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Chloramphenicol[1] (Chloromycetin). VI. A Synthetic Approach

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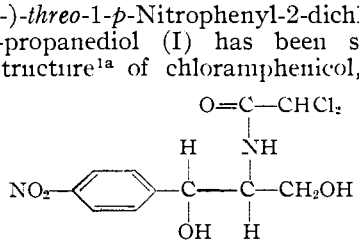
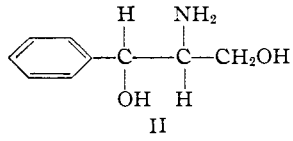
D-(-)-*threo*-1-*p*-Nitrophenyl-2-dichloroacetamido-1,3-propanediol (I) has been shown to be the structure[1a] of chloramphenicol, an antibiotic produced by a soil actinomycete.[2,3] Although subsequent attempts to synthesize I were successful,[4] the present authors were encouraged to search for additional syntheses suitable for large scale production and/or for preparing analogs of chloramphenicol.



I



II

Consideration of I elicits the fact that *dl-threo*-1-phenyl-2-amino-1,3-propanediol (II) is an intermediate of fundamental importance to the synthesis of the antibiotic since it is known that the triacetyl derivative of II can be nitrated in the para-position.[4]

The *p*-nitro derivative of II has been converted to chloramphenicol by resolution and dichloroacetylation of the D-(-)-isomer.[4] Separation of the diastereoracemates, *dl-erythro*-1-phenyl-2-amino-1,3-propanediol and *dl-threo*-1-phenyl-2-amino-1,3-propanediol, has been accomplished.[4]

A possible approach to II was thought to be through acetophenone which would, perhaps, allow addition of essential elements (acylamido and hydroxymethyl) of II to be followed by reduction of the keto group to a hydroxyl as illustrated by the sequence III → IV → V → VI → II in the set of reactions.

It is known that acetophenone under the influence of potassium carbonate at room temperature reacts with three molecules of formaldehyde to form an acetal of α-dimethylolacetophenone.[5] However, at 60° in the presence of alkali the reaction may be controlled so that the principal product is β-hydroxypropiophenone.[6] Furthermore, propiophenone undergoes only monohydroxymethylation in the presence of potassium carbonate to form α-methyl-β-hydroxypropiophenone.[5] The reaction proceeds slowly, requiring several days for the formation of appreciable quantities of the propiophenone derivative. The procedures of the investigators mentioned above[5,6] when applied to α-acylamidoacetophenone produced only low yields of the desired product, but chiefly methylene-bis-derivatives or polyhydroxymethylated compounds. It was found that the desired monohydroxymethylation was obtained when the condensing agent was sodium bicarbonate.

α-Benzamidoacetophenone (IV, R = phenyl) was prepared from isonitrosoacetophenone[7] by reduction with stannous chloride[8] followed by benzoylation of either the tin complex salt[9] itself or the α-aminoacetophenone hydrochloride obtained from the tin complex salt by treatment with hydrogen sulfide.[8,10,11] α-Aminoacetophenone hydrochloride may be prepared quite easily from phenacyl halides through their hexamethylenetetramine salts.[12,13] The authors found that larger quantities of α-acylamidoacetophenones may be prepared conveniently through α-nitroacetophenone as described in the experimental section.

The first attempt to hydroxymethylate α-benzamidoacetophenone consisted of mixing the ketone in methanol with one equivalent of paraformaldehyde and a small quantity of potassium carbonate. On shaking the mixture at room temperature a clear solution was formed within a few minutes and shortly thereafter a crystalline solid precipitated. The product was a high-melting compound with very little water or alcohol solubility which analysis indicated to be 1,5-diphenyl-2,4-dibenzamido-1,5-pentadione (XI).

On repeating the reaction and pouring the solution as soon as it was obtained into ice and water, the desired compound (V) was obtained in an impure state. Purification was difficult and analyses were not satisfactory. However, when the reaction was executed in the presence of sodium bicarbonate in place of potassium carbonate, V was obtained in an excellent state of purity. Under these conditions there was no

(1) Chloroamphenicol has been assigned as a generic name for the compound D-*threo*-N-(1,1'-dihydroxy-1-*p*-nitrophenylisopropyl)-dichloroacetamide for which Parke, Davis and Co. has adopted "Chloromycetin" as its trademark.

