Synthesis of Cyclic Herpes Simplex Virus Peptides Containing 281–284 Epitope of Glycoprotein D-1 in *Endo*- or *Exo*-position

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Abstract: We have prepared two types of cyclopeptides containing the ^{281}DPVG^{284} sequence from the 276–284 region of glycoprotein gD-1 of the Herpes simplex virus (HSV). The syntheses were performed by solid phase methodology using MBHA or BHA resin and orthogonal protection schemes. Head-to-side-chain cyclization included the N-terminal part of the epitope, while side-chain-to-side-chain lactam bridge formation resulted in a peptide containing a C-terminal cycle. Peptides elongated by Cys at the N-terminal of the sequence were also prepared. Boc chemistry using Fmoc and OFm orthogonal protection was applied for on-resin cyclization. Based on the orthogonality of Bzl and cHex esters under a 1 M TMSOTf-thioanisole/ TFA cleavage condition, a new approach for the cyclization on BHA-resin has also been developed. Preliminary studies on solution conformation of the cyclic peptides by CD spectroscopy indicated the importance of the location and the size of the cycle within the epitope sequence. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptide cyclization on solid phase; cyclic epitope peptides of Herpes simplex virus gD protein; TMSOTf cleavage; orthogonality of cyclohexyl and benzyl esters; CD of cyclic peptides

INTRODUCTION

Linear peptides are highly flexible molecules that can adopt a multitude of conformations in solution, only a few of which are responsible for their biological activity (e.g. immunoreactivity, hormone–receptor interaction). The main reason for the growing interest in cyclic peptides is that they provide a possibility to induce preferred spatial arrangement that may reproduce the bioactive conformation and thus result in enhanced binding and improved pharmacological metabolic properties [1–3]. Since epitope sequences are frequently localized in β-turn or loop regions of a protein, the cyclic peptides can mimic the native secondary structure better than the linear form and thus become more appropriate candidates for the development of synthetic antigens.

Herpes simplex virus (HSV), with its two closely related serotypes (HSV-1 and HSV-2), is one of the most common infectious agents in humans. Glycoprotein D (gD) represents a major immunogenic component of the virion envelope [4,5]. Based on prediction analysis of the primary structures of gD from HSV-1 (gD-l) the 268–284 region has been selected for epitope localization studies in our laboratory. Peptides corresponding to this region were prepared, conjugated to the branched polypeptide AK (poly[Lys(DL-Ala$_m$)]), where $m \sim 3$ and used as synthetic immunogens for protection experiments in HSV-1 infected BALB/c and CBA mice. These studies indicated the presence of an immunodominant antigenic determinant at the C-terminal part of this (276–284) region [6–8]. Immunological characterization of truncated peptides of this region suggested the [281]DPVG[284] tetramer sequence as the epitope core [7–9].

Two cyclic versions of [276]SALLEDPVG[284] nonapeptide were designed consisting of either a head-to-side-chain lactam ring, where the epitope core motif is situated essentially outside of the cycle (*exo*-form) (**I**) or a side-chain-to-side-chain lactam ring between the γ-carboxyl group of Glu and the ε-amino group of a Lys residue attached to the C-terminal of the sequence. In the latter case the DPVG motif is a part of the 6-residue lactam ring (*endo*-form) (**II**). Analogues containing cysteine at the N-terminal were also prepared for further conjugation of the cyclic peptides to polymeric carriers (**III**, **IV**) (Figure 1).

There are various protection schemes available for the solid phase synthesis of cyclic peptides [10]. One of the most commonly used is the orthogonal combination of Fmoc and OFm side chain protecting groups with Boc chemistry [11,12]. These fluorenylmethyl-type protecting groups can be cleaved selectively by 20% piperidine or 2% DBU in DMF [13]. After coupling agent-promoted lactam bridge
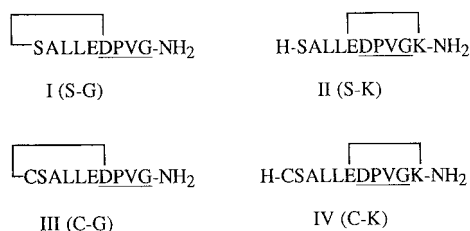
formation, the cyclic peptide can be released from the resin by HF treatment. It has been observed that both cyclohexyl esters and BHA-attached peptide chains are stable in 1 M TMSOTf-thioanisole/ TFA cleavage mixtures [9,14]. Based on these findings we have proposed an alternative synthesis route, namely the use of benzyl and cyclohexyl groups as orthogonal ω-carboxyl protection on BHA-resin using Boc chemistry [15]. In this strategy 'hard acid' deprotecting reagent removes the benzyl-type protecting groups except 4-methylbenzyl from cysteine in 30 min [16].
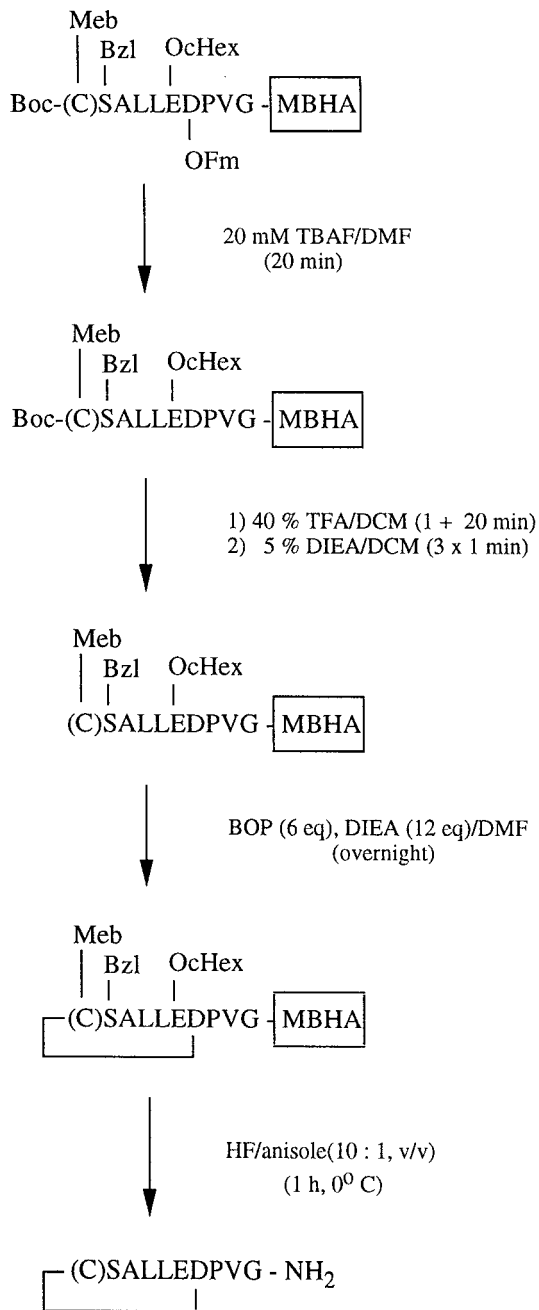
In this paper we describe and compare the two synthetic approaches and some side reactions. Preliminary studies to characterize solution conformation of *endo*- and *exo*-cyclic HSV peptides were also performed by using CD spectroscopy.

