Nucleosides. XXII.[1] Pyrimidine Nucleosides of 4-Amino-4-deoxy-β-D-galactopyranose

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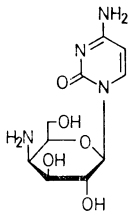
(Received January 14, 1974)

In a four step sequence from 1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-4-*O*-mesyl-α-D-glucose (**4**), 4′-amino-4′-deoxy-β-D-galactosyl-cytosine (**1**) was synthesized, involving stannic chloride catalyzed nucleosidation with bis(trimethylsilyl)-N^4-acetyl-cytosine, azidolysis of the 4′-sulfonyloxy group, deblocking and reduction (overall yield: 48%). An alternate, though preparatively less satisfactory approach was used for the preparation of the uracil analogue **2**, which started from β-D-glucosyl-cytosine or uracil (**9** or **15**) and proceeded *via* standard blocking and deblocking reactions to the 4′-*O*-mesylglucosyl-uracil (**20**) and was concluded by azidolysis (**20, 21**), removal of protecting groups and reduction. Structural and configurational assignments evolved from the mode of preparation as well as from spectroscopic data, most conveniently from the acetyl resonances of the peracetylated nucleosides **8** and **24**, which are in accord with those of their galactosyl and 3-acetamido-3-deoxy-galactosyl counterparts (Table).

Because of the unique structural features associated with the aminoacyl-aminohexosyl-cytosine group of antibiotics, considerable attention has been given to an assessment of their structure-activity relationships.[3] The variety of analogs modified in the carbohydrate portion being rather limited, it was of interest to evaluate analogs for their biological relevance, which have a "1,4-*cis*-arrangement" of nucleobase and amino-acid component, *i.e.* 4-aminogalacto-configuration in the sugar unit, rather than the 1,4-*trans*-disposition present in gougerotin and blasticidin S. We have, by consequence, initiated studies towards a preparatively satisfactory access to 4-amino-galactosyl-pyrimidine nucleosides **1** and **2**, the results being subject of this communication.



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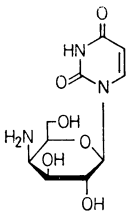

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In design, the synthetic scheme was based upon the efficient preparation of 4-amino-4-deoxy-D-galactose and derivatives by a route involving direct displacement of a 4-mesyloxy group by azide ion in a suitably blocked D-glucose derivative.[4] Nevertheless, when utilizing this route for the preparation of 4-amino-galactosyl nucleosides, several synthetic possibilities remain with respect to the stage at which the aglycon is preferably introduced:

(i) Nucleoside synthesis with an *a priori* prepared 4-aminosugar derivative, *e.g.* with tetra-*O*-acetyl-4-acetamido-4-deoxy-D-galactopyranose by the fusion method or the Friedel-Crafts catalyzed silyl procedure.

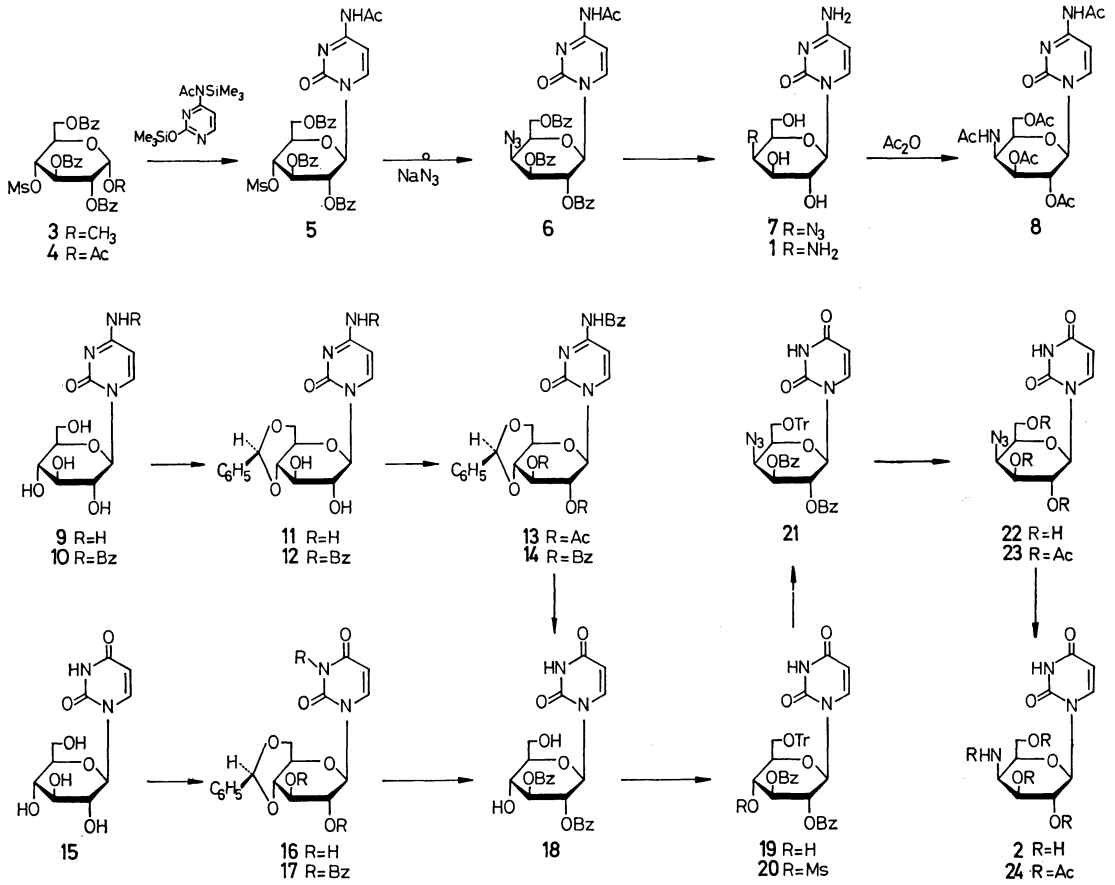
(ii) Nucleosidation at the outset, *e.g.* conversion of the respective glucopyranosyl nucleoside *via* a series of reactions into a suitably blocked 4′-*O*-mesylate followed by azidolysis, deblocking and reduction.

