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Carbacyclic Peptide Mimetics as VCAM–VLA-4 Antagonists

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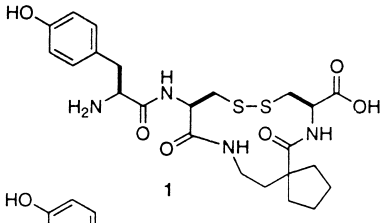
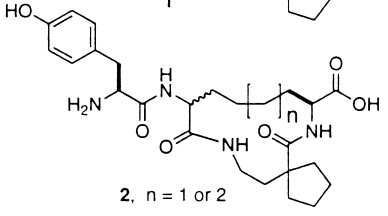
Abstract—Substitution of carbon for sulfur in a potent 13-membered cyclic disulfide containing peptide was accomplished via an intramolecular Wittig reaction and resulted in a series of 'carba' analogues. Potency in the VCAM–VLA-4 assay was sensitive to ring size and lower than that of the parent disulfide.

Vascular cell adhesion molecule-1 (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 which might be useful for the treatment of asthma or rheumatoid arthritis.

In the accompanying paper,[4] we described the development of a class of spirocyclic disulfides derived from tetrapeptides, typified by **1**, which are potent inhibitors of the VCAM–VLA-4 interaction. Since these compounds are of relatively simple structure with MW < 600, we chose to investigate whether further manipulation might lead to orally active VCAM–VLA-4 antagonists. We hypothesized that the carboxyl group could be masked as a prodrug ester and one or both ring amide bonds might be modified by *N*-alkylation without loss of conformational control. Furthermore, the previous experience of the Tanabe[5] and Genentech[6] groups suggested that exploration of potentially more drug like replacements for the N-terminal tyrosine should be fruitful.



1



2, n = 1 or 2

We were also concerned that the disulfide moiety might constitute a stability liability. Previous work on somatostatin[7] and atrial natriuretic factor[8] indicated that carbon may be substituted for the sulfur atoms in cyclic disulfides without loss of agonist activity. Our molecular modeling efforts suggested that the impact of carbon substitution on the orientation of the N- and C-terminal vectors and the relative position of the five-membered ring of 2 was minor. Thus, we were encouraged to prepare the carbacyclic analogues **2**, to determine the effect of this replacement on VCAM–VLA-4 binding activity.

Results and Discussion

Target compounds were prepared by a Wittig strategy involving as the key step, an intramolecular cyclization between an aldehyde and a phosphonoglycine derivative. The starting materials were prepared as shown in Scheme 1, beginning with preparation of the pentenyl- and hexenyl-glycine derivatives **5a** and **5b** via alkylation of

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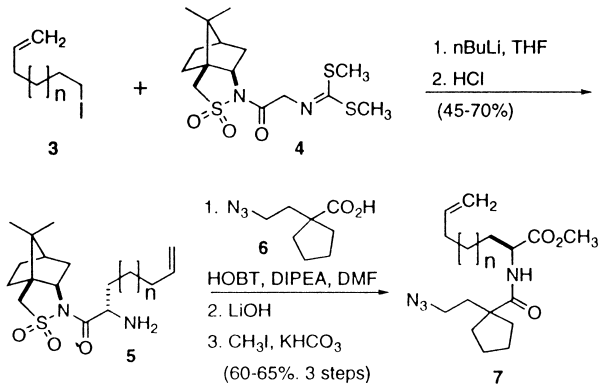
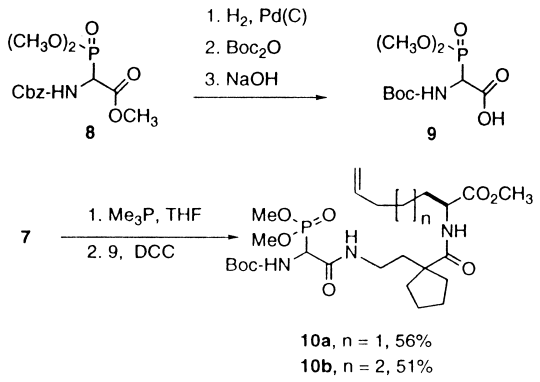


Scheme 1.

