t-Butyl Benzotriazol-1-yl Carbonate and Benzyl Benzotriazol-1-yl Carbonate. Now Reactive Amino Protective Reagents for t-Butoxycarbonylation and Benzyloxycarbonylation of Amines and Amino Acids

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New amino protective reagents, t-butyl benzotriazol-1-yl carbonate and benzyl benzotriazol-1-yl carbonate, for t-butoxycarbonylation and benzyloxycarbonylation of amines and amino acids have been developed. t-Butyl benzotriazol-1-yl carbonate reacts rapidly and cleanly with various amines and amino acids to afford N-Boc amines and N-Boc amino acids in high yields and benzyl benzotriazol-1-yl carbonate is also found to be very effective in the benzyloxycarbonylation of amino acids.

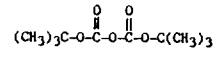
Introduction

The t-butoxycarbonyl (Boc) group is one of the most important amino protective groups along with benzyloxycarbonyl (Cbz) group in peptide synthesis.[1] Since t-butyl chloroformate is only fairly stable above 10°C,[2] it is difficult to isolate t-butyl chloroformate as a pure form in high yields. Thus, considerable efforts have been devoted to the development of a variety of useful and reliable reagents for the preparation of N-Boc amino acids during last 30 years.[1]

Boc-azide (I), which has caused occasionally severe accidents due to the explosive nature of the reagent, has been widely used for many years.[3] The reactive and stable di-t-butyl dicarbonate (II)[4] and 2-(t-butoxycarbonyloxyimino)-2-phenylacetonitrile (III)[5], which are commercially available, are very effective and widely used reagents among many reagents for this purpose. Furthermore, 4-dimethylamino-1-t-butoxycarbonylpyridinium chloride (IV),[6] a water soluble and relatively stable reagent, has been known to be the most reactive among previously known reagents.[7] In view of the great importance of the t-butoxycarbonyl group in peptide synthesis, a great need still exists for an efficient and stable reagent for the t-butoxycarbonylation of amines and amino acids.

In connection with our research program toward the development of active esters and related compounds,[8] we have recently reported that t-butyl benzotriazol-1-yl carbonate (BBC, V) is exceedingly effective in the t-butoxycarbonylation of amino acids.[9] This paper describes a full detail of the preparation of BBC and benzyl benzotriazol-1-yl carbonate (BZBC), and their use for the t-butoxycarbonylation and benzyloxycarbonylation of amines and amino acids.

I



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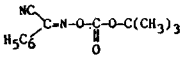

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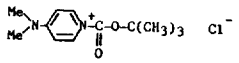


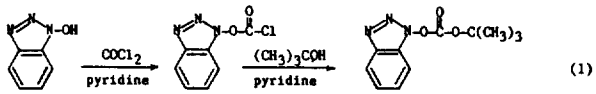
IV

Results and Discussion

t-Butyl Benzotriazol-1-yl Carbonate (BBC) and Benzyl Benzotriazol-1-yl Carbonate (BZBC).

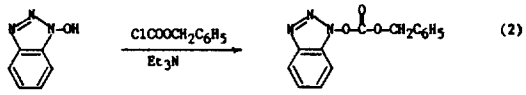
Benzotriazol-1-yl chloroformate was prepared by adding a solution of an equimolar amount of 1-hydroxybenzotriazole and pyridine in methylene chloride to the solution of an excess amount of phosgene in toluene under cooling (-20~-10°C). The resulting product was relatively unstable and was mainly decomposed to 1-hydroxybenzotriazole during workup with cold water. Thus, benzotriazol-1-yl chloroformate was used in a crude form. BBC could be prepared readily by the reaction of benzotriazol-1-yl chloroformate with equimolar amounts of t-butyl alcohol and pyridine in methylene chloride at room temperature for 2 h (eq. 1). BBC was obtained in 85% yield as a stable crystalline compound and could be kept at room temperature over a long period of time without any decompositions.

