TRITIUM LABELLED 2-TRIFLUOROACETAMIDOBENZENESULFONYL FLUORIDE

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Summary

Here, we report the synthesis of high specific activity tritiated 2-trifluoroacetamidobenzenesulfonyl fluoride- a potent elastase inhibitor. Preparation of a ring iodinated precursor followed by palladium on $CaCO_3$ catalyzed deiodination with deuterium and tritium gas afforded high levels of isotope incorporation in both cases.

Key Words: 2-trifluoroacetamidobenzenesulfonyl fluoride, 5-iodo-2-trifluoroacetamidobenzenesulfonyl fluoride, catalytic reduction, deuterium, tritium.

Introduction

It is now generally agreed that an imbalance in the elastase-antielastase system in the lung is responsible for the destructive parenchymal process that leads to pulmonary emphysema, a significant cause of disability, morbidity and mortality in the United States.[1] Among the various proteases present in man, leukocyte elastase is suspected by many investigators to be the primary destructive agent in emphysema. The classification of human leukocyte elastase as a serine protease, belonging to the same family of enzymes such as trypsin, chymotrypsin and porcine pancreatic elastase, has facilitated the derivation of the active catalytic site of elastase and thus permitted a rational design of synthetic elastase inhibitors.[2] Using oligopeptide chloromethylketone elastase inhibitor, *in vivo* studies have shown their beneficial effect in the prevention of elastase in-

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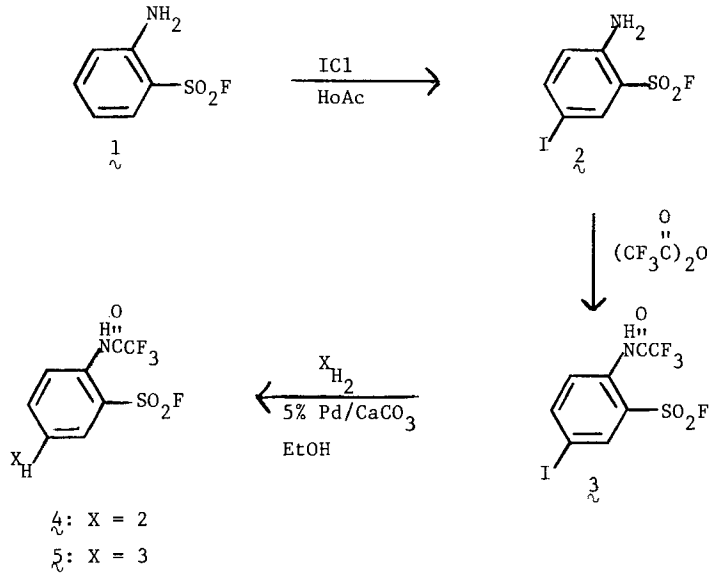
duced emphysema in hamsters and mice.[3,4] However, its toxicity in animal studies[5] precludes its application in humans indicating the need for screening alternative agents for potential use in man.

Sulfonyl fluorides inhibit serine proteases by reacting with the active site serine residue to form a sulfonyl derivative. Yoshimura, et al. have recently shown that introduction of fluoracyl groups into a sulfonyl fluoride structure leads to a series of potent and specific irreversible elastase inhibitors in vitro.[6] We report here, the synthesis of tritiated 2-trifluoroacetamidobenzenesulfonyl fluoride for the performance of pharmacokinetic studies prior to the application of the compound for extensive testing in the elastase-induced model of emphysema in hamsters.

