Chemistry & Biology, Vol. 12, 163–180, February, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.chembiol.2005.01.011

Exploring New Chemical Space by Stereocontrolled Diversity-Oriented Synthesis

Review

Prabhat Arya,[1,2,*] Reni Joseph,[1,3]
Zhonghong Gan,[1] and Bojana Rakic[1,2]
[1]Chemical Biology Program
Steacie Institute for Molecular Sciences
National Research Council of Canada
100 Sussex Drive
Ottawa, Ontario K1A 0R6
Canada
[2]Department of Chemistry
University of Ottawa
10 Marie Curie
Ottawa, Ontario K1N 6N5
Canada

Natural products that act as highly specific, small-molecule protein-binding agents and as modulators of protein-protein interactions are highly complex and exhibit functional groups with three-dimensional and stereochemical diversity. The complex three-dimensional display of chiral functional groups appears to be crucial for exhibiting specificity in protein binding and in differentiating between closely related proteins. The development of methods that allow a high-throughput access to three-dimensional, skelatally complex, polycyclic compounds having few asymmetric diversity sites is essential and a highly challenging task. In the postgenomic chemical biology age, in which there is a great desire to understand protein-protein interactions and to dissect protein networking-based signaling pathways by small molecules, the need for developing "stereocontrolled, diversity-oriented synthesis" methods to generate natural product-like libraries is of utmost importance.

Introduction

Synthetic organic chemistry is a powerful discipline that plays a pivotal role in biomedical research. It provides access to new chemical entities such as natural product analogs, natural product-like scaffolds, and unnatural structures that can be used as small-molecule chemical probes for understanding biological events [1, 2]. The synthetic community has long been engaged in developing efficient methods to generate these architecturally complex natural products and their more simple analogs. In the past few decades, this has driven the need for developing efficient stereo- and enantioselective synthetic methods to keep up with the growing demand for having three-dimensional, architecturally complex molecules readily available [3].

Combinatorial chemistry came on board with the promise of providing rapid access to a wide variety of small molecules, either by solution- or solid-phase synthesis [4–6]. In general, this area of research has been successful in accessing rather simple compounds. Developing high-throughput synthesis methods leading to architecturally complex natural products and their simple analogs is a daunting task, resulting in little success to date [7–10]. Although solution-phase combinatorial chemistry has been utilized [11], solid-phase synthesis, either by parallel or split-and-mix synthesis, appears to be more useful in library generation [12].

Over the years, three-dimensional, architecturally complex natural products have been widely utilized as small-molecule probes for understanding protein function [13]. Imbedded in these natural products are a number of highly diverse, chiral functional groups acting as potential sites for protein binding. With the growing demand for having access to small molecules for use as highly specific modulators of protein function and protein-protein interaction-based signaling pathways, the need for charting a chemical space currently occupied by natural products has also grown. Diversity-oriented synthesis is aimed at reaching this objective, i.e., to populate the chemical space that is currently not available by the conventional combinatorial chemistry [14].

In the postgenomic chemical biology age, understanding complex biological processes by small-molecule probes is a highly desirable activity. In contrast to the classical biochemical tools, the use of chemical probes is highly attractive for understanding dynamic biological processes because of the probe's ability to induce subtle, and generally reversible, changes in protein dynamics [15–21]. Obtaining rapid access to the newly generated stereochemically and skeletally diverse natural product-like derivatives is a limiting factor [22, 23]. Consequently, for example, biological research that involves signaling pathways based on protein-protein interactions has not achieved much success in the past [24]. A lack of useful chemical probes available as specific modulators of these pathways has been a contributing factor to this limited success [25].

The term "diversity-oriented synthesis (DOS)" was coined by Stuart Schreiber, and DOS is aimed at generating natural product-like compounds in a high-throughput manner [14, 23]. In particular, DOS is aimed at obtaining natural product-like compounds that are (i) easy to access, (ii) rich in dense, chiral functional groups, and (iii) rich in stereochemically and three-dimensional, skeletally diverse architectures. DOS was developed to address the growing need for having rapid access to skeletally diverse natural product-like derivatives that could be further utilized in library generation. Unlike the traditional combinatorial approaches that were focused on the library generation of aromatic and heterocyclic products, building three-dimensional, structurally complex stereo- and enantioselective reactions on solid phase is one of the major areas in DOS. In general, the libraries generated by DOS are utilized as small-molecule chemical probes for understanding cellular processes and are not biased toward a specific biological target. Within the scope of this review, compounds that belong to the chemical space currently oc-

---

*Correspondence: prabhat.arya@nrc.ca
[3]Present address: MDS, Inc., 2350 Cohen Street, Saint-Laurent, Montréal, Québec H4R 2N6, Canada.

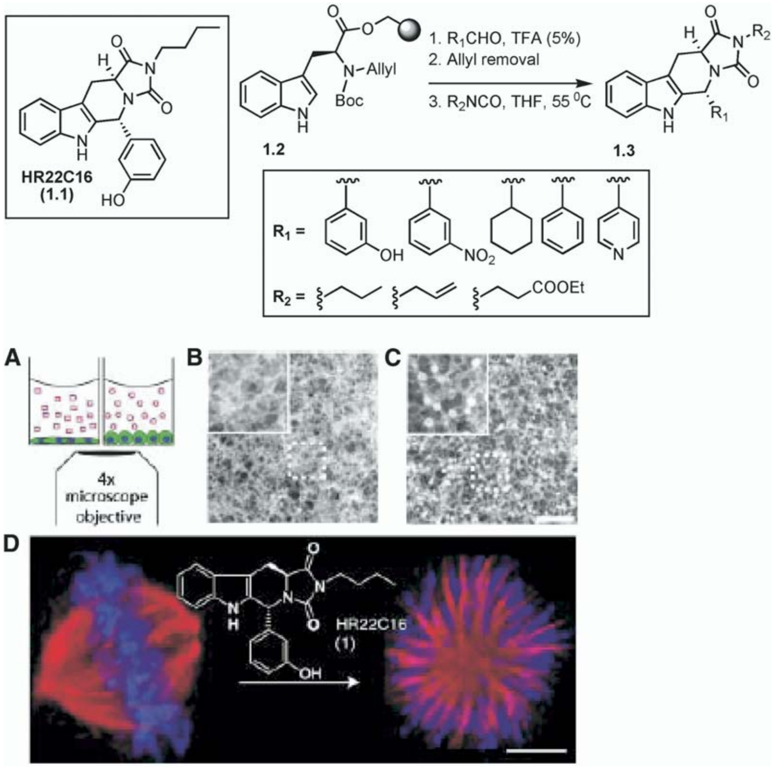

Figure 1. Kapoor and Coworkers' Solid-Phase Synthesis of HR22C16 Analogs that Utilized a Stereocontrolled Pictet-Spengler Approach

Taken from the article by Hotha et al. [30].

(A) Schematic representation of the assay. Vertebrate cells (BS-C-1) in multiwell plates are stained for the actin cytoskeleton and are imaged from below by using a 4× objective.

(B) The effect of a compound that causes no morphological change.

(C and D) (C) The effect of HR22C16 and (D) the configuration of microtubules in a (left) control cell and a (right) HR22C16-treated cell.

cupied by natural products may be considered as natural product-like compounds.

The goal of this review is to highlight the differences between two approaches, (i) natural product-guided combinatorial chemistry, in which natural product analogs of individual natural products are obtained [26–29], and (ii) DOS-based combinatorial chemistry, in which three-dimensional, skeletally diverse natural product-like derivatives are synthesized. This is by no means intended to be an extensive review covering these two topics. Using selected examples, the objective here is to reflect upon the fundamental differences in design strategies and different expectations of both research efforts.

Natural Product-Guided Combinatorial Chemistry: Libraries of Analogs of Individual Natural Products

With the goal of obtaining simple analogs, the unique structural architectures of bioactive natural products present tremendous challenges in developing a combinatorial chemistry program. Based on the fact that natural products have been providing a major source of lead compounds in drug discovery research, there is a growing interest in developing combinatorial chemistry (high-throughput solution- or solid-phase synthesis) that utilizes the core structures.

An application of a stereocontrolled Pictet-Spengler reaction on solid phase was reported by Kapoor and coworkers [30]. Using a high-throughput, microscopy-based, cellular screen, the discovery of a small molecule (HR22C16 [**1.1**]; see Figure 1) as an inhibitor of cell division led to the development of a solid-phase library of its analogs. One of the library members was found to be 9-fold more potent as an inhibitor of Eg5 protein binding agent than the parent compound HR22C16. In their approach (Figure 1), the amino acid derivative anchored onto the solid support, **1.2** (see Figure 1), was subjected to N-Boc removal. The secondary amine was then reacted with a variety of aldehydes, giving the tricyclic *trans* product as the major diastereomer (9:1 ratio). Upon allyl deprotection and reaction with several isocynates, the tetracyclic hydantoin products (**1.3**, Figure 1) were obtained in good yields after release from the solid support.

An application of the domino reaction to obtain several analogs of the natural product emetine (**2.1**, all **2.X** compounds are shown in Figure 2) was reported by Tietze and coworkers [31]. They utilized an enantioselective hydrogenation to obtain enantiomerically pure tetrahydroisoquinolines (**2.3** and **2.4**) from **2.2**. The domino reaction of **2.5** with Meldrum acid (**2.6**) and the enol ether (**2.7**) provided the lactone (not shown in the figure). Upon treatment with base and after hydrogenation, the benzoquinolizidine skeleton (**2.8**) was obtained as a mixture of diastereomers. These compounds were separated by column chromatography. Upon further reaction of **2.8** with 2-(3,4-dimethoxyphenyl)ethylamine (**2.10**), **2.8** provided the corresponding imines (**2.11**) via amide formation by Bischler-Napieralski reaction. The imines were then subjected to enantioselective hydrogenation, giving the stereocontrolled products, **2.12** and **2.13**. Thus, an application of a domino reaction combined with enantioselective hydrogenation provided a solution-phase library synthesis of several analogs of emetine alkaloid.

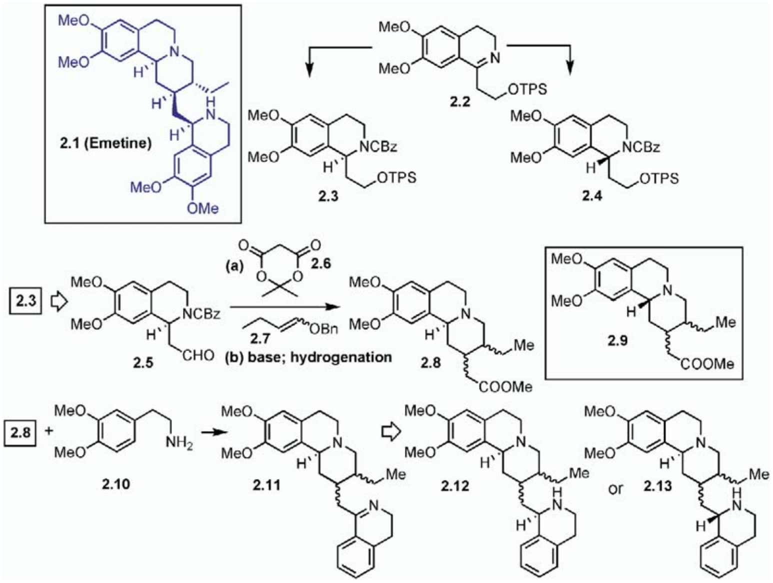

Figure 2. Application of a Domino Reaction Combined with Enantioselective Hydrogenation to Obtain Emetine Analogs by Tietze and Coworkers

See [31].

