SYNTHESIS OF 3-METHYL-16-IODO-[CARBOXYL-^{14}C] HEXADECANOIC ACID (MIHA)

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Summary

Carbon-14 labelled β-methyl-iodohexadecanoic acid (MIHA) was obtained in a four step sequence using Na^{14}CN, starting from 15-tetrahydropyranyloxy-2-methyl-pentadecanol-p-toluene sulfonate **7**. The specific radioactivity was 55 mCi/mmol and the radiochemical yield 41% of the theoretical maximum.

Key Words: Labelled fatty acid, iodo-[^{14}C]-fatty acid, 3-methyl-hexadecanoic acid

INTRODUCTION

Fatty acids can be used to evaluate myocardial cell viability after acute ischemia. However linear fatty acids are not suitable for such analysis (1) since they are oxidized too rapidly in the mitochondria. β-Methyl-pentadecanoic acid (2) is a structurally modified fatty acid whose oxidation is slow enough to allow scintigraphic analysis of its myocardial distribution (3). In order to investigate the myocardic metabolism of fatty acids, ^{14}C labelled 3-methyl-16-iodo-hexadecanoic acid (MIHA) was synthesized from [^{14}C] sodium cyanide (10 mCi). The starting material is prepared from dibromodecane and the protected propargylic alcohol. The labelled compound **11** is obtained via the sequence described in Scheme 1.

EXPERIMENTAL

Infrared spectra were recorded on thin film using a Perkin Elmer Spectrophotometer model 1310. ^{1}H NMR spectra were obtained as $CDCl_3$ solutions using a Bruker AC 200 spectrometer. Radioactivity counting was performed on an Intertechnic ABA SL 40 Liquid Beta Scintillation Spectrometer. Thin layer chromatography (TLC) utilized silicagel. Solvents were redistilled just before utilization from $LiAlH_4$ for THF and from calcium hydride for hexamethylphosphoric triamide (HMPT). [^{14}C] NaCN (10 mCi, 55.9 mCi/mmol) was purchased from Isotec France.

3-Tetrahydropyranyloxy prop-1-yne 1 (4)

In a round bottom flask fitted with a condenser and a magnetic stirrer, a mixture of propargylic alcohol (11.2 g, 0.2 mol), dihydropyrane (16.8 g, 0.2 mol) and hydrochloric acid (0.2 mol) was stirred and cooled in an ice bath for 3 h. The extracted ether layer was washed with a saturated Na_2CO_3 solution (2 x 50 ml), with water (2 x 50 ml) then dried over anhydrous Na_2SO_4.

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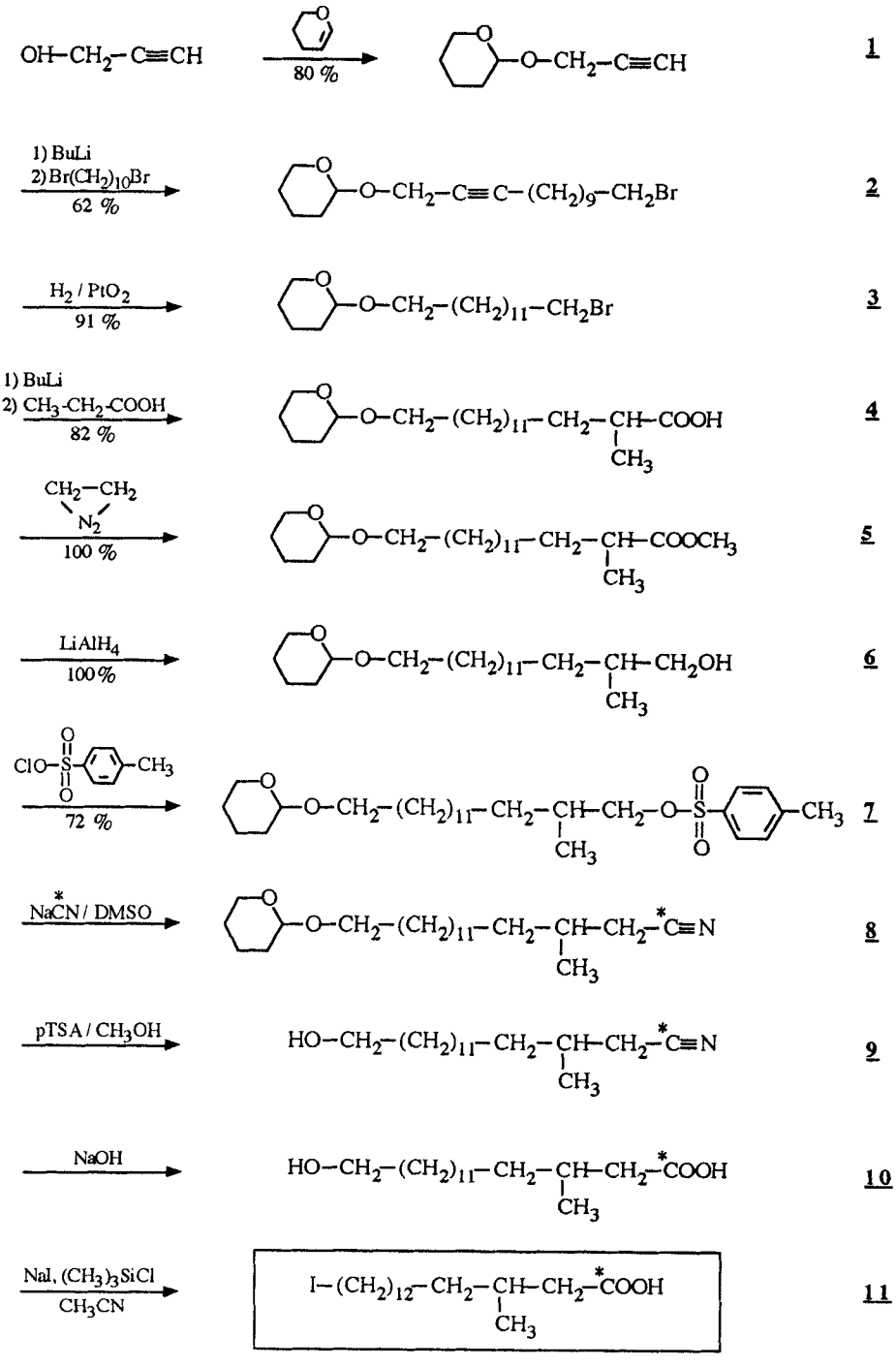
OH–CH2–C≡CH
80 %
1
1) BuLi
2) Br(CH2)10Br
62 %
2
H2 / PtO2
91 %
3
1) BuLi
2) CH3-CH2-COOH
82 %
4
CH2–CH2
N2
100 %
5
LiAlH4
100 %
6
ClO–S–CH3
72 %
7
NaCN / DMSO
8
pTSA / CH3OH
9
NaOH
10
NaI, (CH3)3SiCl
CH3CN
11

