under the conditions $\sum_i f_i = 1$ and $1 \geqslant f_i \geqslant 0$, where $f_i$ is the molar fraction of the i-th secondary structure, $[\theta]_\lambda^{exp}$ is the experimental CD spectrum and $[\theta]_{i\lambda}$ is the reference CD spectrum for the i-th structure. The values of $[\theta]_\lambda^{exp}$ and $[\theta]_{i\lambda}$ were taken every 1 nm in the wavelength range from 205 to 240 nm. The calculations were performed on a Hewlett-Packard 9830 A computer.

## CONCLUSION

1. The secondary structures of the reserve proteins from cotton seeds have been determined by the CD method.

2. It has been established that the conformations of the main globulin components of cotton seeds change according to the conditions of their storage.

3. It has been shown that the secondary structure of the 11S globulin differs for seeds from the cotton plant varieties Tashkent 1 and 108-F.

## LITERATURE CITED


1. S. I. Asatov, É. G. Yadgarov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 54 (1978).
2. M. A. Kuchenkova, É. F. Redina, N. L. Ovchinnikova, and P. Kh. Yuldashev, Khim. Prir. Soedin., 687 (1977).
3. I. A. Bolotina, V. O. Chekhov, V. Yu. Lugauskas, A. V. Finkel'shtein, and O. B. Ptitsyn, Mol. Biol., 14, 891 (1980).
4. I. A. Bolotina, V. O. Chekov, V. Yu. Lugauskas, and O. B. Ptitsyn, Mol. Biol., 14, 902 (1980).
5. I. A. Bolotina, V. O. Chekhov, V. Yu. Lugauskas, and O. B. Ptitsyn, Mol. Biol., 15, 167 (1981).
6. K. G. Kumar, L. V. Venkataraman, and A. G. A. Rao, J. Agric. Food. Chem., 28, 518 (1980).
7. A. L. Li, R. A. Rafikov, Z. S. Yunusova, I. A. Bolotina, T. S. Yunusov, and G. P. Moiseeva, Khim. Prir. Soedin., 349 (1984) [in this issue].
8. S. I. Asatov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 791 (1977).
9. S. S. Yunusova and T. S. Yunusov, Khim. Prir. Soedin., 770 (1981).
10. T. Devenyi and J. Gergely, Amino Acids, Peptides, and Proteins, Elsevier, New York (1974).


# ACTIVATION OF A CARBOXY GROUP BY DIALKYL PYROCARBONATES. SYNTHESIS OF SYMMETRICAL ANHYDRIDES AND ARYL ESTERS OF N-PROTECTED AMINO ACIDS USING DI-tert-BUTYL PYROCARBONATE AS CONDENSING REAGENT


V. F. Pozdnev and M. Yu. Chernaya



UDC 547.466.493



It has been shown that di-tert-butyl pyrocarbonate can be used as a condensing reagent in the production of anhydrides and some aryl esters of carboxylic acids. The synthesis of anhydrides and of phenyl, p-nitrophenyl, β-naphthyl, and quinolin-8-yl esters of N-protected amino acids is described.


Di-tert-butyl pyrocarbonate (DBPC) which we have proposed as a reagent for the introduction of the tert-butoxycarbonyl (BOC) amino-protective grouping into amino acids [1, 2] has recently found wide use not only for obtaining N-BOC derivatives of amino acids [3, 4] but also for the tert-butoxycarbonylation of compounds of various classes. DBPC comparatively readily acylates hydrazides [5, 6] and, in the presence of alkali, the mercapto group of cysteine [2] and the phenolic hydroxyl of tyrosine [7].


Institute of Biological and Medicinal Chemistry, Academy of Medical Sciences of the USSR, Moscow.


Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 357-362, May-June, 1984. Original article submitted April 14, 1983.

