60. Improved Synthesis of O-Phosphohomoserine

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Summary. O-Phosphohomoserine (**PHS**) was prepared in an overall yield of 49% by treatment of N-benzyloxycarbonyl-homoserine *p*-nitrobenzyl ester with diphenylphosphoryl chloride in pyridine, followed by catalytic hydrogenolysis. Both L- and D,L-phosphohomoserine were found to be 100% hydrolyzed by alcaline phosphatase from *E. coli*.

Pea seedlings in the presence of [^{35}S]-H_2S give rise to a labeled compound that was identical with synthetic S-Thr[2])[3]) on TLC. in four different solvent systems [1]. We consider the possibility that **PHS** might be a natural precursor of S-Thr. In order to test this hypothesis *in vitro* we had to synthesize the substrate, **PHS**.

Previous workers have prepared **PHS** a) using enzymes from yeast [3], b) from trichloroacetic acid extracts of *L. casei* [4], or, c), from derepressed mutants of *B. subtilis* [5]. During the course of the present work *Fickel & Gilvarg* [6] reported the chemical synthesis of **PHS** in 17% yield.

Chemical synthesis of **PHS** (*cf.* [3]) is always hampered by the fact that Hse forms a lactone very readily. In the present work attempted transesterfication of acyl-Hse lactone with ethanol and catalytic amounts of ethoxide only gave unstable mixtures (*cf.* [6]). Treatment of Z-Hse lactone (**IV**) with hydrazine gave rise to crystalline Z-Hse hydrazide (**V**); however phosphorylation of this derivative with $POCl_3/H_2O$ [7] resulted in elimination of the hydrazide group, regenerating the lactone[4]). The corresponding phenylhydrazide (**VI**) was smoothly phosphorylated with DPP-Cl/pyridine (**VII**) [8] but selective deprotection with $FeCl_3$ [9] and catalytic hydrogenolysis [8] was unsatisfactory.

The desired compound was finally obtained in acceptable yield by treating Z-Hse-OBZLN (**I**) [10] with DPP-Cl/pyridine (**II**) [8], followed by catalytic hydrogenolysis [8]. It was characterized by elemental analysis, NMR. spectrum, and electrophoresis. Our **PHS** (**III**) obtained from L-Hse was optically active, but the rotation did not agree with the value given in the literature [4]. *Flavin & Slaughter* [11] have made similar observations.

Racemisation tests using alcaline phosphatase were inconclusive because we found that the enzyme catalyzed the hydrolysis of both L- and D,L-**PHS** to the same

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[1]) Taken in part from the Doctoral Dissertation to be submitted by *J. Schnyder*, Present address: Research Institute, *Wander* SA, 3001 Bern, Switzerland.

[2]) Standard abbreviations [2] are used for amino acids and protecting groups. In addition, S-Thr stands for thiothreonine, Hsc for homoserine, **PHS** for O-phosphohomoserine, **BZLN** for *p*-nitrobenzyl, **DPP** for diphenylphosphoryl.

[3]) Our synthetic thiothreonine is a mixture of L-thiothreonine and L-thio-allothreonine.

[4]) See *J. Schnyder & M. Rottenberg*, Helv. *58*, 521 (1975).

extent, *i.e.*, 100%. This is in contrast to the strict chiral stereospecificity reported by *Fickel & Gilvarg* [6].

Experimental part

General: Melting points (uncorrected) were determined in a *Culatti* apparatus. Optical rotations were measured with a *Perkin-Elmer* 141 photoelectric polarimeter in a 1 dm tube. The specific rotations given are accurate to within ± 1°. NMR. spectra were recorded on a *Varian* T-60, and the UV. spectra on a *Perkin-Elmer* 402 instrument. NMR. spectroscopic data as δ-values (ppm) with $\delta = 0$ for tetramethylsilane or 2,2,3,3-tetradeuterio-3-(trimethylsilyl) propionic acid, sodium salt. Coupling constants J are given in Hz (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, $d \times d$ = doublet of doublets, br. = broad).

Material. All solvents and reagents were either redistilled or recrystallized. D,L-Hse was from *Schuchardt*, Germany, L-Hse and all other chemicals were from *Fluka*, Switzerland.

TLC. was run either on silica gel 0.25 mm: *Merck*, Germany, or on cellulose MN 300: *Macherey & Nagel*, Switzerland, 0.3 mm, layered by hand, with the following solvent systems (*v/v*): A) benzene/EtOAc 2:1; B) $CHCl_3$/acetone 3:1; C) benzene/EtOAc/MeOH 6:3:2; D) *n*-BuOH/AcOH/H_2O 4:1:1; E) (for cellulose), *n*-BuOH/acetone/diethylamine/H_2O 10:10:2:5. The Rf-values should be regarded merely as a general indication of chromatographic behavior.

High-voltage electrophoresis was run on a *Locarte* (London) instrument (*Whatman* No. 1; 0.2N lutidine-acetate, pH 6.0; 60 V/cm; 120–130 mA; 30 min. Spots were revealed by spraying with ninhydrin, molybdate [12] and by a modification [13] of the procedure of *Reindel & Hoppe* [14].

