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A modified approach to C-14-labeled 2-(3,4-difluorophenoxy)-5-fluoronicotinic acid and other halogen-substituted analogs†

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A modified approach to a carbon-14-labeled pyridine ring system was developed based on the electrocyclic ring-closure of 1,4,4-trisubstituted butadiene. The new method was applied to prepare 2-(3,4-difluorophenoxy)-5-fluoro-[2-^{14}C] nicotinic acid and other halogen-substituted analogs. The targeted compound was isolated with a radiochemical purity of >98% and a specific activity of 53 mCi/mmol from four radiochemical steps, starting from ethyl [1-^{14}C] cyanoacetate in an overall radiochemical yield of 39%.

Keywords: carbon-14; nicotinic acid; pyridine ring formation; aryl ether formation

Introduction

2-(3,4-Difluorophenoxy)-5-fluoronicotinic acid (**1**, Figure 1) is a key intermediate for preparing some drug candidates. A C-14-labeled compound **1** was required in the synthesis of a radioactive isotope-labeled drug candidate in order to support absorption, distribution, metabolism and elimination studies of the drug. According to our preliminary metabolic studies, the carbon-14 labeling in the circled area was most likely metabolically stable. Therefore, we focused on preparation of the C-14-labeled compound **1** at either the carbonyl group or pyridine ring (Figure 1).

Prior to our involvement, Pfizer process chemists have synthesized the compound **1** in four steps as shown in Scheme 1.[1] A commercially available compound **2** was first treated with H_2SO_4/EtOH to form the ethyl ester **3** in 85% yield. The selective dechlorination of the dichloro-substituted ester **3** with Lindlar's reagent afforded the desired mono-chloride **4** in 65% yield after crystallization from isopropyl alcohol.[1,2] The product **4** was then coupled with 3,4-difluorophenol **5** in the presence of a base to afford the ether **6** in 72% yield. Finally, the ester **6** was hydrolyzed by NaOH in toluene to furnish the target **1** in 89% yield. The original synthetic route is not useful for introducing a C-14 into the desired position. Therefore, we have explored several new synthetic approaches to C-14-labeled compound **1** or **4**. In this paper we wish to report a modified approach to labeled pyridine ring synthesis based on the electrocyclic ring-closure of 1,4,4-trisubstituted butadiene. We also discuss its application for preparing 2-(3,4-difluorophenoxy)-5-fluoro-[^{14}C] nicotinic acid and other halogen-substituted analogs.

Experimental

General: All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. LC-MS data were obtained on a Water Micromass LCZ mass spectrometer with flow injection analysis and electrospray ionization (ESI). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 400 MHz instrument. Chemical purity of all labeled compounds was determined by HPLC and LC-MS. Purifications were done by flash column chromatography on Biotage Flash 40 system. Quantitation of radioactivity of C-14-labeled compounds was performed using a Packard 2200CA liquid scintillation analyzer, with Scintiverse BD cocktail used throughout. Commercial reagents and solvents were purchased from Aldrich and used as-received unless otherwise noted. [^{14}C] ethyl cyanoacetate (250 mCi, 53 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. The intermediate **4** and unlabeled compound **1** were provided by Chemical R&D, Sandwich Lab, Pfizer Inc.

(Z)-3-dimethylamino-2-fluoroacrylaldehyde (18)

To a solution of sodium fluoroacetate (10.0 g, 100 mmol) in dry DMF (80 ml) was added oxalyl chloride (28.0 g) at such a rate that the reaction temperature was kept below 10°C. The mixture was stirred for 30 min at 0°C, and then for another 30 min at 60°C. After the reaction mixture was cooled down to 0°C, triethylamine (27.6 ml) was added slowly while the reaction temperature was kept below 10°C. The resulting mixture was stirred on an ice bath for 30 min and at 50–55°C for another 30 min. After cooling down to room temperature, the reaction

