UNIVERSITA' DEGLI STUDI DI SALERNO
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Synthesis and properties of linear and cyclic peptoids

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Tutor: Prof. Francesco De Riccardis **PhD candidate: Chiara De Cola**
Co-tutor: Prof. Irene Izzo
Coordinatore: Prof. Gaetano Guerra

Abstract

Peptoids: A Promising Class of Peptidomimetics.

If we shift the chain of α-CH group by one position on the peptide backbone, we produced the disappearance of all the intra-chain stereogenic centers and the formation of a sequence of variously substituted *N*-alkylglycines (figure **1.2**).

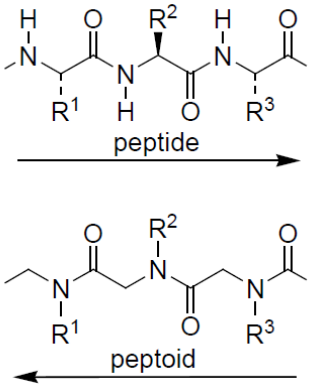

Figure 1.2. Comparison of a portion of a peptide chain with a portion of a peptoid chain.

Oligomers of *N*-substituted glycine, or peptoids, were developed by Zuckermann and co-workers in the early 1990's[1]. They were initially proposed as an accessible class of molecules from which lead compounds could be identified for drug discovery.

Peptoids can be described as mimics of α-peptides in which the side chain is attached to the backbone amide nitrogen instead of the α-carbon (figure **1.2**). These oligomers are an attractive scaffold for biological applications because they can be generated using a straightforward, modular synthesis that allows the incorporation of a wide variety of functionalities[8]. Peptoids have been evaluated as tools to study biomolecular interactions,[8] and also hold significant promise for therapeutic applications due to their enhanced proteolytic stabilities[2] and increased cellular permeabilities[3] relative to α-peptides.

Biologically active peptoids have also been discovered by rational design (*i.e.*, using molecular modeling), and were synthesized either individually or in parallel focused libraries[4]. For some applications, a well-defined structure is also necessary for peptoid function to display the functionality in a particular orientation, or to adopt a conformation that promotes interaction with

[1] 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 and P. A. Bartlett, *Proc. Natl. Acad. Sci. U. S. A.*, **1992**, *89*, 9367–9371.
[2] S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr, W. H. Moos, *Bioorg. Med. Chem. Lett.*, **1994,** 4, 2657–2662.
[3] Y. U.Kwon and T. Kodadek, *J. Am. Chem. Soc.*, **2007**, 129, 1508–1509.
[4] T. Hara, S. R. Durell, M. C. Myers and D. H. Appella, *J. Am. Chem. Soc.*, **2006**, 128, 1995–2004.

other molecules. However, in other biological applications, peptoids lacking defined structures appear to possess superior activities over structured peptoids.

This introduction will focus primarily on the relationship between peptoid structure and function. A comprehensive review of peptoids in drug discovery, detailing peptoid synthesis, biological applications, and structural studies, was published by Barron, Kirshenbaum, Zuckermann, and co-workers in 2004[4]. Since that time, significant advances have been made in these areas, and new applications for peptoids have emerged. In addition, new peptoid secondary structural motifs have been reported, as well as strategies to stabilize those structures. Lastly, the emergence of peptoid with a tertiary structures has driven chemists towards new structures with peculiar properties and side chains. Peptoid monomers are linked through polyimide bonds, in contrast to the amide bonds of peptides. Unfortunately, peptoids do not have the hydrogen of the peptide secondary amide, and are consequently incapable of forming the same types of hydrogen bond networks that stabilize peptide helices and *β*-sheets.

The peptoids oligomer backbone is achiral; however stereogenic centers can be included in the side chains to obtain secondary structures with a preferred handedness[4]. In addition, peptoids carrying *N*-substituted versions of the proteinogenic side chains are highly resistant to degradation by proteases, which is an important attribute of a pharmacologically useful peptide mimic[4].

1.2 Peptoids' Applications.

The well-defined helical structure associated with appropriately substituted peptoid oligomers can be employed to construct compounds that closely mimic the structures and functions of certain bioactive peptides. In this paragraph, are shown some examples of peptoids that have antibacterial and antimicrobial properties, molecular recognition properties, of metal complexing peptoids, of catalytic peptoids, and of peptoids tagged with nucleobases.

1.2.1 Antibacterial and antimicrobial properties

The antibiotic activities of structurally diverse sets of peptides/peptoids derive from their action on microbial cytoplasmic membranes. The model proposed by Shai–Matsuzaki–Huan[5] (SMH) presumes alteration and permeabilization of the phospholipid bilayer with irreversible damage of the critical membrane functions. Cyclization of linear peptide/peptoid precursors (as a mean to obtain conformational order), has been often neglected[6], despite the fact that nature offers a vast

[5] (a) Matsuzaki, K. Biochim. Biophys. Acta 1999, 1462, 1; (b) Yang, L.; Weiss, T. M.; Lehrer, R. I.; Huang, H. W. Biophys. J. 2000, 79, 2002; (c) Shai, Y. Biochim.Biophys. Acta 1999, 1462, 55.

[6] Chongsiriwatana, N. P.; Patch, J. A.; Czyzewski, A. M.; Dohm, M. T.; Ivankin, A.; Gidalevitz, D.; Zuckermann, R. N.; Barron, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **2008,** 105, 2794.

assortment of powerful cyclic antimicrobial peptides[7]. However, macrocyclization of *N*-substituted glycines gives circular peptoids[8], showing reduced conformational freedom[9] and excellent membrane-permeabilizing activity[10].

The magainins exhibit highly selective and potent antimicrobial activity against a broad spectrum of organisms[5]. As these peptides are facially amphipathic, the magainins have a cationic helical face mostly composed of lysine residues, as well as hydrophobic aromatic (phenylalanine) and hydrophobic aliphatic (valine, leucine and isoleucine) helical faces. This structure is responsible for their activity[4].

Peptoids have been shown to form remarkably stable helices, with physical characteristics similar to those of peptide polyproline type-I helices. In fact a series of peptoid magainin mimics with this type of three-residue periodic sequences, has been synthesized[4] and tested against *E. coli* JM109 and *B. subtulis* BR151. In all cases, peptoids are individually more active against the Gram-positive species. The amount of hemolysis induced by these peptoids correlated well with their hydrophobicity. In summary, these recently obtain results demonstrate that certain amphipathic peptoid sequences are also capable of antibacterial activity.

