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3-Pentyl (Pen) Group as a New Base-Resistant Side Chain Protecting Group for Tyrosine

József Bódi, Yuji Nishiuchi,* Hideki Nishio, Tatsuya Inui and Terutoshi Kimura

Peptide Institute, Inc., Protein Research Foundation, 4-1-2 Ina, Minoh-shi, Osaka 562-8686, Japan

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Abstract

The 3-pentyl (Pen) group is a new base-resistant protecting group for tyrosine. It is sufficiently stable in 50% TFA/DCM and completely stable towards 20% piperidine/DMF but is readily cleavable by the standard HF procedure without the formation of any significant amount of alkyltyrosine rearrangement product.

Keywords: Amino acids and derivatives; Protecting groups; Cleavage reactions; Migration.

Convergent synthesis involving the preparation and coupling of protected peptide segments is one of the most advantageous approaches for synthesizing large peptides or proteins. For the detachment of protected peptide segments from solid supports, there must be a highly compatibility between the protecting groups of the segment and the anchoring group which serves to attach the growing peptide to the resin. In the Fmoc/^{t}Bu strategy, highly acid-labile resins, such as the 2-chlorotrityl resin, have been successfully employed for this purpose [1]. Among several linkers reported for the convergent strategy using Boc/Bzl chemistry, base-labile linkers are profitable for the practical peptide synthesis since they require no special equipment (e.g. photolysis reactor for photolabile linkers [2]) and no strict control of the reaction conditions (e.g. those for the cleavage of allyl-type linkers [3]). By employing base-labile linkers such as fluorenylmethyl-based resins [4] to prepare protected peptide segments, the side-chain protecting groups must be completely stable during detachment of the segments from the resin by treatment with nucleophiles such as morpholine or piperidine in DMF, which is the standard Fmoc deprotection condition. The commonly used BrZ group for the Tyr residue is known to be the most susceptible protecting group to these base-catalyzed conditions. Several attempts have been made to develop base-resistant protecting groups

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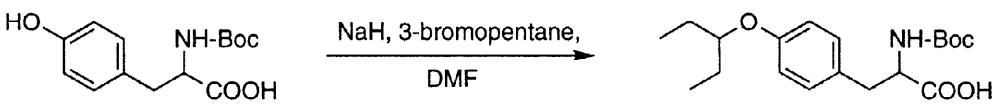
compatible with Boc chemistry for the phenolic hydroxyl group of tyrosine, but none of those reported has gained wide practical application. The recently reported carbamate type 2,4-dimethylpent-3-yloxycarbonyl (Doc) protecting group [5] was found to be far more stable towards piperidine than the commonly used BrZ group. However, this group is lost under standard Fmoc cleavage conditions (Table 1). The ether-type protecting groups are known to be stable under mild basic conditions. Nevertheless, the use of benzyl ether for tyrosine is generally avoided as it causes excessive migration of the benzyl group to the aromatic ring in the final acidolytic deprotection step [6] (Table 2). Another group suggested to be suitable as a protecting group for tyrosine is the cyclohexyl (cHex) ether group [7]. It is stable in 50% TFA/DCM but is readily cleavable under standard HF conditions without the formation of any significant amount (<0.5%) of the rearranged cyclohexyltyrosine side product. However, this group has not gained wide application in practical peptide synthesis because of the difficulty of synthesizing the protected Tyr derivative.

Based on the above considerations, we designed the 3-pentyl ether (Pen) group as an open chain analog of cyclohexyl ether to protect the hydroxyl group of tyrosine. The Pen group can be introduced to the phenolic hydroxyl group of tyrosine in a one-step reaction by chemoselective monoalkylation of the N^{α}-protected tyrosine disodium salt using 3-bromopentane in DMF according to the procedure reported by Mendelson *et al.* [8]. The optical purity of Boc-Tyr(Pen)-OH[1] synthesized in this manner (Scheme 1) was >99.8% as determined by Marfey's method [9] after removing the protecting groups.



Scheme 1

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[1]Boc-Tyr(Pen)-OH.DCHA: m.p. 107-108 °C. Crystallization from ether-hexane. $[\alpha]_D^{26}$ +28.8° (c=1, methanol). Elemental analysis: Found: C 69.74; H 9.80; N 5.21%. Calcd for $C_{31}H_{52}N_2O_5$: C 69.84; H 9.85; N 5.20%.

