Synthesis of Sequential Polypeptides Containing L-Isoleucine for Assignment of the Far-i.r. Band Characteristic of Isoleucyl in a Peptide α-Helix

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Sequential co-polypeptides containing L-isoleucine and L-methionine or L-alanine $(Met\text{-}Ile\text{-}Met)_n$, $(Ala\text{-}Ile\text{-}Ala)_n$, $(Ala\text{-}Ala\text{-}Ile\text{-}Ala)_n$, and $(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$ have been synthesized by polycondensation of peptide *N*-hydroxysuccinimide (ONSu) esters with corresponding sequences of amino acids. The tripeptide ONSu esters were prepared by the conventional stepwise method for peptide synthesis, while the tetrapeptide ONSu esters were synthesized by a so-called 'back-up procedure' from the tripeptide ONSu esters. The sequential co-polypeptides with the α-helical conformation showed a common far-i.r. band near 453 cm^{-1}, the strength of which changed in accordance with a change in the proportion of L-isoleucine in the polypeptides. Thus, the band at near 453 cm^{-1} was assigned to that characteristic of L-isoleucine in a peptide with an α-helical conformation.

In a previous paper,[1] we reported the assignment of a far-i.r. absorption band at 409 cm^{-1} as characteristic of L-methionine in a peptide with an α-helical conformation by analysing the far-i.r. spectra of sequential co-polypeptides of this amino acid with L-alanine and L-leucine. This assignment is based on the fact that the far-i.r. band was commonly observed for both polypeptides containing L-methionine and L-alanine or L-leucine, and that its strength changed as the proportion of L-methionine residue present changed.

In this study, we have extended this approach for assignment of far-i.r. bands characteristic of amino acids to L-isoleucine, which has an interesting conformational preference: i.e., it does not give rise to an α-helical conformation but, instead, forms a homopolypeptide with a β-structure. This is a result of the steric hindrance arising from side-chain branching at the β-carbon. L-Isoleucine may be incorporated, however, into the α-helices of proteins[2] and co-polypeptides.[3] Thus, in order to obtain α-helical polypeptides containing L-isoleucine, we have synthesized sequential co-polypeptides containing this amino acid together with either L-methionine or L-alanine which have a very strong tendency to form stable α-helices.[4] Since the far-i.r. bands characteristic of L-methionine and L-alanine in peptides with the α-helical conformation have been clearly assigned,[1,5] a new band characteristic of L-isoleucine in such polypeptides could be found by analysis of the far-i.r. spectra of these α-helical sequential co-polypeptides.

Results and Discussion

Sequential co-polypeptides containing L-isoleucine and L-methionine or L-alanine $(Met\text{-}Ile\text{-}Met)_n$, $(Ala\text{-}Ile\text{-}Ala)_n$, $(Ala\text{-}Ala\text{-}Ile\text{-}Ala)_n$, and $(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$ were prepared by polycondensation of peptide active *N*-hydroxysuccinimide (ONSu) esters having corresponding sequences of amino acids. The peptide ONSu esters having tripeptide sequences were prepared by the conventional procedure for peptide synthesis: stepwise synthesis of protected tripeptide ethyl esters, saponification of the ethyl ester of the protected tripeptide derivatives to give free acids, and active esterification of the tripeptide free acids with HONSu in the presence of dicyclohexylcarbodi-imide (DCC). The tetrapeptide ONSu esters were prepared by a modification of the conventional procedure. Since tetrapeptide derivatives containing L-isoleucine and L-alanine are poorly soluble in solvents of low polarity, saponification of the ethyl ester, followed by the active esterification of the resulting free acids, must be carried out in a polar solvent such as *N,N*-dimethylformamide (DMF); in such solvents, however, there is an increased danger of racemization of the C-terminal L-alanine. The tetrapeptide ONSu esters were, therefore, prepared by a so-called 'back-up procedure',[6] which involves rapid reaction by the mixed anhydride method, of an *N*-protected amino acid with a tripeptide ONSu ester. By this procedure the tripeptide derivatives which are soluble in less polar solvents were both saponified and esterified, under conditions where risk of racemization was reduced.

