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Working with Hazardous Chemicals

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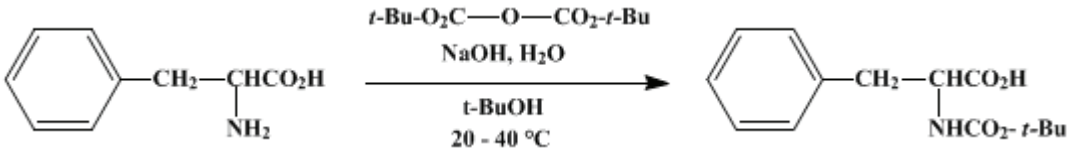
These paragraphs were added in September 2014. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

DOI:10.15227/orgsyn.063.0160

Organic Syntheses, Coll. Vol. 7, p.70 (1990); Vol. 63, p.160 (1985).

tert-BUTOXYCARBONYLATION OF AMINO ACIDS AND THEIR DERIVATIVES: *N-tert*-BUTOXYCARBONYL-l-PHENYLALANINE

[L-Phenylalanine, *N*-[(1,1-dimethylethoxy)carbonyl]-]



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Checked by Thomas von Geldern, Mark A. Sanner, and Clayton H. Heathcock.

1. Procedure

A 4-L, four-necked, round-bottomed flask, equipped with an efficient stirrer, a dropping funnel, reflux condenser, and thermometer is charged with a solution of 44 g (1.1 mol) of sodium hydroxide in 1.1 L of water. Stirring is initiated and 165.2 g (1 mol) of L-phenylalanine (Note 1) is added at ambient temperature, and then diluted with 750 mL of *tert*-butyl alcohol (Note 2). To the well-stirred, clear solution (Note 3) is added dropwise within 1 hr, 223 g (1 mol) of di-*tert*-butyl dicarbonate (Note 4). A white precipitate appears during addition of the di-*tert*-butyl dicarbonate. After a short induction period, the temperature rises to about 30–35°C. The reaction is brought to completion by further stirring overnight at room temperature. At this time, the clear solution will have reached a pH of 7.5–8.5. The reaction mixture is extracted two times with 250 mL of pentane, and the organic phase is extracted three times with 100 mL of saturated aqueous sodium bicarbonate solution. The combined aqueous layers are acidified to pH 1–1.5 by careful addition of a solution of 224 g (1.65 mol) of potassium hydrogen sulfate in 1.5 L of water (Note 5). The acidification is accompanied by copious evolution of carbon dioxide. The turbid reaction mixture is then extracted with four 400-mL portions of ethyl ether (Note 6). The combined organic layers are washed two times with 200 mL of water, dried over anhydrous sodium sulfate or magnesium sulfate, and filtered. The solvent is removed under reduced pressure using a rotary evaporator at a bath temperature not exceeding 30°C (Note 7). The yellowish oil that remains is treated with 150 mL of hexane and allowed to stand overnight (Note 8). Within 1 day the following portions of hexane are added with stirring to the partially crystallized product: 2 × 50 mL, 4 × 100 mL, and 1 × 200 mL. The solution is placed in a refrigerator overnight; the white precipitate is collected on a Büchner funnel and washed with cold pentane. The solid is dried under reduced pressure at ambient temperature to constant weight to give a first crop. The mother liquor is evaporated to dryness leaving a yellowish oil, which is treated in the same manner as described above, giving a second crop (Note 9). The total yield of pure white *N-tert*-butoxycarbonyl-L-phenylalanine is 207–230 g (78–87%), mp 86–88°C, $[\alpha]_D^{20}$ + 25.5° (ethanol, *c* 1.0) (Note 10).