Analytical samples for elemental analysis were dried at room temperature and 0.01 Torr for 12–24 h. The analyses were carried out by Mikroanalytisches Laboratorium, Elbach über Engelskirchen, Germany.

Standard preparative procedures. All reactions in organic solvents were run under exclusion of moisture. The rotary evaporations were carried out under reduced pressure not exceeding 40°. All products were checked by NMR. and IR. spectra.

Z-Hse-OBZLN(Benzyloxycarbonyl-homoserine p*-nitrobenzyl ester)* (**I**). L-Hse $[\alpha]_{436}^{25} = -15.42°$ ($c = 1$, H_2O); m.p. 183,5–185° was processed exactly according to [11] except that for acylation the solvent water was replaced by H_2O/dioxane 3:1. Yield 56%, m.p. 82–88° (L-form) and 81–82,5°/102–104° (D,L-form), $[\alpha]_D^{25} = -19.55°$ ($c = 2$, MeOH). – UV. (ethanol): 268 (9408). The literature [10] records m.p. 85° (L-form), $[\alpha]_D^{25} = -19.8°$ and a yield of 64% and UV. (ethanol): 270 (9450) for Z-L-Thr-OBZLN [15]. Rf 0.28 (A), 0.43 (B), 0.78 (C), 0.86 (D).

Z-Hse(DPP)-OBZLN(Benzyloxycarbonyl-O-diphenylphosphoryl-homoserine p*-nitrobenzyl ester)* (**II**). 2.35 g (6 mmol) of **I** in 10 ml anhydrous pyridine was treated in an ice bath with a total of 2.6 ml (12 mmol) DPP-Cl in 0.2 ml portions. A precipitate formed at this stage, presumably pyridine-HCl. The mixture was stirred for 4 h at room temperature. After addition of 0.3 ml H_2O the clear solution was left for another 30 min. Excess pyridine was removed by evaporation. The residue was taken up in EtOAc, and washed with H_2O, 1N HCl, and H_2O until neutral. The organic layer was dried (Na_2SO_4), filtered, and taken to dryness. The residue after drying in a desiccator at *ca.* 15 Torr was obtained as an oil (4.3 g). Rf 0.56 (A), 0.57 (B), 0.83 (C), 0.83 (D).

O-Phosphohomoserine (**III**) 4.3 g of **II** was dissolved in 50 ml glacial acetic acid and 8 ml 2N HCl. 500 mg PtO_2 catalyst was added, and the mixture was shaken or stirred in an atmosphere of H_2 until no more H_2 was consumed (7 days). During hydrogenation small portions of H_2O had to be added to remove the gummy clots that formed. The catalyst was filtered off. AcOH and HCl were removed by repeated evaporation. To remove *p*-amino-benzylalcohol the solution was filtered on a column of Dowex 50-H^+ W × 8, 20/50 mesh, and **PHS** eluted with H_2O. It was crystallized from H_2O/EtOH/ether. 707 mg (61.7% from **I**). After one more recrystallisation from the same solvents the compound had: m.p. 166.5° and 185° (dec.) (L-form) and 197–199.5° (dec.) (D,L-form). $[\alpha]_D^{25} = +4.21°$, $[\alpha]_{546}^{25} = +4.79°$, $[\alpha]_{436}^{25} = +10.0°$ ($c = 2.4$, H_2O). The literature [4] records m.p. 178° (L-form) and $[\alpha]_D^{25} = +6.25°$. Ionophoretic mobility: 13.0 cm (phosphoserine, 15.0 cm; phosphothreonine, 13.5 cm).

Test for racemisation. L- and D,L-Hse in H_2O was titrated to pH 8.2 with NaOH, and made 10 mM. 50 µl of these solutions were incubated for 1 h with *alcaline phosphatase* from *E. coli*.

(Type III, *Sigma* No. P 4252) in 100 µl 0.1 M tris HCl, pH 8.2, 37°. 10 µl of these reaction mixtures was submitted to analysis of inorganic phosphate [16].

Calc. for 100% hydrolysis	1.066 µg P_i
Found for D,L-phosphoserine	0.95 µg P_i
Found for D,L-**PHS**	0.95 µg P_i
Found for L-**PHS**	1.0 µg P_i

As a control 100 µl each of the 10 mM solutions of L- and D,L-**PHS** were subjected to the same conditions of the phosphate determination [16].

Found from L-**PHS**	0.02 µg P_i
Found from D,L-**PHS**	0.02 µg P_i

NMR. (D_2O/NaOD 2:1): 1.85 (m/2H/—CH_2—); 3.4 ($d \times d/J$ = 5.5, J = 6.0/1H/—N—CH—); 3.85 (pseudo q[5])/J = 6/2H/—OCH_2—); 5.0 (s/water).

