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Synthesis of *O*-Methyl-L-serine and N^{α}-*tert*-Butyloxycarbonyl-*O*-methyl-L-serine

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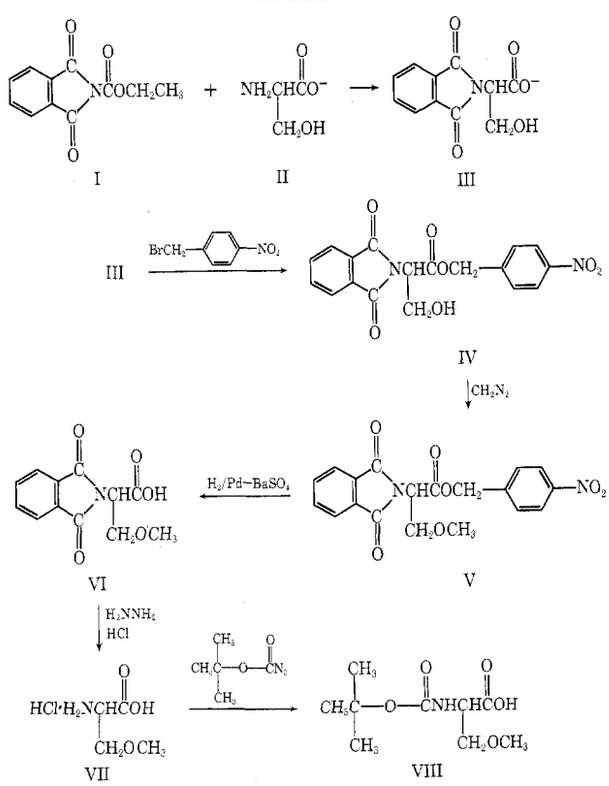
O-Methyl-L-serine and N^{α}-tert-butyloxycarbonyl-*O*-methyl-L-serine were synthesized from L-serine *via* the intermediate N^{α}-phthaloyl-L-serine *p*-nitrobenzyl ester. The ether was made with diazomethane and protecting groups were removed by catalytic hydrogenolysis and hydrazinolysis. Optical purity of the *O*-methyl-L-serine was established. The methyl ether function was stable to the conditions used for acid hydrolysis of peptide bonds and to the acidic conditions used in solid-phase peptide synthesis.

O-Methyl-L-serine was required as an amino acid analog of serine and threonine in the solid-phase peptide synthesis of some new derivatives of ribonuclease A. It was of interest because the hydroxyl groups of serine and threonine can act either as proton donors or acceptors in hydrogen bond formation in proteins, while the methoxyl group of *O*-methyl-L-serine should function only as a proton acceptor. *O*-Methyl-DL-serine was prepared by Schlitz and Carter[3] *via* mercuration of methyl acrylate. Resolution into *O*-methyl-L-serine and N^{α}-acetyl-*O*-methyl-L-serine was achieved by hydrolysis of the N^{α}-acetyl derivative with hog renal acylase.[4] In addition, *N*-phthaloyl-*O*-methyl-L-serine was prepared by Fles and Belenović[5] by resolution of *N*-phthaloyl-*O*-methyl-DL-serine *via* the brucine salt. This paper is concerned with the development of a more convenient synthetic route starting with L-serine.

Four routes were examined: first, a two-step synthesis in which L-serine was acylated to Boc-L-serine followed by methylation of the alcoholic function with methyl iodide in sodium–liquid ammonia at −40°; second, a three-step synthesis in which Boc-L-serine was converted to the *p*-nitrophenyl ester and then methylated with diazomethane (this would have the advantage that the nitrophenyl ester could be used directly for peptide synthesis); third, a four-step synthesis in which Boc-L-serine was converted to the *p*-nitrobenzyl ester with *p*-nitrobenzyl bromide, then methylated with diazomethane, and finally hydrogenated to give Boc-*O*-methyl-L-serine. In preliminary experiments these first three methods were generally unsatisfactory, resulting in poor yields and giving products that were difficult to purify.

