Carbohydrate Research, 66 (1978) 95–101

SYNTHESIS OF 2,4-DIACETAMIDO-2,4,6-TRIDEOXY-D-GALACTOSE*

AVRAHAM LIAV, ISRAEL JACOBSON, MATITYAHU SHEINBLATT†, AND NATHAN SHARON‡
Department of Biophysics, The Weizmann Institute of Science, Rehovoth (Israel)
(Received October 31st, 1977; accepted for publication in revised form, January 15th, 1978)

ABSTRACT

Treatment of benzyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy-4-*O*-(methylsulfonyl)-α-D-glucopyranoside (**1**) with sodium azide in hexamethylphosphoric triamide gave the 4-azido-α-D-*galacto* derivative (**2**), which was converted into benzyl 2,4-diacetamido-3-*O*-benzyl-2,3,6-trideoxy-α-D-galactopyranoside (**3**) by hydrogenation and subsequent acetylation. Hydrogenolysis of **3** at atmospheric pressure afforded benzyl 2,4-diacetamido-2,4,6-trideoxy-α-D-galactopyranoside (**4**), which was acetylated to give the 3-*O*-acetyl derivative (**5**). The n.m.r. spectrum of **5** was in agreement with the assigned structure and different from that of benzyl 2,4-diacetamido-3-*O*-acetyl-α-D-glucopyranoside (**9**), which was prepared from the known benzyl 2,4-diacetamido-3-*O*-benzyl-2,4,6-trideoxy-α-D-glucopyranoside. Catalytic hydrogenolysis of **4** gave 2,4-diacetamido-2,4,6-trideoxy-D-galactose (**6**).

INTRODUCTION

The isolation, in 1959, of a 2,4-diamino sugar[1,2] from a polysaccharide[3] of *Bacillus licheniformis* (*Bacillus subtilis*) ATCC 9945 served as the starting point of our extensive investigations on this class of unusual compounds. The diamino sugar, subsequently named bacillosamine, was identified as 2,4-diamino-2,4,6-trideoxy-D-glucose[4], and its 2,4-diacetamido derivative was synthesized[5,6]. We have also synthesized several other diamino sugar derivatives, including 2,4-diacetamido-2,4,6-trideoxy-L-altrose, -L-idose, and -L-talose[7], as well as 3,4-diacetamido-3,4,6 trideoxy-L-glucose[8].

Very recently, 2,4-diamino-2,4,6-trideoxy-D-glucose has been identified by Wilkinson[9] as a component of the lipopolysaccharide from *Pseudomonas aeruginosa* NCTC 8505. A closely related, perhaps identical, amino sugar had previously been found in the C substance of *Pneumococcus*[10].

*Dedicated to Dr. Allene Jeanes on the occasion of her retirement.
†Katzir Fellow, on leave of absence from the Department of Chemistry, Tel Aviv University.
‡Established Investigator of the Chief Scientist's Bureau, Israel Ministry of Health.

In this report we describe the synthesis of 2,4-diacetamido-2,4,6-trideoxy-D-galactose from benzyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy-4-*O*-(methylsulfonyl)-α-D-glucopyranoside[5,6], which served as the key intermediate in our synthesis of 2,4-diacetamido-2,4,6-trideoxy-D-glucose.

Recently, the synthesis and conformational analysis of methyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-α-D-idopyranoside have been reported by Paulsen and Koebernick[11]. It was therefore of interest to study the n.m.r. spectrum of benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-α-D-galactopyranoside and of the corresponding 2,4-diacetamido-3-*O*-acetyl-α-D-*gluco* derivative, which has also been synthesized in the course of this work.

The only other 2,4-diamino sugars that have been synthesized are 2,4-diamino-2,4-dideoxy-D-galactose and -D-glucose[12].