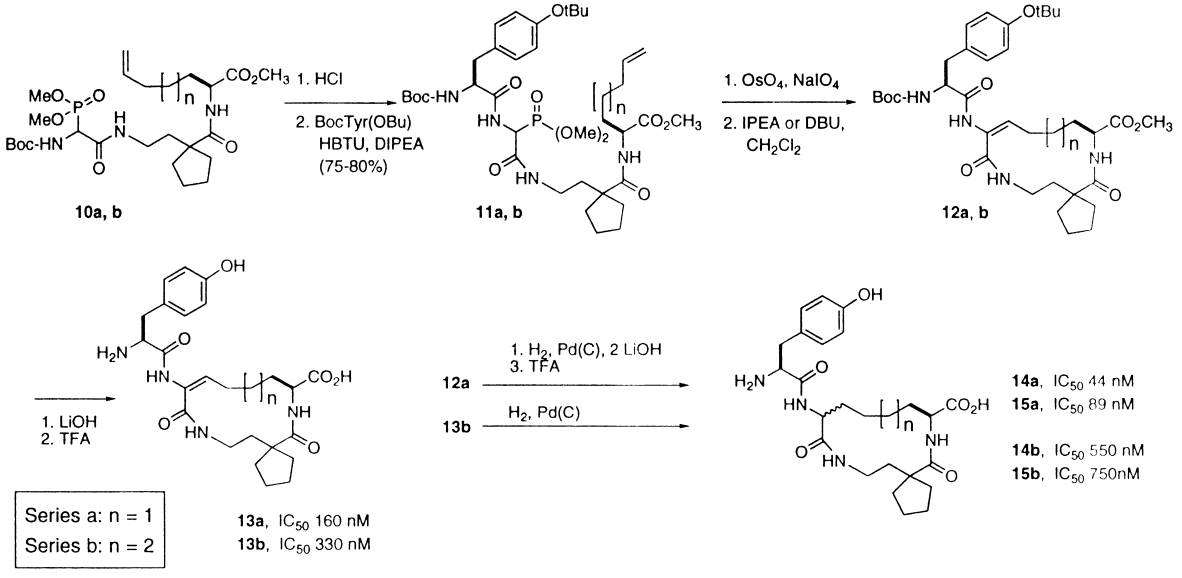


Scheme 2.

the sulfonimide **4** according to Oppoizer's general procedure.[9] This material was coupled with azido acid **6**[4] under standard conditions, the sulfonimide was cleaved by treatment with LiOH and the carboxyl group was esterified to give the azide **7** in good yield. This azide was reduced to the corresponding amine with trimethylphosphine in THF followed by a water quench and the product was used immediately. Coupling with the unstable acid **9** proceeded well when mediated by DCC in the absence of base to give the key olefin **10** (Scheme 2).

To complete the synthesis, the Boc group was removed with methanolic HC1 and the resulting amine was coupled with Boc-Tyr under standard conditions to give **11**. We found that osmium tetraoxide/sodium periodate gave a cleaner conversion of **11** to the corresponding aldehyde than treatment with ozone. The reaction was followed by TLC with verification of the presence of the aldehyde by NMR. This aldehyde was not isolated, but treated immediately with DBU in methylene chloride to give the protected cyclic peptides **12a** and **12b** in moderate (43–62%) yield for the two steps. Successive deprotection then afforded the homologous olefins **13a** and **13b**. NMR and RP-HPLC data suggest that a single olefin isomer predominated, which we presume to be the *trans*-isomer. Hydrogenation afforded in each case, a mixture of isomers, which could be separated by RP-HPLC to give **14** and **15**, which are isomeric at the newly formed chiral center (Scheme 3). Both isomers gave NMR and MS data consistent with the assigned structures although we were unable to assign their absolute stereochemistry.

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-β_i antibody and HRP-conjugated anti-mouse IgG: chromogenic substrate (K-Blue).[10] Both isomers in the 13-membered ring series **14a** and **15a** were relatively potent in the VCAM–VLA-4 binding assay (IC_{50} 44 and 89 nM, respectively) and were approximately 10-fold more potent than the corresponding 14-member ring homologues **14b** and **15b**. The olefins **13a** and **13b** were in between with the higher potency again observed with the 13-member ring compound. In this assay system, the



Scheme 3.

prototype disulfide containing 13-member ring compound **1** had an IC_{50} of 0.5 nM. Thus, while activity and sensitivity to ring size was retained, the transition from a cyclic disulfide to a carbacyclic system led to a pronounced decrease in potency.

References and Notes

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