Review

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Reviews

Solution- and Solid-Phase Strategies for the Design, Synthesis, and Screening of Libraries Based on Natural Product Templates: A Comprehensive Survey

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I. Introduction

The use of natural products in the treatment of human illnesses can be traced back to the origins of early folk medicines more than 4000 years ago.[1] Yet, with medicinal chemistry, the 20th century has witnessed a departure from natural products as the sole inspiration for drug discovery. Organic synthesis provides medicinal chemists with powerful tools to design artificial drugs, i.e., novel molecules nonexistent in nature. Although several synthetic drugs possess structures with little resemblance to that of natural products, these compounds are often much superior as therapeutic agents than their natural counterparts. By accelerating the synthesis and screening of an ever larger number of synthetic analogues, combinatorial chemistry has greatly impacted the drug discovery process.[2] Despite this success, compounds originating from natural sources still play a major role in drug therapy. In fact, as much as half the drugs on the market are direct descendants of natural products.[3] The influence of natural products in drug development has been reviewed recently by Newman, Cragg, and Snader.[1] As their review article describes historical and modern aspects of this field supported with specific cases, it would be redundant to elaborate on the many benefits of natural products as medicinal drugs herein.

In the past few years, there has been an increasing interest in the design of small molecule libraries based on natural products as templates. Many laboratories have been attracted to the challenges of improving upon nature by discovering

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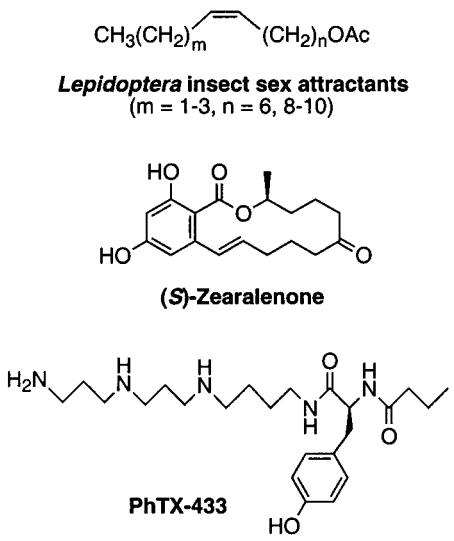

Figure 1. Selected examples of natural products made by solid-phase total synthesis.

analogues with therapeutic potential superior to that of the natural products that inspired them. In the design of a parallel library based on a specific natural product as a lead compound, the combinatorial chemist is confronted with many of the same choices that must be addressed for a library based on unnatural compounds or templates:

(1) Should the library be synthesized in solution or by solid-phase chemistry? Can a total synthesis approach be envisaged, or is it preferable to plan a hemisynthesis from an advanced intermediate?

While the practical advantages of solid-phase chemistry are well appreciated, it is not a risk-free approach either. So far, the total synthesis of only a small number of non-peptidic natural products has been achieved on solid support from simple precursors. Moreover, perhaps with the exception of Nicolaou's synthesis of epothilone A,[4] only relatively small molecules have been constructed by solid-phase total synthesis. This includes examples like early studies by Leznoff on insect pheromones,[5] and more recently, the synthesis of (*S*)-zearalenone by Nicolaou and co-workers,[6] our laboratory's syntheses of insect acylpolyamine toxins HO-416b and PhTX 433 (Figure 1),[7] and a few other natural products which are described in this review. Whereas most libraries of synthetic analogues have focused on rather simple molecules such as small heterocycles,[8] most natural products display much higher complexity. Thus, despite the tremendous progress realized in solid-phase chemistry in the past decade,[9,10] library design based on a multistep total synthesis strategy remains a highly challenging endeavor. For a given solid-phase synthesis, as the number of operations increases, so does the possibility of generating resin-bound impurities, therefore creating potential complications toward final product purification. In addition, spectroscopic characterization of supported products can be cumbersome, and many transformations are still very difficult to realize on solid support. Indeed, there are no universal supports and linkers that behave ideally for all types of chemistries. Despite these limitations, the practical advantages of using solid-phase approaches are very attractive. As solid supports, techniques, and instruments improve, continuous impetus on solid-phase strategies is expected. In the meantime, to make libraries from very complex natural product templates, the use of hemisynthetic approaches often make for a good compromise between solution-phase and solid-phase strategies. This review describes several examples of such strategies where an advanced natural product intermediate is synthesized in solution, then attached to the support and used as scaffold for combinatorial diversification at several positions. As the natural product template is diversified at a rather late stage in the overall synthetic scheme, an obvious limitation of the hemisynthetic approach is that several skeletal modifications and crucial positions can no longer be diversified. This often leaves simple functional group modifications (amides, amines, esters) as the only possible means for combinatorial derivatization. In reality, as discussed in the next section, this limitation is not always detrimental.

(2) Which sites on the natural product template should be diversified, and what size should the library be? Which type of screening format should be used, discrete compounds or mixtures?

These design considerations are not much different whether a natural product or a synthetic compound is used as the template for library design. In the former instance, a natural product becomes a lead compound from which a library of analogues is generated in order to improve the biological properties. Despite having a priori an active compound as a starting point, a rational approach to library design is only possible in cases where prior QSAR data is available or when structural information on the complex between the natural product bound to its biomolecule target is known. This sort of knowledge can help define potential positions for diversification and dictate the type and number of building blocks. These factors will in turn influence the size of the resulting focused library. In the absence of structural information and/or a pharmacophoric profile, it is desirable in an initial exploratory library to optimize size, diversify as many positions as possible according to the synthetic plan employed, and use representative sets of building blocks. However, as discussed in the previous section, the same issues of molecular complexity associated with natural products, such as the taxoids and the sarcodiyctins, often impose limits on the scope of diversity that can be introduced. For example, while it may be desirable to derivatize an isolated methylene site on the core structure with different carbon-containing side chains, it may also be extremely difficult to design and effectively carry out the required synthetic plan. Finally, unless a traceless linker[11] or a cyclization-release strategy is employed, the choice of linker required in solid-phase approaches may affect the ability to diversify one or more sites on the template. In several cases it is necessary to sacrifice a carboxyl, hydroxyl, or amino group for the purpose of immobilizing the template to the support. This prevents most possibilities for solid-phase diversification at the attachment site unless cleavage of the linker itself is designed to be a diversity generating operation. Derivatization of the attachment site can often be done in solution, however, following cleavage of the products from the support.

In the past few years, mini-reviews focusing solely on

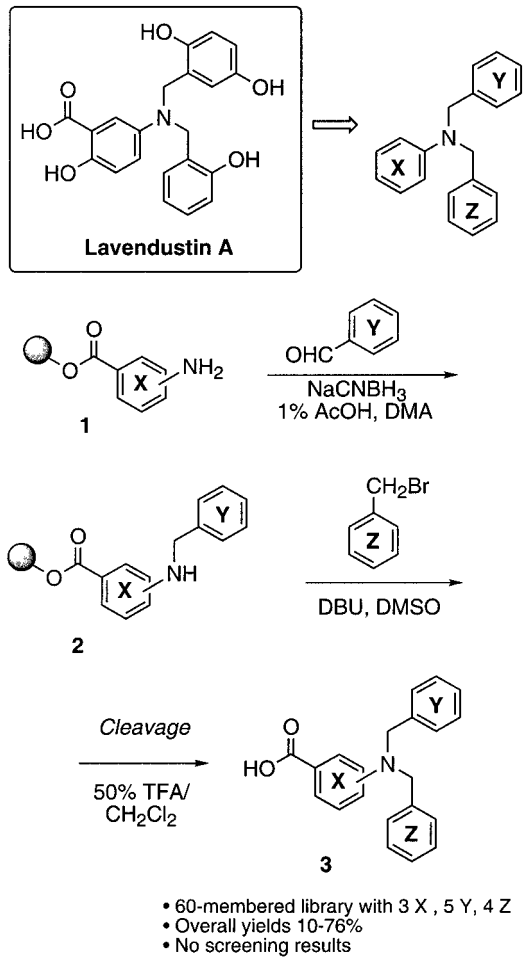

Figure 2. Lavendustin-based library (solid-phase).[19]

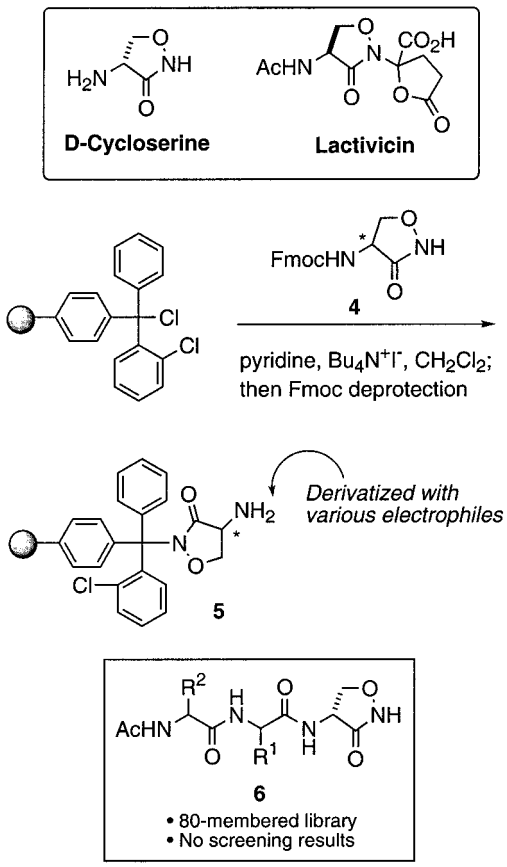

Figure 3. Cycloserine-based library (solid-phase).[21]

solid-phase approaches to natural product based libraries have appeared.[12,13] In this comprehensive review, we have surveyed both solution- and solid-phase approaches to the design of such libraries. We have made all possible efforts to describe relevant work from the journal literature, up to the end of 2000, reporting libraries whose design is truly inspired by a specific non-peptidic natural product. Thus, "natural-product looking" libraries,[14] libraries of hybrid structures, or others fashioned from a simplified mimetic of a natural product[15] were not reviewed, and neither were libraries of the ubiquitous biopolymers (peptides, nucleic acids, oligosaccharides[16]). Finally, avid enthusiasts on the topic can also consult a recent review on combinatorial biosynthesis, a fascinating and promising biological approach to the generation of libraries of novel natural products.[17]

The contents of this review are organized successively according to the approximate order of size and complexity for the natural product template used. Results from biological assays are provided whenever reported in the original literature.

II. Natural Product Templates

A. Amines (Lavendustin A). Anilines and benzylamines constitute an important pharmacophore present in several bioactive substances. Lavendustin A and its various O-methylated analogues, for instance, are potent inhibitors of tyrosine kinase.[18] The simple framework of lavendustin A can be dissected into three aromatic subunits and is thus particularly well amenable to combinatorial library generation by a total synthesis approach (Figure 2).[19] Green has synthesized lavendustin A on solid support with various linkers (Wang, Rink, hydroxymethyl) using reductive alkylation and substitution chemistries.[19a] To make a library of 60 analogues from three, five, and four components of the respective subunits X, Y, and Z, the carboxyl group of subunit X was first attached to Wang resin. Then the Fmoc-protected aniline was liberated to give **1**, which was reacted with various methoxylated benzaldehyde components (Y) by reductive alkylation. The resulting secondary amines **2** were then reacted with benzylbromides to install subunit Z. The final products (**3**) were cleaved from the resin (50% TFA/CH_2Cl_2) and isolated in rather modest to good yields (10−76%) and purities. Although no screening results of these O-methylated analogues of lavendustin A were reported, this work provided one of the earliest examples of a natural product based combinatorial library.

B. Cycloserine. D-Cycloserine is a small natural product isolated from the fermentation broths of *Streptomyces orchidaceus*, *Streptomyces garyphalus*, and *Streptomyces lavendulus* and shows activity as a broad spectrum antibiotic.[20] Its enantiomer, L-cycloserine, is featured as a subunit in the antibiotic lactivicin, isolated from *Empedobacter lactamgenus* and *Lysobacter albus* cultures. A report from Gordeev and co-workers explores the solid-phase combinatorial synthesis of cycloserine analogues (Figure 3).[21] To derivatize both enantiomers, the endocyclic amine of the commercially available Fmoc-protected derivatives **4** was

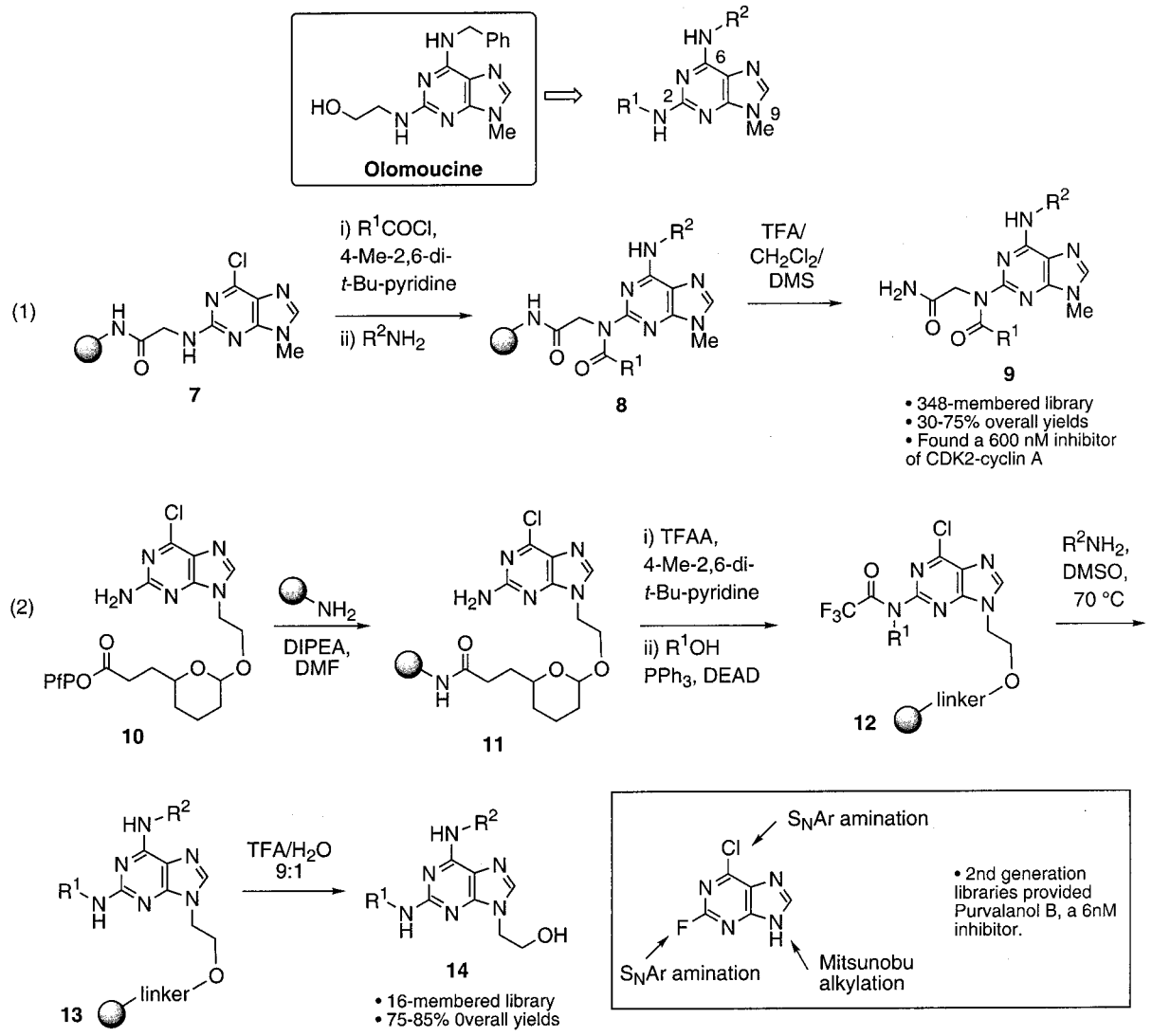

Figure 4. Trisubstituted purine libraries (solid- and solution-phase).[25−28]

attached onto the solid support via the highly acid labile 2-chlorotrityl linker. Following cleavage of the Fmoc carbamate, the exocyclic amine **5** was left exposed to derivatization by various electrophiles. Combinatorial applications of this methodology were demonstrated by the parallel synthesis of an 80-membered dipeptide-cycloserine library **6** made by diversifying amino acid building blocks that were coupled onto the exocyclic amine. The limitation of this approach, however, is that only the exocyclic amine can be derivatized and it does not allow for the solid-phase synthesis of lactivicin analogues.

