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SYNTHESIS OF A POTENT, REVERSIBLE INHIBITOR OF INTERLEUKIN-1β CONVERTING ENZYME

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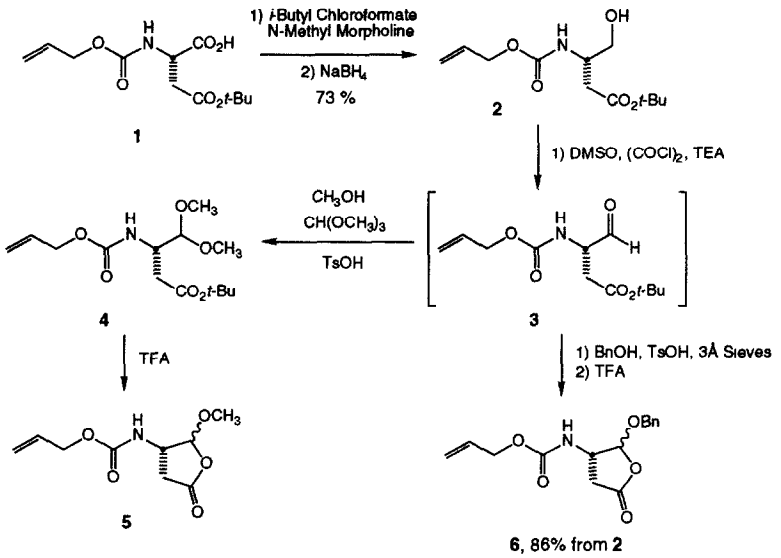
Abstract: An efficient synthesis of L - 709,049, a potent inhibitor of Interleukin-1β converting enzyme (ICE) is reported. The aspartic acid aldehyde moiety is protected as the corresponding O-benzylacylal which can be conveniently coupled to AcTyrValAla and hydrogenolyzed to afford the desired product.

Interleukin-1β (IL-1β) is a cytokine implicated in the pathogenesis of chronic and acute inflammatory disease. Evidence that IL-1β activity is an excellent target for therapeutic intervention has recently been obtained in studies employing a natural 22 kD IL-1 receptor antagonist,[1-4] soluble IL-1 receptor,[5] and specific anti IL-1 receptor antibodies.[6] Kostura[7] and Black[8] have identified an endogenous proteolytic activity capable of cleaving the biologically inactive precursor of IL-1β (pre-IL-1β) to the mature form at the natural Asp^{116} - Ala^{117} site. An attractive strategy for the prevention of IL-1 mediated disease is to interfere with this processing step. Recently, we have demonstrated that this activity is associated with a heterodimeric cysteine protease which we refer to as Interleukin-1β Converting Enzyme or ICE.[9] We have further demonstrated that inhibition of ICE in whole human blood by a potent, reversible inhibitor prevents processing of IL-1β to the active form.[9] We describe here an efficient synthesis of that inhibitor, L - 709,049.

Peptide aldehydes are potent, reversible, transition state inhibitors of cysteine proteases.[10] This class of compound thus provides ideal targets for inhibition of ICE. Substrate specificity data has established that ICE requires four residues to the N-terminal side of the scissile bond (P_4-P_1) in order to process a substrate.[9,11,12] In human pre-IL-1β, those residues are Tyr–Val–His–Asp. In addition, we have found that an alanine for histidine substitution in the P_2 position effects a modest increase in V_{max}/K_m for substrates.[9] Accordingly, we elected to synthesize the tetrapeptide aldehyde AcTyrValAlaAspCHO.

