Design, synthesis and evaluation of β-lactam antigenic peptide hybrids; unusual opening of the β-lactam ring in acidic media†

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β-Lactam peptides were envisioned as conformational constraints in antigenic peptides (APs). Three different β-lactam tripeptides of varying flexibility were prepared in solution and incorporated in place of the central part of the altered melanoma associated antigenic peptide Leu_{27}-Melan-A_{26-35} using solid phase synthesis techniques. Upon TFA cleavage from the solid support, an unexpected opening of the β-lactam ring occurred with conservation of the amide bond. After adaptation of the solid phase synthesis strategy, β-lactam peptides were successfully obtained and both opened and closed forms were evaluated for their capacity to bind to the antigen-presenting class-I MHC HLA-A2 protein system. None of the closed β-lactam peptides bound to HLA-A2, but their opened variants were shown to be moderate to good HLA-A2 ligands, one of them being even capable of stimulating a Melan-A-specific T cell line.

Introduction

The incorporation of conformationally constrained β-turn mimetics in therapeutically relevant peptide structures has long been considered as a valuable approach in the design of peptidomimetics with enhanced affinity and/or improved metabolic stability compared to those of their peptide parents.[1] In particular, since their first utilization by Freidinger in 1980, dipeptide lactams are among the most popular β-turn mimetics.[2,3] In this context, our current efforts towards the design of antigenic peptidomimetics led us to envision the use of the β-lactam scaffolds **I–III**[4,5] (Fig. 1) as surrogates of the central part of a tumor-associated antigenic peptide (AP) and to assess the consequences of such structural modifications on the immunogenicity of the resulting peptidomimetics.

Tumor-associated APs, usually 8 to 10 amino acids long,[6] are generated in cancer cells by proteolysis of self over-expressed proteins in the proteasome and are then exposed to the cell surface by class-I major histocompatibility (MHC-I) complexes to be screened by circulating $CD8^+$ cytotoxic T lymphocytes.[7] Recognition of these peptide-MHC-I (pMHC-I) complexes by T cell receptors (TCRs) is the causal event in the triggering of T cell-mediated immune responses that result in the destruction of antigen-presenting cells. Given the pivotal role played by APs in launching this cellular defense mechanism, it is not surprising that these small peptides have been considered by immunologists as promising leads in the development of immunotherapeutic drugs, as well as synthetic vaccines. Unfortunately, the poor biostability and globally unsatisfactory pharmacokinetic properties inherent to their peptidic nature have precluded the general use of APs as therapeutic agents. Thus, designing bioresistant antigenic peptidomimetics capable of engaging both MHC-I and TCR proteins in the formation of complexes inducing enhanced immune responses remains at the forefront of modern immunology.

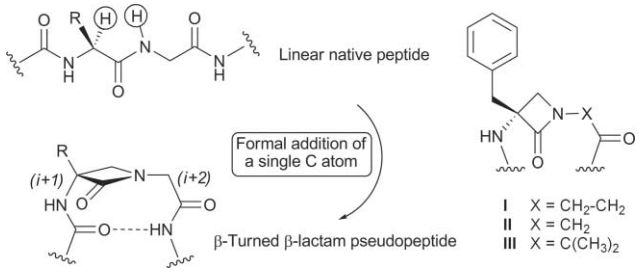

Fig. 1 β-lactam scaffolds envisioned as conformationally constrained surrogates of antigenic peptide central segments.

The structural data that have been gathered by biophysicists in this field over the last decade have provided us with a precious understanding of the mode of binding of APs to MHC-I proteins and of their molecular recognition by TCRs.[8] APs that efficiently bind onto and in between the α-helical units of the MHC-I protein cleft usually present anchoring amino acid residues at position 2 (P2) and at their C-terminus, whereas their central portion (P4–P7) bulges out of the peptide binding cleft, sometimes even zigzaging to interact with the TCR components (Fig. 2). Most previous attempts in designing heteroclitic APs were essentially aimed at developing MHC-I high affinity ligands so as to improve immunogenicity.[9] Based on the X-ray structure of the MHC-I human leucocyte antigen (HLA)-A2 protein complex bearing

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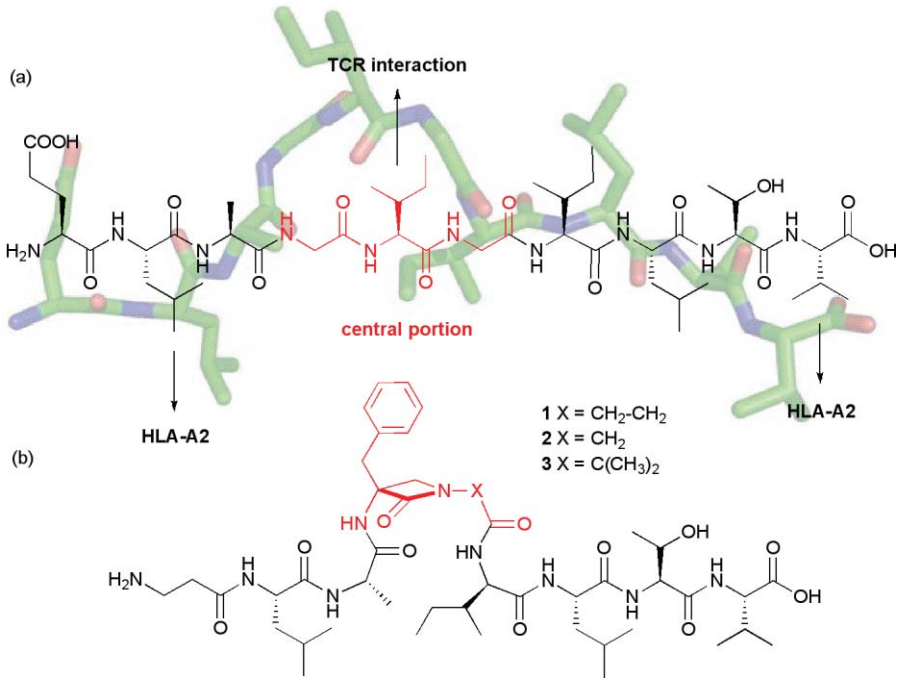


Fig. 2 (a) ELA peptide sequence showing HLA-A2 anchor and TCR-interacting residues, superimposed with its HLA-A2-bound X-ray structure.[10,11] (b) Sequence of the envisioned β-lactam-containing peptidomimetics (**1–3**).

the altered melanoma-associated peptide epitope Leu_{27}-Melan-$A_{26–35}$ (ELAGIGILTV, hereafter referred to as ELA),[10] we recently reported the successful replacement of the central segment GIGI (*i.e.*, P4–P7) of this AP by a nonpeptidic functionalizable scaffold without compromising HLA-A2 binding.[11] Some of the ELA peptidomimetics thus designed even managed to efficiently stimulate Melan-A-specific T cell clones.[11] On the basis of these results, we surmise that the replacement of the ELA central residues by a β-lactam motif such as **I**, **II** or **III** (Fig. 1) thus incorporated in ELA pseudopeptides **1–3** (Fig. 2) could advantageously serve the purpose of mimicking their hydrophobic bulge.

Herein, we disclose the design and solid-phase synthesis of these β-lactam-containing ELA mimetics, together with an unexpected opening of the β-lactam ring during the release of the peptidomimetics from the solid support. The three β-lactam-containing peptidomimetics thus generated, as well as their opened variants, were then evaluated for their binding affinity to the HLA-A2 molecule and for their capacity to activate Melan-A-specific T cells.

