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FIRST DIRECT FLUORINATION OF TYROSINE-CONTAINING BIOLOGICALLY ACTIVE PEPTIDES

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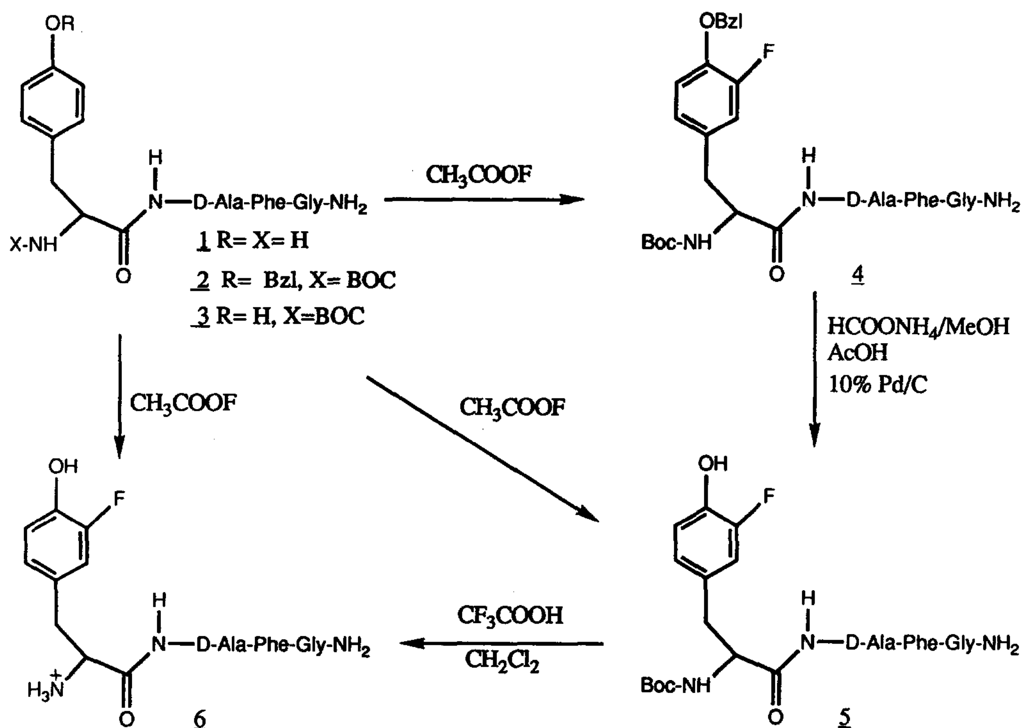
Abstract: Using acetyl hypoflurite a regiospecific electrophilic fluorination of the tyrosine ring of the N-terminal tetrapeptide amide (Tyr-D-Ala-Phe-Gly-NH_2) sequence of the opiate peptide dermorphin has been achieved in good yields.

Fluorinated analogues of biologically active compounds are important biochemical and pharmacological agents.[1] Fluoro compounds often mimic their nonfluorinated parents with respect to enzyme and receptor recognition, yet can drastically change the potency and receptor selectivity of the molecule.[2] In addition, fluorinated molecules have been used as markers that can be unequivocally detected using either ^{19}F NMR[3] or energy loss spectroscopic techniques.[4] Furthermore, a variety of ^{18}F labelled compounds are finding increasing application in positron emission tomography (PET).[5] Consequently, there is a constant need and search for new, efficient and relatively fast ways to introduce fluorine into biologically important molecules. Although many methods have been developed for fast and efficient incorporation of ^{18}F and ^{19}F into various compounds of medical importance, such methods have not been practical for direct incorporation into peptides. We are especially interested in the investigation of the effect of fluorination of the phenolic ring of the Tyr residue in opiate peptides on the affinity and selectivity of these peptides for various subtypes of opiate receptors (e.g. μ, δ and k) and the consequent effects on biological activities. Another aim of this research is to develop receptor-specific and metabolically stable fluorinated analogues for possible use as PET scanning agents.

Dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) is a μ-specific opiate peptide, isolated from the frog skin of the genus Phylomedusae. The N-terminal tetrapeptide amide Tyr-D-Ala-Phe-Gly-NH_2 (1) retains a substantial part of the activity of the parent compound. This peptide has been the subject of a variety of modifications that have resulted in many potent and receptor specific analogues.[6] We have chosen this peptide sequence for our initial fluorination studies.

Acetyl hypofluorite (CH_3COOF) is an electrophilic fluorinating agent which has been found to be an efficient, stereo and regiospecific reagent for the fluorination of a variety of

organic compounds[7]. We report here the first direct fluorination of a peptide. This fluorination with acetyl hypofluorite proceeds rapidly, under mild conditions and in good yield.



