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RADIOSYNTHESIS OF 1-[^{11}C] POLYHOMOALLYLIC FATTY ACIDS

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SUMMARY

A facile retro-synthesis involving the radical chain decarboxylation of the N-hydroxypyridine-2-thione esters of both arachidonic and docosahexaenoic acid was utilized to synthesize (all Z)-1-bromononadeca-4,7,10,13-tetraene, and (all Z)-1-bromoheneicosa-3,6,9,12,15,18-hexaene in 60% overall yield. The corresponding polyhomoallylic magnesium bromides were carbonated with [^{11}C]CO_2 to afford the 1-[^{11}C]polyhomoallylic labeled fatty acids in good yield in less than 35 minutes. The final radiochemical purities were found to be in excess of 95% by radio-HPLC.

Keywords: 1-[^{11}C] Labeled fatty acid, 1-[^{11}C]Arachidonic acid, 1-[^{11}C]Docosa-hexaenoic acid, Radical chain decarboxylation bromination, (all Z)-1-Bromononadeca-4,7,10,13-tetraene, (all Z)-1-Bromoheneicosa-3,6,9, 12,15,18- hexaene.

INTRODUCTION

9,10-[^{3}H]Palmitic, 1-[^{14}C]arachidonic and 1-[^{14}C]docosahexaenoic acids have been shown to be appropriate fatty acids for the study of phospholipid metabolism. *Ex-vivo* experiments in rats have clearly demonstrated that each of these fatty acids are rapidly and selectively incorporated into brain phospholipids. For example, the rate of incorporation of arachidonic acid into brain is related to its synthesis and / or turnover into the sn-2 positions of phosphatidyl inositol and choline while palmitic acid is related to its turnover into the sn-1 positions of phosphatidyl choline (1-3). In an effort to extend this work to the *in vivo* evaluation of regional brain phospholipid metabolism using PET, we have developed a remote radiosynthesis for 1-[^{11}C]arachidonic acid (**1a**) and 1-[^{11}C]docosahexaenoic acid (**1b**) (see Fig 1) (4a,b). Arachidonic acid has been labeled in the C-1 position using both ^{13}C and ^{14}C labeled cyanide as well as ^{14}C labeled CO_2 (5-7).

Typically, the precursors for the radiolabeling step are constructed by multistep syntheses of polyyne intermediates (8,9). These polyyne intermediates are converted to Z polyenes by their subsequent reduction over Lindlar catalyst. Such multistep schemes suffer from low overall yields and difficulties in purification of desired product from the by-products formed during the reduction. Furthermore, because these polyunsaturated

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compounds are difficult to maintain in a highly purified state, a more efficient method for their routine preparation was desirable.

We report a facile retro-synthesis for the preparation of both (all Z)-1-bromononadeca-4,7,10,13-tetraene (**2a**) and (all Z)-1-bromoheneicosa-3, 6, 9, 12, 15, 18-hexaene (**2b**). In addition we report the formation of the corresponding polyhomoallylic magnesium bromides (Grignards) and their subsequent carbonylation with $[^{11}C]CO_2$.

RESULTS AND DISCUSSION

Chemistry

SYNTHETIC SCHEME

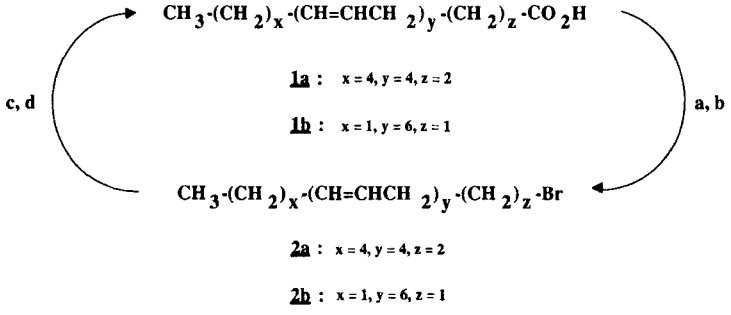

Fig. 1: a) Tetramethylammonium hydroxide / $(CO)_2Cl_2$ / toluene; b) Sodium N-Hydroxy-pyridine-2-thione / $BrCCl_3$ / Δ; c) Mg^o / Et_2O / >>>; d) $[^{11}C]CO_2$ / Et_2O

