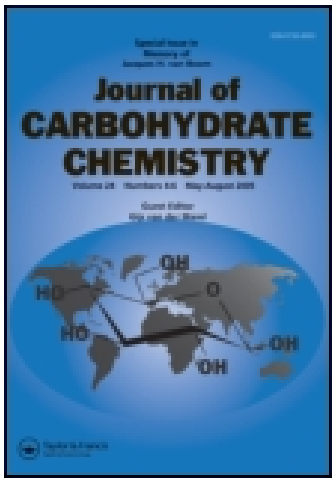

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NEW EVIDENCE FOR ISOMERISM OF THE FORMAZYL GROUP. SYNTHESIS OF SELECTIVELY PROTECTED 2-DEOXY-GALACTOSE FORMAZANS

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ABSTRACT

A new application of the formazyl activation provided selectively protected new derivatives of both 2-deoxy-D-galactose and 2-acetamido-2-deoxy-D-galactose formazans from 6-amino-6-deoxy-D-galactose 6,4-cyclic carbamate (**1**) under very simple conditions. The ^{1}H NMR spectrum of the acetylated 2-deoxy derivative **7** revealed an equilibrium between chelated and unusual non-chelated forms of the formazan moiety in solution.

INTRODUCTION

Beginning in the 1960s, several attempts have been made to elucidate the fine structure of the formazyl ring of sugar formazans.[2] Based on NMR spectra and circular dichroism studies, the formazan ring had been thought to exhibit either tautomeric or mesomeric equilibria between pseudoaromatic cyclic forms. An open phenylazo-phenylhydrazone structure of the formazan, however, was not consid-

ered. Recently, the aim of the synthesis of selectively protected 6-amino-6-deoxy-galactosamine and 6-amino-2,6-dideoxygalactose derivatives, potential building blocks of aminoglycoside antibiotics,[3] led us to recognition of the unexpected tautomeric equilibria between pseudoaromatic chelate and open phenylazo-phenylhydrazone forms in solution.

The synthesis was based on the successful exploitation of the activating effect of the formazyl group. As we reported earlier,[4] regiospecific and stereoselective replacement of the AcO-2 substituent of various acyclic aldose formazans was achieved with nucleophiles under mild conditions. Thus, we succeeded in introducing an acetamido group[5,6] or a hydrogen atom[7] onto C-2 of various aldose formazans. Subsequent decomposition of the formazyl group provides a new method for the synthesis of aldonic acid derivatives substituted at C-2.[6]

RESULTS AND DISCUSSION

Our attempts to synthesise the target compounds were initiated from 4-*O*, 6-*N*-carbonyl-6-amino-6-deoxy-α-D-galactopyranose **1**,[8] easily prepared from 6-azido-6-deoxy-D-galactose with triphenylphosphine–carbon dioxide. The corresponding red-coloured acyclic formazan **3** was obtained by a conventional procedure[9] *via* phenylhydrazone **2**, which was acetylated with Ac_2O-pyridine to its tri-*O*-acetate **4**. Compounds **2, 3** and **4** are new crystalline derivatives.

The formazan character of **3** and **4** was supported by maxima at 424 and 460 nm, respectively, in their visible spectra as well as by a characteristic singlet of the formazyl NH at δ 12.68 ppm in the ^{1}H NMR spectrum of **4** (Table 1). During the reactions the 4,6-cyclic-carbamate moiety remained intact, as was revealed by the unchanged $\nu_{C=O}$ band of the carbamoyl group at ~1670 cm^{-1} in compounds **1–4**.

Treatment of compound **4** with ammonia in aqueous ethanol afforded the new crystalline 2-acetamido derivative **5**, which was acetylated to give the di-*O*-acetate **6**. The one-pot formation of **5** can be explained by nucleophilic 1,4 elimination-addition process and subsequent O→N acetyl migration, as it was suggested in analogous cases.[5] Evidence for the structures was provided by their visible, IR and NMR spectra. It is noteworthy, that in the ^{13}C NMR spectra of acetyl derivatives **4** and **6**, C-4, one of the bridge-heads of the carbamate ring, resonated at lower field (74.61 and 75.18 ppm, respectively) than the other secondary carbon atoms (C-2,3,5) of the molecules (60–70 ppm). On the other hand, in the ^{1}H NMR spectra (Table 1) of the same compounds, the signal of H-4 appeared at higher field (~4,7 ppm) than those (5.25–6.37 ppm) of the other protons (H-2,3,5).

