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Synthetic Studies on an Antitumor Antibiotic, Bleomycin. XII.¹⁾ Preparation of an L-2,3-Diaminopropionic Acid Derivative as a Synthetic Intermediate

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Three methods have been developed for the preparation of (*S*)-3-amino-2-*tert*-butoxycarbonylamino-*propionamide* suitably protected for use in the total synthesis of bleomycin.

Keywords—bleomycin; L-2,3-diaminopropionic acid; L-asparagine; L-serine; Hofmann rearrangement; Mitsunobu reaction; (*S*)-3-amino-2-*tert*-butoxycarbonylamino-*propionamide*

In the course of biological and biochemical studies of bleomycin (BLM),²⁾ a synthetic approach to the pyrimidine moiety (1) of BLM was considered.²⁾ As described in a previous paper,^{1a)} we have developed a two-step and high-yielding synthesis of 2-formylpyrimidine (2). According to our strategy, the next stage is the formation of a Schiff-base from the aldehyde (2) and 2,3-diaminopropionamide (3). We wish to report herein a practical method for the synthesis of (*S*)-3-amino-2-*tert*-butoxycarbonylamino-*propionamide* (Boc-L-DAPA) (3), in which the two amino groups are suitably differentiated by protection for further elaboration. It should be mentioned here that 2,3-diaminopropionic acid is also an important moiety in several monocyclic β -lactam antibiotics of current interest, such as nocardicin³⁾ and monobactam.⁴⁾

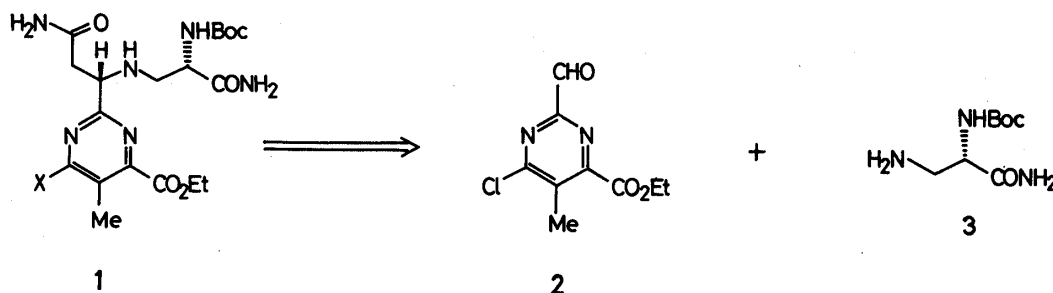


Chart 1

2,3-Diaminopropionic acid is commercially available and can also be obtained from L-aspartic acid by means of the Schmidt reaction.⁵⁾ However, the conversion of L-2,3-diaminopropionic acid to Boc-L-DAPA seems difficult because the two amino groups are located too closely to be distinguished from each other.^{1a)} Thus, the Hofmann rearrangement was preferred to the Schmidt reaction. Karrer and Schlosser^{6a)} and Schneider^{6b)} have reported the Hofmann rearrangement of *N*-acetyl-L-Asn (4) and *N*-benzyloxycarbonyl-L-Asn (6) giving rise to the imidazolidin-2-ones 5 and 7, respectively. In these rearrangements, the intermediary

isocyanates were trapped intramolecularly by acetamide and the *Z*-amino group at the α -position. In the case of Tos-asparagine (**8**), although the formation of the diaminopropionic acid derivative (**9**) has been reported,⁷⁾ the removal of the tosyl group requires conditions that are too severe for the case of BLM.

