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SYNTHESIS OF [1-^{11}C]-2-OCTYNOIC ACID, [1-^{11}C]-2-DECYNOIC ACID AND [1-^{11}C]-3-(R,S)-METHYLOCATANOIC ACID

as potential markers for PET studies of fatty acid metabolism

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SUMMARY

[1-^{11}C]-2-Octynoic acid, [1-^{11}C]-2-decynoic acid and [1-^{11}C]-3-(R,S)-methyloctanoic acid have been synthesized in order to evaluate these compounds as PET (Positron Emission Tomography) tracers for imaging *in vivo* medium-chain acyl-CoA dehydrogenase and medium-chain fatty acid utilization. The synthesis was performed by the Grignard reaction between alkylmagnesium bromides and [^{11}C]CO_2. The radiochemical yields of [1-^{11}C]-2-octynoic acid, [1-^{11}C]-2-decynoic acid, and [1-^{11}C]-3-(R,S)-methyloctanoic acid were 10, 7 and 1% based on the [^{11}C]CO_2 used, respectively. Radiochemical purity was >99% in all cases.

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Key Words: Positron Emission Tomography (PET), [1-^{11}C]-2-Octynoic acid, [1-^{11}C]-2-Decynoic acid, [1-^{11}C]-3-(R,S)-Methyloctanoic acid.

INTRODUCTION

It is well known that fatty acids are the important substrates for energy metabolism in some tissues, especially in the heart, liver, and kidney. It has also been reported that the brain has enzymes for beta-oxidation[1]. The first step of beta-oxidation is catalyzed by acyl-CoA dehydrogenases specified to each class of the fatty acids, namely short-, medium- and long- chain ones. Medium-chain fatty acids like octanoic acid and 3-bromooctanoic acid enter the mitochondrion by a carnitine-independent mechanism[2], are metabolized to acyl-CoA and are mainly dehydrogenated by medium-chain acyl-CoA dehydrogenase (MCAD)[3]. Investigating the potential of [1-^{11}C]octanoate (OA) as a tracer for Positron Emission Tomography (PET), we suggested that OA would be useful for imaging cerebral fatty acid metabolism[4,5]. Yamamura et al. reported the usefulness of OA as a tracer of fatty acid metabolism in the liver[6]. But the use of OA as a tracer to measure fatty acid utilization is restricted by the intricacy of its metabolism which makes it quite difficult to analyze the pharmacokinetics. Octanoic acid analogs labeled with a positron emitter, which are trapped in a particular step on their metabolic pathways, seemed to be more preferable for diagnostic imaging of fatty acid metabolism, getting rid of the difficulty with OA. The acetylenic thioester, 2-octynoyl-CoA was reported as a mechanism-based suicide inhibitor of MCAD[7,8]. It was reported that beta-oxidation of a carboxylic acids which have a

methyl group on the beta carbon atom are prevented on the way[9]. [1-^{11}C]-3-(R,S)-Methylheptadecanoic acid and [^{123}I]-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid were used as tracers in studies on myocardial fatty acid metabolism.

Based on these considerations, we chose [1-^{11}C]-2-octynoic acid (OCY), [1-^{11}C]-2-decynoic acid (DCY) and [1-^{11}C]-3-(R,S)-methyloctanoic acid (BMOA) as candidates of PET tracers for imaging the activity of a particular step of fatty acid beta-oxidation in mitochondrion. The present paper describes the preparation of OCY, DCY and BMOA ready-to-use in biodistribution, metabolic, and imaging studies.