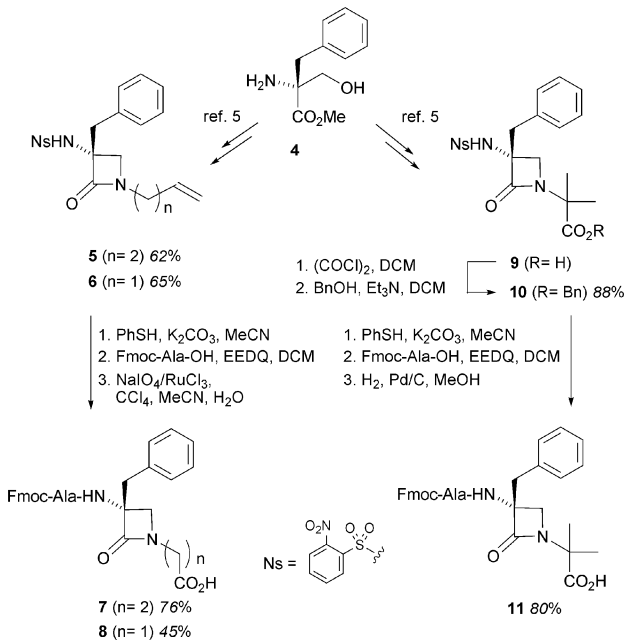
Results and discussion

Design of β-lactam scaffolds

Three different amino acids (Xaa) were selected to occupy the β-turn (i+2) position in the central -(β-lactam)-Xaa- scaffolds **I–III** of pseudopeptides **1–3** (Fig. 1 and 2). On the basis of previous investigations stressing that TCR loops are sufficiently flexible to accommodate rather bulky and hydrophobic central side-chains on hapten-modified peptides[12] and of results from our preliminary *in silico* docking study (data not shown), a benzyl group was selected in place of the *sec*-butyl central isoleucine side-chain to equip the β-lactam motif of the three scaffolds **I–III**. The methylene-composed β-alanine (in **1**) and glycine (in **2**) were chosen to maintain some degree of flexibility in the peptidic backbone in order to allow the resulting constructs to best orient themselves within the HLA-A2 binding cleft. Alternatively, the inclusion of a constrained aminoisobutyric acid (Aib) unit, known to be an efficient inducer of 3_{10} helix,[13] was also examined (in **3**). Moreover, our previous results[11] prompted us to install these β-lactam segments in place of the $G_{29}I_{30}G_{31}$ tripeptide of ELA so as to best mimic the zigzag conformation of its central portion (Fig. 2),[10] while maintaining adequate interactions of the anchoring residue side chains at P2 and P10 within the HLA-A2 binding cleft. Furthermore, our preliminary *in silico* docking study of the three envisaged D-α-benzylserine-derived β-lactam–peptide hybrids (Scheme 1)[2,14] indicated that a substitution by the D-enantiomer of isoleucine at P7 would be necessary to enable the appropriate orientation of the N-terminal part of these β-lactam pseudopeptides (data not shown). Finally, the N-terminal glutamate residue (P1) was replaced by a β-alanine, as such a substitution has previously been shown to be beneficial for biostability without compromising neither the binding to the HLA-A2 protein nor the recognition by Melan-A specific T-cell clones.[11,15]

Solid-phase synthesis of β-lactam pseudopeptides

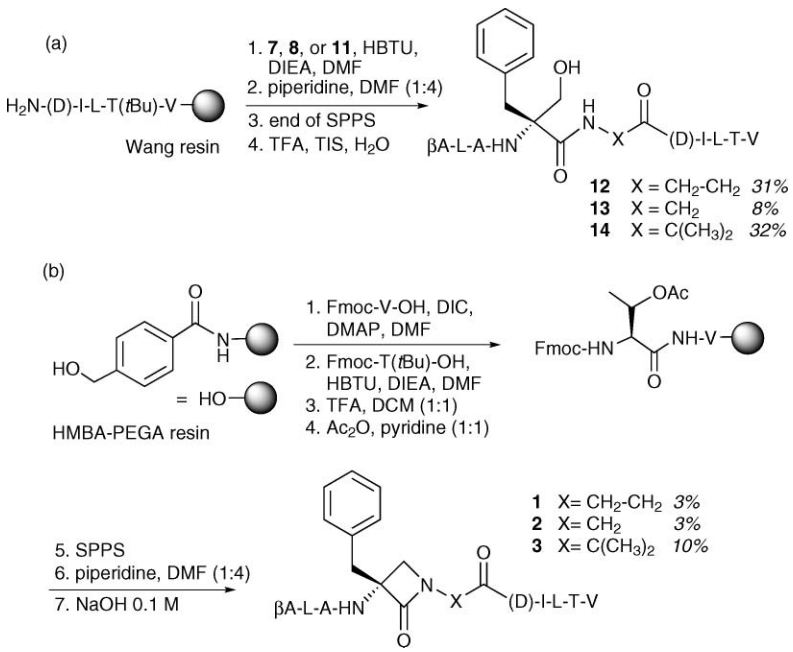
The β-lactam cores **5**, **6** and **9** were prepared from methyl D-α-benzylserinate **4** following previously described procedures (Scheme 1).[5] In the case of tripeptide scaffolds **7** and **8**, allylic and homoallylic amine moieties were respectively chosen as precursors of the glycine and β-alanine residues. The oxidative cleavage of their double bond by $NaIO_4$ conveniently afforded the desired carboxylic acid functions. All three *N*-Fmoc-protected tripeptide scaffolds **7**, **8** and **11** were installed on a D-Ile-Leu-Thr-Val peptide



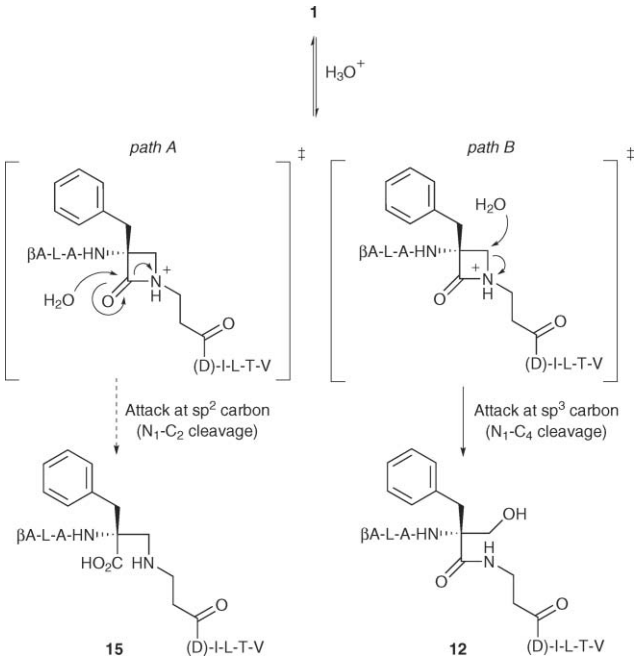
Scheme 1 Synthesis of Fmoc-Ala-β-lactam-Xaa-OH tripeptide scaffolds with C-terminal β-alanine (**7**), glycine (**8**) and Aib (**11**) residues. All yields quoted correspond to overall transformations.

construct supported on a Wang resin by following standard Fmoc-based solid-phase peptide synthesis (SPPS) protocols. Removal of the Fmoc-protecting group, addition of the two remaining amino acids (*i.e.*, leucine and β-alanine) and release of the expected β-lactam–peptide hybrids from the resin with concomitant removal of all protecting groups completed the syntheses (Scheme 2a).

