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α-Amino-β-hydroxy-γ-lactam for Constraining Peptide Ser and Thr Residue Conformation

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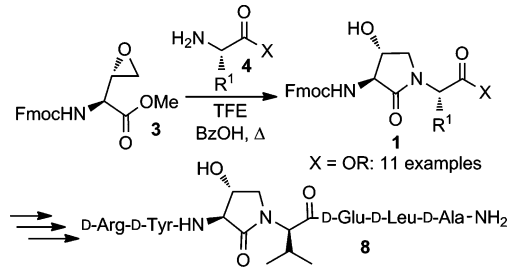
ABSTRACT



α-Amino-β-hydroxy-γ-lactam 1 is a peptide mimic in which the Ser/Thr residue ω-, ψ-, and χ-dihedral angle geometry all are constrained by the 5-membered lactam ring. Lactams 1 were made by employing *N*-(Fmoc)oxiranylglycine 3 as a bis-electrophile in TFE with cat. BzOH to sequentially alkylate and acylate a variety of amino acid derivatives in one pot. Solid-phase synthesis of β-hydroxy-γ-lactam 8, an analogue of the IL-1 modulator 101.10, was achieved using this method for studying Ser/Thr geometry.

Serine and threonine play important roles in peptide activity and secondary structure. For example, the phosphorylation and glycosylation of the β-hydroxyl group of these amino acid residues in proteins is vital for cellular signaling and function.[1] Moreover, hydrogen bonding to the side-chain hydroxyl group may stabilize peptide secondary structure. Constrained Ser and Thr analogues are attractive targets for exploring the impact of their conformation on peptide biology.[2] For example, 3-hydroxyproline mimics Ser and Thr with constrained ϕ- and χ-dihedral angles (Figure 1). The β-turn inducing ability of 3-hydroxyproline and its occurrence in bioactive peptides underscores the importance of this structural motif.[3−5]

Complementing the conformational effects of β-hydroxyproline, α-amino-β-hydroxy-γ-lactam would constrain the *C*-terminal amide and ψ- and χ-dihedral angles (Figure 1).[6] Specifically, the side-chain gauche (+) and (−) isomers of Ser/Thr are locked in by the lactam, which in χ space,[7]

(1) (a) Glycosylation review: Steen, P. V. D.; Rudd, P. M.; Dwek, R. A.; Opdenakker, G. *Crit. Rev. Biochem. Mol. Biol.* **1998**, *33*, 151. (b) Phosphorylation review: Pinna, L. A.; Ruzzene, M. *Biochim. Biophys. Acta* **1996**, *1314*, 191.

(2) (a) Jenkins, C. L.; Bretsher, L. E.; Guzei, I. A.; Raines, R. T. *J. Am. Chem. Soc.* **2003**, *125*, 6422. (b) Rao, M. H. V. R.; Pinyol, E.; Lubell, W. D. *J. Org. Chem.* **2007**, *72*, 736.

(3) For β-turns in 3-Hyp peptides: Chakraborty, T. K.; Srinivasu, P.; Rao, R. V.; Kumar, S. K.; Kunwar, A. C. *J. Org. Chem.* **2004**, *69*, 7399.

