Peter E. Nielsen (Ed.)

Pseudo-peptides in Drug Discovery

WILEY-VCH Verlag GmbH & Co. KGaA

Peter E. Nielsen (Ed.)

Pseudo-peptides in Drug Discovery

Further Titles of Interest

Chi-Huey Wong (Ed.)

Carbohydrate-based Drug Discovery

2003. ISBN 3-527-30632-3

M. Demeunynck, C. Bailly, W. D. Wilson (Eds.)

DNA and RNA Binders

2002. ISBN 3-527-30595-5

N. Sewald, H.-D. Jakubke

Peptides: Chemistry and Biology

2002. ISBN 3-527-30405-3

K. C. Nicolaou, R. Hanko, W. Hartwig (Eds.)

Handbook of Combinatorial Chemistry

2002. ISBN 3-527-30509-2

Peter E. Nielsen (Ed.)

Pseudo-peptides in Drug Discovery

WILEY-VCH Verlag GmbH & Co. KGaA

Prof. Dr. Peter E. Nielsen
Department of Medical Biochemistry and Genetics
University of Copenhagen
The Panum Institute
Blegdamsvej 3c
2200 Copenhagen N
Denmark

This book was carefully produced. Nevertheless, editor, authors and publisher do not warrant the information contained therein to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany

All rights reserved (including those of translation in other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such, are not to be considered unprotected by law.

Printed in the Federal Republic of Germany
Printed on acid-free paper

Cover Grafik-Design Schulz, Fußgönheim
Composition K+V Fotosatz GmbH, Beerfelden
Printing Strauss Offsetdruck GmbH, Mörlenbach
Bookbinding Litges & Dopf Buchbinderei GmbH, Heppenheim

ISBN 3-527-30633-1

Contents

Preface *IX*

List of Contributors *XI*

1 **Versatile Oligo(*N*-Substituted) Glycines: The Many Roles of Peptoids in Drug Discovery** *1*
James A. Patch, Kent Kirshenbaum, Shannon L. Seurynck, Ronald N. Zuckermann, and Annelise E. Barron
1.1 Introduction *1*
1.2 Peptoid Synthesis *3*
1.2.1 Solid-Phase Synthesis *3*
1.2.2 Sub-monomer Solid-Phase Method *4*
1.2.3 Side Reactions *5*
1.2.4 Post-Synthetic Analysis *5*
1.3 Drug Discovery via Small-Molecule Peptoid Libraries *6*
1.3.1 Peptoid Drugs from Combinatorial Libraries *6*
1.3.2 Peptoid Inhibitors of RNA-Protein Interactions *6*
1.4 Peptoid-Based Drug Delivery and Molecular Transporters: Cellular Uptake *8*
1.4.1 Peptoid Mimics of HIV-Tat Protein *9*
1.4.2 Cellular Delivery of Nucleic Acids *9*
1.5 Peptoid Mimics of Peptide Ligands *11*
1.6 Peptoids with Folded Structure *13*
1.6.1 Restricting Conformational Space *13*
1.6.2 Peptoid Helices *14*
1.6.2.1 CD and NMR Studies of a Helical Peptoid Pentamer with α-Chiral Aromatic Side Chains *14*
1.6.2.2 CD Studies of Longer Peptoid Helices Containing α-Chiral Aromatic Side Chains *16*
1.6.2.3 Structural Studies of Peptoids with Aliphatic Side Chains by CD, NMR, and X-ray Crystallography *17*
1.6.2.4 Summary *18*
1.6.3 Protein-mimetic Structures *18*

1.7 Biomimetic Peptoid Structures for Therapeutic Applications *19*
1.7.1 Peptoid Mimics of Antibacterial Peptides *19*
1.7.2 Peptoid-Based Mimics of Lung Surfactant Proteins *21*
1.7.3 Collagen-based Structures Containing Peptoid Residues *23*
1.8 Obstacles to the Development of Biomedically-useful Peptoids *25*
1.8.1 Enhance the Diversity of Secondary Structure in Peptoid Foldamers *25*
1.8.2 Improve Understanding of Peptoid Sequence/Structure Relationships *25*
1.8.3 Translate Bioactive Peptide Sequences into Bioactive Peptoid Sequences *25*
1.8.4 Develop Peptoids with Stable Tertiary Structure *26*
1.8.5 Develop Peptoid Shuttles for Intracellular Import of Xenobiotic Agents *26*
1.8.6 Optimize Pharmacological Profile of Oligopeptoids *26*
1.9 Conclusion *27*
1.10 References *27*

2 β-Peptides, γ-Peptides and Isosteric Backbones: New Scaffolds with Controlled Shapes for Mimicking Protein Secondary Structure Elements *33*
Gilles Guichard
2.1 Introduction *33*
2.2 Molecular Organization in β-Peptide Oligomers *34*
2.2.1 Historical Background *34*
2.2.2 β-Amino Acids versus α-Amino Acids: An Enormous Increase in Chemical Diversity *40*
2.2.3 Helical Folds *49*
2.2.3.1 The 3_{14}-Helix *50*
2.2.3.2 The 12/10- (10/12-) Helix *65*
2.2.3.3 The 2.5_{12}-Helix *68*
2.2.3.4 The 2_8-Helix *73*
2.2.4 Extended β-Peptide Strands, Turns and Formation of Sheet Structures *76*
2.3 Molecular Organization in γ-Peptide Oligomers *82*
2.3.1 Preparation of γ-Amino Acid Monomers for γ-Peptide Synthesis *84*
2.3.2 Helical Folds *88*
2.3.3 Turn and Sheet Structures *92*
2.4 Biological Activities of β- and γ-Peptides *96*
2.4.1 Biological Stability *96*
2.4.2 Bioactive Peptides Based on Helical Scaffolds *97*
2.4.3 Bioactive Peptides Based on Open-Chain β-Turn Mimetics *100*
2.4.4 Cell Penetrating β-Peptides *102*
2.5 Isosteres *104*

