methyllithium in ether–benzene, followed by dehydration of the tertiary alcohol formed and aromatization at 300–330 °C for 30 min over Rh-on-$Al_2O_3$.[20] The melting point and mixture melting point of **6** prepared by the two routes was 167–168 °C.

**Registry No.**—**1**, 50-32-8; **5**, 63104-32-5; **6**, 63104-33-6; **8**, 1714-29-0; **9**, 63104-34-7; **11**, 63104-35-8; **12**, 63104-36-9; 3-(1-pyrenyl)butanol, 63104-37-0; 3-(1-pyrenyl)butanol mesylate, 63104-38-1; mesyl chloride, 124-63-0; 3-(1-pyrenyl)butanoic acid, 63104-39-2; ethyl acetate, 141-78-6; 1-acetylpyrene, 3264-21-9; 3-(1-pyrenyl)-2-butenoic acid, 63104-40-5; 2,5-dimethylfuran, 625-86-5.

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# Isonicotinyloxycarbonyl, a Novel Amino Protecting Group for Peptide Synthesis[1]


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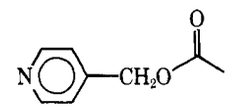
*Received March 23, 1977*


The isonicotinyloxycarbonyl (iNoc) group is an acid-stable protecting group for the protection of the $\epsilon$-amino group of lysine. iNoc can be removed by reduction with Zn under mild acidic conditions or by catalytic hydrogenation. The combined physical and chemical properties of iNoc offer unique advantages for its use as a lysine protecting group.


We[2] and others[3,4] have observed undesired loss of the benzyloxycarbonyl protecting group used for the protection of the $\epsilon$-amino group of lysine during acid-catalyzed removal of the $\alpha$-amine protecting group, *tert*-butyloxycarbonyl. Methods for more selective removal of the *tert*-butyloxycarbonyl group in the presence of benzyloxycarbonyl have been proposed.[5] Protecting groups for lysine of greater acid stability[6] or more acid labile $\alpha$-amino protecting groups[7] have been used to avoid these problems. The former approach has been successfully used in solid-phase peptide synthesis.[6] For a variety of reasons it is less satisfactory for synthesis in solution.[8] These improvements still rely on kinetic differences in the rates of removal of two acid labile protecting groups cleaved by different mechanisms. Cleavage of the more labile protecting groups generally proceeds predominantly via an $S_N1$ pathway, while the more stable protecting groups follow an $S_N2$ pathway. Thus, modifications in removal conditions by the introduction of nucleophilic scavengers or solvent changes may result in a loss of selectivity. We have noted such decreased selectivity for removal of the *tert*-butyloxycarbonyl group in the presence of the benzyloxycarbonyl group.[9] For this reason, we preferred an $\epsilon$-amino lysine protecting group which is completely stable to acid, but which can be smoothly removed under mild conditions, for instance, reductively. Such a protecting group would assure absolute stability when the *tert*-butyloxycarbonyl is cleaved with acid. Conversely, such an $\epsilon$-amino protecting group could be removed reductively without affecting a butyloxycarbonyl group.

Although kinetic selectivity for protecting group removal has, for example, been successfully applied in a synthesis of human insulin,[10] there are advantages to tactics based on a choice of protecting groups removed by chemically different methods. The known protecting groups which would offer chemical selectivity do not fulfill all of our other requirements for a lysine protecting group.[11] We have discussed our criteria in detail elsewhere.[8] These include (1) stability under conditions employed in peptide synthesis, (2) removal under unique and mild chemical conditions, (3) stability under conditions for the removal of other protecting groups, and (4) capability to increase rather than decrease the solubility of large peptides in polar solvents.

