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An improved synthesis of [24-^{14}C]cholic acid, and its application to the synthesis of [^{14}C]SCH 209702 (Syn3). Synthesis of [$^{2}H_8$]SCH 209702

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An efficient synthesis of [24-^{14}C]cholic acid from potassium [^{14}C]cyanide has been developed. The key intermediate, 23-chloro-3α, 7α, 12α-triformyloxynorcholane, was synthesized by degradation of triformyl protected cholic acid. Different degradation conditions were explored. The synthesis of [^{14}C]SCH 209702 from 23-chloro-3α, 7α, 12α-triformyloxynorcholane and potassium [^{14}C]cyanide is described. The synthesis of [$^{2}H_8$]SCH 209702 from [$^{2}H_4$]cholic acid is also presented.

Keywords: [24-^{14}C]cholic acid; bile acid; SCH 209702; Syn3; carbon-14; synthesis

Introduction

SCH 209702 (Syn3) is a synthetic molecule that enhances transfer of therapeutic agents, such as nucleic acids, into cells.[1–4] [^{14}C]SCH 209702 was prepared to support drug disposition studies. [$^{2}H_8$]SCH 209702 was synthesized for use as an internal standard in a bioanalytical liquid chromatography/tandem mass spectrometry (LC/MS/MS) method. Syn3 consists of a disaccharides core linked to two cholic acid molecules via a polyamine tether. We labeled it as cholic acid.

Cholic acid is a major bile acid and synthetic methods for cholic acid may apply to other bile acid syntheses. The synthesis of bile acids labeled with the stable isotopes carbon-13 and deuterium at different positions has been reported.[5–11] However, the synthetic methods for incorporating radioactive isotopes into bile acids are less developed. Radominska-Pyrek and co-workers reported the synthesis of tritium labeled 3-monohydroxylated bile acids with varying side chain lengths by reducing appropriate unsaturated intermediates with sodium [^{3}H]borohydride.[12] In 1954, Bergstrom and co-workers synthesized carbon-13 and carbon-14 labeled cholanic, lithocholic, deoxycholic, chenodeoxycholic, and cholic acids by reacting labeled potassium cyanide with the norbromides.[13] The norbromides were prepared by bromine degradation of the silver salts of the corresponding bile acids (Hunsdiecker reaction).[14] The silver salts must be pure and thoroughly dry, which is not easy to achieve as the silver salt is often heat sensitive. Numerous modifications have been introduced to simplify the Hunsdiecker reaction. An improved [^{14}C]cholic acid synthesis, and its use in synthesis of [^{14}C]SCH 209702, is reported here.

Results and discussion

Knapp synthesized 3α-acetoxy-24-nor-23-bromo-5β-cholane, 3β-acetoxy-24-nor-23-bromo-5α-cholane, and 3β-acetoxy-20-bromo-5α-pregnane with 20–50% yield by bromine degradation of the mercury salts of the corresponding bile acids (Cristol-Firth modified Hunsdiecker reaction).[15] Our first attempt to make norbromide from cholic acid by using this method (Scheme 1) was not successful; no product was obtained.

Tserng and Klein synthesized 24-^{13}C-labeled cholic acid and other bile acids by using formyl derivatives of norbile acids that were made using a modified lead tetraacetate procedure.[7] We adopted this method and 23-chloro-3α, 7α, 12α-triformyloxynorcholane was synthesized with good yield (Scheme 2).

[^{14}C]Cholic acid was synthesized from carbon-14 labeled potassium cyanide and formylated norchloride **4** with a moderate yield (Scheme 3).

[^{14}C]SCH 209702 (Syn3) was prepared from [^{14}C]cholic acid with compound **7** using standard amide coupling condition (Scheme 4).

The coupling reaction was sluggish and gave crude product of [^{14}C]SCH 209702 with ~45% radiochemical purity (RCP) by thin-layer chromatography (TLC). The purification was problematic. An attempt to purify [^{14}C]SCH 209702 by flash chromatography on silica gel (eluting with 0–100% CH_3OH/CH_2Cl_2 gradient) was not successful; the compound was lost on the silica gel. This was probably because the ratio of silica gel to compound was too high. Partial purification on a size exclusion gel, Sephadex LH-20, and followed by preparative high-performance liquid chromatography (HPLC) (twice) gave pure [^{14}C]SCH 209702 with low yield.

