

Chapter 1

Dynamic Combinatorial Chemistry: An Introduction

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Darwin was the first to recognize (or at least the first to publish) the observation that nature employs an incredible strategy for the development and optimization of biological entities with a dizzying array of traits. From the macroscopic (i.e., giraffes with long necks) to the molecular (i.e., enzymes with exquisitely well-defined substrate specificity) level, nature generates populations of molecules (or giraffes) and tests them for fitness against a particular selection scheme. Those that make it through the selection process are rewarded with the ability to successfully reproduce (amplification), generating new populations that undergo essentially open-ended cycles of selection and amplification.

In the laboratory, biologists have directly benefited from the ability to co-opt Darwinian evolution: the polymerase chain reaction (PCR) [1] and the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [2,3] process are obvious examples of the selection and amplification of nucleic acids (and there are many others). Protein- or peptide-targeted approaches are also now commonplace: phage display, for example, has become a standard method [4]. In contrast, until recently chemists have had no such “evolutionary” advantage: while combinatorial chemistry brought about the advent of the synthesis and screening of libraries (populations) of compounds (or, more precisely, the *intentional* synthesis and screening of libraries since the properties of mixtures of compounds had been evaluated through the centuries as part of natural products chemistry, or inadvertently through the synthesis of mixtures), such methods are only a single cycle through the evolutionary process.

No amplification step occurs, and the next step requires intervention by the chemist, in the form of synthesizing a new set of compounds (part of what we view as traditional medicinal chemistry).

Dynamic combinatorial chemistry (DCC) arose out of chemists' desire to couple selection and amplification steps to library production. In essence, DCC relies on the generation of a library of compounds under reversible conditions, and allowing that library to undergo selection based on some desired property. We discuss the components of this process in greater detail later in this chapter (and throughout the rest of the book). However, DCC built on a number of different lines of investigation, and it is useful to first discuss a few of these DCC antecedents in order to understand the intellectual foundations of the field. Perhaps the first recognition of a binding-induced selection process was that of Pasteur, who noted that crystals of tartaric acid could be sorted into mirror-image forms. Although the analytical technique here was certainly something one would not want to extend to large libraries (Pasteur sorted crystals by hand!), enantiomeric selection based on optimizing crystal-packing forces nonetheless demonstrated one component of the DCC process.

Many more recent experiments arose out of the body of researchers studying the molecular origins of life. Two areas of particular interest have been the origins of chirality and replication. Building on work by Miller and Orgel [5], Joyce et al. demonstrated in 1984 that diastereomerically pure nucleotides would assemble on a complementary nucleic acid strand efficiently, but the presence of nonchirally pure materials would dramatically inhibit the assembly process [6]. An important DCC precursor—and evolution of Joyce and Orgel's studies—was reported by Goodwin and Lynno in 1992 [7]. This work demonstrated that trinucleotides bearing either a 5'-amino group or a 3'-aldehyde could be induced to assemble reversibly on a DNA template via formation of an imine. Subsequent work published in 1997 incorporated imine reduction into the process [8], effectively allowing single-stranded DNA to be used as a catalyst for the production of a DNA-like secondary amine. A somewhat more complex variation of the Pasteur experiment involves spontaneous resolution under racemizing conditions (SRURC) of systems such as bromofluoro-1,4-benzodiazepinooxazole, shown in Fig. 1.1 [9]. Crystallization of this compound from a racemic, rapidly equilibrating methanolic solution can lead to amplification of either enantiomer via the production of single-enantiomer crystals.

Product templating and re-equilibration of product mixtures have also been studied extensively in the molecular recognition community. For example, Gutsche and coworkers examined the base-mediated production

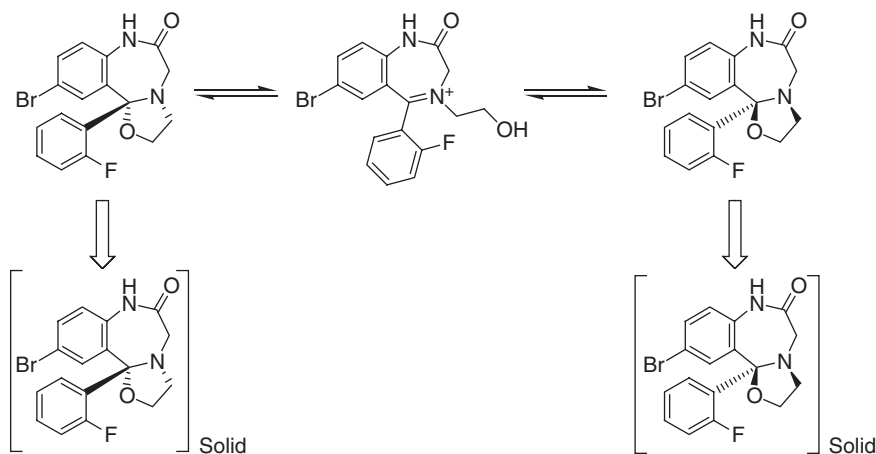


Figure 1.1 SRURC of a bromofluoro-1,3-benzodiazepinooxazole.

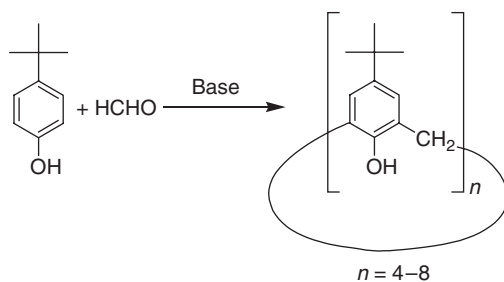


Figure 1.2 Base-mediated synthesis of calixarenes.

of calixarenes from *para*-alkylphenols and formaldehyde (Fig. 1.2), and observed that product distributions were altered based on a large number of factors [10]. Of particular interest to DCC, the authors described calix[4]arenes as arising via a thermodynamically controlled process, in part via ring contraction of calix[8]arenes and calix[6]arenes. Thus, this may be regarded as an example of a dynamic self-selection process.

Molecular recognition is obviously a critical component (and often the primary goal) of DCC-based molecular discovery, and the molecular recognition community was instrumental in developing experiments that directly prefigured the development of DCC. Two examples from the Lehn group are illustrative. In 1990, Lehn and coworkers reported that mixtures of tartrate-based compounds could be induced to form liquid

crystalline phases [11]. This recognition-driven supramolecular assembly was hypothesized to occur via formation of a triple-helix structure, mediated by nucleic acid-like hydrogen-bonding interactions. Three years later, the same group reported a particularly spectacular example of recognition-mediated self-sorting (Fig. 1.3) [12]. On treating an equimolar mixture of **1**, **2**, **3**, and **4** with excess $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{BF}_4$ in CD_3CN , a highly complex ^1H NMR spectrum was initially observed. This was found to gradually resolve itself into a spectrum dominated by the presence of the self-selected complexes **5**, **6**, **7**, and **8** (although small amounts of other complexes remained). Self-sorting among ligands predisposed to bind different metals was also observed when **9** and **10** were mixed with copper and nickel salts. Again, the authors initially observed production of a highly complex mixture, which resolved over time to consist primarily of copper complex **11** and nickel complex **12**.

With these selected examples as context, it became clear to several laboratories in the mid-1990s that one should be able to combine reversible formation of compounds (exchange processes) and a selection method with the then rapidly developing field of combinatorial chemistry to produce equilibrating libraries that would evolve based on some selection process. Thus, dynamic combinatorial chemistry or DCC, as it came to be called,¹ evolved from a number of lines of research into the diverse and vibrant field it is today.

1.1. The Components of a Dynamic Combinatorial Library Experiment

The design of any DCC experiment involves several components, loosely aligned with the components of a system undergoing Darwinian evolution (Fig. 1.4): (1) a library of building blocks (components of a population), (2) a reversible reaction (analogous to a mutagenesis method or reproduction), (3) a selection mechanism, and (4) an analytical method. The relatively short history of DCC has seen many innovative approaches to

¹Other terms have been employed for this general concept, including “self-assembled combinatorial libraries,” “constitutional dynamic chemistry,” and “virtual combinatorial libraries”. “Dynamic combinatorial chemistry” and “dynamic combinatorial library” seem to have found the broadest usage, while “virtual combinatorial library” is perhaps best reserved for conditions under which library members form at concentrations below detection limits in the absence of target (e.g., Reference 81).

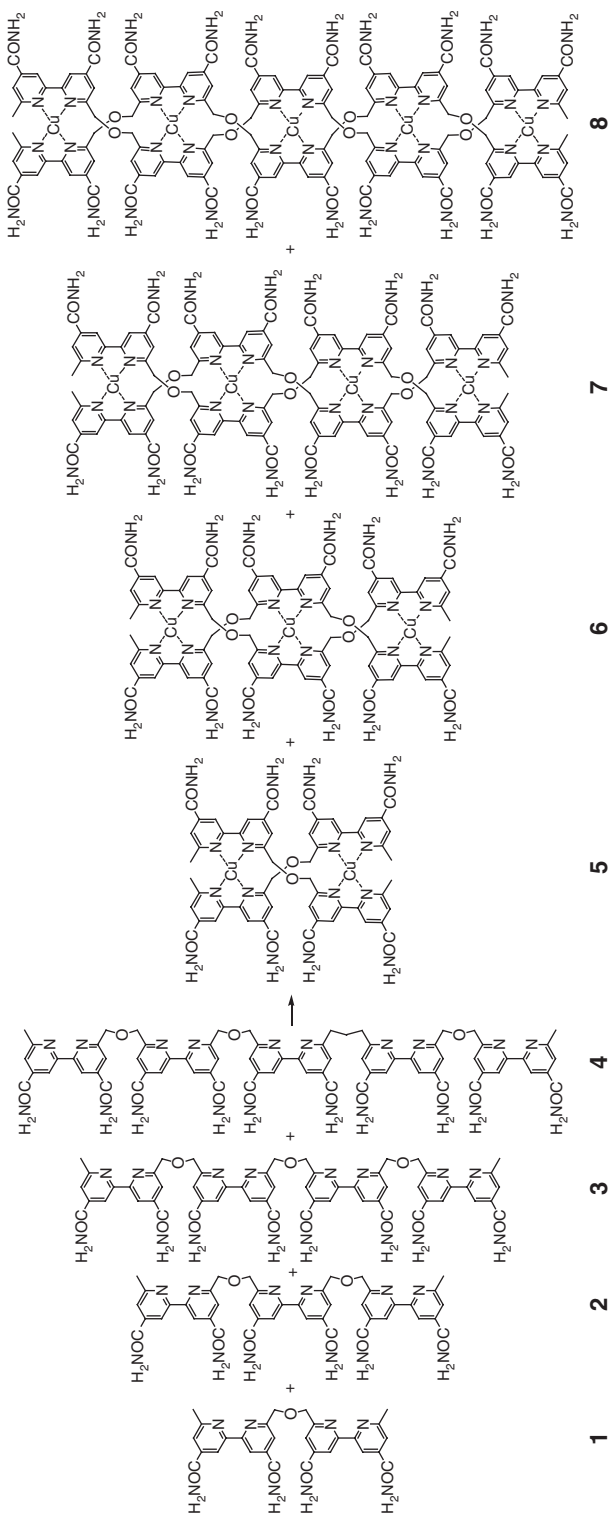


Figure 1.3 Self-selection in coordination complex formation.

