Nucleosides. LXXIV. Synthetic Studies on Nucleoside Antibiotics. 8. Syntheses of 1-[4-Deoxy-4-(sarcosyl-D-seryl)amino-β-D-glucopyranosyl]cytosine and Related Analogs of Gougerotin[1]

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The synthesis of 1-[4-deoxy-4-(sarcosyl-D-seryl)amino-β-D-glucopyranosyl]cytosine (**6a**), an analog of gougerotin, is described. Condensation of 1-(4-amino-4-deoxy-β-D-glucopyranosyl)cytosine (**1**) with *N*-Cbz-D-serine in aqueous acetonitrile in the presence of dicyclohexylcarbodiimide gave the 4′-*N*-Cbz-seryl derivative **3a** as the main product. Hydrogenation of **3a** afforded the 4′-seryl nucleoside (**4a**) which was reacted with *N*-Cbz-sarcosine. After removal of the Cbz group by reduction, **6a** was obtained in ~50% overall yield from **1**. Detailed examination of the acid hydrolysate of **6a** showed that little, if any, racemization of the seryl moiety occurred during the synthesis of **6a**. The sarcosyl-D-alanyl (**6b**) and sarcosyl-D-phenylalanyl (**6c**) analogs were also synthesized using the active ester procedure.

Previous reports from this laboratory described the syntheses of 1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine[2] (C-substance) and 1-(4-amino-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranosyl)cytosine,[3] derivatives related to the nucleoside moieties of gougerotin and blasticidin S.[4] This paper deals with the synthesis of 1-[4-deoxy-4-(sarcosyl-D-seryl)amino-β-D-glucopyranosyl]cytosine (**6a**) and related derivatives as part of our program designed toward the total synthesis of these antibiotics and/or analogs thereof and their biological evaluation.

The most direct approach to the synthesis of gougerotin analogs (**6**) would be to link a protected sarcosyl-D-serine to the 4′-amino group of the aminoglucosylcytosine (**1**). Both *tert*-butoxycarbonyl (Boc) and benzyloxycarbonyl (Cbz) derivatives of sarcosyl-D-serine[5] and their *O*-acetyl derivatives, however, in our hands failed to condense with nucleoside **1** by use of a number of reported procedures including the dicyclohexylcarbodiimide[7] (DCC), azide,[8] mixed anhydride,[8,9] oxidation–reduction,[10] and other methods. The sarcosyl-D-serine derivatives failed even to form an active ester with *p*-nitrophenol, although *O*-acetyl-*N*-Cbz-serine could be converted to its crystalline *p*-nitrophenyl ester in good yield.[11]

Reaction of **1** with 2 equiv of *N*-Cbz-*O*-acetyl-D-serine *p*-nitrophenyl ester (**7a**) in dimethyl sulfoxide (DMSO) (without base) gave the *N*,*N*′-disubstituted derivative (**2a**) in good yield. Treatment of crude **2a** with Dowex-1 (OH^-) selectively removed the seryl group on cytosine as well as causing de-*O*-acetylation of D-serine. After hydrogenolysis of the Cbz group, the desired D-seryl nucleoside (**4a**) was obtained. It was, however, very difficult to purify **4a**. The nmr of the crude product revealed contamination by alanyl derivatives. Apparently the acetoxy group was eliminated during the Dowex-1 treatment introducing the α–β double bond which was reduced during debenzyloxycarbonylation by hydrogenation. However, application of this active ester procedure to *N*-Cbz-D-alanine and *N*-Cbz-D-phenylalanine gave the corresponding products (**4b** and **4c**) in high yield and in pure state. Reaction of **4b** and **4c** with the *p*-nitrophenyl ester of *N*-Cbz-sarcosine in DMSO followed by Dowex-1 (OH^-) treatment and hydrogenolysis gave the corresponding dipeptidyl nucleosides (**6b** and **6c**) in good yield.

