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# Toward High-Throughput Synthesis of Complex Natural Product-Like Compounds in the Genomics and Proteomics Age

## Review

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### Summary

In the age of high-throughput biology, novel genes and proteins are emerging quickly. The need for developing organic synthesis-derived methods that allow rapid access to polyfunctional, complex natural product-like compounds is growing constantly, largely because these small-molecule-based compounds serve as *smart*, powerful tools both in understanding the roles and functions of emerging biological targets and in validating their biological responses. Developing asymmetric synthesis-derived organic reactions on solid phase allows the synthesis of complex natural product-like compounds in a high-throughput manner. Solid phase organic synthesis is now commonly utilized in the library synthesis of rather simple compounds (i.e., compounds with no multiple stereogenic centers). With few exceptions, the synthesis of complex natural product-like derivatives is still in its infancy. Some recent efforts made in this area indicate opportunities yet to be explored.

### Introduction

The use of plant extracts in the search for biologically active natural products is a powerful approach in the identification of lead compounds and, even today, is commonly practiced in drug discovery-derived research programs [1, 2]. In several cases, natural products have served as good starting points in developing new drug-like candidates. After a positive biological response from a given plant extract, the challenge is then to isolate and assign the structure of the natural product responsible for the activity. This could be a time-consuming and challenging exercise!

Stereo- and enantioselective organic synthesis is a powerful discipline that allows the synthesis of complex, chiral, enantiomerically pure, polycyclic, natural products as well as natural product-like derivatives. These small-molecule-based compounds serve as useful chemical probes for understanding the roles and functions of biological targets. They also provide a good starting point in the design and synthesis of compounds that could act as promoters or inhibitors of protein-protein interactions. Such compounds might include small-molecule-based agents with specific bindings to enzymes as well as proteins.

Most natural products known to modulate (i.e., pro-

mote or inhibit) protein-protein interactions by interacting with enzymes and proteins are chiral, highly complex in nature, and possess several stereogenic centers as well as diverse functional groups [3–5]. These properties make them ideal as small-molecule candidates that could selectively bind to enzymes or proteins. From a synthesis point of view, natural products present tremendous challenges for developing efficient synthetic methods. During the past two decades, challenges in the syntheses of complex natural products have been a driving force in the development of stereo- and enantioselective methods through the use of chiral reagents and chiral catalytic reactions [6]. Over the years, one thing has clearly emerged: complex natural-product synthesis is tedious work. This leads to a slow process for the identification of drug-like candidates.

As chemists seek to overcome the time factor in total synthesis, the use of solid phase methodologies is gaining momentum because purification steps can be avoided, allowing the possibility of developing automated synthetic processes [7–12]. In general, it is easy to explore a solid phase method for the development of a parallel, automated process leading to high-throughput synthesis [13–15]. To obtain complex natural products or their derivatives by solid phase high-throughput synthesis, we must have efficient stereo- and enantioselective-based solid phase methods. This is an active area of research, in which the community involved is constantly rising to the challenges of synthesizing complex derivatives on solid phase that can be subjected to automation for high-throughput syntheses. In recent years, several solid phase-based methods leading to the high-throughput synthesis of rather simple compounds having no multiple stereogenic centers have been developed. *At present, highly functionalized, chiral, polycyclic derivatives, medium- to large-ring-size derivatives, and macrolides etc. are still beyond the reach of stereo- and enantioselective, solid phase method development.* For example, complex natural products such as taxol, FK506, rapamycin, and vinblastine (see Figure 1) have proven to be effective as highly potent, bioactive, clinically useful natural products. However, with very few exceptions, the degree of complexity that can be achieved by stereo- and enantioselective solution phase organic synthesis is still beyond the limits of solid phase for developing high-throughput synthesis [16–23].

In this genomics and proteomics age, high-throughput technologies are advancing biology-driven research on several fronts. The ultimate goal is to benefit from the high-throughput technologies by gaining an understanding of the roles and functions of new biological targets and developing highly selective, improved drug-like candidates. One such approach is to use small molecules as chemical probes for the disruption (i.e., activation or inactivation) of protein-protein interactions [24–25]. In contrast to mutation-derived research efforts to understand the functions of a given protein, small molecules can directly be used to modulate protein functions. This

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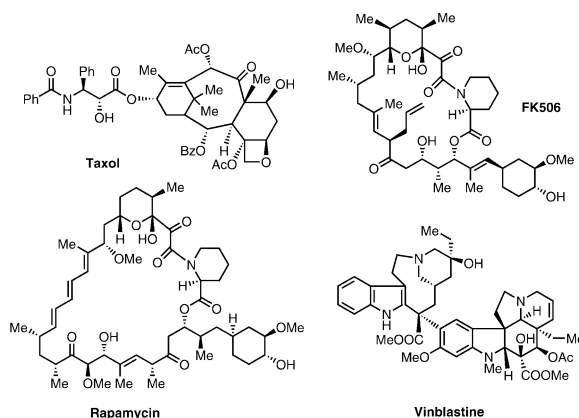


Figure 1. A Few Examples of Bioactive, Complex Natural Products that Have Been Challenging Targets for the Synthetic Community

process is commonly known as chemical genetics [26–30]. The success of the chemical genetics approach largely depends upon the availability of large sets of structurally complex, natural product-like compounds having diverse functionality. The chemical genetics approach is free from the undesired side effects that one may encounter during mutational experiments [26]. With speed increasing constantly in high-throughput biology, there is a growing need for the development of solid support, stereoselective reaction-based methods that can further be utilized in the high-throughput synthesis of complex, highly functionalized, natural product-like derivatives. Very recently, several research groups have taken the challenge of developing stereoselective reaction-based methods on solid phase and of utilizing them for the high-throughput syntheses of complex natural product-like derivatives. Some of these examples are covered in this review article.

### Highly Functionalized Polycyclic Derivatives

Over the years, natural products have been utilized as small molecule-based chemical probes in the modulation of biological targets, i.e., in the precise control of specific cellular processes. If the availability of small molecule-based chemical probes is to keep up with emerging high-throughput biology, there is a growing need for the development of solid phase methods for obtaining highly functionalized, natural product-based chiral templates for further use in the development of high-throughput syntheses.

Toward this goal, Schreiber et al. [31–32] developed a highly efficient, multi-step synthesis to obtain enantiomerically pure template **2.4** (Figure 2) from shikimic acid, **2.1**. Shikimic acid was first converted into both enantiomers of epoxy-cyclohexenol carboxylic acid derivative **2.2**, which was then coupled to a photocleavable linker immobilized onto Tentagel-S-NH<sub>2</sub> poly(ethylene glycol)-polystyrene copolymer. Treatment of the resin-bound epoxy-cyclohexenol derivative, **2.3**, with various nitron carboxylic acids under esterification conditions and a subsequent intramolecular cycloaddition reaction yielded, via a tandem acylation/stereoselective 1,3-dipolar cycloaddition reaction, the tetracyclic scaffold **2.4** with com-

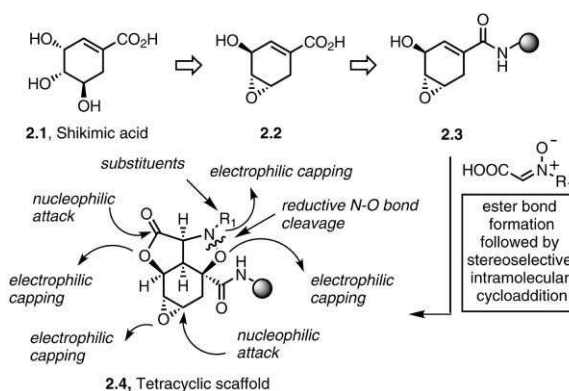


Figure 2. In the Area of Diversity-Oriented Synthesis, Schreiber et al.'s Approach to Obtaining a Highly Functionalized Tetracyclic Template Leading to Natural Product-Like Small-Molecule Libraries

plete regio- and stereoselectivity. The tetracyclic derivative **2.4** is rigid and densely functionalized, allowing it to undergo a variety of organic transformations without the use of protecting groups. Upon treatment with a variety of organic and organometallic reagents, this template can be utilized for obtaining highly functionalized bicyclic and tricyclic derivatives as indicated in Figure 2. In an example shown in Figure 3, the tetracyclic template anchored onto solid support, **3.1**, was subjected to a Cu(I) or Pd(II)-mediated carbon-carbon bond-forming reaction with an iodo-aryl moiety of the template. This reaction gave product **3.2**. Upon reaction with different amines, the lactone ring was opened and gave the carboxylamide derivative, **3.3**, having a free hydroxyl group. The hydroxyl derivative, **3.3**, was acylated under standard acylation reactions to give compound **3.4** in high yields. Several key reactions were well optimized before any library synthesis was undertaken. Via a split-and-mix solid phase synthesis method, the tetracyclic tem-

