Imine-derived macrocyclic peptides

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The potential of peptides as therapeutics is corroborated by more than 60 marketed derivatives, an increasing number of peptides under clinical trials evaluation (>150, 25 phase III, 5 regulatory) and still a bigger number under preclinical studies.[1,2] The evolution in the field has moved from the initial research on endogenous human peptides, to peptides from other sources (i.e marine products), and to totally synthetic cyclic and macrocyclic derivatives.
Here we will distinguish between cyclic peptides,[3] which used amino acid functional groups to allow the cyclization (head-to-tail, side-chain-to-side-chain, etc), and macrocyclic peptides in which the macrocyclization takes place through a non-peptide linker, joining two (or more) functional groups of the peptide. The growing attention to this latter type of compounds is highlighted by the interest of many pharmaceutical and biotech companies in recent years and by the increasing number of publications on this topic.[4-6] Cyclic and macrocyclic peptides represent an equidistant situation between small-molecule drugs and biotherapeutics or biologic drugs (vaccines, antibodies, gene therapies).[4,5] They are synthetically accessible, as small-molecules, and therefore suitable for medicinal chemistry optimization programs directed to fine-tune solubility, potency, selectivity and metabolic stability. However, their intermediate size could allow the modulation of therapeutically relevant protein-protein interactions (PPIs), more difficult to achieve with small-molecule drugs. Since PPIs are crucial in many biological processes and contribute to numerous pathological conditions, such as cancer, pathogen-host PPI networks facilitating infections, diabetes or neurodegenerative diseases, among others, cyclic and macrocyclic peptides could represent invaluable chemical tools on the road to PPI modulation and to treat unmet medical needs.[5]

Though there are many different ways of building macrocyclizations,[4,5] in this short compendium, I will comment on a few recent publications about imine-based macrocyclic peptide derivatives, to illustrate the usefulness of the imine moiety as a unique source of molecular diversification within this type of peptide derivatives (Figure 1).

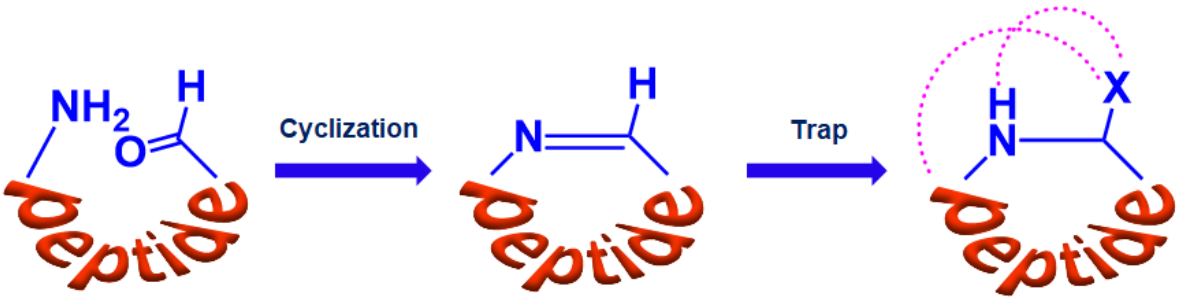

Figure 1. Schematic representation of imine-based macrocyclic peptides

The first attempt to the isosteric replacement of a native amide ligation group by an imine-like moiety in a cyclopeptide was described by Geyer's group.[7] They explored the thermodynamic macrolactamization in aqueous solution of a Tyrocidine A (TycA) linear derivative through the N-terminal Phe residue and a C-terminal leucinal to the corresponding imine bond. Surprisingly, the stereoselective formation of the intermediate hemiaminal Ψ[CH(OH)NH]–TycAs derivative was observed, and its unexpected stability was attributed to a hydrogen bonding network with other groups of the macrocyclic peptide.

A similar exploration of imino macrocyclization in aqueous media to non-ribosomal peptide natural products was described by the group of Phil Baran (>40 macrocyclic peptides).[8] Since the initially prepared linear peptide aldehydes showed a variable propensity to autocyclize, they force a thermodynamic macrocyclization through imine trapping. Thus, the use of a Strecker protocol with KCN afforded α-aminonitriles, susceptible of further transformation to α–aminoamides, while reductive amination procedures ($NaBH_3CN$) give rise to secondary amines, which can be additionally modified though acylation or alkylation. Further diversification at the imine bond can be achieved through intramolecular trapping using suitable N-terminal amino acids. Consequently, tetrahydro-β-carbolines and 4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridines can be obtained under Picted-Spengler conditions with N-terminal Trp and His residues, respectively, while other nucleophilic side-chain, like those in Cys and Thr provided thiazolidine and oxazolidine heterocyclic rings. Some of these saturated heterocycles can be further oxidized to the corresponding aromatic systems (i.e., β-carboline, thiazol). These imine/trapping procedures were applicable to a wide range of linear peptides, including functionalized side-chain residues (Arg, Asp, Gln, Cys(StBu), Lys, Tyr), and diverse cyclic compounds containing from

5 to 10 amino acid residues (15-32 atoms) were prepared. Additionally, the cyclization can be modulated through the incorporation of N-Me and non-proteinogenic D-residues, but conformational studies by NMR and CD suggest that the efficacy of the intramolecular imine formation is essentially independent on the innate preorganization of linear substrates. Finally, the described protocols were applied to the synthesis of some naturally occurring macrocyclic peptides (koranimine, lugdunin, sanguinamide A and scytonemide A) and related analogues, proving their versatility and generality.

