Nonapeptide Analogues Containing (R)-3-Hydroxybutanoate and β-Homoalanine Oligomers: Synthesis and Binding Affinity to a Class I Major Histocompatibility Complex Protein

Sorana Poenaru,‡,§ J. José R. Lamas,† Gerd Folkers,§ José A. López de Castro,† Dieter Seebach,*† and Didier Rognan*‡

Laboratory for Organic Chemistry, Swiss Federal Institute of Technology, Universitaetstrasse 16, CH-8092 Zürich, Switzerland, Centro de Biología Molecular “Severo Ochoa”, Facultad de Ciencias, Universidad Autonoma de Madrid, E-28049 Madrid, Spain, and Department of Pharmacy, Swiss Federal Institute of Technology, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

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Crystal structures of antigenic peptides bound to class I MHC proteins suggest that chemical modifications of the central part of the bound peptide should not alter binding affinity to the MHC restriction protein but could perturb the T-cell response to the parent epitope. In our effort in designing nonpeptidic high-affinity ligands for class I MHC proteins, oligomers of (R)-3-hydroxybutanoate and/or β-homoalanine have been substituted for the central part of a HLA-B27-restricted T-cell epitope of viral origin. The affinity of six modified peptides to the B*2705 allele was determined by an in vitro stabilization assay. Four out of the six designed analogues presented an affinity similar to that of the parent peptide. Two compounds, sharing the same stereochemistry (R,R,S,S) at the four stereogenic centers of the nonpeptidic spacer, bound to B*2705 with a 5–6-fold decreased affinity. Although the chiral spacers do not strongly interact with the protein active site, there are configurations which are not accepted by the MHC binding groove, probably because of improper orientation of some lateral substituents in the bound state and different conformational behavior in the free state. However we demonstrate that β-amino acids can be incorporated in the sequence of viral T-cell epitopes without impairing MHC binding. The presented structure–activity relationships open the door to the rational design of peptide-based vaccines and of nonnatural T-cell receptor antagonists aimed at blocking peptide-specific T-cell responses in MHC-associated autoimmune diseases.

Introduction

Class I major histocompatibility complex (MHC)-encoded proteins play a key role in the intracellular immune surveillance by selectively binding to intracellular peptide antigens and presenting them at the cell surface to T-cell receptors (TCRs) of cytotoxic T-lymphocytes (CTL). Due to the genetically encoded discrepancy between the limited number of class I alleles (about six) expressed by each individual and the infinite number of potential antigenic peptides (usually nonamers), class I MHC molecules must bind diverse sets of foreign peptides with a broad specificity but a high affinity. Numerous structural data on class I MHC–peptide complexes are nowadays available at the three-dimensional level and provide an explanation for that paradigm. The 27 reported X-ray structures (for nine different class I MHC molecules) illustrate a peptide-independent recognition in which both terminal ends of the peptide backbone are tightly bonded to conserved residues of the MHC binding groove. Allele specificity is ensured by the interaction of anchoring side chains,3,4 usually at positions P2, P3 (Pn standing for position n), and the C-terminus with polymorphic pockets of the host MHC protein. The central part of the bound peptides (from positions 4 to 8) generally zigzags or bulges out of the binding groove and thus allows variation in the length of the bound peptides (from 8 to 11 amino acids). Systematic peptide mutation and X-ray structure of MHC–peptide–TCR ternary complexes show that this central part whose conformation is not complementary to that of the MHC protein is the major contact area for α/β TCRs that trigger the T-cell response to the foreign peptide.

The tight association observed between MHC expression and susceptibility or resistance to autoimmune disorders led us to consider class I MHC proteins as particularly interesting targets for the selective immunotherapy of autoimmune diseases. At least two ways of shunting the T-cell response to autoantigens using small-molecular-weight molecules have been proposed. The first one involving MHC blockade by a high-affinity
B27-binding nonapeptides while enhancing 5-fold the enzymatic stability of esterases and peptidases. Recent reports on the remarkable analogues should have very poor in vivo pharmacokinetic properties inherent to their peptidic nature preclude their general use as immunosuppressors. Thus, there is a need for designing high-affinity nonpeptide ligands for class I MHC proteins. Rather few variations around the canonical nonapeptide structure have been described up to date. Peptides bearing unnatural L- or D-α-amino acids at MHC-anchoring positions, reduced peptide bond pseudopeptides, or aminoalkanoates, phenanthridine derivatives, or amino ester gave acid 5 whose benzyl ester group was cleaved by \( \text{HCl} \) (ether (satd); (f) Boc-Ala-OH, HOBt, EDC, Et3N, CHCl3; (g) H2, Pd/C, MeOH; (h) HCl/MeOH; (i) TFA/CH2Cl2, (j) TFA/CH2Cl2, 1:1.

Results and Discussion

Chemistry. Synthesis of the derivative 11 was achieved using a fragment-type coupling strategy (Scheme 1). Boc-protected β-homalanine and benzyl 3-hydroxybutanoate (2) were coupled using DCC and DMAP as activating reagents to give 3. Deprotection of the amino group under acidic conditions gave the amino ester 4, whereas hydrogenolysis of the benzyl ester gave acid 5. Coupling of 4 with the commercially available Boc-protected alanine under the HOBT/EDC peptide conditions gave the fully protected derivative 6 whose benzyl ester group was cleaved by \( \text{H}_2 \) (Pd/C) to yield acid 7. \(^1\)H NMR measurements led to assignment of all signals to the corresponding protons of the amino acid, of the HB unit, and of the β-HAla moiety.

To obtain the second fragment 9 with free amino and protected carboxy group, the acid 5 was coupled with H-Lys(Z)-OBn, using the HOBT/EDC strategy to give 8 (80%), treatment of which with a saturated HCl/dioxane solution yielded the corresponding HCl salt 9. Compound 10, consisting of six building blocks, was then
obtained in 90% yield by coupling of 7 with 9. Subsequent deavage of the Boc protecting group led to the amino ester 11 which was used for the next coupling with arginine, without further purification (Scheme 4).

