SYNTHESIS OF THREE PEPTIDES FROM HLA-A AND HLA-B ANTIGENS*

Miroslava ŽERTOVÁ and Zdenko PROCHÁZKA

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received January 17, 1991
Accepted March 9, 1991

Linear nonapeptides of the sequence 79–87 of HLA-A32 (ref.[1]), HLA-Aw24 (ref.[2]), HLA-Bw58 (ref.) or HLA-B27·2 (refs[4,5]) (*I*) or the same sequence of HLA-B27·1 or HLA-B27·3 (refs[4,5]) (*II*) or this sequence of HLA-B44 (ref.[6]) (*III*) were synthesized. The syntheses of all three peptides** were performed by solid phase technique on the benzhydrylamine resin. As the α-amino protecting group we have used tert-butyloxycarbonyl group. For the side chain protection we have used: tosyl (Arg), 2,6-dichlorobenzyl (Tyr) and benzyl (Thr). Protected amino acids were coupled by N,N′-dicyclohexylcarbodiimide (DCC) and N-hydroxybenzotriazole (HOBt) in dimethylformamide. Side chain protecting groups were cleaved simultaneously with the cleavage of the peptide from the resin by the liquid hydrogen fluoride and compounds were purified by HPLC. The point worth mentioning in this synthesis is the extremely difficult coupling of Ala (or Leu) to N-terminal Leu in position 4.

H-Arg-X-Y-Z-Arg-Tyr-Tyr-Asn-Gln-NH_2

I, X = Ile Y = Ala Z = Leu
II, X = Thr Y = Leu Z = Leu
III, X = Thr Y = Ala Z = Ala

EXPERIMENTAL

Analytical methods and instrument in this work as well as solid-phase peptide synthesis protocol were the same as those described in ref.[8]. The synthesis was monitored by bromophenol blue method[9].

Protected Peptide Resins

Benzhydrylamine resin (UCB, 0·6 mmol/g, 1·5 g) was suspended in dichloromethane and after washing with 5% diisopropylethylamine in dichloromethane and with dimethylformamide it was

* Part CCXXII in the series Amino Acids and Peptides; Part CCXXI: Collect. Czech. Chem. Commun. *56*, 1963 (1991).

** All the chiral amino acids, mentioned in this work, are of the L-series. The nomenclature and symbols of the amino acids and peptides obey the published recommendations[7].

coupled with 4 molar excess of Boc-Gln-OH, N-hydroxybenzotriazole and dicyclohexylcarbodiimide in dimethylformamide. Coupling was interrupted after 3 h, the resin was washed consequently by dimethylformamide (3 × 10 ml) and dichloromethane (3 × 10 ml) and free amino groups were acetylated in the standard way. Substitution of Gln on the resin was determined by amino acid analysis — 0·37 mmol/g.

The following procedure was performed according to general scheme[8] (starting from the point 1.). Protected derivatives were used in the following order: Boc-Asn-OH, Boc-Tyr(2,6-diCl-Bzl)-OH (ref.[10]), Boc-Tyr(2,6-diCl-Bzl)-OH and Boc-Arg(Tos)-OH. Finally, the Boc-group was cleaved from the pentapeptide-resin, resin was washed and dried (1·9 g). At this point the resin was divided (peptide resin A).

Part of this resin (1·26 g, 0·37 mmol) was coupled according to general scheme with Boc-Leu-OH.H_2O. Boc-group was cleaved. The resin washed and dried (1·3 g). At this point the resin was again divided (peptide-resin B).

Arginyl-Isoleucyl-Alanyl-Leucyl-Arginyl-Tyrosyl-Tyrosyl-Asparaginyl-Glutamine Amide (*I*)

Peptide-resin B (0·65 g, 0·185 mmol) was coupled according to general scheme with Boc-Ala-OH, Boc-Ile-OH and Boc-Arg(Tos)-OH. Alanine coupling had to be performed twice overnight and the next coupling had to be prolonged to 6 h. Finally, the Boc-group was cleaved, resin was washed and dried (0·77 g) and treated with liquid hydrogen fluoride (10 ml, 30 min, 0°C) in the presence of anisole (1 ml). Unprotected nonapeptide, together with the resin, was triturated with ether and ethyl acetate after evaporation of hydrogen fluoride, filtered off, washed with ethyl acetate and then free peptide was extracted successively by acetic acid, 50% acetic acid, water and lyophilizate (0·250 g) was purified by HPLC. Elution using a gradient from 15 to 30% methanol in 0·05% trifluoroacetic acid in 60 min and lyophilization of the corresponding fractions afforded 48·9 mg (22%) of the product, pure according to HPLC (k 2·49; methanol–0·05% trifluoroacetic acid 40 : 60). $E_{2.4}^{Gly}$ 1·40, $E_{5.7}^{His}$ 0·77. $[\alpha]_D$ −31·0° (c 0·1; 1M-AcOH). Amino acid analysis: Asp 0·99, Glu 1·16, Ala 1·02, Ile 0·93, Leu 1·04, Tyr 2·07, Arg 1·79. According to elemental analysis the lyophilizate contains 87·5% of peptide tris-trifluoroacetate (for $C_{54}H_{86}.N_{16}O_{13}.3$ TFA (1 537·5) calculated: 16·40% N; found: 14·35% N). FAB MS (m/z): 1 196 ($M + H^+$).

