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Solid phase synthesis of peptide dimers and trimers linked through an N-terminal lysine residue

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Abstract—Peptide dimers and trimers linked through the ε-amino group of an N-terminal lysine residue can be prepared by cross linking peptide chains on the solid phase with a di- or trifunctional carboxylic acid, followed by cleavage from the resin.

1. Introduction

Multivalent interactions are extremely important throughout biology.[1] For example, multivalent interactions govern cell–cell adhesion processes and antibody–antigen recognition, while ligand-induced receptor dimerisation and oligomerisation is a well established regulatory mechanism in signal transduction.[2] A current area of interest is the synthesis of oligomeric peptides for use as ligands in the study of these multivalent biological interactions.[3,4] The simplest way to produce dimeric and higher order peptide constructs is to use either the C- or N-terminus as the site for linker attachment.[5] Whilst this approach has yielded valuable results in some systems, these termini are often crucial for binding and their derivatisation can produce a ligand with greatly reduced affinity.[6] In such cases derivatisation of a side-chain is a viable alternative and can afford ligands which retain biological activity and have undiminished affinity for their receptor.[7,8] Whilst the synthesis of multimeric peptides linked through a C-terminal side-chain can be achieved by the simultaneous stepwise growth of peptide chains from a resin-bound polyfunctional linker, the synthesis of systems linked through an N-terminal side-chain is not so straightforward due to the requirement that peptide synthesis be performed in a C to N direction. Tam has prepared multiple antigen peptides linked through an N-terminal cysteine via the solution phase condensation of peptide fragments, although work of this nature poses considerable synthetic challenges.[9–11] An alternative approach, which overcomes the problem of working with peptide fragments in solution, is to perform the dimerisation process on resin through the reaction of two peptide monomers bound to the same polymer bead. Recently, Conde-Frieboes[3] and Schreiber[4] have shown that such site–site interactions can be exploited for the on-resin dimerisation of amino acids and short peptides (up to four residues) by olefin metathesis, but extending this chemistry to produce trimers and higher order constructs would not be straightforward. Herein we report the synthesis of peptide dimers and trimers linked through the ε-amino group of an N-terminal lysine residue, utilising site-site interactions on the solid phase to cross link peptide chains with di- and trifunctional carboxylic acids.

2. Results and discussion

To explore the scope of the chemistry we studied the dimerisation of peptides varying between 4 and 8 residues, with linker units spanning 8 to 23 atoms. The peptides were synthesised on Wang resin (loading level 0.63–0.90 mmol/g) using standard Fmoc solid phase synthesis protocols. The N-terminal lysine residue was incorporated as the α-Boc, ε-Fmoc derivative; Fmoc removal with piperidine afforded the free amine for cross linking. For simplicity we chose amide bond formation as the cross linking reaction, using di- and tricarboxylic acids **1**–**5** (Fig. 1). Initially we studied the two linker units **1** and **2** based on an ethylene glycol backbone. Linker **1**, 3,6-dioxaoctanedioic acid, is commercially available, while **2** was synthesised in 36% overall yield by bisalkylation of hexaethylene glycol with *tert*-butyl bromoacetate under phase transfer conditions, followed by *tert*-butyl ester cleavage with trifluoroacetic acid.[12]

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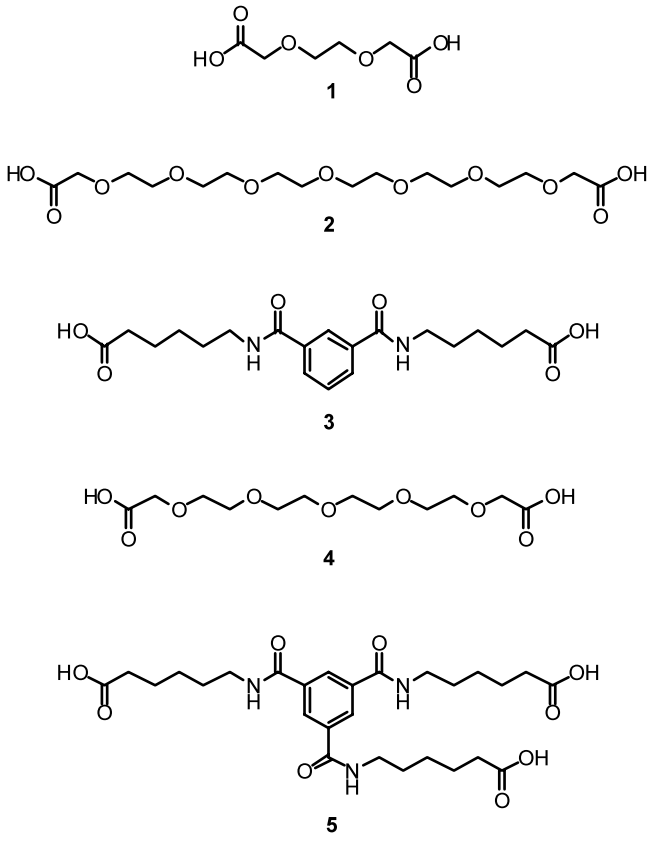