Much to our surprise, a structural analysis of the resulting compounds (*i.e.*, **12–14**) by electrospray ionization mass spectrometry (ESIMS) indicated molecular masses exceeding by 18 Da those expected for the desired β-lactam pseudopeptides **1–3**. We rapidly concluded that these observations were likely due to a conventional hydrolysis of the β-lactam N_1–C_2 amide bond during the final aqueous TFA cleavage step (Scheme 2a), which would then furnish β-amino acid-containing peptides such as **15** from **1** (Scheme 3, path A). However, a careful examination of the data we collected from standard 1D ^{1}H and 2D ^{1}H–^{1}H TOCSY NMR experiments performed on compound **12** clearly indicated that 8 amidic protons were present in the molecule (and not 7 as in **15**), and that the "extra" amidic proton was correlated with one of the methylene protons of the *endo*-β-alanine residue (see ESI† for details). These data strongly suggest that it is instead the N_1–C_4 bond of the β-lactam ring that suffered hydrolytic cleavage (Scheme 3, path B), hence leading to the D-α-benzylserine-containing peptides **12–14** shown in Scheme 2. Cleavage of the N_1–C_4 bond has been previously observed or forced in β-lactam systems bearing an electron-donating group at C_4.[16–19] For example, the hydrogenolysis of 4-arylazetidin-2-ones is a very well know methodology developed by Ojima for the preparation of α-amino acids and peptidomimetics.[17] More recently, McMurray and co-workers described the N_1–C_4 bond opening of 4-(4′-hydroxyphenyl)-2-azetidinones under basic or acidic conditions.[18] In parallel, Alcaide and co-workers reported novel N_1–C_4 bond breakages on β-lactams equipped with non-aryl carbanionic moieties at C_4.[19] Moreover, according to the results of detailed studies of acid-catalyzed hydrolyses,[20] N-protonated β-lactams intermediates undergo nucleophilic addition (Ad_N) of a water molecule at the carbonyl sp^2 carbon atom along a classical Bürgi–Dunitz trajectory,[21] followed by rupture of the N_1–C_2 bond by elimination (E). In the case of our β-lactams, however, the Bürgi–Dunitz trajectory of approach is hindered by the α,α-substituents on both sides of the azetidinone ring, thus energetically penalizing the Ad_N-E pathway (see path A in Scheme 3). Conversely, the unsubstituted β-methylene sp^3 C_4 atom hence becomes more vulnerable to an S_N2 attack by a water molecule that



Scheme 2 Solid-phase synthesis of β-lactam–peptide hybrids **1–3** (b) and their opened variants **12–14** (a).



Scheme 3 Proposed mechanism (path B) for the unusual N_1–C_4 β-lactam ring opening observed during aqueous TFA treatment.

thus opens up the β-lactam ring with conservation of the amide bond (see path B in Scheme 3). To the best of our knowledge, the ring opening reactions we thus observed leading to **12–14** are the first examples of nucleophilic N_1–C_4 bond cleavage occurring at an unsubstituted C_4 center of β-lactam rings.[22]

To gather additional information about this unusual N_1–C_4 bond breakage, we also exposed the non-immobilized Aib-containing tripeptide scaffold **11** and the methyl ester of its nosylated precursor **9**[5] to the same aqueous acidic conditions (see ESI† for details). Interestingly, whereas the C_3-amidated β-lactam **11** was also rapidly converted into its N_1–C_4-opened variant in quantitative yield, as confirmed by ^{1}H NMR analysis showing notably a characteristic downfield shift for the methylene protons at C_4,[23] its C_3-nosylated analogue **9** remained intact. These observations raise the question of whether the presence of an amide substitutent at C_3 is compulsory for mediating the N_1–C_4 bond breakage. The significance of such a "peptide-like" factor in favoring this unusual β-lactam ring opening will be assessed in future studies aimed at delineating further the scope of the reaction.

At this stage and for the sake of the continuation of this study, other conditions of release of our pseudopeptide targets from the solid support had thus to be found to avoid this unprecedented β-lactam ring opening. A solution was found by replacing the Wang resin by an amino PEGA resin bearing a base-labile hydroxymethylbenzoic acid (HMBA) linker (Scheme 2b).[24] The *tert*-butyl protection of the threonine (Thr) side-chain was removed by a TFA treatment, and immediately followed by an acetylation step. The rest of the synthesis, including the installation of the β-lactam scaffolds **7**, **8** and **11**, was carried out by standard SPPS techniques. After removal of the N-terminal β-Ala Fmoc protecting group, pseudopeptides were released by treating the resin for 3 h with a 0.1 M aqueous solution of NaOH, which also enabled cleavage of the Thr acetate protecting group.

The high hydrophobicity of the resulting crude pseudopeptides caused us some difficulties during their purification by HPLC, notably because of their tendency to aggregate. Consequently, the three targeted pseudopeptides **1–3** were obtained in pure forms in relatively poor yields (Scheme 2b), but nevertheless in largely sufficient quantities to handle their immunological evaluation.

HLA-A2 binding

Having thus in hand both the β-lactam-containing ELA mimetics **1–3** and their opened variants **12–14**, we investigated their relative capacity to bind to the HLA-A2 protein *via* the T2 surface binding assay (see Experimental section). Two peptides were used as references: ELA as a positive control and a nonapeptide of sequence GAGAGAGAG (termed GAG) containing no HLA-A2 anchor residues as a negative control. Hence, any peptide with a binding affinity equal to or lower than GAG can be considered as a very weak ligand or non-binding entity. The T2 binding experiments revealed that the opened pseudopeptides **12–14** bind relatively well to HLA-A2, whereas no binding was surprisingly observed with the β-lactam-bearing peptidomimetics **1–3** (Fig. 3). Importantly, compound **14** emerged as an extremely strong HLA-A2 ligand, showing an apparent binding strength superior to that of ELA.

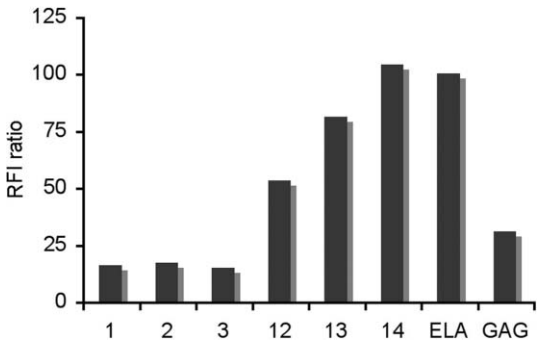


Fig. 3 Normalized HLA-A2 binding affinities for β-lactam-containing ELA mimetics **1–3** and their opened variants **12–14** relative to that of ELA (100) expressed as relative fluorescence intensities (RFI) from HLA-A2-binding surface antibodies.

One possible explanation for the lack of binding of the β-lactam-bearing constructs **1–3** is that their built-in conformational constraints in fact altered the orientation of the anchor residues within the HLA-A2 binding pockets. In contrast, the opened β-lactam–peptide hybrids **12–14**, which possess a D-α-benzylserinate at their core, appeared to fold well inside the HLA-A2 groove. These HLA-A2 binding data also reveal that the substitution by a D-isoleucine residue at P7 does not affect the binding efficiency of these opened pseudopeptides, since all three compounds expressed affinity in the same range as that of the ELA control. Moreover, the fact that the best HLA-A2 ligand **14** possesses an Aib residue suggests that the rotational restriction this residue likely imposes contributes to a proper positioning of the pseudopeptide to best match the HLA-A2 binding requirements. The structure–affinity relationship trend observed with the three pseudopeptides **12–14** is in agreement with this hypothesis, since their binding affinity decreases with an increase of their flexibility. Thus, compound **12**, which possesses a more flexible sequence-embedded β-alanine residue in place of

the more constrained Aib next to the D-α-benzylserine, exerts a two-fold weaker binding affinity compared to that of **14**.

Given the remarkably good binding affinity **14** for HLA-A2, we then decided to evaluate the thermal stability of the resulting complex, since it is now well established that a highly stable pMHC-I complex is more likely to productively interact with circulating T cells.[25] Measurements of thermal stability based upon circular dichroism-monitored protein denaturation have previously been performed for a number of pMHC-I complexes,[26] and in general, a higher melting temperature (T_m) correlates with tighter peptide binding. As shown in Fig. 4, **14** forms a complex with HLA-A2 that is more stable at physiological temperatures than that formed with the native Melan-A_{26-35} decapeptide (EAAGIGILTV), but somewhat less stable than that formed with ELA. The thermal stabilities thus measured have globally a good concordance with the binding affinities measured by the T2 surface binding assay. Notwithstanding the disappointing behavior of our initially designed β-lactam-ELA mimetics vis-à-vis the HLA-A2 protein, the good binding results obtained with pseudopeptides **12–14** then prompted us to investigate their capacity to specifically activate T cells.

