A Unified Approach to Peptin Antibiotics

Marco A. Ciufolini* [a,b], Delphine Valognes [a] and Ning Xi [b]

[a] Université Claude Bernard Lyon 1 and Ecole Supérieure de Chimie, Physique, Electronique de Lyon (CPE Lyon) 43, Bd. du 11 Novembre 1918, 69622 Villeurbanne cedex, France
[b] Department of Chemistry, Rice University, 6100 Main Street, Houston, TX 77005, USA

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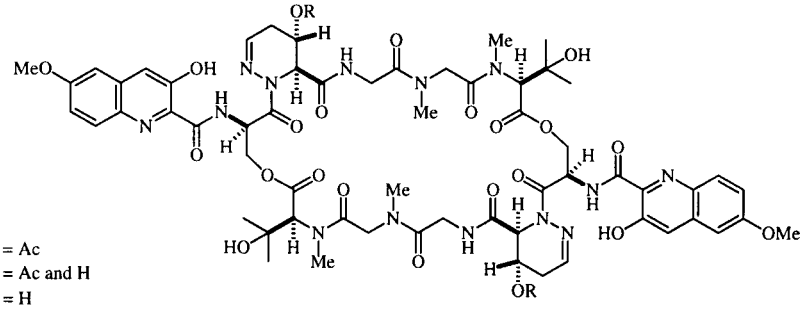
Luzopeptins (Figure 1) [1] and quinoxapeptins (Figure 2) [2] are strong inhibitors of reverse transcriptase, and as such they are of potential interest as lead compounds for the development of new anti-HIV agents [3]. These natural products constitute what we describe as the "peptin" family of antibiotics. Their architecture consists of a dimeric macrocyclic decadepsipeptide core containing two highly unusual subunits, piperazic acid (PIZ) and L-*N*-methyl-3-hydroxyvaline (MHV), in addition to common amino acids such as glycine, sarcosine, and D-serine. This macrocyclic structure is largely invariant across the peptin family except for the oxidation state of the piperazic acid unit. The hydroxy group of D-serine engages the MHVcarboxyl to form the depsi linkage, whereas the serine α-amino group is acylated with a quinaldic acid (luzopeptins) or quinoxaline-2-carboxylic acid (quinoxapeptins) residue.

Boger and collaborators have recently announced the first syntheses ever of luzopeptins A-C and of quinoxapeptins [4]. Our own interest in developing a unified approach to these molecules has now resulted in the total synthesis of luzopeptins E2 (Figure 3) [5]. A summary of this work is provided herein.

As shown in Figure 4, a generic peptin is envisioned to result through acylation of the amino groups of the L-serine subunits with a suitable quinaldic acid or quinoxaline 2-carboxylic acid and adjustment of the oxidation state of the piperazic acid components. The resulting retrosynthetic intermediate (Figure 5) may be created either by macrolactonization or by macrolactamization. Either approach requires two segments: a dipeptide of piperazic acid and L-serine and a tripeptide consisting of L-*N*-methyl-3-hydroxyvaline, sarcosine, and glycine. The latter tripeptide is present in all peptins and it is thus referred to as the "universal" tripeptide.

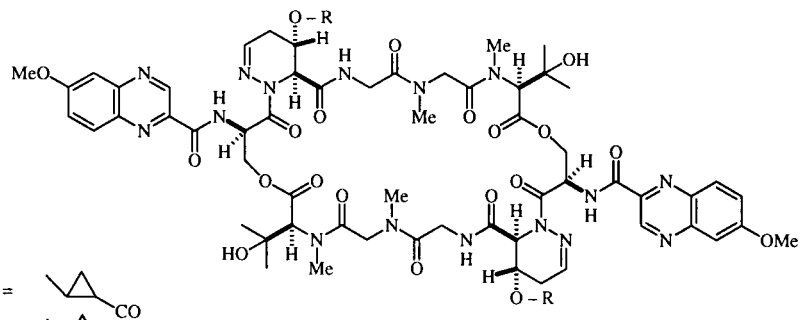
Luzopeptins **A-C**



A: R = Ac
B: R = Ac and H
C: R = H

Figure 1.

Quinoxapeptins **A-B**



A: R =
B: R = and Ac

Figure 2.

Luzopeptin E2

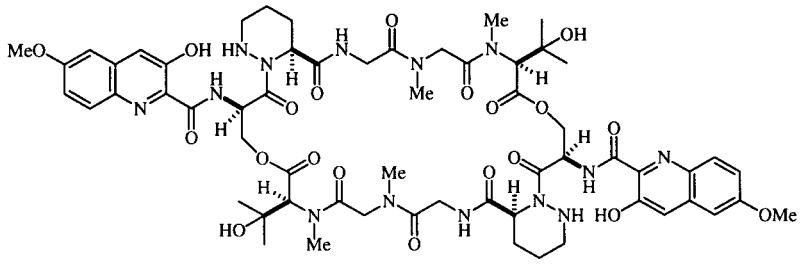


Figure 3.

