting new ligands for a set of different proteins.

Starting the discovery process from fragments with low inhibitory activity has proven to be a successful strategy. A recent study was based on *N*-acetyl-D-glucosamine, a low-affinity inhibitor for hen egg-white lysozyme (HEWL), an enzyme that cleaves polymers of *N*-acetyl-D-glucosamine units.[331] An imine DCL was created from two amines (amine-functionalized *N*-acetyl-D-glucosamine and D-glucose) and six aromatic aldehydes. HEWL shifted the composition of the library toward two out of twelve adducts. A clear preference of HEWL for *N*-acetyl-D-glucosamine over other D-glucose derivatives was observed. One of the amplified species (Chart 18) showed much higher inhibitory activity

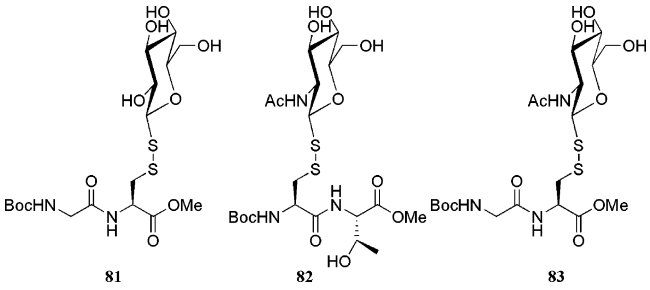
Chart 18. Successful HEWL Activity Inhibitor Discovered in a DCL[331]



than *N*-acetyl-D-glucosamine and was able to compete with chitobiose, a known successful inhibitor of the enzyme.

In a recent study, a DCL of two disulfide building blocks based on glycoside and dipeptide moieties was screened against the protein wheat germ agglutinin (WGA).[334] WGA is a protein used for detecting the naturally occurring reversible modification of a serine or threonine residue with a molecule of *N*-acetylglucosamine, a process involved in the regulation of protein activity. In presence of the template WGA, *N*-acetylglucosamine-containing species were amplified, with molecule **83** being amplified over 2-fold. ESI-MS analysis of the DCL also showed the presence of an **83**−WGA complex (Chart 19).

Chart 19. Disulfide Building Blocks (81 and 82) and Amplified Species 83 in a DCL Screened against Wheat Germ Agglutinin[334]



Frequently, molecules of biological interest are ditopic: they have two (similar or different) units that are involved in the recognition of a biomolecule, separated by a spacer of variable geometric and electrostatic properties. Dynamic combinatorial chemistry can be helpful to optimize the length, geometry, or electrostatics of this spacer once the recognition units are known. If the latter are equipped with appropriate exchange functions and used in conjunction with different spacers, they can form a library that, in the presence of the biomolecule to recognize, will shift toward the library member with the optimum spacer. This stratagy has been used by Nicolaou et al. to select the optimum spacer between two vancomycin molecules.

Vancomycin, a last-resort antibiotic, acts by strongly binding to the D-Ala-D-Ala sequence of peptidoglycan, thus inhibiting the synthesis of the bacterial cell wall. Vancomycin is known to dimerize in the absence and, with a higher association constant, in the presence of D-Ala-D-Ala. Synthetic covalent dimers of vancomycin exhibit higher affinities than monomers. Several novel vancomycin dimers were synthesized and screened for activity in a DCL-type assay.[202,203] Tethers of variable length, functionalized for disulfide exchange or alkene metathesis, were grafted onto a vancomycin unit. When exchange was performed in the

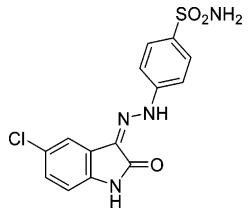
presence of the target peptide sequence, amplification of some of the dimeric library members was observed. By subjecting the amplified and other library members to tests for antibacterial activity, it was demonstrated that the amplified species did indeed exhibit the highest activity.

Another interesting feature of this work is the fact that systems for two different exchange reactions were designed in parallel and compared. The alkene libraries proved to be more sensitive to tether length, and they were also deemed to be more biocompatible than disulfides, which can easily undergo exchange in the presence of thiolate anions such as reduced glutathione or other reduced cysteine-containing peptides and proteins.

Note that the authors of this work termed their approach target-accelerated combinatorial synthesis (TACS) rather than dynamic combinatorial chemistry. No control experiments were carried out to demonstrate whether the product mixture is under thermodynamic or kinetic control.

Scientists at Astex Therapeutics have developed an approach called dynamic combinatorial crystallography (DCX).[335] This builds on their previous work, where protein crystals are soaked in solutions containing small-molecule fragments (M_W = 100−200). Successful binders are then detected in their binding sites by X-ray crystallography. In DCX, protein crystals are soaked with DCLs of fragments to find the best binder. In the published system, a crystal of cyclin-dependent kinase 2 (CDK2) was soaked with a DCL of 30 hydrazones. The electron-density maps of the soaked and unsoaked crystals showed that a single library member (Chart 20) had

Chart 20. A Cyclin-Dependent Kinase 2 Inhibitor Identified by Dynamic Combinatorial Crystallography[335]



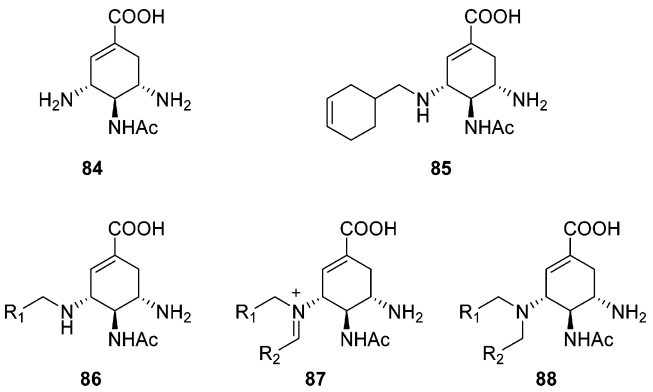
bound to the ATP-binding grove of the kinase. An assay showed this compound to be a potent inhibitor (IC_{50} = 30 nM).

Milanesi et al. have targeted the calcium transducer protein calmodulin with a DCL prepared from five different monothiols that are potential low-affinity binders.[336] The resulting DCL contained 15 disulfides, two of which were found to be amplified in the presence of calmodulin. The selected library members both contained sulfonamide groups.

4.3.1.4. Toward High-Throughput Screening. Over the last decade, traditional combinatorial chemistry has been gearing up for the parallel screening of very high numbers of compounds in a comparatively short span of time requiring extensive automation. Dynamic combinatorial chemistry may prove to be an attractive alternative because it takes away some of the need for high-throughput since a DCL screens itself by increasing the amounts of the wanted compounds while suppressing their inferior counterparts. However, DCLs containing more than 100 library members are still rare, and at present, it is not clear how large a DCL can be made while still allowing hits to be identified.

Work on relatively large imine libraries has been carried out by the Therascope group (now part of Alantos).[112,142] The target in these studies was neuraminidase, an enzyme involved in the propagation of the influenza virus. Libraries potentially containing over 40 000 members were screened against the enzyme.[142] These libraries were built around a central scaffold **84** bearing two primary amines (Chart 21).

Chart 21. Central Scaffold 84, Stable and Transient Library Members 86−88, and Successful Inhibitor 85[142]



The scaffold closely resembles the central core of several known neuraminidase inhibitors and presents a carboxylate and an acetamide group, which play important roles in the recognition of the active site of the enzyme. Commercially available simple aldehydes were reacted with the scaffold. In situ reduction of the resulting imines by $NaBH_3CN$ yields secondary amines **86**, which can form quaternary iminium species **87** with an additional aldehyde, before being reduced to the final tertiary amines **88**. Thus four different substituents can be grafted on every scaffold, generating from a set of 20 aldehydes a library with more than 40 000 potential members. The experimental conditions were chosen such that the amine products can only be detected if they are amplified. Not more than a couple of hits were identified from each of these libraries including compound **85**, which was a successful inhibitor of the enzyme.[337]

Another study on neuraminidase inhibitors was published later[112] using the same scaffold in the presence of ketones rather than aldehydes as building blocks. Although the adduct of one of the ketones had an approximately 500-fold increase in potency as an inhibitor as compared to the previously identified aldehyde adduct **85**, activities of related molecules known as potent inhibitors remained unmatched. While one can argue to what extent the experimental setup allows for the formation of significant quantities of bis-imines, the results from these studies are encouraging.

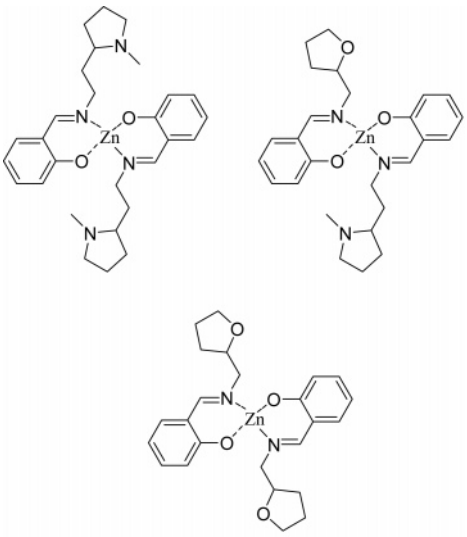
4.3.2. Targeting Nucleic Acids

Unlike the 20-letter alphabet of peptides, the language of nucleic acids mainly operates with four different units that team up in fairly well-defined base-pairing patterns. While the gross behavior of duplex DNA is well-studied, quadruplex DNA folding is still controversial, and the folding of RNA to a large extent still remains unpredictable. Interactions of other types of molecules with a given DNA or RNA are very subtle and manifold, and their prediction remains a major challenge. In section 4.3.2.1, we will describe DCL studies on non-nucleic acid molecules that interact with nucleic acids. Nucleic acid−nucleic acid interactions will be discussed in section 4.3.2.2.

4.3.2.1. Ligands for Duplex or Quadruplex DNA. Studies of DCLs with metal complexes binding to DNA and RNA were carried out by Miller and co-workers.[24,218,238] In their first two publications, zinc(II) salicylaldimine com-

plexes were used.[24,238] The imine ligands are formed by condensation of salicylaldehyde with a primary amine. Zinc is coordinatively labile and allows ligand exchange at the same time as stabilizing the imine bond. Initially the authors studied a system of salicylaldimine complexes targeting oligo d(A·T) DNA immobilized on a cellulose resin.[24] A library of up to 21 bis-salicylaldimine complexes was made from six different amines. After initial equilibration in the absence of template, the mixture was allowed to re-equilibrate on the DNA-functionalized affinity column. Nonbound species were eluted from the column, hydrolyzed, derivatized, and analyzed by HPLC, indirectly revealing three new potential ligands for DNA (Chart 22).

Chart 22. Bis(salicylaldimine)−Zinc Complexes That Were Retained on Immobilized d(A·T) DNA[24]



In this method of analysis, amplification of a specific library member is reflected in a decrease of the concentration of the corresponding amine(s) in the eluted solution. This approach might pose problems in libraries where several species are amplified or in more complex systems (e.g., with metal centers with three different ligands), because only constitutional but no structural information on the amplified library member is obtained.

A third study[218] by the same group used the coordination chemistry of Cu(II) to compare affinities to an RNA hairpin and its homologous DNA sequence. In this work, the ligands were static molecules; all diversity was created by exchange of the bi- or tridentate ligands based on salicylamides of amino acids, forming complexes with one or two ligands in a square planar arrangement. The comparison of the two templates proved very interesting: one library member with a more than 300-fold preference of RNA over the homologous DNA was identified.

