Synthesis of Novel Tritium labeled Oxamyristic Acids

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SUMMARY

Syntheses of [^{3}H]-labeled 13-oxa (5) and 6-oxa (9) myristic acids with specific activity of 137 Ci/mmol and 105 Ci/mmol respectively, starting from the corresponding acetylenic precursors, 12-methoxy-10-dodecynoic acid (4) and 5-(3-octynyloxy)pentanoic acid (8) are described. The precursor 4 is readily obtained in high yield by alkylation of the lithium salt of methyl propargyl ether with 9-iodononanoic acid, whereas 8 is obtained by alkylation of 3-octyn-1-ol with *t*-butyl-5-bromovalerate under phase transfer conditions, followed by acid catalysed removal of the *t*-butyl group.

Key Words: [^{3}H]-13-oxamyristic acid, [^{3}H]-6-oxamyristic acid, 12-methoxy-10-dodecynoic acid, 3-octyn-1-ol, phase transfer catalysis, cell biology.

INTRODUCTION

Myristoyl-CoA:protein *N*-myristoyl transferase (NMT, E.C. 2.3.1.97) catalyzes the transfer of the myristoyl moiety (14:0) from myristoyl-CoA to the amino terminal glycine residue of several cellular and viral proteins[1]. Recent studies[2,3] have revealed that covalent linkage of myristate to certain retroviral proteins, including the *gag* polyprotein precursor of human immunodeficiency virus-1 (HIV-1), is essential for viral assembly and replication. Certain N-myristoylated proteins (e.g. the tyrosine kinase p60$^{v\text{-}src}$) are also involved in the malignant

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transformation of cells[4,5]. Several oxygen containing analogs of myristic acid derived by isosteric replacement of one or more methylene groups by oxygen atoms have been synthesized[6] and when converted to their respective CoA derivatives were found to be good substrates for NMT *in vitro*[6] and *in vivo*[7,8]. Of these, 13-oxa (1) and 6-oxa (2) myristic acids have exhibited promising antiviral activity in cell culture[7]. To further investigate the functional consequences of incorporation of these novel myristate analogs into N-myristoylproteins in the cell, we have synthesized their radiolabeled derivatives. This report describes the syntheses of novel acetylenic analogs of O-13 and O-6 myristic acids that allow for high specific incorporation of tritium by catalytic reduction.

