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Synthesis of 4-Denitro-4-azido-chloramphenicol

A Photochemically Activatable Analog of Chloramphenicol

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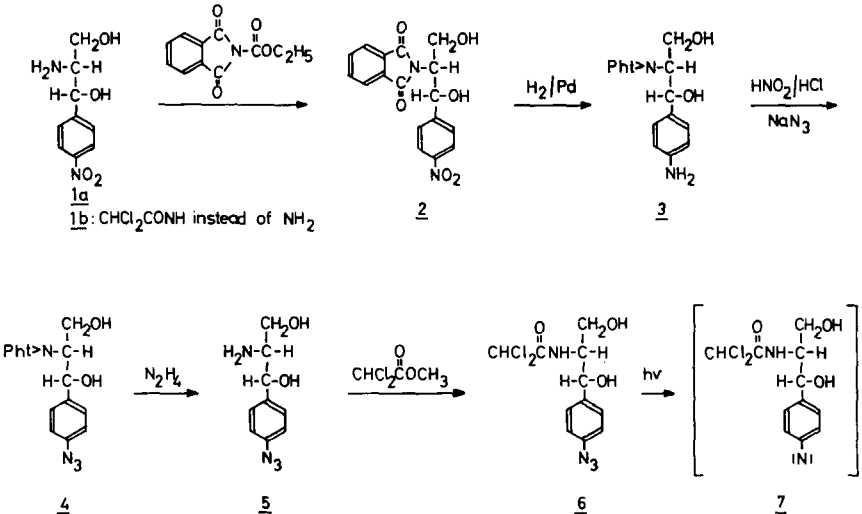
The synthesis of 4-denitro-4-azido-chloramphenicol is described. Phthalylation of D-threo-(1R:2R)-1-(4-nitrophenyl)-2-amino-1.3-propanediol with *N*-ethoxycarbonyl-phthalimide yields D-threo-(1R:2R)-1-(4-nitrophenyl)-2-phthaloylamino-1.3-propanediol. On catalytic hydrogenation, the latter compound is converted to D-threo-(1R:2R)-1-(4-aminophenyl)-2-phthaloylamino-1.3-propanediol, diazotization of which, followed by displacement of the diazonium group by azide ion, gives D-threo-(1R:2R)-1-(4-azidophenyl)-2-phthaloylamino-1.3-propanediol. Hydrazine dephthalylates that compound to give D-threo-(1R:2R)-1-(4-azidophenyl)-2-amino-1.3-propanediol. By esterification of this azide with methyl dichloroacetate D-threo-(1R:2R)-1-(4-azidophenyl)-2-dichloroacetylamino-1.3-propanediol, "4-denitro-4-azido-chloramphenicol" is formed. This substance photolyses on irradiation with uv light to a reactive nitrene, which is expected to form covalent linkages at its ribosomal binding site, and thus, help to elucidate the mode of action of the antibiotic chloramphenicol in protein biosynthesis.

INTRODUCTION

The antibiotic chloramphenicol (**1b**) is a strong, reversible inhibitor of protein biosynthesis (*1–3*). It is thought to bind to the ribosome near the peptidyl transferase center.



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To get detailed information about its mode of action, chloramphenicol has been modified at several positions (*4*). A recently published modification of the structure involved substitution of the dichloroacetyl moiety by a highly reactive iodoacetyl group (*5*). This was expected to lead to a covalent linkage of the properly oriented molecule to its binding region, and that this occurred was demonstrated by the isolation of labeled ribosomal proteins after reaction with the iodo derivative. This method of labeling is called affinity labeling, and the modified bioactive molecule is an affinity label.

We now describe a new chloramphenicol analog from which an affinity label can be generated *in situ* at its regular binding position. It is expected that the affinity label will be covalently linked to its binding protein without affecting other proteins. The chloramphenicol analog contains an aromatic azido group instead of a nitro group. Aryl azides (*6*) are suitable for such a purpose in biosystems since they are chemically stable at 37°C, but are activated on irradiation with uv light to reactive nitrenes, which are not very susceptible to photolytic rearrangements. These nitrenes are able to attack carbon/hydrogen single bonds by insertion, and carbon/carbon double bonds by addition reactions. Nitrenes are also reactive towards substitutents with lone pairs of electrons (*7*). The photolytically generated affinity label may bind covalently to its receptor molecule and therefore, this method is called "photoaffinity labeling" (*6*).

In this paper, we wish to report the complete synthesis of 4-denitro-4-azido-chloramphenicol (**6**) (*8*). This chloramphenicol derivative is a very stable compound under normal conditions, but photolyses readily on irradiation with uv light.