The fourth synthesis involved six steps from L-serine but the high yields and easy purification made it the procedure of choice (Scheme I). Phthaloylation of L-serine (II) was carried out in good yield and with retention of optical purity by the method of Nefkens[6,7] using *N*-ethoxycarbonylphthalimide (I) in aqueous sodium carbonate. The *N*-phthaloyl-L-serine (III) was converted to the *p*-nitrobenzyl ester (IV) by a procedure similar to that used by

Scheme I



Shields and Renner[8] for the synthesis of *N*-benzyloxycarbonyl-L-serine *p*-nitrobenzyl ester. IV was methylated under mild conditions with diazomethane in the presence of fluoroboric acid as catalyst[9,10] to give V, which was converted to *N*-phthaloyl-*O*-methyl-L-serine (VI) by catalytic hydrogenolysis. *O*-Methyl-L-serine hydrochloride (VII) was obtained in 53% overall yield from L-serine by hydrazinolysis with hydrazine hydrate and precipitation of the phthalylhydrazide with HCl. Finally, N^{α}-Boc-*O*-

methyl-L-serine (VIII) was prepared from Boc-azide by the Schwyzer[11] method.

The O-methyl-L-serine prepared by this procedure was shown to be optically pure (<0.1% of D isomer) by the chromatographic method of Manning and Moore.[12] The methyl ether was stable to treatment with 1:3 (v/v) trifluoroacetic acid–methylene chloride for 72 hr at 25° and to HF for at least 4 hr at 0°. Such stability is important if peptides containing this analog are to be synthesized by the solid-phase method. The methyl ether was also stable to hydrolysis by 3 N p-toluenesulfonic acid under conditions where proteins are completely hydrolyzed. O-Methyl-L-serine can be separated and quantitated on the ion-exchange column of the amino acid analyzer.

Experimental Section

Melting points were obtained on a Thomas-Hoover melting point apparatus and are uncorrected. Thin layer chromatography was performed with Analtech silica gel GF plates (0.25 mm); compounds were visualized with 254-nm light or spraying with ninhydrin in acetone followed by heating. Nuclear magnetic resonance (nmr) spectra were measured with a Varian Associates A-60 spectrometer; chemical shifts are expressed in parts per million (ppm) downfield from internal standard tetramethylsilane.

N-Phthaloyl-L-serine (III). L-Serine (18.9 g, 0.18 mol) and 19.1 g (0.18 mol) of sodium carbonate were dissolved in 135 ml of water, followed by 41.6 g (0.19 mol) of N-carboethoxyphthalimide (I). The reaction mixture was stirred at room temperature for 2.5 hr. After filtration, 6 N HCl was added to pH 1.8 and phthaloyl-L-serine (III) was extracted with ethyl acetate. The organic phase was washed with cold water and a saturated solution of sodium chloride, dried over anhydrous $MgSO_4$, and filtered. Thin layer chromatography of the filtrate on silica gel [solvent I, chloroform–methanol–acetic acid (85:10:5), or solvent II, 1-butanol–formic acid–water (75:15:10)] showed a single ninhydrin-positive and ultraviolet-absorbing spot corresponding to N-phthaloyl-L-serine. R_f values from thin layer chromatography of N-phthaloyl-L-serine and L-serine were 0.32 and 0.06 for solvent I and 0.88 and 0.50 for solvent II, respectively.

N-Phthaloyl-L-serine p-Nitrobenzyl Ester (IV). p-Nitrobenzyl bromide (41.0 g, 0.19 mol) was dissolved in the ethyl acetate solution (1 l.) of N-phthaloyl-L-serine from the above reaction and 25.0 ml (0.18 mol) of triethylamine was added. The solution was refluxed for 20 hr, let cool to room temperature, and stored at −20° overnight. The solution was filtered on a Büchner funnel and the crystalline product was washed with 1 N sodium bicarbonate and water and dried under high vacuum, yield 43.1 g (65%), mp 179.5–180.8°. An analytical sample was dried under vacuum at 100° over P_2O_5 for 20 hr, $[\alpha]^{25}D$ −46.1° (c 0.8, DMF).

Anal. Calcd for $C_{18}H_{14}N_2O_7$: C, 58.38; H, 3.81; N, 7.57. Found: C, 58.38; H, 3.91; N, 7.52.

R_f values from thin layer chromatography on silica gel: solvent I, chloroform–acetic acid (95:5), N-phthaloyl-L-serine p-nitrobenzyl ester, 0.59; N-phthaloyl-L-serine, 0.19; p-nitrobenzyl bromide, 0.94; solvent II, chloroform–methanol (50:1), N-phthaloyl-L-serine p-nitrobenzyl ester, 0.54.