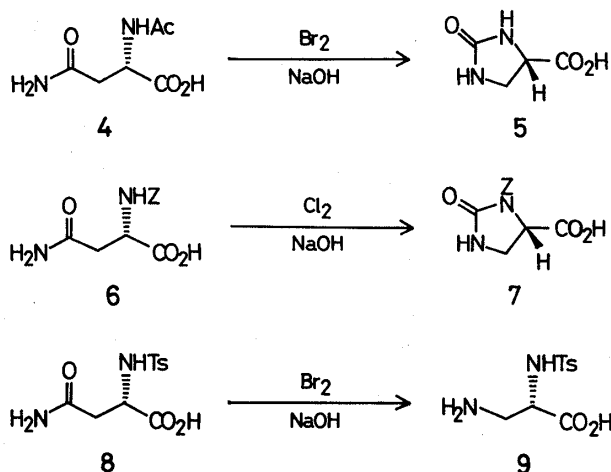


Chart 2

It was considered that a bulky α -amino-protecting group would prevent the formation of the cyclic urea through intramolecular addition to isocyanate. The *tert*-butoxycarbonyl group seemed most suitable in this regard and it can readily be removed under mild acidic conditions in the BLM synthesis.¹⁾ Thus, Boc-L-asparagine (**10**) was added to NaOBr solution, which had been prepared from bromine and aqueous NaOH at -10°C . The reaction mixture was then heated at 70°C to effect decarboxylation, followed by direct treatment with benzyl chloroformate at pH 8.5. Without purification, the resulting protected 2,3-diaminopropionic acid was treated with diazomethane to afford an ester (**11**) $[\alpha]_{\text{D}}^{20} -9.2^\circ$ ($c=1.0$, MeOH) in 12% overall yield.

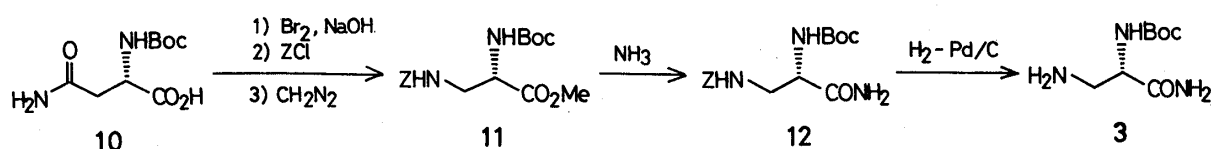


Chart 3

Ammonolysis of the ester (**11**) afforded the carboxamide (**12**) in 73% yield and upon subsequent hydrogenolysis (H_2 -Pd/C) Boc-L-DAPA (**3**) was obtained in quantitative yield. To examine the degree of racemization during these operations, the Boc-L-DAPA thus obtained was converted to the known L-2,3-diaminopropionic acid⁸⁾ by acid hydrolysis. The crude product obtained by direct concentration of the reaction mixture showed $[\alpha]_{\text{D}}^{20} +21.3^\circ$ ($c=1.0$, 1 N HCl), confirming that there was essentially no racemization. Thus, in this method, purification of the intermediary amino acid and doubly-protected acid is not necessary, and the procedure can easily be carried out on a large scale. Although the total yield of Boc-L-DAPA is not get fully satisfactory, the experimental simplicity of the procedure compensates for this.

The next method to obtain Boc-L-DAPA is based on selective cleavage of the α -amino-

protecting group of the diaminopropionic acid derivative. Schneider and Takagi reported that, on treatment of 2,3-bis(benzyloxycarbonylamino)propionic acid (**13**) with thionyl chloride, the cyclic anhydride (**14**) was formed through intramolecular participation of the α -benzyloxycarbonylamino group, and 3-*N*-benzyloxycarbonyl-L-2,3-diaminopropionic acid (**15**) was obtained in 45% yield by acid hydrolysis of **14**.^{6b,9)} By applying this method, we could obtain the 2-*N*-*tert*-butoxycarbonyl-3-*N*-benzyloxycarbonyl-L-2,3-diaminopropionic acid methyl ester (**11**) in 75% yield. The ester (**11**) thus obtained showed an optical rotation value $[[\alpha]_D^{20} - 9.2^\circ (c = 1.34, \text{MeOH})]$ similar to that of the product obtained by the first procedure.