BZBC was conveniently prepared by the reaction of benzyl chloroformate with equimolar amounts of 1-hydroxybenzotriazole and triethylamine at 0°C for 0.5 h (eq. 2). BZBC was obtained in an essentially quantitative yield as colourless crystals and showed no sign of decomposition when kept at room temperature for one month.



(1)

HOBT BBC



(2)

BZBC

t-Butoxycarbonylation of Amines and Amino Acids

First, reaction of BBC with a variety of structurally different amines was studied and some of experimental results are summarized in Table 1. Reaction of simple primary amines such as benzylamine and n-propylamine proceeded to completion within 20 min at room temperature in various solvents such as methylene chloride, acetonitrile, tetrahydrofuran, and p-dioxane to afford the corresponding t-butyl carbamates in high yields.

Simple secondary amines such as diethylamine and piperidine were smoothly t-butoxycarbonylated in acetonitrile at room temperature, whereas t-butoxycarbonylation of sterically hindered secondary amine like 2,6-dimethylpiperidine proceeded with difficulty in methylene chloride at

room temperature, yielding 35% of N-(t-butoxycarbonyl)-2,6-dimethylpiperidine along with 36% of the starting material in 24 h. We have found that the use of dimethylformamide as a solvent is very effective in the t-butoxycarbonylation of hindered amines. Thus, 2,6-dimethylpiperidine and N-isopropylcyclohexylamine were completely converted to the corresponding t-butyl carbamates at 50°C within 3 h. The superiority of dimethylformamide as a solvent has been further demonstrated in the t-butoxycarbonylation of aniline. Reaction of aniline with BBC in methylene chloride, acetonitrile, or p-dioxane did not proceed to an observable extent, even after prolonged stirring for 24 h at room temperature, whereas the reaction in dimethylformamide proceeded smoothly to yield N-(t-butoxycarbonyl) aniline in 90% yield at room temperature in 5 h. Similarly, imidazole was cleanly t-butoxycarbonylated at room temperature in 6 h.

Reaction of various amino acids with BBC was also examined and BBC was found to be highly reactive toward amino acids. For example, when BBC was added to the solution of L-proline and triethylamine in aqueous p-dioxane, the t-butoxycarbonylation occurred almost instantly and was complete within 10 min at room temperature, indicating that BBC is one of the most reactive reagent among various reagents currently available for the t-butoxycarbonylation of amino acids.

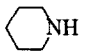
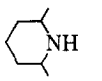
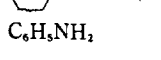
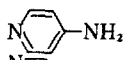
In order to find out optimum conditions for the t-butoxycarbonylation of amino acids, we briefly examined the effects of bases and solvents by use of L-proline as a model compound. With aqueous p-dioxane as the solvent, the effect of four bases was examined at room temperature and it was found that the yield of N-Boc proline was the highest using 1.5 equiv of triethylamine (99%): sodium carbonate (89%), sodium bicarbonate (69%), and sodium hydroxide (63%). Furthermore, the reaction occurred much more rapidly using triethylamine (less than 10 min) than using sodium carbonate (30 min), sodium bicarbonate (1 h), or sodium hydroxide (1 h). Although aqueous p-dioxane and aqueous dimethylformamide were found to be equally effective in the t-butoxycarbonylation of L-proline, aqueous p-dioxane was slightly better than aqueous dimethylformamide in terms of the rapidity of the reaction.

On the basis of these results obtained, the standard condition employed for the t-butoxycarbonylation of amino acids with BBC involved the use of 1.5 equiv of triethylamine and aqueous p-dioxane at room temperature. As shown in Table 2, the t-butoxycarbonylation of several amino acids used in this study was complete within 10 min at room temperature and the corresponding N-Boc amino acids were isolated in high yields. The identities of N-Boc amino acids were confirmed by comparison of mp, NMR, and IR data with reported data. $[\alpha]_D$ values of N-Boc amino acids obtained in this study were in good agreement with reported values within the limit of experimental errors.[7,10]

Benzyloxycarbonylation of Amino Acids.