(iii) Introduction of the nucleobase at some intermediate stage, *e.g.* at that of a suitably blocked 4-*O*-mesyl-glucose.

Since certain preliminary experiments with the first approach, which might be surmised to be the most direct one, presented some preparative complications when employing the stannic chloride catalyzed silyl nucleosidation,[5,6] the second and third reaction path was deemed more appropriate and used for the preparation of **1** and **2**.

Cytosine Nucleosides. A 4-*O*-mesyl-glucose derivative suitable for nucleosidation *via* the Friedel-Crafts catalyzed silyl procedure[5,6] proved to be 1-*O*-acetyl-2,3,6-tri-*O*-benzoyl-4-*O*-mesyl-α-D-galactopyranose (**4**), obtained from its readily accessible methyl glycoside **3**[4] by boron trifluoride catalyzed acetolysis.[7] When reacted in dichloroethane with N^4-acetyl-bis(trimethylsilyl)-cytosine in the presence of stannic chloride (12 hr, 60 °C), crystalline nucleoside **5** was obtained in high yield (81%). The secondary C-4′-mesyloxy function in **5** was readily displaced by sodium azide in hexamethylphosphoric triamide (20 hr, 80 °C), to afford the blocked azidogalactosyl nucleoside. Subsequent de-*O*-benzoylation by methanolic ammonia to the free azido-nucleoside **7**, followed by hydrogenation over palladium-on-charcoal then yielded the desired 4′-amino-4′-deoxy-β-D-galactosyl-cytosine (**1**) as an analytically and chromatographically pure sirup, that could not be induced to crystallize, yet gave a crystalline tetraacetyl derivative (**8**). The yields in this four-step sequence from **4** *via* **5**, **6**, and **7** to **1** being consistently high, the overall yield amounts to a quite satisfactory 48%.

In addition, a number of new crystalline derivatives of β-D-glucopyranosylcytosine (**9**) were prepared and although they could not directly be utilized for the synthesis of **1** (yet of **2**, see below), are placed on record as readily accessible, preparatively useful intermediates. Thus, selective benzoylation of the N^4-amino function in the nucleobase was readily achieved by refluxing **9** with benzoic anhydride in methanol, to afford highly crystalline **10** in 74% yield, further characterized as its tetra-*O*-acetyl derivative. By treatment with benzaldehyde/zinc chloride, **9** as well as **10** were converted into their 4′,6′-*O*-benzylidene derivatives **11** and **12**, respectively, the conversion **9**→**11**, however, proceeding

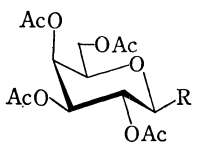
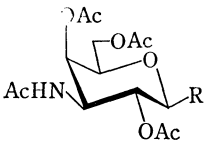
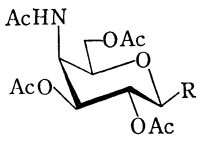


rather sluggishly due to the low solubility of the educt in the reaction mixture. Subsequent acylation of **12** with acetic anhydride or benzoyl chloride in the presence of pyridine proceeded smoothly to afford the 2′,3′-di-*O*-acyl derivatives **13** and **14**. However, as expected from the ready hydrolytic deamination of N^4-acylated cytosine and cytidine derivatives,[8,9] a de-*O*-acetalization in benzylidene-nucleosides **11**—**14** cannot be achieved without concomitant removal of the benzoylamido-function to yield the respective uracil nucleosides. Thus, brief treatment of **14** with 80% acetic acid at 80—90 °C, gave in an 81% yield 2′,3′-di-*O*-benzoyl-β-D-glucosyluracil (**18**), identical with the same product prepared by another route (*cf.* below).

Uracil Nucleosides. In analogy to the efficacious four-step preparation of **1** from 4-*O*-mesyl-glucose (**4**), for a synthesis of 4-aminogalactosyluracil (**2**) the nucleosidation of **4** with bis(trimethylsilyl)uracil followed by introduction of the 4′-amino function must be considered to be the most appropriate and should be feasible without complications. Yet, prior to this, we had followed approach (ii) for a synthesis of **2**, comprising eight steps from readily accessible[10] β-D-glucopyranosyl-uracil (**15**). The conversion of **15** into its 2′,3′-di-*O*-benzoyl derivative (**18**) was readily accomplished by treatment with benzaldehyde/zinc chloride (to **16**), acylation with benzoyl chloride/pyridine to the N^3,2′,3′-tribenzoyl derivative **17** and deacetalization with 80% acetic acid (1 hr, 80 °C) to **18**, which was also obtained from the cytosine nucleoside **18** (*vide supra*). Subsequent tritylation of the primary hydroxyl function in **18**, followed by mesylation gave the key intermediate **20**, suitably blocked for an ensuing S_N2-displacement of its 4′-mesyloxy function by sodium azide in HMPT (20 hr, 90 °C). Deblocking in **21** by treatment with sodium methoxide/methanol and, subsequently, with 80% acetic acid afforded the free azidogalactosyl nucleoside **22**. Due to difficulties in crystallizing **22**, it was directly hydrogenated to yield 4′-aminogalactosyl-uracil (**2**) as an amorphous substance. The peracetyl derivatives of **22** and **2**, however, were obtained in crystalline, readily characterizable form, as was the case with all other intermediates in the conversion of **15** into **2** over eight steps. Not unexpected, though, the preparative utility of the reaction sequence **15**→**2** is considerably inferior to the four-step approach employed in the cytosine series for the synthesis of **1**.

