

Developing a general strategy for the solid supported synthesis of heterocycles: Applications to the generation of molecular diversity and drug discovery

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Abstract: The development of a general strategy for the generation of molecular diversity in the form of novel, non-amide based heterocyclic structures is described. The generation of diverse peptide and peptidomimetic libraries, the automation of these strategies and computational approaches to diversity generation are also discussed. The main focus of this lecture is the progression of these concepts into a strategy for small molecule library generation, and hence the generation of small molecule therapeutic leads.

Introduction

Advances in molecular biology, pharmacology and automation have now made routine the rapid testing of minute amounts of novel compounds.[1] This has provided a huge boon to established pharmaceutical companies, who have found lead molecules for novel assays in the archives of chemicals amassed through many decades of traditional organic synthesis. This can be confirmed by simply examining recent reports of non-peptidic lead structures for various new, exciting biological targets.[2] In contrast, biotechnology companies, with often state-of-the-art biological programs but generally a small medicinal chemistry cohort, do not possess these vast chemical compound collections, and often find themselves at a competitive disadvantage *vis a vis* the “traditional” drug discovery companies with regard to discovering lead structures using modern screening techniques. It is not surprising therefore, that these biotechnology companies have initially led the development of generating molecular diversity in the form of chemical libraries to enhance their drug discovery programs.[1] Early efforts took the form of generating libraries of peptides and oligonucleotides. In recent years, due to generally unfavorable bioavailability and stability, the concepts initially brought forward by these pioneering studies on biopolymers has markedly stimulated the field of molecular diversity as it moves now to the synthesis of more druglike, heterocyclic molecules.[1,3]

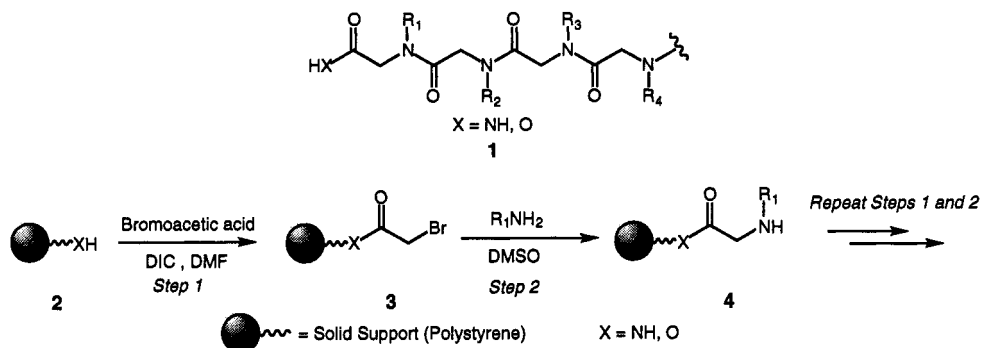
The application of molecular diversity techniques to drug discovery is a multidisciplinary effort, requiring the skills of people of varied backgrounds, ranging from computational chemistry to engineering to organic synthesis to molecular biology, and is therefore difficult to adequately describe in this short format.[1,3] It is the objective of this talk to provide an account of one aspect of the Chiron method for molecular diversity-based drug discovery, namely, the development of a general synthesis strategy for the generation of non-oligomeric, heterocyclic molecules familiar to the medicinal/organic chemist.

“Peptoids” as a Tool for the Generation of Molecular Diversity

In order to rapidly explore a chemical alternative to natural biopolymers, and to attempt to ameliorate some of the unfavorable pharmacological properties generally associated with these types of molecules, the Chiron group has developed the automated synthesis of “peptoids”, a class of compounds which can be thought of as oligomeric N-substituted glycines (NSGs, **1**), but which could, in principle, encompass a wide variety of amino acid or other oligomeric backbones.[4]

