Chem. Pharm. Bull. 29(5)1390—1397(1981)

Synthesis of the Eicosapeptide corresponding to the N-Terminal Amino Acid Sequence of the Major Component of Human Lymphoblastoid Interferon[1)]

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(Received November 11, 1980)

An eicosapeptide, H-Ser-Asp-Leu-Pro-Gln-Thr-His-Ser-Leu-Gly-Asn-Arg-Arg-Ala-Leu-Ile-Leu-Leu-Ala-Gln-OH, corresponding to the N-terminal amino acid sequence of the major component of human lymphoblastoid interferon was synthesized by a conventional method. The final deprotected peptide was purified by gel filtration on Sephadex G-25 and then by partition chromatography on Sephadex G-25. The eicosapeptide at a dose of 100 μg/ml inhibited the incorporation of ^{3}H-thymidine into DNA of PHA-induced lymphocytes by about 15%.

Keywords——human lymphoblastoid interferon; PHA-induced lymphocyte transformation; HOBT-DCC procedure; HONB-DCC procedure; ^{3}H-thymidine incorporation

Interferons are glycoproteins that are produced by various vertebrate cells in response to viral infection or some other stimuli.[2–5)] Interferons have been shown to inhibit DNA synthesis in lymphocytes stimulated by T- and B-cell mitogens.[2–4)] Despite its potential as an antiviral and antitumor agent,[6)] interferon (discovered in 1957[7)]) has remained uncharacterized for 23 years as regards its primary structure. Since the structure of the N-terminal

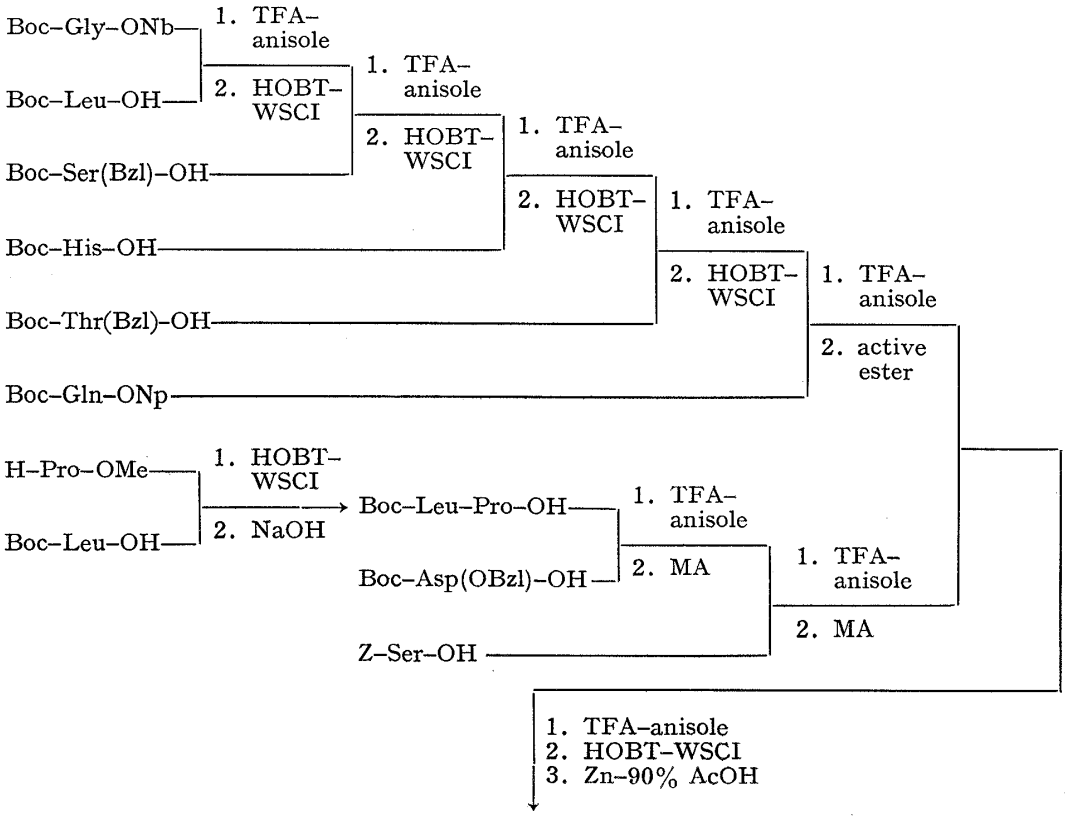
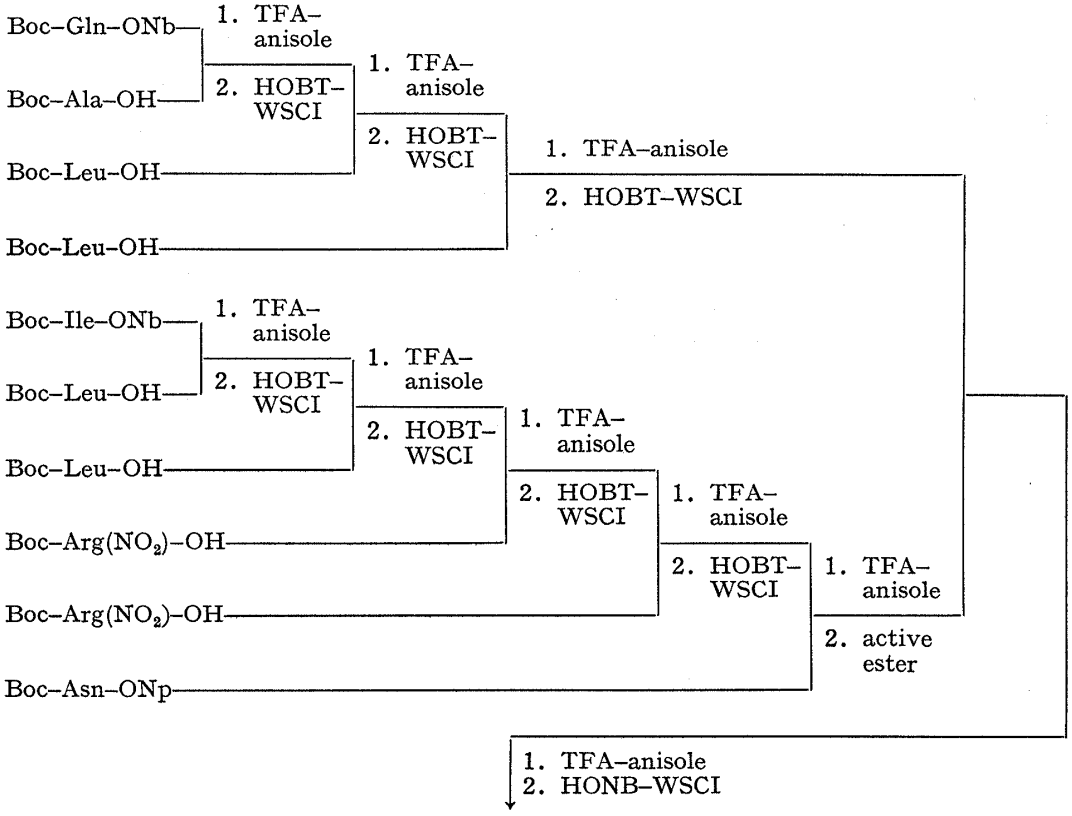