The syntheses of peptide ONSu esters protected by the 2-nitrophenylsulphenyl (Nps) group are illustrated by the successful synthesis of Nps-Ala-Ile-Ala-ONSu (**6**) and Nps-Ala-Ala-Ile-Ala-ONSu (**8**). L-Alanine ethyl ester hydrochloride (**1**) was allowed to react with Nps-L-isoleucine dicyclohexylammonium salt in the presence of DCC to give a dipeptide derivative Nps-L-isoleucyl-L-alanine ethyl ester (**2**), which was treated with hydrochloric acid in dioxane to yield L-isoleucyl-L-alanine ethyl ester hydrochloride (**3**). The hydrochloride (**3**) was allowed to react with Nps-L-alanine dicyclohexylammonium salt to give Nps-L-alanyl-L-isoleucyl-L-alanine ethyl ester (**4**). The ethyl ester of (**4**) was saponified in tetrahydrofuran–methanol by sodium hydroxide to give a free acid Nps-L-alanyl-L-isoleucyl-L-alanine (**5**), which was treated with HONSu in the presence of DCC in tetrahydrofuran to give an active ester Nps-L-alanyl-L-isoleucyl-L-alanine ONSu ester (**6**).

The tetrapeptide active ester Nps-L-alanyl-L-alanyl-L-isoleucyl-L-alanine ONSu ester was prepared from (**6**) by the back-up procedure. The tripeptide ONSu ester (**6**) was treated with hydrochloric acid in dioxane to give a tripeptide ONSu ester hydrochloride (**7**), which was allowed to react with Nps-L-alanine in the presence of isobutyl chlorocarbonate to give the tetrapeptide ONSu ester (**8**) (see Scheme).

Each intermediate and each final product was purified by recrystallization until chromatographically pure. Results of the syntheses and analytical data of the products are listed in Table 1.

Sequential co-polypeptides were synthesized by polycondensation of the peptide ONSu esters. The Nps-peptide ONSu esters listed in Table 1 were treated with hydrochloric acid to give peptide ONSu ester hydrochlorides, which were dissolved in dimethyl sulphoxide at a high concentration. Addition of triethylamine to the concentrated solution of the peptide ONSu esters initiated the polycondensations. After 2 days, the polymers, obtained by dilution of the polymerization systems with water, were collected on a glass filter, washed with methanol, and reprecipitated from dichloroacetic acid with diethyl ether. Results of the syntheses and analytical data of the polymers are summarized in Table 2.

HCl·H-Ala-OEt (1)
↓ Nps-Ile-OH·DCHA, DCC
Nps-Ile-Ala-OEt (2)
↓ HCl
HCl·H-Ile-Ala-OEt (3)
↓ Nps-Ala-OH·DCHA, DCC
Nps-Ala-Ile-Ala-OEt (4)
↓ OH^-, H^+
Nps-Ala-Ile-Ala-OH (5)
↓ HONSu, DCC
Nps-Ala-Ile-Ala-ONSu (6)
↓ HCl
HCl·H-Ala-Ile-Ala-ONSu (7)
↓ Nps-Ala-OH, iBoc-Cl
Nps-Ala-Ala-Ile-Ala-ONSu (8)

Scheme

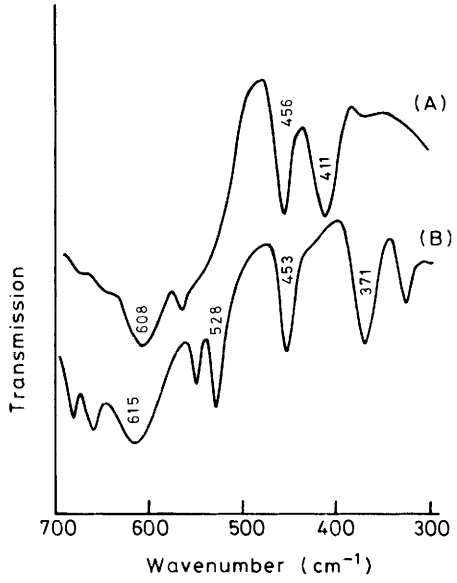

Figure 1. Far-i.r. spectra of sequential co-polypeptides with an α-helical conformation containing L-isoleucine with L-methionine or L-alanine: (A), $(Met\text{-}Ile\text{-}Met)_n$; (B), $(Ala\text{-}Ile\text{-}Ala)_n$.

