A general approach for the non-stop solid phase synthesis of TAC-scaffolded loops towards protein mimics containing discontinuous epitopes†

Cristina Chamorro,[a] John A. W. Kruijtzer,[a] Marina Farsaraki,[a] Jan Balzarini[b] and Rob M. J. Liskamp*[a]

Received (in Cambridge, UK) 3rd October 2008, Accepted 11th November 2008
First published as an Advance Article on the web 17th December 2008
DOI: 10.1039/b817357e

In this Communication, the access to three different peptide loops attached to a small triazacyclophane (TAC) scaffold molecule for the mimicry of discontinuous epitopes present in, for example, antibodies is described for the first time.

One of the greatest challenges in the construction of protein mimics is mimicry of the discontinuous epitope nature of proteins. Discontinuous epitopes are peptide segments that are located relatively closely in a protein's spatial structure, but can be located very distantly in its amino acid sequence (Fig. 1). This is in contrast to continuous epitopes, which consist of a single contiguous strand of amino acids.

Discontinuous epitopes are abundantly present in proteins and are especially prominent at protein–protein interaction sites. The most outstanding examples are probably the discontinuous epitopes comprising the complementarity determining region (CDR) loops of antibodies (see, for example, Fig. 2). However, many other protein–protein interactions include discontinuous epitopes, for example, those present in the crucial HIV-gp120–CD4 interactions (Fig. 2), in which we are interested.[1]

These examples might lead to the fair assumption that when an adequate mimicry of discontinuous epitopes can be realized, efficient synthetic vaccines of, for example, gp120, or even mimics of antibodies ("synthetic antibodies"), come within reach.

Crucial for an adequate mimicry of discontinuous epitopes in a protein is the availability of a molecular scaffold that is capable of replacing most of the protein, and providing a proper arrangement, orientation and perhaps even pre-organization of the peptide segments that comprise the discontinuous epitopes (Fig. 3). Another important characteristic that is required for adequate mimicry is that the peptide segments on the molecular scaffold should be a part of *cyclic* peptides or peptide *loops*, since the discontinuous epitopes, for example those in the CDRs, are present as *loops*. In some cases, linear peptides might be satisfactory, and we have

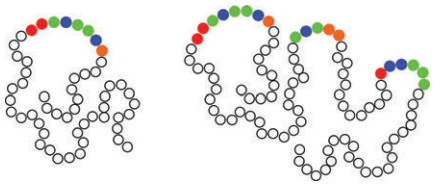


Fig. 1 (Sequential) continuous and discontinuous epitopes.

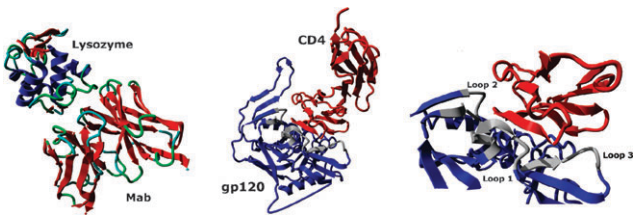


Fig. 2 The CDR loops involved in the interaction of Mab (1G7J) and a lysozyme (left), and the gp120–CD4 interaction (middle and right; overview and detail, respectively).[1]

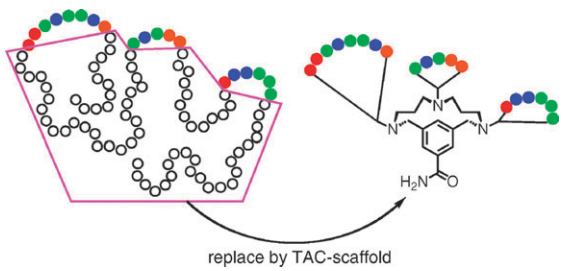


Fig. 3 The replacement of most of a protein by a triazacyclophane (TAC) scaffold and the mimicry of its discontinuous epitopes.

shown that this is indeed possible.[2,3] However, for the best possible loop mimicry, one would expect a *cyclic* peptide to be superior. A loop structure would also provide more pre-organization than a linear peptide, which might also be beneficial for its interactions.

From a chemical point of view, the molecular scaffold should be robust, conveniently synthesized and preferably accessible on a multigram scale. Furthermore, it is essential that the different molecular fragments, *i.e.* the peptides, can be introduced in an equally facile manner. Last but not least, a handle for solid phase attachment or for the attachment of auxiliary groups (*e.g.* a label) should be present.

