Enriching chemical space with diversity-oriented synthesis: parallel synthesis of low molecular weight acyclic and heterocyclic compounds from resin-bound polyamides

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Abstract
Diversity-Oriented Synthesis (DOS), also known as combinatorial synthesis, is a process, by which multiple compounds are generated simultaneously, in a predictable fashion using techniques that involve parallel chemical transformations. Through diversity-oriented synthesis organic chemists are now able to achieve more structural complexity than in the early days of combinatorial chemistry. Combinatorial chemistry techniques offer unique enhancement in the potential identification of new and/or therapeutic candidates. Over the past fifteen years in the design and diversity oriented synthesis of low molecular weight acyclic and heterocyclic combinatorial libraries derived from amino acids, peptides and/or peptidomimetics are described. Employing a “toolbox” of various chemical transformations, the “libraries from libraries” concept has enabled the continued development of an ever-expanding, structurally varied, series of organic chemical libraries.

Keywords: chemical transformations; DOS; peptides.
1. Introduction

One of the central objectives of organic and medicinal chemistry is the design, synthesis, and production of molecules having value as human therapeutic agents. Historically, the identification of such compounds has been carried out using compounds from plant and animal tissue extracts, microbial broth extracts, as well as individual compound collections resulting from fifty years of effort by synthetic chemists in academic and pharmaceutical organizations. Compounds having multiple stereocenters and complex, natural product-like properties are now being increasingly prepared. The preparation of structurally complex and diverse compounds results in a broader expansion of chemical space and facilitates effective probing of biological space. Such structural complexity is clearly significant because many of the small molecules known to disrupt protein-protein interactions are complex, natural products or natural product-like compounds, known to disrupt protein-protein interactions are complex and diverse compounds results in a broader expansion of chemical space and facilitates effective probing of biological space. Such structural complexity is clearly significant because many of the small molecules known to disrupt protein-protein interactions are complex, natural products or natural product-like compounds, increasing the potential for therapeutic development. Diversity-oriented synthesis approaches have created a vast new source of molecular diversity for the potential identification of lead compounds. Merrifield’s solid phase synthetic methods presented in 1963[1, 2] were accelerated by techniques developed by Frank, Geysen, and Houghten for the combinatorial synthesis of oligonucleotides and peptides on cellulose disks[3, 4] pins and standard resins in mesh packets (the “teabag” approach)5 in 1983, 1984 and 1985, respectively [5]. The versatility and increased capability afforded by the teabag approach, first developed for solid phase peptide synthesis, has led to the identification of a wide range of bioactive peptides, including novel antibacterials, potent opioid receptor agonists and antagonists, inhibitors of melittin’s hemolytic activity, antigenic peptides recognized by monoclonal antibodies, and potent endothelin antagonists[6, 7]. The next level of successful diversity generation involved the use of soluble mixture-based synthetic combinatorial libraries (SCLs) made up of tens of millions of compounds. These libraries have been successfully used for the de novo identification of potent analgesics[8-12] antimicrobial agents[13, 14] enzyme inhibitors, antitumoral, and highly specific antigenic determinants of B-cells and T-cells [15]. Along with linear peptide sequences, our laboratory and other groups have also synthesized combinatorial libraries of cyclic peptides [16-23]. In the last decade, the focus of combinatorial chemistry has shifted to libraries of small molecule compounds having molecular weights of 500 Dalton or less [24, 25]. Employing a “toolbox” of various chemical transformations, including alkylation, oxidation, reduction, acylation, and the use of a variety of multifunctional reagents, we present examples of the use of the “libraries from libraries” concept for the development and generation of an ever-expanding, structurally varied, series of organic chemical libraries.

2. Synthesis of low molecular weight acyclic compounds

Early work from our laboratory has shown the utility of mixture-based chemical libraries of small molecules for the de novo identification of highly active antimicrobial compounds, novel antitumor agents [8] and potent analgesics [7]. These libraries represent chemical collections of low molecular weight heterocyclic and acyclic compounds. The diversity of these chemical structures, as well as the large number of compounds making up each class of structures, greatly increases the probability of identifying compounds having useful chemical characteristics.

