USE OF DI-tert-BUTYL PYROCARBONATE TO OBTAIN N-tert-BUTOXYCARBONYL DERIVATIVES OF AMINO ACIDS

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The tert-butoxycarbonyl amino-protective group (BOC group) is widely used in synthetic bioorganic chemistry, particularly in peptide chemistry [1]. However, tert-butoxycarbonylating reagents are still difficultly accessible under laboratory conditions, which is due to the necessity of using phosgene [2] or carbon oxysulfide [3] for their synthesis. As a tert-butoxycarbonylating reagent we have proposed di-tert-butyl pyrocarbonate (BOC_2O) [4]. It was found previously [5] that BOC_2O can be obtained by the preparatively simple reaction of sodium tert-butyl carbonate with trichloroacetyl chloride. Later, it was established that in place of trichloroacetyl chloride it is better to use 3,5-dinitrobenzoyl chloride. The yield of BOC_2O is increased and 3,5-dinitrobenzoic acid is readily regenerated from the wash-waters.

The present paper gives the results of an investigation of the possibility of using BOC_2O for the synthesis of N-tert-butoxycarbonyl derivatives of amino acids (BOC-amino acids), including some trifunctional acids. As a N-tert-butoxycarbonylating agent BOC_2O has a number of advantages over the $BOCN_3$ [6] and BOCF [7] that are widely used for this purpose. In addition to its accessibility, ease of purification and stability, an important advantage is the fact that in its reaction with amines no byproducts are formed other than CO_2 and tert-butanol $(tert\text{-}C_4H_9CO)_2O + H_2NR \rightarrow tert\text{-}C_4H_9OCONHR + tert\text{-}C_4H_9OH + CO_2$. Consequently, neither the correction of the pH of the reaction medium, as with the use of $BOCN_3$ [6] and BOCF [7], nor complex purification from phenol (where BOCOAr is used [9]) is required. All this makes BOC_2O an extremely promising reagent for obtaining BOC derivatives of compounds of various classes. The reaction of BOC_2O with salts of amino acids in aqueous ethanolic solution begins after a short induction period (2-3 min) and is accompanied by the vigorous evolution of CO_2. With a 20-50% molar excess of BOC_2O after only an hour the initial amino acid has reacted completely and can no longer be detected on a chromatogram. The yields of BOC amino acids are high and are determined only by the losses on isolation; the constants correspond to those given in the literature [6, 7] (Table 1).

Lysine and histidine were acylated with two equivalents of BOC_2O. With an equimolar ratio of the reactants, lysine and histidine form a mixture of mono- and di-BOC derivatives with the initial amino acids. The di-BOC-lysine was characterized in the form of its salt with dicyclohexylamine (DCHA), and the di-BOC-histidine in the form of the crystalline N-hydroxysuccinimide ester. To obtain N^{ε}-BOC-lysine we used a solution of the copper salt of lysine [8] in aqueous pyridine. BOC_2O reacts only slowly with asparagine in aqueous ethanolic solution at 20°C. Consequently, this reaction was performed in aqueous dimethylformamide (DMFA) at 40-45°C. Under the reaction conditions selected, tryptophan reacts with BOC_2O only at the α-amino group. In tyrosine, the phenolic hydroxyl also reacts to some extent, so that the N^{α}-BOC-tyrosine obtained is contaminated with a small amount of N^{α},O-di-BOC-tyrosine [7].

EXPERIMENTAL

The work was performed with amino acids of the L series. Ascending thin-layer chromatography was performed on "Silufol" plates in the following solvent systems: 1) benzene–acetone–acetic acid (100:50:2) (Rf_1), and 2) chloroform–methanol–25% ammonia (5:3:1) (Rf_2). The solvent front migrated 6-6.5 cm. The BOC derivatives of amino acids were revealed with ninhydrin at 120°C.

