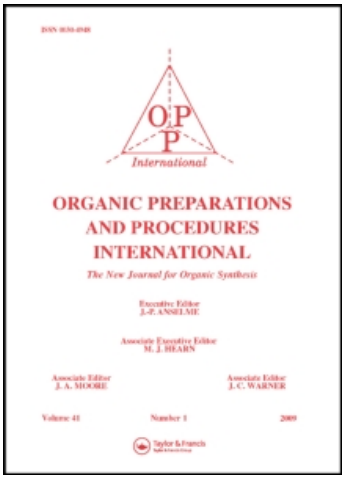

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Leszek Krzyzanowski[a]; Barbara Rzeszotarska[b]
[a] Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa, Poland [b] Institute of Chemistry, Pedagogical University, Opole, Poland

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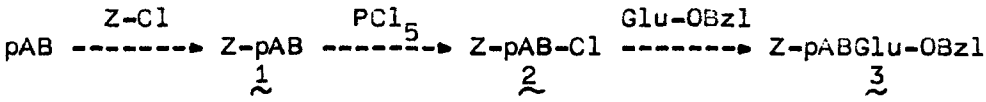
SYNTHESIS OF N-(p-CARBOBENZOXYAMINO-BENZOYL)-L-GLUTAMIC ACID α-BENZYL ESTER

Leszek Krzyżanowski† and Barbara Rzeszotarska††*

†Nencki Institute of Experimental Biology
Polish Academy of Sciences, ul.Pasteura 3, Warszawa 22,POLAND

††Institute of Chemistry
Pedagogical University, ul. Oleska 48, 45-052 Opole, POLAND

Oligo-γ-glutamyl chain lengths in natural folates are determined first by cleavage of the folates to the corresponding N(-p-aminobenzoyl)-oligo-γ- glutamic acid)s (pAB-Glu$_n$) followed by co-chromatography of these simpler components with authentic carrier compounds.[1] Up to now, the authentic carrier compounds were obtained from the splitting of synthetic N-pteroyl-oligo-γ-(glutamic acid)s.[2] We undertook the synthesis of the carrier pABGlu$_n$ from pAB and Glu and chose benzyl as a permanent protecting group; the synthesis of the key compound, N-(p-carbobenzoxyaminobenzoyl)-L-glutamic acid α-benzyl ester (Z-pABGlu-OBzl) is described herein (79% overall yield).



Z-pAB was obtained by treatment of pAB with 20% excess on Z-Cl in aqueous NaOH. Mixed anhydride, Z-pAB-O-Z (4) formed as a by-product of the reaction was hydrolysed *in situ* by NaOH in aqueous acetone. The present preparation of Z-pAB provides a simpler, faster and more efficient route than that

previously reported.[3] Z-pAB-Cl was obtained with PCl_5 according to Fu[4] who used Z-pAB-Cl *in situ*; we isolated the acid chloride in good yields as a stable crystalline compound. Only the acylation of Glu diesters with Z-pAB-Cl is known[4] but that not of monoesters. Thus, we had to determine conditions for the successful preparation of Z-pABGlu-OBzl (the reaction was monitored by TLC; see Table). Coupling Z-pAB-Cl with Glu-OBzl in DMF in the conventional manner using two equivalents