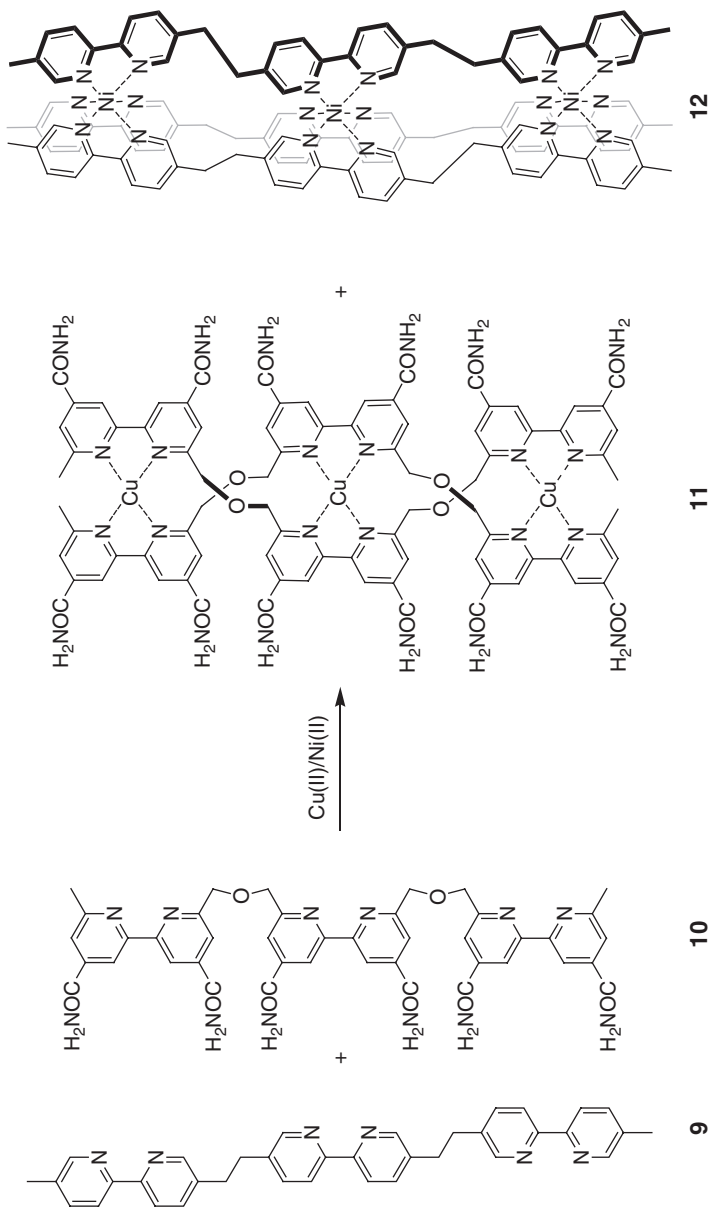


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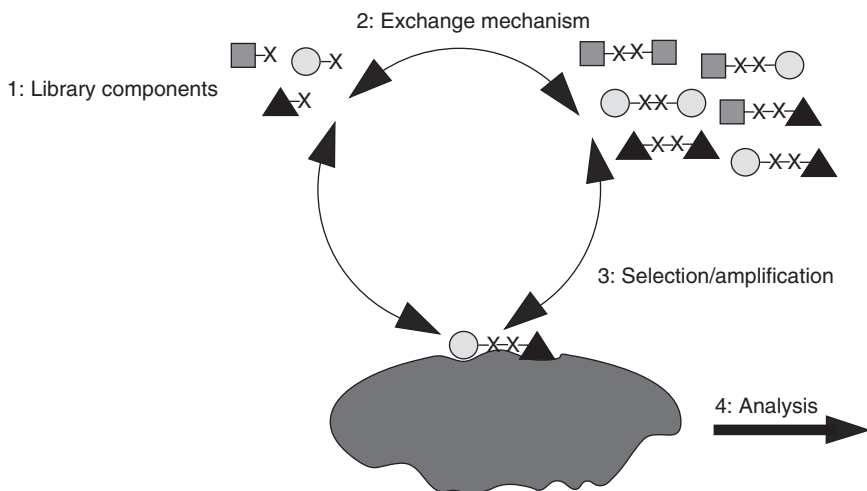


Figure 1.4 The basic structure of a DCC experiment.

each of these areas. They are all interrelated, making it somewhat difficult to discuss them in the linear fashion required by a chapter in a book. However, we will attempt to do so, by way of introduction to the field. This is not intended to be an exhaustive catalog of all dynamic combinatorial library (DCL) experiments, but rather an introduction to each topic via selected examples. More examples can, of course, be found in further chapters of this book.

1.2. Considerations in Choosing an Exchange Reaction

Chemists (and particularly synthetic organic chemists) have been trained to view synthetic reactions through one set of criteria: reactions should be irreversible and highly selective (but general). In contrast, DCC requires one to view candidate exchange reactions with a different set of criteria in mind. Most obviously, reactions must be reversible. Several other criteria are listed in the following text. Some of these constraints are particularly important when one is working with biomolecular targets.

1.2.1. Conditions Under Which Exchange Can Be Made to Occur

An obvious, but important criterion for selecting a scrambling reaction is that equilibration must occur under conditions compatible with the target.

For self-selection experiments and selection in the presence of an organic “guest,” this is generally a simple criterion to satisfy. However, biomolecules dramatically narrow the available conditions: the reaction must ideally occur at room temperature and in buffered aqueous solution. Both of these conditions can be (at least in principle) attained by physically separating the scrambling reaction from the biomolecule against which the library is selected.

1.2.2. Rate of Exchange

The rate of exchange ideally needs to be fast enough that equilibrium is reached within a convenient interval, but slower than binding to the target. One certainly wants equilibrium to be reached faster than the target degrades, if biomolecular binding is the goal. In some cases, interesting things can occur in a very slow regime; for example, studies on folding-driven oligomerization by Moore and coworkers [13–15], in which imine metathesis was used as the exchange mechanism, required as much as 19 weeks or more to reach equilibrium [16], depending on the composition of the library.

1.2.3. Ability to Halt Equilibration

Once an equilibrium distribution of the library has been reached, one generally wants to be able to analyze this distribution in order to determine what compound has been amplified. This requires “freezing” the populations of individual library members such that the analytical method does not alter the composition of the library. Methods for halting (or at least greatly reducing the rate of) equilibration can include changes in temperature, changes in pH (disulfide exchange, imine metathesis, acetal formation), turning off of the light (*cis/trans* olefin isomerization), ablation of the reactive functionality (imine reduction), or removal of catalyst (olefin metathesis and other transition metal-catalyzed processes).

1.2.4. Selectivity

Talking about selectivity in the context of a combinatorial library seems odd, and indeed, from the perspective of generating maximum diversity, it is critical that the reaction is nonstereoselective (stereorandom) and non-substrate selective (general). However, it is important that reaction occurs only with desired functional groups on library constituents rather than with target functionality, or library functionality, leading to irreversible formation of a product.

Fulfilling all of these criteria is difficult, and to date only a very small subset of the reactions available for chemical synthesis has been employed in DCC experiments. In the following sections, we will discuss representative examples of exchange reactions that have proven successful; many others are described in other chapters of this book. Discovery of new types of exchange reactions remains one of the most important challenges in the field.

1.2.5. Disulfide Exchange

Disulfide exchange has proven to be one of the simplest, most robust, and most widely used methods for library equilibration. Extensive studies by the Whitesides group [17] and others in the late 1970s and early 1980s established that thiolate–disulfide exchange was facile in aqueous solution at slightly above neutral pH, but slow at neutral pH and below. The first use of disulfide exchange in a DCL, of which we are aware, was reported by Hioki and Still in 1998 [18]. Building on prior work in Still's laboratory on the design and synthesis of artificial receptors for peptides [19], the authors first examined the disproportionation of compound **13** in chloroform in the presence of 2 mol% thiophenol and triethylamine (Fig. 1.5). In the absence of target resin-bound peptide, equilibrium was reached at 35% **13S**–SPh and 65% PhS–SPh and **13S**–**S13**. However, after incubation with resin-bound Ac(D)Pro(L)Val(D)Val, the equilibrium shifted to 95% PhS–SPh and **13S**–**S13**, a change in K_{eq} from 3.8 to 360. Challenging the selection process with a somewhat more subtle mixture, Hioki and Still next examined the disproportionation of the mixed disulfide **13S**–**S14** in the presence of 10 mol% **14SH** and triethylamine. Although the shift in equilibrium composition was not quite as large in this case (evidence for some peptide-binding ability on the part of receptors including **14SH** in their makeup), it was still definitive: 75% **13S**–**S13** on the resin phase (bound to the peptide), and 85% **14S**–**S14** in solution.

Since this initial report, disulfide chemistry has become perhaps the most widely employed method of component exchange in DCLs. Disulfide exchange is rapid, and conducted under conditions ideal for library selection in the presence of biomolecules. It is highly suitable for even very complex libraries, as in the >11,000-compound resin-bound DCLs targeting RNA binding developed by the Miller group (described in detail in Chapter 3) [20,21], and in a >9000-compound solution-phase DCL reported by Ludlow and Otto [22], described in greater detail in the following text in the context of analytical methodology.

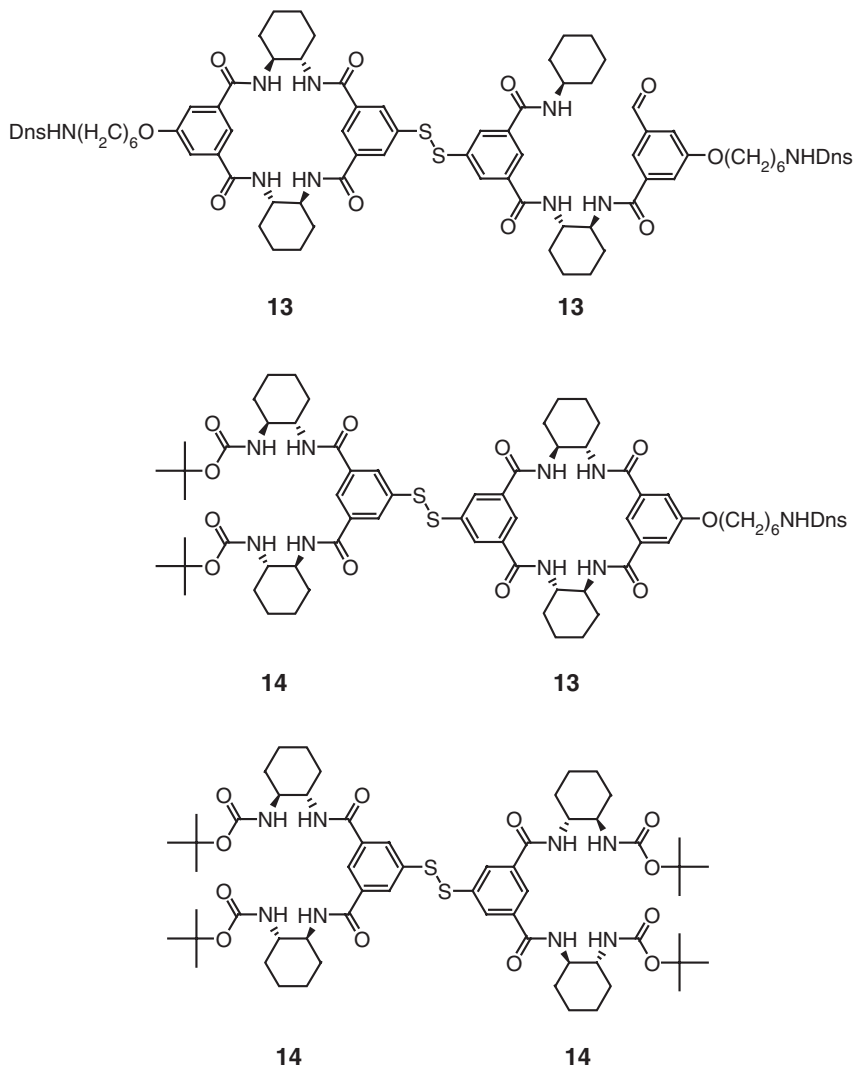
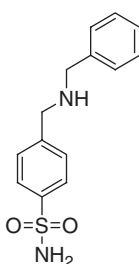


Figure 1.5 Disulfide-containing receptors for peptides prepared by Hioki and Still.