$CH_3(CH_2)_4C{\equiv}C\,{}^{11}COOH$ [1-^{11}C]-2-Octynoic acid

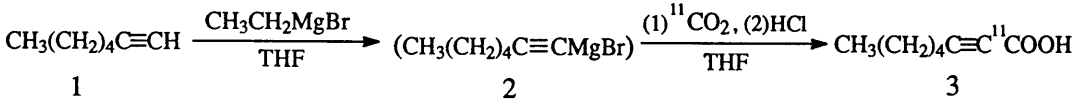
$CH_3(CH_2)_6C{\equiv}C\,{}^{11}COOH$ [1-^{11}C]-2-Decynoic acid

$CH_3(CH_2)_4\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2\,{}^{11}COOH$ [1-^{11}C]-3-(R,S)-Methyloctanoic acid

RESULT AND DISSCUSSION

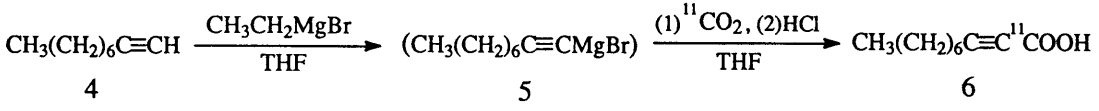
The synthetic pathways of 2-octynoic acid, 2-decynoic acid and 3-(R,S)-methyloctanoic acid are shown in Scheme1, 2, and 3. For synthesis of 2-octynoic acid **3**, 1-heptyne **1** was treated with EtMgBr, CO_2 and 6 M HCl, successively, to give **3** in 80% yield[10,11]. 2-Decynoic acid **6** was prepared similarly starting from 1-nonyne in 65% yield. The synthesis of 3-(R,S)-methyloctanoic acid **11** began with 2-methyl-1-hepten **7**. Treatment of **7** with $NaBH_4$, $BF_3{\cdot}O(C_2H_5)_2$, and then with H_2O_2-NaOH (aq), successively, gave 2-methyl-heptanol **8** in 14% yield[12]. Bromination of **8**

Scheme 1



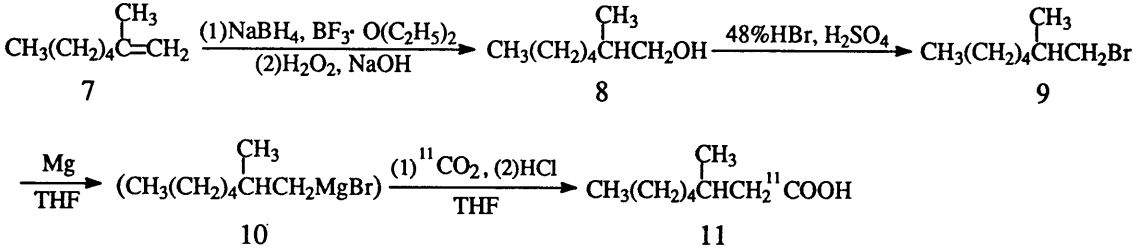
1 2 3

Scheme 2



4 5 6

Scheme 3



7 8 9

10 11

with 48%HBr and H_2SO_4 afforded 2-methyl-1-bromoheptane **9** in 42% yield[13]. Carboxylation of **9** with Mg, CO_2 and 6 M HCl gave 3-(R,S)-methyloctanoic acid **11** in 10% yield[14].

The method used for ^{11}C-carboxylation was similar to that reported previously[15]. Briefly, [^{11}C]carbon dioxide was bubbled into a 0.3 M solution of each Grignard reagent **2**, **5**, **10** in THF and the reaction mixture was purified by reverse-phase high performance liquid chromatography (RP-HPLC). Total synthesis times were 30-35 min and radiochemical yields were 1-10% based on the [^{11}C]CO_2 used (not corrected for the radioactive decay). Radiochemical purity of the product was confirmed by analytical RP-HPLC which showed a single radioactive peak of the same retention time as that of the authentic sample in each case. The radiochemical yield of BMOA was lower than those of OCY and DCY. Accordingly, in an attempt to increase the radiochemical yield of BMOA, the reaction mixture was heated at 100 ℃ for about 2 min, after [^{11}C]CO_2 was bubbled through the Grignard reagent, which, however, only led to a complex reaction mixture, leaving a large amount of [^{11}C]CO_2 unreacted. This result prompted us to a kinetic study on the Grignard reaction with [^{11}C]CO_2[16].

