Scaffold Diversity

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Scaffold Diversity Synthesis and Its Application in Probe and Drug Discovery

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Scaffold diversity is a crucial feature of compound collections that has a huge impact on their success in biological screenings. The synthesis of highly complex and diverse scaffolds, which could be based on natural products, for example, is an arduous task that requires expertise in various aspects of organic synthesis and structural analysis. This challenge has been addressed by a number of synthesis designs, which employ natural products as a source of scaffold diversity, transform suitably designed common intermediates into various molecular frameworks, or entail highly concise synthetic routes to a number of distinct and complex scaffolds. In this Minireview, we highlight recent synthetic developments towards the construction of diverse and complex scaffolds and the application of the resulting compound collections in drug and probe discovery.

1. Introduction

The art of organic synthesis provides chemists with a great toolbox for the creation of novel structures with new properties and thereby prodigiously impacts on human life.[1] Since Wöhler's synthesis of urea, the field has witnessed tremendous developments in the construction of small to complex molecules of natural origin and has crucially supported the medical sciences.[2] Since 2000, aside from its traditional importance to drug discovery, organic synthesis has faced further challenges with regard to the delivery of small-molecule probes for improving our understanding of different biological processes.[3] The use of small molecules in chemical biology, nanomedicine, and chemical genetics, for example, has undoubtedly broadened the horizons of chemical synthesis.[4] At the heart of these applications lies the search for biologically active small molecules, which are often identified through biological screenings of compound libraries. Lessons learnt from disappointing high-throughput screenings (HTS) of combichem libraries, which largely comprise structurally similar molecules, in the mid-1990s marked the beginning of divergent chemical synthesis approaches,[5] in particular the diversity-oriented synthesis (DOS) strategy pioneered by Schreiber and co-workers.[6] The idea was to expand the existing chemical space with new synthetic molecules, hoping to identify novel and better drug and probe molecules.[7] In the words of Burke and Schreiber:[6a] *"In an ideal DOS pathway all of the products of one diversity-generating process are substrates for another, thus making it possible to use split-pool synthesis to access combinatorially matrices of building blocks, stereochemical isomers, and even molecular skeletons."* DOS thus made a smarter use of combichem techniques to quickly access new, diverse, and complex molecules.[8] Meanwhile, biology-oriented synthesis (BIOS), a concept developed by the Waldmann group, emerged as an interesting but arguably more challenging concept that aimed on the one hand to identify natural product (NP) based common ligands for functionally diverse proteins embodying similar ligand-binding sites[9] and on the other hand to explore compound collections based on biologically prevalidated scaffolds[10] in chemical biology and medicinal chemistry.[11] The quality of compound libraries thus began to gain attention.[12] The focus of synthetic chemists moved to the diversity and complexity of the core structures or scaffolds present within a compound collection.[13]

The scaffold is the core molecular framework that provides the basic shape, rigidity, or flexibility of a molecule,[14] and exposes various substituents over its periphery in

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three-dimensional space, which can interact with diverse biological targets. Scaffold diversity is therefore the most important feature of a compound library that determines its success in screening endeavors,[15] in particular when identifying bioactive molecules in unbiased phenotypic screenings or modulators of ill-defined or novel biological targets where rational ligand design is rather arduous. In such scenarios, a compound collection rich in scaffold diversity stands better chances to identify hit and lead molecules.[16]

Interestingly, and worryingly too, organic synthesis has exploited only a small percentage of the plausible chemical space.[17] The precarious structural space that the pharmaceutical industry has been scrutinizing in HTS campaigns further adds to the capriciousness of drug discovery.[18] The lack of efficient synthetic access to a large number of NP-based scaffolds, which could provide a potential source of lead molecules in probe and drug discovery, remains a major reason for the fact that commercial libraries of limited structural diversity and complexity are still preferred for screening purposes.[15c,19] The generation of compound collections with highly complex and appropriately decorated molecular scaffolds requires an approach that integrates the logic and methodology of NP total or divergent total syntheses and the efficacy of combichem methods. At the same time, concise complexity-building transformations of easily accessible substrates could provide practical synthetic solutions to building compound collections of structurally rich small molecules.

Scaffold diversity has been an important goal in pharmaceutical research as well as in synthesis approaches, such as DOS or lead-oriented synthesis, developed in academic research groups.[20] However, when the target scaffolds are highly complex, the rules of these approaches are often not easy to implement in synthesis designs. For instance, the de novo synthesis of a compound collection based on the structure of a complex NP scaffold might not be feasible in a few steps (5–6) as demanded by DOS. Molecular complexity often renders even simple transformations extremely challenging to optimize. Therefore, parallel synthesis or split-pool methods might not be feasible with structurally distinct and differently decorated scaffolds. In this Minireview, we focus on the recent developments in building compound collections that are based on complex molecular scaffolds, in particular on NP structures. We also briefly present emerging trends in divergent and concise synthesis design that afford structurally diverse and complex scaffolds. Some applications of the resulting compound collections in medicinal chemistry and chemical biology are also highlighted. In some of the presented examples, an overlap with other divergent synthesis approaches may be unavoidable; however, we did not include examples from already extensively reviewed topics, such as DOS. Readers interested in the various other divergent synthesis approaches and their applications are referred to the cited references.[8,20,21]

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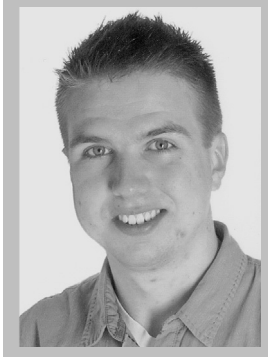

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Kamal Kumar was born in Amritsar, India. He studied Pharmaceutical Sciences at Guru Nanak Dev University, Amritsar, and later completed his Ph.D. under the supervision of Prof. M. P. S. Ishar at the same university. He moved to the MPI Dortmund in 2004, and since 2006, he has been leading a group in the Department of Chemical Biology at the same institute. His research interests include the development of concise methods for the synthesis of natural-product-based libraries, cascade reactions, catalytic and asymmetric cycloaddition/annulation reactions, and probing biological functions with small molecules.

1.1. Scaffolds and Scaffold Diversity

Defining the term "scaffold" is very subjective and may depend on both the chemist and the context in which it is discussed. For a synthetic chemist, a scaffold might be a ring system or a target framework for which a synthetic strategy is designed, whereas a medicinal chemist would define a scaffold as the core structure required for a given pharmacological activity, that is, a pharmacophore. Murcko et al. defined a scaffold as a framework derived from molecules by removing side-chain atoms while preserving the atoms in the ring systems or linking ring systems and the sp^2 atoms directly bonded to these atoms.[22] Each molecule can be further dissected into rings as sub-structures of $n+1$ levels, which are numbered sequentially from level 0 (one single remaining ring) up to level n (the whole molecule). The level $n-1$ is termed "Murcko framework" or "Murcko scaffold" (Figure 1). A scaffold tree is a hierarchical arrangement of ring systems or scaffolds. For instance, in the scaffold tree of lysergic acid amide (**I**), removing the amido and methyl side chains reduces it to Murcko scaffold **II**.[22] Further dissection of the complex Murcko scaffold **II** by cleaving linker bonds between the rings provides different levels of scaffold simplification (Figure 1a).[23] A different terminology is used in the cyclic skeleton (CSK)[24] concept. Here, aside from the removal of appendages, all non-hydrogen atoms are converted into sp^3 carbon atoms (**VI**; Figure 1a). Thus, every CSK represents a range of equivalent scaffolds that differ only in their heteroatom scaffold substituents (e.g., benzene and pyridine). Yet another definition that is popular among medicinal chemists is that the scaffold is the largest central cyclic system, while the smaller ring substituents (decorations) do not belong to the scaffold.[25]

Waldmann and co-workers developed an approach for the structural classification of NPs (SCONP) that is based on the hierarchical classification of scaffolds that consist of ring systems and their aliphatic linkers but not other chains.[10b] The scaffold tree enables the organization of scaffolds into a tree-like pattern, as scaffolds are simplified by the removal of one ring at a time until the parent scaffold, as the simplest unit, is reached. Chromones, for instance, are the parent scaffold of flavones and isoflavones (Figure 1b). Priority rules were defined to ensure that less characteristic, peripheral rings are removed first to afford well-defined chemical entities, thus making the classification chemically intuitive to chemists designing synthetic plans (Figure 1c).

In a generally accepted definition that is meaningful to compound libraries, a molecular scaffold may be described as the minimum core structure that represents a compound collection and defines the spatial arrangement of the substituents or appendages. Different scaffolds can place the same functionality in different positions in a three-dimensional space and thus are the key to interactions with different protein targets.[25] In fact, a scaffold provides more than the spatial orientation of appended functional groups. Even simple carbocyclic scaffolds without any hydrogen-bond acceptors and donors can interact with other molecules, such as proteins, through van der Waals interactions, hydrophobic effects, or non-classical hydrogen bonds[26,27] and thus have a greater role in modulating biological activity. A compound collection rich in scaffold diversity provides ample opportunities for interactions between small compounds and macromolecules.

Analyzing the scaffold diversity of compound databases has multiple applications in medicinal chemistry and drug discovery, including library design, compound acquisition, virtual screening, and the assessment of structure–activity relationships. However, such an analysis of compound collections is not a trivial task and depends on the specific approach applied to generate the scaffolds as well as on the

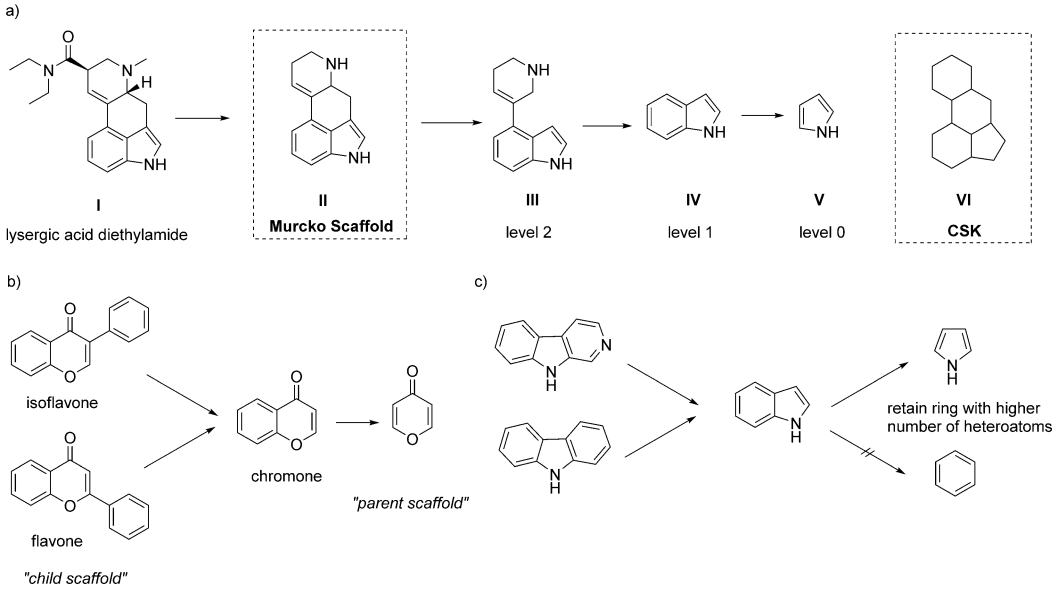


Figure 1. a) Scaffold-tree representation of lysergic acid diethylamide with different scaffold levels and a cyclic skeleton (in the right box). b) Generation of simplified NP scaffolds and c) one of the thirteen rules guiding the parent–child assignment in the newly developed scaffold-tree-generating algorithm.

size of the database. There are different computational methods to derive the scaffold of a molecule in a systematic and consistent manner and to analyze scaffold diversity. However, a detailed discussion of these methods is beyond the scope of this Minireview, and readers are referred to the cited references for further information.[19,28] Briefly, the number of scaffolds is recorded along with the number of scaffolds that is only represented by one compound in the library (i.e., singletons). The scaffold frequency count, that is, the fraction of scaffolds relative to the dataset size (number of scaffolds divided by the size of the database), and the fraction of singletons relative to the dataset size provide an assessment of the scaffold diversity. The distribution and diversity of different scaffolds in compound collections can also be analyzed using measures such as the Shannon entropy (SE) approach or cumulative scaffold recovery (CSR) curves.[29,30] Whereas SE provides a measure for the distribution of scaffolds in a library, in CSR, the fraction of compounds is plotted against the fraction of scaffolds, and the gradient provides the degree of scaffold diversity for a given compound collection. The parameter F_{50} enumerates the fraction of scaffolds that represent 50% of the molecules of a given library; the lower the value, the higher the diversity.

Molecular fingerprinting and related techniques that turn molecules and their properties into a sequence of bits that can be easily compared with those of other molecules, are common and popular methods in drug discovery and virtual screening to assess the features of a given compounds.[31–33] Extended connectivity fingerprints (ECFPs) can be used to analyze scaffold structures. In ECFP_6, for instance, all structural features within the range of six bonds of each scaffold atom will be included in the fingerprint and can be interpreted as substructures.[34,35] Thus the fingerprint covers the complete molecular environment. The Tanimoto coefficient is a measure of the similarity of different fingerprints, ranging from zero for no similarity to one for identical structures.[36]

A novel two-dimensional scaffold fingerprint (SFP) was presented by Rabal et al.[37] for mining ring fragments (Figure 2). This method not only encodes the usual 2D and 3D descriptors, such as shape, topology, heteroatoms, bridges, spirocyclic centers, diversity points, sp^3 carbon atoms, and chirality, but also pharmacophoric features, such as the number of hydrogen-bond donors and acceptors and their relative orientation (vectors), as they play a critical role in drug discovery. SFP can be used 1) to identify alternative chemotypes, including bioisosteres, to a reference ring either in a visual mode or by running quantitative similarity searches and 2) in chemotype-based diversity selection.

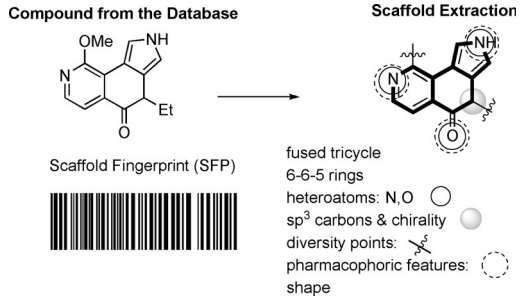


Figure 2. Generation of a scaffold fingerprint from a structure, indicating which parameters are influenced by the corresponding structural features.

2. Synthetic Approaches to Scaffold Diversity

The molecular architectures of NPs are intriguing and challenging synthetic targets that inspire many organic chemists to take up tedious and time-consuming total synthesis projects. Often, these endeavors are unable to provide adequate numbers and amounts of NPs and their derivatives for screening purposes. Therefore, modern probe- and drug-discovery programs have resorted to employ a significant number of accessible synthetic chemical libraries for HTS campaigns.[38] However, the failure of this approach to deliver the expected returns has turned the focus back onto the structural complexity and diversity of the compound libraries. To this end, mainly two types of synthesis objectives have been targeted. In the first one, structural diversity around a given class or type of scaffold is targeted. For instance, synthetic methods that transform an available NP into different scaffolds not only diversify its molecular complexity, but also afford novel structures with structural features of the parent NP embedded in a novel molecule. In the second case, the aim is to build rather unbiased scaffold diversity, but in a very concise and efficient manner. For instance, synthesis designs based on cascade or domino reactions afford diverse scaffolds with varying molecular complexity. In many of these cases, a designed common precursor or intermediate enables different inter- and intramolecular reactions leading to highly complex and diverse frameworks. With this approach, scaffold diversity is built up in a quick and efficient manner.

