

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

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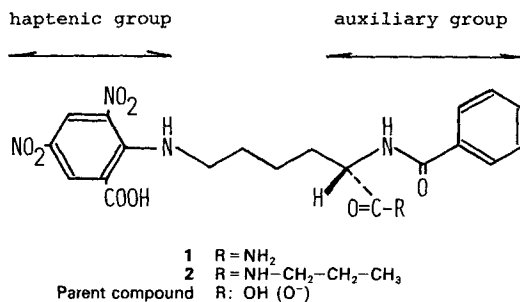
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Summary

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-lysineamide 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-lysineamide (**1**) where steric hindrance is considered to be negli-



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

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

Derivative injected intravenously	μ mol 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 μ g 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 μ g–100 μ g were run and 0.1–0.2 μ g 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-lysineamide. N_α -Boc-L-lysineamide (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 $\text{CHCl}_3/\text{CH}_3\text{OH}$ (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-lysineamide (1). N_ϵ -(2-*tert*-Butoxycarbonyl-4,6-dinitrophenyl)- N_α -Boc-L-lysineamide (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 Et₃N 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 Et₃N to keep the pH at 9. After 20 h the solution was evaporated, the residue taken up in 15 ml CH₂Cl₂ and reacted with 2-(diethylamino)ethylamine for 4 h. After dilution with 200 ml CH₂Cl₂, the solution was extracted with 250 ml 0.1M HCl and 250 ml H₂O, dried (Na₂SO₄) and evaporated *in vacuo* at 40°. The residue (230 mg) was chromatographed on a preparative TLC silica gel plate with CHCl₃/CH₃OH (7:3): 140 mg product. TLC (BuOH/AcOH/H₂O 4:1:1): R_f 0.79 (UV, +; yellow, +; Nh, -); (CHCl₃/CH₃OH 4:1): R_f 0.10 (UV, +; yellow, +; Nh, -). Electrophoresis (paper 2043b/mgl, Schleicher and Schuell; 0.05M PO₄³⁻, pH 7.4; 30 min): 40 mm anodic, homogeneous. IR (CH₂Cl₂): 3450m, 2980m, 2950m, 2690m, 2540w, 2440m, 2320s, 1730m, 1710m, 1690m, 1520s, 1420s, 1370m, 1260m, 1160s, 1050m, 900s. Anal. calc. for C₂₀H₂₁N₅O₈ (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-L-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 Et₃N and 2.63 mmol L-aminopropane. After 1 h, stirring was continued at r.t. for 18 h whereupon the suspension was diluted with 400 ml CH₂Cl₂ and extracted in a spray column extractor [6] with 0.1M HCl (2 l), H₂O (1 l), 0.3M K₂CO₃ (2 l) and H₂O 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 (CH₂Cl₂): 3440m, 2940m, 2870w, 1720m, 1710m, 1670s, 1510–1490s, 1365m, 1225m, 1165m.

N_ε-(2-tert-Butoxycarbonyl-4,6-dinitrophenyl)-N_α-Boc-L-lysyl-L-aminopropane. N_α-Boc-N_ε-Z-L-lysyl-L-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/SiO₂ (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 Et₃N. After 8 h, 150 ml CH₂Cl₂ was added and the solution extracted with 250 ml 0.1M HCl and 250 ml H₂O. The org. phase was dried (Na₂SO₄) and left after evaporation *in vacuo* 1.5 g yellow oil which was chromatographed on a 100 g silica gel 60 column with CHCl₃/CH₃OH (9:1). The pooled fractions gave after evaporation *in vacuo* 850 mg solid product. TLC (B): R_f 0.90 (UV, +; yellow, +). IR (CH₂Cl₂): 3435m, 2965m, 2940m, 2880w, 1715m, 1690m, 1675s, 1610s, 1525s, 1510s, 1455m, 1370m, 1330s, 1145s, 1095m.

N_ε-(2-Carboxy-4,6-dinitrophenyl)-N_α-benzoyl-L-lysyl-L-aminopropane (2). N_ε-(2-tert-Butoxycarbonyl-4,6-dinitrophenyl)-N_α-Boc-L-lysyl-L-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-lysyl-L-lysine. The crude product (650 mg) was chromatographed on a 80 g silica gel 60 column with CHCl₃/CH₃OH (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/H₂O 4:1:1): R_f 0.84 (UV, +; yellow, +). Electrophoresis (paper 2043b/mgl, Schleicher and Schuell; 0.05M PO₄³⁻, pH 7.4; 30 min): 39 mm anodic, homogeneous. IR (CH₂Cl₂): 3390w, 3080w, 2960w, 2930w, 2870w, 1675m, 1625s, 1575s, 1530s, 1485m, 1440m, 1360m, 1320s, 1175m, 1090m, 890m.

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