1.2.6. Imine Metathesis and Related Processes

As we have already mentioned, the ability of imine formation to serve as a useful reaction in templated systems was observed by Lynn et al. in the early 1990s. Use of imine metathesis in DCC was first described by Huc and Lehn in 1997 in a library targeting the production of carbonic

anhydrase inhibitors [23]. In this case, reduction of the imines with sodium cyanoborohydride was employed to halt library equilibration. The authors noted that because of the 18 lysine ϵ -amino groups, in addition to the terminal amine, it was necessary to use an excess (15-fold) of starting amines in order to limit reaction between starting library aldehydes and the enzyme. Equilibration of the library in the presence of target carbonic anhydrase and NaBH_3CN was allowed to proceed for 14 days. HPLC analysis revealed strong amplification of compound **15**; this amplification did not occur in the presence of a competitive carbonic anhydrase inhibitor.



Imine metathesis has continued to be a popular exchange reaction for DCLs. Various groups have found novel systems in which the reaction can be applied, as well as interesting ways to halt the equilibration. For example, Wessjohann and coworkers have demonstrated that Ugi reactions can efficiently halt equilibration of an imine DCL, combining an irreversible diversification process with a reversible library selection [24]. Xu and Giuseppone have integrated reversible imine formation with a self-duplication process [25], and Ziach and Jurczak have examined the ability of ions to template the synthesis of complex azamacrocycles [26]. The mechanistically related reactions of hydrazone [27] and oxime [28] exchange have also been explored as suitable foundations for DCL experiments.

Another process mechanistically related to imine exchange is the dynamic production of pyrazolotriazinones reported in 2005 by Wipf and coworkers [29]. After first verifying that reaction of either **16** or **17** with equimolar quantities of isobutyraldehyde and hydrocinnamaldehyde at 40°C in water (pH 4.0) resulted in the same 3:7 mixture of **16** and **17** at equilibrium (Fig. 1.6, Eq. 1), the authors demonstrated that a library could be generated by reaction of pyrazolotriazinone **16** with a series of aldehydes (Fig. 1.6, Eq. 2). Direct metathesis of pyrazolotriazinones was also demonstrated, as was reaction with ketones. Importantly, equilibration was halted by raising the pH to 7.

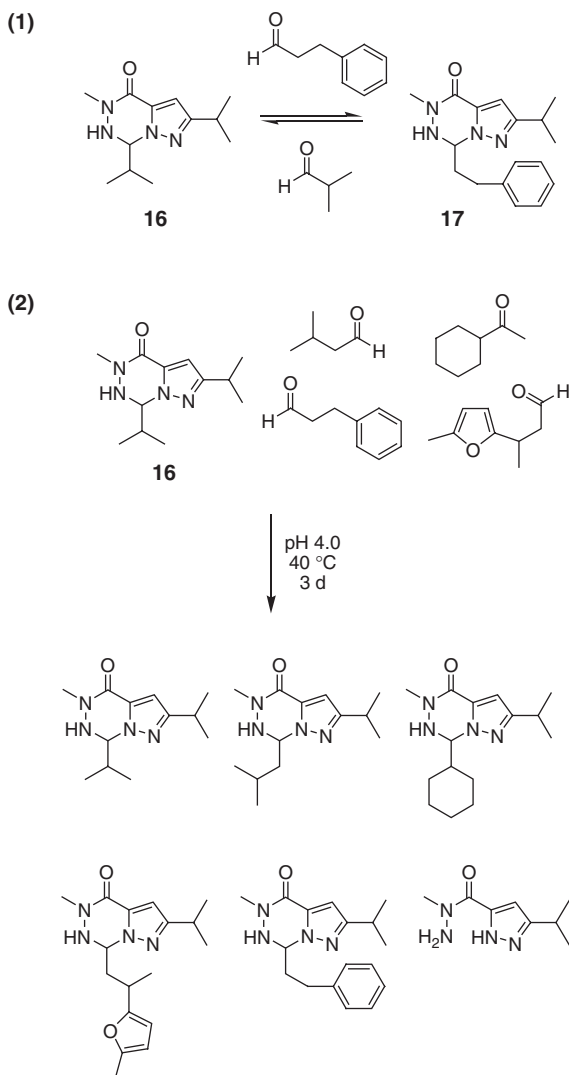


Figure 1.6 Pyrazolotriazinone metathesis (Wipf and coworkers).

1.2.7. Acetal Exchange

The reaction of aldehydes with alcohols to form acetals is rapid and reversible, and both the rate and the position of acetal–aldehyde equilibria can be affected by the pH of the reactant solution [30–32]. Thus far, however, relatively few studies have made use of transacetalization as

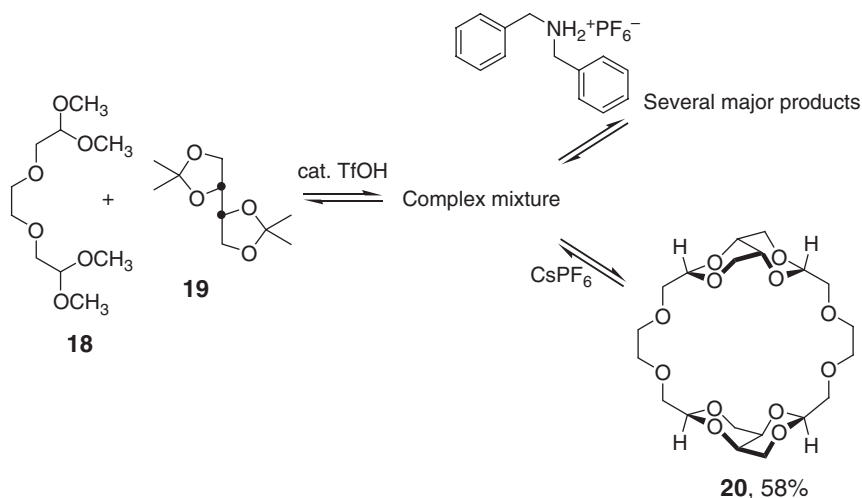


Figure 1.7 Transacetalization as a DCC exchange mechanism.

an exchange reaction in DCC. An initial demonstration of guest-induced equilibrium shifting in a library of acetals undergoing exchange was provided by Stoddart and coworkers in 2003 [33]. Treatment of a deuteriochloroform solution of diacetal **18** and the D-threitol-derived acetonide **19** (rather than threitol directly because of threitol's low solubility in organic solvents) with catalytic TfOH initiated production of a library of cyclic and oligomeric acetals (Fig. 1.7). Addition of the hexafluorophosphate salt of dibenzylamine caused the population of species in the library to shift, attaining equilibrium after 3 days at 45°C. Although the authors reported a much simpler mixture, consisting primarily of [2+2] “macropolycycles” (i.e., cyclic structures derived from two molecules of **18** and two of **19**), NMR spectroscopy indicated that several isomers were present. In contrast, library selection conducted in the presence of CsPF₆ produced cyclic acetal **20** as the primary product, in 58% yield.

Dynamic transacetalization experiments targeting cyclophane formation have also been described by Mandolini and coworkers [34]. Production of a cyclic polyether DCL by direct reaction of triethylene glycol and 4-nitrobenzaldehyde has been reported by Berkovich-Berger and Lemcoff; amplification of small macrocyclic members of the library by ammonium ion was observed [35]. With these few examples demonstrating feasibility, we can anticipate increased use of transacetalization in future DCC efforts.

1.2.8. *Transesterification*

The Sanders group provided several early examples of thermodynamic self-selection from libraries, employing transesterification as the exchange reaction. In one example, the cholic acid methyl ester derivative **21** was induced to form an equilibrating mixture of linear and cyclic oligomers via refluxing in toluene in the presence of potassium methoxide–crown ether complex (Fig. 1.8) [36]. Equilibrium mixtures derived from cholic acid derivatives bearing $R_2 = \text{MEM}$, $R_1 = \text{OB}n$, or $R_1 = R_2 = \text{PMB}$ strongly favored production of the cyclic trimer over that of other cyclic oligomers; $R_1 = R_2 = \text{H}$ also yielded cyclic dimer. Related studies from the Sanders group likewise explored equilibrium selections derived from transesterification of cinchona alkaloids [37,38].

A closely related process is the equilibration of thioesters, explored by the Gellman group in the context of evaluating peptide stability [39]. Larsson and Ramström have also employed thioester exchange in the context of libraries targeting hydrolases [40], while Sanders, Otto, and colleagues have demonstrated that thioester exchange can operate in tandem with disulfide exchange [41]. Importantly, one can also decouple the thioester and disulfide exchange processes to allow for independent staging of the two.

1.2.9. *Metal-Catalyzed Allylic Substitution*

Metal-catalyzed allylic substitution reactions have been a mainstay of synthetic chemistry because of their ability to proceed irreversibly and with high selectivity [42]. It is also feasible, however, to produce analogous systems that are completely reversible and nonselective, or ideally situated for use in DCC. These are essentially metal-catalyzed transesterification reactions, with the added feature of potentially providing stereochemical scrambling (and selection) as well as constitutional variation. An early example of this was provided in 2000 by Kaiser and Sanders [43]. In the absence of a template, reaction of diallyl diacetate **22** with a dicarboxylic acid in the presence of catalytic Pd(0) produced a negligible amount of the cyclized compound **23** (Fig. 1.9). However, when templated with 1,3-bis(4-pyridyl) benzene, yield of the cyclic structure increased to roughly 10%, independent of the dicarboxylic acid used.

