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Trisubstituted (*E*)-Alkene Dipeptide Isosteres as β-Turn Promoters in the Gramicidin S Cyclodecapeptide Scaffold

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Abstract

A concise synthesis of a gramicidin S analogue with trisubstituted (*E*)-alkene dipeptide isostere (TEADI) replacements at both ^{D}Phe-Pro positions was realized. Conformational analysis demonstrated that TEADIs can serve as type II β-turn promoters in a cyclic scaffold and successfully mimic a proline residue.

Peptides demonstrate a wide range of diverse physiological properties as hormones, enzyme inhibitors, growth promoters, signaling pathways modulators, antimicrobial agents, etc.[1] In order to overcome the limited bioavailability of peptides,[2] we are studying the synthesis and pharmacological evaluation of bioisosteric replacements of the amide bond.[3] The relatively rigid trisubstituted (*E*)-alkene dipeptide isosteres (ψ[(*E*)-C(R)=CH], R≠H, TEADIs) maintain ω-angle planarity and represent useful structural surrogates of hydrolytically labile amide bonds.[4] In addition, TEADIs were found to have potential as β-turn promoters in acyclic sequences in our previous studies.[5] In a continuation of this work, we have now started to introduce these building blocks into biologically active cyclic peptide sequences as surrogates of the powerful turn-inducing ^{D}Phe-Pro sequence[6] and evaluate their potential as β-turn promoters as well modulators of biological and metabolic properties.[7]

Since its discovery in 1942,[8] the cyclodecapeptide antibiotic Gramicidin S (GS, *cyclo*[(Val-Orn-Leu-^{D}Phe-Pro-)$_2$]) has served as an inspiration for the design of antibacterial agents and antimicrobial peptides, as well as a model system for conformational mimicry.[9] GS is therefore a particularly significant target for the evaluation of alkene peptide isosteres as surrogates for the type II' β-turn inducing sequence.

The ^{D}Phe-Pro reverse turn is a critical feature of the rigid amphipathic antiparallel β-pleated sheet conformation of GS.[10,11] In earlier work,[7] we were able to replace the Leu-^{D}Phe peptide bond in GS with a ψ[(*E*)-C(CF_3)=CH] isostere with minimal perturbation of secondary structure and biological activity, while the corresponding ψ[(*E*)-C(CH_3)=CH] isostere failed to maintain the β-pleated sheet conformation according to CD and NMR analyses. A different situation presents itself when the ^{D}Phe-Pro peptide bond is replaced. Since the ^{D}Phe carbonyl group is not involved in intramolecular H-bonding or dipolar interactions, a ψ[(*E*)-C(CH_3)=CH] surrogate should be as effective as a trifluoromethylated congener in conformationally preorganizing the chain. The restricted backbone rotation imposed by the $A^{1,3}$-strain across the trisubstituted alkene, and, to a lesser extent, the $A^{1,2}$-strain experienced by substituents

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attached to the alkene, should be sufficient for both methyl and trifluoromethyl groups to impose the reverse turn. Furthermore, neither alkene isostere provides an NH hydrogen bond donor group that can lead to the stabilization of γ-turns or other competitive backbone folding patterns that would interfere with the desired β-turn motif. These properties lead, theoretically, to a close match of isostere and ^{D}Phe-Pro features (Figure 1)[5b] We now report an experimental confirmation of this hypothesis.

Due to the lability of phenylacetaldimines, the sulfinyl adduct **3**[12] was employed in the organometallic allylation reaction (Scheme 1).[13] Using our hydrozirconation/Zr⇨Zn transmetalation methodology,[14,15] the alkenylzinc species derived from the chiral internal alkyne **2**[13b] was added to **3**, affording the allylic amide **4** in 64% yield as a ~1:1 mixture of diastereomers. Deprotection of the TBDPS group with TBAF provided the primary alcohol **5**. The two diastereomers could not be separated after conversion of **5** to the corresponding acetates;[3f,7] however, a two-step oxidation with Dess-Martin periodinane,[16,17] followed by coupling with valine methyl ester provided *pseudo*-tripeptides **6** and **7** which were separated by preparative C_{18} RP HPLC.[18]

Saponification of *pseudo*-tripeptide **7** followed by fragment coupling with dipeptide H-Orn (Cbz)-Leu-OMe in the presence of EDC as a coupling reagent afforded the *pseudo*-pentapeptide **8** in 96% yield over two steps. We initially envisioned a one-pot dimerization-cyclization of **8**; however, this approach resulted exclusively in the formation of cyclized *pseudo*-pentapeptide. In contrast, the stepwise coupling proceeded smoothly to give the *pseudo*-decapeptide **9** in excellent yield. Saponification of **9** and stepwise removal of the Boc protecting group followed by macrolactamization afforded the desired *bis*-Cbz-protected GS analogue **10** in 50% yield after preparative C_{18} RP HPLC purification (Scheme 2).

