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DIRECT PHOTOCHEMICAL TRIFLUOROMETHYLATION OF HISTIDINE-CONTAINING PEPTIDES

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Abstract: Photochemical trifluoromethylation of the imidazole ring of histidine in the tripeptide Glp-His-Pro-NH_2 (TRH) has been achieved to furnish a mixture of the isomers, 2- and 4(5)-CF_3-Im-TRH, which have been separated by reverse-phase HPLC and characterized.

Histidine plays an important, and often critical, role in the biological activities of many peptide hormones, neuropeptides, peptide antibiotics and enzymes. The physico-chemical properties of the imidazole ring, viz its acid base properties, aromatic nature, ability to act as a hydrogen bond donor as well as acceptor, and ring tautomerism make histidine a unique proteinogenic amino acid. Consequently, modification of this amino acid or its replacement by other amino acids in bioactive peptides can have profound effects on their biological activities.[1] We have already demonstrated that replacement or ring substitution of His can lead to loss of bioactivity[2] or, of even greater interest and value, in the dissociation of multiple activities.[1a-1f] Thus, the introduction of a 4(5)-fluoro substituent into Glp-His-Pro-NH_2 (1, TRH) results in differential effects on pituitary-associated and centrally mediated cardiovascular activities of TRH.[1a, 1b]

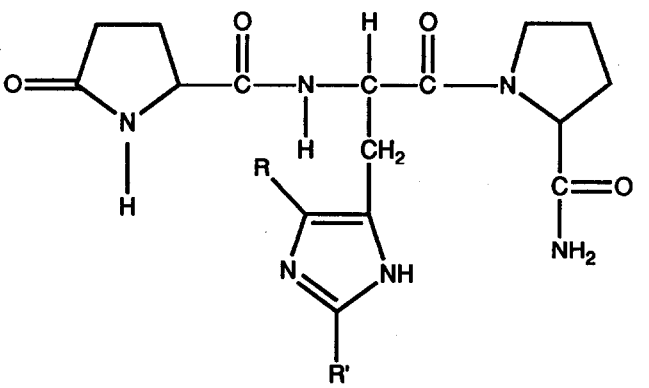
While exploring additional imidazole-ring substituents for TRH, we were led to consider the trifluoromethyl group. Not only is this group larger and more lipophilic than fluorine but, when attached to C-2 or C-4(5) of the imidazole ring, possesses the unique property of undergoing facile loss of fluoride ion at mildly basic pH.[3] The resulting difluorodiazafulvenes are extremely reactive and will add many nucleophiles; in the presence of H_2O or NH_3, e.g., the ultimate product will contain a COOH or CN group, respectively. Thus, a peptide containing a ring-trifluoromethylated His may have interesting biological activities per se, may serve as precursor for numerous other ring-substituted analogues, and provides the potential for covalent affinity labeling of an enzyme or receptor binding site.

2-CF_3-His had first been prepared from His by a four-step process involving ring opening

and reclosure.[4] Following the demonstration that mixtures of 2-CF_3- and 4(5)-CF_3-imidazoles are readily obtained by photochemical trifluoromethylation of imidazoles (with CF_3I and Et_3N in MeOH),[5] this method was used to prepare the isomeric CF_3-histidines.[5b] For peptide synthesis, these amino acids can be converted into N^{α}-Boc derivatives[5b] or even better, Boc-His may itself be subjected to photochemical trifluoromethylation.[6] Thus, 4(5)-CF_3-Im-TRH (2) and 2-CF_3-Im-TRH (3) were prepared by the solution phase method of peptide synthesis.[6]

These analogues show interesting pharmacological and TRH-receptor binding properties. For example, 2 has little affinity (K_i= 569 μM) for "high affinity" TRH receptors while the isomeric analogue 3 shows much higher binding (K_i= 4.1 μM).[1c] On the other hand, both these analogues are 2-3 times more potent than TRH in releasing PRL in vivo and are equipotent to TRH (1) in their effects on the cardiovascular system in rats.[1c]

These results prompted us to explore the possibility of effecting direct photochemical trifluoromethylation of His-containing peptides. Such a method would be of great utility for easy access to trifluoromethylated analogues (and transformation products thereof) of a variety of bioactive peptides. As a first example we herein report our results of direct photochemical trifluoromethylation of His in the tripeptide Glp-His-Pro-NH_2 (1, TRH).



