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Combinatorial Chemistry: Libraries from Libraries, the Art of the Diversity-Oriented Transformation of Resin-Bound Peptides and Chiral Polyamides to Low Molecular Weight Acyclic and Heterocyclic Compounds

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Combinatorial chemistry has deeply impacted the drug discovery process by accelerating the synthesis and screening of large numbers of compounds having therapeutic and/or diagnostic potential. These techniques offer unique enhancement in the potential identification of new and/or therapeutic candidates. Our efforts over the past 10 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, including alkylation, oxidation, reduction, acylation, and the use of a variety of multifunctional reagents, the "libraries from libraries" concept has enabled the continued development of an ever-expanding, structurally varied series of organic chemical libraries.

I. Introduction

The philosophical and practical underpinnings inherent in combinatorial chemistry are broadly based on the solid-phase chemistry approaches first introduced by Merrifield for the synthesis of peptides.[1,2] Merrifield's approaches were accelerated by techniques developed by Frank, Geysen, and Houghten for the combinatorial synthesis of oligonucleotides and peptides on cellulose disks,[3] pins,[4] and standard resins in mesh packets (the "tea-bag" approach)[5] in 1983, 1984, and 1985, respectively. The versatility and increased capability afforded by the tea-bag 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.[7] These libraries have been successfully used for the de novo identification of potent analgesics,[9−12] antimicrobial agents,[13,14] enzyme inhibitors,[7] 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 past decade, the focus of combinatorial chemistry has shifted to libraries of small molecule compounds having molecular weights of 500 Da or less.[24−45] 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"[46−48] concept for the development and generation of an ever-expanding, structurally varied series of organic chemical libraries.

II. Synthesis of Low Molecular Weight Acyclic Compounds

The preparation of structurally complex and diverse compounds results in a broader population of chemical space and facilitates effective probing of biological space. 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-

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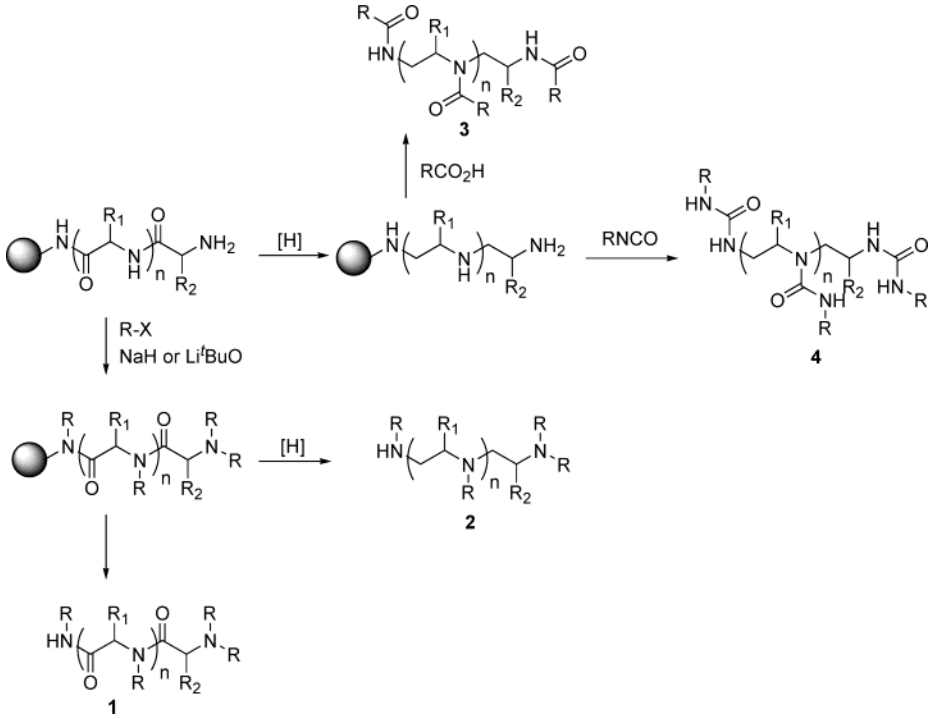
SCHEME 1. Generation of Peptidomimetic and Small Molecule Libraries from Resin-Bound Peptides