Although benzyl chloroformate has been widely used for the preparation of N-Cbz amino acids, it is thermally unstable and decompose to yield carbon dioxide and benzyl chloride when it is stored over a long period of time. In view of en-

Table 1. Preparation of N-Boc Amines[a]

Amine	Reaction Condition: Solvent	Temp, °C	Time, h	Isolated Yield, %[b]
$C_6H_5CH_2NH_2$	CH_2Cl_2	r.t.	0.25	95
	CH_3CN	r.t.	0.25	97
	THF	r.t.	0.25	92
	p-Dioxane	r.t.	0.25	90
$CH_3CH_2CH_2NH_2$	CH_3CN	r.t.	0.2	97
$(CH_3CH_2)_2NH$	CH_3CN	r.t.	1.5	91
	CH_3CN	r.t.	0.5	94
	DMF	r.t.	0.2	86
	CH_2Cl_2	r.t.	30	35(36)
	DMF	r.t.	24	65
	DMF	50	2.5	68
	DMF	50	2	60
$C_6H_5NH_2$	CH_2Cl_2	r.t.	24	0(95)
	CH_3CN	r.t.	24	0
	p-Dioxane	r.t.	24	0
	DMF	r.t.	5	90
	DMF	r.t.	1.5	63
	DMF	r.t.	6	95

[a] The reaction was carried out on 1-3 mmol scale of amine with 1.0 equiv of the reagent. [b] The numbers in parentheses indicate the recovery of the starting material and the yields were based on the amine.

Table 2. Preparation of N-Boc and N-Cbz Amino Acids in Aqueous p-Dioxane[a]

Amino Acid	N-Boc Amino Acid: Yield, %	[Mp, °C]	$[\alpha]_D^b$	N-Cbz Amino Acid: Yield, %	[Mp, °C]	$[\alpha]_D^b$
Pro	90	133-135	-60.7 (1,AcOH)	89	75-77	-58.3 (1.7, AcOH)
Phe	86	221-222[b]	+25.2 (1, MeOH)	98	86-88	-5.4 (1, AcOH)
Met	85	137-139[b]	+16.2 (1.8, MeOH)	95	65-67	-19.7 (1.4, MeOH)
Thr	93	150-152[b]	+9.2 (0.4, MeOH)	96	syrup	-5.2 (1, AcOH)
Ser	85	140-142[b]	+13.1 (0.9, DMF)	97	119-120	+3.4 (0.7, AcOH)
Val	98	syrup	-5.0 (1.1, AcOH)	96	57-60	+1.1 (1, EtOH)
Ala	91	syrup	-19.2 (0.5, AcOH)	90	83-85	-12.8 (0.8, AcOH)
Tyr	85	212-215[b]	+3.2 (2.4, AcOH)			
Leu	99	69-72[c]	-23.2 (2.4, AcOH)			
Try	86	137-138	-17.9 (0.9, AcOH)			

[a] Mp and $[\alpha]_D$ values if the known compounds were within the limit of error in comparison with the reported data and $[\alpha]_D$ values were measured at room temperature (15-25°C). See references 7, 10 and 11 for the reported data. [b] Dicyclohexylammonium salt. [c] Monohydrate.

courging results obtained with BBC, we turned our attention to BZBC for the introduction of benzyloxycarbonyl group in amino acids under mild conditions. Thus, the reaction of several amino acids with BZBC was briefly studied under the almost same conditions employed in the t-butoxycarbonylation of amino acids with BBC and the results are summarized in Table 2.

