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Potent Integrin Antagonists from a Small Library of RGD-Including Cyclic Pseudopeptides

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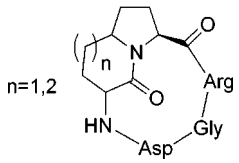
ABSTRACT



A small library of cyclic RGD pseudopentapeptides incorporating stereoisomeric 6,5- and 7,5-fused bicyclic lactams was synthesized with the aim of developing active and selective integrin antagonists. The solid-phase synthesis and activity of these RGD derivatives is described. The approach led to two of the most active known inhibitors of $\alpha_V\beta_3$ receptor.

The integrins are α/β heterodimeric cell surface receptors which play a major role in cell−cell and cell−matrix adhesive interactions.[1] The β_3 class of the integrin family has gained special attention in recent medicinal chemistry.[2] The $\alpha_V\beta_3$ receptor is expressed on the surface of a variety of cell types and is implicated in many pathological processes, such as tumor metastasis, angiogenesis, and osteoporosis.[3] The $\alpha_{IIb}\beta_3$ integrin is mainly involved in the platelet aggregation process, which is responsible for thrombosis.[4] Therefore, selective inhibition of specific integrin β_3 subtypes is of great pharmaceutical interest, offering therapeutically promising perspectives for different diseases. For instance, compounds that bind $\alpha_V\beta_3$ integrins selectively have been shown to inhibit angiogenesis and are currently under active investigation as potential antitumor drugs and for the treatment of osteoporosis.[3,5]

Many integrins, including those of the β_3 class, recognize the tripeptide sequence Arg-Gly-Asp (RGD) in their extracellular matrix adhesive proteins.[1,3] Integrin selectivity appears to derive from recognition of different conformations of the RGD tripeptide determinant, as well as of additional binding sites. Cyclic RGD peptides have been developed by different groups as active and selective integrin antagonists.[3,6,7,8] The conformational constraint imposed by the cyclic template has been shown to be a valuable tool in the indirect determination of the bioactive conformation. To explore the spatial requirements of the antagonist pharmacophore for the selective inhibition of either $\alpha_{IIb}\beta_3$ or $\alpha_V\beta_3$, an efficient procedure of "spatial screening" was developed by Kessler et al.[3,9] The procedure, based on the synthesis of stereoisomeric cyclic peptide libraries, led to the highly active

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$\alpha_V\beta_3$-selective first-generation cyclic pentapeptide cyclo(-Arg-Gly-Asp-D-Phe-Val-). Extensive modifications of this lead structure with different peptidomimetics and carbohydrate scaffolds have been performed with the aim of obtaining a further reduction of the flexibility, and new potent antagonists were identified.[10−12] Distinct turn mimetics[13] and sugar amino acids[11] were introduced by replacing the D-Phe-Val dipeptide, which should adopt a βII′-turn backbone geometry and hence force the RGD sequence into a kinked, $\alpha_V\beta_3$-selective conformation.

In the course of our studies on peptide secondary structure mimics, we reported the synthesis[14] and conformational analysis[15] of a series of 1-aza-2-oxobicyclo[*X*.3.0]alkane amino acids (Figure 1), which can be regarded as conformationally restricted substitutes for Ala-Pro dipeptide units. All these bicyclic lactams include a natural L-Pro residue, but vary in the lactam ring size and in the configuration at the bridgehead atom and at the NH_2-bearing carbon.

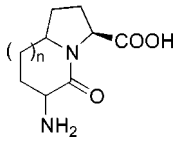

Figure 1. Azabicyclo[*X*.3.0]alkane amino acids.

Computational and spectroscopic studies have revealed that these bicyclic scaffolds can mimic reverse-turn motifs, although there is a dependence of the turn-inducing ability on the lactam ring size and stereochemistry.[15] The replacement of the D-Phe-Val dipeptide in the lead structure c(RGDfV) with such azabicycloalkane scaffolds showing different reverse-turn mimetic properties could constrain the RGD sequence into different conformations and possibly provide the required activity and selectivity for integrin antagonism. To further address the process of spatial screening, a small library of RGD-containing cyclic pseudopentapeptides incorporating stereoisomeric 6,5- and 7,5-fused bicyclic lactams **1**−**8** (Figure 2) was synthesized. The solid-phase synthesis of these RGD cyclic pseudopeptides and their in vitro ability to compete for the binding of ^{125}I-echistatin to purified $\alpha_V\beta_3$ are reported.