Chart 1. Synthesis of the Protected Decapeptide (positions 1—10)

sequence (positions 1—20) of the major component of human lymphoblastoid interferon was determined by Anifinsen and Hood[8] in 1980, we have synthesized this eicosapeptide, H–Ser–Asp–Leu–Pro–Gln–Thr–His–Ser–Leu–Gly–Asn–Arg–Arg–Ala–Leu–Ile–Leu–Leu–Ala–Gln–OH, by a conventional method and examined its effect on DNA synthesis in lymphocytes stimulated by PHA. In the present study, protecting groups of amino acid derivatives, Z–Ser, Arg(NO_2), Ser(Bzl), Thr(Bzl) and Asp(OBzl), but not Gln-ONb, were removed by treatment with hydrogen fluoride.[9] These protecting groups survive mostly intact during TFA treatment for the removal of the Boc group, employed as a temporary α-amino protecting group. First, the N-terminal decapeptide, Z–Ser–Asp(OBzl)–Leu–Pro–Gln–Thr(Bzl)–His–Ser(Bzl)–Leu–Gly–OH (XI), was synthesized according to Chart. 1. The protected pentapeptide ester, Boc–Thr(Bzl)–His–Ser(Bzl)–Leu–Gly–ONb (IV) was synthesized stepwise by the HOBT–DCC procedure[10] starting from Boc–Gly–ONb. After the TFA-anisole treatment of IV, the resulting pentapeptide ester was condensed with Boc–Gln–ONp to give the protected hexapeptide, Boc–Gln–Thr(Bzl)–His–Ser(Bzl)–Leu–Gly–ONb (V). H–Pro–OMe HCl was condensed with Boc–Leu–OH by the HOBT–DCC procedure to give Boc–Leu–Pro–OMe (VI) and then the methyl ester group of VI was cleaved with 1N NaOH. The protected tetrapeptide, Z–Ser–Asp(OBzl)–Leu–Pro–OH (IX) was synthesized in a stepwise manner by the MA procedure[11] starting from Boc–Leu–Pro–OH (VII). The hexapeptide V was treated with TFA-anisole and the product was also condensed with the tetrapeptide IX by the HONB–DCC procedure to minimize undesirable racemization[12] to provide Z–Ser–Asp(OBzl)–Leu–Pro–Gln–Thr(Bzl)–His–Ser(Bzl)–Leu–Gly–ONb (X), from which the *p*-nitrobenzyl ester was removed by treatment with Zn in 90% acetic acid (AcOH).[13] Traces of metal contamination were removed by treatment with 1% EDTA. The synthetic scheme for the protected C-terminal decapeptide, Boc–Asn–Arg(NO_2)–Ala–Leu–Ile–Leu–Leu–Ala–Gln–ONb (XXI), is illustrated in Chart 2. The protected tetrapeptide ester, Boc–Leu–Leu–



Boc–Asn–Arg(NO_2)–Arg(NO_2)–Ala–Leu–Ile–Leu–Leu–Ala–Gln–ONb

Chart 2. Synthesis of the Protected Decapeptide (positions 11—20)