Retrosynthetic Considerations

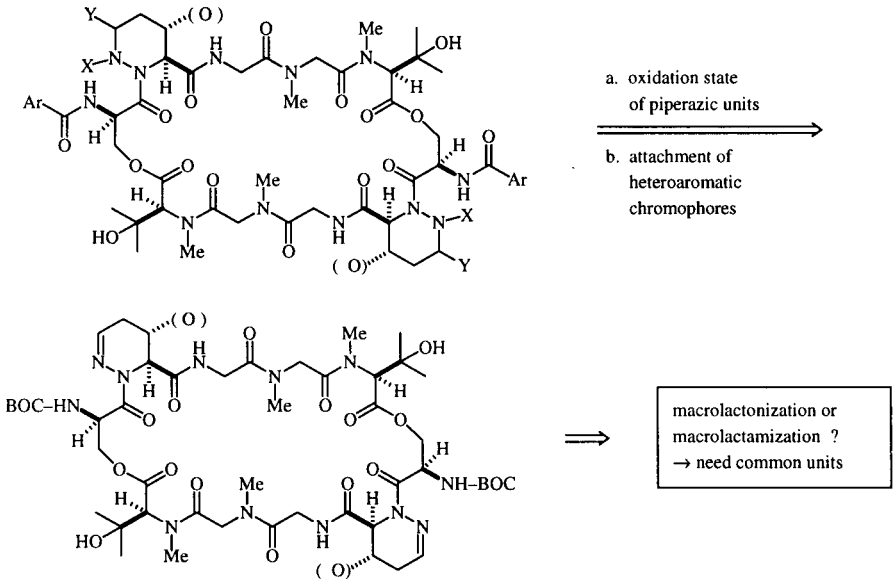


Figure 4.

Required Segments

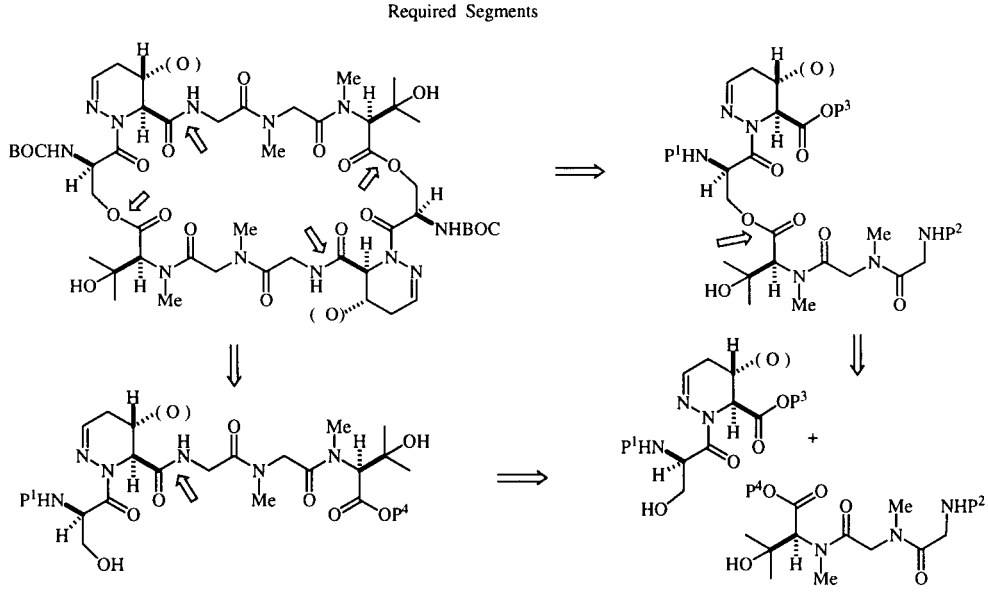


Figure 5.

Esters of L-*N*-methyl-3-hydroxyvaline and of its peptides are sensitive to basic agents, which may cause elimination of the β-hydroxy group and/or retroaldol loss of acetone (Figure 6). Therefore, the universal tripeptide must be prepared as an ester that should preferably be cleavable under neutral conditions. An allyl ester satisfied this requirement. Additionally, various *N*-acyl derivatives of the sarcosine-glycine dipeptide that would serve to acylate the amino unit of MHV displayed mediocre solubility in common organic solvents, complicating coupling operations.

The search for an *N*-blocking group that would alleviate these problems led us to identify the azidoacetyl unit as a convenient analog of glycine. The azido group presents the additional advantage that it may be reduced to an amine also under neutral conditions. The universal tripeptide is thus best manufactured with C-terminus as an allyl ester and the N-terminus as an azide. Details of the synthesis are given in Figures 7 and 8, while Figure 9 demonstrates the technique utilized for selective deblocking of the C- or N-terminus of the peptide [6].

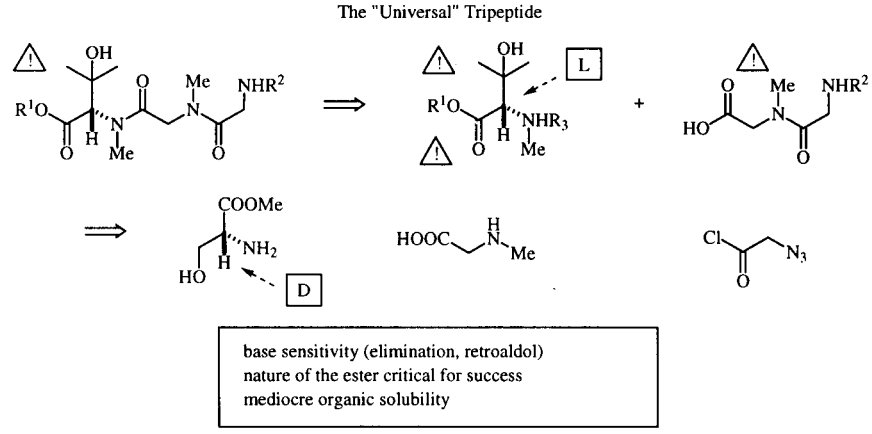


Figure 6.