1.2.2 Molecular Recognition

Peptoids are currently being studied for their potential to serve as pharmaceutical agents and as chemical tools to study complex biomolecular interactions. Peptoid–protein interactions were first demonstrated in a 1994 report by Zuckermann and co-workers,[8] where the authors examined the high-affinity binding of peptoid dimers and trimers to G-protein-coupled receptors. These groundbreaking studies have led to the identification of several peptoids with moderate to good affinity and, more importantly, excellent selectivity for protein targets that are implicated in a range

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[7] Interesting examples are: (a) Motiei, L.; Rahimipour, S.; Thayer, D. A.; Wong, C. H.; Ghadiri, M. R. *Chem. Commun*. **2009**, 3693; (b) Fletcher, J. T.; Finlay, J. A.; Callow, J. A.; Ghadiri, M. R. *Chem. Eur. J.* **2007**, 13, 4008; (c) Au, V. S.; Bremner, J. B.; Coates, J.; Keller, P. A.; Pyne, S. G. *Tetrahedron* **2006,** 62, 9373; (d) 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; (e) Casnati, A.; Fabbi, M.; Pellizzi, N.; Pochini, A.; Sansone, F.; Ungaro, R.; Di Modugno, E.; Tarzia, G. *Bioorg. Med. Chem. Lett*. **1996**, 6, 2699; (f) Robinson, J. A.; Shankaramma, C. S.; Jetter, P.; Kienzl, U.; Schwendener, R. A.; Vrijbloed, J. W.; Obrecht, D. *Bioorg. Med. Chem.* **2005**, 13, 2055.

[8] (a) Craik, D. J.; Cemazar, M.; Daly, N. L. Curr. Opin. Drug Discovery Dev. 2007, 10, 176; (b) Trabi, M.; Craik, D. J. Trend *Biochem. Sci.* **2002**, 27, 132.

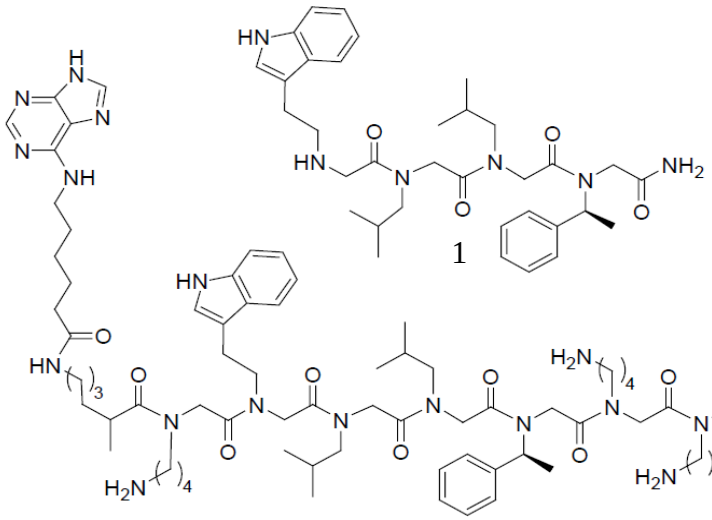
[9] (a) Maulucci, N.; Izzo, I.; Bifulco, G.; Aliberti, A.; De Cola, C.; Comegna, D.; Gaeta, C.; Napolitano, A.; Pizza, C.; Tedesco, C.; Flot, D.; De Riccardis, F. *Chem. Commun*. **2008**, 3927; (b) Kwon, Y.-U.; Kodadek, T. *Chem. Commun*. **2008**, 5704; (c) Vercillo, O. E.; Andrade, C. K. Z.; Wessjohann, L. A. *Org. Lett*. **2008**, 10, 205; (d) Vaz, B.; Brunsveld, L. *Org. Biomol. Chem.* **2008**, 6, 2988; (e) Wessjohann, L. A.; Andrade, C. K. Z.; Vercillo, O. E.; Rivera, D. G.. *In Targets in Heterocyclic Systems*; Attanasi, O. A., Spinelli, D., Eds.*; Italian Society of Chemistry*, **2007;** Vol. 10, pp 24–53; (f) Shin, S. B. Y.; Yoo, B.; Todaro, L. J.; Kirshenbaum, K. *J. Am. Chem. Soc*. **2007**, 129, 3218; (g) Hioki, H.; Kinami, H.; Yoshida, A.; Kojima, A.; Kodama, M.; Taraoka, S.; Ueda, K.; Katsu, T. *Tetrahedron Lett*. **2004**, 45, 1091.

[10] (a) Chatterjee, J.; Mierke, D.; Kessler, H. *Chem. Eur. J.* **2008,** 14, 1508; (b) Chatterjee, J.; Mierke, D.; Kessler, H. *J. Am. Chem. Soc*. **2006**, 128, 15164; (c) Nnanabu, E.; Burgess, K. *Org. Lett.* **2006**, 8, 1259; (d) Sutton, P. W.; Bradley, A.; Farràs, J.; Romea, P.; Urpì, F.; Vilarrasa, J. *Tetrahedron* **2000,** 56, 7947; (e) Sutton, P. W.; Bradley, A.; Elsegood, M. R.; Farràs, J.; Jackson, R. F. W.; Romea, P.; Urpì, F.; Vilarrasa, J. *Tetrahedron Lett.* **1999**, 40, 2629.

of human diseases. There are many different interactions between peptoid and protein, and these interactions can induce a certain inhibition, cellular uptake and delivery. Synthetic molecules capable of activating the expression of specific genes would be valuable for the study of biological phenomena and could be therapeutically useful.

In 2007, Kodadek and co-workers[11] identified the first chemical modulator of the proteasome 19S regulatory particle (which is part of the 26S proteasome, an approximately 2.5 MDa multi-catalytic protease complex responsible for most non-lysosomal protein degradation in eukaryotic cells). A "one bead one compound" peptoid library was constructed by split and pool synthesis. Each peptoid molecule was capped with a purine analogue in hope of biasing the library toward targeting one of the ATPases, which are part of the 19S regulatory particle. Approximately 100 000 beads were used in the screen and a purine-capped peptoid heptamer (**1**, Figure **1.1**) was identified as the first chemical modulator of the 19S regulatory particle. In an effort to evidence the pharmacophore of **1**[12] (by performing a "glycine scan", similar to the "alanine scan" in peptides) it was shown that just the core tetrapeptoid was necessary for the activity.

Interestingly, the synthesis of the shorter peptoid **1** gave, in the experiments made on cells, a 3- to 5-fold increase in activity relative to **2**. The higher activity in the cell-based essay was likely due to increased cellular uptake, as **1** does not contain charged residues.



1

2

Figure 1.1. Purine capped peptoid heptamer (**1**) and tetramer (**2**) reported by Kodadek preventing protein degradation

[11] H. S. Lim, C. T. Archer, T. Kodadek *J. Am. Chem. Soc*., **2007,** 129, 7750.
[12] H.S. Lim, C. T. Archer, Y. C. Kim, T. Hutchens, T. Kodadek *Chem. Commun*., **2008**, 1064.