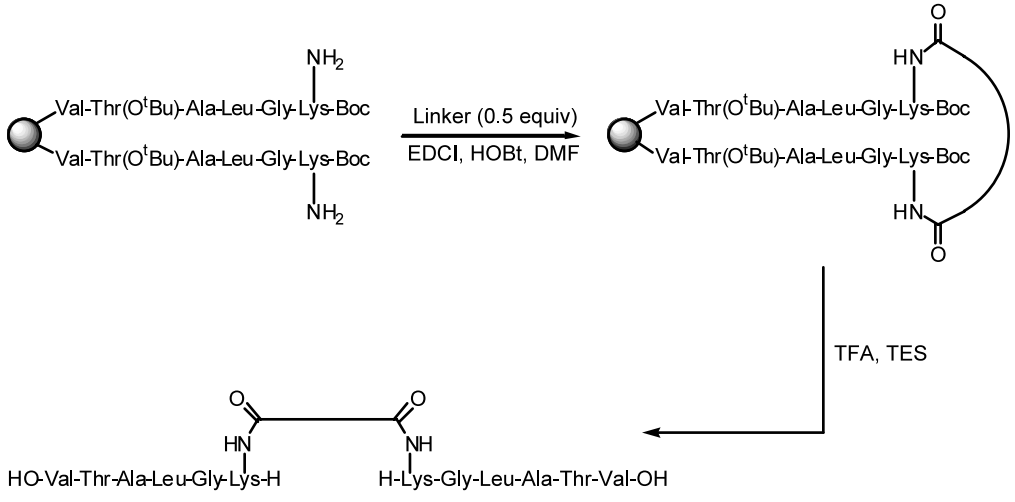
Figure 1.

Dimerisation experiments (Scheme 1) were performed in a fritted glass vessel[13] with agitation by nitrogen gas. The linker (0.5 equivalents based on the initial loading level of the resin), EDCI (5 equivalents) and HOBt (5 equivalents) were agitated with the peptide-loaded resin in DMF for 3 days. The crude dimer was cleaved from the resin using a mixture of trifluoroacetic acid (TFA) and triethylsilane (TES), and purified by reverse phase HPLC. As can be seen from the results summarised in Table 1, both of the linkers were successful in cross linking the peptide chains in acceptable yields.

Having demonstrated the viability of on-resin dimerisation of peptides of various lengths, we went on to explore the possibility of forming a trimer on the solid phase. The difficulty in synthesising branched oligoethylene glycol derivatives led us to explore linkers of a more modular nature consisting of linking arms radiating out from an aromatic core. Aminocaproic acid was chosen as the linking arms, with simple benzene carboxylic acids at the core. For comparison with our earlier work, we first studied the bifunctional linker **3**, synthesised in 91% yield by the condensation of aminocaproic acid and isophthaloyl dichloride under Schotten–Baumann conditions.

The peptide chosen for dimerisation and trimerisation studies was the nonameric sequence Lys-Leu-Val-Pro-Met-Val-Ala-Thr-Val, based on the HLA-A*0201-restricted immunodominant epitope Asn-Leu-Val-Pro-Met-Val-Ala-Thr-Val, residues 495–503 of the matrix protein, pp65, of human cytomegalovirus (HCMV).[14] HCMV is a very common opportunistic infection in immunocompromised individuals, and $pp65_{495–503}$ is the most widely recognised epitope. The dimer was synthesised in 33% yield (29% isolated) via the method outlined previously (Table 2). Incorporation of the aromatic ring and two amide functions reduces the linker flexibility, but this does not significantly affect the dimerisation yield; dimerisation with ethylene glycol based linker **4**,[12] comparable in length to **3**, afforded a similar yield of product.

Trifunctional linker **5** was synthesised in 90% yield from aminocaproic acid and 1,3,5-benzenetricarbonyl trichloride by the Schotten–Baumann procedure used earlier, and trimerisation attempted using 3 equivalents of resin bound peptide epitope per equivalent of linker. Under the previously successful conditions, employing EDCI and HOBt as coupling reagents, resin cleavage afforded a complex mixture of products containing less than 5% of the desired trimer. Happily, changing the coupling reagents to HATU and HOAt resulted in a cleaner HPLC profile and a much improved 35% yield



Scheme 1.

