Synthesis of a Peptide Lactone, *N*-(3-Hydroxypicolinyl)-threonyl-D-leucyl-prolylsarcosyl-leucyl-alanyl-alanine Threonine Lactone

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The synthesis of a peptide lactone, *N*-(3-hydroxypicolinyl)-threonyl-D-leucyl-prolylsarcosyl-leucyl-alanyl-alanine Threonine Lactone (**21**) is described. The *t*-butoxycarbonyl group of *t*-butyl *O*-(*t*-butoxycarbonyl-alanyl)-*N*-benzyloxycarbonyl-threonyl-D-leucyl-prolylsarcosinate (**12**) was deblocked selectively with formic acid in good yield. The coupling of **12** with the azide derived from *t*-butoxycarbonyl-leucyl-alanine hydrazide (**15**) with isopentyl nitrite gave a heptapeptide ester **17**. Deblocking, cyclization, and hydrogenation gave a heptapeptide lactone **20** which was coupled with 3-hydroxypicolinic acid yielding **21**.

In a previous paper,[1] it was reported that the *t*-butoxycarbonyl group was cleaved selectively in the presence of the *t*-butyl ester group using 85% formic acid and the application of this selective deprotection method for the synthesis of peptides.

In this paper, the usefulness of this method for the synthesis of the peptide lactone[2] is reported.

In the past several years, the structures of a number of new antibiotics have been reported in the literature with the common feature of a lactone that is formed from the carboxyl function of an amino acid with the hydroxyl group of an amino acid. Moreover, in most of the cases, the amino function of the latter is acylated by a heterocyclic acid.[3-6] Examples of this class of compounds are the antibiotic actinomycin,[7] etamycin,[5] echinomycin,[8] *etc.* Synthetic approaches in this field of naturally occurring peptide lactone antibiotics have been limited only to actinomycin[9-11] and etamycin.[12]

Because of the difficulties of synthesis, the authors are interested in finding a new method of synthesizing this class of peptides in the utility of the selective deblocking method[1] described previously. For this purpose an attempt was made to synthesize a peptide lactone **21** as a model (Fig. 1).

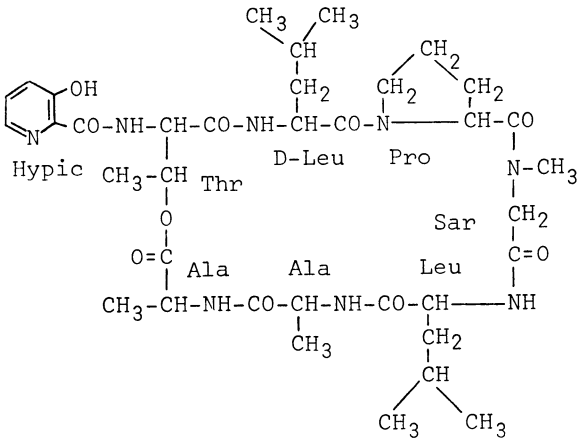
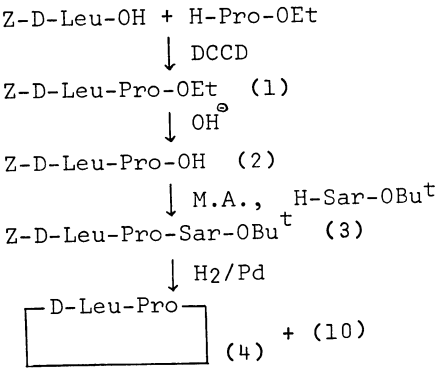


Fig. 1.

In the synthesis of a peptide lactone, it is usual to prepare initially the ester bond followed by cyclization by the formation of an amide bond. For example, in the case of 6-proline-staphylomycin S[13] and etamycin,[12] initially the linear peptide ester containing a 3-hydroxypicolinic acid moiety was prepared and then cyclization was provided by the amide bond formation. However a new route was designed in which the formation of peptide lactone **19** is obtained by cyclization of the linear peptide ester **18** and finally a 3-hydroxypicolinic acid moiety is introduced into the amino group of threonine, as illustrated in Scheme 2. This synthetic approach has not been reported in the literature to date as far as the present authors are aware, because it is necessary to use various protecting groups in this case.

The amide bond between sarcosine and leucine was selected for the cyclization step because of the stability of sarcosine toward racemization. For this approach, it was necessary to synthesize a chain that would include the desired ester bond between alanine and threonine (Scheme 2).

