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14 Automated Synthesis of Nonnatural Oligomer Libraries: The Peptoid Concept

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14.1 Introduction

Historically, the most successful approach for the discovery of lead structures for drug development has been the broad screening of natural products. Lead compounds for pharmaceuticals have been obtained from plants, marine organisms, fungi or other microorganisms. In addition, the screening of collections of organic compounds or the modification of bioactive peptides have been used for the generation of drug candidates [1, 2].

Over the past few years, automated and combinatorial methods for the rapid generation of diverse mixtures ("libraries") of peptides, nucleic acids and organic molecules have been developed [3, 4]. Highly diverse sets of large collections of biooligomers or organic compounds should have an increased probability to comprise a few molecules that bind to a specific pharmaceutical target like an enzyme or a receptor.

The approach used here for the generation of molecular diversity [5] is based on the fully automated, high-through synthesis of *N*-substituted glycine (NSG) peptoid libraries. Equimolar mixtures of nonnatural oligomers are generated with precise control over the composition of the synthesized libraries. The diverse peptoid mixtures are screened in the solution phase to obtain binding data that are as accurate as possible.

14.2 Criteria and Goals for the Generation of Molecular Diversity

To generate a large number of different compounds in a short period of time, automation of the synthetic process is highly desirable. A modular approach for the generation of molecular diversity limits the number of the required synthetic manipulations and thus greatly simplifies automation. Consequently, it was decided to take advantage of a simple, high-yielding linking chemistry for the assembly of oligomeric structures.

The building blocks for the intended modular approach should have a wide variety of structurally and functionally diverse side chains. Structural redundancy within components of the library can be avoided by rational selection of the incorporated monomers. The use of readily available building blocks for the oligomers would overcome the costly and time-consuming process of synthesizing large amounts of monomers prior to assembly of the libraries.

Polymer-supported chemistry is particularly attractive for the generation of diverse mixtures with equimolar concentrations of the individual components. In contrast to solution phase chemistry, the attachment of the substrate to a matrix allows for the easy separation of the substrate and ensures that:

(a) all reagents can be washed away after each synthesis step is completed. This allows the use of excess reagents or even the repetition of each reaction step to drive each reaction to completion. Consequently, equimolarity can be achieved despite some differences in the reactivity of the individual substrates and reagents;

(b) the substrate molecules are spatially separated from one another ("pseudo-dilution") [6]. Consequently, intermolecular side reactions are suppressed efficiently, resulting in more homogeneous reaction products. Moreover, reagents with multiple reaction sites react more selectively with a polymer-bound substrate (e.g., the reaction of a primary amine, a symmetrical diamine, etc., with a resin-bound electrophile often provides a single reaction product, as compared to a more complex reaction mixture for the same reaction using an electrophile in solution).

(c) automation can be developed for almost all manipulations;

(d) by recombining and splitting the resin after each reaction step, combinatorial methods can be used for the generation of a large number of compounds. A suitable resin splitting scheme would allow for the synthesis of equimolar mixtures with precise control over the composition of the libraries. Mixture equimolarity greatly simplifies the evaluation of binding data [7] because all assays will reflect normalized affinities of the tested pools.

When synthetic libraries are used for the discovery of biologically active compounds, all initial assay data are generated from pools of compounds rather than single molecules. Therefore, the molecular diversity approach to drug discovery requires the retrogressive identification of the bioactive components. This has been accomplished (a) by affinity selection [7, 8], (b) by iterative resynthesis of successively smaller subpools [9], (c) by attaching a tag that codes for the synthesis of the molecule [10–13] or (d) by having the molecules attached to a matrix and using the location ("address") on the matrix [14] or microsequencing [15] for their identification.

The use of a linker that attaches the potential ligand to a matrix or tag, however, holds the risk of altering the structural characteristics of the individual molecules. Iterative resynthesis may be more labor-intensive than a decoding process, but allows for the identification of bioactive, structurally unmodified components from complex, diverse mixtures.

As a consequence of the availability of optimized protocols for solid phase peptide and nucleotide synthesis, all the initial work on synthetic molecular diversity was accomplished with peptide libraries [16]. In general, however, peptides suffer from poor oral bioavailability and rapid metabolic inactivation [17]. Therefore, the design of unnatural analogs of peptides [18] that possess increased plasma stability, better absorption characteristics and can be used for controlled oligomerization appeared particularly promising.

14.3 The Peptoid Approach

Peptoids are nonnatural oligomers that contain *N*-substituted glycines (NSGs) as their structural motif [19]. Peptoid oligomers are achiral and possess comparable spacing of the side chains (and the amide bonds) as their natural analogs (Fig. 14-1). In addition, the nature of the peptoid backbone is very similar to the nature of the peptide backbone. However, peptoids are devoid of amide protons, which decreases their overall polarity and should increase their oral bioavailability. Since they contain only tertiary amide bonds, they are not subject to degradation by common proteases [20].

Figure 14-1. General structure of an NSG pentapeptoid. The arrows indicate some major differences of NSG peptoids as compared to peptides.

Since this novel class of molecules is based on an oligomeric glycine backbone, peptoids were expected to have a similar conformational profile as glycine. The unsubstitued α-carbon should allow almost unhindered rotation about the phi and psi angles. In addition, both *cis*- and *trans*- conformers of the amide bond should be accessible at room temperature. However, the substituent on the nitrogen is expected to confer different structural properties on peptoids that might limit their flexibility.

