PYRIMIDINE NUCLEOSIDES OF 2-AMINO-2-DEOXY-D-GALACTOSE*

M. L. WOLFROM†, H. B. BHAT, AND P. J. CONIGLIARO
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 (U. S. A.)
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ABSTRACT

2-Deoxy-2-(*p*-methoxybenzylidene)amino-D-galactose was acetylated, and the resulting product was treated with hydrochloric acid, giving 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranose hydrochloride (**3**). Treatment of **3** with trifluoroacetic anhydride yielded 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranose (**4**), which was converted into 3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl bromide (**5**) by a previously published procedure. Condensation of **5** with bis(trimethylsilyl)cytosine yielded 1-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranosyl]cytosine, which was deacylated with methanolic ammonia to give 1-(2-amino-2-deoxy-β-D-galactopyranosyl)cytosine. Condensation of **5** with bis(trimethylsilyl)thymine yielded 1-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranosyl]thymine (**8**), which was deacylated with methanolic ammonia to give 1-(2-amino-2-deoxy-β-D-galactopyranosyl)thymine (**9**). Condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride with bis(trimethylsilyl)thymine gave 1-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)thymine (**11**), but in substantially lower yield than **8**. Compound **11** was also obtained by acetylation of **9**.

INTRODUCTION

In an earlier paper[1], the synthesis of the anomeric 9-(2-amino-2-deoxy-D-galactopyranosyl)adenines was reported. The present article describes the synthesis of two pyrimidine nucleosides of 2-amino-2-deoxy-D-galactose, namely, 1-(2-amino-2-deoxy-β-D-galactopyranosyl)cytosine and 1-(2-amino-2-deoxy-β-D-galactopyranosyl)thymine. For this synthesis, trifluoroacetyl was employed as the amino-protecting group, and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl bromide (**5**) was utilized for the condensation reactions. This bromide (**5**) had previously been prepared from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α- and -β-D-galactopyranose, which had been obtained by acetylation of

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2-deoxy-2-(trifluoroacetamido)-D-galactose[1]. In the work now described, **5** was prepared as follows. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranose (**4**) was prepared from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranose hydrochloride (**3**), synthesized by a method similar to that employed by Bergmann and Zervas[2] for preparing 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride. Compound **4** was then converted into the bromide **5**.

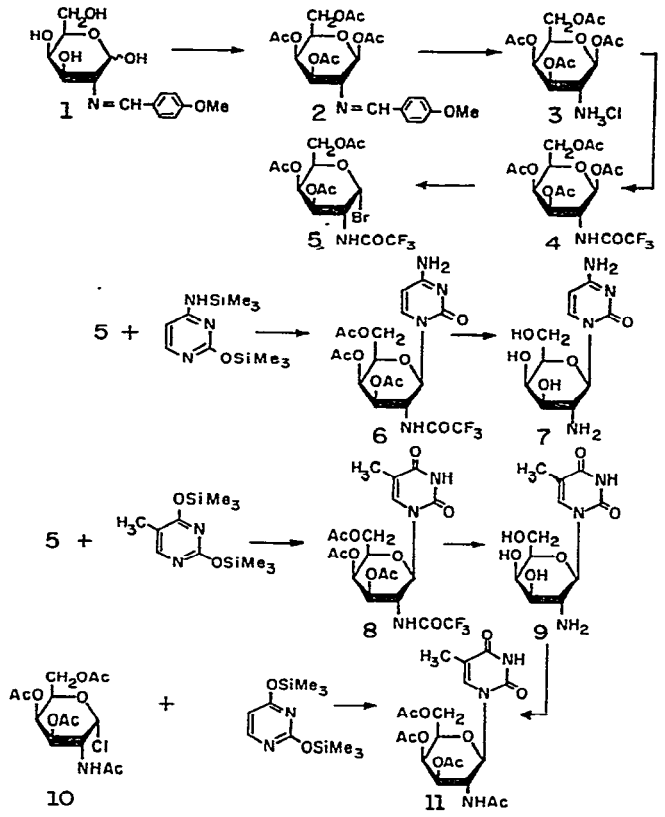
DISCUSSION

2-Deoxy-2-(*p*-methoxybenzylidene)amino-D-galactose[3] (**1**) was acetylated with acetic anhydride–pyridine to give crystalline 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylidene)amino-β-D-galactopyranose (**2**) in 70% yield. Treatment of **2** with hydrochloric acid in hot acetone afforded crystalline 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranose hydrochloride (**3**) in 80% yield. Compound **3** was treated with trifluoroacetic anhydride and pyridine, to give 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranose[1] (**4**), in 80% yield. 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl bromide (**5**) was prepared from **4** by treatment with hydrogen bromide in acetic acid by a published procedure[1].

