Nucleosides LXXXII. Synthetic Studies on Nucleoside Antibiotics. 12. Synthesis of 1-[3,4-Dideoxy-4-(sarcosyl-D-seryl)amino-β-D-ribohexopyranosyl]cytosine, an Analog of Gougerotin (1)

T. M. K. Chiu, D. H. Warnock, K. A. Watanabe and J. J. Fox

Memorial Sloan-Kettering Cancer Center,
Sloan-Kettering Division of Graduate School of Medical Sciences
Cornell University, New York, New York 10021

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1-[3,4-Dideoxy-4-(sarcosyl-D-seryl)amino-β-D-ribohexopyranosyl]cytosine (**3**), a 3′-deoxy-5′-hydroxymethyl analog of gougerotin, was synthesized from 3-deoxy-β-D-*xylo*hexopyranose (3-deoxy-D-galactose)tetraacetate (**4**).

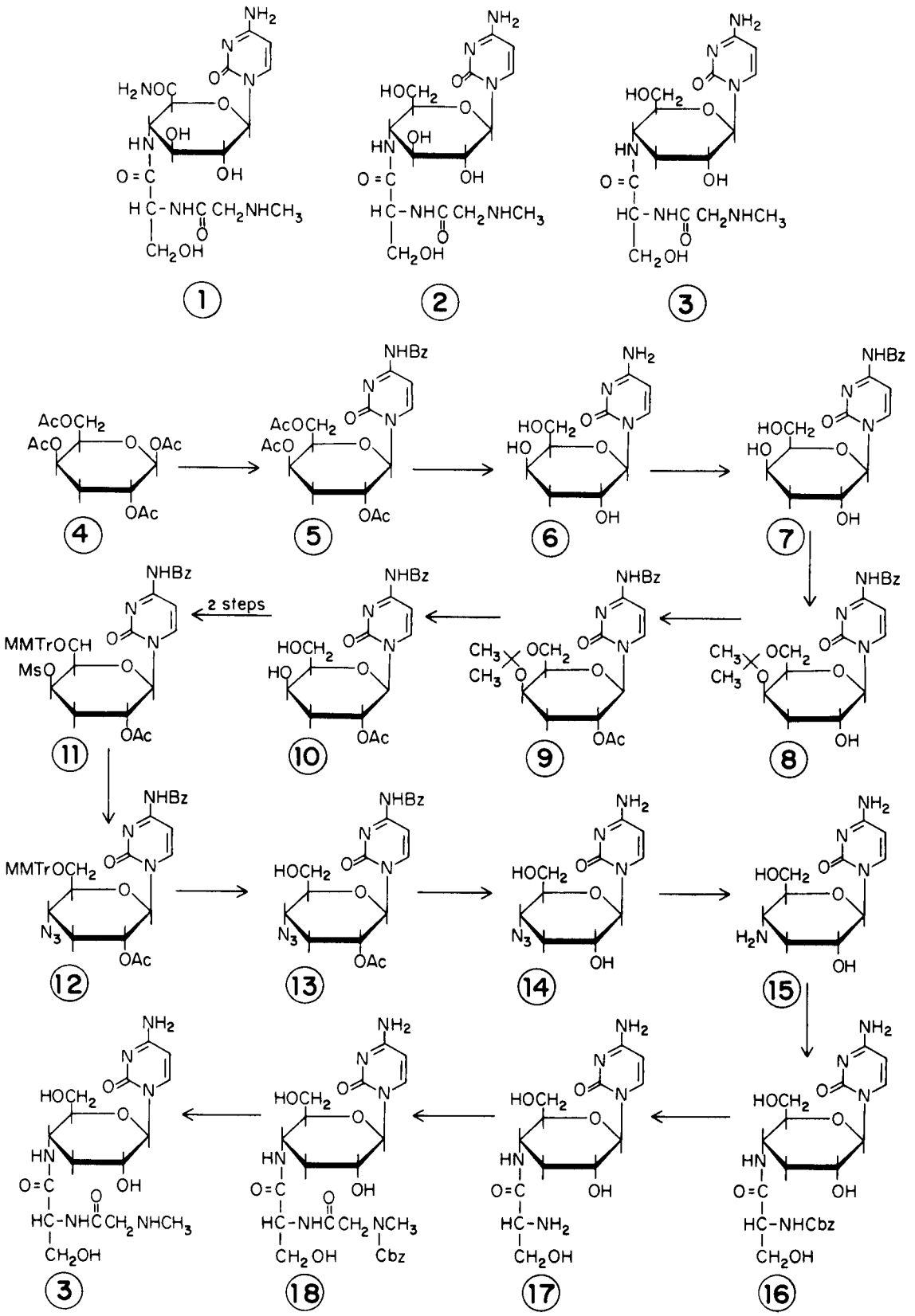
In a recent report, we described the total synthesis (2) of the nucleoside antibiotic, gougerotin (**1**) as well as the synthesis and biological activity (3) of certain analogs modified either in the dipeptide moiety or on the 5′-carboxamide moiety (4) or both. We have shown that the 5′-hydroxymethyl analog (4) of gougerotin (**2**) retains considerable capacity (relative to gougerotin) to inhibit protein synthesis (*in vitro*) on the tRNA-ribosomal level (3). In order to gain information on the role of secondary sugar hydroxyl of gougerotin in the protein synthesis inhibition process, a 3′-deoxy analog (**3**) of **2** was prepared. The synthesis of **3** is the subject of this report.