During further investigations of the chemical properties of DBPC we have observed that in aprotic solvents (benzene, ethyl acetate) and in the presence of pyridine at the ordinary temperature (10-20°C), DBPC is capable of reacting with carboxylic acids. In this process, $CO_2$ is liberated and mixed anhydrides of the carboxylic acids and of tert-butyl carbonate are formed. Anhydrides of this type have been obtained previously by a transacylation reaction from mixed anhydrides of aromatic acids and tert-butyl carbonate [8]. However, it is impossible to obtain the mixed anhydrides in the individual state from DBPC, since, as they accumulate in the reaction mixture, they react with the initial acid with the formation of symmetrical anhydrides. At an equimolar ratio of the initial reactants, a mixture of anhydrides is formed (according to TLC). The spots of the anhydrides were identified by comparison with samples obtained by indirect syntheses and by staining specific reagents (see the Experimental part). If, however, the acid and the DBPC are taken in a ratio of 2:1, the symmetrical anhydrides of the carboxylic acids remain the main reaction product and can be isolated in high yields. Under these conditions, the DBPC is converted completely into $CO_2$ and tert-butanol which is inert under the reaction conditions and does not interfere with the formation and isolation of the anhydrides.

$$RCO_2H \cdot Py + (t\text{-}BuOCO)_2O \rightarrow [RCO-O-COOBu\text{-}t] + t\text{-}BuOH + CO_2$$
$$\downarrow RCO_2H \cdot Py$$
$$(RCO)_2O + t\text{-}BuOH + CO_2$$

The fundamental role of pyridine in the process of forming anhydrides of acids with the aid of DBPC must be mentioned. Its replacement by triethylamine or N-methylmorpholine proved to be ineffective. In this case, the pyridine is obviously not merely a base but also a catalyst [9], not being neutralized or consumed in the reaction, so that it can be added in an amount less than the stoichiometric amount. By using DBPC it is possible to obtain both anhydrides of monofunctional acids (cinnamic anhydride is obtained with a yield of 93%) and also anhydrides of N-acylated amino acids (for example, the anhydride of BOC-L-aspartic acid; yield 75%). Other examples are given in Experimental part.

The possibility of using DBPC as a condensing agent is not limited to the synthesis of anhydrides. It has been found that if phenols which, under the given conditions, do not react with DBPC or react with it more slowly than with the acid are added to a mixture of an acid and DBPC in the presence of pyridine, the acylation of the phenol by the carboxylic acid takes place. We do not yet have definite information concerning the routes and mechanism of this reaction. The acylation of phenols may either be performed by the mixed anhydride or it may proceed through a stage of the formation of symmetrical anhydrides. In the preparative respect, the method of obtaining certain aryl esters of N-protected amino acids using the DBPC-pyridine system has shown good results. The synthesis can be performed by mixing all the starting materials directly. In addition to carboxylic acid, under these conditions aryl tert-butyl carbonates are formed as impurities, and their amount rises with an increase in the acidity of the phenols. However, in the preparation of phenyl, naphthyl, or quinolin-8-yl esters the amounts of carbonates produced as impurities are small (1-5%) and the yields of esters in individual cases are greater than 90%. In the preparation of p-nitrophenyl esters of N-protected amino acids, more carbonate is formed and it frequently interferes with the crystallization of the ester. The amount of carbonate can be diminished if the nitrophenol is added to the reaction mixture not at once but in small portions. In this case, nitrophenyl esters can be obtained with yields of up to 80%.

As follows from a consideration of the results presented in Table 1, the yields of esters of phenols are, in the majority of cases, comparable with the yields obtained by using other condensing reagents. The physiochemical characteristics of the products obtained also agree well with those published in the literature. In the preparative respect, the use of DBPC for the synthesis of aryl esters is particularly convenient in those cases where the esters crystallize from the reaction mixture in the process of formation. Since in this case no sparingly soluble byproducts whatever (as, for example, dicyclohexylurea from dicyclohexylcarbodiimide) are formed, isolation of the esters is substantially simplified.

