SUPPLEMENTAL DATA

Synthesis of unlabeled cholylsarcosine (CSar) for use as reference material

Chemicals and materials

Cholic acid, sarcosine methyl ester hydrochloride, diethyl cyanophosphonate (DEPC), triethylamine (Et_3N), dimethylformamide (DMF; dry) were obtained from Sigma-Aldrich Ltd. and used without further purification. Dichloromethane (DCM), ethylacetate (EtOAc), hexane, methanol (MeOH) were obtained as analytical grade from VWR International Ltd. Flash chromatography was carried out using Merck silica gel 60 (230-400 mesh). Analytical thin layer chromatography (TLC) was performed using *pre*-coated aluminium-backed plates (Merck Kieselgel 60 F254) and visualized with 5% aqueous H_2SO_4 under heating.

Coupling of sarcosine methyl ester to cholic acid to give CSar methyl ester

A solution of cholic acid (2.1 g; 5.1 mmol) and sarcosine methyl ester hydrochloride (0.7 g; 5.0 mmol) in dry DMF (35 mL) was cooled to 0 ^{o}C, and DEPC (0.83 mL; 5.5 mmol) was added. Dry Et_3N (3.5 mL; 25 mmol) was then added dropwise over 10 min, and the mixture was stirred under argon for 45 min at 0 ^{o}C, then overnight at room temperature. Full conversion of cholic acid was observed by TLC (10% methanol in DCM; R_f = 0.25). The precipitated salts were filtered off and the filtrate was concentrated by reduced pressure to give a yellow oil. The crude product was purified by silica gel chromatography (eluent: 8% EtOAc in hexane) to give CSar methyl ester as a white solid (0.97 g; 39%). ^{1}H NMR (Varian AS 400 running at 400 MHz, $CDCl_3$, 25 ^{o}C, 8 mg/mL, chemical shifts (δ) are reported relative to residual signals of $CDCl_3$ (δ = 7.26)). Two sets of resonances (rotamers) were observed in a 4:1 ratio. When resolved, δ of the minor resonance are given in square brackets: δ 4.12 (s, 2H) [4.06 (d, *J* = 3.9 Hz, 2H)], 3.97 (m, 1H), 3.84 (m, 1H), [3.78

(s, 3H)] 3.72 (s, 3H), 3.44 (m, 1H), 3.08 (s, 3H) [2.96 (s, 3H)], 2.41 (m, 1H), 2.17-2.23 (m, 5H), 1.25-1.91 (m, 20H), 1.10 (m, 1H), 1.01 (d, *J* = 6.1 Hz, 3H), 0.88 (s, 3H), 0.68 (s, 3H) ppm.

Hydrolysis of CSar methyl ester to give CSar

To the CSar methyl ester (50 mg; 0.10 mmol) was added methanolic KOH (5% w/w; 2 mL) and the solution was stirred in a closed vial at 70 ^{o}C for 30 min. Full conversion of the methyl ester was observed by TLC (25% methanol in DCM; R_f = 0.05). The solvent was evaporated under a stream of N_2 gas to give a clear oil. The oil was re-dissolved in water (5 mL) and the solution was acidified with 5% aq. H_2SO_4 under stirring. The acidic opaque solution was left at room temperature to allow for a white precipitate to form. After filtration and drying under vacuum, pure unlabeled CSar was obtained as a white solid (27 mg; 56%). ^{1}H NMR (Varian AS 400 running at 400 MHz, CD_3OD, 25 ^{o}C, 7 mg/mL, chemical shifts (δ) are reported relative to residual signals of CD_3OD (δ = 3.31)). Two sets of resonances (rotamers) were observed in a 2.5:1 ratio. When resolved, δ of the minor resonance are given in square brackets: δ [4.18 (s, 2H)] 4.09 (s, 2H), 3.95 (m, 1H), 3.80 (m, 1H), 3.35 (m 1H), 3.12 (s, 3H) [2.94 (s, 3H)], 2.49 (m, 1H), 2.22-2.35 (m, 4H), 1.72-2.01 (m, 8H), 1.30-1.71 (m, 12H), 1.14 (m, 1H), 1.06 (d, *J* = 6.5 Hz, 3H) [1.01 (d, *J* = 6.4 Hz, 3H)], 0.97 (m, 1H), 0.92 (s, 3H), 0.72 (d, *J* = 4.8 Hz, 3H) ppm. ESI-MS (Bruker Daltonics HCT Plus (ion trap), negative ionization mode, capillary voltage +4.5 kV): 478.3 m/z $[M-H]^-$.

Optimization of reaction conditions for the radiosynthesis of [*N*-methyl-^{11}C]cholylsarcosine (^{11}C-CSar)

In the presented three-step radiosynthesis of [*N*-methyl-^{11}C]cholylsarcosine (^{11}C-CSar), the first two steps (i.e. methylation and coupling) were performed as a one-pot procedure in DMSO at 60 ^{o}C with PMP as the auxiliary base and DEPC as the coupling reagent. Under these conditions, the reaction proceeded to give ^{11}C-CSar methyl ester with a radiochemical yield (RCY) of 20%. This yield was determined by analytical HPLC (retention time of ^{11}C-CSar methyl ester: 4.6 min) of the crude reaction mixture using the Luna® column with 40% acetonitrile in aqueous NaH_2PO_4 (70 mM) as eluent (isocratic, 2.5 mL/min) and obtained after optimization of reaction parameters including solvent, concentration, auxiliary base, temperature and reaction time as described below:

Solvent.

A screening of solvents revealed that DMF provided a similar RCY as DMSO (19%), while MeCN failed to provide any ^{11}C-CSar methyl ester presumably due to the low solubilities of the glycine methyl ester hydrochloride and CA in this solvent.

Concentrations.

Changes in the ratios of the reagents were not observed to have any significant effect on the RCY.

Auxiliary base.

As an auxiliary base, TMP performed similar (20% RCY) to PMP, while Et_3N resulted in a slightly lower RCY (15%). Addition of PMP in two portions (1.5 μL for the methylation and 3.5 μL for the coupling) rather than one (5 μL) did not improve the RCY.

Reaction temperature.

A lower RCY was observed when the reaction was carried out at room temperature (6%) rather than at 60 ^{o}C. However, no improvement in RCY was observed when the reaction was performed at 80 ^{o}C and at higher temperature significant decomposition of reagents was observed.

Reaction time.

Reaction times (up to 20 min) for the methylation or coupling did not improve the overall RCY of the two-step one-pot preparation of the ^{11}C-CSar methyl ester.

Our choice of using DEPC was based on previously reported syntheses of bile acids conjugated (*1,2*). No other coupling reagents than DEPC were tested in this study.

The final deprotective hydrolysis of the isolated ^{11}C-CSar methyl ester using aqueous NaOH (0.25 M) proceeded with full conversion at room temperature for 2 min to give ^{11}C-CSar.

References

1. Momose T, Tsubaki T, Iida T, Nambara T. An improved synthesis of taurine- and glycine-conjugated bile acids. *Lipids.* 1997;32:775-778.

2. Willemen Hendra M, Vermonden T, Marcelis Antonius TM, Sudhölter Ernst JR. *N*-Cholyl amino acid alkyl esters − A novel class of organogelators. *European J Org Chem.* 2001;2001:2329-2335.