2.1. Creating Diversity Based on Natural Product Scaffolds

Most of the NPs produced by microorganisms or plants were actually not produced to bind human proteins; however, owing to conservation and similarities in the structural domains of human drug targets and the targets for these NPs, these secondary metabolites have a privileged status. Many of the currently marketed drugs, particularly in the fields of oncology and infectious diseases, are either NPs or of NP origin.[39] The structural differences between combichem molecules, drugs, and NPs clearly indicate that NPs interrogate a different and broader chemical space.[40] NPs embody stereochemically and sp^3-rich as well as 3D complex scaffolds and exhibit potent biological activities with desirable pharmacological profiles. These characteristics of NPs have fueled great interest in producing synthetic libraries that retain the structural features of NPs in various manners and can be explored[41] in drug and probe discovery.[5b,42] To avoid the tedious de novo construction of complex NP-based molecules, easily accessible NPs can themselves be used as a source for the synthesis of novel molecular scaffolds. The following examples represent some of the brilliant synthetic efforts for the transformation of NPs that were reported in recent years

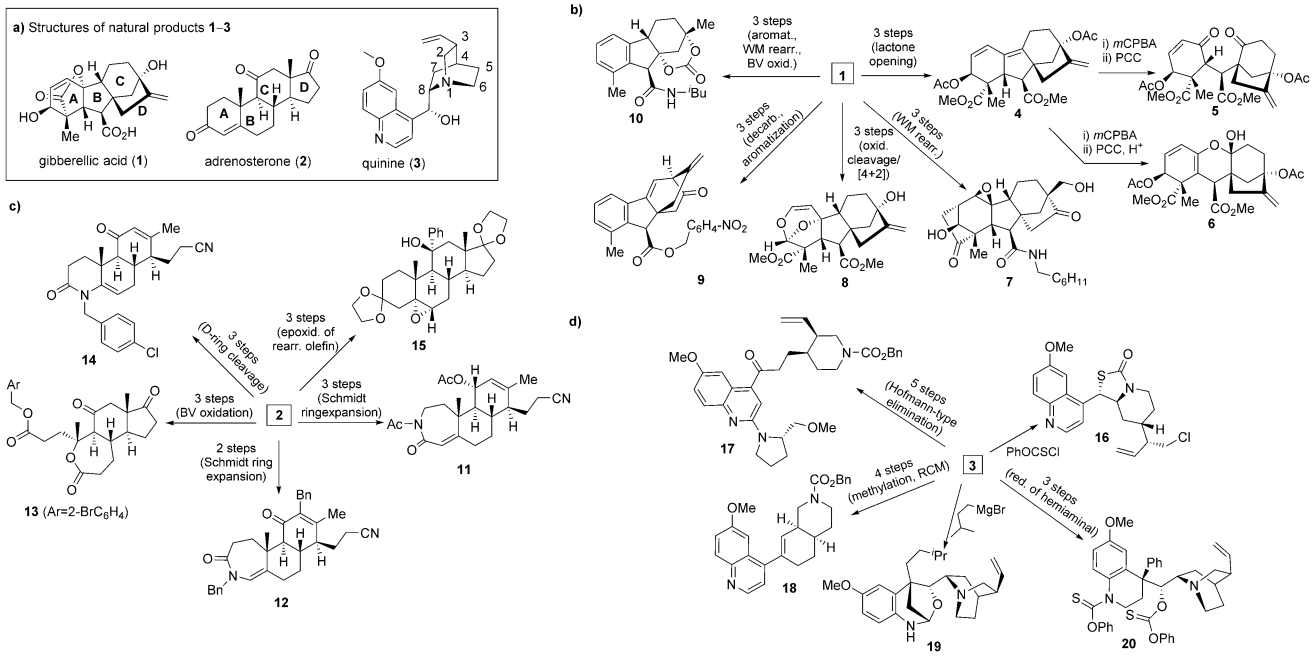
and have not only raised the bar for the quality of molecules in compound collections but have also added a fresh impetus to the concept of building NP-based and -derived scaffold diversity.

Hergenrother and co-workers reported[43] a novel synthesis approach wherein the core ring structures of readily available and structurally complex NPs were selectively rearranged by ring-distortion reactions and, in an average of three chemical steps, transformed into markedly diverse and distinct core scaffolds (Scheme 1). The scaffolds thus generated were inherently rich in structural as well as stereochemical complexity. The approach took cognizance of how nature constructs diverse NPs from common intermediates[44] and was influenced by previously developed processes around these NPs. In this first divergent scaffold-diversity synthesis employing NPs, gibberellic acid, adrenosterone, and quinine were each converted into a series of novel and diverse scaffolds by combinations of ring-cleavage, ring-expansion, ring-fusion, and ring-rearrangement reactions (Scheme 1).

Gibberellic acid (**1**) is a plant hormone and contains a tetracyclic diterpene core with a fused lactone, two allylic alcohols, an exocyclic olefin, and a carboxylic acid unit. These functional groups can facilitate the selective and independent functionalization of each ring of the core structure by a variety of reactions that distort the ring system and afford novel scaffolds. Opening of the lactone on gibberellic acid (**1**) and derivatization of the carboxylic acid and hydroxy groups afforded tetracyclic triene **4** (Scheme 1b). Interestingly, the tetrasubstituted olefin in **4** yielded two distinct scaffolds, **5** and **6**, under neutral and acidic oxidative conditions, respectively. Rearrangement of the fused lactone ring in **1** under basic conditions followed by amidation and epoxidation produced compound **7** by a Wagner–Meerwein (WM) rearrangement. In another three-step transformation, oxidative cleavage of the vicinal diol generated after cleavage of the lactone moiety and olefin isomerization in ring A was followed by intramolecular [4+2] cycloaddition affording acetal **8**. In two different sets of three-step reaction sequences that involve the decarboxylation and aromatization of **1** or a WM rearrangement of **1** followed by a Baeyer–Villiger (BV) oxidation as key reactions, scaffolds **9** and **10**, respectively, were efficiently built up.

Adrenosterone (**2**), a steroid hormone produced in the adrenal cortex of mammals, contains five contiguous stereogenic centers. Four of the carbocyclic rings of **2** are functionalized with an enone or ketone. These key functional groups provide synthetic handles to transform **2** into a set of diverse frameworks in few synthesis steps. By exploiting ring-expansion processes, namely a Schmidt reaction and a BV oxidation, and selective ring-opening reactions, five distinct and complex scaffolds (**11**–**15**) with functional groups for further modifications were generated (Scheme 1c).

The alkaloid quinine (**3**), with two distinct ring systems and a tertiary amine, a secondary alcohol, an olefin, and a quinoline unit as key functional groups, is an ideal candidate to create diverse molecular scaffolds by the ring-distortion approach. Selective cleavage of the quinuclidine ring at the N1–C2 bond with *O*-phenyl chlorothionoformate triggered an in situ diastereoselective rearrangement of the free alcohol unit leading to thiocarbamate **16**. Acid-catalyzed Hofmann-type elimination of **3** with cleavage of the N1–C8 bond afforded scaffold **17** in few steps when (*S*)-2-(methoxymethyl)pyrrolidine was used as the external nucleophile. Alternatively, ring-closing metathesis (RCM) of a common inter-



Scheme 1. Hergenrother's ring-distortion strategy to build NP-derived diverse scaffolds (key reactions are given in parentheses). a) NPs employed. b–d) Generation of diverse molecular scaffolds from gibberellic acid (**1**; b), adrenosterone (**2**; c), and quinine (**3**; d). Bn = benzyl, *m*CPBA = *meta*-chloroperbenzoic acid, PCC = pyridine chlorochromate.

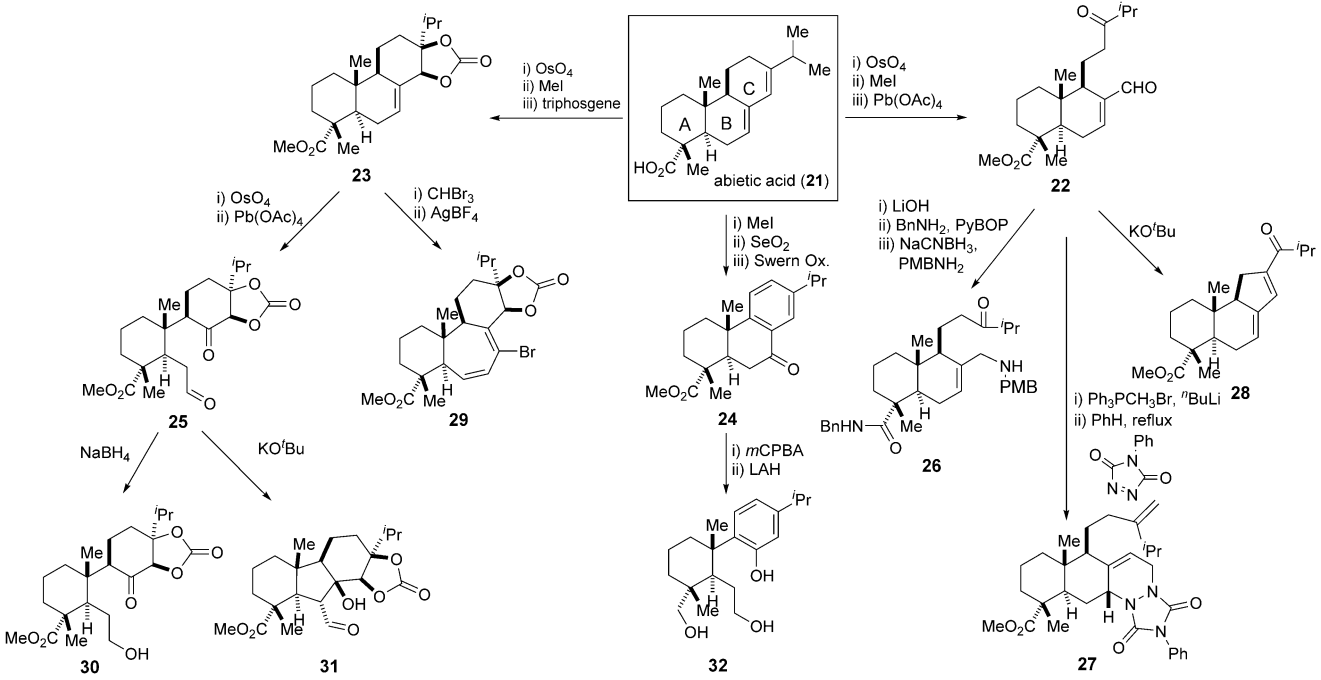
mediate led to [4.4.0] bicyclic scaffold **18** (Scheme 1 d). In an interesting mode of cyclization, nucleophilic addition of isoamylmagnesium bromide to the pyridine ring in **3** was followed by formation of a cyclic hemiaminal, thus affording **19** as a single diastereomer. Reduction of a similar hemiaminal formed by addition of phenylmagnesium chloride to **3** followed by bis-acylation led to scaffold **20** as the major product (Scheme 1 d).

Twelve of the 49 scaffolds thus generated were further functionalized by coupling reactions with various building blocks to produce additional 119 library members, demonstrating the utility of these scaffolds in building focused compound collections. This collection of NP-derived molecules was found to be richer in stereochemical content (an average of 5.2 vs. 0.2 stereocenters) and bond saturation (average C_{sp^3} content: 0.59 vs. 0.23), and had a hydrophobicity that was over ten times lower that that of the commercial ChemBridge library (2.9 vs. 4.0 average *c* log *P*, where *P* is the octanol/water partition coefficient). The molecular fingerprints of the scaffolds showed very low pair-wise similarities (Tanimoto coefficients), indicating high structural diversity in the compound collection.

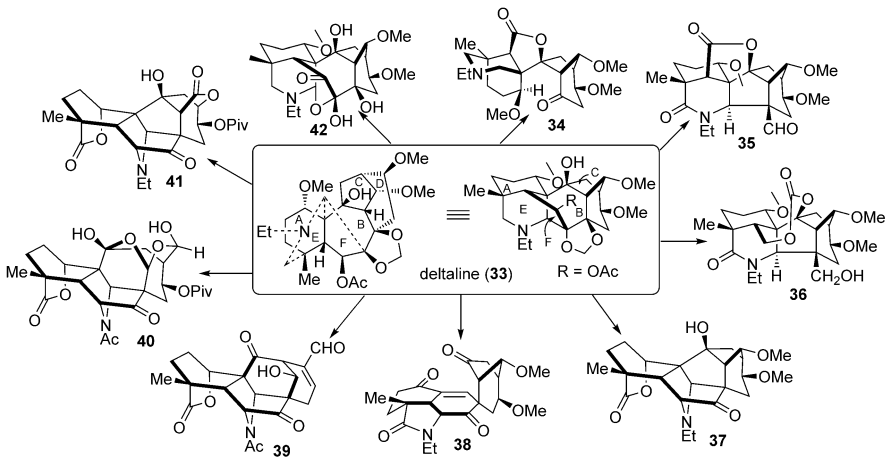
Hergenrother and co-workers applied a similar complexity-to-diversity approach to another NP, abietic acid (Scheme 2).[45] Reaction sequences were designed to rapidly modulate the core ring system of abietic acid (**21**). To this end, under oxidative reaction conditions, simple ring-expansion and -contraction transformations were utilized to deliver the new scaffolds **22**–**26**. Additional one or two transformations on these scaffolds yielded NP-like molecules **27**–**32**, which contain novel scaffolds amenable for the synthesis of compound collections. A collection of 84 complex compounds with ring systems of lower and higher orders in relation to the parent NP scaffold were generated, covering a large chemical space around the given NP structure.

The C19 diterpenoid alkaloid deltaline (**33**; Scheme 3) is a nicotinic acetylcholine receptor antagonist and structurally a staggeringly complex molecule. Deltaline is based on a hexacyclic, cage-like, densely substituted scaffold and poses a formidable synthetic challenge for the complexity-to-diversity approach. Wang and co-workers successfully adapted the ring-distortion approach to transform deltaline into 32 highly complex, diverse, and unique frameworks in less than ten synthetic steps each on the milligram to multi-gram scale.[46] Some of the 32 scaffolds constructed from deltaline (**34**–**42**) are depicted in Scheme 3. What kind of scaffold-transforming reactions can be used on a given NP depends on the functional groups of the NP. For deltaline, which is a highly oxygenated NP with more than one hydroxy group, a pinacol rearrangement, lactonization, Grob fragmentation, alcohol-to-ketone oxidation, and BV oxidation were performed to obtain novel ring systems. It is highly remarkable that simple methods and reagents can enable efficient and facile chemical transformations in rather complex molecular settings.

