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AN ACYLIMINIUM ION ROUTE TO CIS AND TRANS "ANTI" Phe-Gly DIPEPTIDE MIMETICS

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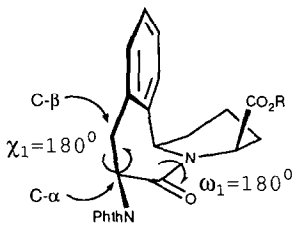
Abstract: The synthesis of two series of constrained mimetics of the dipeptide phenylalanyl-glycine oriented in the "anti" conformation (X_1=180°) are described. These benzolactams differ about the geometry of the internal amide bond (ω_1=0° or 180°). Solution NMR studies support specific conformational assignments for these mimetics.

Receptor affinity and metabolic stability of peptides can be enhanced by restricting the conformational flexibility of amino acid side chains to highly populated and hence energetically favorable orientations. The "anti" trans[1] mimetic of phenylalanyl-leucine[2] has been used to design potent long acting inhibitors of angiotensin I-converting enzyme.[3,4] Our long term goal is to develop a tool set of conformationally restricted dipeptide mimetics to probe the effect of the side chain conformation on binding affinity of peptide ligands to their corresponding pharmacological receptors or biological targets. This paper describes the use of acyliminium ion cyclizations as an entrance into the trans and cis isomeric forms 2 and 3 of the conformationally constrained "anti"-Phe-Gly dipeptide (X_1=180°).



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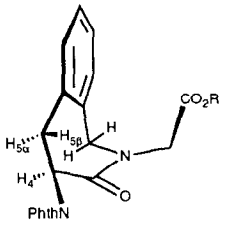

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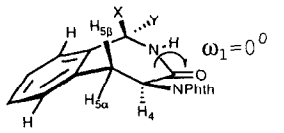


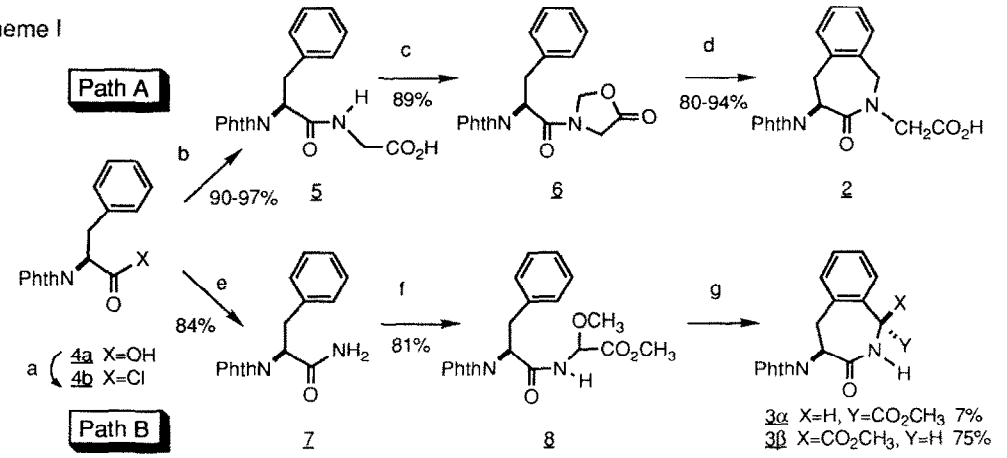
3α X=H, Y=CO_2R
3β X=CO_2R, Y=H_1

The synthesis of benzolactam 2, representing a mimic of the "anti" trans orientation of phenylalanylglycine, was performed as outlined in Scheme I, path A. N-Phthaloyl-L-phenylalanine 4a was converted to acid chloride 4b by treatment with α,α-dichloromethyl methyl ether.[5] Coupling of acid chloride 4b with glycine under Schott-Baumann conditions[6] afforded dipeptide 5 in 90-97% overall yield. Condensation of 5 with paraformaldehyde in toluene at reflux containing p-toluenesulfonic acid with azeotropic removal of water provided enantiomerically pure oxazolidinone 6 (89%, mp=179-181°C, $[\alpha]_D^{20}$=+147.3 (c=0.7, $CHCl_3$)). Cyclization of oxazolidinone 6, under a variety of acidic conditions ($(PO_3H)_n$, 100°C; CH_3SO_3H; CF_3SO_3H; or $TMSOSO_2CF_3$), provided the benzolactam carboxylic acid 2 in 80-94% yield as a mixture of enantiomers.[7]