Recently Barton et al. reported that carboxylic acid esters of N-hydroxypyridine-2-thione undergo radical chain decarboxylation and that in the presence of bromotrichloromethane form nor-alkyl bromides (10). We were able to apply this work to the retro-synthesis of the (all Z) bromotetraene, **2a**, and the (all Z) bromohexaene, **2b**. The tetramethylammonium salts of **1a** and **1b** were reacted with oxalyl chloride in toluene to give the corresponding acid chlorides. The crude acid chlorides were used directly in the next step without further purification. The N-hydroxypyridine-2-thione esters were generated *in situ* and after reflux in bromotrichloromethane were purified by flash chromatography yielding pure **2a** and **2b** in ~60% overall yield based on the starting polyhomoallylic fatty acids. Sonication of refluxing ether solutions of **2a** or **2b** over magnesium provided small scale preparations of the Grignards in the 0.5 - 1.0 mmole range. Analysis of the quenched reaction mixture by GC/MS(EI) revealed that all of the bromide was consumed in less than 4 hours. The use of more activated forms of magnesium were not necessary. Attempts to use tetrahydrofuran instead of ether as solvent substantially decreased the shelf-life of the Grignard reagents. The bromo compounds **2a** and **2b** could be stored for up to 3 months in amberized vials over copper wire at -20 °C. Periodic analysis by TLC (Silica / hexane; visualization conc. by H_2SO_4 / Δ) showed that some decomposition occurs with time. This polar material was removed by filtering through silica with hexane. However, after extended storage even chromatographic purification failed to give bromo material which could be converted to the desired homoallylic magnesium bromides of **2a** and **2b** as evidence by its lack of reactivity with CO_2. Spectral analysis of **2a** and **2b** revealed no apparent differences when compared to spectra of freshly prepared material.

Radiochemistry

The method used for ^{11}C carbonation was similar to those already reported (11-14). The [^{11}C]CO_2 was trapped at -186 °C and upon warming eluted (helium; 20 mL/min) in less than a 60 mL volume. The trapping efficiency for freshly prepared 0.25M Grignard solutions of **2a** were 67.8 ± 14.5%; n = 12 at room temperature. The trapping efficiency decreased to 54.4 ± 6.76 % n = 5 after storage for 18 hours. Lower concentrations, increased flows or lower temperatures were also found to adversely effect the reactivity of these Grignards. The Grignard of **2b** was found not only to be less reactive toward [^{11}C]CO_2 (trapping efficiency 50.5 ± 8.2%) but also less stable than the Grignard of **2a** and had to be used within a few hours after its preparation. Instead of concentrated acid to quench the reaction, a 10% NH_4Cl solution was found to be sufficient for the solubilization of the magnesium salts making it possible to eliminate the need for liquid-liquid extraction. Drying of the ether by passage over a drying column composed of high surface area diatomaceous earth, minimized breakthrough of product on the alumina column. The major by-products, resulting from the quenching of the Grignards were eluted from the alumina column with a 50 mL ether wash. The desired product was found to elute within 20-25 mL with 1% glacial acetic acid-ether. Residual activity left in the reaction vial, drying column, alumina column and ether wash were minor (see **Table 1**).

TABLE 1. ACTIVITY BALANCE FOR ARACHIDONIC ACID

FRACTION	% OF TOTAL (EOB)
5 Å SIEVE	32.2
RX'N VESSEL	4.1
DRYING COLUMN	1.8
ALUMINA COLUMN	12.8
ETHER WASH	8.7
COLLECTED PRODUCT	40.4

See Fig 1. for a description of radiosynthetic apparatus. The percentages shown above were based on freshly prepared Grignards of **2a** (n = 12).