Several scaffolds for peptides have been described in the literature, of which the cyclic peptide, template assisted

[a] *Medicinal Chemistry & Chemical Biology, Utrecht Institute of Pharmaceutical Sciences, Faculty of Science, Utrecht University, P.O. Box 80082, NL-3508 TB Utrecht, The Netherlands. E-mail: r.m.j.liskamp@uu.nl; Fax: +31 30-2536655; Tel: +31 30-2537396; www.pharm.uu.nl/medchem*
[b] *Rega Institute for Medical Research, K.U. Leuven, Minderbroederstraat 10, B 3000 Leuven, Belgium*

† Electronic supplementary information (ESI) available: Abbreviations and experimental details. See DOI: 10.1039/b817357e

synthetic protein (TASP), is probably the most outstanding.[4] This scaffold was introduced by Mutter and Vuilleumier in their seminal work almost twenty years ago.[5] Although there have been many successful applications of TASP,[4] we have developed—in view of the above mentioned requirements—the TAC scaffold, which can be conveniently synthesized on a large scale.[6] So far, this scaffold has been used for the construction of synthetic receptor molecules,[7] in an effective mimic of the papain inhibitory protein,[2] in an approach towards a synthetic vaccine of pertussis[3] and towards a mimic of the copper-binding site of a protein.[8]

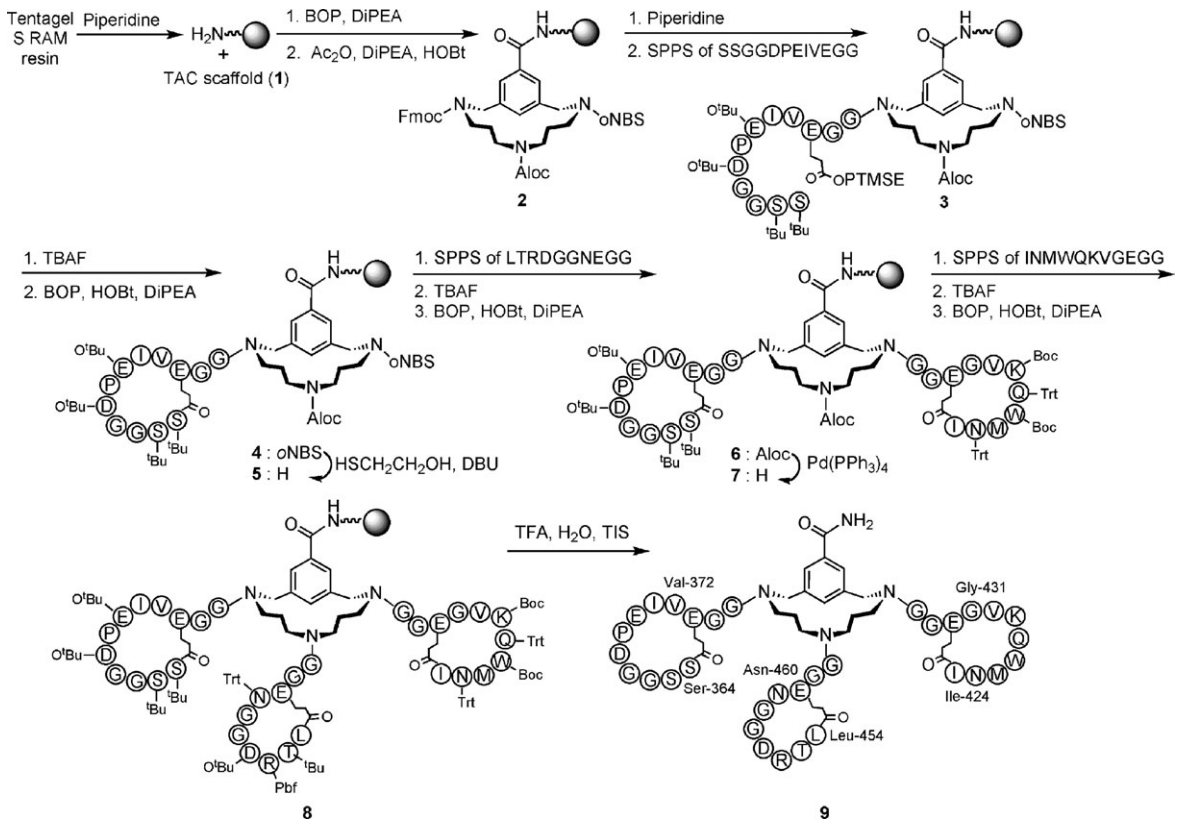
In this Communication, we wish to describe a general approach for the introduction of cyclic peptides onto the TAC scaffold in a continuous solid phase synthesis. This method provides access to protein mimics that contain up to three different discontinuous epitopes, as are present in, for example, antibodies (Fig. 3).