Tetra-*O*-acetyl-3-deoxy-β-D-*xylo*hexopyranose (**4**), the starting material for this synthesis, was prepared in 5 steps from diisopropylidene-α-D-glucofuranose by the modified (6) procedure of Prokop and Murray (5). Condensation of **4** with bis(trimethylsilyl)-*N*-benzoylcytosine in the presence of stannic chloride in dichloroethane (7) gave the protected nucleoside **5** in 90% yield. After deacylation of **5** with sodium methoxide in methanol, the product (**6**) was selectively benzoylated at N^4 with benzoic anhydride in methanol (8) to afford crystalline **7**. Isopropylidenation of **7** gave **8** which was isolated in quantitative yield. [Benzylidenation of **7** gave the desired benzylidene compound but in a very poor yield]. Compound **8** was acetylated to **9** and then treated with 80% aqueous acetic acid at room temperature (9) to afford **10** in 90% yield. Compound **10** was reacted with di-*p*-methoxytrityl chloride (MMTrCl) (10) in pyridine at 80° and the reaction was followed by thin layer chromatography (tlc). After dimethoxytritylation was complete, the reaction mixture was cooled to 0° and the reaction mixture was treated with mesyl chloride. Crystalline mesylate **11**, isolated in *ca.* 90% yield, was treated with sodium azide in hexamethylphosphoric triamide (HMPT) and the mixture was stirred at 80° for 6 hours. The crystalline azide **12** was obtained in 90% yield. Detritylation of **12** in 80% aqueous acetic acid afforded **13** which was deacylated to the free nucleoside (**14**) with sodium methoxide in methanol. Reduction of the azide of **14** in the presence of palladium-on-carbon gave compound **15** in quantitative yield.

Compound **15** was treated with *N*-benzyloxycarbonyl (Cbz)-D-serine and dicyclohexylcarbodiimide (DCC) in a mixture of water and acetonitrile to give crude **16** which was not purified but hydrogenolyzed in the presence of 10% palladium-on-carbon catalyst. The serylamino derivative (**17**) was isolated in crystalline form in *ca.* 80% yield from **15**. Similar treatment of **17** with Cbz-sarcosine and DCC gave the protected peptidyl nucleoside (**18**) in *ca.* 70% yield. Hydrogenolysis of the Cbz group of **18** afforded the crystalline sarcosyl-D-serylamino derivative (**3**) in quantitative yield.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are corrected. The proton magnetic resonance spectra (pmr) were recorded on a Varian A-60 spectrometer using DMSO-d_6 as solvent and tetramethylsilane as internal standard. Chemical shifts are reported in parts per million (δ). Values for coupling constants (hertz) are first order. Optical rotations were determined on a Keston polarimeter attachment to a Beckman DU spectrophotometer set at 589 mμ. Microanalyses were performed by Galbraith Laboratories Inc., Knoxville, Tennessee. Uv spectra were recorded on a Unicam SP 500 spectrometer.



