Synthesis of RGD Analogues Containing α-Trifluoromethylaspartic Acid as Potential Fibrinogen Receptor Antagonists

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The total synthesis of a peptide containing α-trifluoromethylaspartic acid at the P^3 position is described.

The binding of fibrinogen to its platelet receptor GP IIb/IIIa is mediated by the sequence RGD (Arg-Gly-Asp) and represents a contributing factor in the platelet-mediated thrombus formation. As this natural sequence constitutes an attractive goal for an antithrombotic therapy, many classes of RGD peptide mimetics have been developed from search of conformationally constrained fibrinogen receptor analogues with increasing potency.[1–4]

Since it is known that the incorporation of α, α-disubstituted amino acids into key positions of peptides is a useful approach to stabilize secondary structure,[7] our attention has been focused on α-trifluoromethylamino acid-containing peptides. In fact, owing to the considerable steric hindrance and the tendency to form hydrogen bonds, α-trifluoromethyl substitution often induces tight turn structures, that lead to increased stabilization.[8]

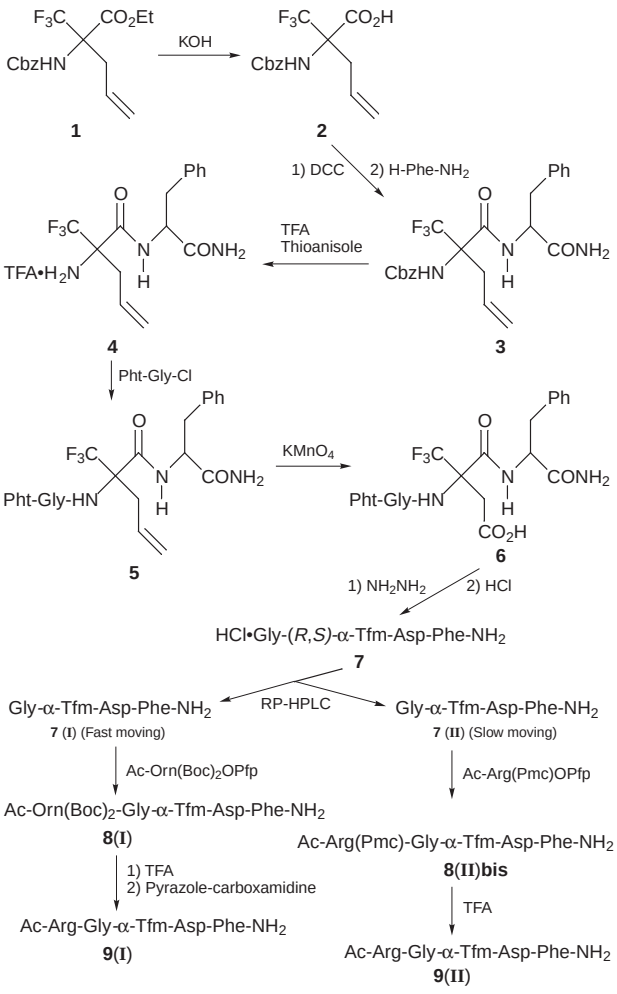
Incorporation of α-trifluoromethylamino acids (Tfm-Xaa) into peptides represents a synthetic challenge, because the reactivity of the carboxylic and especially of the amino functions is decreased and standard methods of peptide synthesis can not be readily applied. Moreover, the choice of synthetic methods depends on the position of the Tfm-amino acid residue incorporated into the peptide chain.

A systematic study has been undertaken for the synthetic feasibility of bioactive peptides (RGD analogues) containing an α-Tfm-amino acid functionality at different positions. In a previous work, tetrapeptides containing α-Tfm-arginine at the N-terminal position have been obtained;[15] in the present article we describe the incorporation of α-Tfm-aspartic acid at the P^3 position of the peptide chain.

The feasibility of different coupling methods for building the peptide chain was studied and the different procedures used are described below.

