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CHLOROTRIMETHYLSILANE-PHENOL AS A MILD DEPROTECTION REAGENT FOR THE TERT-BUTYL BASED PROTECTING GROUPS IN PEPTIDE SYNTHESIS

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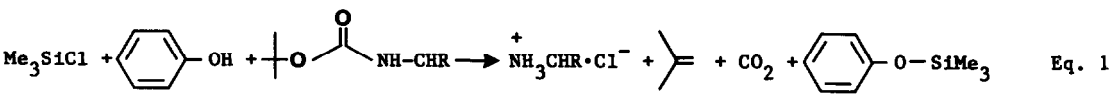
Abstract - Efficient deprotection of the tertbutyl urethane group by 1 M Me_3SiCl- 1 M and 3 M-phenol reagents is described.

The tert-butyl based groups are the most prominently used protecting groups in peptide synthesis. Among the tert-butyl protecting groups, the tert-butyl urethane of the amino acids as represented by the N^{α}-butyloxycarbonyl (Boc) groups is conventionally removed selectively by 50% CF_3CO_2H/CH_2Cl_2 without affecting the integrity of the side chain benzyl (Bzl)-based protecting groups. However, the possibility of trace amount of trifluoroacetic acid forming termination side products during the coupling steps have led us to explore alternative deprotecting methods that would avoid this side reaction.

Organosilicon derivatives such as Me_3SiClO_4 and Me_3SiI have been explored for the cleavage of tertbutyl protecting groups. However, both reagents suffer poor selectivity when benzyl protecting groups are present[1]. The lack of selectivity could be in part attributed to the strong leaving groups, and a trimethylsilyl derivative with a poorer leaving group as found in Me_3SiCl may offer the needed selective cleavage of Boc vs Bzl. Our initial effort to increase this selectivity with different concentrations of Me_3SiCl (0.1 - 3 M) in CH_2Cl_2 was unsuccessful and the rate of the Boc group cleavage was uniformly sluggish ($t_{1/2}$ > 14 h). However, in the presence of 1 to 3 M phenol, the cleavage rate effected by 1 M Me_3SiCl accelerated 100-300 fold and provided the required efficiency for the complete removal of the Boc group in 0.2 to 1 h (Eq. 1). More importantly, the range of selectivity of the cleavage between Boc and benzyl group is high. These properties of Me_3SiCl-phenol are sufficient to permit repeated removal of the Boc group in successive and repetitive steps for peptide synthesis.



Eq. 1

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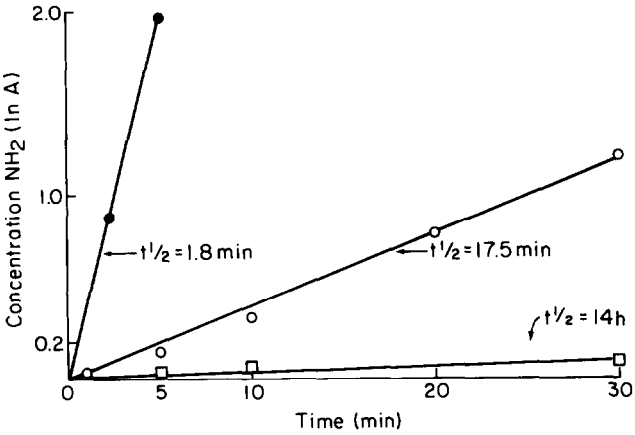

Fig. 1 - Relative deprotection rates of Boc-Val-OCH_2-resin to Val-OCH_2-resin with 1 M $(CH_3)_3SiCl$ in CH_2Cl_2 (□), 1 M phenol (○) and 3 M phenol(●).