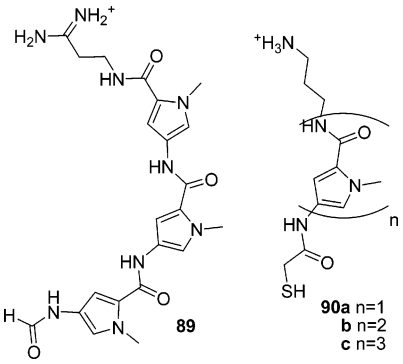
McNaughton and Miller have recently developed an interesting new method in which they immobilize the building blocks on a resin and combine these with the same building blocks in solution.[338] Starting from monothiol building blocks, based on known sequence-specific DNA binders, a DCL of disulfides was prepared and screened using a fluorescently modified DNA template. The beads that contain library members that have affinity for the template will light up, allowing relatively straightforward identification of the building blocks that are part of good binders.

G-Quadruplex DNA is believed to play an important role in various cellular functions and is known to have a crucial role in the aging process of cells, and as such, it is a promising target for cancer treatment. Indeed, stabilization of quadruplexes by binding with a suitable ligand can inhibit the activity of telomerase, an enzyme that interrupts the aging process, making cancer cells immortal. The large planar aromatic surface of the four guanine systems can be complexed by various ligands including acridones. Stabilization of the quadruplex system can be enhanced by linking the π-stacking molecule to a unit that binds to one of the lateral grooves in which the backbone phosphates and the guanine faces not involved in base pairing are exposed.

In a recent study by the Balasubramanian group, an acridone unit bearing positive charges and a thiol group and a thiol-functionalized oligopeptide were used as building blocks.[192] In the presence of a glutathione redox buffer, a library of six disulfides was produced. In the absence of the template, the ratios of the building blocks were roughly statistical. However, in the presence of a G-quadruplex unit, there was a dramatic shift toward two species: the expected acridone−peptide dimer but also the homodimer of the peptide building block. The binding mode of the peptide homodimer remains unknown.

In a more recent paper by the same group, monothiols containing one, two, or three pyrrole units were used, which were inspired by distamycin **89**, a high-affinity binder for both duplex and quadruplex DNA (Chart 23).[193] In presence

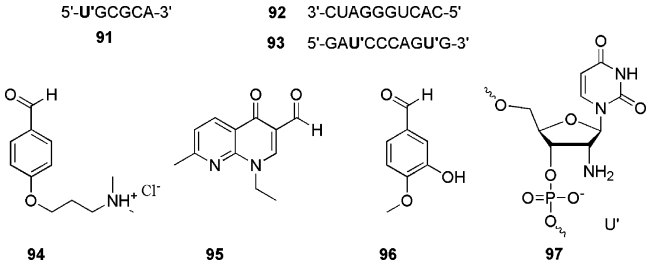
Chart 23. Distamycin and Thiol Building Blocks Inspired by Its Structure[193]



of duplex or quadruplex DNA, homo- and heterodimers of building blocks **90b** and **90c** were strongly amplified. Glutathione redox buffer was used to decrease the self-association of building blocks, which would compete with binding of the dimers to the target. Duplex DNA melting temperatures were determined in the presence of the selected library members to gauge their relative binding affinities. The observed melting temperatures were correlated with the amplification factors.[377]

4.3.2.2. Enhancing Nucleic Acid−Nucleic Acid Recognition. The Rayner group has performed studies aimed at modifying an existing nucleic acid to enhance its affinity for a complementary nucleic acid. Early work involved a self-complementary hexadeoxyribonucleotide **91**, containing a 2′-deoxy-2′-aminoribose **97** at the 5′-terminus (Chart 24).[145] The modified DNA was reacted with a set of three aromatic aldehydes **94**−**96** that could stabilize the duplex structure by stacking on the outer base pairs while forming imine bonds with the amine functions of the terminal U′. The resulting unstable imines were trapped by reduction by $NaBH_3CN$. One main product was obtained, and a control experiment with a noncomplementary DNA sequence (i.e.,

Chart 24. DNA Sequences 91−93 of Which 91 and 93 Contain a Modified Aminosugar Base U′ (97), Which Can Condense with Aldehydes 94−96 To Give a Small DCL of Imines[145]



one that would not form a duplex) and measurements of the corresponding amines confirmed that the amplified product was indeed selected on the basis of its stabilizing effect on the hairpin structure.

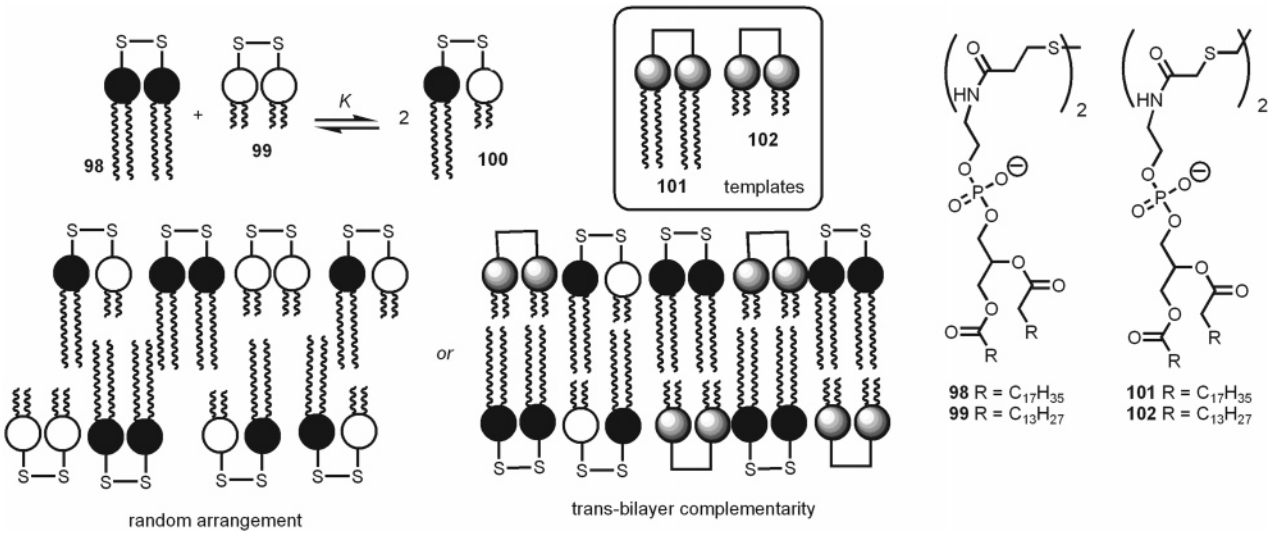
After this preliminary experiment, Rayner and co-workers expanded their strategy to a more complex and therapeutically interesting system: a short RNA aptamer that binds to the transactivating region (TAR) RNA hairpin element of HIV-1, forming a so-called kissing complex.[145] The goal was to increase the stability of the kissing complex by modifying the binding RNA. An amino-substituted RNA aptamer was reacted with the previous three aromatic aldehydes in the presence of the relevant part of the TAR sequence. Interestingly, the adduct with the same aldehyde as in the previous experiment was formed preferentially, and a slight stabilization of the kissing complex was detected from a rise in its melting temperature.

The same approach was used in a second publication by the same group.[339] Whereas the previous study had demonstrated that a functionalized complex can choose the building block that gives the best stabilization of the complex, it was now shown that, in the presence of multiple sites for fixation of the building block on the complex, the most stabilizing adduct was selectively formed. A decaribonucleotide containing two 2′-deoxy-2′-aminoribose-modified nucleotides **93** was reacted with a set of three different aldehydes **94**−**96** in the presence and in the absence of the decaribonucleotide complementary to the modified one (**92**). After separation of the library members by HPCL, the amplified member was identified by mass spectrometry. Digestion with an exonuclease revealed the position of the adduct. Finally, conventional synthesis of some of the individual adducts and melting point measurements confirmed that the most stabilizing adduct had indeed been formed.

4.4. Dynamic Combinatorial Approaches to Aggregation

In principle, the self-assembly of selected members of a DCL into an aggregate (Figure 1b) will influence the library composition, favoring those species that can form the most stable aggregates. This approach has received very little attention thus far as a tool for the development of, for example, new amphiphilic molecules. However, the Regen group has published an extensive series of studies on nearest-neighbor recognition within bilayer aggregates that is conceptually closely related. The nearest-neighbor approach relies on disulfide exchange between the lipid components of a bilayer to generate an equilibrium mixture. For instance, bilayer vesicles that are made from a mixture of two different lipid dimers, A−S−S−A and B−S−S−B, are allowed to equilibrate to give the two starting materials and the mixed disulfide, A−S−S−B. When the bilayer is homogeneous, A and B will mix perfectly and a random distribution of disulfide products will be obtained. However, should A, B, or both have a tendency to form separate domains within the bilayer, then the equilibrium position will be shifted toward the formation of homodimeric species. Thus analysis of the product mixture allows conclusions to be drawn regarding the way the thiol-modified phospholipid (or sterol molecules) are organized in the bilayer. Specific topics that have been addressed using this approach include the influence of cholesterol,[33,340−349] the nature of the headgroup,[350−352] unsaturation of the alkyl chains,[353,354] the nature of the linkage between the glycerol unit and the alkyl chains (ester or ether),[355,356] sphingolipids,[357,358] headgroup hydration,[359] peptides,[360,361] the extent of cooperativity in demixing,[362] and most recently the extent of trans-bilayer organization.[363−365] Because a comprehensive overview over this extensive body of work is beyond the scope of this review, we will here only mention one particularly appealing recent example that illustrates an interesting type of template effect on lipid organization. The Regen lab has studied the equilibrium among disulfide dimers **98**−**100** (Scheme 52) in the absence and presence of the fixed dimers **101** and **102** in order to

Scheme 52. The Use of Disulfide Exchange to Probe the Trans-Bilayer Organization of Lipids[363]



assess the extent of trans-bilayer organization. In the absence of the fixed dimers, the lipids mix randomly giving an equilibrium constant K close to 4 M^{-1}. However, upon replacement of the long dimer **98** with the long template **101**, a substantially reduced value of K was obtained indicating a shift of the equilibrium in the direction of the homodimers. A similar shift was observed when the short dimer **99** was replaced by the short template **102**. These results were interpreted as evidence for the existence of complementary trans-bilayer organization of the type shown in Scheme 52.

4.5. Dynamic Combinatorial Studies of Folding

Reversible reactions can be used to monitor folding into secondary or tertiary structures by proteins or nucleic acids, as well as by synthetic polymers or oligomers. In these systems, there is no formal template molecule; molecular recognition takes place intramolecularly. Thus far the size of the "libraries" used in these studies has been rather limited, and it is less the discovery of unpredictable species that is sought than the confirmation of empirical or theoretical predictions and the establishment of proof-of-principle. Work on the use of reversible chemistry to study folded structures has been published on three main classes of molecules: peptides (section 4.5.1), nucleotides (section 4.5.2), and synthetic polymers (section 4.5.3).

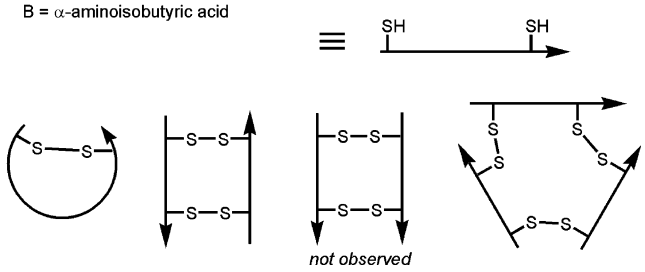
4.5.1. Folding of Peptides

Pons and co-workers have studied the product distribution obtained upon oxidizing a palindromic peptide containing two cysteine residues near the start and end of the sequence (Chart 25).[366,367] In principle, a mixture of products can be

Chart 25. Upon Oxidation of a Peptide Containing Two Cysteine Residues, Different Structures Are Produced Depending on the Extent of Helicity of the Peptide[366,367]

Ac-Cys-B-Lys-Leu-His-Ala-Glu-Leu-Ser-Ser-Leu-Glu-Ala-His-Leu-Lys-B-Cys-NH_2

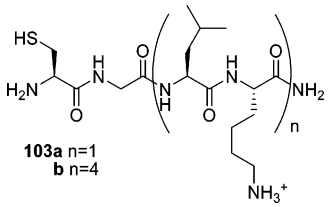
B = α-aminoisobutyric acid



expected ranging from a hairpin monomer with an intramolecular disulfide bond to parallel and antiparallel dimers, two different cyclic trimers, and larger cyclic oligomers. Under thermodynamic control, the product distribution was strongly dependent on the concentration of trifluoroethanol, a known promotor of α-helix formation. At low concentrations of trifluoroethanol, the hairpin monomer was the dominant product, whereas at higher concentrations (50% v/v), one of the cyclic trimers was preferred. Intermediate concentrations gave a mixture of products in which the antiparallel dimer was the major component, while no parallel dimer was observed. Thus, depending on the extent of secondary structure formation, specific different peptide frameworks are favored and formed selectively.