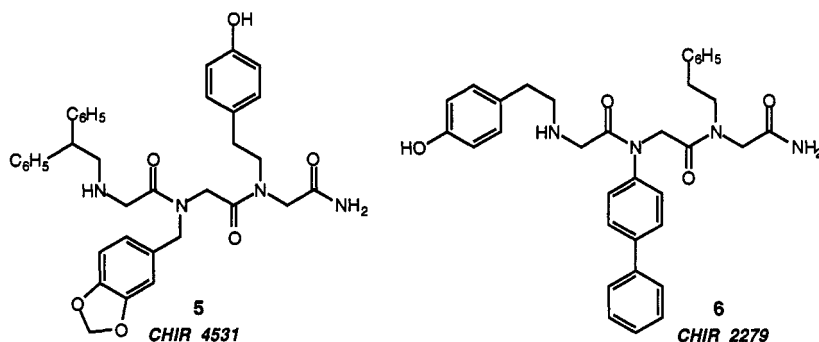
Early studies used the somewhat tedious method of preparing individual N-substituted Fmoc glycine derivatives and employing these in traditional solid phase peptide synthesis,[4] but this approach was quickly superseded by implementation of the much more practical “sub-monomer” (e.g., **2** to **4**)

synthesis method.[5] This has proven to be a very efficient method for the production of these molecules and--due to its iterative nature--can be readily applied to automated synthesis methods.

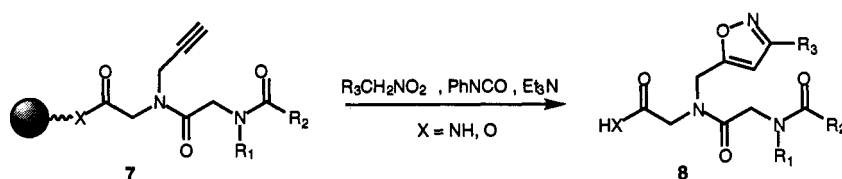


One of the most attractive features of this strategy is the way in which it incorporates the wide diversity represented by available (either commercially or proprietary) primary amines into the synthesis. Since there are $>10^3$ amines commercially available, in principle 10^6 and 10^9 dimer and tetramer peptoids, respectively, can be prepared from commercially available materials, clearly an impractical task. To make the exploration of this vast potential combinatorial library feasible, a paradigm was required to select a minimum set of these amines which preserves the maximum diversity of the library.[6]

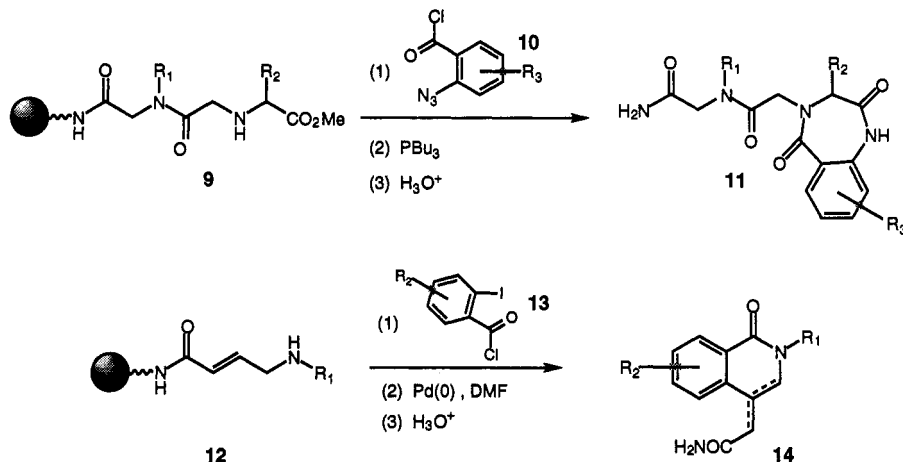
Towards this objective, the Chiron group has developed sophisticated statistical-based computational methods aimed at aiding in the design of libraries.[6,7] In a powerful illustration of the potential of this type of synergistic computational/experimental approach, the sub-monomer synthesis of a library of NSG peptoids designed using this type of computational approach (combined with some degree of medicinal chemistry intuition) led to the discovery of CHIR 2279 (**5**) and CHIR 4531 (**6**), ligands which have nanomolar affinity for the α -adrenergic and μ -opiate receptors, respectively.[7]



