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Potent New Leucine Aminopeptidase Inhibitor of Novel Structure Synthesised by a Modified Wadsworth–Emmons (Horner) Wittig Procedure

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Abstract—The use of a leucine-derived α-keto-β-aldehyde (glyoxal) as a substrate in the Horner–Emmons (Wadsworth) Wittig reaction has enabled the synthesis of (*Z*)-7-methyl-5(*S*)-amino-4-oxo-methyl-oct-2-eneoate. This novel compound is a potent inhibitor ($K_i = 76$ nM) of leucine aminopeptidase and provides an interesting new template for the development of metallopeptidase inhibitors. © 2000 Elsevier Science Ltd. All rights reserved.

Metallopeptidases, such as the matrix metalloproteases (MMPs), angiotensin converting enzyme (ACE) and carboxypeptidase A, constitute an important family of peptidolytic enzymes.[1]

One such group of metallopeptidases which have received considerable attention are the aminopeptidases. These enzymes selectively remove the N-terminal residue from peptides and proteins, which in many instances profoundly affects the biological activity of the target molecule.[2] For example, aminopeptidases have been shown to cleave enkephalins and vasoactive peptides[3] and more recently, methionine aminopeptidase has been shown to be a promising target for the development of anti-angiogenic agents for the treatment of cancer.[4] Whilst there have been numerous reports of aminopeptidase inhibitors based on the chelating groups mentioned above, the majority of medicinal chemistry programs have concentrated on the development of modified analogues of bestatin, which contains an unusual β-amino-α-hydroxyamino acid residue.[5]

We now wish to report the fortuitous discovery of an exceptionally potent new inhibitor of leucine aminopeptidase. This new compound, (*Z*)-7-methyl-5(*S*)-amino-4-oxo-methyl-oct-2-eneoate, the *cis* isomer of leucine α-keto-β-unsaturated methyl ester (**3**) may have applicability, not only for the development of new aminopeptidase inhibitors but also in the wider field of other metalloenzyme inhibitors.

We have previously described the synthesis of *N*-α-protected amino acid and peptide derived α-keto-β-aldehydes, generated by in situ oxidative cleavage of α-diazoketone precursors[6] as inhibitors of the serine, cysteine and threonine proteases[7,8] and have also endeavoured to utilize these compounds as substrates or synthons as replacements for aldehydes and/or ketones in selected reactions.[9] This paper reports an interesting example of such an approach, namely, the utilization of an amino acid derived α-keto-β-aldehyde as an alternative substrate in the Wadsworth–Emmons (Horner) modification of the Wittig olefination reaction.[10] This reaction, in which a phosphorane ylide is replaced by a phosphonate ylide, has attracted considerable attention for synthetic transformations[11] as these ylides are considerably more reactive than their phosphorane counterparts. As a result, such agents can readily react with ketone and aldehydic carbonyl groups unlike phosphorane ylides which are generally inert with the former.

N-α-Boc-(L) leucine was converted to a diazomethyl ketone derivative by the general method of McKervey and Ye.[12] Treatment of this compound with dimethyldioxirane in moist acetone[6] leads to formation of the corresponding α-keto aldehyde analogue (Boc-Leu-COCHO).

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Treatment of Boc-Leu-COCHO with 1 equivalent of trimethylphosphonoacetate and *n*-butyllithium led to the generation of the (*E*) and (*Z*) forms of Boc-Leu-α-keto-β-unsaturated-methyl ester (**1**) and (**2**). It was somewhat surprising that, despite the additional reactivity of phosphonate ylides, subsequent analysis revealed that reaction had only occurred at the aldehydic carbonyl group (Fig. 1).[13]

NMR analysis of olefinic proton signals allowed us to calculate a (*Z*):(*E*) isomer ratio of 16:84. Separation of the isomers afforded both products as yellow oils. The structural characteristics of (**3**) led us to consider this compound as an entirely new potential metal chelating moiety as the configuration of the two carbonyls coupled with the likely conjugation of the enedione ester system would result in physiochemical characteristics ideally suited to binding the active site zinc atom of leucine aminopeptidase. Removal of the *N-tert* butoxycarbonyl protecting group thus afforded an agent with a number of pharmacophores known to be essential for the successful inhibition of leucine aminopeptidase:

1. The presence of a free amino group — essential for substrate binding to aminopeptidase-like enzymes.
2. A bulky aliphatic side chain (isobutyl) which can occupy the primary specificity pocket (S_1)[14] of the enzyme.
3. A potential metal chelating moiety — to coordinate the active site zinc atom.