Due to the well-understood chemistry and excellent synthetic purity and yields obtained during the solid phase synthesis of peptides, our primary efforts have been directed toward the synthesis and design of acyclic and heterocyclic compounds using resin-bound amino acids, peptides and peptidomimetics as starting materials [7, 37]. A range of peptide and peptidomimetic libraries have been modified using a variety of chemical reagents (acylation, alkylation, reduction, etc.) to generate an ever-expanding range of chemical diversities having strikingly different physicochemical properties relative to their starting libraries[46-49]. Such new libraries can, in turn, be used to generate further libraries. The continually expanding combination of such chemical alterations permits the creation of a “toolbox” of chemical transformations for the generation of immense diversities of compounds. Thus, for the last decade, this strategy has been successfully used to design and generate a range of novel solid phase chemistries. We initially developed efficient strategies for the generation of peptidomimetic libraries by the alkylation and/or reduction of the amides of existing short peptide libraries[46-51]. Initial examples of this approach, termed “libraries from libraries,” are shown in Scheme 1 in which peralkylation and/or exhaustive reduction of the amide bonds in peptides yield completely different classes of compounds such as peptidomimetics 1 and polyamines 2. Polyamines were treated with different carboxylic acids or isocyanates to afford following cleavage of the resin the corresponding per-acylated amines 3, or polyisocyanates 4, respectively.
A vital modification of the peptide backbone was the exhaustive reduction of amide bonds leading to chiral polyamines [50, 51]. Polyamines have been shown to be pharmacologically unique compounds [52]. They are also ideally suited to bind DNA. Multiple amine functionalities are common in drugs active within the central nervous system. They constitute one of a few classes of compounds capable of interacting with the three natural biopolymers: proteins, nucleic acids and oligosaccharides[52, 53]. Polyamines interact strongly with nucleic acids and play an important role in their biosynthesis and metabolism[54]. A variety of mixture-based combinatorial libraries made up of different numbers of polyamines, as well as large arrays of individual polyamines have been synthesized in our laboratory [7, 50]. Those libraries have been screened internally and in collaboration with outside collaborators. Examples of reported activities include antimalarial, antitubercular, HIV inhibitory and antitumoral activities [7, 8, 55, 56]. The exhaustive reduction of peptides and chiral polyamides on solid-supports, introduced by our laboratory, has been utilized in a wide range of synthetic procedures. Typical reaction conditions for the solid-phase reduction of polyamides consist of the 72 hour treatment of resin-bound peptides with BH$_3$-THF at 65°C [50,51] The generated resin-bound borane-amine complexes are then disproportion-ate following overnight treatment with neat piperidine at 65°C. Peralkylated chiral amines and/or selectively alkylated amines have been successfully generated by exhaustive reduction of peralkylated peptides and peptidomimetics. Resin-bound chiral polyamines have been further used as templates for the generation of chiral acyl-polymamines, poly-N-aclamines, polyureas and polythioureas libraries[37, 57-59].

Iii. Synthesis of heterocyclic compounds from resin-bound amino acids and/or resin-bound-short peptides.

The reaction of the N-terminal amino group of a resin-bound dipeptide with carbonyldiimidazole or thio-carbonyldiimidazole led to an intermediate isocyanate (or isothiocyanate) that further reacts intramolecularly to form the five-membered ring hydantoin 5 (or thiohydantoin 6 (Scheme 2)) [59]. We have also synthesized branched hydantoins 7 and branched thiohydantoins 8 starting from resin-bound orthogonally protected diamino acids (amino acids having two amine functionalities). Following coupling of the second amino acid and hydantoin (or thiohydantoin) formation, the side chain of the diamino acid was deprotected and the free amino group then N-acylated with a range of carboxylic acids or isocyanates to yield, following cleavage of the solid support, the corresponding branched hydantoins (or thiohydantoins).

