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THE SYNTHESIS AND EVALUATION OF PEPTIDYL ASPARTYL ALDEHYDES AS INHIBITORS OF ICE.

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Abstract.
The tetrapeptide aldehyde Ac-Tyr-Val-Ala-AspH (1, L-709,049) has been reported to be a potent reversible inhibitor of Interleukin-1β Converting Enzyme (ICE). We have prepared a series of analogs of 1, in order to explore the active site of ICE. The effects of truncation, methylation of the amide nitrogens and modification of the aldehyde group of 1 are presented.

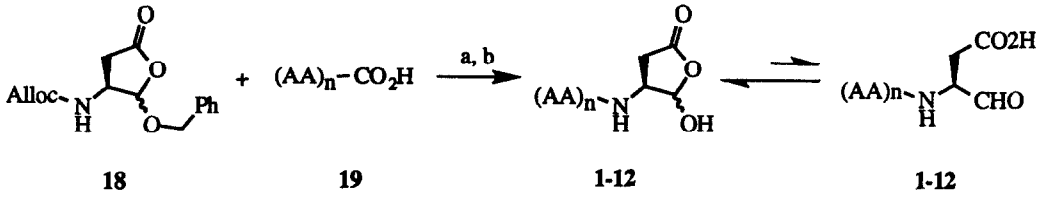
Introduction.
Interleukin-1β (IL-1β) is a potent inflammatory cytokine.[1] Interleukin-1β Converting Enzyme (ICE) is an intracellular cysteine protease which specifically processes the 31 kDa inactive precursor of IL-1β to the mature, active 17.5 kDa form (mIL-1β) at the Asp^{116}-Ala^{117} site.[2-6] Inhibitors of ICE have potential as therapeutic agents for the treatment of chronic inflammatory disease states.

Substrate specificity studies[2-6] have shown that P_4 to P_1 (Tyr-Val-His-Asp) are required to optimize V/K. Further, while P_2 tolerates a variety of amino acid side chain residues, P_1 must be Asp. Two laboratories have reported that peptide C-terminal aldehydes are potent ICE inhibitors, i.e. Ac-Tyr-Val-Ala-Asp-H (1, L-709,049)[7] and Z-Val-Ala-Asp-H.[8] In addition, it has been shown that truncation of P_4, P_3 and P_2 results in a significant decrease in enzyme inhibition potency.[8]

In the course of our research we have prepared a series of aldehyde-based compounds to explore the S_2 - S_4 subsites, and we have N-methylated all positions from S_1 to S_4 in order to explore the significance of the amide N-H groups. We have further prepared a number of compounds in which the aldehyde moiety was modified.

Chemistry.
Aldehydes **1-12** were prepared by coupling the O-benzylacylal **18** to various peptides **19** as described by Chapman[7] (Scheme 1). The diiodo-Tyr compound **2** was prepared in 30% yield by treatment of **1** with benzyltrimethylammonum dichloroiodate (1-).[9] The peptides or amino acids **19** for compounds **1-10** were prepared by standard solution phase chemistry utilizing EDC and HOBT as coupling agents.

Scheme 1.