1.4.3 Metal Complexing Peptoids

A desirable attribute for biomimetic peptoids is the ability to show binding towards receptor sites. This property can be evoked by proper backbone folding due to:

1) local side-chain stereoelectronic influences,

2) coordination with metallic species,

3) presence of hydrogen-bond donor/acceptor patterns.

Those three factors can strongly influence the peptoids'secondary structure, which is difficult to observe due to the lack of the intra-chain C=O···H–N bonds, present in the parent peptides.

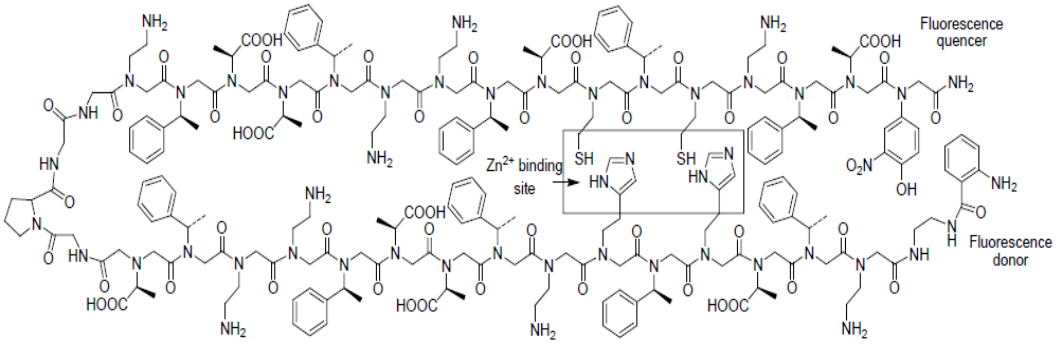
Most peptoids' activities derive by relatively unstructured oligomers. If we want to mimic the sophisticated functions of proteins, we need to be able to form defined peptoid tertiary structure folds and introduce functional side chains at defined locations.

Peptoid oligomers can be already folded into helical secondary structures. They can be readily generated by incorporating bulky chiral side chains into the oligomer[22,13-14]. Such helical secondary structures are extremely stable to chemical denaturants and temperature[13]. The unusual stability of the helical structure may be a consequence of the steric hindrance of backbone φ angle by the bulky chiral side chains[15].

Zuckermann and co-workers synthesized biomimetic peptoids with zinc-binding sites[8], since zinc-binding motifs in protein are well known. Zinc typically stabilizes native protein structures or acts as a cofactor for enzyme catalysis[16-17]. Zinc also binds to cellular cysteine-rich metallothioneins solely for storage and distribution[18]. The binding of zinc is typically mediated by cysteines and histidines[50-51]. In order to create a zinc-binding site, they incorporated thiol and imidazole side chains into a peptoid two-helix bundle.

Classic zinc-binding motifs, present in proteins and including thiol and imidazole moieties, were aligned in two helical peptoid sequences, in a way that they could form a binding site. Fluorescence resonance energy transfer (FRET) reporter groups were located at the edge of this biomimetic structure in order to measure the distance between the two helical segments and probe and, at the same time, the zinc binding propensity (**3**, Figure **1.2**).

[13] Wu, C. W.; Kirshenbaum, K.; Sanborn, T. J.; Patch, J. A.; Huang, K.; Dill, K. A.; Zuckermann, R. N.; Barron, A. E. *J. Am. Chem. Soc.* **2003**, *125*, 13525–13530.
[14] Armand, P.; Kirshenbaum, K.; Falicov, A.; Dunbrack, R. L., Jr.; Dill,K. A.; Zuckermann, R. N.; Cohen, F. E. *Folding Des.* **1997**, *2*, 369–375.
[15] K. Kirshenbaum, R. N. Zuckermann, K. A. Dill, *Curr. Opin. Struct. Biol.* **1999**, *9*, 530–535.
[16] Coleman, J. E. *Annu. Re*V. *Biochem.* **1992**, *61*, 897–946.
[17] Berg, J. M.; Godwin, H. A. *Annu. Re*V. *Biophys. Biomol. Struct.* **1997**, *26*, 357–371.
[18] Cousins, R. J.; Liuzzi, J. P.; Lichten, L. A. *J. Biol. Chem.* **2006**, *281*, 24085–24089.



3

Figure 1.2. Chemical structure of **3**, one of the twelve folded peptoids synthesized by Zuckermann, able to form a Zn^{2+} complex.

Folding of the two helix bundles was allowed by a Gly-Gly-Pro-Gly middle region. The study demonstrated that certain peptoids were selective zinc binders at nanomolar concentration.

The formation of the tertiary structure in these peptoids is governed by the docking of preorganized peptoid helices as shown in these studies[19].

A survey of the structurally diverse ionophores demonstrated that the cyclic arrangement represents a common archetype equally promoted by chemical design[22f] and evolutionary pressure**.** The conformational ordering of peptoids' achiral polyimide backbone is dictated by stereoelectronic effects caused by N- (and C-) substitution[22f] and/or by cyclization. In particular, the prediction and the assessment of the covalent constraints induced by macrolactamization appears crucial for the design of conformationally restricted peptoid templates as preorganized synthetic scaffolds or receptors.

In 2008 were reported the synthesis and the conformational features of cyclic tri-, tetra-, hexa-, octa and deca- *N*-benzyloxyethyl glycines (**4-8**, figure **1.3**)[21a].

[19] B. C. Lee, R. N. Zuckermann, K. A. Dill, *J. Am. Chem. Soc.* **2005**, *127*, 10999–11009.