MATERIALS AND METHODS

Protected amino acids were obtained from Bachem (Bubendorf, Switzerland), Advanced Chem. Tech. (Louisville, KY, USA), Novabiochem (Laufelfingen, Switzerland) and Propeptide (Vert Le Petit, France). MBHA and BHA resin were from Novabiochem and Bachem, respectively. BOP (Novabiochem or Bachem), TBTU (Richelieu Biotechnologies, St. Hyancinte, QC, Canada), DCC and HOBt (Fluka, Buchs, Switzerland) were used as coupling reagents. Chemicals for cleavage, HF, TMSOTf, TFA, TBAF and piperidine, as well as scavengers (anisole, thioanisole, m- and p-cresol) and DIEA were Fluka products. DTT was purchased from Reanal (Budapest, Hungary).

Synthesis

Method A. Head-to-side-chain cyclopeptides (**I**, **III**; Figure 1) were synthesized on an MBHA resin (0.28 mmol/g) by Boc chemistry in the manual mode (Scheme 1). Fluorenylmethyl protection was used for Asp, cyclohexyl for Glu, benzyl for Ser and 4-methylbenzyl for Cys. The resin was submitted to the following protocol for the incorporation of each protected amino acid derivative: (i) Boc removal (40% TFA/DCM, 1 + 20 min); (ii) DCM wash (5 × 1 min); (iii) neutralization (5% DIEA/DCM, 3 × 1 min); (iv) DCM wash (4 × 1 min); (v) coupling with three equivalents of Boc-amino acid and DCC/HOBt in 4:1 (v/v) DCM-DMF for 60 min; (vi) DMF (1 × 1 min) then DCM wash (3 × 1 min); ninhydrin assay [17]. Single coupling was sufficient in all cases. After completing the

Figure 1 Schematic representation of cyclopeptides containing the [281]DPVG[284] epitope sequence from glycoprotein D of HSV in *endo*- (**II**, **IV**) or in *exo*- (**I**, **III**) position.

I (S-G)   II (S-K)

III (C-G)   IV (C-K)

SALLEDPVG-NH$_2$ H-SALLEDPVGK-NH$_2$

CSALLEDPVG-NH$_2$ H-CSALLEDPVGK-NH$_2$

Meb
| Bzl   OcHex
| | |
Boc-(C)SALLEDPVG - MBHA
|
OFm

↓ 20 mM TBAF/DMF
(20 min)

Meb
| Bzl   OcHex
| | |
Boc-(C)SALLEDPVG - MBHA

↓ 1) 40 % TFA/DCM (1 + 20 min)
2) 5 % DIEA/DCM (3 x 1 min)

Meb
| Bzl   OcHex
| | |
(C)SALLEDPVG - MBHA

↓ BOP (6 eq), DIEA (12 eq)/DMF
(overnight)

Meb
| Bzl   OcHex
| | |
⌐(C)SALLEDPVG - MBHA
└──────────┘

↓ HF/anisole(10 : 1, v/v)
(1 h, 0° C)

