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The Design and Synthesis of Potent Cyclic Peptide VCAM–VLA-4 Antagonists Incorporating an Achiral Asp-Pro Mimetic

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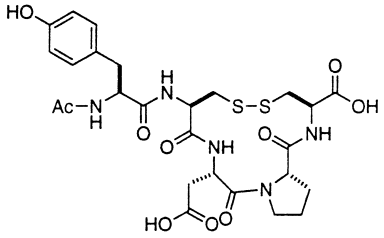
Abstract—The Asp-Pro sequence of the cyclic peptide Ac-HN-Tyr-Cys*-Asp-Pro-Cys*-OH (**1**) could be replaced with the achiral dipeptide mimetic l-(2-aminoethyl)cyclpentylcarboxylic acid with retention of potent inhibition of the VCAM–VLA-4 interaction.

Vascular cell adhesion molecule-l (VCAM-l), a member of the immunoglobulin (Ig) supergene family, is expressed on activated, but not resting, endothelium. The principal receptor for VCAM-1, the integrin very late antigen 4 (VLA-4, $\alpha_4\beta_1$), is expressed on many lymphocytes including circulating eosinophils, basophils, and monocytes, but not neutrophils. Antibodies to either protein are effective at inhibiting leukocyte infiltration and preventing tissue damage in several animal models of inflammation.[1] Peptides derived from the connecting segment 1 (CS1) sequence of fibronectin have also been shown to block VCAM–VLA-4 interactions and to block allergen induced airway responses in a sheep model of asthma.[2,3] Thus, we are interested in discovering orally active VCAM–VLA-4 antagonists that might be useful for the treatment of asthma or rheumatoid arthritis.

Recent work described structure–activity work on a series of cyclic peptide VCAM–VLA-4 antagonists which led to the synthesis of the potent lead pentapeptide **1**.[4] This effort highlighted the importance of the C-terminal carboxyl group, demonstrated that the aspartic acid was not important for activity, and showed that the Asp-Pro dipeptide could be replaced by linear spacers with only a modest decrease in potency. Unpublished results from our laboratories additionally suggested that the proline residue contributed to binding through a hydrophobic interaction with VLA-4. Thus, we were intrigued by the possibility of combining these results in the design of Asp-Pro dipeptide replacements which incorporate both a hydrophobic portion capable of mimicking the proline interaction as well as a suitable spacer to maintain the appropriate geometry and conformational constraints of the cyclic peptide ring system.



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As a starting point for this effort, we employed a NMR based conformational model of the ring system of **1** using the closely related peptide 1-[(9-oxo-fluorenone)carbonyl]-Arg-Cys*-Asp-thiaPro-Cys*-OH.[5] While the position of the N-terminal residue was indeterminent in these structures, the ring is well defined, forming essentially two families of conformers, which differ in the directionality of the disulfide linkage. Since the ring conformation seems well conserved, we proceeded by displaying a single member of the family and manually building in potential spacers using the molecular modeling package Sybyl. Each new structure was minimized, subjected to a short molecular dynamics run and reminimized using the maximin function within Sybyl. The suitability of individual mimetics was evaluated visually by inspecting their overall effect on ring conformation.

As a result of this effort, a series of l-(aminoalkyl)cyclopentane carboxylic acids was designed as easily

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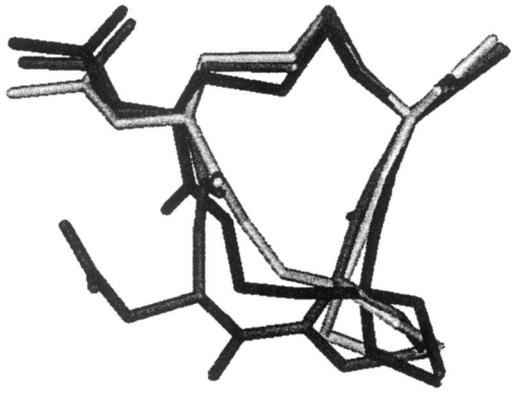
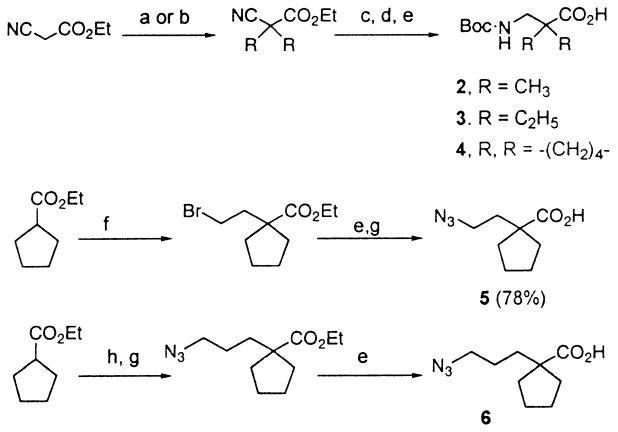


Figure 1. Overlay of the NMR structure of core ring system of 1-FCA-Arg-Cys*-Asp-thiaPro-Cys*-OH[9] (grey) and two spirocyclic systems where the Asp-Gly is substituted with 1-(aminomethyl)cyclopentane carboxylic acid (light grey) or 1-(2-aminoethyl)cyclopentane carboxylic acid (dark grey).

accessible, achiral dipeptide mimetics. As illustrated in Figure 1, modeling experiments suggested that a derivative incorporating 1-(2-aminoethyl)cyclopentane carboxylic acid linker **11** offered the best opportunity to maintain the ring conformation. Shorter spacers caused a considerable shift in the vectors and thus changed the relationship between the critical C-terminal carboxyl group and the N-terminal tyrosine. It is interesting to note that while **1** has a 14-membered ring, the analogue **11** is one atom smaller.

