Peptides. VI. Some Oxime Carbonates as Novel *t*-Butoxycarbonylating Reagents[1)]

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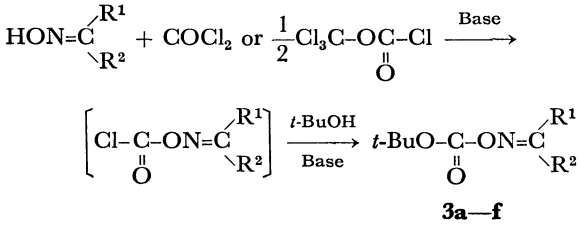
Several *t*-butoxycarbonyl derivatives of oximes were prepared through the corresponding oxime chloroformates. Of these, diethyl (*t*-butoxycarbonyloxyimino)malonate and 2-(*t*-butoxycarbonyloxyimino)-2-phenylacetonitrile were utilized for the preparation of *t*-butoxycarbonylamino acids under various conditions. The results are summarized in a table.

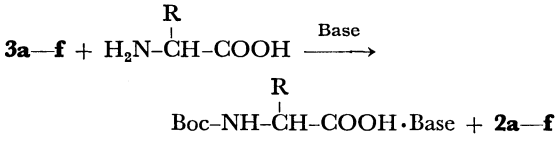
The *t*-butoxycarbonyl (Boc) group is one of the most important amino-protecting groups in peptide synthesis, and there have been many reagents proposed for its introduction.[2-18)] The most popular and still widely utilized one is *t*-butyl azidoformate (**1**),[2)] though it is toxic and relatively less reactive and should be prepared just before use. Most of the substitutes for **1** are, with a few exceptions, less attractive because of disadvantages such as difficult access or insufficient reactivity of the reagents, or cumbersome elimination of by-products from Boc-amino acids. The present authors wish to propose a new promising reagent, 2-(*t*-butoxycarbonyloxyimino)-2-phenylacetonitrile (**3c**), which is an easy-to-prepare, stable, and highly reactive crystalline material and affords contaminant-free Boc-amino acids in high yields by following a conventional procedure.

In the course of studies on the application of *N*-hydroxy compounds to peptide synthesis in our laboratory, it was noticed that some oximes could be suitable as the activator and carrier of Boc groups. Oximes are usually regarded as unstable compounds which are easily hydrolyzed or rearranged. However, certain ketoximes which possess electron-withdrawing substituents and have no hydrogen atoms on the α-carbon atoms are relatively stable and can be converted into their Boc derivatives through the corresponding chloroformates.



Scheme 1.

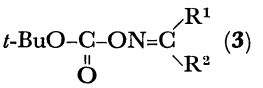
In practice those oximes were allowed to react first with phosgene or trichloromethyl chloroformate in the presence of *N*,*N*-dimethylaniline or pyridine in inert solvents, and the resultant chloroformates were treated *in situ* with *t*-butyl alcohol and pyridine to give Boc-oximes. Some of the derivatives prepared are listed in Table 1. Of these, diethyl (*t*-butoxycarbonyloxyimino)malonate (**3a**) was the most reactive one; it readily introduced the Boc group to amino acids within 1 to 3 h. Compound **3c** was a stable crystalline material and moderately reactive among the compounds listed in Table 1; it completed the reaction within 4 to 5 h. The other cardonates were so unstable that mainly **3a** and **3c** were subjected to further studies.



Scheme 2.

An advantage of **3c** over **3a** is the easy removal of 2-(hydroxyimino)-2-phenylacetonitrile (**2c**) from the reaction mixture. When **3a** is used it requires critical pH adjustment to 7.0 for the removal of diethyl (hydroxyimino)malonate (**2a**) from the reaction mixture. To simulate extraction conditions for the removal of **2a** and **2c**, their ethyl acetate solutions were shaken with water in the presence of sodium hydroxide, sodium hydrogencarbonate, or triethylamine. The oxime **2c** transferred only with sodium hydroxide but not with the others, suggesting an easy way for complete removal of **2c** from the reaction mixture. Both **3a** and **3c** were quite stable to competitive hydrolysis by sodium hydroxide or triethylamine in dioxane–water at room