The isonicotinyloxycarbonyl protecting group (1) appeared

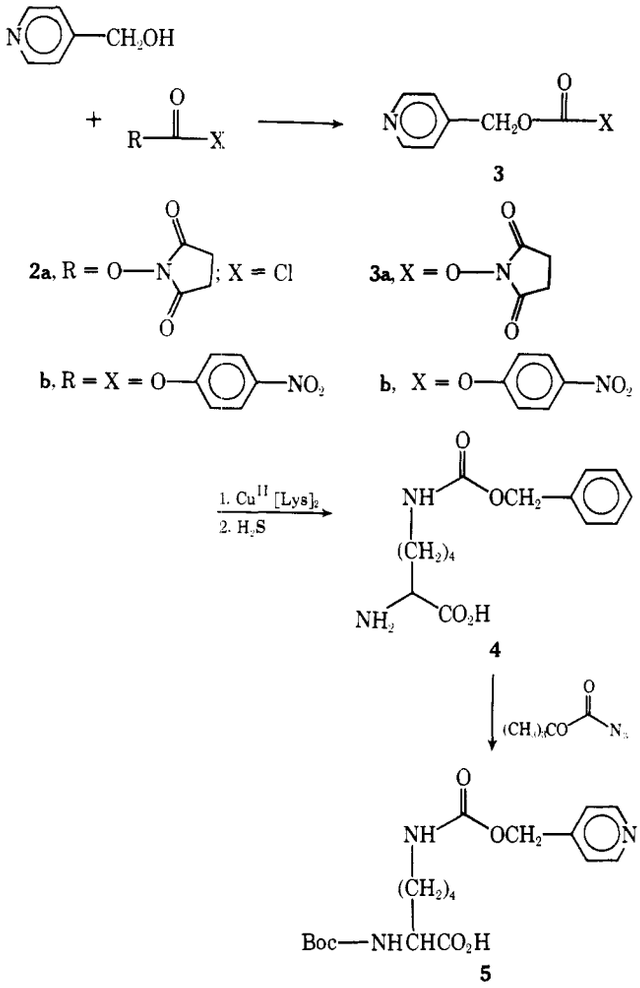


1

to offer the desired combination of properties to meet these criteria. The pyridine ring should make 1 highly stable under acidic conditions, while facilitating reductive removal, as was the case for the carboxyl protecting, 4-picolyl esters of Young.[12]

Isonicotinyl *p*-nitrophenyl carbonate (**3b**) was prepared by the reaction of bis(nitrophenyl) carbonate (**2b**) with 4-hydroxymethylpyridine in the presence of *N*-methylmorpholine

Scheme I



(Scheme I). The *p*-nitrophenol salt of **3b** of purity satisfactory for the synthesis of **4** was obtained on evaporation of the reaction solution. Our earlier studies had utilized isonicotinyl *N*-succinimido carbonate (**3a**). This reagent is more difficult to prepare and is less stable than **3b**. Either reagent can be used in reaction with the copper(II) complex of lysine to give $N^{\epsilon}$-iNoc-Lys (**4**). $N^{\alpha}$-Boc-$N^{\epsilon}$-iNoc-Lys (**5**) was prepared by reaction of **4** with either *tert*-butyl succinimido carbonate or *tert*-butyl azidoformate.

The isonicotinyloxycarbonyl group can be selectively removed from **5** by the action of either zinc dust in aqueous acetic acid or by catalytic hydrogenation using 5% Pd/C, giving clean conversion to $\alpha$-Boc-Lys. In contrast, treatment of **5** with anhydrous HF for 1 h at 18 °C results in a clean conversion to $\epsilon$-iNoc-Lys (**4**) with no detectable lysine being formed. The stability of $\epsilon$-iNoc-Lys under a variety of conditions commonly encountered in peptide synthesis is summarized in Table I. It is clear that this protecting group can be safely employed in synthetic tactics involving any acid labile protecting group, except possibly those requiring HBr for removal. The data in this table also give a view of the range of conditions under which the protecting group may be removed. Thus, complete removal of iNoc can be expected with zinc under acidic but not basic conditions. Sn(II) is found to be too weak a reducing agent to remove iNoc. A more precise definition of the ease of reduction of iNoc was obtained by studies using cyclic voltametry. Two sharp reduction waves were observed at −1.95 and −2.25 V (relative to Ag/AgCl). Controlled potential electrolysis at −2.02 V indicated a two-electron reduction from which 4-picoline and lysine were isolated. Reduction at −2.35 V resulted in a lower yield of lysine, suggesting that the wave at −2.25 V represents a side reaction. The reduction potential

