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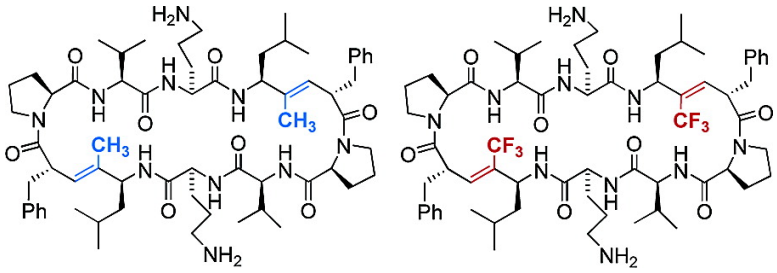

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Electrostatic versus Steric Effects in Peptidomimicry: Synthesis and Secondary Structure Analysis of Gramicidin S Analogues with (*E*)-Alkene Peptide Isosteres

Jingbo Xiao,[§] Bernard Weisblum,[#] and Peter Wipf*[,§]

Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, and Pharmacology Department, University of Wisconsin Medical School, 1300 University Avenue, Madison, Wisconsin 53706

Received February 16, 2005; E-mail: pwipf@pitt.edu

Transition state mimicry of the tetrahedral intermediate of peptide bond hydrolysis in serine, cysteine, and aspartic protease inhibitors has been very successful,[1] but effective ground state isosteric and isoelectronic analogues of the amide bond have yet to be developed.[2] Rigid (*E*)-alkenes provide a suitable fit for the $C_i(\alpha)-C_{i+1}(\alpha)$ distance (3.8 Å) in the peptide linkage,[3] and we have developed stereospecific syntheses of trisubstituted (*E*)-alkene dipeptide isosteres (TEADIs) that are conformationally preorganized into β-turns.[4] Furthermore, we have postulated that a CF_3-substituted (*E*)-alkene (μ = 2.3 D) allows for an improved mimicry of the electrostatic potential surface of the parent amide bond as well as its large dipole moment (μ = 3.6 D).[5] We now describe the synthesis and conformational evaluation of *cyclo*[(-Val-Orn-Leu-ψ[(*E*)-C(R)=CH]-^{D}Phe-Pro-)$_2$], with R=CF_3 and CH_3, which represent the first alkene peptide analogues of the cyclodecapeptide antibiotic Gramicidin S (GS). The location of the amide bond replacements at a critical hydrogen bond acceptor position in the β-hairpin motif was selected in order to probe the effects of polar versus steric peptide bond mimicry (Figure 1).

GS is a broadly utilized scaffold for the study of the effects of turn inducers and peptide mimetics.[6] The rigid, amphipathic antiparallel β-pleated sheet is held in place by two type II′ β-turns (at both ^{D}Phe-Pro positions) and four intramolecular hydrogen bonds between the valine and leucine residues.[7] GS displays antibiotic activity against a wide spectrum of both Gram-negative and Gram-positive bacteria, as well as against several pathogenic fungi.[8]

The preparation of TEADIs **6a** and **6b** was facilitated by a new transition metal based methodology for synthesis of allylic amides (Scheme 1).[9] Hydrozirconation[10] of **2a**[11] with Cp_2ZrHCl followed by transmetalation to Me_2Zn and addition of *N*-Boc-isovaleraldimine afforded the allylic amide **3a** as a 1:1.5 mixture of diastereomers (desired favored), which were separated after desilylation and acetylation.[12] Deprotection of **4a** and oxidation provided the methylated TEADI **6a**. The trifluoromethylated **6b** was prepared using an indirect route. Hydrozirconation−iodination of stannyl alkyne **2b**[11] afforded the vinyl iodide which was subjected to lithium−halogen exchange and addition to imine to provide **3b** as a 1:1.5 mixture of diastereomers (desired favored). Iododestannylation of **3b**, separation of the diastereomers, and Cu-mediated CF_3-coupling[13] followed by deacetylation and two-step oxidation[14] gave the CF_3-substituted TEADI **6b**.

