

Communications to the Editor

Design and Synthesis of a C₇ Mimetic for the Predicted γ -Turn Conformation Found in Several Constrained RGD Antagonists¹

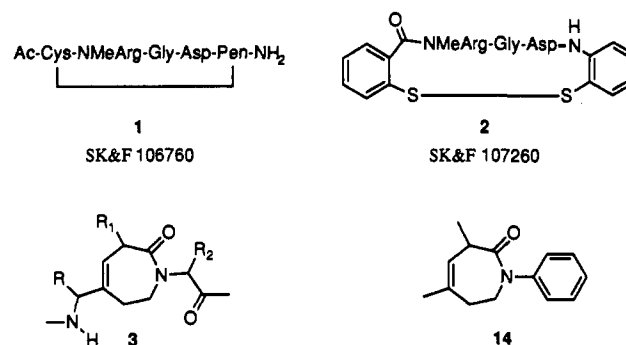
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Synthetic peptides containing the Arg-Gly-Asp (RGD) sequence inhibit binding of fibrinogen to its receptor (GP-IIb/IIIa) thereby inhibiting platelet aggregation and thrombus formation.² Previous reports from these laboratories have outlined the development of peptide fibrinogen receptor antagonists culminating in the synthesis of two cyclic disulfide analogues, 1³ and 2.⁴ In order to more thoroughly define an antagonist pharmacophore for the GPIIb/IIIa receptor, these constrained cyclic peptides have been studied extensively by ¹H NMR and

molecular modeling.⁵ The ¹H NMR spectra of 2 at low temperature show two slowly exchanging conformations. The Arg-Gly-Asp region of the major form is characterized by an extended glycine residue and a C₇ conformation⁶ at aspartic acid, while the minor component exhibits a similar conformation at glycine and a conformation at aspartic acid that is near α_R , similar to that found in a type I β -turn.⁵ The most probable conformation of the minor form of 2 closely agrees with a structure determined by X-ray crystallography.⁵ The ¹H NMR data also suggest that the dominant conformation of the Arg-Gly-Asp region in 1 is similar to that found in the major component of 2.⁵ In order to differentiate the two conformations about the aspartic acid residue and thereby gain insight into the biologically active conformation of 2, we decided to constrain the γ -turn conformation about the aspartic acid residue with a secondary structure mimetic.⁷ Our previous work has shown that the C₇ mimetic 3 locks three residues of a peptide chain into a γ -turn conformation.^{8,9} The syntheses of the RGD analogues 4 and 5, incorporating the appropriate C₇ mimetic, were initiated to test the contribution of the γ -turn conformation to the antagonist pharmacophore. Analogues 4 and 5 were chosen as initial targets to evaluate the contribution of the predicted γ -turn conformation on the biologically active conformation without the additional constraint of the cyclic disulfide.



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(1) This paper is dedicated to Professor Ralph Hirschmann on the occasion of his 70th birthday.

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Entry into the template for the C₇ mimetic ring system 3 is based on the key trisubstituted olefin 7 obtainable in three steps from the commercially available substituted

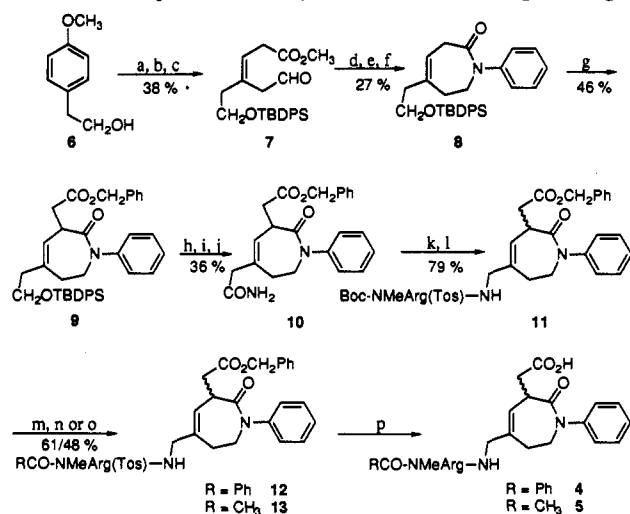
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Scheme I. Synthesis of C₇ Mimetic Containing Analogue

anisole 6 as shown in Scheme I.^{8,10} Reductive amination of 7 with aniline followed by hydrolysis and cyclization gave 8 which incorporated the backbone portion of the C₇ mimetic. The central (*i* + 1) "aspartic acid" side chain was introduced via the alkylation of the anion of 8 with benzyl bromoacetate to give 9.¹¹ Selective deprotection of the alcohol in 9 followed by oxidation and aminolysis gave the primary amide 10. Modified Hofmann rearrangement¹² of 10 followed by BOP coupling¹³ to BocN-MeArg(Tos)-OH^{3b} gave the fully protected peptide 11. Selective removal of the N-terminal Boc protection and treatment with benzoyl chloride or acetyl chloride gave 12 and 13, respectively. Final deprotection of 12 and 13 with anhydrous HF yielded 4 and 5, respectively, each as an inseparable set of diastereoisomers.¹⁴

The biological results for the two mimetic containing analogues 4 and 5 are summarized in Table I. The data indicate that both 4 and 5 are potent inhibitors of platelet aggregation *in vitro*^{3b} and retain nanomolar affinity for isolated human GPIIb/IIIa receptor,^{3b} showing an antag-

(10) Compound 7 was isolated as a 80:20 mixture of the desired unsaturated aldehyde and the saturated aldehyde which resulted from overreduction during the initial Birch reaction. The saturated material was separated by chromatography after cyclization to 8.