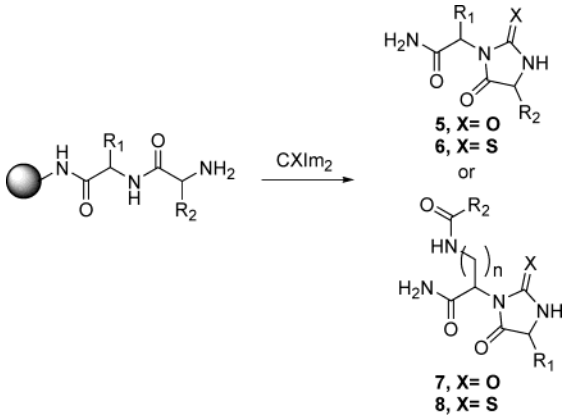
phase synthesis of peptides, our primary efforts have been directed toward the synthesis and design of acyclic and heterocyclic compounds using resin-bound amino acids, peptides, and peptidomimetics as starting materials.[7,37] In a continuation of our early efforts, a range of peptide and peptidomimetic 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 past decade, this strategy has been successfully used to design and generate a range of novel solid-phase chemistries. For low molecular weight acyclic compounds, 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,"[48] 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**.

Resin-bound peptides were successfully peralkylated in the presence of lithium *tert*-butoxide and alkyl halides such as methyl iodide, ethyl iodide, benzyl bromide and naphthylmethyl bromide to provide the corresponding per-*N*-alkylamides. 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.[52] 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 incollaboration with outside investigators. 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 h treatment of resin-bound peptides with BH_3−THF at 65 °C.[50,51] The generated resin-bound borane−amine complexes are then disproportionated 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 acylpolyamines, poly-*N*-acylamines, polyureas, and polythioureas libraries.[37,57−59]

III. Synthesis of Heterocyclic Compounds from Resin-Bound Amino Acids and/or Resin-Bound Peptides

A simple case study illustrating the synthesis of a range of heterocyclic compounds from resin-bound amino acids and/or resin-bound peptides as starting materials is shown in Scheme 2. Thus, the reaction of the N-

SCHEME 2. Synthesis of Hydantoin and Thiohydantoin Libraries from Resin-Bound Dipeptides



terminal amino group of a resin-bound dipeptide with carbonyldiimidazole or thiocarbonyldiimidazole led to an intermediate isocyanate (or thioisocyanate) that further reacts intramolecularly to form the five-membered ring hydantoin **5** (or thiohydantoin **6**).[59] To increase the diversity of this class of compounds, 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).

Treatment of resin-bound amino acids with an isothiocyanate provided a thiourea that, in the presence of $HgCl_2$ and a primary or secondary amine, generates the resin-bound guanidines. Cleavage from the solid support with HF generates the 2,3,4-trisubstituted 4*H*-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-aminoimidazolidin-4-ones **10** (Scheme 3).[60,61]

Starting from resin-bound acylated dipeptides, the bicyclic [3,5,7]-1*H*-imidazo[1,5-*a*]imidazol-2(3*H*)-ones **11** were obtained following the treatment of the resin-bound acylated dipeptides with phosphorus oxychloride ($POCl_3$) (Scheme 4). The best results were obtained using 15 equiv of freshly distilled $POCl_3$ at 100 °C in dioxane for 18 h.[62] Under the same reaction conditions, resin-bound N-acylated dipeptide amides having tryptophan as the C-terminal amino acid generated, following 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]