The fragment assembly of TEADIs **6** and H-Pro-Val-Orn(Cbz)-OMe (**10**) was accomplished using EDC as a coupling reagent to provide the pentapeptides **7**. Stepwise coupling proceeded smoothly to afford the linear decapeptides **8**. Saponification of **8** followed by *N*-Boc removal and macrolactamization afforded the desired GS

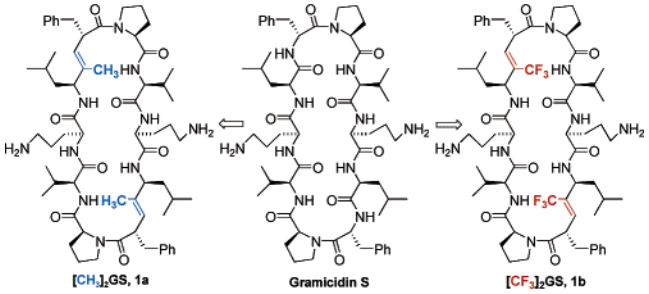
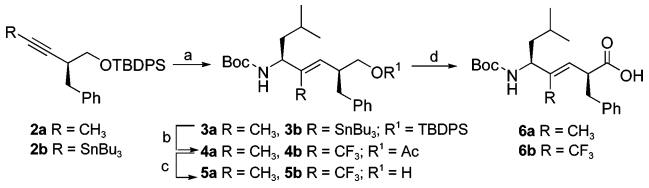


Figure 1. Gramicidin S and two (*E*)-alkene isostere analogues.

Scheme 1. Synthesis of TEADI Building Blocks **6a** and **6b**[a]



[a] (a) **3a**: Cp_2ZrHCl, Me_2Zn, *N*-Boc-isovaleraldimine, 70%; **3b**: (i) Cp_2ZrHCl, I_2, 80%; (ii) ^{t}BuLi, *N*-Boc-isovaleraldimine, 70%. (b) **4a**: (i) TBAF, 70%; (ii) Ac_2O, TEA, DMAP, 92%; **4b**: (i) NIS, 80%; (ii) TBAF, 84%; (iii) Ac_2O, TEA, DMAP, 99%; (iv) $FSO_2CF_2CO_2Me$, CuI, 92%. (c) K_2CO_3, MeOH; **5a**, 90%; **5b**, quant. (d) (i) **6a**: Dess−Martin periodinane; **6b**: TEMPO, trichloroisocyanuric acid; (ii) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene.

analogues **9a** and **9b** (Scheme 2). Variable temperature NMR was used to probe the degree of intramolecular hydrogen bonding in **9a**, **9b**, and Orn-δ-NHCbz-protected GS (**Cbz$_2$GS**). The amide NH protons in leucine and valine residues of all derivatives had temperature coefficients between −4 and 0 ppb/K, which supported intramolecular hydrogen bonding at these sites.[15] Moreover, NOESY studies showed interstrand NH(Leu)-NH(Val) and NH-(Leu)-Hα(Orn) nOe in all three compounds.

As expected, CD spectra of **9a** and **9b** were more highly perturbed over the natural product (Figure 2). The presence of a negative band at ∼205−225 nm for **9b** and **Cbz$_2$GS** is consistent with a combination of type II′ β-turn and β-sheet conformations.[16] In contrast, the CD spectra of **9a** have a negative band centered at 204 nm followed by a positive band at 220 nm, consistent with a disordered peptide conformation.[16] Overall, spectroscopic analysis of the solution conformation of **9a** and **9b** confirmed that the electronic properties of the trifluoromethyl group promoted a preference for the native pseudo type II′ β-turns and backbone β-sheet and resulted in superior mimicry of the conformational properties of the parent cyclopeptide.

In the solid state, **9b** indeed adopts the pleated antiparallel β-sheet conformation with two hydrogen bonds (1.96 and 2.00 Å, respec-

[§] University of Pittsburgh.
[#] University of Wisconsin Medical School.

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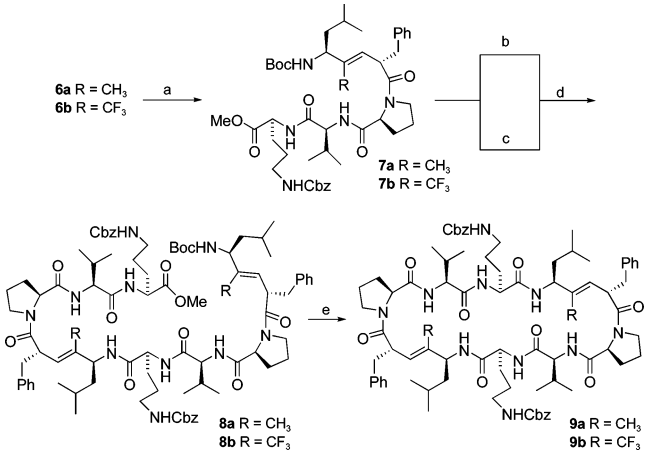
Scheme 2. Synthesis of GS Analogues **9a** and **9b**[a]