Structural and Configurational Assignments. Since well-defined synthetic procedures have been used throughout as well as readily characterizable starting materials (**4**, **9**, and **15**, respectively), the structures and configurations of the newly synthesized nucleosides already follow from the mode of preparation. Sustaining evidence is furnished by NMR data, most conveniently from the acetyl resonances in $CDCl_3$ or DMSO-d_6 of the peracetylated nucleosides. As is clearly apparent from the empirical principles laid down in the "acetyl resonance rule",[11,12] hexopyranosyl nucleosides with axial *O*-acetyl substituents give a larger separation of signals in the 8 τ range than *e.g.* glucosyl derivatives[13] with an equatorial orientation of groups.

Table 1. Relevant NMR-data in DMSO-d_6 and $CDCl_3$, and rotations of peracetyl-β-D-galactopyranosylpyrimidines

Sugar moiety	R[a]		DMSO-d_6[b] H-1′[c]	a-OAc	e-OAc 6′-OAc a-NHAc	e-NHAc	$CDCl_3$[b] H-1′[c]	a-OAc	e-OAc 6′-OAc a-NHAc	e-NHAc	$[\alpha]_D$ (solvent, °C)
	U	(I)	3.96	7.83	8.00 8.06 8.10	—	3.83	7.80	7.95 7.99 (2)	—	+32 (CH_3OH, 22)[e]
	U(O⁴-Et)	(II)	4.03	7.83	8.02 8.06 8.10	—	3.89	7.81	7.93 8.00 (2)	—	+59 ($CHCl_3$, 21)[f]
	C	(III)	3.94	7.82	8.01 8.06 8.10	—	3.87	7.82	7.97 8.02 8.04	—	+48 (CH_3OH, 23)[e]
	C(N⁴-Ac)	(IV)	3.81	7.82	7.88[d] 8.02 8.06 8.10	—	3.92	7.80	7.73[d] 7.97 8.01 8.03	—	?
	U	(V)	4.08	7.84	8.02 8.09	8.23	3.90	7.81	7.97 8.00	8.08	+35 (CH_3OH, 23)[g]
	U(O⁴-Et)	(VI)	3.91	7.82	8.02 8.12	8.22	3.84	7.80	7.97 8.00	8.08	+80 (CH_3OH, 23)[g] +87 ($CHCl_3$, 23)[g]
	U	(**24**)	4.06	—	8.02 8.05 (2) 8.08	—	3.91	—	7.94 7.97 (2) 8.02	—	+14 (CH_3OH, 23)
	C(N⁴-Ac)	(**8**)	3.91	—	7.88[d] 8.02 (2) 8.08 8.10	—	3.86	—	7.71[d] 7.95 (2) 7.99 8.02	—	+16 ($CHCl_3$, 20)

a) Abbreviations used: U=uracilyl-1; U(O⁴-Et)=4-ethoxypyrimidinonyl-1; C=cytosinyl-1; C(N⁴-Ac)=N^4-acetyl-cytosinyl-1. b) Except for some values in $CDCl_3$ (G. Bambach, Diplomarbeit, Techn. Hochschule Darmstadt, 1968) the NMR data for compounds I—VI are derived from Ref.[11] c) Observed as doublets with $J_{1',2'}$=8—9 Hz. d) N^4-Acetyl resonance of the cytosine moiety. e) F.W. Lichtenthaler, G. Bambach, and P. Emig, *Chem. Ber.*, **102**, 1003 (1969). f) G. E. Hilbert, *J. Amer. Chem. Soc.*, **59**, 330 (1937). g) F. W. Lichtenthaler, G. Bambach, and U. Scheidegger, *Chem. Ber.*, **102**, 991 (1969).

In DMSO-d_6 and in $CDCl_3$, the peracetylated galactosyl-nucleosides I—IV as well as their 3′-aminoanalogs V[14] and VI show one distinct lowfield resonance in the 7.80—7.85 τ range (*cf.* Table), clearly attributable to the axial C-4′-acetoxy group. The 4′-aminogalactosyl-pyrimidines **8** and **24**, however, were expectedly devoid of a resonance within the axial range, since acetamido resonances of axial orientation fall together with those for equatorial and C-6′-signals.[11] Equally distinct is the presence of an equatorial acetamido function in 3′-aminogalactosyl nucleosides V and VI, appearing at τ 8.08 in $CDCl_3$, yet at 8.23 in DMSO-d_6, the separation from the equatorial acetoxy groups being larger by about 0.1 ppm in the more polar solvent, undoubtedly due to different solvation of the acetamido group. This solvent effect is also observed—yet in reverse direction—with the N^4-acetyl resonances of the cytosine nucleosides IV and **8**, exhibiting lowfield resonances at τ 7.88 in DMSO-d_6, yet at 7.73 and 7.71, resp., in $CDCl_3$. Worthy of note with all nucleosides in the Table 1 is also the appearance of an acetyl resonance in the upper equatorial range (τ 8.09—8.10 in DMSO-d_6) which is to be assigned to the respective 2′-acetoxy resonances, being in the range of diamagnetic shielding by the nucleobase.

