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ABSTRACT

To probe the interactions between major histocompatibility class-II molecules and the amide bonds of the antigenic peptide main-chain, we synthesized ethylenic and reduced analogues of HEL(52-61), an immunogenic peptide for murine major histocompatibility class-II IA^k restricted T cell clones. The synthesis of the corresponding ethylenic analogue of HEL(52-61) in position 53-54 was performed by coupling the Fmoc-protected tripeptide Asp-Tyr-Ψ[E,CH=CH]Gly with HEL(55-61). Biological tests showed that the ethylenic peptide was presented by major histocompatibility class-II IA^k molecule and recognised by HEL(52-61) specific T cell clones. The corresponding reduced peptide of HEL(52-61) at position 53-54, neither stimulated T cell clones nor competed with the natural peptide. These results show that ethylenic pseudopeptides may be used as probes to dissect the role of the hydrogen bonding between the peptide main-chain and MHC residues and help at the design of more stable immunogenic peptides while reduced pseudopeptides might not be appropriate.

INTRODUCTION

Major Histocompatibility Complex Class-II (MHC-II) molecules bind peptides 11 to 23 residues long derived mainly from cell surface, secreted and endocytosed proteins (8). The MHC-II-peptide complexes are expressed at the cell surface, where they can be recognized by the T cell receptor (TcR) of CD4 T cells. The usual function of CD4 T lymphocytes is to secrete lymphokines and to provide help for humoral immune response (Th lymphocytes). A given peptide-MHC complex can be recognised by several distinct TcRs which interact with overlapping but not identical surface of the complex (3).

Crystallographic studies of MHC-II-peptide complexes have shown that the allele specific interaction stems from several pockets in the MHC groove mainly constituted of polymorphic residues that are regularly spaced and have a specificity for anchoring particular side-chains of the peptide amino acids. In addition, there are 10 to 13 hydrogen bonds between the peptide main-chain and conserved side-chain residues of the groove which account for the great variability of the peptide sequence permissive for MHC-II binding and for the allele independent binding of some peptides (7,19). Few recent studies have been carried out to probe the role of peptide main-chain modifications in the context of peptide-MHC-I or MHC-II molecule interactions (4,9,10,13,20).

We have used as a model, the 52-61 moiety of the hen egg white lysozyme HEL(52-61) **1**, an immunogenic peptide for murine MHC-II IA^k restricted T cell clones. Our initial strategy to uncover the rules governing the association of peptides with the MHC-II molecules and their recognition by the TcR was to substitute each position of HEL(52-61) by natural or unnatural amino acids. We established that efficient binding to MHC-II molecules requires not only few anchors residues correctly interspaced, but a complex, non random, combination of residues with appropriate orientation of the peptide main-chain and some crucial side-chains (12). To probe the

interactions of the peptide main-chain with the MHC-II IA^k molecule, we have chosen to replace several amide bonds of our model HEL(52-61) by reduced or *trans* ethylenic bonds so as to remove selectively potential hydrogen bondings between peptide and the MHC IA^k molecules.

We report the synthesis of an *trans* carbon-carbon double bond analog in position 53-54 and three reduced peptide bond analogs of HEL(52-61) and we describe the biological activity of these peptides using MHC-II IA^k restricted T-cell clones. In the absence of structural data on hydrogen bonding between HEL(52-61) and MHC-II IAk molecule, the 53-54 peptide bond was chosen for ethylenic and reduced bond substitutions because the respective role of residues 53 and 54 in HEL(52-61) binding ot TcR recognition have not been completely clarified (12) and also that synthesis of the ethylenic dipeptide TyrΨ[E,CH=CH]Gly was easily accessible (11).

MATERIALS AND METHODS

Fluorenyl-oxy-carbonyl(Fmoc)-protected amino acid derivatives were purchased from Bachem (Bubendorf, Switzerland), France Biochem (Meudon, France) and Néosystem (Strasbourg, France). Solvents and reagents (obtained from Merck [Darmstadt, Germany], Néosystem [Strasbourg, France], Sigma-Aldrich chimie [St. Quentin Fallavier, France]) were reagent grade and used without further purification.

