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SYNTHESIS OF A NEW SUBSTRATE FOR EXO-GALACTOFURANOSIDASES, 2-HYDROXY-4-NITROBENZENE 1-YL β-D-GALACTOFURANOSIDE

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1,2,3,5,6-Penta-*O*-acetyl β-D-galactofuranose was synthesized by peracetylation of D-galactose in boiling pyridine followed by fractional crystallization. The first crops of crystals were 1,2,3,5,6-penta-*O*-acetyl β-D-galactopyranose, then mother liquor was concentrated and seeded with some crystals of 1,2,3,5,6-penta-*O*-acetyl β-D-galactofuranose. The crystalline mass obtained was recrystallized from ethanol. Optical rotation was a constant guide along the preparation of the furanosic precursor, due to the fact that only pentaacetate-ß-furanose is levorotatory of the four D-galactose pentaacetate isomers. Bromination of penta-*O*-acetyl β-D-galactofuranose with hydrogen bromide produced the glycosylation donor, 1-bromo 1-deoxy 2,3,5,6-tetra-*O*-acetyl α-D-galactofuranose. The aglycon, 1,2-dihydroxy 4-nitrobenzene, was prepared by acidic hydrolysis of 2-hydroxy 5-nitrophenyl sulfate, synthesized at its turn by reacting 4-nitrophenol and potassium persulfate in a strongly alkaline solution conferred by potassium hydroxide. All intermediates and products were structurally characterized by chemical and physical methods.

INTRODUCTION

Studies concerning the enzymes acting on furanosides, either for the understanding of furanosides metabolism or for their use as analytical tools, are strongly dependent on the elaboration of enzymatic substrates that allow identification, isolation and biochemical characterization of the enzymes. At least four types of enzymes are involved in the metabolism of D-galactofuranosides. In the anabolic sense, D-galactofuranose is produced by isomerisation of UDP-α-galactopyranose to UDP-α-galactofuranose, the biocatalyst being a UDP-galactopyranose mutase.[1,2] Once formed, D-galactofuranose is linked by transferases in monofuranosides, oligo- or polysaccharides.[3-5] The catabolism of furanosides is accomplished by two groups of enzymes: *exo*-galactofuranosidases and *endo*-galactofuranosidases. *Exo*-galactofuranosidases remove carbohydrates moieties one by one from the non-reducing end, the product being exclusively monosaccharides.[6,7] *Endo*-galactofuranosidases cleave glycosidic bonds inside the polysaccharidic chain, the products being oligosaccharides of variable chain length.[8,9]

Although furanosides field is an increasing one, relatively few substrates have been synthesized, and even fewer used, till now. 4-Nitrophenyl β-D-galactofuranoside is one of the most intensively used substrate for *exo*-galactofuranosidases and its use is based on the characteristic absorption of visible radiation by the released aglycon. It was synthesized by two routes: (a) from penta-*O*-benzoyl D-galactofuranose (β-isomer or αβ-mixture of isomers) and 4-nitrophenol in the presence of catalytic amounts of *p*-toluenesulfonic acid;[10] (b) from penta-*O*-acetyl-β-D-galactofuranose and 4-nitrophenol by a tin chloride catalyzed reaction followed by *O*-deacetylation.[11] 4-Methylcoumarin-7-yl β-D-galactofuranoside was synthesized from penta-*O*

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benzoyl αβ-D-galactofuranose and tetrabutylammonium salt of 4-methylcoumarin in a tin(IV) chloride promoted glycosylation.[12]

In this paper a new substrate for *exo*-β-D-galactofuranosidases, 2-hydroxy-4-nitrobenzene 1-yl β-D-galactofuranoside (4-nitrocatechol 1-yl β-D-galactofuranoside), has been synthesized. Glycosylation donor was tetra-*O*-acetyl α-D-galactofuranosyl bromide, the acceptor being the di-potassium salt of 1,2-dihydroxy 4-nitrobenzene (4-nitrocatechol).

