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Synthesis of linear tuftsin analogues modified at the ε-amino group of lysine

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ABSTRACT

In this Letter, eight tuftsin analogues, seven of which are novel, are presented. All the linear tuftsin analogues contain an isopeptide bond. Modification of the tuftsin chain was based on the introduction of simple amino acids such as valine, glycine, alanine and β-alanine into the peptide chain at the ε-amino group of lysine. The peptides were synthesized by a solid-phase method using the standard Fmoc procedure. Simultaneous deprotection of the peptide side chain and liberation from the resin was achieved using TFA, and the free novel tuftsin analogues were purified and characterized.

Tuftsin (H-^{289}Thr-Lys-Pro-Arg^{292}-OH) is a tetrapeptide of natural origin that was first isolated in 1970 at Tufts University by Najjar and Nashioka.[1] It is present in mammalian blood and its source is from immunoglobulin molecules. Tuftsin is localized in a heavy chain of immunoglobulin G and is liberated by the action of two specific enzymes, leukokininase (Lys^{288}-Thr^{289}) and spleen tuftsin endocarboxypeptidase (Arg^{292}-Gly^{293}).[1–6] Tuftsin can activate several elements of the immune system such as granulocytes and macrophages. It has been investigated not only in bacterial infections caused by *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and *Serratia marcescens* but also as a co-administration agent with antibiotics against opportunistic infections and has demonstrated beneficial results.[7–13] In spite of the fact that it possesses antibacterial and antiviral features, tuftsin is also an antitumour and antifungal agent. However, the peptide is unstable in plasma and its half-life in blood is 16 min.[2,3] Therefore, it is important to synthesize new analogues of tuftsin which are more resistant to proteolytic degradation. Numerous different modifications of the tuftsin chain which have improved the biological activity have been reported to date. These include changing the length of the central chain and modifying the sequence of amino acids in the primary structure of tuftsin.[3,4,6] Promising results were dependent on peptide resistance against enzymatic cleavage.[3–5,14] Interestingly, recent research has proved that retro-tuftsin analogues significantly surpass tuftsin in both stability and activity.[5] To date, many retro-tuftsin analogues that have demonstrated very encouraging results have been synthesized but there is still wide scope for further research.[4,6] In current studies, tuftsin analogues containing an isopeptide bond have shown increased resistance in relation to tuftsin. The introduction of an additional residue at the ε-amino group of lysine via –NH–CO– formation has led to the isopeptide bond becoming stronger than the peptide bond in the central chain.[2,14]

Encouraged by the results obtained, we envisaged that introduction of an isopeptide bond between the ε-amino group of lysine and the carboxylic group of a simple amino acid into tuftsin and retro-tuftsin would improve the biological properties of the peptides.

In the present Letter, we describe linear tuftsin analogues that were prepared manually using a Fmoc/*t*-Bu procedure. All the peptides fall into two series: tuftsin and retro-tuftsin analogues. Each group includes four analogues containing a modification at the ε-amino group of lysine. The introduction of a simple amino acid such as alanine, β-alanine, valine or glycine at the ε-amino group of lysine allowed us to form the isopeptide bond. However, H-Thr-Lys(Ala)-Pro-Arg-OH has been described earlier and was synthesized for the first time by Mezö's group using the classic solution-phase method.[14]

The present synthesis was started by anchoring the first residue with a carboxylic group to a solid support. Therefore, direct attachment of Fmoc-AA-OH to Wang resin at the C-terminus was achieved by 4-dimethylaminopyridine (DMAP)-catalyzed esterification.[15] The loading of the first residue was determined by HPLC analysis and the difference in mass of the dried resin. The method of elongation of the peptide chain was based on a two-step procedure involving deprotection and coupling (Scheme 1). The Fmoc group was removed in the presence of 30% piperidine (PIP). Both

