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SYNTHESIS OF ^{13}C-ENRICHED AND ^{14}C-LABELLED DITHIOPYR

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SUMMARY

This report describes the synthesis of the ^{13}C-enriched and ^{14}C-labelled title compound starting from isotopically labelled isovaleraldehyde.

Key Words: Synthesis, isovaleraldehyde, carbon-14, carbon-13, herbicide, dithiopyr

INTRODUCTION

2-(Difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarbothioic acid, S,S-dimethyl ester (8) (common name dithiopyr) is an experimental herbicidal candidate used for weed control in transplanted rice and in turf. For the environmental chemistry, animal, and plant metabolism studies, ^{14}C-labelled and ^{13}C-enriched samples of the title compound were required. This paper describes the synthetic and analytical procedures utilized in the preparation of these compounds and their synthetic precursors.

RESULTS AND DISCUSSION

The synthetic pathway for preparing ^{14}C-labelled and ^{13}C-enriched 8 is shown in Scheme I. Labelled isovaleraldehyde was used as the starting material, thereby placing the ^{14}C and ^{13}C labels at

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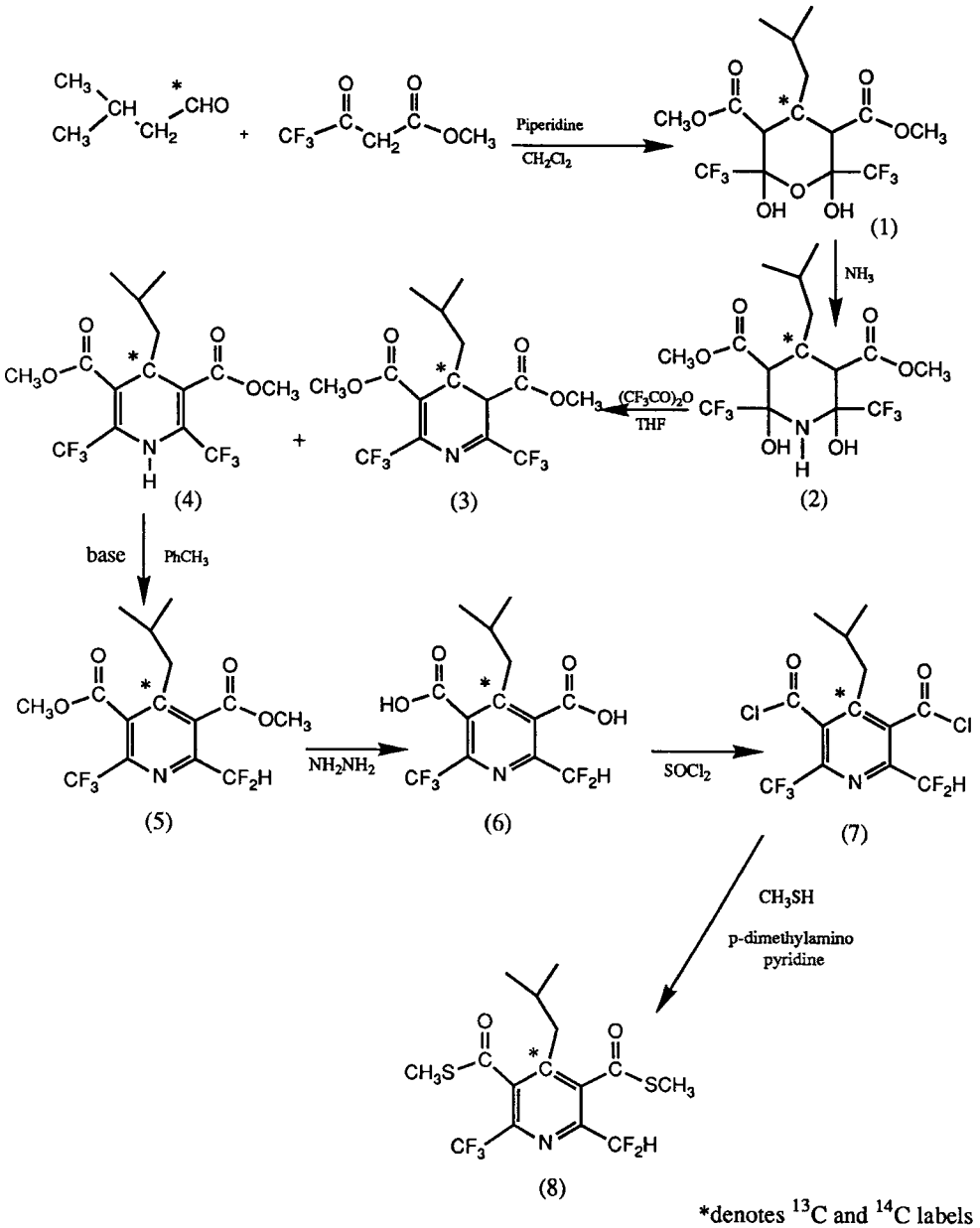
the 4-positions of the pyridine ring. The synthesis of 8 has been previously described[1], and similar procedures were used for the syntheses of labelled compounds. The synthetic approach involved a Hantzsch pyridine synthesis[2], followed by hydrolysis of the methyl esters and conversion to the bis thiolo ester. Reaction of isovaleraldehyde-1-^{14}C with methyl 4,4,4-trifluoroacetoacetate in the presence of a catalytic amount of piperidine gave a mixture of diastereomeric dihydroxydihydropyran 1. Treatment of this dihydropyran with anhydrous ammonia in THF afforded a mixture of diastereomeric dihydroxypiperidine 2. Dehydration with trifluoroacetic anhydride in refluxing dichloromethane gave a mixture of isomeric dienes, 3-^{14}C and 4-^{14}C. Dehydrofluorination[3] of this mixture with N,N-diisopropylethylamine in refluxing toluene afforded ^{14}C-labelled dimethyl ester 5. Hydrolysis of the methyl esters was accomplished by heating with hydrazine hydrate[4] giving the diacid 6-^{14}C. Treatment of 6-^{14}C with refluxing thionyl chloride effected conversion to the diacid chloride 7-^{14}C. Finally, reaction of the diacid chloride with methanethiol and 4-dimethylamino pyridine[5] afforded dithiopyr, 8-^{14}C. The ^{14}C-labelled sample was prepared in an overall yield of 24.6%, starting with 2.23 curies of isovaleraldehyde-1-^{14}C. This reaction sequence afforded 4.85 g of the desired product as a white crystalline solid. The chemical purity was determined to be 98.8% by gas-liquid chromatography (GLC). The radiochemical purity was established as 98.6% by high-pressure liquid chromatography analysis with monitoring of collected fractions by liquid scintillation counting (HPLC/LSC). The specific activity was determined to be 29.24 mCi/mmol. The sample of ^{13}C-enriched 8 was prepared using the same sequence described for the preparation of the ^{14}C-labelled compound in an overall yield of 43%, starting with isovaleraldehyde-1-^{13}C. This reaction sequence afforded 9.20 g of the desired product as a white crystalline solid. The chemical purity was determined to be 98.6% by GLC, and the isotopic enrichment was established as 99 atom % by gas-liquid chromatography/chemical ionization mass spectrometry (GC/CI-MS).

