Amino Acids and Peptides. XXVII.[1] Synthesis of a Decapeptide Sequence (A_1–A_{10}) of Rubredoxin

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A synthesis is described for the protected N-terminal decapeptide (A_1–A_{10}) fragment of rubredoxin, methyl N^{α}-*tert*-butyloxycarbonyl-L-methionyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate.

The simplest of the nonheme iron proteins that are so important in numerous biological electron-transport reactions are the rubredoxins.[2,3] The rubredoxin from *Micrococcus aerogenes* possesses a linear array of 53 amino acid residues and contains one iron that is bound to the four cysteines in the molecule (Figure 1).[4] The chelate structure of this protein has been investigated by various methods, but the results are not definitive.[5–8] A proposed archetype correlation between the rubredoxins and the ferredoxins is of great interest,[9] particularly in view of the fundamental, evolutionary nature of the ferredoxins.[10] Thus, a synthesis of the "active-site" of rubredoxin, and, ultimately, of the complete protein would be of general value.

Rubredoxin contains glycyl residues at positions 10, 19, 27, 42, and 44; therefore, it was decided to prepare smaller fragments that could be united in these locations without fear of racemization. Alternatively, prolyl residues at positions 15, 20, 23, and 39 would provide a similar protective feature. Since two of the four cysteines occur before position 10, it is necessary to develop protecting groups on these particular residues that are compatible with the remainder of the molecule. In view of these considerations, the synthesis of the protected N-terminal decapeptide (A_1–A_{10}) of rubredoxin is now described at this time (Chart I).

Beginning at the *C*-carboxyl end, N^{α}-benzyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteine (I)[11,12] and methyl glycinate hydrochloride (II)[13] were coupled with *N*,*N*′-dicyclohexylcarbodiimide (DCCI)[14] to yield methyl N^{α}-benzyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (III). Treatment of the dipeptide III with hydrogen bromide in acetic acid cleaved the N^{α}-protecting group and furnished the corresponding hydrobromide salt IV. Next, a DCCI condensation between N^{α}-benzyloxycarbonyl-L-threonine (V)[15] and methyl L-leucinate hydrochloride (VI)[16] afforded methyl N^{α}-benzyloxycarbonyl-L-threonyl-L-leucinate (VII). Removal of the N^{α} group of dipeptide VII through catalytic hydrogenation produced oily methyl L-threonyl-L-leucinate (VIII). The second cysteinyl subunit was now introduced in the form of N^{α}-*tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteine (IX), purified as the corresponding dicyclohexylammonium salt X. This particular derivative is new and should have many applications in the peptide field. The acid IX and the amine VIII were joined by DCCI to give methyl N^{α}-*tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucinate (XI). Mild base hydrolysis of the tripeptide XI then formed the corresponding acid XII. A mixed anhydride reaction[17] between XII and IV supplied methyl N^{α}-*tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (XIII). Addition of trifluoroacetic acid to the pentapeptide XIII eliminated the N^{α} group and generated the corresponding trifluoroacetate salt XIV.

Turning to the next major subunit, methyl γ-*tert*-butyl-L-glutamate hydrochloride (XV)[18] and N^{α}-benzyloxycarbonyl-L-phenylalanine (XVI)[19] were coupled by DCCI to obtain methyl N^{α}-benzyloxycarbonyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XVII). Hydrogenolysis of the N^{α}-protecting group led to the corresponding amine XVIII. N^{α}-Benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysine (XIX),[20,21] prepared by a different procedure and purified as the dicyclohexylammonium salt XX,[13] was incorporated into XVIII with the aid of DCCI to yield methyl N^{α}-benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XXI). Hydrogenolysis of the N^{α} group furnished the corresponding amine XXII, which on addition of *p*-nitrophenyl N^{α}-benzyloxycarbonyl-L-glutaminate (XXIII)[22] afforded methyl N^{α}-benzyloxycarbonyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XXIV). Removal of the N^{α} group by hydrogenation produced the tetrapeptide amine XXV. A mixed anhydride reaction[17] between compound XXV

