Selective Deprotection of the N^{α}-*tert*-Butyloxycarbonyl Group in Solid Phase Peptide Synthesis with Chlorotrimethylsilane and Phenol†

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The repetitive deprotection of the N^{α}-*tert*-butyloxycarbonyl group during solid phase peptide synthesis was found to be efficient and quantitative by the use of a mild new reagent containing 1 M chlorotrimethylsilane and 1 M phenol in dichloromethane. Kinetic studies showed that the half-life for the reaction at 22 °C with Boc-Val-resin was 17.5 min, a 40-fold increase over the rate in the absence of phenol. The reaction is not due to the presence of HCl in the reagent. The selectivity between the removal of the N^{α}-*tert*-butyloxycarbonyl group and benzylic esters, ethers, and carbonate side chain protecting groups was $>10^5$ and relative to the anchoring benzyl ester bond to the resin support it was 6×10^3. This is a marked improvement over the selectivity of the conventional 50% trifluoroacetic acid in CH_2Cl_2 deprotecting agent and significantly reduces the accumulated byproducts resulting from losses of benzylic groups. The cleavage of the *tert*-butyl urethane was first order in Me_3SiCl and second order in C_6H_5OH. The preferred reagent is 1 M Me_3SiCl–3 M C_6H_5OH–CH_2Cl_2 and the deprotection time is 20 min ($t_{1/2}$ = 1.8 min for Boc-Val-OCH_2-resin). Evidence for the mechanism of the reaction was deduced. Several peptides, including Leu-enkephalin, [valine-5]-angiotensin II, and glucagon were successfully synthesized in high yields and excellent purity by the stepwise solid phase method using this new reagent.

A general objective for the differential acid deprotection in peptide synthesis is to increase the chemoselectivity between the N^{α}-amino group and side chain protecting groups.[1a,b] The conventional strategy in solid phase peptide synthesis makes use of *tert*-butyloxycarbonyl (Boc) for N^{α} protection, which is selectively removed by trifluoroacetic acid in the presence of benzyl side chain protecting groups.[2] The loss of these benzyl esters, ethers, and urethanes during each acidic deprotection cycle is usually tolerable (0.02–0.1%), but the loss of peptide chains from the resin by cleavage of the benzyl ester anchoring bond derived from chloromethyl-resin (0.7–2% per cycle) is higher than desired, particularly for the synthesis of long peptides.[3] The development of more acid-stable protecting groups and resin linkages offers one way to overcome this problem.[4a,b] We wish to describe here an alternative method to minimize these losses. It involves a mild and more selective organosilane reagent that is especially suitable for the repetitive deprotection steps of solid phase synthesis.

The potential use of organosilicon derivatives as deprotecting reagents in peptide synthesis has not been explored extensively despite the reports on the cleavage of Boc groups by trimethylsilyl perchlorate[5] and trimethylsilyl trifluoromethanesulfonate[6] and the use of Me_3SiI and Me_3SiCl + NaI for the cleavage of ethers and esters.[7a–c] Since the strong complexing nature of organosilicon derivatives toward carbamates would produce an effect analogous to protonation by acid, the possibility of deprotection of the Boc group under neutral or mildly acidic conditions with such reagents is particularly appealing. In general, deprotection of primary alkyl esters and carbamates by trimethylsilyl reagents requires an aprotic solvent[5–7] and, in the presence of a strongly nucleophilic counterion such as iodide, results in a rapid S_N2 cleavage mechanism. For that reason the selectivity for Boc relative to Bzl groups is not high.[7c] In addition, silane derivatives containing a very good nonnucleophilic leaving group such as $Me_3SiOClO_3$ or $Me_3SiOSO_2CF_3$ are powerful silylating agents.[5–7]

We have therefore investigated the removal of Boc groups by reagents containing chlorotrimethylsilane under conditions that proceed by an S_N1 mechanism and that do not result in silylated byproducts. Since the reaction did not occur readily in aprotic solvents such as dioxane, dichloromethane, or toluene, we decided to examine protic and slightly acidic compounds.[8a,b] It was found that the addition of phenol not only enhanced the reactivity of Me_3SiCl in chlorinated or aromatic solvents, but also improved the selectivity of removal of the N^{α}-Boc group in the presence of benzyl-derived protecting groups (eq 1). We have now studied the kinetics and scope of Me_3SiCl–phenol as a deprotecting agent in solid phase peptide synthesis.

† Abbreviations: Boc, *tert*-butyloxycarbonyl; Bpoc, 4-biphenylylisopropyloxycarbonyl; Bzl, benzyl; CTMS, chlorotrimethylsilane; Dnp, 2,4-dinitrophenyl; Tfa, trifluoroacetyl; Tos, 4-toluenesulfonyl; Z, benzyloxycarbonyl.

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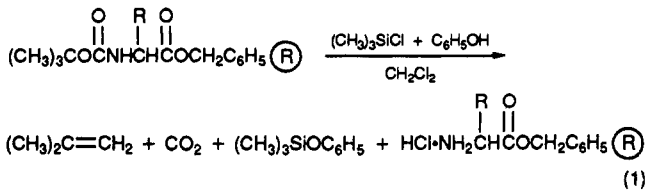

(1)

Results

A. Kinetic Studies of Deprotection of the N^{α}-*tert*-Butyloxycarbonyl Group. Samples of Boc-Val-OCH_2-resin derived from esterification of Boc-Val-OH to chloromethyl-copoly(styrene-1%-divinylbenzene) resin[9a,b] were treated under various conditions with Me_3SiCl, with or without phenol, in CH_2Cl_2. To reduce the possibility of silylation of the newly liberated amine, the HCl·Val-OCH_2-resin products were washed successively with phenol, 2 M phenol–2 M H_2O–CH_2Cl_2, phenol, and CH_2Cl_2 before neutralization with 5% diisopropylethylamine–CH_2Cl_2. Washes with phenol in tetrahydrofuran or dioxane were also found to be effective. The extent of deprotection was determined by the quantitative ninhydrin test,[10] which measured the liberated α-amino group. Two investigators measured the deprotection rate of two samples each and the mean deviation of k_1 (Figures 1 and 2) was less than 5%.

The rate of deprotection of the Boc group from Boc-Val-OCH_2-resin at 22 °C by a large excess of 1 M Me_3SiCl in CH_2Cl_2 was found to obey pseudo-first-order kinetics, $k_1 = 1.5 \times 10^{-5}$ s^{-1}, but required at least 48 h for complete removal of the Boc group and was too slow to be useful. $MeSiCl_3$ was no better and was inconvenient to handle. Solvents such as tetrahydrofuran or dioxane were less effective than CH_2Cl_2. However, the addition of phenol (1 M final concentration) greatly accelerated the reaction ($k_1 = 6.6 \times 10^{-4}$ s^{-1}; $t_{1/2} = 17.5$ min and the reaction went to completion within 1 h. In the absence of Me_3SiCl, the Boc group was completely stable for 7 days in 1 M phenol–CH_2Cl_2.

The deprotection was complete with as little as 2 equiv of Me_3SiCl (0.008 M) in 1 M phenol–CH_2Cl_2 in solid phase deprotection (Table I), but the rate was not satisfactory until the concentration of Me_3SiCl reached 1 M. In all subsequent experiments at least 20 molar excess of 1 M Me_3SiCl was used. In the normal solid phase synthesis of a peptide on 1 g of resin (0.5 mmol/g), 10 mL of the 1 M Me_3SiCl–1 M C_6H_5OH–CH_2Cl_2 reagent will provide a 20-fold molar excess of Me_3SiCl with respect to the N^{α}-Boc protecting group.

To gain a better insight into the mechanism of the removal of the Boc group from Boc-Val-OH by the Me_3SiCl–phenol reagent, the dependency of the rate on the concentrations of Me_3SiCl and phenol was measured. The reaction was clearly first order in Me_3SiCl (Figure 1). It was found, however, to be second order in phenol (Figure 2). In the absence of phenol, the observed rate of appearance of valine was slow ($k_1 = 1.5 \times 10^{-5}$ s^{-1}) but it increased smoothly as the square of the phenol concentration from 5.2×10^{-4} s^{-1} at 0.5 M to 150×10^{-4} s^{-1} at 3 M phenol.