The NSG scaffold afforded by sub-monomer synthesis is extremely amenable to further functionalization and can be easily transformed into alternate scaffolds, which more closely resemble traditional drug-like molecules. For example, incorporation of unsaturation(s) in the side chains of NSG dimers and trimers (using the sub-monomer approach with unsaturated amines, e.g., **7**) allows for addition of 1,3-dipolar species such as nitrile oxides, affording isoxazoles such as **8** in good yields.[8] This has been termed a "post synthesis modification", and is completely analogous to the post-translational modification of proteins employed by biological systems. Immobilization of one of the reactants on the solid support plays an important role in the success of this chemistry, as large excesses of nitrile oxide can be used to drive the reaction to completion with little concern given to the contamination of the cycloadduct with material derived from reagent dimerization, since these contaminants are easily washed off the resin prior to acid catalyzed cleavage of cycloadduct from the resin.



Zuckermann and Goff have shown that the NSG backbone can be transformed into benzodiazepinediones and isoquinolones, as well.[9,10] The synthesis of these "hybrid" molecules are shown below. The critical step in the synthesis of the benzodiazepinediones **11** is the intramolecular Wittig-type reaction of the iminophosphorane produced by azide reduction by tributylphosphine,[9] while the crucial step in the synthesis of the isoquinolines **14** involves the intramolecular Heck reaction of an aryl iodide onto the crotonic acid derived peptoid; this precursor is produced by replacement of the bromoacetic acid by bromocrotonic acid in the initial submoner steps.[10] Since diversity can easily be introduced in these reactions, these reactions are excellent means for the production of libraries for screening in pharmacological assays.

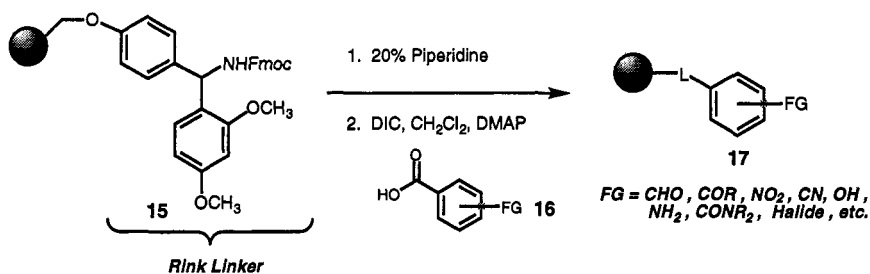


General Strategy for Non-Amide Based Solid-Supported Heterocycle Synthesis

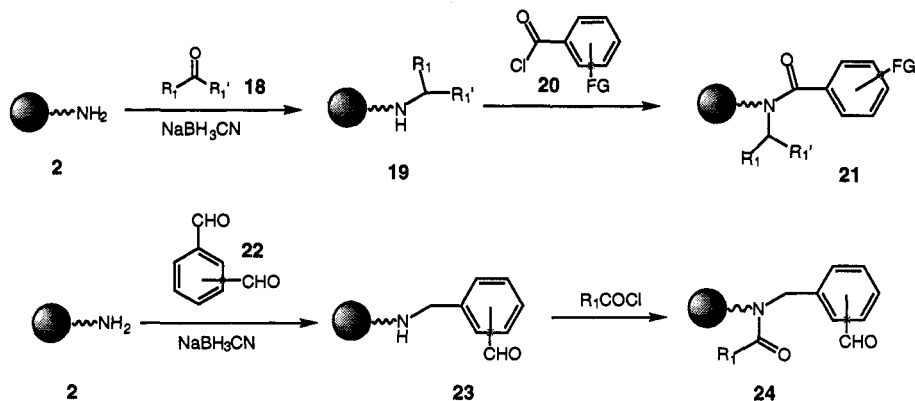
We have been attempting to integrate the basic principles delineated by these groundbreaking studies into a general strategy for the generation of diverse, non-amide based heterocyclic molecules. To do this we are utilizing solid supported synthesis methods. This has permitted dramatic increases in the speed of synthesis through both simplification of work-up, and automation. Additionally, the freedom to use, and wash away, large excesses of reagents, permits some reactions that are wholly impractical in solution-phase. Three important objectives were also established at the outset of our studies. First, we wished to develop rapid, efficient syntheses of structurally diverse, novel ring systems on polymer support (the generation of novel structures which are outside the coverage of existing patents being a priority). We also wished to synthesize structures which will have a high probability of favorable ADME (Absorption, Distribution, Metabolism and Excretion) properties to facilitate further development once a lead has been discovered. Finally, we felt that the syntheses in any such strategy should be modular and convergent in nature, incorporating as many readily accessible building blocks as possible into the synthesis with minimal protecting group transformations and synthetic manipulations. This latter objective highlights one of the most important facets of the Chiron peptoid work--namely, that the facile integration of readily available building blocks, in this case amines in NSG synthesis, permits the rapid synthesis of large libraries, and thus a more thorough exploration of diversity space.