Figure 14-2 shows the Ramachandran plots for a common model of a peptide (Ac–Ala–NHMe) as compared to a common model of a peptoid (Ac–Sar–NMe_2) [21]. These maps were generated using the SM2-AM1 semiempirical method with an implicit solvent term [22, 23]. Figure 14-2a (left) shows the traditional Ramachan-

dran plot [24] for Ac–Ala–NHMe. As expected, the beta-sheet region in the upper left-hand corner of the map is seen as a large area of conformations within 2 kcal/mol of the minimum. The right-handed alpha helix (phi = 100, psi = 60) is only 0.5 kcal/mol higher in energy than the beta-sheet region. The minimum corresponding to the C_7-axial conformation (phi = +60, psi = −60) is approximately 2 kcal/mol higher in energy than the other two minima.

Figure 14-2b (right) shows the Ramachandran plot for the *trans*-amide conformer of Ac–Sar–NMe_2. Since the NSG peptoid backbone contains no stereocenter, there is an axis of symmetry in this plot. The minima for peptoids are clearly in a different region of the map than are the minima for peptides. The lowest energy conformation for Ac–Sar–NMe_2 corresponds to a phi angle = ±90° and a psi angle that is 180°. This is also true for the conformer with a *cis*-amide bond (data not shown). A second minimum, about 2 kcal/mol higher in energy, is found with phi and psi angles of approximately (−120, +90) and (+120, −90). A third minimum at (−60, −60) is 3 kcal/mol higher in energy.

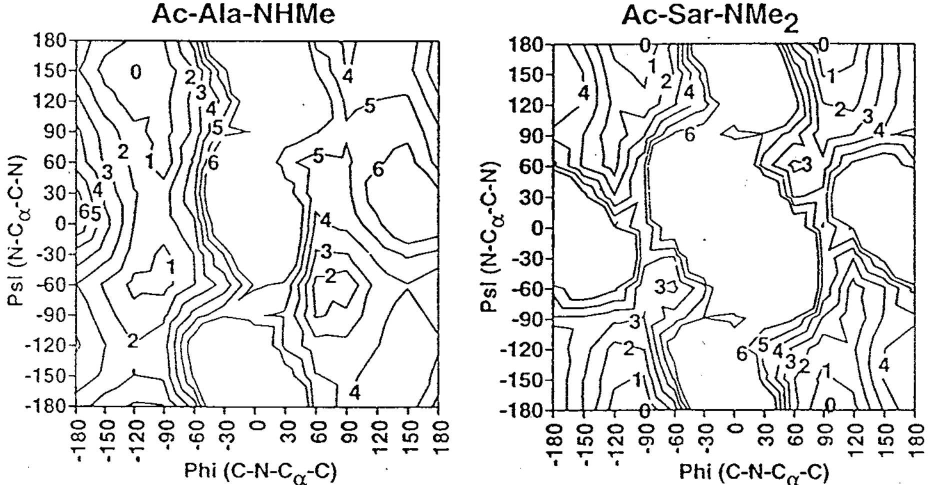


Figure 14-2. Ramachandran plots of Ac–Ala–NHMe (left) and the *trans*-amide conformer of Ac–Sar–NMe_2 (right).

It is striking that the energy minima are so well defined. This shows that peptoids possess a different conformational profile than glycine. The different minimum energy positions suggest that peptoids sample a variety of backbone conformations that are inaccessible to peptides. The side chains are expected to be presented in entirely different manners as well.

A direct comparison of the structural features of peptides and peptoids is shown in Fig. 14-3. The oligomers are aligned in a way that maintains the relative orientation of the amide–oxygen atoms and the side chains.

N-terminus

peptides

C-terminus

peptoids

Figure 14-3. Sequence alignment of peptides and peptoids. To maintain the relative orientation of the amide-oxygen atoms and the side chains, the direction of the peptoid chain had to be reversed.

14.4 Synthesis of NSG Peptoids

The initial approach for the synthesis of NSG peptoids was analogous to conventional solid phase peptide synthesis and based on the condensation of *N*-Fmoc-protected, *N*-substituted glycines [19]. Using the standard Fmoc protocol and PyBOP or PyBroP activation of the monomers, oligomeric peptoids were assembled in good yields and high purity. The NSG monomers had to be synthesized prior to assembly of the libraries and were obtained by alkylation of primary amines with electrophiles (e.g., haloacetic acids and acrylamides) or by reductive amination of primary amines with aldehydes (e.g., glyoxylic acid) [19].

DIC, DMF

R_1—NH_2

DMSO

Figure 14-4. Synthesis of NSG peptoids using the submonomer method.

In a recent synthetic advance, it was possible to improve the efficiency of the synthesis for a large variety of NSG peptoid oligomers. Regarding NSG peptoids as alternating copolymers of primary amines and acetate units, a protocol was developed that allows for the assembly of peptoids from the readily available building blocks ("submonomers") bromoacetic acid and primary amines [25]. As shown in Fig. 14-4, NSG peptoid oligomers can be synthesized using acylation reactions and S_N2-reactions in an alternating fashion, without the need to synthesize *N*-Fmoc-protected monomers.

