

Synthesis of Deuterium Labeled FAD-C44

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SUMMARY

A concise synthetic methodology for selective deuterium labeling at the tertiary centers of the fatty acid dimer, FAD-C44 **1** is described. Labeling methodology involves three steps and the deuterium is introduced at the tertiary carbons selectively by bromination of FAD-C44 followed by reduction with NaCNB^2H_3 and SnCl_2 .

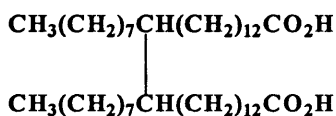
KEY WORDS: Fatty acid dimer, FAD-C44, Deuterium, Boron tribromide, Sodium cyanoborodeuteride

INTRODUCTION

FAD-C44 **1** is a dimer of erucic acid, a 22 carbon unsaturated fatty acid. It is useful as a building block in certain polymeric drug delivery systems⁽¹⁾. In the context of pharmacokinetic and metabolic studies of FAD-C44 a tritium labeled compound was desired. The labeling was required at the tertiary carbons of FAD-C44 since it is expected to be the most conserved site during the mammalian metabolic processes. A number of synthetic methods are available for tritium labeling of aromatic substrates and suitably activated carbons i.e., olefinic, acetylenic and methylene α to carbonyl groups. Tritium labeling of unactivated aliphatic carbons by direct substitution is less well known and more

challenging. In this paper we report regioselective labeling of FAD-C44 with ^2H at the tertiary carbons, as a model study for selective tritium labeling of unactivated tertiary aliphatic carbons.

FAD-C44 is industrially synthesized in two steps from erucic acid⁽²⁾. The first step is a clay catalyzed dimerization of erucic acid under high pressure and high temperature, followed by complete hydrogenation to saturated dimer FAD-C44 **1**. Since the dimerization is a nonselective process, FAD-C44 is a mixture of isomers.



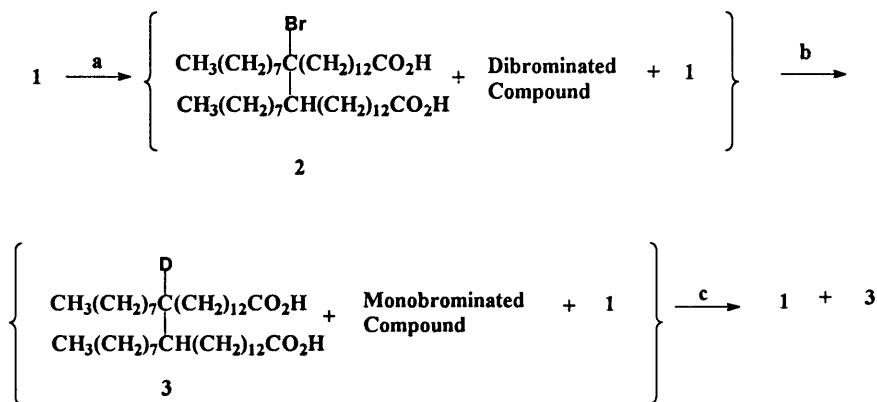
1 and isomers

RESULTS AND DISCUSSION

The ^2H labeling of FAD-C44 in principle can be accomplished either by synthesis of FAD-C44 from the labeled precursors or directly labeling the FAD-C44. We chose the latter process for labeling FAD-C44 since such a method would be shorter, more efficient and would avoid carrying the labeled material over longer synthetic sequence. The direct labeling of FAD-C44 was planned *via* bromination followed by reduction of tertiary bromide **2** with a deuterated reducing agent (scheme 1). FAD-C44 was brominated under aliphatic electrophilic conditions with BBr_3 and Br_2 and the resulting material was investigated for the presence of bromination products by electrospray ionization mass spectroscopy (ESMS) and NMR. Although the TLC of the reaction mixture on silica gel did not reveal any new product, the ESMS of the reaction material displayed three molecular ions at m/z 755, 835 and 675 corresponding to monobrominated **2**, dibrominated and unreacted starting material respectively. The intensities of these

molecular ions indicated the relative ratios of monobrominated, dibrominated and starting material to be 47:10:43 respectively. ^{13}C NMR of the brominated product displayed a new signal in the downfield region at δ 65 ppm which disappeared in the DEPT experiment, indicating the bromination at the tertiary carbon of FAD-C44. If the bromination had occurred on the secondary or primary carbons the resulting carbon signal would be expected to show up in the DEPT experiment. In addition, a triplet at δ 2.42 ppm in ^1H NMR corresponding to methylene α to brominated tertiary carbon showed long range correlation with quaternary carbon at δ 65 ppm confirming the tertiary bromination.