Bryonolic acid (**43**), a pentacyclic triterpenoid isolated from the common zucchini in multi-gram quantities (1.34 % yield by mass), and lanosterol (**44**), an intermediary product in the biological transformation of squalene into cholesterol, have a unique double bond between the steroidal B and C rings (Scheme 4). Tochtrop and co-workers systematically explored the oxidative cleavage of protected bryonolic acid (**43**) as well as lanosterol (**44**) by exploiting the alkene moiety to rearrange the carbocyclic skeletons of these abundant NPs



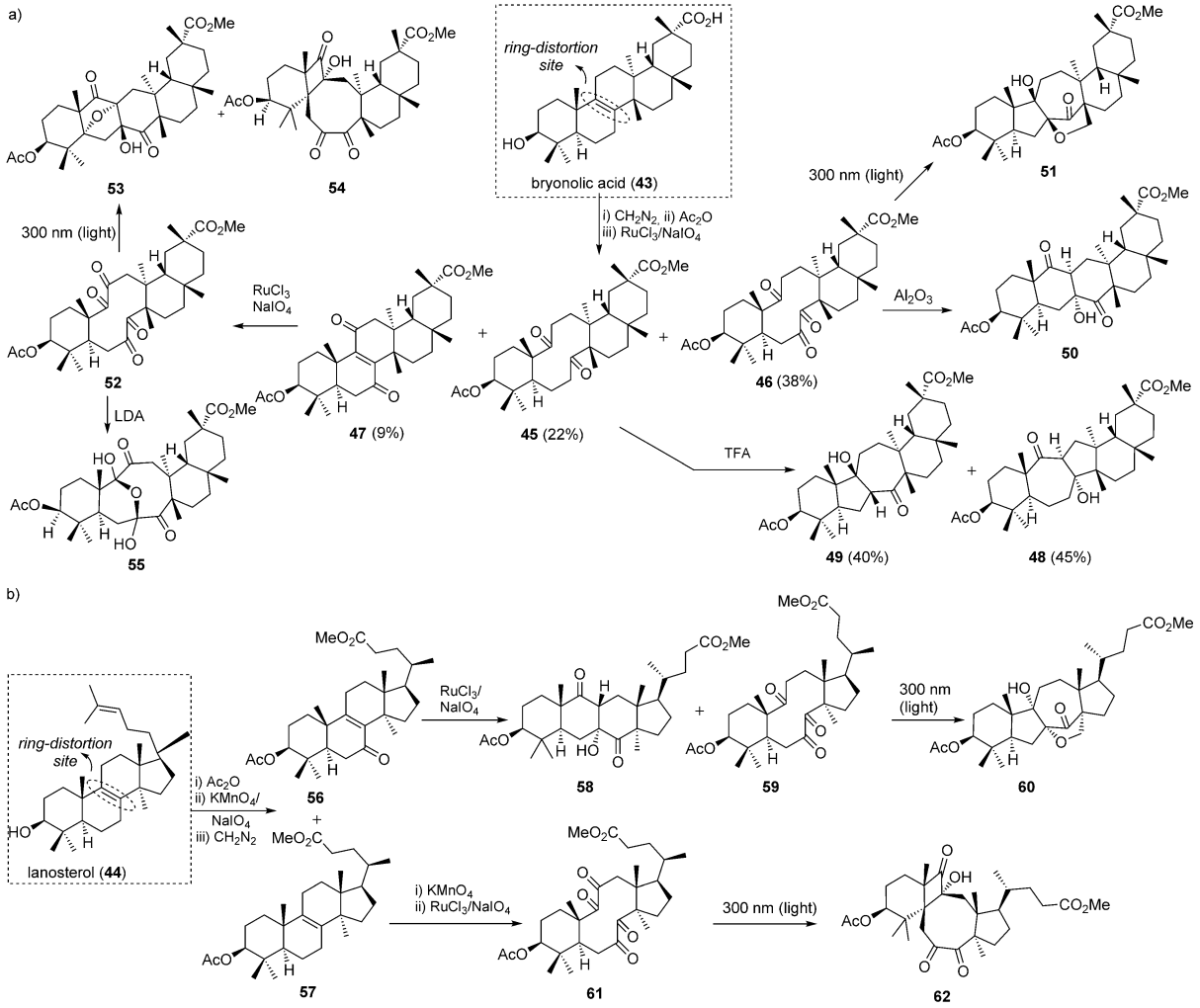
Scheme 2. Ring-distortion approach to build diverse molecular scaffolds from abietic acid. PMB = *para*-methoxybenzyl, PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate.



Scheme 3. Ring-distortion approach to generate diverse molecular scaffolds from deltaline. Piv = pivaloyl.

to generate scaffold diversity.[47] Two types of chemical approaches were applied to the di- and triketones **45**–**47**, which were generated from acylated bryonolic acid or its derivatives by oxidative cleavage of the double bond between the steroidal B and C rings using ruthenium chloride (Scheme 4a). First, the phototransformation potential of bis(ketones) **46** and **52** was exploited in a Norrish–Yang reaction to form the novel scaffolds **51**, **53**, and **54**. Second, transannular aldol reactions under different reaction conditions were expediently performed on these polycarbonyl substrates to afford molecules with NP-derived scaffolds (**48**–**50**, **55**; Scheme 4a).

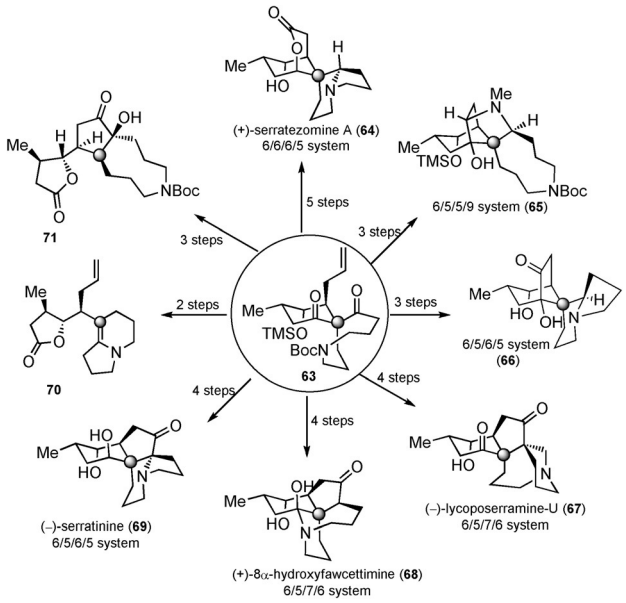
The ring-fusion olefin in the protected lanosterol derivatives **56** and **57** was cleaved under oxidative conditions to afford the corresponding carbonyl compounds **59** and **61**, which underwent similar photochemical transformations to afford the unique complex scaffolds **60** and **62** (Scheme 4b). Although the yields for some of these phototransformations



Scheme 4. Ring diversification of polyketones derived from a) bryonolic acid (**43**) and b) lanosterol (**44**). LDA = lithium diisopropylamide, TFA = trifluoroacetic acid.

were relatively low, the field of photochemistry is currently receiving great interest and has enormous potential to provide highly unique molecular frameworks that cannot be built by other conventional synthetic means.

Resorting to a NP as a substrate to build different molecular scaffolds is inherently advantageous over the de novo construction of complex molecules. This approach naturally shortens the synthesis, and therefore even the moderate yields of some difficult transformations, such as photochemical reactions, can be accommodated. Furthermore, inheriting the structural features as well as the molecular properties of NPs enormously raises the potential of NP-derived molecules as modulators of different biological functions. A major limitation of this strategy remains the accessibility of NPs, in particular of those that are rich in chemical functionalities that facilitate scaffold diversity. Advances in NP isolation techniques as well as in preparative biosynthesis could provide further sources of complex molecules that can be employed in scaffold diversity synthesis.



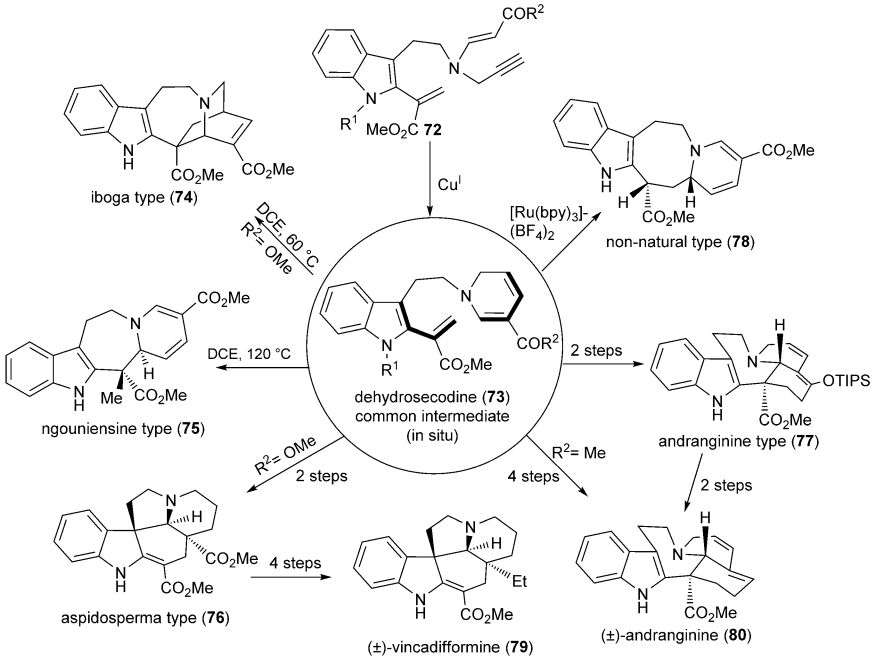
Scheme 5. Synthesis of Lycopodium alkaloid based molecules. Boc = *tert*-butoxycarbonyl, TMS = trimethylsilyl.

2.2. Emerging Strategies towards the Concise and Efficient Construction of Complex and Diverse Scaffolds

In recent years, a surge in the demand for compound libraries based on scaffolds that are missing from or ill-represented by compound collections already available to the pharmaceutical industry has been observed. To access biologically relevant and novel chemical space that could greatly influence and speed up the discovery of new medicines, the pharmaceutical industry has shown its willingness to share risks and collaborate with academic research partners. A common interest and goal is the development of short, feasible, and affordable as well as to some extent scalable syntheses of structurally rich compound libraries. Many research groups have thus developed concise synthetic routes to complex molecular frameworks. In fact, providing feasible synthetic access to a number of NP-based scaffolds could fill the vacuum generated by many pharmaceutical companies discontinuing their NP research. An emerging trend in this direction utilizes a carefully designed common precursor that can be subjected to different cyclization/cycloaddition reactions to construct diverse scaffolds.

Inspired by the functional-group pairing patterns in Lycopodium alkaloids, X. Lei et al. designed a common spirocyclic intermediate **63** to generate structurally complex and diverse scaffolds (Scheme 5).[48] The identification of different functional-group pairings on bicyclic substrate **63** was key to successfully build different highly complex and NP-like scaffolds. Remarkably, ten highly complex alkaloid NP-like scaffolds that also included four NPs, namely (+)-serratezomine A (**64**), (−)-serratinine (**69**), (+)-8α-hydroxyfawcettimine (**68**), and (−)-lycoposerramine-U (**67**), were synthesized in a concise and parallel process (Scheme 5).[49]

The way that biosynthetic strategies employ divergent intramolecular cyclizations of a common intermediate to produce diverse arrays of scaffolds inspired Mizoguchi et al. to employ dehydrosecodine (**73**; Scheme 6) as a common



Scheme 6. Biosynthesis-inspired approach to furnish diverse alkaloidal scaffolds. bpy = bipyridine, DCE = 1,2-dichloroethane, TIPS = triisopropylsilyl.

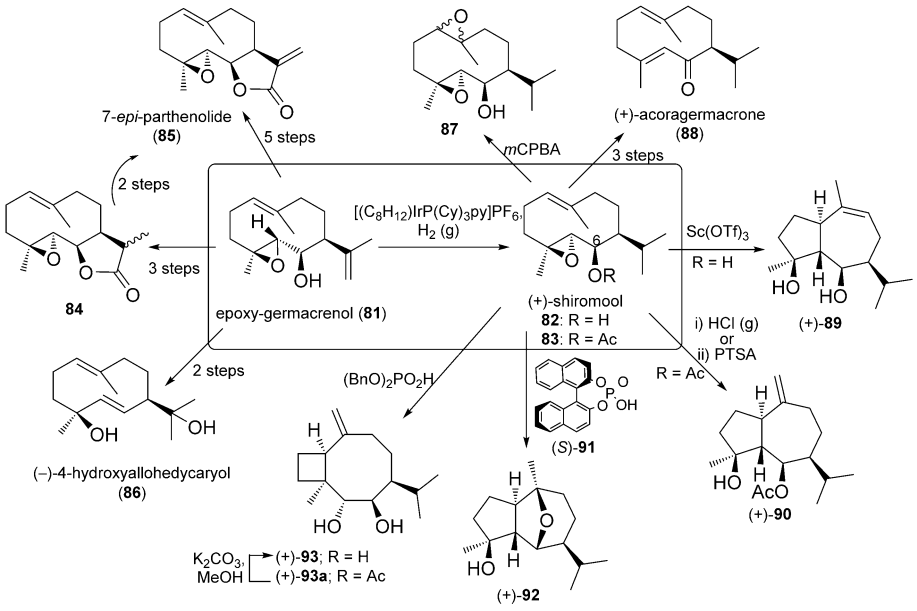
intermediate to build different polycyclic indole scaffolds.[50] Dehydrosecodine has been proposed as an intermediate in the biosynthesis of many indole alkaloid NPs.[51] Therefore, the polyunsaturated structure of dehydrosecodine has pre-encoded chemical information to build the architectural complexity as well as the scaffold variation of the cognate alkaloids.[51] The synthesis was designed to generate the reactive and oxidation-prone intermediate **73** in situ from a precursor enyne **72** (Scheme 6). Different cyclization and cycloaddition reactions were conducted with this common intermediate, affording highly complex NP-like molecules either directly from **73** or in few more steps (Scheme 6). The divergent and concise method provided access to four naturally occurring scaffolds (**74**–**77**) and a non-natural variant **78**, each within six to nine steps from tryptamine. The versatile reactivity of intermediate **73**, which undergoes different annulation reactions, was further demonstrated in the short synthesis of the NPs (+)-vincadifformine (**79**) and (+)-andranginine (**80**; Scheme 6). This approach thus enabled the systematic diversification of the skeletal, stereochemical, and functional-group properties of naturally occurring alkaloids without much structural simplification.

In another biosynthesis-inspired synthesis of scaffold diversity, Baran and co-workers explored various skeleton-modifying transformations on two key intermediates, namely epoxy-germacrenol (**81**) and (+)-shiromool (**82**), which were generated in few steps from commercially available farnesol and afforded germacrene-type NPs as well as polycyclic sesquiterpene frameworks (Scheme 7).[52] In three simple steps, that is, acetylation, hydroboration, and oxidation of the formed diol, 11,13-dihydro-*epi*-parthenolide (**84**) was obtained as a mixture of diastereomers. α-Bromination of lactone **84** followed by dehydrobromination gave rise to 7-*epi*-parthenolide (**85**). Hydroxyallohedycaryol (**86**) was obtained in a four-step sequence from **81** by homoallylic alcohol directed epoxidation, chemoselective reduction of the less hindered epoxide, mesylation, and reductive elimination of the corresponding epoxy mesylate with lithium naphthalenide.

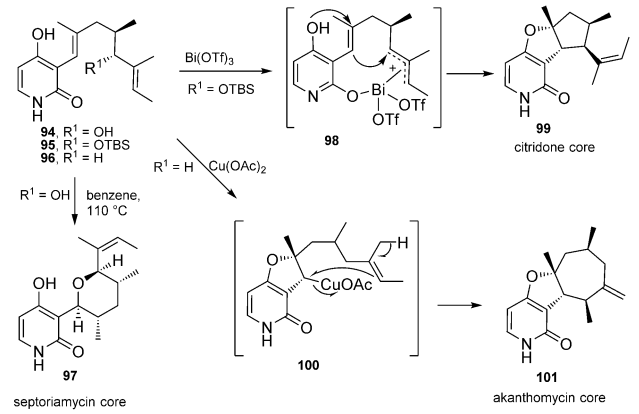
Chemoselective hydrogenation of the isopropylidene side chain in **81** using Crabtree's catalyst afforded (+)-shiromool (**82**) in good yield. Epoxidation of **82** with *m*CPBA led to a 4:1 diastereomeric mixture of the natural scaffold **87** in excellent yield. A three-step reaction sequence starting from acetylated (+)-shiromool (**83**) delivered (+)-acoragermacrone (**88**, Scheme 7). After successfully generating the sesquiterpenes **84**–**88**, the guaiane framework was constructed in the compounds (+)-**90**, (+)-**92**, and (+)-**93** by means of various acid-mediated and -catalyzed transformations of common (+)-shiromool derivatives (Scheme 7). This synthetic campaign illustrates an important example of a divergent synthetic process generating a series of NPs and structural variants thereof that are rich in stereochemistry and functional groups.

In 2011, Zografos and Fotiadou described a unique route to the structurally diverse pyridone alkaloids based on a common synthetic scaffold (Scheme 8).[53] The cores of the NPs akanthomycin, septoriamycin A, and citridone A were prepared by highly selective and novel carbocyclization reactions from derivatives of a common intermediate (**94**–**96**). For instance, intermediate **94** generated the septoriamycin core **97** in 66% yield upon heating to 110°C through a 1,5-hydride shift followed by intramolecular Michael addition. With a minor change in substrate, that is, with a protected hydroxy group (**95**), citridone core **99** was obtained upon treatment with bismuth triflate (probably via bismuth amide complex **98**) in 54% yield. In the meantime, when compound **96**, which bears no such substituent ($R^1 = H$), was reacted with copper(II) acetate, a completely different pathway was promoted, leading to the akanthomycin core **101** by a radical 7-*endo*-dig cyclization of intermediate **100**.