The required cyclopentane carboxylic acids **2**–**6** were synthesized as outlined in Scheme 1. Incorporation into the cyclic peptides shown in Table 1 involved standard HBTU mediated peptide coupling of these acids with 1-tritylcysteine derivatives to give the intermediates **7** (P represents Boc or Fmoc protection) which could be cyclized by treatment with I_2 in methylene chloride-methanol. The remaining steps required routine deprotection, coupling with the appropriate tyrosine derivatives and final deprotection as outlined in Scheme 2. Alternatively the linear peptide could be fully elaborated to incorporate



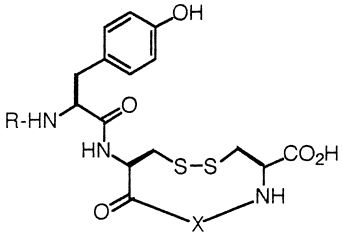
Scheme 1. a. NaH, R-I, THF, 0 °C. b. $Br(CH_2)_4Br$, K_2CO_3, CH_3CN, heat, 4 d. c. H_2, 5% Rh on Alumina. d. Boc_2O. e. NaOH, dioxane. f. nBuLi, THF, −65 °C, then $Br(CH_2)_2Br$. g. NaN_3, DMF, 50 °C, 5 h. h. nBuLi, THF, −65 °C, then $Br(CH_2)_3Br$.

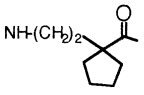
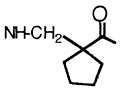
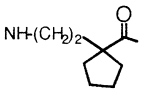
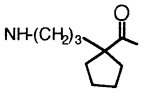
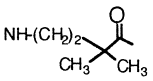
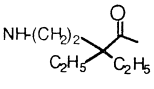
Tyr, and then cyclized. Final deprotection then yielded compound **8**.

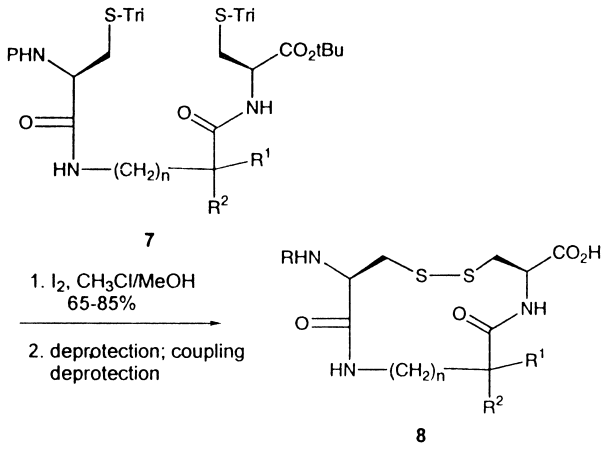
Compounds were assayed for VLA-4 antagonist activity using a solid-phase, dual antibody ELISA in which VLA-4 derived from Ramos cells was allowed to compete for bound recombinant human VCAM in the presence of serial dilutions of test compound. VLA-4 bound to VCAM- 1 was detected by a complex of anti-β1 antibody and HRP-conjugated anti-mouse IgG: chromogenic substrate (K-Blue).[6] A secondary, cell based assay was also run in which fluorescently labeled Ramos cells were allowed to compete for immobilized VCAM. As the data summarized in Table 1 indicate, **9** in which aminoethylcyclopentyl moiety replaces the Asp-Pro of **1** was 2- and 15-fold more potent than the parent compound **1** in the solid phase and cell based assays, respectively.

The similar potencies of *N*-acetyl derivative **9** and the unsubstituted N-terminal amine **11** indicate that activity among compounds in this series is relatively insensitive to

Table 1.



No.	R	X	Solid phase assay IC_{50} (nM)	Ramos cell assay IC_{50} (nM)
1	Ac	Asp-Pro	1.8	200
9	Ac		0.70	12
10	H		5.0	
11	H		0.50	24
12	H		19	480
13	H		112	
14	H		3.9	



Scheme 2.

substitution on the N-terminus. Comparison of a series of analogues possessing free N-terminal nitrogen atoms, in which the ring size is varied is in accord with our modeling predictions. Thus, **11**, which possesses a 13-membered ring, was 10-fold more potent than the 12-member ring analogue **10**, which incorporates 1-(aminomethyl)cyclopentane carboxylic acid and 20-times more potent than the homologue **12**, which incorporates l-(3-aminopropyl)cyclopentane carboxylic acid. In order to determine whether the cyclopentane ring was required, we prepared the analogues **13** and **14** which were constructed from 4-amino-2,2-dimethyl- (**2**) and 4-amino-2,2-diethylbutanoic acid (**3**), respectively. Both compounds were active, although somewhat less potent than the corresponding cyclopentyl derivative **11**. The higher potency of the bulkier diethyl analogue could be the influence of increased hydrophobicity or conformational control as a consequence of the gem-dialkyl effect.

In conclusion, aminoethylcyclopentane carboxylic acid was incorporated into **1** as an Asp-Pro mimetic. It not only provided the proper hydrophobic contacts while preserving the general conformation of the macrocycle, but actually provided an enhancement in potency while markedly simplifying the structure of the macrocyclic system.

References and Notes

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