Reaction of **4** with sodium borohydride in dry 2-methoxyethanol was expected to give 3,5-di-*O*-acetyl-4-*O*,6-*N*-carbonyl-6-amino-2,6-dideoxy-D-*lyxo*-hexose formazan **7**, as a single new compound. The visible and IR spectra of the product were consistent with the structure **7**, however, in its ^{1}H NMR spectrum (Table 1) two sets of signals separated in a ratio of 2:1.

The most characteristic feature of the spectrum was the duplication of the formazyl N—H singlet in the low-field region. The signal of double intensity at 11.11

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Table 1. ^{1}H NMR Chemical Shifts (δ ppm) and Coupling Constants (*J*, Hz) for Compounds **4**, **5**, **6**, **7a** and **7b**

	4	**5**	**6**	**7a**	**7b**
H-2a	6.37	5.82	5.98	3.55	3.86
H-2b	—	—	—	2.99	3.05
H-3	5.64	4.42	5.44	5.47	5.21
H-4	4.76	4.31	4.67	4.54	4.30
H-5	5.27	4.39	5.25	5.29	5.54
H-6a	3.62	3.52	3.59	3.58	3.52
H-6b	3.44	3.39	3.38	3.44	3.41
NH (formazyl)	12.68	12.04	12.34	11.11	9.27
NH (carbamate)	6.39	7.42	6.68	6.13	6.08
NH (acetamido)	—	6.28	6.50	—	—
Ar-H-2′	7.54	7.76	7.55	7.54	7.83, 7.47
Ar-H-3′	7.38	7.48	7.41	7.41	7.47, 7.35
Ar-H-4′	7.21	7.28	7.23	7.22	7.42, 7.01
CH_3 (OAc)	1.90; 2.04		1.93; 2.00	1.88; 2.10	1.89; 2.12
CH_3 (NAc)	2.21	2.11	2.15	—	—
$J_{2a,2b}$	—	—	—	15.0	15.0
$J_{2a,3}$	—	—	—	3.8	6.2
$J_{2b,3}$	2.5	2.9	2.8	7.5	4.4
$J_{3,4}$	9.6	8.1	9.2	8.8	7.2
$J_{4,5}$	1.1	5.7	1.8	1.3	1.2
$J_{5,6a}$	3.8	3.2	3.5	3.4	3.3
$J_{5,6b}$	1.4	1.9	1.7	1.5	1.5
$J_{6a,6b}$	13.4	12.6	13.4	13.1	13.0
$J_{6b,NH}$	4.3	—	—	4.2	4.3
$J_{2,NH}$	—	4.0	9.7	—	—

ppm corresponds to the proton of the pseudoaromatic N—H···N chelate ring, represented by **7a**. At the same time, the singlet of lower intensity at 9.27 ppm was assigned to the N—H proton of the open phenylazo-phenylhydrazone structure **7b**.

Recently, we found a similar non-chelated formazan structure in the solid state as proved by the X-ray crystal structure of a 2-azido-2-deoxy-sugar formazan.[10] The stability of the open structure in the crystal lattice could be attributed to intermolecular N—H···O bonds between the phenylhydrazone moiety and the carbonyl oxygen of AcO-6 of other molecules.

Compound **7** has provided the first example for the equilibrium between the pseudoaromatic formazan ring and the open phenylazo-phenylhydrazone structure of an aldose formazan in solution. In this case, the formation of the open structure is, probably, allowed by the small size of the two H-atoms at C-2 and stabilized by inter- or intramolecular N—H···O bonding similar to the above mentioned case.

