112. Monohaptenic N_α-Benzoyl-L-lysine Derivatives as Anaphylactogens: the Importance of the Unsubstituted Carboxyl Group

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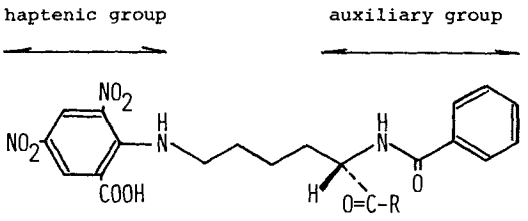
Summary

N_α-Benzoyl-L-lysine with a 2-carboxy-4,6-dinitrophenyl (Dncp) haptenic group on the ε-amino function is a potent anaphylactogen in the guinea pig. We prepared N_ε-Dncp-N_α-benzoyl-L-lysinamide and N_ε-Dncp-N_α-benzoyl-L-lysyl-1-aminopropane where the carboxyl group of lysine is blocked. Both compounds were non-elicitors of anaphylaxis.

Monohaptenic drugs and simple chemicals do not normally elicit anaphylactic and other immediate-type hypersensitivity reactions in sensitized individuals. Among the exceptions which nevertheless do, are molecules that contain, in addition to the haptenic moiety, special auxiliary groups. An effective auxiliary group is constituted *e.g.* by phenyl residues in conjunction with carboxyl groups in selected positions [1] [2]. We have already reported the biological result that, in a series of compounds with the 2-carboxy-4,6-dinitrophenyl (Dncp) group as the hapten, N_ε-Dncp-N_α-benzoyl-L-lysyl-1-aminopropane (**2**) is virtually devoid of anaphylactogenic capacity, whereas the parent compound with the unsubstituted carboxyl group, N_ε-Dncp-N_α-benzoyl-L-lysine is an effective anaphylactogen, as potent or more potent as other similar derivatives [3].

Since the somewhat bulky propylamine substituent might sterically hinder the required interaction between auxiliary group and supposed receptor, we also prepared N_ε-Dncp-N_α-benzoyl-L-lysinamide (**1**) where steric hindrance is considered to be negli-



1 R = NH_2
2 R = NH–CH_2–CH_2–CH_3
Parent compound R: OH (O^-)

[1]) Part of Ph.D. thesis of *R.G.*, University of Bern (1982).

Table. *Elicidation of Passive Cutaneous Anaphylaxis in the Guinea Pig with* N_ε*-Dncp-*N_α*-benzoyl-*L*-lysine Derivatives*

Derivative injected intravenously	μmol injected per animal	No. of positive/all animals	Average diameter of blueing (mm) in sites sensitized by antiserum *CT-23* dilution			
			1/40	1/320	1/1280	1/5120
N_ε-Dncp-N_α-benzoyl-L-lysine amide (**1**)	0.1	0/2	neg.	neg.	neg.	neg.
	1.0	0/4	neg.	neg.	neg.	neg.
	4.0	0/2	neg.	neg.	neg.	neg.
N_ε-Dncp-N_α-benzoyl-L-lysine[a])	0.1	4/4	[b])	27	22	11

[a]) Included for comparison.
[b]) Overreaction.

gible. This compound again was a non-elicitor of passive cutaneous anaphylaxis, used as a model, and gave clearly negative reactions in the tests up to high doses in anti-Dncp sensitized guinea pigs *(Table)*. This result emphasizes the importance of the carboxyl group, and possibly its charge as carboxylate, in mediating anaphylaxis of the type studied here.

Since compounds of low or lacking activity should be tested not only at the usual, but also at high dose, it becomes particularly important to remove traces of reactive reagent used for introducing the haptenic group. The reagent generally used for this purpose has been 2-chloro-3,5-dinitrobenzoic acid [4] but it was sometimes found difficult to remove its excess from the product to a degree which would not interfere with high-dose testing. It is to be noted that intravenous doses exceeding 4 μg of the chloro acid per guinea pig will already show slightly positive reactions in the passive cutaneous anaphylaxis test. We therefore used *tert*-butyl 2-chloro-3,5-dinitrobenzoate [5] which is highly lipophilic and can be readily removed on silica gel columns. Indeed, the products **1** and **2** did not show, after silica gel chromatography, *tert*-butyl 2-chloro-3,5-dinitrobenzoate or 2-chloro-3,5-dinitrobenzoic acid on thin layer chromatography (TLC) plates, where 50 μg–100 μg were run and 0.1–0.2 μg of the chloro derivatives could be detected under 254 nm irradiation.

