172. (*p*-Phenylazophenyl)-isopropyloxycarbonyl, a New Protecting Group for Peptide Synthesis

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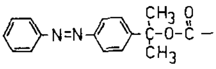
Summary. The preparation and properties of thirty two N(α)-[2-(*p*-phenylazophenyl)-isopropylcarbonyl]-amino-acids and derivatives are described. The new coloured protecting group (AZOC-) can be easily and selectively removed with mild acid treatment, much the same as the 2-(biphenyl)-isopropyloxycarbonyl (BPOC-)group. It is introduced *via* quite stable and yet reactive, crystalline intermediates, AZOC-OPh and AZOC-N_3.

The aim of this work was to develop a new amino-protecting group that combines certain advantages of earlier coloured groups [1] with those of the 2-(*p*-diphenyl)-isopropyloxycarbonyl group (DPOC-, BPOC-)¹) [2] or, for that matter, of the 2-phenyl-isopropyloxycarbonyl group (PPOC-) [2] [4]. The new 2-(*p*-phenylazophenyl)-isopropyloxycarbonyl-group has, as an N(α)-protecting group for amino-acids, the following properties:



$C_6H_5-N{=}N-C_6H_4-\underset{CH_3}{\overset{CH_3}{C}}-O-\overset{O}{\overset{\|}{C}}-$

AZOC-

1) it can be introduced *via* stable, yet reactive, and crystalline intermediates such as its phenyl ester (AZOC-OPh) or acid azide (AZOC-N_3);

2) it can be removed under practically the same mildly acidic conditions and with the same yields and velocities as the BPOC-group, leaving other (side-chain) protecting groups intact;

3) the colour facilitates the operations of purification and isolation by chromatographic and distribution techniques [$\lambda_{max} = 320$–330 nm, $\varepsilon = 2.10^5$–4.10^5 in ethanol];

4) the colour allows for an exact estimation of the reaction time needed for a complete removal of the protective group prior to a next coupling step (this could be an essential advantage for solid-phase synthesis).

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¹) Abbreviations according to the recommendations of the "IUPAC-IUB Commission on Biochemical Nomenclature", *p.e.* Eur. J. Biochemistry *1*, 375 (1967), and [3].