## EXPERIMENTAL

We used N-protected amino acids from Reanal (Hungary). DBPC was obtained from sodium tert-butyl carbonate and trichloroacetyl chloride [10]. The compositions of the reaction mixtures, the processes of isolation and purification, and the individualities of the compounds obtained were checked by thin-layer chromatography on Silufol UV-254 plates. The

TABLE 1

| Initial acid | Yield of ester, % | mp, °C | $[\alpha]_D^{20}$, deg | Literature figures: yield, % | Literature figures: mp, °C | Literature figures: $[\alpha]_D^{20}$, deg |
|---|---|---|---|---|---|---|
| Phenyl esters of $N^{\alpha}$-protected L-(amino acids) (c 1; $C_2H_5OH$) | | | | | | |
| Z-Ala | 88 | 93—95 | —49,2 | 86 | 94—96 | —48,2 [15] |
| Z-Gly | 86 | 66—67 | | 88 | 67—68 | [15] |
| Boc-Cys (Bzl) | 85 | 86—87 | —30,1 | 94 | 81 | —27 [16] |
| Boc-Met | 92 | 72—73 | —45,1 | 86 | 72 | —43 [16] |
| Boc-Pro | 54 | 60—61 | —64,6 | 63 | 63 | —66 [16] |
| Boc-Trp | 74 | 150—151 | —13,3 | 96 | 153 | —11.6 [16] |
| p-Nitrophenyl esters of $N^{\alpha}$-protected L-(amino acids) (c 1; DMF) | | | | | | |
| Z-Pro | 70 | 90—91 | —67,7 | 65 | 94—96 | —68 2 [18] |
| Z-Val | 82 | 62—63 | —24,5 | 60 | 62—64 | —25 [19] |
| Boc-Ala | 60 | 82—83 | —52,3 | 30 | 83 | —52,5 [20] |
| Boc-Cys (Bzl) | 65 | 94—95 | —40 3 | 81 | 95—96 | —37,5 [21] |
| Boc-Gly | 64 | 63—64 | | 77 | 66—68 | [20] |
| Boc-Leu | 65 | 92—93 | —48,2 | 65 | 94—95 | —48 [21] |

spots were detected on the plates visually under a UV lamp or, in the case of the BOC derivatives of amino acids and their esters and anhydrides, they were detected by spraying with a 5% solution of ninhydrin in a mixture of n-butanol and formic acid (9:1) followed by heating at ∿100°C; the spots of anhydrides were detected by spraying the plates with a 0.5 M solution of $NH_2OH$ in methanol, heating at 50°C, and then spraying with an acidified solution of $FeCl_3 \cdot 6H_2O$ in methanol. Compounds with a tert-butoxy group were identified by spraying the plates with a solution of mercury sulfate in dilute sulfuric acid (Dengès' reagent) followed by heating at 60-70°C (yellow spots). Benzyloxycarbonyl derivatives of amino acids were detected by spraying with a 1 M solution of hydrobromic acid in acetic acid, heating at 100°C, and then spraying with a solution of ninhydrin in n-butanol. For the nonspecific detection of amino compounds we used the o-tolidine reagent after chlorination. Below we give the $R_f$ values in the following solvent mixtures: 1) cyclohexane—ethyl acetate (3:1) and 2) benzene—methyl ethyl ketone (4:1). Angles of optical rotation were determined on a Perkin-Elmer 241 polarimeter (Sweden). The melting points of the substances were determined in open capillaries in an instrument with electrical heating and are uncorrected.

In the standard working up of the reaction mixtures, solutions in ethyl acetate were washed with water, with 5% $KHCO_3$, with water, with 5% $H_2SO_4$ solution, with water, and with saturated NaCl solution and were dried with $Na_2SO_4$ and evaporated in a rotary evaporator at 40°C.

The final products were dried in vacuum over $P_2O_5$ and KOH at room temperature for 16-20 h.

Cinnamic Anhydride. A solution of 1.5 g of cinnamic acid, 1.3 ml of DBPC, and 0.4 ml of pyridine in 10 ml of ethyl acetate was stirred for 7 h and was then concentrated in vacuum to incipient crystallization; the residue was diluted with ether (∿20 ml) and kept in the refrigerator and the resulting precipitate was filtered off and washed with cooled ether; after drying it amounted to 1.3 g (93%) of cinnamic anhydride with mp 136-137°C. $R_{f_1}$ 0.43.