⌐ (C)SALLEDPVG - NH$_2$
└─────────────┘

Scheme 1   Outline of the synthesis of ([C]SALLED)PVG-
NH$_2$ cyclopeptide on MBHA resin containing the
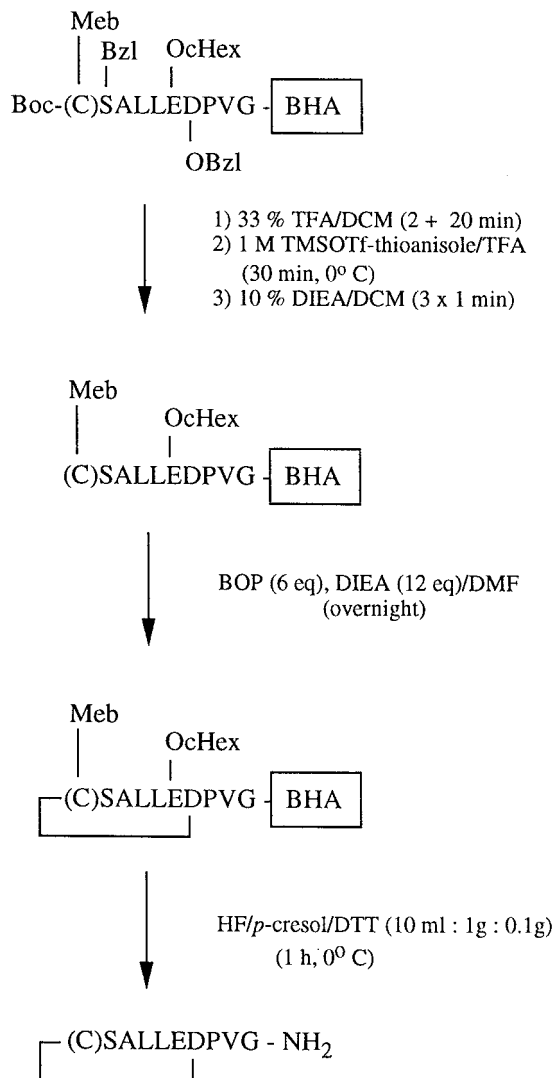^{281}DPVG^{284} sequence in *exo-* (**I**, **III**) position.

synthesis, fluorenylmethyl protection from the side
chain of Asp was cleaved either with (a) 20% pipe-
ridine in DMF (2 + 20 min) or (b) 0.2 M TBAF in DMF
(20 min) [18] and the resin was washed with DMF
and DCM. The N-terminal Boc group was removed
with 40% TFA in DCM (1 + 20 min) and neutraliza-
tion was performed with 5% DIEA in DCM. For

cyclization six equivalents of BOP reagent [19] in
DMF in the presence of DIEA (12 equivalents) was
used for 16 h.

Side-chain-to-side-chain cyclopeptides (**II**, **IV**, Fig-
ure 1) were synthesized by a similar strategy (capac-
ity of MBHA resin was 0.2 mmol/g) (Scheme 2). The
side chain of the C-terminal lysine was protected by

Meb
| Bzl   OcHex
| | |
Boc-(C)SALLEDPVGK - MBHA
| |
OFm   Fmoc

↓ 20 mM TBAF/DMF
(20 min)

Meb
| Bzl   OcHex
| | |
Boc-(C)SALLEDPVGK - MBHA

↓ BOP (6 eq), DIEA (12 eq)/DMF
(overnight)

Meb
| Bzl   OcHex
| | |
Boc-(C)SALLEDPVGK - MBHA
└──────────┘

↓ 1) 40 % TFA/DCM
2) 5 % DIEA/DCM

Meb
| Bzl   OcHex
| | |
(C)SALLEDPVGK - MBHA
└──────────┘

↓ HF/anisole(10 : 1, v/v)
(1 h, 0° C)
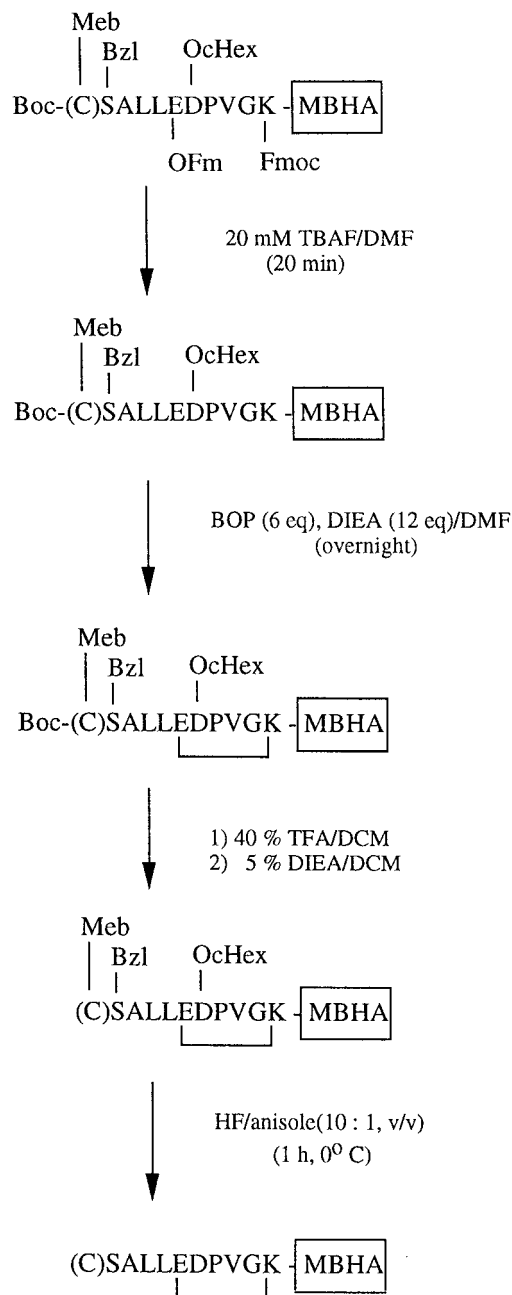
(C)SALLEDPVGK - MBHA
└──────────┘

Scheme 2   Outline of the synthesis of [C]SALL(EDPVGK)-
NH$_2$ cyclopeptide on MBHA resin containing the
^{281}DPVG^{284} sequence in *endo-* (**II**, **IV**) position.