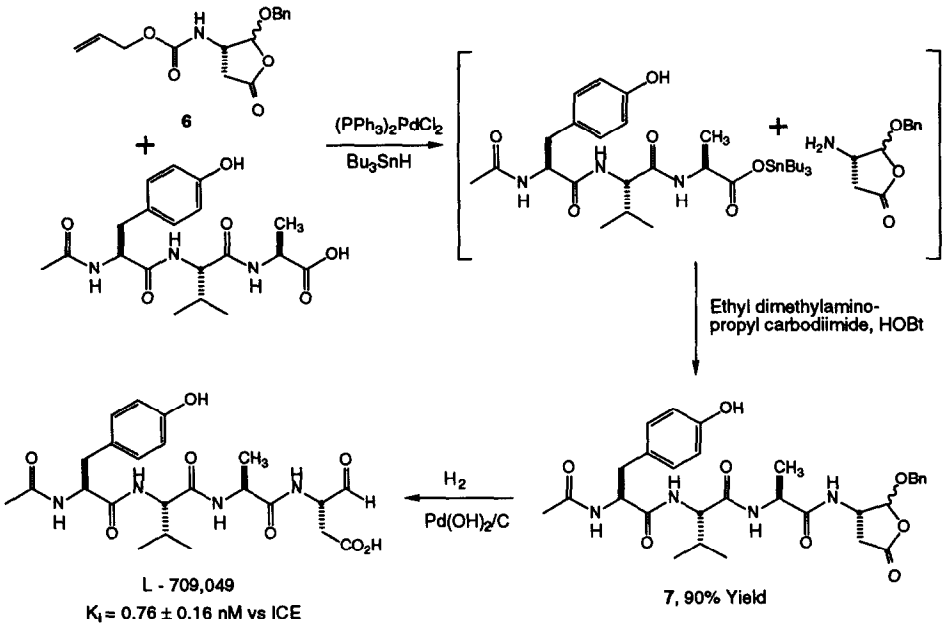
Scheme I



Our initial plan was to protect the aspartic acid aldehyde as the corresponding dimethy' acetal *tert*-butyl ester, couple to AcTyrValAla, and sequentially deprotect. The protected aspartic acid was synthesized as described in Scheme I. Commercially available aspartic acid β-*tert*-butyl ester was N-protected with allylchloroformate in the presence of sodium bicarbonate. The carboxylic acid was then converted to a mixed anhydride with isobutyl chloroformate and N-methylmorpholine, and reduced to the corresponding alcohol with sodium borohydride. The alcohol was oxidized to the aldehyde under Swern conditions,[13] and immediately treated with methanol, trimethyl orthoformate, and *p*-toluenesulphonic acid to provide the desired fully protected aspartic acid aldehyde **4**. At this stage, we wanted to verify that this precursor could be easily deprotected. Treatment of **4** with TFA rapidly and quantitatively afforded the O-methylaclyal **5**. Acid hydrolysis of **5** afforded the desired aldehyde acid, but we were unable to accomplish this reaction cleanly and in high yield. Since we could obtain the O-methylacylal so cleanly, however, we felt that the corresponding O-benzylacylal might be accessible, and would be an ideal precursor to aspartic acid aldehyde. Thus, Alloc-aspartic acid β-*tert*-butyl ester carboxaldehyde generated as before was treated with benzyl alcohol and *p*-toluenesulphonic acid in the presence of 3Å molecular sieves. After 16 hours, addition of trifluoroacetic acid to this solution effected cyclization to the desired O-benzylacylal which was isolated as a 1:1 mixture of diastereomers.

Completion of the synthesis of L - 709,049 is described in Scheme II. Removal of the Alloc protecting group could be accomplished using Bu_3SnH and a catalytic amount of $(PPh_3)_2PdCl_2$ in wet dichloromethane. Unfortunately, the resulting amine proved to be difficult to isolate and purify,

Scheme II



being somewhat volatile as well as being both acid and base sensitive. We circumvented this problem in the following way. Dangeles has demonstrated that removal of an Alloc group using Bu_3SnH and $(PPh_3)_2PdCl_2$ requires the presence of a proton donor such as water or acetic acid.[14] We reasoned that use of AcTyrValAla as the proton source for Alloc removal, followed by addition of a coupling reagent should effect *in situ* conversion to the desired tetrapeptide without isolation of the intermediate amine. In practice this reaction sequence works quite well. When O-benzylacylal **6** is treated with Bu_3SnH and $(PPh_3)_2PdCl_2$ in the presence of 1.1 equivalents of AcTyrValAla, the Alloc group is rapidly removed. Addition of hydroxybenzotriazole and ethyl dimethylaminopropyl carbodiimide (EDC) to this mixture cleanly affords the desired tetrapeptide **7** in 90% yield.[15] Hydrogenolysis of this tetrapeptide O-benzylacylal using Pearlman's catalyst in methanol followed by silica-gel chromatography affords the desired ICE inhibitor, L - 709,049.[16]

L - 709,049 is a potent, reversible inhibitor of ICE with a K_i of 0.76 ± 0.16 nM.[9] Using this compound we have established that inhibition of ICE in whole human blood prevents secretion of biologically active IL-1β.[9] We have also modified L - 709,049 to act as a ligand for affinity purification of ICE to homogeneity from crude THP-1 cell lysates.[9] The efficient synthesis of L - 709,049 which we have described will make this useful tool readily available to the scientific community.