Solid-supported organic synthesis has become one of the most actively investigated areas of modern organic synthesis. It is becoming apparent that nearly every organic reaction can be performed on a solid support, and that, in fact, some reactions are much more effective on solid support than in solution (though the opposite situation is certainly encountered, as well).[11] Some of the advances in this area from Chiron's laboratories will be discussed in detail during this lecture.

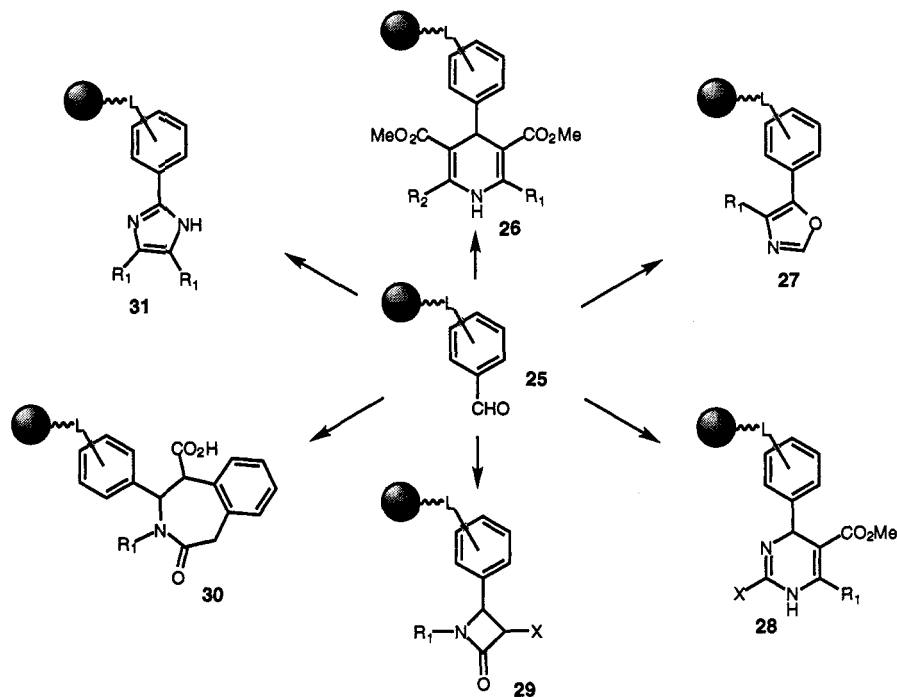
Our synthesis strategy has as its philosophical antecedents the post-synthesis modification of Pei and Moos (*vide supra*) and Houghten's concept of a "library from a library".[8,12] The initial stage of our strategy involves the immobilization of a molecule containing a reactive functional group on the polymer; this functional group--which may be an aldehyde, aromatic ketone, or other moiety -- then functions as the cornerstone for elaboration into various heterocyclic ring systems. This immobilization might be as simple as linking the functionalized molecule to the resin by amide bond coupling reaction (**15** to **17**) or might involve reaction of this functionality via a simple organic transformation such as a Wittig type reaction or aldol condensation to afford a synthon which can subsequently be used in a variety of transformations. The nature of this initiating group depends entirely upon the subsequent chemistry, but comparisons can be made between the linking of these functional groups to the solid support and the set of 20 common amino acids used as building blocks in peptide synthesis.



