4

NEW ADVANCES IN DIVERSITY-ORIENTED SYNTHESIS

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4.1 INTRODUCTION: SMALL MOLECULES AND BIOLOGY

Humanity has a significant dependence on small molecular mass chemical entities, so-called small molecules [1]. The use of small molecules to selectively modulate biological systems represents the basis for medicinal chemistry (wherein molecules are used to chemically modify disease states) and underpins the field of chemical biology (wherein molecules are used as "probes" to investigate biological processes) [1–3]. However, the discovery of new biologically active small molecules represents a significant challenge [4]. In cases where modulators of a specific biological molecule or family of molecules are sought, it is often possible to rationally design (or rationally select) small molecule binding partners using information about the structure of the target or a known native ligand [2, 4–6]. However, there are many situations in which a "rational" design or selection process is not appropriate, desired, or indeed possible. For example, it may be the case that the precise nature of the biological target is unknown (e.g., a phenotypic screen) or that no natural ligands have been identified or that a novel mode of binding to a particular target is sought [2, 4–6]. Such scenarios necessitate a different approach, namely, the screening of collections (libraries) of small molecules in order to identify those with the desired properties [4, 7]. Clearly, the molecular composition of such libraries (i.e., the nature of the compounds present) is a crucial consideration [2, 4, 8].

Broadly speaking, the molecules comprising such screening collections may be obtained from two main sources: natural (i.e., natural products) or nonnatural (chemical synthesis) [4]. Nature undoubtedly represents an extremely important source of biologically active

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small molecules [9, 10]. Natural products have been used medicinally for millennia and still provide many lead compounds and drugs today [4, 11]. However, there are well-documented drawbacks associated with the use of screening libraries consisting solely of natural products [2, 9, 10, 12]. Thus, deliberate chemical synthesis represents an important alternative source of small molecule screening libraries [2, 4].

4.2 THE NEED FOR STRUCTURAL DIVERSITY IN SYNTHETIC SMALL MOLECULE SCREENING COLLECTIONS

Between the late 1980s and mid-1990s, a "brute-force" approach toward small molecule library synthesis was typically adopted, enabled by the development of combinatorial chemistry-type methods that allow extremely large compound collections (literally millions of compounds in some cases) to be efficiently produced [6]. Such libraries could then be rapidly screened for biological activity using high-throughput methods. It was assumed that such collections would yield a plethora of drug leads, simply as a consequence of the sheer volume of molecules examined [6]. However, the expected surge in productivity did not materialize; indeed, libraries of this sort have had limited success in the discovery of new small molecules with useful biological activity [13]. This disappointing degree of productivity has been largely ascribed to the relative lack of structural diversity within the libraries, that is, a lack of structural variation between the compounds [6, 14]. Put simply, these libraries were comprised of very large numbers of molecules, but they all had very similar structures. Why should this be an issue? Biological macromolecules are, on a molecule scale, large three-dimensional (3D) environments with certain defined potential binding regions [4]. A given biological macromolecule will therefore only interact with those small molecules that have a complementary 3D binding surface; that is, nature "sees" small molecules as 3D surfaces of chemical information [2, 4, 15, 16]. Thus, the biological activity of any given small molecule is intrinsically related to its 3D shape [2, 17]. This in turn is directly controlled by its molecular structure [2, 6]. The more structural diversity within a small molecule library, the greater the range of distinct molecular shapes present; consequently, the library as a whole should display a broader range of biological activities [2, 17, 18]. It is now widely acknowledged that the structural diversity of a small molecule library plays a crucial role in determining its success (in terms of the hit frequency) in any screening process [2, 19, 20]. Indeed, the overall functional (biological) diversity of a library is directly correlated with its structural diversity, which in turn is proportional to the amount of chemical space that the library accesses [2, 13, 21]. Clearly, high functional diversity is of value in "unbiased" screens where the precise nature of the biological target is unknown (e.g., a phenotypic screen) [2]. Furthermore, the presence of multiple structural classes within a molecular library that is being screened against a single target also increases the probability of discovering a molecule capable of binding to that target in a novel (previously undefined) fashion [4, 22].

There are four principal components of structural diversity typically identified in the literature [2]: (i) appendage (building-block) diversity, variation in structural moieties around a common scaffold; (ii) functional group diversity, variation in the functional groups present; (iii) stereochemical diversity, variation in the orientation of potential macromolecule-interacting elements; and (iv) scaffold (skeletal) diversity, presence of

many distinct molecular scaffolds (scaffold in this context can be taken to mean the core rigidifying structural feature of a molecule).

Of these, it is the scaffold diversity that has the largest impact upon library functional diversity [2]. The shape space coverage of any compound set stems mainly from the nature and geometries of the central scaffolds (with the peripheral substituents being of minor importance). Substantial shape space coverage (i.e., a high level of molecular shape diversity) is known to be correlated with broad biological activity [2, 17, 18]. Indeed, small multiple-scaffold libraries are widely regarded as being superior to large single-scaffold libraries in terms of biorelevant diversity [2, 17, 18]. The scaffold diversity of a library can be considered as a surrogate measure for shape diversity and thus overall functional diversity [2].

Structural complexity is also generally regarded as a desirable characteristic in the small molecules comprising screening collections. It has been argued that structurally complex molecules are more likely to exhibit selective interactions with biological macromolecules [2, 23].

The combinatorial-type libraries of the late 1980s to mid-1990s were typically generated by combining a number of building blocks in different ways, using the same synthetic methods [4]. This approach usually resulted in the variation of substituents around a common scaffold; thus, such libraries typically possessed low levels of scaffold diversity, which goes someway to explaining their aforementioned poor performances in many biological screens [4, 13]. Recent years have witnessed a growing realization of the importance of structural (scaffold) diversity in small molecule screening libraries [2]. However, a lack of scaffold diversity is still apparent in many of the synthetic collections that are commercially available or employed by pharmaceutical companies [24]. For example, it was recently reported that 83% of the core ring scaffolds found in natural products are unrepresented among commercially available compound collections [25]. The continuing decline in drug discovery successes can be attributed, at least in part, to this deficiency in current compound collections [2, 6, 13]. Overall, the universe of organic chemistry is dominated by an astonishingly small percentage of molecular scaffolds [6, 24]. An illustration of this point is provided by a recent study of all known cyclic molecules, which showed that a large percentage of the compounds were represented by only a small percentage of scaffolds (0.25% of the molecular scaffolds were found in 50% of known compounds) [26]. This scenario demonstrates a clear need for novel or atypical molecular scaffolds in small molecule screening libraries [24].

The lack of scaffold diversity, and thus functional diversity, in many typical screening sets can be partly attributed to the nature of medicinal chemistry research over the course of the last few decades, which has typically focused upon a limited set of biological targets [6]. Indeed, approximately 29% of all drugs are enzyme modulators, and nearly 36% act upon G-protein-coupled receptors [19, 27]. Consequently, typical commercially available and proprietary compound libraries are predominately comprised of compounds that satisfy predefined criteria for the modulation of such "traditional" targets (e.g., the Lipinski "rule of 5" criteria for orally bioavailable drugs) [2, 22, 28], that is, what can be regarded as traditional "drug-like" molecules, which are typically relatively "flat" and structurally simple, being based around aromatic core structures and containing a high proportion of sp^2-hybridized carbon atoms and few stereogenic centers [23, 29, 30]. Unsurprisingly, libraries of this sort are heavily weighted toward biologically active chemical space (i.e., the chemical space accessed by known biologically active

molecules) [2]. By definition, this is a fruitful region of chemical space for the discovery of biologically useful molecules. However, there are large swathes of potentially biologically relevant chemical space underexplored by such libraries [2, 13]. Compounds in such areas may display activity against a chosen biological target through novel, even unexpected modes of action [2, 13]. Furthermore, the exploration of uncharted regions of chemical space is vitally important in the context of intellectual property considerations and patentability, as it is likely that the low-hanging fruit within the boundaries of known bioactive chemical space have already been picked [2, 31].