B. Relative Stabilities of *tert*-Butyl Ethers, Esters, and Urethanes to the Me_3SiCl–Phenol Reagent. The relative rates of cleavage of four representative *tert*-butyl protecting groups in CH_2Cl_2 solution were measured simultaneously by mixing Bpoc-Ser(Bu^t), Bpoc-Asp(OBu^t), Bpoc-Tyr(Bu^t), and Boc-Leu, together with Bpoc-Ala, as an internal standard, in the 1 M Me_3SiCl–1 M phenol–CH_2Cl_2 reagent and analyzing aliquots for free amino acid at various time intervals. The Bpoc group was removed within seconds and did not interfere with the analysis of *tert*-butyl group removal. The reaction was quenched by addition of buffer, and the samples were

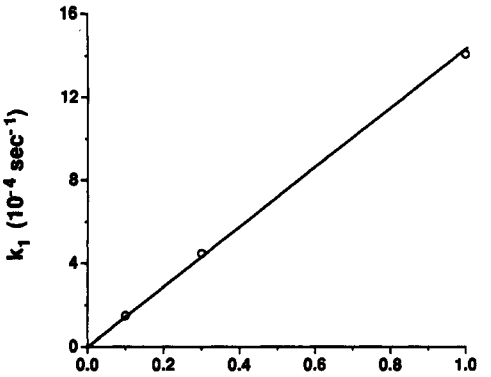

Figure 1. Dependence of the rate of deprotection of Boc-Val by Me_3SiCl–phenol–CH_2Cl_2 on the concentration of Me_3SiCl. The concentration of phenol was 1 M throughout and the ratio of phenol to Boc-Val was 20:1.

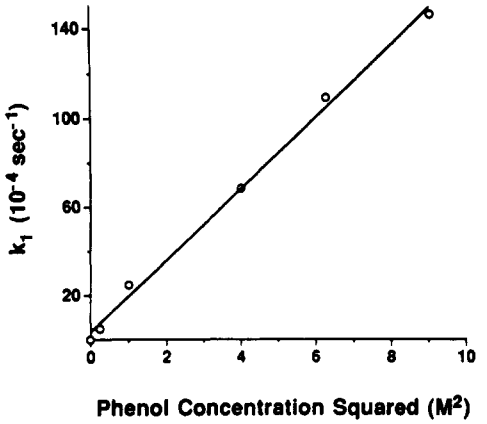

Figure 2. Dependence of the rate of deprotection of Boc-Val by Me_3SiCl–phenol–CH_2Cl_2 on the concentration of phenol. The concentration of Me_3SiCl was 1 M throughout and the ratio of Me_3SiCl to Boc-Val was 10:1.

Table I. Effect in 1 M C_6H_5OH–CH_2Cl_2 of Me_3SiCl Concentration and Molar Excess on Rate and Extent of Boc Removal

	Boc deprotection (%)[a] at Me_3SiCl concentration (M) and mole ratio relative to Boc[b]				
time (h)	0.004 (1:1)	0.008 (2:1)	0.016 (4:1)	0.08 (20:1)	1.0 (20:1)
0.25	–	–	4.5	~5	47.4
0.5	–	–	8.3	~10	70.8
1	–	–	16.4	~20	100
2	–	33.4	38.8		100
4	40.9	64.0	62.5		
6	44.4	78.0	76.8		
24	45.9	100	100		

[a] Determined by ninhydrin analysis. [b] Boc-Val-OCH_2-resin; 179 mg (0.057 mmol) was suspended in 15 ml of 1 *M* phenol in CH_2Cl_2 containing increasing amounts of Me_3SiCl.

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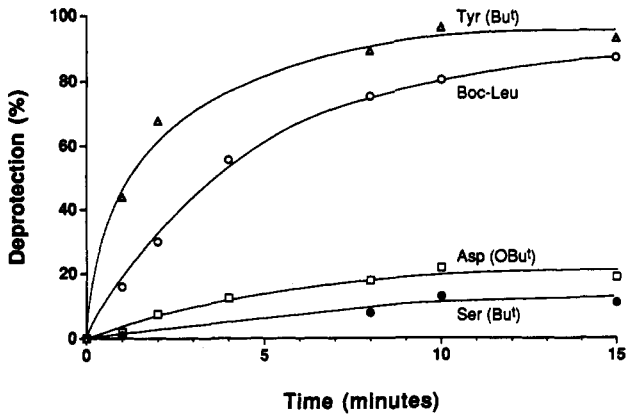

Figure 3. Deprotection of *tert*-butyl groups by 1 M Me_3SiCl–1 M phenol–CH_2Cl_2. The free amino acids were derivatized with *o*-phthalaldehyde and analyzed by fluorescence on a C_{18} reverse-phase HPLC column.

Table II. Stability of Benzyl Side Chains to the 1 *M* Me_3SiCl–Phenol–CH_2Cl_2 Reagents after 120 h at 22 °C

	side chain deprotection (%)				
	1 M phenol		3 M phenol		50% TFA/CH_2Cl_2
sample	120 h[a]	per 1-h cycle[b]	120 h	per 20-min cycle	per 20-min cycle
Boc-Tyr(BrZ)-OH	0.27	0.0022	0.25	0.0007	0.008
Boc-Glu(OBzl)-OH	0.14	0.0017	0.19	0.0005	0.009
Boc-Ser(Bzl)-OH	0.5	0.0042	0.7	0.0022	0.013
Z-Ala-OH	1.2	0.010	3.6	0.01	0.15

[a] Free, unprotected amino acid after 120 h of continuous treatment. Quantitated on the amino acid analyzer and corrected for very low levels of free amino acid in the starting sample. See Experimental Section for details. [b] The cycle time for the 1 M phenol reagent was 1 h and for the 3 M phenol it was 20 min. These are times for 100% removal of the Boc group under the two conditions.

converted to fluorescent derivatives with *o*-phthalaldehyde and quantitated on a C_{18} reverse-phase HPLC column[11] (Figure 3). The pseudo-first-order rate constants, k_1, were 96×10^{-4} s^{-1} for tyrosine *tert*-butyl ether, 31×10^{-4} s^{-1} for N^{α}-(*tert*-butyloxycarbonyl)leucine, 3.6×10^{-4} s^{-1} for aspartic acid β-*tert*-butyl ester, and 0.88×10^{-4} s^{-1} for serine *tert*-butyl ether. Thus, the phenolic ether was removed most rapidly, followed by the urethane, the ester, and the aliphatic ether. The corresponding half-lives were 1.2, 3.7, 32, and 131 min, respectively. Note that these rates are for amino acid derivatives in solution and are faster than those for resin-bound amino acids.

C. Stability of Benzylic Side Chain Protecting Groups and the Benzyl Ester Linkage to the Resin Support. The stabilities of four representative benzylic side chain protecting groups in Tyr(BrZ), Glu(OBzl), Ser(Bzl), and Z-Ala were determined following treatment for 5 days at room temperature with the 1 M Me_3SiCl–1 M C_6H_5OH–CH_2Cl_2 reagent (Table II). The deprotected amino acids were measured by the *o*-phthalaldehyde method. The benzyl ester, ether, and carbonate groups were extremely stable under these conditions. After 120-h treatment only 0.1–0.5% free amino acids were found, indicating average losses per 1-h deprotection cycle of 0.004% or less in the 1 M phenol reagent and 0.002% or less per 20-min cycle in 3 M phenol. The urethane group in N^{α}-Z-Ala was more labile, with a loss of 0.01%/cycle.