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Experimental Part

General. See [5]. For passive cutaneous anaphylaxis the same anti-Dncp-bov. gamma globulin antiserum from the rabbit was used as in [5]. Starting materials were obtained from *Bachem AG*, Bubendorf and from *Fluka AG*, Buchs.

N_ε-*(2-*tert*-Butoxycarbonyl-4,6-dinitrophenyl)-*N_α*-Boc-*L*-lysinamide.* N_α-Boc-L-lysinamide (800 mg, 3.26 mmol) in 7 ml DMF was added to 986 mg (3.26 mmol) *tert*-butyl 2-chloro-3,5-dinitrobenzoate in 3 ml DMF and kept for 6 h at r.t. and pH 9 by adding Et_3N. The solution was diluted with 150 ml CH_2Cl_2 and extracted with 200 ml 0.1M HCl and 250 ml H_2O. The org. phase was dried (Na_2SO_4) and evaporated *in vacuo* at 40°. The residue was chromatographed on a 100 g silica gel *60* column with $CHCl_3/CH_3OH$ (95:5): 640 mg residue from the pooled fractions. TLC (B): R_f 0.56 (UV, +; yellow, +).

N_ε-*(2-Carboxy-4,6-dinitrophenyl)-*N_α*-benzoyl-*L*-lysinamide* (**1**). N_ε-(2-*tert*-Butoxycarbonyl-4,6-dinitrophenyl)-N_α-Boc-L-lysinamide *(640 mg, 1.8 mmol)* was kept in 10 ml ice cold 90% trifluoroacetic acid for 1 h.

The acid was removed *in vacuo* and the residue taken up in 5 ml DMF and neutralized with Et_3N in the cold. The 1,2,3-benzotriazol-1-ol ester of benzoic acid, obtained from a dicyclohexylcarbodiimide condensation, (400 mg, 1.8 mmol) was added using Et_3N to keep the pH at 9. After 20 h the solution was evaporated, the residue taken up in 15 ml CH_2Cl_2 and reacted with 2-(diethylamino)ethylamine for 4 h. After dilution with 200 ml CH_2Cl_2, the solution was extracted with 250 ml 0.1M HCl and 250 ml H_2O, dried (Na_2SO_4) and evaporated *in vacuo* at 40°. The residue (230 mg) was chromatographed on a preparative TLC silica gel plate with $CHCl_3/CH_3OH$ (7:3): 140 mg product. TLC (BuOH/AcOH/H_2O 4:1:1): R_f 0.79 (UV, +; yellow, +; Nh, –); ($CHCl_3/CH_3OH$ 4:1): R_f 0.10 (UV, +; yellow, +; Nh, –). Electrophoresis (paper *2043b*/mgl, *Schleicher* and *Schuell;* 0.05M PO_4^{3-}, pH 7.4; 30 min): 40 mm anodic, homogeneous. IR (CH_2Cl_2): 3450*m*, 2980*m*, 2950*m*, 2690*m*, 2540*w*, 2440*m*, 2320*s*, 1730*m*, 1710*m*, 1690*m*, 1520*s*, 1420*s*, 1370*m*, 1260*m*, 1160*s*, 1050*m*, 900*s*. Anal. calc. for $C_{20}H_{21}N_5O_8$ (459.4) C 52.29, H 4.61, N 15.24; found: C 52.68, H 4.87, N 14.85.