C. Purines (Olomoucine). The adenine derivative olomoucine was isolated by Meijer and co-workers from natural sources and was found to inhibit several cyclin dependent kinases up to micromolar level.[22] For instance, it is a selective competitive inhibitor of CDK2/cyclin A with an IC_{50} of 7 μM. Cyclin dependent kinases, such as CDK2, play a vital role as regulators of cell cycle events including DNA replication and cellular division.[23] These enzymes are suspected to be implicated in several diseases including cancer (there is high incidence of genetically altered CDK's in tumor cells), and thereby stimulate intense efforts in the discovery of potent and selective inhibitors. A crystal structure of the olomoucine-CDK2 complex revealed that the purine-based molecule occupies the adenine binding pocket of ATP although the ring structure lies in a drastically different orientation.[24] Upon examination of olomoucine's structure (Figure 4), it was reasoned that diversification of substituents appended at C6, C2, and N9 of the purine scaffold may allow a significant improvement in the binding affinity and selectivity. This prompted Schultz and coworkers to plan a semirational library approach to the discovery of improved inhibitors of CDK's using the purine scaffold, a rather common heterocyclic unit among biologically relevant molecules. Two solid-phase approaches toward spatially addressable bidimensional libraries were described in the first report by this laboratory (Figure 4).[25] As shown in reaction 1, a 6-chloro-substituted purine scaffold functionalized with a glycinamide spacer, prepared in solution, was coupled to Rink linker-derivatized pins supported onto racks formatted for 96-well microtiter plates. From **7**, the first combinatorial operation was carried out by acylation of the exocyclic amine. Then, aromatic substitution on C6 with primary and secondary amines provided the second element of diversity in intermediates **8** (shown in Figure 4 only for the case of primary amines). In this case, deacylation

of R^1CO- was minimized by effecting the amination in a DMF/DMSO solvent system at 4 °C. Cleavage from the pins provided 2-(acylamino)-6-amino purine analogues **9** in 30−75% overall yields with few or no purine-containing impurities. A library of 348 members was synthesized using 5 acid chlorides and 58 amines. The compounds were screened in solution using a microtiter-based assay for protein kinase activity. One member was found to possess inhibitory activity 10 times superior (600 nM) to oloumycine. An alternate synthetic design allowed attachment of **10** to solid support via the N9 position (reaction 2). The resulting resin-bound scaffold **11** was combinatorialized with Mitsunobu alkylation of the C2 trifluoroacetanilide. Aromatic amination at C6 of **12** with subsequent aminolysis of the trifluoroacetamide provided 2-hydroxyethyl purine analogues **14** in good yields and high purities after cleavage from the resin. The same laboratory significantly expanded the scope of this chemistry in a second-generation approach allowing diversification of the N9 position as well.[26] To this end, various solution-phase and solid-phase approaches from 2-fluoro-6-chloropurine as the template were successfully demonstrated, resulting in the discovery of purvalanol B (2-(1*R*-isopropyl-2-hydroxyethylamino)-6-(3-chloro-4-carboxyanilino)-9-isopropylpurine), a highly potent (6 nM) inhibitor of the human CDK2-cyclin A. This analogue was shown to be highly selective among more than 22 human purified kinases tested. Similar 1,6,9-trisubstituted purine libraries were also evaluated toward other types of targets and screens, including carbohydrate sulfotransferase inhibition,[27] and whole cell chemical genomics assays.[28] In particular, one analogue found from these various libraries, myoseverin (bis-2,6-(4-methoxybenzylamino)-9-isopropylpurine), was recently shown to induce the reversible fission of mice C2C12 multinucleated myotubes (muscle cells) into mononucleated fragments, a process that is known to occur only in amphibians.[29] This discovery, which could find eventual therapeutic applications such as tissue regeneration in humans, provides a powerful example on the enormous potential of natural product based libraries.

D. Oligoheterocycles (Distamycin A). The development of small molecules capable of regulating gene expression is a promising therapeutic approach despite being hampered in part by the difficulty of rationally designing tight DNA-binding, sequence selective agents. Distamycin is a natural antibiotic which has been characterized as a DNA minor groove binder.[30] Boger and co-workers have designed a solution-phase library of 2640 compounds inspired by the structure of distamycin A (Figure 5).[31] To this end, they employed a linear strategy using the EDCI/DMAP promoted coupling of *tert*-butyloxycarbonyl-protected amines, as mixtures, to assemble triamide compounds made of heterocyclic subunits A, B, C, respectively randomized with 10, 11, and 12 components. Compound isolation was performed using acid/base liquid−liquid extraction techniques. Library members were made in two sets of 132 mixtures of 10 compounds on reaction scales allowing multiple assays, and they were obtained with >95% purity. For screening, a rapid high-throughput assay for DNA binding was developed based on the loss of fluorescence from a target oligonucleotide presaturated with ethidium bromide. Deconvolution of the

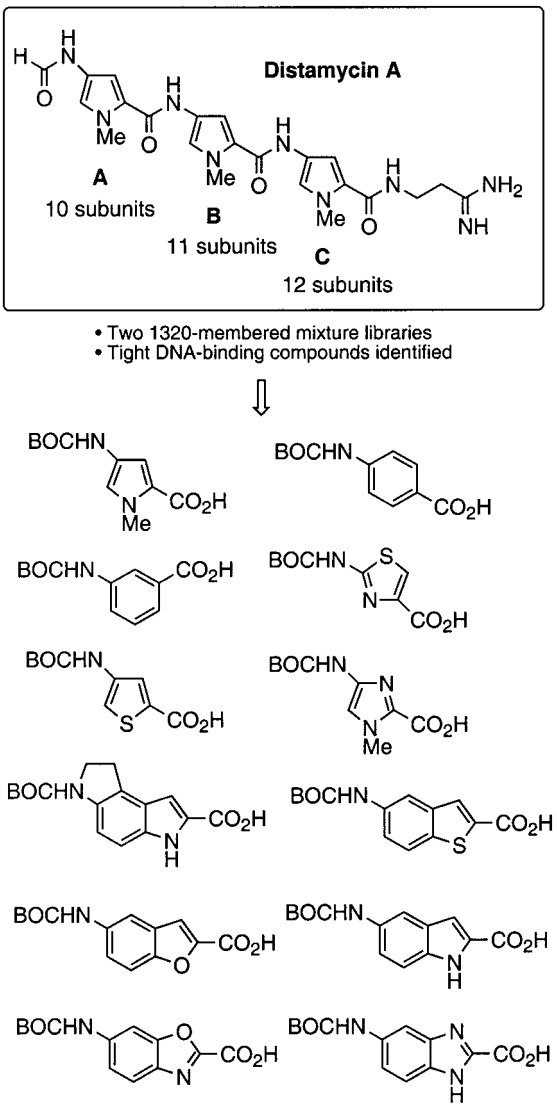

Figure 5. Distamycin-based libraries (solution).[31]

most potent mixtures by resynthesis and evaluation of individual compounds ultimately revealed ligands 1000 times more cytotoxic (IC_{50} 29 nM in the L1210 cytotoxicity assay) compared to distamycin A. Selected library members were also found to bind poly[dA]-poly[dT] with comparable affinity, and one compound demonstrated high affinity (K_d = 4.5 μM) to the androgen response element (ARE)-consensus sequence, a GC base-pair interrupted five-base-pair AT-rich site relevant to the expression of a gene implicated in cases of prostate cancers that are unresponsive to hormone antagonists.[32] The screening technique developed therein is also applicable to the evaluation of a single compound against libraries of hairpin oligonucleotides, hence providing qualitative and possibly quantitative determination of both binding affinity and sequence selectivity. The same group also compared this initial approach with two 1000-membered positional scanning libraries of similar analogues.[33] Although the latter strategy is synthetically less demanding, the assay was less sensitive in distinguishing between active library members. These studies, inspired from the natural product distamycin A, demonstrate that combinatorial high-throughput approaches can help accelerate the discovery of selective DNA-binding molecules.

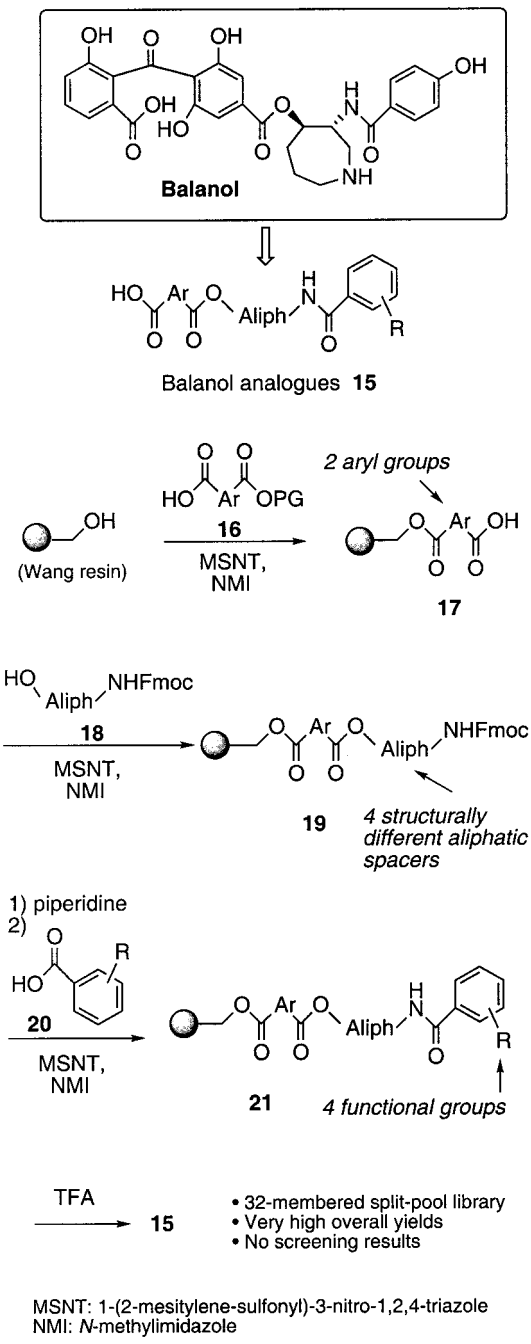

Figure 6. Balanol-based library (solid-phase).[36]

E. Balanol. Protein kinase C (PKC) is a serine/threonine phosphatase which in its activated form is implicated in a variety of severe disease processes such as cancer, inflammation, and cardiovascular dysfunctions.[34] Balanol, a fungal metabolite, possesses high activity for inhibiting PKC, thus it is a potential lead for development as a therapeutic agent.[35] However, significant optimization lies ahead as it lacks specificity toward other kinases. To address this problem, a combinatorial solid-phase synthesis of balanol analogues **15** has been accomplished by Nielsen and co-workers by means of the standard split-pool method, based on a strategy of combining three crucial components (Figure 6).[36] To this end, two monoprotected diacids **16**, four protected amino alcohols **18**, and four benzoic acid derivatives **20** were used as three relatively accessible building blocks to provide the molecular diversity. They were successively coupled in a linear fashion

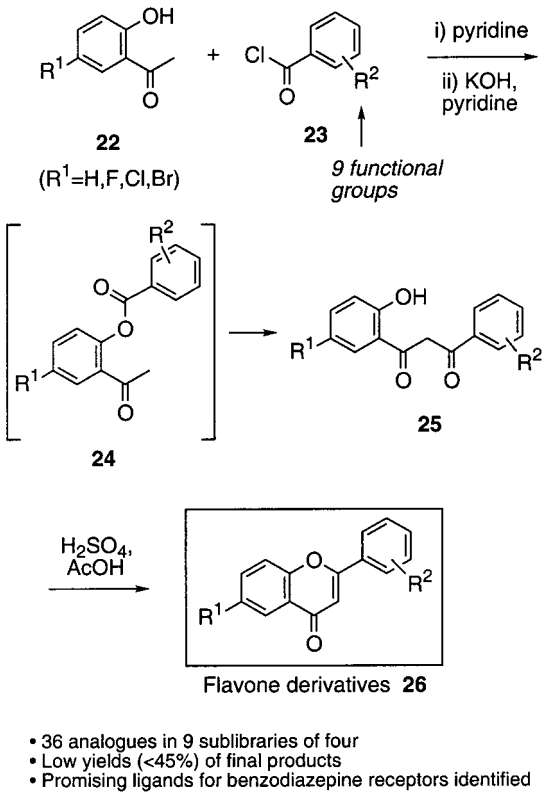

Figure 7. Flavonoid library (solution).[38]

by stepwise MSNT/NMI-mediated esterification and amidation reactions to generate, after cleavage from the support, a 32-membered library of structural analogues of balanol (**15**). Although no screening data were reported, this approach shows that libraries of complex natural products can be constructed by assembling simple building blocks through routine transformations that are well adapted to solid-phase synthesis.

F. Flavones. Several naturally occurring flavonoids are ligands to the central benzodiazepine receptors (BDZ-Rs) and have been found to possess moderate anxiolytic activity in vivo.[37] To find ligands with higher affinity, a library of flavone analogues was synthesized in solution by Paladini and co-workers.[38] In a simple two-step sequence, mixtures containing equimolar amounts of four 5′-substituted-2′-hydroxyacetophenones **22** were independently reacted in pyridine with one of nine different benzoyl chlorides **23** (Figure 7). Addition of hydroxide base transformed intermediate **24** to β-diketone **25**. The latter were then cyclized under acidic conditions into flavone derivatives **26**. Individual library members from the nine mixtures were separated by HPLC. The binding affinity to rat cerebral cortex BDZ-Rs was assayed by displacement of ^{3}H flunitrazam from synaptosomal membranes from different brain structures. This study revealed several analogues with binding affinities in the range of 17−23 nM, thereby showing that flavonoids with substitutions at 6- and 3′- positions exhibit promising biological properties. Thus, although this simple synthetic route provided only low yields of products, its application to the generation of a solution-phase library was successful.

G. Benzofurans (Kramerixin). Kramerixin is an antifungal neolignan isolated from extracts of *Krameria ixena* (from Puerto Rico) and *Krameria triandra* (from Peru).

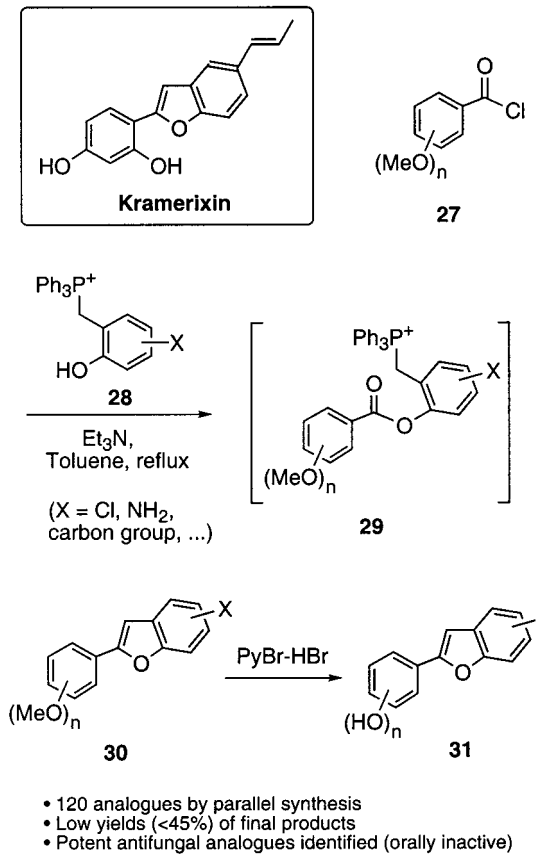

Figure 8. Benzofuran library (solution).[39]

Structurally, this natural product is characterized by a benzofuran nucleus with a pendant phenolic substituent. It has been shown to possess a broad antifungal activity against a number of organisms and is in fact nearly as potent in vitro as amphotericin B. Fecik and co-workers employed parallel synthesis instruments to prepare a library of as many as 120 analogues by a convergent solution-phase strategy where the nature and number of substituents X and the phenolic moiety were varied (Figure 8).[39] Key to the synthesis is the tandem base promoted reaction of acid chlorides **27** with benzylidenephosphonium-containing phenols **28** followed by an intramolecular Wittig reaction on **29** to establish the furan ring of **30**. Demethylation of the methoxy groups afforded the final phenolic compounds **31**. Overall yields obtained on model compounds are rather modest, but the reactions were performed on a scale allowing the isolation of approximately 100 mg of pure material. Variations on the heterocyclic ring were also examined and provided benzothiofurans, benzimidazoles, benzothiazoles, and indoles, although the latter were made using Fischer indole synthesis. Upon biological screening for antifungal activity, several potent analogues were identified, with one (the 5,7-dichloro analogue with the conserved 2,4-dihydroxyphenyl furan substituent) providing improved activity as compared to kramerixin (1.25 μg/mL vs 3.12 μg/mL inhibition of respiration of *C. albicans*). This analogue and two others were further studied in vivo, but they failed in oral tests carried out on infected mice.

H. Benzopyrans (Chalcones, Pyranocoumarins, Chromene Glycosides). In a recent series of detailed accounts, Nicolaou and co-workers described the design, chemical

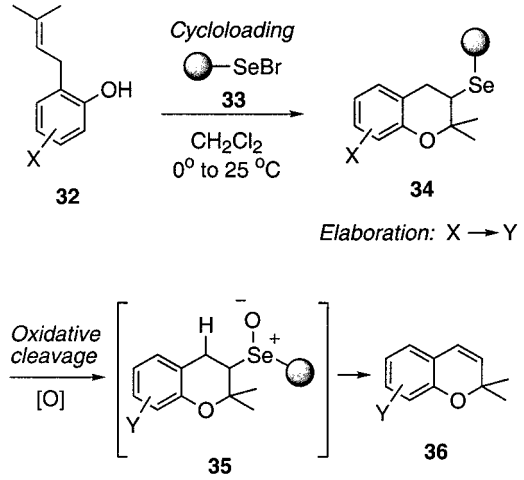

Figure 9. Synthesis of benzopyran scaffolds using the selenenyl bromide resin technology.

optimization, and eventual synthesis of natural product-like libraries centered around the 2,2-dimethyl-2*H*-benzopyran unit,[40−42] a structural motif that has been labeled a *privileged structure*. The concept of *privileged structures* is used to describe common structural motifs that are capable of interacting with a variety of unrelated biomolecular targets.[43] Libraries of analogues based upon structural types such as these have been found particularly useful in the discovery of small molecules active toward biological targets that are either poorly understood or completely unknown. Current examples of privileged structures include benzodiazepines, benzoazepines, benzamidines, biphenyltetrazoles, spiropiperidines, indoles, and benzylpiperidines.[44] The benzopyran motif discussed in this case is found in more than 4000 compounds including many bioactive natural products and pharmaceutically designed compounds, and it is therefore an excellent choice for combinatorial derivatization.