Formation of secondary structure was shown by the Balasubramanian group in a self-sorting DCL experiment using two β-sheet-forming oligopeptide building blocks.[191] Both building blocks, **103a** and **103b** (Chart 26), have

Chart 26. Building Blocks Used in a Small β-Sheet-forming DCL[191]

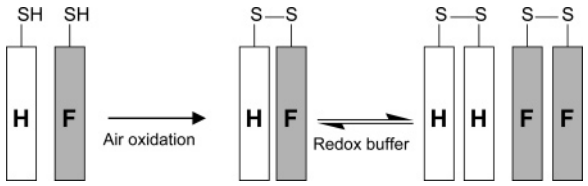


103a n=1
b n=4

alternating Leu-Lys sequences and a cysteine at one end, but the lengths of the Leu-Lys repeats were different. The Leu-Lys sequence is known to be prone to form β-sheets. A glutathione buffer was used to mediate disulfide exchange. In the absence of glutathione buffer, oxidation by atmospheric oxygen was too fast for the library to equilibrate, and product formation occurred under kinetic control without any sign of self-recognition of the building blocks. However, under thermodynamic control in the presence of the redox buffer, the library converged toward the almost exclusive formation of the homodimers of the peptides driven by the β-sheet formation of peptide dimer of **103b**. The formation of the homodimers was demonstrated by NOE data that indicated the formation of β-sheets.

Self-sorting on the basis of tertiary structure formation was observed in a DCL containing two types of α-helix-forming peptides.[190] One of the peptides was designed such that a series of leucine residues were offset by one helical turn. The second type had the same sequence, except that all leucines had been replaced by hexafluoroleucine residues. Each peptide was equipped at one extremity with a flexible Gly-Gly linker followed by a cysteine residue, allowing the formation of peptide dimers by oxidation of the cysteine thiol groups into disulfides (Scheme 53). Kinetically formed

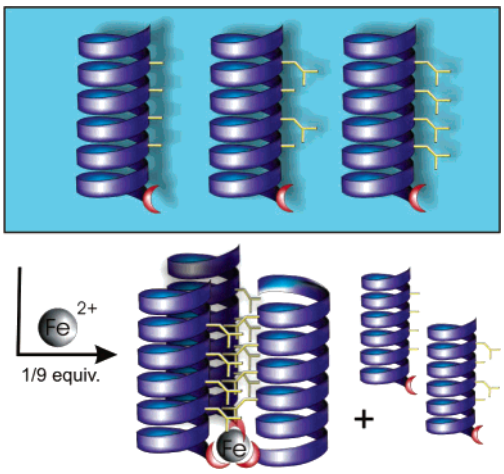
Scheme 53. Thermodynamically Controlled Self-Sorting of α-Helical Peptides[190]



heterodimers disproportionated in the presence of a glutathione redox buffer into two separate homodimeric α-helix bundles.

McLendon and co-workers have explored a similar idea, but using a radically different setup.[221,222,368] The authors have studied the folding of trihelix bundles driven by hydrophobic interactions. They used three α-helix-forming oligopeptides functionalized with a 2,2′-bipyridine that can form stable trimers by coordinating to a d^6 octahedral metal center. The helices differ in hydrophobicity by replacement of four alanine residues by zero, two, or four leucine residues (Scheme 54). If Fe(II) is used as the metal center, the kinetic lability of the coordination chemistry allows dynamic exchange of the ligands. With limiting Fe(II) concentration, preferential formation of the most stable peptide bundles made from the most hydrophobic helices is observed. The identities of the selected species were confirmed by various MS experiments. The stability of coordinatively stable Ru-

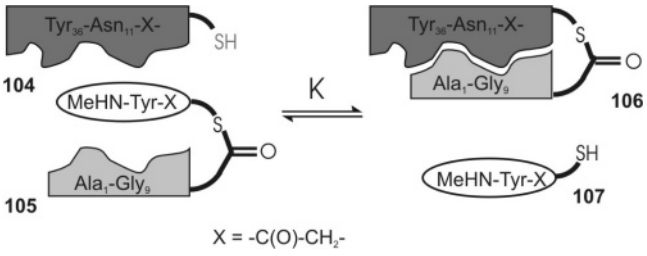
Scheme 54. Selection of the Most Stable α-Helix Bundle from a Small DCL of Iron Complexes[221]



(II) analogues was measured by unfolding the bundles using guanidinium chloride and paralleled the relative stabilities of the DCL members.

Woll and Gellman recently published a clever approach that allows the energy of folding to be estimated.[100] They took bovine pancreatic polypeptide, in which two domains, the C-terminal α-helical one and the *N*-terminal proline-rich one, were interacting in the folded structure of the polypeptide but separated by a loop of a few residues that were solvent-exposed and contributed little to the folded form. By replacing one amide bond in this loop by a thioester bond (as well as changing a few side chains that had little effect on folding, mainly for solubility reasons), they obtained a polypeptide that essentially conserved the structure of the native one. However, the thioester linkage now allowed the two domains of the peptide to be exchanged (Scheme 55).

Scheme 55. Thioester Equilibrium in Which Folding Favors Formation of the Bovine Pancreatic Polypeptide 106[100]



When the folded peptide **106** was allowed to equilibrate with thiol **107**, some of the nonfolded thioester **105** was produced, together with thiol fragment **104**. The stabilization of the bovine pancreatic polypeptide **106** through the noncovalent interactions that drive its folding also determined the position of the thioester equilibrium. By precisely measuring the concentrations of the different components at equilibrium, the free energy of folding could be deduced (assuming that the two thioester bonds have the same energy and that there are no interactions between the tyrosine thiol and the proline-rich domain in **105**).

4.5.2. Folding of Nucleotides

The folding of G-rich peptide oligonucleotides into PNA quadruplex structures in DCLs was reported recently by the Balasubramanian group.[189] PNA was chosen rather than DNA because it is easier to functionalize with amino acids. The tetranucleotides TTTT and TGGG were functionalized with amino acid sequences at both termini in order to provide good solubility, flexibility, and a thiol group for the exchange reaction (Chart 27). Upon oxidation under kinetic control,

Chart 27. Thiol Building Blocks Used for Assessing the Influence of G-Quadruplex Formation on the Equilibrium Distribution[189]

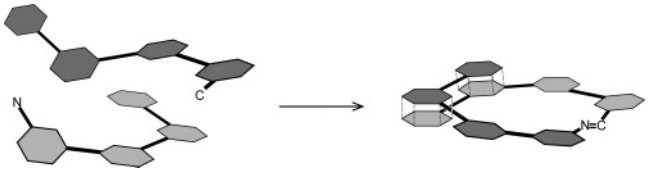
Lys-TTTT-Gly-Gly-Cys	Lys-TGGG-Gly-Gly-Cys
$\mathbf{T}_{SH}$	$\mathbf{G}_{SH}$

the dimers $\mathbf{T}_{SS}\mathbf{T}$, $\mathbf{G}_{SS}\mathbf{T}$, and $\mathbf{G}_{SS}\mathbf{G}$ were formed in an essentially statistical ratio. However, under thermodynamic control, and in the presence of potassium ions, self-sorting occurred, and a dimerization of $\mathbf{G}_{SS}\mathbf{G}$ was observed. MS, UV−vis melting experiments, and D/H-exchange NMR studies confirmed that an intermolecular complex of two $\mathbf{G}_{SS}\mathbf{G}$ entities was formed. Similarly, when potassium was replaced by sodium or lithium, less or no self-sorting was observed, and DCLs equilibrated at temperatures above the quadruplex melting temperature did not show any amplification. The authors also demonstrated that nucleobase recognition occurs prior to disulfide formation.

4.5.3. Folding of Synthetic Polymers

The richness of secondary and tertiary structures encountered in biopolymers has inspired chemists to create synthetic analogues that have an intrinsic tendency to adopt ordered structures in solution. Moore's research group has used reversible bonds to assemble monomers into folding oligomers (Scheme 56).[138−140,369] Curved monomers based on a

Scheme 56. Imine-Linked Building Blocks Forming Helical Assemblies

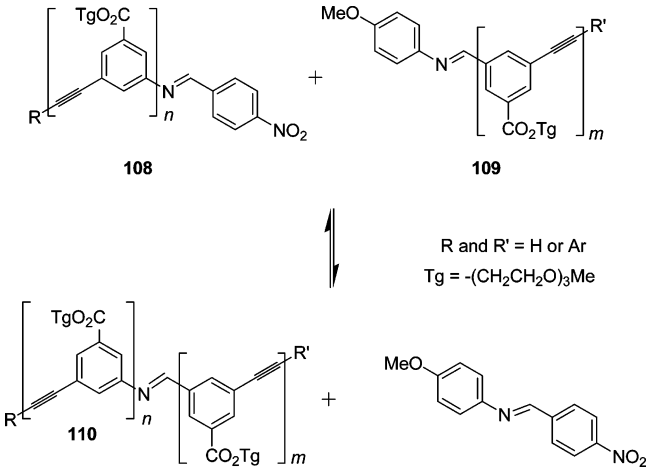


common structural motif with one or two linkers have been assembled into dimers and polymers that can adopt helical conformations. In most of the studies, equilibration of the products is achieved through imine metathesis in organic solvents, catalyzed by oxalic acid, although recently a study using pyridine−paladium coordination chemistry has also been published.[230]

In the first paper of the series, the authors studied the influence of the length of the curved motifs on the folding properties using monofunctionalized building blocks (Scheme 57).[138] The building blocks are based on different numbers of repeats (n = 1, 3, or 5 and m = 1, 3, or 5) of the *m*-phenylene ethynylene unit functionalized at one end with an amine (**108**) or an aldehyde (**109**), which are condensed into imines with sacrificial aldehyde and amine capping groups. Conformational studies had predicted that six aromatic units were necessary to form a full helical turn and that extra aromatic units could induce the formation of a helix, which would be stabilized by intrahelix solvophobic interactions. The imine linker was predicted to have a geometry similar to that of an alkyne linkage and would therefore not disrupt helix formation.

Using NMR studies, it was confirmed that imines with two or five aromatic units did not exhibit any helicity and

Scheme 57. Building Blocks Used for Folding Studies[a]



[a] Tg = triethoxymethyl; n = 1, 3, or 5; m = 1, 3, or 5.[138]

that 8-, 10-, or 12-mers formed helices of increasing stabilities. Under thermodynamic control, the shorter oligomers gave only little formation of the curved dimer **110**, whereas the helix-forming building blocks showed a very strong shift away from the "capped" starting materials. Furthermore, helix formation was not observed in chloroform but was readily noticeable in the more polar acetonitrile, which favors solvophobic interactions within the molecule.