(4) For example, empedopeptin: (a) Elhammer, A. P.; Stachelhaus, T. U.S. Patent 2009124539, 2009. Pneumocandin B_0: (b) Schwartz, R. E.; Sensin, D. F.; Joshua, H.; Wilson, K. E.; Kempf, A. J.; Goklen, K. A.; Kuehner, D.; Gailliot, P.; Gleason, C.; White, R.; Inamine, E.; Bills, G.; Salmon, P.; Zitano, L. *J. Antibiot.* **1992**, *45*, 1853. Telomycin: (c) Sheehan, J. C.; Mania, D.; Nakamura, S.; Stock, J. A.; Maeda, K. *J. Am. Chem. Soc.* **1968**, *90*, 462. Revised structure: (d) Katrukha, G. S.; Maevskaya, S. N.; Silaev, A. B.; Lomonosov, M. V. *Bioorg. Khim.* **1977**, *3*, 422. Cyclothialidine: (e) Nakada, N.; Shimada, H.; Hirata, T.; Aoki, Y.; Kamiyama, T.; Watanabe, J.; Arisawa, M. *Antimicrob. Agents Chemother.* **1993**, *37*, 2656. Mauritine K: (f) Singh, A. K.; Pandey, M. B.; Singh, V. P.; Pandey, V. B. *J. Indian Chem. Soc.* **2007**, *84*, 781. Plusbactin A_3: (g) Wohlrab, A.; Lamer, R.; VanNieuwenhze, M. S. *J. Am. Chem. Soc.* **2007**, *129*, 4175.

(5) Cyclopeptide natural products review: Pomilio, A. B.; Battista, M. E.; Vitale, A. A. *Curr. Org. Chem.* **2006**, *10*, 2075.

(6) Toniolo, C. *Int. J. Pept. Protein Res* **1990**, *35*, 287, and references therein.

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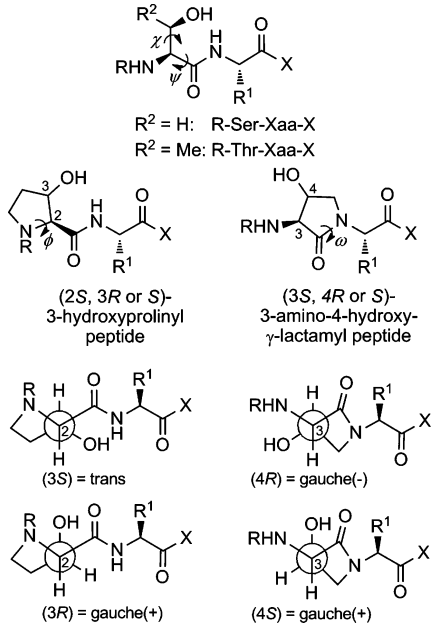


Figure 1. Constraint of χ-dihedral angles in 3-hydroxyproline and α-amino-β-hydroxy-γ-lactam mimics of Ser/Thr residues.

complements the gauche (+) and trans isomers available to β-hydroxyproline, contingent on stereochemistry.[8]

α-Amino-β-hydroxy-γ-lactams have been investigated as *N*-methyl-D-aspartate receptor agonists (i.e., **1a**, Figure 2),[9a]

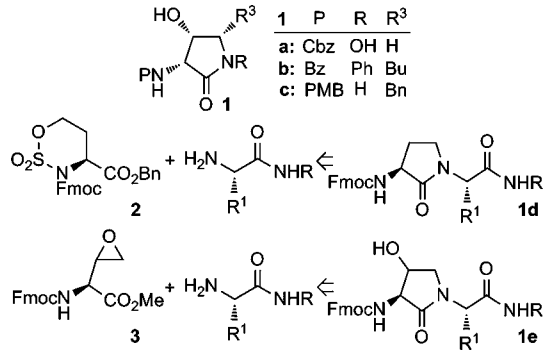


Figure 2. Precedence for α-amino-β-hydroxy-γ-lactams in medicinal chemistry.[9] Recently reported lactam synthesis with sulfamidate **2**[10] and proposed synthesis with epoxide **3**.[11]

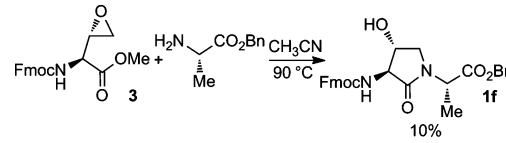
antiinflammatory agents (**1b**),[9b] and HIV-protease inhibitors (**1c**);[9d] however, methodology is lacking for the assembly of this motif on amino acid residues.[9]

We have recently demontrated that the parent α-amino-γ-lactam (Agl) residue can be introduced into peptides by employing dioxooxathiazinane **2** to alkylate and acylate amines, such as the N-terminal of a resin-bound peptide chain to yield γ-lactam **1d** (Figure 2).[10] In considering the construction of Agl's β-hydroxy counterpart **1e**, Rapoport's use of *N*-(Cbz)oxiranylglycine as a building block in alkaloid synthesis (i.e., pentostatin/coformysin aglycons[11] and mitomysin analogues)[12] inspired the application of this bis-electrophile for the synthesis of peptide mimics **1e** bearing the α-amino-β-hydroxy-γ-lactam moiety.

