Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **43**, 243-250 (2000)

Synthesis of ^{14}C - Labeled FAD-C44

Christopher Bieniarz* and Kornepati V. Ramakrishna

Advanced Drug Delivery Department, AP4, Abbott Laboratories,

100 Abbott Park Road

Abbott Park, IL 60064-3500

Terence P. Kelly

NEN Life Science Products, Inc, Custom Synthesis Group, 549 Albany

Street,

Boston, MA 02118

SUMMARY

Synthesis of ^{14}C -labeled FAD-C44 **1** at the tertiary carbon by the dimerization of ^{14}C labeled erucic acid **2** is described. Labeling at the C-14 position of erucic acid is achieved by a convergent approach involving the Wittig coupling of ^{14}C-labeled nonyl aldehyde **4** and the Wittig reagent **3**.

KEY WORDS: ^{14}C-labeled FAD-C44, erucic acid, Wittig reagent, montmorillonite K-10 and dimerization.

INTRODUCTION

The copolymer of FAD (Fatty Acid Dimer)-C44 and sebacic acid has been developed as an implantable and biodegradable matrix for the controlled delivery of gentamicin to treat chronic bone infections *i.e.*, osteomyelitis[1].

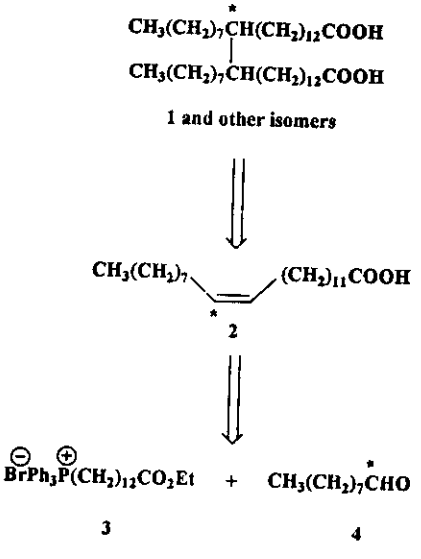
CCC 0362–4803/2000/030243–08$17.50

Received 14 April 1999
Revised 8 July 1999
Accepted 23 July 1999

FAD-C44 **1** labeled at the tertiary centers with ^{14}C was required for metabolic studies. The tertiary carbon position was chosen as the labeling site since it is expected to be the most conserved site during metabolism[2]. Earlier we reported methodology for the selective deuterium labeling at the tertiary carbons of FAD-C44[3]. In this paper we describe an efficient and simple synthetic approach for the incorporation of ^{14}C labels at the tertiary positions of FAD-C44.

RESULTS AND DISCUSSION

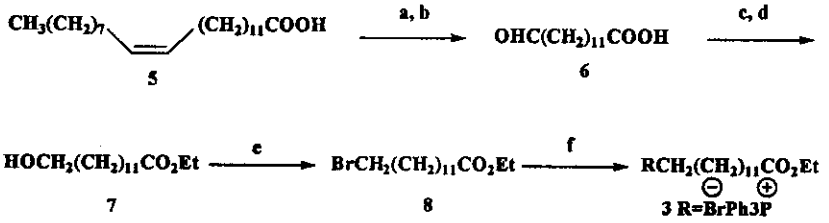
FAD-C44 **1**, a 44 carbon branched dicarboxylic fatty acid is commercially synthesized by the C-C dimerization of a naturally available 22 carbon unsaturated fatty acid, erucic acid **5**[4]. Since the dimerization is a non-selective process, FAD-C44 is a mixture of isomers. Accordingly, the synthetic strategy (Scheme 1) employed for the incorporation of ^{14}C at the tertiary centers of FAD-C44 involved the synthesis of ^{14}C-labeled erucic acid **2**, which was prepared by a convergent approach involving the coupling of the Wittig reagent **3** and ^{14}C nonyl aldehyde **4**.



