A Publication
of Reliable Methods
for the Preparation
of Organic Compounds

Working with Hazardous Chemicals

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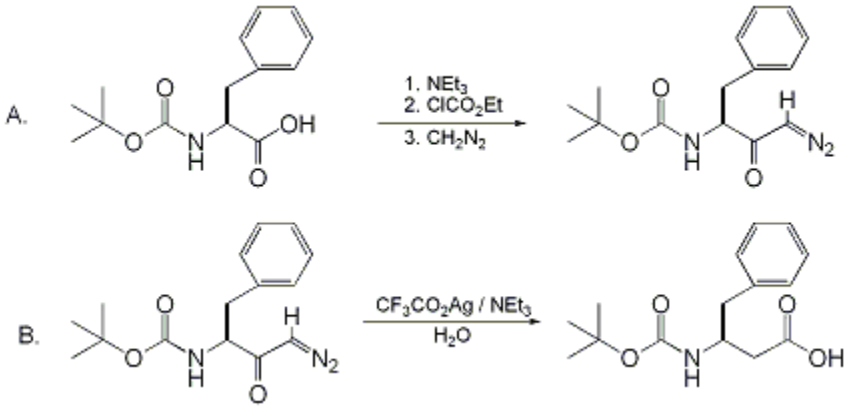
September 2014: The paragraphs above replace the section "Handling and Disposal of Hazardous Chemicals" in the originally published version of this article. The statements above do not supersede any specific hazard caution notes and safety instructions included in the procedure.

DOI:10.15227/orgsyn.079.0154

Organic Syntheses, Coll. Vol. 10, p.194 (2004); Vol. 79, p.154 (2002).

(S)-3-(tert-BUTYLOXYCARBONYLAMINO)-4-PHENYLBUTANOIC ACID

[[Benzenebutanoic acid, β-[[(1,1-dimethylethoxy)carbonyl]amino]-, (S)-]



Submitted by Michael R. Linder, Steffen Steurer, and Joachim Podlech[1] .
Checked by Frédéric Berst and Andrew B. Holmes.

1. Procedure

Caution! Diazomethane should be handled in an efficient fume hood behind a protection shield because of its toxicity and the possibility of explosions.

A. (S)-3-(tert-Butyloxycarbonylamino)-1-diazo-4-phenylbutan-2-one . A 1-L, three-necked, round-bottomed flask is equipped with a magnetic stirring bar, nitrogen gas inlet, bubble counter and a rubber septum on the center neck. The apparatus is dried under a rapid stream of nitrogen with a heat gun. After the flask is cooled to room temperature, the rate of nitrogen flow is reduced and Boc-phenylalanine (25.0 g, 94.2 mmol, Note 1) and anhydrous tetrahydrofuran (250 mL, Note 2) are added. The flask is immersed in an ice-water bath and triethylamine (13.1 mL, 94.0 mmol, Note 3) is added. After 15 min ethyl chloroformate (9.45 mL, 94.0 mmol,Note 4) is added. The reaction mixture is stirred for another 15 min, and a white precipitate of triethylammonium chloride appears; the stirring is then stopped. The septum is replaced by a funnel Note (Note 5). An ethereal solution of diazomethane (about 125 mL, Note 6) is added through the funnel, stirring is resumed for about 5 seconds and the nitrogen stream is stopped. After 45 min, the remainder of the diazomethane solution (about 85 mL) is added. The cooling bath is removed and the solution is allowed to react for 3 hr without stirring. With stirring, 75 mL of 0.5 N acetic acid is added carefully to destroy unreacted diazomethane and saturated aqueous sodium bicarbonate solution (75 mL) is added carefully. The aqueous layer is separated in a separatory funnel and the organic layer is washed with saturated aqueous sodium chloride (75 mL). The organic layer is dried over magnesium sulfate , filtered, and the solvents are removed under vacuum on a rotary evaporator. The crude product is placed under high vacuum for 3 hr (Note 7). The crude material is used directly in the next step (Notes 8, 9).

B. (S)-3-(tert-Butyloxycarbonylamino)-4-phenylbutanoic acid . A 500-mL, three-necked flask is equipped with a nitrogen gas inlet, bubble counter, septum and a magnetic stirring bar. The flask is carefully wrapped in aluminum foil (to exclude light during the reaction). The crude diazo ketone from the preceding step is dissolved in tetrahydrofuran (380 mL, Note 10) and added to the flask under an atmosphere of nitrogen. De-ionized water (38 mL) is added, the flask is immersed in a dry ice-acetone bath, and the solution is cooled to −25°C (temperature of the acetone cooling bath) for 30 min. Silver trifluoroacetate (2.72 g, 12.3 mmol, Note 11) is placed in a 50-mL Erlenmeyer flask and quickly dissolved in triethylamine (39 mL, 279 mmol, Note 3). The resulting solution is added to the diazo

ketone solution in one portion (via syringe). The solution is allowed to warm to room temperature overnight. Evolution of nitrogen starts at a bath temperature of about −15°C.