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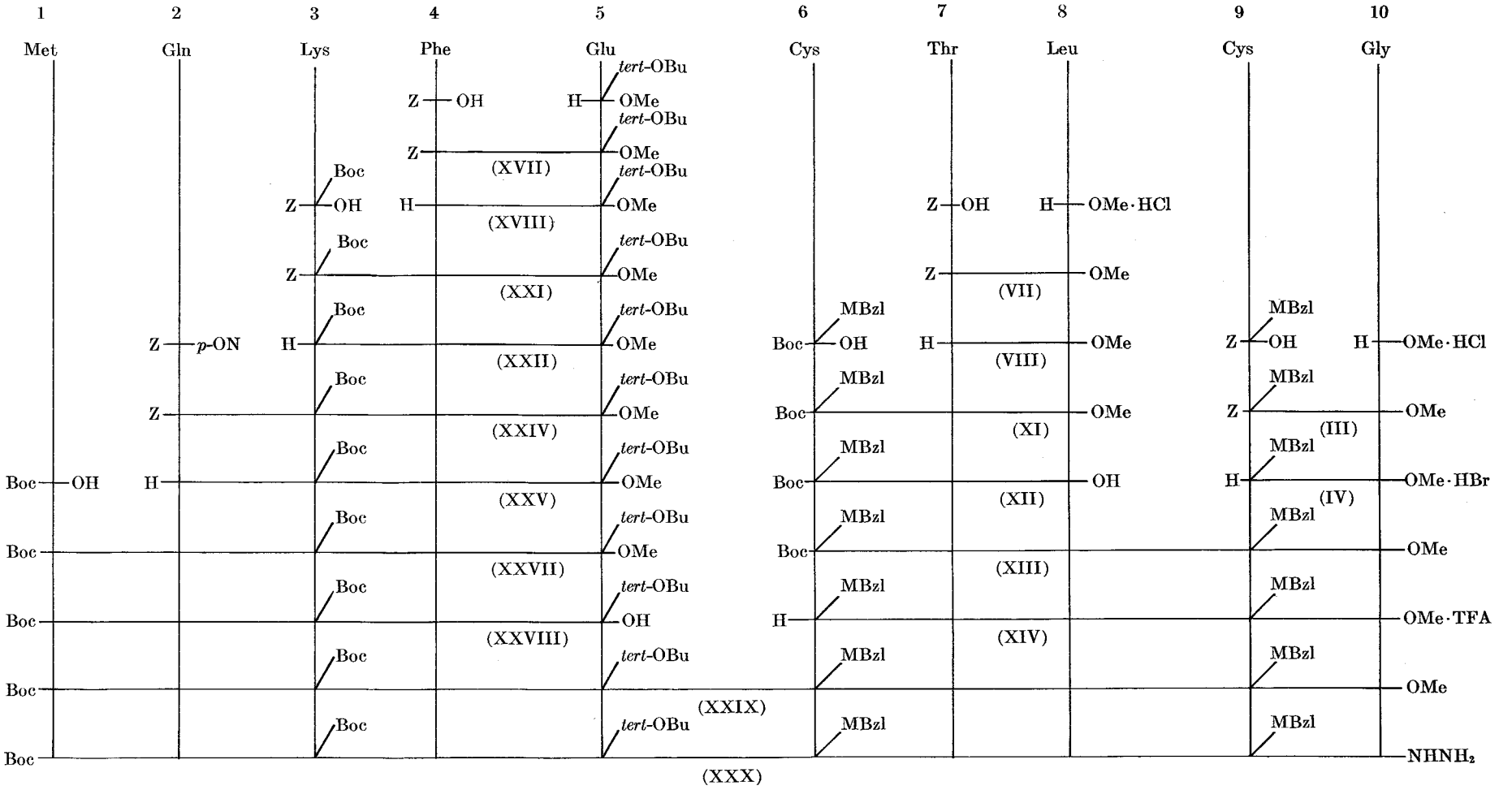
CHART I

SCHEMATIC DIAGRAM OF THE SYNTHESIS OF THE PROTECTED A_1–A_{10} DECAPEPTIDE (XXIX) AND THE DECAPEPTIDE HYDRAZIDE (XXX) OF RUBREDOXIN[a]



[a] Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; HBr, hydrobromide; HCl, hydrochloride; TFA, trifluoroacetate; MBzl, *p*-methoxybenzyl; *tert*-OBu, *tert*-butyl ester; OMe, methyl ester; $NHNH_2$, hydrazide; *p*-ON, *p*-nitrophenyl.

