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STRUCTURE AND ANOMERIC CONFIGURATION OF THE 3,6-ANHYDRO-OSAZONE DERIVATIVES OBTAINED FROM D-*lyxo*-HEXULOSE PHENYLOSAZONE AND D-*xylo*-HEXULOSE PHENYLOSAZONE*

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ABSTRACT

Dehydration of D-*lyxo*-hexulose phenylosazone or D-*xylo*-hexulose phenylosazone afforded 3,6-anhydro-D-*lyxo*-hexulose phenylosazone (**2**) as the preponderant isomer from both. The identity of **2** was obtained by acetylation, and comparison of the products. Acetylation with acetic anhydride–pyridine afforded the same di-*O*-acetyl derivative, and further acetylation afforded the same *N*-acetyldi-*O*-acetyl derivative (**5**). Refluxing **2** with copper sulfate afforded a *C*-nucleoside analog, namely, 2-phenyl-4-α-D-threofuranosyl-1,2,3-osotriazole (**6**). The anomeric configuration of **2**, **5**, and **6** was determined by n.m.r. spectroscopy. The mechanism of the dehydrative cyclization-process, and the conformational stability of **2**, are discussed.

INTRODUCTION

The dehydrative cyclization of monosaccharide phenylosazones with methanolic sulfuric acid solution[2–4] provides a simple method for the preparation of *C*-aldofuranosyl derivatives useful for the synthesis of *C*-nucleoside analogs. The main problem militating against the extensive use of this reaction in the field of *C*-nucleosides has been the anomeric configuration of the products. Circular dichroic, spectral studies[5] have been used for this purpose. Recently[1], extensive studies on the anomeric configuration, using both circular dichroism and high-resolution, n.m.r. spectroscopy, showed conflicting results in some cases, and this was attributed to the mutarotation exhibited by the chelated ring-structure of osazones.

It became evident that, for correct assignment of the anomeric configuration, chiroptical properties should be supported by n.m.r.-spectral results. High-resolution, n.m.r.-spectral studies on a series of 3,6-anhydro-osazone derivatives of hexoses[6] and heptoses[7] showed that n.m.r. spectroscopy provides a reliable means for identifying the anomeric configuration. In the present work, dehydration of D-*lyxo*-hexulose phenylosazone (D-galactose phenylosazone) and D-*xylo*-hexulose phenylosazone

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*Studies on Anhydro-osazones, Part II. For Part I, see ref. 1.
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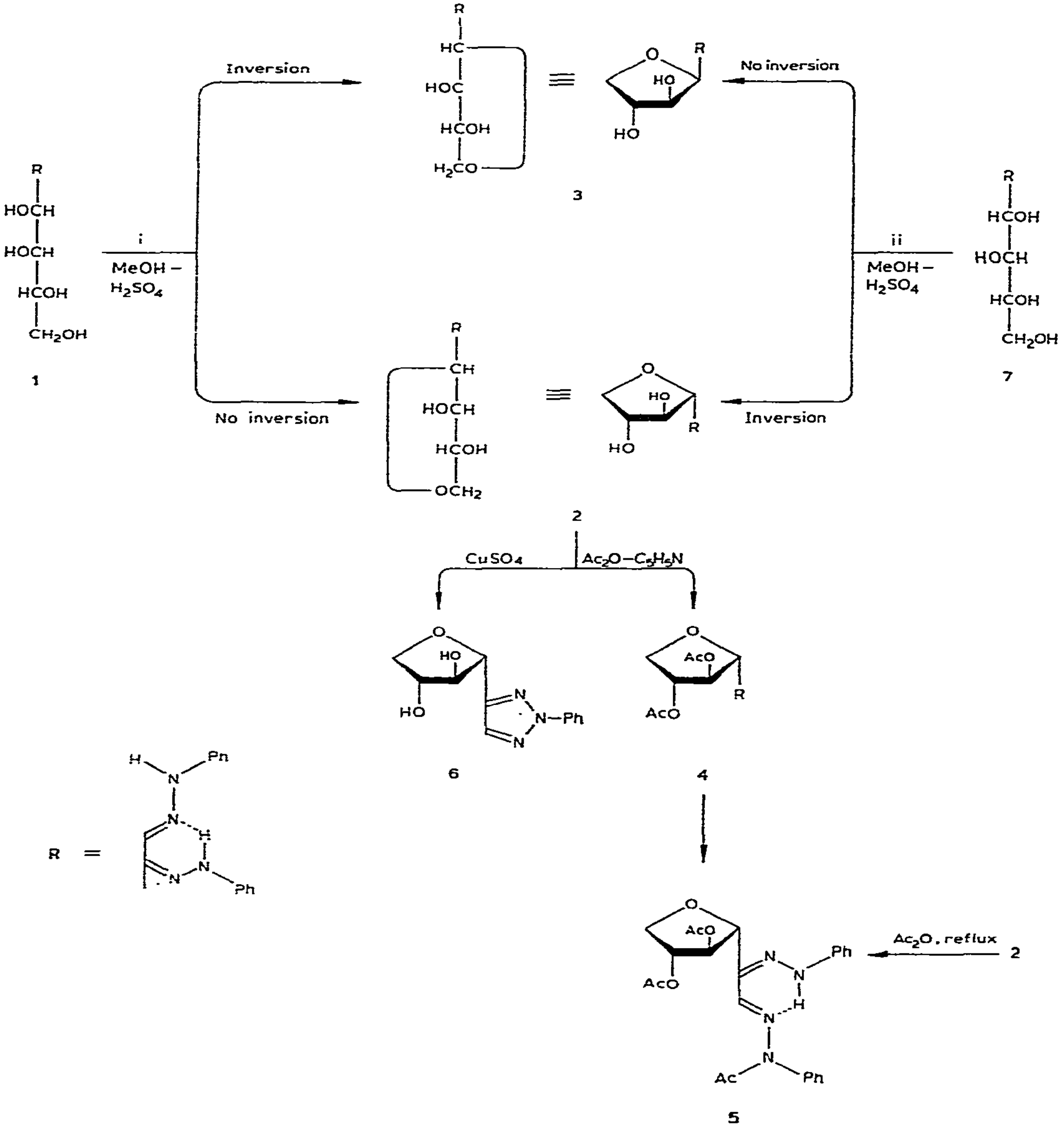
(D-gulose phenylosazone) with methanolic sulfuric acid was studied, and the anomeric configuration of the product was ascertained by n.m.r. spectroscopy.

