Preparation of β-methyl p-iodophenyl pentadecanoic acid (BMIPP) and ^{125}I-BMIPP

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Abstract A method with several steps superior to literature has been developed for the preparation of β-methyl p-iodophenyl pentadecanoic acid(BMIPP). The synthesis and physical properties of BMIPP are described. It is characterized by IR, ^{1}HNMR, elemental analysis and MS. ^{125}I-BMIPP can be prepared by three methods: direct labeling, solid-state transfer labeling and Cu(I) assisted labeling. Cu(I) assisted labeling is simple and not necessary to purify before clinical use. It can fulfil the requirements for kit labeling.

Keywords β-methyl p-iodophenyl pentadecanoic acid, Fatty acid, ^{125}I-BMIPP, Radio labeling yield (*RLY*)

CLC numbers R817.9, O621.3$^+$5, O623.61

1 INTRODUCTION

It is known that the heart uses many kinds of substrates for its energy metabolism, especially fatty acids, they account for more than 70% of myocardial energy. If we use photon emitting nuclides to radiolabel fatty acids, we can assess myocardial metabolism. Previous investigations have shown that radiolabeled fatty acids, such as ^{11}C-palmitate and ^{123}I-IPPA, provide clinically useful images of the heart and have a sensitivity comparable to thallium for the diagnosis of cornary artery diseases (CAD)[1,2]. β-methyl-p-iodophenyl pentadecanoic acid(BMIPP) is a methyl-branched long- chain fatty acid, which is an analogue of IPPA. A methyl group was introduced at the beta-carbon to achieve prolonged retention of the tracer in myocardium. Now it is of widespread interest for evaluating myocardial metabolism with SPECT[3,4], and has been widely used to test the viability of myocardium abroad[5]. In this work, we developed a synthetic method superior to literature[6]. We synthesized BMIPP with domestic materials and radiolabeled BMIPP with ^{125}I by three methods. Among these three methods, Cu(I) assisted labeling is simple and its radiolabeling yield(*RLY*) can be over 95%. It can be made into a kit form.

2 MATERIALS AND METHODS

2.1 Instruments

YANADIMOTO melting point instrument (uncorrected, made in Japan), FT-IR spectrometer (made in USA), PE2400 elemental analyzer (made in USA), Varian Model

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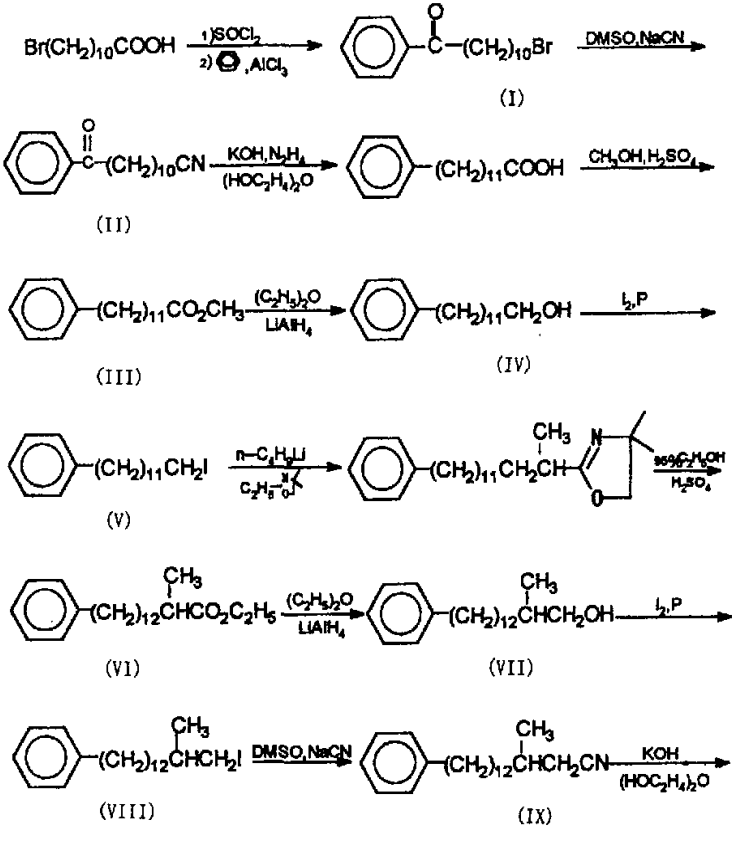
AM-400 Proton Nuclear Magnetic Resonance Spectrometer (made in USA), Packard Cobra γ-counter(made in USA).