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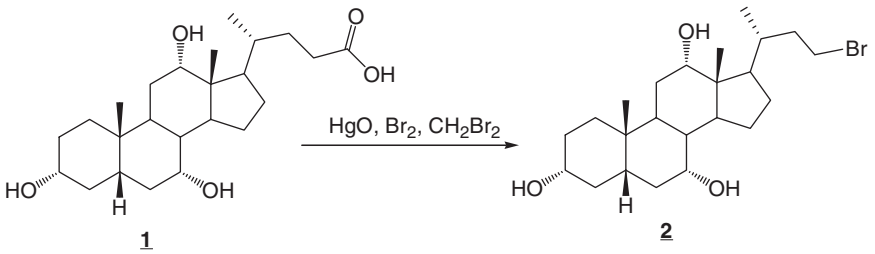
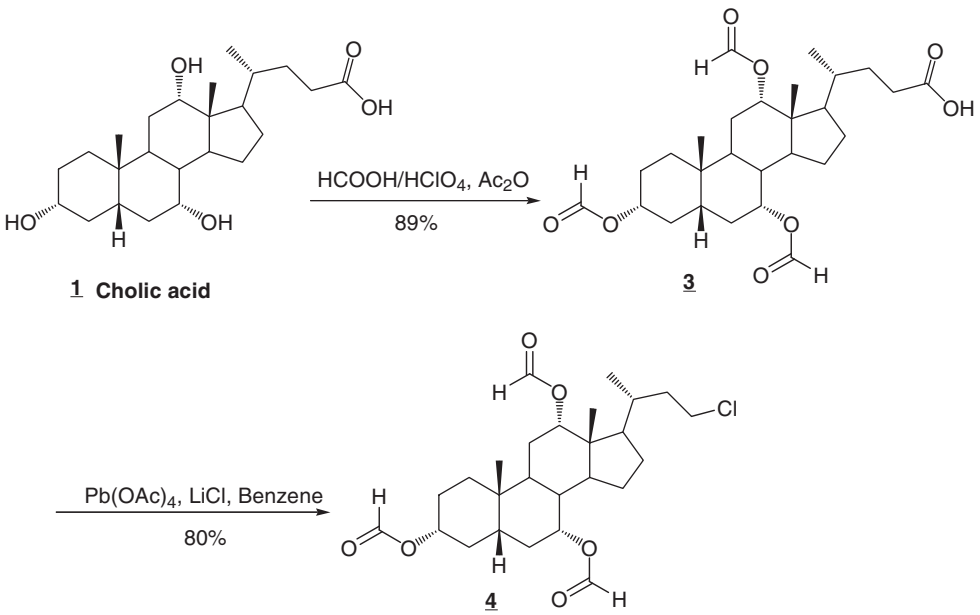


