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N-Hydroxy-amide Analogues of MHC-Class I Peptide Ligands with Nanomolar Binding Affinities

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Abstract: A novel class of major histocompatibility complex class I (MHC-I) ligands containing an N-hydroxy-amide bond was designed on the basis of the natural epitope SIINFEKL, and synthesized on solid phase. The capacity of these compounds to bind to the MHC-I molecule H-2K^b and to induce T cell responses was analysed in comparison with the corresponding glycine containing variant of SIINFEKL. Binding to the MHC molecule was diminished by the N-hydroxy group at positions 2 and 3 of the oligomer and improved in the case of positions 4, 5, 6 and 7. No change was seen for position 1. The efficacy of T cell stimulation was strongly reduced by the modification of all positions except for position 1. A complete loss of activity was found for the N-hydroxy variant in positions 4 and 6. N-Hydroxy amide-containing peptides displayed an enhanced stability to enzymatic degradation. This new class of MHC ligand can become instrumental as immunomodulatory reagent in various disease situations. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: N-hydroxy peptides; peptides; ligands; MHC; solid-phase synthesis

INTRODUCTION

Several natural products contain the ψ[CO-N(OH)] amide modification. N-Hydroxyamide functionalities play a fundamental role in siderophores, which are potent iron chelators presenting often acetylated N-hydroxy side-chains of lysine and ornithine residues, to bind and transport the metal ion essential for growth and proliferation of cells [1,2]. Hydroxamates show interesting activity such as inhibition of metalloproteases like thermolysin, and as anticancer agents preventing cell proliferation [3–5]. Inside a peptide backbone, the N-hydroxy-amide group acts as a strong proton donor and induces conformational modifications. It also increases the stability to enzymatic degradation in comparison with the cognate amide group [6–8].

Until now the insertion of the ψ[CO-N(OH)] modification into small peptides was carried out in solution using N-hydroxy-amino acid residues [9,10] while on solid phase only a few examples for the synthesis of C-terminal peptide hydroxamic acids were reported [11,12]. In order to explore the interesting features of the N-hydroxy-amide group using automated solid phase synthesis and combinatorial techniques [13], we developed a method for the preparation of N-hydroxy peptide ligands for major histocompatibility complex class I (MHC-I) molecules.

MHC I complexes are heterotrimers, composed of two protein subunits and a peptide. The peptides of 8–10 amino acids are generated inside the cell by

Abbreviations: MHC, major histocompatibility complex; BSA, bovine serum albumin; DMEM, Dulbecco s modified Eagle's medium; FITC, fluorescein isothiocyanate; TcR, T cell receptor, Alloc, allyloxycarbonyl; Bzl, benzyl; NMM, N-methyl morpholine; DIC, diisopropylcarbodiimide; TIS, triisopropylsilane; DIPEA, diisopropylethylamine; HOBt, 1-hydroxybenzotriazole; DBU, 1,8-diazabicyclo[5.4.0]undecen-7-ene; HATU, O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAt, 7-aza-1-hydroxybenzotriazole; CPY, carboxypeptidase Y; APM, aminopeptidase M.

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proteolytic digest and are, after transport into the endoplasmic reticulum, inserted into the MHC molecules as the initial step of specific T cell-mediated immune responses [14]. The peptides are crucial for the stabilization of the MHC protein [15]. Cell lines with genetic defects in the gene for the transporter proteins required for delivery of the peptide to the MHC precursors generate labile peptide-free MHC I that can be loaded externally with synthetic peptides [16]. The loading of empty MHC I molecules with external peptides or peptidomimetics has opened the way to the investigation of e.g. T cell antagonists for the therapy of autoimmune diseases and of synthetic vaccines [17–21].

