Design, Synthesis, and Evaluation of a Pyrrolinone−Peptide Hybrid Ligand for the Class II MHC Protein HLA-DR1

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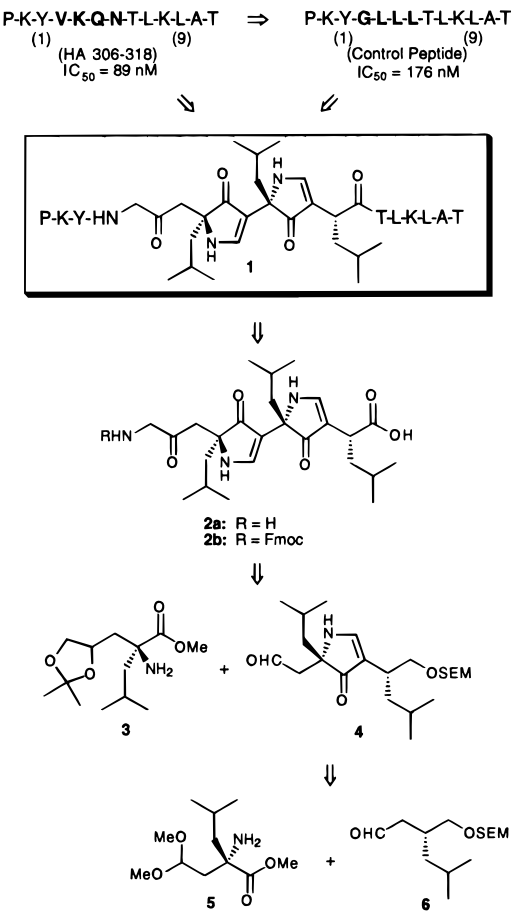
In 1992, we reported the design and synthesis of nonpeptide β-strand peptidomimetics based on the 3,5,5-pyrrolin-4-one scaffold.[1a] These polypyrrolinones, which are stable to proteases, adopt extended conformations both in solution and in the solid state that mimic the antiperiplanar disposition of the carbonyls and side chains of peptidal β-strands. More recently, we successfully exploited the pyrrolinone scaffold to construct potent, bioavailable, small-molecule inhibitors of the HIV-1 protease.[1b−d] In this communication, we disclose the design and synthesis of a pyrrolinone-based peptide hybrid which proved to be a competent ligand for the rheumatoid arthritis-associated class II major histocompatibility complex protein HLA-DR1, thereby further demonstrating the utility of the pyrrolinone scaffold.

The primary role of the class II major histocompatibility complex (MHC), an extracellular polymorphic[2] membrane-bound protein widely dispersed on B lymphocytes, macrophages, and other specialized antigen-presenting cells,[3] is to present antigenic peptides[4] derived from extracellular pathogens and toxins for inspection by CD4 T cells of the immune system. Inhibition of this process holds considerable promise for the treatment of numerous autoimmune diseases.[5]

Scheme 1



The recent X-ray crystal analysis by Wiley and co-workers[6a,b] revealed that the HLA-DR1 MHC class II protein binds the influenza virus hemagglutinin peptide fragment PKYVKQN-TLKLAT (HA 306-318)[6b] in an extended, β-strand-like conformation. The presence of anchor residues at positions 1 and 9 (Scheme 1) in conjunction with a complex network of hydrogen bond interactions between the HA 306-318 backbone and the amino acid side chains in the DR1 MHC binding site are postulated to account for a large portion of the binding energy.[4,7]

On the basis of alanine scans of HA 306-318 and related 7-mer peptide ligands,[8] we postulated that the side chains of the **VKQN** sequence in HA 306-318 could be altered without significantly affecting HLA-DR1 binding. Indeed, control peptide PKY**GLLL**-TLKLAT bound to the HLA-DR1 protein with an IC_{50} of 176 nM, comparable to an IC_{50} of 89 nM for the native HA 306-318 peptide. On the basis of this information, we speculated that bispyrrolinone **2a** (Scheme 1) might serve as a viable replacement for the amide backbone of the **VKQN** sequence of HA 306-318. Molecular modeling revealed that the global low-energy conformations of the bispyrrolinone segment of **1** and the corresponding region of HA 306-318 bound to HLA-DR1 would be quite similar. Moreover, with the exception of a hydrogen bond between Gln-9 of HLA-DR1 and the N-H at position 4 of HA 306-318, the hydrogen bonding network was predicted to remain intact. Integral to this design was the expectation that Fmoc derivative **2b** would permit use of Fmoc-based solid-phase peptide synthesis to construct the hybrid ligand **1**.

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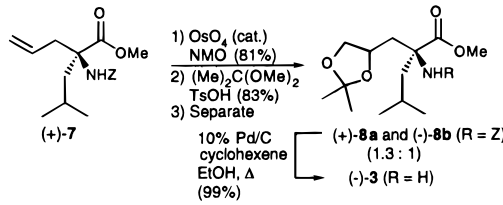
The synthesis of bispyrrolinone **2b** (Scheme 1) was envisioned to involve two iterations of our now well-established pyrrolinone synthetic protocol,[1] beginning with the construction of amino ester **3** (Scheme 2). Treatment of (+)-**7**,[9] available from D-leucine via

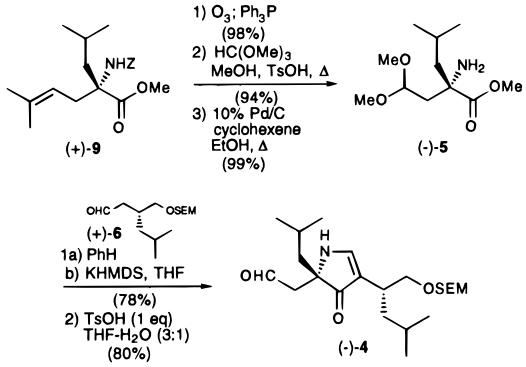
Scheme 2



the Seebach/Karady oxazolidinone,[1,10] with OsO_4 and NMO[11] provided a mixture (1.3:1) of diastereomeric diols. Protection as the acetonides followed by separation and transfer hydrogenolysis[12] of the less polar diastereomer (+)-**8a**[9] gave (−)-**3**.[9]