2. Notes

1. L-Phenylalanine puriss. from Fluka AG or Tridom Chemical Inc. was used.
2. All the solvents and reagents used were of pure grade and obtained from Fluka AG.
3. At this stage, the reaction mixture has a pH of 12–12.5.
4. Di-*tert*-butyl dicarbonate can be prepared according to Pope, B. M.; Yamamoto, Y.; Tarbell, D. S. *Org. Synth., Coll. Vol. VI* **1988**, 418 or purchased from Fluka AG. Di-*tert*-butyl dicarbonate melts at 22–24°C; this compound can be liquified by immersing the reagent bottle in a water bath with a maximum temperature of 35°C. Commercial material is 97–98% pure; a total of 223 g must be employed.
5. It is recommended that acidification be carried out at a temperature of 0–5°C.
6. Ethyl or isopropyl acetate may also be used as extraction solvents for less lipophilic *N-tert*-butoxycarbonyl amino acids.
7. Evaporation should be performed first at 10–20 mm, then at a pressure less than 1 mm in order to remove the *tert*-butyl alcohol completely. Remaining small quantities of *tert*-butyl alcohol lead to

difficulty in crystallization.
8. Seeding or scratching with a glass rod helps to induce crystallization.
9. Normally it is not worthwhile to isolate a third crop, which is of lower purity.
10. *N*-*tert*-Butoxycarbonyl-L-phenylalanine prepared by this method is obtained in a very pure state. Thin-layer chromatography shows a single spot and a content of less than 0.05% free amino acid. Acylation of lipophilic amino acids with excess di-*tert*-butyl dicarbonate may result to some extent in formation of the corresponding *N*-*tert*-butoxycarbonyl dipeptide.

3. Discussion

In recent years the *tert*-butoxycarbonyl (Boc) group has achieved a leading role as a protective group for the amino moiety of amino acids in peptide synthesis.[2] At one time the most widely used *tert*-butoxycarbonylating agent was the hazardous[3 4 5 6] and toxic *tert*-butyl azidoformate.[7 8] Di-*tert*-butyl dicarbonate[9 10 11] is a highly reactive and safe reagent of the "ready-to-use" type that reacts under mild conditions with amino acids,[9,12 13 14 15 16 17 18 19 20] peptides,[21 22 23] hydrazine and its derivatives,[24 25] amines,[26 27 28 29 30 31 32] and CH-acidic compounds[33] in aqueous organic solvent mixtures to form pure derivatives in very good yields. Acylation with di-*tert*-butyl dicarbonate proceeds normally without strict pH control. The procedure given here demonstrates a suitable large-scale and safe preparation of an *N*-*tert*-butoxycarbonylamino acid with extremely simple experimental operations. Table I shows some other Boc-amino acids and derivatives prepared by this method. *N*-*tert*-Butoxycarbonyl-L-phenylalanine has also been prepared by acylation of L-phenylalanine with other *tert*-butoxycarbonylating agents: *tert*-butyl 4-nitrophenyl carbonate,[34] *tert*-butyl azidoformate,[35 36 37] *tert*-butyl 2,4,5-trichlorophenyl carbonate,[38] *tert*-butyl pentachlorophenyl carbonate,[39] *tert*-butyl 8-quinolyl carbonate,[40] *tert*-butyl chloroformate,[41 42] *tert*-butyl fluoroformate,[43] *tert*-butyl phenyl carbonate,[44] *N*-*tert*-butoxycarbonyl-1*H*-1,2,4-triazole,[45] *tert*-butyl 4,6-dimethylpyrimidyl-2-thiol carbonate,[46] *N*-*tert*-butoxycarbonyloxyimino-2-phenylacetonitrile,[47] *tert*-butyl α-methoxyvinyl carbonate,[48] *tert*-butyl aminocarbonate (*tert*-butoxycarbonyloxyamine).[49] Recently, a simple method for the 4-dimethylaminopyridine-catalyzed *tert*-butoxycarbonylation of various types of amides has been reported.[50]