Flow Chart

N^4-Benzoyl-1-(2,3,6-tri-*O*-acetyl-3-deoxy-β-D-*xylo*hexosyl)cytosine (**5**).

Bis(trimethylsilyl)-N^4-benzoylcytosine was prepared from *N*-benzoylcytosine (13.6 g.) and hexamethyldisilazane (15 ml.) and trimethylsilyl chloride (15 ml.) in toluene (11) and dissolved in dry 1,2-dichloroethane.

To a solution of **4** (12.5 g.) in 1,2-dichloroethane (600 ml.) was added tin tetrachloride (16 ml.) and the mixture was stirred for 20 minutes. The dichloroethane solution of bis(trimethylsilyl)-N^4-benzoylcytosine was added, and the reaction mixture was stirred overnight at room temperature, and then at 50° for 1 hour. The reaction mixture was diluted with dichloromethane (300 ml.) and poured slowly into a saturated sodium bicarbonate solution (1000 ml.). The suspension was filtered through a celite pad. The organic layer of the filtrate was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was crystallized from ethanol to give 16.4 g. (90%) of compound **5**; m.p. 216-219° $[\alpha]_D^{27}$ -34 (c, 1.0, chloroform): nmr (deuteriochloroform); H-1′, δ = 6.09 (doublet), $J_{1',2'}$ = 9.5 Hz (establishing the *beta* configuration). The uv spectrum of **5** was similar to that for N^4-benzoylcytidine (5).

Analytical sample was obtained after recrystallization from ethanol, m.p. 220-223°.

Anal. Calcd. for $C_{23}H_{25}N_3O_9$: C, 56.67; H, 5.17; N, 8.62. Found: C, 56.40; H, 5.17; N, 8.62.

1-(3-Deoxy-β-D-*xylo*hexopyranosyl)cytosine (**6**).

To a suspension of **5** (24.5 g.) in methanol (200 ml.) was added 1*N* sodium methoxide in methanol (2.5 ml.), and the mixture was stirred for 3 hours at room temperature. The clear solution obtained was neutralized with Amberlite IRC-50 (H^+) (20 ml.). The resin was filtered and the filtrate was evaporated. The residue was partitioned between water (100 ml.) and ether (100 ml.). The aqueous layer was washed with ether (100 ml.) and evaporated to dryness. Crude **6** was obtained as a powder (13 g.) which was sufficiently pure for the next step.

A small amount of the crude **6** was dissolved in a small amount of water and the solution was acidified to *p*H ~ 1 with concentrated hydrochloric acid. After extraction with ether, the acidic solution was evaporated to near dryness, then diluted with ethanol. Pure **6** separated as its hydrochloride salt as colorless needles; m.p. 135-137° $[\alpha]_D^{27}$ -33 (c, 1.0, water).

Anal. Calcd. for $C_{10}H_{15}N_3O_5 \cdot HCl$: C, 40.89; H, 5.49; N, 14.31; Cl, 12.07. Found: C, 40.78; H, 5.43; N, 14.28; Cl, 12.17.

N^4-Benzoyl-1-(3-deoxy-β-D-*xylo*hexopyranosyl)cytosine (**7**).

A mixture of crude **6** (12 g.) and benzoic anhydride (25 g.) in methanol (800 ml.) was refluxed. During the course of refluxing two further additions of 25 g. portions of benzoic anhydride were made at 2 hour intervals. After the final addition, the solution was refluxed for 2 hours, then concentrated to dryness. The residue was partitioned between dichloromethane (500 ml.) and water (500 ml.). The organic layer was extracted with water (2 x 500 ml.). The water extracts were combined with the original water layer and concentrated to ~ 300 ml. Compound **7** crystallized and was filtered, (20 g., 62%) m.p. 141-143°.

