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# ENZYMATIC SYNTHESIS OF NUCLEOPEPTIDES

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In order to determine the possibility of the enzymatic synthesis of stereoregular nucleopeptides [1] including residues of natural nucleo amino acids and some protein amino acids, we have investigated the reaction of $N^{\alpha}$-acyl-DL-nucleo amino acids (I-V) with the amides of L-leucine and L-methionine in the presence of chymotrypsin or carboxypeptidase Y.

Information on the constants and yields of compounds obtained previously (I-V) is given below (TLC on Silufol UV-254 plates; systems: 1) iso-$C_3H_7OH$–25% $NH_4OH$–$H_2O$ (14:1:5); and 2) n-$C_4H_9OH$–$CH_3CO_2H$–$H_2O$ (4:1:1)).

| Compound | mp, °C | $R_f$ system 1 | $R_f$ system 2 | Yield, % |
|---|---|---|---|---|
| Ac-*DL*-Aal-OMe (I) | 190 | 0 66 | 0,15 | 73 |
| Ac-*DL*-Ual-OMe (II) | 219 | 0,60 | 0,20 | 82 |
| Ac-*DL*-Tal-OMe (III) | 230 | 0,73 | 0.44 | 70 |
| Boc-*DL*-Aal-OMe (IV) | 187 | 0,88 | 0,73 | 60 |
| Boc-*DL*-Ual-OMe (V) | 197 | 0,80 | 0,70 | 70 |

After a series of experiments, we found that the incubation of the esters (I-V) with the amine of L-leucine or L-methionine and chymotrypsin in 0.2 M carbonate buffer (pH 10.0) containing 20% of a 1:1 mixture of dimethylformamide (DNFA) and dimethyl sulfoxide led to the enzymatic synthesis of stereoregular nucleopeptides. At 20°C, the reaction was complete in 8 h and the yield of nucleopeptides amounted to 60-90%. In experiments with carboxypeptidase Y, the yields of nucleopeptides were low.

As a result of the experiments performed we achieved the enzymatic synthesis of the stereoregular nucleopeptides (VI-XII):

VI. Ac-*L*-Ual-L-*L*eu-$NH_2$
VII. Ac-*L*-Aal-L-*L*eu-$NH_2$
VIII. Ac-*L*-Tal-L-Leu-$NH_2$
IX. Boc-*L*-Aal-*L*-Leu-$NH_2$
X. Boc-*L*-Aal-*L*-Met-$NH_2$
XI. Boc-*L*-Ual-*L*-Leu-$NH_2$
XII. Boc-*L*-Ual-*L*-Met-$NH_2$

For subsequent use in nucleopeptide synthesis, compounds (IX-XII), each containing a N-protective Boc group readily eliminated on acidolysis, are particularly promising. Information on the constants of these compounds is given below (system 3: $CHCl_3$–$CH_3OH$ (1:1); electrophoretic mobility in electrophoresis on LKB-3276 paper (240 V, 2 h) in systems 1) 1 N $CH_3CO_2H$ (pH 2.5), and 2) 0.05 M aqueous $(C_2H_5)_3NH_2CO_3$ (pH 8.7); reference standard: H-Aal-OH in the case of compounds (IX) and (X), and H-Ual-OH in the case of compounds (XI) and (XII):

| Compound | mp, °C | $R_f$ in system 1 | $R_f$ in system 2 | $R_f$ in system 3 | EM in system 1 | EM in system 2 | $[\alpha]_D^{20}$, deg (in DMFA) |
|---|---|---|---|---|---|---|---|
| IX | 177 | 0 75 | 0 70 | 0,34 | 1,03 | 0,25 | —30 (*c* 1,0) |
| X | — | 0,76 | 0,62 | 0,30 | 0,73 | 0,30 | —24 (*c* 0,95) |
| XI | 172 | 0,87 | 0 77 | 0,60 | 0,75 | 0 32 | —52 (*c* 0,1) |
| XII | 157 | 0,85 | 0,73 | 0,58 | 1,09 | 0,40 | —55 (*c* 0,25) |

The structures of the compounds obtained were confirmed by the results of acid hydrolysis and UV and mass spectra, and also by independent chemical synthesis. The optical purity of

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the nuclear peptides synthesized was shown by measuring the circular dichroism of chromophoric derivatives of them [2].

Thus, we have shown for the first time the possibility of the enzymatic synthesis of stereoregular nucleopeptides from derivatives of racemic nucleoamino acids.

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