In 2000 the Miller group provided a proof-of-principle study of Pd pi-allyl chemistry for library selection in the presence of a biomolecule [44]. In this approach, Pd(0) chemistry was employed to generate a library of cyclopentene-1,4-diesters in halogenated solvent (Fig. 1.10). This was allowed to equilibrate across a dialysis membrane with an enzyme target (pepsin) in buffered aqueous solution. LC-MS analysis of the library allowed identification of compound **24** as a library member amplified in the presence

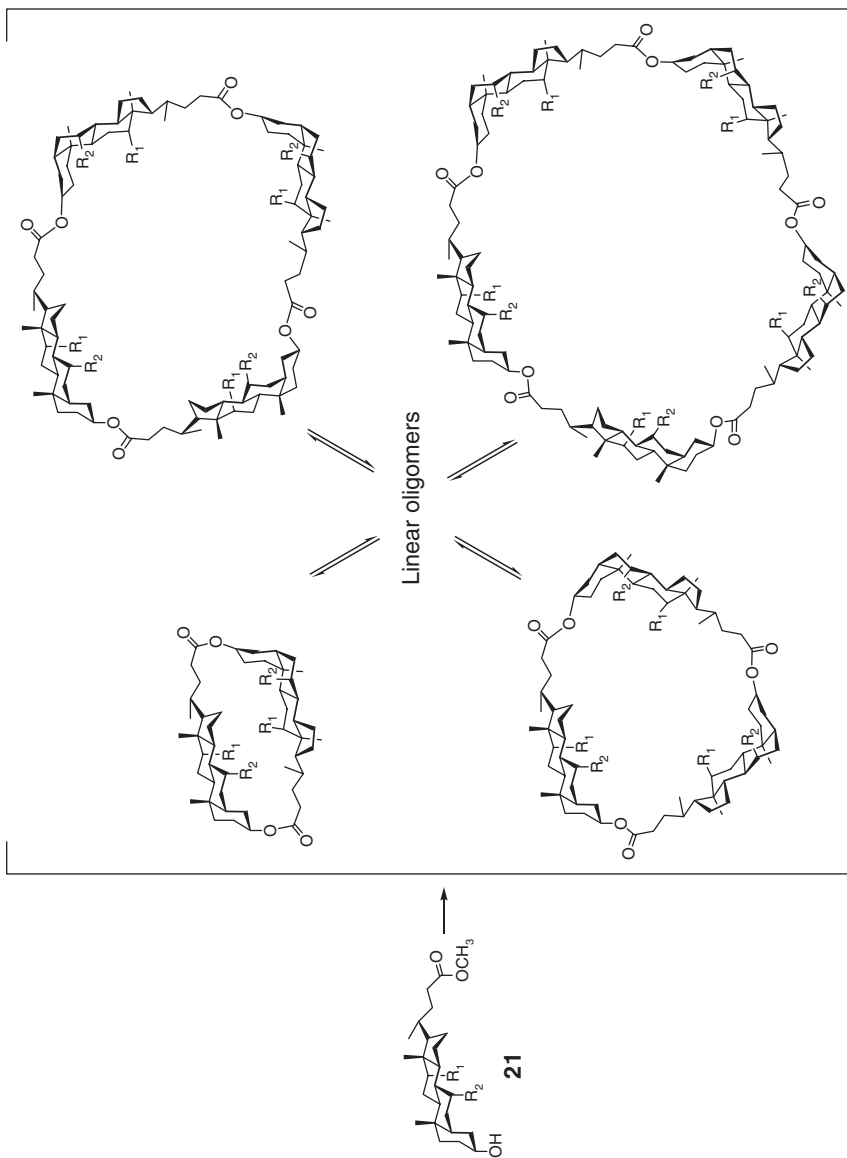


Figure 1.8 Transesterification DCL (Sanders group).

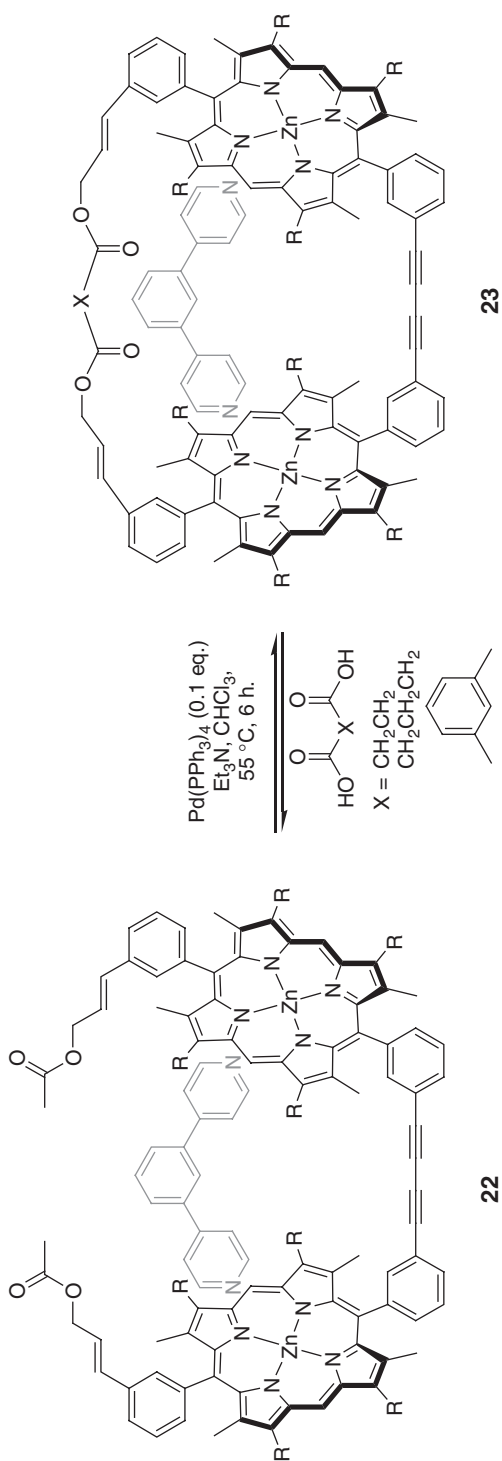


Figure 1.9 Pd pi-allyl-mediated self-selection (Sanders).

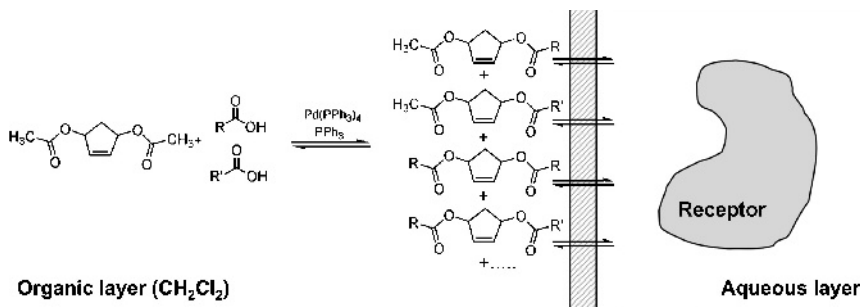
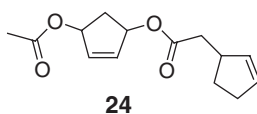


Figure 1.10 Biphasic pi-allyl Pd-based DCC selection for receptor binding.



of the enzyme. An obvious challenge in these systems is the role played by $\log P$ in the library selection process. Future studies in this area would include tuning of the solubility of library building blocks to provide sufficient solubility in chloroform for rapid exchange chemistry, while retaining the ability to remain in aqueous solution and bind to the target receptor. A recent demonstration of Pd-mediated pi-allyl sulfonylation in water [45] suggests that future “water-only” selection experiments may be possible.

1.2.10. Olefin Metathesis

Olefin metathesis is notable as one of the few exchange reactions of carbon–carbon bonds employed to date (the Diels–Alder reaction is the other primary example; *vide infra*). An early use of the metathesis reaction for capturing an equilibrating mixture of self-assembled structures was provided by the Ghadiri group. The cyclic peptide cyclo[–(L-Phe-D-(CH₃)NAla-L-Hag-D-(CH₃)NAla)2–] (**25**, where L-Hag is L-homoallylglycine) was designed to allow for interconversion between diastereomeric hydrogen-bonded dimers **26a** and **26b** (Fig. 1.11). As anticipated by the authors, treatment of a chloroform solution of **25** with Grubbs’ first-generation catalyst provided cyclized structure **27** in 65% yield.

The Ghadiri work set the stage for later experiments employing olefin metathesis in a library selection.² The Nicolaou group reported the first of

²As well as similar demonstrations of covalent capture of hydrogen-bonded dimers (e.g., see Reference 46).

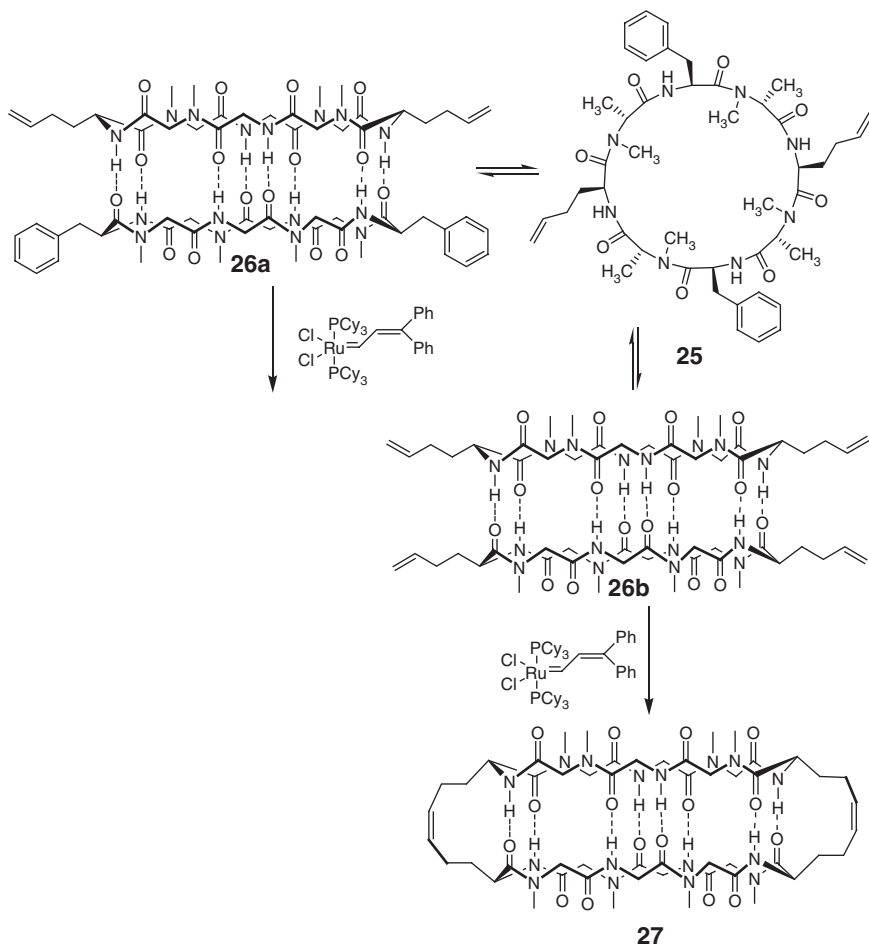


Figure 1.11 Covalent capture of peptide macrocycle dimer (Ghadiri).

these in 2000, in a study focused on the production of vancomycin dimers. A particularly interesting aspect of this work was that both olefin metathesis and disulfide exchange were examined, thus allowing a comparison of the results with the two. Although not intended as DCL experiments per se (the work was described as “target-accelerated synthesis”, and no attempt was made to examine whether libraries reached equilibrium), the experiments nonetheless provided an interesting demonstration of the potential DCC application of cross-metathesis. Initial experiments established the ability of either Ac-D-Ala-D-Ala or Ac₂-L-Lys-D-Ala-D-Ala to accelerate the production of vancomycin dimer **29** via cross-metathesis of **28** (Fig. 1.12); subsequent library experiments allowed optimization of the tether linking

