Heterovalent selectivity and the combinatorial advantage

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Libraries of monovalent compounds can be reacted with each other to give libraries of bivalent ones. If those reactions are efficient, and if the products do not need to be purified, large numbers of bivalent compounds can be produced rapidly, and one might say there is a ''combinatorial advantage'' to doing so. However, selective formation of heterobivalent products must be possible otherwise statistical mixtures will form. This *tutorial review* describes methods that will give heterobivalent compounds almost exclusively. Although there are relatively few methods that will give that desired selectivity, such methods are becoming increasingly important as the potential applications of bi- and multivalent compounds emerge.

1. Introduction

Fig. 1 presents a hypothetical situation of the type that forms the focus of this article. A chemist has prepared five compounds specifically designed for a given purpose (Fig. 1a). In this review, these are referred to as ''monovalent compounds''. The problem is to join these monovalent compounds in all possible combinations to give a library of molecules that here will be called ''bivalent compounds''. If a bivalent molecule consists of two identical fragments, then it is ''homobivalent'', whereas ''heterobivalent'' is the name given here to ones that are formed from different components (Fig. 1b). The bivalent compounds must be formed in a one-compound-per-well format (no mixtures allowed), and the monovalent fragments must be covalently joined together (no dynamic combinatorial libraries) to form the bivalent ones.

The core of the problem is shown in Fig. 1b. It is easy to design chemical methods that allow monovalent compounds to combine into homobivalent ones. However, it is hard to envisage chemistry that allows mixing of two non-identical compounds to form only the heterobivalent product; this review calls that phenomenon ''heterobivalent selectivity''.

Fig. 1c illustrates why heterobivalent selectivity can be useful in combinatorial chemistry. If it can be attained then it would be possible to combine the monovalent molecules in all the possible ways to form a library of bivalent molecules that is much larger than the number of monovalent starting materials. The number of monovalent molecules considered to this point, five, is modest. However, if more monovalent molecules become available then the number of possible bivalent molecules increases dramatically (Fig. 1d). Five monovalent molecules can only give 15 bivalent products, but 100 monovalent molecules could be combined to make 5,050 possible bivalent ones.

In practice there are other stipulations that make heterobivalent selectivity more difficult to achieve. If every bivalent compound must be purified *via*, for instance, HPLC, then the combinatorial advantage is likely to be insignificant relative to the purification time. Consequently, even though the monovalent components may be required to have reactive functionalities, these cannot be protected because removal of

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Kevin Burgess is a professor at Texas A & M University, where he has been since September 1992. His research interest focuses on peptidomimetics for mimicking or disrupting protein–protein interactions, fluorescent dyes for multiplexing in biotechnology, and asymmetric organometallics catalysis. All these projects are pertinent to high throughput and combinatorial chemistry. It was the first project that provided motivation to write this review because we are particularly interested in forming libraries of bivalent peptidomimetics.