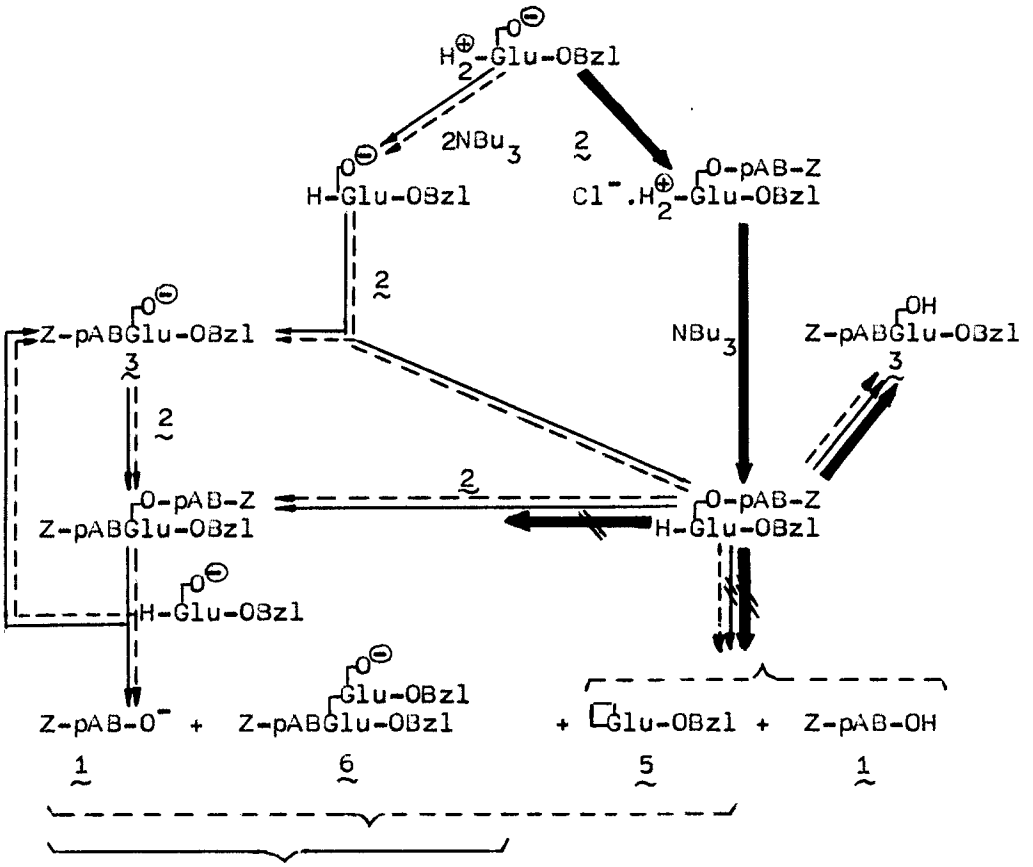
TABLE. *Influence of Solvent, Species and Amount of t-Amine on By-product Formation in Z-pAB-Cl Coupling with Glu-OBzl*

Exp.	Solvent	Equivalents NMM	Equivalents NBu_3	Z-pAB 1	By-products □Glu-OBzl[6] 5	By-products Z-pAB-Glu(Glu-OBzl)-OBzl[7] 6
1	DMF	2		+	+	+
2	DMF		2	+	+	+
3	Dioxane	2		+	-	+
4	Dioxane		2	+	-	+
5	Dioxane	1[a]		-	-	-
6	Dioxane		1[a]	-	-	-

a) Introduced after Glu-OBzl had dissolved.

of either N-methylmorpholine (NMM) or tri-n-butylamine (NBu_3) led to the desired compound and by-products: Z-pAB (1), pyroglutamic acid benzyl ester (□Glu-OBzl 5) and N-(p-carbobenzoxyaminobenzoyl)-L-glutamyl-(Cγ)-L-glutamic acid α,α'—dibenzyl ester [Z-pABGlu(Glu-OBzl)-OBzl 6; Exp. 1 and 2; see Scheme of probable reaction routes (---►)]. The next couplings were performed in dioxane dried over sodium. □Glu-OBzl was not formed and Z-pAB amount decreased (Exp. 3 and 4; the route ——►).

Still better results were achieved in a unconventional manner using one equivalent of tertiary amine only (Exp. 5 and 6; the route ➡). The amine should be introduced into the reaction medium upon dissolution of Glu-OBzl. The above procedu-



re practically suppresses the formation of by-products and thus allowed the formation of Z-pABGlu-OBzl in high yield.

EXPERIMENTAL SECTION

General.- Solvents were evaporated in vacuo on a rotary evaporator at a bath temperature not exceeding 30°. Mps uncorrected were determined an a Boetius apparatus. ^{1}H NMR spectra were taken on a 100 MHz Tesla spectrometer BS 567 in dimethylsulfoxide (TMS internal standard) and IR were obtained on a Zeiss spectrometer Specord 71. TLC was performed on silica gel pla-

tes (DC Alufolien Kieselgel 0.25 Merck 5553) in the following solvent systems: A = chloroform-methanol-acetic acid (95:5:3), B = chloroform-methanol-concd ammonia (6:5:1), C = benzene-pyridine-acetic acid (20:2:1), D = isopropanol-water (7:4). Spots were visualized with ninhydrin, chlorine-tolidine reagent and bromocresol green.