The chemical shifts of all amide protons in **10** were assigned using a combination of COSY, NOESY, HMQC and HMBC data sets collected in DMSO-d_6 at 338 K, since some amide ^{1}H NMR signals were obscured at 298 K. Variable temperature NMR was applied to probe the conformation of **10** in solution and determine the level of intramolecular hydrogen bonding (Table 1). Temperature shift coefficients for **10** were in close agreement with the values for *bis*-Cbz-protected GS (**Cbz$_2$GS**).[7] The NH shifts of Leu and Val residues in **10** showed small temperature coefficients of −1.9 and −1.8 ppb/K, respectively, whereas Orn-NH and ^{D}Phe-NH were solvent exposed, thus indicating a hydrogen bonding array typical for an antiparallel β-pleated sheet conformation.[19] Furthermore, NOESY spectra showed transannular Leu-NH–Val-NH and Val-NH–^{D}Phe-Hαcontacts for **10** in agreement to what was found for **Cbz$_2$GS** (Figure 2). The resulting ten-membered intramolecular H-bonding interaction between the valine amide NH and the carbonyl group of the leucine residue is typical for a type II' β-turn.[20]

Further confirmation of the close match between the secondary structures of **10** and **Cbz$_2$GS** was provided by circular dichroism (CD) spectra in EtOH (Figure 3). The strong negative band at ~205–225 nm and a shoulder in the region of 225–235 nm for both **10** and **Cbz$_2$GS** is consistent with a combination of a type II' β-turn and a β-sheet conformation in these compounds.[21] This result, along with the data from NOESY and variable temperature NMR experiments, further confirmed that the methyl (*E*)-alkene dipeptide isostere replacements at the former ^{D}Phe-Pro positions strongly promoted the archetypical architecture of the parent peptide, gramicidin S. An MMFF-minimized structure for **1** that is in agreement with all experimental data is shown in Figure 4.[22]

Our previous biological studies showed that free amine functions on the ornithine side chains were necessary to retain the antibacterial, antifungal, and hemolytic activities of GS.[7] The Cbz protecting groups in **10** were successfully removed by hydrogenolysis in the presence of 10%

Pd/C in a 0.02 M HCl/MeOH solution, without concomitant reduction of the trisubstituted (*E*)-alkene moieties (Scheme 3). As expected, the hydrochloride salt of **1** also exhibited functional mimicry of the natural product, with an MIC of ~20 μg/mL against *Bacillus subtilis*, and thus equipotent with GS hydrochloride (MIC ~15 μg/mL in the same assay).

The development of proline mimics is of considerable current interest, due to the helix-breaking and unique conformational properties of this amino acid residue.[23] We have now demonstrated that a trisubstituted (*E*)-alkene dipeptide isostere can serve as a bioisosteric replacement for the ^{D}Phe-Pro type II' β-turn in the cyclopeptide antibiotic gramicidin S. The solution conformational analysis and the biological assay of analogues **10** and **1**, respectively, provides a strong validation of our design principles. Furthermore, the hydrozirconation/Zr⇨Zn transmetalation/imine addition methodology was key to a rapid synthetic access to the target compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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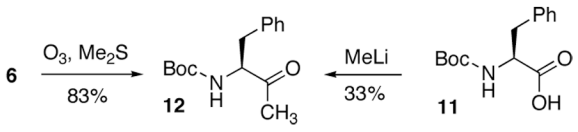
18. The structural assignment of **6** and **7** was based on the ozonolysis product **12**, which was identical to the methylation product obtained from Boc-^{D}Phe-OH (**11**) by HPLC co-injection (Pace RD, Kabalka GW. J. Org. Chem 1995;60:4838.).