Arginyl-Threonyl-Leucyl-Leucyl-Arginyl-Tyrosyl-Tyrosyl-Asparaginyl-Glutamine Amide (*II*)

Peptide-resin B (0·65 g, 0·185 mmol) was coupled according to general scheme with Boc-Leu-OH, Boc-Thr(Bzl)-OH and Boc-Arg(Tos)-OH. Leucine derivative had to be coupled again overnight. Peptide was cleaved and purified in the same way as given above. Yield 30·4 mg (13·4%) of the k 2·24; methanol–0·05% trifluoroacetic acid 40 : 60. $E_{2.4}^{Gly}$ 1·43, $E_{5.7}^{His}$ 0·85 $[\alpha]_D$ −34·7° (c 0·1; 1M-AcOH). Amino acid analysis: Asp 1·02, Thr 0·94, Glu 1·18, Leu 1·80, Tyr 2·08, Arg 1·98. According to elemental analysis the lyophilizate contains 85·5% of peptide tris-trifluoroacetate (for $C_{55}H_{88}N_{18}O_{14}.3$ TFA (1 567·5) calculated: 16·08% N; found: 13·75% N). FAB MS (m/z): 1 226 ($M + H^+$).

Arginyl-Threonyl-Alanyl-Alanyl-Alanyl-Arginyl-Tyrosyl-Tyrosyl-Asparaginyl-Glutamine Amide (*III*)

Peptide-resin A (0·63 g, 0·185 mmol) was coupled according to general scheme with Boc-Ala-OH, Boc-Ala-OH, Boc-Thr(Bzl)-OH and Boc-Arg(Tos)-OH. Peptide was cleaved and purified as

shown above. Yield 43·3 mg (20·5%), k 0·96; methanol–0·05% trifluoroacetic acid 30 : 70. $E_{2.4}^{Gly}$ 1·40, $E_{5.7}^{His}$ 0·81. $[\alpha]_D$ −45·3° (c 0·1; 1M-AcOH). Amino acid analysis: Asp 1·01, Thr 0·97, Glu 1·19, Ala 2·06, Tyr 1·92, Arg 2·04. According to elemental analysis the lyophilizate contains 83·4% of peptide tris-trifluoroacetate (for $C_{49}H_{76}N_{18}O_{14}.3$ TFA (1 483·3) calculated: 17·00% N; found: 14·18% N). FAB MS (m/z): 1 141·8 ($M + H^+$).

REFERENCES

1. Wan A. M., Ennis P., Parham P., Holmes N.: J. Immunol. *137*, 3671 (1986).
2. N.Guyen C., Sodoyer R., Trucy J., Strachan T., Jordan B. R.: Immunogenetics *21*, 479 (1985).
3. Ways J. P., Coppin H. L., Parkham P.: J. Biol. Chem. *260*, 11924 (1985).
4. Ezquerra A., Bragado R., Vega M. A., Strominger J. L., Woody J., Lopez de Castro J. A.: Biochemistry *24*, 1733 (1985).
5. Vega M. A., Ezquerra A., Romo S., Aparicio P., Bragado R., Lopez de Castro J. A.: Proc. Natl. Acad. Sci. U.S.A. *82*, 7394 (1985).
6. Kottmann A. H., Seemann G. H. A., Guessow H. G., Roos M. H.: Immunogenetics *23*, 396 (1986).
7. *Biochemical Nomenclature and Related Documents.* International Union of Biochemistry, London 1978.
8. Žertová M., Procházka Z., Bláha I., Barth T., Slaninová J., Maletínská L., Lebl M.: Collect. Czech. Chem. Commun. *56*, 3000 (1990).
9. Krchňák V., Vágner J., Šafář P., Lebl M.: Collect. Czech. Chem. Commun. *53*, 2542 (1988).
10. Yamashiro D., Li C. H.: J. Am. Chem. Soc. *95*, 1310 (1973).

Translated by the author (Z.P.).