TABLE I
BOC-AMINO ACIDS PREPARED BY ACYLATION WITH DI-*tert*-BUTYL DICARBONATE

Boc-Amino Acids[a]	Solvent[b]	Base	Time (hr)[c]	Yield, (%)	mp, (° C)	$[\alpha]_D^{20}$	Remarks
Boc-Ala-OH	A	NaOH	16	92–94	82–83	−25.5 (acetic acid, *c* 2.0)	pH 8.0[e]
Boc-β-Ala-OH	A	NaOH	16	85–86	76–77		
Boc-Arg-OH	B	—	15	88	159–160 (dec)	−6.8 (acetic acid, *c* 1.0)	Extraction with *n*-butyl alcohol
Boc-Arg (NO_2)-OH[d]	B	NaOH	15	82	107	−22.0 (pyridine, *c* 2.0)	pH 8.5[e]
Boc-Asn-OH	C	NaOH	18	80–81	176 (dec)	−7.2 (dimethylformamide, *c* 2.0)	5 hr, 45–50°C
Boc-Asp (OBzl)-OH	A	NaOH	16	81–89	101–102	−19.7 (dimethylformamide, *c* 2.0)	pH 8.0[e]
Boc-Cys (Bzl)-OH	B	NaOH	15	65	86–87	−43.4 (acetic acid, *c* 1.0)	
$(Boc\text{-}Cys\text{-}OH)_2$	D	NaOH	16	85	143–145 (dec)	−115.6 (acetic acid, *c* 2.0)	

Boc-Gln-OH	E	NaOH	18	76	125 (dec)	−3.4 (ethanol, *c* 2.0)	pH 8.0[e]
Boc-Glu (OBzl)-OH[f]	B	NaOH	15	86	142–143	+13.2(methanol, *c* 1.0)	pH 8.5–9[e]
Boc-Gly-OH	A	NaOH	16	96	87–88		
Boc-His (Boc)-OH	A	$KHCO_3$	18	75	170 (dec)	+19.5 (chloroform, *c* 2.0)	
Boc-Ile-OH	A	NaOH	16	78	69–71	+2.8 (acetic acid, *c* 2.0)	
Boc-Leu-OH[g]	A	NaOH	18	96	85–87	−24.7 (acetic acid, *c* 2.0)	
Boc-Lys (Boc)-OH[f]	A	NaOH	16	82	138–139	+6.1 (dimethylformamide, *c* 1.5)	
Boc-Lys (CBZ)-OH	A	NaOH	18	96	Oil		
Boc-Met-OH	A	NaOH	18	60[h]	50–51	−22.8 (methanol, *c* 1.3)	
Boc-Met-OH[f]	A	NaOH	18	85	139–140	+18.2 (ethanol, *c* 2.0)	
Boc-Pro-OH	A	NaOH	12	95	134–135	−60.6 (acetic acid, *c* 2.0)	
Boc-Ser-OH	A	NaOH	16	66–82	86–88	−3.6 (acetic acid, *c* 2.0)	pH 8.5–9[e]
Boc-Ser (Bzl)-OH	B	NaOH	16	90	62–63	+19.2 (80% ethanol, *c* 2.0)	pH 8.5–9[e]
Boc-Thr-OH	A	NaOH	16	85	71–73	−8.2 (acetic acid, *c* 1.0)	
Boc-Trp-OH[i]	A	NaOH	16	96	137–138 (dec)	−18.2 (dimethylformamide, *c* 1.0)	
Boc-Trp-(FOR)-OH[f]	F	Et_3N	48	61	158–159[k]	+36.0 (ethanol, *c* 2.0)	
Boc-Tyr-OH	A	NaOH[l]	24	75	137[m]	+2.6 (acetic acid, *c* 1.0)	
Boc-Tyr-OH[f]	A	NaOH[l]	24	84	216	+2.6 (acetic acid, *c* 1.0)	
Boc-Tyr (Bzl)-OH	B	NaOH	18	70	110–111	+27.6 (ethanol, *c* 1.0)	pH 10.4[e]
Boc-Tyr (2,6-Cl_2-Bzl)-OH	A	NaOH	24	48	104 (dec)	+20.6 (ethanol, *c* 2.0)	
Boc-Val-OH	A	NaOH	16	85	76–78	−7.5 (acetic acid, *c* 1.0)	

[a]The amino acids used, with the exception of β-alanine and glycine, were of L-configuration The abbreviations used for amino acids and their protecting substituents concur with E. Wünsch.[2]

[b]Solvent systems: A: *tert*-butyl alcohol–water; B: dioxane–water; C: dimethylformamide–water; D: methanol–water; E: acetonitrile–water; F: dimethylformamide.