Table. *Analytical data of some N(α)-AZOC-amino-acids and derivatives*

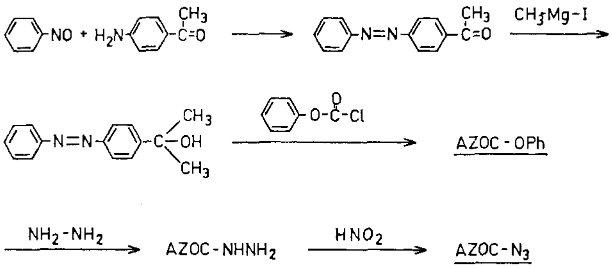
No.	N(α)-AZOC-L-amino-acid	Calc. Mol.-wt.	Empirical formula	Yield Method	M.p. °C	$[\alpha]_D^{25}$ solvent ($c = 1$)	Microanalysis Calc. Found			
							C %	H %	N %	S %
1	· Ala · DCHA	454.55	$C_{25}H_{34}N_4O_4$	70%	146– 9	– 11.0 (MeOH)	66.06	7.54	12.33	
				A			65.90	7.38	12.59	
2	· Arg(NO_2) · DCHA	666.82	$C_{34}H_{50}N_8O_6$	68%	129–131	+ 2.8 (MeOH)	61.24	7.56	16.80	
				A			60.52	7.38	16.81	
3	· Arg(TOS) · CHA	693.87	$C_{35}H_{47}N_7O_6S$	59%	131–133	– 1.9 (MeOH)	60.59	6.83	14.13	4.62
				A			60.74	7.08	13.24	4.66
4	· Asn	398,60	$C_{20}H_{22}N_4O_5$	50%	167– 8	– 2.8 (MeOH)	60.29	5.57	14.06	
				A			60.21	5.55	13.75	
5	· Asp · 2 DCHA	762.00	$C_{44}H_{67}N_5O_6$	73%	158– 60	+ 10.1 (MeOH)	69.33	8.86	9.19	
				A			69.60	8.86	8.91	
6	· Asp(OBZL) · CHA	588.38	$C_{33}H_{40}N_4O_6$	60%	156– 7	+ 31.5 ($CHCl_3$)	67.38	6.81	9.52	
				A			67.53	6.92	9.56	
7	· Cys(ACM) · CHA	557.71	$C_{28}H_{39}N_5O_5S$	68%	82– 84	+ 18.8 (MeOH)	60.3	7.05	12.56	5.75
				C			60.26	7.12	12.37	5.79
8	· Glu · 2 CHA	611.75	$C_{33}H_{49}N_5O_6$	80%	193– 6		64.78	8.07	11.45	
				A			64.45	8.01	11.49	
9	· Glu(OBZL) · CHA	602.70	$C_{34}H_{42}N_4O_6$	45%	156– 7	+ 27.5 ($CHCl_3$)	67.74	7.02	9.30	
				A			67.66	7.11	9.25	
10	· Gln · DCHA	593.82	$C_{33}H_{47}N_5O_5$	32%	–102	+ 4.3 ($CHCl_3$)	66.74	7.99	11.79	
				A			66.43	7.81	11.49	
11	· Gly	341.97	$C_{18}H_{19}N_4O_4$	70%	128– 30	0 (MeOH)	63.39	5.60	12.29	
				A			63.63	5.71	12.25	
12	· His	421.46	$C_{22}H_{23}N_5O_4$	44%	151–152	+ 1.8 (MeOH)	62.70	5.50	16.62	
				C						
13	· His(BZL)	511.55	$C_{29}H_{29}N_5O_4$	50%	200	+105.4 ($CHCl_3$)	68.09	5.71	13.69	
				A			68.12	5.71	13.70	
14	· His(DNP) · DCHA	769.88	$C_{40}H_{49}N_8O_8$	65%	132–134		62.40	6.42	14.55	
				A						
15	· Ile · CHA	496.62	$C_{28}H_{40}N_4O_4$	52%	158– 60	+ 2.9 ($CHCl_3$)	67.73	8.12	11.28	
				A			67.96	8.07	11.17	
16	· Leu · CHA	496.62	$C_{28}H_{40}N_4O_4$	60%	168– 71	0 ($CHCl_3$)	67.73	8.12	11.20	
				A			67.89	8.14	11.34	

Table (continuation)

