Working with Hazardous Chemicals

The procedures in *Organic Syntheses* are intended for use only by persons with proper training in experimental organic chemistry. All hazardous materials should be handled using the standard procedures for work with chemicals described in references such as "Prudent Practices in the Laboratory" (The National Academies Press, Washington, D.C., 2011; the full text can be accessed free of charge at http://www.nap.edu/catalog.php?record_id=12654). All chemical waste should be disposed of in accordance with local regulations. For general guidelines for the management of chemical waste, see Chapter 8 of Prudent Practices.

In some articles in *Organic Syntheses*, chemical-specific hazards are highlighted in red “Caution Notes” within a procedure. It is important to recognize that the absence of a caution note does not imply that no significant hazards are associated with the chemicals involved in that procedure. Prior to performing a reaction, a thorough risk assessment should be carried out that includes a review of the potential hazards associated with each chemical and experimental operation on the scale that is planned for the procedure. Guidelines for carrying out a risk assessment and for analyzing the hazards associated with chemicals can be found in Chapter 4 of Prudent Practices.

The procedures described in *Organic Syntheses* are provided as published and are conducted at one’s own risk. *Organic Syntheses, Inc.*, its Editors, and its Board of Directors do not warrant or guarantee the safety of individuals using these procedures and hereby disclaim any liability for any injuries or damages claimed to have resulted from or related in any way to the procedures herein.

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

![Chemical Structure](image)


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, [α]_{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. At one time the most widely used tert-butoxycarbonylating agent was the hazardous tert-butyl azidoformate. Di-tert-butyl dicarbonate is a highly reactive and safe reagent of the "ready-to-use" type that reacts under mild conditions with amino acids, peptides, hydrazine and its derivatives, and CH-acidic compounds 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.