A second publication shows two additional noteworthy results.[369] First, the position of the imine linkage in the helix does not significantly affect the helix stability, confirming that the imine is a suitable structural analogue of the alkyne linker. Second, the influence of helix length on the shift in the equilibrium was investigated. The maximum equilibrium shift was achieved when the contact between the "amine" and the "aldehyde" building blocks corresponded to one helical turn, that is, six aromatic units. Elongation of the building blocks such that they were already forming a helix by themselves did not further increase the shift in equilibrium.

In subsequent studies, the authors designed systems for polymeric self-assembly of helices.[139,140] Symmetrical oligo(*m*-phenylene ethynylene) units with two capping amine (**111**) or aldehyde (**112**) functions were used (Scheme 58).

When the bis-amine and the bis-aldehyde building blocks each had three aromatic units ($n_1 = n_2 = 1$), no polymer formation was observed. Instead, the building blocks formed cyclic dimers that stack on top of each other and form columnar assemblies.

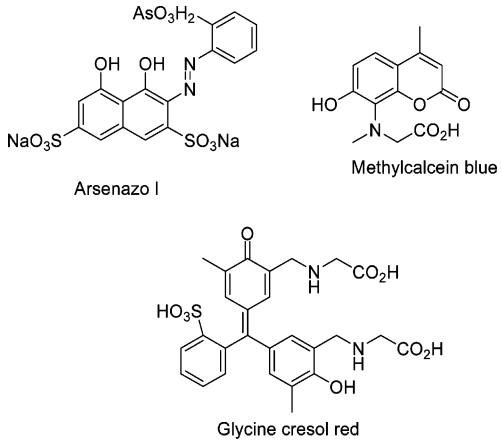
Monomers of different lengths form helical polymers **113**. The degree of polymerization of the monomers was measured in solvents of different polarities. As expected, polymer length increases as polarity increases, because helix formation is more strongly favored in polar solvents.

Building blocks with R = Me appeared to have an enhanced propensity to form helices. However, it was also noticed that equilibration times became very long (over 25 weeks), because imine metathesis is kinetically difficult in strongly preorganized assemblies. Because high temperatures disfavor helix formation but speed up metathesis, reaction conditions were optimized by varying the temperature.

4.6. Sensors

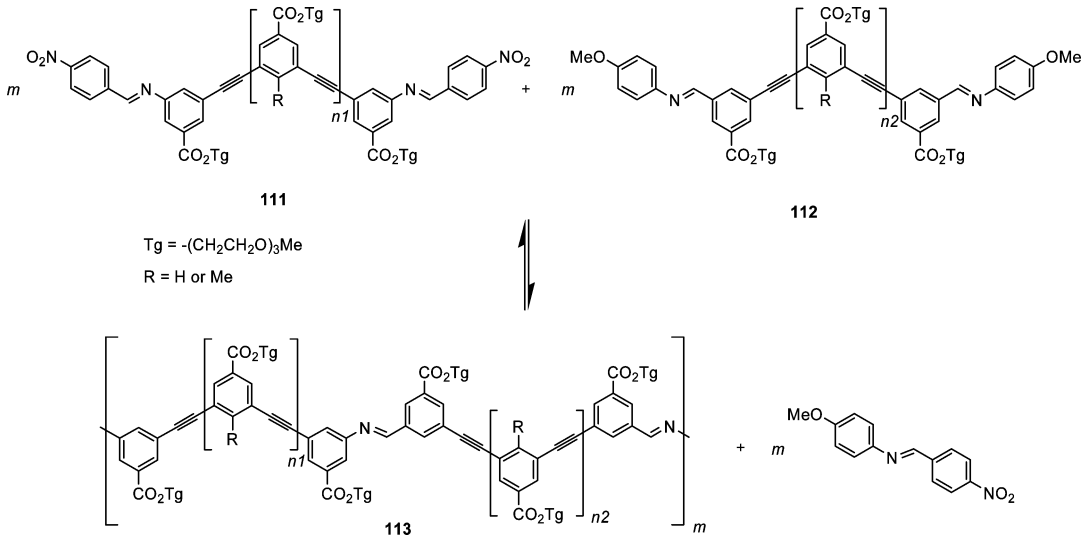
Buryak and Severin described the first examples of the use of dynamic combinatorial libraries for the development of sensors.[370] Their libraries are mixtures of metal−dye complexes, based on the chelating dyes shown in Chart 28

Chart 28. Dyes Used in Combination with Ni(II) and Cu(II) To Generate a DCL That Was Used as a Sensor for Dipeptides[370]



and Ni(II) and Cu(II), which report on the analyte through changes in the overall UV spectrum of the solution. The authors use a chemometrics analysis of the response of the

Scheme 58. Imine Metathesis Reaction for Reversible Polymerization of Helix-Forming Monomers[139,140]



entire library to added dipeptide analytes to identify the peptides.

5. Theoretical Studies of Dynamic Combinatorial Libraries (DCLs)

Development of the DCL concept has largely been driven by experimental work. Many of these have been "proof-of-concept" studies. While strong amplification of selected library members has been observed in many cases, most studies have only sampled relatively small libraries. This raises the question of what will happen as one increases the number of building blocks in a library. On one hand, the probability of creating a better binder rises, but on the other hand, the challenge of detecting such a species in an ever larger sea of competing structures may become difficult. Another intriguing question is what will happen when several different binders have similar affinities. How will such subtle differences be reflected in the efficiency of amplification of these species? Qualitative concepts such as Le Chatelier's principle and a notion of "amplification of the fittest",[371] although sufficient to inspire the early development of the DCL concept, do not provide easy answers to such questions.

The general approach toward gaining a better insight into the behavior of dynamic libraries has been to construct scenarios and explore their behavior. This involves designing a system of equilibrium processes based on our chemical knowledge and then calculating the distribution of products using the initial concentrations of building blocks and equilibrium constants as input. A variety of techniques have been used to facilitate these calculations: generic mathematics software (MathCAD), steady-state searching using off-the-shelf kinetics software, and custom-written software (DCLSim) using known equilibrium-finding algorithms (COMICS/COGS).

An early study by Eliseev and Nelen was based on their experimental system whereby equilibration and template-binding are separated into two compartments, and the unbound library members are pumped between these (Figure 6; for a more detailed description, see section 4.1.1).[276] To address whether their recycling method was equivalent to equilibration in the presence of a template, two mathematical models were constructed. One used kinetic calculations to consider the effects of photoisomerization on the mixture of library members and equilibrium calculations to consider the binding of a stationary mixture of library members to the immobilized ligands. The other simultaneously considered the equilibria involved in both photoisomerization and ligand binding. Both models gave product distributions that were in accord with experiment, confirming that their system produced the same final product distribution as would be reached by equilibrating in the presence of template. Interestingly, the final result of the recirculating system was found to be independent of the degree of isomerization achieved in each cycle, showing that it was not necessary to wait for the isomerization to reach equilibrium before starting a binding cycle.

The equilibrium calculations were also used to evaluate the applicability of the approach to larger libraries, containing 5, 10, and 15 components. Libraries of more than 15 components were not considered due to the increasing complexity of the calculations. A simple model of binding affinity was chosen, whereby the binding constant of each component (K_{weak}) was the same, except for one with a higher binding constant (K_{strong}) (Figure 9). By consideration of a

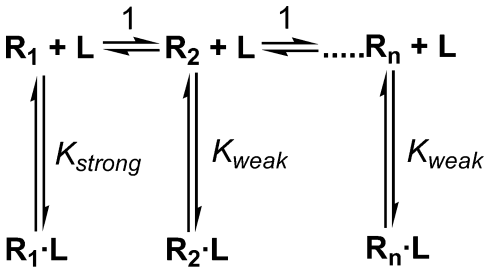


Figure 9. Equilibrium model used by Eliseev and Nelen.[276]

number of scenarios, some trends emerged. For example, in a library of n members, where $K_{strong}/K_{weak} = n$, the best component could be amplified with a total yield of approximately 30%.

A theoretical study by Moore and Zimmerman considered libraries that contain more than two different binding constants (Figure 10).[286] As in the Eliseev−Nelen model,

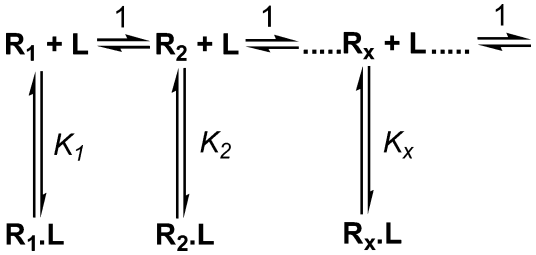


Figure 10. Equilibrium model used by Moore and Zimmerman.[286]

all species can interconvert into all other species, and in the absence of any binding, all species have identical concentrations. In Moore's model, the binding constants are represented by a continuous distribution function: a normal distribution in log K. A modified distribution function containing the effects of template binding was derived, showing the distribution of binding constants (again modeled as a continuum) in the equilibrated library. Comparison of these functions shows that the mean binding constant shifts by slightly more than 2 orders of magnitude. From this, they conclude that the template effect is only of limited usefulness.

We have produced a refinement of the Moore−Zimmerman model, considering the DCL not as a continuum represented by a single equation, but as a collection of discrete species.[372] As in the Moore−Zimmerman model, the distribution of binding constants over the library members is based on a log-normal distribution, but they are now randomly assigned to each of the individual library members. Computer simulations of these idealized DCLs were conducted using specially developed software (DCLSim). Analysis of the results showed that the bulk of the library (the weak to moderate binders) was well-approximated by the Moore−Zimmerman model (red line in Figure 11). However, this approximation broke down for the very best compounds (blue bars in Figure 11). For example, in a typical simulated 10 000-compound DCL, the best binder was amplified from a tiny concentration to become 8% of the library material, thus essentially accounting for the entire tail-end of the shifted distribution in the Moore−Zimmerman model. As expected, increasing the size of the DCL caused the concentration of the best binder to be reduced as a consequence of spreading the building blocks out over an increasing number of library members. However, this drop in concentration is not as sharp as expected on the basis of the above argument, because larger libraries tend to produce

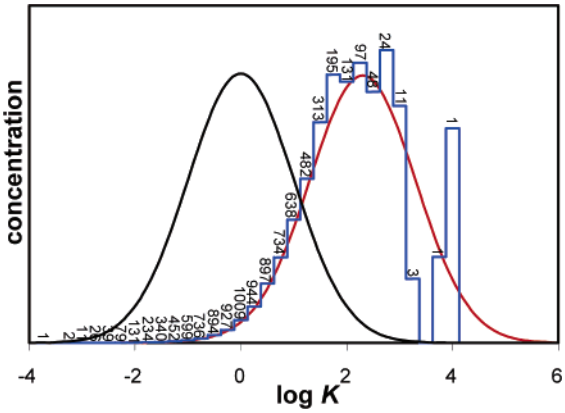


Figure 11. Histograms representing the composition of a continuous DCL in the absence (black) and presence (red) of a template and a typical simulated DCL containing 10 000 compounds (blue). The blue bars represent affinity classes and are labeled with the number of compounds in the affinity class.[372]

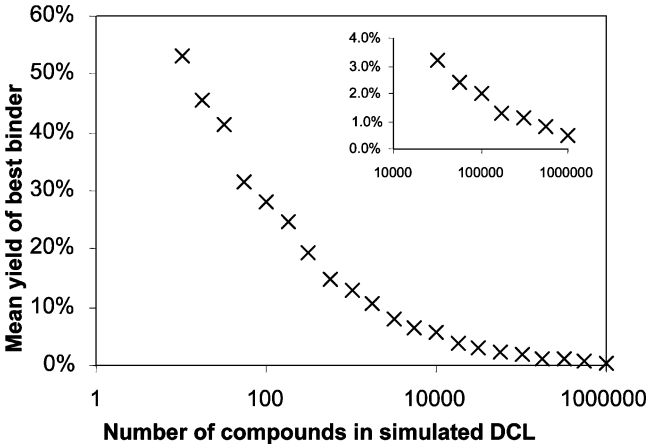


Figure 12. Yields of the best binders as a function of library size. Each data point represents the average from 100 simulated DCLs (averaging is necessary due to the statistical variations resulting from the fact that in any individual simulation binding constants are assigned randomly).[372]

better binders, which are more amplified. Figure 12 shows the percentage of the library material that ends up in the best binder as a function of the number of compounds in the library.