The preparation of the deprotected tripeptide (*t*-butyl D-leucyl-prolylsarcosinate (**10**)) was attempted in two different ways. Catalytic hydrogenation of *t*-butyl benzyloxycarbonyl-D-leucyl-prolylsarcosinate (**3**) derived from the coupling of benzyloxycarbonyl-D-leucyl-proline (**2**) with *t*-butyl sarcosinate, gave a tripeptide ester **10**, but D-leucyl-proline anhydride (**4**) was always produced as a by-product (Scheme 1). Therefore, it was



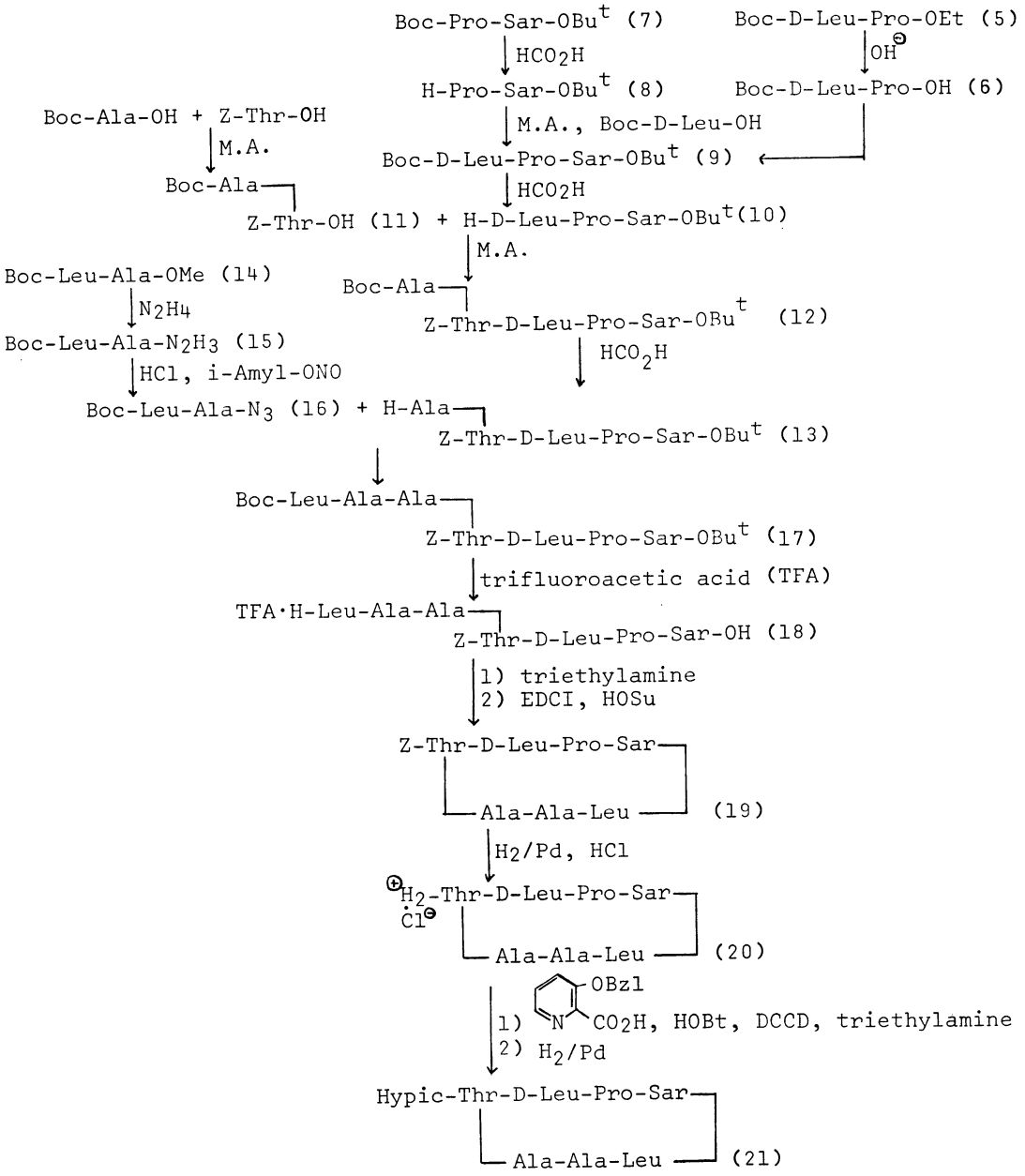
Scheme 1.

thought that the selective deblocking method[1] may be superior for this purpose. The cleavage of the *t*-butoxycarbonyl group of the protected dipeptide ester **7** by formic acid gave a dipeptide ester **8** in a 90% yield, which was condensed with *t*-butoxycarbonyl-D-leucine to give the desired protected tripeptide ester **9** in a 96% yield. Alternatively, compound **9** was obtained only in the low yield of 59% by coupling of *t*-butoxycarbonyl-D-leucyl-proline (**6**) with *t*-butyl sarcosinate. The cleav-

age of the *t*-butoxycarbonyl group of the protected tripeptide ester **9** by formic acid gave a tripeptide ester **10** in an 86% yield with no by-products such as anhydride.