Anhydride of N-tert-butoxycarbonyl-L-aspartic Acid. A solution of 0.93 g of BOC-aspartic acid, 1.0 ml of DBPC, and 0.2 ml of pyridine in 5 ml of ethyl acetate was stirred for 5 h and was then diluted to 25 ml with ethyl acetate, washed with 5% $H_2SO_4$, with water, and with saturated NaCl solution, dried with $Na_2SO_4$, and evaporated. The residue was crystallized from a mixture of acetone, ether, and hexane to give 0.65 g (75%) of the anhydride of BOC-L-aspartic acid with mp 133-134°C, $[\alpha]_D^{20}$ — 39.0° (c 1; $CH_3COOH$) [12].

Anhydride of N-Benzyloxycarbonyl-L-phenylalanine. A solution of 1.0 g of benzyloxycarbonyl-L-phenylalanine, 0.5 ml of DBPC, and 0.2 ml of pyridine in 10 ml of benzene was stirred for 16 h and was then diluted with hexane to 25 ml and was kept in the refrigerator until crystallization had ended. The precipitate was filtered off, washed with cooled hexane, and dried. This gave 0.6 g (60%) of the anhydride of benzyloxycarbonyl-L-phenylalanine with mp 139-140°C, $[\alpha]_D^{20}$ + 29.5° (c 1; $CHCl_3$) [13, 14].

Anhydride of N-Benzyloxycarbonyl-L-valine. This was obtained in a similar manner to the preceding example. Yield 73%, mp 99-101°C, $[\alpha]_D^{20}$ + 19.5° (c 1; $CHCl_3$) [14].

Phenyl Ester of N-Benzyloxycarbonyl-L-phenylalanine. A solution of 1.5 g of benzyloxycarbonyl-L-phenylalanine, 0.6 g of phenol, 1.5 ml of DBPC, and 0.5 ml of pyridine in 10 ml of benzene was stirred for 3 h and was left for 12 h, during which time a crystalline precipitate formed. The mixture was diluted with 15 ml of petroleum ether, and the precipitate was filtered off, washed with cooled hexane, and dried to give 1.8 g (96%) of the phenyl ester of benzyloxycarbonyl-L-phenylalanine with mp 107-107.5°C, $[\alpha]_D^{20}$ − 18° (c 1; $C_2H_5OH$) [15]. In a repeat experiment, the yield of product was 88%, mp 108-109°C.

Diphenyl Ester of N,N'-Di-tert-butoxycarbonyl-L-cystine. A solution of 2.2 g of N,N'-di-BOC-L-cystine, 3.0 ml of DBPC, 1.2 g of phenol, and 0.5 ml of pyridine was stirred for 5 h and was left for 12 h. Then it was diluted with ethyl acetate to 30 ml, and after the standard working-up procedure, the residue was crystallized from a mixture of ether and hexane. This gave 2.4 g (81%) of the diphenyl ester of di-BOC-L-cystine with mp 157-158°C, $[\alpha]_D^{20}$ − 103° (c 1; $C_2H_5OH$). $R_f^{0.35}$. Found, %: C 56.5; H 6.3; N 5.0. $C_{28}H_{36}N_2O_8S_2$. Calculated, %: C 56.7; H 6.1; N 4.7.

Other phenyl ethers of N-protected amino acids were obtained similarly, and the results are given in Table 1.

β-Naphthyl Ester of N-tert-Butoxycarbonyl-L-phenylalanine. A solution of 1.3 g of BOC-L-phenylalanine, 0.7 g of β-naphthol, 1.5 ml of DBPC, and 0.3 ml of pyridine in 10 ml of benzene was stirred for 4 h, and then 0.2 g of β-naphthol was added and the mixture was left for 12 h. After this, it was diluted with ethyl acetate to 30 ml and worked up by the second procedure to give a residue which was crystallized from cyclohexane. This gave 1.9 g (97%) of product with mp 106-110°C. After recrystallization from a mixture of ether and hexane the yield was 1.6 g (82%), mp 117-118°C, $[\alpha]_D^{20}$ − 14.7° (c 1; DMF). $R_{f_1}$ 0.52. Found, %: C 73.3; H 6.5; N 3.7. $C_{24}H_{25}NO_4$. Calculated, %: C 73.6; H 6.4; N 3.6.