Results and Discussion. 1. Synthesis. The plan for the synthesis of the RGD cyclic pseudopeptides called for solid-phase synthesis of the linear peptide sequences, using the 9-fluorenylmethoxycarbonyl (FMOC) protection strategy, followed by cyclization and side-chain deprotection in solution.

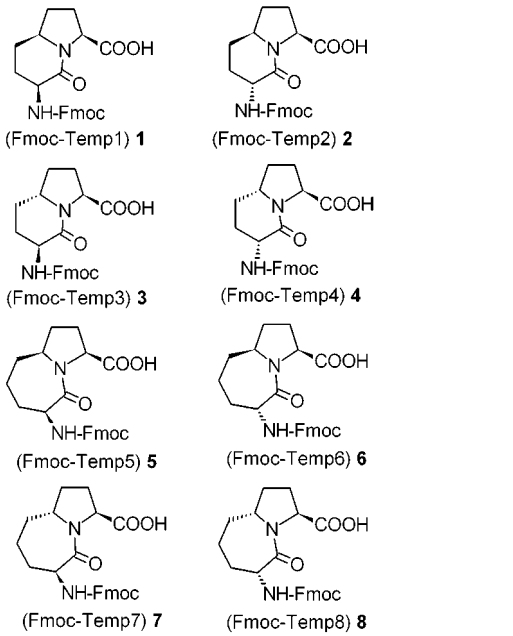

Figure 2. Bicyclic lactam templates *N*-Fmoc-[Temp]-OH **1**−**8**.

To minimize steric hindrance at the cyclization site, cyclization between the Gly and Asp residues was envisaged, and the linear peptides **9**−**16** (Figure 3) were chosen as the target linear sequence. The Arg and Asp side chains were protected using the Pmc (2,2,5,7,8-pentamethylchroman-6-sulfonyl) and tBu groups, respectively. Both these protecting groups are removed under acidic conditions and thus are compatible with the FMOC strategy. In turn, this choice suggested the use of SASRIN resin[16] as the solid support. The SASRIN linker is highly labile under mildly acidic conditions (1% TFA in DCM), and cleavage of the peptide can be achieved without affecting the side-chain protection.

The bicyclic lactams Temp1−Temp8 were prepared by following our published procedure[14] and were protected at the free amino end as fluorenyl carbamates, using Fmoc-*O*-succinimmide (Fmoc-*O*-NSu). The *N*-Fmoc-[bicyclic lactam]-OH compounds **1**−**8** (Figure 2) were obtained in quantitative yield, ready for use in solid-phase chemistry.

The sequence for the solid-phase synthesis is outlined in Scheme 1. *N*-Fmoc-glycine was anchored to SASRIN by the DIC/HOBt/DMAP protocol, followed by capping with Ac_2O and DMAP. Deprotection of the amino groups was achieved under standard conditions, with a 20% piperidine solution in DMF. Condensation of the *N*-Fmoc-Arg(Pmc)-OH residue was performed using DIC/HOAt and subsequent capping of the free amino groups with acetylimidazole in dichloro-