No.	N(α)-AZOC-L-amino-acid	Calc. Mol.-wt.	Empirical formula	Yield Method	M.p. °C	$[\alpha]_D^{25}$ solvent) ($c = 1$)	Microanalysis Calc. Found			
							C %	H %	N %	S %
17	· Lys(BOC) · CHA	612.79	$C_{33}H_{50}N_5O_6$	60% A	95– 99	+ 1.4 (MeOH)	64.68 64.34	8.23 8.06	11.43 10.97	
18	· Lys(Z) · DCHA	727.90	$C_{42}H_{57}N_5O_6$	60% A	65	+ 2.4 (MeOH)	69.31 69.60	7.89 7.99	9.62 9.67	
19	· Met · CHA	515.67	$C_{27}H_{39}N_4O_4S$	78% A	140– 1	+ 6.2 (MeOH)	62.88 63.12	7.62 7.64	10.86 10.85	6.22 6.07
20	· Orn(BOC) · DCHA	679.87	$C_{38}H_{57}N_5O_6$	60% A	160– 1	+ 19.8 (MeOH)	67.13 67.38	8.45 8.45	10.30 10.34	
21	· Phe · DCHA · $^1/_2$ 2-Propanol	642.83	$C_{37}H_{48}N_4O_4$ $+^1/_2(C_3H_8O)$	72% B	185– 6	+ 22.8 (MeOH)	71.93 71.87	8.15 8.05	8.72 8.93	
22	· Pro · CHA	480.56	$C_{27}H_{36}N_4O_4$	80% A	186– 8	+ 4 (MeOH)	67.45 67.43	7.54 7.41	11.65 11.79	
23	· Ser	371.39	$C_{19}H_{21}N_3O_5$	46% C	126–128	– 9.0 (MeOH)	61.45 61.41	5.70 5.74	11.32 11.31	
24	· Ser(Bu^t) · CHA	526.65	$C_{29}H_{42}N_4O_5$	65% A	177– 84	+ 17.8 (MeOH)	66.14 66.09	8.04 8.10	10.64 10.61	
25	· Ser(BZL) · CHA	560.38	$C_{32}H_{40}N_4O_5$	45% A	139– 40	+ 32.3 ($CHCl_3$)	68.58 68.55	7.14 7.24	10.00 9.90	
26	· Thr(Bu^t) · CHA	540.67	$C_{30}H_{44}N_4O_5$	70% A	208– 10	+ 0.7 (MeOH)	66.64 66.65	8.20 8.27	10.36 10.42	
27	· Thr(BZL) · CHA	574.70	$C_{33}H_{42}N_4O_5$	54% A	178– 80	+ 27.2 (MeOH)	68.96 68.86	7.37 7.48	9.75 9.76	
28	· Trp · CHA	561.74	$C_{33}H_{39}N_5O_4$	78% C	100–102	+ 4.4 (MeOH)	69.56 69.46	6.90 7.05	12.29 11.94	
29	· Tyr · DCHA	628.81	$C_{37}H_{48}N_4O_5$	49% C	112–114	+ 39.4 (MeOH)	70.67	7.69	8.91	
30	· Tyr(BZL) · CHA	636.79	$C_{38}H_{44}N_4O_5$	52% A	145–146	+ 45.4 (MeOH)	71.67 71.54	6.96 7.01	8.80 8.90	
31	· Tyr(Bu^t) · CHA	603.78	$C_{35}H_{47}N_4O_5$	52% A	158–161	+ 63.0 (MeOH)	69.62 69.37	7.85 7.75	9.28 9.61	
32	· Val · CHA	482.60	$C_{27}H_{38}N_4O_4$	50% A	162– 3	+ 4 ($CHCl_3$)	67.19 67.16	7.94 7.96	11.61 11.70	

Thus, the ease and specificity of removal distinguish AZOC-favourably from the earlier coloured groups (*p.e.* PZ-) its colour offers an advantage over the BPOC-group. The free AZOC-amino-acids appear to be somewhat more stable than BPOC-amino-acids.

The synthesis of reactive intermediates was carried out as follows:



Phenyl-[2-(*p*-phenylazophenyl)-isopropyl]-carbonate (AZOC-OPh) is a stable crystalline solid. NMR. indicated no deterioration at room temperature after 3 months. It reacts readily either with the benzyl-trimethylammonium (Triton B) salts of amino-acids [2] in dimethylformamide (DMF), procedure B, or preferably with the tetramethyl-guanidine (TMG) salts in dimethylsulfoxide (DMSO), procedure A. Excess quantities of AZOC-OPh can often be recovered unchanged from the reaction mixtures.

2-(*p*-Phenylazophenyl)-isopropyloxycarbonyl-azide, AZOC-N_3, is also crystalline and quite stable (*p.e.* for months at 0°). It was reacted with amino-acids in DMSO + TMG, procedure C.

The amino-acid derivatives are quite stable as such, however, most of them were converted to and stored as either cyclohexylamine (CHA) or dicyclohexylamine (DCHA) salts (see Table).

Experimental Part

M.p. were determined in open capillary tubes and are not corrected. The usual isolation procedure of products from a reaction mixture comprises extraction into an organic solvent (ethyl acetate, ether, dichloromethane, or chloroform), washing of the organic phase with aqueous acid (mostly 1M citric acid), transfer of acidic products into aqueous alcali and back into fresh organic phase when required, washing with water, drying with conc. NaCl-solution followed by anhydrous sodium- or magnesium-sulfate, filtration, and evaporation of the solvent in a rotatory evaporator at 30–40° under reduced pressure (10 to 0.01 Torr). Analytical samples were dried at 20° and 0.001 Torr for 24 hours.

*2-(*p*-Phenylazophenyl)-2-propanol.* *p*-Phenylazo-acetophenone [5] was prepared from nitrosobenzene and *p*-amino-acetophenone according to the general procedure used earlier [1]; yield 50%. The crystalline compound was reacted in ether with methyl-magnesium-iodide to produce 2-(*p*-phenylazophenyl)-2-propanol in 70% yield (after crystallization from ether/petroleum ether), m.p. 85–8°.