The derivative 20, with HB and β-HAla incorporated in different sequence (Scheme 2), was synthesized by application of the same fragment coupling strategy. The fragment 15 was synthesized in a linear fashion, coupling first Boc-β-HAla-OH (11) with H-Lys(Z)-OBn to give dipeptide 12 (85%) which was deprotected to give the HCl salt 13 (HCl in dioxane). The amino functionality, set free in situ by the base present in the reaction mixture, was coupled with 11 to give the fully protected compound 14 (74% from 12). The Boc group of 14 was cleaved to yield 15. The fragment 17 was obtained by ester formation between the hydroxy dimer 16 and Fmoc-protected alanine (using DCC, DMAP), and cleavage of the tert-butyl ester group (50% TFA) gave the desired compound 18. It should be noted that by using a small amount of DMAP (0.05 equiv), no racemization was observed upon coupling. The amino functionality, generated by in situ deprotonation of the HCl salt 15, was coupled with the acid group of 18 (HOBt/EDC) to give the fully protected compound 19 in 92% yield. The Fmoc protecting group was then cleaved (20% diethylamine in DMF) to give the amino ester 20. It is noteworthy that the backbone of compound 19 varies from that of 10 only by the respective positions of HB and β-HAla in the sequence. However their respective solubilities in organic solvents are very different. Compound 10 is highly soluble in chlorinated
Scheme 4 were allowed to react with Boc-Arg(NO$_2$)-
de-protection step, preparative HPLC purification still
some additional impurities were formed. After the last
stage. Thus, we have carried the byproduct all along
it was impossible to purify the intermediates at this
by the Merrifield group, the observed loss of the Z group,
However, considering the much harsher conditions used
solubility in any solvent tested so far. For example, the
$^1$H NMR spectra of protected
nonapeptides analogues. The NO$_2$ and Z protecting
previously prepared, starting from ($S$)- or ($R$)-Boc-Ala-
oligomers ($S$)-HAla tetramers can only
building block linked with the C-terminus (PC) has ($S$)- or ($R$)-Boc-Ala-
configurations. Surface areas were calculated using the MS
29b, 29d (gray
BaSO$_4$ as catalyst. Subsequent treatment with TFA
led to cleavage of the Boc groups to give, after HPLC
purification, the desired nonapeptide analogues 27, 28, and 29a-d which were used for binding assays.

**Binding Affinity to HLA-B*2705.** The binding
affinities of the modified peptides clearly show that the
chirality of the spacer is important for recognition of the B*2705 protein. Compounds in which the chiral building block linked with the C-terminus (PC) has ($R$)-configuration (27, 29a-b) were all potent ligands with

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**Table 1.** Binding and Analytical Properties of Ligands 27, 28, and 29a–d

<table>
<thead>
<tr>
<th>Compd</th>
<th>Sequence</th>
<th>$C_{50}$ ($\mu$mol)</th>
<th>HPLC (t$_{R}$, min)$^b$</th>
<th>MS$^+$ (M + 1)$^c$</th>
</tr>
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<tbody>
<tr>
<td>HIV gp120$^d$</td>
<td>Gly-Arg-Ala-[Phe-Val-Thr-Ile-Gly]-Lys</td>
<td>2.8</td>
<td>19.5</td>
<td>774.1</td>
</tr>
<tr>
<td>A68P1$^e$</td>
<td>Glu-Val-Ala-Pro-Pro-Glu-Tyr-His-Arg</td>
<td>n/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Gly-Arg-Ala-[S($\beta$)-HAla]-Lys$^a$</td>
<td>2.8</td>
<td>16.4</td>
<td>771.9</td>
</tr>
<tr>
<td>28</td>
<td>Gly-Arg-Ala-[(S$^\beta$)-HAla-R-HB$_2$]-Lys$^a$</td>
<td>17.0</td>
<td>19.5</td>
<td>774.1</td>
</tr>
<tr>
<td>29a</td>
<td>Gly-Arg-Ala-[(R$^\beta$)-HAla]-Lys$^a$</td>
<td>2.8</td>
<td>15.4</td>
<td>772.6</td>
</tr>
<tr>
<td>29b</td>
<td>Gly-Arg-Ala-[(S$^\beta$)-HAla-R$^\beta$]-HAla]-Lys$^a$</td>
<td>4.8</td>
<td>15.4</td>
<td>771.9</td>
</tr>
<tr>
<td>29c</td>
<td>Gly-Arg-Ala-[(S$^\beta$)-HAla]-Lys$^a$</td>
<td>6.0</td>
<td>15.3</td>
<td>772.0</td>
</tr>
<tr>
<td>29d</td>
<td>Gly-Arg-Ala-[(R$^\beta$)-HAla]-[(S$^\beta$)-HAla]-Lys$^a$</td>
<td>30.0</td>
<td>15.4</td>
<td></td>
</tr>
</tbody>
</table>

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$a$ Concentration of ligand at which HLA-B*2705 fluorescence (measured by FMC analysis with an anti-B27 monoclonal antibody) on RMA-S cells was half the maximum obtained with that compound (see Experimental Section). $^b$ HPLC purification using a gradient of A (0.1% trifluoroacetic acid in water) and B (acetonitrile): 0–100% B, 60 min. $^c$ MALDI-TOF spectra, recorded on a Bruker Biflex instrument (Bruker-Franzen Analytik, Bremen, Germany) in linear mode. $^d$ HIV-1 glycoprotein 120 (314–322). $^e$ Self-peptide eluted from the HLA-A68 allotypes. $^f$ No detectable binding at 10$^{-4}$ M. $^g$ $\beta$-homoalanine; HB, 3-hydroxybutanoate.
Replacing ester by amide groups in the spacer (cf. 29b) Figure 2. Three-dimensional structure of HLA-B*2705 in complex with the MHC binding groove.34 Analogues bearing a moiety of (S)-configuration next to PC were less active, especially compounds 28 and 29d sharing the same sequence of (R,R,S,S)-configuration of the spacing oligomers (Table 1). However, an (S)-chiral spacer attached to PC does not necessarily prevent binding (see compound 29c, Table 1). Furthermore, it seems that certain configurations of the four spacing monomers are detrimental to binding. Thus, the two weakest binders (28, 29d) share the same (R,R,S,S)-configuration at positions 4 to 7. Replacing ester by amide groups in the spacer (cf. 27 with 29b and 28 with 29d) did not affect binding for both high-affinity and low-affinity ligands. This result is in agreement with the available X-ray structure56 of a B*2705–nonapeptide complex, showing weak contributions of peptide bonds, located between the P4 and P8 positions, to the binding of a nonapeptide to HLA-B27.

Apart from binding potencies, it should be noticed that HB-containing compounds 27 and 28 are probably still sensitive to esterases, although stability studies on these compounds have not been performed yet. We also expect that the replacement of HB oligomers by β-amino acids in analogues 29a–d enhances the resistance of the modified peptides to enzymatic degradation.