RESULTS

Synthesis of 4-Denitro-4-azido-chloramphenicol

Commercially available D-threo-(1R:2R)-2-amino-1-(4-nitrophenyl)-1.3-propanediol (**1a**) (*1*, *9*), possessing the same optical configuration at C-1 and C-2 as the antibiotic chloramphenicol (**1b**), was used as starting material for the synthesis.

To convert the nitro group at C-4 in the aromatic ring into an azido group, it was first necessary to protect the aliphatic 2-amino group. As the azido group is sensitive towards acid, the phthaloyl-protecting group was chosen, which can be easily removed under mild alkaline conditions.

The condensation of the amino group in (**1a**) with phthalic acid anhydride by heating it in glacial acetic acid (*10*) always resulted in impure material, as shown by tlc (silica gel, chloroform/methanol, 95:5). Therefore, another phthaloylation method had to be used. It has been shown that phthaloylation of amino acids with *N*-ethoxycarbonylphthalimide (*11*) under alkaline conditions (sodium carbonate or sodium hydroxide solution) at room temperature yields the expected *N*-phthaloyl derivatives almost quantitatively. However, the use of this method for the preparation of (**2**) resulted in poor yields and the formation of side products, which were detected by tlc (silica gel, chloroform/methanol, 95:5). By investigation of the reaction conditions with bases of higher and lower p*K*-values it was found that weak bases such as sodium hydrogen carbonate or sodium acetate led to the best results: Treatment of compound (**1a**) with *N*-ethoxycarbonyl-phthalimide in dioxane/water containing 0.17 *M* sodium hydrogen carbonate gave (**2**) in 72% yield. The 4-nitro group of (**2**) was reduced by hydrogenation in meth-

anolic solution at normal pressure with palladium-on-charcoal as catalyst. The amorphous compound (**3**), obtained almost quantitatively, gave a positive ninhydrin test, and migrated as a single spot on tlc (silica gel, chloroform/methanol, 95:5). The combustion analysis was in agreement with the structure (**3**). The ^1H nmr spectrum showed proton signals of an A_2B_2 pattern that were shifted downfield from 8.25; 7.68 for compound (**2**) to 7.05; 6.58 ppm for the amino derivative (**3**).

Conversion of the 4-amino group of (**3**) into an azido group was accomplished by diazotization of (**3**) with nitrous acid, leading to the diazonium salt that was not isolated, but converted directly into the azido compound by nucleophilic displacement of the diazonium group with azide ion. After extraction of the aqueous solution and chromatography on silica gel in chloroform/methanol (95:5), colourless prisms of D-threo-(1R:2R)-1-(4-azidophenyl)-2-phthaloylamino-1.3-propanediol (**4**) were obtained. A strong absorption at 2100 cm^{-1} in the ir spectrum, resulting from the stretching mode of the azide in (**4**), indicated the presence of the azido group in the molecule.

Dephthalylation of (**4**) was accomplished by hydrazinolysis in ethanol solution at room temperature overnight. The o-phthaloyl-*N*.*N'*-hydrazine was easily removed by precipitation after acidification with acetic acid, and D-threo-(1R:2R)-1-(4-azidophenyl)-2-amino-1.3-propanediol (**5**) was isolated in 65% yield. Compound (**5**) migrates on electrophoresis (silica gel, 0.1 *M* sodium citrate, pH 6.5) as a single spot and gives a purple colour with ninhydrin.

Suspension of the azide (**5**) in methyl dichloroacetate and heating for 1 hr at 90°C leads to D-threo-(1R:2R)-1-(4-azidophenyl)-2-dichloracetylamino-1.3-propanediol (**6**), "4-denitro-4-azido-chloramphenicol." After column chromatography (silica gel, chloroform/ethanol, 97:3) and crystallization, the pure analog (**6**) was obtained in 70% yield as yellowish prisms. The ir spectrum shows a strong absorption at 2100 cm^{-1}, indicating the presence of the azido group; the uv spectrum of **6** in methanol shows maxima at 253 and 288 nm. Its combustion analysis agrees with the calculated formula, and the mass spectrum shows a mole peak $m/e = 218$. Compound (**6**) migrates with an $R_f = 1.14$ relative to chloramphenicol on tlc plates (silica gel, chloroform/methanol, 9:1).

Under the conditions used for the synthesis of the chloramphenicol derivative (**6**), no racemization is possible since the asymmetric centers do not participate in the reactions (*12*). This was confirmed by the optical rotations obtained for all intermediates.

The chloramphenicol derivative photolyses readily by irradiation with uv light to give a red–brown reaction product. Under these conditions, native chloramphenicol (**1b**) does not show an appreciable photoreaction.

