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Working with Hazardous Chemicals

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These paragraphs were added in September 2014. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

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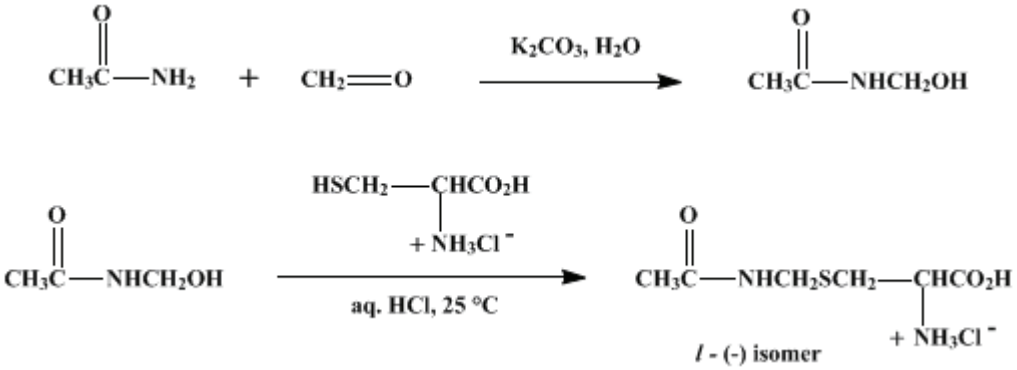
THIOL PROTECTION WITH THE ACETAMIDOMETHYL GROUP: *S*-ACETAMIDOMETHYL-L-CYSTEINE HYDROCHLORIDE

[L-Cysteine, *S*-[(acetylamino)methyl]-, monohydrochloride]



Submitted by John D. Milkowski[1], Daniel F. Veber[2], and Ralph Hirschmann[1].
Checked by A. Pearce and G. Büchi.

1. Procedure

A. N-*(Hydroxymethyl)acetamide*. In a 2-l., round-bottomed flask, 100 g. (1.70 moles) of acetamide (Note 1) is added to a solution of 10 g. (0.072 mole) of anhydrous potassium carbonate in 137 g. (1.7 moles) of an aqueous 36–38% solution of formaldehyde (Note 2). The mixture is swirled, heated on a steam bath for 3 minutes, and allowed to stand overnight at room temperature. Several pieces of crushed dry ice are added (Note 3), after which the mixture is evaporated under reduced pressure with a heating bath kept below 40° (Note 4). A 128-g. portion of anhydrous sodium sulfate is added to the remaining colorless oil, which may have some precipitated salt suspended in it. After several hours the oil is dissolved in 1 l. of acetone, the suspended drying agent and salts are filtered, and the filtrate (Note 5) is dried further with additional anhydrous sodium sulfate. The suspension is filtered, and the clear filtrate is evaporated under reduced pressure. The yield of *N*-(hydroxymethyl)acetamide, a colorless hygroscopic oil at this point, is 148–151 g. (98–100%) (Note 6). The oily product, which may solidify (Note 7) on standing for several days, is used directly in step B.

B. S-*Acetamidomethyl*-L-*cysteine hydrochloride*. A 1-l., round-bottomed flask is charged with 127 g. (1.43 moles) of *N*-(hydroxymethyl)acetamide, 228 g. (1.30 moles) of L-cysteine hydrochloride monohydrate (Note 8), and 350 ml. of water. The resulting solution is swirled and cooled in an ice bath as 50 ml. of concentrated hydrochloric acid is slowly added (Note 9). The flask is flushed with nitrogen, capped with a nitrogen-filled balloon, and allowed to stand for 1–2 days at room temperature. The progress of the reaction is monitored by TLC (Note 10). When L-cysteine hydrochloride is no longer detectable, the solution is evaporated under reduced pressure at a bath temperature of *ca.* 40°. The remaining solid is suspended in a small amount of absolute ethanol, and the mixture is again carefully evaporated to avoid bumping. This entrainment procedure with absolute ethanol is repeated several times to remove traces of water. The dry solid is dissolved in the minimum amount of methanol (Note 11), and anhydrous diethyl ether is added until the cloud point is reached. The cloudy solution is allowed to stand in a refrigerator at *ca.* 4–5° for 1 week, during which the crystalline mass is broken up several times. The white crystalline product is collected, washed with ether, and dried under reduced pressure, yielding 152–190 g. (51–64%) of *S*-acetamidomethyl-L-cysteine hydrochloride, dec. 159–163°, $[\alpha]_D^{25}$ −30.7° (*c* = 1, water) (Note 12),(Note 13),(Note 14).

