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An efficient laboratory-scale preparative method for [1-^{13}C]glycocholic acid

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A breath test using [1-^{13}C]glycocholic acid as a substrate is a potential diagnostic method for small intestinal bacterial overgrowth syndrome. [1-^{13}C]Glycocholic acid has been thus synthesized in an excellent yield from ethyl [1-^{13}C]glycinate hydrochloride in a one-pot reaction. This method is suitable for the preparation of the labeled compound on a laboratory scale, which helps to perform extensive clinical studies of the breath test.

Keywords: stable labeled synthesis; carbon-13; glycocholic acid; laboratory scale; one-pot reaction; breath test

Introduction

Small intestinal bacterial overgrowth (SIBO) occurs as a complication of a postgastrectomic state, chronic pancreatitis, liver cirrhosis, Crohn's disease, gastric achlorhydria, blind loop syndrome, small intestinal diverticulosis, and so on.[1] This syndrome causes abdominal pain, chronic diarrhea, nausea, anemia, abdominal bloating, and constipation. Although the gold standard for the diagnosis of SIBO is to culture aspirates from the small bowel, it is invasive, troublesome, and time-consuming. Fromm *et al*. and Sherr *et al*. have independently reported on a noninvasive diagnostic procedure for SIBO by using a [1-^{14}C]glycocholic acid breath test.[2] This method is based on the fact that the ingested [1-^{14}C] glycocholic acid can be deconjugated by the action of the intestinal bacteria to produce [1-^{14}C]glycine, which is rapidly absorbed and converted into $^{14}CO_2$ and then excreted in exhaled breath. The serious drawback of this method is that the long half-life of radioactive ^{14}C makes it undesirable for use in infants, children, and pregnant women.

To solve this problem associated with the use of ^{14}C, a breath test using [1-^{13}C] or [1,2-$^{13}C_2$]glycocholic acid was reported by Solomons *et al*.,[3] where the labeled compounds (250–600 mg) were ingested and carbon dioxide in exhaled breath was analyzed by mass spectrometer. Recently, breath tests using ^{13}C-labeled substrates, which are noninvasive and convenient, and applicable to a wide range of clinical diagnostic procedures were widely employed.[4] These tests are based on $^{13}CO_2/^{12}CO_2$ ratio analysis in exhaled breath, using a mass spectrometer or infrared spectrometer. Compact, easily accessible, and inexpensive devices for $^{13}CO_2/^{12}CO_2$ ratio analysis by infrared spectrometry are now available, which can contribute to the utilization promotion of the breath tests.

In the previous report by Solomons *et al*., the labeled compounds were prepared according to the method reported by Lack *et al*.,[5] where ^{13}C-labeled ethyl glycinate and cholic acid were condensed using *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as a coupling reagent. Although the EEDQ method was subsequently improved by Tserng *et al*.,[6] it is still time-consuming and requires a complicated reaction work-up. Thus, the EEDQ method is insufficient to prepare large amounts of the labeled compound for clinical studies. In this paper, we present a more efficient method suitable for laboratory-scale preparation of [1-^{13}C]glycocholic acid.

Results and discussion

At first, we tried to prepare [1-^{13}C]glycocholic acid by using Tserng's procedure. Ethyl [1-^{13}C]glycinate hydrochloride was prepared quantitatively from [1-^{13}C]glycine by treatment with a solution of two equivalent moles of hydrogen chloride in ethanol–ethyl acetate followed by concentrating under reduced pressure. The hydrogen chloride solution was prepared by the addition of acetyl chloride into ethanol at room temperature. In our experiments using Tserng's method, a gummy material appeared during the reaction work-up, and it interfered with the crystallization of the product. This result made it difficult to obtain the product in a constant yield, unfortunately. This problem seemed to be due to the low solubility of the reagents and/or reaction products in ethyl acetate. 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM) is an effective reagent for the condensation of carboxylic acids with amines to give the corresponding amide, and it can work in alcoholic solvents.[7] When ethyl [1-^{13}C]glycinate hydrochloride (1.0 eq.), cholic acid (1.0 eq.), and DMT-MM (1.1 eq.) were simply mixed in ethanol in the presence of potassium carbonate

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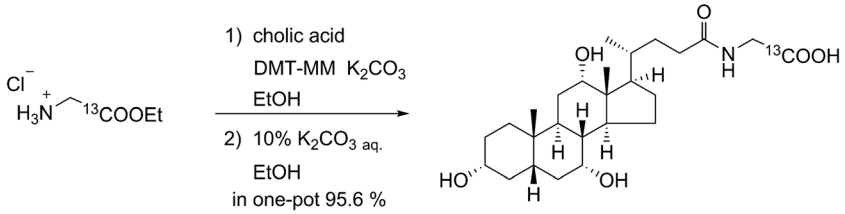

Scheme 1. Preparation of [1-^{13}C]glycocholic acid.