The synthetic strategy used to obtain the diastreomeric peptides **9(I)** and **9(II)** is illustrated in Scheme 1. The building block **1**, as a precursor of α-Tfm-aspartic acid, was synthesized by methods already described in the literature.[8] The allylic side chain was maintained throughout the first synthetic steps leading to the pseudopeptide **5**, thus avoiding the problem of *β*-carboxy protection. The precursor dipeptide **4** was prepared in three steps from **1**: (1) alkaline hydrolysis of the ester function to give **2** (yield 97%); (2) formation of the oxazolone with DCC, followed by ring opening with phenylalanine amide to provide **3** (yield 90%); (3) cleavage of the N–Cbz bond with TFA–thioanisole (yield 76% after purification by flash chromatography). Attempts to transform **2** directly into the corresponding Leuchs anhydride[10] by treatment with thionyl chloride led to modest yields owing to the volatility of the anhydride.

To achieve N-elongation of an α-Tfm-amino acid in good yield represents a challenging problem; however, we could readily prepare **5** in 94% yield *via* phthaloyl glycine chloride the risk of racemization using glycine being absent. Regioselective oxidative cleavage of the double bond with $KMnO_4$ proceeded smoothly giving the *N*-phthaloyl-tripeptide **6** in quantitative yield. After deblocking the *N*-phthaloyl residue by hydrazine, purification and separation of the two diastereomeric forms of compound **7** were performed by preparative RP-HPLC. In fact, separation



Scheme 1

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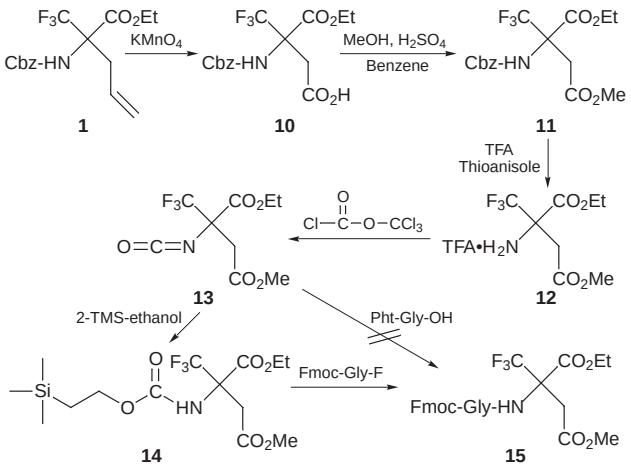

Scheme 2

of the two isomers **7(I)** and **7(II)** was very easy at this stage, whereas it proved difficult for the final tetrapeptides. Therefore, the last steps of the synthesis were carried out in parallel for each isomer; two alternative synthetic pathways affording peptides **9(I)** and **9(II)**, respectively, were followed in order to compare the yields. The method used for peptide **9(I)** proved to be the more convenient; the second method was affected by lower yields owing to the undesired cyclization of the carboxy-activated Ac-Arg(Pmc)-OPfp, leading to the α-amino-lactam.

An alternative method (illustrated in Scheme 2) was also investigated for the N-elongation of α-Tfm-aspartic acid *via* isocyanate. While formation of the amide bond by direct reaction of the isocyanate with Pht-Gly-OH failed in our hands, we could obtain the precursor dipeptide **15** in rather low yield (30%) *via in situ* deprotection/coupling of 2-trimethylsilylethoxycarbonyl (Teoc) protected α-Tfm-aspartic acid diester **14** with Fmoc-Gly-F (as described recently by Sewald *et al.*[12]). However, the coupling of the latter peptide with phenylalanine still has to be studied.

In summary, we have described an efficient approach to the incorporation of an α-Tfm-amino acid at a central position of a peptide chain.

Techniques used for the characterization of intermediates and final compounds: ^{1}H, ^{13}C and ^{19}F NMR, MS, elemental analyses

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