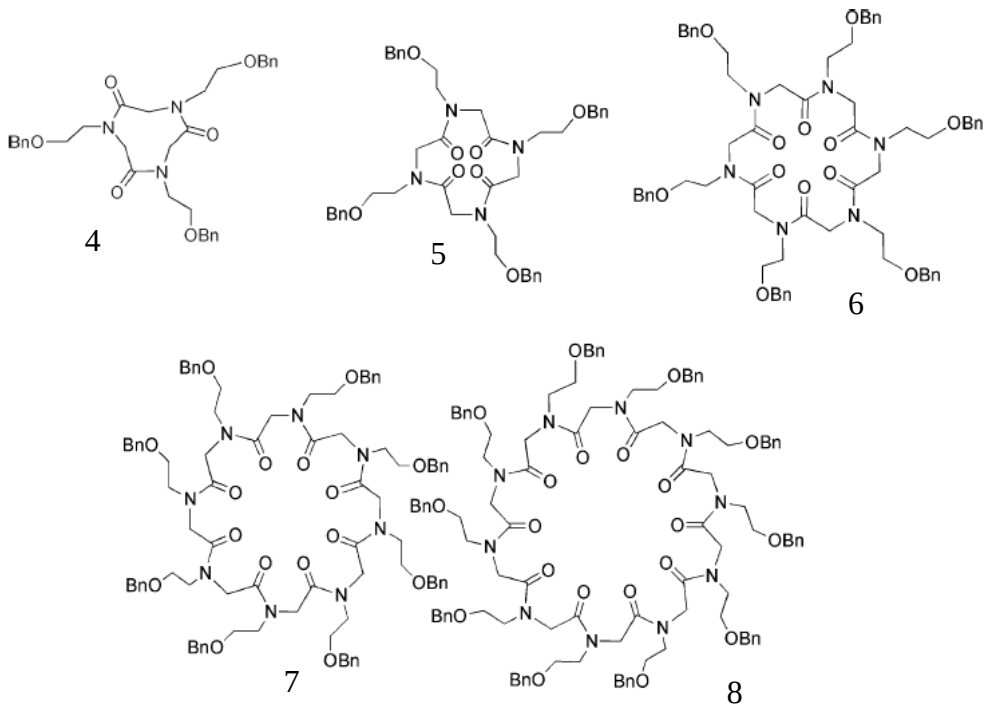


Figure 1.3. Structure of cyclic tri-, tetra-, hexa-, octa and deca- *N*-benzyloxyethyl glycines.

It was found, for the flexible eighteen-membered *N*-benzyloxyethyl cyclic peptoid **32**, high binding constants with the first group alkali metals (*K*a ~ 106 for Na^+, Li^+ and K^+), while, for the rigid *cis–trans–cis–trans* cyclic tetrapeptoid **5**, there was no evidence of alkali metals complexation. The conformational disorder in solution was seen as a propitious auspice for the complexation studies. In fact, the stepwise addition of sodium picrate to **6**, induced the formation of a new chemical species, whose concentration increased with the gradual addition of the guest. The conformational equilibrium between the free host and the sodium complex, resulted in being slower than the NMR-time scale, giving, with an excess of guest, a remarkably simplified ^{1}H NMR spectrum, reflecting the formation of a 6-fold symmetric species (Figure **1.4**).

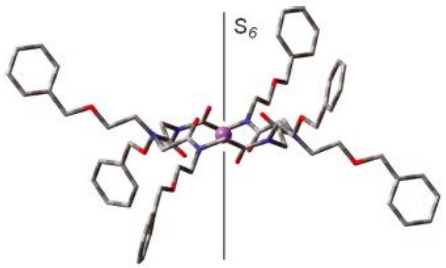


Figure 1.4. Picture of the predicted lowest energy conformation for the complex **32** with sodium.

A conformational search on **6** as a sodium complex suggested the presence of an S_6-symmetry axis passing through the intracavity sodium cation (Figure **1.4**). The electrostatic (ion–dipole) forces stabilize this conformation, hampering the ring inversion up to 425 K. The complexity of the r.t. ^{1}H

NMR spectrum recorded for the cyclic **7**, demonstrated the slow exchange of multiple conformations on the NMR time scale.

Stepwise addition of sodium picrate to **7**, induced the formation of a complex with a remarkably simplified ^{1}H NMR spectrum. With an excess of guest, we observed the formation of an 8-fold symmetric species (Figure **1.5**).

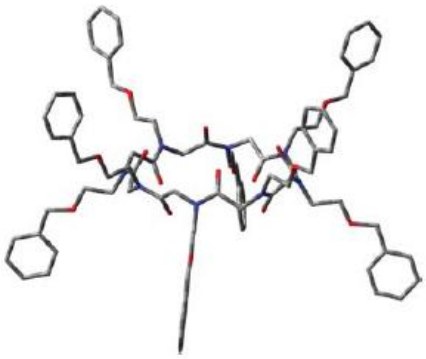


Figure 1.5. Picture of the predicted lowest energy conformations for **33** without sodium cations.

Differently from the twenty-four-membered **7**, the *N*-benzyloxyethyl cyclic homologue **8** did not yield any ordered conformation in the presence of cationic guests. The association constants (Ka) for the complexation of **6, 7** and **8** to the first group alkali metals and ammonium, were evaluated in H_2O–$CHCl_3$ following Cram's method (Table **1.1**) [20]. The results presented in Table **1.1** show a good degree of selectivity for the smaller cations.

host 32			
Picrate salt	R[a]	K_a [M^{-1}]	$-\Delta G^\circ$ [kcal/mol]
Li^+	0.17	0.95×10^6	8.1
Na^+	0.35	3.3×10^6	8.9
K^+	0.24	0.94×10^6	8.1
Rb^+	0.126	0.41×10^6	7.7
Cs^+	0.085	0.21×10^6	7.2
NH_4^+	0.18	0.38×10^6	7.6
host 33			
Picrate salt	R[a]	K_a [M^{-1}]	$-\Delta G^\circ$ [kcal/mol]
Li^+	0.14	0.66×10^6	7.9
Na^+	0.17	0.73×10^6	8.0
K^+	0.26	1.1×10^6	8.2
Rb^+	0.25	1.3×10^6	8.3
Cs^+	0.29	15×10^6	8.4
NH_4^+	0.25	0.65×10^6	7.9
host 34			
Picrate salt	R[a]	K_a [M^{-1}]	$-\Delta G^\circ$ [kcal/mol]
Li^+	0.13	0.58×10^6	7.9
Na^+	0.14	0.55×10^6	7.8
K^+	0.23	1.2×10^6	8.3
Rb^+	0.19	0.73×10^6	8.0
Cs^+	0.21	0.77×10^6	8.0
NH_4^+	0.18	0.39×10^6	7.6

[a] [Guest]/[host] in $CHCl_3$ layer at equilibrium.

Table 1.1 R, K_a, and ΔG° for cyclic peptoid hosts **6, 7** and **8** complexing picrate salt guests in $CHCl_3$ at 25 °C; figures within ±10% in multiple experiments, guest/host stoichiometry for extractions was assumed as 1:1.

[20] K. E. Koenig, G. M. Lein, P. Stuckler, T. Kaneda and D. J. Cram, *J. Am. Chem. Soc.*, **1979**, 101, 3553.

The ability of cyclic peptoids to extract cations from bulk water to an organic phase prompted us to verify their transport properties across a phospholipid membrane.

The two processes were clearly correlated although the latter is more complex implying, after complexation and diffusion across the membrane, a decomplexation step.[21-22] In the presence of NaCl as added salt, only compound **6** showed ionophoric activity while the other cyclopeptoids are almost inactive.

Cyclic peptoids have different cation binding preferences and, consequently, they may exert selective cation transport. These results are the first indication that cyclic peptoids can represent new motifs on which to base artificial ionophoric antibiotics.