N-Phthaloyl-O-methyl-L-serine p-Nitrobenzyl Ester (V). To 800 ml of ether, 200 ml of 40% KOH was added and mixture was cooled to 5°. To this solution 66 g of N-nitroso-N-methylurea[10] was added in small portions over a period of several minutes with continued cooling and stirring. The deep yellow ether layer was decanted into 200 ml of ether containing KOH pellets and allowed to stand for 3 hr at 5° to remove dissolved water. The ether solution, containing approximately 0.4 mol of diazomethane, was decanted and used without distillation. The N-phthaloyl-L-serine p-nitrobenzyl ester (11.5 g, 0.031 mol) was dissolved in a minimum volume of dichloromethane at 5° (approximately 2 l.). Fluoroboric acid (0.1 ml) was diluted to 5 ml with ether. A 0.75-ml aliquot (0.22×10^{-3} mol) was added to the reaction mixture. The diazomethane solution was added in 250-ml aliquots (about 0.1 mol) every 5 hr.

The extent of the reaction was followed by thin layer chromatography on silica gel [solvent, chloroform–methanol (50:1); R_f values, N-phthaloyl-L-serine p-nitrobenzyl ester, 0.54; N-phthaloyl-O-methyl-L-serine p-nitrobenzyl ester, 0.79]. Starting material was absent after 20 hr. The solution was evaporated to dryness; the resulting oil was triturated with ether, reevaporated to dryness, and dried under high vacuum over P_2O_5 for 20 hr to give a white powder (11.9 g, 100%). An analytical sample was twice crystallized from ether–petroleum ether and dried for 16 hr under high vacuum at 100° to furnish the methyl ether V as a white powder: mp 126.2–126.8°; $[\alpha]^{25}D$ −48.4° (c 1.0, CH_2Cl_2); nmr ($CDCl_3$) 3.34 (s, 3 H, OCH_3), 4.11 (d, 1 H, J = 6.4 Hz, $CH^{\beta 1}$), 4.15 (d, 1 H, J = 8.8 Hz, $CH^{\beta 2}$), 5.27 (d of d, 2 H, $J_{\alpha,\beta 1}$ = 6.4, $J_{\alpha,\beta 2}$ = 8.8 Hz, CH^{α}), 5.31 (s, 2 H, CO_2CH_2), 7.47 (d, 2 H, J = 8.5 Hz, aromatic near CH_2), 7.81 (AB quartet, 4 H, J = 4 Hz, phthalimide), and 8.17 ppm (d, 2 H, J = 8.5 Hz, aromatic near NO_2).

Anal. Calcd for $C_{19}H_{16}N_2O_7$: C, 59.37; H, 4.20; N 7.29. Found: C, 59.37; H, 4.36; N, 7.29.

N-Phthaloyl-O-methyl-L-serine (VI). The ester V (10.0 g) was dissolved in 300 ml of glacial acetic acid. To this solution was added 2 g of 5% palladium on $BaSO_4$. Hydrogenation was carried out for 24 hr at a pressure of 50 psi. The catalyst was filtered off and washed several times with 50% acetic acid and the combined filtrates were evaporated to dryness *in vacuo* at 40°. Water (25 ml) was added to the residue and the solution was reconcentrated. This procedure was repeated several times. The residue was dissolved in 500 ml of ethyl acetate and extracted with seven 100-ml portions of a saturated sodium bicarbonate solution. The combined aqueous phase was acidified to pH 2.0 with 6 N HCl and extracted with five 200-ml portions of ethyl acetate. The combined organic phase was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness to give an oil. An analytical sample was crystallized from hot water and dried under high vacuum over P_2O_5 for 20 hr at 65° to give long, needle-like, white crystals: mp 98.0–99.0° (lit.[5] mp 101–102°); $[\alpha]^{25}D$ −66.9° (c 1.0, ethyl acetate) [lit.[5] $[\alpha]^{20}D$ −48.1° (c 2.0, ethanol)].

Anal. Calcd for $C_{12}H_{11}NO_5$: C, 57.83; H, 4.45; N, 5.46. Found: C, 57.78; H, 4.58; N, 5.58.