Sesquiterpenoid dysidiolide (**3.5**, all **3.X** compounds are shown in Figure 3) is a naturally occurring inhibitor of the dual specificity Cdc25 protein phosphatase family that plays a crucial role in the regulation of the cell cycle. It has a decalin-type framework that carries a lipophilic side chain terminating in an olefin, and a hydrophobic side chain incorporating an alcohol and a γ-hydroxybutenolide moiety. For the solid-phase synthesis of 6-epi-dysidiolide [32], polymer bound diene, **3.1**, was subjected to Diels-Alder reaction conditions at −78°C with triglic aldehyde, which had been converted into a quasi C_2 symmetric, chiral acetal in order to enhance the stereoselectivity. After hydrolytic removal of the chiral auxiliary, aldehyde **3.2** was obtained as a mixture of four isomers formed in the ratio 87 endo:4 endo′:9 exo:0.1 exo′. The carbon chain of aldehyde **3.2** could then be elongated in a two-step sequence of the Wittig reaction and hydrolysis of the enol ether formed therein. However, the reaction did not go to completion on the solid support. An alternative route employing the corresponding phosphonium chloride and KO-*t*-Bu as a base resulted in clean transformation, and further hydrolysis of the enol ether was carried out with pyridinium para-toluenesulfonate (PPTS)/tetrahydrofuran (THF)/H_2O. Nucleophilic addition of furyllithium to resin bound aldehyde **3.3** resulted in **3.4** as a 2:1 mixture of diastereomers. Subsequently, furan **3.4** was oxidized to a butenolide derivative with singlet oxygen in the presence of a mild base. Finally, release of product **3.5** from the polymeric support was achieved in a traceless manner by performing an olefin metathesis with 10 mol% Grubbs' catalyst.

The next goal was to obtain the analogs of dysidiolide that differ from the natural product in chain length and in the substitution pattern. Dysidiolide analogs with a shortened carbon chain were obtained via the addition of 3-lithiofuran to aldehyde **3.6**, thus giving compound **3.7**. This was then subjected to oxidation with bengal rose and subsequent cleavage from the solid support by olefin metathesis. Subsequent oxidation with *ortho*-iodoxybenzoic acid resulted in the corresponding ketone, **3.9**. In a second series of the experiments, dysidiolide with a double bond between the anellated ring and the hydroxyl butenolide (**3.12**) was also synthesized. Treatment of **3.6** with the ylide obtained from furylmethyltriphenylphosphonium bromide by reaction with KO-*t*-Bu in THF at room temperature resulted in **3.11**. The furan ring was oxidized with singlet oxygen, and the olefinic dysidiolide analog **3.12** was released by olefin metathesis. Finally, compound **3.8** was obtained by a Grignard reaction of **3.6** with 3-bromomethylfuran and Mg in THF. The product of the Grignard reaction (**3.8**) was then subjected to oxidation and cleavage from the solid support by olefin metathesis. Subsequent oxidation resulted in **3.10**. The same reaction sequence was also carried out on the homoaldehyde system. The dysidiolide analogs were then investigated as inhibitors for the protein phosphatase Cdc25c and in cellular cytotoxicity assays. Most of the analogs exhibit inhibition properties. In conclusion, a natural product-derived library was obtained through a multistep synthesis on solid phase, and this library delivered several potent, biologically active compounds.

1α, 25-dihydroxyvitamin D_3 (**4.1**, all **4.X** compounds are shown in Figure 4) is a steroid hormone and a bioactive metabolite of vitamin D_3. This compound is known to exhibit several biological activities that range from the regulation of calcium and phosphorus metabolism, to modulation of cell differentiation and proliferation, to regulation of the immune system. To generate several analogs of this steroid hormone in order to study its biological functions, Takahashi and coworkers [33, 34] developed the solid-phase synthesis of the vitamin D_3 skeleton. A key strategy in their approach was to immobilize the CD ring system onto the solid support by using the primary hydroxyl group, **4.2**. This scaffold could be coupled first to the A ring moiety, **4.3**, and, finally, to the side chain extension with the use of Grignard **4.4**. The primary hydroxyl group was immobilized onto the solid support by using a sulfonylchloride

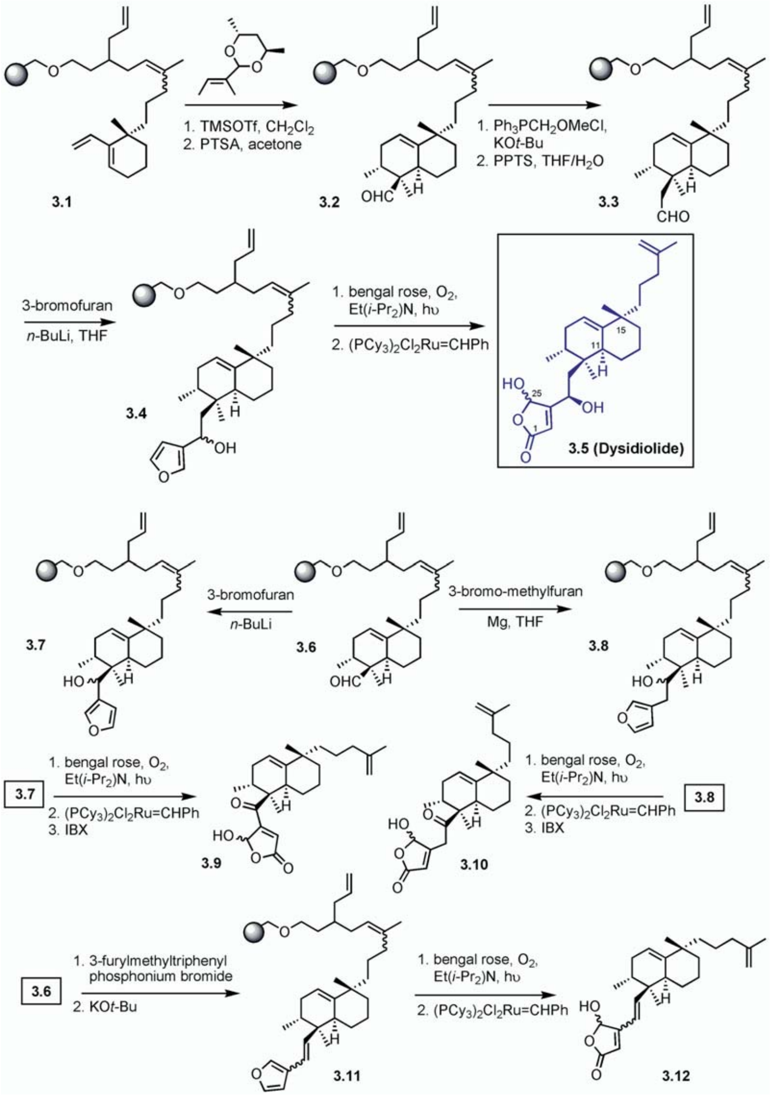

Figure 3. Waldmann and Coworkers' Solid-Phase Synthesis of Dysidiolide-Derived Protein Phosphatase Inhibitors

See [32].

linked to a polystyrene resin. As shown in Figure 4, the CD ring building block having a free hydroxyl group, **4.5**, was prepared by solution synthesis. It was then reacted with chlorosulfonyl polystyrene resin, **4.6**, giving the immobilized CD ring skeleton, **4.7**. Compound **4.7** was then subjected to a Horner-Wadsworth-Emmons reaction with **4.8** to obtain the coupled product, **4.9**. Finally, upon reaction with the Grignard reagent prepared from **4.10**, followed by deprotection under mildly acidic conditions, compound **4.11** was obtained. By varying the CD ring scaffold, by varying the A ring skeleton, and by using different side chains, a library of 72 compounds was synthesized by utilizing the radiofrequency-encoded IRORI technology.

Saframycin A (**5.1**, all **5.X** compounds are shown in Figure 5) is a structurally complex alkaloid and belongs to a member of a series of natural antiproliferative agents that contains a cyanopiperazine scaffold or its functionalized equivalent [35]. Myers and coworkers [36, 37] developed a solid-phase synthesis to obtain rapid access to saframycin analog **5.2**. The synthesis began with attachment of the antimorpholino nitrile (**5.3**) onto the solid support. The silylation of the primary hydroxyl group with 4-(chlorodiisopropyl-silyl)polystyrene provided the resin bound intermediate, **5.4**. Selective deprotection of the *tert*-butyldimethylsilyl ether group and subsequent removal of the Fmoc group with piperidine afforded the phenolic amine. Upon treatment with *N*-protected α amino aldehyde, **5.5**, the phenolic amine provided the corresponding resin-supported imine. Warming the imine in a saturated solution of anhydrous lithium bromide in 1,2-dimethoxyethane induced a stereoselective Pictet-Spengler cyclization reaction, resulting in the *cis*-tetrahydroisoquinoline derivative, **5.6.** The secondary amine group of tetrahydroisoquinoline intermediate **5.6** formed in the Pictet-Spengler cyclization was then reductively methylated. Subsequent deprotection of the phenol and of the primary amine produced an intermediate, **5.7**, which was then subjected to a second Pictet-Spengler cyclization reaction upon exposure to *N*-Fmoc glycinal. The resulting *bis*-tetrahydroisoquinoline derivative, **5.8**, was formed

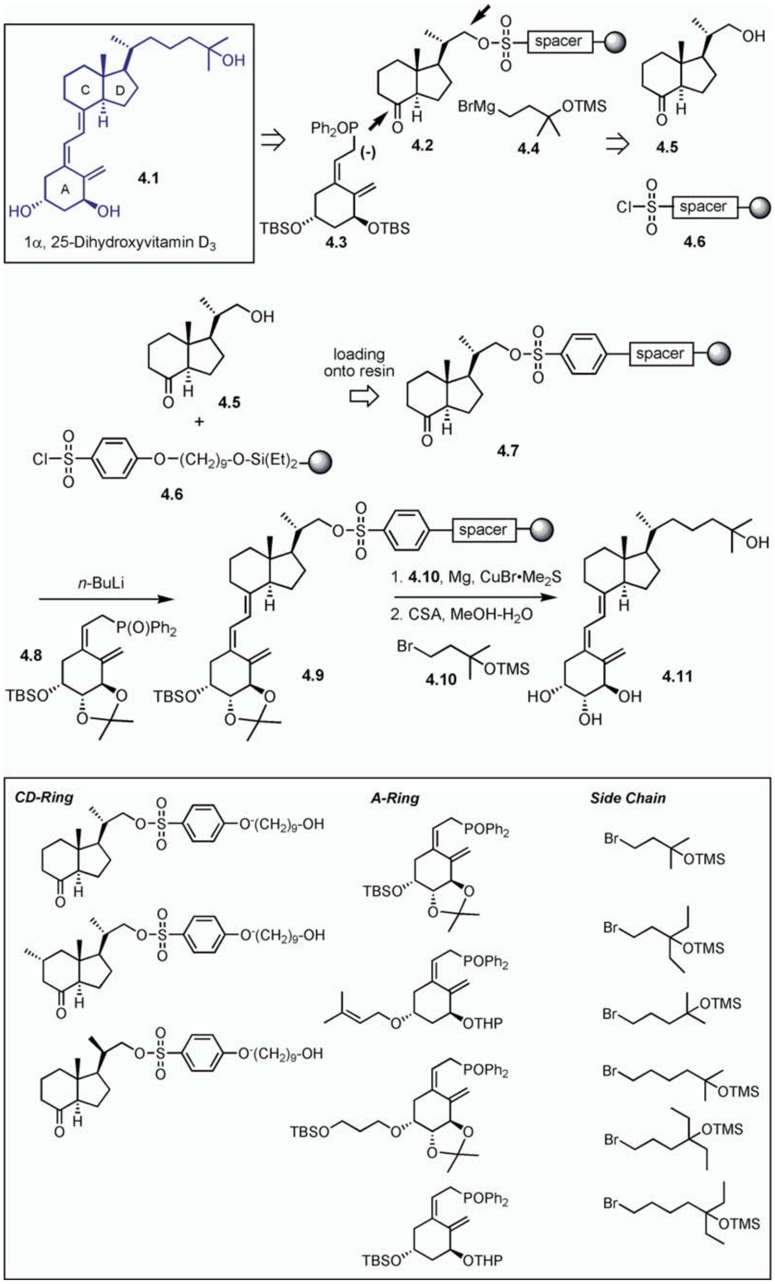

Figure 4. Takahashi and Coworkers' Solid-Phase Approach to Vitamin D$_3$
See [33, 34].

in quantitative yield with the required *cis* stereochemistry in the newly formed ring. In the key step, the *bis*-tetrahydroisoquinoline intermediate, **5.8**, was subjected to cyclization autorelease [38, 39] by warming at 55°C in the presence of $ZnCl_2$ for 1.5 hr. This transformation is presumed to involve (i) reversible morpholinium ion formation through an expulsion of cyanide, (ii) internal capture by cyclization of the secondary amino group formed in the final Pictet-Spengler cyclization reaction, and (iii) subsequent extrusion of the resin bound morpholine linker through its secondary point of attachment (i.e., the amino group). The newly liberated iminium ion is then captured in solution by the cyanide, providing the saframycin analog, **5.9.** A key feature of this reaction is the distereospecificity. Further diversity was introduced by employing a range of alkyl bromides in the ***N***-alkylation of resin **5.7** and varying the aldehyde reactant in the second Pictet-Spengler cyclization reaction. Thus, a 16-membered library of saframycin A analogs was prepared by parallel synthesis.