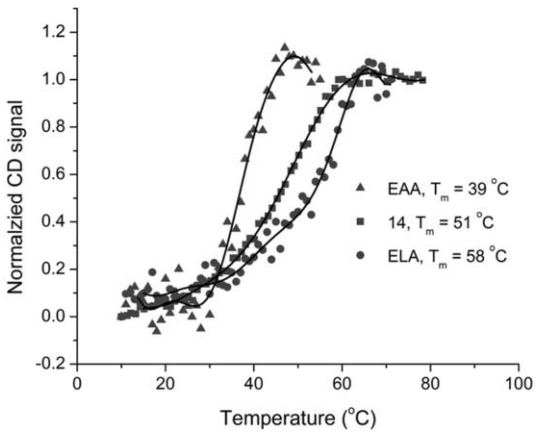


Fig. 4 Thermal stability measurements of complexes of HLA-A2 with EAA, ELA, and **14**.

ELA-specific T cell stimulation

The capacity of T cells to functionally recognize pseudopeptides **12–14** in a HLA-A2-restricted context was determined by performing an intracellular cytokine stain (ICS) to measure the level of cytokines secreted by activated T cells *via* flow cytometry. Thus, briefly, a Melan-A-specific T cell line was generated by stimulating HLA-$A2^+$ peripheral blood mononuclear cells (PBMC) with 10 μM of the native Melan-A_{26-35} epitope (*i.e.*, EAA). The PBMC were cultured for 10 days in T cell media, which resulted in approximately 15% of the total T cell population staining with a Melan-A-HLA-A2 tetramer (data not shown). The T cell line was then divided into 1×10^5 cell samples, pulsed with 500 μM of either EAA used as a positive control or **12–14**, washed and incubated overnight in an ICS assay. The T cell line was then examined by flow cytometry for expression of the lysosomal-associated membrane protein (LAMP) CD107a[27] and the stimulatory cytokine interferon-γ (INFγ); both produced by the tetramer gated T cells (Fig. 5). Once again, **14** emerged as the most interesting compound, as it was the only one to elicit

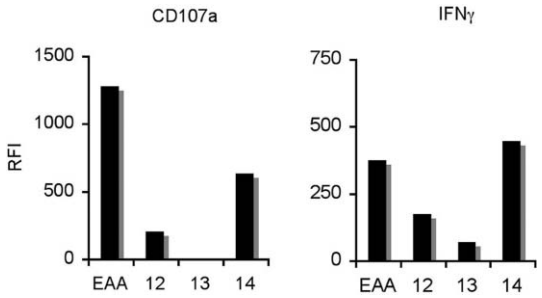


Fig. 5 Flow cytometry detection of secretion of the stimulatory cytokine INF-γ and the LAMP CD107a after stimulation of Melan-A-specific T cells with EAA (native peptide used as positive control) or peptides **12–14** at 500 μM.

a CD107a response in tetramer gated T cells, albeit in weaker amounts in comparison to the response elicited by the native peptide EAA. Furthermore, **14** elicited an IFNγ response in the same range as that of EAA. These data clearly indicate that a peptidomimetic structure such as **14** can specifically stimulate T cells in a MHC-I-restricted context.

Conclusion

None of the designed β-lactam-ELA peptidomimetics displayed HLA-A2 binding affinity, but the unprecedented acid-catalyzed opening of their β-lactam ring led to the serendipitous discovery of the HLA-A2 binding and T cell stimulation capacities of their opened variants. Taken together, the results described herein provide us with highly informative insights into the possibilities offered to medicinal chemists to modify the structures of MHC-I-restricted antigenic peptides with the aim of better stimulating T cells. The simple fact that the T cell stimulating pseudopeptide **14** exhibits binding properties similar to those of ELA suggests that the same type of molecular interactions upon binding to the HLA-A2 protein are at play. Future work will address these issues through X-ray crystallographic studies of the **14**–HLA-A2 complex.

Experimental

General

All reagents were purchased from either Aldrich, Acros, or Fluka. Amino acids, Wang resin, amino-PEGA resin, and HBTU were purchased from Novabiochem (Switzerland). Tetrahydrofuran (THF) and diethyl ether (Et_2O) were dried through PS-MD-2 columns. Extra pure dichloromethane (CH_2Cl_2), hexane (Hex) and ethyl acetate (EtOAc) were bought from Sharlau. All other organic solvents were of analytical quality and Milli-Q (Millipore) water was used for reverse phase (RP) HPLC analyses and purifications.

All solution-phase reactions were carried out under nitrogen atmosphere in oven- or flame-dried glassware with magnetic stirring and dry solvents were used. Solid-phase peptide syntheses were performed on a Chemspeed ASW100 automated synthesizer, in double-jacket glass reactors.

RP-HPLC analyses were performed on a Thermo system using a Chromolith performance RP-18e column (4.6 × 100 mm, 5 μm) with P1000 XR pumps. The mobile phase was composed of 0.1%

(v/v) TFA-H_2O (Solvent A) and 0.1% TFA-CH_3CN (Solvent B), unless otherwise noted. A gradient elution (0–10 min: 100% to 50% A) was applied at a flow rate of 3 mL min^{-1}. Column effluent was monitored by UV detection at 214 and 254 nm using a Thermo UV 6000 LP diode array detector. Semi-preparative purifications of peptides were performed on a Varian PrepStar system with SD-1 Dynamax® pumps, using a Microsorb C18 column (21.4 mm × 250 mm, 100 Å pore size, 5 µm). The mobile phase was similar as for the analytic system, unless otherwise notified. A gradient elution (0–40 min: 90% to 50% A) was applied at a flow rate of 20 mL min^{-1}. Column effluent was monitored by UV detection at 214 and 254 nm using a Varian UV-Vis Prostar 325 diode array detector.

Purification of reaction products was carried out by flash chromatography using silica gel 60 (230–400 mesh) and the indicated solvents. Optical rotation were obtained using a Perkin-Elmer 243B polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded using Bruker Avance spectrometers DPX-400 and DPX-500. Chemical shifts (δ) are given in ppm and J values are given in Hz. Mass spectra were obtained either under electron impact (EI, 70 eV) or electrospray ionization (ESI) conditions after direct injection (HRMS) or using GC-MS coupling (column: fused silica gel, 15 m, 0.25 mm, 0.25 nm phase SPB-5). Peptides were characterized by electrospray ionization low- and high-resolution (ESI, HRMS) obtained from the Mass Spectrometry Laboratory at the European Institute of Chemistry and Biology (IECB), Pessac, France.