Macrocycles are an important class of organic molecules with a variety of impressive biological activities. In fact, a number of macrocycles are currently in development or already approved by the FDA for clinical use.[54] The biological activities of naturally occurring macrocycles may result from their preorganization ability as well as their flexibility, which facilitates the interaction with and binding to biological targets. With regard to macrocycles, scaffold diversity results from variations either in the ring size and the substituents around the ring or in the nature of the bonds forming the macrocycle, and can provide ample opportunities for interactions with biological targets.[55] Aiming to construct diverse mac-



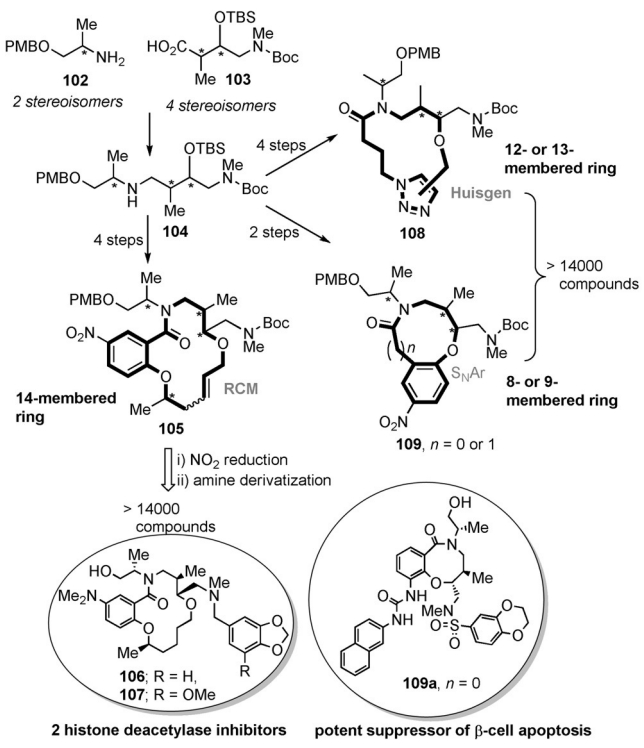
Scheme 7. Collective enantioselective synthesis of germacrenes and related scaffolds. Cy = cyclohexyl, PTSA = *para*-toluenesulfonic acid, py = pyridine, Tf = trifluoromethanesulfonyl.



Scheme 8. Synthesis of septoriamycin and analogues of citridone and akanthomycin. TBS = *tert*-butyldimethylsilyl.

rocycles of various sizes and medium-sized rings, in particular, Marcaurelle et al. reported impressive synthesis efforts, which led to the discovery that certain macrocyclic lactones are histone deacetylase inhibitors.[56]

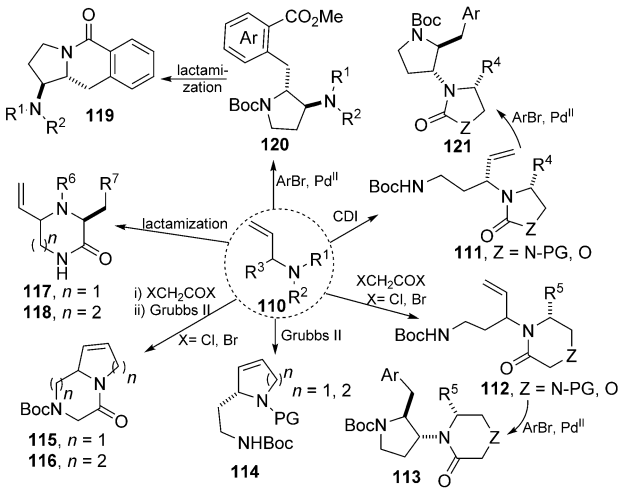
Initially, a series of asymmetric *syn* and *anti* aldol reactions were performed to produce four stereoisomers of a Boc-protected γ-amino acid (**103**; Scheme 9). The coupling of **103** with either of the two stereoisomers of O-PMB-protected alaninol (**102**) followed by amide reduction led to the eight stereoisomers of amine **104** (Scheme 9). To build different macrocycles, **104** was subjected to three different reactions for intramolecular ring formation: nucleophilic aromatic substitution (S_NAr), Huisgen [3+2] cycloaddition, and RCM. This strategy provided a variety of skeletons ranging from eight- to fourteen-membered rings (Scheme 9). The 48 scaffolds thus generated were subjected to further derivatizations using SynPhase Lantern technology, affording more than 30000 compounds. Furthermore, the use of this library in a variety of biochemical and cell-based screens led to the discovery of several low-micromolar histone deacetylase (HDAC) inhibitors.[56a] Another related library (with 6488 members) afforded a potent eight-membered macrolactam as a suppressor of β-cell apoptosis (Scheme 9).[57] Importantly, the presence of all stereoisomers in the screening collection was crucial for the preliminary determination of the structural and stereochemical requirements for cellular activity, leading to an approximately threefold increase in activity compared to the initial hit molecule.



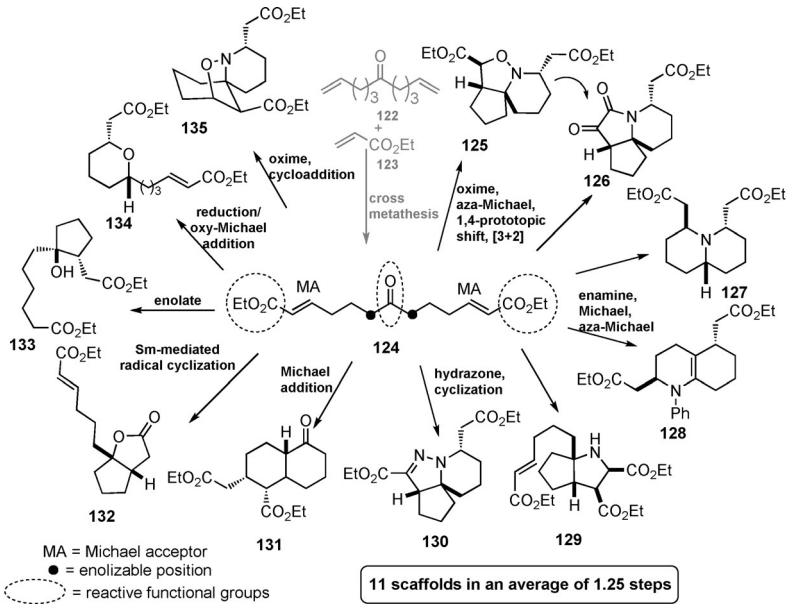
Scheme 9. Marcaurelle's approach to diverse macrocycles.

Nelson and co-workers, who had already developed a lead-oriented synthesis approach to produce molecules with drug-like molecular properties,[20] extended this approach to a short synthesis of diverse lead-like scaffolds, by employing substituted chiral allylic amines (**110**) as common substrates for different cyclization reactions leading to a range of different heterocycles (Scheme 10).[20a] For instance, reactions of appropriately functionalized **110** with carbonyl diimidazole (CDI) afforded molecules of type **111**. Reactions of **110** with α-haloacetyl halides delivered various derivatives **112**. Similarly, Pd-catalyzed aminoarylations of **110** led to scaffolds **120** and (via **111** and **112**) **121** and **113**. Whereas lactamization of **110** yielded **117** and **118** and (via **120**) **119**, RCM afforded scaffolds **114–116**. A number of distinct molecular scaffolds with lead-like molecular properties were thus synthesized. Notably, the scaffolds were suitably functionalized for further chemical derivatization and modification (Scheme 10).

Targeting diverse sp^3-rich three-dimensional scaffolds, Stockman et al. devised a two-directional approach employing a symmetric linear "rope-like" ketodienolate, which was then subjected to twelve separate tandem reactions to build twelve NP-like scaffolds. The scaffolds thus generated were already equipped with functional groups for further elabo-



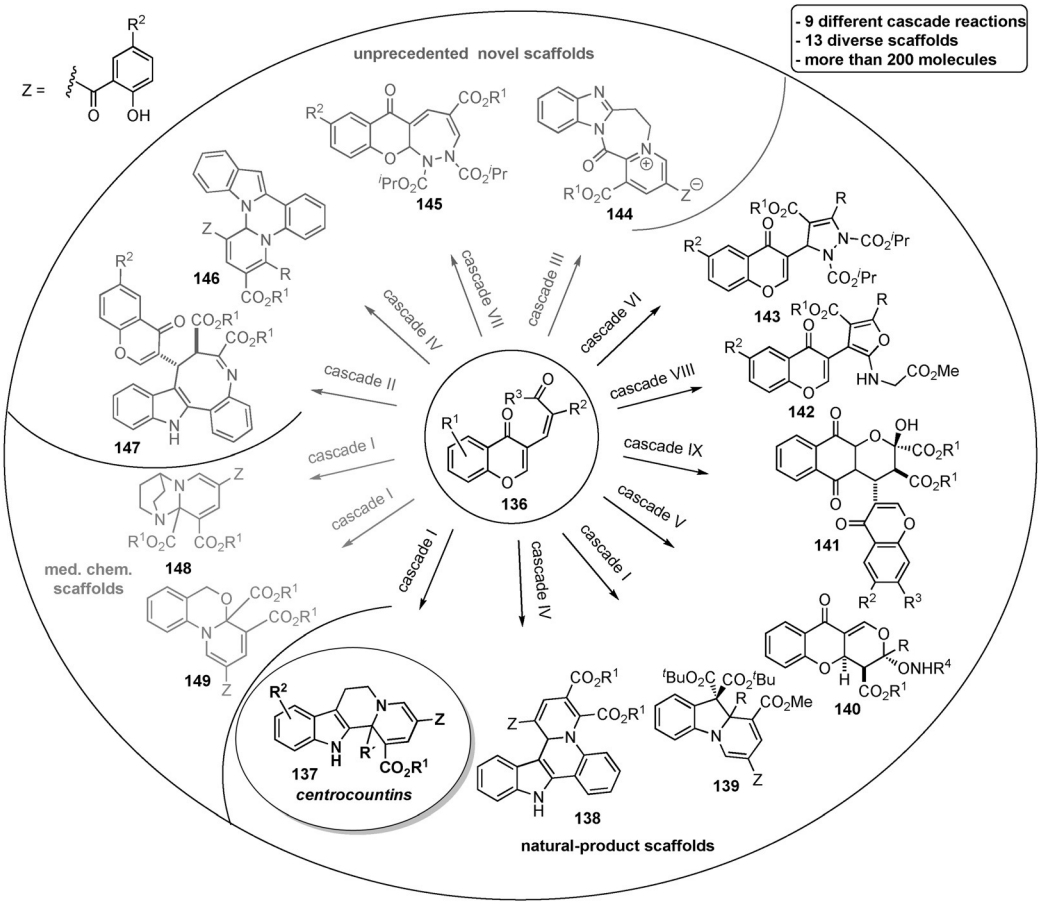
Scheme 10. Nelson's unified synthesis approach to afford diverse lead-like compounds. PG = protecting group.



Scheme 11. Stockman's two-directional approach to transform a symmetric linear substrate into diverse sp^3-rich scaffolds.

ration (Scheme 11).[58] The central ketone moiety in **124** was transformed into a range of nucleophilic functional groups (e.g., oxime, imine, azomethine ylide, or enolate), which then reacted with the tethered α,β-unsaturated esters at the termini of the chain (e.g., various [3+2] cycloadditions or amine/enolate conjugate addition).[59]

In another cascade reaction based design, our group explored the various electrophilic moieties of a common precursor to generate scaffold diversity.[60] Various mono- and bis-nucleophiles as well as nucleophilic zwitterions were identified during reaction optimization and successfully reacted with **136** to yield thirteen diverse scaffolds (Scheme 12). The nucleophile employed determines the type of domino reaction that occurs as well as the scaffold type that is formed. Eleven nucleophiles yielded thirteen diverse scaffolds in nine different domino reactions (**137**–**149**). These novel chemotypes represent a broad chemical space and embody the structural fingerprints of NPs and medicinally important compounds. Employing tryptamine as a bis-nucleophile led to the discovery of the centrocountins (**137**), mitotic modulators that target the centrosomal proteins nucleophosmin and Crm-1 (Scheme 12; see also Figure 6).[61]

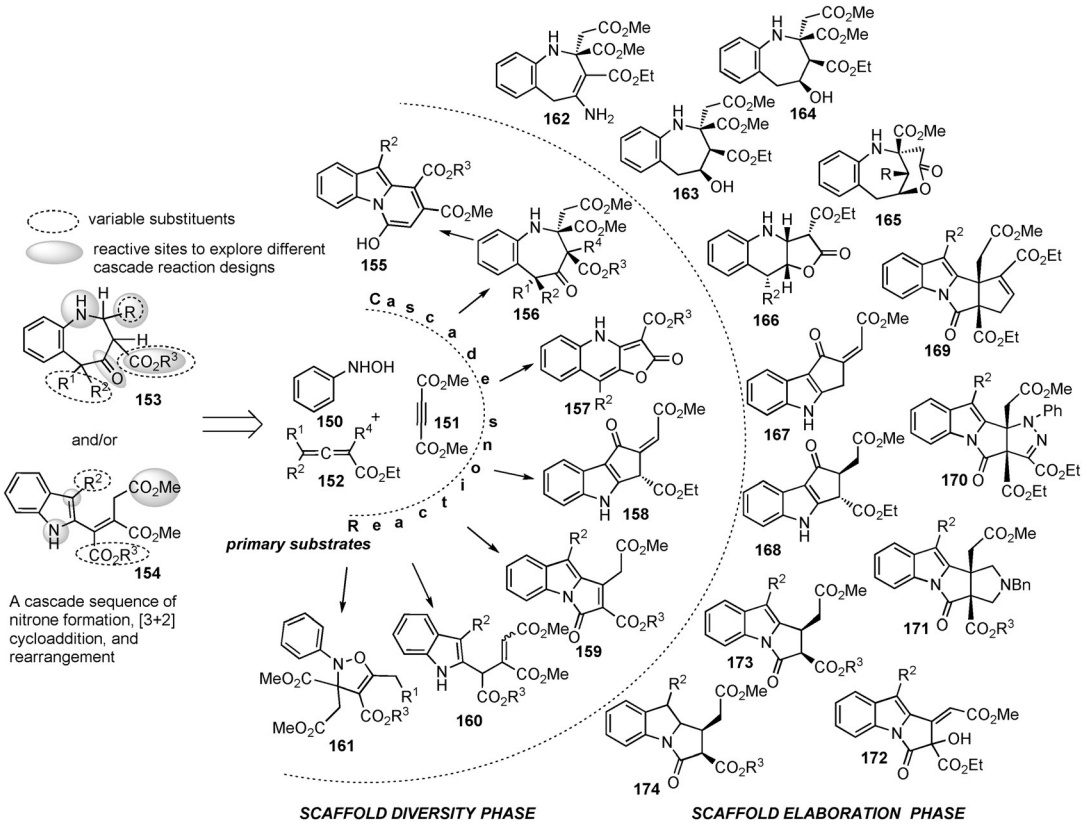


Scheme 12. Branching cascade strategy to build scaffold diversity.