The spiroketal moiety is present in several bioactive natural products, such as spongistatins (**6.1,** all **6.X** compounds are shown in Figure 6) and okadaic acid (**6.3**). Therefore, it is not surprising that this scaffold has received attention from the combinatorial chemistry community. In a recent study reported by Waldmann and coworkers [40], asymmetric, aldol reaction-mediated, solid-phase synthesis [41, 42] was utilized in the

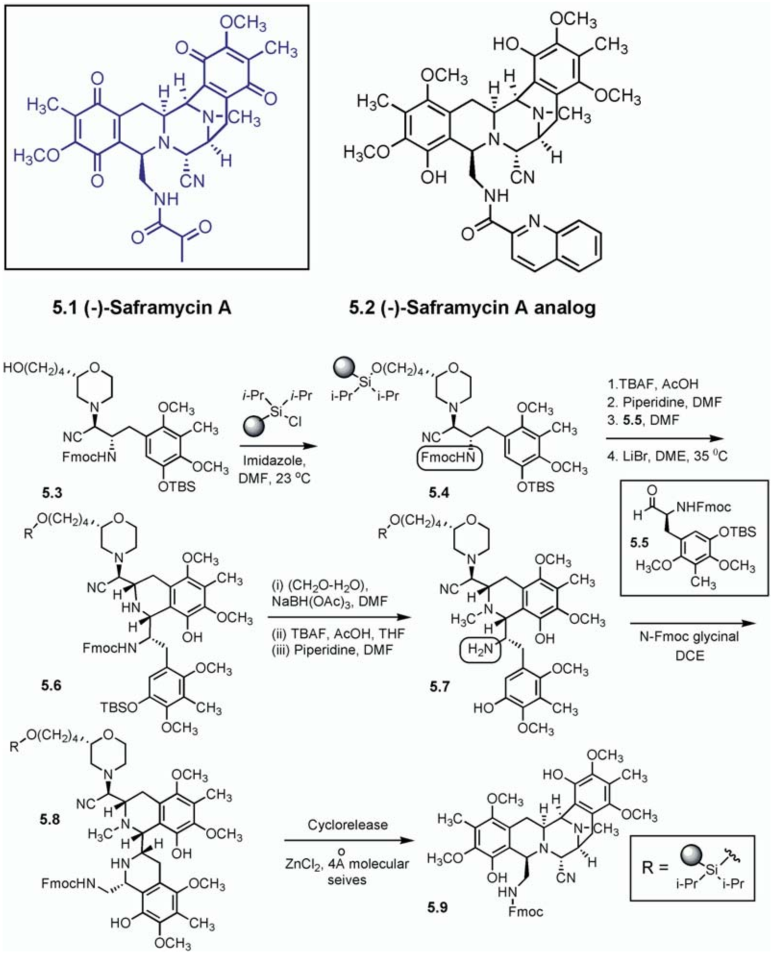

Figure 5. Myers and Coworkers' Enantioselective Solid-Phase Synthesis of Saframycin Analogs

See [36, 37].

generation of a library of spiroketal derivatives. Chiral boron Z-enol ether **6.6** mediated a *syn*-aldol reaction with an aldehyde anchored on the solid support, providing product **6.7**. The E-Enol ether, **6.8**, was then generated by using established methods from aldol literature such that upon reaction with various aldehydes, only the *anti*-aldol products were obtained (only two examples are shown, **6.13** and **6.14**). Following functional group modification, **6.8** could be converted into different spiroketals, **6.9–6.12**. Thus, a combination of asymmetric *syn*- and *anti*-aldol reactions on solid phase was utilized in generating different spiroketals and is a powerful approach in the generation of complex spiroketal-derived libraries.

Diversity-Oriented Synthesis-Based Combinatorial Chemistry: Libraries of Natural Product-like Compounds

The combinatorial chemistry examples discussed in the previous section allow access to small-molecule analogs of a given bioactive natural product, which tends to populate the chemical space currently occupied by the existing natural products. Parallel to this approach, the goal of diversity-oriented synthesis is to populate the unexplored chemical space by developing methods that allow rapid access to natural product-like compounds having three-dimensionally complex architectures. To meet some of the emerging challenges that involve the understanding of protein networking-based signaling pathways, the need for small molecules that are capable of specifically modulating protein function and protein-protein interactions is likely to grow. The combinatorial chemistry program in DOS utilizes stereo- and enantioselective organic synthesis tools and is expected to provide small molecules that are rich in stereochemically defined polyfunctional groups and in three-dimensional, conformationally diverse natural product-like skeletons. Stuart Schreiber's team is the main ambassador of the DOS approach that was introduced to the synthetic community in late 90's. This area of research is a relatively new direction in the combinatorial chemistry community. Selected examples from several research groups discussed herein are indicative of a growing interest in this area and different strategies that have been successfully developed to date.

A recent example of DOS used to obtain small molecules having stereochemically defined, chiral functional groups and conformationally diverse structures was reported by Schreiber and coworkers [43]. The development of successful methods on solid phase by the use of alkylsilyl macrobeads (500–560 μm) was then utilized in library generation [44]. The furaldehyde was immobi-

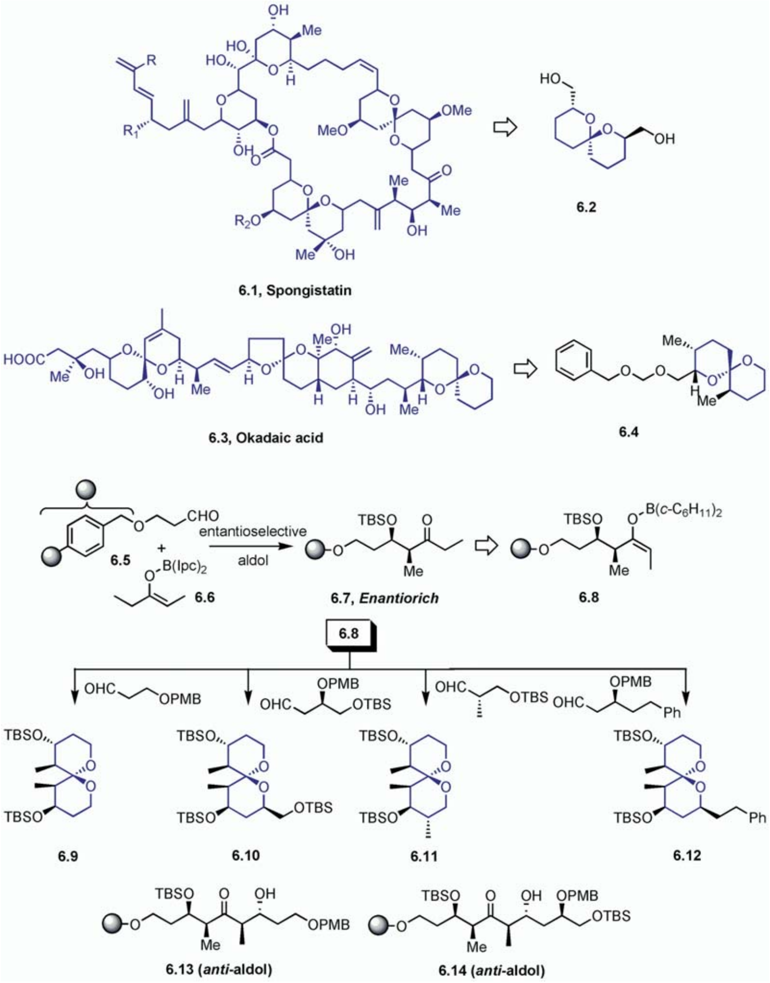

Figure 6. Bioactive Spiroketal Moieties Embedded in Spongistatin and Okadaic Acid

Waldmann and coworkers' [40] enantioselective, aldol-based solid-phase synthesis of the spiroketal library.

lized onto the macrobead-based solid support (**7.1**, all **7.X** compounds are shown in Figure 7), which was then subjected to Evans asymmetric aldol reaction conditions, giving the aldol product, **7.2**. In a different experiment, the aldehyde was subjected to chain extension, leading to the protected allylic alcohol derivative, **7.3**, required for the Sharpless asymmetric dihydroxylation reaction to obtain **7.4**. Compounds **7.2** and **7.4** are useful intermediates to obtain skeletally diverse derivatives. For example, an opening of the substituted furan ring could result in a linear skeleton having a *trans*-olefinic moiety, **7.6**. The NBS-mediated oxidative ring expansion of the aldol product, **7.2**, provided the stereochemically defined pyranone derivative, **7.7**, after the PPTS-catalyzed dehydration. Under similar oxidative ring expansion conditions, diol **7.4** was converted into the bicyclic derivative, **7.8**. Thus, starting from a common furaldehyde starting material, several skeletally diverse compounds could be obtained that utilize the substituted furan moiety as a source for creating the skeletal diversity. After these reactions were developed on solid phase by using alkylsilyl macrobeads, they were then used to generate a library by split-and-mix synthesis approach. Figure 7 also outlines an extension of this approach that was further tried on bromobenzofuraldehyde, **7.10**. The incorporation of the bromo functional group on the furan moiety provides an additional site that could be further subjected to structural diversification. Overall, this is an excellent example of stereocontrolled DOS in which small-molecule libraries have been obtained that combine the use of different building blocks and an approach to obtain stereochemically and skeletally diverse natural product-like architectures.