Molecular Modeling of B*2705–Ligand Complexes. To find a rational explanation for the weak binding of compounds with (R,R,S,S)-configuration of the chiral spacer, a 500-ps molecular dynamics (MD) study of the binary complexes between B*2705 and three ligands was undertaken. Compound 29b was chosen as representative of the high-affinity peptides, whereas 29d was selected as representative of weak binding ligands. The parent HIV peptide was selected as reference. The trajectory of the three solvated complexes was stable after 350 ps, with rms deviations of the protein backbone from the starting X-ray coordinates of ca. 1.5 Å (data not shown). We previously used atomic fluctuations of the bound ligands, as a criterion, for discriminating high-affinity from low-affinity peptides.24,33,57 In the present case, they were very similar for ligands 29b and 29d. Thus, subtle differences must cause the very different binding affinities of the two modified peptides. In fact, recording the frequency of the MHC–ligand hydrogen bonds allows to distinguish the two modified peptides. High-affinity ligands (HIV gp120, 29b) present many more hydrogen bonds to the HLA-B27 binding groove than the weak binding compound 29d (Figure 1A). Strong H-bonds with a frequency higher than 50% were remarkably identical in both cases, but medium H-bonds (with frequencies between 25% and 50%) are significantly in favor of 29b. A very similar pattern has already been observed for a set of four natural peptides binding to two closely related HLA-B27 alleles.33 The major differences between the two nonnatural complexes could be correlated with the H-bond-donating strength of the N-terminus, well-known to significantly contribute to the binding free energy of nonapeptides to class I MHC proteins.58 The buried surface areas of each monomer of the protein-bound ligand were also very similar with the exception of two residues P5, and PC (Figure 1B). P5 corresponds to the second unit of the spacer (R→HAla in both cases). Depending on its environment in the sequence of the modified peptide, it is more or less deeply buried in the HLA-B27 binding groove. With compound 29b, the P5 position is significantly deeper inside the groove than with compound 29d (compare Figure 2A,B). However, this feature is unlikely to induce nearly a 10-fold difference in the binding affinity of the corresponding ligands. The C-terminal amino acid (Lys) also shows a better fit in the case of the high-affinity ligand (Figure 1B). As the C-terminal residue is an important anchor to B*2705,56 this structural difference should also contribute to the improved binding of 29b versus 29d.

However, the computed properties of the two analogues bound to their target protein can only explain a part of the experimentally determined difference of binding affinities. The modeling study presented here takes into account only enthalpic contributions to the binding of each ligand to HLA-B*2705. As desolvation energies and rotational/translational entropy losses upon binding (assuming a conserved binding mode of the two compounds) should be very similar due to the structural analogy of all modified peptides listed in Table 1, the 10-fold decreased binding of two analogues (28, 29d), having the same configuration, may be due to different association rates and different conformational populations in the free state. This feature has already been experimentally described for two related PEPs,34 for which the length of the polyester spacer varies. Hydrophobic β-peptides are known to have conformations strongly depending on the chirality of their residues and on the nature of their side chains.38,59 The (R,R,S,S)-configuration of four chiral monomers in low-affinity ligands might lead to a conformational space arrangement in the free state that is different from that of high-affinity compounds (27, 28, 29a–c). The higher “strain energy” necessary to bring ligands 28 and 29d from the free to the bound state may partially contribute to the weaker binding of these two analogues. Unfortunately, simulating the free ligands, although computationally easier, is very risky because they adopt no stable secondary structure, as concluded from their CD or NMR spectra.

Conclusion
Replacing the central amino acids of class I MHC-binding peptides by (R)-3-hydroxybutanoate and (or) β-homoalanine oligomers leads to still high-affinity ligands. Up to now, β-amino acids have hardly been used in medicinal chemistry. Some natural β-amino acids (taurine, β-aminobutyric acid, β-aminoisobutyric acid)
have been reported as agonists of the inhibitory glycine receptor.60 Substituted β-amino acids have also been described as fibrinogen receptor Glb/IIα antagonists.61 β-Lactamase inhibitors,62 μ-opioid receptor agonists,63 or enkephalin-degrading enzyme inhibitors.64 Furthermore, various β-amino acids are found in natural antibiotics, fungicides, and antineoplastic compounds.65 However, to the best of our knowledge, this is the very first report of biologically active molecules containing β-amino acid oligomers. The present study demonstrates that β-amino acids are valuable tools, indeed, for designing peptidomimetics of bioactive peptides. By contrast to most α-amino acid surrogates, the H-bonding properties of backbone atoms, the backbone direction, or the side chain directionality might be similar in natural and β-peptides, at the condition that the β-peptide can adopt the biologically active conformation of its natural α-peptide analogue. Thus, if all side chains are not mandatory for biological activity, β-amino acids and, more generally, β-peptides undoubtedly represent new promising tools in medicinal chemistry. In the special case of class I MHC ligands, one might imagine to use β-amino acids for replacing MHC anchors and/or TCR contact residues. Such altered peptides may thus lead to peptide-based vaccines and TCR antagonists, which would be stable to all common peptide degradations tested so far, including pronase, 20S proteasome, and proteinase K.

**Experimental Section**

**Abbreviations:** (R)-β-homoalanine (R-β-HAla), (S)-β-homoalanine (S-β-HAla), dicyclohexylcarbodiimide (DCC), diisopropylethylamine (DIEA), 4-(dimethylamino)pyridine (DMAP), dimethylformamide (DMF), N-[3-(dimethylamino)propyl]-N'-ethylcarbodiimide hydrochloride (EDC), 1-hydroxy-1H-benzotriazole (HOBt), trifluoroacetic acid (TFA), trifluoroethanol (TFE), (R)-3-hydroxybutanoyloxy (R-HB).

**Chemistry.** Dichloromethane (CH2Cl2) was dried over 4Å molecular sieves. Solvents for chromatography and workup procedures were distilled from Sikkon (anhydrous CaSO4, molecular sieves). Solvents for chromatography and workup procedures were distilled from Sikkon (anhydrous CaSO4, molecular sieves). Microanalytical Laboratory of the Laboratorium für Organische Chemie, ETH-Zürich (only analyses above 0.4% were given).