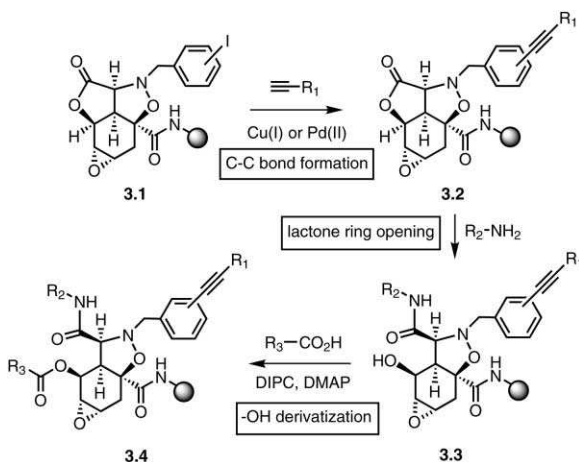


Figure 3. An Example of Diversity-Oriented Synthesis in which the Tetracyclic Template **3.1** Has Been Utilized for the Introduction of Three Diversity Sites

Diversity sites include the Pd(II)-mediated carbon-carbon bond formation from the iodobenzene moiety, an amine-mediated opening of the lactone ring, and the derivatization of -COOH and -OH groups.

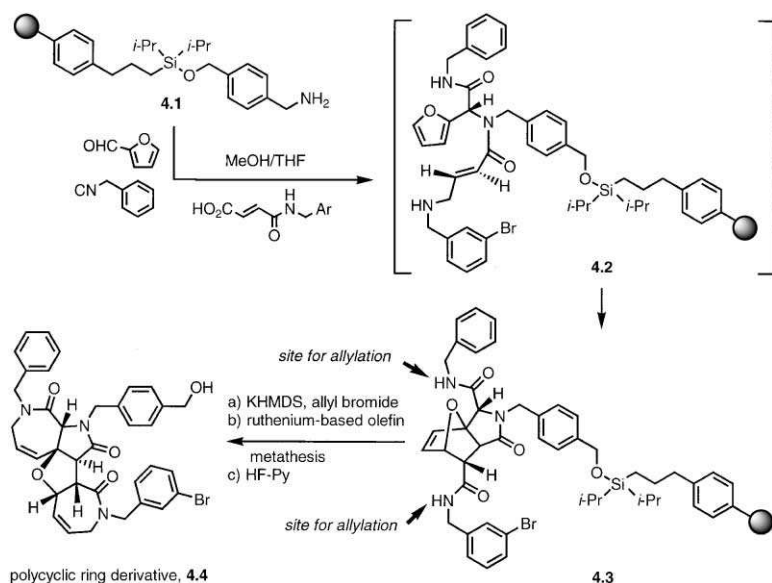


Figure 4. Another Example of the Diversity-Oriented Synthesis of Natural Product-Like Compounds

Here, Schreiber and coworkers have utilized a Ugi reaction followed by the ring closing metathesis approach to obtain 4.3, the bis-allyl derivative of the Ugi product.

plate 2.4 was utilized in developing a stereoselective synthesis of a library exceeding 2,000,000 compounds. It is interesting to note that the library synthesis plan did not incorporate functional-group protection and deprotection. Usually, this is a daunting task when it comes to the total synthesis of complex molecules.

The library was then tested in several miniaturized cell-based assays in a search for cell-permeable small molecules as protein binding agents. For example, several members of this library were found to activate a reporter gene in mink lung cells. The driving force in the library synthesis plan was the development of a stereoselective synthesis of a highly functionalized tetracyclic derivative, 2.4, which can undergo several simple organic transformations, i.e. nucleophilic and acylation reactions etc. This is the first example of a complex natural product-based library synthesis, in which the development of enantiomerically pure template was achieved by the use of a highly functionalized chiral starting material.

Another method developed by Schreiber and coworkers [33] is the pairwise use of complexity-generating reactions in diversity-oriented organic synthesis (Figure 4). The key step in this plan is the use of a Ugi four-component reaction [34] to give the product capable of undergoing an intramolecular cycloaddition reaction followed by subjection to ring opening-closing metathesis. The stereoselective synthesis of highly complex, polycyclic derivatives by a Ugi reaction followed by an intramolecular Diels-Alder reaction and the ring opening-closing olefin metathesis led to the 7-5-5-7-membered fused tetracyclic derivative 4.4 on solid phase. The free hydroxyl group of the Fmoc-protected amine derivative was immobilized onto polystyrene beads containing a carbon-silicon linker. The release of the Ugi product from the resin was achieved by the standard cleavage of the silicon-oxygen bond via HF in pyridine. The resin bound amine 4.1 was treated with excess furfural, benzylisocyanide, and fumaric acid (3-bromobenzyl) monocarboxamide to give the complex Ugi

product, 4.3, after an intramolecular Diels-Alder reaction via the intermediate derivative, 4.2. Bis-allylation of the secondary amides was achieved by reaction with allyl-bromide and potassium hexamethyldisilazane. This was then subjected to ring opening-closing metathesis in the presence of the ruthenium catalyst. The resin was treated subsequently with HF-pyridine to give the desired polycyclic compound 4.4 in high yield. The stereoselective Ugi reaction, combined with intramolecular Diels-Alder and the olefin ring opening-closing metathesis, has proven to be a highly efficient strategy for one-pot preparation of highly functionalized, natural product-like compounds. The paper describes only the stereoselective method developed on solid phase and does not discuss the library synthesis based upon the method developed by the researchers.

Recently, Schreiber et al. [35] also developed a method for the split-pool synthesis of 1,3-dioxanes (see Figure 5). Their method led to arrayed stock solutions of single compounds sufficient for multiple phenotypic and protein binding assays. The 1,3-dioxane structure was selected for split-pool synthesis because it is a rigid core that can be synthesized in a stereoselective manner with high purity in the presence of diverse ancillary functional groups. The  $\gamma$ ,  $\delta$ -epoxyalcohol derivatives were attached onto the polystyrene solid support through a diisopropylphenylsilyl ether linkage in 90% of the theoretical yield. The epoxyalcohol-derivatized resin, 5.1, was pooled and split into 30 vessels with a diverse set of secondary amine and thiol building blocks to generate 90 different 1,3-diol derivatives in near-quantitative yields. The solid-supported 1,3-diol derivatives were pooled, split into two portions, and reacted with Fmoc-aminodimethyl acetal building blocks in 0.05 M HCl in dioxane and trimethylsilyl chloride (TMSCl) to furnish 180 Fmoc amino-1,3-dioxane in an 85%–95% yield. The resins were pooled and treated with piperidine to affect the Fmoc removal, and the solid-supported amines were split and reacted with 10 electrophilic reagents to generate 1800 amides, ureas, thioureas, and sulfonamides.

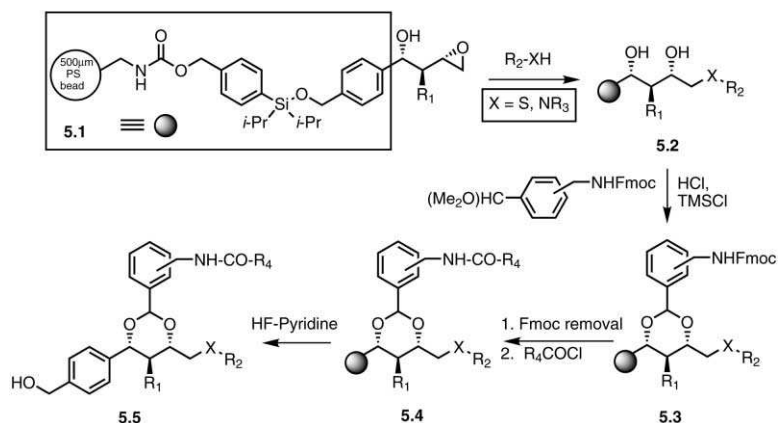


Figure 5. An Example of the Stereoselective Synthesis of a 1,3-di-OH Syn-Derivative, 5.2, from the Chiral Epoxide 5.1

This reaction led to the synthesis of chiral, 1,3-dioxane derivatives on solid support.

