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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.

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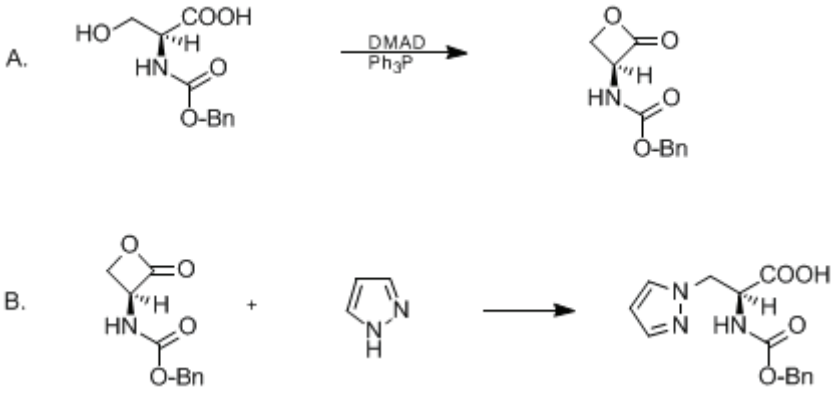
SYNTHESIS OF N-PROTECTED α-AMINO ACIDS FROM N-(BENZYLOXYCARBONYL)-L-SERINE VIA ITS β-LACTONE: N^{α}-(BENZYLOXYCARBONYL)-β-(PYRAZOL-1-YL)-L-ALANINE

[Serine, N-carboxy-, β-lactone, benzyl ester, L-]



Submitted by Sunil V. Pansare, Gregory Huyer, Lee D. Arnold, and John C. Vederas[1].
Checked by M. Jones, G. L. Olson, and David L. Coffen.

1. Procedure

A. *N-(Benzyloxycarbonyl)-L-serine β-lactone*.[2] [3] A 2-L, three-necked, round-bottomed flask is equipped with a magnetic stirring bar, an argon inlet adaptor, a low temperature thermometer, and a rubber septum (Note 1). The flask is charged with tetrahydrofuran (1.1 L) and triphenylphosphine (42.1 g, 160 mmol, (Note 2)). The triphenylphosphine is dissolved with stirring and the flask is then cooled to −78°C with a dry ice-acetone bath (Note 3). Distilled dimethyl azodicarboxylate (17.7 mL, 160 mmol, d = 1.33 g/mL at 25°C) is added dropwise with a syringe over 10 min (*Caution*, (Note 4)). The resulting pale yellow solution is stirred at −75° to −78°C for 10 min, at which point a milky white slurry is obtained. The rubber septum on the flask is quickly replaced with a 1-L, pressure-equalizing dropping funnel containing a solution of N-(benzyloxycarbonyl)-L-serine (38.3 g, 160 mmol) in tetrahydrofuran (240 mL), (Note 2), (Note 5) which is added dropwise to the mixture over 30 min. After completion of the addition, the mixture is stirred at −75° to −77°C for 20 min, the cooling bath is removed, and the mixture is slowly warmed with stirring to room temperature over 2.5 hr (Note 6). The solvent is removed on a rotary evaporator at 35°C. The residual pale yellow syrup is dried briefly (15 min) under high vacuum (~0.2 mm) and suspended in hexane/ethyl acetate (4/1, 20 mL). Ethyl acetate (30 mL) is added to give a solution which is applied to a 10 × 23-cm column of flash silica gel[4] (800 g) packed in hexane/ethyl acetate (4/1). The flask and the sides of the column are rinsed with additional ethyl acetate (20 mL), and this is added to the column which is then eluted with hexane/ethyl acetate (4/1, 2.6 L). The solvent is changed to hexane/ethyl acetate (3/2) and 500-mL fractions are collected. Concentration of fractions 6–10 on a rotary evaporator gives 15.5 g (44%) of analytically pure N-(benzyloxycarbonyl)-L-serine β-lactone (Note 7). Fractions 11–14 contain slightly impure β-lactone. The solid obtained from concentration of these fractions on a rotary evaporator is dissolved in dichloromethane (50 mL) and precipitated by addition of hexane (50 mL) followed by cooling to −20°C (0.5 hr). The process is repeated twice to afford additional β-lactone (1.25 g). The total yield is 16.8 g (47%) (Note 8).

