Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 23, pp. 2825-2828, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00494-7

Peptide Mimics of Glycylproline as Inhibitors of Prolidase

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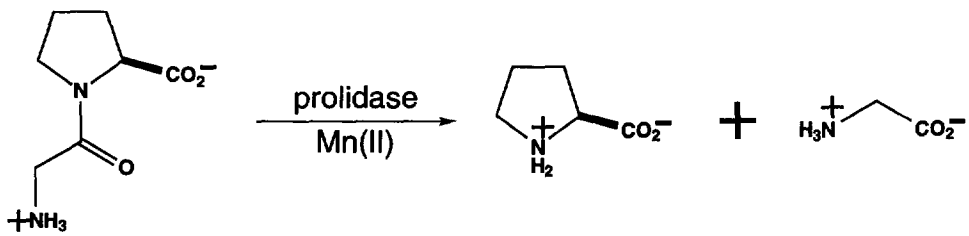
Abstract: Aminoketone **1**, a Ψ[CO-CH] dipeptide mimic of glycylproline, is a potent, competitive inhibitor (K_i = 270 ± 24 nM) of porcine kidney prolidase, a Mn(II)-dependent dipeptidase, whereas the homologous aminoketone **2** and its non-cyclic analogue δ-amino-levulinic acid, **3**, are significantly less inhibitory ($K_i \approx 1$ mM).

Prolidase (EC 3.4.13.9), discovered in 1937,[1] is a manganese-dependent dipeptidase which hydrolyzes glycylproline to glycine and proline (see Scheme I).[2] The primary physiological role of prolidase is thought to be the recycling of proline in the metabolism of collagen.[3] Prolidase activity has been detected in the brain,[4] implicating this activity in the regulation of the concentration of proline, which is demonstrably neuroactive, in the brain tissue.[4d] In humans, a genetic deficiency of prolidase results in a complex clinical syndrome which includes chronic ulcerative dermatitis and mental retardation.[5]

Scheme I



Though the precise mechanism by which prolidase effects peptide bond hydrolysis is not clear, a proposed mechanism has been outlined by Mock and Liu.[6] Prolidase hydrolyzes the transoid peptide bond specifically[7] and several inhibitors have been reported which are competitive with respect to the Gly-Pro substrate.[8] Perhaps most intriguing is the observation of Radzicka and Wolfenden[9] that phosphoenol pyruvate (PEP) strongly inhibits this enzyme at concentrations which approximate the intracellular concentration of PEP. Mock and Liu[10] have recently shown that inhibition of prolidase by PEP is biphasic and they postulated a negative cooperativity between the enzyme monomers, so as to avoid inactivation of prolidase by extant PEP.

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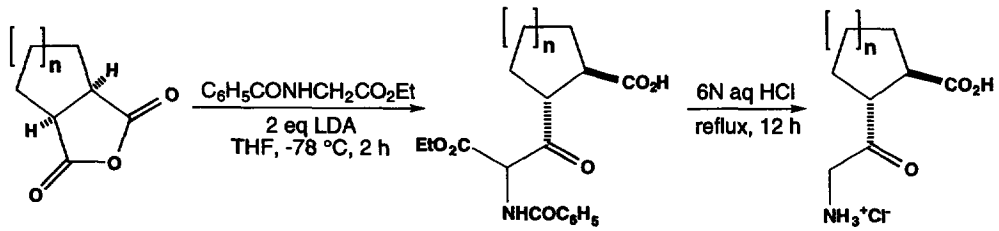
We have prepared a bisubstrate analogue of Gly-Pro in which the pyrrolidine nitrogen atom has been replaced by a methine carbon to give cyclopentane **1**, as well as its homologue **2**. Similar ketomethylene isosteres have been used as hydrolysis-resistant inhibitors of peptidases and proteases.[11] For example, tripeptide Bz-Phe-Gly-Pro is an inhibitor (IC_{50} = 9.4 μM) of angiotensin converting enzyme, but its ketomethylene analogue (Bz-Phe-Ψ[CO-CH_2]-Gly-Pro) was significantly more potent (IC_{50} = 0.07 μM).[12] Below we describe the syntheses and inhibition constants of aminoketones **1**, **2**, and **3** (δ-aminolevulinic acid) against porcine kidney prolidase.