Thus, Schreiber and coworkers developed a split-pool 1,3-dioxane synthesis that led to the production of arrayed stock solution compatible with multiple phenotypic and protein binding assays by using a chemically robust silicon linker that is crucial for performing multiple assays with individual compounds derived from a single bead synthesis.

Verdine et al. [36] reported in 2001 a solid phase library synthesis of heterodimerizers having chiral tetrahydrooxazepine (THOX) derivatives as a part of the molecule attached to AP 1867, a ligand that targets FK 506 binding protein (Figure 6). The goal was to develop such libraries in search of cell-permeable, small molecule-based ligands as chemical inducers of protein dimerization. In other words, they were looking for small molecules that are capable of inducing the dimerization of macromolecules. The tetrahydrooxazepine (THOX) was synthesized as shown in Figure 6. The chiral template, a THOX semirigid core, has three diversity elements, two on the ring and one on the side chain. This presented the possibility that the N-O bond of the THOX might be reductively cleaved to convert the semirigid cyclic derivative to a more flexible acyclic compound containing additional diversity sites. Three stereogenic centers on the THOX unit can be independently varied via asymmetric synthesis, thus giving rise to all eight possible stereoisomers. The presence of uncharged heteroatoms on the THOX

unit at physiological pH balances the hydrophobicity of the molecule and thus favors its solubility in aqueous media and transit across cell membranes. The THOX structure was synthesized by a convergent approach in which two independently diversifiable monomers were coupled via a Mitsunobu reaction followed by ring closing metathesis to produce exclusively the hetero-coupled product with the *cis* double bond orientation. At this stage, the AP 1867 was attached onto the moiety in the penultimate step. The presence of the olefin in the THOX ligand should enable further functionalization through reduction, epoxidation, and dihydroxylation, etc. Thus, Verdine and coworkers demonstrated for the first time a library constructed for the purpose of inducing the association of two proteins, only one of which has a known small-molecule ligand.

#### Biomimetic Approach to Highly Functionalized Polycyclic Derivatives

Nature is extraordinarily efficient and precise during the biosynthesis of secondary metabolites and has generated, through an evolutionary process, a vast variety of natural products. As discussed earlier, examples of natural products such as Taxol, FK-506, vinblastine, and rapamycin are known to associate strongly and specifically to proteins. The goal of uncovering small molecules that bind to proteins in a highly specific manner and

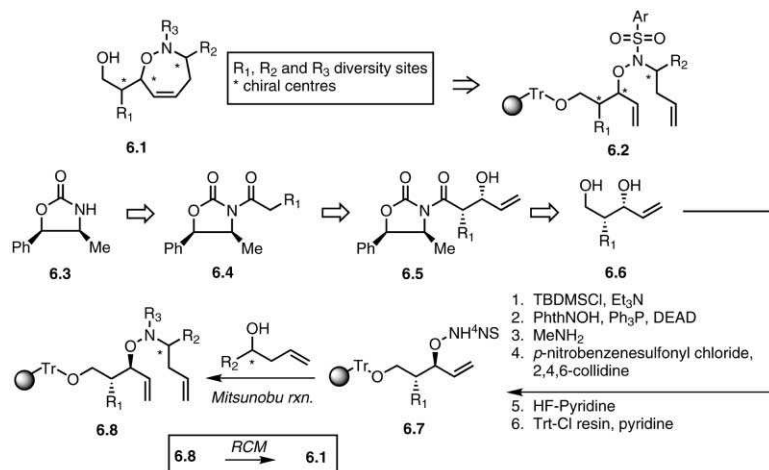


Figure 6. To Obtain a Small Molecule-Based, Heterodimeric Compound Library, Verdine and Coworkers Utilized Evans' *Syn* Aldol Methodology to Synthesize the Intermediate 6.6.

After a few modifications, the derivative 6.8 anchored onto solid support was subjected to ring-closing metathesis, giving the tetrahydrooxazepines.

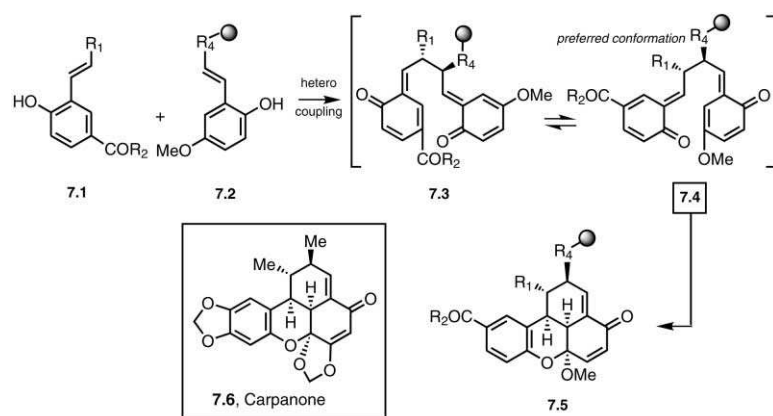


Figure 7. Shair and Coworkers Developed a Biomimetic-Type Approach that Involves the Cycloaddition Reaction with Electron-Rich and Electron-Deficient Phenolic Derivatives

This strategy was proven to be highly efficient for the synthesis of natural product-like (i.e., *carpanone*) polycyclic derivatives.

with high affinity has presented a major challenge to construct libraries of molecules that take advantage of the structural features of natural products while incorporating diverse elements of functionality. Rather than devising new synthetic routes to natural product-like molecules, it would be more efficient to emulate the process that nature has developed to access these privileged structures.

The first biosynthetic process reported is the remarkable construction of a natural product, *Carpanone* (Figure 7, 7.6), from the homodimerization of two simple phenolic precursors under control of a variant of the phenolic oxidative coupling pathway. Chapman et al. [37] replicated this biosynthesis by exposing the phenol to Pd(II) salts. To broaden the scope of this biomimetic synthesis, with the goal of constructing a split-pool synthesis library of carpanone-like molecules more diverse than those isolated from nature, Shair and coworkers [38] developed a novel reaction that would result in intermolecular oxidative heterodimerization of dissimilar *o*-hydroxystyrenes. They accomplished this by electronically differentiating the phenols and immobilizing the more reactive phenol onto the solid support in order to minimize side reactions. An oxidative dimerization with  $\text{PhI}(\text{OAc})_2$  and a subsequent intramolecular inverse electron-demand Diels-Alder cycloaddition, controlled by the differential electronic nature of the two aromatic partners (see compounds 7.1 and 7.2), through transition

state 7.4, provided the carpanone-based tetracyclic derivative, 7.5, in a single operation.

Shair et al. [39] very recently demonstrated the use of a biomimetic-based diversity-oriented synthesis to discover Galanthamine-like molecules (Figure 8) with biological properties beyond those of the natural product, Galanthamine. As first articulated by Barton [40], a single precursor, Norbelladine, was converted via specific oxidative phenolic coupling pathways to an entire class of natural products including the Crimines, Galanthamines, Lycoranes, and Pretazetines. Each compound is structurally different and elicits a different biological response. Shair and coworkers utilized these characteristics in developing a biomimetic synthesis-based method on solid phase to obtain a chiral template for the diverse libraries of complex molecules.