B. *N^{α}-(Benzyloxycarbonyl)-β-(pyrazol-1-yl)-L-alanine*.[2,3,5] [6] [7] A 500-mL, single-necked, round-bottomed flask is equipped with a magnetic stirring bar, a rubber septum and an argon inlet (Note 1). The flask is charged with N-(benzyloxycarbonyl)-L-serine β-lactone (15 g, 68 mmol) and anhydrous acetonitrile (240 mL, (Note 2)). The cloudy mixture is stirred and solid pyrazole (4.9 g, 72 mmol) (Note 9) is added. The rubber septum is quickly replaced with a reflux condenser and an argon inlet adaptor, and the reaction mixture is heated in an oil bath at 52–54°C for 24 hr. The solvent is removed on a

rotary evaporator to leave a white solid that is dried under vacuum for 30 min. Sodium hydroxide (1 N, 69 mL) is added, the suspension is diluted with distilled water (350 mL) and the mixture is stirred vigorously for 5 min. It is then extracted with dichloromethane (3 × 100 mL) to remove unreacted pyrazole and side products. The aqueous phase is cooled in an ice bath to ca. 4°C, and concentrated hydrochloric acid is added with stirring to bring the pH to 1 (about 10 mL required). The resulting precipitate is filtered, washed with water (ca. 75 mL), air dried, and then completely dried in a desiccator over phosphorus oxide (P_2O_5) at 0.2 mm for 12 hr. This material is recrystallized from ethyl acetate (350 mL) to give 7.3 g of pure product (37% yield). Concentration of the mother liquor on a rotary evaporator and recrystallization from the minimum volume of ethyl acetate gives an additional 1.1 g of analytically pure N^{α}-(benzyloxycarbonyl)-β-(pyrazol-1-yl)-L-alanine. The total yield is 8.4 g (43%) (Note 10).

2. Notes

1. The glass components of the apparatus are dried overnight in a 120°C oven, and then assembled and maintained under an atmosphere of dry argon or nitrogen before use. It is essential to complete the purification of the β-lactone as rapidly as possible because this compound is unstable in the crude reaction mixture.
2. Triphenylphosphine (obtained from General Intermediates of Canada) and N-(benzyloxycarbonyl)-L-serine (obtained from Sigma Chemical Company) were dried under reduced pressure over P_2O_5 for 72 hr and 24 hr, respectively. Acetonitrile was refluxed over calcium hydride (CaH_2) for ca. 10 hr and distilled from CaH_2 before use. Tetrahydrofuran was distilled from sodium benzophenone ketyl directly into the glassware (under argon) the day before and stored under argon overnight until used.
3. The temperature of the solution should be about −75°C before dimethyl azodicarboxylate is added.
4. Dimethyl azodicarboxylate (manufactured by Tokyo Kasei Kogyo Co., Japan) was purchased from CTC Organic, 792 Windsor Street, Atlanta, GA 30315. (See warning, p. 837). *Overheating of dimethyl azodicarboxylate should be avoided because of the danger of explosion*. Distillation should be conducted from a temperature-controlled bath in the hood behind a safety shield. The material used distilled at 71–72°C (2 mm), at a bath temperature of 84–86°C. It is important that the addition of this compound to the reaction mixture be carried out at a constant rate without interruption because it tends to freeze in the syringe needle. The checkers explored the use of diethyl azodicarboxylate because of its lower cost and wider availability. However, the corresponding hydrazine derivative is more difficult to separate from the β-lactone product of this step. The use of a very slight excess of azodicarboxylate ester (a few percent relative to triphenylphosphine) prevents reaction of triphenylphosphine with the β-lactone product.[8]
5. The solution of dried N-(benzyloxycarbonyl)-L-serine was made up separately in an addition funnel under an atmosphere of argon. This avoids complications that may arise if the funnel is prefitted on the reaction vessel.
6. The reaction vessel was placed in a water bath at room temperature after the temperature of the mixture was ca. 15°C.
7. The reaction usually works better on a small scale (25 mmol) and a yield of 60% or more is usually obtained. The flash chromatography column was eluted so that the solvent level dropped 1 cm/13 sec. This corresponds to an approximate rate of 362 mL/min. The concentration and purification of the reaction mixture should be carried out as quickly as possible on the same day. Although storage of the concentrated reaction mixture at −20°C overnight results in substantial decomposition of the β-lactone, column fractions containing pure β-lactone (after chromatography) can be stored at 4°C overnight and concentrated on the following day.
The β-lactone is readily visualized by TLC (Merck, Kieselgel 60 F_{254}, 0.25-mm thickness, hexane/ethyl acetate (55/45) as solvent system) under UV, or by using bromocresol green spray (0.04% in EtOH, made blue by NaOH) followed by heating of the plate to detect the β-lactone as a yellow spot on a blue background.
8. The product exhibits the following properties: mp 133–134°C; $[\alpha]_D^{22}$ −26.5° (CH_3CN, *c* 1); IR cm^{-1}: 3355, 1847, 1828, 1685, 1530, 1268; ^1H NMR (360 MHz, CD_2Cl_2) δ: 4.4 (m, 2 H, CH-CH_2-O), 5.0–5.1 (m, 1 H, N-CH-CO), 5.12 (s, 2 H, OCH_2Ph), 5.5–5.7 (br, s, 1 H, NH), 7.3–7.4 (s, 5 H, ArH); EI-MS: M^+ 221.0681 (221.0688 calcd for $C_{11}H_{11}NO_4$). Anal. Calcd for $C_{11}H_{11}NO_4$: C, 59.71; H, 4.97, 6.33. Found: C, 59.66; H, 4.92; N, 6.32.