The main difficulty in the synthesis of the seryl nucleoside **3a** is the protection of the hydroxyl group of the amino acid. With an *O*-acyl protecting group some β elimination always occurred. The *O*-benzyl protecting group also failed due to reduction of the 5,6 double bond of cytosine during the subsequent reductive debenzylation step. We found, however, that when *N*-Cbz-D-serine itself was treated with **1** and DCC in aqueous tetrahydrofuran (THF) or aqueous acetonitrile, the *N*-Cbz-D-seryl nucleoside (**3a**) was obtained together with a small amount of **2** (R = OH). Treatment of the mixture of **3a** and **2a** with Dowex-1 (OH^-) followed by hydrogenolysis gave pure D-seryl nucleoside (**4a**) in good yield. Reaction of **4a** with *N*-Cbz-sarcosine and DCC resulted in the formation of *N*-Cbz-sarcosyl-D-seryl nucleoside (**5a**) contaminated with some N^4-acylated material. Treatment of this mixture with Dowex-1 (OH^-) followed by hydrogenolysis afforded 1-[4-deoxy-4-(sarcosyl-D-seryl)-amino-β-D-glucopyranosyl]cytosine (**6a**) as colorless microcrystals in ~50% overall yield from **1**. (Compound **6a** differs structurally from gougerotin only by the presence of a 5′-hydroxymethyl group instead of a 5′-carboxamide function). The uv spectrum of **6a** as a function of pH is similar to that for cytidine, showing that the amino group of the aglycon is unsubstituted. The ir spectrum showed the absence of an ester linkage, thus ruling out the possibility of a linkage of

(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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(5) Recently Lichtenthaler, *et al.* [*Tetrahedron Lett.*, 2061 (1970)] reported the synthesis of this compound by the same procedure that we reported for the preparation of its racemate.[6]

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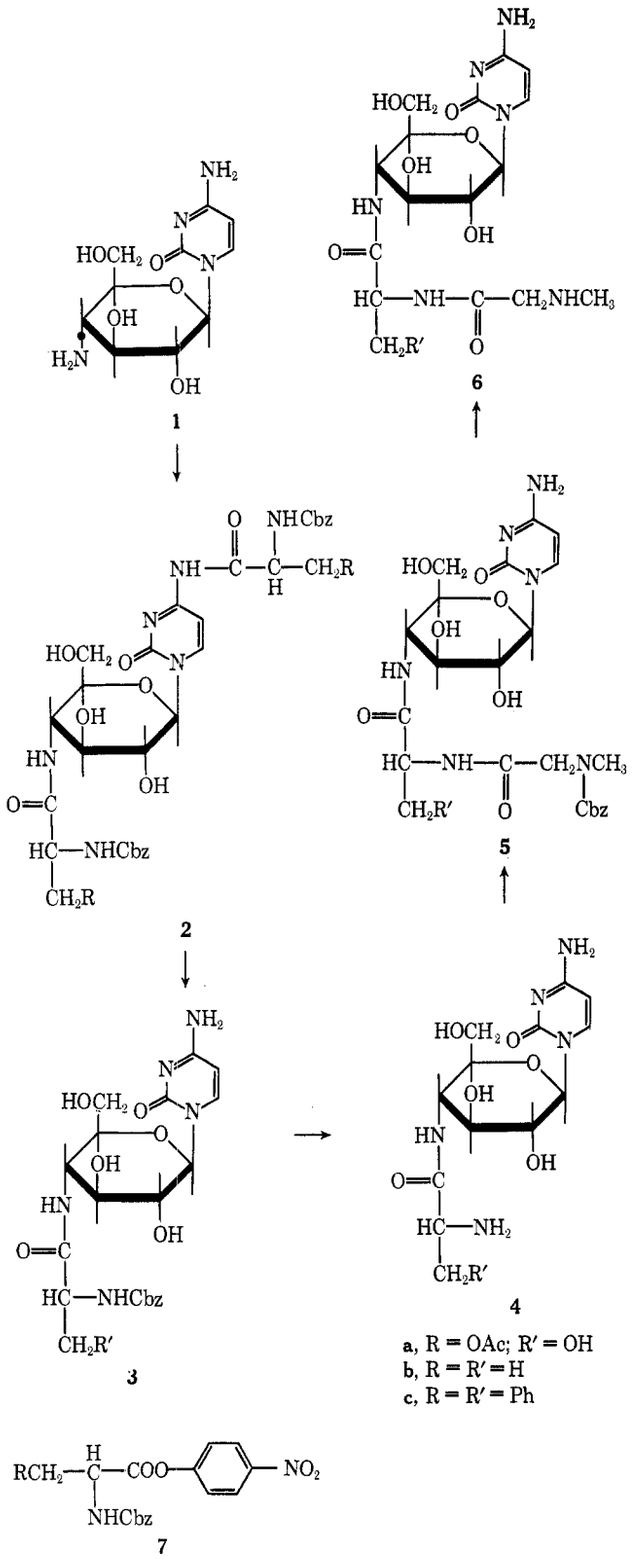