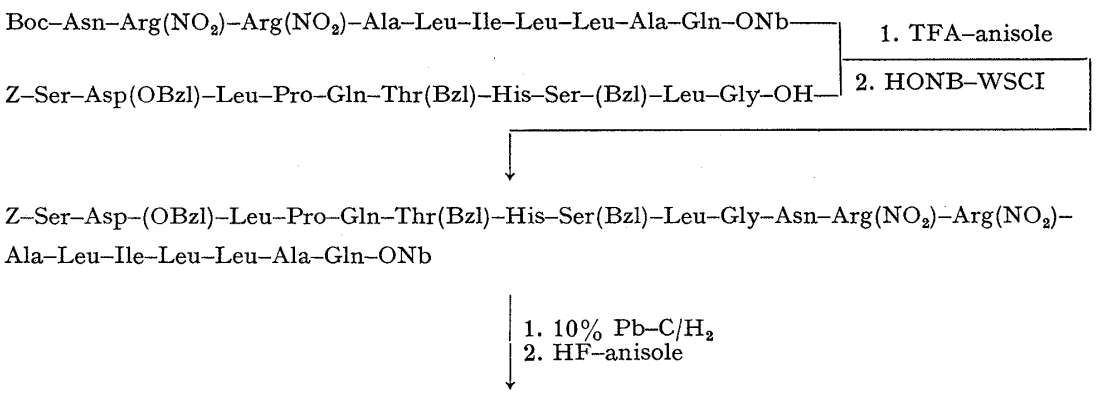


Chart 3. Synthesis of the N-terminal Human Lymphoblastoid Interferon Fragment (positions 1—20)

Ala–Gln–ONb (XIV), was synthesized stepwise by the HOBT–DCC procedure starting from Boc–Gln–ONb. The protected pentapeptide ester, Boc–Arg(NO_2)–Arg(NO_2)–Ala–Leu–Ile–ONb (XVIII) was also synthesized stepwise by the HOBT–DCC procedure starting from Boc–Ile–ONb. After the TFA-anisole treatment of XVIII, the resulting pentapeptide ester was condensed with Boc–Asn–ONp to give the protected hexapeptide, Boc–Asn–Arg(NO_2)–Arg(NO_2)–Ala–Leu–Ile–ONb (XIX), from which the *p*-nitrobenzyl ester group was removed by treatment with Zn in 90% AcOH. The tetrapeptide XIV was treated with TFA-anisole to remove the Boc group and the resulting free base of XIV was condensed with the hexapeptide XX by the HONB–DCC procedure, minimizing racemization during the coupling reaction to afford Boc–Asn–Arg(NO_2)–Arg(NO_2)–Ala–Leu–Ile–Leu–Leu–Ala–Gln–ONb (XXI). Racemization might occur during the condensation due to the bulkiness of Ile in the C-terminus. We took advantage of the well-known fact that D-allo-Ile is easily separable from Ile on an amino acid analyzer.[14] The presence of D-allo-Ile in an acid and enzymatic hydrolysate of the eicosapeptide XXIII was not detected. The Boc group of the decapeptide XXI was removed by treatment with TFA-anisole, and the resulting free base was coupled with the decapeptide

Table I. Effect of the Eicosapeptide (positions 1—20) of the Major Component of Human Lymphoblastoid Interferon on the Stimulation of T-Lymphocytes by PHA

Peptides	Dose (μg/ml)	^{3}H–Thymidine incorporation (cpm)
——*a*)		587 ± 45
——*b*)		46898 ± 2380
Eicosapeptide XXIII*b*)	100	39366 ± 1961
(H–Ser–Asp–Leu–Pro–Gln–Thr–His–Ser–Leu–Gly–Asn–Arg–Arg–Ala–Leu–Ile–Leu–Leu–Ala–Gln–OH)	300	33302 ± 2145
	600	26641 ± 2412
	900	21942 ± 1898
Glucagon fragment*b*,*c*)	100	45897 ± 2286
(H–His–Ser–Gln–Gly–Thr–Phe–Thr–Ser–Asp–Tyr–Ser–Lys–Tyr–Leu–OH)	300	46729 ± 2110
	600	45960 ± 2419
	900	44796 ± 1981
	2000	45003 ± 2124

a) PHA−.
b) PHA+.
c) Control.

XI by the HONB–DCC procedure to afford Z–Ser–Asp(OBzl)–Leu–Pro–Gln–Thr(Bzl)–His–Ser(Bzl)–Leu–Gly–Asn–Arg(NO_2)–Arg(NO_2)–Ala–Leu–Ile–Leu–Leu–Ala–Gln–ONb (XXII). N-Methyl-2-pyrrolidone was used as a solvent because of the poor solubility of the amino component. The protected eicosapeptide XXII was hydrogenated over 10% Pd-charcoal in aqueous AcOH for 20 hr and then treated with hydrogen fluoride in the presence of anisole.[9] The free peptide, precipitated by adding dry ether, was converted to the corresponding acetate by treatment with Amberlite CG-4B (acetate form) and then treated with 1 N NH_4OH for 30 min. The latter treatment was performed because of the reversible N→O shift at the Ser and Thr residues during the hydrogen fluoride treatment.[15,16] Finally, the product was purified by gel-filtration on Sephadex G-25 using 1% AcOH, followed by partition column chromatography on Sephadex G-25 according to Yamashiro.[17] The eicosapetide (XXIII) thus obtained was found to be homogeneous by paper chromatography in two different solvent systems. The amino acid compositions in an acid hydrolysate of XXIII agreed well with the theoretical values. Despite the presence of a Pro residue,[18] complete digestion of the peptide with aminopeptidase (AP–M)[19] made it possible to identify Gln and Asn residues in this peptide. These results indicate that the synthetic eicosapeptide possesses a high degree of homogeneity and the L-configuration of constituent amino acid residues. Incubation of T-lymphocytes stimulated by PHA with the eicosapeptide at concentrations ranging from 100 to 900 μg/ml cell culture, resulted in a 15—50% decrease in ^3H-thymidine incorporation into DNA (Table I). In contrast, glucagon fragment (positions 1—14)[20] used as a control had no inhibitory activity on the lymphocyte transformation by PHA at a dose of 2 mg/ml. These results suggest that the N-terminal eicosapeptide of the major component of human lymphoblastoid interferon might be important for the inhibition of DNA synthesis in lymphocytes stimulated by PHA.

