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Synthesis of a Thioether Analog of the Macrocyclic Tripeptide K-13

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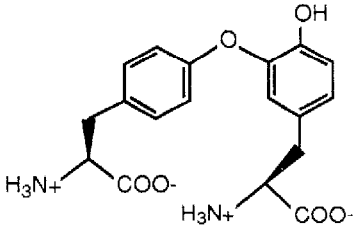
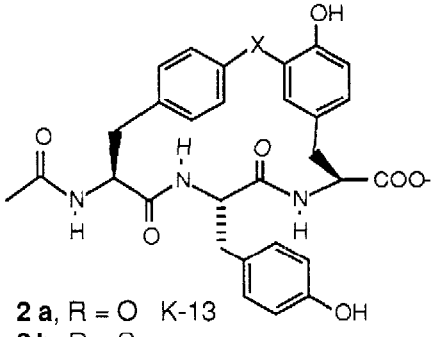
Summary Starting from tyrosine and phenylalanine, a sulfur-containing analog of K-13 (**2a**), a naturally occurring the angiotensin I converting enzyme (ACE) inhibitor was synthesized. The diphenyl thioether moiety was constructed by an $S_{RN}1$ reaction. The conformation of the product was probed by NMR and modeling, and a ground state conformation is proposed.

In recent years, a large number of peptidic natural products containing the novel amino acid isodityrosine (1) have been isolated. When such a sidechain-linked amino acid is built into a peptide, then the result of the sidechain linkage is a decrease in the conformational heterogeneity of the peptide. If rigidification is the primary function of the link, then structurally similar linkages could serve as well. In the case of diphenyl ethers such as isodityrosine, a synthetically convenient replacement for O is S.[1] Although lengthened C-S bonds, a compressed C-S-C bond angle and lowered C-C-S-C rotational barriers distinguish phenyl ethers from phenyl thioethers, the overall geometries of the two moieties are similar and the S/O replacement in these materials can be a useful strategy for analog synthesis. In this letter, we describe such a replacement in the macrocyclic tripeptide K-13 (**2a**).[2] Thus we synthesize the thioether analog (**2b**) and report its conformational and biological properties.

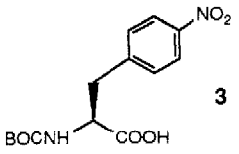


1 Isodityrosine



2a, R = O K-13
2b, R = S

As shown on the following page, the synthesis of **2b** began with BOC-L-*p*-nitrophenyl-alanine (**3**). Standard peptide coupling with L-tyrosine methyl ester (DCC, HOSu, N-methylmorpholine, CH_2Cl_2) gave **4** in 95% yield. Reduction to **5** (H_2, Pd black, MeOH; 96%)[3] followed by diazonium ion chemistry[4] (a. $NaNO_2$/HCl; b. KSCSOEt) produced the xanthate **6**. Finally, saponification (NaOH, MeOH) of the ester/xanthate, and reduction (Ph_3P, H_2O) of disulfides formed during the previous step then gave thiol acid **7** in 60% overall yield from **5**. The thiol acid was purified by flash



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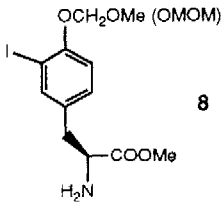

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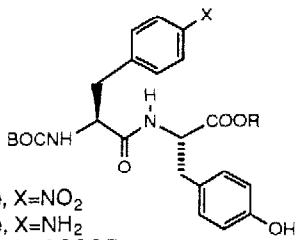
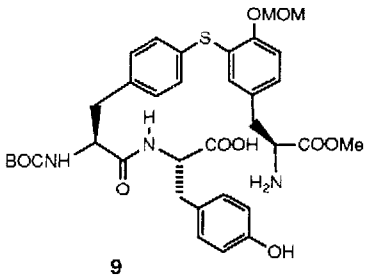


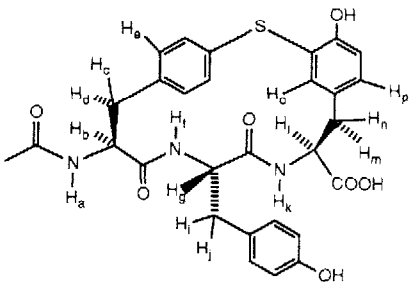
4, R=Me, X=NO_2
5, R=Me, X=NH_2
6, R=Me, X=SCSOEt
7, R=H, X=SH



9

chromatography and formed an air-stable solid upon azeotropic drying with toluene. Photoinitiated $S_{RN}1$ coupling[1] of **7** and **8**[5] in liquid ammonia provided **9** in 40% yield. Unreacted iodotyrosine **8** was isolated from the coupling reaction in 34% yield. The phenol of residue 2 appears to be responsible for the only modest yield of the coupling since a related $S_{RN}1$ coupling of **8** with the C-terminal glycine analog of **7** proceeds in 75% yield.

Macrolactamization of **9** then followed in 73% yield using diphenylphosphoryl azide (DMF, N-methylmorpholine, 5.0 mM). Finally TFA/anisole removed the BOC and MOM protecting groups, acetic anhydride (pH 7) provided the N-terminal acetamide and NaOH saponified the C-terminal methyl ester to give **2b** (85% overall). That saponification had not affected the product was shown by reesterification with CH_2N_2 to regenerate the **2b** precursor.