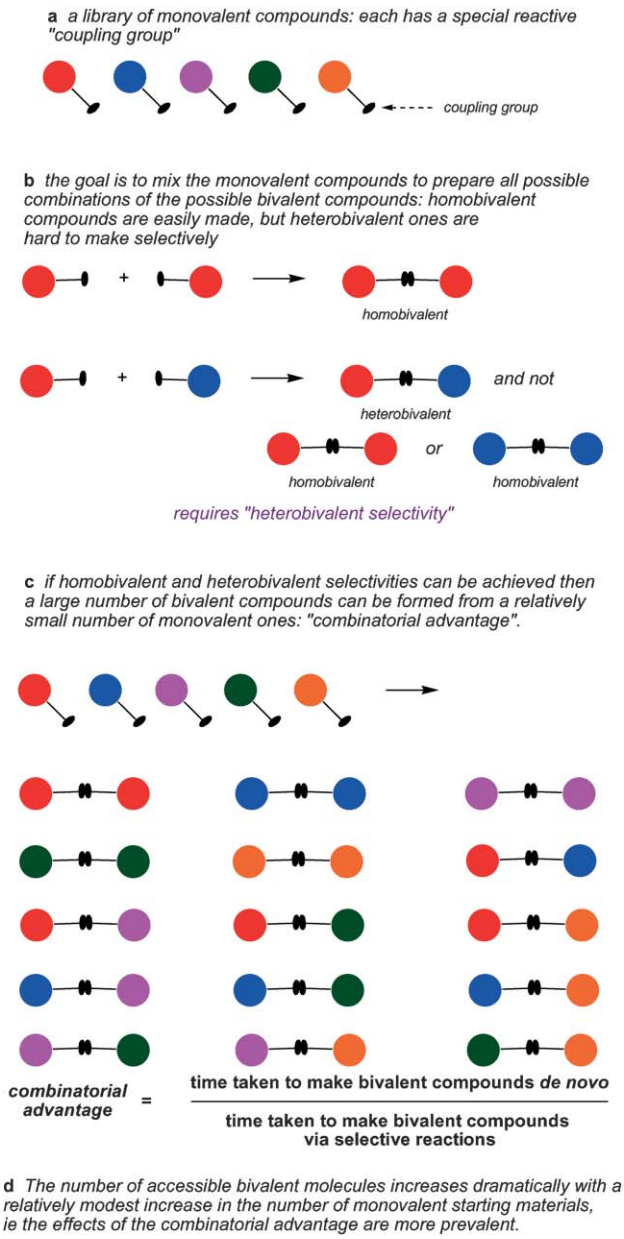


Fig. 1 **a** A library of monovalent molecules, each with a functional group designated for the coupling reaction to form bivalent molecules; **b** forming heterobivalent molecules without contamination from homobivalent ones is hard; **c** heterobivalent selectivity is the key to making libraries of bivalent molecules; and, **d** the impact of the combinatorial advantage of making bivalent molecules increases dramatically with the number of monomers involved.

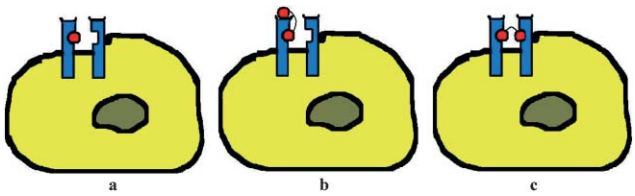


Fig. 2 Monovalent and bivalent ligands (red) binding cell surface receptors (blue). **a** A monovalent molecule might block a protein ligand from dimerizing, then it is likely to be an antagonist; **b** a bivalent molecule that binds two sites on the same receptor is likely to be an even more effective antagonist, because it should have higher affinity; and, **c** a bivalent molecule that spans cell surface receptors could potentially function in the same way as a bivalent protein ligand, *i.e.* it would be an agonist.

reagents for deprotection and protecting group by-products almost invariably necessitates significant purification. Similarly, reagents to bring about couplings can only be tolerated if they give no by-products, otherwise significant purification steps are needed. Thus, what is required are chemical methods that allow selective formation of covalent bonds to form heterobivalent molecules with a high degree of purity on combination, ideally just by mixing aliquots of solutions, so that the crude products can be used/assayed *as is*, *i.e.* without purification.†

The discussion above argues that formation of bivalent compounds is an effective approach in combinatorial chemistry, but are bivalent molecules important compounds to be making? In some cases, the answer is definitely affirmative. For instance, cell surface receptors that are activated by ligand-induced dimerization might interact with bivalent molecules in such a way that two receptors are spanned giving an agonistic effect. Conversely, a bivalent molecule might bind one receptor molecule with relatively high affinity (two enthalpic contributions, and a favorable entropic one) to give molecules that have more potent antagonistic properties than any of the monovalent building blocks (Fig. 2).

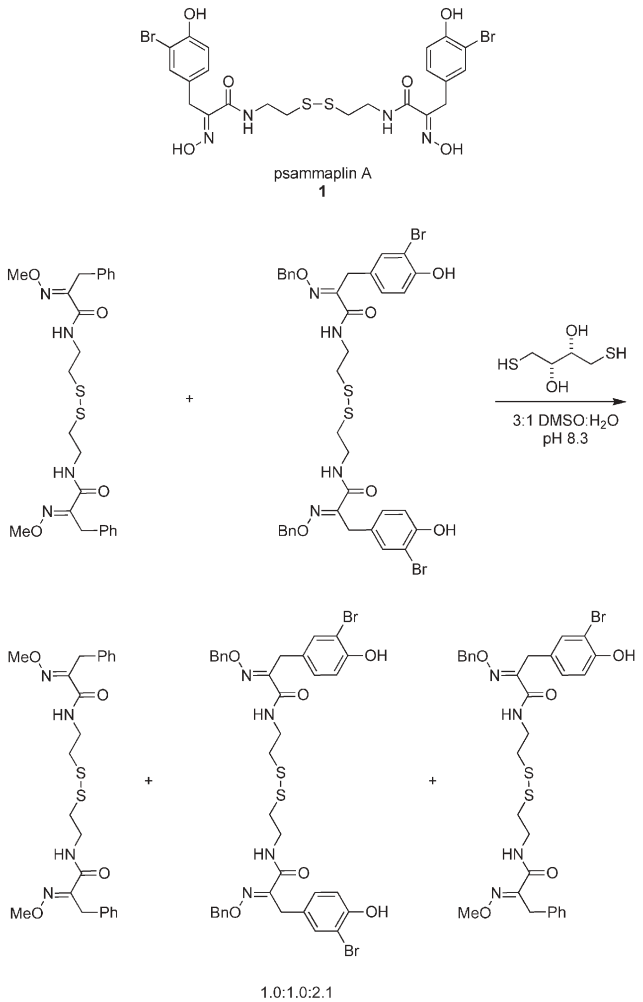
Another application of bivalent molecules is in the formation of potential catalysts. Monovalent molecules might each contain one binding center, and they could be combined to give ones with two, *i.e.* chelating systems. Consequently, bivalent molecules could be valuable in medicinal chemistry,[1,2] pharmacology,[3] and catalysis.