2.5.1 Example 1: Oligomers of α-Aminooxy Acids as β-Peptide Mimetics *105*
2.5.2 Example 2: *N,N′*-Linked Oligoureas as γ-Peptide Mimetics *107*
2.6 Conclusion *112*
2.7 References *113*

3 Regulation of Gene Expression with Pyrrole-Imidazole Polyamides *121*
Peter B. Dervan, Eric J. Fechter, Benjamin S. Edelson, and Joel M. Gottesfeld
3.1 Introduction *121*
3.2 Pairing Rules *122*
3.3 Hairpin and Cycle Motifs *125*
3.3.1 H-Pin and U-Pin Motifs *125*
3.3.2 The β/Ring Pair *127*
3.4 Binding Site Size *128*
3.5 The Importance of β-Alanine *129*
3.6 Solid Phase Methods *131*
3.7 Benzimidizole Ring Systems for Minor Groove Recognition *132*
3.8 Sequence Specific Alkylation of DNA *133*
3.9 Inhibition of Gene Expression *135*
3.10 Gene Activation *140*
3.11 Nucleosomes *141*
3.12 Nuclear Uptake *144*
3.13 DNA Detection *145*
3.14 Future Directions *145*
3.15 Acknowledgments *147*
3.16 References *147*

4 Peptide Nucleic Acid (PNA): A Pseudo-peptide with DNA-like Properties *153*
Peter E. Nielsen, Uffe Koppelhus, and Frederik Beck
4.1 Introduction *153*
4.2 Chemistry *155*
4.3 DNA Recognition *157*
4.4 Drug Discovery *159*
4.4.1 RNA Targeting (Antisense) *160*
4.4.2 DNA Targeting (Antigene) *164*
4.4.3 Protein Targeting *166*
4.4.4 Cellular Delivery *166*
4.4.5 Pharmacology *167*
4.5 Nucleic Acid Detection and Analysis *168*
4.6 Nanotechnology *168*
4.7 Pre-RNA World *168*
4.8 Epilogue *170*
4.9 Acknowledgments *170*
4.10 References *170*

Appendix 180

5 **α-Helical Peptide Nucleic Acids (αPNAs)** 193
Philip P. Garner, Subhakar Dey, and Yumei Huang
5.1 Introduction 193
5.2 The αPNA Design Concept 196
5.3 Synthesis of αPNAs 200
5.3.1 Nucleoamino Acid Synthesis 200
5.3.2 Solid Phase Synthesis of αPNA 202
5.3.3 Dimer Formation and Fluorescence Labeling of αPNA 204
5.4 αPNA-Nucleic Acid Binding Studies 205
5.4.1 αPNA Secondary Structure 205
5.4.2 Thermal Denaturation Studies 206
5.4.2.1 *N*-Acetylated b1 and b2 αPNAs 207
5.4.2.2 Disulfide-Linked αPNA Dimers 208
5.4.2.3 Hydrophobic *N*-Capped αPNAs 209
5.4.3 Gel Electrophoresis Studies 210
5.4.4 CD Studies on αPNA-DNA Complexes 211
5.5 Pharmacological Studies 214
5.5.1 Bioavailability of αPNA 214
5.5.2 Biostability of αPNA 216
5.6 Conclusions and Prospects 217
5.7 Addendum 218
5.8 Acknowledgements 219
5.9 References 219

6 **DNA and RNA-cleaving Pseudo-peptides** 223
Alessandro Scarso and Paolo Scrimin
6.1 Introduction 223
6.2 Cleavage of a Phosphate Bond 223
6.3 Systems which Utilize an Oxidative Mechanism 225
6.4 Systems Based on a Hydrolytic Mechanism 227
6.4.1 DNA, Oligo- and Polynucleotides, and Synthetic Models as Substrates 227
6.4.2 RNA, Polyribonucleotides and Synthetic Models as Substrates 231
6.5 Conclusions 238
6.6 References 238

Subject Index 241

Preface

Peptides are used extensively by Nature for a variety of signalling functions in both unicellular and multicellular organisms including man. For example, many peptide hormones and analogous short peptides exert their action by binding to membrane receptors. Peptides may also show biological activities in the form of nerve toxins, antibacterial agents or general cell toxins. Therefore, it is no wonder that medical drug discovery has extensively exploited peptides as lead compounds. This development was further accelerated by the development of very effective methods for solid phase synthesis of peptides, and in particular the development of combinatorial methods for synthesizing and screening peptide libraries.

However, most natural peptides are composed of L-form α-amino acids and because of the ubiquitous prevalence of peptidases they have limited biostability, and consequently low bioavailability. Thus, a novel field of peptidomimetics has emerged in drug discovery, in attempts to design non-peptide compounds mimicking the pharmacophore and thus the activity of the original peptide.

This field has also inspired the development of a range of pseudo-peptides, that is polyamides composed of amino acids other than α-amino acids. These include for instance peptoids, β-amino acid oligomers and also compounds such as peptide nucleic acids and DNA binding polyamides, all of which share the amide (peptide) chemistry with natural peptides.

The present book attempts to present the state of the art in the rapidly expanding field of pseudo-peptides, in particular relating to (long term aims of) drug discovery. Many chemists are realizing the power and versatility of “peptide” chemistry, and the large structural and functional space attainable using this technology. Hopefully the book will inspire new developments.