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Experimental Section

N-Allyloxycarbonyl-3-amino-4-hyroxybutanoic acid *tert*-butyl ester (2). To a solution of N-allyloxycarbonyl (S)-aspartic acid β-*tert*-butyl ester (2.00 g, 7.32 mmol) in 50 mL of tetrahydrofuran (THF) at 0 °C, was added N-methyl morpholine (NMM, 885 μL, 8.05 mmol) followed by isobutyl chloroformate (IBCF, 997 μL, 7.68 mmol). After 15 min, this mixture was added to a suspension of sodium borohydride (550 mg, 14.55 mmol) in 50 mL of THF and 12.5 mL of methanol at –78 °C. After 2 h at –78 °C, the mixture was quenched with acetic acid, diluted with 1:1 ethyl acetate:hexane, and washed 3 times with dilute sodium bicarbonate. The organics were dried over sodium sulfate, filtered, and concentrated. The residue was purified by MPLC on silica-gel (35x350 mm column, 30% ethyl acetate/hexane) to give 1.39 g (5.34 mmol, 73%) of the desired product as a colorless oil: ^{1}H NMR (200 MHz, CD_3OD) δ 5.9 (m, 1H), 5.28 (br d, 1H, J = 17 Hz), 5.15 (br d, 1H, J = 9 Hz), 4.52 (br d, 2H, J = 6 Hz), 3.98 (m, 1H), 3.48 (ABX, 2H, J = 5, 6, 11 Hz), 2.53 (dd, 1H, J = 5, 16 Hz), 2.32 (dd, 1H, J = 9, 16 Hz), 1.43 (s, 9H).

N-Allyloxycarbonyl-4-amino-5-benzyloxy-2-oxotetrahydrofuran (6). To a solution of dimethylsulphoxide (1.86 mL, 26.16 mmol) in 30 mL of freshly distilled dichloromethane at –45 °C was added oxalyl chloride (1.24 mL, 14.27 mmol). After 5 min, a solution of N-allyloxycarbonyl-3-amino-4-hydroxybutanoic acid *tert*-butyl ester **II** (3.07 g, 11.89 mmol) in 20 mL of dichloromethane was added. After 15 min, diisopropyl ethylamine (6.21 mL, 35.67 mmol) was added and the mixture stirred at –23 °C for 30 min. The mixture was diluted with ethyl acetate and washed with water, 1 N sodium hydrogen sulfate, and three times with water. The organics were dried over sodium sulfate, filtered, and concentrated. The resulting colorless oil was dissolved in 7 mL of dichloromethane and 6.5 mL of benzyl alcohol. To this solution was added ~1 g of 3Å molecular sieves followed by a catalytic amount of *p*-toluenesulphonic acid. After 16 h, trifluoroacetic acid (~8 mL) was added, and the mixture stirred for 30 min and concentrated. The mixture was diluted with ethyl acetate and filtered through Celite®. The organics were then washed three times with dilute sodium bicarbonate, dried over sodium sulfate, filtered, and concentrated. The residue was purified by MPLC on silica-gel (35x350 mm column, using 20% ether in hexane as eluent until the benzyl alcohol came off, and then 10% ethyl acetate in 1:1 dichloromethane/hexane to elute the products) to afford 2.99 g (10.26 mmol, 86%) of the title compound as a mixture of two diastereomers which crystallized on standing: ^{1}H NMR (400 MHz, CD_3OD) δ 7.3 (m, 5H, Ar-H), 5.89 (m, 1H, C<u>H</u>=CH_2), 5.61 (d, 0.5H, C<u>H</u>OBn), 5.47 (d, 0.5H, C<u>H</u>OBn), 5.28 (br d, 1H, CH=CH<u>H</u>), 5.18 (br d, 1H, CH=C<u>H</u>H), 4.82 (2d's, 1H, C<u>H</u>$_2$Ph), 4.67 (2d's, 1H, C<u>H</u>$_2$Ph), 4.52 (m, 3H, C<u>H</u>$_2$OCO, C<u>H</u>N), 3.02 (dd, 0.5H, C<u>H</u>HCO_2), 2.74 (dd, 0.5H, CH<u>H</u>CO_2), 2.61 (dd, 0.5H, CH<u>H</u>CO_2), 2.45 (dd, 0.5H, CH<u>H</u>CO_2).