The key feature in the synthesis of each class of benzopyran derivative is the initial cycloloading of various *ortho*-prenylated phenols **32** onto a novel selenenyl bromide polystyrene resin[45] (**33**) to provide the supported benzopyran scaffold **34** (Figure 9). Oxidative release from the support through *syn*-elimination of the intermediate selenoxide **35** provides the desired benzopyran products **36**. In addition to its chemical stability, linkage of the scaffold via the pyran ring provides the advantage of allowing the derivatization of all four remaining positions on the aromatic ring. To test and optimize the chemistries that will be employed in a large library synthesis, a series of focused libraries were produced, all containing either a true natural product or existing designed structures.[40]

In one natural product based library, a chalcone family of compounds was synthesized in parallel fashion by condensing a variety of 11 aldehydes (**38**) to a set of three resin-bound benzopyrans **37** containing a methyl ketone substituent (Figure 10). Spontaneous dehydration of the resulting β-hydroxy ketones led to the supported chalcone framework **39** which was cleaved from the resin by hydrogen peroxide promoted selenoxide elimination to give the final products **40**. Included in the 26-membered library were three biologically active natural compounds: lonchocarpin, 4-hydroxylonchocarpin, and 4-hydroxy-3-methoxylonchocarpin in 91%,

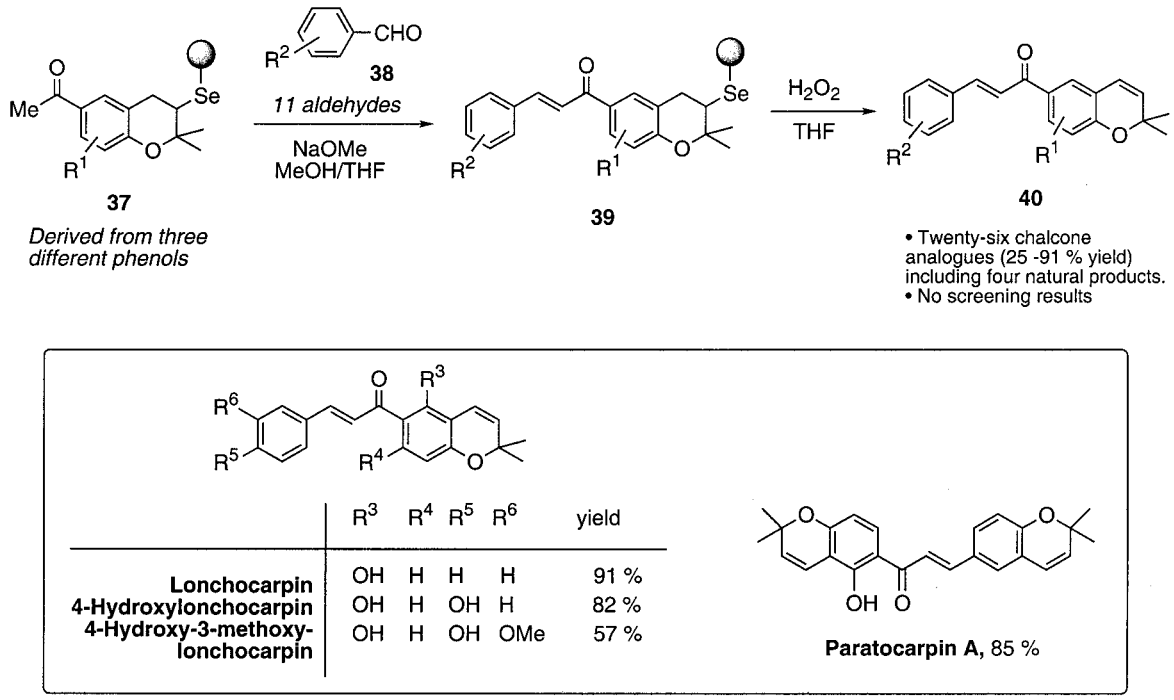

Figure 10. Chalcone library (solid-phase).[40]

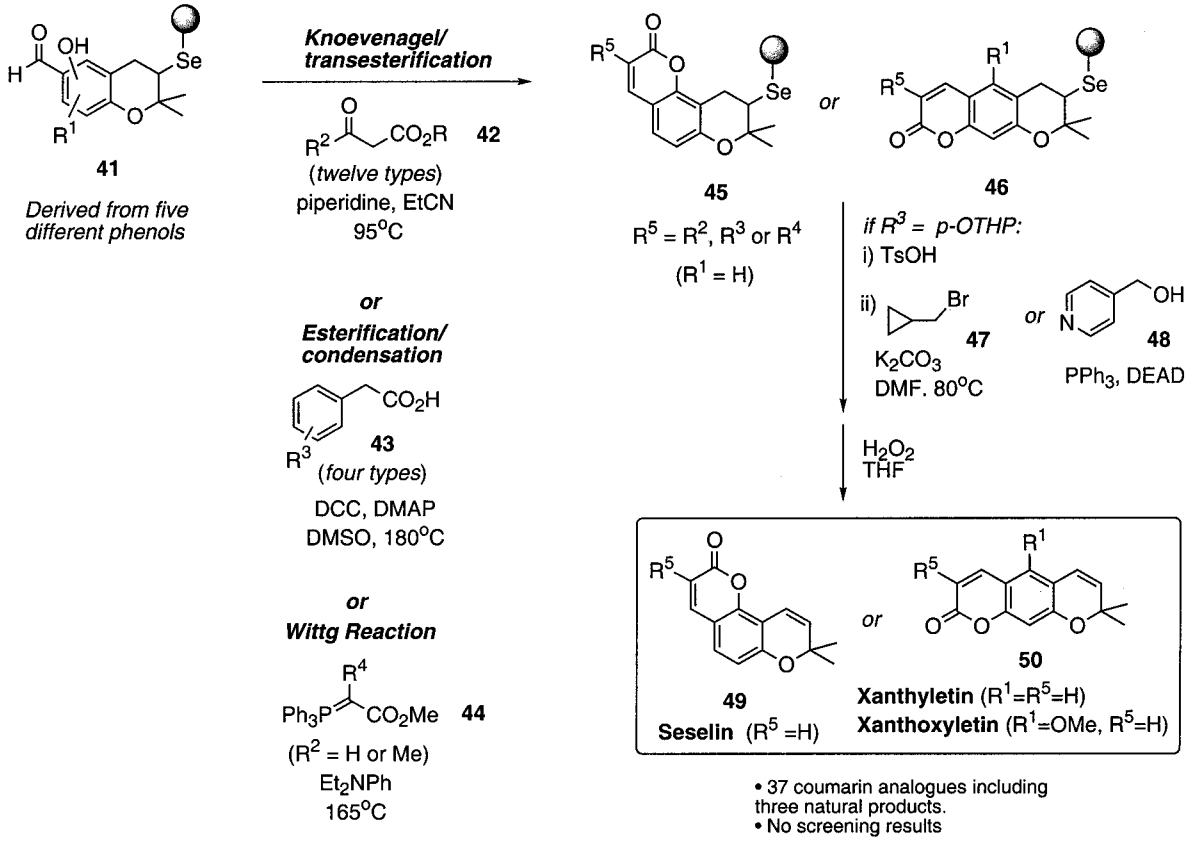

Figure 11. Pyranocoumarin library (solid-phase).[40]

82%, and 57% overall yields, respectively. Each of these compounds are components extracted from Cubé resin obtained from the roots of *Lonchocarpus utilis* and *Lonchocarpus urucu*, and they have all been shown to interrupt mitochondrial electron transport leading to the inhibition of NADH:ubiquinone oxidoreductase (complex I).[46] Also present in the library was paratocarpin A (85% overall yield), a compound reported to inhibit tumor invasion activity in the MCF-7/6 breast cancer cell line.[47] Yields of the remaining analogues ranged between 25 and 90%.

Another natural product inspired library was made based on frameworks of linear and angular pyranocoumarins (Figure 11). The library was prepared by a split-pool strategy with the aid of radio frequency encoded combinatorial (REC) chemistry using IRORI SMART (single or multiple addressable radio frequency tag) memory and storage MacroKans.[48]

The MacroKans are polypropylene microreactors which contain the resin and a radio frequency (RF) tag capable of emitting, receiving, and storing RF signals that record the synthetic history of each compound thus providing its identity. For this library, a set of five benzopyrans **41** containing an *o*-hydroxy aldehyde were prepared and then elaborated using three different reaction conditions. The first reaction was the treatment of **41** with a set of 12 aryl, alkyl, or alkoxy β-ketoesters **42** resulting in the Knoevenagel condensation and concomitant transesterification leading to the formation of pyranocoumarins **45** or **46** depending on the relative position of the hydroxy substituent on the aromatic ring. Alternatively, **45** and **46** were formed by coupling benzopyran **41** to four different phenylacetic acids **43** or by Wittig reaction with two stabilized phosphoranes **44**. Any phenol substituents on the scaffold were further derivatized by alkylation with bromide **47** or by a Mitsunobu reaction with alcohol **48**. Cleavage by hydrogen peroxide yielded a library of 37 pyranocoumarin analogues. Within the library were seselin, xanthyletin, and xanthoxyletin which are all naturally isolated from the Sri Lankan rutaceae plant[49] and are reported to exhibit cytotoxic, antiviral, and anti-platelet aggregation activities.[50]

The incorporation of a carbohydrate moiety onto the benzopyran scaffold was achieved as a way to obtain more desirable solubility properties and cellular targeting abilities required during biological screening assays. To this end, the authors synthesized sugar-containing analogues derived from a naturally occurring chromene glycoside isolated from *Ageratum conyzoides*[51] (Figure 12). Here, trichloroacetimidates of three sugars, D-glucose, D-xylose, and L-rhamnose, **52** were coupled onto three types of phenol containing scaffolds **51** to afford selectively the β-glycosides **53**. Deacetylation followed by cleavage from the resin gave the chromene glycoside natural product plus eight analogues **54** with purity greater than 90% in all cases.

In addition to the natural product libraries described above, Nicolaou and co-workers go further to demonstrate the utility of the selenoether linker by synthesizing libraries that are centered on existing pharmaceutically designed molecules. These include a known inhibitor of aldosterone biosynthesis, and an inhibitor of phosphodiesterase IV. The success of each library along with other preliminary studies confirmed the chemical robustness of the linker, surviving a variety of temperatures, solvents, and reagents.

With the reliability and versatility of the selenoether linker proven, they were now prepared to embark on a more ambitious 10 215-membered library based on the same benzopyran template.[41] For this they introduced the IRORI NanoKans,[52] miniaturized versions of the MicroKans which instead use an optical encoding system rather than an RF system. The advantage of the NanoKans, aside from its lower cost, is that its smaller size allows for the synthesis of larger split-pool libraries up to a size of 100 000 members. The synthesis began as shown in the previous figures with the cycloloading of nine *ortho*-prenylated benzaldehydes onto the selenenyl bromide resin. The supported scaffold was then elaborated further via three types of reactions: (1) the addition onto the aldehyde function of 20 different aryl or

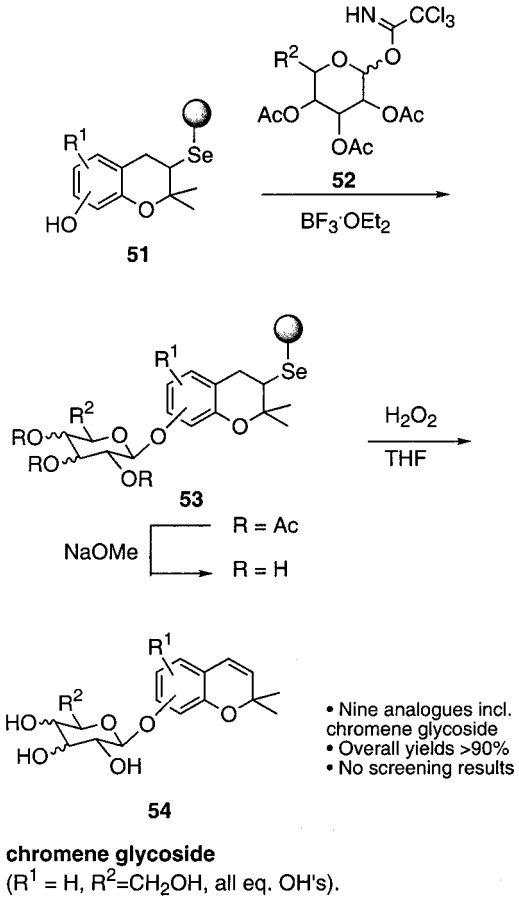

Figure 12. Chromene glycoside library (solid-phase).[40]

alkyl Grignard reagents and organolithiums as well as by $NaBH_4$; (2) reductive amination by 20 different primary amines, including anilines; and (3) a Knoevenagel condensation with 15 varieties of phenylacetonitriles. Continuation of the library involved straightforward transformations of functionalities created in the first round. Each building block employed in the library was chosen based on its varying electronic properties and lipophilicity, and they were individually tested prior to synthesizing the library to ensure that they reacted effectively with the benzopyran scaffold. Each reaction sequence was also evaluated in a series of test reactions in order to foresee any problems that may occur in the preparation of the actual library. During the library synthesis itself, a series of measures were taken to monitor the quality of certain library members. One important measure was the tracking of a select number of NanoKans, each containing an individual library member, to ensure reactions completeness at each step. At the end of the synthesis, 500 library members were cleaved and analyzed, and of these, 347 were found to have a purity of approximately 80% or higher by HPLC. It should be noted that these types of quality control measures are important in a library synthesis since the presence of significant impurities can hamper biological screening assays by causing false positives or other complications. At the completion of the benzopyran library, each NanoKan was sorted individually into cleavage plates (96 NanoKans per plate), releasing about 1−2 mg of compound in each of the individual 96 wells. Direct cleavage into a 96-well microtiter plate format facilitates both library analysis and biological screening. No

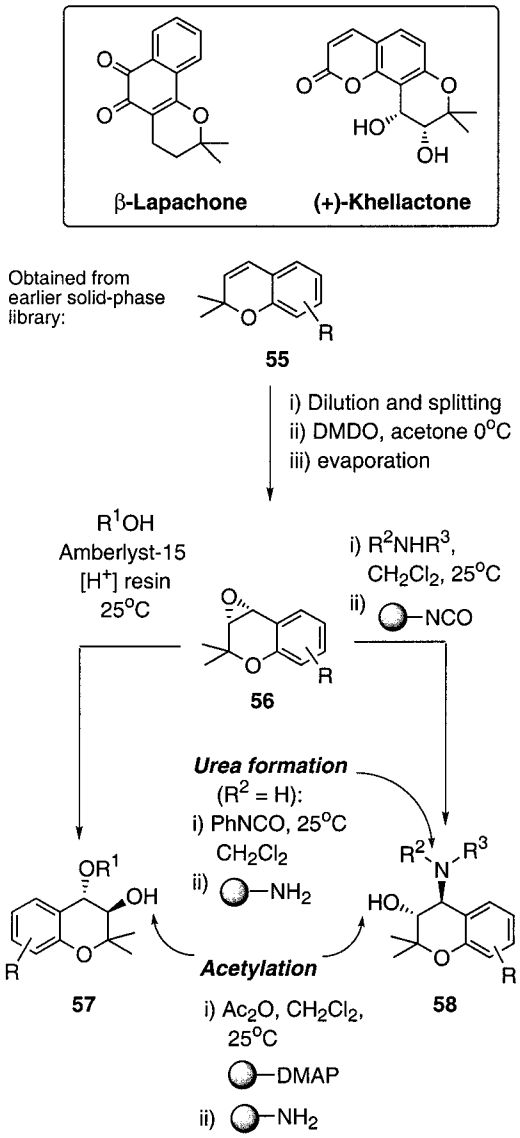

Figure 13. Parallel solution-phase modifications of benzopyran libraries.[42]

screening results are reported in this article, although they should be expected shortly as indicated by the authors.

Aside from the benzopyrans themselves, there are 8000 additional natural and designed analogues in where the pyran olefin is modified. In many cases, the consequence is either enhanced biological activity over the parent benzopyran or complete alteration of its biological properties. Examples of natural products with a modified benzopyran moiety are shown in Figure 13. For instance, *β*-lapachone exhibits a range of antineoplastic activities,[53] while (+)-khellactone is known to display cytotoxic and antivirial activities.[54] The Nicolaou group developed a "library-from-library" strategy[55] where an existing benzopyran library would be further derivatized at the pyran olefin thereby increasing the structural diversity.[42] To test this strategy, two small model libraries were made (50- and 120-membered libraries) starting from defined benzopyran scaffolds **55** (Figure 13). The latter have been released from the selenenyl resin into 96-well plates in which a parallel solution-phase procedure was used to epoxidize the olefin using dimethyl dioxirane (DMDO) to give **56**. After evaporation of the excess DMDO, the epoxide was opened with either an alcohol (H_2O, methanol, ethanol, or 2-propanol) to provide **57**, or with an amine (*n*-$BuNH_2$, pyrrolidine, or morpholine) to give **58**. Excess volatile alcohols and amines were simply evaporated after the reaction, while excess morpholine, which is nonvolatile, was scavenged by polymer-bound methyl isocyanate. The hydroxyl that forms upon epoxide opening was derivatized by an electrophile, in this case with acetic anhydride in the presence of polymer-supported 4-(*N*-benzyl-*N*-methylamino)pyridine. Excess acetic anhydride was scavenged by polymer-supported tris(2-aminoethyl)amine. The amine function on the scaffold was also treated with an isocyanate to give the urea with the excess isocyanate being removed by the polymer-supported tris(2-aminoethyl)amine. Purities of selected members were found to be at least 80% by ^{1}H NMR analysis. Overall, these extensive studies centered on the benzopyran framework demonstrate that a single linker technology, when applied to the right framework, can be developed into a vast scope of applications.