RESULTS

2-Hydroxy 5-nitrophenylsulfate (4-nitrocatechol sulfate) prepared in this paper had m. p. 245-250 ^{o}C (decomp.) and gave a red color with a neutral solution of ferric chloride. Its IR spectra presented the characteristic absorption band of sulfate ester at 1254 cm^{-1}; this band dissapeared after its conversion into 4-nitrocatechol by acidic hydrolysis. When incubated with arylsulphatase from *Helix pomatia* in acetate buffer,[13] it was cleaved to 4-nitrocatechol. 1,2-Dihydroxy 4-nitrobenzene (4-nitrocatechol) obtained by acidic hydrolysis had m. p. 175 ^{o}C and its dibenzoate had m. p. 156 ^{o}C, after recrystallization from an ethanol-water mixture. NMR spectra of di-acetate derivative has confirmed the envisaged structure.

The degree of acetylation of D-galactose was monitored by TLC in comparison with the native sugar. Peracetylation was proved by IR spectra (lack of hydroxyl absorption band at 3400-3500 cm^{-1}). On the other hand, excellent NMR spectral data, as a consequence of the purity degree achieved, have been obtained for 1,2,3,5,6-penta-*O*-acetyl β-D-galactofuranose, the quality of the spectra being evident by comparison with similar data from literature.[14-16] It showed a levorotatory action, $[\alpha]_D^{23}$ –41^{o} in $CHCl_3$.

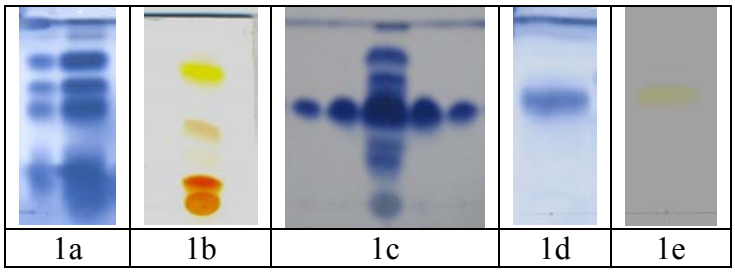

Fig. 1 – TLC analysis of separation steps of 4-nitrocatechol 1- yl ß-D-galactofuranoside. 1a, 1b, total glycosylation mixture; migration of both plates with SS I; visualization of 1a and 1b with mostain and sodium hydroxide, respectively. 1c, lanes 1, 2, 4, 5, fractions from column chromatography; migration SS II, visualization with mostain. 1d, 1e, fractions containing the purified glycoside; migration SS IV, visualization with mostain and sodium hydroxide, respectively.

By TLC analysis of glycosylation mixture, eight to ten bands appeared by mostain visualization (Fig. 1a). When a similar plate was exposed to sodium hydroxide, one or two yellow bands and two or three red bands appeared (Fig. 1b). A prior visualization in UV light of the latter plate revealed the same number and the same pattern of bands as the alkaline solution, although their tone was different: the faster bands, that became yellow at alkaline pH, presented a deep blue color in UV light, a nuance that perfectly matched the absorption presented by the tetraacetate of 4-nitrophenyl ß-D-galactopyranoside; the slower bands, that became red at alkaline pH, presented a brownish color in UV light. These compounds were quantitatively recovered in the chloroformic phase when glycosylation mixture was partitioned between this solvent and water.

After repeated column chromatography on silica gel, by using the whole glycosylation mixture as a reference (Fig. 1c), a compound has been isolated which showed one band in four solvent systems, by alternative visualization under UV light, exposing to alkaline pH or reaction with mostain (Fig. 1d and 1e). This compound had the following properties: it strongly absorbed the UV light in a manner very similar to 4-nitrophenyl ß-D-galactopyranoside. Acidic hydrolysis of the glycoside afforded D-galactose (anthrone) and 4-nitrocatechol (colorimetric determination) in the molar ratio 1:1. ^{1}H and ^{13}C NMR spectra have confirmed the chemical data.