Another drawback associated with the aforementioned structural bias in typical synthetic small molecule screening sets is their lack of efficacy against "underexploited" drug targets. Overall, there are only approximately 500 distinct targets of the current pharmacopoeia [29, 32]. It is widely acknowledged that this represents only a tiny fraction of the potential targets that could impact on human health [2, 6, 29]. There are many biological molecules and processes that bear little resemblance to the molecular drug targets exploited in present-day drug therapy (examples include transcription factors and processes such as protein–protein interactions and protein–DNA interactions). These targets and processes have historically been considered "undruggable," that is, considered difficult, if not impossible, to modulate using small molecules [2, 6, 29, 33]. However, recent years have witnessed a growing realization that such targets and processes are indeed tractable to small molecule modulation; it is simply that the candidate molecules comprising typical commercially available and proprietary compound collections are not well suited to the task (i.e., they lack the necessary structural features) [6, 25, 29, 33]. Many of these classically "undruggable" biological macromolecules and processes are now validated targets for the treatment of a variety of human diseases, offering exciting (and largely untapped) therapeutic potential [2, 6, 29, 33]. However, in order to address these targets, new small molecule collections that span regions of bioactive chemical space not accessed by more "traditional" compound libraries are required [2, 6].

4.3 DIVERSITY-ORIENTED SYNTHESIS OF NEW STRUCTURALLY DIVERSE COMPOUND COLLECTIONS

4.3.1 General Principles of Diversity-Oriented Synthesis

Diversity-oriented synthesis (DOS) can be considered as a concept (or indeed a guiding principle [34]) in small molecule library synthesis that was born out of a desire to address the problems associated with the use of natural products and "traditional" commercially available compound collections in biological screens [2]. The aim of a DOS campaign is to deliberately generate a structurally, and thus functionally, diverse collection of structurally complex small molecules that efficiently interrogates wide regions of biologically active chemical space (i.e., with a relatively small number of total compounds per library), including known and previously "untapped" areas [2]. The molecules comprising such collections should ideally be nonnatural (i.e., structurally novel), but natural product-like in terms of their capability to interact with, and thus modulate biological systems (i.e., they should be structurally complex and rich in functionality and 3D structural features, with a higher level of saturation compared to more "typical" drug-like compounds) [2, 23, 35, 36]. DOS libraries are usually created without a

particular target in mind. Instead, collections of small molecules with disparate biological properties are desired; in principle, the screening of such a collection should provide hits against a broad range of biological targets with increased frequency and decreased cost relative to less diverse libraries [8, 29, 34, 36, 37].

The term DOS was first used by Schreiber and coworkers in two seminal publications in 2000 [36, 37]. These reports highlighted the potential value of small molecule libraries with high levels of structural diversity and used the term "DOS" to describe the concept of synthesizing such collections in a deliberate fashion. However, it is reasonable to argue that many of the ideas behind DOS existed for some time before these reports [4, 8]. In 1997, Spaller et al. published a review article [38] in which they suggested that combinatorial libraries may be considered to fall into two classes: (i) "focused" libraries, where a number of similar compounds each based on the same privileged structure are generated with a specific, known biological target in mind; and (ii) "prospecting" libraries, where an entirely novel lead compound is desired, so the aim is to prepare and screen a large number of structurally varied molecules in an attempt to find a lead with a novel mode of action [4, 8]. The syntheses of these so-called "prospecting" libraries can conceivably be considered to be early examples of DOS, in that the goal was to achieve high levels of structural diversity and thus bioactive chemical space coverage, as is the case in contemporary DOS campaigns [4, 8]. However, it was not until the term "DOS" was first coined in 2000 that the strategies and ideas underpinning modern diversity-driven synthesis began to become more formalized, and the field of DOS has evolved rapidly since [4, 8].

4.3.2 Achieving Structural Diversity: The Importance of Scaffold Diversity

Traditionally, DOS campaigns have sought to address the four principal types of structural diversity mentioned previously [2]. As alluded to earlier, one can consider there to be a hierarchy within these diversity aspects that is based on both their relative perceived "value" and also synthetic tractability [4]. Appendage diversity in a compound set is generally accepted as the easiest to achieve but is of the least importance in terms of generating functional diversity across the compound set [4]. On the other hand, it is widely accepted that the incorporation of scaffold diversity into a compound collection is the most important in terms of producing functional diversity (vide supra) [2, 4]. Indeed, there are many examples of DOS strategies that focus almost entirely upon the generation of diverse molecular scaffolds [4, 39, 40]. The efficient generation of multiple molecular scaffolds in a DOS campaign is generally viewed as crucial in order to achieve high levels of library structural diversity (and thus large chemical space coverage) in an *efficient* manner (i.e., with the fewest number of molecules) [2]. However, this presents a formidable synthetic challenge.

4.3.3 Synthetic Principles in DOS

Broadly speaking, there are two basic approaches for the generation of scaffold diversity in a DOS context (Fig. 4.1). The first is a "branching" approach, where divergent reactions are carried out on a substrate to afford a number of different compounds with distinct molecular scaffolds. The second is a "folding" approach, where strategically positioned functional groups are reacted together ("paired") in an intramolecular fashion and so "fold" a substrate into distinct molecular scaffolds. This approach typically involves the

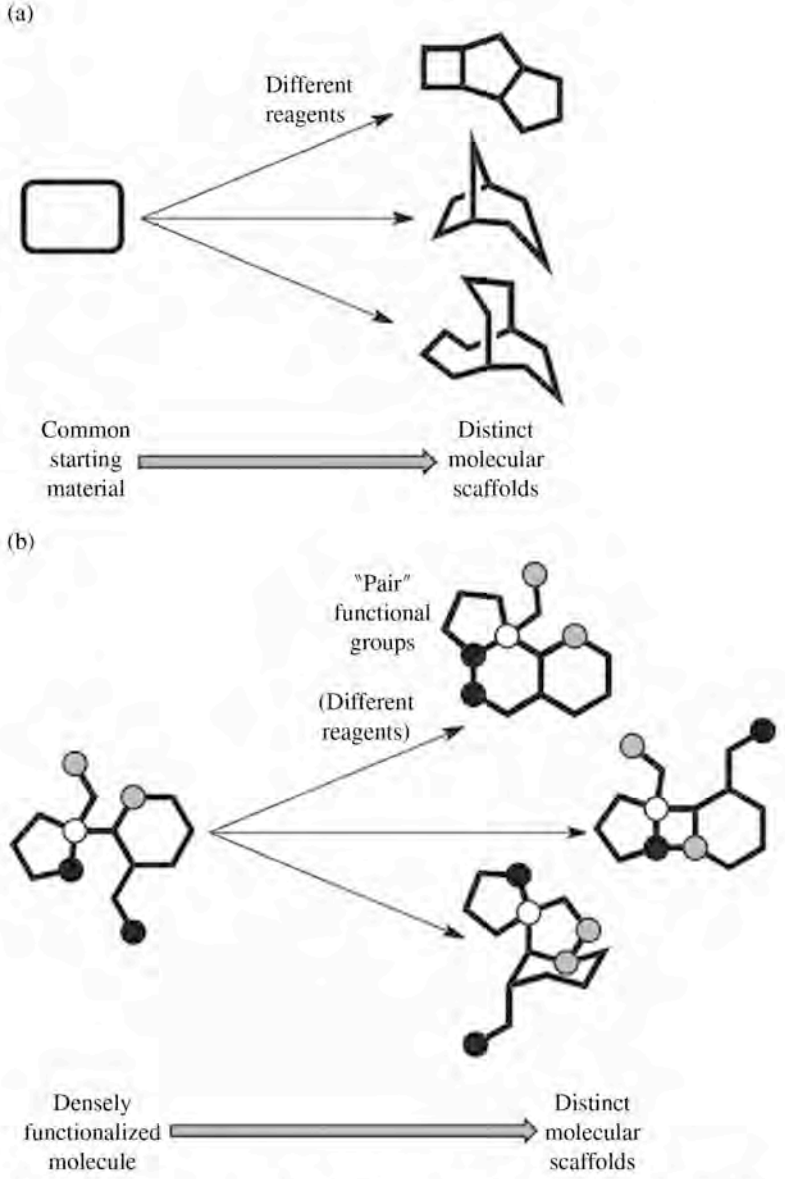


FIGURE 4.1 General approaches for generating scaffold diversity in DOS. (a) The "branching" approach. (b) The "folding" approach.

use of either a densely functionalized molecule, where different functional groups can be reacted together under different reaction conditions to yield multiple scaffolds from one substrate, or different starting materials and common reaction conditions, such that each starting material will furnish a product based around a different scaffold. These two approaches toward scaffold diversification are not orthogonal, and most modern DOS strategies will incorporate aspects of both [2, 4, 19]. Other diversity aspects (appendage, functional group, and stereochemical) can be introduced into the compound libraries

through variation in the starting materials and/or reagents used. Stereochemical diversity can also be incorporated if new stereocenters are created in any reactions [2, 35].