Chromatography generally refers to flash silica gel 60 (Fluka 40–63 mm) and TLC (Merck Kieselgel 60 F254 plates), detection UV and Ninhydrin. HPLC analyses were carried out on a C18 analytical column on a Knauer HPLC system (pump type 64, EuroChrom 2000 integration package, degaser, UV detector (variable-wavelength monitor)) using a linear gradient of (A) 0.1% CF3COOH in H2O and (B) MeCN at a flow rate of 1 mL/min with UV detection at 220 nm. HPLC purification was carried out on a C8 preparative column on a Knauer HPLC system (pump type 64, programmer 50, UV detector (variable-wavelength monitor)) using a gradient of (A) 0.1% CF3COOH in H2O and (B) MeCN at a flow rate of 4 mL/min with UV detection at 214 nm. Retention times (tR) are given in min.

**General Procedure A: Amino Acid Coupling.** The free amine or the appropriate salt (1 equiv) was dissolved in CH2Cl2 or 50% CH2Cl2/DMF (0.1 M) under argon and cooled to 0 ºC. The reaction mixture was treated with a base (Et3N or DIEA, 3 equiv), HOBt (1.25 equiv), the acid (1 equiv), and EDC (1.25 equiv) were then successively added. The reaction mixture was allowed to warm to room temperature and then stirred for 18 h. The mixture was diluted with CH2Cl2 and washed with 1 N HCl, saturated NaHCO3 solution, and brine. The organic layer was dried over anhydrous MgSO4, filtered, and concentrated. The resulting residue was purified on silica gel to afford the pure product.

**General Procedure B: Amino Acid Coupling.** The free amine or the appropriate salt (1 equiv) was dissolved in DMF (0.15 M) under argon and cooled to 0 ºC. The reaction mixture was treated with DIEA (3 equiv), HOBt (1.25 equiv), the acid (1 equiv), and EDC (1.25 equiv) were then successively added. The product was precipitated by the addition of a saturated NaHCO3 solution. The precipitate was washed several times with saturated NaHCO3, 1 M KHSO4 solutions and H2O and dried 24 h under high vacuum to give the crude product which was utilized in the next step without further purification.

**General Procedure C: Boc Cleavage Using a HCl Solution.** Under argon and at 0 ºC, the Boc-protected compound was dissolved in a saturated HCl/EtO or dioxane) solution. The mixture was stirred for 18 min to 1 h and then evaporated. The resulting HCl salt was precipitated in ether, dried under high vacuum, and used for the next step without further purification.

**General Procedure D: Boc Cleavage Using a TFA Solution.** Under argon and at 0 ºC, the Boc-protected compound was dissolved in a TFA/CH2Cl2 (50–100%) solution. The mixture was stirred for 10 min to 1 h and then evaporated. The resulting TFA salt was precipitated in Et2O, dried under high vacuum, and used for the next step without further purification.

**General Procedure E: Final Deprotection.** The fully protected compound was dissolved in TFE/CH3COOH (3/1), and a catalytic amount of 10% Pd/BaSO4 was added. The apparatus was evacuated and flushed three times with H2, and the mixture was stirred under an atmosphere of H2 for ca. 15 h. The mixture was then filtered though Celite, concentrated, and precipitated from Et2O. The resulting yellow-white CH3COOH salt was then treated with concentrated TFA. After 15 min, the crude product was precipitated from Et2O and purified by HPLC.

**Boc-S-β-HAla-R-HB-OBn (3).** To a solution of the (R)-3-hydroxybutanoyloxy benzyl ester (2) (1.90 g, 9.8 mmol) in CH2Cl2 (20 mL) was added a solution of the acid 1 (2.00 g, 9.8 mmol) in CH2Cl2 (30 mL) under argon, cooled to −5 ºC. DCC (2.12 g, 10.3 mmol) and DMAP (0.09 g, 0.49 mmol) were added. The resulting mixture was allowed to warm to room temperature and then stirred for 24 h. The mixture was diluted with Et2O and washed with 1 N HCl, saturated NaHCO3 solution, and brine. The organic layer was dried over anhydrous MgSO4, filtered, and concentrated. The residue was purified on silica gel (20% Et2O/pentane) and gave compound 3 (3.25 g, 88%) as a colorless oil. 1H NMR (300 MHz, CDCl3): δ 7.37–7.33 (m, 5H ar), 5.36–5.26 (m, 1H, CH), 5.14 (AB, J = 12.1, 1H, OCH2Ph), 5.09 (AB, J = 12.1, 1H, OCH2Ph), 5.09–4.99 (m, 1H, NH), 4.10–3.96 (m, 1H, CHN), 2.66 (dd, ABX, J = 7.5, 15.6, 1H, CHO), 2.54 (dd, ABX, J = 5.3, 15.6, 1H, CH2), 2.43 (dd, ABX, J = 5.3, 14.9, 1H, CH2CHN), 1.27 (dd, ABX, J = 5.9, 14.9, 1H, CH2CHN), 1.43 (s, 9H, tBu), 1.29 (d, J = 6.2, 3H, Me of HB), 1.17 (d, J = 6.5, 3H, Me of β-HAla), 1C NMR (75 MHz, CDCl3): δ 171.00, 170.42, 155.36, 135.93, 132.94, 128.60, 67.58, 66.65, 43.60, 40.77, 28.46, 20.31, 19.96. FAB-MS: m/z 279 (26, 3M + 1)+, 308 (64, M + 1)+, 280 (100).

**HCl-S-β-HAla-R-HB-OBn (4).** According to general procedure C, compound 3 (227 mg, 0.66 mmol) was treated with a saturated HCl/EtO solution (6 mL). The resulting HCl salt 4 was obtained as a white precipitate and used in the next coupling step without further purification.

**Boc-S-β-HAla-R-HB-OH (5).** The benzyl-protected compound 3 (400 mg, 2.9 mmol) was dissolved in MeOH (20 mL); catalytic amounts of 10% Pd/C (90 mg) and acetic acid (0.1 mg)

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**References:**

60. Substituted β-amino acids have also been described as fibrinogen receptor Glb/IIα antagonists.

61. β-Lactamase inhibitors.

62. μ-opioid receptor agonists.

63. Or enkephalin-degrading enzyme inhibitors.

64. Furthermore, various β-amino acids are found in natural antibiotics, fungicides, and antineoplastic compounds.

65. However, to the best of our knowledge, this is the very first report of biologically active molecules containing β-amino acid oligomers. The present study demonstrates that β-amino acids are valuable tools, indeed, for designing peptidomimetics of bioactive peptides.
ml) were added. The apparatus was evacuated and flushed three times with H2, and the mixture was stirred under an atmosphere of H2 for ca. 8 h. Subsequent filtration through Celite and concentration under reduced pressure yielded the acid 5 (558 mg, 84%) as a colorless oil which was identified by NMR and used for the next coupling step without purification.