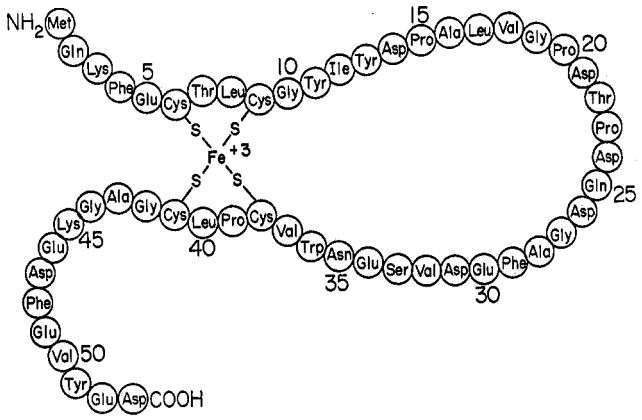


Figure 1.—The amino acid sequence of the rubredoxin from *Micrococcus aerogenes*.

and N^{α}-*tert*-butyloxycarbonyl-L-methionine (XXVI),[20,23] gave methyl N^{α}-*tert*-butyloxycarbonyl-L-methionyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XXVII). Mild base hydrolysis of the pentapeptide XXVII then formed the corresponding acid XXVIII.

Finally, a DCCI condensation between XXVIII and XIV in the presence of *N*-hydroxysuccinimide[24] supplied the desired decapeptide, methyl N^{α}-*tert*-butyloxycarbonyl-L-methionyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (XXIX). Addition of hydrazine to the methyl ester XXIX resulted in the formation of the corresponding hydrazide XXX. Further work in this series is in progress and will be discussed at a future date.

Experimental Section[25]

Methyl N^{α}-Benzyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (III).—N^{α}-Benzyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteine (3.75 g, 0.01 mol) and methyl glycinate hydrochloride (1.25 g, 0.01 mol) in dichloromethane (60 ml) was treated with triethylamine (1.01 g, 0.01 mol) and the solution was cooled to −10°. *N*,*N*′-Dicyclohexylcarbodiimide (2.06 g, 0.01 mol) was added and the resulting mixture was stirred vigorously overnight, while slowly warming to room temperature. The solvent was evaporated and the residue dissolved in ethyl acetate (200 ml). After filtration of the *N*,*N*′-dicyclohexylurea the solution was washed with dilute hydrochloric acid (1 *N*, two 60-ml portions), water (one 60-ml portion), saturated sodium bicarbonate solution (two 60-ml portions), and water (two 60-ml portions), and then dried and evaporated. The residue was crystallized from ethyl acetate–petroleum ether and recrystallized from carbon tetrachloride (4.02 g, 90%): mp 104–105°; $[\alpha]^{25.0}$D −57.9° (*c* 1.00, *N*,*N*-dimethylformamide).

Anal. Calcd for $C_{22}H_{26}N_2SO_6$ (446.53): C, 59.19; H, 5.87; N, 6.27; S, 7.17. Found: C, 59.88; H, 5.98; N, 6.24; S, 7.17.

Methyl *S*-*p*-Methoxybenzyl-L-cysteinylglycinate Hydrobromide (IV).—A solution of methyl N^{α}-benzyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (5.36 g, 0.012 mol) in acetic acid (15 ml) was mixed with 32% hydrogen bromide in acetic acid (15 ml). After 1 hr ether (300 ml) was added with swirling and the precipitated salt was collected and dried over sodium hydroxide in a vacuum desiccator for 12 hr.

Methyl N^{α}-Benzyloxycarbonyl-L-threonyl-L-leucinate (VII).—N^{α}-Benzyloxycarbonyl-L-threonine (5.07 g, 0.02 mol) and methyl L-leucinate hydrochloride (3.63 g, 0.02 mol) in dichloromethane (100 ml) was treated with triethylamine (2.02 g, 0.02 mol) and the solution was cooled to −10°. *N*,*N*′-Dicyclohexylcarbodiimide (4.12 g, 0.02 mol) was added and the resulting mixture was stirred vigorously overnight, while slowly warming to room temperature. The reaction was worked up in the usual fashion to obtain an oil, which solidified on standing (7.00 g, 92%): mp 48–53°; $[\alpha]^{25.0}$D −36.2° (*c* 1.25, chloroform).