I am extremely grateful to the friends and colleagues who have made this project possible by investing their time and expertise.

Copenhagen, October 2003 *Peter E. Nielsen*

List of Contributors

ANNELISE E. BARRON
Department of Chemical Engineering
Northwestern University
2145 Sheridan Road
Tech E136
Evanston, IL 60208
USA
a-barron@northwestern.edu

FREDERIK BECK
GEA A/S
Kanalholmen 8–12
DK 2650 Hvidovre
Denmark

PETER B. DERVAN
Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, CA 91125
USA
dervan@caltech.edu

SUBHAKAR DEY
Department of Chemistry
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106
USA

BENJAMIN S. EDELSON
Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, CA 91125
USA

ERIC J. FECHTER
Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, CA 91125
USA

PHILIP P. GARNER
Department of Chemistry
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106
USA
ppg@cwru.edu

JOEL M. GOTTESFELD
Department of Molecular Biology
The Scripps Research Institute
10550 North Torrey Pines Road
La Jolla, CA 92037
USA
joelg@scripps.edu

Gilles Guichard
Institut de Biologie Moléculaire
et Cellulaire
UPR 9021 CNRS
Université Louis Pasteur Strasbourg
15, rue Descartes
67084 Strasbourg Cedex
France
g.guichard@ibmc.u-strasbg.fr

Yumei Huang
Department of Chemistry
Case Western Reserve University
10900 Euclid Avenue
Cleveland, OH 44106
USA

Kent Kirshenbaum
Department of Chemistry
New York University
100 Washington Square East
Room 1001
New York, NY 10003
USA

Uffe Koppelhus
Department of Medical Biochemistry
and Genetics
University of Copenhagen
The Panum Institute
Blegdamsvej 3c
DK 2200 Copenhagen N
Denmark

Peter E. Nielsen
University of Copenhagen
The Panum Institute
Blegdamsvej 3c
DK 2200 Copenhagen N
Denmark
pen@imbg.ku.dk

James A. Patch
Department of Chemical Engineering
Northwestern University
2145 Sheridan Road
Tech E136
Evanston, IL 60208
USA

Alessandro Scarso
Department of Organic Chemistry
and ITM-CNR Padova Section
University of Padova
Via Marzolo 1
35131 Padova
Italy

Paolo Scrimin
Department of Organic Chemistry and
ITM-CNR Padova Section
University of Padova
Via Marzolo 1
35131 Padova
Italy
paolo.scrimin@unipd.it

Shannon L. Seurynck
Department of Chemical Engineering
Northwestern University
2145 Sheridan Road
Tech E136
Evanston, IL 60208
USA

Ronald N. Zuckermann
Bioorganic Chemistry
Chiron Corp.
4560 Horton St.
Emeryville, CA 94608
USA

1

Versatile Oligo(*N*-Substituted) Glycines: The Many Roles of Peptoids in Drug Discovery

James A. Patch, Kent Kirshenbaum, Shannon L. Seurynck, Ronald N. Zuckermann, and Annelise E. Barron

1.1

Introduction

Despite their wide range of important bioactivities, polypeptides are generally poor drugs. Typically, they are rapidly degraded by proteases *in vivo*, and are frequently immunogenic. This fact has stimulated widespread efforts to develop peptide mimics for biomedical applications, a task that presents formidable challenges in molecular design. Chemists seek efficient routes to peptidomimetic compounds with enhanced pharmacological properties, which retain the activities of their biological counterparts. Since peptides play myriad roles in living systems, it is likely that no individual strategy will suffice. Indeed, a wide variety of different peptidomimetic oligomer scaffolds have been explored [1]. In order to address multiple design criteria for applications ranging from medicinal chemistry to materials science, researchers have worked to identify a non-natural chemical scaffold that recapitulates the desirable attributes of polypeptides. These include good solubility in aqueous solution, access to facile sequence-specific assembly of monomers containing chemically diverse side chains, and the capacity to form stable, biomimetic folded structures.

Among the first reports of chemically diverse peptide mimics were those of (*N*-substituted) glycine oligomers (peptoids) [2]. Sequence-specific oligopeptoids have now been studied for over a decade, and have provided illustrative examples of both the potential of peptidomimetics and the obstacles faced in translating this potential into clinically useful compounds. We begin this chapter with a summary of the desirable attributes of peptoids as peptide mimics, along with a description of strategies for their chemical synthesis. Throughout the remainder of the chapter, we present an overview of biomedically relevant studies of peptoids with an emphasis on recently reported results. The chapter includes discussion of peptoid combinatorial libraries, folded peptoid structures, and biomimetic peptoid sequences. Finally, we conclude by suggesting promising avenues for future investigations.