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Table III. Stability of the Benzyl Ester Anchoring Bond to the Resin in the 1 M Me_3SiCl–1 M C_6H_5OH–CH_2Cl_2 Reagent after 168 h at 22 °C

	amino acid lost from resin (%)		
sample	by ninhydrin analysis of resin after 168 h	by aminjo acid analysis of filtrate after 168 h	av loss per 1-h cycle
Boc-Val-resin	21.6	28.0	0.15
Boc-Leu-resin	14.2	16.1	0.09
Boc-Phe-resin	6.6	7.1	0.04

These low values are in direct contrast with those for samples treated in 50% TFA/CH_2Cl_2, where an average of >10% cleavage of these groups was observed in 120 h. This is 15–30 fold greater than with the new reagent.

The losses of amino acids anchored as benzyl esters[12] to the conventional styrene–divinylbenzene resin, derived from chloromethyl-resin, were determined by measuring both the loss of amino acid into the filtrate and the amount of free amine remaining on the resin by ninhydrin analysis (Table III). The data showed that the losses in the Me_3SiCl–phenol reagent for Val, Leu, and Phe were 0.17, 0.01 and, 0.04%/cycle, respectively. The selectivity of deprotection of N^{α}-Boc relative to the benzyl ester bond to the resin was 5.7×10^3. Relative to N^{α}-Z it was 1.1×10^5, and relative to side chain benzyl esters or benzyl ethers it was $(2.8–9.7) \times 10^5$. Even more importantly, when these values were compared with the corresponding data from 50% TFA/CH_2Cl_2 deprotection experiments it was found that the selectivity for Boc removal was 5–10 times better with the Me_3SiCl–phenol reagent.

D. Evidence That the Activity of the Me_3SiCl–Phenol Reagent is Not Due to HCl. It is well known that Boc groups can be removed from peptides within 10 min by 1 M HCl in HOAc[13] or 4 M HCl in dioxane,[14] and the possibility that the 1 M Me_3SiCl–1 M phenol reagent might simply be a source of free HCl (eq 2) had to be considered. All the evidence now indicates that this is

$$Me_3SiCl + C_6H_5OH \xrightarrow{CH_2Cl_2} Me_3SiOC_6H_5 + HCl \quad (2)$$

not the case, and in addition it eliminates the possibility that traces of HCl may significantly accelerate the reaction.

When the reactions were run in the presence of 0.1 or 1 M triethylamine, it was found that the deprotection was slowed somewhat but was not prevented. Similar conclusions were reached by Jung and Lyster,[7b] and Ho and Olah,[7c] who had previously shown that the cleavage of ethers by Me_3SiI was not due to the presence of HI. In addition, Me_3SiI has been used for the transesterification of esters under mild and neutral conditions and it was shown that the reaction was not due to HI generated in the reaction mixture.[7d]

Hammett indicators showed that the pK_a of 4 M HCl in dioxane is about −2, whereas the pK_a of 1 M phenol in CH_2Cl_2 is ~10. Immediately after mixing equal volumes of 2 M Me_3SiCl and 2 M phenol in CH_2Cl_2, the acidity immediately increased to pK_a ~2, but did not continue to increase with time, suggesting that HCl was not being produced according to eq 2, but that a complex between Me_3SiCl and phenol was responsible for the pK_a shift.

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Table IV. Estimation of HCl Concentration by Conductivity[a]

dioxane (ml)	4 M HCl–dioxane (ml)	added HCl M	time (min)	conductivity (μS)
1	0	0	0	1.8 ± 0.02
0.9	0.1	0.05	0	4.7 ± 0.2
0.7	0.3	0.15	0	12.1 ± 0.5
0.4	0.6	0.30	0	22.4 ± 0.8
0	1.0	0.50	0	42.9 ± 1.7
1	0	0	10	1.8 ± 0.02
1	0	0	30	2.7 ± 0.2
1	0	0	60	2.0 ± 0.2
1	0	0	240	2.2 ± 0.2

[a] 2 M Me_3SiCl–CH_2Cl_2 (2 ml) and 2 M phenol–CH_2Cl_2 (2 mL) were rapidly mixed, and after the indicated time at 22 °C were added to a premixed solution containing 3 mL of CH_3CN and 1 mL of dioxane containing 0–4 M HCl. The conductivity of each preparation was measured immediately after mixing on a Radiometer conductivity meter CDM 3.

The level of free HCl in an anhydrous mixture of Me_3SiCl and phenol could be estimated by conductivity measurements (Table IV). The conductivity of freshly prepared 1 M Me_3SiCl–1 M C_6H_5OH in CH_2Cl_2 (measured in the presence of 27.5% CH_3CN and 12.5% dioxane) was only 1.8 μS and did not increase significantly with time. Comparison with a standard curve prepared by addition of increasing amounts of anhydrous HCl showed that the reagent produced a concentration of less than 0.005 M HCl after 4 h standing at 22 °C. This is too low for the observed deprotection rate of the Boc group to be attributed to the generation of HCl. For example, the pseudo-first-order rate constant for removal of the Boc group by 0.016 M HCl plus 1 M phenol in CH_2Cl_2 was 6.8×10^{-5} s^{-1}, and by 1 M HCl plus 1 M C_6H_5OH in CH_2Cl_2 it was 6.0×10^{-3} s^{-1}. Therefore, if the observed deprotection rate (3.1×10^{-3} s^{-1}) for Boc-Val in 1 M Me_3SiCl plus 1 M C_6H_5OH were to be attributed entirely to the generation of HCl, it would require the occurrence of ~50% reaction of the large excess of Me_3SiCl and phenol within minutes in order to give the necessary concentration of HCl, and that was not found.

E. The Preparation of Anhydrous, HCl-Free Reagent. The water content of the reagent grade crystalline phenol was nominally 0.5%. To measure the water content and to obtain a water-free reagent a gravimetric method based on a reaction with anhydrous Na_2CO_3 was developed.

$$C_6H_5OH + H_2O + Na_2CO_3 \xrightarrow{CH_2Cl_2} C_6H_5OH + Na_2CO_3 \cdot H_2O \quad (3)$$

A 4 M solution of phenol in CH_2Cl_2 was prepared and a weighed 2-fold excess of anhydrous Na_2CO_3 was added. The mixture was stirred 20 h at room temperature, centrifuged, and washed several times with dry ether, and the residue was dried for 2 h in vacuo at 25 °C. A weighed sample was combusted, and from the carbon content the percent water was calculated to be 0.49%, in good agreement with the expected value. The resulting dry phenol gave a negative test for water when treated again with Na_2CO_3. The presence of this small amount, 0.49%, of water in the 1 M Me_3SiCl–1 M C_6H_5OH–CH_2Cl_2 reagent did not make a significant change in the rate of deprotection of Boc-Val-OCH_2-resin. The observed half-lives were 17.5 min for the 0.49% H_2O reagent and 19.5 min for the anhydrous reagent.

The HCl content of a freshly prepared solution of 4 M Me_3SiCl in CH_2Cl_2 was determined by a related procedure.

$$HCl + Na_2CO_3 \xrightarrow[Me_3SiCl]{CH_2Cl_2} NaCl + {}^1/_2CO_2 + {}^1/_2Na_2CO_3 \cdot H_2O \quad (4)$$

A weighed excess of anhydrous Na_2CO_3 was added to the Me_3SiCl solution, and the suspension was stirred for 20 h at room temperature. After centrifuging, washing thoroughly with ether, and drying, the Cl content of a weighed sample was determined gravimetrically as AgCl. The measured Cl was equivalent to 0.35% of the Me_3SiCl, which means that only 0.0035 M HCl in the final 1 M Me_3SiCl–1 M phenol reagent would be derived from this source. There was no increase in Cl content with time, showing that Me_3SiCl and Na_2CO_3 do not react significantly under these conditions. When a 0.2 M HCl solution in CH_2Cl_2 was analyzed, the recovery of Cl was 0.195 M (97.5%), indicating that the analytical method was satisfactory. The deprotection rate constants k_1 with HCl-free reagent or reagent containing 0.0035 M HCl were indistinguishable.

