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DIMETHYLPHOSPHINYL (Dmp): A NEW PROTECTING GROUP OF TYROSINE SUITABLE FOR PEPTIDE SYNTHESIS BY USE OF BOC-AMINO ACIDS

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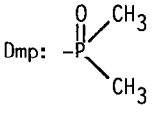
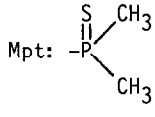
Summary: Use of dimethylphosphinyl (Dmp) group as a side-chain phenolic OH protecting group of tyrosine in peptide synthesis was studied. The Dmp group is resistant to trifluoroacetic acid and hydrogenolysis and removed by fluoride ion or liquid HF.

The formation of 3-benzyltyrosine from O-benzyl tyrosine in the total deprotection step by HF of protected peptides containing tyrosine is one of the most hazardous side reactions in peptide synthesis.[1] Recently "low HF" cleavage procedure was reported to be useful to suppress this side reaction.[2,3] However, a substantial solution of this problem should be made by development of a new protecting group which would not yield any active cationic species during cleavage. In this paper we would like to report the use of the Dmp group which fulfils this requirement.

We have already reported the use of dimethylphosphinothioyl (Mpt) group for the protection of amine,[4] phenolic hydroxyl[5] and thiol[6,7] functions of amino acids. The Mpt group could protect the tyrosine moiety effectively and be removed without any side reaction, but the presence of sulfur atom in the group and its basic and not necessarily mild removal conditions have restricted its versatile use. The Dmp group as the most labile one among a series of phosphinyl groups containing no sulfur atom was expected to overcome these problems.





Dimethylphosphinyl chloride (Dmp-Cl), a reagent for the introduction of the Dmp group, is obtained as crystals of Mp 70-72°C by treating tetramethyldiphosphine disulfide[8] with thionyl chloride.[9] The Dmp-Cl is sensitive to moisture, but storable in a sealed tube.

When benzyloxycarbonyl-L-tyrosine benzyl ester (Z-L-Tyr-OBzl) in chloroform was treated with 3 equiv. of Dmp-Cl in presence of triethylamine Z-L-Tyr(Dmp)-OBzl (**1**), Mp 98-99°C; $[\alpha]_D^{25}$ -14.4°(c1, MeOH), was obtained in 76% yield.[10] In a similar manner t-butyloxycarbonyl-L-tyrosine benzyl ester (Boc-L-Tyr-OBzl) gave Boc-L-Tyr(Dmp)-OBzl (**2**), Mp 117-118°C; $[\alpha]_D^{20}$ -8.0° (c1, EtOH), in 80% yield. Compound **1** could be catalytically hydrogenolyzed to afford O-Dmp-L-tyrosine, which was isolated as an acetate (**3**), Mp 180°C(dec); $[\alpha]_D^{28}$ -6.8°(c1, AcOH), in 90% yield. Hydrogenolysis of **2** gave Boc-L-Tyr(Dmp)-OH (**4**), Mp 75°C; $[\alpha]_D^{20}$ +18.5°(c1, EtOH), in 70% yield. Compound **4** was also available by t-butyloxycarbonylation of **3**, but the product obtained by this way contained a small amount of Boc-L-Tyr-OH probably because of partial hydrolysis of the O-Dmp bond in an aqueous base solution.

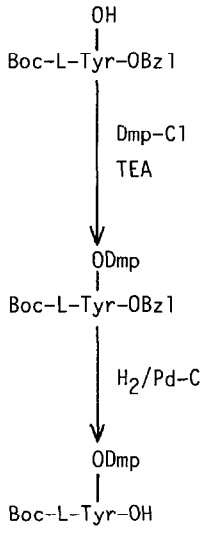


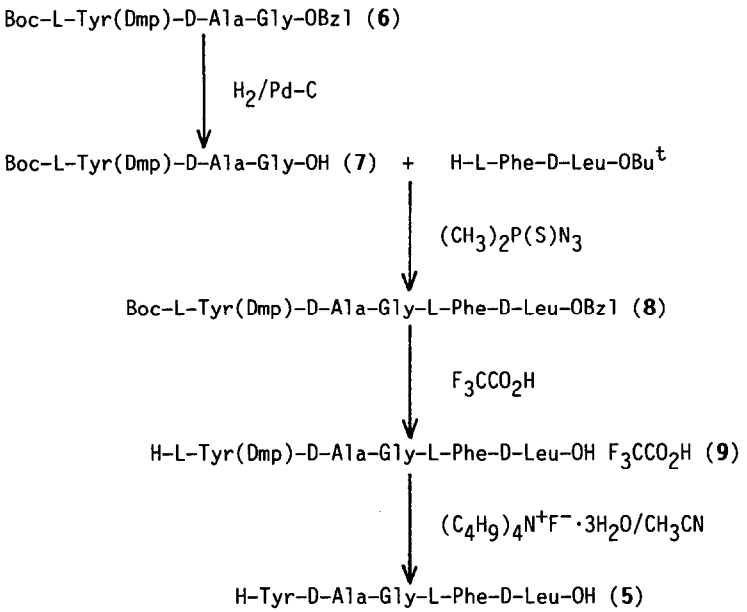
Table. Stability and Removal of the O-Dmp Group

Reagent	Conditions	Compound	Reaction
1M HCl/MeOH	RT, 24h	**1**	stable
1M HCl/AcOH	RT, 24h	**1**	stable
F_3CCO_2H	RT, 24h	**1**	stable
HBr/AcOH	RT, 24h	**1**	stable
H_2/Pd-C	RT	**1**	stable
liquid HF	0°C, 1h	**1**	complete cleavage
1M TEA/MeOH	RT, 7h	**1**	complete cleavage
0.1M NaOH	RT, <5min	**1**	complete cleavage
5% aq. $NaHCO_3$	RT, 5h	**3**	partial cleavage*
20% H_2NNH_2/MeOH	RT, <5min	**1**	complete cleavage
50% piperidine/DMF	RT, 6h	**1**	complete cleavage
$(C_4H_9)_4N^+F^-\cdot 3H_2O/CH_2Cl_2$	RT, <5min	**1**	complete cleavage

*)About 10 % of tyrosine was detected by amino acid analyzer.