Although commonly utilized in classical synthesis projects, the use of enanantioselective catalysis in DOS has not been explored extensively. To this goal, Stavenger and Schreiber [45] reported the enantioselective synthesis of 2H-pyran derivatives on solid phase that utilized the C_2-symmetry, chiral, bis(oxazoline)-derived Lewis acids (**8.4** and **8.5**, all **8.X** compounds are shown in Figure 8). In their approach, the vinyl ethers anchored onto encoded alkylsilyl macrobeads (**8.2**, **8.3**, **8.8**, and **8.9**) were reacted with the ketoester (**8.1**), giving different 2H-pyrans (**8.6**, **8.7**, **8.10**, and **8.11**). The use of encoded split-and-mix technology on macrobeads allowed the authors to obtain a library of 4320 compounds. The enantioselective cycloaddition reaction worked well on solid phase, and the products were obtained in good yields and with high %enantiomeric ex-

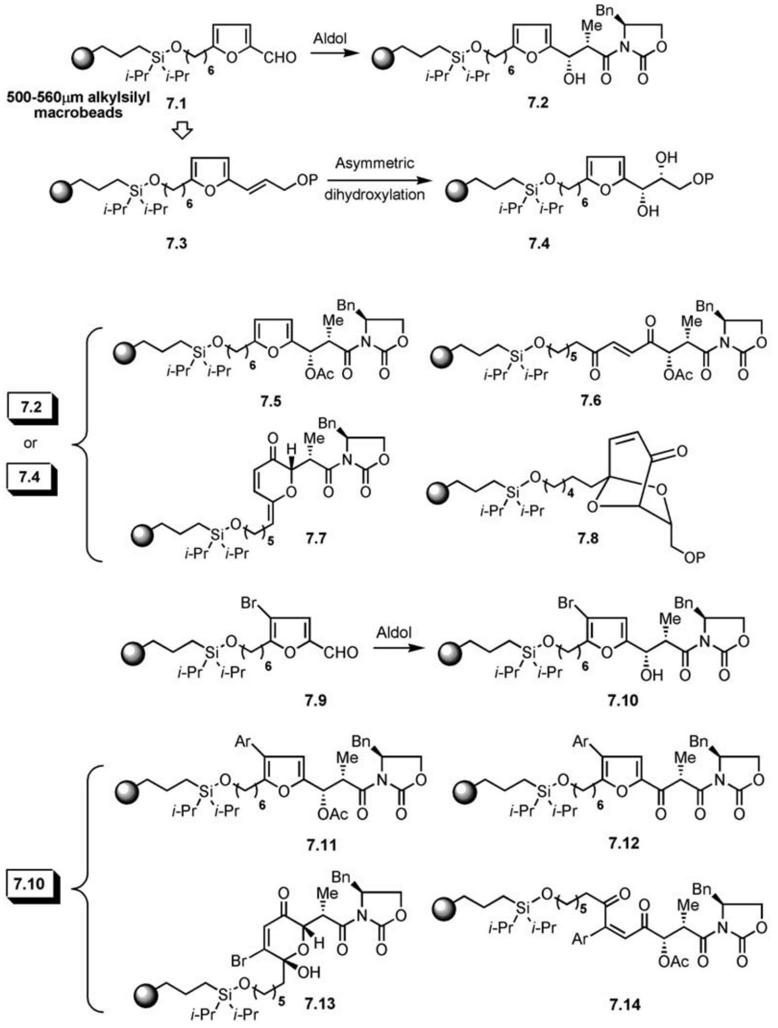

Figure 7. Schreiber and Coworkers' DOS Approach to Obtain Skeletally Diverse Natural Product-like Compounds

See [43].

cess (%ee). Along with other compounds, this library was arrayed and tested for the inhibition of a transcription factor that requires the disruption of either DNA-protein or protein-protein interactions. This DOS library was tested for small-molecule binding to modulate the transcriptional activity of Hap3p [46]. This is a subunit of the yeast hap2/3/4 transcriptional factor complex and is involved in aerobic respiration and the nutrient response signaling network. The small-molecule microarray was probed with purified Hap3p-GST fusion protein, and the color detection was achieved by using a labeled antibody against the GST portion of the fused protein. Interestingly, two DOS library members (see Figure 8) were identified as positive compounds. In a control experiment to examine the binding with the GST protein, one of the two compounds showed positive results. Finally, the binding of a DOS library member (Heptamide A) was confirmed by surface plasmon resonance studies.

In another study, Schreiber and coworkers reported the diversity-oriented synthesis of a library of medium-sized rings containing an atrop-diastereodefined biaryl moiety [47, 48]. Natural products such as Vancomycin (structure not shown) and Pterocaryanin C (**9.1**, all **9.X** compounds are shown in Figure 9) contain asymmetric biaryl moieties within 10- and 12-membered rings. Aldehyde **9.3** was prepared through loading onto the alkylsilyl macrobeads. This was then subjected to reductive amination, followed by alkylation of the secondary amine to obtain **9.4**. Having two brominated aryl moieties in **9.4**, the stage was now set to explore the carbon-carbon bond-forming reaction. Thus, after a halogen metal exchange reaction, **9.4** was then subjected to conditions used to generate the cyclic cuprate intermediate (structure not shown). Upon oxidation, this intermediate provided **9.5**, an atropstereocontrolled product having an asymmetric biaryl moiety (diastereomeric ratio = 6:1 [P:M]) with a 10-membered ring, on solid phase. Interestingly, upon heating compound **9.5**, the authors were able to reverse the atropdiastereomeric ratio to 1:7 (P:M). Using encoded split-and-mix technology, a library of approximately 1400 compounds was obtained. This library was used to explore the effects of small molecules on the development of (i) 7-day-old *Arabidopsis thaliana* seedlings and (ii) Zebrafish embryos. As shown in Figure 9, one library member exhibited inhibition of plant development, whereas its other diastereomer showed very weak inhibition ef-

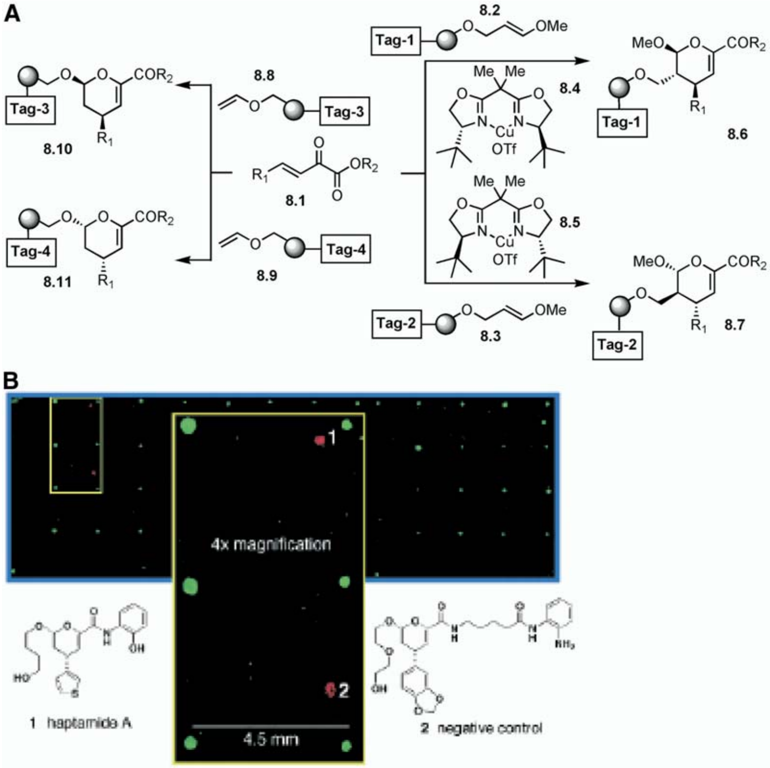

Figure 8. Schreiber and Coworkers' Approach to 2H-Pyran Derivatives

(A) An application of enantioselective Lewis acid catalysis on solid phase to obtain a library of substituted 2H-pyrans.

(B) Small-molecule microarray of compounds obtained from a substituted 2H-pyran library (taken from [46]).

See [45, 46].

fects. This effect presumably arises from the inhibition of chlorophyll and/or carotenoid biosynthesis. In another assay, one of the library members was found to inhibit the development of Zebrafish embryos at 100 nM. By the second day, they exhibited lower pigmentation, weak hearts, abnormal brains, and misshapen jaws. In one example, the M-diastereomer had an effect on the cardiovascular system, whereas the P-diastereomer showed no effect at all.

With the goal of obtaining small molecules having skeletally diverse structures with stereodefined functional groups, Schreiber and coworkers [49, 50] developed a boronic ester annulation strategy. The transesterification of unsaturated boronic esters with allylic or propargylic alcohols (**10.2** and **10.5**, all **10.X** compounds are shown in Figure 10), prepared from aldehydes (**10.1**), transiently provided mixed organoboronic esters (**10.3** and **10.6**) that could then be trapped by using ring-closing metathesis (RCM) to afford cyclic boronic esters (**10.4** and **10.7**). Oxidation, cycloaddition, and allene-forming hydroxyalkylation of the resulting cyclic boronic esters further illustrate the diversity-generating, branching pathways. For example, 3-hydroxybenzaldehye loaded onto 500–600 μm alkylsilyl-derivatized polystyrene macrobeads (**10.8**) was subjected to 1-propynylmagnesium bromide to yield propargylic alcohol, **10.9**. Upon treatment of **10.9** with the unsaturated boronic ester, a mixed organoboronic ester resulted, which was then trapped by RCM to afford the dienylic boronic ester, **10.10**. Stereoselective Diels-Alder cycloaddition of **10.10** provided access to polycyclic derivative **10.11**. Finally, the resin was cleaved by using HF-pyridine to yield polycyclic derivative **10.12**. This boronic ester approach to diversity-oriented synthesis yielded complex structures containing multiple rings, four stereogenic centers, and unsaturated units in just four steps. As an extension, the allenylation reaction of cyclic boronic acid was also investigated. Annulation product **10.13** was converted into trisubstituted allene, **10.14**, by simple heating. A closed transition state was postulated as leading to the formation of **10.14** as the major isomer. This reaction was then tried on solid support by using alkylsilyl macrobeads. Compound **10.16** reacted with the immobilized 3-hydroxylbenzaldehyde, **10.15**, to produce product **10.17** upon cleavage from the support. The successful demonstration of an allenylation reaction on macrobead-based solid phase could further be utilized in the library generation by split-and-mix synthesis protocols. It is interesting to note the stereocontrolled nature of this reaction when tried on solid phase.

In another example, Schreiber and coworkers [51] developed an efficient stereoselective synthesis of tricyclic compounds by exploring Ferrier and Pauson-Khand reactions [52–54] on a glycal template. This methodology was utilized in a stereoselective synthesis of a library of 2500 compounds aided by a split-and-mix synthesis strategy and a "one-bead, one-stock solution" technology platform. Solid-phase synthesis was performed on 500–560 μm polystyrene, alkylsilyl-derivatized macrobeads. The initial starting compound for the loading, **11.4** (all **11.X** compounds are shown in Figure 11), was synthesized according to the reaction shown in Figure 11. Ferrier reaction of 3,4,6-tri-*O*-acetyl-D-glucal (**11.1**) with (*S*)-1[tert-butyldiphenylsilyl)oxy]-3-buytyn-2-ol (**11.2**) gave the pseudoglucal, **11.3**, as the

