The chemical synthesis of peptidomimetic libraries

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Molecular diversity as a source of potential drug candidates has been an area of tremendous growth during the past year. The field has been dominated by investigations featuring diverse peptide libraries, generated by both chemical and biological methods. Yet the many undesirable properties of peptides as drugs, such as poor oral availability and low in vivo stability, have prompted chemists to develop methods for the efficient synthesis of conformationally constrained peptide, biopolymer and non-polymeric compound collections.

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Introduction

The systematic screening of diverse compound collections from microbial fermentation broths, plants, marine organisms, and synthetic compound collections has yielded a substantial number of pharmaceutical lead compounds in drug discovery programs in recent years [1]. These screening strategies rely not on the knowledge of a target’s structure but rather on the random testing of a sufficiently large number of chemical entities. The success of these screening programs suggests that a highly diverse set of chemical structures generated by rapid combinatorial synthesis could similarly provide drug candidates.

Within the past two years, molecular biologists and peptide chemists have developed schemes for the rapid generation and screening of diverse peptide libraries [2]. Peptides are an ideal class of molecules for the generation of combinatorial libraries because they are composed of 20 reasonably diverse amino acid building blocks which can be efficiently assembled either by chemical synthesis or by transcription/translation of DNA. In several cases, peptide libraries have provided moderate- to high-affinity ligands to cellular receptors (S Fong et al., unpublished data) [3,4•] and to proteins [5–7] with no known natural peptide ligand. However, peptides are less than ideal when considered as drug candidates. Poor oral availability and low in vivo stability are among the undesirable features of peptides [8].

In order to avoid these problems, chemists are now applying the methodologies used with synthetic peptide libraries [9,10•,11,12•,13,14•] to collections of cyclic peptides, novel biopolymers, and even non-polymers. New synthesis technologies and chemistries that facilitate the synthesis and screening of diverse chemical libraries have recently been developed. One of the most significant of these is the ‘resin-splitting’ mixture synthesis method, a polymer-supported multiple synthesis procedure that allows a high degree of control over the composition of a peptide mixture [15]. Mixtures are generated by dividing a solid support into equal portions, coupling a different amino acid to each portion, and then combining the portions. Equimolarity of mixtures is ensured as competition between the amino acids is eliminated. The method generates for each particle of solid support one polymer sequence that can facilitate screening [12•], and can generate mixtures containing more than one million different components [4•,11,12•]. The synthesis procedure has recently been fully automated [16••], and should be extendable to a variety of new chemistries.

Chemistries that are amenable to combinatorial library synthesis would ideally have the following characteristics: be polymer-supported to facilitate the resin-splitting method; be assembled in high yield with automatable chemistry; and allow the incorporation of a wide variety of chemical functionalities. In addition, the chemistry should generate classes of compounds that are biostable and bioavailable, and have the appropriate conformational properties.

Cyclic peptides

Peptides that are cyclic often show increased resistance to enzymatic degradation [10] and constrained flexibility compared with the linear form. There are several recent examples of peptides where the increased rigidity induced by cyclization has led to enhanced receptor-binding affinity; these include potent cyclic RGD antithrombotics [17•], cyclic oxytocin antagonists [18], and cyclic α-melanotropin analogues [19].

In order to generate libraries of cyclic peptides, it is important that the cyclization reaction can be performed in high yield and with a minimum of additional manipula-

Abbreviation

Fmoc—9-fluorenlymethoxycarbonyl.
Polymeric diversity

Polymers are well suited for the generation of chemical diversity, as relatively few monomers (e.g. the standard 20 amino acids) can be combined with a common linking chemistry to generate a tremendous number of compounds. This combinatorial approach has recently been applied to non-peptide polymers to allow the generation of diverse peptidomimetic libraries. Such polymers would be expected to display an even more diverse array of side chain functionalities than peptides, have different structural characteristics, be resistant to enzymatic degradation, and perhaps exhibit better bioavailability.

Amide polymers

The chemistry of amide bond formation has been used to generate a variety of polymers with alternative peptide backbones. A straightforward approach has been taken by Wang and coworkers [26] in which a large set of peptides containing ε-amino acids were synthesized and assayed for receptor binding. Hagihara and coworkers [27**] reported the synthesis of vinylogous polypeptides, in which an (E)-ethenyl unit was inserted between the carbonyl carbon and the α-carbon (Fig. 1). Trimeric vinylogous peptides and tetrameric hybrid peptides were synthesized by solution-phase chemistry and shown using X-ray crystallography to possess novel secondary structures. The vinylogous amino acid monomers were readily prepared by the homologation of amino aldehydes protected at their amino termini, and coupled by conventional amide-bond formation chemistry.