Melting points were performed on a Köfler hot-stage apparatus (Wagner and Munz, Munich, Germany) and were not corrected. IR spectra were carried out on a Perkin-Elmer FT-IR 1600 spectrophotometer (Saint-Quentin-Yvelines, France). Optical rotations were determined with a Roussel-Jouan type 71 digital polarimeter (Jouan, Paris, France). ^1H NMR spectra were recorded on a Brucker AC 200 (200MHz) spectrometer (Bruker Spectrospin, Wissembourg, France) in $CDCl_3$ with tetramethylsilane as internal reference. Mass spectrometry was performed by fast atom bombardment (FAB) using the

facilities of the Service Central d'Analyse du C.N.R.S. (Solaize, France). Ascending TLC was performed on precoated plates of silica gel 60 F_{256} (Merck, Darmstadt, Germany). Products were located by UV (254nm) or with iodine vapour and purified by silica gel chromatography (Matrex silica 60Å 35-70μ, Grace Amicon, Épernon, France).

Synthesis of aminoaldehydes

The aldehydes used in this study were synthetized according to the method of Fehrentz and Castro (5).

Synthesis of peptides

Solid-phase synthesis of the peptides on p-alkoxybenzylalcohol resin (Wang resin) was carried out manually with an in-house apparatus. Loading of first residue on resin was performed as described by Lu et al (17). Side-chain protecting groups were OtBu for Asp, Ser, Tyr and Mtr for Arg. Couplings were mediated by Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) / 1-hydroxybenzotriazole (HOBt) / diisopropylethylamine (DIEA) mixture in dimethylformamide (DMF) for all amino acids unless Asn which was incorporated as tetrafluorophenyl ester derivative. Removal of the Fmoc group before each coupling was effected by a 20% solution (v/v) of piperidine in DMF for 4+7min. Resin washing was carried out by repeated application of DMF, isopropanol and DCM. Monitoring was done using the Kaiser test. The peptides were released from resins by treatment at room temperature with 20ml/g of a freshly prepared solution of Reagent K (15) for 15hrs so as to achieve complete cleavage of the Mtr protecting group. The peptides were precipitated from the cleavage solution after filtration of the resin, by addition of cold diethyl ether (100ml/g), dissolved in 0.1% trifluoroacetic acid (TFA) /30% acetonitrile (CH_3CN)/H_2O and finally lyophilized. Purification of the crude peptides were performed on a Kontron HPLC 400 system (Saint-Quentin-Yvelines, France)

(2 HPLC pump 420 , UV detector 432) equipped with a Vydac 218TP1022 C18 reverse-phase preparative column (Hesperia, CA, USA) (250 · 22 mm, 10 μm particle size, 300Å pore size), with detection at 210nm. Crude peptides were purified with a binary solvent system (system 1), where A was triethylammonium phosphate (TEAP) pH 2.25 and solvent B was 60% CH_3CN/TEAP pH 2.25. Pure fractions were pooled and desalinated with a binary solvent system (system 2), where A was TFA 0.1%/H_2O and solvent B 0.08%TFA /60% CH_3CN / H_2O. Screening and final purity was assessed on the same chromatograph by analytical RP-HPLC on a Vydac 218TP54 C18 column (Hesperia, CA, USA) (250 · 4.6mm, 5μm particle size, 300Å pore size). The peptides were characterized by Fast atom bombardment mass spectrometry in thioglycerol unless otherwise stated.

