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^{99m}Tc Labeled Myocardial Imaging Agents of Long-chain Fatty Acid: An Intermediate Synthesis Process Research

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Myocardial fatty acid metabolic imaging agent can be used for studying the myocardial ischemia positioning, measurements reflect changes in myocardial metabolic function and the detection of myocardial cell survival, provide a reliable basis for the clinical diagnosis of cardiac disease. In this paper, we study the synthesis of fatty brominated products BSE, which is the intermediates of novel ^{99m}Tc-labeled fatty acid metabolic imaging agent. The final yield of the product was 38 %, NMR spectroscopy confirmed very consistent the hydrogen peak displacement and integral spectrum area with the goal of formula. This synthetic route is convenient, simple, economic and has a potential value of industrial applications.

Key Words: ^{99m}Tc, Myocardial metabolic agent, Imaging, Radioactivity.

INTRODUCTION

^{99m}Tc-labeled imaging agent used to diagnose diseases at home and abroad, many studies have been carried out. Unlike the B ultrasound, CT, MRI, it combines form and function as a whole, it can not only show the organ morphology but also understand organ function. Although in recent years there have been many competing diagnostic methods, however, the function of the nuclear medicine imaging still a popular inspection method. In 1999, Yamamura[1] synthesized ^{99m}Tc-MAMA-HA, this is the first confirmed *in vivo* fatty acid β-oxidation of the derivative, which is the development potential imaging agent. On this basis, Magata[2] and Wisneski[3] increase the chain length of fatty acids, synthesis of ^{99m}Tc-MAMA-HDA and ^{99m}Tc-MAMA-DA, biological experiment results show ^{99m}Tc-MAMA-HAD has higher heart/blood ratio. Since then, a lot of exploration and improvement to imaging agent ^{99m}Tc labeled myocardial fatty acid metabolism are made.

Fatty acids is the most important material for the normal myocardial cells. The concentration of the fatty acids in myocardial cells is 20 times higher than in the plasma concentration. In the fasting, 80 % the energy requirement in myocardial is from the free fatty acids. When myocardial ischemia, myocardial fatty acid metabolism and clear have downward trend. Therefore, myocardial fatty acid metabolism imaging agent can be used on myocardial ischemia positioning. The measurement reflect changes in myocardial metabolic function, detect myocardial cell survival, provide reliable clinical diagnosis of cardiac disease[4].

^{99m}Tc-labeled fatty acids (Fig. 1), after the modification and exploration of different points, different core and different chain length, there are not satisfied with the results, the study stopped at the biodistribution, did not use in the imaging experiments. The main reason is its biological activity by the labeled fatty acids change larger.

How to reduce the labeled fatty acids bioactive change, improve the absorption and retention time of the imaging agent

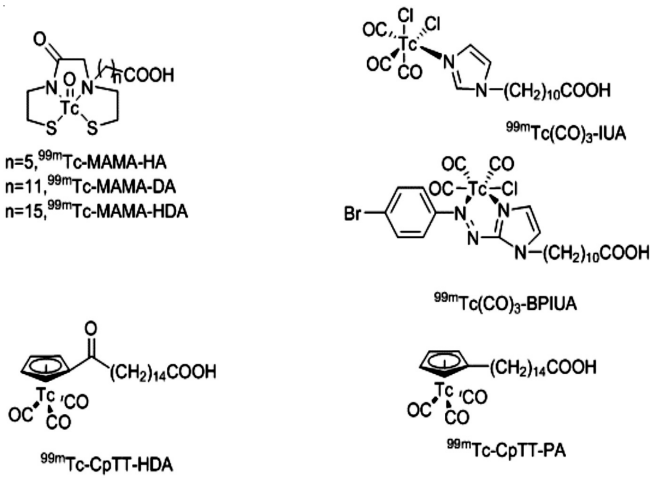


Fig. 1. Several ^{99m}Tc labeled fatty acid metabolic imaging agent

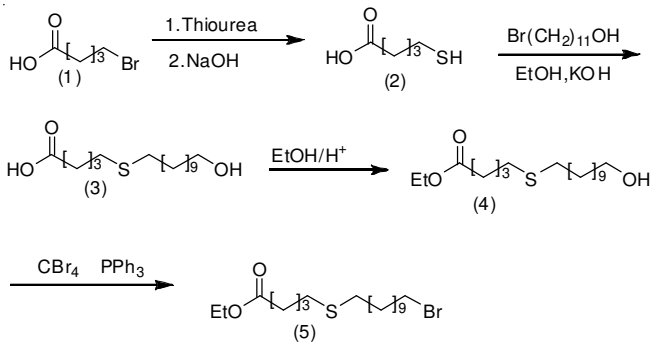
in the myocardium, improving the ratio of heart/blood, heart/lung and heart/liver is the key. The metallic core and the size of the spatial structure of the ligand compound, as well as the fatty acid chain length and structure directly affect the biological activity of the fatty acid. Small space structure of the metal core and the ligand compound, as well as modification of the structure of the fatty acid carbon chain is the development direction of ^{99m}Tc-labeled fatty acid metabolic imaging agent.