Me_3SiCl does react readily with sodium bicarbonate in CH_2Cl_2 according to eq 5.

$$Me_3SiCl + NaHCO_3 \xrightarrow{CH_2Cl_2} Me_3SiOH + NaCl + CO_2 \quad (5)$$

Two 1 M Me_3SiCl–1 M phenol reagents were prepared by mixing equal volumes of 2 M Me_3SiCl in CH_2Cl_2 and 2 M phenol in CH_2Cl_2. In one reagent the crystalline phenol containing 0.49% H_2O was used and for the second reagent water-free phenol, prepared by the Na_2CO_3 procedure, was used. Immediately after mixing, Na_2CO_3 was added to each reagent and after 20 h stirring they were centrifuged washed, dried, and analyzed for Cl. They both indicated the presence of 0.056 M HCl. Similar solutions were prepared, but without the Na_2CO_3 treatment, and, within 5 min, were analyzed by NMR. Each reagent contained 0.06 M $Me_3SiOC_6H_5$. In addition, Me_3SiOH was present in the H_2O-containing solution, but not in the H_2O-free solution. We conclude from these experiments that a low level of H_2O in the reagent does promote, within minutes, the reaction:

$$2Me_3SiCl + H_2O \rightarrow (Me_3Si)_2O + 2HCl \quad (6)$$

but has no effect on the reaction in eq 2. It appears that the formation of 6% of $Me_3SiOC_6H_5$ and HCl is very fast and does not increase with time and is probably a consequence of an impurity in the reagent. We believe pure Me_3SiCl and phenol do not react significantly in CH_2Cl_2 at room temperature within 20 h.

F. Removal of Excess Reactants after Deprotection of the Boc Group by the Me_3SiCl–Phenol Reagent. One of the practical difficulties encountered in developing the new reagent was the removal of excess reagents after the reaction. Washing with phenol in CH_2Cl_2 and then CH_2Cl_2 was not very effective, but it was found that essentially complete removal was achieved by washing with 10% phenol in glacial acetic acid. The danger of carryover of small amounts of HOAc was great, however, and peptide chain termination by acetylation during the next coupling step was a potential hazard that was actually observed. This problem was overcome by omitting the HOAc and adding a small amount of water. This wash solution was 2 M phenol–2 M H_2O–CH_2Cl_2. An alternative procedure was use of 4% H_2O in dimethylformamide. These wash solutions effectively removed Me_3SiCl and its hydrolysis products.

G. Evidence for Formation of Phenoxytrimethylsilane as a Byproduct of the Deprotection of Boc-Amino Acids by Me_3SiCl and Phenol. Although Me_3SiCl and phenol do not spontaneously react at an appreciable rate in CH_2Cl_2 at room temperature, they do react in the presence of a Boc-amino acid to give phenoxytrimethylsilane. The product could be separated by gas chromatography and identified and quantitated by mass spectrometry or NMR.

Mass Spectrometry. Four solutions were prepared and, after removal of CH_2Cl_2 and Me_3SiCl by a stream of N_2, were injected onto a gas chromatographic column in line with an electron impact mass spectrometer: (1) equal volumes of 2 M C_6H_5OH in CH_2Cl_2 and 2 M Me_3SiCl in CH_2Cl_2 were mixed and immediately flushed and injected; (2) 2 M C_6H_5–CH_2Cl_2 and 2 M Me_3SiCl–CH_2Cl_2 were mixed and after standing for 30 min at 25 °C were flushed and injected; (3) 2 M phenol in CH_2Cl_2 and 2 M Me_3SiCl in CH_2Cl_2 were mixed and refluxed for 7 h, cooled, flushed, and injected; (4) equal volumes of 2 M phenol in CH_2Cl_2 and 2 M Me_3SiCl in CH_2Cl_2 were mixed and Boc-valine was added to 0.1 M. After 30 min at 25 °C the mixture was flushed and injected. The GC eluates were monitored by an ionization detector.

The GC/MS of solutions 1 and 2 showed phenol, but only low levels of $Me_3SiOC_6H_5$ (ratio ~1:0.06) which did not increase with time. Solution 3 showed peaks for phenol and $Me_3SiOC_6H_5$ in a ratio of ~1:2 (~67% reaction). Reaction mixture 4 gave peaks corresponding to phenol and $Me_3SiOC_6H_5$ in a ratio of 1:0.16. After correcting for the 0.06 M reagent blank, the $Me_3SiOC_6H_5$ produced in the deprotection reaction was approximately equivalent (0.1 M) to the starting Boc-valine, indicating that it was a stoichiometric product of the deprotection reaction.

Nuclear Magnetic Resonance. Solutions similar to the above solutions 1–4 were prepared in CD_2Cl_2 and the methyl proton resonances were followed with time at 25 °C in the 360-MHz spectrometer. The singlet for $(CH_3)_3SiCl$ at 0.410 ppm relative to TMS was unchanged after 1 h in solutions 1 and 2. In addition, a small, well-resolved, new peak at 0.250 ppm corresponding to $Me_3SiOC_6H_5$ appeared at a concentration of 0.06 M. It did not change during the 1-h observation. Solution 3 showed the 0.250 ppm resonance at a concentration of ~0.7 M. In reaction mixture 4 the 0.250 ppm peak increased with time and the *tert*-butyl protons of Boc-Val decreased. The final concentration of $Me_3SiOC_6H_5$ was 0.16 M, giving an increase of 0.10 M, again indicating that the product was formed stoichiometrically as the Boc group was removed. Under these conditions the HCl–Val precipitated and was not seen.

The deprotection reaction was repeated using 0.15 M Boc-Val-OMe, 0.18 M chlorotrimethylsilane and 0.5 M phenol and the rate was monitored with proton NMR (Figure 4). Again it can be seen that the $(CH_3)_3SiOC_6H_5$ peak slowly increased while the *tert*-butyl protons of the Boc-Val-OMe decreased. In addition, the two doublets for the protons of the two Boc-valine methyl groups decreased with time, while the overlapping doublets of the protonated valine methyl groups appeared. To determine rate constants, this deprotection experiment was repeated using 0.1 M Boc-Val-OMe + 1 M Me_3SiCl + 1 M C_6H_5OH (Figure 5). The pseudo-first-order rate constant for the formation of phenoxytrimethylsilane,

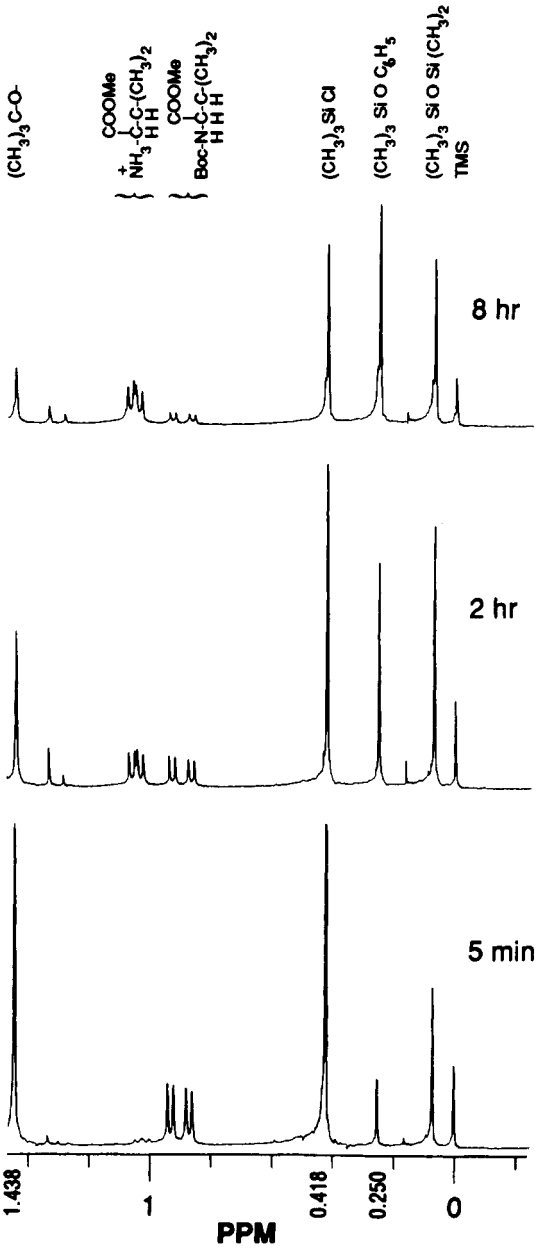

Figure 4. Proton NMR spectra of the deprotection reaction mixture of 0.15 M Boc-Val-OMe + 0.18 M chlorotrimethylsilane + 0.50 M phenol in CD_2Cl_2.

measured at 0.250 ppm, was 0.036 min^{-1}, and k, for the decrease in *tert*-butyl protons at 1.438 ppm, was 0.037 min^{-1}.