Table 1. Results of radiochemical synthesis

Compound	Radiochemical yield*	Synthetic time	Radiochemical purity	Retention time
[1-^{11}C]-2-Octynoic acid	10%	30 min	99%	3.65 min
[1-^{11}C]-2-Decynoic acid	7%	30 min	99%	5.58 min
[1-^{11}C]-3-(R,S)-Methyloctanoic acid	4%	35 min	99%	5.77 min

* Based on the [^{11}C]CO_2 used, without correction for the radioactive decay.

Thus, we obtained injectable solution of OCY, DCY, and BMOA with high radiochemical purity. Preliminary animal studies with OCY, DCY, and BMOA showed that they might be useful for imaging fatty acid metabolism.

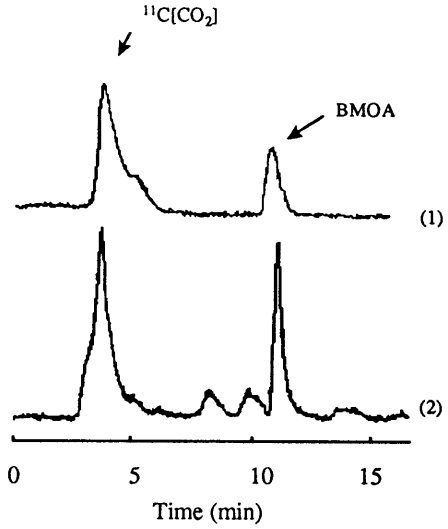


Figure 1. Preparative HPLC radiochromatogram of the reaction mixture

(1) reacted for 3 min at r.t., (2): reacted for total 5 min and heated at 100 ℃ for the last 2 min

EXPERIMENTAL

Materials and Methods

The reagents used were purchased from commercial suppliers. THF was freshly distilled over $LiAlH_4$ under argon. All other reagents were used without further purification. NMR spectra were recorded on a Varian Gemini 300. Results are reported in ppm (δ) using tetramethylsilane as an internal standard.

[^{11}C]Carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in a nitrogen gas target using a cyclotron (CYPRIS HM-18; Sumitomo Heavy Industries Co., Ltd., Tokyo Japan). The

[^{11}C]CO_2 produced was trapped in a stainless steel spiral immersed in liquid argon. For the carbon-11 labeling, we modified a commercial automated synthesis apparatus (Sumitomo Heavy Industries Co., Ltd.) and used. The radiochemical purity of the labeled products was determined by RP-HPLC using a Cosmosil 5C_{18}-AR column (150 mm x 4.6 mm i.d.; Nacalai Tesque, Co., Ltd., Kyoto, Japan) with 0.1% HCl in 55% CH_3CN.

Synthesis of authentic compounds

2-Octynoic acid(3)

The reaction vessel was purged with argon until completion of the preparation of **2**. Magnesium turnings (2.43 g, 100 mmol) and THF (20 ml) were placed in a reaction vessel (100 ml), and to this a small amount of iodine was added. After 5 min, ethyl bromide (11.22 g, 103 mmol) was added dropwise during 5 min and then the mixture was heated under reflux for 1 h. After the solution was cooled to room temperature, a solution of 1-heptyne (14.71 g, 19 mmol) in dry THF (20 ml) was added to it dropwise for 25 min and the mixture was heated under reflux for 1h. The reaction mixture containing **2** was cooled to room temperature and to this was added a lump of Dry-Ice (ca. 10 g). The mixture was stirred for a further 15 min and then the reaction was quenched by addition of 6 M HCl (10 ml). The reaction mixture was extracted with diethyl ether and the ether layer was separated and extracted with a saturated $NaHCO_3$ solution. The aqueous layer was then acidified to pH 2 with 6 M HCl and re-extracted with AcOEt. The AcOEt layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give 3 (5.47 g, 80%) as an oil.

^{1}H-NMR ($CDCl_3$) δ : 0.91 (3H, t), 1.24-1.44 (4H, m), 1.60 (2H, m), 2.42 (2H, m), 11.83 (1H, S).

