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SYNTHESIS OF 14(*R,S*)-[^{18}F]FLUORO-6-THIA-HEPTADECANOIC ACID (FTHA)

Timothy R. DeGrado
Institut für Chemie 1, Forschungszentrum Jülich,
D-5170 Jülich, FRG

SUMMARY

No-carrier-added (n.c.a) 14(*R,S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid (FTHA) has been synthesized for evaluation as a PET tracer for myocardial long chain fatty acid utilization. The n.c.a. nucleophilic radiofluorination of benzyl 14(*R,S*)-tosyloxy-6-thia-heptadecanoate in acetonitrile utilized (cryptate 2.2.2/K)$_2$CO$_3$ for anion activation. The resulting [^{18}F]fluoro-ester was quantitatively hydrolyzed with addition of aqueous KOH and the product purified by reversed phase HPLC. The radiochemical yield of purified FTHA was 35-65% (n=5) with a synthesis time of 50 min. Radiochemical purity was >99%.

Key words: ^{18}F, nucleophilic radiofluorination, thia fatty acid

INTRODUCTION

Positron-emitter labeled long chain fatty acids (LCFAs) have been developed for non-invasive PET studies of LCFA utilization in the heart (for a review, see 1). [1-^{11}C]Palmitate (CPA) has been extensively studied as a natural tracer of palmitate utilization. However, the interpretation of myocardial time-activity curves of CPA is complicated by the rapidity and multiplicity of clearance processes involved. The β-methyl substituted analog of palmitate, β-methyl-[1-^{11}C]heptadecanoic acid has been proposed to provide a longer myocardial retention of the radiolabel as a consequence of inhibited β-oxidation (2). In light of the favorable properties of

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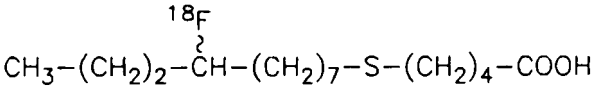
^{18}F ($T_{1/2}$=110 min, low β^+ energy) for PET imaging, ^{18}F-labeled fatty acids were devloped. The first were straight chain LCFA analogs with carrier [^{19}F]fluoride added (3,4). No-carrier-added (n.c.a.) syntheses of 6- and 7-[^{18}F]-labeled palmitate (5), ω-^{18}F-labeled straight chain (6-7), and ω-^{18}F-labeled methyl-substituted LCFA analogs have also been reported (8-10).

Recent biochemical studies show the CoA thioesters of 4-thia fatty acid analogs to be potent inhibitors of β-oxidation (11). Although the mechanism of inhibition is not known, β-oxidition of 4-thia fatty acids may proceed to 4-thia-2-enoyl-CoA thioesters which could possibly bind to acyl-CoA dehydrogenases within the mitochondrion (cf. 12). To determine whether an ^{18}F-labeled, even-substituted thia LCFA analog could be metabolically trapped in myocardium, we have synthesized 14(*R,S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid (FTHA) in no-carrier-added form.



FTHA

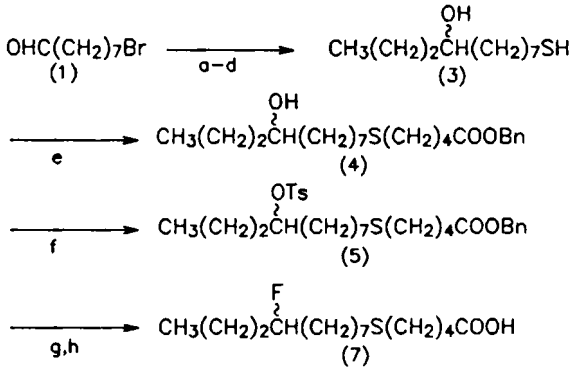
RESULTS AND DISCUSSION

The n.c.a. nucleophilic radiofluorination of benzyl 14(*R,S*)-tosyloxy-6-thia-heptadecanoate **5** in acetonitrile utilized (cryptate 2.2.2/K)$_2CO_3$ for anion activation (7). Incorporation of [^{18}F]fluoride was 40-82%. The resulting [^{18}F]fluoro-ester (FTHA-Bn) was quantitatively hydrolyzed with the addition of aqueous KOH and purified by reversed-phase HPLC (Table 1). Radiochemical yield of purified FTHA was 35-65% (n=5) with a synthesis time of 50 min.

Other than [^{18}F]fluoride, only minor radiochemical impurities (< 5%) were observed in the HPLC radiochromatograms. A large fraction of the unincorporated [^{18}F]fluoride was retained on the HPLC column, the remainder eluting with the void volume or bleeding slowly from the column. For experiments that were sensitive to the small [^{18}F]fluoride impurity, the product fraction was passed through a silica gel SEP-PAK cartridge (400 mg, Millipore) before evaporation of solvent. This reduced the contamination of [^{18}F]fluoride typically from 0.2% to <.03%. The radiochemical purity of FTHA, as monitored up to 4 hr after synthesis by

analytical reversed-phase HPLC, was >99%.