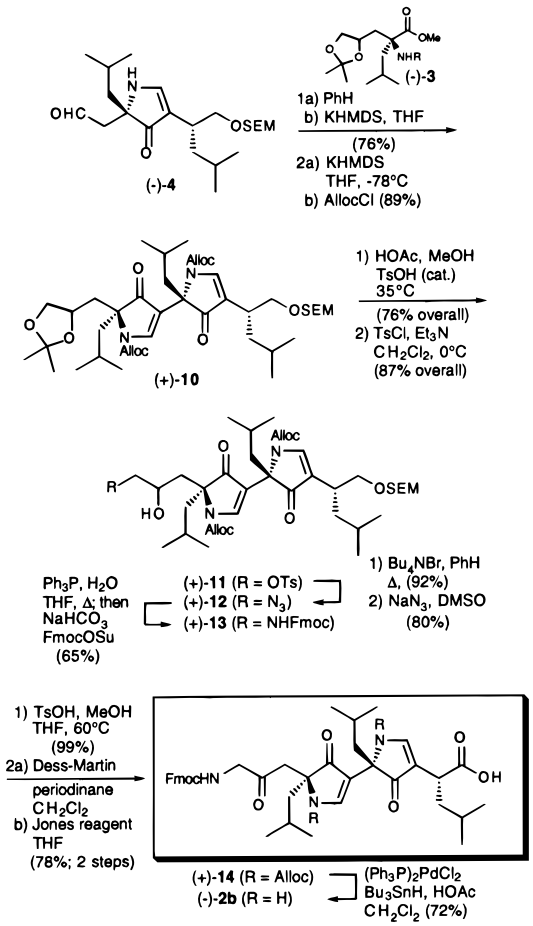
Preparation of aldehyde **4** (Scheme 3) entailed ozonolysis of (+)-**9**,[9,13] followed by protection of the derived aldehyde and transfer hydrogenolysis[12] to provide amino ester (−)-**5**.[9] Condensation with aldehyde (+)-**6**,[9,14] to form the imine, followed in turn by treatment with KHMDS (4 equiv) and acid gave aldehyde (−)-**4**;[9] the overall yield was 57% (five steps).

Scheme 3



A second iteration of the pyrrolinone protocol employing (−)-**3** and (−)-**4**, followed by protection of the pyrrolinone nitrogens with the Alloc moiety, furnished (+)-**10**[9] (Scheme 4). Removal of the acetonide and conversion of the primary alcohol in turn to the tosylate, bromide, and azide provided (+)-**12**.[9] Reduction (Ph_3P)[15] followed by in situ protection of the resulting amine using FmocOSu then gave (+)-**13**.[9] A two-step oxidation with the Dess−Martin periodinane[16] and Jones reagent, following removal of the SEM protecting group, provided keto-acid (+)-**14**.[9] Removal of the Alloc protecting groups[17] completed the synthesis of bispyrrolinone (−)-**2b**.[9]

Scheme 4



Construction of the TLKLAT peptide and incorporation of bispyrrolinone (−)-**2b** proceeded smoothly on Wang resin employing Fmoc-based solid-phase peptide synthesis. Removal of the N-terminal Fmoc group, addition of the remaining amino acids (PKY), and liberation of the pyrrolinone−peptide hybrid from the resin with concomitant protecting group removal completed the synthesis of **1**.[18]

Affinity binding experiments[19] revealed that pyrrolinone−peptide hybrid **1** was indeed a competent ligand for HLA-DR1, having an IC_{50} of 137 nM, compared with 89 nM for the HA 306-318 peptide and 176 nM for the control peptide. The similar binding affinity of **1** relative to HA 306-318 is intriguing given that the N-terminal pyrrolinone, due to displacement of the backbone N-H, is unable to form a hydrogen bond with Gln-9 of the class II MHC HLA-DR1 protein in contrast to HA 306-318. The similar binding affinity is also of interest when one considers that the carbonyl at position 2 of **1**, required only for synthetic purposes, is not expected to be as effective a hydrogen bond acceptor as the backbone amide at position 2 of HA 306-318. Notwithstanding these considerations, the similar binding affinities of **1** and the native and control peptides suggest that additional compensating interactions may be formed. We hope to address this issue through X-ray crystallographic studies of the hybrid ligand bound to the class II MHC protein HLA-DR1.

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Supporting Information Available: Spectroscopic and analytical data for **1**, **2b**, **3**−**6**, **8a**, **8b**, and **10**−**14** and selected experimental procedures (17 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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(18) The assigned structure was in accord with its amino acid analysis as well as appropriate parent ion identification by mass spectrometry.
(19) The binding affinity of the pyrrolinone−peptide hybrid **1** to HLA-DR1 was assessed using a scintillation proximity assay (SPA) protocol in which an ^{125}I-peptide was competitively displaced from HLA-DR1 (isolated from LG2 cells, purified, and coupled to SPA beads via an LB3.1 antibody). See: Ito, K.; Bian, H.-J.; Molina, M.; Han, J.; Magram, J.; Saar, E.; Belunis, C.; Bolin, D. R.; Arceo, R.; Campbell, R.; Falcioni, F.; Vidovic, D.; Hammer, J.; Nagy, Z. A. *J. Exp. Med.* **1996**, *183*, 2635.