the amino acid to any hydroxyl group. The nmr spectrum was also consistent with structure **6a**. Acid hydrolysis (6 *N* HCl) of compound **6a** according to Iwasaki[12] gave the amino nucleoside **1**, sarcosine, and D-serine. The D-serine was isolated in crystalline form and characterized by paper chromatography, melting point, and optical rotation. The mother liquors from the D-serine crystallizations were combined and subjected to preparative paper chromatography. The optical rotation obtained for the serine was $-13°$ (lit.[12] $-14°$), clearly indicating that very little, if any, racemization of the D-seryl moiety had occurred during the synthesis of **6a**.

Upon completion of these studies, we noted a recent communication by Lichtenthaler, *et al.*,[13] describing the synthesis of **6a** in 40% yield by coupling **1** with *N*-Boc-sarcosyl-D-seryl azide in DMF followed by trifluoroacetic acid treatment. The physical constants given by these authors[13] [decomposition above 155°, $[\alpha]^{23}D$ $+5°$ (*c* 0.6, H_2O)], however, differ appreciably from those obtained in our preparation of **6a** [decomposition at 210–250°, $[\alpha]^{23}D$ $+44°$ (*c* 0.6, H_2O)], suggesting that their D-seryl moiety may be racemized. However, we have also prepared a paper chromatographically homogeneous, solid sarcosyl-DL-seryl nucleoside from racemic serine by the same method used for our synthesis of **6a**. This diastereoisomeric mixture again showed different physical properties [decomposition at 172–189°, $[\alpha]^{23}D$ $+11°$ (*c* 0.8, H_2O)] from their material. Since they[13] gave no other physical constants or other supporting evidence, it is difficult to rationalize their data with compound **6a**. Indeed, as stated above, in our hands we could not isolate the desired product from the condensation of **1** and *N*-Boc- or *N*-Cbz-sarcosyl-D-seryl azide in various solvents or solvent systems. It should be noted that the azide procedure is known[14] to produce side products.

We have also prepared **6a** by the active ester procedure and purified it by preparative paper chromatography. Nucleoside **6a** thus prepared possessed a smaller optical rotation ($[\alpha]^{23}D$ $+33°$) than that obtained above by the DCC method ($[\alpha]^{23}D$ $+44°$), which may indicate some racemization of the D-seryl moiety in this preparation. Acid hydrolysis (6 *N* HCl) of the sarcosyl-D-alanyl derivative (**6b**) prepared by the activated ester process, however, gave D-alanine indicating that little racemization, if any, occurred with this derivative. We assume also that the sarcosyl-D-phenylalanyl analog (**6c**) prepared by the same procedure is essentially optically pure. Apparently, the seryl derivative **6a** shows greater tendency toward racemization than the alanyl derivatives when the activated ester procedure is employed.

Experimental Section

Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich., and by Galbraith Laboratories, Inc., Knoxville, Tenn. Melting points are corrected. Nmr spectra were taken on a Varian A-60 spectrometer. Samples for elemental analyses were dried *in vacuo* at 78° for *ca.* 24 hr unless specified otherwise. The presence and quantity of solvent of crystallization (other than water) were determined by nmr. All compounds with analyses described below contained water, as shown by nmr. A quantitative estimate of the amount of water of crystallization was deduced from the elemental analyses.

1-[4-Deoxy-4-(*N*-benzyloxycarbonyl-D-alanyl)amino-β-D-glucopyranosyl]cytosine (3b).—A mixture of **1**[2] (272 mg, 1 mmol) and the *p*-nitrophenyl ester of *N*-benzyloxycarbonyl-D-alanine (**7b**, 688 mg, 2 mmol) in DMSO (2 ml) was stirred for 20 hr at room temperature. Methanol (10 ml) and then ether (200 ml) were added to the mixture, which was stirred for 1 hr. The supernatant was decanted and the gummy residue was washed with ether (three 30-ml portions). The gummy residue (**2b**, λ_{max}^{EtOH}