1,2-Di-*O*-acetyl 4-nitrobenzene. NMR spectra of peracetylated 4-nitrocatechol disclosed the following signals:

^{1}H-NMR ($CDCl_3$; δ, ppm; *J*, Hz): 8.12 (s, H-3), 8.15 (d, 8.8 Hz, H-5), 7.39 (d, 8.8 Hz, H-6), 2.33 and 2.34 (s, 6H, C*H*$_3$ groups of acetate).

^{13}C-NMR ($CDCl_3$): 147.43 (C-1), 145.46 (C-2), 119.66 (C-3), 142.35 (C-4), 121.89 (C-5), 124.07 (C-6), 20.50 and 20.62 (*C*H$_3$ groups of acetate), 167.28 and 167.56 (*C*O groups of acetate).

1,2,3,5,6-Penta-*O*-acetyl β-D-galactofuranose. ^{1}H-NMR ($CDCl_3$; δ, ppm; *J*, Hz): 6.12 (s, H-1β), 5.11 (d, 1.6 Hz, H-2), 5.02 (dd, 2.0 Hz, 1.6 Hz, H-3), 4.28 (d, 1.2 Hz, H-4), 5.28 (H-5), 4.25 (d, 4.0 Hz, H-6b), 4.13 (dd, 6.8 Hz, 4.4 Hz, H-6a), 1.99, 2.06, 2.06, 2.07 (s, 15H, C*H*$_3$ groups of acetates).

^{13}C-NMR ($CDCl_3$): 98.96 (C-1), 80.44 (C-2), 76.19 (C-3), 82.04 (C-4), 69.11 (C-5), 62.39 (C-6), 20.47, 20.50, 20.52, 20.65 and 20.83 (*C*H$_3$ groups of acetates), 168.89, 169.26, 169.62, 169.85 and 170.36 (*C*O groups of acetates).

2'-*O*-Acetyl-4'-nitrobenzene 1'-yl β-D-(2,3,5,6-tetra-*O*-acetyl)galactofuranoside. ^{1}H-NMR ($CDCl_3$; δ, ppm; *J*, Hz): 5.76 (s, H-1β), 5.35 (H-2), 5.11 (m, 3.6 Hz, H-3), 4.42 (t, 4.8 Hz, H-4), 5.41 (m, 3.6 Hz, H-5), 4.31 (dd, 8.0 Hz, 4.4 Hz, H-6a), 4.16 (dd, 7.2 Hz, 4.8 Hz, H-6b), 8.00 (s, H-3'), 8.13 (d, 9.2 Hz, H-5'), 7.28 (d, 9.2 Hz, H-6') 2.00, 2.14, 2.15, 2.16 (s, 12H, C*H*$_3$ groups of acetates in sugar), 2.35 (s, 3H, C*H*$_3$ groups of acetate in 4-nitrocatechol).

^{13}C-NMR ($CDCl_3$): 104.00 (C-1), 81.02 (C-2), 76.12 (C-3), 82.12 (C-4), 69.08 (C-5), 62.25 (C-6), 20.43, 20.52, 20.62, 20.69 and 20.80 (*C*H$_3$ groups of acetate), 152.82 (C-1'), 146.06 (C-2'), 119.43 (C-3'), 142.63 (C-4'), 122.82 (C-5'), 123.62 (C-6'), 169.49, 169.84, 169.92 and 170.42, (*C*O groups of acetate from carbohydrate) 167.89 (*C*O groups of acetate from 4-nitrocatechol).

DISCUSSION

As long as both hydroxy groups of 4-nitrocatechol are free or when they are linked to substituents that are sensitive to alkaline pH, these compounds assume red color in alkaline environment, while the blocking of at least one phenolic group prevents the compound to swerve to red, the color assumed at strongly alkaline pH being yellow. Such behaviour has been assigned to 2-hydroxy 5-nitrophenyl sulfate and constitutes the principle of its use as substrate for (aryl)sulfatases, this compound being extremely resistant in alkaline solution.[13] Glycosidic linkage with phenolic aglycons is also relatively stable in alkaline solution and on this property is based the use of 4-nitrophenol-glycosides as substrates for a series of exo-glycosidases: glucosidases, galactosidases, hexosaminidases, arabinosidases, etc.[17] Contrary to inorganic ester or glycosidic linkages, organic ester bonds are extremely labile to alkaline treatment and this property is widely used to remove ester protecting groups by Zemplen deacylation or to simplify the structure of glycolipids or phospholipids by the so-called mild alkaline treatment.[17-19]

