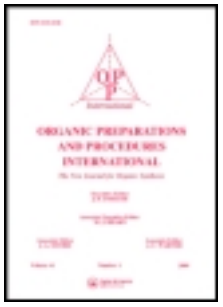

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THE EFFICIENT SYNTHESIS OF FMOC-L-HOMOGLUTAMINE

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(07/23/96)

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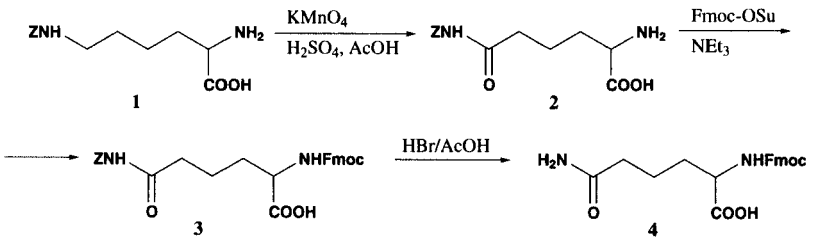
Homoglutamine ($H_2NCH(CH_2)_3CONH_2)CO_2H$, Hgn), like asparagine is a close homologue of glutamine. Its residue has been incorporated into a number of peptide analogues replacing glutamine or asparagine residues. Using Boc-strategy, Liberek *et al.*[1] synthesized analogues of oxytocine and vasopressine containing the Hgn-moiety which possessed interesting biological activity. Potent analogues of substance P were also obtained.[2] Growing interest in homoglutamine peptides and flourishing Fmoc[3] strategy of peptide synthesis prompted us to elaborate a method of Fmoc-Hgn-OH synthesis. The compound obtained is sufficiently soluble to be used in "continuous-flow" mode[4] of peptide synthesis and to the best of our knowledge is not available commercially.

The title compound, Fmoc-Hgn-OH (**4**), was synthesized from N-ε-benzyloxycarbonyl lysine (**1**), an inexpensive material, in a three-step procedure involving C-oxidation of Lys(Z)-OH ε-methylene unit resulting in amide protected homoglutamine (**2**), followed by Fmoc introduction with formation of **3** and acidolytic removal of benzyloxycarbonyl group.



Z-benzyloxycarbonyl

The oxidation of ε-methylene unit in lysine derivatives is well-known.[5-7] We employed the method of oxidation with permanganate in aqueous solution elaborated by Kasprzykowska and Liberek[5] to accomplish the first step of the synthesis.

Since the N-acylamide group present in the oxidation product is susceptible to both basic and acidic hydrolysis leading to dicarboxy amino acid and benzyl carbamate,[8] the introduction of Fmoc was carried out under mild conditions using Fmoc-OSu (9-fluorenylmethylsuccinimidyl carbonate) in the presence of triethylamine[9] in aqueous acetonitrile. These conditions allowed us to overcome the problems mentioned above as well as the very poor solubility of the substrate in aqueous solutions at pH 5-9. Although the reaction product was contaminated with some by-products, it could be used in the next step without additional purification. Pure Fmoc-Hgn(Z)-OH may be obtained, however, by two crystallization from AcOEt-Et_2O-*n*-hexane system. Removal of Z-amide protecting group can be achieved either by catalytic hydrogenation or by acidolysis. Both methods were employed with similar results. However, acidolysis is the easier and simpler method. The

product obtained can be purified by crystallization from ethanol (elemental analysis and spectral characteristics are consistent).

To summarize, we have elaborated a simple procedure for the synthesis of Fmoc-homoglutamine. The procedure does not involve chromatographic purifications. None of the intermediates require complicated work-up and the whole process can be accomplished within a few hours.