**Table I. Stability of the Isonicotinyloxycarbonyl Group**

| Reagent or conditions | Time, h | Stability[a] |
|---|---|---|
| HF (liquid) (18 °C) | 1 | Stable |
| HCl/ethyl acetate (satd) (20 °C) | 1 | Stable |
| Trifluoroacetic acid (20 °C) | 1 | Stable |
| HCl/ethyl acetate (satd) + 10% mercaptoethanol (20 °C) | 1 | Stable |
| HBr/acetic acid (30%) (20 °C) | 1 | ~10% removal |
| Zn (dust) bicarbonate 0.1 N pH 8 (20 °C) | 1 | ~10% removal |
| 50% aqueous acetic acid (100 °C) | 0.5 | Stable |
| $SnCl_2$ in 50% aqueous acetic acid | 1 | Stable |
| Zn dust (HCl activated) in 50% acetic acid | 1.5 | Complete removal |

[a] Determined by TLC analysis of total reaction.

of −1.95 V for the iNoc group is more positive than that required for the removal of the benzyloxycarbonyl group (−2.90 V).[13]

An example of the removal of the iNoc protecting group from $\epsilon$-iNoc-Lys$^3$-substance P is described in the Experimental Section, for illustrative purposes. None of the amino acids present in this peptide show any reaction under the conditions for protecting group removal. The use of the iNoc protecting group has also been reported for the synthesis of somatostatin[14] and analogues.[15] The stability of the indole of tryptophan was also demonstrated by removal of the protecting group of $\epsilon$-iNoc-Lys (Zn/50% aqueous HOAc) in the presence of an equimolar amount of Boc-Trp-Ser-Tyr-OEt. No detectable change occurred in the UV absorbance of the peptide at 280 m$\mu$. We have observed oxidation of sulfur-containing peptides if high-speed stirring is used in the presence of air and zinc dust. Cysteine is oxidized to cysteic acid and methionine to the sulfone. This process may be metal catalyzed. Under optimal conditions for protecting group removal (as described in the Experimental Section), no side reactions have been observed with any of the genetically coded amino acids.

Use of the isonicotinyloxycarbonyl protecting group would appear to offer a solution to the problems which have been encountered when benzyloxycarbonyl has been used for the protection of the $\epsilon$-amine of lysine. It is stable to most of the conditions commonly employed in peptide synthesis, including the strongly acidic conditions often employed for removal of peptides from resin supports in the solid-phase method. The new protecting group is cleanly removed under mild conditions, which can be safely applied in the presence of all functional groups commonly encountered in peptide synthesis. The presence of a basic functionality also offers potential advantages for purification of products by electrophoresis or ion-exchange chromatography, as emphasized by Young et al. in the use of picolyl esters.[12,16] In our experience, the isonicotinyloxycarbonyl protected peptides have shown favorable solubility properties, often a crucial practical consideration in the synthesis of large molecules by classical methods.

## Experimental Section

**Preparation of Isonicotinyl *p*-Nitrophenyl Carbonate (3b).** (a) Bis(*p*-nitrophenyl) carbonate (152 g, 0.67 mol) was dissolved in 1600 mL of methylene chloride. A solution of 4-pyridylcarbinol (63.5 g, 0.58 mol) (azeotropically dried using benzene to remove water of hydration) in 500 mL of methylene chloride was added dropwise over 30 min with stirring, followed by a solution of *N*-methylmorpholine (50 g) in 150 mL of methylene chloride. The solution was allowed to stir for 2.5 days. TLC (silica gel, $CHCl_3$) showed that this length of time is required for complete reaction. The resulting solution was washed as follows: two 2-L portions of $H_2O$, one 2-L portion of 0.1 N

$H_2SO_4$, four 2-L portions of saturated $NaHCO_3$, and one 2-L portion of saturated NaCl. The organic phase was dried over $Na_2SO_4$, filtered, and evaporated to dryness in vacuo. The solid residue was dissolved in a minimum of warm (30 °C) ethyl acetate. The free base of isonicotinyl *p*-nitrophenyl carbonate crystallized on standing overnight, 53 g, mp 104–106 °C. (Anal. Calcd for $C_{13}H_{10}N_2O_5$: C, 56.93; H, 3.68; N, 10.22. Found: C, 56.80; H, 3.69; N, 10.16.) Addition of hexane to the supernatant gave 45 g of the nitrophenol salt (mp 112–113 °C). Further addition of hexane gave a third crop, which was also the nitrophenol salt (37 g). The total yield from these three crops was 76%. The NMR and IR spectra of both the free base and nitrophenol salt were consistent with the assigned structures.