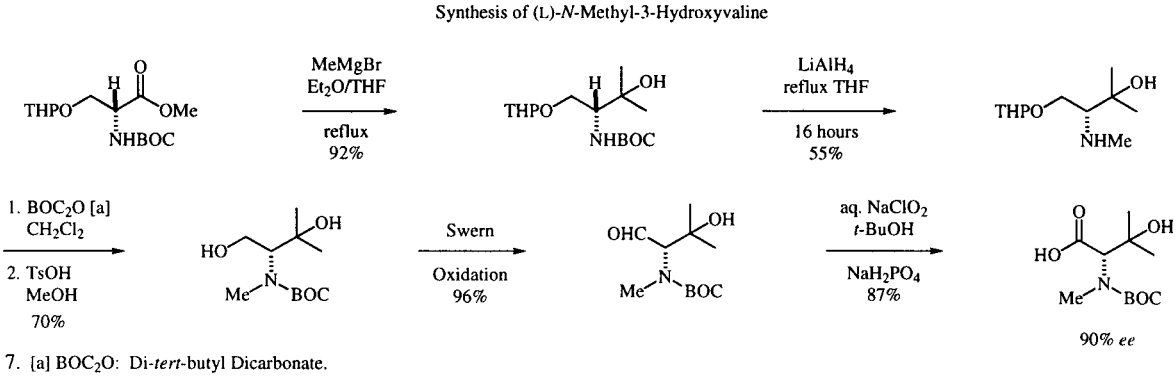


Figure 7. [a] BOC_2O: Di-*tert*-butyl Dicarbonate.

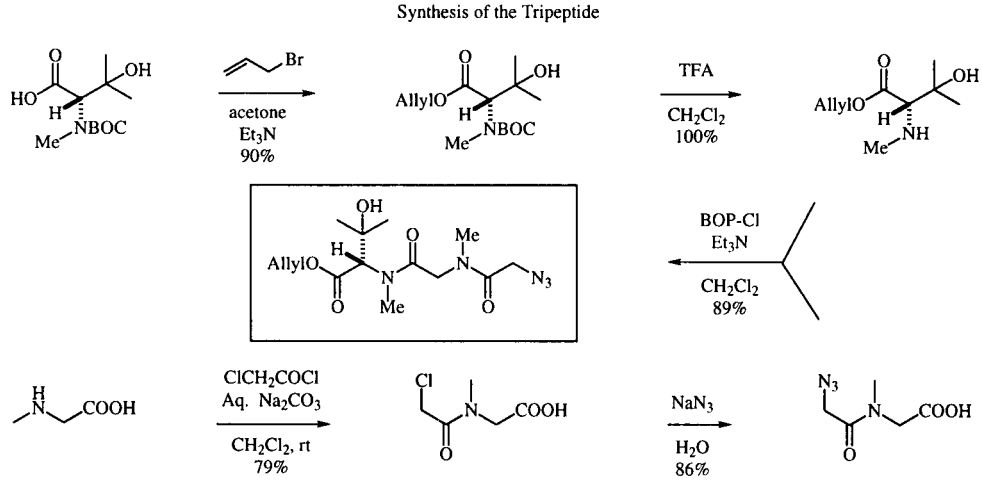


Figure 8.

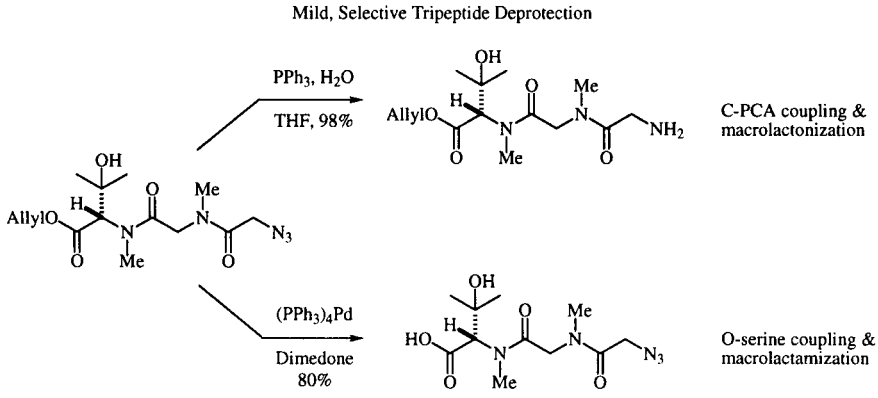


Figure 9.

The piperazic acid component of luzopeptin E2 was prepared starting with the product of Schreiber ozonolysis of cyclopentene. This aldehydoester was advanced to the target molecule by a sequence involving Gennari-Evans-Vederas hydrazination as a key step. For reasons that will become apparent shortly, hydrazination was performed with dibenzyl azodicarboxylate and the intermediate bis-CBZ hydrazinoester was subjected to transprotection to furnish a terminal mono-BOC-derivative. This material may be easily cyclized to a piperazic acid proper under acidic conditions (Figure 10) [7]. A similar logic governed the synthesis of oxygenated piperazic acids found in luzopeptins A-C and quinoxapeptins. As shown in Figures 11-13, a suitable β-hydroxyester was made in scalemic form by bakers' yeast reduction of a β-ketoester. Hydrazination and transprotection produced a terminal BOC-derivative of the hydrazinoester which, again, cyclized to a piperazic acid upon acid treatment [8].