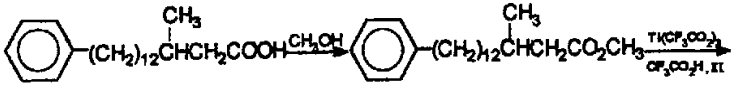
2.2 Reagents

11-bromoundecanoic acid, 4,4-dimethyl-2-ethyl-oxazoline, n-butyllithium, and thallium trifluoroacetate were prepared by ourselves, thionyl chloride, dimethyl formamide, $LiAlH_4$, trifluoroacetic acid, benzene, anhydrous aluminum chloride, dimethyl sulfoxide, sodium cyanide, potassium hydroxide, diethylene glycol, hydrazine hydrate(85%), phosphorus, Vitamin C, gentisic acid, copper sulfate and iodine were from Shanghai Chemical Co. And all were of chemical and analytical grade. ^{125}I-NaI solution was from China Institute of Atomic Energy, Beijing.

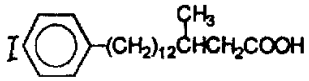
2.3 The preparation of BMIPP

The reaction formulae of the preparation of BMIPP are as follows.





(BMPPA)



(BMIPP)

2.3.1 11-Bromo-1-phenylundecan-1-one (I)

To a three-necked bottle were added dry 11-bromoundecanoic acid(54 g, 0.2 mol), thionyl chloride (20 mL) and dimethyl formamide (0.5 mL), the mixed solution was stirred and refluxed for 1h. The resulting brown solution was cooled to 10°C, 250 mL of dry thiophene-free benzene and anhydrous Aluminum chloride were added in a small portion. Then the mixture was continuously refluxed for 2 h. After cooling, pour it into 300 mL H_2O. Benzene layer was separated and the inorganic layer was extracted by benzene for three times. Combining the organic layer and evaporating, the residue was distilled under vacuum to give 39 g (60%) of colorless crystal(bp:220°C/2kPa, mp:49~51°C, IR: 3030 cm^{-1} (-CH), 1680 cm^{-1} (C=O), 770, 690 cm^{-1} (-ph)).

2.3.2 11-cyano-1-phenylundecan-1-one (II)

To a three-necked bottle were added I(65 g, 0.2moL), 200 mL of dimethyl sulfoxide and sodium cyanide(0.22mol), the resulting solution was stirred at 90°C for 4 h. The mixture was cooled to room temperature and poured into 400 mL of H_2O. After filtering, the solid was recrystallized by petroleum to give 49 g (90%) of white crystal II (mp: 56~57°C, IR:1680 cm^{-1} (C=O), 2245 cm^{-1} (C≡N)).

2.3.3 Methyl 12-phenyldodecanoate (III)

A mixture of II(56 g, 0.2 mol), 85% hydrazin hydrate (0.3 mol), Potassium hydroxide (1.0 mol) and 250 mL of diethylene glycol was refluxed for 2 h. The mixture was distilled untill the solution reached a temperature of 210°C and then heated under reflux for 4h till no gas was sent off. The resulting solution was acidified by 12N HCl and white precipitate was formed. After filtering, the solid was mixed with 250 mL of methanol and 10 mL of con. H_2SO_4. The mixture was refluxed for another 4 h and poured into 300 mL of H_2O and extracted 3 times by Et_2O. Et_2O was removed and the residue was distilled under vacuum to give 53 g(90%) of colorless liquid III(bp: 196°C/2kPa, IR:1730 cm^{-1}(-$COOCH_3$)).

2.3.4 12-phenyldodecan-1-ol (IV)

III(30 g, 0.1 mol) was added dropwise to a stirred suspension of $LiAlH_4$(0.1mol) in 200 mL of anhydrous Et_2O. The resulting mixture was refluxed for 4 h and was carefully poured into ice-water, acidified by HCl and extracted several times with Et_2O. The combined Et_2O was dried over anhydrous Na_2SO_4, and the solvent was removed. The residue

was recrystallized by petroleum to give 24 g(90%) of white crystal IV(mp:40~41°C, IR:3500 cm^{-1}(-OH)).