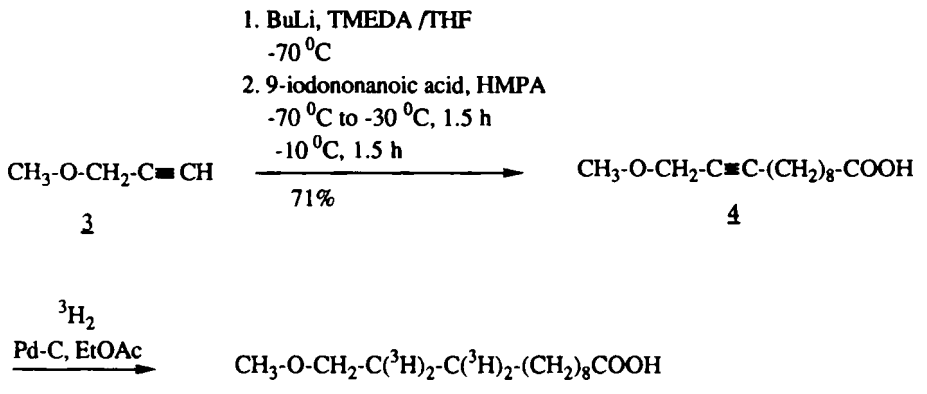
CH_3-O-$(CH_2)_{11}$-COOH 1

CH_3-$(CH_2)_7$-O-$(CH_2)_4$-COOH 2

RESULTS AND DISCUSSION

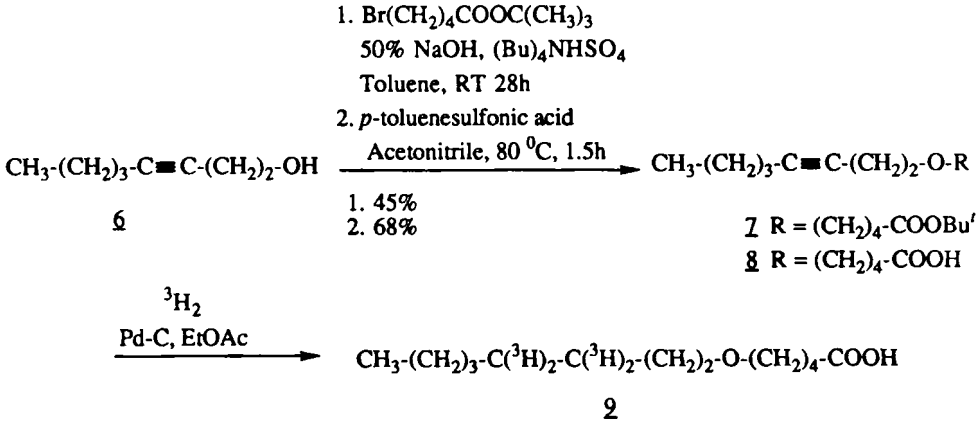
Our approach to the synthesis of title compounds 5 and 9 involved the preparation of the respective acetylenic acids, 4 and 8, as outlined in schemes I and II. Coupling of lithium acetylide with ω-iodoalkanoic acid in hexamethylphosphoramide in the presence of ethylenediamine is reported[9] to generate the corresponding ω- alkynoic acid in high yield. However, application of this procedure using methyl propargyl ether (3) and 9-iodononanoic acid[10] in the presence of butyllithium and tetramethylethylenediamine, furnished 4 in low yield (38%). Therefore an improved methodology was developed which consisted of reacting three equivalents of 3 and one equivalent of 9-iodononanoic acid using butyllithium and tetramethylethylenediamine in a tetrahydrofuran-hexamethylphosphoramide (3:2 v/v) solvent mixture. In this instance product 4 was obtained in 71% yield. The acetylenic acid 4 was then subjected to catalytic reduction over palladium-carbon in the presence of carrier free tritium gas and the product was purified by reverse phase thin-layer chromatography to give 13-oxamyristic acid 5, labeled on C-10 and C-11 with a specific activity of 137 Ci/mmol and 99.2% radiochemical purity. The mass spectrum of 5 gave a peak at 239 (M+H) which is consistent with incorporation of four tritium atoms per molecule of 5 (MW of 1 is 230).

Scheme I



The synthesis of 6-oxamyristic acid 8 (Scheme II) entailed the preparation of acetylenic ether 7. While the preparation of 7 should, in principle, be achievable by the classical Williamson ether synthesis, it has recently been shown that simple alkoxides react with halovalerate to yield only olefinic or ester exchange products instead of the desired ethers[11]. For example, the reaction of 5-ethoxypentane-1-ol with *t*-butyl-5-iodovalerate in the presence of sodium hydride in tetrahydrofuran gave a mixture of ethoxypentyl-5-iodovalerate (32%) and ethoxypentyl-4-pentenoate (22%), and similar reaction with methyl-5-bromo- valerate furnished ethoxypentyl-5-bromovalerate (63%). This problem has been circumvented by the application of phase transfer methodology[12]. Thus, the known 3-octyn-1-ol 6[13], was reacted with *t*-butyl-5-bromovalerate[14] under phase transfer conditions using tetrabutylammonium

Scheme II



hydrogensulphate as the catalyst to obtain the *t*-butyl ester (7) in 45% yield. The *t*-butyl group was cleaved by refluxing a solution of 7 in acetonitrile in the presence of *p*-toluene-sulfonic acid to afford the acetylenic acid 8[15]. This product was then subjected to catalytic reduction by tritium gas over palladium-carbon[16] to furnish 9,10 labeled O-6 myristic acid 9. The tritiated material was purified by high performance liquid chromatography on a Techsphere column using a linear gradient of methanol-triethylammonium formate to give 9 with a specific activity of 105 Ci/mmol and 99.2% radiochemical purity. The mass spectrum of 9 revealed a peak at 237(M+H) which indicates the incorporation of three tritium atoms per molecule of 9 (MW of 2 is 230).