H. Search for a Silyl Urethane Intermediate in the Deprotection Reaction. The suggestion has been made that the first step in the reaction between Me_3SiCl and Boc-amino acid ester might be a coordination between Si and the carbonyl oxygen of the urethane, followed by rapid loss of *tert*-butyl carbonium ion to give the silyl urethane derivative (eq 7), which would subsequently be

$$Me_3SiCl + Me_3COCONHCHRCOOR' \rightarrow Me_3SiOCONHCHRCOOR' + (CH_3)_3C^+ \quad (7)$$

decomposed by phenol in a rate-limiting step to give the final products. ^1H NMR was not suitable to study the reaction because the chemical shifts of the methyl protons in $Me_3SiOC_6H_5$ were not resolved from those of Me_3Si-

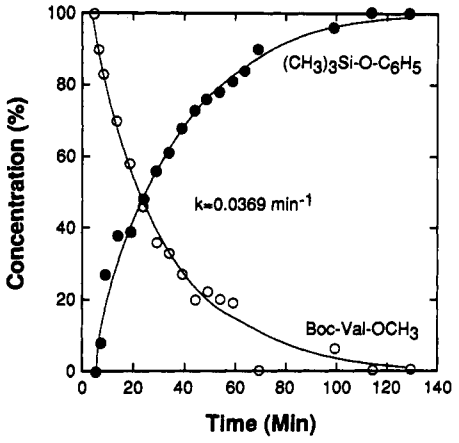

Figure 5. Kinetics of the 1 M Me_3SiCl–1 M phenol–0.1 M Boc-Val-OMe–CH_2Cl_2 reaction. The appearance of phenoxytrimethylsilane (●) was measured by following the increase in peak size of the nine methyl protons at 0.250 ppm downfield from TMS. The decrease in Boc-Val-OMe was followed (○) by the decrease in the *tert*-butyl protons at 1.438 ppm.

OCONHCHRCOOR′. However, ^{29}Si NMR provides good resolution of the relevant Si compounds. $(CH_3)_3Si$-$OCON(CH_3)_2$[15] was used as a reference silyl urethane. The ^{29}Si chemical shift of this compound was 19.858 ppm downfield from TMS, whereas the shift for phenoxytrimethylsilane was 18.660 ppm, Me_3SiCl was 31.245 ppm, and $(Me)_3SiOSiMe_3$ was 7.132 ppm. The deprotection of Boc-Val-OMe by 1 M phenol was followed by Si NMR, but no resonance corresponding to a silyl urethane intermediate could be detected. The only new Si product seen was $Me_3SiOC_6H_5$. When the model trimethylsilyl *N,N*-dimethylcarbamate was examined by silicon NMR, it was found that the 19.858 ppm resonance was stable and remained unchanged in 1 M phenol for 20 h. The phenoxysilane would have been resolved because its addition gave a second peak at 18.66 ppm. In the presence of 1 M phenol + 1 M Me_3SiCl the model urethane decomposed completely before the first time point could be taken. This explains why, during the deprotection of Boc-Val-OMe by 1 M Me_3SiCl–1M phenol–CH_2Cl_2, we were unable to detect any silyl urethane intermediate.

An alternate mechanism for the deprotection reaction can be envisioned, which could be tested by use of Boc-[^{15}N]valine methyl ester. Thus, the formation of an intermediate was considered in which the proton on ^{15}N (8.15 ppm) would be replaced by the Me_3Si group, which would then be removed by phenol to give the observed products. However, during 20 h no replacement of the

$$(CH_3)_3SiCl + Boc{-}Val{-}OCH_3 \xrightarrow{\text{phenol}}$$

$$(CH_3)_3COC(=O){-}N(Si(CH_3)_3){-}CH(R)COOCH_3 \xrightarrow{C_6H_5OH}$$

$$(CH_3)_2C{=}CH_2 + CO_2 + (CH_3)_3SiOC_6H_5 + Cl^-H_3N^+CHRCOOCH_3 \quad (8)$$

^{15}N proton was detected.

I. Synthesis of Test Peptides by Use of the Me_3SiCl–Phenol Reagent. 1. Leucyl-alanyl-glycyl-valine.[13] This tetrapeptide was synthesized by a dicyclohexylcarbodiimide protocol using standard manual solid phase procedures except that the removal of the N^{α}-Boc group at each cycle was by the new 1 M Me_3SiCl–3 M phenol–CH_2Cl_2 reagent and washing was with 4% H_2O–DMF. After deprotection and neutralization with 10% DIEA in DMF, couplings were with 3 equiv of Boc-amino acid and 3 equiv of DCC in CH_2Cl_2 for 1 h. Couplings were monitored by the quantitative ninhydrin reaction[10] and repeated if necessary. For this peptide only the leucine required a second coupling. The peptide-resin was cleaved with 90% HF–10% *p*-cresol, 0 °C, 1 h. The crude, cleaved peptide showed only one significant peak, at 4.2 min, in the analytical HPLC system (Figure 6a), and after preparative HPLC the product was homogeneous and showed the correct amino acid analysis.

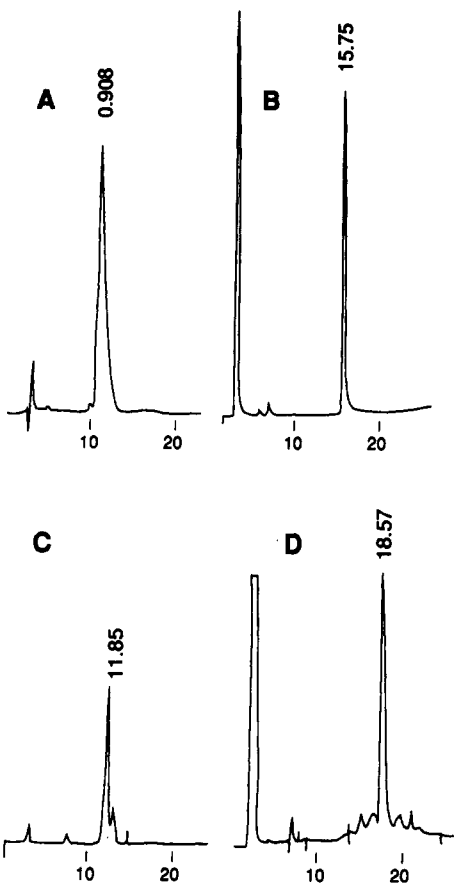

Figure 6. Reverse-phase HPLC on unpurified samples of (A) Leu-Ala-Gly-Val; (B) Leu-enkephalin; (C) [Val^5]angiotensin; (D) glucagon. The analytical column was a 5-μm, 0.46 × 25 cm Vydac 218TP54, run in a gradient of CH_3CN–H_2O containing 0.05% TFA, monitored at 210 nm.

Ion-exchange analysis of the crude cleaved peptide showed that LAGV was 99.7% of the total peptides. The single-deletion peptides AGV, LGV, and LAV were 0.2, 0.05, and 0.03%, respectively, indicating very low levels of deletions, which were comparable to previous results using 50% TFA deprotection of Boc-amino acids attached in benzyl ester linkage to the OCH_2-Pam-resin.[4a,b]

2. Leu-enkephalin.[16] The protected pentapeptide-resin, Boc-Tyr(BrZ)-Gly-Gly-Phe-Leu-OCH_2-resin was synthesized by the same procedure as just described for LAGV. The data are shown in Figure 6b. The results on this peptide were also quite satisfactory. The main peak represented over 90% of the total peptide, and after preparative HPLC the product was homogeneous by

(15) Knausz, D.; Meszticky, A.; Szakacs, L.; Csakvari, B. *J. Organomet. Chem.* **1983**, *256*, 11–21.