The formation of the ester bond between alanine and threonine was mediated by the mixed anhydride method. The product was separated from the reaction mixture by gel filtration on Sephadex LH-20 to give the desired ester **11** in a 64% yield, which was isolated as dicyclohexylammonium salt. The coupling of the peptide **11** with the tripeptide *t*-butyl ester **10** using the mixed anhydride method gave the pentapeptide ester **12** in an 80% yield.

The selective cleavage of compound **12** by formic acid, a key step in the elongation of the peptide bond to the *N*-terminus, was realized successfully to give the desired deprotected pentapeptide ester **13** in good yield of 87%, which was condensed with the azide **16** derived from *t*-butoxycarbonyl-leucyl-alanine hydrazide (**15**) with isopentyl nitrite[14] to give a 91% yield of the desired heptapeptide ester **17**. A short treatment of the linear heptapeptide ester **17** with anhydrous trifluoroacetic acid is sufficient to remove both of the amino- and the carboxyl-protecting groups to give a trifluoroacetate **18** in a 93% yield. After neutralization with triethylamine, cyclization of the deprotected peptide ester was achieved in a highly dilute solution using five times excess amounts of EDCI and *N*-hydroxysuccinimide,[15] followed by separation of the reaction mixture by preparative TLC to give the desired peptide lactone **19** in good yield (45%). A mass spectrum of compound **19** showed the expected molecular weight. Debenzyloxycarbonylation of the heptapeptide lactone **19** was carried out in an acidic medium containing hydrogen chloride to avoid intramolecular acylation, the so-called O→N acyl



Scheme 2. Synthesis of Peptide Lactone.

migration.[16-18]

The introduction of a 3-hydroxypicolinic acid moiety to the *N*-terminus was at first carried out by the reaction of the deprotected cyclic peptide **20** with the 3-benzyloxypicolinic acid *p*-nitrophenyl ester[19] with slow neutralization, followed by catalytic hydrogenation giving only a poor yield of the desired acylated peptide lactone (20%). Alternatively, condensation of the heptapeptide lactone **20** with the active ester, derived from 3-benzyloxypicolinic acid[19] and HOBt using dicyclohexylcarbodiimide under the same reaction conditions (as described above) gave the desired cyclic octapeptide ester **21** in good yield (68%). The presence of 3-hydroxypicolinic acid was confirmed by spectroscopically[12,13] and by the color reaction[20] of the hydrolysate with an aqueous ferric chloride solution.

As mentioned above, the selective deblocking method has proved useful for the synthesis of a peptide lactone.

Experimental

All melting points are uncorrected. The NMR, IR, and UV spectra were recorded on a JEOL JNH-60, a JASCO IRA-1 spectrometer and a Union Giken SM-401 spectrometer, respectively. The optical rotation values were measured with a JASCO DIP-SL polarimeter.

Z-D-Leu-Pro-OEt (1). To a solution of benzyloxycarbonyl-D-leucine (13.3 g, 0.05 mol) and ethyl prolinate hydrochloride (7.57 g, 0.05 mol) in chloroform (80 ml), triethylamine (5.5 g, 0.055 mol) was added at 0 °C, followed, after 1 h, by dicyclohexylcarbodiimide (10.3 g, 0.05 mol). The reaction mixture was stirred for 3 h below 0 °C and allowed to stand overnight at room temperature. After a precipitate of dicyclohexylurea was filtered off, the filtrate was concentrated to dryness under reduced pressure. The residue was dissolved in ethyl acetate and the organic layer was washed with 1M-hydrochloric acid, 10% sodium hydrogencarbonate and water, and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to give an oily product. Yield, 16.3 g (83.6%).

Z-D-Leu-Pro-OH (2). To a solution of **1** (16.3 g, 41.8 mmol) in EtOH (50 ml), 46 ml of 1M-aqueous sodium hydroxide was added with stirring at 0 °C for 3 h, followed by standing overnight at room temperature. The EtOH was removed *in vacuo* and water was added to the reaction mixture. The aqueous residue was extracted with ethyl acetate, and the alkaline aqueous layer was adjusted to pH 3 to afford a crude crystalline product (12.9 g, 81.2%) with a melting point of 98—102 °C, which was recrystallized from ethyl acetate–hexane. Yield, 10.81 g (68%); mp 103—104 °C; $[\alpha]_D^{27}$ −21.3° (1.05, abs EtOH). Found: C, 60.03; H, 7.38; N, 7.21%. Calcd for $C_{19}H_{26}O_5N_2 \cdot H_2O$: C, 59.98; H, 7.38; N, 7.36%.