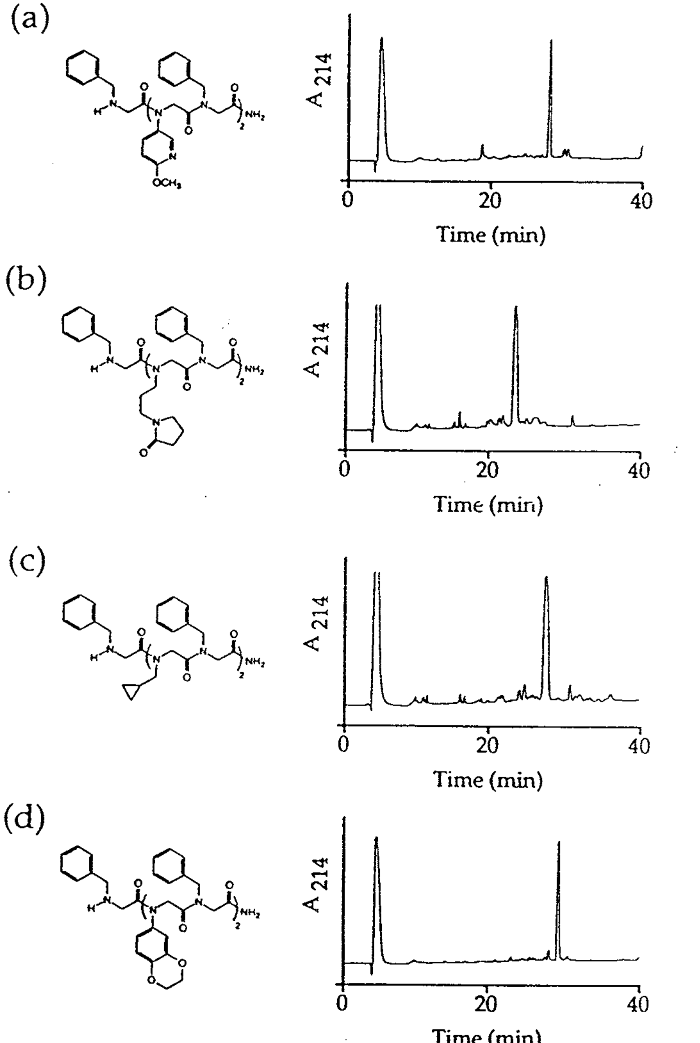
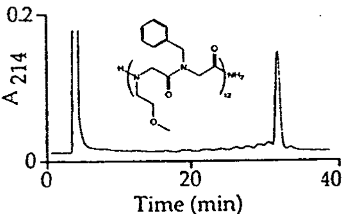


Figure 14-5. HPLC-chromatograms of four crude peptoid pentamers; each sample gave the expected peak in the ESI mass spectrum (data not shown). Prior to incorporation into a peptoid library, each amine was tested for its ability to incorporate into a pentamer.

The submonomer approach requires the protection of side chain functionalities like carboxyl-, hydroxyl-, amino- and thiol-groups; phenols can be used without protection. Using optimized reaction conditions, it was possible to assemble homo- and heteropentamers in greater than 80% purity from a wide variety of commercially available amines (Fig. 14-5). Moreover, a 24-mer (*N*-methoxyethylglycine-*N*-benzylglycine)$_{12}$, was obtained in 60% yield (crude material) and in >70% purity [26], thus further demonstrating the efficiency of the method (Fig. 14-6).



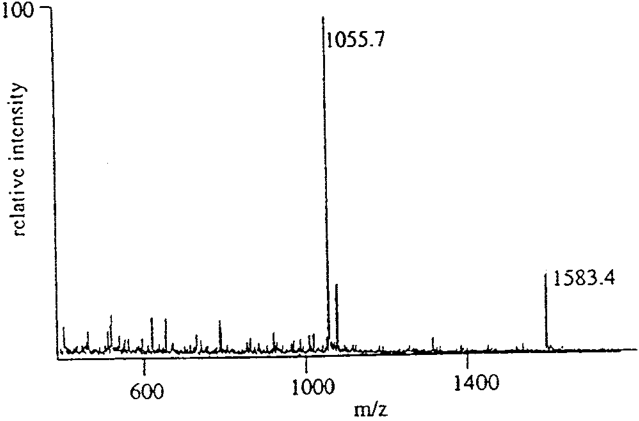


Figure 14-6. HPLC-chromatogram and ESI mass spectrum of a crude NSG-peptoid 24-mer *N*-methoxyethylglycine-*N*-benzylglycine)$_{12}$. The mass spectrum shows the peaks corresponding to the doubly and triply charged species.

In summary, the submonomer protocol allows for the efficient synthesis of equimolar mixtures of NSG peptoid oligomers with a wide variety of side chain functionalities. The high-yielding, reproducible linking chemistry, combined with the structural and functional diversity of the monomers that can be incorporated, renders the peptoid approach ideally suited for the automated generation of molecular diversity.

14.5 Automated Synthesis of Equimolar Peptoid Mixtures

The high-throughput synthesis of diverse peptoid libraries is performed by a robotic workstation of our own design [27]. The apparatus is capable of performing all the required synthetic and resin-splitting manipulations. The key features of the instrument have been described in detail [27]; some of the most significant characteristics can be summarized as follows.

(i) The workstation consists of a Zymate XP robot that is interfaced with a Macintosh computer [28]. The robotic arm delivers solvents and reagents from pressurized lines into a 6 × 6 array of reaction vessels (Fig. 14-7).