Meb
| Bzl OcHex
| | |
Boc-(C)SALLEDPVG - | BHA |
|
OBzl

1) 33 % TFA/DCM (2 + 20 min)
2) 1 M TMSOTf-thioanisole/TFA
   (30 min, 0° C)
3) 10 % DIEA/DCM (3 x 1 min)

Meb
| OcHex
| |
(C)SALLEDPVG - | BHA |

BOP (6 eq), DIEA (12 eq)/DMF
(overnight)

Meb
| OcHex
| |
┌ (C)SALLEDPVG - | BHA |
└─────────────┘

HF/p-cresol/DTT (10 ml : 1g : 0.1g)
(1 h, 0° C)

┌ (C)SALLEDPVG - NH$_2$
└─────────────┘

Scheme 3 Outline of the synthesis of ([C]SALLED)PVG-NH2 cyclopeptide on BHA resin containing the ^{281}DPVG^{284} sequence in *exo*- (**I**, **III**) position.

the Fmoc group. In this case the ω-COOH groups of Asp and Glu were protected by cyclohexyl and fluorenylmethyl groups, respectively. Fmoc and OFm protections were removed with 0.2 M TBAF in DMF (20 min), followed by cyclization with BOP/DIEA in DMF overnight. The N-terminal Boc group was removed after cyclization, prior to HF cleavage. For the preparation of peptide Ac-C-K, the N-terminal Cys was deprotected before cyclization and acetylated with acetic anhydride in the presence of DIEA in DMF.

Method B. Cyclic peptides were synthesized on BHA resin (0.25 mmol/g) by Boc chemistry using cyclohexyl- and benzyl-groups as orthogonal carboxyl

protection. For the synthesis of *exo*-cyclic versions (**I**, **III**; Figure 1), benzyl side chain protection was applied for aspartic acid and cyclohexyl for glutamic acid (Scheme 3). N-terminal Boc group was removed from the protected linear peptide with 33% TFA/DCM (2 + 20 min) prior to 'hard acid' deprotection. Benzyl-groups from Asp and Ser by 1 M TMSOTf-thioanisole in TFA (10 ml total) in the presence of 0.2 ml m-cresol were removed in 30 min at 0°C. After washing with DCM neutralization was carried out with 10% DIEA in DCM (3 × 1 min). Cyclization was performed with six equivalents of BOP and 12 equivalents of DIEA in DMF for 16 h. Cyclic peptides were removed from the resin with an HF/p-cresol/DTT (10 ml : 1 g : 0.1 g) mixture in 1.5 h at 0°C.

During the synthesis of side-chain-to-side-chain cyclic peptides (**II**, **IV**; Figure 1) on BHA resin (0.21 mmol/g) ClZ was used for the protection of ε-amino group of Lys, cHex for Asp, Bzl for Glu and Fmoc-Cys(Trt)-OH was introduced as last residue (Scheme 4). In the case of compound **IV** the side chain of Ser was protected by the benzyl-group, while for compound **II** the N-terminal amino acid was coupled as Fmoc-Ser(*t*Bu)-OH. All protecting groups, except for Fmoc and cHex were removed by 1 M TMSOTf-thioanisole/TFA mixture (see above) in 30 min at 0°C. The cyclization was performed with BOP/DIEA in DMF as described above. The Fmoc group was removed with 20% piperidine in DMF (2 + 20 min) prior to the HF-cleavage (10 ml HF, 1 g p-cresol and 100 mg DTT, 1.5 h at 0°C).

All products were purified by RP-HPLC and the pure cyclopeptides were characterized by HPLC, amino acid composition and MS (Table 1).

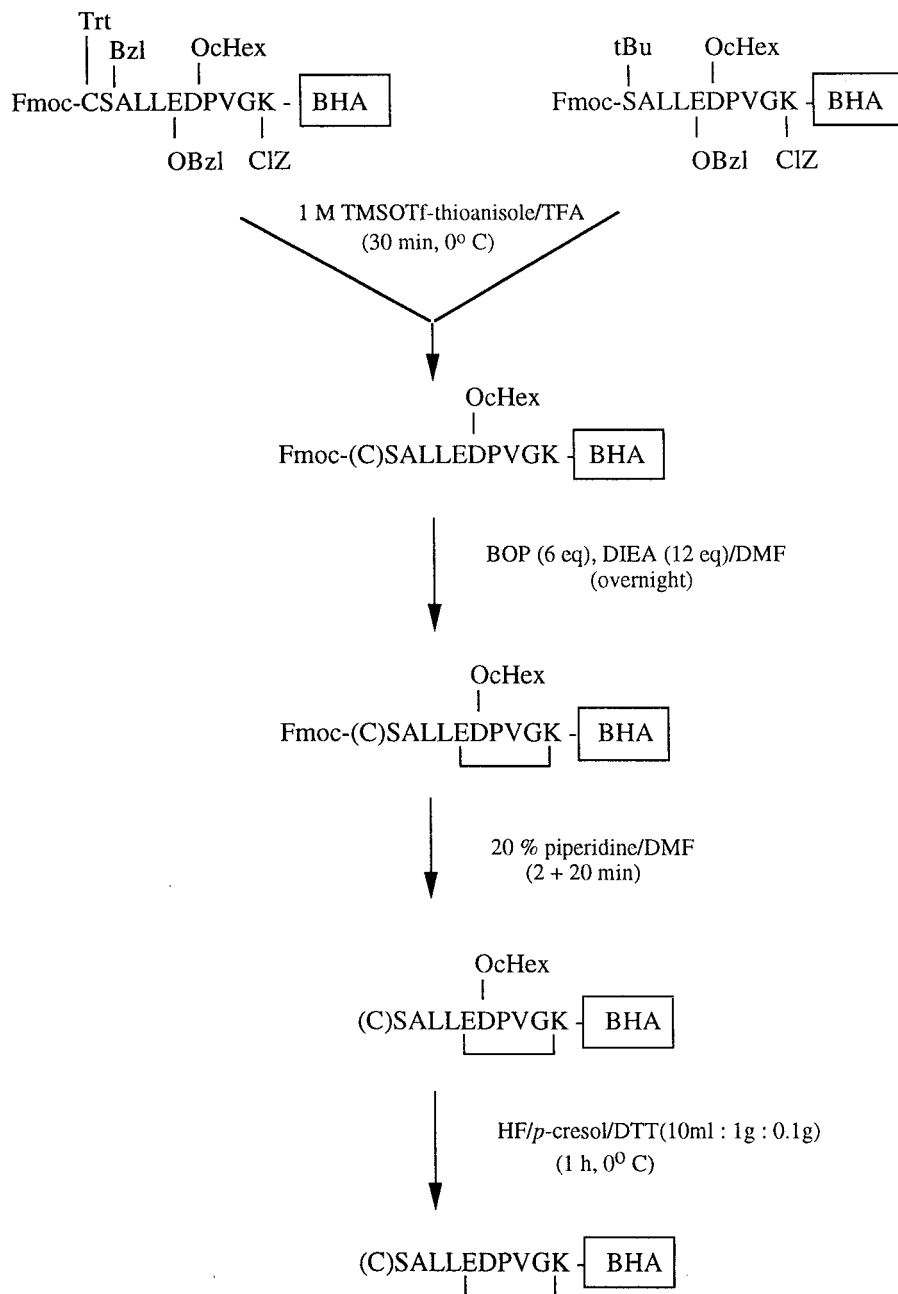## High Performance Liquid Chromatography (HPLC)