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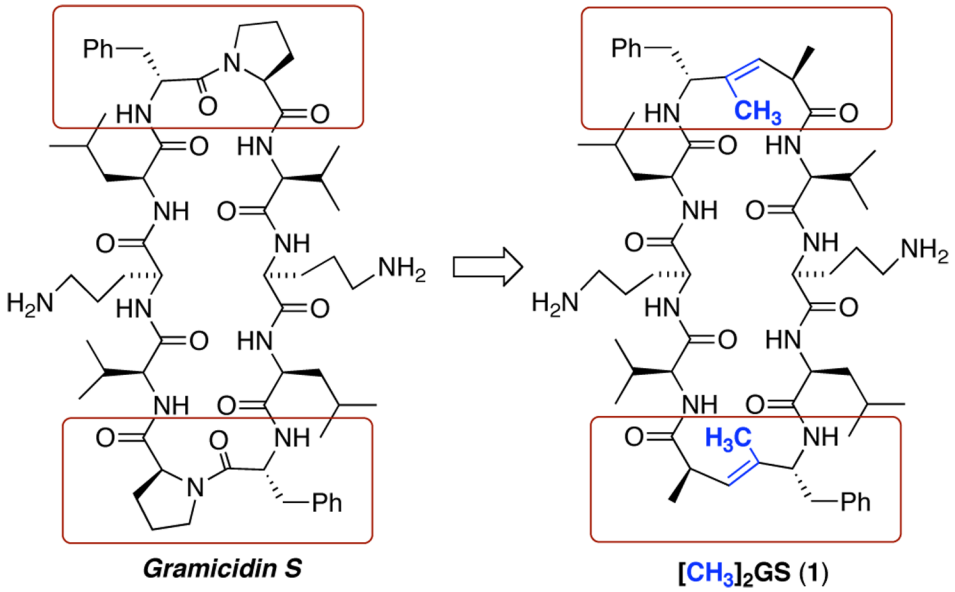


Figure 1.
GS and analogue with ψ[(*E*)-C(CH_3)=CH] peptide bond surrogates.