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mixture was then poured on to a mixture of 100 ml of ice water and 130 ml of saturated aqueous potassium carbonate. The resulting mixture was stirred at 80°C for 30 min. After cooling down to room temperature, the mixture was extracted with methylene chloride (3 × 50 ml). The combined extracts were washed with water (2 × 50 ml) and dried over sodium sulfate. Solvent was evaporated in vacuo and the residue was purified by flash chromatography (silica gel column, eluted with hexane/ethyl acetate/methanol 50/50/1) to afford the titled compound **18** (4.68 g, 40%) as an oil. ^{1}HNMR[6] ($CDCl_3$): δ 2.85 (s, CH_3), 2.92(s, CH_3), 6.10 (d, J_{FH} = 27 Hz, HC = C), 8.55 (dd, $J_{F\text{-}H}$ = 20.0 Hz, $J_{H\text{-}H}$ = 0.80 Hz, COH).

(2E, 4Z)-ethyl-2-[^{14}C] cyano-5-(dimethylamino)-4-fluoro-penta-2,4-dienoate (22)

A solution of (Z)-3-(dimethyl) amino-2-fluoro-acrolein **18** (600 mg, 5.13 mmol), [^{14}C] ethyl cyanoacetate (250 mCi, 4.71 mmol, 53 mCi/mmol), piperidine (30 μl), and acetic acid (65 μl) in toluene (5 ml) was refluxed for 4 h. Water formed during the reaction was removed by Dean–Stark distillation. Solvent was evaporated *in vacuo* to give the crude product, which was purified by flash chromatography (silica gel column, eluted with Hexane/Ethyl Acetate 1/1) to afford the titled compound **22** as a light yellow solid (155 mCi, 53 mCi/mmol, 620 mg, 62%). ^{1}HNMR ($CDCl_3$): δ 1.31 (t, CH_3), 3.19 (s, CH_3 × 2), 4.23 (q, CH_2), 6.20 (d, 1H), 7.20 (d, 1H); LC-MS (ESI): *m/z* 213 (M+H). HRMS (unlabeled material **19** prepared by the same procedure, ESI) *m/z* Found 212.0960, calcd for $C_{10}H_{13}FN_2O_2$: 212.0961.

Ethyl 2-chloro-5-fluoro-[2-^{14}C]nicotinate (23)

Dry hydrogen chloride gas was passed through a suspension of compound **22** (343.6 mg, 1.62 mmol, 85.9 mCi, 53 mCi/mmol) in 7 ml of 4 M HCl in dioxane for 20 min. The mixture was then stirred for 5 h at 40°C. Solvent and excess HCl gas was removed under reduced pressure and the residue was taken up in 20 ml of methylene chloride. The methylene chloride solution was washed with aqueous 10% $NaHCO_3$ solution (2 × 5 ml), brine (2 × 5 ml), and dried over $MgSO_4$. Solvent was removed under reduced pressure to give the crude product, which was applied to flash chromatography (silica gel, hexane/THF = 1/4) to afford the titled compound **23** (302.6 mg, 79.0 mCi, 53 mCi/mmol, 92%) as a colorless oil. ^{1}HNMR ($CDCl_3$)[1]: δ 1.41 (t, CH_3), 4.42 (q, CH_2), 7.90 (dd), 8.38 (d). LC-MS (ESI): *m/z* 204 (M+H).