(1a) Rebstock, Crooks, Controulis and Bartz, THIS JOURNAL, **71**, 2458 (1949).

(2) Bartz, *J. Biol. Chem.*, **172**, 445 (1948).

(3) Carter, Gottlieb, David and Anderson, *Science*, **107**, 113 (1948).

(4) Controulis, Rebstock and Crooks, THIS JOURNAL, **71**, 2463 (1949).

(5) Fuson, Ross and McKeever, *ibid.*, **60**, 2935 (1938).

(6) Schörnig, *Bib. Sci. and Ind. Rept.*, **9**, 212 (April 16, 1948).

(7) Claisen and Manasse, *Ber.*, **20**, 2194 (1887).

(8) Rupe, *ibid.*, **28**, 251 (1895).

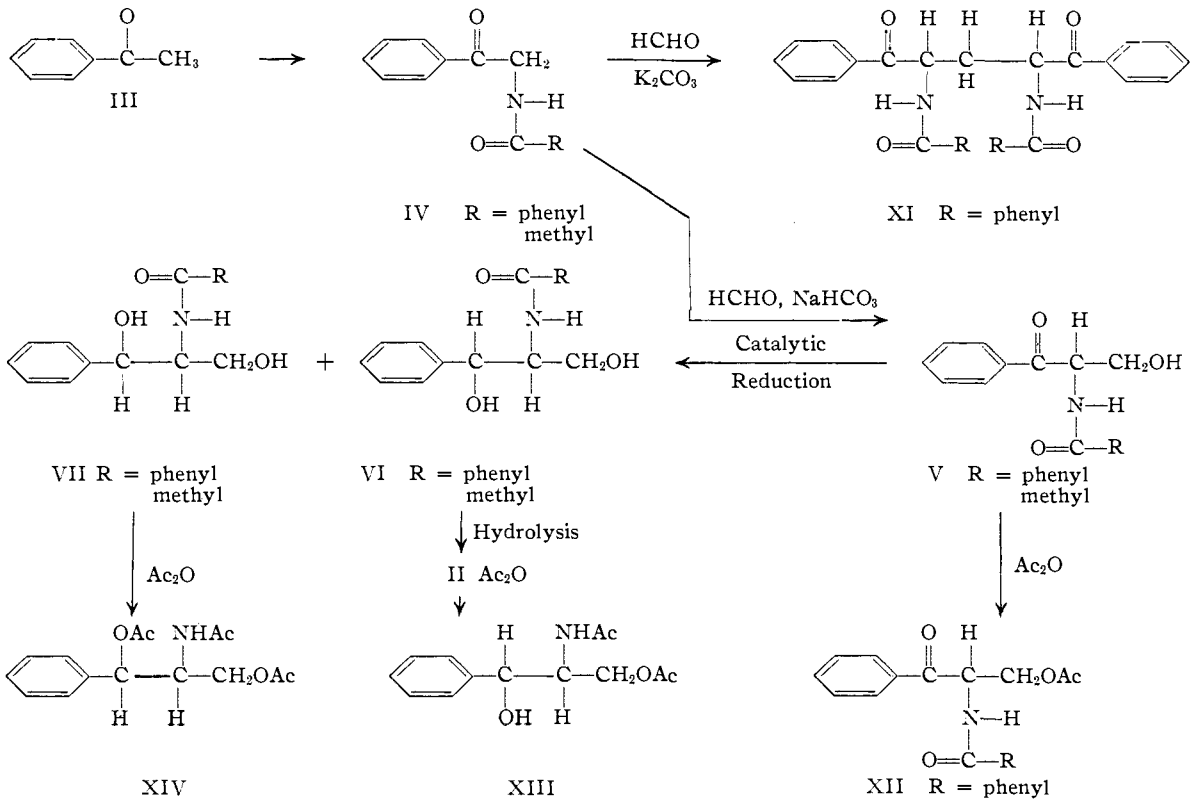
(9) Lister and Robinson, *J. Chem. Soc.*, 1297 (1912).

(10) Robinson, *ibid.*, 2167 (1909).

(11) Gabriel, *Ber.*, **43**, 134 (1910).

