SYNTHESIS OF α,β-DI[^{3}H]-2-FLUORO-L-HISTIDINE

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SUMMARY

The potent antimetabolite, 2-fluoro-L-histidine, has been synthesized from 2-fluoroimidazole with nonexchangeable ^{3}H labeling in the side chain. 2-Fluoro-4-iodoimidazole was N-tritylated, was subjected to metal-halogen exchange with n-butyllithium, and the carbanion formylated with DMF. The imidazole-4-aldehyde was condensed with hippuric acid, the azlactone was solvolyzed with methanol, and the trityl group was removed by selective reduction with PtS_2. The resulting 2-fluoro-α-benzamidoacrylic ester was reduced with tritium gas and the blocking groups were removed successively with base and with acylase I to produce the L-enantiomer of the free amino acid analogue.

Key words: 2-Fluoro-L-histidine, fluoroimidazoles, azlactone synthesis, tritiation, metal-halogen exchange, catalytic detritylation.

INTRODUCTION

In 1971, we developed a photochemical variant of the classical Schiemann reaction which permitted the first synthesis of ring-fluorinated imidazoles, including 2-fluoro-L-histidine (1,2). This amino acid possesses a wealth of interesting and useful biological properties, including the ability to be incorporated into both mammalian (3) and bacterial (4) protein in place of histidine. The same analogue inhibits bacterial growth (4), virus replication (2), leukocytopoiesis (antileukemic) (5), enzyme induction (6) and maturation of malaria parasites (7). The study of incorporation into protein (3-6), and its possible connection with one or more of these antimetabolic expressions,

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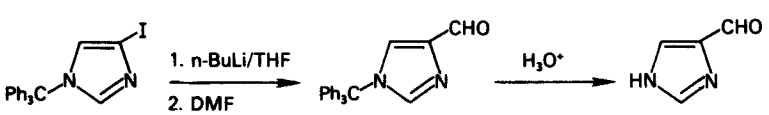
has been hampered by the unavailability of material radiolabeled at a chemically and biochemically stable position. Earlier, we had labeled 2-fluoro-L-histidine (2-FHIS) by taking advantage of the ease of exchange of hydrogen at C-4 of the imidazole ring above pH 9 (6,8). Unfortunately, only relatively low activities can be achieved by exchange with tritiated water. A more serious complication, however, was the fact that tritium could be lost almost as easily as it was incorporated. Thus, isolation, purification and analysis of labeled biological materials required avoidance of even modestly alkaline conditions. Strong acid conditions are equally harmful, since hydrolytic replacement of fluorine (an acid-catalyzed process) (9) labilizes the tritium at low pH. Nevertheless, the use of proteolytic enzymes for protein digestion allowed us to demonstrate the incorporation of 2-FHIS in several protein-synthesizing systems (4,6).

