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# P3 and P4 position analysis of vinyl ester pseudopeptide proteasome inhibitors

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**Abstract**—Two small libraries of tripeptidic-based vinyl ester derivative proteasome inhibitors were synthesized and tested, starting with the Hmb-Val-Gln-Leu-VE prototype. The P3 and P4 positions were investigated with a complete set of amino acid residues, some of which showed remarkable selective inhibition of the trypsin-like (β2) subunit. In both positions, aromatic and hydrophobic residues were preferred.


The proteasome is a multicatalytic protease complex, which represents the central enzyme of intracellular protein degradation in prokaryotes and eukaryotes. It is involved in many biological processes, including the removal of abnormal, misfolded or improperly assembled proteins, and has a key role in the stress response, cell cycle control and differentiation. In addition, metabolic adaptation and generation of peptide antigens for presentation by major histocompatibility complex (MHC) class I molecules to $CD8^+$ cytotoxic T cells[1,2] are linked to an ubiquitin- and ATP-requiring protein degradation pathway involving the 26S proteasome (2.4 MDa). The 26S proteasomes are made up of a cylinder-shaped multimeric protein complex, whose core and proteolytic chamber is the 20S proteasome, capped at each end by a regulatory component termed 19S. The 20S proteasome consists of four stacked rings, where each of the two inner rings is composed of seven different β subunits. Each β-ring contains three different proteolytically active sites: the β1 subunit, which contains a post-acidic (PGPH) active site, the β2 subunit, which is associated with a trypsin-like (T-L) activity, and the β5 subunit, which has a chymotrypsin-like (ChT-L) proteolytic function. All the proteolytic sites utilize an N-ter-

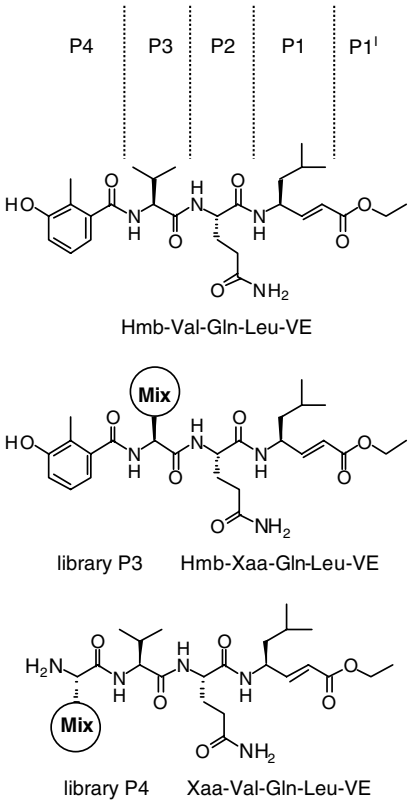


**Figure 1.** Structures of the reference inhibitor and libraries P3 and P4. Variable positions that contain a mixture of amino acids are indicated as Mix.