(ii) The individual reactions are performed on polystyrene beads with acid-labile linkers. The resin is placed into 36 1.5 × 10 cm fritted glass reaction vessels. Bubbling with argon is used to ensure proper agitation of the reagents and the resin particles. Moreover, the argon provides a blanket that shields the reaction mixture from water and oxygen.

(iii) The reaction vessels are mounted on a custom-designed aluminum block that can be heated to 100 °C. Reagents and solvents are added through the open top of the reaction vessels. Argon delivery to, and solvent removal from, the reaction vessels is accomplished by applying vacuum or argon pressure to the bottom of the reaction

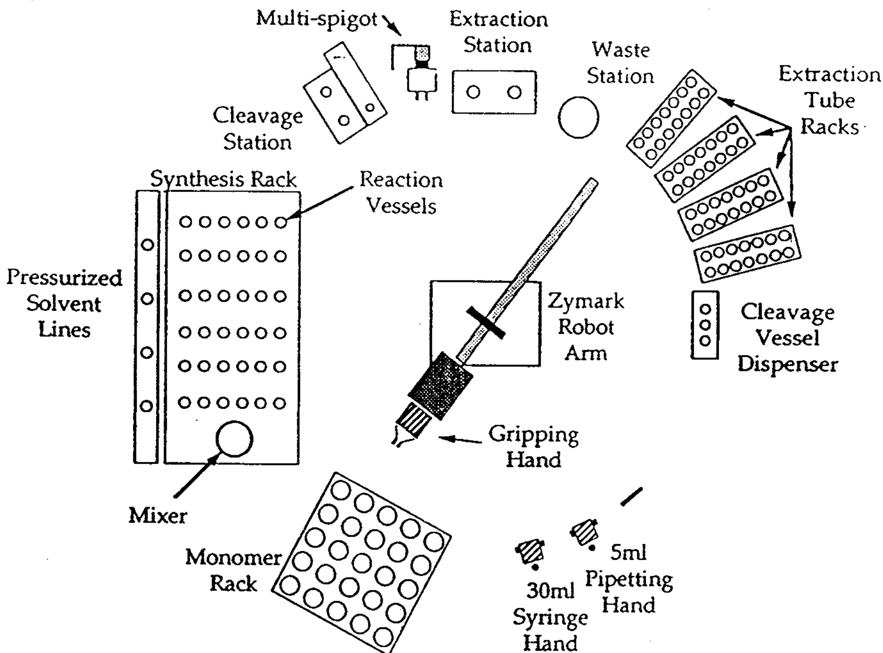


Figure 14-7. Schematic view of the automated workstation for the synthesis of equimolar peptoid mixtures and the integrated cleavage/deprotection station.

vessels. The latter operations are controlled by three-way Teflon solenoid valves that are interfaced with the Macintosh computer.

(iv) The distribution and recombining of the solid support ("resin splitting") is performed after generating a free-flowing, isopycnic slurry of the resin in 1,2-dichloroethane/DMF. Resin splitting that allows for precise control over the composition of the libraries is accomplished using the scheme summarized in Fig. 14-8. Syringes are used to measure equal volumes of the slurry into the reaction vessels so that equimolar amounts of resin are transferred. By repeating each of the resin transfer steps three times, virtually qunatitative transfer of the resin particles is achieved.

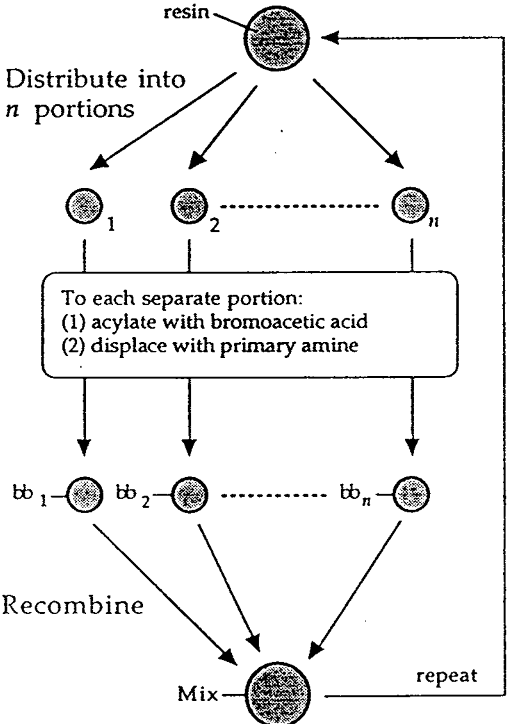


Figure 14-8. Resin-splitting scheme for the generation of equimolar peptoid mixtures.

After completion of the synthesis, the peptoid libraries are detached from the solid support using standard, trifluoroacetic acid-based cleavage protocols. An automated cleavage station has been developed that greatly increases the library throughput [29]. Prior to assay, manual lyophilization of the crude samples is required.

14.6 Rational Approaches for Library Design and the Generation of Structural Diversity

More than 13000 primary amines are listed in the *Available Chemicals Database* [30]. Over 1000 of these are inexpensive (priced less than $5.00/g) and suitable for submonomer synthesis. In a set of peptoid trimers, more than 10^9 different combinations can be generated with these inexpensive amines alone. If the synthesis of tetramers or modifications of the *C*- and *N*-termini are also taken into account, the number of possible combinations greatly exceeds that which can be handled by automated synthesis or automated binding assays. To limit the library synthesis to a reasonable number for assays and deconvolution, some *a priori* selections have to be made.