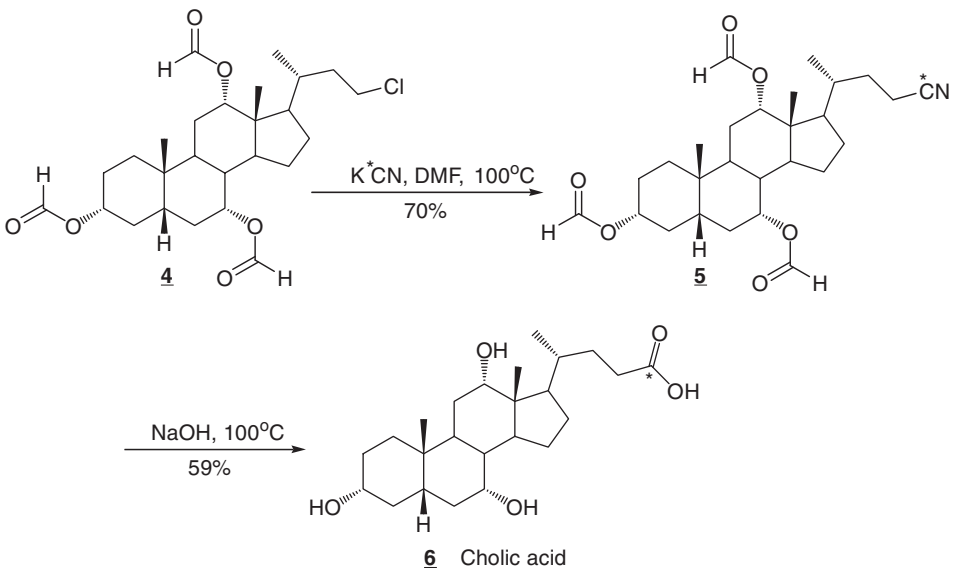
1 2

Scheme 1. Synthesis of the Norbromide



1 Cholic acid **3** **4**

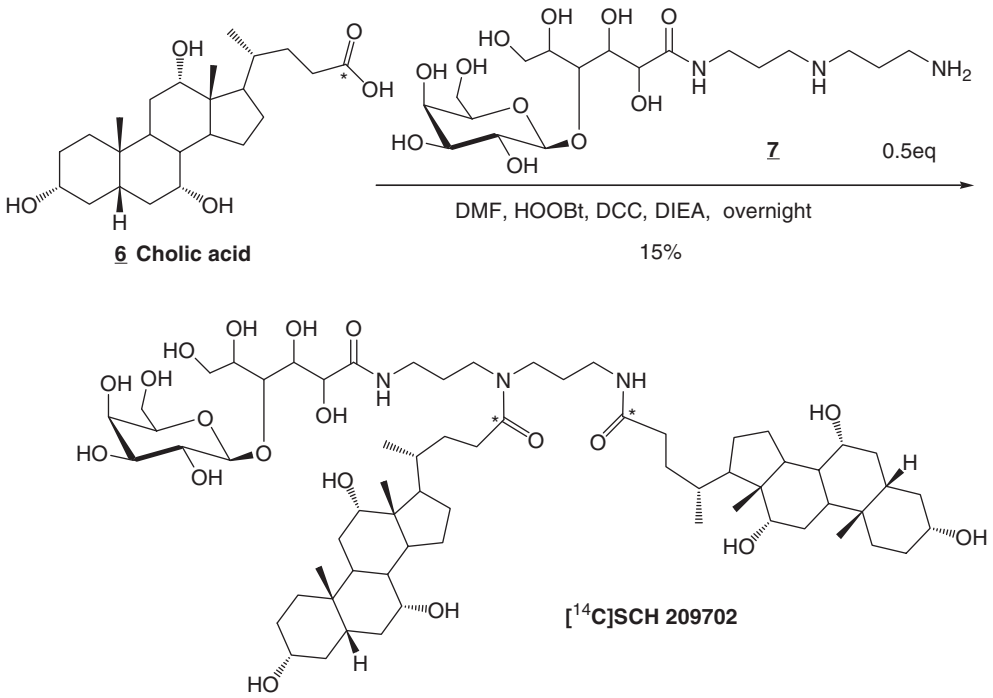
Scheme 2. Synthesis of 23-Chloro-3α,7α,12α-triformyloxynorcholane