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**Scheme 1.** Generation of peptidomimetic and small molecule libraries.
Starting from resin-bound amino acids, the treatment of the N-terminal amine with isothiocyanate provided a thiourea that, in the presence of HgCl₂ and a primary or secondary amine, generated the resin-bound guanidines. Cleavage from the solid support with HF generates the 2, 3, 4-trisubstituted 4H-imidazolones 9 following an intramolecular cyclization via an “Edman-like” degradation (Scheme 3). The reaction of resin-bound thiourea with Mukayama’s reagent resulted in the formation of the carbodiimide intermediate that undergoes intramolecular cyclization to yield, following cleavage of the solid support, the 2-amino imidazolidin-4-ones 10 (Scheme 3) [60,61].

Functional amino acid side chains were also used for the synthesis of different heterocyclic compounds. The parallel synthesis of 1,3,4,7-tetra substituted perhydro-1,4-diazepine-2,5-diones 13 (Scheme 5) was performed starting from the Fmoc-protected resin-bound 4-butyln ester of aspartic acid.

The treatment of resin-bound acylated dipeptides with freshly distilled phosphorous oxychloride (POCl₃) led to the formation of bicyclic [3,5,7]-1H-imidazo[1,5-a]-imidazol-2(3H)-ones 11 (Scheme 4) [62]. Under the same reaction conditions, and starting from resin-bound N-acylated dipeptides having tryptophan (or Trp analog) as the C-terminal amino acid generated following treatment with...
(POCl₃) and double cyclodehydration under Bischler-Napieralski conditions [63] the fused tricyclic imidazopyridoindole 12. A large number of individual imidazopyridoindoles was obtained following hydrogen fluoride (HF) cleavage (Scheme 4) [64].

Following deprotection of the Fmoc group, the amine was reductively alkylated. An Fmoc amino acid was then coupled, and a second reductive alkylation was performed following Fmoc deprotection. The t-buty group was cleaved and an intramolecular amidation occurred in the presence of HATU to afford the desired trisubstituted diazepinediones.

Separately, starting from resin-bound Fmoc-Cys(Trt)-OH, the solid-phase synthesis of 2,4,5-trisubstituted thiomorpholin-3-ones 14 was achieved following deprotection of the side chain, reductive alkylation, and intramolecular amide formation (Scheme 5). Similarly, starting from resin-bound cysteine and following reaction of the deprotected cysteine side chain with 2-fluoro-5-nitrobenzoic acid, followed by reductive alkylation and intramolecular cyclization, the 1,4-benzothiazepin-5-one derivatives 15 were obtained in good yield and high purity (Scheme 6) [66].

### IV. Synthesis of diazacyclic compounds from resin-bound chiral polyamines

Following exhaustive reduction of the resin-bound polyamides, the resulting chiral polyamines can be used as templates for the solid phase synthesis of highly diverse individual compound arrays and mixture-based combinatorial libraries [7,50,67-81]. Various disubstituted heterocyclic compounds were prepared from resin-bound N-acylated amino acid amides (Scheme 7). Thus, following reduction of the two amides, the resulting resin-bound diamines was treated with commercially available bifunctional reagents such as carbonyldiimidazole, thiocarbonyldiimidazole, cyanogen bromide, oxalyldiimidazole, malonyl chloride, and benzyl isocyanatidocarbonate to afford following HF cleavage, the corresponding disubstituted imidazolinones 16, imidazolidinethiones 17, cyclic guanidines 18, diketopiperazines 19, piperazines 20, diazepinediones 21, and triazinediones 22, respectively.

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**Scheme 5.** Synthesis of diazepinediones from resin-bound aspartic acid.

**Scheme 6.** Synthesis of thiomorpholinones and benzothiazepines from resin-bound cysteine.
Scheme 7. Synthesis of trisubstituted diaza- and triaza-cyclic compounds from resin-bound diamines.