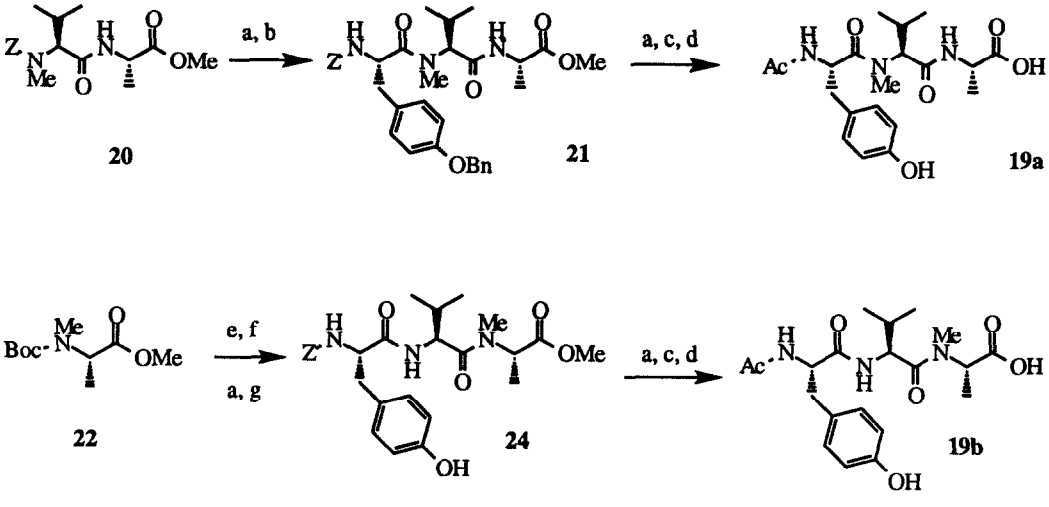


18 **19** **1-12** **1-12**

a) Bu_3SnH, $(PPh_3)_2PdCl_2$; EDC, HOBT; b) H_2, Pearlman's catalyst

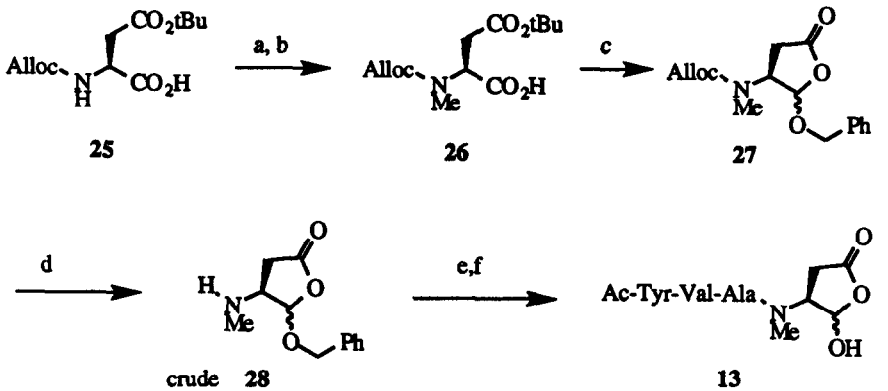
The N-methyl amide bonds were formed in **11-13** using PyBroP as the coupling agent. The N-methylated tripeptide intermediates **19a-19b** for compounds **11** and **12** were prepared as shown in Scheme 2. The N-methylated Asp aldehyde **13** was prepared as shown in Scheme 3. The alanine α-carbon in **17** racemized under the coupling conditions. The NMR data for aldehydes **1-13** is consistent with the cyclic hemiacetal form.[7,8] The semicarbazone **14** was prepared using a method described by Graybill et al.[8] The synthesis of lactam **16** and lactone **17** are shown in Scheme 4.

Scheme 2.



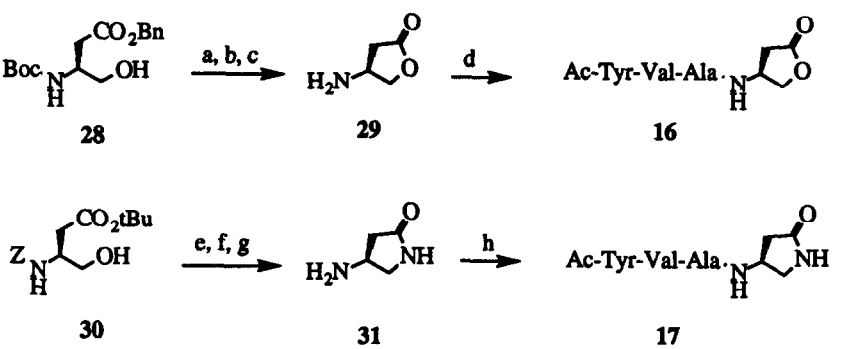
a) H_2, Pd/C, 1M HCl/EtOH; b) PyBroP, DIPEA, Z-Tyr(OBn)-OH, 43%; c) Ac_2O, Pyr, 67%; d) NaOH aq., MeOH, 99%; e) TFA; f) PyBroP, DIPEA, Z-Val-OH, 58 %; g) PyBroP, DIPEA, Z-Tyr-OH, 53%

Scheme 3.