(*R*)-3-Benzyl-1-(but-3-enyl)-3-(2-nitrophenylsulfonamido)-azetidin-2-one (5). To a solution of methyl (*R*)-2-benzylserinate **4**[5] (1.05 mmol, 219 mg) in CH_3CN (40 mL) were added 2-nitrobenzenesulfonyl chloride (NsCl, 2.31 mmol, 511 mg) and $KHCO_3$ (5.25 mmol, 525 mg). The reaction mixture was stirred overnight under reflux. The temperature was decreased to 50 °C, then homoallylic amine hydrochloride (1.57 mmol, 169 mg) and $KHCO_3$ (1.57 mmol, 157 mg) were added and the solution was allowed to stir at 50 °C until full consumption of the *N*-nosyl aziridine. The reaction mixture was concentrated and the residue was purified by flash chromatography, eluting with EtOAc–Hex (3 : 1), to furnish the expected methyl (*R*)-2-benzyl-3-(but-3-enylamino)-2-(2-nitrophenylsulfonamido)-propanoate (340 mg, 73%) as a yellow solid, mp 55–56 °C. $[\alpha]_D^{25}$ −23.8 (*c* 1.05 in CH_2Cl_2); ν_{max}(KBr)/cm^{-1} 3339 (NH), 1738 (C═O), 1540 (NO_2); δ_H(500 MHz; $CDCl_3$) 1.98–2.03 (2 H, m, NCH_2CH_2), 2.33–2.38 (1 H, m, NCH_2CH_2), 2.43–2.48 (1 H, m, NCH_2CH_2), 3.00 (1 H, d, *J* 12.5, CH_2Ph), 3.07 (1 H, d, *J* 12.5, CH_2Ph), 3.31 (1 H, d, *J* 13.4, CCH_2NH), 3.43 (1 H, d, *J* 3.4, CCH_2NH), 3.61 (3 H, s, OCH_3), 4.95–4.98 (2 H, m, $CH{=}CH_2$), 5.56–5.65 (1 H, m, $CH{=}CH_2$), 7.25–7.29 (5 H, m, Ph), 7.70–7.77 (2 H, m, Ns), 7.94 (1 H, d, *J* 7.5, Ns), 8.17 (1 H, d, *J* 7.5, Ns); δ_C(125 MHz; $CDCl_3$) 171.7, 147.4, 136.7, 135.9, 134.8, 133.0, 132.7, 130.3, 129.9, 128.3, 127.2, 125.3, 116.3, 68.2, 53.5, 52.5, 48.5, 41.3, 34.1; HRMS (ESI) calcd. for $C_{18}H_{20}N_3O_6S$ [M − C_3H_5]$^+$ 406.1073, found 406.1071.

This sulfonamide (0.62 mmol, 279 mg) was dissolved in THF (15 mL) at −10 °C and a 1 M solution of LiHMDS (1.55 mmol, 1.6 mL) in THF was slowly added. The resulting solution was stirred for 20 min, quenched with a saturated solution of $NaHCO_3$ (15 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to give a residue, which was purified by flash chromatography, eluting with EtOAc–Hex (2 : 1), to furnish **5** (220 mg, 85%) as a yellow oil. $[\alpha]_D^{25}$ −100.6 (*c* 0.6 in CH_2Cl_2); ν_{max}(KBr)/cm^{-1} 3421 (NH), 1750 (C═O), 1556 (NO_2); δ_H(500 MHz, $CDCl_3$) 2.08–2.12 (2 H, m, NCH_2CH_2), 3.12 (2 H, s, CH_2Ph), 3.12–3.21 (2 H, m, NCH_2CH_2), 3.44 (1 H, d, *J* 5.5, lactam CH_2), 3.72 (1 H, d, *J* 5.5, lactam CH_2), 5.00–5.05 (2 H, m, $CH{=}CH_2$), 5.59–5.67 (1 H, m, $CH{=}CH_2$), 5.89 (1 H, s, NH), 7.23–7.29 (5 H, m, Ph), 7.70–7.78 (2 H, m, Ns), 7.88 (1 H, d, *J* 8.0, Ns), 8.17 (1 H, d, *J* 7.5, Ns); δ_C(125 MHz, $CDCl_3$) 166.0, 147.4, 135.4, 134.6, 133.5, 132.9, 131.0, 130.0, 128.8, 127.7, 125.3, 117.1, 70.0, 52.8, 40.8, 40.7, 31.5; HRMS (ESI) calcd. for $C_{20}H_{22}N_3O_5S$ [M+H]$^+$ 416.1280, found 416.1296.

***N*-Fmoc-Ala-(β-lactam)-β-Ala-OH (7).** To a solution of **5** (0.38 mmol, 157 mg) in CH_3CN (20 mL) were added K_2CO_3 (0.38 mmol, 52 mg) and thiophenol (PhSH, 0.75 mmol, 0.077 mL). The mixture was allowed to stir for 3 h at room temperature. The suspension was filtered through a celite pad and the mixture was concentrated to dryness. The residue was used without further purification. It was dissolved in CH_2Cl_2 (20 mL) at −10 °C. *N*-Fmoc-Ala-OH (0.44 mmol, 138 mg) and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 0.67 mmol, 164 mg) were added successively and the suspension was allowed to warm up to room temperature overnight. The reaction mixture was then diluted with CH_2Cl_2, washed with 1 M HCl, dried over $MgSO_4$, and evaporated to give a residue, which was purified by flash chromatography, eluting with EtOAc–Hex (1 : 2), to furnish the expected *N*-Fmoc-Ala-(β-lactam)-but-3-ene (172 mg, 87%) as a white solid, mp 65–67 °C. $[\alpha]_D^{25}$ −20.4 (*c* 1.18 in CH_2Cl_2); ν_{max}(KBr)/cm^{-1} 3298 (NH), 1745, 1666 (C═O); δ_H(500 MHz, $CDCl_3$) 1.35 (3 H, br s, CH_3), 2.04 (2 H, s, NCH_2CH_2), 3.03–3.09 (1 H, m, NCH_2CH_2), 3.13–3.20 (2 H, m, CH_2Ph), 3.22–3.26 (1 H, m, NCH_2CH_2), 3.38 (1 H, d, *J* 5.2, lactam CH_2), 3.54 (1 H, br s, lactam CH_2), 4.19–4.21 (2 H, m, Ala CH + Fmoc CH), 4.37–4.42 (2 H, m, Fmoc CH_2), 4.96–4.99 (2 H, m, $CH{=}CH_2$), 5.21 (1 H, br s, NH), 5.51–5.59 (1 H, m, $CH{=}CH_2$), 6.64 (1 H, s, NH), 7.23–7.77 (13 H, m, Fmoc, Ph); δ_C(125 MHz, $CDCl_3$) 172.4, 167.4, 155.8, 143.7, 141.3, 134.7, 134.6, 130.2, 128.5, 127.7, 127.2, 127.0, 125.0, 120.0, 116.9, 67.6, 67.1, 50.8, 47.1, 40.8, 38.8, 31.4, 18.6; HRMS (ESI) calcd. for $C_{32}H_{33}N_3O_4Na$ [M+Na]$^+$ 546.2369, found 546.2365.

This compound (0.20 mmol, 103 mg) was then dissolved in a mixture of CCl_4–CH_3CN–H_2O (2 : 2 : 3 (v/v/v), 28 mL) at 0 °C. $NaIO_4$ (2.95 mmol, 634 mg) and $RuCl_3$ (3.94 µmol, 1 mg) were added. The resulting emulsion was stirred at room temperature for 2 h, after which time the organic phase of this reaction mixture was diluted with CH_2Cl_2 and separated, and the aqueous phase was acidified using 1 M HCl. After extraction of the aqueous phase with CH_2Cl_2, the combined organic layers were evaporated to dryness to give a residue, which was purified by flash chromatography, eluting with MeOH–CH_2Cl_2 (1 : 10), to furnish **7** (92 mg, 87%) as a white solid, mp 116–117 °C. $[\alpha]_D^{25}$ −0.2 (*c* 1.25 in CH_3OH); (Found: C, 68.26; H, 6.01; N, 7.76. $C_{31}H_{31}N_3O_6$ requires C, 68.75; H, 5.77; N, 7.76%); ν_{max}(KBr)/cm^{-1} 3301 (NH), 1743 (C═O); δ_H(500 MHz, CD_3OD) 1.30 (3 H, d, *J* 7.0, CH_3), 2.08 (2 H, br s, CH_2COOH), 3.10–3.16 (2 H, m, CH_2Ph), 3.22–3.28 (2 H, m, NCH_2), 3.38 (1 H, d, *J* 4.5, lactam CH_2), 3.53 (1 H, br s, lactam CH_2), 4.14 (1 H, q, *J* 7.0, Ala CH), 4.20 (1 H, t, *J* 6.7, Fmoc CH), 4.36 (2 H, d, *J* 6.7, Fmoc CH_2), 7.22–7.30 (5 H, m, Ph), 7.31 (2

H, t, *J* 7.5, Fmoc), 7.39 (2 H, t, *J* 7.5, Fmoc), 7.65 (2 H, d, *J* 6.0, Fmoc), 7.79 (2 H, d, *J* 7.5, Fmoc); δ_C(125 MHz, CD_3OD) 175.4, 174.7, 170.1, 158.2, 145.2, 142.6, 135.7, 131.4, 129.4, 128.8, 128.3, 128.1, 126.2, 120.9, 68.7, 67.9, 51.7, 51.5, 48.4, 39.3, 38.0, 32.7, 18.2; HRMS (ESI) calcd. for $C_{31}H_{31}N_3O_6Na$ $[M+Na]^+$ 564.2111, found 564.2128.