[c]The reaction was generally carried out at room temperature after the exothermic starting period had subsided. Progress of the reaction was monitored by TLC. Reaction times are not optimized.

[d]Crystallizes with 15% solvent (ethyl acetate).

[e]pH control is necessary.

[f]Dicyclohexylamine salt.

References and Notes

1. Fluka AG, Chemische Fabrik, CH-9470 Buchs, Switzerland.
2. Wunsch, E. In "Methoden der Organischen Chemie" (Houben-Weyl), 4th ed.; Thieme: Stuttgart, 1975; Vol. 15.
3. Fenlon, W. J. *Chem. Eng. News* **1976**, *54*, 3;
4. Koppel, H. C. *Chem. Eng. News* **1976**, *54*, 5;
5. Feyen, P. *Angew. Chem*. **1977**, *89*, 119; *Angew. Chem., Int. Ed*. **1977**, *16*, 115;
6. *WARNING: Org. Synth., Coll. Vol. VI* **1988**, 207.
7. Insalaco, M. A.; Tarbell, D. S. *Org. Synth., Coll. Vol. VI* **1988**, 207, 9–12;
8. Carpino, L. A.; Carpino, B. A.; Crowley, P. J.; Giza, C. A.: Terry, P. H. *Org. Synth. Coll. Vol. V* **1973**, 157–159.
9. Pozdnev, V. F. *Khim. Prir. Soedin*. **1974**, 764–767; *Chem. Abstr*. **1975**, *82*, 156690d;
10. Pope, B. M.; Yamamoto, Y.; Tarbell, D. S. *Org. Synth., Coll. Vol. VI* **1988**, 418;
11. Pozdnev, V. F.; Smirnova, E. A.; Podgornova, N. N.: Zentsova, N. K.; Kalei, U. O. *Zhur. Org. Khim*. **1979**, *15*, 106–109; *Chem. Abstr*. **1979**, *90*, 168196a.
12. Fauchère, J. L.; Leukart, O.; Eberle, A.; Schwyzer, R. *Helv. Chim. Acta* **1979**, *62*, 1385–1395;
13. Do, K. W.; Thanei, P.; Caviezel, M.; Schwyzer, R. *Helv. Chim. Acta* **1979**, *62*, 956–964;
14. Hubbuch, A.; Danho, W.; Zahn, H. *Liebigs Ann. Chem*. **1979**, 776–783;
15. Hofmann, K.; Finn, F. M.; Kiso, Y. *J. Am. Chem. Soc*. **1978**, *100*, 3585–3590;
16. Pozdnev, V. F. *Zhur. Obshch. Khim*. **1978**, *48*, 476–477; *Chem. Abstr*. **1978**, *89*, 24739m;
17. Pozdnev, V. F. *Bioorg. Khim*. **1977**, *3*, 1605–1610; *Chem. Abstr*. **1978**, *88*, 62595y;
18. Moroder, L.; Hallett, A.; Wünsch, E.; Keller, O.; Wersin, G. *Hoppe-Seyler's Z. Physiol. Chem*. **1976**, *357*, 1651–1653; *Chem. Abstr*. **1977**, *86*, 107005h;
19. Pozdnev, V. F. *Khim. Prir. Soedin*. **1980**, 379; *Chem. Abstr*. **1980**, *93*, 204996j;
20. Scott, J. W.; Parker, D.; Parrish, D. R. *Synth. Commun*. **1981**, *11*, 303–304;