The synthesis of the cis-Phe-Gly mimetic 3α, was performed as outlined in Scheme I, path B. The aminal 8 was a convenient acyliminium ion source enroute to lactam 3α.[8] Acid chloride 4b was converted to amide 7[9] with ammonium hydroxide. Condensation of 7 with methyl glyoxylate

hemiacetal[10] and p-toluenesulfonic acid in chloroform at reflux with azeotropic removal of water gave aminal 8 (81%). Acid catalyzed cyclization (CF_3SO_3H, CH_2Cl_2, 23°C) gave lactams 3β (75%, mp=180-182°C, $[\alpha]_D^{20}$=-60.5 (c=1.02, $CHCl_3$)) and 3α (7%). The stereochemical and conformational assignment for 3β was made on the basis of the ^1H NMR and 2D NOE NMR.[11]

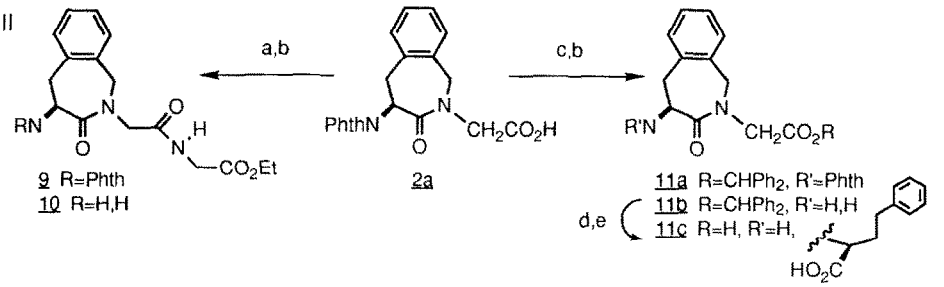
Scheme I



(a) Cl_2CHOCH_3, 60°C, 13h; (b) $H_2NCH_2CO_2H$, Na_2CO_3, CH_3COCH_3/H_2O; (c) $(CH_2O)_n$, $C_6H_5CH_3$, reflux; (d) Lewis acid (see text), p-TsOH·H_2O; (e) NH_4OH; (f) $HOCH(OCH_3)CO_2CH_3$, p-TsOH·H_2O, $CHCl_3$, reflux; (g) CF_3SO_3H, CH_2Cl_2, 23°C, 17h.

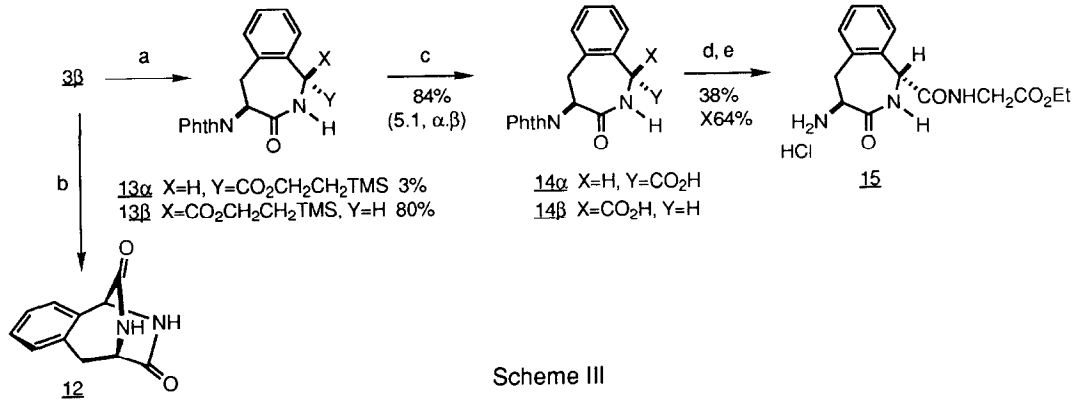
The utility of constrained mimetics as tools to probe the binding requirements of peptides of interest is dependent on their compatibility with standard peptide coupling and deprotection methods. Lactam acid 2a was coupled directly with glycine ethyl ester (EEDQ, CH_2Cl_2, 23°C, 18h) to give protected tripeptide 9 in 80% yield (Scheme II). Dephthaloylation of 9 (1.1 eq. NH_2NH_2, EtOH, 23°C, 48h) afforded the tripeptide ester 10 in 65% yield after chromatography (9:1:0.5 $CH_2Cl_2/CH_3OH/NH_4OH$). Alternatively, lactam acid 2a was converted to its benzhydryl ester 11a (Ph_2CN_2, CH_2Cl_2) and dephthaloylated to afford amino ester 11b in 90% overall yield. Amine 11b was elaborated to diacid 11c using standard methods.[2] Diacid 11c was a modest inhibitor of angiotensin converting enzyme (K_i = 1 x 10^{-8} M) relative to the analogous inhibitor (K_i = 4 x 10^{-12} M)[4] incorporating the more constrained mimetic 1.