Experimental

Melting points are uncorrected. Rotations were determined in a Atago Polax (cell length: 10 cm). The amino acid compositions of the acid and enzymatic hydrolysates were determined with a JEOL JLC-8AH amino acid analyzer (one-column system). Solvents were removed by evaporation *in vacuo* at 35 to 40° in a rotary evaporator. Boc groups of the protected peptides were removed by treatment with TFA-anisole. The resulting amino components were chromatographed on filter paper, Toyo Roshi No. 51, at room temperature. Rf^1 values refer to the Partridge system[21] and Rf^2 values refer to BuOH–pyridine–AcOH–H_2O (30: 20: 6: 24).[22] Venous blood from normal subjects was withdrawn into heparinized syringes and sedimented at room temperature. Aminopeptidase (3501, Aminopeptidase 210520) was purchased from the Protein Research Foundation, Osaka, Japan. The glucagon fragment (positions 1—14) was synthesized in our laboratory.[20]

Boc-Leu-Gly-ONb (I)——Boc–Gly–ONb (3.1 g) was treated with TFA (7 ml)- anisole (0.7 ml) at room temperature for 30 min, then excess TFA was removed by evaporation. The residue was washed with dry ether and then dried over KOH pellets *in vacuo*. The product was dissolved in THF (15 ml) and the solution was neutralized with Et_3N (1.5 ml). To this ice-chilled solution of Boc–Leu–OH (2.8 g) in THF (10 ml), HOBT (1.5 g) and WSCI (1.8 g) were added, and the mixture was stirred at 0° for 16 hr. The reaction mixture was extracted with ethyl acetate (EtOAc) and then washed successively with 1 N citric acid, H_2O, 1 N $NaHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was reprecipitated from EtOAc and *n*-hexane: oily material, 3.2 g (76%), $[\alpha]_D^{30}$ −30.1° (c=1.0, DMF), Rf^1 0.78, Rf^2 0.88, single ninhydrin-positive spot. *Anal.* Calcd for $C_{20}H_{29}N_3O_7 \cdot H_2O$: C, 54.41; H, 7.08; N, 9.52. Found: C, 54.22; H, 7.29; N, 9.34.

Boc-Ser(Bzl)-Leu-Gly-ONb (II)——I (3 g) was treated with TFA (5 ml)- anisole (0.5 ml) and the deprotected peptide isolated as described above was dissolved in DMF (15 ml). To this ice-chilled solution, Et_3N (1.1 ml), Boc–Ser(Bzl)–OH (2.2 g), HOBT (1 g) and WSCI (1.2 g) were successively added. After being stirred at 0° for 16 hr, the mixture was extracted with EtOAc and then washed successively with 1 N citric acid, H_2O, 1 N $NaHCO_3$ and H_2O, dried over $MgSO_4$ and concentrated *in vacuo*, then *n*-hexane was added to the residue and the precipitate formed was filtered off *in vacuo*: 3.5 g (81%), mp 55—61°, $[\alpha]_D^{30}$ −28.4° (c=1.0, DMF), Rf^1 0.84, Rf^2 0.94, single ninhydrin–positive spot. *Anal.* Calcd for $C_{30}H_{40}N_4O_9$: C, 59.99; H, 6.71; N, 9.33. Found: C, 60.25; H, 6.84; N, 8.97.

Boc-His-Ser(Bzl)-Leu-Gly-ONb (III)——This compound was prepared from II (3000 mg), HOBT (744 mg), Boc–His–OH (1400 mg) and WSCI (854 mg) essentially as described for the preparation of II. The

product was reprecipitated from EtOAc and ether: 3.3 g (89%), mp 55—58°, $[\alpha]_D^{30}$ −27.3° (c=1.0, DMF), Rf^1 0.74, Rf^2 0.86, single ninhydrin- and Pauly-positive spot. *Anal.* Calcd for $C_{36}H_{47}N_7O_{10}\cdot H_2O$: C, 57.21; H, 6.54; N, 12.97. Found: C, 56.99; H, 6.32; N, 12.73.

