Synthesis of Chloramphenicol via an Enzymatic Enantioselective Hydrolysis

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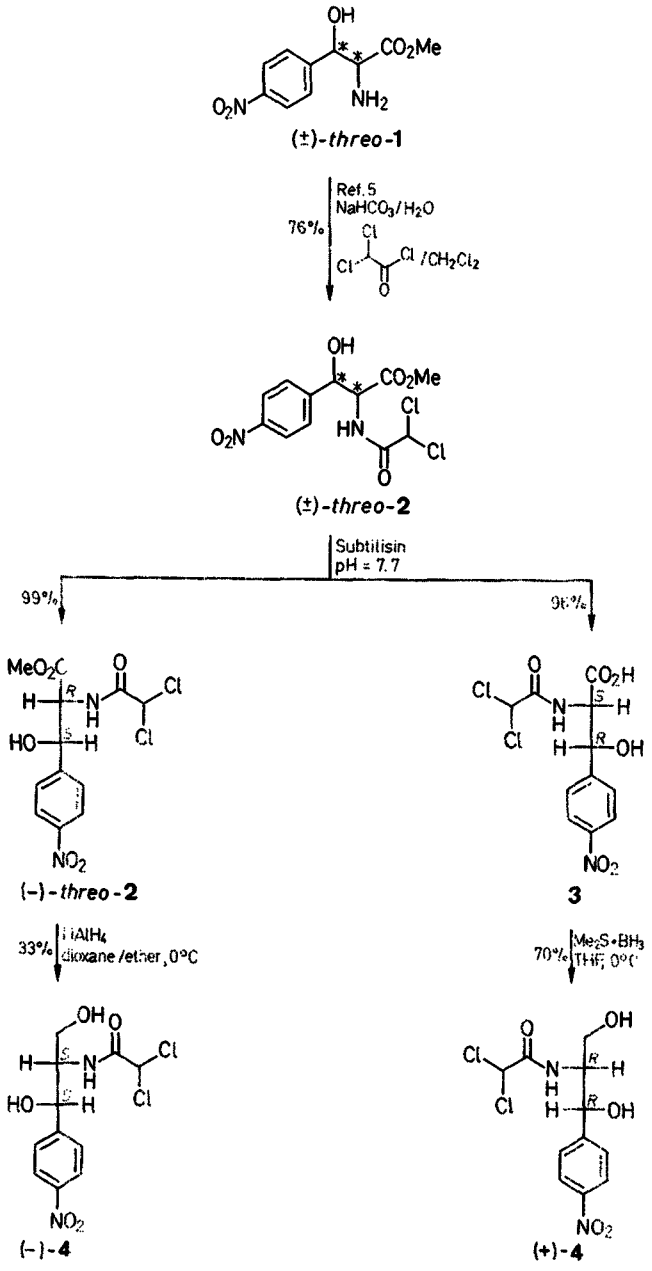
An efficient method for the preparation of the antibiotic chloramphenicol [(+)-**4**] is described. Methyl (±)-*threo*-*N*-dichloroacetyl-*β*-(4-nitrophenyl)serinate [(±)-*threo*-**2**], readily obtainable on a preparative laboratory scale, was enantioselectively hydrolyzed by Subtilisin. This enzymatic kinetic resolution gave the corresponding (2*S*,3*R*)-acid **3** and the unhydrolyzed (2*R*,2*S*)-ester (−)-*threo*-**2** in high yield and high optical purity. Reduction of the (2*S*,3*R*)-acid **3** by borane–methyl sulfide complex gave chloramphenicol [(+)-**4**]. Reduction of the (2*R*,3*S*)-ester (−)-*threo*-**2** by lithium aluminum hydride gave the enantiomer (−)-**4** chloramphenicol.

Chloramphenicol, the first of the broad-spectrum antibiotics, was initially isolated from cultures of various Streptomyces strains.[1–3] It was also the first antibiotic industrially produced by chemical synthesis rather than by fermentation.[4] We report here a simple chemoenzymatic preparation of chloramphenicol.

The *threo* isomer of 4-nitrophenylserinate **(1)** was first obtained by condensation of 4-nitrobenzaldehyde with methyl glycinate according to the procedure of Ehrhart et al.[5] Compound **1** was then acylated with dichloroacetyl chloride in dichloromethane

to give compound **2** in 76% yield.[5] The dichloroacetyl derivative (±)-*threo*-**2** was treated with Subtilisin to give the unreactive (2*R*,3*S*)-ester (−)-*threo*-**2** and the (2*S*,3*R*)-acid **3**. Both compounds are obtained in nearly quantitative yields and high optical purities (ee > 97%). Enantiomeric excess (ee) values were determined by ^{1}H-NMR analysis using tris[(heptafluoropropylhydroxymethylene)-*d*-camphorato] europium(III) as a chiral shift reagent.[6] A small sample of acid **3** was converted to its methyl ester before determination of the optical purity. Well resolved signals were obtained for the ester methyl protons.