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303 and 249 mμ, λ_{min}^{EtOH} 275 and 227 mμ) was stirred with Dowex-1 (OH^-) (4 g) in methanol (150 ml). As soon as uv absorption at 303 mμ disappeared, the resin was removed and washed several times with methanol and water. The combined filtrate and washings were concentrated to dryness to give crude **3b** (322 mg, 67%), which on crystallization from water gave a white powder: mp 190–194° dec; $[\alpha]^{23}D$ +24° (*c* 1.25, 66% ethanol); uv $\lambda_{max}^{pH\ 6.8}$ 267, 237, $\lambda_{min}^{pH\ 6.8}$ 252, 228, $\lambda_{max}^{pH\ 1}$ 277, $\lambda_{min}^{pH\ 1}$ 240 mμ; nmr (D_2O) H-6, δ 7.74 (1 H, d), benzyl 7.44 (5 H, s), H-5, 6.12 (1 H, d), H-1′, 5.75 (1 H, d), benzyl CH_2, 5.17 (2 H, s), alanine CH, 4.22 (1 H, q), alanine CH_3, 1.46 (3 H, d).

Anal. Calcd for $C_{21}H_{27}N_5O_8 \cdot H_2O$: C, 50.91; H, 5.89; N, 14.13. Found: C, 50.44; H, 5.89; N, 14.06.

1-[4-Deoxy-4-(D-alanyl)amino-β-D-glucopyranosyl]cytosine (4b).—A solution of **3b** (1.6 g) in 50% ethanol was hydrogenated over 10% palladium on carbon catalyst (100 mg) for 15 min with an initial pressure of *ca.* 2 atm. Filtration from the catalyst and subsequent concentration of the filtrate gave a white powder, which was purified by reprecipitation from hot ethanol and ethanol (1.03 g, 79%): mp 233–240° dec; $[\alpha]^{23}D$ +22° (*c* 1, H_2O); nmr (D_2O) H-6, δ 7.70 (1 H, d), H-5, 6.07 (1 H, d), H-1′, 5.66 (1 H, m), alanine CH_3, 1.23 (3 H, d).

Anal. Calcd for $C_{13}H_{21}O_6N_5 \cdot {}^1/_2C_2H_5OH \cdot H_2O$: C, 43.74; H, 6.82; N, 18.22. Found: C, 43.46; H, 6.35; N, 18.47.

1-[4-Deoxy-4-(*N*-benzyloxycarbonylsarcosyl-D-alanyl)amino-β-D-glucopyranosyl]cytosine (5b).—The procedure described for **3b** was followed using **4b** (343 mg, 0.89 mmol) and *N*-benzyloxycarbonylsarcosine *p*-nitrophenyl ester[14] (688 mg, 2 mmol) in DMSO (1 ml). After treatment of the crude condensation product with Dowex-1 (OH^-), the colorless powder was crystallized from ethanol (300 mg, 58% dried at 100° for 24 hr *in vacuo*): mp 210–212°; $[\alpha]^{23}D$ +35° (*c* 0.6, 66% ethanol); nmr (pyridine-d_5–D_2O) H-6, δ 7.68 (1 H, d), benzyl, 7.38 (5 H, s), H-1′, 6.40 (1 H, d), H-5, 6.03 (1 H, d), benzyl CH_2, 5.25 (2 H, s), NCH_3, 3.10 (3 H, s), alanine CH_3, 1.66 (3 H, d).

Anal. Calcd for $C_{24}H_{32}O_9N_6 \cdot 2H_2O$: C, 49.31; H, 6.21; N, 14.38. Found: C, 49.17; H, 6.09; N, 14.48.

1-[4-Deoxy-4-(sarcosyl-D-alanyl)amino-β-D-glucopyranosyl]cytosine (6b).—Compound **5b** (300 mg) in 50% ethanol (50 ml) was hydrogenated over 10% palladium on carbon (50 mg) for 5 min at the initial pressure of *ca.* 2 atm. The compound obtained was purified by reprecipitation from hot methanol and ethanol: 200 mg (85%, after drying at 64° *in vacuo* for 24 hr); mp 93–95° dec; $[\alpha]^{23}D$ +33° (*c* 1, H_2O); nmr (D_2O) H-6, δ 7.76 (1 H, d), H-5, 6.08 (1 H, d), H-1′, 5.68 (1 H, m), NCH_3, 2.40 (3 H, s), alanine CH_3, 1.42 (3 H, d); uv $\lambda_{max}^{pH\ 6.8}$ 267, 235, $\lambda_{min}^{pH\ 6.8}$ 253, 225, $\lambda_{max}^{pH\ 1}$ 275, $\lambda_{min}^{pH\ 1}$ 240 mμ.

Anal. Calcd for $C_{16}H_{26}O_7N_6 \cdot 1{}^1/_2C_2H_5OH \cdot 1{}^1/_2H_2O$: C, 43.96; H, 6.94; N, 18.09. Found: C, 44.08; H, 6.39; N, 17.70.

