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RGD mounted on an L-proline scaffold

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Abstract—The construction of an L-proline scaffold that enforces a defined beta-turn loop for RGD is reported. A key feature was the use of SASRIN (super acid sensitive resin) that allowed solid-phase synthesis of the tetrapeptide. A HATU-induced cyclization of the sequence was successful, followed by a single acid-promoted deprotection of the final product.

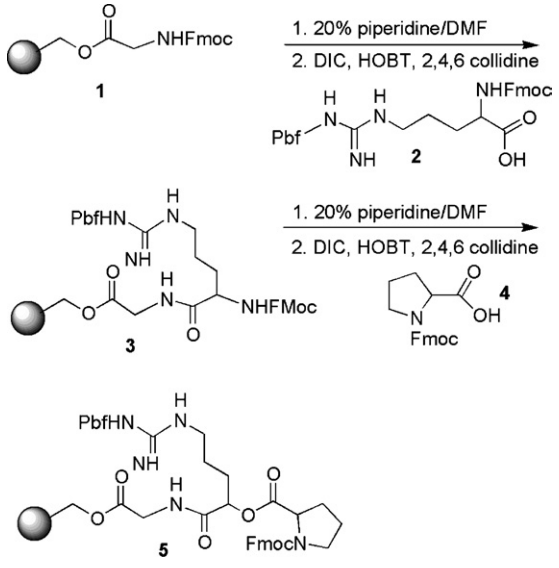
Integrins are proteins involved in several important cellular processes, including cell-signaling, cell–cell adhesion, apoptosis and cell-matrix adhesion.[1] Overexpressed in tumor cells, integrins can bind to short amino acid sequences that have the potential to be attractive antitumor agents.[2] The consensus binding sequence in numerous integrin ligands is the tripeptide RGD, the single letter amino acid code for arginine-glycine-aspartic acid.[3]

There have been several RGD motifs extensively studied using both ^{1}H NMR and crystallographic methods.[4] RGD has also been mounted on various scaffolds including polymers.[5] Overall, these structures provide insight into the fine details of the integrin-ligand recognition sequence.

The interaction between the peptides and their receptor targets commonly involves beta-turn structures for the ligands on the surface of proteins.[6] Recent studies indicate the importance of cyclic beta-turns in the design of RGD peptides to increase affinity.[7] We report here, the design of an L-proline scaffold that enforces a defined beta-turn loop for RGD. This new synthetic approach will involve mounting an acyclic RGD to span the distance between carbonyl and amine of the chiral proline ring. In this article, we show how the tetrapeptide was formed on solid support and, more importantly, how the cyclization off-support was achieved.

We selected SASRIN (super acid sensitive resin), for the initial solid-phase synthesis portion of our route.[8] With 0.5 mmol g^{-1} loading capacity, SASRIN is related to Wang resin. The major difference, besides structural differences, is the method of cleavage. Wang resin requires much harsher cleavage conditions often requiring 50% TFA. This is not compatible with our strategy because of potential cleavage of the protecting groups on arginine and aspartic acid. Conversely, SASRIN cleavage requires only 1% TFA in methylene chloride that allows side chain protecting groups to survive these conditions.

Starting with SASRIN-Gly-NHFmoc (**1**), as shown in Scheme 1, the solid-phase synthesis was key to



Scheme 1. The synthesis of tripeptide **5**.

Keywords: RGD; SASRIN resin; Beta-turn mimetic.
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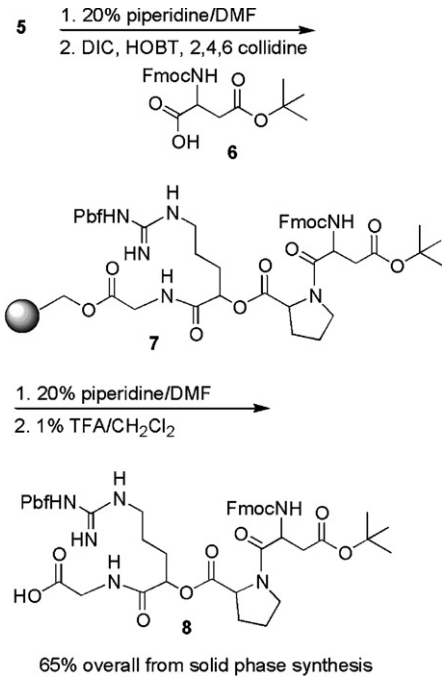
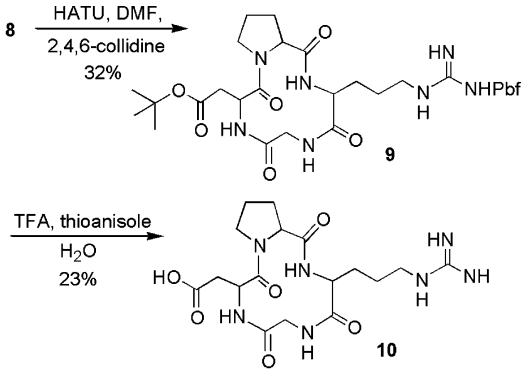


Scheme 2. The synthesis of acyclic tetrapeptide **8**.



Scheme 3. Cyclization to prepare **10**.

constructing the linear tetrapeptide in a small number of repeated and similar steps for each peptide bond. First, deprotection of an Fmoc to a free amine was performed with piperidine and second, a coupling reaction with diisopropylcarbodiimde (DIC), 1-hydroxybenzotriazole (HOBt), and 2,4,6-collidine was performed, as shown in Schemes 1 and 2. All the reagents in the coupling reactions were used in 3-fold excess. Yields of the steps were all over 95% based on the recovery of the bead.

Tetrapeptide **7** was subjected to mild cleavage conditions (Schemes 2 and 3) with 1% trifluoroacetic acid in methylene chloride to give **8**. After cyclization with HATU and HOBt, **9** was obtained and subsequently treated with a mixture of trifluoroacetic acid, thioanisole, anisole, and water (90:5:3:2). After purification by reverse-phase HPLC the isolated cyclic peptide **10** was synthesized in 23% yield.

In conclusion, a successful synthesis of a cyclic RGD on a proline scaffold was achieved. Noteworthy was the use of SASRIN and a single final deprotection with 1% TFA.

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Supplementary data

Full characterization of all new compounds. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.04.029.

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