2. Notes

1. The submitters obtained acetamide from Merck & Company, Inc. Acetamide was purchased by the checkers from the Fisher Scientific Company.
2. A 37% solution of formaldehyde in water is available from the Aldrich Chemical Company, Inc.
3. The submitters state that the failure to add dry ice at this point may result in greatly reduced yields. The purpose of the dry ice is presumably to lower the pH of the solution by converting potassium carbonate to potassium bicarbonate.
4. When the mixture was heated above 40° by the submitters, it became discolored and an insoluble precipitate was formed.
5. The filtrate may be cloudy.
6. Apparent yields in excess of the theoretical amount may be observed, owing to the presence of a small portion of water. The oily product may be dried at high vacuum over phosphorous pentoxide for several days.
7. A melting point of 50–52° is reported for *N*-(hydroxymethyl)acetamide.[3]
8. The submitters purchased L-cysteine hydrochloride monohydrate from Schwartz/Mann Division, Becton, Dickinson, and Company, Mountain View Avenue, Orangeburg, New York 10962. The checkers used material supplied by Aldrich Chemical Company, Inc.
9. The pH of the solution is *ca.* 0.5.
10. The balloon was removed briefly while aliquots were taken. The flask was flushed again with nitrogen and the balloon was then replaced. TLC analyses were carried out on glass plates coated with silica gel G purchased from Analtech, Newark, Delaware. With a 10:2:3 (v/v/v) solution of 1-butanol, acetic acid, and water as developing solvent, the R_f values for the product and L-cysteine hydrochloride are 0.19 and 0.25, respectively.
11. The submitters dissolved the solid in methanol at room temperature; however, the solid obtained by the checkers was not very soluble under these conditions. Consequently, the material was dissolved in approximately 2–3 l. of methanol by gentle warming on a steam bath.
12. The product obtained by the checkers had $[\alpha]_D^{25}$ −28° (*c* = 1, water); IR (Nujol) cm.$^{-1}$: 1715 (C=O), 1580 (C=O); ^{1}H NMR (DCl in D_2O), δ (multiplicity, number of protons, assignment): 2.05 (s, 3H, C*H*$_3$), 3.2–3.4 (m, 2H, C*H*$_2$CH), 4.3–4.5 (m, 1H, CH$_2$C*H*), 4.39 (s, 2H, NC*H*$_2$S).
13. On several occasions the product isolated by the submitters was contaminated with L-cystine dihydrochloride, which was not easily removed by recrystallization. In this event the product was converted to the zwitterionic form and recrystallized in the following manner: The pH of a solution of the product in water was adjusted to 6 with aqueous 2.5 *N* potassium hydroxide, and the solution was evaporated to dryness under reduced pressure at *ca.* 40°. The residue was dissolved in a minimum amount of hot water, and two volumes of 95% ethanol were added to precipitate *S*-acetamidomethyl-L-cysteine monohydrate, dec. 187°, $[\alpha]_{589}^{25}$ −42.5° (*c* = 1, water).
14. The following unchecked procedure for liberating L-cysteine from *S*-acetamidomethyl-L-cysteine was provided by the submitters as a model for removing the *S*-acetamidomethyl group from peptides. The pH of a solution of 96.1 mg. (0.500 mmole) of *S*-acetamidomethyl-L-cysteine in 10.0 ml. of water is adjusted to 4.0 with aqueous 0.25 *N* hydrochloric acid. The solution is stirred, 159.3 mg. (0.5000 mmole) of mercury(II) acetate is added, and the pH is readjusted to 4.0 by adding more 0.25 *N* hydrochloric acid. The resulting suspension is stirred for 1 hour at room temperature then diluted with an equal volume of water. Hydrogen sulfide gas is introduced to complete the precipitation of mercury from solution, the mixture is filtered, and the aqueous filtrate is evaporated to dryness under reduced pressure. TLC analysis on the residue as described in (Note 10) showed the presence of L-cysteine and the absence of *S*-acetamidomethyl-L-cysteine.

