Nucleosides. XLIII. 3′-Amino-3′-deoxyhexopyranosyl Nucleosides. V. Studies on the Preparation of Aminoacyl Derivatives of Amino Sugar Nucleosides[1]

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1-(3-Deoxy-3-glycylamido-β-D-glucopyranosyl)uracil (**5a**), as well as the 3′-sarcosylamido (**5b**), L-alanylamido (**5c**), and D-phenylalanylamido (**5d**) analogs, was prepared in a four-step synthesis from 1-(3-amino-3-deoxy-β-D-glucopyranosyl)uracil (**1**). The syntheses involved the coupling of the per-O-acetylated derivative (**2**) of nucleoside (**1**) with *N*-carbobenzoxy derivatives of the corresponding amino acids to form **3**. Selective removal of the acetyl functions followed by catalytic removal of the carbobenzoxy groups yielded the aminoacyl nucleosides (**5**). A facile synthesis of a mixture of 1-(3-deoxy-3-nitro-β-D-hexopyranosyl)cytosine hydrochlorides (**11**) from cytidine was achieved by the metaperiodate–nitromethane procedure. Reduction of **11** gave the known 1-(3-amino-3-deoxy-β-D-glucopyranosyl)cytosine (**12**) as well as the *manno* isomer (**13**). The structure of the *manno* isomer was proved by chemical degradative procedures. Similarly, 1-(3-deoxy-3-nitro-β-D-glucopyranosyl)-N^4-benzoylcytosine (**19**) was prepared from N^4-benzoylcytidine. Attempts to prepare the cytosine analogs of **5** from **12** were not successful.

Several cytosine nucleoside antibiotics have been discovered which contain 4-amino-4-deoxy-D-hexopyranosyl moieties and amino acid or dipeptide residues.[3] In two of these antibiotics, blasticidin S and gougerotin, the 4′-amino group bears the amino acid or dipeptide in acyl linkage. As part of a long-range program toward the synthesis and biological evaluation of analogs of blasticidin S and gougerotin, we report herein studies on the preparation of α-aminoacyl derivatives linked to the 3′-amino group of 1-(3-amino-3-deoxy-β-D-glucopyranosyl)pyrimidines.

The easily accessible 1-(3-amino-3-deoxy-β-D-glucopyranosyl)uracil[4] (**1**, Figure 1) was used in model studies to determine the most effective method of preparing a nucleoside containing a "glycopeptide" linkage. The mixed anhydrides of the amino acids used (*e.g.*, of *N*-carbobenzoxyglycine, $CboNHCH_2C(=O)O-C(=O)OCH_3$) were prepared *in situ* by standard procedures.[5,6] Attempts to condense the mixed anhydride of *N*-carbobenzoxyglycine with **1** in 75% aqueous[7] *N,N*-dimethylformamide (DMF) led to a mixture of products along with unreacted starting material as the major component. This was unexpected since Baker, *et al.*,[8] succeeded by this procedure in synthesizing a number of α-aminoacyl derivatives of the aminonucleoside derived from the antibiotic, puromycin.

The syntheses of compounds containing a glycopeptide linkage between a glycosyl amine and amino acid derivatives have been reported.[9-14] In all of these examples, 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine, rather than the unblocked sugar, was used as the starting material. It was deemed advisable therefore to use suitably protected nucleosides for these condensation reactions. 1-(3-Amino-3-deoxytri-*O*-acetyl-β-D-glucopyranosyl)uracil hydrochloride (**2**)[4] was condensed with *N*-carbobenzoxy derivatives of sarcosine, glycine, L-alanine, and D-phenylalanine *via* the mixed anhydride method in anhydrous chloroform using either methyl or ethyl chloroformate. Whereas ethyl chloroformate had been recommended over the corresponding methyl ester as a reagent affording higher yields,[6] better yields were obtained when the methyl ester was used. In the syntheses of the glycine and L-alanine nucleosides (**3a,c**) the reaction mixture contained another ultraviolet absorbing component when ethyl chloroformate was used. Compounds **3a–d** were obtained in crystalline form by trituration of the residue, following evaporation of the solvent, with

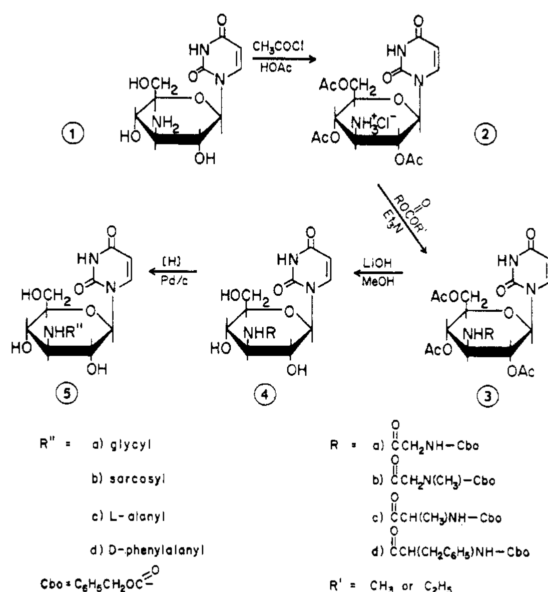

Figure 1

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cyclohexane–chloroform (or ethanol). The L-alanine derivative (**3c**) obtained contained cyclohexane of crystallization. The presence of the solvent and its molar quantity reported in the analysis of **3c** was confirmed by nmr. Attempts to remove the solvent completely led to decomposition of the nucleoside. It is interesting to note that when this same reaction (**2** → **3**) was run using the unprotected 3′-amino-3′-deoxyglucopyranosyluracil (1), the reaction did not proceed smoothly and pure product could not be isolated.