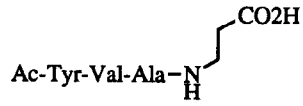
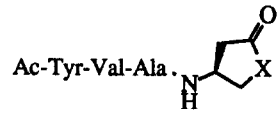
a) MeI, Ag_2O, DMF, 72%; b) NaOH aq., Acetone, 99%; c) see reference 7, 63%; d) Bu_3SnH, $(PPh_3)_2PdCl_2$, CH_2Cl_2; e) PyBroP, DIPEA, Ac-Tyr-Val-Ala-OH (19c), 40%; f) H_2, Pd/C, MeOH.

Scheme 4.



a) H_2, Pd/C, EtOH, 80%; b) p-TsOH, toluene, 70%; c) TFA; d) HBTU, DIPEA, Ac-Tyr-Val-Ala-OH, 88%; e) $(PhO)_2PON_3$, Ph_3P, DEAD, 47%; f) $HS(CH_2)_3SH$, IPA, $NaBH_4$, 60%[10]; g) H_2, Pd/C, MeOH, 100%; h) EDC, HOBT, DIPEA, Ac-Tyr-Val-Ala-OH, 32%

Table. Enzyme Data

Number	Aldehyde	Ki (μM)
1 (L-709,049)	Ac-Tyr-Val-Ala-Asp-H	0.006
2	Ac-I2-Tyr-Val-Ala-Asp-H	0.0075
3	Ac-Phe-Val-Ala-Asp-H	0.042
4	DCA-Val-Ala-Asp-H	0.028
5	Ac-Val-Ala-Asp-H	1.7
6	Ac-Ala-Asp-H	>20
7	Ac-Tyr-Ala-Asp-H	2.1
8	Ac-Tyr-Val-Asp-H	2.4
9	Ac-Tyr-Asp-H	>20
10	Ac-NMe-Tyr-Val-Ala-Asp-H	0.12
11	Ac-Tyr-NMe-Val-Ala-Asp-H	50
12	Ac-Tyr-Val-NMe-Ala-Asp-H	0.059
13	Ac-Tyr-Val-Ala-NMe-Asp-H	>20
14	Ac-Tyr-Val-Ala-Asp-H semicarbazone	0.5
15		>20
16 (X=O)		>20
17 (X=NH)		>20

Protocol for ICE Assay.

The visible ICE assay was run in a 96-well microtiter plate, using a reader with kinetic capability.[11] 65 µl of assay buffer (10mM Tris, 1 mM DTT, 0.1% CHAPS @pH 7.6), 10 µl of ICE (40 nM), 5 µl of DMSO containing the inhibitor, and 20 µl of 400 µM substrate were combined in a total reaction volume of 100 µl. The final concentration of substrate (Suc-Tyr-Val-Ala-Asp-pNA) was 80 µM. Buffer, ICE, and inhibitor were added to the wells, and the components incubated at room temperature for 15 minutes. Substrate was added and the release of pNA was monitored at 405 nm at 37 °C for 20 minutes. The Km of the substrate is 18 µM. Ki values were calculated from rate vs [inhibitor] plots by a nonlinear least squares fit of the data to the equation of Henderson for tight binding competitive inhibition.[12] A commercial program, KineTic (BioKin Ltd.) was used for this procedure.

Results and Conclusions.

The data in the Table demonstrates that removal of P_4 results in a considerable loss of inhibitory activity (on the order of 100-fold) while further truncation results in >1,000-fold loss of activity.[8] Removal of the para-OH from the tyrosine ring in P_4 results in only a seven-fold loss of activity. Any modification of the aldehyde group either diminishes activity greatly (compound **14**) or completely (**15-17**).

N-methylation of the P_1 or P_3 amide nitrogens results in complete loss of activity, while N-methylation at P_2 or P_4 is tolerated, with a mild (10 to 20-fold) loss of activity. These results suggest that the P_2 and P_4 amide nitrogens do not form strong hydrogen bonds with ICE and may be reasonable locations for substitution, while the P_1 and P_3 amides may be forming hydrogen bonds. This pattern is consistent with the high-resolution crystal structure of the complex of compound **1** with ICE.[13, 14]

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