I. Fumiquinazolines. The synthesis of fumiquinazoline analogues on solid support illustrates an elegant biomimetic approach to the solid-phase synthesis of a quinazoline skeleton fused to two amino acids via a diketopiperazine-like fashion.[56] These alkaloid natural products, isolated as fungal metabolites,[57] have exhibited cytotoxicity against P388 leukemia cells, suggesting that their scaffold could serve as a template to generate analogues with improved medicinal potential. A model parallel library of 27 compounds was made in order to demonstrate the generality of the synthetic strategy (Figure 14). Five sets of anthranilic acids **60** were coupled to three Wang resin-bound amino acids **59** (L-alanine, L-leucine, and L-phenylalanine) to afford intermediates **61**. Acylation of the aniline was achieved using L and D-Fmoc amino acid chlorides (**62**) to furnish the tripeptide **63**. Treatment of the latter with triphenylphosphine, in the presence of iodine and Hünig's base, resulted in a dehydrative cyclization to the oxazine intermediates **64**. Removal of the Fmoc group followed by refluxing in acetonitrile led to the cyclization-release of the fumiquinazoline analogues **65**. The use of a cyclorelease strategy can be very profitable because side products from incomplete reactions cannot undergo the cyclization and cleavage reaction and thus remain attached to the leftover resin. Except for an anilide from 5-nitroanthranilic acid which failed to undergo the dehydrative cyclization, the purity of the crude materials ranged from 74 to 98%, while crude yields were between 17 and 90%. All *cis*-quinazolines (made by using L-Fmoc amino acid chloride) were always accompanied by a small amount of the sterically more favored trans diastereomer due to epimerization of the R^3 methine during the cyclization step. No results from biological screening for this library have been reported to date.

J. Indolyl Diketopipezines. Fumitremorgin, which is isolated from the fermentation broth of fungus *Aspergillus fumigatus*,[58] is a natural product from a group of tremorgenic mycotoxins with indolyl diketopiperazine core. These natural products interfere with the mechanisms responsible for the release of CNS neurotransmitters and also act as inhibitors for the mammalian cell cycle. Such properties confer to these

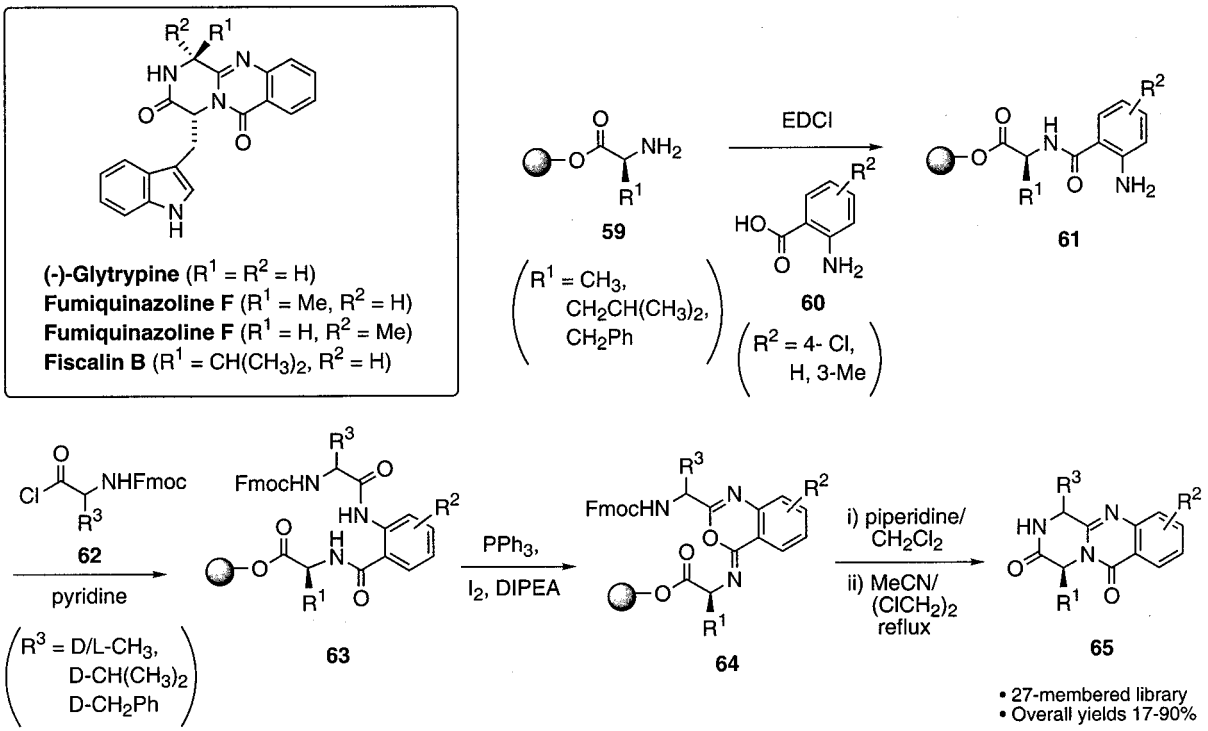

Figure 14. Fumiquinazoline-based library (solid-phase).[56]

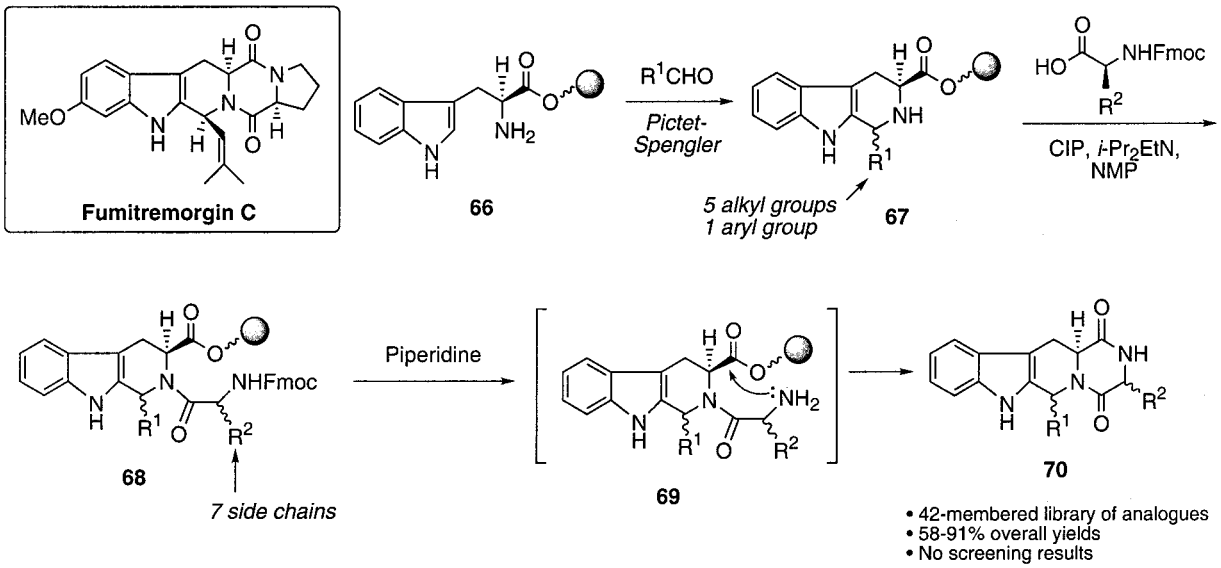

Figure 15. Indolyl diketopiperazine based library (solid-phase).[59]

compounds potential use as molecular probes in CNS receptor studies and particularly as leads for cancer chemotherapy. Recently, an efficient solid-phase parallel synthesis toward structural analogues of these mycotoxins has been developed by Koomen and co-workers utilizing a cyclization-release reaction as a final step (Figure 15).[59] Starting with resin-bound L-tryptophan (**66**), a 42-member combinatorial library (as mixtures of four diastereoisomers due to epimerization of the second amino acid) of indolyl diketopiperazine class of natural products was prepared by parallel synthesis technique via a Pictet−Spengler condensation, amide coupling, and cyclization reactions. The resulting R^1 and R^2 groups provide substantial structural modifications on the resulting analogues, with changes affecting the main carbon core of the natural mycotoxins. From **68**, the key final step involving simultaneous formation of the diketopiperazine ring and release of the products from the support provided final products **70** in 50−99% yields with purities ranging from 58% to 91%. The design strategy of this small bidimensional library relied heavily on the key Pictet−Spengler condensation (**66** to **67**) and the use of tryptophan as substrate. Recently, a similar solid-phase approach to the same class of natural products has been described under improved conditions, allowing the use of a larger variety of aldehydes for the Pictet−Spengler reaction.[60] Both of these routes, however, may not be readily applicable to the solid-phase synthesis of libraries of some oxygenated analogues such as the cyclotryprostatins A−D.[61]

K. Indolactams. A library of indolactam analogues was generated around the molecular framework of (−)-indolactam

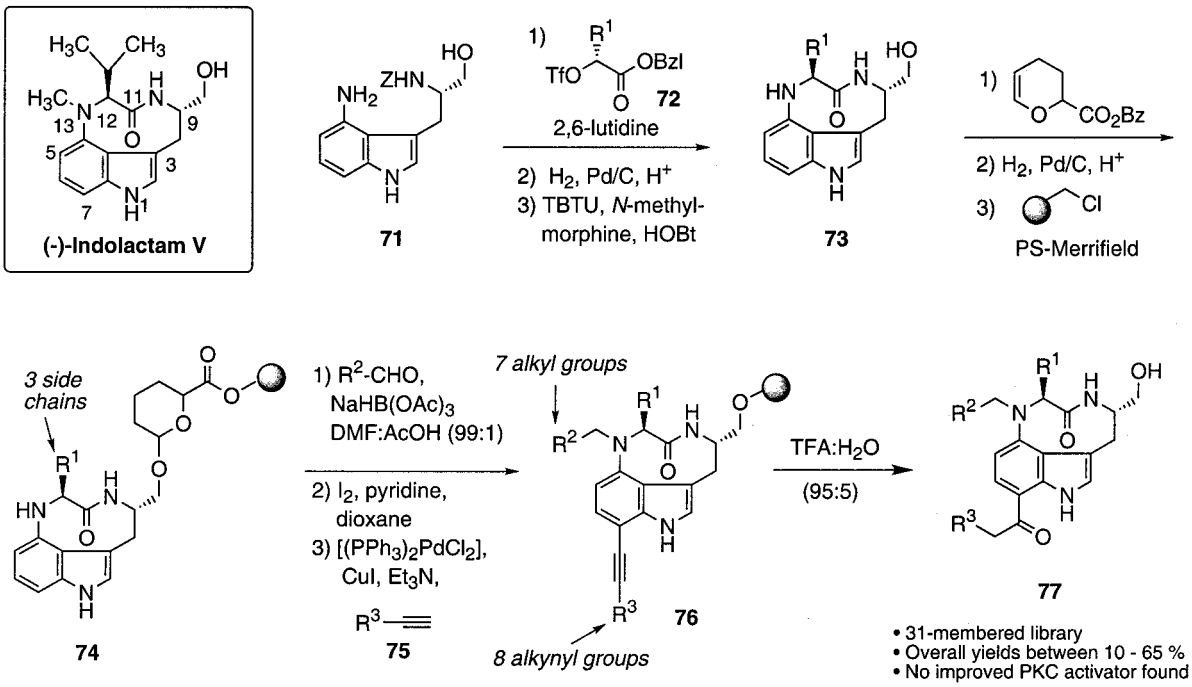

Figure 16. Indolactam library (solid-phase).[62]

V (Figure 16),[62] a known protein kinase C activator and the core structure of the tumor promoting teleocidins.[63] As described in the earlier library of Balanol analogues, protein kinase C (PKC) is an important enzyme involved in signal transduction pathways that regulate numerous normal and abnormal cellular responses.[34] Altered PKC activity has been implicated in many diseases such as cancer, asthma, and disorders of the central nervous system. Consequently, modulators of PKC activity, such as indolactam V, are considered promising leads for drug discovery. In the design of their library, Waldmann and co-workers have taken into account the influence of the substituents at C12 and N13 on the conformation of the nine-membered lactam ring (i.e., cis−trans conformation of the amide), which is known to affect the binding ability of the indolactams to PKC.[64] In addition, substituents at C7 are known to mediate cellular membrane binding of the indolactam, while the free hydroxyl at C14 is required for maintaining the biological activity.[65] The library, therefore, consists of analogues **77** wherein the substituents R^1, R^2, and R^3 at C12, C7, and N13 are diversified both in solution and on solid support using the C9 hydroxymethyl group as an anchor onto the polymeric resin (Figure 16). To this end, the 4-aminoindole **71** was prepared in solution and used as a central intermediate. Introduction of the R^1 substituent as well as formation of the lactam ring to give **73** were performed, again in solution, using three different α-hydroxy acid ester triflates **72**. Attachment of intermediates **73** to the resin was effected using a THP-based linker. This was followed by the combinatorial incorporation of the R^2 group via reductive amination of N13 on **74** with seven aldehydes, and the R^3 group via Sonogashira coupling with eight alkynes **75**, thereby affording supported indolactams **76**. Overall yields of library members **77** after cleavage from the resin ranged from 10 to 65%. To investigate if the indolactam analogues were capable of PKC activation, 11 library members were assayed for their effect on Swiss 3T3 cells. PKC activation causes phosphorylation of the major PKC substrate, MARCKS (myristoylated alanine-rich C kinase substrate), resulting in the translocation of the substrate from the membrane into the cytosol.[66] Detection of MARCKS in the cytoplasm by Western blot analyses revealed that all 11 analogues induced PKC activity, although they were less active than indolactam V itself and the phorbol ester PDB—another naturally occurring PKC activator. However, the varying activity between different indolactam library members opens the possibility for investigating new structure−function relationships. One apparent limitation to this approach is the somewhat involved screening procedure for PKC activity that may restrict the size of future indolactam libraries. Alternatively, more convenient assays could be employed.

L. Mappicine. The tetracyclic natural compounds (s)-mappicine and its ketone analogue nothapodytine are metabolites isolated, respectively, from *Mappa foetida*[67] and *Nothapodytes foetida*[68] (Figure 17). Of the two, mappicine ketone currently generates more interest as a result of its antiviral activity, in the micromolar range, against herpes (HSV) and human cytomegalovirus (HCMV).[69] Unfortunately, such levels of potency are not quite practical toward pharmaceutical applications. In addition, there is a lack of available structure−activity relationships, making the mappicine template an attractive choice of target for library generation. To this end, DeFrutos and Curran have developed an elegant parallel solution-phase strategy based on a key radical cascade annulation.[70] In fact, they employed an improved variant of a process previously applied by the same group to the related camptothecin class of antitumor agents.[71] Through convergent assembly of the polycyclic structure in only two consecutive steps at the late stage of a total synthesis approach, the authors designed their library from three simple building blocks, each bringing one diversity element. This strategy helps minimizing purification steps

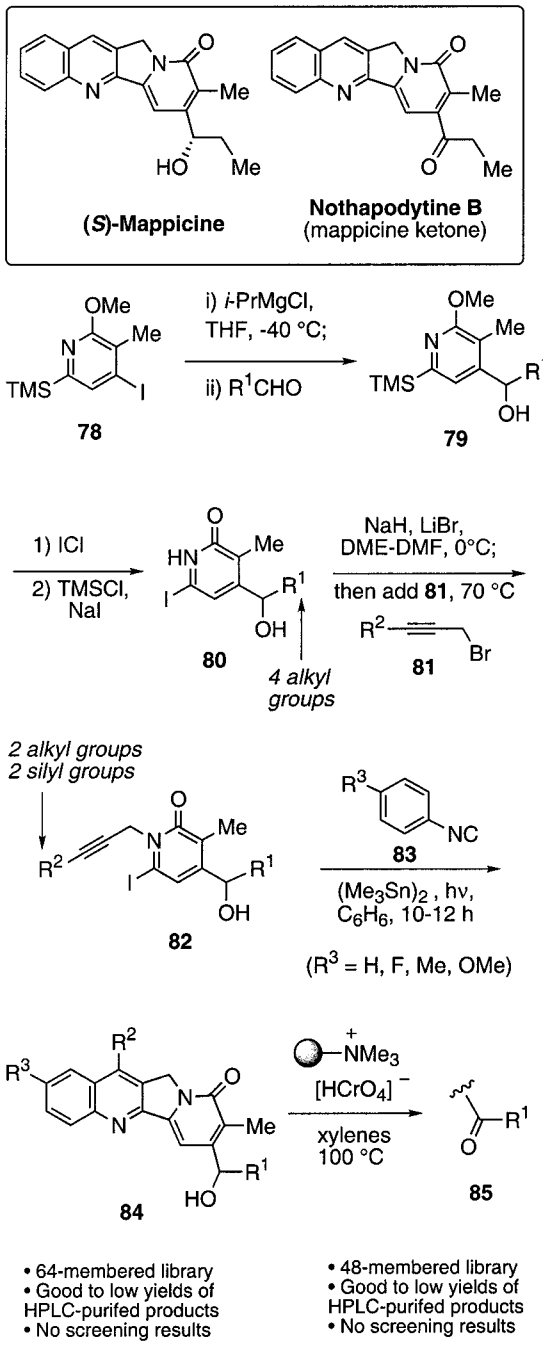

Figure 17. Mappicine-based library (solution).[70]

and is thus particularly favorable for automation. A subset of 16 iodopyridones **82** were prepared independently by the N-alkylation of four precursors **80** with four propargylic bromides **81** (Figure 17). The benzylic alcohol unit in iodopyridones **80** was established by Grignard reagent formation from **78** and addition to four aldehydes to give **79**. In the second diversity generating operation, solutions of each of the 16 iodopyridones **82** were added by the robotic liquid handler to four separate isonitriles **83**, and the resulting parallel mixtures were irradiated with a sunlamp in the presence of a radical initiator. The 64 racemic mappicine analogues **84**, actually made in four libraries of 16, were purified by the robot through solid-phase extraction with prepacked silica gel cartridges, and ultimately isolated after solvent concentration in 60−80% yields and 70−90% purity.