The bromide **5** was condensed with bis(trimethylsilyl)cytosine[4,5] by the fusion technique[4,6], to give amorphous 1-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranosyl]cytosine (**6**), initially isolated as the crystalline hydrate in 74% yield. The protected nucleoside **6** was deacylated with methanolic ammonia at room temperature to give crystalline 1-(2-amino-2-deoxy-β-D-galactopyranosyl)cytosine (**7**) in 91% yield. The anomeric assignments of **6** and **7** were made on the basis of n.m.r. spectroscopy. The n.m.r. spectrum of **7** (measured in deuterium oxide) showed a distinct doublet at τ 4.42, having a coupling constant of 9.4 Hz. The magnitude of this coupling constant is indicative[7,8] of a *trans*-diaxial relationship between H-1 and H-2 of the sugar moiety. This relationship is found in the favored conformation of the β-D-anomer (**7**).

Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-α-D-galactopyranosyl bromide (**5**) with bis(trimethylsilyl)thymine[4,5] by the fusion technique[4,6] gave crystalline 1-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranosyl]thymine (**8**) in 80% yield. This compound was deacylated with methanolic ammonia at room temperature, to give amorphous 1-(2-amino-2-deoxy-β-D-galactopyranosyl)thymine (**9**) in 83% yield. The anomeric assignments for **8** and **9** were also made on the basis of n.m.r. spectroscopy. The n.m.r. spectrum of **9** (measured in deuterium oxide) showed a distinct doublet at τ 4.48, having a coupling constant of 9.4 Hz. The magnitude of this coupling constant is indicative[7,8] of a *trans*-diaxial relationship between H-1 and H-2 of the glycosyl group, and this showed that **9** was the β-D anomer.

The use of *N*-acetyl as the protecting group was also investigated for the synthesis of **9**. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride[9]



(**10**) was condensed with bis(trimethylsilyl)thymine[4,5] by the fusion technique[4,6], to give crystalline 1-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-thymine (**11**). However, the yield obtained was only 40%, which is substantially lower than that (80%) obtained by the condensation of **5** with bis(trimethylsilyl)-thymine. Acetylation of 1-(2-amino-2-deoxy-β-D-galactopyranosyl)thymine with acetic anhydride–pyridine also yielded **11**.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover apparatus. Specific rotations were determined with a 2-dm polarimeter tube. Infrared spectra were recorded with a Perkin–Elmer Infracord spectrometer. Ultraviolet spectra were recorded with a Bausch and Lomb Spectronic 505 spectrometer. N.m.r. spectra were recorded by Dr. T. Radford with a Varian A-60 n.m.r. spectrometer. X-Ray powder diffraction data give interplanar spacings in Ångstroms for CuKα radiation. Relative intensities were estimated visually: m, moderate; s, strong; v, very; w, weak. The stronger lines are numbered (1, strongest); multiple numbers

indicate approximately equal intensities. T.l.c. was performed with Desaga equipment by use of Silica Gel G (E. Merck, Darmstadt, Germany), activated at 110°. Indication was effected by sulfuric acid; unless otherwise noted, proportions for developers are given by volume. Evaporations were performed under diminished pressure. Microanalyses were made by W. N. Rond.

*1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylidene)amino-β-*D*-galactopyranose* (**2**). — 2-Deoxy-2-(*p*-methoxybenzylidene)amino-D-galactose[3] (**1**, 2.3 g) was dissolved, by addition in small portions, in a stirred mixture of acetic anhydride (10 ml) and pyridine (12 ml) kept below 25° by cooling in an ice bath. The mixture was kept for 24 h at room temperature, and then poured into ice and water (100 ml). The crystalline product which formed was collected, and recrystallized from methanol; yield 2.6 g (70%), m.p. 174–176°, $[\alpha]_D^{21}$ +62.5° (*c* 1.9, chloroform); λ_{max}^{KBr} 5.75 (*O*-acetyl carbonyl), 6.1, 6.2, 6.6 (C=N, aryl C=C), 8.0–8.2 (ester), 6.85, 7.3, 7.6, 8.5, 9.4, 9.7, 10.5, 11.1, and 11.9 μm; X-ray powder diffraction data: 9.41 s, 8.27 vs (1), 6.97 s, 5.95 s, 5.64 s, 5.25 s, 4.77 vs (2), 4.40 s, 3.99 vs (3), 3.62 s, 3.39 s, 3.23 s, 3.13 s, and 2.99 s.