(*R*)-3-Benzyl-1-(prop-2-enyl)-3-(2-nitrophenylsulfonamido)-azetidin-2-one (6). To a solution of methyl (*R*)-2-benzylserinate **4**[5] (1.9 mmol, 398 mg) in CH_3CN (40 mL) were added NsCl (4.18 mmol, 926 mg) and $KHCO_3$ (9.50 mmol, 951 mg), and the reaction mixture was stirred overnight under reflux. The temperature was decreased to 50 °C, allylic amine hydrochloride (2.87 mmol, 0.215 mL) and $KHCO_3$ (2.87 mmol, 287 mg) were added and the solution was allowed to stir at the same temperature until full consumption of the *N*-nosyl aziridine. The reaction mixture was concentrated and the residue was purified by flash chromatography, eluting with Hex–EtOAc (3 : 1), to furnish the expected methyl (*R*)-2-benzyl-3-(prop-2-enylamino)-2-(2-nitrophenylsulfonamido)-propanoate (570 mg, 69%) as a yellow solid, mp 53–55 °C. $[\alpha]_D^{25}$ −21.8 (*c* 1.00 in CH_2Cl_2); (Found: C, 55.72; H, 5.29; N, 9.42. $C_{20}H_{23}N_3O_6S$ requires C, 55.42; H, 5.35; N, 9.69%); ν_{max}(KBr)/cm^{-1} 3340 (NH), 1738 (C═O), (NO_2); δ_H(500 MHz, $CDCl_3$) 2.92–2.98 (2H, m, CH_2CHCH_2), 3.05–3.08 (2H, m, NCH_2C), 3.39 (2H, dd, *J* 13.8 and 30.2, CCH_2Ph), 3.62 (3H, s, OCH_3), 4.95–5.03 (2H, m, CH_2CHCH_2), 5.54–5.73 (1H, m, CH_2CHCH_2), 7.14–7.26 (5H, m, Ph), 7.66–8.17 (4H, m, Ns); δ_C(125 MHz, $CDCl_3$) 171.6, 147.3, 136.2, 136.0, 134.6, 133.1, 132.7, 130.2, 129.8, 128.2, 127.1, 125.3, 115.8, 68.4, 52.9, 52.6, 51.7, 41.4; *m*/*z* (ESI) 434 (MH^+, 100%).

This sulfonamide (1.31 mmol, 570 mg) was dissolved in THF (15 mL) at −10 °C and a 1 M solution of LiHMDS (3.3 mmol, 3.3 mL) in THF was slowly added. The resulting solution was stirred for 20 min, quenched with a saturated solution of $NaHCO_3$ (15 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to give a residue, which was purified by flash chromatography, eluting with Hex–EtOAc (2 : 1), to furnish **6** (493 mg, 94%) as a yellow oil. $[\alpha]_D^{25}$ −88.6 (*c* 0.75 in CH_2Cl_2); ν_{max}(KBr)/cm^{-1} 3350 (NH), 1745 (C═O), 2922 (NO_2); δ_H(500 MHz, $CDCl_3$) 3.12 (2H, s, CH_2Ph), 3.38 (1H, d, *J* 5.6, lactam CH_2), 3.68 (1H, d, *J* 5.4, lactam CH_2), 3.68–3.70 (2H, m, CH_2CHCH_2), 5.02–5.09 (2H, m, CH_2CHCH_2), 5.39–5.45 (1H, m, CH_2CHCH_2), 5.90 (1H, s, N*H*), 7.22–7.27 (5H, m, Ph), 7.69–8.17 (4H, m, Ns); δ_C(75 MHz, $CDCl_3$) 166.0, 147.4, 135.4, 133.5, 133.4, 133.0, 131.0, 130.9, 130.1, 128.8, 127.7, 125.3, 118.7, 70.1, 52.3, 44.2, 40.7; *m*/*z* (ESI) 402 (MH^+, 100%).

***N*-Fmoc-Ala-(β-lactam)-Gly-OH (8).** To a solution of **6** (0.30 mmol, 120 mg) in CH_3CN (10 mL) were added K_2CO_3 (0.30 mmol, 41 mg) and PhSH (0.60 mmol, 0.06 mL). The mixture was allowed to stir overnight at room temperature. The suspension was filtered through a celite pad and the filtrate was concentrated to dryness. The residue was used without further purification. It was dissolved in CH_2Cl_2 (10 mL) at −10 °C. *N*-Fmoc-Ala-OH (0.45 mmol, 140 mg) and EEDQ (0.54 mmol, 134 mg) were successively added. The solution was then allowed to warm up to room temperature and stirring was maintained overnight. The reaction mixture was then diluted with CH_2Cl_2, washed with 1 M HCl, dried over $MgSO_4$ and evaporated to give a residue, which was purified by flash chromatography, eluting with MeOH–DCM (1 : 10), to furnish the expected *N*-Fmoc-Ala-(β-lactam)-prop-2-ene (101 mg, 66%) as a white solid, mp 75–76 °C. $[\alpha]_D^{25}$ −19.3 (*c* 2.3 in CH_2Cl_2); (Found: C, 72.82; H, 6.14; N, 8.66. $C_{31}H_{31}N_3O_4$ requires C, 73.06; H, 6.13; N, 8.25%); ν_{max}(KBr)/cm^{-1} 3298 (NH), 1748, 1720, 1672 (C═O); δ_H(500 MHz, $CDCl_3$) 1.37 (3H, d, *J* 6.0, CH_3), 3.16 (2H, s, CH_2Ph), 3.33 (1H, d, *J* 5.7, lactam CH_2), 3.50 (d, 1H, *J* 4.9, lactam CH_2), 3.55 (1H, dd, *J* 15.5 and 6.2, CH_2), 3.76 (1H, dd, *J* 15.4 and 4.5, CH_2), 4.16–4.21 (1H, m, Ala C*H*), 4.27–4.36 (1H, m, Fmoc C*H*), 4.39 (2H, d, *J* 3.0, Fmoc CH_2), 4.90 (1H, dd, *J* 28.5 and 2.0, CH_2), 5.00 (1H, dd, *J* 15.5 and 1.5, CH_2), 5.28–5.35 (1H, m, C*H*), 5.44 (1H, d, *J* 7.6, N*H*), 6.99 (1H, s, N*H*), 7.22–7.32 (7H, m, Fmoc Ph), 7.39 (2H, t, *J* 12.0, Fmoc), 7.57 (2H, d, *J* 12.0, Fmoc), 7.75 (2H, d, *J* 12.5, Fmoc); δ_C(125 MHz, $CDCl_3$) 172.4, 167.3, 155.9, 143.7, 141.3, 134.5, 130.9, 130.2, 128.5, 127.7, 127.2, 127.0, 125.0, 120.0, 118.4, 67.7, 67.1, 50.4, 50.2, 47.1, 44.1, 38.8, 18.6; HRMS (ESI) calcd. for $C_{27}H_{26}N_2O_3$ $[M-C_4H_5NO]^+$ 426.1943, found 426.1948.