As a consequence for the conformational flexibility of the backbone, an NSG peptoid library will be capable of accessing a variety of conformational states. Therefore, the individual side chains can be arranged in many different orientations. However, structural and functional redundancy of NSG peptoid libraries can be kept to a minimum by maximizing diversity among the monomeric building blocks. In addition, this strategy should suppress the occurence of "false positives" in the assays that could arise from the combined effects of several structurally related ligands (with only moderate affinity for each individual ligand).

Maximally dissimilar building blocks are used for the synthesis of NSG peptoid libraries that are intended for random screening. Due to the conformational flexibility around the α-carbon atoms, and the accessibility of both *cis*- and *trans*-amide bonds, even a peptoid library consisting of a limited number of dissimilar building blocks should be able to cover a significant sector of a conformational and functional continuum. For these reasons, random screening of peptoid libraries should have a good chance of success even if the number of the incorporated, diverse monomers is limited.

Following a semirational, target-oriented approach, libraries can be directed towards specific enzymes or receptors. This can be accomplished by including side chains containing functional units, e.g., transition-state analogs for the generation of lead structures for enzyme inhibitors. Alternatively, monomers that contain structural units which are known to commonly occur in ligands for certain classes of receptors can be used to bias the library. Maximally dissimilar monomers are also incorporated into these libraries to present the biased elements in many different geometries and chemical environments.

Computational tools are used to determine and measure similarity and dissimilarity between monomers by computing a variety of structural and functional properties including lipophilicity, shape, branching and "atom layer properties" (roughly speaking, the number of bonds separating any functionality from the closest atom of the backbone) [31]. Each building block is represented by a vector

of 16 numerical descriptors that summarize these structural and functional characteristics.

Sets of monomers that are highly similar to a known pharmacophore, or to one another, are obtained by simply rank ordering every member of the pool by the Euclidean distance from a reference structure. Maximally dissimilar compounds are found with D-optimal design [32]. The algorithm chooses subsets of points from a larger pool that are well spread out and largely orthogonal in property space, i.e., that are maximally dissimilar. Any number and combination of monomers can initially be included (to serve as starting points) in the set. Subsequently, additional points that best fill out the set to a specified size will be selected by the optimizer.

In summary, strategies and methods have been developed for selecting monomer subsets for peptoid library synthesis. Using computational tools, we maximize dissimilarity within the monomeric building blocks, thereby increasing the structural diversity and minimizing the structural overlap in our libraries. The methods and tools described here can also be used in biased libraries, combining approaches that rely on intuition, experience and on the incorporation of known pharmacophores with computational strategies for the generation of molecular diversity.

14.7 Peptoid Ligands with Nanomolar Affinity for Adrenergic and Opiate Receptors

14.7.1 Design of a Biased Library for 7-Transmembrane/G-Protein Coupled Receptors

A substantial amount of therapeutically relevant drugs bind to 7-transmembrane/G-protein coupled receptors [33]. Since amino acid sequences of this class of receptors are highly homologous [34], ligands to this receptor family frequently share common pharmacophores. An analysis of structural elements of known drugs revealed that most of the ligands contained at least one hydroxyl or phenol group as hydrogen donor and one hydrophobic, aromatic group [35].

For the synthesis of a biased peptoid library, three sets of building blocks were selected from commercially available amines [35]. Set "A" was composed of 4 aromatic, hydrophobic amines, set "O" of 3 amines with side chains containing hydroxyl or phenol groups, and set "D" was designed to comprise 17 maximally diverse amines (Table 14-1). In addition, the *N*-termini of the NSG peptoids were either left as a free amine or capped as an acetamide or cyclohexylurea; the *C*-terminus of all compounds was fixed as a primary amide. The complete library contained 3672 peptoid trimers (18 combinatorial mixtures of $3 \times 4 \times 17 = 204$ compounds). Moreover, all possible dimers were included into the library.

Table 14-1. Each of the 18 peptoid pools was made from all combinations drawn from the six permutations of three sets of amines, times three different *N*-terminal endings. Thus, each trimer contained at least one hydroxylic and one aromatic group.

Cap, R_1, R_2, R_3, O, NH_2			
N-Terminal Capping Groups			
H Free Amine	O H_3C Acetamido	O N H Cyclohexylureido	
Hydroxyl Set of Sidechains (O)			
$-CH_2CH_2CH_2CH_2OH$ Nhyb	$-CH_2CH_2OH$ Nhser	$-CH_2CH_2$- -OH Nhtyr	
Aromatic Set of Sidechains (A)			
Nbiph	$-CH_2CH_2$- Nhphe	$-CH_2$- Nnap	$-CH_2$- Ndpe
Diverse Set of Sidechains (D)			
$-CH_3$		$-CH_2CH_2COOH$	
$-CH_2(CH_2)_3CH_3$ Npen	$-CH(CH_2CH_3)_2$	$-CH_2CH_2OCH_3$	
$-CH_2CH_2N$ O	$-CH_2CH_2CH_2NH_2$	$-CH_2CH_2OH$	$-OCH_3$ N
Nbiph	O Npop	$-CH_2$- O O Nmdb	$-CH_2$- $-OCH_3$ OCH_3 Ndmb
	$-CH_2$- O	$-CH_2$ O O H_2N	$-CH_2$- CH_3 CH_3

14.7.2 Identification of Peptoid Ligands with Nanomolar Affinity

The equimolar NSG peptoid mixtures were screened for their ability to competitively inhibit binding of high-affinity radioligands to 7-transmembrane/G-protein coupled

receptors. Assays were performed in aqueous solution containing 0.1 to 1% DMSO at a concentration of 100 nM for each individual compound [35].