21. Bullesbach, E. E.; Naithani, V. K. *Hoppe-Seyler's Z. Physiol. Chem*. **1980**, *361*, 723–734; *Chem. Abstr*. **1981**, *94*, 16076f;
22. Gattner, H. G.; Danho, W.; Behn, C.; Zahn, H. *Hoppe-Seyler's Z. Physiol. Chem*. **1980**, *361*, 1135–1138; *Chem. Abstr*. **1980**, *93*, 239908j;
23. Naithani, V. K.; Gattner, H. G. *Hoppe-Seyler's Z. Physiol. Chem*. **1981**, *362*, 685–695; *Chem. Abstr*. **1981**, *95*, 220291x.
24. Pozdnev, V. F. *Zhur. Org. Khim*. **1977**, *13*, 2531–2535; *Chem. Abstr*. **1978**, *88*, 89063h;
25. Dolinskaya, S. I.; Pozdnev, V. F.; Chaman, E. S. *Khim. Prir. Soedin*. **1974**, 266–267; *Chem. Abstr*. **1974**, *81*, 49994q.
26. Pirkle, W. H.; Simmons, K. A.; Boeder, C. W. *J. Org. Chem*. **1979**, *44*, 4891–4896;
27. Pozdnev, V. F. *Khim. Prir. Soedin*. **1980**, 408–409; *Chem. Abstr*. **1980**, *93*, 186700b;
28. Smith, M. A.; Weinstein, B.; Greene, F. D. *J. Org. Chem*. **1980**, *45*, 4597–4602;
29. Muchowski, J. M.; Venuti, M. C. *J. Org. Chem*. **1980**, *45*, 4798–4801;
30. Hofmann, K.; Finn, F. M.; Kiso, Y. *J. Am. Chem. Soc*. **1978**, *100*, 3585–3590;
31. Jones, N. F.; Kumar, A.; Sutherland, I. O. *Chem. Commun*. **1981**, 990–992;
32. Ernest, I. *Helv. Chim. Acta* **1980**, *63*, 201–213;
33. Rachon, J.; Schollkopf, U. *Liebigs Ann. Chem*. **1981**, 99–102.
34. Anderson, G. W.; McGregor, A. C. *J. Am. Chem. Soc*. **1957**, *79*, 6180–6183.
35. Schwyzer, R.; Sieber, P.; Kappeler, H. *Helv. Chim. Acta* **1959**, *42*, 2622–2624;
36. Schnabel, E. *Liebigs Ann. Chem*. **1967**, *702*, 188–196;
37. Ali. A.; Fahrenholz, F.; Weinstein, B. *Angew. Chem*. **1972**, *84*, 259; *Angew. Chem., Int. Ed*. **1972**, *11*, 289.
38. Broadbent, W.; Morley, J. S.; Stone, B. E. *J. Chem. Soc. (C)* **1967**, 2632–2636.
39. Fujino, M.; Hatanaka, C. *Chem. Pharm. Bull*. **1967**, *15*, 2015–2016.
40. Rzeszotarska, B.; Wiejak, S. *Liebigs Ann. Chem*. **1968**, *716*, 216–218.
41. Sakakibara, S.; Honda, I.; Takada, K.; Miyoshi, M.; Ohnishi, T.; Okumura, K.; *Bull. Chem. Soc. Jpn*. **1969**, *42*, 809–811;
42. Klengel, H.; Schumacher, K. J.; Losse, G. Z. *Chem*. **1973**, *13*, 221–222; *Chem. Abstr*. **1973**, *79*,

