Synthesis S. Collins et al. Short Review

Diversity-Oriented Synthesis of Macrocycle Libraries for Drug Discovery and Chemical Biology

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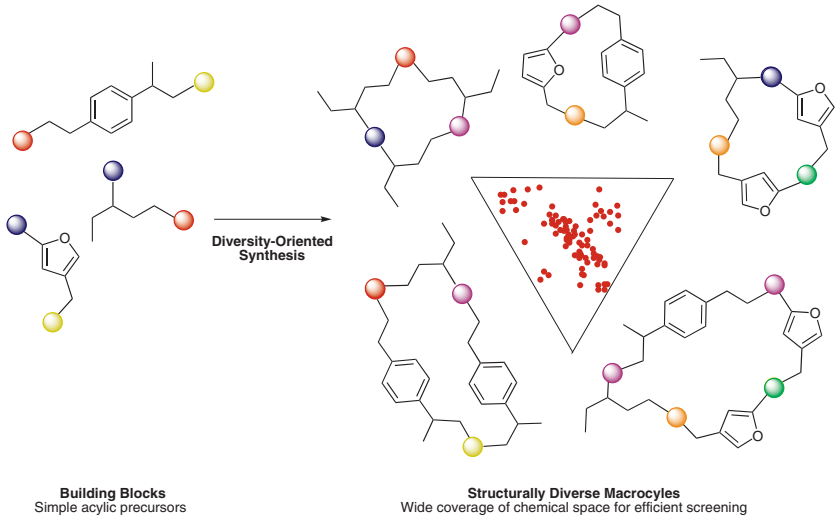

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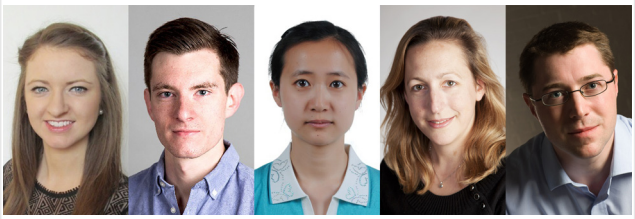
Abstract The identification of new bioactive small molecules is increasingly reliant upon the synthesis and screening of chemical libraries. The extent of structural diversity and the proportion of unique scaffolds in a library are commonly acknowledged to be the most important factors in determining its success in identifying new biologically relevant compounds. Particularly important in this respect are macrocycles, which display unique physicochemical attributes and are used in many clinical applications. Despite these advantages, macrocycles remain under-represented in many contemporary screening collections, predominantly due to their synthetic intractability. Diversity-oriented synthesis is a powerful method for the construction of deliberately diverse collections of small molecules, and many research groups are working to apply its principles to the synthesis of structurally and functionally diverse macrocyclic libraries. In this short review we introduce why macrocycles are promising chemotypes in screening libraries, especially for challenging biological targets such as protein–protein interactions, and we review a collection of strategies developed in our laboratory for the diversity-oriented synthesis of macrocycle libraries. We analyse a selection of the macrocycle collections generated using these approaches and conclude with our perspective on future directions of the field.

Key words diversity-oriented synthesis, diversity, macrocycles, small molecule libraries, cheminformatics

1 Introduction

1.1 Chemical Libraries in Drug Discovery and Chemical Biology

Modern medicine relies heavily on the identification of small molecules capable of selectively perturbing the function of biological systems.[1–3] The general approach taken to identify these chemical entities is to screen vast libraries (compound collections) and then to elaborate hits in medicinal chemistry campaigns.[4] Accordingly, the success of such screening endeavours is dependent upon the molecular composition of the libraries used.[5] Ideally the population of a chemical library will exhibit diverse molecular size and shape, as well as a variety of heteroatoms and functional groups.[6] It has also been shown that compounds of intermediate (stereochemical) complexity are generally the best selective binders of a given protein, so screening collections should also display a certain structural complexity.[7] These qualities are not always observed in 'traditional' commercial and proprietary screening collections.[8] The various synthetic strategies employed for the synthesis of these large compound collections for high-throughput screening has resulted in the accumulation of countless similar screening collections, which have had little impact in hit identification.[9,10] It is even argued that the over-eager adoption of combinatorial synthesis may have hindered progress in drug discovery by encouraging adherence to the Lipinski *rule of five*,[11] by restricting research to validated biological targets,[12–14] and by limiting the stereochemical complexity achieved in final libraries.[7] The need for unbiased and structurally complex libraries for the identification of bio-



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logically relevant small molecules therefore continues to challenge chemists to re-evaluate the strategies employed for library synthesis.[15–17]

1.2 Macrocycles in Screening Collections

Eager to explore the vast areas of chemical space not thought to be accessible with typical small molecules collections, many research groups have focused their attention on the application of macrocycles in drug discovery.[18,19] These cyclic organic structures, comprising twelve or more atoms in a ring, represent a significant yet under-represented class of biologically relevant compounds in contemporary screening collections.[20] The biological attributes of macrocycles are thought to stem from a combination of structural features including the size (somewhere between small molecules and biologics) and conformational pre-organisation of the ring architecture.[19,21] Relative to small molecules, the large surface area offers an extended binding surface, thereby increasing the likelihood of making multiple contacts with a macromolecular target.[22] In addition, it is theorised that the structural pre-organisation of the ring architecture minimises the entropic cost upon binding to a protein by virtue of reduced rotational freedom, thus contributing to an increased binding affinity relative to acyclic analogues.[19,22]

It is worth noting that the use of macrocycles as therapeutic agents is not a new phenomenon, with a variety of natural and semi-synthetic variants being used medicinally for decades (Figure 1).[19,23] Currently there are at least one hundred approved drugs containing macrocyclic ring systems, and these represent some of the most effective clinical drugs available to date.[19,24–27] The vast majority of these marketed macrocyclic drugs are derived from natural products, often with little or no modification.[19] This contrasts greatly with the origin of typical small molecule therapeutics, where at least half have been discovered from synthetic libraries rather than natural scaffolds.[28] This disparity is unsurprising considering the paucity of macrocycle chemotypes in compound collections, reported to be as low as just 1% in some industrial libraries.[20] The lack of macrocycles can be attributed to a multitude of factors, for example, until recently such structures were not considered to be 'drug-like' according to Lipinski's rules, and so were likely to be rejected early on in drug discovery campaigns.[19,29] Furthermore general methods for the synthesis and modification of macrocycles have been few in number and quite impractical—probably a hangover of their oversight in medicinal chemistry programs.[19]

Recently, a wealth of reports describing advances in selective macrocyclisation chemistry has facilitated the target-oriented synthesis of a variety of macrocycles of biological interest.[23] In many cases, however, the synthetic strategies designed to access such structures have been tailored to specific targets and often cannot be applied generally. Consequently, progress in methods for the synthesis of structurally diverse and complex macrocycle libraries has been limited. This has restricted efforts to identify novel bioactive chemotypes through modern approaches, such as chemical genomics,[1,30] and the simultaneous generation of complex and functionalised macrocycles thus remains a significant challenge.

