

AUTOMATED TOOLS FOR THE PRODUCTION OF NON-NATURAL MOLECULAR DIVERSITY

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Novel and efficient oligomerization chemistry has been combined with robotic synthesis technology to rapidly generate equimolar biopolymer mixtures. Diverse libraries of peptoids (N-substituted glycines) are routinely generated and screened against a variety of pharmaceutically relevant receptors as part of a drug discovery program. Details of peptoid synthesis by the solid-phase submonomer method are described, as well as a description of the robotic synthesizer, and elements of library design.

INTRODUCTION

The screening of large compound collections has traditionally been a fruitful approach toward the identification of potential therapeutic agents (1). Recent efforts to dramatically accelerate the rate of drug discovery (2) have focused on the rapid synthesis of peptide and nucleic acid libraries as a source of molecular diversity. These compound collections are readily accessible and have been screened against a variety of molecular targets. Although these libraries have provided novel, high-affinity ligands to a variety of antibodies and other proteins, the natural biopolymers often lack the pharmaceutical properties such as oral bioavailability and *in vivo* stability that are necessary for clinical development (3). Thus, the utility of these methods for the discovery of viable drug candidates can often require the crucial step of converting the biopolymer lead into a more "synthetic" structure. In order to avoid this difficult step, we have extended the molecular diversity concept to non-natural polymer libraries.

Through a combination of automated synthesis technology and novel oligomerization chemistry, we have developed a rapid method for the discovery of drug candidates. Equimolar mixtures of oligo (N-substituted)glycines are robotically synthesized and screened in solution against a variety of pharmaceutically relevant receptors on a routine basis in our laboratories.

AUTOMATED EQUIMOLAR MIXTURE SYNTHESIS

When screening a mixture of compounds for biological activity, the quantitative interpretation of the data is greatly simplified if it is known that the mixture composition is equimolar in all components (4). Mixture equimolarity can be readily attained by a modification (5) of solid-phase synthesis methods. In the resin-splitting method, equimolar mixtures are generated by dividing a solid support into equal portions, performing a different chemical reaction on each portion, and then combining

the portions. Equimolarity of mixtures is ensured since competition between different reactions is eliminated. Thus, any compounds that can be made by a multi-step solid-phase synthesis can be used for the combinatorial synthesis of libraries.

In order to facilitate a high-throughput screening program based on the screening of equimolar mixtures in solution, we have developed instrumentation (6) that fully automates the resin-splitting procedure. The equimolar mixture synthesizer was designed around a Zymark robotic arm and consists of an array of 36 fritted reaction vessels, a resin mixing vessel, a series of solenoid valves, a rack to store up to 25 monomer solutions, pressurized solvent/reagent bottles, a computer controller and an integrated cleavage/deprotection station (7) (Fig. 1). The distributing and recombining of the solid support as per the resin-splitting method is achieved by the robotic transfer of the resin as a slurry between the mixer and the reaction vessel array. All other aspects of solid phase synthesis such as reagent addition, resin mixing and resin washing have been automated as described (6). After a synthesis has been completed, oligomers are processed by an automated cleavage/deprotection station to release the compound into solution. Samples require manual lyophilization prior to assay.

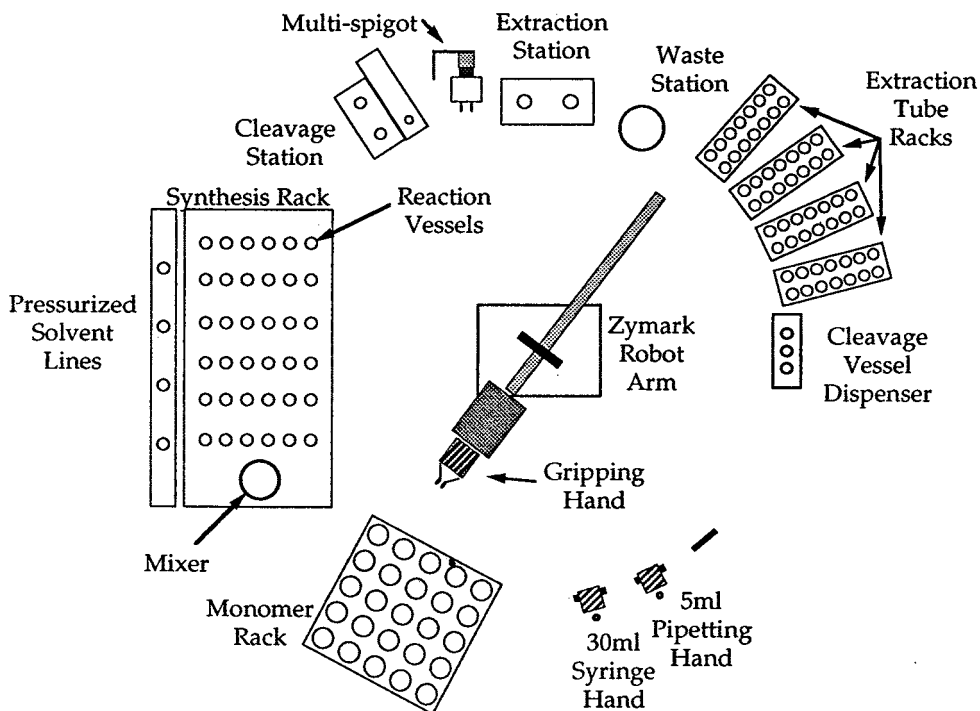


Figure 1. Schematic of the equimolar mixture synthesizer and integrated cleavage/deprotection station.