Table 1. *t*-Butoxycarbonylated oximes



	R^1	R^2	Yield (%)	Mp (°C)	Recrystd. from
3a	COOEt	COOEt	77.2	oil	—
3b	$COCH_3$	COOEt	57.0	oil	—
3c	CN	C_6H_5	65.0	84—86	methanol
3d	CN	C_6H_4-Cl(*p*)	6.7	91—92	methanol
3e	CN	1-naphthyl	31.1	90—92	methanol
3f	C_6H_5	C_6H_5	70.6	131—133	toluene–pet. ether

Table 2. Yields of Boc-amino acids and reaction conditions employed[a]

Boc-Amino Acid	Reagent	Base (equiv)	Solvent[b]	Reaction time (h)	Oxime extraction	Yield (%)	Mp (°C)	Ref.
Boc-Ala-OH	**3a**	TEA 1.2	A*	1	ether	84.1	82—84	19
-Ala-OH	**3c**	TEA 1.5	B*	4	ether	80.3	82—84	
-Arg(NO_2)-OH	**3a**	$NaHCO_3$ 1.5	A[c]	3	EtOAc	70.0	115—116(dec)[d]	31
-Arg(NO_2)-OH	**3c**	TEA 1.5	B*	1[e]	ether	80.0	123—125	19
-Asn-OH	**3c**	TEA 1.5	C	20	EtOAc	85.8	173(dec)	18
-CyS(Bzl)-OH	**3c**	TEA 1.5	C	3	EtOAc	94.0	65—67	19
-Glu$(OH)_2$	**3c**	TEA 1.5	C	3	EtOAc	78.4	103—105(dec)	18
-Gly-OH	**3c**	TEA 1.5	C	2	EtOAc	86.9	86—88	18
-Gly-OH	**3c**	TEA 1.5	C*	40 min[f]	EtOAc	81.4	86—88	
-Ile-OH·DCHA	**3c**	NaOH 1.0	A*	3	ether	89.2	123—125	9
-Leu-OH·1/2H_2O	**3c**	TEA 1.5	C*	3	EtOAc	72.0	78—84	19
-Leu-OH·1/2H_2O	**3c**	TEA 1.5	C	3	ether	99.1	78—82	
-Met-OH·DCHA	**3c**	TEA 1.5	C*,[g]	3	EtOAc	82.1	137—139	19
-Phe-OH	**3b**	TEA 1.0	A	2	EtOAc	54.0	84—86	19
-Phe-OH·DCHA	**3c**	TEA 1.5	C[h]	2	C_6H_6	65.5	222—223	20
-Phe-OH·DCHA	**3c**	TEA 1.5	C	5	ether	98.2	221—223	
-Phg-OH	**3a**	TEA 1.5	D*	3	EtOAc	86.0	87—89.5	21
-Pro-OH	**3c**[i]	TEA 1.5	E*	1.5	C_6H_6	87.8	132—133	19
-Pro-OH	**3f**	TEA 1.5	F*	24	EtOAc	13.0	132—133	
-Thr-OH·DCHA	**3c**	FEA 1.5	C	3	EtOAc	99.7	151—153	19
-Trp-OH	**3c**	TEA 1.5	C	3	EtOAc	98.6	137—138(dec)	19
-Tyr-OH·DCHA	**3c**	NaOH 2.0[j]; TEA 1.0	C	4	EtOAc	81.8	212(dec)	9

a) The amino acids used, with the exceptions of glycine and D-phenylglycine, are of L-configuration. The reactions were carried out in 10 mmol scale with 1.1 equiv of the reagents at 20 to 25 °C unless stated otherwise. All products were purified following descriptions in the literatures, and optical rotations observed were within the limit of error in comparison with reported values. b) Five to 10 ml of organic solvents and water per 10 mmol amino acid were used unless cited below. Those with asterisks were removed *in vacuo* before the extraction of oximes; A: *t*-butyl alcohol/water, B: acetone/water, C: dioxane/water, D: methanol/water (70/70 ml), E: methanol/dioxane/water (15/5/10 ml), and F: dioxane/chloroform/water (20/10/10 ml). c) 40/100 ml. d) The difference of the melting points of the two N^{α}-Boc-N^{G}-nitro-L-arginine preparations will be due to polymorphism. e) Warmed for a while at 50 °C. f) At 40 °C. g) 14/20 ml. h) 30/20 ml. i) An equiv of **3c** was used. j) Sodium hydroxide was neutralized before the extraction.