The tosyloxy-ester **5** was prepared in several synthetic steps according to Scheme 1. This compound was an oil and stable when refrigerated. Non-radioactive standards of fluorinated fatty acid **7** and benzyl ester **6** were prepared via fluorination of **5** with tetraethylammonium fluoride.



a) $CH_3(CH_2)_2MgBr$, THF; b) H^+; c) $(NH_2)_2CS$, DMSO; d) aq. KOH, Δ;
e) $Br(CH_2)_4COOBn$, K_2CO_3, DMF; f) $(Ts)_2O$, C_5H_5N;
g) TEAF, CH_3CN; h) KOH, $EtOH/H_2O$

Scheme 1

EXPERIMENTAL

General

Chemicals were of analytical grade unless otherwise noted. DMF and DMSO were stored over molecular sieves. THF was dried by distillation from $LiAlH_4$. Dry CH_3CN (<0.003% H_2O) was obtained commercially (Merck). ^{1}H-NMR spectra were recorded with a Varian Model-390 90 MHz spectrometer using $CDCl_3$ as solvent (Me_4Si, 0.00 ppm). R_f values refer to thin layer chromatography (TLC) performed on silica gel (Merck) with the solvent system noted. Routine column chromatography was performed under normal pressure with silica gel (Merck, 70-325 mesh).

11-Bromo-4(*R,S*)-undecanol (2)

8-Bromo-1-octanal **1** was prepared in 76% yield from 8-bromo-1-octanol according to the method of Corey et al. (13). The aldehyde was isolated by column chromatography (hexane/ether 3:1). TLC (hexane/ether 3:1) R_f=0.7. IR 1730 cm^{-1}. The aldehyde was allowed to react with propyl magnesium bromide in dry THF in the classical

manner for a Grignard reaction. Hydrolysis of the resulting magnesium alkoxide was performed by the slow addition of ice-diluted HCl. The organic phase was separated, dried over Na_2SO_4, and evaporated under reduced pressure. The product **2** was isolated as an oil by column chromatography (hexane/ether 1:2) in 95% yield. TLC (hexane/ether 1:2) R_f=0.6. NMR 0.94 (t, 3H, C(1)H_3), 1.40 (m, 16H, CH_2), 3.5 (m, 3H, C(11)H_2, C(4)H). Anal.- Calc. for $C_{11}H_{23}BrO$: C, 52.59; H, 9.23; Br, 31.8. Found: C, 53.23; H, 9.22; Br, 32.0.

11-Mercapto-4(*R,S*)-undecanol (**3**)

To a solution of **2** (13 g, 52 mmol) in DMSO (15 ml) at 24 °C was added thiourea (55 mmol). After 20 hr, 2N KOH (10 ml) was added and the mixture was heated at 80 °C for 5 min. The mixture was acidified (HCl) and extracted twice with ether (40 ml). The combined ether phases were washed successively with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The product **3** (7 g, 66% yield) was isolated as a colorless oil by column chromatography (hexane/ether 1:3). TLC (hexane/ether 1:3) R_f=0.65. NMR 0.94 (t, 3H, C(1)H_3), 1.40 (m, 16H, CH_2), 2.51 (quartet, 2H, C(11)H_2), 3.64 (m, 1H, C(4)H). Anal.- Calc. for $C_{11}H_{24}OS$: C, 64.65; H, 11.84; O, 7.83; S, 15.7. Found: C, 64.10; H, 12.08; O, 7.82; S, 15.6.

Benzyl 14(*R,S*)-hydroxy-6-thia-heptadecanoate (**4**)

To a solution of benzyl 5-bromopentanoate (2.7 g, 10 mmol, ref. 14) in dry DMF (100 ml) at 24 °C was added the mercaptan **3** (2.3 g, 9.9 mmol) and K_2CO_3 (0.7 g, 5 mmol). After reaction under argon for 20 hr, the mixture was acidified with ice-diluted HCl and extracted with ether (2 x 50 ml). The combined ether fractions were washed successively with dilute $NaHCO_3$, water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The product **4** (1.6 g, 40% yield) was isolated by column chromatography (hexane/ether 1:2) and recrystallized from hexane to give white flakes. M.p.= 31.7 °C. TLC (hexane/ether 1:2) R_f=0.5. NMR 0.92 (t, 3H, C(17)H_3), 1.40 (m, 20H, CH_2), 2.4 (m, 6H, C(2)H_2, C(5)H_2, C(7)H_2), 3.69 (m, 1H, C(14)H), 5.10 (s, 2H, -CH_2-ϕ), 7.32 (s, 5H, aryl). Anal.- Calc. for $C_{23}H_{38}O_3S$: C, 70.01; H, 9.71; O, 12.2; S, 8.12. Found: C, 69.65; H, 9.76; O, 12.1; S, 8.07.