(b) 4-Pyridylcarbinol (13.12 g, 0.12 mol), which was azeotropically dried with benzene, and bis(*p*-nitrophenyl) carbonate (27.2 g, 0.12 mol) were dissolved in 200 mL of $CH_2Cl_2$ and the mixture was stirred for 3 days at 20 °C. The mixture was filtered to remove a small amount of insoluble material and the solution was evaporated to dryness. The foamy solid was dissolved in 500 mL of ethyl acetate and crystallized by the addition of about 200 mL of hexane to give 26.2 g (80% yield) of the *p*-nitrophenyl salt of **3b** (mp 108–111 °C).

**Preparation of ε-iNoc-Lys (4).** A solution of 7.44 g (0.044 mol) of $CuCl_2 \cdot 2H_2O$ in 160 mL of $H_2O$ was added to a solution of 14.56 g (0.08 mol) of L-lysine hydrochloride in 160 mL of $H_2O$, and the solution was adjusted to pH 9.5 by the addition of 50% aqueous NaOH. Isonicotinyl *p*-nitrophenyl carbonate (22 g, 0.08 mol) was added over a period of 20 min, with stirring. The reaction mixture was stirred at 20 °C for 26 h (heavy precipitate, vigorous stirring required), after which acetic acid was added to pH 7.2. The copper complex of ε-isonicotinyloxycarbonyl lysine was isolated by filtration and washed with $H_2O$ (slow filtration). This material was dissolved in 10% aqueous acetic acid (900 mL) and treated with $H_2S$ to precipitate CuS. The mixture was filtered through Celite and the filtrate evaporated to dryness in vacuo. The product (22 g) contains small amounts of free lysine and *p*-nitrophenol. This material (20 g) was dissolved in water (150 mL) and sufficient Dowex 1 × 2 ($OH^-$) added to obtain pH 6.6, thus adsorbing residual *p*-nitrophenol. The solution was applied to a 1400-mL column of IRC-50 ($NH_4^+$) and eluted with 20% ethanol in 0.1 N $NH_4OAc$. The first four ninhydrin positive fractions (500 mL each) contained a single component as indicated by TLC (silica gel, $CHCl_3$–MeOH–$H_2O$, 50:40:10). These were combined and evaporated to dryness in vacuo, and the residue was crystallized from water–ethanol to yield 13.3 g (47%), mp 235–236 °C, $[\alpha]^{24}_D$ −7.32° (*c* 0.7, 2 N HOAc). Anal. Calcd for $C_{13}H_{19}N_3O_4$: C, 55.50; H, 6.81; N, 14.94. Found: C, 55.45; H, 6.79; N, 14.93. *Note*: If the copper complex of ε-iNOC-Lys is thoroughly washed with water, clean product is obtained by crystallization of the free ε-iNoc-Lys without ion exchange chromatography. Thorough washing is made difficult, however, by the physical nature of the complex.

***N*-α-Boc-ε-iNoc-Lys (5).** ε-iNoc-Lys (24 g, 0.085 mol) was suspended in 645 mL of dimethylformamide. Tetramethylguanidine (9.89 g, 0.086 mol) was added, followed by dropwise addition of *tert*-butyl azidoformate (12.12 g, 0.085 mol) over a period of 45 min, with stirring. An additional 9.89 g of tetramethylguanidine was added, and the solution was stirred 18 h at 20 °C. The resulting solution was evaporated to dryness in vacuo at a temperature below 35 °C and the residue dissolved in 300 mL of $H_2O$. The solution was adjusted to pH 4.2 (product oils out) by the addition of dilute $H_2SO_4$ and flushed with a stream of nitrogen in a hood for a period of 1 h. The mixture was extracted four times with ethyl acetate (400 mL). The organic phase was dried over $Na_2SO_4$, filtered, and evaporated in vacuo to a small volume. When crystals started to form the evaporation was stopped and the solution heated to dissolve the crystals. Product crystallized on standing at room temperature overnight. After filtration, the crystals were washed with cold ethyl acetate–hexane and dried in vacuo at 20 °C (22.5 g). A second crop gave an additional 5.4 g: total yield 86%; mp 130–131 °C; $[\alpha]^{24}_D$ 7.49° (*c* 0.6, 2 N HOAc). Anal. Calcd for $C_{18}H_{27}N_3O_6$: C, 56.68; H, 7.14; N, 11.02. Found: C, 56.62; H, 6.90; N, 10.72.

