ORGANIC LETTERS

2007
Vol. 9, No. 8
1549−1552

α-Peptide/β-Peptoid Chimeras

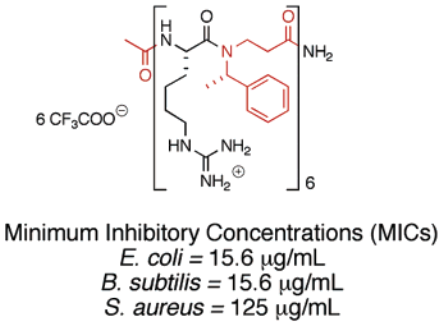
Christian A. Olsen,*,†,§ Gitte Bonke,† Line Vedel,† Anne Adsersen,† Matthias Witt,‡ Henrik Franzyk,† and Jerzy W. Jaroszewski†

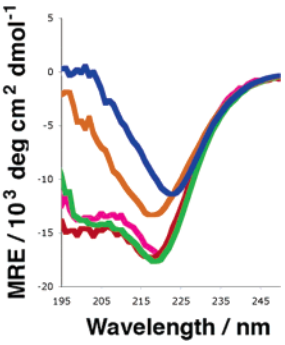
Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark, and Bruker Daltonik GmbH, Fahrenheitstrasse 4, D-28359 Bremen, Germany

cao@farma.ku.dk

Received February 7, 2007

ABSTRACT





We describe the synthesis and characterization of the first generation of oligomers consisting of alternating repeats of α-amino acids and chiral *N*-alkyl-β-alanine (β-peptoid) residues. These chimeras are stable toward proteolysis, non-hemolytic, and possess antibacterial activity comparable to well-known antimicrobial agents. Moreover, the chimeras exhibit length-dependent, concentration-dependent, solvent-dependent, and ion-strength-dependent ellipticity, indicating the presence of a secondary structure in solution. Thus, α-peptide/β-peptoid oligomers represent a promising novel peptidomimetic backbone construct for biologically active ligands.

Natural antimicrobial peptides (AMPs) have been developed through evolution as host-defense systems of animals and plants[1,2] as well as fungi,[3] providing immunity to attack by a broad spectrum of microorganisms. Many AMPs adopt amphipathic secondary structures displaying positively charged domains believed to bind to negatively charged sites, which are more abundant on the outer surface of microbial membranes as compared to cytoplasmic membranes of higher animals, thus facilitating selectivity for microbes.[4−7]

Peptides with a therapeutic potential such as plectasin may be produced on an industrial scale by a fungal expression system.[3] Low molecular weight octapeptide lead compounds have been discovered by high-throughput synthesis and screening of libraries based on the natural AMP bactenecin.[8] Nevertheless, an inherent weakness associated with antimicrobial peptides constructed from natural α-amino acids (**1**, Figure 1) is their instability toward proteases, which severely diminishes their bioavailability. This intrinsic obstacle has been circumvented by the design of synthetic foldamers, i.e., biomimetic oligomers that exhibit a high propensity for adopting specific secondary structures,[9,10] for example,

† University of Copenhagen.
§ Current e-mail: caolsen@scripps.edu.
‡ Bruker Daltonik GmbH.