The preparative-scale enzyme-catalyzed hydrolysis was performed in a buffered aqueous solution at pH 7.7. The enzymatic reaction was indicated by the decrease of pH, which was maintained at its initial value by the addition of a diluted sodium hydroxide solution. The reaction was monitored by the consumption of the base and stopped completely after 50% conversion, as expected for a highly selective enantiomer differentiation. The unhydrolyzed ester was recovered from the reaction mixture by extraction with ethyl acetate at initial pH. The product acid, with was present in the aqueous phase as sodium salt was isolated after acidification to pH 2 and extraction with ethyl acetate. Other usual hydrolases (chymotrypsin, bromelain, papain, lipases, esterases) were not active or gave products of low optical purity.

Reduction of the (−)-*threo*-**2** with lithium aluminum hydride gave the (*S*,*S*)-diol (−)-**4**, the optical antipode of chloramphenicol, in 33% yield. Reduction of the (2*S*,3*R*)-acid **3** by borane-methyl sulfide complex[7,8] gave the (*R*,*R*)-diol (+)-**4** in 70% yield. All the physical and spectral data of compound (+)-**4** are identical to those of an authentic sample of chloramphenicol.[9,10]

Subtilisin (EC 3.4.21.14, Carlsberg, protease type VIII), authentic chloramphenicol, and Eu(hfc)$_3$ were purchased from Aldrich Chem. Co. Optical rotations were measured with a Jasco DIP-360 digital polarimeter. IR spectra were obtained using a Beckman 4250 spectrophotometer. NMR studies were performed on a Varian XL-200 spectrometer using TMS as internal standard. Mass spectra were obtained using a Hewlett Packard 5992 GC/MS system.

Methyl (±)-*threo*-*N*-Dichloroacetyl-β-(4-nitrophenyl)serinate (2):[5]
A solution of dichloroacetyl chloride (2.9 mL, 30 mmol) in CH_2Cl_2 (30 mL) is added dropwise to a cold suspension of methyl (±)-*threo*-β-(4-nitrophenyl)serinate[5] (**1**; 4.80 g, 20 mmol) and $NaHCO_3$ (3.36 g, 40 mmol) in distilled water (50 mL). The mixture is stirred overnight at room temperature. CH_2Cl_2 (250 mL) is added, and the organic layer is separated, dried ($MgSO_4$), and concentrated at reduced pressure to a volume of 50 mL. *n*-Pentane is added until a crystalline solid begins to form. The resulting suspension is chilled overnight and filtered to give **2** as a colorless solid; yield: 5.32 g (76%); mp 151–152°C (Lit.[5] mp 152°C).

IR (KBr): ν = 3430, 3305, 1725, 1690, 1660, 1540, 1350, 1290, 1190, 1060, 1000, 850 cm^{-1}.

^{1}H-NMR (acetone-d_6): δ = 3.78 (s, 3H, OCH_3); 4.20 (m, 1H, OH); 4.43 (dd, 1H, J_1 = 1 Hz, J_2 = 5 Hz, CHN); 5.58 (d, 1H, J = 1 Hz, CHO); 6.43 (s, 1H, $CHCl_2$); 7.77 (d, 2H$_{arom}$, J = 4 Hz); 7.92 (d, 1H, J = 5 Hz, NH); 8.20 (d, 2H$_{arom}$, J = 4 Hz).

MS (70 eV): m/z = 292 (M^+ − 59).

Enzymatic Resolution of Methyl (±)-*threo*-*N*-Dichloroacetyl-β-(4-nitrophenyl)serinate](±)-*threo*-2]:
A solution of Subtilisin Carlsberg (200 mg, 2000 units) in phosphate buffer (10 mL, pH 7.7) is added to a suspension of (±)-*threo*-**2** (3.60 g, 10 mmol) in the same solvent (200 mL). Hydrolysis is indicated by a decrease of pH (measured with a pH meter), which is maintained at its initial value by the addition of a 0.5 N NaOH solution. The reaction comes to a near standstill after 4.5 h (50% conversion). The unhydrolyzed ester is then removed from the aqueous layer by extraction with EtOAc (3 × 100 mL). The aqueous phase is acidified with 1 N HCl to pH 2, and the acid **3** is extracted with EtOAc (3 × 100 mL). Both the organic phases are separately dried ($MgSO_4$), and evaporated to yield the unhydrolyzed ester (−)-*threo*-**2**; yield: 1.797 g (99%); $[\alpha]_D^{25}$ −17.1° (*c* = 0.5, MeOH); and the acid **3**; yield: 1.259 g (96%); mp 168–170°C (dec); $[\alpha]_D^{25}$ +23.4° (*c* = 0.1, MeOH) [Lit.[11] mp 168°C (dec)].

IR (KBr): ν = 3340, 1700, 1690, 1650, 1530, 1340, 1285, 1100, 1060, 1010, 850 cm^{-1}.