We extended our approach to the parallel solid-phase synthesis of the trisubstituted heterocyclic analogs starting from selectively N-alkylated resin-bound triamines. The free N-terminal amino functionalities of resin-bound amino acids were protected with triphenylmethyl chloride (TrtCl). The secondary amides linked to the solid support were then selectively alkylated in the presence of lithium t-butoxide and an alkyl halide. As anticipated, alkylation of the amide nitrogen of the resin linkage dramatically increased the acid sensitivity of the MBHA resin-bound peptide, excluding the use of Boc-amino acids in further couplings. Therefore, Fmoc-amino acids were employed in subsequent couplings. Following Fmoc removal and N-acylation of the resin-bound dipeptide, exhaustive reduction of the amide bonds using borane in tetrahydrofuran again yielded the desired resin-bound chiral triamine 23 having two available secondary amines and one tertiary amine [51].

Treatment of the triamine with the previously described bifunctional reagents yielded, following HF cleavage, the corresponding trisubstituted heterocyclic compounds. Scheme 8 illustrates the approach for the solid phase synthesis of the triamine template 23. Following the strategy described in Scheme 8, various heterocyclic SCLs 24 were prepared in positional scanning format containing the aforementioned heterocycles. The solid phase synthesis of bicyclic guanidine 25 from resin-bound triamines (three secondary amines), which itself was derived from an N-acylated dipeptide. Following addition of thiocarbonyldiimidazole to form the cyclic thioureas described above, the presence of a third secondary amine permits the spontaneous formation of the bicyclic guanidines via a highly active intermediate.
Scheme 8. Synthesis of diazacyclic compounds and bis-cyclic guanidines from resin-bound reduced acylated dipeptides

Starting from glutamine-containing resin-bound N-acylated dipeptides, and using the same strategy, tethered urea-linked bicyclic guanidines 26 and N-acyl amino-linked bicyclic guanidines 27 were prepared [75]. As outlined in Scheme 9, following exhaustive reduction and selective protection of the primary amine with a trityl group, treatment of the three secondary amines with isocyanate generated the resin-bound bicyclic guanidine. Following trityl deprotection, the free primary amine was acylated with a wide range of carboxylic acids to yield, following HF cleavage, the N-acyl amino-linked bicyclic guanidines 27. The urea-linked bicyclic guanidine 26 library was obtained following trityl deprotection, coupling of an amino acid, isocyanate treatment, and final HF cleavage (Scheme 9).

Scheme 9. Synthesis of urea tethered bis-cyclic guanidines.
We have also designed and prepared resin-bound polyamines for the synthesis of a variety of “bis”-heterocyclic compounds. Starting from resin-bound Fmoc-Lys (Boc)-OH (Scheme 10), the Fmoc group was cleaved and the resulting free amine was N-acylated with a variety of carboxylic acids. Following cleavage of the Boc group and subsequent coupling of a Boc-amino acid and acylation, exhaustive reduction of the amide bonds on the solid support was performed using the same conditions described above. Treatment of the resin-bound tetraamines with carboxylidiimidazole, thiocarboxylidiimidazole, cyanogen bromide and oxalyldiimidazole resulted in the formation of the energetically favored five and six-membered rings, corresponding to the bis-cyclic ureas 28, bis-cyclic thioureas 29, bis-cyclic guanidines 30, bis-diketopiperazines 31 and bis-piperazines 32[83]. Cleavage from the solid support with hydrogen fluoride, followed by extraction and lyophilization, yielded the desired bis-heterocyclic compounds in excellent yield and high purity.

Extending the above mentioned approaches to larger polyamines, tripeptide amides were synthesized using conventional Boc/Bzl chemistry. Following coupling of the third amino acid and Boc deprotection, the resin-bound tripeptide was exhaustively reduced with borane in THF to yield resin-bound tetraamines containing three secondary amines and one terminal primary amine (Scheme 11). The resin-bound tetra-amine was then treated with bifunctional reagents. Excellent product purities were obtained following their treatment with thiocarboxylidiimidazole and cyanogen bromide, which afforded, following HF cleavage, the bis-cyclic thiourea 33 and bis-cyclic guanidines 34. Kinetically, as expected, the primary amine reacts first with the thiocarboxylidiimidazole, which then favors intermolecular cyclization with the adjacent secondary amine to yield a cyclic urea due to the formation of the energetically favored five-membered ring. The two remaining secondary amines then further react with a second molecule of thiocarboxylidiimidazole to yield the second heterocycle.