EXPERIMENTAL

All boiling points and melting points are uncorrected. To monitor the progress of selected reactions and to assay the purity of the products, a Varian model 3700 gas chromatograph equipped with a flame ionization detector and 3% DC-200 on Chromosorb W column (80-100 mesh, 6 ft X 2 mm); a Waters HPLC system equipped with a Waters model U6K injector, Waters model 481 UV detector, Waters model 680 controller, Waters model 510 solvent pumps, and a Waters radial compression module with NOVA-PAK C_{18}, cartridge (5μ, 10 cm X 8 mm); and a Varian VXR-300 NMR spectrometer were utilized. Liquid scintillation counting (LSC) was performed on Tracor

SCHEME I



*denotes ^{13}C and ^{14}C labels

Analytic Mark III Model 6881 counters which were interfaced with a Monsanto-developed data acquisition and processing software system. The radiochemical purity of the ^{14}C-labelled sample was determined by HPLC analysis with monitoring of collected fractions by LSC. The mass spectra of the isotopically labelled samples were obtained on a Finnigan 4515 quadrupole mass

spectrometer. The samples were introduced into the mass spectrometer using a Finnigan 9610 gas chromatograph, equipped with a J&W Scientific DB-5 Capillary column (30 m X 0.32 mm). Analyses were performed in the positive ion mode. All synthetic and analytical operations were initially performed with unlabelled compounds and the structure of unlabelled intermediates and products were confirmed spectroscopically and by comparison with authentic samples. Isovaleraldehyde-1-^{14}C was purchased from New England Nuclear, Boston, Massachusetts. Isovaleraldehyde-1-^{13}C was purchased from Merck Sharp & Dohme Isotopes, Quebec, Canada. All other solvents and reagents were reagent grade and obtained from readily available commercial sources.