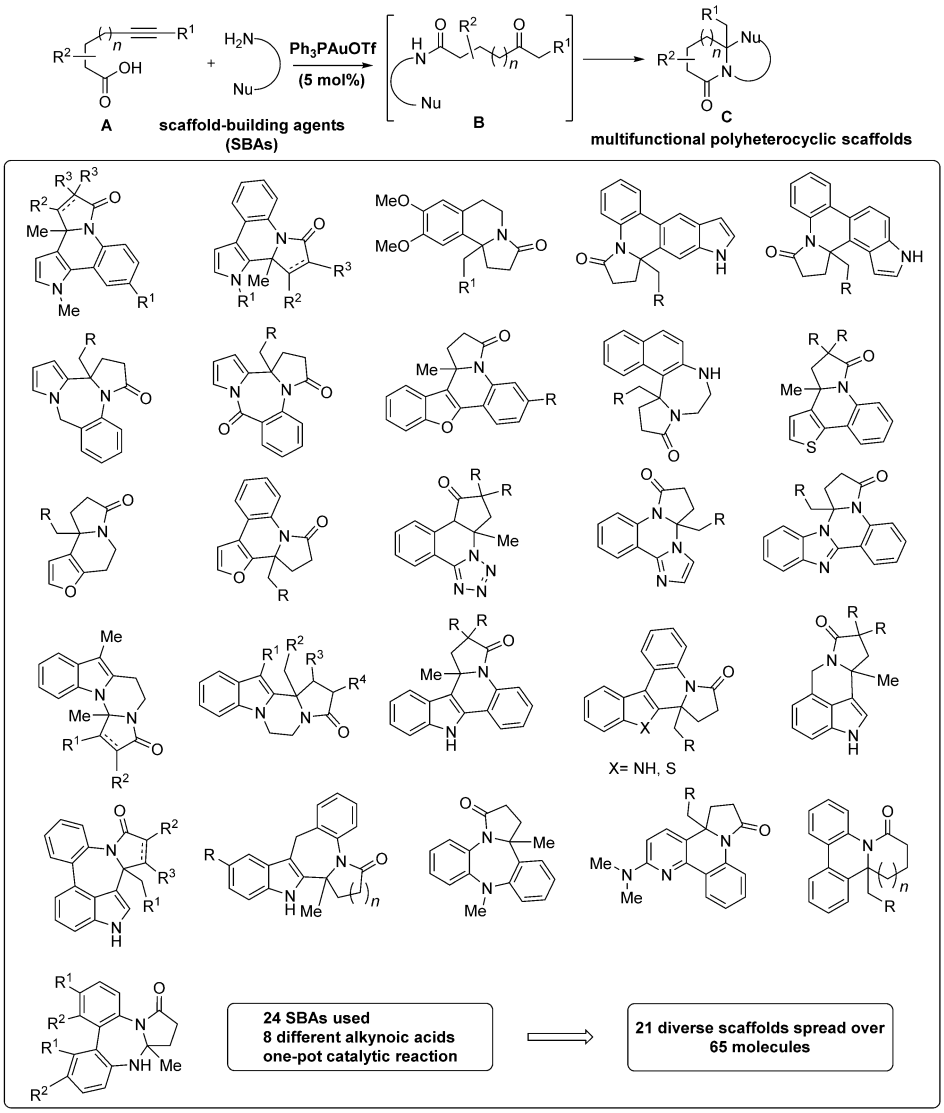
In most of the synthetic approaches that exploit a common intermediate to deliver different scaffolds, multistep processes are required to build these suitably functionalized substrates. A less-travelled alternative synthetic route employs simple primary substrates to build structral diversity and complexity. Our group recently reported a de novo branching-cascade approach that is based on cascade reactions of commercially available substrates to build scaffold diversity.[62] Interestingly, these simple substrates did not contain any of the ring systems contained in the diverse scaffolds formed by the domino reactions. In that sense, this approach resembles the de novo biosynthesis of diverse NPs from acyclic primary substrates. Our strategy was based on the fact that a nitrone (formed in situ) can be transformed into either a benzazepinone (**153**) or a vinylindole (**154**) under different reaction conditions (Scheme 13). The different reactive sites available on these intermediates could be exploited in diverse cyclization reactions by simply modifying the reaction conditions. Thus the three primary substrates *N*-phenylhydroxylamine (**150**), dimethyl acetylenedicarboxylate (**151**), and allene carboxylate **152** yielded seven distinct molecular frameworks in different cascade reactions under optimized reaction conditions in the "scaffold diversity phase" (Scheme 13). In the scaffold elaboration phase, four of these scaffolds were transformed into complex molecular frameworks by single-step transformations. With three primary substrates, a compound collection of 61 molecules representing 17 diverse scaffolds was synthesized (Scheme 13), which was then analyzed in biological assays (see Figure 5 and accompanying text).

Patil and co-workers designed a catalytic branching-cascade strategy that delivered drug-like polyheterocycles (Scheme 14).[63] The reaction of a common precursor, alkynoic acid **A**, with several scaffold-building agents (SBAs; variables) in the presence of a suitable metal catalyst led to the formation of various ketoamide intermediates **B**. When subjected to different metal-catalyzed cyclization cascade reactions, the latter afforded various heterocyclic scaffolds **C**. With their relay catalytic branching cascade (RCBC) approach, a collection of 65 molecules representing 21 diverse scaffolds was generated (Scheme 14). The collection also contained molecules that are highly selective against *Mycobacterium smegmatis*.[23]

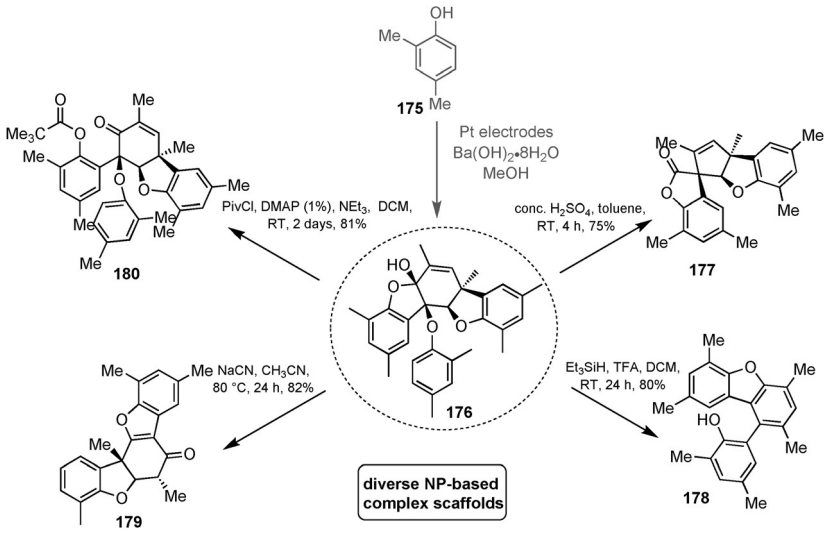
In a very innovative and interesting approach that leads to structurally rich and diverse NP scaffolds, Waldvogel and coworkers employed an electrochemical transformation to build an intermediate (**176**) that was utilized to generate various complex molecular scaffolds (Scheme 15).[64] Anodic oxidation of 2,4-dimethylphenol (**175**) on platinum electrodes with $Ba(OH)_2{\cdot}8H_2O$ in methanol as the electrolyte afforded oxygen-rich compound **176**. Further simple transformations (one to two steps) of **176** led to fourteen compounds based on four distinct scaffolds with almost exclusive selectivity and in reasonable yields.



Scheme 13. Our de novo branching-cascade approach for the construction of novel and diverse scaffolds.



Scheme 14. Patil's RCBC approach to scaffold diversity.



Scheme 15. Waldvogel's approach to build NP-based scaffold diversity. DCM = dichloromethane, DMAP = 4-dimethylaminopyridine.

3. The Potential of Scaffold-Rich Compound Collections in Drug and Probe Discovery

The diverse and complex nature of the biological targets of small molecules requires a similar level of structural diversity and complexity in the screening libraries. A scaffold-rich library is essentially a library of libraries with good representatives for each and every scaffold that it contains. Scaffold diversity bequeaths functional diversity and thus leads to a library of compounds with different biological functions, which provides an ideal platform for medicinal-chemistry and probe-discovery research projects. Although the potential of recently synthesized scaffold-rich libraries in drug and probe discovery will emerge slowly over time, some interesting cases described below strongly support the development of various approaches for scaffold-diversity synthesis.

A scaffold-rich macrocyclic compound collection synthesized by Marcaurelle and coworkers (see Scheme 9) was biologically evaluated by various groups.[56,57] Fang et al. screened a library of 100000 molecules to discover small molecules that interact with MCL1 (myeloid cell leukemia),[65] an anti-apoptotic protein.[66–68] The binding mode of the initial hit was established by a fluorescence polarization assay, which measured the ability of ligands to competitively bind in the presence of the NOXA BH3 peptide, and later validated by biophysical methods, such as differential scanning fluorimetry, isothermal titration calorimetry, and differential scanning calorimetry. Initial structure–binding relationship studies considering the configuration as well as the substitution of the scaffold led to the identification of compound **181** (see Figure 4), which gave an IC_{50} value as low as 4.5 μM in a fluorescence polarization assay. The binding mode was ultimately determined by X-ray structure analysis of the macrolactam as well as other well validated MCL1 ligands

co-crystallized with a designed MCL1 protein construct (Figure 3a). Lactam **181** is the first ligand that binds MCL1 without engaging R263 and takes up a hydrophobic binding pocket that was not occupied by other MCL1 ligands (Figure 3b). This finding opens up new possibilities for the discovery and design of potent MCL1 inhibitors.

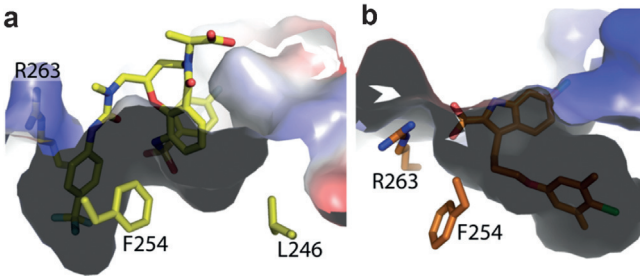


Figure 3. Crystal structures of a) compound **181** co-crystallized with MCL1, revealing a new binding mode, and b) a well-known MCL1 indole inhibitor bound to MCL1. Reproduced from Ref. [65] with permission. Copyright 2014, ACS.

In another project, modulators of scavenger receptor class B type I (SR-BI), the receptor for high-density lipoprotein cholesterol (HDL-C) particles, were sought by Dockendorf et al.[69] SR-BI is an important target receptor involved in the hepatic uptake of HDL-C and a co-receptor for the entry of pathogens into hepatocytes, including the hepatitis C virus (HCV) and malaria (*Plasmodium*). Aside from its recognized role in lipid metabolism and infectious diseases, it also has a strong impact on immune response and female fertility. In a DiI–HDL-C uptake assay,[69] measuring the transfer of the fluorescent lipid DiI from HDL-C particles to CHO cells overexpressing SR-BI, potent inhibitors of SR-BI were identified from the macrocycle library.[7b] Modification of the eight-membered lactam hits led to the soluble and non-toxic compound **182** (Figure 4), which was found to possess a superior combination of potency (average $IC_{50} = 100 \pm 20$ nM) and solubility (79 μM in PBS) and was designated as a probe for SR-BI.

The notion that the scaffold diversity of a compound collection results in functional diversity[70] was validated by exposing the compound collection that we had generated by the de novo branching-cascade strategy to two phenotypic cell-based assays (Scheme 13).[62] In the first study, approximately sixty molecules with over seventeen scaffold structures were screened for their ability to modulate the hedgehog pathway.[71] This pathway plays a fundamental role during animal embryonic and post-embryonic development as well as in tumorigenesis as aberrant hedgehog signaling is detected in various cancers. Therefore, small-molecule modulators of the hedgehog pathway are well sought after for drug discovery as well as chemical-biology investigations. Screening the compound collection identified three molecules with three different scaffolds, namely **156a**, **157b**, and **159c**, as inhibitors of hedgehog signaling with IC_{50} values of 0.79, 0.84, and 0.16 μM, respectively (without influencing cell viability; Figure 5a,b). The purmorphamine-induced expression of the hedgehog target gene Ptch1 in NIH/3T3 cells was suppressed by these compounds (Figure 5c). Furthermore, several hedgehog inhibitors operate by binding and inhibiting the transmembrane protein Smoothened (Smo). However, **156a**, **157b**, and **159c** failed to displace BODIPY–cyclopamine from Smo, and thus most likely do not bind to this receptor (Figure 5d). These small molecules thus are vital starting points for the development of probes that can illuminate the role of this signaling pathway in various disease states and hedgehog-inhibition-based therapeutics.[72]

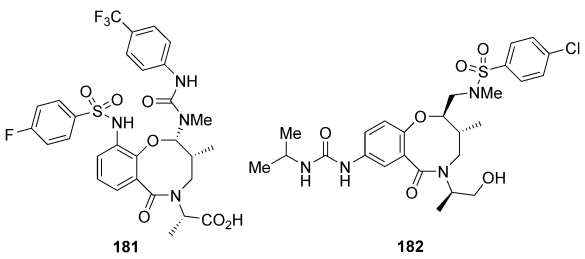


Figure 4. Macrocyclic molecules that interact with MCL1 (**181**) and SR-BI (**182**).

Another high-content phenotypic assay that monitored changes in the cytoskeleton and DNA in HeLa cells revealed that compound **158** causes cytoskeleton impairment (Figure 5f) and mitotic arrest (Figure 5e) followed by apoptosis. Treatment with **158** for 48 hours reduced the viability of HeLa cells with an IC_{50} value of 3.87 ± 0.01 μM. In a competition experiment, compound **158** displaced (colchicine could not) fluorescent BODIPY–FL–vinblastine from tubulin in a concentration-dependent manner with a half-maximal effective concentration (EC_{50}) of 0.67 ± 1.51 μM (Figure 5g), and therefore most likely binds to the vinca alkaloid binding site in tubulin.

The branching-cascade strategy developed by our group delivered tetrahydroindolo[2,3-*a*]quinolizines **137** (Scheme 12), which were found to be mitotic inhibitors. Later, our group, in collaboration with the Waldmann group, reported a twelve-step cascade synthesis of this class of molecules, which were called centrocountins.[61a] A phenotypic screen of indoloquinolizines that looked for mitotic arrest in BSC-1 and HeLa cells showed that compound **137a** (centrocountin 1; Figure 6a) causes the formation of multiple mitotic spindles and the accumulation of mitotic cells (Figure 6g,h). Further investigations revealed that centrocountin 1 was a modulator of centrosome integrity, inducing fragmented and supernumerary centrosomes, chromosome congression defects, multipolar mitotic spindles, acentrosomal spindle poles, and multipolar cell division. Target identification by means of a chemical proteomics approach revealed that pulldown (PD) probe **183** (*R* enantiomer), but not control probe **184** (*S* enantiomer; Figure 6b), binds to the nucleolar and centrosomal protein nucleophosmin (NPM), which is involved in the regulation of centrosome duplication during mitosis and also promotes ribosome biogenesis.