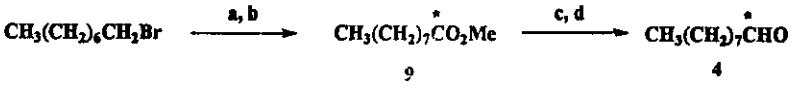
Scheme 1 (* indicates radiolabeled carbon)

The bromoester **8** required for the synthesis of the Wittig synthon **3** was prepared starting from commercially available erucic acid **5** (Scheme 2). Hydroxylation of erucic acid **5** with 40% H_2O_2 and formic acid followed by cleavage of the intermediate diol with $NaIO_4$ yielded the carboxyaldehyde **6** in 60% yield. Reduction of **6** followed by esterification with ethanol afforded the hydroxy ester **7** in 72% yield; this was then smoothly converted to bromoester **8** with Ph_3P and NBS in 88% yield. Finally the Wittig synthon **3** was prepared in 83% yield by refluxing bromoester **8** and Ph_3P in toluene.

The carboxylation of the 1-octylmagnesium bromide followed by treatment with an ether solution of diazomethane afforded the methyl ester **9** which was then sequentially reduced with LAH and then oxidized with PCC to yield the desired ^{14}C nonyl aldehyde **4** in 60% yield (Scheme 3).

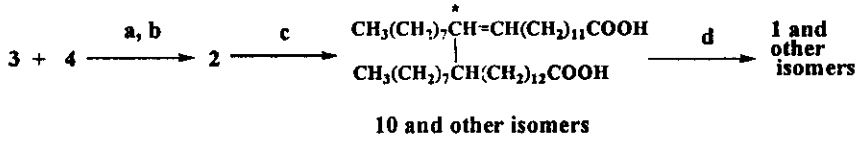


Scheme 2 a) 40% H_2O_2, HCOOH, 50 °C to room temperature, 18h, NaOH, 2h (72%), b) $NaIO_4$, room temperature, 18h (60%), c) $NaBH_4$, EtOH, 30 min (95%) d) H_2SO_4 (cat.), EtOH, reflux, 4h (72%), e) Ph_3P, NBS, room temperature, 2h (88%); f) Ph_3P, toluene, 120 °C, 72h (83%)



Scheme 3 a) Mg, $^{14}CO_2$; b) CH_2N_2; c) $LiAlH_4$; d) PCC

Finally the coupling of the Wittig reagent **3** with ^{14}C nonyl aldehyde **4** gave ^{14}C erucic acid. Following the literature procedure[4], erucic acid was dimerized with montmorillonite-K10 at 60 psi and 275 °C in the presence of 2% water to afford the unsaturated FAD-C44 **10**. Finally hydrogenation of **10** afforded the desired ^{14}C compound labeled at the tertiary centers of FAD-C44 **1** (0.72 mCi/776 mg).



Scheme 4. a) LDA; b) KOH, methanol, reflux; c) 10% montmorillonite-10K, 2% water, 60 psi, 275 °C, 6h; d) 10% Pd/C, EtOAc, 4 atm.

In conclusion, we have developed a simple and efficient convergent approach to the site specific ^{14}C labeling at the tertiary centers of the branched dicarboxylic fatty acids. ^{14}C labeled erucic acid **2**, the radiolabeled precursor to FAD-C44 **1** was readily synthesized by the Wittig coupling of ^{14}C-labeled nonyl aldehyde **4** and the Wittig reagent **3**. The labeled monomer **1** of the polyanhydride drug delivery matrix allowed efficient monitoring of the metabolic fate of the matrix in animal models.

EXPERIMENTAL

Materials and Methods

Erucic acid was obtained from Unichema North America (Chicago, IL). All the other chemicals were purchased from Aldrich (Milwaukee, WI). ^{1}H and ^{13}C NMR were recorded on Varian-400 Unity or Varian-500 Unity spectrometers. Electrospray ionization mass spectra were obtained on Finnigan-TSQ700 or Finnigan-SSQ7000 spectrometers with $MeOH/CH_2Cl_2/H_2O$ (65:35:10) as a flow solvent.