(16) Hughes, J.; Smith, T. W.; Kosterlitz, H. W.; Fothergill, L. A.; Morgan, B. A.; Morris, H. R. *Nature* **1975**, *258*, 577–579.

analytical HPLC. Furthermore, the purified product was indistinguishable from the product synthesized using 50% TFA deprotection.

3. [Valine-5]angiotensin II.[17] This octapeptide contains four trifunctional amino acids and is a better test of the new Me_3SiCl–phenol deprotecting reagent than the previous two shorter peptides. The protected peptide-resin was Boc-Asp(OBzl)-Arg(Tos)-Val-Tyr(BrZ)-Val-His(Dnp)-Pro-Phe-OCH_2-resin. The synthetic protocol was the same as for the other peptides except that Boc-Arg(Tos) and Boc-His(Dnp) were coupled by DCC in the presence of 3 equiv of 1-hydroxybenzotriazole, and the deprotection reagent was 1 M Me_3SiCl–3 M phenol in CH_2Cl_2. Washing for the removal of Me_3SiCl was with 2 M phenol–2 M H_2O–CH_2Cl_2.

Following assembly of the chain, the Dnp group was removed from histidine by treatment of the protected peptide-resin with 10 equiv of 0.2 M thiophenol in DMF for 1 h.[18] The yellow suspension was filtered and thoroughly washed, and the treatment was repeated. Only a trace of additional color was removed. The protected peptide-resin (350 mg) was cleaved and deprotected by treatment with the low-high HF procedure.[19] Washing with ether, extraction into 10% acetic acid, and lyophilization gave 110 mg of crude product. By analytical HPLC (Figure 6c) the content of angiotensin was ~82%. It was separated on a preparative reverse-phase C_{18}-silica HPLC column (2.5 × 25 cm) with a linear gradient of solution B into solution A (1.5 L each) (Figure 6c). Solution A contained 0.05% TFA in H_2O and solution B contained 75% H_2O, 25% CH_3CN, and 0.05% TFA. A narrow cut of peak 1 (1.20 L to 1.40 L) gave 60 mg of angiotensin (70% recovery), which had a good amino acid analysis and was homogeneous by analytical HPLC.

4. Glucagon. This 29-residue peptide was synthesized by the general procedure described for angiotensin II. The sequence of the protected peptide-resin is Boc-His(Dnp)-Ser(Bzl)-Glu-Gly-Thr(Bzl)-Phe-Thr(Bzl)-Ser(Bzl)-Asp(OBzl)-Tyr(Bzl)-Ser(Bzl)-Lys(ClZ)-Tyr(Bzl)-Leu-Asp(OBzl)-Ser(Bzl)-Arg(Tos)-Arg(Tos)-Ala-Gln-Asp(OBzl)-Phe-Val-Gln-Trp(CHO)-Leu-Met-Asn-Thr(Bzl)-OCH_2-copoly(styrene–1%-divinylbenzene). The stepwise deprotection of the N^{α}-Boc group was with the 1 M Me_3SiCl–3 M phenol–CH_2Cl_2 reagent (20 min). Washing to remove Me_3SiCl was with CH_2Cl_2 (2 × 1 min) and 4% H_2O–DMF (3 min). Following removal of the Dnp group[18] and cleavage and deprotection by the low-high HF method,[19] approximately 75% of the product was in the HPLC peak corresponding to natural glucagon (Figure 6d). Homogeneous glucagon with a good amino acid analysis was obtained after preparative HPLC.

Discussion

One of the major objectives of the development of a new silicon-containing reagent for removal of the N^{α}-Boc group was to enhance the chemical selectivity of the deprotection step during a long repetitive stepwise synthesis.

For that purpose we decided to examine a silane derivative that contained a poor leaving group of low nucleophilicity, and we selected Me_3SiCl. Since it did not react readily with the N^{α}-Boc group in aprotic solvents such as dioxane, tetrahydrofuran, toluene, or dichloromethane, we examined protic and slightly acidic solvents. Neither acetic acid nor propionic acid (1 M in CH_2Cl_2) was satisfactory in promoting the reaction, but phenol very markedly accelerated the deprotection rate.

The new Me_3SiCl–phenol reagent appears to offer an alternative approach to increased selectivity of removal of the Boc group relative to the simple benzyl ester anchoring bond. A pseudo-first-order rate constant of 6.6×10^{-4} s^{-1} ($t_{1/2}$ = 17.5 min) was found for the deprotection of Boc-Val-OCH_2-resin in 1 M Me_3SiCl–1 M phenol–CH_2Cl_2, which was 40 times faster than in Me_3SiCl–CH_2Cl_2 without phenol. Similarly, the rate of deprotection of Boc-Val in homogeneous solution (no resin) with 1 M Me_3SiCl–1 M C_5H_6OH–CH_2Cl_2 was accelerated 220-fold (k_1, 3.1×10^3 s^{-1}) relative to the rate of 1 M Me_3SiCl–CH_2Cl_2 without phenol. On the basis of these data, the deprotection condition selected was a 2-min prewash with the reagent followed by a 60-min treatment with a fresh aliquot of reagent. The results showed that in each synthetic cycle the removal of Boc was complete and the loss of peptide chain was less than 0.02%, which gave a selectivity ratio of about 5000. The ratios for simple side chain benzyl esters and ethers relative to the N^{α}-Boc group were between 10^5 and 10^6. Thus, the selectivity for removal of Boc vs benzyl groups was 5–10 times better than with TFA.

Since the kinetics showed that the deprotection rate depended on the square of the phenol concentration, the time required for removal of the Boc group in 3 M phenol is much shorter than in 1 M phenol. We now prefer to use 1 M Me_3SiCl–3 M phenol in CH_2Cl_2 for 20 min as our standard deprotection reagent.

Because anhydrous HCl in phenol or other solvents readily removes the Boc group, we were much concerned that the function of Me_3SiCl and phenol was simply to provide a source of HCl. However, it was shown that phenol and Me_3SiCl do not react appreciably at room temperature to produce HCl. The absence of a significant amount of HCl in the reagent was indicated by several different techniques, including conductivity, NMR, mass spectrometry, kinetic experiments, and a method based on reaction of HCl with Na_2CO_3 followed by a gravimetric AgCl analysis. In addition, the enhanced specificity of the reagent toward *tert*-butyl urethanes relative to *tert*-butyl ethers or esters or toward benzyl derivatives suggests that the cleavage reaction is not simply due to the presence of HCl, where the known selectivity is not as high.