Boc-Thr(Bzl)-His-Ser(Bzl)-Ser(Bzl)-Leu-Gly-ONb (IV)——This compound was prepared from III (3000 mg), Boc–Thr(Bzl)–OH (1300 mg), HOBT (600 mg) and WSCI (682 mg) essentially as described for the preparation of II. The product was reprecipitated from EtOAc and ether: 2.8 g (75%), mp 86—89°, $[\alpha]_D^{30}$ −19.5° (c=1.0, DMF), Rf^1 0.79, Rf^2 0.87, single ninhydrin- and Pauly-positive spot. *Anal.* Calcd for $C_{47}H_{60}N_8O_{12}$: C, 60.76; H, 6.51; N, 12.06. Found: C, 60.42; H, 6.82; N, 11.90.

Boc-Gln-Thr(Bzl)-His-Ser(Bzl)-Leu-Gly-ONb (V)——IV (465 mg) was treated with TFA (2 ml)- anisole (0.2 ml) as described above. Boc–Gln–ONp (202 mg) was added to a solution of this product in DMF (5 ml), followed by Et_3N to keep the solution slightly alkaline. After 24 hr at room temperature, the reaction mixture was diluted with 1 N NH_4OH (1 ml) with stirring to saponify the unchanged p-nitrophenyl ester. After 1 hr, the mixture was extracted with EtOAc and washed successively with 1 N NH_4OH, H_2O, 1 N citric acid and H_2O. The solution was dried over $MgSO_4$, concentrated to a small volume, and petroleum ether was added to the residue. The precipitate was reprecipitated from EtOAc and n-hexane: 284 mg (54%), mp 78—83°, $[\alpha]_D^{30}$ −18.5° (c=1.0, DMF), Rf^1 0.78, Rf^2 0.84, single ninhydrin- and Pauly-positive spot. *Anal.* Calcd for $C_{52}H_{68}N_{10}O_{14}$: C, 59.08; H, 6.48; N, 13.25. Found: C, 59.01; H, 6.29; N, 13.47.

Boc-Leu-Pro-OMe (VI)——This compound was prepared from H–Pro–OMe HCl (1.7 g), Boc–Leu–OH (2.7 g), HOBT (1.5 g) and WSCI (1.8 g) essentially as described for the preparation of II. The product was reprecipitated from EtOAc and petroleum ether: oily material, 2.4 g (69%), $[\alpha]_D^{30}$ −41.6° (c=1.0, DMF), Rf^1 0.66, Rf^2 0.81, single ninhydrin-positive spot. *Anal.* Calcd for $C_{17}H_{30}N_2O_5$: C, 59.63; H, 8.83; N, 8.18. Found: C, 59.83; H, 9.07; N, 8.41.

Boc-Leu-Pro-OH (VII)——VI (2.4 g) was dissolved in dioxane (20 ml) and 1 N NaOH (7.7 ml) was added. The mixture was stirred for 1 hr at room temperature and the solution was concentrated to small volume *in vacuo*. The residue was diluted with H_2O and the aqueous solution was washed with EtOAc. The aqueous layer was acidified to Congo red with 3 M citric acid. The oily product was extracted with EtOAc and the extract was washed with H_2O, dried over $MgSO_4$ and concentrated *in vacuo*, then n-hexane was added to the residue and the precipitate formed was filtered off *in vacuo*: 1.4 g (61%), mp 55—57°, $[\alpha]_D^{30}$ −44.9° (c=1.0, DMF), Rf^1 0.67, Rf^2 0.52, single ninhydrin-positive spot. *Anal.* Calcd for $C_{16}H_{28}N_2O_5\cdot H_2O$: C, 55.47; H, 8.73; N, 8.09. Found: C, 55.83; H, 8.99; N, 7.88.

Boc-Asp(OBzl)-Leu-Pro-OH (VIII)——The above protected dipeptide (1.3 g) was treated with TFA (5 ml)-anisole (0.5 ml) as usual and n-hexane was added. The resulting oil was dried over KOH pellets *in vacuo*, and dissolved in THF (5 ml) containing N-methylmorpholine (0.4 ml. To this ice-chilled solution, a solution of the mixed anhydride (prepared from 1.4 g of Boc–Asp(OBzl)–OH with 0.4 ml of ethylchlorocarbonate and 0.4 ml of N-methylmorpholine at −10°) in THF (5 ml) and acetonitrile (5 ml) was added. The solution was stirred at 4° for 8 hr, then concentrated and the residue was diluted with EtOAc. The solution was washed successively with 1 N citric acid and H_2O, dried over $MgSO_4$ and concentrated. The residue was reprecipitated from EtOAc and n-hexane: 1.3 g (62%), mp 54—56°, $[\alpha]_D^{30}$ −37.8° (c=1.0, DMF), Rf^1 0.81, Rf^2 0.86, single ninhydrin-positive spot. *Anal.* Calcd for $C_{27}H_{39}N_3O_8$: C, 60.77; H, 7.37; N, 7.88. Found: C, 60.53; H, 7.47; N, 7.94.

Z-Ser-Asp(OBzl)-Leu-Pro-OH (IX)——Compound VIII (1300 mg) was treated with TFA (6 ml)-anisole (0.6 ml) in the usual manner and the deprotected peptide was dissolved in THF (8 ml) containing N-methylmorpholine (0.28 ml). To this ice-chilled solution, a solution of the mixed anhydride (prepared from 658 mg of Z–Ser–OH with 0.29 ml of ethylchlorocarbonate and 0.28 ml of N-methylmorpholine at −10°) in THF (4 ml) and acetonitrile (4 ml) was added. The mixture was stirred at 4° for 8 hr, then concentrated, and the residue was diluted with EtOAc. The solution was washed as described above and then precipitated from EtOAc and ether: 794 mg (50%), mp 91—93°, $[\alpha]_D^{30}$ −7.3° (c=1.0, DMF). *Anal.* Calcd for $C_{33}H_{42}N_4O_{10}$: C, 60.54; H, 6.47; N, 8.56. Found: C, 60.65; H, 6.81; N, 8.45.

