Radiolabeling of N-Succinimidyl-3-[^{125}I]iodobenzoate intermediate

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Abstract Organostannanes were important precursors which was easy to radioiodinate. N-Succinimidyl-3-(tri-n-butylstannyl) benzoate (STB) was radiolabeled using Iodogen to get radioactive N-Succinimidyl-3-iodobenzoate ($S^{125}IB$) with 96% of high radiochemical yield. The optimization of labeling condition was explored in this study. $S^{125}IB$ was stable at room temperature in dark. Cold SIB was prepared as a standard and IR and NMR results were given in this article.

Keywords Organostannanes, Radioiodination, Stability, Iodogen

CLC numbers TL92^{+}3, R817.8

1 Introduction

Radiohalogenation reactions using organometallic intermediates have been studied extensively. Halogen-reactive organometallic compounds including aromatic compounds have been studied as a means of efficiently, rapidly, and site-specifically incorporating radiohalogens into small molecules. But in various kinds of organometallic compounds, organostannes were the best candidates for labeling because they can react quickly (in 5 min) and efficiently.[1] Its application offers a brand new way for radioiodinated pharmaceuticals. Such as in 1987, Zalutsky and its co-worker synthesized STB as an intermediate and then radioiodinated using t-butylhydro-peroxide (TBHP) and N-chlorosuccinimide (NCS) oxidant.[2] As a conjugation reagent, $S^{125}IB$ is easily radioiodinated and very stable in dark at room temperature. Moreover, from its structure, we can deduce that the deiodination will not occur in theory. In addition, as there are no hydroxyl groups in aromatic ring, hydroxylation of enzyme would not happen in the radiolabeled compounds.[1]

In our lab, STB was chosen for radioiodination using Iodogen method and radioactive SIB was prepared successfully.

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2 Experimental

2.1 Synthesis of cold SIB

The synthesis route of cold N-Succinimidyl-3-iodobenzoate (SIB) is shown in Fig.1.

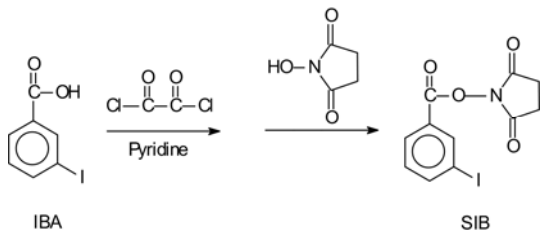

Fig.1 Synthesis of cold SIB.

In detail, 0.35g of pyridine was added to a flask with 1g of meta-iodobenzoic acid (IBA) in toluene and stirred, followed by adding 0.42 mL of oxalyl chloride. Stirred for 30min at room temperature. After heated to remove excess oxalyl chloride, the solution was cooled to room temperature. 0.46g of N-hydroxysuccinimide (NHS) was added and refluxed for 30 min. The product was separated through silica gel-flash chromatography (from 100% petroleum ether to 70% petroleum ether and 30% ethyl acetate). The product was traced by TLC (GF_{254}, 30% ethyl acetate in hexane) and then collected the fractions containing SIB. The R_f of SIB was 0.23.

2.2 Radiolabeling of $S^{125}IB$ from STB(Fig.2)

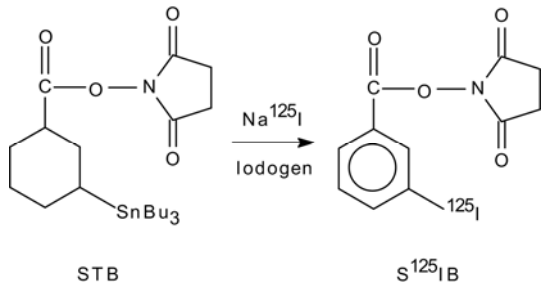

Fig.2 Preparation of $S^{125}IB$ using Iodogen.

50 μL of STB (0.25 g/L) and 60 μL of Iodogen (Sigma) (0.33 g/L) were added to a 1 mL glass vial coated with 1 μL no carrier-added $Na^{125}I$ (13.4 GBq/mL, >99.95%, Amersham), and vortexed for 5 min at room temperature. The labeling efficiency was described as the ratio of radioactivity of $S^{125}IB$ to the added radioactivity and determined by TLC[3] (GF_{254}, 30% ethyl acetate in hexane).

2.3 Separation of $S^{125}IB$

$S^{125}IB$ was isolated by Sep-Pak silica gel.[4] Briefly, a Sep-Pak silica gel cartridge was first saturated with hexane, and the reaction mixture was loaded on the column with 300 μL of hexane. Following elution with 40 mL of hexane and 25 mL of 8% ethyl acetate in hexane, the product of radioactive $S^{125}IB$ was isolated in 12~15 mL of 30% ethyl acetate in hexane. Then the 30% ethyl acetate effluent containing $S^{125}IB$ was collected and the solvent was evaporated to dryness with nitrogen.

2.4 Quality control of $S^{125}IB$

$S^{125}IB$ was dissolved in anhydrous acetonitrile. The radiochemical purity of $S^{125}IB$ was determined by TLC and HPLC. TLC (GF_{254}, 30% ethyl acetate in hexane) examination showed the t_R of product was 0.23 which coincided with cold SIB, and no significant STB (R_f 0.5) was found.

HPLC was performed with RP C18 column, the mobile phase was 65% methanol/35% water (*V*/*V*, 0.1%TFA), flow rate was 1mL/min, uv254 detector and radiodetector were used to detect the radioactive $S^{125}IB$, at the same time, cold SIB was injected as standard. The t_R of $S^{125}IB$ was 7.83 min and coincided with the cold SIB standard (Fig.3).