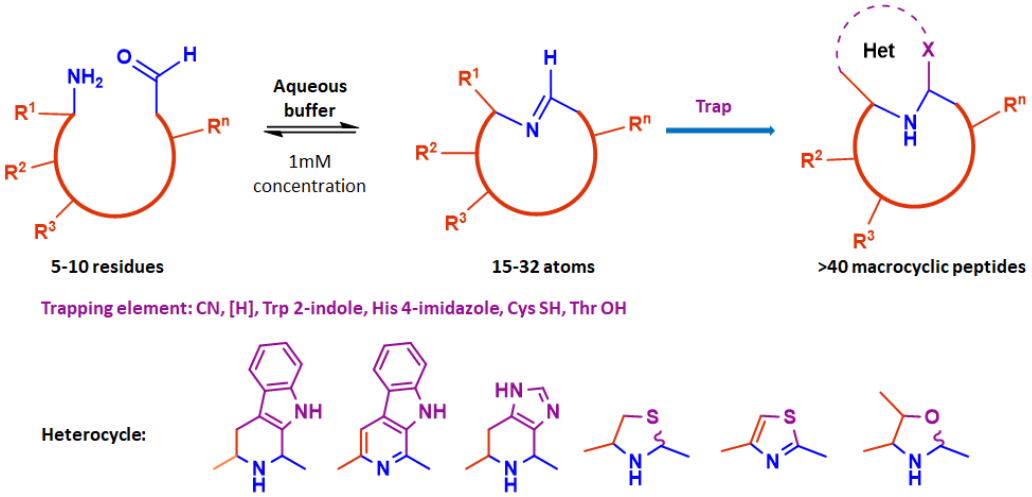

Figure 2. Imino macrocyclization approach to non-ribosomal peptide natural products

In the same line, Raj's group developed what they call "CyClick" strategy for the macrocyclization of peptides.[9] This approach consists in the reaction of the N-terminal amino group (residue i) with a C-terminal aldehyde/ketone (residue n), and ulterior intramolecular imine trapping by the amide NH of i+1 residue, to afford stable, chiral 4-imidazolinone-containing macrocycles (Figure 3). The reaction proceeds smoothly in the presence of dimethylaminopiridine (DMAP), as a macrocyclization facilitator, in mixtures of H_2O and DMF(1:1) at room temperature, does not require high-dilution conditions (no formation of dimers or oligomers), and no significant epimerization is observed. Different 4-imidazolinone-derived macrocyclic peptides have been prepared, with diversity in size (from 12 to 23 atoms in the macrocycle), and virtually all amino acid residues, including amino functionalized side-chains (Lys). When Pro is at the i+1 position, the corresponding fused bicyclic 1H-pyrrolo[1,2-c]imidazole-1-one macrocyclic peptides were obtained. NMR studies revealed a turn-like conformation around the heterocyclic five-membered ring. 4-Imidazolinone-containing macrocycles are stable over a range of pHs, and more protected toward proteolysis compared to the corresponding linear counterparts, thus constituting attractive compounds for interrogating biological systems.

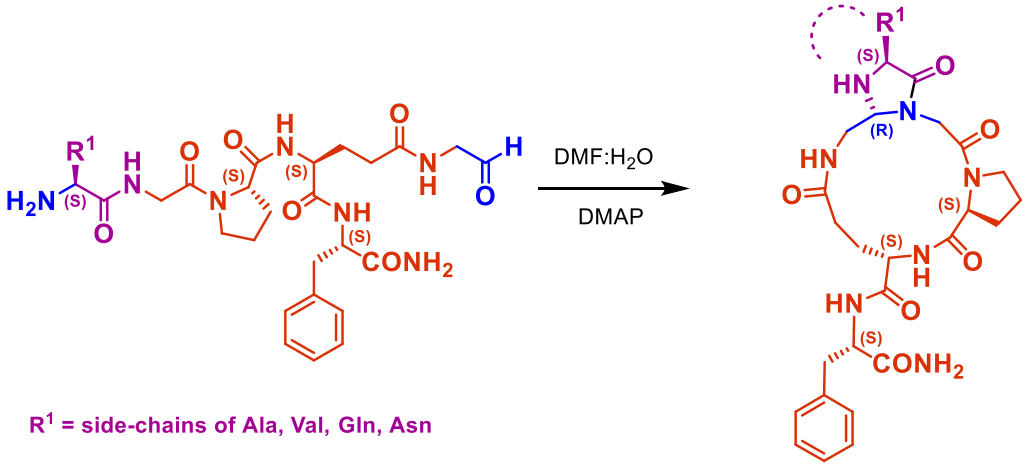

Figure 3. Representative examples of 4-imidazolinone-containing macrocycles (**CyClick**)