DISCUSSION

Dehydration of D-*lyxo*-hexulose phenylosazone (**1**) by refluxing with methanolic sulfuric acid solution[2-4] (and monitoring of the reaction by t.l.c.) afforded 3,6-anhydro-D-*lyxo*-hexulose phenylosazone (**2**) as the preponderant product. Compound



2 is formed from **1** without inversion in the configuration of C-3 (C-1 of the aldosyl group formed; see Scheme 1). Its n.m.r. spectrum (80 MHz) showed the anomeric proton as a doublet at δ 5.08 ($J_{1',2'}$ 4.2 Hz). The small value of $J_{1',2'}$ observed for **2** is consistent[8] with a trans arrangement for H-1′ and H-2′, *i.e.*, the α-D-*threo* configuration of the glycosyl group. These n.m.r.-spectral results are in agreement with the c.d.-spectral results[5] for compound **2**. The minor isomer **3**, which is formed from **1** with inversion in the configuration of C-3, and was detected[9] in small proportion, could also be detected, by t.l.c. using different solvent-mixtures (solvents *A*, *B*, and *C*), as a trace in the reaction products.

Acetylation of **2** with acetic anhydride–pyridine afforded the di-*O*-acetyl derivative **4**. Its infrared (i.r.) spectrum showed a band at 1730 cm^{-1} corresponding to the *O*-acetyl groups. Further acetylation of **2**, by prolonged treatment with acetic anhydride–pyridine, or by refluxing with acetic anhydride, resulted in acetylation of the nonchelated, imino proton of the C-1 hydrazone residue, with the formation of the *N*-acetyl-di-*O*-acetyl derivative **5**. Its i.r. spectrum showed bands, at 1760 and 1705 cm^{-1}, respectively attributable to the *O*-acetyl and *N*-acetyl groups. Additionally, the n.m.r. spectrum of **5** showed the two *O*-acetyl groups as two singlets, each of three-proton intensity, at δ 1.87 and 2.0, and the *N*-acetyl group as a broad singlet of three-proton intensity at δ 2.40. The anomeric proton of **5** was seen as a doublet at δ 4.38 ($J_{1',2'}$ 4.1 Hz).