The reversible binding of NPM to **183** further confirmed it as the target protein (Figure 6c). NPM in a complex with the nuclear export receptor Crm1 regulates centrosome duplication. Further elaboration of the affinity PD experiment by

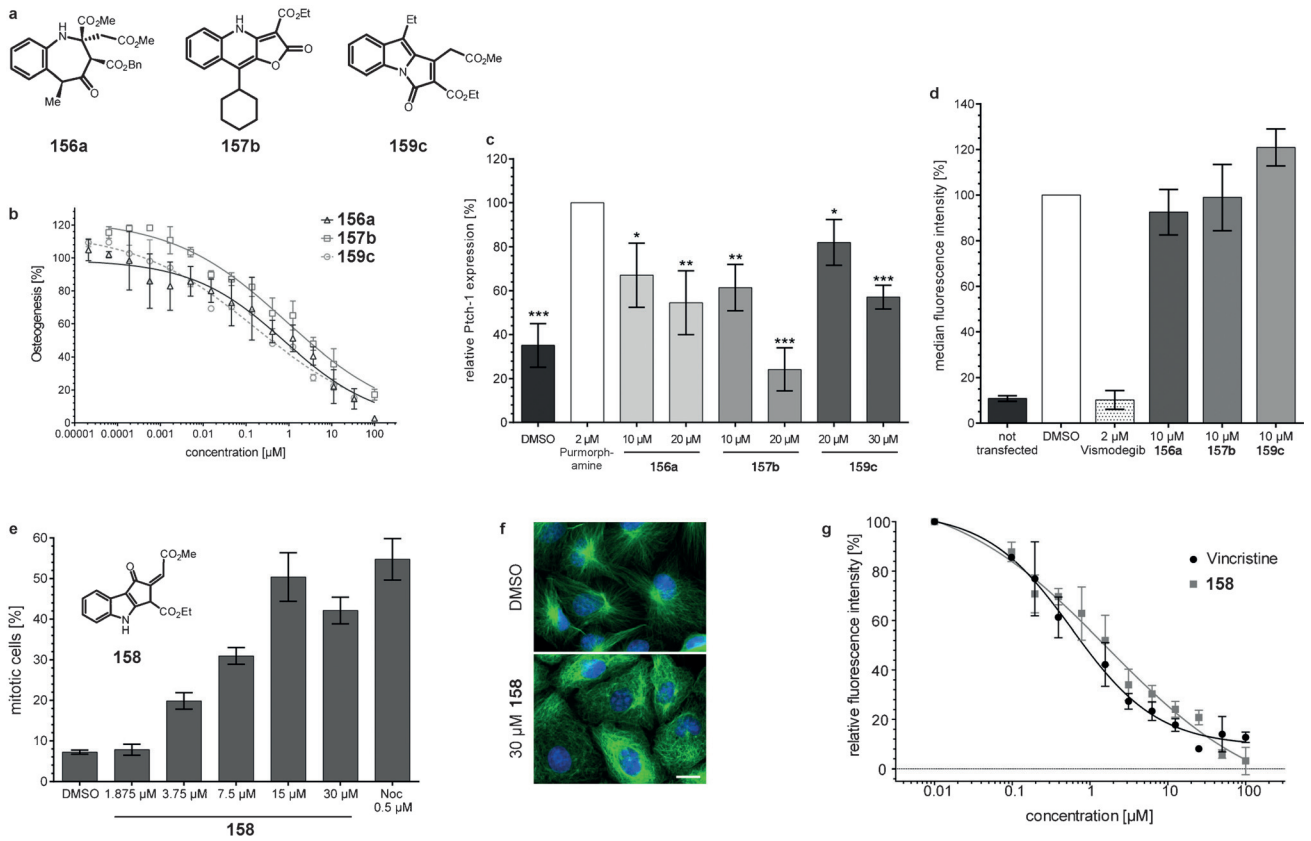
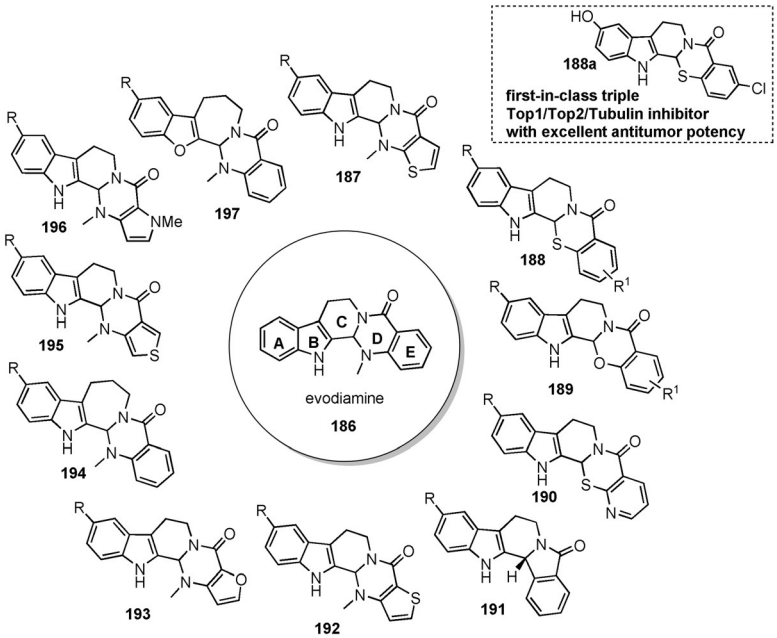


Figure 5. Biological evaluation of active molecules synthesized by the de novo branching-cascade approach. a) Chemical structures of **156a**, **157b**, and **159c**. Effect of the addition of **156a**, **157b**, and **159c** b) on purmorphamine-mediated osteogenesis in C3H10T1/2 cells based on the activity of alkaline phosphatase, c) on the relative expression of Ptch-1, and d) on the binding of BODIPY–cyclopamine to Smo. e) The concentration-dependent percentage increase in mitotic cells suggesting that **158** induces mitotic arrest. f) Cytoskeleton impairment caused by **158** in HeLa cells. g) Displacement of BODIPY–FL–vinblastine from tubulin by **158**.

immunoblotting with a Crm1-specific antibody revealed the binding of Crm1 to the immobilized centrocountin probe (Figure 6c). Evidence for the direct interaction between indoloquinolizine probe **185** and NPM, as well as Crm1, in HeLa cells was obtained by fluorescence lifetime imaging (FLIM) experiments (Figure 6d–f). The scaffold-diversity synthesis thereby not only provided potential anticancer small molecules but also showed that NPM is a potential therapeutic target for anticancer drug discovery.[73]

Recently, the NP evodiamine (**186**) inspired the scaffold-diversity synthesis of eleven indole polyheterocycles and their derivatives (Scheme 16).[74] Although each scaffold was built by multistep syntheses, the design was to cover a broader chemical space matching the pentacyclic core of the diversely bioactive natural product evodiamine (Scheme 16). Many of the evodiamine-based molecules exhibited good to excellent antitumor activities against different cancer cell lines. Compound **188a** (Scheme 16), in particular, was highly potent and showed excellent in vitro and in vivo antitumor efficacies with low toxicity. Target profiling studies revealed this molecule to be the first-in-class triple topoisomerase I/topoisomerase II/tubulin inhibitor. The identifi-



Scheme 16. Synthesis of diverse scaffolds inspired by the natural product evodiamine.

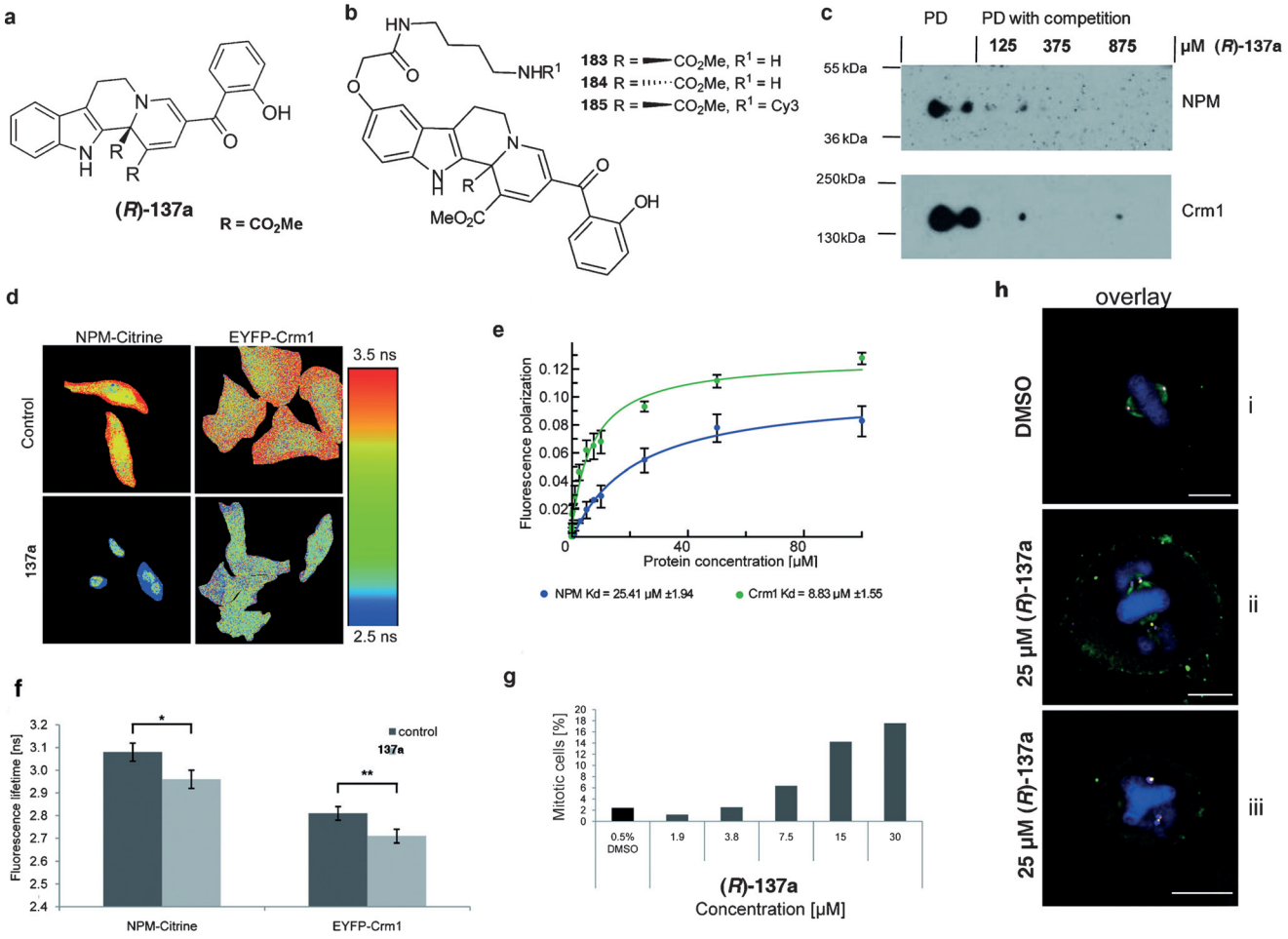


Figure 6. Biological evaluation of centrocountin 1 (**137a**) and identification and validation of target proteins. Structures of a) centrocountin 1 (**137a**) and b) probes **183**–**185**, which were used in PD experiments. c) Immunodetection of NPM and Crm1 binding to immobilized **183**. d) FLIM showing the specific binding of NPM–citrine and EYF–Crm1 (donor) to Cy3-labeled **185** (acceptor). e) Binding of **185** to NPM and Crm1 as determined by fluorescence polarization. f) Decrease in the donor lifetime in the presence of **137a** as compared to the control (see part (d)). g) Dose-dependent accumulation of mitotic HeLa cells upon treatment with compound (*R*)-**137a**. h) Z projections of two image stacks of HeLa cells incubated with DMSO or (*R*)-**137a**, depicting multiple defects on spindle formation, centrosomes, and centrioles.

cation of a series of highly potent antitumor molecules with promising features as drug candidates in this study further encourages chemists looking for novel bioactive molecules to synthesize NP-scaffold-based compound collections.

In contrast to the evolutionary optimization of NPs, which is driven by the functional benefits to their host organism, the synthesis of molecules and the analysis of their activity are often isolated developments. Inspired by nature's function-oriented synthesis designs, Nelson and co-workers developed an activity-directed synthesis approach.[75] The versatility of rhodium carbenes, which were formed from diazo compounds, to build different ring systems by different cyclization reactions was exploited to enrich the collection, which was then assayed to identify androgen receptor agonists. For the reactions, a 96 well plate was used, and twelve differently substituted α-diazo arylamide substrates were employed in combination with different rhodium catalysts and solvents, leading to 96 different reaction conditions. After 48 hours, metal contaminants and the solvents were removed, and the reaction mixtures were tested in an assay to identify the androgen receptor agonist properties of the products by FRET (Förster resonance transfer) techniques. The four substrates that yielded highly active product mixtures were then used for the next round of reaction/screening (round 2) together with two that had not shown reactivity as negative controls. Solvent and catalyst were varied, and in this screening round, the concentration of the compounds was reduced by a factor of ten to identify more potent molecules. The third reaction setup included the two most active substrates from the previous round as well as four structurally similar substrates along with various solvents and catalysts. In this round, the concentration was again reduced by a factor of ten. The eight reactions whose product mixtures exhibited the best activity were then scaled up, and the major products were isolated and identified (Figure 7).

Three distinct products tested at different concentrations for their androgen receptor agonist activities displayed EC_{50} values of 340–470 nM. Whereas β-lactams **198** and **200**

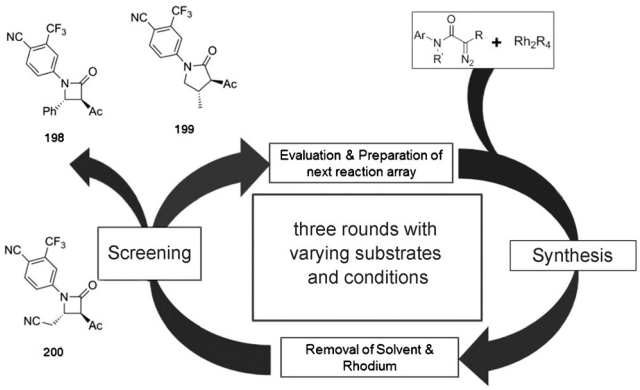


Figure 7. Activity-directed synthesis approach developed by Nelson and co-workers. For details see the main text.

were identified to be full agonists of the androgen receptor, γ-lactam **199** was a partial agonist. Interestingly, none of the two scaffolds that these compounds represent had previously been annotated with androgen agonist activity. The activity-directed synthesis approach is highly useful for identifying interesting starting points for drug and probe discovery.

4. Outlook

Despite the fact that the pharmaceutical industry has moved beyond the brute-force approach towards more effective drug-discovery strategies over the past decades, the productivity challenges that result from unmet medical needs[76] remain substantial.[77] New and recurring drug-resistant infections,[78] aggressive forms of different cancers,[79] and an increasing number of new druggable protein targets[80] identified in genome studies desperately call for a pipeline rich in structurally novel bioactive small molecules. Even in this post-genomic era, our understanding of human biology remains limited. More knowledge about the various aspects of disease- and non-disease-state biology would equip us well to meet the challenges of drug discovery. A number of chemical probes that can shed light on intricate signaling pathways and other complex biological functions are urgently required. Unfortunately, only a few probe molecules that can be universally used for biological investigations have been discovered thus far.[81] The chemical space that could provide bioactive molecules with novel modes of action to deliver first-in-class drug candidates and/or chemical probes needs to be identified, accessed, and explored. The quest for modulators of biological functions therefore calls for coherent synthetic efforts to create structurally rich compound collections that yield functionally diverse small molecules.

Notably, despite the recent developments in the synthesis of diverse scaffolds of natural or non-natural (novel) origin, efforts to suitably represent each scaffold in a library are often lacking, and therefore, such libraries tend to consist of many singletons and poorly decorated scaffolds. A sparse representation of a large number of molecular frameworks is less likely to deliver any structure–activity relationships and therefore hit confirmations. An awareness for the desired and undesired structural features in a small-molecule library is equally important. In particular, when a compound collection developed by an academic research group is a side product of their original research goals, for instance, a reaction discovery, they usually do not focus on improving the structural features or molecular properties of the library. The removal or modification of undesired features, at least for some of the compounds, would help identifying false positives. An academia–industry collaboration[82] can easily address these issues and avoid the repeated identification of wrong starting points. For example, the European lead factory (ELF) consortium is a unique collaboration of academic and industrial partners working towards building a joint European compound library (JECL) that is based on novel and complex scaffolds yet with more drug-like molecular properties. The ELF aims to deliver innovative starting points for drug discovery and methods to advance our biological understanding by screening the JECL.[83]

Natural products have historically been a rich source of biologically active molecules, and their chemical space needs to be further explored. The generation of structurally rich and diverse NP-based or -derived compound libraries is a formidable challenge that may benefit from cutting-edge expertise in NP total or divergent total synthesis. Examples of successful scalable syntheses of various natural products indicate the possibility of totally synthetic analogues of complex natural products being part of future compound collections for various screenings.[52,84] Methods to manipulate biosynthetic designs and deliver structurally modified complex natural products or their precursors for divergent synthesis strategies would help the scientific community engaged in discovery research to come out of the current productivity crisis.[85] Simple modifications of a functional group can be extremely challenging on a complex molecule, such as a natural product.[86] Time is also ripe for new chemoselective functional-group transformations that can be applied in complex three-dimensional settings.