Amide-bond formation has also been used to synthesize oligonucleotide analogues. Huang and coworkers [28] have reported the (solution-phase) synthesis of nylon-based acyclic nucleic acid oligomers up to six residues in length (Fig. 2a). Nucleic acid surrogates based on a peptide backbone have been reported in which serine residues are modified at the side-chain hydroxyl group with a nucleobase and are oligomerized with glycine as alternating copolymers (Fig. 2b) [29]. The solution-phase synthesis of tetrameric structures was achieved. Similarly, the side-chain hydroxyl groups of serine and threonine have been modified with N-acetyl galactose, allowing the solid-phase synthesis of multiply glycosylated peptides [30]. Oligonucleotide analogues of up to 10 residues in length based on an N-aminoethylglycine backbone (Fig. 2c) have been synthesized by solid-phase methods [31†]. These polyamide nucleic acids were shown to bind to complementary DNA strands with high affinity.

Peptide analogues in which the side chains are substituted at the nitrogen atom rather than at the α-carbon have recently been reported (Fig. 3a) [32••]. These peptoid oligomers (oligomeric N-substituted glycines) appear to have excellent resistance to proteases. The peptoids were prepared by solid-phase chemistry from a set of Nα-Fmoc-N-substituted (Fmoc, 9-fluorenylethoxycarbonyl) glycine monomers whose side chains closely resemble those of the natural amino acids. The recently reported synthesis of N-substituted glycine oligomers from readily available primary amine and α-halocetic acid 'submonomers' (Fig. 3b) avoids the use of Nα-Fmoc-
protected monomers entirely [35••]. The solid-phase submonomer method was used to synthesize efficiently several oligomers with a variety of 'non-natural' side-chain structures up to 25 residues in length.

Non-amide polymers

The synthesis of functionalized oligomers without amide bonds has also been reported. Nowick and coworkers [34•••] developed an oligoureide scaffold that is stabilized by internal hydrogen bonding, which may serve to orient side chains in a parallel fashion (Fig. 4a). Trimeric compounds were synthesized by solution-phase chemistry, in which a wide variety of side-chain structures were readily introduced as isocyanates. Smith and coworkers [35•] have synthesized 3,5-linked pyrrolin-4-one oligomers which mimic the β strand conformation of peptides (Fig. 4b). Tetrameric compounds were synthesized by solution-phase methods and were characterized by X-ray crystallography.

Non-polymeric diversity

The combinatorial synthesis of non-polymeric organic compounds would allow the rapid screening of 'drug-like' chemical structures. These systems are characterized by the display of a number of side chains around a structured cyclic scaffold. The advantage of libraries of this type is that any lead identified from it will already have many desirable characteristics (such as conformational rigidity and biostability) built into it. This may avoid the step of converting a lead generated from a peptide library into a peptidomimetic drug candidate, and may thus be a better starting point for drug discovery. Bunin and Ellman [36••] have demonstrated this concept by developing a solid-phase method for the general and expedient synthesis of 1,4-benzodiazepine derivatives (Fig. 5a). Each derivative is prepared from three pieces: a 2-amino-benzophenone, an amino acid and an alkylating agent. Ten different benzodiazepine analogs were prepared in excellent overall yields. Hirschmann and coworkers [37•] have reported the use of β-D-glucose as a scaffold upon which four to five side chain functional groups were appended (Fig. 5b). Potent ligands for G-protein-coupled receptors have been discovered using this template.

Future directions

The past two years have seen rapid progress in the synthesis and screening of synthetic peptide libraries. There is currently a trend by chemists toward the synthesis of diverse compound libraries that are less peptide-like and more drug-like. In the near future, there will be wider use of polyamide-based oligomer libraries that contain non-natural amino acids, D-amino acids, cyclization sites, N-alkylation and other conformational constraints. The development of novel biopolymer and non-polymeric diversity systems will continue to be an area of tremendous growth. Chemistries that are amenable to high-throughput solid-phase synthesis will be particularly useful. Mass screening of chemical libraries can require substantial quantities of synthetic building blocks, so movement toward both miniaturization [13] and chemistries that use readily available building blocks [33••] is likely.

The screening of diverse non-peptide libraries will surely present new analytical problems in identifying ligands.
of interest, as they are not likely to be characterizable by conventional peptide analyses. One solution to this problem is to encode the sequence of novel building blocks that comprise a biopolymer or peptide mimetic with a covalently attached chemical tag, e.g. a sequence of amino acids or nucleotides, which can be readily analyzed using conventional methods. Kerr and coworkers [38] have demonstrated the use of amino acid triplets to encode a library of 200 peptides that contain non-natural amino acids. Brenner and Lerner [39] have proposed the use of oligonucleotides as the coding sequence. This procedure of tagging will allow selection experiments to be performed on very large libraries because the coding sequence can be amplified by PCR. These coding strategies do not, however, allow the free ligand to be assayed as the ligands must either be attached to a solid phase or to the chemical tag. The identity of the highest-affinity binders of a free ligand mixture must still be determined by iterative resynthesis procedures [40] or by affinity selection followed by mass spectrometry [41].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest


2. PAVIA MR, SAWYER TK, MOON WH: The Generation of Molecu-

3. O'NEIL KT, HOESS RH, JACKSON SA, RAMACHANDRAN NS, MOUSA SA, DUGRADO WE: Identification of Novel Peptide Antago-

4. HOUGHTEN RA, COOLEY CT: The Use of Synthetic Peptide 

A diverse library of one million free hexapeptides is screened in a competitive opioid receptor binding assay. Through a screening process involving several cycles of iterative resynthesis, several enkephalin analogs are identified.