R_f values from thin layer chromatography on silica gel: solvent I, chloroform–methanol (50:1), N-phthaloyl-O-methyl-L-serine p-nitrobenzyl ester, 0.89; N-phthaloyl-O-methyl-L-serine, 0.11; solvent II, chloroform–acetic acid (95:5), N-phthaloyl-O-methyl-L-serine, 0.40.

O-Methyl-L-serine Hydrochloride (VII). The oily N-phthaloyl-O-methyl-L-serine (about 0.026 mol) obtained in the above reaction was dissolved in a minimum volume of methanol (about 300 ml) and 1.75 ml (0.052 mol) of hydrazine hydrate (95%) was added. The solution was stirred at room temperature for 20 hr, at which time the absence of an N-phthaloyl-O-methyl-L-serine spot was observed on thin layer chromatography on silica gel [solvent, chloroform–methanol–acetic acid (85:10:5)]: R_f values phthaloyl hydrazide, 0.57; N-phthaloyl-O-methyl-L-serine, 0.65 (both observed as ultraviolet-positive spots); O-methyl-L-serine hydrochloride, 0.01 (ninhydrin positive). The solution was filtered to remove phthaloyl hydrazide and the filtrate was evaporated to dryness *in vacuo*. The residue was dried for 16 hr under high vacuum over concentrated H_2SO_4 to remove the last traces of hydrazine. The phthaloyl hydrazide was precipitated by addition of 150 ml of 0.5 N HCl. After cooling for 2 hr at 4° the solution was filtered and the filtrate was freeze-dried to a white powder. The residue was triturated with 500 ml of acetone, let stand at −20° overnight, and filtered. The solid material was washed with cold acetone and dried under high vacuum, yield of O-methyl-L-serine hydrochloride after hydrogenolysis and hydrazinolysis 3.30 g (82%), overall yield from L-serine 53%. An analytical sample was twice crystallized from absolute ethanol–ether and dried under high vacuum over P_2O_5, first at room temperature and then at 56° for 10 hr, mp 197° dec, $[\alpha]^{25}D$ +17.9° (c 0.4, methanol).

Anal. Calcd for $2(C_4H_9O_3N)\cdot HCl$: C, 34.98; H, 6.97; N, 10.19; Cl, 12.91. Found: C, 35.47; H, 6.94; N, 10.24; Cl, 12.71.

R_f value from thin layer chromatography on silica gel [solvent, 1-butanol–acetic acid–water (65:15:20)], O-methyl-L-serine hydrochloride, 0.36.

Nmr analysis in trifluoroacetic acid showed 3.60 (s, 3 H, OCH_3), 4.18 (d, 2 H, J = 4.0 Hz, OCH_2), and 4.7 ppm (m, 1 H, CH).

Optical Purity of O-Methyl-L-serine. L-Leucine-N-carboxyanhydride was coupled[12] with O-methyl-L-serine and O-methyl-DL-serine and the separation of the diastereomeric dipeptides was performed on a 0.9 × 60 cm column of Beckman AA 15 resin on the Beckman 120B amino acid analyzer at a flow rate of 66 ml/hr at 56°. A 0.2 N sodium citrate buffer, pH 3.20, was used for column elution. This buffer was prepared by addition of concentrated HCl to the pH 3.49 buffer concentrate supplied by Beckman. The elution time of the dipeptides and amino acids are shown in Table I. Less than 0.1% of D isomer was present in the synthetic O-methyl-L-serine.

Acid Stability of O-Methyl-L-serine. The use of O-methyl-L-

Table I
Elution Conditions of Dipeptides and Amino Acids

Dipeptide	Elution pH	Peak position, min
L-Leu-L-Ser(Me)	3.20	264
L-Leu-D-Ser(Me)	3.20	222
L-Leu	3.20	325
DL-Ser(Me)	3.20	72

Table II
Recovery of *O*-Methyl-L-serine after Acid Hydrolysis with 3 *N* Toluenesulfonic Acid Containing 0.2% Indole

Hydrolysis time, hr	Recovery of *O*-methyl-L-serine, %
0	100
22	97.5
48	94.2
72	91.6

serine in peptide synthesis requires that the methyl ether be acid stable. The stability to various acidic conditions was determined by quantitative amino acid analysis. After a 72-hr exposure to 25% trifluoroacetic acid–75% dichloromethane at 25°, 100% of the methyl ether was recovered. Anhydrous HF for 1 hr at 0° is generally sufficient for cleavage of peptides from resin supports. Recovery of the methyl ether was 100% after treatment with HF for 4 hr at 0°.