It is also found that the benzyloxycarbonylation with BZBC occurred almost instantly in aqueous p-dioxane at room temperature and the corresponding N-Cbz amino acids were isolated in excellent yields. The identities of N-Cbz amino acids were also confirmed by mp, NMR, and IR data and $[\alpha]_D$ values with reported data.[7e,11]

Experimental

NMR spectra were recorded with a Varian T-60A spectrometer and chemical shifts are expressed as δ units relative to tetramethylsilane. Infrared spectra were recorded on a Perkin-Elmer 267 and the frequences are given in reciprocal centimeters. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected and optical rotations were determined on an Autopol III automatic polarimeter.

Preparation of t-Butyl Benzotriazol-1-yl Carbonate.

To a stirred solution of phosgene (2.5M, 20 m*l*, 50 mmol) in toluene at −20°C was dropwise added a solution of 1-hydroxybenzotriazole (1.35g, 10 mmol) and pyridine (830 mg, 10.5 mmol) in methylene chloride (30 m*l*) and the resulting solution was stirred at −20∼−10°C for 1 h. After an excess amount of phosgene and most of solvents were removed in vacuo, methylene chloride (10 m*l*) was added to the reaction mixture and a solution of t-butyl alcohol (750 mg, 10 mmol) and pyridine (830 mg, 10.5 mmol) in methylene chloride (10 m*l*) at −20 °C was added to the flask. The reaction mixture was allowed to warm to room temperature over 2 h and washed with water, saturated sodium bicarbonate, and dried over anhydrous magnesium sulfate. After evaporation of solvents under reduced pressure, the crude product was crystallized from methylene chloride-petroleum ether to give BBC (1.98g, 85%). g, 85%). mp 90-91 °C; NMR($CDCl_3$)δ 1.92 (s, 9H), 7.22-8.15 (m, 4H); IR(KBr) 1755 cm^{-1}. Anal. Calcd for $C_{11}H_{13}N_3O_3$: C, 56.16; H, 5.57; N, 17.86. Found: C, 55.77; H, 5.42; N, 17.53.

Preparation of Benzyl Benzotriazol-1-yl Carbonate.

To a solution of benzyl chloroformate (1.43 m*l*, 10 mmol) and 1-hydroxybenzotrizole (1.35 g, 10 mmol) in methylene chloride (30 m*l*) at 0°C was added triethylamine (1.4 ml, 10 mmol). After being stirred for 30 min at 0°C, the reaction mixture was diluted with methylene chloride (20 m*l*), washed with saturated sodium bicarbonate, dried over anhydrous magnesium sulfate, and evaporated to dryness. The crude product was crystallized from methylene chloride-petroleum ether to give BZBC (2.47 g, 90%). mp 127-129°C; NMR($CDCl_3$) δ 5.72 (s, 2H), 7.42 (s, 5H), 7.18-8.32 (m, 4H); IR(KBr) 1750 cm^{-1}. Anal. Calcd for $C_{14}H_{11}N_3O_3$: C, 61.07; H, 4.03; N, 15.27. Found: C, 61.57; H, 3.93; N, 15.05.

General Procedure for the preparation of t-Butyl Carbamates.

To a stirred solution of t-butyl benzotriazol-1-yl carbonate (1 mmol) in acetonitrile or appropriate solvents was added an amine and the solution was stirred at room temperature or 50°C until completion of the reaction. The reaction was diluted with methylene chloride, washed with saturated sodium bicarbonate and water, dried over anhydrous magnesium sulfate, and evaporated to dryness. The crude product was purified by distillation with a Kugelrohr apparatus or crystallization. The products obtained here are known compounds and physical and spectral data of the products were in good agreement with reported data.[10e]

General Procedure for the Preparation of N-Boc and N-Cbz Amino Acids.

