Methodologies for Generating Solution-Phase Combinatorial Libraries

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Received September 14, 1999

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1. Introduction

In the past 15 years combinatorial chemistry, linked to high-throughput screening techniques, has been developed into a powerful technology for the drug discovery process and has resulted in a variety of biologically active agents.[1a,b] The combinatorial strategies have also been utilized in other scientific areas such as material science, catalysts, and biosynthesis.[1c−g] A variety of solid-phase combinatorial approaches have been developed and successfully used for the generation of oligomeric and small heterocyclic libraries. Solid-phase combinatorial chemistry indeed has been widely utilized for drug discovery, especially for effective lead optimization, because the compounds attached to solid supports are easily isolated. Solution-phase combinatorial approaches have recently become of interest as an alternative drug discovery avenue for lead discovery and optimization. The key advantages of solution-phase combinatorial approaches include the following: (1) an unlimited number of reactions can be used, therefore, providing maximal structural diversity, (2) an unlimited reaction scale allows for the generation of sufficient quantities of libraries to be derived into different diverse libraries and tested in a broad range of assays, (3) large excesses of reagents and solvents, typically required in solid-phase chemistry, are not needed in solution-phase chemistry, (4) there is no need for linker manipulation, attachment to and detachment from resin; therefore, the reaction sequences for library generation are shorter, (5) soluble intermediates and final products can be obtained directly for purification and assays, (6) it is easy to develop and monitor solution-phase reactions, and (7) it is an efficient way for lead discovery and optimization from single-compound and complex libraries. The great interest of these methods is illustrated in Figure 1 by the sharp increase of the number of papers devoted to solution-phase combinatorial chemistry and methodologies published between 1994 and 1998.

Solid-phase combinatorial approaches and their progress have been extensively reviewed in recent years.[2,3] A couple of review articles focused on solid-phase strategies with very brief introduction into solution-phase combinatorial chemistry.[4] Merritt and Storer[5] reviewed their work at GlaxoWelcome as well as others' on solution-phase combinatorial chemistry. Darvas and co-workers[6] in Hungary briefly described their solution-phase combinatorial chemistry approach and provided some examples for heterocyclic library generation. Gayo[7] recently summarized the purification strategies of solution-phase combinatorial libraries. Over a hundred papers on solution-phase combinatorial methodologies, contributed from approximately 50 different pharmaceutical companies and academic research groups, have been published. The number of publications in 1998 has increased 100% compared with 1997. Because of the potential advantages of solution-phase combinatorial methodologies over solid-phase approaches, it is likely to become more popular for drug discovery. We

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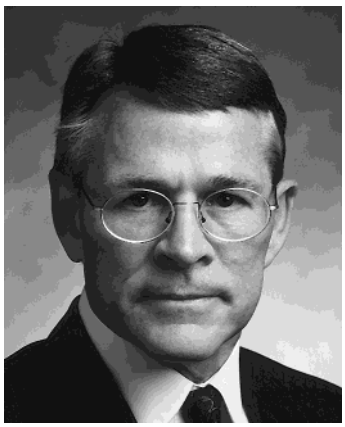
10.1021/cr990014r CCC: $35.00
Published on Web 07/20/2000

Haoyun An received his Ph.D. degree in 1992 with Professor Jerald S. Bradshaw in synthetic organic chemistry at Brigham Young University. After two years working as a research scientist at IBC Advanced Technologies in Utah, he completed his postdoctoral research on synthetic and bioorganic chemistry with Professor Sidney M. Hecht at the University of Virginia. He has been working on combinatorial and antisense drug discovery at Isis Pharmaceuticals since 1995. He is a member of the American Chemical Society. He has authored or co-authored 40 publications and is an inventor on 10 patents. His research interests include total synthesis of natural product antibiotics, Bleomycins and RNA cleaving agents as well as their analogues, and synthesis of amino acid derivatives, peptides, dincleotides, macrocyclic/heterocyclic superchelating ligands. Recently he has been focusing on combinatorial drug discovery, based on novel pharmacophores, and new modifications of nucleosides and nucleotides.



Dan Cook, a keep-fit fanatic, was born in 1944 in the farm community of Clovis, NM. On his way to his Doctorate degree in Organic Chemistry, under the direction of Raymond N. Castle, he attended the New Mexico Military Institute, Eastern New Mexico University, University of New Mexico, and Brigham Young University. He completed an industrial postdoctoral fellowship with Roland K. Robins at ICN Pharmaceutical in Costa Mesa, CA, in 1970–1972, spent 11 years as a medicinal chemist at Warner-Lambert/Parke-Davis Pharmaceutical Research Division in Ann Arbor, MI, and one year at Eastman Kodak Research Laboratories in Rochester, NY, before moving to Malvern, PA, with the Eastman Pharmaceutical Division/Sterling Research Group. In 1989, he joined Isis Pharmaceuticals in Calsbad, CA, as Director of Medicinal Chemistry. He now serves as Vice President, Research Chemistry, at Isis Pharmaceuticals. His research interests are in the areas of heterocycles, nucleosides, nucleotides, and oligonucleotides as they relate to medicinal applications.

believe that this review will be of significant value to the drug discovery community.

This review covers the methodologies for generating a variety of solution-phase combinatorial libraries including parallel single-compound libraries, indexed and mixture combinatorial libraries, complex libraries generated from unsymmetrical and constrained symmetrical scaffolds, and libraries produced by

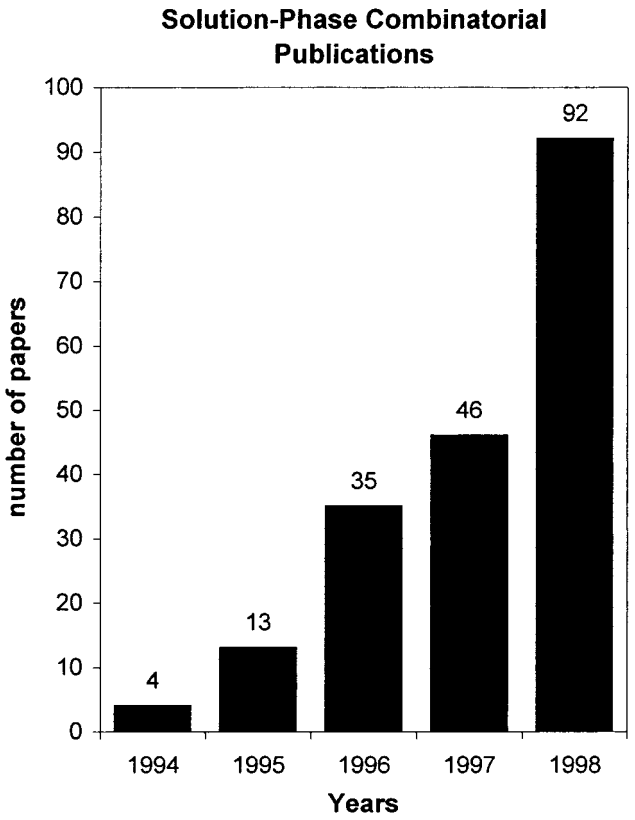


Figure 1.

chemoenzymatic and multicomponent reaction (MCR) approaches up to the beginning of 1999. Biological activities, discovered from libraries generated by solution-phase methodologies, and deconvolution processes of related libraries are briefly summarized. We have also discussed the purification and characterization/confirmation of solution-phase combinatorial libraries as a separate section because of its obvious importance to the progress of solution-phase combinatorial methodologies.

2. Parallel Single-Compound Libraries

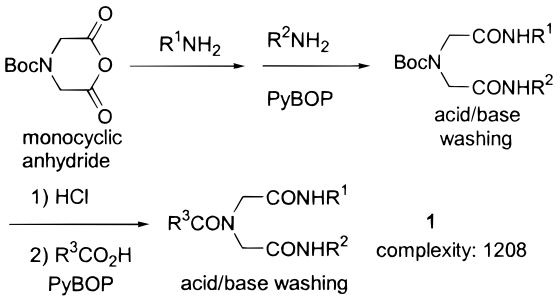
Combinatorial chemistry has been recognized as an efficient tool for accelerating the drug discovery process.[8] Since 1996, much academic and industrial research effort has been directed to parallel solution-phase organic synthesis for generating single-compound libraries. However, parallel combinatorial library synthesis is still in the development stage. Most of the published work focuses on the development of organic reactions for further automated library generation. Several groups have applied their developed approaches and reactions to automated or semi-automated parallel synthesis. Automated solution-phase synthesizers have not been developed as advanced as and have not been utilized as routine as solid-phase synthesizers. Several groups have focused on method development, which might be widely applicable in the future, while others have focused on lead optimization based on known pharmacophores. The latter approach takes advantage of a parallel approach and will actually accelerate the lead optimization process for drug candidates. This section will focus on various types of libraries, method

development, and biological results observed. Purification and characterization methods used will be briefly described along with library generation and will be summarized in the later section.

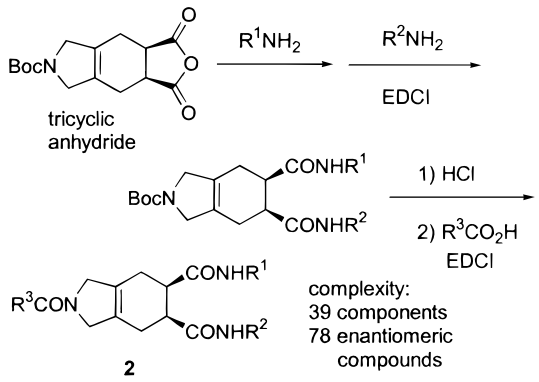
A. Amide-Type Libraries

Boger and co-workers[9−12] developed a parallel solution-phase approach for the generation of triamide libraries from monocyclic anhydride (Scheme 1) and constrained tricyclic anhydride (Scheme 2)

Scheme 1[9−11]



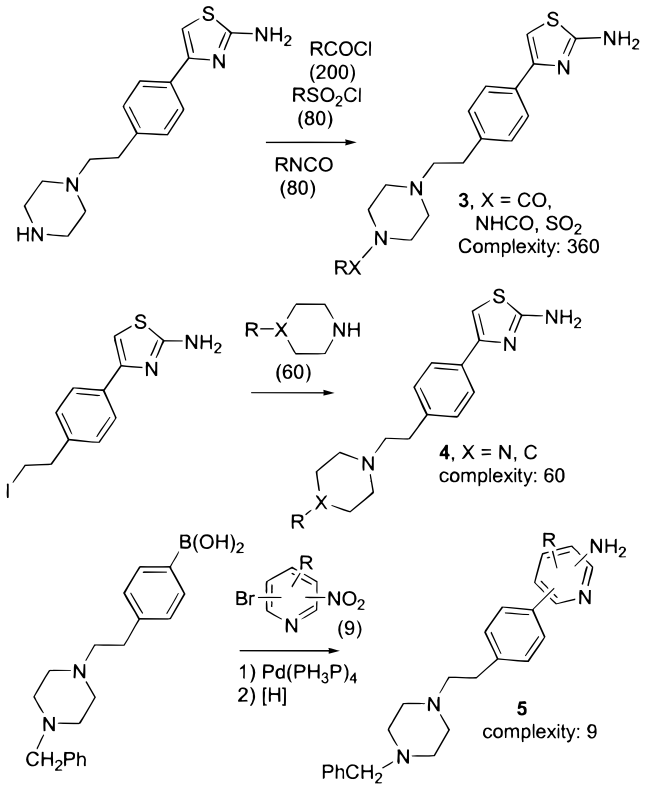
Scheme 2[10−12]



templates in four linear steps. Both cyclic anhydride templates were generated in situ and reacted with primary amines (R^1-NH_2) to introduce the first amide group. The carboxylic acid group released in the first step was further reacted with other primary amines (R^2-NH_2) to provide corresponding diamides. After removing the *t*-Boc protecting group, secondary amines, thus obtained, were similarly coupled with a series of carboxylic acids to provide final triamide libraries **1**. Three linear libraries with a complexity of 1208 compounds were generated from the monocyclic anhydride template. Ring opening of the tricyclic anhydride template provided a constrained bicyclic triamide library **2** with a complexity of 78 enantiomeric compounds. A general liquid−liquid extraction process with acid and base provided single-compound libraries in 5−150 mg scales with 85−98% purity. Other reactive nucleophiles (e.g., R−OH, R−SH, etc.) could also be used in the first two steps, and other acylating or alkylating agents or electrophiles (e.g., $R-SO_2Cl$, R−X, R−N=C=O, R−N=C=S, etc.) could be used in the last step. Further utilization of other electrophiles and/or nucleophiles would significantly increase the diversity of the libraries. The intermediates resulting from these reaction sequences may also be used directly for biological screening. This is one of the major advantages for solution-phase combinatorial chemistry compared with solid-phase approaches. The authors also took advantage of the cyclic anhydride templates and produced more complicated libraries (see section 3).

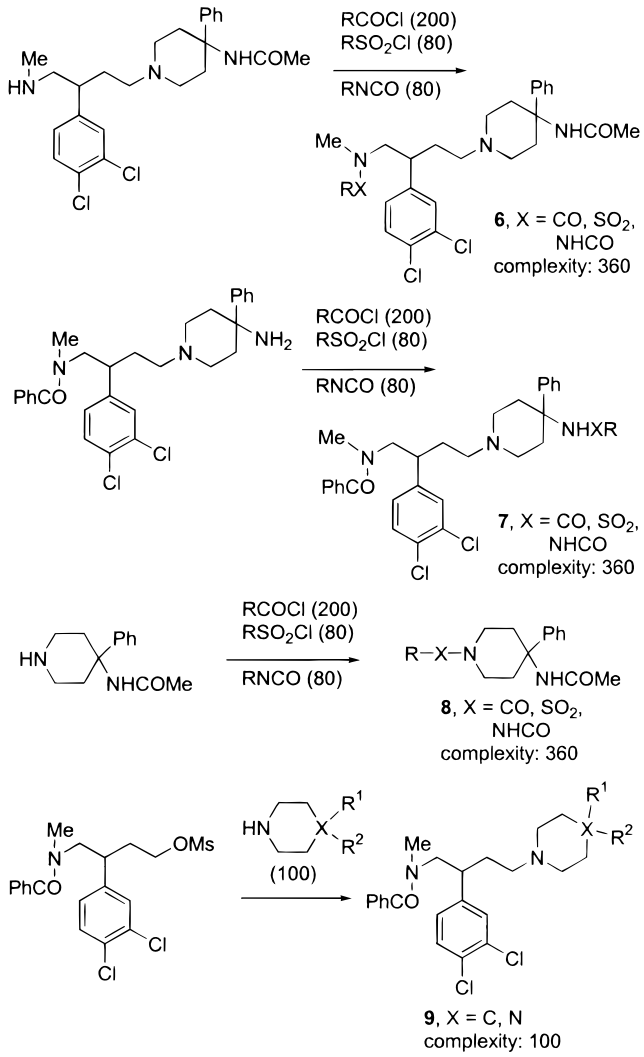
Selway and co-workers[13] at Pfizer reported a series of parallel single-compound libraries **3**−**9** (Schemes 3 and 4) for lead optimization studies and subsequent

Scheme 3[13]



SAR (structure−activity relationship) studies, which provided promising results. Libraries **3**−**5**, generated by a semi-automated method, were designed to perform SAR studies to optimize a known aminothiazole lead and to improve activity against herpes simplex virus (HSV-1 helicase ATPase). The piperazine substrate was treated in a parallel fashion with 200 carboxylic acid chlorides, 80 sulfonyl chlorides, and 80 isocyanates, providing library **3** containing 360 different amide, urea, and sulfonamide derivatives. Library **4** containing 60 compounds was obtained by the reaction of the iodo compound with 60 different piperidines and piperazines. Suzuki reaction conditions were used for the preparation of library **5** by the palladium-mediated coupling of boronic acid with bromonitroheterocyclic compounds followed by hydrogenation. Testing 429 compounds from libraries **3**−**5** provided an active compound which was 18 times more potent in the HSV helicase plaque reduction assay than original lead compounds. The authors also reported the generation of amide-type libraries **6**−**9** based on the structure of the known NK_2 antagonist (Scheme 4). The same series of carboxylic acid chlorides, sulfonyl chlorides, and isocyanates were attached on different positions of an active structure using the same procedures to provide

Scheme 4[13]

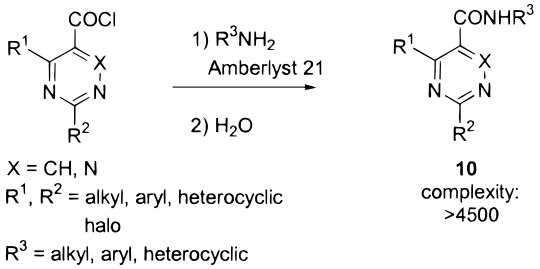


libraries **6**−**8**. One hundred different piperazines and piperidines were reacted with a mesylate derivative to give library **9**. Libraries **6**−**9** were prepared to examine the SAR of four key positions of the original lead compound. SAR studies and biological screening results were also discussed.

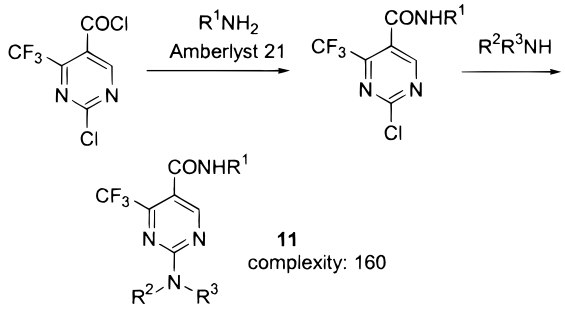
In a similar manner, several other single-compound libraries have been prepared (schemes not provided). Xie and co-workers[14] developed a robotics-driven solution-phase combinatorial method. Substituted piperazines and piperidines were reacted in a parallel fashion with alkyl halides and acyl halides to provide amine and amide libraries containing 1086 alkyl/aryl piperazine and 835 alkyl/acyl piperidine derivatives. A water-soluble base, 1,1,3,3-tetramethylguanidine, was effectively utilized to trap the acid generated from the reaction. The base and its salt were easily removed by a simple aqueous-phase extraction. Five percent of the library members were analyzed by GC-MS (gas chromatography−mass spectrometry) techniques and showed over 70% purity. Carrol and co-workers[15] reported the synthesis of an *N*-substituted piperidine library with a complexity of 288 compounds by a parallel solution-phase approach to search for an opioid κ receptor. Garr and co-workers described the design and the parallel synthesis of a 20 000-compound library by a high-throughput synthesis method based on aminopiperidine, piperazine, and aminobenzylamine scaffolds (scheme not provided).[16,17]

Suto and co-workers at Signal Pharmaceuticals[18−20] reported the parallel generation of amide libraries using a basic ion-exchange resin, Amberlyst 21, as a scavenger to remove excess acyl halides (Schemes 5 and 6). Approximately 30 aromatic and heterocyclic carboxylic acid chlorides, prepared in situ, were reacted with 160 commercially available amines. The reactions were carried out in a parallel microtiter format of 80 compounds per plate. A slight excess (1.05 equiv) of acid chloride was used in each reaction in the presence of Amberlyst 21 resin. Library members were analyzed by TLC (thin-layer chromatography), and 20 random samples per plate were analyzed by HPLC with >85% purity. The heterocyclic amide library **10**, generated by this method, represented a total complexity of >4500 compounds.[18,19] Carboxylic acid chlorides could also be reacted with various alcohols, thiols, and other nucleophiles to generate ester and other types of libraries. The 2-chloro-4-trifluoromethylpyrimidine-5-carboxylic acid chloride has two different reactive sites (Scheme 6).[19,20] By using the same procedure developed by Suto's group, the pyrimidine acid chloride was reacted with 160 heterocyclic, aliphatic, and aromatic amines providing a library with 160 pyrimidine amide compounds. Analyses of 15 random samples gave 60−90% yields with >85% purity. The authors also took advantage of different reactive sites on the pyrimidine ring. Eleven 2-chloropyrimidine amides were further reacted with various amines to provide a series of disubstituted pyrimidine derivatives **11**. The purpose of this research is lead optimization and subsequent preliminary SAR studies. A compound from this research was identified as a potent inhibitor of NF-κB and AP-1, which was active in an animal model.