temperature. Compound **3c** dissolves only partly in a solvent–water mixture at an initial stage of the reaction, but the mixture usually becomes homogeneous within 1 h. Yields of Boc-amino acids and conditions employed for each reaction are summarized in Table 2. In the case of L-tyrosine, two equivalents of sodium hydroxide and an equivalent of triethylamine were employed to obtain a clear solution, and a small amount of a side-product, *N,O*-bis-Boc-L-tyrosine, was detected on a thin layer chromatogram. However, the side-product could be eliminated effectively from *N*-Boc-L-tyrosine through salt formation with dicyclohexylamine. To secure a high yield and the purity of the products, other solvents than dioxane should be removed before the extraction of **2a** or **2c** from the reaction mixture. Omission of the removal of dioxane did not cause any trouble unless a large excess of it was used. The selection of a suitable solvent for the removal of **2a** or **2c** may be important

Table 3. Oxime carbonates prepared for the introduction of other amino protective groups

$$R^3\text{-O-}\overset{\overset{O}{\|}}{C}\text{-ON=}C\begin{matrix}R^1\\R^2\end{matrix}$$

	R^3	R^1	R^2	Yield (%)	Mp (°C)	Recrystd. from
3g	C_6H_5-CH_2-	CN	C_6H_5	61.9[a]	73—75	EtOAc/Hex.[c]
3h	*p*-MeO-C_6H_4-CH_2-	CN	C_6H_5	35.5[b]	112—113	EtOAc/Hex.
3i	Cl_3C-CH_2-	CN	C_6H_5	87.2[a]	82—84	MeOH
3j	*c*-C_3H_5-CH(Me)-	CN	C_6H_5	18.1[b]	65—67	MeOH
3k	*p*-MeO-C_6H_4-CH_2-	COOEt	COOEt	80.0[a]; 78.4[d]	syrup	—

a) Prepared from the corresponding alkyl or aralkyl chloroformates with the oximes by following the conventional procedure.[28] b) Prepared by following a similar procedure to that described for **3c**. c) Hexane. d) Prepared by following the procedure for **3a**.

for the Boc-amino acids with lipophilic side chains. For example, in the preparation of Boc-L-phenylalanine with **3c**, the use of ethyl acetate or benzene lowered the yield, whereas the use of ether gave a satisfactory result. Possible contaminants, **2a** and **2c**, were detected on fluorescent indicator-impregnated silica gel plates.

In comparison with **1** and 2-(*t*-butoxycarbonylthio)-4,6-dimethylpyrimidine (**4**),[18] **3c** showed a much higher reactivity. In the preparation of Boc-glycine, for example, **3c** completed the reaction within 2 h at room temperature,[1] whereas both **1** and **4** required more than 20 h at 40 °C and room temperature, respectively. Furthermore, **3c** is less toxic (LD_{50} orally in mice: 8000 mg/kg) and the by-product **2c** is easily recovered and recycled.

This procedure is wholly applicable to the preparation of other *N*-protected amino acids, and the reagents prepared for such purposes are listed in Table 3. Examples of *N*-protected amino acids, prepared by similar procedures to those described in the experimental section, are *N*-benzyloxycarbonyl-L-serine[32] (87.8%), *N*-(*p*-methoxybenzyloxycarbonyl)-L-phenylalanine[33] (64.6%), *N*-(2,2,2-trichloroethoxycarbonyl)-D-phenylglycine (mp 141—143 °C, 92.1%),[34] and *N*-(1-cyclopropylethoxycarbonyl)-D-phenylglycine (mp 94—96 °C, 88.5%).[34] The 1-cyclopropylethoxycarbonyl group was easily removed under the conditions required for the Boc group.

Experimental

The capillary melting points were observed on a Hoover "Uni-Melt" apparatus and are uncorrected. Precoated silica gel 60 F_{254} plates (Merck) were used for thin layer chromatography (TLC).