There are a large variety of MHC allomorphs which have a similar structure but which contain different allele-specific pockets within their peptide binding groove. We used the natural SIINFEKL epitope which binds with high affinity to the murine MHC H-2K^b [22,23] as template for the design of a novel class of MHC ligand. Seven modified SIINFEKL peptides were prepared by systematically replacing each amino acid position by an N-hydroxy-glycine residue (Table 1). The related peptide analogues derived from a glycine positional scanning were synthesized employing multiple peptide synthesis [24]. The effect of N-hydroxy-glycine and glycine substitution on the enzymatic stability, the MHC binding and the TcR recognition of SIINFEKL was evaluated.

MATERIALS AND METHODS

General

All reagents including amino acids and solvents were purchased from Fluka (Buchs, Switzerland), Aldrich (Millwaukee, USA), Novabiochem (Laubelfingen, Switzerland) and Merck (Darmstadt, Germany). Aminopeptidase M and carboxypeptidase Y were obtained from Boehringer Mannheim Biochemicals (Mannheim, Germany).

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC 250 spectrometer. Chemical shifts are given as parts per million (δ) relative to tetramethylsilane. Infrared absorption spectra were recorded with a Perkin-Elmer model 580 B spectrophotometer equipped with a Perkin-Elmer model 3600 1R data station. Melting points were determined using a Laborlux 12 apparatus and were not corrected. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter equipped with a Haake thermostat and a 10 cm pathlength cell. Field desorption mass spectrometry (FD-MS) was performed with a MAT 711A modified, AMD Intectra instrument (Harpstedt, Germany).

Synthesis of derivatives

Alloc-L-Ile-OH. N-Alloc-amino acids were prepared according to Dangles *et al.* [25]. To H-Ile-OH (1.3 g; 10 mmol) in dioxane–water (1:1) (20 ml), at 0°C, a

Table 1 Characterization of the N-Hydroxy Peptides

	Sequences	Method[a]	HPLC t_R (min)[b]	ESI MS[c] found	ESI MS[c] calcd.	Yield (%)[d]
6a	H-Ser-Ile-Ile-Asn-Phe-Glu-ψ[CO-(NOH)]-Gly-Leu-OH	f.c.	18.5	908.5	907.0	34
6b	H-Ser-Ile-Ile-Asn-Phe-ψ[CO-(NOH)]-Gly-Lys-Leu-OH	f.c.	19.6	907.5	906.0	20
6c	H-Ser-Ile-Ile-Asn-ψ[CO-(NOH)]-Gly-Glu-Lys-Leu-OH	s.-by-s.	13.5	889.5	888.0	30
6d	H-Ser-Ile-Ile-ψ[CO-(NOH)]-Gly-Phe-Glu-Lys-Leu-OH	s.-by-s.	17.2	922.5	921.0	54
6e	H-Ser-Ile-ψ[CO-(NOH)]-Gly-Asn-Phe-Glu-Lys-Leu-OH	s.-by-s.	15.6	923.5	922.0	35
6f	H-Ser-ψ[CO-(NOH)]-Gly-Ile-Asn-Phe-Glu-Lys-Leu-OH	s.-by-s.	15.9	923.5	922.0	25
6g	ψ[H-(NOH)]-Gly-Ile-Ile-Asn-Phe-Glu-Lys-Leu-OH	s.-by-s.	18.0	949.5	948.0	65

[a] f.c., fragment condensation; s.-by-s., step-by-step coupling.
[b] Linear gradient of A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile, 10–100% B in 45 min at 0.3 ml/min flow rate.
[c] Electrospray mass spectrometry, $[M+H]^+$ peak.
[d] After RP-HPLC purification.