As recently shown (*13*), irradiation of native chloramphenicol bound to ribosomes can result in covalent coupling of the antibiotic to ribosomal proteins. However, the reaction, which is not fully understood, led to an unspecific labeling pattern. This probably results from the low quantum yield of this photoactivation process. In contrast, the chloramphenicol analog (**6**), because of its high light sensitivity, is expected to readily form covalent linkages only at its regular binding site.

One way to detect the chloramphenicol-binding protein is to introduce a radioactive atom into the photoactivatable molecule. This can be done by condensation of [^{14}C]dichloroacetate with (**5**) in the last step of synthesis and investigations of this type are in progress.

EXPERIMENTAL

Melting points were determined on the Monoskop (Reichert, Austria) and are not corrected. Optical rotations were measured with the Perkin–Elmer 141 spectropolarimeter. The nmr spectra were taken with a Bruker HX-60 spectrometer and are reported in δ-values (ppm) relative to tetramethylsilane as internal standard. The uv spectra were measured with the Zeiss PMQ II or the Shimadzu uv 200 spectrometer, respectively, and the ir spectra with the Perkin–Elmer infracord spectrometer. Microanalyses were performed by Mikroanalytisches Labor Beller, Göttingen.

Chromatography

Column chromatography was performed on silica gel 60 (70–230 mesh, Merck, Darmstadt) using a LKB fraction collecter. Because of the sensitivity of the azide compounds to uv light, no uv detection unit was used. Instead, compounds were separated by thin-layer chromatography on silica gel plates (Merck, Darmstadt) and detected by uv_{260} absorption or ninhydrin reaction.

Electrophoresis

Thin-layer electrophoresis was performed on thin-layer plates (Woelm, F 254) in a Desaga TLE-double chamber. D-threo-(1R:2R)-1-(4-nitrophenyl)-2-amino-1.3-propanediol (**1a**) (*1*, *7*) was purchased from Sigma Chemical Company, St. Louis, Mo.

D-threo-(1R:2R)-2-phthaloylamino-1-(4-nitrophenyl)-1.3-propanediol (**2**)

To a stirred solution of 2.12 g (10 mmole) D-threo-(1R:2R)-2-amino-1-(4-nitrophenyl)-1.3-propanediol (**1a**) in 30 ml dioxane, a solution of 840 mg (10 mmole) sodium hydrogen carbonate in 30 ml water and solid *N*-ethoxycarbonyl-phthalimide (2.19 g, 10 mmole) were added. After 30 min of stirring at room temperature, the solution was acidified with 20 ml of 1 *N* hydrochloric acid and then diluted with 50 ml of water. Evaporation to one-half of the volume led to crystallization of (**2**). After filtration and drying *in vacuo*, 2.47 g (72.2%) colorless crystals were obtained; mp 227–229°C; tlc (chloroform/methanol, 95:5) $R_f = 0.7$; uv λ_{max} (methanol) 228, 272 nm (ε, 35 100, 10 600); $[\alpha]^{20}D$ -44.4° (*c*, 0.9, methanol); ^{1}H-nmr (DMSO-d_6) 4.07 (*m*, 2H), 4.82 (*t*, 1H, $J = 6$ Hz), 5.17 (*dd*, 1H, $J = 4.5$ Hz), 5.92 (*d*, 1H, $J = 4.5$ Hz), 7.68 (*d*, 2H, $J = 8$ Hz), 7.88 (*s*, 4H), 8.25 (*d*, 2H, $J = 8$ Hz) ppm. *Anal.* Calcd for $C_{17}H_{14}N_2O_6$ (342.31): C, 52.65; H, 4.12; N, 8.18. Found: C, 59.75; H, 4.28; N, 8.23.

D-threo-(1R:2R)-2-phthaloylamino-1-(4-aminophenyl)-1.3-propanediol (**3**)

An amount of 1.03 g (3 mmole) of compound (**2**) was dissolved in 150 ml methanol and hydrogenated in the presence of Pd/charcoal (10% Pd) under 760 mm Hg. The catalyst was removed by filtration and the solvent evaporated. After adding ether and additional evaporation, yellowish foam was obtained in almost quantitative yield. Compound (**3**) gave a positive ninhydrin test and migrated on tlc plates (silica gel,

chloroform/methanol, 95:5) with an R_f-value of 0.4. uv λ_{max} (methanol) 221, 236, 242, 292 nm (ε, 34 300; 21 400; 21 700; 3500). *Anal.* Calcd for $C_{17}H_{16}N_2O_4$ (312.33): C, 65.28; H, 5.16; N, 8.97. Found: C, 64.65; H, 5.28; N, 8.80.