2. Illustrative non-selective methods for formation of bivalent compounds

Developments in this area really built on non-selective methods that give statistical mixtures of homo- and heterobivalent products. These are not our main concern, but a few well-known cases are described here to form a foundation for the discussion of selective methods.

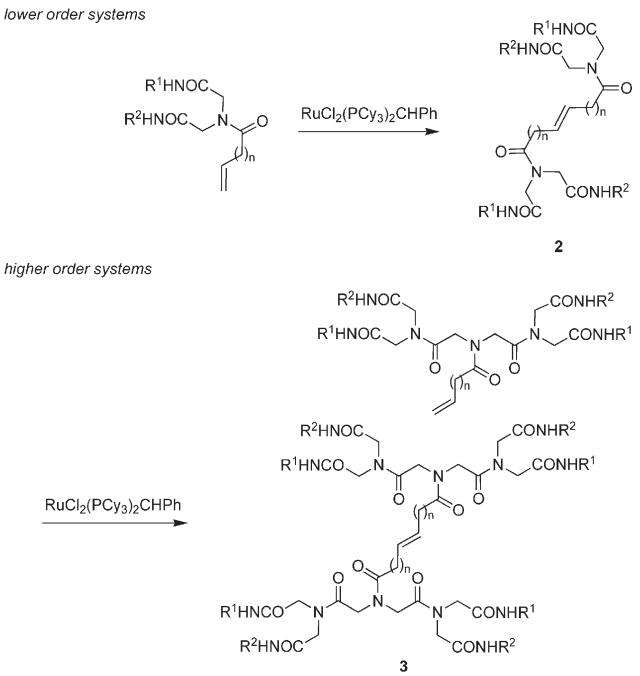
Non-selective methods for assembly of homo- and heterobivalent molecules are most powerful if they are compatible with a broad spectrum of other organic functional groups.

† We stress that the definitions given in this article are designed to be used in the context outlined here. Others may use the words "monovalent", "bivalent", and "combinatorial advantage" to describe other types of compounds and situations.



Scheme 1 Combinatorial disulfide exchange.

This is because monomers with reactive sites can be used without protection. Formation of disulfide bonds is a tried and tested method of this kind. The mild oxidative conditions required are tolerated by most reactive functionalities. Nicolaou and co-workers used "combinatorial disulfide exchange" to prepare analogs of the anti-bacterial compound psammaplin A **1**;[4] Scheme 1 gives an illustrative example. The products tend to form as near statistical mixtures (in favor of the heterobivalent material), thus the method is non-selective. An advantage of the method is that the disulfide starting materials are readily obtained (88 were used in this work), and the chemistry to generate the products is facile. Further, the target upon which this work is based is ideal for this approach because it has a disulfide bond. There are several drawbacks to combinatorial disulfide exchange, however, that may discourage others from using it in cases where the target is less ideal. For instance, each mixture is contaminated with dithiothreitol and its oxidation products. The product mixtures were initially tested without prior isolation of the components. This is a relatively efficient process but the researchers had to rely upon relatively slow purification methods (preparative TLC) to isolate and identify the active component since there is no convenient way to repeat the syntheses to obtain a given disulfide product selectively.



Scheme 2 Syntheses of "multivalent monomeric and dimeric" ligands *via* cross-metathesis reaction.