Deacetylation of monosaccharide acetates may be performed by either acid[15] or base[16a] catalysis. Treatment of the nucleoside **3a** with alcoholic hydrogen chloride led to the incomplete removal of the acetate groups and refluxing the reaction solution led to some cleavage of the peptide bond. The Zemplen method[16a] of base catalysis had proved suitable for the complete removal of acetate groups without affecting the carbobenzoxy groups of amino acid derivatives. Since a large quantity of base may cause side reactions with the carbobenzoxy groups,[16b] complete removal of all the blocking groups by alkaline saponification was avoided. All four nucleosides of **3** were deacetylated in anhydrous methanol using a trace of lithium hydroxide. The lithium was removed by Dowex 50 (H^+) resin and a crystalline compound **4d** was obtained in the case of the D-phenylalanine derivative and amorphous compounds **4a–c** in the case of the other aminoacyl nucleosides.

The two most widely used methods for removing carbobenzoxy groups are by catalytic hydrogenation, usually over palladium on charcoal,[17,18] and by hydrogen bromide in glacial acetic acid.[17,19] Use of the latter method on **4a** led to rapid evolution of gas and the formation of a highly colored solution. Difficulties were encountered in the attempted isolation of product. It should also be noted that anhydrous hydrogen halides in glacial acetic acid are known to form poly-O-acylated glycosyl halides from free sugars.[20] The carbobenzoxy group was removed without difficulty by subjecting the deacetylated nucleosides of **4** to hydrogenolysis over 10% palladium on charcoal. All the aminoacyl aminonucleosides, **5a–d** were obtained as amorphous solids which did not crystallize. All these compounds exhibited only one spot on silica gel thin layer chromatography. To prove that acyl migration did not occur during the conversions of 2 → **5**, the final product **5a** was treated with sodium metaperiodate. Compound **5a** was inert toward this oxidant, consistent with the structure shown in Figure 1.

Prior to testing the applicability of the above reactions to the corresponding cytosine analog (**12**, Figure 2) it was desired to find a more facile synthesis for this compound. A multistep synthesis of **12** from **1** had been reported.[4] A more direct synthesis of **12** was achieved from cytidine. Cytidine (**6**) was oxidized with sodium metaperiodate to its dialdehyde **7**, which was condensed with nitromethane in aqueous alkaline medium. The solution (containing **8**) was deionized and neutralized with an acid resin. However, the product could not be isolated as the free nucleosides **9** and **10** without the occurrence of extensive decomposition. The mixture of 3′-nitro nucleoside hydrochlorides, **11**, was isolated upon the addition of hydrochloric acid followed by removal of solvent and trituration of the crude material with acetone–methanol to a crystalline compound. Raney nickel hydrogenations of the free nucleoside or of its hydrochloride salts yielded two compounds. The major component[21] was shown to be 1-(3-amino-3-deoxy-β-D-glucopyranosyl)cytosine (**12**) identical with an authentic sample prepared by an independent route.[4] The second component, which was present in only $^1/_{20}$ of the amount of the *gluco* isomer, was shown to be the *manno* isomer **13**. Ion exchange chromatography of the mixture of nucleosides separated the two components.

Proof of the structure of **13** was obtained by acetylation of **13** (Figure 2) with acetic anhydride in pyridine to give a sirup **14** in quantitative yield. The nmr spectrum of **14** in DMSO-d_6 showed a signal for the anomeric proton at $\tau \cong 3.8$ with $J_{H_1'-H_2'} \approx 2$ cps indicative of an axial–equatorial arrangement (*manno* configuration) for these protons. Partial deacetylation of **14** gave the crystalline N-acetyl derivative **15**. Nucleoside **13** was hydrogenated over platinum oxide[22] to the hexahydro derivative **16** which was isolated as a syrup. Treatment of **16** with methanolic hydrogen chloride afforded **17** which was not isolated. Acetylation of **17** gave crystalline methyl 3-acetamido-3-deoxy-tri-*O*-acetyl-α-D-mannopyranoside (**18**), identical with regard to melting point, mixture melting point, and infrared spectrum with an authentic sample.[23]

As mentioned above, isolation of free nitro nucleosides **9** and **10** was not possible owing, presumably, to some interaction between the nitro group of the sugar moiety and the heterocyclic base. Therefore, as an alternate route to **12**, N[4]-acylated derivatives of cytidine were examined in the dialdehyde–nitromethane reaction. *N*[4]-Acetylcytidine[24] was found to be too unstable in alkaline medium to be of value as starting material. *N*[4]-Benzoylcytidine[24] proved to have much greater stability under these conditions. Oxidation of this nucleoside with metaperiodate followed by condensation with nitromethane gave the crystalline 3′-nitro nucleoside (**19**) in 25% yield which was a single component as indicated by thin layer chromatography. Hydrogenation of **19** over Raney nickel followed by chromatographic examination of the reaction mixture showed that several products had formed, one of which was the *gluco* isomer (**12**), indicating that some dibenzoylation had occurred. The mixture, which was not readily separable, was subjected to debenzoylation with methanolic ammonia. The major product formed was the *gluco* isomer (**12**) along with numerous minor components.