In conclusion, this report provides a short and facile route to novel, biologically important oxamyristic acids radiolabled to high specific activity. The labeled compounds described above, have enabled cell biology studies examining the incorporation of 5 and 9 into retroviral *gag* polyproteins[17].

EXPERIMENTAL

All ^{1}H and ^{13}C NMR spectra were recorded using a Varian 300 MHz spectrometer. Low resolution FAB mass spectra were recorded on VG40-250T instrument and high resolution FAB data were obtained on a Finnigan MAT-90 instrument using a Cs incident beam at 25 keV; compounds were dissolved in a nitrobenzyl alcohol/LiI matrix and peaks were matched to CsI as the standard. FTIR spectra were measured on a Nicolet 170SX spectrophotometer. Starting materials and reagents were purchased from Aldrich Chemical Company and used without further purification.

12-Methoxy-10-dodecynoic acid 4

To a solution of *N,N,N',N'*-tetramethylethylenediamine (TMEDA) (1.35 g, 0.116 mol) in THF (6 mL) at -70 °C was added a solution of BuLi (2.4 M in hexane, 4.8 mL, 0.115 mol) and the mixture was stirred under argon atmosphere for 30 min. To this solution was added dropwise, methyl propargyl ether (0.74g, 0.0106 mol). After stirring the reaction mixture for 45 min. at -70 °C, a solution of 9-iodononanoic acid (1.0g, 0.0035 mol) in HMPA (4 mL) was added dropwise and the resulting mixture stirred at -30 °C for 1.5 h and -10 °C for 1.5 h, at which point TLC (EtOAc/Hexane 1:4) revealed complete conversion to the products. The mixture was

cooled to -30 °C and a cold solution of 1N HCl (25 mL) was added followed by dilution with EtOAc (50mL). The organic layer was washed with 1N HCl (10 mL), water (3 x 25 mL), dried (Na_2SO_4) and concentrated to give a thick oil (0.9 g.). This material was purified by flash chromatography (silica gel, EtOAc/Hexane 3:7)to give 4 as a low melting solid; yield: 0.6g. (75%); R_f = 0.47 (EtOAc/Hexane 2:3).

IR (neat): υ = 2930, 2860, 2230 (C≡C), 1710 (C=O), 1460,1100 (C-O), 860 cm^{-1}.

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.32 (m, 8H, CH_2); 1.51(m, 2H, CH_2); 1.64 (m, 2H, CH_2); 2.22 (m, 2H, CH_2); 2.35 (t, 2H, J = 7.42Hz, CH_2); 3.37 (s, 3H, OCH_3); 4.08 (s, 2H, OCH_2).

^{13}C-NMR ($CDCl_3$/TMS): δ = 18.67, 24.58, 28.51, 28.67, 28.81, 28.91, 29.02, 34.02, 57.26, 60.16, 75.64, 87.14, 179.98.

HRMS: *m/z*, $C_{13}H_{22}O_3Li$ calc.: 233.1729; found: 233.1734 (M^+ + Li).

12-Methoxy-10,10,11,11-t_4-dodecanoic acid 5

The acid 4 (30 mg, 0.13 mmol) was dissolved in benzene (3 mL) and subjected to catalytic reduction using tritium gas (20 Ci) in the presence of 10% Pd-Carbon (10 mg) for 3 h. The catalyst was filtered, washed with ethanol, and the combined filtrate and ethanol washings were concentrated. The resulting residue was repeatedly dissolved in ethanol and concentrated to remove excess and exchangable tritium. This material (4.5 Ci) was purified on Whatman PLKC-18F plates using methanol/water/acetic acid (70:30:0.5 v/v) as the eluent. The plates were dried, and the major radioactive band (determined by autoradiography) was removed, extracted with ethanol and filtered. The filtrate was concentrated *in vacuo* to give 5 (1.75 Ci, 137 Ci/mmol). Radiochemical purity (99.2%) was ascertained by reverse phase HPLC on a Techsphere C-18 column using a methanol/dioxane/triethylammonium formate (62:5:33) gradient: R_t = 15 min.