1 R = R' = H, TRH

2 R = CF_3, R' = H [4(5)-CF_3-Im-TRH]

3 R = H, R' = CF_3 [2-CF_3-Im-TRH]

When a solution of 1 in MeOH containing CF_3I (1 eq.) and Et_3N (1 eq.) was irradiated with UV light (254 nm) for 72 h, TLC indicated, in addition to the starting material, the presence of a faster moving spot which had the same R_f as 2 and 3. The mass spectrum (CI, NH_3) of the crude material showed $(M+1)^+$ ions at m/z = 431 and 362 for 2 and/or 3 and 1, respectively. The crude material was subjected to column chromatography over silica gel and two major fractions were collected. The slower moving material was found to be 1 (TLC). RP HPLC analysis of the faster moving fraction revealed two major peaks (in a 2:1 ratio) with retention times of 14.82 and 15.41 min (Beckman Ultrasphere C_{18} column, 5μ, 0.46 X 25 cm, solvent gradient, 0% B-80% B in 40 min (A= 0.05% TFA-H_2O, B= 70% CH_3CN-H_2O containing 0.05% TFA). The two peaks together accounted for 70% of the partially purified material and their retention times corresponded to the retention times of authentic 2 and 3. Mass spectrum (CI, NH_3) of this mixture showed the $(M+1)^+$ ion at m/z = 431. By use of a Beckman Ultrasphere RP semipreparative column (C_{18}, 5μ, 1 X 25 cm) and isocratic elution (10% B), we were able to separate the two isomers in mg quantities. The combined isolated yield of the products was 20%. The faster moving isomer coeluted with authentic 4(5)-CF_3-Im-TRH (2) and the two peptides gave similar mass spectra [(CI, NH_3), m/z 431 $(M+1)^+$, 303 [cyclo{4(5)-CF_3-Im-His-Pro}+1]$^+$, 129 (Glp-NH_2+1)$^+$, 146 (Glp-NH_2+18)$^+$, 115 (Pro-NH_2+1)$^+$]. The slower moving isomer coeluted with authentic 2-CF_3-Im-TRH (3), and the two peptides gave similar mass spectra [(CI, NH_3), m/z 431 $(M+1)^+$, 303 [cyclo(2-CF_3-Im-His-Pro)+1]$^+$, 129 (Glp-NH_2+1)$^+$, 115 (Pro-NH_2+1)$^+$]. The ^1H and ^{19}F NMR spectra of the products matched those of the authentic isomers: ^{19}F NMR (D_2O, relative to TFA), 2, +16.21 and 3, +12.15 ppm.

The following procedure is representative for the photochemical trifluoromethylation reaction: to a chilled (-30 °C) solution of 1 (362 mg, 1 mmol) in MeOH (5 mL) in a 18 mL quartz tube is added Et_3N (0.14 ml, 1 mmol) and a methanolic solution of CF_3I (1 equivalent). The tube is filled with glass beads to exclude air, stoppered and irradiated in a Rayonet photoreactor RPR 208 equipped with 4 (15 watts each) UV lamps (254 nm). After 72 h, the solvent is evaporated under reduced pressure. The residue is chromatographed on a column of silica gel. Elution is carried out first with $CHCl_3$ and then successively with 5%, 10%, 15% and 20% MeOH-$CHCl_3$. The fractions are checked by TLC. The fractions containing the mixture of 2 and 3 are combined and evaporated to dryness under reduced pressure. The two products are separated and purified by HPLC on a Beckman Ultrasphere semipreparative RP column as described above.

This work demonstrates that the imidazole ring of His-containing peptides can be trifluoromethylated photochemically with CF_3I and that the two positional isomers can be

separated by HPLC. The value of this approach as a general method in peptide modification is enhanced by our preliminary observations that photochemical trifluoromethylation of imidazole (His) occurs more readily than that of benzene (Phe) or of indole (Trp) and is competitive with that of phenol (Tyr).[5b, 7] The scope of this procedure for peptides with other sensitive side chains is under investigation.

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7. Unpublished results.

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