The rotational values for the peracetylated nucleosides, which should be similar in sign and magnitude, if the sugar-nucleobase orientations are identical, show a considerably better conformity as their glucoanalogs.[13] Although farreaching conclusions may not be drawn therefrom, it is interesting to note that the ethoxypyrimidinone nucleosides II and VI have the highest rotational values (+59 and +87° in chloroform, *cf.* Table 1), whilst, curiously, the 4′-aminogalacto derivatives exhibit the lowest.

Experimental

Melting points were determined on a Bock Monoskop, and are uncorrected. Spectral measurements were effected with Perkin-Elmer 125 (IR), Perkin-Elmer 137 (UV), and Varian A-60A (NMR) instruments. Thin layer chromatography on Kieselgel F_{254} plastic sheets (Merck, Darmstadt) was used to monitor the reactions and to ascertain the purity of the reaction products; developers employed (A) benzene–ethyl acetate (4:1); (B) benzene–ethyl acetate–methanol (12:7:1); (C) ethyl acetate–ethanol–water (15:2:1). The spots were visivalized by UV light, by iodine vaporing or by spraying with 80% aqueous sulfuric acid and charring at 110 °C for 5 min. Column chromatography was carried out on silica gel 70—230 mesh ("Kieselgel 60", Merck,

Darmstadt).

1-O-Acetyl-2,3,6-tri-O-benzoyl-4-O-mesyl-α-D-glucopyranose (4). To a solution of 10.0 g (17 mmol) of methyl 2,3,6-tri-*O*-benzoyl-4-*O*-mesyl-α-D-glucopyranoside (3)[4] in acetic anhydride (200 ml) was added 20 ml of ethereal BF_3-etherate and the mixture was kept at 60 °C for 12 hr, followed by pouring into ice-water under vigorous stirring. The aqueous phase was decanted several times to be replaced by new ice-water. After 2 hr, chloroform (500 ml) was added and the organic phase was separated, dried over Na_2SO_4 and evaporated to dryness. The residue, after charcoal treatment in hot methanol (150 ml), was crystallized gradually on standing at ambient temperature and was collected after standing overnight in a refrigerator: 5.9 g (47%), mp 175—178 °C, after sintering from 150 °C on; $[\alpha]_D^{20}$ +126° (*c* 1, $CHCl_3$); NMR ($CDCl_3$) τ 3.40 (d, 1, $J_{1,2}$=3.5 Hz, H-1), 3.86 (t, 1, $J_{2,3}=J_{3,4}$=10 Hz, H-3), 4.53 (q, 1, J=3.5 Hz and 10 Hz, H-2), 4.77 (t, 1, $J_{3,4}=J_{4,5}$=10 Hz, H-4), 7.08 (s, 3, OMs), 7.84 (s, 3, OAc).

Found: C, 59.09; H, 4.63; S, 5.39%. Calcd for $C_{20}H_{28}O_{12}S$: C, 58.82; H, 4.61; S, 5.22.

Cytosine Nucleosides (1, 5—14). *N⁴-Acetyl-1-(2',3',6'-tri-O-benzoyl-4'-O-mesyl-β-D-glucopyranosyl)cytosine (5)*: To a solution of 3.0 g (4.9 mmol) of mesylate **4** in 1,2-dichloroethane (100 ml) was added 6 g of molecular sieve,[18] stannous tetrachloride (1.5 ml) and, after 15 min, 1.65 (1.1 molar equiv.) of *N⁴,O*-bis(trimethylsilyl)-*N⁴*-acetyl-cytosine[19] and the mixture was heated to 60° for 12 hr. Subsequently, 750 ml of dichloromethane was added and the solution was extracted with two 200 ml portions of saturated $NaHCO_3$-solution,[20] followed by washing with water (2×200 ml). The organic phase was dried (Na_2SO_4), and evaporated to dryness *in vacuo*, to leave a crystalline residue which was filtered and thoroughly washed with ethanol: 2.8 g (81%) of crystals; mp 240—241 °C (decomp.); $[\alpha]_D^{20}$ +24° (*c* 1.2, dimethylformamide); uniform by tlc (D); λ_{max}^{MeOH} 233, 283, and 330 nm.

Found: C, 57.63; H, 4.48; N, 5.85; S, 5.46%. Calcd for $C_{34}H_{31}N_3O_{12}S$: C, 57.87; H, 4.43; N, 5.96; S, 5.54%.

N⁴-Acetyl-1-(4'-azido-2',3',6'-tri-O-benzoyl-4'-deoxy-β-D-galactopyranosyl)cytosine (6): A suspension of 2.3 g (3.1 mmol) of mesyl derivative **5** and sodium azide (1.0 g, 5 molar equiv.) in hexamethylphosphoric acid triamide (20 ml) was heated at 80 °C for 20 hr with vigorous stirring. The yellow mixture, containing some undissolved sodium azide, was poured into ice-water (100 ml), and the crystalline, colorless precipitate was filtered off: 1.8 g (88%) of the crude, chromatographically homogenous (tlc in A) product which was used for the ensuing reactions. A small amount was recrystallized from chloroform-methanol to give the analytical sample of mp 228 °C (decomp.) and $[\alpha]_D^{20}$ +121° (*c* 1, $CHCl_3$); NMR ($CDCl_3$) τ 0.33 (s, 1, NH), 2.0—3.0 (m, 17, H-5, H-6 and 3 C_6H_5); 3.77 (m, 1, H-1'), 8.13 (s, 3, NHAc).