MS (DCI/NH_3): *m/z* = 239.

t- Butyl-5-bromovalerate:

To a solution of dicyclohexylcarbodiimide (6.25 g, 0.03 mol) and *t*-butanol (9.43 g, 0.127 mol) in dry dichloromethane (40 mL) at 0 °C was added dropwise a solution of 5-bromovaleric acid (5.0 g, 0.028 mol) and 4-dimethylaminopyridine (1.2 g. 0.01 mol) in dichloromethane

(10 ml). The reactants were stirred for 1 h at 0 °C followed by 16 h at room temperature. The mixture was cooled, filtered and the filtrate was concentrated to give a syrup which was dissolved in EtOAc (50 mL). 1N HCl (50 mL) was added and the solution stirred at room temperature for 30 min and filtered again. The organic layer was washed with saturated $NaHCO_3$ (3 x 25 ml), water (3 x 25 mL), dried (Na_2SO_4) and concentrated to give a colourless liquid which was purified by flash chromatography (silica gel, EtOAc/Hexane, 5:95) to give *t*-butyl-5-bromovalerate[14] as a colorless liquid; yield: 5.0 g (77%); bp 93 °C/2.1 mbar (Lit [14] bp not reported).

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.45 (s, 9H, *t*-butyl); 1.75 (m, 2H, CH_2); 1.9 (m, 2H, CH_2); 2.25 (t, 2H, *J* = 7.2 Hz); 3.41 (t, 2H, *J* = 6.6 Hz).

FABMS: *m/z*, 237 (M+H); 183; 165; 137.

t-Butyl-5-(3-Octynloxy)pentanoate 7

A mixture of 6 (0.12 g, 0.95 mmol) and *t*-butyl-5-bromovalerate (0.48 g, 2.0 mmol) in 50% aqueous NaOH (0.6 mL) and toluene (0.5 mL) containing tetrabutylammonium bisulphate (0.1 g, 0.3 mmol) was stirred vigorously at room temperature for 28 h. The reaction mixture was poured into ice cold water (25 mL) and extracted with EtOAc (20 mL). The organic phase was washed with cold 1N HCl (10 mL), water (3 x 10 mL) and dried (Na_2SO_4). After removal of the solvent, the resulting syrup was purified twice by flash chromatography (silica gel, EtOAc/Hexane 5:95) to furnish the *t*-butyl ester 7 as a colorless syrup; yield: 0.12 g (45%); R_f = 0.59 (EtOAc/Hexane 2:3).

IR (neat): υ = 2960, 2930, 2860, 1730 (ester), 1460, 1370, 1260, 1160, 1120, 860 cm^{-1}.

^{1}H NMR ($CDCl_3$): δ = 0.9 (t, 3H, CH_3); 1.4 (m, 4H, CH_2); 1.44 (s, 9H, *t*-butyl); 1.65 (m, 4H, CH_2); 2.15 (m, 2H, CH_2); 2.24 (m, 2H, CH_2); 2.42 (m, 4H, CH_2); 3.5 (m, 2H, CH_2).

^{13}C NMR ($CDCl_3$): δ = 13.55, 18.39, 20.09, 21.76, 21.86, 28.07, 29.00, 31.04, 35.23, 69.52, 70.43, 79.93, 81.28, 172.89.

FABMS: *m/z*, 289 (M+Li), 283 (M+H), 227 (M+H-C_4H_9).

HRMS: *m/z*, $C_{17}H_{30}O_3Li$ calc.: 289.2355; found: 289.2351 (M+Li)

5-(3-Octynyloxy)pentanoic acid 8

A mixture of the ester 7 (0.12 g, 0.43 mmol) and *p*-toluenesulfonic acid (0.015 g, 0.08 mmol) in acetonitrile (1 ml) was heated to reflux for 1.5 h under argon. After removal of the solvent, the residue was purified by flash chromatography (silica gel, EtOAc/Hexane 1:4) to afford 8 as a colorless liquid; yield: 0.065 g (68%); R_f = 0.41 (silica gel, EtOAc/Hexane 2:3).

^{1}H NMR ($CDCl_3$/TMS): δ = 0.9(t, 3H, *J*= 7.1Hz, CH_3), 1.44 (m,4H, CH_2), 1.7 (m, 4H, CH_2) , 2.14 (m, 2H, CH_2), 2.42 (m, 4H, CH_2), 3.50 (m, 4H, OCH_2).