1-[4-Deoxy-4-(*N*-benzyloxycarbonyl-D-phenylalanyl)amino-β-D-glucopyranosyl]cytosine (3c).—Compound 1 (1.36 g, 5 mmol) and *N*-benzyloxycarbonyl-D-phenylalanine *p*-nitrophenyl ester[15] (4.2 g, 10 mmol) in DMSO (10 ml) were treated in the same manner as **3b**. The product obtained was recrystallized from methanol and dried at 100° for 24 hr *in vacuo* (2.4 g, 82%): mp >300°; $[\alpha]^{23}D$ +20° (*c* 0.6, 66% ethanol); nmr (DMSO) H-6, δ 7.66 (1 H, d), aromatic, 7.28 (10 H, s), H-5, 5.78 (1 H, d), H-1′, 5.56 (1 H, d), benzyl CH_2, 4.96 (2 H, s).

Anal. Calcd for $C_{27}H_{31}O_8N_5 \cdot H_2O$: C, 56.26; H, 5.78; N, 12.28. Found: C, 56.14; H, 5.81; N, 12.25.

1-[4-Deoxy-4-(D-phenylalanyl)amino-β-D-glucopyranosyl]cytosine (4c).—Compound **3c** (1.3 g) was hydrogenated in the usual manner in the presence of 10% palladium on carbon. Compound **5c** (890 mg, 88%) was obtained as a white powder: mp 263–264° (sintered at 183–189°); $[\alpha]^{23}D$ +13° (*c* 1, H_2O); nmr (DMSO) H-6, δ 7.62 (1 H, d), aromatic, 7.25 (5 H, s), H-5, 5.78 (1 H, d), H-1′, 5.55 (1 H, d).

Anal. Calcd for $C_{19}H_{23}O_6N_5 \cdot 1{}^1/_2H_2O$: C, 51.12; H, 6.32; N, 15.69. Found: C, 50.80; H, 6.08; N, 15.58.

1-[4-Deoxy-4-(*N*-benzyloxycarbonylsarcosyl-D-phenylalanyl)-amino-β-D-glucopyranosyl]cytosine (5c).—From 418 mg of **4c** and 688 mg of *N*-benzyloxycarbonylsarcosine *p*-nitrophenyl ester, 431 mg of **5c** (70%) was obtained as a white powder: mp 243–247° dec; $[\alpha]D$ +13° (*c* 0.5, 66% ethanol); nmr (DMSO) H-6, δ 7.62 (1 H, d), aromatic 7.33 and 7.23 (total 10 H), H-5, 5.77 (1 H, d), H-1′, 5.52 (1 H, d), benzyl CH_2, 5.03 (2 H, s), NCH_3, 2.72 (3 H, s).

Anal. Calcd for $C_{30}H_{36}O_9N_6 \cdot 1{}^1/_2H_2O$: C, 55.29; H, 6.03; N, 12.89. Found: C, 55.57; H, 5.52; N, 12.97.

1-[4-Deoxy-4-(sarcosyl-D-phenylalanyl)amino-β-D-glucopyranosyl]cytosine (6c).—Hydrogenation of compound **5c** (351 mg) gave 242 mg of **6c** (84%) which was crystallized from 2-propanol–ethanol (1:1). After it had been dried at 100° *in vacuo* for 24 hr, **6c** had mp 221–231° dec (sintered at 116°); $[\alpha]^{23}D$ +19° (*c* 1, H_2O); nmr (DMSO) H-6, δ 7.66 (1 H, d), aromatic 7.28 (5 H, s), H-5, 5.81 (1 H, d), H-1′, 5.55 (1 H, m), NCH_3, 2.25 (3 H, s).

Anal. Calcd for $C_{22}H_{30}O_7N_6 \cdot {}^1/_2C_3H_7OH \cdot H_2O$: C, 52.41; H, 6.73; N, 15.60. Found: C, 52.07; H, 5.95; N, 15.69.

***N*-Benzyloxycarbonyl-D-alanine *p*-Nitrophenyl Ester (7b).**—The compound was prepared from *N*-benzyloxycarbonyl-D-alanine and *p*-nitrophenol in 83% yield by the method of Marchiori, *et al.*,[16] mp 77–78°, $[\alpha]^{23}D$ +54° (*c* 0.8, 66% ethanol). (The L isomer[16] had mp 77–78°, $[\alpha]^{20}D$ −41°.)