The utility of Fmoc protection compelled the synthesis of *N*-(Fmoc)oxiranylglycine methyl ester (2*S*,2′*S*)-**3**.[13] The higher boiling 2,4-dichlorotoluene, instead of xylenes, for pyrolysis of *N*-(Fmoc)Met(O)-OMe gave the vinylglycine precursor in 2 h instead of 2−3 days.[13,14] Epoxidation gave **3** as a 4:1 mixture of diastereomers, from which a 9:1 mixture was isolated by flash chromatography[15,16] and used subsequently to give mixtures of lactams **1**, which were separated by flash chromatography.[16,17]

Epoxide **3** reacted with Ala-OBn to produce lactam **1f** in 10% yield (Scheme 1).[18] Little improvement was obtained

Scheme 1. Initial γ-Lactam Synthesis



in attempts to yield lactam **1f** using acid catalysis.[19] Epoxide ring opening was accelerated using fluorinated alcohol solvents.[20] In 2,2,2-trifluoroethanol (TFE), *N*-(Fmoc)oxiranylglycine **3** and Ala-OBn reacted at 80 °C affording γ-lactam **1f** in 65% yield within 12 h (Figure 3). With the

(7) Hruby, V. J.; Li, G.; Haskell-Luevano, C.; Shenderovich, M. *Biopolymers* **1997**, *43*, 219.

(8) An astute reviewer noted that the actual ground state conformation of the Figure 1 structures will likely be intermediate between the idealized Newman-projection staggered and eclipsed confomers due to the constraint of the five-membered ring.

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(10) (a) Jamieson, A. G.; Boutard, N.; Beauregard, K.; Bodas, M. S.; Ong, H.; Quiniou, C.; Chemtob, S.; Lubell, W. D. *J. Am. Chem. Soc.* **2009**, *131*, 7917. (b) Boutard, N.; Jamieson, A. G.; Ong, H.; Lubell, W. D. *Chem. Biol. Drug Des.* **2010**, *75*, 40.

(11) Truong, T. V.; Rapoport, H. *J. Org. Chem.* **1993**, *58*, 6090.

(12) Shaw, K. J.; Luly, J. R.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 4515.

(13) *N*-(Fmoc)vinylglycine-OMe precursor: Organ, M. G.; Xu, J.; N'Zemba, B. *Tetrahedron Lett.* **2002**, *43*, 8177.

(14) Vinylglycine synthesis: (a) Afzali-Ardakani, A.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 4817. (b) Carrasco, M.; Jones, R. J.; Kamel, S.; Rapoport, H. *Org. Synth.* **1992**, *70*, 29. (c) Meffre, P.; Vo-Quang, L.; Vo-Quang, Y.; Le Goffic, F. *Synth. Commun.* **1989**, *19*, 3457. (d) Review: Berkowitz, D. B.; Charette, B. D.; Karukurichi, K. R.; McFadden, J. M. *Tetrahedron: Asymmetry* **2006**, *17*, 869.