All three models discussed so far are highly idealized in that they have assumed that all of the library members can be interconverted on a simple 1:1 basis, that is, each library member can transform into any other library member without needing to pick up or shed any of its building blocks. In reality, the interconversion of library members is more restricted and has to obey the mass balances for every building block.

To address issues that arise from the competition for different building blocks between the members of a DCL, Severin simulated small (often minimal) model DCLs.[374,375]

An initial study considered a library of 10 trimers made from equimolar amounts of three building blocks: **A**, **B**, and **C**.[374] Depending on the stabilization endowed upon selected library members some arguably counterintuitive results were obtained (Table 3). Stabilization of two of the trimers, **ABC** and **AAA**, by a factor of 1000 resulted in the amplification of only the **ABC** trimer. The **AAA** trimer concentration actually went down. In other cases, it was observed that some trimers could be amplified despite not being stabilized. Such

Table 3. Calculated Equilibrium Distributions for a Series of Model DCLs Showing the Responses to the Stabilization of Various Library Members[374]

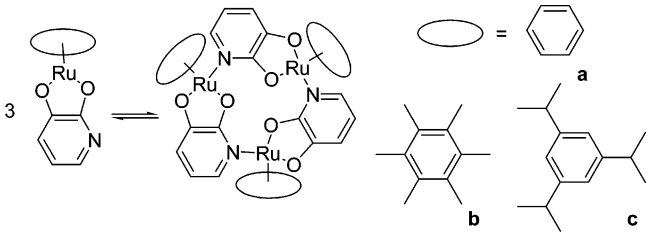
				relative stability relative concentration (%)					
AAA	**AAB**	**ABC**	**ABB**	**BBB**	**BBC**	**BCC**	**CCC**	**AAC**	**ACC**
1 3.7	1 11.1	1 22.2	1 11.1	1 3.7	1 11.1	1 11.1	1 3.7	1 11.1	1 11.1
1000 **28.1**	1 0.5	1 6.8	1 3.4	1 7.2	1 21.5	1 21.5	1 7.2	1 0.5	1 3.4
1 0.0	**1000** **47.7**	1 2	1 0.5	1 1.9	1 11.1	1 21.2	1 13.5	1 0.1	1 1.9
1 0.0	1 0.1	**1000** **99.6**	1 0.1	1 0	1 0.1	1 0.1	1 0.0	1 0.1	1 0.1
1000 0.4	1 0.0	**1000** **98.6**	1 0.1	1 0.1	1 0.3	1 0.3	1 0.1	1 0.0	1 0.1
900 0.1	**600** 2.1	**300** **45.7**	**300** **22.8**	1 0.5	1 1.6	1 1.6	1 0.5	**600** 2.1	**300** **22.8**

false positives are probably unique to very small libraries (vide infra).

The source of this apparently perverse behavior is that if the stability of the library as a whole is considered, then it may be more advantageous for the library to produce many complexes of moderately high stability than a few of high stability and more of low stability. While this analysis considers differences in the intrinsic stability of the library members, the results are equally applicable to template binding.

The theoretical analysis was accompanied by an experimental system that confirmed that there is not always a direct correlation between the relative amounts of compounds that are in thermodynamic equilibrium and their individual thermodynamic stabilities. Trimeric hosts were assembled using reversible ruthenium coordination chemistry (Scheme 59), and studied by NMR and X-ray crystallography. The

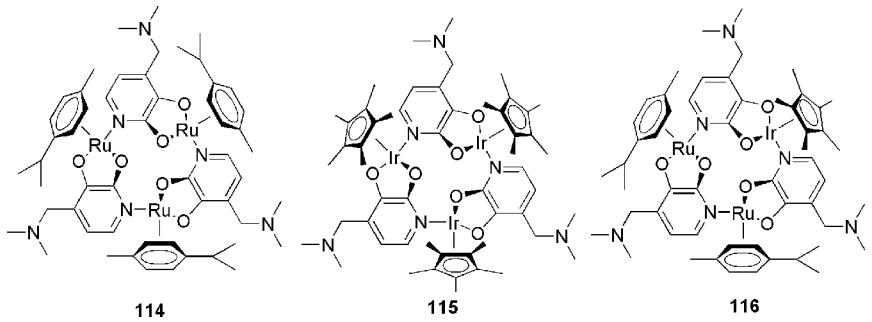
Scheme 59. An Experimental System that Revealed the Effect of Stability Differences on the Distribution of Products in a Model DCL[374]



presence of the **b** and **c** moieties was found to destabilize the complexes; for example, the Ru−Ru distance in the **aaa** trimer (5.32 Å) was found to be shorter than the corresponding distance in the **bbb** trimer (5.46 Å). When mixed libraries were made, using two or three building blocks, the heterotrimeric species were found to dominate the libraries, despite the fact that the **aaa** trimer is the most stable product.

A follow-up article by Severin explores these paradoxical effects in more detail again using a set of minimal model DCLs.[375] The preference to form hetero compounds (containing different building blocks) over homo compounds (containing only one type of building block) under specific conditions, as was found earlier, was confirmed in a smaller DCL (**AA**, **AB**, **BB**). Also a selection bias toward smaller oligomers was observed (again under specific "experimental"

Chart 29. Lithium Receptor Complexes Used To Make DCLs To Demonstrate the Effect of Template Concentrations on the Distribution of Products[225]



conditions). Simulations were conducted with a range of concentrations and affinities, further charting the effects.

Clearly, these effects potentially complicate the analysis of real libraries, and some remedies are proposed that prevent such complications from arising. For instance, using a method for separating the bound and unbound library members can eliminate "false positives". Furthermore, by designing libraries such that they are dominated by free monomers, one can establish a much better correspondence between amplification and stability independent of the exact conditions. It was noted that this can be achieved by using a limiting supply of a *spacer* (such as the ruthenium metal ion in Scheme 59) to connect the building blocks. Another solution suggested by the simulations is the use of a substoichiometric concentration of template or the use of an analytical technique that only observes the template-bound species. Finally, an evolutionary system, whereby equilibration in the presence of a template is alternated by the removal of unbound library members, was proposed to be another useful strategy for avoiding possible problems associated with identifying the best binders in a DCL.

Saur and Severin have tested the influence of the template concentration on the correlation between amplification and binding semiquantitatively. The authors studied lithium-binding by ruthenium and iridium complexes.[225] DCLs were produced by mixing **114** and **115** (Chart 29) and allowing them to scramble. Lithium sulfate was added to the DCLs, and the binding was studied by ^{7}Li NMR. When a small amount of lithium was added, it was found to bind preferentially to **114**, indicating that **114** was the preferred receptor for Li^{+}. When successively larger excesses of lithium were added, up to 40 equiv, the lithium was increasingly found to be bound to **116**, demonstrating that adding excessive amounts of template can indeed cause DCLs to select suboptimal library members.

In a recent experimental study, Severin and co-workers have demonstrated a method for improving the correlation between amplification and affinity by using self-sorting to generate a DCL consisting two partially orthogonal sublibraries.[376] One sublibrary contains competing high-affinity receptors, whereas the other sublibrary contains only low-affinity receptors and can thus act as a reservoir of building blocks.

The pioneering work by Severin focused exclusively on small model libraries, and while it clearly highlighted some scenarios in which DCLs can produce counterintuitive results, it remained unclear whether such scenarios are commonplace or perhaps only freak events resulting from particularly unfortunate distributions of binding constants/stabilities over the library members. It was also unclear to what extent the effects observed in small equilibrium mixtures translate to larger more realistic DCLs.

To address these questions, we have conducted an extensive series of computer simulations on DCLs containing seven building blocks, which combined to form 28 dimers, 84 trimers, and 210 tetramers.[373] Binding constants were randomly assigned to the library members.[372] Using our DCLSim software, we were able to simulate the product distributions of the resulting libraries and analyze the relationship between binding constants and amplification factors[377] within each of the libraries quantitatively. Some typical results are shown in Figure 13 and illustrate that the

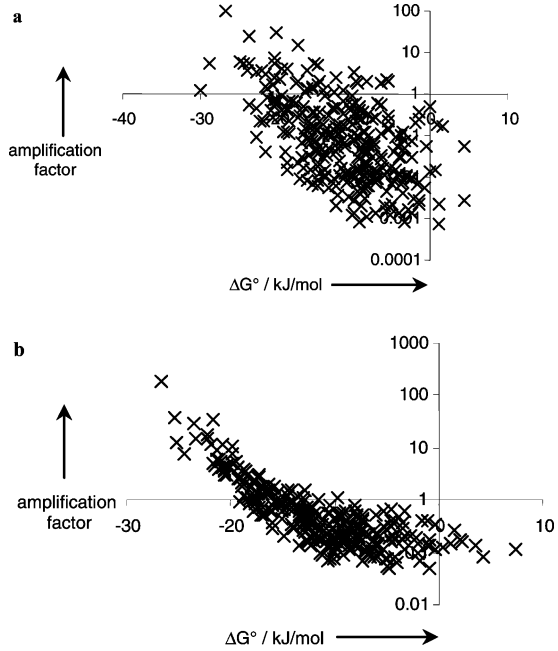


Figure 13. The relationship between amplification and free energy of binding for all binders in two randomly generated DCLs that differ only in the way the binding constants are distributed over the various hosts. In both DCLs, the total concentration of the building blocks and the concentration of the template is 10 mM. R^2 values for the correlation between free energy and the logarithm of the amplification factor[377] (taking only significantly amplified hosts into account) are 0.24 and 0.72, respectively.[373]

binding energy−amplification factor correlation can be highly variable, depending heavily on how binding constants happen to be distributed over the library members. In particular, it was notable that if a DCL happened to contain a few strongly binding dimeric species (even if those were not the library members with the highest affinity), as in the library represented by Figure 13a, the correlation was

significantly worse than that in DCLs that lacked such species (Figure 13b).

We also noted that the correlation between binding energy and amplification was strongly dependent on the "experimental" conditions. Analysis of the results of simulations carried out using 289 different combinations of template and building block concentrations (Figure 14) confirmed Sev-

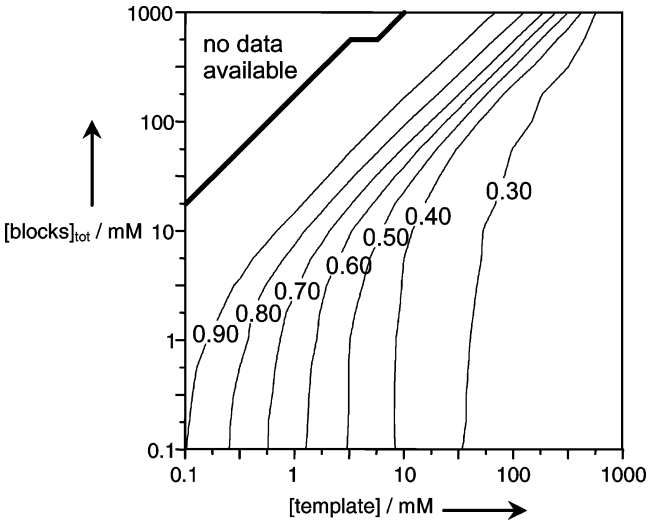


Figure 14. Correlation between binding affinity and amplification in simulated DCLs as a function of template and total building block concentration. The numbers indicate the correlation coefficient (averaged over 50 simulations).[373]

erin's prediction[375] that the template concentration is an important parameter. In particular, in more concentrated libraries, reducing the amount of template *relative to the amount of building blocks* improved the correlation, so a similar effect could be achieved by increasing the building block supply. In more dilute libraries, the building block supply made no difference, and only the *absolute* concentration of template affected the correlation. Note that we never observed strong amplification of a poorly binding library member under any of the experimental conditions, suggesting that the risk of encountering false positives is limited to very small libraries. Thus some, but not all of the observations made by Severin on small libraries can be extrapolated to large DCLs.