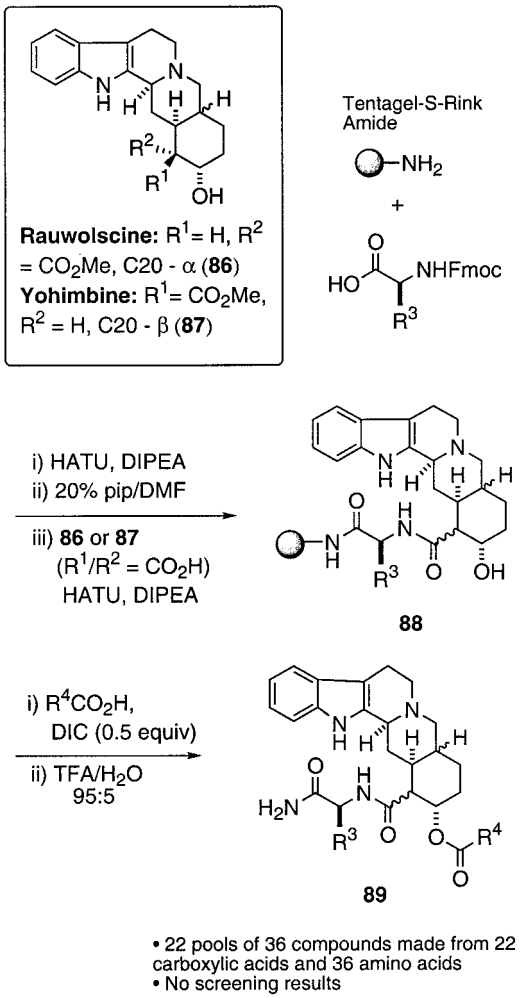

Figure 18. Rauwolfia alkaloids library (solid-phase).[72]

A major portion of these library members was further purified by serial HPLC, providing reduced yields of analogues nonetheless in quantities and purity potentially suitable for biological screening. Using the library-from-library concept,[55] 48 mappicine analogues **84** were oxidized with a supported chromate reagent, providing the corresponding mappicine ketones **85** after automated HPLC purification. No biological screening was reported for any of these libraries.

M. Rauwolfia Alkaloids (Rauwolscine and Yohimbine). An early demonstration of combinatorial modifications to an existing natural product template was illustrated in the derivatization of the *Rauwolfia* alkaloids yohimbine (**86**) and rauwolscine (**87**) (Figure 18).[72] These alkaloids have known activity as antihypertensive agents, antiarrythmics, and as adrenoceptor antagonists,[73] making them attractive targets for library synthesis. These particular alkaloids were good candidates for combinatorial derivatization by satisfying the following criteria: (1) being commercially available in large quantities, allowing for the optimization of reactions prior to library synthesis; (2) containing a point of attachment onto the solid support, which can be accomplished via the E-ring ester after conversion to the acid; (3) containing functionality within the core skeleton which can be modified combinatorially; and (4) being stable to the reaction conditions that will be employed during library synthesis. The E-ring substituents on both yohimbine and rauwolscine were modi-

fied by using various resin-bound α-amino acids to load the core scaffold by carboxylate derivatization (Figure 18). The resulting intermediates **88** were derivatized with a range of carboxylic acids to acylate the secondary hydroxyl group on solid support. Split-pool libraries were made with each alkaloid using 36 amino acids and 22 carboxylic acids to afford, after resin cleavage, 792 analogues of type **89**. The library of yohimbine analogues was left unencoded. Thus, to simplify library analysis and eventual screening, the second pooling step was avoided, affording 22 pools of 36 compounds. The presence of each of the 36 compounds in a given pool was verified by electrospray mass spectrometry, revealing all 36 corresponding molecular ions. The rauwolscine library, on the other hand, was encoded using a binary encoding strategy that uses a series of secondary amine tags attached to an orthogonally differentiated linker. This feature allows the tags to be cleaved off from an individual resin bead and then subsequently derivatized with dansyl chloride and analyzed by HPLC with fluorescence detection.[74] A limitation of this library approach based on modifying an advanced natural product scaffold, however, is that it only permits diversification at the E-ring and no other sites within the alkaloid skeletal core. On the other hand, the simplicity of this strategy makes it an attractive one for assembling natural product libraries when the core scaffold is readily available and can be easily attached to a polymeric support. Screening results for these yohimbine and rauwolscine-based libraries have not been reported yet.

N. Muscone. Muscone has no particular therapeutic virtues but it is an important chemical component in the perfume industry and is used in a large number of fine cosmetic products. A small library of racemic muscone analogues has been generated by Nicolaou and co-workers using a solid-phase approach and an elegant cyclorelease strategy that takes advantage of an intramolecular ketophosphonate-aldehyde condensation (Horner−Emmons−Wadsworth reaction) (Figure 19).[75] A novel phosphonate-functionalized support (**91**) was used for the attachment of long alkenoate chains **90**. The formation of β-ketophosphonates **92** was followed by olefin cross metathesis with two different alkenols, then oxidation to aldehydes **93**, and final cyclization and simultaneous release of the intermediates **94** from the solid support by means of intramolecular HEW reaction. The final steps involved parallel solution-phase transformations using organocuprate conjugate addition on the enone of **94**, followed by hydrogenation, to yield (DL)-muscone and its modified analogues **95**. The molecular diversification was reflected in the R^1, R^2, and R^3 moieties. The synthesis of the prototypical library of 12 members was accomplished using SMART/REC technology and a sort-pool strategy.[48] Aside from the advantages of the cyclorease strategy mentioned above, in the current macrocyclization the polymeric support creates a very dilute reactant environment resulting in the absence of dimers and other higher order macrocyclic impurities. Conceptually, this work shows that libraries of analogues of macrocyclic natural products can be made successfully by solid-phase methods.

O. Steroids. In view of their widespread use as pharmaceutical drugs there is no doubt that steroidal compounds

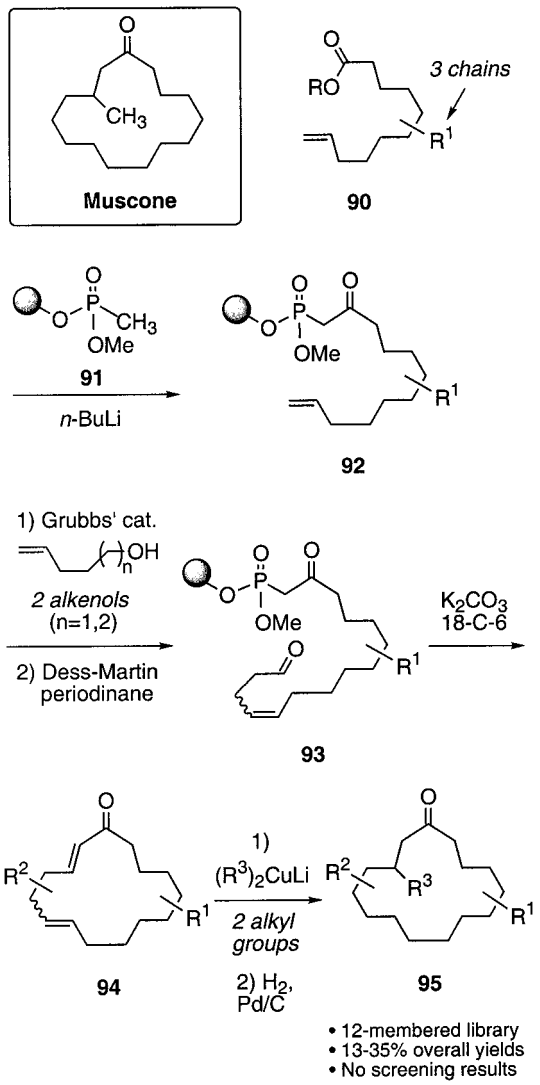

Figure 19. Muscone-based library (solid-phase).[75]

constitute a classical example of privileged natural products. The range of biological activity demonstrated by steroids is extensive, and as a result a large number of derivatives, whether natural or synthetic ones, are currently employed in several therapeutic treatments including antiinflammatory and antihormone applications. Although they were not inspired by natural steroids as leads, but rather by closely related synthetic analogues, Poirier and co-workers have designed several solution- and solid-phase sequences to diversify steroid-based templates by hemisynthetic strategies. For instance, a model solid-phase library of 7α-alkylamide estradiol derivatives was developed as a mean to discover potential antagonists of estrogen receptors.[76] In a more recent account from that laboratory, solid-phase protocols using simple but efficient chemistries were optimized in order to access libraries of analogues A−D (Figure 20).[77] These structures were chosen as a result of previous SAR studies. Whereas analogues of type B and C target steroid sulfatase enzyme, analogues A were designed as potential inhibitors of type III 17β-hydroxysteroid dehydrogenase (17β-HSD). Indeed, synthetic 3α-hydroxy-androstane analogues with hydrophobic 3β-alkyl-substituents have been identified to act as potent inhibitors of type III 17β-HSD with IC_{50} values as low as 57−147 nM.[78] As a proof of concept toward eventual

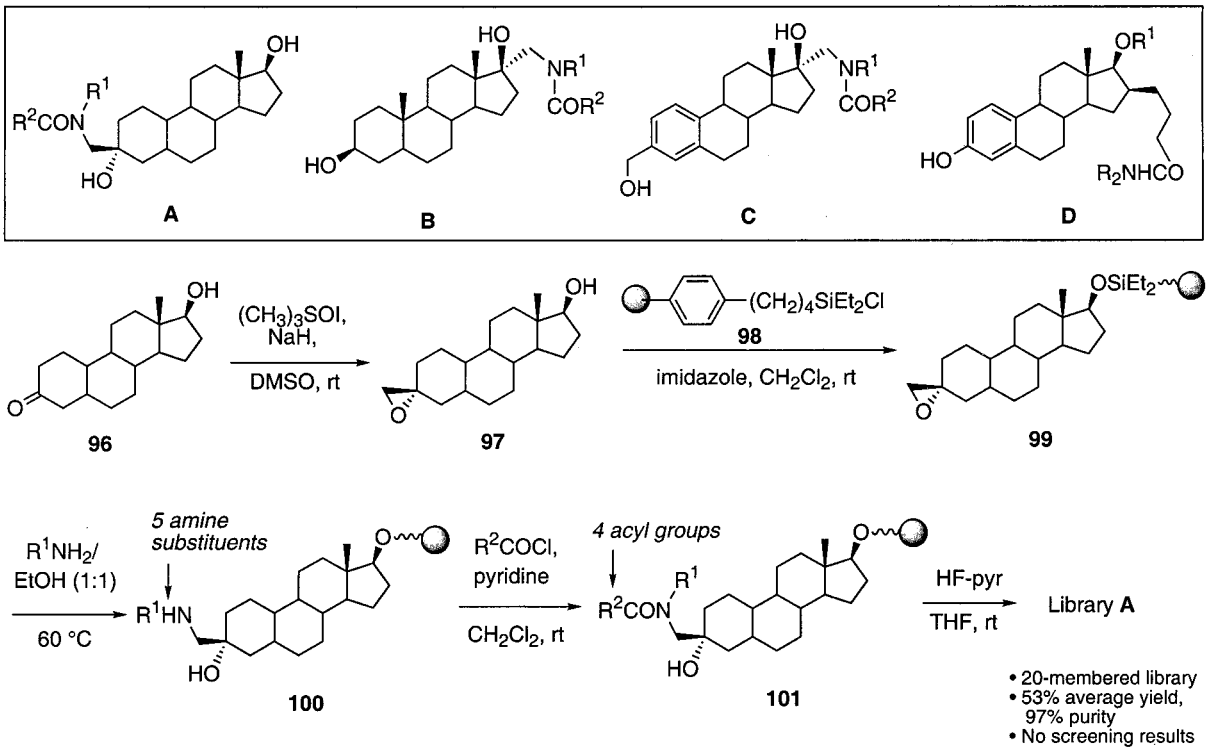

Figure 20. Steroidal libraries (solution- and solid-phase).[77]

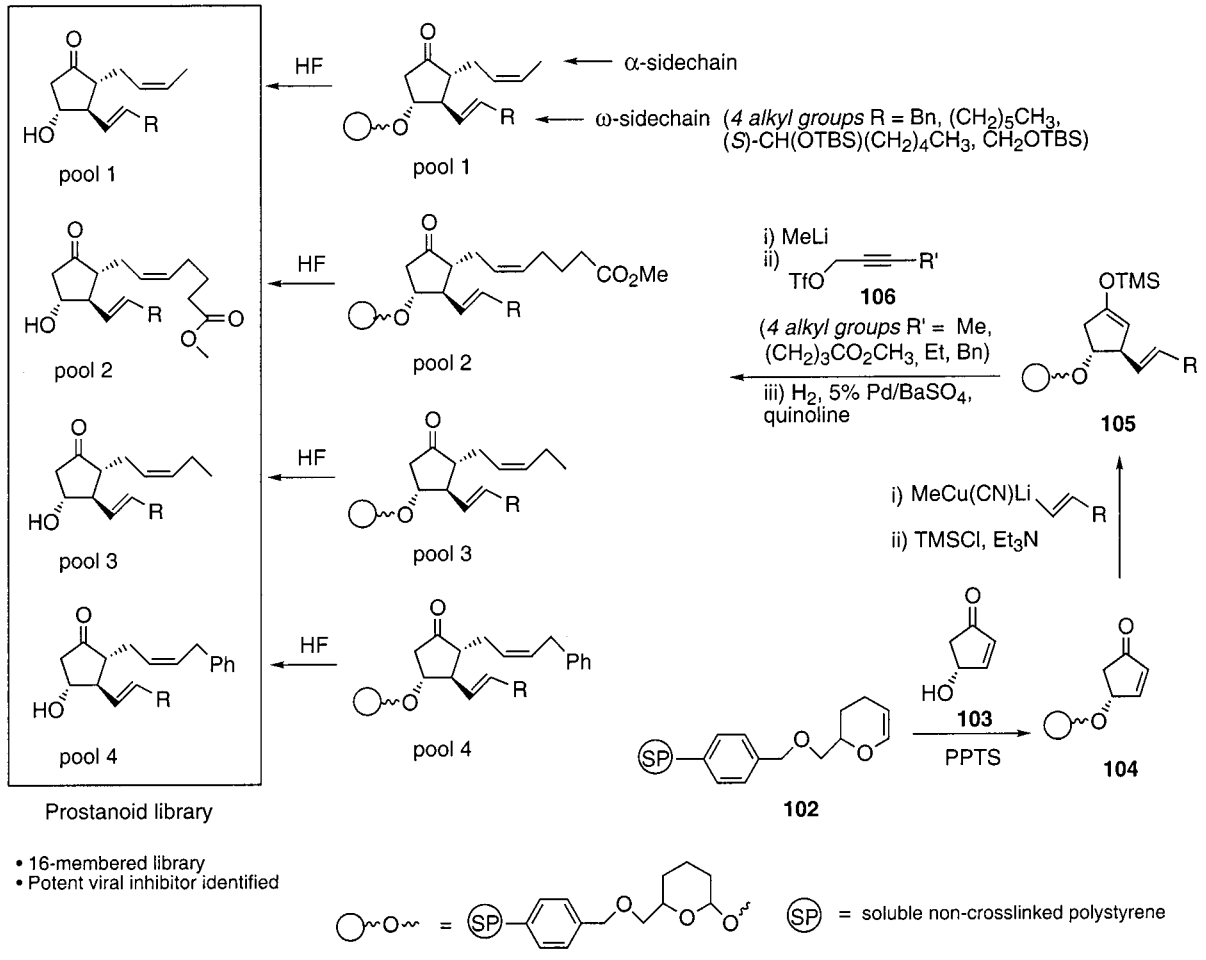

Figure 21. Prostanoid library (soluble polymer support).[85]

elaboration of large parallel libraries, a small prototypical library of 20 analogues of type A was prepared by a solid-phase synthetic strategy similar to an earlier solution-phase approach (Figure 20).[79] Dihydrotestosterone (**96**) was first

