Novel Cyclic Biphenyl Ether Peptide β-Strand Mimetics and HIV-Protease Inhibitors

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K-13 (**1**)[1] and OF4949-IV (**2**)[2] are natural inhibitors of the metalloproteases ACE and aminopeptidase (Chart 1). Since these two inhibitors lack the metal-binding functionality (sulfydryl, hydroxamic acid, or additional carboxyl) usually needed for tight-binding inhibition of metalloproteases,[3] a novel mechanism of interaction with the target enzymes is suggested. Many cyclic biphenyl ether peptides exist in nature,[4] but this cyclic 17-membered ring conformation has been studied only once. Still and Hobbs[5] showed by NMR and molecular mechanics calculations that the peptide backbone of the cyclic thioether K-13 analog **3** exists primarily as an extended β-structure in organic solvents. Here, we show that the solution and solid state conformations of synthetic analogs[6] **4**−**6** are extended β-strand structures[7] so that the 17-membered cyclic biphenyl ether peptide system is a conformationally restricted β-strand mimetic. This system can be used to create a new class of protease inhibitor.

NMR and X-ray data establish that peptides **4** and **5** in solution, and tripeptide hydrochloride **6** in the solid state, adopt extended β-strand structures. DQF-COSY,[8] TOCSY,[9] and NOESY[10] experiments performed on cyclic biaryl ether tripeptides **4** and **5** show *J* coupling constants (*J*, NH−CαH) and $d_{\alpha N}(i, i + 1)$ NOE connectivities (Table 1) expected for an extended β-strand structure.[11] These results are almost identical to the data obtained for the thioether analog **3**.[5] Further, the X-ray crystal structure[12] of the *para*-, *meta*-substituted biaryl ether hydrochloride **6** shows that the tripeptide backbone torsion angles, ψ and φ, are similar to the torsion angles of a β-sheet

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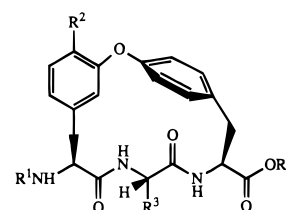
Chart 1
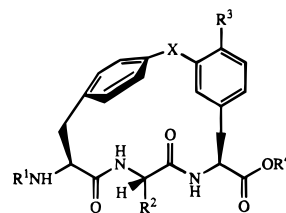
1: R[1] = Ac; R[2] = 4-OH-Bn (Tyr); R[3] = OH; R[4] = H; X= O, **K-13**

3: R[1] = Ac; R[2] = 4-OH-Bn (Tyr); R[3] = OH; R[4] = H; X= S

4: R[1] = Boc; R[2] = s-butyl (Ile); R[3] = H; R[4] = CH₃; X= O

6: R[1] = H.HCl; R[2] = Bn (Phe); R[3] = H; R[4] = CH₃; X= O

10: R[1] = H.HCl; R[2] = s-butyl (Ile); R[3] = H; R[4] = CH₃; X= O

2: R[1] = H; R[2] = OH; R[3] = CH₂CONH₂; R[4] = H, **OF4949-IV**

5: R[1] = CBz; R[2] = H; R[3] = s-butyl (Ile); R[4] = CH₃

11: R[1] = H.HCl; R[2] = H; R[3] = s-butyl (Ile); R[4] = CH₃

Table 1. Coupling Constant and NOE Data from the NMR Conformational Analysis of K-13 and OF4949 Analogs

NOE cross peaks	NOE cross peaks
H_kH_g, H_bH_f, H_lH_o, H_aH_c, H_aH_d, H_qH_c, H_qH_d	H_kH_g, H_bH_f, H_aH_c, H_qH_m, H_sH_n, H_dH_p

	J observed	K-13[1]		J observed	OF4949-II[2a]
H_a-H_b	11.0	--	H_a-H_b	7.3	--
H_b-H_c, H_d	11.9, 5.1	11.9, 5.4	H_b-H_c, H_d	12.9, 6.7	3.0, 5.5
H_f-H_g	4.7	--	H_f-H_g	8.8	--
H_k-H_l	7.0	--	H_k-H_l	9.9	--
H_l-H_m, H_n	3.6, 7.0	3.4, 7.5	H_l-H_m, H_n	12.8, 3.9	13.0, 4.0
H_g-H_i	6.7	5.3	H_g-H_i	5.6	4.0

motif.[13] The crystal structure for **6** superimposed on the enzyme-bound conformation of the HIV protease inhibitor JG-365[14] illustrates how closely the solid state comformation of **6** mimics the enzyme-bound β-strand structure of the HIV protease inhibitor (Figure 1, crossed stereoview). These results suggest