Z-Ser-Asp(OBzl)-Leu-Pro-Gln-Thr(Bzl)-His-Ser(Bzl)-Leu-Gly-ONb (X)——V (200 mg) was treated with TFA (2 ml)-anisole (0.2 ml) as described above. To an ice-chilled solution of the resulting hexapeptide ester trifluoroacetate in DMF (3 ml), IX (136 mg), HONB (38 mg) and WSCI (32 mg) were added, followed by addition of N-methylmorpholine[23] to keep the solution slightly alkaline. After 36 hr at 0°, the reaction mixture was poured into 1 N $NaHCO_3$ with stirring. The precipitate thus formed was washed successively with 1 N $NaHCO_3$, H_2O, 1 N citric acid and H_2O. The precipitate was reprecipitated from MeOH and H_2O: 175 mg (53%), mp 107—109°, $[\alpha]_D^{30}$ −14.3° (c=1.0, DMF). *Anal.* Calcd for $C_{87}H_{106}N_{15}O_{24}$: C, 59.85; H, 6.12; N, 12.04. Found: C, 60.05; H, 6.33; N, 11.78.

Z-Ser-Asp(OBzl)-Leu-Pro-Gln-Thr(Bzl)-His-Ser(Bzl)-Leu-Gly-OH (XI)——Zn dust (22 mg) was added to a solution of X (116 mg) in 90% AcOH (3 ml). The mixture was stirred at 0° for 30 min, then at room temperature for 90 min. The solution was filtered, then concentrated *in vacuo* and the residue was extracted with BuOH. The extract was washed successively with 1 N citric acid, 1% EDTA and H_2O and concentrated *in vacuo*. The residue was reprecipitated from MeOH and ether: 51 mg (48%), mp 121—128°, $[\alpha]_D^{30}$ −31.5° (c=1.0, DMF), *Anal.* Calcd for $C_{80}H_{101}N_{14}O_{22}$: C, 59.65; H, 6.32; N, 12.18. Found: C, 59.41; H, 6.23; N, 11.94.

Boc-Ala-Gln-ONb (XII)——This compound was prepared from Boc–Gln–ONb (6 g), Boc–Ala–OH (3 g), HOBT (2 g) and WSCI (2.4 g) essentially as described for the preparation of II. The product was reprecipitated from EtOAc and *n*-hexane: 5.1 g (80%), mp 129—131°, $[\alpha]_D^{30}$ −20.0° (c=1.0, DMF), Rf^1 0.61, Rf^2 0.70, single ninhydrin-positive spot. *Anal.* Calcd for $C_{20}H_{28}N_4O_8$: C, 53.09; H, 6.24; N, 12.38. Found: C, 53.01; H, 6.11; N, 12.13.

Boc-Leu-Ala-Gln-ONb (XIII)——This compound was prepared from XII (2.3 g), Boc–Leu–OH (1.4 g), HOBT (744 mg) and WSCI (854 mg) essentially as described for the preparation of II: 1.9 g (66%), mp 136—144°, $[\alpha]_D^{30}$ −25.1° (c=1.0, DMF), Rf^1 0.60, Rf^2 0.83, single ninhydrin-positive spot. *Anal.* Calcd for $C_{26}H_{39}N_5O_9$: C, 55.20; H, 6.95; N, 12.38. Found: C, 54.81; H, 7.13; N, 12.24.

Boc-Leu-Leu-Ala-Gln-ONb (XIV)——This compound was prepared from XIII (566 mg), Boc–Leu–OH (274 mg), HOBT (148 mg) and WSCI (170 mg) essentially as described for the preparation of II: 411 mg (61%), mp 141—144°, $[\alpha]_D^{30}$ −33.8° (c=1.0, DMF), Rf^1 0.71, Rf^2 0.89, single ninhydrin-positive spot. *Anal.* Calcd for $C_{32}H_{50}N_6O_{10}$: C, 56.62; H, 7.43; N, 12.38. Found: C, 56.32; H, 7.02; N, 12.06.

Boc-Leu-Ile-ONb (XV)——This compound was prepared from Boc–Ile–ONb (5.2 g), Boc–Leu–OH (4 g), HOBT (2.1 g) and WSCI (2.4 g) essentially as described for the preparation of II: oily material, 4 g (59%), $[\alpha]_D^{30}$ −15.3° (c=1.0, DMF), Rf^1 0.57, Rf^2 0.68, single ninhydrin-positive spot. *Anal.* Calcd for $C_{24}H_{37}N_3O_7$: C, 60.11; H, 7.78; N, 8.76. Found: C, 59.87; H, 8.10; N, 8.53.