Two important conclusions can be derived from our simulations of large libraries: (i) unless excessive amounts of template are used, good binders have a high probability of being significantly amplified; (ii) any significantly amplified compound is essentially guaranteed to be a good binder.

We have recently reported the first quantitative experimental study into the effect of template concentration on amplification efficiency in a situation where several synthetic receptors compete for a small number of building blocks.[378] This study specifically addressed the competition between a homotrimer and a heterotrimer, as well as the competition between a homotrimer and a homotetramer. Theory predicts that in the presence of excess template, hetero compounds are favored over homocompounds and small oligomers are favored over large oligomers (this is because in both cases, from a fixed supply of building blocks it is possible to produce more of the former than of the latter). An experimental confirmation of this behavior was indeed observed, but we had to select the template carefully so that it had comparable affinities for the competing receptors. More selective templates, such as those shown in Scheme 40 (section 4.1.2.7), tended to remain selective for their best binders over a wide range of experimental conditions. Introducing template **117** into a library made from building blocks **31** and **32** resulted in the amplification of receptors **37** and **38** (Figure 15). At low template concentration, the

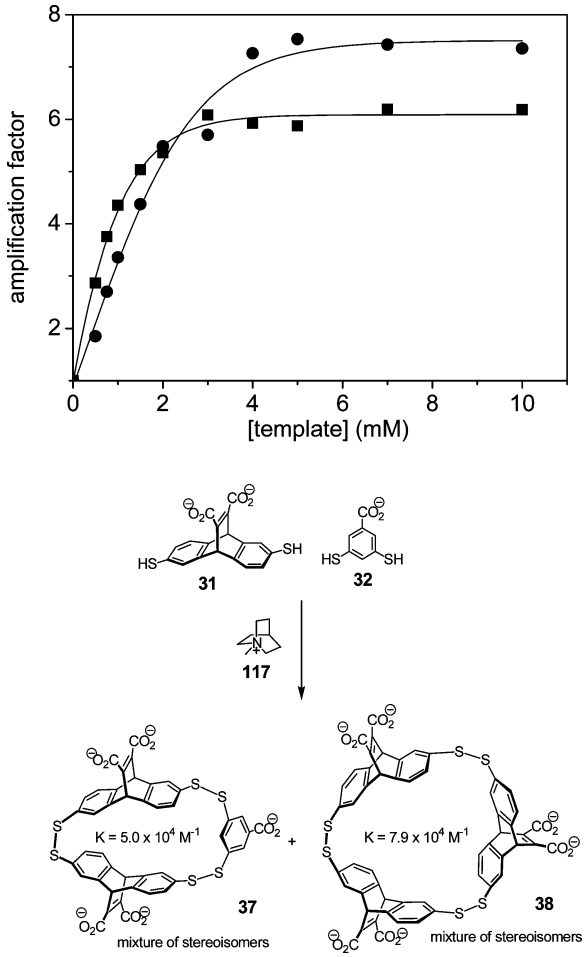


Figure 15. Amplification factors[377] for receptors **37** (●) and **38** (■) as a function of the concentration of the template **117**, showing that at low template concentration the best binder is most amplified, whereas upon increasing the template concentration, the somewhat weaker binder is amplified the most.[378]

amplification factor[377] for **38** was higher than that for **37**, reflecting the fact that **38** is a somewhat better binder than **37** ($K = 7.9 \times 10^4$ M^{-1} versus 5.0×10^4 M^{-1}, respectively).[378] However, upon increase of the template concentration, **37** becomes the most amplified compound because the system can gain more binding energy by producing more of this weaker binding heterotrimer.

Thus, depending on the experimental conditions, libraries producing the "wrong" answers (by a small margin) can be produced, but we had to look hard to find them. Moreover, even if the most amplified compound may not be the best binder, it is very likely to be one of the better binders in the system. Therefore these effects are not a significant limitation to the dynamic combinatorial approach.

6. Related Approaches

The dynamic combinatorial experiments discussed above involve the generation of an equilibrium mixture and the observation of an increase in concentration of some of the

library members as a result of molecular recognition, either intramolecularly or intermolecularly or following interaction with a template in the equilibrium system. However, a number of systems have been reported that also aim at achieving the selection of high-affinity compounds from diverse mixtures and that involve reversibility in some sense but that deviate from the above blueprint. These include systems in which equilibration and template binding are separated, systems in which the reversible template binding process is followed by an irreversible reaction on the selected strong binders, and systems in which synthesis and degradation of library members is spatially separated.

6.1. Separation of Equilibration and Template Binding

As discussed in more detail in section 4.1.1, Eliseev and Nelen have developed an elegant flow system that allows the spatial separation of equilibration (in their case based on photochemical cis−trans isomerization) and binding to an (immobilized) template. Cycling the DCL several times through the setup produced template-induced shifts in the product distribution that were very similar to what would have been achieved had equilibration been carried out in the presence of template. While this approach allows selection and equilibration to be carried out under different, possibly incompatible, conditions, it does increase the complexity and duration of the experiment.

Lehn and co-workers have reported three studies that used hydrazone exchange chemistry to prepare DCLs targeting acetylcholinesterase, HPr kinase/phosphatase, and the plant lectin concanavalin A. Because hydrazone exchange generally requires acidic conditions, which are incompatible with the protein targets, equilibration had to be carried in the absence of the target. Screening was performed after the libraries were neutralized and no longer dynamic and was based on enzymatic assays. Hits were identified through a "dynamic" deconvolution process (Figure 16):[162−164] A parent

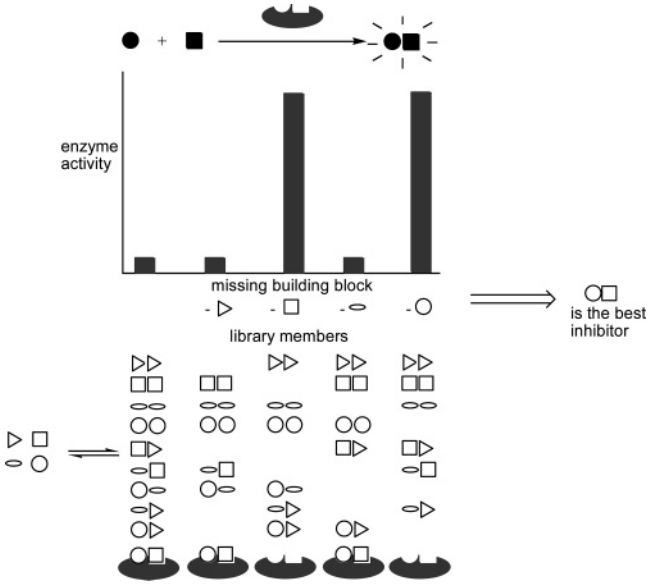
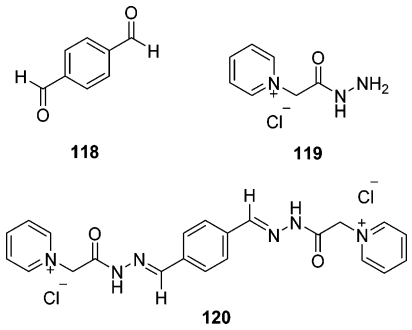


Figure 16. Cartoon representation of dynamic deconvolution.

library is prepared using all of the building blocks, and in addition, a set of sublibraries is constructed, each lacking a single building block. By comparison of the activity of the parent and the sublibraries, it is often possible to deduce which building blocks are necessary for high activity and thus to identify the best library members.

In the work targeting acetylcholinesterase, a DCL was prepared by mixing a set of four hydrazides, four monoaldehydes, and five symmetrical dialdehydes to generate 66 possible hydrazones.[162] Independently, 36 of these (all the monohydrazones and symmetrical dihydrazones) were synthesized and profiled for acetylcholinesterase inhibition, using a standard acetylthiocholine/Ellmann's reagent assay. Of these compounds, **120** (Chart 30) was found to have the

Chart 30. The Best Building Blocks and Library Member Selected from DCLs Using a Deconvolution Strategy[162]

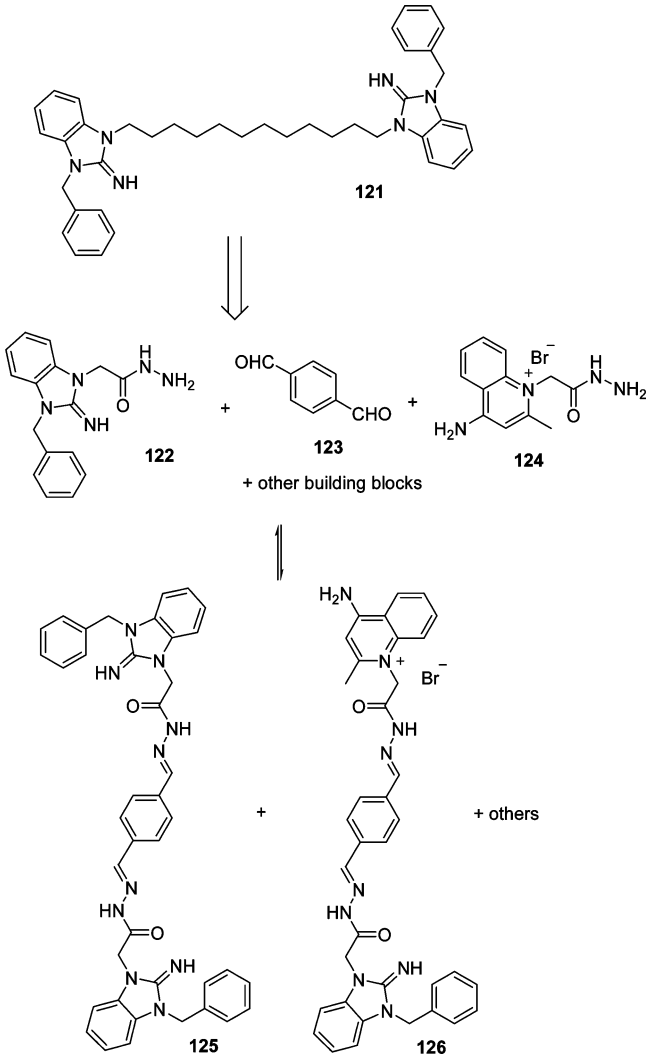


greatest inhibitory effect. The deconvolution strategy was then pursued, making one large combined library and 13 sublibraries. Enzyme assays in the presence of these libraries showed strong inhibition of the enzyme, except for two sublibraries, those that were missing the building blocks **118** and **119**, the building blocks corresponding to the previously identified best receptor **120**. The IC_{50} of **120** was found to be 2.3 nM, more potent than the best existing inhibitor (1,12-bis(pyridinium)dodecane, $IC_{50} = 16.0$ nM).

In the work targeting HPr kinase/phosphatase (HPrK/P) from *Bacillus subtilis*, a set of 21 building blocks (16 monohydrazides, two monoaldehydes, and three dialdehydes) was designed, based around the known inhibitor **121** (Scheme 60).[163] The DCL produced upon mixing these building blocks contained 440 potential library members. The complete library was screened for HPrK/P inhibition and found to be effective. A set of sublibraries was then screened, and it was found that inhibition was strongly dependent on the building blocks **122** and **123**. This suggested that **125** would be the best inhibitor. This was confirmed after resynthesizing and assaying **125** for its inhibitory activity ($IC_{50} = 17\ \mu M$), which was found to be similar to that of the original **121** ($IC_{50} \approx 10\ \mu M$). Another new but somewhat less efficient inhibitor was identified in the form of **126** (containing **124**, which was one of the other building blocks that had been found to positively contribute toward the inhibitory activity of the libraries).