Anal. Calc. for $C_{22}H_{27}NO_{10}$: C, 56.76; H, 5.81; N, 3.01. Found: C, 56.70; H, 5.88; N, 3.31.

*1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-β-*D*-galactopyranose hydrochloride* (**3**). — Compound **2** (2.47 g) was dissolved in acetone (25 ml), the solution was heated to boiling, and hydrochloric acid (5M, 1 ml) was quickly added to the boiling solution, with stirring. The crystalline product that formed was filtered off, and washed with ether; yield 1.70 g (80%); m.p. 231–234° (dec.), $[\alpha]_D^{19}$ +32° (*c* 2.9, water); λ_{max}^{KBr} 3.2–3.4 (NH_3^+), 5.7 (*O*-acetyl carbonyl), 8.1–8.3 (ester), 6.6, 7.3, 8.0, 9.3, 10.3, 11.0, and 11.7 μm; X-ray powder diffraction data: 10.40 vs (1), 7.50 s, 5.37 vs (2), 4.98 s, 4.37 s, 3.88 s, 3.49 s, 3.23 vs (3), 3.01 m, 2.79 m, 2.65 m, and 2.21 m.

Anal. Calc. for $C_{14}H_{22}ClNO_9$: C, 43.86; H, 5.74; Cl, 9.83; N, 3.65. Found: C, 43.87; H, 5.95; Cl, 10.07; N, 3.35.

*Preparation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-*D*-galactopyranose*[1] (**4**). — Compound **3** (1.5 g) was suspended in dichloromethane (30 ml), pyridine (1.5 ml) added, with stirring, and trifluoroacetic anhydride (1.5 ml) was added to the stirred mixture. After being stirred for an additional 30 min, the solution was washed with water, dried (sodium sulfate), and evaporated to a syrup. Crystallization of this syrup from ether–hexane gave a white, crystalline material; yield 1.5 g (80%); m.p. and m.m.p. with authentic material[1] 131–132°; the X-ray powder diffraction data were identical with those for the compound previously prepared in this laboratory[1].

*1-[3,4,6-Tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-*D*-galactopyranosyl]-cytosine* (**6**). — Compound **5** (2.0 g), prepared from **4** by treatment with hydrogen bromide in acetic acid according to a published procedure[1], was dissolved in benzene (15 ml), and bis(trimethylsilyl)cytosine[4,5] was added. After thorough mixing, the solvent was evaporated, and the resulting syrup was heated under diminished pressure (water aspirator) for 20 min at 130–140°. The product was cooled to room tempera-

ture, added to 80% aqueous ethanol (100 ml) containing sodium hydrogen carbonate (1.0 g), and the mixture heated at 60°, with stirring. The solvent was evaporated, the residue was extracted with chloroform (160 ml), and the extract was dried (sodium sulfate), concentrated to 15 ml, and ether (100 ml) added. The resulting precipitate was filtered off, and crystallized from moist methanol–isopropyl ether, to give the crystalline monohydrate, yield 1.63 g (74%); m.p. 168–172° (with softening above 155°); X-ray powder diffraction data: 13.76 vs (1), 9.99 m, 8.61 vs (3), 7.69 m, 7.04 m, 6.28 vs (2), 5.77 m, 4.96 m, 4.67 s, 4.44 s, 4.26 vw, 4.10 s, 3.91 s, 3.74 s, 3.55 w, 3.42 s, 3.21 w, 3.02 w, 2.80 w, 2.71 vw, 2.60 vw, 2.49 vw, and 2.37 w.

Anal. Calc. for $C_{18}H_{21}F_3N_4O_9 \cdot H_2O$: C, 42.19; H, 4.49; N, 10.93. Found: C, 41.95; H, 4.69; N, 10.62.