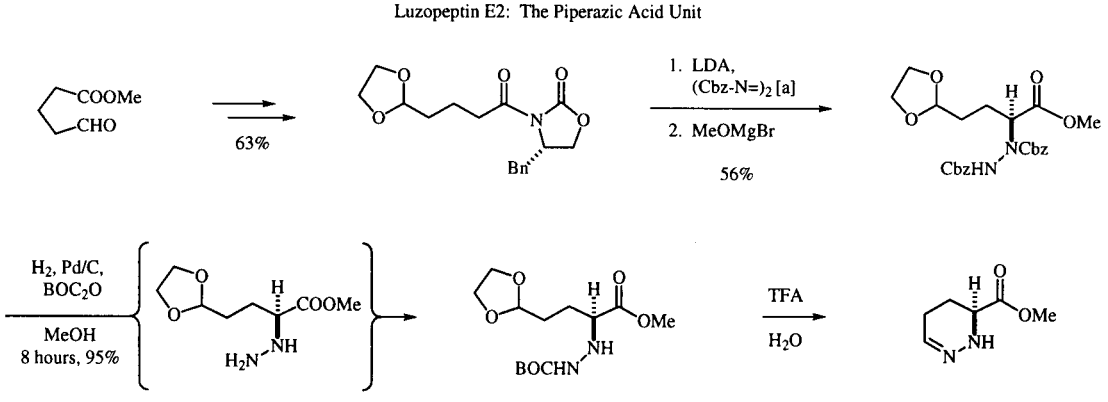


Figure 10. [a] (Cbz-N=)$_2$: Dibenzyl Azodicarboxylate.

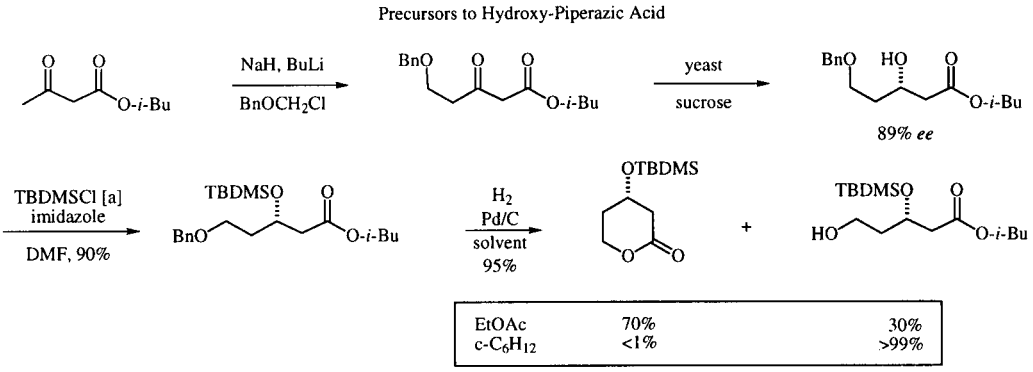


Figure 11. [a] TBDMSCl: *tert*-Butyldimethylsilyl Chloride.

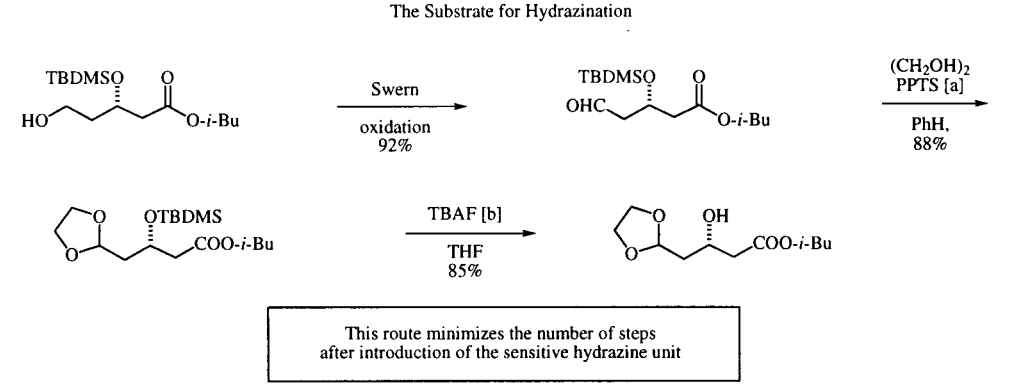


Figure 12. [a] PPTS: Pyridinium *p*-Toluenesulfonate; [b] TBAF: Tetrabutylammonium Floride.

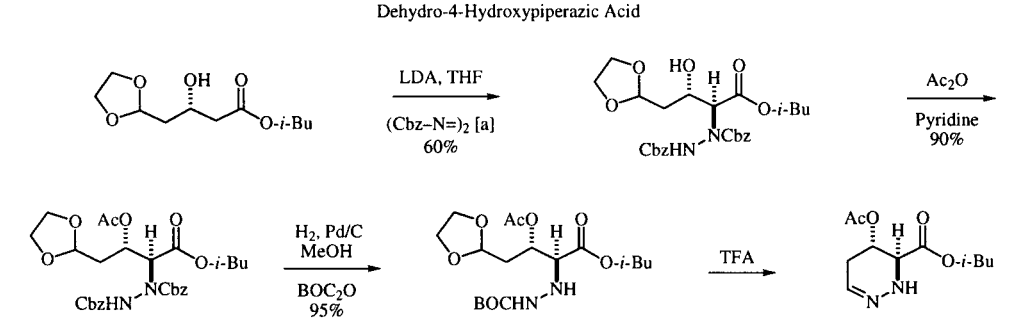


Figure 13. [a] $(Cbz\text{-}N=)_2$: Dibenzyl Azodicarboxylate.