RESULTS AND DISCUSSION

Treatment of benzyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy-4-*O*-(methylsulfonyl)-α-D-glucopyranoside[6] (**1**) with sodium azide in hexamethylphosphoric triamide gave the 4-azido-α-D-*galacto* derivative (**2**) in good yield. Hydrogenation of **2** under mild conditions, followed by acetylation, gave the corresponding diacetamido derivative **3**. Selective removal of the 3-benzyl group was achieved by catalytic hydrogenolysis and benzyl 2,4-diacetamido-2,4,6-trideoxy-α-D-galactopyranoside (**4**) was obtained in satisfactory yield. Acetylation of **4** by acetic anhydride and pyridine afforded the 3-*O*-acetyl derivative (**5**).

In a previous study[6], we described the synthesis of benzyl 2,4-diacetamido-3-*O*-benzyl-2,4,6-trideoxy-α-D-glucopyranoside (**7**). It was of interest to compare the n.m.r. spectrum of the *galacto* derivative **5** just described with that of the *gluco* analog. Compound **7** was therefore selectively hydrogenolyzed to give benzyl 2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranoside (**8**), and the latter was converted into the 3-*O*-acetyl derivative **9**. The partial ^{1}H-n.m.r. spectra of **5** and **9** in chloroform *d* solution are shown in Figs. 1a and 1b, respectively. Assignment of the spectra was accomplished by successive double-irradiation (homonuclear decoupling). An example of decoupled spectra (compound **5**) is illustrated in Fig. 2. In this instance, considerable overlapping of the signals of H-2, H-4, and the higher-field AB doublet of the benzylic methylene protons occurs (as shown in Fig. 2A). Nevertheless, it was possible by this technique to identify the foregoing spectral lines unequivocally. Irradiation of H-3 (Fig. 2B) quite considerably decreases the multiplicity of the spectrum, leaving for H-2 the multiplicity arising from H-1 and the adjacent NH proton, and for H-4 its interactions with H-5 and the adjacent NH proton. In Fig. 2C, the effect of irradiation of H-1 is shown. The results of the irradiation of the high-field and low-field portions of the NH signals are illustrated in Figs. 2D and 2E, respectively. The n.m.r. data for the compounds examined are summarized in Tables I and II. These data show that the two 2,4-diacetamido-3-*O*-acetyl derivatives **5** and **9** exist mainly in the $^4C_1$ (D) conformation in solution, in contrast to the analogous 2,4-

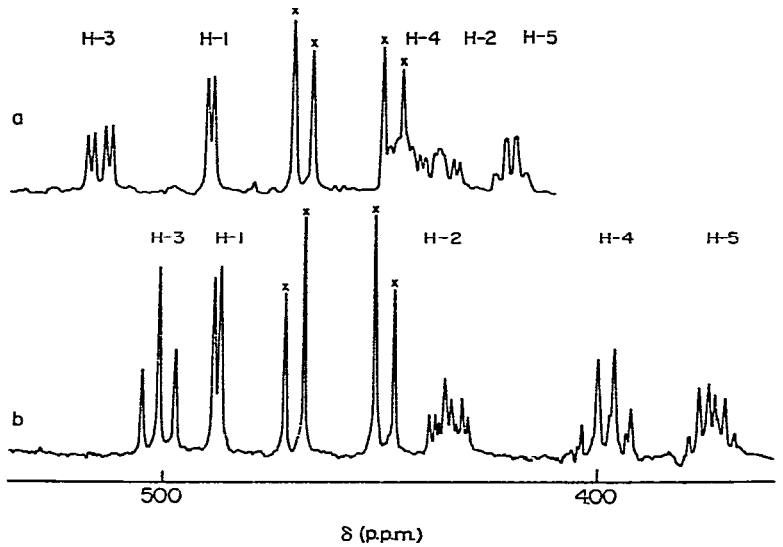


Fig. 1. (a) Partial ^{1}H-n.m.r. spectrum of benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-α-D-galactopyranoside (**5**); (b) the spectrum of the analogous α-D-*gluco* derivative (**9**). (The spectra were recorded at 270 MHz in chloroform-*d*. The symbol X indicates the AB pair of doublets of the benzylic CH_2 protons.)