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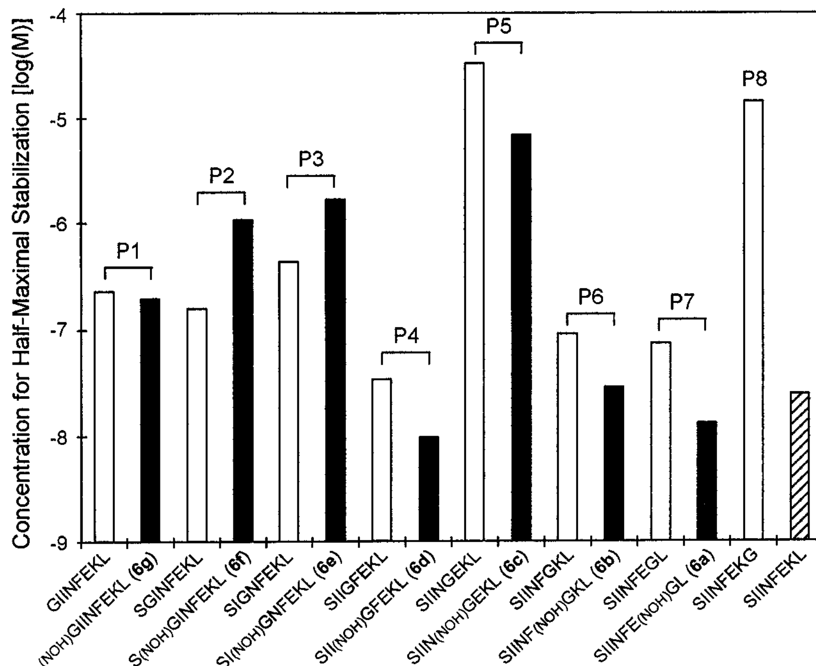

Figure 1 Concentration for the half-maximal stabilization of the murine MHC-molecule H-2K^b by the N-hydroxy amide peptides **6a–g** compared with the glycine scanning peptides and SIINFEKL. P1–P8 correspond to the amino acid positions from the N-terminus.

solution of 2 M NaOH (10 ml; 20 mmol) was slowly added. Alloc-Cl (1.09 ml; 10 mmol) in dioxane (10 ml) was dropped in within 30 min. After stirring for 2 h at 0°C and 16 h at room temperature, dioxane was evaporated and the solution was washed twice with Et_2O and acidified with 5% $KHSO_4$. The product was extracted with AcOEt and washed twice with water. The solvent was evaporated affording a pale yellow oil. Yield: 97%; $[\alpha]_D^{20} = +2.6°$ (c = 0.5, MeOH); IR (film) 3320, 1708, 1524 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.96 (s, 1H, COOH), 6.35 and 5.35 (2d, 1H, αNH *cis* and *trans*), 5.90 (m, 1H, CH Alloc), 5.33 (m, 1H, CH Alloc), 5.22 (m, 1H, CH Alloc), 4.59 (m, 2H, CH_2 Alloc), 4.36 (m, 1H, αCH), 1.93 (m, 1H βCH), 1.19 (m, 2H, γCH_2), 0.97–0.89 (2t, 6H, CH_3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 156.17, 132.51, 117.91, 65.98, 58.20, 37.73, 24.80, 15.43, 11.52; MS (FD): *m*/*z* 216 [MH$^+$].

Alloc-L-Phe-OH. Oil. Yield: 95%; $[\alpha]_D^{20} = +5.4°$ (c = 0.5, MeOH); IR (film) 3325, 1703, 1520 cm^{-1}; ^{1}H NMR (250 MHz., $CDCl_3$) δ 9.37 (s, 1H, COOH), 7.31 (m, 5H phenyl), 6.35 and 5.33 (2d, 1H, αNH *cis* and *trans*), 6.01 (m, 1H, CH Alloc), 5.33 (m, 2H, 2CH Alloc), 4.76 (m, 1H, αCH), 4.64 (m, 2H, CH_2 Alloc), 3.19 (m, 2H βCH_2); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 175.87, 155.79, 135.58, 132.42, 129.31, 128.63, 127.18, 117.93, 65.98, 54.52, 37.74; MS (FD): *m*/*z* 249 [M$^+$].

