Synthesis and Evaluation of Novel Activated Mixed Carbonate Reagents for the Introduction of the 2-(Trimethylsilyl)ethoxycarbonyl(Teoc)-Protecting Group

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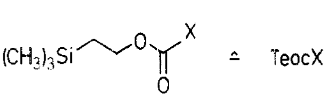
The synthesis of 1-[2-(trimethylsilyl)ethoxycarbonyloxy]benzotriazole (Teoc-OBt) and 1-[2-(trimethylsilyl)ethoxycarbonyloxy]pyrrolidin-2,5-dione (Teoc-OSu) in high yields is reported. Both compounds are crystalline compounds that react with amino acids under Schotten-Baumann conditions to produce Teoc-amino acids in high yields. The synthesis of nine new Teoc-amino acid derivatives is reported. Analysis of the products obtained during the synthesis of Teoc-Phe before and after crystallization as the cyclohexylamine salt revealed that ~ 5% of the corresponding dipeptide, Teoc-Phe-Phe-OH was produced when Teoc-OBt was used as the acylating reagent. In contrast, no dipeptide was detected when Teoc-OSu was utilized as the acylating reagent for the synthesis of the Teoc-amino acid derivatives studied. The use of Teoc-OSu should facilitate the use of the Teoc-group in peptide synthesis.

The use of silicon-based reagents for the protection of labile functionalities in all aspects of synthetic chemistry has continued apace in recent years.[1] Such protecting groups have been used primarily for the masking of hydroxy functionalities (alcohols, diols, and carboxylic acids), whilst only scant attention has been paid to their use in the masking of amino functionalities, despite their apparent potential in the field of peptide synthesis for "orthogonal" protection. The initial communication on the 2-(trimethylsilyl)ethoxycarbonyl (Teoc)-protecting group[2] for *N*-protection passed comparatively unheeded[3] until two reports on its use in peptide and amino acid chemistry appeared.[4,5] These studies further illustrated the usefulness of the Teoc-group, its stability under basic and hydrogenolytic conditions, its cleavage by tetraalkylammonium fluorides, strong acid, and Lewis acids,[6] and also the gaseous nature of the cleavage by-products, but suggested that the introduction of the Teoc group could be troublesome. The protecting group has been introduced in moderate yield using 2-trimethylsilylethyl 4-nitrophenyl carbonate (Teoc-ONp, **1**) (now commercially available[7]) and also by means of an adduct of 2-trimethylsilylethanol with *N*,*N*'-carbonyldiimidazole (Teoc-Im, **2**).[8,9]



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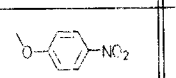
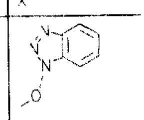
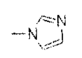
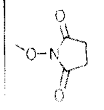
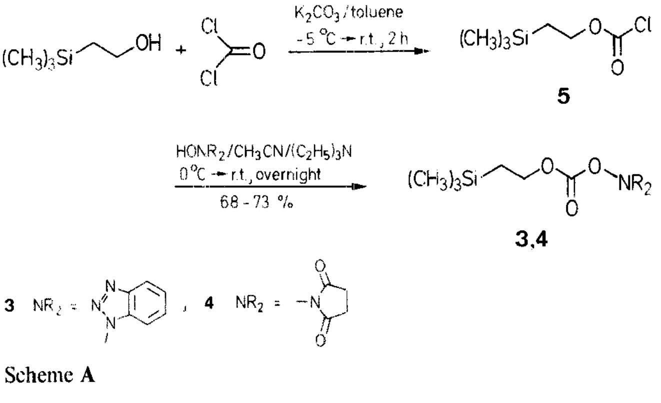
1		3	
2		4	
		5	Cl