The conformations of the sequential co-polypeptides thus obtained were analysed both by i.r. spectroscopy in the amide I and II region and *X*-ray powder diffraction measurements. All the polypeptides showed the amide I band at 1 651 to 1 656 cm^{-1} and the amide II band at 1 541 to 1 544 cm^{-1}; these are characteristic of α-helical or random coil conformations of polypeptides. The polypeptides showed an *X*-ray diffraction pattern having two prominent peaks characteristic of the α-helix for polypeptides, and the strongest peak that is assigned to the (100) plane of the hexagonal unit cell of the peptides[7] was observed at $2\theta = 8.4$, 9.5, and 10.2° for $(Met\text{-}Ile\text{-}Met)_n$, $(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$ and $(Ala\text{-}Ala\text{-}Ile\text{-}Ala)_n$, and $(Ala\text{-}Ile\text{-}Ala)_n$, respectively. These results suggest clearly that the sequential co-polypeptides containing L-isoleucine adopt the α-helical conformation.

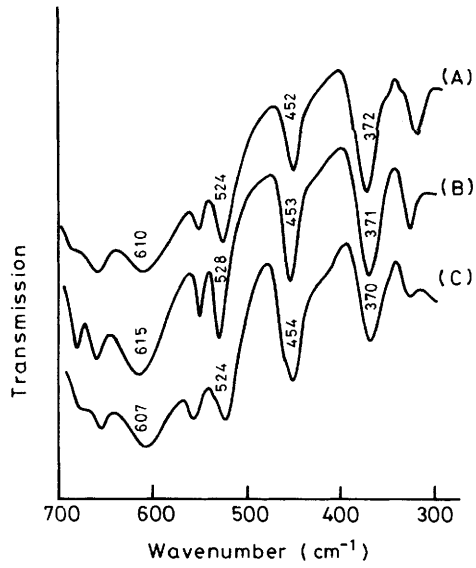

Figure 2. Far-i.r. spectra of sequential co-polypeptides with an α-helical conformation containing L-isoleucine and L-alanine: (A), $(Ala\text{-}Ala\text{-}Ile\text{-}Ala)_n$; (B), $(Ala\text{-}Ile\text{-}Ala)_n$; (C), $(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$.

Figure 1 shows far-i.r. spectra of two kinds of sequential co-polypeptides containing L-isoleucine $(Met\text{-}Ile\text{-}Met)_n$ and $(Ala\text{-}Ile\text{-}Ala)_n$ which adopt the α-helical conformation. A co-polypetide consisting of L-isoleucine and L-methionine showed a far-i.r. spectrum having strong bands at 608, 456, and 411 cm^{-1}. The band at 608 cm^{-1}, amide V band, is characteristic of the α-helix[8] and the band at 411 cm^{-1} is characteristic of L-methionine in this conformation.[1] Another co-polypeptide consisting of L-isoleucine and L-alanine showed a far-i.r. spectrum having bands at 615, 528, 453, and 371 cm^{-1}. The band at 615 cm^{-1} is characteristic of the amide V with the α-helix, and the bands at 528 and 371 cm^{-1} are characteristic of L-alanine in peptides of this conformation.[5] Both co-polypeptides showed a new band at near 455 cm^{-1}. This band, common for different polypeptides containing L-isoleucine as a component, may be assigned to that characteristic of L-isoleucine in peptides with an α-helical conformation. This possible assignment was demonstrated by analysis of far-i.r. spectra of sequential co-polypeptides containing different proportions of L-isoleucine to L-alanine $(Ala\text{-}Ala\text{-}Ile\text{-}Ala)_n$, $(Ala\text{-}Ile\text{-}Ala)_n$, and $(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$, which are shown in Figure 2. These co-polypeptides showed similar far-i.r. spectra having common bands at 607—615, 524—528, 452—454, and 370—372 cm^{-1}. It should be noted that the strength of the band near 453 cm^{-1} increased in accordance with increasing proportion of the L-isoleucine residue in the polypeptides from $(Ala\text{-}Ala\text{-}Ile\text{-}Ala)_n$ to $(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$, and the bands at near 525 and 371 cm^{-1} characteristic of the L-alanine residue with the α-helical conformation decreased relatively in accordance with the decreasing proportion of this amino acid residue. These facts obtained by analyses of the far-i.r. spectra shown in Figures 1 and 2 clearly show that the band at 453 cm^{-1} is characteristic of L-isoleucine in peptides with an α-helical conformation.

Experimental

M.p.s are uncorrected and were determined using a Yamato MP-21 apparatus. Optical rotations at the sodium D line were measured with a Jasco DIP-SL polarimeter. *X*-Ray powder diffractions were recorded with Rigaku GF-2012 *X*-ray diffractometer. I.r. spectra in the amide I and II, and far-i.r.