TABLE I

CHEMICAL-SHIFT DATA[a]

Proton	**2**	**3**	**4**	**5**	**9**
H-1	4.81	4.91	4.92	4.89	4.88
H-2	4.43	4.20	4.09	4.36	4.34
H-3	3.67	3.63	3.97	5.14	5.01
H-4	3.70	4.06	4.32	4.48	4.16
H-5	3.86	4.06	4.16	4.19	3.74
NH(C-2)	5.28	5.43	5.78	5.80	5.56
NH(C-4)		6.13	6.03	6.26	5.28
CH_2(C-1)	4.68–4.64	4.79–4.75	4.70–4.65	4.69–4.65	4.72–4.68
	4.45–4.41	4.37–4.33	4.50–4.45	4.48–4.44	4.51–4.47
CH_2(C-3)	4.57–4.53	4.65–4.60			
	4.38–4.34	4.46–4.41			
OAc				2.08	2.01
NAc	1.80	2.08	2.11	1.99	1.93
		1.92	1.98	1.92	1.90
CH_3(C-6)	1.18	1.14	1.14	1.11	1.22

[a]Measured in p.p.m. from internal tetramethylsilane.

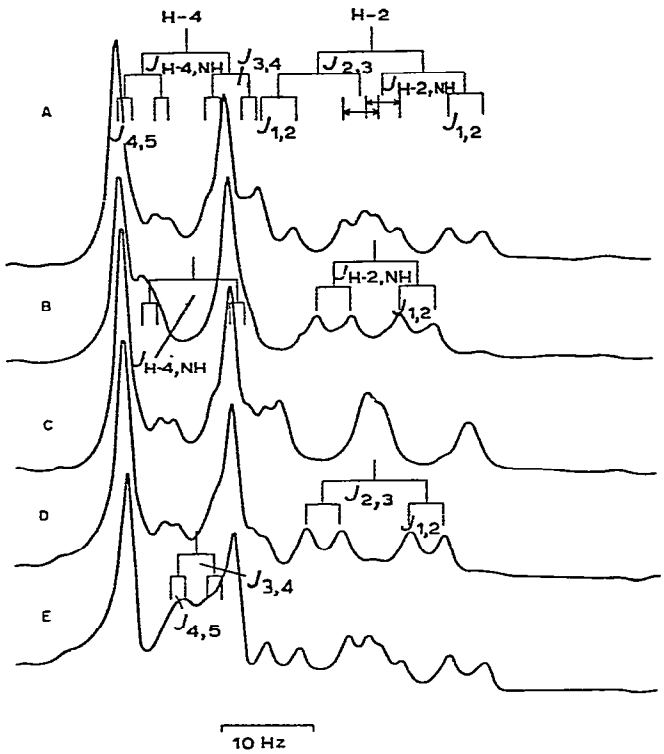


Fig. 2. The enlarged H-2 and H-4 portion of the 270-MHz, ^{1}H-n.m.r. spectrum of compound **5** (A) and the multiplicities of these signals after double irradiation (B–E).

TABLE II

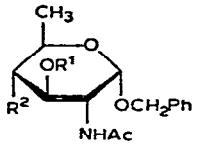
VICINAL, SPIN–SPIN COUPLING-CONSTANTS

Coupling (Hz)	2	3	4	5	9
$J_{1,2}$	3.8	3.8	3.8	3.7	3.5
$J_{2,3}$	10.2	11.2	10.8	11.5	10.4
$J_{3,4}$	3.4	4.2	3.8	4.1	10.4
$J_{4,5}$	1.2	~1.0	1.2	~0.5	10.3
$J_{5,6}$	6.5	6.2	6.4	6.3	6.2
$J_{NH,H-2}$	8.9	8.6	8.4	9.7	9.6
$J_{NH,H-4}$		10.1	8.9	9.1	9.4

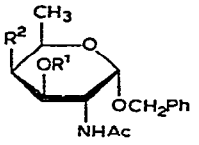
diacetamido-β-L-*ido*[7], 2,4-diacetamido-β-L-*altro*[7], and 2,4-diacetamido-α-D-*ido*[11] derivatives, where both chair conformations are present.