Z-D-Leu-Pro-Sar-OBu^t (3). To a solution of **2** (7.24 g, 0.02 mol) in dry tetrahydrofuran (40 ml) was added triethylamine (2.02 g, 0.02 mol), followed, after 2 min, by isobutyl chloroformate (2.73 g, 0.02 mol) at −15 °C with stirring. A solution of *t*-butyl sarcosinate (2.90 g, 0.02 mol) in dry tetrahydrofuran (20 ml) was added. The solution was stirred for 2 h below 0 °C and allowed to stand overnight. The solvent was evaporated under reduced pressure and the residual oil was partitioned between ethyl acetate and water. The organic layer was washed with 1M-hydrochloric acid, 10% sodium hydrogencarbonate and water, and dried over anhydrous sodium sulfate. The ethyl acetate was evaporated *in vacuo* to give an oily product. Yield, 8.30 g (84.9%). M^+ 489.

Boc-D-Leu-Pro-OEt (5). *t*-Butoxycarbonyl-D-leucine monohydrate (8.34 g, 33.5 mmol) was dissolved in ethyl acetate (30 ml) and dry tetrahydrofuran (15 ml), and then to this solution was added dicyclohexylcarbodiimide (6.89 g, 33.5 mmol) at 0 °C. After 20 min, a solution of ethyl prolinate (4.97 g, 33.5 mmol) in ethyl acetate (10 ml) was added for a period of 20 min and the reaction mixture was stirred for 2 h at 0 °C and allowed to stand overnight at room temperature. After dicyclohexylurea was filtered off, the filtrate was concentrated to dryness *in vacuo* and the residue was dissolved in ethyl acetate. The organic layer was washed with 1M-hydrochloric acid, 10% sodium hydrogencarbonate and water, and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to give an oily product. Yield, 10.8 g (90.6%).

Boc-D-Leu-Pro-OH (6). To a solution of **5** (10.8 g, 30.3 mmol) in EtOH (31 ml) was added 32 ml of 1M-aqueous sodium hydroxide (32 mmol) for a period of 40 min with stirring at −2 °C. The solution was stirred for 2.5 h below 0 °C and allowed to stand overnight. The EtOH was removed *in vacuo* and water was added to the reaction mixture. The aqueous residue was treated with ethyl acetate, and the alkaline aqueous layer was adjusted to pH 3 to afford an oily product, which was extracted with ethyl acetate. The ethyl acetate was removed *in vacuo* to give an oily product in quantitative yield, which was isolated in the form of a crystalline substance, dicyclohexylammonium salt, from ethyl acetate–hexane; 12.58 g (81.6%); mp 168.0—170.5 °C. Recrystallization from ethyl acetate gave a pure crystalline product with a melting point of 172—173 °C; yield, 11.98 g (77.7%); $[\alpha]_D^{31}$ −23.0° (1.02, abs EtOH). Found: C, 65.91; H, 9.92; N, 8.17%. Calcd for $C_{28}H_{51}O_5N_3$: C, 65.97; H, 9.92; N, 8.24%.

For the conversion to the free acid, the dicyclohexylammonium salt 3.50 g (6.87 mmol) was dissolved in a mixture of water and ethyl acetate. After the acidification with 1M-hydrochloric acid (8 ml) the precipitate of dicyclohexylammonium hydrochloride was filtered off. The ethyl acetate extract was dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo*. Yield, 2.24 g (99.6%).

Boc-Pro-Sar-OBu^t (7). To a solution of *t*-butoxycarbonyl proline (1.29 g, 6 mmol) in dry tetrahydrofuran (5 ml) and ethyl acetate (10 ml) were added *t*-butyl sarcosinate (870 mg, 6 mmol) and dicyclohexylcarbodiimide (1.24 g, 6 mmol) with stirring at 0 °C. The reaction mixture was allowed to stand overnight at room temperature. The dicyclohexylurea precipitate was filtered off and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in ethyl acetate and the organic layer was washed with 1M-hydrochloric acid, 10% sodium hydrogencarbonate and water. After drying over anhydrous sodium sulfate, the solvent was removed *in vacuo*. A crude crystalline substance (1.66 g, 80%) with a melting point of 70—75 °C was obtained, which was recrystallized from heptane. Yield, 1.48 g (72.7%); mp 73—75 °C. The second crop was obtained from the main solution, 60 mg; mp 72—73 °C. Total yield, 1.54 g (76.8%); $[\alpha]_D^{28}$ −52.1° (1.06, abs EtOH); NMR (CCl_4): δ 1.37 (s, 9H), 1.44 (s, 9H). Found: C, 59.63; H, 9.02; N, 8.08%. Calcd for $C_{17}H_{30}O_5N_2$: C, 59.62; H, 8.83; N, 8.18%.