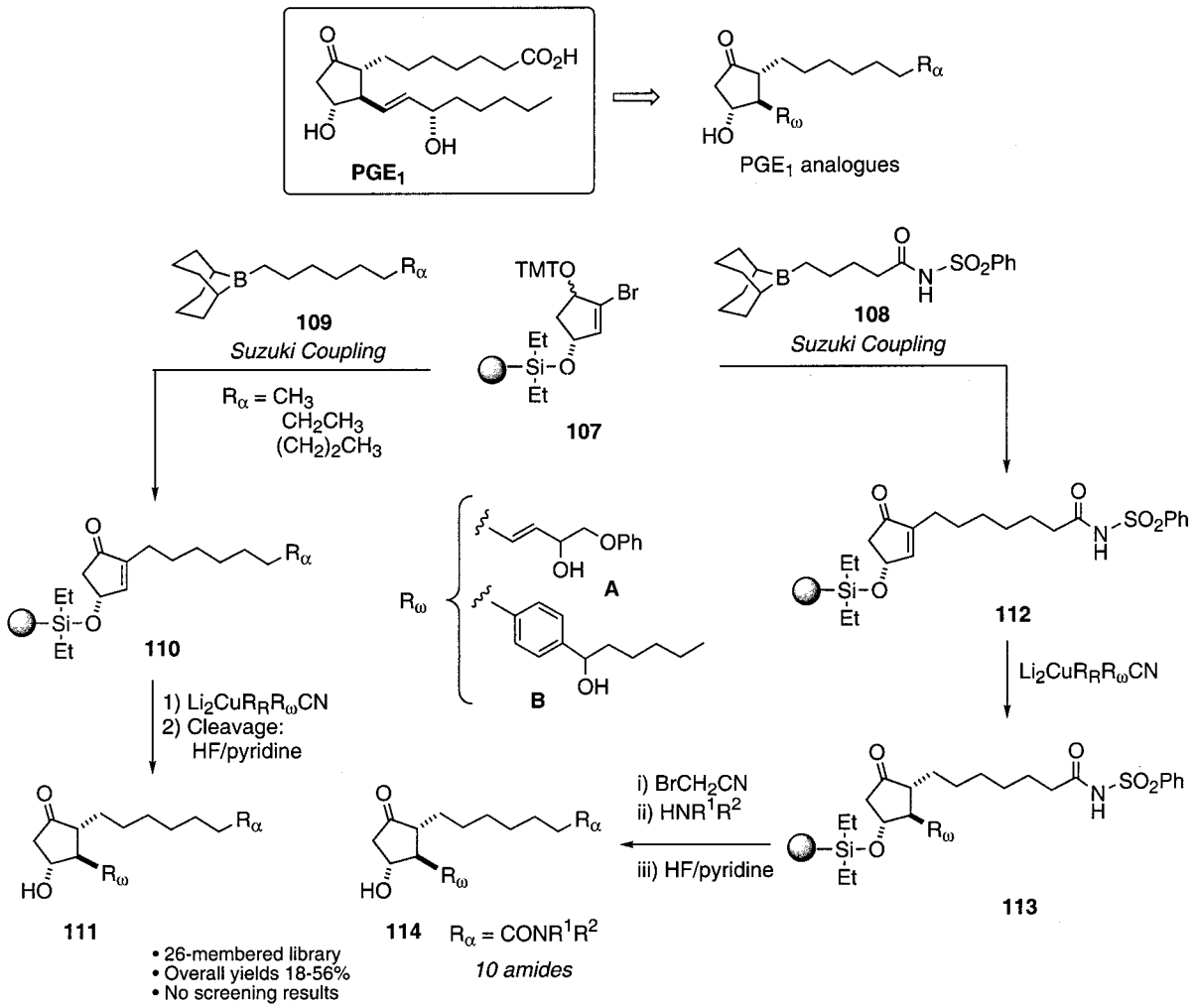

Figure 22. Prostanoid library (solid-phase).[89,90]

transformed into the sensitive α-oxirane derivative **97** using a known transformation.[80] The latter was then safely loaded through the 17β-hydroxy functionality onto resin **98**[81] (activated by reaction of diethylsilane-derivatized polystyrene with 1,3-dichloro-5,5-dimethylhydantoin). The first level of diversity was generated by aminolysis of supported epoxide **99** with four different primary amines. The resulting secondary amines of intermediates **100** were each acylated with five acid chlorides to give amides **101** which provided the bidimensional library **A** in 53% average overall yield and 97% purity after cleavage from the resin. Only building blocks with hydrophobic side chains were employed at both positions. No screening results have been disclosed yet for this particular library. Steroid derivatives have also been employed as templates for the generation of libraries of synthetic peptide-based receptors for small peptides[82] and as general scaffolds for combinatorial chemistry.[83]

P. Prostanoids. The natural prostaglandins are extremely potent mammalian natural products which take part in inflammation, tissue repair, immune response, and many other physiological processes.[84] Recently, the synthesis of a small prostaglandin library and its evaluation for inhibition of cytomegalovirus (CMV) have been accomplished by Janda and co-workers in order to find improved analogues.[85] The authors utilized a "parallel-pool" strategy combining split-pool and parallel synthesis techniques to construct a 16-member prostaglandin library. This was achieved on a soluble polymeric support by means of Noyori's three-component convergent methodology (Figure 21), which had been previously demonstrated by the same group in the total synthesis of prostaglandin E methyl ester.[86] Herein, the same type of support was used.[87] It is derived from non-cross-linked chloromethylated polystyrene with a tetrahydropyranyl linker and possesses excellent solubility properties (soluble in THF, CH_2Cl_2, $CHCl_3$, and EtOAc, and insoluble in water and MeOH from which it can be precipitated for filtration purposes). These properties allowed reaction and product analysis to be performed via standard techniques used in solution-phase chemistry. This way, several advantages of homogeneous methods (high reactivity, lack of diffusion problem, and ease of characterization) are merged with those of heterogeneous solid-supported chemistry (utilization of excess reagents and easy separation of products by precipitation of the polymer-bound products). The functionalized cyclopentenone core **103** was linked to THP resin linker **102**, followed by assembly of ω- and α-side chains by the stereoselective addition of four diverse cuprate reagents to a resin-bound cyclopentenone (**104**). This was followed by alkylation of the resulting silyl enol **105** with the appropriate propargylic triflates **106** and alkyne cis-hydrogenation. These operations led to a library of 16 prostaglandin derivatives distributed in four pools, each containing a constant α-side

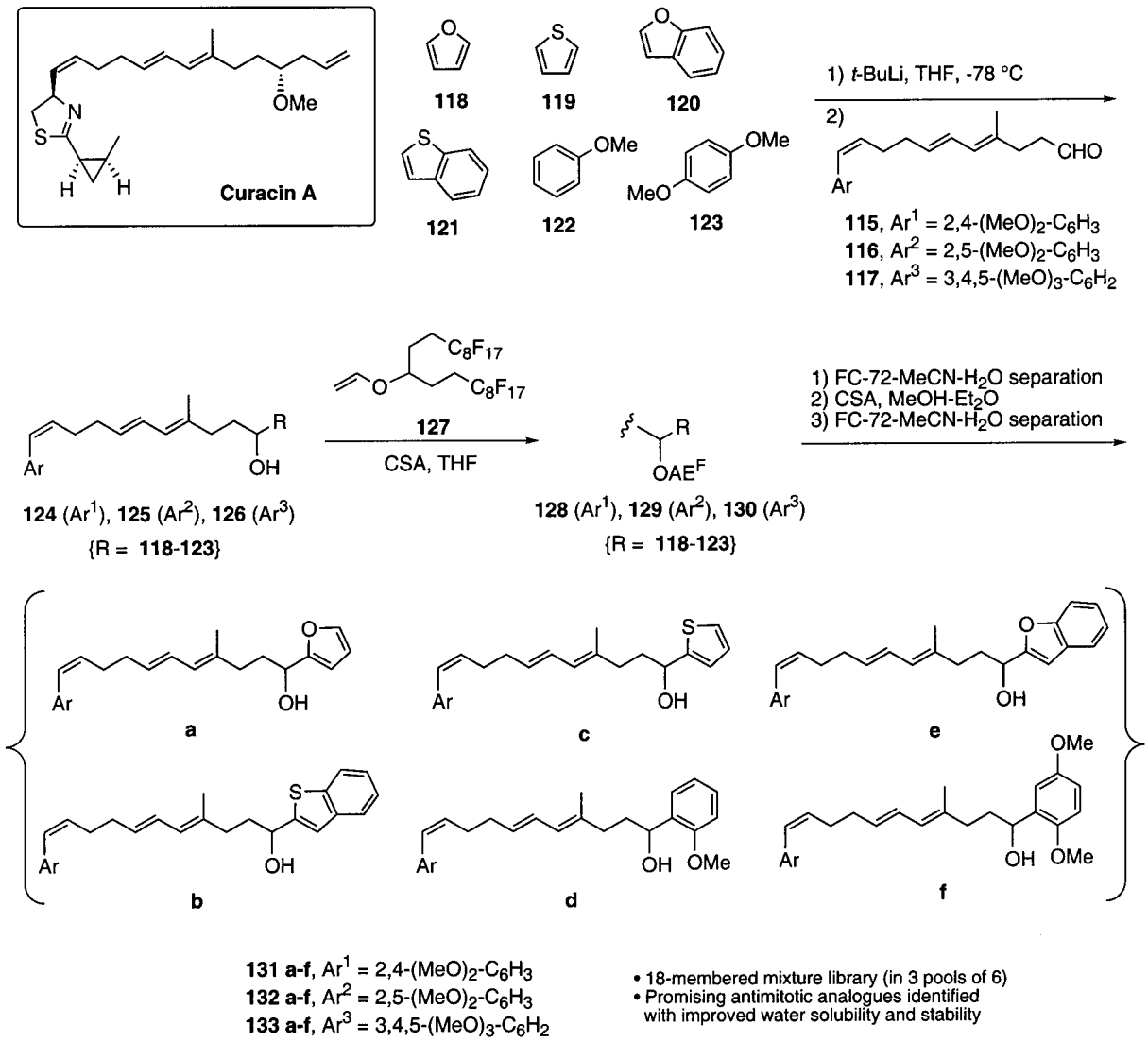

Figure 23. Curacin-based library (solution).[95]

chain with four different ω-side chains (desilylated). These pools were screened for inhibition of murine CMV growth in NIH 3T3 cells, and pool 1 was found to induce a significant inhibition of viral titer. Subsequently, each member of this pool was individually prepared and examined for antiviral activity. The prostaglandin analogue with ω-chain R = $(CH_2)_5CH_3$ proved to be the most potent and promising compound (leftover viral activity: 2%). These findings are highly significant because clinically available antiviral agents are not only limited but also quite inefficacious. It is anticipated that a larger second-generation library of prostaglandins can be constructed by the "parallel-pool" approach and screened for other targets to find even more effective lead compounds as antiviral agents. Recently, the same group has also reported a similar synthetic route to cyclohexane-based analogues.[88]

Using their previous solid-phase synthetic approach to diverse E- and F-series prostaglandins,[89] Ellman and coworkers have used a standard insoluble polymeric support for generating analogues by rapid parallel synthesis.[90] Twenty-six PGE_1 analogues were prepared to target the TA202 mutant of the prostaglandin EP_3 receptor.[91] The discovery of highly active prostaglandins toward the TA202 mutant might enable researchers to probe it selectively in animal studies without activation of other receptors, thus providing an excellent tool in determining the physiological role of the EP_3 receptor. Although all naturally occurring prostaglandins contain a C1 carboxylic acid on the α-chain, previous QSAR studies with the TA202 mutant EP_3 have revealed that the binding affinity to their methyl esters was comparable to the free acids.[91] Thus the library was made with the α-side chains that were either aliphatic without the carboxylic acid or with an amide that would be more biostable that the corresponding ester derivative (Figure 22). Incorporation of the aliphatic α-chain onto the core scaffold **107**, to give cyclopentenones **110**, was achieved by a Suzuki cross-coupling with three different alkylboranes **109**. The latter were prepared in situ via the hydroboration of the corresponding alkenes with 9-BBN. For the lower ω-side chain, two types of side chains, **A** and **B**, were used in the library. The ω-side chain **A** is known to exhibit some selectivity toward EP_3, while the ω-side chain **B** has shown activity toward EP_2, making it potentially useful in binding studies against mutants of this receptor. Both ω-chains were inserted via the conjugate addition by higher order cuprates onto the enone **110**. Cleavage of the prostanoid from the

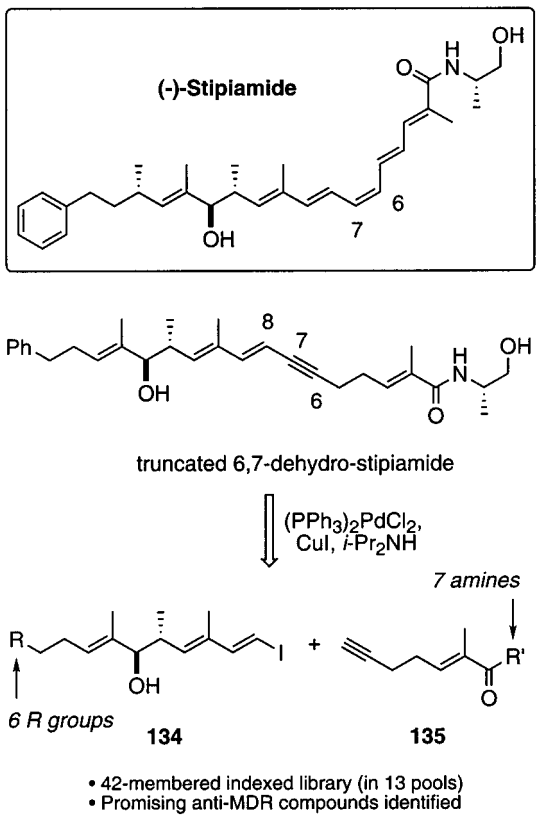

Figure 24. Stipiamide-based library (solution).[98]

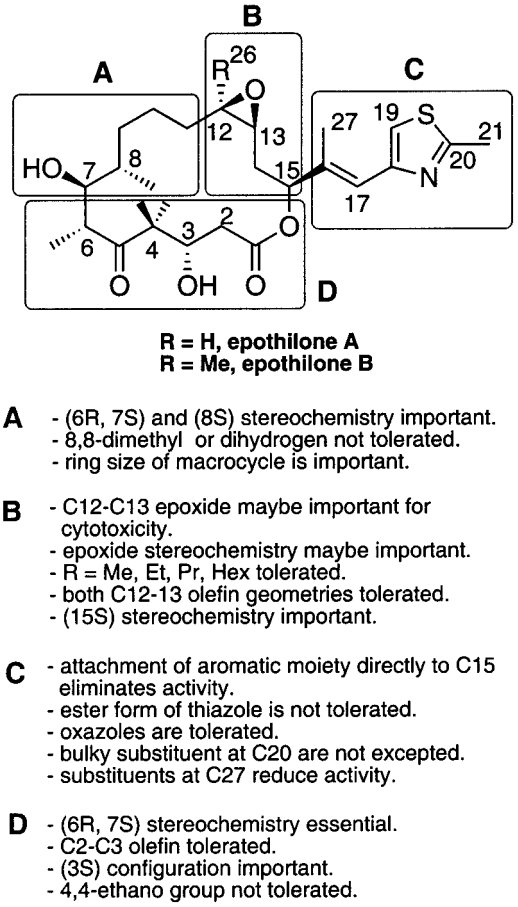

Figure 25. Structure−activity relationships for the epothilones.

triethylsilane linker was effected by exposure to HF/pyridine, affording six trans-substituted products **111** in high selectivity. Synthesis of the prostanoids with the amide α-side chain began with the Suzuki coupling of the 9-BBN-adduct of the *N*-acylsulfonamide chain **108** to vinylic bromide **107**. After organocopper conjugate addition of the *ω*-chains (**A** and **B**) onto the enone of resulting coupling product **112**, the two supported *N*-acylsulfonamide products **113** were activated for displacement by *N*-cyanomethylation. They were then treated independently with 10 diverse amines to give 20 supported amide products which were cleaved off from the resin to provide prostanoids **114**. Overall yields of the 26 PGE_1 analogues **111** and **114** ranged from 18 to 56%. All solid-phase reactions described above were performed on a semiautomated synthesizer to expedite library synthesis. Screening results of this PGE_1 library against EP_3 mutant receptors have yet to be reported. This work, along with the work of Janda and co-workers, illustrates how libraries of natural products with significant changes in the carbon skeleton can be synthesized on polymeric support by the efficient use of organometallic reagents and building blocks.