3. Discussion

The present procedure provides a convenient method for preparing *S*-acetamidomethyl-L-cysteine hydrochloride.[4] The zwitterionic form may be obtained readily from the hydrochloride by the procedure described in (Note 13), by ion-exchange chromatography,[5] or by precipitation from 2-propanol with pyridine.[6] *S*-Acetamidomethyl-L-cysteine has also been prepared from *N*-(hydroxymethyl)acetamide under anhydrous conditions in liquid hydrogen fluoride[4] and in trifluoroacetic acid.[7] The preparation of *N*-(hydroxymethyl)acetamide described in Part A is based on the procedure of Einhorn.[3]

The acetamidomethyl group serves as a useful thiol-protecting group for cysteine during peptide synthesis.[4,7,8,9] The protecting group is stable to the conditions generally prevailing in peptide synthesis,

including not only typical solution and solid-phase procedures but also reactions carried out in liquid hydrogen fluoride.[4] Peptides containing *S*-acetamidomethyl-L-cysteine generally have good water solubility[8] and are not prone to racemization at the α-position of the cysteine residue.[4] The acetamidomethyl protecting group may be easily removed by reaction with either mercury(II) acetate at pH 4, as described in (Note 14), or iodine.[7,8] *S*-Acetamidomethylation of the cysteine residues of proteins may be accomplished in liquid hydrogen fluoride, and the group may be removed by reaction with mercury(II) ion.[4] The thiol group of β-mercaptopropionic acid has also been protected by formation of the *S*-acetamidomethyl derivative.[7]

This preparation is referenced from:

- Org. Syn. Coll. Vol. 6, 252

References and Notes

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Appendix
Chemical Abstracts Nomenclature (Collective Index Number); (Registry Number)

phosphorous pentoxide

ethanol (64-17-5)

potassium carbonate (584-08-7)

hydrochloric acid,
hydrochloride (7647-01-0)

Acetamide (60-35-5)

acetic acid (64-19-7)

methanol (67-56-1)

ether,
diethyl ether (60-29-7)

formaldehyde (50-00-0)

hydrogen sulfide (7783-06-4)

sodium sulfate (7757-82-6)

nitrogen (7727-37-9)

mercury(II) acetate (1600-27-7)

mercury (7439-97-6)

hydrogen fluoride (7664-39-3)

1-butanol (71-36-3)

iodine (7553-56-2)

acetone (67-64-1)

pyridine (110-86-1)

potassium hydroxide (1310-58-3)

2-propanol (67-63-0)

potassium bicarbonate (298-14-6)

trifluoroacetic acid (76-05-1)

mercury(II) ion L-cysteine hydrochloride (52-89-1)

L-cysteine hydrochloride monohydrate (7048-04-6)

L-cystine dihydrochloride (90350-38-2)

cysteine,
L-cysteine (52-90-4)

β-mercaptopropionic acid (107-96-0)

S-acetamidomethyl-L-cysteine monohydrate

S-acetamidomethyl-L-cysteine

L-Cysteine, S-[(acetylamino)methyl]-, monohydrochloride (28798-28-9)

N-(hydroxymethyl)acetamide (625-51-4)

S-Acetamidomethyl-L-cysteine hydrochloride