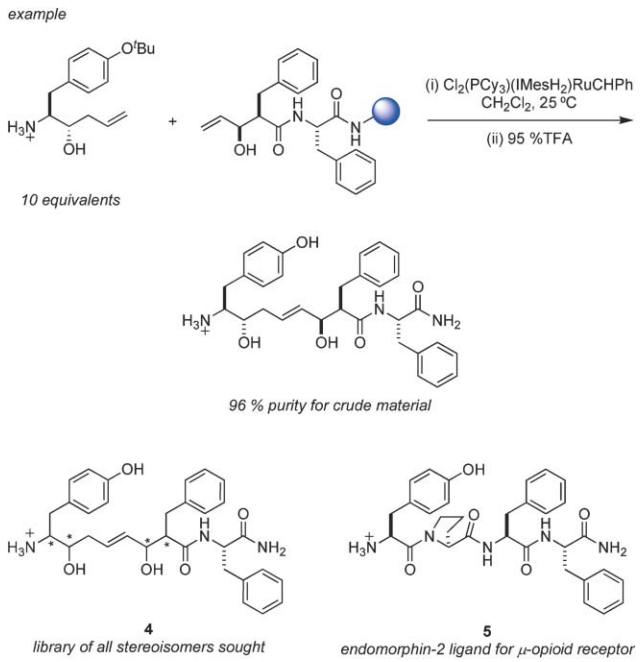
Of course, it is possible to prepare unsymmetrical disulfides *via* differential protection schemes. This has been done to give heterobivalent compounds selectively (for example in work by Spatola),[5] but extensive manipulation of protecting groups is required for this type of approach.

Cross metathesis reactions are also a good choice for non-selective formation of homo- and heterobivalent molecules, since they are compatible with most functional groups. One of the earliest examples of this application was by Boger *et al.*[6,7] They used simple solution phase coupling and extraction methodology to form "multivalent monomers and dimers" like **2** and **3**, as shown in Scheme 2. How one accesses the "valency" of these libraries depends on how that term is applied. Compounds **2** for instance, could be regarded as tetravalent (four amide termini) or bivalent (two combinations of amides containing the R^1 and R^2 functionalities). To attempt to define valency in this particular case may not be productive, but everyone should agree that the compounds **2** are of a lower order than the systems **3**. Both compound types are accessible using similar coupling methodologies. Compounds of these types have been shown to possess interesting biological activities (*e.g.* as mimics of erythropoietin).[8,9]

Other groups have also used alkene metathesis reactions to form homobivalent dimers, but *via* routes that can give no selectivity for heterobivalent systems.[10–14]

3. Cross metathesis for selective formation of heterobivalent compounds

All the methods described above are non-selective, but it is possible to use many reactions to generate heterobivalent molecules *selectively* if one component is anchored on a solid phase at low loading. Verdine and co-workers have done this (Scheme 3) to prepare all stereoisomers of the opiate



Scheme 3 Selective formation of heterodimeric mimics of endomorphin-2 *via* cross-metathesis.

peptidomimetics **4** of the established ligand **5**, *via* an alkene metathesis route.[15]

4. Heterobivalent compounds *via* oxime formation

One way to circumvent the issue of heterobivalent selectivity is to prepare the library of monovalent starting materials twice, giving identical copies except that they have complementary coupling groups (Fig. 3a). This ensures heterobivalent selectivity, but increases, and possibly doubles, the amount of work necessary to obtain the monovalent compounds. More commonly, researchers might take two different libraries with complementary coupling groups and link them together as shown in Fig. 3b. If less monovalent monomers are involved, then the numbers of compounds that can be made are correspondingly less. Both the contributions described in this section feature the approach in Fig. 3b.

Many reactions can give heterobivalent dimers if the starting materials are designed with appropriately reactive groups that can be combined selectively in the presence of other functionalities in the molecule. If that functionality is minimal, or is protected (but this is undesirable, see above), then many reactions can be used to form bivalent compounds selectively. One method of this kind is Ellman's *O*-alkoxyhydroxylamine/carbonyl approach;[16] this relies on the efficiency of oxime formation for syntheses of bivalent compounds. An advantage of using this reaction is that it proceeds in high yields and other reagents are not required to bring about the coupling. The main disadvantage is that unprotected amine and aldehyde/ketone-side chains are unlikely to be compatible. Curiously, though Ellman and co-workers were the first to use this reaction type to combine pharmacophores but they did so only with a symmetrical linker hence statistical mixtures of homo- and heterobivalent compounds were produced (Scheme 4).