Analytical RP-HPLC was performed on a Knauer (Bad Homburg, Germany) HPLC system using a Vydac C$_4$ column (250 × 4.6 mm i.d.) with 5 µ silica (300 Å pore size) (Hesperia, CA, USA) as a stationary phase and linear gradient elution with eluent A = 0.1% TFA in water and eluent B = 0.1% TFA in acetonitrile–water (8:2, v/v) as mobile phase. The gradient used was as follows: 0 min 15% B, 5 min 15% B and 35 min 75% B. The flow rate was 1 ml/min. The analysis was carried out at ambient temperature with detection at λ = 220 nm. The samples were dissolved in eluent A or in the 1:1 (v/v) mixture of eluent A and B. The crude products were purified on a semipreparative Phenomenex Jupiter C$_{18}$ column (250 × 10 mm i.d.) with 10 µ silica (300 Å pore size) (Torrance, CA, USA). The flow rate was

Table 1   Characterization of the HSV Cyclo Epitope Peptides

Compounds (method)	Yield	FAB-MS $[M+H]^+$		Amino acid analysis[a] (Calc.) Found									HPLC[b]
	(%)	Calc.	Found	Asp (1)	Ser (1)	Glu (1)	Pro (1)	Gly (1)	Ala (1)	Val (1)	Leu (2)	Lys (1)	R_t (min)
C-G (A)	18.3	984.5	984.4	1.05	0.91	1.06	1.07	0.99	1.00	0.95	1.97	—	18.9
C-K (A)	11.6	1112.6	1112.5	1.04	0.93	1.07	1.09	0.98	0.99	0.96	1.98	0.98	16.9
Ac-C-K (A)	19.2	1154.6	1154.3	1.04	0.93	1.06	1.07	1.00	0.98	0.96	1.98	0.98	18.3
S-G (A)	6.5	881.5	881.5	1.03	0.94	1.07	1.08	1.00	1.02	0.94	1.98	—	17.6
S-K (A)	10.8	1009.6	1009.4	1.05	0.92	1.06	1.06	0.98	1.00	0.95	1.99	0.98	15.1
C-G (B)	20.1	984.5	984.3	1.02	0.90	1.06	1.07	0.98	0.99	0.96	1.98	—	19.0
C-K (B)	5.5	1112.6	1112.6	1.06	0.90	1.08	1.05	0.97	1.02	0.93	1.96	0.99	16.8
S-G (B)	6.2	881.5	881.5	1.02	0.93	1.06	1.06	0.97	1.03	0.95	1.95	—	17.6
S-K (B)	12.0	1009.6	1009.3	1.04	0.95	1.07	1.11	0.96	1.04	0.96	1.93	0.99	15.1

[a] Acid hydrolysis (6 M HCl, 110°C, 24 h).
[b] HPLC conditions: Vydac C_4 column (4.6×250 mm) with 5 μm silica (300 Å pore size); gradient elution: 0 min 15% B, 5 min 15% B, 35 min 75% B; eluents: 0.1% TFA in water (eluent A) and 0.1% TFA in acetonitrile/water = 80/20 (v/v) (eluent B); flow rate: = 1 ml/min; detection $\lambda = 220$ nm.

Trt
| Bzl   OcHex
| | |
Fmoc-CSALLEDPVGK - | BHA |
        | |
      OBzl   ClZ

tBu   OcHex
| |
Fmoc-SALLEDPVGK - | BHA |
      | |
    OBzl   ClZ

1 M TMSOTf-thioanisole/TFA
(30 min, 0° C)

OcHex
|
Fmoc-(C)SALLEDPVGK - | BHA |

BOP (6 eq), DIEA (12 eq)/DMF
(overnight)

OcHex
|
Fmoc-(C)SALLEDPVGK - | BHA |

20 % piperidine/DMF
(2 + 20 min)

OcHex
|
(C)SALLEDPVGK - | BHA |

HF/*p*-cresol/DTT(10ml : 1g : 0.1g)
(1 h, 0° C)

(C)SALLEDPVGK - | BHA |

Scheme 4 Outline of the synthesis of [C]SALL(EDPVGK)-NH$_2$ cyclopeptide on BHA resin containing the ^{281}DPVG^{284} sequence in *endo-* (**II**, **IV**) position.

4 ml/min using the same eluents with a linear gradient from 20% B to 65% B in 30 min.