p-Carbobenzoxyaminobenzoic Acid (1).- To a vigorously stirred solution of pAB (13.70 g, 0.1 mol) in 1N NaOH (100 ml) cooled at 0°C were added Z-Cl (0.12 mol) and 2N NaOH (60 ml) at such a rate that the temperature did not exceed 25°C. After 6 hrs, acetone was added to produce a homogeneous mixture and 2N NaOH (30 ml) was introduced. After overnight standing, the acetone was evaporated and the aqueous solution extracted twice with ethyl acetate, placed in a large Erlenmeyer flask, vigorously stirred and acidified with 1N HCl at pH 1. The precipitate was dissolved in ethyl acetate, the organic layer was extracted with brine. Concentration of the organic layer afforded 25.57 g (94%) of product, mp. 224-225° (dec.), lit.[3] mp. 217-218° (dec.). R_f: A - 0.47, B - 0.65, C - 0.43, D -0.75. Anal. Calcd for $C_{15}H_{13}NO_4$: C,66.41; H,4.83; N,5.16

Found: C,66.41; H,4.72; N,5.14

Mixed Anhydride of p-Carbobenzoxyaminobenzoic Acid with Benzyl Carbonate (4).- This compound can be isolated as a by-product from Z-pAB synthesis. After 6 hrs acylation according to the foregoing procedure, the white precipitate was filtered, washed with water, dried and crystallized from diethyl ether-petroleum ether mp. 124-126°. R_f: A - 0.80, C - 0.46. ^1H NMR: δ 5.53 (s, 2H, CH_2), 5.70 (s, 2H, CH_2), 7.76 (s, br, 10H, 2 x C_6H_5), 8.02, 8.31 (d, d, A_2B_2, 4H, $J_1 = J_2 = 8.9$ Hz, C_6H_4), 10.71 (s, 1H, NH) ppm. IR(KBr): 3335 (NH), 1803 (CO), 1728 (CO), 1695 (CO Ia.b.), 1600 (C=C in Ar), 1532 (C-N-H

IIa.b.), 1222 (C-O-C), 1055 (C-O-C), 691 ($PhCH_2$) cm^{-1}.

Anal. Calcd for $C_{23}H_{19}NO_6$: C,68.14; H,4.72; N,3.46

Found: C,68.23; H,4.62; N,3.43

p-Carbobenzoxyaminobenzoylchloride (2).- To a stirred suspension of PCl_5 (2.29 g, 11 mmol) in anhydrous diethyl ether (30 ml) cooled at 0° was added Z-pAB (2.72 g, 10 mmol). After 1 h, anhydrous petroleum ether (60 ml) was introduced and after further 15 min. the white precipitate was filtered, washed with petroleum ether, spread out in a form of thin layer and left in the open air until smell of PCl_5 disappeared to give 2.55 g (90%) of product, mp. 128-130°; which may be stored in a vacuum desiccator over P_2O_5 for many months without change in its mp.

^1H NMR: δ 5.26 (s, 2H, CH_2), 7.46 (s, br, 5H, C_6H_5), 7.71, 8.01 (d, d, A_2B_2, 4H, $J_1 = J_2 = 8.5$ Hz, C_6H_4), 10.24 (s, 1H, NH) ppm. IR(KBr): 3365 (NH), 1728 (CO), 1707 (CO Ia.b.), 1584 (C=C in Ar), 1530 (C-N-H IIa.b.), 1205 (C-O-C), 1040 (C-O-C), 691 ($PhCH_2$) cm^{-1}.

Anal.Calcd for $C_{15}H_{12}ClNO_3$: C,62.18; H,4.18; Cl,12.24; N,4.84

Found: C,61.89; H,4.08; Cl,12.02; N,5.05

N- p-Carbobenzoxyaminobenzoyl -L-Glutamic Acid α-Benzyl Ester (3).- To a stirred suspension of Glu-OBzl[8] (2.37g, 10 mmol) in anhydrous dioxane (10 ml), was added Z-pAB-Cl (2.89 g, 10 mmol); Glu-OBzl dissolved after about 10 min. Then, the mixture was placed on a water bath and NBu_3 (2.38 ml, 10 mmol) added at such a rate that the temperature did not exceed 20°. After further 10 min., 0.1N HCl (100 ml) was introduced and the white precipitate was filtered and washed with water to

give 4.75 g (95%) of crude product which was crystallized from ethyl acetate to afford 4.65 g (93%) of product, mp. 184-187°. R_f: A - 0.27, B - 0.80, C - 0.24, D - 0.80, $[\alpha]_{578}^{21}$ = - 3.0°, $[\alpha]_{D}^{20}$ = - 2.9° (c 2, methanol; Zeiss polarimeter Polamat A).