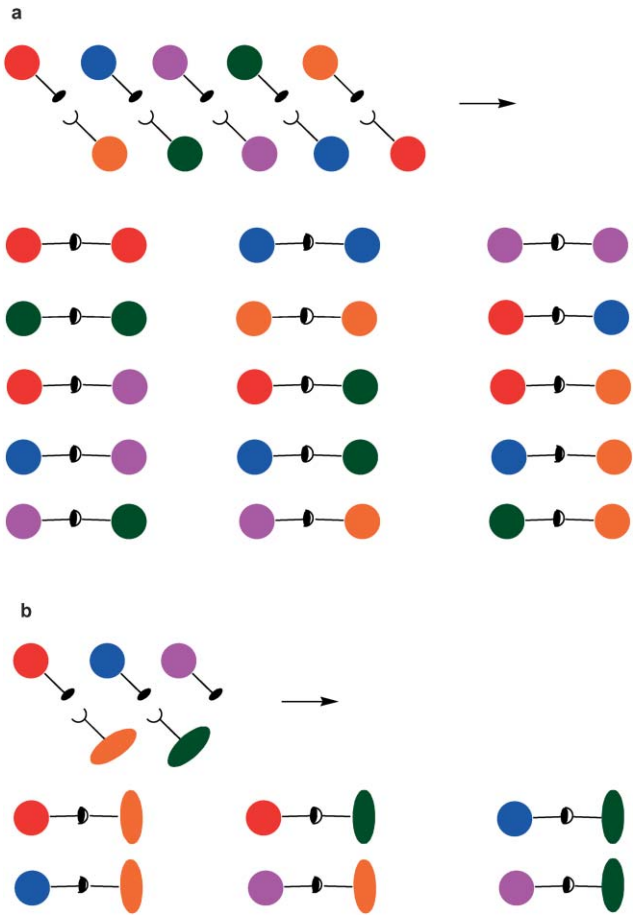
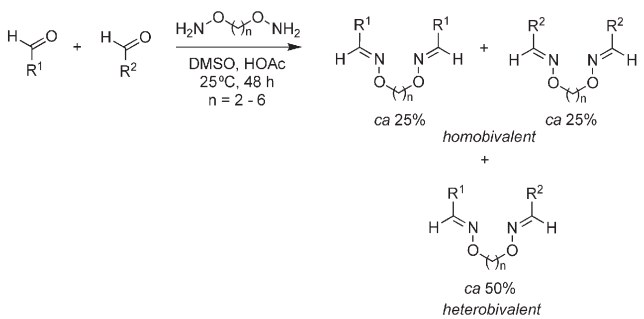
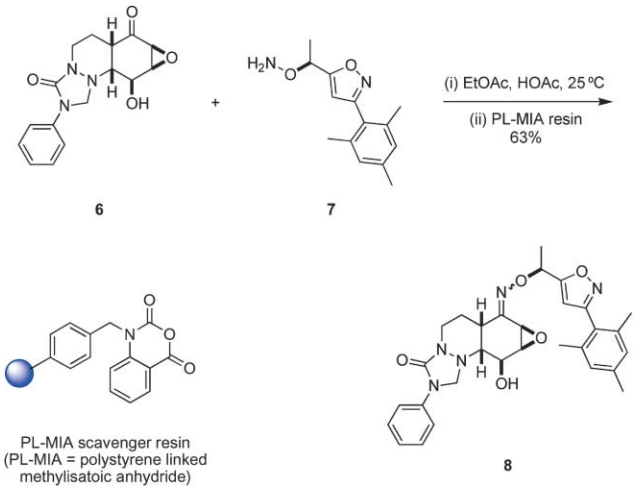


Fig. 3 **a** If each monovalent compound is prepared twice, with a given coupling and with a complementary one, then this is more work but heterobivalent selectivity is not an issue; alternatively, **b** two different libraries, each featuring a complementary coupling group, can be combined.

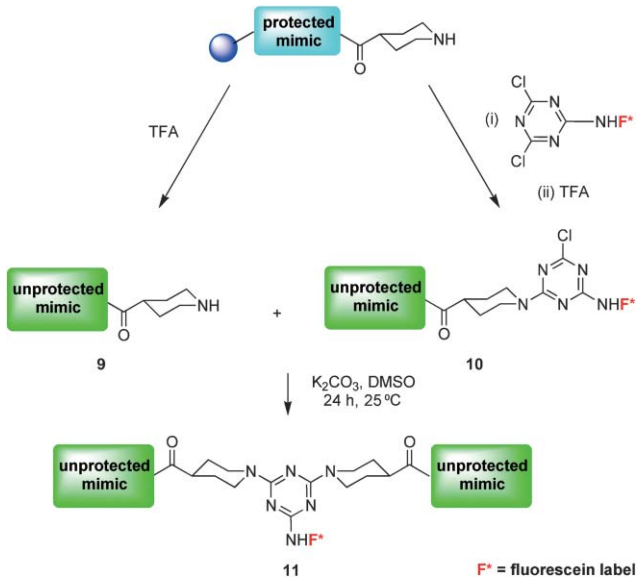
In an approach similar to the one described above, Porco and co-workers used a library of *O*-alkylhydroxylamines to react with a set of aldehydes/ketones.[17] However, unlike the previous work, this strategy necessarily gives heterobivalent compounds. The monomer sets used in this work were considerably more complex than those used in many other library syntheses, and this illustrates the considerable scope for tolerance of other functionalities in the molecules. A restriction of this approach is that the number of accessible combinations



Scheme 4 Ellman's synthesis of a library of oxime-linked homo- and heterodimers.



