

SYNTHESIS OF IODINE-125 LABELLED ω -IODOUNDECYL CHOLESTERYL ETHER

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SUMMARY

ω -[¹²⁵I]Iodoundecyl cholesteryl ether has been synthesized via a hydroboration-iodination sequence. The syntheses can be used to prepare high specific activity (no-carrier-added) reagents.

KEY WORDS: Iodine-125, Organoborane, Cholesteryl Alkenyl Ether,
 ω -[¹²⁵I]Iodoundecyl Cholesteryl Ether.

INTRODUCTION

Radioactively labelled markers have been used extensively to determine the metabolic and pharmacokinetic behavior of liposomes(1) and lipoprotein particles(2). A variety of ³H- or ¹⁴C-labelled nonmetabolizable and non-exchangeable marker compounds have been reported(3,4). However, the in vivo use of these compounds is rather limited. ¹²⁵I-labelled analogs of phospholipids have been used successfully in vitro(5,6) but these compounds are metabolized in vivo, making long term studies difficult(5,6). We(3) and others(2) previously reported that ³H-labelled cholesteryl alkyl ethers are nonmetabolizable and nonexchangeable markers for liposomes both in vitro and in vivo.

We wish to report a convenient synthesis of ω -[¹²⁵I]iodoundecyl cholesteryl ether via a hydroboration-iodination sequence, Figure I. The

scheme was developed in our laboratory and had been used previously to prepare high specific activity (no-carrier-added) reagents(7,8). ω -[^{125}I]-Iodoundecyl cholesteryl ether is currently being evaluated for use as a liposomal marker in our laboratories.

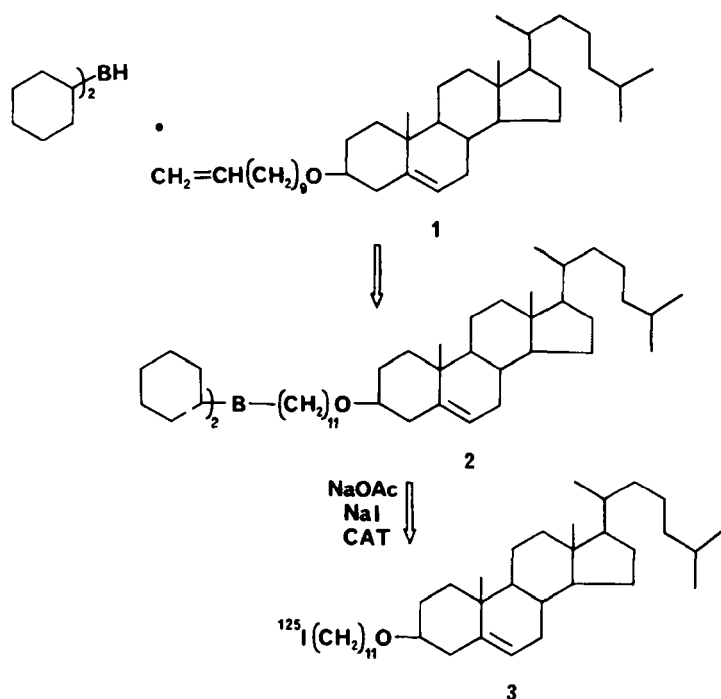


Figure I

EXPERIMENTAL

Melting points are uncorrected. NMR spectra were recorded on a JEOL-FX90Q spectrometer and referenced to Me_4Si . Radioactivity was determined by scintillation counting on a Beckman Biogamma II counter. Air and moisture sensitive borane solutions were transferred using oven-dried hypodermic syringes. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, Tennessee.

Reagents.

Sodium iodide-125 (Amersham and ICN) was diluted with aqueous sodium iodide (1M) to achieve a specific activity of 2 mCi/mole. THF was dried (CaH_2) and distilled over LiAlH_4 and stored under nitrogen. $\text{BH}_3 \cdot \text{THF}$ was prepared according to published procedure(9). Dicyclohexylborane was prepared via the hydroboration of cyclohexene with $\text{BH}_3 \cdot \text{THF}$ (10). 10-Undecen-1-ol (Aldrich) was used as received.