The optical purity was determined as previously described,[3] and corresponds within experimental error to that of the starting material. N-(Benzyloxycarbonyl)-L-serine obtained from Sigma typically contains 0.75–2.80% of the D-isomer.
9. Pyrazole was obtained from Aldrich Chemical Company, Inc.
10. The reaction usually is more successful on a smaller scale and yields up to 70% can be obtained. The product exhibits the following properties: mp 168–169°C, $[\alpha]_D^{22}$ −53.5°; (DMF, *c* 1); IR (KBr disc) cm^{-1}: 3350, 1745, 1696, 1534, 1260; ^1H NMR (360 MHz, CD_3OD) δ: 4.4–4.5 (m, 1 H, CH), 4.60–4.70 (m, 2 H, CH_2N), 5.08 (s, 2 H, OCH_2Ph), 6.25 (t, 1 H), 7.3 (s, 5 H, -Ph), 7.48 (d, 1 H), 7.52 (d, 1 H); MS: FAB MS in glycerol m/z 290 (MH^+, 36%). Anal. Calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53. Found: C, 57.86; H, 5.25; N, 14.36.

Waste Disposal Information

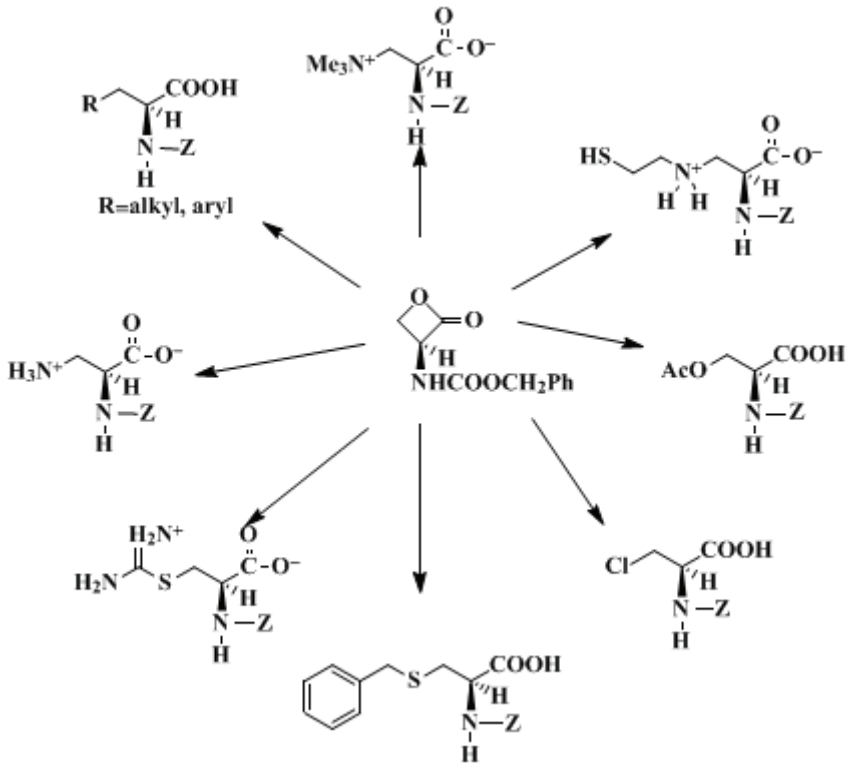
All toxic materials were disposed of in accordance with "Prudent Practices in the Laboratory"; National Academy Press; Washington, DC, 1995.