Scheme 5 Selective formation of heterobivalent dimers using oxime bond formation.



Scheme 6 Synthesis of triazine-linked homo- and heterobivalent peptidomimetics.

is less than in some of the other methods to be considered. This is because in Scheme 5, for example, ketone **6** cannot be combined with other carbonyl-based monomers, only with the reactive amine **7** and others like it (and *vice versa*). Secondly, excess of the hydroxylamine component was used to drive the reactions to completion; this necessitated use of a scavenger resin to remove the unreacted nucleophile, and some fraction of this component is lost in each reaction. Further, the composition of the library is complicated by *cis*:*trans* isomers about the oxime functionality.

5. Selective formation of heterobivalent compounds *via* triazine chemistry

The concept to selective formation of bivalent molecules that was developed in our laboratories is illustrated in Fig. 4. A linker scaffold is to be used to couple two monovalent fragments together. Selectivity can be achieved if the rate of addition of the linker scaffold with the first monovalent compound is significantly greater than the rate of addition of the second. Conceptually, this is similar to the route described in Fig. 3a. It is potentially efficient because the samples of each of the monovalent molecules need only be modified in the same way, *via* one step, to achieve the desired heterovalent selectivity. Complementary sets of monovalent molecules do not have to be synthesized *de novo* to facilitate construction of the bivalent libraries.

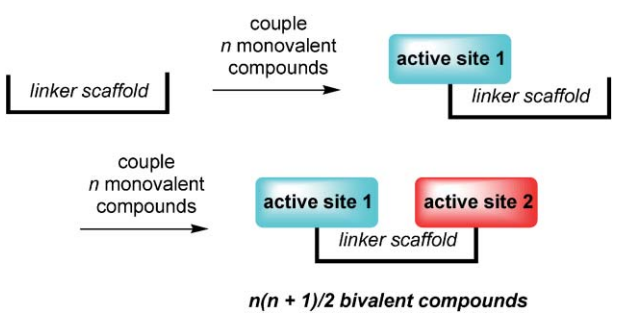


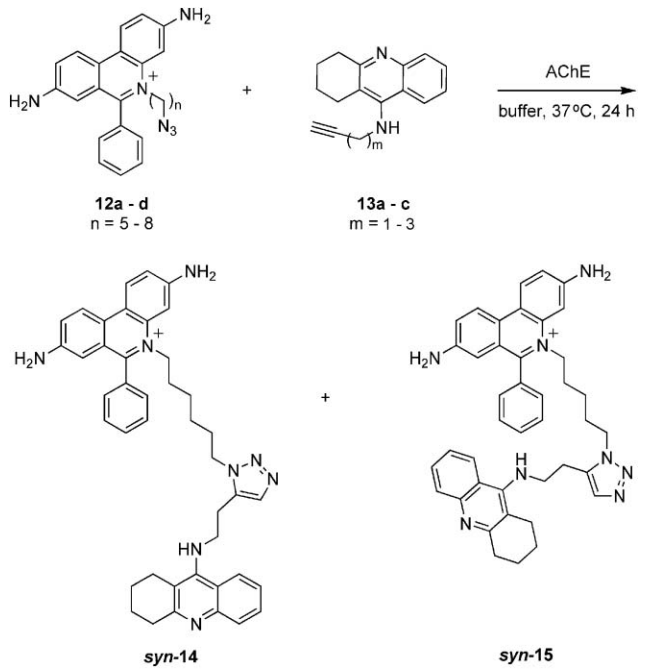
Fig. 4 Generation of bivalent compounds using stepwise additions to a linker molecule. This linker must add one monovalent compound relatively quickly, then another at a slower rate.

This method was put into practice *via* the route shown in Scheme 6.[18] It comprises of a synthesis (here on solid phase) of a monomer with a reactive piperidine functionality; the choice of this nucleophile was critical. Each sample in the library was divided into two. Half was reacted with a dichlorotriazine, effectively converting this nucleophilic component into a weak electrophile. These samples were then combined in solution with the nucleophilic components to give the bivalent molecules. This strategy has several advantages. First, *unprotected* monovalent components can be used in the coupling, even for reactive side chains such as the Lys amine, Arg guanidine, Ser alcohol, and Tyr phenol. Second, the monovalent components can be combined in all permutations. Third, the electrophilic carbon on the triazine fragment can be used to support another group of interest (a fluorescein label in Scheme 6). A disadvantage of this approach is that the linker region is currently restricted to triazine fragments: no others have been reported to date.