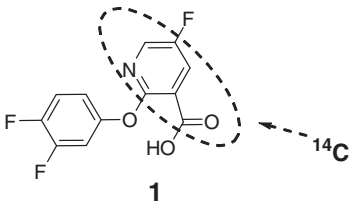


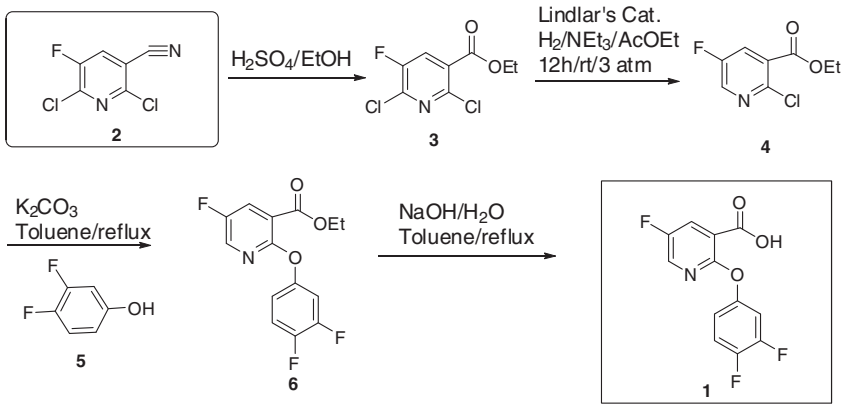
Figure 1. 2-(3, 4-difluorophenoxy)-5-fluoronicotinic acid with the desired labeling area.

Ethyl 2-(3, 4-difluorophenoxy-5-fluoro-[2-^{14}C] nicotinate (24)

A suspension of ethyl 2-chloro-5-fluoro-[2-^{14}C]nicotinate **23** (282 mg, 1.39 mmol, 73.7 mCi, 53 mCi/mmol), 3,4-difluorophenol (312 mg, 2.38 mmol), potassium carbonate (309 mg), and dihydrogen dichlorobis(di-t-butylphosphinito-KP)palladium (6 mg) in toluene (5 ml) was refluxed for 24 h and filtered after cooling down to room temperature. Solvent was evaporated *in vacuo* to give the crude product, which was directly purified by flash chromatography (silica gel column, eluted with hexane/ether 95/5) to afford the titled compound **24** (312 mg, 55.7 mCi, 53 mCi/mmol, 75.6%) as a colorless solid. ^{1}HNMR ($CDCl_3$)[1]: δ 1.43 (t, CH_3), 4.45 (q, CH_3), 6. 91 (m), 7.02 (m), 7.20 (m), 8.15 (d), 8.20 (q), 8.20(d). LC-MS (ESI): *m/z* 298 (M+H).

2-(3, 4-Difluorophenoxy-5-fluoro-[2-^{14}C] nicotinic acid (25)

A solution of **24** (297 mg, 53.0 mCi, 1.0 mmol) in dioxane (1.8 ml) was treated with sodium hydroxide (44 mg, 1.1 mmol) in 0.37 ml of water. The resulting reaction mixture was heated at 100°C for 15 h. Solvent was removed under reduced pressure and the residue was taken up in 20 ml of water. The aqueous solution was extracted with ether (8 ml × 2) and then was acidified to pH 1 to 2 with 2 N HCl. The white precipitate was collected by filtration, washed with water, and dried under vacuum to provide the titled compound **25** (238.5 mg, 87%, 53 mCi/mmol, 47.0 mCi) as an off-white solid. ^{1}HNMR ($CDCl_3$)[1]: δ 6.92 (m), 7.05 (m), 7.22 (m), 8.16 (d), 8.20 (q), 8.22 (d). LC-MS (ESI): *m/z* 270 (M+H). HPLC (column, YMC ODS-AQ, 250 × 4.6 mm; mobile phase A: 0.05%TFA in water, mobile phase B: 0.05%TFA in acetonitrile; gradient: 20 min from 40%A–60%B to 5%A–95%B; flow-rate: 1 ml/min; UV detector: 254 nm; a Flow-one was used as the radioactivity detector; retention time: 8.5 min) and was found to be greater than 98% chemically and radiochemically pure. The



Scheme 1. A known synthesis of 2-(3, 4-difluorophenoxy)-5-fluoronicotinic acid **1**.

H-NMR and mass spectrum of **25** were compared to those of authentic non-labeled standard and found to be consistent.[1]