Figures 14 and 15 illustrate the first cluster of serious problems that we encountered during the present venture. Piperazic acids, especially the unsaturated variants, proved to be extremely unreactive toward acylation of the inner nitrogen atom [9]. Fortunately, the terminally BOC-protected precursors to PIZ proved to be amenable to *N*-acylation; however, the nitrogen atom destined to accept an acyl unit displayed abnormally poor nucleophilicity, and it reacted only with acid chlorides (Figure 15). This mandated the use of a serinyl chloride to prepare the requisite PIZ-serine dipeptide.

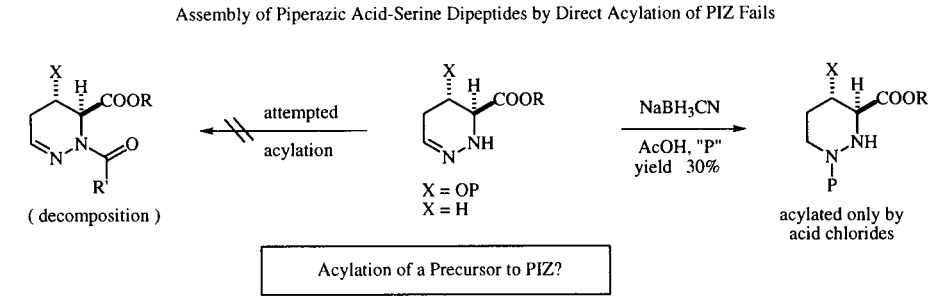


Figure 14.

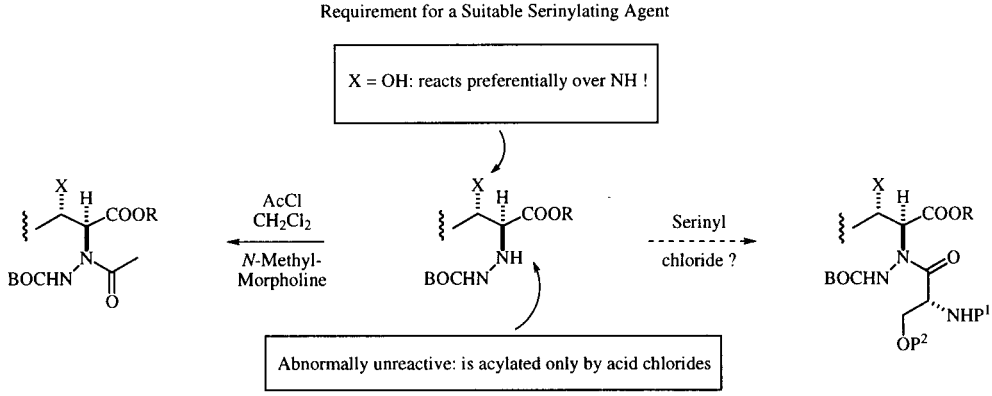


Figure 15.

Various serinyl chlorides (Figure 16) proved to be wholly unsatisfactory for the requisite operation, due to the ease of β-elimination of the protected oxygen functionality, and consequent polymerization of the resulting dehydroamino acid, under the conditions of the acylation step. Suppression of this undesirable reactivity was finally accomplished through the agency of a Baldwin stereoelectronic effect [10]. Thus, serinyl and threoninyl chlorides wherein the oxygen and nitrogen functionalities are part of an oxazolidinone ring proved to be well behaved, yet highly reactive, acylating agents (Figure 17) [11]. The hypothetical β-elimination of the oxygen functionality in these reagents would amount to a reverse "5-endo-trig" process, and it is thus strongly disfavored. The new serinyl chlorides proved to be highly successful in the acylation of appropriately crafted forerunners of piperazic acids, as shown in Figures 18 and 19. However, whereas piperazic acids of the type found in luzopeptin E2 were fully tolerant of the Grieco-Kunieda-type conditions leading to release of the oxazolone ring (Figure 18), the luzopeptins A-C/quinoxapeptins-type oxygenated piperazic acids, in free form or as esters, were sensitive to β-elimination of the oxygen functionality under similar conditions. Oxazolone cleavage was nonetheless possible if the piperazic unit was present in the amide form (Figure 20).

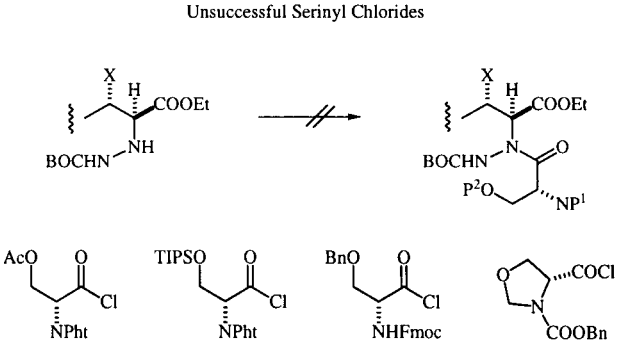


Figure 16.