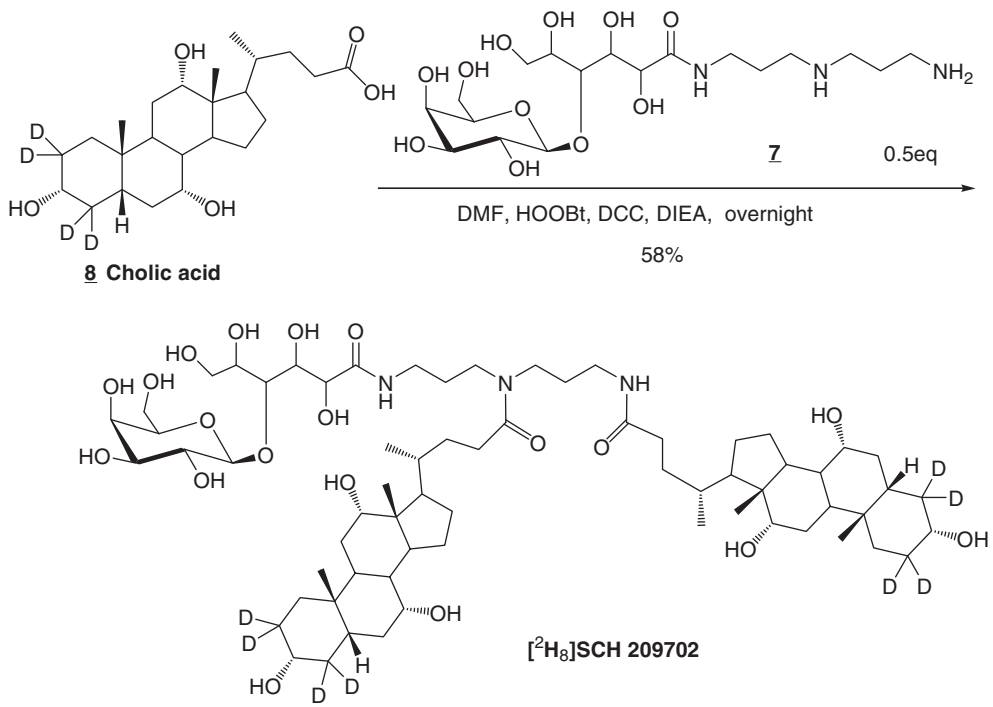
4 **5** **6** Cholic acid

Scheme 3. Synthesis of [^{14}C]cholic acid

[$^{2}H_8$]SCH 209702, needed as an internal standard for an high-performance liquid chromatographyl tandem mass spectrometry (HPLC/MS/MS) bioanalytical method, was synthesized by coupling compound **7** with commercially available [$^{2}H_4$]cholic acid (Scheme 5). It was found that the compound could be partially purified by flash chromatography on silica gel if the ratio of silica gel to compound was low. The compound was further purified by recrystallization from ethanol/acetone. The cleaner reaction and easier purification of [$^{2}H_8$]SCH 209702 gave much higher yield than [^{14}C]SCH 209702.



Scheme 4. Synthesis of [^{14}C]SCH 209702 (Syn3)



Scheme 5. Synthesis of [$^{2}H_{8}$]SCH 209702

Experimental

General

Potassium [^{14}C]cyanide was purchased from Amersham Biosciences. Compound **7** was obtained from Schering-Plough Research Institute, Chemical Development. [$^{2}H_{4}$]cholic acid was purchased from C/D/N Isotopes Inc. 23-chloro-3α, 7α, 12α-triformyloxynorcholane was prepared according to the procedure given in the literature.[7] All remaining reagents and solvents were purchased from Aldrich and used as received. Radioactivity measurements were performed on a Packard 2200CA liquid scintillation analyzer using Scintiverse BD as liquid scintillation cocktail. TLC was performed with Whatman LK6DF (silica gel 60) 5 × 20 cm, 0.25 mm plates. The plates were scanned on a Bioscan 1000 linear analyzer. Mass Spectra were acquired on the

JEOL MStation magnetic sector mass spectrometer operating in the fast atom bombardment (FAB) ionization mode. Proton nuclear magnetic resonance (^{1}H-NMR) (500 MHz) spectra was obtained on a Varian spectrometer.

[^{14}C]SCH 209702 was purified by HPLC systems 1 and 2. HPLC was conducted on a Waters Delta Prep 4000 system with Waters 486 tunable absorbance detector. [^{14}C]SCH 209702 and [$^2H_8$]SCH 209702 were analyzed by HPLC system 5. HPLC was conducted on a Waters 600 multisolvent delivery system with Waters 2487 dual channel absorbance detector. RCP was determined by a Radiomatic 525TR radioflow detector with Flo-Scint III liquid scintillation cocktail. The following systems were used:

System 1: Zorbax SB C18, 250 mm × 9.4 mm, 215 nm; 0.03% trifluoroacetic acid (TFA) in CH_3CN: 0.03% TFA in water (40:60) for 20 min followed by a step gradient to 0.03% TFA in CH_3CN, 4.0 ml/min.

System 2: Zorbax SB C18, 250 mm × 9.4 mm, 215 nm; 0.05 M triethylammonium acetate (TEAA) (pH 4.0): CH_3OH (15:85) for 30 min, 5.0 ml/min.

System 3: Agilent SB C18, 100 mm × 3.0 mm, 210 nm; 0.05 M TEAA (pH 4.0): CH_3OH (20:80) for 20 min followed by a step gradient to methanol, 0.5 ml/min.

System 4: Advantage Armour C18, 150 mm × 3.0 mm, 280 nm; methanol: 0.05 M TEAA (pH 4.0) (80:20) isocratic for 15 min followed by a step gradient to methanol, 0.8 ml/min.

System 5: Agilent Extend C18, 150 mm × 3.0 mm, 220 nm; CH_3OH: 0.05 M NH_4OH (pH 9.0) (80:20), 0.5 ml/min.

Synthesis of 23-[^{14}C]-cyano-3α, 7α, 12α-triformyloxynorcholane (5)

23-Chloro-3α, 7α, 12α-triformyloxynorcholane **4** (824 mg, 1.70 mmol) and potassium [^{14}C]cyanide (88.5 mCi, 1.61 mmol) were suspended in anhydrous dimethylformamide (DMF) (7 ml). The reaction was heated to 100°C and stirred under nitrogen for 3 h, cooled to room temperature, and quenched with water (28 ml). The resulting brown precipitate was filtered, washed with water (2 × 5 ml), and dried under vacuum to give 62.0 mCi (70%) of compound **5** with 87% RCP. Radio-TLC: R_f (acetone:-benzene = 5:95) 0.49. The crude product was directly used in the next step without further purification.