Benzyl 14(*R,S*)-tosyloxy-6-thia-heptadecanoate (**5**)

The hydroxy-ester **4** (0.44 g, 1.11 mmol) was dissolved in CH_2Cl_2 (5 ml) at 0 °C. Pyridine (1.4 mmol) and p-toluenesulfonic

anhydride (1.4 mmol) were added and the mixture was allowed to react for 4 hr. The mixture was diluted with ether (10 ml) and washed successively with dil. HCl, dil. $NaHCO_3$, water and brine, dried over Na_2SO_4, and the solvent evaporated under reduced pressure. The product **5** (0.56 g, 92% yield) was isolated by column chromatography (hexane/ethyl acetate 3:1), giving a colorless oil. TLC (hexane/ethyl acetate 3:1) R_f=0.6. NMR 0.92 (t, 3H, C(17)H_3), 1.40 (m, 20H, CH_2), 2.4 (m, 9H, C(2)H_2, C(5)H_2, C(7)H_2, (tosyl) CH_3), 4.57 (m, 1H, C(14)H), 5.09 (s, 2H, -CH_2-ϕ), 7.5 (m, 9H, aryl). Anal.- Calc. for $C_{30}H_{44}O_5S_2$: C, 65.66; H, 8.08. Found: C, 65.28; H, 8.08.

Benzyl 14(*R,S*)-fluoro-6-thia-heptadecanoate (6)

Tetraethylammonium fluoride hydrate (5 mmol) was dried by azeotropic distillation with dry CH_3CN (2 x 20 ml). A solution of the tosylate **5** (1 mmol) in dry CH_3CN (10 ml) was added and the mixture was allowed to react at 24 °C for 20 hr. After evaporation of the solvent, water (20 ml) and ether (20 ml) were added. The organic phase was dried over Na_2SO_4 and the solvent evaporated. The product **6** (24% yield) was isolated by HPLC (Table 1). TLC (hexane/ethyl acetate 3:1) R_f=0.8. NMR 0.92 (t, 3H, C(17)H_3), 1.40 (m, 20H, CH_2), 2.45 (m, 6H, C(2)H_2, C(5)H_2, C(7)H_2), 4.11 (m, 0.5H, C(14)H), 4.75 (m, 0.5H, C(14)H), 5.09 (s, 2H, -CH_2-ϕ), 7.32 (s, 5H, aryl).

14(*R,S*)-Fluoro-6-thia-heptadecanoic acid (7)

The fluoro-ester **6** (1 mmol) was hydrolyzed by treatment with 15 ml EtOH/0.2N aq. KOH (1:1) at 24 °C for 20 hr. After acidification (HCl), the crude product was extracted into ether (25 ml). The ether phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product **7** was isolated by preparative HPLC (Table 1) and recrystallized from hexane. M.p.= 50.3 °C. TLC (hexane/ethyl acetate/acetic acid 75:25:2) R_f=0.35. NMR 0.92 (t, 3H, C(17)H_3), 1.40 (m, 20H, CH_2), 2.45 (m, 6H, C(2)H_2, C(5)H_2, C(7)H_2), 4.13 (m, 0.5H, C(14)H), 4.75 (m, 0.5H, C(14)H).

^{18}F-labeling procedure

To a 20 ml glassy carbon vessel was added Kryptofix 2.2.2 (10 mg, 25 μmol), CH_3CN (0.5 ml) and 20 μl of a 9% K_2CO_3 solution in water. [^{18}F]Fluoride, produced via proton bombardment of $H_2{}^{18}O$ (>95 atom %), was then added, the vessel placed into a 90-95 °C oil bath, and the solvent evaporated under a stream of helium. The residue was

Table 1. Preparative reverse-phase HPLC capacity factors (k') of LCFA analogs and esters (Nucleosil, C-18 (10μ), 250 x 20 mm; MeOH/H_2O/AcOH (88:12:0.4); flow: 10 ml/min)

Compound	k'
Benzyl 14-tosyloxy-6-thia-heptadecanoate (**5**)	3.2
Benzyl 14-fluoro-6-thia-heptadecanoate (**6**, FTHA-Bn)	3.5
14-Tosyloxy-6-thia-heptadecanoic acid	2.1
14-Fluoro-6-thia-heptadecanoic acid (**7**, FTHA)	2.4

further dried by azeotropic distillation with CH_3CN (2 x 0.3 ml). A solution of the benzyl ester **5** (25 μmol) in dry acetonitrile (0.5 ml) was added and a water-cooled condensor was placed atop the reaction vessel. Reaction time was 15 min. The incorporation of [^{18}F]fluoride was monitored by radio-TLC (hexane/ethyl acetate 3:1). R_f values were 0.0 and 0.8 for [^{18}F]fluoride and [^{18}F]fluoro-ester, respectively.

Subsequent hydrolysis of the resulting [^{18}F]fluoro-ester was performed in the same vessel by the addition of 0.3 ml 0.2N KOH and continued heating at 90-95 °C for 5 min. The mixture was cooled, acidified with concentrated acetic acid (30μl), and applied to the preparative HPLC column (Table 1). After evaporation of solvent under reduced pressure, the residue was typically formulated in a 0.9% NaCl solution containing 4-6% albumin for *in vivo* studies in small animals. Alternatively, FTHA may be formulated in patient's serum for clinical use (7).

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