The compounds forming red bands (Fig. 1b) are di-*O*-acetyl 4-nitrocatechol (the fastest one), *O*-acetyl 4-nitrocatechol and 4-nitrocatechol (the slowest one). In fact, some of them become red instantly while others could be contemplated while growing red in time of seconds after alkalinization, as a direct proof of the *in situ* alkaline hydrolysis of acetate esters. Acylation products of aglycon – a reaction similar to *in vivo* acylation by transacylases – are well known for glycosylation reactions when protecting groups are esters.[20]

A relatively abundant product was obtained, with the following properties: in native state it migrated in the first third of the plate with SS I and SS IV. It presented no color with sodium hydroxide and no absorption in UV light, however, it got intensely colored with mostain. After Zemplen deacylation and neutralization with Dowex 50 WX2 (H^+) it could not be moved from the start by TLC, with all solvent systems used. According to these properties, it could be either unreacted sugar or ß-D-galactofuranosyl-ß'-D-galactofuranosyl (a trehalose type furanosic disaccharide), but further studies were not undertaken on it.

After repeatedly achieved column chromatography (Fig. 1c), a product was isolated giving one single spot by TLC in UV light, by mostain visualization and by visualization with sodium hydroxide (Fig. 1d, 1e), in all solvent systems used. It strongly absorbed UV light in a manner of remarcable resemblance with 4-nitrophenyl ß-D-galactopyranoside, and different from the absorption of free phenol. In alkaline solution it turned yellow, and this constituted a

proof that at least one hydroxyl group of 4-nitrocatechol was blocked by a linkage resistant to alkaline pH. Its migration as a monosaccharide by TLC, in comparison with 4-nitrophenyl ß-D-galactopyranoside, was the first evidence that at least one hydroxyl group of 4-nitrocatechol was free. After acidic hydrolysis of this glycoside, a red color appeared, when the pH was brought to alkaline value. This product contained D-galactose (anthrone method) and 4-nitrocatechol (spectrophotometric determination, 510 nm) in the molar ratio 1:1.

There is a good agreement between chemical and NMR data. The chemical shift of methyl group of acetates (2.33, 2.34 ppm) in 4-nitrocatechol diacetate, in comparison with the similar groups of penta-*O*-acetyl β-D-galactofuranose (1.993, 2.056, 2.061, 2.072 ppm) shifted *downfield*. The same phenomenon has been observed in case of estrone acetate[18] and D,L-α-tocopheryl acetate.[21] A similar value to 4-nitrocatechol diacetate was found for the conjugate synthesized in this paper (2.35 ppm). Were both hydroxy groups in 4-nitrocatechol glycosylated, only values around 2.00 ppm would have been found. At the same time, in ^{13}C NMR spectra, the signal for C=O group of acetate in 4-nitrocatechol diacetate (167.28 and 167.56) clearly shifted *upfield* in comparison with similar values of penta-*O*-acetyl β-D-galactofuranose (169.26, 169.62, 169.85 and 170.36). A distinct value (167.89 ppm), together with other values (169.49, 169.84, 169.92 and 170.42 ppm), has been found for the glycoconjugate synthesized by us. The signal for H-1 (s) and C-1 (104.00 ppm), as well as the other signals in both types of spectra, clearly indicated the furanosic ring and β-configuration of D-galactose. Consequently, the compound synthesized in this paper is 2'-hydroxy-4'-nitrobenzene 1'-yl β-D-(2,3,5,6-tetra-*O*-acetyl)galactofuranoside. By acetylation it was converted to 2'-*O*-acetyl-4'-nitrobenzene 1'-yl β-D-(2,3,5,6-tetra-*O*-acetyl)galactofuranoside and by Zemplen deacylation it gave 2'-hydroxy-4'-nitrobenzene 1'-yl β-D-galactofuranoside (4-nitrocatechol 1-yl β-D-galactofuranoside) (Fig. 2).