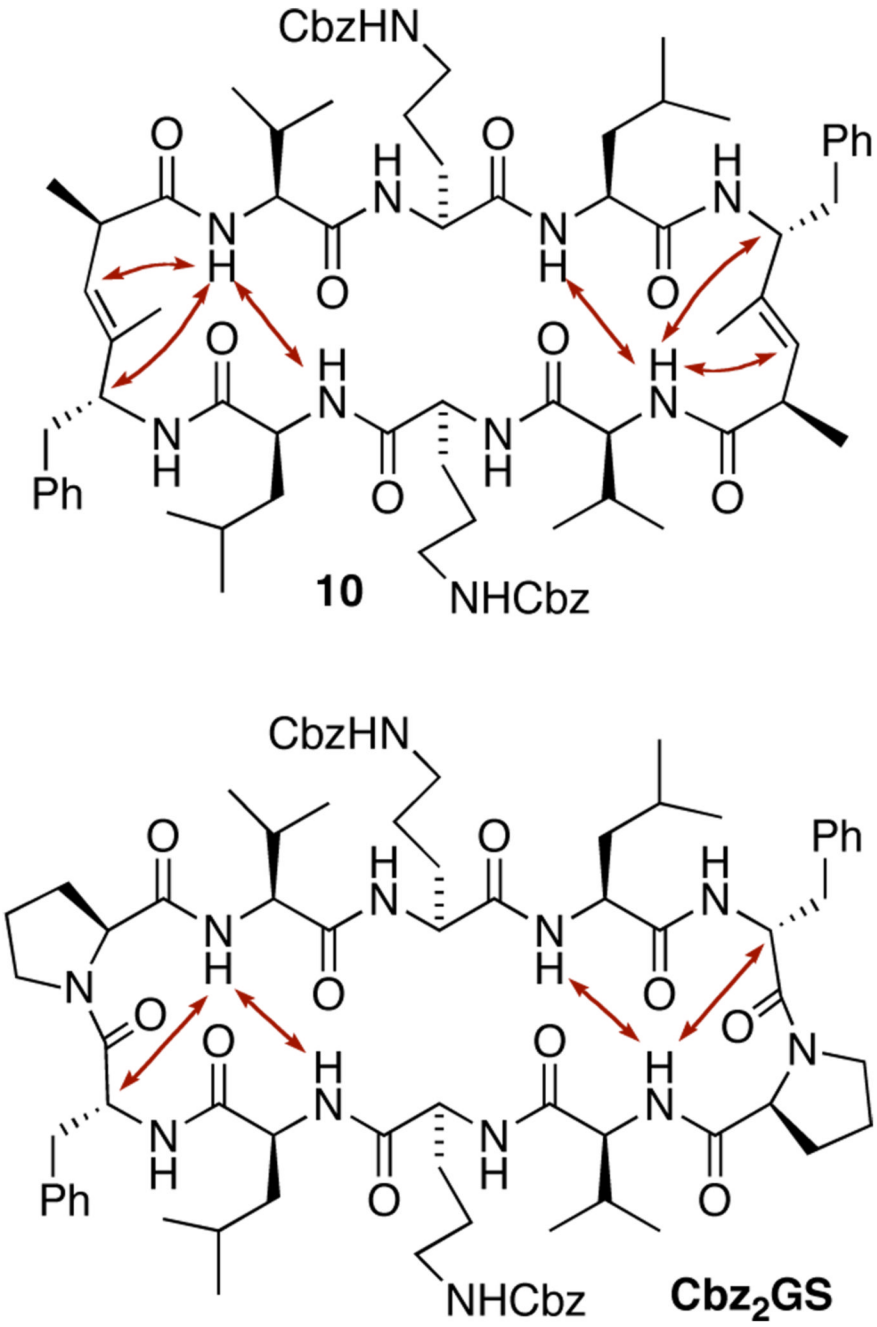


Figure 2.
Observed NOEs for **10** and **Cbz$_2$GS**.

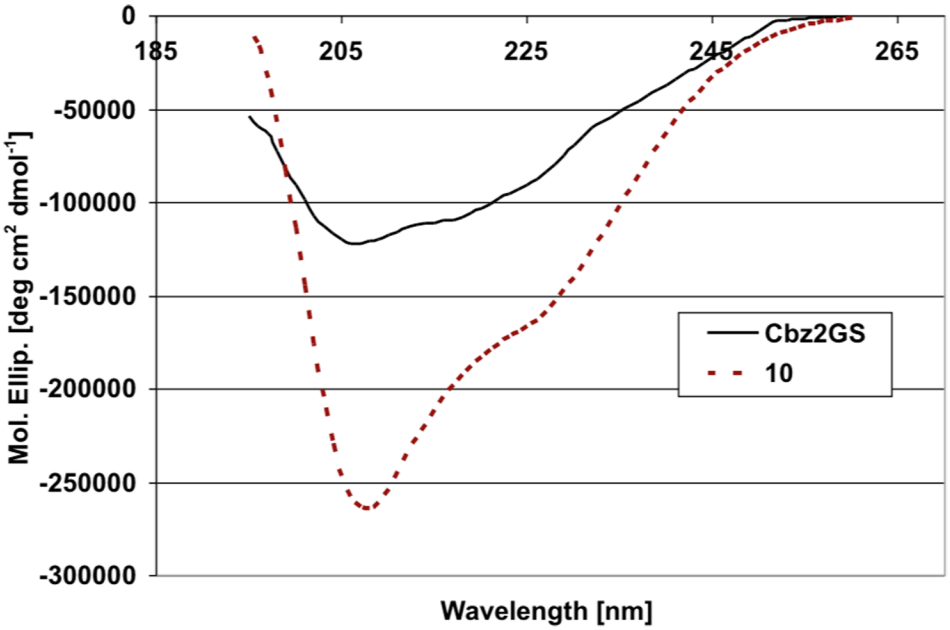


Figure 3.
CD Spectra of **10** and **Cbz$_2$GS** in EtOH.