All 18 pools were tested for inhibition of [^{3}H]-prazosin binding [36] to an α_1-adrenergic receptor preparation. The pool that showed greatest inhibition of [^{3}H]-prazosin binding was subjected to the subsequent cycle of deconvolution. In the third round of deconvolution, CHIR 2279 (Fig. 14-9) was resynthesized in pure form and showed competitive inhibition of [^{3}H]-prazisin binding with a $K_i = 5 \pm 3$ nM. CHIR 2276 and CHIR 2283, two closely related peptoids, also inhibited [^{3}H]-prazosin binding at nanomolar concentrations ($K_i = 310$ nM for CHIR 2276 and $K_i = 140$ nM for CHIR 2283; structures not shown) [35].

Similarly, the 18 NSG peptoid pools were assayed for inhibition of [^{3}H]-DAMGO (μ-specific) binding [37] to opiate receptors. After deconvolution and individual resynthesis of the three most potent compounds [35], CHIR 4531 (Fig. 14-9) was shown to inhibit [^{3}H]-DAMGO binding with a $K_i = 6 \pm 2$ nM. Two related peptoids, CHIR 4537 and CHIR 4534, had K_i values of 31 nM and 46 nM (structures not shown).

CHIR 2279 CHIR 4531

Figure 14-9. Structures of CHIR 2279 and CHIR 4531, two nanomolar ligands for 7-transmembrane/G-protein coupled receptors.

14.7.3 Discussion

Using a relatively small, biased library of NSG peptoid trimers, it was possible to identify novel, low-molecular weight ligands with high affinity for two pharmaceutically relevant receptors. Although an analysis of common pharmacophores for 7-transmembrane/G-protein coupled receptors served as the starting point for library design, the newly discovered ligands represent an entirely novel class of biologically active molecules. The peptoid trimers CHIR 2279 and CHIR 4531 are

capable of competively inhibiting binding of endogenous ligands to their receptors, despite their complete lack of chiral elements.

Both CHIR 2279 and epinephrine/norpinephrine, the endogenous ligands for the α_1-adrenergic receptor, contain a substituted tyramine derivative; however, the hydroxyl group of CHIR 2279 apparently is not required for biological acitvity, as evidenced by the high affinity of its deshydroxy analog (K_i = 4 nM). Other than that, CHIR 2279 shares few structural elements with either epinephrine/norepinephrine or prazosin (Fig. 14-10).

CHIR 4531 inhibits binding of the natural ligands morphine and Met-enkephalin to the μ-opiate receptor. Although some structural elements, like the substituted tyramine or a hydrophobic ring, are present in CHIR 4531 and the two natural ligands (Fig. 14-10), the different backbone, the lack of chirality and the nature of the aromatic side chains of CHIR 4531 clearly distinguish the peptoid lead from the natural ligands.

The discovery of novel, high affinity ligands for 7-transmembrane/G-protein coupled receptors is the result of a multidisciplinary effort. Key factors that con-

(a)

epinephrine : R = CH_3
norepinephrine: R = H

prazosin

(b)

morphine

Met-enkephalin

Figure 14-10. Structures of known ligands for the α_1-adrenergic receptor and the μ-opiate receptor.

tributed to success were a general, highly reproducible protocol for the synthesis of nonnatural oligomers with a large variety of structurally and functionally different side chains; the availability of suitable automation for synthesis and deconvolution of equimolar peptoid mixtures; and efficient strategies for library design under consideration of the conformational characteristics of NSG peptoids.

14.8 Summary

Since the concept of molecular diversity was introduced into pharmaceutical research, the research field has quickly expanded. Initially, the generation of diverse libraries was accomplished with biomolecules like peptides and nucleic acids, but more and more examples of libraries of non-natural oligomers and organic molecules have been described [38].

The ultimate goal of pharmaceutical research is the discovery of small molecule drug leads and drug canditates. To realize this goal, the structural diversity of the libraries is maximized. Computational tools have been developed that allow for the minimization of structural and functional redundancy of the building blocks that are incorporated. These methods can be applied to "diversify" libraries for random screening and to reduce overlap around constant units in biased libraries. In addition, synergistic effects of similar ligands (with only moderate affinity for each of the ligands) are kept to a minimum.

Suitable automation allows for the high-throughput synthesis of equimolar peptoid mixtures with precise control over the composition of the libraries. A high-yielding, reproducible protocol has been developed for the synthesis of diverse, unnatural oligomers from inexpensive, readily available building blocks.

To obtain binding data that are as accurate as possible, all the libraries are screened in solution phase. Iterative resynthesis of successively smaller subpools is used for the identification of bioactive components. Like library production, the deconvolution process is greatly accelerated by automation. By using this approach for lead identification, the covalent attachment of molecules to a matrix or a tag which could structurally interfere with the binding to the pharmaceutical target is avoided.

