Proc. Nat. Acad. Sci. USA
Vol. 69, No. 3, pp. 730–732, March 1972

New Method to Prepare *N*-*t*-Butoxycarbonyl Derivatives and the Corresponding Sulfur Analogs from di-*t*-Butyl Dicarbonate or di-*t*-Butyl Dithiol Dicarbonates and Amino Acids

(blocking groups/peptide synthesis)

D. STANLEY TARBELL, YUTAKA YAMAMOTO, AND BARRY M. POPE

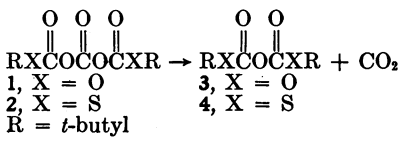
Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37203

Contributed by D. Stanley Tarbell, January 17, 1972

ABSTRACT **Di-*t*-butyl dicarbonate and one of its dithiol analogs, practical methods of preparation for which are given, react with amino-acid esters to form the *N*-*t*-butoxycarbonyl (*t*-BOC) derivatives and the thiol analogs in good yield under mild conditions. The thiol analogs are stable to acidic conditions, which rapidly remove the *t*-BOC group itself. *t*-Butyl trimethylsilyl carbonate forms a $(CH_3)_3Si$ ether from a *N*-thiol-*t*-BOC serine methyl ester. The *N*-thiol-*t*-BOC group can be removed from the —NHCOSR (R = *t*-butyl) by heating with peroxide–acetic acid.**

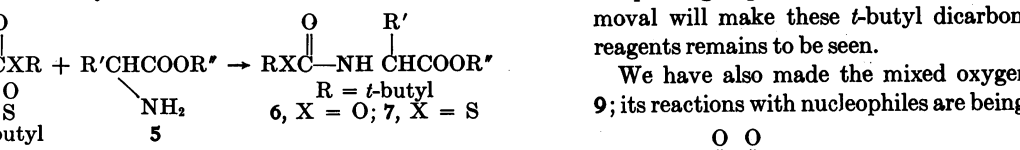
Action of the dicarbonates described above has not been attended by racemization in the cases examined. The two dicarbonates may be useful as agents for selective blocking and deblocking of amino or other groups.

Recent studies (1, 2) in this laboratory have made the tricarbonates **1** and **2** readily available. From them, without isolation of the tricarbonates if desired, the corresponding *dicarbonates* **3** and **4** are readily prepared by treatment with tertiary amines or by mild heating (3).



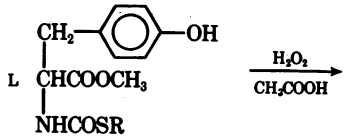
The ready availability of the sulfur and oxygen dicarbonates (for nomenclature, see ref. 2.) suggested that their behavior with α-amino acids should be studied, because the expected reaction (4, 5) would lead to the *t*-butoxycarbonyl (*t*-BOC) derivatives, or their sulfur analogs (thiol-*t*-BOC); the *t*-BOC group is a standard protecting group for the amino group in peptide syntheses. The reaction of **3** and **4** should allow synthesis of such derivatives under mild conditions, and might have useful applications in selective alteration of enzymes or proteins.

The expected reaction (4, 5) took place readily; the amino-acid ester hydrochloride was usually used, neutralized with bicarbonate, and heated briefly in tetrahydrofuran (THF) or chloroform to give the product in good yield. Free glycine was also used successfully.



Some of the derivatives prepared in model studies are shown in Table 1.

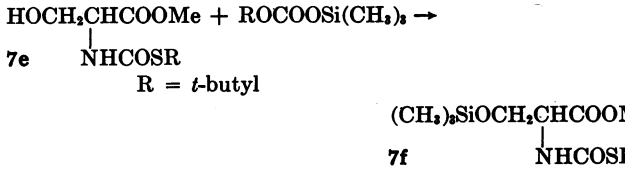
The advantage of the *t*-BOC group in peptide syntheses lies in its rapid removal, by mild acid, presumably via alkyl–oxygen cleavage and a carbonium ion mechanism. The thiol analogs **7**, however, as would be predicted from much work in this and other laboratories (see ref 1 for references), are stable to acid conditions, such as formic acid or CF_3COOH, that cleave the *t*-BOC group completely. The thiol analog **7** can be cleaved by Kollonitsch's (6) method with peracids, without racemization.



