APPLICATION OF PENTAFLUOROPHENYL ESTERS OF BOC-AMINO ACIDS IN SOLID PHASE PEPTIDE SYNTHESIS

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Owing to their high reactivity, pentafluorophenyl esters of BOC-amino acids can be well applied in solid phase peptide synthesis. With their application we synthesized the protected tetrapeptide ACTH 11—14. The synthesis is compared with other methods.

In the first years of the application of solid phase peptide synthesis DCC was almost exclusively used as condensing agent in the peptide coupling reactions; only the coupling of N-acylglutamine and asparagine was performed by active esters. When the numerous disadvantages of the DCC-method had become evident, various active esters, mostly *p*-nitrophenyl esters (*p*-ONP), were often successfully applied in solid phase peptide synthesis [1—3]. A great advantage of the active ester coupling is to give generally purer products than DCC-condensation, furthermore the excess active ester can be regenerated. However, the use of *p*-ONP has also shortcomings, because, according to KARLSSON [4] and LOSSE [5], quantitative reaction cannot be reached even after 2×16 hours coupling. BODANSZKY [6] found, that in solid phase peptide synthesis *o*-nitrophenyl esters (*o*-ONP) are more reactive than *p*-nitrophenyl- and pentachlorophenyl esters, probably owing to steric effects.

As the active esters applied hitherto in solid phase synthesis were not sufficiently reactive, we tried to use for solid phase peptide synthesis the pentafluorophenyl esters of high reactivity, successfully applied in classical peptide synthesis by KISFALUDY and KOVÁCS [7]. First, we prepared *o*- and *p*-nitrophenyl esters as well as pentafluorophenyl esters of several BOC-amino acids (see Table I) then we stated [8] by reaction kinetic measurements that the pentafluorophenyl esters of BOC-amino acids are much more reactive in solid phase peptide synthesis, than *o*-ONP and *p*-ONP. In our kinetic measurements we always used the concentration generally applied in solid phase peptide synthesis, because only results obtained in this way can be utilized in synthetic work. For comparing the reactivity of different active esters, two data were used: (*a*) the time ($t_{1/2}$) necessary for 50% reaction, (*b*) the degree of coupling reached after 16 hours reaction time with 3fold excess of the active ester. The results are given in Table II.

According to Table II, the difference between the reactivities of *o*- and *p*-nitrophenyl esters is not high, and in some cases the *p*-nitrophenyl esters prove to be more reactive. The deviation of these results from the measurements of BODANSZKY [6]