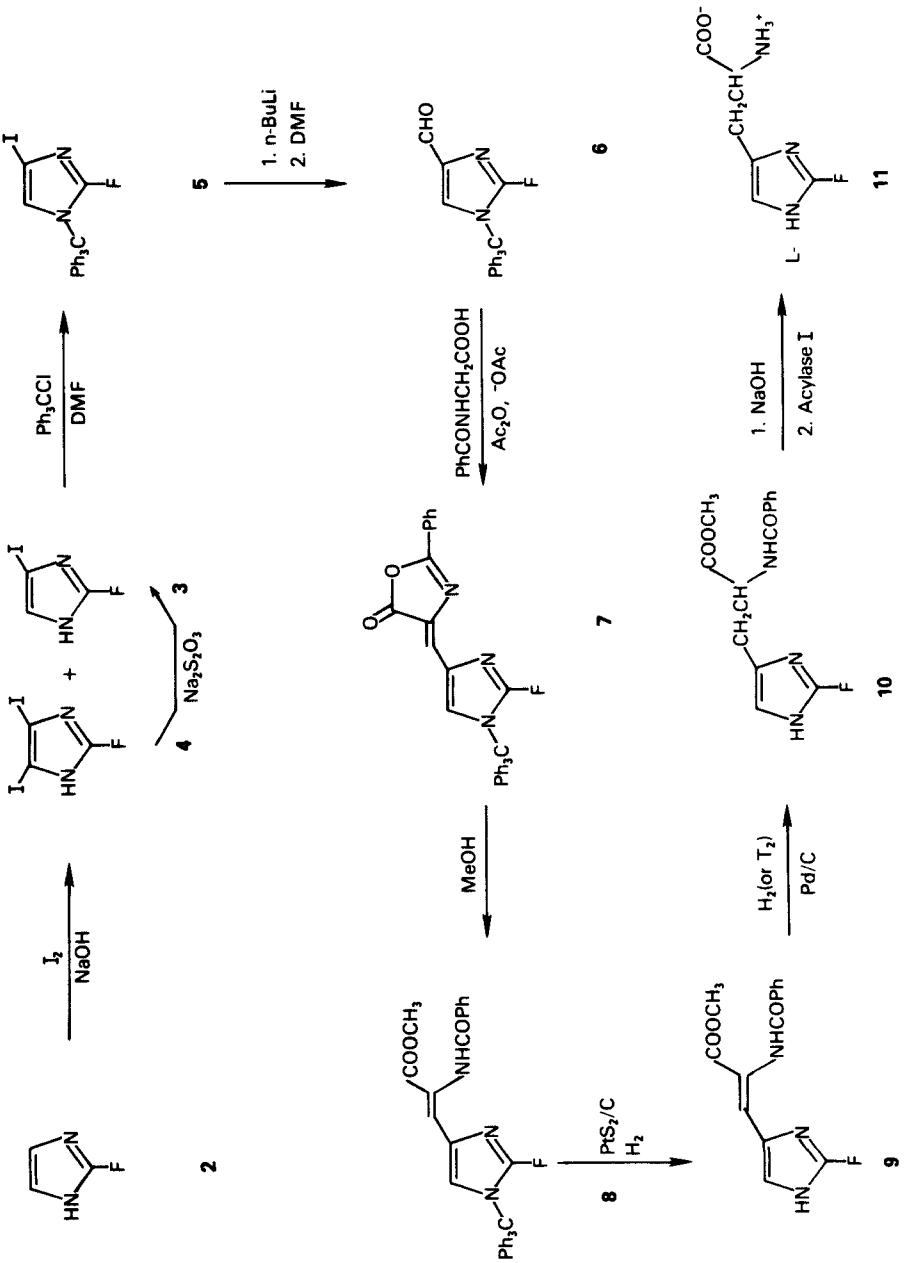
Various alternative apporaches to labeled 2-FHIS were considered or tried. Routes beginning with labeled histidine were rejected since the conversion of histidine to 2-FHIS requires six steps, elaborate manipulations and a low overall yield (2). Efforts to label the side chain of 2-FHIS by formation and hydrolysis of an azlactone were unsuccessful (10). In a study of lithiated imidazole species as reactive intermediates, we developed a facile synthesis of 1-trityl-4-imidazolecarboxaldehyde (1) by lithium-halogen exchange (Scheme 1) (11). We have now applied this procedure to the corresponding 2-fluoroimidazole (2, Scheme 2), and have used the resulting 6 as a key intermediate in the synthesis of side-chain tritiated 2-FHIS (11) with high specific activity. In this synthesis, tritium was introduced by catalytic reduction (Pd) of the dehydroamino acid (9) derived from 6.

Scheme 1



1

Scheme 2


I2
NaOH
Na2S2O3
Ph3CCl
DMF
1. n-BuLi
2. DMF
PhCONHCH2COOH
Ac2O, -OAc
MeOH
PtS2/C
H2
H2(or T2)
Pd/C
1. NaOH
2. Acylase I
L-
2
3
4
5
6
7
8
9
10
11

RESULTS

2-Fluoroimidazole was prepared as previously reported (12) and was iodinated to give a mixture of 2-fluoro-4-iodoimidazole (3) and 4,5-diiodo-2-fluoroimidazole (4). This mixture was reduced to the monoiodo derivative with sodium sulfite (13). After tritylation (5) and lithium-halogen exchange (n-butyllithium, THF, -60°C), immediate quenching of the reaction mixture with dimethylformamide gave 2-fluoro-1-tritylimidazole-4-carboxaldehyde (6) in good yield. The aldehyde was condensed with hippuric acid in the usual fashion to give the azlactone (7). This azlactone was opened by methanolysis and the resulting ester (8) was subjected to detritylation by controlled hydrogenolysis on PtS_2/carbon; under these conditions, no reduction of the double bond was observed. Catalytic reduction of 9 (Pd) gave 2-fluoro-N-benzoyl-DL-histidine methyl ester (10). The sensitivity of 2-fluoroimidazoles to both acid and base limited our options in deblocking. Saponification of the methyl ester proceeded smoothly; the benzoyl group was removed enzymatically, a process which also resulted in resolution of the product. 2-Fluoro-L-histidine (11) was separated from N-benzoyl-2-fluoro-D-histidine by HPLC.