HRMS m/z: Calcd for $C_8H_{12}O_2$: 140.0837, Found: 140.0850

2-Decynoic acid (6)

The reaction vessel was purged with dry argon until completion of the preparation of **5**. Magnesium turnings (0.38 g, 16 mmol) and THF (20 ml) were placed in a reaction vessel (10 ml), and to this a small amount of iodine was added. After 5 min, ethyl bromide (1.74 g, 16 mmol) was added dropwise for 10 min and then the mixture was heated under reflux for 1 h. After the solution was cooled to room temperature, a solution of 1-nonyne (3.98 g, 32 mmol) in dry THF (20 ml) was added dropwise for 25 min and the mixture was heated under reflux for 1 h. The reaction mixture containing **5** was cooled to room temperature and to this was added a lump of Dry-Ice (ca.20 g). The reaction mixture was stirred for a further 15 min, and then the reaction was quenched by addition of 6 M HCl (15 ml). The reaction mixture was extracted with diethyl ether and the ether layer was extracted with a saturated $NaHCO_3$ solution. The aqueous layer was acidified to pH 2 with 2 M HCl and re-extracted with AcOEt. The AcOEt layer was then washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give **3** (1.47 g, 65%) as an oil.

^{1}H-NMR ($CDCl_3$) δ : 0.88 (3H, t), 1.26-1.42 (8H, m), 1.59 (2H, m), 2.35 (2H, m), 10.48 (1H, s).

HRMS m/z: Calcd for $C_{10}H_{16}O_2$: 168.1150, Found: 168.1150.

2-(R,S)-Methyl-1-heptanol (8)

Boron trifluoride diethyl etherate (9.86 ml, 80.2 mmol) was added to a mixture of $NaBH_4$ (2.25 g, 60 mmol) and 2-methyl-1-heptene 7 (25.00 g, 223 mmol) in dry THF (75 ml) with stirring, and the mixture stirred for 1 h at 20 ℃ under nitrogen. Then, H_2O (15 ml), 3 M NaOH (25 ml) and 30% H_2O_2 (25 ml) were added to the reaction mixture, successively. The resulting mixture was then saturated with NaCl and extracted with ether. The extract was dried over $MgSO_4$ and concentrated under reduced pressure and the residue was purified by silica gel chromatography (solvent: hexane-ethyl acetate 5:1) to give 8 (4.33 g, 15%) as an oil.

^{1}H-NMR($CDCl_3$) δ : 0.86-0.92 (6H, m), 1.03-1.45 (8H, m), 1.59 (1H, m), 2.35 (1H, br), 3.39-3.48 (2H, m).

HRMS m/z: Calcd for $C_8H_{18}O$: 130.1358, Found: 130.1351.

2-(R,S)-Methyl-1-bromoheptane (9)

2-(R,S)-Methyl-1-heptanol 8 (1.00 g, 7.7 mmol) was added to a mixture of H_2SO_4 (0.7 ml) and 48% HBr (1.8 ml), and the mixture was heated at 100 ℃ for 5 h with stirring. The reaction mixture was extracted with ether and the extract was washed with a saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (solvent: hexane-ethyl acetate 5:1) to give 9 (0.63 g, 42%) as an oil.

^{1}H-NMR ($CDCl_3$) δ : 0.89 (3H, t), 0.99 (3H, d), 1.16-1.50 (8H, m), 1.79 (1H, m), 3.29-3.42 (2H, m).

HRMS m/z: Calcd for $C_8H_{17}{}^{79}Br$: 192.0514, Found: 192.0513.

