Pergamon

Tetrahedron Letters, Vol. 37, No. 33, pp. 5989-5990, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01255-5

Silicon Tetrachloride and Phenol as N^{α}-t-Butoxycarbonyl Group Deprotecting Agent in Solid Phase Peptide Synthesis

Kadlebal M. Sivanandaiah*, Vommina V. Suresh Babu and Beechanahalli P. Gangadhar

Department of Studies in Chemistry, Central College, Bangalore University, Bangalore-560 001, India

Abstract : The combination of 1M silicon tetrachloride and 3M phenol serves as an efficient N^{α} -t-butoxycarbonyl deblocking agent in solid phase peptide synthesis, the duration of the cleavage being 10 min. This is demonstrated by the synthesis of the naturally occurring μ-receptor selective opioid heptapeptide, dermorphin. Copyright © 1996 Elsevier Science Ltd

t-Butoxycarbonyl (Boc) group is extensively used for temporary protection of the amino group in solid phase peptide synthesis (SPPS). Its subsequent removal is effected by acidolysis. A major concern of peptide chemists is the repetitive acidolysis that becomes mandatory in this approach and the consequent side reactions that result. In recent years, instead of acidolysis, some organo-silicon reagents have been tried. Lott et al.[1] have observed that iodotrimethylsilane can bring about the cleavage of Boc group from several protected amino acids and peptides. The cleavage was followed by NMR. However, its application for the synthesis of any specific peptide was not reported by them. We have earlier described the utility of iodotrichlorosilane, prepared from $SiCl_4$ and NaI, for Boc deprotection during SPPS of oxytocin[2]. Merrifield and co-workers[3,4] observed that 1M chlorotrimethylsilane and 3M phenol can be employed for Boc group deprotection in SPPS. This communication outlines the use of the combination of 1M $SiCl_4$ and 3M phenol in CH_2Cl_2 for efficient Boc group deprotection in SPPS. The reagent is inexpensive and is easily prepared by mixing freshly distilled $SiCl_4$ and phenol in CH_2Cl_2. The cleavage of Boc group from Boc-Phe-OCH_2-C_6H_4-resin was effected by combinations of 1M $SiCl_4$ with 1M phenol, 2M phenol and 3M phenol and the duration of cleavage in these cases was found to be 45 min, 30 min and 10 min respectively as estimated by the picrate method[5]. Subsequently further deprotection experiments were carried out employing 20 mmoles of $SiCl_4$ and 60 mmoles of phenol in CH_2Cl_2 (20 ml) per gram of protected aminoacyl resin with an esterification level of about 0.5 mmole /g.

The stability of different amino and carboxyl protecting groups to 1M $SiCl_4$-3M phenol has been studied by the addition of the reagent to the amino acid derivative and stirring at room temperature. The reaction was monitored by thin layer chromatography. It was observed that Boc group was cleaved completely in 10 min, while the N^{α}-9-fluorenylmethoxycarbonyl, N^{α}-benzyloxycarbonyl, benzyl ester and benzyl ether groups were not cleaved to any noticeable extent even after 18 hr. The reagent does not also affect the side chain protecting groups such as nitro group of nitroarginine, ethyl and methyl carboxylic esters after continuous treatment for 18 hr.

In order to determine the stability of the benzyl ester linkage between the C-terminal amino acid and the solid support, Boc-Phe-OCH_2-C_6H_4-resin with an esterification level of 0.24 mmole/g was treated with the reagent for 40 hr. During this period, the reagent was replaced at intervals of 10 hr and the reaction was carried out under nitrogen atmosphere. A known quantity of the resin was withdrawn at intervals of 10 min, 10 hr, 20 hr, 30 hr and 40 hr and the amino acid content on the resin was estimated by the picrate method[5]. No cleavage of the aminoacyl resin bond was observed in about 20 hr. After 40 hr treatment the cleavage was estimated to be about 2%.

That the mixture of $SiCl_4$ and phenol does not act as a source of HCl that causes Boc deprotection was shown by the slow introduction of one equivalent of 2% triethylamine in CH_2Cl_2 to the reaction mixture containing the reagent and Boc-Phe-OCH_2-C_6H_4-resin. It was observed that the deprotection, though slow, was not prevented as indicated by Kaiser's qualitative ninhydrin test[6]. The reaction presumably involves an intermediate complex, (Cl_3Si---O-C_6H_5), similar to the one proposed by Merrifield and co-workers[3].