Table I

Physical properties and analytical data of active esters of BOC-amino acids

Active ester	Solvent for recrystallization	Melting point °C	Formula; (MW)	Elemental analysis Calcd.	Found
BOC-Phe-*o*-ONP	ethanol	143—144	$C_{20}H_{22}O_6N_2$ (386.4)	C 62.2 H 5.7	62.5 6.0
BOC-Phe-*p*-ONP	ethanol	125—126	$C_{20}H_{22}O_6N_2$ (386.4)	C 62.2 H 5.7	62.0 5.9
BOC-Phe-OPFP	ethyl acetate—petroleum-ether	108—109	$C_{20}H_{18}O_4NF_5$ (431.3)	C 55.7 H 4.2	55.5 4.4
BOC-Leu-*o*-ONP	ether—hexane	52—54	$C_{17}H_{24}O_6N_2$ (352.4)	C 57.9 H 6.9	57.8 7.1
BOC-Leu-*p*-ONP	ethanol—water	85—86	$C_{17}H_{24}O_6N_2$ (352.4)	C 57.9 H 6.9	58.1 6.7
BOC-Leu-OPFP	oil	oil	$C_{17}H_{20}O_4NF_5$ (397.3)	C 51.5 H 5.1	51.9 5.4
BOC-Asn-*o*-ONP	DMF—water	143—145	$C_{15}H_{19}O_7N_3$ (353.3)	C 51.0 H 5.4	51.3 5.6
BOC-Asn-*p*-ONP	ethanol	162—163	$C_{15}H_{19}O_7N_3$ (353.3)	C 51.0 H 5.4	51.4 5.6
BOC-Asn-OPFP	ether—hexane	119—121	$C_{15}H_{15}O_5N_2F_5$ (397.3)	C 45.5 H 3.8	46.1 4.2
BOC-Asn(Xa)-*o*-ONP	DMF—water	139—141	$C_{28}H_{27}O_8N_3$ (533.6)	C 63.0 H 5.2	63.4 5.4
BOC-Asn(Xa)-*p*-ONP	DMF—water	149—152	$C_{28}H_{27}O_8N_3$ (533.6)	C 63.0 H 5.2	63.7 5.3
BOC-Asn(Xa)-OPFP	ethyl acetate—hexane	160—162	$C_{28}H_{23}O_6N_2F_5$ (579.2)	C 58.2 H 4.0	58.4 4.0
BOC-Gln(Xa)-*o*-ONP	DMF—water	115—117	$C_{29}H_{29}O_8N_3$ (547.6)	C 63.5 H 5.3	64.0 5.5
BOC-Gln(Xa)-*p*-ONP	DMF—water	109—111	$C_{29}H_{29}O_8N_3$ (547.6)	C 63.6 H 5.3	64.1 5.7
BOC-Gln(Xa)-OPFP	ethyl acetate—hexane	149—150	$C_{29}H_{25}O_6N_2F_5$ (593.2)	C 58.8 H 4.2	59.2 4.4
BOC-Gly-*o*-ONP	ethanol	97—98	$C_{13}H_{16}O_6N_2$ (296.3)	C 52.7 H 5.4	53.1 5.6
BOC-Gly-*p*-ONP	ethanol—water	78—80	$C_{13}H_{16}O_6N_2$ (296.3)	C 52.7 H 5.4	53.0 5.5
BOC-Gly-OPFP	ethyl acetate—hexane	75—76	$C_{13}H_{12}O_4NF_5$ (341.3)	C 45.7 H 3.5	45.8 3.8
BOC-Tyr(Bzl) OPFP	ethyl acetate—hexane	121—123	$C_{27}H_{24}O_5NF_5$ (537.5)	C 60.4 H 4.5	60.8 4.6
BOC-Pro-OPFP	hexane	48—51	$C_{16}H_{16}O_4NF_5$ (381.3)	C 50.4 H 4.2	50.3 4.1
BOC-Val-OPFP	hexane	58—60	$C_{16}H_{18}O_4NF_5$ (383.3)	C 50.1 H 4.7	50.4 4.9
Z-Lys(BOC)-OPFP	ether—hexane	68—70	$C_{25}H_{27}O_6N_2F_5$ (546.5)	C 55.0 H 5.0	55.0 4.8

Table II

Results of kinetic measurements

Amino acid	Active ester in equivalents	$t_{1/2}$ (hours)			Coupling (%) after 16 hrs reaction		
		p-ONP	*o*-ONP	OPFP	*p*-ONP	*o*-ONP	OPFP
BOC-Phe	1	11.5	3.2	0.4	—	—	—
	3	1.0	0.5	0.3	68	80	96
BOC-Leu	1	23.0	18.0	—	—	—	—
	3	2.5	1.5	1.0	54	82	97
BOC-Asn	1	4.5	6.0	—	—	—	—
	3	1.5	1.0	0.5	67	75	88
BOC-Val	3	—	—	1.5	—	—	92
BOC-Gly	1	24.0	9.3	—	—	—	—
	3	2.8	1.4	1.1	55	70	95
BOC-Ile	3	—	—	2.4	—	—	77
BOC-Pro	3	—	—	0.3	—	—	98
BOC-Met	3	—	—	2.1	—	—	82
BOC-Ala	3	—	—	1.2	—	—	94
BOC-Cys(Bzl)	3	—	—	0.6	—	—	96
BOC-Tyr(Bzl)	3	2.0	2.1	1.4	63	60	91
BOC-Asn(Xa)	3	2.6	2.8	1.7	65	58	86
BOC-Gln(Xa)	3	6.0	2.9	1.5	52	60	88

may be explained by the much higher concentrations applied in our method, and perhaps by the difference of polymers. It is worth mentioning that the $t_{1/2}$ values of pentafluorophenyl esters exceed 1 hour only in few cases. The difference in reactivities of active esters is even more apparent from the comparison of the percentage of coupling after 16 hours. This value often does not reach 60%, and never exceeds 90% for nitrophenyl esters, while for pentafluorophenyl esters it is never lower than 80% and often as high as 95—97%.