(1) Tossi, A.; Sandri, L.; Giangaspero, A. *Biopolymers* **2000**, *55*, 4−30.
(2) Zasloff, M. *Nature* **2002**, *415*, 389−395.
(3) Mygind, P. H.; Fischer, R. L.; Schnorr, K. M.; Hansen, M. T.; Sönksen, C. P.; Ludvigsen, S.; Raventós, D.; Buskov, S.; Christiensen, B.; De Maria, L.; Taboureau, O.; Yaver, D.; Elvig-Jørgensen, S. G.; Sørensen, M. V.; Christensen, B. E.; Kjærulff, S.; Frimodt-Møller, N.; Lehrer, R. I.; Zasloff, M.; Kristensen, H.-H. *Nature* **2005**, *437*, 975−980.
(4) Matsuzaki, K. *Biochem. Biophys. Acta* **1999**, *1462*, 1−10.
(5) Shai, Y. *Biochem. Biophys. Acta* **1999**, *1462*, 55−70.
(6) Yang, L.; Weiss, T. M.; Lehrer, R. I.; Huang, H. W. *Biophys. J.* **2000**, *79*, 2002−2009.
(7) Pujals, S.; Fernández-Carneado, J.; López-Iglesias, C.; Kogan, M. J.; Giralt, E. *Biochem. Biophys. Acta* **2006**, *1758*, 264−279.
(8) Hilpert, K.; Volkmer-Engert, R.; Walter, T.; Hancock, R. E. W. *Nat. Biotechnol.* **2005**, *23*, 1008−1012.
(9) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180.
(10) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4011.

10.1021/ol070316c CCC: $37.00
Published on Web 03/13/2007

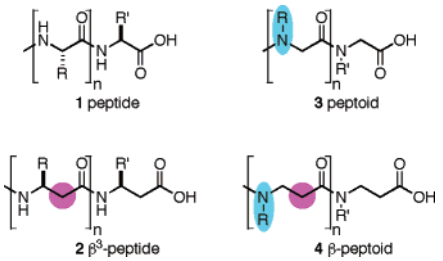


Figure 1. Backbone structures of natural α-peptides and main peptidomimetic designs.

oligomers of *β*-amino acids[11] or α-chiral *N*-alkylated glycines, i.e., *β*-peptides (**2**) and peptoids (**3**), respectively (Figure 1).[11,12] Mimicry of the non-hemolytic antimicrobial activity of the medium-sized natural host-defense peptide magainin[13] with *β*-peptides,[14−16] peptoids,[17] *N*,*N*′-linked oligoureas,[18] and arylamide oligomers[19] has been reported. Furthermore, cationic *N*-alkylated *β*-alanine oligomers (*β*-peptoids, **4**, Figure 1) showing a moderate inhibitory activity against *E. coli* have recently been reported,[20] and self-assembling cyclic D,L-α-peptide nanotubes have proved to be efficient antimicrobial agents in mice.[21]

Recently, the first synthesis and characterization of homomeric *β*-peptoid oligomers with α-chiral phenethyl side chains were described by Arvidsson's group.[22] Here, we present the synthesis, CD characterization, and antimicrobial activity of this chiral *N*-(*S*)-phenethyl-*β*-alanine (*β*-*N*spe) motif within a chimeric framework including α-amino acid residues.