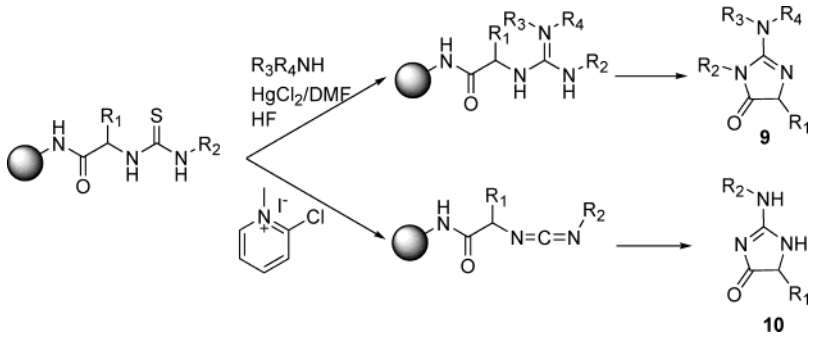
Functional amino acid side chains were also used for the synthesis of different heterocyclic compounds. The parallel synthesis of 1,3,4,7-tetrasubstituted perhydro-l,4-diazepine-2,5-diones **13** (Scheme 5) was performed starting from the Fmoc-protected resin-bound *tert*-butyl ester of aspartic acid. 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 *tert*-butyl group was cleaved and an intramolecular amidation occurred in the presence of HATU to afford the desired 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).[65] Similarly, starting from the same resin-bound amino acid and following reaction of the deprotected cysteine side chain with 2-fluoro-5-nitrobenzoic acid, followed by reductive alkylation and intramolecular cyclization, the l,4-benzothiazepin-5-one derivatives **15** were obtained in good yield and high purity (Scheme 5).[66]

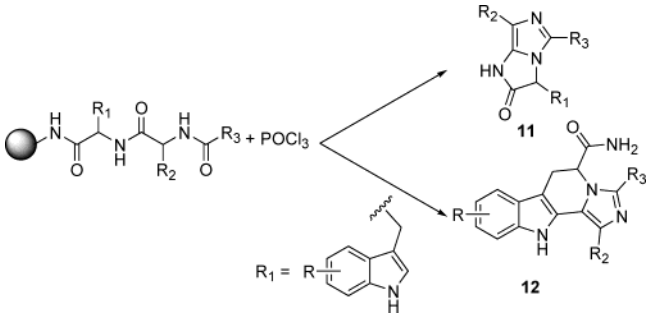
IV. Synthesis of Heterocyclic Compounds from Resin-Bound Polyamines

Following exhaustive reduction of the resin-bound peptides or 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 6). Thus, following reduction of the two amides, the resulting resin-bound intermediate containing two secondary amines was treated with commercially available bifunctional reagents including carbonyldiimidazole, thiocarbonyldiimidazole, cyanogene bromide, oxalyldiimidazole, malonyl chloride, and benzyl isocyanatidocarbonate to form,

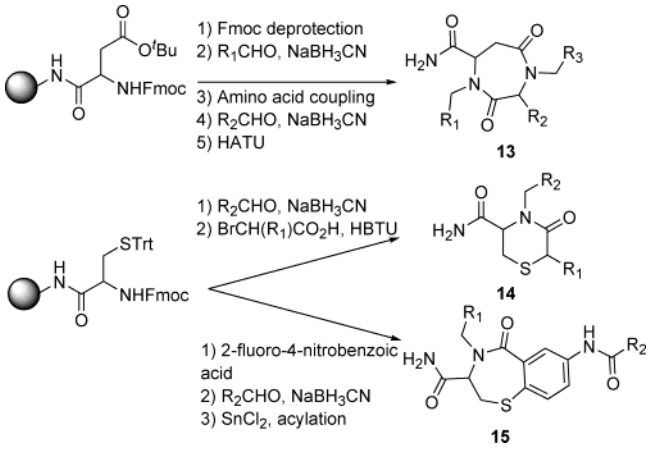
SCHEME 3. Synthesis of Aminoimidazolones from Resin-Bound Amino Acids



