Phosphonocysteine and Phosphonohomocysteine; Synthesis and Isolation

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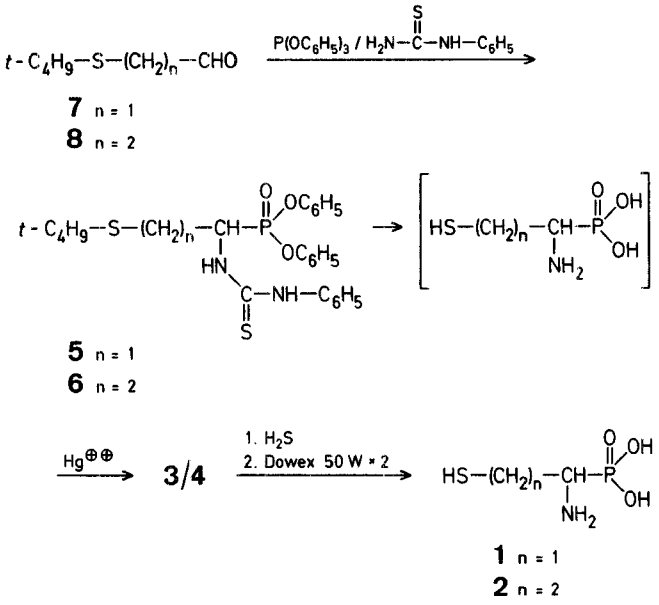
Although the synthesis of phosphonic analogs of cysteine and homocysteine has attracted the attention of this[1,2] and other laboratories[4,5] attempts to isolate phosphonocysteine **(1)** and phosphonohomocysteine **(2)**, which contain free mercapto groups, have so far been unsuccessful in our hands[1,2]. We present now the results of our recent work on the synthesis of compounds **1** and **2** and a method for their isolation via the mercury salts **3** and **4**, respectively.

The reaction of (*t*-butylthio)-acetaldehyde **(7)** with triphenyl phosphite and *N*-phenylthiourea following the method of Ref.[1,2,3] affords diphenyl 2-(*t*-butylthio)-1-(*N'*-phenylthioureido)-phosphonate **(5)** which can be fully hydrolyzed (at O, S, and N) by treatment with hydrobromic acid in aqueous acetic acid to give phosphonocysteine **(1)**. This product can be isolated in the following way: The mixture obtained on hydrolysis of **5** is treated with aqueous mercury sulfate to give the crystalline salt **3** which, according to microanalysis, possesses the formula $C_2H_6NO_3PS \cdot Hg \cdot H_2O$. Treatment of a suspension of **3** in water with hydrogen sulfide liberates free phosphonocysteine **(1)** which upon ion-exchange chromatography is isolated in crystalline form (platelets). A similar sequence can be applied to the synthesis of phosphonohomocysteine **(2)** starting from 3-(*t*-butylthio)-propanal **(8)**; in this case, the mercury salt **4** used for the isolation of **2** is assumed to have the formula $C_6H_{20}N_2O_6P_2S_2 \cdot Hg_3$.



In analogy to a cysteine synthesis from 2,2-dimethyl-2,5-dihydro-1,3-thiazole and hydrogen cyanide[6], we tried the synthesis of phosphonocysteine **(1)** from the same reagent and diethyl phosphite but obtained product **1** in only 5% yield.

All melting points were measured on a Boetius apparatus and are uncorrected. The purities of products **1** and **2** were determined by G.L.C. analysis, from the results of T.L.C. on cellulose, and from the integrated ^{1}H-N.M.R. spectra. Mass spectra were obtained on a LKB-2091 Spectrometer at 15 eV ionizing energy. Samples were introduced via Direct Inlet System. I.R. spectra were measured on a Zeiss-Jena UR-10 Spectrometer. ^{1}H-N.M.R. spectra were taken at 80 MHz on a Tesla BS-487 Spectrometer. ^{31}P-N.M.R. spectra were recorded on a Jeol C-60 H Spectrometer equipped with Heterospin Decoupler SNH-SP-HC at 24.3 MHz. Negative chemical shift values (from H_3PO_4) are reported for compounds absorbing at higher fields than H_3PO_4.