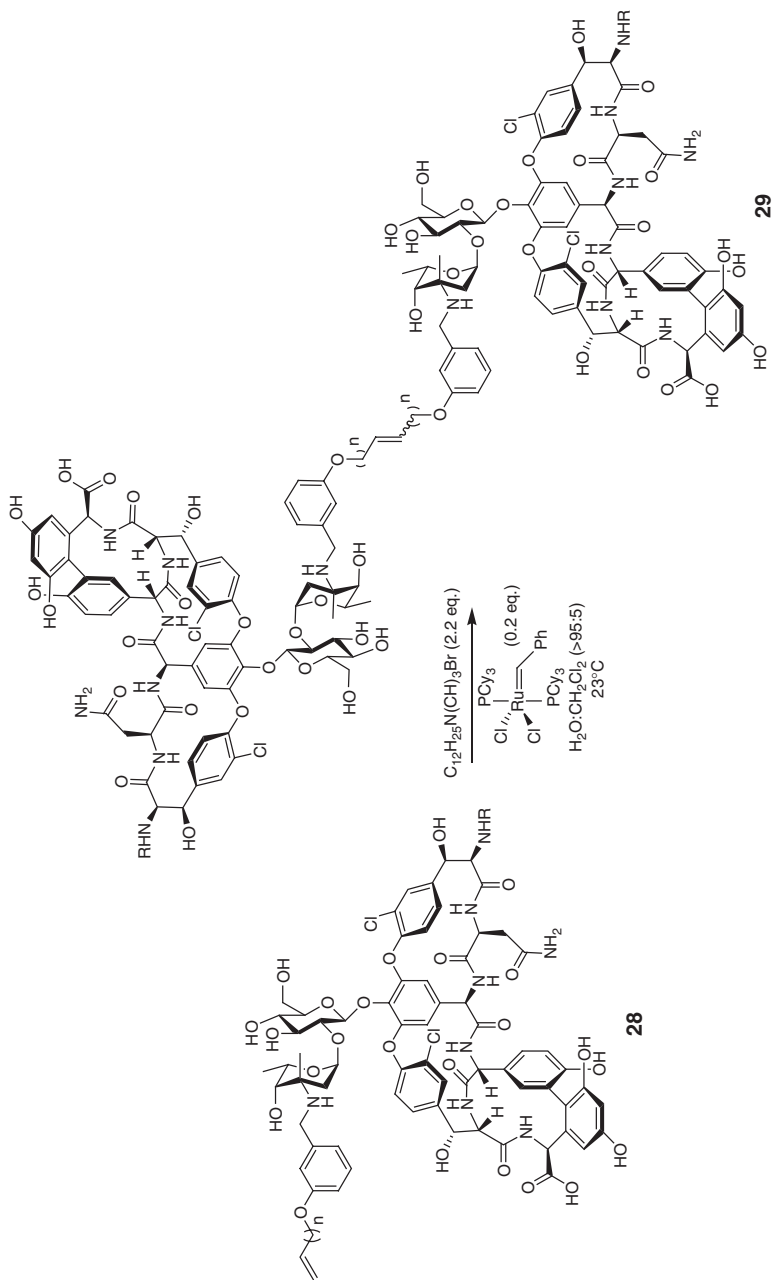


Figure 1.12 Target-accelerated synthesis of vancomycin dimers via olefin metathesis ($n = 2$ or 4).

the two halves of the dimers. Importantly, amplification (or acceleration) of dimers based on their affinity for the peptide targets correlated well with bactericidal activity. A recent DCC experiment targeting vancomycin analogs has been reported employing resin-immobilized peptides (by analogy to the work of Hioki and Still) by Chen et al. [47].

Like all reversible reactions, systems employing olefin metathesis are subject to self-selection. This was examined in the context of self-metathesis of simple allyl- and homoallylamides by McNaughton et al. [48]. In that report, both the yield of self-metathesis products and the ratio of *cis*- and *trans*-olefin isomers produced depended strongly on remote functionality on the homoallylamide. A 2005 study by Nolte, Rowan, and coworkers [49], focused on the templated production of porphyrin “boxes,” provides an interesting case study in the need to also carefully consider issues of catalyst reactivity in the design of metathesis-based DCC experiments. As shown in Fig. 1.13, the authors first subjected zinc porphyrin derivative **30** to Grubbs’ second-generation metathesis catalyst in the presence of tetrapyrrolylporphyrin template **31** (TPyP). Contrary to expectation, this provided only a low yield of the desired TPyP-coordinated cyclic tetramer, instead of providing a complex mixture of products. The low yield was attributed to coordination of TPyP to the ruthenium catalyst. In contrast, treatment of **30** with Grubbs’ first-generation catalyst to produce a library of cyclic and linear oligomers, followed by re-equilibration of the library in the presence of the TPyP template, yielded the desired structure in substantially higher yield.

1.2.11. Alkyne Metathesis

Alkyne metathesis, a mechanistic cousin of alkene metathesis, has thus far found only limited exploration. In 2004, Zhang and Moore reported that precipitation-driven alkyne metathesis reactions could efficiently produce arylene ethynylene macrocycles [50]. This was explored further in a 2005 report, verifying that the products obtained were indeed the result of thermodynamic self-selection [51].

1.2.12. Diels–Alder Reaction

Joining olefin metathesis on the very short list of exchange reactions involving carbon–carbon bonds, the Diels–Alder reaction was studied in 2005 by Lehn and colleagues [52]. As the authors note, most Diels–Alder reactions proceed only in the forward direction at room temperature, with retro Diels–Alder reactions typically requiring elevated temperatures. Careful tuning of the diene and dienophile, however, can alter this significantly. In particular, reactions of substituted fulvenes (**32**) with diethylcyanofumarate (**33**) were

found to reach an equilibrium mixture of cycloadduct products and starting materials “within seconds” of mixing at room temperature in chloroform (Fig. 1.14). Reversibility of the reaction was established through a series of diene exchange reactions.

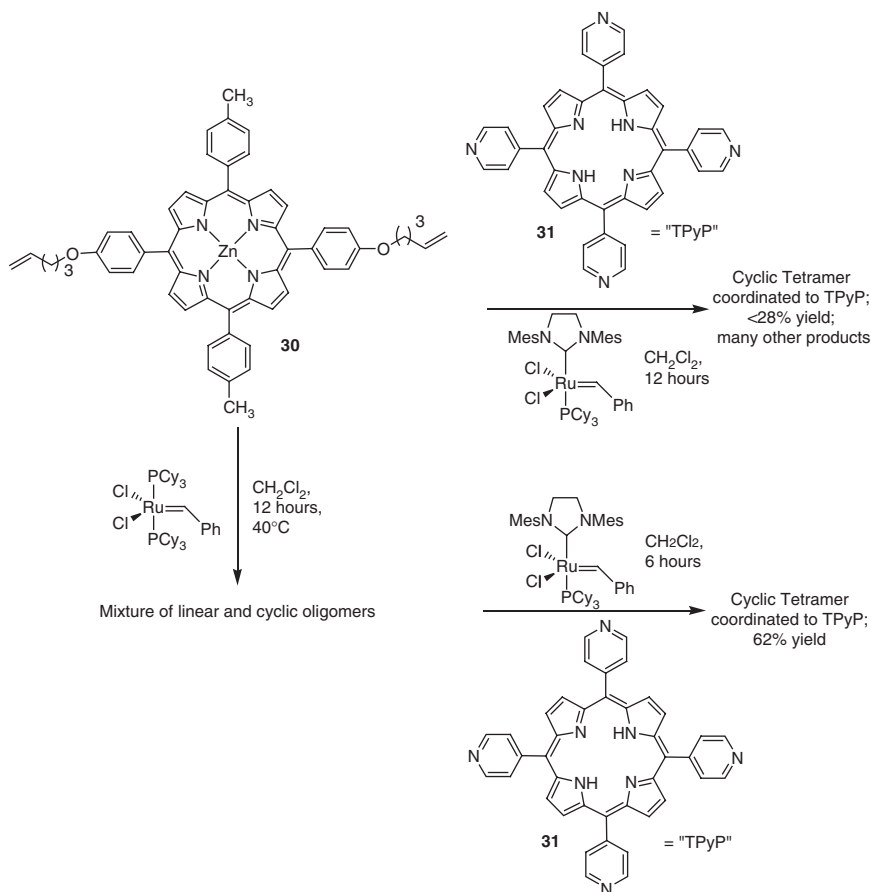


Figure 1.13 Self-selection of molecular boxes via olefin metathesis.

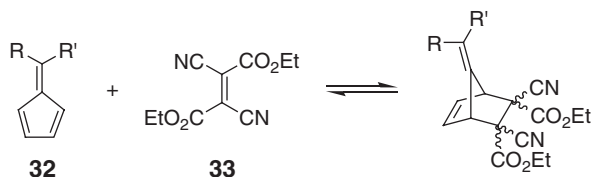


Figure 1.14 Reversible Diels-Alder reaction of substituted fulvenes with diethylcyanofumarate.

Bennes and Philp have employed a simple DCL based on a reversible Diels–Alder reaction to study kinetic versus thermodynamic selectivities, as well as concentration-dependent compensatory effects in a DCL self-selection process [53]. Rate constants and equilibrium constants for the reaction of dienophiles **34**, **35**, and **36** (Fig. 1.15) with diene **37** in CDCl_3 were first established. These confirmed molecular modeling predictions that cycloaddition between **37** and the dienophile **34** bearing a two-carbon spacer provided the most thermodynamically stable product, presumably because of an ionic or a hydrogen-bonding interaction between the carboxylic acid and amidopyridine moieties. Interestingly, reaction between **37** and the one-carbon spacer dienophile **34** had the highest rate, however (kinetically favored product). Running the reaction as a DCL provided the counterintuitive observation that maximum selectivity for the thermodynamic product **38** was obtained at low conversion. This is hypothesized to result from a compensatory effect: as dienophile **35** is depleted from the pool of available reactants, more of the less thermodynamically stable products are formed simply because of differences in the concentration of reactant dienophiles. This effect has also been studied extensively by the Severin and Otto groups (among others) and is discussed further in the following sections.

1.2.13. Photochemical Isomerization

While the vast majority of DCC experiments have focused on equilibration of constitutionally distinct library members, methods for the

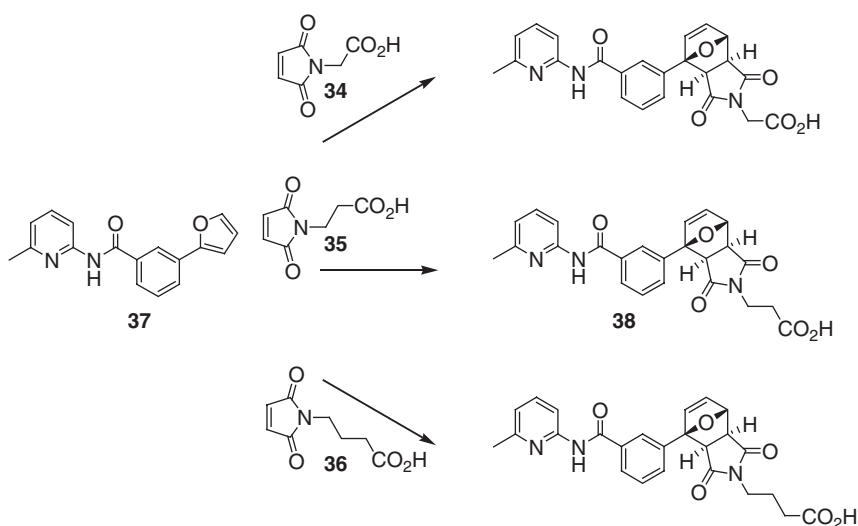


Figure 1.15 Recognition-mediated selectivity in a reversible Diels–Alder DCL.