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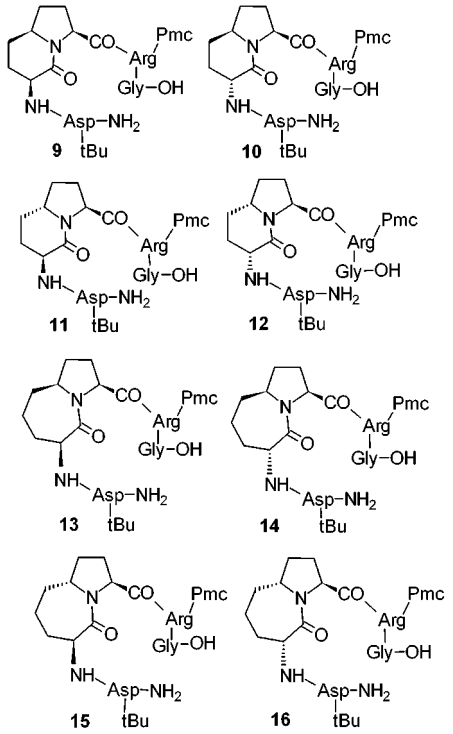
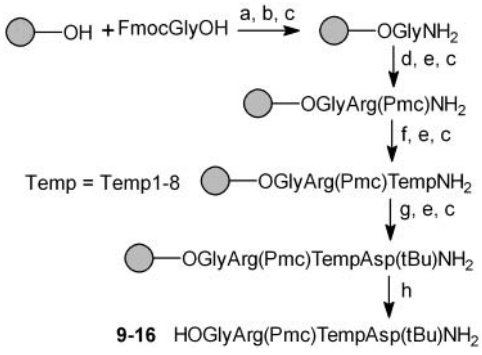


Figure 3. Linear pseudopeptides H_2N-Asp(tBu)-Temp-Arg(Pmc)-Gly-OH **9**−**16**.

methane. After deprotection of the Fmoc group, coupling of the templates **1**−**8** was accomplished with the potent Carpino's condensation system HATU/HOAt/2,4,6-collidine.[17] Owing to their high synthetic value, the templates were used in a stoichiometric amount with respect to the resin. After capping with acetylimidazole and deprotection, the *N*-Fmoc-Asp(tBu)-OH was introduced on the sterically hindered amino group of the templates using HATU/HOAt/2,4,6-collidine and a large excess of reagents. After capping

Scheme 1. Synthesis of the Protected Linear Peptides **9**−**16**[a]

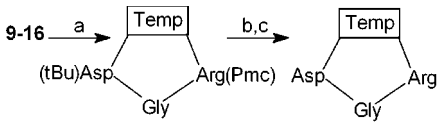


[a] (a) DIC/HOBt/DMAP, DMF; (b) Ac_2O, DMAP; (c) Pip/DMF 20%; (d) FmocArg(Pmc)OH, HOAt/DIC, DCM/DMF 2:1; (e) AcIm, DCM; (f) FmocTempOH, HATU/HOAt/2,4,6-collidine, DMF/DCM 3:1; (g) FmocAsp(tBu)OH, HATU/HOAt/2,4,6-collidine, DMF/DCM 3:1; (h) TFA/DCM 1%.

with acetylimidazole and nitrogen deprotection, the linear pseudopeptides **9**−**16** were cleaved from the resin by treatment with a 1% solution of TFA in dichloromethane. Filtrates were immediately neutralized with pyridine to avoid side-chain deprotection. Crude products were purified by size-exclusion chromatography (XAD-2 resin) to give linear peptides **9**−**16** in 30−67% overall yields.

The peptides were cyclized in a DMF solution (0.05 M) with HATU/HOAt/2,4,6-collidine at room temperature (Scheme 2). Products **17**−**23** (Figure 4) were obtained from

Scheme 2. Cyclization of **9**−**16** and Deprotection[a]



17 (Temp=Temp1)
n.r. (Temp=Temp2)
18 (Temp=Temp3)
19 (Temp=Temp4)
20 (Temp=Temp5)
21 (Temp=Temp6)
22 (Temp=Temp7)
23 (Temp=Temp8)

24 (Temp=Temp1)
25 (Temp=Temp3)
26 (Temp=Temp4)
27 (Temp=Temp5)
28 (Temp=Temp6)
29 (Temp=Temp7)
30 (Temp=Temp8)

[a] (a) HATU/HOAt/2,4,6-collidine, DMF; (b) TFA, thioanisole, 1,2-ethanedithiol, anisole 90:5:3:2; (c) ion-exchange chromatography (AMBERLITE IRA-93 resin).

