Preparation of N^{α}-(2-Nitrophenylthio)-N^{ε}-acyl Lysine Derivatives

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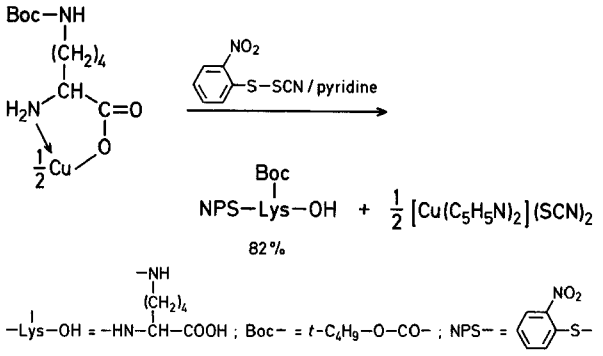
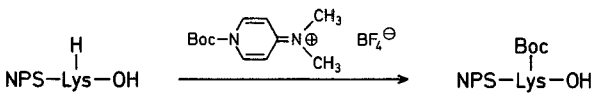
Lysine derivatives having two different *N*-protecting groups are generally[1] prepared by a three-step reaction sequence: selective acylation of the ε-amino group of the lysine-copper complex, removal of copper from the resultant N^{ε}-acylated complex, and acylation of the free α-amino group.

We have found that in the case of the preparation of N^{α}-(2-nitrophenylthio)-N^{ε}-acyl lysine derivatives the tedious step of removal of copper from the intermediate N^{ε}-acyl lysine complex can be easily and rapidly accomplished *in situ* during the introduction of the 2-nitrobenzenesulfenyl group by using 2-nitrobenzenesulfenyl thiocyanate in pyridine as *N*-sulfenylating agent[2]; the copper is removed as an insoluble complex with pyridine and thioacyanate ion. The reaction is illustrated by the preparation of N^{α}-(2-nitrophenylthio)-N^{ε}-(*t*-butoxycarbonyl)-L-lysine:

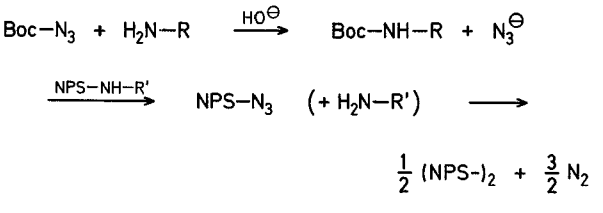


The procedure has been applied to the preparation of N^{α}-(2-nitrophenylthio)-N^{ε}-*t*-butoxycarbonyl-[3] and N^{α}-(2-nitrophenylthio)-N^{ε}-benzyloxycarbonyl-L-lysine[4] derivatives.

N^{α}-(2-Nitrophenylthio)-NN^{ε}-*t*-butoxycarbonyl-lysine can also be prepared by acylation of the free ε-amino group of N^{α}-(2-nitrophenylthio)-lysine[2] with 1-(*t*-butoxycarbonyl)-4-dimethylaminopyridinium tetrafluoroborate[5]:



In this case, however, the usual acylating agent, *t*-butoxycarbonyl azide[6], cannot be used because of the incompatibility of the 2-nitrobenzenesulfenyl group with the azide ion formed during the reaction. In fact, we isolated from the reaction mixture an appreciable amount of bis-[2-nitrophenyl] disulfide.



Kessler and Iselin[7] have shown that the 2-nitrobenzenesulfenyl group can be removed from the *N*-protected amino acid by azide ion in acidic solutions. Our finding that a similar cleavage reaction occurs under basic conditions shows that the azide coupling method gives rise to unwanted side reactions in the case of *N*-(2-nitrophenylthio)-protected amino acids.

N^{ε}-*t*-Butyloxycarbonyllysine has been obtained[8] from its copper complex by treatment with hydrogen sulfide. We found that the copper can be removed from the complex by chelating agents[9]. While only modest yields are obtained by use of EDTA, because of unfavorable solubilities of the N^{ε}-*t*-butyloxycarbonyllysine, this derivative can be obtained in good yield by use of Chelex 100 resin.