Scheme 5[18,19]



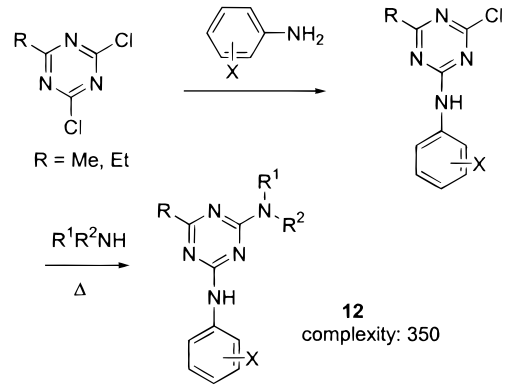
Scheme 6[19,20]



B. Heterocyclic Libraries from Heterocycles

Di- and trihalogenated heterocycles have been employed as reactive scaffolds for library generation. Two or more heteroatoms, especially nitrogen atoms, in the heterocyclic rings activate the halides for nucleophilic substitution. These halogen atoms can be sequentially and selectively replaced with a variety of nucleophiles to provide diverse libraries based on heterocyclic scaffolds. Several research groups produced some useful libraries from polyhalogenated heterocycles. Whitten and co-workers[21] at Neurocrine Biosciences generated a triazine library **12** by a parallel synthetic method for the optimization of corticotropin releasing factor$_1$ (CRF) receptor antagonists (Scheme 7). 2-Methyl- and 2-ethyl-4,6-dichlo-

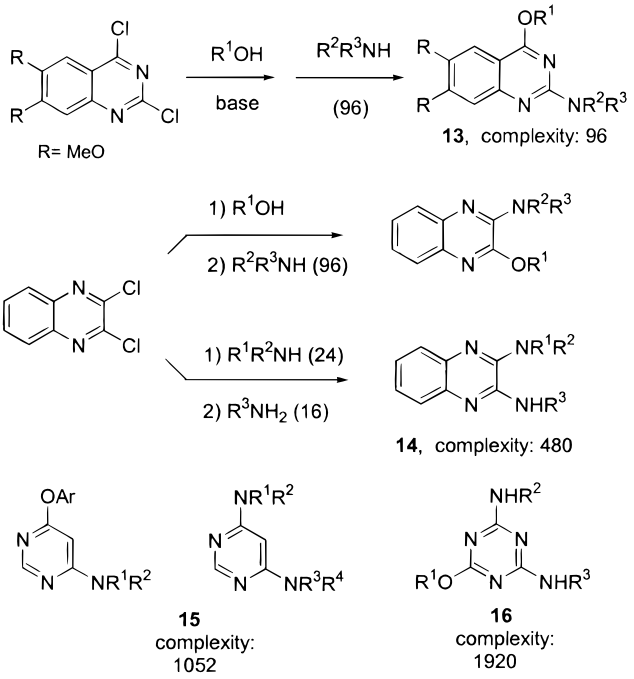
Scheme 7[21]



rotriazine derivatives were reacted with different aniline derivatives, followed by another nucleophilic substitution with secondary amines providing library **12** containing 350 triazine analogues. Several compounds were isolated in 70−95% purity as determined by GC-MS analysis. A potent CRF receptor antagonist (K_i = 57 nM) was identified from this library. Further optimization of the active compound resulted in a more potent CRF receptor antagonist with a K_i value in the low nanomolar range.[22]

Johnson and co-workers[23] at Arris Pharmaceuticals reported the generation of substituted heterocyclic libraries from polyhalogenated pyrimidine, triazine, quinazoline, and quinoxaline derivatives (Scheme 8). The 2,4-dichloro-6,7-dimethoxyquinazoline was first reacted with an alcohol to introduce the first group and then reacted in a parallel fashion with 96 different secondary amines to provide library **13** containing 96 quinazoline derivatives. The quinozaline library **14** was prepared from 2,3-dichloroquinoxaline by sequential substitution with an alcohol or amine first and then with amines. Similarly, phenols or secondary amines were reacted with 2,4-dichloropyrimidine. The resulting monochloropyrimidine derivatives were then reacted with secondary or primary amines to provide library **15** containing 1052 pyrimidine derivatives. Cyanuric chloride (2,4,6-trichlorotriazine) was reacted with alcohols providing monosubstituted dichlorotriazines. The dichlorotriazine derivatives were then sequentially reacted with two series of amines to give trisubstituted triazine library **16** with a complexity of 1920 compounds. A total of 3648 di- and trisubstituted heterocyclic

Scheme 8[23]



compounds were obtained by automated solution-phase parallel synthesis. A liquid−liquid-phase extraction process was used for the purification of library members, and the samples were analyzed by electrospray MS.

Coffen and co-workers[24] at ArQule described a procedure for the sequential and selective derivatization of cyanuric chloride with carbohydrates, dipeptides, aminimides, and α-ketoamides generating triazine derivatives. The libraries were examined as protease inhibitors (Figure 2). Library **17** was con-

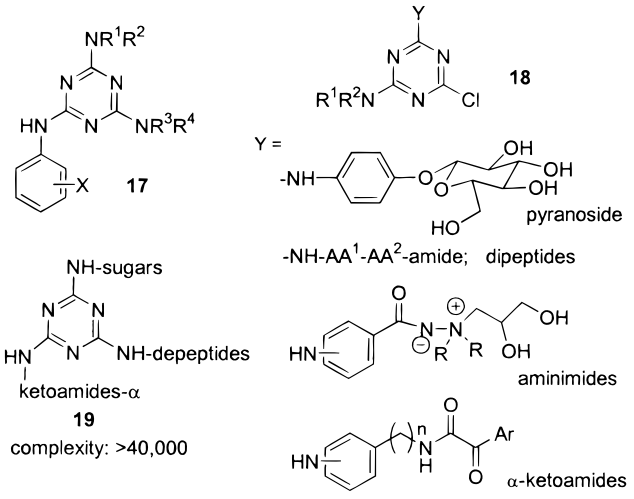
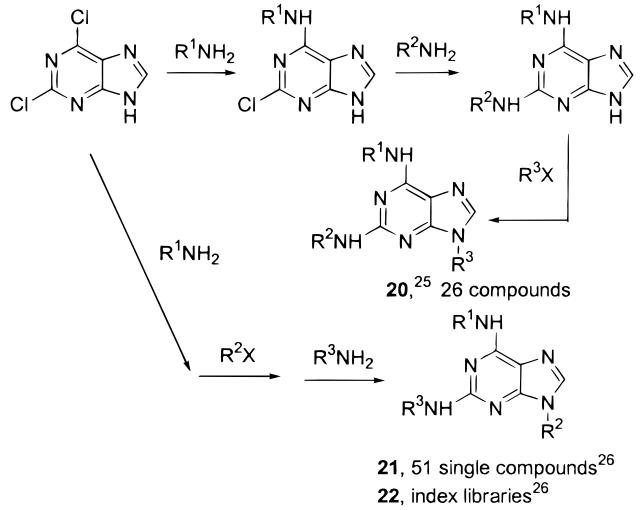


Figure 2.[24]

structed in a parallel array by sequentially attaching anilines and then amines. In library **18**, anilines or other amines were incorporated at the one position of the triazine ring while carbohydrate, dipeptide, or α-ketoamide moieties were attached at another position of the triazine ring. For library **19**, three reactive sites on the triazine ring were all substituted by carbohydrates, dipeptides, and α-ketoamides. More

than 40 000 triazine derivatives were synthesized by an automated parallel process. Incorporation of biologically relevant moieties on core structures should increase opportunities to find related biologically active compounds. Indeed, a series of hits have been identified for the inhibition of protease factor Xα and plasmin. The 2,6-dichloropurine was sequentially and selectively reacted with two different sets of amines and/or hydrazine providing disubstituted purine derivatives (Scheme 9).[25] The *N*-9 position of purine was

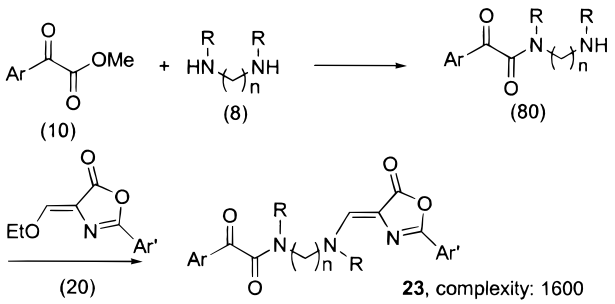
Scheme 9[25,26]



then substituted by aryl or alkyl groups providing the corresponding trisubstituted purine library **20**. Lum and co-workers also generated a 51-member single-compound library **21** from 2,6-dichloropurine by a different reaction sequence.[26] After R^1NH_2 was reacted at position 6 of purine, the alkylation of position 9 was performed before the final nucleophilic subsitution on position 2. This same reaction sequence was also used for the generation of 14 intermediate and final indexed libraries **22**, each containing 5−25 compounds, by reacting five reagents at the same time. This purine template, containing electrophilic and nucleophilic sites for functionalization, should be applicable to the generation of large diverse libraries by automated systems.

The ArQule's group[27] also produced oxazolone libraries by parallel solution-phase combinatorial methods. The condensation of α-ketoesters and α,ω-diamines provided 80 α-ketoamides (Scheme 10)

Scheme 10[27]

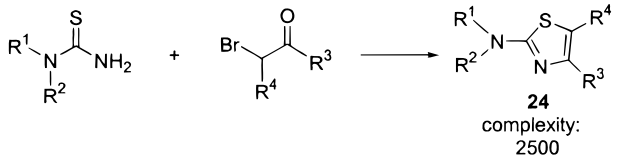


which were reacted in a parallel fashion with 20 ethoxymethyleneoxazolones, providing library **23** with 1600 aminomethyleneoxazolone derivatives.[27] The library was designed for potential serine protease inhibitors. Some of library members were analyzed by HPLC-MS techniques.

C. Heterocyclic Libraries from Non-Heterocycles

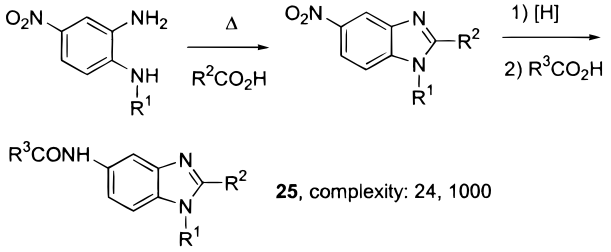
Solution-phase parallel combinatorial approaches have been developed to generate a variety of other heterocyclic libraries directly from nonheterocyclic molecules. Watson and co-workers[28] described a convenient one-step procedure for the preparation of 2-aminothiazole libraries (Scheme 11). An initial

Scheme 11[28]



illustrative library **24** of 20 compounds was generated by the reaction of thiourea and α-bromoketone derivatives. Library members, without purification, were characterized by NMR and MS spectrometric techniques. On the basis of the procedure developed, a library of 2500 aminothiazole compounds was prepared for lead optimization. Carroll and co-workers[29] developed a parallel solution-phase procedure for the preparation of etonitazene-related benzimidazole libraries **25** (Scheme 12). Variation of R^1 and

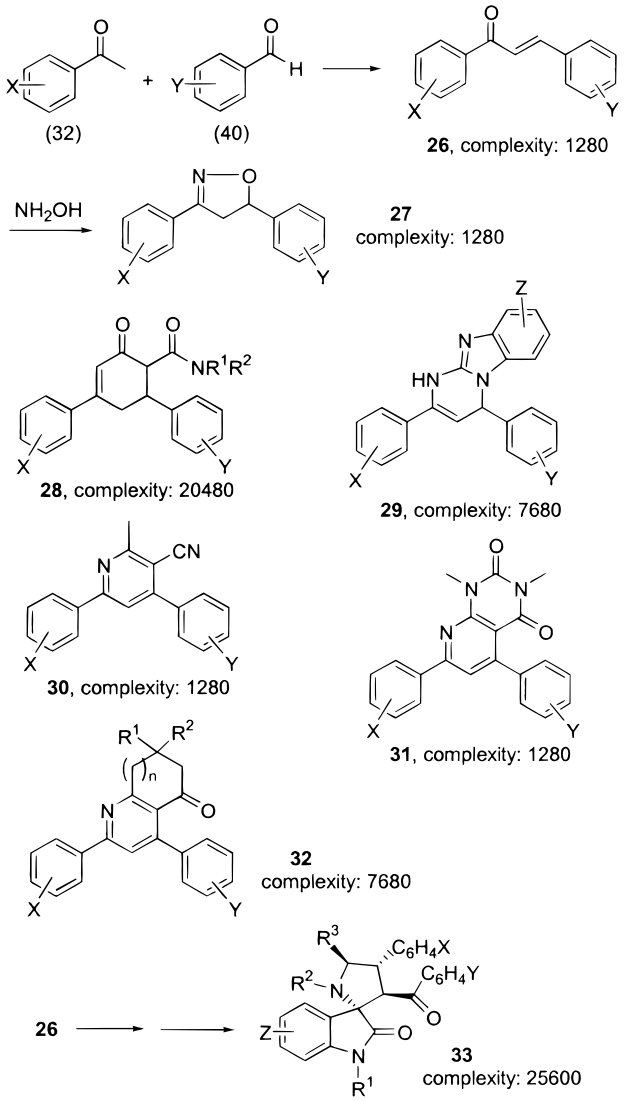
Scheme 12[29]



R^2 has been studied and discussed. An important discovery of this procedure was using elevated temperatures for the cyclization step to shorten the reaction time from days to minutes. The procedure has been utilized for the preparation of a 1000-compound library.

Coffen and co-workers at ArQule utilized cycloaddition reactions to generate heterocyclic libraries (Scheme 13).[30,31] The condensation of aryl aldehydes and acetophenones gave chalcone library **26** with a complexity of 1280. Library **26** was used as a synthon for the further construction of five- and six-membered heterocyclic systems. Library **26** was treated with hydroxyamine to give isoxazoline library **27**. The Michael addition of 1,3-dicarbonyl compounds with chalcone library **26** provided cyclohexenone library **28** with 4-fold functional diversity. The reaction of **26** with six aminobenzimidazoles provided tetrahydropyrimidine library **29** containing 7680 compounds. The Hantzsch cyclization of library **26** with ethyl 3-aminocrotonate provided tetrasubstituted pyridine library **30**. Library **26** was reacted with 6-amino-1,3-

Scheme 13[30,31]



dimethyl uracil to provide a fused ring system of library **31**. Library **32** with a fused ring system was also prepared from library **26** and the corresponding *β*-aminocyclic ketones. The spiro-pyrrolidine-2,3′-oxindole library **33** was prepared by the reaction of 80 chalcone compounds of library **26** with intermediate ylides, generated from 16 isatins and 20 amino acids, through a 1,3-dipolar addition of chalcones. The structure of a spiro-type compound was confirmed by X-ray structural analysis. On the basis of chalcone library **26**, other five- and six-member and spiro-system-fused chalcone libraries were generated with a total complexity of over 70 000 compounds. Random samples from libraries were analyzed by NMR, HPLC, and MS spectrometric techniques.

Hydantoin library **34** was synthesized in 70−90% yields from amino acid esters, aromatic isocyanates, and alcohols.[32] Fifty compounds were characterized by spectroscopic analyses. Several 5′-alkoxyhydantoin derivatives were also prepared by a solid-phase approach in lower yields (23−72%) compared with the solution-phase approach. Ganesan and co-workers[33,34] reported hydantoin and thiohydantoin libraries **35** with 3-fold diversity, represented by R^1, R^2, and R^3. This library was prepared by the reductive amination of aldehydes with unprotected amino acid esters, followed by the reaction with isocyanates or thioisocyanates. The cyclization of the resulting intermediates in the final step provided over 700 distinct compounds in 52−98% yields.

Other heterocyclic libraries **36**−**46** (Figure 3) were

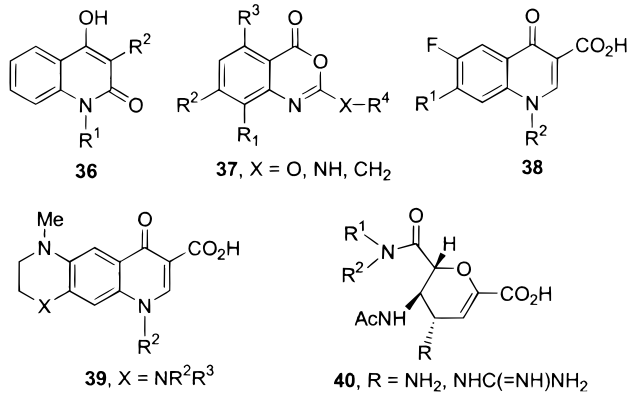


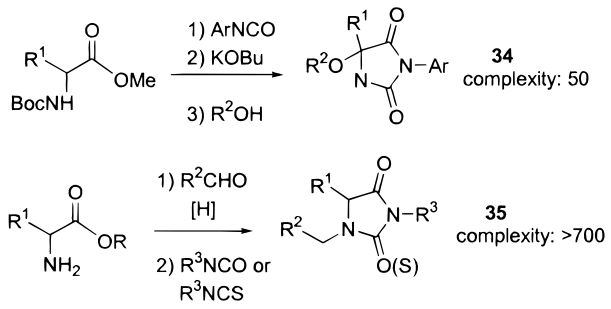
Figure 3. Other heterocyclic libraries.[35−39]

prepared through different types of cyclization reactions by a parallel solution-phase approach.[35−39] 4-Hydroxyquinolin-2(1*H*)-one library **36** was obtained in 80−96% yields by a Claisen-type condensation of the corresponding benzene derivatives.[35] Anthranilic acid derivatives were cyclized with isocyanates or chloroformates to provide benzoxazinone library **37**.[36] A number of lead compounds, showing inhibition against HSV-1 protease, were identified from this library. Mitscher and co-workers[37,38] reported the preparation of quinolone libraries **38** as antiinfective agents by solution-phase parallel synthesis. The cyclization of benzoyl acetate ester derivatives with amines provided bicyclic quinolone library **38**. Aromatic nucleophilic substitution followed by cyclization gave tricyclic library **39**. A number of derivatives were synthesized in a parallel manner, and representative compounds were fully characterized. The compounds were examined for antiinfective activity. Amino- and guanidino-substituted library **40** with a complexity of 80 compounds was generated by an automated parallel synthesis.[39] The tertiary amide compounds of library **40**, containing one or more small alkyl groups, are highly potent inhibitors of the influenza A virus enzyme.