In spite of the fact that the number of papers dealing with D-galactopyranosides is by two or three order of magnitude bigger than the similar number concerning D-galactofuranosides, molecular diversity of the latter ones is practically identical with molecular diversity of D-galactopyranosides. Numerous polysaccharides containing D-galactofuranose have been found in both pathogenic microorganisms,[7,22-24] as well as in beneficial ones.[25,26] A special natural reagent for galactofuranosylation, UDP-α-D-galactofuranose, has been found in microorganisms producing furanosides.[27,28] Subsequently, it was revealed that UDP-α-D-galactofuranose is produced by isomerisation of UDP-α-D-galactopyranose by UDP-α-D-galactopyranose mutase.[1,28] Neutral glycosphingolipids are represented by three compounds: longiside (β-D-Gal*f*-3-α-D-Gal*p*-1'Cer),[3] agelagalastatin (α-D-Gal*f*-2-β-D-Gal*f*-3-α-D-Gal*p*-1'Cer),[4] ectyoceramide (β-D-Gal*f*-1'Cer).[29] Gangliosides having relatively complicated structures were isolated from marine organisms: *Acanthaster planci* (two)[30] and *Asterina pectinifera*.[31] Glycoglycerolipids are exemplified by both α configuration – *sn* 1,2-di-*O*-acyl 3-α-D-galactofuranoside glycerol, in *Acholeplasma axanthum*[32] and ß configuration, *sn* 1,2-di-*O*-acyl 3-β-D-galactofuranoside glycerol in *Mycoplasma mycoides*,[33] *Bacteroides symbioticus*,[34] *Bifidobacterium bifidum*,[35] *Arthrobacter globiformis*.[36] A unique galactofuranosylated poly(glycerophosphate) lipoteichoic acid has been isolated from a *Streptococcus* sp. closely related to *Streptococcus pneumoniae*.[37] An interesting feature of furanosic ring is its existence in glycoglycerolipids with the structure *sn* 1-β-D-galactofuranoside 2,3-di-*O*-acyl glycerol, that have been isolated till now exclusively from *Archaebacteria*.[38] Furanosylated sterylglycolipids are illustrated by two crossasterosides from the starfish *Crossaster papposus*, (24R)-24-ethyl-5α-cholestane-3ß, 6ß, 8, 15α,16ß, 29-hexaol 29-*O*-[2-*O*-methyl-ß-D-xylopyranosyl-(1-2)-ß-D-galactofuranoside] and its 4ß-hydroxy analogue[39] and by indicoside A, (24R)-24-methyl-5α-cholestane-3ß, 6α, 7α, 8, 15β, 24, 28-heptol-ß-D-(5-*O*-methyl)galactofuranoside, from the starfish *Astropecten indicus*.[40] Helminthosporoside toxin C from *Helminthosporium sacchari* possesses a variable number of β-D-galactofuranosic residues and these structures represent the group of sesquiterpenglycolipids (polyprenylglycolipids).[15] Lecythophorin – a representative of resorcinolglycolipids and an interspecific mediator – has also D-galactofuranose in its molecule.[41] Glycoproteins based on D-galactofuranose are represented by α-D-galactosidase from *Aspergillus niger*[42,43] and by phospholipase C and phytase of *A. fumigatus*.[44]