Novel, Robust Serinyl/Threoninyl Chlorides

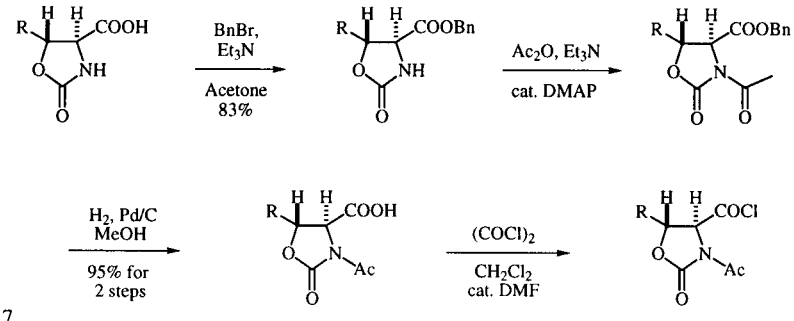


Figure 17.

Successful Serinylation of PIZ Units - I

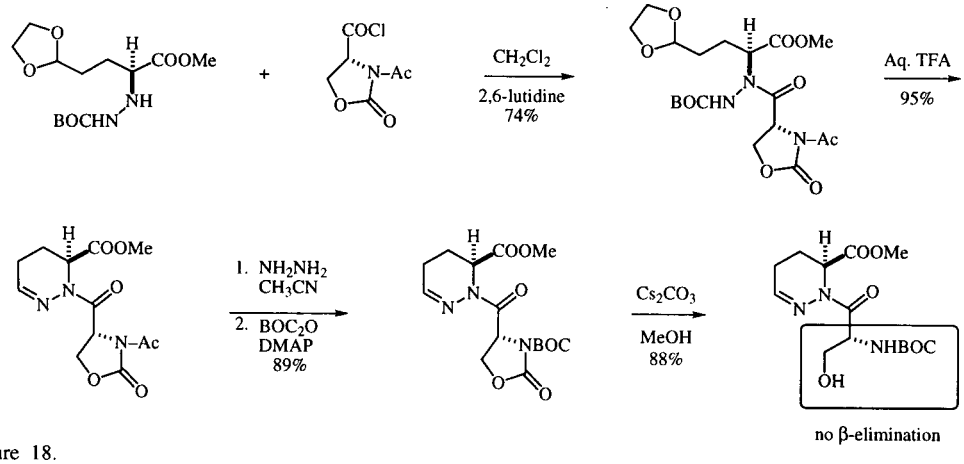


Figure 18.

Successful Serinylation of PIZ Units - II

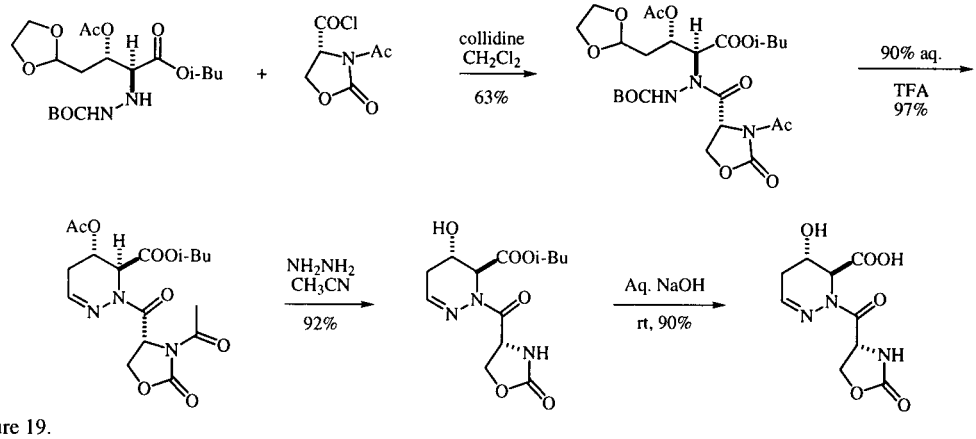


Figure 19.

Reactivity of PIZ Amides *vs.* PIZ Esters

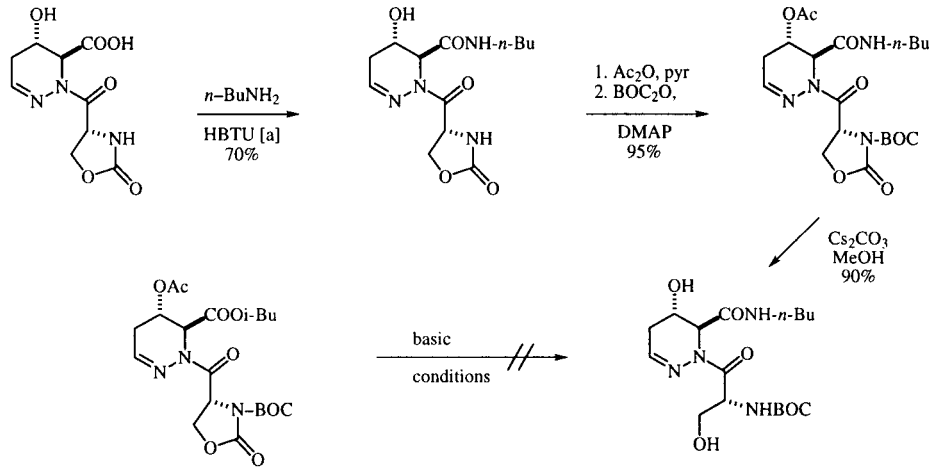


Figure 20. [a] HBTU: *O*-Benzotriazol-1-yl-*N*,*N*,*N*',*N*'-Tetramethyluronium Hexafluorophosphate.