Synthesis of $Tyr^{53}\Psi$[E,CH=CH]Gly^{54}HEL(52-61) **6**

*N*α-(*tert*-Butoxycarbonyl)-(O-*tert*-butyl)-L-tyrosinal **2**:

Colorless oil, yield=74.4%; $[\alpha]^{20}_D$ -28 (c 0.1, EtOH) (litt. (11) $[\alpha]^{20}_D$ -23 (c 1 MeOH)). IR (film, ν cm^{-1}) 3346 (νNH), 2976, 1711 (νCO), 1608, 1507, 1366, 1162, 898. ^1H NMR (200MHz, DMSO-d_6, δ ppm): 1.25 (s, 9H, $OC(CH_3)_3$); 1.33 (s, 9H, $OC(CH_3)_3$); 2.65 (dd, J=14Hz and 10Hz, 1H, $CH_2\beta$); 3.03 (dd, J=14Hz and 4.5Hz, 1H, $CH_2\beta$); 4.08 (m, 1H, CHα); 6.86 (d, J=8.36Hz, 2H, H2+H6); 7.12 (d, J=8.36Hz, 2H, H3+H5); 7.29 (d, J=7.9Hz, 1H, NH); 9.51 (s, 1H, CHO).

S-trans-2-*tert*-Butoxycarbonylamino-1-(4-*tert*-Butoxyphenyl)-6-(trimethylsilyl)-hex-3-en-5-yne **3 a** and *S-cis*-2-*tert*-Butoxycarbo-nylamino-1-(4-*tert*-Butoxyphenyl)-6-(trimethylsilyl)-hex-3-en-5-yne **3b**:

Synthesis of compounds **3 a** and **3b** were carried out from **2** and phosphonium salt $Br^-Ph_3^+CH_2C{\equiv}CSi(Me)_3$ according to Hann and coworkers (11).

Spectral data of **3a** and **3b**:

Compound **3a**: colorless oil, yield=53%. $[\alpha]^{20}_D$ +4.5 (c 0.088, EtOH) (litt. (11) $[\alpha]^{20}_D$ +6 (c 1.0, MeOH)). IR (film, ν cm^{-1}) 3343 (νNH), 2975, 2137 (νC≡C), 1690 (νCO), 1608, 1506, 1365, 1248, 1162, 842. ^{1}H NMR (200MHz, $CDCl_3$, δ ppm): 0.17 (s, 9H, $Si(CH_3)_3$); 1.33 (s, 9H, $OC(CH_3)_3$); 1.39 (s, 9H, $OC(CH_3)_3$); 2.77 (d, J=5.14Hz, 2H, $CH_2\beta$); 4.42 (m, 1H, CHα); 5.59 (d, Jtrans=16.08Hz, 1H, =CH); 6.13 (dd, Jtrans = 15.97Hz and 5.44Hz, 1H, =CH); 6.99 (d, J=8.5Hz, 2H, H2+H6); 7.19 (d, J=8.52Hz, 2H, H3+H5).

Compound **3b**: colorless oil, yield=6%. $[\alpha]^{20}_D$ +62 (c 0.158, EtOH) (litt. (11) $[\alpha]^{20}_D$ +98 (c 1.0, MeOH)). IR (film, ν cm^{-1}) 3364 (νNH), 2976, 2147 (νC≡C), 1702 (νCO), 1608, 1506, 1365, 1250, 1163, 843. ^{1}H NMR (200MHz, $CDCl_3$, δ ppm): 0.21 (s, 9H, $Si(CH_3)_3$); 1.33 (s, 9H, $OC(CH_3)_3$); 1.40 (s, 9H, $OC(CH_3)_3$); 2.88 (m, 2H, $CH_2\beta$); 4.71 (m, 1H, CHα); 5.50 (d, Jcis=11.18Hz, 1H, =CH); 5.85 (m, 1H, =CH); 6.91 (d, J=8.5Hz, 2H, H2+H6); 7.06 (d, J=8.5Hz, 2H, H3+H5).

S-trans-5-*tert*-Butoxycarbonylamino-6-(4-*tert*-Butoxyphenyl)-6-(trimethylsilyl)-hex-3-enoic acid **4**:

Synthesis of compound **4** was carried out from **3a** according to Hann and coworkers (11).