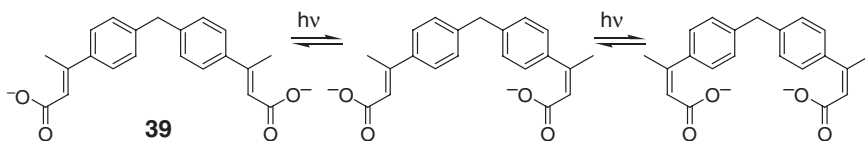


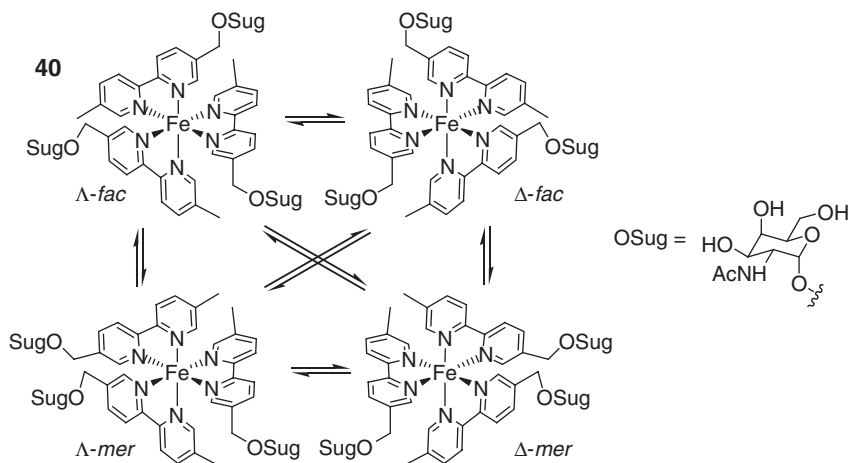
Figure 1.16 Photochemical isomerization (Eliseev).

equilibration of configurational isomers are also of interest. An early example of DCC from the Eliseev group employed photochemical *cis/trans* isomerization as the exchange reaction [54]. As shown in Fig. 1.16, photolysis of dicarboxylate **39** yields a mixture of three isomers, with a photostationary state of 17:31:52 *trans/trans:cis/trans:cis/cis*. Subjecting this mixture to 30 cycles of irradiation followed by passage through an affinity column bearing guanidinium groups (as the selection process), and subsequent elution of material on the column, yielded a substantially altered mixture: 2:13:85 *trans/trans:cis/trans:cis/cis*.

Surprisingly, some 11 years would elapse before another example of the use of photochemical *cis/trans* isomerization as a diversity-generating reaction in DCC would appear in the literature. In a 2008 report [55], Ingerman and Waters described the use of azobenzene photoisomerization and hydrazone exchange as a “doubly dynamic” system (further examples of multiexchange systems are presented below). Unlike the Eliseev work, photochemical equilibration was carried out in the presence of the target. As the authors note, photoequilibration converts the library to a photostationary state rather than a thermodynamic minimum, but binding to a particular library member can alter the distribution of products in the photostationary state just as readily as binding can alter the distribution of a thermodynamic equilibrium.

1.2.14. Metal Coordination

The ability of metal coordination to influence the distribution of materials formed in a labile mixture was recognized as early as 1927, in a pair of studies examining the self-condensation of aminobenzaldehydes [56,57]. As discussed above, many other experimental antecedents of DCC centered on observation of self-selection processes occurring during the formation of coordination complexes, and it is therefore not surprising that transition metal complexes capable of facile ligand exchange have been the subject of library experiments. A particular challenge in this case is that one must choose the coordination carefully, as many coordination complexes are too labile to permit simple analysis postequilibration. Indeed, some early



experiments from our group involved complexes whose existence could only be inferred based on analysis of stable derivatives [58,59]. However, more “cooperative” systems have been reported by others. For example, a 1997 report from Sakai, Shigemasa, and Sasaki explored the lectin-mediated selection of carbohydrate-based ligands from an equilibrating mixture of Fe(II) complexes [60]. In the presence of Fe(II), bipyridyl carbohydrate derivative **40** forms an equilibrating mixture of stereoisomeric complexes, as shown in Fig. 1.17. Introduction of *Vicia villosa* B4 lectin causes this equilibrium to shift in favor of the Δ -mer isomer (from 29% of the mixture to 85%), which is best able to bind to the carbohydrate-binding site. In this case, individual complexes were sufficiently stable to permit analysis by HPLC.

Buryak and Severin have described the use of dynamic libraries of Cu(II) and Ni(II) complexes as sensors for tripeptides [61]. A notable aspect of this work is that as isolation of the metal complexes is not necessary (sensing is accomplished by observing changes in the UV-vis spectrum), potential concerns over the lability of coordination complexes do not apply. Specifically, three common dyes [Arsenazo I (**41**), Methyl Calcein Blue (**42**), and Glycine Cresol Red (**43**), Fig. 1.18] were mixed with varying ratios and total concentrations of Cu(II) and Ni(II) salts in a 4×5 array. Previous work had demonstrated that these conditions produced equilibrating mixtures of 1:1 and 2:1 homo- and heteroleptic complexes [62]. These arrays were able to clearly and unambiguously differentiate tripeptides based on the differential pattern of response. The Severin laboratory has

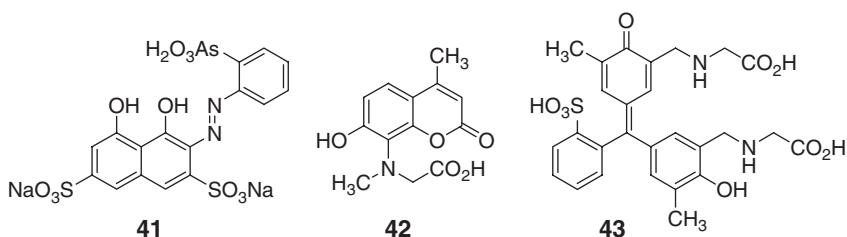


Figure 1.18 Dyes employed in the construction of Ni(II)/Cu(II) coordination DCLs for tripeptide sensing (Buryak and Severin).

successfully employed a similar strategy (which they call a “Multicomponent Indicator Displacement Assay”, or MIDA) for nucleotide sensing [63] and as molecular timers [64].

Complex cage structures produced by the reversible assembly of pyridine 2-carboxyaldehyde, biphenyl amines, and iron salts have been described by the Nitschke group [65]. Interestingly, these were found to be capable of capturing hydrophobic solvent molecules as guests and carrying them into aqueous solution. Addition of a competing amine set off an imine exchange reaction that “unlocked” the cage complex, liberating the guest solvent.

1.2.15. Enzyme-Mediated Processes

Enzymes can also be brought to bear as catalysts for effecting scrambling reactions. This can be particularly useful in cases where the bond breaking/making process of interest is one not generally viewed as labile under conditions amenable to standard DCL experiments. For example, an early demonstration of DCC was provided by Swann et al., who employed thermolysin, a bacterial metalloprotease, as transamidation catalyst. Mixing H₂N-Tyr-Gly-Gly-COOH and H₂N-Phe-Leu-COOH with thermolysin resulted in the production of H₂N-Tyr-Gly-Gly-Phe-Leu-COOH, as well as other unidentified peptides. Incubation of this system with a target (fibrinogen, separated from the thermolysin solution by a dialysis membrane) amplified a fibrinogen-binding peptide relative to the rest of the mixture.

Enzyme-mediated chemistry can also inspire the development of novel nonenzymatic catalysts. Stahl, Gellman and collaborators at Wisconsin have taken on the challenge of developing transamidation catalysts, successfully identifying Al(III) complexes capable of equilibrating mixtures of tertiary carboxamides with secondary amines [66,67]. For example, treatment of an equimolar mixture of **44** and **45** with 2.5 mol% of an aluminum catalyst in

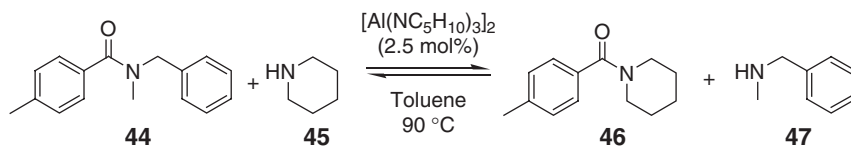


Figure 1.19 Aluminum-mediated transamidation in toluene.

toluene at 90°C rapidly affords a thermodynamic mixture of transamidated species **46** and **47** (Fig. 1.19). The exchange rate is first order in catalyst concentration, and independent of the concentrations of amine and amide. While the conditions employed are obviously incompatible with biomolecule-directed DCC, this nonetheless represents an important step forward and sets the stage for the development of catalysts capable of functioning closer to room temperature.

1.2.16. Multiple Exchange Reactions

One can in principle combine different exchange reactions in the same system in order to further increase the structural diversity accessed by the library. However, as this compounds the problem of selectivity (i.e., one now has two or more reactions that must exclusively involve one pair of functional groups), there are very few examples thus far of the practical implementation of this concept. An early, highly intriguing example was described by Lehn and coworkers in 2001 [68]. In this system, imine exchange (acyl hydrazone formation) and reversible metal coordination were employed in library generation.

The ability of boronic acids to serve as components of DCLs has been recognized for some time. For example, both the Shinkai [69] and Shimizu [70] groups have explored the properties of reversibly formed, oligomeric structures produced by reaction of bifunctional boronic acids with diols. In a recent example, the Severin group has demonstrated assembly of macrocycles via imine formation combined with the reversible reaction of boronic acids with diols (Fig. 1.20) [71]. Reaction of 3-formylphenylboronic acid, 1,4-diaminobenzene, and pentaerythritol provided macrocycle **48** as the primary characterizable product in 44% yield. Increasing the complexity of the system by addition of tris(2-aminoethyl)amine (tren) unfortunately produced a material of insufficient solubility to permit characterization, but changing the boronic acid from 3-formyl to the 4-formyl isomer allowed isolation and characterization of the cryptand **49**. Pushing the complexity of the self-selection process still further, Severin and coworkers mixed 3-aminophenylboronic acid, pentaerythritol, 3-chloro-4-formyl pyridine, and

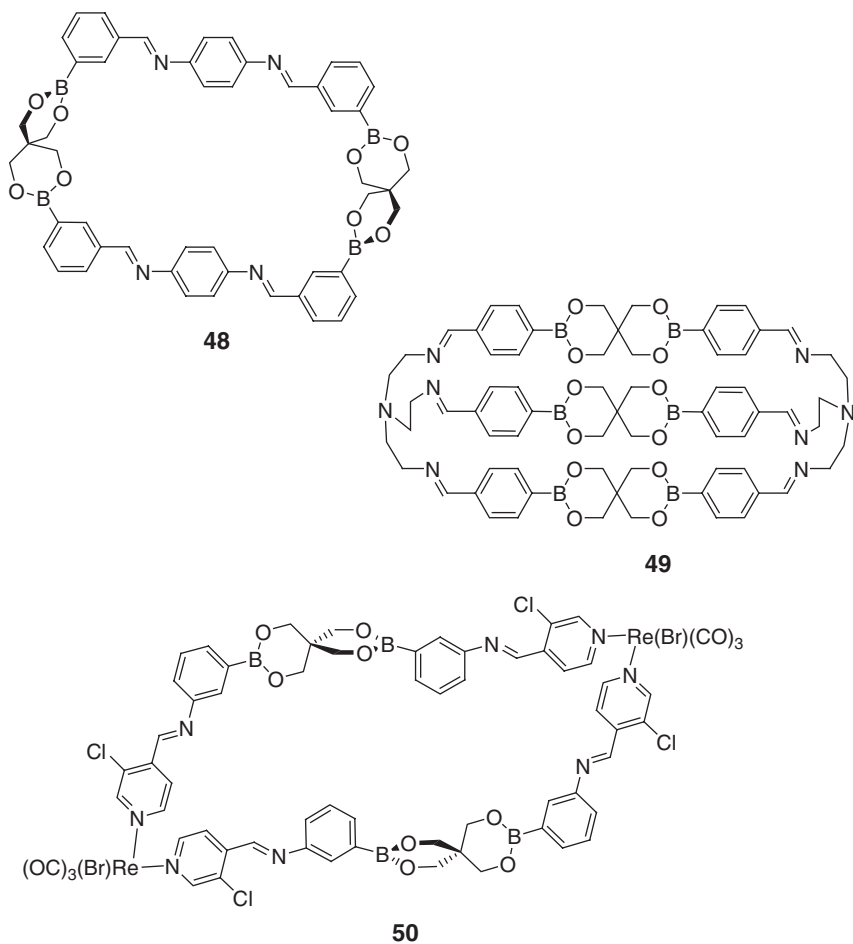


Figure 1.20 Multicomponent exchange.

