Synthesis of 1,2,5,6-tetra-O-acetyl-3-O-allyl αβ-D-galactofuranose, a Versatile Precursor of Pyranosylated and Furanosylated Glycoconjugates

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Dissolution of D-galactose in N,N-dimethylformamide and then acetonation by boiling with acetone and anhydrous copper sulfate, produced a mixture of 1,2-5,6-di-O-isopropylidene-α-D-galactofuranose and 1,2-3,4-di-O-isopropylidene-α-D-galactopyranose. The first isomer was separated by column chromatography on silica gel and allylated in N,N-dimethylformamide with allyl bromide in the presence of NaH. A smaller portion of di-isopropylidene furanose was acetylated and another one was left aside per se. Then the terminal isopropylidene group from all three compounds was selectively removed by stirring with dilute acetic acid. The newly formed hydroxy groups were peracetylated and then the other isopropylidene group was removed, and the reaction products peracetylated. All intermediates as well as the final products were characterized chemically and by ^{1}H NMR spectroscopy. As an application, the allylated tetra-acetylated sugar was converted to 2,5,6-tri-O-acetyl-3-O-allyl aβ-D-galactofuranosyl bromide and the latter product used for galactofuranosylation of cholesterol.

Keywords: 1,2-5,6-Di-O-isopropylidene 3-O-allyl α-D-galactofuranose, 3-O-allyl-D-galactose, D-galactopyranose, NMR spectra

A vast number of glycoconjugates, as represented by glycolipids and glycoproteins, contain C-3 substituted D-galactopyranose in their molecule [1]. A common structural motif to all sulfatides – mono- [2], di- [3,4], tri- [5-8], tetra- [9], pentahexosyl-ceramide [10], is sulfo-3-D-galactopyranose. Sialic acid, linked in the same manner, has been found in all subgroups of gangliosides: hematosides, ganglio, globo, lacto, neolacto [11]. The same structural motif of sulfate ester has been found in glycoglycerolipids [12,13]. D-Galactopyranose substituted on C-3 with a neutral sugar or sialic acid constitutes a structural characteristic of numerous glycoproteins, proteoglycans, glycosaminoglycans [1,14,15]. The same structural fragment was characterized practically in all blood-groups antigenic determinants, both in glycolipids and glycoproteins [1,14]. Fatty acyl linked on C-3 of D-galactose has been found in neutral glycosphingolipids produced by organisms living at temperatures of 0 - – 5 °C [16].

A comfortable approach to this structural motif is the synthesis of 1,2-5,6-Di-O-isopropylidene α-D-galacto-furanose, its allylation at C-3 (or the use of another substituent that is orthogonal with isopropylidenic groups) and then, by simultaneous removing of the two isopropylidenic substituents, the molecule spontaneously adopts pyranosic ring; direct glycosylation on C-3, followed by removing of isopropylidene groups, was also accomplished [17,18].

Other natural compounds contain D-galactofuranose substituted on C-3 with a fatty acid [19], or a neutral sugar [20-22]. It must be underlined that a series of natural furanosides contain D-galactopyranose substituted on C-3 with D-galactofuranose [23-25], or L-arabinofuranose [26]; D-galactofuranose substituted on C-3 with D-galactofuranose has also been found [21].

In this paper, the synthesis of the main compound for the initiation and development of a new group of glycolipids has been described. After allylation, 1,2-5,6-di-O-isopropylidene-α-D-galactofuranose was managed so as to preserve it locked as furanosic ring. Subsequently, it can be substituted by sialic acid, sulfate, neutral sugars, etc.

Experimental part

Materials and methods

D-Galactose (D-Gal) was from Sigma. Pyridine, acetic anhydride, allyl bromide, N,N-dimethylformamide (DMF), sulfuric acid, silicagel for column chromatography, ready-to-use plates for thin layer chromatography were from Merck, Germany. Organic solvents and cupric sulfate were purchased from Chimopar, Bucharest. All reagents used were either from Fluka or from Merck and they were of p. a. grade.

Isopropylidenation of D-Gal (fig. 1) was made as described by [27,28] and separation of the two isomers, 1,2-3,4-di-O-isopropylidene-α-D-galactopyranose and 1,2-5,6-di-O-isopropylidene α-D-galactofuranose, as [29]. Allylation was accomplished according to [30], with allyl bromide in the presence of sodium hydride. Selective de-isopropylidenation of terminal Ipd group was made with dilute acetic acid at room temperature according to [27]. All chemical reactions were followed by TLC [28,29]. The following solvent systems (SS) were used: toluene-ethanol (3:1, v/v) (SS I) and chloroform-methanol (25:1, v/v) (SS II). Visualization was made by dipping the plates in a solution of amonium molybdate, sulfuric acid and cerium(IV) sulfate (mostain), or in an orcinol/$FeCl_3$ reagent, followed by heating in both cases.