Compound **4**: waxy solid, yield=53%.IR (KBr, ν cm^{-1}) 3352 (νNH), 1713 (νCO), 1608, 1505, 842. ^{1}H NMR (200MHz, $CDCl_3$, δ ppm): 1.32 (s, 9H, $OC(CH_3)_3$); 1.38 (s, 9H, $OC(CH_3)_3$); 2.77 (d, 2H, J=6.72Hz, 2H, $CH_2\beta$); 3.05 (d, 2H, J=5.62Hz, 2H, $C\underline{H_2}COOH$); 4.40 (m, 1H, CHα); 5.58 (m, 2H, 2 =CH); 6.90 (d, J=8.48Hz, 2H, H2+H6); 7.05 (d, J=8.48Hz, 2H, H3+H5).

Tripeptide *N*α-Fmoc-Asp-O-*tert*-Butyl-TyrΨ[E,CH=CH]Gly-OH **5**:

Compound **4** (0.15g, 4.03 10^{-4} mol) was added to TFA (1.5ml) at room temperature. After 45mn the solvent was removed i*n vacuo*. The residue was treated with N-Fmoc-Asp-(OtBu)-OSu (0.17g, 3.34 10^{-4} mol) and 0.13ml of DIEA (7.28 10^{-4} mol) in 5ml DMF . After stirring for 2h30 at room

temperature, 10% aqueous HCl was added. The solid formed was subsequently triturated in diethyl ether/dichloromethane (DCM). After concentration *in vacuo*, compound **5** was obtained as a white solid (0.193g, yield=78%).

Compound **5**: $[\alpha]^{20}_{D}$ -5 (c 0.109, THF). IR (KBr, ν cm^{-1}) 3323 (νNH), 2978, 1718 (νCO), 1654 (νC=C), 1515, 1450, 1368, 1249, 1155, 760,741. ^{1}H NMR (200MHz, DMSO-d_6, δ ppm): 1.35 (s, 9H, $OC(CH_3)_3$); 2.61 (m, 2H, $CH_2\beta$); 2.91 (m, 2H, CH=CH-C$\underline{H_2}$-COOH); 3.60 (m,2H, C$\underline{H_2}$COOtBu); 4.30 (m, 5H, 3H Fmoc+CHα Tyr+CHα Asp); 5.49 (m, 2H, 2 =CH); 6.62 (d, J= 8.3Hz 2H, H2+H6); 6.96(d, J=8.39Hz, 2H, H3+H5); 7.36 (m, 4H, 4H Fmoc); 7.54 (d, J=8.54Hz, 1H, NH); 7.71 (m, 2H, 2H Fmoc); 7.89 (d, J=7.77Hz, 2H, 2H Fmoc) MS FAB (3-nitrobenzylalcohol, positive mode): 637 $[M+Na]^+$, 615 $[M+H]^+$, 559 $[M-tBu+2H]^+$.

Tyr^{53}Ψ[E,CH=CH]Gly^{54}-HEL(52-61) **6**:

The tripeptide **5** (0.117g, 1.91 10^{-4}mol, 1.27eq) was coupled by solid phase (Wang resin subst. 0.9mmol/g) onto a HEL(55-61) moiety (1.5 10^{-4}mol) after deprotection of the Fmoc terminal protecting group, in DMF (4ml) with dicyclohexylcarbodiimide (1.27eq) and HOBt (1.27eq) for 15hrs at room temperature. At this time, the Kaiser test showed a nearly complete coupling reaction. The resin was cleaved after deprotection of the terminal Fmoc protecting group, for one night to yield crude peptide. HPLC purification was performed with system 1 (15 to 55%B in A over 45') followed by system 2 (15 to 45%B in A over 30'). Lyophilisation of pure fractions afforded peptide **6** (0.008g, yield=4.5%). Purity control was performed by analytical HPLC and shown a single peak.

MS FAB (thioglycerol+H_2O+acetic acid 5%, positive mode): 1161.7 $[M+H]^+$.