The rate enhancement of the Boc deprotection reaction was strongly dependent on the phenol concentration. Kinetic experiments using the quantitative ninhydrin test[2] for the determination of the free amine showed that the $t_{1/2}$ for the removal of the Boc group of Boc-Val-, Boc-Leu-, Boc-Phe- or Boc-Ala-OCH_2-resin in solid-phase peptide syntheses by a 1 M Me_3SiCl in 1 M phenol in CH_2Cl_2 was about 17 min (Fig. 1). Complete removal of the Boc was observed in all cases in 1 h when compared with the control experiments using 50% CF_3CO_2H/CH_2Cl_2. Increasing phenol concentration from 1 to 3 M accelerated the rate of the Boc removal 10 fold and completed the deprotection of the Boc-group in 20 min. Similar deprotection rates were observed with Boc-amino acids not bound to resin supports. For example, the deprotection rates ($t_{1/2}$) of Boc-Val-OH, Boc-Leu-OH and Boc-Ala-OH were found to be about 2 min as those observed in the solid-phase condition. We found that a 1 M Me_3SiCl-3 M phenol in CH_2Cl_2 solution was most suitable for solid-phase peptide synthesis[3], and a total deprotection time of 20 min would generally be sufficient for the removal of the Boc groups[4].

The mechanism by which rate enhancement of Me_3SiCl in the deprotection of the Boc with phenol occurs has not been determined. However, such a rate enhancement was not observed with other solvents such as dioxane, acetonitrile and acetic acid. It is possible that, based on our results, the cleavage reagent responsible for the Boc deprotection reaction is a Me_3SiCl-phenol complex (Fig. 2), which provides an acidic phenolic proton due to the strong Si-O bond. The postulate of such a complex would explain the strong dependence of the cleavage rate on phenol and the dramatic rate enhancement of the proposed reagents.

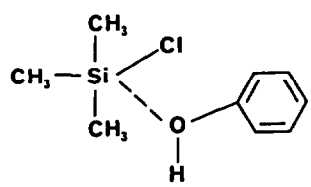

Fig. 2 - Proposed $(CH_3)_3SiCl$-Phenol complex that provides an acidic phenolic proton.

The selectivity of the reagent showed that in prolonged treatment (120 h) of the benzyl protected amino acids the loss of the benzyl protecting groups was minimal (Table I). The cleavage of benzyl-group from Tyr(Bzl), Ser(Bzl), Glu(OBzl) and Lys(ClZ) in a 20 min deprotecting cycle by 1 M Me_3SiCl- 3 M phenol in CH_2Cl_2 was found to be in the ratio of 10^4 to 10^5 and is generally acceptable for the Boc-benzyl strategy of the peptide synthesis. Furthermore the loss due to premature cleavage of amino acids from the Boc-Val-, Boc-Leu- and Boc-Phe-O-CH_2-resins derived from esterification of the commercial chloromethyl-copoly-(styrene-1%-divinylbenzene) resin ranged from 0.04 to 0.17% in a 20 min deprotection cycle.

To obtain the best results from the proposed reagents, freshly prepared 4 M Me_3SiCl in CH_2Cl_2 and 4 M phenol also in CH_2Cl_2 were mixed (2.5 volumes 4 M-Me_3SiCl + 7.5 vol 4 M-phenol) before the application for the deprotection reactions to avoid the slow reaction of the silyl chloride with phenol to form the silylphenyl ether and HCl. Kinetic NMR experiments showed that Me_3SiCl-phenol was relatively stable in phenol and remained essentially unchanged after 24 h. Conductivity measurements and halide determinations also confirmed that there was little HCl in the solution in the 1 M Me_3SiCl-phenol solution in CH_2Cl_2. Halide determination after direct base neutralization of the resulting HCl in a mixture of 1 M $(CH_3)_3SiCl$-3 M phenol after 24 h showed less then 0.07 mol % of HCl formed. Furthermore, the rate of deprotection of 1 M $(CH_3)_3SiCl$-3 M phenol was not significantly impeded by the presence of sodium carbonate in the reaction mixture. These experiments provided strong evidence that HCl is not the responsible reagent for the cleavage of the Boc group. The use of phenol in combination of Me_3SiCl has several distinct advantages.