1.2.5 Catalytic Peptoids

An interesting example of the imaginative use of reactive heterocycles in the peptoid field, can be found in the "foldamers" mimics. "Foldamers" mimics are synthetic oligomers displaying conformational ordering. Peptoids have never been explored as platform for asymmetric catalysis. Kirshenbaum reported the synthesis of a library of helical "peptoid" oligomers enabling the oxidative kinetic resolution (OKR) of 1-phenylethanol induced by the catalyst TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) (figure **1.14**)[23].

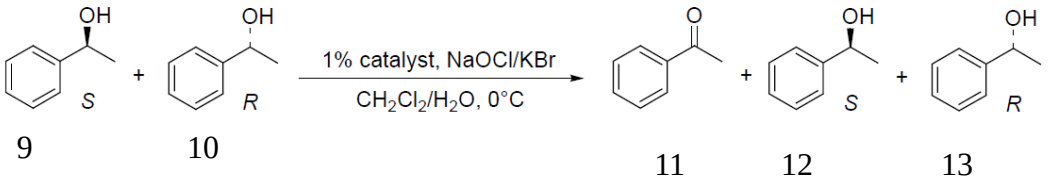


Figure 1.6 Oxidative kinetic resolution of enantiomeric phenylethanols **12** and **13.**

The TEMPO residue was covalently integrated in properly designed chiral peptoid backbones which were used as asymmetric components in the oxidative resolution.

The study demonstrated that the enantioselectivity of the catalytic peptoids (built using the chiral (*S*)- and (*R*)-phenylethyl amines) depended on three factors: 1) the handedness of the asymmetric environment derived from the helical scaffold, 2) the position of the catalytic centre along the peptoid backbone, and 3) the degree of conformational ordering of the peptoid scaffold.

[21] R. Ditchfield, *J. Chem. Phys.*, **1972**, 56, 5688.
[22] K. Wolinski, J. F. Hinton and P. Pulay, *J. Am. Chem. Soc*., **1990**, 112, 8251.
[23] G. Maayan, M. D. Ward, and K. Kirshenbaum, *Proc. Natl. Acad. Sci. USA*, **2009**, 106, 13679.

1.4.6 PNA and Peptoids Tagged With Nucleobases.

Nature has selected nucleic acids for storage (DNA primarily) and transfer of genetic information (RNA) in living cells, whereas proteins fulfill the role of carrying out the instructions stored in the genes in the form of enzymes in metabolism and structural scaffolds of the cells. However, no examples of protein as carriers of genetic information have yet been identified.

Self-recognition by nucleic acids is a fundamental process of life. Although, in nature, proteins are not carriers of genetic information, pseudopeptides bearing nucleobases, denominate "peptide nucleic acids" (PNA, **14**, figure **1.7**),[4] can mimic the biological functions of DNA and RNA (**15** and **16**, figure **1.7**).

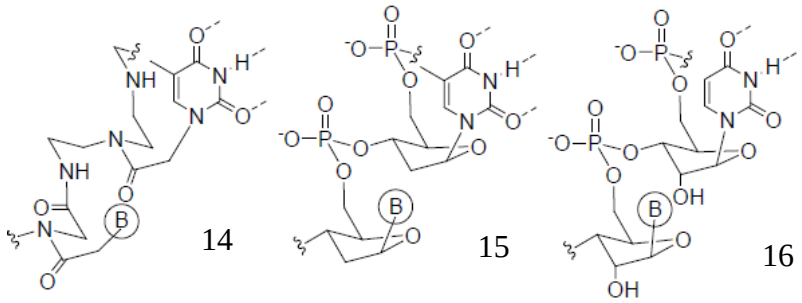


Figure 1.7. Chemical structure of PNA (**14**), DNA (**15**), RNA (**16**). B = nucleobase

The development of the aminoethylglycine polyamide (peptide) backbone oligomer with pendant nucleobases linked to the glycine nitrogen *via* an acetyl bridge now often referred to PNA, was inspired by triple helix targeting of duplex DNA in an effort to combine the recognition power of nucleobases with the versatility and chemical flexibility of peptide chemistry[4]. PNAs were extremely good structural mimics of nucleic acids with a range of interesting properties:

- ✓ DNA recognition,
- ✓ Drug discovery:
    1. RNA targeting
    2. DNA targeting
    3. Protein targeting
    4. Cellular delivery
    5. Pharmacology
- ✓ Nucleic acid detection and analysis,
- ✓ Nanotechnology,
- ✓ Pre-RNA world.

The very simple PNA platform has inspired many chemists to explore analogs and derivatives in order to understand and/or improve the properties of this class DNA mimics. As the PNA backbone

is more flexible (has more degrees of freedom) than the phosphodiester ribose backbone, one could hope that adequate restriction of flexibility would yield higher affinity PNA derivates.

The success of PNAs made it clear that oligonucleotide analogues could be obtained with drastic changes from the natural model, provided that some important structural features were preserved.

The PNA scaffold has served as a model for the design of new compounds able to perform DNA recognition. One important aspect of this type of research is that the design of new molecules and the study of their performances are strictly interconnected, inducing organic chemists to collaborate with biologists, physicians and biophysicists.

An interesting property of PNAs, which is useful in biological applications, is their stability to both nucleases and peptidases, since the "unnatural" skeleton prevents recognition by natural enzymes, making them more persistent in biological fluids.[24] The PNA backbone, which is composed by repeating *N*-(2 aminoethyl)glycine units, is constituted by six atoms for each repeating unit and by a two atom spacer between the backbone and the nucleobase, similarly to the natural DNA. However, the PNA skeleton is neutral, allowing the binding to complementary polyanionic DNA to occur without repulsive electrostatic interactions, which are present in the DNA:DNA duplex. As a result, the thermal stability of the PNA:DNA duplexes (measured by their melting temperature) is higher than that of the natural DNA:DNA double helix of the same length.

However, despite the excellent attributes, PNA has two serious limitations: low water solubility[25] and poor cellular uptake[26].

Many modifications of the basic PNA structure have been proposed in order to improve their performances in term of affinity and specificity towards complementary oligonucleotide sequences. A modification introduced in the PNA structure can improve its properties generally in three different ways:

i) improving DNA binding affinity;

ii) improving sequence specificity, in particular for directional preference (antiparallel vs parallel) and mismatch recognition;

iii) improving bioavailability (cell internalization, pharmacokinetics, etc.).

Structure activity relationships showed that the original design containing a 6-atom repeating unit and a 2-atom spacer between backbone and the nucleobase was optimal for DNA recognition. Introduction of different functional groups with different charges/polarity/flexibility have been

[24] Demidov V.A., Potaman V.N., Frank-Kamenetskii M. D., Egholm M., Buchardt O., Sonnichsen S. H., Nielsen P.E., *Biochem. Pharmscol.* **1994**, *48*, 1310.