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[1] a) K. C. Nicolaou, *Angew. Chem. Int. Ed.* **2013**, *52*, 131–146; *Angew. Chem.* **2013**, *125*, 141–157; b) K. C. Nicolaou, *Proc. R. Soc. London Ser. A* **2014**, *470*, 20130690.

[2] a) K. C. Nicolaou, *Angew. Chem. Int. Ed.* **2014**, *53*, 9128–9140; *Angew. Chem.* **2014**, *126*, 9280–9292; b) K. C. Nicolaou, T. Montagnon, *Molecules That Changed the World*, Wiley-VCH, Weinheim, **2008**.

[3] a) W. L. Jorgensen, *Angew. Chem. Int. Ed.* **2012**, *51*, 11680–11684; *Angew. Chem.* **2012**, *124*, 11848–11853; b) V. J. Hruby, *J. Org. Chem.* **2009**, *74*, 9245–9264; c) C. M. Dobson, *Nature* **2004**, *432*, 824–828; d) C. J. O'Connor, H. S. G. Beckmann, D. R. Spring, *Chem. Soc. Rev.* **2012**, *41*, 4444–4456; e) K. Hübel,

T. Lessmann, H. Waldmann, *Chem. Soc. Rev.* **2008**, *37*, 1361 – 1374.
[4] a) C. J. O'Connor, L. Laraia, D. R. Spring, *Chem. Soc. Rev.* **2011**, *40*, 4332 – 4345; b) W. R. J. D. Galloway, D. R. Spring, *Nature* **2011**, *470*, 43 – 43; c) E. Lenci, A. Guarna, A. Trabocchi, *Molecules* **2014**, *19*, 16506 – 16528.
[5] a) L. M. Mayr, P. Fuerst, *J. Biomol. Screening* **2008**, *13*, 443 – 448; b) D. J. Newman, G. M. Cragg, K. M. Snader, *J. Nat. Prod.* **2003**, *66*, 1022 – 1037.
[6] a) M. D. Burke, S. L. Schreiber, *Angew. Chem. Int. Ed.* **2004**, *43*, 46 – 58; *Angew. Chem.* **2004**, *116*, 48 – 60; b) S. L. Schreiber, *Science* **2000**, *287*, 1964 – 1969.
[7] a) E. Comer, J. A. Beaudoin, N. Kato, M. E. Fitzgerald, R. W. Heidebrecht, M. duPont Lee IV, D. Masi, M. Mercier, C. Mulrooney, G. Muncipinto, A. Rowley, K. Crespo-Llado, A. E. Serrano, A. K. Lukens, R. C. Wiegand, D. F. Wirth, M. A. Palmer, M. A. Foley, B. Munoz, C. A. Scherer, J. R. Duvall, S. L. Schreiber, *J. Med. Chem.* **2014**, *57*, 8496 – 8502; b) C. Dockendorff, P. W. Faloon, J. Pu, M. Yu, S. Johnston, M. Bennion, M. Penman, T. J. Nieland, S. Dandapani, J. R. Perez, B. Munoz, M. A. Palmer, S. L. Schreiber, M. Krieger, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 2100 – 2105; c) W. R. J. D. Galloway, A. Isidro-Llobet, D. R. Spring, *Nat. Commun.* **2010**, *1*, 80.
[8] a) D. S. Tan, *Nat. Chem. Biol.* **2005**, *1*, 74 – 84; b) D. S. Tan, M. A. Foley, M. D. Shair, S. L. Schreiber, *J. Am. Chem. Soc.* **1998**, *120*, 8565 – 8566; c) O. Kwon, S. B. Park, S. L. Schreiber, *J. Am. Chem. Soc.* **2002**, *124*, 13402 – 13404; d) M. D. Burke, E. M. Berger, S. L. Schreiber, *Science* **2003**, *302*, 613 – 618.
[9] a) A. Nören-Muller, I. Reis-Correa, H. Prinz, C. Rosenbaum, K. Saxena, H. J. Schwalbe, D. Vestweber, G. Cagna, S. Schunk, O. Schwarz, H. Schiewe, H. Waldmann, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10606 – 10611; b) H. Waldmann, *Drugs Future* **2009**, *34*, 24 – 25; c) W. Wilk, T. J. Zimmermann, M. Kaiser, H. Waldmann, *Biol. Chem.* **2009**, *391*, 491 – 497; d) R. S. Bon, H. Waldmann, *Acc. Chem. Res.* **2010**, *43*, 1103 – 1114; e) W. Wilk, T. J. Zimmermann, M. Kaiser, H. Waldmann, *Biol. Chem.* **2010**, *391*, 491 – 497; f) S. Wetzel, R. S. Bon, K. Kumar, H. Waldmann, *Angew. Chem. Int. Ed.* **2011**, *50*, 10800 – 10826; *Angew. Chem.* **2011**, *123*, 10990 – 11018.
[10] a) S. Renner, W. A. L. van Otterlo, M. D. Seoane, S. Mocklinghoff, B. Hofmann, S. Wetzel, A. Schuffenhauer, P. Ertl, T. I. Oprea, D. Steinhilber, L. Brunsveld, D. Rauh, H. Waldmann, *Nat. Chem. Biol.* **2009**, *5*, 585 – 592; b) P. E. A. Schuffenhauer, S. Roggo, S. Wetzel, M. A. Koch, H. Waldmann, *J. Chem. Inf. Model.* **2007**, *47*, 47 – 58; c) L. Arve, T. Voigt, H. Waldmann, *QSAR Comb. Sci.* **2006**, *25*, 449 – 456.
[11] a) J. D. Umarye, T. Lessmann, A. B. Garcia, V. Mamane, S. Sommer, H. Waldmann, *Chem. Eur. J.* **2007**, *13*, 3305 – 3319; b) W. Wilk, A. Nören-Müller, M. Kaiser, H. Waldmann, *Chem. Eur. J.* **2009**, *15*, 11976 – 11984; c) S. Basu, B. Ellinger, S. Rizzo, C. Deraeve, M. Schürmann, H. Preut, H. D. Arndt, H. Waldmann, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6805 – 6810; d) A. Nören-Müller, W. Wilk, K. Saxena, H. Schwalbe, M. Kaiser, H. Waldmann, *Angew. Chem. Int. Ed.* **2008**, *47*, 5973 – 5977; *Angew. Chem.* **2008**, *120*, 6061 – 6066; e) A. P. Antonchick, S. Lopez-Tosco, J. Parga, S. Sievers, M. Schürmann, H. Preut, S. Höing, H. R. Schöler, J. Sterneckert, D. Rauh, H. Waldmann, *Chem. Biol.* **2013**, *20*, 500 – 509; f) P.-Y. Dakas, J. A. Parga, S. Höing, H. R. Schöler, J. Sterneckert, K. Kumar, H. Waldmann, *Angew. Chem. Int. Ed.* **2013**, *52*, 9576 – 9581; *Angew. Chem.* **2013**, *125*, 9755 – 9760; g) R. Narayan, J. O. Bauer, C. Strohmann, A. P. Antonchick, H. Waldmann, *Angew. Chem. Int. Ed.* **2013**, *52*, 12892 – 12896; *Angew. Chem.* **2013**, *125*, 13130 – 13134; h) H. Takayama, Z. J. Jia, L. Kremer, J. O. Bauer, C. Strohmann, S. Ziegler, A. P. Antonchick, H. Waldmann, *Angew. Chem. Int. Ed.* **2013**, *52*, 12404 – 12408; *Angew. Chem.* **2013**, *125*, 12630 – 12634; i) T. Voigt, C. Gerding-Reimers, T. N. T. Tuyen, S. Bergmann, H. Lachance, B. Schölermann, A. Brockmeyer, P. Janning, S. Ziegler, H. Waldmann, *Angew. Chem. Int. Ed.* **2013**, *52*, 410 – 414; *Angew. Chem.* **2013**, *125*, 428 – 432; j) T. J. Zimmermann, S. Roy, N. E. Martinez, S. Ziegler, C. Hedberg, H. Waldmann, *ChemBioChem* **2013**, *14*, 295 – 300.
[12] A. Reayi, P. Arya, *Curr. Opin. Chem. Biol.* **2005**, *9*, 240 – 247.
[13] a) P. A. Clemons, J. A. Wilson, V. Dancik, S. Muller, H. A. Carrinski, B. K. Wagner, A. N. Koehler, S. L. Schreiber, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6817 – 6822; b) J. Y. Cui, J. Hao, O. A. Ulanovskaya, J. Dundas, J. Liang, S. A. Kozmin, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6763 – 6768; c) S. L. Schreiber, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6699 – 6702.
[14] P. Ertl, S. Jelfs, J. Mühlbacher, A. Schuffenhauer, P. Selzer, *J. Med. Chem.* **2006**, *49*, 4568 – 4573.
[15] a) G. L. Thomas, E. E. Wyatt, D. R. Spring, *Curr. Opin. Drug Discovery Dev.* **2006**, *9*, 700 – 712; b) E. E. Wyatt, S. Fergus, W. R. J. D. Galloway, A. Bender, D. J. Fox, A. T. Plowright, A. S. Jessiman, M. Welch, D. R. Spring, *Chem. Commun.* **2006**, 3296 – 3298; c) A. A. Shelat, R. K. Guy, *Nat. Chem. Biol.* **2007**, *3*, 442 – 446.
[16] W. R. J. D. Galloway, D. R. Spring, *Diversity-Oriented Synth.* **2012**, 21 – 28.
[17] A. H. Lipkus, Q. Yuan, K. A. Lucas, S. A. Funk, W. F. Bartelt, R. J. Schenck, A. J. Trippe, *J. Org. Chem.* **2008**, *73*, 4443 – 4451.
[18] J. W. Scannell, A. Blanckley, H. Boldon, B. Warrington, *Nat. Rev. Drug Discovery* **2012**, *11*, 191 – 200.
[19] M. Krier, G. Bret, D. Rognan, *J. Chem. Inf. Model.* **2006**, *46*, 512 – 524.
[20] a) R. G. Doveston, P. Tosatti, M. Dow, D. J. Foley, H. Y. Li, A. J. Campbell, D. House, I. Churcher, S. P. Marsden, A. Nelson, *Org. Biomol. Chem.* **2015**, *13*, 859 – 865; b) T. James, P. MacLellan, G. M. Burslem, I. Simpson, J. A. Grant, S. Warriner, V. Sridharan, A. Nelson, *Org. Biomol. Chem.* **2014**, *12*, 2584 – 2591; c) P. MacLellan, A. Nelson, *Chem. Commun.* **2013**, *49*, 2383 – 2393; d) T. James, L. Simpson, J. A. Grant, V. Sridharan, A. Nelson, *Org. Lett.* **2013**, *15*, 6094 – 6097; e) R. Doveston, S. Marsden, A. Nelson, *Drug Discovery Today* **2014**, *19*, 813 – 819.
[21] a) R. J. Spandl, M. Diaz-Gavilan, K. M. G. O'Connell, G. L. Thomas, D. R. Spring, *Chem. Rec.* **2008**, *8*, 129 – 142; b) P. Arya, R. Joseph, Z. H. Gan, B. Rakic, *Chem. Biol.* **2005**, *12*, 163 – 180; c) A. Nadin, C. Hattotuwagama, I. Churcher, *Angew. Chem. Int. Ed.* **2012**, *51*, 1114 – 1122; *Angew. Chem.* **2012**, *124*, 1140 – 1149; d) D. J. Foley, R. G. Doveston, I. Churcher, A. Nelson, S. P. Marsden, *Chem. Commun.* **2015**, *51*, 11174 – 11177; e) G. L. Thomas, R. J. Spandl, F. G. Glansdorp, M. Welch, A. Bender, J. Cockfield, J. A. Lindsay, C. Bryant, D. F. Brown, O. Loiseleur, H. Rudyk, M. Ladlow, D. R. Spring, *Angew. Chem. Int. Ed.* **2008**, *47*, 2808 – 2812; *Angew. Chem.* **2008**, *120*, 2850 – 2854; f) A. Robinson, G. L. Thomas, R. J. Spandl, M. Welch, D. R. Spring, *Org. Biomol. Chem.* **2008**, *6*, 2978 – 2981; g) D. M. Marsden, R. L. Nicholson, M. E. Skindersoe, W. R. J. D. Galloway, H. F. Sore, M. Givskov, G. P. C. Salmond, M. Ladlow, M. Welch, D. R. Spring, *Org. Biomol. Chem.* **2010**, *8*, 5313 – 5323; h) A. Isidro-Llobet, T. Murillo, P. Bello, A. Cilibrizzi, J. T. Hodgkinson, W. R. J. D. Galloway, A. Bender, M. Welch, D. R. Spring, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6793 – 6798; i) M. Díaz-Gavilán, W. R. J. D. Galloway, K. M. G. O'Connell, J. T. Hodgkingson, D. R. Spring, *Chem. Commun.* **2010**, *46*, 776 – 778; j) C. Serba, N. Winssinger, *Eur. J. Org. Chem.* **2013**, 4195 – 4214.
[22] G. W. Bemis, M. A. Murcko, *J. Med. Chem.* **1996**, *39*, 2887 – 2893.
[23] X. Q. Lewell, A. C. Jones, C. L. Bruce, G. Harper, M. M. Jones, L. M. Mclay, J. Bradshaw, *J. Med. Chem.* **2003**, *46*, 3257 – 3274.
[24] Y. J. Xu, M. Johnson, *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 181 – 185.
[25] P. Ertl, *J. Chem. Inf. Model.* **2014**, *54*, 1617 – 1622.
[26] R. C. Johnston, P. H. Cheong, *Org. Biomol. Chem.* **2013**, *11*, 5057 – 5064.