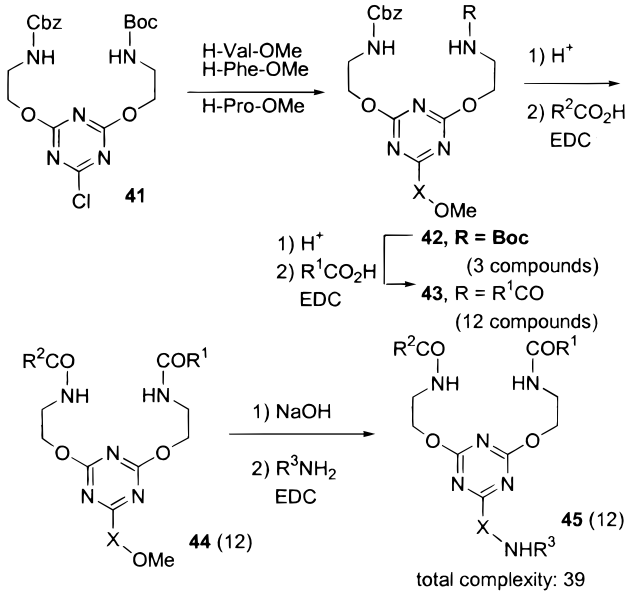
D. Other Heterocyclic Libraries

Falorni and co-workers[40,41] designed and synthesized orthogonally protected polyfunctionalized heterocyclic scaffolds which were then converted to heterocyclic libraries by a parallel synthetic procedure. Disubstituted chlorotriazine **41** was reacted with three amino acid methyl esters to provide three orthogonally protected triazine compounds **42** (Scheme 15). After selective deprotection of **42**, the resulting amines were coupled with four carboxylic acids (R^1-COOH) to give **43**. Selectively removing the Cbz (benzyloxycarbonyl) group and subsequent coupling with another set of four carboxylic acids (R^2COOH) provided triazine derivatives **44**. The esters of **44** were hydrolyzed and reacted with a set of four amines (R^3NH_2), giving the final triazine library **45**

Scheme 14[32−34]

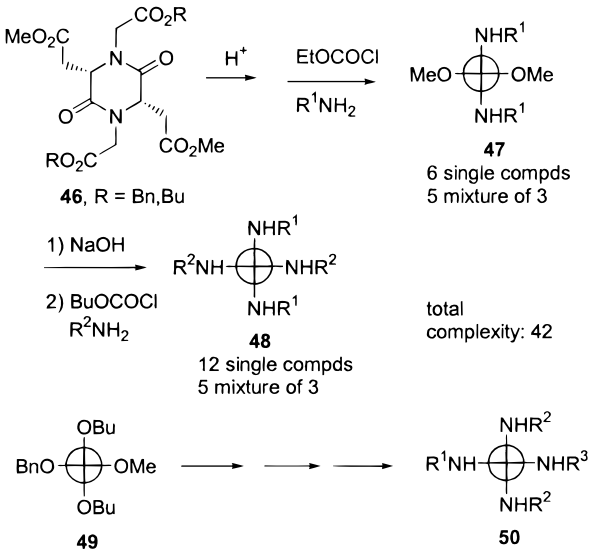


Scheme 15[40]



with 3-fold functional diversity. Additional libraries may be made by this procedure from this as well as other polyfunctionalized scaffolds. Falorni's group also synthesized protected diketopiperazine tetracarboxylic acids **46** and **49** (Scheme 16)[41] as scaffolds for

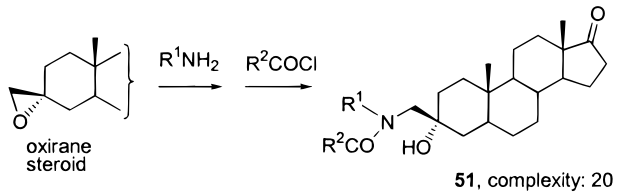
Scheme 16[41]



library generation. Protected compounds **46** were selectively deprotected and coupled with six amines (R^1NH_2). The resulting diamide library **47** was deprotected and coupled with another set of five amines (R^2NH_2) to give tetraamide library **48** containing 12 single compounds. Five sets of two amines (R^1NH_2) were used in the first coupling step to give five mixtures of three diamide sublibraries **47**. Sublibraries **47** were further coupled in a parallel fashion with five other amines (R^2NH_2) to give five complex libraries **48** each containing three compounds. By this protocol, 42 di- and tetraamide compounds were generated based on diketopiperazine scaffold **46**. Compound **49**, orthogonally protected by three different protecting groups, was also synthesized and used for the preparation of library **50**.

A parallel solution-phase combinatorial approach has also been used for the generation of a steroidal library (Scheme 17).[42] The steroidal oxirane was

Scheme 17[42]



reacted with amines (R^1NH_2) providing ring-opened ethanolamines. Regioselective acylation of secondary amines with carboxylic acid chlorides gave the corresponding 20-member steroidal library **51**. Library members were obtained in 50−98% yields with high purity and characterized by NMR and MS spectrometric techniques. This procedure could also be used to synthesize more diverse steroidal libraries as well as other nonsteroidal molecules. The reductive amination of ethanolamines with aldehydes and ketones provided substituted ethanolamine libraries by high-throughput parallel synthesis (scheme not provided).[43] Ion exchange chromatographic purification provided the library in 60−99% yields with high purity.

Several other solution-phase combinatorial approaches have also been described. Vogel summarized the combinatorial Diels−Alder approach for the synthesis of antitumor anthracycline derivatives (scheme not provided).[44] Solution-phase combinatorial approaches were also used to synthesize natural products in a parallel manner to derivatize intermediates or final products. For example, Nicolaou and co-workers[45] applied a solution-phase parallel synthetic approach to the synthesis of sarcodictyin derivatives (scheme not provided). A 26-member combinatorial library of paclitaxel C7 esters was generated by an automated two-step procedure in 60−94% yields (scheme not provided).[46] The resulting paclitaxel derivatives were purified by silica gel chromatography and fully characterized. A parallel array solution-phase combinatorial approach has been effectively utilized by Brady and co-workers for batch optimizations of a two-step esterification/deprotection reaction sequence (scheme not provided).[47] Subsequently, a 600-member library, prepared using the optimized reaction conditions, provided more potent inhibitors for *N*-His(D381E) ICE (interleukin-1β converting enzyme). Hird and co-workers recently

reported a "split−split" multiple-synthesizer approach for the generation of a pyrazole library.[48] To eliminate duplication in the multistep synthesis, this approach established a cascade of matched synthesizers in which each synthesizer performed one step and provided feedstock for the next instrument. A four-step synthesis for a 960-member library was carried out in 1045 reactions instead of 9600 reactions by a totally parallel method.

Solution-phase parallel combinatorial approaches are very important tools for accelerating the drug discovery process. Theoretically, all known organic reactions can be used for generating different types of diverse libraries. However, the development of solution-phase automated synthesizers has not advanced as much as that of solid-phase automated synthesizers. More and more chemical reactions will be developed for combinatorial application in the laboratory and then applied on robotics automation. While numerous parallel single-compound libraries have been generated for lead optimization, few libraries have been generated for lead discovery.

3. Positional Scanning or Indexed Combinatorial Libraries

Houghten and co-workers[2] explored and synthesized positional scanning and other heterocyclic mixture libraries by a solid-phase approach. Solution-phase combinatorial chemistry has also been employed to generate mixture libraries containing a pool or a mixture of compounds. Mixture libraries are further classified as complex libraries and positional scanning or indexed libraries. The later was first named as positional scanning or deletion synthesis mixture libraries. These types of libraries may be considered more applicable for lead discovery than the previously discussed parallel approaches. These approaches are also used for lead optimization. Indexed libraries are discussed in this section, and complex libraries will be presented in sections 4 and 5. Figure 4 illustrates the generation of two-dimen-

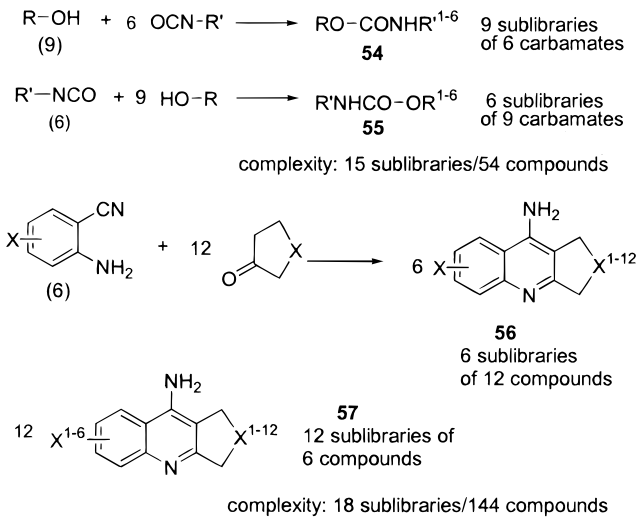
Figure 4. Two-dimensional indexed libraries.

sional positional scanning or indexed combinatorial libraries. Each of *m* molecules (RX) having one reactive site is reacted with a mixture of *n* other molecules (R′Y) to provide *m* sublibraries **52** each containing *n* compounds with variation of only R′ groups. When another type of molecule R′Y is fixed, *n* numbers of sublibraries **53**, each containing *m* compounds with variation of only R groups, are obtained. This indexing combinatorial approach was utilized for the generation of libraries by Pirrung[49−51] and Smith.[52] Positional scanning or indexed libraries offer several advantages over solid-phase and solution-phase parallel approaches. Small mixture libraries can be assembled simultaneously in a simple chemical process from multiple subunits and then screened directly. The number of compounds prepared is much greater than the number of chemical steps required; therefore, this approach is fast and relatively inexpensive. Screening two-dimensional matrix sublibraries should identify the most active sublibraries, which can be resynthesized; therefore, the deconvolution procedure can be simplified. The positional scanning or indexed combinatorial library approach is not limited to a two-dimensional matrix. In multicomponent condensation (MCC) reactions, for example, 3CC, three- or multidimensional matrix indexed libraries may be prepared by varying one component and fixing other components for multiple runs. Polyfunctionalized scaffolds, protected orthogonally or having different types of functional groups, can also be utilized for the preparation of multidimensional matrix indexed libraries. Multidimensional matrix indexed libraries significantly increase the diversity of libraries.

A. Positional Scanning or Indexed Libraries

Pirrung and co-workers[49−51] produced a series of carbamate and tetrahydroacridine two-dimensional indexed libraries **54**−**57** (Scheme 18). Each of the

Scheme 18[49−51]

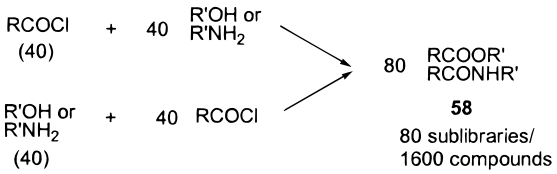


nine alcohols (ROH) was reacted in a parallel manner with an equimolar mixture of six isocyanates (R′NCO), providing nine sublibraries **54** each containing six carbamates. By fixing isocyanate subunit R′NCO, each of the six isocyanates was reacted with an equimolar mixture of nine alcohols, providing six indexed sublibraries **55** each containing nine carbamates. The resulting 15 indexed sublibraries contained 54 carbamate compounds. A similar two-dimensional matrix approach was also used to prepare heterocyclic libraries **56** and **57** (Scheme 18). Fixing six cyanoaniline subunits and varying 12 ketones provided six indexed sublibraries **56** each containing 12 tetrahydroacridine derivatives. Reversing the sequence of fixing and varying subunits gave 12 indexed sublibraries **57**. Eighteen indexed sublibraries **56**/**57**, containing 144 tetrahydroacridines, were screened for their inhibition of acetylcholinesterase. Selected compounds from several active pools were

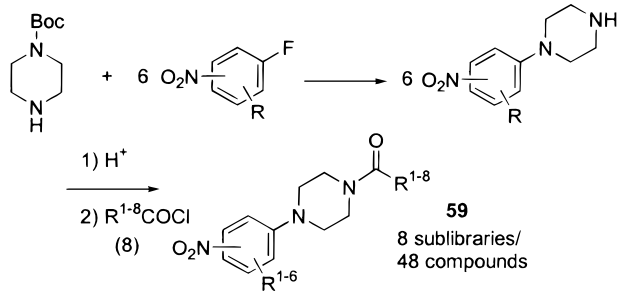
resynthesized to provide an active compound with 10-fold greater potency than the parent lead compound.

Smith and co-workers[52] reported the synthesis of 80 two-dimensional indexed sublibraries **58** (Scheme 19). Forty carboxylic acid chlorides and 40 nucleophiles, including amines and alcohols, were selected for the amide/ester library construction. As described above for the two-dimensional matrix indexed library approach, acid chlorides and nucleophiles (amines/alcohols) were subsequently employed as fixed reagents to generate 80 indexed sublibraries **58** each containing 40 compounds with a total complexity of 1600. Two sublibraries were analyzed by GC-MS techniques, which identified at least 25 of the 40 expected products in each sublibrary. Screening these sublibraries for the NK_3 receptor and matrix metalloproteinase-1 provided several active samples. Resynthesis of the single compounds from active pools provided several lead compounds. Zhu and co-workers[53] produced one-dimensional indexed libraries based on piperazine (Scheme 20). *t*-Boc-protected piperazine was reacted with a mixture of six nitro-substituted phenyl fluorides to give a one-dimensional indexed library containing six compounds. The deprotected intermediate library was acylated in a parallel manner with eight carboxylic acid chlorides. The resulting eight indexed sublibraries **59** contained 48 arylpiperazine amides. All of the steps proceeded in high yields and were monitored by TLC. Aqueous acid/base workup gave sublibraries with 88−95% purity.

Scheme 19[52]

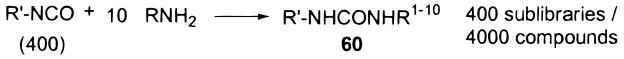


Scheme 20[53]



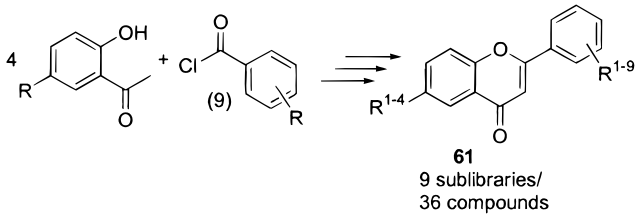
Kaldor and co-workers[54] at Eli Lilly reported the discovery of antirhinoviral leads from two-dimensional indexed combinatorial libraries. Fixing isocyanate and reacting with a mixture of 10 different primary or secondary amines provided indexed library **60** each containing 10 different compounds (Scheme 21). A resin-supported scavenger, aminomethylpolystyrene, was employed to remove excess isocyanate. A representative indexed library, having an equimolar mixture of 10 ureas, was reasonably pure and sufficient for biological assays. A similar parallel array process using different isocyanates and different sets of amines afforded 400 indexed sublibraries. The libraries with significant antirhinoviral activity and low to moderate cytotoxicity were resynthesized as single compounds and reassayed. Two compounds had potent antirhinoviral activity.

Scheme 21[54]



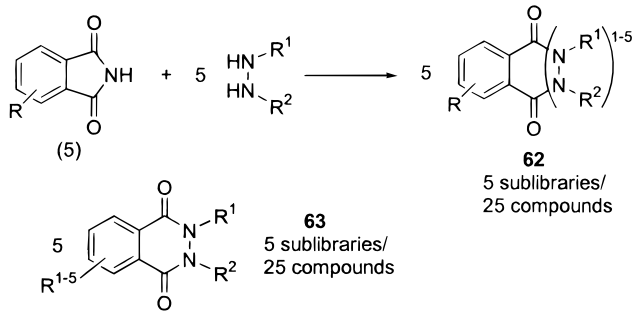
Marder and co-workers[55] prepared nine indexed sublibraries by a one-pot three-step procedure (Scheme 22). Nine substituted benzoyl chlorides were reacted in a parallel fashion with a mixture of four 2-hydroxyacetophenone derivatives, affording nine one-dimensional indexed sublibraries **61** each containing 36 flavone derivatives, which were separated by HPLC. One sublibrary contained approximately equal amounts of all members in the range of 23% to 26% of the total content. Evaluation of their binding affinity for benzodiazepine receptors provided four active substances with K_i values in the nanomolar range.

Scheme 22[55]

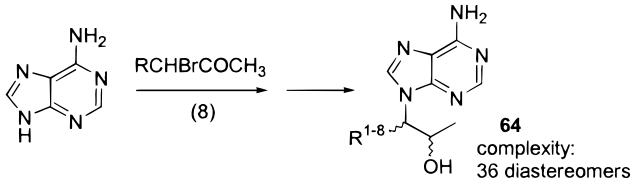


Nielsen and co-worker[56] reported the generation of phthalhydrazide indexed libraries (Scheme 23). Five different phthalimides were employed as fixed reagents and separately reacted with a mixture of five substituted hydrazines to afford five indexed sublibraries **62**, each containing five phthalhydrazide derivatives. When five hydrazine derivatives were fixed and reacted in a parallel fashion with a mixture of five phthalimides, five indexed sublibraries **63** were obtained. The reaction of five phthalimides and five hydrazines in one pot also provided a full complex library containing 33 isomeric compounds. These indexed libraries were also resolved into individual compounds by HPLC. An adenine indexed library **64** was prepared to demonstrate pulsed ultrafiltration/electrospray mass spectrometry (PUF/ESMS) for examining mixture libraries (Scheme

Scheme 23[56]

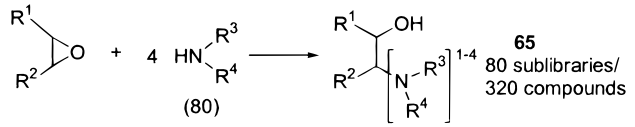


Scheme 24[57]



24).[57] A mixture of eight α-bromoketones was reacted with adenine followed by the reduction of the resulted ketones. The resulting one-dimensional indexed library **64** contained 36 diastereomers because of the two chiral centers generated in the process. Compared with the computer-reconstructed mass chromatograms, the mass spectrum of library **64** indicated seven sets of products out of eight at approximately equal abundance. Lum and co-workers also synthesized 14 indexed libraries **22** based on purine scaffolds (Scheme 9).[26] Ganesan and co-worker[58] prepared an *β*-amino alcohol library **65** in solution (Scheme 25). Lithium perchlorate promoted ring

Scheme 25[58]

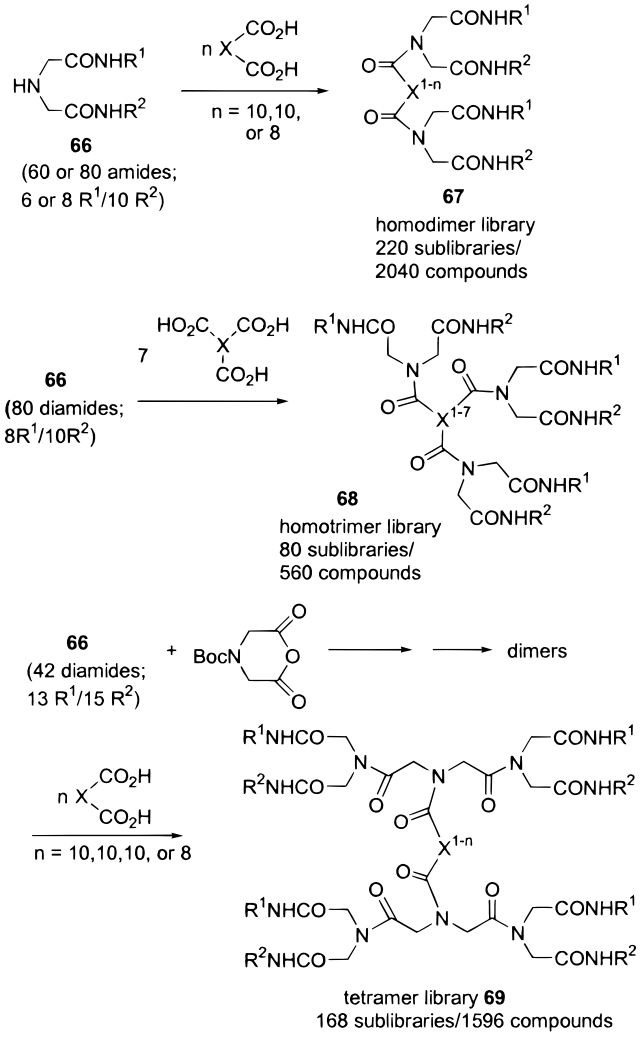


opening of an epoxide by four amines affording a one-dimensional indexed library of four amino alcohols. One epoxide was reacted with 80 sets of four amines, providing 80 sublibraries **65** with a complexity of 320 compounds. Over 6000 different *β*-amino alcohols were prepared by this method from a variety of epoxides. Representative indexed sublibraries exhibited the four expected product peaks.