Anal. Calcd for $C_{18}H_{28}N_2O_6$ (380.45): C, 59.98; H, 7.42; N, 7.36. Found: C, 60.14; H, 7.44; N, 7.22.

Methyl L-Threonyl-L-leucinate (VIII).—Oily methyl N^{α}-benzyloxycarbonyl-L-threonyl-L-leucinate (8.36 g, 0.022 mol) in methanol (300 ml) containing 10% palladium-on-charcoal catalyst (0.80 g) was hydrogenated for 5 hr. The catalyst was removed by filtration and evaporation of the solvent gave an oil (5.20 g, 96%): R_f 0.24 [acetic acid–butanol–water (1:4:1); ninhydrin positive].

N^{α}-*tert*-Butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteine (IX).—*tert*-Butyl azidoformate (21.45 g, 0.15 mol) was added dropwise over a 30-min period to a stirred suspension of *S*-*p*-methoxybenzyl-L-cysteine (24.10 g, 0.10 mol) in *N*,*N*′-dimethylformamide (350 ml) and 1,1,3,3-tetramethylguanidine (23.0 g). A clear solution was obtained on the addition of a few drops of dilute sodium hydroxide (1 *N*). After 4 days at room temperature, the solvent was evaporated and the residue was worked up in the usual fashion to obtain an oil (30.7 g, 90%).

N^{α}-*tert*-Butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteine Dicyclohexylammonium Salt (X).—A solution of N^{α}-*tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteine in ether on treatment with dicyclohexylamine yielded the corresponding dicyclohexylammonium salt. Crystallization from ethyl acetate–petroleum ether afforded white needles: mp 132°; $[\alpha]^{25.0}$D −14.6° (*c* 1.00, chloroform).

Anal. Calcd for $C_{28}H_{46}N_2SO_5$ (522.75): C, 64.34; H, 8.87; N, 5.36; S, 6.74. Found: C, 63.90; H, 8.30; N, 5.39; S, 6.58.

Methyl N^{α}-*tert*-Butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucinate (XI).—A solution of N^{α}-*tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteine (7.17 g, 0.021 mol) and freshly prepared methyl L-threonyl-L-leucinate (5.17 g, 0.021 mol) in dichloromethane (150 ml) was cooled to −10° and *N*,*N*′-dicyclohexylcarbodiimide (4.33 g, 0.021 mol) was added and the resulting mixture was vigorously stirred overnight, while slowly warming to room temperature. The reaction was worked up in the usual fashion to obtain a residue, which was purified by silica gel column chromatography employing chloroform–methanol (97:3) as the developer. The resulting foam could not be crystallized (9.10 g, 76%): mp 38–42°; $[\alpha]^{25.0}$D −40.5° (*c* 1.00, chloroform).

Anal. Calcd for $C_{27}H_{43}N_3SO_8$ (569.73): C, 56.91; H, 7.61; N, 7.38; S, 5.63. Found: C, 57.17; H, 7.67; N, 7.40; S, 5.33.

N^{α}-*tert*-Butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucine (XII).—The aforementioned tripeptide (6.84 g, 0.012 mol) was treated with a solution of sodium hydroxide (1 *N*, 15 ml) in methanol (50 ml) for 30 min, and then water (40 ml) was added and the bulk of the methanol was removed by evaporation. After washing with ether (two 30-ml portions), acidification of the aqueous phase with citric acid solution precipitated an oily product. This material was taken into ethyl acetate (three 75-ml portions) and the combined organic phases was washed with water (two 75-ml portions) and dried. Evaporation gave a white solid (5.82 g, 87%).

