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A NOVEL AND RAPID PEPTIDE SYNTHESIS

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We report a rapid stepwise peptide synthesis in solution,based on the reactivity of pentafluorophenyl esters[1].After each step the reactants in excess may be removed and the intermediates purified,thus allowing full control of the reaction.Each cycle consists of six steps:1.coupling /1o-3o min./ 2. removal of excess of ester /5-1o min./ 3.purification /1o-15 min./ 4. drying and evaporation /5-1o min./ 5. removal of N-terminal protection /5 min./ 6. solution of the free peptide and pH adjustment /1o min./.

The couplings were carried out with BOC-aminoacid pentafluorophenylesters[2] in excess of 1-2 equivalents,which effect a nearly quantitative acylation in 1o-3o min.-depending on the peptide bond formed.N,-N-Dialkylaminoethylamines can be used for the removal of the excess of active esters[3],These compounds quickly react with the esters and the amides formed are soluble in a weak acidic solution such as 1o% citric acid and thus are easily removed.These extractions also allow for purification,since the occasionally unreacted amino component - if soluble - is also removed.The next purification step is extraction with 5% $NaHCO_3$ removing acidic components.Another way to remove excess of active ester,particularly from higher insoluble peptides,is to triturate the evaporated reaction mixture with organic solvents.Various solvents /dioxan,chloroform etc./ saturated with hydrogen halide,are used for the removal of the N-terminal protecting group.The widely used HBr/acetic acid cannot be used due to acetylation.The peptide salt - precipitated and washed with dry ether - is then dissolved in a suitable solvent and the pH is adjusted to 7.5-8.5 with a tertiary base.Every step,including the extractions,can be controlled by t.l.c.It follows that for the protection of side chains and the C-terminal end,protecting groups should be used which are stable during deprotection of the N-terminal groups.

As can seen from Table 1 each cycle takes 65-85 minutes i.e.in one day 7-10couplings

can be carried out.Furthermore,should any problem be indicated by t.l.c.,it is possible to stop at any stage so as to modify the synthesis or purify the intermediates. A possible development of the method is the combination of fragment condensation.

Peptides prepared by this method are summarized in Table 1.

TABLE 1

Peptides	Number of cycles	Yield %	Required time/min.	Physical constants[d]
BOC-Phe-Pro-Pro-Phe-Phe-Val-Pro-Pro-Ala-Phe-OMe	7[a]	5o/89/[b]	51o/73/[c]	Pro:4,2;Val:1,o;Ala:1,o Phe:3,8;R_f^2:o,6
BOC-Trp-Glu/ONB/-His /DNP/-Phe-OMe[e]	3	75/91/	24o/8o/	Mp.:1o8-114C°;R_f^1:o,6;/α/$_D$= 16,9°±o,4;c=1;dioxan
BOC-Arg/NO_2/-Lys/Tos/-Pro-OMe[e]	2	76/87/	13o/65/	Mp.:9o-92C°;R_f^1:o,5;/α/$_D$= -35,8°±o,4;c=1;ethanol
BOC-Cys/Bzl/-Tyr/BOC/-Ile-Gln-Asn-Cys/Bzl/-Pro-Leu-Gly-NH_2[f]	7	39/87/	6oo/86/	Mp.:257-258C°;R_f^3:o,75;/α/$_D$= -46,4;c=1,2;DMF
Z-Asp/OBzl/-Arg/NO_2/-Val-Tyr /Bzl/-Ile-His/DNP/-Pro-Phe-ONB[f]	7	43/89/	6oo/85/	Mp.:18o-182C°;R_f^1:o,6;/α/$_D$= -15,2°±o,4;c=o,6;DMF

[a]BOC-Pro-Pro-OPFP was used twice.[b]The average yields/cycle are given in parentheses.[c]The average required time/cycle are given in parentheses.[d]Following solvent systems were used: /1/Ethylacetate-/pyridine:acetic acid:water=2o:6:11/=9:1;/2/Ethylacetate-/pyridine: acetic acid:water=2o:6;11/ = 4:1?/3/Ethylacetate-/pyridine:acetic acid:water= 3:2; [e]The analyses were correct.[f]After the stepwise deprotection and a simple purification the free peptides showed full biological activity compared with authentic samples.

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