B. Trimer and Tetramer Positional Scanning and Deletion Synthetic Libraries

Boger and co-workers at the Scripps Research Institute developed a parallel solution-phase combinatorial approach for the synthesis of single-compound libraries (see section 1).[9−12] Boger's group also prepared several series of iminodiacetic acid mixture libraries (deletion synthesis libraries as termed by authors) with higher order, as well as large complex libraries (Schemes 26 and 27).[59−64] Sixty or eighty diamides **66** were coupled in parallel and combination procedures with three mixtures of *n* (*n* = 10, 10, 8) dicarboxylic acids to afford 220 homodimer sublibraries **67**. Each sublibrary contained 8 or 10 compounds with a total complexity of 2040 compounds (Scheme 26).[59,60] The sublibraries were purified by liquid−liquid- and liquid−solid-phase extraction procedures to provide an average yield of 57%. The matrix characterization of these sublibraries by MS and ^{1}H NMR spectrometric analyses confirmed the constitution of the mixtures. A comparison of one sublibrary with a reconstituted mixture, prepared by combining an equimolar amount of individual components, established the integrity of the sublibrary. Eighty diamides **66** were also coupled in a parallel manner with a mixture of seven tricarboxylic acids

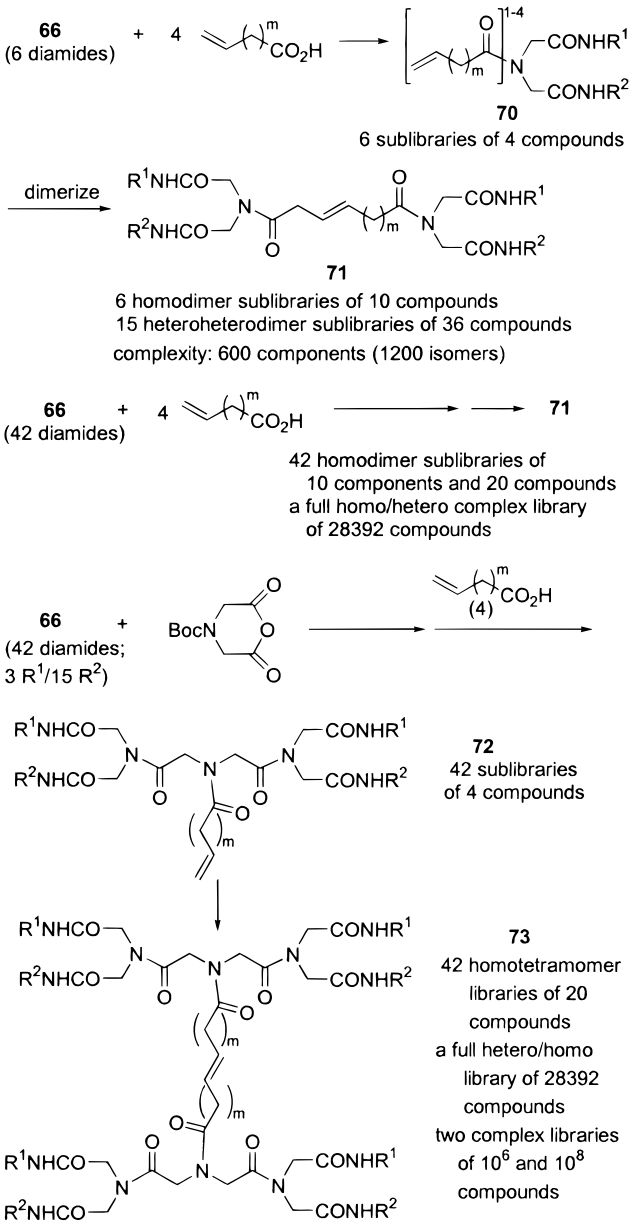
Scheme 26[59−61]



to give 80 homotrimer sublibraries **68**. Each of these sublibraries contained seven compounds with a total complexity of 560 compounds.[60,61] Forty-two diamides **66** were first reacted in pairs and then in a parallel manner with *t*-Boc-protected iminodiacetic anhydride under the coupling conditions described in Scheme 1 to give the corresponding dimers. After deprotection, the resulting 42 dimers were coupled in a parallel manner with four sets of *n* (*n* = 10, 10, 10, 8) dicarboxylic acids, providing 168 tetramer sublibraries **69** with a complexity of 1596 compounds. On the basis of screening results, the members of an active sublibrary were resynthesized, providing a weak antagonist of an endogenous ligand binding to a target receptor. The structure−activity relationship (SAR) was explored based on the lead compound by preparing a large number of single compounds.

An olefin metathesis reaction was utilized for the construction of dimer and tetramer mixture libraries (Scheme 27).[62−64] Six diamides **66** were reacted in a parallel manner with a mixture of four *ω*-alkenecarboxylic acids to provide six pools **70** of four compounds. The dimerization of **70** under olefin metathesis reaction conditions afforded six homodimer and 15 homo-/heterodimer sublibraries **71**. Twenty-one sublibraries of **71** contained 600 components and represented a complexity of 1200 isomeric com-

Scheme 27[62−64]



pounds.[62,63] These sublibraries were obtained in 40−80% yields by a simple liquid−liquid-phase extraction with acid/base. The mass spectrum of a representative sublibrary exhibited the molecular ion peaks corresponding to each of all library members. The ^{1}H NMR comparison of a sublibrary with a single library member provided a clear picture for the purity of the mixture.[63] Similarly, 42 diamides **66** were also used as starting precursors for the preparation of 42 homodimer sublibraries **71** with a complexity of 840 compounds. In addition, dimerization of the resulting 42 pools of olefin-substituted triamides randomly in a single step afforded a full homo-/heterodimer complex library **71** of 28 392 compounds. Forty-two diamides **66** were further reacted with *t*-Boc-protected iminodiacetic anhydride and *ω*-alkenecarboxylic acids to provide 42 homodimer pools **72** each containing four compounds. These pools were then subjected in a parallel fashion to the olefin metathesis reaction providing the corresponding 42 tetramer sublibraries **73** with a complexity of 840 compounds. The random combinatorialization of 42 pools **72** under the same conditions in a single step provided a full homo-/heterodimerized tetramer complex library **73** with a complexity of 28 392 compounds (Scheme 27).[63] Two large complex libraries of 10^6 and 10^8 tetramers, as an alkene-reduced form of **73**, were also constructed using the same strategy from four *ω*-alkenecarboxamides and 20 or 10 amines.[64] The combination of the positional scanning and deletion deconvolution protocols provided a powerful method for lead identification from large complex libraries. Potent active compounds in a mouse leukemia cytotoxic assay were found with IC_{50} values of 0.6−0.8 *μ*g/mL. The generation of large complex libraries demonstrated the power of combinatorial chemistry for drug discovery. A variety of complex libraries made from constrained and unsymmetrical scaffolds are further discussed in the following two sections.

4. Complex Libraries from Unsymmetrical Scaffolds

A. SPSAF Combinatorial Strategy and Unsymmetrical Scaffolds

Parallel single-compound combinatorial approaches described in section 2 provide an effective strategy for lead optimization based on known pharmacophores. Indexed combinatorial libraries described in section 3 provide an effective lead optimization approach and may also be used for lead discovery. Generating and screening large complex libraries should enhance the power of combinatorial chemistry to provide new pharmacophores. Libraries obtained by the random combination of a selected set of functional groups with different reactive sites are defined as complex libraries. For example, two or more different functional groups or reagents randomly react with two or more other different reagents or different reactive sites on a scaffold to give a complex library. This approach takes full advantage of the random combination of all possible functional groups, reagents, and reactive sites; therefore, the resulting complex libraries contain all possible combined compounds. Researchers from Isis Pharmaceuticals recently described a combinatorial approach for the generation of complex libraries by simultaneously reacting a mixture of functionalities with several different reactive sites on an unsymmetrical core structure (scaffold) in solution.[65−68] This solution-phase simultaneous addition of functionalities (SPSAF) combinatorial approach was designed primarily for lead discovery by incorporating informationally rich functionalities into various diverse scaffolds. In addition to advantages of solution-phase combinatorial chemistry described in the Introduction, the SPSAF approach also provides a maximum number of compounds in one reaction step. A library can be converted to different libraries by parallel chemical transformations, known as "library from library". Derivatizing the last fixed position of complex libraries with different functional groups provides more complex libraries, therefore, allowing the initial SAR study directly on libraries instead of only single compounds.

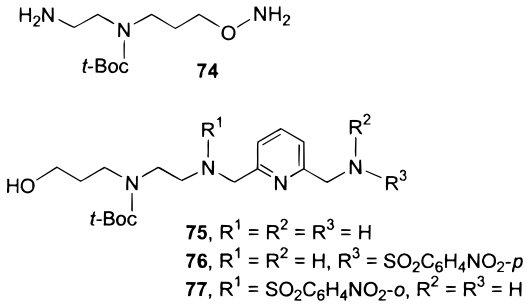


Figure 5. Unsymmetrical linear scaffolds.[69−71]

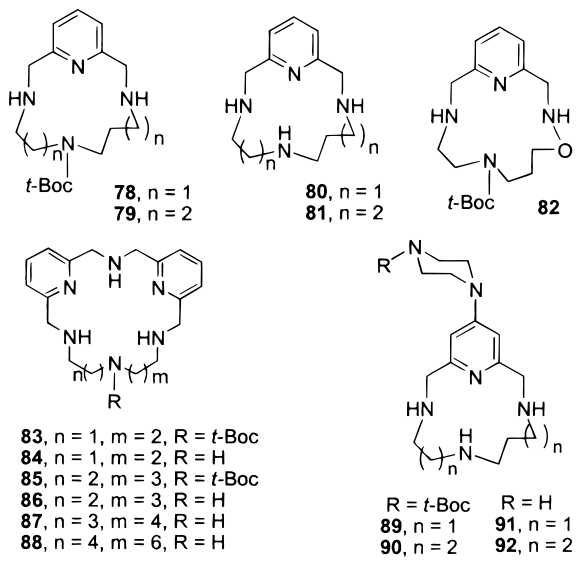


Figure 6. Unsymmetrical polyazamacrocyclic scaffolds.[65−68,72−74]

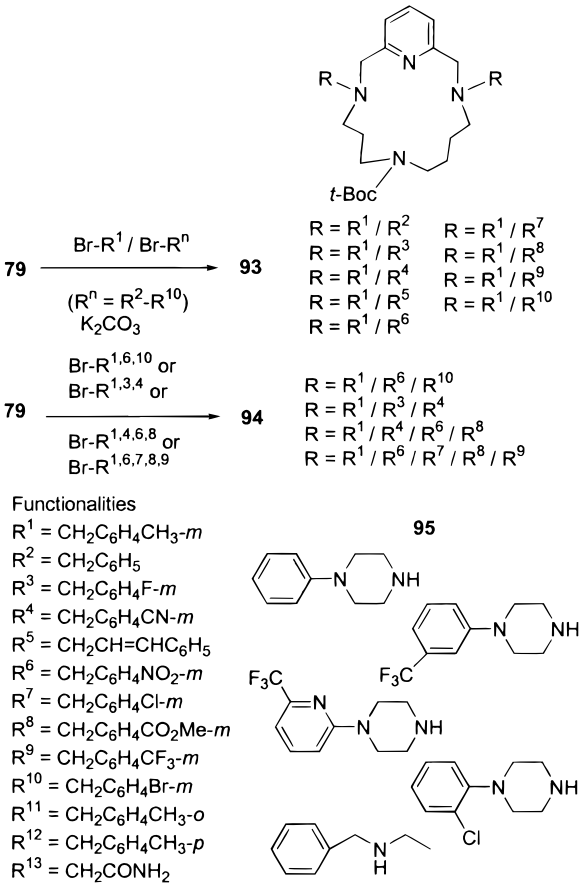
A variety of unsymmetrical scaffolds (Figures 5 and 6), representing a wide range of shapes, including linear, polyazamacrocyclic, and heterocyclic materials, were designed and synthesized. Unsymmetrical linear polyamine scaffold **74** was used as a starting material for the synthesis of more sophisticated scaffolds **75**.[69,70] The orthogonally protected scaffolds **76** and **77** were synthesized for further deconvolution of active libraries generated from scaffold **75**.[70,71]

In addition to the above linear scaffolds, a series of conformationally constrained, unsymmetrical polyazamacrocyclic scaffolds **78**−**92** were also designed and synthesized to explore the different effects of structural space, conformational rigidity, and ring size on their biological activity (Figure 6).[65−68,72−74] The novel cyclization, orthogonal protection, and selective deprotection strategies were utilized for the synthesis of scaffolds **78**−**82**. These strategies also serve as a very efficient way for the synthesis of other polyazamacrocyclic scaffolds. To study the ring size effects on the biological results, unsymmetrical 19−26-membered polyazamacrocyclic scaffolds **83**−**88** (Figure 6)[73] having two pyridine moieties were synthesized by the same strategies. The piperazine-substituted scaffolds **89**−**92**[74] were also synthesized by the similar cyclization and deprotection strategies. The libraries derived from these scaffolds provided a variety of interesting biological activities (see section 4.C).

B. Generation of Complex Libraries

For the combinatorialization of scaffolds **74**−**92** with a variety of functionalities (electrophiles) by simultaneous addition of the selected sets of electrophiles, an approximately equal reactivity of each electrophile is required to obtain undistorted libraries. The *meta*-substituted benzylic bromides (Br−R^{1-10}) (Scheme 28) are expected to have approxi-

Scheme 28[66,75,76]



mately the same reactivity because the different substituents on the *meta*-position have a minimal contribution to the electrophilicity compared with substituents on *ortho*- and *para*-positions. The nitrogenous nucleophiles on the scaffolds are expected to have approximately the same nucleophilicity. Therefore, the libraries generated by simultaneous reaction of these electrophiles with the scaffolds are expected to contain all possible compounds in approximately the same amounts. To confirm this hypothesis, model studies were performed by preparing a series of small libraries from scaffold **79** and 10 benzylic bromides (Br−R^{1-10}) (Scheme 28).[66] The electrophile Br−R^1 was chosen as a standard and combined into pairs with each of the other electrophiles Br−R^{2-10} to separately react with scaffold **79**. In this manner, nine different and comparative small libraries **93**, each containing four compounds, were obtained after chromatographic purification. The quality and structure of these pure libraries were confirmed by TLC, ^{1}H NMR, and ESI (electrospray ionization) MS spectral data. For example, the MS spectrum of library **93** (R = R^1/R^2) provided three

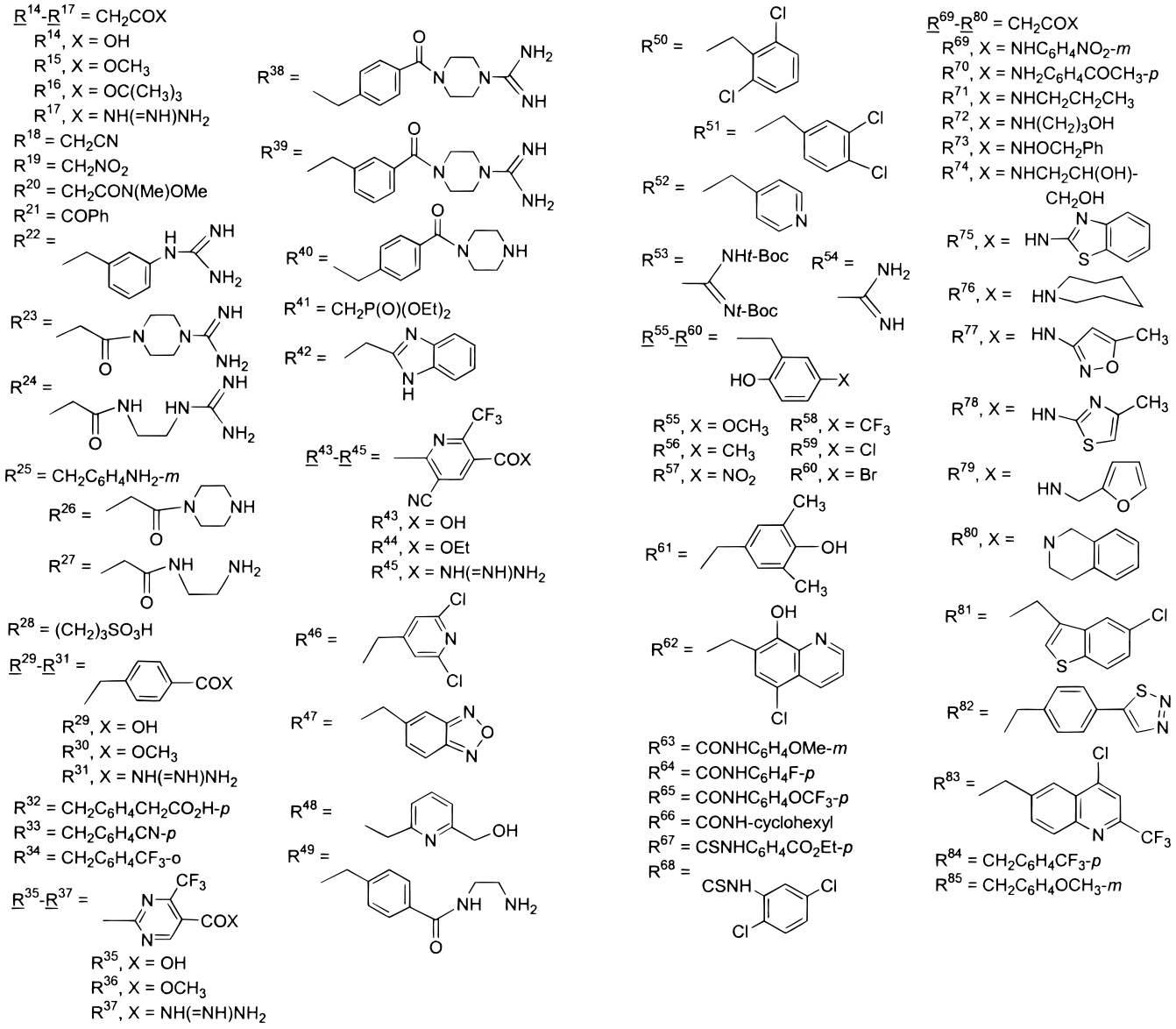


Figure 7. Functionalities for libraries.

peaks at 529, 543, and 557 $(M + 1)^+$ in the ratio of approximately 1:2:1. These three peaks correspond to three theoretical molecular weights of four compounds in the library.