Removal of the benzyl group from **4** by catalytic hydrogenolysis was remarkably sluggish. When hydrogenolysis was carried out at 65 lb.in^{-2} in the presence of palladium–charcoal catalyst for several days, almost no reaction occurred. The benzyl group could be completely removed only when hydrogenolysis was performed in an

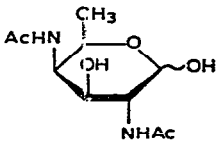
autoclave at 300 lb.in^{-2}. The free diacetamido sugar (**6**) obtained was purified by paper chromatography.



1 R^1 = CH_2Ph; R^2 = OMs
7 R^1 = CH_2Ph; R^2 = NHAc
8 R^1 = H; R^2 = NHAc
9 R^1 = Ac; R^2 = NHAc



2 R^1 = CH_2Ph; R^2 = N_3
3 R^1 = CH_2Ph; R^2 = NHAc
4 R^1 = H; R^2 = NHAc
5 R^1 = Ac; R^2 = NHAc



6

EXPERIMENTAL

General methods. — Melting points were measured in capillary tubes on a Büchi apparatus and are not corrected. Optical rotations were determined with a Perkin–Elmer 141 polarimeter. N.m.r. spectra were recorded with Brucker 90- or 270-MHz spectrometers operating in the Fourier-transform mode, with tetramethylsilane as the internal standard and chloroform-*d* as solvent. I.r. spectra were recorded with a Perkin–Elmer 237 spectrometer. Columns were prepared with silica gel (E. Merck, No. 7734) and t.l.c. was performed with silica gel-coated aluminum sheets (E. Merck). Chromatograms were sprayed with dilute sulfuric acid. Evaporations were conducted *in vacuo*. Light petroleum refers to the fraction boiling at 40–60°.

*Benzyl 2-acetamido-4-azido-3-*O*-benzyl-2,4,6-trideoxy-α-*D*-galactopyranoside* (**2**). — A suspension of benzyl 2-acetamido-3-*O*-benzyl-2,6-dideoxy-4-*O*-(methylsulfonyl)-α-D-glucopyranoside[6] (**1**, 3.5 g) and sodium azide (3.5 g) in hexamethylphosphoric triamide (140 ml) was heated for 24 h at 135° under nitrogen. The mixture was then cooled and poured into water (3 liters). The resulting precipitate was filtered off, dissolved in ethyl acetate, and washed with water. The organic layer was evaporated and the residue crystallized from chloroform–light petroleum to give the title compound (2.0 g, 65%), which migrated as a single spot in t.l.c. (R_F 0.85 in 9:1 chloroform–methanol); m.p. 204–205°, $[\alpha]_D^{22}$ +132° (*c* 0.75, chloroform).

Anal. Calc. for $C_{22}H_{26}N_4O_4$: C, 64.4; H, 6.4; N, 13.7. Found: C, 64.4; H, 6.3; N, 13.7.

*Benzyl 2,4-diacetamido-3-*O*-benzyl-2,4,6-trideoxy-α-*D*-galactopyranoside* (**3**). — A solution of **2** (1.3 g) in methanol (400 ml) was hydrogenated at atmospheric pressure in the presence of 10% palladium–charcoal catalyst (0.6 g) for 6 h. The catalyst was filtered off and washed with methanol. The solvent was evaporated to give a solid that was acetylated with pyridine (40 ml) and acetic anhydride (2 ml). The mixture was evaporated and the residue was extracted with ethyl acetate. The extract was treated with charcoal, the charcoal was filtered off, and the filtrate was evaporated. The resulting residue was crystallized from benzene–light petroleum to give **3** (0.95 g, 74%), which was homogeneous in t.l.c. (R_F 0.57 in 9:1 chloroform–methanol); m.p. 85–90°, $[\alpha]_D^{22}$ +182° (*c* 0.85, chloroform).