The strategies summarized here have been applied both to the synthesis of random mixtures and to the synthesis of libraries that are focused towards specific pharmaceutical targets. The design and synthesis of an NSG peptoid library which was biased towards 7-transmembrane/G-protein coupled receptors resulted in the discovery of novel, low-molecular weight ligands with nanomolar affinity for the α_1-adrenergic and the μ-opiate receptor. Since achiral, low-molecular weight peptoid trimers have the ability to competively inhibit binding of potent, endogenous ligands, the peptoid approach holds enormous potential for the discovery of novel lead structures for drug development.

14.9 Experimental Procedures

14.9.1 Standard Protocol for the Synthesis of NSG Peptoids with *C*-terminal Amides Using the Submonomer Method [40]

14.9.1.1 Bromoacetylation of Rink-Amide-Resin and the *N*-terminal Amine of an NSG Peptoid Chain

After standard deprotection of *N*-Fmoc-Rink amide resin [39] with 20% piperidine in DMF, the resin (96 mg, 0.52 mmol/g) was extensively washed with DMF (5 × 1 ml) and drained. A 0.6 M solution of bromoacetic acid in DMF (830 μl) and subsequently a 3.2 M solution of diisopropylcarbodiimide in DMF (200 μl) was added, and the resin was agitated with argon. The reaction mixture was agitated at room temperature for 30 minutes, washed with DMF (3 × 1 ml), and the reaction was repeated once under identical conditions.

14.9.1.2 Displacement of the Bromide of Resin-Bound Bromoacetamides with Primary Amines

The resin-bound bromoacetamide was washed with DMSO (3 × 1 ml), and 1.0 ml of 2.0 M solution of the primary amine in DMSO was added. For amines with poor solubility in DMSO such as tyramine, a 1.0 M solution of the amine in DMSO was used instead. The reaction mixture was agitated with argon for 2 h at room temperature. Subsequently, the resin was washed with DMF (3 × 1 ml).

14.9.1.3 Cleavage of the Peptoid/Peptoid Mixture from the Solid Support

After all synthetic manipulations were completed, the resin was washed with DMF (3 × 1 ml), dichloromethane (3 × 1 ml) and dried. 95% aqueous TFA (5.0 ml) was added to the resin particles, and the mixture was stirred for 20 min at room temperature. The solid support was removed by filtration and washed with water (2 × 2.5 ml). The crude samples were lyophilized, followed by relyophilization from glacial acetic acid.

References

[1] R. Hirschmann, Medicinal chemistry in the golgen age of biology: Lessons from steroid and peptide research. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1278–1301.
[2] A. A. Patchett, Excursion in drug discovery. *J. Med. Chem.* **1993**, *36*, 2051–2058.
[3] M. A. Gallop, R. W. Barrett, W. J. Dower, S. P. A. Fodor, E. M. Gordon, Applications of combinatorial technologies to drug discovery. 1. Background and peptide combinatorial libraries. *J. Med. Chem.* **1994**, *37*, 1233–1251.

[4] E. M. Gordon, R. W. Barrett, W. J. Dower, S. P. A. Fodor, M. A. Gallop, Applications of combinatiorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions. *J. Med. Chem.* **1994**, *37*, 1385–1401.

[5] W. H. Moos, G. D. Green, M. R. Pavia, Recent advances in the generation of molecular diversity. *Annu. Rep. Med. Chem.* **1993**, *28*, 315–324.

[6] S. A. Kates, N. A. Sole, C. R. Johnson, D. Hudson, G. Barany, F. A. Albericio, Novel, convenient, three-dimensional orthogonal strategy for solid-phase synthesis of cyclic peptides. *Tetrahedron Lett.* **1993**, *34*, 1549–1552 and references cited therein.

[7] R. N. Zuckermann, J. M. Kerr, M. A. Siani, S. C. Banville, D. V. Santi, Identification of highest-affinity ligands by affinity selection from equimolar peptide mixtures generated by robotic synthesis. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 4505–4509.

[8] J. M. Kerr, S. C. Banville, R. N. Zuckermann, Identification of antibody mimotopes containing non-natural amino acids by recombinant and synthetic peptide library affinity selection methods. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 463–468.

[9] R. A. Houghten, C. Pinilla, S. E. Blondelle, J. R. Appel, C. T. Dooley, J. H. Cuervo, Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery. *Nature (London)* **1991**, *354*, 84–86.

[10] S. Brenner, R. A. Lerner, Encoded combinatorial chemistry. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 5381–5383.

[11] J. M. Kerr, S. C. Banville, R. N. Zuckermann, Encoded combinatorial peptide libraries containing non-natural amino acids. *J. Am. Chem. Soc.* **1993**, *115*, 2529–2531.

[12] M. C. Needles, D. G. Jones, E. H. Tate, G. L. Heinkel, L. M. Kochersperger, W. J. Barrett, M. A. Gallop, Generation and screening of an oligonucleotide-encoded synthetic peptide library. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10700–10704.

[13] M. H. J. Ohlmeyer, R. N. Swanson, L. W. Dillard, J. C. Reader, G. Asouline, R. Kobayashi, M. Wigler, W. C. Still, Complex synthetic chemical libraries indexed with molecular tags. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10922–10926.

[14] S. P. A. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu, D. Solas, Light-directed, spatially addressable parallel chemical synthesis. *Science* **1991**, *251*, 767–773.

[15] K. Lam, S. Salmon, E. Hersh, V. Hruby, W. Kazmiersky, R. A. Knapp, A new type of synthetic peptide library for identifying ligand-binding activity. *Nature (London)* **1991**, *354*, 82–84.