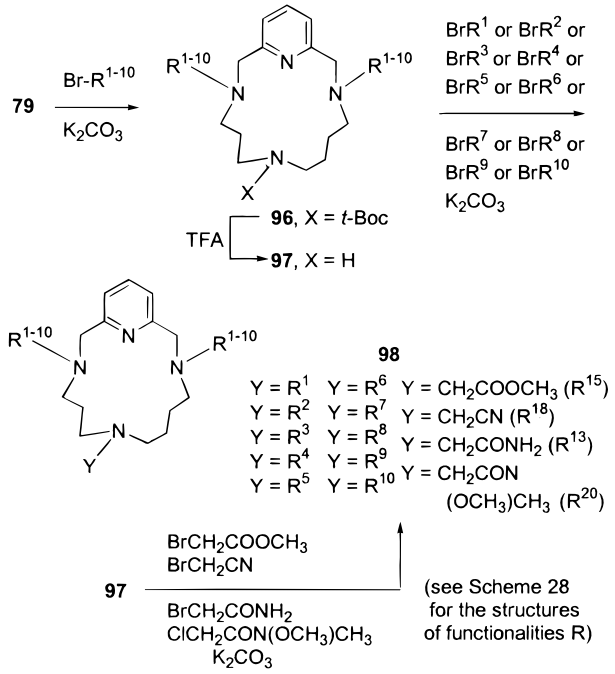
Libraries **93** were all analyzed by capillary zone electrophoresis (CZE) technique. A representative CZE profile of **93** ($R = R^1/R^7$) showed four well-separated peaks at almost the same heights for four expected compounds.[66] The contents of these four compounds have a deviation of 7−15%. This relative ratio is appropriate for biological screening purposes. Other model libraries gave similar CZE profiles. These results indicated that α-bromo-*m*-xylene (Br−R^1) showed approximately the same reactivity as each of the other tested electrophiles. Therefore, all tested electrophiles have approximately the same reactivity under the described reaction conditions. To further substantiate the above conclusion, model libraries **94** were also prepared by the reaction of scaffold **79** with four sets of three, four , and five different electrophiles (Scheme 28) under the same reaction, workup, and purification conditions.[66] Libraries **94** exhibited appropriate TLC, ^{1}H NMR, and ESI MS spectra. The CZE profile of library **94** ($R = R^1/R^6/R^{10}$) indicated that the area percentages of nine peaks deviated 0.2−12% from the expected value. The results further indicated that these electrophiles have similar reactivity, and the possible reactivity difference exhibited little influence on final libraries.

Phenyl piperazine scaffolds **95** were utilized to prepared model libraries, which were studied by ESI MS and CZE techniques (Scheme 28).[75] The results indicated that CZE and ESI MS are useful tools for monitoring and analyzing combinatorial libraries to ensure that all members of a designed library are equally represented. The reactivities of other electrophiles corresponding to the functionalities listed in Figure 7 were studied by their comparative libraries and grouped into different sets.

A variety of complex libraries were synthesized by combinatorializing the unsymmetrical polyazamacrocyclic, linear, and heterocyclic scaffolds (Figures 5 and 6), having 2−4 different reactive sites, with over 80 diverse functionalities using the SPSAF combi-

natorial approach. The "fix-last" strategy was used for the synthesis of libraries from mono-*t*-Boc-protected scaffolds, which would benefit the further deconvolution of active libraries and SAR studies directly on libraries. Some larger libraries were prepared from the unprotected scaffolds to increase the diversity. Scheme 29 illustrates a representative

Scheme 29. Preparation of complex libraries[65,66]



example for the generation of libraries **96**−**98** by the "fix-last" strategy. The mono-*t*-Boc-protected scaffold **79** was reacted with a mixture containing equimolar amounts of 10 benzylic bromides Br−R^{1-10} (2.4 equiv total) in one pot.[65,66] The resulting *t*-Boc-protected library **96** was obtained in 94% yield after chromatographic purification. Library **96** containing 100 (10^2) compounds was verified by ^{1}H NMR and ESI MS spectral data. Deprotection of library **96** with trifluoroacetic acid (TFA) gave the corresponding library **97** in 93% yield after chromatographic purification. The secondary amine in library **97** was further reacted in a parallel fashion with 10 different functionalities Br−R^{1-10} under similar conditions as described above, affording 10 different sublibraries **98** (Y = R^{1-10}) in 60−98% yields after preparative thin layer chromatographic (PLC) purification.

With the key intermediate library **97** containing one reactive site in hand, initial structure−activity relationship (SAR) studies were performed on the mixture by introducing different functionalities at the fixed position (Y).[66] The reaction of **97** with other electrophiles under similar reaction and purification conditions afforded the corresponding libraries **98** (Y = $R^{13,15,18,20}$) in 95−98% yields. All libraries described above were prepared in large quantities (200−3000 mg), monitored by TLC, purified by chromatographic techniques, and confirmed by their ^{1}H NMR and ESI MS spectral data. The availability of key intermediate libraries as well as final sublibraries for broader screening and further reactions is a major advantage of the SPSAF process.

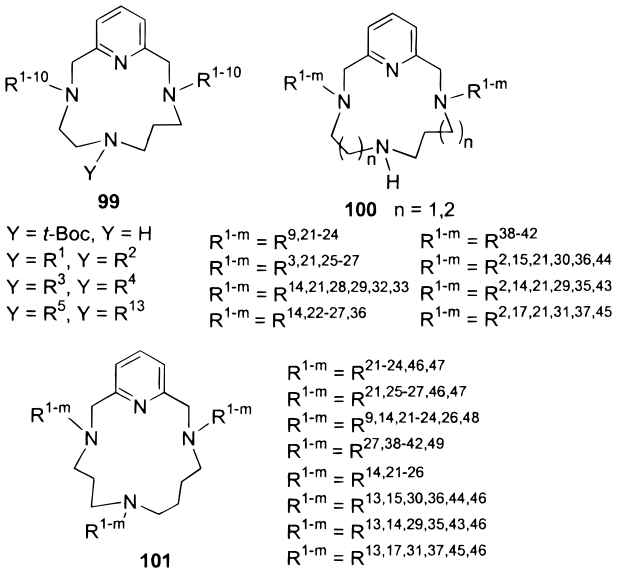


Figure 8. Polyazamacrocyclic libraries.[72]

The same strategy was also used for the preparation of libraries **99** from scaffold **78** and 10 benzylic bromides (Br−R^{1-10}) (Figure 8).[72] Each of libraries **99** contains 100 compounds. With the intermediate libraries **97** and **99** (Y = H) available, one can readily prepare many other libraries by attaching other functional groups on the fixed position (Y) to explore additional structural space and biological activities. This represents the advantage of the "fix-last" strategy. Libraries **100**, containing various polar functionalities (Figure 7), were prepared from scaffolds **78** and **79** (Figure 6) to increase the diversity and polarity of the resulted libraries.[72] On the basis of the "library from library" concept, two ester libraries in **100** (n = 1, 2; $R^{1-m} = R^{2,15,21,30,36,44}$) were hydrolyzed to the corresponding new acid libraries **100** (n = 1, 2; $R^{1-m} = R^{2,14,21,29,35,43}$) and were also reacted with guanidine to provide the corresponding new sets of acylguanidine libraries **100** (n = 1, 2; $R^{1-m} = R^{2,17,21,31,37,45}$). The same sets of functionalities employed for libraries **100** were also utilized to combinatorialize scaffold **81** providing the corresponding polar libraries **101**. As described above, carboxylic acid and acylguanidine libraries were also derived from the corresponding ester libraries in the **101** series. The complexities of **100** ranged from 25 to 64, while the complexities of **101** ranged from 216 to 343 because the numbers of combinatorial sites on scaffolds **78**/**79** and **81** are different. From the generation of polar libraries **100** and **101**, it can be concluded that the diversity, complexity, polarity, total number of libraries, and, consequently, the properties of libraries can be easily designed and controlled. Therefore, the vast numbers of diverse libraries can be used to explore a wide range of biological activities and accelerate drug discovery.

Piperazine-substituted scaffolds **89**−**92** (Figure 6) were designed and synthesized to explore three-dimensional structural effects compared with scaffolds **78**−**81**. Scaffold **90** was combinatorialized with 10 bromides (Br−$R^{1-4,6-10,18}$) by the SPSAF approach as demonstrated in Scheme 29 to provide library **102**

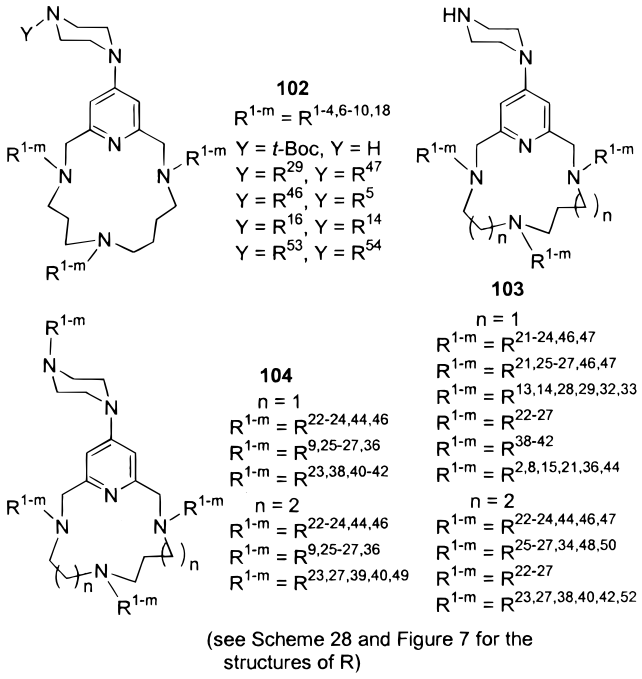


Figure 9. Polyazamacrocyclic complex libraries.[74]

(Y = H) containing 1000 compounds (Figure 9).[74] Different functional groups were attached at the fixed 4′-piperazinyl position (Y) to increase diversity. Library **102** (Y = H) was reacted with 1,3-bis(*tert*-butoxycarbonyl)-2-methylthiopseudourea to give *t*-Boc-protected guanidine library **102** (Y = R^{53}), which was then deprotected to provide library **102** with an amidine group (R^{54}) at the fixed position (Y). To increase the polarity and diversity of libraries, scaffolds **89** and **90** (Figure 6) with 13- and 15-membered ring systems were combinatorialized with different sets of six functionalities to give final polar libraries **103** with hydrogen (H) at the fixed position (Y) (Figure 9).[74] Each of these libraries contained 216 (6^3) compounds. Unprotected scaffolds **91** and **92** with four reactive sites (Figure 6) were separately combinatorialized with three sets of functionalities (five in each set). Deprotection of *t*-Boc-protected libraries provided the corresponding final libraries **104** (Figure 9). Each of these libraries contained 625 (5^4) compounds.

In addition to 13-, 14-, and 15-membered macrocyclic scaffolds described above, polyazadipyridinophane scaffolds **83**−**88** (Figure 6), having 19−26-membered ring systems, were also designed and synthesized to further study large ring-size effects on the biological activity of resulting libraries.[73] Libraries **105** (Figure 10) were prepared from scaffold **83** by the SPSAF approach as illustrated in Scheme 29.[73] Substituted phenolic functionalities R^{55-62} (Figure 7) were successfully introduced to the fixed position (Y) by the modified Mannich reaction of **105** (Y = H) with the corresponding phenolic derivatives to give phenolic libraries **106** (Y = R^{55-62}). Each of libraries **105** and **106** contained 1000 compounds (10^3). Libraries **107**, each having a complexity of 625 (5^4) compounds, were prepared from unprotected scaffold **84** (Figure 6) as described above after deprotecting the corresponding intermediate *t*-Boc-protected libraries. Isocyanate and thioisocyanate derivatives were also used for the preparation of library **107** having urea and thiourea functional groups R^{64-68} (Figure 7). Isocyanates and thioisocyantes can be used together with bromide functionalities by the SPSAF approach, which was illustrated by library **107**. Introduction of urea/thiourea functional groups expanded the application of the SPSAF approach for the preparation of other types of libraries by mixing different types of functional groups.

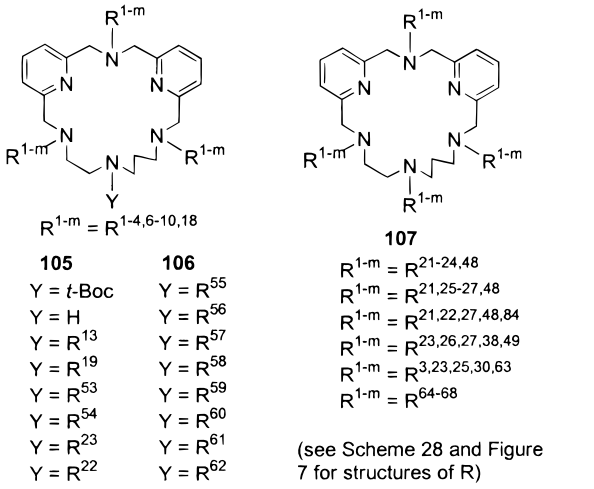


Figure 10. Polyazamacrocyclic complex libraries.[73]

Figure 11 lists the libraries **108**−**110** generated from unsymmetrical linear scaffolds **74** and **75** (Figure 5). Scaffold **75** was combinatorialized with six benzylic bromides (Br−$R^{1-3,6,8,9}$) (Scheme 28) by the SPSAF approach to provide libraries **108** (Y = *t*-Boc).[70] After deprotection, intermediate library **108** (Y = H) was reacted in a parallel fashion with Br−$R^{1-3,6,8,9,15,18,13}$ and Cl−R^{20} (Scheme 28 and Figure 7), providing other corresponding libraries **108** with different functional groups at the fixed position (Y). Model studies based on scaffold **75** and the preparation of libraries **108** indicated that the two primary amine and one secondary amine reactive sites can be effectively combinatorialized by the SPSAF approach to provide high-quality libraries. Libraries **108** have a complexity of 126.[70] Libraries **109** (Figure 11) were prepared as described above by combinatorializing unsymmetrical linear scaffold **74** with three

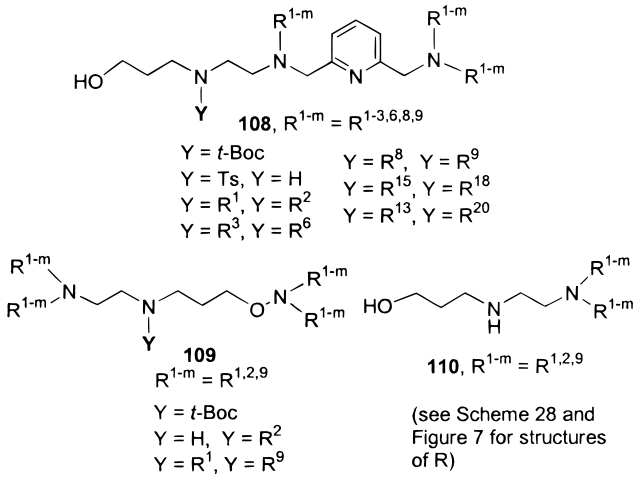


Figure 11. Linear complex libraries.[69,70]

benzylic bromides (Br−$R^{1,2,9}$).[69] The reduction of **109** (Y = H) with borane cleaved the hydroxyamine N−O bond to provide a different library **110**. Pyrimidine and purine libraries were also generated by the SPSAF approach, and several libraries and compounds exhibited antibacterial activity.[77,78]

C. Biological Evaluation and Deconvolution

The libraries described above were tested in bacteria *Streptococcus pyogenes* and *Escherichea coli imp*$^-$ antigrowth assays and HIV-1 tat/TAR (transactivater/transactivating responsive) protein−RNA disrupting scintillation proximity assays (SPA) by high-throughput screening. When selected benzyls were used as functional groups, six libraries of **99** series made from the 13-membered polyazaphane scaffold exhibited potent antibacterial activities in the MIC (minimum inhibitory concentration) range of 1−25 μM for two bacterial assays.[72] Less active libraries were seen in the corresponding libraries **96**−**98** made from the 15-membered scaffold.[66,72] The libraries with hydrogen, *m*-cyanobenzyl (R^4), and cinnamyl (R^5) groups at the fixed position (Y) from both 15- and 13-membered scaffolds were active against two bacteria. Clearly, the ring size had an important effect on biological activity, with the libraries from the more rigid 13-membered scaffold having broader and more potent activities. A molecular modeling study indicated that the intramolecular hydrogen bond, observed only in the compound with the 13-membered ring, orients the aromatic groups to the most favored stacked conformation.[72] Therefore, the different activities may be due to the overall shape difference and conformational preference. Initial biological results for diverse and polar libraries **100** and **101** (Figure 8) were also briefly discussed, and a crude rank order of activity among functionality sets (R^{1-m}) was observed as guanidine > amines > amines > acids.[72]

Biological studies of libraries **102**−**107** (Figures 9 and 10), made from piperazinyl-substituted scaffolds **89**−**92** and dipyridine scaffolds **83** and **84** (Figure 6), indicated that libraries **102** with hydrogen and amidine (R^{54}) groups at the fixed position (Y) exhibited antibacterial activity against *S. pyogenes* and *E. coli* with MICs of 4−20 and <20 μM.[74] Libraries **103** having more polar and diverse functional groups showed more activities. The active libraries were more potent against Gram-positive bacterium *S. pyogenes* than Gram-negative bacterium *E. coli*. *t*-Boc-protected libraries as well as acid libraries were not active in all assays. Libraries **104** made from the 13-membered scaffold **91** exhibited more potent activity than the similar libraries made from the 15-membered scaffold **92**. Guanidine and amine libraries in **103** and **104** series disrupted HIV-1 tat/TAR protein−RNA interaction with IC_{50}s as low as 80 and 90 nM.