The solvent was evaporated in vacuum and the main product was distilled under reduced pressure. Pure compound **1**, $Eb_{7mm\ Hg}$ = 75°C, was obtained as a colorless liquid. Yield = 80%. ^1H NMR (δ ppm, $CDCl_3$): 1.6 (m, 6H, 3(CH_2) pyrane); 2.4 (t, 1H, =CH, J =2.0 Hz); 3.5-3.8 (m, 2H, CH_2-O pyrane); 4.2 (dd, 2H, O-CH_2-C=, J_g = 15.8 Hz, J_a = 2.4 Hz); 4.8 (t, 1H, -OCHO-, J = 2.8 Hz).

1-Bromo-13-tetrahydropyranyloxy-tridec-11-yne 2 (4,6)

In a 250 ml three necked flask compound **1** (5.6 g, 40 mmol) was dissolved in a solution of 40 ml of freshly distilled THF. The mixture was cooled to -10°C and butyl lithium (1.6 M in hexane, 26 ml) was injected slowly with a syringe. The stirring was maintained for 3 h at room temperature and the solution was transferred under argon to a dropping funnel and added dropwise to a solution of dibromodecane (24 g, 80 mmol) in THF (100 ml) for 2 h. The temperature was maintained at 20-25°C. The reaction progress was monitored by TLC with silicagel using toluene as eluent. The mixture was hydrolyzed by a saturated solution of NH_4Cl (200 ml) and extracted with pentane (4 x 100 ml). The organic layer was washed with water (2 x 100 ml) and dried over Na_2SO_4 and the solvent was evaporated in vacuum. Pure product was obtained by silica column chromatography and eluted with toluene giving 8.9 g of **2** as a light yellow oil. Yield = 62%. ^1H NMR (δ ppm, $CDCl_3$): 1.5-2.0 (m, 22H); 2.2 (m, 2H, C=C-CH_2); 3.4 (t, 2H, CH_2Br, J = 7.0 Hz); 3.5-3.8 (m, 2H, CH_2O pyrane); 4.2 (dd, 2H, O-CH_2-C=, J_g= 15.2 Hz, J_v = 2.1 Hz); 4.8 (t, 1H, -OCHO-, J = 1.0 Hz).

1-Bromo-13-tetrahydropyranyloxy-tridecane 3 (6)

Compound **2** (4.5 g, 12 mmol) was dissolved in anhydrous diethyl ether (200 ml), stirred and reduced by platinum dioxide catalyst (50 mg) under a hydrogen atmosphere for 2 h. The solvent was removed and the crude product was purified using silica column chromatography (eluent: toluene) giving compound **3** as a colorless oil. Yield 91%. ^1H NMR (δ ppm, $CDCl_3$): 1.5-2.0 (m, 28H); 3.4 (t, 2H, CH_2Br, J = 7.0 Hz); 3.5-4.0 (m, 4H, CH_2O); 4.6 (m, 1H, -OCHO-).