Amino Acid Analysis

The amino acid composition of peptides was determined by amino acid analysis using a Beckman (Fullerton, CA, USA) Model 6300 analyser. Prior to analysis samples were hydrolysed in 6 M HCl in sealed and evacuated tubes at 110°C for 24 h or at 160°C for 1 h.

Mass Spectrometry

Fast Atom Bombardment Mass Spectrometry (FAB-MS). FAB mass spectra were obtained on a VG-ZA-2SEQ tandem mass spectrometer (Fisons, UK) equipped with a Cs$^+$ ion gun (30 keV). The peptide
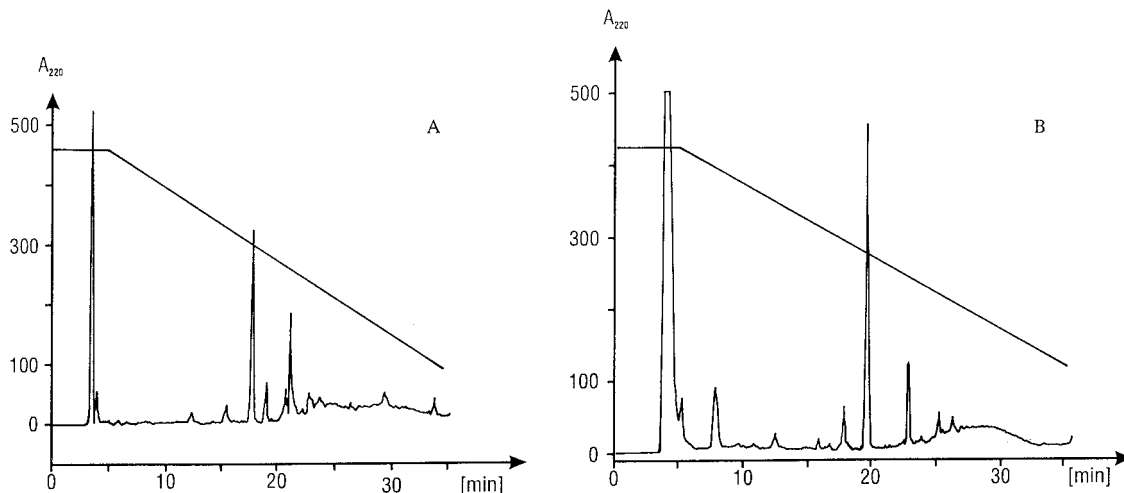
Figure 2   Analytical RP-HPLC profile of the crude cyclopeptide (SALLED)PVG-NH$_2$ (A) and (CSALLED)PVG-NH$_2$ (B). Elution was performed using a gradient: 0 min 15% B, 5 min 15% B and 35 min 75% B [eluent A: 0.1% TFA in water, eluent B: 0.1% TFA in acetonitrile: water 8:2 (v/v)] at a flow rate of 1 ml/min, detection at $\lambda = 220$ nm. A 20 μl volume of sample (1 mg/ml in eluent A) was injected.

samples were dissolved in DMSO and mixed with glycerol matrix.

MALDI-TOF Mass Spectometry. MALDI-TOF analysis was performed on Lasermat 2000 spectrometer (Finningan).

Circular Dichroism (CD)

CD spectra were recorded on a Jobin-Yvon (Longjumaeu, France) Dichrographe Mark VI in quartz cells with a path length of 0.1 cm and 0.02 cm at room temperature. Trifluoroethanol (TFE), distilled water and a 50% mixture (v/v) of TFE and water were used as solvents. The concentrations of solutions were 0.5–1.0 mg/ml. The CD band intensities are expressed as $[\Theta]_{MR}$ values. Spectra were measured from 185–280 nm.

RESULTS AND DISCUSSION

Synthesis

The synthesis of cyclic epitope peptides containing a lactam ring at the N- or C-terminal part of the 276–284 region of HSV gD-1 was attempted by solid phase methodology on MBHA and BHA resins using Boc chemistry as outlined in Schemes 1–4.

Two strategies were used for the preparation of *exo*- and *endo*-peptides (Figure 1). In the first case cyclic peptides were produced on MBHA resin. The OFm group was removed from the peptide-MBHA

resin by piperidine/DMF followed by Boc deprotection to provide free β-COOH of Asp and free α-NH$_2$ for cyclization. This was performed with BOP reagent in DMF in the presence of DIEA. Since a ninhydrin test was positive even after 2 days, BOP was replaced by TBTU [20] and cyclization was repeated. A negative ninhydrin assay was observed after 16 h. The product obtained by this route was cleaved from the resin in anhydrous HF in the presence of anisole. MALDI-TOF mass spectrometric analysis of the crude products provided main peaks (M + H$^+$ = 1065.6 for S-G and 1168.8 for C-G). These were assigned to the Asp-piperidide [21] of the linear peptide blocked by the tetramethylguanidinium (Tmg) group [22] on its N-terminal. These findings confirmed that the apparently very sluggish acylation rate observed for cyclizations after piperidine deprotection was in fact the result of piperidide formation at the β-COOH of Asp. Consequently this prevented any lactam formation. Resorting to TBTU as an alternative coupling agent, in the absence of any available carboxyl group, Tmg capping of the N-terminus was promoted. These reactions resulted in the incorrect interpretation of the negative ninhydrin test as evidence of complete cyclization. Once again, these results reinforce the need for detailed analytical control of all reactions carried out on solid phase.