To a stirred solution of L-amino acids (2 mmol) and triethylamine (3 mmol) in aqueous p-dioxane (10 m*l*) at room temperature was added t-butyl benzotriazol-1-yl carbonate (2 mmol) or benzyl benzotriazol-1-yl carbonate (2 mmol). After being stirred at room temperature for 10 min, the reaction mixture was concentrated under reduced pressure, acidified with 3% aqueous oxalic acid (pH 3.5), and extracted three times with ethyl acetate. The combined extracts were washed with saturated cupric sulfate and dried over anhydrous magnesium sulfate. Evaporation of solvents afforded the crude products, which could be further purified by recrystallization.

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Characterization of Korean Clays and Pottery by Neutron Activation Analysis (I). Characterization of Korean Porcelainsherds

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Data on the concentration of Na, K, Sc, Cr, Fe, Co, Cu, Ga, Rb, Cs, Ba, La, Ce, Sm, Eu, Tb, Lu, Hf, Ta, and Th obtained by neutron activation analysis have been used to characterize Korean porcelainsherds by multivariate analysis. The mathematical approach employed is principal component analysis (PCA). PCA was found to be helpful for dimensionality reduction and for obtaining information regarding (a) the number of independent causal variables required to account for the variability in the overall data set, (b) the extent to which a given variable contributes to a component and (c) the number of causal variables required to explain the total variability of each measured variable.

Introduction

The combination of the advances in multielement analysis and the development in the application of mathematical methods for the analysis of data has enabled valuable information to be extracted from analytical data, that would not have been readily accesible otherwise.[1-3] Archaeology is one of the major beneficiaries of such a combination approach,[4] the other notable areas of application being environmental science[5] and forensic science.[6] Pattern recognition (PR) techniques[1,2] have a significant role for the extraction of information from analytical data.

This paper reports on the work done in the development and application of principal component analaysis (PCA) for the grouping of Korean ancient porcelain-sherds, using elemental abundance data generated by present authors. The results obtained by PCA in the present work have been discussed in relation to those reported previously using other PR techniques such as single linkage and minimal spanning tree.[4]

Principal Component Analysis (PCA)

PCA is an approach akin to factor analysis and is known in pattern recognition literature as Karhunen-Loeve transformation. The set of data on N samples with M variables measured (N × M data) can be represented as a set of N points in M dimensional space. For convenience, the initial variables can be preprocessed and represented as deviation from their respective means so that all the variables are equally weighted. The new variables will be

$$Z_{ij} = (X_{ij} - \overline{X}_j) \quad (1)$$

for $i = 1, 2, 3, \cdots N$ and $j = 1, 2, 3, \cdots M$.

The product $Z' \cdot Z/N$ gives the variance-coveriance(VC) matrix S and is taken to represent the dispersion in the original data set. The objective of PCA is to generate abstract causal variables (factors) from matrix S using Eckart-Young theorem or by considering the projections of the N points in M dimensions onto some orthogonal axes such that the variances for the projected points are maximum in their respective directions.

The first principal component is a vector such that the perpendicular projections of the N points on to that vector have the largest possible variance. The second principal component is a vector such that it is orthogonal to the first and the projections of the N points onto it account for the largest fraction of remaining variance.

The new component variables Y_{ij} are given by the linear combination,

$$Y_{ij} = k1 \cdot Z1 + k2 \cdot Z2 + \cdots + km \cdot Zm \quad (2)$$

In matrix notion $Y = Z \cdot K$, where $Z(N,M)$ is the original data matrix, $K(M,P)$ is the matrix of coefficients and $Y(N,P)$ is the matrix of component scores. P is the rank of the VC matrix.

Derivation of the Coefficients in PCA.

N points are assumed to be projected onto a vector in M dimensional space such that the projections will have the maximum variance. For a vector to fit this condition in a M dimensional space, it is required that the sum of the squares of its direction cosines should be unity.

Let $K1$ be the $(M \times 1)$ vector of the direction cosines such that,

$$K1' \cdot K1 = 1 \quad (3)$$

The projection of a point Z_{ij} onto this vector is given by,

$$Y_{ij} = Z_{ij} \cdot K1 \quad (4)$$