**Boc-Ala-S-Hala-R-HB-OBn (6).** According to general procedure A, to a solution in CH2Cl2 (26 mL) of HCl salt 4 (1 equiv, 2.61 mmol) was added Et3N (1.45 mL, 10.4 mmol). HOBt (440 mg, 3.3 mmol), Boc-Ala-OH (850 mg, 1.8 mmol), and then EDC (623 mg, 3.3 mmol) were successively added to the reaction. The residue was purified by recrystallization (Et2O/pentane, 2/5) to give compound 6 (994 mg, 85%) as a white solid. 1H NMR (300 MHz, CDCl3): δ 7.35–7.32 (m, 5H ar), 6.70 (br d, J = 6.8, 1H, NH), 5.33–5.27 (m, 1H, CHO), 5.12 2H, OCH2Ph), 5.12–5.06 (m, 1H, NH), 4.36–4.22 (m, 1H, CHN), 4.18–4.06 (m, 1H, CHN), 2.74–2.66 (m, 1H, CHO), 2.42 (d, J = 5.3, 2H, CH2CHN), 1.44 (s, 9H, tBu), 1.34–1.29 (m, 6H, 2 Me), 1.17 (d, J = 6.8, 3H, Me).

13C NMR (75 MHz, CDCl3): δ 170.87, 135.80, 128.86, 128.67, 128.87, 67.87, 66.78, 42.02, 40.69, 40.33, 28.38, 19.98, 19.66, 18.64. FAB-MS: m/z 906 (6, (M + Na)+), 884 (32, (M + 1)+), 784 (100). Anal. (C39H39NO13S2) C, H, N.

**TFA-H-Ala-(S-Hala-R-HB)-Lys(Z)-OBn (11).** According to general procedure D, compound 10 (654 mg, 0.74 mmol) was treated with a CH2Cl2/TFA (1:1) solution (6 mL). After 30 min, the reaction was completed and the solvent was evaporated. The TFA resulting salt 11 was obtained in almost quantitative yield as a white precipitate (from Et2O) and used in the next coupling step without further purification.

**Boc-S-Hala-Lys(Z)-OBn (12).** According to general procedure B, a solution in DMF (50 mL) of the HCl salt of Lys-(Z)-OBn (2.00 g, 4.9 mmol) was added Et3N (2.04 mL, 14.7 mmol). HOBt (0.83 g, 6.1 mmol), the acid Boc-Hala-OH (1) (1.00 g, 4.9 mmol), and then EDC (1.17 g, 6.1 mmol) were successively added to the reaction. The residue was purified by recrystallization (ethyl acetate/hexane, 20:1) to give compound 12 (2.30 g, 85%) as a white solid. 1H NMR (300 MHz, CDCl3): δ 7.37–7.26 (m, 10H ar), 6.48–6.36 (m, 1H, NH), 5.22–5.10 (m, 3H, OCH2Ph, NH), 5.09 (s, 2H, OCH2Ph), 4.95–4.87 (m, 1H, CHN), 4.62–4.56 (m, 1H, CHN), 4.00–3.90 (m, 1H, CHN), 3.17–3.10 (m, 2H, CH2CHN2), 2.46–2.32 (m, 2H, CH2CHN, 1.90–1.60 (m, 2H, CH2), 1.50–1.25 (m, 4H, CH2), 1.42 (s, 9H, tBu), 1.16 (d, J = 6.8, 3H, Me). 13C NMR (75 MHz, CDCl3): δ 172.44, 155.78, 128.88, 128.78, 128.62, 128.37, 67.29, 66.74, 52.41, 48.18, 42.52, 40.41, 31.61, 29.30, 28.46, 22.15, 20.60. FAB-MS: m/z 556 (28, (M + 1)+), 456 (100).

**HCl-H-S-Hala-Lys(Z)-OBn (13).** According to general procedure C, compound 12 (1.00 g, 1.8 mmol) was treated with a saturated HCl/dioxane solution (20 mL). After 30 min, the reaction was completed and the solvent was evaporated. The resulting HCl salt 13 was obtained in almost quantitative yield as a white precipitate (from Et2O) and used in the next coupling step without further purification.

**Boc-(S-Hala)-Lys(Z)-OBn (14).** According to general procedure A, to a solution in DMF (5 mL) of the HCl salt 13 (1 equiv, 1.8 mmol) was added DIEA (0.92 mL, 5.44 mmol), HOBT (304 mg, 2.2 mmol), the acid Boc-Hala-OH (1) (365 mg, 1.8 mmol), and then EDC (430 mg, 2.2 mmol) were successively added to the reaction. The residue was purified by recrystallization (CH2Cl2/hexane, 20:1) to give compound 14 (850 mg, 74%) as a white solid. 1H NMR (300 MHz, CDCl3): δ 7.37–7.26 (m, 10H ar), 7.12–7.01 (m, 10H ar), 6.48–6.36 (m, 1H, CHN), 5.25–5.09 (m, 4H, 2 CH2), 4.95–4.87 (m, 1H, CHN), 4.62–4.56 (m, 1H, CHN), 4.00–3.90 (m, 1H, CHN), 3.17–3.10 (m, 2H, CH2CHN2), 2.46–2.32 (m, 2H, CH2CHN, 1.90–1.60 (m, 2H, CH2), 1.50–1.25 (m, 4H, CH2), 1.42 (s, 9H, tBu), 1.16 (d, J = 6.8, 3H, Me). 13C NMR (75 MHz, CDCl3): δ 172.44, 155.78, 128.88, 128.78, 128.62, 128.37, 67.29, 66.74, 52.41, 48.18, 42.52, 40.41, 31.61, 29.30, 28.46, 22.15, 20.60. FAB-MS: m/z 556 (28, (M + 1)+), 456 (100).

**HCl-(S-Hala)-Lys(Z)-OBn (15).** According to general procedure C, compound 15 (712 mg, 1.1 mmol) was treated with a saturated HCl/dioxane solution (10 mL). After 30 min, the reaction was completed and the solvent was evaporated. The HCl resulting salt 15 was obtained in almost quantitative yield as a white precipitate (from Et2O) and used in the next coupling step without further purification.