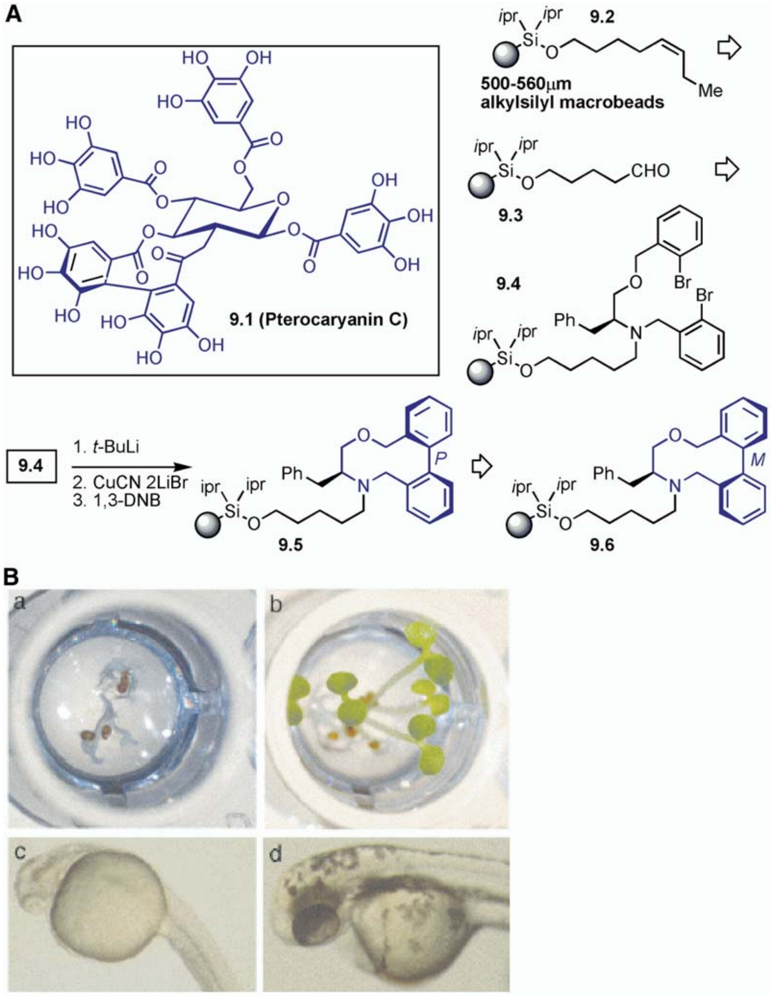

Figure 9. Schreiber and Coworkers' Approach

(A) A library of small molecules containing atrop-diastereodefined, medium-sized rings. (B) (a and b) Plant and (c and d) Zebrafish assay: (a) 7-day-old *Arabidopsis* seedlings germinated on agar containing 1% DMSO and a small-molecule library member (10 μm). (b) 7-day-old control experiment. (c) Synchronized Zebrafish embryos upon treatment with a small-molecule library member (100 nM); these embryos are slow to develop, and they exhibit lower than normal pigmentation, weak hearts, abnormal brains, and misshapen jaws. (d) Synchronized embryos upon treatment with the same library member (5 μm); Zebrafish look indistinguishable from untreated controls (taken from [48]). See [47, 48].

α anomer exclusively, whereas the Ferrier reaction with the (*R*)-isomer gave a mixture of both the α and β isomers in a ratio of 5:1 from which the α anomer could be isolated by column chromatography. Removal of the *tertiary*-butyldimethylsilyl protecting group, followed by protection of the primary hydroxyl functionality as 4-butyloxybenzyl (BOB) ether and deacetylation, resulted in BOB ether **11.4**. The BOB group can be easily deprotected by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone without harming the encoding tags. Ether **11.4** was then loaded onto 500–600 μm polystyrene, alkylsilyl-derivatized macrobeads, giving **11.5**. The first solid-phase diversity step (R_1) is the functionalization of the 4-hydroxy group of the pseudoglucal, **11.5**. Phenylisocyanate reacted quantitatively to afford the carbamate, and deprotection of the BOB group afforded the alcohol **11.6**, which was the second point of diversity. The hydroxyl group was first converted to the triflate and then treated with primary amine to afford the secondary amine. Reaction of the resulting secondary amine with different acylation agents generated compound **11.7** with the third diversity point. Pauson-Khand reaction on **11.7** resulted in the tricyclic α,β-unsaturated ketone, **11.8**, which was further subjected to a hetero-Michael reaction to insert the fourth point of diversity, yielding **11.9**. Finally, treatment of macrobeads with HF-pyridine resulted in the tricyclic compound, **11.10**, with four diversity points.

With the goal of obtaining skeletal diversity, Schreiber and coworkers [55] explored the use of a triene in cycloaddition reactions. The triene synthesis followed the work of the Fallis group [56], in which indium-mediated alkylation is the key step. The substituted hydroxyl group could then undergo elimination, leading to the desired triene. For model studies, 4-hydroxy-3-methoxybenzaldehyde was loaded onto the alkylsilyl macrobeads, giving compound **12.1** (all **12.X** compounds are shown in Figure 12). Upon subjection to indium-mediated alkylation, followed by hydroxyl elimination, the triene anchored onto a solid support (**12.2**) was obtained. This triene was then subjected to one of two reactions. In one study, the triene, **12.2**, was subjected to a cycloaddition reaction with a disubstituted dienophile that gave the *bis*-cycloaddition product, **12.5**, with complete stereocontrol. Interesting to note was the observation that the reaction of the triene, **12.2**, with a trisubstituted dienophile gave only monocycloaddition product **12.3**. This compound could further be resubjected to cycloaddition with different dienophiles, giving product **12.4**. This method is highly

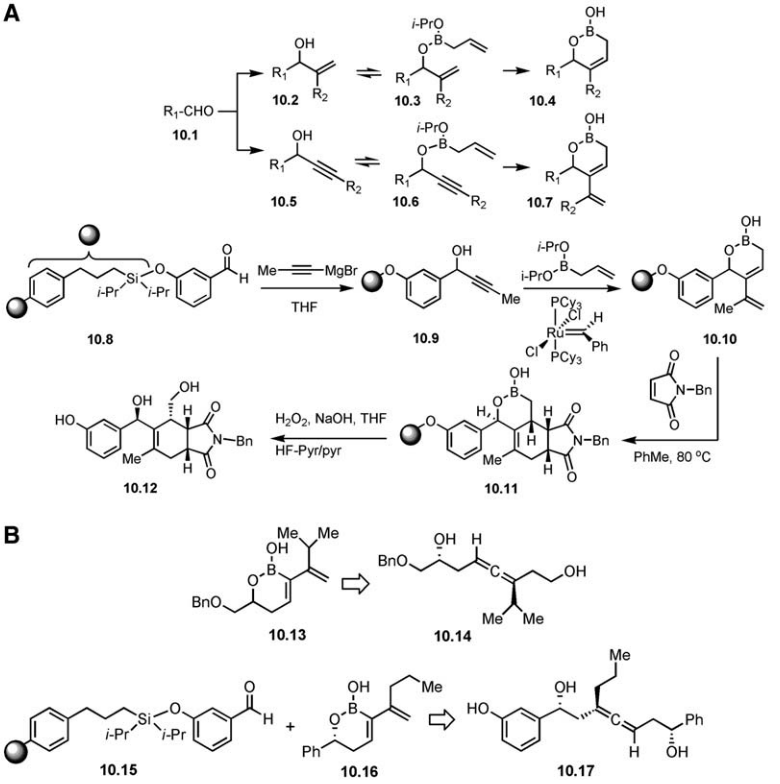

Figure 10. Schreiber and Coworkers' Boronic Ester Annulation/Diels-Alder Approach

See [49, 50].

practical, and by using the split-and-mix synthesis protocol, an encoded library of 29,400 compounds was obtained from different aldehydes and di-, tri-, and tetrasubstituted dienophiles. As depicted in Figure 12, this library was immobilized on glass slides to obtain a small-molecule microarray [57]. In a typical experiment, the aminopropyl silane group, **12.6**, was coupled with *p*-toluenesulfonylhydrazone to obtain **12.7**. Upon reaction with NaOMe, **12.7** gave the reactive diazobenzylidene-derivatized glass surface (**12.8**) to which a small molecule having an acidic proton, **12.9**, could then be immobilized. The arrayed library was then probed with the Cy5-calmodulin fusion protein to explore the bindings. Few library members were found to be active. The binding measurements of these small molecules (see **12.10**, **12.11**, and **12.12**) were confirmed in solution by

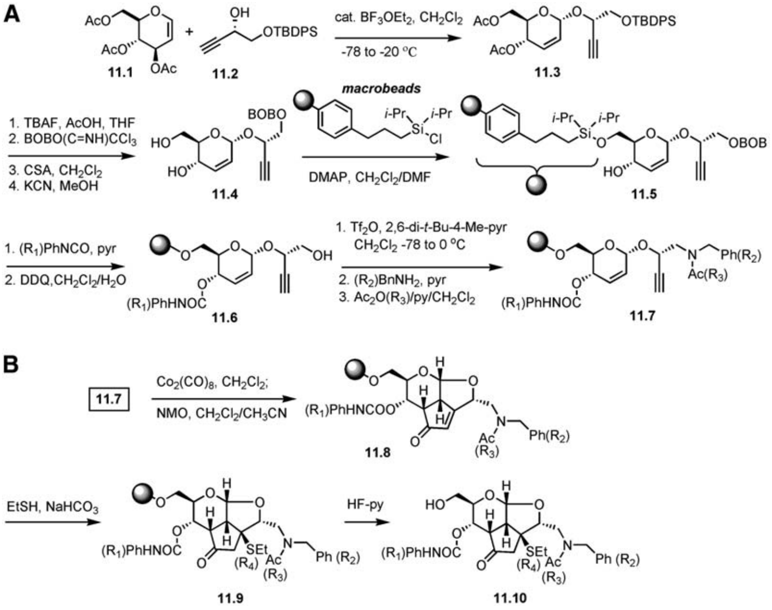

Figure 11. Schreiber and Coworkers' DOS Approach to Obtain Polycyclic Compounds from Glycals

See [51].

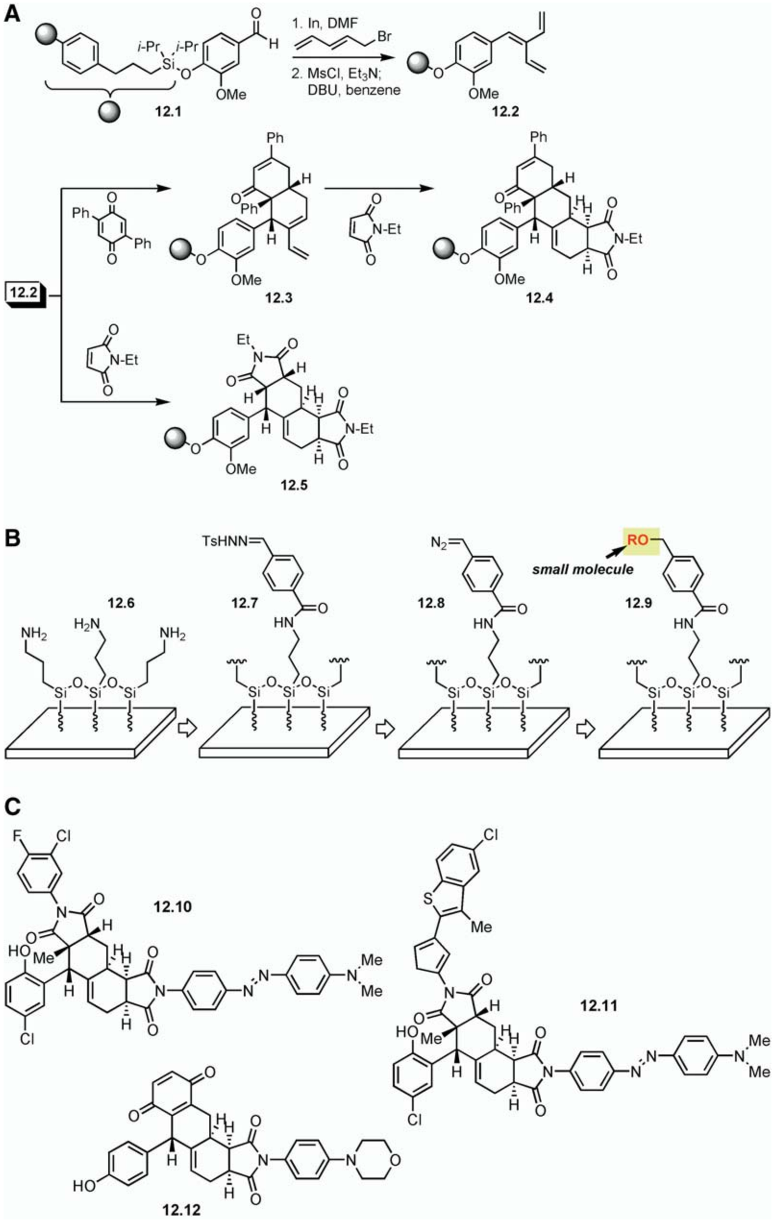

Figure 12. An Asymmetric Cycloaddition Approach by Schreiber and Coworkers

(A) A cycloaddition approach with triene to obtain polycyclic compounds.