Table 1. Analytical data for the *N*-protected monomers

Compound	Yield[a] (%)	M.p. (decomp.) (°C)	R_F	$[\alpha]_D$[e]	Found (%) (Required) C	H	N
Nps-Met-Ile-Met-ONSu	52	143—146	0.62[b]	−17.4	48.3 (48.5)	5.65 (5.75)	11.0 (10.9)
Nps-Ala-Ile-Ala-ONSu	53	195—197	0.56[c]	−36.0	50.0 (50.5)	5.6 (5.55)	13.4 (13.4)
Nps-Ala-Ile-Ile-Ala-ONSu	39	195—199	0.71[d]	−35.8	53.2 (52.8)	6.4 (6.3)	13.3 (13.2)
Nps-Ala-Ala-Ile-Ala-ONSu	43	205—208	0.56[d]	−49.6	50.0 (50.5)	5.8 (5.7)	14.0 (14.1)

[a] The yields are the values from the starting amino acid ethyl esters. [b] Eluant: tetrahydrofuran–benzene (1 : 2). [c] Eluant: tetrahydrofuran–benzene (1 : 1). [d] Eluant: tetrahydrofuran–benzene (2 : 1). [e] (*c* 0.5 In *N*,*N*-dimethylformamide).

Table 2. Analytical data for the sequential polypeptides.

Polypeptide	Yield (%)	[η] in DCA	Found (%) C	H	N	Required (%) C	H	N
$(Met\text{-}Ile\text{-}Met)_n$	88	0.27	51.3	7.8	11.3	51.2	7.7	11.2
$(Ala\text{-}Ile\text{-}Ala)_n$	92	0.28	55.8	8.3	16.4	56.6	8.2	16.5
$(Ala\text{-}Ile\text{-}Ile\text{-}Ala)_n$	78	0.25	57.6	8.6	14.7	58.7	8.7	15.2
$(Ala\text{-}Ala\text{-}Ile\text{-}Ala)_n$	80	0.20	54.1	8.2	16.7	55.2	8.0	17.2

regions were recorded for KBr disks with a Jasco A-702 spectrophotometer controlled by a microcomputer Jasco DP-A330.

Nps-Ile-Ala-OEt.—L-Alanine ethyl ester hydrochloride (15.4 g, 0.1 mol) and 2-nitrophenylsulphenyl-L-isoleucine dicyclohexylammonium salt (46.5 g, 0.1 mol) were dissolved in chloroform (300 ml). The solution was cooled at −10 °C and DCC (22.7 g, 0.11 mol) was added with stirring. The reaction mixture was then stirred for 2 h at −10 °C and for 2 h at room temperature. It was then diluted with ethyl acetate (300 ml) and the resulting crystals were filtered off. The filtrate was concentrated under reduced pressure to give a solid, which was dissolved in ethyl acetate (400 ml). The undissolved crystals of the urea were filtered off and the filtrate was washed with 5% citric acid, water, 5% $NaHCO_3$, and water, and then dried (Na_2SO_4). Evaporation of the solvent gave crystals, which were recrystallized from ethyl acetate–hexane (35.6 g, 93%), m.p. 126—127 °C, R_F 0.71 (ethyl acetate–benzene 1 : 1), $[\alpha]_D$ −94.2° (*c* 0.5 in tetrahydrofuran) (Found: C, 53.5; H, 6.6; N, 11.1. $C_{17}H_{25}N_3O_5S$ requires C, 53.25; H, 6.6; N, 11.0%).

Nps-Ala-Ile-Ala-OEt.—Nps-Ile-Ala-OEt (30.6 g, 0.08 mol) was dissolved in 3M-hydrochloric acid in dioxane (54 ml). To the solution was added diethyl ether (200 ml) and hexane (200 ml) to precipitate the dipeptide ethyl ester hydrochloride. The system was then allowed to stand overnight in a refrigerator when the oily precipitate crystallized. The crystals were collected on a glass filter, washed with diethyl ether until they were no longer yellow, and then dissolved in chloroform (300 ml). 2-Nitrophenylsulphenyl-L-alanine dicyclohexylammonium salt (33.8 g, 0.08 mol) was added to the solution which was then cooled to −10 °C. To the solution was added DCC (16.5 g, 0.08 mol). The solution was stirred for 3 h at −10 °C and for 2 h at room temperature. The resulting crystals were filtered off, and the filtrate was washed with 5% citric acid, water, 5% $NaHCO_3$, and water, and dried (Na_2SO_4). Evaporation of the solvent gave a solid, which was recrystallized from tetrahydrofuran–hexane (29.8 g, 82%), m.p. 193—194 °C, R_F 0.72 (tetrahydrofuran–benzene, 1 : 1), $[\alpha]_D$ −81.3° (*c* 0.5 in tetrahydrofuran) (Found: C, 53.2; H, 6.6; N, 12.3. $C_{20}H_{30}N_4O_6S$ requires C, 52.85; H, 6.65; N, 12.3%).