1-[4-Deoxy-4-(*N*-benzyloxycarbonyl-D-seryl)amino-β-D-glucopyranosyl]cytosine (3a).—To a mixture of compound 1 (1.36 g, 5 mmol) and *N*-benzyloxycarbonyl-D-serine (2.2 g, 9 mmol) in water (2 ml) was added DCC (2.2 g) in acetonitrile (10 ml) and the solution was stirred for 20 hr at room temperature. Dicyclohexylurea was filtered and washed with 50% aqueous methanol (50 ml). The combined filtrate and washings were stirred with Dowex-1 (OH^-) (20 ml) for 10 min and filtered. The filtrate was stirred with Amberlite IRC-50 (H^+) for 30 min, filtered, and washed with 50% methanol. The combined filtrate and washings were evaporated to *ca.* 75 ml and left overnight at room temperature. The precipitate (probably *N*-Cbz-D-seryl dicyclohexylurea according to nmr and ir) was filtered and the filtrate was evaporated to dryness. The residue was coevaporated several times with ethanol until colorless microcrystals were obtained: 1.78 g (71%); mp 166–170°; $[\alpha]D$ +27° (0.9, 1:2 EtOH–H_2O); nmr (DMSO) H-6, δ 7.70 (1 H, d), aromatic, 7.38 (5 H, s), H-5, 5.95 (1 H, d), H-1′, 5.60 (1 H, d), benzyl CH_2, 5.10 (2 H, s).

Anal. Calcd for $C_{21}H_{27}O_9N_5 \cdot H_2O$: C, 49.31; H, 5.71; N, 13.69. Found: C, 49.58; H, 5.55; N, 14.23.

1-[4-Deoxy-4-(D-seryl)amino-β-D-glucopyranosyl]cytosine (4a).—Compound **3a** (1.41 g) was dissolved in a mixture of water (45 ml) and ethanol (10 ml) and the mixture was shaken for 15 min in a hydrogen atmosphere (initial pressure of about 2 atom) in the presence of *ca.* 250 mg of 10% palladium on carbon. The catalyst was filtered and washed with water. The combined filtrate and washings were evaporated to dryness. The residue was treated with hot ethanol for 10–15 min until microcrystals were obtained; yield 960 mg (96%). The compound did not have a definite melting point but browned at 243° and decomposed at 271–274°: $[\alpha]D$ +29° (0.8, H_2O); nmr (D_2O) H-6, δ 7.73 (1 H, d), H-5, 6.08 (1 H, d), H-1′, 5.68 (1 H, d).

Anal. Calcd for $C_{13}H_{21}O_7N_5 \cdot {}^1/_2H_2O$: C, 42.39; H, 6.02; N, 19.01. Found: C, 42.13; H, 5.79; N, 18.78.

1-[4-Deoxy-4-(*N*-benzyloxycarbonylsarcosyl-D-seryl)amino-β-D-glucopyranosyl]cytosine (5a).—Compound **4a** (720 mg, 2 mmol) was treated with *N*-benzyloxycarbonylsarcosine (890 mg, 4 mmol) and DCC (880 mg) in a mixture of water (1.5 ml) and acetonitrile (6 ml) for 20 hr. Dicyclohexylurea was filtered and washed with 3:7 methanol–water (50 ml). To the filtrate and washings was added acetonitrile (*ca.* 15 ml) until a clear solution was obtained. Dowex-1 (OH^-) (5 ml) was added to the solution and stirred for 5 min and the resin was washed with 3:7 methanol–water (25 ml). The combined filtrate and washings were treated with Amberlite IRC-50 (H^+) (10 ml) for 15 min and filtered. The filtrate was evaporated to dryness and the residue was partitioned between water (70 ml) and chloroform (30 ml). The insoluble solid and chloroform layer were discarded. The aqueous layer was evaporated and the residue was co-evaporated with ethanol until colorless microcrystals were obtained. The yield of the product was 784 mg (71%): mp 163–166° (eff without coloring) and dec 236–239°; $[\alpha]D$ +35° (*c* 1.1, 1:2 EtOH–H_2O); nmr (DMSO) H-6, δ 7.63 (1 H, d), aromatic, 7.35 (5 H, s), H-5, 5.80 (1 H, d), H-1′, 5.57 (1 H, d), benzyl CH_2, 5.07 (2 H, s), NCH_3, 2.88 (3 H, s).