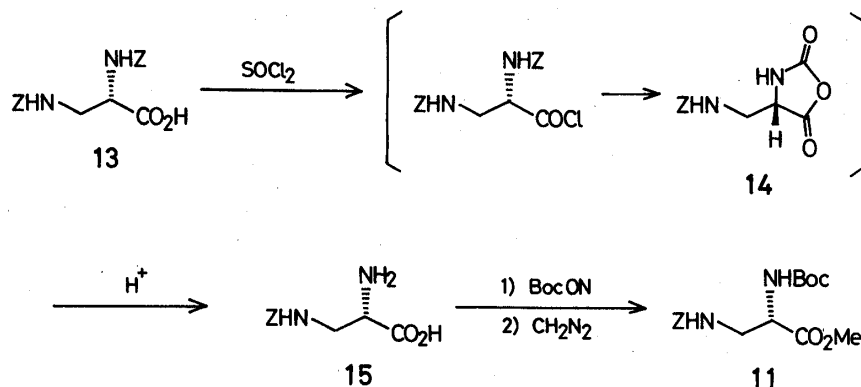


Chart 4

Although these two methods provide optically pure Boc-L-DAPA without racemization, the overall yields remain to be improved. Therefore, we further investigated an alternative route to Boc-L-DAPA. We thought of using Boc-L-serine as a starting material; substitution of the hydroxyl group with an amino group should give the L-2,3-diaminopropionic acid in a most straightforward manner. In this type of reaction, the choice of both leaving group and nitrogen nucleophile seems to be critical, since Benoiton observed complete racemization in the reaction of the β -chloro-L-alanine derivative (**16**) with potassium phthalimide.¹⁰⁾ This reaction is thought to proceed through the conjugate addition of the imide anion to α -aminoacrylate rather than through direct substitution. Furthermore, it is also known that the β -tosyloxy-L-alanine methyl ester (**18**) affords the dehydroalanine derivative (**19**) by means of a reaction with diethylamine.¹¹⁾

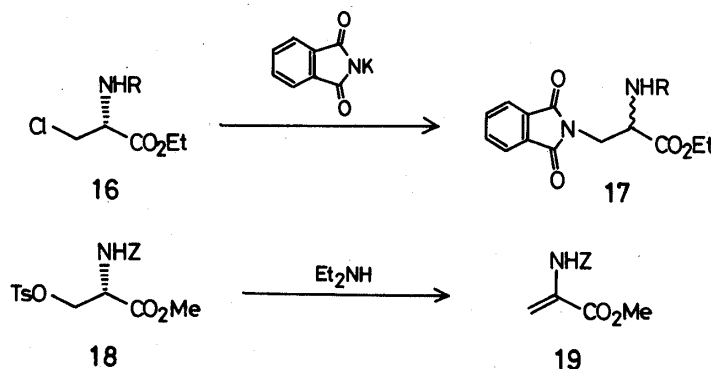


Chart 5

These two examples suggest that β -elimination takes place much faster than substitution under basic conditions. Thus, the desired substitution product should be formed under neutral or acidic media. The Mitsunobu reaction¹²⁾ was considered most promising for our purposes, since the hydroxyl group is activated with triphenylphosphine (TPP) and diethyl azo-

dicarboxylate (DEAD) to react with weakly acidic compounds, such as a carboxylic acid, an imide, and hydrazoic acid. Thus, the Boc-L-serine methyl ester (**20**) was treated with TPP (1.2 eq), DEAD (1.2 eq), and hydrazoic acid (1.2 eq as a benzene solution) in tetrahydrofuran (THF) to afford the desired azide (**21**) in 73% yield.¹³⁾ This reaction is a new application of the Mitsunobu reaction, because there have been no earlier examples of the reaction at such a labile hydroxyl group β to a carbonyl group. Hydrogenolysis of **21** followed by the protection of the free amino group with benzyl chloroformate provided the known compound (**11**) $[\alpha]_D^{20} - 9.18^\circ$ ($c = 1.0$, MeOH) in high overall yield.

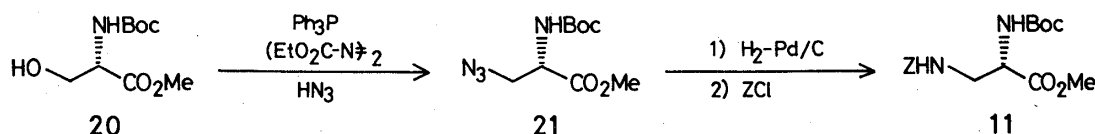


Chart 6

We have thus investigated three methods for the preparation of Boc-L-DAPA. The last method, utilizing the Mitsunobu reaction, is the most practical and can be efficiently employed in the total synthesis of bleomycin.^{1c,g,i)}

Experimental

The melting points were measured on a Yamato MP-21 apparatus and are uncorrected. The nuclear magnetic resonance (NMR) spectra were obtained on a JEOL FX-100 spectrometer, and the chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS). The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. The infrared (IR) spectra were recorded on a JASCO DS-402G spectrometer. The mass spectra (MS) were measured with a JEOL JMS-01 SG-2 mass spectrometer. The optical rotations were measured with a JASCO DIP-181 digital polarimeter.