Alloc-L-Asn(Trt)-OH. Recrystallized from Et_2O-petroleum ether. Yield: 77%; m.p. 192–195°C; $[\alpha]_D^{20} = -18.3°$ (c = 0.5, MeOH); IR (KBr) 331 1, 1726, 1698, 1641, 1523 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.19 (m, 16H Trt, NHCO), 6.00 (d, 1H, αNH), 5.85 (m, 1H, CH Alloc), 5.24 (m, 2H, 2CH Alloc), 4.52 (m, 2H, CH_2 Alloc), 4.38 (m, 1H, αCH), 2.82 (m, 2H βCH_2); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 172.80, 170.51, 156.14, 143.89, 132.33, 128.62, 128.03, 127.25, 117.94, 71.22, 66.02, 50.58, 38.54; MS (FD): *m*/*z* 458 [M$^+$].

Alloc-L-Glu(tBu)-OH. Oil. Yield: 70%; $[\alpha]_D^{20} = -11.0°$ (c = 0.5, MeOH); IR (film) 3325, 1723, 1528 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.18 (s, 1H, COOH), 6.40 and 5.61 (2d, 1H, αNH *cis* and *trans*), 5.87 (m, 1H, CH Alloc), 5.23 (m, 2H, 2CH Alloc), 4.55 (m, 2H, CH_2 Alloc), 4.36 (m, 1H, αCH), 2.34 (m, 2H, γCH_2), 2.17 (m, 1H, βCH_2), 1.98 (m, 1H βCH_2), 1.42 (s, 9H, *t*Bu); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 175.70, 172.44, 156.11, 132.42, 117.88, 81.09, 65.98, 53.28, 31.55, 27.98, 27.29; MS (FD): *m*/*z* 288 [MH$^+$].

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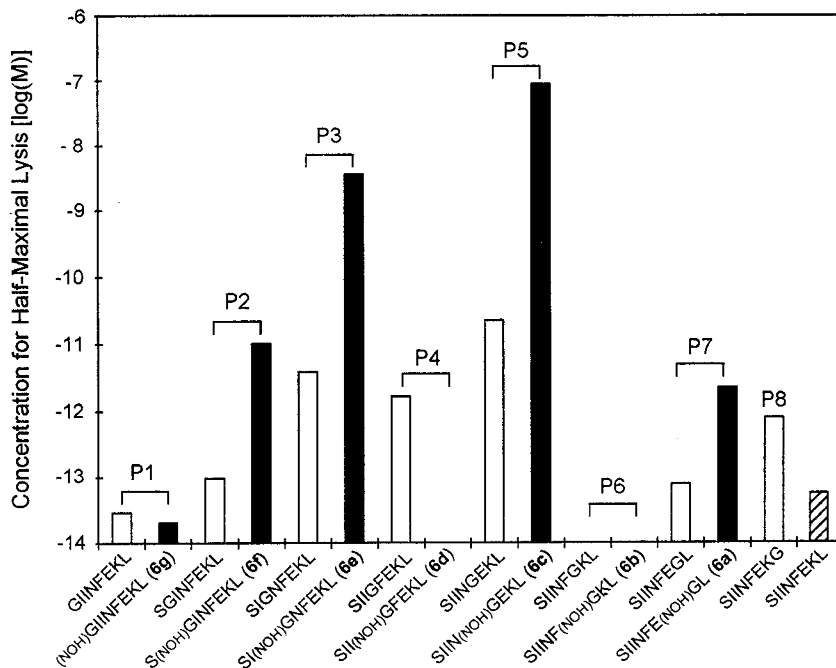

Figure 2 Influence of the N-hydroxy amide modification in compounds **6a–g** on the H-2K^b restricted stimulation of the SIINFEKL specific T cell clone 4G3, compared with the corresponding glycine analogues and to the natural epitope.

