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A NOVEL AND EFFICIENT METHOD FOR CLEAVAGE OF PHENACYL ESTERS BY ZINC REDUCTION WITH ACETYLACETONE AND PYRIDINE[1)]

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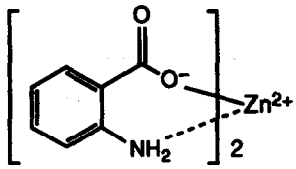
Summary: The phenacyl esters of peptide segments were efficiently cleaved by zinc reduction using a chelating compound, acetylacetone, in the presence of pyridine.

The phenacyl (Pac, C_6H_5-CO-CH_2-) ester,[2)] which can be selectively cleaved by reduction with zinc-acetic acid, has been used as a carboxy protecting group and its usefulness has been shown by the successful synthesis of long chain peptides[3)] by the segment condensation method, where the segments were protected as the Pac ester at the carboxyl termini. However, the cleavage reaction of the Pac ester by the conventional zinc-acetic acid method becomes difficult as the chain of the peptide segment is elongated because of the following reasons. Firstly, the solubilities of the peptide segments significantly decrease in the typical solvents for the reaction such as dimethylformamide (DMF) or N-methyl-2 -pyrrolidone (NMP), containing acetic acid. Addition of dimethylsulfoxide (DMSO) as a co-solvent to dissolve them results in a significant reduction in the rate of the reaction compared to the rate without DMSO. Secondly, in a deprotection reaction of a segment of poor solubility, a precipitation of zinc salts of the resulting acid frequently occurs which seriously disturbs the progress of the reaction and makes the isolation of the target acid difficult. Thus an efficient method for cleavage of the Pac ester is required which can be extensively applied to the synthesis of long chain peptides.

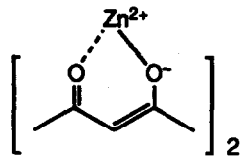
We have already reported that the zinc reduction with anthranilic acid in the presence of pyridine efficiently cleaves the Pac ester of the segments in the total synthesis of the 53 amino acid peptide urogastrone (h-EGF).[4) 5)] Anthranilic acid not only works as a proton source but also traps zinc ions by chelation as shown (**1**). The role of such a chelating compound as (**1**) is that it removes the zinc ions continuously from the reaction media and drives the reaction equilibrium toward completion by regenerating a fresh surface of zinc metal. However, the removal of excess anthranilic acid from the product is not always easy because it is not volatile and sparingly soluble in water. We therefore began studies to

find other co-reagents superior to anthranilic acid for reductive cleavage of the Pac ester by zinc reduction.

The co-reagent is required to have the following characteristics: first, it must have an acidic proton to be used for reduction; second, it should form a stable complex with zinc.

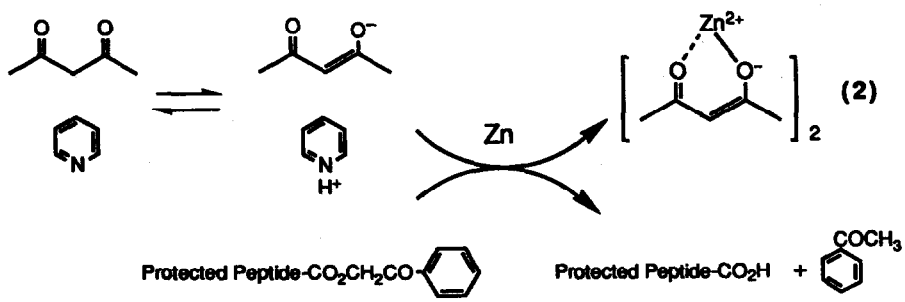


(1)



(2)

We have found that the β-diketone acetylacetone in combination with pyridine to be the most efficient reagent. Acetylacetone has an acidic proton and forms a stable complex with zinc ion, known as zinc acetoacetonate (2).[6] The reaction was conducted with pyridine and its presence was essential to the success of the reaction. Since acetylacetone is a weak acid (pKa=9.0), the protonated pyridine (pKa=5.2, a slightly weaker acid than acetic acid, pKa=4.74) is the actual proton source. The reaction pathway is shown in the following scheme.



A typical procedure is as follows (Entry 1 in Table 1): The Pac protected segment of h-EGF, corresponding to the amino acid sequence from ^{13}Tyr to ^{26}Leu, Boc-(13-26)-OPac [7] (33.3g) was dissolved in a mixed solvent of NMP and DMSO (4:1, 500ml) at 35°C, and pyridine (20ml) and acetylacetone (10eq) were added. Then activated zinc dust [8] (33g) was added and the mixture was stirred under nitrogen for 6h at 35°C . After filtration and concentration under vacuum, 0.5N hydrochloric acid was added to the residue. The precipitates were filtered, washed with water and ether, and dried to give 29.1 g of Boc-(13-26)-OH (yield 90.1%).