Since CH_2Cl_2 is a better solvent for the reaction than dioxane or toluene and neither acetic acid nor propionic acid is a useful substitute for phenol, it appears that the role of phenol is not simply to increase the polarity of the solvent or to provide an acidic environment. Its ability to associate with the silane and to provide a proton source appears to be one of the important features of this reaction. A second important feature is based on the fact that silicon contains low-lying vacant 3d orbitals and, therefore, has a strong tendency to complex with amines,[20] halides,[21] and election-donating oxygen compounds. Complexes between Me_3SiCl and pyridine or triethylamine are known to cleave *N*-benzyloxycarbonyl amino acids,[20] while $Me_3SiOClO_3$[5] or $Me_3SiOSO_2CF_3$[6] containing good leaving

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(19) Tam, J. P.; Heath, W. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1983**, *105*, 6442–6455.
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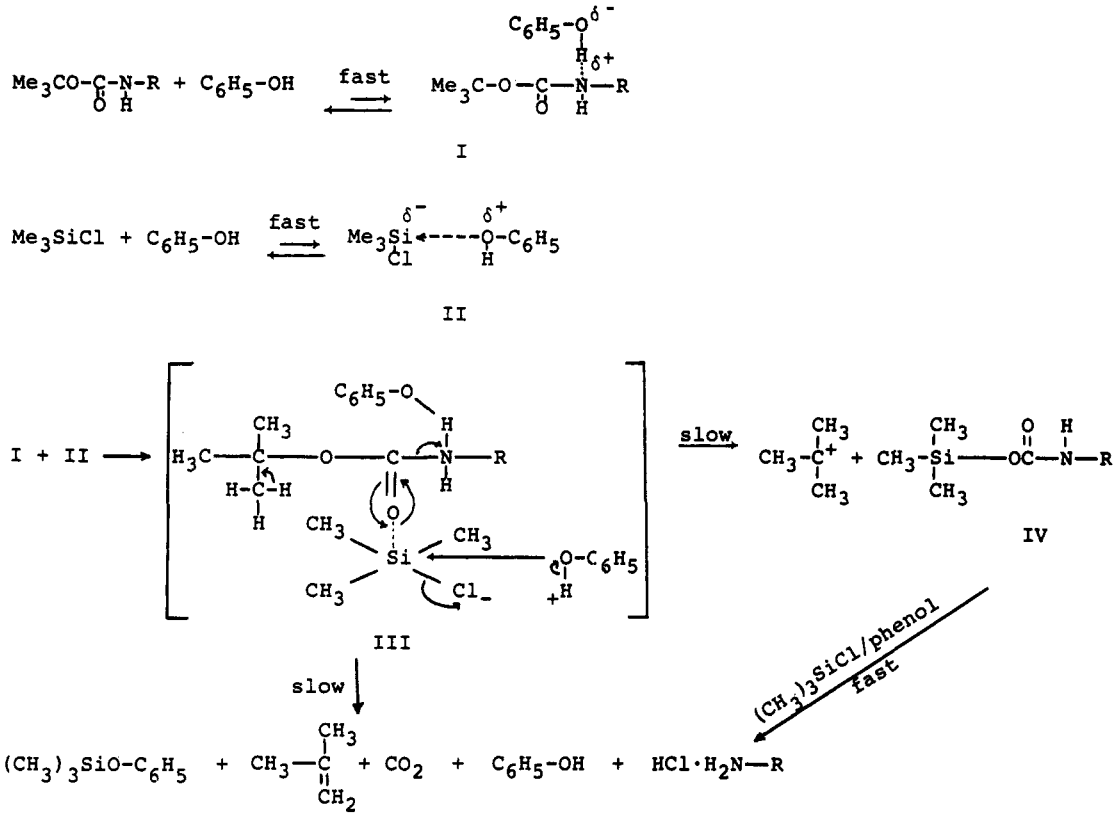
(21) Lott, R. S.; Chauhan, V. S.; Stammer, C. H. *J. Chem. Soc. Chem. Commun.* **1979**, 495–496.

Scheme I



groups can cleave Boc groups by S_N1 mechanisms. Me_3SiCl, containing a poor leaving group, is ineffective alone toward *tert*-butyl groups, but in the presence of NaI even *tert*-butyl ethers and esters are cleaved, via an S_N2 mechanism.[7]

On the basis of our current information, we can offer some tentative suggestions for the mechanism of the deprotection of the Boc group by the Me_3SiCl–phenol reagent. A reasonable route for the reaction would involve the rapid formation of an equilibrium complex between Me_3SiCl and phenol and another between the Boc-amino acid and phenol, followed by a rate-limiting bimolecular reaction between them (Scheme I). The intermediate **III** might decompose directly to products in one step or give rise to the silyl urethane **IV**, which would rapidly decompose in a second step. The reaction would be initiated by donation of electrons from the carbonyl oxygen to silicon and this intermediate would be stabilized by donation of an electron pair from nitrogen to the proton of phenol. The resulting positive carbonyl carbon would promote the dissociation of the *tert*-butyl carbonium ion. This would be followed by loss of CO_2 and generation of phenoxytrimethylsilane from the second equivalent of phenol. The alternative to this one-step bimolecular reaction might be a two-step sequence in which a covalent silyl urethane **IV** is formed, with release of the *tert*-butyl ion. The decomposition of the silyl urethane, if it is actually formed, would be very fast. Whether one or two distinct steps are involved, the rate-determining step would be first order in Me_3SiCl and second order in phenol as observed. There is proton NMR evidence for the generation of both **I** and **II** and the data from decomposition of trimethylsilyl *N,N*-dimethylcarbamate by Me_3SiCl and phenol show it to be very rapid, but the NMR experiments have not yet clearly distinguished between the one and two step mechanisms.

The Me_3SiCl–phenol deprotection reagent was tested on several small peptides that contained a number of benzyl-based side chain protecting groups and were attached to the polystyrene support by a simple benzyl ester. This avoided the need for more complicated anchoring bonds. The test peptides included Leu-Ala-Gly-Val, Leu-enkephalin, [Val^5]angiotensin, and glucagon. In each case quite satisfactory results were obtained. The crude cleaved peptide mixture, as determined by HPLC, contained greater than 75% of the desired product, and homogeneous peptide could be obtained by simple chromatographic procedures. The reagent provides a novel approach to the design of more selective deprotection procedures. It is expected to minimize losses of side chain protecting groups, and the accompanying side reactions, and also to decrease losses of peptide chains from the resin during the synthesis of long and complex peptides.

Experimental Section

Materials. Chlorotrimethylsilane was purchased from Petrarch, Inc. (packaged under nitrogen) and from Fluka Chemical Co. (purity >99%). The compound is a colorless liquid, bp 57.6 °C. After opening, it was stabilized against moisture by addition of calcium hydride. Caution! Chlorotrimethylsilane is a cancer suspect agent and should be handled in a well-ventilated hood. Methyltrichlorosilane (Petrarch) (bp 66–67 °C) was handled similarly. Phenol (crystalline, reagent ACS grade) was obtained from Fisher Scientific and was stored in a brown bottle in a desiccator at room temperature.

Dichloromethane was distilled from Na_2CO_3. Trifluoroacetic acid (Halocarbon) contained <0.05% H_2O and no measurable anhydride. Diisopropylethylamine (Aldrich Chemical Co.) was distilled from CaH_2, bp 129 °C. Dimethylformamide, acetonitrile, dioxane, and glacial acetic acid were reagent grade and used without further purification. Anhydrous HF (Matheson) was handled in a fluorocarbon apparatus (Toho, Osaka). Anhydrous 4 N HCl in dioxane was obtained in sealed ampoules from Aldrich.

Boc-amino acids were from Protein Research Foundation, Osaka. Chloromethylated styrene–divinylbenzene resin beads, 1% crosslinked, were from Lab Systems, Inc. (San Mateo, CA). Trimethylsilyl *N,N*-dimethylcarbamate[15] was purchased from Fluka.

Preparation of Pure Phenoxytrimethylsilane. According to Langer et al.,[22] a mixture of 7.48 g phenol and 11.8 g of CTMS was refluxed for 7 h, and then after addition of 20 mL of CH_2Cl_2 the reflux was continued an additional 4 h. The solvent was removed in a rotary evaporator and the residue distilled at 0.68 bar. The forerun fraction up to 134 °C analyzed for C, 69.73; H, 7.45. The second fraction of 0.68 bar, bp 134–136 °C was the pure product. Anal. Calcd for $C_9H_{21}SiO$: C, 65.06, H, 8.43, found C, 65.20; H, 8.46. GC-MS calcd and found 166.081.

^{29}Si NMR of the 1 M $(CH_3)_3SiOC(O)N(CH_3)_2$–1 M Phenol–CD_2Cl_2 Solution. Phenol (55 mg) was dissolved with light warming in 0.4 mL of CD_2Cl_2, and 10.1 mg of $(CH_3)_3SiOC(O)N(CH_3)_2$ was added. The mixture was transferred to a 500-MHz NMR spectrometer and ^{29}Si NMR spectra were recorded at 5, 10, 15, 20, 30, and 60 min. The peak area at 19.858 ppm remained unchanged during this time. When a small amount of the $Me_3SiOC_6H_5$ was added, an additional peak appeared at 18,660 ppm.