The ^{1}H and ^{13}C NMR spectra of synthesized compounds were measured in $CDCl_3$ containing TMS. One-dimensional NMR experiments were performed on a Bruker Avance

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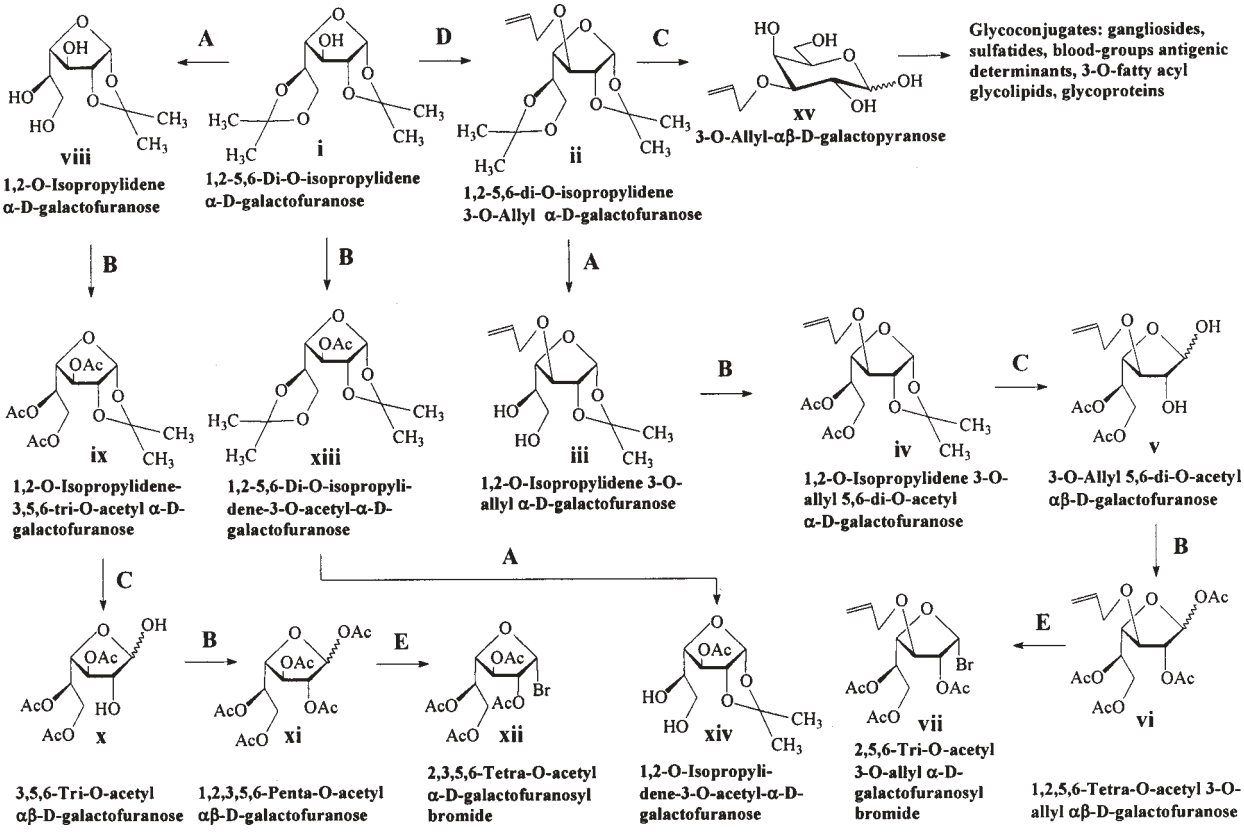

Fig. 1. Synthesis of three galactofuranosylation reagents starting from 1,2-5,6-di-O-isopropylidene α-D-galactofuranose. A: acetic acid-water, 4/6 (v/v), room temperature; B: Ac_2O-pyridine, 1/2 (v/v); C: acetic acid-water, 8/2 (v/v), boiling; D: DMF, NaH, allyl bromide; E: 33 % HBr in glacial acetic acid

DRX 400 spectrometer using 400 and 100 MHz for the ^1H and ^{13}C frequencies, respectively. The ^1H-^1H correlation spectroscopy (COSY) and ^1H-^{13}C heteronuclear multiple quantum coherence (HMQC) experiments were carried out with an inverse probe.