Synthesis of [^{14}C]cholic acid (6)

NaOH (7 ml, 10% wt/wt) was added to a solution of 23-[^{14}C]-cyano-3α, 7α, 12α-triformyloxynorcholane **5** (62.0 mCi) in ethanol (7 ml) and refluxed under nitrogen for 48 h. The mixture was cooled to room temperature, diluted with water (35 ml), and washed with Et_2O (2 × 40 ml). The aqueous phase was acidified to pH 2 with HCl (2.0 M) and extracted with EtOAc (4 × 40 ml). The combined organics was concentrated *in vacuo* and purified by silica flash chromatography (gradient 1–30% CH_3OH, 1% HOAc/CH_2Cl_2) to give 36.6 mCi (59%) of 100% radiochemically pure product **6** as light yellow solid. Radio TLC: R_f (benzene:-dioxane:HOAc = 10:5:1) 0.45.

Synthesis of [^{14}C]SCH 209702 (Syn3)

To a solution of [^{14}C]cholic acid (29.9 mCi, 0.454 mmol) in anhydrous DMF (4.0 ml), *N*-3-hydroxy-1, 2, 3-benzotriazin-4(3 H)-one (110 mg, 0.674 mmol), *N*, *N*-diisopropylethylamine (110 µl, 0.611 mmol), and dicyclohexylcarbodiimide (126 mg, 0.611 mmol) were added. The reaction was stirred at room temperature for 15 min and compound **7** (115 mg, 0.244 mmol) was added rapidly (compound **7** is very hygroscopic). The mixture was warmed to 50°C, stirred under nitrogen for 4 h, cooled to room temperature, and stirred overnight. The reaction was quenched with water (18 ml), concentrated *in vacuo*, and the crude product was initially purified by Sephadex LH-20 column (CH_3OH:CH_2Cl_2 = 50:50). The product was further purified by preparative HPLC twice (systems 1 and 2) to give 4.4 mCi (15%) of 99.5% radiochemically pure [^{14}C]SCH 209702 by HPLC systems 3 and 4. [^{14}C]SCH 209702 coeluted with the unlabeled SCH 209702 standard in the above HPLC systems. The specific activity of [^{14}C]SCH 209702 is 100.4 mCi/mmol. Fast atom bombardment-mass spectrometry (FAB-MS): *m/z* 1257 $(M+H)^+$, 1279 $(M+Na)^+$. Major fast atom bombardment-tandem mass spectrometry (FAB-MS/MS) fragments of the protonated molecular ion are consistent with the unlabeled standard.

Synthesis of [$^2H_8$]SCH 209702

To a solution of [$^2H_4$]cholic acid (385 mg, 0.933 mmol) in anhydrous DMF (8.0 ml), N-3-hydroxy-1, 2, 3-benzotriazin-4(3H)-one (173 mg, 1.06 mmol), *N*, *N*-diisopropylethylamine (200 µl, 1.15 mmol), and dicyclohexylcarbodiimide (219 mg, 1.06 mmol) were added. The reaction was stirred at room temperature for 15 min, and compound **7** (200 mg, 0.424 mmol) was added. Then the reaction was heated to 50°C, stirred under nitrogen for 4 h, cooled to room temperature, and stirred for an additional 22 h. The reaction was quenched with water (5.0 ml), concentrated *in vacuo*, and the crude product was initially purified by a 10 g silica gel SepPak column eluting with CH_3OH:CH_2Cl_2 (gradient methanol 5–50%). The resulting product was dissolved in ethanol (8 ml) at 60°C, and added dropwise to acetone (30 ml) under stirring. The solid was collected, washed with acetone (4 × 2 ml), and dried to constant weight under vacuum to give 310 mg (58%) of 99.9% pure [$^2H_8$]SCH 209702 as determined by HPLC system 5. FAB-MS: *m/z* 1261 $(M+H)^+$, 1283 $(M+Na)^+$. ^{1}H-NMR (500 MHz, dimethylsulfoxide-d6 (DMSO-d6)): δ7.81(m, 1H), 7.68 (m, 1H), 5.18 (d, 1H), 5.60 (d, 1H), 4.75 (m, 2H), 4.62 (m, 1H), 4.46 (m, 2H), 4.28 (d, 2H), 4.26 (d, 1H), 3.78 (s, 2H), 3.68 (s, 2H), 3.60 (s, 4H), 3.58 (m, 1H), 3.53 (m, 1H), 3.48 (t, 2H), 3.39 (t, 1H), 3.20 (m, 3H), 3.14 (d, 2H), 3.10 (dd, 1H), 3.02 (dd, 2H), 2.95 (dd, 1H), 2.30–1.10 (m, 49H), 0.90 (m, 10H), 0.78 (s, 6H), and 0.58 (s, 6H).

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