In 2008, Nielsen and Schreiber identified a common synthetic algorithm that was present in many DOS pathways, referred to as the build/couple/pair (B/C/P) three-phase strategy (Fig. 4.2) [31]. The "build" phase involves the synthesis of starting materials (or building blocks), which are ideally chiral. These building blocks are then coupled together in the "pair" phase to produce densely functionalized molecules; this process provides the basis for the introduction of stereochemical diversity. The "pair" stage is a folding-type process that provides the basis for the introduction of scaffold diversity; intramolecular reactions that join ("pair") functional groups incorporated in the "build" phase are performed, usually under reagent control, to generate diverse molecular scaffolds. The B/C/P algorithm subsequently found widespread application in a DOS context and has been used (often identified explicitly) as the basis for a large number of DOS pathways; indeed, it is arguably the most commonly used synthetic strategy in DOS.

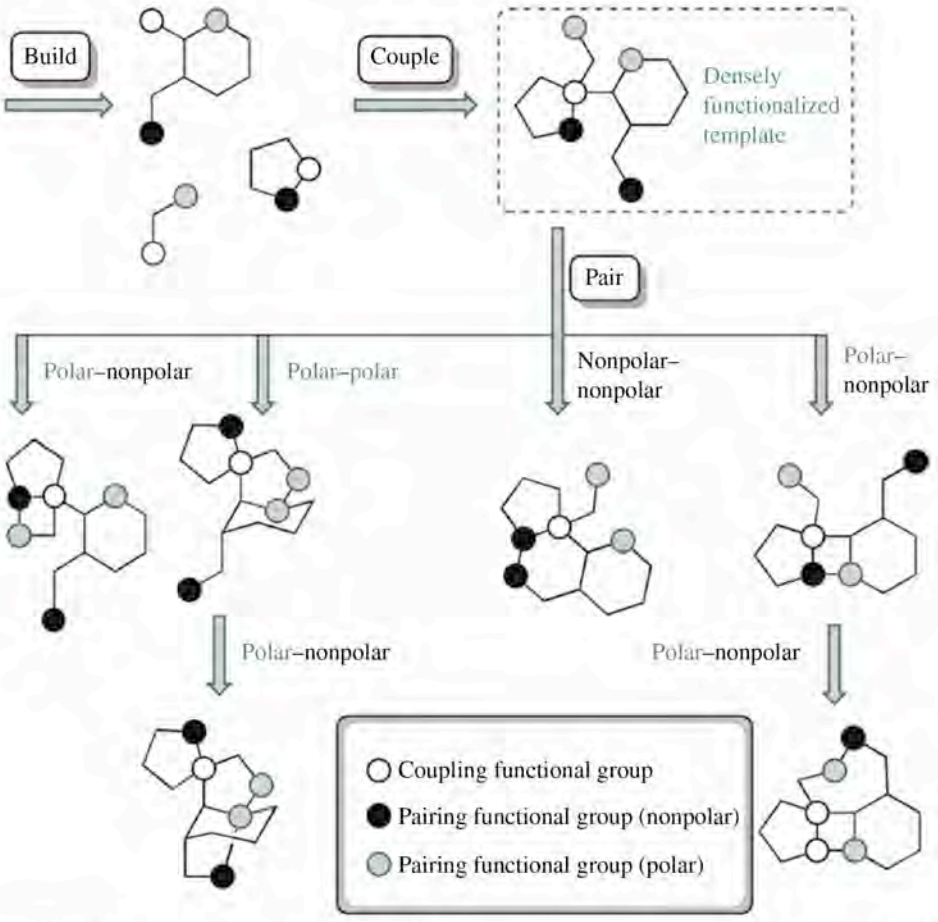


FIGURE 4.2 The build/couple/pair (B/C/P) synthetic algorithm, a common feature in DOS pathways.

4.3.4 Scaffold Diversity and Molecular Type

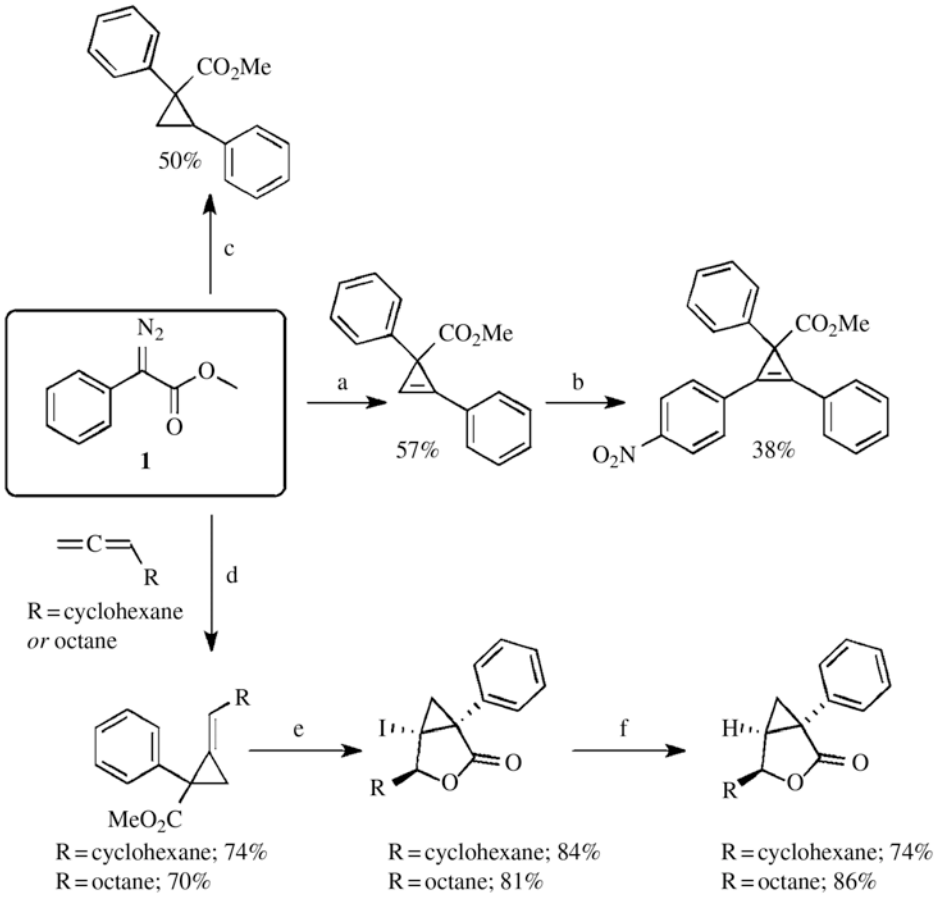
In a broad sense, most DOS programs can be classified into two categories: (i) "undirected" approaches, which do not target a particular "class" of molecule, or (ii) "directed" approaches, which do target a predefined "class" of molecule (e.g., macrocycles) and thus generate libraries composed of molecules that typically contain certain structural motifs or features (e.g., a ring structure of 12 or more atoms in the case of macrocycles). DOS campaigns that fall into this latter category can arguably be described as being slightly more "focused" in nature, but nevertheless, the generation of high levels of diversity (*including* scaffold) is still sought.

In the remainder of this chapter, recent representative examples of DOS campaigns from each of these categories are discussed, with examples taken from our own research and from the wider community. These examples illustrate the current state of the art in DOS.