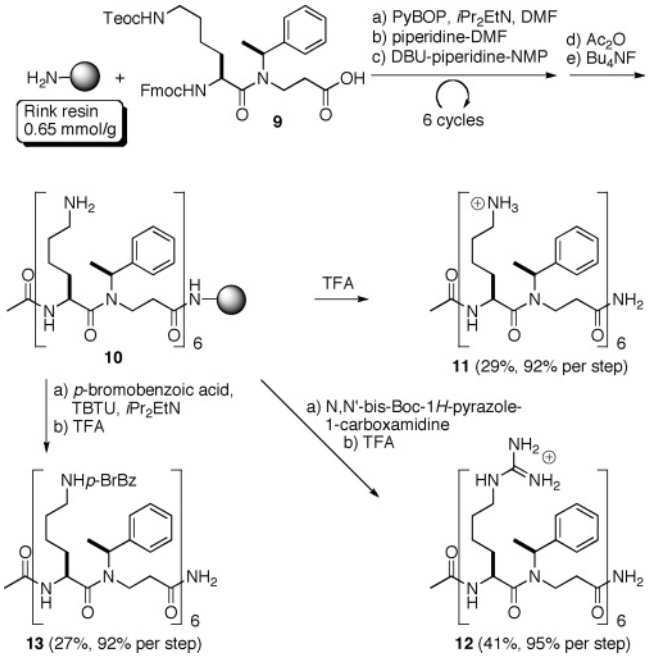
Although the structures of the chiral *β*-peptoid homooligomers appeared to exhibit insignificant chain-length-dependent CD behavior according to Arvidsson's preliminary investigations,[22] we decided to explore oligomers of alternating α-amino acid and *β*-peptoid monomers giving rise to a heteromeric backbone resembling that of α/*β*-peptides,[23−25] but with different side chain positioning. This design extends the structural space available for side chain topology of the constructs. Moreover, additional stabilization of potential secondary structures was expected due to the possibility of forming internal hydrogen bonds, possibly resulting in compounds with attractive physical and biological features. In our initial studies we selected lysine as the α-amino acid residue, since peptide oligomers with cationic sites have previously proved to exhibit antimicrobial activity. Recently, Gellman and co-workers reported that "scrambled" α/*β*-peptide analogues, designed not to exhibit a global amphipathic structure, gave rise to antimicrobials with high membrane selectivity (i.e., low hemolytic activity toward mammalian cell membranes compared to the antibacterial MIC values).[25] Interestingly, our chimeric design, which has alternating repeats of lipophilic and cationic residues, is not expected to adopt globally amphipathic secondary structures either.

Initially, a strategy involving a submonomer SPS of the *β*-peptoid residues was considered; however, preliminary experiments showed that SPPS couplings involving the sterically hindered *β*-peptoid nitrogen atom did not perform satisfactorily. Hence, a versatile dimeric building block (**9**) was prepared in solution in three steps on gram-scale (Supporting Information, Scheme S1). The 2-(trimethylsilyl)-ethoxycarbonyl (Teoc) group was chosen for side chain protection to allow on-resin derivatization of the side chain amino groups upon oligomer assembly. Attachment of the dimer **9** to a Rink amide resin followed by elongation by SPPS, *N*-terminal acetylation, and removal of the Teoc groups, afforded resin **10** (Scheme 1). The possibility of post-assembly on-resin side chain functionalization was demonstrated by guanidinylation and acylation to give **12** and **13**, respectively. This approach indeed furnished good overall isolated yields of the oligomers (27−41%).

The chimeras were evaluated by CD spectroscopy in several solvents and at various concentrations (Figure 2). The CD curves of **11**−**13** show mean residue molar ellipticities (MREs) around 219 nm (−15000 to −20000 deg cm^2 dmol^{-1}) that are much higher than those observed for unordered (random coil) peptides, but in the same range as those found for *β*-peptides and peptoids with a high helix content. In all these structures, the observed Cotton effects are exhibited at very similar wavelengths, and hence are expected to be due to a common structural feature, namely a helical polyamide backbone interacting with the side chain chromophores via exciton coupling. Interestingly, **11** exhibited the same trend in solvent effect on the CD spectra as that previously observed for water-soluble peptoids, where the molar ellipticity in methanol was diminished as compared to phosphate buffer (pH 7), while addition of 20% of 2,2,2-trifluoroethanol (TFE) to the aqueous medium resulted in