The structure of the dibrominated product could not be determined from the spectral data available and thus remains unknown. Since the introduction of deuterium label was required at the tertiary position, conditions for the chemoselective reduction of tertiary bromide were chosen so as not to affect any primary or secondary bromides, if present. The selective reduction was accomplished by reducing brominated material with NaCNB^2H_3 in presence of $\text{SnCl}_2^{(6)}$. The ^2H NMR of the reduced material displayed a single

Scheme 1^a

^a Reagents: a. BBr_3 , Br_2 ; b. NaCNB^2H_3 , Et_2O ; c. NaBH_4 , DMSO .

peak for deuterium at δ 2.1 ppm consistent with introduction of ^2H at the tertiary carbon indicating the formation of deuterated product **3**. The ESMS of the reduced material displayed m/z ions at 675, 677 and 755 corresponding to starting material, deuterated FAD-C44 and brominated product respectively. Thus, partially reduced material containing brominated product was completely reduced to FAD-C44 enriched with deuterated FAD-C44 **3** with NaBH_4 in $\text{DMSO}^{(6)}$. The ESMS of the reduced material showed molecular ions at m/z 675 and 677 corresponding to FAD-C44 and deuterated FAD-C44.

In conclusion, we developed a concise synthetic methodology for labeling tertiary carbons of fatty acid dimer by utilizing sequential substitution reactions - aliphatic electrophilic bromination⁽⁷⁾ followed by reduction with NaCNB^2H_3 . Since sodium cyanoborotritide is commercially available, the present methodology can be readily applied to tritiation of similar structures at the tertiary carbons.

EXPERIMENTAL

Materials and Methods

FAD-C44 was obtained from Unichema North America (Chicago, IL). BBR_3 , NaCNB^2H_3 and NaBH_4 were purchased from Aldrich (Milwaukee, WI). ^1H and ^{13}C NMR were recorded on Varian-400 Unity or Varian-500 Unity. Electrospray ionization mass spectra were obtained on Finnigan-TSQ700 or Finnigan-SSQ7000 with $\text{MeOH}/\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (65:35:10) as a flow solvent.

Bromination of FAD-C44. 1.0 M BBR_3 in CH_2Cl_2 (1.3 mL, 1.3 mmol) was added to a solution of Br_2 (0.1 mL, 1.9 mmol) in CH_2Cl_2 (3.0 mL) and heated at 55 °C. After 15 min a solution of FAD-C44 (1.0 g, 1.5 mmol) in CH_2Cl_2 (2.0 mL) was added to the reaction mixture and heating was continued for 2.0 h. The reaction mixture was cooled to room

temperature and stirred over night. Ice cold water (6.0 mL), 1.0 M NaHSO₃ (10.0 mL) and CH₂Cl₂ (3.0 mL) were successively added to the red colored reaction solution with stirring. Organic layer was separated, dried over MgSO₄, filtered, concentrated and the oily residue obtained was chromatographed on silica gel (Hexane/Et₂O/AcOH 80:20:4) to afford 0.932 g of brominated FAD-C44 as light brown oily material. MS (ESMS) *m/z* (relative intensity) 675 (M-H, 43%), 755 (M-H, 47%) and 833 (M-H, 10%).

Reduction of brominated FAD-C44 material with NaCNB²H₃. SnCl₂ (47 mg, 0.25 mmol) was added to a suspension of NaCNB²H₃ (32 mg, 0.5 mmol) in Et₂O (20 mL) under N₂ and stirred at room temperature. After 10 minutes a solution of brominated FAD-C44 material (190 mg.) in Et₂O (4.0 mL) was added dropwise to the reaction flask. The reaction was stirred under N₂ at room temperature for 16 h. After the addition of H₂O (10.0 ml) to the reaction flask, the contents were transferred to the separatory funnel. Et₂O layer was separated and water layer was further extracted with Et₂O (5.0 ml x 2). Organic layers were combined, washed with brine, dried over MgSO₄, filtered and concentrated to brown oily material which was purified on silica gel (Hexane/Et₂O/AcOH 80:20:4) to yield 152 mg of partially deuterated FAD-C44 as a pale yellow oily material. MS (ESMS) *m/z* (relative intensity) 675 (M-H, 46%), 677 (M-H, 48%) and 755 (M-H, 6%).

Reduction of FAD-C44. NaBH₄ (76 mg, 0.1 mmol) was added portion wise to a solution of partially deuterated FAD-C44 (76 mg.) in anhydrous DMSO (1.0 mL) at room temperature under N₂ atmosphere. Reaction was heated at 90 °C under nitrogen for 17 h. Reaction mixture was diluted with H₂O (3.0 mL), acidified with 1.0 N HCl and extracted with CH₂Cl₂ (5.0 mL x 2). Combined organic layers were washed with H₂O, brine and dried over anhydrous MgSO₄ and concentrated to yield 17.0 mg of partially deuterated FAD-C44 as a light brown oily material. MS (ESMS) *m/z* (relative intensity) 675 (M-H, 43%), 677 (M-H, 47%). ¹H NMR (CDCl₃) 0.82-0.93 (m, 6H), 1.18-2.00 (m, 70H), 1.22-1.26 (m, 4H), 2.38(t, 6H). ¹D NMR (CDCl₃) 2.1 (t).

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