The solution is transferred to a 1-L, round-bottomed flask and the reaction vessel is rinsed with ethyl acetate (2 × 10 mL). The solution is evaporated to dryness with a rotary evaporator and the residue is stirred for 1 hr with saturated aqueous sodium bicarbonate ($NaHCO_3$) solution (100 mL, Note 12). The black mixture is transferred into a 1-L separatory funnel with water (150 mL) and ethyl acetate (200 mL), and the mixture is shaken well. The clear aqueous layer is separated and put aside, leaving an organic phase containing a suspension of black solid. Brine (30 mL) is added to the organic phase and the resulting mixture is shaken vigorously. Saturated, aqueous $NaHCO_3$ solution (30 mL) is added, the medium is shaken again, and the layers are separated. The black solid is carried away with the aqueous phase, which is now combined with the first-separated aqueous phase. The organic layer is washed with three additional portions of saturated aqueous $NaHCO_3$ solution (30 mL each) and all the aqueous layers are combined. The first organic layer is put aside and not used further. The combined aqueous layers containing a black suspension are extracted with ethyl acetate (50 mL) and the ethyl acetate layer is then back-extracted with two portions of saturated aqueous $NaHCO_3$ solution (25 mL each), which are combined with the original aqueous layers. The ethyl acetate is put aside and not used further. All the combined aqueous layers are extracted again with 50 mL of ethyl acetate , which is washed with saturated aqueous $NaHCO_3$ solution (2 × 20 mL, Note 13). The organic layer is put aside and not used further. All the combined aqueous layers are then transferred to a 2-L, round-bottomed flask equipped with a magnetic stirring bar and about 10 drops of Congo Red indicator (Note 14) and ethyl acetate (100 mL) are added. The flask is immersed in an ice-water bath, the solution is stirred and 5 N (17.5 wt %) hydrochloric acid is added dropwise through an addition funnel until the color of the indicator changes from red to blue (Note 15). The solution is placed in a 1-L separatory funnel and the organic layer is separated. The aqueous layer is additionally extracted with three portions of ethyl acetate (100 mL each, Note 16). The combined organic layers are dried over magnesium sulfate and evaporated on a rotary evaporator. Residual ethyl acetate is azeotropically removed by adding dichloromethane (10 mL) three times and evaporating on the rotary evaporator. Trifluoroacetic acid and traces of solvent are removed under high vacuum (Note 17). The product crystallizes slowly to essentially pure material (16.9-17.1 g, 57.6-61.2 mmol, 61-65%) and can be recrystallized (diethyl ether/light petroleum 1 : 1; about 100 mL) to yield 12.1 g product (43.3 mmol, 46%, Notes 18, 19).

2. Notes

1. Boc-phenylalanine was obtained from Aldrich Chemical Co., Inc. (The submitters obtained their sample from Bachem).
2. Tetrahydrofuran was dried over sodium/benzophenone and freshly distilled before use.
3. Triethylamine was freshly distilled from calcium hydride .
4. Ethyl chloroformate was freshly distilled before use.
5. A short stem, flame-polished funnel of diameter ca. 12.5 cm, free of any scratches or broken edges, was used to prevent spontaneous decomposition of diazomethane .
6. Diazomethane was prepared by the method described (de Boer, Th. J.; Backer, H. J. *Org. Synth., Coll. Vol. IV* **1963**, 250) using a special diazomethane generator, which can be purchased from Aldrich Chemical Company, Inc. (Diazald kit Z10,025-0). The diazomethane solution was prepared by slow distillation of a reaction mixture, which was prepared by adding first a solution of 21.5 g of N-methyl-N-nitroso-p-toluenesulfonamide dissolved in 200 mL of ether to a solution of 6 g of potassium hydroxide , 10 mL of water, 35 mL of 2-(2-ethoxyethoxy)ethanol and 10 mL of ether, followed by a final addition of about 30 mL of ether until the distillate was colorless. All operations involving diazomethane were carried out behind a blast shield and special attention should be paid to the safety instructions made in the above reference.
7. The crude diazo ketone is first obtained as a viscous yellow oil, which slowly solidifies under high vacuum. The checkers always handled the solid material behind a safety shield.
8. The crude diazo ketone (30.8-33.4 g) always contains about 10% of Boc-L-phenylalanine methyl ester formed by esterification of Boc-L-phenylalanine with diazomethane . This material can be carried through the synthesis and is removed during Step B.
9. The checkers purified the diazo ketone (1.5 g) for characterization purposes by dissolution in the minimum quantity of boiling diethyl ether (ca. 2 mL) to which was added boiling hexane (ca. 40 mL).