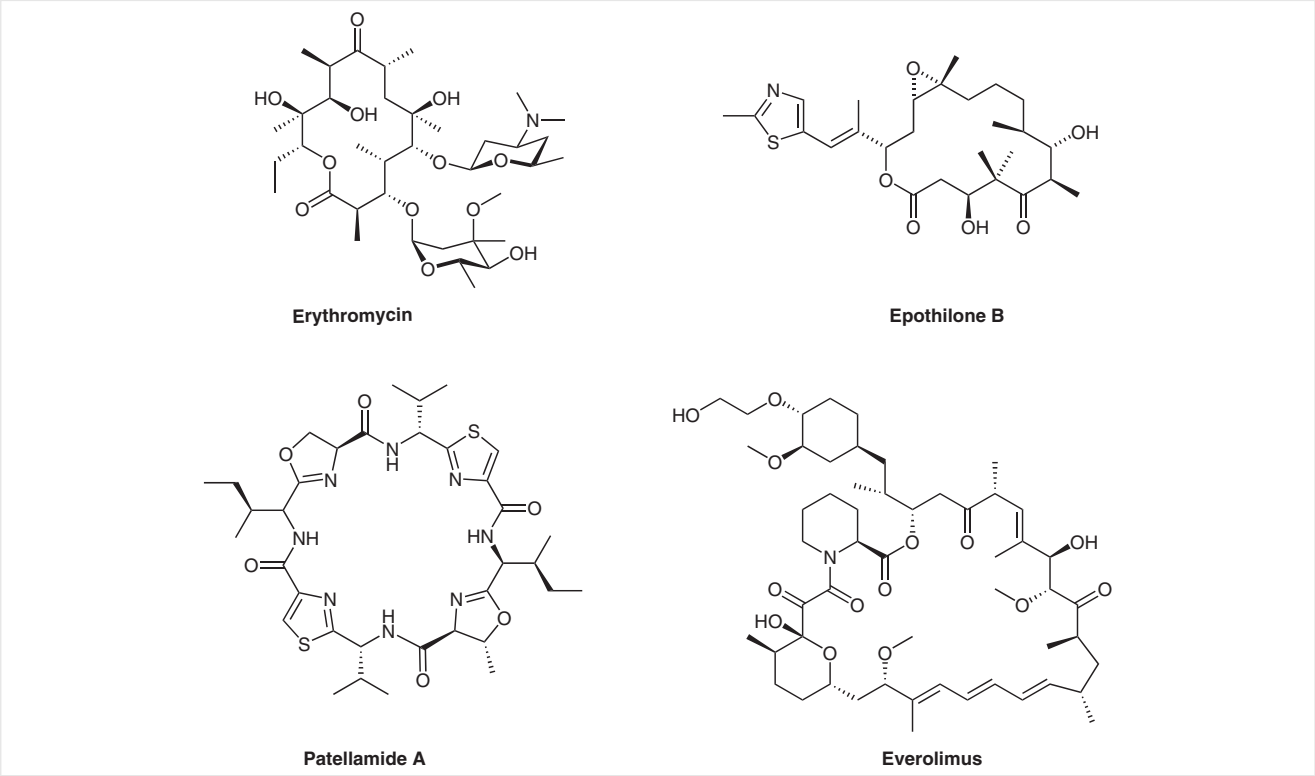


Figure 1 Chemical structures of some therapeutic macrocycles: erythromycin, macrolide antibiotic; epothilone B and patellamide A, anti-cancer therapeutics; everolimus, immunosuppressant

1.3 Diversity-Oriented Synthesis

One strategy toward the efficient generation of structurally complex and functionally diverse chemical libraries is diversity-oriented synthesis (DOS), a major research area in our group.[7,31,32] First introduced by Schreiber,[33] this approach is used to generate purposely diverse collections of compounds for the identification of novel and biologically active chemical entities. The s*tructural diversity* sought after in this approach is commonly taken to comprise four common principal components:[6]

(1) *Appendage diversity*: variation in structural moieties around a common scaffold.

(2) *Functional group diversity*: variation in the functional groups present in a given scaffold.

(3) *Stereochemical diversity*: variation in the orientation of potential macromolecule-interacting elements.

(4) *Scaffold diversity*: presence of many distinct molecular skeletons. In this context, this can be taken to mean the combined rigidifying elements of a macrocycle.

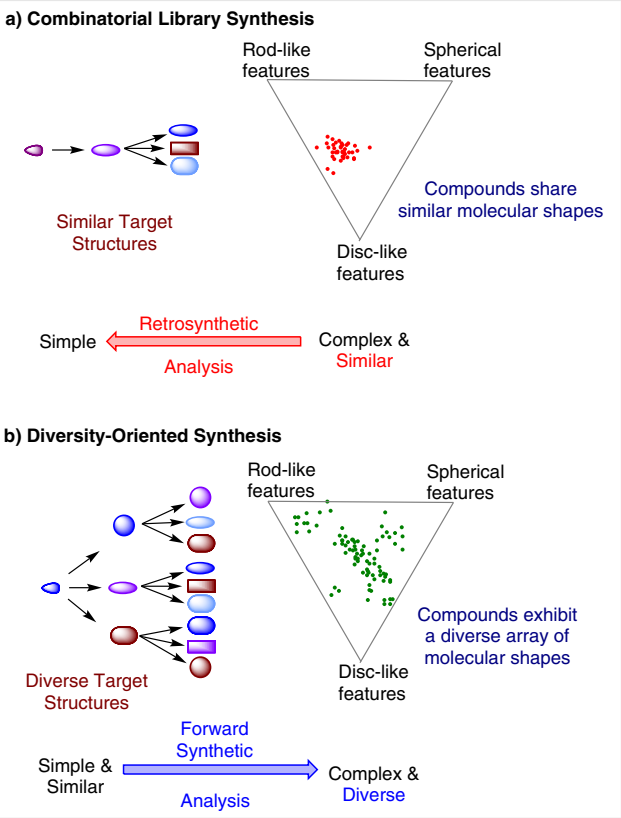
Whilst these parameters may all contribute toward the quality and nature of a compound's interaction with a biological target, scaffold diversity is generally acknowledged to be the most important metric in determining how efficiently a library interrogates chemical space.[34] The biological activity of a given molecule is intrinsically related to its three-dimensional shape, which is governed in turn by its molecular scaffold. Consequently, the greater the degree of structural diversity achieved within a certain library, the higher the probability of identifying new biologically active compounds (Scheme 1).[8,35]

In this short review we discuss the strategies developed in our laboratory toward the diversity-oriented synthesis of macrocyclic libraries, and touch briefly on a selection of alternative approaches taken by other research groups. We assess the structural diversity of some of these libraries with reference to drug and natural product collections, discuss alternative methods for macrocycle library synthesis, and conclude with our perspective on the future of the field.

2 Build/Couple/Pair

2.1 Strategy Overview

Many of our DOS strategies are built upon the application of the build/couple/pair (B/C/P) method.[36] This strategy involves three principal steps in the generation of libraries of skeletally diverse and stereochemically defined compounds. Briefly, the *build* step involves the asymmetric syn-



Scheme 1 (a) Combinatorial library synthesis, and (b) diversity-oriented synthesis; a comparison of the planning strategies used and the degree of molecular shape diversity generated using these methods. Adapted from Spring et al.[6]

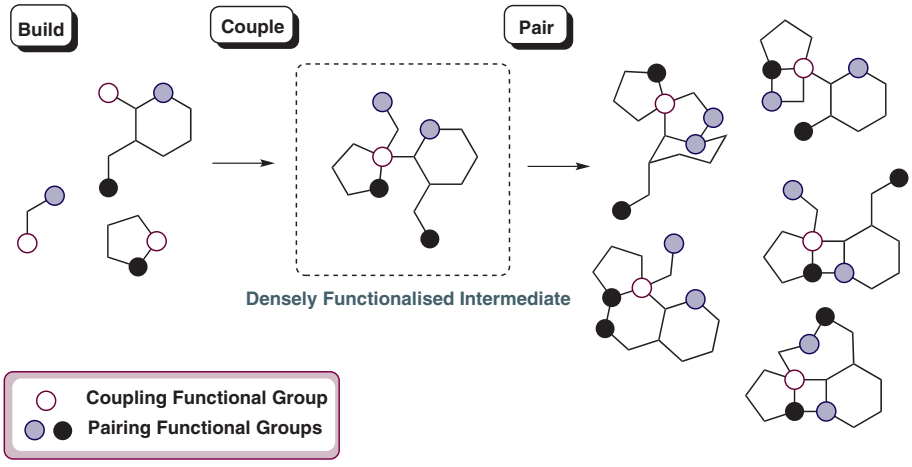
thesis of strategically designed precursor components. These building blocks can then be linked in the *couple* phase, resulting in the formation of complex and densely functionalised intermediates. Judicious functionalisation within each intermediate facilitates regio- and chemoselective ring closure in the *pair* step, yielding structurally and spatially diverse macrocycles (Scheme 2).[37,38]

Within the context of DOS, the B/C/P strategy is the most prominent choice for macrocycle library synthesis to date. The popularity of this approach is likely the result of multiple factors. The method is modular and concise, which allows for easy modification and diversification at each stage. Structural variation of building blocks and many diversity-generating coupling reactions can be explored to expand the number and complexity of macrocyclic scaffolds populating the library. Alternatively, more discrete functionalisation can be carried out on particular species to incorporate further appendage diversity to an existing library (for example, after the identification of one or more hits). Another advantage is the ability to generate an entire matrix of stereoisomers, which expedites stereo/structure–activity relationship (SSAR) studies—an important feature of modern drug development programs.[39–41] Since its conception, a number of research groups have expanded upon the original B/C/P strategy,[36,41,42] and by introducing opportunities to generate structural complexity at each step of the synthesis, chemists have been able to design and generate diverse macrocycle screening collections with much success. Herein we discuss the development and application of this and related strategies.