Table 1. Results of dimerisation experiments

Peptide sequence	Linker	Yield (%)[a]	R_t (min)[b]	m/z[c]	
				Calculated	Found
Lys-Ala-Thr-Val	**1**	49	8.77	977.55	977.6
Lys-Ala-Thr-Val	**2**	43	12.13	1197.69	1198.4
Lys-Gly-Leu-Ala-Thr-Val	**1**	31	15.83	1317.77	1317.7
Lys-Gly-Leu-Ala-Thr-Val	**2**	51	17.37	1537.90	1538.4
Lys-Val-Phe-Gly-Leu-Ala-Thr-Val	**1**	34	23.23	1810.04	1809.5
Lys-Val-Phe-Gly-Leu-Ala-Thr-Val	**2**	32	24.03	2030.17	2030.2

[a] Yield of dimer determined by reverse phase HPLC of crude product.
[b] HPLC retention time (method A); for conditions see Section 3.
[c] $[M+H]^+$ ion from MALDI-TOF mass spectrum of purified peptide dimer.

Table 2. Results of dimerisation and trimerisation experiments for Lys-Leu-Val-Pro-Met-Val-Ala-Thr-Val

Linker	Yield (%)		R_t (min)[a]	m/z[b]	
	Crude	Isolated		Calculated	Found
3	33	29	22.60	2270.33	2270.3
4	41	25	23.80	2188.26	2188.2
5	35	28	25.03	3365.97	3366.0

[a] HPLC retention time (method B); for conditions see Section 3.
[b] $[M+H]^+$ ion from MALDI-TOF mass spectrum of purified peptide dimer or trimer.

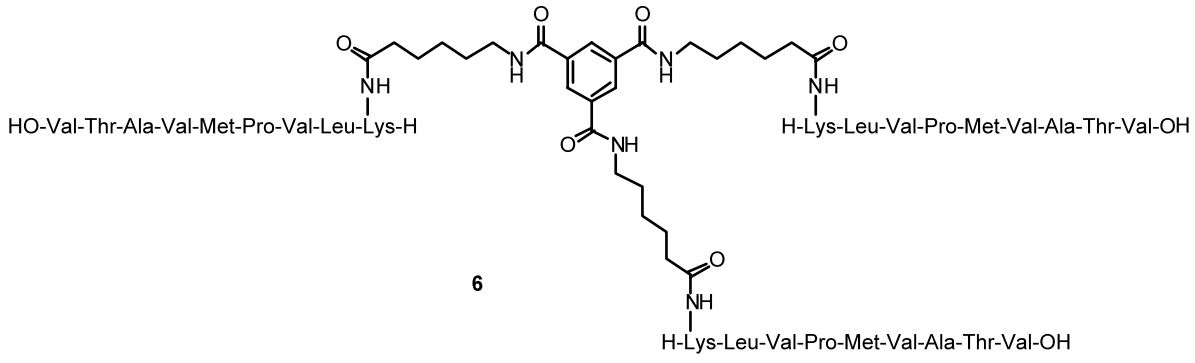


Figure 2.

(28% isolated) of trimer **6** (Fig. 2). The trimer was purified to >99% purity by reverse phase HPLC and characterised by MALDI-TOF mass spectrometry (Fig. 3).

In conclusion, we have discovered a simple and convenient route to dimeric and trimeric peptides linked through an N-terminal lysine residue. Multimeric peptides of this type may be of value in the study of receptor-mediated oligomerisation processes and in the development of novel vaccines.

3. Experimental

Typical dimerisation procedure: In a manual peptide synthesis vessel, the linker (0.05 mmol) was dissolved in anhydrous DMF (3 mL) and preactivated by stirring with EDCI (0.25 mmol) and HOBt (0.25 mmol) for 20 min. After this period resin bound peptide (0.1 mmol, pre-swollen for 30 min in DMF (2 mL)) was added, and the mixture agitated by a stream of nitrogen gas for 3 days. The supernatant was removed by filtration and the resin was washed with DMF (4×5 mL), DCM (4×5 mL), and MeOH (4×5 mL) and dried in vacuo. A cleavage cocktail of TFA and TES (95:5, 2 mL) was added and the resin agitated for 2 h. The resin was removed by filtration, washed with TFA and TES (95:5, 3 mL) and the combined filtrates evaporated to dryness. The residue was triturated with cold diethyl ether and the resulting solid dissolved in water and lyophilised. Purification was effected by reverse phase HPLC by either: method A (C_{18} Phenomenex Luna column, 10 μm particle size, 250×4.6 mm; gradient elution from 12:88 acetonitrile:water+0.05% TFA to 45:55 acetonitrile:water+0.05% TFA over 30 min, flow rate 1 mL/min); or method B (C_{18} Phenomenex Luna column, 10 μm particle size, 250×21.2 mm; gradient elution as above, flow rate 10 mL/min).