H-Pro-Sal-OBu^t (8). Compound **7** (300 mg, 0.877 mmol) was dissolved in 7 ml of 85% formic acid, and after the solution had been maintained for 4.5 h at 19 °C, the solvent was removed *in vacuo*. The residual oil was dissolved in chloroform and ammonia was passed through the solution. The precipitate of ammonium formate was filtered off and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in ethyl acetate and an insoluble material was filtered off. The organic layer was dried over anhydrous sodium sulfate and removed *in vacuo* to give an oily product.

Yield, 190 mg (89.6%); NMR (CCl_4): δ 1.45 (s, 9H).

Boc-D-Leu-Pro-Sar-OBu^t (9). *Method A*: To a solution of **6** (2.24 g, 6.84 mmol) in dry tetrahydrofuran (15 ml) was added triethylamine (690 mg, 6.84 mmol) and the mixture was cooled to −15 °C. To the solution, isobutyl chloroformate (934 mg, 6.84 mmol) was added followed, after 5 min, by a solution of *t*-butyl sarcosinate (1.00 g, 6.89 mmol) in dry tetrahydrofuran (1 ml). The reaction mixture was stirred for 2 h at 0 °C and allowed to stand overnight at room temperature. The solvent was removed *in vacuo* and the residue was partitioned between ethyl acetate and water, the organic layer was washed with 1 M-hydrochloric acid, 10% sodium hydrogencarbonate and water, and then dried over anhydrous sodium sulfate. The ethyl acetate was removed *in vacuo* to give a crude oil, which was subjected to column chromatography on silica gel using hexane–ethyl acetate (2: 1 v/v). Yield, 1.83 g (58.8%). NMR (CCl_4): δ 1.38 (s, 9H), 1.43 (s, 9H); M^+ 455.

Method B: To a stirred solution of *t*-butoxycarbonyl-D-leucine monohydrate (195 mg, 0.783 mmol) in dry tetrahydrofuran (2 ml) at −10 °C, was added triethylamine (79 mg, 0.783 mmol) followed by isobutyl chloroformate (107 mg, 0.783 mmol). After 5 min a solution of **8** (190 mg, 0.783 mmol) in dry tetrahydrofuran (1 ml) was stirred for 3.5 h below 0 °C and allowed to stand overnight at room temperature. The solvent was evaporated *in vacuo* and the residue was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with 1 M-hydrochloric acid, 10% sodium hydrogencarbonate and water. After drying over anhydrous sodium sulfate, the ethyl acetate was removed *in vacuo* to give a pure oil. Yield, 340 mg (95.7%). The physical properties of this oil were similar to those of the product obtained according to method A.

H-D-Leu-Pro-Sar-OBu^t (10). *Catalytic Hydrogenation Method*: Catalytic hydrogenation of **3** (1.00 g, 2.04 mmol) with palladium (50 mg) in EtOH (25 ml) afforded the desired tripeptide (**10**) contaminated with a ninhydrin-negative compound. It was found that this compound was D-leucylproline anhydride (**4**), which was confirmed by its IR spectrum and elemental analysis. mp 148—149 °C; $[\alpha]_D^{21}$ −105.5° (0.50, abs EtOH). Found: C, 62.67; H, 8.44; N, 13.04%. Calcd for $C_{11}H_{18}O_2N_2$: C, 62.83; H, 8.63; N, 13.32%.

Selective Cleavage Method: Compound **9** (3.36 g, 7.41 mmol) was dissolved in 100 ml of 85% formic acid, and after the solution had been maintained for 3.5 h at 18 °C, the solvent was removed *in vacuo*. The residual oil was dissolved in chloroform, and ammonia was passed through it. After a short cooling, the precipitate was filtered off and the filtrate was evaporated *in vacuo*. The residual oil was dissolved in ethyl acetate and an insoluble material was filtered off. The organic layer was dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to give an oily product. Yield, 2.24 g (85.9%). NMR (CCl_4): δ 1.44 (s, 9H); M^+ 355.