To prevent piperidide formation during the removal of a OFm group from the side chain of Asp, the use of TBAF in DMF was investigated (Scheme 1). In this case the cyclization using BOP reagent
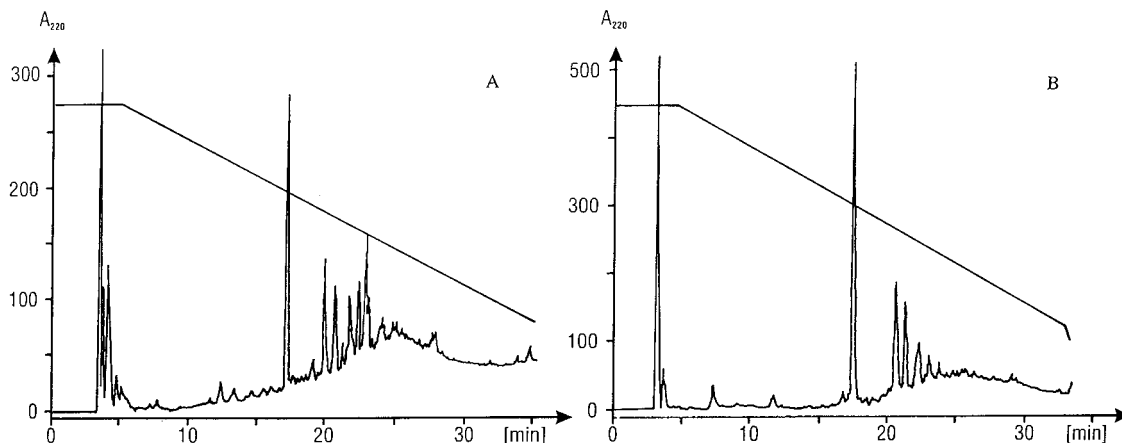
Figure 3   Analytical RP-HPLC profile of the crude cyclopeptide CSALL(EDPVGK)-NH$_2$ (A) and Ac-CSALL(EDPVGK)-NH$_2$ (B). Elution was performed using a gradient: 0 min 15% B, 5 min 15% B and 35 min 75% B [eluent A: 0.1% TFA in water, eluent B: 0.1% TFA in acetonitrile: water 8:2 (v/v)] at a flow rate 1 ml/min, detection at $\lambda = 220$ nm. A 20 μl volume of sample (1 mg/ml in eluent A) was injected.

and DIEA was completed in 16 h. Cyclic peptides were cleaved from MBHA resin with anhydrous HF, purified by HPLC and resulted in the expected compounds. The yields of the purified ($\sim 95\%$ by analytical HPLC) products were between 6 and 20% based on the initial substitution of the resin. As shown by the yields (Table 1) and HPLC traces (Figure 2) of the crude products, lactam formation at the N-terminus was more efficient for the analogue with N-terminal Cys residue (**III**) containing a 7-residue ring than for the one with a 6-residue ring (**I**).

At the C-terminus, the cyclization reactions (Scheme 2) were essentially not dependent on the presence of the N-terminal Cys. Interestingly, replacing Boc by acetyl at the N-terminus (Ac-C-K) gave a higher yield of cyclic peptide (Table 1 and Figure 3).

An alternative synthetic approach to the cyclic peptides of this study (Method B) took advantage of the fact that the cyclohexyl ester protection of Asp is orthogonal to benzyl-type groups under acidolysis with TMSOTf/TFA (Schemes 3 and 4). In this procedure BHA resin was used. Prior to the cyclization the N-terminal Boc group was removed with 33% TFA/DCM followed by the cleavage of benzyl protection from the side chain of Asp with 1 M TMSOTf-thioanisole/TFA procedure at 0°C for 30 min. Under this cleavage condition the cyclohexyl group, the peptide–BHA resin bond as well as Meb of Cys residue remain intact (Scheme 3). Cyclization reaction using six equivalents of the BOP reagent in the presence of DIEA (12 equivalents) was completed overnight. The yield of the HF-cleaved and purified

products was fairly good (6–20%). Similar to the observation described above, the 7-member ring containing the intramolecular lactam bond was formed with higher yields.

The synthesis of **II** (S-K) and **IV** (C-K) peptides containing the lactam bridge on the C-terminus between the γ-carboxyl group of Glu and the ε-amino group of the C-terminal Lys was carried out by Boc chemistry, but the N-terminal amino acid
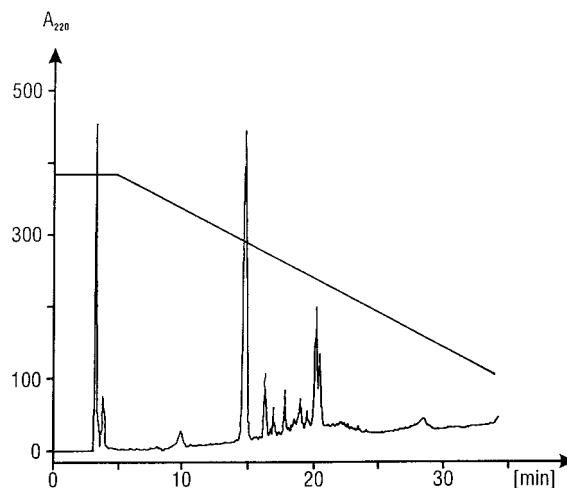

