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Synthesis of "CHO-CO-Peptides" by N-terminal Acylation with a Glyoxylyl Equivalent

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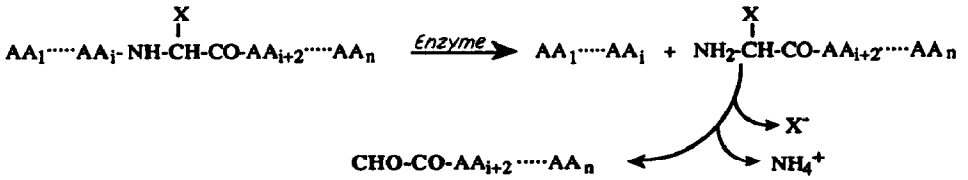
Abstract: *Activated esters of glyoxylic acid di-isopropylthioacetal were prepared by conventional procedures and used in the Solid Phase Peptide Synthesis of N-glyoxylyl-peptides (CHOCO-Peptides).*

The introduction of a 2-heterosubstituted-2-amino acid into a peptide sequence has been recently the subject of increasing attention[1-4]. These α-substituted glycine-containing peptides indeed illustrate an attractive approach either in the development of peptide delivery systems[5,6] or in the design of mechanism-based enzyme inhibitors[7]. As illustrated in scheme 1 following enzyme-mediated cleavage, the peptide of interest releases the N-terminal fragment $AA_1...AA_i$ together with the C-terminal sequence which spontaneously eliminates X^-.

Scheme 1



Previous studies have illustrated the portage transport strategy (X= fluoro-uracil or sulfanilic acid)[5] but the suicide character of an α-phenylthio or α-anilino substituted glycine containing peptides was not demonstrated. However no attempt has been made to investigate the potentialities of the N-glyoxylyl-peptides (CHO-CO-peptides) spontaneously generated from the C-terminal fragment resulting from enzyme cleavage. Since the aldehyde function is present in aqueous solution as the hydrate form[8], it could mimick the transition state developped during the catalysis of proteolytic enzymes. If literature provides numerous examples of carboxy-terminal peptide aldehydes as such inhibitors[9-11] the inhibitory properties of peptides bearing an amino-terminal aldehyde function was never investigated.

Although direct oxidation of serine and threonine containing peptides with periodate has been described[12, 13] to afford CHO-CO-peptides, we selected a glyoxylic derivative as a convenient building block suitable for Solid Phase Peptide Synthesis (SPPS) using Fmoc strategy. If Bis (Boc-amino)-acetic acid pentafluorophenyl ester[14,15] was a very efficient acylating agent in peptide synthesis, the glyoxylamide derivatives could not survive the drastic conditions required for resin cleavage and side-chain deprotection. The 3-Hydroxy-1,2,3-benzotriazin-4(3H)-one ester of di-isopropylthio-acetic acid 1 was therefore synthesized[16] in good yield using classical activation procedures[17].

Introduction of glyoxylate ester dithioacetal could then be performed at the last step of solid phase peptide synthesis[18] (Scheme 2) as demonstrated by the obtention of the aldehyde-protected CHO-CO-peptides overlapping the Matrix/Capsid sequence of the Human Immunodeficiency Virus protease substrate[20] (VSQNFPIV). At this stage these peptide derivatives can be handled and purified as usual. Deprotection of the aldehyde function occurred smoothly using N-bromosuccinimide in acetonitrile/H_2O[21].

Scheme 2

The *in vitro* evaluation of these derivatives which can be selectively generated from α-amino or α-hydroxy-containing peptides is currently under investigation.

REFERENCES AND NOTES

Abbrevations: Boc: *t*-butyloxucarbonyl; Fmoc: 9-fluorenylmethyloxycarbonyl; NBS: N-bromo-succinimide.

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14. Bis-(Boc-amino)-acetic acid: Mp: 198°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.36 (s, 18H); 5.16 (t, 1H, J=7.4); 6.88 (bs, 2H). ^{13}C NMR (DMSO d_6, 50 MHz): δ 28.33, 59.22, 78.94, 154.9, 170.51. Mass spectroscopy (chemical ionization, NH_3): m/z 291, MH, 308, M+NH_4. Bis-(Boc-amino)-acetic acid pentafluorophenylester: Mp: 148°C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ1.37 (s, 18H); 5.16 (t, 1H, J=7.4); 5.98 (bs, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 27.91, 59.6, 79.8, 154.5, 170.25.
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18. Acylation with four equivalents of compound 1 was performed on the peptide obtained by standard SPPS Fmoc strategy[19] and purified by C_{18} Reverse Phase HPLC (CH_3CN/H_2O, 0.05% TFA) before deprotection of the aldehyde function. (Yield) Mass spectroscopy (FAB) m/z: (*i*-PrS)$_2$-CH-CO-PIV-NH_2: (70)% 517 M+H, 539 M+Na; (*i*-PrS)$_2$-CH-CO-FPIV-NH_2: (65%) 664 M+H, 686 M+Na; (*i*-PrS)$_2$-CH-CO-NFPIV-NH_2: (68%) 778 M+H, 800 M+Na; (*i*-PrS)$_2$-CH-CO-QNFPIV-NH_2: (70%) 906 M+H, 928 M+Na; (*i*-PrS)$_2$-CH-CO-SQNFPIV-NH_2: (60%) 993 M+H, 1015 M+Na; (*i*-PrS)$_2$-CH-CO-VSQNFPIV-NH_2: (56)% 1092 M+H, 1114 M+Na.
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21. Deprotection of aldehyde function was performed with two equivalents of NBS in CH_3CN/H_2O 80/20[22] at 0°C. Peptides were purified by C_{18} Reverse Phase HPLC (CH_3CN/H_2O, 0.05% TFA). (Yield)Mass spectroscopy (FAB) m/z: CHOCO-PIV-NH_2: (63%) 401 M+H_2O+H, 423 M+H_2O+Na; CHOCO-FPIV-NH_2: (60%) 548 M+H, 570 M+Na; CHOCO-NFPIV-NH_2: (54%) 662 M+H_2O+H, 684 M+H_2O+Na; CHOCO-QNFPIV-NH_2: (42%) 794 M+Na; CHOCO-SQNFPIV-NH_2: (31%) 881 M+Na; CHOCO-VSQNFPIV-NH_2: (36%) 958 M+H, 976 M+H_2O+H, 998 M+H_2O+Na.
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