Synthesis of N-hydroxy Peptides

The N-hydroxy amide ligands were synthesized on Wang resin (54 mg, 20 mmol) (RAPP Polymere, Tübingen, Germany). The amino acids of the C-terminal part of the oligomers were introduced using Fmoc/*t*Bu strategy [24]. Fmoc-protected amino acids were activated with DIC/HOBt in DMF and coupled in 10-fold excess for 90 min. The N-terminal protecting group was cleaved by piperidine/DMF 1:1 (v/v) within 30 min. Bromoacetic acid was preactivated with DIC in DMF for 30 min and coupled as symmetric anhydride in 10-fold excess for 2 h (twice). BzlO-$NH_2 \times$ HCl was neutralized with DIPEA in DMF and added to the resin in 50-fold excess. The mixture was shaken at room temperature for 12 days. Alloc-protected amino acid was activated with HATU/DIPEA in DMF and coupled in 10-fold excess for 20 h (twice). After Alloc cleavage with $Pd[P(Ph_3)]_4$ (1.5 eq) in $CHCl_3$/AcOH/NMM (2 ml) for 2 h, the N-hydroxy peptides were elongated using either step-by-step N-Alloc-amino acids, activated with DIC/HOBt in DMF and coupled in 10-fold excess for 90 min, or by fragment condensation. Peptide fragments were activated with HATU/HOAt/$CuCl_2$/DIPEA in DMF and coupled in 2-fold excess for 24 h. After the cleavage from the resin with a 9:2:1 mixture of TFA/DCM/TIS (1.1 ml) within 3 h, O-benzyl group was removed by catalytic hydrogenolysis with Pd/C in MeOH. Fully protected peptides for fragment condensation were synthesized on a multiple peptide synthesizer (SMPS 350, Zinsser Analytic, Frankfurt; Software Syro, MultiSynTech, Bochum, Germany) using the same conditions as described above, and obtained by cleavage from trityl resin (25 mg, 20 μmol) (PepChem, Tübingen, Germany) using 40% hexafluoroisopropanol in dichloromethane [26]. The terminal amino acid (serine) was introduced as *N*-Boc-protected derivative. After the cleavage from the resin, precipitation, washing with cold diethyl ether and lyophilization from *tert*-butyl alcohol–water 4:1 (v/v), the crude compounds **6a–g** were analysed and purified by RP-HPLC using a Nucleosil 300 C_{18} column (5 μm particle size; 250 × 2 mm or 250 × 8 mm; Grom, Herrenberg, Germany). The glycine positional scanning compounds were also prepared on the multiple peptide synthesizer using trityl resin (25 mg, 20 μmol) as reported above.

The identity of all oligomers was verified by electrospray mass spectrometry (ESI-MS) on a API III triple quadrupole mass spectrometer equipped with an IonSpray™ source (Perkin-Elmer-Sciex, Thornhill, Canada).

Stabilization Assay

The MHC stabilization assay is described in detail elsewere [17]. Briefly, 100000 peptide transporter-

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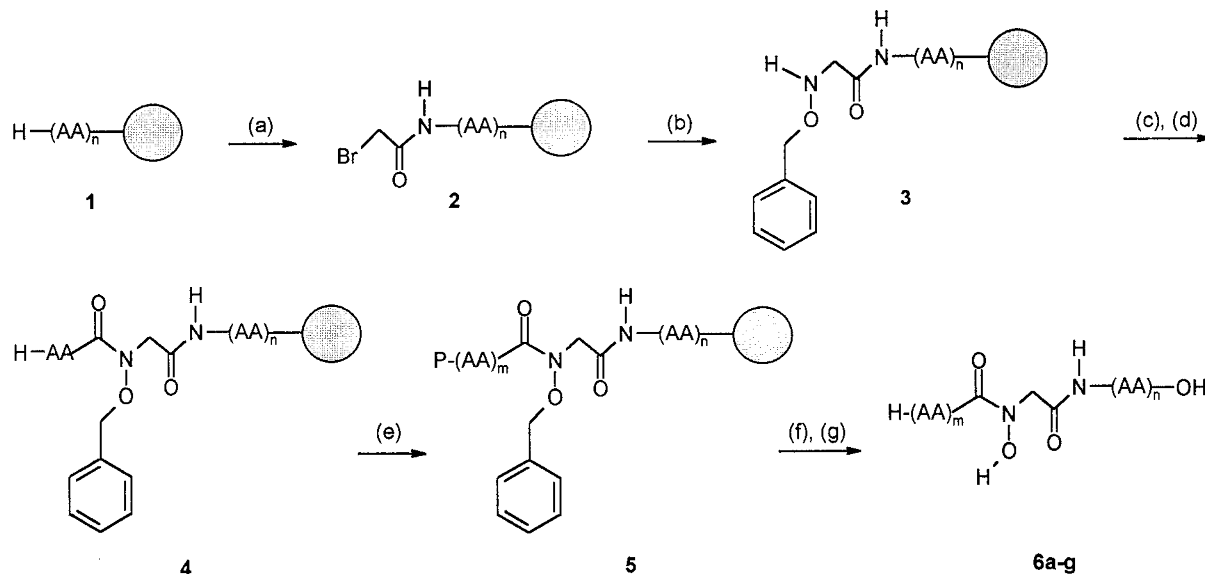