NON-NATURAL DIVERSITY CHEMISTRY

In order for a class of molecules to be suitable for the generation of diverse libraries, several criteria must be met (8). First of all, the compounds should be accessible by solid-phase synthesis to allow the resin-splitting method of equimolar mixture synthesis to be used. Secondly, molecules with an oligomeric architecture allow a variety of chemical functionalities to be incorporated into a molecule with a *single linking chemistry*. This greatly simplifies automation of the library synthesis. The chemistry used to couple the monomers must be high-yielding, and must be general enough to allow the inclusion of a wide variety of sidechain structures. Importantly, the monomers used must be readily available to facilitate the high-throughput synthesis of libraries; otherwise the most significant task in library production can easily become the preparation of large monomer stocks by a team of synthetic chemists. Finally, the molecules generated should be hydrolytically and enzymatically stable.

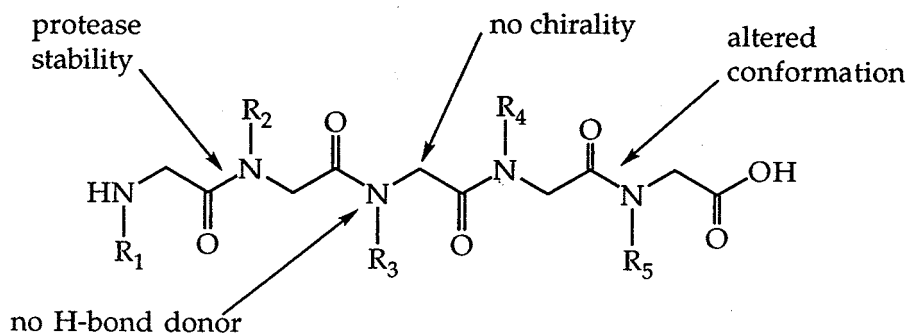
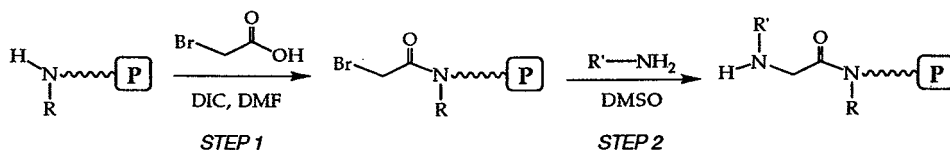


Figure 2. Structure of an N-substituted glycine peptoid pentamer and some major differences as compared to a peptide.

Our efforts have focused on the synthesis of (N-substituted)glycine (NSG) peptoid oligomers (9) because they fit the criteria listed above. These oligomers are structurally similar to peptides, but have several major differences as outlined in Figure 2. The original method (9) for the synthesis of oligomeric NSG peptoids was analogous to standard solid-phase methods for peptide synthesis. Specifically, N^{α} -Fmoc-protected monomers were coupled to the secondary amino group of the resin-bound peptoid chain, followed by removal of the Fmoc group. A disadvantage of this approach, however, was the necessity of preparing suitable quantities of diverse sets of protected N-substituted glycine building blocks.

In a recent synthetic advance (10), however, it was found that each N-substituted glycine monomer could be assembled from two readily available "submonomers" in the course of extending the NSG polymer (Scheme I). Each cycle of monomer addition consists of two steps, an acylation step and a nucleophilic displacement step - there is no N^{α} -deprotection step. Thus, oligomeric NSGs can be considered as alternating condensation copolymers of a haloacetic acid and a primary amine. The α -haloacetyl

submonomer is common to all cycles of chain extension; each R-NH₂ submonomer is simpler in structure and many are commercially available. Thus, oligo NSG synthesis was dramatically simplified.



Scheme I

The solid-phase submonomer synthesis method has been used to synthesize a wide variety of NSG peptoid oligomers in our laboratories (10). Crude HPLC analyses of some representative pentapeptoids are shown in Figure 3. Primary amines that are not too sterically hindered or too electron deficient can successfully be incorporated into oligomers. For example, primary amines that are hindered at the α carbon, like diphenylmethylamine and *t*-butylamine, do not work as submonomers, but the less congested amines, like 2,2-diphenylethylamine, isobutylamine and cyclopentylamine, can be efficiently incorporated into pentamers. Primary amines with low nucleophilicity like anilines can also be incorporated into pentamers as long as they are not substituted with electron withdrawing groups. Amines that contain reactive sidechain functionalities, such as aliphatic hydroxyl groups, 1° and 2° amines, carboxyl and thiol groups, need to be protected. The most straightforward strategy is to use a standard TFA-labile resin-linkage with TFA-labile sidechain protecting groups.