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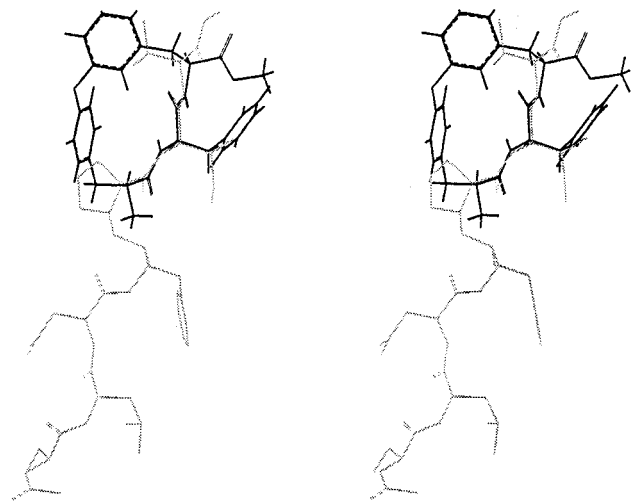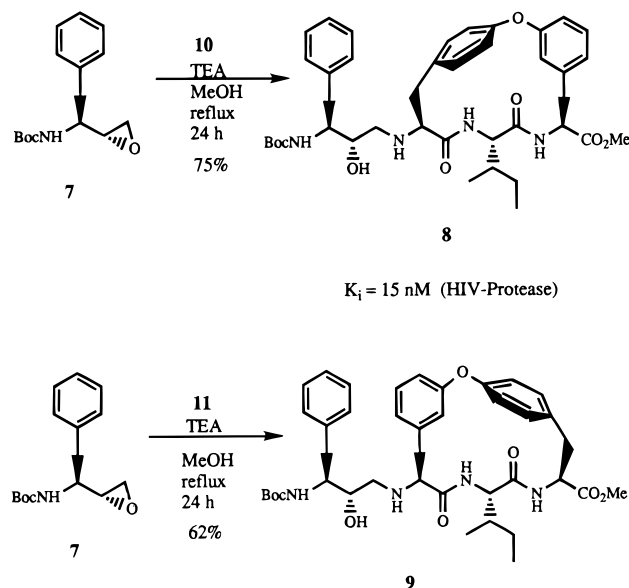
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Figure 1. Crystal structure of *para*-, *meta*-substituted ring compound **6** overlaid on enzyme-bound conformation of JG-365.

Scheme 1. Epoxide Ring-Opening Reactions in the Formation of (*R*)-HEA Inhibitors of HIV-Protease Bearing a Cyclic Biaryl Ether β-Strand Scaffold

that preorganization of the peptide portions of K-13 and OF4949-IV into β-strand structures contributes to the efficient inhibition of these two metalloproteases.

Since many protease inhibitors adopt an extended β-strand conformation when bound to the enzyme,[3] we wished to determine if the 17-membered cyclic biphenyl ether peptide system could be used to construct inhibitors of other classes of proteases. Inhibitors **8** and **9** (Scheme 1) were designed as potential HIV-protease inhibitors in which the P1′ and P3′ side chains are connected via the ether oxygen. A comparison of the P1′ and P3′ tripeptide **6** superimposed on the enzyme-bound structure of JG-365 (Figure 1) illustrates that the P1′ and P3′ side chain residues serve as conformationally restricted analogs
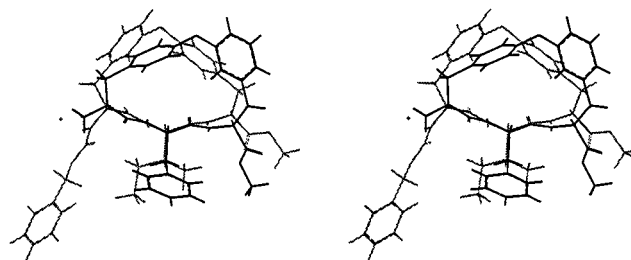

Figure 2. Comparison of the side chain topography in peptidyl biphenyl ethers. The energy-minimized structure of **5** derived from NMR data and the X-ray structure of **6** were superimposed by overlapping their respective peptide backbones.

of binding elements. The hydroxyethylamine (HEA) moiety is known to inhibit aspartic proteases by a transition state analog mechanism.[3,14,16]

The hydroxyethylamine isosteres were synthesized by reaction of amines **10** and **11** with the known epoxide **7**[15,16] to afford the two HEA biphenyl ethers **8** and **9** in 75% and 62% yield, respectively (Scheme 1). Compounds **8** and **9** were assayed for inhibition of HIV proteolytic cleavage of substrate[17] and were found to inhibit HIV-protease with K_i values of 15 and 900 nM, respectively.

It is important to note that although compounds **8** and **9** are nanomolar inhibitors of HIV-protease, they are neither equipotent nor topographically identical due to the differences in the P1′−P3′ side chain ring substitution pattern. The two ring systems reside in distinctly different conformational space, which is clearly seen when the peptide backbones of the two systems are superimposed (Figure 2, crossed stereoview). This disparate biphenyl ether topography may be responsible for the differences in biological activity observed for these ring systems. The *para*-, *meta*-substituted ether **8** is 60-fold more potent inhibitor of HIV protease than the *meta*-, *para*-substituted ether **9**, suggesting that the side chain conformation adopted by the *para*-, *meta*-substituted ring system is a better fit in the active site of the protease. It is evident that the side chain ring substitution pattern must be taken into consideration while designing inhibitors containing the biphenyl ether moiety.

These results show that the cyclic biaryl ether tripeptides can be utilized to create inhibitors of other protease systems. Addition of the known hydroxyethylamine isostere to the cyclic biphenyl ether ring system has transformed a metalloprotease inhibitor system into an aspartic protease inhibitor, and we believe that replacement of the transition state isostere by other moieties at either end of the tripeptide chain will lead to inhibitors of other proteases and β-strand binding proteins.

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