The new synthetic approach, with a successful combination of the phosphinimine reaction and formazyl activation, has resulted in conservation and simultaneous and selective protection of the O-4 and N-6 substituents of 6-amino-6-de-

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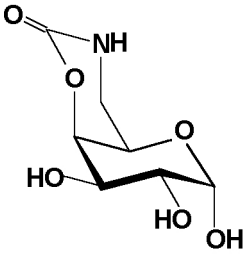

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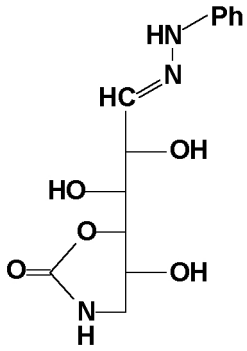

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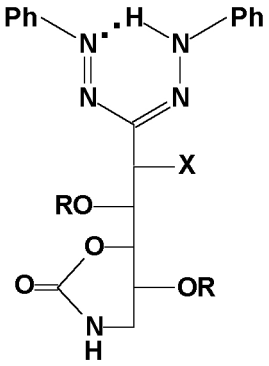
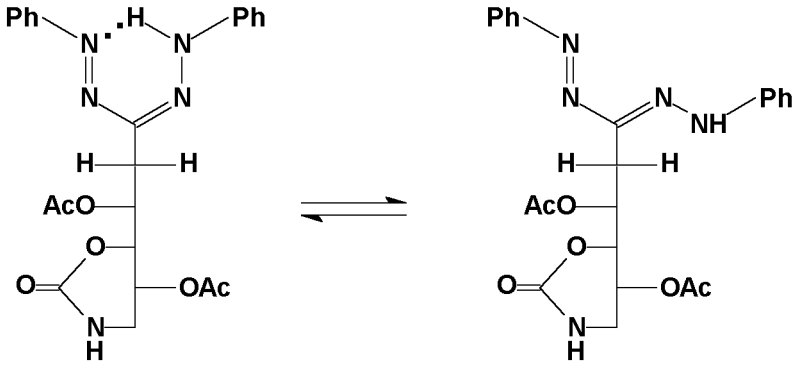


3 : R = H, X = OH
4 : R = Ac, X = OAc
5 : R = H, X = NHAc
6 : R = Ac, X = NHAc
7 : R = Ac, X = H



7a 7b

oxy-D-galactose by a cyclic carbamate. The unchanged configuration and conformation of the 6-membered carbamate cycle during all the transformations has been indicated by the ^{1}H NMR spectra (Table 1) of compounds **4–7**. The theoretically interesting cyclic-acyclic isomerism of the formazan moiety found in the case of **7** will be further investigated.

EXPERIMENTAL

General Methods. TLC was performed on Silica Gel 60 F_{254} (E. Merck) plates developed with eluents *A* (EtOAc—1,4-dioxane—AcOH 5:5:0.3); *B,* ($CHCl_3$—MeOH 9:1); *C*, (EtOAc—CH_2Cl_2 2:1). Spots were detected visually and by exposure to UV light. Column chromatography was carried out on silica

gel (E. Merck, 0.020–0.043 mesh). IR spectra (KBr) were recorded with a Nicolet 205 FT spectrometer, UV spectra with an HP 8452 A spectrometer in EtOH—H_2O (19:1) solution. NMR spectra were determined on Varian XL-100 and Varian XLAA-400 spectrometers using Me_4Si as an internal standard. Assignments were confirmed by proton-proton homocorrelated and carbon-proton heterocorrelated spectra.

4-*O*,6-*N*-Carbonyl-6-amino-6-deoxy-D-galactose Phenylhydrazone (2). To a filtered solution of 6-amino-6-deoxy-D-galactose 6,4-cyclic carbamate **1**, (1.78 g, 8.67 mmol)[8] in distilled water (26 mL) was added a filtered solution of phenylhydrazine hydrochloride (1.78 g, 8.7 mmol) and sodium acetate trihydrate (1.45 g) in distilled water (14 mL). Within a few minutes white crystals started to separate. The reaction mixture was left to stand at room temperature for 2 hours and at 0°C overnight, then filtered and washed with chilled EtOAc. The yellowish-white crude phenylhydrazone (**2**, 2.32 g, 91%), mp 209–211°C, was pure enough for further reaction. A sample (1.0 g) was crystallized twice from hot ethanol giving white needles (0.72 g), mp 212–214°C; R_f 0.29 (solvent *A*).