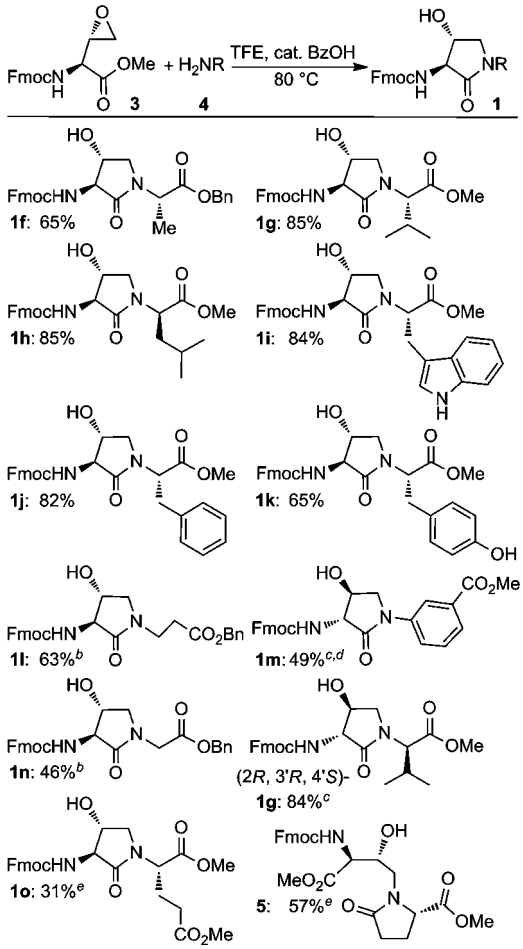


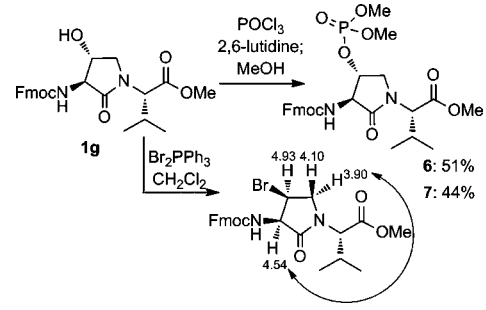
Figure 3. Amino acid scope in dipeptide synthesis. Key: (a) epoxide (2*S*, 2′*S*)-**3** (50 μmol, 9: 1 mixture with (2*S*, 2′*R*)-**3**), **4** (150−180 μmol), BzOH (15 μmol), and TFE (0.3 mL) were heated at 80 °C until TLC showed that **3** was consumed (2−24 h); (b) 40 °C; (c) (2*R*, 2′*R*)-**3** used; (d) 2.5 equiv of BzOH; (e) Glu(OMe)-OMe gave **1o** and **5**.

more sterically encumbered Val-OMe as substrate, however, the reaction required 2.5 days at 80 °C. Monitoring (^{1}H NMR, TLC, HPLC−MS) revealed rapid formation and buildup of linear intermediate from epoxide opening, suggesting annulation was the slower step. In TFE, catalytic benzoic acid (0.3 equiv) promoted γ-lactam formation within 1 day (**1g**, Figure 3). The TFE/catalytic BzOH combination proved effective with a variety of α-amino esters (e.g., **1h**−**j**, Figure 3). The nucleophilic phenol of unprotected Tyr-OMe was tolerated (**1k**). β- and γ-amino ester substrates, benzyl β-alaninate and methyl *m*-aminobenzoate, gave, respectively, 63% and 49% yields of **1l** and **1m**. Lower reaction temperature (40 °C) mitigated Fmoc deprotection using Gly-OBn to make **1n**. The methyl ester side chain of dimethyl glutamate competed in the annulation to **1o** producing pyroglutamate **5**. Enantiomeric (2*R*,2′*R*)-**3** reacted with D-Val-OMe providing access to (2*R*,3′*R*,4′*S*)-**1g**.

The configurational lability of **3** was examined by heating to 80 °C for 1 day, revealing 3% epimerization of the α-center and 3% racemization, which may be rationalized by the reversible ring opening of the oxiranyl moiety.[15,21] Moreover, when Val-OMe reacted with **3** under standard reaction conditions, HPLC analysis of the crude revealed that ca. 10% epimer was incorporated into the corresponding γ-lactam product **1g**.