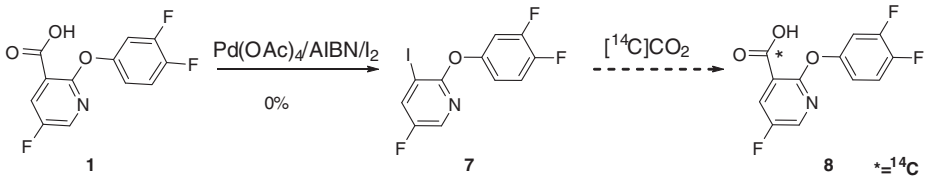
Results and discussion

We first attempted a typical approach to introduce C-14 into the carbonyl group connected to the pyridine ring (Schemes 2 and 3). As the unlabeled compounds **1** and **4** were available from our Chemical Process Lab, we investigated the shortest approach, including decarboxylation, halogenations and ^{14}C-carboxylation or ^{14}C-cyanation to the labeled acid **1** or ester **4**. Thus, the acid **1** was treated with de-carboxylation-iodination reagents, $Pd(OAc)_4$/AIBN/I_2. Unfortunately, no desired iodo-substituted compound **7** was isolated besides the starting material **1** (Scheme 2). Then, the available ester **4** was hydrolyzed to the acid **9** by F_3CCO_2H/HCl in 82% yield. Other reagents such as NaOH and HCl aqueous solution were also applied in the hydrolysis reaction but gave poor yields due to side reactions occurring at the 2-chloro group of pyridine ring system. In this case, the oxidative de-carboxylation-iodination reaction of the acid **9** with Pd $(OAc)_4$/AIBN/I_2 afforded the desired iodo compound **10** in a 54% isolated yield. However, the conversion of the iodo group to carboxyl group by a standard procedure[3] was not straightforward and no desired acid **9** was isolated. We then attempted the cyanation of the iodo compound **10** with CuCN in DMF and isolated the desired compound **11** in only 10% yield but a major by-product in 43% yield, which was identified as 2,3-dicyano-5-fluoropyridine **12**.

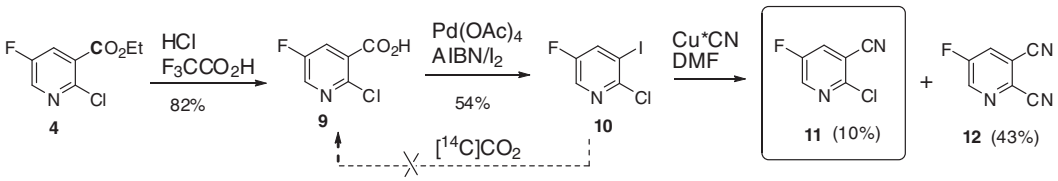
Our results indicated that the chloro group at position 2 of pyridine was very active, and once a cyano group was introduced into the position 3 the chloro group at the position 2 became even more active. The cyanation reaction did not stop at the mono-substitution stage under the tested reaction condition.

Without success of the first approach, we then explored a method to introduce C-14 into the pyridine ring system. In 1990, Shroeder reported an synthetic approach to ethyl 2-chloro-nicotinate **15**.[4] This two-step synthesis included condensation of unsaturated aldehyde **13** with cyanoacetate in the presence of piperidine and acetic acid and cyclic ring-closure of butadiene derivative **14** in ethanolic HCl (Scheme 4).

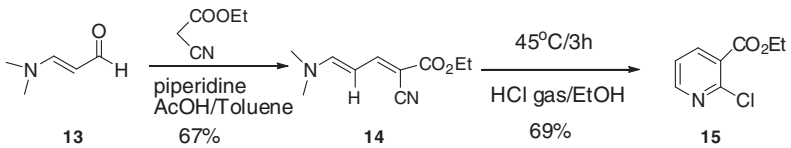
Based on the known synthetic route, if we could introduce a fluoro group into the position 2 of 1-dimethylamino-propenal **13**, this approach might lead to our desired compound **4**. So, (Z)-3-dimethylamino-2-fluoroacrylaldehyde **18** was prepared by