EXPERIMENTAL

Thiourea, sodium hydroxide, potassium hydroxide, dichloromethane, chloroform, anhydrous ethanol, anhydrous sodium sulfate, concentrated sulfuric acid, bromo undecanol, methanol, ethyl acetate, carbon tetrabromide (CP, Beijing Chemical Plant), triphenylphosphonium (AR, Beijing Chemical Reagent Company), bromovaleric acid (CP, Tianjin Tiantai Fine Chemicals Co. Ltd.), *n*-hexane (AR, Beijing Chemical Plant), deionized water (Institute of High Energy Physics, Chinese Academy of Sciences).

Electronic balance (MP120-1 capacity 120 g/0.001 g); magnetic stirrer (GSP-79-03, Tai xian Jiangsu province Electronic Instrument factory; rotary evaporator (R201, Shanghai Shen Shengsheng Biotechnology Co. Ltd.); heater (R201, Shanghai Shen Shengsheng Biotechnology Co. Ltd.); silica gel plates (GF_{254}, Qingdao Marine Chemical Plant) .

Synthesis



5-Bromovaleric acid (**1**); 5-Sulfanylvaleric Acid (**2**); 17-Hydroxy-6-thia-heptadecanoic Acid (**3**); Ethyl 17- Hydroxy-6-thia-heptadecanoate (**4**); Ethyl 17-Bromo-6-thia-heptadecanoate (**5**)

Fig. 2. Synthetic route

RESULTS AND DISCUSSION

Preparation of thiols: The purity of 5-bromo valerate has great impact on the follow-up reaction. Adding an appropriate amount of *n*-hexane and recrystallized, 5-bromopentanoic acid change from a pale yellow solid powder into a white solid powder, so we can get pure product.

10 g of 5-bromo valeric acid with 6.3 g thiourea immiscible in 100 mL of ethanol, heated to reflux and stirred at 100 ºC for 16 h until the mixture was cooled to room temperature, the solvent was evaporated to dryness under reduced pressure; adding an alkali 7.5 M NaOH (Na_2CO_3, $NaHCO_3$) and then the heating was continued under reflux (100 ºC) for 6 h; after the mixture was cooled to room temperature.

Acidified with 2.5 M H_2SO_4 and adding large quantities of methylene chloride for extraction, the organic phase (lower layer) was separated using a separatory funnel, washed several times repeatedly with dichloromethane, the organic phase was separated and placed in a beaker, add the excess of anhydrous Na_2SO_4 dried; filtered off Na_2SO_4, the solvent was evaporated to dryness under reduced pressure to give the mercaptan product.

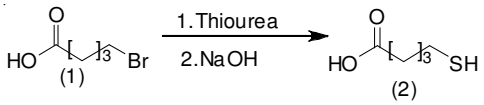


Table-1 shows that the yield of the product with the addition of an alkali solution and the reaction time has a relationship: NaOH > Na_2CO_3 > $NaHCO_3$, the stronger the alkaline, the higher the yield. Table-2 shows that: 6 h is the optimum reaction time, the reaction was not complete in short reaction time, alkali in the solution will continue to react with the product in long reaction time, therefore, the strong base and the reaction time was 6 h are the best experimental conditions.

TABLE-1
RELATIONSHIP BETWEEN THE YIELD OF THE PRODUCT AND THE BASICITY

Alkali	Reaction time (h)	Quantity (g)	Yield (%)
$NaHCO_3$	6	4.32	58
Na_2CO_3	6	5.04	68
NaOH	6	5.81	78

TABLE-2
RELATIONSHIP BETWEEN THE YIELD OF THE PRODUCT AND REACTION TIME

Alkali	Reaction time (h)	Quantity (g)	Yield (%)
NaOH	4	5.35	72
NaOH	6	5.81	78
NaOH	8	5.52	74

Synthesis of fatty acids: Weigh 2.9 g mercaptan product of the previous step 5-sulfanyl valeric acid (2) with 2.5 g KOH solid immiscible in 85 mL of anhydrous ethanol was added 5.4 g bromo undecanol, heat reflux at 60, 100, 135 ºC and stirred 12 h. After the reaction, mixture was treated with 2.5 M of H_2SO_4 acidified. After acidification, the solvent was evaporated to dryness under reduced pressure. 40 mL of distilled water was added to dissolve, repeatedly washed several times with chloroform and the organic phase (lower layer) was separated using a separatory funnel; separated organic phase was placed in a beaker and dried over anhydrous Na_2SO_4, filtered off Na_2SO_4, the solvent was evaporated under reduced pressure. Add 60 mL of methanol, placed at -15 ºC, recrystallization, the product is a white solid.