Table I. Relative lability of Boc and benzyl protecting groups in 1 M $(CH_3)_3SiCl$-3 M phenol in CH_2Cl_2[a].

Compound	mol % cleaved in 20 min[b]	Group cleaved
Boc-Val-OCH_2-resin	100	Boc
Boc-Phe-OCH_2-resin	100	Boc
Boc-Leu-OCH_2-resin	100	Boc
Val-OCH_2-resin	0.08	benzyl-ester-resin
Tyr(Bzl)	0.007[c]	Bzl
Gly(Bzl)	0.005	Bzl
Ser(Bzl)	0.003	Bzl
Z-Ala	0.1	Z

[a] Pseudo first order reaction rates, all measurements were determined at room temperature,

[b] determined by quantitative ninhydrin test,

[c] determined by amino acid analysis.

Phenol is an excellent swelling solvent for the peptidyl resins in solid-phase peptide synthesis and the addition of phenol to the Me_3SiCl deprotecting reagent would provide a slightly acidic condition that prevents silylation of the resulting free amine. To remove

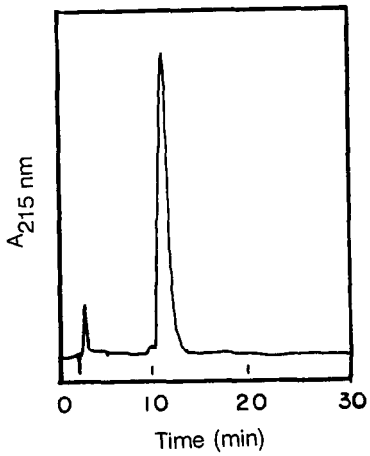

Fig. 3 - C_{18} reverse phase HPLC of crude and unpurified Leu-Ala-Gly-Val.

any possibility of N^{α}-silylation, the resins were washed with moist DMF (4% H_2O) solution to decompose unwanted silylation products after the removal of the Boc group.

To test the efficacy of the new deprotecting reagent, a tetrapeptide, Leu-Ala-Gly-Val, was synthesized by the stepwise method on a chloromethyl resin using 1 M Me_3SiCl- 3 M phenol as the repetitive deprotection reagent to cleave the Boc group with a deprotection time of 20 min[3,4]. Careful analysis by ion-exchange and by C_{18} reverse phase chromatography in six syntheses revealed that the purity of the desired product, Leu-Ala-Gly-Val, were obtained in 95% yield or greater and the levels of the deletion and termination products were comparable to the control syntheses using CF_3CO_2H as the deprotecting agent (Fig. 3). Furthermore, we have gone on to use this reagent for the synthesis of several complex peptides including a 29 residue peptide, glucagon. The results through parallel synthesis with the conventional CF_3CO_2H deprotection method showed slight improvement by the new reagent.

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4. A typical synthetic protocol in the solid-phase synthesis using 1 M $(CH_3)_3SiCl$-3 M phenol and Boc-Val-OCH_2-resin (0.6 mmol/g, 1 g) is as follows (20 ml of solvents were used throughout the synthesis): (1) CH_2Cl_2 2 x 1 min, (2) 1 M $(CH_3)_3SiCl$-3 M phenol 1 x 3 min and 1 x 17 min total 20 min (mixing 5 ml of 4 M $(CH_3)_3SiCl$ in CH_2Cl_2 and 15 ml of 4 M phenol in CH_2Cl_2), (3) CH_2Cl_2 2 x 1 min, (4) DMF-H_2O (96:4,v/v) 2 x 2 min, (5) DMF 2 x 2 min, (6) DMF-DIEA 2 x 2 min, (7) CH_2Cl_2 3 x 1 min, (8) Boc-amino acid (3 equiv, 1.8 mmol) and followed by DCC (3 equiv, 1.8 mmol), (9) CH_2Cl_2 3 x 1 min.
5. Abbreviations used: DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DMF, dimethylformamide, ClZ, 2-chlorobenzoxycarbonyl.

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