Anal. Calc. for $C_{24}H_{30}N_2O_5$: C, 67.6; H, 7.1; N, 6.6. Found: C, 67.3; H, 7.1; N, 6.6.

Benzyl 2,4-diacetamido-2,4,6-trideoxy-α-D-galactopyranoside (**4**). — A solution of **3** (4.0 g) in ethyl alcohol (1 liter) was hydrogenated in the presence of 10% palladium–charcoal catalyst (2 g) for 24 h. T.l.c. examination (9:1 chloroform–methanol) showed 2 spots: unchanged starting material and the product. The catalyst was filtered off and washed with ethyl alcohol, and the filtrate was evaporated. The residue was dissolved in ethyl alcohol (1 liter) and hydrogenated at atmospheric pressure in the presence of the palladium catalyst (2.5 g) for an additional 24 h. Removal of the catalyst and concentration of the filtrate afforded crystalline **4** (2.65 g, 84%); m.p. 230°, $[\alpha]_D^{22}$ +146° (*c* 0.65, chloroform); R_F 0.71 in 3:1 chloroform–ethanol.

Anal. Calc. for $C_{17}H_{24}N_2O_5$: C, 60.7; H, 7.2; N, 8.3. Found: C, 60.7; H, 7.2; N, 8.3.

Benzyl 2,4-diacetamido-3-O-acetyl-2,4,6-trideoxy-α-D-galactopyranoside (**5**). — A solution of **4** (250 mg in pyridine (15 ml) and acetic anhydride (2.5 ml) was heated for 24 h at 65°. The mixture was then evaporated to give a solid. Recrystallization from ether–light petroleum gave the product (150 mg, 53%), which was homogeneous in t.l.c. (2:1 acetone–methanol); m.p. 106–108°, $[\alpha]_D^{22}$ +124° (*c* 0.6, chloroform).

Anal. Calc. for $C_{19}H_{26}N_2O_6$: C, 60.3; H, 6.9; N, 7.4. Found: C, 60.3; H, 6.9; N, 7.4.

2,4-Diacetamido-2,4,6-trideoxy-D-galactose (**6**). — A solution of benzyl 2,4-diacetamido-2,4,6-trideoxy-α-D-galactopyranoside (**4**, 0.5 g) in methanol (80 ml) and water (40 ml) was hydrogenated at 65 lb.in.$^{-2}$ in the presence of 10% palladium–charcoal catalyst (0.5 g) for 2 days. T.l.c. indicated that no change in the starting material had occurred. The mixture was then transferred to an autoclave, and the hydrogenolysis was carried out at 300 lb.in.$^{-2}$ for 3 days at 40°. The catalyst was filtered off and washed with 50% aqueous methanol. The filtrate was evaporated, and the residue was dissolved in the minimal volume of methanol. Addition of ether gave a precipitate that was washed and dried, to give 0.3 g (82%) of a crude material migrating as a single spot on a paper chromatogram (4:1:1 1-butanol–ethanol–water); it failed to crystallize. A sample (90 mg) was purified by preparative, paper chromatography (Whatman No. 3) in 4:1:1 1-butanol–ethanol–water. The pure product obtained from the chromatogram was freeze-dried, and the residue was crystallized from methanol–ether to give 45 mg (41%) of the title compound: m.p. 177–178°, $[\alpha]_D^{22}$ +3° (*c* 0.6, 1:1 methanol–water, equil.).

Anal. Calc. for $C_{10}H_{18}N_2O_5 \cdot H_2O$: C, 45.4; H, 7.6; N, 10.6. Found: C, 45.8; H, 7.5; N, 10.4.

