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The Synthesis of Some N-Phosphoryldipeptide Aldehydes

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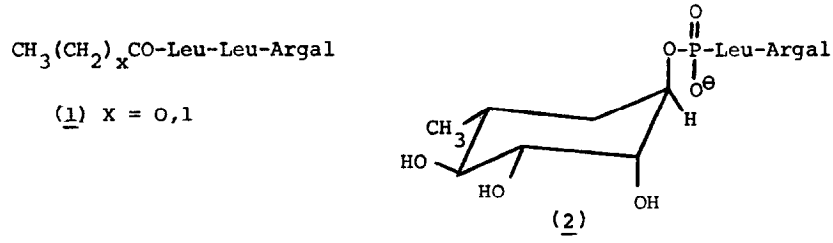
Abstract: Four N-phosphoryldipeptide aldehydes have been synthesized and tested *in vitro* against thermolysin and trypsin. Their structures exemplify a combination of leupeptin and phosphoramidan structures into one molecule which then shows enzyme inhibition.

This paper reports the synthesis of some small N-phosphorylated peptide aldehydes analogous to both leupeptin (1) (1) and phosphoramidon (2) (2). These compounds are microbial peptides which have been shown to be excellent inhibitors of acrolysin (3) (thermolysin-like specificity) and acrosin (1) (trypsin-like specificity), enzymes directly involved in the penentration of the ovum by spermatazoa. They are therefore of interest as antifertility agents. It appeared that combination of these structures into one molecule might produce compounds inhibitory to both the metallo-type enzyme



(2)

(thermolysin) requiring the phosphate group for binding and the arginine specific protease (trypsin) requiring the argininal (4) terminus. Since a report (3) in 1975 indicated that the rhamnose moiety of phosphoramidon (2) was unnecessary for protease inhibitory activity, we report here the synthesis of a series of four N-phosphoryl dipeptide aldehydes in which argininal is present at the C-terminus. These compounds were active against both thermolysin and trypsin *in vitro* but they have not yet been tested as antifertility agents.

The general reaction sequence used in the syntheses is shown in the Scheme. Clearly, the amino blocking and deblocking steps of our sequence

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Table I

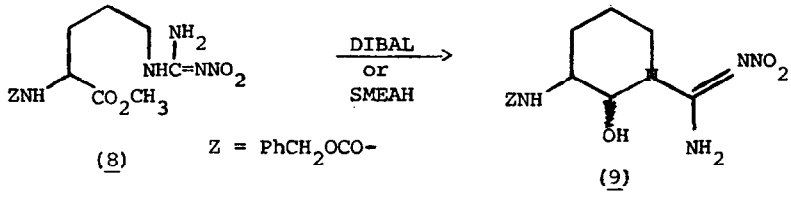
BOC-X-Arg(NO_2)·OMe (3)

		Yield[a]	M.P. (Solv.)	$[\alpha]_D^{24}$ [b]
3a	BOC-Leu-Arg(NO_2)·OMe	93	Amor.	-22.9°
3b	BOC-Phe-Arg(NO_2)·OMe	85	Amor.	-8.2°
3c	BOC-Tyr(Bzl)-Arg(NO_2)·OMe	96	108-112°(EtOAc)	-2.1°
3d	BOC-Ala-Arg(NO_2)·OMe	70	160-162°(EtOAc)	-24.5°

[a] All compounds showed acceptable elemental, infrared and NMR analyses.

[b] C = 0.8 - 1.1 in MeOH

might be avoided if the N-terminal amino acid were phosphorylated and coupled with an arginine derivative instead of preparing the N-BOC dipeptides (3) as shown. The longer route presented in Scheme 1 gave much better yields and purer products (Table I) than the shorter one using N-phosphorylated compounds. We investigated thoroughly the direct reduction of the esters 4a-d with diisobutyl aluminum hydride (DIBAL) as reported by Ito (5), et al. In our hands, this method, as described, failed to give any of the aldehydes (6b-d), and the use of excess DIBAL gave a product which was apparently the alcohol. We were, however, able to prepare the cyclic carbonolamine (9) by DIBAL and sodium bis-methoxyethoxy)-aluminum hydride (SMEAH)(6) reduction of Z-Arg(NO_2)·OMe (8) in 36 and 43% yields, respectively.



(8) Z = $PhCH_2OCO$- (9)

It appears that the dibenzyloxyphosphoryl group in 4b-d was responsible for the failure of the DIBAL ester reduction that we observed. Since the lactam of Z-Arg(NO_2)·OH had been previously reduced with lithium aluminum hydride (LAH) to the cyclic carbinolamine which is in equilibrium with the acyclic aldehyde, we extended that work by using the milder reducing agent DIBAL to reduce the presumed lactam intermediate. When the acid, Z-Arg(NO_2)·OH was treated with carbonyl diimidazole (CDI) followed by DIBAL, the carbinolamine (9) was produced in 63% yield after chromatographic purification (7). The dipeptide, Z-Phe-Arg(NO_2)·OH, was also readily reduced to Z-Phe-Arg(NO_2)al in 42% by this CDI/DIBAL procedure. When extended to the phosphorylated dipeptide acids 5a-d the aldehydes (carbinolamines) (6a-d) were

