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DESIGN, SYNTHESES AND POTENTIATING ACTIVITIES AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* OF CYCLIC ANALOGS OF LY301621

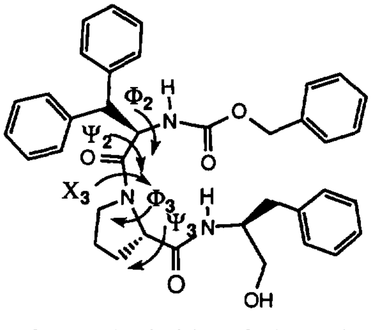
Clark N. Eid*, Thalia I. Nicas, Deborah L. Mullen, Richard J. Loncharich, and Jonathan W. Paschal

Infectious Diseases Research, Lilly Research Laboratories
Eli Lilly and Company, Indianapolis, IN 46285 USA

Abstract. Previous SAR studies of the diastereomers of LY301621 suggested the importance of a β-turn conformation for biological activity. In the present study, cyclic analogs were designed and synthesized that possess Type II and II' β-turns. Their biological activity will be discussed. © 1997 Elsevier Science Ltd.

Introduction. The unnatural tripeptide **LY301621** increases (potentiates) the activity of methicillin against methicillin resistant *Staphylococcus aureus* (MRSA), but is a relatively weak stand-alone inhibitor of bacterial growth (Figure 1 and Table 1).[1] Four of the eight possible diastereomers exist in Type II and II' β-turns and are active potentiating agents. The remaining four diastereomers can not form β-turns and are inactive. To obtain further information about the influence of the β-turn topography upon activity, we synthesized several analogs that constrained the β-turn by means of a cyclic configuration.

Figure 1. LY301621 shown in β-turn conformation.



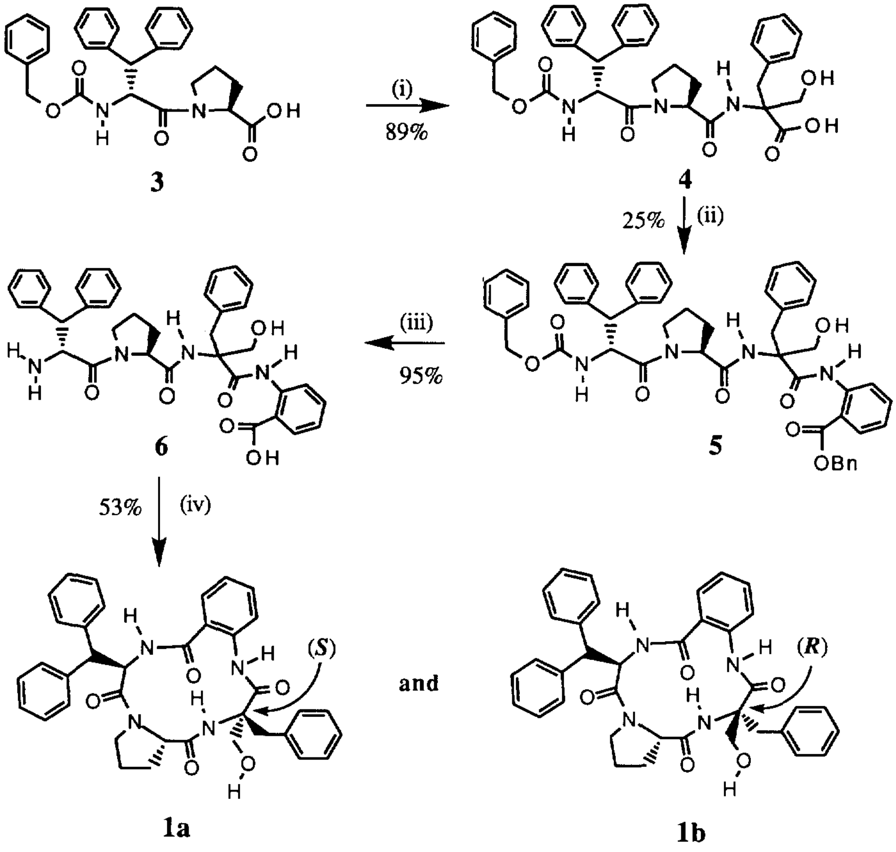
Cyclizations were designed to maintain the terminal lipophilic aryl moieties and the β-turn topography along the -(D)-DPA-(L)-Pro- residues.

Chemistry. The decision of what molecular framework should be used to bridge the gap between the N- and C-termini, while maintaining important recognition elements and a Type II or II' β-turn secondary structure, was based upon earlier SAR observations.[1,2] We believed the type II β-turn of **LY301621** positioned these terminal lipophilic groups close together and that they could interact with a common lipophilic binding pocket.

The first cyclic analog required an α-hydroxymethyl phenylalanine substitution at the C-terminus linked through an amide bond to a 2-amino benzoyl group substituted at the N-terminus. Molecular models and preliminary force field optimization of the proposed 13-membered ring analog **1a** revealed a Type II β-turn that had a startlingly good overlap with the β-turn structure of **LY301621** (Table 1). Analog **1b** did not have a β-turn topography but still had a reasonable overlay with **LY301621** to warrant its investigation.