2.3.5 12-phenyldodecyl iodide (V)

A mixture of IV(26.2 g, 0.1 mol), iodine(15.24 g, 0.06 mol) and phosphorus(3.2 g, 0.1 mol) was heated at 150~160°C for 40 h. The mixture was poured into H_2O and filtered, the filtrate was extracted by Et_2O and was concentrated in vacuum to afford a brown residue. The residue was dissolved in petroleum and applied to silica column. The column was eluted with petroleum and the eluate was dried over anhydrous Na_2SO_4. The solvent was removed in vacuum to afford V, and the crude V can be put into the next reaction without being purified.

2.3.6 Ethyl 14-phenyl-α-methyltetradecanoate (VI)

n-butyllithium solution(100 mL, 1.2 mol/L) was cooled to −60°C, then 2-ethyl-4, 4-dimethyl-2-oxazoline(51.8 g, 0.14 mol in 50 mL of THF) was added dropwise to the above solution. After the resulting mixture was stirred at −60°C for 1h, the temperature was raised to −35°C, and the crude V in 50 mL THF was added, keep the reaction at this temperature for another 1 h. Then rise to room temperature. The mixture was poured into H_2O, acidified to neutral and extracted 3 times by Et_2O. After Et_2O was removed, the residue was refluxed with 200 mL of H_2O and extracted by Et_2O. The combined Et_2O was dried over anhydrous Na_2SO_4, and the solvent were removed. The residue was distilled in vacuum to afford 30 g(85%) of yellow liquid VI(bp: 224°C/2kPa, IR:1730 cm^{-1}(-$COOC_2H_5$)).

2.3.7 14-phenyl-α-methyl tetradecan-1-ol (VII)

VI(34.6 g, 0.1 moL) in 50 mL of anhydrous Et_2O was added dropwise to a stirred suspension of $LiAlH_4$(4 g, 0.1 mol) in 200 mL of anhydrous Et_2O as described for IV. After the solvent was removed, The residue was distilled in vacuum to give 28g(90%) of colorless liquid VII(bp: 210°C/2kPa, IR:3500 cm^{-1}(-OH)).

2.3.8 14-phenyl-α-methyl tetradecyl iodine (VIII)

A mixture of VII(30.4 g, 0.1 mol), iodine(15.24 g, 0.06 mol) and phosphorus(3.2 g, 0.1 mol) was heated at 150~160°C for 40 h. The mixture was poured into H_2O and filtered as described for V. The solvent was removed in vacuum to give VIII and the crude VIII can be put into the next reaction without being purified.

2.3.9 14-phenyl-α-methyl tetradecane nitrile (IX)

A mixture of the crude VIII(41.4 g, 0.1 mol), 200 mL of dimethyl sulfoxide and sodium cyanide (0.22 mol) was stirred at 90°C for 4 h, and was cooled to room temperature, poured into 400 mL of H_2O, extracted 3 times by Et_2O, After the solvent was removed, the residue was distilled in vacuum to afford 22 g(70%) of colorless liquid IX(bp: 206°C/2kPa, IR:2245 cm^{-1}(-CN)).

2.3.10 β-methyl-15-phenyl pentadecanoic acid (BMPPA)

A mixture of IX(31 g, 0.1 mol), 200 mL of ethylene glycol and KOH(0.5 mol) was refluxed till no NH_3 was sent off. The mixture was cooled to room temperature, poured into H_2O, and acidified by HCl to white solid was precipitated. After filtering, the solid was recrystallized by CH_3COCH_3 to afford 30 g(90%) of white solid BMPPA (mp: 38~39°C IR: 1700 cm^{-1}(-COOH), 1500 cm^{-1}(-ph), Theo Anal: C, 79.52%, H, 10.84%. Found: C, 79.07%, H, 9.74% ^{1}HNMR ($CDCl_3$), δ (10^{-6}): 0.96 (d. 3H, $CHCH_3$), 1.27 (s, 22H, CH_2), 1.95(s, 1H, CH), 2.25(d, 2H, $CHCH_2CO_2$), 2.58(t, 2H, phCH_2), 7.27 (s, 5H, Ar-H);

MS, m/e: 332(M^+, 8), 314(M^+–H_2O, 11), 104(M^+–$H_3C(CH_2)_9CH(CH_3)CH_2COOH$,18), 92($M^+HC(CH_2)_{10}CH(CH_3)CH_2COOH$, 34), 91($M^+$–$(CH_2)_{11}CH(CH_3)CH_2COOH$, 100).

