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A New and Simple Synthesis of N-Succinimidyl-4-[$^{127/125}$I] Iodobenzoate Involving A Microwave-Accelerated Iodination Step

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SUMMARY

A short and simple method for the preparation of N-succinimidyl-4-iodobenzoate **3** and its ^{125}I analogue that is widely used for radioiodination of proteins is described. The method involves the reaction of *in situ* generated trimethylsilyl iodide [^{127}I or ^{125}I] from trimethylsilyl chloride with N-succinimidyl-4-[3,3-(1,4-butanediyl)-triazene] benzoate **2** available from the condensation of N-hydroxysuccinimide and 1-(4-carboxyphenyl)-3,3-(1,4-butanediyl)-triazene **1** in the presence of dicyclohexyl carbodiimide (DCC). The new triazene **1** was prepared by precipitating the diazonium ion of 4-aminobenzoic acid with pyrrolidine. A microwave-mediated rate acceleration was observed during the iodination step and by applying this procedure the labelled compound **3** was obtained in 81% radiochemical yield in 8 minutes.

KEYWORDS: N-succinimidyl-4-iodobenzoate, iodination step, trimethylsilyl iodide, triazene intermediates, *in vivo* deiodination, microwave irradiation.

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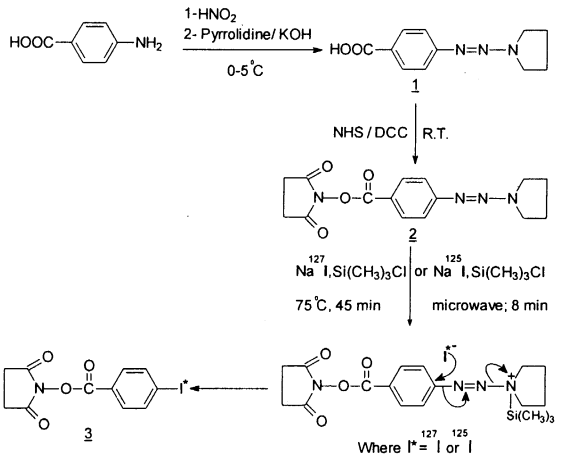
INTRODUCTION

Due to the importance of radioiodinated proteins for diagnosis and therapy of tumors, their synthesis has been the subject of many investigations (1). Earlier methods are based on direct radioiodination using $Na^{125}I$ in the presence of oxidants such as chloramine-T (2), iodogen (3) or N-bromosuccinimide (4) which presumably involves substitution of the iodine ortho to the hydroxyl groups of tyrosine residues of proteins. In addition to the instability of some proteins toward oxidizing agents (5), the radiolabelled compounds synthesized by this method undergo various degrees of *in vivo* deiodination due to their similarities to thyroid hormones (6) and weakening of the carbon-iodine bond by the ortho hydroxyl group (7). An alternative approach, which is suitable for proteins capable withstanding harsh oxidizing conditions, but which still suffer from *in vivo* deiodination, is attachment of radioiodinated small molecules such as the Bolton Hunter (8), Wood's (9) or SU-Jeng reagents (10) to the free functional groups of proteins. One of the most widely used techniques for labelling of proteins which by comparison with other methods eliminates the problems associated with their uses and/or synthesis is the reaction of proteins with N-succinimidyl-3- or -4-[^{125}I]iodobenzoates which are prepared by iododestannylation of the corresponding N-succinimidyl-3- or -4-(tri-n-butylstannyl)benzoates (11, 12). The disadvantages of these methods are the time consuming synthesis of the tin precursor, the lengthy reaction sequences, the use of hazardous reagents, essential use of inert atmosphere and difficulties in separation of products from the reaction mixtures. In view of the drawbacks of the existing methods for the preparation of **3** introduction of a new method is of practical importance. In this paper a short and convenient synthesis of **3** is reported.

RESULT AND DISCUSSION

In order to overcome the limitations of the reported methods a short and efficient synthesis of **3** appeared to be iodination of N-succinimidyl-4-[3,3-(1,4-butanediyl)-triazene]benzoate **2** with NaI which has been successfully applied for the preparation of aryl iodides from aniline via triazene intermediates (13).