(11) (a) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219−3232. (b) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiv.* **2004**, *1*, 1111−1239.
(12) Kirshenbaum, K.; Barron, A. E.; Goldsmith, R. A.; Armand, P.; Bradley, E. K.; Truong, K. T. V.; Dill, K. A.; Cohen, F. E.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4303−4308.
(13) Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5449−5453.
(14) Arvidsson, P. I.; Ryder, N. S.; Weiss, H. M.; Hook, D. F.; Escalante, J.; Seebach, D. *Chem. Biodiv.* **2005**, *2*, 401−420.
(15) Hamuro, Y.; Schneider, J. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1999**, *121*, 12200−12201.
(16) Porter, E. A.; Wang, X.; Lee, H.-S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565. Erratum: *Nature* **2000**, *405*, 298.
(17) Patch, J. A.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 12092−12093.
(18) Violette, A.; Fournel, S.; Lamour, K.; Chaloin, O.; Frisch, B.; Briand, J.-P.; Monteil, H.; Guichard, G. *Chem. Biol.* **2006**, *13*, 531−538.
(19) Tew, G. N.; Liu, D.; Chen, B.; Doerksen, R. J.; Kaplan, J.; Carroll, P. J.; Klein, M. L.; DeGrado, W. F. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5110−5114.
(20) Shuey, S. W.; Delaney, W. J.; Shah, M. C.; Scialdone, M. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1245−1248.
(21) Fernandez-Lopez, S.; Kim, H.-S.; Choi, E. C.; Delgado, M.; Granja, J. R.; Khasanov, A.; Kraehenbuehl, K.; Long, G.; Weinberger, D. A.; Wilcoxen, K. M.; Ghadiri, M. R. *Nature* **2001**, *412*, 452−456.
(22) Norgren, A. S.; Zhang, S.; Arvidsson, P. I. *Org. Lett.* **2006**, *8*, 4533−4536.
(23) De Pol, S.; Zorn, C.; Klein, C. D.; Zerbe, O.; Reiser, O. *Angew. Chem., Int. Ed.* **2004**, *43*, 511−514.
(24) Hayen, A.; Schmitt, M. A.; Ngassa, F. N.; Thomasson, K. A.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 505−511.
(25) Schmitt, M. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2004**, *126*, 6848−6849.

Scheme 1. SPPS of Oligomers **11**−**13**



increased molar ellipticity.[26] The CD intensity in neat TFE resembled that in the aqueous buffer, while the spectrum obtained in neat acetonitrile was considerably less intense. Compounds **12** and **13** gave CD spectra similar to those of **11**, but **13** was only tested in the organic solvents due to its insolubility in water.[27] The normalized CD spectrum of an *N*-acylated dipeptide building block did not by itself exhibit the characteristic saddle-shaped curve (Supporting Information, Figure S2).

The water-soluble oligomers retained remarkably strong CD spectra in aqueous media as compared to the trend observed for β^3-peptides when going from structure-promoting organic solvents (e.g., MeOH and TFE) to aqueous solutions.[16] This property has previously been observed for peptoids[26] and β-peptides with conformationally restrained backbone elements[16,28] or side chain macrodipoles.[29] Also, addition of 1 M NaCl to the phosphate buffer affected the shape of the CD curve, diminishing the intensity of the saddle section and increasing the Cotton effect around 195 nm considerably (Figure 2a, orange). The salt effect observed for this chimera parallels that observed for α-helical peptides due to charge screening by counterions.[30]

The concentration effect on the CD behavior was investigated in phosphate buffer (Figure 2b) and in MeOH

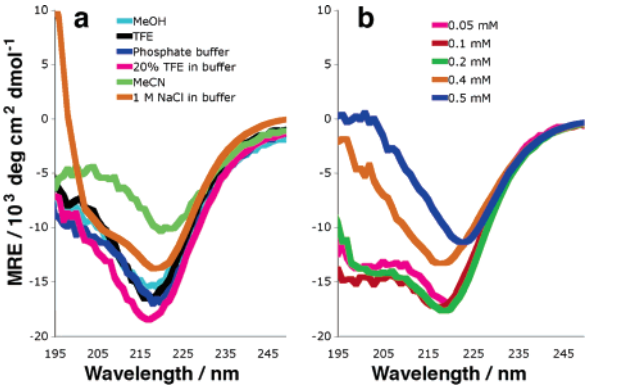


Figure 2. (a) CD spectra of compound **11** (60 μM) in various solvents. (b) CD-spectra of chimera **11** in phosphate buffer (pH 7) at various concentrations. The solutions were prepared from lyophilized hexakis(trifluoroacetates) of the peptide. MRE = mean residue ellipticity.