N^{α}-(2-Nitrophenylthio)-N^{ε}-*t*-butoxycarbonyl-L-lysine:

Method A; from N^{ε}-*t*-Butoxycarbonyl-L-lysine-Copper Complex: The copper complex of N^{ε}-*t*-butoxycarbonyl-L-lysine[8] (27.7 g, 100 mmol) was suspended in pyridine (250 ml). 2-Nitrobenzenesulfenyl thiocyanate[10] (25.4 g, 120 mmol) was added in small portions over a period of 2 hr to the stirred reaction mixture at room temperature. Stirring was continued for 30 min. The reaction mixture was then diluted with water (600 ml) and covered with ether (250 ml). The pH of the mixture was adjusted to 2–3 with 5 *N* sulfuric acid. The ethereal phase was separated and the aqueous phase extracted with ether (3×). Most of the insoluble copper complex formed during the reaction remained in the aqueous phase. The combined ethereal phases were freed from insoluble copper compounds by filtration, washed with water, and dried with magnesium sulfate. Upon addition of dicyclohexylamine (22 ml, 110 mmol), the dicyclohexylaminium salt of N^{α}-(2-nitrophenylthio)-N^{ε}-*t*-butoxycarbonyl-L-lysine crystallized out; yield: 48 g (82%). The product was recrystallized from absolute ethanol; yield: 42.5 g (73%); m.p. 194–195°; $[\alpha]_D^{22}$: −35.8° (c = 1.5, chloroform) (Ref. [3], m.p. 194–195°; $[\alpha]_D^{22}$: −43.4°).

We have persistently obtained $[\alpha]_D$ values of the order of −36° for this derivative prepared according to the described method[3] or using our two independent routes.

Method B; from *N*-(2-Nitrophenylthio)-L-lysine: 1-*t*-Butoxycarbonyl-4-dimethylaminopyridinium tetrafluororoborate[5] (7 g, 22 mmol) was added to a stirred solution of *N*-(2-nitrophenylthio)-L-lysine[2] (6.6 g, 22 mmol) in 1 *N* sodium hydroxide solution (22 ml). After 15 min, the pH which had decreased from 11.8 to 8.4, was readjusted to 10.2 with 1 *N* sodium hydroxide solution and one more equivalent (7 g, 22 mmol) of 1-*t*-butoxycarbonyl-4-dimethylaminopyridinium tetrafluoroborate was added. The mixture was stirred for a further 15 min and was then diluted with water. The pH was adjusted to 2–3 and the product extracted and isolated as its dicyclohexylaminium salt as described under Method A; yield: 10.1 g (79%); m.p. 192–194°; $[\alpha]_D^{22}$: −35.6° (c = 1.5, chloroform).

N^{α}-(2-Nitrophenylthio)-N^{ε}-benzyloxycarbonyl-L-lysine Dicyclohexylaminium Salt:

The compound was prepared as described above (methdo A) for the N^{ε}-*t*-butyloxycarbonyl Derivative; yield: 65%; m.p. 186–187°; $[\alpha]_D^{20}$: −28.1° (c = 0.7, dimethylformamide) (Ref. [4], m.p. 184–187°; $[\alpha]_D$: −29.1°).

N^{ε}-*t*-Butoxycarbonyl-L-lysine:

The copper complex of N^{ε}-*t*-butoxycarbonyl-L-lysine[8] (5.54 g, 20 mmol) was ground to a fine powder and added to pyridine (150 ml; the compound dissolved only partially). Chelex 100 (Bio-Rad chelating resin in sodium form; 70 ml wet resin, 1.1 equiv) was added in portions to the stirred mixture over a period of 3 hr. Water (150 ml) was added and stirring was continued overnight. The clear blue solution was filtered free from resin, the resin washed with water, and the combined filtrates were passed through a small column of Chelex resin (∅ 2 cm, height 8 cm). The colorless eluate was evaporated to dryness at 50° under reduced pressure. The residue was dissolved in water (50 ml) and the pH

adjusted to 6.2 with 5 *N* sulfuric acid. The product precipitated; yield: 3.2 g (65%). From the mother liquor, after evaporation to a smaller volume, a second crop was obtained (0.88 g); total yield: 4.08 g (83%); m.p. 235–255°; $[\alpha]_D^{23}$: +6.8° (c = 0.88, 2 *N*-ammonia); $[\alpha]_D^{23}$: +13.2° (c = 2.5, 0.1 *N* hydrochloric acid). The product was homogeneous on T. L. C. [Ref.[3,8], m.p. 237–355°; $[\alpha]_D^{26}$: +4.7° (2 *N* aqueous ammonia), and m.p. 238°; $[\alpha]_D^{24}$: +14.4° (0.1 *N* hydrochloric acid)].

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