Table II

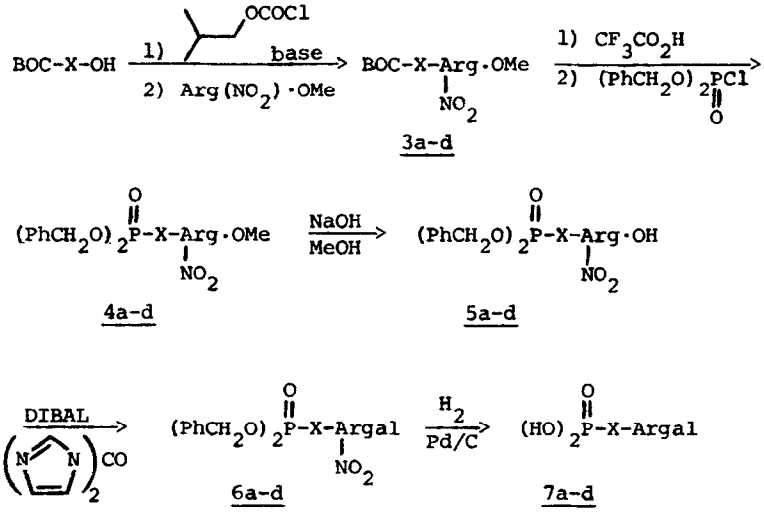
$(PhCH_2O)_2P(O)$-X-Arg(NO_2)·OMe (4)

		Yield[a]	M.P.(solv.)	$[\alpha]_D^{24}$ [b]
4a	$(PhCH_2O)_2P(O)$-Leu-Arg(NO_2)·OMe	43	134-136° (acetone/ether)	-16.6°
4b	$(PhCH_2O)_2P(O)$-Phe-Arg(NO_2)·OMe	31	Amor.	-5.6°
4c	$(PhCH_2O)_2P(O)$-Tyr(Bzl)-Arg(NO_2)·OMe	31	Amor.	-1.5°
4d	$(PhCH_2O)_2P(O)$-Ala-Arg(NO_2)·OMe	24	Amor.	-18.4°

[a] All compounds showed acceptable elemental, infrared and NMR analyses.

[b] C = 0.8 - 1.1 in MeOH

formed in quite acceptable yields (Table III). Hydrogenolysis of these products removed the benzyl blocking groups and the nitro function to give the final phosphoryl dipeptide aldehydes (7a-d). (Table IV). The final products (7a-d) gave positive dinitrophenylhydrazine and Sakaguchi tests for the aldehyde and guanidine functions, respectively. As mentioned by Umezawa (8) they showed two spots on tlc due apparently to anomerlike stereoisomers formed during argininal ring closure. All compounds were checked for purity in at least three tlc systems.



BOC=$(CH_3)_3COCO$ (a) Leu, (b) Phe, (c) Tyr(Bzl), (d) Ala

Table III

$(PhCH_2O)_2P(O)$-X-Argal (6)
NO_2

		Yield[a]	$[\alpha]_D^{24}$[b]
6a	$(PhCH_2O)_2P(O)$-Leu-Arg(NO_2)al	52	-24.5°
6b	$(PhCH_2O)_2P(O)$-Phe-Arg(NO_2)al	41	-6.8°
6c	$(PhCH_2O)_2P(O)$-Tyr(Bzl)-Arg(NO_2)al	26	-2.1°
6d	$(PhCH_2O)_2P(O)$-Ala-Arg(NO_2)al	52	-22.0°

[a] All compounds were amorphous solids, gave a positive 2,4-DNPH test on tlc and showed the correct infrared and NMR parameters.

[b] C = 0.8 - 1.1 in MeOH

The four aldehydes (7a-d) prepared were tested as inhibitors of thermolysin and trypsin by the methods described in the experimental section. The results (Table IV) show that the combination of two different molecular functionalities, each specifically inhibitory to distinct types of enzymes, can be combined to form one molecule inhibitory to both enzyme types. It appears that the presence of an aromatic ring enhanced the inhibition of thermolysin while aliphatic residues increased the inhibition of trypsin. We find this an intriguing aspect.

Inhibition Studies

Trypsin or thermolysin was incubated in the presence of varying concentrations of the test compound in the same buffer used in assay but containing

Table IV

$(HO)_2P(O)$-X-Argal (7)

		Yield	$[\alpha]_D^{24}$ b	Ki x 10^{-4}	
				Thermolysin[c]	Trypsin[d]
7a	$(HO)_2P(O)$-Leu-Argal	82	-6.1°	6.1	5.0
7b	$(HO)_2P(O)$-Phe-Argal	87	-13.5°	1.3	11
7c	$(HO)_2P(O)$-Tyr-Argal	94	+1.8°	0.5	10
7d	$(HO)_2P(O)$-Ala-Argal	97	-3.2°	5.0	4.4

[a] All compounds were amorphous solids, gave a positive 2,4-DNPH test on tlc and showed the correct infrared and NMR parameters.

[b] C = 0.8 - 1.1 in MeOH

[c] Ki for phosphoramidon inhibition of thermolysin[3] is 7.4 x 10^{-8}M.

[d] Ki for leupeptin inhibition of trypsin[1] is 1.3-3.8 x 10^{-7}M.

no substrate. Samples of enzyme were assayed with the desired substrate and % residual activity compared to control calculated. Percent residual activities versus concentration of inhibitor were evaluated by linear regression analysis and the Ki corresponding to 50% inhibition was determined directly from those analyses.

Assay Methods

Trypsin was assayed according to the method of Schwart (9) monitoring increase in absorbance at 253 nm in a Bausch and Lomb spectronic 200 UV spectrophotometer. Bz-Arg·OEt was 5 x 10^{-4} M in 5 x 10^{-2} M tris HCl pH 8.6 buffer containing 5 x 10^{-2} $CaCl_2$. Thermolysin was assayed according to the method of Feder (10) monitoring decrease in absorbance at 345 nm. Fagla was 5 x 10^{-4} M in 5 x 10^{-2}M HEPES pH 7.0 buffer containing 5 x 10^{-2}M $CaCl_2$.

Acknowledgement

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