Materials. Trichloromethyl chloroformate was obtained from Hodogaya Chemical Co., Ltd., Tokyo. Diethyl (hydroxyimino)malonate (**2a**),[22] ethyl 2-(hydroxyimino)acetoacetate (**2b**),[23] 2-(hydroxyimino)-2-(*p*-chlorophenyl)acetonitrile (**2d**),[24] benzophenone oxime (**2f**),[25] 1-cyclopropylethanol,[29] and *p*-methoxybenzyl chloroformate[30] were prepared according to the literature.

2-(Hydroxyimino)-2-phenylacetonitrile (2c). Into an ice-cooled solution of benzyl cyanide (117 g, 1 mol) and sodium hydroxide (40 g, 1 mol) in methanol (300 ml) was introduced gaseous methyl nitrite[26] which was generated from a suspension of sodium nitrite (83 g) in a mixture of methanol (53 ml) and water (50 ml) by dropwise addition of a mixture of concentrated sulfuric acid (32 ml) and water (65 ml). The mixture was stirred for 2 h at room temperature and concentrated to dryness. The residue was dissolved in water and washed with toluene twice. The aqueous layer was acidified with concentrated hydrochloric acid to precipitate **2c**: 120 g (82%); mp 119—124 °C (lit,[27] mp 126—128 °C); TLC, R_f=0.50 (chloroform–methanol (9: 1)). This was used in the next step without further purification.

2-(Hydroxyimino)-2-(1-naphthyl)acetonitrile (2e). This was prepared from 2-(1-naphthyl)acetonitrile by the procedure described above. The syrupy product obtained in 36.2 % yield was used in the next step without purification.

Diethyl (t-Butoxycarbonyloxyimino)malonate (3a). To a well-stirred solution of phosgene (151 g, 1.53 mol) in benzene (800 ml) was added dropwise a solution of **2a** (289 g, 1.53 mol) and *N*,*N*-dimethylaniline (DMA) (186 g, 1.53 mol) in benzene (800 ml) at 5—6 °C over the period of 2.5 h under nitrogen atmosphere. The mixture was stirred for 2 h at 5—6 °C, and for 18 h at room temperature. To the resultant mixture was added dropwise a mixture of *t*-butyl alcohol (225 g, 3.06 mol) and pyridine (Pyr) (486 ml, 6.12 mol) at 5—6 °C over the period of 1.5 h under nitrogen. Stirring was continued for 18 h at room temperature. After filtration of precipitates the filtrate was washed with cold 1M hydrochloric acid (1 l×6) and cold water (1 l×3), and stirred with sodium hydrogencarbonate solution (70 g in 1 l) for 3 h at room temperature. The organic phase was washed with 5% sodium carbonate solution (100 ml×2), water (200 ml×2) and saturated sodium chloride solution (100 ml), and dried over magnesium sulfate with 15 g of charcoal. Evaporation gave 341 g (77.2%) of **3a**. IR (neat, cm^{-1}) 1800, 1750; nuclear magnetic resonance (NMR) (in CCl_4, δ(ppm)) 1.39 (3H, triplet, J=6.5 Hz), 1.41 (3H, triplet, J=6.5 Hz), 1.59 (9H, singlet), 4.34 (4H, quartet, J=6.5 Hz).

Ethyl 2-(t-Butoxycarbonyloxyimino)acetoacetate (3b). This was prepared from **2b** by a procedure similar to that described in the preparation of **3a**, except for the use of Pyr in the place of DMA. IR (neat, cm^{-1}) 1780, 1730, 1690; NMR (in CCl_4, δ(ppm)) 1.37 (3H, triplet, J=7 Hz), 1.57 (9H, singlet), 2.48 (3H, singlet), 4.34 (2H, quartet, J=7 Hz).

2-t-Butoxycarbonyloxyimino-2-phenylacetonitrile (3c). To a stirred solution of trichloromethyl chloroformate (6.7 ml, 0.055 mol) or an equivalent of phosgene (0.11 mol) in benzene (30 ml) was added dropwise a solution of **2c** (14.6 g, 0.1 mol), DMA (12.0 g), and dioxane (5 ml) in benzene (100 ml) at 3—5 °C. The mixture was stirred for 6 h at room temperature and allowed to stand overnight. To the resultant mixture was added a solution of *t*-butyl alcohol (11.1 g) and Pyr (16.0 ml) in benzene (20 ml) at 5—10 °C. The mixture was allowed to react for 3 h at the same temperature, then for 4 h at room temperature, and to stand overnight. The mixture was then washed with water, 1 M hydrochloric acid, water, 5% sodium hydrogencarbonate solution, and water again, and dried over magnesium sulfate. After evaporation of the solvent, the residue was triturated with 20 ml of 90% aqueous methanol, filtered, washed with 30 ml of the same solvent, and dried to give 17.0 g of **3c**: mp 84—86 °C; TLC, R_f=0.74 (chloroform–methanol (9: 1)).

