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Solid-Phase Synthesis of Cyclic RGD-Furanoid Sugar Amino Acid Peptides as Integrin Inhibitors

Renate M. van Well,[a] Herman S. Overkleeft,[a] Gijsbert A. van der Marel,[a] Dan Bruss,[b] Gaétan Thibault,[c] Phillip G. de Groot,[d] Jacques H. van Boom[a] and Mark Overhand[a,*]

[a]*Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300 RA Leiden, The Netherlands*
[b]*Department of Chemistry, Central College, 812 University, Pella, IA 50219, USA*
[c]*Laboratory of Cell Biology of Hypertension, Clinical Research Institute Montreal, 110 Pines Avenue West, Montreal, Quebec, Canada H2W 1R7*
[d]*Department of Haematology, University Medical Center, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands*

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Abstract—The solid-phase synthesis of cyclic RGD peptides containing either one or two furanoid sugar amino acids (SAAs) is reported. Using a cyclization-cleavage approach five peptides were successfully assembled and consecutively tested on their ability to bind to the integrin receptors $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$. The cyclic tetrapeptide c[RGD-SAA] (**1**) showed the most promising activity in an inhibition assay with an IC_{50} of 1.49 μM for the $\alpha_v\beta_3$ receptor and 384 nM for the $\alpha_{IIb}\beta_3$ receptor.

Integrin receptors are a family of heterodimeric transmembrane glycoproteins, which are involved in cell–cell and cell–matrix interactions. Two members of this family, the $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ receptor, have been studied extensively in the past two decades. It has been shown that the $\alpha_v\beta_3$ receptor plays a key role in angiogenesis, tumor metastasis and acute renal failure,[1] while the $\alpha_{IIb}\beta_3$ receptor mediates platelet aggregation.[2] The latter process may lead to blood clot formation which, in turn, may cause severe pathological disorders such as myocardial infarction, unstable angina or ischemic stroke. Both receptors recognise the consensus sequence Arg-Gly-Asp (RGD) of their natural ligands with a high degree of selectivity.[1,2] A determining factor responsible for this selectivity is thought to be the conformation of the RGD motif.[3]

The search for inhibitors of integrin receptors led to the discovery of several cyclic and linear RGD containing peptidic constructs showing potent antagonistic activity.[4] It is generally accepted that cyclic RGD peptides display a higher activity compared to the linear counterparts. This beneficial effect may be due to the fact that cyclic peptides are conformationally less flexible and metabolically more stable.[5] For example, a more pronounced secondary structure could be induced by the incorporation of a rigid scaffold [e.g., penicillamine, *m*-(aminomethyl) benzoic acid, 5-aminomethyl-2-thiophene acetic acid] into cyclic RGD peptides.[6] On the other hand, it is well established now that sugar amino acids (SAAs) are promising tools in eliciting conformational restraints in oligosaccharide analogues[7] and peptides.[8] The merit of using a pyranoid SAA as a conformationally restricted dipeptide isostere in the synthesis of cyclic RGD analogues was nicely demonstrated by Kessler et al. in the development of efficient inhibitors of both the $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ receptor.[9]

A recent contribution from our laboratory revealed that cyclic peptides, containing one or two furanoid SAA units, as well as hydrophobic amino acids (i.e., Gly, Ala and Phe), were readily accessible by cyclization-cleavage of the corresponding linear sequences immobilised on a solid support via the base labile *p*-nitrobenzophenone oxime linker.[10]

Here we wish to report that the Kaiser's oxime approach can also be applied successfully for the preparation of the furanoid SAA containing cyclic RGD peptides **1**–**5** (see Fig. 1). Furthermore, the affinity of

*Corresponding author. Tel.: +31-71-527-4483; fax: +31-71-527-4307; e-mail: overhand@chem.leidenuniv.nl

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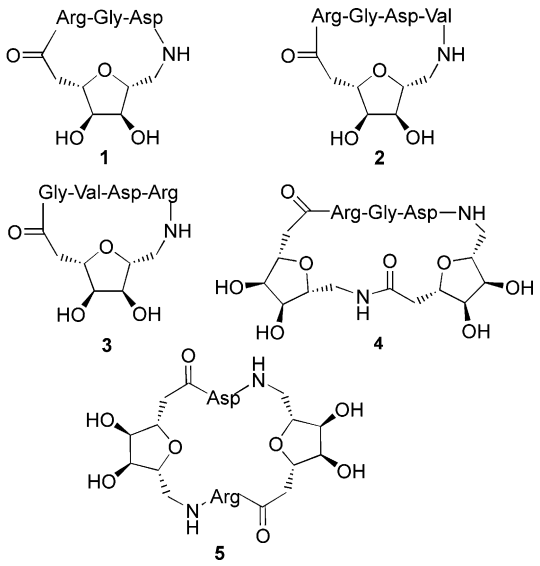
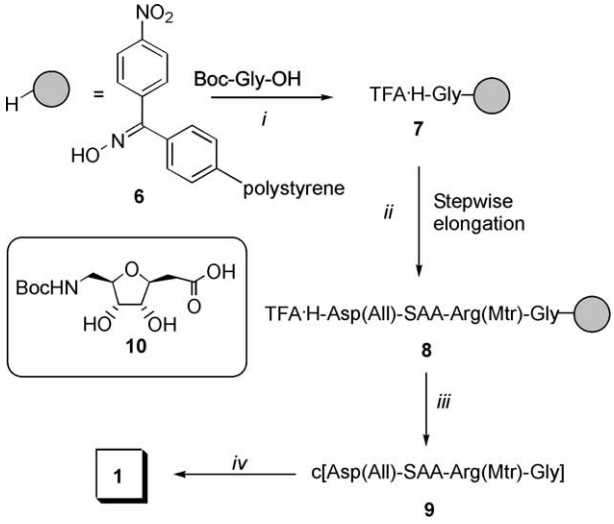


