Bioorganic & Medicinal Chemistry Letters, Vol.3, No.4, pp. 707-710, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

Synthesis of N_α-Fmoc-N_ε-Nvoc-Lysine and Use in the Preparation of Selectively Functionalized Peptides

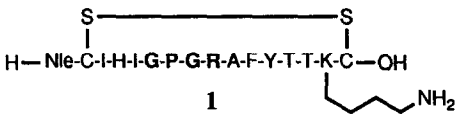
Victoria K. Rusiecki*[1] and Susan A. Warne

Department of Medicinal Chemical Research
Merck Research Laboratories
P.O. Box 2000
Rahway, NJ 07065-0900

(*Received in USA* 21 *January* 1993)

Abstract: *N_α-Fmoc-N_ε-Nvoc -lysine 2 has been prepared and used in the solid phase peptide synthesis (SPPS) of selectively functionalized HIV-1 peptides. The removal of the Nvoc group, from the protected peptide, has been shown to be compatible with the presence of a reactive maleimide linker which is useful for conjugation of peptides to proteins containing thiol groups.*

There is high interest in the preparation of an AIDS vaccine, which might develop a broad-based antibody (Ab) response in humans. Putney[2] and co-workers have shown that HIV-1 contains a highly conserved region at the gp120 V3 loop tip, to which a neutralizing response is elicited and is known as the principal neutralizing determinant (PND) of HIV-1. Antibodies which recognize the G-P-G-R residues[3] at the V3 loop tip have been shown to neutralize HIV-1 isolates *in vivo*[4] and prevent infection in chimpanzees.[5] Thus as part of our effort to prepare cyclic V3-domain related haptens we have synthesized peptide **1**, whose sequence is related to the MN strain of the virus, for conjugation to an immunogenic protein carrier to test its ability to raise neutralizing Ab's against the HIV-1 virus.

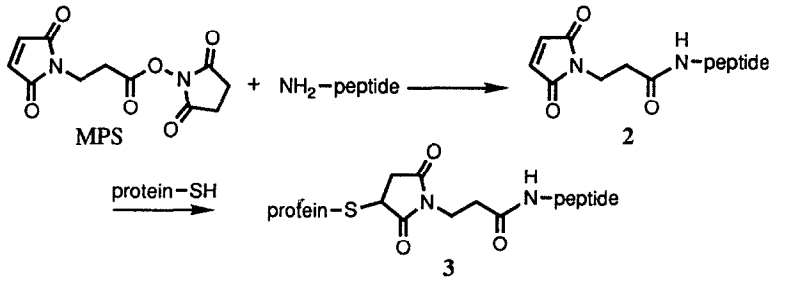


1

Our conjugation strategy involved attachment of a maleimide linker to the amino terminus of the peptide epitope with 3-maleimidopropionic acid *N*-hydroxysuccinimide ester (MPS) forming **2**, followed by addition with a thiolated protein carrier[6] providing the desired conjugate **3** (Scheme 1). In addition to the N-terminal group, immunogen **1** also contains a lysine residue whose ε-amino group can react with MPS. A method was required for its protection to allow exclusive derivatization at the peptide amino-terminus by MPS.

It was found that the maleimide group was degraded by most of the common methods[7] of peptide protecting group removal (anhydrous HF,TFA/CH_2Cl_2, or bases). Thus, a protected lysine derivative was

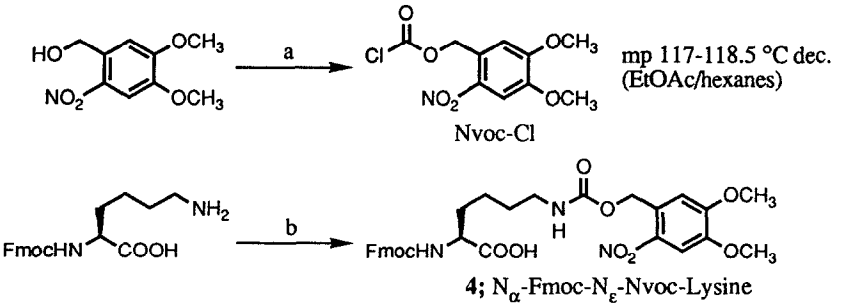
Scheme 1



needed for use in solid phase peptide synthesis with a deprotection scheme that was compatible with the reactive maleimide. A photocleavable protecting group seemed appropriate for our needs, and we were able to demonstrate that maleoyl-peptides were stable to broad spectrum illumination. The 6-nitroveratryloxy[8] (Nvoc) group has recently been implemented in the photolithographic preparation of peptides[9] and Schultz[10] has demonstrated its photo-deprotection from Nvoc-aminoacyl dinucleotides under mildly acidic conditions. Therefore, N_α-Fmoc-N_ε-Nvoc-lysine **4** was prepared for incorporation into peptide **1**.