The synthesis of the proposed cyclic analogs began from intermediate 3[1] and followed typical peptide coupling protocols (Figure 2). The protected dipeptide 3 was coupled to (*R*/*S*) α-hydroxymethyl phenylalanine[3] with EDCI/HOBT to yield tripeptide 4 as a mixture of two diastereomers. The diastereomers could not be easily separated and were coupled as a 1:1 mixture with 2-aminobenzoic acid benzyl ester using EDCI/HOBT to give 5. Again, the separation of diastereomers proved difficult so the 1:1 mixture was hydrogenated to remove the protecting groups, yielding 6 as a 1:1 mixture of diastereomers. Separation of the diastereomeric peptides was not attempted. Ring closure was accomplished using PyBOP/DIEA in DMF/DCM and gave an easily separable mixture of diastereomers **1a** and **1b**. The absolute stereochemistries were assigned by ^{1}H NMR.[4]

Figure 2. Synthesis of cyclic analogs **1a** and **1b**.



Reagents: (i) EDCI/HOBT, DCM, (*R*/*S*) α-hydroxymethyl-phenylalanine (ii) EDCI/HOBT, DCM, 2-amino-benzoic acid benzylester (iii) $H_2/Pd(OH)_2$, EtOH (iv) PyBOP/DIEA, DMF/DCM.

Both **LY301621** and its enantiomer are active potentiating agents.[1] Therefore, the enantiomers of **1a** (**1c**) and **1b** (**1d**) were synthesized following the same route using the enantiomer of intermediate **3**, Cbz-(L)-DPA-(D)-Pro-OH.

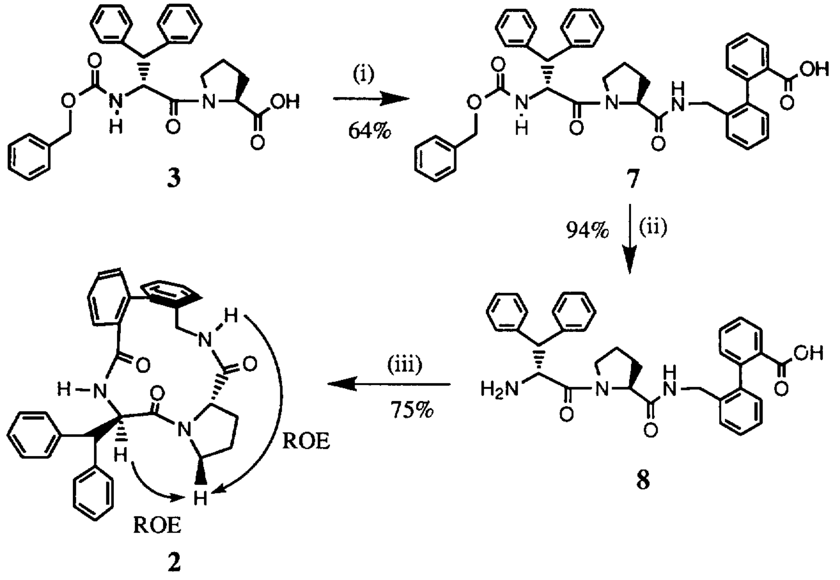
Superposition of the AM1 calculated structures of **LY301621** and **1a** shows a RMS difference for the 48 β-turn atoms of these two structures is 0.12 Å, while the RMS difference for the 8 β-turn atoms is 0.11 Å.

The extremely low values illustrates just how well the cyclic analog mimics the β-turn conformation of the prototype compound **LY301621**. The solution conformation, using the FELIX computer program and ROE data, is in good agreement with the computer generated model.

The second cyclic analog utilized a 2,2'-substituted biphenyl to bridge between the N- and C-termini of H-(D)-DPA-(L)Pro-OH. Molecular models and preliminary force field optimization of the proposed 13-membered ring analog **2** showed a reasonable overlap with the β-turn structure of **LY301621.**

The synthesis of the proposed cyclic analog began from intermediate **3** and followed typical peptide coupling protocols (Figure 3). The protected dipeptide **3** was coupled to (+/-) 2-aminomethyl-2'-carboxybiphenyl[5] with EDCI/HOBT yielding tripeptide **7**. The Cbz group was removed using $H_2/Pd(OH)_2$ to provide unnatural tripeptide **8**. Ring closure was accomplished using PyBOP/DIEA in DMF/DCM and gave **2** as a single diastereomer.[6]

Figure 3. Synthesis of biphenyl bridged cyclic analog **2**.