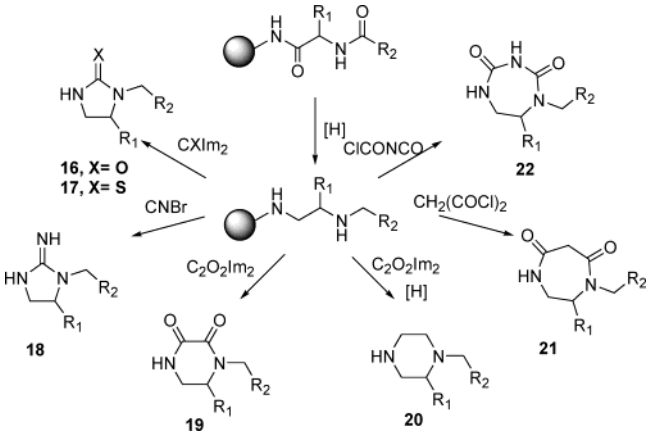
SCHEME 4. Synthesis of Imidazoimidazolones and Imidazopyridoindoles from Resin-Bound Acylated Dipeptides



SCHEME 5. Synthesis of Diazepinediones, Thiomorpholinones, and Benzothiazepines from Resin-Bound Aspartic Acid and Resin-Bound Cysteine



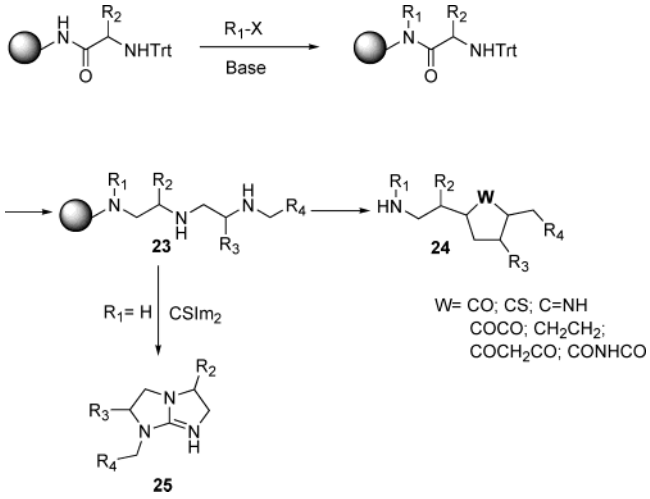
SCHEME 6. Synthesis of Disubstituted Diaza- and Triazacyclic Compounds from Resin-Bound Reduced Acylated Amino Acids



after HF cleavage, the corresponding disubstituted imidazolinones **16**, imidazolidinethiones **17**, cyclic guanidines **18**, diketopiperazines **19**, piperazines **20**, diazepinediones **21**, and triazinediones **22**, respectively.

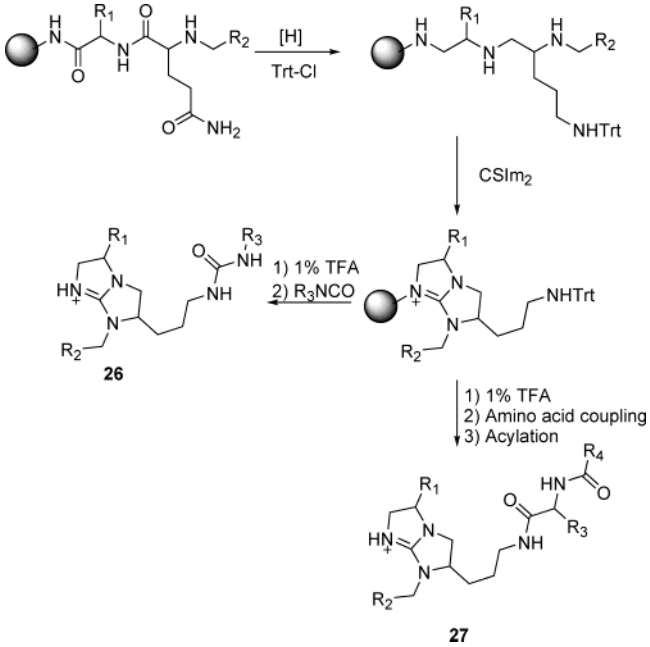
To increase the diversity of each heterocyclic pharmacophore, we extended our approach to the combinatorial synthesis of the trisubstituted heterocyclic analogues starting from selectively N-alkylated resin-bound triamines. The free N-terminal amino functionalities of resin-bound amino acids were protected with triphenylmethyl chloride (TrtCl). The secondary amides linked to the solid support were then selectively alkylated in the presence of lithium *tert*-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 7 illustrates the approach for the solid-phase synthesis of the triamine template **23**. Following the strategy described in Scheme 7, various heterocyclic SCLs **24** were prepared in positional scanning format[82] containing the aforementioned heterocycles. A similar strategy was used for the solid-phase synthesis of bicyclic guanidine **25** from a triamine having three available 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 7. Synthesis of Trisubstituted Diaza- and Triazacyclic Compounds from Resin-Bound Reduced Acylated Dipeptides