Scheme II



4a (n = 1) **5a** (n = 1) **1** (n = 1)
4b (n = 2) **5b** (n = 2) **2** (n = 2)

The synthesis of **1** starts with *cis*-cyclopentane-1,2-dicarboxylic acid anhydride (**4a**), which is treated with the dianion derived from double deprotonation (2 equiv LDA in THF, -78 °C, 2 h) of ethyl hippurate[13] (see Scheme II). The product of this condensation (**5a**), obtained in 80% yield, is a complex mixture of stereoisomers and tautomers. Acid-catalyzed ester hydrolysis, decarboxylation, and benzamide hydrolysis (6N aq HCl, reflux, 12 h, 50% yield) yields aminoketone **1**.[14] Similarly, *cis*-cyclohexane-1,2-dicarboxylic acid anhydride (**4b**) affords aminoketone **2** in 53% overall yield after the same sequence.[15]

The final hydrolysis of **5b** affords **2** with the thermodynamically preferred *trans* geometry, as evidenced by the vicinal coupling between the methine protons (J = 11.4 Hz). However, vicinal coupling constants on cyclopentanes are poor indicators of stereochemistry. We initially assumed that **1** was *trans* based on thermodynamic arguments; in fact, all efforts to produces *cis*-**1** by milder methods yielded a compound indistinguishable from **1**, presumably because of facile epimerization. The stereochemistry of **1** was finally determined by a detailed evaluation of the coupling constants of **1** ($J_{1,2}$ = 8.9, $J_{2,3}$ = $J_{2,4}$ = 7.6, $J_{1,6}$ = $J_{1,7}$ = 7.6 Hz) and NOE experiments. Protons H1 (methine α to ketone) and H2 (methine α to carboxylate) have nearly equal, large coupling constants with all vicinal partners, reminiscent of prostaglandins in which the ring methine protons are *trans*.[16] This stereochemistry is corroborated by observed NOEs (indicated in Figure 1), reflecting the *trans* relationship of the substituents in **1**.

Figure 1. Observed NOEs for aminoketone **1** are indicated by arrows.

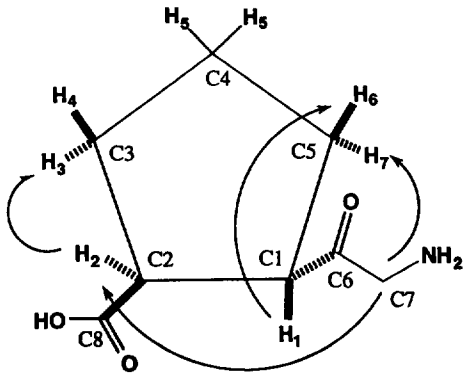


Table 1. ^{1}H and ^{13}C assignments for **1**, obtained by HMQC, HMBC, and homonuclear decoupling experiments.

^{1}H	δ (ppm)	^{13}C	δ (ppm)
1	3.27	1	52.0
2	3.00	2	46.2
3	1.92	3	30.6
4	1.74	4	26.0
5,5'	1.62	5	29.9
6	1.98	6	205.5
7	1.68	7	176.4
8	4.03, 3.93	8	47.0