Reagents: (i) EDCI/HOBT, DCM, (+/-) 2-aminomethyl-2'-carboxybiphenyl (ii) $H_2/Pd(OH)_2$, EtOH (iii) PyBOP/DIEA, DMF/DCM

Computational Method. All force field calculations were performed with the CHARMM (Chemistry at HARvard Molecular Mechanics) program[7] on a Silicon Graphics workstation. The Molecular Simulations Inc all atom parameter set (PARM.PRM) and topology file (AMINOH.RTF) were used for all minimizations.[8] In addition, to further our understanding of the β-turn geometry and thermodynamic stabilities of these unnatural cyclic peptides, we studied each molecule using semiempirical[9] calculations. Each of the peptide structures were fully optimized with the AM1 semiempirical method[10] using the AMPAC program[11] on a Silicon Graphics workstation.

Table 1. Torsion angles (°) and ß-turn topology from force field calculations. The torsion angles in parenthesis are from AM1 semiempirical calculations.

Compound	Φ_2	Ψ_2	χ_3	Φ_3	Ψ_3	**ß-Turn Topology**
LY301621	61 (66)	-126 (-109)	180 (176)	-81 (-84)	-12 (-22)	Type II'
1a	56 (68)	-117 (-108)	180 (175)	-75 (-79)	1 (-9)	Type II'
1b	55 (84)	-124 (-95)	180 (175)	-75 (-95)	-6 (-49)	---------
1c	-56 (-68)	117 (108)	-180 (-175)	75 (79)	-1 (9)	Type II
1d	-55 (-84)	124 (95)	-180 (-175)	75 (95)	6 (49)	---------
2	76 (88)	-104 (-96)	163 (154)	-67 (-61)	-13 (-32)	Type II'

Biological evaluation. All cyclic analogs were assayed for their potentiating activity against MRSA (Table 2). Analog **1b** was marginally active at 100 µg/mL while the other analogs were not active. Synergy in the much more sensitive disk assay was observed for **1b**, **1c**, and **1d**.

Table 2. Biological activity.

Compound	Minimal Synergizing Concentration[i] (µg/mL)	Minimal Inhibitory Concentration vs MRSA[ii] (µg/mL)	Minimal Inhibitory Concentration vs MSSA[iii] (µg/mL)	Synergy with Methicillin in Disk Assay[iv]
LY301621	12.5	>100	>100	+
1a	>100	>100	>128	-
1b	100	>100	>100	+
1c	>128	>128	>128	+
1d	>128	>128	>128	+
2	>100	>100	>100	-

i,ii,iii Determined by broth microdilution in Phenol Red Sucrose broth. Bacterial inoculum of 10^5 bacteria/mL, incubated 18 h at 35 °C.

i) Minimum conc. required to allow inhibition of growth of *S. aures* strain 447 in the presence of 4 µg/mL of methicillin.

ii) Minimum conc. required to allow inhibition of growth of the methicillin-resistant *S. aures* strain 447.

iii) Minimum conc. required to allow inhibition of growth of the methicillin-sensitive *S. aures* strain 446.

iv) Ability of 40 µg of compound to enlarge the zone of inhibition produced by 100 µg of methicillin when compounds are delivered on filter-paper disks placed 0.3 to 1 cm apart on an agar plate seed with *S. aures* strain 447 and grown overnight. "+", synergy; "-", no synergy.

Discussion. Five cyclic analogs of the lead compound **LY301621** were synthesized. Two of these, **1a** and **1c**, maintained Type II and II' β-turn topography and other important recognition features. The best fitting cyclic analog, **1a**, differs mostly from **LY301621** at the C-terminus where the benzyl and the hydroxymethyl groups are restricted to a subset of the total space available to **LY301621**'s phenylalanine alcohol. Surprisingly, only the cyclic analog, **1b**, which does not maintain Type II or II' β-turn topography, is marginally active. At the very least, these results confirm that the -(D)-DPA-(L)-Pro- section of **LY301621**, locked into a Type II β-turn, is not solely responsible for activity. Further interpretation is difficult since the location and the mode of action are unknown and the assays are conducted on whole cells, which present additional challenges such as transport to the active site.

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4. The stereochemistries of **1a** and **1b** were solved using homonuclear decoupling, ROESY, and comparison to **LY301621**'s spectra. In both diastereomers, there is a ROE between the (*S*) proline α-proton and the amide proton of the α-hydroxymethyl phenylalanine. In compound **1a**, there is another ROE between the α-hydroxymethyl phenylalanine's amide proton and its benzylic protons. This situation requires the benzyl group to be oriented in the same direction resulting in an *S* configuration for the α-hydroxymethyl phenylalanine residue. Compound **1b** has a ROE between the α-hydroxymethyl phenylalanine's amide proton and its hydroxymethyl methylene protons, indicative of the ***R*** stereochemistry.
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6. Although AM1 force field calculations indicated a ΔH_f of about 1 kcal/mol for the two tortionally asymetric diastereomers, we do not know the energy barrier of interconversion. Low temperature (-60 °C) ^{1}H NMR in $CDCl_3$ and ROE experiments indicate that only one diastereomer was present. The NMR data was consistent with the AM1 calculated lowest energy structure. We hypothesize that the diastereomers of **8** readily interconvert at room temperature and that only one can adopt a favorable conformation for ring closure.
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