Q. Curacin. The marine natural product curacin A (Figure 23), an antimitotic agent isolated in 1994 from *Lyngbya majuscula*,[92] was found to promote arrest of the cell cycle at the G2/M checkpoint.[93] Like taxotere, sarcodictyins, and colchicine, curacin A inhibits tubulin polymerization. In fact, it inhibits binding of colchicine (a well-known tubulin binding molecule) in a competitive fashion, thus it most likely binds to the same site of tubulin.[93] According to the few SAR studies on analogues of curacin A, even subtle structural changes can lead to a drastic reduction in the compound's activity.[94] To fulfill tight requirements toward pharmaceutical development, there remains a need for the development of active analogues showing improved bioavailability and chemical stability as compared to the natural lead. The thiazoline moiety of curacin A is the major cause of the molecule's instability. Thus, in the design of their library,[95] Wipf, Day, and co-workers chose to replace this unit for stable electron-rich arenes which are reminescent of the trimethoxyphenyl ring A of colchicine. Thus, three key building blocks, **115**−**117**, with the isolated *cis*-alkene were made by way of a Wittig reaction between the corresponding benzaldehydes Ar^{1-3}-CHO and a common alkylphosphonium precursor (Figure 23). While keeping the crucial diene unit, the plan was to diversify the opposite end containing the homoallylic ether termini into a subset of hydrophobic benzylic alcohols. The library was prepared in three mixtures of six compounds by reacting the three aldehydes **115**−**117** separately with an equimolar mixture (3 equiv each) of lithiated arenes **118**−**123** in solution phase. The crude mixtures of benzylic alcohol products **124**−**126** were purified from residual arenes by attachment of a "fluorous tag".[96] To this end, vinyl ether **127** was reacted with mixtures **124**−**126** under catalytic acid conditions to give, respectively, **128**−**130**, and after simple liquid−liquid extractions with perfluorinated solvent FC-72, followed by hydrolysis and elimination of the fluorinated acetal, pure final mixtures **131**−**133** were obtained. This straightforward protocol using fluorous-phase chemistry facilitates product purification by allowing elimination of all nonfluorous impurities.[97] The fluorous solvent employed is immiscible with water and most

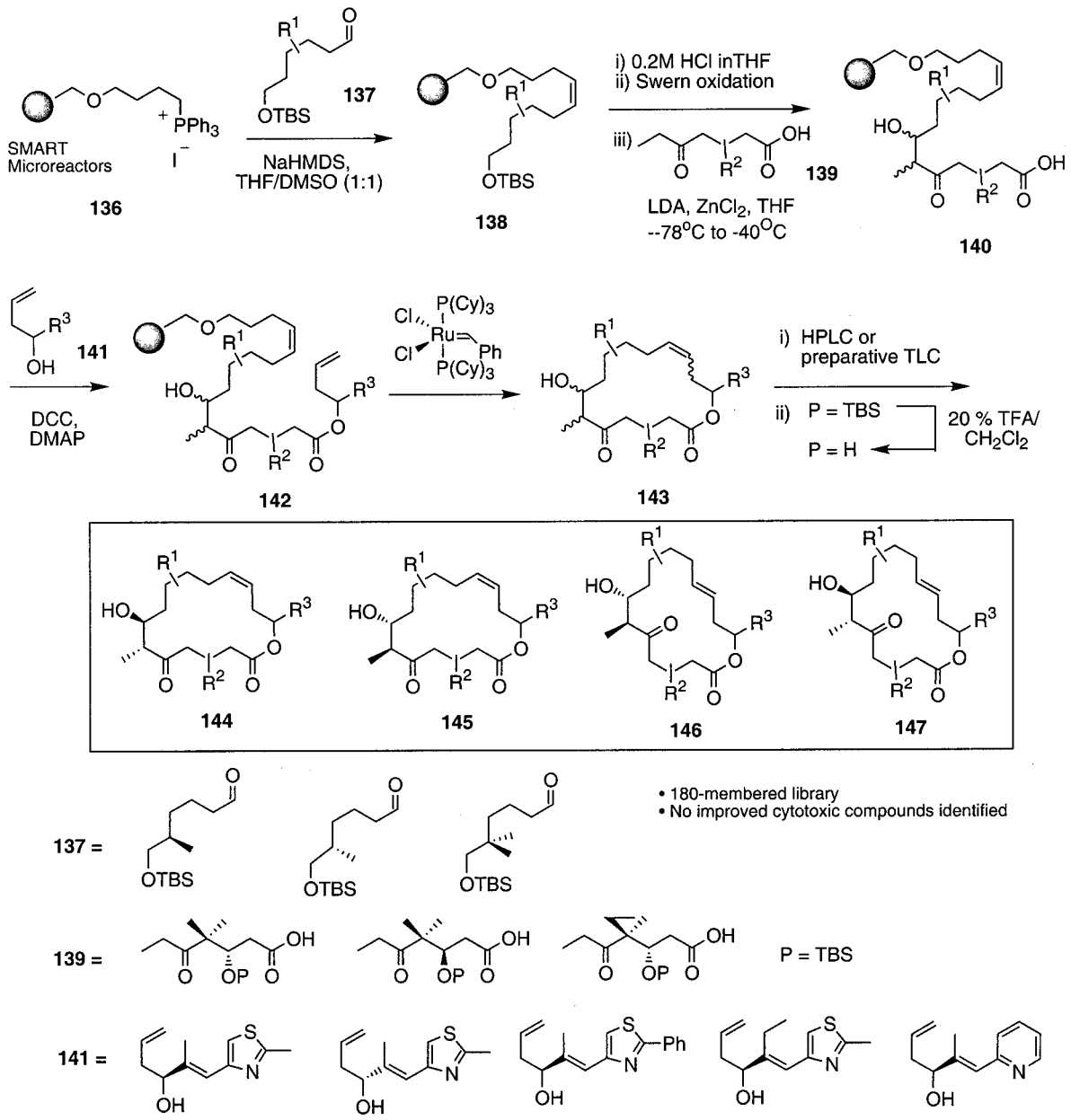

Figure 26. Epothilone-based library (solid-phase).[101]

organic solvents, and it solubilizes only these compounds which possess a fluorous tag. One mixture library, **133**, showed significant inhibition of [^{3}H]colchinine binding to tubulin. This mixture also inhibited cell proliferation at submicromolar concentration. The six components **133a**−**f** were resynthesized individually in order to determine their specific potencies. Their biological evaluation successfully validated the composite data from the mixture library and revealed the high potency of compounds **133a**−**d** at altering the cell cycle in living human carcinoma cells. Through several other assays, two compounds, **133c** and **133e**, emerged as the most promising analogues. They both showed inhibition of tubulin polymerization with an IC_{50} of ca. 1 μM, an average growth inhibition activity GI_{50} of ca. 250 nM, inhibition of [^{3}H]colchicine binding to tubulin, and were found to block mitotic progression at nanomolar concentrations. Overall, the bioactivity of these analogues rivals that of curacin A although they possess the added benefits of superior water solubility and stability as compared to the natural lead. In addition, this work also provides evidence that the use of a soluble mixture library approach can be highly advantageous.

R. Polyenes (Stipiamide). An interesting example on the potential applications of mixture-based solution-phase libraries was reported by Andrus and co-workers.[98] These authors had previously identified a simplified analogue of (−)-stipiamide with improved multidrug resistance (MDR) reversing properties and much reduced toxicity. This truncated 6,7-dehydro derivative (Figure 24) is potent (ED_{50} 4 μM) with a variety of drugs with resistant human breast cancer MCF7-adrR cells and was found to bind to the P-glycoprotein expressed at the surface of these cells.[99] There is no crystal structure or other information available on the requirement for PGP binding. Therefore, a stipiamide-based template constitutes a good choice for the design of a combinatorial library. The two end sites were chosen as diversification points so as to facilitate the synthesis of mixture libraries using preformed vinyl iodide and amide

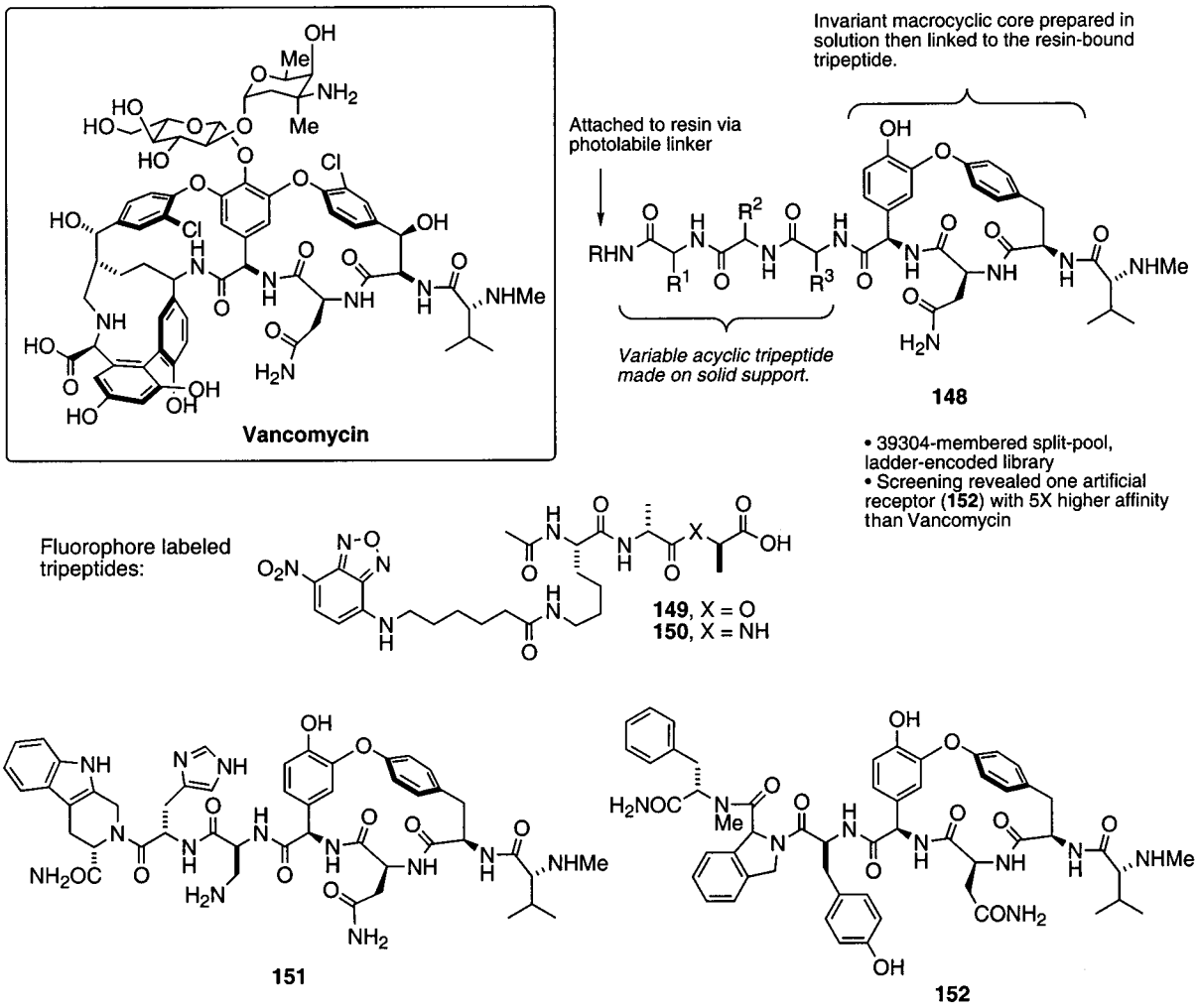

Figure 27. Vancomycin-based receptor library (solid-phase).[102]

fragments **134** and **135** (Figure 24). The chemistry employed to make the more complex fragment **134** is rather lengthy and time limiting. As a result, the library is relatively small but the diverse end groups were selected to give a representative range of structures with polar and apolar functionalities. The authors choose to assemble the two sublibraries of components by formation of the C8−C7 bond via a Sonogashira cross-coupling reaction. This way, similar coupling rates between the two sublibraries were assured. Library assembly was effected through an indexed manner in which each of the six left side fragments were coupled with an equimolar mixture of amide fragments. Similarly, each of the seven amide fragment were kept constant and coupled with an equimolar mixture of left-end fragments. Thus, 42 compounds were accessed in only 13 coupling operations, and each compound is encountered in only two of the 13 pools, allowing facile identification of hits from the matching activity of individual pools. This feature helps minimize the risks of losing a potent compound diluted in a pool containing much weaker ones. The 13 pools, made on a scale sufficient to allow multiple MDR assays and eventual isolation of hits, were screened using adriamycin resistant MCF-7adr cells, with added adriamycin. Discrete hits are assumed to arise where active pools overlap. The most active pools (ED_{50} = 1.3 μM) contained compounds with R = naphthyl, and in the amide dimension (R′ group) the hydroxy amide ((*S*)-alaninol) and morpholine. Individual compounds were isolated by radial chromatography and tested as before. As expected, library members with R = Ph or naphthyl, and R′ = alaninol were among the most active along with a few other promising ones. The role of both polyene end groups and the interaction of the new MDR agents with Pgp were evidenced by ATPase and photoaffinity displacement assays. This study confirms the viability of indexed mixture library approaches as applied to a complex natural product based template.

S. Epothilones. Epothilones A and B, isolated from myxobacteria, are the latest in the line of highly potent anticancer agents. They have generated a considerable amount of excitement due to their extraordinary potency over multidrug resistant tumor cells.[100] As with paclitaxel, these macrolactones cause cancer cell death by inducing tubulin polymerization into microtubules and by microtubule stabilization, thus preventing cell division. However, their most impressive feature is their 2000 to 5000-fold higher potency over paclitaxel against a number of multidrug resistant cells. For these reasons, there have been extensive studies in their biology and chemical synthesis.[100] The synthesis of epothilone analogues has helped improve the understanding of the structure−activity relationships which are summarized in Figure 25. Although hundreds of analogues have been prepared, mostly using solution-phase chemistry, one par-

ticular library of analogues worth mentioning in the context of this review is the library synthesized by Nicolaou and co-workers (Figure 26).[101] It is, to date, the only epothilone library made using solid-phase combinatorial techniques and is itself an elegant illustration of natural product synthesis on solid support.[4] The split-pool library was synthesized using building blocks designed to help probe new structure−activity relationships by providing the various functionalities and stereochemistries that may influence the molecule's anticancer potency. Library synthesis began with a phosphonium salt resin (**136**) contained inside a SMART microreactor. In each microreactor was assembled a macrolactone precursor **142** via Wittig reaction with one of the three aldehydes **137**, followed by an aldol reaction involving one of the three dianions of various ketoacids **139**, and then the esterification with either one of the five homoallylic alcohols **141**. Ring closing metathesis on the resulting intermediates **142** was employed to simultaneously close the ring by olefination and release the products **143** from the resin. Within each microreactor was obtained a mixture of four diastereomeric 12,13-desoxyepothilones A analogues **144**−**147** which were separated by chromatography and then epoxidized in solution to deliver the final epothilone library. The library was subjected to both tubulin polymerization assays as well as cytotoxicity assays against breast cancer cells (MCF7 cell line) and paclitaxel-resistant ovarian cells (1A9PTX10 and 1A9PTX22 cells). Although no synthetic analogue from this library exceeded the activity of epothilone B, the more potent of the natural epothilones, the results add to previous studies regarding the structural requirements for biological activity (Figure 25). On the standpoint of synthetic design, this library demonstrates the feasibility of a split-pool, solid-phase total synthesis approach to rapidly assemble complex building blocks, prepared earlier in solution, into highly diverse libraries of a complex natural product.

T. Vancomycin. The increasing number of vancomycin-resistant bacteria has drawn several researchers including Ellman and co-workers into developing synthetic antibacterials based on some of the key elements of this potent antibiotic (Figure 27).[102] The mode of action of vancomycin is the inhibition of peptidoglycan biosynthesis in the bacterial cell wall by binding to the terminal peptide sequence L-Lys-D-Ala-D-Ala, the precursor to peptidoglycan cross-linking. Vancomycin resistance arises from a mutation of the normal tripeptide sequence to L-Lys-D-Ala-D-Lac, where the terminal D-alanine is replaced by D-lactate.[103] A combinatorial approach to synthetic receptor molecules (**148**) targeting vancomycin-resistant bacteria was therefore designed to bind to the mutated sequence L-Lys-D-Ala-D-Lac (Figure 27). The right-hand binding pocket of vancomycin was preserved to retain key hydrogen bonding sites and hydrophobic interactions[104] while the left-hand side was replaced with a variable tripeptide unit. The latter was left acyclic to allow free rotation and to prevent an unfavorable electrostatic interaction between the phenylalanine carbonyl of vancomycin and the oxygen of the lactate group. The invariant macrocyclic core was prepared in solution, and to simplify its synthesis the vancomycin E-ring chlorine and the sugar moiety were omitted. Previous binding studies on analogues lacking these

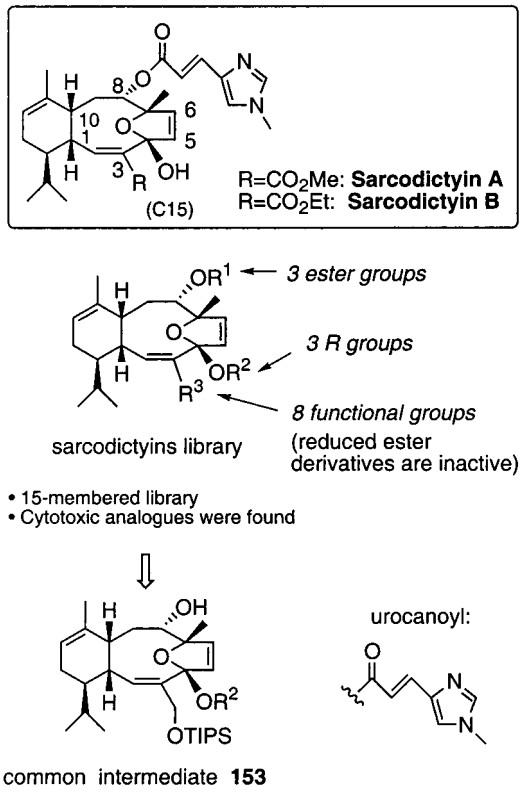

Figure 28. Sarcodictyin-based library (solution).[109]

groups have indicated that they contribute less than 2- to 3-fold in binding of vancomycin to L-Lys-D-Ala-D-Ala.[105] A split-pool library of 39 304 receptors **148** from 34 amino acid building blocks was then prepared on solid support using a ladder encoding technique and was followed by attachment of the macrocyclic template. The amino acids chosen for the tripeptide unit were based on the side chains displayed in the proteinogenic amino acids and on their degree of rigidity which they can give to the tripeptide. The resin bound library was then screened against both *N*-Ac_2-L-Lys-D-Ala-D-Ala (**150**) and *N*-Ac_2-L-Lys-D-Ala-D-Lac (**149**) labeled with the fluorophore nitrobenzodioxazole. The identity of an active receptor on a resin bead was determined by mass ladder sequencing using matrix-assisted laser desorption ionization mass spectrometry (MALDI).[106] Consensus sequences arising from the screening led to receptors **151** and **152** as candidates for further evaluation in solution. By microcalorimetry, both exhibited slightly lower binding affinity toward *N*-Ac_2-L-Lys-D-Ala-D-Ala than vancomycin, yet they showed greater binding affinity toward *N*-Ac_2-L-Lys-D-Ala-D-Lac, with **152** having approximately 5 times greater affinity. The significance of these results is that receptors less structurally complex than vancomycin can exhibit comparable and even enhanced binding affinity toward the same target. It also represents the first successful example on the identification of synthetic receptors that bind to a small molecule target in aqueous solution.