An analytical sample was recrystallized from methanol-water (1:9); m.p. 144-147° $[\alpha]_D^{27}$ -37 (c, 1.0, methanol).

Anal. Calcd. for $C_{17}H_{19}N_3O_6$: C, 56.51; H, 5.30; N, 11.63. Found: C, 56.68; H, 5.48; N, 11.42.

N^4-Benzoyl-1-(3-deoxy-4,6-*O*-isopropylidene-β-D-*xylo*hexosyl)cytosine (**8**).

A mixture of **7** (10 g.), *p*-toluenesulfonic acid (1.0 g.) and 2,2-dimethoxypropane (40 ml.) in acetone (400 ml.) was shaken for 4 hours at room temperature, then neutralized with sodium bicarbonate powder (3.0 g.). After removal of insoluble salts, the filtrate was evaporated to dryness. The residue was crystallized from ethanol-water to give 11.0 g. of **8**; m.p. 122-124°.

Anal. Calcd. for $C_{20}H_{23}N_3O_6 \cdot 2/3H_2O$: C, 58.10; H, 5.93; N, 10.16. Found: C, 57.81; H, 5.78; N, 9.93. The presence of a small amount of water in the analytical sample was detected by an nmr spectrum taken in anhydrous DMSO-d_6.

N^4-Benzoyl-1-(2-*O*-acetyl-3-deoxy-4,6-*O*-isopropylidene-β-D-*xylo*hexosyl)cytosine (**9**).

A mixture of **8** (11.0 g.) and acetic anhydride (4.0 ml.) in pyridine (13 ml.) was kept standing overnight at room temperature. Compound **9** (5.2 g.) precipitated was filtered and washed with ether. The filtrate was diluted with ether to give another crop (1.7 g.) of **9**. The combined crops of **9** were recrystallized from ethanol; m.p. 226-228°; $[\alpha]_D^{27}$ -34 (c, 1.0, methanol).

Anal. Calcd. for $C_{22}H_{25}N_3O_7 \cdot 1/3H_2O$: C, 58.79; H, 5.75; N, 9.34. Found: C, 59.01; H, 5.65; N, 8.96.

N^4-Benzoyl-1-(2-*O*-acetyl-3-deoxy-β-D-*xylo*hexopyranosyl)cytosine (**10**).

A mixture of **5** (4.5 g.), glacial acetic acid (4.5 ml.) and water (2 ml.) was stirred at room temperature for 18 hours then at 40° for 3 hours. The solution was co-evaporated several times with ethanol to give a colorless solid which was recrystallized from ethanol to give 3.7 g. (92%) of **10**; m.p. 188-191°.

Anal. Calcd. for $C_{19}H_{21}N_3O_7 \cdot 2/3H_2O$: C, 54.94; H, 5.42; N, 10.12. Found: C, 55.23; H, 5.39; N, 9.65. The presence of a small amount of water in the analytical sample was detected by nmr spectrum taken in DMSO-d_6.

N^4-Benzoyl-1-(2-*O*-acetyl-3-deoxy-6-*O*-dimethoxytrityl-4-*O*-mesyl-β-D-*xylo*hexosyl)cytosine (**11**).

A mixture of **10** (3.6 g.) and 4,4′-dimethoxytrityl chloride (3.4 g.) in pyridine (25 ml.) was heated at 80° for 2 hours. Another charge of dimethoxytrityl chloride (0.3 g.) was added and the mixture was heated for another 2 hours. Then the reaction mixture was cooled to ~ 0° and mesyl chloride (1.0 ml.) was added. After 18 hours at ~ 0° the mixture was poured into an ice-water mixture with stirring. The precipitate was filtered, washed with water, then dissolved in a small amount of dichloromethane. Compound **11** was obtained by reprecipitation from the dichloromethane solution with ether and petroleum ether to give 5.1 g. of colorless microcrystals, m.p. 131-134°.