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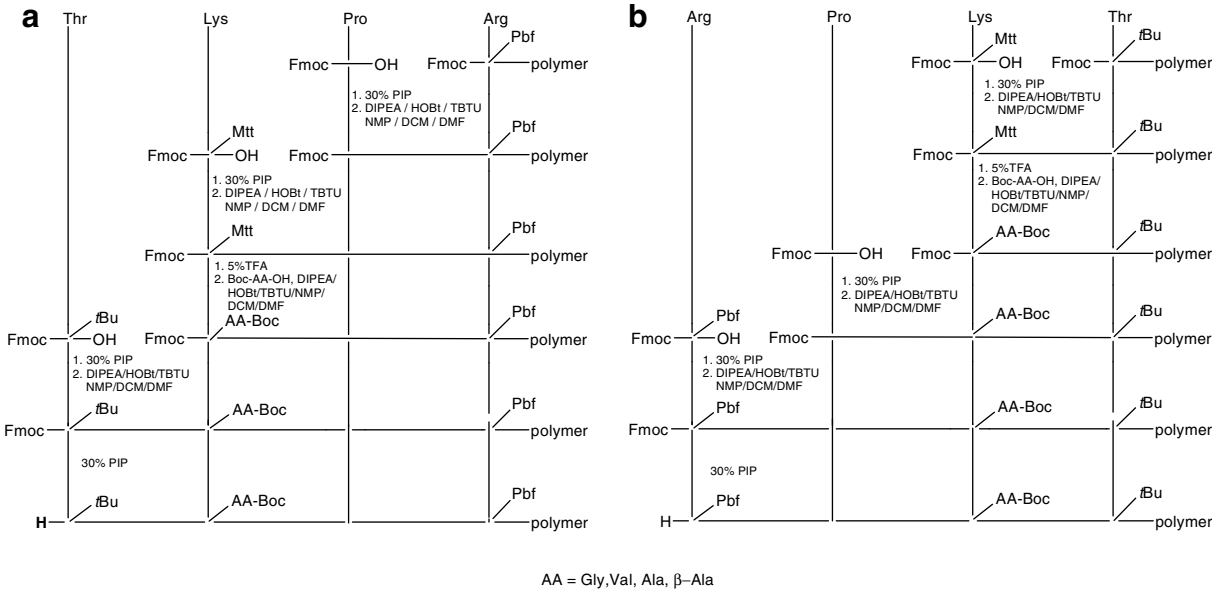

Scheme 1. Preparation of (a) H-Thr(*t*Bu)-Lys(AA-Boc)-Pro-Arg(Pbf)-polymer and (b) H-Arg(Pbf)-Pro-Lys(AA-Boc)-Thr(*t*Bu)-polymer.

peptide and isopeptide bond formations were carried out with *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) as coupling agent and *N*-hydroxybenzotriazole (HOBt) as an additive to suppress racemisation in the presence of *N*,*N*-diisopropylethylamine (DIPEA) (Fmoc- or Boc-AA-OH/HOBt/TBTU/DIPEA, 1:1:1:2). To build peptides containing a –NH–CO– bond, we used a lysine residue protected at the ε-amino group with a 4-methyltrityl (Mtt) group. The Mtt group was removed selectively using 5% TFA in DCM. The ease of removal of the Mtt group was dependent upon the amino acid sequence.[16] Retro-tuftsin analogues were more susceptible to deprotection of the Mtt group compared to tuftsin analogues. The degree of difficulty in removing the Mtt group resulted in the deprotection of peptide-resins **1a–d** being repeated several times. The free ε-amino group of lysine was linked to the appropriate Boc-AA-OH residue using the same coupling method. The main products in this procedure were tuftsin analogues containing modifications at the ε-amino group of lysine; however, we also observed trace tetrapeptides (tuftsin or retro-tuftsin). The appearance of tetra- and pentapeptides was confirmed by MS and RP-HPLC analysis.

Cleavage of the peptide from the resin was accomplished using the standard procedure (TFA/TIS/H_2O, 95:2.5:2.5, v/v/v) to give the free analogues. The crude peptides **1,2a–d** were purified by RP-HPLC, linear gradient 0–100% in 30 min. The process was repeated in a few cases to obtain highly pure peptides. The RP-HLPC profiles for a pentapeptide, **1a** are presented in Figure 1.