On the basis of these data it seemed probable that pentafluorophenyl esters can be succesfully used in solid phase synthesis; we proved their applicability by the synthesis of the protected tetrapeptide Z-Lys(BOC)-Pro-Val-GlyOMe (ACTH 11—14). In the synthesis the reaction of the active esters BOC-Val-OPFP (in 6x excess), BOC-Pro-OPFP (in 3x excess), Z-Leu(BOC)-OPFP (in 6x excess) with the glycyl-polymer was successively used. We obtained 95—97% coupling after 10—12 hours in all cases. The tetrapeptide was split off from the resin in methyl-ester form, yielding ~80% rough product, which, purified by crystallization, gave 70% pure, chromatographically homogeneous protected tetrapeptide.

Our method was compared with other methods of solid phase peptide synthesis and with the repetitive excess mixed anhydride (REMA) method. We synthesized the ACTH 11—14 sequence mentioned above according to MERRIFIELD's original description, using 3x excess BOC-amino acids and DCC for each coupling. The rough product contained more impurities than that obtained with our method; it could be, however, comparatively easily purified by extraction. Compared with the active

ester method the synthesis of this tetrapeptide by simple DCC-condensation gave a lower yield and required longer purification. However, applying 3-nitrophthalic anhydride for preventing the formation of failure sequences [9], the DCC-condensation could also be performed with good yield, giving a relatively pure end-product. Details of this synthesis will be described elsewhere [10].

For comparison, we tried also to apply the REMA-method [11] in the synthesis of the ACTH 11—14. As the sequence contains valine and proline, this method did not prove to be suitable for the synthesis of this tetrapeptide.

Summarizing the results of kinetic measurements and the methods of peptide sysntheses described, it can be stated that the BOC-amino acid pentafluorophenyl esters, owing to their high reactivity, can be well applied in solid phase peptide synthesis. The synthesis of ACTH 11—14 can be performed also with DCC method, the active ester method gives, however, better results.

Experimental

Pentafluorophenyl esters of BOC-amino acids

10 mmole BOC-amino acid and 10 mmole pentafluorophenol were dissolved in 25 ml ethyl acetate, (in the case of derivatives of glutamine and asparagine with addition of some dimethylformamide). The solution was cooled to 0°C, then 10 mmole DCC solved in 5 ml ethyl acetate was added, the solution was stirred at 0°C for 1 hr. The DCU, precipitated after standing at −10°C for 30 min, was filtered off and the solution evaporated in vacuum. The rough product was triturated with *n*-hexane and purified by recrystallization (see Table I).

o- and p-Nitrophenyl esters of BOC-amino acids

5 mmole BOC-amino acid and 6 mmole nitrophenol was dissolved in 10 ml ethyl acetate (in the case of derivatives of glutamine and asparagine in DMF—tetrahydrofuran mixture). Further process was as for pentafluorophenyl esters.

Kinetic measurements

The first charge of the glycyl-polymer prepared from MERRIFIELD-polymer (FLUKA 63 871) by the method described in [12] was 0.15 mmole/g. The BOC protecting group was split off with 25% trifluoroacetic acid (TFA) in CH_2Cl_2. The active esters were used in two different quantities (see Table II). The rate of the coupling reaction was followed by amino-acid analysis, as the measurement of the liberated phenol reagent did not give reliable results.