Boc-Ala—

Z-Thr-OH (11). *t*-Butoxycarbonyl-alanine (7.56 g, 40 mmol) and triethylamine (4.04 g, 40 mmol) were dissolved in dry tetrahydrofuran (12 ml), and the solution was cooled to −5 °C. Isobutyl chloroformate (5.44 g, 40 mmol) was added, followed, after 5 min of stirring in the cold bath, by a solution of benzyloxycarbonyl threonine (20.24 g, 80 mmol) and triethylamine (12.12 g, 120 mmol) in dry tetrahydrofuran (80 ml). The reaction mixture was allowed to stand with stirring at room temperature. The salt was filtered off and washed well with ethyl acetate. The filtrate was concentrated to dryness, and the residual oil was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with 1 M-hydrochloric acid and water and dried over anhydrous sodium sulfate. The ethyl acetate was evaporated *in vacuo* to afford an oily product. The crude reaction mixture so obtained was separated by gel filtration on Sephadex LH-20, using chloroform as the elution solvent to give the desired product (16 g), which was isolated in the form of a crystalline product, dicyclohexylammonium salt, from ethyl acetate. Yield, 15.45 g (63.8%) with a melting point of 165—166 °C; $[\alpha]_D^{32}$ −10.5° (1.04, abs EtOH). Found: C, 63.15; H, 8.62; N, 6.83%. Calcd for $C_{32}H_{51}O_8N_3$: C, 63.45; H, 8.49; N, 6.93%.

Boc-Ala—

Z-Thr-D-Leu-Pro-Sar-OBu^t (12). Triethylamine (674 mg, 6.67 mmol) and **11** (2.83 g, 6.67 mmol) were dissolved in dry tetrahydrofuran (30 ml) and the solution was cooled to −15 °C with stirring. Isobutyl chloroformate (911 mg, 6.67 mmol) was added, followed, after 5 min of stirring in the cold bath, by a solution of **10** (2.24 g, 6.36 mmol) in dry tetrahydrofuran (20 ml). The reaction mixture was kept at −10 °C for 1 h, then at 0 °C for 1.5 h and allowed to stand overnight at room temperature. The residual oil was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with 1 M-hydrochloric acid, 10% sodium hydrogencarbonate and water and dried over anhydrous sodium sulfate. Evaporation afforded a crude oily product (4.51 g) and this oil was subjected to column chromatography on silica gel using ethyl acetate–hexane (2: 1 v/v) to give an 80% yield of the desired product (3.88 g); $[\alpha]_D^{28}$ −36.4° (1.20, abs EtOH); NMR (CCl_4): δ 1.37 (s, 9H), 1.47 (s, 9H); M^+ 761. Found: C, 59.60; H, 7.91; N, 8.95%. Calcd for $C_{38}H_{59}O_{11}N_5 \cdot 1/2H_2O$: C, 59.20; H, 7.84; N, 9.06%. Amino acid analysis showed the presence of threonine, sarcosine, proline, leucine and alanine in ratios of 0.8: 1.2: 1.3: 0.8: 1.0.

Boc-Leu-Ala-OMe (14). Methyl alaninate hydrochloride (3.14 g, 25 mmol) was suspended in chloroform (30 ml), followed by the addition of triethylamine (2.78 g, 27.5 mmol) at 0 °C. To the solution, dicyclohexylcarbodiimide (5.15 g, 25 mmol) were added. After cooling in a cold bath for 2 h, the reaction mixture was allowed to stand overnight. Dicyclohexylurea was filtered off and the organic layer was washed with 1 M-hydrochloric acid, 10% sodium hydrogencarbonate and water and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to give a crystalline substance, (6.80 g, 86%) with a melting point of 113—115 °C, which was recrystallized from benzene–hexane. Yield, 6.36 g (80.5%); mp 113—115 °C; $[\alpha]_D^{28}$ −48.3° (1.20, abs MeOH). Found: C, 57.10; H, 8.84; N, 8.67%. Calcd for $C_{15}H_{28}O_5N_2$: C, 56.94; H, 8.92; N, 8.85%.

Boc-Leu-Ala-N_2H_3 (15). To a solution of **14** (3.9 g, 12.3 mmol), was added hydrazine monohydrate (2.4 g, 48 mmol) in MeOH (10 ml), and the solution was allowed to stand overnight at room temperature. Evaporation *in vacuo* afforded the crude product (3.77 g, 96.7%) with a melting point of 175—177 °C, which was recrystallized from ethyl acetate. Yield, 3.46 g (88.7%); mp 178—179 °C; $[\alpha]_D^{24}$ −51.9° (0.94, abs MeOH). Found: C, 52.90; H, 8.89; N, 17.37%. Calcd for $C_{14}H_{28}O_4N_4$: C, 53.14; H, 8.92; N, 17.74%.