[25] (a) U. Koppelhus and P. E. Nielsen, *Adv. Drug. Delivery Rev*., **2003**, *55*, 267; (b) P. Wittung, J. Kajanus, K. Edwards, P. E. Nielsen, B. Nordén, and B. G. Malmstrom, FEBS Lett., **1995**, 365, 27.

[26] (a) E. A. Englund, D. H. Appella, Angew. Chem. Int. Ed., **2007**, *46*, 1414; (b) A. Dragulescu-Andrasi, S. Rapireddy, G. He, B. Bhattacharya, J. J. Hyldig-Nielsen, G. Zon, and D. H. Ly, *J. Am. Chem. Soc*., **2006**, *128*, 16104; (c) P. E. Nielsen, Q. Rev. Biophys., **2006**, 39, 1; (d) A. Abibi, E. Protozanova, V. V. Demidov, and M. D. Frank-Kamenetskii, Biophys. J., **2004**, *86*, 3070.

described and are extensively reviewed in several papers[27,28,29]. These studies showed that a "constrained flexibility" was necessary to have good DNA binding.

1.7 Aims of the work

The objective of this research, is to gain new insights in the use of peptoids as tools for structural studies and biological applications. Five are the themes developed in the present thesis:

1. **Carboxyalkyl Peptoid PNAs**. N^{γ}-carboxyalkyl modified peptide nucleic acids (PNAs), containing the four canonical nucleobases, were prepared *via* solid-phase oligomerization. The inserted modified peptoid monomers (figures **1.8**: **17** and **18**) were constructed through simple synthetic procedures, utilizing proper glycidol and iodoalkyl electrophiles.

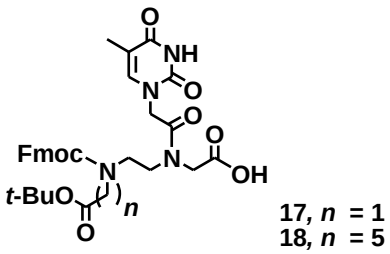


Figure 1.8 Modified peptoid monomers

Synthesis of PNA oligomers was realized by inserting modified peptoid monomers into a canonical PNA, by this way four different modified PNA oligomers were obtained (figure **1.9**).

GTAGAT*$_{50}$CACT–Gly–NH$_2$,	**19**
G T*$_{50}$AGAT*$_{50}$CAC T*$_{50}$–Gly–NH$_2$,	**20**
GTAGAT*$_{51}$CACT–Gly–NH$_2$,	**21**
G T*$_{51}$AGAT*$_{51}$CAC T*$_{51}$–Gly–NH$_2$,	**22**

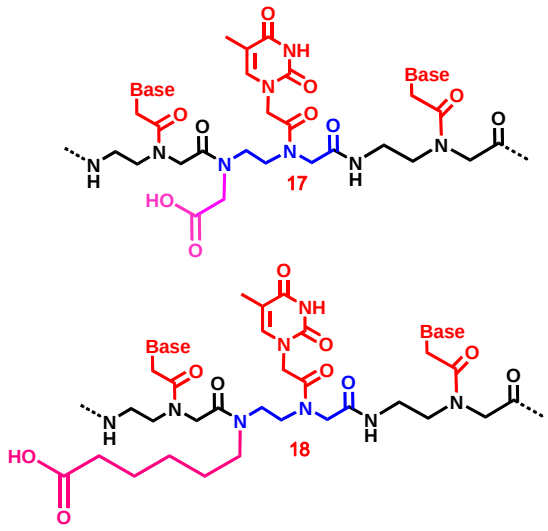


Figure 1.9. Modified PNA.

[27] a) Kumar, V. A., *Eur. J. Org. Chem.*, **2002**, 2021-2032. b) Corradini R.; Sforza S.; Tedeschi T.; Marchelli R.; *Seminar in Organic Synthesis*, Società Chimica Italiana, **2003**, 41-70.
[28] Sforza, S.; Haaima, G.; Marchelli, R.; Nielsen, P.E.. *Eur. J. Org. Chem*. **1999**, 197-204.
[29] Sforza, S.; Galaverna, G.; Dossena, A.; Corradini, R.; Marchelli, R. *Chirality*, **2002**, *14*, 591-598.

Thermal denaturation studies performed, in collaboration with Prof. R. Corradini from the University of Parma, with complementary antiparallel DNA strands, demonstrated that the length of the N^{γ}-side chain strongly influences the modified PNAs hybridization properties. Moreover, multiple negative charges on the oligoamide backbone, when present on γ-nitrogen C_6 side chains, proved to be beneficial for the oligomers water solubility and DNA hybridization specificity.

2. **Structural analysis of cyclopeptoids and their complexes**. The aim of this work was the studies of structural properties of cyclopeptoids in their free and complexed form (figure **1.10**: **23, 24** and **25**).

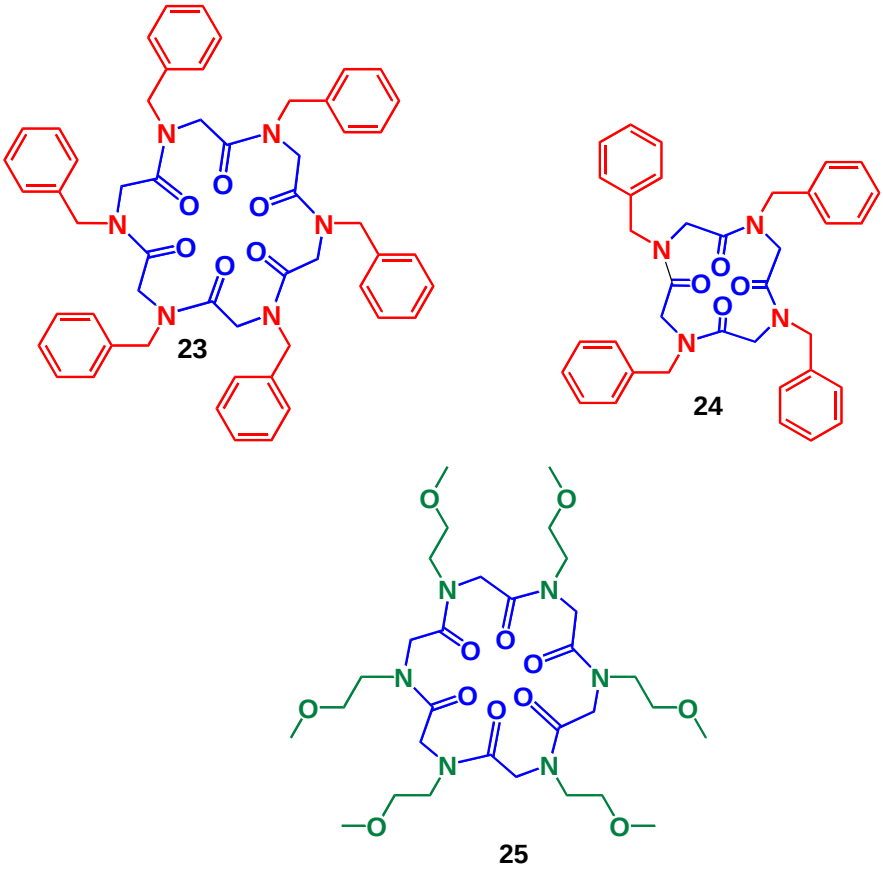


Figure 1.10. *N*-Benzyl-cyclohexapeptoid **23**, *N*-benzyl-cyclotetrapeptoid **24** and *N*-metoxyethyl-cyclohexapeptoid **25**.