Potentiometric measurements: The potentiometric measurements of acid-basic equilibria were carried out at 25 °C and ionic strength of $\mu = 0.100$ controlled by the addition of potassium nitrate. The measurements were made with a Elpo Typ 512-N pH Meter fitted with a combined glass/calomel electrode. Calibration of the pH meter electrode system was carried out using standard buffer solutions. The concentrations of amino acids in the experimental solutions were kept at 0.0127 molar for **1** and 0.01 molar for **2**.

Thiomercurometric Titration of the Mercapto Group: Determination of molecular equivalents of mercapto groups is carried out in aqueous ammonia/ethanol according to Ref.[7] using 2-(hydroxymercuri)-benzoic acid (0.0005 molar in water) and dithiofluorescine as indicator. The concentration of mercury reagent is determined directly before use by titration against triphenylmethanethiol as standard.

Triphenyl phosphite, *t*-butanethiol, and *N*-phenylthiourea were commercial products. The aldehydes **7** and **8** were prepared from *t*-butanethiol and acrolein or bromoacetal, respectively according to Ref.[1,2], and distilled before use.

Table 1. Compounds **1–4** prepared

Compound	Yield[a] [%]	m.p. [°C] (Lit. value)	T.L.C.[b] R_f value	Molecular formula (Mol weight)[c]	Microanalyses	C	H	N	P	S
1	42	251.5–252.5°	0.18[d]	$C_2H_8NO_3PS$ (157.1)	calc.	15.32	5.12	8.92	19.76	20.18
		(228–234°)[5]	0.43[e]	(161.1)[g]	found	15.33	4.87	9.35	19.76	20.06
			0.32[f]	(154.5)[h]						
2	53	253–255°	0.24[d]	$C_3H_{10}NO_3PS$ (171.1)	calc.	21.05	5.88	8.19	18.15	18.65
			0.56[e]	(173.6)[g]	found	21.19	6.05		18.52	18.71
				(174.3)[h]						
3[i]	61	248.5–250°		$C_2H_6NO_3PS \cdot Hg \cdot H_2O$ (373.7)	calc.	6.44	2.15	3.75	8.33	8.57
					found	6.32	2.35	3.73	8.03	7.75
4[j]	72	246.5–250.5°		$C_6H_{20}N_2O_6P_2S_2 \cdot 3\,Hg$ (943.85)	calc.	7.63	2.14	2.97	6.56	6.79
				(471)[k]	found	8.00	2.26	2.92	6.75	5.52

[a] Overall yields, based on **7** or **8**, respectively. The yields of the individual steps were: **7**→**5** (88%)→**3** (62%)→**1** (77%) and **8**→**6** (90%)→**4** (72%)→**2** (82%).

[b] Cellulose plates DC (E. Merck); indicator: 0.5% ninhydrine in ethanol.

[c] The calculated mol weights and thereby the molecular formulas were in good correlation with the results of the microanalyses, except for compound **4**.

[d] Butanol/acetic acid/water (12/3/5).

[e] Pyridine/acetic acid/water (10/7/3).

[f] Isopropanol/aqueous ammonia (25%)/water (7/1/1).

[g] Molecular equivalent derived from thiomercurimetric determination of mercapto groups.

[h] Molecular equivalent derived from potentiometric titration (first inflection point).

[i] I.R. (KBr): ν = 3700–2000 bs, 1620, 1520, 1430, 1380, 1300, 1280–980 bs, 920 cm^{-1}.

[j] I.R. (KBr): ν = 3700–2000, 1610, 1520, 1280, 1070, 940 cm^{-1}.

[k] Mol weight derived from microanalytical data.