2.2 Typical B/C/P Strategies

2.2.1 Peptidomimetic Library Generation

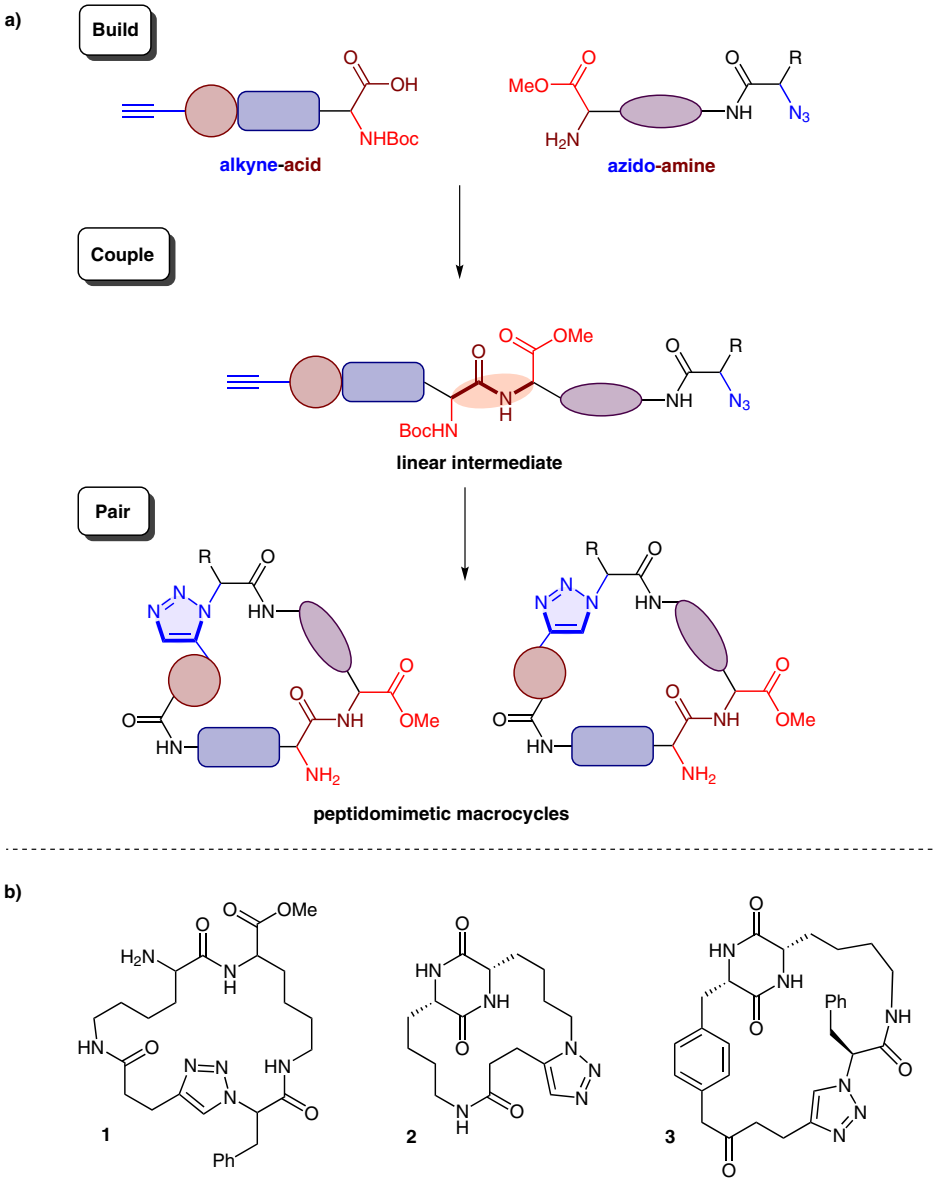
Our early work investigating the use of DOS strategies for macrocycle synthesis focused on developing libraries that incorporated peptidomimetic scaffolds (Scheme 3, a).[38] Natural and synthetic peptides are widely used in medicine.



Scheme 2 A general schematic of the build/couple/pair strategy in diversity-oriented synthesis. Adapted with permission from Nielsen, T. E.; Schreiber, S. L. *Angew. Chem. Int. Ed.* **2008**, *47*, 48. © Wiley-VCH, 2008.

As a result of the improved stability and cell permeability that cyclisation imparts on such small molecules,[18,43] the presence of a core peptidic architecture is common in many clinical macrocyclic drugs.[19,43,44] The limited bioavailability of natural peptides can still however hamper their development as viable drug candidates.[45] This has therefore prompted studies into the development of synthetic peptidomimetic macrocycles.[46,47]

The target peptidomimetic scaffolds (**1**–**3**) (Scheme 3, b), were designed to incorporate amino acid moieties linked by a triazole ring—a common amide biostere.[48] The amino acid derived building blocks contained complementary functionalities for both the *couple* and *pair* stages in the form of an alkyne-acid and an azido-amine. These orthogonally-protected precursors were synthesised from commercially available materials and coupling afforded the linear intermediates with ease. Such intermediates were then cyclised in the *pair* phase to form the respective peptidomimetic macrocycles. Prior to this work, regioselective formation of 1,5- over 1,4-disubstituted triazoles in the presence of neighbouring chiral centres had not been well described in the literature. We were able to identify conditions to effect regioselective copper- or ruthenium-catalysed azide–alkyne cycloaddition reactions (CuAAC and RuAAC), and generate the desired 1,4- and 1,5-disubstituted products respectively, without degradation of the stereochemical in-

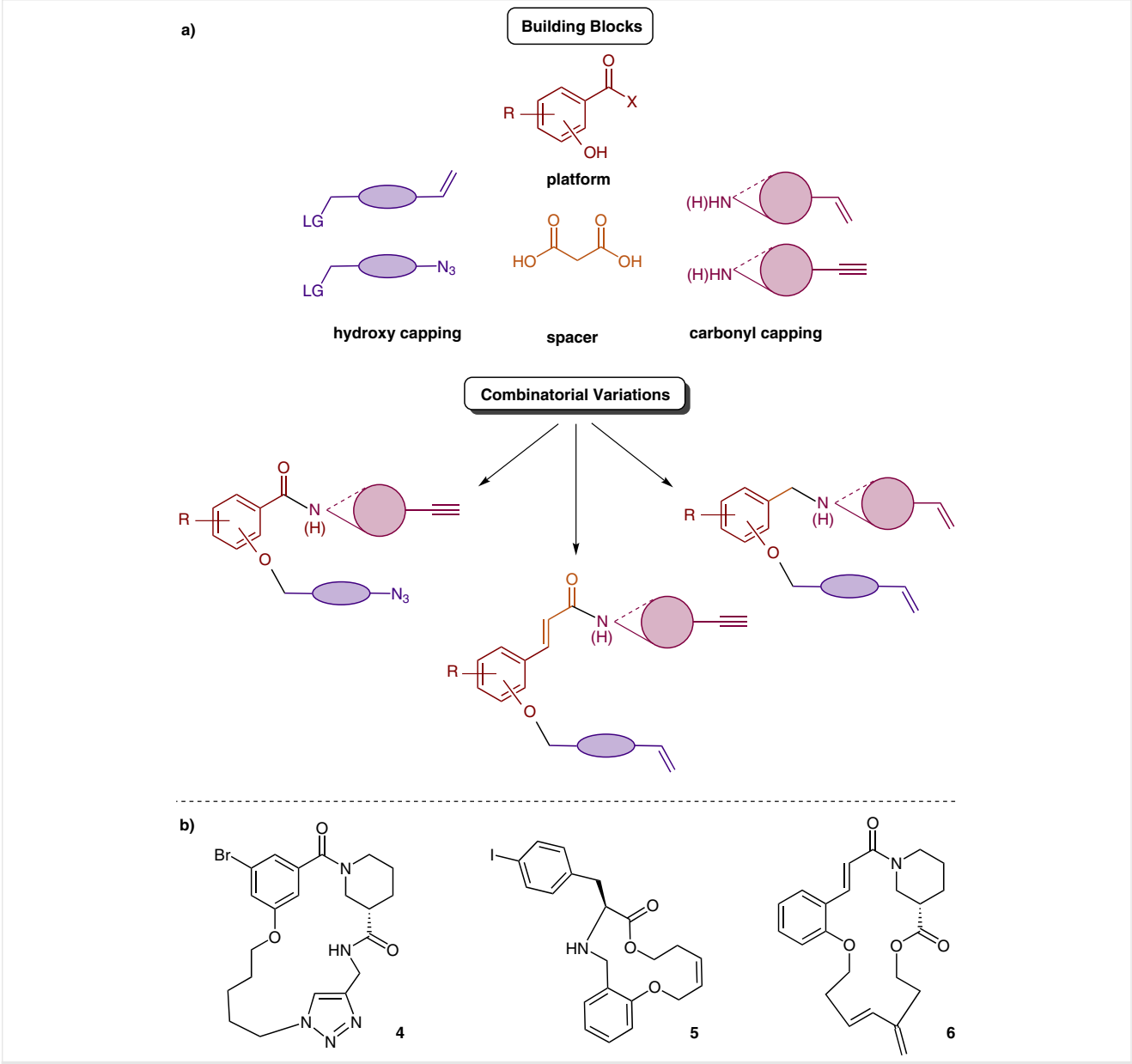


Scheme 3 (a) The general strategy for the diversity-oriented synthesis of a peptidomimetic macrocycle library. (b) Representative library members.[38]

tegrity of adjacent chiral centres. We have found these conditions to be widely applicable and have made extensive use of this methodology in later work—demonstrating the synergy of fundamental research in DOS and its application in translational research. For library development, diketopiperazine (DKP) units were also introduced into the macrocyclic scaffolds. These cyclic dipeptide motifs are found in a variety of natural products and have also been incorporated in a plethora of drug candidates, where they can be used to orient side chains or functional groups in the three-dimensional directions required to imitate peptide secondary structures.[49–51] In this work, DKP units were introduced to act as conformational mimics of linear peptides with the added benefits conferred by cyclisation as discussed earlier.