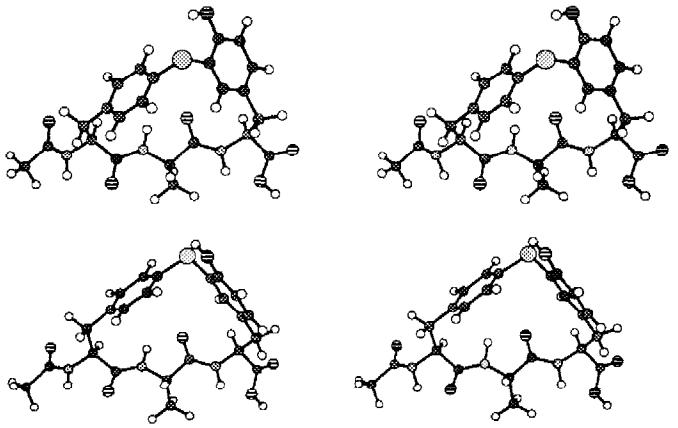


NOE (intensity)
H_b H_f (strong)
H_g H_k (strong)
H_a H_c (strong)
H_a H_d (weak)
H_e H_c (strong)
H_e H_d (strong)
H_k H_i (strong)
H_k H_j (strong)
H_b H_e (moderate)
H_l H_o (moderate)
H_o H_m (moderate)
H_p H_n (moderate)

NMR analysis of **2b** was consistent with a structure having substantial conformational homogeneity. For example, the α- and β-hydrogens of residues 1 and 3 show coupling constants which are distinct from the average values obtained for conformationally mobile systems. Thus J_{bc}=11.5, J_{bd}=5.3, J_{lm}=2.8 and J_{ln}=7.9 Hz. In contrast, the coupling constants for the acyclic sidechain of residue 2 are consistent with conformational averaging (J_{gi}=5.6, J_{gj}=6.3 Hz). We found little variation in coupling constants when the solvent was changed from d_6-DMSO to d_4-methanol. NOE measurements (see diagram above) also suggested only limited flexibility. Thus H_m

showed an NOE with H_o but not H_p, and H_n showed an NOE with H_p but not H_o. NOE's between H_b/H_f and H_g/H_k, and NH/CH_α couplings constants (J_{ab}=7.4, J_{fg}=5.2 and J_{kl}=7.5 Hz) are consistent with a β sheet-like conformation of the peptide backbone.

To find possible conformations of **2b**, we carried out a 5000-step Monte Carlo conformational search[6] in which all torsions (excluding those of the benzene rings and amides) were varied. To simplify the search, we modeled L-tyrosine-2 by L-alanine. We used the OPLS force field[7] with an aqueous solvation model[8] based on approximate solvent accessible surface areas. Considering conformers within the lowest 3 kcal/mol, the search converged in ~1000 steps. In all, we found 2, 3 and 8 conformers within 1, 2 and 3 kcal/mol of the global minimum. The two lowest energy conformers differ by only 0.4 kcal/mol (equal energy if aromatic hydrogens are included) and are shown below in stereo.



The second of the two lowest energy conformers is most consistent our NMR data. Hydrogens were first constructed on the united atom molecular mechanics structures found in the conformational search. In the second of the structures shown above, all hydrogen pairs having moderate or strong NOE's were <3.0Å. Coupling constants were calculated using standard equations ($NH\text{-}CH_\alpha$[9] and $CH_\alpha\text{-}CH_\beta$[10]) and weighted by the Boltzmann populations of the conformers based on OPLS energies. As shown in the table below, the correlation is good. The calculated $NH\text{-}CH_\alpha$ backbone J's are within experimental error. The $CH_\alpha\text{-}CH_\beta$ coupling constants for **2b** and K-13 (**2a**) are also very similar and suggest that **2a** and **2b** have similar conformational properties.

	2b, J Observed	**2b**, J Calculated	**2a**, J Observed[2]
H_a - H_b	7.4	7.3	11
H_b - H_c, H_d	11.5, 5.3	11.8, 3.6	11.8, 5.3
H_f - H_g	5.2	5.4	11
H_k - H_l	7.5	7.2	11
H_l - H_m, H_n	2.8, 7.9	1.6, 10.7	3.1, 7.8
H_g - H_i, H_j	5.6, 6.3	-	5.3, 6.0

Finally, our sulfur analog **2b** is an effective ACE inhibitor with a potency ~1/10 that of K-13 (**2a**) itself. In an assay carried out at Merck Sharp & Dohme, **2a** and **2b** had IC_{50}'s of 0.09 (±0.02) and 1.04 (±0.03) μM respectively against ACE from albino rabbit lung.[12]

In conclusion, we have found the $S_{RN}1$ reaction to provide a mild and effective method for linking peptide sidechains to produce isodityrosine analogs. In the case of our thio-K-13, both experimental and theoretical results indicate a structure having only a few low-energy conformers. The correspondence between the independent NMR measurements and the conformational search results is notable.[13]

Notes and References

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5. Tyrosine derivative **8** was prepared in 80% yield from commercial L-monoiodotyrosine by esterification (MeOH, HCl), N-protection (FmocOSu), O-protection (NaH, $MeOCH_2OCH_2Cl$) and Fmoc removal (Et_2NH).
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11. The K-13 spectrum reported was measured in d4-methanol where amide hydrogens H_a, H_f and H_k are exchanged for deuteriums.
12. The ACE assay was based on the method of M.L. Cohen and K.D. Kurz, *J. Pharmac. Exp. Ther.*, **220**, 63 (1982).
13. We thank Drs. S. Pitzenburger, A. Patchett and P. Mallorga at Merck for their assistance with the NOE measurements and ACE inhibition assays. We also thank Professor D.A. Evans for providing us with spectra and a sample of K-13. We acknowledge support by NIH grant GM37255.

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