Methyl (S)-3-Benzyloxycarbonylamino-2-tert-butoxycarbonylaminopropionate (11) by the Hofmann Rearrangement of Z-L-Asparagine—Br₂ (10 ml, 0.2 mol) was added at to a solution of NaOH (40.0 g, 1 mol) in H₂O (330 ml), at -10°C . The mixture was stirred for 15 min at the same temperature, then *N*-tert-butoxycarbonyl-L-asparagine (**10**) (39.5 g, 0.17 mol) was added in small portions. Decarboxylation occurred heating at 70°C for 1 h. After the evolution of CO₂ had ceased, the reaction mixture was cooled to room temperature, and benzyl chloroformate (30 g, 0.18 mol) in Et₂O (44 ml) was added to the solution, which had first been adjusted to pH 8.5 by adding 1 N NaOH. After being stirred overnight, the reaction mixture was adjusted to pH 10.0. The solution was washed with AcOEt, acidified to pH 2.0 with cold 1 M citric acid and extracted with AcOEt. The extract was washed with water, dried over Na₂SO₄ and concentrated. The oily residue was dissolved in Et₂O, treated with an Et₂O solution of CH₂N₂ and concentrated under reduced pressure to give an oily substance, which was purified by chromatography on silica gel. Fractions eluted with C₆H₆-AcOEt (5:1) afforded **11** (7.4 g, 12%), which solidified upon standing, mp $44-45^\circ\text{C}$, $[\alpha]_D^{20} - 9.2^\circ$ ($c = 1.0$, MeOH). IR (neat): 3360, 3020, 2980, 1755, 1715, 1520 cm⁻¹. ¹H-NMR (CDCl₃) δ : 1.44 (9H, s), 3.58 (2H, m), 3.74 (3H, s), 4.38 (1H, dd), 5.11 (2H, s), 5.29 (1H, br t), 5.52 (1H, br d), 7.35 (5H, s). ¹³C-NMR (CDCl₃) δ : 171.14 (s), 156.66 (s), 155.35 (s), 136.39 (s), 128.40 (d), 128.01 (d $\times$ 2), 80.21 (s), 66.90 (t), 52.48 (d), 42.83 (q), 28.26 (t), 14.13 (q). Anal. Calcd for C₁₇H₂₄N₂O₆: C, 57.94; H, 6.87; N, 7.95. Found: C, 57.69; H, 6.91; N, 7.78. MS m/e : 353 ($M^+ + 1$), 297, 280.

(S)-3-Benzyloxycarbonylamino-2-tert-butoxycarbonylaminopropionamide (12)—A solution of **11** (6.75 g, 17.7 mmol) in MeOH (100 ml) was saturated with NH₃ at -20°C and allowed to stand overnight at room temperature. The white solid obtained by removal of the solvent was recrystallized from MeOH to give **12** as a white powder (3.29 g). The mother liquor afforded a further 1.05 g of **12** (total 73% yield). mp $163.5-165.0^\circ\text{C}$, $[\alpha]_D^{20} - 4.0^\circ$ ($c = 1.0$, MeOH). IR (KBr): 3380, 3340, 3190, 3040, 2980, 1705, 1660, 1525 cm⁻¹. ¹H-NMR (CD₃OD) δ : 1.42 (9H, s), 3.43 (2H, dd $\times$ 2), 4.24 (1H, dd), 5.10 (2H, s), 7.34 (5H, s). Anal. Calcd for C₁₆H₂₃N₃O₅: C, 56.96; H, 6.87; N, 12.46. Found: C, 56.90; H, 6.81; N, 12.49. MS m/e : 337 (M^+), 293, 281, 264.

(S)-3-Amino-2-tert-butoxycarbonylaminopropionamide (3)—A solution of **12** (500 mg, 1.48 mmol) in MeOH (50 ml) was stirred overnight with 10% palladium on carbon (50 mg) under a hydrogen atmosphere. The palladium catalyst was filtered off and the filtrate was concentrated to give **3** as a colorless foam (299 mg, 99%), $[\alpha]_D^{20} + 5.6^\circ$ ($c = 1.0$, MeOH). IR (CHCl₃): 3420, 2990, 1710, 1690, 1595, 870 cm⁻¹. ¹H-NMR (CD₃OD) δ : 1.46 (9H, s), 2.80 (1H, dd, $J = 7.0, 13.6$ Hz), 2.96 (1H, dd, $J = 5.4, 13.6$ Hz), 4.06 (1H, dd, $J = 5.4, 7.0$ Hz). ¹³C-NMR (CD₃OD) δ : 175.87 (s),

157.50 (s), 80.45 (s), 57.75 (d), 44.34 (t), 28.56 (q). MS m/e : 204 (M^+), 174, 159, 148, 130.