Peptoids are an archetypal and relatively conservative example of a peptidomimetic oligomer (Tab. 1.1). In fact, the sequence of atoms along the peptoid backbone is identical to that of peptides. However, peptoids differ from peptides in the manner of side chain appendage. Specifically, the side chains of peptoid oligo-

Tab. 1.1 Comparison of key characteristics of peptides and peptoids

	Peptides	***Peptoids***
Identity	Sequence-specific polymers of amino acids	Sequence-specific polymers of *N*-substituted glycines
Synthesis	Solid-phase. Polymerization of *N*-protected α-amino acids (Fmoc or Boc).	Solid-phase sub-monomer synthesis
Number of side chains?	20+	Derived from hundreds of available primary amines
Secondary structures	Helices (3^{10}, α), β-sheets	Helices
Structural stabilization	Intra-chain hydrogen bonds	Steric and electronic repulsions
Thermal stability of structures?	Up to ~40 °C	>75 °C
Structural stability to solvent environment?	May denature in high salt, organic solvent, or at pH extremes	Generally stable to salt, pH, and organic solvent
In vivo stability	Rapidly degraded (proteolysis)	Stable to proteolysis, excreted whole in urine

mers are shifted to become pendant groups of the main-chain nitrogen atoms (Fig. 1.1). The presentation of peptide and peptoid side chains is roughly isosteric, potentially allowing for suitable mimicry of the spacing between the critical chemical functionalities of bioactive peptides. Peptoid monomers are linked through polyimide bonds, in contrast to the amide bonds of peptides (with the sole exception of proline residues, which are also -imino acids). Peptoids lack the hydrogen of the peptide secondary amide, and are thus incapable of forming the same types of hydrogen bond networks that stabilize peptide helices and β-sheets, respectively. The peptoid oligomer backbone is achiral; however, chiral centers can be included in the side chains to obtain secondary structures with a preferred handedness [3, 4]. In addition, peptoids carrying *N*-substituted versions of the proteinogenic side chains are highly resistant to degradation by proteases [5], which is an important attribute of a pharmacologically useful peptide mimic.

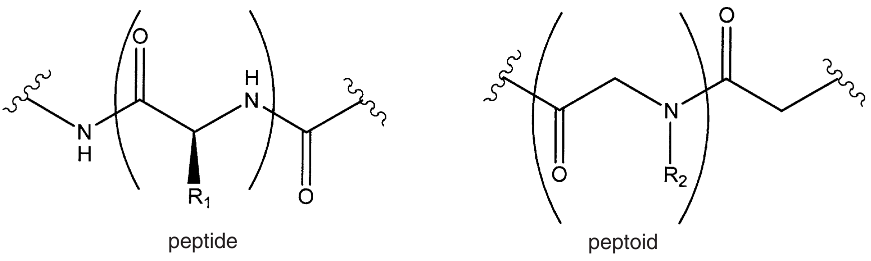

Fig. 1.1 Comparison of the primary structure of peptide and peptoid oligomers

The efficient solid-phase synthesis of peptoids (Section 1.2) enables facile combinatorial library generation. In the "sub-monomer" synthetic strategy, peptoid monomers are synthesized by a two-step process from a haloacetic acid and a primary amine. A wide variety of amines are commercially available, which facilitates the incorporation of chemically diverse side chains. High synthetic yields typically attained at each synthetic step permit the propagation of peptoid chains to substantial lengths. For instance, peptoids up to 48 residues long have been synthesized with reasonable yields of the full-length target sequence [6].

1.2
Peptoid Synthesis

1.2.1
Solid-Phase Synthesis

Sequence-specific heteropolymers, as a class of synthetic molecules, are unique in that they must be made by chemical steps that add one monomer unit at a time. Moreover, to create truly protein-like structures, which typically have chain lengths of at least 100 monomers and a diverse set of 20 side chains (or more), extremely efficient and rapid couplings under general reaction conditions are necessary. For these reasons, solid-phase synthesis is typically used, so that excess reagents can be used to drive reactions to completion, and subsequent reaction work-ups are quite rapid.

A common feature of most solid-phase oligomer syntheses (e.g. peptide, oligonucleotide, peptide nucleic acid, β-peptide, etc.) is that they are made by a two-step monomer addition cycle. First, a protected monomer unit is coupled to a terminus of the resin-bound growing chain, and then the protecting group is removed to regenerate the active terminus. Each side chain group requires a separate N^{α}-protected monomer. The first oligopeptoids reported were synthesized by this method, for which a set of Fmoc-protected peptoid monomers was made [2].

Specifically, the carboxylates of N^{α}-Fmoc-protected (and side chain-protected) N-substituted glycines were activated and then coupled to the secondary amino

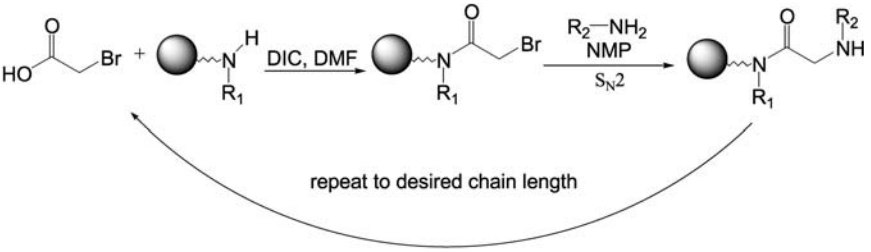

Fig. 1.2 Solid-phase sub-monomer peptoid synthesis

group of a resin-bound peptoid chain. Removal of the Fmoc group was then followed by addition of the next monomer. Thus, peptoid oligomers can be thought of as condensation homopolymers of *N*-substituted glycine. There are several advantages to this method [7], but the extensive synthetic effort required to prepare a suitable set of chemically diverse monomers is a significant disadvantage of this approach. Additionally, the secondary *N*-terminal amine in peptoid oligomers is more sterically hindered than the primary amine of an amino acid, which slows coupling reactions.

1.2.2
Sub-monomer Solid-Phase Method

A major breakthrough came in 1992 when a much more efficient method of peptoid synthesis was invented [8]. In this method, each *N*-substituted glycine (NSG) monomer is assembled from two readily available “sub-monomers” in the course of extending the NSG oligomer [9]. This method is known as the sub-monomer method, in which each cycle of monomer addition consists of two steps, an acylation step and a nucleophilic displacement step (Fig. 1.2). Thus, peptoid oligomers can also be considered to be alternating condensation copolymers of a haloacetic acid and a primary amine. This method is unique among solid-phase oligomer syntheses in that there are no protecting groups used in elongating the main chain. As in the original method, the direction of oligomer synthesis utilizing these sub-monomers occurs in the carboxy to amino direction.