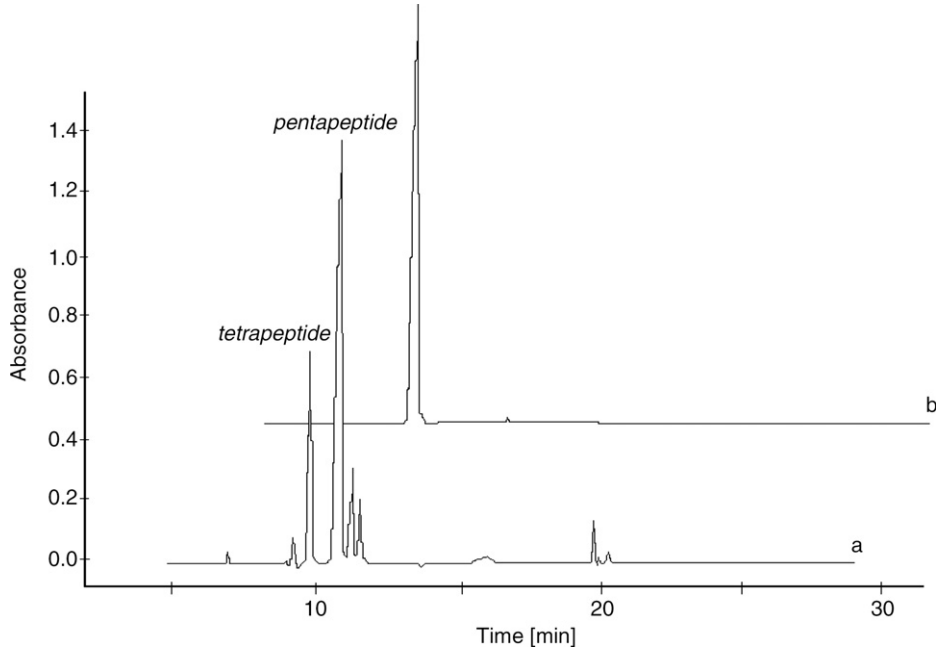

Figure 1. RP-HLPC Chromatogram of (a) crude and (b) pure pentapeptide, **1a**.

Table 1
Characteristics of tuftsin analogues

	Peptides	R_t (min)	Purity[a] (%)	Yield[b] (%)	[M+H]$^+$		Formula	Elemental analysis	
					Calcd	Found		Calcd	Found
1a	H-Thr-Lys(Gly)-Pro-Arg-OH	10.19	95	61	557.99	558.0	$C_{23}H_{42}N_9O_7$	C, 49.75; H, 7.59; N, 22.59	C, 49.95; H, 7.62; N, 22.70
1b	H-Thr-Lys(Val)-Pro-Arg-OH	10.16	94	62	600.07	600.4	$C_{26}H_{47}N_9O_7$	C, 52.27; H, 8.06; N, 21.01	C, 52.40; H, 8.10; N, 21.10
1c	H-Thr-Lys(β-Ala)-Pro-Arg-OH	10.49	95	60	572.01	572.1	$C_{24}H_{44}N_9O_7$	C, 50.63; H, 7.75; N, 22.04	C, 50.70; H, 7.85; N, 22.15
1d	H-Thr-Lys(Ala)-Pro-Arg-OH	10.58	91	58	572.01	572.1	$C_{24}H_{44}N_9O_7$	C, 50.63; H, 7.75; N, 22.04	C, 50.60; H, 7.90; N, 22.25
2a	H-Arg-Pro-Lys(Gly)-Thr-OH	11.08	92	59	557.99	558.1	$C_{23}H_{42}N_9O_7$	C, 49.75; H, 7.59; N, 22.59	C, 49.80; H, 7.62; N, 22.61
2b	H-Arg-Pro-Lys(Val)-Thr-OH	11.10	95	61	600.07	600.1	$C_{26}H_{47}N_9O_7$	C, 52.27; H, 8.06; N, 21.01	C, 52.42; H, 8.10; N, 21.10
2c	H-Arg-Pro-Lys(β-Ala)-Thr-OH	10.90	93	62	572.01	572.3	$C_{24}H_{44}N_9O_7$	C, 50.63; H, 7.75; N, 22.04	C, 50.70; H, 7.85; N, 22.15
2d	H-Arg-Pro-Lys(Ala)-Thr-OH	10.80	90	65	572.01	572.2	$C_{24}H_{44}N_9O_7$	C, 50.63; H, 7.75; N, 22.04	C, 50.80; H, 7.92; N, 22.22

[a] Purity was estimated according to integration of the RP-HPLC chromatogram.
[b] The yield for the pure peptide.

All the compounds were homogenous by TLC and were characterized by MS, elemental analysis and analytical RP-HPLC (Table 1) and by HPLC qualitative amino acid analysis. In RP-HPLC, the tuftsin analogues were eluted with a linear gradient of 0–90% of solvent (0.1% TFA/CH_3CN/H_2O, 80:20, v/v) over 30 min with a flow rate of 1 ml/min with monitoring at 266 nm. All compounds **1,2a–d** gave satisfactory analytical results.

In summary, we have described the solid-phase synthesis, and characterization of tuftsin analogues **1,2a–d**. The introduction of an additional residue at the ε-amino group of lysine resulted in the new bond becoming more stable than the peptide bond in the central chain.[9,14] The products will be assayed for their biological properties and the results will be described elsewhere.

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