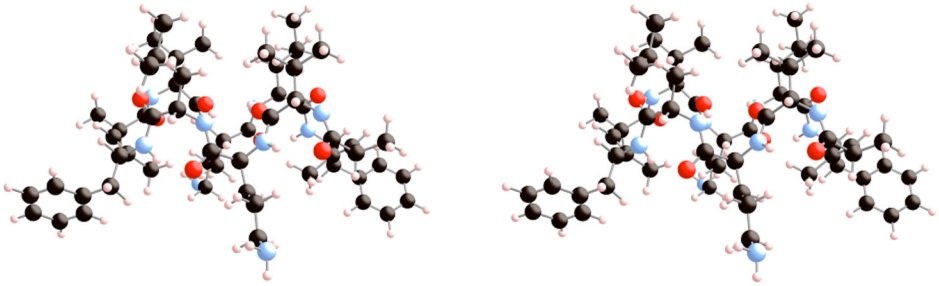
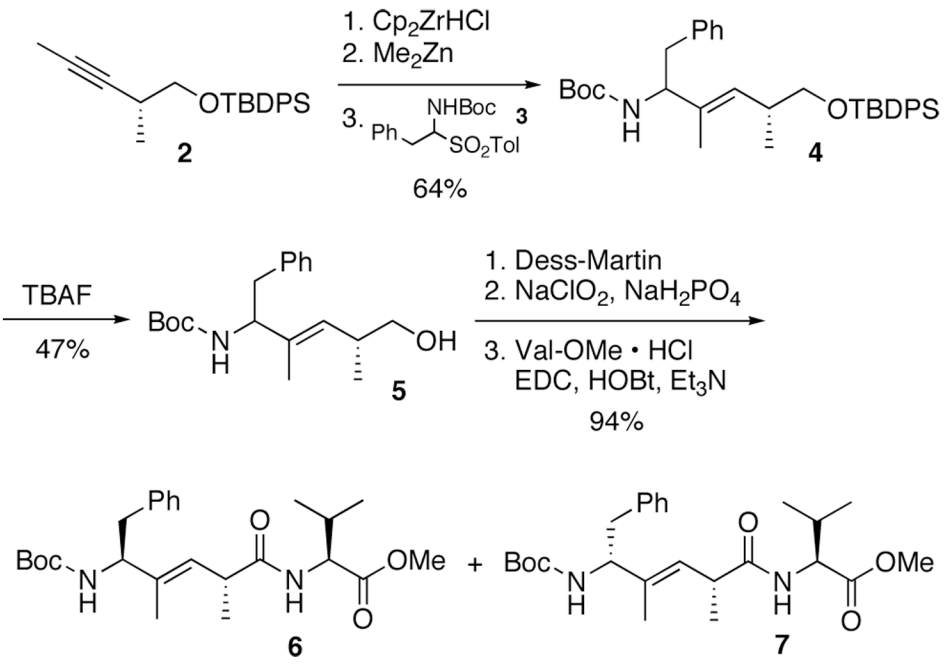
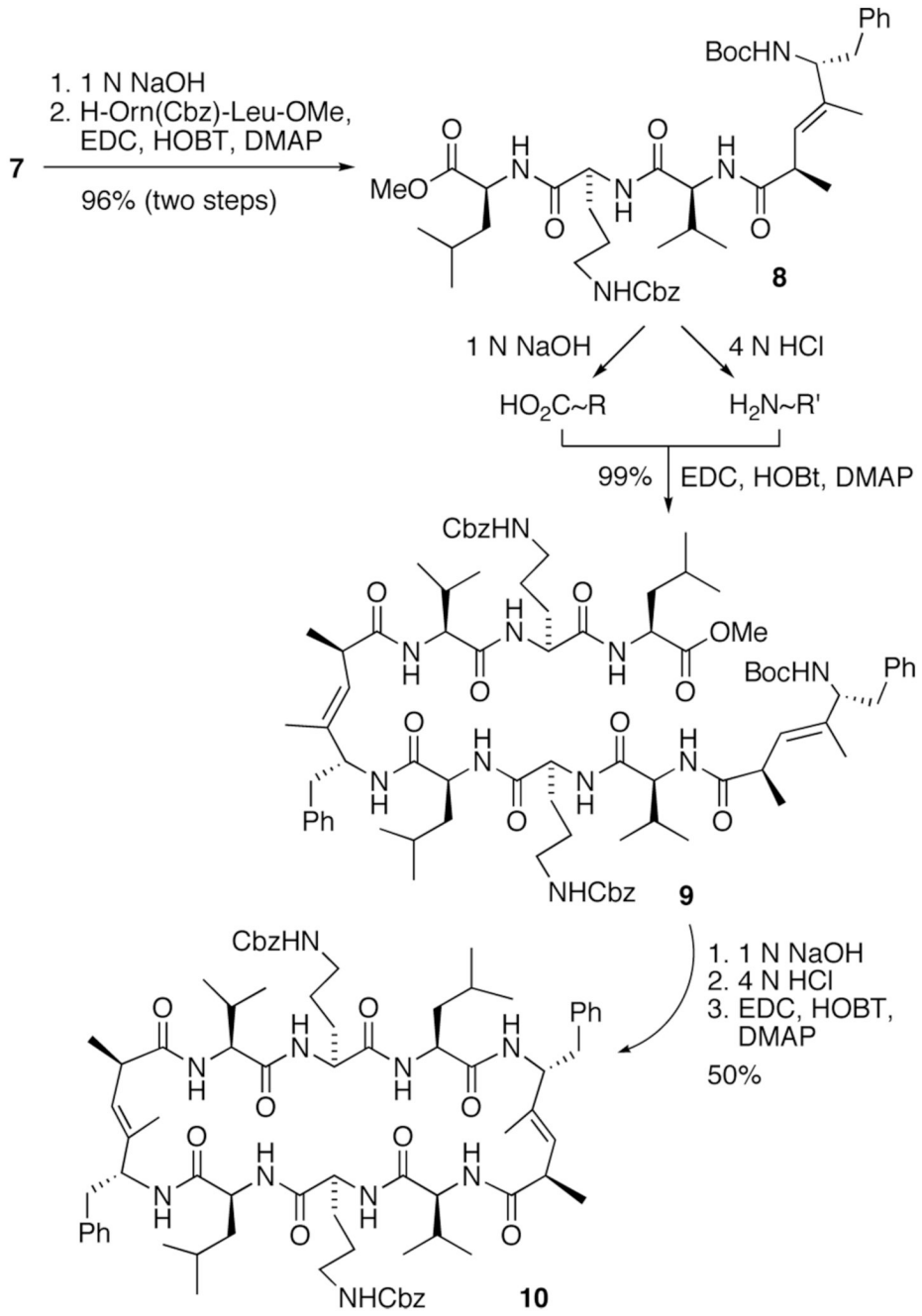