Found: C, 60.65; H, 4.30; N, 12.75%. Calcd for $C_{33}H_{28}N_6O_9$: C, 60.74; H, 4.43; N, 12.87%.

1-(4'-Azido-4'-deoxy-β-D-galactopyranosyl)cytosine (7): To a suspension of **6** (1.6 g, 2.5 mmol, of crude product as described above) in methanol (20 ml) was added, with stirring, 0.1 ml of sodium methoxide solution, prepared from 1 g of sodium and 10 ml of methanol. A clear solution was not obtained, since product (**7**) started to precipitate immediately. After 3 hr stirring and standing overnight at ambient temperature, the precipitate was filtered (500 mg, 61%). Deionization of the filtrate and evaporation to dryness afforded a second crop (120 mg, 15%) of crude product, homogeneous by tlc (in C), which was used for hydrogenation (s. below).

A 150 mg portion was recrystallized from little methanol (standing for 1—2 days due to slow crystallization) to give the analytical sample of mp 180—182 °C (effervesc.); $[\alpha]_D^{20}$ −34° (*c* 1, H_2O); NMR (D_2O) τ 2.28 and 3.90 (two d, 1, $J_{5,6}$=7.5 Hz, H-6 and H-5), 4.36 (d, 1, $J_{1,2}$=8 Hz, H-1'), other protons give complex multiplets.

Found: C, 39.98; H, 5.00; N, 27.94%. Calcd for $C_{10}H_{14}N_6O_5$: C, 40.27; H, 4.73; N, 28.18%.

1-(4'-Amino-4'-deoxy-β-D-galactopyranosyl)cytosine (1): To a prehydrogenated suspension of 10% Pd/C (100 mg) in water (30 ml) was added 450 mg of crude azido-nucleoside **7**, and the hydrogenation was continued. After 2 hr, tlc (in C) showing the absence of starting material, the catalyst was removed and the filtrate was evaporated *in vacuo* (bath temperature below 25 °C) to give 380 mg (89%) of **1** as a sirup, that could not be induced to crystallization from the usual solvents. The analytical sample was dried at 0.1 mm for 12 hr.

Found: C, 43.95; H, 6.14; N, 20.14%. Calcd for $C_{10}H_{16}N_5O_5$: C, 44.11; H, 5.92; N, 20.58%.

N⁴-Acetyl-1-(4'-acetamido-2',3',6'-tri-O-acetyl-4'-deoxy-β-D-galactopyranosyl)cytosine (8): Sirupy **1** (250 mg) was refluxed for 30 min in 1:1 pyridine-acetic anhydride, followed by evaporation to dryness *in vacuo* (finally 0.1 mm). The remaining sirup was subjected to purification on a silica gel column (2×20 cm) with ethyl acetate. The residue, obtained after concentration of the eluate, was dissolved in ethyl acetate (3 ml) and precipitated by addition of ether: 380 mg (86%); mp 174—177 °C, after sintering from 165 °C on; $[\alpha]_D^{20}$ +16° (*c* 1, $CHCl_3$); NMR (DMSO-d_6) τ 0.87 (s, 1, cytosine-NH), 1.54 and 2.73 (two 1 H-d, $J_{5,6}$=7.5 Hz, H-6 and H-5); 1.86 (d, 1, $J_{4',NH}$=10 Hz, C-4'-NH), 3.91 (m, 1, H-1'), 4.63 (narrow (6 Hz) m, 2, H-2' and H-3'), 5.5 (broad m (25 Hz), 2, H-4' and H-5'), 5.95 (m, 2, CH_2), acetyl resonances *cf.* Table.

Found: C, 49.64; H, 5.27; N, 11.63%. Calcd for $C_{20}H_{26}N_4O_{10}$: C, 49.79; H, 5.39; N, 11.62%.

N⁴-Benzoyl-1-(β-D-glucopyranosyl)cytosine (10): To solution of 5.0 g (0.18 mol) of 1-(β-D-glucopyranosyl)cytosine (**9**)[22] in methanol (500 ml) was added 4.2 g (1 molar equiv.) of benzoic anhydride and the mixture was refluxed. After 1 and 3 hr, respectively, another molar equiv. of benzoic anhydride was added, followed by additional 4 hr of reflux. Filtration and evaporation to dryness *in vacuo* left a white residue, which was thoroughly extracted with ether for removal of benzoic acid and excessive benzoic anhydride. The ether-insoluble residue (3.2 g, mp 173—177 °C) was recrystallized twice from ethanol to yield 2.55 g (74%) of **10** as colorless prisms of mp 191—192 °C; $[\alpha]_D^{20}$ +28° (*c* 1, dimethylformamide).

Found: C, 54.06; H, 4.99; N, 11.12%. Calcd for $C_{17}H_{19}N_3O_7$: C, 54.11; H, 5.08; N, 11.14%.

N⁴-Benzoyl-1-(tetra-O-acetyl-β-D-glucopyranosyl)cytosine: A solution of 200 mg of **10** in 1:1 pyridine-acetic anhydride (10 ml) was kept at ambient temperature overnight followed by concentration to dryness and several reevaporations from ethanol. The solid residue was recrystallized from ethanol to give needles (150 mg, 52%) of mp 248—252 °C; NMR ($CDCl_3$) τ 7.93, 7.95, 8.01, and 8.03 for acetoxy resonances.