Nps-Ile-Ile-Ala-OEt.—This compound was prepared by the same procedure as for Nps-Ala-Ile-Ala-OEt from Nps-Ile-Ala-OEt and Nps-Ile-OH·DCHA (78%), m.p. 199—200 °C, R_F 0.84 (tetrahydrofuran–benzene, 1 : 1), $[\alpha]_D$ −80.3° (*c* 0.5 in tetrahydrofuran) (Found: C, 56.0; H, 7.4; N, 11.1. $C_{23}H_{36}N_4O_6S$ requires C, 55.6; H, 7.3; N, 11.3%).

Nps-Ala-Ile-Ala-OH.—To a solution of Nps-Ala-Ile-Ala-OEt (22.7 g, 0.05 mol) in tetrahydrofuran–methanol (2 : 1) (150 ml) was added 1M-sodium hydroxide (50 ml). The mixture was then stirred for 1 h at room temperature. After addition of diethyl ether (100 ml), the organic layer was separated off and the aqueous layer was acidified with 10% citric acid and extracted with ethyl acetate (2 × 150 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to give an oil, which crystallized upon addition of hexane. The crude product was recrystallized from ethyl acetate (20.3 g, 95%), m.p. 197—202 °C (decomp.); R_F 0.63 (ethyl acetate–methanol 1 : 1), $[\alpha]_D$ −75.2° (*c* 0.5 in tetrahydrofuran) (Found: C, 49.7; H, 6.2; N, 12.8. $C_{18}H_{26}N_4O_6S$ requires C, 50.7; N, 6.15; N, 13.1%).

Nps-Ile-Ile-Ala-OH.—Nps-Ile-Ile-Ala-OEt (24.8 g, 0.05 mol) was saponified by the procedure described above to give the free acid (20.8 g, 89%), m.p. 206—208 °C (decomp.), R_F 0.60 (ethyl acetate–methanol, 1 : 1), $[\alpha]_D$ −74.4° (*c* 0.5 in tetrahydrofuran) (Found: C, 53.5; H, 7.0; N, 11.8. $C_{21}H_{32}N_4O_6S$ requires C, 53.8; H, 6.9; N, 12.0%).

Nps-Ala-Ile-Ala-ONSu.—Nps-Ala-Ile-Ala-OH (17.0 g, 0.04 mol) and *N*-hydroxysuccinimide (9.2 g, 0.08 mol) were dissolved in tetrahydrofuran (200 ml) and the solution was cooled at −10 °C. DCC (9.8 g, 0.048 mol) was then added to the solution with stirring and the latter was continued for 3 h at −10 °C and overnight at 0 °C. The solution was diluted with diethyl ether (400 ml) to give a precipitate, which was collected on a glass filter, washed with diethyl ether, and dried. The crude product

was dissolved in DMF and insoluble crystals were removed by filtration. To the filtrate was added diethyl ether (500 ml) to give crystals. The product was recrystallized from DMF–diethyl ether (15.3 g, 73%).

Nps-Ile-Ile-Ala-ONSu.—This active ester was prepared from Nps-Ile-Ile-Ala-OH by the same procedure as above (71%), m.p. 189—193 °C (decomp.), R_F 0.73 (tetrahydrofuran–benzene 1:1); $[\alpha]_D$ −50.0° (*c* 0.5 in DMF) (Found: C, 53.1; H, 6.3; N, 12.3. $C_{23}H_{35}N_5O_8S$ requires C, 53.1; H, 6.2; N, 12.4%).