(12) Mannich and Hahn, *ibid.*, **44**, 1545 (1911).

(13) Jacobs and Heidelberger, *J. Biol. Chem.*, **21**, 455 (1915).



tendency for the diketone (XI) to form even when the solution was allowed to stand for several days.

The reduction of α-benzamido-β-hydroxypropiophenone (V, R = phenyl) was accomplished by hydrogenation in methanol with Raney nickel catalyst. Separation of the diastereoracemates (VI and VII) was carried out by recrystallization from ethanol and water. An alternate method involves hydrolysis of the entire hydrogenation product and conversion of the free bases to the diacetates. The diacetyl derivative (XIII) of the *dl-threo* base (II) is the more insoluble racemate.

Further experience indicated that the use of α-acetamidoacetophenone[14,15,16] (IV, R = methyl) offers some advantage over the benzamido derivative as an intermediate. α-Acetamidoacetophenone was prepared from the tin complex salt of α-aminoacetophenone and condensed with formaldehyde in the presence of sodium bicarbonate. The reaction proceeds satisfactorily in water at 35° and is essentially complete within two hours. The α-acetamido-β-hydroxypropiophenone (V, R = methyl) precipitates from the aqueous solution as the monohydrate.

Hydrogenation was accomplished by the same procedure used for the reduction of the benzamido derivative. Separation of VI and VII (R = methyl) was carried out by recrystallization from ethyl acetate, the *dl-threo*-racemate being the more insoluble derivative. The *dl-erythro*-racemate (VII) may be isolated by conversion to the triacetyl derivative, the *dl-erythro*-racemate (XIV) being precipitated with ether.

Experimental[17]

1-Phenyl-2-nitroethanol (VIII).—The sodium salt[18] prepared from 320 g. (5.25 moles) of nitromethane and 530 g. (5.0 moles) of benzaldehyde was filtered and washed with a cold solution of 250 ml. of methanol and 250 ml. of ether. The product was added in portions with stirring to a mixture of 303 g. (5.05 moles) of glacial acetic acid, 2 l. of water and 1 kg. of chipped ice. The mixture was kept below 5° during the addition and for thirty minutes thereafter. A yellow oil separated as the lower layer.

The aqueous layer was decanted and extracted with three 500-ml. portions of ether. The extracts were combined with the oily layer along with an additional 500 ml. of ether and washed twice with 10% sodium bisulfite solution, twice with 5% sodium bicarbonate solution and finally with water. The ether solution was dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* (water-pump at 40°). The residue weighed 651 g. (78%).

Anal. Calcd. for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.23; H, 5.38; N, 8.47.

α-Nitroacetophenone (IX).—To a 5-l. flask fitted with a mechanical stirrer, a thermometer and a large bore funnel were added 1.5 l. of water, 750 ml. of glacial acetic acid and

(14) Gabriel, *Ber.*, **43**, 1283 (1910).
(15) Pictet and Gams, *ibid.*, **43**, 2388 (1910).
(16) Wolfheim, *ibid.*, **47**, 1442 (1914).
(17) The analytical data were determined by Mrs. Patricia Ramey and Mr. C. E. Childs of this Laboratory.
(18) Rosenmund, *Ber.*, **46**, 1037 (1913).

157 g. of potassium dichromate. The mixture was stirred until the dichromate was in solution. The solution was cooled to 15° following the addition of 78.5 ml. of concentrated sulfuric acid. One hundred and sixty-seven grams (1.0 mole) of 1-phenyl-2-nitroethanol was added in one portion, and the resulting dark-colored mixture was allowed to warm up while being stirred. The temperature was maintained at 20–25° for twenty-four hours.

The mixture was filtered and the crystalline product was washed thoroughly with cold water. The yield was 118 g. (72%); m. p. 105°. Extension of the reaction time to forty-eight hours increased the yield to 80%.

Anal. Calcd. for $C_8H_7NO_3$: C, 58.17; H, 4.27. Found: C, 58.17; H, 4.15.