Figure 8 outlines the Amaryllidaceae alkaloid pathway by mimicking the oxidative phenolic coupling reaction that takes place in nature with the hypervalent iodine reagent. By using a simple orthogonal protecting group strategy, one can then direct a common dienone intermediate to cyclize on nitrogen to generate Crimine- or Galanthamine-type structures after selective liberation of the phenolic moiety. Subsequent split pool-derived organic synthesis on two core systems generated a structurally rich Amaryllidaceae alkaloid-based library. The library synthesis commenced with attachment of tyrosine derivatives to 500–600  $\mu\text{m}$  high-capacity poly-

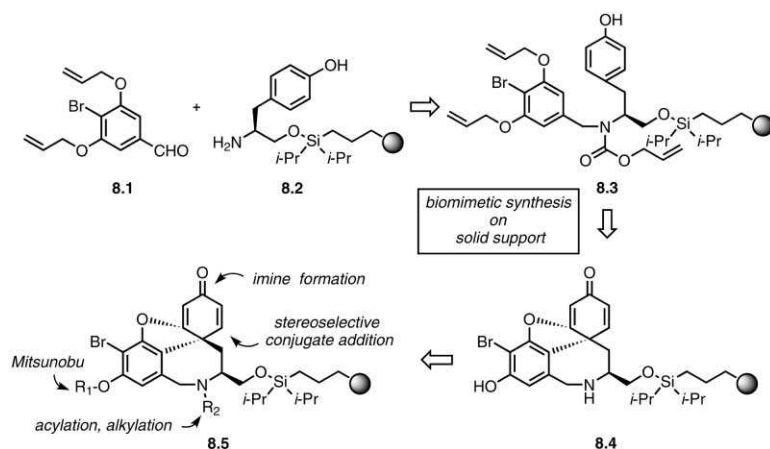


Figure 8. Another Example of a Biomimetic-Type Approach, reported by Shair and Coworkers, in which the Oxidation of the Phenolic Moiety and Subsequent Hetero-Michael-Type Reactions on Solid Phase Are the Key Steps

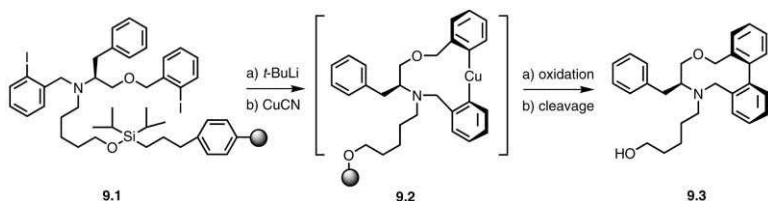


Figure 9. Schreiber and Coworkers Developed a Solid-Phase Asymmetric Synthesis of Medium-Ring Derivatives Having a Chiral Biaryl Moiety

This synthesis was accomplished from the chiral di-iodoaryl derivative by application of the chemistry developed by Lipshutz and coworkers.

styrene beads through the Si-O bond to generate derivative **8.5**. The reductive amination followed by protecting-group adjustments yielded compound **8.3** anchored onto solid support. Upon exposure to  $\text{PhI}(\text{OAc})_2$ , **8.3** afforded the oxidative derivative **8.4**. The quinone derivative, **8.4**, was then converted to compound **8.5** via Pd-mediated deprotection and spontaneous intramolecular hetero Michael-type reaction, giving the cyclic derivative, **8.6**. The diversity steps were accomplished by (a) the phenolic hydroxyl group alkylation, (b) an intermolecular Michael-type reaction with thiols in the presence of *n*-BuLi, (c) an imine formation from the carbonyl group, and (d) the secondary amine alkylation or acylation. It is interesting to note that the intermolecular Michael-type reaction with various thiol-based nucleophiles is highly diastereoselective and gives a single diastereomer as the product. The product was finally cleaved from the solid support by the use of HF-pyridine, and the library was then screened via a cell-based phenotypic assay. A new natural product-like derivative was identified as a potent inhibitor of VSVG-GFP movement from the Golgi apparatus to the plasma membrane. However, Galanthamine, as such, had no observed effects on the secretory pathway.

### Highly Functionalized Medium Ring to Macrocyclic Derivatives

Schreiber and coworkers [41] developed a novel method toward diversity-oriented stereoselective syntheses of biaryl- or bis(aryl)metal-containing medium rings [42]. Their plan was to incorporate within the derivative a biaryl group that, in addition to having medium-sized rings, was chiral. The key reaction was the formation of a medium-sized ring mediated by copper that led to the synthesis of asymmetric biaryl-derived natural product-like molecules on solid phase. The asymmetric biaryl coupling was achieved from the corresponding chiral diiodo derivative, **9.1** (Figure 9), anchored onto the solid support. The driving force for the asymmetric aryl-aryl bond formation was the presence of a chiral group in compound **9.1**. Presumably, treatment of **9.1** with *tert*-butyl-lithium followed by CuCN gave the cyclic cuprate, **9.2**, which yielded the biaryl derivative, **9.3**, upon exposure to 1,3-dinitrobenzene. The reaction conditions utilized could also be applied to several electron-rich and electron-poor aromatic rings such as thiophenes, pyridines, etc. The method developed by Schreiber and coworkers is an attractive approach for obtaining asymmetric medium-sized ring derivatives on solid phase.

Another novel method developed by Schreiber and coworkers [43] was a strategy toward macrocyclic ring closure and functionalization aimed at split-pool syntheses.

The ring closure substrate, **10.4** (Figure 10), was prepared by simultaneous or sequential acylation of a 1,2-aminoalcohol derivative with 4-pentenoic acid or its 2-substituted derivatives. The ring-closing metathesis reaction was then performed in the presence of the Grubbs catalyst to produce compound **10.6** in moderate to excellent yields. To further illustrate the use of bifurcating reaction pathways that produce different backbone scaffolds, Schreiber and coworkers went on to further macrocyclic ring formation on solid phase and to explore reactions on the macrocycle under macrocyclic control. Functional groups such as olefins and carbonyl groups that are present in the macrocycle could then undergo various macrocyclic-based stereocontrolled reactions (i.e., epoxidation, enol ether reactions, etc). Finally, to demonstrate the feasibility of performing the ring-closing metathesis reaction sequence on a solid support, Schreiber et al. used a traceless linker to synthesize the macrocyclic derivative, **10.8**, with excellent purity after cleavage with 10% TFA.

Protein kinase C (PKC) plays a critical role in numerous cellular processes, such as growth, differentiation, secretion, apoptosis, and cancer development [44, 45]. Studies have showed that altered PKC activity has been linked to cancer, asthma, rheumatoid arthritis, diabetic complications, psoriasis, and disorders of the central nervous system [46, 47]. Thus, the synthesis of modulators (i.e., activators or inhibitors) of PKC activity may help us to gain insightful information regarding the structural-activity relationship (SAR) of PKC enzymes and

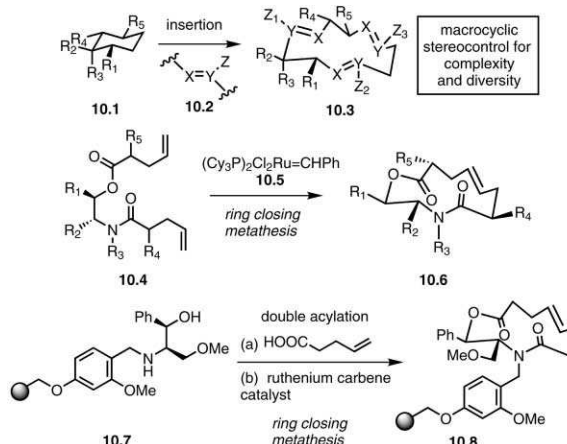


Figure 10. Using Ring-Closing Metathesis as a Key Step, Schreiber and Coworkers Reported the Synthesis of Macrocyclic Derivatives, **10.8**, on Solid Phase

These derivatives could further be subjected to macrocyclic control-derived, stereoselective transformations.

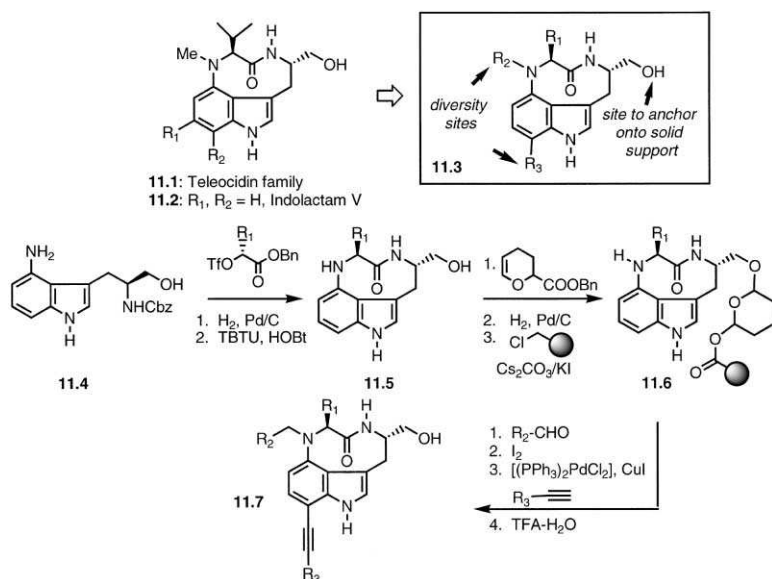


Figure 11. Based upon the *Indolactam* Natural Product, a Small-Molecule Library Synthesis Was Reported by Waldmann and Co-workers

The reductive amination and coupling of the iodoaryl moiety with different alkynes were two key steps.

could have implication in the development of new drug-like candidates.