Table 2. Spectral Data of Compounds **1** and **2** or Their Derivatives

Compound	I.R. (KBr) ν [cm^{-1}]	^{1}H-N.M.R. (F_3C—COOH/TMS) δ [ppm]	^{31}P-N.M.R. (H_2O/H_3PO_{4ext}) δ [ppm]	M.S. of *O,O,S,N*-Tetrakis[trimethylsilyl] Derivative: Molecular formula	*m/e*
1	3600–2000 bs, 1730, 1655, 1555, 1430, 1230, 1155, 1050, 915	1.50–2.25 (m, 1H, SH); 2.63–4.75 (m, 3H, CH_2—CH); 6.83–8.40 (m, 3H, NH_3)	14.5[a] 19.5[b]	$C_{14}H_{40}NO_3PSSi_4$ (445.8)	445 (M^+, 0.2%), 430 (M−15, 2.4), 340 (M−105, 19.4), 326 (M−119, 31.6), 220 (M−225, 100)
2	3650–2000 bs, 1610, 1520, 1410, 1250, 1175, 1110, 1090, 1055, 1025, 960, 930	1.25–2.00 (m, 1H, SH); 2.00–2.71 (m, 2H, CH_2—CH_2—SH); 2.71–3.50 (m, 2H, CH_2—CH_2—SH); 3.63–4.87 (m, 1H, CH); 7.02–8.38 (m, 3H, NH_3)	16.6[a] 21.1[b]	$C_{15}H_{42}NO_3PSSi_4$ (459.85)	459 (M^+, 3.5%), 444 (M−15, 2.4), 354 (M−105, 2.9), 340 (M−119, 15.0), 327 (M−183, 20.5), 234 (M−225, 100)

[a] 5% Solution in 2 normal aqueous potassium hydroxide; signal of $H_2L^{3\ominus}$ form of acids **1** and **2**.

[b] 5% Solution in 10 normal hydrochloric acid; signal of $H_5L^{\oplus}$ form of acids **1** and **2**.

Table 3. Acid Dissociation Constants of Phosphonocysteine **(1)**, Phosphonohomocysteine **(2)**, and Their Carboxylic Analogs

Compounds	Negative logarithm of equilibrium constant				Ref.
	pK_1	pK_2	pK_3	pK_4	
Phosphonocysteine **(1)**	2.45	5.26	8.90	11.01	
Phosphonohomocysteine **(2)**	2.50	5.72	9.50	10.95	
Cysteine	1.71	8.33	10.78		8
		8.30	10.40		9
Homocysteine	2.22	8.87	10.86		10

Phosphonocysteine (1) or Phosphonohomocysteine (2):

Triphenyl phosphite (18.6 g, 0.06 mol) is added in one portion to a stirred solution of the aldehyde **7** or **8** (0.05 mol) and *N*-phenylthiourea (9.06 g, 0.06 mol) in glacial acetic acid (50 ml). Stirring is continued for 1 h, the mixture allowed to stand at room temperature overnight, and then diluted with water (5 ml). After 2 h, the precipitated phosphonate **5** or **6** is filtered off and washed with acetic acid/water (1/1). The crude phosphonate **5** or **6** is dissolved in a mixture of glacial acetic acid (50 ml) and 40% hydrobromic acid (100 ml) and this solution is heated at reflux temperature for 16 h. The resultant mixture is cooled to room temperature, diluted with water (100 ml), extracted with benzene (2 × 50 ml), and evaporated to dryness under reduced pressure. The solid residue is dissolved in methanol (125 ml), and a solution of mercury sulfate (30 g) in sulfuric acid (30 g) + water (125 ml) is added. After 3 h, the precipitated salt **3** or **4** is isolated by suction, washed with water (2 × 30 ml), and dried to constant weight in a desiccator over solid phosphorus pentoxide and potassium hydroxide under reduced pressure. [The characteristics of **3** and **4** thus obtained are given in Table 1]. The salt **3** or **4** (10 g) is suspended in water (100 ml). This suspension is saturated with hydrogen sulfide and stirred at room temperature for 3 h. The precipitated solid is filtered off, washed with water (2 × 30 ml), and the combined filtrate is concentrated in vacuo to a volume of ~25 ml. The solution is passed through a Dowex 50W × 2 column and the fractions containing **1** or **2** (ninhydrine or thiomercurimetric test) are collected. The combined fractions containing **1** or **2**, respectively, are concentrated under reduced pressure to a volume of ~5 ml and compounds **1** or **2** are precipitated with ethanol (25 ml).

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