**Fmoc-Ala-(R-HB)-OtBu (17).** To a solution of the hydroxy derivative 16 (1 equiv, 4.4 mmol) in CH2Cl2 (40 mL) was added Fmoc-Ala-OH (1.45 g, 4.4 mmol) under argon, and the mixture was cooled to 0 °C. DMAP (95 mg, 0.74 mmol), and DMAP (0.04 g, 0.22 mmol) were added, and the resulting mixture was allowed to warm to room temperature and then stirred for 24 h. The mixture was diluted with Et2O and washed with 1 N HCl, saturated NaHCO3 solution, and brine. The organic layer was dried over anhydrous MgSO4, filtered, and concentrated. The residue was purified on silica gel (Et2O/
pentane, 2(3) to give compound 17 (700 mg, 30%) as a white foam. 1H NMR (300 MHz, CDCl3): δ 7.77–7.75 (m, 2H ar), 7.61–7.59 (m, 2H ar), 7.42–7.37 (m, 2H ar), 7.34–7.26 (m, 2H ar), 6.49 (d, J = 7.2, NH), 5.38–5.22 (m, 2H, CHO), 4.44–4.32 (m, 3H, CH2, CH2 of Fmoc), 4.25–4.20 (m, 1H, CH of Fmoc), 2.69–2.37 (m, 4H, CH2, CHNO), 1.43 (s, 9H, tBu), 1.30 (d, J = 6.2, 3H, Me), 1.29 (d, J = 6.2, 3H, Me), 1.26 (d, J = 6.5, 3H, Me), 1.12 (t, J = 6.6, 3H, tBu). The presence of the desired compound was obtained in almost quantitative yield as a yellow oil and used in the next coupling step without further purification.

Fmoc-Ala-(R-HB)-OH (18). According to general procedure C, compound 17 (600 mg, 1.1 mmol) was treated with a TFA/CH2Cl2 (1:1) solution (6 mL). After 30 min, the reaction was completed and the solvent was evaporated. The acid 18 was obtained in almost quantitative yield as a yellow oil and used in the next coupling step without further purification.

Fmoc-Ala-(R-HB)-S-(S-/β-HAla)-Lys(Z)-OBn (19). According to general procedure C, to a solution in CH2Cl2/DMF (1:1, 12 mL) of the HCl salt 15 (1 equiv, 1.10 mmol) was added DIEA (0.75 mL, 4.40 mmol). HOBt (185 mg, 1.37 mmol), the acid 18 (1 equiv, 1.10 mmol), and then EDC (262 mg, 1.37 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-(S-/β-HAla)-Lys(Z)-OBn (11 mg, 90% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (0.55 mL), according to the general procedure D, gave the TFA salt 22b, which was used without further purification.

Fmoc-Ala-(R-HB)-S-(S-/β-HAla)-Lys(Z)-OBn (20). The Fmoc-protected compound 19 (520 mg, 0.52 mmol) was dissolved in DMF/NET3 (9:1, 4 mL) under argon and cooled to 0 °C. The mixture was stirred for 1–2 h, and concentration under reduced pressure yielded the crude amine 20 which was identified by NMR and used without further purification.

Fmoc-Ala-(R-HB)-S-(S-/β-HAla)-Lys(Z)-OBn (22a). According to general procedure B, to a solution in DMF (11 mL) of HCl•H2O-Lys(Z)-OBn (442 mg, 1.09 mmol) was added DIEA (0.56 mL, 3.27 mmol), HOBt (194 mg, 1.36 mmol), the acid 18 (1 equiv, 1.10 mmol), and then EDC (260 mg, 1.36 mmol) were successively added to the reaction. The residue was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-(S-/β-HAla)-Lys(Z)-OBn (723 mg, 82% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (3.6 mL), according to the general procedure D, gave the TFA salt 22a, which was used without further purification.

Fmoc-Ala-(R-HB)-S-(S-/β-HAla)-Lys(Z)-OBn (22b). According to general procedure B, to a solution in DMF (2 mL) of HCl•H2O-Lys(Z)-OBn (62 mg, 0.15 mmol) was added DIEA (0.08 mL, 0.45 mmol). HOBt (26 mg, 0.19 mmol), the acid Boc-(S-/β-HAla)-Lys(Z)-OBn (22a) (1 equiv, 0.15 mmol), and then EDC (36 mg, 0.19 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-(S-/β-HAla)-Lys(Z)-OBn (11 mg, 90% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (0.55 mL), according to the general procedure D, gave the TFA salt 22b, which was used without further purification.

Fmoc-Ala-(R-HB)-S-(S-/β-HAla)-Lys(Z)-OBn (22c). According to general procedure B, to a solution in DMF (15 mL) of HCl•H2O-Lys(Z)-OBn (515 mg, 1.27 mmol) was added DIEA (0.65 mL, 3.81 mmol), HOBt (214 mg, 1.59 mmol), the acid Boc-(S-/β-HAla)-Lys(Z)-OBn (22a) (1 equiv, 1.27 mmol), and then EDC (304 mg, 1.59 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-(S-/β-HAla)-Lys(Z)-OBn (819.1 mg, 79% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (4 mL), according to the general procedure D, gave the TFA salt 22c, which was used without further purification.

Fmoc-Ala-(R-HB)-S-(S-/β-HAla)-Lys(Z)-OBn (22d). According to general procedure B, to a solution in DMF (11 mL) of HCl•H2O-Lys(Z)-OBn (442 mg, 1.09 mmol) was added DIEA (0.56 mL, 3.27 mmol). HOBt (184 mg, 1.36 mmol), the acid Boc-(R-/β-HAla)-S-(S-/β-HAla)-Lys(Z)-OBn (21d) (1 equiv, 1.09 mmol), and then EDC (260 mg, 1.36 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-(R-/β-HAla)-S-(S-/β-HAla)-Lys(Z)-OBn (796 mg, 87% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (3.9 mL), according to the general procedure D, gave the TFA salt 22d, which was used without further purification.
According to general procedure B, to a solution in DMF (10 mL) of the TFA salt 22d (770 mg, 0.93 mmol) was added DIEA (0.64 mL, 3.72 mmol), HOBt (157 mg, 1.16 mmol), Boc-Ala-OH (211 mg, 1.12 mmol), and then EDC (215 mg, 1.16 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-Ala-(R/β-HAla)-(S/β-HAla)-Lys(Z)-OBn (490 mg, 60% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (2.2 mL), according to the general procedure D, gave the TFA salt 23d, which was used without further purification.