Libraries **105** (Figure 10),[73] having hydrogen and guanidine groups (R^{22}, R^{23}) at the fixed position, exhibited potent antibacterial activity against *S. pyogenes* and *E. coli imp*$^-$ with MICs of 2−20 μM and tat/TAR activity. The libraries with *t*-Boc, *t*-Boc-protected, phenolic (**106**), and other functional groups at the fixed position (Y) were not active. The results indicate that increasing the polarity of libraries is essential. Indeed, all polar libraries **107**,[73] containing guanidine, amine, hydroxyl, and amide functional groups, exhibited antibacterial activity with MICs of 2−50 μM. The most active library from this series exhibited antibacterial activity for two assays with MICs of 2−5 μM. One library exhibited the best activity in tat/TAR SPA with an IC_{50} of 80 nM. Screening linear libraries **108**−**110** provided similar conclusions. Libraries having hydrogen or functional groups R^2, R^{18}, R^{13}, and R^{20} (Figures 7 and 11) at the fixed position (Y) exhibited antibacterial activity with MICs of 1−5 to 12−25 μM.[69,70]

The deconvolution procedure is a strategy designed to quickly identify active lead compounds from active libraries. Lead compounds can be identified by systematically deconvoluting an active library chemically or by an HPLC fractionation strategy. A 1638-member linear library has been iteratively deconvoluted by a chemical synthesis strategy, providing a series of potent antibacterial compounds with high selectivity for Gram-positive bacteria over Gram-negative.[71] The first-round iterative sublibraries **108** (Figure 11), each containing 126 compounds, have different functional groups at the fixing position A (Figure 12). The active library **108** (Y = H) with

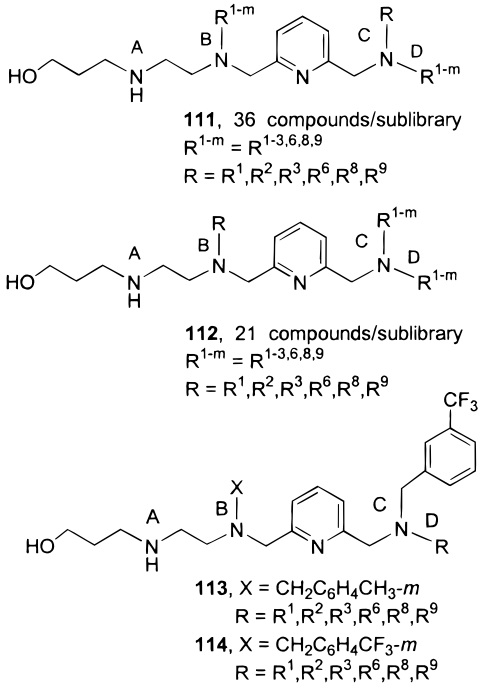


Figure 12. Sublibraries and single compounds.[70,71]

hydrogen at position A was selected to be deconvoluted. The positional scanning procedure was employed for the second-/third-round deconvolution starting from the orthogonally protected scaffolds **76** and **77**.[71] Screening the second- and third-round sublibraries **111** and **112** provided two series of single active compounds **113** and **114** (Figure 12).[71]

Single compounds **113** and **114** resulting from the complete deconvolution were screened against a tier II panel of bacteria consisting of *S. pyogenes*, *S. pyogenes* (wild type), *S. aureus*, *E. faecalis*, *E. coli*

imp$^-$, *E. coli* (wild type), *K. pneumoniae*, *P. vulgaris*, and *P. aeruginosa* as well as yeast *C. albicans* for an indication of specificity.[71] The data indicated that the deconvoluted compounds **113** and **114** have significantly more potent activity and greater selectivity for Gram-positive bacteria compared to the corresponding compounds with uniform functional groups. The deconvoluted compounds exhibited potent, highly selective activities for Gram-positive bacteria *S. pyogenes*, *S. pyogenes* (wild type), *S. aureus*, and *E. faecalis* with MIC values of 1−6 μM over the tested Gram-negative bacteria (MIC > 100 μM). They also exhibited very high specificity for bacteria compared with the yeast *C. albicans* (MIC > 100 μM). The combination of iterative and positional scanning procedures provided a useful deconvolution strategy to identify lead compounds from active libraries.[71]

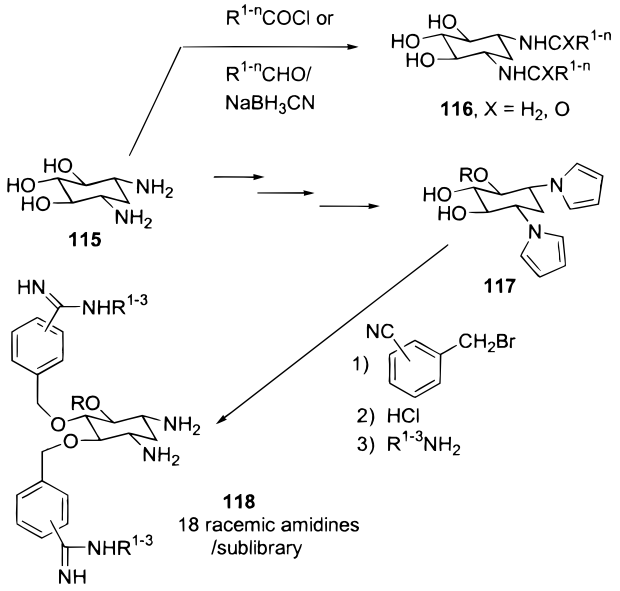
An HPLC fractionation strategy[79] has been developed for the deconvolution of complex libraries generated by the SPSAF combinatorial approach. This rapid deconvolution strategy greatly increased the efficiency for identifying lead compounds from active complex libraries and expanded the application of the SPSAF approach for the discovery of new leads and new pharmacophores. One of the polyazapyridinophane libraries **100** (n = 1) (Figure 8) containing 25 compounds was separated into eight fractions by HPLC chromatography.[79] The fractions were dried and screened for their ability to inhibit the growth of *E. coli imp*$^-$ and *S. pyogenes*. A single fraction 8 inhibited the growth of *S. pyogenes* over 90% at 5 μM, while fractions 7 and 8 demonstrated high inhibition against *E. coli* at 20 μM. Mass spectrometric analysis demonstrated that fraction 8 contained a single compound with a molecular mass of 537.3 Da. The structure of this compound was established as a polyazaphane derivative of scaffold **80** (Figure 6) having two *m*-trifluoromethylbenzyl groups (R^9) on two pyridine-2,6-methylamine nitrogen positions and a hydrogen at the fixed position (Y). A library containing 100 compounds was also separated by HPLC. The active fraction containing 11 compounds was further separated by different solid-support columns to find the active compound.[79]

One of the polar libraries **103** containing 216 compounds, inhibiting transcription/ translation at 5 μM and bacteria at 10 μM, was screened by a mass spectrometry-based assay.[80] The assay provided the most stable complex between 27-mer RNA and library members. Dissociation of the complex generated a ligand (753.5 Da) which was consistent with the structures of six possible isomeric compounds in the library, having the combination of two functional groups on three positions. This assay provides the fast identification of RNA-binding ligands from complex libraries and greatly simplifies the deconvolution procedure. Utilizing the SPSAF combinatorial approach to generate diverse complex libraries, combined with the rapid HPLC fractionation and mass spectrometry-based RNA-binding deconvolution strategies to identify lead compounds, offers an alternative avenue, complimentary to solid-phase combinatorial approaches, and will greatly accelerate the drug discovery process.

D. Other Complex Libraries from Unsymmetrical Scaffolds

Other investigators have utilized similar combinatorial approaches described in the previous section for the generation of complex libraries from unsymmetrical scaffolds. Wuonola and co-worker[81] reported complex libraries generated from 2-deoxystreptamine **115** to mimic aminoglycosides in a search for the inhibitors of protein−RNA interactions. Compound **115** was acylated with different mixtures of carboxylic acid chlorides or reacted with aldehydes followed by reduction to give the corresponding amide or amine complex libraries **116** (Scheme 30). A series

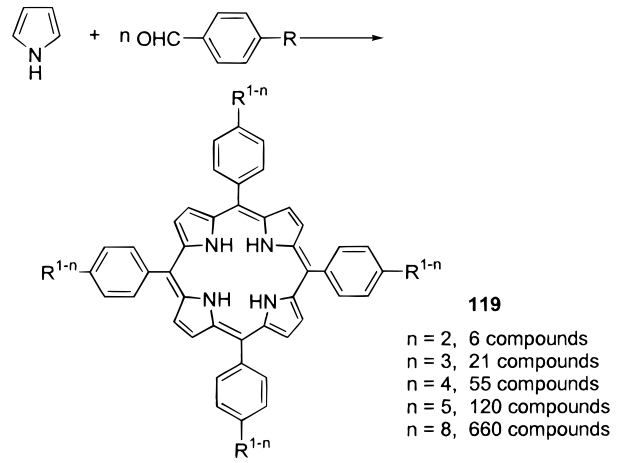
Scheme 30[81]



of compounds **117** with different R groups was prepared from **115** and then reacted with cyanobenzyl bromides. The resulting cyano compounds were reacted with mixtures of amines to give amidine complex libraries **118**. When one cyano compound and three amines were used in this process, the complex sublibrary **118** contained 18 amidines. The libraries were purified by chromatography and confirmed by mass spectrometry. By using these procedures, several thousand compounds were generated from the 2-deoxystreptamine scaffold **115**.

Richert and co-workers prepared tetraphenylporphyrin complex libraries **119** by a solution-phase combinatorial approach (Scheme 31).[82] Mixed-aldehyde (n = 2, 3, 4, 5, 8) Rothemund cyclization, followed by DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) oxidation, provided libraries **119** with complexities of 6, 21, 55, 120, and 660 tetraporphyrin derivatives in yields of 9−30%. The libraries were treated with a silica gel/chloroform suspension at 60 °C to remove byproducts. Two libraries **119**, containing 21 and 55 compounds (n = 3, 4) with ester groups, were hydrolyzed quantitatively to new libraries. The mass spectrum of a 55-member library exhibited most of the 35 nonisobaric molecular ion peaks. The more complex libraries were confirmed by matching the peak patterns of calculated and experimental mass spectra. It was concluded that the low-yielding

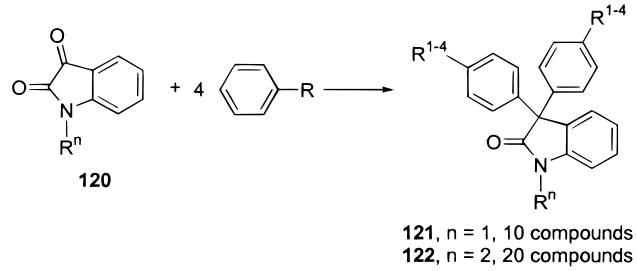
Scheme 31[82]



solution-phase reactions are not likely to effect the quality of complex libraries. The authors indicated that a modern anticancer treatment modality was successfully performed with a complex mixture of porphyrins. The application of complex libraries directly in biological systems is of considerable interest for future research.

Klumpp and co-workers[83] reported the preparation of 3,3-diaryloxindole complex libraries (Scheme 32).

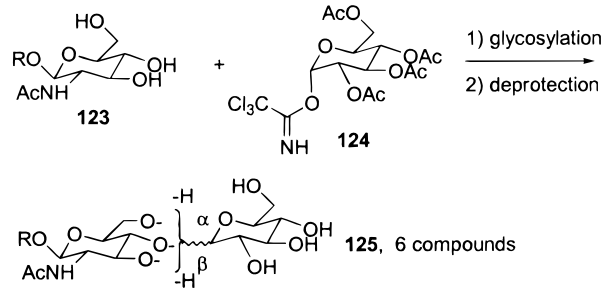
Scheme 32[83]



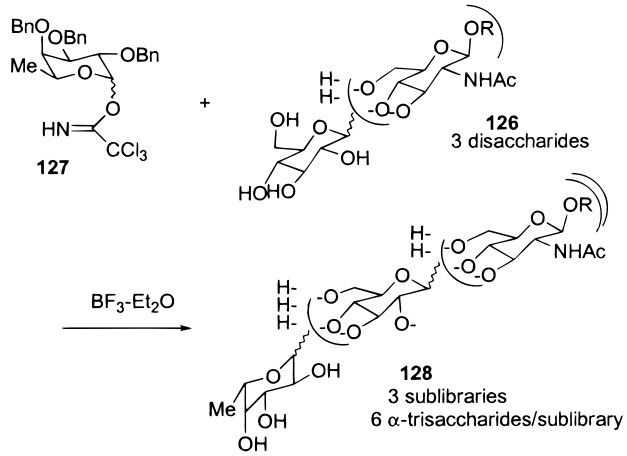
When *N*-methylisotin **120** was reacted with a mixture of four different aromatic substrates, a complex library **121** was obtained, which contained 10 nonisobaric products. The GC-MS analysis of the library provided 10 peaks. When a mixture of two isotins (R = Me, Ph) was combinatorialized with four aromatic substrates, a complex library **122** was formed. These 12 library members in **122** were confirmed by GC-MS analysis. The method for the preparation of library **122** doubled the complexity of the library, which would be utilized to generate other complex libraries.

Oligosaccharide complex libraries have been generated by different approaches.[84] Hindsgaul and co-workers developed a solution-phase random glycosylation strategy to prepare disaccharide (Scheme 33) and trisaccharide (Scheme 34) complex libraries.[85] Three hydroxyl groups of the unprotected glycoside **123** were reacted with peracetylated galactopyranosyl imidate **124** (Scheme 33).[86] Deprotection of the intermediate library gave library **125** containing all six possible anomeric disaccharides (α/β). The HPLC analysis of **125** indicated six peaks with a distribution of 2−36%. The same strategy was also used to generate trisaccharide complex libraries **128** (Scheme

Scheme 33[85,86]



Scheme 34[85,87]



34).[87] Three disaccharides **126**, each having five different hydroxy groups, were α-fucosylated in a parallel manner with tribenzylfucopyranosyl imidate **127**. Deprotection of the intermediate sublibraries provided three sublibraries **128**. Each of sublibraries **128** contained six possible α-anomeric trisaccharides with distributions ranged from 8% to 23%. β-Anomers (5−20%) were observed in those sublibraries. These libraries can be generated in one step to obtain all possible compounds instead of multistep manipulating different protecting groups in a parallel single-compound synthesis. Utilizing all available mono- and disaccharide building blocks would generate millions of disaccharides, trisaccharides, and higher order oligosaccharides. Therefore, the random glycosylation strategy could significantly accelerate the initial phase of a search for new ligands. Boons and co-workers[86] reported the assembly of branched and linear trisaccharide libraries using the latent-active glycosylation strategy (scheme not shown). A mixture library was purified by size exclusion chromatography and verified by mass spectrometric analysis.

Sanders and co-workers reported an unusual macrocyclic library **131** (Figure 13).[89] Thermodynamically controlled transesterification of cinchonidine **129** and quinine monomer **130** provided a macrocyclic complex library **131**. ES MS analysis indicated that the library contained 11 compounds. One heterodimer, all four trimers, and six tetramers were observed in MS. Panunzio and co-workers[90] reported a solution-phase library of perhydrooxazin-4-ones (scheme not provided). In addition to normal six-membered cyclic compounds, four side products in the libraries were also identified, analyzed, and characterized.

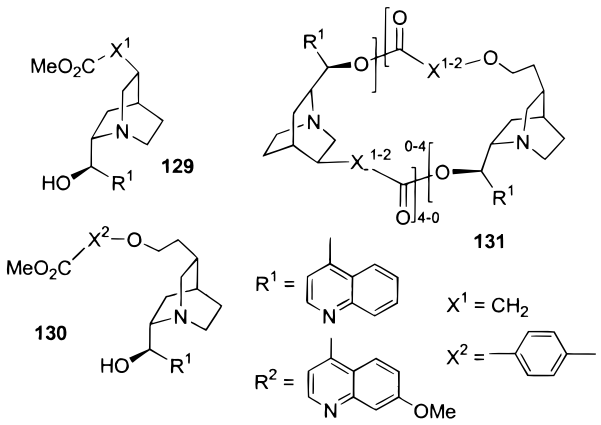
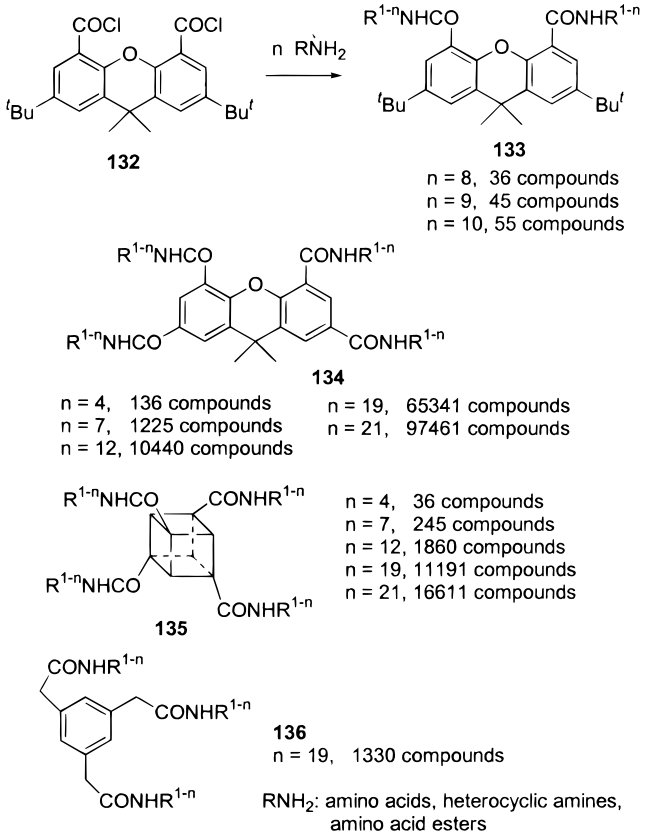


Figure 13.[89]

5. Complex Libraries from Constrained Symmetrical Scaffolds

Rebek and co-workers[91−94] synthesized rigid core molecules as scaffolds or templates for complex library generation. One scaffold possessing multiple reactive functional groups was reacted with a mixture of small reactive organic molecules. The resulting complex library, obtained in one step, presumably contained all possible library members. A series of amino acid derivatives and other heterocyclic amines having primary amino groups were utilized as building blocks. The xanthenedicarboxylic acid dichloride **132** was combinatorialized with a 2-equiv mixture containing equimolar amounts of 8, 9, or 10 selected amino building blocks (Scheme 35). The reaction mixture was washed with citric acid and aqueous sodium hydrogen carbonate to provide library **133** containing 36, 45, or 55 compounds. When esters of amino acids were used as building blocks, the resulting libraries were then hydrolyzed to give final water-soluble acid libraries. The libraries were precipitated from ether/hexanes to give white powders. This represents an attractive method for generating large complex libraries even though the complexity of libraries decreased from 64 (8^2), 81 (9^2), and 100 (10^2) for unsymmetrical scaffolds to 36, 45, and 55 because of the symmetry of the core template. The same procedure and building blocks were utilized for the generation of libraries **134**−**136**. Xanthene and cubane tetracarboxylic acid tetrachlorides were separately combinatorialized with 4-equiv mixtures containing equimolar amounts of 4, 7, 12, 19, and 21 building blocks. Xanthene libraries **134** contained 136, 1225, 10 440, 65 340, and 97 460 compounds corresponding to the sizes of building block sets. Cubane libraries **135** contained fewer compounds corresponding to the same numbers of building blocks because the cubane scaffold has a higher symmetry than the xathene scaffold. Library **136** having 1330 compounds was generated from the corresponding tricarboxylic acid trichloride and a set of 19 building blocks. Several libraries, obtained from the methyl ester of amino acids, were converted to the corresponding hydrazide and hydroxyl amide libraries by hydrazinolysis and hydroxy aminolysis.

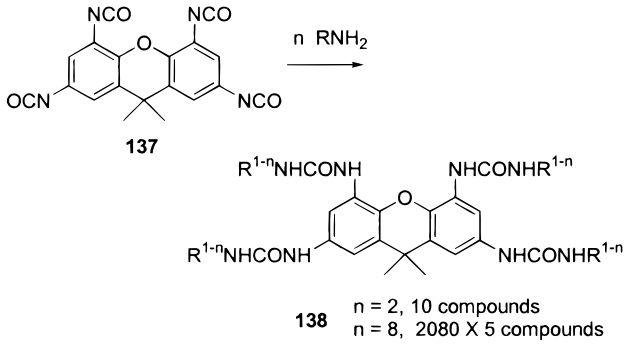
Scheme 35[91−94]



The ESI mass spectrum of a 55-member library depicted 43 peaks, 78% of the total mass number. Screening the complex libraries described above indicated that xanthene-based library **134** ($n = 19$) significantly reduced enzyme activity. An iterative deconvolution procedure was used to identify lead compounds from the library. These 19 building blocks were sequentially subtracted in a seven-round synthesis of sublibraries. The final active compounds were identified and synthesized. This library generation and iterative screening procedure may be considered as a valuable tool in searching for lead compounds and a useful means of exploring the structure landscape.