2.2.2 B/C/P Building Block Platform

We have also investigated the use of a core building block platform to which various other precursor units could be added in a combinatorial-like fashion (Scheme 4).[52] Here the core aromatic unit bears both a nucleophilic hydroxy group and an electrophilic carbonyl group. These serve as



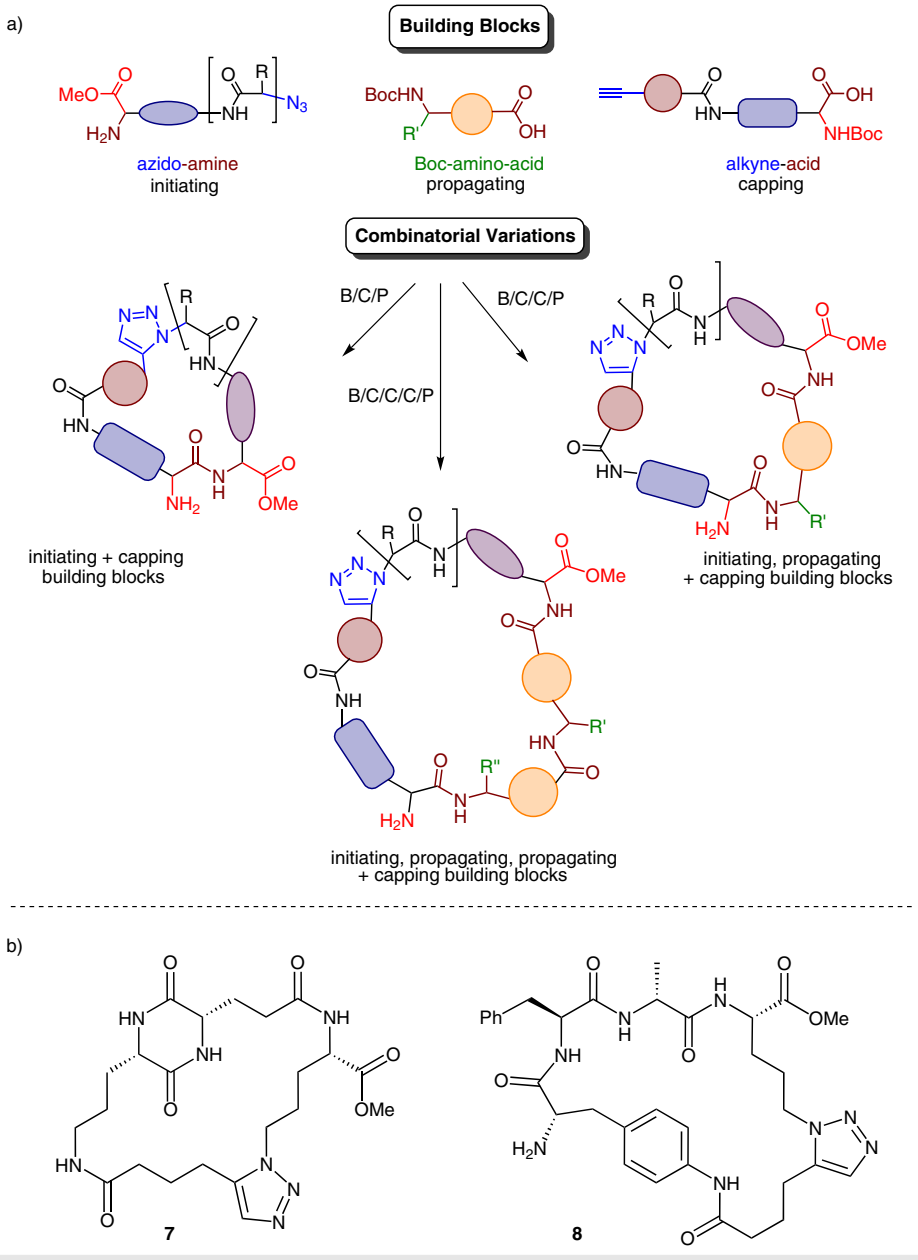
Scheme 4 (a) A B/C/P building block platform strategy. Combinatorial-like variation of the hydroxy and carbonyl capping groups affords a variety of macrocyclic scaffolds. LG = leaving group, e.g., halogen. (b) Representative library members.[52]

sites for the attachment of variable 'hydroxy capping' or 'carbonyl capping' building blocks respectively. Successive functionalisation of the phenolic platform at these positions resulted in the formation of acyclic precursors, in which the linking motifs had been installed for the final macrocyclisation step. The incorporation of 'spacers' could also be used to expand the macrocyclic ring-size range achieved in the final library. Variation of the capping building blocks or the core aryl platform itself (for example, by the inclusion of a halogen for late-stage diversification via cross-coupling) demonstrates the potential of this modified B/C/P approach for further development and applications.

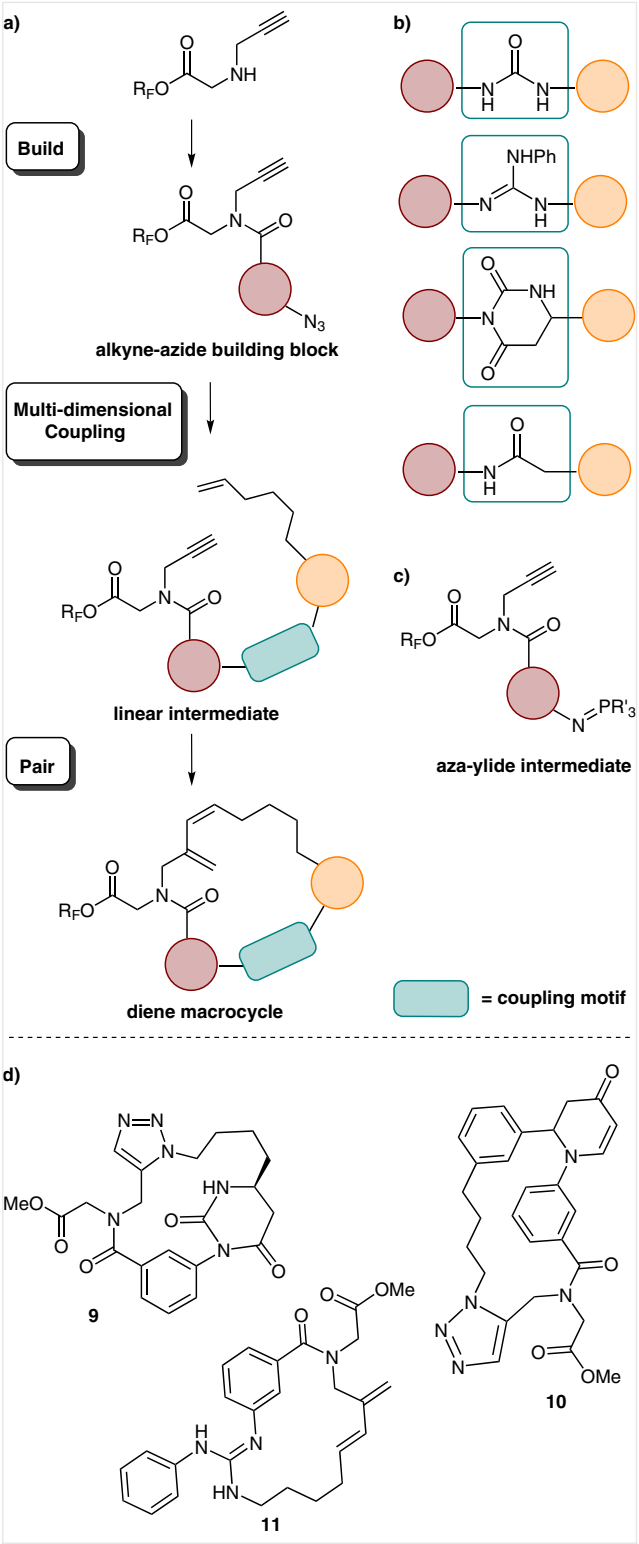
2.3 Advanced B/C/P Strategies

2.3.1 Multiple Couplings

To investigate how we could elaborate on the conventional B/C/P approach, we built upon a strategy reported by



Scheme 5 (a) Multiple coupling B/C/P strategies employing initiating, propagating and capping building blocks. Combinatorial variations of the three categories of building blocks provided access to a wide range of macrocyclic structures. (b) Representative library members.[38]



Scheme 6 (a) An overview of the multi-dimensional coupling DOS approach. (b) A selection of coupling motifs generated using this approach. (c) An aza-ylide intermediate as a branching point. (d) Representative library members.[42] $R_F = -(CH_2)_2C_8F_{17}$.