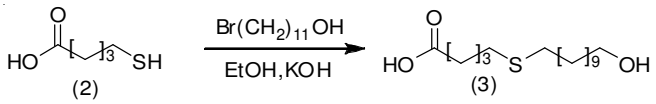


Table-3 shows that the yield and the reaction temperature has a relationship. When heated at temperature 100 ºC, yield relatively highest, the temperature is controlled in ethanol reflux rate of 60 drops/min. Reflux rate is too fast (the temperature is too high) or too low (too low temperature) will affect the progress of the reaction, reducing the yield, temperature of 100 ºC, the yield is relatively satisfactory.

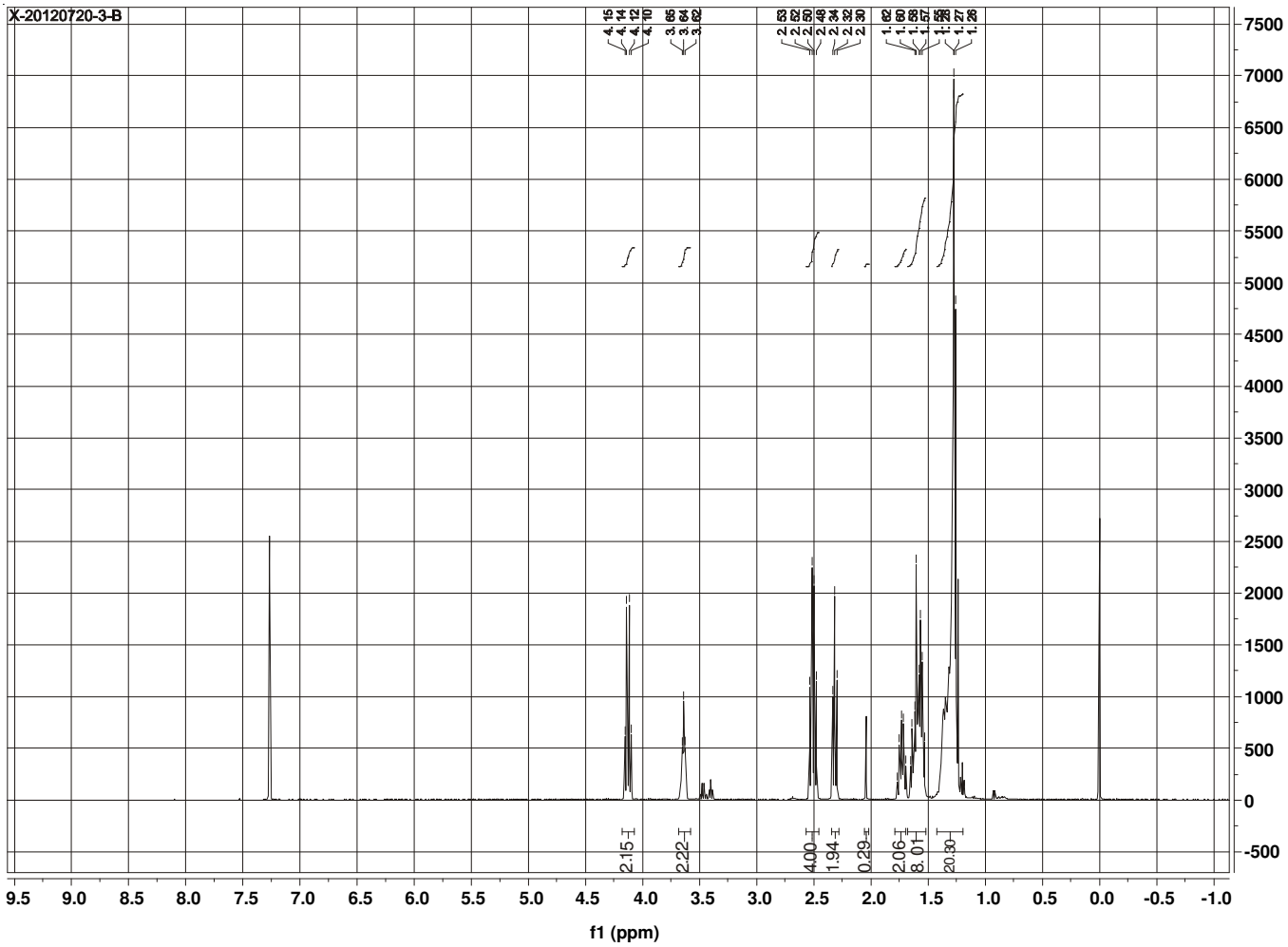
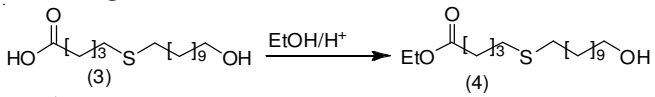