2.3.11 β-methyl-15-(p-iodophenyl) pentadecanoic acid (BMIPP)

BMPPA (10 g, 0.03 mol) was refluxed in 50 mL methanol containing 0.2mL con. H_2SO_4 for 2 h. The mixture was poured into H_2O, and extracted by Et_2O. The solvent was removed and the solid was recrystallized by CH_3COCH_3 to give 9.0 g(87%), IR spectrum demonstrated that it was methyl 15-phenyl-3-methyl pentadecanoate. Methyl 15-phenyl-3-methyl pentadecanoate(1.0 g, 3 mmol) and thallium trifluoroacetate(2.0 g) in 20 mL of trifluoroacetic acid were stirred under red light at room temperature for 5 days. Potassium iodide (1.5 g) in 10 ml water was added, and the resulting mixture was stirred for 15 min. Followed by the addition of sodium thiosulfate (1 g) the mixture was stirred for another 15 min. Poured into 100 mL water and filtered. The crude precipitate was recrystallized from CH_3COCH_3 to give 0.85g(60%) of yellow needle crystal BMIPP(mp: 48~50°C, IR: 1700 cm^{-1}(-COOH), 1500 cm^{-1}(-ph). Theo Anal: C, 57.64%, H, 7.64%. Found: C, 56.92%, H, 7.64%. ^{1}HNMR ($CDCl_3$), δ (10^{-6}): 0.96(d, 3H, $CHCH_3$), 1.27(s, 22H, CH_2), 1.95(s, 1H, CH), 2.25(d, 2H, $CHCH_2CO_2$), 2.58(t, 2H, $phCH_2$), 6.92(d, 2H, Ar-H), 7.57(d, 2H, Ar-H): MS, m/z: 458(M^+, 12), 314(M^+–I, OH, 30), 313(M^+–I, H_2O, 100), 217(M^+–I, $HCCH_2CH(CH_3)CH_2COOH$, 76), 131(M^+–I, $CH(CH_2)_7CH(CH_3)CH_2COOH$, 32), 92($M^+$–I, $CH(CH_2)_{10}$ $CH(CH_3)CH_2COOH$, 100).

2.4 The preparation of ^{125}I-BMIPP

^{125}I-BMIPP can be prepared by the following three methods:

2.4.1 Direct labeling[6]

BMPPA (1.0 mg) was dissolved in 1 mL thallium trifluoroacetate and allowed to stand at room temperature for 1 h. $Na^{125}I$(18.5 MBq) was added to the insitu parathallium complex and sealed to react for 1 h at 60°C to yield ^{125}I-BMIPP, and extracted by chloroform or hexane (3×1 mL) to purify.

2.4.2 Solid-state transfer labeling[7]

BMIPP (0.5 mg) and ammonium sulfate (1.5 mg) were dissolved in ethanol (0.5 mL). After the addition of $Na^{125}I$(18.5 MBq), the solution was dried under a steam of nitrogen and allowed to react for 2 h at 170°C to form ^{125}I-BMIPP. Cooled to room temperature and extracted by chloroform or hexane to purify.

2.4.3 Cu(I) assisted labeling[8]

Preliminary radiolabeling showed that *RLY* of ^{125}I-BMIPP could reach 95%, so we made orthogonal design to choose the optimal iodolabeling condition. On the base of initial labeling, five of the following six influence factors were fixed, and the sixth factor was varied from a certain range, then *RLY* was determined. To a clean penicillin vial containing 1.0 mg BMIPP, VitC(1~20 mg), gentisic acid(1~5 mg), copper sulfate (20~100 μg), 400μL of an oxygen-free ethanol solution(concentration from 20%~100%) and $Na^{125}I$(18.5 MBq) were added. The vial was sealed and heated at different temperature for different times to yield ^{125}I-BMIPP.

2.5 Determination of radiochemical purity (*RCP*) and *RLY*

2.5.1 Silica gel sheets with the solvent of 5% ethanol in ether. R_f value for ^{125}I-BMIPP

and ^{125}I were 0.9~1.0 and 0.0, respectively.

2.5.2 Silica gel plates (soaked in HSA) with the solvent of 2.5% methanol in methylene chloride. R_f value for ^{125}I-BMIPP and ^{125}I were 0.6~0.7 and 0.0, respectively.