Nelson et al.,[53] whereby the generation of increasingly complex building blocks allowed for multiple subsequent coupling steps (Scheme 5, a). In this work we incorporated a variety of coupling steps before the ring-closing reaction to increase the number of distinct molecular scaffolds accessible from a common intermediate. These extra stages required elaboration of the precursors in the *build* phase. In contrast to the earlier work where only two building block partners were needed, the advanced B/C/C/P and B/C/C/C/P approaches required additional building blocks—so-called 'propagating units'. These extra Boc-protected amino acids could be used to elongate the linear precursor prior to the *pairing* step, allowing for the generation of increasingly large and complex structures (Scheme 5, b).[54]

This study generated a library of over 200 peptidomimetic macrocycles, featuring an unprecedented number of distinct molecular scaffolds.[54] The strategy is advantageous in terms of the associated increase in both scaffold diversity and size, with the larger structures believed to be able to target increasingly large binding interfaces.[22]

2.3.2 Multi-Dimensional Coupling

With an efficient multistep B/C/P strategy in hand for the preparation of peptidomimetic macrocycles, we next focused on extending the strategy to include non-peptidic scaffolds (Scheme 6, a).[42] Such compounds are significantly under-represented in chemical libraries and offer the opportunity to explore new areas of biologically relevant chemical space.[19] Again, however, the development of libraries populated by these kinds of macrocycles is often complicated by synthetic intractability. We envisioned that tailoring the building blocks used in the previous B/C/P approaches would provide access to a non-peptidic macrocycle library. Additionally, we reasoned that the multi-dimensional coupling strategy could be expanded further to take advantage of the pluripotent reactivity in the intermediate species.[55] For example, it was proposed that building blocks endowed with an azide moiety could be converted into aza-ylide intermediates for subsequent in situ coupling reactions with a selection of electrophiles (Scheme 6, a and Scheme 7). Such reactions would generate diverse and complex coupling motifs, and in this way the scaffolds of the resulting compound library would be defined not only by building blocks, but also the linking motifs installed during their construction (Scheme 6, b).

The alkyne–azide building blocks were first synthesised from commercially available materials. Treatment under aza-Wittig reactions conditions afforded pluripotent aza-ylide intermediates, which were then reacted with a variety of electrophiles including isocyanates, acyl chlorides, aldehydes and carbon dioxide to afford a range of linear products (Scheme 6, b).[42] These were either purified directly, such as the amide generated through the reaction with acid chlorides or trapped and subjected to further diversifica-

tion (Scheme 7). For example, imine **15** formed by the aza-ylide/aldehyde reaction, could generate either amine **16** through reduction with sodium borohydride, or dihydropyridinone **17** via an aza-Diels–Alder reaction with Danishefsky's diene.

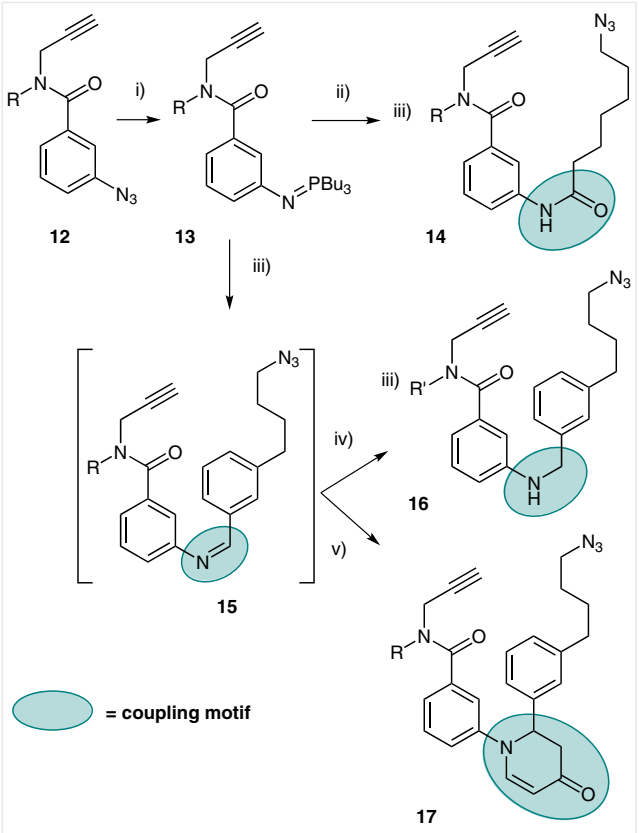
An important feature of these complexity-generating coupling steps is the concomitant installation of the second synthetic handle in the form of an azide or an alkene. These functionalities were utilised in the final *pair* step through an intramolecular reaction with the terminal alkyne. Again we were able to utilise our regioselective CuAAC and RuAAC methodology to form 1,4- or 1,5-disubstituted triazoles, respectively. Ring-closing enyne metathesis was also achieved easily. Cleavage of the fluorous tag (R_F; installed early in the synthesis to facilitate purification) provided another opportunity to introduce appendage diversity by means of transesterification, amidation, hydrolysis or reduction (discussed later). Taken together, this resulted in a final library of 73 macrocyclic compounds, typified by structures **9**–**11**, comprising 59 distinct macrocyclic scaffolds with a natural-product-like level of structural diversity.[42]

Encouraged by these results, we reasoned that new methods for the generation of multiple linking motifs from common precursors would expedite and improve the scope of the library diversity that is achievable. Organocatalysis is an exciting area of research in organic chemistry and, in particular, N-heterocyclic carbenes (NHCs) constitute a simple, cheap and green class of catalysts for stereocontrolled C–C and C–heteroatom bond formation.[56–58] Inspired by the opportunities that this presented, we therefore designed a new strategy for the DOS of a collection of non-peptidic macrocycles utilising the organocatalytic pluripotency of aldehydes and their derivatives (Scheme 8).[59]

The building blocks used in this study fell into two categories: aldehydes and their 'alophile' coupling partners. The aldehydes were easily prepared from commercial materials, and further modification afforded the corresponding alophile building blocks. With these in hand, we were able to demonstrate the wide but chemoselective reactivity of NHCs to carry out a variety of coupling reactions between the two components. The resultant linear intermediates could be paired through ring-closing metathesis following the single B/C/P protocol or extended with further coupling reactions to generate increasingly large and complex structures. In all, this approach afforded 51 macrocycles bearing 48 unique scaffolds—all of which originated from just five simple building blocks.

2.3.3 Post-Pairing Modification

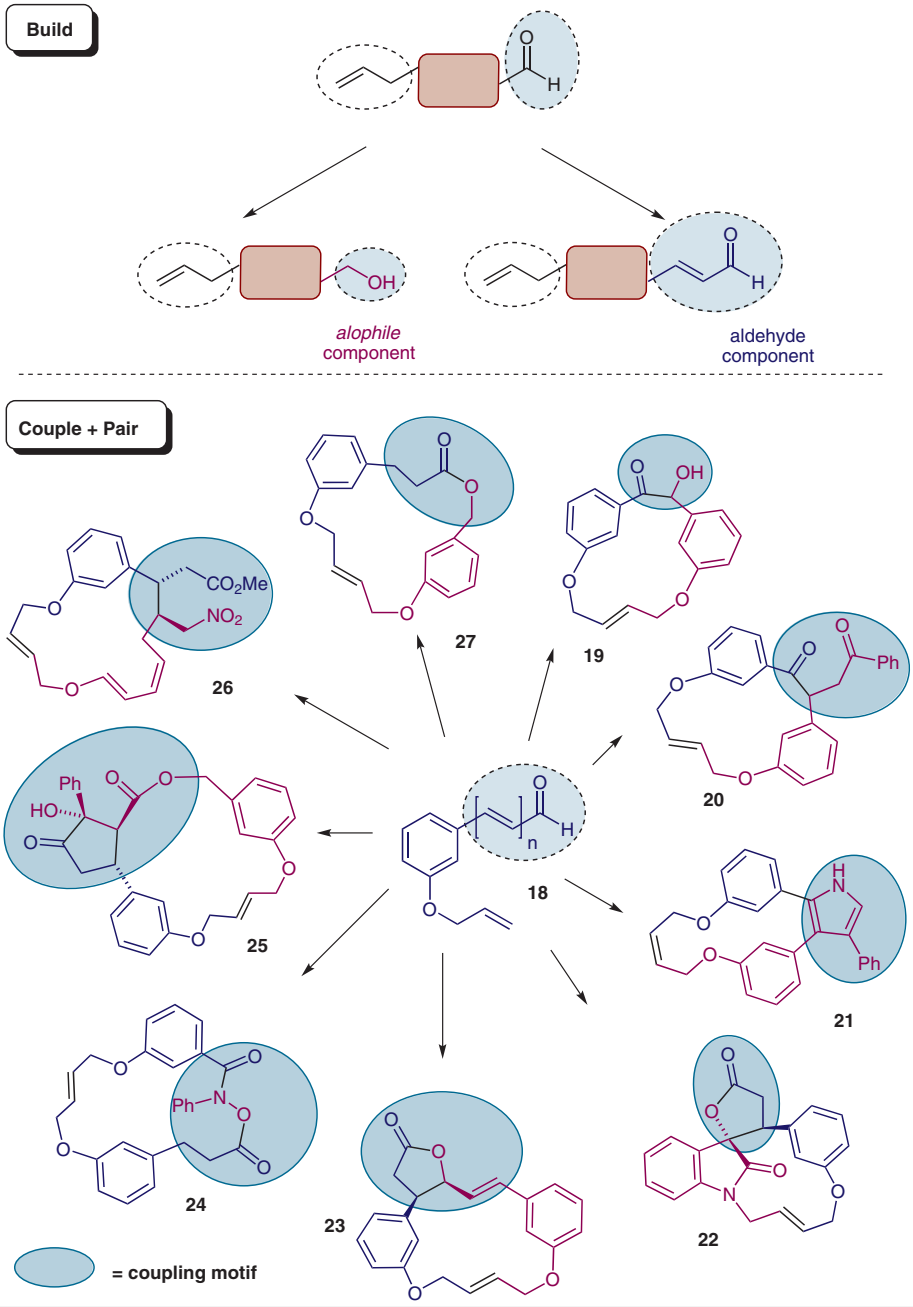
The final step explored in a number of the aforementioned DOS strategies was post-pairing modifications. Although this step is somewhat loosely defined, various approaches toward late-stage diversification of macrocycles have been reported in our work. These can be categorised into two general methods: elaboration of functional groups not comprising the molecular scaffold (Scheme 9, a), or the reaction of latent functionality within the macrocyclic core itself (Schemes 9, b and c).



