N^{in}-Diphenylphosphinothioyltryptophan, a Useful Derivative for Peptide Synthesis by the Methanesulphonic Acid–Thioanisole System and Fluoride Ion Deprotection Methods

Yoshiaki Kiso,* Tooru Kimura, Masanori Shimokura, and Takatomo Narukami

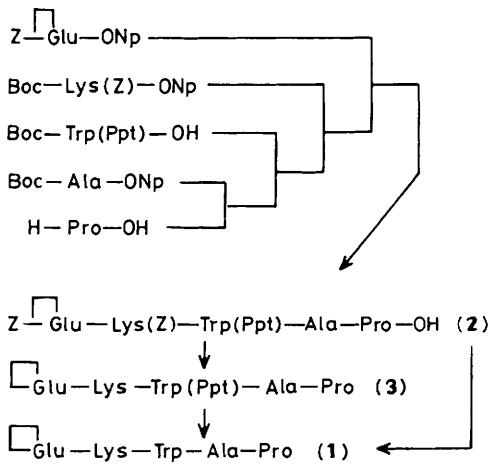
Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan

The diphenylphosphinothioyl (Ppt) group attached at the N^{in} function of tryptophan can be smoothly removed not only by 0.25 M methanesulphonic acid–thioanisole in trifluoroacetic acid, but also by tetra-n-butylammonium fluoride trihydrate in dimethylformamide without any side reaction; this new Trp(Ppt) derivative is successfully applied to the synthesis of bradykinin potentiating peptide 5a by the solution method, as well as by the solid phase method using a fluoride ion deprotection method.

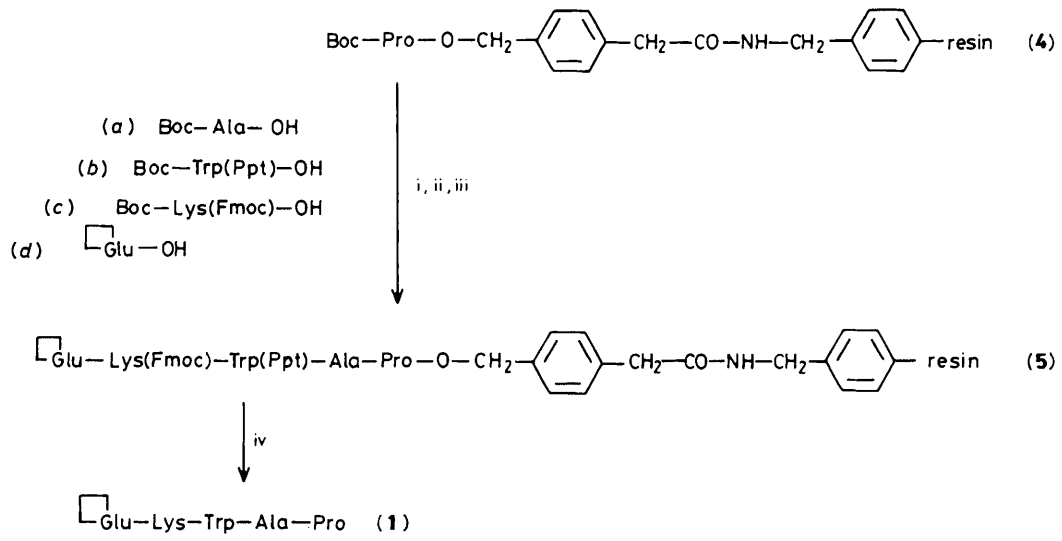
Protection of the indole moiety of tryptophan is often required because it is susceptible to oxidative degradation and to alkylation when acidic conditions are used for removal of protecting groups in peptide synthesis.[1,2] We now report that the diphenylphosphinothioyl (Ppt) group[3,4] attached at the N^{in} atom of the tryptophan indole ring can be smoothly removed not only by methanesulphonic acid (MSA)[5]–thioanisole in trifluoroacetic acid (TFA), but also by tetra-n-butylammonium fluoride trihydrate (TBAF) in dimethylformamide (DMF), without any side reaction.