Methyl N^{α}-*tert*-Butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucinyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (XIII).—A solution of *N*-*tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucine (5.56 g, 0.01 mol) in tetrahydrofuran (30 ml) was cooled to −20° and treated in turn with *N*-methylmorpholine (1.01 g, 0.01 mol) and isobutyl chloroformate (1.37 g, 0.01 mol). After 4 min, a solution of methyl *S*-*p*-methoxybenzyl-L-cysteinylglycinate in tetrahydrofuran (40 ml) and water (5 ml), freshly prepared by dissolving the corresponding hydrobromide IV in tetrahydrofuran (20 ml) and trimethylamine (3 ml) and evaporating to dryness, was added and stirred for 30 min at −20°, followed by allowing the reaction to warm to room temperature over 2 hr. The solvent was evapo-

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(25) All melting points were determined on a Reichert "Thermopan" unit and are uncorrected. Evaporations were performed under reduced pressure (water pump) with a rotatory apparatus at minimum temperature, while high-boiling solvents were removed at vacuum pressure (0.2–0.5 mm). Magnesium sulfate was used for drying purposes. Acetonitrile and *N*,*N*-dimethylformamide were spectroscopic quality; other solvents were reagent grade and petroleum ether had bp 30–60°. Microanalyses were furnished by Galbraith Laboratories, Knoxville, Tenn.

rated and the residue was dissolved in ethyl acetate (300 ml) and worked up in the usual fashion. The product was purified by silica gel column chromatography employing chloroform–methanol (97:3) as the developer. The resulting foam could not be crystallized (6.63 g, 78%): mp 112–115°; $[\alpha]^{25.0}D$ −55.1° (*c* 1.00, chloroform).

Anal. Calcd for $C_{40}H_{59}N_5S_2O_{11}$ (850.08): C, 56.52; H, 7.00; N, 8.24; S, 7.54. Found: C, 56.59; H, 7.30; N, 8.14; S, 7.96.

Methyl *S*-*p*-Methoxybenzyl-L-cysteinyl-L-threonyl-L-leucinyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate Trifluoroacetate (XIV).—Methyl N^{α}-*tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucyl-*S*-*p*-methoxybenzyl-L-cysteineglucinate (1.36 g, 0.0016 mol) was dissolved in trifluoroacetic acid (10 ml). After 30 min at room temperature, the solution was evaporated and the residue was dried in a vacuum desiccator over sodium hydroxide for 5 hr.

Methyl N^{α}-Benzyloxycarbonyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XVII).—A solution of N^{α}-benzyloxycarbonyl-L-phenylalanine (2.99 g, 0.01 mol) and methyl γ-*tert*-butyl-L-glutamate hydrochloride (2.54 g, 0.01 mol) in dichloromethane (75 ml) was treated with triethylamine (1.01 g, 0.01 mol) and cooled to −10°. *N*,*N*′-Dicyclohexylcarbodiimide (2.06 g, 0.01 mol) was added and the resulting mixture was stirred vigorously overnight, while slowly warming to room temperature. The reaction was worked up in the usual fashion to obtain an oil, which solidified on standing (4.88 g, 98%): mp 67–68°; $[\alpha]^{25.0}D$ +10.0° (*c* 2.00, chloroform).

Anal. Calcd for $C_{27}H_{34}N_2O_7$ (498.59): C, 65.06; H, 6.87; N, 5.62. Found: C, 65.86; H, 7.11; N, 5.80.

Methyl L-Phenylalanyl-γ-*tert*-butyl-L-glutamate (XVIII).—A solution of methyl N^{α}-benzyloxycarbonyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (5.98 g, 0.012 mol) in methanol (250 ml) containing 10% palladium-on-charcoal catalyst (0.80 g) was hydrogenated for 2 hr. The catalyst was removed by filtration and evaporation of the solvent gave a thick oil (3.71 g, 85%): R_f 0.57 [chloroform–methanol (95:5); ninhydrin positive].

N^{α}-Benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysine (XIX).—A mixture of N^{α}-benzyloxycarbonyl-L-lysine (28.03 g, 0.10 mol) in *N*,*N*′-dimethylformamide (220 ml) was warmed to 40° and then cooled to room temperature, and 1,1,3,3-tetramethylguanidine (23.0 g) was added, followed by *tert*-butyl azidoformate (21.45 g, 0.15 mol) dropwise over 30 min. The clear solution stood for 4 days at room temperature, after which the solvent was evaporated and the residue was partitioned between ethyl acetate (300 ml) and citric acid solution (100 ml). The aqueous layer was extracted with ethyl acetate (100 ml) and the combined organic phases were washed with water (three 100-ml portions), dried, and evaporated to give a thick oil (35.0 g, 92%).