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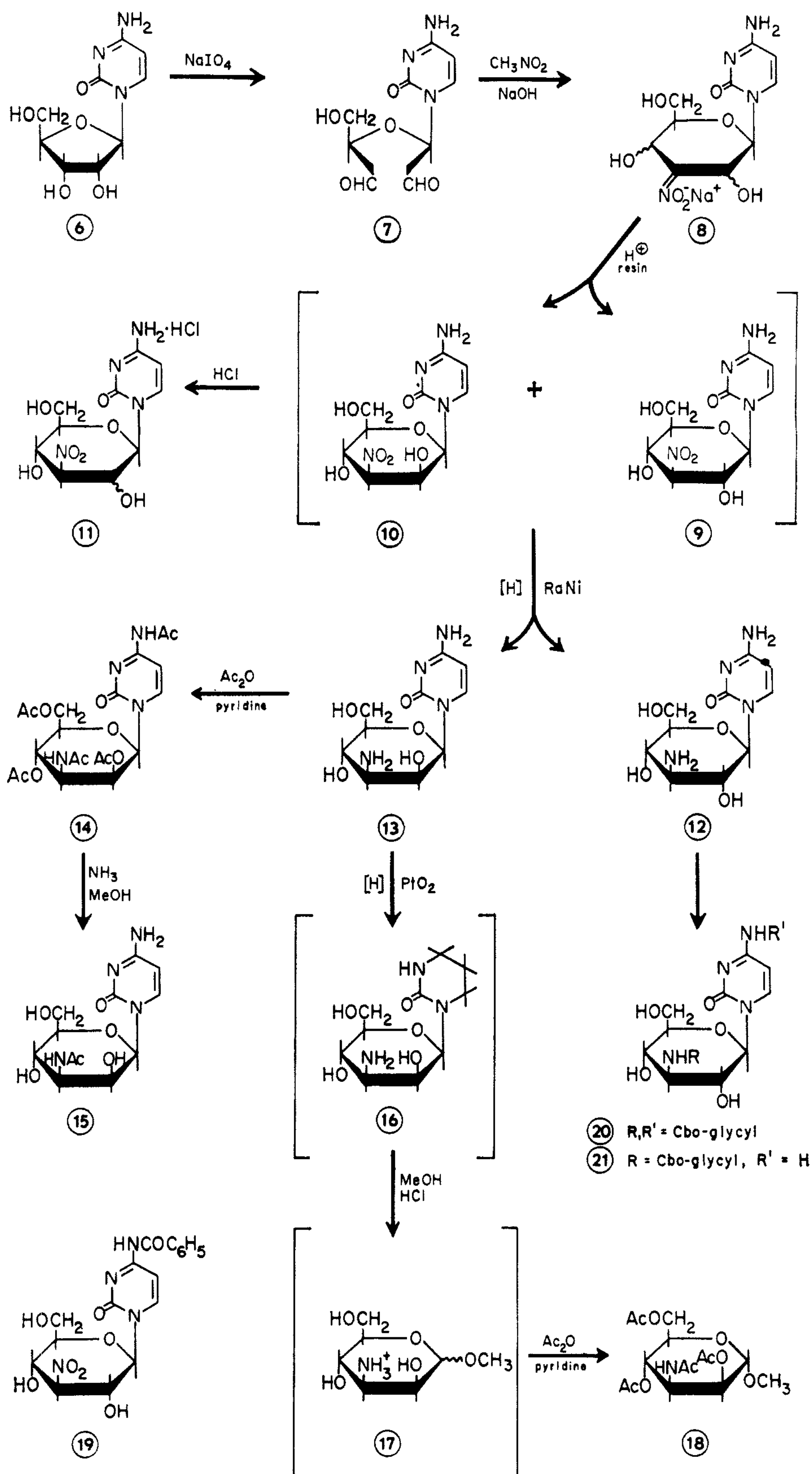

Figure 2

An attempt to convert the *gluco* nucleoside **12** to its per-O-acetylated derivative led to the formation of an intractable hygroscopic product(s) which was unsuitable for further aminoacylation reactions. It was necessary, therefore, to attempt acylation of the amino group of **12** directly with a suitable amino acid derivative. The procedure of Baker, *et al.*,[8] was applied to the reaction of **12** with the mixed anhydride of *N*-carbobenzoxyglycine in 75% aqueous DMF.[7] Ultraviolet absorption spectroscopic examination of the reaction mixture indicated that no acylation occurred at the N^4 position (absence of selective absorption at ~305 mμ). Thin layer chromatographic examination of the mixture showed the presence of two components,