On being refluxed with copper sulfate, compound **2** afforded a *C*-nucleoside derivative, namely, 2-phenyl-4-α-D-threofuranosyl-1,2,3-osotriazole (**6**). The 360-

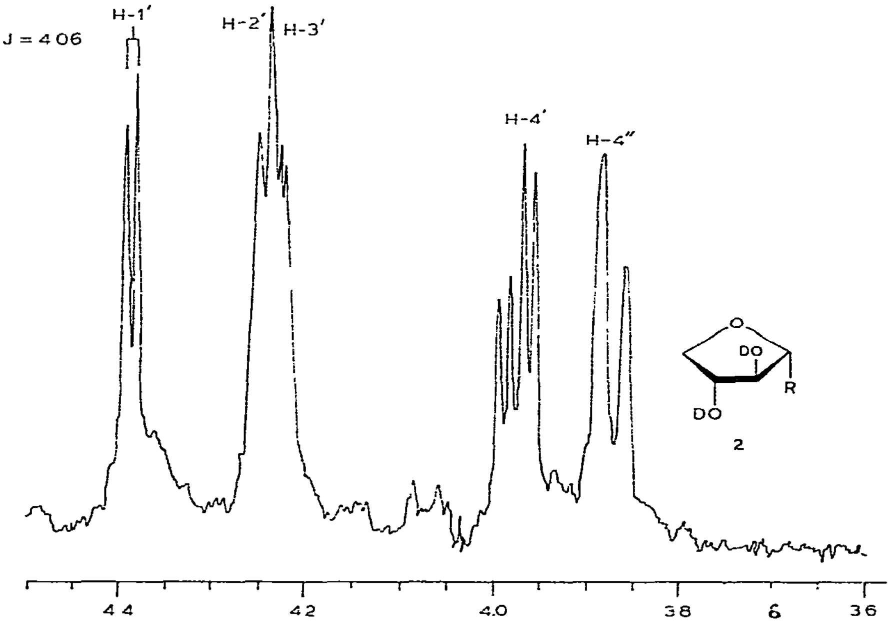


Fig. 1. N.m.r. spectrum, at 360 MHz, of 3,6-anhydro-D-*lyxo*-hexulose phenylosazone (**2**) in CD$_3$CO$_2$D (high resolution of the sugar moiety); R = bis(phenylhydrazone) residue.

MHz, high-resolution, n.m.r. spectrum of **6** showed the anomeric proton shifted to lower field, far from the glycosyl protons, as a doublet at δ 4.702 ($J_{1',2'}$ 2.87 Hz). This small value of the coupling constant supports the α-D-*threo* configuration assigned to compound **2**.

In order to obtain detailed knowledge about the steric course, and the mechanism, of the dehydration process, D-*xylo*-hexulose phenylosazone (**7**) was dehydrated with methanolic sulfuric acid solution (with monitoring of the reaction by t.l.c.), and the products obtained were compared with those from compound **1**. Dehydration of **7** afforded the same two 3,6-anhydro-osazones as had been obtained from **1**, having the same mobility in t.l.c. using different solvent mixtures (solvents *A*, *B*, and *C*), and compound **2** was also obtained as the preponderant isomer from **7**. Its 360-MHz, n.m.r. spectrum (see Fig. 1) showed the anomeric proton as a doublet at δ 4.389 ($J_{1',2'}$ 4.06 Hz), in accord[8] with the α-D-*threo* configuration assigned through Route i. Acetylation with acetic anhydride–pyridine afforded the di-*O*-acetyl derivative **4**, having the same R_F value in t.l.c., and the same melting and mixed melting point. Further acetylation afforded the same *N*-acetyldi-*O*-acetyl derivative **6**, having the same melting and mixed melting point. On being refluxed with copper sulfate (Route ii), compound **2** afforded the *C*-nucleoside osotriazole **6**, having the same mobility in t.l.c., and melting and mixed melting point.

The formation of **2** as the preponderant isomer by both Routes i and ii (without inversion from **1**, and with inversion from **7**) constitutes direct proof for the stereospecificity of the dehydration process. Formation of the 3,6-anhydro ring takes place in the sterically favored direction, with the production of the preponderant isomer having a trans relationship between the bis(hydrazone) residue and the 4-hydroxyl group. The minor isomer, having a cis relationship between the bis(hydrazone) residue and the 4-hydroxyl group, is formed by cyclization in the sterically disfavored direction. A mechanism for this process, suggested by El Khadem[10], involves racemization, and formation of the 2-(phenylazo)-2-ene intermediate.