3 RESULTS AND DISCUSSION

3.1 we have made some improvement in the preparation of BMIPP based on the literature[6]. During the synthesis of V and VIII, we used simple iodine method instead of the reported method, in which the source material iodotrimethylsilane is expensive and must be imported. In this simple iodine method, the yield of the key sequence has significantly increased. The total yield is 43% from IV to BMPPA (literature[6]: 26%), so the cost of synthesis is greatly decreased. All data of IR, ^{1}HNMR, MS and melting point of the terminal product BMPPA and BMIPP were in accordance with those of literature[6].

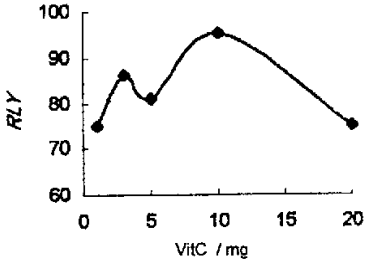


Fig.1 *RLY* of ^{125}I-BMIPP at different amount of VitC

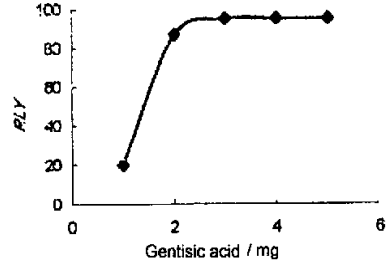


Fig.2 *RLY* of ^{125}I-BMIPP at different amount of gentisic acid

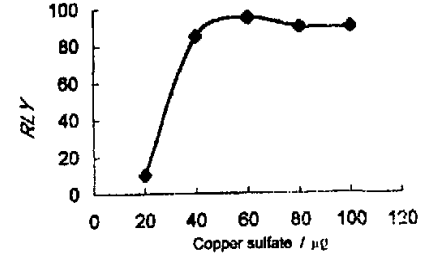


Fig.3 *RLY* of ^{125}I-BMIPP at different amount of copper sulfate

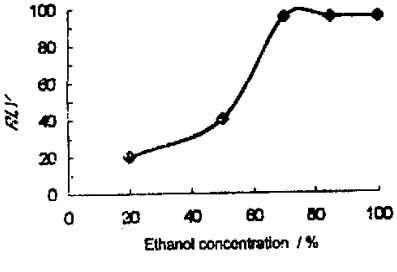


Fig.4 *RLY* of ^{125}I-BMIPP at different amount of ethanol concentration

3.2 Direct labeling of BMIPP using an organic thallium intermediate had some advantages such as low temperature, shortage of reaction time and high labeling efficiency (higher than 90%). But from the chemical point of view, the compound of thallium is

central toxic. It can't meet the real kit labeling condition because it is necessary to purify before clinical use. The *RLY* of solid-state transfer labeling of BMIPP was only 65% and the *RCP* was over 98% after purification. It can't be made into a kit form either because of its low *RLY*.

3.3 In order to overcome the central toxicity of direct labeling and the harsh terms and low yield of solid-state transfer labeling, we use Copper (I) as catalyst and radiolabel BMIPP[8]. A *RLY* of 95% can be obtained(see Fig.1. to Fig.6.).

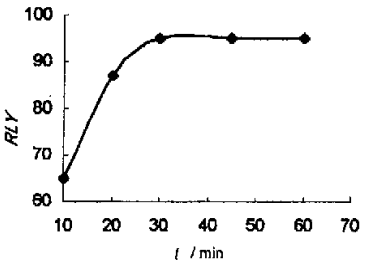


Fig.5 *RLY* of ^{125}I-BMIPP at different reaction time

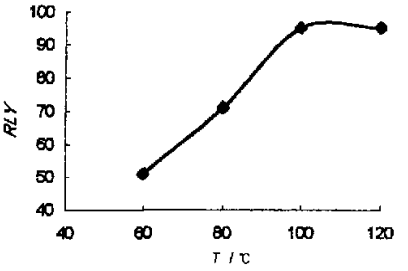


Fig.6 *RLY* of ^{125}I-BMIPP at different reaction temperaute

From Fig.1 to Fig.6, the optimal labeling condition was: VitC 10 mg, gentisic acid over 3 mg, copper sulfate over 60μg, concentration of an oxygen-free ethanol solution over 70%, reaction time over 30 min and reaction temperature over 80°C. Cu(I) assisted labeling is a simple new chemistry applicable on iodinated aryl compounds, based on the nucleophilic exchange assisted by Cu(I). This method can fulfill the requirements for a good kit preparation of iodine labeled radiopharmaceuticals. Presently we have made several kit preparations and these kits prepared ^{125}I-BMIPP have been used with success in animal studies.

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