one of which was starting material **12**. Attempts to separate these by ion-exchange chromatography were not successful. Attempts to apply the *N*,*N'*-dicyclohexylcarbodiimide method[25] afforded difficulties in the removal of the *N*,*N'*-dicyclohexylurea formed and gave a mixture which contained an N^4-acylated nucleoside (presumably **20**). N^4-acylation of **12** also occurred when the activated ester procedure[26] (using the *p*-nitrophenyl ester of *N*-carbobenzoxyglycine) was used. The *p*-nitrophenol generated in the reaction was removed by column chromatography with Dowex-1 (acetate) and the peptide linkage on the exocyclic amine was cleaved with dilute alkali. Separation of the product from starting material by ion-exchange chromatography gave an amorphous solid (presumably **21**) which exhibited one spot on paper electrophoresis (borate buffer, pH 9.2). Hydrogenolysis of **21** over 10% palladium on charcoal caused, unexpectedly, the reduction of the aglycon as evidenced by the loss of selective absorption in the ultraviolet spectrum. Attempts to circumvent this reduction by the use of hydrogen bromide–glacial acetic acid gave a low yield of a colored intractable mixture. It is evident that alternate procedures will be necessary for the synthesis of the cytosine analogs of **5**.

Experimental Section[27]

1-(3-*N*-Carbobenzoxysarcosylamido-3-deoxy-tri-*O*-acetyl-β-D-glucopyranosyl)uracil (3b).—1-(3-Amino-3-deoxy-tri-*O*-acetyl-β-D-glucopyranosyl)uracil hydrochloride (**2**)[4] (1.2 g, 2.9 mmoles) was added to 7 ml of dry chloroform. Solution took place upon the addition of 0.81 ml (5.8 mmoles) of triethylamine. A second solution was prepared containing *N*-carbobenzoxysarcosine[28] (0.65 g, 2.9 mmoles), triethylamine (0.41 ml, 2.9 mmoles), and chloroform (8 ml). Both solutions were cooled to 0° in an ice bath. Ethyl chloroformate (0.23 ml, 2.9 mmoles) was added to the second solution and the mixture was stirred for 20 min at 0°. The solution containing the nucleoside was added to the mixed anhydride and the mixture was stirred for an additional 15 min at 0° and then at room temperature overnight. The solution was extracted with 0.2 *N* hydrochloric acid, followed by a saturated solution of sodium bicarbonate and finally with water containing a few crystals of sodium chloride (to prevent emulsion formation). The chloroform layer was dried over anhydrous magnesium sulfate. The solvent was evaporated *in vacuo* and cyclohexane was added to the residue along with a few milliliters of chloroform. The ratio of cyclohexane to chloroform was no less than 10:1. The mixture was boiled on a water bath for ~10 min. This procedure changed the gummy residue into a crystalline compound: yield 1.1 g (63%); mp 120–125° (effervescent). Recrystallization of this substance was attempted without success. Silica gel thin layer chromatography (chloroform–methanol, 5:1) showed one spot along with a slight trace of impurity.

Anal. Calcd for $C_{27}H_{32}N_4O_{12}$: C, 53.64; H, 5.34; N, 9.27. Found: C, 54.02; H, 5.55; N, 9.18.

1-(3-*N*-Carbobenzoxy-D-phenylalanylamido-3-deoxytri-*O*-acetyl-β-D-glucopyranosyl)uracil (3d).—Compound **2** (0.84 g, 2.0 mmoles) was added to 7 ml of dry chloroform. Solution took place upon the addition of 0.56 ml (4 mmole) of triethylamine. A second solution was prepared containing *N*-carbobenzoxy-D-phenylalanine[28] (0.60 g, 2.0 mmole) and chloroform (8 ml). Both solutions were cooled in an ice bath. Ethyl chloroformate (0.16 ml, 2.0 mmole) was added to the latter solution and the mixture was stirred for 20 min in an ice bath. The solution containing the nucleoside was added and the mixture was stirred for an additional 15 min at 0° and then overnight at room temperature. The chloroform solution was extracted with 0.2 *N* hydrochloric acid, followed by a saturated solution of sodium bicarbonate and finally with water containing a few crystals of sodium chloride. The chloroform layer was dried over anhydrous magnesium sulfate. After removal of solvent, the residue was triturated with boiling cyclohexane–ethanol (in ratio of no less than 10:1) to yield 650 mg (48%) of crystals, mp 246–247°. Silica gel thin layer chromatography (chloroform–methanol 5:1) indicated only one spot.

Anal. Calcd for $C_{33}H_{36}N_4O_{12}$: C, 58.23; H, 5.33; N, 8.23. Found: C, 58.12; H, 5.53; N, 8.08.

1-(3-*N*-Carbobenzoxyglycylamido-3-deoxy-tri-*O*-acetyl-β-D-glucopyranosyl)uracil (3a).—Compound **2** (0.42 g, 1.0 mmole) was added to 5 ml of dry chloroform. The nucleoside dissolved upon the addition of triethylamine (0.28 ml, 2.0 mmoles). A second solution was prepared containing *N*-carbobenzoxyglycine[28] (0.21 g, 1.0 mmole), triethylamine (0.14 ml), and chloroform (5 ml). The solution was cooled to 0° in an ice bath.