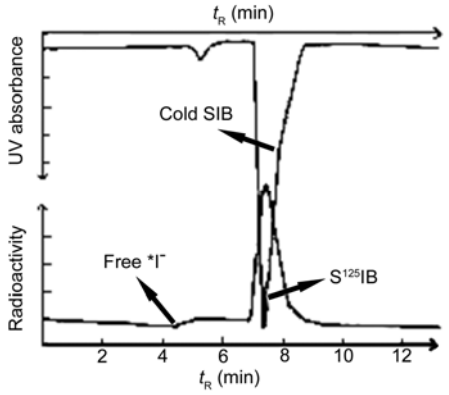

Fig.3 HPLC profile of $S^{125}IB$.

2.5 In vitro stability of $S^{125}IB$

$S^{125}IB$ was stored at 4 °C in dark, its stability was detected by TLC at different time points.

2.6 Optimization of labeling conditions

In order to optimize the radioiodination of STB, effects of reaction time (*t*), amount of Iodogen, and mole ratio of STB on $Na^{125}I$ were studied.

3 Results and discussion

3.1 Synthesis of cold SIB

In the preparation of cold SIB, 0.86 g of white solid was obtained with a yield of 65%. It was characterized by ^{1}HNMR and IR.[2]

^{1}HNMR: 2.95 (s, 4H, $-CO-CH_2CH_2-CO-$); 7.24 (t, 1H, C4-H); 8.01 (d, 1H, C5-H); 8.16 (d, 1H, C6-H); 8.50 (s, 1H, C2-H)

IR (KBr): ν_{max} (cm^{-1}): 1770, 1733, 1596, 1563, 1471, 1421, 1413, 1214, 1072, 994, 730, 603.

3.2 Quality control of $S^{125}IB$

HPLC was an efficient method to separate $S^{125}IB$ and other impurities. From Fig.3 we can see that the t_R of $S^{125}IB$ was consistent with the SIB standard and its RCP was about 99%.

3.3 In vitro stability of $S^{125}IB$

The RCP of $S^{125}IB$ was determined after storing 1, 2, 3, 4, 5 d respectively and the data were shown in Fig.4. $S^{125}IB$ was stable at room temperature in dark, above 99% of radiochemical purity was achieved after 5 d, and no significant radioactive impurities were found.

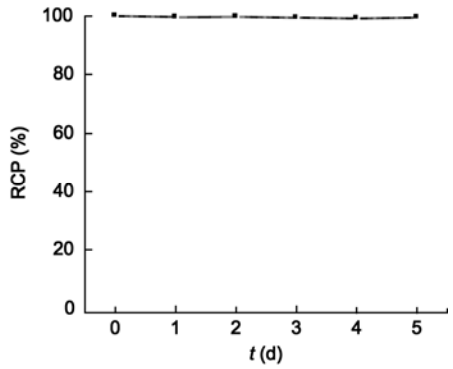

Fig.4 The stability of S^{125}IB by determining RCP.

3.4 Optimization of labeling conditions

In the preparation of S^{125}IB, when mole ratio of STB to Na^{125}I was 3~8, the labeling efficiency was about 95%. About 10 μg (7~14 μg) of Iodogen was suitable for the reaction (Fig.5). The reaction was successfully completed in 5 min at room temperature and the yield could reach 96%. More than 5 min was not necessary for this reaction.

4 Conclusions

Radioiodination of the organostannane in our lab was performed using frequently-used oxidants such as Iodogen. In fact, it is convenient to prepare radioactive S^{125}IB from STB using Iodogen, about 96% of RCY was achieved. The labeling condition was optimized in several sides including the Iodogen dosage, the mole ratio of reatant and reaction time. When mole ratio of STB to Na^{125}I was 3~8, the Iodogen dosage was about 10 μg, the reaction was successfully completed in 5 min at room temperature and the yield could reach 96%. The product of S^{125}IB was very stable and TLC results showed above 99% of RCP after stored in dark at room temperature for 5 days. Stannylated compounds were good reagents and easy to radioiodinate using Iodogen, the whole procedure was simple to operate and high specific activity could be obtained.

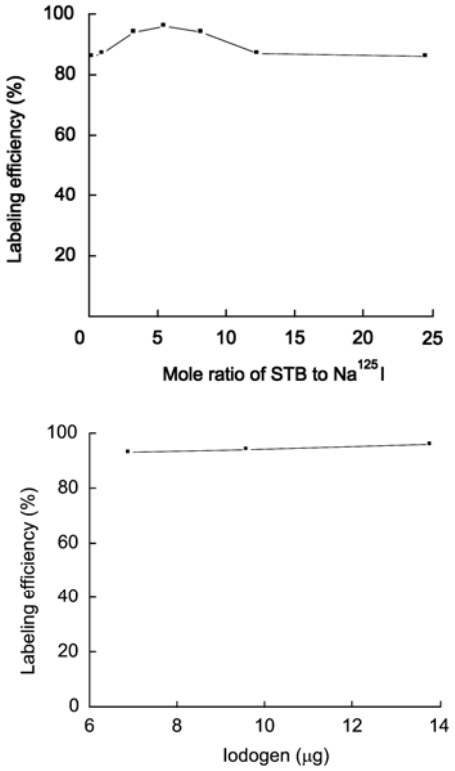

Fig.5 Optimization of radioiodination of STB.

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