In a recent work, the group of Herbert Waldmann describes the PepNats strategy, consisting in the combination of hot loop-derived peptides with natural product-inspired structures leading to hybrid derivatives (Figure 4).[10] Again, the imine macrocyclization was used as the key synthetic intermediate, to be trapped in this case through 1,3-dipolar cycloadditions. The whole process takes place on solid-phase, enabling the stereoselective preparation of a library of diverse peptide macrocycles (>60 components). As a common element, all the initial linear peptides incorporate a Lys(Mtt) residue at n-1 position, which upon orthogonal deprotection, reacted with N^{ε}-4-formylbenzoic acid, to provide the aldehyde function

for the Schiff base formation with the N-terminal Gly α–NH_2 group (TEA, trimethyl orthoformate, DMF). The imine function was then entrapped by azomethine ylide generation (LiBr, TEA) and reaction with different dipolarophiles (maleimide, 3-benzylidenindolin-2-one and 5-benzylidenpirimidinetrione derivatives) to the corresponding cycloadducts (pyrrolidine, di-pyrrolidine, pyrrolidinyl-spirobarbiturate, pyrrolidinyl-spirooxindole).

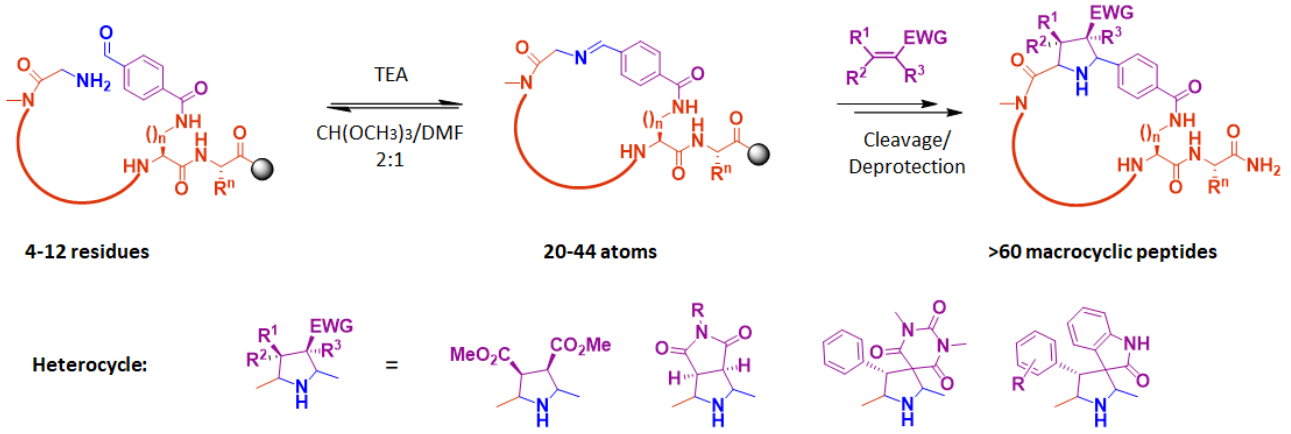

Figure 4. Schematic chart of **Pep-Nat** approach to macrocyclic peptides

Final PepNats, ranging from 4 to 12 diverse amino acid residues within the macrocycle (20-44 atoms), were obtained in reasonable yields after cleavage from the resin, concomitant side-chains deprotection, and semipreparative HPLC purification. NMR studies with selected macrocyclic peptides indicated that the solution conformational preferences of PepNats are dictated both by the configuration and the structure **of** the created heterocycle, factors also influencing their activities as PPI modulators. Accordingly, for the iNOS-SPSB2 protein–protein interaction, major (2*R*,3*S*,4*R*,5*S*) diastereomers are preferred over minor (2*S*,3*R*,4*S*,5*R*) isomers, and the di-pyrrolidine (R = Me, Ph) and the pyrrolidinyl-spirobarbiturate NP-inspired scaffolds led to low nanomolar inhibitors. Similarly, major diasteroisomers displayed higher binding affinity toward melanocortin receptors than minor equivalents, with the 3,3′-pyrrolidinyl-spirooxindole as the best NP-inspired moiety. The extraordinary results obtained with the library of PepNats opens the opportunity to apply the combination of NP-inspired scaffolds with new peptide hot loops in the search of other PPI modulators of biological relevance.

The success of the methodologies described here would inspire and stimulate future related processes for imine-derived macrocyclization, given that many chemical transformations of the imine functional group remain to be explored in this context.

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