Quinolin-8-yl Ester of N-Benzyloxycarbonyl-L-alanine. A solution of 2.24 g of benzyloxycarbonyl-L-alanine, 1.7 g of 8-hydroxyquinoline, 2.3 ml of DBPC, and 0.4 ml of pyridine in 15 ml of ethyl acetate was stirred for 16 h, and diluted with ethyl acetate to 30 ml, and, after the standard working up procedure, the residue was crystallized from a mixture of diethyl ether and petroleum ether. After drying, 2.8 g (80%) of the quinolin-8-yl ester of benzyloxycarbonyl-L-alanine was obtained with mp 99-100°C, $[\alpha]_D^{20}$ − 68° (c 1; DMF). $R_{f_1}$ 0.60 [17].

Quinolin-8-yl Ester of N-Benzyloxycarbonyl-L-phenylalanine. A solution of 3.0 g of benzyloxycarbonyl-L-phenylalanine, 1.5 g of 8-hydroxyquinoline, 2.3 ml of DBPC, and 0.5 ml of pyridine in 15 ml of benzene was stirred for 7 h. A crystalline precipitate formed. The mixture was diluted with 20 ml of petroleum ether, and the precipitate was filtered off, washed with a mixture of ether and hexane, and dried to give 3.6 g (84%) of a product with mp 141-142°C, $[\alpha]_D^{20}$ − 69.7 (c 1; DMF). The filtrate was evaporated in vacuum and the residue was crystallized to give another 0.4 g of the quinolin-8-yl ester of benzyloxycarbonyl-L-phenylalanine with mp 140-141°C. The total yield was 93%. $R_{f_2}$ 0.62 [17].

Quinolin-8-yl Ester of N,O-Di-tert-butoxycarbonyltyrosine. A solution of N,O-di-BOC-L-tyrosine, 0.8 g of 8-hydroxyquinoline, 1.5 ml of DBPC, and 0.4 ml of pyridine in 10 ml of benzene was stirred for 12 h and was then diluted with 20 ml of ethyl acetate and, after the standard working-up procedure, the residue was crystallized from a mixture of diethyl ether and petroleum ether. This gave 1.8 g (71%) of the quinolin-8-yl ester of N,O-di-BOC-L-tyrosine with mp 102-103°C, $[\alpha]_D^{20}$ − 3.13 (c 1; DMF). $R_{f_2}$ 0.72. Found, %: C 60.9; H 6.5; N 5.4. $C_{28}H_{32}N_2O_7$. Calculated, %: C 61.2; H 6.3; N 5.5.

Quinolin-8-yl Ester of N-tert-Butoxycarbonyl-D-tryptophan. A solution of 1.5 g of BOC-D-tryptophan, 0.8 g of 8-hydroxyquinoline, 1.3 ml of DBPC, and 0.5 ml of pyridine in 20 ml of benzene was stirred for 16 h, whereupon a precipitate formed. The mixture was diluted with ether, and the precipitate was filtered off, washed with ether, and dried to give 2.0 g (92%) of the desired product, with mp 186-187°C, $[\alpha]_D^{20}$ + 65.8° (c 1; DMF). $R_{f_2}$ 0.25. Found, %: C 69.6; H 5.9; N 9.6. $C_{25}H_{26}N_3O_4$. Calculated, %: C 69.4; H 6.0; N 9.7.