L-Tyr methyl ester, of unchanged rotation.

This difference in rate of cleavage by acid between the oxygen and sulfur analogs **6** and **7** might allow preferential deblocking of amino groups in complex molecules.

The yield of isolated material in Table 1 was usually high, although some of the *t*-BOC derivatives **6** (as esters) were difficult to purify. With 1 mol of dicarbonate, no diacylated material was obtained from serine or tyrosine. The trimethylsilyl ether of the serine thiol derivative was prepared as below (7).



Tests of acidic hydrolysis of *N*-*t*-BOC methyl ester of Tyr showed no appreciable racemization during the process.

It is well known that the diethyl analog **8** of **3**, usually called diethyl pyrocarbonate, reacts with various enzymes, proteins (8), and with adenosine and adenine (9). Whether

8, R = Et.

differences in reactivity of **8**, **3**, and **4** with amino or other nucleophilic groups and differences in their relative rates of removal will make these *t*-butyl dicarbonates **3** and **4** useful reagents remains to be seen.

We have also made the mixed oxygen–sulfur dicarbonate **9**; its reactions with nucleophiles are being investigated.

9, R = *t*-butyl

Modified procedure for preparation of di-*t*-butyl tricarbonate (1) and di-*t*-butyl dicarbonate (3) ref. 1

Potassium *t*-butoxide (44.8 g, 0.4 mol) was dissolved in 550 ml of freshly distilled THF at room temperature. Dry CO_2

Abbreviations: THF, tetrahydrofuran.

was passed through the solution, which was cooled with an ice–salt bath (−15 to about −20°) with vigorous stirring for 30 min. A solution of about 20 ml of phosgene in 85 ml of benzene was added dropwise. The reaction mixture was stirred for an additional hour, while the bath temperature was maintained at −15° to about −20°. Dry nitrogen was bubbled into the cold solution for 45 min.

The resulting solution was evaporated to about 100 ml in a rotary evaporator under reduced pressure by a rotary pump, while being kept below 0°.

The precipitate was removed by suction filtration through a fritted-glass filter funnel of medium porosity, which had previously been cooled with ice-cold pentane. The precipitate was washed with pentane and the solution was completely evaporated at a temperature below 0° in a rotary evaporator under reduced pressure by a rotary pump to give 33.7 g (64.1%) of colorless fine solid of **1**.

In 75 ml of CCl_4, 20 g of unrecrystallized tricarbonate was dissolved, and 0.1 g of freshly sublimed 1,4-diazobicyclo-[2.2.2]octane (Dabco) was added. This mixture was stirred at room temperature for 45 min, then the solvent was evaporated by a rotary evaporator.

After the Dabco was removed by sublimation, the solution was distilled to give 14.8 g (89%) of di-*t*-butyl dicarbonate, bp 50–51° (0.3 mm), whose spectroscopic properties agreed with reported values (1).

Modified procedure for preparation of di-*t*-butyl dithiol dicarbonate (4) via di-*t*-butyl dithiol tricarbonate (2)

Sodium hydride (6 g, in 50% mineral oil dispersion) was washed with three 50-ml portions of THF and suspended in 250 ml of THF. A solution of 9 g of *t*-butyl mercaptan in 30 ml of the same solvent was added dropwise with stirring; the resulting mixture was refluxed for 1 hr and cooled with an ice–salt bath (−15° to about −20°). Dry carbon dioxide was passed into the mixture for 30 min with vigorous stirring. A solution of about 9 g of phosgene in 25 ml of benzene was added dropwise. The reaction mixture was stirred for an additional 30 min, while the bath temperature was maintained at −15° to about −20°. The NaCl was removed by filtration as before, the filtrate and washings were completely removed by evaporation with an oil pump, and the resulting oily tricarbonate was heated at a bath temperature of 85–90° until evolution of CO_2 ceased. Distillation gave 8.9 g (71%, overall, from the mercaptan) of material, bp 82–83° (0.5 mm) with the spectroscopic properties previously reported for the dithiol dicarbonate **4**.