1,2-5,6-Di-O-isopropylidene α-D-galactofuranose (i). ^1H-NMR ($CDCl_3$;δ, ppm; J, Hz): 5.84 (d, 3.9, H-1a), 4.51 (dd, 1.3, 2.6, H-2), 3.86-3.79 (m, H-4, H-6'), 4.38-4.30 (m, H-5), 4.09-4.02 (m, H-3,6), 1.52, 1.43, 1.35, 1.33, 4s (four Me groups of Ipd), 2.35, brs (OH of C-3).

1,2-5,6-Di-O-isopropylidene 3-O-allyl α-D-galactofuranose (ii). ^1H-NMR ($CDCl_3$; δ, ppm; J, Hz): 5.82 (d, 4.1, H-1a), 4.54 (q, 1.3, 2.7, H-2), 4.10 (m, H-3), 4.04 (m, H-4), 4.28 (m, H-5), 3.82-3.73 (m, H-6,6'), 5.92-5.82 (m, H_2C=CH), 5.33-5.19 (m, H_2C=CH), 3.98 (m, =CH–CH_2O).

1,2-O-Isopropylidene 3-O-allyl α-D-galactofuranose (iii). ^1H-NMR ($CDCl_3$;δ, ppm; J, Hz): 5.87 (d, 4.1, H-1α), 4.59 (dd, 1.0, 3.1, H-2), 4.03 (m, H-3), 3.96-3.94 (m, H-4), 4.08 (m, H-5), 3.84-3.63 (m, H-6,6'), 5.89-5.81 (m, H_2C=CH), 5.33-5.19 (m, H_2C=CH), 3.96 (m, =CH–CH_2O), 1.51, 1.32 (2s, two Me groups of Ipd), 2.78 (OH of C-6), 2.14 (OH of C-5).

1,2-O-Isopropylidene 3-O-allyl 5,6-di-O-acetyl α-D-galactofuranose (iv). ^1H-NMR ($CDCl_3$; δ, ppm; J, Hz): 5.80 (d, 3.9, H-1α), 4.54 (dd, 1.3, 2.7, H-2), 4.30 (dd, 3.8, 8.2, H-5), 4.18-3.99 (m, H-3,4,6), 3.79 (dd, 1.2, 3.8, H-6'), 2.09, 2.04 (2 s, two Me group of acetate), 5.84-5.79 (m, H_2C=CH), 5.34-5.19 (m, H_2C=CH), 4.15 (m, =CH–CH_2O), 1.55, 1.34 (2s, two Me groups of Ipd).

3-O-Allyl 5,6-di-O-acetyl αβ-D-galactofuranose (v). ^1H-NMR ($CDCl_3$; δ, ppm; J, Hz): 5.30 (d, 2.2, H-1α), 5.28 (s, H-1β), 4.34-4.05 (m, H-2,5), 3.93 (dd, 1.4, 5.3, H-3), 3.84 (t, 5.2, H-4,6), 3.71 (d, 2.7, H-6'), 5.88-5.82 (m, H_2C=CH), 5.32-5.22 (m, H_2C=CH), 4.12 (m, =CH–CH_2O), 2.12, 2.05 (2 s, two Me group of acetate).

1,2,5,6-Tetra-O-acetyl-3-O-allyl αβ-D-galacto -furanose (vi). ^1H-NMR ($CDCl_3$; δ, ppm; J, Hz): 6.31 (d, 4.5, H-1α), 6.17 (s, H-1β), 4.34-4.29 (m, H-2,5), 4.22-4.03 (m, H-3), 3.82 (d, 4.5, H-4,6,6'), 5.85-5.81 (m, H_2C=CH), 5.35-5.14 (m, H_2C=CH), 4.11 (m, =CH–CH_2O), 2.13, 2.12, 2.10, 2.06 (4s, Me groups of acetate).

1,2-O-Isopropylidene α-D-galactofuranose (viii). ^1H-NMR ($CDCl_3$; δ, ppm; J, Hz): 5.90 (d, 4.0, H-1α), 4.57 (d, 4.0, H-2), 4.24 (d, 2.4, H-3,5), 3.99 (d, 3.0, 4.7, H-6), 3.88-3.64 (m, H-4), 3.47 (d, 8.0, H-6'), 1.50, 1.31 (2s, Me groups of Ipd).