(-)-Indolactam V, the core structure of tumor-protecting teleocidins [48], has generated vast interest as the key compound for structural-diversity synthesis (Figure 11). Herein, Waldmann et al. describe an efficient synthesis for a library of indolactam analogs. This synthesis uses a combination of solution and solid phase approaches [49–51]. For the design of the library, Waldmann et al. have taken into consideration the substituents at R<sub>1</sub>, and R<sub>2</sub> (see compound 11.3), which are known to influence the conformation of the nine-member lactam ring. Furthermore, substitution at R<sub>3</sub> is known to mediate membrane binding, and the hydroxyl group in the lactam ring is critical for biological activity. Hence, indolactam analogs 11.3 (with diversity at R<sub>1</sub>, R<sub>2</sub>, and R<sub>3</sub>) may lead to the identification of more potent PCK modulators.

Diversity at R<sub>1</sub> was introduced by alkylation of the aromatic amine with three different  $\alpha$ -hydroxy acid ester triflates. The triflate derivatives were displaced by aminoindole 11.4 in dichloromethane after the removal

of the Cbz group under hydrogenolysis and amide bond formation for which TBTU was the coupling reagent. This gave indolactam derivative 11.5. Attachment of indolactams 11.5 onto the solid support was effected with a tetrahydropyran linker by conversion of 11.5 into the corresponding acetals. Removal of the benzyl ester group and subsequent coupling to chloromethyl polystyrene beads by esterification via CsCO<sub>3</sub> resulted in substrate/linker/resins 11.6. This resin-bound indole was *N*-alkylated by reductive amination (aldehydes and NaHB(OAc)<sub>3</sub>) for incorporation of R<sub>2</sub>. The diversity at R<sub>3</sub> was introduced by Sonogashira coupling with acetylenes to give alkyne derivatives. Finally, the resin was cleaved (TFA/H<sub>2</sub>O) to give compound 11.7 in overall yields ranging from 10% to 65%.

### Benzopyran and Indole-Derived Natural Product-Like Derivatives

The 2,2-dimethylbenzopyran moiety has been the center of several libraries designed by Nicolaou's group. This

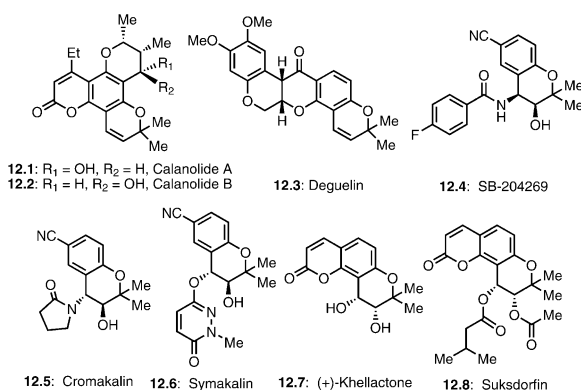


Figure 12. Several Bioactive, Polyphenolic-Derived Natural Products Contain 2,2-Dimethylbenzopyran Moieties

A few examples of this category are shown in this figure.

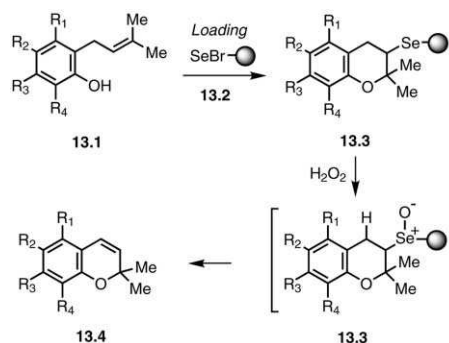


Figure 13. Nicolaou and Coworkers Have Shown that *Ortho*-Prenylated Phenols upon Reaction with Selenium-Functionalized Resin Give 2,2-Dimethylbenzopyran Derivatives Anchored onto Solid Support

The cleavage that forms the support could be achieved under oxidative conditions (e.g., H<sub>2</sub>O<sub>2</sub>), giving 3,4-dehydro-benzopyrans.



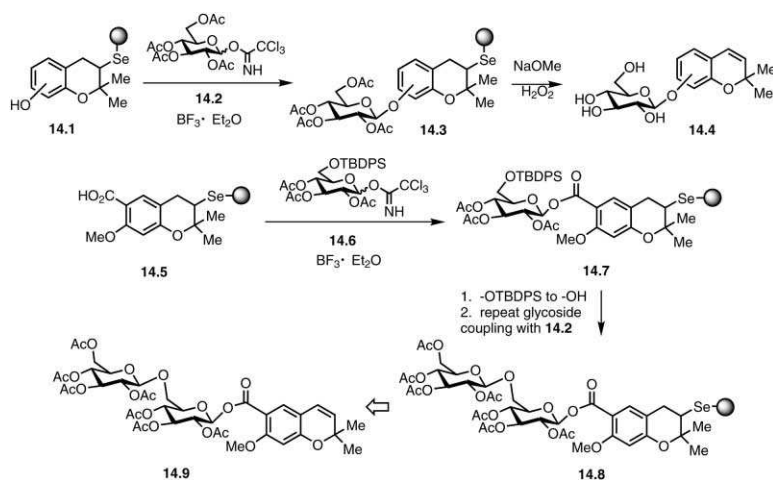


Figure 14. The Benzopyran Derivative Having a Phenolic -OH Anchored onto Solid Support, 14.1, Could be Glycosylated under the Standard Glycoside Bond-Forming Reaction Conditions

functionality is contained in numerous classes of natural products, such as flavanoids, comarins, rotenoids, stilbenoids, chromene glycosides, etc. A number of these compounds have potential application in medicine as outlined in Figure 12. The benzopyran unit is not only found in many classes of natural products but it is also contained in many pharmaceutically designed compounds. Hence, it is an ideal candidate for combinatorial derivatization.

The synthesis of natural product-like libraries centered around the 2,2,-dimethylbenzopyran unit involves the use of selenium-functionalized resins as shown in Figure 13. Cycloloading of several *ortho*-prenylated phenols, 13.1, with selenyl bromide polystyrene resin, 13.2, provided the benzopyran scaffold, 13.2 [52–54]. Oxidative cleavage ( $\text{H}_2\text{O}_2/\text{THF}$ ) of the intermediate, 13.3, provided the corresponding benzopyran derivatives 13.4 in good yields. This strategy not only acts as a robust tether via the pyran ring but it also allows for further functionalization of the aromatic ring regardless of its electronic environment. To investigate the variability of this technique, Nicolaou and coworkers selected a series of focused libraries (natural products or natural

product-like compounds) for combinatorial synthesis of chalcones and pyranocoumarins [55–58].

Chromene glycoside, a natural product isolated from *Angeratum conyzoides*, was another target pursued by Nicolaou and coworkers [54]. The researchers believed that the incorporation of a benzopyran scaffold onto the sugar moiety would enhance the solubility and cellular target capabilities during biological screening processes. Hence, trichloroacetimidates of different sugars, such as D-glucose, D-xylose, and L-rhamnose (only one example is shown, 14.2 [Figure 14]) upon reaction with the phenolic hydroxyl group under Lewis acid-catalyzed conditions afforded selectively the  $\beta$ -glycosides 14.3. Deacetylation (NaOMe, THF:MeOH, 5:1, 24 hr) followed by cleavage under standard oxidative condition gave the natural products, chromene glycosides (14.3), in greater than 90% yields.