The successful conclusion of this phase of the work heralded the beginning of research directed toward the establishment of a method for macrocyle formation. The universal tripeptide and the piperazic acid-serine dipeptide were combined as required for either a macrolactonization or a macrolactamization approach to the cyclopeptide core of the natural products. A significant number of experiments ultimately revealed that macrolactamization represented by far the best technique for ring formation. With this information in hand, we launched the final attack on luzopeptin E2.

As alluded to earlier, basic reagents are particularly damaging to the universal tripeptide. The sequence leading to an advanced intermediate for macrolactamization thus commenced with transprotection of the piperazic acid dipeptide, initially prepared as a methyl ester. Prior experience with the universal tripeptide induced us to examine an allyl ester of the PIZ containing segment. However, this blocking group displayed unusual lability for reasons that are not fully clear at this time. Additional experimentation defined a crotyl ester as the optimal compromise between stability and ease of removal under neutral conditions. As indicated in Figure 21, the crotyl derivative of the PIZ-serine dipeptide was esterified [4] with the free acid version of the universal tripeptide to afford a pentapeptide that constitutes the monomeric precursor to the macrocycle. Early cyclization protocols involving stepwise linking of two such subunits in a head-to-tail mode could be greatly simplified upon discovery of a remarkable property of the monomeric intermediate. Thus, deblocking of

Macrolactamization Route to Luzopeptin E2: Requirement for a Suitable PIZ Ester

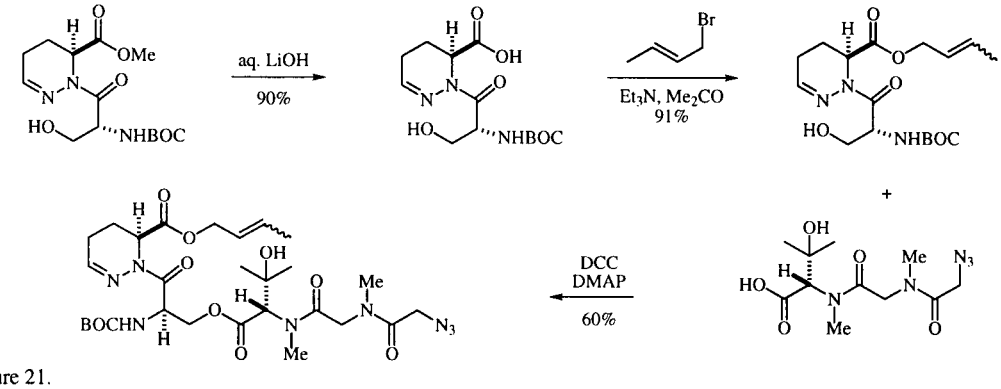


Figure 21.

N and C termini and treatment of the corresponding free amino acid with DCC, DMAP and HOBt (Figure 22) resulted in direct formation of a cyclodimer as the major product in 25% chromatographed yield for the overall 3-step sequence (Figure 23). A small amount of cyclic monomer (10%) was also obtained, together with unreacted starting aminoacid (15-20%) and oligomeric materials.

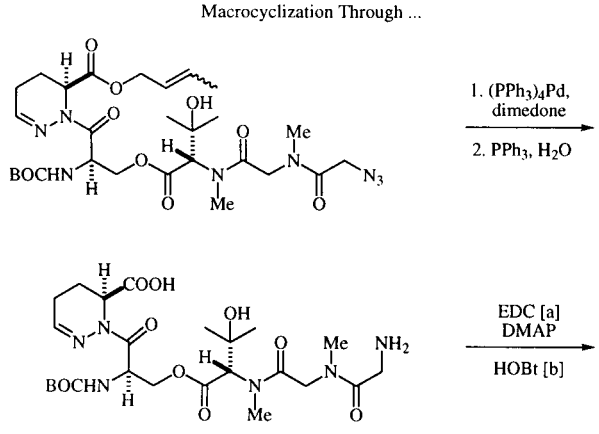


Figure 22. [a] EDC: 1,2-Dichloroethane; [b] HOBt: 1-Hydroxybenzotriazole.

The synthesis of luzopeptin E2 was completed by release of the BOC protecting groups on the serine units and installation of the quinaldoyl chromophores prior to reduction of the imino linkage in the unsaturated piperazic acid with sodium cyanoborohydride (Figure 24).

The peptin effort provided us with an opportunity to study the properties of individual components of the molecule and those of a number of segments of the macrocyclic core in considerable detail. We wish to conclude this overview of our research by highlighting two aspects of these investigations that we regard as particularly significant.

We have devoted much attention to the chemistry of piperazic acids, and a summary of recent findings in this area has been published [12]. Dipeptides incorporating these hydrazino acids display unusual conformational properties. Thus, while proline and pipecolinic acid produce *N*-acyl derivatives that exist as mixtures of rotamers (nmr), piperazic acids, especially the unsaturated variants, form *N*-acyl derivatives that exist as single rotamers in a temperature range from -90° to +120°. Experimental and computational methods suggest that the rotational energy barrier in these systems is large (Figure 25). On the other hand, the favorable (= sole!) conformer of *N*-acyl piperazic acids possesses the vector properties of a peptide turn, suggesting that PIZ structures might be useful as rigid