3. **Cationic cyclopeptoids as potential macrocyclic nonviral vectors.** The aim of this work was the synthesis of three different cationic cyclopeptoids (figure **1.11**: **26, 27** and **28**) to assess their efficiency in DNA cell transfection, in collaboration with Prof. G. Donofrio of the University of Parma.

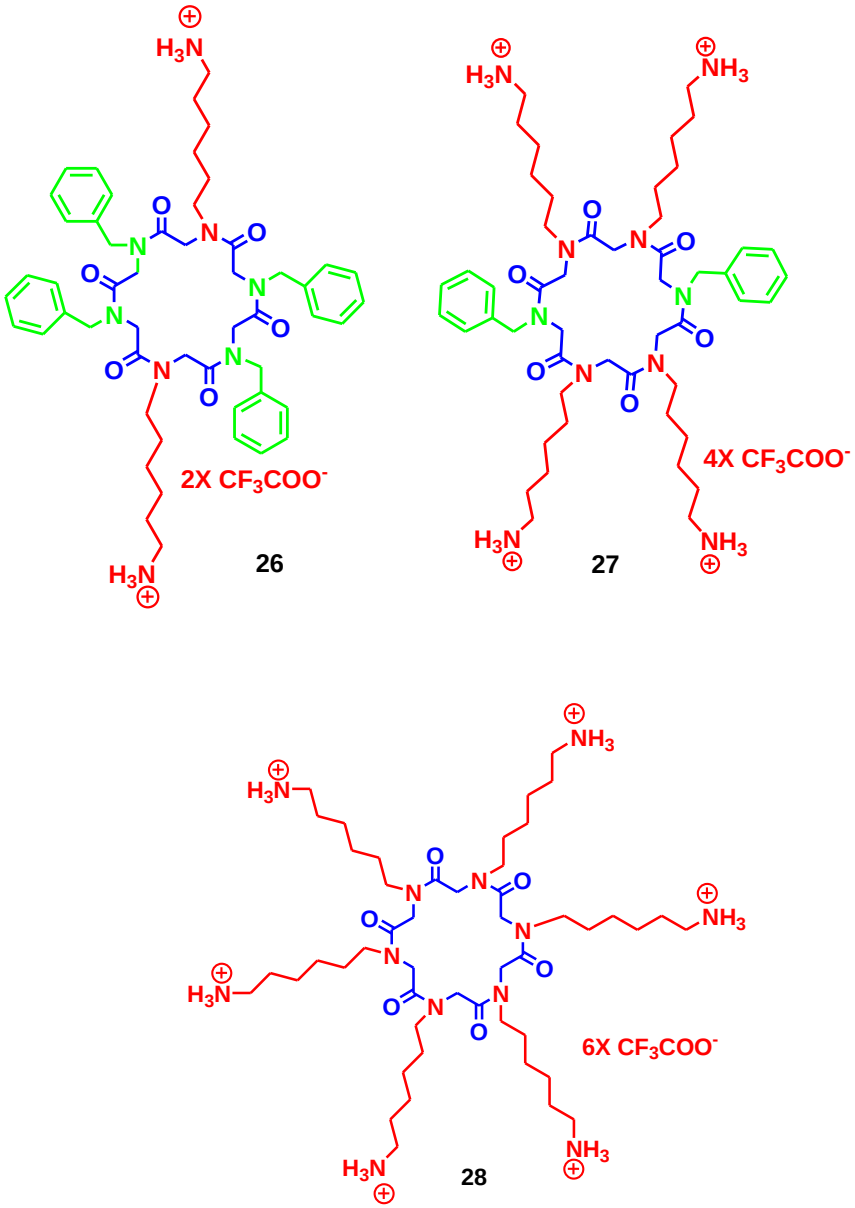


Figure 1.11. Di-cationic cyclohexapeptoid **26,** Tetra-cationic cyclohexapeptoid **27**, Hexa-cationic cyclohexapeptoid **28.**

4. **Complexation with Gd^{3+} of carboxyethyl cyclopeptoids as possible contrast agents in MRI.** Three cyclopeptoids **29**, **30** and **31** (figure **1.12**) containing polar side chains, were synthesized and, in collaboration with Prof. S. Aime, of the University of Torino, the complexation properties with Gd^{3+} were evaluated.

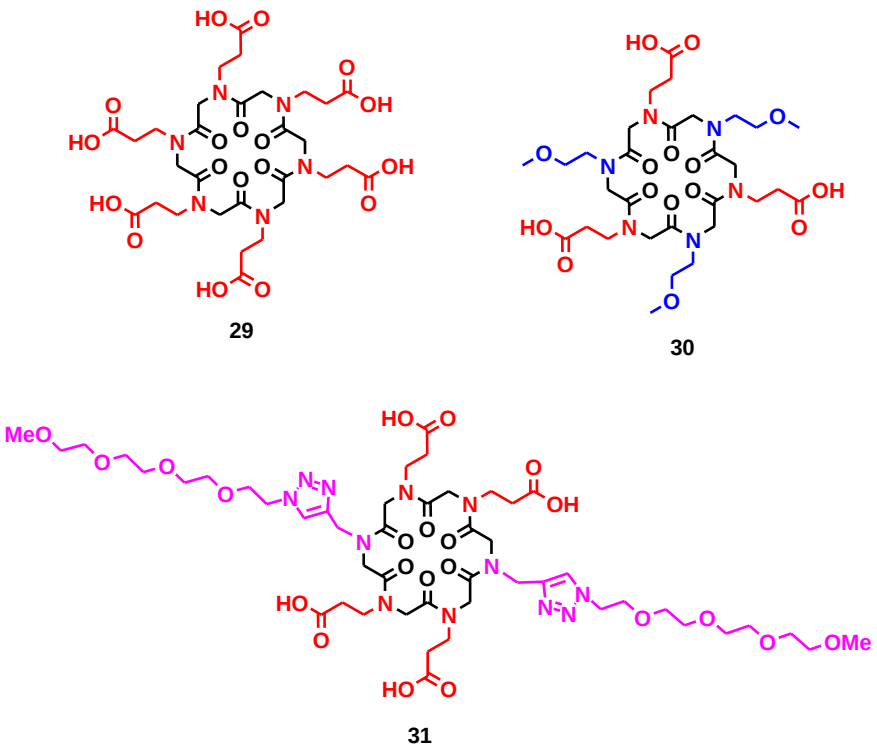


Figure 1.12. Hexacarboxyethyl cyclohexapeptiod **29**, Tricarboxyethyl ciclohexapeprtoid **30** and tetracarboxyethyl cyclopeptoids **31**.