EXPERIMENTAL*

Iodination of 2-Fluoroimidazole. To a solution of 2.64 g (20 mmol) of 2-aminoimidazole sulfate (Aldrich Chemical Co.) in 100 ml of chilled 50% fluoroboric acid (-20 to -10°C) was added a solution of 1.66 g (24 mmol) of sodium nitrite in 3 ml of water. The solution was irradiated for 3 h at -60 to -40°C, at which time the diazonium chromophore (313 nm) had disappeared (12). The mixture was neutralized to pH 5 (at -20 to -10°C) with cold, 10 N sodium hydroxide and was extracted with four 250-ml portions of ether. The combined extracts were dried (Na_2SO_4) and evaporated (at 20-25°C). The residual material was dissolved in 100 ml of chloroform; to the solution was

*Analyses and mass spectral measurements were performed by the Microanalytical Services and Instrumentation Section of this laboratory, under the direction of Dr. D. F. Johnson. Identities and homogeneities of all compounds were verified by mass spectra and tlc.

added 7.6 g (30 mmol) of iodine and 40 ml of 2 N sodium hydroxide. The mixture was stirred vigorously for 1 h at ambient temperature and was then acidified to pH 4 with 3 N hydrochloric acid. The chloroform layer was separated and the aqueous layer (and solid) were extracted with three 250-ml portions of ether. The combined organic extracts were washed with 50 ml of 10% sodium hydrosulfite, with 200 ml of water, were dried (Na_2SO_4) and evaporated to give 2.6 g of a mixture of 3 and 4.

The total product was dissolved in 40 ml of methanol and a solution of 1.07 g (8.5 mmol) of sodium sulfite in 20 ml of water was added. The suspension was stirred for 2 days at ambient temperature until 4 was no longer evident by tlc. The reaction mixture was concentrated to ca 30 ml, the colorless solid was collected, dried and recrystallized from ether-petroleum ether to give 1.35 g (32% based on 2-aminoimidazole) of 3, mp 125-126°C dec. Anal. Calcd for $C_3H_2FIN_2$: C, 17.00; H, 0.95; N, 13.22. Found: C, 17.11; H, 0.92; N, 13.23.

1-Trityl-2-fluoro-4-iodoimidazole (5). To an ice-cold solution of 1.35 g (6.37 mmol) of 3 and 2.13 g (7.63 mmol) of trityl chloride in 20 ml of dry DMF was added 1.06 ml (7.63 mmol) of triethylamine under argon. The solution was stirred for 20 h at ambient temperature and was poured into 150 ml of water. The mixture was stirred for 0.5 h, the colorless precipitate was collected, dried and recrystallized from n-hexane (or a small volume of methanol) to give 1.95 g (67.5%) of 5, mp 174-175°C. Anal. Calcd for $C_{22}H_{16}FIN_2$: C, 58.17; H, 3.55; N, 6.17. Found: C, 58.17; H, 3.49; N, 6.20.

1-Trityl-2-fluoroimidazole-4-carboxaldehyde (6).[#] To a solution of 454 mg (1 mmol) of 5 in 5 ml of dry THF (-78°C) was added 750 µl (1.2 mmol) of a 1.6 M solution of n-butyllithium in hexane (under an argon atmosphere). The mixture was stirred for 30 sec and an excess of dry DMF (500 µl) was added. The solution was stirred for 20 min at -78°C, for 1 h at ambient temperature,

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[#] After Completion of our work, we learned of an alternative approach to 6, involving direct lithiation (t-butyllithium) of 2-fluoro-1-tritylimidazole (ref. 14).

[+] Condensation at higher temperatures gave dark, complex mixtures.

and was then poured into 50 ml of ice-water. The reaction mixture was neutralized (pH 6) with 2 N hydrochloric acid, was extracted with three 30-ml portions of ether, and the combined extracts were dried (Na_2SO_4) and evaporated. The residual material was recrystallized from ether-petroleum ether to give 212 mg (60%) of 6, mp 176-177°C; NMR ($CDCl_3$) δ 7.0-7.4 (m, 16, phenyls and H-5), 9.68 (s, 1, CHO). Anal. Calcd for $C_{23}H_{17}FN_2O$: C, 77.51; H, 4.81; N, 7.86; F, 5.33. Found: C, 77.57; H, 4.97; N, 7.83; F, 5.26.