Given the success with these three sugars, a more complex target, namely macrophylliside heptaacetate containing a disaccharide, was undertaken. Treatment of compound 14.5 with trichloroacetimidate 14.6 and  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  for 12 hr at  $-40\text{--}0^\circ\text{C}$  resulted in the formation of glycoside derivative 14.7 ( $\beta$ -anomer). Selective deprotection of the silyl group was accomplished with HF-pyridine. Finally, the second sugar unit was coupled under similar conditions, followed by cleavage from the

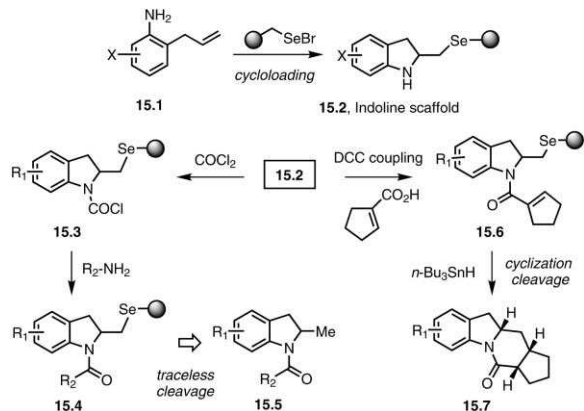


Figure 15. Nicolaou and Coworkers Reported the Synthesis of the Indoline Template Anchored onto Solid Support, 15.2, from *Ortho*-Allylaniline upon Reaction with Selenenyl Bromide Resin in the Presence of the Lewis Acid

The cleavage of the resin could be achieved under free radicals-mediated reaction conditions ( $n\text{Bu}_3\text{SnH}$ , catalytic AIBN).

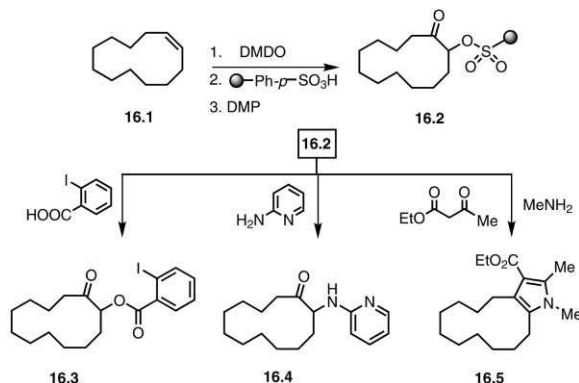


Figure 16.  $\alpha$ -Sulfonated Keto Derivatives were Synthesized on Solid Phase by Nicolaou and Coworkers

In the approach of Nicolaou and coworkers, the epoxide ring is opened upon treatment with the immobilized sulfonic acid.

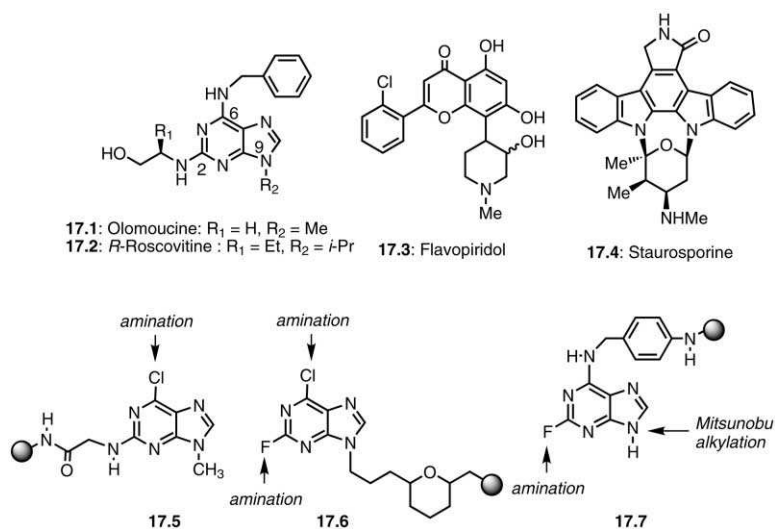


Figure 17. A Few Examples of Bioactive, Natural Products as Kinase Inhibitors

resin to give hepta-*O*-acetyl-macrophylloside (**14.9**) as a single anomer in good yield. Having developed a reliable and versatile solid phase technique based on selenoether linker for the synthesis of many natural and pharmaceutically designed benzopyran analogs, Nicolaou et al. then prepared a 10,000-member natural product-like compound library (the structures are not shown). For this study, they used the IRORI NanoKans technology, which uses an optical encoding system rather than the RF system used in the MicroKans system.

The success of the selenium-based resin for synthesizing many benzopyran compounds has led Nicolaou et al. to develop a novel strategy for synthesizing various heterocyclic indoline derivatives [59]. The synthetic approach involved substituted *C*-allylaniline **15.1** (Figure 15), which was cycloloated onto the polystyrene-based selenenyl bromide resin via a 5-*exo* trig-cyclization to give indole scaffold **15.2**. This scaffold was reacted with phosgene to afford the acyl chloride **15.3**, which was then treated with various amines to obtain **15.4**. Finally, the selenium-based resin was cleaved in a traceless manner (*n*-Bu<sub>3</sub>SnH/AIBN radical-mediated cleavage) to

give 1-methyl indoline, **15.5**. This class of compounds has provided several drug candidates such as antineoplastic sulfonamides, 5-hydroxytryptamine receptor antagonists (5-HT<sub>3</sub>), and muscarine receptor agonists and antagonists. Additional complexity was generated in the indoline derivative by the coupling of compound **15.2** with vinyl carboxylic acid (e.g., 1-cyclopentene-1-carboxylic acid). The free radical-mediated cleavage generated a carbon-centered radical that immediately reacted in a Michael-type reaction with the alkene functionality and formed the tetracyclic indoline framework **15.7**.

Nicolaou et al. [60] have also developed a novel one-pot synthesis of  $\alpha$ -sulfonated ketones from olefins, both in solution and on solid phase. The protocol involved synthesizing the immobilized variant of *p*-TsOH and using olefinic substrates as  $\alpha$ -sulfonate ketones. Both *cis*- and *trans*-olefins were loaded with equal efficiency onto the solid support. The initial step in this approach involved epoxidation of the olefin by DMDO (see Figure 16); the solution was then treated with *p*-TsOH in CH<sub>2</sub>Cl<sub>2</sub>, resulting in the formation of an  $\alpha$ -hydroxytosyl derivative. The addition of DMP led to  $\alpha$ -tosyloxy ketone **16.2**.

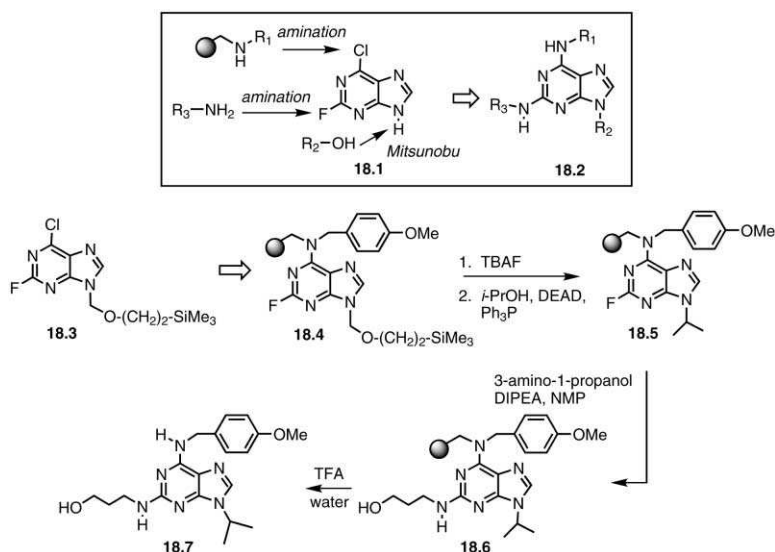


Figure 18. Solid-Phase Approaches by Schultz and Coworkers for the Synthesis of Purine-Based, Natural Product-Like Small-Molecule Libraries

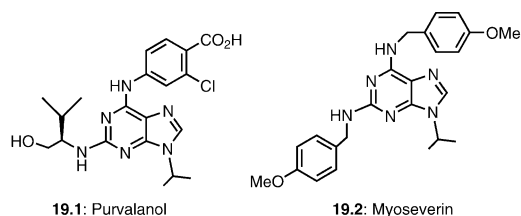


Figure 19. Examples of Purine-Based Natural Products, such as Purvalanol and Myoseverin, with CDK2 Cyclin-Inhibitory Effects

The highly stable  $\alpha$ -sulfonyl ketones when released by various reagents (i.e., *o*-iodobenzoic acid, *o*-aminopyridine, and ethylacetoacetate) led to a variety of molecular frameworks (e.g., 16.3, 16.4, and 16.5) in good yields.