Boc-Ala-Leu-Ile-ONb (XVI)——This compound was prepared from XV (3.2 g), Boc–Ala–OH (1.4 g), HOBT (1 g) and WSCI (1.1 g) essentially as described for the preparation of II: oily material, 2.9 g (78%), $[\alpha]_D^{30}$ −22.1° (c=1.0, DMF), Rf^1 0.80, Rf^2 0.88, single ninhydrin-positive spot. *Anal.* Calcd for $C_{27}H_{42}N_4O_8$: C, 58.89; H, 7.69; N, 10.18. Found: C, 58.53; H, 8.03; N, 10.02.

Boc-Arg(NO_2)-Ala-Leu-Ile-ONb (XVII)——This compound was prepared from XVI (789 mg), Boc–Arg(NO_2)–OH (502 mg), HOBT (212 mg) and WSCI (244 mg) essentially as described for the preparation of II: 930 mg (85%), mp 84—88°, $[\alpha]_D^{30}$ −23.4° (c=1.0, DMF), Rf^1 0.65, Rf^2 0.68, single ninhydrin-positive spot. *Anal.* Calcd for $C_{33}H_{53}N_9O_{11}$: C, 52.72; H, 7.11; N, 16.77. Found: C, 52.91; H, 7.39; N, 16.85.

Boc-Arg(NO_2)-Arg(NO_2)-Ala-Leu-Ile-ONb (XVIII)——This compound was prepared from XVII (752 mg), Boc–Arg(NO_2)–OH (351 mg), HOBT (149 mg) and WSCI (171 mg) essentially as described for the preparation of II. The product was reprecipitated from MeOH and ether: 568 mg (60%), mp 87—88°, $[\alpha]_D^{30}$ −30.3° (c=1.0, DMF), Rf^1 0.77, Rf^2 0.89, single ninhydrin-positive spot. *Anal.* Calcd for $C_{39}H_{64}N_{14}O_{14}$: C, 49.15; H, 6.77; N, 20.58. Found: C, 48.73; H, 6.63; N, 20.68.

Boc-Asn-Arg(NO_2)-Arg(NO_2)-Ala-Leu-Ile-ONb (XIX)——This compound was prepared from XVIII (477 mg) and Boc–Asn–ONp (200 mg) essentially as described for the preparation of V. The product was reprecipitated from MeOH and ether: 311 mg (58%), mp 117—124°, $[\alpha]_D^{30}$ −38.7° (c=1.0, DMF), Rf^1 0.50, Rf^2 0.76, single ninhydrin-positive spot. *Anal.* Calcd for $C_{43}H_{70}N_{16}O_{16}\cdot H_2O$: C, 47.59; H, 6.69; N, 20.56. Found: C, 47.46; H, 7.04; N, 20.32.

Boc-Asn-Arg(NO_2)-Arg(NO_2)-Ala-Leu-Ile-OH (XX)——XIX (356 mg) was dissolved in 90% AcOH (4 ml) and then treated with Zn dust (110 mg) essentially as described for the preparation of XI. The product was extracted with EtOAc and the extract was washed successively with 1 N citric acid, 1% EDTA and H_2O. The EtOAc layer was concentrated *in vacuo* and the residue was dissolved in DMF (2 ml). The solution was poured into 1 N citric acid with stirring and the precipitate thereby formed was washed successively with 1 N citric acid and H_2O. The product was reprecipitated from MeOH and ether: 150 mg (48%), mp 128—136°, $[\alpha]_D^{30}$ −22.1° (c=1.0, DMF), Rf^1 0.51, Rf^2 0.69, single ninhydrin-positive spot. *Anal.* Calcd for $C_{36}H_{65}N_{15}O_{14}$: C, 46.39; H, 7.03; N, 22.55. Found: C, 46.11; H, 7.35; N, 22.29.

Boc-Asn-Arg(NO_2)-Arg(NO_2)-Ala-Leu-Ile-Leu-Leu-Ala-Gln-ONb (XXI)——This compound was prepared from XIV (85 mg), XX (128 mg), HONB (24 mg) and WSCI (21 mg) essentially as described for the preparation of X. The product was reprecipitated from DMF and 1 N citric acid: 115 mg (62%), mp 133—137°, $[\alpha]_D^{30}$ −24.8° (c=1.0, DMF), Rf^1 0.72, Rf^2 0.82, single ninhydrin-positive spot. *Anal.* Calcd for $C_{63}H_{105}N_{21}O_{21}$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.28; H, 6.89; N, 19.96.

Z-Ser-Asp(OBzl)-Leu-Pro-Gln-Thr(Bzl)-His-Ser(Bzl)-Leu-Gly-Asn-Arg(NO_2)-Arg(NO_2)-Ala-Leu-Ile-Leu-Leu-Ala-Gln-ONb (XXII)——XXI (25 mg) was treated with TFA (0.5 ml)-anisole (0.1 ml) as described above. To an ice-chilled solution of this product in N-methyl-2-pyrrolidone (1 ml), XI (27 mg), HONB (4 mg) and WSCI (4 mg) were added, followed by N-methylmorpholine to keep the solution slightly alkaline. After 48 hr at −10°, the reaction mixture was poured into 1 N $NaHCO_3$ with stirring. The precipitate thus formed was washed successively with 1 N $NaHCO_3$, H_2O, 1 N HCl and H_2O. The dried product was recrystallized from hot EtOH: 30 mg (64%), mp 138—145°, $[\alpha]_D^{30}$ −18.7° (c=0.4, DMF), *Anal.* Calcd for $C_{131}H_{190}N_{34}O_{36}$: C, 55.85; H, 6.80; N, 16.91. Found: C, 55.57; H, 6.94; N, 17.20.