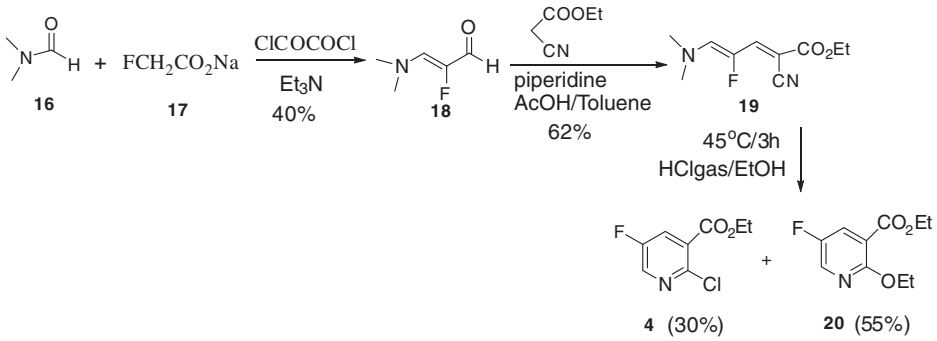
Scheme 2. Attempted synthesis of [^{14}C] **1** from unlabeled **1**.



Scheme 3. Attempted synthesis of [^{14}C] **4** from the unlabeled **4**.



Scheme 4. A known approach to ethyl 2-chloro-nicotinate **15**.



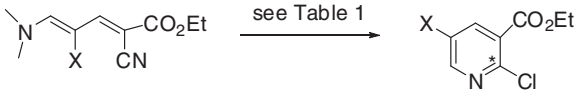
Scheme 5. Synthesis of ethyl 2-chloro-5-fluoro-nicotinate **4**.

reacting an excess of DMF with 2-fluoroacetic sodium salt and oxalyl chloride in the presence of triethylamine according to the literature method.[5] The condensation of the aldehyde **18** with ethyl cyanoacetate in presence of piperidine/acetic acid produced the desired fluoro-substituted diene **19** in 62% yield. However, the cyclic ring-closure with HCl gas in EtOH gave mixture of the desired product **4** in 30% yield and a by-product **20** in 55% yield. The latter, a major by-prodcut, resulted from the nucleophilic substitution of the chloro group at the position 2 of pyridine with EtOH. Our result further indicates that the introduction of fluoro group into the pyridine ring system increases the nucleophilic reactivity of the chloro group (Scheme 5).

As the solvent, ethanol, acted as a nucleophile in the cyclic ring-closure reaction, it should be replaced by other polar non-nucleophilic solvents, such as THF and dioxane, to avoid the side reaction. Indeed, using THF as a solvent the cyclic ring-closure reaction of the diene **19** in the presence of HCl gas produced the desired compound **4** in 55% yield (see entry 2 in Table 1). Furthermore, the yield was greatly improved from 55 to 93% by using 4 M HCl in dioxane as a solvent (see entry 2, 3, and 4 in Table 1). Using the optimized ring-closure condition, we examined the chloro- and bromo-substituted dienes (see entry 5 and 6 in Table 1) and were able to prepare 5-Cl- and 5-Br-substituted pyridine derivatives in very good yields.[6]

In addition, to increase the chemical yield and decrease the reaction time, we studied both palladium catalyst[7] and base[8] promoted the O-arylation reactions (Table 2). In the original large scale process, the chloride **4** reacted with phenol 5 in toluene at refluxing temperature for 72 h to afford the product **6** in 72% yield, while in our small scale process, we only obtained 44% yield using the same condition (entry 2 in

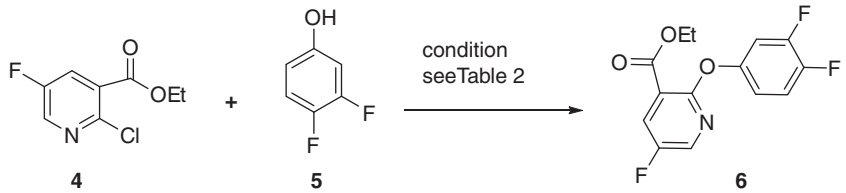
Table 1. Modification of cyclic ring-closure of halogen substituted dienes



Entry	*X*	Solvent	Condition	Yield (%)
1	F	EtOH	HCl (gas) 40°C/5 h	30
2	F	THF	HCl (gas) 40°C/5 h	55
3	**F**	**Dioxane**	**4 M HCl 40°C/5 h**	**93**
4	**F***	**Dioxane**	**4 M HCl 40°C/5 h**	**92**
5	Br	Dioxane	4 M HCl 40°C/5 h	63
6	Cl	Dioxane	4 M HCl 40°C/5 h	82

Note: F* = carbon-14-labeled material.