L-2,3-Diaminopropionic Acid by Acid Hydrolysis of (S)-3-Amino-2-tert-butoxycarbonylamino-2-aminopropionamide (3)—A solution of **3** (437 mg, 2.15 mmol) in 1 N HCl (5 ml) was heated at 50 °C for 5 h. Evaporation of the solvent under reduced pressure gave 315 mg of crude 2,3-diaminopropionic acid, $[\alpha]_D^{20} + 21.28^\circ$ ($c = 1.0$, 1 N HCl).

Methyl (S)-3-Benzoyloxycarbonylamino-2-tert-butoxycarbonylamino-2-aminopropionate (11) from (S)-3-Benzoyloxycarbonylamino-2-aminopropionic Acid (15)—Triethylamine (46 mg, 0.45 mmol) and Boc-ON (81 mg, 0.33 mmol) in dioxane (0.2 ml) were added to a solution of (S)-3-benzoyloxycarbonylamino-2-aminopropionic acid (**15**)^{6b,9} (71 mg, 0.3 mmol) in H₂O (0.2 ml). The reaction mixture was stirred overnight, then poured into H₂O, and washed with AcOEt. The aqueous solution was acidified to pH 2.0 with cold 1 M citric acid, and extracted with AcOEt. This extract was washed with H₂O, dried over Na₂SO₄, and concentrated to give crude (S)-3-benzoyloxycarbonylamino-2-tert-butoxycarbonylamino-2-aminopropionic acid. This was dissolved in Et₂O, treated with an Et₂O solution of CH₂N₂, and concentrated under reduced pressure to give an oily substance which was purified by chromatography on silica gel to afford **11** (75 mg, 70%). $[\alpha]_D^{20} - 9.2^\circ$ ($c = 1.34$, MeOH).

Methyl (S)-3-Azido-2-tert-butoxycarbonylamino-2-aminopropionate (21) from Boc-L-Serine Methyl Ester by Mitsunobu Reaction—Diethyl azodicarboxylate (7.18 g, 41.2 mmol) in THF (20 ml), a 1.0 M benzene solution of hydrazoic acid (40 ml, 40 mmol), and *N*-tert-butoxycarbonyl-L-serine methyl ester (**20**) (7.72 g, 35.2 mmol) in THF (30 ml) were added in that order to a solution of triphenylphosphine (11.10 g, 42.3 mmol) in THF at -78 °C. The mixture was stirred overnight at room temperature. After removal of the solvent, the residue was chromatographed on silica gel (eluted with *n*-hexane: Et₂O = 2:1) to afford **21** (6.25 g, 73%). $[\alpha]_D^{20} + 36.7^\circ$ ($c = 1.02$, CHCl₃). IR (CHCl₃): 3430, 2980, 2210, 1745, 1710 cm⁻¹. ¹H-NMR (CDCl₃) δ : 1.45 (9H, s), 3.63–3.86 (2H, m), 3.79 (3H, s), 4.32–4.56 (1H, m), 5.33 (1H, br s). MS m/e : 244 (M^+).

Methyl (S)-3-Benzoyloxycarbonylamino-2-tert-butoxycarbonylamino-2-aminopropionate (11) from Methyl (S)-3-Azido-2-tert-butoxycarbonylamino-2-aminopropionate (21)—A solution of **21** (6.25 g, 25.6 mmol) in MeOH (65 ml) was stirred overnight with 10% palladium on carbon (625 mg) under a hydrogen atmosphere. The palladium catalyst was filtered off and the filtrate was concentrated to give the crude amine. Trituration with a small amount of THF afforded white crystals (4.14 g, 74%). mp 139–140 °C, $[\alpha]_D^{20} - 38.8^\circ$ ($c = 1.08$, MeOH). A mixture of the amine (52.3 mg, 0.24 mmol), triethylamine (40 μ l, 0.29 mmol) and benzyl chloroformate (41 μ l, 0.29 mmol) in CH₂Cl₂ was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel to give **11** (84.8 mg, quant.). $[\alpha]_D^{20} - 9.18^\circ$ ($c = 1.0$, MeOH).

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