(11) Attempts to alkylate the hydroxymethyl-substituted cyclic lactam, which were successful in similar cases, gave only a diene side product formed via the initial acylation of the dianion at oxygen followed by elimination.

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(14) The diastereoisomers of 4 and 5 were identical under several sets of chromatographic conditions. Alkylation with an optically active benzyl ester (iodo (+)-methylbenzylacetate) followed by the same reaction sequence allowed for separation of 11 into two diastereoisomers. Unfortunately, acylation and final deprotection caused partial racemization at *i* + 1 in the two diastereoisomers of 5 as determined by ¹H NMR analysis.

Table I. Affinity and Antiaggregatory Activity

compound	antiaggregatory activity canine PRP/ADP: ^{3b} IC ₅₀ (μM) ^a	binding inhibition human GPIIb/IIIa: ^{3b} K _i (μM) ^b
1 (SK&F 106760)	0.36 ± 0.04	0.175 ± 0.025
2 (SK&F 107260)	0.09 ± 0.02	0.004
4	1.11 ± 0.16	0.106 ± 0.007
5	0.72 ± 0.28	0.271 ± 0.003
Ac-Arg-Gly-Asp-Ser-NH ₂	91.3 ± 0.10	37.01 ± 0.28

^a Inhibition of platelet aggregation in canine platelet-rich plasma induced by ADP. ^b Inhibition of [³H]-107260 binding to purified GPIIb/IIIa isolated from human platelets that were reconstituted in liposomes.

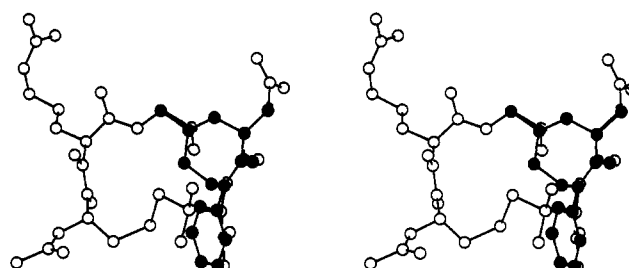


Figure 1. Overlay of mimetic fragment 14 onto the solution conformation of 1.

onist profile similar to that of the cyclic peptide 1. These results suggest that the γ -turn conformation locked by the mimetic found in 4 is consistent with an antagonist pharmacophore for the GPIIb/IIIa receptor.

In order to examine more closely the overlap of this C₇ mimetic onto the proposed γ -turn region of the cyclic peptides 1 and 2, the lowest energy conformation of 14,^{15,16} a simplified version of the C₇ mimetic contained in 4 and 5, was calculated.¹⁷ This conformation of 14 can be readily superimposed on the γ -turn containing solution conformation of the cyclic antagonist 1 (Figure 1). The excellent fit of the mimetic is consistent with the comparable affinity of 4 and 1 for the GPIIb/IIIa receptor. Although mimetic in 4 fits the γ -turn conformation found in 2 equally well, it exhibits a lower affinity for the GPIIb/IIIa receptor. This partial loss of affinity can be rationalized by a variety of other factors. For example, the mimetic containing analogue 4 may lack a conformational constraint or receptor interaction that is present in the antagonist 2. It is also possible that even though the C₇ mimetic in 4 successfully constrains the backbone of the molecule in a γ -turn conformation, the variation of functional groups inherent in the C₇ mimic design either eliminates an important binding element of the ligand or introduces a new, unfavorable interaction with the receptor. Further work to examine these and other possible explanations are currently being pursued.

In conclusion, we have successfully designed and synthesized a conformational mimetic that replaces the

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(16) The lowest energy conformation was calculated using the multiconformer and Batchmin utility found in MacroModel Version 3.1x, Still, W. C. Department of Chemistry, Columbia University, New York, NY 10027. The ϕ_{i+1} and ψ_{i+1} angles of the mimetic 14 were varied at 30° resolution with a closure distance (bond between bridge methylenes) of 1-5 Å. The resulting multiconformer run generated 633 conformations which were then minimized utilizing a MM2 force field in Batchmin.

(17) The calculated low energy conformation of 14 was found to be consistent with the conformation of other similarly substituted C₇ mimetics which have been determined by X-ray crystallography (see refs 8 and 9).

C-terminal region of a RGD antagonist leading to both a high affinity ligand of the GPIIb/IIIa receptor and an potent inhibitor of in vitro platelet aggregation.

Registry No. 1, 126053-71-2; 2, 136620-00-3; 4 (diastereomer 1), 142841-16-5; 4 (diastereomer 2), 142865-90-5; 5 (diastereomer 1), 142841-17-6; 5 (diastereomer 2), 142865-91-6; 6, 702-23-8; 7, 142841-18-7; 8, 142841-19-8; 9, 142841-20-1; 10, 142841-21-2; 11

(diastereomer 1), 142841-22-3; 11 (diastereomer 2), 142841-26-7; 12 (diastereomer 1), 142841-23-4; 12 (diastereomer 2), 142841-27-8; 13 (diastereomer 1), 142841-24-5; 13 (diastereomer 2), 142841-28-9; 14, 142841-25-6; TBDPS-Cl, 58479-61-1; PhNH₂, 62-53-3; BrCH₂CO₂CH₂Ph, 5437-45-6; Boc-NMeArg(Tos)-OH, 108695-16-5; PhCOCl, 98-88-4; AcCl, 75-36-5; Ac-Arg-Gly-Asp-Ser-NH₂, 122207-62-9; H-Arg-Gly-Asp-OH, 99896-85-2.