N-(N-Acetyltyrosinyl-valinyl-alaninyl)-4-amino-5-benzyloxy-2-oxotetrahydrofuran (7). To a solution of N-allyloxycarbonyl-4-amino-5-benzyloxy-2-oxotetrahydrofuran (1.00g, 3.45 mmol) and N-acetyltyrosinyl-valinyl-alanine (1.83g, 3.79 mmol) in 10 mL each of dichloromethane and DMF was added ~50 mg of $(PPh_3)_2PdCl_2$ followed by tri-n-butyltin hydride (1.02 mL, 3.79 mmol) dropwise over two min. An additional 800 μL of tri-n-butyltin hydride was added dropwise until the color of the reaction mixture had turned dark orange. Hydroxybenzotriazole (932 mg, 6.9 mmol) was added, and the mixture cooled to 0 °C. Ethyl dimethylaminopropyl carbodiimide (794 mg, 4.14 mmol) was added and the mixture allowed to warm slowly to ambient temperature. After 16 h, the mixture was diluted with ethyl acetate and washed three times with dilute hydrochloric acid, and three times with dilute sodium bicarbonate. To prevent precipitation of the tetrapeptide, the organic solution was not dried but concentrated. The residue was purified by MPLC on silica-gel (35x350 mm column, using a gradient of 1% pyridine in dichloromethane to 1% pyridine and 20% isopropanol in dichloromethane as eluent) to afford 1.80 g (90%) of the title compound as a colorless solid consisting of a 1:1 mixture of two diastereomers: ^{1}H NMR (400 MHz, CD_3OD) δ 7.5-7.2 (m, 5H, Ar-H), 7.04 (d, 1H, J = 8.58 Hz, Ar-H), 7.03 (d, 1H, J = 8.57 Hz, Ar-H), 6.67 (d, 2H, J = 8.57 Hz, Ar-H), 5.64 (d, 0.5H, J = 6.02 Hz, CHOBn), 5.45 (d, 0.5H, J = 1.16 Hz, CHOBn), 5.0-4.8 (m. 2H, OCH_2Ph, partially obscured by CD_3OH), 4.74-4.55 (m, 1.5H, CHN), 4.36 (m, 0.5H, CHN), 4.30 (q, 0.5H, J = 7.15 Hz, $CHCH_3$), 4.21 (q, 0.5H, J = 7.10 Hz, $CHCH_3$), 4.13 (d, 0.5H, J = 7.42 Hz, $CHCH(CH_3)_2$), 4.07 (d, 0.5H, J = 7.18 Hz, $CHCH(CH_3)_2$), 3.1-2.9 (m, 1.5H, CHHAr, $CHHCO_2$), 2.60 (dd, 0.5H, $CHHCO_2$), 2.45 (dd, $CHHCO_2$), 2.04 (M, 1H, $CH(CH_3)_2$), 1.91 (s, 1.5H, CH_3CO), 1.89 (s. 1.5H, CH_3CO), 1.33 (d, 1.5H, J = 7.14 Hz, $CHCH_3$), 1.27 (d, 1.5H, J = 7.34 Hz, $CHCH_3$), 1.0-0.9 (4d's, 6H, $CH(CH_3)_2$).

N-(N-Acetyl-tyrosinyl-valinyl-alaninyl)-3-amino-4-oxobutanoic acid (L - 709,049). To a solution of 900 mg of N-(N-Acetyltyrosinyl-valinyl-alaninyl)-4-amino-5-benzyloxy-2-oxotetrahydrofuran in 20 mL of methanol was added ~200 mg of 20% $Pd(OH)_2/C$. After 2.5 hours under hydrogen, the mixture was filtered and concentrated. The product was purified by MPLC on silica-gel (22x300 mm column, eluting with a gradient of dichloromethane to 8% formic acid and 32% methanol in dichloromethane) to afford 476 mg of the title compound as a colorless solid: ^{1}H NMR (400 MHz, CD_3OD, mixture of diastereomeric hemiacetals in this solvent) δ 7.04 (d, 2H, J = 8.30 Hz, Ar-H), 6.67 (d, 2H, J = 8.30 Hz, Ar-H), 4.58 (m, 2H, $CHOH(OCD_3)$, CHN), 4.33 (m, 1H, CHN), 4.24 (m, 1H, CHN), 4.14 (d, 1H, J = 7.28 Hz, $CHCH(CH_3)_2$), 3.01 (dd, 1H, J = 5.49, 14.02 Hz, CHHAr), 2.77 (dd, 1H, J = 9.03, 14.01 Hz, CHHAr), 2.63 (m, 1H, $CHHCO_2$), 2.48 (m, 1H, $CHHCO_2$), 2.05 (m, 1H, $CH(CH_3)_2$), 1.90 (s, 3H, CH_3CO), 1.33 (2d's, 3H, J = 7.15 Hz, $CH(CH)_3$), 0.94 (t, 6H, J = 6.96 Hz, $CH(CH_3)_2$); FAB Mass spectroscopy shows M+1 = 493, M+Li = 499, M+Na = 515; Analysis calculated for $C_{23}H_{32}N_4O_8 \cdot 1.5\ H_2O$: C, 53.17 H, 6.79 N, 10.78; found: C, 52.80 H, 6.81 N, 10.61.

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15. This *in situ* deprotection/coupling sequence has allowed us to couple several highly reactive amines to peptides in excellent yields. We will report these results in due course.

16. Interestingly, 400 MHz ^{1}H NMR in CD_3OD shows the presence of two diastereomeric hemiacetals and no epimerization at the α-carbon. We can detect no free aldehyde present in protic solvents suggesting a plausible reason for the stability of the C_α stereocenter.