Characteristic properties of the O-Dmp group as a side chain OH protecting group of tyrosine were checked by use of compounds **1** and **3** and results were summarized in the above table. The most convenient reagent for the removal of the Dmp group would be tetrabutylammonium fluoride trihydrate, which was shown to be effective in the removal of the Mpt[7] and diphenylphosphinothioyl[11] groups protecting the thiol function of cysteine. Crystalline water is necessary in this cleavage reaction which proceeds hydrolytically. Contrast to the easiness of removal under neutral and basic conditions the Dmp group showed strong resistance to the acidic reagents including trifluoroacetic acid. Catalytic hydrogenolysis could be performed without any problem in the presence of the Dmp group remaining the Dmp group intact. These properties of the Dmp group would offer a new tactics in the synthesis of tyrosine containing peptides by the Boc strategy. Usefulness of the Dmp group was exemplified in the synthesis of [D-Ala^2, D-Leu^5]enkephalin (**5**).

Solution phase synthesis of **5** was carried out by the [3+2] segment coupling manner. Compound **2** was activated as the mixed anhydride with use of dimethylphosphinothioyl chloride[12] and coupled with H-D-Ala-Gly-OBzl to give Boc-L-Tyr(Dmp)-D-Ala-Gly-OBzl (**6**) in 82% yield. From **6** the benzyl ester residue was selectively removed by catalytic hydrogenolysis on Pd-C to afford N-terminal segment, Boc-L-Tyr(Dmp)-D-Ala-Gly-OH (**7**), in 90% yield. Compound **7** was coupled with C-terminal dipeptide segment, H-L-Phe-D-Leu-OBu^t, by using dimethylphosphinothioyl azide[13] to give Boc-L-Tyr(Dmp)-D-Ala-Gly-L-Phe-D-Leu-OBu^t (**8**) in 64% yield. Final deprotection was performed in two steps. Boc and t-butyl ester groups were removed by treatment with trifluoroacetic acid to yield O-Dmp-pentapeptide **9**, which was precipitated by ether and treated with tetrabutylammonium fluoride in acetonitrile at room temperature for 1h. The Dmp group was removed completely to give **5** in 93% yield from **8**.

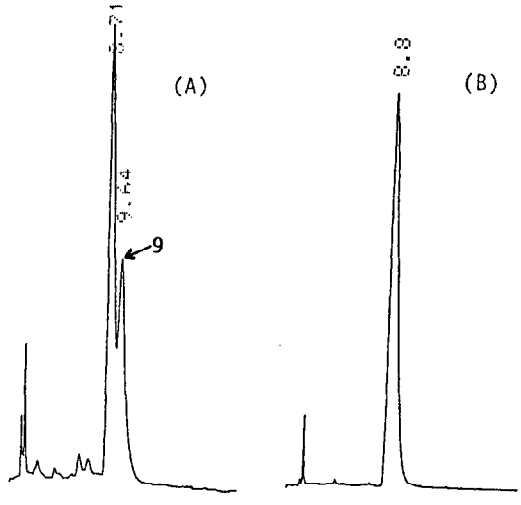


Removability by liquid HF is another attractive feature of the new protecting group. The Dmp group of **1** was cleaved completely by liquid HF under the standard reaction conditions (0° C, 1h) to give 96% recovery of tyrosine by an amino acid analyzer. This result suggests possibility of use of the Boc derivative **2** instead of Boc-L-Tyr(Bzl)-OH in solid phase synthesis. In order to ascertain this solid phase synthesis of **5** was also tried.

All operations of the solid phase synthesis were performed on the Beckman model 990 peptide synthesizer. Starting from Boc-D-Leu-resin (polystyrene-1%-divinylbenzene, Leu content 0.75 mequiv/g) Boc-amino acids were incorporated by the oxidation-reduction condensation method using tris(p-methoxyphenyl)phosphine and 2,2'-dipyridyl disulfide as the reagents.[14] The protected pentapeptide resin thus obtained was treated with liquid HF at 0°C for 1h. Although HPLC analysis of crude products shown in Figure revealed the presence of the incomplete deprotection product **9**, the Dmp group of which was removed completely during isolation procedures by preparative TLC in a solvent system of chloroform: methanol: aq. NH_3 (65:25:4) to give pure **5** in 65% yield.

Figure. HPLC Profiles of Crude (A) and Purified (B) **5**

HPLC conditions: column, Unisil Q C18 (4x150 mm); temp, 25°C; solvent system, 0.01 M HCl:CH_3OH=50:50(v/v); flow rate, 1.0 ml/min; detection, 210 nm.



Both samples of **5** obtained by solution and solid phase methods showed biological activity in in vitro isolated preparations identical with that of the authentic sample. Thus, the Dmp group was shown to be useful for the synthesis of peptides containing tyrosine.

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