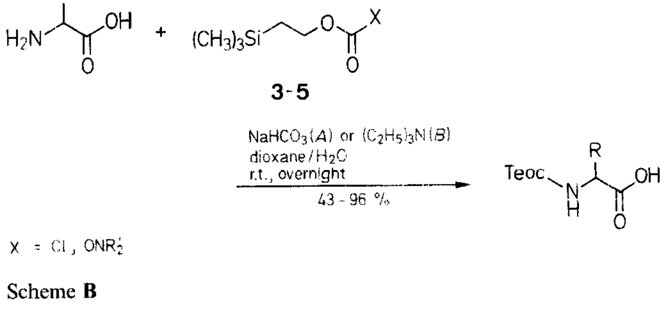
Our need to synthesize peptides containing four mutually orthogonal protecting groups led us to reexamine the preparation of Teoc-protected amino acids. Since attention has been directed recently to the use of mixed carbonate reagents derived from *N*-hydroxysuccinimide and 1-hydroxybenzotriazole for the introduction of a variety of amino-protecting groups,[10-13] we investigated such reagents for the introduction of the Teoc group. We report herein the synthesis of 1-[2-(trimethylsilyl)ethoxycarbonyloxy]benzotriazole (Teoc-OBt, **3**), and 1-[2-(trimethylsilyl)ethoxycarbonyloxy]pyrrolidin-2,5-dione (Teoc-OSu, **4**) and their use as reagents for the introduction of the Teoc group onto amino acids.

Synthesis of 2-trimethylsilylethyl carbonochloridate (Teoc-Cl, **5**), was accomplished in acceptable yield by a modification of the literature procedure.[14] Reaction of commercially available 2-trimethylsilylethanol and a slight excess of phosgene in toluene in the presence of powdered anhydrous potassium carbonate at low temperature to room temperature yielded the desired Teoc-Cl (Scheme A). Reaction of the carbonochloridate **5**, without further purification, with 1-hydroxybenzotriazole hydrate (HOBt) in acetonitrile overnight with triethylamine as the acid scavenger[13] (Scheme A) produced a 65–70% yield of reagent **3**; synthesis of reagent **4** from Teoc-Cl and *N*-hydroxysuccinimide was achieved in 71–73% yield under identical conditions.



Scheme A

Both reagents **3** and **4** are stable, highly crystalline solids. An evaluation of both Teoc-Cl (Method A) and the new reagents (Method B) for the introduction of the Teoc-group under Schotten-Baumann conditions (Scheme **B**) clearly indicated the superiority of the new mixed carbonate reagents over **5** in yield and over **1** in ease of removal of aromatic by-products. It should be noted that the resultant Teoc-amino acids are frequently oils, which can be characterized as their dicyclohexylammonium (DCHA) salts but which are more readily derivatized as their less lipophilic cyclohexylammonium (CHA) salts (*Caution!*). Furthermore, synthesis of a number of previously unreported derivatives indicate the utility of the reagents.



Scheme B

In a more rigorous appraisal of the two novel reagents themselves, however, close chromatographic analysis and detailed high-field NMR examination of the crude reaction mixtures and crystalline derivatives revealed in some cases a more polar impurity in the protected amino acids derived from Teoc-OBt which could only be removed by repeated recrystallization. In the case of Teoc-Phe, this impurity was present to the extent of ~ 5% (200 MHz ^{1}H-NMR) and was found to be the dipeptide Teoc-Phe-Phe by comparison with independently synthesized standards. Contrarily, when Teoc-OSu was used for protective-group introduction, there was never any evidence of impurity.

Oligopeptide formation has been recognized increasingly as a serious side-reaction during *N*-protection of amino acids.[15,16,17] It is most prevalent in the protection of hydrophobic and nonfunctional amino acids, particularly glycine, or when a carbonochloridate is used to introduce the grouping, and it is now well established that the side-product arises via a mixed anhydride intermediate.[10,12,15,16,17] Such contamination has not previously been reported as arising from the use of 1-hydroxybenzotriazole-derived mixed carbonate reagents;[11,13] however, it was noted in the synthesis of Fmoc-Ala via Fmoc-OBt[12] that although the yield was high (84%), an impure product was obtained; this impurity was not further investigated but was presumably oligopeptide. Our observation that no oligopeptide was formed when using Teoc-OSu confirms previous reports on the superiority of *N*-hydroxysuccinimidyl mixed carbonate reagents for the introduction of amino acid *N*-protecting groups.[10,12]

Hence, although both new reagents allow for the high-yielding introduction of the Teoc protective group, the lack of oligopeptide by-product formation in the use of Teoc-OSu combined with its ease of preparation, excellent physical properties, and convenience strongly favors this as the reagent of choice for the introduction of the Teoc functionality. This should facilitate the use of this protective group not only in the field of peptides but also in general synthesis.