^{1}H-NMR ($CDCl_3$): δ = 4.88 (d, 1H, J = 1 Hz, CHN); 5.64 (d, 1H, J = 1 Hz, CHO); 5.80 (m, 1H, OH); 6.44 (s, 1H, $COCHCl_2$); 7.81 (d, 2H$_{arom}$, J = 5 Hz); 7.87 (m, 1H, NH); 8.18 (d, 2H$_{arom}$, J = 5 Hz); 11.17 (m, 1H, CO_2H).

MS (70 eV): m/z = 337 (M^+).

Esterification of a Sample of Acid 3; Methyl (+)-*threo*-*N*-Dichloroacetyl-β-(4-nitrophenyl)serinate [(+)-*threo*-2]:
The acid (51 mg, 0.15 mmol), 2,2-dimethoxypropane (2.0 mL) and a catalytic amount of TsOH (2 mg) are dissolved in dry MeOH (2 mL). The solution is stirred magnetically for 4 h at 55°C under a dry atmosphere. Acetone and MeOH are evaporated, and the oily residue is dissolved in EtOAc (15 mL). The organic layer is washed with water (10 mL), aq. 5% $NaHCO_3$ (10 mL), brine (10 mL), dried ($MgSO_4$), and evaporated; yield: 26 mg (50%); $[\alpha]_D^{25}$ +17.1° (*c* = 0.3, MeOH). Other physical or spectroscopic data are identical to those reported above for this compound.

Enantiomer (−)-4 of Chloramphenicol [(+)-4]:
To a cold solution of ester (−)-*threo*-**2** (350 mg, 1 mmol) in dry dioxane (19 mL) and dry ether (11 mL), is added slowly a suspension of $LiAlH_4$ (38 mg, 1 mmol) in dry ether (5 mL). The reaction is maintained at 0 °C for 30 min and at room temperature for 4 h under a dry atmosphere. A solution of water/dioxane (95:5) (100 mL) is added at 0 °C, and the mixture is stirred for 16 h. The mixture is filtered, and the filtrate is evaporated. The crude product is dissolved in distilled water (50 mL), and the aqueous phase is extracted with EtOAc (3 × 100 mL). The organic phase is dried ($MgSO_4$), and evaporated. The product is further purified by chromatography on a silica gel column using EtOAc as eluent; yield: 108 mg (33%); mp 149–151 °C; $[\alpha]_D^{25}$ −18.4° (*c* = 1.00, EtOH) [Lit.[12] mp 151.5–152.5 °C, $[\alpha]_D^{20}$ −20.0° (EtOH)].

IR (KBr): ν = 3340, 3260, 1685, 1610, 1560, 1340, 1290, 1250, 1060, 850, 820 cm^{-1}.

^{1}H-NMR (acetone-d_6): δ = 3.30 (m, 2H, 2OH); 3.76 (m, 2H, CH_2O); 4.19 (m, 1H, CHN); 5.33 (d, 1H, *J* = 1 Hz, CHO); 6.35 (s, 1H, $CHCl_2$); 7.58 (d, 1H, *J* = 5 Hz, NH); 7.71 (d, 2H_{arom}, *J* = 4 Hz); 8.15 (d, 2H_{arom}, *J* = 4 Hz).

MS (70 eV): *m/z* = 323 (M^+).

Chloramphenicol [(+)-4]:
In a dried nitrogen-filled flask fitted with a septum, the acid **3** (672 mg, 2 mmol) is dissolved in dry THF (100 mL). A 2M THF solution of $Me_2S \cdot BH_3$ (1.5 mL, 3 mmol) is added slowly with a syringe at 0 °C. The mixture is stirred at room temperature for 16 h. MeOH (20 mL) is then added at 0 °C. The solvents are evaporated, and the resulting oil is taken up in EtOAc (200 mL). This organic solution is washed with a solution of aq. 5% $NaHCO_3$ (50 mL), brine (50 mL), dried ($MgSO_4$), and evaporated. Chloramphenicol [(+)-**4**] crystallizes on standing and is recrystallized from water. (The unchanged acid can be recovered by acidification of the aqueous layer and extraction with EtOAc); yield: 446 mg (70%) (98% based upon recovered starting material); mp 149.5–150 °C; $[\alpha]_D^{25}$ +18.3° (*c* = 2.0, EtOH) (Lit.[9,10] mp 149.7–150.7 °C; $[\alpha]_D^{25}$ +18.6° (*c* = 4.86, EtOH) and mp 150–151 °C; $[\alpha]_D^{19}$ +17.9° (*c* = 2.89, EtOH).

IR (KBr): ν = 3340, 3260, 1685, 1615, 1560, 1345, 1290, 1250, 1060, 850, 815 cm^{-1}.

^{1}H-NMR (acetone-d_6): δ = 3.29 (m, 2H, 2OH); 3.76 (m, 2H, CH_2O); 4.20 (m, 1H, CHN); 5.33 (d, 1H, *J* = 1 Hz, CHO); 6.35 (s, 1H, $CHCl_2$); 7.58 (d, 1H, *J* = 5 Hz, NH); 7.71 (d, 2H_{arom}, *J* = 4 Hz); 8.15 (d, 2H_{arom}, *J* = 4 Hz).

MS (70 eV) *m/z* = 323 (M^+).

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