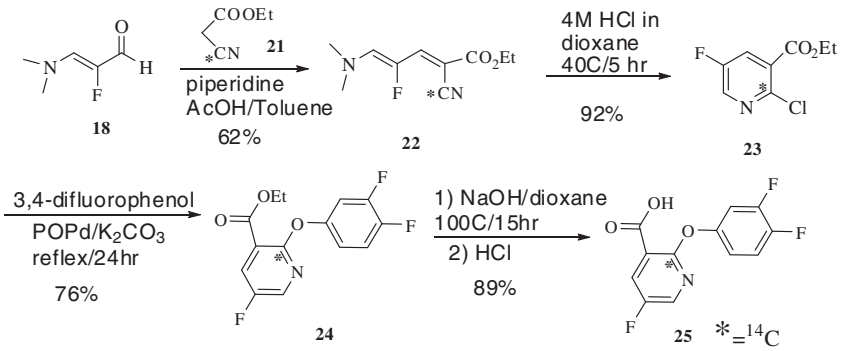
Table 2. Improved process for the synthesis of compound **6**



4 **5** **6**

Entry	Condition	Yield (%)
1	K_2CO_3/toluene/72 h/reflux	72 (kg scale)
2	K_2CO_3/toluene/72 h/reflux	44 (100 mg scale)
3	POPd*/K_2CO_3/toluene/24 h/reflux	76
4	Barton's base#/MeCN/2 h/réflux	68

Note: *POPd = dihydrogendichloro-bis (di-*tert*-butylphosphinito-kP) palladium (2-); #Barton's base = 2-*tert*-butyl-2,2,3,3-tetramethylguanidine.



18 **21** **22** **23** **24** **25**

Scheme 6. Synthesis of 2-(3, 4-difluorophenoxy-5-fluoro-[2-^{14}C] nicotinic acid (**25**).

Table 2). We found that addition of an air-stable Pd catalyst, such as POPd[8] (entry 3 in Table 2), to the ether formation reaction increased the yield from 44 to 76% and decreased the reaction completion time from 72 to 24 h. Furthermore, changing base from K_2CO_3 to Barton's base[9] and solvent from toluene to acetonitrile allowed us to prepare the desired ether **6** in 68% within 2 h.

The radiosynthesis of the compound **25** is shown in Scheme 6. Knoevenagel condensation of aldehyde **18** with commercially available ethyl [^{14}C] cyanoacetate **21** afforded C-14-labeled compound **22** in 62% yield after silica gel chromatography. Using the optimized cyclic ring-closure reaction conditions discussed above, we were able to obtain the desired 2-chloro-5-fluoro-[2-^{14}C] nicotinate in a high isolated yield. The POPd-catalyzed aryl ether formation was completed in 24 h to give the labeled ether **24** in 76% yield. Finally, the labeled compound **24** was hydrolyzed with a NaOH aqueous solution in dioxane at 100°C to furnish the labeled acid **25**. The crude **25** was purified by a hydrochloric acid neutralization process. The radiochemical purity, as measured by analytical HPLC, was $>$98% with a gravimetric specific activity of 53 mCi/mmol (Scheme 6).

Conclusion

In summary, we have developed a new efficient synthesis of the radio-isotope-labeled 2,5-di-substituted pyridine-3-carboxylic acid **25** based on the acid promoted cyclic ring-closure reaction. This four-step synthesis offered 39% overall yield with radiochemical purity of 98% and specific activity of 53 mCi/mmol. We found that the cyclic ring-closure in EtOH gave 55% of a by-product, 2-ethyloxy pyridine derivative, while changing solvent from EtOH to dioxane afforded only desired 2-chloro pyridine derivative in 92%. This new synthetic route for 2-chloro-5-fluoro pyridine derivative was easily applied to make other 5-halogen-substituted pyridine derivative. It could also be expanded to the synthesis of other 5-substituted pyridine derivatives.

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