1. Synthesis of 3,5-Bis(carbomethoxy)-2,6-bis(trifluoromethyl)-2,6-dihydroxy-4-(2-methylpropyl)-4-^{14}C-tetrahydropyran (1).

A mixture of methyl 4,4,4-trifluoroacetoacetate (26.74 g, 157 mmol) and a solution of isovaleraldehyde-1-^{14}C (6.44 g, 74.8 mmol, 2231 mCi, spec. act. = 29.8 mCi/mmol) in dichloromethane (177 mL), together with eight drops of piperidine, was heated at gentle reflux. The reaction was monitored by the disappearance of the isovaleraldehyde-1-^{14}C starting material by GLC. After 24 hr at reflux, the reaction appeared complete by GLC analysis. The reaction mixture was then cooled and concentrated to give a yellow oil (30.10 g, 70.60 mmol, 94%). This material was used in the next reaction without further purification.

2. Synthesis of 3,5-Bis(carbomethoxy-2,6-bis(trifluoromethyl)-2,6-dihydroxy-4-(2-methylpropyl)-4-^{14}C-piperidine (2).

A solution of the yellow oil 1 (30.10 g, 70.60 mmol) in tetrahydrofuran (160 mL) was heated to about 40°C, and anhydrous ammonia from a lecture bottle was bubbled through the solution at a moderate rate for 1.5 hr. Concentration of the solution then gave a yellow oil (29.89 g, 70.30 mmol, 99.5%). This material was used in the next step without further purification.

3. Synthesis of 1,4-Dihydro-2,6-bis(trifluoromethyl)-4-(2-methylpropyl)-3,5-pyridine-4-^{14}C-dicarboxylic acid, dimethyl ester (3) and 3,4-dihydro-2,6-bis(trifluoromethyl)-4-(2-methylpropyl)-3,5-pyridine-4-^{14}C-dicarboxylic acid, dimethyl ester (4).

To a solution of the yellow oil 2 (29.89 g, 70.30 mmol) in dichloromethane (100 mL) was added trifluoroacetic anhydride (42 g, 200 mmol), and the mixture was heated at gentle reflux for 2 hr. Concentration of this solution then gave a yellow oil, which was taken up in dichloromethane

(200 mL). The solution was then washed carefully with saturated sodium bicarbonate (3 X 50 mL), and dried over magnesium sulfate. Filtration and concentration afforded a yellow oil (24.04 g, 61.7 mmol, 88%). This material was used in the next step without further purification.

4. Synthesis of 2-(Difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridine-4-^{14}C-dicarboxylic acid, dimethyl ester (5).

A solution of the mixture of 3 and 4 (24.04 g, 61.7 mmol) and N,N-diisopropylethylamine (8.25 g, 63.8 mmol) in dry toluene (150 mL) was heated at reflux under nitrogen for four days, at which time the reaction was complete by GLC and ^{19}F NMR analyses. The reaction mixture was washed with water (100 mL) and 10% hydrochloric acid solution (100 mL), then dried over magnesium sulfate. Filtration and concentration gave a light brown oil, which was dissolved in a small amount of 10% ethyl acetate in hexane and filtered through a plug of silica gel in a sintered glass funnel with 10% ethyl acetate in hexane (400 mL). Concentration of this solution gave a yellow oil, which was dissolved in hexane (30 mL) and placed in a freezer at 0°C overnight. The resulting solid was collected by vacuum filtration and washed with a small amount of ice-cold hexane to give a white crystalline solid (6.94 g). The mother liquors were then concentrated and subjected to flash column chromatography on silica gel (400 g) using 5% ethyl acetate in hexane as eluent. Those fractions containing 5 were combined, concentrated, and recrystallized to give 3.65 g of additional compound. The first crystalline material was then used in the next reaction without further purification. ^{19}F NMR (282 MHz, $CDCl_3$) δ -65.7 (CF_3), -116.78 (d, J=54 Hz, CHF_2); ^1H NMR (300 MHz, $CDCl_3$) δ 6.74 (t, J=54 Hz, 1H), 3.99 (s, 3H), 3.97 (s, 3H), 2.72 (d, J=7 Hz, 2H), 1.80 - 2.00 (m, 1H), 0.89 (d, J=7 Hz, 6H).