H-Ala—

Z-Thr-D-Leu-Pro-Sar-OBu^t (13). Compound **12** (1.20 g, 1.58 mmol) was dissolved in 60 ml of 85% formic acid and then the solution was maintained for 2 h at 18—19 °C. The solvent was removed *in vacuo* and the residual oil was partitioned between ethyl acetate and water. Sodium hydrogencarbonate was added to the aqueous layer producing alkaline solution, and the isolated oil was extracted with ethyl acetate. After drying the solvent was removed *in vacuo* to give the desired product. Yield, 900 mg (86.5%); NMR (CCl_4):

δ 1.47 (s, 9H); M^+ 661.

Boc-Leu-Ala-Ala-Z-Thr-D-Leu-Pro-Sar-OBu^t (**17**). To a solution of **15** (1.062 g, 3.36 mmol) in dry *N,N*-dimethylformamide (18 ml) was added 2.18 ml of 4.62 M-HCl in dioxane (10.08 mmol) at −50 °C, followed by isopentyl nitrite (0.47 ml, 3.36 mmol). After the temperature had been raised to −20 °C and kept there for 30 min, the solution was again cooled to −50 °C, and triethylamine (1.01 g, 10.08 mmol) was added. A solution of **13** (2.22 g, 3.36 mmol) in dry *N,N*-dimethylformamide (10 ml) was added dropwise at −20 °C. After stirring at 0 °C for 70 h, the solvent was removed *in vacuo* and the residual oil was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with 1M-hydrochloric acid, 10% sodium hydrogencarbonate and water. The solvent was evaporated to dryness under reduced pressure to give a crude product. The crude oil was subjected to column chromatography on silica gel using benzene-MeOH (20: 1 v/v) to afford a 90.5% yield of the desired pure product (2.88 g); $[\alpha]_D^{28}$ −37.5° (1.04, abs EtOH); NMR (CCl_4): δ 1.38 (s, 9H), 1.44 (s, 9H). Found: C, 59.05; H, 8.03; N, 10.53%. Calcd for $C_{47}H_{75}O_{13}N_7 \cdot 1/2H_2O$: C, 59.09; H, 7.92; N, 10.27%. Amino acid analysis showed the presence of threonine, sarcosine, proline, alanine and leucine in ratios of 0.8: 0.7: 1.0: 2.0: 1.5.

Z-Thr-D-Leu-Pro-Sar-Ala-Ala-Leu (**19**). Compound **17** (1.10 g, 1.16 mmol) was dissolved in 15 ml of trifluoroacetic acid and allowed to stand for 1 h at room temperature. Excess trifluoroacetic acid was removed *in vacuo* and the residue was triturated with dry ether to give a salt (**18**) in a 92.3% yield (975 mg). The salt (600 mg, 0.664 mmol) was suspended in dry dichloromethane (10 ml) and triethylamine (133 mg, 1.32 mmol) was added with ice cooling, after 50 min, followed by evaporation and drying under reduced pressure. The residue was taken up in 1.2 l of dry dichloromethane and cooled to 0 °C. *N*-Hydroxysuccinimide (381 mg, 3.32 mmol) was added and after a short delay, a solution of EDCI (515 mg, 3.32 mmol) in dry dichloromethane (10 ml) was added. The reaction mixture was stirred for 30 h at 0 °C and then the stirring was continued for 60 h at 5 °C. The solution was evaporated *in vacuo* and the residue was partitioned with ethyl acetate and water. The organic layer was washed with 0.1 M hydrochloric acid, 10% sodium hydrogencarbonate and water, dried over anhydrous sodium sulfate, and evaporated. The product was subjected to preparative TLC first using ethyl acetate-EtOH (6: 1 v/v) and once more using benzene-EtOH (5: 2 v/v). The desired product was obtained in a 44.5% yield (233 mg) which was recrystallized from ethyl acetate-hexane to give a crystalline product with a melting point of 176—178 °C. Yield, 224 mg; $[\alpha]_D^{24}$ +59.7° (1.17, abs EtOH); M^+ 771. Found: C, 58.01; H, 7.60; N, 12.41%. Calcd for $C_{38}H_{57}O_{11}N_7 \cdot H_2O$: C, 57.78; H, 7.27; N, 12.41%.