(Supporting Information, Figure S3). No significant concentration-induced changes were observed below 200 μM in either of the two solvents, but at higher concentrations the CD spectra changed in intensity as well as in shape, suggesting that the compounds are monomeric at biologically relevant concentrations (<200 μM). On the other hand, the apparent aggregation at higher concentrations will severely complicate NMR studies.[31]

Next, we wished to assess whether the chain length and the β-peptoid chirality influenced the CD spectra. Hence, compounds (**14**−**20**, Figure 3b) were obtained by oligomer-

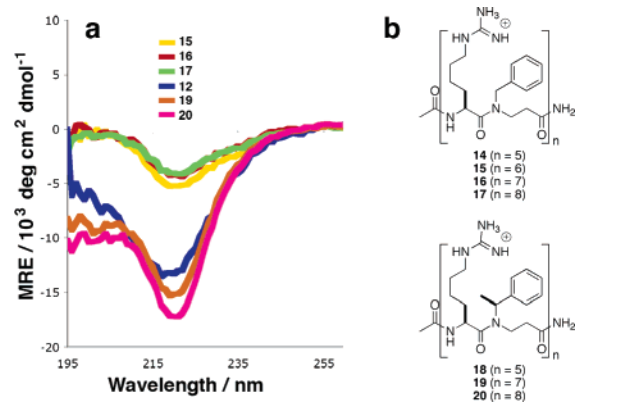


Figure 3. (a) CD spectra of compounds **12**, **15**−**17**, **19**, and **20** (60 μM) in MeOH. The solutions were prepared from lyophilized trifluoroacetate salts of the peptides. (b) Structures of compounds **14**−**20**.

ization on Rink amide resin with use of pre-guanidinylated building blocks **24a**,**b** (Supporting Information, Scheme S2). The CD spectra showed that lack of β-peptoid chirality (as in **14**−**17**) resulted in a substantial drop in the ellipticity amplitudes at 219 nm, which indicates a low degree of

(26) Sanborn, T. J.; Wu, C. W.; Zuckermann, R. N.; Barron, A. E. *Biopolymers* **2002**, *63*, 12−20.
(27) See the Supporting Information (Figure S1) for normalized CD spectra of **12** and **13** in various solvents.
(28) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J., Jr.; Gellman, S. H. *Nature* **1997**, *387*, 381−384.
(29) Hart, S. A.; Bahadoor, A. B. F.; Matthews, E. E.; Qiu, X. J.; Schepartz, A. *J. Am. Chem. Soc.* **2003**, *125*, 4022−4023.
(30) For examples, see: (a) Kojima, S.; Kuriki, Y.; Sato, Y.; Arisaka, F.; Kumagai, I.; Takahashi, S.; Miura, K.-i. *Biochem. Biophys. Acta* **1996**, *1294*, 129−137. (b) Smith, J. S.; Scholtz, J. M. *Biochemistry* **1998**, *37*, 33−40.

folding for these compounds. On the other hand, a comparison of the CD spectra of compounds **12** (dodecamer), **19** (tetradecamer), and **20** (hexadecamer) clearly revealed that the minima at 219 nm increased in amplitude with increasing chain length (Figure 3a). This trend contrasts the behavior of β-peptoid homooligomers described recently by Arvidsson and co-workers, where no significant chain-length effect on CD spectra was observed. This suggests that hydrogen bonding from the α-amino acid residues contributes to the stabilization of a secondary structure apparently present in solutions of the chimeras.