Boc–Trp(Ppt)–OMe,[4] m.p. 125—126.5 °C, $[\alpha]_D^{31}$ −8.0° (*c* 0.6, DMF), was prepared in 87% yield from Boc–Trp–OMe (Boc = t-butoxycarbonyl) and Ppt–Cl (1.5 equiv.) in CH_2Cl_2 in the presence of tetra-n-butylammonium hydrogen sulphate (0.01 equiv.) and pulverized NaOH (2.5 equiv.)[2,4,6] at 0 °C for 2 h. Hydrolysis of Boc–Trp(Ppt)–OMe by use of 1M aq. NaOH in tetrahydrofuran (THF) gave the corresponding carboxylic acid, Boc–Trp(Ppt)–OH, m.p. 127—129 °C, $[\alpha]_D^{31}$ +17.4° (*c* 1, MeOH), in 73% yield.

The N^{in}-Ppt group was stable to TFA–anisole and $SiCl_4$–anisole/TFA[7] at 25 °C for 1 h, while it was quantitatively cleaved under relatively mild conditions, *e.g.* 0.25 M-MSA[5]–0.25 M-thioanisole in TFA (0 °C, 90 min) and 0.25 M-trifluoromethanesulphonic acid (TFMSA)[8]–0.25 M-thioanisole in TFA (0 °C, 30 min) in the presence of *m*-cresol and ethane-1,2-dithiol. Under these acidic conditions, the presence of thioanisole[9] was essential for the complete deprotection of Trp(Ppt). In the absence of thioanisole, the deprotection of the N^{in}-Ppt group was incomplete even after 24 h under strongly acidic conditions, *e.g.* 1 M-TFMSA. It is noteworthy that the N^{in}-Ppt group could be removed under mild conditions using fluoride ion. The rate of cleavage was dependent



Scheme 1. Application of Trp(Ppt) to the synthesis of BPP5a.



Scheme 2. Solid phase synthesis using a fluoride ion deprotection method. *Reagents and conditions:* For each amino acid (*a*)—(*d*): i, MSA (0.5 M in CH_2Cl_2–dioxane, 9 : 1); ii, DIPEA in CH_2Cl_2; iii, DIPCDI–HOBt (in CH_2Cl_2–DMF). iv, $Bu^n_4NF{\cdot}3H_2O$ in DMF.

on the solvent, *i.e.*, the N^{in}-Ppt was removed by 0.1 M-TBAF in DMF or dimethyl sulphoxide at 25 °C within 10 min, and in THF or MeCN the cleavage was complete within 30 min. In MeOH or CH_2Cl_2, the cleavage reaction was incomplete even after 12 h. By use of 0.5 M-KF with 0.5 M-18-crown-6 in MeCN at 25 °C, the N^{in}-Ppt group was cleaved in 3 h.

In order to demonstrate the usefulness of Trp(Ppt), bradykinin potentiating peptide 5a (BPP5a)[10] was synthesized by the conventional solution method (Scheme 1). Using the *p*-nitrophenyl ester (ONp) and the mixed anhydride method, (**2**) was assembled, and prior to each coupling, TFA–anisole was used to remove the Boc group. The N^{in}-Ppt group survived intact under the various conditions employed during this synthesis and sufficiently protected the indole moiety by its electron-withdrawing property. The protected peptide (**2**) thus obtained was deprotected with 0.5 M MSA–TFA (for 2 h) or 0.3 M TFMSA–TFA (for 1 h) in the presence of thioanisole (0.3 M), *m*-cresol (0.3 M), and ethane-1,2-dithiol (0.25 M) at 0 °C, and purified by gel filtration on Sephadex G-10, followed by fast protein–liquid chromatography (f.p.l.c.) on a PEP-ODS WP-300 Å column. The purified BPP5a (**1**)† was obtained in good yield, 71 and 63% from the deprotection and purification steps, respectively. Alternatively, (**2**) was first treated with $SiCl_4$–anisole/TFA[7] at 25 °C for 1 h to remove the benzyloxycarbonyl (Z) groups, and then (**3**) was treated with 0.5 M TBAF–DMF at 25 °C for 30 min to deprotect the N^{in}-Ppt group. The product was purified as described above, and the homogeneous peptide (**1**) was obtained in 73% yield (from the deprotection and purification steps). The peptides synthesized by the three different deprotecting procedures possessed properties identical with an authentic sample.