Combinatorialization of xathene tetraisocyanate **137** (Scheme 36) with two amino acid esters afforded a tetraurea library **138** containing 10 compounds.[95] An HPLC analysis of this model library gave 10 distinct peaks corresponding to the 10 possible compounds in the library. Scaffold **137** was also

Scheme 36[95,96]



reacted with five sets of eight amino acid esters providing five sublibraries **138** each having a complexity of 2080 tetraurea compounds. A subtractive deconvolution procedure was used, and two DNA intercalators were identified from the large library.[96] This strategy could be used to generate additional complex libraries from other multiple functionalized scaffolds synthesized by the same laboratory.[97]

Lansbury and co-worker[98] constructed a complex library using carbohydrates as building blocks (Figure 14) and a 1,3,5-benzene tricarboxylic acid trichloride as a scaffold. A mixture of three glycosamino esters was utilized to prepare a complex library **139** containing 10 glycotides. Mass spectrometry confirmed the existence of 9 out of 10 library members. Ten diketopiperazine amide complex libraries **47** and **48** were generated from the corresponding multiple carboxylic acid scaffold (Scheme 16).[41]

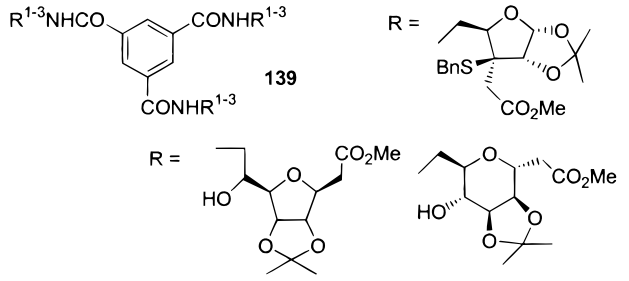


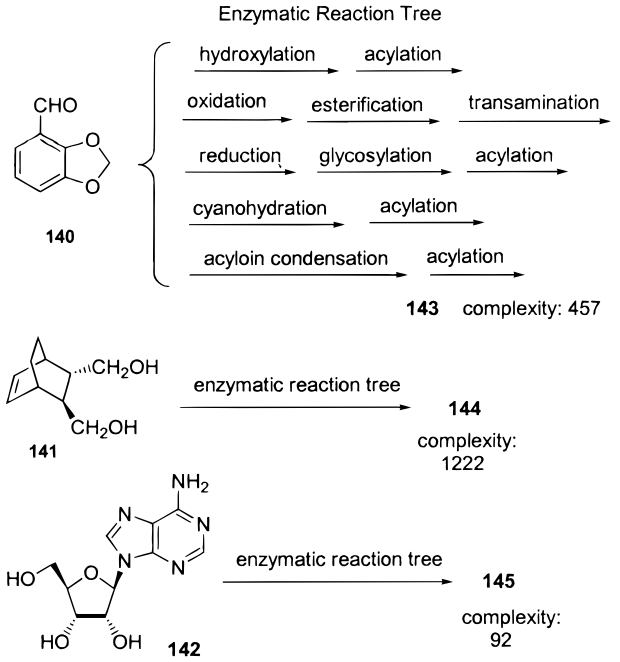
Figure 14.[98]

6. Libraries by Chemoenzymatic Approach

Chemoenzymatic synthesis, also known as biocatalysis, has emerged as a new combinatorial approach to generate libraries.[99−103] This approach provides a method for synthesizing libraries of organic compounds from existing lead compounds in solution. The reactions are catalyzed by enzymes and microorganisms with high specificity and regioselectivity under mild conditions without formation of byproducts. Enzymes accept a wide variety of substrates with different functional groups. Different types of enzymes recognize different functional groups or structural elements; therefore, different enzymes are used to modify functional groups and extend the carbon skeleton. Three major categories of 50 different types of enzymatic reactions are available, and nearly all organic molecules are theoretically potential substrates for combinatorial library generation. The chemoenzymatic combinatorial approach can be used to generate multischemed parallel single-compound and complex mixture libraries ranging from small organic molecules to polyfunctional structures and complicated natural products.

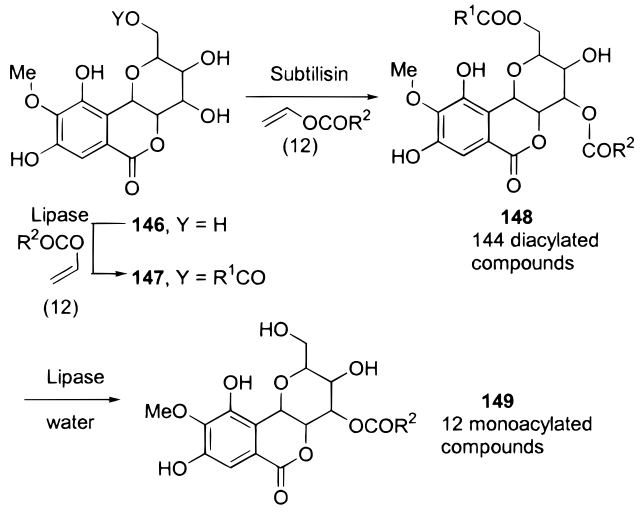
Khmelnitsky and co-workers at EnzyMed selected compounds **140**−**142**, having different spatial structures, as substrates to demonstrate library generation by chemoenzymatic synthesis (Scheme 37).[99] Over 50 available enzymes were employed for 12 different types of reactions for modification of substrate **140**. Library **143** containing 457 derivatives was generated in a parallel fashion through this enzymatic reaction tree. The bicyclo compound **141** and adenosine **142** were also modified by similar enzymatic reaction trees. The resulting libraries **144** and **145** have complexities of 1222 and 92 compounds, respectively. This approach has also been used for the modification of natural products. For example, acylating hydroxyl groups at different positions of taxol provided a library of 200 taxol derivatives (scheme not provided).[99−102]

Scheme 37[99]



EnzyMed's group also reported regioselective, enzymatic acylation of polyhydroxylated flavoid, bergenin **146**, for the generation of libraries (Scheme 38).[100,101,103] After screening more than 50 enzymes,

Scheme 38[100,101]

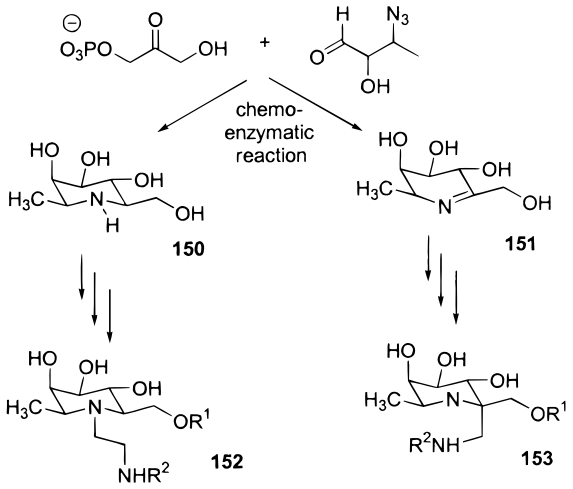


11 were identified and selected for acylation of bergenin **146**. The primary hydroxyl group at position 11 of **146** was acylated in a parallel manner with 12 different activated acylating agents by using a mixture of four lipases as enzymatic catalysts. The resulting 12 monoacylated compounds **147** were subjected to a Subtilisin-catalyzed acylation by 12

acylating agents. The secondary hydroxyl group at position 4 was acylated to provide library **148** containing 144 diacylated bergenin derivatives. The acyl group at position 11 of **148** was regeoselectively hydrolyzed by lipase in the presence of water to give 14 monoacylated bergenin derivatives **149**. This chemoenzymatic acylation tree was performed in a 96-well plate format.

Wong and co-workers[104,105] at the Scripps Research Institute utilized chemoenzymatic strategies to synthesize iminocyclitol derivatives **150** and **151** (Scheme 39). Several parallel single-compound libraries **152**

Scheme 39[104,105]



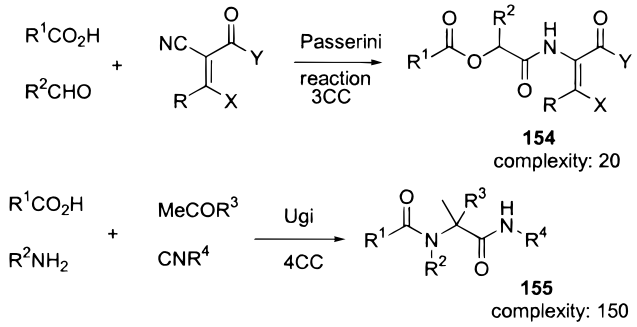
and **153** were generated from scaffolds **150** and **151**. More potent and selective fucosidase inhibitors were identified. Scaffolds **150** and **151** may be further utilized for iminocyclitol library generation and for further searching of new fucosidase and fucosyltransferase inhibitors.

7. Libraries by Multicomponent Reaction (MCR) Approaches

The MCRs utilize three or more starting materials in a single reaction vessel to give a new product which incorporates the components of all starting materials. A number of three- to seven-component reactions (3−7CRs) are available to rapidly generate diverse libraries. The MCR approach has been applied to the generation of parallel single-compound and complex mixture librries by solid- and solution-phase methods.[106] The laboratories of Ugi[107−111] and Armstrong[112,113] published extensively on the application of MCRs for library preparation. The readers can refer to the above articles for MCRs published before 1997. The current review covers the combinatorial libraries generated in solution by the MCR approach published since 1997.

To perform SAR studies of antitumor antibiotics, azinomycins A and B, Armstrong and co-workers[114] synthesized a dehydroamino acid library **154** using a Passerini three-component condensation (3CC) approach in solution (Scheme 40). Thus, the reaction of selected carboxylic acids, aldehydes, and isocyanides provided 20 single compounds in 30−90% yields after chromatographic purification. Five variable

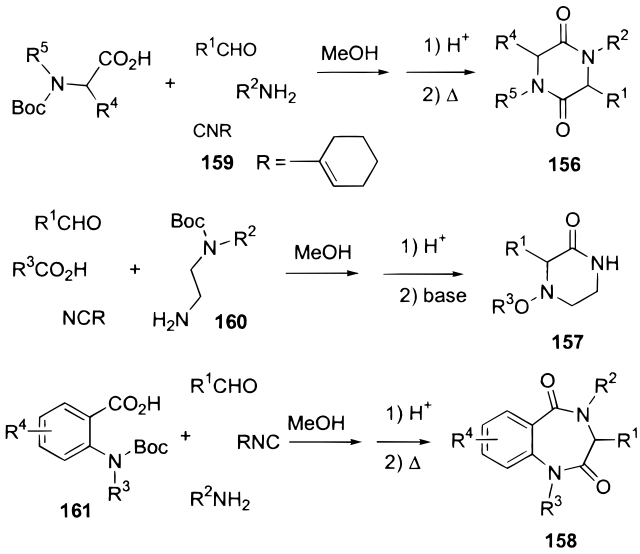
Scheme 40[114,115]



groups were introduced to increase the diversity of the library. The peptidomimetic library **155** was generated by an Ugi four-component reaction (4CR) (Scheme 40).[115] Several α-methylated amino acids were synthesized in solution. A solid-phase method was also utilized to generate the similar libraries but in lower yields compared with the solution-phase procedure.

Hulme and co-workers utilized Ugi 4CC reactions to generate diketopiperazine, ketopiperazine, and 1,4-benzodiazepine-2,5-dione libraries **156**−**158** by a solution-phase approach (Scheme 41).[117−120] The Ugi

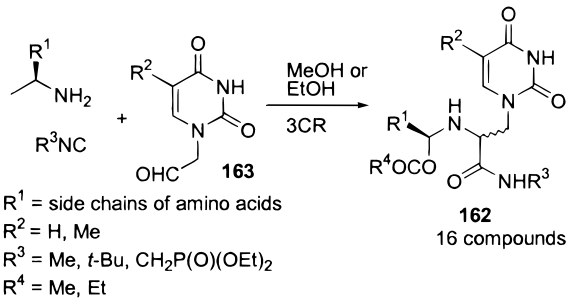
Scheme 41[117,120]



4CC reaction of selected amino acids, aldehydes, amines, and isocyanates generated linear dipeptide derivatives. When the "convertible isonitrile"[116] 1-isocyanocyclohexene (**159**) was used, the resulting linear peptide was treated with base and then cyclized to the diketopiperazine library **156**.[117] The diketopiperazine derivatives were obtained in 60−80% yields. When *t*-Boc-diamines (**160**) were used as the amine components in the 4CC reaction, cyclization of the linear dipeptide provided acylated ketopiperazine library **157**.[118] The yields of this procedure were about 85%. This strategy was also extended to the seven-membered ring system. Aminophenyl carboxylic acid derivatives (**161**) were used as acid components for the 4CC reaction, and then the resulting dipeptides were cyclized to the corresponding benzodiazopinedione library **158**.[119,120] A series of 1,4-benzodiazopine-2,5-dione derivatives were obtained in various yields.

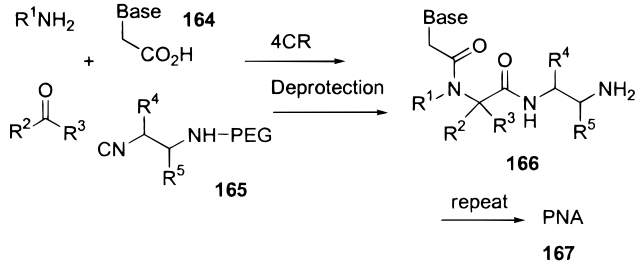
Multicomponent reactions for peptide bond formation have also been utilized to attach peptide moieties to nucleobases and glycosides. Dömling and coworkers reported the synthesis of nucleobase library **162** in 90% yields by the reaction of nucleobase-derived aldehydes (**163**) with amino acids and isocyanates (Scheme 42).[121,122] Sixteen representative

Scheme 42[121,122]



nucleobase compounds **162** were synthesized and characterized. When nucleobase-derived carboxylic acids **164** and *N*-protected isocyanide derivatives **165** were used as building blocks, the four-component reaction with amines and aldehydes/ketones afforded different types of nucleobase-substituted peptides **166** after deprotection (Scheme 43).[123] Further repetition

Scheme 43[123]

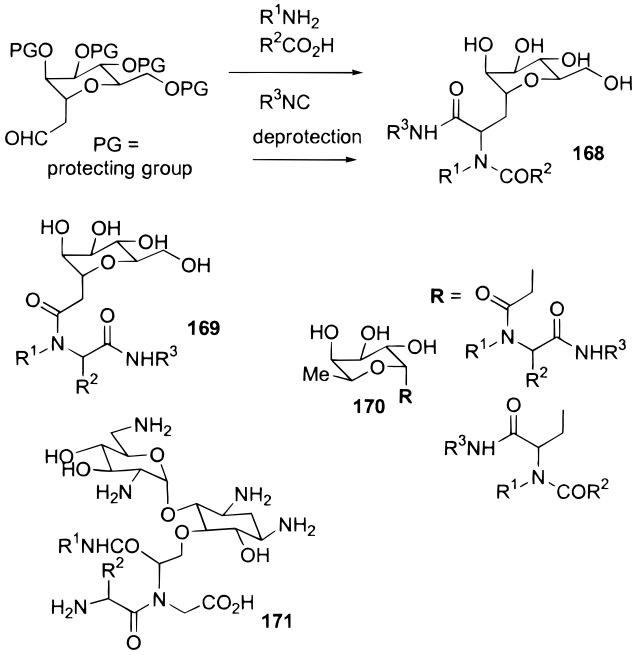


of the 4CC reaction of **166** with acids, aldehydes, and isocyanides gave peptide nucleic acid (PNA) library **167**. This method has been developed for the generation of combinatorial PNA libraries.

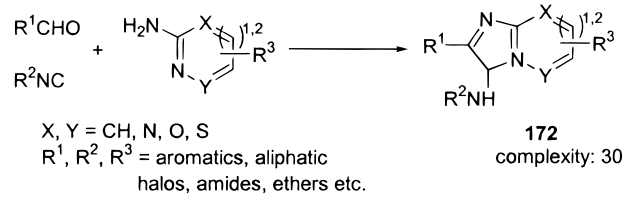
Wong and co-workers[124] reported the synthesis of C-linked glycopeptide libraries, which may serve as sialyl Lewis X mimetics. Thus, when glyco-derived aldehydes and carboxylic acids were used as building blocks, the four-component reaction provided glycopeptide libraries **168** and **169** after deprotection (Scheme 44). Methyl-substituted glycopeptide libraries **170** were also prepared by the same procedure. A neomycin B mimetic library **171** was synthesized using a four-component reaction by reacting an aminoglycoside aldehyde with an isocyanide, amino acid, and poly(ethylene glycol) (PEG)-linked amine (Scheme 44).[125] The PEG-linked products were filtered from the reaction mixture and then cleaved to afford aminoglycoside derivatives **171**. This strategy takes advantage of solution-/solid-phase chemistry and multicomponent reactions for the generation of diverse libraries.

Multicomponent condensation (MCC) reactions have been successfully utilized for the synthesis of heterocyclic compound libraries. Bienayme and coworker[126] developed a method to synthesize fused

Scheme 44[124,125]

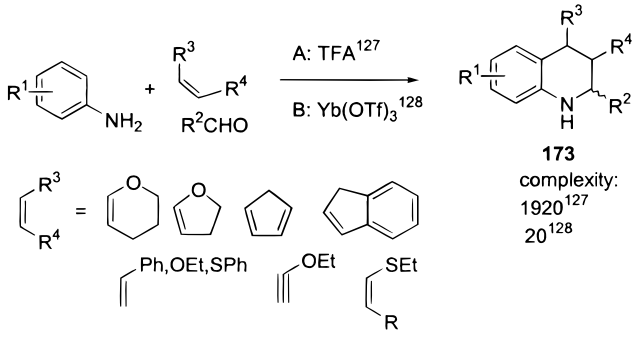


Scheme 45[126]



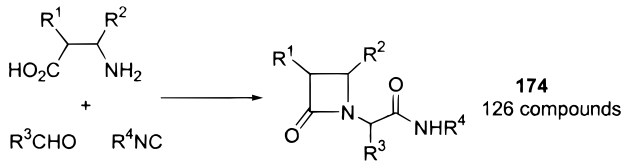
3-aminoimidazole libraries by a three-component condensation reaction (Scheme 45). Heteroaromatic amidines, such as 2-aminosubstituted pyridines or pyrimidines, were reacted with isocyanides and aldehydes in the presence of a catalytic amount of protic acids to give the heterocyclic-fused 2-aminoimidazole library **172** in 50−98% yields. Electron-poor amidines gave low yields. This procedure was developed by preparing 30 representative heterocyclic-fused compounds. Parallel and mixture libraries consisting of 30 000 compounds were then prepared using this procedure. Baudelle[127] and Kobayashi[128] described the preparation of tetrahydroquinoline library **173** in high yields by different three-component condensation reactions of alkenes having electron-donating groups on aniline and aldehyde moieties (Scheme 46). Baudelle and co-workers[127] also

Scheme 46[127,128]



synthesized 1920 pairs of diastereoisomers under TFA conditions. Representative library members were purified by flash chromatography and characterized. Kabayashi and co-workers[128] utilized ytterbium triflate as a catalyst for a three-component reaction to synthesize β-amino ester compounds from silyl enolates, amines, and aldehydes. This method was then extended to other alkenes to provide 20 tetrahydroquinoline derivatives **173**. The four-component reaction was also used for the generation of monocyclic β-lactam dipeptide library **174** as potential elastase inhibitors (Scheme 47).[129] Thus, the

Scheme 47[129]



reaction of seven β-amino carboxylic acid derivatives with six isocyanides and three aldehydes gave a library of 126 β-lactam dipeptides in which library members were obtained in 50−90% yields and confirmed by ES MS.

