Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVIII, No. 2

SYNTHESIS OF ω-LABELLED FATTY ACIDS

C. S. Tamvakopoulos and V. E. Anderson*

Department of Chemistry, Brown University
Providence, Rhode Island 02912

SUMMARY

A synthetic procedure for producing isotopically ω-labelled fatty acids is described. The isotopic label is introduced by reducing an ω-tosylate with either labelled sodium borohydride or labelled lithium dimethyl cuprate.

Key Words: ω-labelled fatty acids, [16-^{2}H]hexadecanoic acid

INTRODUCTION

Isotopically labelled fatty acids are used in studies of fatty acid oxidation (1,2), lipid biosynthesis and studies of biological membranes (3). Although isotopic labelling at C-1 and C-2 or C-8 and C-9 of fatty acids is relatively facile, the generation of ω-labelled fatty acids has been more difficult. The ω-labelled fatty acids are useful in studies of the physical properties of amphiphilic membranes or micelles where the environment of the ω-carbon is in question, and in studies of fatty acid oxidation where an ω-label would be present in all intermediates with a constant specific activity. The synthetic scheme developed in this paper allows the incorporation of any of the possible ω-labels (^{13}C, ^{2}H, ^{3}H, ^{14}C), in three standard high yielding procedures.

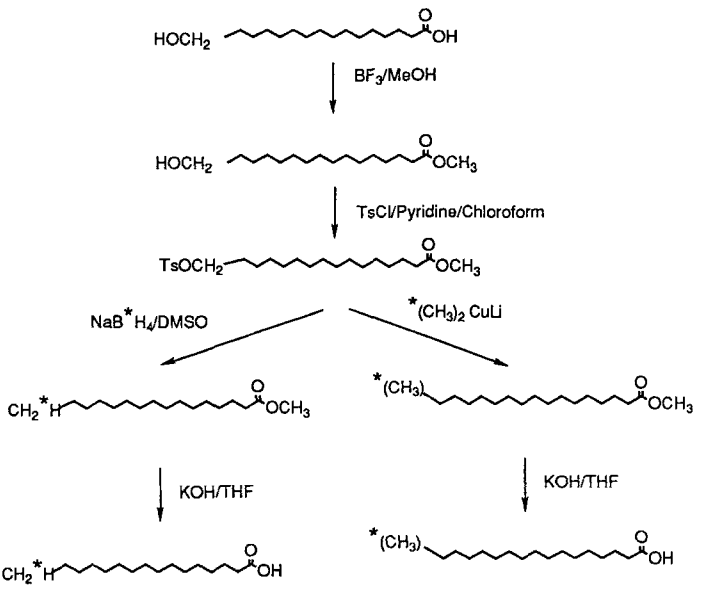
The synthesis involves the sequential esterification of a commercially available ω-hydroxy fatty acid with BF_3-Methanol (4), tosylation of the ω-hydroxy methyl ester with p-toluenesulfonyl chloride in chloroform and

0362–4803/90/020187–05$05.00

Received July 14, 1989
Revised August 29, 1989

pyridine (5) and reduction of the tosylate with either labelled sodium borohydride (6) or lithium dimethyl cuprate (7). The synthesis is summarized in scheme A1:

SCHEME A1



EXPERIMENTAL

Methyl 16-hydroxypalmitate: 16-Hydroxypalmitic acid (Sigma) was converted to the methyl ester by dissolving 100-200 mg in three ml of BF_3-Methanol (4). The mixture was refluxed on a steam bath for 3 minutes. The reaction mixture was added to 30 ml of petroleum ether and extracted 3 times with 20 ml of distilled water. The ether layer was dried with anhydrous sodium sulfate and evaporated under a stream of dry nitrogen. ^1H NMR ($CDCl_3$) δ 1.3 (broad singlet 22H), δ 1.6 (broad multiplet, 4H), δ 2.25 (t, 2H), δ 3.6 (t, 2H) δ 3.65 (s, 3H) . Isolated yields were routinely over 80%.

Methyl 16-tosylpalmitate: The tosylate was formed by the method of Kabalka et al (5). Methyl 16-hydroxypalmitate (0.1 g, 350 µmol) was dissolved in 350 µl of chloroform-d and cooled in an ice bath (0 ^oC),

pyridine-d_5 (60 µl, 742 µmol) and p-toluenesulfonyl chloride (0.1 g, 525 µmol), were then added. The reaction mixture was stirred at 0 °C for 3 hours. The progress of the reaction was followed by monitoring the disappearance of the triplet at 3.6 ppm and the appearance of a triplet at 4.0 ppm in the ^1H NMR. Ethyl ether (1 ml) and water (250 µl) were added and the organic layer was washed successively with 2 N HCl, 5% $NaHCO_3$, and sodium phosphate buffer at pH 6.5. The organic layer was dried with anhydrous sodium sulfate and the ethyl ether was evaporated under a stream of dry nitrogen. Yield, 0.115 g, 75%. ^1H NMR ($CDCL_3$) δ 1.3(22H), δ 1.6(4H), δ 2.25 (2H), δ 2.5 (s, 3H), δ 3.65 (s, 3H CH_3), δ 4.0 (t, 2H CH_2O), δ 7.35 (d, 2H) and δ 7.8 (d, 2H).