In the first step, a resin-bound secondary amine is acylated with bromoacetic acid, in the presence of *N,N*-diisopropylcarbodiimide. Acylation of secondary amines is difficult, especially when coupling an amino acid with a bulky side chain. The sub-monomer method, on the other hand, is facilitated by the use of bromoacetic acid, which is a very reactive acylating agent. Activated bromoacetic acid is bis-reactive, in that it acylates by reacting with a nucleophile at the carbonyl carbon, or it can alkylate by reacting with a nucleophile at the neighboring aliphatic carbon. Because acylation is approximately 1000 times faster than alkylation, acylation is exclusively observed.

The second step introduces the side chain group by nucleophilic displacement of the bromide (as a resin-bound α-bromoacetamide) with an excess of primary amine. Because there is such diversity in reactivity among candidate amine sub-monomers, high concentrations of the amine are typically used ($\sim$1–2 M) in a polar aprotic solvent (e.g. DMSO, NMP or DMF). This S_N2 reaction is really a mono-alkylation of a primary amine, a reaction that is typically complicated by over-alkylation when amines are alkylated with halides in solution. However, since the reactive bromoacetamide is immobilized to the solid support, any over-alkylation side-products would be the result of a cross-reaction with another immobilized oligomer (slow) in preference to reaction with an amine in solution at high concentration (fast). Thus, in the sub-monomer method, the solid phase serves not only to enable a rapid reaction work-up, but also to isolate reactive sites from one another. However, the primary advantage of the method is that each primary amine is much simpler in structure than a protected full monomer. The fact that several hundred primary amines are commercially available greatly facilitates peptoid synthesis.

Most primary amines that are neither sterically hindered nor very weak nucleophiles will incorporate into the peptoid chain in high yield. However, protection of reactive side-chain functionalities such as carboxyl, thiol, amino, hydroxy and other groups may be required to minimize undesired side reactions [10]. Acid-labile protecting groups are preferred, as they may be removed during peptoid cleavage from solid support with trifluoroacetic acid (TFA). The mild reactivity of some side-chain moieties toward displacement or acylation allows their use without protection in some cases (e.g. indole, phenol). In these cases, the side chain may become transiently acylated during the acylation step and will subsequently revert back to the free side chain upon treatment with the amine in the displacement step. Heterocyclic side-chain moieties such as imidazole, pyrazine, quinolines and pyridines may also become transiently acylated. However, since these groups are more nucleophilic they are susceptible to alkyation by the activated bromoacetic acid. In these cases, clean incorporation can be achieved by replacing bromoacetic acid with chloroacetic acid [11].

1.2.3

Side Reactions

There are a few competing side reactions that are unique to the synthesis of peptoid oligomers. For example, peptoid dimer synthesis often leads to formation of the cyclic diketopiperazines instead of the linear molecule [12]. Sub-monomers whose side chains bear a nucleophile three or four atoms from the amino nitrogen, are also prone to cyclizations after bromoacetylation. We have found the submonomers trityl-histamine, 2-(aminomethyl)benzimidazole, and 2-aminoethylmorpholine fall into this category. Electron-rich benzylic side chains, such as those derived from *p*-methoxy-1-phenylethylamine or 2,4,6-trimethoxybenzylamine, will fall off the main chain during the post-synthetic acidolytic cleavage with TFA.

1.2.4

Post-Synthetic Analysis

Like peptide oligomers, peptoids can be analyzed by HPLC and by mass spectrometry. They can be sequenced by Edman degradation [13] or by tandem mass spectrometry [14] since, like polypeptides, they conveniently fragment along the main chain amides [15, 16].

1.3
Drug Discovery via Small-Molecule Peptoid Libraries

Because of their ease of synthesis and their structural similarity to peptides, many laboratories have used peptoids as the basis for combinatorial drug discovery. Peptoids were among the first non-natural compounds used to establish the basic principles and practical methods of combinatorial discovery [17]. Typically, diverse libraries of relatively short peptoids (<10 residues) are synthesized by the mix-and-split method and then screened for biological activity. Individual active compounds can then be identified by iterative re-synthesis, sequencing of compounds on individual beads, or indirect deduction by the preparation of positional scanning libraries.

1.3.1
Peptoid Drugs from Combinatorial Libraries

Early on, peptoid trimers that were nanomolar ligands for the opiate and α_1-adrenergic receptors were identified by *in vitro* receptor binding experiments [17] (Fig. 1.3). These ligands were discovered from a library of 3500 trimers by iterative re-synthesis, where activity was followed through successive rounds of re-synthesis of smaller pools. These compounds showed some *in vivo* activity, despite their poor pharmacokinetic properties [18]. Trimeric peptoid libraries were also screened for antimicrobial activity in whole cell assays, which yielded compounds with modest activities against *S. aureus* and *E. coli* [19]. Very large libraries of peptoid trimers ($\sim$350,000 compounds) were screened against a variety of agrochemical targets to provide a sub-micromolar antagonist of the nicotinic acetylcholine receptor, following iterative deconvolution [20]. Positional scanning libraries of peptoid trimers were used to discover noncompetitive antagonists of the vanilloid receptor subunit 1 (VR1) [21]. Another research group prepared a library of 328,000 peptoid trimers, and used iterative deconvolution to discover micromolar ligands for both the melanocortin type 1 (MC1) and gastrin-releasing peptide/bombesin receptors [22]. Finally, a small family of peptoid trimers was generated in order to mimic the Agouti-related protein, including one member that showed micromolar affinity for the melanocortin type 4 receptor [23].