Found: C, 63.69; H, 5.71; N, 11.20%. Calcd for $C_{13}H_{14}O_3N_2$: C, 63.40; H, 5.73; N, 11.38%. IR (Nujol, cm^{-1}) 1785; NMR (in $CDCl_3$, δ(ppm)) 1.62 (9H, singlet), 7.2—8.2 (5H, multiplet).

2-(t-Butoxycarbonyloxyimino)-2-(p-chlorophenyl)acetonitrile (3d). This was prepared from **2d** (37.5 mmol) by a procedure similar to that described above except that chloroformylation was done by the use of DMA in a mixture of dioxane (10 ml), tetrahydrofuran (10 ml), and dichloromethane (70 ml). (See Table 1).

Found: C, 55.80; H, 4.65; N, 10.07; Cl, 12.62%. Calcd for $C_{13}H_{13}O_3N_2Cl$: C, 55.62; H, 4.67; N, 9.98; Cl, 12.63%. IR (Nujol, cm^{-1}) 1790; NMR (in $CDCl_3$, δ(ppm)), 1.63 (9H, (singlet), 7.50 and 7.90 (2H, each, AB Quartet, J=4.5 Hz).

2-(t-Butoxycarbonyloxyimino)-2-(1-naphthyl)acetonitrile (3e). A similar procedure to that described above was followed in toluene. IR (Nujol, cm^{-1}) 1790.

Found: C, 68.85; H, 5.38; N, 9.40%. Calcd for $C_{17}H_{16}O_3N_2$: C, 68.90; H, 5.44; H, 9.46%.

(t-Butoxycarbonyloxyimino)diphenylmethane (3f). A similar procedure to that described above was employed. IR (Nujol, cm^{-1}) 1770; NMR (in $CDCl_3$, δ(ppm)), 1.48 (9H, singlet), 7.17—7.65 (10H, multiplet).

General Procedures for the Introduction of t-Butoxycarbonyl Group. a) By the Use of 3a: To a solution of L-alanine (0.89 g, 10 mmol) and triethylamine (TEA) (1.68 ml, 1.2 mmol) in a mixture of water (5 ml) and *t*-butyl alcohol (5 ml) was added

3a (3.2 g, 11 mmol). The mixture was stirred for 1 h at room temperature. After evaporation of *t*-butyl alcohol and addition of ether and 5% sodium hydrogencarbonate solution, the mixture was adjusted to pH 7.0 precisely with a citric acid solution. The aqueous layer was separated, overlaid with ether, and adjusted to pH 7.0 again. The aqueous layer separated was acidified with citric acid solution and extracted with ethyl acetate (EtOAc). The extract was treated in the usual manner to give *N*-*t*-butoxycarbonyl-L-alanine: 1.59 g (84.1%); mp 82—84 °C. Conditions employed in the other examples are presented in Table 2.

b) By the Use of **3c**: To a solution of L-tryptophan (2.05 g, 10 mmol) and TEA (2.10 ml, 15 mmol) in water (6 ml) was added dioxane (6 ml) and crystalline **3c** (2.71 g, 11 mmol) at room temperature. The mixture became homogeneous within 1 h and stirring was continued for two more hours. After addition of water (15 ml) and EtOAc (20 ml), the aqueous layer was separated, washed with EtOAc (20 ml), acidified with 5% citric acid solution, and extracted with EtOAc. The extract was treated in the usual manner to give *N*-*t*-butoxycarbonyl-L-tryptophan: 3.00 g (98.6%); mp 137—138 °C (decomp). Other amino acids were allowed to react by the same procedure, unless stated otherwise in Table 2. Dicyclohexylammonium salts were crystallized by the addition of dicyclohexylamine (1.8 g per 10 mmol amino acid) to an ether solution of *t*-butoxycarbonylamino acids.

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