Synthesis of reduced peptides Tyr^{53}Ψ[CH_2-NH]Gly^{54}HEL(52-61) **10**, Gly^{54}Ψ[CH_2-NH]Ile^{55}HEL(52-61) **11** and Ile^{55}Ψ[CH_2-NH]leu^{56}HEL(52-61) **12**:

Reduced peptides **10**, **11** and **12** were synthetized according to Sasaki and Coy (21) with sodium cyanborohydride ($NaBH_3CN$, 3eq) in 0.1% acetic acid/DMF and the corresponding Fmoc aminoaldehydes **7**, **8** and **9** for 2 hrs. Coupling with aminoaldehyde **9** was carried out twice (3eq then 1.5eq) owing to a first incomplete coupling. Purity controls were performed by analytical HPLC and showed a single peak for **11** and **12** and a peak splitting for **10**.

*N*α-(fluoren-9-ylmethyloxycarbonyl)-(O-*tert*-butyl)-L-tyrosinal **7**:

White solid, yield=64%. m.p.: 56°C (dec.). $[\alpha]^{20}_D$ -26.4 (c 0.144, EtOH). IR (KBr, ν cm^{-1}) 3331 (νNH), 30036, 2924, 1721 (νCO), 1608, 1505, 1449, 1237, 1161, 758, 740. ^1H NMR (200MHz, DMSO-d_6, δ ppm): 1.21 (m, 6H, $C(CH_3)_3$); 2.67 (dd, J=14Hz and 10.4Hz, 1H, CH_2β); 3.09 (dd, J=14Hz and 4.1Hz, 1H, CH_2β); 4.20 (m, 4H, CHα+3H Fmoc); 6.82 (d, J=8.4Hz, 2H, H3+H5); 7.1 (d, J=8.4Hz, 2H, H2+H6); 7.35 (m, 4H, 4H Fmoc); 7.66 (d, J=7.2Hz, 2H, 2H Fmoc); 7.89 (d, J=7.1Hz, 2H, 2H Fmoc); 9.54 (s, 1H, CHO).

*N*α-(fluoren-9-ylmethyloxycarbonyl)-glycinal **8**:

Semi-solid paste, yield=57%. IR (film, ν cm^{-1}) 3409 (νNH), 3065, 2949, 1713 (νCO), 1529, 1449, 1248, 759, 740. ^1H NMR (200MHz, DMSO-d_6, δ ppm): 3,84 (d, J=5.6Hz, 2H, CH_2); 4.24 (t, J=6.1Hz, 1H, H Fmoc); 4.35 (d, J=6.5Hz, 2H, CH_2 Fmoc); 7.37 (m, 4H, 4H Fmoc); 7.72 (d, J=7Hz, 2H, 2H Fmoc); 7.9 (d, J=7.4Hz, 2H, 2H Fmoc); 9.47 (s, 1H, CHO).

*N*α-(fluoren-9-ylmethyloxycarbonyl)-L-Isoleucinal **9**:

White solid, yield=40%. m.p.: 100°C. $[\alpha]^{20}_D$ +1.46 (c 0.1274, EtOH). IR (KBr, ν cm^{-1}) 3335 (νNH), 2964, 2949, 1713 (νCO), 1520, 1450, 1247, 758, 740. ^1H NMR (200MHz, DMSO-d_6, δ ppm): 0.82 (m, 6H, $2CH_3$); 1.18 (m, 1H, $CHC\underline{H_2}CH_3$); 1.36 (m, 1H, $CHC\underline{H_2}CH_3$); 1.90 (m, 1H, $C\underline{H}CH_2CH_3$); 3,91 (t, J=6.66Hz, 1H, CHα); 4.26 (t, J=5.8Hz, 1H, H Fmoc); 4.34 (d, J=5.76Hz, 2H, CH_2 Fmoc); 7.35 (m, 4H, 4H Fmoc); 7.73 (d, J=7.28Hz, 2H, 2H Fmoc); 7.89 (d, J=7.22Hz, 2H, 2H Fmoc); 9.5 (s, 1H, CHO).

Tyr$^{53}\Psi$[CH_2-NH]Gly^{54}HEL(52-61) **10**: MS FAB (positive mode): 1164.7 $[M+H]^+$.