5. Synthesis of 2-(Difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridine-4-^{14}C-dicarboxylic acid (6).

A solution of crystalline 5-^{14}C (6.94 g, 18.8 mmol) in hydrazine hydrate (20 mL) was stirred under nitrogen at gentle reflux for 6.5 hr. The yellow reaction mixture was then cooled, concentrated HCl (25 mL) was added, and the solution was extracted with ether (4 X 50 mL). The combined ether solutions were dried over magnesium sulfate, filtered and concentrated to give a yellow semisolid (6.20 g, 18.2 mmol), whose structure was consistent with the desired product by ^{19}F and ^1H NMR. This material was then used in the next reaction without further purification. ^{19}F NMR (282 MHz, $CDCl_3$) δ -65.0 (s, CF_3), -116.82 (d, J=54 Hz, CHF_2); ^1H NMR (300 MHz,

$CDCl_3$) δ 6.88 (t, J=54 Hz, 1H), 2.87 (d, J=7 Hz, 2H), 2.00 - 2.15 (m, 1H), 0.91 (d, J=7 Hz, 3H).

6. Synthesis of 2-(Difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridine-4-^{14}C-dicarbonyl chloride (7).

A solution of 6-^{14}C (6.20 g, 18.2 mmol) in thionyl chloride (50 mL) was heated at gentle reflux under nitrogen for 16 hr, at which time the reaction was complete by ^{19}F NMR. The solution was then concentrated to give a yellow-brown oil, which was dissolved in hexane (50 mL) and filtered through a plug of silica gel in a sintered glass funnel with hexane (300 mL). Concentration of this solution gave a colorless oil (6.05 g, 16 mmol) whose structure was consistent with the desired product by ^{19}F and ^{1}H NMR, and was used in the next reaction without further purification. ^{19}F NMR (282 MHz, $CDCl_3$) δ −63.9 (s, CF_3), -114 (bs, CHF_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.81 (t, J=54Hz, 1H), 2.88 (d, J=7Hz, 2H), 2.01-2.18(m, 1H), 0.95 (d, J=7Hz, 6H).

7. Synthesis of 2-(Difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridine-4-^{14}C-dicarbothioic acid, S,S-dimethyl ester (8).

A solution of 7 (6.05 g, 16 mmol), methanethiol (10 g, 208 mmol), triethylamine (4.50 g, 44.5 mmol), and 4-dimethylaminopyridine (20 mg) in dichloromethane (50 mL) and toluene (25 mL) was stirred at room temperature for 2 hr. The solution was then washed with 1 N HCl (50 mL), followed by saturated sodium chloride (50 mL), and dried over anhydrous magnesium sulfate. Filtration and concentration gave an oily residue, which was dissolved in 10% ethyl acetate in hexane (100 mL) and filtered through a plug of silica gel in a sintered glass funnel using 10% ethyl acetate in hexane (400 mL). Concentration of the resulting solution gave a white crystalline solid (6.03 g), which was recrystallized twice from hexane (24 mL) to give the desired product as a white crystalline solid (4.85 g). The chemical purity of this sample was determined by GLC to be 98.8% by comparison with an unlabelled analytical standard. The radiochemical purity was established as 98.6% by HPLC/LSC analysis, and the specific activity was determined to be 29.24 mCi/mmol. Analysis of this material by GLC/MS gave the expected parent peaks and isotopic cluster. ^{19}F NMR (282 MHz, $CDCl_3$) δ −63.1 (s, CF_3), -114 (bs, CHF_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (t, J=54Hz, 1H), 2.77 (d, J=7Hz, 2H), 2.60 (s, 3H), 2.58 (s, 3H), 1.85 - 2.00 (m, 1H), 0.84 (d, J=7Hz, 6H)

8. Synthesis of 2-(Difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridine-4-^{13}C-dicarbothioic acid, S,S-dimethyl ester (8-^{13}C).

This compound was prepared by the same procedures described for the preparation of the ^{14}C samples. After recrystallization of the final product this sequence gave the desired product as a white crystalline solid (9.20 g, 44% overall yield). The chemical purity of this sample was determined by GLC analysis to be 98.6% by comparison with an unlabelled analytical standard. The ^{13}C isotopic enrichment was found to be 99 atom% by GLC/MS analysis, which also gave expected parent peaks and isotopic cluster. ^{19}F NMR (282, MHz, $CDCl_3$) δ −63.1 (s, CF_3), -114 (bs, CHF_2); ^{1}H NMR (300MHz, $CDCl_3$) δ 6.74 (t, J=54Hz, 1H), 2.76-2.80 (m, 2H), 2.60 (d, 3H), 2.58 (s, 3H), 1.85-2.00 (m, 1H), 0.84 (d, J=3Hz, 6H).

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