Drying of this compound for 24 h in a desiccator under vacuum (water aspirator) gave the anhydrous form as a white, amorphous** powder; m.p. 168–172° (with softening above 155°), $[\alpha]_D^{22}$ +24 ±1° (*c* 1.3, chloroform); λ_{max}^{KBr} 3.1 (NH, NH_2), 5.7–5.8 (*O*-acetyl and *N*-trifluoroacetyl carbonyl), 6.05, 6.44, 6.6, 6.7 (cytosine, NH), 8.1–8.3 (ester), 8.65 (CF), 7.32, 8.95, 9.25, 9.55, 10.54, 10.8, 11.6, 12.7, and 13.8 μm; λ_{max}^{EtOH} 206 (ε 17,400), 245 (ε 8,840), and 270 nm (shoulder, ε 8,100).

Anal. Calc. for $C_{18}H_{21}F_3N_4O_9$: C, 43.73; H, 4.28; N, 11.33. Found: C, 43.80; H, 4.37; N, 11.14.

This compound was homogeneous by t.l.c. with 3:1 acetone–chloroform or 10:1 ethyl acetate–methanol as the developer.

Examination of the mother liquors from the crystallization of **6**, by t.l.c. with 3:1 acetone–chloroform or 10:1 ethyl acetate–methanol as the developer, gave no evidence of a second anomer.

1-(2-Amino-2-deoxy-β-D-galactopyranosyl)cytosine (**7**). — Compound **6** (0.24 g) was dissolved in 40 ml of methanol presaturated at 0° with ammonia, and the solution was kept for 24 h at room temperature, concentrated to ~5 ml, and ether (50 ml) added. The white, flocculent precipitate was filtered off, and crystallized from methanol; yield 0.12 g (91%); m.p. 233–237° (with softening above 170° and swelling above 180°), $[\alpha]_D^{20}$ +67 ±1.5° (*c* 1.5, water); λ_{max}^{KBr} 2.9–3.1 (OH, NH_2), 6.05, 6.2, 6.6, 6.75 (cytosine), 7.3, 7.8, 8.35, 9.3, 11.3, and 12.83 μm; $\lambda_{max}^{H_2O}$ 203 (ε 15,000), 237 (ε 7,750), and 270 nm (ε 8,300); $\lambda_{max}^{0.1M\ HCl}$ 212 (ε9,100) and 276 nm (12,100); n.m.r. spectrum (deuterium oxide): τ 6.0–6.82 (sugar-ring protons), 5.30 (solvent), 4.42 (distinct doublet, $J_{1,2}$ 9.4 Hz, H-1′), 4.93, (doublet, *J* 7.5 Hz, H-5), and 2.29 (doublet, *J* 7.5 Hz, H-6); X-ray powder diffraction data: 10.46 m, 6.84 s (1), 5.95 w, 5.52 s (2), 5.22 w, 5.02 s, 4.70 s, 4.24 s, 3.83 s (3), 3.60 m, 3.47 s (3), 3.33 m, 3.14 vw, 2.98 w, 2.86 s, 2.76 m, 2.64 m, 2.51 w, 2.36 w, 2.22 vw, 2.15 vw, 2.08 vw, and 1.93 w.

Anal. Calc. for $C_{10}H_{16}N_4O_5$: C, 44.11; H, 5.92; N, 20.58. Found: C, 44.38; H, 6.13; N, 20.61.

This compound was homogeneous by t.l.c. with methanol as the developer.

1-[3,4,6-Tri-O-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranosyl]-

**It was concluded that this compound was amorphous because it gave a blank, or foggy, X-ray powder diffraction pattern.

thymine (**8**). — Compound **5** (2.5 g) was dissolved in chloroform (20 ml), and bis-(trimethylsilyl)thymine[4,5] (5.0 g) was added. After thorough mixing, the solvent was evaporated, and the residue was heated for 20 min at 120–130°. After being cooled to room temperature, the product was poured into methanol (100 ml), and the mixture was heated for 15 min at 60°, with stirring. The solvent was evaporated, and the residue was extracted with chloroform (200 ml). The extract was washed with water, dried (sodium sulfate), and evaporated to a pale-yellow glass which was crystallized from chloroform–isopropyl ether by slow evaporation, to give a white, crystalline material; yield 2.2 g (80%), m.p. 174–179° (with softening above 160°), $[\alpha]_D^{21}$ +40 ±1° (*c* 3.0, chloroform); λ_{max}^{KBr} 3.1 (NH), 5.7–5.9 (*O*-acetyl and *N*-trifluoroacetyl carbonyl), 5.9, 6.42, 6.9 (NH, thymine), 8.1–8.3 (ester), 8.65 (CF), 7.3, 9.25, 9.55, 10.66, 11.2, 12.7, and 13.68 μm; λ_{max}^{EtOH} 212 (ε 10,300) and 262 nm (ε 9,100); X-ray powder diffraction data: 8.76 s (1), 8.47 m, 7.76 m, 6.94 m, 6.15 w, 5.63 m, 4.98 vw, 4.80 m, 4.48 w, 4.33 s, 4.20 s (2), 3.83 s (3), 3.76 w, 3.11 vw, and 2.84 vw.