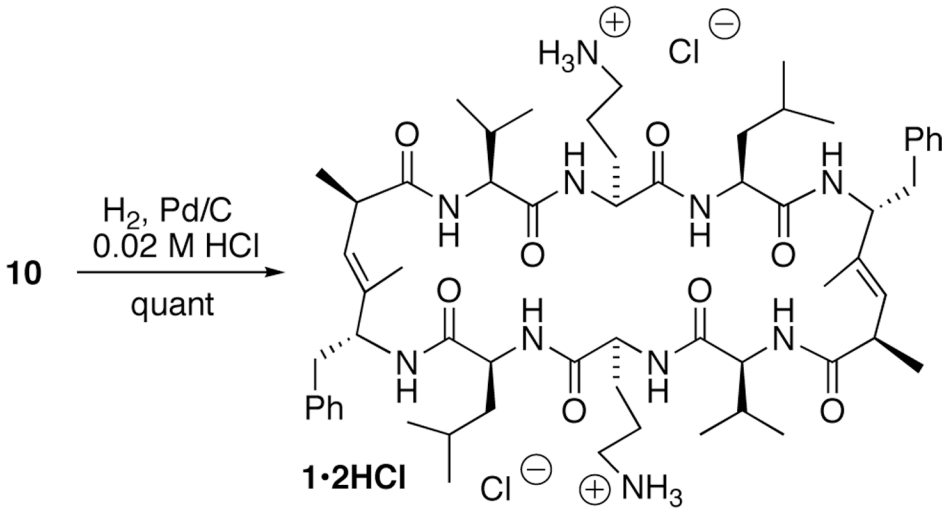
Figure 4.
Stereoview of the minimized structure of **1** derived from an MMFF conformational search algorithm.



Scheme 1.
Synthesis of Boc-^{D}Phe-ψ[(*E*)-C(CH_3)=CH]-Pro-Val-OMe



Scheme 2.
Fragment condensation and synthesis of *pseudo*-cyclodecapeptide **10**



Scheme 3.
Synthesis of the hydrochloride salt of $[CH_3]_2$GS (**1•2HCl**)

Table 1

Temperature Shift Coefficients (Δδ/ΔT) of Amide Protons in DMSO-d_6 [ppb/K] for **10** and **Cbz$_2$GS**

	Orn-NH	Leu-NH	Orn-δ-NH	Val-NH	^{D}Phe-NH
10	−4.8	−1.9	−6.3	−1.8	−5.9
Cbz$_2$GS	−6.7	−3.8	−8.6	−2.2	−11.8