O-Methyl-L-serine was heated in 6 *N* HCl for 24 hr at 110°. Amino acid analysis indicated a 64% hydrolysis of the methyl ether to serine. However, hydrolysis at 110° with 3 *N* *p*-toluenesulfonic acid containing 0.2% indole as described by Liu and Chang[13] resulted in very small losses of *O*-methyl-L-serine (Table II) under conditions where proteins are completely hydrolyzed. Currently 4 *N* methanesulfonic acid[13,14] at 110° for 22 hr is being used routinely for this purpose.

Chromatographic Separation of *O*-Methyl-L-serine. The *O*-methyl-L-serine hydrochloride cochromatographed with the *O*-methyl-DL-serine standard on the Beckman AA 15 resin in a 0.9 × 60 cm column of the amino acid analyzer under two sets of conditions. *O*-Methyl-L-serine elutes in the identical position of threonine using the standard conditions of amino acid analysis, that is, 56° and a first buffer of pH 3.49. To separate *O*-methyl-L-serine from threonine, the incorporation of 1-propanol in the first buffer is required. The first six amino acids of the standard amino acid mixture, aspartic acid, threonine, serine, glutamic acid, and proline, are separated from each other and from *O*-methyl-L-serine using a temperature of 76° and a 0.2 *N* sodium citrate buffer, pH 2.90, containing 7% by volume 1-propanol. This buffer was prepared by addition of concentrated HCl to the pH 3.49 buffer concentrate supplied by Beckman and addition of alcohol during dilution. The conditions for the complete amino acid analysis of protein hydrolysates containing *O*-methyl-L-serine will be reported in subsequent papers on the solid-phase synthesis of *O*-methyl-L-serine-containing peptides.

N^{α}-*tert*-Butyloxycarbonyl-*O*-methyl-L-serine (VIII). A mixture of *O*-methyl-L-serine hydrochloride ($C_4H_9O_3N \cdot \frac{1}{2}HCl$, 1.56 g, 11.4 mmol), Boc azide (2.90 ml, 20 mmol), magnesium oxide (1.2 g, 30 mmol), dioxane (30 ml), and water (15 ml) was stirred at 40–45° for 36 hr. The reaction mixture was cooled and the magnesium oxide was removed by filtration and washed twice with 50 ml of water. The combined filtrate and washings were extracted three times with ether. The aqueous phase was chilled with ice and acidified with 0.5 *N* HCl to pH 2.0 on the pH-Stat. The aqueous phase was extracted with six portions of ethyl acetate (75 ml). The combined organic phase was washed with a saturated sodium chloride solution, dried over anhydrous $MgSO_4$, and evaporated to dryness *in vacuo*. The residue was dissolved in 50 ml of ethyl acetate, cyclohexylamine was added, and the N^{α}-*tert*-butyloxycarbonyl-*O*-methyl-L-serine cyclohexylamine salt was crystallized by the addition of ether. The product was filtered and dried over P_2O_5 for 20 hr under high vacuum: yield 2.5 g (80%); mp 153.4–154.5; $[\alpha]^{25}D$ + 19.8° (*c* 1.0, methanol).

Anal. Calcd for $C_{15}H_{30}N_2O_5$: C, 56.58; H, 9.50; N, 8.80. Found: C, 56.69; H, 9.48; N, 9.01.

R_f values from thin layer chromatography on silica gel [solvent, chloroform–methanol–acetic acid (85:10:5)]: N^{α}-Boc-L-serine (CH_3), 0.76; N^{α}-Boc-L-serine, 0.45.

Registry No.—I, 22509-74-6; II, 56-45-1; III, 4702-14-1; IV, 51293-43-7; V, 51293-44-8; VI, 51293-45-9; VII, 51293-46-0; VIII cyclohexylamine salt, 51293-48-2; *p*-nitrobenzyl bromide, 100-11-8; *N*-nitroso-*N*-methylurea, 684-93-5.

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