**Example of Removal of the iNoc Protecting Group. Synthesis of Substance P.** Arg-Pro-Lys(iNoc)-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-$NH_2$[17] (298 mg) was dissolved in 3 mL of 50% aqueous acetic acid, and 300 mg of HCl-activated zinc dust[18] was added. The reaction was stirred at room temperature for 1.5 h, at which time an aliquot (5 μL) was removed, diluted to 100 μL with water, and treated with $H_2S$ to remove Zn(II). TLC analysis (silica gel using $CHCl_3$–MeOH–2% aqueous NaCl 55:40:10) indicated a single product and about 25% remaining starting peptide. The remaining reaction solution was diluted with 3 mL of 50% aqueous acetic acid, and an additional 300 mg of zinc dust was added. After stirring an additional 1.5 h, TLC analysis as above showed complete conversion to substance P. No by-products were detected by TLC. The remaining zinc dust was separated by decantation and washed several times with 50% aqueous acetic acid by decantation. The combined solution and washes were diluted to a volume of 20 mL and Zn(II) precipitated as ZnS by treatment with $H_2S$. ZnS was removed by centrifugation and washed five times with 10-mL portions of 50% aqueous acetic acid. The combined solution and washes were evaporated to dryness in vacuo, and the gummy residue was triturated with ethyl acetate to give a solid which was dried in vacuo to give 239 mg (90%) of substance P. Amino acid analysis after enzymatic degradation using pronase and aminopeptidase-M showed: $Lys_{1.02}$, $Arg_{0.99}$, $Glx_{2.08}$,[19] $Pro_{2.01}$, $Gly_{1.03}$, $Met_{0.93}$, $Leu_{0.99}$, $Phe_{1.96}$. ε-iNoc-Lys, which is eluted at the same time as histidine on the short column of the amino acid analyzer, was not detectable in the hydrolyzate.

**Electrolysis of ε-iNoc-Lys.** A cyclic voltammogram of ε-iNoc-Lys was obtained using a cyclic universal programmer, Model PAR 175, and a coulometer, Model PAR 179 (Princeton Applied Research Corp.). Two sharp reduction waves at $E_{pc}$ = −1.95 and −2.25 V (vs. Ag/AgCl) were measured at a hanging mercury drop electrode in 0.1 M tetra-*n*-butylammonium iodide in 80% DMF and 20% $H_2O$. For controlled potential electrolysis, ε-iNoc-Lys (0.101 g, $35 \times 10^{-4}$ mol) was dissolved in a mixture of $H_2O$ (5 mL) and 0.1 M tetrabutylammonium iodide in DMF (25 mL). A mercury pool (10 mL) was used as cathode and a platinum wire was used as anode with an Ag/AgCl reference electrode. Electrolysis at −2.02 V for 40 min resulted in precipitation of lysine ($1.16 \times 10^{-4}$ mol), 64% as determined by amino acid analysis. Amino acid analysis of the solution showed an additional $3.7 \times 10^{-5}$ mol of lysine (total lysine, 84%) and $10^{-5}$ mol of unreacted ε-iNoc-Lys. Gas chromatographic analysis of the solution showed the presence of 4-picoline. A similar electrolysis at −2.35 V gave only 20–25% yield of lysine.

**Removal of iNoc by Hydrogenation.** *N*-α-Boc-ε-iNoc-Lys (**5**) (10 mg) was dissolved in 1 mL of 5% aqueous acetic acid. Pd/C (10%; 10 mg) was added, the suspension was purged with nitrogen, and hydrogen was bubbled through the solution for 10 min. Analysis by TLC showed complete removal of the iNoc protecting group.

**Acknowledgment.** We wish to acknowledge the skilled technical assistance of Mr. James E. Deak and the assistance of Mr. Carl Homnick in obtaining the amino acid analyses.

**Registry No.**—**1**, 1007-48-3; **3b**, 32919-24-7; **4**, 42561-68-2; **5**, 42417-16-3; bis(*p*-nitrophenyl) carbonate, 5070-13-3; 4-pyridylcarbinol, 586-95-8; L-lysine hydrochloride, 10098-89-2.

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