

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

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The antibiotics we rely upon to treat bacterial infections are quickly becoming ineffective due to the emergence of multi-drug resistant pathogens¹. 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, vancomycin². 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

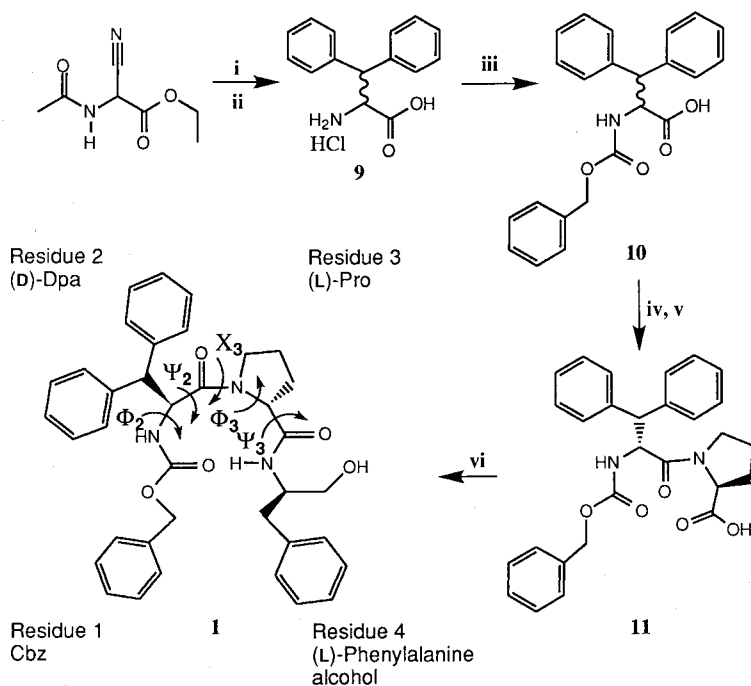
increases (potentiates) the activity of methicillin against MRSA (Table 1). It is also a relatively weak stand-alone inhibitor of bacterial growth.

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 acetate³. 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 ¹H NMR nOe experiments in DMSO-*d*₆/D₂O 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 -turn⁴. 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 illustrated⁴.



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/H₂O/NaHCO₃, 98% before crystallization of (D,L). vi: EDCI, HOBT, (L)-Phenylalanine alcohol, 85%.

Table 1. Biological activity of diastereomers.

Compound	Minimal synergizing concentration ^a ($\mu\text{g/ml}$)	Minimal inhibitory concentration vs. MRSA ^b ($\mu\text{g/ml}$)	Minimal inhibitory concentration vs. MSSA ^c ($\mu\text{g/ml}$)	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 $4\mu\text{g/ml}$ of methicillin. Minimal inhibitory concentration of methicillin in this isolate is about $2000\mu\text{g/ml}$.

^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 $40\mu\text{g}$ compound to enlarge the zone of inhibition produced by $100\mu\text{g}$ 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 ($^\circ$), 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) program⁵⁾ on a Silicon Graphics workstation. The Molecular Simulations Inc. all atom parameter set (PARM.PRM) and topology file (AMINO.HRTF) 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 method⁷⁾ using the AMPAC program⁸⁾ 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 weak stand-alone 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.

References

- 1) TRAVIS, J.: Reviving the antibiotic miracle? *Science* 264: 360~362, 1994
- 2) NOBLE, W. C.; Z. VIRANI & R. G. CREE: Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* MCTC 12207 to *Staphylococcus aureus*. *FEMS Microbiology Letters* 72: 195~198, 1992
- 3) CHENG, L.; C. A. GOODWIN, M. F. SCULLY, V. V. KAKKAR, G. CLAESON: Substrate-related phosphono-peptides, a new class of thrombin inhibitors. *Tetrahedron Letters* 32: 7333~7336, 1991
- 4) BALL, J. B.; R. A. HUGHES, P. F. ALEWOOD, P. R. ANDREWS: β -Turn topography. *Tetrahedron* 49: 3467~3478, 1993

- 5) BROOKS, B. R.; R. E. BRUCCOLERI, B. D. OLAFSON, D. J. STATES, S. SWAMINATHAN & M. CHARMM KARPLUS: A program for macromolecular energy, minimization, and dynamics calculations. *J. Comp. Chem.* 4: 187~217, 1983
- 6) Molecular Simulations Inc. Parameter file and topology file for CHARMM version 22. Parameter file copyright 1993 and release February 1994. Topology file copyright 1992 and release September 1994
- 7) DEWAR, M. J. S.; E. G. ZOEBISCH, E. F. HEALY & J. J. P. STEWART: AM1: A new general purpose quantum mechanical molecular model. *J. Am. Chem. Soc.* 107: 3902, 1985
- 8) AMPAC 5.0, copyright 1994 Semichem, Inc., 7128 Summit, Shawnee, KS 66216