Reagents were used as commercially available, except for: hexane, distilled to b.p. 68–70 °C; triethylamine, redistilled from phthalic anhydride followed by calcium hydride; *N*-hydroxysuccinimide, recrystallized from ethyl acetate. "Acetonitrile" refers to HPLC-grade reagent. Melting points were determined using a Fisher-Johns apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer Model 241 automatic polarimeter (1.0 dm). ^{1}H-NMR spectra were recorded on deuteriochloroform solutions using either a Varian EM-390 (90 MHz) or a Bruker WP-200 (200 MHz) spectrometer. Chemical shifts are reported in ppm downfield from an external tetramethylsilane (TMS) standard or relative to an internal dioxane standard (3.71 ppm downfield from TMS).

2-Trimethylsilylethyl Carbonochloridate (Teoc-Cl, 5):

Warning! Synthesis of this reagent involves the use of phosgene, a highly toxic material. All manipulations should therefore be performed in a very well-ventilated hood.

To a solution of 2-trimethylsilylethanol (2.0 g, 17 mmol) in toluene (10 ml) at −10 to −5 °C is added powdered anhydrous potassium carbonate (2 g). To this vigorously stirred suspension is added dropwise over 15–30 min a 12.5% solution of phosgene in toluene (20 ml). On completion of the addition, the mixture is stirred at low temperature for 30 min, the ice bath removed, and the mixture stirred for a further 1 h at room temperature. After 1 h, nitrogen is bubbled through the mixture, *with the hood closed*, to remove the majority of the excess phosgene and the mixture put on a rotary evaporator at a temperature less than 40 °C to remove the last traces of phosgene. The solids are then filtered off through a pad of anhydrous magnesium sulfate, and washed with dry

Table 1. *N*-[2-(Trimethylsilyl)ethoxycarbonyl]-amino Acids (Teoc-Amino Acids) Prepared

Teoc-Amino Acid	Salt Derivative[a] Prepared for Isolation	Method	Yield (%)	m.p. (°C) (softens at)	Recrystallization Solvent[b]	R_f (solvent system)[c]	$[\alpha]_D^{25}$ (conc.) (methanol)	Molecular Formula[d] or m.p. (°C) reported
L-Phe	DCHA	A	45	112.5–113.5	A	0.26 (A)	+26.6° (2.1)	$C_{27}H_{46}N_2O_4Si$ (490.75)
	CHA	A	66	132–139 (125)	B	0.26 (A)	+28.2° (2.0)	$C_{21}H_{36}N_2O_4Si$ (408.6)
	CHA	B	88					
L-Ala	CHA	A	46	125–131 (115)	C	0.16 (A)	−0.5° (2.0)	$C_{15}H_{32}N_2O_4Si$ (332.5)
	CHA	B	86					
D-Ala	CHA	A	43	125–132 (115)	C	0.16 (A)	+0.7° (2.0)	$C_{15}H_{32}N_2O_4Si$ (332.5)
	CHA	B	92					
L-Val	CHA	A	50	127–133	D	0.23 (A)	−2.0° (3.7)	$C_{17}H_{36}N_2O_4Si$ (360.6)
	CHA	B	95					
L-Met	–	B	92	97–98	E	0.18 (A)	+13.8° (2.2, chloroform)	$C_{11}H_{23}NO_4SSi$ (293.4)
L-Ser	CHA	B	88	120–123 (110)	C	0.67 (B)	+12.8° (1.9)	$C_{15}H_{32}N_2O_5Si$ (348.5)
L-Trp	–	B	88	97–100 (90)	F	0.83 (B)	−23.4° (1.8, DMF)[e]	100–105[4]
L-Pro	DCHA	B	63	138–139 (128)	D	0.16 (A)	−27.4 (1.1)[f]	145–148[4]
L-Glu	CHA[g]	B	92	170–178 (158)	G	0.74 (B)	+7.8 (2.1)	$C_{20}H_{40.5}N_{2.5}O_6Si$[g] (441.1)
L-Asp	CHA[g]	B	84	134–142 (126)	H	0.70 (B)	+12.3 (2.4)	$C_{19}H_{38.5}N_{2.5}O_6Si$[g] (426.9)
N$^\alpha$-Boc-L-Lys	–	B	96[h]	foam[i]				