Scheme II



(a) EEDQ, $H_2NCH_2CO_2Et$, CH_2Cl_2, 18h; (b) NH_2NH_2, CH_3CH_2OH, 23°C, 48h; (c) Ph_2CN_2, CH_2Cl_2
(d) Ethyl (R)-4-phenyl-2-trifluromethanesulfonyloxybutyrate, proton sponge; (e) LiOH, HCl

Further modification of 3β was not straightforward (Scheme III). Attempted removal of the phthalimide protecting group with hydrazine afforded diketopiperazine 12. This observation supports the assignment of a cis relationship between the ester and protected amine functions in 3β. Deprotection and coupling at the β-carboxyl moiety of 3β was complicated by the hindered steric environment about the methyl ester functionality and the sensitivity of the phthalimido protecting group to standard methyl ester hydrolytic conditions ($LiOH/H_2O/THF$, LiI/DMF). Conversion of methyl ester 3β to the β-trimethylsilylethyl esters 13β (80%) and 13α (3%) was effected with $Ti(OEt)_4$ and $TMSCH_2CH_2OH$ in THF at reflux for 4.25h.[12] Fluoride ion cleavage of silyl ethyl ester 13β (TBAF, THF, 23°C, 3h) occurred predominantly with epimerization at C-6 resulting in a 5:1 ratio of α/β acids 14 in 84% yield.[13] This propensity for the α-carboxyl group to adopt an equatorial orientation is consistent only with the conformation of the seven membered ring 3β determined by our NOE experiments.[14]



Scheme III

(a) $Ti(OEt)_4$, $TMSCH_2CH_2OH$, THF, reflux, 4.25h; (b) NH_2NH_2, CH_3OH, 23°C; (c) TBAF, THF, 23°C, 3h; (d) i-butyl chloroformate, N-methylmorpholine, THF, -10°C ---> 23°C; $H_2NCH_2CO_2Et$, THF, reflux, 20h.

The α-carboxylic acid 14α was coupled to glycine ethyl ester via its mixed anhydride[15] in refluxing THF for 20h (38%).[16] Removal of the phthalimido moiety with hydrazine in ethanol followed by treatment with etheral HCl gave the hydrochloride salt of "anti"-cis-Phe-Gly-Gly-OCH_2CH_3 (15) in 64% yield.

In conclusion, methodology has been developed for the synthesis of two isomeric "anti"-Phe-Gly dipeptide mimics utilizing acyliminium ion cyclization in the key step. The "anti"-trans mimetic 2 is available as a racemate in good yield and can be easily incorporated into a peptide sequence. The "anti"-cis compound 3 is more difficult to incorporate into a peptide sequence. Exploration of the scope and limitations of the technology leading to "anti" trans peptide mimetics described here continues. Further studies to develop cyclization conditions which will not promote the epimerization of 2 are under way.