Scheme 1 Solid phase synthesis of the N-hydroxy peptides. (a) Bromoacetic acid/DIC in DMF; (b) BzlO-NH_2 × HCl/DIPEA in DMF; (c) Alloc-AA-OH/HATU/DIPEA in DMF; (d) $Pd[P(Ph_3)]_4$ in $CHCl_3$/AcOH/NMM; (e) Alloc-AA-OH/DIC/HOBt, or fragment condensation (HATU/HOAt/$CuCl_2$/DIPEA in DMF); (f) TFA/DCM/TIS; (g) H_2, Pd/C′ in MeOH. AA, amino acid; AA_n = Leu, Lys(Boc), Glu(*t*Bu), Phe, Asn(Trt), Ile, Ile from the C-terminus; AA_m = Ser(*t*Bu), Ile, Ile, Asn(Trt), Phe, Glu(*t*Bu) from the N-terminus; P, protecting group; Alloc-AA-OH = Ile, Asn(Trt), Phe, Glu(*t*Bu).

deficient RMA-S cells cultured for 16 h at 26°C, were incubated for 30 min at room temperature in a total volume of 100 μl of DMEM plus BSA (0.1% (w/v)) with the peptides and their N-hydroxy analogues in serial 3-fold dilutions. Following 1 h incubation at 37°C to allow denaturation of peptide-free MHC molecules, cells were stained with the monoclonal antibody B8.24.3 which detects conformationally intact H-$2K^b$ and FITC-labelled goat anti mouse IgG and IgM (H + L) (Jackson ImmunoResearch Inc., West Grove, USA). The level of stabilized H-$2K^b$ was analysed by flow cytometry with a FACScan® (Becton Dickinson, Heidelberg, Germany). The ligand concentrations required for half-maximal H-$2K^b$ stabilization were calculated after linearization of the data according to the formalism of the occupancy concept and linear regression extrapolation as described elsewhere [17].

Cytotoxicity Assay

The chromium release assay, described in detail elsewere [27], was used to measure peptide and N-hydroxy peptide dependent stimulation of T cell clone 4G3. Briefly, target cells (RMA-S) were incubated 16 h at 26°C, labeled with ^{51}Cr, washed five times and incubated with various concentrations of the peptides and N-hydroxy-amide analogues for 30 min at 26°C in 96-well U-bottom plates. 4G3 cells were added and incubated for 5 h at 37°C. ^{51}Cr released from the cells was measured with a gamma counter. Percent specific lysis was calculated as specific lysis = (experimental release − spontaneous release)/(total release − spontaneous release).

Enzymatic Degradation

Hydrolysis studies were performed with the substrates and the enzymes dissolved in 20 mM (pH 7.0) and 60 mM (pH 7.2) phosphate buffer for carboxypeptidase Y [28] and aminopeptidase M [29], respectively. Samples of 80 μl were taken at different time points after addition of 5 μl of 1 M HCl, to stop the enzymatic activity, and analysed by RP-HPLC and ESI-MS. Aminopeptidase M was purchased as an $(NH_4)_2SO_4$ suspension with an activity of 20 units/ml. The degradation kinetics of the oligomers followed an exponential decay.