15-Tetrahydropyranyloxy-2-methyl-pentadecanoic acid 4 (6)

In a round bottom flask and under an inert atmosphere, diisopropylamine (2.8 ml, 20 mmol) was dissolved in anhydrous THF (80 ml). The temperature was maintained at -10°C and butyl lithium (12.5 ml, 20 mmol) was injected. Propanoic acid (0.75 ml, 10 mmol) in THF (5 ml) was added dropwise. A white precipitate appeared and after 10 min. HMPT (14 ml) was added with a syringe. The mixture became clear and yellow colored. After about 20 min, a solution of the bromine derivative **3** (3.6 g, 10 mmol) in THF was added quickly and the temperature increased to 50°C. The reaction was left overnight and monitored by TLC using silicagel and n-hexane-ethyl acetate (7/3) as eluent.The crude product was extracted with pentane (4 x 100 ml), washed with water (4 x 100 ml) and dried. The purification was performed using silica column chromatography (eluent: hexane/ethyl acetate 7/3). Yield = 82 %. The pure compound **4** was a white solid powder. M.p. = 35°C. ^1H NMR (δ ppm, $CDCl_3$): 1.2 (d, 3H, CH_3, J = 7.0 Hz), 1.2-1.8 (m, 30H, CH_2); 2.3-2.6 (m, 1H, CH-COO); 3.2-4.1 (m, 4H, CH_2O); 4.6 (m, 1H, -OCHO-).

Methyl-15-tetrahydropyranyloxy-2-methyl-pentadecanoate 5 (4)

The main acid (1.0 g, 2.8 mmol) was esterified using an excess of diazomethane in ether freshly prepared from Diazald (Aldrich). The solvent was evaporated and the ester **5** was obtained in

quantitative yield as a colorless oil. ^{1}H NMR (δppm, $CDCl_3$): 1.1 (d, 3H, $C\underline{H}_3$-CH, J = 7.0 Hz); 1.2-1.8 (m, 30H, CH_2); 2.3-2.5 (m, 1H, CH_3-C$\underline{H}$); 3.3-4.0 (m, 4H, CH_2O); 3.7 (s, 1H, OCH_3); 4.6 (m. 1H, -OCHO-).

15-Tetrahydropyranyloxy-2-methyl-pentadecan-1-ol 6 (6)

To a solution of $LiAlH_4$ (4.8 mmol) in anhydrous ether (50 ml) in a three necked flask fitted with a magnetic stirrer, the above ester 5 (0.82 g, 2.4 mmol) was added during the course of 30 min. The mixture was refluxed for 30 min and the reaction was monitored by TLC (eluent: heptane/ethyl acetate 8/2). The white precipitate was filtered and washed with diethyl ether. The resulting product was dried and evaporated in vacuo to give a colorless oil in quantitative yield. ^{1}H NMR (δppm, $CDCl_3$): 0.9 (d, 3H, CH_3, J = 6.7 Hz); 1.2-1.7 (m, 32H, CH_2); 1.8 (m, 1H, C$\underline{H}$-CH_3); 3.3-4.0 (m, 4H, CH_2O); 4.6 (m, 1H, -OCHO-).

15-Tetrahydropyranyloxy-2-methyl-pentadecanol-p-toluenesulfonate 7 (6)

A mixture of tosyl chloride (0.84 g, 4.4 mmol) and alcohol 6 (0.75 g, 2.2 mmol) in triethylamine (20 ml) was maintained at 0°-5°C for 3 days. The reaction was controlled by TLC using silica gel and heptane-ethyl acetate (8/2) as eluent. The hydrolysis was carried out in 12N HCl (10 ml) and the organic layer was extracted with dichloromethane (3 x 20 ml), and washed with water (3 x 20 ml). The solid tosylate 7 was purified using silica column chromatography and hexane-ethyl acetate 8/2 as eluent. Yield = 72 %. ^{1}H NMR (δ ppm, $CDCl_3$): 0.9 (d, 3H, $CHC\underline{H}_3$, J = 6.8 Hz); 1.3 (m, 30H, CH_2); 1.8 (m, 1H, C$\underline{H}CH_3$); 2.5 (s, 3H, $C_6H_4CH_3$); 3.3-4.0 (m, 4H, CH_2O); 4.6 (m, 1H, -OCHO-); 7.3 and 7.8 (dd, 4H, C_6H_4, J = 8.2 Hz).