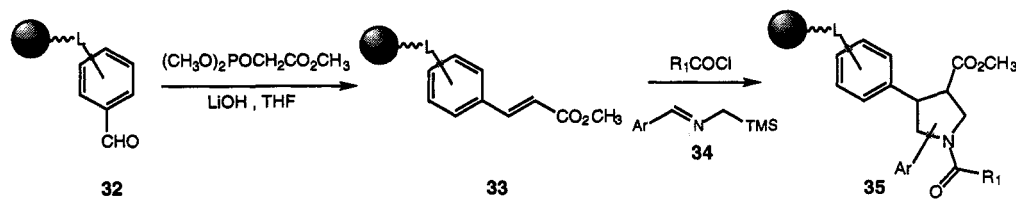
Diversity can also be installed in this step, as shown below. The amine functionality from the support linker (illustrated in this case with commercially available Rink [13] linker) can be easily and cleanly functionalized by using reductive amination chemistry, meaning that a wide variety of aldehydes and ketones can be incorporated into the synthesis at this position.[14] Following reductive amination, acylation with a moiety containing the functional group to be propagated then proceeds smoothly (**19** to **21**). This allows for considerable diversity generation prior to installation of the heterocyclic scaffold. Alternatively, the functional group itself can be installed using this reductive amination strategy; for example, we have found that reductive amination of dialdehydes such as **22** affords selectively the product of monoreductive amination, **23**, in good yields.[14] Acylation of this with various carboxylic acid derivatives then allows for considerable diversity generation in this step. It should be noted that while the solution phase analogue of this reaction provides a very complex mixture of products, immobilizing one of the reactants on the resin affords complete control over product distribution. Leznoff has used a similar strategy to monoprotect symmetrical diols, among other reactions.[15]



Once immobilized onto the solid support, the next stage of our strategy involves the elaboration of these synthons into a variety of heterocyclic ring systems. An example of this propagation sequence for support bound aromatic aldehydes is shown below. A series of operationally simple one- and two-step, convergent protocols are performed which efficiently transform these support-bound aromatic aldehydes into heterocyclic systems such as pyrrolidines, pyrimidines, β -lactams, and imidazoles, among others. Diversity is added as components are incorporated into the synthesis. For example, support-bound imines can be easily formed by condensation of immobilized aldehydes with large excesses of amines; reaction of these imines with acid chlorides in a Staudinger condensation leads to very efficient β -lactam formation (**29**).[16] Similarly, in a modified Biginelli condensation, reaction of this aldehyde with guanidines (and amidines) and β -ketoesters efficiently leads to dihydropyrimidines (**25** to **28**).[17] Reaction with β -ketoesters and amines gives the dihydropyridines **26**,[18] while condensation with amines and α -diketones affords imidazoles in good yields.[18] Similar arrays of reactions can be performed with other functional groups, as well, such as ketones, α,β -unsaturated carbonyl compounds, benzoin, nitroaromatics, etc., permitting access to a huge "library" of diverse ring systems. Computational considerations similar to those described above can also be implemented in order to obtain the most satisfactory sampling of diversity space.