Discussion

Potential sites for nonexchangeable tritium labelling of 2-trifluoroacetamidobenzenesulfonyl fluoride are limited to the aromatic ring. This procedure for aromatic tritiation is normally carried out by palladium catalyzed reductive dehalogenation of the corresponding aryl halide which is promoted by addition of an aprotic base such as triethylamine.[7] There being some concern regarding possible interaction of the substrate sulfonyl fluoride with triethylamine, the sulfonyl fluoride was initially mixed with a slight excess of triethylamine in THF to assess compatibility. Upon immediate examination by TLC, only a much lower R_f spot was evident relative to the sulfonyl fluoride, indicating an undesirable interaction of the sulfonyl fluoride with the triethylamine. Therefore, it became necessary to carry out the aryl dehalogenation in the absence of triethylamine or similar base. According to the literature,[8] aryl chlorides and bromides are not dehalogenated in the absence of base, whereas aryl iodides are dehalogenated to some extent in neutral media. Therefore, 5-iodo-2-trifluoroacetamidobenzenesulfonyl fluoride (3) (Figure 1) was prepared by iodine monochloride iodination[9] of o-aminobenzenesulfonyl fluoride (1) followed by N-acylation with trifluoroacetic anhydride according to the procedure of Yoshimura[6] for the synthesis of the noniodinated compound. Upon exposure of 3 to 1.0 atm of hydrogen gas in both THF and absolute ethanol at room temperature in the absence of base, no deiodination oc-

FIGURE 1



1 2 3

4: X = 2

5: X = 3

curred. It is reported that catalytic aryl dehalogenation may also be promoted by the use of an appropriate catalyst on a basic support material.[8] Upon exposure of 3 in absolute ethanol to 1.0 atm of hydrogen gas at room temperature in the presence of 5% Pd on $CaCO_3$, complete deiodination to the desired product occurred as evidenced by a lower R_f material corresponding to authentic product and by ^1H-NMR. Under similar conditions, no reaction occurred in THF. Exposure to deuterium gas under similar conditions afforded 2-trifluoroacetamido-[5-^2H]benzenesulfonyl fluoride (4) in 45% yield. Mass spectral evidence of deuterium incorporation indicated d_0 = 9.77%, d_1 = 90.04% and d_4 = 0.20%. Apparently, the palladium catalyst caused a small degree of hydrogen incorporation involving the protic solvent. This lack of label scrambling into other positions was further evidenced by ^2H-NMR (CCl_4) which showed the aromatic deuterium singlet at δ 7.48 relative to CD_3CN at δ 1.93 and no other signals were evident. Using the same conditions with 5.0 Ci of carrier free tritium gas, 40 mCi (5.3%) of 2-trifluoroacetamido-[5-^3H]benzenesulfonyl fluoride (5) was obtained with a specific activity of 10 Ci/mmole (38 mCi/mg). The apparent low yield is attributable to radiolytic decomposition of the high specific activity product during reaction and purification on silica gel.

Experimental Procedures

All chemicals were used as obtained from the manufacturer. Melting points were obtained on a Thomas Hoover Melting Point Apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a JEOL FX-60 60 MHz FT spectrometer using $CDCl_3$ (TMS) as solvent and ^{2}H-NMR spectra on a Bruker 250 MHz spectrometer using CCl_4 (CD_3CN) as solvent. Ultraviolet spectra were obtained on a Cary 15 UV spectrometer using methanol as solvent. Radiopurity was determined using a Packard Radioscanner Model 7201. Tritium was counted using a Packard Liquid Scintillation Counter, Model 3255 (internal standard) with Scintiverse® (Fisher) counting solution. Silica gel plates (UV) were used for TLC analyses. Elemental compositions of novel compounds were determined by high resolution mass spectrometry using an AEI MS-902 mass spectrometer.