Fig. 3. Fatty acid bromide spectrum

TABLE-3
RELATIONSHIP BETWEEN THE YIELD OF THE PRODUCT AND TEMPERATURE

Temperature	Reaction time (h)	Quantity (g)	Yield (%)
60	12	0.9	31
100	12	1.7	59
135	12	1.1	38

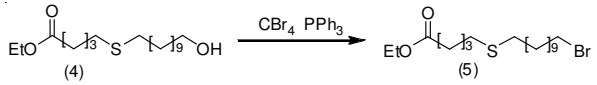
Esterification reaction of fatty acids: Weigh 2 g fatty acids product of the previous step 17-hydroxy-6-thia-heptadecanoic acid (**3**) and 2 mL of concentrated H_2SO_4 placed in 150 mL of absolute ethanol, heated stirred at reflux 100 ºC, after the reaction, heating was stopped and stirring was continued overnight. The solvent was evaporated to dryness under reduced pressure; which was dissolved in 100 mL of ethyl acetate, washed with 100 mL of deionized water once, the organic phase (upper layer) was separated using a separatory funnel and the separated organic phase was placed in a beaker, dried with anhydrous Na_2SO_4, filtered off Na_2SO_4, solvent was evaporated under reduced pressure and purified by flash column chromatography (3:1 hexane/ethyl acetate) obtain a fatty acid esterified product as a white solid.



^{1}H NMR chemical shifts and integrated area of analysis, the hydrogen atoms in the chemical shifts and integral area and molecular corresponding relationship has been marked with italics and boldface. The results are as follows: ^{1}H NMR ($CDCl_3$): δ = 1.24-1.32 (m, 17H, 7 × CH_2, $COOCH_2CH_3$), 1.53-1.77 (m, 8H, CH_2CH_2OH, $CH_2CH_2SCH_2CH_2$, $CH_2CH_2COOCH_2CH_3$), 2.32 (t, *J* = 7.3, 2H, $CH_2COOCH_2CH_3$), 2.48-2.53 (m, 4H, CH_2SCH_2), 3.65 (t, *J* = 6.2, 2H, CH_2OH), 4.12 (q, *J* = 7.1, 2H, COOC H_2CH_3).

From Fig. 3 we can see, this product is indeed the desired product of a fatty acid bromide (yield ~ 59 %).

Synthesis of the imaging agents of the long chain fatty acid intermediates-BSE: Weigh 0.332 g product of the previous step Ethyl 17-Hydroxy-6-thia-heptadecanoate (**4**), add CBr 40.996 g, dichloromethane reagent 50 mL, 0 ºC (ice-water) nitrogen stirring, add triphenyl phosphorus (PPh3) 0.788 g and allowed to react overnight. After drying under reduced pressure and purified by flash column chromatography (10:1 hexane/ethyl acetate), we can get the crude product as a pale yellow oil.



^{1}H NMR chemical shifts and integrated area of analysis, the hydrogen atoms in the chemical shifts and integral area and molecular corresponding relationship has been marked with italics and boldface. The results are as follows: ^{1}H NMR ($CDCl_3$): δ = 1.20-1.35 (m, 17H, 7 × CH_2, $COOCH_2CH_3$), 1.55-1.75 (m, 8H, CH_2CH_2Br, $CH_2CH_2SCH_2CH_2$, $CH_2CH_2COOCH_2$