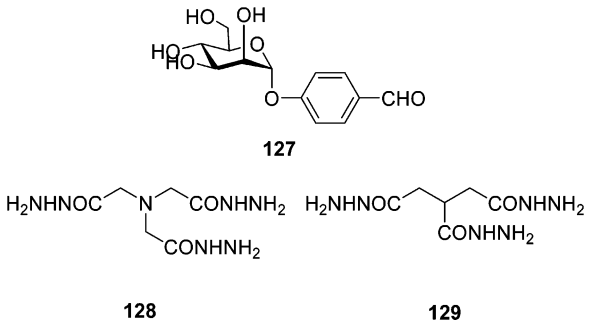
A third study examined binding to concanavalin A (Con A), a plant lectin.[164] A series of six carbohydrates were derivatized to give six aldehydes. A single monohydrazide, five dihydrazides, and three trihydrazides were chosen to act as scaffolds, onto which the carbohydrate binding units would be arranged. Together, these components are capable of forming 474 different species. A library was formed from all of the components and found to strongly inhibit the binding of ConA, using an enzyme-linked lectin assay. A series of sublibraries was then screened, and those lacking the building blocks **127**, **128**, or **129** (Chart 31) were found to have a significantly reduced inhibitory activity. Because only one carbohydrate was found to be essential to inhibition,

Scheme 60. The Best Building Blocks and Library Members Selected from a DCL Targeting HPr Kinase/Phosphatase by Dynamic Deconvolution[a]



[a] Building block designs were derived from the known inhibitor **121**.[163]

Chart 31. Building Blocks for Dynamic Deconvolution Studies of Lectin Binding[164]



a second set of sublibraries, containing only **127** and all but one of the scaffolds, was made and profiled, showing significantly reduced inhibition in the **128**-free library. The compound **(127)$_3$128** was then synthesized and evaluated and shown to have an IC_{50} of 22 μM, 36-fold better than the reference compound, methyl-α-D-mannoside (IC_{50} = 0.8 mM).

Poulsen and Bornaghi have recently used alkene metathesis mediated by an immobilized version of the first-generation Grubbs' catalyst to develop a DCL of alkenes.[379] They screened the pre-equilibrated libraries for affinity for carbonic anhydrase II.

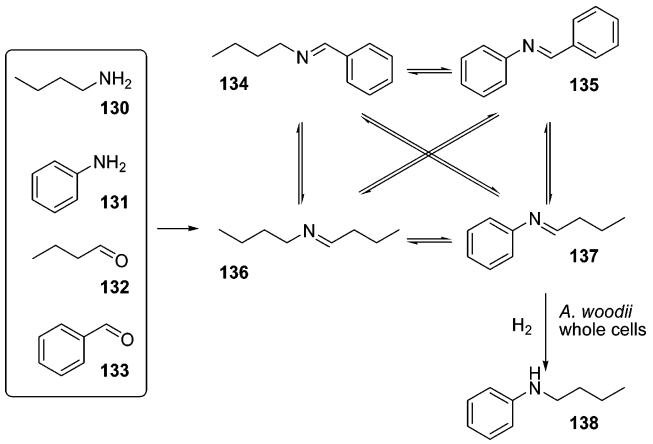
Note that the systems described above do not take advantage of the adaptive properties of DCLs because equilibration and screening are carried out separately. Thus, similar results could arguably be achieved by using traditional kinetically controlled combinatorial libraries.

6.2. DCLs in Which Template Binding Is Followed by an Irreversible Reaction

Very recently, dynamic combinatorial chemistry has been used to identify enzyme substrates by observing the buildup of reaction products. This is a powerful new strategy for the purpose of screening enzymes for new reactivities or for profiling the substrate specificity of a known enzyme-catalyzed reaction.

A screen for imine reductase activity was implemented using the building blocks **130**−**133** to make a small DCL of imines (**134**−**137**; Scheme 61).[380] This library was then

Scheme 61. Screening a DCL of Imines for Imine Reductase Substrates[380]



incubated with hydrogen in the presence and absence of whole cells of the bacterium *Acetobacterium woodii*, a strict anaerobe, to allow reduction of the imines to take place. Analysis of the resultant mixtures revealed that butylaniline **138** (made by reduction of **137**) had been produced. None of the reduction products could be found in the absence of live cells. Interestingly, when caffeate, a known inducer for C=C reduction, was added to the live cells, phenylbenzylaniline (from **135**) was also found. These results suggest that *A. woodii* possesses at least two different imine reductase activities. Thus new substrates for imine reductase enzymes were discovered, providing a lead in the isolation of this previously unknown class of enzymes.

The Ramström group has reported a library based on thioester exchange targeting the enzyme acetylcholinesterase.[99,381] A notable feature of the reaction is that both thioesters and thiols take part in the reaction; however, carboxylates do not. Therefore, if a thioester in a thioester-exchange DCL is hydrolyzed, the thiol produced is returned to the equilibrating mixture, whereas the carboxylate accumulates. By observation of this accumulation, the rate of hydrolysis of the various library members can be observed (Figure 17).

This system was implemented using a DCL made using thiocholine and a series of acylsulfanylpropionates, which equilibrated to give all possible acylsulfanylpropionates and

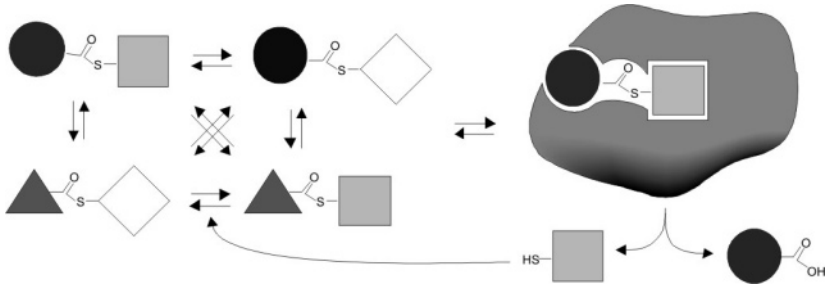


Figure 17. Combining an irreversible enzymatic reaction with reversible thioester chemistry to investigate substrate affinity.[99]

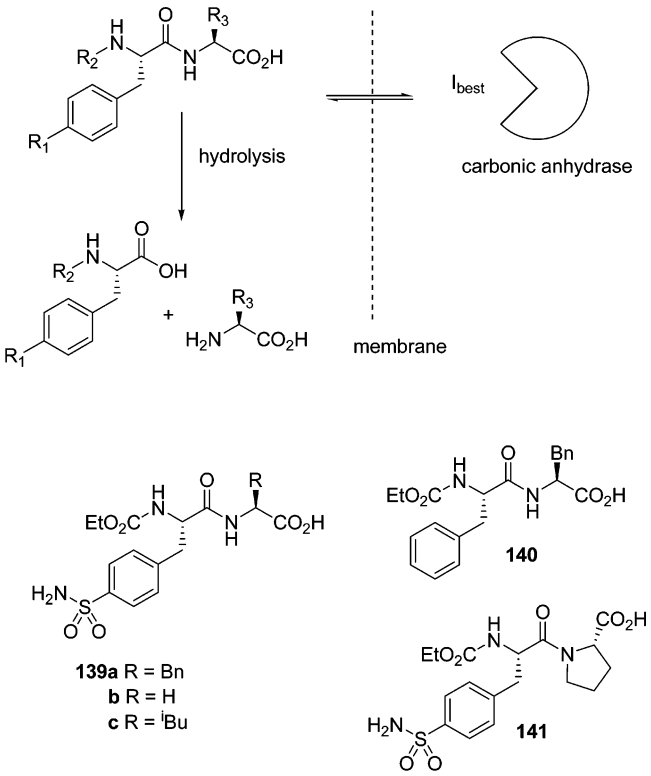
acylthiocholines. On introduction of acetylcholinesterase, acetic and propionic acid were formed most readily ($t_{1/2}$ = 210 and 270 min, respectively). After the substantial hydrolysis of acetylthiocholine and propionylthiocholine, butyric acid formation began and proceeded slowly ($t_{1/2}$ = ca. 1100 min). The pentoyl and hexoyl species remained unhydrolyzed. These results were found to be in accord with the known substrate specificity of acetylcholinesterase.

6.3. Pseudo-Dynamic Combinatorial Chemistry

In pseudo-dynamic combinatorial chemistry some of the concepts of dynamic combinatorial chemistry are combined with *irreversible* chemistry with the aim of achieving high degrees of selectivity of production of the best binders. This approach has been pioneered by Gleason, Kazlauskas, and co-workers. En route to pseudo-dynamic combinatorial chemistry, they first developed a selective destruction method for screening a presynthesized combinatorial library.[382] In this protocol, all library members are slowly destroyed using an irreversible reaction. Reversible binding of the library members to a template protects them from this reaction, the best binders being the best protected. In the experimental setup, the destruction reaction (enzyme-catalyzed) and the template (an enzyme target) were separated using a membrane, which allows the diffusion of the library members while keeping the two enzymes apart (Scheme 62). Diffusion across the membrane is rate-limiting, so the membrane masks any substrate selectivity of the destruction enzyme, allowing the rate of destruction to be determined by the partitioning between bound and unbound library members. As the reaction proceeds, the more weakly bound library members are destroyed, leaving the better binders behind; this process is similar to a kinetic resolution. As with a kinetic resolution, as the reaction proceeds, the selectivity of the process can increase without limit but at the expense of the final yield of product.

The experimental system consisted of four sulfonamide−dipeptide carbonic anhydrase inhibitors (**139a**−**c** and **141**) and a noninhibitory dipeptide **140** (Scheme 62). The protease Pronase was used as the destructive enzyme. In an initial experiment, the noninhibitor **140** and the inhibitor **139a** were placed in the system. After 6 h, the ratio of **139a** to **140** in the inner chamber was found to be 3.7:1. This increased to 20:1 after 12 h. In a control experiment without Pronase, the final ratio was found to be 1.75:1. In the second experiment, the inhibitors **139a** and **139b** were used together with an excess of carbonic anhydrase (1.6:1 CA/dipeptides). After 193 h and the hydrolysis of 83% of the dipeptides, the **139a**/**139b** ratio was 3.8:1, well in excess of the binding constant ratio (2.1:1). A further experiment used **139a** and **139c** with the dipeptides in excess (1:2.1 CA/dipeptides). After 6 h, 93% of **139c** and 58% of **139a** was hydrolyzed,

Scheme 62. Selective Destruction of Library Members That Are Not Bound by the Target Enzyme[382]

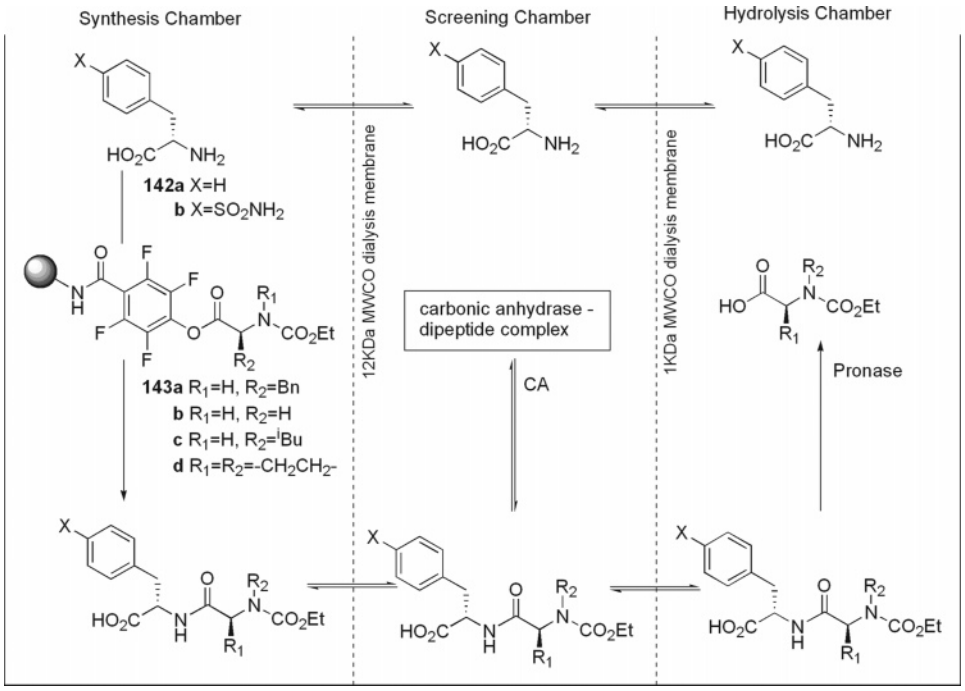


giving a concentration ratio of 6:1, greater than the 3.7:1 ratio of binding constants. Finally, all five dipeptides **139**−**141** were tested together, giving a rapid cleavage of **140** and the disappearance of **139a**−**c** and **141** at "rates which corresponded to their binding constants".