† $[\alpha]_D^{31}$ −72.4° (*c* C.6, 1M-AcOH); t.l.c. (silica, Bu^nOH: AcOH: pyridine: H_2O 4:1:1:2), R_f 0.30; h.p.l.c. [Chemcosorb 5-ODS-H, 4.6 × 150 mm, MeCN (10—60%, 30 min) in 0.1% aq. TFA, 0.7 ml min^{-1}], retention time 13.61 min; satisfactory elemental analyses were obtained for $C_{30}H_{41}N_7O_7{\cdot}CF_3CO_2H{\cdot}2.5H_2O$. Amino acid ratios (4M MSA hydrolysate): Glu 1.05, Ala 1.00, Lys 1.00, Trp 1.02, Pro 1.04.

Since the N^{in}-Ppt group has been found to be smoothly deprotected by TBAF, we applied Trp(Ppt) to the solid phase synthesis of BPP5a by a new strategy using a fluoride ion deprotection method (Scheme 2). A solid support with a *p*-(carbamoylmethyl)-benzyl ester linkage (Pam)–resin (**4**)[11] was used, and, in combination with the acid labile Boc group for N^{α}-protection, new amino acid derivatives bearing protecting groups removable with fluoride ion were employed, *i.e.*, Boc–Trp(Ppt)–OH and Boc–Lys(Fmoc)–OH (Fmoc = 9-fluorenylmethyloxycarbonyl).[12] In preliminary experiments, the N^{ε}-Fmoc group was removed by 0.1 M-TBAF in DMF at 25 °C within 2 min,[13,14] and proline was obtained from Pro–Pam–resin in 95% yield (calculated by amino acid analyser) from the cleavage reaction with 0.1 M-TBAF in DMF at 25 °C for 3 h.[14]

The protected peptide–resin (**5**) was prepared from Boc–Pro–Pam–resin (**4**) (Pro, 0.76 mequiv. g^{-1}) using a Biosearch 9500 automated synthesizer and di-isopropylcarbodi-imide (DIPCDI)–1-hydroxybenzotriazole (HOBt) activation. To remove the Boc group, a new reagent [MSA (0.5 M in CH_2Cl_2)–dioxane, 9 : 1][15] was used, and 5% *N,N*-di-isopropylethylamine (DIPEA) in CH_2Cl_2 was used for neutralization. In preliminary experiments, the N^{ε}-Fmoc group, N^{in}-Ppt group, and Pam–resin ester linkage were stable under those conditions employed in the solid phase synthesis. The protected peptide–resin (**5**) thus obtained was treated with 0.1 M-TBAF in DMF at 25 °C for 3 h, to cleave the peptide from the resin and to deprotect the N^{ε}-Fmoc and N^{in}-Ppt groups. After the resin‡ had been separated from the solution by filtration and washed with 50% aqueous acetic acid, the combined filtrate and washing were concentrated *in vacuo*. The product was purified as previously described, and the homogeneous peptide (**1**) thus obtained (overall yield 63% based on starting Boc–Pro–Pam–resin) possessed properties identical with an authentic sample. These excellent results

‡ The result (peptide content, 0.07 mequiv. g^{-1}) of amino acid analyses of 12 M HCl–propionic acid hydrolysate indicated that the cleavage of peptide–resin was successful.

show the potential of the method for both solution and solid phase peptide syntheses.

Received, 3rd November 1987; Com. 1602

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