(1.1 eq.), the reaction smoothly proceeded at room temperature. The resulting reaction mixture was directly subjected to hydrolysis by potassium carbonate to constantly give [1-^{13}C]glycocholic acid in an excellent yield (Scheme 1).

The present simple and convenient method using a one-pot reaction enabled the laboratory-scale preparation of pure [1-^{13}C] glycocholic acid with a minimal loss of relatively high cost of [1-^{13}C]glycine, which contributes to clinical studies of the breath test for the diagnosing of SIBO.

Experimental

General

[1-^{13}C]glycine (enrichment specification 99%) was purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA) and used as received. The DMT-MM was prepared from 2-chloro-4,6-dimethoxy-1,3,5-triazine and 4-methylmorpholine according to the method of Kunishima *et al.*[8] Cholic acid, 2-chloro-4,6-dimethoxy-1,3,5-triazine and 4-methylmorpholine were purchased from Tokyo Chemical Industry Co., Ltd.(Tokyo, Japan) and was used as received. Other reagents and solvents were obtained from Wako Pure Chemical Industries, Ltd.(Osaka, Japan).

Melting point was measured with a Yanako MP-J3 melting point apparatus and was uncorrected. Infrared (IR) spectrum was recorded with a JASCO FT-IR-4100 type A spectrometer. ^{1}H and ^{13}C NMR spectra were taken with a Bruker BioSpin AVANCE 500 spectrometer. Chemical shifts were given on δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). High-resolution electrospray ionization mass spectrum (ESIMS) was obtained with a Bruker Daltonics micro TOF-Q spectrometer.

[1-^{13}C]Glycocholic acid

To a solution of ethyl [1-^{13}C]glycinate hydrochloride (4.22 g, 30.0 mmol) in ethanol (150 mL) were added cholic acid (12.3 g, 30.0 mmol), potassium carbonate (4.56 g, 33.0 mmol), and DMT-MM (9.13 g, 33.0 mmol), followed by stirring at room temperature for 4 h. To the reaction mixture was added a 10% aqueous solution of potassium carbonate (150 mL) and refluxed for 15 min. The resulting clear solution was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in water (200 mL) and acidified with 1.0 mol/L hydrochloric acid. The resulting crystalline precipitate was collected and washed with water, followed by drying under reduced pressure to obtain crude crystals. The crude crystals were recrystallized from ethanol–ethyl acetate to afford [1-^{13}C]glycocholic acid (13.4 g, 95.6% yield): colorless needles (mp 166–7°C); IR (KBr) υ_{max} 3407, 2935, 1655 cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.43 (s, 1H, COOH), 8.07 (t, J = 5.8, 1H, NH), 4.30 (d, J = 3.6, 1H, OH), 4.08 (d, J = 3.3, 1H, OH), 3.98 (d, J = 3.3, 1H, OH), 3.78 (br s, 1H), 3.71 (t, J = 5.8, 2H), 3.61 (br s, 1H), 3.19 (m, 1H), 2.25–2.10 (m, 3H), 2.00 (m, 2H), 1.83–1.60 (m, 6H), 1.50–1.13 (m, 11H), 0.97 (m, 1H), 0.93 (d, J = 6.5, 3H), 0.84 (m, 1H), 0.81 (s, 3H), 0.59 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ 172.9, 171.4 (strong), 70.9, 70.3, 66.2, 46.1, 45.7, 41.4, 41.3, 40.7, 40.2, 35.2, 35.0, 34.8, 34.3, 32.1, 31.5, 30.3, 28.5, 27.2, 26.1, 22.7, 22.5, 17.0, 12.3; ESIMS (negative mode) *m/z* 465.3032 (calcd for $C_{25}^{13}CH_{42}NO_6^-$, 465.3051).

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Conflict of Interest

The authors did not report any conflict of interest.

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