RESULTS AND DISCUSSION

On the basis of the MHC class I H-$2K^b$-restricted SIINFEKL epitope a series of N-hydroxy-amide-containing peptides was designed to study the influence of the NOH modification on the MHC-complex formation and the T cell recognition.

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The introduction of the N-hydroxy-glycine building block was achieved starting from the resin bound C-terminal peptide sequence **1** (Scheme 1). The free amino group was acylated with bromoacetic anhydride, affording **2**. The bromide was substituted using O-benzylhydroxylamine and **3** was subsequently coupled with an N-Alloc-amino acid using O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) as activating agent [30]. The attempt to use N-Fmoc-amino acids was not successful. The Fmoc-cleavage, using either piperidine or 1,8-diazabicyclo[5.4.0]undecen-7-ene (DBU) in DMF, afforded a complex mixture of byproducts, that has not been characterized. The N-Alloc-protecting group was removed using tetrakistriphenylphosphine palladium catalyst [31], yielding the O-benzyl-N-hydroxy-amide resin bound peptide **4**. The N-terminal part of the analogues was built up using either step-by-step N-Alloc-amino acid couplings, or by fragment condensation [32]. The last amino acid (serine) was introduced as Boc-Ser(*t*Bu)-OH. In the case of the replacement of serine by N-hydroxy-glycine (Table 1, **6g**), the bromide substitution was carried out with N-hydroxylamine. The products **5** were cleaved from the resin with 90% TFA in DCM, containing 5% triisopropylsilane as scavenger. The O-benzyl group was removed in solution by catalytic hydrogenolysis with Pd/C in MeOH, yielding the series **6a–g**. The N-hydroxy-amide analogues were purified by RP-HPLC and characterized by electrospray mass spectrometry (Table 1).

The preparation of these peptidomimetics on a solid support is relatively straightforward and gives fairly pure products. The critical step appears to be the nucleophilic substitution by O-benzylhydroxylamine, which requires several days even when a high excess of the reagent is used as reported for N-hydroxy amino acid synthesis [10].

The N-hydroxy peptide analogues were analysed for their stability to enzymatic degradation. Table 2 summarizes the results of the incubation of some N-hydroxy derivatives with two different exopeptidases, aminopeptidase M (APM) and carboxypeptidase Y (CPY), using the glycine-containing analogues and the natural epitope SIINFEKL as controls. Compound **6f** possessing an N-hydroxyamide bond at the N-terminus is completely resistant to APM while the peptides are rapidly digested. SIINFEKL is stable to CPY, most likely because of the presence of the -Lys-Leu- sequence at the C-terminal part of the peptide. This was confirmed by the resistance of SIINFGKL to enzymatic degradation. When substituting Lys with Gly, the C-terminal residue and glycine were readily cleaved off. In the case of compound **6a** the presence of the NOH group increased the stability to enzymatic degradation and the two C-terminal residues were smoothly removed. Leucine and N-hydroxy-glycine are cleaved more than four and three times slower than the parent peptide, respectively.

Several studies with peptides, reduced bond and retroinverso pseudopeptides had shown that the affinity between the MHC and its peptide ligand is important for the stability of the complex [18–21,33,34]. The oligomers **6a–g** with the ψ[CO-N(OH)] peptide modification were tested for their ability to stabilize the murine MHC molecule H-2K^b. Earlier studies on the structural requirement of peptide binding to H-2K^b have shown that the posi-