Reduction of α-Nitroacetophenone. (a) **By Stannous Chloride.**—A solution was prepared by gently warming a mixture of 430 g. (1.9 moles) of stannous chloride dihydrate and 660 ml. of concentrated hydrochloric acid in a 3-l. flask fitted with a reflux condenser. With mild cooling 450 ml. of 95% ethanol was added. When the solution had cooled to 25°, 99 g. (0.6 mole) of α-nitroacetophenone was added in one portion, and the resulting mixture heated to reflux for two hours.

The mixture was cooled to 5° and filtered. The crystalline solid was washed with cold, 95% ethanol and dried *in vacuo* over sulfuric acid; yield 167 g. (92%), m. p. 250°.

(b) **By Metallic Tin.**—A solution of 800 ml. of concentrated hydrochloric acid and 800 ml. of 95% ethanol was prepared in a 3-l. flask equipped with a stirrer and a reflux condenser. The solution was cooled to 30° and 99 g. (0.6 mole) of α-nitroacetophenone and 112 g. (0.95 mole) of granular tin (20–50 mesh) were added. The mixture was heated to reflux on a steam-bath and refluxing was continued for thirty minutes after all of the tin had dissolved. The clear, yellow solution was cooled to 5° and the crystalline product filtered off. The solid was washed with cold 95% ethanol and dried; yield 148 g. (82%), m. p. 245°. The yield was increased when tin recovered from stannic chloride solutions by the addition of zinc dust was employed.

α-Benzamidoacetophenone (IV, R = phenyl).—Although a previous publication[10] warns that difficulties are encountered unless great care is taken in preparing α-acylamidoacetophenone from the tin complex salt of α-aminoacetophenone (X), the following procedure was quite successful for the preparation of α-benzamidoacetophenone.

In a 3-l. flask equipped with an efficient stirrer, a thermometer and a dropping funnel were placed 55.5 g. (0.092 mole) of the tin complex salt of α-aminoacetophenone and 750 ml. of water (40°). The mixture was stirred until a clear solution was obtained. The solution was cooled to 0° and 31 g. (0.22 mole) of benzoyl chloride was added. The mixture was stirred vigorously while 150 g. of cold, 30% aqueous sodium hydroxide was added rather rapidly, the temperature remaining below 10°. The cold mixture was stirred for thirty minutes after the addition was complete.

The mixture was filtered and the solid product washed thoroughly with water, dilute hydrochloric acid and again with water. The filter-cake was dissolved in 225 ml. of hot 95% ethanol, charcoaled and filtered. The hot filtrate was diluted with 225 ml. of hot water. On cooling, the crystalline product precipitated. It was filtered off and dried; yield 41 g. (98%), m. p. 123–125° (recorded m. p.[11] 123°).

1,5-Diphenyl-2,4-dibenzamido-1,5-pentadione (XI).—Twelve grams (0.05 mole) of α-benzamidoacetophenone, 1.5 g. of paraformaldehyde and 1 g. of potassium carbonate were mixed together with 100 ml. of methanol. When the mixture was shaken at room temperature, a clear solution was formed within five to ten minutes. After a total of twenty minutes a solid precipitated. The solid was removed by filtration, washed with water and dilute hydrochloric acid and dried; yield 11 g. (90%), m. p. 195–200°. One gram of product was recrystallized with little loss of material by solution in 100 ml. of hot 95% ethanol and cooling, m. p. 208–210°.

Anal. Calcd. for $C_{31}H_{26}N_2O_4$: C, 75.90; H, 5.34; N, 5.71. Found: C, 76.01; H, 5.55; N, 5.68.

α-Benzamido-β-hydroxypropiophenone (V, R = phenyl). (a) **Using Potassium Carbonate as a Catalyst.**—Twelve grams (0.05 mole) of α-benzamidoacetophenone was mixed in 100 ml. of methanol with 1.6 g. of paraformaldehyde and 0.3 g. of potassium carbonate. The mixture was shaken vigorously at room temperature, and after eight minutes a clear solution was formed. The solution was poured at once into a mixture of water and ice. A gummy material precipitated which solidified within a short time. The dried product weighed 12 g., m. p. 100–125°. Recrystallization from aqueous methanol did not greatly improve the melting point. The analysis was unsatisfactory.

Anal. Calcd. for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.62. Found: C, 72.66; H, 5.56.