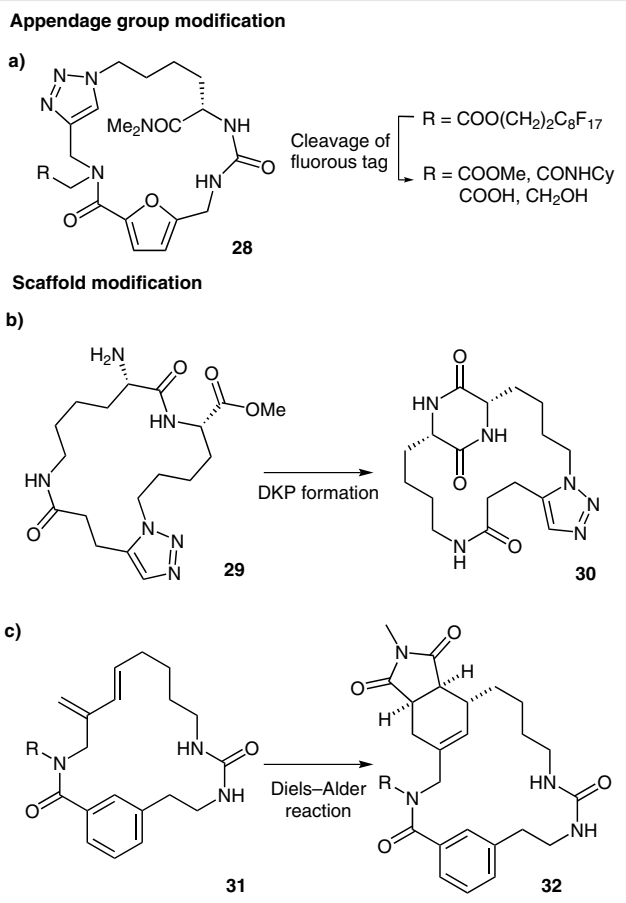
Scheme 7 A multidimensional coupling strategy. Azide **12** is converted into aza-ylide **13**, which may be reacted in situ to generate complex linking motifs comprising part of the macrocyclic scaffold. Imine **15** may undergo further diversification to increase the number of unique scaffolds accessible from a common intermediate. *Reagents*: (i) PBu_3, THF; (ii) 7-azidoheptanoyl chloride; (iii) 3-(4-azidobutyl)benzaldehyde; (iv) $NaBH_4$, MeOH; (v) Danishefsky's diene, AgOTf. R = $CH_2COO(CH_2)_2C_8F_{17}$, R′ = CH_2COOMe.

The first of these diversifying strategies was briefly addressed in the multi-dimensional coupling route (Scheme 6),[42] whereby modifications at the site of the fluorous tag in **28** gave access to various appendage isomers (Scheme 9, a). Similarly, strategic incorporation of functional handles early in the synthetic route provides the opportunity to modify appendages on the core structure later in the synthesis. Such modifications result in increased functional group or appendage diversity in the final library, which may be advantageous for hit-to-lead campaigns. These diversity parameters are believed, however, to be the least significant contributors to the shape diversity exhibited by an overall compound collection.[6] In this respect, post-pairing modifications that fall into the latter category—that is those that

alter the macrocyclic core itself—are capable of generating superior scaffold diversity in a given library. This can be demonstrated by the incorporation of the DKP moiety in the peptidomimetic libraries through reaction of **29** (Schemes 3, 5 and 9, b).[38,54] Similarly, diene **31** (synthesised in the multi-dimensional coupling strategy library; Schemes 6 and 9, c)[42] served as a substrate for further scaffold modification through Diels–Alder reactivity. The resulting compounds differ from their original scaffolds in terms of modular flexibility and available functionality for biological interaction, thereby expanding the scope of chemical space explored by the complete library.



Scheme 8 A pluripotent building block B/C/P strategy. A small collection of aldehydes and their derivatives could be coupled using a variety of complexity-generating organocatalytic reactions and subsequently paired via alkene metathesis. n = 0 or 1.[59]



Scheme 9 Examples of post-pairing modifications in some reported strategies. (a) Appendage group modification; cleavage of the fluorous tag yields a collection of appendage isomers. (b)/(c) Scaffold modification; diketopiperazine (DKP) formation and Diels–Alder cycloaddition generate macrocycles with new connectivity and distinct molecular scaffolds. R = $CH_2COO(CH_2)_2C_8F_{17}$ in (c)

2.4 Two-Directional Synthesis

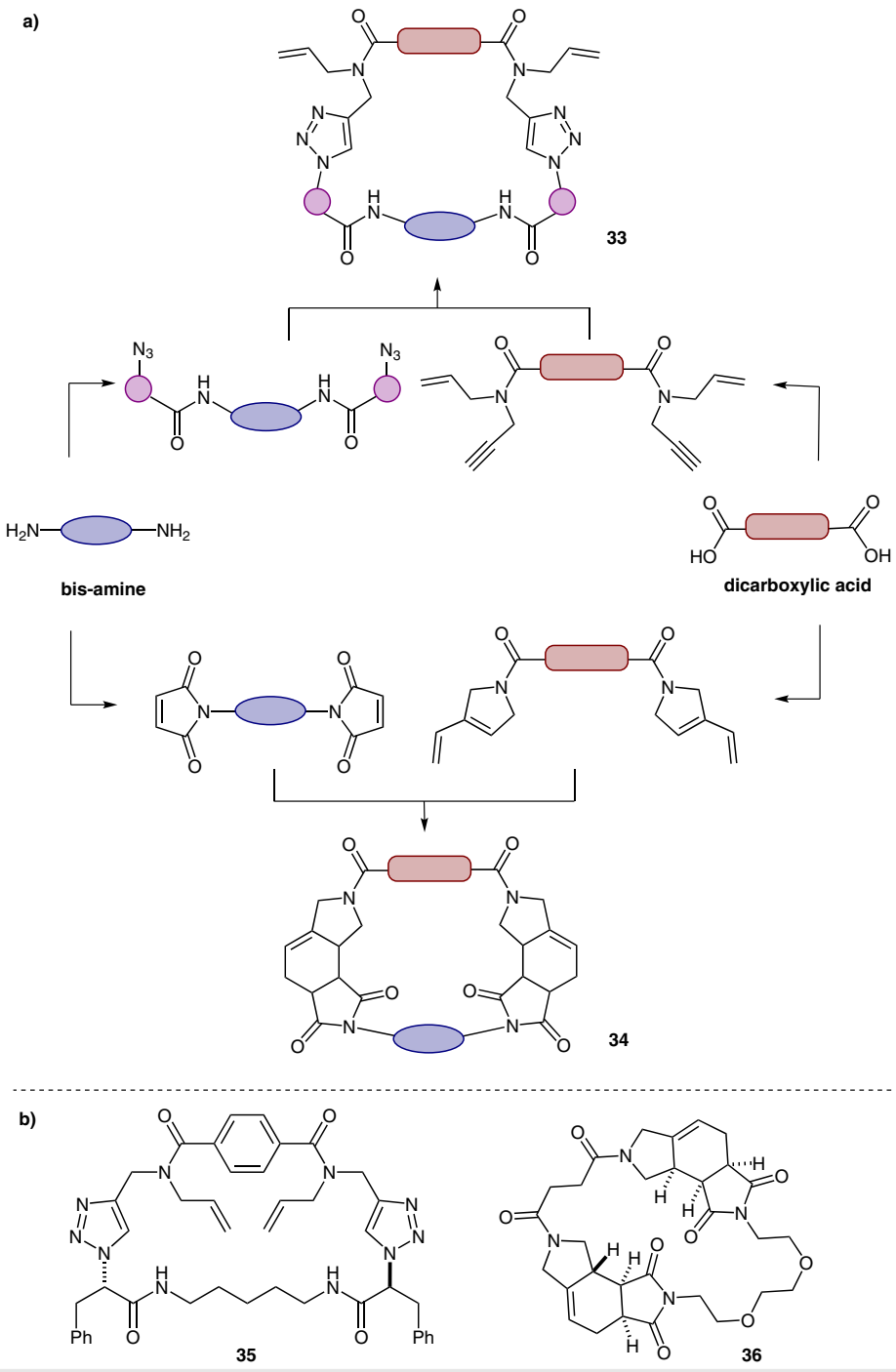
Whilst not technically a B/C/P approach, two-directional synthesis[55] embraces the concept of introducing dense functionality in the precursor *build* phase, as outlined in early B/C/P plans (Scheme 10, a). In this approach the symmetrical and bifunctional precursors may be paired directly or derivatised further to afford additional functionally distinct building blocks. These may then be paired through complexity-generating methods, yielding diverse molecular scaffolds from a few common precursors.