The authors point out some potential limitations of the technique. One is that the destruction enzyme needs to have a broad spectrum of activity. As with enzyme-catalyzed DCLs, severe differences in substrate specificity for the hydrolytic enzyme could bias the results. Another is the need for stoichiometric amounts of the target. Because destruction rather than amplification takes place, only a limited amount of library material remains at the end of the experiment. It has been pointed out by Severin[375] that this technique could usefully be applied to a DCL that has been previously equilibrated in the presence of a template. Selective destruction after amplification would accentuate the differences in affinity between the amplified library members.

By addition of an irreversible synthesis reaction to a selective destruction experiment, a closer approximation to a DCL is achieved, a pseudodynamic combinatorial library (pDCL), where the reversible reactions involved in dynamic combinatorial chemistry are replaced by pairs of irreversible

Scheme 63. Pseudo-Dynamic Combinatorial Chemistry[383]



reactions.[383] This decoupling of synthesis and destruction allows an extra degree of adjustability, which may be used to improve the selectivity of the experiment.

The experimental setup is based on the selective destruction system, but now a third synthesis chamber is added (Scheme 63). Instead of using presynthesized dipeptides, two sets of building blocks are used. One set, **142a** and **142b**, are used as amines in a peptide coupling reaction and are placed into the solution at the start of the experiment. The other building blocks (**143a**−**d**) are added as polymer-supported active esters, which are added in batches to the synthesis chamber at time intervals. In the synthesis chamber, these may react to give an eight-membered pDCL of dipeptides. The four adducts containing **142a** had no detectable effect on the action of carbonic anhydrase, whereas the other four were inhibitory, with K_i values in the ratio of 7.9:5.1:2.3:1. In the hydrolysis chamber, the dipeptides are hydrolyzed, liberating the amine, which can then diffuse back to the synthesis chamber. The carboxylates, however, take no further part in the reactions and accumulate during the experiment.

After some experimentation with the conditions, it was found that by addition of batches of the active esters at 16 h intervals, very high selectivity could be achieved. After seven cycles (112 h), the strongest binding peptide dominated the mixture, with a ratio of >100:1 and a yield of 29% relative to the amount of CA.

It should be noted that the system presented is not fully equivalent to a DCL because only the amines and not the carboxylates are recycled. The obvious next step[384] is to construct a pDCL where all of the library members are recycled (perhaps by using EDC- or DCC-mediated peptide couplings), which will reveal whether the high selectivities achieved using the existing system can also be reached in a system with full recycling.

Intriguingly, a model of prebiotic peptide synthesis and degradation by Wächtershäuser and co-workers bears a strong resemblance to a pDCL.[385] Under conditions resembling the environment around volcanic vents containing carbon monoxide and colloidal (Fe,Ni)S, both peptide synthesis and degradation were observed, leading to the speculation that the system could produce a "dynamic chemical library", with all of the usual implications for selection. Furthermore, the authors hint that the peptides may affect the catalytic properties of the transition metal compounds, allowing for the possibility of a positive feedback loop.

7. Prospects

Our current understanding of noncovalent interactions and molecular conformations is still insufficient to encompass all the subtleties that are involved in molecular recognition and folding of complex molecules. Consequently, the process of creating new synthetic receptors or new ligands for biomolecules by design is not as successful as we would like it to be. Because this situation is likely to persist for some time to come, selection approaches remain important for improving our fundamental understanding of noncovalent interactions, as well as for developing synthetic molecules that participate in molecular recognition. Dynamic combinatorial chemistry appears ideally situated as a tool in this area. It combines the best features of supramolecular error checking with the possibility for covalent robustness.

In the last decade, dynamic combinatorial chemistry has developed from scratch into a recognized tool for exploring systems that rely on molecular recognition. A significant and increasing number of reversible chemistries with which to construct DCLs is now at our disposal (section 2), the most popular of which are imine exchange, hydrazone exchange, and disulfide exchange. However, for work under physiological conditions, we are still mostly restricted to imine exchange, disulfide exchange, thioester exchange, and, in restricted areas, reversible enzymatic reactions (see section 4.3). Further expansion of the repertoire of reversible reactions that can be used for making DCLs is clearly desirable. A potentially ideal exchange reaction is alkene metathesis (section 2.1.6).[202,203] The workhorses for this reaction (the Grubbs' catalysts) show high selectivity for

alkenes (which are rare in biomolecules) but do not affect most other functional groups. However improving lifetimes of these catalysts and their water solubility will be necessary. For nonbiological applications, many other reactions involving reversible condensations (e.g., to give small heterocycles, porphyrinogens, and calixarenes) have as yet unexplored potential.

DCLs are complex equilibrium mixtures that pose significant analytical and theoretical challenges. Some perhaps counterintuitive behavior has been observed, particularly with regards to how the Gibbs energy associated with molecular recognition is related to the subsequent amplification of selected library members (section 5). However, computer simulations have provided clear guidance on how to avoid potential pitfalls and reliably identify the best binders in a dynamic combinatorial screen.

Dynamic combinatorial chemistry has been applied successfully in the development of synthetic receptors and has proven to be a practical method that not only allows the identification of new receptors but also provides a synthetic route toward these complex molecules (section 4.1). While most of the work has been focused on macrocyclic receptors, the technique allows much more complex architectures to be screened or discovered that are difficult to access using traditional kinetically controlled methods of synthesis (section 4.1.3). When extended to molecular encapsulation, this may lead to new applications in drug delivery, as some of the most popular reversible chemistries in dynamic combinatorial chemistry (hydrazone and disulfide exchange) also happen to be the workhorses for making cleavable drug-carrier conjugates.[317]

Dynamic combinatorial chemistry also holds potential for catalysis. The first studies have demonstrated that modestly active supramolecular catalysts can be obtained by screening DCLs for affinity for transition-state analogues (see section 4.2). While the related work on catalytic antibodies appears to indicate that the efficiency exhibited by enzymes may well remain beyond our reach, it should at least be possible to develop catalysts that exhibit (enantio)selectivity using dynamic combinatorial chemistry.

Developing ligands for biomolecules is another major application of dynamic combinatorial chemistry that has met with considerable success (section 4.3), despite the fact that this approach is probably more experimentally demanding than developing synthetic receptors. Reversible reactions need to be carried out in the presence of the biomolecule target, which is required in stoichiometric amounts since the biomolecule acts as the template for the formation of the ligand. The limited solubility or availability of the target usually implies that the concentration of building blocks needs to be low, increasing the demand on the reversible chemistry as well as the analytical chemistry.

Fields in which dynamic combinatorial chemistry has definite potential but has not yet made the same impact as in the above areas are those of molecular aggregation (section 4.4) and macromolecule folding (section 4.5). In particular, the area of molecular aggregation (i.e., the self-assembly of library members into larger structures) has received very little attention. Because aggregation can be viewed as an arrested state of phase separation (i.e., parts of the molecules want to phase separate from the solvent but other parts of the same molecule prevent this process from going to completion by forming an interface between the solvent and the "non-soluble" parts of the molecule), one operates near the edge of the building block solubility almost by definition. Consequently, in a combinatorial experiment, there will be a significant risk that some of the library members are actually insoluble, draining the equilibrium to form a fully phase-separated product.

In the field of macromolecule conformation or folding some proof-of-principle studies have started to appear that are based on equilibrium mixtures containing only a few components, paving the way for future work on more complex mixtures. Such studies would enable direct and quantitative[100] comparisons to be made between stabilities of differently folded structures, which would be instrumental in discovering particularly favorable folds.

While DCLs are powerful tools to lead to individual molecules with interesting recognition properties, the libraries themselves are also fruitful subjects of study because they represent complex networks of molecules that may exhibit unique properties that emerge from the system, rather than from the individual molecular constituents.[7] In particular, when linked to an irreversible reaction, complex dynamic mixtures may lead to new insights into molecular evolution. This perhaps represents the beginnings of a "systems chemistry" akin to systems biology.

In summary, DCLs allow tackling of complexity in terms of isolated molecules, as well as interacting mixtures of these. The technique has demonstrated its practicality in identifying molecules with unusual binding properties and in providing an attractive synthetic route to complex molecules that are not easily accessible by other means. While some of the synthetic challenges have been removed, these are to some extent replaced by analytical challenges. However, rapid recent developments in analytical chemistry mean we will be able to cope with these increasingly well. Thus the field of dynamic combinatorial chemistry is in full swing: the foundations have been established, and even though they may not quite be finished, exciting applications of the technique in a wide range of areas are already emerging.

8. List of Abbreviations

AChE	acetylcholinesterase
Con A	concanavalin A
CA	carbonic anhydrase
CSD	Cambridge Structural Database
DABCO	1,4-diazabicyclo[2.2.2]octane
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DCL	dynamic combinatorial library
DCX	dynamic combinatorial X-ray crystallography
DTT	dithiothreitol
EA	ethacrinic acid
EDC	*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide
ESI-MS	electrospray ionization mass spectrometry
ESI-FTICR-MS	electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry
HEWL	hen egg-white lysozyme
HPLC	high-performance liquid chromatography
HPrK/P	HPr kinase/phosphatase
ITC	isothermal titration calorimetry
JS	Jacobsen and Stockmayer
LC-MS	liquid chromatography mass spectrometry
pDCL	pseudo-dynamic combinatorial library
TCEP	tricarboxyethylphosphine
TAR	transactivating region
TSA	transition-state analogue

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10. Notes and References

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(2) This retains the original architectural meaning whereby wooden templates were used to aid the creation of stone or brick arches in a building. In medicinal and synthetic organic chemistry or where complex three-dimensional topologies are created, and frequently in coordination chemistry, a "template" refers to a point or a framework around which a larger structure is created, and it is an intrinsic component of the final product.
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(17) On that day, Jeremy Sanders gave a conference lecture in which he described the stereoselective acceleration of a bimolecular reversible Diels—Alder reaction within the cavity of a cyclic porphyrin trimer (Walter, C. J.; Anderson, H. L.; Sanders, J. K. M. *J. Chem. Soc., Chem. Commun.* **1993**, 458—460). Over lunch afterwards, Chris Hunter and Fraser Stoddart pointed out the limitations of the approach: the complex multistep synthesis of the host followed by careful design of a highly specific diene—dienophile pair might have been successful in this case, but it was hardly a general solution to the problem of how to achieve effective molecular recognition and catalysis in supramolecular chemistry. It was clear that one had to build proof-reading into the receptor synthesis so that only successful molecules would survive. This feedback effect would be reminiscent of the way that the mammalian immune system amplifies successful antibodies. By the end of that day, most of the essential features of our current thinking in dynamic combinatorial chemistry—thermodynamically controlled bond-breaking and -making, templating, selection, and amplification—had been sketched out.
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