Methyl chloroformate (0.08 ml, 1.0 mmole) was added to the solution and the mixture was stirred in an ice bath. The solution containing the nucleoside was added and the mixture was stirred for an additional 20 min at 0° and then overnight at room temperature. The chloroform solution was extracted with 0.2 *N* hydrochloric acid followed by a saturated solution of sodium bicarbonate and then with water containing a few crystals of sodium chloride. The chloroform layer was dried over anhydrous magnesium sulfate. Trituration of the residue remaining after evaporation of the solvent with boiling cyclohexane–chloroform, as in previous procedures (*vide supra*), yielded crystalline material, 250 mg (42%), mp 171–191°. Recrystallization of this compound was attempted without success. Silica gel thin layer chromatography (chloroform–methanol, 5:1) indicated only one spot with a trace impurity.

Anal. Calcd for $C_{26}H_{30}N_4O_{12}$: C, 52.88; H, 5.11; N, 9.48. Found: C, 52.28; H, 5.32; N, 9.33.

1-(3-*N*-Carbobenzoxy-L-alanylamido-3-deoxy-tri-*O*-acetyl-β-D-glucopyranosyl)uracil (3c).—The procedure employed was the same as that used for preparation of the glycine derivative (**3a**) except that *N*-carbobenzoxy-L-alanine[28] (0.23 g, 1.0 mmole) was used. This yield of product was 250 mg (39%), mp 135–155°. Silica gel thin layer chromatography (chloroform–methanol 5:1) indicated only one spot.

Anal. Calcd for $C_{27}H_{32}N_4O_{12}\cdot{}^1/_2C_6H_{12}$: C, 55.71; H, 5.92; N, 8.66. Found: C, 55.55; H, 5.93; N, 8.33.

1-(3-*N*-Carbobenzoxysarcosylamido-3-deoxy-β-D-glucopyranosyl)uracil (4b).—Compound **3b** (100 mg, 0.17 mmole) was dissolved in 10 ml of anhydrous methanol. Following the addition of 1–3 mg of anhydrous lithium hydroxide the solution, protected from moisture, was allowed to stir for 24 hr at room temperature. Lithium ion was removed by the addition of a small amount of Dowex-50 (H^+) resin and stirring the slurry for about 15 min. Evaporation of the solvent left an amorphous solid which defied attempts at crystallization. The yield of **4b** was 50 mg (64%). The melting point was not definitive. Silica gel thin layer chromatography taken in three separate systems all indicated only one compound. The systems were as follows: benzene–ether–ethanol (5:3:2); chloroform–methanol (5:1); and *t*-butyl alcohol–2-butanone–water–triethylamine (4:3:2:1).

Anal. Calcd for $C_{21}H_{26}N_4O_9$: C, 52.71; H, 5.48; N, 11.70. Found: C, 52.66; H, 5.77; N, 11.39.

1-(3-*N*-Carbobenzoxyglycylamido-3-deoxy-β-D-glucopyranosyl)uracil (4a).—The procedure employed was the same as that used for the preparation of the sarcosine derivative **4b** except that compound **3a** (100 mg, 0.17 mmole) was used. The product was isolated as an amorphous solid 50 mg, (63%). The melting point was indefinite. Silica gel thin layer chromatography (*t*-butyl alcohol–2-butanone–water–triethylamine, 4:3:2:1) indicated only one spot.

Anal. Calcd for $C_{20}H_{24}N_4O_9\cdot{}^1/_2H_2O$: C, 50.74; H, 5.32; N, 11.83. Found: C, 51.17; H, 5.31; N, 11.82.

1-(3-*N*-Carbobenzoxy-L-alanylamido-3-deoxy-β-D-glucopyranosyl)uracil (4c).—The procedure employed was the same as that used for the preparation of the sarcosine derivative **4b** except that compound **3c** (100 mg, 0.16 mmole) was used. The product was isolated in a yield of 60 mg (79%) as an amorphous solid. The melting point was indefinite. Silica gel thin layer chromatography (*t*-butyl alcohol–2-butanone–water–triethylamine, 4:3:2:1) indicated only one spot.

Anal. Calcd for $C_{21}H_{26}N_4O_9\cdot{}^1/_2H_2O$: C, 51.73; H, 5.48; N, 11.49. Found: C, 51.77; H, 5.55; N, 11.80.

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(27) Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and Spang Microanalytical Laboratories, Ann Arbor, Mich.

(28) Cyclo Chemical Corp. Purity was "grade II."

1-(3-*N*-Carbobenzoxy-D-phenylalanylamido-3-deoxy-β-D-glucopyranosyl)uracil (4d).—Compound **3d** (100 mg, 0.15 mmole) was added along with anhydrous lithium hydroxide (2 mg) to 10 ml of anhydrous methanol. The mixture, protected from moisture, was stirred for 24 hr during which time complete solution did not take place. Silica gel thin layer chromatography (chloroform–methanol, 5:1) of the dissolved portion of the reaction mixture indicated complete removal of the acetyl blocking groups. The mixture was evaporated to dryness *in vacuo* and the solid was washed with methanol to remove the lithium hydroxide present. The product was isolated as white microcrystals which were sparingly soluble in methanol: 70 mg (84%); mp 222–226°. Silica gel thin layer chromatography of the compound indicated only one spot.

Anal. Calcd for $C_{27}H_{30}N_4O_9 \cdot {}^1/_2H_2O$: C, 57.54; H, 5.82; N, 9.94. Found: C, 57.68; H, 5.59; N, 9.59.