[a] (a) H-Pro-Val-Orn(Cbz)-OMe (**10**), EDC, HOBt, DMAP; **7a**, 94% from **5a**; **7b**, 77% from **5b**; (b) 1.0 N NaOH; (c) 4.0 N HCl in dioxane; (d) EDC, HOBt, DMAP; **8a**, 97% from **7a**; **8b**, 85% from **7b**; (e) (i) 1.0 N NaOH; (ii) 4.0 N HCl in dioxane; (iii) EDC, HOBt, DMAP; **9a**, 42%; **9b**, 42% (after reverse phase semi-prep HPLC purification).

Table 1. Tabular Display of Amide Proton Temperature Shift Coefficients ($\Delta\delta/\Delta T$) of **9a**, **9b**, and **Cbz$_2$GS** in DMSO-d_6 [ppb/K]

	Cbz$_2$GS	**9a**	**9b**
Orn-NH	−6.7	−7.0	−4.6
Leu-NH	−3.8	−3.1	−2.9
Orn-δ-NH	−8.6	−8.4	N/A[a]
Val-NH	−2.2	−0.4	−0.01
^{D}Phe-NH	−11.8	N/A	N/A

[a] Signal obscured in ^{1}H NMR.

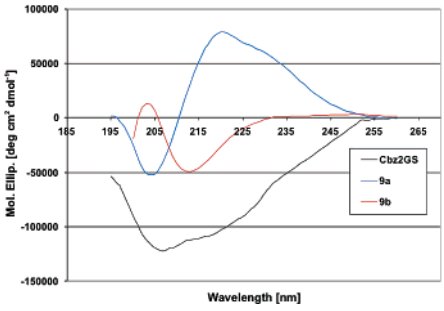


Figure 2. Circular dichroism spectra of **9a**, **9b**, and **Cbz$_2$GS** in EtOH.

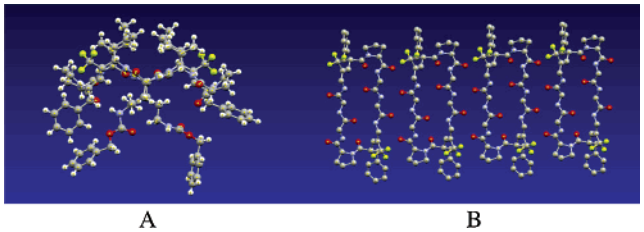


Figure 3. X-ray structure of **9b**: (A) side view; (B) top view of four molecules (side chains were truncated for clarity).

tively) between NH(Leu) and C═O(Val) that is typical of GS (Figure 3). The ^{D}Phe-Pro type II′ β-turn has an ideal set of dihedral angles ($\phi_2 = 137°$, $\psi_2 = -95°$, $\phi_3 = -82°$, $\psi_3 = -5.7°$) and is apparently not perturbed by the adjacent CF_3-substituted (*E*)-alkene.[17] The plane of the double bond is, however, twisted by 70° away from the interior of the β-turn compared to that of the analogous amide, leading to a fluorine−hydrogen distance of 4.2 Å to NH(Val), most likely due to the bulk of the CF_3 group which cannot easily be accommodated inside the hairpin. The rest of the peptide backbone, including the remaining two intramolecular hydrogen bonds, is only minimally perturbed by this movement of the plane of the trisubstituted alkene. The ornithine side chains extend in an orthogonal fashion away from the perimeter of the β-sheet, a feature that is essential for antibiotic activity.[6,8] Indeed, the Orn-δ-amino deprotected **1a** and **1b** demonstrated functional mimicry of the natural product with MICs of 5−15 μg/mL, equivalent to GS, against *Bacillus subtilis*.[18]

In conclusion, a concise synthetic strategy was used for the first preparation of GS analogues with trisubstituted (*E*)-alkene peptide bond replacements. Solution and solid state conformational analysis demonstrated that the bistrifluoromethylated analogue **9b** was a superior mimic of the natural product, whereas the incorporation of methyl groups into the alkene peptide isostere **9a** led to a far greater perturbation of the secondary structure features of GS. The difference between CF_3- and CH_3-substitution can be explained by the enhanced electrostatic carbonyl group mimicry of the former function.

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Supporting Information Available: Crystal information files (CIF) for compounds **5a** and **9b**. Experimental procedures, ^{1}H and ^{13}C spectra for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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