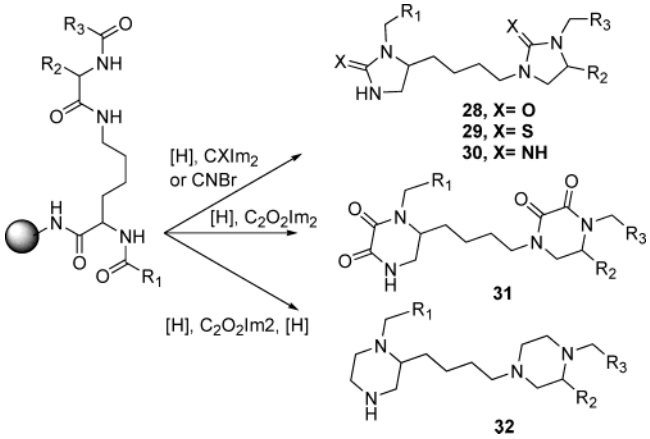


Tethered urea-linked bicyclic guanidines **26** and *N*-acylamino-linked bicyclic guanidines **27** were achieved starting from glutamine-containing resin-bound N-acylated dipeptides.[75] Following exhaustive reduction and selective protection of the primary amine with a trityl group, treatment of the three secondary amines with thiocarbonyldiimidazole 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*-acylamino-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 8).

SCHEME 8. Synthesis of Tethered Libraries from Resin-Bound Reduced Acylated Dipeptides

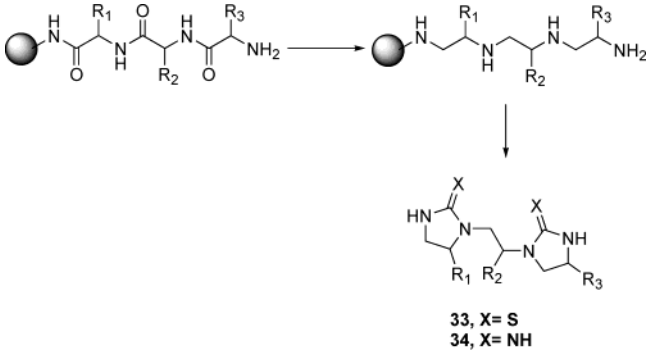


SCHEME 9. Synthesis of Bis-heterocyclic Compounds from Resin-Bound Orthogonally Protected Lysine



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, 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 carbonyldiimidazole, thiocarbonyldiimidazole, 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** (Scheme 9).[83] Cleavage from the solid support with hydrogen fluoride, followed by extraction and lyophilization, yielded

SCHEME 10. Synthesis of Bis-heterocyclic Compounds from Resin-Bound Tripeptides



the desired bis-heterocyclic compounds in excellent yield and high purity.

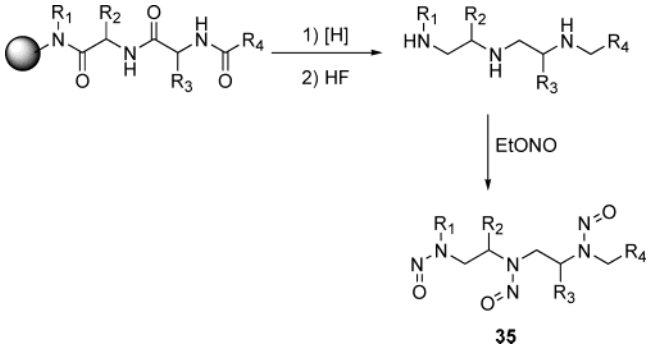
Extending the above-mentioned approaches to larger polyamines, tripeptide amides were synthesized using conventional Boc/Bzl chemistry. Following coupling of the third amino acid and Boc deprotection, the resin-bound tripeptide was exhaustively reduced with borane in THF to yield resin-bound tetraamines containing three secondary amines and one terminal primary amine (Scheme 10). The resin-bound tetra-amine was then treated with bifunctional reagents. Excellent product purities were obtained following their treatment with thiocarbonyldiimidazole 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 thiocarbonyldiimidazole, 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 thiocarbonyldiimidazole to yield the second heterocycle.