Anal. Calcd. for $C_{41}H_{43}N_3O_{11}S$: C, 62.82; H, 5.27; N, 5.36; S, 4.09. Found: C, 63.59; H, 5.27; N, 4.55; S, 3.63. Though the compound did not give satisfactory elemental analyses, the nmr spectrum (in deuteriochloroform) was consistent with structure **11**: acetyl CH_3 at δ = 1.97 (3H); mesyl CH_3 at δ = 2.87 (3H), anisyl CH_3 at δ = 3.78 (6H), H2′ at δ = 5.21 (1H), H1′ at δ = 6.04 (1H, $J_{1',2} \cong 9.0$). This compound was used for the next step without further purification.

N^4-Benzoyl-1-(2-*O*-acetyl-4-azido-3,4-dideoxy-6-*O*-dimethoxytrityl-β-D-*ribo*hexosyl)cytosine (**12**).

A mixture of **11** (4.7 g.), sodium azide (2.4 g.) and HMPT (15 ml.) was heated at 70° with stirring for 6 hours. The reaction mixture was poured into an ice-water mixture (250 ml.) with stirring. The precipitate was filtered, washed with water to give crude **12** which was suspended in water (200 ml.), stirred and filtered. Colorless solid of **12** (4.0 g.) was obtained, m.p.

101-108°. The nmr spectrum (deuteriochloroform) indicates the presence of approximately one mole of HMPT; acetyl CH_3 at δ = 1.99 (3H), HMPT CH_3 at δ = 2.57 and 2.72, anisyl CH_3 at δ = 3.77 (6H), H2′ at δ = 5.10 and H1′ at δ = 5.97 ($J_{1',2'} \cong 9.0$ Hz).

The product analyzed best with one mole of HMPT.

Anal. Calcd. for $C_{40}H_{38}N_6O_8 \cdot C_6H_{18}N_3OP$: C, 60.65; H, 6.20; N, 13.84. Found: C, 61.16; H, 5.84; N, 12.69.

N^4-Benzoyl-1-(2-*O*-acetyl-4-azido-3,4-dideoxy-β-D-*ribo*hexosyl)-cytosine (**13**).

Compound **12** (4.2 g.) was dissolved in a mixture of acetic acid (10 ml.) and water (1.5 ml.) and the solution was stirred at room temperature for 1 hour. After evaporation of the solvent *in vacuo*, the remaining acetic acid was removed by repeated additions of ethanol, followed by evaporation. The residue was triturated well with ether and filtered. Compound **12** (2.4 g.) was obtained as a colorless powder.

This product was contaminated with a small amount of HMPT and was used directly in the next step.

1-(4-Azido-3,4-dideoxy-β-D-*ribo*hexosyl)cytosine (**14**).

Compound **13** (2.2 g.) was dissolved in methanol (20 ml.) saturated wtih ammonia at 0°. The flask was sealed and left at room temperature for 24 hours. The solvent was evaporated to dryness and the residue was chromatographed on a column (10 x 5 cm.) of 65 g. of silica gel G (9:1, chloroform-methanol). The eluate was monitored by tlc (silica gel GF_{254}) and the major fraction was collected and evaporated to dryness to give **14** as a colorless powder, 805 mg., m.p. 231-233° dec., $[\alpha]_D^{27}$ +36 (c, 1.0, water).

Anal. Calcd. for $C_{10}H_{14}N_6O_4$: C, 42.55; H, 5.00; N, 29.77. Found: C, 42.63; H, 5.04; N, 29.63.

1-(4-Amino-3,4-dideoxy-β-D-*ribo*hexopyranosyl)cytosine (**15**).

Compound **14** (740 mg.) was dissolved in water (50 ml.) and hydrogenated over 10% palladium-on-carbon (~ 100 mg.) for 30 minutes with the inital pressure of 33 lbs. The catalyst was removed and the filtrate was evaporated to dryness. The residue was co-evaporated several times with ethanol to yield crystalline **15**, 660 mg. (98%). An analytical sample was recrystallized from methanol; m.p. 254-256° dec.

Anal. Calcd. for $C_{10}H_{16}N_4O_4$: C, 46.87; H, 6.29; N, 21.86. Found: C, 46.64; H, 6.25; N, 21.72.