The hydroxy group was further elaborated (Scheme 2). Phosphorylated dipeptide **6** was made from alcohol **1g** using

Scheme 2. Phosphorylation and Bromination of γ-Lactam[a]



[a] Double-headed arrow represents NOESY correlations.

$POCl_3$ and 2,6-lutidine, followed by a methanol quench. Dehydroxybromination of **1g** with PPh_3Br_2 occurred with inversion, providing access to lactam **7**. The stereochemistry of **7** was assigned by examining the relative intensity of the magnetization transfer between the lactam α-proton and the other ring hydrogens.[17]

Lactam dipeptide has been employed in solid-phase synthesis of peptide mimics.[22] A more modular approach was examined to install directly α-amino-β-hydroxy-γ-lactam onto the N-terminal of solid-supported peptide. Peptide 101.10 (rytvela) is an allosteric modulator of the interleukin 1 (IL-1) receptor, which has potential clinical applications

(15) The enantiomeric purity of **3** was ascertained by chiral SFC chromatography to be of >96%. The major diasteriomer was assigned by conversion of (2*S*,2′*S*)-*N*-(Cbz)oxiranylglycine methyl ester into **3** under hydrogenative conditions: Dzubeck, V.; Schneider, J. P. *Tetrahedron Lett.* **2000**, *41*, 9953.

(16) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(17) See the Supporting Information for details.

(18) (2*S*, 2′*S*)-*N*-(Cbz)oxiranylglycine methyl ester has been reported to form lactams on reactions with H_2NOBn and Gly; see ref 9a and: Tashiro, T.; Fushiya, S.; Nozoe, S. *Chem. Pharm. Bull.* **1988**, *36*, 893.

(19) For example, BF_3•Et_2O, 34%; TsOH•H_2O, 46%.

(20) (a) Philippe, C.; Milcent, T.; Crousse, B.; Bonnet-Delpon, D. *Org. Biomol. Chem.* **2009**, *7*, 2026. (b) Das, U.; Crousse, B.; Kesavan, V.; Bonnet-Delpon, D.; Bégué, J.-P. *J. Org. Chem.* **2000**, *65*, 6749. (c) Westermaier, M.; Mayr, H. *Chem.—Eur. J.* **2008**, *14*, 1638. (d) Review: Bégué, J.-P.; Bonnet-Delpon, D.; Crousse, B. *Synlett* **2004**, *1*, 18.

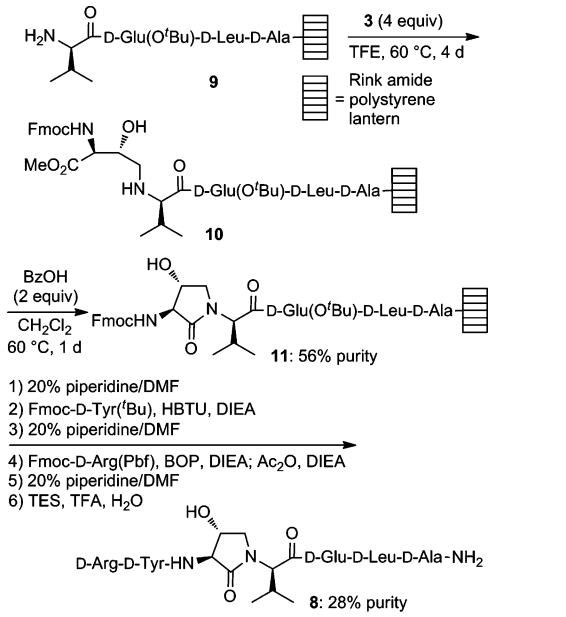
(21) (2*S*,2′*S*)-*N*-(Cbz)oxiranylglycine methyl ester β-eliminated to afford 3-(Cbz-amino)furan-2(5*H*)-one.[12] Reversible β-elimination may explain racemization.