Aminoketone **1** was determined[17] to be a strong competitive inhibitor of porcine kidney prolidase (K_i = 270 ± 24 nM), whereas **2**, a higher homologue of **1**, proved to be a weak non-competitive inhibitor (K_i = 1.1 ± 0.074 mM). δ-Aminolevulinic acid, **3**, which contains all the functionality of **1** and **2** except the carbocycle, is also a weak non-competitive inhibitor of prolidase (K_i = 0.96 ± 0.086 mM). Compounds **1** and **2** are expected to be inhibitory because (i) they closely approximate the shape and charge of the natural substrate, (ii) the tetrahedral methine carbon which replaces the nitrogen atom of pyrrolidine ring may approximate the anticipated pyramidalization of the nitrogen as it approaches the transition state,[18] and (iii) the ketone may combine with an active site nucleophile to form a hemi-ketolate ligand for the presumed active site Mn(II) ion.[19]

The most curious aspect of the present results is the large difference in the inhibitory activity of **1** and **2**. Specifically, **1** is roughly 3,000 times better as an inhibitor than **2**. The only structural difference between **1** and **2** appears to be the size of the carbocycles. However, examination of the K_i values of CBZ-L-proline (90 μM) vs. CBZ-L-pipecolate (93 μM)[8a] and *trans*-DL-cyclopentanedicarboxylic acid (0.51 μM) vs. *trans*-DL-cyclohexanedicarboxylic acid (0.13 μM)[8b] suggests that the enzyme cannot readily discriminate between five- and six-membered rings. One possible explanation is that the aminoketone serves as a bidentate ligand for the active site Mn(II) ions[6] and that the geometric requirements for this ligation are strict. A second possibility is that the relatively facile enolization of **1** allows an even more potent but synthetically inaccessible *cis*-**1** to form and bind. By the same reasoning, the enol tautomer of **1** itself might serve as the active site ligand. Further work is needed to test the validity of these conjectures.

Acknowledgments: This work was supported in part by grants from the NIH and the American Cancer Society (CH-454). The authors wish to acknowledge valuable insights offered by the referee.

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14. **1**: m.p. 174-5 °C ^{1}H-NMR (D_2O, 300 MHz) δ = 1.6-2.2 (6H, m), 3.15 (1H, q, 8.3 Hz), 3.35 (1H, q, 8.3 Hz), 4.15 (2H, ABq, J = 18.5 Hz) ppm. ^{13}C-NMR (D_2O, 75 MHz) δ = 27.5, 32.1, 32.2, 48.3, 49.3, 54.4, 181.4, 208.8 ppm. IR (KBr) ν = 2970 (br), 1720, 1695, 1593, 1475, 1417 cm^{-1}. MS (FAB/glycerol) m/z = 172 amu (M+H).
15. This compound was reported earlier by Kimoto, S.; Okamoto, M.; Ueno, M.; Ohta, S.; Nakamura, M.; Niiya, T. *Chem. Pharm. Bull.* **1970**, *18*, 2141. **2**: m.p. 194-6 °C. ^{1}H-NMR (D_2O, 300 MHz) δ = 1.1-1.5 (4H, m), 1.82 (2H, m), 2.0-2.2 (2H, m), 2.67 (1H, dt, J = 3.6, 11.3 Hz), 2.88 (1H, dt, J = 3.6, 11.3 Hz), 4.19 (2H, ABq, J = 18.5 Hz) ppm. ^{13}C-NMR (D_2O, 75 MHz) δ = 27.2, 30.6, 30.9, 46.7, 51.6, 181.9, 210.6 ppm. IR (KBr) ν = 3073 (br), 1720, 1471, 1192 cm^{-1}. MS (FAB/glycerol) m/z = 186 amu (M+H).
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17. Porcine kidney prolidase was obtained from Sigma and assayed by continuous spectrophotometry,[9] following the disappearance of the substrate Gly-Pro at 228 nm in K$^+$MES buffer (10 mM, pH 6.0) at 25 °C. K_i values were estimated using Grafit 3.0 (Erithacus Software: Leatherbarrow, R. J. *Trends Biochem. Sci.* **1990**, *15*, 455). The complete kinetic data were analyzed with competitive, non-competitive, and un-competitive inhibition models, and the best fitting model selected.
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(*Received in USA* 5 *September* 1995; *accepted* 16 *October* 1995)