To 20 mg (3 μmole) glycyl-polymer 3 μmole=1 equivalent or 9 μmole=3 equivalents active ester of BOC-amino acid dissolved in 0.2 ml DMF was added. After 2, 10, 30 min, and 1, 2, 4, 8, 16, 24 hours the suspension was filtered, the unreacted active ester was washed off 3× with DMF, 2× with CH_2Cl_2, 2× with methanol and 1× with ether. The dipeptidyl-resin was hydrolysed in vacuum in a sealed tube with a mixture of 0.5 ml cc. HCl and 0.5 ml acetic acid for 8 hrs or with 3*M* mercapto-

ethanesulfonic acid (MES) in acetic acid. The hydrolysate was filtered, and condensed. (When applying MES the pH was adjusted to 2.20 by 4*N* NaOH). The residue was dissolved in 1.00 ml buffer (pH 2.20) and the amino-acids were analyzed with an automatic amino-acid analyser Type Hd-1200 E.

Solid phase synthesis of Z-Lys(BOC)-Pro-Val-Gly-OMe

(*a*) *By DCC-condensation.* The synthesis was performed with 12 g (= 4.8 mmole) glycyl-resin, using subsequently the BOC-valine (in 4× excess), BOC-proline and Z-Lys(BOC)OH (both in 3× excess). CH_2Cl_2 was used as solvent for coupling and subsequent washing. The BOC protecting groups were split off by 25% TFA in CH_2Cl_2. The coupling reactions were checked by amino-acid analysis. (Repetition of the coupling reaction proved not necessary in any case). The peptide was split off from the resin with BEYERMAN's method [13]: the polymer was suspended in 150 ml methanol, and 30 ml diisopropylethylamine was added. After 20 hrs. shaking the resin was filtered off, washed with 50 ml methanol and the methanol distilled off in vacuum. The oily residue obtained was triturated with ether, yielding 2.8 g (87%) amorphous material, which was chromatographycally inhomogeneous. Further purification (boiling in ether, crystallization from ethyl acetate—petroleum ether) gave 1.76 g (55%) pure product, m.p. 109—111 °C. Amino-acid analysis: Gly 1.00; Pro 1.1; Val 0.96; Lys 0.95. $C_{32}H_{49}O_9N_5$ (674.75); Calc.: C 59.33; H 7.62; N 10.79. Found: C 58.90; H 7.80; N 10.65%.

(*b*) *By application of pentafluorophenyl esters of BOC-amino acids.* The synthesis was made with 5 g (= 2 mmole) glycyl-resin using subsequently BOC-Val-OPFP (in 6× excess), BOC-Pro-OPFP (in 3× excess) and Z-Lys(BOC)-OPFP (in 6× excess). A 2:1 mixture of CH_2Cl_2 and DMF was used as solvent for coupling and subsequent washing. Coupling time was 10—12 hrs, the reaction was checked by the semiquantitative KAISER test [14] and by amino-acid analysis. Repeated coupling was not necessary. The BOC-groups were split off by 25% TFA in CH_2Cl_2. The tetrapeptide was cleaved from the resin with BEYERMAN's method (see preceding paragraph). 1.1 g (80%) rough material was obtained and purified by crystallization from ethyl acetate—petroleum ether, yielding 0.94 g (70%) chromatographically pure tetrapeptide, m.p. 110—113 °C.

Amino-acid analysis: Gly 1.00; Pro 1.08; Val 0.92; Lys 1.05.

$C_{32}H_{49}O_9N_5$ (674.75); Calc.: C 59.33; H 7.62; N 10.79. Found: C 59.05; H 7.80; N 10.52%.

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ПРИМЕНЕНИЕ ПЕНТАФТОРФЕНИЛОВЫХ ЭФИРОВ ВОС-АМИНОКИСЛОТ В СИНТЕЗЕ ПЕПТИДОВ В ТВЁРДОЙ ФАЗЕ

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Пентафторфениловые эфиры ВОС-аминокислот вследствие их большой реактивности с успехом употребляются в синтезе пептидов в твёрдой фазе. С применением этих соединений нами был синтезирован защитный тетрапептид АСТН 11—14. Этот синтез сравнивался с другими методами синтеза.