6. Selective production of heterobivalent compounds *via* 2 + 3 cycloadditions

A conspicuously different strategy to make heterobivalent compounds was devised in the Sharpless/Finn/Kolb lab. They used two monomer sets, one group of terminal alkynes and another consisting of azides, and set out to combine them *via* 2 + 3 cycloadditions. Just as in Ellmans' and Porco's oxime-based methodology, the two monomer sets used were distinct and different, so this approach is conceptually represented by Fig. 3b: it is not ideal for construction of very big libraries.

The feature that sets this work apart from all the others mentioned in this review is that the reactions were done in the presence of the target enzyme (acetylcholinesterase {AChE}[19–21]



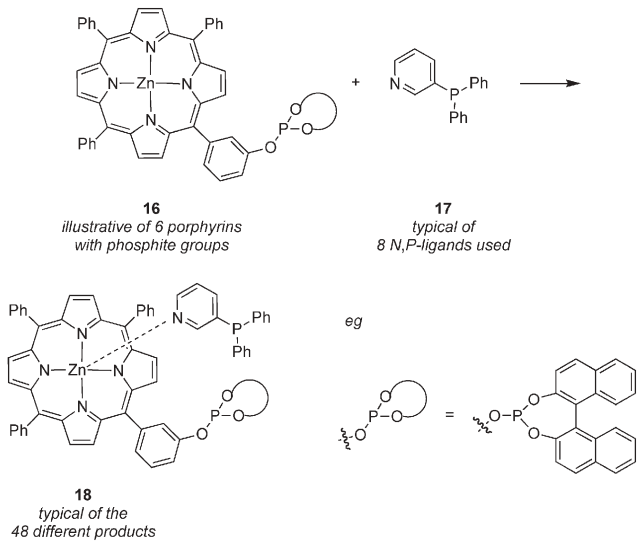
Scheme 7 *In situ* multicomponent 2 + 3 enzyme-assisted cycloaddition.

and carbonic anhydrase[22]). Only combinations of monovalent components that could be simultaneously incorporated into an enzyme deep cavity would be forced into close proximity. In observations that many would find surprising, only such combinations appear to react. Thus, the approach is combinatorial in the spirit of potentially bringing together large numbers of monovalent molecules to form bivalent ones, but it is economical insofar as only the interesting bivalent compounds are actually formed. For instance, 12 *syn*- and 12 *anti*-products could have been generated in the particular reaction shown in Scheme 7, but, in fact, only two were detected by LC-MS,[20] and both were shown to have significant dissociation constants for binding the enzyme.

All the experiments performed using enzyme templates to facilitate syntheses of bivalent compounds that bind the enzyme are "proof of concept" situations. In the earliest experiments, both the monovalent fragments were known to bind the enzyme. Those experiments really established which of the linker fragments would *not* allow the two monovalent molecules to bind simultaneously. In later cases, one monovalent component that was previously known to bind was combined with other fragments that were not.[21] This methodology has never been reported to work for two libraries wherein both monovalent fragments are not derived from small molecules that were known to bind.

7. Production of heterobivalent metal–ligand libraries

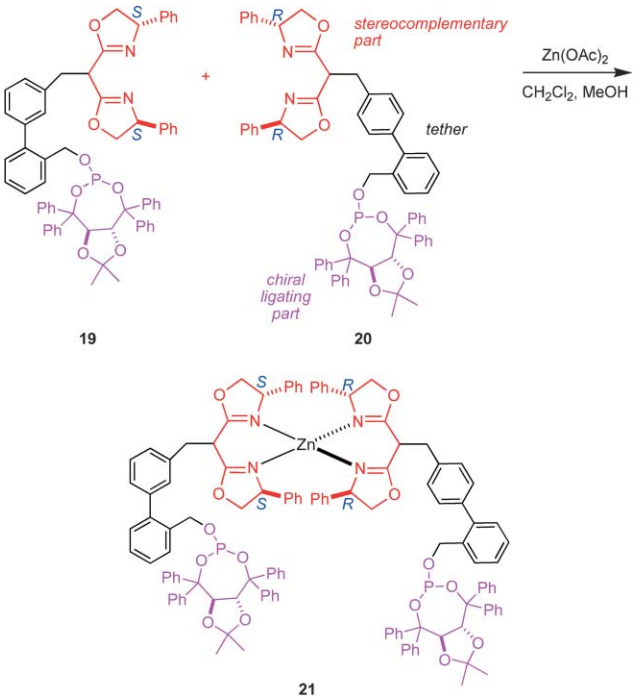
Formation of libraries of ligands as a basis for discovery of new homogeneous transition metal catalysts is not an easy task, but it can sometimes be facilitated by selective syntheses of heterobivalent entities. Reek and co-workers have pioneered