This compound (0.098 mmol, 50 mg) was then dissolved in a mixture of CCl_4–CH_3CN–H_2O (2 : 2 : 3 (v/v/v), 14 mL) at 0 °C; $NaIO_4$ (0.98 mmol, 210 mg) and $RuCl_3$ (2 μmol, 0.4 mg) were added. The resulting emulsion was stirred at room temperature for 2 h, after which time the organic phase of this reaction mixture was diluted with CH_2Cl_2 and separated, and the aqueous phase was acidified with 1 M HCl. After extraction with CH_2Cl_2 and EtOAc, the combined organic layers were evaporated to give a residue, which was purified by flash chromatography, eluting with MeOH–CH_2Cl_2 (1 : 7), to furnish **8** (35 mg, 68%) as a white solid, mp 120–121 °C. $[\alpha]_D^{25}$ −7.4 (*c* 1.5 in CH_3OH); ν_{max}(KBr)/cm^{-1} 3391 (NH, OH), 1743, 1614 (C═O). δ_H(500 MHz, CD_3OD) 1.28 (3H, d, *J* 7.0, CH_3), 3.31 (2H, m, $PhCH_2$), 3.60 (1H, d, *J* 5.5, lactam CH_2), 3.62–3.71 (3H, m, lactam CH_2 + NCH_2), 4.14 (1H, q, *J* 6.8, Ala C*H*), 4.21 (1H, t, *J* 6.0, Fmoc C*H*), 4.31–4.38 (2H, m, Fmoc CH_2), 7.15–7.33 (7H, m, Fmoc + Ph), 7.39 (2H, t, *J* 7.5, Fmoc), 7.67 (2H, d, *J* 7.0, Fmoc), 7.80 (2H, d, *J* 7.5, Fmoc); δ_C(125 MHz, CD_3OD) 175.3, 170.0, 158.1, 145.2, 142.6, 136.7, 131.2, 129.3, 128.8, 128.2, 128.0, 126.2, 120.9, 69.1, 67.9, 53.5, 51.7, 39.4, 18.2; HRMS (ESI) calcd. for $C_{30}H_{29}N_3O_6Na$ $[M+Na]^+$ 550.1954, found 550.1942.

Ns-(β-Lactam)-Aib-OBn (10). To a solution of Ns-(β-lactam)-Aib-OH **9**[5] (1.07 mmol, 479 mg) in CH_2Cl_2 (15 mL) at 0 °C were added oxalyl chloride (1.60 mmol, 140 μL) and a catalytic amount of DMF. The solution was stirred at 0 °C for 1 h, then at room temperature for another 1 h. The reaction mixture was concentrated and kept under vacuum for 1 h. This freshly prepared acyl chloride was then diluted with CH_2Cl_2 (15 mL) and benzyl alcohol (3.21 mmol, 0.34 mL) and triethylamine (6.43 mmol, 0.9 mL) were added at 0 °C. The mixture was allowed to warm up to room temperature and stirring was maintained overnight. The mixture was then acidified with 1 M HCl, extracted with CH_2Cl_2, dried over $MgSO_4$ and evaporated to give a residue, which was purified by flash chromatography, eluting with EtOAc–Hex (1 : 3), to furnish **10** (506 mg, 88%) as a white solid, mp 92–93 °C. $[\alpha]_D^{25}$ −77.1 (*c* 0.99 in CH_2Cl_2); (Found: C, 60.01; H, 4.58; N, 7.95. $C_{27}H_{27}N_3O_7S$ requires C, 60.32; H, 5.06; N, 7.82%); ν_{max}(KBr)/cm^{-1} 3357 (N*H*), 1759, 1738 (C═O), 1540 (NO_2); δ_H(500 MHz, $CDCl_3$) 1.45 (3H, s, CH_3), 1.46 (3H, s, CH_3), 3.05 (1H, d, *J* 14.0, C*H*Ph), 3.09 (1H, d, *J* 14.0, C*H*Ph), 3.48 (1H, d, *J* 5.3, lactam CH_2), 3.75 (1H, d, *J* 5.3, lactam CH_2), 5.15 (1H, d,

J 12.3, OC*H*Ph), 5.17 (1H, d, *J* 12.3, OC*H*Ph), 5.85 (1H, s, NH), 7.17–7.34 (10H, m, 2Ph), 7.67–8.12 (4H, m, Ns); δ_C(125 MHz, $CDCl_3$) 172.2, 165.1, 147.3, 135.3, 133.5, 133.4, 132.9, 131.0, 130.0, 128.7, 128.6, 128.4, 128.3, 128.1, 127.6, 125.2, 68.1, 67.3, 59.0, 51.5, 40.4, 23.7, 23.5; *m*/*z* (ESI) 538 (MH^+, 100%).

***N*-Fmoc-Ala-(β-lactam)-Aib-OH (11).** To a solution of **10** (0.75 mmol, 403 mg) in CH_3CN (10 mL) were added K_2CO_3 (0.75 mmol, 103.6 mg) and PhSH (1.5 mmol, 0.15 mL). The mixture was allowed to stir for 3 h at room temperature. The suspension was filtered through a celite pad and the filtrate was concentrated to give a residue, which was used without further purification. It was dissolved in CH_2Cl_2 (50 mL) at −10 °C. *N*-Fmoc-Ala-OH (0.90 mmol, 280 mg) and EEDQ (1.35 mmol, 333 mg) were successively added. The solution was then allowed to warm up to room temperature and stirring was maintained overnight. The reaction mixture was then diluted with CH_2Cl_2, washed with 1M HCl, dried over $MgSO_4$ and evaporated to give a residue, which was purified by flash chromatography, eluting with MeOH–CH_2Cl_2 (1 : 10), to furnish the expected *N*-Fmoc-Ala-(β-lactam)-Aib-OBn (390 mg, 80%) as a white solid, mp 98–99 °C. $[\alpha]_D^{25}$ −20.1 (*c* 1.01 in CH_2Cl_2); (Found: C, 72.73; H, 6.306; N, 6.56. $C_{39}H_{39}N_3O_6$ requires C, 72.54; H, 6.09; N, 6.51%); ν_{max}(KBr)/cm^{-1} 3292 (NH), 1734, 1717 (C═O); δ_H(500 MHz, $CDCl_3$) 1.26–1.33 (3H, m, Ala CH_3), 1.38 (3H, s, Aib CH_3), 1.46 (3H, s, Aib CH_3), 3.12–3.17 (2H, m, CH_2Ph), 3.42 (1H, d, *J* 5.0, lactam CH_2), 3.59 (1H, br d, *J* 3.0, lactam CH_2), 4.14–4.21 (2H, m, Fmoc C*H* + Ala C*H*), 4.35–4.41 (2H, m, Fmoc CH_2), 5.09–5.14 (3H, m, OCH_2Ph + Ala N*H*), 6.47 (1H, s, N*H*), 7.22–7.25 (5H, m, Ph), 7.31–7.33 (7H, m, Ph, Fmoc), 7.40 (2H, t, *J* 7.5, Fmoc), 7.57 (2H, d, *J* 7.0, Fmoc), 7.77 (2H, d, *J* 7.5, Fmoc); δ_C(125 MHz, $CDCl_3$) 172.4, 172.2, 166.6, 155.8, 143.8, 141.3, 135.4, 134.9, 130.2, 128.6, 128.4, 128.3, 128.1, 127.7, 127.2, 127.1, 125.0, 120.0, 67.2, 67.1, 65.8, 59.0, 49.7, 47.1, 38.7, 23.6, 23.55, 18.5; HRMS (ESI) calcd. for $C_{39}H_{40}N_3O_6$ $[M+H]^+$ 646.2917, found 646.2938.