137466g.
43. Schnabel, E.; Herzog, H.; Hoffmann, P.; Klauke, E.; Ugi, I. *Liebigs Ann. Chem*. **1969**, *716*, 175–185.
44. Ragnarsson, U.; Karlsson, S. M.; Sandberg, B. E.; Larsson, L. E. *Org. Synth., Coll. Vol. VI* **1988**, 203.
45. Bram, G. *Tetrahedron Lett*. **1973**, 469–472.
46. Nagasawa, T.; Kuriowa, K.; Narita, K.; Isowa, Y. *Bull. Chem. Soc. Jpn*. **1973**, *46*, 1269–1277.
47. Itoh, M.; Hagiwara, D.; Kamiya, T. *Bull. Chem. Soc. Jpn*. **1977**, *50*, 718–721.
48. Kita, Y.; Haruta, J.; Yasuda, H.; Fukunaga, K.; Shirouchi, Y.; Tamura, Y. *J. Org. Chem*. **1982**, *47*, 2697–2700.
49. Harris, R. B.; Wilson, I. B. *Int. J. Pept. Prot. Res*. **1984**, *23*, 55–60.
50. Grehn, L.; Gunnarsson, K.; Ragnarsson, U. *Acta Chem. Scand*. **1986**, *B40*, 745–750.

Appendix
Chemical Abstracts Nomenclature (Collective Index Number); (Registry Number)

Ethyl or isopropyl acetate

Boc-Ala-OH

Boc-β-Ala-OH

Boc-Arg-OH

Boc-Arg(NO_2)-OH

Boc-Asn-OH

Boc-Asp(OBzl)-OH

Boc-Cys(Bzl)-OH

(Boc-Cys-OH)$_2$

Boc-Gln-OH

Boc-Glu(OBzl)-OH

Boc-Gly-OH

Boc-His(Boc)-OH

Boc-Ile-OH

Boc-Leu-OH

Boc-Lys(Boc)-OH

Boc-Lys(CBZ)-OH

Boc-Met-OH

Boc-Pro-OH

Boc-Ser-OH

Boc-Ser(Bzl)-OH

Boc-Thr-OH

Boc-Trp-OH

Boc-Trp-(FOR)-OH

Boc-Tyr-OH

Boc-Tyr(Bzl)-OH

Boc-Tyr(2,6-Cl_2-Bzl)-OH

Boc-Val-OH

ethanol (64-17-5)

acetic acid (64-19-7)

ethyl acetate (141-78-6)

methanol (67-56-1)

ethyl ether (60-29-7)

acetonitrile (75-05-8)

sodium hydroxide (1310-73-2)

chloroform (67-66-3)

sodium bicarbonate (144-55-8)

sodium sulfate (7757-82-6)

carbon dioxide (124-38-9)

potassium hydrogen sulfate (7646-93-7)

n-butyl alcohol (71-36-3)

pyridine (110-86-1)

Glycine (513-29-1)

Pentane (109-66-0)

β-Alanine (107-95-9)

magnesium sulfate (7487-88-9)

dioxane (123-91-1)

L-phenylalanine (63-91-2)

dimethylformamide (68-12-2)

hexane (110-54-3)

tert-butyl alcohol (75-65-0)

Boc-amino

tert-Butyl azidoformate (1070-19-5)

N-tert-butoxycarbonyloxyimino-2-phenylacetonitrile (58632-95-4)

tert-Butyl phenyl carbonate (6627-89-0)

tert-butyl chloroformate

tert-butyl 2,4,5-trichlorophenyl carbonate (16965-08-5)

Di-tert-butyl dicarbonate (24424-99-5)

tert-butyl fluoroformate

tert-butyl α-methoxyvinyl carbonate

N-tert-Butoxycarbonyl-L-phenylalanine,
L-Phenylalanine, N-[(1,1-dimethylethoxy)carbonyl]- (13734-34-4)

tert-butyl 4-nitrophenyl carbonate (13303-10-1)

tert-butyl pentachlorophenyl carbonate (18942-25-1)

tert-butyl 8-quinolyl carbonate

N-tert-butoxycarbonyl-1H-1,2,4-triazole (41864-24-8)

tert-butyl 4,6-dimethylpyrimidyl-2-thiol carbonate

tert-butyl aminocarbonate,
tert-butoxycarbonyloxyamine