As a promising target, we selected a mimic of the discontinuous epitope formed by the three loops of gp120 interacting with the CD4 receptor.[1] An effective mimic of this ensemble might provide access towards a synthetic vaccine candidate or even lead to a (protein) mimic of gp120, which might be capable of interfering with the binding of HIV-gp120 to the cellular CD4 receptor. Thus, based on the X-ray structure of the gp120–CD4 complex and using data from relatively straightforward molecular modelling, we selected the amino acid loops ^{364}SSGGDPEIV^{372}, ^{424}INMWQKVG^{431} and ^{454}LTRDGGN^{460} of gp120 for incorporation onto the TAC scaffold.[1,9]

Crucial to the success of an approach to sequentially introduce three peptide loops onto the TAC scaffold in a continuous solid phase synthesis procedure is the availability of an additional orthogonal protecting group, which can be cleaved in the presence of the TAC protecting groups whenever cyclization to the individual peptide loop has to take place. We found that the PTMSE group introduced by Wagner and Kunz was highly suitable for this purpose, and completely orthogonal to the *o*-NBS, Aloc and amino acid side chain protecting groups.[10] Therefore, it could be successfully applied to the synthesis of cyclic peptides, independent of their length and sequence.

Thus, as a hinge for cyclization, a glutamic acid residue containing the PTMSE group was used, *i.e.* Fmoc–Glu(OPTMSE)–OH, which could be prepared on a multigram scale from commercially available Cbz–Glu–OBzl.

This set the stage for the non-stop solid phase synthesis of TAC-scaffolded loops derived from gp120 towards a protein mimic containing discontinuous epitopes (Scheme 1). After loading Tentagel S-RAM resin with the Fmoc–*o*-NBS–Aloc-protected TAC scaffold, **1**,[6] construction of the first loop using automatic peptide synthesis was begun by first introducing two glycine residues as a spacer.[11] This was followed by synthesis of the peptide arm sequence corresponding to residues 364–372 in loop 1 of gp120, leading to **3**. After removal of the PTMSE group with TBAF, an on-resin tail-to-side chain cyclization to **4** was achieved using BOP.[12] Next, *o*-NBS removal by β-mercaptoethanol and DBU to give **5** was followed by automatic peptide synthesis of the second peptide



Scheme 1 The non-stop solid phase synthesis of TAC-scaffolded loops towards protein mimics containing discontinuous epitopes

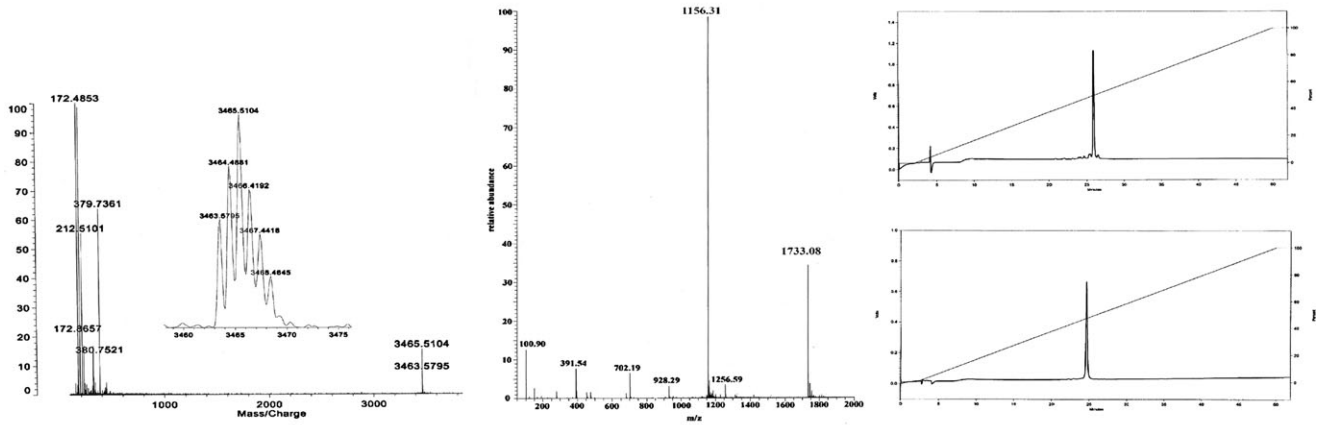


Fig. 4 The characterization of protein mimic **9** by MALDI-TOF (left), ES-MS (middle) and analytical HPLC (top right: TFA, acetonitrile, water; bottom right: NH_4OAc, acetonitrile, water). Mono-isotopic mass $[M + H]^+$ calculated for $C_{149}H_{228}N_{45}O_{49}S$: 3463.65; found: 3463.58.

arm, comprising residues 424–431 in loop 2 of gp120. Upon completion, the PTMSE group was removed, and cyclization was effected with BOP to form the second loop mimic in **6**.[12] Introduction of the third loop took place after palladium-assisted cleavage of the Aloc group, leading to **7**, and successful introduction of the third peptide arm, comprising residues 454–460, was realized. The final cyclization was performed after deprotection of the glutamic acid hinge, and the resulting resin-bound gp120 discontinuous epitope mimic, **8**, was ready for cleavage and simultaneous deprotection. Thus, after acidolysis, the free gp120 mimic, **9**, was obtained as its trifluoroacetate salt in high purity (>99%) after purification by preparative HPLC (Fig. 4).[13]

The identity of **9** was confirmed by MALDI-TOF and ES-MS. The overall yield of the discontinuous epitope mimic by this non-stop solid phase approach was 0.17% (*ca.* 1.5 mg), which corresponds to an average yield of 97% per step (see the ESI†).