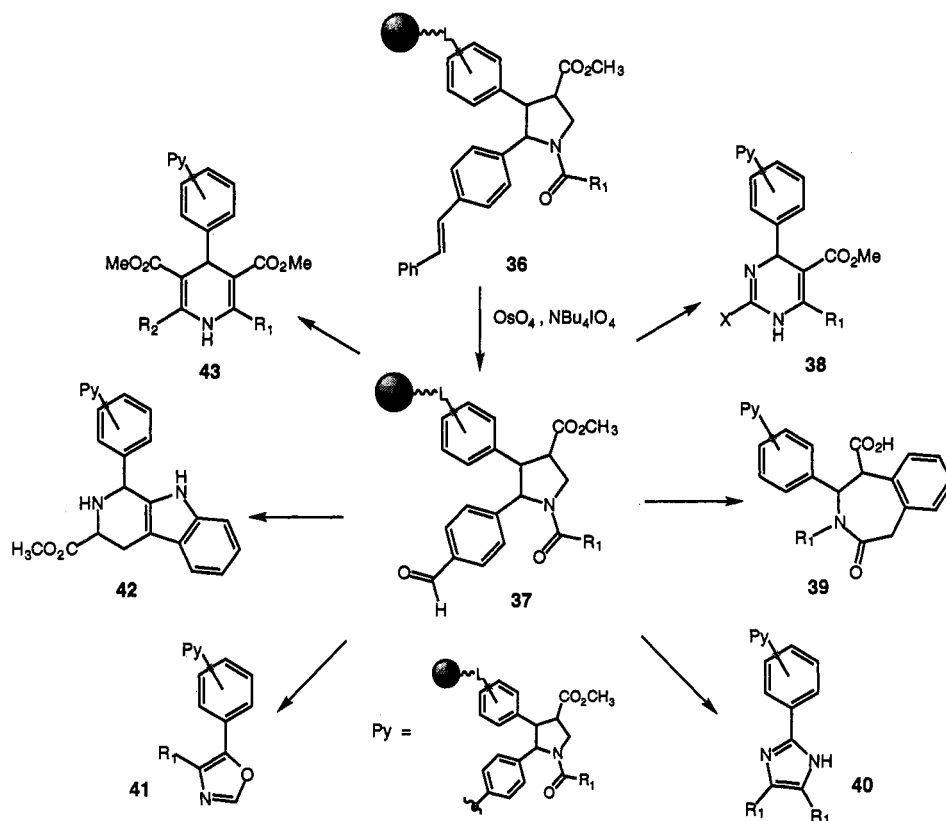


The third stage of our strategy involves exploitation of the functional groups embedded into the newly generated scaffolds. This small set of functional groups is amplified into a larger set of functionalities *via* a series of transformations similar to those described earlier. This is perhaps best illustrated in the synthesis of the structurally novel pyrrolidines given below. Following immobilization of the aryl aldehyde on the solid support, a Horner-Emmons olefination affords the resin bound cinnamate **33** in high yield. This is the initiation step. Cinnamate **33** can now be readily elaborated into a variety of heterocyclic systems. For the purposes of this example, we show the propagation of this synthon into the pyrrolidines.[18] Reaction of the cinnamate with an N-acylazomethine ylide, generated by treatment of the aryl (trimethylsilyl)methylimine **34** with an acid chloride according to the protocol of Achiwa [19], affords the pyrrolidines **35** as a mixture of diastereomers in very high yields. Diversity can be introduced in each component in this cycloaddition, i.e., the cinnamate, the aldehyde and acid chloride. Even more diversity can be easily introduced by further manipulation of the carboxylate functionality (e.g., saponification and subsequent amide bond formation).



The amplification of this scaffold is shown below. If the azomethine ylide in this cycloaddition is derived from 4-stilbenylbenzaldehyde, the resulting pyrrolidine adducts **36** can then be amplified by simply oxidizing (NBu_4IO_4 , OsO_4) (18) the stilbenyl moiety to the aromatic aldehyde **37**. Aldehyde **37** can then be again transformed *via* a series of simple one- and two-step reaction protocols into the array of novel heterocyclic systems shown below. In this way, the chemistry developed for one class of heterocycles can be easily adapted for the synthesis of new classes of molecules, and a variety of novel structures easily generated. The number of total steps in these sequences are held to below 8-10 and the reactions have deliberately been chosen to be operationally simple and easily amenable to automation.

It has been the aim of this lecture to describe the evolution of a general strategy for the synthesis of novel heterocyclic molecules which expands upon the principles first laid out during early studies on the synthesis of amide-based libraries. While the field of molecular diversity is developing at an astonishing pace, and practitioners must be ready to embrace new, emerging technologies at any time, we feel that we have now in place a strategy which allows for the efficient production of structurally novel, diverse libraries which will provide novel lead structures in our drug discovery program.



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