Found: C, 55.51; H, 4.94; N, 7.75%. Calcd for $C_{25}H_{27}N_3O_{11}$: C, 55.62; H, 4.99; N, 7.70%.

1-(4',6'-O-Benzylidene-β-D-glucopyranosyl)cytosine (11): Anhydrous zinc chloride (4.0 g) and 2.5 g (9.2 mmol) of glucosylcytosine (**9**) in 20 ml of benzaldehyde were stirred at ambient temperature until completely dissolved (5 days). The mixture was then stirred into ice-water (400 ml) and the crystalline product was filtered off. Recrystallization from methanol gave 1.4 g (42%) of **11**, decomposing from 270 °C on,

$[\alpha]_D^{20}$ −48° (*c* 1, $CHCl_3$).

Found: C, 56.43; H, 5.20; N, 11.60%. Calcd for $C_{17}H_{19}N_3O_6$: C, 56.50; H, 5.30; N, 11.63%.

N⁴-Benzoyl-1-(4′,6′-O-benzylidene-β-D-glucopyranosyl)cytosine (12): A mixture of zinc chloride (1.2 g), **10** (1.2 g, 3.2 mmol) and benzaldehyde (12 ml) was stirred overnight at room temperature followed by pouring into ice-water (200 ml) with vigorous stirring. *n*-Hexane (200 ml) was added whereafter the product started to crystallize. Filtration, washing with cold ethanol and recrystallization from methanol afforded 850 mg (61%) of colorless prisms of mp 245—248 °C; $[\alpha]_D^{20}$ −11° (*c* 1, dimethylformamide).

Found: C, 61.86; H, 4.96; N, 9.01%. Calcd for $C_{24}H_{23}N_3O_7$: C, 61.93; H, 4.98; N, 9.03%.

N⁴-Benzoyl-1-(2′,3′-di-O-acetyl-4′,6′-O-benzylidene-β-D-glucopyranosyl)cytosine (13): A solution of **12** (465 mg, 1 mmol) in 1:1 pyridin-acetic anhydride (10 ml) was kept for 2 hr at 80 °C and subsequently stirred into ice-water (150 ml). The crystalline solid was filtered off after 1 hr and recrystallized from ethanol: 360 mg (65%) of **13** as colorless needles; mp 262—264 °C; $[\alpha]_D^{20}$ −37° (*c* 1, $CHCl_3$).

Found: C, 60.98; H, 5.02; N, 7.62%. Calcd for $C_{28}H_{27}N_3O_9$: C, 61.20; H, 4.95; N, 7.65%.

N⁴-Benzoyl-1-(2′,3′-di-O-benzoyl-4′,6′-O-benzylidene-β-D-glucopyranosyl)cytosine (14): Benzoyl chloride (2.0 ml) was added to 1.18 g (2.5 mmol) of **12** in pyridine (40 ml) and the mixture was heated to 80 °C for 6 hr. Since starting material could still be detected by tlc (in B), another 2.0 ml of benzoyl chloride was added followed by heating to 100 °C for 5 hr. The mixture was then evaporated to dryness *in vacuo* (finally 0.1 mm) and the residue was dissolved in chloroform (150 ml) followed by washings, successively, with 1 M NaOH, water, 2 M HCl and again water. Drying over Na_2SO_4 and removal of the solvent left a residue which was recrystallized from ethanol: 1.32 g of **14** as colorless prisms; mp 271—273 °C; $[\alpha]_D^{20}$ −26° (*c* 1, $CHCl_3$).

Found: C, 67.43; H, 4.92; N, 5.96%. Calcd for $C_{38}H_{31}N_3O_9$: C, 67.46; H, 5.06; N, 6.21%.

Uracil Nucleosides (2, 15—24). *1-(4′,6′-O-Benzylidene-β-D-glucopyranosyl)uracil (16)*: A suspension of 10.0 g (36.4 mmol) of 1-(β-D-glucopyranosyl)uracil (**15**)[10] and 11 g of freshly molten zinc chloride in 115 ml of benzaldehyde was shaken for 30 hr. The resulting clear solution was stirred into ice water (700 ml), from which was decanted after 1 hr of vigorous stirring. The viscous sirup was brought to crystallization by trituration with petroleum ether, filtered off and recrystallized from methanol: 4.5 g (72%) of **16** as colorless needles, uniform by tlc (C); mp 282—284 °C; $[\alpha]_D^{32}$ −10° (*c* 1, dimethylformamide).

Found: C, 56.54; H, 5.21; N, 7.85%. Calcd for $C_{17}H_{18}N_2O_7$: C, 56.35; H, 5.01; N, 7.73.

1-(2′,3′-Di-O-benzoyl-4′,6′-O-benzylidene-β-D-glucopyranosyl)-N³-benzoyluracil (17): To a suspension of 9.2 g (25.4 mmol) of **17** in pyridine (360 ml) was added with cooling 30 ml of benzoyl chloride and the mixture was stirred at ambient temperature for 24 hr. After removal of pyridine by evaporation (1 mm, bath temperature 50 °C), the brownish residue was taken up in chloroform and washed, successively, with N sodium carbonate, water, 0.1 M hydrochloric acid, and water. Treatment with activated carbon, drying over Na_2SO_4 and removal of chloroform *in vacuo* left a residue, which crystallized on solution in little hot chloroform and addition of methanol. Recrystallization from methanol afforded 10.1 g (59%) of colorless prisms; mp 217—219 °C; $[\alpha]_D^{32}$ +3.7° (*c* 1, chloroform).