The purine ring is a common structural motif found in numerous biological molecules. Olomoucine, which contains this motif, is a natural inhibitor of several cyclin dependent kinases (CDK). These enzymes, including CDK 2, regulate many biological events such as cell growth, DNA replication, and cellular division. In addition, there is evidence that high levels of genetically altered CDKs are found in tumor cells. Given the importance of these enzymes, synthesis of CDK inhibitors has traditionally been an area of active research. Some of these earlier inhibitors (17.2–17.4), along with the natural product, olomoucine, 17.1, are shown in Figure 17. However, inhibition by these derivatives is usually at the micromolar level.

In order to stimulate intense efforts in the discovery of more potent and selective CDK inhibitors, various groups envisioned, based on several X-ray crystallographic studies, that diversification at C-2, C-6, and N-9 positions of the purine ring may lead to such realization. Schultz et al. have developed several solution and solid phase strategies [61, 62] for the initial determination of structural-activity relationships (SARs) against these enzymes. The different strategies of solid phase approach involved linking the solid support to one of the purine positions as shown in derivatives 17.5–17.7. This strategy suffers from the loss of one position (C-2, C-6, or N-9) from further diversification. To overcome this problem, Schultz et al. decided to immobilize the resin at C-6 (see, Figure 18, 18.1 and 18.2).

In this synthesis, reductive amination of 2,3-dithio-1-propane-derivatized 4-methylbenzhydramine (MBHA) with primary amine gave resin bound amines, which were reacted with the purine derivative and achieved diversity at the C6 position (18.4). Functionalizations at N-9 (18.5) and C-2 (18.6) were accomplished via Mitsunobu and amination chemistry, respectively. This approach suffers from two significant drawbacks: (1) functionalization with a secondary amine is not possible at C6, and (2) bulky groups do not undergo amination at C2; however, the problems can be solved with solution phase strategies developed by Schultz et al.

To date, using 2-amino-6-chloropurine (18.3) as the template for various solution and solid-phase chemistry, Schultz and coworkers have synthesized several purine libraries with structural diversity at C-2, C-6, and N-9. Biological assays have led to the discovery of purvalanol B (2-(1-*R*-isopropyl-2-hydroxy-ethyl-amino-6-(3-chloro-

4-carboxyanilino)-9-isopropylpurine) 19.1 (Figure 19), a potent (6 nM) inhibitor of CDK 2-cyclin A in humans. Furthermore, other purine analogs have been shown to inhibit both cellular proliferation at specific phases of the cell cycle and the growth of cells involved in specific tumors. One particularly interesting compound, Myoseverin (bis-2,6-(4-methoxybenzyl-amino)9-isopropylpurine), 19.2, has the capability to induce reversible fission of myotubes (muscle cells) in mice into mononucleated fragments. This may have implication in the processes of tissue regeneration and wound healing in humans. The purine libraries have also been used for other targets and screens, such as carbohydrate transferase inhibitors. Thus, Bertozzi et al. were able to identify several lead compounds as inhibitors of carbohydrate sulfotransferases [63].

### Summary

To summarize, natural products are commonly utilized as small molecule-based chemical probes in modulation (i.e., as activators or deactivators) of protein-protein interactions. They are also used as specific binding agents to enzymes and proteins. With the rapid rise in advances in high-throughput biology, there is a great need for development of efficient, stereo- and enantioselective solid phase methods that could eventually lead to the syntheses of complex natural product-like compounds in a high-throughput manner. In this genomics and proteomics age, the synthetic community has been challenged to synthesize complex derivatives that may go beyond natural products, preferably in a high-throughput manner!

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### References

- Hankel, T., Brunne, R.M., Muller, H., and Reichel, F. (1999). Statistical investigation into the structural complementarity of natural products and synthetic compounds. *Angew. Chem. Int. Ed.* 38, 643–647.
- Newmann, D.J., Cragg, G.M., and Snader, K.M. (2000). The influence of natural products upon drug discovery. *Nat. Prod. Rep.* 17, 215–234.
- Mann, J. (2001). Natural product as immunosuppressive agents. *Nat. Prod. Rep.* 18, 417–430.
- Crews, C.M. (2000). Small molecule inhibitors of the cell cycle. *Curr. Opin. Chem. Biol.* 4, 47–53.
- Hinterding, K., Alonso-Diaz, D., and Waldmann, H. (1998). Organic synthesis and biological signal transduction. *Angew. Chem. Int. Ed.* 37, 688–749.
- Nicolaou, K.C., Vourloumis, D., Winssinger, N., and Baran, P.S. (2001). The art and science of total synthesis at the dawn of the twenty-first century. *Angew. Chem. Int. Ed.* 39, 44–122.
- Franzen, R.G. (2000). Recent advances in the preparation of heterocycles on solid support: a review of the literature. *J. Comb. Chem.* 2, 195–214.
- Lorbach, B.A., and Kurth, M.J. (1999). Carbon-carbon bond forming solid-phase reactions. *Chem. Rev.* 99, 1549–1581.
- Sammelson, R.E., and Kurth, M.J. (2001). Carbon-carbon bond forming solid-phase reactions. Part II. *Chem. Rev.* 101, 137–202.