EXPERIMENTAL SECTION

^{1}H NMR spectra were recorded on a Tesla BS 567A 100 MHz spectrometer. ^{13}C NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer. HPLC-RP chromatography was performed on 5500 Vista Varian liquid chromatograph using Kromasil KR100-5C8, 250x4.6 mm column. Following gradients were applied: X: 45→85% B in 20 min; Y: 60→100% B in 20 min. (solvent A: 0.1% aqueous TFA, solvent B: acetonitrile), λ = 254 nm. TLC was performed using Merck aluminium foils coated with silica containing UV indicator in following solvent systems: A = BuOH/HCOOH/H_2O 4:1:1, B = AcOEt/*n*-hexane/AcOH 20:10:0.5, C = $CHCl_3$/MeOH/AcOH 17:2:1. Elemental analysis was performed on EA 1108 Elemental Analyser, Carlo Erba Instruments. Optical rotations were determined by using a Jasco J-20 spectropolarimeter. HPLC grade acetonitrile was purchased from Merck, TEA and HBr/AcOH from BDH and the other chemicals were purchased from Polish Chemicals POCh. ε-Z-Lysine **1** was synthesized according to a reported procedure.[10]

N$^\delta$-Benzyloxycarbonylhomoglutamine, (H-Hgn(Z)-OH, 2).- H-Lys(Z)-OH (10.00 g, 35.6 mmol) was dissolved in 440 mL of 1.8M H_2SO_4 in 50% AcOH and placed in an ice bath. Well powdered $KMnO_4$ (17.10 g, 108 mmol) was added portionwise over 20 min. The mixture was stirred (magnetic stirrer) for 0.5 hr and then was decolorized by the addition of saturated solution of Na_2SO_3. The pH of the solution was then increased to 5 by adding 25% aqueous ammonia. The suspension was stirred for one additional hour in an ice bath then the precipitate was collected. The product was washed thoroughly with water, methanol and diethyl ether to yield 4.62 g (44%) of colorless solid , mp. 172-173°. The compound may be crystallized from hot water but is usually sufficiently pure to be used in the next step. R_f (A) = 0.69. ^{1}H NMR (TFA): δ 1.90 (m, 4H, $CH_2^{\beta,\gamma}$), 2.55 (t, 2H, CH_2^{δ}), 4.20 (m, 1H, CH^{α}), 5.10 (s, 2H, CH_2-Ar), 7.10 (s, 5H, C_6H_5), 7.25 (m, 3H, NH_3^+).

Anal. Calcd for $C_{14}H_{18}N_2O_5$: C, 57.16; H, 6.16; N, 9.52. Found: C, 56.99; H, 6.05; N, 9.44

N$^\alpha$-Fmoc-N$^\delta$-benzyloxycarbonylhomoglutamine, (Fmoc-Hgn(Z)-OH, 3).- To a vigorously stirred suspension of H-Hgn(Z)-OH (4.41g, 15 mmol) in 100 mL of acetonitrile/water 1:1 was added 21 mL (15 mmol) of triethylamine and 5.06 g (15 mmol) of Fmoc-OSu. The pH was maintained at 8.5-9 by addition of another equivalent of triethylamine. The suspension was stirred until the pH stabilized (approx. 3 hrs). Acetonitrile was evaporated, 50 mL of water was added and the mixture was acidified with 5M HCl. The aqueous phase was extracted with ethyl acetate (3 x 50 mL), washed with brine, dried over magnesium sulfate and evaporated. The solid residue was suspended in hexane/diethyl ether 1:1 mixture, filtered off and washed with hexane. The crude product (7.05g, 91%) was used in the next step without further purification. An analytical sample was obtained after two crystallizations from AcOEt-Et_2O-*n*-hexane system, mp. 129-132°. $[\alpha]^{20}_{589}$ = -7.5° (c = 1, DMF). TLC : R_f (B)= 0.19.

HPLC : RT (Y) = 7.1 min. ^{1}H-NMR (DMSO-d_6/$CDCl_3$ 1:2): δ 1.77 (m, 4H, $CH_2^{\beta,\gamma}$), 2.57 (t, 2H, CH_2^{δ}), 4.10-4.47 (m, 4H, CH^{α}, H9 + CH_2 /Fmoc/), 5.15 (s, 2H, CH_2 /Z/), 6.32 (d, 1H, NH^{α}), 7.25-7.82 (m, 14H, Ar + NH^{ε}), 10.04 (s, 1H, COOH).