3-Methyl-16-tetrahydropyranyloxy-hexadeca-[^{14}C]nitrile 8

In a 50 ml three necked flask fitted with a magnetic stirrer and under argon a mixture of $Na^{14}CN$ (1.5 eq., 10 mCi, 55.9 mCi/mmol) and inert NaCN (8.8 mg, 0.179 mmol) was dissolved in DMSO (1 ml) in a 80°C water bath. Tosylate 7 (0.059 g, 0.119 mmol) in DMSO (2 ml) was added. The reaction was monitored by TLC using silicagel (eluent: petrol ether/ethyl acetate 8/2) and finished in 2 days. The temperature of the reaction was brought back to 0°C and hydrolyzed with a saturated NH_4Cl solution (4 ml). The nitrile 8 was extracted with petroleum ether (3 x 5 ml) and the organic layer was washed with water (3 x 5 ml) and evaporated in vacuum. ^{1}H NMR (δppm, $CDCl_3$) of the stable compound: 1.0 (d, 3H, $CHCH_3$, J = 6.7 Hz); 1.3 (m, 28H, CH_2); 1.6 (m, 2H, C$\underline{H}_2$CH); 1.8 (m, 1H, $CH_2C\underline{H}$); 2.2 (dd, H, CH_2CN, J_g = 16.5 Hz, J_v = 6.5 Hz); 2.3 (2dd, 1H, CH_2CN, J_g = 16.5 Hz, J_v = 6.0 Hz); 3.2-4.0 (m, 4H, CH_2O); 4.6 (m, H, -OCHO-).

3-Methyl-16-hydroxy-hexadeca-[^{14}C]nitrile 9

From the crude labelled nitrile 8 dissolved in methyl alcohol (2 ml), p-toluenesulfonic acid (5 mg) was added, with stirring for 2 h at room temperature. The reaction was followed by TLC (eluent: dichloromethane-THF 95/5). The ^{1}H NMR of the corresponding stable product was characterized by the loss of signals at 3.2-4.0 ppm and 4.6 ppm.

3-Methyl-16-hydroxy-[carboxyl-^{14}C]hexadecanoic acid 10

The deprotected nitrile **9** was directly treated in the same flask with a 40% NaOH solution (3 ml). The mixture was refluxed for 3 days, then cooled to 0°C and acidified with concentrated HCl (3 ml). The reaction was monitored by silcagel TLC (eluent: CH_3Cl-CH_3OH 95/5). The solution was extracted with diethyl ether (3 x 5 ml) and the organic layer was washed with water (3 x 5 ml) then filtered through a 1PS Whatman filter paper and evaporated in vacuo. ^{1}H NMR (δ ppm, $CDCl_3$) of the stable product: 1.0 (d, 3H, $CHCH_3$, J = 6.6 Hz); 1.3 (m, 22H, CH_2); 1.6 (m, 2H, CH_2CH); 1.8 (m, 1H, $CHCH_3$); 2.2 (dd, 1H, CH_2COOH, J_v = 8.0 Hz, J_g = 14.8 Hz); 2.3 (2dd, 1H, CH_2COOH, J_v = 6.0 Hz, J_g = 14.8 Hz); 3.6 (t, 2H, CH_2OH, J = 6.6 Hz).

3-Methyl-16-iodo-[carboxyl-^{14}C]hexadecanoic acid 11 (MIHA)

The obtained hydroxy acid **10** was dissolved in freshly distilled acetonitrile (4 ml). Trimethylsilane (0.07 mmol) and NaI (0.4 mmol) were added and the mixture was heated to 70°C. The iodination was monitored by TLC using CH_3Cl-CH_3OH (95/5) as eluent. The reaction was complete after 4 h. After cooling, a solution of 10% $Na_2S_2O_3$ was added until complete decoloration of the reaction product. The resulting mixture was extracted with diethyl ether (4 x 5 ml) and the organic layer washed with a solution of 10% $Na_2S_2O_3$ (4 x 5 ml) and filtered through a 1PS Whatman filter paper. The final purification was carried out using TLC on silicagel (300 mm thickness) and CH_3Cl-CH_3OH (95/5) as eluent. The final product 11 (MIHA) was a white solid, m.p. = 49-50°C. Total yield = 41 %. Radioactivity = 2.70 mCi. Specific radioactivity = 55 mCi/mmol. ^{1}H NMR (δ ppm, $CDCl_3$) of the stable product: 1.0 (d, 3H, $CHCH_3$, J = 6.7 Hz); 1.3 (m, 22H, CH_2); 1.85 (m, 2H, CH_2); 2.2 (dd, 1H, CH_2COOH, J_v = 8.0 Hz, J_g = 14.8 Hz); 2.4 (2dd, 2H, CH_2COOH, J_v = 5.9 Hz, J_g = 14.8 Hz); 3.2 (t, 2H, ICH_2, J = 7.0 Hz).

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