1-[4-(*N*-Benzyloxycarbonyl-D-seryl)amino-3,4-dideoxy-β-D-*ribo*-hexopyranosyl]cytosine (**16**).

To a mixture of **15** (370 mg., 1.78 mmoles) and *N*-Cbz-D-serine (540 mg., 2.43 mmoles) in water (1.5 ml.) was added DCC (660 mg.) in acetonitrile (6 ml.). The mixture was shaken for 24 hours at room temperature. Dicyclohexylurea was removed and washed with 50% aqueous methanol (15 ml.). The combined filtrate and washings were stirred with Dowex-1 (OH^-) (3 ml.) for 15 minutes and filtered. The filtrate was evaporated to dryness, and the residue was co-evaporated several times with ethanol and triturated with ether (5 ml. x 3). Compound **16** (850 mg.) was obtained as a white powder which was contaminated with a small amount of dicyclohexylurea and was used directly in the next step.

1-[3,4-Dideoxy-4-(D-seryl)amino-β-D-*ribo*hexopyranosyl]cytosine (**17**).

Compound **16** (800 mg.) dissolved in a 1:3 ethanol-water mixture (70 ml.) was hydrogenated in the presence of 10% palladium-on-carbon (~ 150 mg.) for 20 minutes with an initial hydrogen pressure of 33 lbs. The catalyst was filtered and washed with water. The combined filtrate and washings were evaporated to dryness. The residue was co-evaporated several times with ethanol until microcrystals were obtained (513 mg., 89%). Recrystallization from methanol gave an analytical sample: m.p. 140-143°, $[\alpha]_D^{27}$ +54 (c, 1.0, methanol).

Anal. Calcd. for $C_{13}H_{21}N_5O_6 \cdot C_2H_5OH$: C, 46.27; H, 6.98; N, 17.98. Found: C, 46.15; H, 6.59; N, 17.81. The presence of one mole of ethanol of crystallization was shown by nmr spectrum (in deuterium oxide) of the analytical sample.

1-[4-(*N*-benzyloxycarbonylsarcosyl-D-seryl)amino-3,4-dideoxy-β-D-*ribo*hexopyranosyl]cytosine (**18**).

To a mixture of **17** (563 mg., 1.65 mmoles) and Cbz-sarcosine (700 mg., 2.88 mmoles) in water (1.5 ml.) was added DCC (630 mg.) in acetonitrile (6 ml.). The mixture was shaken for 24 hours at room temperature. Dicyclohexylurea was filtered and washed with 50% aqueous methanol (40 ml.). The combined filtrate and washings were evaporated to near dryness and the residue was triturated well with ether (15 ml. x 3). The colorless powder obtained was dissolved in 60% aqueous methanol and the solution was stirred with Dowex-1 (OH^-) (6 ml.) for 15 minutes. The resin was filtered and washed with 60% methanol. The combined filtrate and washings were evaporated to near dryness and the residue was triturated with ether (15 ml. x 3). Compound **18** (617 mg.) was obtained as a colorless powder which was not further purified but used directly in the next step.

1-[3,4-Dideoxy-4-(sarcosyl-D-seryl)amino-β-D-*ribo*hexopyranosyl]-cytosine (**3**).

Compound **18** (513 mg.) was dissolved in 50% aqueous ethanol (50 ml.) and hydrogenated over 10% palladium-on-carbon for 20 minutes with an initial hydrogen pressure of 33 lbs. The catalyst was filtered and washed with water. The combined filtrate and washings were evaporated to dryness. The residue was co-evaporated several times with ethanol until microcrystals were obtained. The product (417 mg.) thus obtained was analytically pure; m.p. 214-217° dec., $[\alpha]_D^{27}$ -48 (c, 1.0, water).

Anal. Calcd. for $C_{16}H_{26}N_6O_7 \cdot 2H_2O \cdot 1.5\ C_2H_5OH$: C, 43.92; H, 7.56; N, 16.18. Found: C, 44.01; H, 7.41; N, 15.89. The presence of water and 1.5 moles of ethanol in the analytical sample was demonstrated by nmr spectrum (DMSO-d_6).

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(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service (Grant No. CA 08748).

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