9 and **11**−**16** and isolated by flash cromatography in 26−70% yields. On the contrary, peptide **10** failed to cyclize

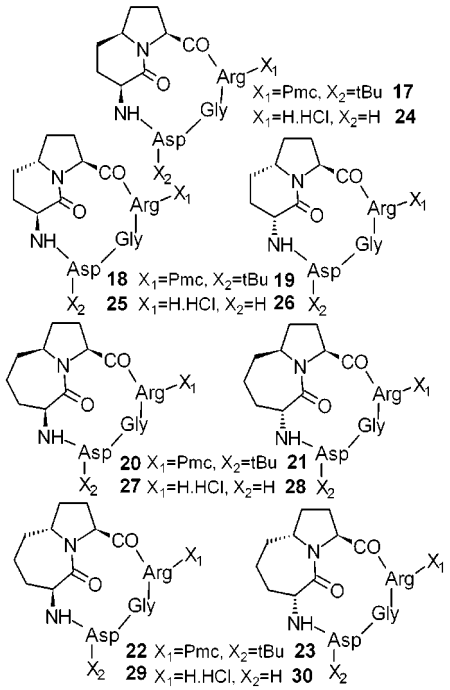


Figure 4. Protected cyclic pseudopeptides cyclo[-Temp-Arg(Pmc)-Gly-Asp(tBu)-] **17**−**23** and RGD cyclic pseudopeptides cyclo(-Temp-Arg-Gly-Asp-) **24**−**30**.

Table 1. Inhibition of ^{125}I-Echistatin Binding to $\alpha_V\beta_3$ Receptor[a]

compound	IC_{50} (nM) ± SD	K_i (nM) ± SD
echistatin	0.28 ± 0.08	0.26 ± 0.07
vitronectin	44.1 ± 17.0	40.7 ± 15.7
fibronectin	835.4 ± 287.0	771.1 ± 264.9
c(RGDfV)	195.9 ± 16.8	157.8 ± 13.5
24	206.9 ± 8.7	191.0 ± 8.0
25	97.3 ± 7.5	89.8 ± 6.9
26	14.3 ± 4.7	11.5 ± 3.8
27	245.2 ± 43.0	226.3 ± 39.7
28	2478.4 ± 373.2	2287.8 ± 344.5
29	412.5 ± 94.1	332.3 ± 75.8
30	3.7 ± 0.6	3.0 ± 0.5

[a] The IC_{50} values were calculated as the concentrations of compounds required for 50% inhibition of echistatin binding and were estimated by the Allfit program. The K_i of the competing ligands were calculated according to the Cheng and Prusoff equation.[19] Values are the mean ± standard deviation of triplicate determinations.

under the above conditions. Changing the base, from collidine to DIPEA, and warming the reaction at 40 °C did not change the result of the reaction.

Side-chain deprotection was achieved in quantitative yields by treating **17**−**23** with TFA, in the presence of ion scavengers (Scheme 2). The resulting TFA salts were transformed into the corresponding chlorides **24**−**30** (Figure 4) by ion-exchange chromatography and were tested and stored as chloride salts.

2. Biological Evaluation. The RGD cyclic pseudopeptides **24**−**30** were examined in vitro for their abilities to compete with ^{125}I-echistatin for binding to the purified $\alpha_V\beta_3$ receptor (Table 1). It has been demonstrated that both purified and membrane-bound integrin $\alpha_V\beta_3$ bind with very high affinity to echistatin, which can be antagonized efficiently by linear and cyclic RGD-containing peptides.[18] Among the seven peptides tested, compounds **26** and **30** showed the highest affinity to $\alpha_V\beta_3$ and inhibited echistatin binding to $\alpha_V\beta_3$ with a K_i of 11.5 ± 3.8 and 3.0 ± 0.5 nM, respectively. Interestingly, the affinities of these RGD cyclic pseudopeptides for the $\alpha_V\beta_3$ integrin in this kind of assay were almost 10 and 50 times higher than the affinity of the lead structure c(RGDfV) used as reference compound.

$\alpha_{IIb}\beta_3$ receptor binding assays, endothelial cells adhesion inhibition tests, and in vivo investigations in angiogenesis models are currently in progress. Effects of the structural constraint introduced by the bicyclic templates on the conformation of the RGD sequence will be also reported in due course.

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Supporting Information Available: Full experimental procedures for syntheses and receptor binding assays. Characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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