Chemical and Radiochemical Purity.

The chemical and radiochemical purity of the products were determined by thin layer chromatography using silica gel plates (250 μ , Whatman K-5). An authentic sample of the unlabelled product was applied adjacent to the reaction mixture. The radioactivity was associated with a spot having an R_f value identical to that of the unlabelled material.

ω -Undecylenyl Tosylate.

10-Undecen-1-ol (5.72 g, 33.6 mmol) was placed in a 100 mL flask containing chloroform (50 mL) and then cooled to 0°C. Pyridine (10.68 mL, 10.44 g, 132 mmol) was then added to the flask followed by addition of *p*-toluenesulfonyl chloride (13.0 g, 68 mmol) in five portions. The progress of the reaction was monitored by TLC (4% ether/petroleum ether; product R_f = 0.3). After 8 hrs, the chloroform solution was successively washed with aqueous 5% NaHCO_3 , 2N HCl and water. The chloroform solution was then dried over MgSO_4 and solvent removed under reduced pressure. The crude tosylate (13.96 g) was purified by column chromatography on a silica gel column (90 g, 60 cm x 2.7 cm). The product was obtained as a thick liquid (80% benzene/petroleum ether eluant) in nearly quantitative yields. ^1H nmr (CDCl_3): δ 2.15-1.23(m,16H, CH_2), 2.44(s,3H, CH_3), 4.02(t,2H,-OCH $_2$), 6.05-5.6(m,1H,CH), 7.8-7.3(m,4H,Ar-H). ^{13}C -NMR (CDCl_3): δ 139.05 and 114.13(olefinic carbons), 70.65(-OCH $_2$). Anal. Cald. for $\text{C}_{18}\text{H}_{28}\text{O}_3\text{S}$: C,66.66; H,8.64; S,9.88. Found: C,66.69; H,8.94; S,10.14.

ω -Undecenyl Cholesterol Ether (1).

Potassium metal (0.82 g, 21 mmol) and dry benzene (50 mL) were added to a flame-dried, nitrogen flushed, 250 mL round-bottomed flask fitted with

a condenser, magnetic stirring bar and a septum-capped side arm. The mixture was heated to 95°C in an oil bath for 30 min. Cholesterol (10.44 g, 27 mmol) was then added to the fine suspension of potassium in benzene followed by addition of additional dry benzene (20 mL). The reaction mixture was refluxed overnight with constant stirring. Undecylenyl tosylate (7.45 g, 23 mmol) in dry benzene (15 mL) was then added to the flask followed by additional benzene (25 mL). The reaction mixture was refluxed for 48 hrs under a nitrogen atmosphere. [The progress of the reaction was monitored by tlc (solvent 4% ether/petroleum ether)]. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (75 g, 45 x 2.75 cm). Pure undecylenyl cholesteryl ether 8.6 g, 76% yield was obtained using 5% ether/petroleum ether as eluant. The product crystallized as needles, mp 60°C (ethanol). $^1\text{H-NMR}$ (CDCl_3): δ 0.68, 0.84, 0.90, 0.95, 1.0 (s, 5 x CH_3), 2.4-1.1 (m, 28H, alkyl-H), 3.12 (pentuplet, 1H, $-\text{OCH}$), 3.45 (t, 2H, $-\text{OCH}_2$), 5.07-4.87 (m, 2H, $=\text{CH}_2$), 5.35 (bs, 1H, cyclic olefin), 6.05-5.6 (m, 1H, $=\text{CH}$). $^{13}\text{C-NMR}$ (CDCl_3): δ 141.27, 139.29, 121.4 and 114.20 (olefinic carbons), 79.05 ($-\text{OCH}$), and 68.27 ($-\text{OCH}_2$). Anal. Calcd. for $\text{C}_{38}\text{H}_{66}\text{O}$: C, 84.76; H, 12.27. Found: C, 84.47; H, 12.25.

Hydroboration of ω -Undecenyl Cholesteryl Ether with Dicyclohexylborane.