Scheme 8 Bivalent supramolecular ligands formed by selective *N*-to-*Zn* coordination.

a way to do this using zinc porphyrin derivatives mixed with pyridyl- or imidazoylphosphines, *e.g.* **16** and **17** in Scheme 8.[23] The pivotal feature of this approach is that the zinc atom in porphyrins like **16** has a much higher affinity for the *N*-donor site of the pyridine in **17** than for either of the *P*-donor sites. Mixing the two components therefore causes them to self assemble into coordination complexes that bring the two *P*-donor sites together into a single molecule. Consequently, large molecules like **18** were formed, all having proximal, non-coordinated *P*-donor sites that are capable of acting as bidentate ligands. This approach has been used, for instance, to complex rhodium for hydroformylation catalysts.[23–25]

The strategy described in Scheme 8 exploits the combinatorial advantage of forming bivalent ligands (though not to the maximum since the components in each sub-library cannot be combined) but it still is reasonably labor intensive. If the bivalent molecules prepared were also optically active, and have reasonable structures for asymmetric catalysis, then the effort expended in preparing the monomer fragments would be further justified. Work by Takacs *et al* does just that: it uses self-assembly on coordination to give optically active bivalent ligands. The centerpiece of this approach is extremely elegant. Mirror image forms of the same bidentate bisoxazoline ligand were tethered to different optically pure *P*-donor fragments, *e.g.* **19** and **20** (Scheme 9). Heterobivalent ligands are formed selectively when aliquots of these are mixed in the presence of Zn, because the stereocomplementary nature of the bisoxazolines drives them to come together like two people holding hands: left hand clasping right, not right in right or left in left. The bidentate ligands like **21** formed in this process were tested in a palladium-mediated allylation reaction. Fifty ligands were made and tested. Remarkably, when the enantioselectivities were plotted in ascending order, a near linear plot was obtained indicating very subtle changes in the ligand structure have marked effects on the stereoselectivity. It is also interesting that the optimal ligand found (**21**) is not a symmetrical system, but a heterobivalent one. Homobivalent

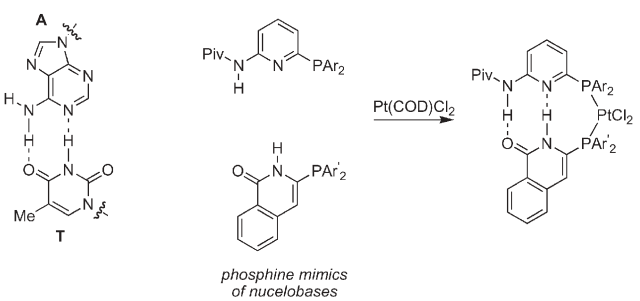


Scheme 9 Stereocomplementarity used to form heterobivalent ligands.

ligands formed from either component were significantly less effective.[26]

Two other areas of organometallic research may be considered to involve selective formation of heterobivalent systems, but only when loosely defined. For instance, zinc complexes have been formed that simultaneously coordinate one of a small library of bidentate *O,O*-alkoxide ligands, and another component which is an *N,N*-diimine ligand; these were then used to mediate addition of diethyl zinc to aldehydes.[27] However, this is more accurately described as selective formation of heteroleptic complexes rather than selectivity for heterovalent molecules, and this situation is ubiquitous in organometallic chemistry. The second contribution involves assembly of bivalent molecules by hydrogen bonds to give bidentate phosphine ligands (Scheme 10).[28] These ligands, however, are not covalently bonded, so they are, strictly speaking, beyond the scope of this article.

All three approaches outlined in this section involve combining *different* monomer sets, hence they are of the less



Scheme 10 H-Bonding to drive selective self-assembly of bidentate ligands.

efficient type summarized in Fig. 3b. If libraries of complexes are formed *via* reversible heterovalent selectivity, then these are dynamic combinatorial reactions and are beyond the remit of this review. However, the chemistry described in this section is probably essentially irreversible because homobivalent forms are disfavored, and we feel it is relevant.

8. Conclusions

Chemists tend to divide "selectivity" into regio-, chemo-, diastereo-, and/or enantio-forms. However, in combinatorial chemistry there is a parameter that does not fit any of these terms well. Here we call it *heterovalent selectivity*. There are probably more examples of selective formation of heterovalent compounds than the ones we have collected here, but it is extremely difficult to be comprehensive. This is because the chemical community has not, until recently, appreciated why this form of selectivity could be important, hence papers simply do not highlight reactions that could be used for selective formation of heterovalent molecules. However, the *combinatorial advantage* of rapidly assembling libraries of heterovalent molecules is real, and their applications are widespread. Heterovalent selectivity is an area that may therefore become a more topical issue in the future.

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