Chlorotrimethylsilane–Phenol–CH_2Cl_2 Reagents. A 2 M stock solution of Me_3SiCl was prepared by diluting 25.4 mL of Me_3SiCl to 100 mL with CH_2Cl_2. A 2 M stock solution of phenol was prepared by dissolving 18.8 g phenol in CH_2Cl_2 and, after the cool solution warmed to room temperature, diluting to 100 mL with CH_2Cl_2. These stock solutions were stable for several days. The final reagent was prepared by mixing equal volumes of the 2 M Me_3SiCl and 2 M phenol solutions. NMR at 360 MHz showed resonances at 3.5 ppm for CH_2Cl_2, 0.418 ppm for Me_3SiCl, and 0.250 ppm for $Me_3SiOC_6H_5$ at a concentration of 0.06 M. In addition, a peak at 0.065–0.072 ppm was observed. It was identified as hexamethyl–disiloxane, $Me_3SiOSiMe_3$. The latter was formed by reaction of Me_3SiCl with the 0.5% H_2O in the commercial phenol. It was present at a concentration of 0.02 M, and 0.04 M HCl was simultaneously produced. No disilane was present in the reagent prepared from anhydrous phenol. During the Boc deprotection reaction, the concentration of the disiloxane remained unchanged.

The 1 M Me_3SiCl–3 M phenol reagent was prepared by mixing 1 volume of 4 M Me_3SiCl in CH_2Cl_2 and 3 volumes of 4 M phenol in CH_2Cl_2.

To prepare the 2 M phenol–2 M H_2O–CH_2Cl_2 washing solution, phenol (18.8 g) and H_2O (3.6 mL) were mixed and diluted to 100 mL with CH_2Cl_2.

Peptide Synthesis. Boc-Aminoacyl-OCH_2-resins were prepared from Boc-amino acids (10.5 mmol) and $ClCH_2$-copoly(styrene–1%-divinylbenzene) (10 g, 0.35 mmol/g) by addition of 10.5 mmol of powdered and dried KF in 50 mL of dry DMF. After 24 h gentle paddle stirring at 75 °C, the resin was filtered and washed with DMF, MeOH, H_2O, and MeOH. A sample was deprotected in 50% TFA–CH_2Cl_2, washed, neutralized with 5% DIEA–CH_2Cl_2, and washed with CH_2Cl_2. The degree of substitution was determined by the quantitative ninhydrin method.[10]

Peptide chain assembly was carried out in a 20-mL reaction vessel on a manual shaker by standard solid phase procedures[2] with 3 equiv of Boc-amino acid and 3 equiv of dicyclohexylcarbodiimide in CH_2Cl_2 for 1 h for all amino acids except Boc-His(Dnp) and Boc-Arg(Tos) where 3 equiv of 1-hydroxybenzotriazole were also added and coupling was for 2 h. The coupling reactions were monitored by the ninhydrin method.[10] If indicated, a second coupling was carried out.

Deprotection. After each cycle of coupling and washing of the Boc-peptide-resin, the Boc group was removed by pretreatment with 15 mL (per 1 g resin) of the 1 M Me_3SiCl–3 M phenol–CH_2Cl_2 reagent for 5 min and then with a second 15-mL portion for 15 min. The washing protocol was as follows. The filtered resin was washed two times with 15 mL of CH_2Cl_2, shaken 3 min with 15 mL of 4% H_2O–DMF, filtered, shaken for 3 min with 10% DIEA–DMF, filtered, and washed 3 × 1 min with 3 × 15 mL of CH_2Cl_2. The resulting hydrochloride of the peptide-resin was neutralized with 10% DIEA–DMF and washed with CH_2Cl_2 in preparation for the next coupling cycle.

Kinetic Studies for N^{α}-*tert*-Butyloxycarbonyl Removal. Samples of Boc-Val-OCH_2-resin (100 mg, 0.60 mmol/g) derived from esterification of Boc-Val-OH to chloromethyl resin (Lab Systems, CA) by the KF method were treated with 5 mL of deprotecting reagent under various conditions as described in Tables I and II. Samples were retrieved at different time intervals, washed, and neutralized, and the free amine was determined by the quantitative ninhydrin method in which $\epsilon = 1.5 \times 10^4$ M^{-1} cm^{-1} was used in all calculations. Rates were calculated from first-order plots.

Kinetic Studies for Benzylic Group Removal. Samples (~10 mg each) of Boc-Tyr(BrZ), Boc-Glu(OBzl), Boc-Ser(Bzl), and Z-Ala were treated with 20 mL of 1 M Me_3SiCl–phenol reagent in CH_2Cl_2. Each deprotecting reagent also contained 1 mg of Boc-Ala as internal standard. After 120 h, aliquots of sample were taken and the amino acids were determined by the amino acid analysis according to Jones et al.[11]

Estimation of the Water Content of Phenol. Forty milliliters of a 4 M solution of phenol (15.09 g) in dry CH_2Cl_2 was treated for 20 h with 500 mg of anhydrous Na_2CO_3 (4.717 mmol, 11.32% C) and the mixture was filtered, washed with CH_2Cl_2, and dried. The solid Na_2CO_3 + $Na_2CO_3 \cdot H_2O$ was analyzed for carbon, found 9.68%. From this it could be calculated that 88.2 mg of water had been removed, giving a value of 0.59% H_2O in the phenol.

Estimation of Free HCl in the Reagents. The analysis was based on the reaction of HCl with Na_2CO_3, followed by microelemental analysis for chlorine as AgCl.

a. Analysis of a Standard HCl Solution. A 4 M solution of HCl in dioxane was diluted 1 to 20 with CH_2Cl_2. This 0.2 M HCl solution (5.00 mL) was stirred for 20 h with 106 mg of Na_2CO_3. The dried solid was analyzed for 29.00% Cl (corrected for a 0.29% blank), giving an HCl concentration of 0.197 M and indicating that the procedure was satisfactory.

b. Free HCl in 1 M Me_3SiCl in CH_2Cl_2. Freshly prepared 1 M Me_3SiCl–CH_2Cl_2 (5.00 mL) was treated with 106 mg of anhydrous Na_2CO_3. The solid was filtered, washed with CH_2Cl_2, dried in vacuo, and analyzed for Cl. Found: 0.75% Cl (corrected for reagent blank), indicating that only 0.2% of the chlorine in the Me_3SiCl was present in solution as chloride.

c. Estimation of Free HCl in the 1 M Me_3SiCl–1 M Phenol–CH_2Cl_2 Reagent. Solutions of 2 M Me_3SiCl in CH_2Cl_2 and 2 M phenol in CH_2Cl_2 were separately freed of HCl and H_2O by the Na_2CO_3 procedure. Equal 2.5-mL volumes were mixed and treated with 106 mg of Na_2CO_3 for 20 h. Ether (40 mL) was added and the solid was separated by centrifugation. After three washes with ether, the solid was dried in vacuo and analyzed for chlorine. Found: Cl 9.20 ± 0.04% (after correction for a 0.30% reagent blank), from which a concentration of 0.056 M HCl could be calculated. Therefore, only 5.6% of the Me_3SiCl reacted with phenol to give HCl.

d. Estimation of the Me_3SiCl Content of the 2 M Me_3SiCl–CH_2Cl_2 Reagent. Although Me_3SiCl is stable to Na_2CO_3, it is decomposed by $NaHCO_3$, yielding NaCl + Me_3SiOH + CO_2. One milliliter of 2 M Me_3SiCl–CH_2Cl_2 was treated with 367.9 mg of $NaHCO_3$ for 20 h. The solid was filtered, washed, and dried in vacuo, leaving the NaCl and excess $NaHCO_3$. Analysis for chloride gave 21.94% Cl, from which it could be calculated that the concentration of Me_3SiCl in the reagent was 1.97 M.

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