Compound **2** was prepared by esterification of N-hydroxysuccinimide with 1-(4-carboxyphenyl)-3,3-(1,4- butanediyl)-triazene **1** in the presence of DCC (Scheme 1).



Scheme 1: Synthesis of N-Succinimidyl-4-[$^{127/125}$I]iodobenzoate

The new air and light stable triazene intermediate **1** was prepared by addition of pyrrolidine to the diazonium ion solution of 4-aminobenzoic acid. While attempted iodination of **2** by the reported method (13) for decomposition of aryl triazenes to aryl iodides through the reaction with sodium iodide in hydrochloric acid or trifluoroacetic acid at 5°C could not be accomplished due to the hydrolysis of the succinimidyl moiety, treatment of **2** with *in situ* generated trimethylsilyl iodide from trimethylsilyl chloride which has been used for transformation of aromatic dialkyl triazenes to aryl iodides (14) gave the unlabelled product **3** in 90% yield (method A). Additional experiments revealed that the activation of this reaction by exposure to microwave irradiation reduced the reaction time to 8 min (method B). These findings led us to use the microwave irradiation for radioiodination of **2** which requires a simple and rapid method for the small scale preparations and under these conditions the labelled compound **3** was obtained in 81% radiochemical yield.

EXPERIMENTAL

All chemicals used were of reagent grade. Anhydrous solvents were dried over an appropriate drying agent and freshly distilled. The progress of reactions was

monitored on Whatman MK6F (250 μm) thin layer chromatographic (TLC) silica gel micro-plates. ^{1}H NMR spectra were recorded on a Bruker AM-300 spectrometer using $CDCl_3$ as solvent. Chemical shifts (δ) are reported in ppm downfield with respect to tetramethylsilane as internal standard. Melting points were recorded on a Buchi capillary apparatus and are uncorrected. Elemental analysis was performed as a commercial service by the Department of Chemistry, University of Alberta, Canada. Radiochemical products were purified by HPLC on a μbond radial-pak C-18 analytical column. The commercially available radioiodide [^{125}I] in 0.1N NaOH solution (Amersham) was extracted with freshly distilled 2-butanone (4×3 mL) and dried over $CaSO_4$. Evaporation of the solvent under reduced pressure afforded anhydrous sodium iodide [^{125}I].

1-(4-Carboxyphenyl)-3,3-(1,4-butanediyl)-triazene **1**: A cold solution of sodium nitrite (1.15 g, 21.9 mmol) in water (10 mL) was added dropwise to a stirred solution of 4-aminobenzoic acid (3 g, 21.9 mmol) in cold concentrated hydrochloric acid (43.8 mmol) at 0°C. The mixture was stirred at 0°C for an additional 20 min and then transferred to a vigorously stirred solution of pyrrolidine (1.56 g, 22 mmol) in aqueous potassium hydroxide solution (15 mL, 0.22 M) at 5°C. The suspension was stirred for a further 10 minutes and the reaction mixture was then extracted with ether. The ethereal extracts were washed with water and dried over anhydrous $MgSO_4$. After evaporation of the solvent under reduced pressure the residue was crystallized from 95 % ethanol to afford **1** (3.8 g, 80%); mp 194°C; ^{1}H NMR ($CDCl_3$) δ 1.92-2.12 (m, 4H, $H_{2\text{ and }3}$ of pyrrolidine), 3.65-3.95 (m, 4H, $H_{1\text{ and }4}$ of pyrrolidine), 7.49 (d, 2H, J=8.5 Hz, $H_{2\text{ and }6}$ of phenyl), 8.05 (d, 2H, J=8.5 Hz, $H_{3\text{ and }5}$ of phenyl); analysis for $C_{11}H_{13}N_3O_2$, calc. C, 60.26; H, 5.98; N, 19.17; found C, 60.32; H, 6.02 and N, 19.20.