TFA-H-Arg(NO2)2-Ala-(S/β-HAla-R-HB)2-Lys(Z)-OBn (24). According to general procedure A, to a solution in CH2Cl2/DMF (4:3, 8 mL) of the TFA salt 11 (1 equiv, 0.74 mmol) was added DIEA (0.38 mL, 2.22 mmol), HOBt (125 mg, 0.92 mmol), Boc-Arg(NO2)-OH (259 mg, 0.81 mmol), and then EDC (176 mg, 0.92 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification. FAB-MS: 80.46, 68.68, 68.40, 67.22, 65.96, 52.48, 49.54, 42.48, 42.16, 40.46, 31.36, 29.41, 28.39, 24.85, 22.41, 20.02, 19.88, 19.74, 19.66, 10.19. Further treatment with TFA (5 mL of the TFA salt 770 mg, 88% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (2.2 mL), according to the general procedure D, gave the TFA salt 24, which was used without further purification.

TFA-H-Arg(NO2)2-Ala-(R-HB)2-(S/β-HAla)-Lys(Z)-OBn (25). According to general procedure B, to a solution in DMF (6 mL) of the TFA salt 20 (1 equiv, 0.52 mmol) was added DIAE (0.26 mL, 1.55 mmol). HOBt (87 mg, 0.65 mmol), Boc-Arg(NO2)-OH (198 mg, 0.62 mmol), and then EDC (123 mg, 0.65 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-Arg(NO2)2-Ala-(R-HB)2-(S/β-HAla)-Lys(Z)-OBn (409 mg, 77% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (1.4 mL), according to the general procedure D, gave the TFA salt 25, which was used without further purification.

TFA-H-Arg(NO2)2-Ala-(R/β-HAla)-Lys(Z)-OBn (26a). According to general procedure B, to a solution in DMF (9 mL) of the TFA salt 23a (1 equiv, 0.77 mmol) was added DIAE (0.53 mL, 3.08 mmol), HOBt (130 mg, 0.96 mmol), Boc-Arg(NO2)-OH (295 mg, 0.92 mmol) and then EDC (183 mg, 0.96 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-Arg(NO2)2-Ala-(R/β-HAla)-Lys(Z)-OBn (757 mg, 81% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (2.7 mL), according to the general procedure D, gave the TFA salt 26a, which was used without further purification.

TFA-H-Arg(NO2)2-Ala-(S/β-HAla-R-HB)-Lys(Z)-OBn (26b). According to general procedure B, to a solution in DMF (9 mL) of the TFA salt 23b (1 equiv, 0.83 mmol) was added DIAE (0.057 mL, 0.33 mmol), HOBt (14 mg, 0.104 mmol), Boc-Arg(NO2)-OH (32 mg, 0.099 mmol), and then EDC (20 mg, 0.104 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-Arg(NO2)2-Ala-(S/β-HAla-R-HB)-Lys(Z)-OBn (74 mg was confirmed by FAB-MS. Further treatment with TFA (0.5 mL), according to the general procedure D, gave the TFA salt 26b, which was used without further purification.

TFA-H-Arg(NO2)2-Ala-(S/β-HAla)-Lys(Z)-OBn (26c). According to general procedure B, to a solution in DMF (9 mL) of the TFA salt 23c (1 equiv, 0.86 mmol) was added DIAE (0.59 mL, 3.54 mmol), HOBt (145 mg, 1.17 mmol), Boc-Arg(NO2)-OH (329 mg, 1.03 mmol), and then EDC (205 mg, 1.07 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-Arg(NO2)2-Ala-(S/β-HAla)-Lys(Z)-OBn (860 mg, 82% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (3.1 mL), according to the general procedure D, gave the TFA salt 26c, which was used without further purification.

TFA-H-Arg(NO2)2-Ala-(R/β-HAla)-Lys(Z)-OBn (26d). According to general procedure B, to a solution in DMF (9 mL) of the TFA salt 23d (1 equiv, 0.45 mmol) was added DIAE (0.31 mL, 1.81 mmol), HOBt (76 mg, 0.56 mmol), Boc-Arg(NO2)-OH (172 mg, 0.54 mmol), and then EDC (107 mg, 0.56 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-Arg(NO2)2-Ala-(R/β-HAla)-Lys(Z)-OBn (460 mg, 84% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (2.2 mL), according to the general procedure D, gave the TFA salt 26d, which was used without further purification.

H-Gly-Arg-Ala-(S/β-HAla-R-HB)-Lys(OH) (27). According to general procedure B, to a solution in DMF (6 mL) of the TFA salt 24 (1 equiv, 0.50 mmol) was added DIAE (0.26 mL, 1.51 mmol). HOBt (85 mg, 0.63 mmol), Boc-Gly-OBH (97 mg, 0.55 mmol), and then EDC (124 mg, 0.63 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any solvent to be purified. The presence of the desired Gly-Arg(NO2)2-Ala-(S/β-HAla-R-HB)-Lys(OH) (447 mg, 68% crude) as a fine yellow-white powder was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (2.7 mL), according to the general procedure E, gave the TFA salt 27, which was used without further purification.

H-Gly-Arg-Ala-(R-HB)-Lys(Z)-OBn (28). According to general procedure D, to a solution in DMF (5 mL) of the TFA salt 25 (1 equiv, 0.37 mmol) was added DIAE (0.19 mL, 1.11 mmol). HOBt (62 mg, 0.46 mmol), Boc-Gly-OBH (77 mg, 0.44 mmol), and then EDC (88 mg, 0.46 mmol) were successively added to the reaction. The resulting precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Gly-Arg-(NO2)2-Ala-(R-HB)-Lys(Z)-OBn (252 mg, 60% crude) was confirmed by 1H and 13C NMR and MS spectra. Further treatment with TFA (3.1 mL), according to the general procedure E, gave the TFA salt 28, which was used without further purification.
precipitate was purified by HPLC (10–40% B, 30 min), tR 6.2 min, to give lyophilization the pure compound 28 in about 25% yield. 1H NMR (300 MHz, D2O): δ 5.22–5.06 (m, 2H, CH2O), 4.28–4.20 (m, 3H, CHN), 4.16–4.04 (m, 2H, CHN), 3.76 (s, 2H, CH2N), 3.16–3.10 (m, 2H, CH2NH2), 2.93–2.86 (m, 2H, CH2NH2), 2.65–2.49 (m, 2H, CH2CHO), 2.43–2.32 (m, 2H, CH2CHO), 2.36 (d, J = 7.2, 2H, CH2CH2N), 2.26 (d, J = 7.2, 2H, CH2CH2N). Purity by analytical HPLC (0–100% B, 60 min, tR 19.5 min) > 99%.