5. DEVILIN JJ, PANGANIBAN LC, DEVILIN PE: Random Peptide Li-


8. PLATTE RN, NOBRECK DW: Obstacles to Drug Develop-


10. GEYSEN HM, MASON TJ: Screening Chemically Synthesized 

Practical optimization strategies and theoretical limitations are presented for the screening of diverse peptide libraries generated by the multipin method. The authors discuss the "micromote strategy" in which a mixture of all possible hexapeptides are prepared in 400 different pins and then iterative resynthesis is used to identify high-affinity components.


12. LAM KS, HRUBY VJ, LEHN M, KNAPP RJ, KAZMIRSKI WM, HERBH 

A diverse library of resin-bound pentapeptides is synthesized by the resin-splitting method such that each bead contains a unique peptide sequence. The library is used to identify several high-affinity ligands for an anti-β-endorphin monoclonal antibody.

13. FODOR SFA, READ JL, PIRBURG MC, STIPER L, LU AT, SOLAS 

14. ZUCKERMAN RN, KERR JM, SANSI MA, BANVILLE SC, SANTI 
DV: Identification of Highest-Affinity Ligands by Affin-
ity Selection from Equimolar Peptide Mixtures Gen-

The highest-affinity components of an equimolar mixture of free pep-
tides are directly identified by affinity selection with a monoclonal antibody. An equilibrium mixture of the free peptides and free anti-
body is rapidly fractionated by gel filtration. Peptides that elute with the antibody are characterized by mass spectrometry.


16. ZUCKERMAN RN, KERR JM, SANSI MA, BANVILLE SC: Design, Con-

The resin-splitting method for the synthesis of equimolar ligand mix-
tures is automated using a laboratory robot. Detailed description of the apparatus is given as well as the characterization of several peptide mix-
tures.

17. BARKER PJ, BULLINS S, BUNTING S, BURDICK DJ, CHAN KS, DEISHER T, EIBENBROCK C, GADIE TR, GANTZOS R, LEHNMT, ET AL: Cyclic RGD Peptide Analogues as Antiplatelet Anti-

Many cyclic peptide analogues are prepared using a high-yielding thiol-ether-bond-forming reaction. Cyclizations are performed in solution and on-resin to give many potent compounds.


19. AL-OBEID F, DE L CAstrUCCI AM, HADLEY ME, HRUBY VJ: Potent and Prolonged Acting Cyclic Lactam Analogues of α-Melanop-


21. KATES SA, SOLENA, JOHNSON CR, HUDSON D, BARANY G, 
• AUREBECO F: A Novel, Convenient, Three-Dimensional Or-
thogonal Strategy for Solid-Phase Synthesis of Cyclic Pepti-

A cyclic decapeptide is prepared by a convenient on-resin cyclization strategy in which the side chain of an Asp, Glu, Asn, or Gln is used to
link the peptide to the support. A method for the selective deprotection of allyl esters is presented.


33. The synthesis of a novel class of peptidomimetic biopolymers based on an N-substituted glycine backbone is presented. The synthesis of many Fmoc-protected monomers having natural amino acid like side chains, and the solid phase synthesis of several oligomers are demonstrated.


35. The synthesis of oligo(N-substituted glycines) is achieved by the solid-phase assembly of each monomer from two submonomers in the course of polymer formation. The primary amine and halocetatic acid submonomers are readily available and are assembled in high yield. A sequence as long as 25 residues is prepared as well as a number of pentamers containing diverse side-chain structures.


37. The synthesis of 10 benzodiazepine derivatives is accomplished in high yield on a solid support. The suitability of this chemistry for the generation of diverse libraries of non-polymers organic compounds is discussed.


39. A method is developed to encode each non-natural component in a biopolymer library with a unique peptide sequence. The peptide code provides a tag that can be characterized by conventional peptide analysis. An encoded library containing 200 non-natural decapeptides is synthesized by the alternating, parallel synthesis of a branched polymer containing both a binding ligand and a coding peptide.


41. A concept paper, in which the use of a unique oligonucleotide to encode each component of a peptide library is proposed. An advantage of this strategy is that one can perform very large scale in vitro selection experiments because the coding strand can be amplified by PCR.


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