(B) An approach to immobilize small molecules having an acidic proton on an aminopropyl glass surface by Schreiber and coworkers.

(C) Few library members identified as Calmodulin protein binding agents.

See [55, 57].

surface plasmon resonance studies. In this study, the protein was immobilized on the surface, and small molecules were brought in contact in solution.

In another report [58], Schreiber and coworkers disclosed their result of the use of an unstable reactive intermediate (e.g., dihydroisoquinoline) in obtaining skeletally different products. Interesting to note is their finding that, when immobilized onto the solid support, the otherwise reactive dihydroisoquinoline system is quite stable and could be stored at room temperature for a few days. Using their previously described alkylsilyl macrobeads, they obtained compound **13.2** from **13.1** (all **13.X** compounds are shown in Figure 13). This was then subjected to allylation and Grignard reaction, giving the desired intermediate, **13.3.** A few examples of the skeletally different products they obtained (**13.4, 13.5, 13.6,** and **13.7**) are also shown.

Due to its presence in a wide variety of bioactive, alkaloid natural products, the tetrahydroquinoline scaffold is considered to be privileged ([59]; see Chapter 6 on Alkaloids). The extensive usefulness of quinoline- and tetrahydroquinoline-based natural products prompted Arya and coworkers [60] to develop a diversity-oriented synthesis program to obtain rapid access to natural product-like, polycyclic derivatives having this privileged structure. The first milestone toward this objective was to develop an efficient enantioselective synthesis of highly functionalized, tetrahydroquinoline-based β amino acid, **14.1** (all **14.X** compounds are shown in Figure 14). The phenolic hydroxyl group could be used as an anchoring site in solid-phase synthesis. The remaining functional groups could then be utilized to explore the three-dimensional chemical space around this scaffold, thus leading to the synthesis of skeletally dif-

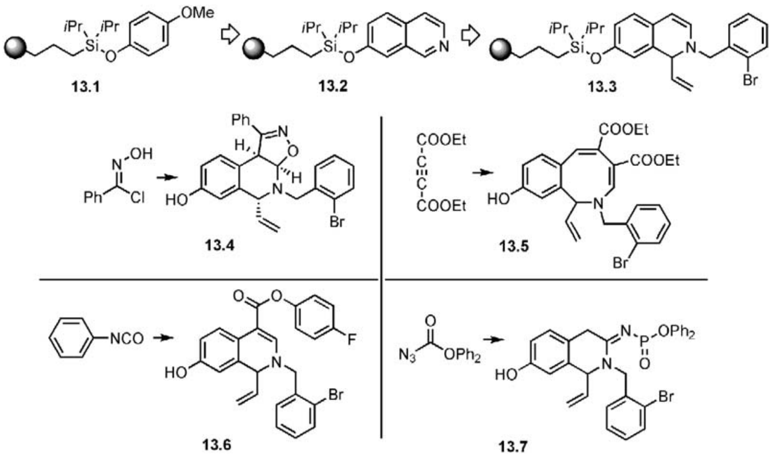

Figure 13. An Application of Immobilized Dihydroisoquinoline to Obtain Skeletally Different Compounds

See [60].

ferent, complex, polycyclic derivatives such as **14.2**, **14.3**, and **14.4**. Tricyclic compound **14.2**, having a 6-membered enamide functional group, could be obtained from **14.1** through an RCM approach. For the library generation, the enamide moiety could then be subjected to asymmetric, diversity-oriented reactions. In another approach, after the modification of the side chain of **14.1**, resulting in the incorporation of the allylic hydroxyl group, an additional 8-membered ring containing an enamide functional group, **14.3**, could be generated. Tetrahydroquinoline-derived tricyclic derivative, **14.3**, could then be further exploited in ring conformation-controlled, asymmetric, diversity-oriented reactions. The incorporation of the medium-sized ring in polycyclic skeletons could be highly attractive for further derivatization on solid phase. Finally, one could envision that the synthesis of the bridged tricyclic derivative, **14.4**, could be derived from a regio- and stereoselective conjugate addition. Thus, using the common precursor, **14.1**, it is possible to obtain skeletally different tricyclic derivatives, **14.2**, **14.3**, and **14.4**, that could further be utilized to generate libraries of functionalized tetrahydroquinoline-derived natural product-like, polycyclic compounds. Although the tetrahydroquinoline moiety is present in all three scaffolds, these scaffolds are structurally distinct and occupy different chemical spaces.

As a model study, Arya and coworkers developed an enantioselective synthesis of tetrahydroquinoline β amino acid [60]. Using an RCM approach, the tetrahydroquinoline-derived tricyclic compound having an enamide functional group was obtained in solution and on solid phase. For the solid-phase synthesis, compound **15.1** (all **15.X** compounds are shown in Figure 15), having a free hydroxyl group, was selected. This compound was synthesized in a similar manner to the studies carried out in the model system. Compound **15.1** was first anchored onto the solid support by using 4-(bromomethyl)phenoxymethyl polystyrene resin (loading 93%) to give **15.2**. Following the *N*-alloc removal and acryloylation, the RCM reaction gave the cyclic enamide product on solid phase. As observed in solution studies, compound **15.4** was obtained as a single diastereomer (attack from the α face) upon reaction with PhSH after cleavage from the solid support (27% overall yield for six steps). The nuclear magnetic resonance (NMR) studies with compound **15.4** showed nOe between C_3-H and $C_{4'}$-H.

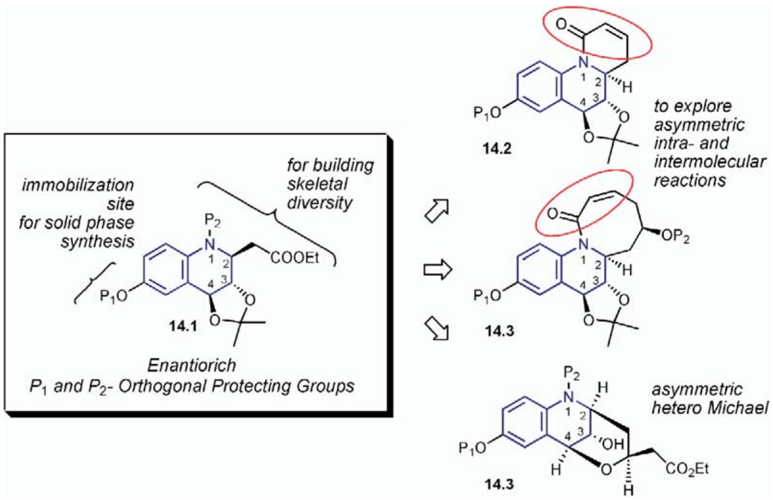

Figure 14. Arya and Coworkers' Stereocontrolled DOS to Obtain Tetrahydroquinoline-Derived, Natural Product-like, Polycyclic Compounds

See [60].

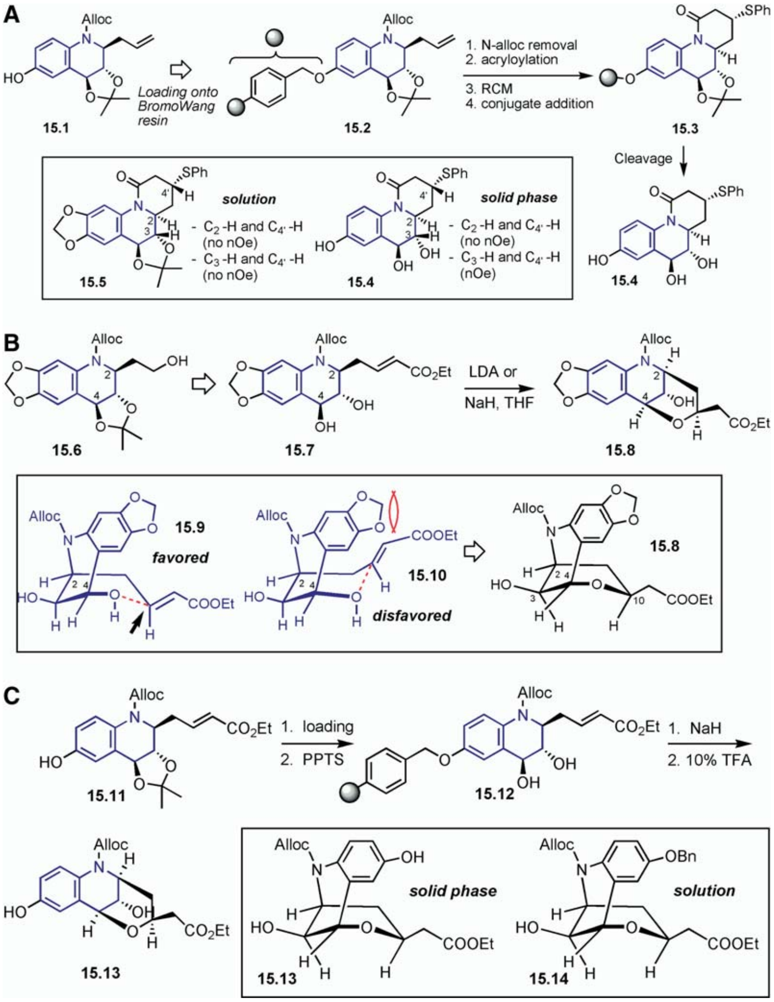

Figure 15. Ring-Closing Metathesis Approach by Arya and Coworkers

(A) RCM approach to obtain natural product-like, polycyclic compounds.

(B) Regio- and stereoselective hetero-Michael approach to tetrahydroquinoline-based, polycyclic compounds.

(C) Stereocontrolled solid-phase synthesis of tetrahydroquinoline-based, natural product-like, polycyclic compounds.

See [60].

In a model study to explore the regio- and stereoselective hetero-Michael approach shown in Figure 15, an enantiorich, tetrahydroquinoline β amino acid was converted through a series of simple transformations into the hydroxyl derivative, **15.6**. With compound **15.7** as the starting material, the stage was now set to explore the asymmetric hetero-Michael reaction. The researchers were pleased to note that this reaction proceeded very smoothly and produced only a single diastereomer, **15.8**, in high yield (84%). The tetrahydroquinoline-based tricyclic derivative, **15.8**, was well characterized by mass spectrometry (MS) and NMR. As observed earlier, this reaction seems to be independent of the choice of the base and is an excellent example of a highly regio- and stereoselective (reaction with benzylic-OH at C_4 only) hetero-Michael reaction. Based upon extensive NMR studies that showed no nOe between C_2-H and C_4-H (note: compound **15.7** showed nOe between C_2-H and C_4-H), a boat-like structure for the newly formed pyran ring, **15.9**, via the boat-like transition state, was proposed. The regio- and stereoselective outcome could be envisioned by a pseudo-axial occupation of functional groups at C-2, C-3, and C-4, allowing a facially selective attack of the oxygen nucleophile onto the conjugated carboxyl ester site.