4.3.5 Examples of DOS Campaigns

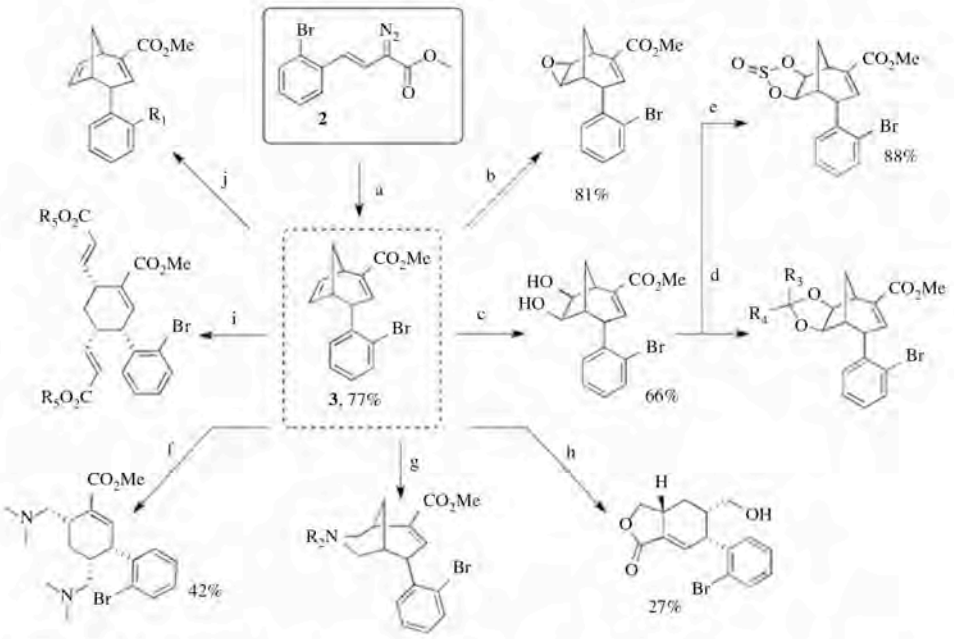
4.3.5.1 "Undirected" DOS Diazoacetates represent very attractive starting units for branching DOS pathways due to their enormous synthetic versatility [35, 41]. A wide variety of different synthetic transformations can be carried out on diazoacetates to yield scaffold-diverse products, which are themselves suitable for further diversification [35]. Ibbeson et al. have recently reported two new branching DOS pathways (Schemes 4.1 and 4.2), each of which utilized a different, readily accessible phenyl diazoacetate compound (**1** or **2**, respectively) as a starting material [42]. The second pathway (Scheme 4.2) used the highly functionalized derivative **3** as a key branch-point intermediate, whose molecular scaffold was modified in a regioselective and multidirectional fashion. The products from both pathways were combined to yield a library of 35 structurally diverse and complex small molecules based around 10 distinct molecular scaffolds. The DOS compounds displayed a high sp^3 content; indeed, computational analyses indicated that the DOS library had a good level of shape diversity, with scaffolds that are drug-like but are more 3D in character than drugs. This is notable, as the lack of three-dimensionality (low sp^3 carbon count) is considered to be one of the major factors why typical small molecule screening collections have performed so poorly when screened against challenging targets such as protein–protein interactions [23, 42].

The DOS library compounds were screened for antimitotic activity against human osteosarcoma cells (U2OS line). The most potent compound was found to be **4**, which gave a large mitotic arrest. The partially saturated analogue of this hit, **5**, was then prepared in a racemic form and found to have a twofold greater antimitotic activity against U2OS cells (Fig. 4.3). Treatment of U2OS cells with compound **5**, which was subsequently termed "dosabulin," also resulted in growth inhibition in the low micromolar range over a time period of 72 h. It was later determined that the biological activity of dosabulin resided in the (*S*)-enantiomer; it was found that cells treated with (*S*)-dosabulin died through apoptosis, while cells treated with (*R*)-dosabulin did not. Further biological studies indicate that (*S*)-dosabulin causes mitotic arrest by the depolymerization of microtubules. The compound was shown to not significantly affect vinblastine binding to tubulin; instead, evidence suggests binding to a site on tubulin vicinal or allosteric to colchicine [42].



SCHEME 4.1 DOS library subset generated from compound **1**. Conditions: (a) phenylacetylene, $Rh_2(OAc)_4$ (1 mol%), CH_2Cl_2; (b) *p*-nitroiodobenzene, $Pd(OAc)_2$ (10 mol%), K_2CO_3, DMF; (c) styrene, $Rh_2(OAc)_4$ (1 mol%), CH_2Cl_2; (d) allene, $Rh_2(OAc)_4$ (1 mol%), CH_2Cl_2; (e) *N*-iodosuccinimide, MeCN–H_2O (2:1), 50°C; (f) Bu_3SnH, AIBN, PhH, 80°C.

Robbins et al. have developed a novel and powerful branching DOS strategy (Scheme 4.3) [43]. Their approach involved the use of symmetrical linear ketone **6** as a starting unit, which could be transformed into 12 distinct scaffolds through an ingenious combination of two-directional synthesis and tandem reactions. The DOS involved the transformation of the central ketone group into various nucleophilic functionalities, which were then able to react with the tethered unsaturated ester groups at the chain termini. In some cases, essentially symmetrical products were produced (e.g., compound **7**). In other cases, tandem reaction sequences were developed in which reaction of the central functionality with one chain end transformed the central functionality such that it was then able to react with the other chain end under a different mode of reactivity, thus creating non-symmetrical 3D molecules from the simple linear substrate (e.g., compound **8**). The authors suggested that the linear ketone could be considered as a "molecular rope," which was then subjected to 12 separate tandem reactions to "tie the rope in knots" and so



SCHEME 4.2 DOS library subset generated from compound **2**. Conditions: (a) cyclopentadiene, $Rh_2(OAc)_4$ (1 mol%), CH_2Cl_2; (b) mCPBA, CH_2Cl_2; (c) OsO_4 (2.5 mol%), NMO, acetone/H_2O (9:1); (d) aldehyde/ketone, CSA (10 mol%), 3Å molecular sieves, CH_2Cl_2; (e) $SOCl_2$, CH_2Cl_2; (f) 2,6-lutidine, NMO, OsO_4 (2.5 mol%), $PhI(OAc)_2$, acetone/H_2O (10:1), then dimethylamine, $NaBH(OAc)_3$, CH_2Cl_2; (g) 2,6-lutidine, NMO, OsO_4 (2.5 mol%), $PhI(OAc)_2$, acetone/H_2O (10:1), then primary amine, $NaBH(OAc)_3$, CH_2Cl_2; (h) 2,6-lutidine, NMO, OsO_4 (2.5 mol%), $PhI(OAc)_2$, acetone/H_2O (10:1), then $NaBH_4$, MeOH; (i) alkene, Hoveyda–Grubbs (II) catalyst (10 mol%), ethylene, PhMe, 100°C; (j) $Pd(OAc)_2$ (10 mol%), boronic acid, PPh_3 (15 mol%), 2N K_2CO_3, PhMe, 90°C.

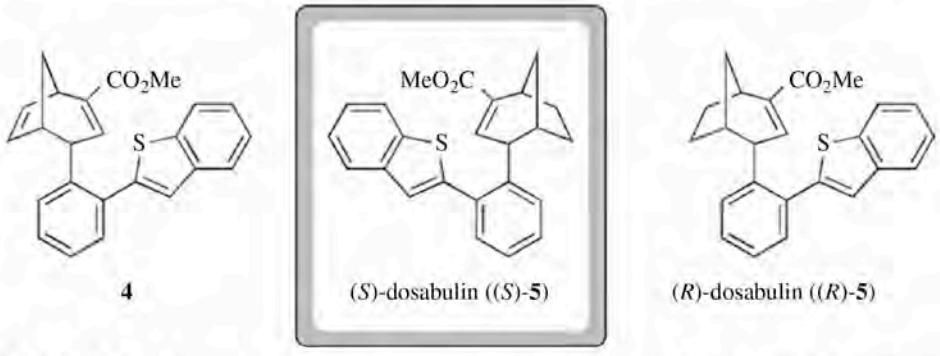
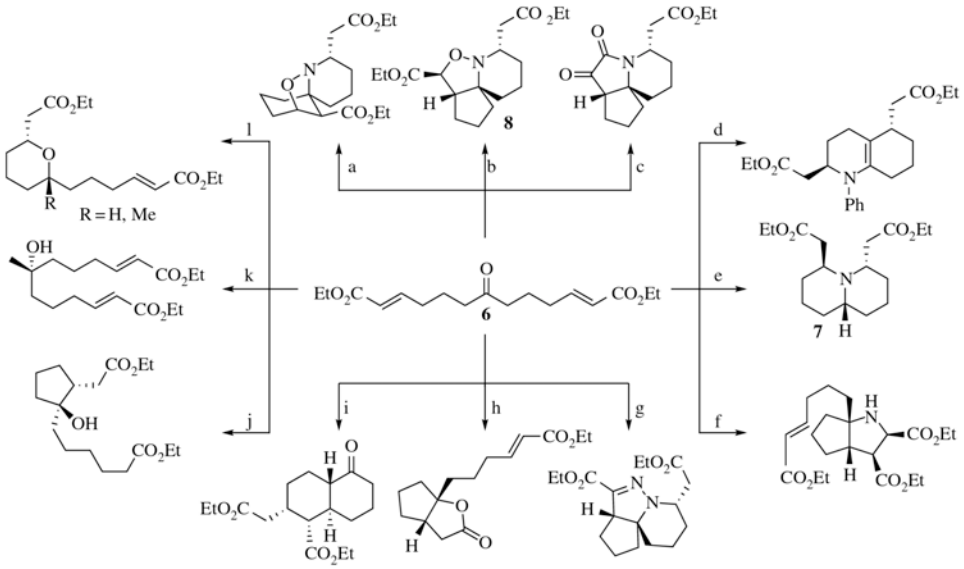


FIGURE 4.3 Compounds with potent antimitotic activity identified through the DOS campaign of Ibbeson et al.