13-oxotridecanoic acid 6. Erucic acid (20 g, 60 mmol) was added to a solution of 30% hydrogen peroxide (10 mL) and formic acid (36 mL) and heated at 55 °C for 1.5 h. The reaction mixture was cooled to room temperature and stirred for 16 h. Excess formic acid was removed *in vacuo* and the thick white residue obtained was cooled in an ice bath and 50% NaOH solution was added slowly dropwise and stirred with a mechanical stirrer for 2h at room temperature. The reaction flask was cooled in an ice bath and acidified to pH 1 with concentrated HCl. The white solid precipitate was filtered washed with water (5 x 50 mL), dried *in vacuo* to yield 15.8 gm of crude diol. To a solution of $NaIO_4$ (19.41 g) in water (180 mL) a suspension of the diol (8.4 g, 23.5 mmol) in chloroform (60 mL) was added and stirred at room temperature for 6 h. The chloroform layer was separated washed with water (2 x 20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated to yield a colorless waxy solid which was purified by silica gel chromatography with hexane:ether: AcOH (93:7:4) to afford 3.17g (60%) of aldehyde 6.

13-hydroxytridecanoic acid. Sodium borohydride (1.05 g, 28 mmol) was added to a solution of aldehyde 6 (3.17 g, 14.0 mmol) in ethanol (30 mL). The reaction was stirred at room temperature for 2h. Ethanol was removed on a rotatory evaporator and the residue was taken up in water (20 mL) and extracted with ether (3 x 10 mL). The combined organic layers were washed with water (2 x 10 mL) and brine (10 mL) and concentrated to yield the hydroxy acid (3.06 g, 95%). ^{1}H NMR ($CDCl_3$) δ 1.23-1.31 (m, 18H), 1.54-1.66 (m, 2H), 2.30 (t, 2H) and 3.61(t, 2H).

13-hydroxyethyltridecanoate 7. To a solution of the hydroxy acid (1.0 g, 4.34 mmol) obtained from the reduction of 6 in ethanol (15 mL), sulfuric acid (2 drops) was added and the reaction was refluxed for 4h. Ethanol was removed *in vacuo* and the residue obtained was taken up in ether (20 mL) and washed with water (2 x 10 mL) and brine (10 mL), dried over anhydrous magnesium sulfate and concentrated. The residue obtained was purified by silica gel chromatography (7:3 hexane:EtOAc) to afford ester 7 (0.8 g,

72%) as colorless oily material. ^{1}H NMR ($CDCl_3$) δ 1.23-1.31 (m, 18H), 1.54-1.68 (m, 5H), 2.30 (t, 2H), 3.62 (t, 2H) and 4.13 (q, 2H).

13-bromoethyltridecanoate 8. A solution of hydroxy ester 7 (2.98 g, 11.55 mmol) and Ph_3P (4.10 g, 15.6 mmol) in THF (30 mL) was cooled in an ice bath and NBS (2.78 g, 15.6 mmol) was added in portions. After 10 min the reaction flask was allowed to warm to room temperature. After 30 min the orange colored reaction mixture was filtered to remove the solid triphenylphosphine oxide; the filtrate was concentrated and the residue obtained was purified by silica gel chromatography (8:2 hexane:ether) to give bromoester 8 as a colorless oily material (3.25 g, 88% yield). ^{1}H NMR ($CDCl_3$) δ 1.23-1.29 (m, 16H), 1.36-1.50 (m, 2H), 1.62 (t, 3H), 1.84 (q, 2H), 2.28 (t, 2H), 3.23 (t, 2H) and 4.17 (q, 2H).

[12-(Ethoxycarbonyl)dodecanyl]triphenylphosphonium bromide 3. A solution of bromoester 8 (4.76 g, 14.8 mmol) and Ph_3P (3.62 g, 14.8 mmol) in toluene (7.0 mL) was refluxed for 72 h. Hexane was then added to the reaction mixture and refluxed for 30 min. The reaction flask was cooled and the upper layer was decanted while retaining the bottom thick colorless oily product which was further washed with hexane (3 x 30 mL) and ether (30 mL). The residue was dried under high vacuum to yield Wittig reagent 3 as a white hygroscopic solid (7.2 g, 83% yield). ^{1}H NMR ($CDCl_3$) δ 1.13-1.36 (m, 19H), 1.53-1.70 (m, 4H), 2.30 (t, 2H), 3.72-3.86 (m, 2H), 4.05-4.18 (q, 2H) and 7.62-7.91 (m, 15H).