Given the large substrate tolerance and biocompatibility of the resulting triazoles, we again recognised CuAAC reactions to be a promising macrocyclisation method. Diels–Alder chemistry was also identified as an apt method for macrocycle formation, providing a means to generate three-dimensional shape (and stereochemical) diversity from two-dimensional and achiral building blocks. The linear intermediates were prepared with the required functionalities symmetrically installed at terminal positions for macrocyclisation. For CuAAC reactions, the necessary bis-enynes and bis-azides were prepared from readily available dicarboxylic acids or diamines respectively. Copper(I)-catalysed ring-closure then afforded compounds of the type **33**. We were able to improve step efficiency in the synthetic protocol by employing the same starting materials for the preparation of the Diels–Alder coupling partners. Here, the linear diamines were used to synthesise the maleimide dienophiles. Similarly, transformation of the CuAAC bis-enynes through ring-closing enyne metathesis afforded the *exo*-diene building blocks. With these precursors in place the cycloaddition reactions were carried out to generate a small collection of stereochemically defined macrocycles of the type **34**. As mentioned, although this is not a B/C/P procedure in the strictest sense, the two-directional approach shares similar underlying principles and advantages. Specifically, this strategy allows combinatorial variation of scaffold elements to be achieved with ease, and can generate scaffold diversity efficiently in a tandem coupling and macrocyclisation step.

3 Alternative Approaches to Macrocycle Library Synthesis

While we have focused thus far on a selection of the developments made in our own laboratory, it is important to recognise the many and important contributions to the field made by other research groups. Schreiber's work on regioselective gold(I)-catalysed coupling reactions was an early example of the power of cascade reactivity in combination with DOS.[60] Shortly afterwards, a report of an aldol-based B/C/P method detailed the construction of a library of medium-ring and macrocyclic structures of various sizes.[61] This approach coupled with the synthesis and testing of a complete stereomatrix of diverse compounds demonstrates the benefit of 'built-in' SSAR facilitated by the B/C/P method in the discovery of a new class of inhibitors of histone deacetylase.[61] Marcaurelle and co-workers have described the construction of stereochemically complex pyran-based macrocycles through the combination of three sets of complementary building blocks in a 'domain shuffling' approach.[39] Diverse molecular shape was achieved within a given combination of building blocks (e.g., a combination of all stereoisomers of the building blocks *A*, *B* and *C* in that order) as well as macrocycles differing only in the connectivity of the same building blocks (e.g., *ABC* vs *ACB*). Other groups have taken rather different approaches for macrocycle synthesis. To overcome the typical shortcomings of macrocyclisation reactions, such as the complicating influence of ring size and need for high dilution conditions, Tan and co-workers adopted a DOS strategy based on the oxidative ring expansion of bicyclic diketones to give macrolact-

ams.[62] A conceptually similar ring-distortion strategy for the synthesis of [*n*]paracyclophanes has been reported by Meyer and Cossy, based upon sequential Diels–Alder/retro-Diels–Alder reactivity.[63] More recently, Unsworth and co-workers have reported a successive ring-expansion method for macrocycle synthesis, in which cyclic β-ketoesters are acylated with linear fragments that undergo deprotection and rearrangement in situ to generate expanded lactams and lactones.[64]



Scheme 10 (a) Two-dimensional synthesis of macrocycles. Modification of the bis-amine and dicarboxylic acid precursors gave access to a range of terminally functionalised linear intermediates, which could be coupled to generate symmetrical macrocycles. (b) Representative library members.[55]

The construction of structurally diverse small-molecule libraries is of course not limited to DOS. Other prominent strategies for the synthesis and discovery of biologically active small molecules include biology-oriented synthesis, which focuses on privileged scaffolds with known biological relevance;[65] lead-oriented synthesis[66] and also activity-directed synthesis, a function-driven approach to hit discovery in which the crude product mixtures of diversifying reactions are screened iteratively to bias synthesis toward bioactive small molecules.[67] Interested readers are directed toward dedicated texts and recent articles detailing alternative methods for macrocycle library synthesis including DNA-templated synthesis, biosynthesis and chemoenzymatic synthesis.[68–72]

4 Discussion and Concluding Remarks

In this article we have described a number of strategies devised to assemble structurally diverse macrocycle collections for biological screening, principally by means of diversity-oriented synthesis. The chemical diversity of such libraries may be assessed using a variety of methods, each with associated advantages and limitations.[73] Chemical similarity screening,[74] for example, can be applied to reduce the size and cost of screening libraries without detriment to its coverage of chemical space.[2] Numerous advances in computation and the field of molecular docking of macrocyclic scaffolds give hope to these kinds of approaches.[74–78] The rigorous application of compound filtering and virtual screening, however, is challenging and must be approached in a way that does not preclude novel discoveries.[79] Shape-based methods for diversity assessment of screening libraries are often used due to the intrinsic relationship between structural diversity and biological activity.[4,34] Principal moments of inertia (PMI) analysis is a popular method for this type of investigation, although its use is subject to some debate.[73] PMI analysis involves the calculation of normalised ratios of principal moments of inertia for the lowest-energy conformation of each library member. The resulting data can be visualised in a triangular plot where the three vertices correspond to spherical, disc-like and rod-like shapes. Using previously reported methods,[34,42] PMI plots were constructed to assess the degree of shape diversity achieved by a selection of the DOS libraries previously discussed (Figure 2). We included two established reference collections in each analysis for comparison: a sample of 40 top-selling drugs[62] and 60 diverse natural products.[80] Generally the collection of drugs (blue triangles) are calculated to reside within the flatter area of the plot with fewer spherical features, while the natural products (green circles) exhibit greater shape diversity despite a similar bias toward two-dimensional shape. Gratifyingly, our macrocyclic DOS collections and the additional reference domain shuffling DOS library[39] (red circles) are seen to achieve significantly greater shape diversity when compared with the top-selling drugs. They are, in fact, more akin to the natural product collection in this regard, thus supporting our claims that the DOS strategies employed can generate compound collections of natural-product-like structural complexity.[38,54,55]

With the ever-increasing adoption of cheminformatics and availability of diversity assessment methods, it is easy to lose sight of the fundamental goals driving this research. Simply put, DOS and related approaches toward library synthesis are designed to facilitate the discovery of novel bioactive small molecules for the development of potential drugs and chemical probes. Like many, our group is particularly interested in identifying compounds that can modulate key protein–protein interactions (PPIs). PPIs are implicated in a variety of cancers and other diseases, and as such, we regularly assay our compound libraries against these targets. High-throughput screening technologies are no longer restricted to industry but are increasingly available to academics,[81] for example, through in-house[82] and open innovation[83,84] facilities aiming to accelerate the identification of novel and biologically active small molecules. Through DOS library screening campaigns undertaken in our laboratory, we have identified various acyclic hits which have been developed into published lead compounds, such as the antimitotic dosabulin and the anti-MRSA agent gemmacin B (Figure 3).[85–87] Discoveries made from *in silico* and *in vitro* screening of DOS macrocycles synthesised in our group[42,52,54,59] form the basis of a number of active research projects; results will be published when appropriate. Other groups have reported similar successes, for example the landmark discovery of robotnikinin,[88,89] an inhibitor of the Sonic Hedgehog protein (Shh), as well as the development of the antimalarial lead ML238.[90,91] Lessons learned from these discoveries can be used to guide future design strategies and highlight potential points for improvement within current studies.