The product does not crystallize until the solution is cooled to −20°C. The crystals are isolated (0.65 g) by filtration under vacuum, washed with hexane , and then recrystallized to give the pure diazo ketone (0.10 g). The product has the following characteristics: mp 96°C, $[\alpha]_D^{20}$ −30.4° (MeOH, *c* 2.57); IR (KBr) cm^{-1}: 699, 1168, 1366, 1498, 1515, 1638, 1702, 2108, 2933, 2979, 3338 ; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.39 (s, 9 H, C_4H_9), 3.05 (m, 2 H, CH_2Ph), 4.40 (br s, 1 H, $CHCH_2Ph$), 5.07 (br s, 1 H, NH), 5.20 (br s, 1 H, CHN_2), 7.17-7.31 (m, 5 H, ArH) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.3, 39.6, 55.5, 59.5, 81.1, 128.0, 129.6, 130.4, 137.3, 156.1, 194.3 . MS (ES^+) m/z (rel intensity) 312.1320 [$(M + Na)^+$, calcd. for $C_{15}H_{19}N_3O_3Na$ 312.1324], 290 [70, $(M + H)^+$]. Anal. Calcd. for $C_{15}H_{19}N_3O_3$: C, 62.3; H, 6.6; N, 14.5. Found: C, 62.3; H, 6.6; N, 14.1.

10. The checkers used distilled, dry tetrahydrofuran (Note 1), whereas the submitters either distilled the tetrahydrofuran without drying, or purchased a pure grade .

11. Silver trifluoroacetate was obtained from Fluka Chemika or Aldrich Chemical Company, Inc. , and used as received.

12. At this stage, the material consists of large, black lumps, which should be broken up with a spatula.

13. These subsequent re-extractions are essential, since this is the most convenient method for the complete removal of the side product Boc-phenylalanine methyl ester .

14. Solid Congo Red was prepared as a well-shaken 1% w/w suspension in ethanol .

15. About 50-60 mL of hydrochloric acid are used. The color change can be obscured by the presence of the black solid, which should be allowed to settle from time to time so that the solution can be clearly viewed. The checkers observed that the pH of the aqueous phase was between 2-3 as shown by universal pH paper strips.

16. After the second extraction with ethyl acetate the pH value of the aqueous layer is shown to be pH 2-3. If necessary more hydrochloric acid is added.

17. Drying over a period of 16 hr at a pressure of 10^{-3} bar (0.75 mm) is usually sufficient.

18. The submitters obtained 17.4 g (66%). The product has the following characteristics: mp 102-103°C (the submitters obtained mp 102-106°C; Fluka catalog 1999/2000 mp 100-104°C). $[\alpha]_D^{20}$ −15.7 (MeOH, *c* 1.84) [Fluka catalog 1999/2000 $[\alpha]_D^{20}$ −17.5° (CH_2Cl_2, *c* 1.00)]; IR (KBr) cm^{-1}: 3330 (br), 2980, 1712 (br), 1053 ; ^1H NMR (400 MHz, $CDCl_3$) δ: 1,40 (s, 9 H, C_4H_9), 2.39-2,60 (m, 2 H, CH_2Ph), 2.79-2.99 (m, 2 H, CH_2COOH), 4.00-4.25 (br m, 1 H, $CHCH_2Ph$), 5.02 (br s, 0.66 H, NH), 5.96 (br s, 0.33 H, NH), 7.10-7.35 (m, 5 H, ArH), 7.70 (br s,1 H, $-CO_2H$) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.7, 37.8, 40.6, 49.1, 80.0, 127.0, 128.9, 129.7, 138.0, 155.6, 176.8 . MS (ES^+) m/z (rel intensity) 302.1369 [$(M + Na)^+$, calcd. for $C_{15}H_{21}NO_4Na$ 302.1368], 280 [65, $(M + H)^+$], 224 (100), 180 (55) . Anal. Calcd. for $C_{15}H_{21}NO_4$: C, 64.5; H, 7.6; N, 5.0. Found C, 64.2; H, 7.6; N, 5.2. Owing to the presence of rotamers the NMR spectra measured at room temperature showed broadened or duplicated signals, and only the more intense carbon resonances have been listed. The proton and carbon spectra of the synthetic sample were identical to those of a commercial (Fluka) sample.