Boc-Tyr(Pen)-OH: Colorless oil. ^{1}H NMR ($CDCl_3$/TMS_{int}): δ = 7.08 (d, J 7.6, 2H Tyr ring 2), 6.82 (d, J 8.6, 2H, Tyr ring 3), 4.98 (d, J 7.6, 1H, NH), 4.57 (m, 1H, α-H), 4.07 (m, J 5.9, 1H, C<u>H</u>$(CH_2CH_3)_2$), 3.13 (m, J 5.3 and 13.9, 1H, β-H), 3.01 (m, J 5.5 and 13.9, 1H, β-H), 1.66 (m, J 7.3 and 13.2, 4H, CH(C<u>H</u>$_2$$CH_3)_2$), 1.41 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.94 (t, J 7.6, 6H, CH(CH_2C<u>H</u>$_3$)$_2$). ^{13}C NMR ($CDCl_3$/TMS_{int}): δ = 176.50 (1C, COOH), 157.83 (1C, NH<u>C</u>OO), 155.40 (1C, Tyr ring 4), 130.39 (2C, Tyr ring 2), 127.48 (1C, Tyr ring 1), 116.03 (2C, Tyr ring 3), 81.31 (1C, <u>C</u>$(CH_3)_2$), 80.20 (1C, <u>C</u>H$(CH_2CH_3)_2$), 54.36 (1C, α-C), 36.97 (1C, β-C), 28.23 (3C, C(<u>C</u>$H_3)_2$), 26.02 (2C, CH(<u>C</u>$H_2CH_3)_2$), 9.53 (2C, CH(CH_2<u>C</u>$H_3)_2$).

The stability of some tyrosine protecting groups was studied by treating Boc-Tyr(X)-OH (X = BrZ, Doc, cHex, Pen) with acids and bases. Samples were taken and analyzed by RP-HPLC. The Pen group was as stable as the commonly used BrZ or the reported cHex group in 50% TFA/DCM (Table 1). Cleavage of the Pen group was not detected in 20% piperidine/DMF for 24 h, while 61% of the Doc protecting groups was cleaved within 1 h at room temperature.[1]

The migration of the pentyl cation to the aromatic ring in strong acid was studied by treating H-Tyr(X)-OH (X = BrZ, Cl_2Bzl, cHex and Pen) with HF under different conditions. The product yield distribution was analyzed by RP-HPLC (Table 2). The migration of the Pen group was considerable (6.9%) in neat HF. However, in the presence of 10% cation scavenger such as anisole or *p*-cresol, the extent of formation of the alkyltyrosine side product could be kept below a significant (0.5%) level, which was comparable to that obtained with BrZ or cHex.

Table 1
Cleavage of side chain protecting group of Boc-Tyr(X)-OH under acidic or basic conditions at room temperature

	Cleavage of X (%)			
Reaction conditions	BrZ	Doc	cHex[a]	Pen
100% TFA, 24 h	0.9	17		1.2
50% TFA/DCM, 24 h	0.4	4	0.4	0.3
20% piperidine/DMF, 1 h	100	61		0[b]

[a]From ref. 7.
[b]24 h.

Table 2
Alkyltyrosine formation during acidolysis of Tyr(X) in HF at -5°C for 60 min

	Formation of 3-alkyltyrosine (%)			
Cleavage conditions	BrZ	Cl_2Bzl	cHex[a]	Pen
HF	0.6	69	6.5	6.9
HF/anisole (9:1)	0.3	23	<0.5	0.5
HF/*p*-cresol (9:1)	0.2	10	2.4[b]	0.5

[a]From ref. 7, reaction conditions at 0°C, 30 min.
[b]In HF/phenol (9:1).

[1]The nucleophile stability of the Doc group is described as the half-life ($t_{1/2}$ = 8 h) in ref. 5, where the model peptide containing Tyr(Doc) on a solid support was treated with 20% piperidine in DMF at room temperature. In the present study, however, the stability of the Doc group was examined in solution using 20% piperidine/DMF at room temperature. The discrepancy in the stability of the Doc group might be arisen from different situations, *i.e.*, solid-phase or solution-phase.

In conclusion, the Pen group was shown to be a suitable new protecting group for protection of the phenolic hydroxyl of tyrosine in peptide synthesis using either Boc or Fmoc chemistry. In particular, the Pen group for tyrosine was proved to be indispensable for preparing the protected peptide segments on the base-labile linkers using the Boc/Bzl strategy for the convergent synthesis.

References

[1] Barlos K, Gatos D, Kapolos S, Papaphhotiu G, Schäfer W, Wenqing Y. Tetrahedron Lett. 1989;30:3947-3950.

[2] Barany G, Albericio F. J. Am. Chem. Soc. 1985;107:4936-4942.

[3] Kunz H, Domgo B. Angew. Chem. Int. Ed. Engl. 1986;27:711-713.

[4] Rabanal F, Giralt E, Albericio F. Tetrahedron. 1995;51:1449-1458.

[5] Rosenthal K, Karlström A, Undén A. Tetrahedron Lett. 1997;38:1075-1078.

[6] Erickson BW, Merrifield RB. J. Am. Chem. Soc. 1973;95:3750-3756.

[7] Engelhard M, Merrifield RB. J. Am. Chem. Soc. 1978;100:3559-3563.

[8] Mendelson WL, Tickner AM. Lantos I. J. Org. Chem. 1983;48:4127-4129.

[9] Marfey P. Carlsberg Res.Commun. 1984;49:591-596.