Cyclohexene (2.2 mmol, 0.22 mL) and dry THF (2 mL) were added to a nitrogen flushed, flame-dried flask (25 mL) fitted with a septum-capped side arm and a magnetic stirring bar. The solution was cooled to 0°C and $\text{BH}_3 \cdot \text{THF}$ (1.1 mmol, 0.42 mL of 2.6 M soln. in THF) was added. The reaction mixture was stirred at 0°C for 2 hr (a white precipitate appeared.) ω -Undecenyl cholesteryl ether (1 mmol, 0.54 g) and THF (5 mL) were then added, the reaction allowed to reach room temperature and stirred for another 4 hrs.

Synthesis of ω -Iodoundecyl Cholesterol Ether (3).

The dicyclohexylborane derivative, (2), (0.7 mmol, 7 mL of 0.1 M soln. in THF) was placed in a flame-dried, nitrogen flushed round-bottomed flask

fitted with a septum-capped side arm. The apparatus was shielded from light. Sodium acetate (0.7 mmol, 0.7 mL of 1M methanolic soln.) was added to the flask followed by sodium iodide (0.7 mmol, 0.7 mL of 1 M aqueous solution and chloramine-T (CAT, 0.7 mmol, 1.4 mL of 0.5 M methanolic solution). The mixture was stirred at room temperature until the color faded (~ 10 min.) and then diluted with 15 mL of water. The product was extracted into ether, the solution dried over MgSO_4 , and the solvent removed. The product was purified by chromatography on silica gel using petroleum ether as eluant. The product crystallized as needles from acetone, 0.34 g (72%), mp 75–76°C. $^1\text{H-NMR}$ ($\text{CDCl}_3\text{-CD}_3\text{COCD}_3$): δ 0.71, 0.84, 0.91, 0.95 and 1.02(s, 5xCH₃), 1.31, 1.81, 2.11 and 2.27(m, 28H, alkyl-H), 3.21(t, 2H, CH₂-I), 3.43(t, 2H, -OCH₂) and 5.34(bs, 1H, O-CH). $^{13}\text{C-NMR}$ ($\text{CDCl}_3\text{-CD}_3\text{COCD}_3$): δ 141.68 and 122.02 (olefinic carbons), 79.6(OCH), 68.63(OCH₂) and 7.63(CH₂I). Anal. Calcd. for $\text{C}_{38}\text{H}_{67}\text{OI}$: C, 68.47; H, 10.06. Found: C, 68.60; H, 10.24.

Synthesis of ω -[^{125}I]Iodoundecyl Cholesteryl Ether (3).

The dicyclohexylborane derivative, 2, (10 mL, 1 mmol, 0.1 M solution in THF) was placed in a nitrogen flushed, dried, septum-capped side arm fitted round-bottomed flask at room temperature. Sodium acetate (1 mL, 1 mmol, 1M methanolic solution), sodium iodide [2 μCi , 1 mmol, 1 mL of a 1M aqueous solution and chloramine-T (2 mL, 1 mmol of a 0.5 M methanolic solution) were added sequentially. The reaction mixture was stirred at room temperature until the color faded (~ 10 min.) and then diluted with 15 mL of water. The product was extracted into ether, the solution dried over MgSO_4 , and the solvent removed under reduced pressure. The product was purified by chromatography on silica gel using petroleum ether (40–60°C) as eluant (0.35 g, 52.6% yield); R_f 0.65 in hexane-ether-methanol-acetic acid (70:30:5:1) agreed with the cold iodide prepared earlier. The radio-chemical yield was 50%.

Synthesis of No-Carried-Added ω -[^{125}I]Iodoundecyl Cholesteryl Ether (3).

The dicyclohexylborane derivative, (2), (5 μmole , 50 μL of a 0.1 M solution in THF) was placed in a small vial (Wheaton) with a small magnetic stirring

bar. Sodium acetate (50 μ L of a 1 M methanol solution), Na¹²⁵I (2 μ L, 2 mCi) and Chloramine-T (75 μ L) were added sequentially. The product was extracted into ether and placed on preparative TLC plate. After development in hexane-ether-methanol-acetic acid (70:30:5:1), the radioactive band corresponding to the product was scraped and then extracted with hexane-ether-methanol (70:30:5). The radiochemical yield was 20%.

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