^{13}C-NMR ($CDCl_3$/TMS): δ = 14.12, 18.93, 20.59, 21.98, 22.43, 29.4, 31.58, 34.26, 70.1, 74.54, 81.93, 180.2.

HRMS: *m/z* , $C_{13}H_{22}O_3Li$ calc.: 233.1729; found: 233.1739 (M+Li).

5-(Octyloxy-3,3,4,4-t_4)pentanoic acid 9

The acid 8 (50 mg, 0.22 mmol) was dissolved in benzene (1 mL) and subjected to catalytic reduction using tritium gas (25 Ci) in the presence of 10% Pd-Carbon (20 mg) for 90 min at room temperature. The catalyst was removed by filtration and washed with ethanol (5 ml). The combined filtrate and ethanol washings were concentrated and resulting residue was repeatedly dissolved in ethanol and concentrated in order to remove excess and exchangable tritium. The material thus obtained (19.9 Ci) was purified by HPLC in four runs using a Sperisorb-ODS column and methanol:water:acetic acid (350:150:1 v/v) as the solvent system, at a flow rate of 3 ml/min. The major radioactive fractions from each run were combined and extracted with toluene (2 x 2 mL). The organic phase was dried ($MgSO_4$) and concentrated to dryness to give 9 (4.59 Ci) of 105 Ci/mmol specific activity. Radiochemical purity (98.8%) was acertained by HPLC on a Techsphere ODS column using a linear gradient of methanol/dioxane/0.04M triethylammonium formate (62:5:33): R_t = 14 min.

MS (DCI/NH_3): *m/z* = 237.

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REFERENCES

1. Towler, D. A., Gordon, J. I., Adams S. P., Glaser, L. *Ann. Rev. Biochem.* 57: 69 (1988).

2. Rhee, S. S., Hunter, E. *J.Virol.* 61:1045 (1987).

3. Buss, J. E., Sefton, B. M. *J. Virol.* 53: 7 (1985).

4. Cross, F. R., Garber, E. A., Pellman, D. Hanafusa, H. *J. Mol. Cell. Biol.* 4:1834 (1984).

5. Kamps, M. P., Buss, J. E., Sefton, B, M. *Proc. Natl. Acad. Sci.. USA.* 82: 4625 (1985).

6. Heuckeroth, R. O., Jackson-Machelski, E., Adams, S. P., Kishore, N. S., Huhn, M., Katoh, A., Lu, T., Gokel, G. W., Gordon, J. I. *J. Lipid Research*, 31: 1121(1990).

7. Heuckeroth, R, O., Gordon, J. I. *Proc. Natl. Acad. Sci. USA.* 86: 5262 (1989).

8. Bryant, M. L., Heuckeroth, R. O., Kimata, J. T., Ratner, L., Gordon, J. I. *Proc. Natl. Acad. Sci. USA.* 86: 8655 (1989).

9. DeJarlais, W. J., Emken, E. A, *Syn. Comm.* 10(9): 653 (1980).

10. Stanko, V. I., Brattsev, V. A., Danilova, G. N., Sedov, V. V., Matveev, V. A., Korsunskii, V. N. *Otkrytiya Izobret Prom. Obraztsy Tovarnye Znaki* 26: 230 (1983); *C. A.* 100(1): 5860y.

11. Katoh, A., Devadas, B., Adams, S. P., Gordon, J. I., Gokel, G. W. *J. Org. Chem.* in press.

12. Freedman, H. H., Dubois, R. A. *Tetrahedron Lett.* 3251(1975).

13. Pop, L., Oprean, I.,Barabas, A., Hodosan, F. *J. Prakt. Chem.* 328: 867 (1986).

14. Warther, J. D., McGovern, T. P. *J. Chromatogr. Sci.* 24(10): 451(1986).

15. All new compounds exhibited satisfactory analytical and/or exact (<5 ppm) mass data.

16. Tritiation of **4** and **8** were carried out at Amersham Corp., Arlington Heights, IL.

17. Johnson, D. R., Cox, A. D., Solski, P. A., Devadas, B., Adams, S. P., Liemgruber, R. M., Heuckeroth, R. O., Buss, J. E., and Gordon, J. I. *Proc. Natl. Acad. Sci. USA.* in press.