1.3.2
Peptoid Inhibitors of RNA-Protein Interactions

Peptoid libraries have also yielded compounds active in the disruption of RNA-protein interactions. Compounds not derived from library syntheses are discussed in Section 1.4.1. A peptoid 9-mer with a number of cationic groups was discovered (Fig. 1.4) after several rounds of mixture deconvolution, that was able to block the interaction of HIV-1 Tat protein with TAR RNA at nanomolar concentra-

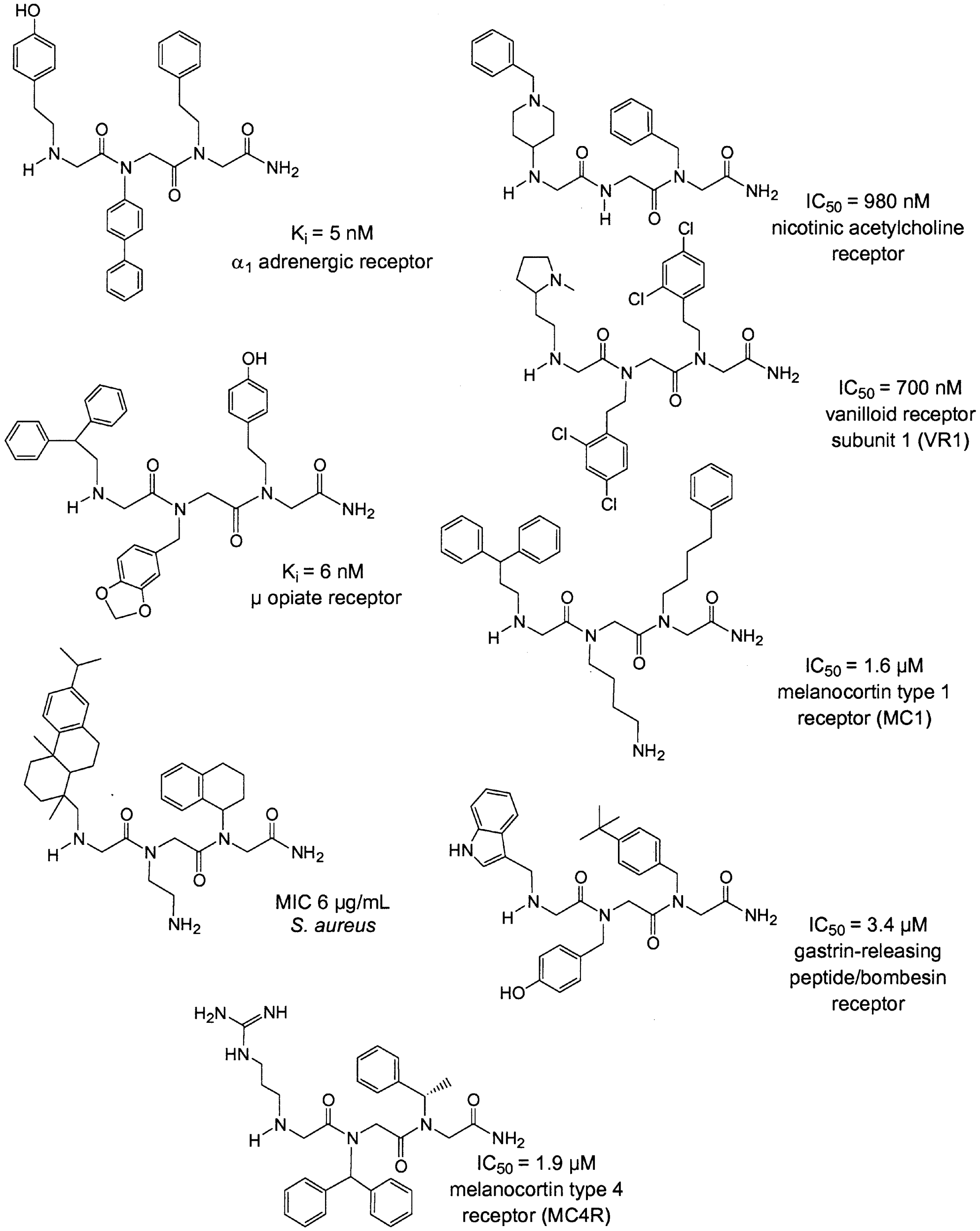
K$_i$ = 5 nM
α_1 adrenergic receptor
IC$_{50}$ = 980 nM
nicotinic acetylcholine receptor
IC$_{50}$ = 700 nM
vanilloid receptor subunit 1 (VR1)
K$_i$ = 6 nM
μ opiate receptor
IC$_{50}$ = 1.6 μM
melanocortin type 1 receptor (MC1)
MIC 6 μg/mL
S. aureus
IC$_{50}$ = 3.4 μM
gastrin-releasing peptide/bombesin receptor
IC$_{50}$ = 1.9 μM
melanocortin type 4 receptor (MC4R)

Fig. 1.3 Various small-molecule peptoid ligands derived from combinatorial libraries