GC/MS(EI) analysis of the final product revealed that only minor amounts of the corresponding nor-alcohols were still present. ^{13}C NMR and GC(FID) analysis of reaction mixtures prepared by saturation of the respective Grignards with CO_2 demonstrated that only the desired all Z isomers were obtained. The entire process from end of bombardment to final formulation was accomplished in less than 35 minutes. The radiochemical yield (yield EOS = 15.9-18.3%) for 1-[^{11}C]docosahexaenoic acid prior to formulation was comparable to that for 1-[^{11}C]arachidonic acid (yield EOS = 23.2 ± 9.0%; n = 12). The same procedures were found to work equally well for preparation of 1-[^{11}C]palmitic acid. Analysis of the final products by reversed-phase HPLC showed the radiochemical purities of each to be in excess of 95%.

EXPERIMENTAL SECTION

Materials and Methods

Arachidonic and docosahexaenoic acids were obtained from Nu-Check Prep.(Elysian, MN), N-hydroxypyridine-2-thione sodium salt and magnesium were obtained from Sigma Chemical, and Alfa Products, respectively. The magnesium was stored in a desiccator over drierite. The diethyl ether was freshly distilled from sodium / benzophenone under dry argon.

IR spectra were obtained on a Nicolet 20-SXB FTIR spectrophotometer.

Both ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra were obtained on a Varian VXR-200 in $CDCl_3$ with 0.3% TMS. All chemical shifts are reported in ppm relative to TMS (0.00 ppm) for ^{1}H spectra and $CDCl_3$ (76.9 ppm) for ^{13}C spectra.

GC/MS were obtained on a HP-5880A Gas Chromatograph interfaced to a HP-5970 Mass Selective Detector. The column used was a HP-1 (0.2 mm x 12 m) capillary column. The analyses were all performed with the following temperature program: injector 175 °C, detector interface 270 °C, and the oven was programmed for 100 °C for 1 minute then 100 °C to 250 °C at 15°/min. The chemical purity of the fatty acids as their corresponding methyl esters (derivitization with Methyl-8 (Pierce Co.)) after the synthesis-labeling cycle were ascertained by GC analysis on a DB-225 column (J&W 50% Cyanopropylphenyl; 30 m x 0.25 mm) isothermally at 245 °C using FID detection. The retention times were determined to be 11.4 and 14.9 min for **1a** and **1b**, respectively.

Analytical TLC were performed on Silica Gel GHLF plates (2.5 x 10 cm, 250 m, Analtech) and silica gel 60 (0.04 - 0.063 mm, Macherey Nagel) was used for flash column chromatography. HPLC was performed on a C-8 Altech/Econosil® analytical column using 90% methanol / 0.1% H_3PO_4 as eluent at 1 mL/min. The effluent was monitored for both UV (215 nm) and radioactivity with a Kratos Spectroflow 783 and Beckman 170 Radioisotope Detector, respectively.

All valves used for the semi-automated $[^{11}C]CO_2$ labeling system were manufactured by Hamilton (Reno, NV). Valves 1 (HV 3-2), 2 (HV 3-2) and 3 (HVX L6-6) were controlled by contact closure while valves 4 (HVX L6-6), 5 (HVX D6-5), and 6 (HVX D6-5) were under computer control via the Hamiliton IVP controller.

Chemistry

1-Bromononadeca-4,7,10,13-tetraene, (2a). To a solution of arachidonic acid (5.17 g; 17.1 mmol) in 330 mL acetonitrile was added tetramethylammonium hydroxide (2.99 g; 16.5 mmol). Upon dissolution, the acetonitrile was evaporated and the residue dried in vacuum. The residue was suspended in toluene, cooled to 0 °C and 15 mL oxalyl chloride gradually added. The mixture was allowed to react at room temperature for 3.5 hrs. The toluene was evaporated and the residue resuspended in hexane (50 mL) and filtered. The precipitate was washed with hexane and the filtrate plus combined washings evaporated. This material was used directly in the next step without further purification.