Nps-Ala-Ala-Ile-Ala-ONSu.—Nps-Ala-Ile-Ala-ONSu (15.7 g, 0.03 mol) was dissolved in 1,1,1,3,3,3-hexafluoropropan-2-ol (30 ml) and 3M-hydrochloric acid in dioxane (20 ml) was added. Addition of diethyl ether (300 ml) to the solution gave crystals of the tripeptide ONSu ester hydrochloride which were collected on a glass filter and washed with diethyl ether. The product was recrystallized from DMF–diethyl ether to give white crystals. The tripeptide ONSu ester hydrochloride thus obtained was dissolved in DMF (100 ml) and the solution was cooled at −10 °C. Separately, Nps-L-alanine (14.5 g, 0.06 mol) was dissolved in tetrahydrofuran (100 ml) and *N*-methylmorpholine (6.6 ml, 0.06 mol) was added. The solution was cooled at −10 °C and to it was added isobutyl chlorocarbonate (7.9 ml, 0.06 mol) with vigorous stirring. The stirring was continued for 5 min at −10 °C. To the solution was added the pre-cooled solution of the tripeptide ONSu ester hydrochloride in DMF followed by dropwise additon of a solution of triethylamine (4.6 ml, 0.033 mol) in tetrahydrofuran (50 ml). After 1 h, the solution was diluted with water (700 ml) and the resulting crystals were collected on a glass filter, washed with water and diethyl ether, and dried *in vacuo*. The crude product was recrystallized from DMF–diethyl ether.

Nps-Ala-Ile-Ile-Ala-ONSu.—This active ester was prepared by the same procedure as above from Nps-Ile-Ile-Ala-ONSu and Nps-L-alanine.

Nps-Ile-Met-OEt.—This compound was prepared by the reaction of L-methionine ethyl ester hydrochloride (21.3 g, 0.1 mol) with Nps-L-isoleucine dicyclohexylammonium salt (46.5 g, 0.1 mol) by the same procedure as for Nps-Ile-Ala-OEt. The crude product was recrystallized from diethyl ether–hexane (90%), m.p. 110—111 °C, R_F 0.72 (ethyl acetate–benzene 1:1), $[\alpha]_D$ −70.6° (*c* 0.5 in tetrahydrofuran) (Found: C, 52.1; H, 6.6; N, 9.7. $C_{19}H_{29}N_5O_5S_2$ requires C, 51.5; H, 6.5; N, 9.5%).

Nps-Met-Ile-Met-OEt.—This tripeptide derivative was prepared by the reaction of L-isoleucyl-L-methionine ethyl ester hydrochloride (26.1 g, 0.08 mol) with Nps-L-methionine dicyclohexylammonium salt (38.6 g, 0.08 mol) by the same procedure as for Nps-Ala-Ile-Ala-OEt (79%), m.p. 157—158 °C, R_F 0.69 (ethyl acetate–benzene, 1:1), $[\alpha]_D$ −39.8° (*c* 0.5 in tetrahydrofuran) (Found: C, 50.8; H, 6.7; N, 9.8. $C_{24}H_{38}N_4O_6S_3$ requires C, 50.2; H, 6.6; N, 9.8%.

Nps-Met-Ile-Met-OH.—Nps-Met-Ile-Met-OEt (28.7 g, 0.05 mol) was saponified by the same procedure as for Nps-Ala-Ile-Ala-OH to give this free acid (92%), m.p. 159—160 °C, R_F 0.72 (ethyl acetate–methanol, 1:1), $[\alpha]_D$ −33.4° (*c* 0.5 in tetrahydrofuran) (Found: C, 48.7; H, 6.2; N, 10.25. $C_{22}H_{34}N_4O_6S_3$ requires C, 48.4; H, 6.2; N, 10.3%).

Nps-Met-Ile-Met-ONSu.—Nps-Met-Ile-Met-OH (16.4 g, 0.03 mol) was esterified with *N*-hydroxysuccinimide (6.9 g, 0.06 mol) in the presence of DCC (6.8 g, 0.033 mol) by the same procedure as for Nps-Ala-Ile-Ala-ONSu (80%).

Synthesis of Polypeptides.—The Nps-peptide ONSu ester (0.01 mol) was dissolved in 1,1,1,3,3,3-hexafluoropropan-2-ol (20 ml) and 3M-hydrochloric acid (7 ml) was added. To the solution was added diethyl ether (300 ml) to precipitate the peptide ONSu ester hydrochloride, which was collected on a glass filter and washed with diethyl ether until it was no longer yellow. The white solid was dissolved at a concentration 1 g/15 ml in dimethyl sulphoxide. To the solution was added triethylamine (1.68 ml, 0.12 mol) with vigorous stirring. The stirring was continued for 2 days at room temperature after which the system was diluted with water (300 ml) to precipitate the polymer. This was collected on a glass filter, washed with methanol and diethyl ether, and dried. The polymer thus obtained was dissolved in dichloroacetic acid (5 ml) and reprecipitated by addition of diethyl ether.

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