Synthesis[11] of 6-nitroveratryl chloroformate (Nvoc-Cl) was modified from the literature procedure[8] substituting a solution of phosgene (CAUTION![12]) in toluene for the gaseous reagent. This modification had little effect on the product yield of the reaction. Next, a solution of Nvoc-Cl was added to N_α-Fmoc-lysine in the presence of base, and reacted overnight at room temperature. Differentially protected **4**[13] was isolated in 75% yield after flash chromatography (Scheme 2).

Scheme 2

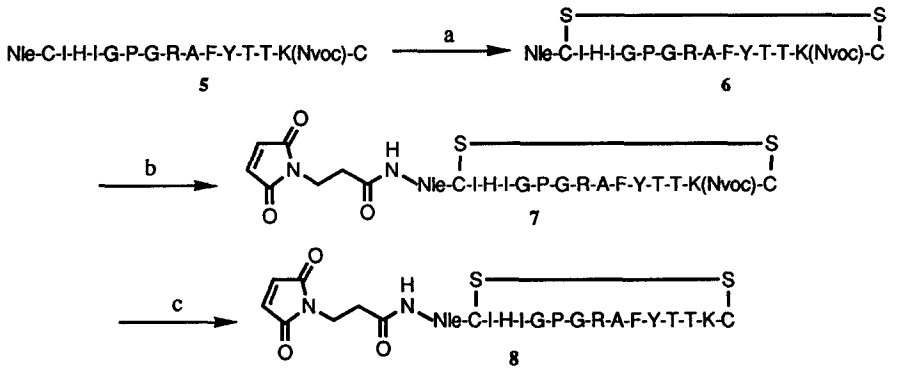


Reagents: a) 4-5 eq. 20% $Cl_2CO/PhCH_3$, dioxane, RT, 4 days, 81%.
b) Dioxane/CH_3CN/H_2O (1:1:1), Na_2CO_3, Nvoc-Cl (in dioxane), RT, 18 h, 75%.

The Lys(Nvoc) protected peptide **5** was synthesized on an ABI 431A peptide synthesizer using Fmoc-chemistry (DCC/HOBt activation) and single amino acid couplings on Wang resin.[14] The peptide was cleaved from the resin (30:1:1 TFA/thioanisole/ 1,2-ethanedithiol) and purified by preparative C_{18} reverse

phase (C_{18} RP) HPLC.[15] Attempts at disulfide cyclization using standard conditions (air/pH>8, or ferricyanide) led to very low mass recoveries of product. This problem was overcome by conducting the cyclization at slightly acidic pH in degassed aqueous DMSO.[16] The crude cyclic product was purified by size exclusion chromatography (Pharmacia LH-20, MeOH) and preparative C_{18} RP-HPLC. The maleimide linker was introduced on the amino terminus by reaction of the cyclic peptide with MPS. Photochemical deblocking of the Lys(Nvoc) protecting group was accomplished by irradiation (450 W Hanovia, pyrex) of an aqueous solution of **7**, thus providing **8** as the sole product of the reaction (Scheme 3).

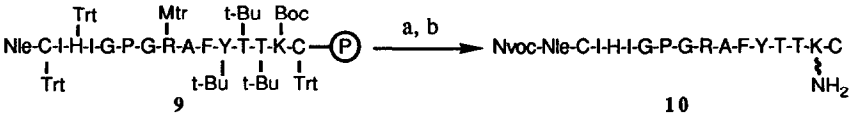
Scheme 3



Reagents: a) degassed 10% DMSO/H_2O (v/v), pH 6.4, 0.33 mM, RT, 1.5 h, 65%.
b) MPS, DIEA, DMF, RT, 1h, 50%.
c) hv (λ>280 nm, pyrex), pH 4.5 KOAc, 1.0 mM, 10 °C, 30 min., 65%.

The position of attachment of the protein carrier to a peptide epitope could effect the antigenicity of the conjugate. Therefore, to fully assess the contribution of this effect we needed to prepare the regioisomeric maleimido-derivative in which the maleimide linker was attached to the lysine residue. This was accomplished by reacting the resin-bound, (N_ε-Boc)Lys protected peptide with Nvoc-Cl providing the N-terminal protected peptide (**10**) after deprotection/cleavage from the resin. This material could be carried on to the N_ε-(maleimidopropanoyl)-Lys analogue of **8** as described above (Scheme 4).

Scheme 4



Reagents: a) 1.1 eq. Nvoc-Cl, CH_2Cl_2, DIEA. b) 30:1:1 TFA/thioanisole/1,2-ethanedithiol.

In conclusion, we have demonstrated that the orthogonal photodeprotection of an Nvoc-protected peptide can be accomplished in the presence of a highly reactive maleimide group. This has allowed us to

vary the site of attachment of these peptides to protein carriers. Biological evaluation of these selectively functionalized cyclic peptides as epitopes in a synthetic HIV-1 vaccine will be reported elsewhere.