19. The checkers also prepared (R)-3-(tert-butyloxycarbonylamino)-4-phenylbutanoic acid from Boc-D-phenylalanine according to the same procedure. The enantiomeric purities of the (S)- and (R)-enantiomers were checked by courtesy of Mr. Eric Hortense (GlaxoSmithKline, Stevenage) separately on the corresponding methyl esters, obtained by treatment of the β-amino acids (40 mg, 0.14 mmol) with polymer-supported carbodiimide (PS-carbodiimide, Argonaut, 250 mg, 0.28 mmol) and 4-dimethylaminopyridine (8 mg, 0.07 mmol) in methanol/CH_2Cl_2 (1.4 v/v, 4 mL) for 18 hr. Subsequent filtration of the resin and purification of the crude ester by preparative reverse phase HPLC [C18 column, 10-cm × 2-cm, gradient elution, MeCN, H_2O, CF_3CO_2H 95:5 v/v (solvent A), H_2O, CF_3CO_2H 99.9:0.1 v/v (solvent B) varying from A:B 20:80 to 95:5 A:B over 20 min at a flow rate 6 mL min^{-1} afforded, after freeze-drying, the methyl ester as a colorless powder (ca. 40 mg). Upon chiral HPLC analysis on a Chiralpak AD column (25 cm, solvent EtOH/heptane 5:95 v/v , flow rate 1.0 mL min^{-1}), the (S)-enantiomer (retention time 9.9 min) exhibited an enantiomeric ratio of 99.5:0.5. The retention time of the (R)-enantiomer was 8.6 min.

Waste Disposal Information

All toxic materials were disposed of in accordance with "Prudent Practices in the Laboratory"; National Academy Press; Washington, DC, 1995.

3. Discussion

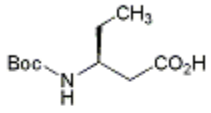
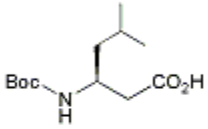
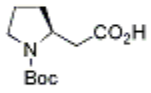
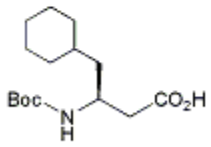
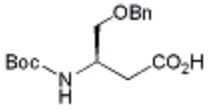
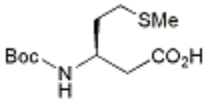
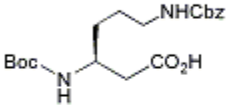
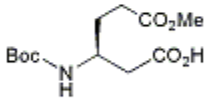
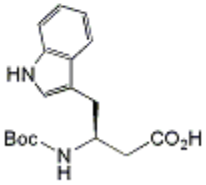
β-Amino acids are useful precursors for the construction of β-peptides,[2] [3] α-substituted β-amino acids[4] and related compounds.[5] They can be prepared enantiomerically pure by homologation of α-amino acids using the Arndt-Eistert method. The suitably protected amino acid is activated as the mixed anhydride and treated with diazomethane to produce the corresponding diazo ketone. Rearrangement in the presence of water furnishes the β-amino acid. Diazomethane contains varying amounts of water, which is able to hydrolyze the activated amino acid. This leads to subsequent methylation by diazomethane to form the methyl ester as a side product. This cannot easily be removed from the diazo ketone, but can be separated during work-up of the homologated amino acids.

Substitution of diazomethane by the less hazardous trimethylsilyl-substituted diazomethane (TMS-CHN_2)[6] is not possible, since TMS-CHN_2 is not acylated by mixed anhydrides.

The diazo ketones that are synthesized as intermediates are not only useful for the preparation of β-amino acids but may serve as versatile starting materials in different reactions,[7] e.g. preparation of 3-azetidinones[8] or 2-aminocyclopentanones.[9]

The procedure described here has been used for the synthesis of further Boc-protected β-amino acids:

Synthesis of Boc-Protected β-Amino Acids

entry	product	yield(%)
1		58
2		36
3		80
4		58
5		44
6		58
7		32
8		74
9		58

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

(S)-3-(tert-Butyloxycarbonylamino)-4-phenylbutanoic acid:
Benzenebutanoic acid, β-[[(1,1-dimethylethoxy)carbonyl]amino]-, (S)- (9); (51871-62-6)

Diazomethane:
Methane, diazo- (8,9); (334-88-3)

(S)-3-(tert-Butyloxycarbonylamino)-1-diazo-4-phenylbutan-2-one:
Carbamic acid,
[3-diazo-2-oxo-1-(phenylmethyl)propyl]-, 1,1-dimethylethyl ester, (S)- (9); (60398-41-6)

Boc-Phenylalanine:
L-Phenylalanine, N-[(1,1-dimethylethoxy)carbonyl]- (9); (13734-34-4)

Triethylamine (8);
Ethanamine, N,N-diethyl- (9); (121-44-8)

Ethyl chloroformate:
Formic acid, chloro-, ethyl ester (8);
Carbonochloridic acid, ethyl ester (9); (541-41-3)

Silver trifluoroacetate:
Acetic acid, trifluoro-, silver(1+) salt (8,9); (2966-50-9)

Trifluoroacetic acid:
Acetic acid, trifluoro- (8, 9); (76-05-1)