LIBRARY DESIGN

The efficient assembly of diverse NSG peptoid oligomers from readily available starting materials coupled with automated equimolar mixture synthesis technology opens up a tremendous new source of accessible compounds. In order to focus this expansive tool, several experimental design limitations must be imposed.

A search of the Fine Chemicals Database reveals over 6000 primary amines that are commercially available. If one sorts these by cost to only include amines that cost less than \$5.00/g then the number is reduced to ~1000 amines. In order to maximize the potential for acceptable bioavailability and absorption characteristics, the molecular weight of the library components are kept below a maximum of 600 Daltons - which limits the oligomer length to tetramers. In theory, then, over 10¹² tetrapeptoids are readily accessible.

In a typical library synthesis, however, the complexity of each pool is often kept below 1000 components in order to simplify the interpretation of assay data (4). For robotics convenience each peptoid library is synthesized from a set of ≤ 25 primary amines. Therefore, computational tools are used to choose dissimilar subsets of 25 amine sidechains to be included in a particular library (11). These sidechains are chosen to cover a wide range of characteristics: charge, hydrophobicity, aromaticity, size, shape, flexibility and degree of branching. Alternatively, libraries can be designed around a particular pharmacophore.

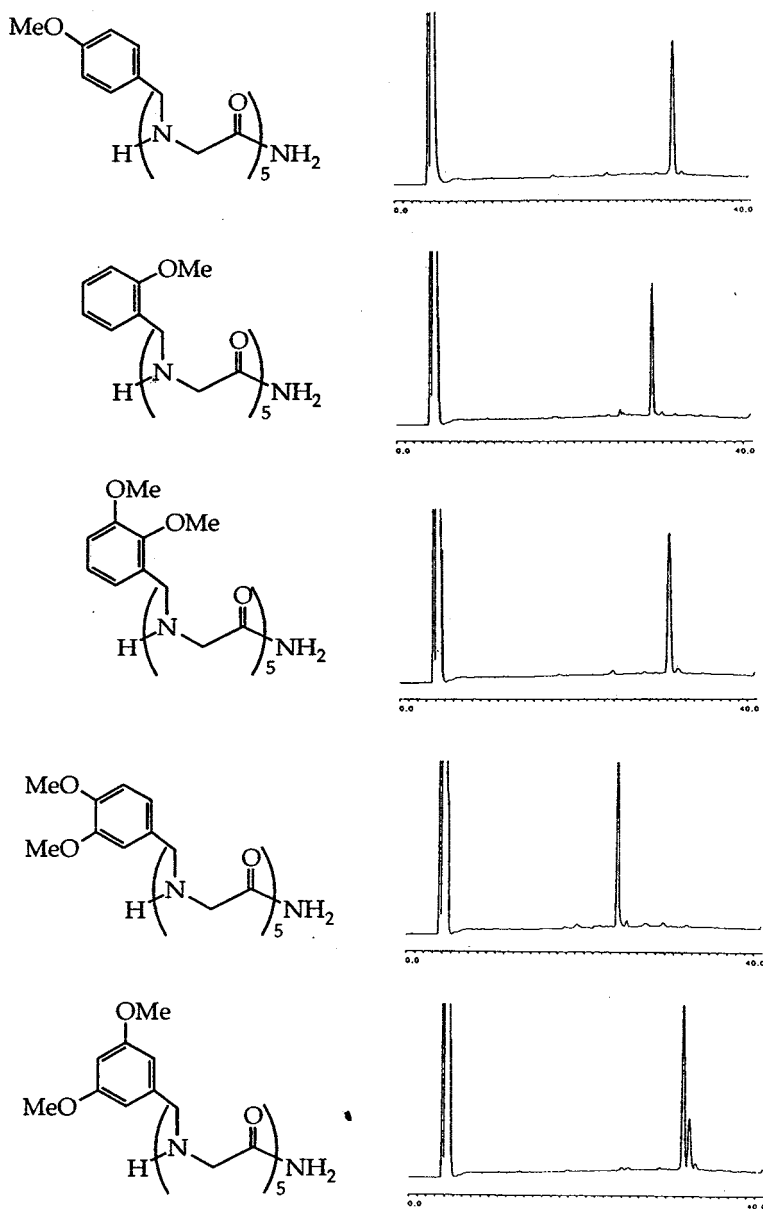


Figure 3. Reverse-phase HPLC traces of five crude pentapeptoids synthesized by the submonomer method. All amines are tested for their ability to incorporate into a pentamer prior to their inclusion in a library. Analyses were performed on a Vydac C18 analytical column with an elution gradient of 0-80% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ containing 0.1% TFA in 40 minutes.

CONCLUSION

The peptoid approach offers a distinct advantage over the use of peptide libraries for drug discovery in that the conversion of a lead into a non-peptide is entirely avoided. The oligo (N-substituted)glycines are synthesized in high yields by a polymer-supported method that uses commercially available starting materials. The chemistry is general enough to allow the incorporation of a wide variety of sidechain structures that are much more diverse than the standard amino acid sidechains. The robotic apparatus fully automates the splitting of resins so that defined mixtures of precise composition can be rapidly prepared.

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