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Electrochemical Removal of the Picolinoyl Group under Mild Acidic Conditions. Application to the Protection of Amines in Peptide Synthesis.

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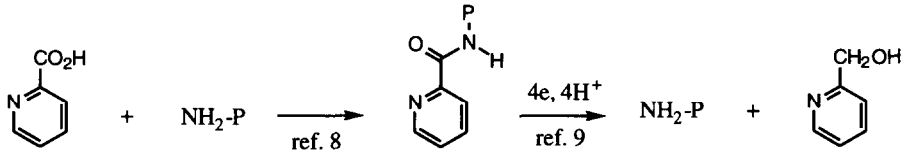
Abstract: The picolinoyl group can be used as a convenient protective group for amines in peptide chemistry. Deprotection is performed by electrochemical reduction under mild acidic conditions.

A few years ago, we discovered that the 3-hydroxy-picolinoyl residue of the antibiotic pristinamycin I_A (PI_A) could be cleaved electrochemically to afford the corresponding amine (M-NH_2), though in moderate yield (50%)[1]. In subsequent studies[2,3], we established the generality of this reaction within a series of analogues of PI_A. In particular, the substitution of the 3-hydroxy-picolinoyl residue by a picolinoyl group led to a cleaner reaction and to higher yield of M-NH_2 (80%)[2]. This result prompted us to wonder whether the picolinoyl group (Pic) could be used as a protective group for amines in peptides, according to scheme 1 :