A solution of arachidonyl chloride (assumed 5.52 g; 17.1 mmol) in 25 mL $BrCCl_3$ was added dropwise over 30 min to a refluxing suspension of sodium N-hydroxypyridine-2-thione (2.80 g; 18.8 mmol) and 4-dimethylaminopyridine (209 mg; 1.71 mmol) in 75 mL $BrCCl_3$. The mixture was allowed to continue to reflux an additional 30 min, cooled, and the $BrCCl_3$ evaporated. The residue was 3 times suspended in hexane and evaporated. A silica flash column (50 mm OD) with hexane gave 3.49 g (60.1%) of pure desired product. ^{1}H NMR δ ($CDCl_3$, TMS) 0.89 (t, J = 6.6 Hz, 3 H, CH_3), 1.13 - 1.50 (m, 6 H, alkyls), 1.92 - 2.17 (m, 4 H, CH_2CH_2Br + $C{=}CCH_2$), 2.35 (apparent q, J = 7.0 Hz, 2 H, $C{=}CCH_2$), 2.89-2.71 (m, 6 H, $C{=}CCH_2C{=}C$), 3.42 (t, J = 6.6 Hz, 2 H, CH_2Br), 5.20-5.48 (m, 8 H, vinyl); ^{13}C NMR ($CDCl_3$, $CDCl_3$) δ 130.4, 129.5, 128.5, 128.2, 128.0, 127.9, 127.9, 127.8, 127.5, 33.0, 32.5, 31.5, 29.3, 27.2, 25.6, 22.5, 14.0; IR (neat) 3012.4, 2959.1, 2926.5, 2854.1, 1435.5, 1243.9, 714.9 cm^{-1}. MS(EI), *m/e* (relative intensity) 267 (0.4), 229(0.5), 227(0.8), 202(2), 200(2), 119(10), 93(27), 79(100), 67(59), 55(39).

1-Bromoheneicosa-3, 6, 9, 12, 15, 18-hexaene, (2b). In an identical fashion docosahexaenoic acid (3.12 g; 9.5 mmoles) afforded 2.03 g (59.0%) of **2b**. ^{1}H NMR δ ($CDCl_3$, TMS) 0.98 (t, J = 7.5 Hz, 3H, CH_3), 2.08 (m, 2 H, CH_2CH_2Br) 2.65 (m, 2 H, $C{=}CCH_2$) 2.85 (m, 10 H, $C{=}CCH_2C{=}C$) 3.38 (t, J = 7.1 Hz, 2 H, CH_2Br) 5.39 (m, 12 H, vinyl); ^{13}C NMR ($CDCl_3$, $CDCl_3$) δ 131.90, 130.72, 128.45, 128.33, 128.17, 127.92, 127.88, 127.73,

127.59, 126.89, 126.26, 32.20, 30.71, 25.71, 25.56, 25.45, 20.46, 14.18; IR (neat) 3015.8, 2965.0, 2932.2, 1437.2, 1265.5, 709.9 cm^{-1}. MS(EI), *m/e* (relative intensity) 333(0.5), 228(1.6), 226(1.4), 215(1.4), 213(1.5), 188(6.3), 186(6.5), 133(7.7), 131(8.4), 91(67.2), 79(100.0), 67(58.4), 55(26.5).

Radiochemistry

1-[^{11}C]Arachidonic Acid (1a) or 1-[^{11}C]-(all *Z*)-4, 7, 10 ,13 ,16 ,19-Docosahexaenoic Acid. (1b) To 0.5 mL anhydrous ether containing 18.2 mg; 0.75 mmol magnesium while sonicating at reflux was added (170 mg: 0.5 mmol) of **2a** or (182 mg: 0.5 mmol) of **2b** over a 30 min period. The reaction was allowed to continue an additional 4 hrs and the reaction mixture brought to a final volume of 2.0 mL. [^{11}C]carbon dioxide in helium was delivered at room temperature to 2 mL of a 0.25 M ether solution of the bromoalkene **2a** or **2b** at a flow rate of 20 mL/min (Fig 2). Exhaust gases including any unreacted [^{11}C]carbon dioxide were either trapped on 5Å molecular sieve or contained in a 8 L waste gas bag. After the addition was complete, as evidenced by no further increase in activity in reaction vial (~ 3-4 min), 0.5 mL 10% NH_4Cl was added to quench the reaction. Bubbling was continued another 60 seconds until partial solution of the precipitated magnesium salts was obtained. The entire contents of the reaction vessel were passed through an extraction column (Chem Elute®, Varian Assoc.) onto a neutral alumina (5.0 g; 1 x 6 cm) column which had been previously conditioned with 50 mL 0.15% glacial acetic acid in Et_2O and 50 mL Et_2O. The vial, extraction column, and alumina column were washed with 50 mL Et_2O. The labeled 1-[^{11}C]arachidonic acid or 1-[^{11}C]docosahexaenoic acid was subsequently eluted with 1.0% glacial acetic acid in Et_2O and the activity collected (~10-15 mL). A small aliquot (5%) of the ether eluate was removed for analysis by radio-HPLC. The remainder of the ether was evaporated to dryness and the residue