Hypic-Thr-D-Leu-Pro-Sar-Ala-Ala-Leu (**21**). Compound **19** (70 mg, 0.09 mmol) was dissolved in abs EtOH (15 ml) containing 0.04 ml of 4.62 M-HCl in dioxane and hydrogenated for 1 h over 10% palladium on charcoal (30 mg). The catalyst was filtered off and the solution was evaporated *in vacuo* to give a salt (**20**). 3-Benzyloxypicolinic acid sesquihydrate hydrochloride[19] (30 mg, 0.1 mmol) was suspended in 2 ml of dichloromethane followed by the addition of triethylamine (40 mg) with ice cooling. After 1 h the solution was evaporated *in vacuo* and dried. The residue was taken up in 1 ml of dry tetrahydrofuran followed by the addition of HOBt (13.5 mg, 0.1 mmol) and dicyclohexylcarbodiimide (20.6 mg, 0.1 mmol) and stirring for 1 h at 0 °C. To the solution prepared above was added a solution of **20** in tetrahydrofuran (1 ml) and a solution of triethylamine (9.1 mg, 0.09 mmol) in dry tetrahydrofuran (5 ml) was added dropwise over a 2.5 h period. The reaction mixture was stirred for 5 h at room temperature and evaporated. The residue was subjected to preparative TLC using benzene-EtOH (100: 35 v/v) to give the protected product in a 68.1% yield (52 mg), which was hydrogenated for 1 h over 10% palladium on charcoal (40 mg). After filtration of the catalyst the solvent was evaporated *in vacuo* to give the desired product in a 64.5% yield (44 mg); $[\alpha]_D^{25}$ +40.8° (0.98, CH_2Cl_2); IR (KBr): 1740, 1630, 1520 cm^{-1}; λ_{max} 304 nm (log ε 3.77).[12,13] Found: C, 54.87; H, 7.12; N, 14.44%. Calcd for $C_{36}H_{54}O_{10}N_8 \cdot 3/2H_2O$: C, 55.01; H, 7.31; N, 14.25%. The product showed a single spot using various solvents with TLC. Amino acid analysis showed the presence of threonine, sarcosine, proline, leucine, and alanine in ratios of 0.9: 1.0: 1.0: 1.9: 2.0.

References

1) H. Kinoshita and H. Kotake, *Chem. Lett.*, **1974**, 631.

2) The abbreviations used in this work are those recommended by the IUPAC-IUB commision on Biochemical Nomenclature, as published in *J. Biol. Chem.*, **247**, 977 (1972): EDCI for 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, M. A. for mixed anhydride method, HOBt for 1-hydroxybenzotriazole, and Hypic for 3-hydroxypicolinyl.

3) H. Vanderhaeghe and G. Parmentier, *J. Am. Chem. Soc.*, **82**, 4414 (1960).

4) M. Bodanszky and M. A. Ondetti, *Antimicro. Agents Chemother.*, **1963**, 360.

5) J. C. Sheehan, H. G. Zachau, and W. B. Lawson, *J. Am. Chem. Soc.*, **80**, 3349 (1958).

6) H. Otsuka and J. Shoji, *Tetrahedron*, **21**, 2931 (1965).

7) H. Brockmann, G. Bohnsack, B. Franck, H. Groene, H. Muxfeldt, and C. Sueling, *Angew. Chem.*, **68**, 70 (1956).

8) W. Keller-Schierlein, M. L. Mihailovic, and V. Prelog, *Helv. Chim. Acta*, **42**, 305 (1959).

9) H. Brockmann and H. Lackner, *Naturwissenschaften*, **47**, 230 (1960).

10) H. Brockmann and H. Lackner, *Naturwissenschaften*, **51**, 381, 407, 435 (1964).

11) J. Meienhofer, *J. Am. Chem. Soc.*, **92**, 3771 (1970).

12) J. C. Sheehan and S. L. Ledis, *J. Am. Chem. Soc.*, **95**, 875 (1973).

13) M. A. Ondetti and P. L. Thomas, *J. Am. Chem. Soc.*, **87**, 4375 (1965).

14) J. Honzl and J. Rudinger, *Collect. Czech. Chem. Commun.*, **26**, 2333 (1961).

15) T. Wieland, C. Birr, and F. Flor, *Ann.*, **727**, 130 (1969).

16) S. Guttmann and R. A. Boissonnas, *Helv. Chim. Acta*, **41**, 1853 (1958).

17) D. Theodoropoulos, H. Bennich, G. Foelsh, and O. Mellander, *Nature*, **184**, 270 (1959).

18) H. Kinoshita and H. Kotake, *Bull. Chem. Soc. Jpn.*, **43**, 3609 (1970).

19) J. C. Sheehan, *J. Org. Chem.*, **31**, 636 (1966).

20) Nippon Bunseki Kagaku Kai, "Bunseki Kagaku Binran," Maruzen, Tokyo (1961), p. 553.