Solid Phase Synthesis of Crystalline Protected Penta-L-tryptophan Methyl Esters

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Synopsis. Solid phase syntheses of protected tryptophan homo-oligomers were accomplished by utilizing dimethylphosphinothioyl(Mpt) group as an N^{α}-amino protecting group which was removed by treatment with triphenylphosphine dihydrochloride. By measurements of the ultraviolet and fluorescence spectra it was ascertained that no modification on the tryptophan indole ring had occurred during these syntheses.

Studies on conformation of tryptophan containing peptides have restriction because of difficulty in synthesizing pure model peptides. The usual synthesis by the use of *t*-butoxycarbonyl (Boc) group as an N^{α}-amino protecting group is not applicable to the synthesis of tryptophan containing peptides without special care because *t*-butylation of the tryptophan indole ring easily occurs during deprotection by trifluoroacetic acid.[1] Occurrence of modification of the indole nucleus can be most conveniently detected by fluorescence spectrum measurement.[2]

Recently we reported that the diphenylphosphinothioyl (Ppt) group was useful for the synthesis of tryptophan containing peptides because Ppt–Cl, a by-product formed in the deprotection step, did not modify the indole ring.[3,4] Lately dimethylphosphinothioyl (Mpt) group was found to be much more easily removable than the Ppt group and especially suitable for solid phase synthesis as shown in the synthesis of Leu^5-enkephalin and its D-Ala^2 analog.[5] In this work solid phase synthesis of tryptophan oligomers by use of Mpt-tryptophan was tried.

The *N*-Mpt group can be removed by the hydrogen chloride reagents used generally in peptide synthesis. As an especially effective reagent for the removal of the Mpt group, hydrogen chloride absorbed in a solution of triphenyl phosphine (TPP) in dichloromethane is recommended. TPP is added; firstly because it can suppress the acidity of HCl through the salt formation with TPP (pK_a 2.3 in 80% ethanol)[6] and secondly because it makes it possible to prepare a storable dichloromethane solution of HCl of practically useful concentration. The effectiveness of the acidity control of HCl by TPP was made clear by comparison of deprotection rates of Boc- and Mpt-di-L-tryptophan methyl esters in the presence or absence of TPP. The results are summerized in Table 1. The rate of removal of the Boc group which needs sufficient proton concentration was markedly lowered by the addition of TPP. On the other hand the removal rate of the Mpt group was affected only slightly.

Table 1. Deprotection rate of R–L-Trp–L-Trp–OCH_3

Reagent	R=Mpt k/h^{-1}	R=Boc k/h^{-1}
0.1 M HCl/CH_2Cl_2	1.90	2.40
0.1 M HCl (0.1 M TPP)/CH_2Cl_2	1.65	0.027

The stability of tryptophan during the course of deprotection of the Mpt group was ascertained by measuring the per cent recovery of tryptophan with an amino acid analyzer. When Mpt-L-tryptophan was deprotected by treating with 0.2 M† HCl (0.2 M TPP)/CH_2Cl_2 a clear colorless solution resulted to give 98% recovery of tryptophan. No ninhydrin-positive spot other than tryptophan was detected by silica gel thin layer chromatography.

Solid phase syntheses of protected penta-L-tryptophan methyl esters were performed on the automatically programmed Beckman model 990 peptide synthesizer.

Table 2. Properties of protected tryptophanhomo-oligomers

Compound	Mp/°C	$[\alpha]_D^{25}$(deg)	Found (Calcd)(%) C	H	N
Mpt–L-Trp–OCH_3	108—109	−2.4 (*c* 1, methanol)	54.31 (54.20)	6.50 (6.12)	9.05 (9.02)
Mpt–(L-Trp)$_2$–OCH_3	153—154	−47.6 (*c* 0.5, methanol)	60.84 (60.47)	6.03 (5.89)	11.35 (11.28)
Mpt–(L-Trp)$_3$–OCH_3	amorphous	−66.8 (*c* 0.5, methanol)	63.21 (63.33)	5.96 (5.76)	12.23 (12.31)
Mpt–(L-Trp)$_4$–OCH_3	amorphous	−65.6 (*c* 0.5, methanol)	63.52 (63.67)[a]	5.87 (5.75)[a]	12.27 (12.63)[a]
Mpt–(L-Trp)$_5$–OCH_3	151—154	−65.0 (*c* 0.5, methanol)	65.11 (64.91)[b]	5.56 (5.73)[b]	13.13 (13.05)[b]
Ac–(L-Trp)$_5$–OCH_3	233—236 (dec)	−33.1 (*c* 0.6, DMF)	67.70 (68.08)[c]	5.58 (5.71)[c]	13.81 (13.69)[c]
Boc–(L-Trp)$_5$–OCH_3	211—213 (dec)	−51.0 (*c* 0.5, methanol)	68.71 (68.91)	5.93 (5.88)	13.04 (13.17)

a) Calcd for $C_{47}H_{49}N_8O_5PS\cdot H_2O$. b) Calcd for $C_{58}H_{59}N_{10}O_6PS\cdot H_2O$. c) Calcd for $C_{58}H_{56}N_{10}O_7\cdot H_2O$.