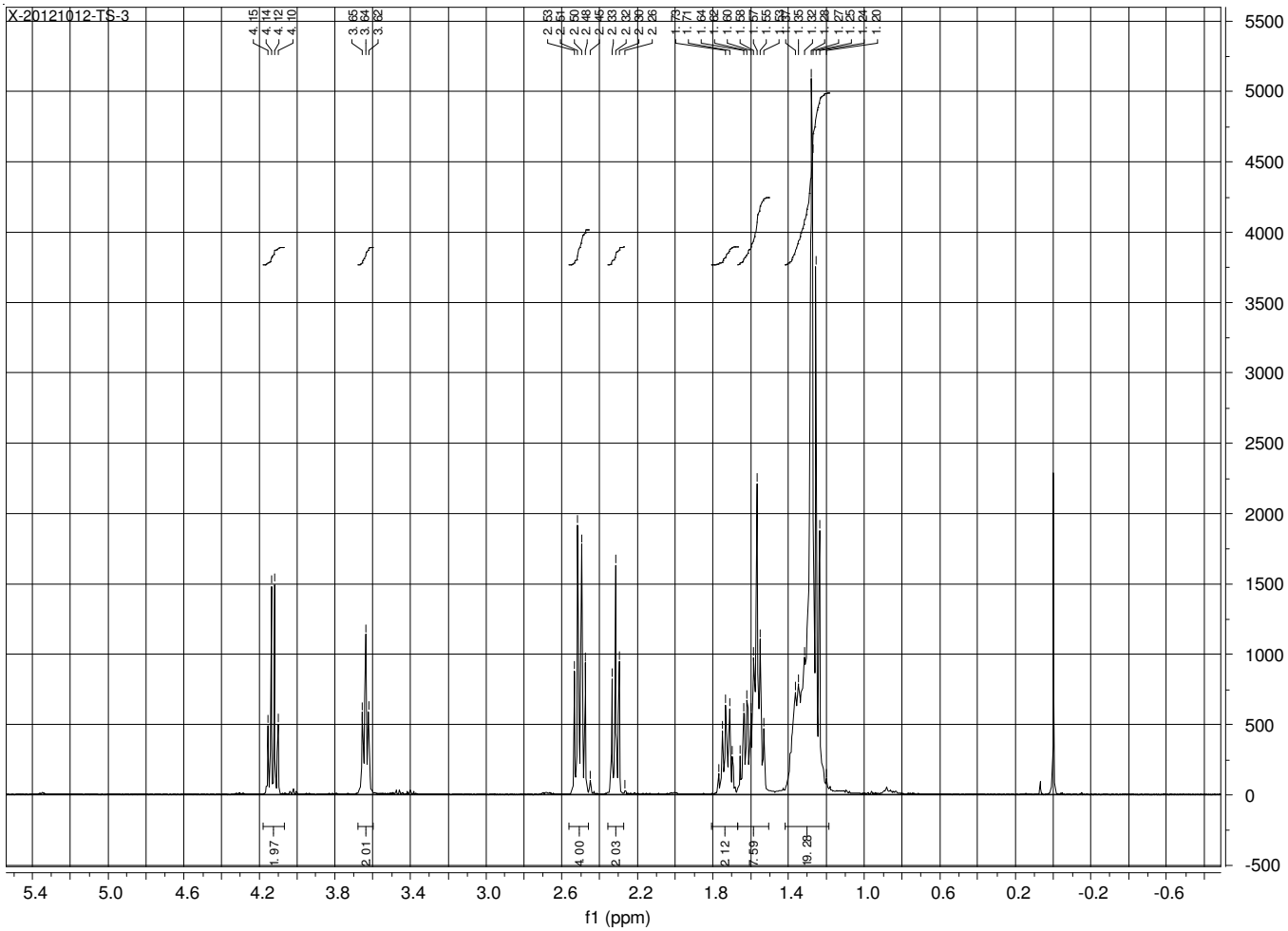


Fig. 4. Fatty acid of brominated products spectrum

CH_3), 2.32 (t, J = 7.3,2 H, $CH_2COOCH_2CH_3$), 2.45-2.53 (m, 4H, CH_2SCH_2), 3.64 (t, J = 6.6, 2H, CH_2Br), 4.14 (q, J = 7.1, 2 H, $COOCH_2CH_3$).

Inferred from Fig. 4 that this product is the intermediates of the final product ^{99m}Tc labeled myocardial fatty acid metabolism imaging agent of BSE, the yield is 38 %.

Conclusion

The purpose of this experiment is to synthesize a fatty acid bromide-BSE. This fatty acid bromide can promote metabolism, likely to be marked, in addition, the carbon chain structure help to simulate in vivo myocardial fatty acid behaviour. Its carbon chain structure contributes to the absorption and retention of the myocardium. Preparation of thiol should add 7.5M alkali NaOH, the reaction time is 6h, the highest yield is 78 %. Fatty acid synthesis in the reflux temperature is 100 ºC, the highest yield is 59 %. In Fatty acids esterification, the reaction time is 6h, the highest yield is 55 %. In synthesis of ethyl 17-bromo-6-thia-heptadecanoate, the order of addition should be noted.

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