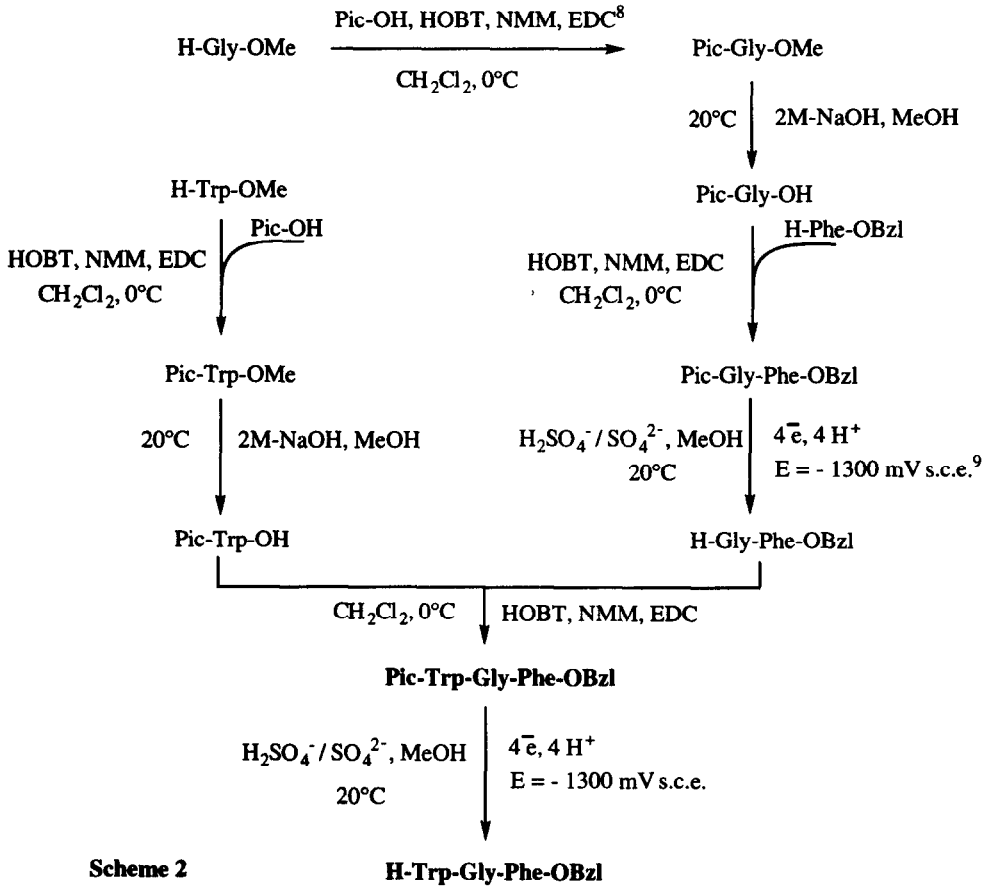
Scheme 1

So far, little attention has been paid to this group as a protective group of the amino function, probably because of the nature of the deprotective step (hydrolysis by Cu^{++} ions)[4]. We envisioned that the electrochemical cleavage would confer to the picolinoyl group all the features of an attractive protecting group: a) stability under conditions used in peptide chemistry; b) improvement of the solubility of the starting peptide in organic solvents; c) stability upon removal of classical protecting groups; d) cleavage in one step under mild conditions. Electrochemical removal of protecting groups has been shown indeed to display significant advantages over conventional methods[5,6]. The mild conditions of this kind of cleavage, and the possibility of modulation of the deprotecting reagent (in this case, the electrode) by a simple variation of the potential, makes electrolysis an appealing approach for the removal of protecting groups, as well as the selective deprotection of multi-protected molecules.

We therefore embarked upon a program aimed at investigating the electrochemical cleavage of various 2-pyridyl carboxamides in order to reassess the interest of this group as a protecting group for amines in peptides.

We report herein the results of this study which demonstrate that the picolinoyl group effectively constitutes a convenient protecting group for these amines.

The N-picolinoyl amines were synthesized in good yields following a Boc or a Pic strategy, alternatively, according to classical procedures of peptide chemistry[7] (see table). The usefulness of the Pic procedure[8] could be demonstrated by the synthesis of the protected tripeptide N-picolinoyl-L-tryptophyl-glycyl-L-phenylalanine benzyl ester (entry 8 of the table) as an example (scheme 2).



Scheme 2

As expected, the attachment of the picolinoyl group improved the solubility of the starting peptides in organic and aqueous solvents. Nevertheless, it did not enhance markedly the solubility of the peptide in a slightly acidic aqueous medium, owing to the unexpectedly low value of pKa found for the protonation equilibrium of the picolinoyl group (pKa = 1.8 ± 0.1). As a consequence, the protected peptides could be purified by washing with mild acidic solutions.

Initially, the reductive cleavage of the picolinoyl group was studied on simple N-protected α-amino esters (entries 1-4 of the table). The best experimental conditions were then applied to N-picolinoyl peptides (entries 5-9). The cyclic voltammogram of Pic-protected peptides in dilute hydroalcoholic (50:50) sulfuric acid solution, at a stationary mercury electrode, showed two distinct reduction peaks Pc_1 and Pc_2, around - 1000 and

-1200 mV vs. saturated calomel electrode (s.c.e.) respectively, the sweep rate being 0.2 V s^{-1}. A detailed mechanistic study of the electrochemical reduction of picolinamides has been reported in reference 2. Following a preparative electrolysis[9], and provided that the potential of the mercury electrode was fixed at - 1300 mV s.c.e., the expected peptide NH_2-P was isolated as the sole product in good yields ranging from 70 to 90 % (see table). Thus, electrolysis turned out to be an efficient method to remove the picolinoyl group.

In order to explore further the scope of this approach with respect to practical applications, we investigated the cathodic behaviour of a series of Nα-Pic protected derivatives of lysine (entries 10-14 of the table). As anticipated, the picolinoyl group could be cleaved selectively in the presence of the tosyl group (entry 10), the Z group (entry 11), or the OBzl group (entries 1-9 and 11) but not the other way round. The tosyl, Z and OBzl groups are indeed more difficult to reduce than the picolinoyl group[5,6]. Interestingly enough, a Boc or a *tert*-butyloxy (OBut) group remained unaffected under the conditions used for the electrochemical removal of the Pic group (entries 12-13). Likewise, the picolinoyl group was found to be stable under all acidic conditions which are used to remove the Boc or the OBut group[10]. In contrast, the presence of a Troc group was found not to be compatible with the cleavage of the Pic group (entry 14). The competitive cleavage of the C-Cl bonds is probably responsible for the low yield of the expected amine. Lastly, the introduction of a 9-fluorenylmethylcarbonyl group (Fmoc) produced an unsoluble peptide under our experimental conditions so that it could not be electrochemically reduced.