$C_{15}H_{16}N_2O$ (240.29) Calc. C 74.98 H 6.71 N 11.66% Found C 74.76 H 6.82 N 11.86%

*Phenyl-[2-(*p*-phenylazo-phenyl)-isopropyl]-carbonate (AZOC-OPh).* To a stirred solution of 7.2 g of 2-(*p*-phenylazo-phenyl)-2-propanol in 40 ml of dichloromethane and 3.6 ml of pyridine at −5° was added dropwise over a period of 30 min a solution of 4.8 ml of phenyl-chloroformate dissolved in 20 ml of dichloromethane. A thick paste was gradually formed after the addition. The reaction mixture was further stirred overnight at 0°. The resulting mixture was now quite clear, except for a small amount of undissolved matter. It was diluted with 100 ml of dichloromethane and the product isolated with the usual procedure. Recrystallization from abs. ethanol: 9.2 g (88%), m.p. 100–3°.

$C_{22}H_{20}N_2O_3$ (360.39) Calc. C 73.32 H 5.59 N 7.77% Found C 73.14 H 5.58 N 8.05%

*2-(*p*-Phenylazo-phenyl)-isopropyloxycarbonyl-hydrazine, AZOC-NHNH*$_2$. 18 ml of hydrazine hydrate were added to the solution of 21.6 g of AZOC-OPh. The mixture was stirred at room temp. for 18 h and then poured into much ice water. The product was isolated by the usual procedure (ether; 1N NaOH; water; NaCl solution; $MgSO_4$). Crystallisation from ether/petroleum ether and diisopropyl-ether: 14.5 g (81%), m.p. 94–96°.

$C_{16}H_{18}N_4O_2$ (298.33) Calc. C 64.37 H 6.08 N 18.77% Found C 64.37 H 6.11 N 18.73%

*2-(*p*-Phenylazo-phenyl)-isopropyl-oxycarbonyl-azide, AZOC N*$_3$. This compound was prepared essentially according to the procedure of *Sieber & Iselin* [2] for BPOC-N_3. Crystallisation from petroleum ether. Yield 91%, m.p. 49–50°. Characteristic IR. absorption bands at 2810–2870, 2120–2160, 1705, 1445, and 1350–1360 cm^{-1}.

$C_{16}H_{15}N_5O_2$ (309.31) Calc. N 22.64% Found 22.70%

Preparation of N(α)-AZOC-amino-acids. – A) *The AZOC-OPh/TMG procedure.* 0.225 g (3 mmol) of glycine in 6 ml of DMSO were treated with 0.38 ml (3 mmol) of tetramethylguanidine (TMG) and 1.08 g (3 mmol) of AZOC-OPh with gentle stirring at 50° for 4 h. The red, crystalline product obtained by the usual isolation procedure was recrystallized from ether: 0.7 g (70%).

B) *The AZOC-OPh/Triton procedure.* The procedure is essentially that of *Sieber & Iselin* [2] for BPOC-derivatives. Example: AZOC · Phe · OH, DCHA, see Table.

C) *The AZOC-N*$_3$*/TMG procedure.* 1.135 g (5 mmol) of S-acetamidomethyl-cysteine hydrochloride were suspended by stirring in 6 ml of dry DMF. 2.5 ml (20 mmol) of tetramethyl-guanidine (TMG) were added, followed by 1.54 g (5 mmol) of solid AZOC-N_3. After 3½ h at 45°, the product obtained by the usual isolation procedure was dissolved in ether and converted to the cyclohexylamine (CHA) salt. Crystallisation from petroleum ether: 1.9 g (68%).

Removal of the AZOC-Group. – Complete conversion of AZOC-glycine (0.1M) to glycine, carbon dioxide, and 2-(*p*-phenylazophenyl)-2-propanol was achieved at room temp. in 5 min with trifluoro-acetic acid/dichloromethane 1.5:98.5 (*v*/*v*), in 45 min with acetic acid/83% formic acid/water 7:1:2 (*v*/*v*), and in 6 h with acetic acid/water 8:2 (*v*:*v*).

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