Following optimization under various concentrations of thiocarbonyldiimidazole, 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 thiocarbonyldiimidazole 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.

V. Combined Solid-/Solution-Phase Synthesis of Chemical Libraries

To expand the "libraries from libraries" concept, we have combined solid-phase and solution-phase syntheses for the synthesis of a nitrosamine library. Thus, starting from resin-bound selectively N-alkylated acylated dipeptide and following exhaustive reduction of the amides, the resulting chiral polyamines (having only secondary amines) were cleaved from the solid-support. Following reaction with ethyl nitrite in solution, the desired nitrosamine compounds **35** were obtained in good yield and high purity (Scheme 11).[86]

SCHEME 11. Combined Solid-/Solution-Phase Synthesis of Nitrosamines from Resin-Bound Acylated Dipeptides



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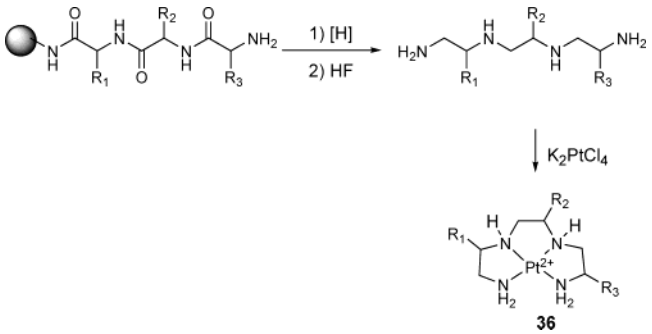
SCHEME 12. Combined Solid-/Solution-Phase Synthesis of Tetraamine−Pt Complexes from Resin-Bound Tripeptides



36

Due to the ability of polyamines to serve as multidentate ligands in the formation of metal coordination complexes, we further extended the "libraries from libraries" concept for the preparation of polyamines in which we were able to combine the benefits of solid phase and solution phase combinatorial approaches for the synthesis of platinum tetraamine coordination complexes **36** (Scheme 12). Individual chiral tetraamines were synthesized by exhaustive reduction of resin-bound tripeptides. Following cleavage of these individual polyamines from the resin, quantitative conversion to the corresponding tetraamine−Pt(II) complexes was achieved by solution phase interaction of the individual chiral tetraamines with K_2PtCl_4. Coordination of platinum(II) with tetraamines converts the two secondary nitrogen atoms to chiral stereocenters, yielding four possible Pt−NH stereoisomers. Depending on steric hindrance and ring torsion, one of the four isomers should be thermodynamically favored. Interestingly, the toxicity of the tetraamine−platinum(II) complexes identified against normal cells were significantly less than the corresponding uncoordinated tetraamines.[87]

Conclusion

The synthetic approaches and combinatorial concepts described above in this short perspective have allowed our laboratory to generate libraries totaling millions of compounds in mixture-based positional scaning formats[82] that are readily deconvoluted following biological screening, thereby greatly increasing the chances of finding useful therapeutic and diagnostic agents. We have found that the use of amino acids and peptides as precursors for the generation of combinatorial libraries simplifies the generation of potentially biologically active compounds. 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 tea-bag technique[5] has been licensed to Irori-Discovery Partners, and millions of tea-bags are now sold as MicroKans. The synthesis and screening of libraries in the "positional scanning" format has greatly decreased the time between screening and the synthesis of individual compounds. Additionally, the application of the "libraries from libraries" concept has permitted the near geometrical 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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