(b) **Using Sodium Bicarbonate as a Catalyst.**—Twelve grams (0.05 mole) of α-benzamidoacetophenone was mixed in 100 ml. of methanol with 3 g. of paraformaldehyde and 0.4 g. of sodium bicarbonate. At room temperature there was very little evidence of reaction. When shaken at 50°, the mixture was converted to a clear solution within a few minutes. This solution remained clear for two days. When poured into a mixture of ice and water, a colorless gum precipitated which quickly solidified. Recrystallization was accomplished by solution in 50 ml. of hot, 95% ethanol and dilution with an equal volume of water, m. p. 134–141°. It was recrystallized a second time from ethyl acetate; yield 10.4 g. (77%), m. p. 142–143°.

Anal. Calcd. for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.62. Found: C, 71.05; H, 5.59.

α-Benzamido-β-acetoxypropiophenone (XII, R = phenyl).—Acetylation of α-benzamido-β-hydroxypropiophenone with a mixture of acetic anhydride and glacial acetic acid at 75° was unsuccessful. However, when 5.38 g. (0.02 mole) of the propiophenone derivative was mixed with 12 ml. of acetic anhydride and one drop of concentrated sulfuric acid, the reaction proceeded satisfactorily. After fifteen minutes at 40° the solution was concentrated *in vacuo* and the residue washed with cold water and dried, m. p. 121–123°. Recrystallization was effected by solution in 50 ml. of hot 95% ethanol and dilution with 25 ml. of water; yield 6.2 g. (99%), m. p. 123–125°.

Anal. Calcd. for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.51. Found: C, 69.72; H, 5.46.

***dl-threo*-1-Phenyl-2-benzamido-1,3-propanediol** (VI, R = phenyl).—The crude product from the condensation of 23.9 g. (0.1 mole) of α-benzamidoacetophenone and paraformaldehyde was mixed with a moderate amount of Raney nickel catalyst in 200 ml. of methanol. Hydrogenation of 50 p. s. i. was complete within two hours. The catalyst was removed and the filtrate was thoroughly concentrated *in vacuo*. The residue was refluxed twice with 250-ml. portions of ether, filtered and dried; yield 16 g., m. p. 127–142°. The product was recrystallized twice by solution in hot, 50% ethanol and slow cooling; yield 5.5 g. (40% based on α-benzamidoacetophenone), m. p. 166–167°. Acid hydrolysis and diacetylation of the free base produced the *dl-threo*-diacetate identical with the derivative prepared by an alternate procedure.[4] The lower-melting *dl-erythro*-racemate may be isolated from the residues.

Anal. Calcd. for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32. Found: C, 71.01; H, 6.33.

***dl-threo*-1-Phenyl-2-amino-1,3-propanediol Diacetate** (XIII).—The crude product from the hydrogenation of 50 g. (0.185 mole) of α-benzamido-β-hydroxypropiophenone was refluxed for three hours with 800 ml. of 6 *N* hydrochloric acid. The mixture was cooled, and the pre-

cipitated benzoic acid was filtered off. The filtrate was extracted twice with moderate quantities of ethyl acetate and concentrated *in vacuo*. The residue was dissolved in 100 ml. of water, made basic with alkali and saturated with potassium carbonate. The mixture was extracted thoroughly with ethyl acetate, and the combined extracts were dried over anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was dissolved in 50 ml. of acetic anhydride and allowed to stand for thirty minutes before being concentrated *in vacuo*. By recrystallization of the residue from 75 ml. of absolute ethanol, 14 g. (60%) of the desired product was obtained, m. p. 166–167°.

Anal. Calcd. for $C_{13}H_{17}NO_4$: C, 62.13; H, 6.82. Found: C, 62.30; H, 6.89.

α-Acetamidoacetophenone (IV, R = methyl).—Earlier procedures[14,15,16] have employed α-aminoacetophenone hydrochloride; whereas the following preparation starts with the α-aminoacetophenone tin complex salt.

To a 3-l. flask equipped with an efficient stirrer, a thermometer and a dropping funnel were added the tin complex salt obtained from 99 g. (0.6 mole) of α-nitroacetophenone and 750 ml. of water (40°). The mixture was stirred until a clear solution was produced which was then cooled to −5°. Seventy-four grams of acetic anhydride was added in one portion, and the resulting mixture was stirred rapidly while a cold solution of 135 g. of sodium hydroxide in 300 ml. of water was added at such a rate as to keep the temperature below 10°. When about 225 ml. of the sodium hydroxide solution had been added and the mixture was almost alkaline, 20 g. of acetic anhydride was poured in. Addition of the hydroxide was completed and stirring was continued for thirty minutes at 0°.