Anal. Calcd for $C_{29}H_{28}N_2O_7$: C, 67.43; H, 5.46; N, 5.42. Found: C, 67.21; H, 5.48; N, 5.39

N^{α}-Fmoc-Homoglutamine (Fmoc-Hgn-OH, 4).- The well-powdered crude product obtained in the previous step (6.18 g, 12 mmol) was treated with 20 mL of 45% HBr/AcOH with vigorous (magnetic) stirring until carbon dioxide evolution ceased (ca 0.5 hr). The mixture was evaporated at **room temperature** and 100 mL of water was added to the viscous residue. The suspension was extracted with ethyl acetate (3 x 100 mL), the combined organic phase was washed several times with brine and dried over $MgSO_4$ for 15 min. After evaporation of the solvent, the residue was triturated with diethyl ether and 3.70 g of the solid was collected. The crude product was recrystallized from ethanol to yield 2.81 g (61%) of coloress crystals of **4**, mp. 169-173° (dec). $[\alpha]^{20}_{589}$ = -14.0° (c = 1, DMF). TLC : R_f © = 0.26. HPLC: RT (X) = 5.4 min. ^{1}H NMR (DMSO-d_6/$CDCl_3$ 1:2): δ 1.78 (m, 4H, $CH_2^{\beta,\gamma}$), 2.27 (t, 2H, CH_2^{δ}), 4.07-4.48 (m, 4H, CH^{α}, H9 + CH_2 /Fmoc/), 6.82 (d, 1H, NH^{α}), 7.25-7.95 (m, 10H, Ar + NH_2). ^{13}C-NMR (DMSO-d_6): δ 21.87 (CH_2^{γ}), 30.46 (CH_2^{β}), 34.57 (CH_2^{δ}), 46.71 (H9 /Fmoc/), 53.77 (CH^{α}), 65.69 (CH_2 /Fmoc/), 120.15, 125.35, 127.14, 127.69, 140.75, 143.86°, aromatic), 156.20 (-O-CO-NH-), 173.96, 174.00 (COOH, $CONH_2$).

Anal. Calcd for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.32. Found: C, 65.56; H, 5.79; N, 7.25

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ALLYLIC AND PHENOLIC PHOSPHATE ESTERS OF DEXANABINOL

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While natural cannabinoids belonging to the (-) *3R, 4R* series bind to specific central and peripheral receptors,[1] the synthetic (+) *3S, 4S* epimers, of which the 5'(1',1'-dimethylheptyl)-7-hydroxy-Δ^6-tetrahydrocannabinol (dexanabinol, HU-211) (**1**) is the benchmark compound, are devoid of any cannabimimetic activity, since they have only negligible affinity to these receptors.[2] On the other hand, **1** proved to be a noncompetitive N-methyl-D-aspartate (NMDA) inhibitor [3] and an effective scavenger of peroxy and hydroxy radicals [4] and has been evaluated as a neuroprotective agent.[5-8] The further development of **1** as a therapeutic agent is somewhat hampered by its very low solubility in water, which complicates the formulation for intravenous administration. Various water-soluble esters of **1** containing polar or permanent charge-bearing moieties [9] including the glycinate salts [10] and salts of amino acid esters containing cyclic nitrogen [11] were evaluated as possible prodrugs or congeners. The syntheses of two phosphate esters of **1** are described herein.

Attempts to synthesize the allylic phosphate **3a** by reaction of dexanabinol with phosphorus oxychloride ($POCl_3$) and pyrophosphoryl chloride under various conditions, with or without base failed, since the 7-chloro derivative or a mixture of various unwanted products (dimers, polymers etc.) resulted. The reaction of **1** with freshly prepared di-*tert*-butylphosphorochloridate [12] in pyridine in the presence of triethylamine at -20° resulted in the 7-(di-*tert*-butyl) phosphate (**2**) which was purified by chromatography; the structure of **2** was unambiguously proven by ^1H and ^{13}C NMR spectroscopy. Reaction of **2** with trifluoroacetic acid in chloroform at room temperature for 30 min., the 7-dihydrogen phosphate **3a** resulted (structure proved by NMR and high resolution mass spectrometry). The *bis* cyclohexylammonium salt **3b** prepared from **3a** and cyclohexylamine in dry methanol and purified by recrystallization from *I*-propanol was used for characterization of the allylic phosphate. The sodium salt **3c** prepared from **3a** and ethanolic NaOH was used for solubility and stability studies (Scheme I).