Anal. Calcd for $C_{13}H_{17}N_3O_5$ (295.30): C, 52.88; H, 5.80; N, 14.23. Found: C, 52.97; H, 5.84; N, 14.11.

4-*O*,6-*N*-Carbonyl-6-amino-6-deoxy-D-galactose *N′*,*N″*-Diphenylformazan (3). A solution of benzenediazonium chloride[9] was prepared from aniline (1.94 g, 20.9 mmol), 1:1 concd hydrochloric acid–water (10.6 mL) and sodium nitrite (1.44 g) in water (3.4 mL), and added dropwise to a solution of **2** (4.8 g, 16.2 mmol) in a mixture of pyridine (77 mL), ethanol (58 mL) and water (19 mL) at −5°C. The red mixture was stirred for 30 min at 0°C and for 30 min at room temperature, then was poured into ice–water (1L) to give a red syrup. On trituration with ice–water and on standing at 0°C a red solid was formed (5.45 g, 84%), mp 184–186°C, which was pure enough for the preparation of compound **4**. Crystallization of a sample of crude **3** (0.31 g) from 2-propanol resulted in red needles (0.21 g), mp 186–187°C; R_f 0.55 (solvent *A*); λ_{max} 424 nm; ν_{max} 3600–3200 (OH, NH), 1670 (CO) cm^{-1}.

Anal. Calcd for $C_{19}H_{21}N_5O_5$ (399.42): C, 57.14; H, 5.30; N, 17.54. Found: C, 57.29; H, 5.42; N, 17.77.

2,3,5-Tri-*O*-acetyl-4-*O*,6-*N*-carbonyl-6-amino-6-deoxy-D-galactose *N′*,*N″*-Diphenylformazan (4). Compound **3** (1.2 g, 3.0 mmol) was acetylated with a mixture of pyridine (6 mL) and acetic anhydride (4 mL) at 0°C for 2 days, then was poured into ice–water (200 mL), to give a red solid; (1.42 g, 90%), mp 167–170°C. Recrystallization from 2-propanol by precipitation with water gave red needles of **4**, (0.99 g, 63%), mp 172–173°C; R_f 0.67 (solvent *B*); λ_{max} 460 nm, ν_{max} 3500–3300 (NH), 1760 (AcO), 1670 (carbamate CO), 1220 (ester COC) cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ170.35, 169.65, 169.32 (CH_3CO); 152.68 (NH*C*O); 147.62 (Ar—C-1′); 139.91 (C-1); 129.35 (Ar—C-3′); 127.35 (Ar—C-4′); 118.69 (Ar—C-2′); 74.61 (C-4); 69.42 (C-2); 68.56 (C-3); 44.54 (C-6); 20.83, 20.52, 20.08 (CH_3CO).

Anal. Calcd for $C_{25}H_{27}N_5O_8$ (525,53): C, 57.14; H, 5.18; N, 13.33. Found: C, 57.39; H, 5.17; N, 13.48.

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2-*N*-Acetyl-4-*O*,6-*N*-carbonyl-2,6-diamino-2,6-dideoxy-D-galactose *N′,N′*-Diphenylformazan (5). Compound **4** (3.0 g, 5.7 mmol) was allowed to stand with a mixture of EtOH (30 mL) and 25% aqueous ammonia solution (30 mL) for 6 h at room temperature and for 24 h at 0°C, while TLC (solvent *A*) indicated complete reaction. The separated solid was filtered, washed with chilled EtOH–H_2O (1:1) to give red needles (1.57 g), mp 187–190°C. Concentration of the mother liquor (bath temperature below 30°C) resulted in a second crop of crystals (0.44 g, total yield 80%). Recrystallization was made by dissolving the solid in hot EtOH—H_2O (9:1, 15 mL) and adding water (1.5 mL) till turbidity. On cooling, red crystals (1.56 g, 62%) separated, mp 193–194°C; R_f 0.23 (solvent *A*); λ_{max} 464 nm; ν_{max} 3600–3200 (OH, NH), 1685–1615 (carbamate CO, amide CO) cm^{-1}.