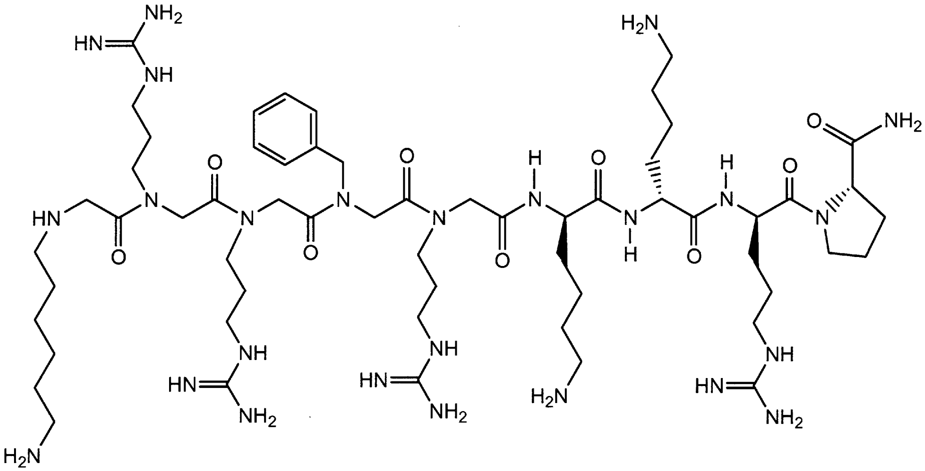

Fig. 1.4 Structure of a peptoid/peptide hybrid that is a submicromolar inhibitor of the HIV-1 Tat/Tar interaction

tions, as well as block HIV-1 replication *in vivo* [24]. Other cationic peptoids with similar side chains, which also inhibit this interaction have been discovered [25].

1.4

Peptoid-Based Drug Delivery and Molecular Transporters: Cellular Uptake

The cellular membrane presents a formidable barrier to drug uptake. In order to exhibit passive diffusion into the cell, drugs must be polar so as to facilitate distribution into the aqueous cellular environment, yet not so polar as to prevent diffusion across the hydrophobic interior of the cellular membrane. In addition, other physical drug characteristics (e.g. molecular weight > ~700 Da) can limit bioavailability. Promising drugs that do not possess the requisite characteristics for passive cellular entry can instead be delivered by novel techniques. For instance, a highly lipophilic drug might be delivered by packaging in liposomes, or a very polar drug might be functionalized with a lipophilic moiety. Similarly, certain large polycationic homopolymers of lysine [26], ornithine [27], and arginine [27] (between 4 and 200 kDa in weight) have been shown to facilitate membrane translocation in cells, and can be covalently ligated to biomolecules to promote cellular entry. However, these large polycations can be toxic *in vivo*, difficult to produce, and expensive.

1.4.1
Peptoid Mimics of HIV-Tat Protein

Alternatively, one interesting drug delivery technique exploits the active transport of certain naturally-occurring and relatively small biomacromolecules across the cellular membrane. For instance, the nuclear transcription activator protein (Tat) from HIV type 1 (HIV-1) is a 101-amino acid protein that must interact with a 59-base RNA stem–loop structure, called the *trans*-activation region (Tar) at the 5′ end of all nascent HIV-1 mRNA molecules, in order for the virus to replicate. HIV-Tat is actively transported across the cell membrane, and localizes to the nucleus [28]. It has been found that the arginine-rich Tar-binding region of the Tat protein, residues 49–57 (Tat_{49-57}), is primarily responsible for this translocation activity [29].

Recently, there has been significant interest in peptidomimetic forms of Tat_{49-57}, not only because of its membrane translocation activity, but as a means of treating HIV infection [1]. Several peptoids, similar in sequence to Tat_{49-57}, have been synthesized with the intention of preventing the HIV-Tat/Tar interaction, and thus preventing HIV replication [24, 25, 30, 31]. However, only recently has this class of peptoids been applied to membrane translocation and drug delivery applications.

Short peptoid-based Tat_{49-57} analogs are more advantageous drug delivery vehicles than large polycationic homopolymers of lysine, ornithine, and arginine. Not only are peptoids more readily synthesized and potentially bioavailable than such large polymers (Section 1.2), they are less likely to be proteolytically degraded [5] or to cause an immunological response than are peptide-based analogs.

Wender and colleagues investigated various truncated and alanine-substituted fluorescently labeled peptoid analogs of Tat_{49-57} in order to determine the requisite structural features for membrane translocation activity in Jurkat cells [32]. They determined that the presence of at least six arginine residues (guanidino moieties) was critical for rapid cell entry, while no specific charge or structural element was important. Correspondingly, they synthesized a series of fluorescently-labeled oligoguanidine peptoids, and compared their capacity for cellular uptake in Jurkat cells with peptide D-arginine oligomers between five and nine residues in length. They identified one compound, *N*-hxg9, which was superior to a D-arginine nonamer in cellular uptake, which in turn was about 100-fold more rapidly translocated across the cell membrane than a fluorescently-labeled Tat_{49-57}.

1.4.2
Cellular Delivery of Nucleic Acids

Peptoids have also shown great utility in their ability to complex with and deliver nucleic acids to cells, a critical step toward the development of antisense drugs, DNA vaccines, or gene-based therapeutics. Most non-viral nucleic acid delivery systems are based on cationic molecules that can form complexes with the polyan-