Methyl [1-^{14}C] nonanoate 9. 1-Bromooctane (7.7 mL, 45 mmol) was reacted with magnesium (1.2 g, 49.5 mmol) in refluxing ethyl ether under argon for 2h. The reaction was cooled in liquid nitrogen and carefully evacuated. Carbon dioxide [^{14}C] (825 mCi) was introduced by vacuum transfer and the reaction was warmed to - 20 °C for 1.5 h. The reaction was quenched with 1N HCl (60 mL) and the product isolated by extraction. The acid was converted to the methyl ester by an ether solution of diazomethane, purified by silica gel flash chromatography (from 100% pentane to 50% pentane: diethyl ether) followed by vacuum distillation to afford the ester 9 as colorless liquid (2.37 g, 92% yield) in 92% radiochemical yield.

[1-^{14}C] - nonanol. To a solution of methyl [1-^{14}C] nonanoate **9** in anhydrous ethyl ether (15 mL), lithium aluminum hydride (13.8 mmol) in ether (13.8 mL) was added dropwise and the reaction mixture was heated at reflux for 2 h. The reaction was cooled in an ice bath and sodium sulfate (1.0 g) was added followed by dropwise addition of a saturated solution of sodium sulfate until the evolution of gas had ceased. Solids were removed by filtration and concentration of the filtrate followed by vacuum distillation of the residue afforded the [1-^{14}C] - nonanol in 91 % yield.

[1-^{14}C] - nonyl aldehyde. Pyridinium chlorochromate (PCC) (5.43 g, 25.2 mmol) was ground to an intimate mix with silica gel (5.43 g) and treated with [1-^{14}C] - nonanol (12.6 mmol) in dichloromethane at room temperature for 45 minutes. The dark reaction mixture was filtered through a pad of silica gel and vacuum distillation of the filtrate afforded [1-^{14}C] - nonyl aldehyde (1.07 g, 60% yield) in 59% radiochemical yield.

[14-^{14}C] Erucic acid 2. To a solution of Wittig reagent **3** (5.65 g, 10 mmol) in THF (22 mL), n-butyllithium (5.7 mL of 1.6 M in hexane, 9.1 mmol) was added dropwise, turning the pale yellow colored solution to dark red. [1-^{14}C] - nonyl aldehyde (0.85 g, 7 mmol) in THF was added to the reaction mixture. Stirring was continued at room temperature for 1.5 h and at 50 °C for 0.5 h. The crude [14-^{14}C] erucic acid ethyl ester obtained was hydrolyzed in refluxing 5% KOH in methanol.The product was purified by silica gel chromatography (hexane: ether: acetic acid 80:20:1) to afford [14-^{14}C] erucic acid **2** (0.43 g, 18% yield) in 16% radiochemical yield.

[14-^{14}C] Erucic acid dimer: [14-^{14}C] Erucic acid (0.43 g) was diluted with non-radioactive erucic acid and the total amount (25g, 75 mmol) was placed in a 75 mL steel reaction vessel equipped with a stir bar. Montmorillonite K-10 (2 gm, 8% by weight) and water (0.5 mL, 2% by weight) were introduced and the reaction vessel was pressurized with nitrogen to 60-80 psi and heated at 275 - 280 °C for 6 h. The unreacted erucic acid was removed by vacuum distillation and the residue was chromatographed on silica gel (hexane: ether: acetic acid 80:20:1) to yield the unsaturated dimer. The

dimer was hydrogenated with 10% Pd/C at 50psi in ethyl acetate for 48 h to afford ^{14}C labeled FAD-C44 (2.47 g) in 5% radiochemical yield.

ACKNOWLEDGEMENTS

One of us (TK) thanks Dr. Puliyur Srinivasan and Dr. Mahendra Suryan for providing NMR and mass spectral data.

REFERENCES

1. a) Domb J.A and Maninar M. - J. Polym. Sci. Part A: Polymer Chemistry. **31**: 1275 (1993). b) Domb J.A. - US Patent. 5, 171, 812 (1992)
2. a) Bergseth S., Poisson J. and Bermer J. Biochim. Biophys. Acta **1042**, 182 (1990)
   b) Singh H., Usher S., Johnson D., and Poulous A. J. of Lipid Res. **31**, 217 (1990)
3. Bieniarz C., Ramakrishna K.V., and Mitchell M. J. Lab. Comp. & Radiopharm. **42**, 17 (1999)
4. Cros G. A., Hasse K. D., Lanne N. C. M., and Vreeswijk J. J. European Patent Application No: 91307505.7 (1991)