Anal. Calcd for $C_{21}H_{24}N_6O_5$ (440.47): C, 57.26; H, 5.49; N, 19.08. Found: C, 57.18; H, 5.56; N, 19.01.

2-*N*-Acetyl-3,5-di-*O*-acetyl-4-*O*,6-*N*-carbonyl-2,6-diamino-2,6-dideoxy-D-galactose *N′,N″*-Diphenylformazan (6). Compound **5** (0.25 g, 0.57 mmol) was acetylated with a mixture of pyridine (2 mL) and Ac_2O (1.5 mL) for 2 days at 0°C. Conventional work-up afforded a red solid (263 mg, 86%), mp 206–208°C, which was purified by column chromatography with a solvent mixture CH_2Cl_2—MeOH (98:2, later 9:1). After concentration, the syrup was dissolved in CH_2Cl_2 and precipitated with CCl_4, to give red crystals (206 mg, 67%), mp 218–219°C; R_f 0.35 (solvent *B*); λ_{max} 462 nm; ν_{max} 3550–3200 (NH), 1747 (AcO), 1717, 1659 (carbamate CO, amide CO) cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ170.36, 170.15, 169.15 (CH_3CO); 152.60 (NH*C*O); 147.44 (Ar—C-1′); 142.07 (C-1); 129.39 (Ar—C-3′); 127.37 (Ar—C-4′); 118.63 (Ar—C-2′); 75.18 (C-4); 70.16 (C-3); 60.66 (C-5); 48.33 (C-2); 44.40 (C-6); 23.53, 20.77, 20.50 (CH_3CO).

Anal. Calcd for $C_{25}H_{28}N_6O_7$ (524,55): C, 57.24; H, 5.38; N, 16.02. Found: C, 57.11; H, 5.46; N, 16.15.

3,5-Di-*O*-acetyl-4-*O*,6-*N*-carbonyl-6-amino-2,6-dideoxy-D-*lyxo*-hexose *N′,N″*-Diphenylformazan (7). A solution of **4** (0.52 g, 1.0 mmol) in dry 2-methoxyethanol (14 mL) was cooled to 0°C, then sodium borohydride (1.2 g, 32 mmol) dissolved in 2-methoxyethanol (14 mL) was added dropwise. The mixture was allowed to stand for 5 h at the same temperature when TLC (solvent *C*) revealed the complete transformation of the starting formazan. After adding AcOH (2 mL) the mixture was poured into ice-water (150 mL), and the red precipitate was filtered to give the crude product (0.40 g). Separation by short-column chromatography with 2:1 EtOAc—CH_2Cl_2 mixture furnished pure, red solid, (0.21 g, 45%), R_f 0.51 (solvent *C*). On the basis of its NMR spectra the product is a 2:1 mixture of **7a** and **7b** in $CDCl_3$ solution at room temperature (see Table 1). ^{13}C NMR (100 MHz, $CDCl_3$): for **7a**: δ170.33, 169.54, (CH_3CO); 152.77 (NH*C*O); 147.92 (Ar—C-1′); 144.23 (C-1); 129.37 (Ar—C-3′); 126.78 (Ar—C-4′); 118.50 (Ar—C-2′); 77.42 (C-4); 68.08 (C-3); 60.77 (C-5); 30.65 (C-2); 44.77 (C-6); 20.82, 20.83, (CH_3CO); for **7b**: δ170.21, 170.04, (CH_3CO); 152.21 (NH*C*O); 151.69 and 151.99 (Ar—C-1′); 143.01 (C-1); 129.17 and 129.42 (Ar—C-3′); 130.71 and 122.52 (Ar—C-4′); 122.81 and 114.60 (Ar—C-2′); 76.87 (C-4); 68.53 (C-3); 60.33 (C-5); 21.32 (C-2); 44.95 (C-6); 20.89, 20.88, (CH_3CO).

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Anal. Calcd for $C_{23}H_{25}N_5O_6$ (467.49): C, 59.35; H, 5.41; N, 18.06. Found: C, 59.62; H, 5.49; N, 17.98.

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