P-Nitrophenyl Ester of N-Benzyloxycarbonyl-L-phenylalanine. A solution of 3.0 g of benzyloxycarbonyl-L-phenylalanine, 1.5 g of p-nitrophenol, 2.3 ml of DBPC, and 0.4 ml of pyridine in 15 ml of benzene was stirred for 5 h, and then another 0.5 g of p-nitrophenol

and 0.5 ml of DBPC were added and the mixture was left for 12 h, whereupon a crystalline precipitate formed. After dilution with petroleum ether, the precipitate was filtered off, and it was washed with a mixture of ether and hexane and was dried to give 3.4 g (81%) of the p-nitrophenyl ester of benzyloxycarbonyl-L-phenylalanine with mp 123-124°C, $[\alpha]_D^{20}$ − 24.9° (c 1; DMF).

p-Nitrophenyl Ester of N-tert-Butoxycarbonyl-O-tert-butyl-L-tyrosine. A solution of 1.9 g of N-BOC-O-tert-butyl-L-tyrosine, 1.7 ml of DBPC, and 0.4 ml of pyridine in 10 ml of ethyl acetate was added dropwise over 45 min, and the mixture was stirred for 4 h and was left for 12 h. Then it was diluted with ethyl acetate to 30 ml and, after the standard working-up procedure and crystallization of the residue from a mixture of diethyl ether and petroleum ether, 1.9 g (81%) of the p-nitrophenyl ester of N-BOC-O-tert-butyl-L-tyrosine was obtained with mp 138-139°C, $[\alpha]_D^{20}$ − 2.8° (c 1; DMF). $R_{f_2}$ 0.33. Found, %: C 63.0; H 6.4; N 5.9. $C_{24}H_{30}N_2O_7$. Calculated, %: C 62.8; H 6.6; N 6.1.

Other p-nitrophenyl esters of N-protected amino acids were obtained similarly, and the results are given in Table 1.

## CONCLUSION

It has been shown that di-tert-butyl pyrocarbonate can be used as a condensing reagent in the preparation of the anhydrides and some aryl esters of carboxylic acids.

## LITERATURE CITED


1. V. F. Pozdnev, Khim. Prir. Soedin., 384 (1971).
2. V. F. Pozdnev, Khim. Prir. Soedin., 764 (1974).
3. V. F. Pozdnev, Bioorg. Khim., 3, 1605 (1977).
4. V. F. Pozdnev, N. N. Podgornova, N. K. Zentsova, G. I. Aukone, and U. O. Kalei, Khim. Prir. Soedin., 543 (1979).
5. S. I. Dolinskaya, V. F. Pozdnev, and E. S. Chaman, Khim. Prir. Soedin., 266 (1974).
6. V. F. Pozdnev, Zh. Org. Khim., 13, 2531 (1977).
7. V. F. Pozdnev, Khim. Prir. Soedin., 536 (1982).
8. V. F. Pozdnev, Zh. Khim. Org. Khim., 14, 1558 (1978).
9. E. M. Cherkasova, S. V. Bogatkov, and Z. P. Golovina, Usp. Khim., 46, 477 (1977).
10. V. F. Pozdnev, E. A. Smirnova, N. N. Podgornova, N. K. Zentsova, and U. O. Kalei, Zh. Org. Khim., 15, 106 (1979).
11. S. Veibel, Identification of Organic Compounds, G. E. C. Gad, Copenhagen (1954).
12. E. Schröder and E. Klinger, Ann. Chem., 673, 208 (1964).
13. E. Wünsch and G. Wendlberger, Chem. Ber., 100, 160 (1967).
14. F. M. F. Chen., K. Kuroda, and N. L. Benoiton, Synthesis, 928 (1978).
15. I. J. Galpin, P. M. Hardy, G. W. Kenner, J. R. McDermott, R. Ramage, J. H. Seely, and R. G. Tyson, Tetrahedron, 35, 2577 (1979).
16. B. Castro, G. Evin, C. Selve, and R. Seyer, Synthesis, 413 (1977).
17. H. D. Jakubke and A. Voigt, Chem. Ber., 99, 2419 (1966).
18. M. Goodman and K. C. Stueben, J. Am. Chem. Soc., 81, 3980 (1959).
19. M. Itoh, Chem. Pharm. Bull., 18, 784 (1970).
20. E. Sandrin and R. A. Boissonas, Helv. Chim. Acta, 46, 1637 (1963).
21. H. C. Beyermann, C. A. M. Boers-Boonekamp, Massen van der Brink, and H. Zimmermanova, Recl. Trav. Chim. Pays-Bas, 87, 257 (1968).