Synthesis of a symmetric branched peptide. Assembly of a cyclic peptide on a small tetraacetate template

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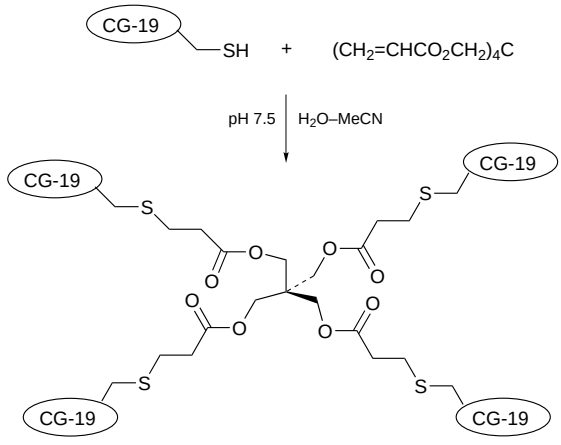
A method for the synthesis of a symmetric peptide dendrimer (MW 8685.0) by assembling four copies of an unprotected Cys-containing cyclic peptide *via* Michael addition onto a small organic template, pentaerythritol tetraacrylate, is described.

Branched peptides in which peptides radiate from a central core are emerging as a new class of artificial proteins with many potential applications.[1–3] Notable examples include the cascade peptide dendrimers found in the design of Multiple Antigenic Peptides (MAPs), in which the core structures are peptidyl templates organized by geometric branching of a bivalent lysine,[2] and TASP, which contains a preorganized, linear lysyl peptide template.[3–5] More recently, small, rigid organic compounds,[6] such as cavitands[7] and Kemp's triacid,[8] have also been exploited as templates for branched peptides. These templates have the advantages that they are readily obtainable and form branched peptides that have pseudo-symmetry. We have report the use of a readily available small organic molecule, pentaerythritol tetraacrylate (PETA), as the tempalte to prepare a symmetrical branched peptide containing four copies of a large cyclic peptide *via* Michael addition of a cysteinyl thiol (Scheme 1).

Together with branched peptide design, efficient methods to accomplish their synthesis are also required. Thus, recent advances in the development of ligation methods using unprotected peptides as building blocks have greatly facilitated their preparation.[9–13] These methods are mainly dependent on either thiol or weak base–aldehyde chemistry.[2] Our scheme for synthesizing symmetric peptide dendrimers involves Michael addition of a cysteinyl thiol to the double bond of the acrylate moiety of PETA. In practice, a two-fold excess (8 equiv.) of peptide with respect to the tetravalent PETA† was found to be adequate to drive the reaction to completion in 45 min, even at a relatively dilute concentration of peptide (1–2 mM). The reaction was performed in H_2O at pH 8 with MeCN (50%) as a cosolvent to increase the solubility of PETA. However, the assembly reaction could also be carried out in 4–6 M guanidine hydrochloride solutions with comparable reaction rates. The only detectable side reaction was the disulfide formation of CG-19, and which could be minimized when a trialkyl phosphine was present in the reaction medium.[12] In 15 min, > 80% of the four-branch peptide dendrimer was formed, together with < 15% of the two- and three-branch intermediates, as monitored by HPLC (Fig. 1). Both the three-branch and four-branch products were isolated from HPLC and analysed by MALDI-TOF MS, which gave expected mass units for both products: 6604.0 (calc. 6602.0) for three branch and 8685.7 (calc. 8685.0) for four-branch (Fig. 1). The tetravalent peptide dendrimer was stable at both acidic and basic pH, and no decomposed product was found after 24 h treatment at pH 2 or 8 as monitored by HPLC.

An advantage of our synthetic scheme is the regioselectivity of Michael addition by a thiol to the acrylate moiety in the presence of other amine nucleophiles. This selectivity permits the use of unprotected, cyclic peptide as the building block for the assemblage on the PETA template. Furthermore, such cyclic peptides with a pendant cysteinyl thiol side chain can be readily synthesized through the intramolecular orthogonal coupling methods developed recently in our laboratory.[16,17] the cyclic peptide CG-19, which is rich in amino side chains, is derived from the sequence of the V3 loop of the gp 120 protein of HIV-1 (III-B). The linear, unprotected peptide precursor was prepared by solid phase synthesis through Boc-benzyl chemistry on a thioester resin.[15] HF cleavage gave the unprotected peptide with an N^{α}-Cys(Npys) and a thiocarboxylic acid at the C-terminus with all other side chains unprotected. Cyclization was achieved *in situ* immediately after HF cleavage in which the C-terminal CO–SH reacted with the N-terminal H-Cys(NPys) residue,



CG-19 = cyclo(CysAlaLysArgIleGlnArgGlyProGlyArgAlaPheValTheIleGly)

Scheme 1

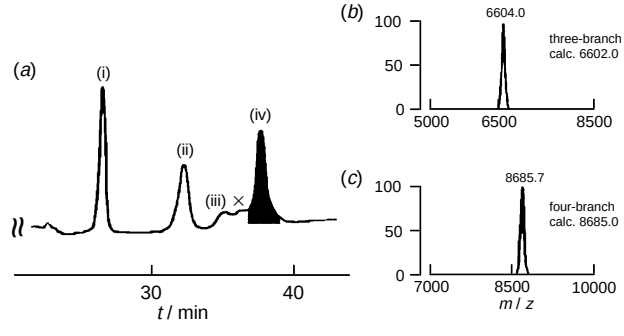


Fig. 1 (*a*) HPLC monitoring of the Michael reaction of the thiol cyclic peptide CG-19 with PETA after 15 min. (*b*) MALDI MS of the 3-branch peptide dendrimer. (*c*) MALDI MS of the four-branch peptide dendrimer. Peaks are (i) cyclic peptide CG-19, (ii) cyclic peptide CG-9 disulfide, (iii) three-branch, (iv) four-branch (x = two-branch). HPLC analyses were run on a Shimadzu system with a Vydac analytical column (0.46 × 25 cm, C_{18} reverse phase) at a flow rate of 1.5 ml min^{-1}, with UV detection at 225 nm and a linear gradient from 20 to 65% buffer B in buffer A in 45 min. Buffer A = H_2O (0.045% TFA); Buffer B = 60% MeCN in H_2O (0.04% TFA).

leading to the formation of an internal Gly–Cys peptide through proximity-driven acyl migration.[15] After purification, a cyclic peptide building block with unambiguous structure and composition was obtained for the subsequent assemblage on PETA. Michael addition of a thiol to the maleimido group has been extensively used for peptide–protein conjugates.[9] However, the hydrolysis of the maleimide at basic pH is a well recognized disadvantage.[9b] Thus, the use of PETA, which forms a more stable and non-maleimide product, is a significant advantage.

The method presented here provides a straightforward and practical approach to preparing branched peptide dendrimers that would be useful for the preparation of specific antibodies as well as for the development of synthetic vaccines. We anticipate that other types of artifical proteins might also be synthesized using this method.

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Footnote and References

† PETA was purchased directly from Aldrich and used after one HPLC purification.

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