H-Ser-Asp-Leu-Pro-Gln-Thr-His-Ser-Leu-Gly-Asn-Arg-Arg-Ala-Leu-Ile-Leu-Leu-Ala-Gln-OH (XXIII)——The protected eicosapeptide XXII (50 mg) was hydrogenated in AcOH (8 ml–H_2O (8 ml) over 10% Pd–charcoal for 20 hr. After removal of the catalyst by filtration with the aid of celite, the filtrate was evaporated to dryness, and then the hydrogenated product was treated with HF (approximately 3 ml) in the presence of anisole (0.3 ml) in an ice-bath for 60 min. After removal of the HF, dry ether was added and the resulting powder was dissolved in H_2O (3 ml). The solution was treated with Amberlite CG-4B (acetate form, approximately 1 g) for 30 min, filtered by suction, and concentrated *in vacuo*. The residue

was mixed with 1 N NH_4OH (3 ml) and the solution was left to stand at 0° for 30 min, then lyophilized. The crude peptide thus obtained was dissolved in 1% AcOH (2 ml), applied to a column of Sephadex G-25 (2.8×60 cm), and eluted with the same solvent. Fractions of 5 ml were collected per 15 min, and the absorption at 230 nm was determined. Fractions corresponding to the front main peak (tube Nos. 46—51) were combined and lyophilized. The resulting powder was dissolved in a small amount of the upper phase of the solvent system consisting of BuOH–AcOH–H_2O (4:1:5, by volume). The solution was subjected to partition column chromatography on Sephadex G-25 (2.8×60 cm) previously equilibrated with the lower phase of the above solvent system. The column was developed with the same upper phase. One 6 ml fraction was collected every 25 min and the absorbancy at 230 nm was determined. The fractions corresponding to the main peak (tube Nos. 34—38) were combined, and evaporated to dryness. The residue was dissolved in 10% AcOH and lyophilized again to give a fluffy white powder: 11 mg (34%), $[\alpha]_D^{30}$ −65.3° (c=0.3, 10% AcOH), Rf^1 0.07, Rf^2 0.30, single ninhydrin- Sakaguchi- and Pauly-positive spot. Amino acid ratios in an acid hydrolysate (6 N HCl, 24 hr): Leu 4.86, Ile 0.89, Gly 1.00, Ala 2.13, Ser 1.81, Thr 0.83, Pro 0.93, Asp 1.83, Glu 1.89, His 0.84, Arg 1.80 (average recovery 84%). Amino acid ratios in an AP-M digest (24 hr): Leu 4.79, Ile 0.84, Gly 1.00, Ala 2.01, Ser 1.85, Pro 0.82, Asp 0.82, (Gln+Asn+Thr) 3.74, His 0.93, Arg 1.89 (average recovery 81%) (Gln and Asn emerged at the same position as Thr and were calculated as Thr).

Inhibition Activity of the Synthetic Eicosapeptide on DNA Synthesis in Lymphocytes stimulated by PHA ——Lymphocytes were separated from whole heparinized blood on a Ficoll-Isopaque gradient as described by Harris *et al.*[24] for T-cell transformation. The cells were cultured in 0.2 ml of RPMI 1640 (Gibco) with 10% FCS (Dainippon Pharmaceutical Co.) in microplates (12×8 wells), and 0.02 ml (final 10 μg/ml) of PHA-P (Difco) was added, with 0.02 ml (final concentration 100—900 μg/ml) of eicosapeptide. Triplicate cultures of each combination (1×10^5 cells per well) were incubated at 37° in a humidified atmosphere of 5% CO_2 in air for 64 hr. Eight hr before harvest, 0.25 μCi per well of ^{3}H-thymidine was added per culture. The amount of thymidine incorporation into DNA was measured in a LKB-1216 liquid scintillation counter. The isotope incorporation into DNA was decreased by about 15% in the presence of 100 μg/ml of the eicosapeptide.

Acknowlcdgement The authors thank the staff of the Division of Cellular Immunology, Special Reference Laboratory Inc., and Prof. K. Kumagai of the Department of Microbiology, Tohoku University, School of Dentistry, for the test of suppression of PHA-induced lymphocyte transformation, and the staff of the Central Analysis Room of the Pharmaceutical Institute, Tohoku University, for elemental analysis.

References and Notes

1) The amino acid residues are of the L-configuration. The abbreviations used to denote amino acid derivatives and peptides are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochem. Biophys. Acta*, **263**, 205 (1972). Other abbreviations: DMF, dimethylformamide; WSCI, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; HOBT, N-hydroxybenzotriazole; HONB, N-hydroxy-5-norbornene-2,3-dicarboximide; MA, mixed anhydride; PBS, phosphate-buffered saline; FCS, fetal calf serum; PHA, phytohemagglutinin; THF, tetrahydrofuran; Et_3N, triethylamine; EDTA, ethylenediaminetetraacetic acid; HF, hydrogen fluoride; Z, benzyloxycarbonyl; Bzl, benzyl; OBzl, benzyloxy; Boc, *tert*-butoxycarbonyl; ONp, *p*-nitrophenoxy; RPMI, Roswell Park Memorial Institute.
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