To test the validity of this hypothesis we also prepared the deprotected (*E*) isomer for kinetic evaluation against LAP. Continuous spectrophotometric measurement and kinetic analysis of LAP-catalysed cleavage of the synthetic substrate L-leucyl aminomethylcoumaryl amide[15,16] in the presence of (**3**), illustrated that this compound was an excellent inactivator of the peptidase ($K_i = 76$ nM). This value compares favourably with previous amino acid derived inactivators, such as glycine hydroxamate and leucinethiol.[17,18] It was pleasing to note that the corresponding (*E*) isomer was completely devoid of inhibitory activity, even at concentrations as high as 200 μM. As a consequence of these observations, we tentatively suggest that this is due to the (*Z*) enedione ester group co-ordinating the active site zinc atom, thus effectively preventing substrate hydrolysis.

In addition to its potential as a molecular probe to delineate the role of leucine aminopeptidase in cellular and biological systems, we also believe that the core (*Z*) α-keto-β-unsaturated alkyl ester structure may be an attractive new pharmacophore for the development of novel metallopeptidase inhibitors.[19]

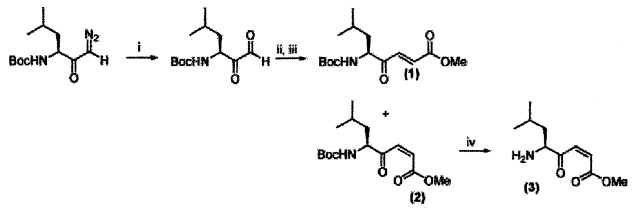

Figure 1. Synthesis of 7-methyl-5(*S*)-amino-4-oxo-methyl-oct-2-eneoate. (i) dimethyldioxirane in moist acetone; (ii) *n*-butyllithium, THF, 0 °C; (iii) $(MeO)_2P(O)CH_2COOMe$, THF, −78 °C; (iv) 50% (v/v) TFA/DCM.

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13. Boc-Leu-CH=CHCOOMe (*Z*) isomer: ^{1}H (300 MHz, $CDCl_3$) 0.95 (6H, d, $J = 5.4$ Hz, $CH(CH_3)_2$), 1.44 (9H, s, $C(CH_3)_3$), 1.60–1.74 (3H, s, OCH_3), 4.47 (1H, m, *CH*(N)CO), 5.00 (1H, br s, N*H*), 6.12 (1H, d, $J = 12.0$ Hz, CH = *CH*COOMe), 6.59 (1H, d, $J = 12.0$ Hz, *CH* = CHCOOMe); (*E*) isomer: ^{1}H (300 MHz, $CDCl_3$) 0.94 (3H, d, $J = 6.6$ Hz, $CH(CH_3)_2$), 0.99 (3H, d, $J = 6.4$ Hz, $C(CH_3)_3$), 1.44 (9H, s, $C(CH_3)_3$), 1.52–1.77 (3H, m, $CH_2CH(CH_3)_2$), 4.56 (1H, m, *CH*(N)CO), 5.03 (1H, m, N*H*), 6.82 (1H, d, $J = 16.1$ Hz, CH = *CH*COOMe), 7.22 (1H, d, $J = 15.8$ Hz, *CH* = CHCOOMe). MS(ES) $(M+H)^+ = 300.23$ (expected 299.17).
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19. Abbreviations used: Boc-*t*-butyloxycarbonyl; LAP leucine aminopeptidase; TFA trifluoroacetic acid.