^{1}H NMR: δ 2.12 (m, 2H, CH_2), 2.42 (t, 2H, J = 6.6 Hz, γCH_2), 4.58 (m, 1H, CH), 5.17 (s, 4H, 2 x CH_2Ph), 7.36 (s, br, 5H, Ph-ester), 7.41 (s, br, 5H, Ph-urethane), 7.61, 7.89 (d, d, $AA'B_2$, 4H, J_{AB} =8.0 Hz, $J_{A'B}$ = 8.5 Hz, C_6H_4), 8.68 (d, 1H, J = 7.0 Hz, αNH), 10.10 (s, 1H, NH), ppm. IR(KBr): 3360 (NH), 3260 (OH), 1735 (CO), 1707 (CO Ia.b.Ar), 1640 (C'O'Ia.b.), 1605 (C=C in Ar), 1532 (C-N-H Ar IIa.b.), 1500 (C'-N'-H' IIa.b.), 1235 (C-O-C), 1055 (C-O-C), 691 ($PhCH_2$) cm^{-1}.

Anal. Calcd for $C_{27}H_{26}N_2O_7 \cdot 1/2H_2O$: C,64.92; H,5.45; N,5.61

Found: C,64.88; H,5.23; N,5.31

REFERENCES

1. D. J. Cichowicz, S. K. Foo and B. Shane, Moll. Cell. Biochem., 39, 209 (1981); R. L. Kisliuk, ibid., 39, 331 (1981); I. Eto and C. L. Krumdieck, Anal. Biochem., 115, 138 (1981).
2. C. L. Krumdieck and C. M. Baugh, Biochem., 8, 1568 (1969); H. A. Godwin, I. H. Rosenberg, C. R. Ferenz, P. M. Jacobs and J. Meienhofer, J. Biol. Chem., 247, 2266 (1972).
3. German Patent 3,016,144 1980 ; Chem. Abs., 94, 121937k (1981).
4. S. C. J. Fu, J. Med. Pharm. Chem., 5, 33 (1962).
5. "Methoden der Organischen Chemie (Houben-Weyl). Synthese von Peptiden", Vol. XV/2, Ed. E. Wünsch, George Thieme Verlag, Stuttgart, 1974, p. 355.
6. ^{1}H NMR: δ 2.22-2.85 (m, 4H, CH_2CH_2), 4.56 (d, d, 1H, J_1 = J_2 = 4.5 Hz, CH), 5.49 (s, 2H, CH_2Ph), 7.70 (s, 5H, C_6H_5),

8.48 (s, 1H, NH) ppm. IR(KBr): 3330 (NH), 2940 (CH), 1735 (CO), 1696 (CO Ia.b.), 1195 (C-O-C), 1020 (C-O-C), 700 ($PhCH_2$) cm^{-1}.

7. ^1H NMR: δ 2.00-2.95 (m, 8H, 2 x CH_2CH_2), 5.46-5.51 (m, 6H, 3 x CH_2Ph), 7.70 (m, 15H, 3 x C_6H_5), 7.93, 8.22 (d, d, $AA'-B_2$, 4H, J_{AB} = 8.0 Hz, $J_{A'B}$ = 8.5 Hz, C_6H_4), 8.77 (d, 1H, J = 7.0 Hz, αNH), 9.08 (d, 1H, J = 7.0 Hz, αNH), 10.43 (s, 1H, NH) ppm. IR(KBr): 3300 (NH), 3030 (CH in Ar), 2940 (CH), 1732 (CO), 1722 (CO), 1705 (CO Ia.b.Ar), 1640 (C'O' Ia.b.), 1610 (C=C in Ar), 1535-1500 (C-N-H IIa.b.), 1218 (C-O-C), 1060 (C-O-C), 695 ($PhCH_2$) cm^{-1}.

8. H. Sachs and E. Brand, J. Am. Chem. Soc., 75, 4610 (1953).

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