REFERENCES AND NOTES

1. The term "anti" refers to the conformation where the torsional angle X_1, between an amino acid's c-β substituent and the c-α amino group is 180°. The terms cis and trans are used here to describe the geometry about the amide bond (i.e. ω_1=180° for trans amides 1 and 2 or 0° for cis amide 3).

2. Flynn, G.A., Giroux, E.L., Dage, R.C. J. Am. Chem. Soc., 1987, 109, 7914-7915.

3. Flynn, G.A., Beight, D.W., Huber, E.W., Bey, P. Tetrahedron Lett., 1990, 31, 815-818.

4. Giroux, E.L., Beight, D.W., Dage, R.C., Flynn, G.A., J. Enzyme Inhibition, 1989, 2, 269-277.

5. The acid was heated at 60°C in excess neat Cl_2CHOCH_3 for 13 hours then evaporated to dryness in vacuo to give the acid chloride as a solid mass.

6. A solution of acid chloride in acetone was added in dropwise fashion to a solution of 2.0 equivalents at glycine and 2.0 equivalents of Na_2CO_3 in 2:1 water/acetone at 25°C. Acidification provided a solid: m.p. 184-185°C, $[\alpha]_D^{20}$=-153.7° (c=0.8, EtOH).

7. Unfortunately, under the conditions explored this cyclization occurred with epimerization at the C-α center derived from phenylalanine. The optical purity of 2 was determined by GC analysis of the N-trifluoroacetyl methyl ester derivative on a Chirasil val column. The conformation was established based on scalar coupling constants ($J_{4,5\alpha}$= 4.6 Hz, $J_{4,5\beta}$= 12.8 Hz) and on the observation of NOE's between $H_{1\alpha}$ and H_4, H_4 and $H_{5\alpha}$, and $H_{5\alpha}$ and its ortho-aromatic proton. No NOE was observed between $H_{5\beta}$ and $H_{1\beta}$.

8. During the preparation of this manuscript, we became aware of a recent report of closely related work presented at the 20th European Peptide Symposium. We would like to thank J.A. Gainer for forwarding us a copy of the cited abstract: (D. Ben-Ishai, A. Rabi, and R. McMurry, Peptides 1988, Proc. 20th Eur. Pep. Sym.)

9. Morely, John, S. J. Chem. Soc. C. 1969, 809.

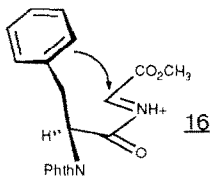
10. Ben-Ishai, D., Bernstein, Z. Tetrahedron 1977, 33, 881.

11. NOE's were observed between $H_{5\alpha}$ and its ortho-aromatic proton and H_1 and its ortho-aromatic proton indicative of a planar relationship between these protons. An NOE was also observed between $H_{5\alpha}$ and H_4. No NOE was observed between $H_{5\beta}$ or $H_{5\alpha}$ and H_1. These observations and the scalar coupling constants observed ($J_{5\alpha,4}$ = 3.6 Hz and $J_{5\beta,4}$ = 12.1 Hz) are consistent with the conformation shown for 3β.

12. (a) Seebach, D.; Hungerbühler, E., Naef, R., Schnurrenberger, P., Weidmann, B. Züger, M. Synthesis, 1982, 1138. (b) Rehwinkel, H.; Steglish, W. Synthesis, 1982, 826.

13. Sieber, P. Helvetica Chimica Acta, 1977, 60, 2711.

14. The initial formation of cyclization product 3β which possesses an axial carboxyl moiety during the iminium ion cyclization of aminal 8 is consistent with preference for transition state 16.



16

15. Torres, J.L., Haro, I., Valencia, G., Garcia-Anton, J.M., Reig, F. Tetrahedron, 1987, 43, 4031 and references cited within.

16. The mixed anhydride did not react with glycine ethyl ester at room temperature. The poor yield reflects the hindered steric environment of this carboxylic acid, therefore, attack at the isobutyl carboxyl group may compete with the desired coupling (TLC indicated that the carboxylic acid was formed in this reaction).