Following optimization under various concentrations of thiocarboxylidiimidazole, it was observed that working at lower concentrations with small excesses of this reagent led to the desired bis-heterocyclic compounds having purities greater than 80% were obtained[84, 85]. Using higher concentrations and larger excesses of the reagent increased the probability that the different amines would both react with thiocarboxylidiimidazole and prevent the cyclization step. In support of our kinetic hypothesis, we found that the treatment of a resin-bound polyamine containing four secondary amines led to the formation of multiple products.

Scheme 10. Synthesis of bis-heterocyclic compounds from resin-bound orthogonally protected Lysine.
V. Solid-Phase Synthesis of New Heterocyclic Azoniaspiro Ring Systems

We developed an efficient approach for the parallel solid-phase synthesis of novel heterocyclic azoniaspiro ring systems. The target compounds, the 1,8,9-trisubstituted-10-oxo-3,9-diaza-6-azoniaspiro-[5.5]undecanes, were obtained starting from resin-bound reduced dipeptides. The azoniaspiro cation was formed by intramolecular attack of a tertiary nitrogen on pendant α-bromocarbonyl. N-3 acylated and N-3 alkylamino carbonyl derivatives of the 1,8,9-trisubstituted-10-oxo-3,9-diaza-6-azoniaspiro[5.5]undecanes were obtained following in solution treatment of the N-3 azoniaspiro derivatives with different carboxylic acids and isocyanates. The strategy leading to the desired 1,8,9-trisubstituted-10-oxo-3,9-diaza-6-azoniaspiro[5.5]undecane 35 is outlined in Scheme 12. Starting from p-methylbenzhydrylamine (MBHA) resin-bound dipeptide and following exhaustive reduction of the amide bonds,[51] the N-terminal primary amine was selectively protected with 2-acetyldimedone (Dde-OH) or triphenylmethyl chloride (Trt-Cl). The two secondary amines were treated with oxalyldiimidazole to generate the corresponding resin-bound 2,3-diketopiperazines. Following cleavage of the Dde or Trt group, the free amine was acylated with phenyl acetic acid. The oxamide and amide groups were then reduced using BH₃·THF to generate the corresponding resin-bound piperazine tethered secondary amine. The secondary amine was then coupled overnight with bromoacetic acid in the presence of diisopropylcarbodiimide (DIC). Following acylation, an intramolecular displacement of the bromo group occurred to yield the resin-bound diastereomeric mixture of the desired 1,8,9-trisubstituted-10-oxo-3,9-diaza-6-azoniaspiro[5.5]undecane 35.[38]

Similarly, an efficient approach for the design and parallel solid-phase synthesis of unique tetrasubstituted oxopiperazinium derivatives was developed [89]. The synthetic procedure involved a 7-step sequence carried out starting from resin-bound dipeptide. The desired compounds were obtained in good yields and high purity. The strategy leading to 1,1,3,4-tetrasubstituted-5-oxopiperazin-1-ium salts 36 is outlined in Scheme 13. Starting from p-methylbenzhydrylamine (MBHA) resin-bound dipeptide, the N-terminal primary amine was protected with triphenylmethyl chloride. The two secondary amines were methylated following treatment of the resin-bound protected dipeptide with lithium tert-butoxide and methyl iodide.

Scheme 11. Synthesis of bis-heterocyclic compounds from resin-bound tripeptides.

Scheme 12. Synthesis of trisubstituted azoniaspiro compounds.
Following cleavage of the trityl protecting group, the free amine was acylated with a variety of carboxylic acids. The amide groups were then reduced using BH$_3$-THF to generate the corresponding resin-bound triamines having two tertiary amines and one secondary amine. The secondary amine was then coupled with bromoacetic acid overnight in the presence of diisopropylcarbodiimide (DIC). Following acylation, an energetically favorable spontaneous intramolecular displacement of the bromo group occurred to yield the resin-bound epimeric mixture of the desired tetrasubstituted-5-oxopiperazinium salt.

Scheme 13. Solid-phase synthesis of tetrasubstituted oxopiperazinium.