N^{α}-Benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysine Dicyclohexylammonium Salt (XX).—A sample of the aforementioned compound was treated with dicyclohexylamine in the usual fashion to obtain the corresponding salt, which was crystallized from 2-propanol: mp 154–155°.

Methyl N^{α}-Benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XXI).—A solution of methyl L-phenylalanyl-γ-*tert*-butyl-L-glutamate (3.64 g, 0.01 mol) and N^{α}-benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysine (3.80 g, 0.01 mol) in dichloromethane (150 ml) was cooled to −10°. *N*,*N*′-Dicyclohexylcarbodiimide (2.06 g, 0.01 mol) was added and the resulting mixture was stirred vigorously overnight, while slowly warming to room temperature. The reaction was worked up in the usual fashion and the product was crystallized from ethyl acetate–ether (5.96 g, 86%): mp 126–127°; $[\alpha]^{25.0}D$ −25.6° (*c* 1.00, chloroform).

Anal. Calcd for $C_{38}H_5N_4O_{10}$ (726.88): C, 62.79; H, 7.49; N, 7.71. Found: C, 63.21; H, 7.55; N, 7.22.

Methyl N^{ϵ}-*tert*-Butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XXII).—A solution of methyl N^{α}-benzyloxycarbonyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (7.27 g, 0.01 mol) in methanol (200 ml) containing 10% palladium-on-charcoal catalyst (0.70 g) was hydrogenated for 15 hr. The catalyst was removed by filtration and evaporation of the solvent left a residue (5.39 g, 91%): R_f 0.57 [acetic acid–butanol–water (1:4:1); ninhydrin positive].

Methyl N^{α}-Benzyloxycarbonyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XXIV).—A solution of *p*-nitrophenyl N^{α}-benzyloxycarbonyl-L-glutamine (4.42 g, 0.011 mol) and methyl N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (5.33 g, 0.009 mol) in *N*,*N*′-dimethylformamide (60 ml) was allowed to stand for 4 days at room temperature. The solvent was evaporated and the residue was crystallized from methanol (5.85 g, 76%): mp 219–221°; $[\alpha]^{25.0}D$ −26.1° (*c* 2.00, *N*,*N*′-dimethylformamide).

Anal. Calcd for $C_{43}H_{62}N_6O_{12}\cdot{}^1/_2H_2O$ (864.03): C, 59.75; H, 7.35; N, 9.73. Found: C, 59.75; H, 7.00; N, 9.30.

Methyl L-Glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XXV).—A solution of methyl N^{α}-benzyloxycarbonyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (5.99 g, 0.007 mol) in methanol (350 ml) containing 10% palladium on charcoal (0.80 g) was hydrogenated for 24 hr. The catalyst was removed by filtration and evaporation of the solvent left a residue (4.64 g, 92%): R_f 0.53 [acetic acid–butanol–water (1:4:1); ninhydrin positive].

N^{α}-*tert*-Butyloxycarbonyl-L-methionine (XXVI).—L-Methionine (44.70 g, 0.30 mol) was converted into N^{α}-*tert*-butyloxycarbonyl-L-methionine, following the procedure described for XIX, to afford a syrup (75.00 g, 100%).

Methyl N^{α}-*tert*-Butyloxycarbonyl-L-methionyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (XXVII).—A solution of N^{α}-*tert*-butyloxycarbonyl-L-methionine (1.60 g, 0.0064 mol) in tetrahydrofuran (30 ml) was cooled to −20° and treated with *N*-methylmorpholine (0.65 g, 0.0064 mol), followed by isobutyl chloroformate (0.87 g, 0.0064 mol). After 4 min, a solution of methyl N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (4.61 g, 0.0064 mol) in *N*,*N*′-dimethylformamide (40 ml) was added and the reaction was stirred for 30 min at −20°, then allowed to slowly warm to room temperature, and stirred for another 2 hr. The solvent was evaporated and the residue was crystallized from methanol (5.12 g, 84%): mp 223–225°; $[\alpha]^{25.0}D$ −22.5° (*c* 1.00, *N*,*N*′-dimethylformamide).