The mixture was saturated with sodium chloride and extracted twice with 500-ml. portions of ethyl acetate. The combined extracts were washed with saturated salt solution, dried over anhydrous magnesium sulfate and concentrated to 200 ml. on a steam-bath. To the hot concentrate was added 180 ml. of ligroin. The mixture was cooled to 5° and filtered. The yield was 67 g. (63% based on α-nitroacetophenone), m. p. 86–87°.

α-Acetamido-β-hydroxypropiophenone (V, R = methyl).—Fifty-three and one-tenth grams (0.3 mole) of α-acetamidoacetophenone was mixed with 150 ml. of water and 45 ml. of 36–38% aqueous formaldehyde in a flask fitted with a stirrer and a thermometer. One-half gram of sodium bicarbonate was added and the mixture was stirred and warmed to 35°. Within five minutes a clear solution was formed. Stirring was continued for two hours while the mixture was allowed to cool to room temperature. At this point an appreciable quantity of solid had precipitated. After thorough cooling the mixture was filtered and the solid product was washed with cold water. A small amount of the crystalline material was dissolved in hot water and allowed to crystallize out slowly. In this manner several large, clear crystals were obtained, m. p. 68–70°. A single crystal was carefully wiped clean with filter paper and analyzed.

Anal. Calcd. for $C_{11}H_{13}NO_3 \cdot H_2O$: C, 58.65; H, 6.71. Found: C, 58.31; H, 6.71.

When the product was dried at 80° *in vacuo*, the melting point increased to 112–115°. The material was recrystallized from benzene. The yield was 48 g. (77%); m. p. 117–119°.

Anal. Calcd. for $C_{11}H_{13}NO_3$: C, 63.75; H, 6.32. Found: C, 63.71; H, 6.25.

***dl-threo*-1-Phenyl-2-acetamido-1,3-propanediol (VI, R = methyl).**—Two hundred and seven grams (1.0 mole) of α-acetamido-β-hydroxypropiophenone was hydrogenated as described above for the corresponding benzamido derivative. The residue obtained by concentration *in vacuo* of the filtrate from the catalyst was refluxed for a few minutes with 600 ml. of ethyl acetate. On cooling slightly, a crystalline precipitate began to form. As soon as the temperature of the mixture reached 30°, the solid product was removed by filtration, washed with ethyl acetate and dried. The yield was 68 g. (65%); m. p. 135–136°. By recrystallization from ethyl acetate the melting point was increased to 136–138°. When mixed with material prepared by an alternate procedure,[4] the melting point was not lowered.

Anal. Calcd. for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23. Found: C, 63.33; H, 7.33.

***dl-erythro*-1-Phenyl-2-amino-1,3-propanediol Triacetate (XIV).**—The monoacetyl derivative, m. p. 108–110°, may be isolated from the residues of the preceding preparation by recrystallization with alcohol and ethyl acetate. However, a better separation is arrived at by conversion to the triacetyl derivative. After removal of the *dl-threo*-1-phenyl-2-acetamido-1,3-propanediol, the ethyl acetate filtrate was concentrated and the residue was refluxed with 250 ml. of acetic anhydride for one hour. The excess solvents were removed *in vacuo*, and the oily residue was mixed with 600 ml. of dry ether. A clear solution was formed and shortly thereafter a crystalline solid precipitated. The mixture was filtered and the product dried; yield 70 g. (47%), m. p. 116–118°.

Anal. Calcd. for $C_{15}H_{19}NO_5$: C, 61.42; H, 6.53. Found: C, 60.98; H, 6.34.

Summary

A procedure for the synthesis of chloramphenicol has been developed. Derivatives of *dl-threo*-1-phenyl-2-amino-1,3-propanediol have been prepared by the monohydroxymethylation of α-acylamidoacetophenone and subsequent catalytic hydrogenation. Previous experience[4] has shown that these derivatives may be converted to chloramphenicol.

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