Tripeptide LY301621 and Its Diastereomers as Methicillin Potentiators against Methicillin Resistant *Staphylococcus aureus*

CLARK N. EID*, NOREEN G. HALLIGAN, THALIA I. NICAS, DEBORAH L. MULLEN THOMAS F. BUTLER, RICHARD J. LONCHARICH and JONATHAN W. PASCHAL

Infectious Diseases Research, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 U.S.A.

CHRISTOPHER J. SCHOFIELD, NICHOLAS J. WESTWOOD and LEIFENG CHENG

The Dyson Perrins Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QY U.K.

(Received for publication November 15, 1996)

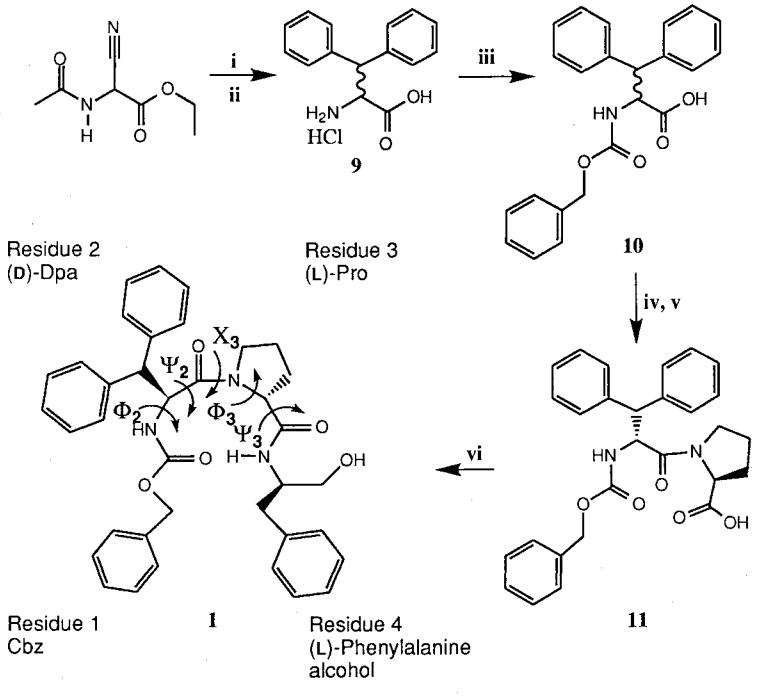
The antibiotics we rely upon to treat bacterial infections are quickly becoming ineffective due to the emergence of multi-drug resistant pathogens[1]. Staphylococci, which commonly cause skin infection, wound infection, and more serious hospital acquired infection, have developed resistance to many drugs, but still remain susceptible to the last-line-of-defense antibiotic, vancomycin[2]. In the course of our continuous screening for new antimicrobials active against methicillin resistant *S. aureus* (MRSA), we discovered the unnatural tripeptide LY301621, **1** (Scheme 1). This unnatural tripeptide increases (potentiates) the activity of methicillin against MRSA (Table 1). It is also a relatively weak stand-alone inhibitor of bacterial growth.

The synthesis of the title compound **1** is outlined in Scheme 1. Alkylation of ethyl acetamidocyanoacetate anion with bromodiphenylmethane, followed by acid reflux, affords racemic diphenylalanine **9** (Dpa). The *N*-terminus is acylated under modified Schotten-Bauman conditions to yield **10**. Racemic **10** was converted to its activated NHS ester and coupled to (L)-proline under basic conditions to give a diastereomeric mixture. Diastereomer **11**, Cbz-(D)-Dpa-Pro-OH, was selectively recrystallized from ethyl acetate[3]. The structure of **11** was confirmed by X-ray crystallography. Lastly, dipeptide **11** was coupled to (*S*)-(−)-2-amino-3-phenyl-1-propanol with EDCI/HOBT to yield **1**.

The remaining seven diastereomers were synthesized following analogous synthetic routes and tested for their potentiating activity against MRSA. Four diastereomers having the configuration (D,L,L) **1**, (L,D,D) **2** (D,L,D) **3**, and (L,D,L) **4** were active potentiating agents. The four other diastereomers having the configuration (D,D,D) **5**, (L,L,L) **6**, (D,D,L) **7**, and (L,L,D) **8** were inactive.