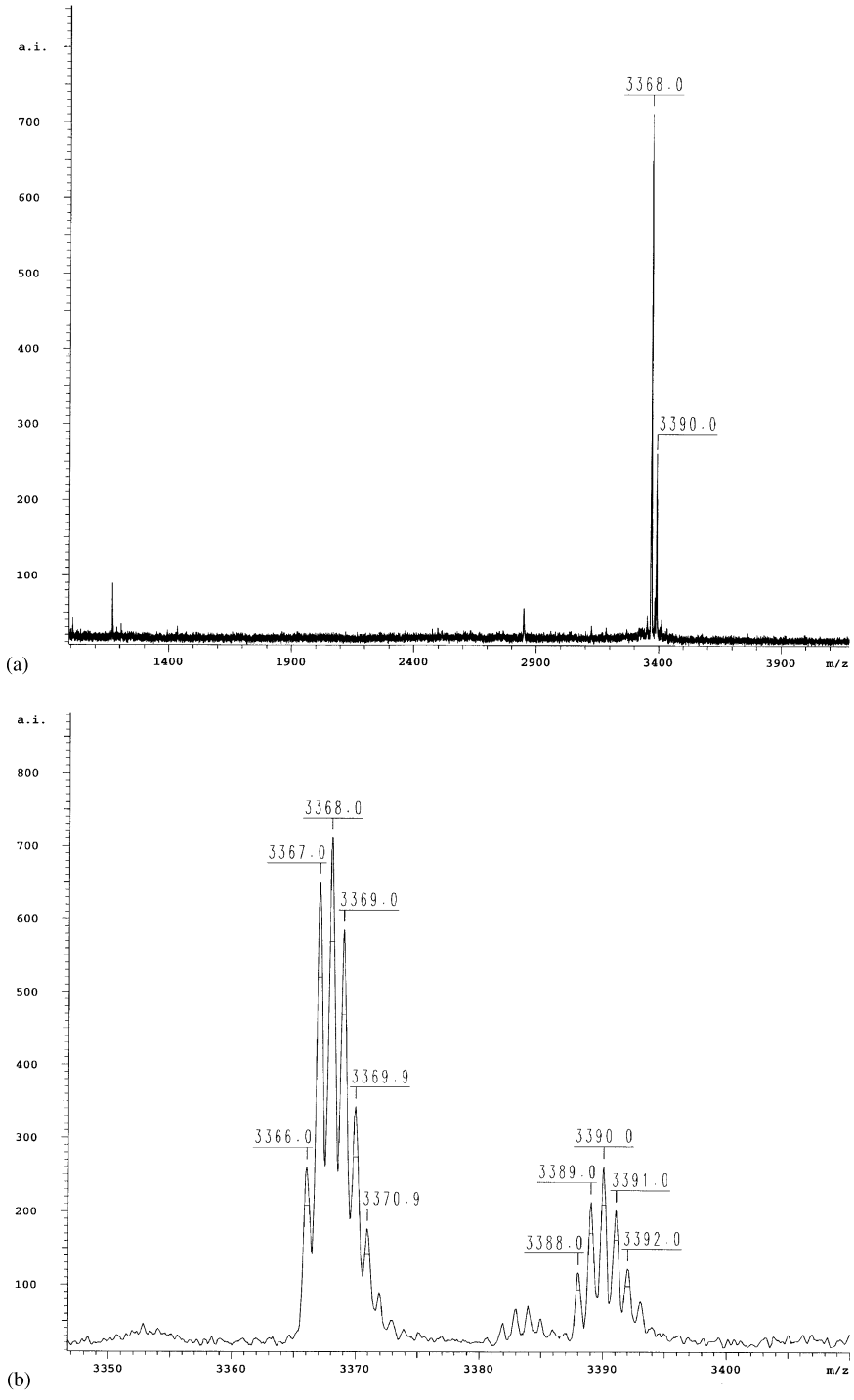


Figure 3. (a) MALDI-TOF mass spectrum of **6**. (b) Expansion showing $[M+H]^+$ and $[M+Na]^+$.

Acknowledgements

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