1-(3-Deoxy-3-sarcosylamido-β-D-glucopyranosyl)uracil (5b).—Compound **3b** (200 mg, 0.33 mmole) was added to 10 ml of anhydrous methanol along with anhydrous lithium hydroxide (3 mg). The mixture was stirred overnight. Silica gel thin layer chromatography (chloroform–methanol, 5:1) indicated the complete loss of starting material. The lithium was removed by the addition of a small amount of Dowex 50 (H^+) resin to the solution and stirring for 15 min. The resin was removed by filtration, the filtrate was evaporated *in vacuo* and the residue was dissolved in 80% aqueous methanol containing 2 drops of glacial acetic acid. The compound was subjected to hydrogenolysis over 10% palladium on charcoal. After 1 hr the catalyst was removed by filtration and the solvent was evaporated *in vacuo*. The residue, a white amorphous solid, was dissolved in 80% aqueous methanol and treated with a small amount of Dowex-3 (OH^-) resin. Evaporation of the solvent yielded a white amorphous solid which, when triturated with methanol, yielded crystalline material: 100 mg (82%); shrinkage point 165–170°, decomposition point ~225°; $[\alpha]^{23}D$ ~0° (*c* 0.6, H_2O). Silica gel thin layer chromatography (*t*-butyl alcohol–2-butanone–water–ammonium hydroxide, 4:3:2:1) indicated only one spot with an R_{AN} value[29] of 0.38.

Anal. Calcd for $C_{13}H_{20}N_4O_7 \cdot {}^3/_2H_2O$: C, 42.05; H, 5.83; N, 15.08. Found: C, 42.40; H, 5.99; N, 15.34.

1-(3-Deoxy-3-glycylamido-α-D-glucopyranosyl)uracil (5a).—Compound **3a** (250 mg, 0.42 mmole) was deacetylated and decarbobenzoxylated according to the procedure employed for the sarcosine derivative **3b**. The product was isolated (70 mg, 48%) as a white amorphous solid which defied attempts at crystallization. The product gave a shrinking point of ~160° and a browning point of ~180°, $[\alpha]^{23}D$ ~0° (*c* 0.8 H_2O). Silica gel thin layer chromatography (*t*-butyl alcohol–2-butanone–water–ammonium hydroxide, 4:3:2:1) indicated only one spot with an R_{AN} value[29] of 0.55.

Anal. Calcd for $C_{12}H_{18}N_4O_7 \cdot 2CH_3OH$: C, 42.64; H, 6.65; N, 14.21. Found: C, 42.07; H, 6.02; N, 14.01.

1-(3-L-Alanylamido-3-deoxy-β-D-glucopyranosyl)uracil (5c).—Compound **3c** (250 mg, 0.29 mmole) was deacetylated and decarbobenzoxylated according to the procedure employed for the sarcosine derivative **3b**. The product was isolated in a yield of 100 mg (71%) as a white amorphous solid which defied attempts at crystallization. The product showed a shrinking point of *ca.* 145°, $[\alpha]^{23}D$ −2.5° (*c* 0.7, H_2O). Silica gel thin layer chromatography (*t*-butyl alcohol–2-butanone–water–ammonium hydroxide, 4:3:2:1) indicated only one spot with an R_{AN} value[29] of 0.75.

Anal. Calcd for $C_{13}H_{20}N_4O_7 \cdot 2CH_3OH$: C, 44.11; H, 6.91; N, 13.72. Found: C, 43.70; H, 6.30; N, 13.40.

1-(3-Deoxy-3-D-phenylalanylamido-β-D-glucopyranosyl)uracil (5d).—Compound **3d** (500 mg, 0.73 mmole) was added to 20 ml of anhydrous methanol along with 5 mg of anhydrous lithium hydroxide. The mixture, protected from moisture, was stirred for 24 hr at room temperature during which time complete solution did not take place. Silica gel thin layer chromatography (chloroform–methanol, 5:1) of the solution indicated that the deacetylation reaction was incomplete. The mixture was stirred for an additional 24 hr in a water bath at 45°. During this period solution occurred and the deacetylation reaction was complete as indicated by thin layer chromatography. Water (5 ml) and 3 drops of glacial acetic acid were added and the solution was subjected to hydrogenolysis over 10% palladium on charcoal for 1 hr. The solvent was evaporated and the residue washed with ethanol to yield 300 mg (95%) of a white crystalline compound: mp *ca.* 180° with slow decomposition; $[\alpha]^{23}D$ +24° (*c* 0.6 in water). Silica gel thin layer chromatography showed one spot with an R_{AN} value[29] of 2.55.

Anal. Calcd for $C_{19}H_{24}N_4O_7 \cdot {}^1/_2H_2O$: C, 53.14; H, 5.87; N, 13.05. Found: C, 53.11; H, 5.69; N, 12.56.