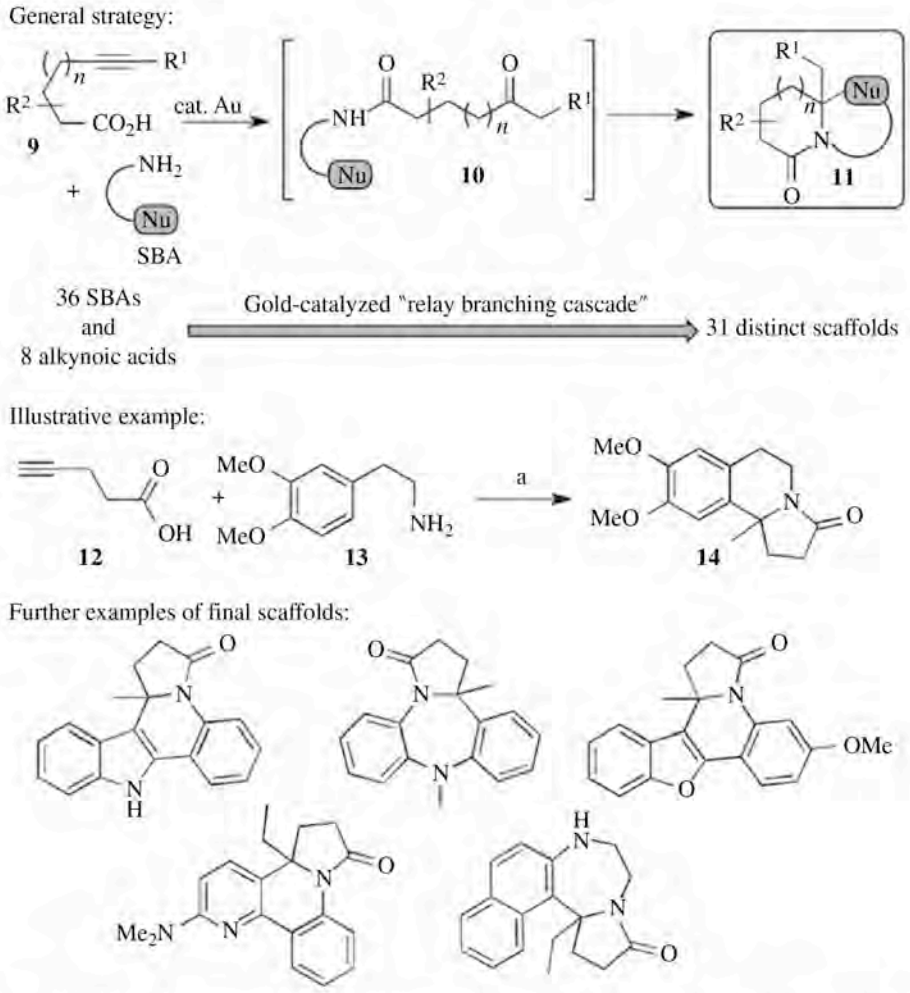
produce a range of molecular scaffolds. This 12-fold branching DOS strategy enabled the very direct and efficient formation of 3D molecular architectures with natural product-like features (polycyclic ring systems, multiple stereocenters, and a diverse range of functional groups) from a single substrate in an impressive 1.25 steps per scaffold on average.



SCHEME 4.3 The 12-fold branching DOS strategy developed by Robbins et al. Conditions: (a) $NH_2OH.HCl$, NaOAc, MeCN, then PhMe, 140°C, microwave; (b) $NH_2OH.HCl$, NaOAc, MeCN, 60°C; (c) $NH_2OH.HCl$, NaOEt, EtOH; (d) $PhNH_2$, $TiCl_4$, CH_2Cl_2; (e) $NaBH_4$, NH_3, EtOH, $Ti(OEt)_4$, then AcOH; (f) DIPEA, $H_2NCH_2CO_2Et$; (g) NH_2NHTs, toluene, reflux; (h) SmI_2 (2 equivalents), THF, MeOH, −78°C; (i) NaH, THF; (j) SmI_2 (5 equivalents), THF, MeOH, −78°C; (k) MeMgBr, THF; (l) superhydride, THF.

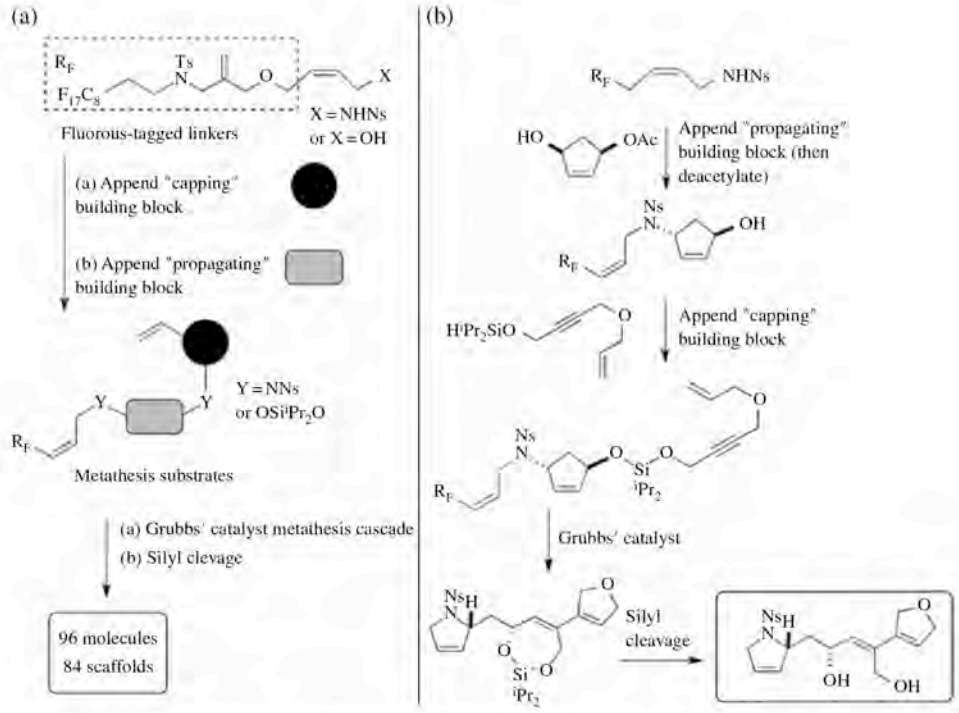
Patil et al. have recently reported a DOS strategy to access diverse molecular scaffolds based around a so-called relay catalytic branching cascade (RCBC) sequence [44]. The general synthetic concept is outlined in Scheme 4.4. Alkynoic acids (of the general form **9**, a common type of substrate) are reacted with several amine-functionalized scaffold building blocks (SBAs; variable) in the presence of a gold catalyst to form keto amides (of the general form **10**), which can then undergo gold-catalyzed cyclization cascades to produce various chiral multifunctional polyheterocyclic scaffolds (of the general form **11**). A specific example is shown in Scheme 4.4, where acid **12** reacts with amine **13** to generate heterocycle **14**. One of the principal advantages of this strategy is that a large number of SBAs are readily available, thus enabling efficient and rapid access to large scaffold diversity. Overall, the authors reported the DOS of 31 distinct scaffolds using 36 different SBAs and eight different alkynoic acids. All final library compounds were rich in functionality, and many contained structural features reminiscent of those found in natural products. This is undoubtedly a very elegant and powerful method for scaffold diversity generation. The authors suggest that it should be possible to expand the scope of this relay catalytic branching DOS strategy through the use of other reactions rather than just gold-catalyzed processes. For example, the authors highlight the fact that there are a multitude of reactions that can be catalyzed by metal-based catalysts and organocatalysts at the same time. Coupled with the ready availability of the substrates required, this approach clearly offers tremendous potential for the DOS of larger libraries of extremely high levels of structural diversity.