For the solid-phase synthesis of **15.13** (Figure 15), compound **15.11** was immobilized onto the resin as in the previous example (loading 86%). The free hydroxyl derivative, **15.12**, obtained after acetonide removal, was subjected to the crucial hetero-Michael reaction. The use of NaH as a base at room temperature provided the expected product, **15.13**, after cleavage from the support. As observed in the solution-phase synthesis (see compound **15.14**), this yielded the unusual regio- and stereoselective hetero-Michael product. The two examples discussed above could be further utilized in the generation of a library of tetrahydroquinoline-based, natural product-like, polycyclic compounds.

As reported by Arya and coworkers [61], the last example of tetrahydroquinoline-derived, polycyclic compounds is the solution- and solid-phase synthesis of tricyclic derivatives having a functionalized 8-membered ring with an enamide functional group. As shown in Figure 16, enantio-rich, tetrahydroquinoline-derived β amino acid **16.1** (all **16.X** compounds are shown in Figure 16) was converted into **16.2**, a starting material for the solid-phase synthesis. This compound was immobilized onto the resin as described before and then subjected to a series of reactions as follows: (i) acetylation, (ii) *N*-alloc removal, and (iii) acryloylation. Upon

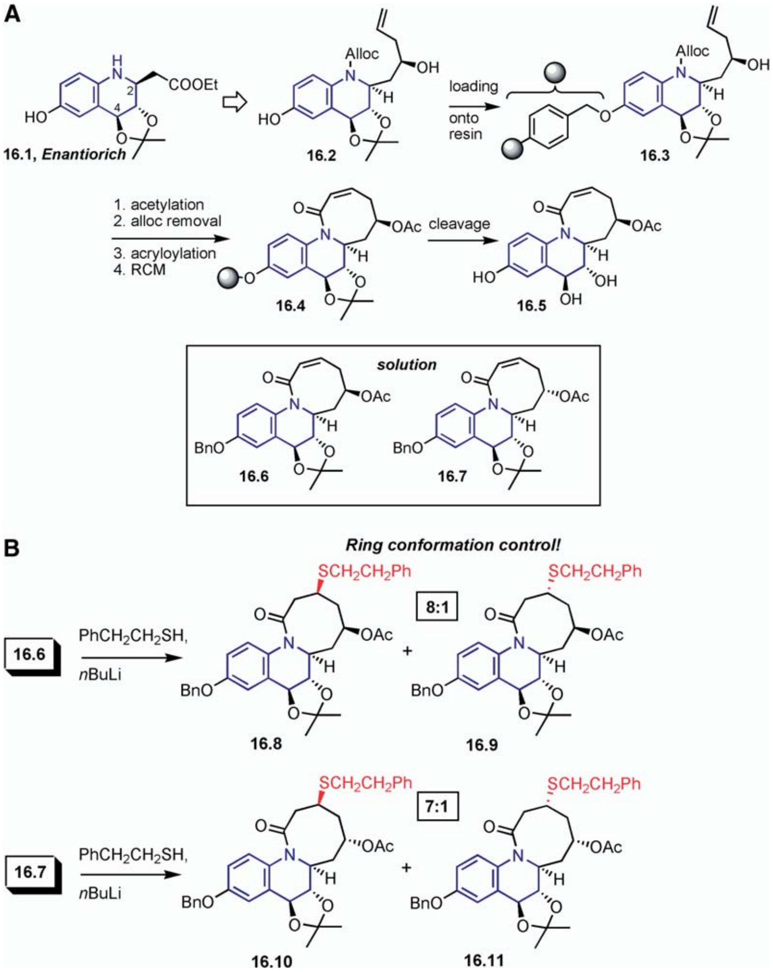

Figure 16. Stereocontrolled Diversity-Oriented Reactions

(A) Tetrahydroquinoline-derived, polycyclic compounds having a functionalized 8-membered ring.

(B) Ring conformation-controlled, asymmetric conjugate addition.

See [61].

subjection to a second generation Grubbs' catalyst, a successful synthesis of a medium-sized ring having an enamide functional group, **16.4**, was achieved. Finally, compound **16.5** was obtained after cleavage from the solid support and was thoroughly characterized by 2D NMR studies after purification by column chromatography. The solution-phase synthesis of the related compounds, **16.6** and **16.7**, was also achieved in parallel for comparison. An example of ring conformation-controlled asymmetric conjugate addition with the thiol as a nucleophile is shown in Figure 16. As expected, the major products, **16.8** and **16.10**, from compounds **16.6** and **16.7** resulted from the approach of the nucleophile from the β face. Once again, the products were well characterized by 2D NMR studies.

With few exceptions [62–65], there are not many examples of library generation of macrocyclic derivatives or small molecules having functionalized medium-sized rings. With the goal of obtaining a library of polycyclic compounds having a functionalized macrocycle, Arya and coworkers [66] reported the solution- and manual-solid-phase synthesis of tetrahydroquinoline-based tricyclic derivatives having a functionalized 10-membered ring. Starting from the enantio-rich compound, **17.1** (all **17.X** compounds are shown in Figure 17), the plan was to immobilize **17.2** onto the solid support, giving **17.3.** RCM with the use of a second generation Grubbs' catalyst was the key reaction in this approach. Obtained from **17.3**, compound **17.4** could then be subjected to RCM, giving the bridged, 10-membered ring having an enamide functional group. This functional group could further be utilized in asymmetric diversity-oriented reactions.

Compound **17.6** (Figure 17), having a free phenolic hydroxyl group, was used for developing a solid-phase synthesis. The solid-phase synthesis was carried out on the bromo-Wang resin (1.70 mmol/g), and the loading of the phenolic derivative, **17.6**, was accomplished nicely (85% loading after cleavage of the product from the solid support). Following immobilization, **17.7** was then subjected to *O*-alloc removal under basic conditions (NaOMe), giving the free secondary hydroxyl group. This derivative was then acylated with pentenoic acid (diisopropylcarbodiimide, 4-dimethylaminopyridine). To obtain a precursor of the RCM, the *N*-alloc group was deprotected, and the free amine was acylated with acryloyl chloride, giving compound **17.8.** When subjected to RCM reaction conditions, compound **17.8** nicely gave the bicyclic product, **17.9**, with a functionalized, bridged, 10-membered ring. Following cleavage from the support and purification by column chromatography, product **17.10** was well characterized by

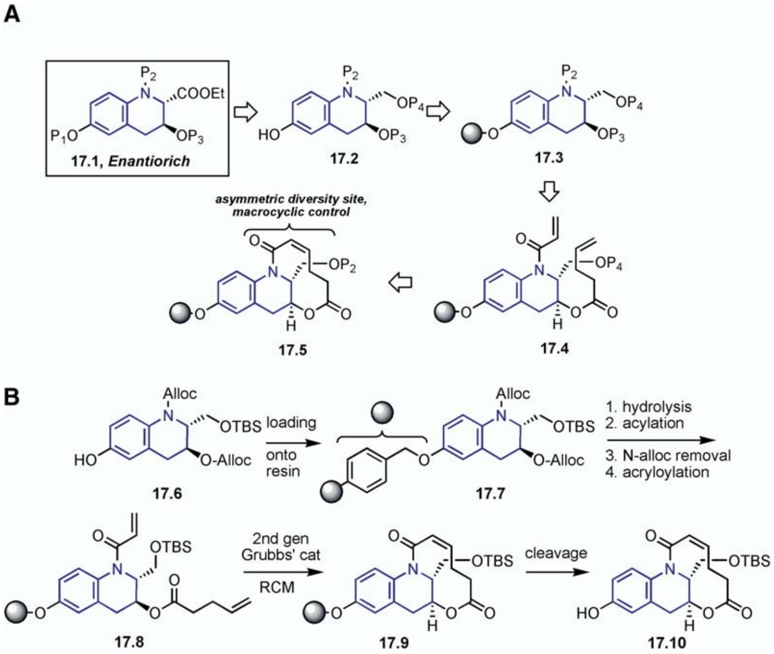

Figure 17. Natural Product-like Compounds with a Bridged, Medium-Sized Ring

(A) Tetrahydroquinoline-based polycyclics having a functionalized, bridged, 10-membered ring.

(B) Solid-phase synthesis of tetrahydroquinoline-based, polycyclic compounds with a bridged, 10-membered ring.

See [66].

NMR and MS. It was therefore possible to obtain on solid phase a natural product-like, tetrahydroquinoline-derived, polycyclic compound having a functionalized, 10-membered ring whose novel scaffold could be utilized in library generation.

Conclusions

With the growing interest in having rapid access to small molecules used as (i) chemical probes for understanding ligand-protein interactions and as (ii) modulators of protein-protein interactions, generating natural product-like small molecules that are skeletally diverse, structurally complex, and rich in stereogenic functional groups is attracting attention. The early days of combinatorial chemistry were originally devoted to obtaining simple, flat, aromatic-based compounds. However, the need to develop efficient stereo- and enantioselective methods that use the tools of modern organic synthesis is now in demand. In the combinatorial chemistry arena, diversity-oriented synthesis is aimed at meeting these challenges. To meet the emerging biological challenges of postgenomic chemical biology, the development of novel, stereocontrolled, diversity-oriented synthetic methods is likely to play a key role in the generation of libraries of small molecules with the desired characteristics. From the examples discussed in this review, it is evident that the synthetic community is prepared to meet these challenges, and it is likely that we will see a rise in activity in this area in the coming years. It will be interesting to see what the future value of exploring the new chemical space that is not currently available to the biological community will be, and how these types of small molecules will compare to the presently available chemical space seen in nature. Whatever the outcome of this venture, the synthetic community is well positioned to undertake some of these challenges.

Acknowledgments

Our sincere thanks to several funding agencies for providing financial support: the National Research Council (NRC) Genomics program, NRC-Vice President Research Funds for Chemical Biology, the National Cancer Institute Canada, and the Canadian Institutes for Health Research. Thanks to the Department of Chemistry, University of Ottawa for providing financial support to B.R. Ayub Reayi and David Nauman are thanked for the critical reading of this article. The authors also sincerely thank both of the reviewers for providing several excellent suggestions that have been useful to revise the original version of the review article. We thank Professors Stuart Schreiber and Taran Kapoor for permission to reprint their published data.

Received: August 3, 2004
Revised: January 22, 2005
Accepted: January 24, 2005
Published: February 25, 2005

References

1. Gura, T. (2000). A chemistry set for life. Nature *407*, 282–284.
2. Schreiber, S.L. (2003). The small-molecule approach to biology. Chem. Eng. News *81*, 51–61.
3. Nicolaou, K.C., Vourloumis, D., Winssinger, N., and Baran, P.S. (2000). The art and science of total synthesis at the dawn of the twenty-first century. Angew. Chem. Int. Ed. Engl. *39*, 44–122.
4. Dolle, R.E. (2002). Comprehensive survey of combinatorial library synthesis: 2001. J. Comb. Chem. *4*, 369–418.
5. Dolle, R.E. (2003). Comprehensive survey of combinatorial library synthesis: 2002. J. Comb. Chem. *5*, 693–753.
6. Dolle, R.E. (2004). Comprehensive survey of combinatorial library synthesis: 2003. J. Comb. Chem. *6*, 623–679.
7. Hall, D.G., Manku, S., and Wang, F. (2001). Solution- and solid-phase strategies for the design, synthesis, and screening of libraries based on natural product templates: a comprehensive survey. J. Comb. Chem. *3*, 125–150.

