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FACILE SYNTHESIS OF D,L-[^{34}S]CYSTEINE HYDROCHLORIDE

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SUMMARY

The title compound was synthesized at the gram level in 66% yield by addition of [^{34}S]thioacetic acid to α-acetamidoacrylic acid, followed by acid hydrolysis.

KEYWORDS: D,L-cysteine, ^{34}S labelling.

INTRODUCTION

During our investigation on the role of cysteine in food systems, we needed isotopically-stable labelled cysteine in gram amounts. The synthesis of L-[^{34}S]cysteine was recently reported (1), by reacting elemental sulfur-34 with benzylmagnesium chloride, then adding to L-β-chloroalanine to produce S-benzylcysteine and subsequently reducing with sodium in ammonia. Unfortunately, no chemical yield was given. A similar synthetic sequence was used in the synthesis of radioactive cystine (2), but the yield of the intermediate S-benzylcysteine was low. Since optically active cysteine was not necessarily required for our study, we decided to develop a more convenient, simple and high-yielding method for the synthesis of racemic [^{34}S]cysteine.

RESULTS AND DISCUSSION

The synthesis of [^{34}S]cysteine was carried out in a two-step procedure, by Michael addition of [^{34}S]thioacetic acid to α-acetamidoacrylic acid and subsequent hydrolysis with 20% HCl, in 66% overall yield. This approach was previously reported for the

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synthesis of unlabelled cystine (3,4), but with the use of a large excess of thioacetic acid. This problem was overcome by conducting the reaction in N,N-dimethylformamide at 90°C, and the yield of N,S-diacetylcysteine was 70%. Lower temperatures required longer reaction times, but at higher temperatures (100°C), an unidentifed compound was formed. Reaction under neat conditions at 90°C also produced N,S-diacetylcysteine, but the yield failed to be reproducible. To avoid any cystine formation, the acid hydrolysis step was carried out under an argon atmosphere, and the residual aqueous HCl of the crude product after evaporation was removed azeotropically with benzene. This facilitated the crystallization of the final compound from ethanol/ether.

The identity and the purity of synthesized cysteine were checked by elemental analysis and spectroscopic techniques (^{1}H-NMR, GC/MS). Analysis by GC/MS on a chiral column of the N,S-bis-pentafluoropropionyl isopropylester derivative of the synthetic [^{34}S]cysteine showed it to be a racemic mixture. The isotopic enrichment of sulfur-34 was the same as for the starting material.

EXPERIMENTAL

[^{34}S]Thioacetic acid (95 atom %) was purchased from ICON Services Inc., Summit, New Jersey (USA). The melting points were uncorrected. Proton NMR spectra were recorded on a Bruker AM360, narrow bore, spectrometer. GC/MS analysis was performed with a Hewlett-Packard HP 5995 instrument connected to a HP RTE-6 data system, equipped with an Alltech Ass. Chirasil-Val column (25 m, 0.25 mm ID). Elemental analysis was performed by G. Nein, Markgraflerstrasse 21, Basel (Switzerland).

N,S-Diacetyl[^{34}S]cysteine

A suspension of α-acetamidoacrylic acid (4.72 g, 36.56 mmol) and [^{34}S]thioacetic acid (2.85 g, 36.54 mmol) in anhydrous DMF (12 ml) was heated at 90°C. After 10 min., the solid went into solution and the heating was continued for further 12 h. The reaction mixture was evaporated under vacuum, and the solid residue was washed by triturating with 20 ml ether. Crystallization of the crude product from chloroform/ether yielded N,S-diacetyl[^{34}S]cysteine (5.27 g, 25.43 mmol, 70%); m.p. 116-118°C. ^{1}H-NMR (DMSO-d6) δ: 12.9 (broad, COOH), 8.29 (d, J 8.4, NH), 4.34 (dt, J 5 and 8.4, Hα), 3.34 (dd, J

5 and 13.6, Hβ), 3.01 (dd, J 8.4 and 13.6, Hβ), 2.33 (s, S-acetyl), 1.83 (s, N-acetyl).

Elemental analysis: Calc. for $C_7H_{11}NO_4{}^{34}S$ (MW 207.23): C 40.58; H 5.36; N 6.76; S 16.41. Found: C 40.70; H 5.49; N 6.85; S 16.17.

[^{34}S]Cysteine hydrochloride

All the operations were conducted under argon. A solution of N,S-diacetyl-[^{34}S]cysteine (3.80 g, 18.34 mmol) in deaerated 20% HCl (40 ml) was refluxed for 5 h. After cooling to room temperature, the reaction mixture was filtered and the fitrate was evaporated under vacuum. The oily residue was triturated with dry benzene (40 ml), which was removed under vacuum. This was repeated three times until a solid was obtained. Crystallization from ethanol/ether afforded [^{34}S]cysteine hydrochloride (2.72 g, 17.04 mmol, 93%); m.p. 139-141°C (dec.). ^{1}H-NMR (D_2O) δ: 4.29 (dd, J 4.3 and 5.6, Hα), 3.13 (ddd, J 4.3, 5.6 and 15.2, 2 Hβ). MS of the N,S-bis-pentafuoropropionyl isopropylester derivative: m/z (relative intensity) 457 (<1) $[M]^+$, 415 (1) $[M - C_3H_6]^+$, 398 (1) $[M - OC_3H_7]^+$, 370 (17) $[M - COOC_3H_7]^+$, 310 (2) $[M - COC_2F_5]^+$, 268 (5) $[M - COC_2F_5 - C_3H_6]^+$, 220 (6) $[M - CH_2{}^{34}SCOC_2F_5 - C_3H_6]^+$, 190 (48) $[M - {}^{34}SCOC_2F_5 - COOC_3H_6]^+$, 147 (5) $[COC_2F_5]^+$, 119 (22) $[C_2F_5]^+$, 105 (10) $[M - 2\ COC_2F_5 - OC_3H_6]^+$, 69 (8) $[CF_3]^+$, 43 (100) $[C_3H_7]^+$.
Elemental analysis: Calc. for $C_3H_8ClNO_2{}^{34}S$ (MW 159.62): C 22.58; H 5.05; Cl 22.22; N 8.78; S 21.31. Found: C 22.44; H 5.18; Cl 22.06; N 8.77; S 21.12.

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