D-threo-(1R:2R)-2-phthaloylamino-1-(4-azidophenyl)-1.3-propanediol (**4**)

A solution of 312 mg (1 mmole) compound (**3**), dissolved in 1.5 ml 2 *N* hydrochloric acid, was cooled to 0–5°C. The diazonium salt was formed by treating the ice-cold solution with 72 mg (1 mmole) sodium nitrite in 1 ml water with stirring. After a total reaction time of 30 min, a solution of 65 mg (1 mmole) sodium azide in 1 ml water was added dropwise. During this step, nitrogen was liberated. The mixture was kept for 1 hr at approximately 0°C, and the reaction product was extracted with ethyl acetate. The organic layer was washed with water, filtered, and the solution was evaporated. The residue was chromatographed on a 13 × 3.5 cm column (silica gel, chloroform/ethanol, 97:3) and fractions containing the major peak were pooled. After evaporation of the solvent, the residue was crystallized from a small amount of methanol by decreasing the temperature to –20°C. 172 mg (44.3%), colorless prisms were obtained; mp 163°C. Compound (**4**) migrated as a single spot on tlc plates (silica gel, chloroform/ethanol, 97:3). ir (KBr): 2 100 cm^{-1} (s), azide; uv λ_{max} (methanol) 243, 250, 290 nm (ε, 20 300; 19 600; 4 200); $[\alpha]^{20}D$ -57.8° (*c*, 1.0, methanol); ^{1}H-nmr (DMSO-d_6) 3.1 (*m*, 1H), 4.1 (*m*, 2H), 4.9 (*m*, 2H), 5.60 (*d*, 1H, J = 4 Hz), 7.17 (*d*, 2H, J = 8 Hz), 7.45 (*d*, 2H, J = 8 Hz), 8.83 (*s*, 4H) ppm. *Anal.* Calcd for $C_{17}H_{14}N_4O_4$ (338.32): C, 60.35; H, 4.17; N, 16.56. Found: C, 60.49; H, 4.16; N, 16.56.

D-threo-(1R:2R)-2-amino-1-(4-azidophenyl)-1.3-propanediol (**5**)

To the solution of 250 mg (**4**) (0.74 mmole) in 30 ml ethanol there was added at room temperature 12.5 ml of 1 *M* hydrazine hydrate in ethanol. The mixture was stored for 20 hr at room temperature. After evaporation of the solvent, the residue was dissolved in 25 ml water, then acidified with acetic acid. The precipitated o-phthaloyl-*N*.*N*'-hydrazine was removed by filtration. After evaporation of the solvent, the residual water was removed by azeotropic evaporation with benzene. After recrystallization from ethyl acetate/low petroleum ether, 134 mg (79.2%) of colorless prisms were obtained; mp 121–123°C. Compound (**5**) gave a positive ninhydrin test and migrated in the tlc electrophoresis with R_f = 0.75 of compound (**1a**). uv λ_{max} (methanol) 253, 288 nm (ε, 15 600; 2 500); ir (KBr): 2100 cm^{-1} (*s*), azide; $[\alpha]^{20}D$ -24.7° (*c*, 1.0, methanol); ^{1}H-nmr (DMSO-d_6) 2.72 (*m*), 3.20 (*m*), 4.47 (*d*, 1H, J = 5 Hz), 7.17 (*d*, 2H, J = 8 Hz), 7.31 (*d*, 2H, J = 8 Hz) ppm. *Anal.* Calcd for $C_9H_{12}N_4O_2$ (208.23): C, 51.91; H, 5.81; N, 26.91. Found: C, 52.35; H, 5.81; N, 26.87.

D-threo-(1R:2R)-2-dichloroacetylamino-1-(4-azidophenyl)-1.3-propanediol (**6**)

A suspension of 100 mg (0.48 mmole) of compound (**5**) in 250 µl methyl dichloroacetate was kept at 90°C for 1 hr. In that time, the substance dissolved. After cooling of the solution, the excess of solvent was removed by extraction with low petroleum ether and the oily residue separated on a 7.5 × 1.5 cm column (silica gel, chloroform/ethanol,

9:1) by chromatography. The substance of the slow migrating main band was isolated after evaporation and the residue crystallized from methylenechloride/low petroleum ether. Yield 107 mg (70.4%) pale yellow prisms; mp 92–94°C; ir (KBr): 2100 cm^{-1} (*s*), azide; uv λ_{max} (methanol) 253, 288 nm (ε, 16 100; 2 500); MS: *m/e* 218. *Anal.* Calcd for $C_{11}H_{12}N_4O_3Cl_2$ (319.16): C, 41.40; H, 3.79; N, 17.56; Cl, 22.22. Found: C, 41.29; H, 3.83; N, 17.50; Cl, 22.30.

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