- Burgess, K., ed. (2000). *Solid Phase Organic Synthesis*. (New York: Wiley-Interscience).
- Dorwald, F.Z. (2000). *Organic Synthesis on Solid Phase-Supports, Linkers, Reactions*. (Weinheim, Germany: Wiley-VCH).
- Watson, C. (1998). Polymer-supported synthesis of non-oligomeric natural products. *Angew. Chem. Int. Ed.* **38**, 1903–1908.
- Dolle, R.E. (2000). Comprehensive survey of combinatorial library synthesis. *J. Comb. Chem.* **2**, 383–433.
- Kirschning, A., Monenschein, H., and Wittenberg, R. (2001). Functionalized polymers—emerging versatile tools for solution-phase chemistry and automated parallel synthesis. *Angew. Chem. Int. Ed.* **40**, 650–679.
- Gordon, E.M., and Kerwin, J.F. Jr., eds. (1998). *Combinatorial Chemistry and Molecular Diversity in Drug Discovery*. (New York: Wiley-Liss).
- Arya, P., and Baek, M.-G. (2001). Natural product-like chiral derivatives by solid phase synthesis. *Curr. Opin. Chem. Biol.* **5**, 292–301.
- Arya, P., Chou, D.T.H., and Baek, M.-G. (2001). Diversity-based organic synthesis in the era of genomics and proteomics. *Angew. Chem. Int. Ed.* **40**, 339–346.
- Hall, D.G., Manku, S., and Wang, F. (2001). Solution- and solid phase strategies for the design and screening of libraries based on natural product templates. *J. Comb. Chem.* **3**, 125–150.
- Wendeborn, S., de Mesmaeker, A., Brill, W.K.-D., and Berteina, S. (2000). Synthesis of diverse and complex molecules on the solid phase. *Acc. Chem. Res.* **33**, 215–224.
- Wessjohann, L.A. (2000). Synthesis of natural-product-based compound libraries. *Curr. Opin. Chem. Biol.* **4**, 303–309.
- Wilson, L.A. (2000). Recent advances in solid-phase synthesis of natural products. In *Solid-Phase Organic Synthesis*. K. Burgess, ed. (New York: Wiley-Interscience), 247–267.
- Weber, L. (2000). High-diversity combinatorial libraries. *Curr. Opin. Chem. Biol.* **4**, 295–302.
- Beroza, P., Suto, M.J. (2000). Designing chiral libraries for drug discovery. *Drug Discov. Today* **5**, 364–372.
- Lenz, G.R., Nash, H.M., Jindal, S. (2000). Chemical ligands, genomics and drug discovery. *DDT* **5**, 145–156.
- Crews, C.M. (1996). Deciphering isozyme function: exploring cell biology with chemistry in the post-genomic era. *Chem. Biol.* **3**, 961–965.
- Schreiber, S.L. (1998). Chemical genetics resulting from a passion for synthetic organic chemistry. *Bioorg. Med. Chem.* **6**, 1127–1152.
- Schreiber, S.L. (2000). Target-oriented and diversity-oriented organic synthesis in drug discovery. *Science* **287**, 1964–1969.
- Crews, C.M., and Splittgerber, U. (2000). Chemical genetics: exploring and controlling cellular processes with chemical probes. *TIBS* **24**, 317–320.
- Stockwell, B.R. (2000). *Frontiers in chemical genetics*. *TIBS* **18**, 449–455.
- Stockwell, B.R. (2000). Chemical genetics: ligand-based discovery of gene function. *Nature Rev. Genet.* **1**, 116–125.
- Tan, D.S., Foley, M.A., Shair, M.D., and Schreiber, S.L. (1998). Stereoselective synthesis of over two million compounds having structural features both reminiscent of natural products and compatible with miniaturized cell-based assays. *J. Am. Chem. Soc.* **120**, 8565–8566.
- Tan, D.S., Foley, M.A., Stockwell, B.R., Shair, M.D., and Schreiber, S.L. (1999). Synthesis and preliminary evaluation of a library of polycyclic small molecules for use in chemical genetic assays. *J. Am. Chem. Soc.* **121**, 9073–9087.
- Lee, D., Sello, J.K., and Schreiber, S.L. (2000). Pairwise use of complexity-generating reactions in diversity-oriented organic synthesis. *Org. Lett.* **2**, 709–712.
- Ugi, I. (1982). From isocyanides via four-component condensation to antibiotic syntheses. *Angew. Chem. Int. Ed. Engl.* **21**, 810–819.
- Stemson, S.M., Louca, J.B., Wang, J.C., and Schreiber, S.L. (2001). Split-pool synthesis of 1,3-dioxanes leading to arrayed stock solutions of single compounds sufficient for multiple phenotype and protein-binding assays. *J. Am. Chem. Soc.* **123**, 1740–1747.
- Koide, K., Finkelstein, J.M., Ball, Z., and Verdine, G.L. (2001). A synthetic library of cell-permeable molecules. *J. Am. Chem. Soc.* **123**, 398–408.
- Chapman, O.L., Engel, M.R., Springer, J.P., and Clardy, J.C. (1971). The total synthesis of carpanone. *J. Am. Chem. Soc.* **93**, 6696–6698.
- Lindsley, C.W., Chan, L.K., Goess, B.C., Joseph, R., and Shair, M.D. (2000). Solid-phase biomimetic synthesis of carpanone-like molecules. *J. Am. Chem. Soc.* **122**, 422–423.
- Pelish, H.E., Westwood, N.J., Feng, Y., Kirschhausen, T., and Shair, M.D., (2001). Use of biomimetic diversity-oriented synthesis to discover galanthamine-like molecules with biological properties beyond those of natural products. *J. Am. Chem. Soc.* **123**, 6740–6741.
- Barton, D.H.R., and Kirby G.W. (1962). Phenol oxidation and biosynthesis. Part V. The synthesis of galanthamine. *J. Chem. Soc.* 806–817.
- Spring, D.R., Krishnan, S., and Schreiber, S.L. (2000). Towards diversity-oriented, stereoselective syntheses of biaryl- or bis(aryl)metal-containing medium rings. *J. Am. Chem. Soc.* **122**, 5656–5657.
- Maier, M.E. (1999). Synthesis of medium-sized rings by ring-closing metathesis reaction. *Angew. Chem. Int. Ed.* **39**, 2073–2077.
- Lee, D.S., Sello, J.K., and Schreiber, S.L. (1999). A strategy for macrocyclic ring closure and functionalization aimed toward split-pool syntheses. *J. Am. Chem. Soc.* **121**, 10648–10649.
- Stillman, B. (1996). Cell cycle control of DNA replication. *Science* **274**, 1659–1664.
- Barinaga, M. (1997). From bench top to bedside. *Science* **278**, 1036–1039.
- Bridges, A.J. (2001). Chemical inhibitors of cell cycle. *Chem. Rev.* **101**, 2541–2571.
- Qiao, L., Wang, S., George, C., Lewin, N.E., Blumberg, P.M., and Kozikowski, A.P. (1998). Structure-based design of a new class of protein kinase C modulators. *J. Am. Chem. Soc.* **120**, 6629–6630.
- Nishizuka, Y. (1996). Studies and perspectives of protein kinase C. *Science* **233**, 305–312.
- Meseguer, B., Alonso-Díaz, D., Griebenow, N., Herget, T., and Waldmann, H. (1999). Natural product synthesis on polymeric supports—synthesis and biological evaluation of an indolactam library. *Angew. Chem. Int. Ed.* **38**, 2902–2906.
- Stieber, F., Grether, U., and Waldmann, H. (1999). An oxidation-labile traceless linker for solid-phase synthesis. *Angew. Chem. Int. Ed.* **38**, 1073–1077.
- Meseguer, B., Alonso-Díaz, D., Griebenow, N., Herget, T., and Waldmann, H. (2000). Solid-phase synthesis and biological evaluation of teleocidin library—discovery of a selective PKC  $\delta$  down regulator. *Chem. Eur. J.* **6**, 3943–3957.
- Nicolaou, K.C., Pfefferkorn, J.A., and Cao, G.-Q. (2000). Selenium-based solid-phase synthesis of benzopyrans I: applications to combinatorial synthesis of natural products. *Angew. Chem. Int. Ed.* **39**, 734–739.
- Nicolaou, K.C., Cao, G.-Q., and Pfefferkorn, J.A. (2000). Selenium-based solid-phase synthesis of benzopyrans II: applications to combinatorial synthesis of medicinally relevant small organic molecules. *Angew. Chem. Int. Ed.* **39**, 739–743.
- Nicolaou, K.C., Snyder, S.A., Bigot, A., and Pfefferkorn, J.A. (2000). Solution and solid phase synthesis of functionalized 3-arylbenzofurans by a novel cyclofragmentation-release pathway. *Angew. Chem. Int. Ed.* **39**, 1093–1096.
- Nicolaou, K.C., Pfefferkorn, J.A., Roecker, A.J., Cao, G.-Q., Barluenga, S., and Mitchell, H.J. (2000). Natural product-like combinatorial libraries based on privileged structures. 1. General principles and solid-phase synthesis of benzopyrans. *J. Am. Chem. Soc.* **122**, 9939–9953.
- Nicolaou, K.C., Pfefferkorn, J.A., Mitchell, H.J., Roecker, A.J., Barluenga, S., Cao, G.-Q., and Lillig, J.E. (2000). Natural product-like combinatorial libraries based on privileged structures. 2. Construction of 10 000-membered benzopyran library by direct split-and-pool chemistry using nanokans and optical sorting. *J. Am. Chem. Soc.* **122**, 9954–9967.
- Nicolaou, K.C., Pfefferkorn, J.A., Mitchell, H.J., Roecker, A.J., Barluenga, S., Cao, G.-Q., and Lillig, J.E. (2000). Natural product-

- like combinatorial libraries based on privileged structures. 3. The “libraries from libraries” principle for diversity enhancement of benzopyran libraries. *J. Am. Chem. Soc.* **122**, 9968–9976.
58. Nicolaou, K.C., Pfefferkorn, J.A., Schuler, F., Roecker, A.J., Cao, G.-Q., and Casida, J.E. (2000). Combinatorial synthesis of novel and potent inhibitors of NADH:ubiquinone oxidoreductase. *Chem. Biol.* **7**, 979–992.
59. Nicolaou, K.C., Roecker, A.J., Pfefferkorn, J.A., and Cao, Q.-C. (2000). A novel strategy for the solid-phase synthesis of substituted indolines. *J. Am. Chem. Soc.* **122**, 2966–2967.
60. Nicolaou, K.C., Baran, P.S., and Zhong, Y.L. (2000). Novel solution- and solid-phase chemistry of  $\alpha$ -sulfonated ketones applicable to combinatorial chemistry. *J. Am. Chem. Soc.* **122**, 10246–10248.
61. Norman, T.C., Gray, N.S., Koh, J.T., Schultz, P.G. (1996). A structural-based library approach to kinase inhibitors. *J. Am. Chem. Soc.* **118**, 7430–7431.
62. Change, Y.-T., Gray, N.S., Rosania, G.R., Sutherlin, D.P., Kwon, S., Norman, T.C., Sarohia, R., Leost, M., Meijer, L., and Schultz, P.G. (1999). Synthesis and application of functionally diverse 2,6,9-trisubstituted purine libraries as CDK inhibitors. *Chem. Biol.* **6**, 361–375.
63. Armstrong, J.A., Portley, A.R., Chang, Y.-T., Nierengarten, D.M., Cook, B.N., Bowman, K.G., Bishop, A., Gray, N.S., Shokat, K.M., Schultz, P.G., and Bertozzi, C.R. (2000). Discovery of carbohydrate sulfotransferase inhibitors from a kinase-directed library. *Angew. Chem. Int. Ed.* **39**, 1303–1306.