Tetrahedron Letters Vol. 21, pp 4263 - 4264 0040-4039/80/1029-4263$02.00/0

SYNTHESIS OF MONO- AND DI-ACYLATED L-2,6-DIAMINO-4-HEXYNOIC ACID POTENTIAL DERIVATIVES FOR PREPARATION OF ^{3}H-LABELLED LYSINE CONTAINING PEPTIDES

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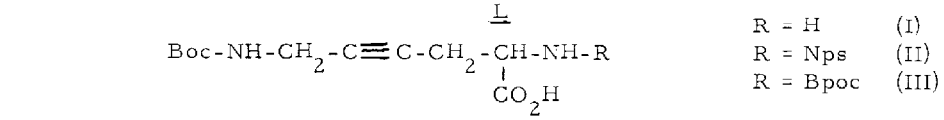
Abstract : The selective acylation of the 6-amino group of 2,6-diamino-4-hexynoic acid was obtained in high overall yield by a simple approach instead of copper complex method.

We have recently studied structure-activity relationships by synthesizing several analogs of the nonapeptide "facteur thymique sérique" or FTS[1,2]. We have thus developed a convenient method for the preparation of the N-acylated derivatives of the unsaturated L-lysine analog, L-2,6-diamino-4-hexynoic acid[3], or L-Dha, in which the two amino groups are acylated by readily and independently removable protecting groups.

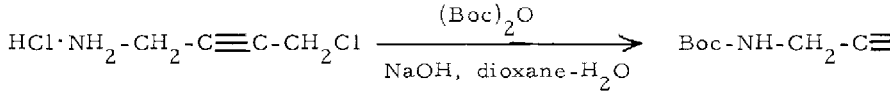
The rather cumbersome classical process of selective acylation of 6-amino group via a copper chelate[4] provided very poor yield of the desired product in this case.

We report here a simple approach to prepare in excellent overall yield L-2-amino-6-t-butoxycarbonylamino-4-hexynoic acid (I) or 6-Boc-L-Dha, a key intermediate for the syntheses of the di-acylated derivatives : L-2-(2-nitrophenylthio)amino-6-t-butoxycarbonylamino-4-hexynoic acid (II) or 2-Nps-6-Boc-L-Dha, and L-2-[2-(biphenyl)isopropoxycarbonyl]amino-6-t-butoxycarbonylamino-4-hexynoic acid (III) or 2-Bpoc-6-Boc-L-Dha.



The introduction of the unsaturated lysine-like amino acid, L-Dha, into peptide chains by using these derivatives can serve for the preparation of radioactive [^{3}H-Lys]-FTS[5] and also of other tritium labelled lysine-containing synthetic peptides.

1-Amino-4-chloro-2-butyne hydrochloride[6] (IV) was treated with di-t-butylcarbonate in dioxane-water (2/1) with 1 equ. NaOH at room temperature for 2 h, to yield 1-t-butoxycarbonylamino-4-chloro-2-butyne (V) in 92% yield, mp 43°C; nmr (90-MHz, $CDCl_3$) : δ 1.43 (s, 9H), 4.00 (m, 2H), 4.15 (m, 2H), 4.85 (m, 1H).



(IV) (V)

Condensation of the sodium salt of diethylacetamidomalonate and (V) in absolute ethanol gave crystalline ethyl-2-acetylamino-6-t-butoxycarbonylamino-2-ethoxycarbonyl-4-hexy-

noate (VI) in 75% yield, mp 78-79°C ; nmr ($CDCl_3$) : δ 1.25 (t, 3H), 1.33 (t, 3H), 1.43 (s, 9H), 2,05 (s, 3H), 3.25 (m, 2H), 3.85 (m, 2H), 4.28 (q, 4H), 4.70 (m, 1H), 7.00 (m, 1H).

$$Boc\text{-}NH\text{-}CH_2\text{-}C{\equiv}C\text{-}CH_2\text{-}C(CO_2C_2H_5)_2NH\text{-}CO\text{-}CH_3 \qquad (VI)$$

Partial saponification of (VI) in ethanol with 1.5 equ. KOH at room temperature for 2 h afforded the monoethyl compound in 90% yield, mp 117-118°C; nmr ($CDCl_3$): δ 1.23 (t, 3H), 1.45 (s, 9H), 2.08 (s, 3H), 3.30 (m, 2H), 3.85 (m, 2H), 4.28 (q, 2H), 4.90 (t, 1H), 7.15 (s, 1H), 8.10 (s, 1H). Decarboxylation of this compound by refluxing in dioxane for 24 h followed by further saponification in ethanol with 1.2 equ. KOH at room temperature for 15 h provided 2-acetylamino-6-t-butoxycarbonyl-4-hexynoic acid (VII) in 80% yield: mp 177-178°C dec (as dicyclohexylammonium salt); nmr ($CDCl_3$) : δ 1.48 (s, 9H), 2.03 (s, 3H), 2.88 (m, 2H), 3.87 (m, 2H), 4.95 (m, 1H), 7.10 (broad s, 1H), 7.89 (s, 1H).

$$Boc\text{-}NH\text{-}CH_2\text{-}C{\equiv}C\text{-}CH_2\text{-}CH(CO_2H)NH\text{-}CO\text{-}CH_3 \qquad (VII)$$

Stereospecific enzymatic hydrolysis of the acetylamino group of (VII) was performed [7] by treatment with porcine kidney acylase I and a trace of $CoCl_2 6H_2O$ in 0.1 M phosphate buffer (pH 7.5, 37°C, 15h) and the desired product (I), 6-Boc-L-Dha, was obtained in 82% yield after purification by column chromatography on silica gel, and elution with 95% ethanol : mp 197-199°C dec; $[\alpha]_D^{21°}$ – 17.5° (c 2.0, H_2O); nmr (D_2O): δ 1.26 (s, 9H), 2.65 (m, 2H), 3.66 (m, 2H). Treatment of (I) with o-nitrophenylsulfenyl thiocyanate [8] and 2-(4-biphenyl)isopropoxycarbonyl azide [9] gave (II) in 75% yield, mp 168-169°C dec (as dicyclohexylammonium salt); $[\alpha]_D^{22°}$ +36.9° (c 1.5, $CHCl_3$); nmr ($CDCl_3$): δ 1.48 (s, 9H), 2.88 (m, 2H), 3.67 (m, 1H), 3.92 (m, 2H), 7.05 (m, 1H), 7.45 (m, 2H), 8.00 (m, 2H) and (III) in 30% yield, mp 154-155°C dec (as dicyclohexylammonium salt); $[\alpha]_D^{22°}$ +37.5 (c 1.5, $CHCl_3$); nmr ($CDCl_3$): δ 1.46 (s, 9H), 1.83 (s, 6H), 2.81 (m, 2H), 3.90 (m, 2H), 4.50 (m, 1H), 7.35-7.80 (m, 10H), respectively. All products gave excellent elemental analyses.

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(Received in France 22 May 1980)