(22) Galaud, F.; Demers, A.; Ong, H.; Lubell, W. D., *Understanding Biology Using Peptides*; Blondelle, S. E., Ed.; Springer: New York, 2006; pp 188−189.

(23) (a) Quiniou, C.; Sapieha, P.; Lahaie, I.; Hou, X.; Brault, S.; Beauchamp, M.; Leduc, M.; Rihakova, L.; Joyal, J.-S.; Nadeau, S.; Heveker, N.; Lubell, W. D.; Sennlaub, F.; Gobeil, F., Jr.; Miller, G.; Pshezhetsky, A. V.; Chemtob, S. *J. Immunol.* **2008**, *180*, 6977. (b) Chemtob, S.; Quiniou, C.; Lubell, W. D.; Beauchamp, M.; Hansford, K. A. US Patent No. 20,060-094,663, 2006. (c) Quiniou, C.; Kooli, E.; Joyal, J.-S.; Sapieha, P.; Sennlaub, F.; Lahaie, I.; Zhuo, S.; Hou, X.; Hardy, P.; Lubell, W.; Chemtob, S. *Semin. Perinatol.* **2008**, *32*, 325.

in inflammation.[23] It was chosen as a challenging target because the Thr to be replaced in lactam **8** preceded a sterically encumbered D-Val residue (Scheme 3). Synphase

Scheme 3. Solid-Phase Synthesis of Constrained Peptide Mimics



lantern-supported vela peptide **9** was reacted with **3** in TFE at 60 °C for 4 days, followed by 1 day in CH_2Cl_2/BzOH. Lactam **11** was assessed to be of 56% purity after TES/TFA/H_2O cleavage of a sliver of the lantern, followed by HPLC−MS analysis; linear alkylation product **10** was the major impurity (9% conversion). After Fmoc group removal with 20% piperidine/DMF, the remaining residues were added using standard solid-phase peptide synthesis.[24] Cleavage of the peptide from the support gave a 16:5:1 mixture of closely eluting isomers. The purity of the major isomer was assessed at 28%, from which 0.6 mg of 96.7% pure isomer assigned as **8** (0.4% yield overall) was isolated along with mixed fractions. Using (2*R*,2′*R*)-**3**, the above synthesis equally produced 0.5 mg of the diasteriomeric (3*R*,4*S*)-lactam counterpart.

In the context of solid-supported peptide synthesis, elaboration of the hydroxy group may allow mimicry with other Ser/Thr residues, attached to carbohydrate, phosphonate, sulfate, ester, and ether moieties. Oxiranylglycine **3** has thus proven effective for the synthesis of α-amino-*β*-hydroxy-*γ*-lactams in the context of structure−activity relationships of Ser/Thr-containing peptides.

Acknowledgment. Financial support from the Natural Sciences and Engineering Research Council of Canada, le Fond Québécois de la Recherche sur la Nature et les Technologies, and the Canadian Institutes of Health Research Team Grant Program (funding no. CTP79848) in G-Protein Coupled Receptor Allosteric Regulation is gratefully acknowledged. We thank the following current/former members of the Département de Chimie, Université de Montréal: Dr. Alexandra Fürtös, Marie-Christine Tang, and Karine Venne for assistance in LC−MS and HRMS analyses, Jad Tannous for conducting SFC analyses, Dr. Phan viet Minh Tan, Dr. Cédric Malveau, and Sylvie Bilodeau for assistance with NMR spectroscopy, and Dr. Luisa Ronga for help with solid-phase peptide synthesis.

Note Added in Proof. In the course of review, the following publication was reported in which a complementary method featuring *N*-(Cbz)oxiranylglycine was employed to make dipeptide building blocks that were inserted into longer peptides: Sicherl, F.; Cupido, T.; Albericio, F. *Chem. Commun.* **2010**, *46*, 1266.

Supporting Information Available: Full details on the preparation and characterization of synthetic products. This material is available free of charge via the Internet at http://pubs.acs.org.

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