8. 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. Engl. *40*, 339–346.
9. Arya, P., and Baek, M.-G. (2001). Natural-product-like chiral derivatives by solid-phase synthesis. Curr. Opin. Chem. Biol. *5*, 292–301.
10. Arya, P., Joseph, R., and Chou, D.T.H. (2002). Toward high-throughput synthesis of complex natural product-like compounds in the genomics and proteomics age. Chem. Biol. *9*, 145–156.
11. Boger, D.L., Desharnais, J., and Capps, K. (2003). Solution-phase combinatorial libraries: modulating cellular signaling by targeting protein–protein or protein–DNA interactions. Angew. Chem. Int. Ed. Engl. *42*, 4138–4176.
12. Dörwald, F.Z. (2000). Organic Synthesis on Solid Phase: Supports, Linkers, Reactions (Weinheim, Germany: Wiley-VCH).
13. Peterson, J.R., and Mitchison, T.J. (2002). Small molecules, big impact: a history of chemical inhibitors and the cytoskeleton. Chem. Biol. *9*, 1275–1285.
14. Schreiber, S.L. (2000). Target-oriented and diversity-oriented organic synthesis in drug discovery. Science *287*, 1964–1969.
15. Schreiber, S.L. (1998). Chemical genetics resulting from a passion for synthetic organic chemistry. Bioorg. Med. Chem. *6*, 1127–1152.
16. Crews, C.M., and Mohan, R. (2000). Small-molecule inhibitors of the cell cycle. Curr. Opin. Chem. Biol. *4*, 47–53.
17. Crews, C.M., and Splittgerber, U. (1999). Chemical genetics: exploring and controlling cellular processes with chemical probes. Trends Biochem. Sci. *24*, 317–320.
18. Stockwell, B.R. (2000). Frontiers in chemical genetics. Trends Biotechnol. *18*, 449–455.
19. Stockwell, B.R. (2000). Chemical genetics: ligand-based discovery of gene function. Nat. Rev. Genet. *1*, 116–125.
20. Crews, C.M. (1996). Deciphering isozyme function: exploring cell biology with chemistry in the post-genomic era. Chem. Biol. *3*, 961–965.
21. Mayer, T.U. (2003). Chemical genetics: tailoring tools for cell biology. Trends Cell Biol. *13*, 270–277.
22. Strausberg, R.L., and Schreiber, S.L. (2003). From knowing to controlling: a path from genomics to drugs using small molecule probes. Science *300*, 294–295.
23. Burke, M.D., and Schreiber, S.L. (2004). A planning strategy for diversity-oriented synthesis. Angew. Chem. Int. Ed. Engl. *43*, 46–58.
24. Arkin, M.R., and Wells, J.A. (2004). Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. Nat. Rev. Drug Discov. *3*, 301–317.
25. Wess, G., Urmann, M., and Sickenberger, B. (2001). Medicinal chemistry: challenges and opportunities. Angew. Chem. Int. Ed. Engl. *40*, 3341–3350.
26. Breinbauer, R., Vetter, I.R., and Waldmann, H. (2002). From protein domains to drug candidates — natural products as guiding principles in the design and synthesis of compound libraries. Angew. Chem. Int. Ed. Engl. *41*, 2878–2890.
27. Ortholand, J.-Y., and Ganesan, A. (2004). Natural products and combinatorial chemistry: back to the furture. Curr. Opin. Chem. Biol. *8*, 271–280.
28. Abel, U., Koch, C., Speitling, M., and Hansske, F.G. (2002). Modern methods to produce natural-product libraries. Curr. Opin. Chem. Biol. *6*, 453–458.
29. Boldi, A.M. (2004). Libraries from natural product-like scaffolds. Curr. Opin. Chem. Biol. *8*, 281–286.
30. Hotha, S., Yarrow, J.C., Yang, J.G., Garrett, S., Renduchintala, K.V., Mayer, T.U., and Kapoor, T.M. (2003). HR22CC16: A potent small-molecule probe for the dynamics of cell divsion. Angew. Chem. Int. Ed. Engl. *42*, 2379–2382.
31. Tietze, L.F., Rackelmann, N., and Sekar, G. (2003). Catalyst-controlled stereoselective combinatorial synthesis. Angew. Chem. Int. Ed. Engl. *42*, 4254–4257.
32. Brohm, D., Philippe, N., Metzger, S., Bhargava, A., Müller, O., Lieb, F., and Waldmann, H. (2002). Solid-phase synthesis of dysidiolide-derived protein phosphatase inhibitors. J. Am. Chem. Soc. *124*, 13171–13178.
33. Doi, T., Hijikuro, I., and Takahashi, T. (1999). An efficient solid-phase synthesis of the vitamin D_3 system. J. Am. Chem. Soc. *121*, 6749–6750.
34. Hijikuro, I., Doi, T., and Takahashi, T. (2001). Parallel synthesis of vitamin D_3 library in the solid phase. J. Am. Chem. Soc. *123*, 3716–3722.
35. Arai, T., and Kubo, A. (1983). Isoquinolinequinones from Actinomycetes and Sponges. In The Alkaloids, A. Brossi, ed. (New York: Academic Press), pp. 56–100.
36. Myers, A.G., and Lanman, B.A. (2002). A solid-supported, enantioselective synthesis suitable for the rapid preparation of large numbers of diverse structural analogues of (-)-saframycin A. J. Am. Chem. Soc. *124*, 12969–12971.
37. Myers, A.G., and Plowright, A.T. (2001). Synthesis and evaluation of bishydroquinone derivatives of (-)-saframycin A: identification of a versatile molecular template imparting potent antiproliferative activity. J. Am. Chem. Soc. *123*, 5114–5115.
38. Plowright, A.T., Schaus, S.E., and Myers, A.G. (2002). Transcriptional response pathways in a yeast strain sensitive to saframycin A and a more potent analog: evidence for a common basis of activity. Chem. Biol. *9*, 607–618.
39. van Maarseveen, J.H. (1998). Solid phase synthesis of heterocycles by cyclization/cleavage methodologies. Comb. Chem. High Throughput Screen. *1*, 185–214.
40. Barun, O., Sommer, S., and Waldmann, H. (2004). Asymmetric solid-phase synthesis of 6,6-spiroketals. Angew. Chem. Int. Ed. Engl. *43*, 3195–3199.
41. Paterson, I., Donghi, M., and Gerlach, K. (2000). A combinatorial approach to polyketide-type libraries by iterative asymmetric aldol reactions performed on solid support. Angew. Chem. Int. Ed. Engl. *39*, 3315–3319.
42. Paterson, I., and Temal-Laib, T. (2002). Toward the combinatorial synthesis of polyketide libraries: asymmetric aldol reactions with α-chiral aldehydes on solid support. Org. Lett. *4*, 2473–2476.
43. Burke, M.D., Berger, E.M., and Schreiber, S.L. (2003). Generating diverse skeletons of small molecules combinatorially. Science *302*, 613–618.
44. Tallarico, J.A., Depew, K.M., Pelish, H.E., Westwood, N.J., Lindsley, C.W., Shair, M.D., Schreiber, S.L., and Foley, M.A. (2001). An alkylsilyl-tethered, high-capacity solid support amenable to diversity-oriented synthesis for one-bead, one-stock solution chemical genetics. J. Comb. Chem. *3*, 312–318.
45. Stavenger, R.A., and Schreiber, S.L. (2001). Asymmetric catalysis in diversity-oriented organic synthesis: enantioselective synthesis of 4320 encoded and spatially segregated dihydropyrancarboxamides. Angew. Chem. Int. Ed. Engl. *40*, 3417–3420.
46. Koehler, A.N., Shamji, A.F., and Schreiber, S.L. (2003). Discovery of an inhibitor of a transcription factor using small molecule microarrays and diversity-oriented synthesis. J. Am. Chem. Soc. *125*, 8420–8421.
47. Spring, D.R., Krishnan, S., and Schreiber, S.L. (2000). Towards diversity-oriented, stereoselective synthesis of biary- or bis(aryl)metal-containing medium rings. J. Am. Chem. Soc. *122*, 5656–5657.
48. Spring, D.R., Krishnan, S., Blackwell, H.E., and Schreiber, S.L. (2002). Diversity-oriented synthesis of biary-containing medium rings using a one bead/one stock solution platform. J. Am. Chem. Soc. *124*, 1354–1362.
49. Micalizio, G.C., and Schreiber, S.L. (2002). A boronic ester annulation strategy for diversity-oriented organic synthesis. Angew. Chem. Int. Ed. Engl. *41*, 152–154.
50. Micalizio, G.C., and Schreiber, S.L. (2002). An alkynylboronic ester annulation: development of synthetic methods for application to diversity-oriented organic synthesis. Angew. Chem. Int. Ed. Engl. *41*, 3272–3275.
51. Kubota, H., Lim, J., Depew, K.M., and Schreiber, S.L. (2002). Pathway development and pilot library realization in diversity-oriented synthesis: exploring Ferrier and Pauson-Khand reactions on a glycal template. Chem. Biol. *9*, 265–276.
52. Geis, O., and Schmalz, H.-G. (1998). New developments in the Pauson-Khand reaction. Angew. Chem. Int. Ed. Engl. *37*, 911–914.
53. Brummond, K.M., and Kent, J.L. (2000). Recent advances in

the Pauson-Khand reaction and related [2+2+1] cycloadditions. Tetrahedron *56*, 3263–3283.
54. Fletcher, A.J., and Christie, S.D.R. (2000). Cobalt mediated cyclisations. J. Chem. Soc. Perkin Trans. I *56*, 1657–1668.
55. Kwon, O., Park, S.B., and Schreiber, S.L. (2002). Skeletal diversity via a branched pathway: efficient synthesis of 29,400 discrete, polycyclic compounds and their arraying into stock solutions. J. Am. Chem. Soc. *124*, 13402–13404.
56. Woo, S., Squires, N., and Fallis, A.G. (1999). Indium-mediated γ-pentadienylation of aldehydes and ketones: cross-conjugated trienes for diene-transmissive cycloadditions. Org. Lett. *1*, 573–575.
57. Barnes-Seeman, D., Park, S.B., Koehler, A.N., and Schreiber, S.L. (2003). Expanding the functional group compatibility of small-molecule microarrays: discovery of novel calmodulin ligands. Angew. Chem. Int. Ed. Engl. *42*, 2376–2379.
58. Taylor, S.J., Taylor, A.M., and, and Schreiber, S.L. (2004). Synthetic strategy toward skeletal diversity via solid-supported, otherwise unstable reactive intermediates. Angew. Chem. Int. Ed. Engl. *43*, 1681–1685.
59. Dewick, P.M. (2002). Medicinal Natural Products: A Biosynthetic Approach (New York: Wiley-Interscience).
60. Arya, P., Durieux, P., Chen, Z.-X., Joseph, R., and Leek, D.M. (2004). Stereoselective diversity-oriented solution and solid-phase synthesis of tetrahydroquinoline-based polycyclic derivatives. J. Comb. Chem. *6*, 54–64.
61. Arya, P., Couve-Bonnaire, S., Durieux, P., Laforce, D., Kumar, R., and Leek, D.M. (2004). Solution- and solid-phase synthesis of natural product-like tetrahydroquinoline-based polycyclics having a medium size ring. J. Comb. Chem. *6*, 735–745.
62. Lee, D., 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.
63. Su, Q., Beeler, A.B., Lobkovsky, E., Porco, J.J.A., and Panek, J.S. (2003). Stereochemical diversity through cyclodimerization: synthesis of polyketide-like macrodiolides. Org. Lett. *5*, 2149–2152.
64. Sello, J.K., Andreana, P.R., Lee, D., and Schreiber, S.L. (2003). Stereochemical control of skeletal diversity. Org. Lett. *5*, 4125–4127.
65. Schmidt, D.R., Kwon, O., and Schreiber, S.L. (2004). Macrolactones in diversity-oriented synthesis: preparation of a pilot library and exploration of factors controlling macrocyclization. J. Comb. Chem. *6*, 286–292.
66. Khadem, S., Joseph, R., Rastegar, M., Leek, D.M., Oudatchin, K.A., and Arya, P. (2004). A solution- and solid-phase approach to tetrahydroquinoline-derived polycyclics having a 10-membered ring. J. Comb. Chem. *6*, 724–734.