3-(R,S)-methyloctanoic acid (11)

Magnesium turnings (0.08 g, 3.3 mmol) and dry THF (3 ml) were placed in a reaction vessel purged with dry argon. To this was added a small amount of iodine. After 5 min, 2-(R,S)-methyl-1-bromoheptane **9** (0.63 g, 3.3 mmol) was added dropwise during 10 min and the mixture was heated under reflux for 1 h. To the resulting solution, after cooling to room temperature, was added a lump of Dry-Ice (ca. 2 g). The reaction mixture was stirred for a further 10 min, and then the reaction was quenched by addition of 6 M HCl (10 ml). The resulting mixture was extracted with diethyl ether and the ether layer was separated and extracted with a saturated $NaHCO_3$ solution. The aqueous layer was acidified to pH 2 with 6 M HCl and re-extracted with diethyl ether. The ether layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give **11** (0.85 g, 10%) as an oil.

^{1}H-NMR($CDCl_3$) δ : 0.88 (3H, t), 0.97 (3H, d), 1.16-1.80 (8H, m), 1.96 (1H, m), 2.10-2.39 (2H, m).

HRMS m/z: Calcd for $C_9H_{18}O_2$:158.1307, Found:158.1303.

Radiochemical synthesis

[1-^{11}C]-2-Octynoic acid (3)

The reaction vessel for the radiochemical synthesis was purged with nitrogen until the preparation of **2** was completed. Magnesium turnings (1.21 g, 50 mmol) and dry THF (50 ml) were added in a reaction vessel (200 ml), and a small amount of iodide was added to it. After 5

min, ethyl bromide (5.61 g, 52 mmol) was added dropwise during 10 min and the mixture was heated under reflux for 1 h, and then cooled to room temperature. To this a solution of 1-heptyne (4.71 g, 50 mmol) in dry THF (20 ml) was added dropwise during 20 min and the mixture was heated under reflux for 1 h. The reaction mixture was cooled to room temperature and diluted to 0.3 M with dry THF. An aliquot (0.5 ml) of the resulting solution of **2** was placed in the reaction vessel of an automated synthesis apparatus and [^{11}C]CO_2 in nitrogen was then passed through the solution at room temperature. After 3 min, 1 M HCl (0.5 ml) was added to the reaction vessel to quench the reaction. The reaction mixture was then purified by RP-HPLC (column: Cosmosil 5C_{18}-AR 250 mm x 10mm i.d.; Nacalai Tesque, Co.,Ltd.; eluent: 0.1% HCl in 60% CH_3CN; flow rate: 5 ml/min; retention time: 8 min) . The eluate containing **3** was collected in a flask (100 ml), containing 7% $NaHCO_3$ aq (1 ml), of a rotary evaporator and concentrated under reduced pressure at 80 ℃ to remove the organic solvent. The residue (3 ml) was filtered through a 0.2 μm sterile filter to give a solution of **3**.

[1-^{11}C]-2-Decynoic acid (6)

Following the procedure for the preparation of [1-^{11}C]-2-octynoic acid, a 0.3 M solution of Grignard reagent (0.5 ml) **5** prepared from magnesium turnings, ethyl bromide, and 1-nonyne was reacted with [^{11}C]CO_2 and then treated with 1 M HCl (0.5 ml). The reaction mixture was purified by RP-HPLC (column: Cosmosil 5C_{18}-AR 250 mm x 10 mm i.d.; Nacalai Tesque, Co.,Ltd.; eluent: 0.1% HCl in 60% CH_3CN; flow rate: 5 ml/min; retention time: 8 min) and the elute containing **6** treated as above to give a sterile aqueous solution of **6**.

[1-^{11}C]-3-(R,S)-methyloctanoic acid (11)

Following the procedure for the preparation of [1-^{11}C]-2-octynoic acid, a 0.3 M solution of Grignard reagent (0.5 ml) **10** prepared from magnesium turnings and **9**, was reacted with [^{11}C]CO_2 and then treated with 1 M HCl (0.5 ml). The reaction mixture was purified by RP-HPLC (column: Cosmosil 5C_{18}-AR 250 mm x 10 mm i.d.; Nacalai Tesque, Co.,Ltd.; eluent: 0.1% HCl in 60% CH_3CN; flow rate: 5 ml/min; retention time: 11 min) and the eluate containing **11** treated as above to give a sterile aqueous solution of **11**.

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