N_α-*Boc*-N_ε-*Z*-L-*lysyl-1-aminopropane*. N_α-Boc-N_ε-Z-L-lysine (1.0 g, 2.63 mmol) in 8 ml DMF were mixed and stirred at 5° with 2.63 mmol 1,2,3-benzotriazol-1-ol, 2.89 mmol *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride, 5.52 mmol Et_3N and 2.63 mmol 1-aminopropane. After 1 h, stirring was continued at r.t. for 18 h whereupon the suspension was diluted with 400 ml CH_2Cl_2 and extracted in a spray column extractor [6] with 0.1M HCl (2 l), H_2O (1 l), 0.3M K_2CO_3 (2 l) and H_2O to neutrality. Removal of the solvent *in vacuo* left 1.09 g oil. TLC (A): R_f 0.67 (UV, +; Nh, +), R_f 0.54 (UV, +, trace); (B): R_f 0.69 (UV, +; Nh, +). IR (CH_2Cl_2): 3440*m*, 2940*m*, 2870*w*, 1720*m*, 1710*m*, 1670*s*, 1510–1490*s*, 1365*m*, 1225*m*, 1165*m*.

N_ε-*(2-*tert*-Butoxycarbonyl-4,6-dinitrophenyl)*-N_α-*Boc*-L-*lysyl-1-aminopropane*. N_α-Boc-N_ε-Z-L-lysyl-1-aminopropane (1.08 g, 2.84 mmol) in 35 ml 2-propanol were hydrogenated for 5 h after addition of 0.1 ml glacial AcOH and 100 mg Pd/C + Pt/SiO_2 (1:1) as catalysts. The suspension was filtered through *Celite* and the filtrate evaporated *in vacuo* below 35° to give 775 mg residue of crude decarbobenzoxylated educt. This material (687 mg, 2.39 mmol) was dissolved in 7 ml DMF and mixed with 797 mg (2.63 mmol) *tert*-butyl 2-chloro-3,5-dinitrobenzoate in 3 ml DMF. The pH was kept at 9 with Et_3N. After 8 h, 150 ml CH_2Cl_2 was added and the solution extracted with 250 ml 0.1M HCl and 250 ml H_2O. The org. phase was dried (Na_2SO_4) and left after evaporation *in vacuo* 1.5 g yellow oil which was chromatographed on a 100 g silica gel *60* column with $CHCl_3/CH_3OH$ (9:1). The pooled fractions gave after evaporation *in vacuo* 850 mg solid product. TLC (B): R_f 0.90 (UV, +; yellow, +). IR (CH_2Cl_2): 3435*m*, 2965*m*, 2940*m*, 2880*w*, 1715*m*, 1690*m*, 1675*s*, 1610*s*, 1525*s*, 1510*s*, 1455*m*, 1370*m*, 1330*s*, 1145*s*, 1095*m*.

N_ε-*(2-Carboxy-4,6-dinitrophenyl)*-N_α-*benzoyl*-L-*lysyl-1-aminopropane* (**2**). N_ε-(2-*tert*-Butoxycarbonyl-4,6-dinitrophenyl)-N_α-Boc-L-lysyl-1-aminopropane (850 mg, 1.53 mmol) was treated with 10 ml 90% trifluoroacetic acid, reacted with the 1,2,3-benzotriazol-1-ol ester of benzoic acid, treated with 2-(diethylamino)ethylamine and subjected to liquid-liquid extraction as described for N_ε-(2-*tert*-butoxycarbonyl-4,6-dinitrophenyl)-N_α-Boc-L-lysinamide. The crude product (650 mg) was chromatographed on a 80 g silica gel *60* column with $CHCl_3/CH_3OH$ (9:1). Evaporation *in vacuo* of the fractions containing the product gave an oil which was dissolved in a small volume of water and lyophilized: 310 mg solid. TLC (BuOH/AcOH/H_2O 4:1:1): R_f 0.84 (UV, +; yellow, +). Electrophoresis (paper *2043b*/mgl, *Schleicher* and *Schuell*; 0.05M PO_4^{3-}, pH 7.4; 30 min): 39 mm anodic, homogeneous. IR (CH_2Cl_2): 3390*w*, 3080*w*, 2960*w*, 2930*w*, 2870*w*, 1675*m*, 1625*s*, 1575*s*, 1530*s*, 1485*m*, 1440*m*, 1360*m*, 1320*s*, 1175*m*, 1090*m*, 890*m*.

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