1,2-5,6-Di-O-isopropylidene 3-O-acetyl α-D-galactofuranose (xiii). 5.90 (d, 3.78, H-1α), 4.87 (d, 2.4, H-2), 4.54 (d, 3.83, H-3,5), 4.37-3.96 (m, H-4,6), 3.80 (dd, 1.8, 6.7, H-6'), 1.54, 1.41, 1.33, 1.30 (4s, four Me groups of Ipd), 2.06 (s, Me group of Ac).

1,2-O-Isopropylidene-3-O-acetyl α-D-galacto -furanose (xiv). ^1H-NMR ($CDCl_3$; δ, ppm; J, Hz): 5.94 (d, 4.0, H-1α), 5.05 (d, 1.4, H-5), 4.64 (d, 4.0, H-2,3), 3.93-3.87 (m, H-4), 4.08 (dd, 1.3, 6.9, H-6), 3.80-3.67 (m, H-6'), 1.52, 1.29 (2s, Me groups of Ipd), 2.08 (s, Me group of Ac), 2.87, 2.45 (C_5-OH, C_6-OH).

1,2,3,5,6-Penta-O-acetyl αβ-D-galactofuranose (xi). ^1H-NMR ($CDCl_3$; δ, ppm; J, Hz): 6.16 (s, H-1β), 6.26 (d, 4.6, H-1α), 5.13 (d, 3.5 Hz, H-2), 5.01 (m, H-3), 4.30 (m, H-4), 5.36 (m, H-5), 4.24 (m, H-6a), 4.07 (m, H-6a), 2.10, 2.08, 2.06, 2.02, 1.96 (5s, Me groups of acetates).

Simultaneous removal of both Ipd residues from 1,2-5,6-di-O-isopropylidene 3-O-allyl α-D-galactofuranose produced 3-O-allyl α*β*-D-galactopyranose, as indicated by NMR spectra of a peracetylated sample of the latter compound.

A small portion of allylated peracetylated sugar was converted to bromide and used for cholesterol galactofuranosylation; formation of a lipidic compound

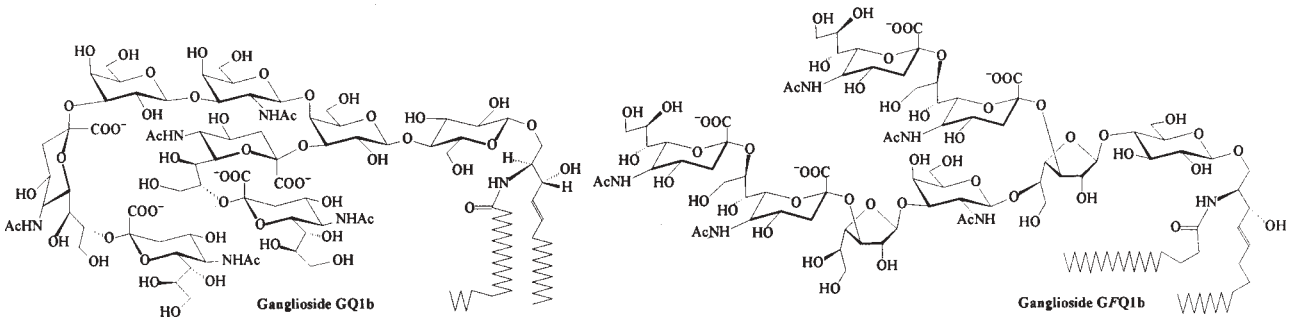

Fig. 2. Comparative structure of the "classical" ganglioside GQ1b and an envisaged ganglioside, G*F*Q1b

after glycosylation and Zemplen de-acylation indicated that the reaction had been accomplished [31].

Results and discussions

Mono- or di-isopropylidenation of D-galactose led to less polar compounds that could be distinguished by TLC from one another and from the initial precursor. SS containing halogenated solvents especially, permitted also to distinguish between the two diisopropylidenated isomers, pyranosic and furanosic. As soon as it was pure, 1,2-5,6-di-O-isopropylidene α-D-galactofuranose presented a spectacular crystallization in the concentration flask, while its isomer, 1,2-3,4-di-O-isopropylidene-α-D-galactopyranose, in the same state, remained as an oil. This criterion is quite valuable and unequivocal, and associated with optical rotation and MS [32] and NMR spectra led to a complete characterization of di-isopropylidenic furanose.