$C_4H_{10}NO_6P$	Calc.	C 24.10	H 5.06	N 7.04	P 15.6%
(199.1)	Found	,, 24.05	,, 5.22	,, 6.91	,, 15.56% (L-form)
	Found	,, 24.06	,, 5.40	,, 7.16	,, 15.46% (D,L-form)

In a synthetic run without isolation of intermediates 0.99 g **PHS** could be secured from 1.23 g of Hse (49.7% overall yield).

N-Benzyloxycarbonyl-homoserine lactone (**IV**). It was prepared according to [3] with the modification that the precipitated product was filtered off directly and recrystallized from EtOAc/ether. Yield 90.6%. M.p. 108.5–109°. Rf 0.45 (A), 0.52 (B), 0.81 (C), 0.77 (D) (Lit. [3]: 84.5% m.p. 93–97°).

N-Benzyloxycarbonyl-homoserine hydrazide (**V**). 470 mg (2 mmol) of **IV** in 6 ml methanol was treated with a total of 171 µl (3.6 mmol) of hydrazine hydrate in small portions. After 6 h at room temperature precipitation of the crystals was completed by adding 5 ml of ether. 517 mg (96.5%) after drying. Recrystallized from H_2O/EtOH/ether it had m.p. 149–150°. Rf 0.02 (A), 0.13 (B), 0.26 (C), 0.58 (D).

$C_{12}H_{17}N_3O_4$ (267.28) Calc. C 53.92 H 6.41 N 15.72% Found C 53.78 H 6.30 N 15.95%

N-Benzyloxycarbonyl-homoserine phenylhydrazide (**VI**). 3 ml (30 mmol) phenylhydrazine was added to 852 mg **IV** in 3 ml CH_2Cl_2. After 18 h at 4° the solvent was evaporated, and the residue was taken up in EtOAc and washed with 1N HCl, and H_2O until neutral. The organic phase was dried (Na_2SO_4), and the solvent removed by evaporation. The residue was crystallized from EtOH/H_2O. Yield 1.2 g (95%), m.p. 83.5–86°. Rf 0.07 (A), 0.13 (B), 0.63 (C), 0.88 (D). – UV. (EtOH): 235.5 and 285 (11100 and 1900) (Lit. [17]: 234 and 281 (10750 and 1550) for Z-Gly-L-Phe phenylhydrazide).

$C_{18}H_{21}N_3O_4$ (343.37) Calc. C 62.96 H 6.16 N 12.24% Found C 62.99 H 6.32 N 12.19%

N-benzyloxycarbonyl-O-diphenylphosphoryl-homoserine phenylhydrazide (**VII**). Compound **VI** was reacted with DPP-Cl as described for compound **II**. The product was crystallized from EtOH/H_2O (63.5% yield), and recrystallized from EtOAc/petrol ether. M.p. 98–99°. Rf 0.19 (A), 0.40 (B), 0.82 (C), 0.90 (D). – UV. (EtOH): 234.5 and 262 (10650 and 2350).

$C_{30}H_{30}N_3O_7P$	Calc.	C 62.61	H 5.25	N 7.3	P 5.38%
(575.56)	Found	,, 62.47	,, 5.18	,, 7.43	,, 5.32%

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61. Hydrazide as a Carboxyl Protecting Group Deprotection by Acidolysis

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Summary. Elimination of the hydrazide group was studied with the model compounds N-benzoyl-glycine hydrazide and N-benzoyl-L-phenylalanine hydrazide, using phosphorus oxychloride, hydrogen bromide or hydrogen chloride in acetic acid, or 60% perchloric acid. It was found that treatment of N-benzoyl-L-phenylalanine hydrazide with perchloric acid gave N-benzoyl-L-phenylalanine in 100% yield and without racemisation.

During an attempted synthesis of phosphohomoserine [1] we found that treatment of Z-Hse hydrazide[2]) with $POCl_3/H_2O$ [2] resulted in elimination of the hydrazide group and formation of the lactone, thus preventing phosphorylation. It was thought that lactone ring formation [3] might be responsible for the ready elimination. In order to test this hypothesis we studied the reaction with other hydrazides that could not form a lactone.

Previous workers have already suggested or used hydrazides [4–6] as carboxyl protective groups. However, their methods of deprotection are either accompanied by side reactions or are troublesome in the isolation stage. We now describe a method that is free from these drawbacks.

Using either Bz-Gly hydrazide (**I**) or Bz-Phe hydrazide (**III**) as model compounds, and $POCl_3/H_2O$; HBr or HCl/AcOH, and $HClO_4$ as acidolytic reagents we tested the deprotection both qualitatively and quantitatively.

With the three reagents, $POCl_3/H_2O$, HCl/AcOH, and HBr/AcOH, elimination of hydrazine went to no more than 94% completion, and was accompanied by partial or total racemisation of Bz-Phe. With $HClO_4$, on the other hand, cleavage of the hy-

[1]) Taken in part from the Doctoral Dissertation to be submitted by *J. Schnyder*. Present address: Research Institute, *Wander* SA., 3001 Bern, Switzerland.

[2]) Standard abbreviations [12] are used for amino acids and protecting groups. In addition, Hse stands for homoserine.