N-succinimidyl-4-[3,3-(1,4-butanediyl)-triazene]benzoate **2**: To a solution of **1** (2.19 g, 10 mmol) in dry THF (100 mL), were added N-hydroxysuccinimide (1.15 g, 10 mmol) and dicyclohexyl carbodiimide (2.06 g, 10 mmol) and the reaction mixture was stirred overnight at room temperature. Then the mixture was filtered and the solvent was evaporated under reduced pressure to give a solid (mp 198-201°C). The crude product was purified by column chromatography over silica gel

using toluene/ethyl acetate (9:1) as eluent to give **2**. (3 g, 95%); mp 200°C; ^{1}H NMR ($CDCl_3$) δ 1.95-2.16 (m, 4H, $H_{2\ and\ 3}$ of pyrrolidine), 2.89 (s, 4H, $COCH_2CH_2CO$), 3.60-4.10 (m, 4H, $H_{1\ and\ 4}$ of pyrrolidine), 7.51 (d, 2H, J=8.5 Hz, $H_{2\ and\ 6}$ of phenyl), 8.08 (d, 2H, J=8.5 Hz, $H_{3\ and\ 5}$ of phenyl), analysis for $C_{15}H_{16}N_3O_4$, calc. C, 59.60; H, 5.33; N, 13.90; found C, 59.74; H, 5.21 and N, 13.79.

N-succinimidyl-4-iodobenzoate **3**:

Method A:

To a solution of **2** (316 mg, 1 mmol) and NaI (300 mg, 2 mmol) in 20 mL acetonitrile was added trimethylsilyl chloride (163 mg, 1.5 mmol) and the mixture was stirred at 75°C for 45 minutes. After evaporation of the solvent under reduced pressure the residue was chromatographed on a silica gel column using ethyl acetate/toluene (1:20) as eluent to give pure **3**. (310 mg, 90%); mp 212°C [Litt. (12) 212-213°C]; ^{1}H NMR (CDCl3) δ 2.89 (s, 4H, $COCH_2CH_2CO$), 7.75 (d, 2H, J=8.24 Hz, $H_{2\ and\ 6}$ of phenyl), 7.82 (d, 2H, J= 8.24 Hz, $H_{3\ and\ 5}$ of phenyl); analysis for $C_{11}H_8NO_4$, calc. C, 60.55; H, 3.69; N, 6.42; found C, 60.64; H, 3.73 and N, 6.36.

Method B:

To a solution of 32 mg (0.1 mmol) of **2** in 2 mL dry acetonitrile in an Erlenmeyer flask were added NaI (30 mg, 0.2 mmol) and trimethylsilyl chloride (16 mg, 0.15 mmol).The mixture was shaken at room temperature for five minutes. Then the flask was placed in a domestic microwave oven and irradiated at 2450 MHz for 8 minutes when TLC analysis showed complete disappearance of the starting material **2**. The crude product obtained after evaporation of the solvent under reduced pressure was purified by preparative TLC on silica gel plates with toluene/ethyl acetate (8:2) as eluent to afford pure **3** (30 mg, 87 %).

N-succinimidyl-4-[^{125}I]iodobenzoate **3**: To a solution of 40 μCi sodium iodide (^{125}I) in acetonitrile (20 μL) in a 1 mL reactival^{Tm} were added 80 μL of 6 mM acetonitrile solution of **2** and 80 μL of 9 mM acetonitrile solution of trimethylsilyl chloride. The mixture was shaken for 5 minutes at room temperature and then the vial was placed in a domestic microwave oven and irradiated at 2450 MHz for 8 minutes. Then the vial was removed from the microwave oven and the reaction mixture was allowed to reach to room temperature. The mixture was analyzed on a

μbond radial-pak C-18 reversed phase column at 260 nm and eluted with methanol and water (55:45) at a flow rate of 0.5 mL/min. The fractions (1.5 mL) were collected every minute and counted on a Beckman γ-scintillation counter. The radiochemical yield was 81%.

CONCLUSION

When compared with a previously reported method (12) for the preparation of **3**, the present approach, which reduced the reaction sequence from 5 to 3 steps with comparable overall (68%) and radiochemical (81%) yields, was faster. Additionally the method required less expensive reagents and purification was easier.

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