N-*t*-BOC glycine ethyl ester (6a)

A typical preparation of compounds in Table 1*A* follows. Glycine ethyl ester hydrochloride (1.395 g, 0.01 mol) was suspended in 20 ml of chloroform, and 0.84 g (0.01 mol) of $NaHCO_3$ in 15 ml of H_2O was added. Sodium chloride (2 g) was added, and then 2.18 g (0.01 mol) of di-*t*-butyl dicarbonate **3** dissolved in a few milliliters of $CHCl_3$; the mixture was refluxed for 90 min. After the solution cooled, it was separated, and the aqueous layer was extracted with $CHCl_3$; the $CHCl_3$ solution was dried and evaporated at room temperature. The colorless product was then distilled at 104–105° (0.15 mm), to give 1.8 g (89%) of *N*-*t*-butoxycarbonyl glycine ethyl ester (**6a**). The IR spectrum (CCl_4) showed -NH stretch at 3440 cm^{-1} and carbonyl bands at 1725 and 1745. The NMR spectrum (CCl_4) in ppm: 1.27 (t, 3H); 1.45 (s, 9H); 3.8 (d, 2H); 4.17 (q, 2H); 5.67 (t, 1H), in satisfactory agreement with the reported values (ref. *e*, Table 1).

As an example of other analytical data for Table 1*A*, *N*-*t*-BOC L-serine methyl ester (**6c**) may be quoted. It showed IR (CCl_4) 3300–3550 (OH and NH), 1750 and 1730 (C=O), and NMR (CCl_4) at 1.43 (s, 9H); 3.73 (s, 3H); 3.8–4.4 (m, 4H) and 5.9 (d, 1H). *Anal.* Calcd. for $C_9H_{17}NO_5$: C, 49.30; H, 7.82: Found: C, 49.15; H, 8.00.

Action of trifluoroacetic acid -H_2O on *N*-*t*-BOC tyrosine methyl ester (6f)

The *N*-*t*-BOC compound **6f** (0.3023 g) was allowed to stand in a mixture of 15 ml of CF_3COOH and 5 ml of H_2O for 15 min at room temperature; the solution was then evaporated, neutralized with 3% NH_4OH, saturated with NaCl, and extracted with $CHCl_3$. The $CHCl_3$ layer was dried and evaporated under reduced pressure to give 0.1995 g (82.8%) of white solid. The solid was recrystallized from ethyl acetate and has a mp of 134–135°C; it showed no depression of melting point in a mixture melting point with authentic L-tyrosine methyl ester. The $[\alpha]^{25}_D$ for the tyrosine methyl ester recovered from the *N*-*t*-BOC derivative, as above, was +26.9° (*C* = 2.36, CH_3OH); the starting material, before treatment with the dicarbonate, showed $[\alpha]^{25}_D$ + 26.5° (*C* = 2.33, CH_3OH).

N-Thiol-*t*-BOC L-tyrosine methyl ester (7c). Method A

To a solution of 2.5 g (0.01 mol) of di-*t*-butyl dithiol dicarbonate in 10 ml of THF was added a solution of 1.95 g (0.01 mol) of L-tyrosine methyl ester in the same solvent with stirring at room temperature. L-Tyrosine ester gradually reacted, evolution of gas was observed, and the reaction mixture was refluxed for 30 min. Removal of solvent under reduced pressure gave a viscous oil, which was crystallized by washing with petroleum ether. Recrystallization from benzene (about 25 ml of benzene solution was concentrated to about 10 ml) gave 2.4 g of colorless crystals, mp 110–112°C. Concentration of the mother liquors and addition of cyclohexane yielded 0.4 g more of the same crystals; total amount of crystals was 2.8 g (90%). The properties were IR (CCl_4); 1745, 1670 cm^{-1}; NMR (CCl_4) 1.50 (*t*-butyl protons). *Anal.* Calcd. for $C_{15}H_{21}NO_4S$: C, 57.86; H, 6.80. Found: C, 57.97; H, 6.71.