Data obtained for **1** from ^{1}H NMR nOe experiments in DMSO-d_6/D_2O positioned the two benzylic methylenes at the *C*- and *N*-termini close together and suggested the possibility of a β-turn configuration. Computational analysis supported the existence of a Type II β-turn[4]. The calculations were expanded to study all other diastereomers in the series (Table 2). Analysis of the energy

Scheme 1. Synthesis of **1** (LY301621). Angles describing the β-turn conformation and residue numbering system are illustrated[4].



i: PH_2CHBr, KO-*t*-Bu, HO-*t*-Bu, r.t., 67%. ii: 20% HCl, reflux, 84%. iii: Cbz-Cl, NaOH, 93%. iv: *N*-hydroxysuccinimide, EDCI, 90%. v: (L)-Pro, acetone/H_2O/$NaHCO_3$, 98% before crystallization of (D,L). vi: EDCI, HOBT, (L)-Phenylalanine alcohol, 85%.

Table 1. Biological activity of diastereomers.

Compound	Minimal synergizing concentration[a] (μg/ml)	Minimal inhibitory concentration *vs.* MRSA[b] (μg/ml)	Minimal inhibitory concentration *vs.* MSSA[c] (μg/ml)	Synergy with methicillin in disk assay[d]
1	12.5	>100	>100	+
2	25.0	>100	>100	+
3	25.0	>100	>100	+
4	12.5	>100	>100	+
5	>100	>100	>100	+
6	>100	>100	>100	NA
7	>100	>100	>100	+
8	>100	>100	>100	+

Determined by broth microdilution in Phenol Red Sucrose broth. Bacterial inoculum of 10^5 bacteria/ml, incubated 18 hours at 35°C.

[a] Minimum concentration required to allow inhibition of growth of *S. aureus* strain 447 in the presence of 4 μg/ml of methicillin. Minimal inhibitory concentration of methicillin in this isolate is about 2000 μg/ml.

[b] Minimum concentration required to allow inhibition of growth of the methicillin-resistant *S. aureus* strain 447.

[c] Minimum concentration required to allow inhibition of growth of the methicillin-sensitive *S. aureus* strain 446.

[d] Ability of 40 μg compound to enlarge the zone of inhbition 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. aureus* strain 447 and grown overnight.
+: Synergy; −: no synergy; NA: not available.

Table 2. Torsion angles (°), β-turn topology, and hydrogen bond strength (kcal/mol) from force field calculations.

Compound	Φ_2	Ψ_2	χ_3	Φ_3	Ψ_3	β-Turn topology	Hydrogen bond strength
1	61 (66)	−126 (−109)	180 (176)	−81 (−84)	−12 (−22)	Type II′	−1.8
2	−61 (−66)	126 (110)	−180 (−176)	81 (85)	12 (21)	Type II	−1.8
3	62 (65)	−120 (−111)	−177 (176)	−77 (−83)	−9 (−20)	Type II′	−2.5
4	−61 (−65)	121 (110)	180 (−176)	78 (85)	7 (16)	Type II	−2.5
5	75 (115)	−122 (−105)	178 (170)	71 (87)	−109 (−68)	—	
6	−74 (−87)	128 (127)	171 (−173)	−63 (−77)	97 (83)	—	
7	74 (114)	−123 (−105)	−176 (170)	54 (86)	−111 (−65)	—	
8	−77 (−99)	137 (109)	174 (−173)	−52 (−71)	120 (90)	—	

All force field calculations were performed with the CHARMM (Chemistry at HARvard Molecular Mechanics) program[5] on a Silicon Graphics workstation. The Molecular Simulations Inc. all atom parameter set (PARM.PRM) and topology file (AMINOH.RTF) were used for all minimization and hydrogen bonding analyses.[6] The torsion angles in parenthesis are from AM1 semiempirical calculations. Each structure was fully optimized with the AM1 semiempirical method[7] using the AMPAC program[8] to a root mean square gradient norm less than 0.01. All stationary points were characterized as local minima on the potential surface by a force calculation.

minimized structures revealed that all active diastereomers (**1**, **2**, **3**, and **4**) could exist in Type II or Type II′ β-turns. The four inactive diastereomers (**5**, **6**, **7**, and **8**) can not form stable β-turns because the force field geometries did not meet the criteria for an ideal β-turn. The optimized structures from semiempirical calculations also indicated that the inactive diastereomers did not form a stable β-turn. With both methods of calculation, the ψ_3 angle deviated largely from the ideal β-turn value of 0°.

Conclusion

The unnatural tripeptide **1** (LY301621) and its diastereomers differently effect the activity of methicillin on MRSA while being themselves relatively week standalone antimicrobials. A structure based ligand design program revealed that a β-turn secondary structure was common only to the active diastereomers. Our efforts to synthesize more potent analogs utilizing this structural information will be reported in due course.

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