<table>
<thead>
<tr>
<th>Boc-Amino Acids Prepared by Acylation with Di-tert-Butyl Dicarbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table I</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Boc-Amino Acids</th>
<th>Solvent</th>
<th>Base</th>
<th>Time (hr)</th>
<th>Yield (%)</th>
<th>mp, (°C)</th>
<th>[α]D</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boc-Ala-OH</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>92–94</td>
<td>82–83</td>
<td>−25.5 (acetic acid, c 2.0)</td>
<td>pH 8.0e</td>
</tr>
<tr>
<td>Boc-β-Ala-OH</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>85–86</td>
<td>76–77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boc-Arg-OH</td>
<td>B</td>
<td>—</td>
<td>15</td>
<td></td>
<td>159–160 (dec)</td>
<td>−6.8 (acetic acid, c 1.0)</td>
<td>Extraction with n-butyl alcohol</td>
</tr>
<tr>
<td>Boc-Arg(NO2)-OH</td>
<td>B</td>
<td>NaOH</td>
<td>15</td>
<td>82</td>
<td>107</td>
<td>−22.0 (pyridine, c 2.0)</td>
<td>pH 8.5e</td>
</tr>
<tr>
<td>Boc-Asn-OH</td>
<td>C</td>
<td>NaOH</td>
<td>18</td>
<td>80–81</td>
<td>176 (dec)</td>
<td>−7.2 (dimethylformamide, c 2.0)</td>
<td>5 hr, 45–50°C</td>
</tr>
<tr>
<td>Boc-Asp(OBzl)-OH</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>81–89</td>
<td>101–102 (dimethylformamide, c 2.0)</td>
<td>pH 8.0e</td>
<td></td>
</tr>
<tr>
<td>Boc-Cys(Bzl)-OH</td>
<td>B</td>
<td>NaOH</td>
<td>15</td>
<td>65</td>
<td>86–87</td>
<td>−43.4 (acetic acid, c 1.0)</td>
<td></td>
</tr>
<tr>
<td>(Boc-Cys-OH)2</td>
<td>D</td>
<td>NaOH</td>
<td>16</td>
<td>85</td>
<td>143–145 (dec)</td>
<td>−115.6 (acetic acid, c 2.0)</td>
<td></td>
</tr>
<tr>
<td>Amino Acid</td>
<td>Solvent</td>
<td>Temperature</td>
<td>Melting Point</td>
<td>Solubility</td>
<td>pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Boc-Gln-OH</td>
<td>E</td>
<td>NaOH</td>
<td>18</td>
<td>76</td>
<td>125 (dec)</td>
<td>−3.4 (ethanol, c 2.0)</td>
<td>pH 8.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boc-Glu (OBzl)-OH</td>
<td>B</td>
<td>NaOH</td>
<td>15</td>
<td>86</td>
<td>142–143</td>
<td>+13.2 (methanol, c 1.0)</td>
<td>pH 8.5–9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boc-Gly-OH</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>96</td>
<td>87–88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boc-His (Boc)-OH</td>
<td>A</td>
<td>KHCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>18</td>
<td>75</td>
<td>170 (dec)</td>
<td>+19.5 (chloroform, c 2.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Ile-OH</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>78</td>
<td>69–71</td>
<td>+2.8 (acetic acid, c 2.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Leu-OH&lt;sup&gt;z&lt;/sup&gt;</td>
<td>A</td>
<td>NaOH</td>
<td>18</td>
<td>96</td>
<td>85–87</td>
<td>−24.7 (acetic acid, c 2.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Lys (Boc)-OH</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>82</td>
<td>138–139</td>
<td>+6.1 (dimethylformamide, c 1.5)</td>
<td></td>
</tr>
<tr>
<td>Boc-Lys (CBZ)-OH</td>
<td>A</td>
<td>NaOH</td>
<td>18</td>
<td>96</td>
<td>Oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boc-Met-OH</td>
<td>A</td>
<td>NaOH</td>
<td>18</td>
<td>60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50–51</td>
<td>−22.8 (methanol, c 1.3)</td>
<td></td>
</tr>
<tr>
<td>Boc-Met-OH&lt;sup&gt;f&lt;/sup&gt;</td>
<td>A</td>
<td>NaOH</td>
<td>18</td>
<td>85</td>
<td>139–140</td>
<td>+18.2 (ethanol, c 2.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Pro-OH</td>
<td>A</td>
<td>NaOH</td>
<td>12</td>
<td>95</td>
<td>134–135</td>
<td>−60.6 (acetic acid, c 2.0)</td>
<td>pH 8.5–9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boc-Ser-OH</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>66–82</td>
<td>86–88</td>
<td>−3.6 (acetic acid, c 2.0)</td>
<td>pH 8.5–9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boc-Ser (Bzl)-OH</td>
<td>B</td>
<td>NaOH</td>
<td>16</td>
<td>90</td>
<td>62–63</td>
<td>+19.2 (80% ethanol, c 2.0)</td>
<td>pH 8.5–9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boc-Thr-OH</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>85</td>
<td>71–73</td>
<td>−8.2 (acetic acid, c 1.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Trp-OH&lt;sup&gt;i&lt;/sup&gt;</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>96</td>
<td>137–138 (dec)</td>
<td>−18.2 (dimethylformamide, c 1.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Trp-(FOR)-OH&lt;sup&gt;f&lt;/sup&gt;</td>
<td>F</td>
<td>Et&lt;sub&gt;3&lt;/sub&gt;N</td>
<td>48</td>
<td>61</td>
<td>158–159&lt;sup&gt;z&lt;/sup&gt;</td>
<td>+36.0 (ethanol, c 2.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Tyr-OH</td>
<td>A</td>
<td>NaOH&lt;sup&gt;z&lt;/sup&gt;</td>
<td>24</td>
<td>75</td>
<td>137&lt;sup&gt;m&lt;/sup&gt;</td>
<td>+2.6 (acetic acid, c 1.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Tyr-OH&lt;sup&gt;f&lt;/sup&gt;</td>
<td>A</td>
<td>NaOH&lt;sup;z&lt;/sup&gt;</td>
<td>24</td>
<td>84</td>
<td>216</td>
<td>+2.6 (acetic acid, c 1.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Tyr (Bzl)-OH</td>
<td>B</td>
<td>NaOH</td>
<td>18</td>
<td>70</td>
<td>110–111</td>
<td>+27.6 (ethanol, c 1.0)</td>
<td>pH 10.4&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boc-Tyr (2,6-Cl&lt;sub&gt;2&lt;/sub&gt;-Bzl)-OH</td>
<td>A</td>
<td>NaOH</td>
<td>24</td>
<td>48</td>
<td>104 (dec)</td>
<td>+20.6 (ethanol, c 2.0)</td>
<td></td>
</tr>
<tr>
<td>Boc-Val-OH</td>
<td>A</td>
<td>NaOH</td>
<td>16</td>
<td>85</td>
<td>76–78</td>
<td>−7.5 (acetic acid, c 1.0)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>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.<sup>2</sup>

<sup>b</sup>Solvent systems: A: tert-butyl alcohol–water; B: dioxane–water; C: dimethylformamide–water; D: methanol–water; E: acetonitrile–water; F: dimethylformamide.

<sup>c</sup>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.

<sup>d</sup>Crystallizes with 15% solvent (ethyl acetate).

<sup>e</sup>pH control is necessary.

<sup>f</sup>Dicyclohexylamine salt.
References and Notes

1. Fluka AG, Chemische Fabrik, CH-9470 Buchs, Switzerland.
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(NO₂)-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-Cl₂-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 (5703-46-8)
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 dicarbanate (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