† 1 M=1 mol dm^{-3}.

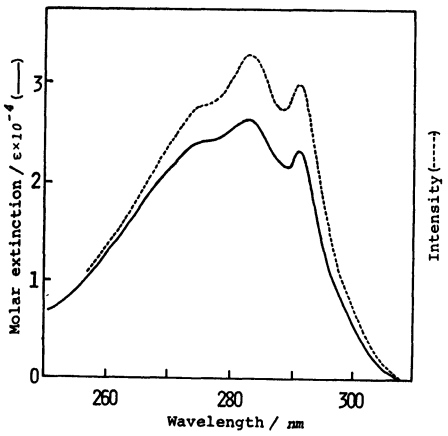

Fig. 1. a) UV spectrum of **1**(——) in methanol. b)Fluorescences excitation spectrum of **1** (emmission at 340 nm) (------) in methanol.

N^{α}-Mpt-penta-L-tryptophan methyl ester (**1**) was separated from the resin support by transesterification.[7] The crude product was purified by gel chromatography on Sephadex LH-20 and recrystallization from ethanol. Pure **1** was obtained in 60% yield as colorless crystals.

In order to know the effect of *N*-terminal group N^{α}-Boc-penta-L-tryptophan methyl ester (**2**) was also synthesized by coupling tetra-L-tryptophan resin with Boc-L-tryptophan. Compound **2** was obtained in 66% yield after the same processes of isolation and purification used in the synthesis of the Mpt derivative. The N^{α}-acetyl (Ac) derivative was obtained in 66% yield by treating unprotected penta-L-tryptophan resin with acetic anhydride and triethylamine.

Among the three types of protected-penta-L-tryptophan methyl esters the Mpt derivative showed the highest solubility in organic solvents such as methanol.

N^{α}-Mpt-tri- and tetra-L-tryptophan methyl esters were synthesized according to the same solid phase procedures. N^{α}-Mpt-mono- and di-L-tryptophan methyl esters were obtained by a liquid phase method. The physical properties and elemental analysis data of all the synthetic peptides are summarized in Table 2.

Chemical purity was checked by high performance liquid chromatography by adopting an ODS support and a linear (0 to 3%) methanol–chloroform gradient for elution. Each peptide appeared as a single peak.

Purity of the peptide **1** was further ascertained by measurements of the ultraviolet and fluorescence spectra. The UV spectrum (Fig. 1a) showed the typical curve of tryptophan with λ_{max} at 283 nm; also the fluorescence excitation spectrum (Fig. 1b) gave the same pattern as the UV spectrum. From these results it was ascertained that no modification on the tryptophan indole ring had occurred during the synthesis. Boc- and Ac-derivatives also showed high purity.

The relationships between the spectroscopic properties and the conformation of these synthetic peptides are now being studied and the results will be published in due course.

Experimental

*N^{α}-Dimethylphosphinothioyl-penta-L-Tryptophan Methyl Ester (Mpt-L-Trp-L-Trp-L-Trp-L-Trp-L-Trp-L-Trp-OCH_3) (**1**).* Chloromethylated copoly(styrene-1% divinylbenzene) resin (0.96 mmol Cl/g, Wako Pure Chemicals Ind., Ltd.) was esterified with N^{α}-Mpt-L-tryptophan by the caesium salt method.[8] The amino acid content of the resin was obtained as 0.56 mmol/g by Dorman method.[9] One gram of the ester resin was placed in the reaction vessel of the Beckman model 990 peptide synthesizer. Mpt group was removed by treating twice for each 30 min with 0.2 M HCl (0.2 M TPP)/CH_2Cl_2. After neutralization with 10% triethylamine in dichloromethane, couplings were mediated with the oxidation-reduction condensation[10] using tris(*p*-methoxyphenyl)phosphine and 2,2′-dithiodipyridine. After four cycles of these procedures N^{α}-Mpt-penta-L-tryptophan was cleaved from the resin support as its methyl ester by treating it five times with 1 M triethylamine in methanol for each 12 h. All the filtrates and methanol washings were combined and evaporated *in vacuo*. The residue was washed with water and dried to give a slightly yellow solid containing minor impurity when analyzed on silica gel by thin layer chromatography; 0.611 g. The solid was dissolved in a small volume of methanol and applied to a Sephadex LH-20 (1.9 cm × 100 cm) column. Methanol eluate (flow rate-1 ml/min) was monitored by UV spectrophotometry at 254 nm and collected as 5 ml fractions. The fractions containing a single component, when detected on silica gel thin layer chromatography, were combined and evaporated to give colorless crystals of **1**; 0.414 g. An analytically pure sample was obtained by recrystallization from ethanol; 0.354 g (60% calculated from N^{α}-Mpt-L-tryptophan resin).

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