This benzyl ester (0.14 mmol, 90 mg) was added to a suspension of 10% Pd/C in MeOH under H_2, which was stirred for 1 h. After filtration through a celite pad and evaporation of the filtrates, the β-lactam **11** (77 mg, 100%) was obtained as a white solid, degradation at 101 °C. $[\alpha]_D^{25}$ −0.3 (*c* 0.49 in CH_3OH); ν_{max}(KBr)/cm^{-1} 3404 (OH, NH), 1728, 1710, 1690, 1679 (C═O); δ_H(500 MHz, CD_3OD) 1.22 (3H, s, Aib CH_3), 1.32 (3H, d, *J* 6.8, Ala-CH_3), 1.36 (3H, s, Aib CH_3), 3.16 (2H, s, CH_2Ph), 3.44 (1H, d, *J* 5.0, lactam CH_2), 3.60 (1H, d, *J* 5.0, lactam CH_2), 4.16 (1H, q, *J* 6.8, Ala C*H*), 4.21 (1H, t, *J* 6.2, Fmoc-C*H*), 4.36 (2H, d, *J* 6.2, Fmoc-CHCH_2), 7.21–7.33 (7H, m, Fmoc+Ph), 7.39 (2H, t, *J* 7.5, Fmoc), 7.66–7.67 (2H, m, Fmoc), 7.80 (2H, d, *J* 7.5, Fmoc); δ_C(125 MHz, CD_3OD) 176.1, 175.5, 168.9, 158.2, 145.2, 142.6, 136.2, 131.5, 129.4, 128.8, 128.2, 126.2, 120.9, 68.0, 67.1, 60.1, 51.7, 50.3, 48.5, 48.4, 39.4, 24.0, 18.3; HRMS (ESI) calcd. for $C_{32}H_{33}N_3O_6Na$ $[M+Na]^+$ 578.2267, found 578.2258.

General procedure for solid-phase peptide synthesis (SPPS). The synthesis of all peptides was carried out on solid phase, following a Fmoc strategy and standard SPPS protocols. Briefly, to a solution of *N*-Fmoc-Val-OH (10 equiv. relatively to resin loading) in CH_2Cl_2 (a few drops of DMF were required to ensure complete dissolution) under N_2 was added di-*iso*-propylcarbodiimide (DIC, 5 equiv.). The reaction mixture was stirred for 20 min at 0 °C. At the same time, the resin was suspended in CH_2Cl_2 and allowed to swell for 20 min. After filtration, the *N*-Fmoc-Val anhydride solution and DMAP (0.1 equiv. relatively to the anhydride) were added, and the resin was shaken 4 h. After filtration, the resin was successively washed twice with DMF, MeOH, and CH_2Cl_2 and again with DMF. The Fmoc protecting group was removed by using twice a solution of piperidine in DMF (1 : 4, v/v) for 3 min, then for 7 min. After filtration, the resin was successively washed as before. The SPPS was continued using *N*-Fmoc amino acids (3 equiv.) in the presence of HBTU (3 equiv.) and DIEA (5 equiv.) in DMF. Each coupling reaction was performed for 45 min, after which time the resin was washed as described above. After final deprotection and cleavage from the resin, all peptides were checked for purity by RP-HPLC and purified by semi-preparative RP-HPLC.

Solid-phase synthesis of peptides 12–14. The H-(D)ILTV peptide construct on Wang resin (300 mg, 0.054 mmol) was split into three equal fractions that were pre-swelled in DMF and to which were respectively added a solution of the β-lactam scaffolds **7**, **8** or **11** (1.5 equiv. relatively to the resin loading) in DMF, a solution of DIEA (2.2 equiv.) in DMF and a solution of HBTU (1.5 equiv.) in DMF. The resins were shaken overnight and were washed as described in the general procedure. After standard removal of the Fmoc-protecting group and introduction of the two last amino acids (*N*-Fmoc-Leu-OH and *N*-Boc-βAla-OH), final deprotection and cleavage from the resins was carried out using a TFA-H_2O-TIS mixture (95 : 2.5 : 2.5, v/v/v) for 4 h. Peptides were precipitated using cold Et_2O and then lyophilized. After purification by semi-preparative RP-HPLC, peptides **12**, **13** and **14** were obtained in correct yields: 31%, 8% and 32%, respectively (see ESI† for characterization data).

Solid-phase synthesis of β-lactam peptide hybrids 1–3. These peptidomimetics were synthesized on an amino-PEGA resin (0.4 mmol g^{-1}). Briefly, *N*-Fmoc-Gly-OH (3 equiv.) and *para*-hydroxymethylbenzoic acid (3 equiv.) were successively introduced, followed by the two first amino-acids (*N*-Fmoc-Val-OH and *N*-Fmoc-Thr(*t*Bu)-OH) as described above. The resin was then suspended in a solution of DCM-TFA (50 : 50, v/v) for 2 h. After filtration, the resin was washed with DCM, MeOH and again with DCM. A solution of pyridine–acetic anhydride (1 : 1, v/v) was then added and the resin was shaken for 30 min. After filtration, the resin was washed as described in the general procedure. After introduction of the two next amino acids (*N*-Fmoc-Leu-OH and *N*-Fmoc-(D)-Ile-OH), the resin was split into three equal fractions. Again, after Fmoc protecting group removal, solutions of **7**, **8** or **11** (1.2 equiv.) in DMF (3 mL), HBTU (1.2 equiv.) and DIEA (3 equiv.) were added to each resin and let react overnight. After washings, the two last amino acids were successively introduced (*N*-Fmoc-Leu-OH and *N*-Fmoc-β-Ala-OH). The last Fmoc protecting group was finally removed and cleavage from the resins was carried out using a 0.1 M NaOH aqueous solution for 3 h. Each resin was filtrated off and washed once with 0.1 M NaOH. The filtrates were then acidified with acetic acid until pH = 4 and lyophilized. After purification by semi-preparative RP-HPLC without any acid in the eluent system to prevent β-lactam ring opening, peptidomimetics **1–3** were respectively obtained in 3%, 3% and 10% yields (see ESI† for characterization data).

Peptide-MHC binding assay

The mutant LCL T-lymphoblastoid hybrid cell line 174xCEM.T2 (referred to as T2 cells) is an antigen-presentation mutant line. The cells express stable HLA-A2 molecules on their surface upon addition of an exogenous HLA-A2-binding peptide. T2 cells were incubated with pseudopeptides **1–3** or **12–14** in AIM V serum media (Invitrogen Co., Carlsbad, California) at 26 °C for 16 h, followed by incubation at 37 °C for 2 h. Quantification of stable surface HLA was analysed by surface staining using the HLA-A2-specific BB7.2 antibody (from the ATCC hybridoma HB-82), and measured as mean fluorescent intensity on a FACSCanto II apparatus (Becton Dickinson, Mountain View, CA).

T cell stimulation

A Melan-A T cell line was pulsed with the endogenous peptide EAAGIGILTV (EAA) and combined with 1 μL mL^{-1} of brefeldin A (Becton Dickinson, Mountain View, CA) to inhibit ER transport. The cells were then incubated for 14 h at 37 °C, washed twice with PBS and stained with 1 μg of antigen presenting cell (APC)-labelled Melan-A-HLA-A2 tetramer for 30 min at 4 °C. The cells were then washed twice using PBS and resuspended in 100 μL of Cytofix/Cytoperm solution (Becton Dickinson, Mountain View, CA). 2μL of anti-INFγ PE-labeled mAb, 2μL anti-CD107a FITC-labeled mAb and 2μL of anti-CD8 PCP-labeled mAb (Becton Dickinson, Mountain View, CA) were added to the cells and incubated for 20 min at 4 °C. The cells were then washed twice with Perm/Wash (Becton Dickinson, Mountain View, CA), resuspended in 100 μL of Cytofix/Cytoperm solution and run on the FACSCantoII (Becton Dickinson, Mountain View, CA).

Thermal stability measurement

Circular dichroism measurements of thermal stability were performed using an Aviv 62DS instrument, monitoring a wavelength of 218 nm as previously described.[28] Solution conditions were 20 mM phosphate and 75 mM NaCl (pH 7.4). Protein concentrations were approximately 10 micromolar. The temperature increment was approximately 0.3 °C min^{-1}. As unfolding of HLA-A2 is irreversible, CD data were fit to a six order polynomial, and the apparent T_m was taken from the first derivative of the fitted curve.

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