Anal. Calcd for $C_{24}H_{32}O_{10}N_6 \cdot {}^1/_2C_2H_5OH \cdot 1{}^1/_2H_2O$: C, 48.85; H, 6.23; N, 13.67. Found: C, 49.13; H, 6.31; N, 13.91.

1-[4-Deoxy-4-(sarcosyl-D-seryl)amino-β-D-glucopyranosyl]cytosine (6a).—Compound **5a** (400 mg) was dissolved in 1:3

(15) T. Yusupov, A. B. Zegelman, L. Radzhabov, and K. T. Poroshin, *Dokl. Akad. Nauk Tadzh SSR*, **11**, 22 (1968); *Chem. Abstr.*, **70**, 115545u (1969).

ethanol–water (40 ml) and hydrogenated in the presence of *ca.* 200 mg of 10% palladium on carbon catalyst for 15 min with the initial pressure of about 2 atm. The catalyst was filtered and washed with water. The combined filtrate and washings were evaporated to dryness, and then the residue was evaporated several times with ethanol until colorless microcrystals were obtained. After drying overnight at 78° *in vacuo*, 287 mg (92%) of product was obtained: mp 136° (sintered) 210–250° dec; $[\alpha]_D$ +44° (*c* 0.6, H_2O), nmr (D_2O) H-6, δ 7.77 (1 H, d), H-5, 6.03 (1 H, d), H-1′, 5.67 (1 H, d), NCH_3, 2.79 (3 H, s).

Anal. Calcd for $C_{16}H_{26}O_8N_6 \cdot H_2O$: C, 42.86; H, 6.29; N, 18.74. Found: C, 43.13; H, 6.36; N, 18.73.

1-[4-Deoxy-4-(sarcosyl-DL-seryl)amino-β-D-glucopyranosyl]-cytosine [mp 172–189° dec, $[\alpha]^{23}_D$ +11° (*c* 0.8, H_2O)] was synthesized from **1**, *N*-Cbz-DL-serine, and *N*-Cbz-sarcosin by the same procedure used for the preparation of **6a**.

Hydrolysis of Compound 6a and Isolation of D-Serine.—Compound **6a** (980 mg) was dissolved in 6 *N* HCl (50 ml) and refluxed gently for 24 hr. After concentration *in vacuo* the residue was taken up in water (50 ml) and passed through a column of Dowex-1 (OH^-) (2.6 × 9.6 cm). The column was washed with water (2 l.) to remove compound **1**. The amino acids were eluted from the column with 1 *N* HCl (100 ml). The acid eluate was evaporated to dryness and the residue was dissolved in a small amount of water and passed through a column of Amberlite IR-45 (OH^-) (2.6 × 10 cm) to remove hydrogen chloride. The column was washed with water (200 ml) and the effluent was evaporated to dryness. Crude D-serine (106 mg) was crystallized from the residue from methanol (5 ml). One recrystallization of the crude product from water–ethanol gave colorless needles: mp 216–217° dec; $[\alpha]^{23}_D$ +7° (*c* 1, H_2O); $[\alpha]^{23}_D$ −14° (*c* 0.9, 1 *N* HCl). The authentic D-serine, mp 214–215° dec, showed the identical optical rotations under the same conditions.

Both the methanolic and water–ethanol mother liquors of the crystallizations were combined and applied to two sheets of Whatman #1 paper (46 × 57 cm) and developed with 88% phenol. The serine bands were extracted with water and evaporated to dryness. The residue (34.7 mg) showed $[\alpha]^{23}_D$ −13° (*c* 0.9, 1 *N* HCl).

Degradation of Compound 6b and Characterization of D-Alanine.—Compound **6b** (890 mg) was hydrolyzed and D-alanine was obtained after essentially the same processing for the isolation of D-serine from compound **2a**. The crude residue from the Amberlite IR-45 column was taken up in 3 ml of water and applied to two sheets (46 × 57 cm) of Whatman #1 paper, developed with 88% phenol, and the alanine bands were eluted with water and evaporated to dryness. The residue (125 mg) had $[\alpha]_D$ −10° (*c* 1, 6 *N* HCl). One recrystallization of the crude sample gave pure D-alanine (65 mg), $[\alpha]_D$ −12° (*c* 1, 6 *N* HCl).