Anal. Calcd for $C_{45}H_{73}N_7SO_{13}$ (952.20): C, 56.76; H, 7.73; N, 10.30; S, 3.37. Found: C, 57.43; H, 7.82; N, 10.30; S, 3.01.

N^{α}-*tert*-Butyloxycarbonyl-L-methionyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamic Acid (XXVIII).—A solution of methyl N^{α}-*tert*-butyloxycarbonyl-L-methionyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamate (1.90 g, 0.002 mol) in methanol (30 ml) and sodium hydroxide (1 *N*, 2 ml) was stirred for 2 hr. After dilution with water (30 ml) the methanol was evaporated, and the mixture was acidified with citric acid solution. The precipitated product was filtered, washed, and purified from methanol–water (1.61 g, 86%): mp 212–215; $[\alpha]^{25.0}D$ −20.6° (*c* 1.00, *N*,*N*′-dimethylformamide).

Anal. Calcd for $C_{44}H_{71}N_7SO_{13}$ (938.17): C, 56.33; H, 7.63; N, 10.45; S, 3.42. Found: C, 55.94; H, 7.69; N, 10.35; S, 3.40.

Methyl N^{α}-*tert*-Butyloxycarbonyl-L-methionyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate (XXIX).—The trifluoroacetate salt XIV was dissolved in tetrahydrofuran (15 ml), treated with trimethylamine (2.0 ml), and evaporated to dryness. Pentapeptide acid XXVIII (1.50 g, 0.0016 mol) and *N*-hydroxysuccinimide (0.28 g, 0.0024 mol) were dissolved in *N*,*N*′-dimethylformamide (20 ml), cooled to −10°, and treated with *N*,*N*′-dicyclohexylcarbodiimide (0.33 g, 0.0016 mol), followed by the above residue in *N*,*N*′-dimethylformamide (10 ml). After stirring for 1 hr at −10°, the reaction was allowed to slowly warm to room temperature followed by stirring for 4 days. The solvent was evaporated and the residue was triturated under water and washed with a saturated solution of sodium bicarbonate. Purification from methanol gave a solid (1.72 g, 64%): mp 244–246° dec; $[\alpha]^{25.0}D$ −31.1° (*c* 1.00, *N*,*N*′-dimethylformamide).

Anal. Calcd for $C_{79}H_{120}N_{12}S_3O_{21}$ (1670.12): C, 56.82; H, 7.24; N, 10.07; S, 5.76. Found: C, 56.52; H, 7.25; N, 9.84; S, 5.90.

N^{α}-*tert*-Butyloxycarbonyl-L-methionyl-L-glutaminyl-N^{ϵ}-*tert*-butyloxycarbonyl-L-lysyl-L-phenylalanyl-γ-*tert*-butyl-L-glutamyl-*S*-*p*-methoxybenzyl-L-cysteinyl-L-threonyl-L-leucyl-*S*-*p*-methoxybenzyl-L-cysteinylglycinate Hydrazide (XXX).—The aforementioned decapeptide XXIX (0.251 g, 0.00015 mol) was dissolved in methanol (150 ml) and *N*,*N*′-dimethylformamide (1.5 ml) and treated with hydrazine hydrate (90%, 3 ml). The reaction was stirred for 1.5 hr and then the solvent was evaporated, and the product was precipitated with water. Crystallization from

methanol gave a solid (0.228 g, 91%): mp 248° (slow decomposition); $[\alpha]^{25.0}D$ −30.6° (*c* 0.50, *N*,*N*′-dimethylformamide).

Anal. Calcd for $C_{78}H_{120}N_{14}S_8O_{20}$ (1670.13): C, 56.01; H, 7.24; N, 11.74; S, 5.76. Found: C, 56.02; H, 7.19; N, 11.79; S, 5.52.

Registry No.—III, 31025-11-3; VII, 31025-12-4; VIII, 31025-13-5; X, 31025-14-6; XI, 31025-15-7; XIII, 31020-53-8; XVII, 31025-16-8; XVIII, 31025-17-9; XX, 2212-76-2; XXI, 31025-19-1; XXII, 31025-20-4; XXIV, 31020-54-9; XXV, 31020-55-0; XXVII, 31020-56-1; XXVIII, 31020-57-2; XXIX, 31020-58-3; XXX, 31020-59-4.