8. Purification and Characterization/Confirmation of Solution-Phase Combinatorial Libraries

A. Purification of Libraries

A variety of purification methods have been developed to purify libraries for biological screening.[130] Functionalized polymers have been used as reagents or scavengers for the purification of solution-phase libraries. Interested readers may refer to the related review articles.[7,131−133] The current review summarizes purification approaches and procedures for solution-phase libraries. High-performance liquid,[134] preparative thin layer, and flash chromatographic techniques have been utilized for the purification of single-compound[134] and complex libraries.[65−74] Liquid−liquid phase extraction and washing reaction mixtures of libraries with water, appropriate base, and/or acid have been used as routine methods to rapidly purify reaction mixtures during workup.[9−12,59−64] The libraries thus obtained are of a significant level of purity for most screening programs. Curran and co-workers[135] developed a fluorous-phase approach to improve the separation and purification efficiency. Perfluorocarbonated compounds are insoluble in water and most organic solvents; therefore, they can be considered as a third phase. Library members linked with a perfluorocarbon tag ($C_6F_{13}CH_2CH_2$ or $C_{10}F_{21}CH_2CH_2$) were easily isolated from inorganic (in aqueous phase) and organic (in organic phase) impurities. Cleaving the perfluorocarbon tag then provided pure compounds. This strategy, known as a three-phase liquid−liquid extraction, has been utilized for the generation and purification of various heterocyclic libraries.[136] Janda and co-workers used poly(ethylene glycol) (PEG) as a liquid polymer support for library generation instead of solid polymer supports.[137] PEG conjugates are soluble in most organic solvents; therefore, the reaction and workup can be done in one phase. After cleavage of the product from PEG, addition of ether precipitates the PEG moiety, leaving the product in solution.

Small molecules, such as sodium 3′-mercapto-1-propanesulfonate (**175**) and potassium sarcosinate (**176**) (Figure 15), have been used as quenchers to

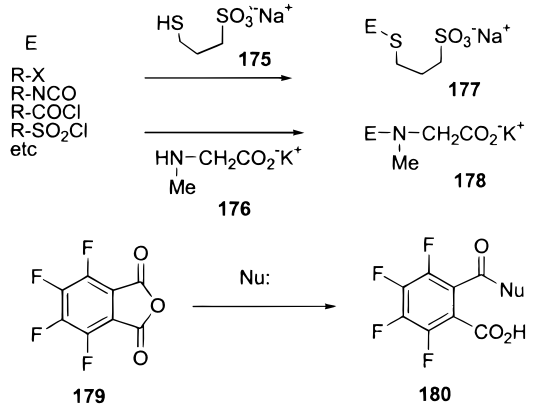


Figure 15. Small molecules as quenchers of electrophiles (E)[68,75,138] and nucleophiles (Nu:).[140,141]

remove excess electrophiles.[68,75,138] After the completion of the combinatorial process, the unreacted electrophiles were quantitatively reacted with quenchers **175** or **176** and the resulting water-soluble salts **177** and **178** were efficiently removed from the reaction mixture by aqueous-phase extraction. This purification method provided single-compound and complex libraries in over 90% purity. Flynn and co-workers[139−142] employed tetrafluorophthalic anhydride (**179**) as a quencher to trap the excess nucleophilic amines or alcohols from reaction mixtures. Resulting acid **180** was further quenched with different amino resins (Figure 15).

Functionalized polymers have been widely utilized for the purification of libraries by solid-phase extraction. Polymers can be used to trap impurities from reaction mixtures leaving the product in solution. They can also be utilized to retain the desired products on solid support. After impurities are removed by washing the reaction mixture with solvents, the desired products are then released providing pure products. Solid-phase extractions for the purification of solution-phase libraries are performed by both noncovalent bond (ionic and hydrophobic) interactions and covalent bond reactions. Suto and co-workers studied a series of ion-exchange resins for trapping intermediates and starting materials (Figure 16).[18,19] The basic anionic exchange resins, Amberlyst and Amberlite series, were used to remove acid impurities for the generation of amide and sulfonamide libraries.[18,19,140−142] The acid cationic exchange resins, e.g., Amberlyst 15 and 120, were successfully employed to remove organic and inorganic base impurities as well as metal cations generated during the library preparation process (Figure 16).[19,144−147] Lawrence and co-workers reported an automated solid-phase extraction process for the purification of amide and ester libraries.[148−150] Amberlyst 15, a strong acid resin, was used to remove the *t*-Boc-protecting group and then to form salts with

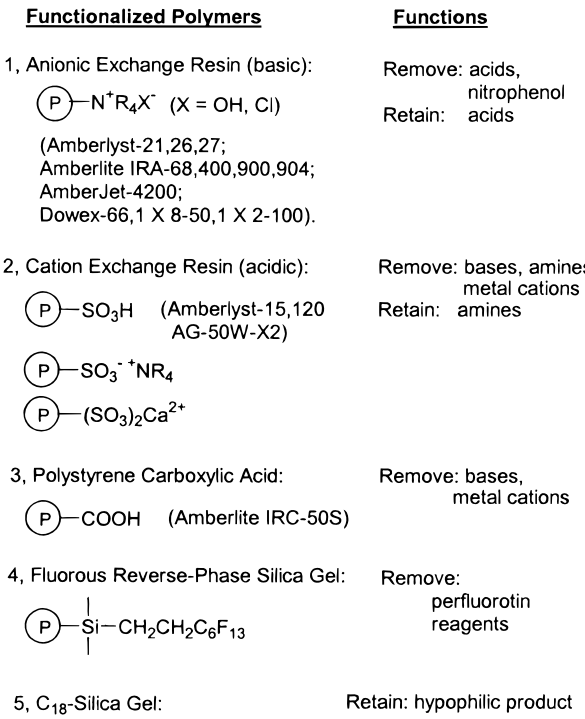


Figure 16. Functionalized polymers for library purification by noncovalent bonds.

deprotected amines to facilitate the generation and purification of amine libraries.[151] Bussolari and co-workers[152] utilized functionalized polymers as scavengers, reagents, and product-capturing agents for the purification of aromatic amine libraries. An amino polymer was used to scavenge an electrophile in the first step. A sulfonic acid resin, AG-50W-X2, was employed to dissociate a boron−amine complex and subsequently capture amino products. Carboxylic acid functionalized Amberlite IRC-50C was also used to remove metal cations and base impurities.[145] Curran and co-workers prepared fluorous reverse-phase silica gel by attaching perfluoro groups on solid support.[153] This hydrophobic solid support was used to trap a perfluoro−tin reagent from reaction mixtures. Hindsgaul and co-workers prepared carbohydrate libraries containing hydrophobic *O*-laurate groups.[154] The hydrophobic carbohydrate compounds retained on the C_{18} silica gel when other impurities were washed away. The products were then easily obtained by changing eluting solvents.

Functionalized polymers were also utilized for library purification by forming covalent bonds with impurities or products. Solid supports having amino groups (Figure 17) were used as scavengers to remove excess electrophiles such as alkyl halides, acid chlorides, sulfonyl chlorides, isocyanates, carboxylic acids, aldehydes, etc., from reaction mixtures.[66,68−75,142,144,155−161] Excess nucleophiles in reaction mixtures were removed by solid supports having electrophilic groups (−NCO, COCl, OCOCl, CHO, etc.).[154−157,161] Solid supports having both nucleophilic or electrophilic groups were also utilized to sequentially remove both electrophilic and nucleophilic impurities from one reaction mixture as needed. Utilizing functionalized polymers as scavengers to remove excess starting materials or impurities gen-

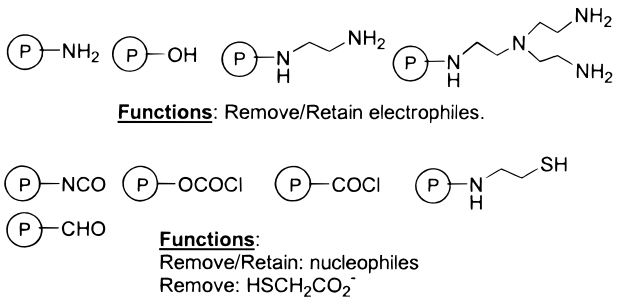


Figure 17. Functionalized polymers for library purification by covalent bonds.

erally provides libraries with purity of over 90%, which is sufficient for biological screening. The mercaptoethylamino polymer was used to remove mercaptoethyl acetic acid by forming the S−S bond.[160] Armstrong and co-workers utilized solid supports having hydroxyl and aryliodo groups to trap peptide and olefin products from reaction mixtures.[161,162] After washing away other impurities, the products were then released from solid supports and obtained in over 95% purity.

Functionalized polymers have also been used as bases to trap inorganic acids, generated from the reaction, and used as catalysts for the reaction (Figure 18). After the reaction is completed, solid

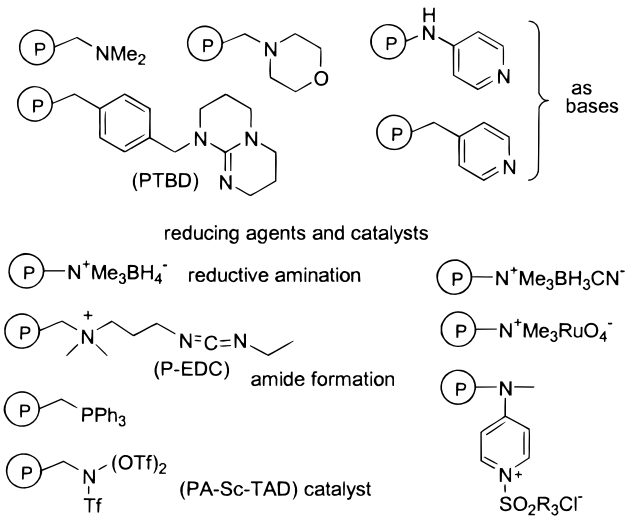


Figure 18. Functionalized polymers as reagents or catalysts.

supports are removed by filtration providing relatively pure compounds. Solid supports having tertiary amine, morpholine, and pyridines were used to remove HCl and dichloroacetic acid.[145,156,157] Ganesan and co-workers[164] have shown that Amberlyst A-26 resin (−NMe_3OH), a strong base, promoted and catalyzed the Dieckmann cyclization for the preparation of hetercyclic libraries. The quaternary ammonium resin (OH form) then formed a salt with the product, therefore, facilitating purification. Polymer-bonded 1,5,7-triazabicyclo[4.4.0]dec-5-ene (PTBD) was utilized to catalyze the alkylation of sulfonamide and phenol derivatives.[165] Polymer-supported borohydride and cyanoborohydride were used for the reductive amination of ketones or aldehydes.[148,166] Polymer-bonded EDC (1-(3-dimethylaminopropyl)-3-ethylcar-

bodiimide) was employed as a coupling agent for the formation of amide, sulfonamide, and anhydride bonds.[60,142,147,158] Polymer-supported perruthenate has been used to oxidize alcohol to aldehyde libraries.[166] A series of polymer-bonded aminosulfonylpyridinium chlorides were prepared and utilized as reagents to form sulfonamides with amines.[166] Aryl ether derivatives were synthesized from phenols and alcohols by using polymer-bonded triphenylphosphine as catalyst.[167] Kobayashi and co-workers[168] prepared (polyallyl)scandium trifylamide ditriflate (PA-Sc-TAD), which was used to catalyze the formation of a heterocyclic library by a three-component condensation approach. Barrett and co-workers described a procedure for the purification of amide and sulfonamide libraries by copolymerizing excess amines.[169] The excess amine in the amide formation procedure was reacted with 1,4-phenylene diisocyanate and pentaethylenehexaamine, and the resulting insoluble polyurea was removed by filtration, providing amide library members in 60−99% yields. The combination of this copolymerization procedure and employing poly(vinylpyridine) as a base provided sulfonamide libraries in over 92% yields. The various procedures discussed above may be employed for the purification of single-compound or complex libraries based on the reaction and impurity types. A large number of different functionalized polymers[131] can be chosen as reagents, trapping agents, scavengers, and catalysts for a variety of reactions as well as utilized for the purification of libraries.

B. Characterization/Confirmation of Libraries

Single-compound libraries can be fully characterized by traditional spectroscopic and combustion analyses. However, most of the reported single-compound libraries were not fully characterized because of the large number and small amount of samples, but representative samples of library members were characterized by NMR, MS, or elemental analyses. Practically, characterization of representative (>20%) library members is sufficient for the determination of library quality. Kassel and co-workers at CombiChem demonstrated an HPLC/MS high-throughput method for characterizing and purifying parallel single-compound libraries.[170,171] Hogan and co-workers at ArQule described a high-throughput analysis and characterization of single-compound libraries by HPLC/UV and MS approaches.[172]

Small indexed libraries of flavone derivatives were separated by HPLC, and representative members in each library were then characterized by mass spectrometry.[55] The GC-MS analysis confirmed the presence of the expected 20 indole derivatives in a mixture library.[83] Boger and co-workers[60−63] have shown a spectroscopic comparison of a single compound with a mixture library containing 10 compounds by NMR. The construction of a sublibrary was confirmed by MS. An and co-workers at Isis Pharmaceuticals[65−75] characterized their complex libraries by NMR, CZE, and ES MS. ^{1}H NMR spectra of libraries depicted the correct ratio of specific protons for libraries with complexity from four to hundreds of compounds.[66,70] Capillary zone electrophoresis analysis of complex libraries containing up to 16 compounds depicted the existence of all library members in approximately the same concentration.[66] Complex libraries having 100−1000 compounds are difficult to analyze by HPLC, CZE, and NMR techniques. Isis' group was able to successfully characterize and confirm the quality of complex libraries by electrospray mass spectrometry and computer simulation approaches. A 100-member complex library exhibited all expected ion peaks, and the abundance pattern of the mass spectrum was consistent with that obtained from the computer simulation.[66] Complex libraries of 4−1000 compounds from Isis were characterized and confirmed by ES mass spectrometry.[65−75] Richert and co-workers[80] demonstrated an LD-TOF mass spectrum of a 55-member complex library, which provided the expected 35 peaks for nonisobaric masses. The mass spectra of more complicated libraries were also compared with the corresponding computer-simulated mass spectra. Several other research groups have used mass spectrometry for the confirmation of other indexed and complex libraries.[58,93,94,98,173,174] Complex libraries are yet to be fully characterized using current analytical techniques that are routinely used for the characterization of single compounds. However, the combination of mass spectrometric techniques and computer simulations provides an attractive process for the characterization and confirmation of complex libraries having up to thousands of compounds.

C. Screening and Deconvolution of Complex Libraries by Mass Spectrometry

Complex libraries have been screened directly to provide active libraries, which have been deconvoluted by iterative and/or positional scanning as well as HPLC fractionation strategies.[174] Eliseev described a target-assisted screening of complex libraries and subsequent identification of active compounds by size-exclusion chromatography, ultrafiltration, and capillary electrophoresis.[176] Mass spectrometry has been utilized to screen and deconvolute complex libraries directly to provide active single compounds with distinct structures. Griffey and co-workers[80] identified bacterial A site RNA binding ligands from a 216-member complex library by a mass−mass spectrometry strategy. Venton and co-workers[57,177] screened indexed libraries using a competitive binding assay based on pulsed ultrafiltration/electrospray mass spectrometry to tentatively identify target receptors. Hsieh and co-workers[178] developed a high-throughput multiple target screening of complex libraries by microfluidic MALDI-TOF MS (matrix-assisted laser desorption ionization time-off flight mass spectrometry) techniques. This approach can be used to simultaneously identify all individual lead compounds with activity against multiple enzyme targets. Vouros demonstrated an efficient approach for selecting active ligands from complex libraries with subsequent identification of lead compounds by mass spectrometry.[179] The active compounds in the mixture were isolated using a biological target molecule by gel filtration on a size exclusion medium.

The mass spectrometry was used to identify the ligands with high affinity to a receptor.

9. Concluding Remarks

In the past 5 years, substantial progress has been achieved on solution-phase combinatorial chemistry for drug discovery. The methodologies for the generation of parallel single-compound libraries have been developed on various small molecular structures. The array preparation of single-compound libraries has been partially automated to generate large numbers of compounds for high-throughput screening. The progress achieved in this area greatly increased the speed of lead optimization. The methodologies for generating indexed and complex libraries from a variety of scaffolds has introduced new approaches for the discovery of novel pharmacophores and has greatly accelerated lead optimization. Chemoenzymatic approaches for library generation has rapidly expanded and shall be effectively utilized for different types of structures by different enzymes. Multicomponent reaction approaches have also rapidly advanced but are still limited in the type of reactions utilized and in the structural diversity. New MCRs shall be explored for generating different types of diverse libraries. Development of library purification strategies is a key issue for solution-phase combinatorial chemistry, and much progress has been achieved. Extraction methods by small molecules, solid-phase, and liquid-phase including fluoro-phase polymers have been widely explored; however, even more advances in functionalized polymers and small molecules are required to effectively generate high-purity libraries. Screening and deconvolution of complex libraries by mass spectrometry will become a major approach in the examination of complex libraries for drug discovery.

Solution-phase combinatorial chemistry has not been developed to the level of solid-phase approaches in the automation areas. Further exploring various reactions and chemically diverse structures, as well as developing fully automated processes, would significantly enhance solution-phase combinatorial approaches for drug discovery.

10. Abbreviations

AA	amino acid
Ac	acetyl
Ar	aryl
Bn	benzyl
t-Boc	*tert*-butoxycarbonyl
Bu	butyl
Cbz	benzyloxycarbonyl
CRF	corticotropin releasing factor
CZE	capillary zone electrophoresis
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DNA	dexoyribonucleic acid
E	electrophile
EDC	1-(3-dimethylaminopropyo)-3-ethylcarbodiimide
ESI	electrospray ionization
Et	ethyl
GC	gas chromatography
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
HSV	herpes simplex virus
ICE	interleukin-1β converting enzyme
LD-TOF	laser desorption time-off flight
MALDI	matrix-assisted laser desorption ionization
MCC	multicomponent condensation
MCR	multicomponent reaction
Me	methyl
MIC	minimum inhibitory concentration
Ms	methane sulfonyl
MS	mass spectrometry
NMR	nuclear magnetic resonance
Nu:	nucleophile
PA-Sc-TAD	(polyally)scandium trifylamide ditriflate
PEG	poly(ethylene glycol)
PG	protecting group
Ph	phenyl
Phe	phenylalanine
PLC	preparative thin-layer chromatography
PNA	peptide nucleic acid
Pro	proline
PTBD	1,5,7-triazabicyclo[4.4.0]dec-5-ene
PUF/ES	pulsed ultrafiltration/electrospray
PyBOP	benzotriazole-1-yl-oxy-trispyrrolindinophosphonium hexafluorophosphate
RNA	ribonucleic acid
SAR	structure−activity-relationship
SPA	scintillation proximity assay
SPSAF	solution-phase simultaneous addition of functionalities
TAR	transactivating responsive
tat	trans-activator
Tf	triflate
TFA	trifluoroacetic acid
TLC	thin-layer chromatography
UV	ultraviolet
Val	valine

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CR990014R