5. **Cyclopeptoids as mimetic of natural defensins[30].** In this work some linear and cyclopeptoids with specific side chains (-SH groups) were synthetized. The aim was to introduce, by means of sulfur bridges, peptoid backbone constrictions and to mimic natural defensins (figure **1.13,** block **I: 32** hexa-linear and related cycles **33** and **34**; block **II: 35** octa-linear and related cycles **36** and **37**; block **III: 38** dodeca-linear and related cycles **39**, **40** and **41**; block **IV: 42** dodeca-linear diprolinate and related cycles **43**, **44** and **45**).

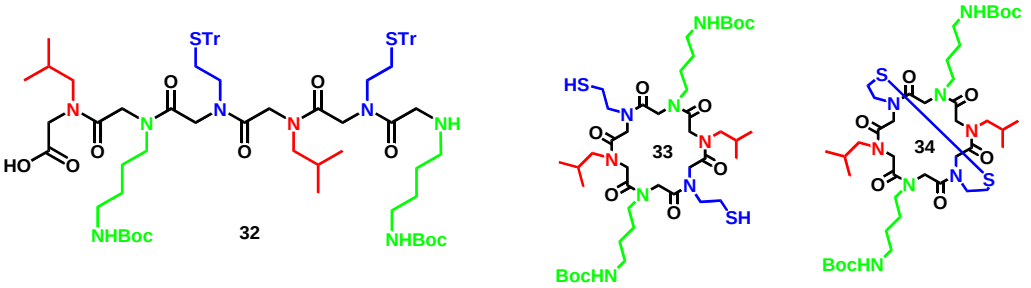


Figure 1.13, block I. Structures of the hexameric linear (**32**) and corresponding cyclic **33** and **34.**

[30] a) W. Wang, S.M. Owen, D. L. Rudolph, A. M. Cole, T. Hong, A. J. Waring, R. B. Lal, and R. I. Lehrer *The Journal of Immunology*, **2010**, 515-520; b) D. Yang, A. Biragyn, D. M. Hoover, J. Lubkowski, J. J. Oppenheim *Annu. Rev. Immunol*. **2004**, 181-215.

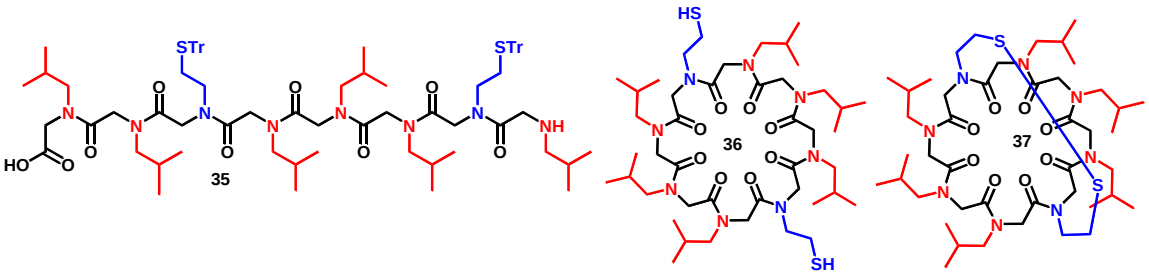


Figure 1.13, block II. Structures of octameric linear (**35**) and corresponding cyclic **36** and **37**

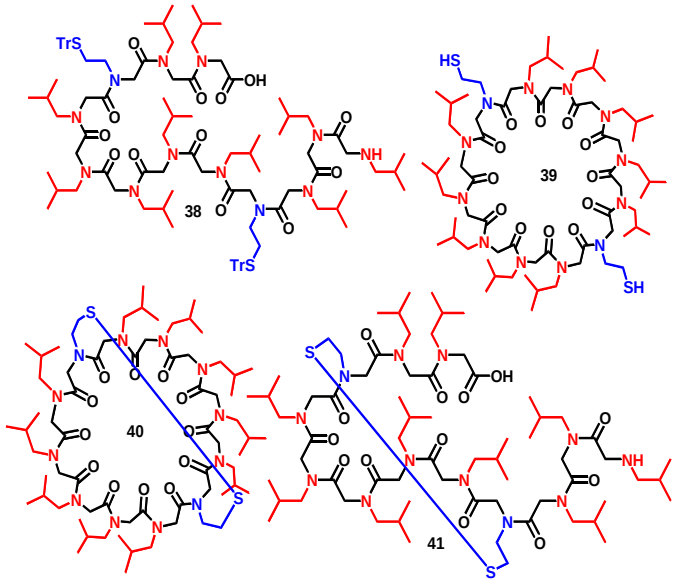


Figure 1.13, block III. Structures of linear (**38**) and corresponding cyclic **39, 40** and **41**

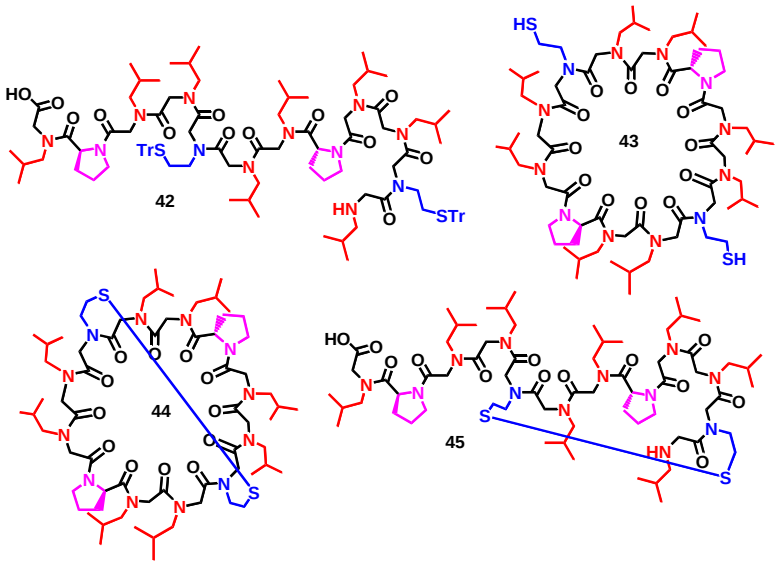


Figure 1.13, block IV. Structures of dodecameric linear diprolinate (**42**) and corresponding cyclic **43, 44** and **45.**