Table 2 Enzymatic Degradation Data for N-Hydroxy Peptides and Parent Peptides

Substrates	Aminopeptidase M[a] k (min^{-1})	Carboxypeptidase Y[b] k (min^{-1})
H-Ser-Ile-Ile-Asn-Phe-Glu-Lys-Leu-OH	$(4.18 \pm 0.34) \times 10^{-2}$	stable
H-Ser-Gly-Ile-Asn-Phe-Glu-Lys-Leu-OH	$(1.49 \pm 0.11) \times 10^{-2}$	—
H-Ser-ψ(CO-NOH)-Gly-Ile-Asn-Phe-Glu-Lys-Leu-OH (**6f**)	stable	—
H-Ser-Ile-Ile-Asn-Phe-Glu-Gly-Leu-OH	—	$(4.07 \pm 0.51) \times 10^{-2}$ [c]
	—	$(2.37 \pm 0.07) \times 10^{-3}$ [d]
H-Ser-Ile-Ile-Asn-Phe-Glu-ψ(CO-NOH)-Gly-Leu-OH (**6a**)	—	$(9.32 \pm 0.44) \times 10^{-3}$ [c]
	—	$(7.24 \pm 1.36) \times 10^{-4}$ [d]

[a] Substrate (*ca.* 1 mg) was incubated with the enzyme (0.1 units) at 24°C in 60 mM phosphate buffer (1.2 ml) at pH 7.2.
[b] Substrate (*ca.* 1 mg) was incubated with the enzyme (20 μg) at 23°C in 20 mM phosphate buffer (1.2 ml) at pH 7.0.
[c] Constant relative to the cleavage of the Leu.
[d] Constant relative to the cleavage of the Gly and of the N-hydroxy Gly, respectively.

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tions 1, 2, 3, 5, and 8 are anchoring the peptide to the MHC groove whereas the side chains at positions 4, 6 and 7 are exposed and potential T cell receptor contact sites [23,35,36]. The binding of the new N-hydroxy-amide ligands was compared with the natural epitope SIINFEKL and the corresponding glycine-containing analogues (Figure 1). The N-hydroxy peptides display stabilizing activities in the nanomolar range as does the natural epitope SIINFEKL. Binding to the MHC molecule was reduced after insertion of N-hydroxy-glycine at the secondary anchor positions 2 and 3 [23] of the oligomer by factor 4–7 and improved in the cases of positions 4, 5, 6 and 7 by factor 3–6. No change was seen for position 1 when compared with the corresponding glycine-containing peptide. Thus, substitution of surface exposed residues at positions 4, 6 and 7 with N-hydroxy-glycine affected binding to H-$2K^b$ positively whereas the substitution of buried residues in the pocket at the N-terminus decreased the binding efficiency [23,37] when compared with glycine peptides. The different proportion of the analogues **6a–g** in the interaction with the MHC molecules might be due to the particular H-bonding characteristics of the N-hydroxy group.

The influence of N-hydroxylation on T cell stimulation was tested using the SIINFEKL specific clone 4G3. The efficacy of T cell stimulation was strongly reduced by the N-hydroxy-glycine modification at all positions except for position 1. N-hydroxy-glycine in primary or secondary anchor amino acids positions (positions 1, 2, 3, 5 and 8) led to a decrease in half maximal lysis of two to 3.5 orders of magnitude (Figure 2) relative to the glycine-containing modification. The side-chains at positions 4, 6 and 7 are the potential contact residues for the TcR [35,37]. A complete loss of activity was found for the N-hydroxy-glycine variant of SIIGFEKL and for the removal of the side-chain at position 6 (Glu [37]). The N-hydroxy-amide bond at position 7 reduced the potency by two orders of magnitude. Particularly interesting is the loss of activity caused by the backbone N-hydroxylation at position 4. Binding to the MHC I molecule is only marginally affected and the absence of the side-chain rules out a direct impact on the interaction with the TcR. Thus, conformational changes in the side-chains at positions 6 and 7 or both are the most probable reason for the lack of T cell response. As a result of such conformational modifications the ligand **6d** is either not recognized anymore or a negative, i.e. antagonistic signal [38], is induced.

CONCLUSIONS

Compounds stable to enzymatic degradation but with high affinities to MHC molecules and differential potencies for T cell stimulation may become important in the development of new treatment strategies of autoimmune diseases that are associated with specific MHC alleles and for construction of novel synthetic vaccines.

Peptidomimetics bearing N-hydroxy-amide bonds have been inserted into and are proven effective in biological systems such as peptide hormone antagonists or enzyme inhibitors and, thus, appear to have a broad spectrum of potential applications.

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