Synthesis of N_α-Fmoc-N_ε-Nvoc-lysine **2**: N_α-Fmoc-lysine (3.685 g, 10 mmol) was dissolved in 1:1:1 H_2O/dioxane/CH_3CN (60 mL) in a foil covered flask and sodium bicarbonate (1.060 g, 10 mmol) was added. Additional amounts of water (10 mL) and CH_3CN (5 mL) were added so that all solids went into solution. Nvoc-chloroformate (2.756 g, 10 mmol) was dissolved in dioxane (20 mL), added dropwise to the reaction mixture (20 min.), and stirred overnight at RT (18 h). The solvent was removed *in vacuo* and the residue was partitioned between 1 N sodium bisulfate (50 mL) and dichloromethane (125 mL) and the aqueous phase washed with additional dichloromethane (125 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The resulting orange oil (9.240 g) was purified by flash chromatography (SiO_2, 5% MeOH/$CHCl_3$/0.1% HOAc), affording **2** (4.540 g, 7.48 mmol, 75%) as a yellow foam which was stable for >6 months at -10 °C in a foil covered flask. R_f=0.3 (10% MeOH/$CHCl_3$ /0.1% HOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (d, 2H, J=7.32), 7.70 (d, 2H, J=7.40), 7.68 (s, 1H), 7.39 (t, 2H, J=7.33), 7.30 (t, 2H, J=7.32), 7.16 (s, 1H), 5.32-5.27 (m, 1H), 5.30 (s, 2H). 4.27-4.18 (m, 2H), 3.90-3.82 (m, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 2.98 (dist. q, 2H), 1.75-1.64 (m, 1H), 1.63-1.52 (m, 1H), 1.95-1.25 (m, 4H) ppm; FAB-MS (Finnegan MAT-90, 3-nitrobenzyl alcohol matrix) M+H=608.

Acknowledgement

We would like to thank Mr. G. Kolodin (MRL, West Point) for performing all amino acid analyses and Dr. L. Colwell and Ms. A. Bernick for obtaining all FAB-MS spectra.

References and Notes

[1] Please address correspondence to Dr. Victoria Rusiecki Lombardo.

[2] Putney, S. D.; LaRosa, G. J.; Davide, J. P.; Weinhold, K.; Waterbury, J. A.; Profy, A. T.; Lewis, J. A.; Langlois, A. J.; Dreesman, G. R.; Boswell, R. N.; Shadduck, P.; Holley, L. H.; Karplus, M.; Bolognesi, D. P.; Matthews, T. J.; Emini, E. A. *Science*, **1990**, *249*, 932.

[3] The standard nomenclature and abbreviations for amino acids and peptides of the IUB/IUPAC are used throughout.

[4] Emini, E. A.; Schleif, W. A.; Nunberg, J. H.; Conley, A. J.; Eda, Y.; Tokiyoshi, S.; Putney, S. D.; Matsushita, S.; Cobb, K. E.; Jett, C. M.; Eichberg, J. W.; Murphy, K. K. *Nature*, **1992**, *355*, 728

[5] For a mechanism of the prevention of infection see: Putney, Scott *Trends Biochem. Sci.*, **1992**, *17*, 191.

[6] Tolman, R. L.; Bednarek, M. A.; Johnson, B. A.; Leanza, W. J.; Marburg, S.; Underwood, D. J.; Emini, E. A.; Conley, A. J. *Int. J. Peptide Protein Res.*, in press.

[7] Bednarek, M.; Rivetna, M.; Bondy, S. S. Unpublished results.

[8] Amit, B.; Sehavi, V.; Patchornik, A. *J. Org. Chem.*, **1974**, *39*, 192.

[9] Fodor, S. P. A.; Read, J. L.; Pirrung, M. C.; Stryer, L.; Lu, A. T.; Solas, D. *Science*, **1991**, *251*, 767.

[10] Robertson, S. A.; Ellman, J. A.; Schultz, P. G . *J. Am. Chem. Soc.*, **1991**, *113*, 2722.

[11] All reactions were performed in either foil-covered or amber glassware in normal fluorescent room light.

[12] CAUTION! Phosgene is extremely toxic. All reactions should be conducted in a well ventilated hood with entrainment of excess phosgene (sodalime trap).

[13] All new compounds were characterized by 400 MHz ^{1}H-NMR, FAB-MS, and amino acid analysis and data was consistent with proposed structures.

[14] Wang, S. S. *J. Am. Chem. Soc.*, **1973**, *95*, 1328.

[15] All HPLC performed on a Waters™ 600E system (H_2O/CH_3CN gradient) using Waters™ Delta-Pak™ C_{18} columns (300Å) and a Waters™ 484 tunable U.V. detector at λ=215 nm.

[16] Tam, J. P.; Wu, C. -R.; Liu, W.; Zhang, J. -W. *J. Am. Chem. Soc.*, **1992**, *113*, 6657.