[16] G. Jung, A. G. Beck-Sickinger, Multiple peptide synthesis methods and their applications. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 367–486.

[17] J. J. Plattner, D. W. Norbeck, "Obstacles to drug developement from peptide leads" in *Drug Discovery Technologies* (Eds.: C. R. Clark, W. H. Moos) Elllis Horwood Limited: Chichester, England, **1990**, pp. 92–126.

[18] R. N. Zuckermann, The chemical synthesis of peptidomimetic libraries. *Curr. Opin. Struct. Biol.* **1993**, *3*, 580–584.

[19] R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, D. C. Spellmeyer, R. Tan, A. D. Frankel, D. V. Santi, F. E. Cohen, P. A. Bartlett, Peptoids: A modular approach to drug discovery. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9367–9371.

[20] S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, W. H. Moos, Comparison of the proteolytic susceptibilities of homologous L-amino acid, D-amino acid and *N*-substituted glycine peptide and peptoid oligomers. *Drug Dev. Res.* **1995**, *35*, 20–32.

[21] D. C. Spellmeyer, unpublished results.

[22] C. J. Cramer, D. G. Truhlar, General parameterized SCF model for free energies of solvation in aqueous solution. *J. Am. Chem. Soc.* **1991**, *113*, 8305, 9901 (E).

[23] C. J. Cramer, D. G. Truhlar, AM1-SM2 and PM3-SM3 Parameterized solvation models for free energies in aqueous solution. *J. Computer Aided Mol. Design* **1992**, *6*, 629-666.

[26] G. N. Ramachandran, V. Sasisekharan, *Adv. Prot. Chem.* **1968**, *23*, 283-438.

[25] R. N. Zuckermann, J. M. Kerr, S. B. H. Kent, W. H. Moos, Efficient method for the preparation of peptoids [oligo(*N*-substituted glycines)] by submonomer solid-phase synthesis. *J. Am. Chem. Soc.* **1992**, *114*, 10646-10647.

[26] R. N. Zuckermann, D. A. Goff, Synthesis of (*N*-substituted) glycine polymers of defined sequence and length. *ACS Polymer Preprint* **1994**, *35*, 975-976.

[27] R. N. Zuckermann, J. M. Kerr, M. A. Siani, S. C. Banville, Design, construction and application of a fully automated equimolar peptide mixture synthesizer. *Int. J. Pept. Protein Res.* **1992**, *40*, 497-506.

[28] R. N. Zuckermann, M. A. Siani, S. C. Banville, Control of the zymate robot with an external computer: construction of a multiple peptide synthesizer. *Lab. Robotics Automation* **1992**, *4*, 183-192.

[29] R. N. Zuckermann, S. C. Banville, Automated peptide-resin deprotection/cleavage by a robotic workstation *Pept. Res.* **1992**, *5*, 169-174.

[30] *Available Chemicals Directory 93.1*, Molecular Design Limited, San Leandro, CA, **1993**.

[31] E. J. Martin, J. M. Blaney, M. A. Siani, D. C. Spellmeyer, A. K. Wong, W. H. Moos, Measuring Diversity: experimental design of combinatorial libraries for drug discovery. *J. Med. Chem.* **1995**, *38*, 1431-1436.

[32] V. V. Federov, *Theory of Optimal Experiments*, Academic Press: New York, 1972.

[33] M. N. Potenza, G. F. Graminski, M. R. Lerner, A method for evaluating the effects of ligands upon G-protein-coupled receptors using a recombinant melanophore-based bioassay. *Anal. Biochem.* **1992**, *206*, 315-322.

[34] W. C. Probst, L. A. Snyder, D. I. Schuster, J. Brosius, S. C. Sealfon, Sequence alignment of the G-protein coupled receptor superfamily. *DNA Cell Biol.* **1992**, *11*, 1-20.

[35] R. N. Zuckermann, E. J. Martin, D. C. Spellmeyer, G. B. Stauber, K. R. Shoemaker, J. M. Kerr, G. M. Figliozzi, D. A. Goff, M. A. Siani, R. J. Simon, S. C. Banville, E. G. Brown, L. Wang, L. S. Richter, W. H. Moos, Discovery of nanomolar ligands for 7-transmembrane G-protein coupled receptors from a diverse (*N*-substituted)glycine peptoid library. *J. Med. Chem.* **1994**, *37*, 2678-2685.

[36] P. B. M. W. M. Timmermans, F. K. Ali, H. Y. Kwa, A. M. C. Schoop, F. P. Slothorst-Grisdijk, P. A. v. Zwieten, Identical antagonist selectivity of central and peripheral alphal-adrenoceptors. *Mol. Pharmacol.* **1981**, *20*, 295-301.

[37] M. G. C. Gillan, H. W. Kosterlitz, Spectrum of the mu-, delta-, and kappa-binding sites in homogenates of rat brain. *Br. J. Pharm.* **1982**, *77*, 461-469.

[38] see refs. 3-5 and references cited therein.

[39] H. Rink, Solid-phase synthesis of protected peptide fragments using a trialkoxy-diphenyl-methylester resin. *Tetrahedron Lett.* **1987**, *28*, 3787-3790.

[40] G. M. Figliozzi, R. Goldsmith, S. C. Ng, S. C. Banville, R. N. Zuckermann, Synthesis of *N*-Substituted glycine peptoid libraries. *Methods in Enzymology.* **1996**, *267*, 437-447.