N-thiol-*t*-BOC L-serine methyl ester (7e). Method B

L-Serine methyl ester hydrochloride (1.55 g) was dissolved in H_2O containing 1 eq of $NaHCO_3$. Di-*t*-butyl dithiol dicarbonate (2.5 g), and enough alcohol to form a homogeneous solution, were added. The reaction was kept at room temperature for several hours and concentrated under reduced pressure. The resulting solution was saturated with NaCl, and extracted with $CHCl_3$, the $CHCl_3$ layer was dried, and solvent was evaporated under reduced pressure. The viscous liquid obtained had reasonable IR and NMR spectra for structure **6c**, but decomposed on attempted distillation, apparently to form a 2-oxooxazolidine with loss of $(CH_3)_3CSH$.

The crude **7e** (1.7 g) was silylated to the *O*-trimethylsilyl compound **7f** by heating at 95–100°C (bath temperature) for 16 hr with stirring with 4.5 g of *t*-butyl trimethylsilyl carbonate (7). The reaction mixture was evaporated with an aspirator. Distillation gave 1.6 g of the *O*-silylated compound **7f**, [71%, bp 112–113°C (0.35 mm)]. The properties were IR

TABLE 1.

A. $RO\overset{O}{\overset{\|}{C}}NH-\underset{R'}{\underset{|}{C}}HCOOR''$. Prepared from the Dicarbonate **3**.
R = *t*-butyl

Compound	Amino acid	R''	Characterization*	mp (solvent) or bp (mm)
6a	Gly	Et	*a, b, e,* known	104–105 (0.15)
6b	D-Try	Et	*a, b, c, g*	166–168 ($CHCl_3$)
6c	L-Ser	Me	*a, b, c*	126 (0.35)
6d	L-Leu	Et	*a, b, c*	97 (0.2)
6e	L-Pro	$C_6H_5CH_2$	*a, b, c*	136 (0.35)
6f	L-Tyr	Me	*a, b, d, f,* known	105–106 (C_6H_{12})
6g	4-OH-L-Pro	Et	*a, b, c*	142 (0.35)
6h	L-Ala	Me	*a, b, c*	60 (0.03)

B. $RS\overset{O}{\overset{\|}{C}}NH\underset{R'}{\underset{|}{C}}HCOOR''$. Prepared from the Dicarbonate **4**.
R = *t*-butyl

No.	Amino acid	R''	Characterization	mp (solvent) or bp (mm)	Method
7a	Gly	H	*a, b, c*	129–130 ($CHCl_3$)	B
7b	Gly	Et	*a, b, c*	113–114 (1.1)	A
7c	L-Tyr	Me	*a, b, c, d,* no *O*-acylation	110–112 (C_6H_6)	A
7d	L-His	Me	*a, b, c, N-α, N*-im derivative	98–99.5 (EtOH–H_2O)	B
7e	L-Ser	Me	Purified through silyl derivative	—	
7f	$(CH_3)_3SiOCH_2\underset{NH_2}{\underset{\|}{C}}HCOOH$	Me	*a, b, c*	112–113 (0.35)	

* (*a*) Correct NMR spectrum; (*b*) correct IR spectrum; (*c*) correct elemental analysis; (*d*) hydrolysis to L-Tyr methyl ester; (*e*) spectra (IR and NMR) agree with those of Tarbell, D. S. & Insalaco, M. A. (1967) *Proc. Nat. Acad. Sci. USA* **57**, 233–235; (*f*) Schrader, E. (1963) Annalen **670**, 133, reports mp of 102–104°C; (*g*) Iselin, B. & Schwyzer, R. (1961) *Helv. Chim. Acta* **44**, 171; mention, but do not characterize, this compound.