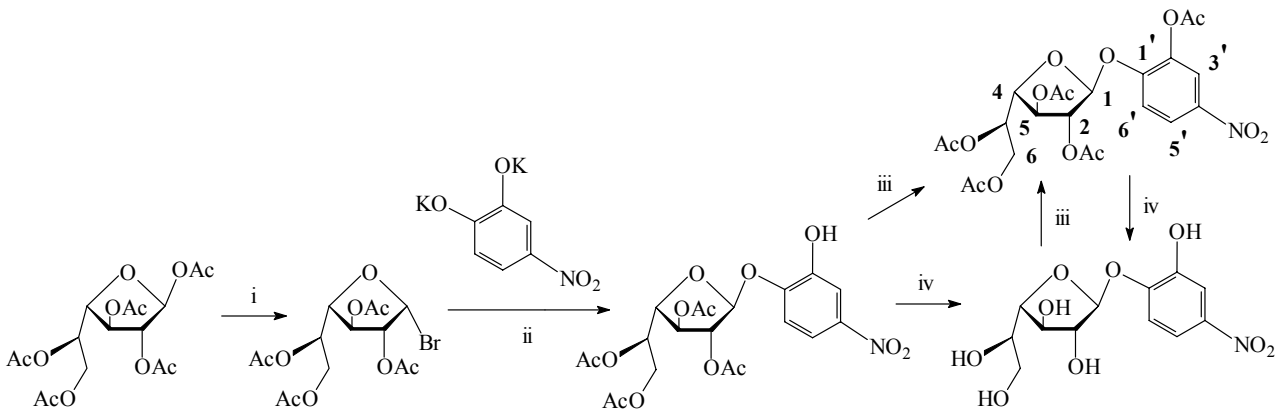

Fig. 2 – Synthesis of 2'-hydroxy-4'-nitrobenzene 1'-yl β-D-galactofuranoside (4-nitrocatechol 1-yl β-D-galactofuranoside) from tetra-*O*-acetyl ß-D-galactofuranosyl bromide and nitrocatechol di-phenoxide. (i) Bromination with HBr 33 % in glacial AcOH. (ii) Stirring overnight in acetone. (iii) Acetylation with Ac_2O-pyridine, 1/2 (v/v). (iv) Sodium methoxide in methanol followed by neutralization with Dowex 50 WX2 (H^+).

EXPERIMENTAL

Materials. $CDCl_3$ containing TMS, D-galactose, 4-nitrophenyl ß-D-galactopyranoside, 1,2-dichloroethane, acetic acid, acetic anhydride, acetone, anthrone, chloroform, diethyl ether, ethanol, methanol, 4-nitrophenol, *p*-toluenesulfonic acid, pyridine, toluene, active carbon, ammonium molibdate, cadmium carbonate, calcium sulfate, $Ce(SO_4)_2$, concd. sulfuric acid, dry zinc chloride, HBr in glacial acetic acid (33 %), molecular sieves (4 Å), potassium hydroxide, potassium persulfate, sodium carbonate, sodium bicarbonate, sodium hydroxide, sodium metal, ferric chloride, ready-to-use thin layer silica plates, silicagel for column chromatography, Celite, Dowex 50 WX2, were either from Merck or from Fluka.

Methods

NMR Spectra Recording. ^1H and ^{13}C NMR spectra of 4-nitrocatechol-di-*O*-acetate, penta-*O*-acetyl ß-D-galactofuranose, and 2'-*O*-acetyl-4'-nitrobenzene 1'-yl β-D-(2,3,5,6-tetra-*O*-acetyl)galactofuranoside were recorded in $CDCl_3$ solution, containing TMS as internal standard. Moreover, the spectra of these compounds were compared with the similar data concerning estrone β-D-galactofuranoside tetraacetate, estrone acetate,[18] α-DL-tocopheryl acetate and α-DL-tocopheryl β-D-galactofuranoside tetraacetate.[21,45]

One-dimensional NMR studies. NMR experiments were performed on a Bruker Avance DRX 400 spectrometer using 400 and 100 MHz for ^1H and ^{13}C frequencies, respectively.

Two-dimensional NMR experiments. The ^1H-^1H correlation spectroscopy (COSY) and ^1H-^{13}C heteronuclear multiple quantum coherence (HMQC) experiments were carried out with an inverse probe.

IR spectra were recorded as KBr pellets or as a solution in Nujol on a Bruker Equinox 55 FT-IR spectrometer.

Acetylation. Compounds were solved in pyridine and stirred overnight with an excess of acetic anhydride, the ratio between acetic anhydride and pyridine was 2/1 (v/v).