The formation of two isomers (as major and minor products) from the two 3-epimeric saccharide phenylosazones **1** and **7** indicates that inversion took place for both compounds, and supports the stereospecificity[10] of the dehydration process. This accords with a cyclization mechanism of the SN2 type having the steric requirements suggested by El Khadem[10]. Another 3-epimeric saccharide phenylosazone[11] showed identical results. A reversible mechanism involving the formation of a carbonium-ion intermediate identical to that suggested for the dehydration of 2-(D-*arabino*-tetritol-1-yl)furans[12] is excluded, as refluxing of a solution of **2** in methanolic sulfuric acid (with monitoring of the reaction by t.l.c.) did not produce spots having the R_F value of the precursor osazones **1** or **7**. The greater stability of **2**, compared to **3**, can be accounted for by the trans,trans arrangement of the groups on C-1′, C-2′, and C-3′ of the Haworth formula for **2**.

In connection with the conformation of the aldofuranosyl moiety of **2**, it is recognized[13,14] in the field of nucleosides that the furanosyl ring is puckered in such a way as to have the carbon meta to the ring-oxygen atom (either C-2 or C-3) farthest

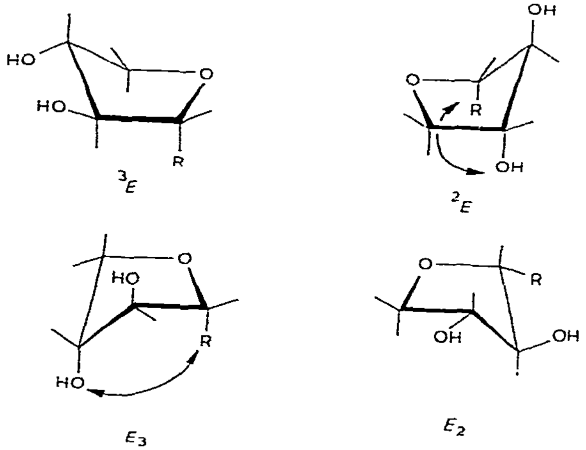


Fig. 2. The envelope conformers of the α-D-threofuranosyl moiety of 3,6-anhydro-D-*lyxo*-hexulose phenylosazone (2).

out of the mean plane of the ring, *i.e.*, ^2E, E_2, ^3E, or E_3. Accordingly, compound **2** can be represented as shown in Fig. 2. Conformers ^2E and E_3 are the less favored conformers[15], due to the presence of all groups in axial orientations, and the presence of a strong, 1,3-*syn*-axial interaction between the bulky bis(hydrazone) residue and the 3-hydroxyl group. On the other hand, conformer E_2 is relatively more stable than ^3E, due to the presence of the maximum number of groups in the stable, equatorial orientation and the absence of 1,3-*syn*-axial interaction. Accordingly, it is to be expected that compound **2** will mostly be present as the E_2 conformer. The $J_{1',2'}$ value observed can alone suffice to indicate[16] the preponderant conformer of a nucleoside. The $J_{1',2'}$ value (4.1 Hz) for **2**, which corresponds to the torsion angle $\phi_{H1',H2'} = 133°$ (as calculated from the Karplus equation[17]), is in agreement with the Dreiding model of the E_2 conformer.

EXPERIMENTAL

General. — Melting points are uncorrected; evaporations were performed under diminished pressure below 60°. Thin-layer chromatography (t.l.c.) was conducted on silica gel (Kiesel gel G, Merck), with solvent *A*, 3:1 benzene–ethanol; solvent *B*, 2:1:1 benzene–chloroform–ethanol; solvent *C*, 4:1:1 benzene–petroleum ether–ethanol, and solvent *D*, 9:1 benzene–ethanol. I.r. spectra were recorded with a Unicam SP 1025 instrument, and n.m.r. spectra with Varian FT (80 MHz) and NTC (360 MHz) instruments, using tetramethylsilane as the internal, reference standard. High-resolution mass spectra were recorded with a Dupont MS 21-492B instrument.

Combustion analyses were performed in the Department of Chemistry, Faculty of Science, Cairo University, Cairo, Egypt.