Methyl [16-^2H]palmitate: Sodium borodeuteride (40 µmol, 98% D, Sigma) was dissolved in 1 ml of dimethyl sulfoxide and added to 20 µmol of methyl 16-tosylpalmitate. The reaction mixture was heated at 85 °C for 2 hours. The progress of the reaction could be followed by disappearance of the triplet at 4.0 ppm in the ^1H NMR if DMSO-d_6 were used as solvent. The reaction mixture was titrated to pH 4-5 with 1 M HCl and the aqueous layer was extracted 3-4 times with petroleum ether. The pooled petroleum ether extracts were dried with anhydrous sodium sulfate and evaporated under a stream of dry nitrogen. Yield, 4.3 mg, 80%. ^1H NMR ($CDCl_3$) δ 0.9 ppm (3H broad multiplet CH_2D), δ at 1.3 and δ 1.6 (26H), δ at 2.25 (t 2H), δ at 3.65 (s 3H). GC\MS (EI+) of the product dissolved in ethyl acetate yielded a 271/270 ratio of 100/8.5 with a retention time identical to authentic methyl palmitate.

Methyl heptadecanoate: The coupling of the primary tosylate to the lithium diorganocuprate reagent is done according to Johnson et al. (7). To about 150 µmol of CuI is added 820 µl of dry freshly distilled ethyl ether and the solution stirred at 0°. Then 180 µl of 1.4 M CH_3Li (250 µmol) are added dropwise over a period of 30 min. The endpoint of the reaction is reached when the solution turns from a bright yellow to a light tan solution. To the lithium dimethylcuprate was added 32.5 mg (74 µmol) of 16-tosyl methyl palmitate dissolved in 1 ml of ethyl ether. The reactants were stirred at 0

^{o}C for two hours. Upon completion of the reaction, a mixture of 10% (v:v) conc. NH_4OH in sat. NH_4Cl is added to the reaction mixture and stirred for 20 min. at 0 ^{o}C. The layers are separated and the ether layer is washed three times with saturated ammonium chloride, dried with anhydrous sodium sulfate, and evaporated under a stream of dry nitrogen. Yield, 13.6 mg, 65%. ^{1}H NMR ($CDCl_3$) δ 0.9 ppm (t, 3H), δ at 1.3 and 1.6 ppm(28H), δ 2.25 (t 3H), δ 3.65 (s 3H). GC\MS(EI+) gives a parent ion peak of m/e 284 eluting 58 seconds after authentic methyl palmitate. No methyl palmitate was detected.

Methyl ester hydrolysis: The methyl esters were routinely hydrolyzed quantitatively in THF (1.5 ml) with 2 M KOH at 60 ^{o}C (overnight). After evaporating the THF under a stream of dry nitrogen, equal volumes of ethyl ether and 1 M HCl were added. The water layer was extracted three times with ethyl ether and the pooled extracts were dried with anhydrous sodium sulfate. The ether was then evaporated under a stream of dry nitrogen.

RESULTS AND DISCUSSION

The need for ω-labelled fatty acids in our studies revealed the lack of a facile published synthesis. Bouloussa et al (8) and Dinh-Nguyen (9) have summarized the schemes available, all of which are longer and less flexible than that described here. The availability of ω-hydroxy fatty acids of various chain lengths made them prime candidates for starting materials. The ^{1}H NMR spectra of the esterification and tosylation reaction mixtures suggested nearly quantitative reaction, obviating the need for purification. The reported yields presumably reflect losses in isolation. The reductive removal of the tosylate by $NaBH_4$ or by dialkyl cuprates are also very high yielding reactions in general (6,7), and as demonstrated. Isotopically labelled (^{14}C, ^{13}C, CH_2T, CD_3) lithium dimethyl cuprate could be easily synthesized from the commercially available methyl iodides by the procedure of Aberhart (10). Although we synthesized methyl heptadecanoic acid, ω-labelled palmitate would be generated by starting with the commercially available 15-hydroxypentadecanoic acid (Wiley Organics, Columbus, OH). By using longer chain dialkyl cuprates a variety of specifically labelled fatty acids could be

generated. Reducing methyl 12-tosyldodecanoate with 1,1-[$^2H_2$]butyl cuprate would generate methyl 13,13-[$^2H_2$]palmitate, which would be unavailable by the current less flexible synthetic schemes.

Acknowledgements: We thank Dr. Wender Wan for assistance with the GC-MS and the National Institutes of Health (GM-36562) for financial support.

REFERENCES

1. Stanley K.K. and Tubbs P.K. - Biochem. J. 150: 77 (1975).

2. Chatzidakis C. and Otto D.A. - Lipids 22: 620 (1987).

3. Stockton G.W., Polnaszek C.F., Tulloch A.P., Hasan F., and Smith I.C.P. - Biochemistry 15: 954 (1976).

4. Metcalfe L.D. and Schmitz A.A. - Anal. Chem. 33: 363 (1961).

5. Kabalka G.W., Varma M., and Varma R. - J. Org. Chem. 51:2386 (1986).

6. Hutchins O.R., Kandasamy D., Dux III F., Maranoff A.C., Rotstein D., Goldsmith B., Burgoyne W., Cistone F., Dalessandro J., and Puglis J. - J. Org. Chem. 43: 2259 (1978).

7. Johnson R.C. and Dutra A.G. - J. Amer. Chem. Soc. 95: 7777 (1973).

8. Bouloussa O., Denhez J.P., and Dizabo P. - J. Labelled Compd. Radiopharm. 23: 127 (1986).

9. Nguyen-Dinh N. - Ark. Kemi 22: 151 (1964).

10. Aberhart D.J. - J. Labelled Compd. Radiopharm. 20: 605 (1983).