In 2009, the research group of Nelson described an elegant "folding" DOS pathway (Scheme 4.5) [40]. This approach involved the attachments of pairs of unsaturated



SCHEME 4.4 DOS strategy to access diverse molecular scaffolds based around a so-called relay catalytic branching cascade (RCBC) sequence. Conditions: (a) $Ph_3PAuOTf$ (5 mol%), CH_2Cl_2, 100°C.

functionalized building blocks (called a "propagating" building block in the center of the molecule and a "capping" building block on the end) to a fluorous-tagged linker (a specific allyl alcohol or allyl amine equivalent) to furnish a wide variety of linear intermediates with a dense array of structural features (appendage, functional group, and stereochemical diversity). Treatment with a suitable metathesis catalyst led to intramolecular cyclization reactions that paired unsaturated functional groups together, thus converting a collection of simple and similar substrates into a dense matrix of skeletally complex and diverse products [2, 45]. The fluorous-tagged linker was also designed to take part in the metathesis cascade, with the final cyclization intended to cleave the linker [45]. Consequently, only cyclized products were released from the fluorous tag during the metathesis process, allowing easy generic purification from uncyclized material by fluorous solid-phase extraction [2, 45]. This DOS strategy can be analyzed in terms of the



SCHEME 4.5 Folding DOS pathway developed by Nelson and coworkers. (a) General synthetic scheme. (b) Synthesis of one final library compound.

B/C/P algorithm: the synthesis of the "capping" and "propagating" building blocks can be considered as the "build" phase, formation of the metathesis substrates constitutes the "couple" phase, and the "pair" phase involves intramolecular cyclization reactions. Overall, the DOS of a library of 96 molecules based on a total of 84 distinct molecular scaffolds was reported. The library also contained high levels of functional group and stereochemical diversity, with a diverse range of different 3D features and functionalization motifs present among the compounds [2]. This is currently the largest number of scaffolds present in a deliberately synthesized DOS library. Recently, Maurya et al. have described an interesting extension to this strategy [46]. They demonstrate that it is feasible to exploit triplets of unsaturated building blocks (i.e., various different "initiating," "propagating," and "terminal" building blocks), thus extending the diversity of molecular scaffolds that can be prepared. This modified strategy thus constitutes a more powerful approach to constructing scaffold diversity.

4.3.5.2 DOS from the "Top Down": Natural Products as Starting Points for the Synthesis of Complex and Diverse Compounds DOS has traditionally been viewed as a guiding paradigm for the generation of complexity and diversity in a de novo fashion; that is, "typical" DOS campaigns aim to generate nonnatural (i.e., novel) molecules that are natural product-like in terms of their complexity and functionalization from structurally simpler starting materials (vide supra) [2]. Recent years have witnessed considerable interest in an alternative "top-down" strategy toward the synthesis of complex and diverse

small molecules, where natural products, which are inherently biased for biological activity and are rich in stereochemical and functional group diversity, are used as the starting points for library synthesis [47]. This strategy can arguably still be considered as a form (or perhaps a subtype) of DOS (e.g., "DOS on natural products"), in the sense that both have the same ultimate aim: the deliberate synthesis of structurally *novel* and diverse (including scaffold diversity) compound libraries that achieve a broad coverage of biologically relevant chemical space.

This approach is exemplified in recent work by Hergenrother and coworkers [48, 49]. They have developed a strategy for the production of complex natural product-like libraries via the controlled application of ring distortion reactions on natural products (i.e., reactions that modify the core ring systems present). The authors refer to this as a "complexity-to-diversity" ("Ctd") approach (Fig. 4.4). In their initial report, three readily available natural products were used as starting substrates: gibberellic acid, adrenosterone, and quinine (Scheme 4.6) [49]. The resulting compound library (totaling over 160 members) was found to exhibit desirable structural properties and high structural diversity. This Ctd approach was subsequently applied to abietic acid, leading to a library of 84 compounds [48].

4.3.5.3 "Directed" DOS of Macrocyclic Scaffolds Macrocyclic rings (ring composed of 12 or more atoms) are the core scaffolds of numerous compounds capable of modulating biological systems in a useful fashion. Indeed, there are over 100 marketed

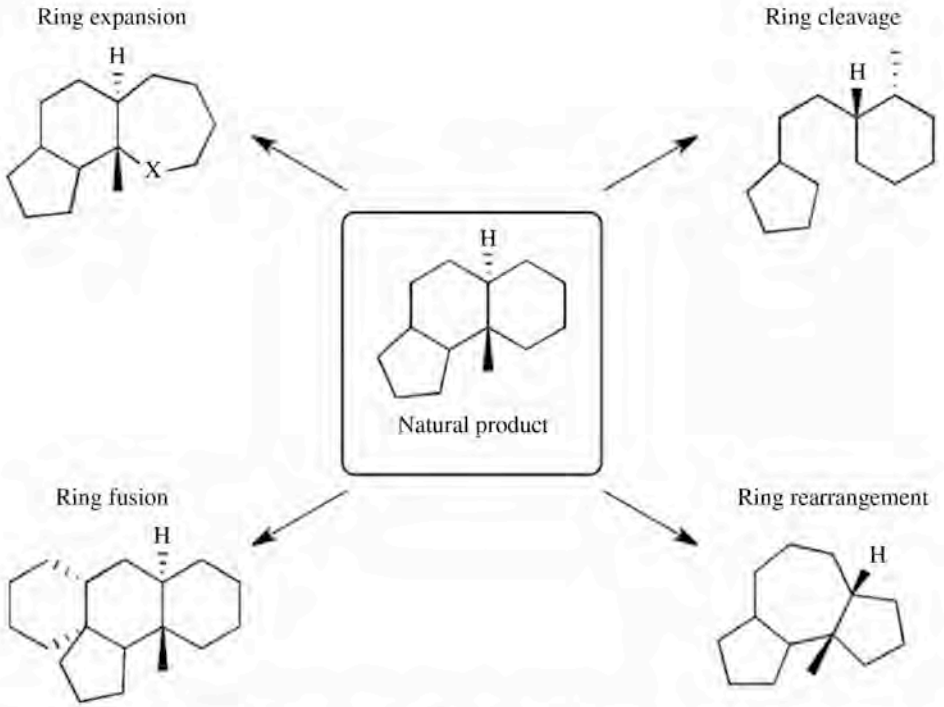
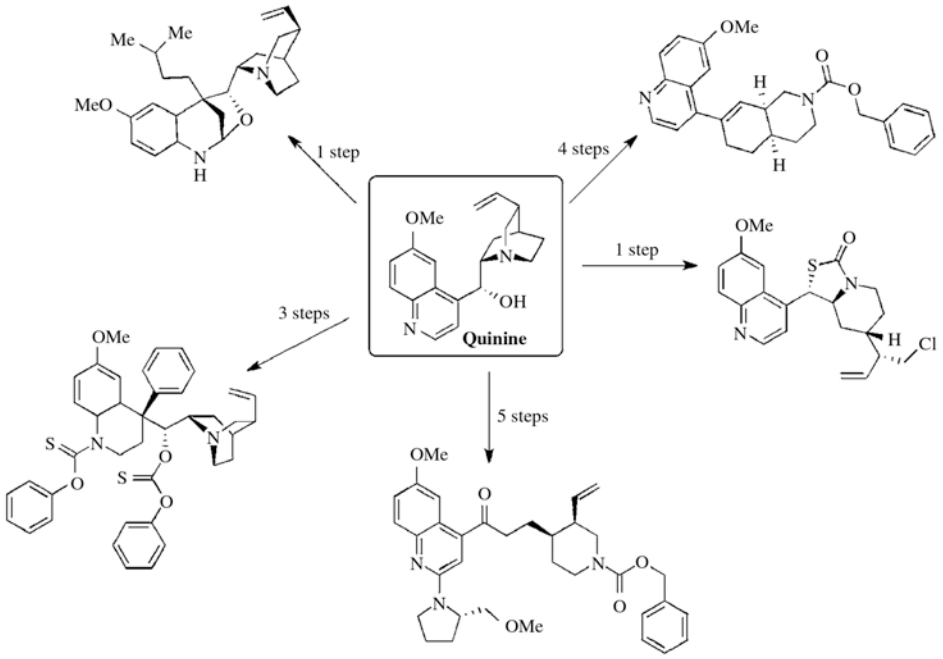