Di-tert-Butyl Pyrocarbonate (BOC_2O). Carbon dioxide was bubbled into a suspension of 1.2 mole of sodium tert-butoxide in two liters of petroleum ether until the increase in weight was 53 g. To the result-

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TABLE 1. N^{α}-BOC-(Amino Acids) [6, 7]

Amino acid	Yield, %	mp, °C	R_{f_1}	$[\alpha]_D^{25}$
Ala	84	80–81	0,51	−25 (ACOH)
Asn	78	177–179	0,13*	
Gly	95	90–92	0,43	
N^{im} – BOCHis	95	Amorphous	0,44	
Leu	96	78–81	0,53†	−30 ACOH
Phe	86	82–83	0,56	
Pro	95	133–134	0,55	
Try	94	138–140	0,54†	
N^{ε} BOCLys·DCHA	70	136–138	0,54‡	
Tyr—DCHA	84	197–201	0,45**	

*From water;
†from aqueous ethanol;
‡from ether;
**contaminated with BOC_2Tyr.

ing suspension of sodium tert-butyl carbonate was added a solution of 0.5 mole of 3,5-dinitrobenzoyl chloride in 200 ml of benzene, and the mixture was stirred at 20°C for 4 h. Then 100 ml of dimethylformamide was added and it was stirred at 20°C for another 2 h and at 50°C for 1 h. Then it was cooled to 15°C, and water was added until the precipitate of salts had dissolved (about 500 ml). The organic layer was separated off, washed with water, and dried with $MgSO_4$. After the solvent had been distilled off (40°C at 10 ml), the residue was distilled in vacuum. This gave 78.0 (71.5%) of di-tert-butyl pyrocarbonate with mp 75-78°C at 3 mm, mp 21-23°C [5]. The wash-waters were acidified with hydrochloric acid, and 96.2 g (91%) of 3,5-dinitrobenzoic acid was filtered off.

General Procedure for the N-tert-Butoxycarbonylation of Amino-Acid Salts with BOC_2O. A solution of 0.5 g of $NaHCO_3$ in 5 ml of water, 10 ml of tert-butanol, and 3 ml of BOC_2O were added to a solution of 10 mmole of amino acid in 10 ml of NaOH. The mixture was stirred at 20°C for 2 h, diluted with water to 50 ml, and extracted with petroleum ether (2 × 20 ml). The aqueous solution was acidified with citric acid and extracted with ethyl acetate (20 + 2 × 15 ml). The extract was washed with brine and dried with $MgSO_4$. After evaporation of the ethyl acetate, the residue was crystallized [6] or converted into crystalline derivatives (see Table 1).

N^{α}-BOC-Asparagine. To a solution of 1.3 g (10 mmole) of asparagine in 10 ml of NaOH were added 10 ml of dimethylformamide and 3 ml (~13 mmole) of BOC_2O, and the mixture was stirred at 45-50°C for 3 h. Then it was evaporated in vacuum (40°C) to the state of a syrup, and this was diluted with water to 50 ml, extracted with ether (2 × 15 ml), acidified with 2 N HCl to pH 3, and left in the refrigerator for 2 h. The resulting precipitate was filtered off, washed with water, and dried in vacuum over $CaCl_2$. This gave 1.8 g (77.5%) of N^{α}-BOC-asparagine with mp 177-179°C, R_{f_1} 0.13 ([7] 176-177°C).

N^{α}, N^{im}-Di-BOC-histidine. By the general method, 1.5 g of histidine and 5.5 ml of BOC_2O yielded, after evaporation of the dried solution, 3.5 g of chromatographically pure di-BOC-histidine with R_{f_1} 0.44 in the form of a dry foam [7].

The N-hydroxysuccinimide ester of di-BOC-histidine was obtained with the aid of dicyclohexylcarbodiimide. After recrystallization from a mixture of diethyl ether and petroleum ether the yield was 78%, mp 113-115°C, R_{f_1} 0.55. Found %: C 52.95; H 6.55; N 12.63. $C_{20}H_{23}N_4O_8$.

Copper Salt of N^{ε}-BOC-Lysine. A solution of the copper salt of lysine [8] (from 18.2 g of lysine) in 70 ml of water was mixed with 40 ml of pyridine and 17 ml of Et_3N and, with vigorous stirring over 30-40 min a solution of 25 ml of BOC_2O in 25 ml of tert-butanol was added. Then the mixture was stirred for another hour and the solidifying reaction mixture was diluted with 200 ml of water and cooled to 10°C, and the precipitate was filtered off. This was resuspended in 150 ml of water, filtered off, washed with water, methanol, and ether, and dried over $CaCl_2$. This gave 16.5 g (60%) of the copper salts of N^{ε}-BOC-lysine, decomp. 255-260°C ([8] 230-240°C). Found %: C 47.68; H 6.92; N 9.7 Cu 11.4. $C_{22}H_{40}N_4O_8Cu$.

SUMMARY

It has been shown that BOC derivatives of amino acids can be obtained by the tert-butoxycarbonylation of amino-acid salts with di-tert-butylpyrocarbonate in aqueous organic solutions.

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