Gly$^{54}\Psi$[CH_2-NH]Ile^{55}HEL(52-61) **11**: MS FAB (positive mode): 1164.6 $[M+H]^+$.

Ile$^{55}\Psi$[CH_2-NH]leu^{56}HEL(52-61) **12**: MS FAB (positive mode): 1164.6 $[M+H]^+$.

T-cell stimulation assay

The T-cell stimulation assay was performed as previously described (12) on MHC-II IAk restricted 3A9 and 2A11 T cell clones. The results were expressed as the concentration of peptide inducing half maximal stimulation of T cells (Stimulating Peptide Concentration, s.p.c.) or reducing by 50 % the sub-optimal stimulation observed in the presence of the natural peptide (Competing Peptide Concentration, c.p.c.) - Table 1.

RESULTS AND DISCUSSION

Synthesis of pseudopeptide **6**, *trans* ethylenic analog of HEL(52-61) implied the obtaining of a suitably protected ethylenic dipeptide TyrΨ[E,CH=CH]Gly. Among the various published methods, we chose the method of Hann and coworkers (11) as shown in Scheme 1. Fmoc-Asp(OtBu)-OH was subsequently coupled to the ethylenic dipeptide **4** as succinimide derivative to obtain pseudotripeptide **5** in 53% yield after purification. The next steps involved the presence of a free hydroxyl group for tyrosine residue. However, it has been shown that coupling with non-protected tyrosine is possible (6). Indeed, the tripeptide **5** was attached by solid phase peptide synthesis onto the HEL(55-61) moiety with the DCC/HOBt procedure (16) for 15hrs and Kaiser test shown a nearly complete coupling reaction - scheme 2. Finally, pseudopeptide **6** was obtained after cleavage from the resin and purification by HPLC in 4.5% yield. It should be stressed that peptide synthesis was not optimized.

Synthesis of reduced peptides **10**, **11** and **12** were synthetized by reductive amination according to Sasaki and Coy (21). Reactions for peptides **10** and **11** were complete as checked with Kaiser test. However for peptide **12**, it was necessary to couple twice with the aminoaldehyde. Preparation of peptides containing a reduced bond isostere with this method is known to generate significant amounts of peptide diastereoisomers especially with aromatic peptides as phenylalanine or tyrosine (14). Indeed, analytical HPLC chromatogram shown a splitting. Diastereoisomeric ratio calculated by ^{1}H NMR shown a 65:35 ratio for peptide **10** (Pr Marraud, personal communication).

Pseudopeptides **6**, **10**, **11** and **12** were tested for their ability to stimulate 2A11 and 3A9 MHC-II IA^{k} restricted T cell clones (12) and/or to compete for presentation of natural HEL(52-61) **1** to these T cells. The introduction of a trans ethylenic bond in 53-54 junction had no major effect on its ability to bind to MHC-II and to be recognised by the 3A9 TcR. Indeed, the *trans* ethylenic pseudopeptide **6** was found to be a good stimulator for 3A9 T cells, requiring only 30 μM to induce half-stimulation. This concentration was only 7-fold higher than that required by its natural counterpart. It could be inferred from these results that *trans* ethylenic isostere **6** adopts a conformation close to that of HEL(52-61) in the MHC groove and that abolition of potential hydrogen bonds between the amide bond Tyr^{53}-Gly^{54} with MHC residues does not drastically impair the binding. Early studies on HEl(52-61)-CMH-II IA^{k} MHC molecule interactions suggested an α-helical conformation for HEL(52-61) (1). However, crystallographic studies of several peptide-MHC-II complexes have shown that the top portion of MHC molecules forms a groove opened at both end in which the peptide fits in a polyproline type II conformation (7). The result obtained with peptide **6** is well consistent with these studies. However, surface exposure of the *trans* ethylenic pseudopeptide was not identical to that of natural HEL(52-61)

peptide. When compared to the natural peptide, the pseudopeptide **6** was a poor stimulator of 2A11 T cells and required a 3,000-fold higher concentration. The 53 residue has been previously described to interact with the TcR (1). The constraint resulting from the *trans* ethylenic bond could have changed the orientation and/or the surface exposure of the 53-Tyr side-chain. This limited conformational change of peptide **6** would then be tolerated by 3A9 TcR but not by 2A11 TcR. Accordingly, replacement of 53-Tyr by a p-NO_2-Tyr residue in HEL(52-61) is tolerated by 3A9 TcR but not by 2A11 TcR (2).