Since these compounds constitute a new backbone design, no reference CD data are available, and thus no exact structural properties can be derived from the CD measurements. In addition, similar CD spectra may arise from very different ensembles of folded secondary structures in solution.[32] Nevertheless, the indications of the overall folding propensity of the chimeras are important, as also reported in the initial characterization of previous novel types of oligomers.[12,22]

In a preliminary evaluation, compounds **11**−**15** were tested for antibacterial activity against one strain of Gram-negative bacteria (*Escherichia coli*) and two strains of Gram-positive bacteria (*Bacillus subtilis* and *Staphyllococcus aureus*). The results are shown in Table 1. Compound **11** was only moderately active against *E. coli* and *B. subtilis*, but the guanidinium-functionalized chimeras exhibited potencies comparable to that of streptomycin and higher than that of magainin-2, a well-known antimicrobial peptide. Importantly, no significant hemolysis of human red blood cells was observed with **11**, **12**, and **15** at concentrations up to 500, 50, and 50 μg/mL, respectively. These results are remarkable considering that these compounds represent the first generation of α-peptide/β-peptoid chimeras. The oligomer **15**, lacking chirality in the β-peptoid residue, was twice as potent as the dodecamer **12**, indicating that this chirality is not essential for antimicrobial activity. This finding is significant, as it extends the range of possible building blocks and possibly simplifies the construct design in future structure−activity investigations in search of chimeras with enhanced potency and membrane selectivity. Compounds **11**, **12**, and **14**−**20** are currently undergoing extensive testing against a broader range of bacteria, and second-generation compounds with different ratios of cationic and lipophilic sites are in preparation; the results of these studies will be reported in due course.

Table 1. Antimicrobial Activities of Chimeras **11**−**15** and Reference Compounds

	MIC in μg/mL[a]		
compd	*E. coli*	*B. subtilis*	*S. aureus*
11	125	62.5	n.a.[b]
12	15.6	15.6	125
13	n.s.[c]	n.s.[c]	n.s.[c]
14	15.6	15.6	125
15	7.8	7.8	31.5
magainin-2	62.5	62.5	n.a.[b]
streptomycin	15.6	15.6	62.5

[a] MIC = minimum inhibitory concentration. [b] Not active (MIC > 200 μg/mL). [c] Not soluble in the medium.

Finally, stability of the compounds toward proteolytic degradation was tested. Incubation of compound **11** with a large excess of trypsin from porcine pancreas for up to 50 h did not result in any detectable degradation (Supporting Information, Figure S4) showing that the α-peptide/β-peptoid chimeras are highly stable toward proteolysis.

In summary, the first generation of oligomers with an alternating α-peptide/β-peptoid backbone construct is described. The compounds were readily prepared in high yields by combined solution- and solid-phase synthesis methods and were stable toward proteolysis. Furthermore, the guanidinium-functionalized analogues proved to be relatively potent antimicrobial agents. Thus, the data reported herein show that β-peptoid residues may be valuable for diversification of peptide analogues, thus opening new avenues in peptidomimetic research.

Acknowledgment. We thank Ms. Dorte Brix, Ms. Katrine Krydsfeldt, Ms. Uraiwan N. Adamsen, and Ms. Birgitte Simonsen (all from University of Copenhagen) for excellent technical assistance. This work was supported by the Danish Medical Research Council (Grant No. 271-06-0126) and by the Danish Technical Research Council (Grant No. 26-04-0248).

Note Added After ASAP Publication: In the Supporting Information, Figure S3 and the caption, there was an error in the version published ASAP on March 13, 2007; the correct version was published on March 14, 2007.

Supporting Information Available: Experimental procedures, characterization data, CD spectra of **12**, **13**, and of an *N*-acetylated chimeric building block as well as concentration dependence data for **11** in MeOH, and proteolysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

OL070316C

(31) Thus far, structural investigations of the synthesized oligomers by NOESY and ROESY experiments proved fruitless due to signal overlap. Hence, efforts to prepare analogues suitable for NMR spectroscopic investigation have been initiated.

(32) Glättli, A.; Daura, X.; Seebach, D.; van Gunsteren, W. F. *J. Am. Chem. Soc.* **2002**, *124*, 12972−12978.