[27] Z. S. Derewenda, L. Lee, U. Derewenda, *J. Mol. Biol.* **1995**, *252*, 248 – 262.
[28] N. Brown, E. Jacoby, *Mini-Rev. Med. Chem.* **2006**, *6*, 1217 – 1229.
[29] A. B. Yongye, J. Waddell, J. L. Medina-Franco, *Chem. Biol. Drug Des.* **2012**, *80*, 717 – 724.
[30] J. L. Medina-Franco, K. Martínez-Mayorga, A. Bender, T. Scior, *QSAR Comb. Sci.* **2009**, *28*, 1551 – 1560.
[31] C. M. Richardson, M. J. Lipkin, D. W. Sheppard, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 2089 – 2095.
[32] P. Willett, *Mol. Inf.* **2014**, *33*, 403 – 413.
[33] A. Cereto-Massagué, M. J. Ojeda, C. Valls, M. Mulero, S. Garcia-Vallvé, G. Pujadas, *Methods* **2015**, *71*, 58 – 63.
[34] D. Rogers, R. D. Brown, M. Hahn, *J. Biomol. Screening* **2005**, *10*, 682 – 686.
[35] S. C. Sukuru, J. L. Jenkins, R. E. Beckwith, J. Scheiber, A. Bender, D. Mikhailov, J. W. Davies, M. Glick, *J. Biomol. Screening* **2009**, *14*, 690 – 699.
[36] J. S. Delaney, *Mol. Diversity* **1996**, *1*, 217 – 222.
[37] O. Rabal, F. I. Amr, J. Oyarzabal, *J. Chem. Inf. Model.* **2015**, *55*, 1 – 18.
[38] D. C. Swinney, J. Anthony, *Nat. Rev. Drug Discovery* **2011**, *10*, 507 – 519.
[39] a) D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2012**, *75*, 311 – 335; b) A. Ganesan, *Curr. Opin. Chem. Biol.* **2008**, *12*, 306 – 317.
[40] a) M. Feher, J. M. Schmidt, *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 218 – 227; b) K. Grabowski, K. H. Baringhaus, G. Schneider, *Nat. Prod. Rep.* **2008**, *25*, 892 – 904.
[41] J. Hert, J. J. Irwin, C. Laggner, M. J. Keiser, B. K. Shoichet, *Nat. Chem. Biol.* **2009**, *5*, 479 – 483.
[42] G. T. Carter, *Nat. Prod. Rep.* **2011**, *28*, 1783 – 1789.
[43] R. W. Huigens III, K. C. Morrison, R. W. Hicklin, T. A. Flood, Jr., M. F. Richter, P. J. Hergenrother, *Nat. Chem.* **2013**, *5*, 195 – 202.
[44] S. E. O'Connor, J. J. Maresh, *Nat. Prod. Rep.* **2006**, *23*, 532 – 547.
[45] R. J. Rafferty, R. W. Hicklin, K. A. Maloof, P. J. Hergenrother, *Angew. Chem. Int. Ed.* **2014**, *53*, 220 – 224; *Angew. Chem.* **2014**, *126*, 224 – 228.
[46] Q. F. Chen, F. P. Wang, X. Y. Liu, *Chem. Eur. J.* **2015**, *21*, 8946 – 8950.
[47] V. A. Ignatenko, Y. Han, G. P. Tochtrop, *J. Org. Chem.* **2013**, *78*, 410 – 418.
[48] J. Zhang, J. Wu, B. Hong, W. Ai, X. Wang, H. Li, X. Lei, *Nat. Commun.* **2014**, *5*, 4614.
[49] a) B. K. Hong, H. H. Li, J. B. Wu, J. Zhang, X. G. Lei, *Angew. Chem. Int. Ed.* **2015**, *54*, 1011 – 1015; *Angew. Chem.* **2015**, *127*, 1025 – 1029; b) H. H. Li, X. M. Wang, B. K. Hong, X. G. Lei, *J. Org. Chem.* **2013**, *78*, 800 – 821; c) H. H. Li, X. M. Wang, X. G. Lei, *Angew. Chem. Int. Ed.* **2012**, *51*, 491 – 495; *Angew. Chem.* **2012**, *124*, 506 – 510.
[50] H. Mizoguchi, H. Oikawa, H. Oguri, *Nat. Chem.* **2014**, *6*, 57 – 64.
[51] J. M. Finefield, D. H. Sherman, M. Kreitman, R. M. Williams, *Angew. Chem. Int. Ed.* **2012**, *51*, 4802 – 4836; *Angew. Chem.* **2012**, *124*, 4886 – 4920.
[52] K. Foo, I. Usui, D. C. G. Götz, E. W. Werner, D. Holte, P. S. Baran, *Angew. Chem. Int. Ed.* **2012**, *51*, 11491 – 11495; *Angew. Chem.* **2012**, *124*, 11659 – 11663.
[53] A. D. Fotiadou, A. L. Zografos, *Org. Lett.* **2011**, *13*, 4592 – 4595.
[54] a) E. M. Driggers, S. P. Hale, J. Lee, N. K. Terrett, *Nat. Rev. Drug Discovery* **2008**, *7*, 608 – 624; b) H. R. Hoveyda, E. Marsault, R. Gagnon, A. P. Mathieu, M. Vezina, A. Landry, Z. G. Wang, K. Benakli, S. Beaubien, C. Saint-Louis, M. Brassard, J. F. Pinault, L. Ouellet, S. Bhat, M. Ramaseshan, X. W. Peng, L. Foucher, S. Beauchemin, P. Bherer, D. F. Veber, M. L. Peterson, G. L. Fraser, *J. Med. Chem.* **2011**, *54*, 8305 – 8320; c) E. Marsault, M. L. Peterson, *J. Med. Chem.* **2011**, *54*, 1961 – 2004; d) M. D. Cummings, T. I. Lin, L. Hu, A. Tahri, D. McGowan, K. Amssoms, S. Last, B. Devogelaere, M. C. Rouan, L. Vijgen, J. M. Berke, P. Dehertogh, E. Fransen, E. Cleiren, L. van der Helm, G. Fanning, K. Van Emelen, O. Nyanguile, K. Simmen, P. Raboisson, S. Vendeville, *Angew. Chem. Int. Ed.* **2012**, *51*, 4637 – 4640; *Angew. Chem.* **2012**, *124*, 4715 – 4718.
[55] a) H. S. G. Beckmann, F. L. Nie, C. E. Hagerman, H. Johansson, Y. S. Tan, D. Wilcke, D. R. Spring, *Nat. Chem.* **2013**, *5*, 861 – 867; b) A. Isidro-Llobet, K. H. Georgiou, W. R. J. D. Galloway, E. Giacomini, M. R. Hansen, G. Mendez-Abt, Y. S. Tan, L. Carro, H. F. Sore, D. R. Spring, *Org. Biomol. Chem.* **2015**, *13*, 4570 – 4580; c) A. Grossmann, S. Bartlett, M. Janecek, J. T. Hodgkinson, D. R. Spring, *Angew. Chem. Int. Ed.* **2014**, *53*, 13093 – 13097; *Angew. Chem.* **2014**, *126*, 13309 – 13313.
[56] a) L. A. Marcaurelle, E. Comer, S. Dandapani, J. R. Duvall, B. Gerard, S. Kesavan, M. D. Lee, H. B. Liu, J. T. Lowe, J. C. Marie, C. A. Mulrooney, B. A. Pandya, A. Rowley, T. D. Ryba, B. C. Suh, J. Q. Wei, D. W. Young, L. B. Akella, N. T. Ross, Y. L. Zhang, D. M. Fass, S. A. Reis, W. N. Zhao, S. J. Haggarty, M. Palmer, M. A. Foley, *J. Am. Chem. Soc.* **2010**, *132*, 16962 – 16976; b) S. Dandapani, J. T. Lowe, E. Comer, L. A. Marcaurelle, *J. Org. Chem.* **2011**, *76*, 8042 – 8048; c) M. E. Fitzgerald, C. A. Mulrooney, J. R. Duvall, J. Q. Wei, B. C. Suh, L. B. Akella, A. Vrcic, L. A. Marcaurelle, *ACS Comb. Sci.* **2012**, *14*, 89 – 96.
[57] D. H. Chou, J. R. Duvall, B. Gerard, H. Liu, B. A. Pandya, B. C. Suh, E. M. Forbeck, P. Faloon, B. K. Wagner, L. A. Marcaurelle, *ACS Med. Chem. Lett.* **2011**, *2*, 698 – 702.
[58] D. Robbins, A. F. Newton, C. Gignoux, J. C. Legeay, A. Sinclair, M. Rejzek, C. A. Laxon, S. K. Yalamanchili, W. Lewis, M. A. O'Connell, R. A. Stockman, *Chem. Sci.* **2011**, *2*, 2232 – 2235.
[59] a) P. Aggarwal, G. Procopiou, D. Robbins, G. Harbottle, W. Lewis, R. A. Stockman, *Synlett* **2012**, 423 – 427; b) T. E. Storr, S. J. Cully, M. J. Rawling, W. Lewis, D. Hamza, G. Jones, R. A. Stockman, *Bioorg. Med. Chem.* **2015**, *23*, 2621 – 2628.
[60] W. Liu, V. Khedkar, B. Baskar, M. Schürmann, K. Kumar, *Angew. Chem. Int. Ed.* **2011**, *50*, 6900 – 6905; *Angew. Chem.* **2011**, *123*, 7032 – 7037.
[61] a) H. Dückert, V. Pries, V. Khedkar, S. Menninger, H. Bruss, A. W. Bird, Z. Maliga, A. Brockmeyer, P. Janning, A. Hyman, S. Grimme, M. Schürmann, H. Preut, K. Hübel, S. Ziegler, K. Kumar, H. Waldmann, *Nat. Chem. Biol.* **2012**, *8*, 179 – 184; b) V. Eschenbrenner-Lux, H. Dückert, V. Khedkar, H. Bruss, H. Waldmann, K. Kumar, *Chem. Eur. J.* **2013**, *19*, 2294 – 2304.
[62] M. Garcia-Castro, L. Kremer, C. D. Reinkemeier, C. Unkelbach, C. Strohmann, S. Ziegler, C. Ostermann, K. Kumar, *Nat. Commun.* **2015**, *6*, 6516.
[63] a) A. H. Bansode, A. C. Shaikh, R. D. Kavthe, S. Thorat, R. G. Gonnade, N. T. Patil, *Chem. Eur. J.* **2015**, *21*, 2319 – 2323; b) N. T. Patil, V. S. Shinde, B. Sridhar, *Angew. Chem. Int. Ed.* **2013**, *52*, 2251 – 2255; *Angew. Chem.* **2013**, *125*, 2307 – 2311.
[64] J. Barjau, G. Schnakenburg, S. R. Waldvogel, *Angew. Chem. Int. Ed.* **2011**, *50*, 1415 – 1419; *Angew. Chem.* **2011**, *123*, 1451 – 1455.
[65] C. Fang, B. D'Souza, C. F. Thompson, M. C. Clifton, J. W. Fairman, B. Fulroth, A. Leed, P. McCarren, L. Wang, Y. Wang, C. Feau, V. K. Kaushik, M. Palmer, G. Wei, T. R. Golub, B. K. Hubbard, M. H. Serrano-Wu, *ACS Med. Chem. Lett.* **2014**, *5*, 1308 – 1312.
[66] J. D. Leverson, H. Zhang, J. Chen, S. K. Tahir, D. C. Phillips, J. Xue, P. Nimmer, S. Jin, M. Smith, Y. Xiao, P. Kovar, A. Tanaka, M. Bruncko, G. S. Sheppard, L. Wang, S. Gierke, L. Kategaya, D. J. Anderson, C. Wong, J. Eastham-Anderson, M. J. Ludlam, D. Sampath, W. J. Fairbrother, I. Wertz, S. H. Rosenberg, C. Tse, S. W. Elmore, A. J. Souers, *Cell Death Dis.* **2015**, *6*, e1590.
[67] W. Pierson, B. Cauwe, A. Policheni, S. M. Schlenner, D. Franckaert, J. Berges, S. Humblet-Baron, S. Schonefeldt, M. J. Herold, D. Hildeman, A. Strasser, P. Bouillet, L. F. Lu, P. Matthys, A. A. Freitas, R. J. Luther, C. T. Weaver, J. Dooley, D. H. Gray, A. Liston, *Nat. Immunol.* **2013**, *14*, 959 – 965.
[68] R. J. Youle, A. Strasser, *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 47 – 59.

[69] D. C. Faloon et al., probe reports from the NIH molecular libraries program, National Center for Biotechnology Information (US), **2010**; available from: http://www.ncbi.nlm.nih.gov/books/NBK133438/.
[70] H.-J. Roth, *Curr. Opin. Chem. Biol.* **2005**, *9*, 293 – 295.
[71] a) S. X. Atwood, A. L. S. Chang, A. E. Oro, *J. Cell Biol.* **2012**, *199*, 193 – 197; b) R. McMillan, W. Matsui, *Clin. Cancer Res.* **2012**, *18*, 4883 – 4888.
[72] D. Amakye, Z. Jagani, M. Dorsch, *Nat. Med.* **2013**, *19*, 1410 – 1422.
[73] E. Colombo, M. Alcalay, P. G. Pelicci, *Oncogene* **2011**, *30*, 2595 – 2609.
[74] S. Z. Wang, K. Fang, G. Q. Dong, S. Q. Chen, N. Liu, Z. Y. Miao, J. Z. Yao, J. Li, W. N. Zhang, C. Q. Sheng, *J. Med. Chem.* **2015**, *58*, 6678 – 6696.
[75] a) G. Karageorgis, S. Warriner, A. Nelson, *Nat. Chem.* **2014**, *6*, 872 – 876; b) G. Karageorgis, M. Dow, A. Aimon, S. Warriner, A. Nelson, *Angew. Chem. Int. Ed.* **2015**, *54*, 13538 – 13544; *Angew. Chem.* **2015**, *127*, 13742 – 13748.
[76] D. H. Drewry, R. Macarron, *Curr. Opin. Chem. Biol.* **2010**, *14*, 289 – 298.
[77] A. L. Hopkins, *Nat. Chem. Biol.* **2008**, *4*, 682 – 690.
[78] D. J. Payne, M. N. Gwynn, D. J. Holmes, D. L. Pompliano, *Nat. Rev. Drug Discovery* **2007**, *6*, 29 – 40.
[79] a) M. N. Pollak, W. D. Foulkes, *Nat. Rev. Cancer* **2003**, *3*, 297 – 303; b) E. Sahai, *Nat. Rev. Cancer* **2007**, *7*, 737 – 749.
[80] a) M. P. H. Stumpf, T. Thorne, E. de Silva, R. Stewart, H. J. An, M. Lappe, C. Wiuf, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 6959 – 6964; b) D. W. Walke, C. S. Han, J. Shaw, E. Wann, B. Zambrowicz, A. Sands, *Curr. Opin. Biotechnol.* **2001**, *12*, 626 – 631.
[81] C. H. Arrowsmith, J. E. Audia, C. Austin, J. Baell, J. Bennett, J. Blagg, C. Bountra, P. E. Brennan, P. J. Brown, M. E. Bunnage, C. Buser-Doepner, R. M. Campbell, A. J. Carter, P. Cohen, R. A. Copeland, B. Cravatt, J. L. Dahlin, D. Dhanak, A. M. Edwards, S. V. Frye, N. Gray, C. E. Grimshaw, D. Hepworth, T. Howe, K. V. M. Huber, J. Jin, S. Knapp, J. D. Kotz, R. G. Kruger, D. Lowe, M. M. Mader, B. Marsden, A. Mueller-Fahrnow, S. Muller, R. C. O'Hagan, J. P. Overington, D. R. Owen, S. H. Rosenberg, B. Roth, R. Ross, M. Schapira, S. L. Schreiber, B. Shoichet, M. Sundström, G. Superti-Furga, J. Taunton, L. Toledo-Sherman, C. Walpole, M. A. Walters, T. M. Willson, P. Workman, R. N. Young, W. J. Zuercher, *Nat. Chem. Biol.* **2015**, *11*, 536 – 541.
[82] Q. Michaudel, Y. Ishihara, P. S. Baran, *Acc. Chem. Res.* **2015**, *48*, 712 – 721.
[83] a) https://www.europeanleadfactory.eu; b) H. Laverty, K. M. Orrling, F. Giordanetto, M. Poinot, E. Ottow, T. W. Rijnders, D. Tzalis, S. Jaroch, *J. Med. Dev. Sci.* **2015**, *1*, 20 – 33.
[84] a) N. Z. Burns, I. N. Krylova, R. N. Hannoush, P. S. Baran, *J. Am. Chem. Soc.* **2009**, *131*, 9172 – 9173; b) A. Mendoza, Y. Ishihara, P. S. Baran, *Nat. Chem.* **2012**, *4*, 21 – 25; c) J. Shi, G. Manolikakes, C. H. Yeh, C. A. Guerrero, R. A. Shenvi, H. Shigehisa, P. S. Baran, *J. Am. Chem. Soc.* **2011**, *133*, 8014 – 8027; d) S. Su, R. A. Rodriguez, P. S. Baran, *J. Am. Chem. Soc.* **2011**, *133*, 13922 – 13925; e) T. J. Maimone, Y. Ishihara, P. S. Baran, *Tetrahedron* **2015**, *71*, 3652 – 3665.
[85] G. T. Carter, P. Crews, *Bioorg. Med. Chem.* **2011**, *19*, 6556 – 6556.
[86] a) M. C. White, *Science* **2012**, *335*, 807 – 809; b) E. M. Stang, M. C. White, *Angew. Chem. Int. Ed.* **2011**, *50*, 2094 – 2097; *Angew. Chem.* **2011**, *123*, 2142 – 2145; c) E. M. Stang, M. C. White, *Nat. Chem.* **2009**, *1*, 547 – 551; d) J. H. Gui, Q. H. Zhou, C. M. Pan, Y. Yabe, A. C. Burns, M. R. Collins, M. A. Ornelas, Y. Ishihara, P. S. Baran, *J. Am. Chem. Soc.* **2014**, *136*, 4853 – 4856.

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