*3,6-Anhydro-*D-lyxo-*hexulose phenylosazone* (**2**). — *Method A. From* D-lyxo-*hexulose phenylosazone* (D-*galactose phenylosazone*, **1**), *by "Route i"*. Compound **1**

(8 g) was boiled for 6 h under reflux with 0.05% methanolic sulfuric acid (500 mL), the reaction being monitored by t.l.c.; after 6 h, t.l.c. (solvent *A*) revealed the absence of the starting osazone and the formation of two (a major and a faint) spots respectively having R_F (0.57. 0.49), (0.74, 0.65). and (0.63, 0.53) in solvents *A*, *B*, and *C*. The solution was then poured into hot water, and the methanol was evaporated under diminished pressure. The precipitate obtained was filtered off, washed with water, and dried; yield 4 g. A portion (1 g) of the mixture was purified by chromatography on a column (2 × 50 cm) of silica gel, with solvent *A* as the eluant. The yellow fractions were collected, and, upon concentration, gave yellow needles of **2**. The compound was recrystallized from dilute methanol, to give chromatographically pure (t.l.c.), yellow needles, m.p. 224–225° (lit.[9] m.p. 220–221°); n.m.r. data (80 MHz, Me_2SO-d_6): δ 3.70–4.31 (m, 4 H, H-4″. 4′,3′,2′), 5.09 (d, H-1′, $J_{1',2'}$ 4.1 Hz), 5.6 (d, 1 H, OH-2′, *J* 5.3 Hz), 5.46 (d, 1 H, OH-3′, *J* 5.7 Hz), 6.71–7.65 (m, 10 H, aromatic protons), 7.86 (s, 1 H, aldimino proton), 10.78 (s, 1 H, nonchelated NH of C-1 hydrazone residue), and 12.33 (s, 1 H, chelated NH of C-2 hydrazone residue); after addition of CD_3CO_2D, the two NH protons and the two OH protons disappeared; accurate measurement of the molecular-ion peak: Found 340.153 (Calc. for $C_{18}H_{20}N_4O_3$, 340.153).

Method B. From D-xylo-*hexulose phenylosazone* (D-*gulose phenylosazone*, **7**), *by "Route ii"*. Compound **7** (2 g) was boiled for 4 h under reflux with 0.05% methanolic sulfuric acid (100 mL), the reaction being monitored by t.l.c.: after 4 h, t.l.c. (solvent *A*) revealed the absence of the starting osazone and the formation of two spots similar to those obtained by Route i, at the same R_F values (solvents *A*, *B*, and *C*). The solution was poured into hot water, and the methanol was evaporated under diminished pressure. The precipitate obtained was filtered off, washed with water, and dried: yield 1.4 g. It was purified by column chromatography on silica gel, with solvent *A* as the eluant. The yellow fractions were collected, and evaporated to dryness, and the residue was recrystallized from dilute methanol, to give chromatographically pure (t.l.c.), yellow needles, m.p. and mixed m.p. (with **2**), 224–225°; n.m.r. data (360 MHz, acetone-d_6): δ 3.80–4.70 (m, 2 OH and 5 sugar protons), 6.80–7.70 (m, 10 H, aromatic protons), 7.867 (s, 1 H, aldimino proton), 9.867 (s, 1 H, nonchelated NH of C-1 hydrazone residue), and 12.406 (s, 1 H, chelated NH of C-2 hydrazone residue). After addition of CD_3CO_2D, the two NH and the two OH protons disappeared: δ 3.87 (bd, 1 H, H-4″), 3.974 (dd, 1 H, H-4′, $J_{3',4'}$ 4.20, $J_{4',4''}$ 9.47 Hz), 4.223–4.252 (m, 2 H, H-3′,2′, $J_{2',3'}$ 2.13 Hz), and 4.389 (d, 1 H, H-1′, $J_{1',2'}$ 4.06 Hz).

Anal. Calc. for $C_{18}H_{20}N_4O_3$: C, 63.50; H, 5.88; N, 16.47. Found: C, 63.49; H, 6.05; N, 16.21.

On boiling a sample of pure **2** with methanolic sulfuric acid for 10 h, monitoring of the reaction by t.l.c. did not show the formation of spots at the R_F values of the osazones **1** or **7**.

The minor isomer **3** was detected as traces in the mother liquor (Routes i and ii), by t.l.c. using solvent mixtures *A*, *B*, and *C*, at R_F 0.49, 0.65, and 0.54, respectively. The spots were further clarified by exposure to iodine.

*2,3-Di-O-acetyl-3,6-anhydro-*D-lyxo-*hexulose phenylosazone* (**4**). — Compound **2** (Route i, 2 g) was acetylated with 1 : 1 acetic anhydride–pyridine (30 mL) for 24 h at room temperature, the solution poured onto crushed ice, and the precipitate collected, washed with water, and dried: yield 2.1 g. It was recrystallized from methanol, to give yellow needles of **4**, m.p. 220–222°, depressed to 208–210° when admixed with **1**; R_F 0.61 (solvent *D*); ν_{max}^{KBr} 1730 (OAc) and 1605 cm^{-1} (C=N).