3. Discussion

A large number of α-amino acids with interesting biological properties occur in nature.[9 10 11] This fact, together with the utility of amino acids as chiral synthons, catalysts, and auxiliaries,[12 13 14] has stimulated extensive interest in their chemical synthesis.[15] Serine is an especially attractive starting material for preparation of other amino acids because both enantiomers are commercially available (in both free and various N-protected forms) with high optical purity at relatively low cost. Recent work has shown that chiral N-protected serine β-lactones are readily formed under modified Mitsunobu conditions and that they react readily with a variety of carbon, nitrogen, oxygen, sulfur, and halogen nucleophiles to afford optically pure N-protected α-amino acids (Scheme 1).[2,3,16 17]

The procedure given here describes the preparation and use of N-(benzyloxycarbonyl)-L-serine β-lactone for the synthesis of a protected β-substituted alanine, N^{α}-(benzyloxycarbonyl)-β-(pyrazol-1-yl)-L-alanine. This compound occurs in watermelon seeds,[18] and has been used as a histidine analog.[6] Its synthesis illustrates how serine β-lactones can provide convenient access to other β-substituted alanines such as mimosine, willardiine, quisqualic acid, and stizolobic acid which occur in higher plants.[9,19] Many previous chemical syntheses of racemic pyrazolylalanine have been published; the best of these routes appear to be from acetamidoacrylic acid (94–96% yield)[20] and from O-acetylserine (40–45%).[7] The racemate has been resolved.[6] The β-(pyrazol-1-yl)-L-alanine synthase enzyme has been purified and used to make the chiral material from O-acetyl-L-serine.[21]

The crystalline N-(benzyloxycarbonyl)-serine β-lactone is easily handled in air at room temperature and can be stored dry at −20°C for many months without decomposition. Solutions of this compound in non-nucleophilic organic solvents (e.g., chloroform, ethyl acetate) or in neutral or slightly acidic water (pH 3–6) are stable for several days. Aqueous base rapidly hydrolyzes the β-lactone.[3,16,17]



Scheme 1

This preparation is referenced from:

- Org. Syn. Coll. Vol. 9, 24

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

silica gel

N^{α}-(Benzyloxycarbonyl)-β-(pyrazol-1-yl)-L-alanine

sodium benzophenone ketyl

quisqualic acid

stizolobic acid

hydrochloric acid (7647-01-0)

ethyl acetate (141-78-6)

acetonitrile (75-05-8)

sodium hydroxide (1310-73-2)

chloroform (67-66-3)

oxygen (7782-44-7)

nitrogen (7727-37-9)

sulfur (7704-34-9)

carbon (7782-42-5)

dichloromethane (75-09-2)

bromocresol

Tetrahydrofuran (109-99-9)

diethyl azodicarboxylate (1972-28-7)

hexane (110-54-3)

argon (7440-37-1)

pyrazole (288-13-1)

calcium hydride (7789-78-8)

triphenylphosphine (603-35-0)

dimethyl azodicarboxylate (2446-84-6)

phosphorus oxide (1314-56-3)

N-(BENZYLOXYCARBONYL)-L-SERINE (1145-80-8)

pyrazolylalanine

acetamidoacrylic acid (5429-56-1)

O-acetylserine,
O-acetyl-L-serine (5147-00-2)

N-(Benzyloxycarbonyl)-L-serine β-lactone,
N-(benzyloxycarbonyl)-serine β-lactone,
Serine, N-carboxy-, β-lactone, benzyl ester, L- (26054-60-4)

β-(pyrazol-1-yl)-L-alanine

mimosine

willardiine