As an alternative to remove a potential hydrogen bond between MHC-II molecule and the ketone of the amide bond, reduced peptide in position 53-54 **10** was also tested for its ability to be presented by IA^k molecules to the same T cells. Even at a concentration of 100 μM, it was unable to stimulate 3A9 and 2A11 T cells. This was likely due to the poor ability of the reduced peptide **10** to bind to MHC-II IA^k molecules since it could not compete with the presentation of the natural peptide. If we compare the activity of peptide **6** and **10**, we expected a better or at least the same binding affinity for reduced peptide **10** owing to the secondary amine which could be involved in a hydrogen bonding. However, reduced peptides are prone to intramolecular hydrogen bond with adjacent carbonyl group (18) because of nitrogen protonation at physiological pH and this mechanism, by stabilizing a folded structure, could account for the inability of peptide **10** to bind to MHC-II A^k molecule. In favour of this hypothesis, reduced peptides at two other positions (**11** and **12**) also displayed a poor MHC binding. For example, 1,500 times more reduced peptide at position 54-55 than natural peptide are required for stimulating 2A11 T cells. If we assume there is no direct effect on the recognition by 2A11 TcR, this would mean that reducing the 54-55 amide bond decrease the binding strength to MHC-II by 3 orders of magnitude. However, it should be stressed that a reduced bond could also bring an

additional conformational flexibility due to the free rotation around C-N bond allowing peptides to take conformations different from those taken by the native peptide. These biological results are in agreement with the study of C.M. Hill and coworkers which showed that introduction of reduced bonds in peptide sequences were generally deleterious for an efficient binding (13).

In conclusion, a trans ethylenic analog of an antigenic peptide was tested for the first time in the context of peptide-MHC-II recognition and found to keep its ability to bind MHC molecule and stimulate Class-II IAk restricted T cell clones while its reduced pseudopeptide counterpart cannot bind the MHC molecule. This indicates that ethylenic pseudopeptides may be used as probes to dissect the role of the hydrogen bonding between the peptide main-chain and MHC residues and help at the design of more stable immunogenic peptides while reduced pseudopeptides might not be appropriate.