Found: C, 67.63; H, 4.42; N, 4.19%. Calcd for $C_{38}H_{30}N_2O_{10}$: C, 67.65; H, 4.48; N, 4.15%.

1-(2′,3′-Di-O-benzoyl-β-D-glucopyranosyl)uracil (18): *A. From* **17** *by Removal of* N-*Benzoyl and* O-*Benzylidene Groups*: A solution of **17** (9.15 g, 13.5 mmol) was refluxed in 80% aqueous acetic acid for 9 hr and the mixture was subsequently taken to dryness *in vacuo* (1 mm), followed by several reevaporations from ethanol-water to remove the benzaldehyde formed. The residue crystallized on trituration with ether and was recrystallized by dissolution in little hot ethanol and addition of ether until turbidity: 4.0 g (61%) of **18**; tlc in A; mp 158—161 °C; $[\alpha]_D^{32}$ +47° (*c* 1, ethanol); τ (DMSO-d_6) 1.9—2.8 (11H-m, H-6 and $2C_6H_5$), 3.91 (1H-d, J=8.5 Hz, H-1′).

Found: C, 60.01; H, 4.73; N, 5.89. Calcd for $C_{24}H_{22}N_2O_9$: C, 59.75; H, 4.60; N, 5.81%.

B. From Cytosine Nucleoside **14** *by Simultaneous De-*O*-benzylidenation and Hydrolytic Deamination*: A solution of **14** (1.60 g, 2.4 mmol) in 80% acetic acid (80 ml) was heated to 80—90 °C for 1 hr, and subsequently filtered upon addition of charcoal. Evaporation to dryness and trituration of the residue with ethanol gave a solid mass, which was filtered and recrystallized from ethanol-ether (as above), to yield 1.13 g (81%) of **18** as colorless needles, identical in mp, rotation, tlc (A) and IR data with the product obtained above.

1-(2′,3′-Di-O-benzoyl-6′-O-trityl-β-D-glucopyranosyl)uracil (19): To a solution of 1.82 g (3.7 mmol) of **18** in pyridine (30 ml) was added 4.0 g (4 molar equiv.) of trityl chloride[23] and the mixture was stirred for 3 days at ambient temperature. Filtration, evaporation to dryness followed by several reevaporations from ethanol-water gave a sirup, which was disolsved in chloroform. Washing with water, drying over Na_2SO_4 and evaporation *in vacuo* left a sirup, which was transfered to a silica gel column (2×20 cm) and eluted with chloroform until the effluent was free of UV active material.[24] The product (**19**) was then eluted with ethyl acetate, followed by evaporation of the eluate to dryness and trituration of the residue with methanol, which induced crystallization. Recrystallization from ethanol-*n*-hexane afforded 2.1 g (78%) of tlc pure (A) crystals of mp 171—173 °C and $[\alpha]_D^{25}$ +46° (*c* 1, $CHCl_3$).

Found: C, 71.16; H, 4.98; N, 4.10%. Calcd for $C_{43}H_{36}N_2O_9$: C, 71.26; H, 5.01; N, 3.87%.

1-(2′,3′-Di-O-benzoyl-4′-O-mesyl-6′-O-trityl-β-D-glucopyranosyl)uracil (20): To a solution of 1.90 g (2.6 mmol) of the trityl derivative **19** in pyridine (60 ml) was added under cooling (0 °C) 1.3 ml (7 molar equiv.) of methanesulfonyl chloride and the mixture was kept in a refrigerator (5 °C) for 24 hr, followed by stirring into ice-water (50 ml). The resulting precipitate was filtered off, washed with water and subjected to purification by chromatography on silica with chloroform. Removal of solvent from the eluate and recrystallization of the residue from ethanol-*n*-hexane gave 1.42 g (66%) of **20** as prisms of mp 152—154 °C; $[\alpha]_D^{25}$ +89° (*c* 1, $CHCl_3$).

Found: C, 65.53; H, 4.58; N, 3.38%. Calcd for $C_{44}H_{38}N_2O_{11}S$: C, 65.82; H, 4.47; N, 3.49%.

1-(4′-Azido-2′,3′-di-O-benzoyl-4′-deoxy-6′-O-trityl-β-D-galactopyranosyl)uracil (21): A mixture of 1.20 g (1.5 mmol) of mesylate **20** and 390 mg of sodium azide (4 molar equiv.) in hexamethyl phosphoric triamide (50 ml) was heated at 90 °C for 24 days, followed by evaporation to dryness *in vacuo* (1 Torr). The sirup was dissolved in ethyl acetate, which was repeatedly washed with water and dried over Na_2SO_4. Removal of the solvent, and purification of the sirup by chromatography on a silica gel column (2×20 cm) with chloroform. The residue obtained on evaporation to dryness of the appropriate eluate, crystallized on trituration with ethanol. Recrystallization from the same solvent yielded 0.88 g (78%) of **21** as colorless prisms; mp 226—227 °C; $[\alpha]_D^{25}$ −37° (*c* 1,

$CHCl_3$).

Found: C, 68.92; H, 4.75; N, 9.52%. Calcd for $C_{43}H_{35}N_5O_8$: C, 68.88; H, 4.71; N, 9.34%.