[a] CHA: cyclohexylamine salt; DCHA: dicyclohexylamine salt.

[b] A: isopropanol/water; B: diisopropyl ether/dichloromethane (20 : 1) + hexane; C: dichloromethane/ether (1 : 1) + hexane; D: diisopropyl ether/hexane; E: hexane; F: chloroform/hexane; G: acetonitrile/water; H: isopropanol/hexane.

[c] TLC solvent systems; A: toluene/acetic acid (10 : 1); B: butanol/acetic acid/water (4 : 1 : 1).

[d] The microanalyses were in satisfactory agreement with the calculated values: C ±0.37, H ±0.26, N ±0.24.

[e] Lit.,[4]: $[\alpha]_D^{25}$: −24.7° (c = 1, DMF).

[f] Lit.,[4] $[\alpha]_D^{25}$: −28.3° (c = 1, methanol).

[g] Microanalysis indicates 1.5 molecules of cyclohexylamine per molecule of Teoc-amino acid.

[h] Lit.,[5] yield: 93%.

[i] ^{1}H-NMR data identical with the reported data.[5]

Table 2. ^{1}H-NMR-Spectral Data of the *N*-Teoc-amino Acids or Their Cyclohexylammonium or Dicyclohexylammonium Salts

Compound	^{1}H-NMR ($CDCl_3/TMS_{ext}$) δ (ppm)
Teoc-L-Phe, DCHA	0.07 (s, 9H); 0.89–1.07 (m, 2H); 0.90–2.15 (m, 20H); 2.70–3.25 (m, 2H); 3.15 (d, 2H, *J* = 6 Hz); 4.05–4.40 (m, 3H); 5.46 (br. d, 1H, *J* = 6 Hz); 7.23 (s, 5H); 8.50 (br. s, 2H)
Teoc-L-Phe, CHA	0.04 (s, 9H); 0.94 (m, 2H); 0.99–2.01 (m, 10H); 2.74 (m, 1H); 3.12 (m, 2H); 3.93–4.39 (m, 3H); 5.38 (d, 1H, *J* = 6 Hz); 7.26 (s, 5H); 7.88 (br. s, 3H)
Teoc-D-Ala, CHA	0.06 (s, 9H); 0.89–1.08 (m, 2H); 1.36 (d, 3H, *J* = 7 Hz); 1.20–2.15 (m, 10H); 2.90 (m, 1H); 3.91–4.26 (m, 3H); 5.59 (br. d, 1H, *J* = 6 Hz); 7.91 (br. s, 3H)
Teoc-L-Ala, CHA	0.07 (s, 9H); 0.90–1.09 (m, 2H); 1.36 (d, 3H, *J* = 7 Hz); 1.28–2.17 (m, 10H); 2.92 (m, 1H); 3.90–4.25 (m, 3H); 5.56 (br. d, 1H, *J* = 7 Hz); 7.94 (br. s, 3H)
Teoc-L-Val, CHA	0.04 (s, 9H); 0.80–1.15 (m, 8H); 1.20–2.27 (m, 11H); 2.91 (m, 1H); 3.86–4.30 (m, 3H); 5.41 (d, 1H, *J* = 8 Hz); 8.04 (br. s, 3H)
Teoc-L-Met	0.04 (s, 9H); 0.99 (m, 2H); 1.90–2.21 (m, 2H); 2.07 (s, 3H); 2.57 (m, 2H); 4.18 (m, 2H); 4.49 (m, 1H); 5.40 (br. m, 1H); 10.86 (br. s, 1H)[b]
Teoc-L-Ser, CHA	0.04 (s, 9H); 0.98 (m, 2H); 1.10–2.10 (m, 10H); 2.94 (m, 1H); 3.71–3.89 (m, 2H); 3.91–4.18 (m, 3H); 6.10 (br. m, 1H); 6.98 (br. s, 3H)
Teoc-L-Glu, 1.5 CHA[a]	0.03 (s, 9H); 0.97 (m, 2H); 2.13 (m, 2H); 2.46 (m, 2H); 4.17 (m, 2H); 4.38 (m, 1H); 5.74 (br. m, 1H); 11.28 (br. s, 2H)[b]
Teoc-L-Asp, 1.5 CHA[a]	0.04 (s, 9H); 1.00 (m, 2H); 3.00 (m, 2H); 4.19 (m, 2H); 4.67 (m, 1H); 5.96 (br. d, 1H, *J* = 6 Hz); 10.11 (br. s, 2H)[b]