Preparation of 1-(3-Deoxy-3-nitrohexopyranosyl)cytosines (11).—Sodium metaperiodate (10.7 g, 0.05 mole) was dissolved in 50 ml of water. Cytidine (6) (12.2 g, 0.05 mole) was added portionwise to the stirred solution which was cooled in an ice bath. Following completion of the addition, enough additional metaperiodate was added to give a positive starch–iodide test, thus ensuring the complete oxidation of the cytidine. The solution was allowed to stir for an additional 0.5 hr and was then poured into *ca.* 800 ml of ethanol. The precipitate was filtered, washed well with ethanol, and discarded. The combined filtrates were evaporated to dryness *in vacuo*. The residue was dissolved in 80 ml of water and nitromethane (3.0 ml, 0.056 mole) was added. Sodium hydroxide (50 ml of 1 *N* solution) was added dropwise. Following the completion of the addition, the solution was stirred for an additional 2 hr at room temperature. The yellow color of the solution deepened with time. Deionization of the solution was effected with an Amberlite IRC-50 (weakly acidic, carboxylic type) resin. Following filtration of the slurry, concentrated hydrochloric acid was added to the filtrate in a quantity sufficient to lower the pH to 1–2. The solvent was removed *in vacuo* leaving a yellow residue. Trituration of this solid with hot ethyl acetate removed most of the color and afforded 17 g of a semicrystalline compound. Trituration of this solid with hot acetone–methanol afforded white crystals of **11** melting at 203–203.5° (effervescent) with browning prior to melting. The product (a mixture of *gluco* and *manno* isomers) gave a positive test for ionic chlorine.

Anal. Calcd for $C_{10}H_{14}N_4O_7 \cdot HCl \cdot 2H_2O$: C, 32.05; H, 5.11; N, 14.95. Found: C, 32.47; H, 4.49; N, 14.75.

1-(3-Amino-3-deoxy-β-D-mannopyranosyl)cytosine (13).—A 500-mg sample of **11** was dissolved in 75 ml of a 2:1 mixture of methanol–water. Activated Raney nickel (1 ml) was used as the catalyst for the hydrogenation. A second 1-ml portion of catalyst was added during the course of the reduction. The progress of the reaction was followed by silica gel thin layer chromatography (chloroform–methanol, 5:1). The reduced product (**12** and **13**) does not migrate in this system. The solvent was removed by evaporation *in vacuo*. The greenish residue was dissolved in a small volume of a slightly ammoniacal solution. Gaseous hydrogen sulfide was passed through the aqueous solution until the precipitation of nickel sulfide was complete. The latter was filtered through a bed of diatomaceous earth and the filtrate was concentrated to a small volume and applied to a 10-mm-diameter Dowex-1 (OH^-) column. The height of the resin bed was *ca.* 200 mm. Elution was performed with water and 5-ml fractions were collected. Fractions 10–16 were combined and evaporated to yield 10 mg (2%) of the *manno* isomer which, after recrystallization from water–methanol, gave colorless needles sintering at 168° and melting with decomposition at 192°, $[\alpha]^{23}D$ +82° (*c* 0.94, H_2O). Paper electrophoresis in borate buffer (pH 9.2, 900 v, 8 hr) gave a cathodic migration of 7.5 cm (the *gluco* isomer = +4.5 *vide infra*). The infrared spectrum of the *manno* nucleoside **13** differed from that of the *gluco*.

Anal. Calcd for $C_{10}H_{16}N_4O_5 \cdot 2H_2O$: C, 38.96; H, 6.49; N, 18.18. Found: C, 39.07; H, 6.57; N, 18.05.

1(3-Amino-3-deoxy-β-D-glucopyranosyl)cytosine (12).—After elution of the *manno* isomer (*vide supra*) fractions 17–48 were combined and concentrated to dryness. The *gluco* isomer **12** was obtained (200 mg) in crystalline form. The properties of this nucleoside were identical with those reported for 1-(3-amino-3-deoxy-β-D-glucopyranosyl)cytosine synthesized previously[4] from compound **1**. In paper electrophoresis (borate buffer, pH 9.2, 900 v, 8 hr), **12** showed a migration of +4.5 cm.

The same products **12** and **13** may be obtained from cytidine without isolation of the 3′-nitro nucleoside salt **11**. In this procedure, the crude mixture of **9** and **10** is hydrogenated directly over Raney nickel. The mixture of 3′-amino nucleosides is then treated in a manner similar to that described above.

1-(3-Acetamido-3-deoxy-β-D-mannopyranosyl)cytosine (15).—The *manno* nucleoside **13** (250 mg, 0.92 mmole) in a mixture of pyridine (25 ml) and acetic anhydride (5 ml) was shaken overnight at room temperature during which time solution took place.

(29) R_{AN} is the ratio of the R_f of the compound in question to the R_f of compound **1** (amino nucleoside). The two compounds are run side by side on the same plate.

The solvent was evaporated *in vacuo* and traces of pyridine and acetic anhydride were removed by azeotropic distillation of toluene. The polyacetate **14** was obtained as a syrup (443 mg, quantitative yield) which did not crystallize.

Compound **14** (400 mg, 0.83 mmole) was dissolved in methanolic ammonia (40 ml) and allowed to stand overnight at room temperature. Removal of the solvent under reduced pressure yielded a sirupy residue which was shaken for 5 min with 20 ml of chloroform and allowed to stand overnight at room temperature. The insoluble tacky crystalline material was separated by filtration and recrystallized from water–ethanol. The yield of **15** was 203 mg (80%), mp 202–225° (sintered), 233–235° (effervescent). The ultraviolet spectrum of the compound was similar to that for cytidine, and an infrared spectrum indicated the lack of ester linkages. A ninhydrin test on **15** was negative.