Other examples are also shown in Table 1 (Entry 2~5). The most remarkable advantage of the present method is that the rate of the deprotection reaction is satisfactory even in

DMSO- containing solvents and thus the method is quite useful for such a long chain segment of poor solubiliy as Entry 1 and 2 in Table 1.[9] The ratio of NMP and DMSO can be varied and DMF as a single solvent can be used. When DMF was used, the reaction was complete within an hour or less (see Entry 3~5 in Table 1). Of other importance, the product carboxylic acid did not form a zinc salt, since zinc ions were removed from the reaction media as zinc acetoacetonate (2). As shown in the note 11 on Entry 3, the zinc-acetic acid method gave only a moderate yield (61%) due to the precipitation of zinc salts from which the target acid could hardly be recovered, while our new method, on the contrary, provided a high yield (98%). In the other four examples, the precipitates of the zinc salts were not observed and the yields were also excellent.

In those segments which have a base-sensitive Asp-Gly part (Entry 1 and 3), the amount of cyclized α– aminosuccinimide peptides formed during the deprotection reaction was estimated to be negligible on HPLC after treatment of the products with HF (0°C, 1h). Since acetylacetone is a volatile reagent and easily removed by washing with ether, the isolation of the product is much easier than the reaction with anthranilic acid. There were no problems in the subsequent segment condensation steps for chain elongation. The use of other chelating agents, for example, ethyl benzoylacetate, dimethylglyoxime, and salicylic acid have failed.

In conclusion, the present new method for the Pac ester cleavage using acetylacetone as a chelating agent is quite efficient and can be applied not only to the synthesis of peptides by the segment condensation strategy but also to synthetic reactions in other fields.

Table 1

Entry	Pac Esters of Protected Peptides[7]	Reaction Conditions[10] Solvent	Temp.	Time	Products[12] Yd.	m.p. (dec)	$[\alpha]_D^{25}$
1	Boc-YCLHDGVCMYIEA--L-OPac (13-26)	NMP-DMSO (4:1)	35°C	6h	90.1%	270~272°C	-15.4° (c1,DMSO)
2	Boc-DKYACNCVVG-OPac (27-36)	NMP-DMSO (2:1)	50°C	4h	95.4%	248~250°C	-16.3° (c1,DMSO)
3	Boc-YCLHDG-OPac[11] (13-18)	DMF	35°C	0.6h	98.0%	183~184°C	-27.2° (c1,DMF)
4	Boc-ERCQYRDL-OPac (40-47)	DMF	45°C	1h	91.0%	168~170°C	-14.7° (c1,DMF)
5	Boc-DKYA-OPac (27-30)	DMF	40°C	1h	92.6%	181~182°C	-19.3° (c1,DMF)

References and Notes

1) A part of this study was presented at the 105th annual meeting of Pharmaceutical Society of Japan (Kanazawa, Japan 1985).
2) J.Hendrickson and C.Kendall,Tetrahedron Lett., 343 (1970).
3) T.Kimura, T.Morikawa, M.Takai. and S.Sakakibara, J. Chem. Soc. Chem. Commun., 340 (1982).
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6) G. Rudolph and M.Henry, Inorganic Chem., 3, 1317 (1964).
7) All these compounds are the protected peptide segments for h-EGF corresponding to the amino acid sequences shown in the parentheses, and the preparations and characterizations of these compounds were described in ref.5.
   One letter symbols for amino acids were used. Side chain protecting groups were as follows: Lys, 2- chlorobenzyloxycarbonyl; Cys, acetamidemethyl; Glu, benzyl; Asp, cyclohexyl; and Tyr, 2,6- dichlorobenzyl; Arg, p-toluenesulfonyl.
8) Washed several times with 5% hydrochloric acid and washed in turn with water, methanol, and ether, and dried.
9) The Pac esters of these segments could not be deprotected by the usual zinc-acetic acid method in the same solvent as those in Table 1 (NMP-DMSO, 4:1 and 2:1, respectively).
10) In all examples, acetylacetone (10eq) and pyridine (4% of the total reaction volume) were used. The amount of acetylacetone was not optimized.
11) In example 3, the zinc-acetic acid method (DMF, 35° C,1 h) gave a lower yield (61%).
12) Elemental analysis of the products were as follows: Ex.1, calcd. for $C_{110}H_{150}Cl_4N_{18}O_{25}S_3 \cdot HCl$: C,55.07; H,6.34; N,10.51. Found: C,54.9; H,6.25; N,10.25. Ex.2, calcd. for $C_{76}H_{107}Cl_3N_{14}O_{21}S_2$: C,52.97; H,6.26; N,11.38. Found: C,52.25; H,6.20; N,11.20. Ex.3, calcd. for $C_{51}H_{69}Cl_2N_9O_{13}S_1 \cdot HCl$: C,53.01; H,6.02; N,10.91. Found: C,52.45; H,6.05; N,10.40. Ex.4, calcd. for $C_{86}H_{116}Cl_2N_{16}O_{22}S_3$: C,54.57; H,6.18; N,11.84. Found: C,52.2; H,6.00; N,11.55. Ex.5, calcd. for $C_{48}H_{60}Cl_3N_5O_{12}$: C,57.34; H,6.01; N,6.97. Found: C,57.10; H,6.00; N,7.00.

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