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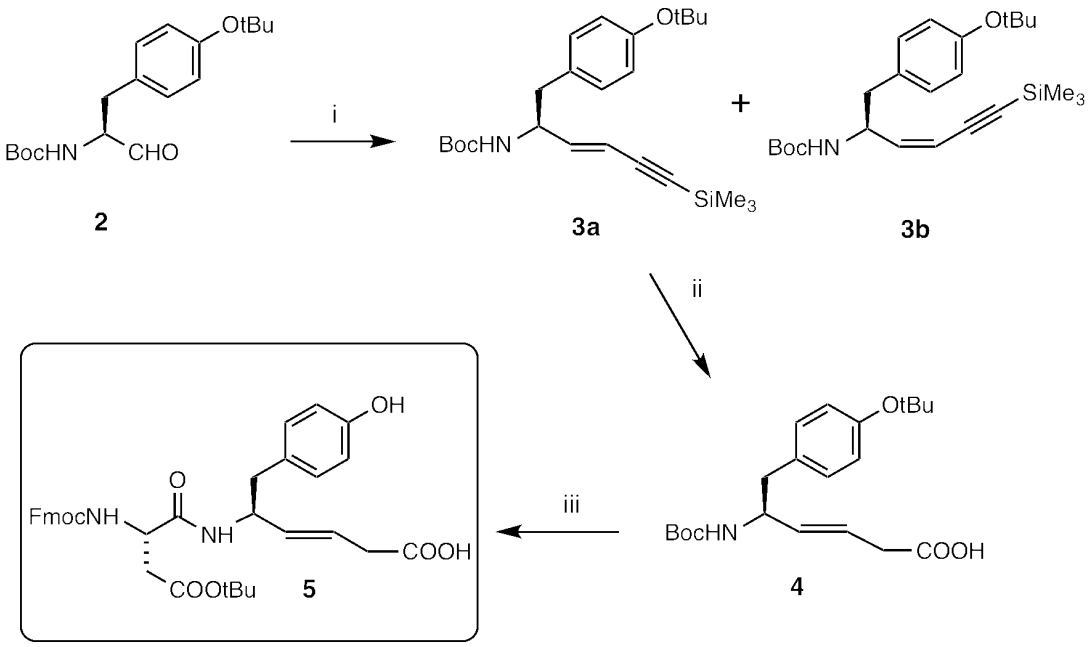
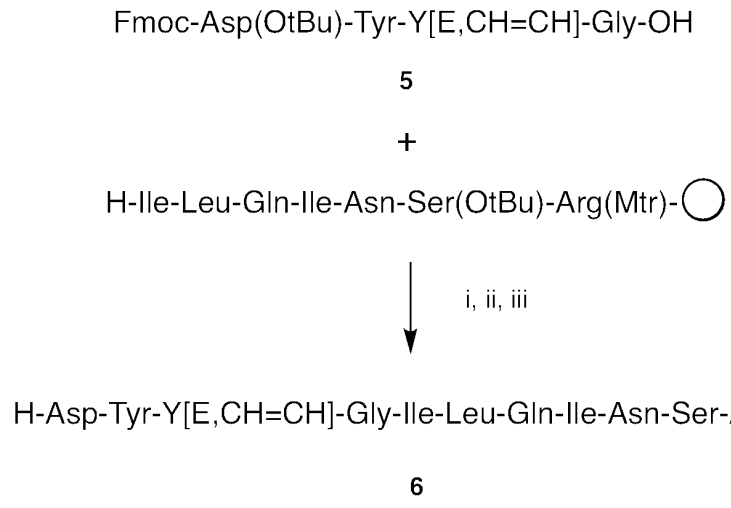


i. $Br^{-}Ph_3P^{+}CH_2C\equiv CSiMe_3$, n-BuLi, THF, yield=59% ii. dicyclohexylborane, H_2O_2, NaOH, yield=53% iii. a) TFA b) N-Fmoc-Asp(OtBu)-O-Su, DIEA, DMF, yield=78%

Scheme 1. Synthesis of protected pseudopeptide **5**.



i. DCC, HOBt, DMF, 15 hours ii. piperidine 20%/DMF iii. reagent K

Scheme 2. Synthesis of pseudopeptide **6**.

Entry	Peptide sequence										2A11 S.P.C.* ± SD	3A9 S.P.C. ± SD	2A11 C.P.C.† ± SD	3A9 C.P.C ± SD
	52	53	54	55	56	57	58	59	60	61				
1	Asp	Tyr	Gly	Ile	Leu	Gln	Ile	Asn	Ser	Arg	8 nM	4 μM	-	-
6	-	- [CH=CH]	-	-	-	-	-	-	-	-	25 μM	30 μM	-	-
10-	-[$CH_2NH_2^+$]-		-	-	-	-	-	-	-	n.s.‡	n.s.	n.c.§	n.c.	
11	-	-	-[$CH_2NH_2^+$]-		-	-	-	-	-	-	12 μM	n.s.	-	n.c.
12	-	-	-	-[$CH_2NH_2^+$]-		-	-	-	-	-	n.s.	n.s.	n.c.	n.c.

* 50% Stimulating Peptide Concentration. † 50% Competing Peptide Concentration.
‡ n.s.: no stimulation at a concentration of at least 100 μM. § n.c.: no competition against HEL(52-61) at a concentration of at least 100 μM.
Data represent the average of two independent experiments.