This compound was homogeneous by t.l.c. with 3:2 chloroform–acetone or 2:1 ethyl acetate–benzene as the developer.

Examination of the mother liquors from the crystallization of **8**, by t.l.c. with 3:2 chloroform–acetone or 2:1 ethyl acetate–benzene as the developer, gave no evidence of a second anomer.

*1-(2-Amino-2-deoxy-β-*D*-galactopyranosyl)thymine* (**9**). — Compound **8** (1.0 g) was deacylated with 120 ml of methanol presaturated at 0° with ammonia, by the procedure described for the preparation of **7**. Attempted crystallization of the product from methanol–ether gave a white, amorphous material; yield 0.47 g (83%); m.p. indefinite between 110 and 190° (dec. 243–245°); $[\alpha]_D^{20}$ +49 ±2° (*c* 1.5, water); λ_{max}^{KBr} 2.9–3.0 (OH, NH_2), 5.9, 6.8 (thymine), 7.3, 7.82, 9.2, 11.3, 12.8, and 13.85 μm; $\lambda_{max}^{H_2O}$ 209 (ε 10,500) and 266 nm (ε 8,870); n.m.r. spectrum (deuterium oxide): τ 8.10 (doublet, *J* 1.5 Hz, thymine CH_3), 5.98–6.98 (sugar-ring protons), 5.3 (solvent), 4.48 (distinct doublet, $J_{1,2}$ 9.4 Hz, H-1'), and 2.35 (multiplet, *J* 1.5 Hz, H-6).

Anal. Calc. for $C_{11}H_{17}N_3O_6$: C, 45.99; H, 5.96; N, 14.63. Found: C, 45.73; H, 5.78; N, 14.88.

*1-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-galactopyranosyl)thymine* (**11**). — *Method A.* 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride[9] (**10**, 1.0 g) was mixed with bis(trimethylsilyl)thymine[4,5], and the mixture was fused at 130–140° under diminished pressure (water aspirator). After being cooled to room temperature, the product was added to methanol (30 ml); the mixture was stirred, and evaporated to dryness, and the residue was extracted with chloroform. The extract was washed, dried (sodium sulfate), and evaporated to a syrup that was crystallized from methanol–ether; yield 0.517 g (40%), m.p. 152–154°, $[\alpha]_D^{20}$ −45 ±1.5° (*c* 2.0, chloroform); λ_{max}^{KBr} 2.9–3.1 (NH), 5.7 (*O*-acetyl carbonyl), 5.95, 6.5, 6.8 (thymine, *N*-Ac), 8.0–8.2 (ester), 7.3, 9.2, 9.6, 10.7, and 11.7 μm; λ_{max}^{EtOH} 208 (ε 14,150) and 263 nm (ε 10,470); X-ray powder diffraction data: 13.00 m, 11.48 w, 9.03 vs (1), 6.51 vs (2), 6.11 m, 5.75 s, 5.47 m, 5.13 s, 4.85 m, 4.60 s, 4.25 m, 3.97 vs (3), 3.82 m, and 3.65 m.

Anal. Calc. for $C_{19}H_{25}N_3O_{10}$: C, 50.10; H, 5.49; N, 9.23. Found: C, 50.12; H, 5.74; N, 8.99.

Method B. Compound **9** (100 mg) was dissolved in a mixture of acetic anhydride (0.5 ml) and pyridine (1.0 ml). After 24 h at room temperature, the solution was poured into water (5 ml), and the mixture was extracted with chloroform (20 ml). The extract was washed, dried (sodium sulfate), and evaporated to a syrup that was crystallized from methanol–ether to give **11**; m.p. and m.m.p. 152–154°. The X-ray powder diffraction data were identical with those for the compound prepared by condensation of **10** with bis(trimethylsilyl)thymine.

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