1-(4'-Azido-2',3',6'-tri-O-acetyl-4'-deoxy-β-D-galactopyranosyl)-uracil (23): To azidogalactoside **21** (750 mg, 1 mmol) in methanol (70 ml) was added 0.5 ml of 4% methanolic sodium methoxide, followed by storage in a refrigerator (5 °C) for 2 days. After neutralization with acetic acid, the mixture was taken to dryness *in vacuo* and the residual sirup was heated in 30 ml of 80% acetic acid for 1 hr (80 °C). Evaporation to dryness *in vacuo*, followed by several reevaporations from ethanol gave a sirup, which upon solution in water was extracted twice with ether. The aqueous phase was evaporated to dryness and the residual **22**, resisting crystallization, was refluxed in acetic anhydride (20 ml) containing potassium acetate (200 mg) for 2 hr. The dark solution was poured in water followed by several extractions with 1:1 chloroform–ethyl acetate. The combined extracts, after drying over Na_2SO_4, were taken to dryness and the solid mass remaining was recrystallized from ethanol, involving charcoal treatment: 285 mg (68%) of **23** as colorless crystals of mp 225—226 °C; $[\alpha]_D^{25}$ −40° (*c* 0.5, dimethylformamide), IR (KBr) cm^{-1} 2154; N_3; NMR (DMSO-d_6) τ −1.73 (broad s, 1, NH) 2.51 (d, 1, $J_{5,6}$=8.5 Hz, H-6), 4.04 (d, 1, $J_{1',2'}$=9.0 Hz, H-1'), 4.29 (d, 1, $J_{5,6}$=8.5 Hz, H-5), 4.36 (dd, 1, J=3 and 9 Hz, H-3'), 4.79 (t, 1, J=9 Hz, H-2'), 7.93, 7.97, and 8.06 (three 3H-s, acetyl resonances).

Found: C, 44.98; H, 4.55; N, 16.41%. Calcd for $C_{16}H_{19}N_5O_9$: C, 45.18; H, 4.50; N, 16.47%.

1-(4'-Amino-4'-deoxy-β-D-galactopyranosyl)uracil (2): To blocked azidogalactosyl-uracil **21** (800 mg, 1.1 mmol) in methanol (80 ml) was added 2 ml of N sodium methoxide/methanol. After being stood at 5 °C for 2 days, the mixture was neutralized with acetic acid followed by evaporation to dryness. After treatment with 80% acetic acid (30 ml) for 1 hr at 80 °C, to effect complete detrytilation, the mixture was evaporated to a sirup, which upon solution in water was extracted twice with ether for removal of methyl benzoate and tritanol. The aqueous layer was taken to dryness, repeatedly reevaporated from ethanol to yield a viscous sirup of **22**, not amenable to crystallization. It was dissolved in 1:1 methanol–water (50 ml) and added to a prehydrogenated suspension of Pd/C in water (200 mg in 20 ml). After 2 hr the hydrogen uptake was complete (tlc in C), the catalyst was filtered off and the filtrate taken to dryness. Solving the residue in warm ethanol (70—80 ml), removal of some insoluble material, and concentration to a volume of about 30 ml afforded after longer standing (1—2 day) 94 mg (67%) of **2** as an amorphous, hygroscopic, ninhydrin-positive product; another 25 mg was isolated by concentration of the mother liquor; mp 155—158 °C, $[\alpha]_D^{23}$ +45° (*c* 0.2, MeOH).

Found: C, 43.73; H, 5.70; N, 15.12. Calcd for $C_{10}H_{15}N_3O_6$: C, 43.96; H, 5.53; N, 15.38.

1-(4'-Acetamido-2',3',6'-tri-O-acetyl-4'-deoxy-β-D-galactopyranosyl)uracil (24): A solution of 200 mg of azidotriacetate **23** in 50 ml of methanol saturated with ammonia was stored in a refrigerator (5 °C) overnight, followed by evaporation to dryness and hydrogenation of the residue in water (50 ml) in the presence of Raney-Nickel T4 catalyst[25] (1 ml). After 2 hr, tlc (C) showing the absence of starting material, the catalyst was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. *N*-Acetylation of the sirupous residue with acetic anhydride (0.5 ml) in 20 ml of methanol (5 hr, 25 °C) and evaporation to dryness with subsequent reevaporations from methanol afforded a sirup, pure by tlc (C), yet resistant to crystallization from the usual solvents.[26] Treatment with 1:1 pyridine–acetic anhydride (20 ml) for 12 hr at ambient temperature, removal of solvents *in vacuo* (finally 0.1 mm), yielded a sirup, which was dissolved in ethyl acetate and extracted with water (3×10 ml). The aqueous extracts were washed with ethyl acetate (2×10 ml) and the combined ethyl acetate solutions were evaporated to dryness. The remaining residue was applied to a silica gel column (2×20 cm) and first eluted with chloroform for removal of impurities, subsequently with ethyl acetate to yield the product. Evaporation of the eluate gave a sirup which crystallized on dissolution in little methanol and addition of benzene: 124 mg (59%) of crystals, pure by tlc (C); mp 165—168 °C after sintering from 145 °C on; $[\alpha]_D^{23}$ +14° (*c* 1, CH_3OH); NMR (DMSO-d_6) τ −1.36 (s, 1, uracil-NH), 1.90 and 4.22 (two d, 1, $J_{5,6}$=8.5 Hz, H-6 and H-5), 1.92 (d, 1, $J_{4',NH}$=9 Hz, C-4'-NH), 4.06 and 4.67 (two m, 1 and 2, ABX system for H-1' and H-2'/H-3'), 5.5 (m, 2, H-4' and H-5'), 6.00 (m, 2, 6'-CH_2); acetyl resonances *cf.* Table 1.

Found: C, 48.89; H, 5.18; N, 9.40%. Calcd for $C_{18}H_{23}N_3O_{10}$: C, 48.98; H, 5.25; N, 9.52%.

Support of this work by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

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