Benzoylation. Compounds were solved in pyridine, the solution was cooled on ice and benzoyl chloride was added in small portions, the ratio between solvent and benzoylatig agent was 10:1. Then pyridine was removed by co-evaporating with toluene. Excess benzoyl chloride was decomposed by adding ice in the reaction mixture and the acidic products formed (HCl, benzoic acid) were removed by partition between chloroform and a saturated solution of $NaHCO_3$.

Synthesis of 1,2-dihydroxy 4-nitrobenzene (4-nitrocatechol). Reaction of 4-nitrophenol with potassium persulfate in a strongly alkaline environment conferred by potassium hydroxide gave 2-hydroxy 5-nitrophenyl sulfate (4-nitrocatechol sulfate);[13] its acidic hydrolysis and partition between water and diethyl ether led to 4-nitrocatechol.[46]

Glycosylation agent and Koenigs-Knorr glycosylation. Penta-*O*-acetyl-ß-D-galactofuranoside was prepared as indicated.[47] By bromination of peracetylated sugar with hydrobromic acid in glacial acetic acid, 1-bromo-1-deoxy-α-D-2,3,5,6-tetra-*O*-acetyl-galactofuranoside was obtained.[48] Glycosylation of 4-nitrocatechol was performed in boiling dry toluene, by using cadmium carbonate as chemical condensing agent.[18,20] Freshly dried molecular sieves (4 Å) and calcium sulfate were used as water scavengers.

Helferich glycosylation was made by boiling the protected sugar with p-toluenesulfonic acid or dry zinc chloride in toluene.[49]

Glycosylation of 4-nitrocatechol as potassium phenoxide with 1-bromo-1-deoxy-α-D-2,3,5,6-tetra-*O*-acetyl-galactofuranoside was accomplished in acetone.[50]

Thin layer chromatography (TLC) was achieved on ready-to-use plates in the following mobile phases: solvent system (SS) I (chloroform-methanol, 19/1, v/v), SS II (toluene-ethanol, 3/1, v/v), SS III (toluene-methanol, 7/1), SS IV (chloroform-methanol-water, 60/25/4, v/v). Visualization of spots was made in three ways: (a) by dipping the plates in a solution called mostain consisting of water, sulfuric acid, ammonium molybdate and $Ce(SO_4)_2$, followed by heating on a hot plate; (b) under UV light in comparison with 4-nitrophenyl ß-D-galactopyranoside or its peracetate; (c) by dipping the plates in a diluted solution of sodium hydroxide.

Reaction mixture after glycosylation in case of Koenigs-Knorr was diluted with chloroform, mixed with Celite, filtered and evaporated to dryness. In case of Helferich method, reaction mixture was neutralized with sodium carbonate, filtered and the solvent removed. The mixture from glycosylation of phenoxide was neutralized with acetic acid, repeatedly mixed with toluene and evaporated by rotavapor.

Either residue was partitioned between water and chloroform, aqueous phase being discarded. Chloroformic

phase was repeatedly chromatographed on silica gel column. The purified product was analyzed for sugar content by anthrone reaction[19] and for 4-nitrocatechol by using molar coefficient of 12500 $cm^2 \cdot mol^{-1}$.[13] A small portion was acetylated for NMR analysis.

Protecting acetate groups were removed by Zemplen deacylation of the product with 0.2 M sodium methoxide followed by neutralization with Dowex 50 WX2 (H^+), filtration and concentration. In this form, the product is ready for enzymatic test.

CONCLUSIONS

Radical sulfation of 4-nitrophenol led to 4-nitrocatechol sulfate and its hydrolysis constitues an efficient route for 4-nitrocatechol preparation.

Glycosylation of 4-nitrocatechol as a double phenoxide or by Koenigs-Knorr or by Helferich method led to 2-hydroxy 4'-nitrobenzene 1'-yl β-D-(2,3,5,6-tetra-O-acetyl)galactofuranoside, providing that a suitable ratio between reagents was adopted.

Chemical means combined with spectral data unequivocally characterized the envisaged product. New NMR spectral data were obtained for 2'-O-acetyl-4'-nitrobenzene 1'-yl β-D-(2,3,5,6-tetra-O-acetyl)galactofuranoside, a new compound for chemical literature.

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