Both 5 and 6 are arbitrarily assigned substitution at C-4 (rather than C-5), based on steric considerations in the tritylation of 3. Unfortunately, H-F coupling across the ring (8) could not be used to verify these assignments since the NMR signals for the trityl protons mask those of the imidazole ring. Only one isomer was found in each case.

Methyl α-N-Benzoylamino-β-[4-(2-fluoroimidazolyl)]acrylate (E or Z) (9). To a solution of 600 mg (1.7 mmol) of 6 in 50 ml of acetic anhydride was added 600 mg (3.3 mmol) of hippuric acid and 350 mg (4.3 mmol) of anhydrous sodium acetate. The suspension was stirred at ambient temperature for one week, at which point 6 was no longer observed by tlc.[+] The reaction mixture was evaporated at 30°C and the residue was dissolved in 200 ml of methanol. The solution was stirred at ambient temperature for 10 days, at which point the azlactone 7 had been consumed (tlc). The mixture was evaporated at 30°C and the residue was extracted with three 100-ml portions of ether. The combined extracts were evaporated and the residual methyl ester 8 was dissolved in 150 ml of methanol containing 100 mg of 5% PtS_2 on carbon. The suspension was stirred under a slight pressure of hydrogen for 2 days, at which point the trityl group had been removed completely. The reaction mixture was filtered with Celite and the filtrate was evaporated. The residue was applied to a preparative HPLC column (Waters 440 HPLC system; Altex Ultrasphere ODS column, 5 μm, 10.0 x 250 mm; mobile phase, MeOH-H_2O (1:1) to 100% MeOH, 30 min linear gradient, 1.5 ml/min; 340 nm UV detector). The eluates between 19 min and 22

min were collected and lyophilized to give 130 mg (27%) of 9: NMR ($CDCl_3$ + CD_3OD) δ 3.87 (s, 3, CH_3), 6.60 (s, 1, β-CH=), 6.83 (s, 1, H-5), 7.4-8.0 (m, 5, phenyl).

N-Benzoyl-2-fluoro-D,L-histidine Methyl Ester (10). A solution of 15 mg of 9 in 70 ml of methanol, containing 10 mg of 5% palladium-on-carbon was stirred under a slight pressure of hydrogen for 20 h at ambient temperature. The solution was filtered through Celite and the filtrate was evaporated. The residue was recrystallized from ether-petroleum ether to give 10, mp 55-60°C; UV (MeOH) λ_{max} 226 nm (ε 9120); NMR ($CDCl_3$) δ 3.06 (m, 2, β-CH_2), 3.69 (s, 3, OCH_3), 4.93 (tt, 1, α-CH), 6.42 (s, 1, H-5) and 7.4-7.9 (m, 5, phenyl).

2-Fluoro-L-histidine (11). A solution of crude 10 in 0.3 ml of 0.5 N sodium hydroxide was kept at ambient temperature overnight. The pH of the solution was then adjusted to 7.0 with 0.1 N sodium phosphate, to give a total volume of ca 3 ml. To the solution was added 3 mg of acylase I (Sigma Chemical Co.) and the mixture was incubated at 37°C for 2 days. The reaction mixture was filtered through a bed of charcoal on Celite and the filtrate was lyophilized. The residue was applied to the HPLC column described under 9 (mobile phase: 0.1 N triethylamine-acetic acid buffer, pH 5.0, to buffer-methanol (7:3) with 60 min linear gradient, 1 ml/min; 254 nm UV detector). The eluates between 15 and 17 min were collected as 11; these fractions were UV negative but positive to Pauly reagent and ninhydrin. N-Benzoyl-2-fluoro-D-histidine was eluted between 57 and 65 min as the fraction which was UV and Pauly positive but ninhydrin negative. The 2-fluoro-L-histidine fractions were combined and lyophilized to give 4 mg of material. The product proved identical (tlc, MS) to that prepared previously by another route (1,2).

Preparation of Tritiated 11. A 50 mg sample of 9 was subjected to catalytic reduction as above using tritium gas (procedure performed by Research Products International) to give 10 containing 90 mCi of tritium, after purification. A 25 mCi sample of labeled 10 was deblocked as above and was purified by HPLC to give 6 mCi of 11 having a calculated specific activity

of 3.4 Ci/mmol (based on quantitation of N-benzoyl-2-fluoro-D-histidine by UV spectrum). Tlc gave a single radioactive spot with an R_f identical to that of authentic 11.

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