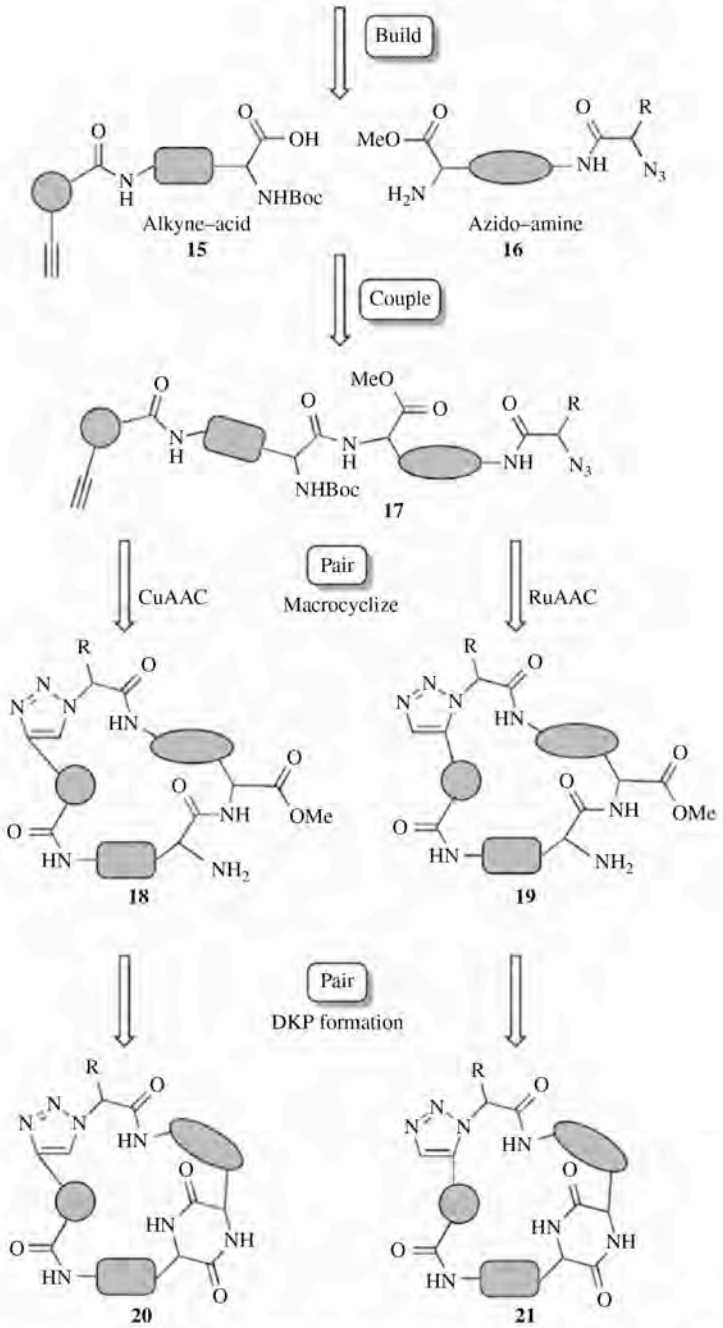
FIGURE 4.4 The complexity-to-diversity (Ctd) approach for small molecule library construction. The controlled application of ring distortion reactions (ring expansion, ring cleavage, ring fusion, or ring rearrangement) on a natural product starting material yields complex natural product-like compounds.



SCHEME 4.6 Application of the complexity-to-diversity (Ctd) approach to the natural product quinine. Reaction sequences of between one and five steps were used to convert quinine into the five structurally complex and diverse structures shown.

macrocyclic drugs derived from natural products [6, 50, 51]. However, there is widely thought to be a lack of *synthetic* macrocycles in drug discovery. This is in part due to concerns about synthetic intractability [6, 52, 53]. Unsurprisingly, therefore, recent years have witnessed intense interest in the development of new synthetic strategies to efficiently access nonnatural macrocycles, and several DOS programs directed toward this structural type have been reported [6, 54–56].

For example, Isidro-Llobet et al. have reported a DOS pathway for the generation of peptidic macrocyclic compounds (i.e., macrocyclic peptidomimetics; Scheme 4.7) [57]. This DOS strategy was based around the B/C/P algorithm [6]. In the "build" stage, two types of chiral building block were prepared using standard methods: (i) "alkyne acids" of the general form **15**, which contain an alkyne and a carboxylic acid, and (ii) "azido amines" of the general form **16**, which contain a free amine and an azide. In the "couple" stage, these building blocks were combined to produce a range of tripeptides **17**. The subsequent "pair" stage, which provided the basis for the generation of scaffold diversity, was comprised of two cyclization steps. The first step involved two variations of the "click"-type 1,3-dipolar azide–alkyne cycloaddition process, which selectively paired the azide and alkyne functionalities of the linear substrates to generate the desired macrocyclic architectures. The copper-catalyzed azide–alkyne cycloaddition (CuACC) provided the 1,4-isomers **18**, and the ruthenium-catalyzed variant (RuAAC) gave the 1,5-isomers **19**. Further diversity was then introduced into the compound set in the second step of the "pair" stage, which involved an intramolecular reaction between the ester and amine functionalities to generate a diketopiperazine (DKP) motif embedded within the macrocyclic frameworks (molecules of the general form **20** and **21**).



SCHEME 4.7 Overview of the strategy developed by Isidro-Llobet et al. for the DOS of macrocyclic peptidomimetics. CuAAC, copper-catalyzed azide–alkyne cycloaddition; RuAAC, ruthenium-catalyzed azide–alkyne cycloaddition. The shaded shapes represent major scaffold-defining elements.

In total, a small proof-of-concept library of 14 compounds based around four different macrocyclic scaffolds was generated (viz., *cis*-DKPs, trans-DKPs, 1,4- and 1,5-triazoles) [6].

Beckmann et al. have recently described an innovative and powerful approach for the DOS of macrocyclic scaffolds based around the use of an "advanced" B/C/P strategy [58]. The classical B/C/P strategy involves the synthesis of building blocks in the "build" phase. These are then combined in the "couple" phase using the same reaction type to forge a conserved linking motif between the building blocks (vide supra). The resulting linear precursors are subsequently subjected to scaffold-defining reactions in the "pair" phase. Beckmann and coworkers reasoned that the structural diversity of these linear precursors could be significantly increased through the utilization of a diverse set of branching reactions in the "couple" phase starting from a pluripotent functional group rather than a sole coupling reaction; this approach, which the authors termed "multidimensional coupling," would lead to the introduction of a diverse set of linking motifs between the building blocks (Fig. 4.5). Thus, the structural diversity of the linear precursors would now be defined not only by the nature of the building blocks used but also by the respective linker motif installed. In principle, multidimensional coupling should lead to a greater structural diversity of linear precursors (from a given set of building blocks) than that achieved using the "classical" B/C/P strategy, which in turn should also lead to a significant increase in the overall structural diversity of the resulting compound library.