The synthetic approach described in this paper allowed reliable and convenient access for the first time to three different peptide loops attached to a small TAC scaffold molecule, leading to a protein mimic containing discontinuous epitopes.

A key issue in this non-stop solid phase peptide synthesis was the splendid synthesis of cyclic peptides, which could be very efficiently achieved using the orthogonal PTMSE group.[10] The PTMSE group could be cleaved under almost neutral conditions by treatment with TBAF in DCM, much more rapidly than the well known TMSE group,[14a] thereby leading to fewer sequence-dependent side reactions, such as aspartimide formation and rearrangements of aspartyl/glutamyl peptides.[14]

Although this protein mimic was not capable of preventing HIV-1 (IIIB) and HIV-2 (ROD) infection in CEM cell cultures,[15] we plan to investigate its properties as a synthetic vaccine, which is certainly one of the most attractive future prospects of this and related protein mimics.

In our opinion, with this approach and approaches under development, we can successfully address the chemical challenges that lie ahead for the construction of protein mimics.

Financial support by the council for Chemical Sciences of the Netherlands Organisation for Scientific Research (CW-NWO) and by the GOA (Krediet nr. 05/19) of the KULeuven. We thank Annemarie Dechesne for recording the mass spectra and Anton Bunschoten for assistance with the molecular modelling. The technical assistance of Mrs Leen Ingels is greatly appreciated.

Notes and references

1 P. D. Kwong, R. Wyatt, J. Robinson, R. W. Sweet, J. Sodroski and W. A. Hendrickson, *Nature*, 1998, **393**, 648.
2 D.-J. van Zoelen, M. R. Egmond, R. J. Pieters and R. M. J. Liskamp, *ChemBioChem*, 2007, **8**, 1950.
3 M. Hijnen, D.-J. van Zoelen, C. Chamorro, F. R. Mooi, P. van Gageldonk, G. Berbers and R. M. J. Liskamp, *Vaccine*, 2007, **25**, 6807.
4 For a recent review, see: Y. Singh, G. T. Dolphin, J. Razkin and P. Dumy, *ChemBioChem*, 2006, **7**, 1298.
5 M. Mutter and S. Vuilleumier, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 535.
6 T. Opatz and R. M. J. Liskamp, *Org. Lett.*, 2001, **3**, 3499.
7 T. Opatz and R. M. J. Liskamp, *J. Comb. Chem.*, 2002, **4**, 275.
8 H. B. Albada, F. Soulimani, B. M. Weckhuysen and R. M. J. Liskamp, *Chem. Commun.*, 2007, 4895.
9 Recently, Eichler *et al.* attached similar, albeit linear, peptides of gp120 for the design of immunogens: R. Franke, T. Hirsch, H. Overwin and J. Eichler, *Angew. Chem., Int. Ed.*, 2007, **46**, 1253.
10 (*a*) M. Wagner and H. Kunz, *Synlett*, 2000, 400; (*b*) M. Wagner and H. Kunz, *Z. Naturforsch., B: Chem. Sci.*, 2002, **57b**, 928.
11 A spacer consisting of two glycine residues was introduced, because originally we experienced problems in attaching cyclic peptides to the TAC scaffold, which we ascribed to steric hindrance.
12 The cleavage and mass analysis of an aliquot of resin showed that the synthesis had successfully reached this stage.
13 Ammonium acetate buffers were used for purification in view of the presence of an Asp–Pro sequence in the first loop, which is prone to cleavage under acidic conditions.
14 (*a*) P. Sieber, *Helv. Chim. Acta*, 1977, **60**, 2711; (*b*) H.-G. Chao, M. S. Bernatowicz, P. D. Reiss, C. E. Klimas and G. R. Matsueda, *J. Am. Chem. Soc.*, 1994, **116**, 1746; (*c*) S. A. Kates and F. Albericio, *Lett. Pept. Sci.*, 1994, **1**, 213; (*d*) J. D. Wade, M. N. Mathieu, M. Macris and G. W. Tregear, *Lett. Pept. Sci.*, 2000, **7**, 107.
15 J. Balzarini, L. Naesens, J. Slachmuijlders, H. Niphuis, I. Rosenberg, A. Holy, H. Schellekens and E. De Clercq, *AIDS*, 1991, **5**, 21.