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Synthesis of 2-Thiouridine and 2-Thioisouridine by Mercuri Procedure[1,2]

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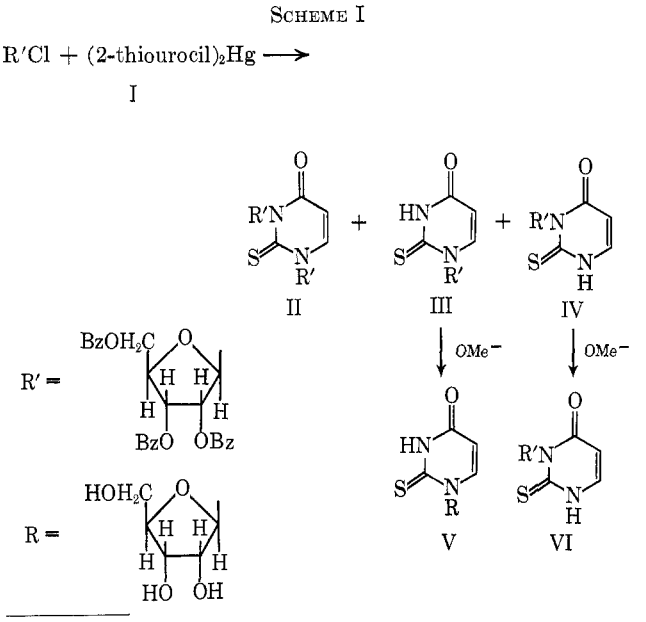
Contrary to an earlier report, (2-thiouracil)$_2$Hg (I) can be obtained from 2-thiouracil and $HgCl_2$. The compound I on treatment with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride formed one disubstituted (II) and two monosubstituted products (III and IV). The compounds III and IV on debenzoylation with sodium methoxide formed 2-thiouridine (V) and 2-thioisouridine (VI), respectively. Compounds V and VI were converted into uridine and isouridine, respectively, by cyanogen bromide. The pK_a's of both V and VI are 8.1.

The facile formation and cleavage of disulfide bonds involving 2-thio- and 4-thiopyrimidines in tRNA has been invoked on several occasions to explain biochemical mechanisms.[3-6] Although 2-thiouracil[7] and 4-thiouridine[8,9] form disulfides readily after iodine treatment, a similar oxidation of 2-thiouridine derivatives was not observed by at least two groups of workers.[10,11] It is possible that such oxidation may take place easily when 2-thiouridine moieties are parts of a macromolecule. In order to study the properties of 2-thiouridines, particularly the formation of a disulfide on oxidation, the chemical synthesis of the compound was undertaken. Of the five different methods of synthesis of 2-thiouridine,[12-16] the one reported by Lee and Wigler[16] based on mercuri condensation was reinvestigated. Several discrepancies were observed, and the characterization and properties of the intermediate blocked nucleosides and the final products are reported.

Lee and Wigler[16] were unable to prepare (2-thiouracil)$_2$Hg (I) from 2-thiouracil and $HgCl_2$ in aqueous solution by the method of Fox, *et al.*[17] In my hands, however, 2-thiouracil did form with mercuric chloride the desired (2-thiouracil)$_2$Hg in 2:1 stoichiometry and in a quantitative yield. It is noteworthy that (2-methylthiouracil)$_2$Hg was previously obtained in high yield directly from 2-methylthiouracil by Scannell and Allen.[18] However, Lee and Wigler[16] claim to have prepared I in 83% yield by prior acetylation of 2-thiouracil followed by mercuric chloride treatment: mp 282–286° dec, λ_{max} (ethanol) 294 nm. On the other hand, I have been unable to prepare 2-thiouracil·HgCl salt using equimolar amounts of 2-thiouracil and $HgCl_2$; elemental analysis of the product indicated the formation of mixtures. The addition of mercuric bromide in this mercuri condensation reaction was not essential and did not improve the yield of 2-thiouridine.

The treatment of I with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride yielded a mixture of blocked nucleosides II, III, and IV separable by chromatography on silicic acid (Scheme I). On debenzoylation with sodium methoxide in methanol, IV gave 2-thioisouridine (VI), identified by the similarities of its uv absorption spectra

SCHEME I



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