Table 1. Examples of heterocyclic libraries.
VI. Examples of identified active compounds from small molecule mixture based libraries

Table 1 summarizes a number of libraries of acyclic and heterocyclic compounds made using resin-bound modified amino acids and/or short peptides.

To illustrate the screening and deconvolution of mixture-based libraries, the tri-substituted bicyclic guanidine positional scan combinatorial library (PS-SCL) [7, 50] was screened at a final concentration of 4 µg/ml in a radio receptor binding assay specific for the kappa opioid receptor [7]. The library was synthesized in positional scan format [7]. Three sublibraries were made. The first sublibrary represents the 49 mixtures in which the R₁ position is defined, the second sublibrary represents R₂ (51 mixtures) and the third sublibrary represents R₃ (41 mixtures). Mixtures exhibiting greater than 80% inhibition (total of 38 mixtures from the three sublibraries) were selected and tested in a dose-response manner. The most active mixtures were chosen, and all possible combinations of the building blocks used for the synthesis of individual compounds. In this case, three building blocks were chosen for R₁, four for R₂ and four for R₃. The forty-eight (3 x 4 x 4) individual bicyclic guanidines synthesized were then tested for activity and found to range from 37 to 10,000 nM. The most active individual bicyclic guanidine identified has an activity of (IC₅₀ = 37 nM). This same bicyclic guanidine PS-SCL has been screened to identify potent antifungal compounds. Each of the 141 mixtures was tested for its ability to inhibit Candida albicans growth in a standard microdilution assay. Half of the generated 32 individual compounds exhibited antifungal activity with minimum inhibitory concentrations (MIC) varying from 3 to 10 µg/ml.

The cyclic urea and thiourea libraries were assayed for their ability to inhibit Candida albicans growth, which is one of the most common opportunistic fungi responsible for infections and is the fungal infection most frequently associated with HIV-positive patients [7]. Greater activities were found for the N-benzylated compounds relative to N-methylated compounds (MIC values of the most active compounds varied from 8 to 64 µg/ml, and 64 to 125 µg/ml, respectively).

In another example, a linear urea library has been prepared by the reaction of a resin-bound amino acid with individual isocyanates, affording the linear ureas in good yields. The library composed of 125,000 linear N, N'-disubstituted ureas was prepared and has been tested for opioid activity in mu, delta, and kappa opioid and sigma receptor binding assays. Following deconvolution of the library, individual compounds with 1-10 nM affinities at the mu and sigma receptors were found. Figure 1 presents examples of the identification of different active compounds from different libraries in a variety of assays.

Fig. 1. Examples of identified active compounds from small molecule mixture based libraries.
Highly active individual small molecule compounds were also identified from the N-benzyl-1,4,5-trisubstituted-2,3-diketopiperazine library and the triphenylurea library in an assay to identify XIAP inhibitors based on the ability of the compounds to overcome the XIAP-mediated suppression of caspases (enzyme depression assay). Two different mixture-based positional scanning libraries made up of more than 30,000 compounds each were screened against *P. falciparum* and *T. brucie rhodesiense*. Both libraries, namely N-methylated 1,3,4-trisubstituted piperazine and the N-methyl trimaine, yielded good inhibitory activity in these assays. Individual N-methyl amines and N-methyl piperazines were synthesized following the standard positional scanning deconvolution approach, and have been prepared for testing against *T. brucie rhodesiense*, and *P. falciparum*. Bis-piperazine and bis-cyclic guanidine compounds demonstrated higher than 90% inhibition in the primary antitubercular assay at low concentrations (MIC < 4 µg/ml).

**Conclusion**

We have described part of our work on the use of amino acids and peptides as precursors for the generation of combinatorial libraries. The use of the teabag approach for parallel array synthesis greatly facilitates the synthesis of the individual compounds, as well as mixture based combinatorial libraries. The application of the “libraries from libraries” concept has permitted the expansion of the number of combinatorial libraries with each new chemical transformation developed. The combination of these techniques advances, not only the synthesis of biologically relevant compounds, but also greatly facilitates the ultimate goal of medicinal chemistry: the identification of individual therapeutically useful molecules.

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**References**