[a] 0.5 mol water of crystallization.

[b] ^{1}H-NMR of free *N*-protected amino acid.

ether. The combined filtrate and washings are evaporated at a temperature not higher than 50 °C. The resultant oily carbonochloridate **5** is used without further purification.

1-[2-(Trimethylsilyl)ethoxycarbonyloxy]benzotriazole (Teoc-OBt) (3) and 1-[2-(Trimethylsilyl)ethoxycarbonyloxy]pyrrolidin-2,5-dione (Teoc-OSu) (4):

2-Trimethylsilylethyl carbonochloridate (3.07 g, 17 mmol) is taken up in acetonitrile (50 ml). The solution is cooled to 0 °C and solid 1-hydroxybenzotriazole monohydrate (3.0 g, 22 mmol) [for the synthesis of **3**] or solid *N*-hydroxysuccinimide (2.6 g, 22 mmol) [for the synthesis of **4**] is added with vigorous stirring followed by a solution of triethylamine (2.0 g, 20 mmol) in acetonitrile (5 ml). The mixture is stirred at low temperature for 15 min, then at room temperature overnight. The mixture is poured into water (200 ml) and extracted with ether (3 × 75 ml). The organic extracts are combined, washed with water (2 × 75 ml), 1 normal hydrochloric acid (75 ml), and again water (2 × 75 ml), dried with magnesium sulfate, and evaporated to dryness. The residue is taken up in boiling hexane (250–350 ml), and the solution allowed to cool. Crystallization is completed by storage at −15 °C.

Teoc-OBt (**3**); yield: 3.19 g (68 %, based on 2-trimethylsilylethanol); colorless plates, m.p. 104–106 °C.

$C_{12}H_{17}N_3O_3Si$ (279.4) calc. C 51.59 H 6.14 N 15.04
found 51.47 6.35 15.43

^{1}H-NMR ($CDCl_3/TMS_{ext}$): δ = 0.11 (s, 9H); 1.29 (m, 2H); 4.69 (m, 2H); 7.60 (m, 1H); 7.84 (m, 1H); 8.08 (m, 1H); 8.28 ppm (m, 1H).

Teoc-OSu (**4**); yield: 3.21 g (73 %, based on 2-trimethylsilylethanol); colorless needles, m.p. 97.5–98.5 °C.

$C_{10}H_{17}NO_5Si$ (259.3) calc. C 46.31 H 6.61 N 5.40
found 46.26 6.58 5.38

^{1}H-NMR ($CDCl_3/TMS_{ext}$): δ = 0.06 (s, 9H); 1.15 (m, 2H); 2.81 (s, 4H); 4.41 ppm (m, 2H).