Registry No.—**3a**, 33780-67-5; **3b**, 33780-68-6; **3c**, 33780-69-7; **4a**, 33780-70-7; **4b**, 33780-71-1; **4c**, 33780-72-2; **5a**, 33780-73-3; **5b**, 33780-74-4; **5c**, 33780-75-5; **6a**, 31883-24-6; **6b**, 33780-77-7; **6c**, 33780-78-8; 1-[4-deoxy-4-(sarcosyl-DL-seryl]amino-β-D-glucopyranosyl]cytosine, 33780-79-9; D-serine, 312-84-5; D-alanine, 338-69-2.

Dioldithiol Analogs of the 1,2,4,5-Cyclohexanetetrols. Chemical and Nuclear Magnetic Resonance Studies[1,2]

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Reaction of 1,4-cyclohexadiene dioxide (cis–trans mixture) with sodium benzylmercaptide gave a mixture from which were isolated three of the four expected isomers of dibenzylmercaptocyclohexanediol. The two possible structures for each product were 4,6-dibenzylmercapto-1,3-cyclohexanediol (**2**) and 2,5-dibenzylmercapto-1,4-cyclohexanediol (**4**). For each structure, one meso and one DL diastereomer (**12** and **8**, or **9** and **10**) would be predicted. For the molecules of either meso isomer, only one (tetraequatorial) chair conformation (**14** or **16**) is predicted. The DL isomer molecules, however, should each be diaxial–diequatorial (**13a** or **15a**) and readily transformed by ring inversion into alternate chair conformations (**13b** or **15b**) indistinguishable from the original. This analysis permitted nmr spectral assignments based on (1) presence of time-averaging effects (DL isomers only); (2) equivalence between corresponding methylene protons at positions 3 and 6 (para isomers only). The assignments are para-DL (14/25), mp 92°; para-meso (15/24), mp 158°; meta-DL (14/36), mp 109°; meta-meso (13/46), unknown. The mp 109° isomer identity was confirmed by chemical correlation with the known *trans*-1,3-cyclohexanediol.

We wish to report nmr configurational proofs for the family of four trans–trans dioldithiols which are analogous to 1,2,4,5-cyclohexanetetrol and derived from 1,3- or 1,4-cyclohexanediol (see formulas **8**, **9**, **10**, and **12**, Scheme I). This family of structural and stereoisomers provides an interesting example of nmr configurational assignments based on conformational analysis and nmr spectroscopy. A similar study on the parent tetrols was previously reported by one of us.[5] Although only the di-*S*-benzyl derivatives were actually examined, the structures and configurations of the parent dioldithiols can now be easily established by simple chemical correlations.[6]

The synthetic and nmr studies here reported are part of a program for preparation of cyclitols and other carbohydrates[7] in which most or all of the oxygen functions will be replaced by sulfur functions (see Acknowledgment).[2b]

(1) Presented to the Division of Organic Chemistry at the 159th National Meeting of the American Chemical Society, Houston, Texas, Feb 1970.

(2) (a) Paper XXXVIII on Alicyclic Carbohydrates; for paper XXXVII, see N. Kurihara, Y. Sanemitsu, M. Nakajima, G. E. McCasland, and L. F. Johnson, *Agr. Biol. Chem.*, **35**, 71 (1971). For paper XXXVI, see G. E. McCasland, M. O. Naumann, and Lois J. Durham, *J. Org. Chem.*, **34**, 1382 (1969). (b) For preceding publication on thio carbohydrates, see G. E. McCasland and A. B. Zanlungo, *Carbohyd. Res.*, **17**, 475 (1971). (c) For preceding paper on (nonalicyclic) carbohydrates, see A. E. Lipska and G. E. McCasland, *J. Appl. Polym. Sci.*, **15**, 419 (1971).

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(5) For studies on the 1,2,4,5-cyclohexanetetrols, see G. E. McCasland, S. Furuta, L. F. Johnson, and J. N. Shoolery, *J. Org. Chem.*, **28**, 894 (1963).

(6) A benzylmercaptocyclohexane is easily converted to a mercaptocyclohexane, with retention of configuration, by reaction with sodium in liquid ammonia. See G. E. McCasland, S. Furuta, and A. Furst, *ibid.*, **29**, 724 (1964), for application of this reaction to mercaptodeoxyinositol derivatives.

(7) A report on sulfur analogs of D-iditol and D-mannitol was presented by G. E. McCasland and A. B. Zanlungo to the Division of Carbohydrate Chemistry at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970. See also G. E. McCasland and A. B. Zanlungo, *Carbohyd. Res.*, **17**, 475 (1971).