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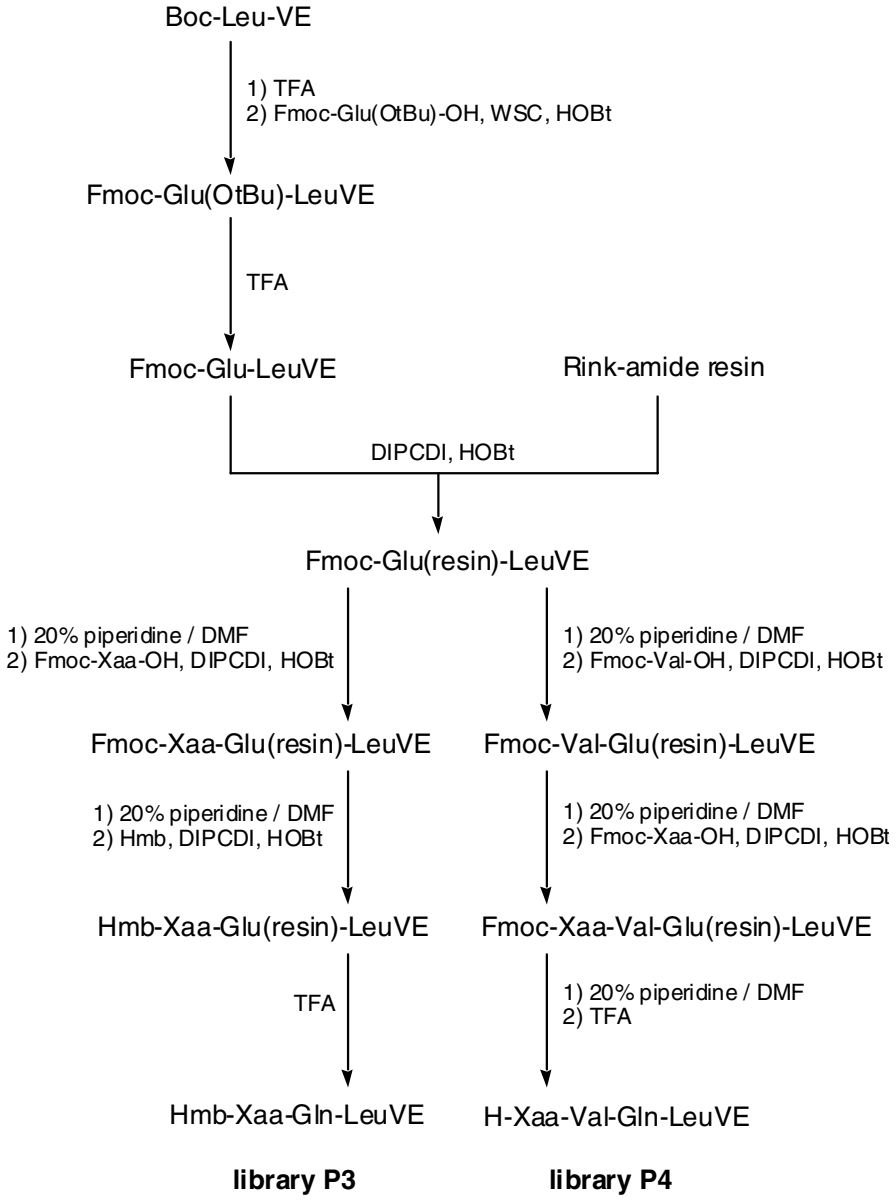
minal threonine residue of β subunits as nucleophile, employing a catalytic mechanism similar to those of the serine proteases.[3]

The development of proteasome inhibitors into novel therapeutic agents represents a stimulating approach in the treatment of many disease states including inflammation and cancer, and for the modulation of immune responses.[4] Proteasome inhibitors are usually short peptides linked to a C-terminal pharmacophore, which is responsible for the interaction with catalytic threonine.[5]

Once synthesized and tested, a series of peptide-based derivatives were found to be the peptidyl portion which derives from a screening of tripeptide sequences.[6] Vinyl ester moiety has been considered a potential substrate for the catalytic threonine.[7] The more efficacious of the series, Hmb-Val-Gln-Leu-VE, showed good inhibition, favourable pharmacokinetic properties, and remarkable selectivity for the trypsin-like activity of the 20S proteasome. These compounds increased the generation and presentation of subdominant MHC class I CTL epitopes without affecting cell viability suggesting that they may find application as immunomodulators.

The following presents a study of the P3 and P4 positions of the reference compound, employing small libraries of pseudotripeptides with the C-terminal ethyl acrylate group. These can function as substrates of catalytic threonine in Michael addition in the same way that has been suggested for the well-known peptide vinyl sulfone inhibitors.[8] As compared to the prototype, both small libraries bear a glutamine in P2 position in order to promote selectivity for the β2 subsite, in similar fashion to that previously carried out for vinyl sulfone derivatives.[9] P3 and P4 libraries contain a complete set of




**Scheme 1.** Synthesis of vinyl ester pseudopeptide libraries P3 and P4.