Figure 4   Analytical RP-HPLC profile of the crude cyclopeptide SALL(EDPVGK)-NH$_2$. Elution was performed using a gradient: 0 min 15% B, 5 min 15% B and 35 min 75% B [eluent A: 0.1% TFA in water, eluent B: 0.1% TFA in acetonitrile: water 8:2 (v/v)] at a flow rate 1 ml/min, detection at $\lambda = 220$ nm. A 20 μl volume of sample (1 mg/ml in eluent A) was injected.
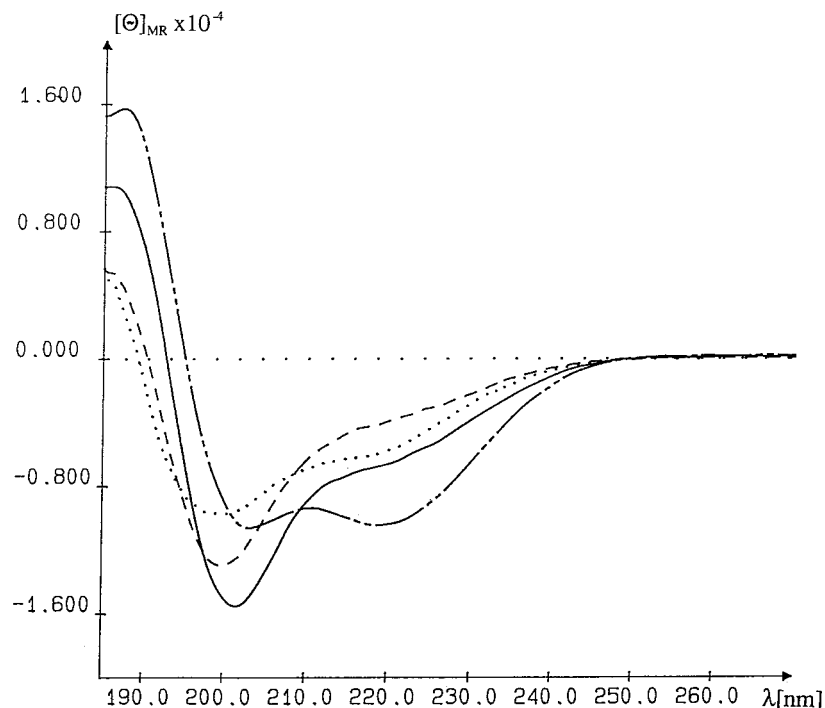
Figure 5   CD spectra of cyclopeptides containing [281]DPVG[284] epitope sequence from glycoprotein D of HSV in *endo*- (**II**, **IV**) or in *exo*- (**I**, **III**) position. The spectra were recorded in TFE, $c = 0.5$–1.0 mg/ml at room temperature. Results are expressed as mean residue ellipticity values $[\Theta]_{MR}$. H-CSALL(EDPVGK)-NH$_2$ (—), H-SALL(EDPVGK)-NH$_2$ (– – –), (CSALLED)PVG-NH$_2$ (– - - –), (SALLED)PVG-NH$_2$ (·······).

was coupled as Fmoc-derivatives (Scheme 4). The protected peptide on BHA resin was treated with 1 M TMSOTf-thioanisole/TFA. In this way all protecting groups were removed except of cyclohexyl on Asp and the N-terminal Fmoc group. After an overnight cyclization with BOP/DIEA the Fmoc group was cleaved with 20% piperidine in DMF followed by HF-cleavage, which removed the unprotected peptides from the resin (Figure 4). The yield of the purified cyclic decapeptide was slightly better than the same analogue synthesized by Method A, however, the yield for the cysteine containing version was low (Table 1). That might be attributed to the presence of unprotected sulfhydryl group of Cys during the cyclization and Fmoc deprotection. According to the yields and the HPLC profile of crude cyclic peptides we can conclude that the free hydroxyl group on the serine residue does not influence the efficiency of the cyclization.

Solution Conformation

In water the CD spectra of cyclic peptide similarly to the native linear peptide H-SALLEDPVG-NH$_2$ [7] reflecting the predominance of open (unordered) con-

formers (data not shown). The CD spectra of four cyclic epitope peptides in TFE are presented in Figure 5. In TFE the class C character of spectra may be indicative for the presence of folded (turn) conformation. Cyclization at the C-terminus (ring formation between E and K in H-CSALL(EDPVGK)-NH$_2$ and in H-SALL(EDPVGK)-NH$_2$) results in a slightly decreased flexibility of the molecule, however these spectra do not show a high population of conformers with fixed turn structural element. This observation might be due to the presence of the unordered N-terminal flanking region (CSALL or SALL, respectively). Lactam bridge formation at the N-terminal part of the molecule [between β-COOH of Asp and N terminal α-amino group in (CSALLED)PVG-NH$_2$ and (SALLED)PVG-NH$_2$] has a more profound effect on solution conformation. Cyclopeptide (CSALLED)PVG-NH$_2$ is flexible in water (data not show), however, the spectrum in both TFE/water (1:1, v/v) and TFE is predominated by folded conformers characterized by bands at $\lambda = 205$ and 222 nm. Interestingly, the 6-amino acid residue containing cyclopeptide (SALLED)PVG-NH$_2$ lacking the N-terminal Cys, shows spectral features corresponding to a relatively small population of

ordered conformers (Figure 5). FT-IR and NMR studies are in progress to localize the position and to identify the type of folded conformers (β- or γ-turn).

CONCLUSIONS

In this communication we report the preparation of a new set of cyclic peptides containing DPVG sequence in *endo*- or in *exo*-position. This class of epitope peptides derived from the glycoprotein D of HSV type 1 represent a new group of compounds that might be suitable for the analysis of the position and size of cycle on antibody recognition. The CD studies of the cyclic HSV epitope peptides indicated that these features have a pronounced effect on solution conformation. However, the identification of folded conformers needs further investigation using FT-IR and/or NMR.

For the synthesis of these compounds a new on-resin cyclization method based on the orthogonality of cyclohexyl and benzyl esters under the TMSOTf-thioanisole/TFA cleavage condition was developed. The comparison of this procedure with an earlier reported technique using OFm/OBzl orthogonality was also studied. The analysis of side reactions (e.g. Asp-piperidide formation observed during the OFm cleavage by piperidine) suggest that the new method could be advantageous and could replace in most cases the OFm/OBzl strategy which needs more expensive amino acid derivatives.

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