N-[2-(Trimethylsilyl)ethoxycarbonyl]-amino Acids (Teoc-Amino Acids); General Procedures:

Method A, using 2-Trimethylsilylethyl Carbonochloridate (Teoc-Cl, **5**): Sodium hydrogen carbonate (3.78 g, 45 mmol) and the amino acid (15 mmol) are added to water (15 ml) and the mixture is stirred at room temperature for 30 min or until the majority of the solids dissolve. To this suspension is added a solution of 2-trimethylsilylethyl carbonochloridate [from 2 g (17 mmol) of 2-trimethylsilylethanol] in dioxane (30 ml). The resultant mixture is stirred vigorously at room temperature overnight, poured into water (50 ml) and extracted with ether (3 × 50 ml), the organic extracts being discarded. The aqueous layer is acidified to pH ~ 2 with saturated potassium hydrogen sulfate solution and extracted with ether (3 × 50 ml). The combined organic extracts are washed with water (3 × 75 ml), dried with magnesium sulfate, and evaporated. The Teoc-amino acids thus obtained are purified by crystallization (if solid) or as their cyclohexylammonium (or dicyclohexylammonium) salts (if they are oils) (Table 1 and 2).

Method B, using the Mixed Carbonate Reagents **3** or **4**: To a stirred suspension of the amino acid (1 mmol) in water (1 ml) is added a solution of triethylamine (1.5 mmol) in dioxane (1 ml) followed by solid Teoc-OBt (**3**; 0.31 g, ~ 1.1 mmol) or Teoc-OSu (**4**; 0.29 g, ~ 1.1 mmol). The mixture is stirred at room temperature overnight, then diluted with water (5 ml), acidified with saturated potassium hydrogen sulfate solution, and extracted with ether (3 × 15 ml). The combined organic layers are washed with water (4 × 20 ml), dried with magnesium sulfate, and evaporated. The resultant Teoc-amino acid is purified as in Method A (Table 1 and 2).

Caution! Cyclohexylamine and dicyclohexylamine have been implicated as possible carcinogens. Care should therefore be taken in the use of these reagents.

N-Teoc-L-methionine; Typical Procedure (Method B, using Teoc-OSu):
To a stirred suspension of L-methionine (0.15 g, 1 mmol) in water (1 ml) is added a solution of triethylamine (0.15 g, 1.5 mmol) in dioxane (1 ml). To the resultant solution is added solid Teoc-OSu (**4**; 0.29 g, 1.1 mmol). The mixture is stirred at room temperature overnight, then diluted with water (5 ml), acidified with saturated potassium hydrogen sulfate, and extracted with ether (3 × 15 ml). The combined organic extracts are washed with water (4 × 20 ml), dried with magnesium sulfate, and evaporated to dryness. The residue is recrystallized from hexane; yield: 0.27 g (92 %); m.p. 97–98 °C.

N-Teoc-L-aspartic Acid, Cyclohexylammonium Salt; Typical Procedure (Method B, using Teoc-OSu):

To a stirred suspension of L-aspartic acid (0.13 g, 1 mmol) in water (1 ml) is added a solution of triethylamine (0.26 g, 2.6 mmol) in dioxane (1 ml). To the resultant solution is added solid Teoc-OSu (**4**; 0.29 g, 1.1 mmol). The mixture is stirred at room temperature overnight, then diluted with water (5 ml), acidified with saturated potassium hydrogen sulfate solution, and extracted with ether (3 × 15 ml). The combined organic extracts are washed with water (4 × 20 ml), dried with magnesium sulfate, and evaporated. To the oily residue is added a solution of cyclohexylamine (0.19 g, 2 mmol) in ether (5 ml) and the solvent evaporated. Crystallization is achieved by taking the residue up in the minimum volume of boiling isopropanol and diluting to faint turbidity with hexane. Slow cooling to room temperature overnight causes the product to crystallize; yield: 0.35 g (84 %); m.p. 134–142 °C (softens at 126 °C).

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