$\text{ReBr}(\text{CO})_5$ to produce macrocycle **50**. This serves as an elegant proof-of-concept for incorporation of three-way orthogonal exchange reactions in DCLs. Several obvious challenges remain, however, as the conditions under which the reactions occur (refluxing THF/benzene) place obvious constraints on the targets that can be employed.

1.3. Library of Building Blocks

Once an exchange reaction has been chosen, the researcher must next choose a set of building blocks for construction of the dynamic library.

Considerations for building blocks in DCC experiments include the following:

Molecular weight: This is a two-part criterion, as both the absolute molecular weight and its uniqueness are important for each building block. The first of these is particularly important in the context of libraries focused on “drug-like” molecules, since one generally wants to keep the total molecular weight low. Uniqueness is critical if one plans to employ mass spectrometry for analysis of library results.

Solubility: Anecdotal evidence from several sources suggests that this is a particular concern. However, it is difficult to predict a priori, particularly in instances where oligomer libraries (rather than simple binary or A/B type libraries) are to be generated.

Structural diversity: One wants to be able to generate the greatest structural diversity possible with the smallest number of components. This both increases the chance of success in a binding-directed experiment (as opposed to a self-selection) and simplifies the analytical challenge.

Unique functionality: This is required for participating in the reversible reaction, and is the converse of the criterion listed above for choosing an exchange reaction. This functionality is carefully chosen to allow production of a binary A/B library, or formation of oligomers or cyclic structures. If multiple exchange reactions are anticipated, this increases the complexity of the design process accordingly.

Many of the issues one needs to consider in the selection of building blocks for DCC are common to all library experiments (including “static” libraries as well as DCC). One generally wants to generate as much structural diversity as possible from the smallest possible number of building blocks; the more diversity among the building blocks, the better the DCL is. If one is using DCC to target molecules for *in vivo* use, either as drugs or as probes, molecular weight can be an important consideration, since bringing DCL fragments together to form dimers (or trimers or oligomers) can obviously escalate molecular weight well beyond the size typically considered drug-like [72]. Molecular weight *uniqueness* is also an important consideration if mass spectrometry is intended as the primary analytical method for the library (discussed further in Chapter 7). Although unique molecular weights of DCL building blocks do not guarantee unique molecular weights for each member of the DCL, they clearly reduce the number of overlapping masses in the final library.

1.4. Selection Mechanisms

We have already discussed many different selection mechanisms in our brief survey of exchange reactions above. These can include ligand–receptor binding, self-selection, physical properties (of a polymer, etc.), and phase selection (binding to a target on solid phase, crystal packing). “Ligand–receptor binding” can be broken down into a number of smaller categories including DNA–small molecule, protein–small molecule, small molecule–small molecule (“host”–“guest”), ion–receptor, and others. It is also possible to combine the selection mechanism with the scrambling reaction in a negative selection, for example, by employing an enzyme capable of selectively destroying some library components. Such methodology is discussed further in Chapters 2 and 6.

1.5. Analytical Methodology

The problem of efficiently identifying “active” compounds in mixtures was noted early in the history of “static” combinatorial chemistry, and led to the development of many elegant strategies including binary encoding and recursive deconvolution. DCC, because of its integrated amplification step, should be less susceptible to the mixture problem. However, in practice, identification of the selected component can still present a challenge, particularly in cases where the theoretical size of the library is large. Typically, analytical strategies fall into one of two broad categories: (1) those independent of the selection scheme and (2) those coupled to the selection scheme.

Selection-independent analysis: In this case, library analysis occurs strictly after and apart from the library selection experiment. Typically, what this means is that the solution resulting from a library is analyzed by HPLC or HPLC-mass spectrometry (HPLC-MS), and compared with the chromatographic trace obtained for an identical library prepared in the absence of target. This provides an internal control for self-selection processes and (hopefully) allows direct identification library members undergoing enhancement through visual inspection. If self-selection is the goal, one simply compares HPLC traces of libraries at different time points.

The challenges of this method have kept the majority of DCLs relatively small. However, Ludlow and Otto recently demonstrated that, in some cases at least, direct HPLC-MS analysis of large libraries is possible [22].

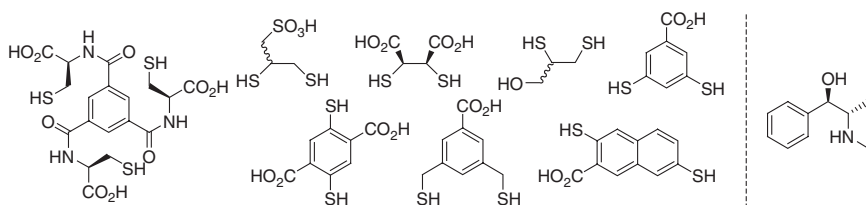


Figure 1.21 Components of a 9000-compound solution-phase DCL.

As shown in Fig. 1.21, a series of di- and tri-thiols were mixed under conditions suitable for disulfide formation and exchange, and allowed to evolve in the presence of an ephedrine template. HPLC-MS analysis of the library mixture after equilibrium had been reached allowed the identification of two heterotetrameric receptors with high ($K = 10^4$) affinity for ephedrine in borate buffer, although it is not clear whether these were in fact the “best” binders in the library.

Selection-coupled analysis/phase segregation: One strategy for simplifying the analytical challenge is to use phase segregation. Three subclasses are possible. In the first of these, a phase transition is part of the selection process. This includes not only the familiar crystallization-induced enantiomeric enrichment discussed above but also the experiments (primarily employed in experiments directed toward the production of novel materials) such as those described by Lehn and coworkers in 2005. In this study, an acylhydrazone library was created from guanosine hydrazide and a mixture of aldehydes (Fig. 1.22); in the presence of metal ions, formation of G-quartet structures led to the production of a gel.

Liquid–liquid phase segregation has been accomplished using two immiscible solvents (i.e., “phase transfer” DCC) by several laboratories. For example, the Morrow group has reported on imine [73] and acylhydrazone [74] DCLs targeting extraction of metal ions from aqueous to halogenated solutions. As discussed above in the context of Pd-mediated transesterification, the Miller group has also contributed to this area.

An alternative formulation of the phase-transfer DCC concept was reported in 2008 by the Sanders group [75]. In this case, thiol monomers were dissolved in water on either side of a U-tube containing chloroform (Fig. 1.23). After allowing the system to reach equilibrium, monomer distribution was identical in both aqueous solutions, and mixed species (e.g., **51**) were observed in the chloroform layer.

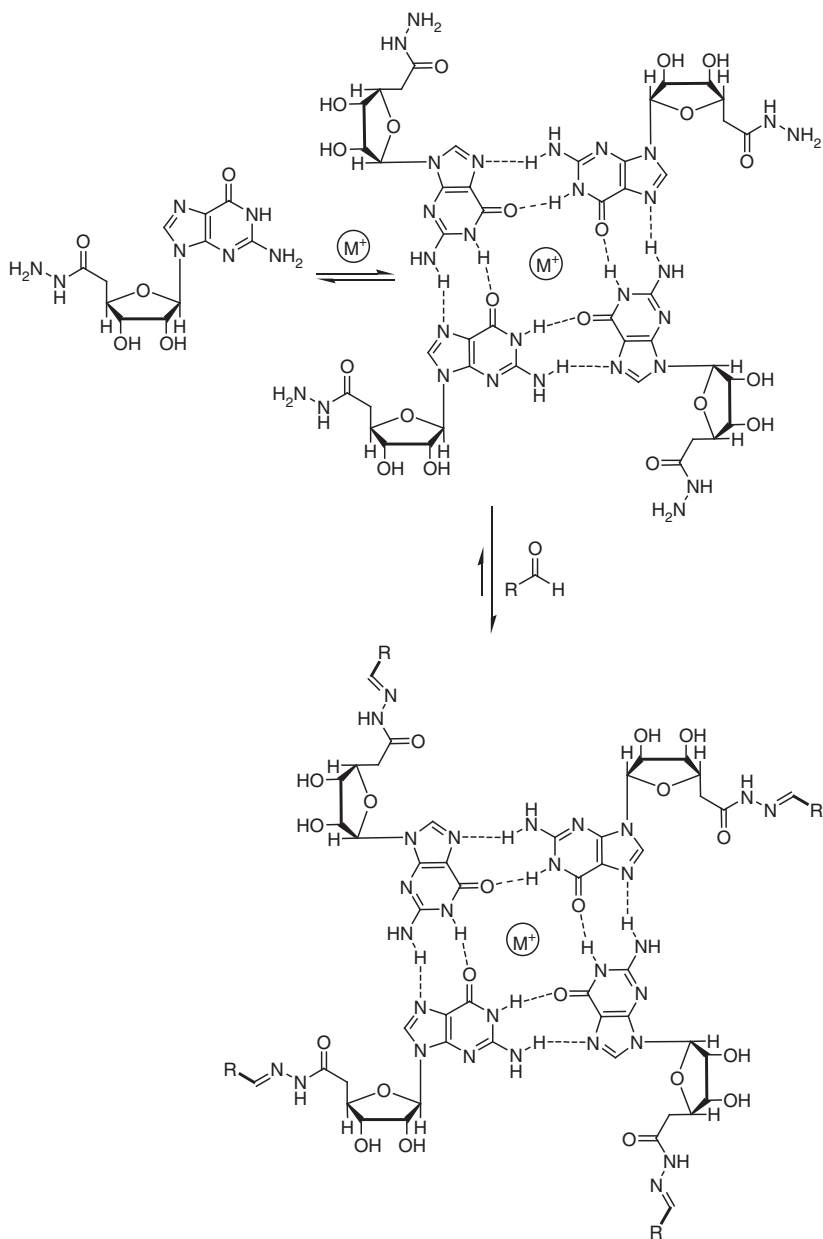


Figure 1.22 Selection by gelation (Lehn and coworkers).