Benzyl 2,4-diacetamido-2,4,6-trideoxy-α-D-glucopyranoside (**8**). — To a solution of benzyl 2,4-diacetamido-3-*O*-benzyl-2,4,6-trideoxy-α-D-glucopyranoside[6] (**7**, 0.3 g) in ethyl alcohol (100 ml) was added 10% palladium–charcoal catalyst (0.15 g) and hydrogen was passed through the mixture for 24 h. T.l.c. (9:1 chloroform–methanol) showed that no reaction had taken place. More catalyst (0.2 g) and ethyl alcohol

(100 ml) were added, and hydrogenation was continued for an additional 24 h. T.l.c. indicated that most of the starting material had reacted to give the desired product. The catalyst was filtered off and the filtrate was evaporated. The residue was dissolved in ethyl alcohol (100 ml) and more catalyst (0.15 g) was added. Hydrogenation was continued for 24 h and the catalyst was removed. Evaporation of the filtrate gave an amorphous residue that was dissolved in 3:1 chloroform–ethanol and fractionated on a column of silica gel by using the same solvent mixture as eluant. A fast-moving component was found to be the starting material. Continued elution with 3:1 chloroform–ethanol gave fractions which, upon removal of the solvent, afforded the title product (R_F 0.57 in 3:1 chloroform–ethanol). Recrystallization from chloroform–light petroleum gave an analytical sample; yield 102 mg (42%), m.p. 222–223°, $[\alpha]_D^{22}$ +163° (*c* 0.6, methanol).

Anal. Calc. for $C_{17}H_{24}N_2O_5$: C, 60.7; H, 7.2; N, 8.3. Found: C, 60.8; H, 7.3; N, 8.2.

Benzyl 2,4-diacetamido-3-O-acetyl-2,4,6-trideoxy-α-D-glucopyranoside (**9**). — A solution of **8** (100 mg) in pyridine (6 ml) and acetic anhydride (0.2 ml) was heated for 3 days at 50°. The mixture was then evaporated and the residue, which still contained unreacted starting-material, was dissolved in 9:1 chloroform–methanol and applied to a column of silica gel. Elution with 9:1 chloroform–methanol gave fractions which, upon removal of the solvents, afforded the desired 3-*O*-acetyl derivative. The latter was recrystallized from methanol–ether to give an analytical sample (50 mg, 44%); m.p. 232°, $[\alpha]_D^{22}$ +99° (*c* 0.6, chloroform).

Anal. Calc. for $C_{19}H_{26}N_2O_6$: C, 60.3; H, 6.9; N, 7.4. Found: C, 59.9; H, 7.1; N, 7.4.

ACKNOWLEDGMENTS

This work was supported, in part, by a contribution from a friend of the Weizmann Institute of Science in Buenos Aires, Argentina. We thank Mrs. Sarah Ehrlich-Rogozinsky and Mrs. Bracha Goldner for performing the microanalyses.

REFERENCES

1 N. SHARON AND R. W. JEANLOZ, *Biochim. Biophys. Acta*, 31 (1959) 277–278.
2 N. SHARON AND R. W. JEANLOZ, *J. Biol. Chem.*, 235 (1960) 1–5.
3 N. SHARON, *Nature*, 179 (1957) 919.
4 U. ZEHAVI AND N. SHARON, *J. Biol. Chem.*, 248 (1973) 433–438.
5 A. LIAV, J. HILDESHEIM, U. ZEHAVI, AND N. SHARON. *J. Chem. Soc. Chem. Commun.*, (1973) 668–669.
6 A. LIAV, J. HILDESHEIM, U. ZEHAVI, AND N. SHARON, *Carbohydr. Res.*, 33 (1974) 217–227.
7 A. LIAV AND N. SHARON, *Carbohydr. Res.*, 30 (1973) 109–126.
8 A. LIAV AND N. SHARON, *Carbohydr. Res.*, 37 (1974) 248–251.
9 S. G. WILKINSON, *Biochem. J.*, 161 (1977) 103–109.
10 D. E. BRUNDISH AND J. BADDILEY, *Biochem. J.*, 110 (1968) 573–582.
11 H. PAULSEN AND H. KOEBERNICK, *Chem. Ber.*, 109 (1976) 90–103.
12 W. MEYER ZU RECKENDORF AND N. WASSILIADOU-MICHELI, *Chem. Ber.*, 105 (1972) 2998–3013.