Figure 1. Target molecules.

the latter cyclic peptides for the integrin receptors $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$, as well as their effect on blood coagulation of human plasma is evaluated.

The intrinsic base-lability of the oxime resin employed in the cyclization-cleavage step requires a strategy based on Boc-chemistry. Accordingly, the known Boc protected furanoid SAA 2,7-dideoxy-3,6-anhydro-7-*tert*-butyloxycarbonyl-amino-D-*allo*-heptonic acid[10,11] (**10** in Scheme 1) in combination with *N*-Boc amino acids were used in the synthesis of the target molecules **1**–**5**. The side chain functionalities in Asp and Arg were protected with an allyl and 4-methoxy-2,3,6-trimethyl-benzenesulfonyl (Mtr) group, respectively. The latter protecting group, normally used in Fmoc-based solid phase peptide synthesis, proved to be compatible with a stepwise



Scheme 1. Reagents and conditions: (i) (a) BOP (5 equiv), DIPEA (6.5 equiv), NMP, 2 h, two times; (b) 25% TFA, 1% TIS, DCM, 5×5 min; (ii) (a) amino acid or SAA (5 equiv), BOP (5 equiv), DIPEA (6.5 equiv), NMP, 45 min; (b) 25% TFA, 1% TIS, DCM, 5×5 min; (iii) DIPEA (2 equiv), AcOH (2 equiv), DMF, 36 h; (iv) (a) $Pd(PPh_3)_2Cl_2$, AcOH, Bu_3SnH, 1 h; (b) 95% TFA, 2.5% H_2O, 2.5% TIS, 16 h.

solid phase peptide synthesis based on Boc- chemistry.[12] The SAA containing RGD peptides **1**–**5** were constructed as outlined in Scheme 1 for the target compound **1**. Thus, loading of the oxime resin **6** with Boc-protected glycine was carried out under the influence of Castro's reagent (BOP) and diisopropylethylamine (DIPEA).[13] Unmasking of the Boc group with 25% TFA in the presence of the cation scavenger triisopropylsilane (TIS) provided the immobilised glycine **7**. Sequential elongation of **7** with Boc-Arg(Mtr)-OH, Boc-SAA-OH and Boc-Asp(All)-OH, using the same coupling and Boc cleavage conditions, yielded the linear immobilised precursor **8**. Cyclization of **8** with concomitant cleavage from the solid support was effected with a 1:1 mixture of DIPEA and acetic acid in DMF to give crude **9** which was purified by HPLC. Deallylation of **9** by palladium(II)-catalysed hydrostannolysis[14] followed by acidolysis of the Mtr group ($TFA/H_2O/TIS$ 95:2.5:2.5) afforded, after HPLC purification, target compound **1** in a low overall yield. (see entry 1, Table 1). In order to improve the yield of **1**, we explored the cyclization efficiency of the linear sequences resulting from using the SAA building block **10** or Boc-Asp(All)-OH in the anchoring step to the oxime resin **6**.[15,16]

Cyclization of the sequence in entry 2, containing a C-terminal SAA unit, proceeded with comparable moderate efficiency as observed for the sequence in entry 1. On the other hand, submission of the linear precursor in entry 3 to the cyclization conditions afforded **1** in a yield of 17%. Compound **2** was obtained in a similar yield by cyclization of the linear precursor in entry 4. The same result was observed for compound **3** starting from the immobilised peptide in entry 5. Remarkably, transformation of the linear peptide in entry 6 containing two SAA residues led to the isolation of compound **4** in 31%. In sharp contrast, attempts to prepare compound **5** from the sequence having a SAA as the first residue attached to the resin (entry 7) proved to be unsuccessful. Gratifyingly, it turned out that the linear intermediate starting with aspartic acid underwent cyclization to give target compound **5** (see entry 8).