Anal. Calcd for $C_{12}H_{18}N_4O_5 \cdot {}^3/_2H_2O$: C, 42.23; H, 6.16; N, 16.42. Found: C, 42.33; H, 6.39; N, 16.41.

Methyl 3-Acetamido-3-deoxy-tri-*O*-acetyl-α-D-mannopyranoside (18).—Compound **13** (97 mg, 0.31 mmole) was hydrogenated at room temperature in 20 ml of water with 89 mg of platinum oxide for a period of 60 hr. The catalyst was filtered and the alkaline filtrate was evaporated to dryness. The hexahydro pyrimidine nucleoside **16**, obtained as a syrup, was dissolved in 15 ml of methanol and the solution was saturated with hydrogen chloride at 0°. The mixture was refluxed gently for 8 hr. The solvent was evaporated under reduced pressure and the resultant syrup was azeotropically dried with three portions of toluene. The residue (containing **17**) was dissolved in a mixture of pyridine (8 ml) and acetic anhydride (2 ml) and was allowed to remain overnight at room temperature. The solvent was evaporated to dryness under reduced pressure followed by an azeotropic removal of traces of acetic anhydride with toluene. The residue was dissolved in chloroform (5 ml) and placed on an acid-washed alumina column packed with benzene. Elution was performed with 150 ml of benzene (which yielded 20 mg of material), followed by 100 ml of a 1:1 benzene–ethyl acetate mixture (which yielded 78 mg of material) and finally with 100 ml of ethyl acetate (which yielded 28 mg of material). Upon evaporation of the second fraction followed by refrigeration, the residue yielded colorless needles. These were recrystallized from ethanol–petroleum ether to give colorless needles of methyl 3-acetamido-3-deoxy-2,4,6-tri-*O*-acetyl-α-D-mannoside (**18**), mp 150–151°. A mixture melting point with an authentic sample[23] showed no depression and their infrared spectra were identical.

1-(3-Deoxy-3-nitro-β-D-glucopyranosyl)-N^4-benzoylcytosine (19).—*N*-Benzoylcytidine[24] (7.0 g, 0.02 mole) was oxidized with sodium metaperiodate (4.28 g, 0.02 mole) in 150 ml of 50% aqueous ethanol. Sufficient additional metaperiodate was added to ensure complete oxidation of the glycol (as indicated by a positive starch–iodide test). The precipitate formed was filtered and washed with ethanol, and the combined filtrates were evaporated *in vacuo*. To a solution of the dialdehyde in 100 ml of 75% ethanol was added nitromethane (1.2 ml, 0.02 mole). Sodium hydroxide (10 ml of 1 *N* solution) was added dropwise and the mixture was stirred for an additional 15 min. Dowex 50 (H^+) was added with stirring along with 25 ml of water. Enough resin was added to lower the pH to *ca.* 4–5. The resin was removed by filtration and washed with 50 ml of water and 75 ml of ethanol. The combined filtrates were concentrated to a small volume. Ethanol was added and removed *in vacuo* repeatedly until crystallization occurred in the yellow solution. (If allowed to evaporate to dryness, the product undergoes decomposition). The mixture was cooled overnight and the precipitate was removed by filtration, washed with ethanol, cooled in a Dry Ice–acetone bath, and then thoroughly washed with ether and dried. The yield of product was 2.0 g (25%), mp 174–177°. Silica gel thin layer chromatography (chloroform–methanol, 5:1) indicated only one spot.

Anal. Calcd for $C_{17}H_{18}N_4O_8 \cdot {}^1/_2H_2O$: C, 49.16; H, 4.62; N, 13.48. Found: C, 48.70; H, 4.89; N, 13.03.

Calculation of Molecular Rotation by Summation of Partial Conformational Contributions. Rotations of the Eight 1,6-Anhydro-β-D-hexopyranoses and Their Triacetates[1]

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The optical rotations of the 1,6-anhydro-β-D-hexopyranoses and their triacetates have been correlated. The rotations are expressed as the algebraic sum of a series of empirical rotatory contributions by various conformational elements of asymmetry; close agreement between the calculated and experimentally determined rotations is observed.

It has been shown by Whiffen[2] that the observed rotation of an optically active molecule can be regarded as an algebraic summation of partial rotatory contributions of various conformational elements of asymmetry. Empirical values were determined that permitted calculation of the rotations of various cyclic sugars and cyclitols with fair accuracy. A more extensive treatment bas been presented by Brewster,[3] and it has been applied to various types of optically active organic compounds.[4,5] The best correlations between the calculated and the experimental values have been observed with compounds that are devoid of absorption bands in the near ultraviolet, and which have predictable, fixed conformations, or for which valid estimates of conformational populations can be made.[6] In the carbohydrate field the calculations are particularly simple in the case of the polyhydroxycyclohexanes,[2,5,7] and discrepancies between observed and calculated rotations have been interpreted in terms of equilibria between different conformers.[2,5,8] Similar comparisons have been made for the methyl D-aldopyranosides,[2,5,9] although the correlations between the observed and calculated values are not precise enough to permit detailed treatment in terms of conformational populations.

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