When a solution of acetyl hypofluorite, prepared from F_2 and NaOAc in $CFCl_3$ was added at room temperature to a solution of Boc-Tyr(Bzl)-D-Ala-Phe-Gly-NH_2 (2)[8] in acetic acid, an immediate reaction took place. RP HPLC analysis of the reaction mixture revealed a major peak (65%), with a slightly longer retention time (29.20 min) than the starting material (28.05 min) and with a shift in λ_{max} from 275 to 270 nm. A similar UV shift was observed in 3-fluorotyrosine when compared to tyrosine. ^{19}F NMR of the reaction mixture exhibited a single fluorine peak at -136 ppm relative to $CFCl_3$. This chemical shift is characteristic of an aromatic fluorine ortho to an oxygen functionality.[7a] In addition, the characteristic AB splitting pattern of the aromatic ring of tyrosine was no longer present in the ^1H NMR spectrum, and the mass spectrum (^{252}Cf) showed the molecular ion plus Na at m/z = 686. The product 4 was purified by preparative HPLC on a reverse phase column (Vydac, C-18, 10 μ, 2.2X 25cm)

using a gradient of 20% B to 100% B (A= 0.05% TFA in H_2O, B= 70% CH_3CN-H_2O containing 0.05 % TFA, flow rate 20 ml/min) in 40 minutes.

Although there are three aromatic rings in this peptide, no fluorination of the benzyloxy group or the phenylalanine residue was detected, emphasizing the degree of selectivity which can be realized with acetyl hypofluorite.

Under the same reaction conditions, Boc-Tyr-D-Ala-Phe-Gly-NH_2 (3) gave a mixture of products. The UV spectra of these products indicated that the phenolic ring had been oxidized. However, by restricting the conversion to 75%, a major product 5 (50%) was obtained. This product exhibited the same shift in λ_{max} to lower wavelength, as in the previous example. The ^{19}F NMR showed a single peak at -137 ppm, the ^1H NMR showed a pattern similar to 3-fluorotyrosine and the mass spectrum showed the molecular ion plus Na at m/z = 596. This product showed identical spectroscopic (UV, mass, NMR) properties and co-eluted in RP HPLC (21.83 min) with 5, the product of debenzylation of 4 (transfer hydrogenation; ammonium formate, MeOH/acetic acid (1%), 10% Pd/charcoal).

The BOC group of 5 was removed by stirring a solution of the fluorinated compound with a 1:1 mixture of CH_2Cl_2 and CF_3COOH for 30 minutes to give the free peptide 6 which was purified by RP HPLC and characterized by mass [m/z = 496 $(M+Na)^+$], ^1H and ^{19}F NMR (-136 ppm) spectroscopy. This peptide was further characterized by sequencing with Edman degradation on a Beckman 890 M sequencer. The PTH derivative of 3-fluorotyrosine [m/z = 317 $(M+H)^+$, CI/NH_3] showed a slightly longer retention time (14.31 min) than the PTH derivative of tyrosine (13.22 min). PTH derivatives of D-Ala and Phe were found in proper sequence and with their expected retention times. To demonstrate further the utility of this fluorinating procedure, completely unprotected peptide 1 was treated with acetyl hypofluorite to give 6 in comparable yields. The product was easily purified by RP HPLC, co-eluted with 6 obtained from 5 (vide supra) and showed identical spectroscopic properties.

The following procedure is representative for the fluorination reaction: acetyl hypofluorite is prepared by bubbling 10% F_2 in N_2 through a suspension of NaOAc in $CFCl_3$ containing one equivalent of AcOH at -78°C. Compound 2 (200 mg, 0.31 mmol) is dissolved in AcOH (4 ml). The solution containing acetyl hypofluorite (1.2 equivalents) is added dropwise at 25°C and the progress of the reaction is monitored by HPLC (Vydac C-18, 5 μ, 0.46 X 25 cm). The acetic acid is removed in a stream of nitrogen and the residual solid is dissolved in acetonitrile-water (7:3), filtered and purified by preparative RP HPLC.

This work demonstrates that acetyl hypofluorite can be used for selective monofluorination of tyrosine containing peptides with or without protection of the phenolic group in the side chain and or of amino groups. The scope of this fluorinating procedure for peptides with other sensitive side chains is under investigation. Since these reactions are rapid,

the present work also suggests that ^{18}F might be efficiently incorporated into certain biologically important tyrosine containing peptides.

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8. Boc-Tyr(Bzl)-D-Ala-Phe-Gly (2) was prepared as a benzylic ester on Merrifield resin (1% crosslinked) using the DCC-HOBT coupling procedure and N^{α}-t-butoxycarbonyl protection with benzyl protection for the Tyr side chain. Ammonolysis of the resin with saturated iPrOH-NH_3 furnished Boc-Tyr(Bzl)-D-Ala-Phe-Gly-NH_2 (2), which was purified by column chromatography (silica gel) and then crystallized from MeOH-EtOAc. Transfer hydrogenation of 2 ($HCOONH_4$/10% Pd-C in MeOH containing 1% AcOH) for 15 minutes furnished 3, which was crystallized from MeOH-Et_2O. Treatment of 3 with TFA-CH_2Cl_2-anisole (5:4:1) at room temperature for 30 min furnished 1, which was purified by preparative RP HPLC (Vydac C-18, 2.2X25 cm). The compounds were checked for purity by RP HPLC and characterized by mass (^{252}Cf) and ^1H NMR spectroscopy.

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