U. Sarcodictyins. The sarcodictyins (Figure 28) were isolated from Mediterranean stoloniferan coral *Sarcodictyon roseum*.[107] Their potent antitumor and Taxol-like mechanism of action involving disturbance of the tubulin-microtubulin interplay resulting in tumor cell death has been recognized.[108] Recently, the generation of a sarcodictyin-based library in

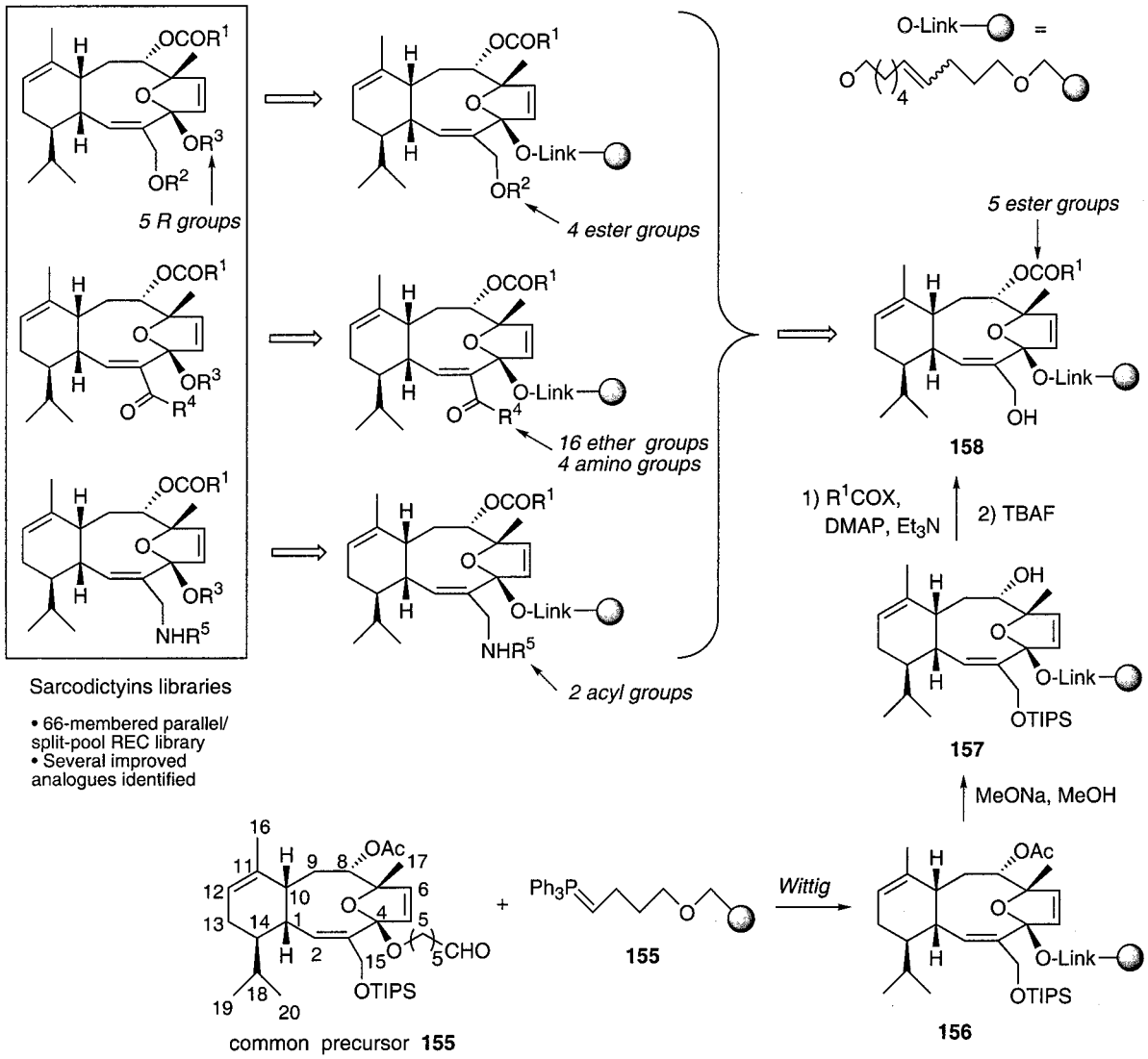

Figure 29. Sarcodictyins library (solid-phase).[111]

solution and its biological evaluation have been accomplished by Nicolaou and co-workers in order to discover analogues possessing bioactivities superior to those of these natural products.[109] To this end, they employed an improved hemisynthetic approach involving the use of more effective protecting groups and hydrogenation catalysts as compared with their first total synthesis of sarcodictyin A.[110] The three side chains of C3, C4, and C8 were diversified because they are suspected to be important functional groups to tune the biological properties (Figure 28). As a result, 15 compounds were generated via a key intermediate (**153**) by a series of standard linear transformations. Though the library was relatively small and lacks a few members (some combination of building blocks are not reported), it integrates many important structural changes. Evaluation of library members for tubulin polymerization showed that the analogue with R^1 = urocanoyl, R^2 = Et, R^3 = CO_2Me was the most active (induction of tubulin polymerization = 85%). Cytotoxicity studies with the parental ovarian carcinoma cell line 1A9 and the Taxol-resistant tumor cell lines PTX10 and PTX22 derived from 1A9 unveiled another compound (R^1 = urocanoyl, R^2 = Me, R^3 = CO_2Et) to be highest in activity (IC_{50} = 2.0 nM for 1A9, 0.6 for 1A9PTX10, and 6.0 for 1A9PTX22). Thus, very subtle structural modifications gave much improved leads in some cases. The obvious inconsistency observed between tubulin polymerization activity and cytotoxicity for several analogues might imply a different mechanism of action. These findings from a library approach confirm that the C8 and C3 side chains of sarcodictyins are indeed crucial for maintaining their bioactivity.

A combination of solid-phase and solution methods for constructing a second-generation combinatorial library of sarcodictyins containing 66 analogues has been reported by the same group (Figure 29).[111] The authors developed a solid-phase hemisynthesis in which a common precursor **155** representing the core of the natural product was attached using a Wittig olefination onto a solid support (**155**) derived from Merrifield resin. Template immobilization was followed by standard chemical manipulations leading to intermediates **158** then to a 57-membered library of sarcodictyin analogues. Both parallel and a split-pool synthesis combining REC chemistry were utilized for this library. The plan for molecular diversification involved mainly the C8 and C15 esters and the C4 ketal functionalities. This work emphasizes that there still are limitations in performing multistep total syntheses of libraries of complex targets on solid

support. Indeed, in this case, construction of the challenging sarcodictyin core had to be performed in solution and solid-phase methods were applied at a late stage. Following cleavage from the resin, all library members were screened for the induction of tubulin polymerization by the filtration colorimetric assay, and selected compounds were tested for cytotoxicity against a number of tumor cells. Several analogues exhibited superior biological activities compared to the natural sarcodictyins, although the most active analogues are the same ones identified by the authors' previous solution-phase approach described above. Further examination of these results confirmed the importance of the C8 ester side chain. Replacing the natural urocanic acid group at C8 with a number of substitutions resulted in considerable loss of biological activity. Therefore, both nitrogen atoms of the natural sarcodictyins may play a role in the mechanism of action. In contrast, ketal substitutions at C4(R^2) were well tolerated. Modifications of the C3 side chain showed that esters were preferred over amides, and reduction of the ester to the alcohol and saturated derivatives thereof was not tolerated. These conclusions regarding structure−property relationships are very valuable for further advances in this area of cancer chemotherapy.

V. Taxoids. Isolated from the stem bark of the western yew *Taxus brevifolia*, paclitaxel (Taxol) has reached a billion-dollar drug status as an anticancer agent which is particularly effective against ovarian and breast cancers.[112] However, there still are limitations for its clinical application such as the rising problem of multiple drug resistant tumor cells and formulation. To search for new analogues with improved properties, the solid-phase synthesis of a taxoid library has been reported by Xiao and co-workers using a REC strategy.[113] Advanced intermediate **159**, derived from commercial baccatin III, was chosen as the taxoid template toward a hemisynthetic approach (Figure 30). This scaffold representing the core structure of the targeted library was attached onto 2-chlorotrityl polystyrene, and the resulting resin **160** was subsequently distributed into 400 porous, SMART microreactors. The 400-membered taxoid library was constructed utilizing the split-pool technique by amidation and esterification reactions. The two secondary hydroxy groups at C2′ and C7 were randomized simultaneously with 20 carboxylic acids, while the C3′-amino group was reacted with another set of 20 carboxylic acids. These three positions were selected as diversity sites for reasons of synthetic simplicity. In addition, incorporation of amide, ester side chains, and other hydrophilic groups at these sites may modulate the biological activities of the paclitaxel analogues and increase solubility. Although biological evaluation of this library was not reported, this work featured the first taxoid library in a discrete format and in multimilligrams/member quantities, by employing the convenient REC technique.

Georg and co-workers have demonstrated an efficient method of using solution-phase combinatorial chemistry in creating paclitaxel derivatives for eventual biological screening.[114] From previous structure−activity studies it has been shown that the C13 side chain, including the C2′ hydroxyl group, are essential for preserving paclitaxel's biological

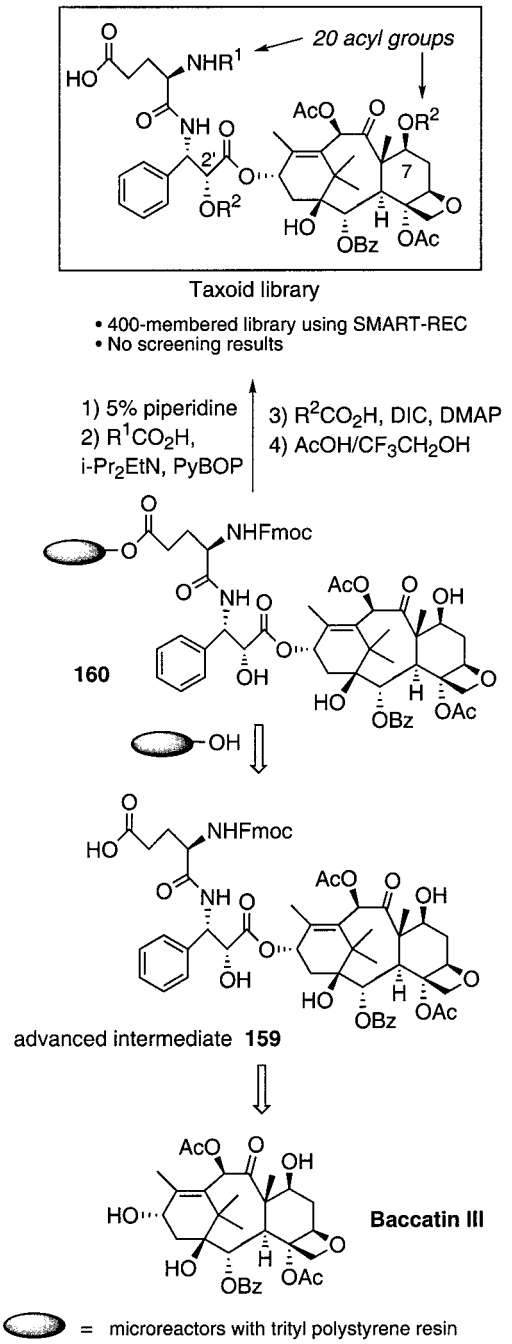

Figure 30. Taxoid library (solid-phase).[113]

activity[115] while changes at C7 and C10 are more tolerated,[116] making them good areas for modifications. In this report the authors designed a library of C7 derivatives for screening against a line of drug resistant human breast cancer cells (MCF7-R) (Figure 31). An automated synthesizer was utilized to perform a routine three-step sequence. Starting from paclitaxel as template for a hemisynthetic approach, the C2′ hydroxyl was selectively protected with a *tert*-butyldimethylsilyl (TBDMS) group, followed by the esterification of the C7 hydroxyl of **161** with 26 different carboxylic acids. Then, desilylation of **162** provided the unidimensional library **163**. In addition to performing the reactions, the synthesizer also automated both the liquid/liquid extractions and the purification through silica gel cartridges thereby eliminating many time-consuming manual

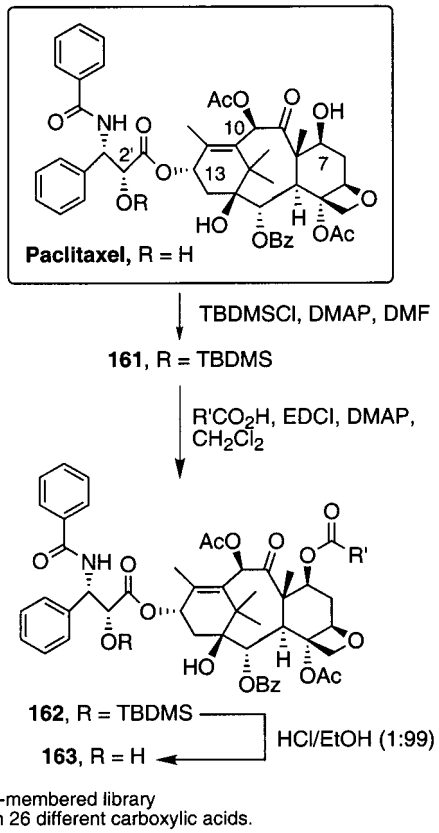

Figure 31. Taxoid library (solution).[114]

steps. The library was subjected to cytotoxicity assays against both the human breast cancer cell line MCF7 and resistant cancer cell line MCF7-R. The Taxol analogues of interest in each assay were those derivatized with acetic acid, *p*-toluic acid valeric acid, and methylthioacetic acid. While each showed approximately 10-fold-reduced cytotoxicity against MCF7 cells, they were slightly more active against MCF7-R ($ED_{50}/ED_{50\ (paclitaxel)}$ between 0.4 and 0.9).

W. Other Natural Products. A number of other libraries have been reported where a general class of natural products is targeted rather than a specific one. In a few examples, a natural product is used rather as a general scaffold for diversity generation. Sometimes, the natural product template needs to be drastically modified for use in the synthesis of directed combinatorial libraries. For example, to circumvent inherent difficulties in the construction of the core structure of the cyclic depsipeptide Hapalosin, a potent but slightly cytotoxic MDR agent, a template mimetic approach using a trifunctionalized arene scaffold was proposed.[15] In other cases, promising classes of natural products have been made on solid support but not yet reported in a library format. Just to mention a few examples, tropane derivatives have been made by solid-phase synthesis by the laboratories of Ellman[117] and Undén.[118] Solid-phase methodologies to access polyketide-type natural products have also been described.[119] Takahashi and co-workers have reported a solid-phase synthesis of the vitamin D_3-system.[120] Recently, Shair and co-workers have described a solid-phase biomimetic synthesis of tetracyclic carpanone-like molecules that could be amenable to the generation of libraries of this benzoxanthenone class of natural products.[121] An approach to the solid-phase synthesis of deglycobleomycin A_5 analogues has also been just disclosed.[122]

III. Concluding Remarks

Although they display a wide range of biological properties, natural products are not created to function specifically as drugs for human therapeutic uses. Nonetheless, the myriad of natural products isolated from various sources provides medicinal chemists with extraordinary rich molecular diversity. This review shows that combinatorial chemistry, whether performed under a solution- or solid-phase approach, can help further extend this diversity through the design of libraries from natural product templates. Moreover, it has already been demonstrated, in particular in the case of purines, curacin A, vancomycin, sarcodiyictins, and other natural product based libraries described herein, that a specific, desirable biological property of a natural product can be improved even with rather small libraries integrating simple functional group modifications. This kind of success will motivate further work in the field. Hopefully, if these results are any indication of the promise held in generating libraries with profound skeletal modifications, such as functionalization of the carbon skeleton, this approach may lead to the discovery of highly valuable drugs. Another lesson to be drawn from this review concerns the use of solid-phase synthesis to elaborate natural product based libraries. While the practical advantages of solid-phase approaches are undeniable, the assembly of complex targets by solid-phase total synthesis remains rather challenging. With no opportunity to remove impurities in the course of a multistep synthesis, the practice of solid-phase chemistry demands careful optimization of reaction selectivity, and efficiency, in order to avoid difficult problems of product purification at the end. The design of natural product based libraries may thus serve as a testing ground for the development of increasingly more efficient solid-phase chemistry techniques, methods, and supports. Clearly, combinatorial chemistry has joined the select group of research fields that finds both inspiration and challenge in natural products.

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