Having target compounds **1**–**5** in hand, their ability to displace ^{125}I-echistatin from the $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ integrin receptors was examined.[17] To this end human skin fibroblasts extracts were used as the source of $\alpha_v\beta_3$ integrins while the $\alpha_{IIb}\beta_3$ integrins were isolated from

Table 1.

Entry	Sequence of immobilized linear precursor	Prod.	Yield[a] (%)
1	TFA·H-Asp(All)-SAA-Arg(Mtr)-Gly-•	**1**	4
2	TFA·H-Arg(Mtr)-Gly-Asp(All)- SAA-•	**1**	3
3	TFA·H-SAA-Arg(Mtr)-Gly-Asp(All)-•	**1**	17
4	TFA·H-Asp(All)-Val-SAA-Arg(Mtr)-Gly-•	**2**	19
5	TFA·H-Arg(Mtr)-SAA-Gly-Val-Asp(All)-•	**3**	16
6	TFA·H-Arg(Mtr)-Gly-Asp(All)-SAA-SAA-•	**4**	31
7	TFA·H-Arg(Mtr)-SAA-Asp(All)-SAA-•	**5**	—
8	TFA·H-SAA-Arg(Mtr)-SAA-Asp(All)-•	**5**	9

[a]Yields were determined after synthesis of the linear precursor, cyclization-cleavage followed by HPLC purification and 2-step deprotection followed by HPLC purification.

crude human platelets. The efficiency (IC_{50}-values) of the cyclic peptides **1–5** to displace ^{125}I-echistatin from both integrin receptors is recorded in Table 2.

Perusal of these data shows that the most promising results were obtained for c[RGD-SAA] (**1**), with an IC_{50} value of 1.49 μM for the $\alpha_v\beta_3$ receptor and 384 nM for the $\alpha_{IIb}\beta_3$ receptor. It can also be seen that c[RGDV-SAA] (**2**) has a lower affinity for the two integrin receptors, while c[RGD-SAA-SAA] (**4**) displayed very modest affinity. In accordance with expectation, a scrambled sequence as in compound **3** did not inhibit ^{125}I-echistatin binding to the $\alpha_v\beta_3$ or the $\alpha_{IIb}\beta_3$ receptor, even at a concentration as high as 1 mM. A similar result was observed for compound **5**, purposely designed to force the Asp and Arg side chains in a specific spatial arrangement. It is not excluded that the induced orientation of the side chains prohibited receptor binding.[3]

The ability of compounds **1–5** to inhibit platelet aggregation was investigated in an assay using ADP stimulated human PRP.[18] The results of these studies, using the known linear tetrapeptide rGDW ($IC_{50} = 2.1$ μM) as a reference, are presented in Figure 2.[19,20] The cyclic peptide c[RGD-SAA] (**1**) showed complete inhibition of platelet aggregation at a concentration of 100 μM. By testing the inhibitory capacity of compound **1** in a range of different concentrations, an IC_{50} value of 6.7 μM was found. On the other hand, an IC_{50} value of 184 μM was established in the case of compound **2**, which only partially inhibited aggregation at 100 μM. Addition of compound **3, 4** or **5** to activated PRP did not influence platelet aggregation at the 100 μM level. These results are in agreement with those obtained with the isolated receptors.

In conclusion, five novel RGD peptides containing either one or two furanoid SAAs (i.e., **1–5**) were synthesised via an efficient solid-phase strategy based on the Kaisers' oxime resin. The cyclic peptide c[RGD-SAA] (**1**) showed promising activity as an inhibitor of the isolated $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ receptors as well as platelet aggregation. Future research will be directed towards the conformational analysis of the active furanoid SAA containing cyclic RGD peptides. The latter structural information may eventually lead to the design of novel antagonists with increased affinity for specific integrin receptors.

Table 2. Efficacy (IC_{50}, μM) of compounds **1–5** to displace ^{125}I-echistatin from $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ receptors

Compd	$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$
1	1.49 ± 0.08	0.384 ± 0.061
2	98.1 ± 12.1	32.2 ± 4.9
3	Inactive[a]	Inactive[a]
4	597 ± 58	198 ± 12
5	Inactive[a]	Inactive[a]

[a]Inactive: no displacement observed at a concentration of 1 mM.

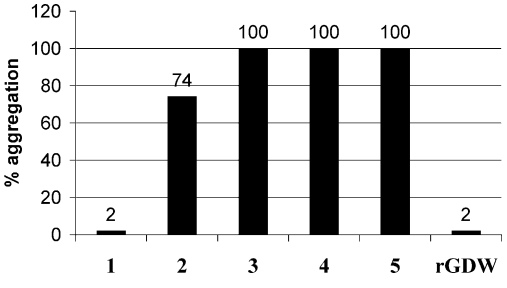


Figure 2. Platelet aggregation at 100 μM concentration of RGD peptides **1–5**.

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