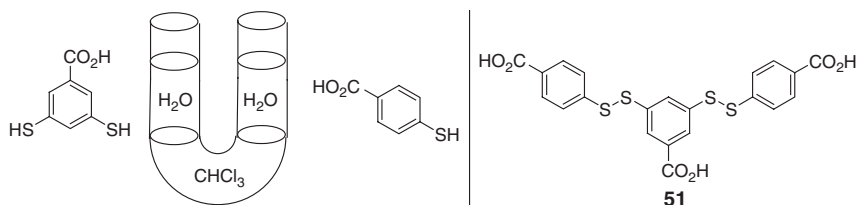


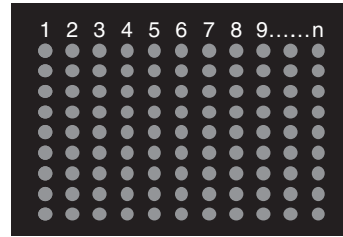
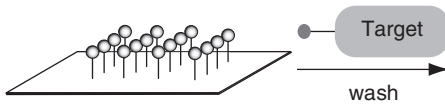
Figure 1.23 Phase-transfer DCC (Sanders).

A second method of incorporating phase into the selection process is to immobilize the target. Essentially an affinity chromatographic method, this allows nonbound library constituents to be washed away, leaving the selected compound(s) bound to resin. Eliseev's guanidine resin and Still's peptide-bearing beads, both discussed above in the context of exchange reactions, are examples of these.

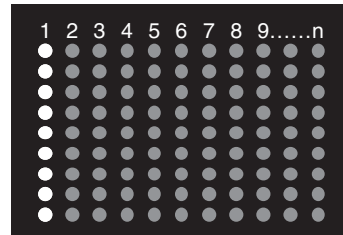
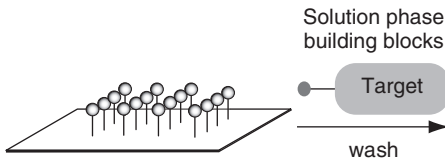
Finally, one can also invert the affinity chromatographic concept and immobilize the library constituents themselves. We have termed this technique "resin-bound DCC" (RBDCC), and have found it to be an exceptionally efficient method for generating and screening large DCLs. The RBDCC method evolved out of an earlier attempt to phase-tag library components, in this case using a microarray format. This is illustrated schematically in Fig. 1.24, using a dimer library A_n-B_m as an example. One would first create an array of all A_n and B_m on the chip (in this case, the y-axis of the array would just be replicate spots of the individual library components; one could also imagine printing a single row). After carrying out a control experiment to verify that individual array-immobilized monomers did not bind to the fluorophore-labeled target, one would introduce an identical set of A_n and B_m in solution and allow the library to evolve in the presence of the target. Once equilibrium was reached, one could then wash the array and directly identify the selected components based on a simple imaging experiment. For example, if only the row corresponding to A_1 showed visible fluorescence, then one would conclude that only A_1-A_1 was the active compound. However, if both the A_1 and B_3 columns fluoresced, one would then have to evaluate three possible binders: A_1-A_1 , A_1-B_3 , and B_3-B_3 . Of course, a major assumption of this method is that immobilization on the chip does not diminish the ability of any library component to participate in binding.

In practice, this array-based method is ineffective, primarily because there is insufficient material on the surface of the array to compete with solution-phase library members. As discussed in Chapter 3, however, implementing the RBDCC concept on resin beads produced a viable method for

Control experiment—monomers don't bind



Dimer 1–1 is the highest affinity molecule:



Dimers 1–1, 1–4, and 4–4 are potential ligands:

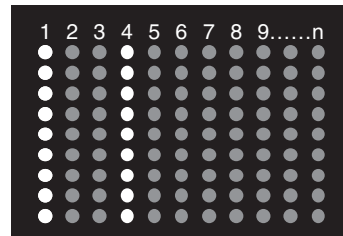
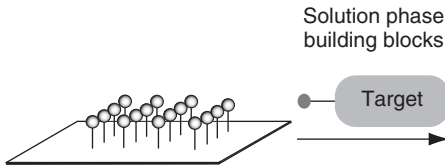


Figure 1.24 Microarray-format DCC (courtesy Brian R. McNaughton).

the generation of large DCLs and simple identification of “best” binders to biological targets. It is still conceivable that one might make the array-based method work through the use of extremely low solution volumes, and this will be an interesting area of exploration for the future.

1.6. Simulation of DCL Behavior

Many of the early “proof of concept” DCC experiments were carried out on a somewhat ad hoc basis. Several authors quickly came to the realization that it would be useful to develop methodology for simulating DCLs, both as a guide to experimental design and as a way to resolve the question of whether screening a DCL really leads to the identification of the

library method with optimum fitness for the selected property (i.e., tightest binder).

The earliest efforts toward simulating the behavior of DCLs and DCL selection processes were reported by Moore and Zimmerman [76]. In this relatively simple model, the authors focused on predicting the behavior of a large population (e.g., 10^{10}) of interconverting copolymer sequences. Using a compilation of binding constants originally developed by Connors for cyclodextrin complexes [77], binding for the theoretical system was modeled as a normal (Gaussian) distribution in $\log K$. Given a standard deviation in the binding constant of one order of magnitude, the model suggested that the mean binding constant for the population (i.e., for the library as a whole) could be shifted by no more than two orders of magnitude by a typical DCC selection process. This in turn led to the overall conclusion that although DCC could be a useful method for identifying lead compounds, it would not provide a practical method of synthesizing ultra-high affinity molecules unless the selection process could be coupled to an amplification method more powerful than equilibrium shifting. Of course, as the largest DCL synthesized to date is on the order of 10^4 (rather than 10^{10}), it is not clear how precisely this model will correlate with common DCC experiments.

While Moore and Zimmerman considered the behavior of a DCL as a whole, more recent efforts by the Otto, Sanders, and Severin laboratories have attempted to model the concentrations of each library component explicitly.³ Notably, these groups have also tested their predictions in the laboratory. The Severin group's efforts in this area began with a 2003 study in which the behavior of libraries undergoing self-selection was modeled [78]. Three types of libraries were considered (Fig. 1.25): type "A", in which one building block undergoes assembly into a library of compounds with variable stoichiometry; type "B", in which multiple building blocks form a library with fixed stoichiometry; and type "C", in which multiple building blocks undergo assembly into a library of variable stoichiometry. Calculations of steady-state concentrations for libraries of types "B" and "C" led to two somewhat surprising predictions. First, the selection of a structure containing a high percentage of one building block would drive formation of structures containing the other building blocks, and second, the selection processes (self or otherwise) naturally favor the production of heteroassemblies.

³Other contemporary reports supplemented experimental data with limited theoretical analysis; as the detailed simulation of library behavior was not the primary focus, they will not be discussed here.

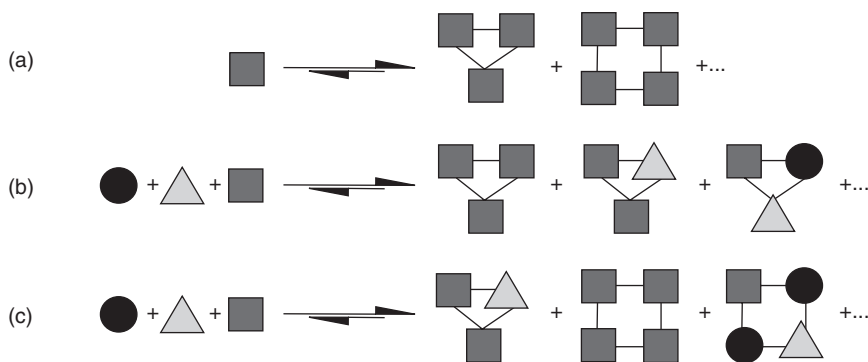


Figure 1.25 Library types examined computationally and experimentally by the Severin group.

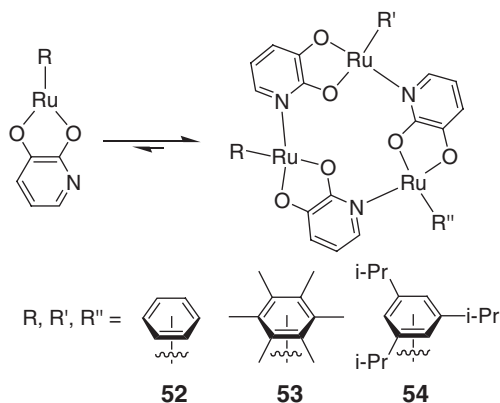


Figure 1.26 Experimental validation of predicted DCL behavior with metal-lomacrocycles.

Experimental confirmation of this predicted behavior was obtained via the examination of DCLs of trinuclear metallamacrocycles formed from ruthenium pyridonate complexes (Fig. 1.26). After first forming the individual homotrimers (i.e., **52–52–52**), these were mixed in various combinations and allowed to undergo exchange. As predicted, the thermodynamically most stable species was not always the most strongly selected.

In a subsequent paper, Severin expanded on these observations by defining boundary conditions under which DCLs can indeed provide the highest amplification of the most stable (or highest affinity) members [79]. The program Gepasi [80] was used to simulate the behavior of several different

library types, including A, B, and C from the previous work as well as a new type, dubbed B*, in which all members of the library have a common subunit. Focusing in particular on target-induced adaptation, Severin found once again that equilibrium library distribution did not necessarily correspond to amplification of the tightest binder. However, this situation could be avoided by working with a relatively low concentration of target. Alternatively, Severin also suggested carrying out the selection in a “virtual” mode (in which the concentrations of library monomers outweigh concentrations of assemblies) as an alternative. Such libraries have been reported by Eliseev [81] and Lehn [82] as providing very large amplification factors for selected compounds.

In a pair of papers published in 2004 and 2005, Corbett, Otto, and Sanders described similar theoretical analyses of relatively simple DCLs, as well as the development and testing of a new tool for simulating DCL behavior, appropriately dubbed DCLSim [83]. The authors described the potential for DCL selections to yield something other than the “fittest” binder as the “tendency of DCLs to maximize the binding interactions in the entire library” [84]. As in the cases described by Severin, this tendency could be combated by careful choice of initial library conditions.

In more recent efforts, the Otto group has reported on the development and use of a related program, dubbed DCLFit, for converting experimentally observed product distributions from selection experiments into estimates of binding constants [85]. Experimental analysis of a number of different guest-templated libraries indicated a strong correlation between amplification factors and binding constants, consistent with the predictions of DCLSim and DCLFit [86]. Corbett, Sanders, and Otto have also noted that DCL experiments combined with simulation constitute an intriguing chemical approach to the study of complex systems [87]. We can anticipate that this “systems chemistry” will prove to be a vibrant field of research in the future. In the near term, such simulations are an important component of validating the DCC concept, as well as serving as critical guides to experimental design.

1.7. Conclusions

While the field of DCC is fairly young, it has extensive roots in other areas of endeavor, particularly in “origins of life” research and in self-assembly strategies from the supramolecular chemistry community. DCC has rapidly built an impressive and diverse set of applications, library types, and selection strategies. However, further research into new reversible reactions is

needed in order to expand the range of structural types accessible to DCC. Efforts to simulate the behavior of DCLs are having a clear impact on the design of selection experiments, and we can anticipate that such studies will continue to represent valuable contributions to the field.

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