Cl H

The utility of this reagent for the synthesis of biologically active peptides is demonstrated by the solid phase synthesis of the μ-receptor selective opioid heptapeptide, dermorphin[7] (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2), using conventional Merrifield resin as solid support. Chain lengthening was carried out using appropriate Boc-amino acid (3 equiv.), N,N'-dicyclohexylcarbodiimide (3 equiv.). and 1-hydroxybenzotriazole (1 equiv.). After each coupling, Boc group was removed by 1M $SiCl_4$ and 3M phenol in CH_2Cl_2 in 10 min. Ammonolysis of the protected heptapeptide-resin gave Boc-Tyr(Bzl)-D-Ala-Phe-Gly-Tyr(Bzl)-Pro-Ser(Bzl)-NH_2 (overall yield 59% based on the amount of first amino acid esterified to the resin). Catalytic transfer hydrogenation[8] of this protected heptapeptide gave the free peptide (HPLC profile A) which was purified over Sephadex G-15 using 1N acetic acid in water. The purity of the peptide was satisfactory as indicated by the HPLC profile B shown in the figure. It had the same physical properties[9] as reported earlier[10] and exhibited the same opioid agonistic activity when studied *in vitro* by guinea pig ileum assay as that of an authentic sample[7].

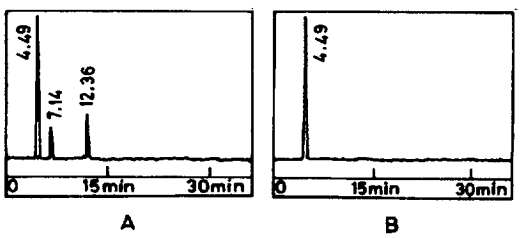

Figure : Analytical RP-HPLC profile of dermorphin (A, crude sample; B, pure peptide). Waters Bondapak C-18 column (3.9 × 300 mm) was used. Elution was carried out with two solvents : A, 20% (v/v) acetonitrile in water ; B, 60% (v/v) acetonitrile in water both containing 4.5 mmoles trifluoroacetic acid and 4.9 mmoles triethylamine. The gradient programme used was linear gradient from 10% to 25% B in 15 min, 25% B for 5 min, linear gradient from 25% to 40% B in 10 min at 1ml / min flow rate. UV, 280 nm.

REFERENCES AND NOTES

1. Lott, R. S.; Chauhan, V. S.; Stammer, C. H. J. Chem, Soc. Chem. Comm. 1979, 495-496.
2. Sivanandaiah, K. M.; Suresh Babu, V. V.; Shankaramma, S. C. Int. J. Peptide Protein. Res. 1995, 45, 377-379.
3. Kaiser, E. Sr.; Tam, J. P.; Kubiak, T. M.; Merrifield, R. B. Tetrahedron Lett. 1988, 29, 303-306.
4. Kaiser, E. Sr.; Picart, F.; Kubiak, T. M .; Tam, J. P.; Merrifield, R. B. J. Org. Chem. 1993, 58, 5167-5175.
5. Gisin, B. F. Anal, Chim. Acta. 1972, 58, 248-249.
6. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I.; Anal. Biochem. 1970, 34, 595-598.
7. Montecucchi, P. C.; De Cartiglione, R.; Erspamer, V. Int. J. Peptide protein Res. 1981, 17, 316-321.
8. Sivanandaiah, K. M.; Gurusiddappa, S. J. Chem. Res. (S), 1979, 108-109.
9. M. P. 156-158° ; $[\alpha]_D^{22}$ + 4.93 (c1, 1N acetic acid) Reported[10] : M. P. 159-160° ; $[\alpha]_D^{25}$ +4.95° (c 1, 1N acetic acid). Paper chromato graphy : R_f (n-butanol : acetic acid : water : pyridine :: 15 : 3 : 12 : 10, by volume) 0.66; R_f (n-butanol : acetic acid : water :: 4 : 1 : 1, by volume) 0.60. Amino acid analysis : Amino acid (theory) found ; Ser (1) 1.03, Pro (1) 0.97, Tyr (2) 2.04, Gly (1) 0.98, Phe (1) 1.01, Ala (1) 0.99. Elemental analysis : Anal. Calcd. for $C_{40}H_{50}N_8O_{10}$ (802.93) : C 59.78, H 6.22, N 13.95 %. Found : 59.67, H 6.4, N 13.86 %.
10. Sivanandaiah, K. M.; Gurusiddappa, S.; Suresh Babu, V. V. Int. J. Peptide Protein Res. 1989, 33, 463-467.

(*Received in UK* 11 *April* 1996; *revised* 26 *June* 1996; *accepted* 28 *June* 1996)