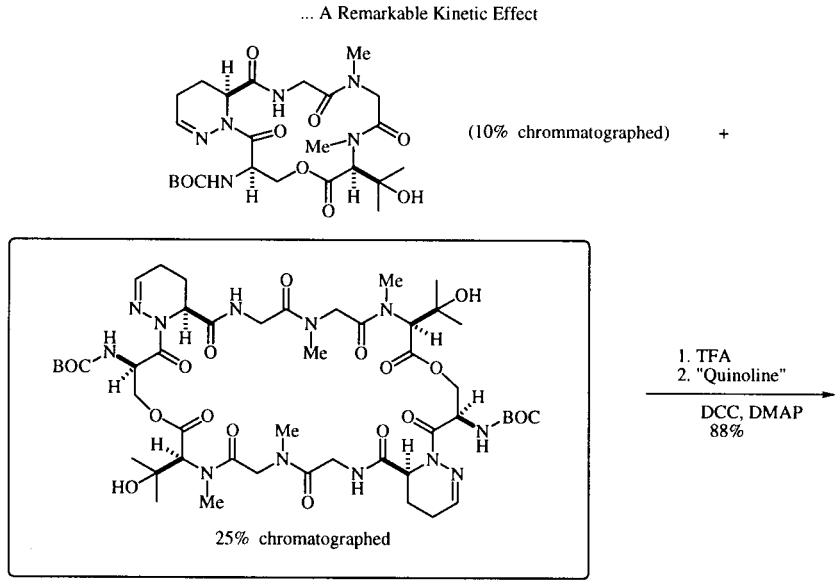


Figure 23.

Total Synthesis of Luzopeptin E2

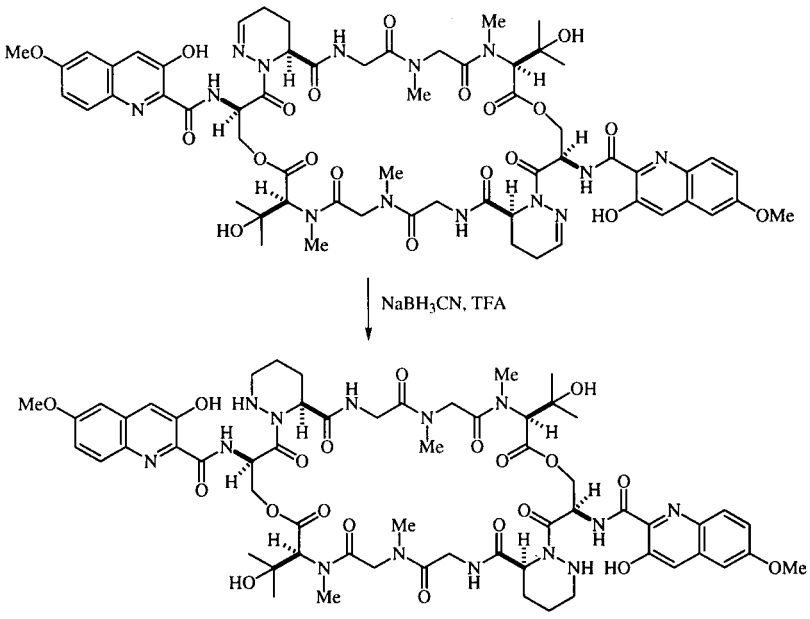


Figure 24.

Unusual Conformational Rigidity of Piperazic Acid Peptides

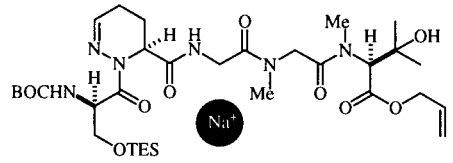
ΔE = 5.7 kcal/mol (MM+, PM3)

^{1}H nmr spectra showed no significant changes from -90° to +120°
SINGLE ROTAMER AROUND TERTIARY AMIDE !

Figure 25.

analogs of proline for the creation of, *e.g.*, artificial β-turns. An even more interesting observation was made during a study of the pentapeptide shown in Figure 26. This material was prepared in connection with research on macrocycle formation through macrolactonization. The compound proved to be an exceptionally effective ligand for sodium ion, with which it forms a crystalline 1:1 complex soluble in water, but insoluble in most organic solvents. The complex was characterized by ^{23}Na nmr and by mass spectrometry. Remarkably, electron impact mass spectra of the complex showed fragment ions containing sodium ion. Moreover, the ability of the peptide to coordinate sodium appeared to *decrease* substantially upon release of the TES group on the serine hydroxyl. It is interesting to speculate whether the pentapeptide may be able to function somehow as a sodium channel mimic, in the sense that reversible modification of a subunit (the serine OH in the present case) may modulate its affinity for sodium ion. These provocative ideas are currently under scrutiny in our group.

Remarkable Affinity of a Pentapeptide for Sodium Ion



Mass Spectra
- FAB: Strong (M+Na)+ peak; No M+ or (M+H)+ peak
- HRMS: $C_{33}H_{58}N_6O_{10}SiNa$
- EI: Fragment peaks contain Na

^{23}Na nmr
- NaCl in pyridine: 0.0 ppm
- Peptide/NaCl in pyridine: 5.7 ppm

Figure 26.

The summary of the luzopeptin effort presented herein underscores the importance to engage in complex synthetic ventures, not only to establish chemical routes to interesting and unusual substances with promising biological activities, not only to make it possible to conduct

detailed structure-activity relationship (SAR) and medicinal chemistry studies, not only to advance the state of the art of organic chemistry, but also and especially to unveil novel and exciting areas of chemical endeavoring. Synthesis remains indeed a uniquely effective vehicle to deliver such new and potentially valuable research leads to the scientific community.

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[*] E-mail: ciufi@cpe.fr

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