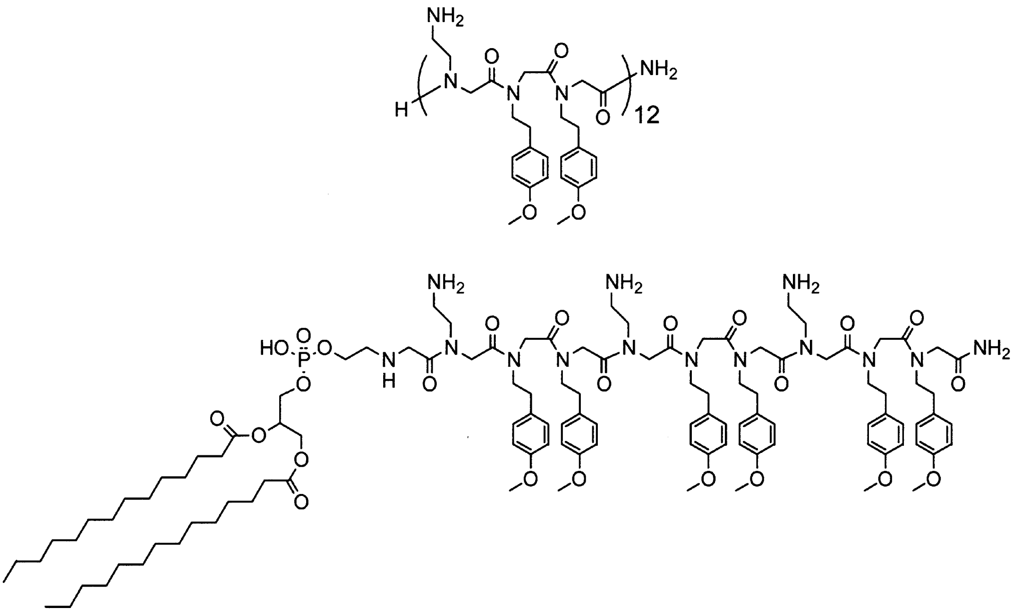

Fig. 1.5 Structure of a 36mer peptoid and the corresponding "lipitoid"

ionic nucleic acid [33]. These cationic materials are typically either polymeric or lipid-based structures, whose performance depends on a balance of factors including overall size, type of cation, density of charge, hydrophobicity, and solubility.

Peptoids are ideally suited to this task because their primary and secondary structures can be precisely predicted and controlled through their sequence. Combinatorial libraries of cationic peptoids were synthesized and evaluated for their ability to condense, protect, and deliver plasmid DNA to cells in culture [6]. An effective, 36mer compound was discovered that contained a repeating cationic trimer motif: cationic-hydrophobic-hydrophobic (Fig. 1.5). In an effort to refine the activity of this compound, solid-phase chemistry was developed to rapidly conjugate lipid moieties to the *N*-termini of peptoids. These cationic peptoid-lipid conjugates (called "lipitoids") were substantially more active in delivering plasmid DNA to cells, and also showed reduced cellular toxicity relative to the lead compound [34]. The most active lipitoids are composed of a natural phosphatidyl ethanolamine lipid conjugated to a 9mer with the same trimeric motif.

Although *in vivo* delivery studies using these reagents are still an early stage, high *in vitro* activity and low toxicity make lipitoids ideal transfection reagents. Recent work has shown that very small structural changes in the lipitoid can result in molecules that efficiently deliver antisense oligonucleotides and RNA.

1.5
Peptoid Mimics of Peptide Ligands

One straightforward approach to the design of biologically-active peptoid sequences is the systematic modification of an active peptide target sequence. For example, constituent amino acids of a target peptide may be substituted by peptoid residues with identical side chains. This modification results in a peptoid oligomer with side chains that are shifted relative to their original positions in the peptide template. An alternative approach is to generate a library of compounds in which site-specific substitutions are made using a diverse set of peptoid monomers, followed by screening to identify the most active compounds. In these methods, the partial substitution of amino acids by peptoid residues generates peptide/peptoid hybrids, which should ideally possess improved pharmokinetic and/or binding properties.

Characterization of peptoid-containing analogs of peptide ligands can provide valuable information. For example, this process can help to elucidate the position of critical residues in the protein target that provide important binding determinants. It can identify molecules with enhanced activity relative to the starting structure and/or with enhanced specificity to a protein target. Finally, it can identify active species with peptoid monomer substitution levels sufficient to grant significantly improved protease stability relative to the original peptide of interest.

The utility of peptoid/peptide hybrids was exemplified by a study of hybrid ligands to proteins containing the Src homology 3 (SH3) domain [35, 36]. As part of their signal transduction activity, these proteins recognize peptides with a PxxP motif (where P = proline, and x = any other amino acid). In general, peptide ligand binding to SH3 domains occurs with low affinity and low specificity. Peptide sequences derived from wild-type protein partners exhibit significant binding to the large family of proteins with SH3 domains. Nguyen *et al.* conducted studies aimed at an improved understanding of the role of proline residues in the core PxxP motif at the binding interface. Previous structural studies had suggested that recognition requires the presence of an *N*-substituted amino acid at proline positions, but that proline itself was not specifically required. Peptide/peptoid hybrids containing a variety of different peptoid substitutions for proline in the core PxxP region were synthesized and evaluated for binding to various proteins containing SH3 domains. It was found that single-site substitutions can have a strong effect on binding affinity. For instance, a hybrid oligomer bearing a dimethoxybenyzl *N*-substitution showed a 100-fold increase in binding affinity over the wild-type peptide (to a K_D of 30 nm), accompanied by a dramatic gain in specificity for binding to the SH3 domain of the protein *N*-Grb2 relative to those of Src and Crk. Multiple substitutions were also well tolerated. For example, an analog of a peptide with partial specificity for Crk was synthesized, in which both proline residues in the PxxP motif were replaced by peptoid monomers. The new hybrid retained strong binding to Crk, but no longer recognized Src or *N*-Grb2 [35]. The Goodman group has similarly investigated the effect of a variety of peptoid substitutions at the proline position in a cyclic hexapeptide analog of somatostatin, cyclo-