The major part of 1,2-5,6-Di-O-isopropylidene α-D-galactofuranose was allylated, a smaller one was acetylated and another one left per se. Allylation or acetylation of 1,2-5,6-di-O-isopropylidene α-D-galactofuranose were both quantitative and both gave rise to less polar compounds, so they could be followed by TLC. Selective removal of terminal Ipd group, although clearly traced by TLC, constituted a critical step: the yield was halved and more cycles de-isopropylidenation-separation were necessary to increase it. The products, analyzed by IR and NMR, met de structural requirements. The newly formed hydroxy groups were acetylated and in this way the sugar was locked as furanosic ring. More energetic conditions – higher concentration of AcOH, warming – could be used to remove the Ipd group from reducing end. The reaction was complete, and the next step, peracetylation, too.

In general, there was a reduced interpherence by NMR spectra between protecting groups used in this paper and sugar atoms. Chemical shifts of Me groups of Ipd (around 1.50 ppm) or of acetate (about 2 ppm) were completely different from all chemical shifts of D-galactose. An interpherence was noticed in case of allyl group, and auxiliary compounds were synthesized and used for its elimination: 1,2-5,6-di-O-isopropylidene 3-O-acetyl α-D-galactofuranose (xiii), 1,2-O-Isopropylidene-3-O-acetyl *a*-D-galactofuranose (xiv) and even 1,2,3,5,6-penta-*O*-acetyl αβ-D-galactofuranose (xi). Initially, a sample of 1,2-5,6-Di-O-isopropylidene α-D-galactofuranose, donated by Dr. Andreas Tauss (Technical University, Graz, Austria), was used as reference compound. On the other hand, 1,2,3,5,6-penta-O-acetyl αβ-D-galactofuranose has been also synthesized by direct acetylation of D-galactose, concomitantly with heating [33,34].

Protecting group on C-3 of D-galactose is removed in the last steps and replaced by protecting groups that are even more susceptible to alkali than acetate, usually levulinoyl [35-38]. Also, glycosylation step is preceded by sulfate or phosphate linkage. In case of gangliosides [36] or blood-group antigenic determinants [39], linkage of sialic acid or neutral sugar are linked before the attachment of aglycon – ceramide, sn-1,2-diacylglycerol, protein, etc.

One can imagine and develop a new group of glycoconjugates by replacing Galp for Galf and linking the substituent – sialic acid, sulfate, phosphate, Galp, GalNAcp, etc., on the same carbon atom, C-3 or C-6. This principle has been suggested in this paper by a ganglioside with a relatively complicated structure, ganglioside GQ1b (Fig. 2). Since gangliosides have a detailed elaborated nomenclature, just letter *F* has been added to the established name of gangliosides. Following the strategy of Carbohydrate Chemistry, a series of new gangliosides could be synthesized: Neu5Acα2-3Galf*β*-1'Cer (G*F*M4), Neu5Acα2-3Galf*β*-4Glcp*β*-1'Cer (G*F*M3), Neu5Acα2-3Galfβ-3GalNAcpβ-5(Neu5Acα2-3)Galfβ-4Glcpβ-1'-Cer (GFGD1a), Galfβ-3GalNAcpβ-5(Neu5Acα2-8Neu5Acα2-3)Galfβ-4Glcpβ-1'-Cer (GFGD1b), Neu5Acα2-8Neu5Acα2-3Galfβ-3GalNAcpβ-5(Neu5Acα2-8Neu5Acα2-3)Galfβ-4Glcpβ-1'-Cer (GFGQ1b), etc.

Glycosphingolipids containing Galf instead of sialic acid have been synthesized: *neo*longiside and *isoneo*longiside [40]; in fact, they contain natural structural motifs [23,24,41,42].

On the other hand, it should be underlined that 1,2-3,4-di-O-isopropylidene-α-D-galactopyranose and 1,2-5,6-di-O-isopropylidene α-D-galactofuranose are suitable for direct and specific methylation on C-6 and C-3, respectively [43]. At the same time, if compound **viii** is perbenzoylated, then isopropylidenic group removed and the produced derivative converted to alkyl glycoside, C-2 can be selectively methylated [44]. Methylation reactions constitute another proof that 1,2-5,6-di-O-isopropylidene α-D-galactofuranose is remarkably versatile.

Conclusions

Di-O-isopropylidene D-galactofuranose is a very adequate derivative for selective linkage on C-3 of substituents that are orthogonal to Ipd, or its substitution with other groups.

A slight but measurable difference of reactivity between 1,2-O-Ipd and 5,6-O-Ipd could be exploited for their successive substitution with adequate substituents.

Once substituted on C-3, the furanosic ring can be turned to pyranosic one, by removing of substituents on C-1 and C-5, or maintained *per se* by an adequate management of substituents.

Allyl group has been used in this paper as a substituent on C-3.

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