RADIOSYNTHETIC APPARATUS

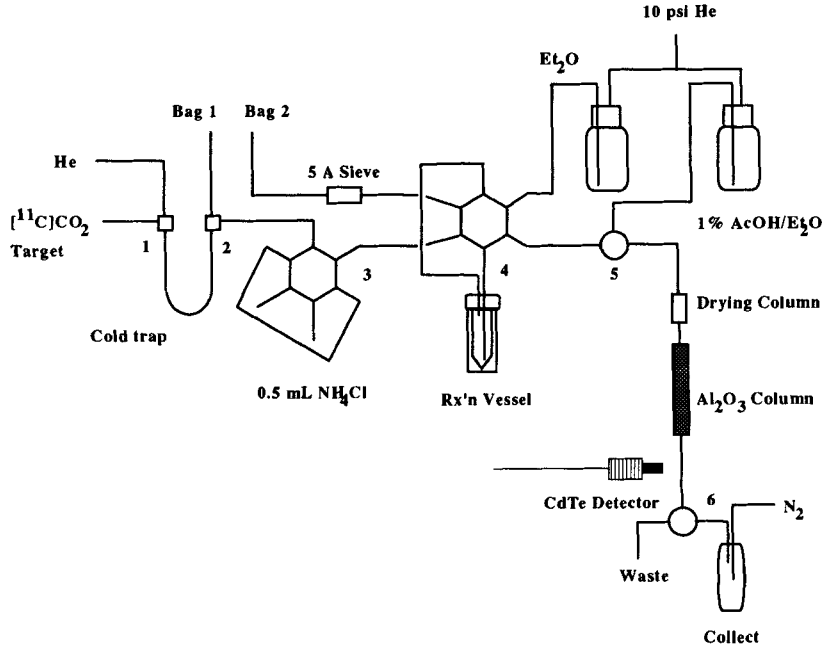

Fig 2 : Valves 1,2 and 3 are controlled by contact closure while valves 4,5, and 6 are under computer control.

formulated the addition of 0.8 mL 7.5% sodium bicarbonate (USP, for Injection) followed by 0.8 mL plasma and 2.5 mL 0.9% saline (USP, for Injection). The mixture was sonicated at 40 °C for 3 min, the solution transferred to a 12 mL syringe through a 0.22 μ filter and brought to a final volume of 10 mL with 0.9% saline.

Chromatographic Analysis The radiochemical purity of the fatty acids was determine by radio-HPLC of a evaporated aliquot of the 1% glacial acetic acid / ether. The analyses were performed on a C-8 Altech/Econosil® column (4.6 x 250 mm) using 90% methanol 10% aqueous 0.1% H_3PO_4 with a flow of 1.0 mL/min The UV adsorbance (215 nm) and radioactivity (NaI) were monitored. The retention time data for **1a**, **1b**, and palmitic acid are give in **Table 2.**

TABLE 2. HPLC RETENTION TIME DATA

Compound	Retention Time (min)
Arachidonic Acid	4.92
Docosahexa-4, 7, 10, 13, 16, 19-enoic Acid	4.82
Palmitic Acid	5.47

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