Anal. Calc. for $C_{22}H_{24}N_4O_5$: C, 62.24; N, 5.70; N, 13.21. Found: C, 62.50; H, 5.82; N, 13.55.

Similar treatment of compound **2** (Route ii) with acetic anhydride–pyridine, processing as described for Route i, and recrystallization from methanol, gave yellow needles, m.p., and mixed m.p. with the product of Route i, 220–222°.

N-*Acetyl-2,3-di-O-acetyl-3,6-anhydro-*D-lyxo-*hexulose phenylosazone* (**5**). — *Method A. Using acetic anhydride–pyridine.* Compound **2** (Route i, 2 g) was treated with 1 : 1 acetic anhydride–pyridine (30 mL) for 7 days at room temperature, poured onto crushed ice, and the precipitate filtered off, washed with water, and dried; yield 2.1 g. It was recrystallized from methanol, to give yellow needles of **5**, m.p. 176–178°; ν_{max}^{KBr} 1760 (OAc), 1705 (NAc), and 1590 cm^{-1} (C=N); n.m.r. data (60 MHz, $CDCl_3$): δ 1.87 and 2.0 (d, 6 H, 2 CH_3CO). 2.4 (bs, 3 H, *N*-$COCH_3$), 3.65–4.16 (m, 2 H, H-4′4″), 4.38 (d, 1 H, H-1′, $J_{1',2'}$ 4.1 Hz), 4.90–5.15 (m, 1 H, H-3′), 5.39–5.55 (dd, 1 H, H-2′), 6.66–7.60 (m, 10 H, aromatic protons), and 12.48 (bs, 1 H, NH).

Anal. Calc. for $C_{24}H_{26}N_4O_6$: C, 61.80; H, 5.62; N, 12.01. Found: C, 62.00; H, 5.88; N, 12.30.

Compound **2** (Route ii) by similar treatment, and recrystallization from methanol, afforded yellow needles, m.p., and mixed m.p. with **5** (Route i), 175–177°.

Method B. By refluxing **2** *with acetic anhydride.* Compound **2** (Route i, 2 g) was refluxed with acetic anhydride (15 mL) for 1 h, and the solution was poured onto crushed ice; the syrupy product solidified on washing repeatedly with water; the solid was filtered off, washed with water, and dried. Recrystallized from methanol, it gave yellow needles, m.p., and mixed m.p. with **5** obtained by Method A (Route i or ii), 178–180°.

*2-Phenyl-4-α-*D-*threofuranosyl-1,2,3-osotriazole* (**6**). — A suspension of compound **2** (2 g) in water (100 mL) was boiled under reflux, with stirring, and a solution of copper sulfate (1.6 g) in water (25 mL) was added dropwise; then, 1-propanol (4 mL) was added, and the mixture was boiled for 6 h, cooled, filtered, and the filtrate extracted with ether (4 × 100 mL). The extract was successively washed with M H_2SO_4, saturated sodium hydrogencarbonate solution, and water, dried, and evaporated. A solution of the residue in methanol was stirred with Amberlite IR-MB cation–anion-exchange resin, the resin was filtered off and washed thoroughly with methanol, and the filtrate and washings were combined, and evaporated to a syrup which was recrystallized from ether, giving **6**; yield 100 mg; m.p. 97–99°, lit.[9] m.p.

102–103° (acetone–petroleum ether); R_F 0.69 (*A*) and 0.58 (*B*); n.m.r. data (360 MHz, Me_2SO-d_6): δ 3.853–3.890 (d, 1 H, H-4″, $J_{4',4''}$ 9.50 Hz), 3.99–4.027 (m, 1 H, H-4′), 4.103–4.12 (t, 1 H, H-3′), 4.17 (bs, 1 H, H-2′), 4.702 (d, 1 H, H-1′, $J_{1',2'}$ 2.87 Hz), 5.022 (d, 1 H, OH), 5.454 (d, 1 H, OH), 7.24–7.46 (m, 3 H, meta and para protons of the phenyl group), and 7.834–7.849 (m, 3 H, ortho protons of the phenyl group, and H-5); after addition of CD_3CO_2D, the OH protons disappeared.

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