Table - *Electrochemical Cleavage of Pyridyl Carboxamides to Amines in Dilute Hydroalcoholic (50:50) Sulfuric Acid Solutions[9] (E = - 1300 mV s.c.e.).*

Entry	Pyridyl Carboxamide	Yield of Formation (%)	Isolated Yield of Amine (%) after Electrochemical Removal (a)
1	Pic-Tyr-OBzl	97	90 (b)
2	Pic-Phe-OBzl	96	87 (b)
3	Pic-His-OBzl	75	83
4	Pic-Trp-OBzl	86	83
5	Pic-Ala-Tyr-OBzl	75	70
6	Pic-Phe-Phe-OBzl	91	80
7	Pic-Ala-Phe-Phe-OBzl	82	76
8	Pic-Trp-Gly-Phe-OBzl	92	84
9	Pic-Leu-Ala-Phe-Phe-OBzl	81	75
10	Tos-Phe-Lys(Pic)-OMe	75	65
11	Nα-Pic-Lys(Z)-OBzl	96	86
12	Nα-Boc-Lys(Pic)-OMe	78	82 (b)
13	Boc-Phe-Lys(Pic)-OMe	80	94
14	Troc-Phe-Lys(Pic)-OMe	78	20 (c)

Abbreviations: tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Z), tosyl (Tos), trichloroethoxycarbonyl (Troc), picolinoyl (Pic), benzyl (Bzl). (a) Significant amounts of 2-hydroxymethylpyridine were lost upon isolation due to its volatility. (b) No noticeable racemization was observed as shown by the comparison of the experimental specific optical rotation with that of the commercially available compound. (c) Low yields of the expected product were obtained along with many by-products resulting from competitive cleavage of C-Cl bonds.

In conclusion, we have demonstrated that the picolinoyl group fulfilled the criteria required for a convenient protecting group for amines in peptide chemistry: the preparation of Pic-protected peptides is easy and proceeds in high yield; the resulting peptide is soluble in organic solvents; the Pic group is stable under strong acidic conditions; its removal by electrolysis at controlled potential, under mild acidic conditions, generally proceeds in good to excellent yields and is respectful of commun protecting groups, except the Troc group. Peptides can be protected and deprotected, under our experimental conditions, without noticeable racemization (entries 1, 2 and 12 of table). We considered that all these features should allow the picolinoyl group to enter into the set of protecting groups used in the chemical manipulation of multi-protected peptides (orthogonal-strategy).

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8. A typical procedure was as follows: A reaction mixture of picolinic acid [(Pic-OH), 0.25g, 2.0 mmol], 1-hydroxy-benzotriazole [(HOBT), 0.27g, 2.0 mmol], N-methylmorpholine [(NMM), 0.20g, 2.0 mmol] and peptide (2.0 mmol) in dichloromethane (50 mL), was stirred and cooled to 0°C in an ice-water bath. A solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride [(EDC), 0.38g, 2.2 mmol] in dichloromethane (40 mL) was added dropwise. After addition, stirring was continued for 2h at 0°C and for an additionnal 2h at 20°C. The reaction mixture was then treated according to the procedure reported in ref. 2.
   Pic-Trp-Gly-Phe-OBzl : ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.05 (m, 2H, Hβ, Phe), 3.35 (m, 2H, Hβ, Trp), 3.75 (m, 2H, Hα, Gly), 4.85 (m, 2H, Hα, Phe and Hα, Trp), 5.05 (m, 2H, CH_2, OBzl), 6.90 to 7.40 [m, 17H, H aromatic, H(5), Pic and NH(Phe and Gly)], 7.65 [d, 1H, H(4), Trp, J = 8 Hz], 7.75 [dt, 1H, H(4), Pic, J = 8 Hz, J = 2 Hz], 8.10 [d, 1H, H(3), Pic, J = 8 Hz], 8.50 [d, 1H, H(6), Pic, J = 5 Hz], 8.70 [m, 2H, NH(amide) and NH(indole), Trp, D_2O exchanged].
9. A typical procedure was as follows: 100 mL of an aqueous sulfuric acid (0.2 mol.L^{-1}) solution was adjusted to pH 2.0 with sodium hydroxide and diluted twofold with methanol. The Pic-protected peptide (0.6 mmol) was dissolved in the resulting hydroalcoholic (50:50) buffered solution and then reduced (E = - 1300 mV s.c.e.) under nitrogen, at 25°C, in a 3-compartment cell (cathode: mercury pool; anode: platinum foil). After exhaustive electrolysis, i.e. when a steady state minimum value of the current was recorded, the hydroalcoholic solution was concentrated to 100 mL under reduced pressure, at 40°C. The resulting solution was neutralized by an aqueous sodium carbonate solution (5 mol.L^{-1}) and extracted with ethyl acetate (200 mL). The organic phase was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure, at 30°C. The residue was chromatographed on silica, to give the expected amine. Its structure was confirmed by comparison with an authentic sample.
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