5-Iodo-2-aminobenzenesulfonyl Fluoride (2). To a solution of 1.0 g (0.006 mol) of o-aminobenzenesulfonyl fluoride (1) in 20 ml of glacial acetic acid was added 0.93 g (0.006 mol) of iodine monochloride[9] in 4.0 ml of glacial acetic acid in one portion. The dark solution was stirred at 90-95°C for 1.0 h, evaporated *in vacuo* and the crude brown solid residue was column chromatographed on silica gel 60 (70-230 mesh) (benzene) to afford 1.2 g (71%) of product as a tan solid; mp = 80-81°C. ^{1}H-NMR ($CDCl_3$, TMS) δ 8.00 [d (J = 2.2 Hz), 1H, Ar-H-6], 7.63 [$^{q}_{AB}$ (J = 9.0, 2.2 Hz, 1H, Ar-H-4], 6.58 [d (J = 9.0 Hz), 1H, Ar-H-3], 5.20 [s, 2H, $-NH_2$].

5-Iodo-2-trifluoroacetamidobenzenesulfonyl Fluoride (3). Using the procedure of Yoshimura,[6] 1.2 g of 2 (0.004 mol) was treated with 5.0 ml (0.035 mol) of trifluoroacetic anhydride and the resulting suspension stirred for 15 min at room temperature. Cold water was slowly added and the solid filtered and recrystallized from water/MEOH to afford 0.6 g (38%) of product as colorless needles; mp = 101-103°C. ^{1}H-NMR ($CDCl_3$, TMS) δ 9.80 [s, 1H, -NH-], 8.28 [m, 3H, ArH_3]; m/e 396.8894 ($C_8H_4F_4INO_3S$ requires 396.8895).

2-Trifluoroacetamido-[5-^{2}H]benzenesulfonyl Fluoride (4). A solution of 66 mg (0.167 mmol) of 3 in 3.0 ml of absolute ethanol was stirred overnight at room

temperature with 32 mg of 5% $Pd/CaCO_3$ under 1.0 atm of deuterium gas. The catalyst was removed by filtration through a Celite column and the filtrate evaporated in vacuo. The residue was chromatographed on two 20 x 20 cm x 0.25 mm silica gel plates (benzene) to afford 10.4 mg (45%) of product as a colorless solid; mp = 85-86°C [lit.[6] (unlabelled) mp = 85-87°C]. The R_f for the iodo precursor was 0.65 and 0.53 for the product. ^{1}H-NMR ($CDCl_3$, TMS) δ 9.87 [s, 1H, -NH-], 8.59 [d (J = 9.0 Hz), 1H, Ar- H-3], 8.1 [s, 1H, Ar-H-6], 7.87 [d (J = 9.0 Hz), 1H, Ar-H-4]. Mass spectrum indicates d_0 = 9.77%, d_1 = 90.04%, d_2 = 0.00%, d_3 = 0.00%, d_4 = 0.20% and d_5 = 0.00%.

2-Trifluoroacetamido-[5-^{3}H]benzenesulfonyl Fluoride (5). A solution of 29.7 mg (0.075 mmol) of 3 in 1.0 ml of absolute ethanol was stirred at room temperature overnight with 23.9 mg of 5% $Pd/CaCO_3$ under 5.0 Ci (0.086 mmol) of carrier free tritium gas. The catalyst was removed by filtration through a Celite-Na_2SO_4 pipet column and evaporated. The residue was immediately dissolved in 10 ml of CH_2Cl_2 and an aliquot diluted and counted to afford 415 mCi of crude product. The CH_2Cl_2 was evaporated in vacuo and the residue chromatographed on two 20 x 20 cm x 0.25 mm silica gel plates (benzene) to afford 40 mCi of product which was ~ 99% radiochemically pure by TLC-radioscan (benzene). The R_f value on the PTLC and analytical TLC plates corresponded to authentic unlabelled material used as a reference standard. The pure product was stored at a concentration of 0.4 mCi/ml in benzene solution at 6-10°C. A 15 ml aliquot of this stock solution was taken and evaporated in vacuo and the residue dissolved in 10 ml of methanol and quantitated by UV spectroscopy (330 nm -250 nm, ε = 2083) using unlabelled 2-trifluoroacetamidobenzenesulfonyl fluoride as a reference standard. The specific activity obtained was 10 Ci/ mmole (38 mCi/mg).

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