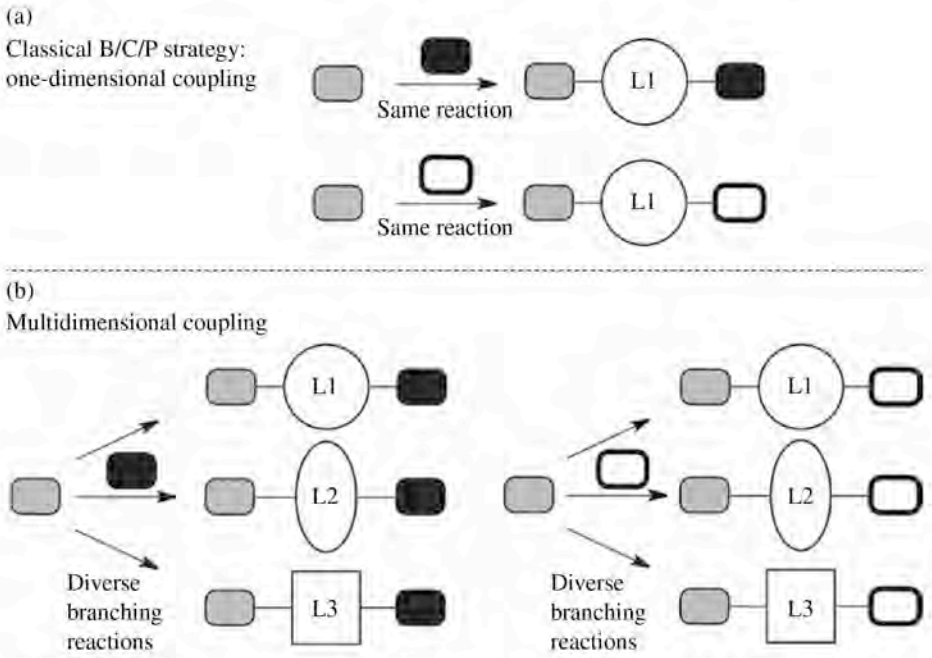
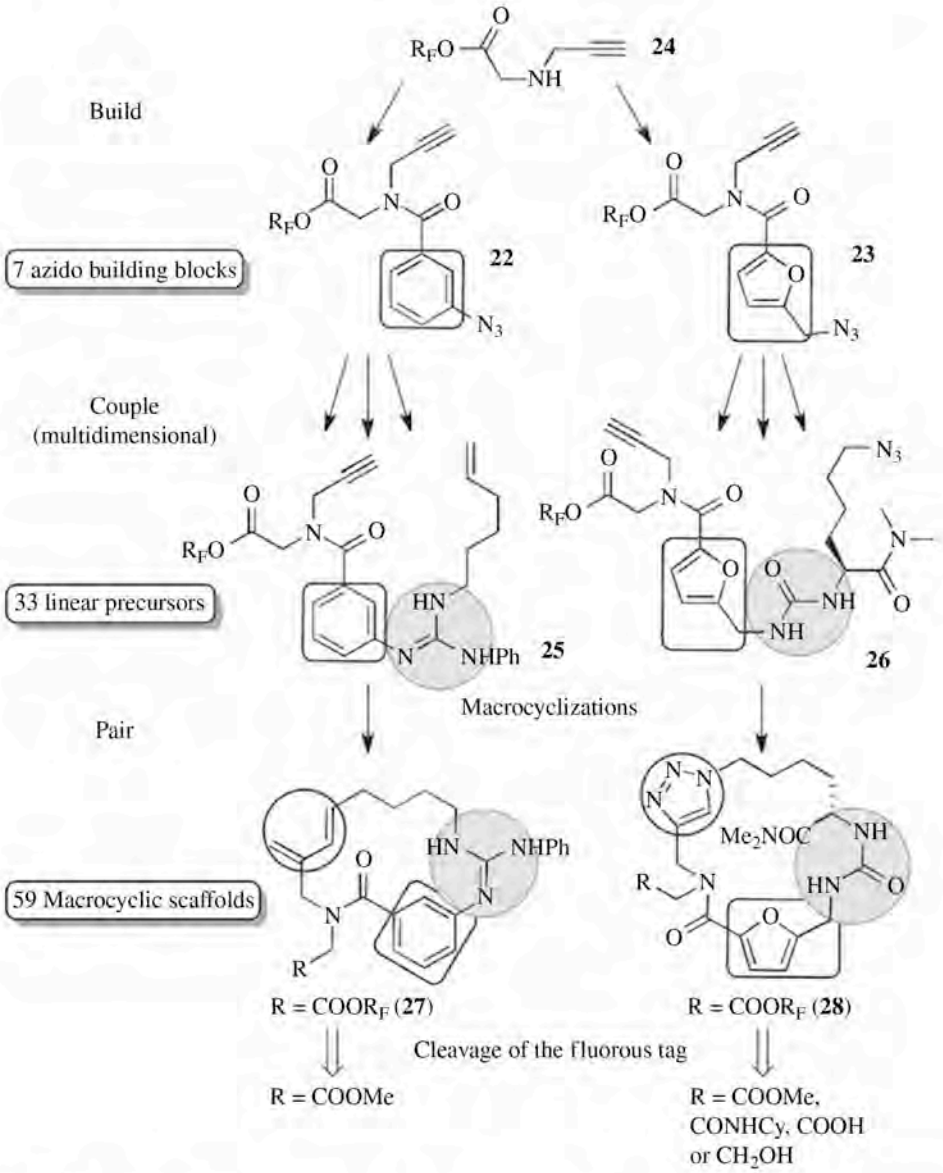


FIGURE 4.5 Schematic comparison of the "couple" phase in a classical B/C/P strategy with the "multidimensional coupling" phase. In this example, one building block (represented by a gray-filled rectangle) can be coupled with two other different building blocks (represented by black-and-white-filled rectangles) via the formation of three possible different linking motifs (L1–L3). (a) In the classical B/C/P strategy, the building blocks are coupled together using only one linking motif (L1). (b) When multidimensional coupling is used, the three different building blocks are coupled using three different linking motifs (L1–L3).



SCHEME 4.8 Outline of the strategy developed by Beckmann et al. for the DOS of macrocycles using multidimensional coupling. The synthesis of two representative macrocyclic scaffolds is shown. The multidimensional coupling process is represented by multiple arrows, but only one reaction product shown. Major scaffold-defining parameters are the variation of the building block (highlighted with a rectangle), the generation of different linking motifs using multidimensional coupling (highlighted with a gray-filled circle), and divergent macrocyclization (highlighted with a circle).

The authors have reported the application of this multidimensional B/C/P strategy to the DOS of a library of nonpeptidic macrocycles with unprecedented scaffold diversity (Scheme 4.8) [58]. The DOS pathway centered around exploitation of the pluripotent

reactivity of azide-derived aza-ylides. In the "build" phase of the DOS, a series of diverse azido building blocks (such as **22** and **23**) were prepared starting from the common fluorous-tagged precursor **24**. In the "multidimensional couple" phase, the azide group was transformed *in situ* into an aza-ylide, which was reacted with a diverse range of electrophiles in aza-Wittig reactions to generate various structural motifs (e.g., guanidine **25** and urea **26**) along with the concomitant installation of either a terminal alkene (as in compound **25**) or a new azide group (as in compound **26**). The azide and alkene groups, together with the initially attached terminal alkyne, served as synthetic handles for macrocyclization in the subsequent "pair" phase. Depending on the functionality present, macrocyclizations were performed either by (i) enyne metathesis (e.g., leading to **27**) or (ii) "click"-type azide–alkyne cycloaddition with copper catalysis (e.g., leading to **28**) or ruthenium catalysis. The presence of a fluorous tag in common precursor **24** allowed standard solution-phase parallel synthesis methods to be coupled with the benefits of fluorous-based purification protocols, allowing the generic purification of products along the DOS pathway by fluorous solid-phase extraction. In the final step of the DOS, the fluorous tag was removed using a diverse range of different methods (transesterification, transamidation, ester hydrolysis, and ester reduction) as a means to incorporate additional appendage and functional group diversity into the library. Overall, through the use of a limited number of structurally diverse building blocks, a DOS of 73 macrocycles based on 59 distinct scaffolds was achieved in no more than 4–5 steps from **24**. This represents an unprecedented level of scaffold diversity in a synthetically derived library of macrocyclic compounds. Computational analysis indicated that the library has a high level of shape diversity and thus overall structural diversity.

4.4. CONCLUDING REMARKS

Small molecule screening collections with high levels of structural, and thus functional, diversity are of undoubted value in the discovery of novel biologically active compounds. DOS has firmly established itself as a guiding paradigm for the efficient generation of such compound collections. The field of DOS has evolved rapidly since its "formal" conception some 14 years ago. As evidenced by the cutting-edge examples highlighted in this chapter, DOS planning algorithms have become considerably more sophisticated and powerful, allowing access to an unprecedented range of molecular structures, including many structural types that are underrepresented in standard small molecule screening collections. Numerous biologically active small molecules have been identified through the screening of DOS libraries, including modulators of classically "undruggable" targets and processes, thus validating the usefulness of DOS [2, 6]. However, there remains significant scope for further developments in the field. From a synthetic perspective, an ongoing challenge is to increase the *efficiency* of diversity (and complexity) construction, that is, to reduce the steps (and thus cost) associated with the generation of a library of structurally diverse and complex small molecules [2]. This can only be addressed by the development of new synthetic methodologies of broad utility [2]. A related and arguably much more fundamental issue is the absolute level of structural diversity that is desirable in new small molecule collections [2]. The goal of a DOS should be to efficiently and specifically access biologically *relevant* chemical space,

rather than chemical space devoid of biologically useful molecules [2]. As evidenced by the disappointing performance of current low-diversity small molecule collections in drug discovery screens, structural diversity is undoubtedly an important feature of small molecule libraries in this regard. However, if a specific biomolecule or family of biomolecules is targeted, some level of library structural bias will be valuable. In addition, a common criticism of DOS is that many of the physiochemical properties of compounds in DOS libraries are not "drug-like" [20]. This may be attributed to the fact that DOS typically places an emphasis upon efficiently generating structural diversity rather than tailoring the properties of the library members. Thus, even if such compounds appear as hits in a screen, they may be unsuitable for development into lead compounds for further testing [20]. Therefore, it could be argued that future DOS campaigns should aim to generate compounds that fall within preselected structural limits. However, it is unclear how this level of structural bias should be defined, and this remains an area of some debate [2].

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