Looking ahead, we anticipate the development of many areas of fundamental and translational research to benefit directly the application of the DOS approach.[92] Progress in synthetic methods will continue to drive opportunities for innovative DOS,[17] particularly in exciting areas such as cascade[93–95] and multicomponent[96,97] reactions. This should ameliorate existing strategies for complexity-generating coupling and macrocyclisation reactions, which often rely on tried-and-tested methods rather than exploring novel synthetic routes.[21,98] There also remains ample opportunity to extend concepts of the multi-dimensional coupling approaches discussed previously. Many groups have investigated ways to further diversify their macrocyclic libraries, through various post-pairing modifications.[41,99] Whilst we have made some initial attempts to carry out such modifications on our DOS macrocycles (Scheme 9), we believe the potential of these diversifying reactions has not yet been realised. The impact on scaffold diversity and physicochem-

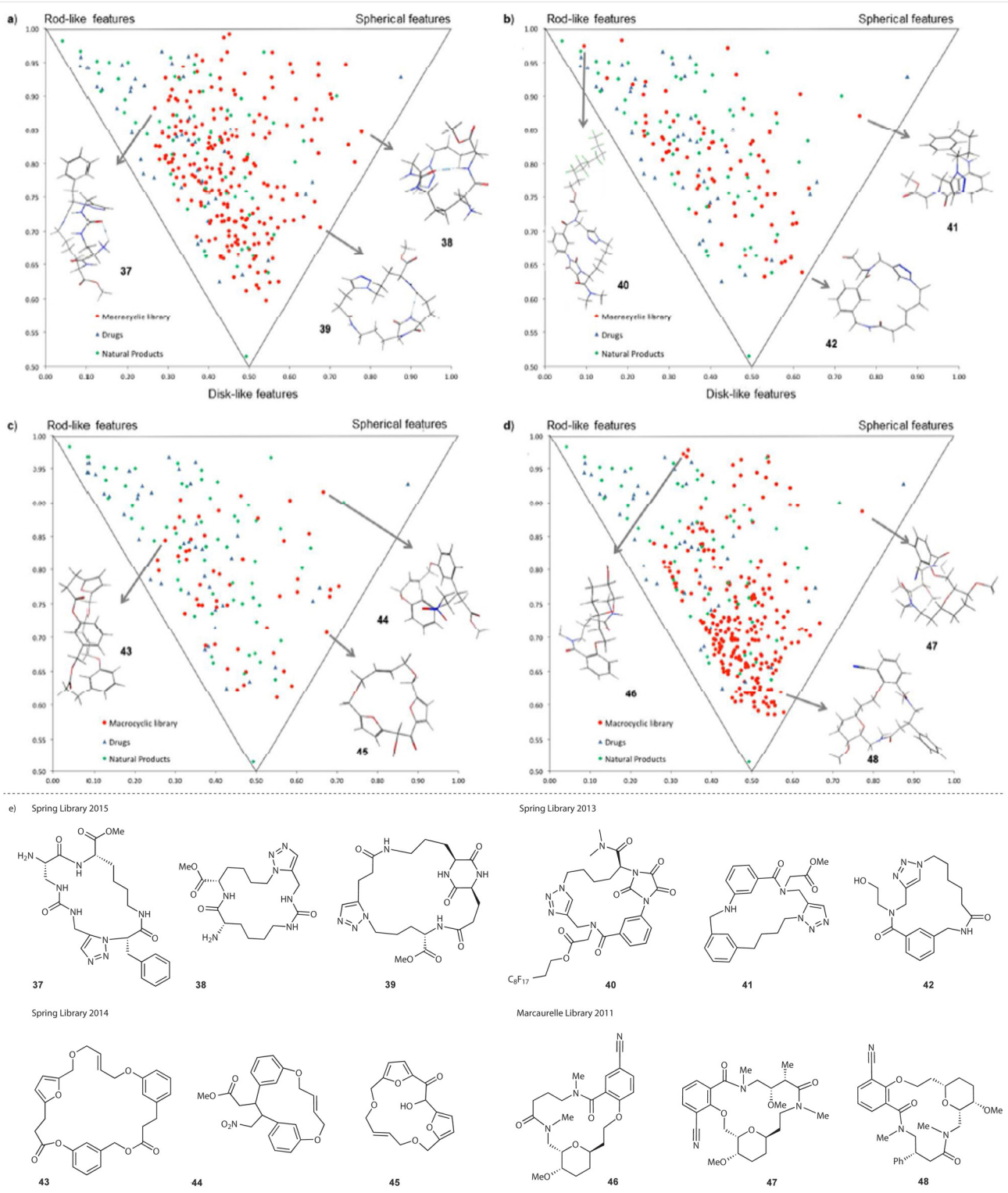


Figure 2 The shape diversity of select DOS libraries (red dots) compared with reference sets of 40 top-selling drugs (blue triangles) and 60 diverse natural products (green circles) by means of principal moments of inertia (PMI) analysis. (a) Combined typical and advanced peptidomimetic library, Spring library 2015.[38,54] (b) Multi-dimensional coupling library, Spring library 2013.[42] (c) Organocatalytic coupling library, Spring library 2014.[59] (d) Fragment-based domain shuffling library, Marcaurelle library 2011.[39] The lowest energy conformation of each library member is plotted between the three extremes of molecular shape type [conformational search by molecular operating environment (MOE) software package]. (e) Chemical structures of representative compounds **37**–**48** highlighted in (a)–(d)

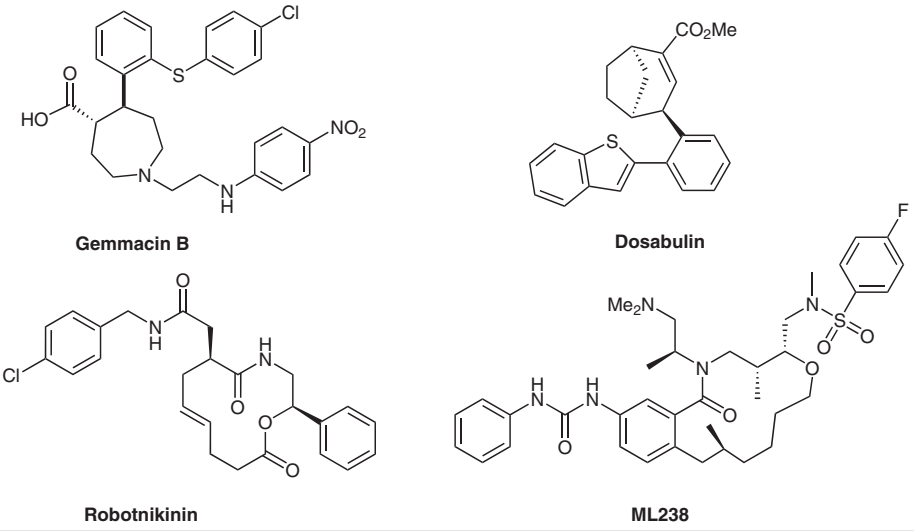


Figure 3 Biologically active compounds discovered by diversity-oriented synthesis

ical properties of the resulting libraries are not well understood, and therefore we are pursuing this area of research currently. We also expect to see advancements in synthetic strategies targeted at enhancing saturation[100] and three-dimensionality.[99] These factors are associated with an increased capacity to probe unexplored areas of chemical space, and also with higher success rates in drug discovery programs.[100,101]

Another research topic that has seen a surge of interest in recent years is the use of natural products as starting points for DOS,[102] and this trend looks likely to continue. Natural products are typically structurally complex (they are particularly rich in unusual ring systems) and functionally dense. As such they possess many synthetic 'handles' for diversification. They therefore represent new opportunities for library synthesis, as demonstrated recently in Hergenrother's complexity-to-diversity strategy and recent developments in the use of carbohydrates as stereochemically rich and densely functionalised building blocks.[103–105] The development of each of these strategies will be guided, in part, by improving compound filtering and selection techniques. Despite their aforementioned drawbacks, these methods remain necessary to minimise problems associated with assay interference (PAINS) or poor absorption, distribution, metabolism, elimination and toxicity (ADMET) properties associated with late-stage attrition.[106] We expect that the techniques employed will continue to be refined, as will new guidelines to better define selection criteria, druggability and drug-likeness.[107,108]

Drug discovery and chemical biology rely on the development of robust synthetic strategies for the generation of chemical libraries that can adequately interrogate biologically relevant chemical space.[109] The DOS approaches described herein represent pertinent examples of such strategies, and continued development of these methodologies inspires optimism for the identification of novel macrocyclic therapeutics and chemical probes.

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