H-Gly-Arg-Ala-(R)-β-Hala-Lys-CH2COOH (29a). According to general procedure B, to a solution in DMF (7 mL) of the TFA salt 26a (1 equiv, 0.68 mmol) was added DIEA (0.47 mL, 2.71 mmol). HOBt (115 mg, 0.85 mmol), Boc-Gly-OH (143 mg, 0.82 mmol), and then EDC (163 mg, 0.85 mmol) were successively added to the reaction. The precipitate was dried under high vacuum and used without further purification as it is not soluble in any flash chromatography or HPLC solvent. The presence of the desired Boc-Gly-Arg-N(O2)-Ala-(R)-β-Hala-Lys-Z-CH2COOH (29a) was confirmed by 1H and 13C NMR and MS spectra.

According to general procedure E, Boc-Gly-Arg-N(O2)-Ala-(R)-β-Hala-Lys-Z-CH2COOH (29a) was dissolved in TFE/CH3COOH (3:1, 3.5 mL) and hydrogenated in the presence of Pd/BaSO4 (10%, 60 mg). The resulting precipitate was purified by HPLC (10–40% B, 30 min), tR 6.8 min, to give lyophilization the pure compound 29b in about 30% yield. 1H NMR (300 MHz, D2O): δ 4.34–4.27 (m, 2H, CHN), 4.24–4.09 (m, 2H, CHN), 3.84–3.81 (m, 2H, CHN), 3.21–3.15 (m, 2H, CH2NH2), 2.99–2.93 (m, 2H, CH2NH2), 2.45–2.41 (m, 2H, CH2CH2N), 2.40–2.26 (m, 6H, CH2CH2N), 1.92–1.57 (m, 8H, CH2), 1.47–1.35 (m, 2H, CH2), 1.31 (d, J = 7.2, 3H, Me), 1.15–1.09 (12H, Me). 13C NMR (75 MHz, D2O): δ 178.59, 176.58, 176.50, 176.08, 175.30, 170.09, 159.82, 56.22, 55.30, 52.71, 46.25, 45.22, 44.78, 43.37, 43.13, 42.00, 32.78, 31.13, 28.99, 27.02, 24.85, 21.16, 21.97, 19.50. FAB-MS: m/z 1542 (17, [2M + 2]+), 771 (100, [M + 1]+). Purity by analytical HPLC (0–100% B, 60 min, tR 15.3 min) > 99%.

H-Gly-Arg-Ala-(S)-β-Hala-Lys-CH2COOH (29d). According to general procedure E, Boc-Gly-Arg-N(O2)-Ala-(S)-β-Hala-Lys-Z-CH2COOH (340 mg, 0.30 mmol) was dissolved in TFE/CH3COOH (3:1, 4 mL) and hydrogenated in the presence of Pd/BaSO4 (10%, 60 mg). The resulting precipitate was purified by HPLC (10–40% B, 30 min), tR 6.8 min, to give lyophilization the pure compound 29d in about 30% yield. 1H NMR (300 MHz, D2O): δ 4.34–4.27 (m, 2H, CHN), 4.24–4.09 (m, 2H, CHN), 3.84–3.81 (m, 2H, CHN), 3.21–3.15 (m, 2H, CH2NH2), 2.99–2.93 (m, 2H, CH2NH2), 2.45–2.41 (m, 2H, CH2CH2N), 2.40–2.26 (m, 6H, CH2CH2N), 1.92–1.57 (m, 8H, CH2), 1.47–1.35 (m, 2H, CH2), 1.31 (d, J = 7.2, 3H, Me), 1.15–1.09 (12H, Me). 13C NMR (75 MHz, D2O): δ 178.59, 176.58, 176.50, 176.08, 175.30, 170.09, 159.82, 56.22, 55.30, 52.71, 46.25, 45.22, 44.78, 43.37, 43.13, 42.00, 32.78, 31.13, 28.99, 27.02, 24.85, 21.16, 21.97, 19.50. FAB-MS: m/z 1542 (17, [2M + 2]+), 771 (100, [M + 1]+). Purity by analytical HPLC (0–100% B, 60 min, tR 15.3 min) > 99%.
From the last SA conformer, 13 counterions (9 Na+ and 4 Cl- ions) were then placed at electrostatic minima to neutralize the protein, using the CION routine of AMBER.66 It was then solvated in a 10 Å thick TIP3P water shell. After the solvent was minimized by 1000 steps of steepest descent, the solvent (water and counterions) was equilibrated by 25-ps MD at 300 K. The solvent was minimized again, and the fully solvated complex was finally relaxed by 1000 steps of steepest descent. The obtained coordinates were then used as a starting point for a 300-ps MD simulation at 300 K. To avoid large drifts from the protein crystal structure, a weak positional harmonic constraint of 0.05 kcal-mol\(^{-1}\)-Å\(^{-1}\) was applied to backbone atoms of B*2705. As the solvent was implicitly taken into account, a constant dielectric function (\(\epsilon = 1\)) was utilized. For the whole trajectory, the same twin cutoff (10−15 Å) was used for calculating nonbonded interactions, and the nonbonded pair list was updated every 10 steps. The SHAKE algorithm was used on hydrogens with a tolerance of 0.00025 Å, a time step of 2 fs, and Berendsen temperature coupling with separate coupling of solute and solvent atoms to the heat bath. Coordinates, velocities, and energies were saved every 0.5 ps. All computations were done using the parallel version of AMBER5.0 implemented on a CRAY J90 cluster and an INTEL paragon machine. The analyses of molecular dynamics trajectories were achieved using in-house routines and the CARNAL module of AMBER.86

**Epitope Stabilization Assay.** The quantitative assay used was previously described.71 Briefly, RMA-S transfectants expressing B*2705 were used. These are murine cells with the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. J. Mol. Biol. 1991, 219, 277−319.

**References**


(34) Holmes, P. A. In Podlech, J.; Seebach, D. On the preparation of Compound 4, 1191, 11


(52) Compound 10 could also be obtained using an alternative synthetic route: coupling of 4 to 5 giving the tetramer Boc-(Boc-HI-A-OBn). Cleavage of the benzyl ester group in order to couple the lysine and subsequent Boc deprotection allow coupling of the N-terminal alanine to give the desired compound.
Nonapeptide Analogue Binding Affinity to MHC Protein


J M 981123L