(liquid film) 3340 cm^{-1} (—NH); 1750, 1670 (C=O). The NMR (CCl_4, ppm): 0.92 (s, 9H); 1.65 (s, 9H); 3.74 (s, 3H); 3.80 (m, 2H); 4.95 (m, 1H); 5.9 (broad, 1H, NH). *Anal.* Calcd. for $C_{12}H_{25}NO_4SSi$: C, 46.87; H, 8.19; N, 4.56. Found: C, 46.71; H, 8.00; N, 4.47.

N-Thiol-*t*-BOC glycine from the free amino acid

A mixture of di-*t*-butyl dithiol dicarbonate (1.7 g) and glycine (0.5 g) in THF–H_2O (2:1, v/v) was refluxed for 18 hr. Treatment of the reaction mixture by method *B* gave 0.95 g (79%) of *N*-thiol-*t*-BOC glycine, mp 129–130°C (from $CHCl_3$). The properties were: IR ($CHCl_3$), 3400–2550 cm^{-1} (broad, —NH and COOH); 1730, 1660 (C=O); NMR ($CHCl_3$); 1.51 (s, 9H); 4.06 (d, J = 5.5, 2H); 6.10 (broad, 1H, NH); 10.05 (s, 1H, COOH). *Anal.* Calcd. for $C_7H_{13}NO_3S$: C, 43.97; H, 6.85, Found: C, 44.10; H, 6.71.

Acidic removal of *N*-thiol-*t*-BOC group from *N*-thiol-*t*-BOC L-tyrosine methyl ester (7c)

This compound was unchanged by treatment with formic acid or by CF_3COOH–H_2O, under conditions that remove the *t*-BOC group.

N-thiol-*t*-BOC L-tyrosine methyl ester (**7c**) (0.2 g) was dissolved in a mixture of acetic acid (2 ml) and 30% H_2O_2 (1 ml). The solution was gently refluxed for 30 min and evaporated on a steam bath under reduced pressure. The resulting reddish-brown oil was cooled with ice and made basic with 3% NH_4OH. The solution was saturated with NaCl and extracted with $CHCl_3$; the $CHCl_3$ layer was dried and evaporated under reduced pressure. Solid (50 mg) was obtained. A sample recrystallized from EtOAc melted at 134–136°C and showed no depression of melting point in mixture with an authentic sample of L-tyrosine methyl ester; the IR spectrum and the optical rotation were identical with an authentic sample, indicating no racemization during formation or removal of the *N*-thiol-*t*-BOC group.

This work was supported by Grant GP-15795 from the National Science Foundation and by Grant AI-08424 from the National Institutes of Health.

1. Dean, C. S., Tarbell, D. S. & Friederang, A. W. (1970) *J. Org. Chem.* **35**, 3393–3397.
2. Dean, C. S. & Tarbell, D. S. (1971) *J. Org. Chem.* **36**, 1180–1183.
3. Compound **3** has been reported in low yield by Howe, J. W. & Morris, L. R. (1962) *J. Org. Chem.* **27**, 1901–1902.
4. Boehm, T. & Mehta, D. (1938) *Chem. Ber.* **71**, 1797–1802.
5. Thoma, W. & Rinke, H. (1959) *Annalen* **624**, 30–36.
6. Kollonitsch, J., Gabor, V. & Hajos, A. (1956) *Chem. Ber.* **89**, 2288–2292, 2293–2301.
7. Yamamoto, Y. & Tarbell, D. S. (1971) *J. Org. Chem.* **36**, 2954–2956.
8. For leading results and references, see Melchior, W. B., Jr. & Fahrney, D. (1970) *Biochemistry* **9**, 251–258.
9. Leonard, N. J., McDonald, J. J., Henderson R. E. L. & Reichmann, M. E. (1971) *Biochemistry* **10**, 3335–3342; Leonard, N. J., McDonald, J. J. & Reichmann, M. E. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 93–98.