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Regioselective acylation of 1,6-anhydro-β-D-manno and galactopyranose catalysed by lipases

N. Junot [a], J.C. Meslin [b] and C. Rabiller [a]*

[a] Laboratoire de RMN et de Réactivité Chimique, URA CNRS 472, 2, rue de la Houssinière

F - 44072 NANTES Cedex 03 (FRANCE)

[b] Laboratoire de Synthèse Organique, URA CNRS 475, 2, rue de la Houssinière

F - 44072 NANTES Cedex 03 (FRANCE)

Abstract *: Pseudomonas fluorescens lipase (Amano) was found to be highly regioselective in the transesterification of 1,6-anhydro-β-D-manno and galactopyranoses (mannosane and galactosane respectively) using vinyl acetate as an acyl donor . As in the case of 1,6-anhydro-β-D-glucopyranose (glucosane), the 4-OH axial group of mannosane is preferred, while the titled lipase catalysed the regioselective transesterification at the 2-axial OH of galactosane. The enzymatic acylation affords monoesters of the 1,6-anhydropyranoses which are difficult to obtain using conventional methods.*

Saccharides, glycosides and glycoconjugates embody several hydroxyl groups of similar reactivity. The modification of only one of these functions is still a fundamental challenge for organic chemists. In the case of pyranosides, the regioselective acylation is of great interest because the highly biodegradable sucro-esters obtained can be used as surfactants in detergent formulations[1] and as emulsifiers in food[2]. These compounds are also very useful synthons in medicinal chemistry and some of them exhibit very interesting liquid crystal properties. Numerous chemical methods are available to achieve the selective acylation, but the processes described involve very tedious and time consuming protection / deprotection steps[3]. Among the methods proposed, enzymes take a particularly important place as these biocatalysts are able to induce organic reactions stereoselectively and regioselectively. Excellent reviews on this topic[4,5] reveal that lipases can be used with success to acylate regioselectively the hydroxy functions in positions 1 and 6 of the furanoses and of the pyranoses. Nevertheless, few papers deal with the ability of the lipases to operate selectively in the 2, 3, and 4 positions when the 1- and 6-hydroxy groups are already protected or absent. Good results have been obtained with L-galacto- and mannopyranoside[6], with 4,6-O-benzylidene-α- and β- D- glucopyranoside[7] and with xylopyranosides[8]. Various lipases of different regioselectivity were also used to deacetylate the 2,3,4 positions of 1,6-anhydro-β-D-glucopyranose thus affording one of the three diesters[9-13]. Similarly Candida Rugosa lipase (CRL) catalyses the hydrolysis of the 2-ester function of a triester of galactosane[14]. Surprisingly the 1,6-anhydro-β-D-hexopyranoses were not used as substrates in the esterification or in the transesterification reactions.

The aim of this work is to present some results on the regioselectivity of the transesterification reactions of 1,6-anhydro-β-D-manno- and galactopyranose (mannosane and galactosane) catalysed by lipases.

RESULTS AND DISCUSSION

In a preliminary paper, we described a high yield regioselective synthesis of the 4-acetylglucosane[15].The choice of the anhydro model was supported by the following considerations :

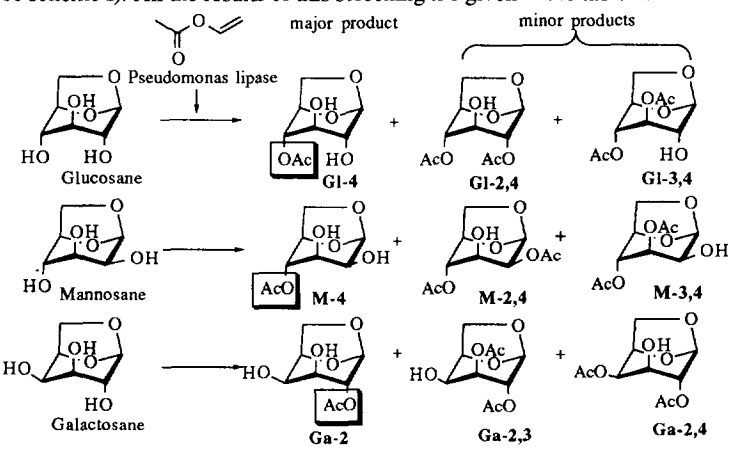
- the conformational rigidity of this kind of compound which is very unfavorable for further isomerisations of the esters obtained.

- the relative simplicity of synthesis[16-19].

- the possibility to prepare the stereoisomers of 1,6-anhydro-β-D-pyranose in order to study the regioselectivity as a function of axial/equatorial position in connexion with steric hindrance, and finally to check if the lipases known for their regioselectivity towards the hydroxyl functions of glycerol (Mucor mieihi, Rhizopus arrhizus, Porcine pancreatic lipase)[20,21] would exhibit a similar trend in the presence of such triol models.

The reactions were carried out at room temperature using vinyl acetate as an acyl donor and solvent. The course of the reaction was monitored by TLC and proton, ^{13}C NMR spectroscopy. The structure of the esters synthesized was determined by comparison with the spectra of the 1,6-anhydro-β-pyranoses[22,23] and with literature data[9,10]. A verification of the attributions was performed by means of the two classical two dimensional NMR spectroscopy experiments (COSY, see figure 1 and C-H correlation).

In order to check the regioselectivity in the transesterification reaction, we studied the behaviour of four common lipases, namely Candida Rugosa (CRL), porcine pancreatic (PPL) Pseudomonas fluorescens (PSL) and Rhizopus arrhizus (RAL). As we already mentioned it in the case of glucosane[15], the rates of the transesterifications of mannosane and of galactosane using vinyl acetate and CRL as a catalyst were relatively fast, but a very poor selectivity was observed. Conversely in similar conditions, PPL and RAL were extremely selective but these enzymes acted very slowly thus affording low conversions over a two weaks period of incubation. An intermediate situation occured with PSL which usually induces both good yield and good regioselectivity (see scheme I). All the results of this screening are given in the table 1.

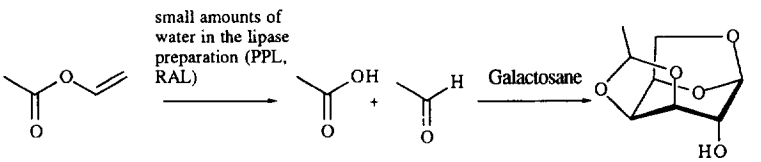


Scheme I : *Lipase catalysed transesterifications of 1,6-anhydro-β-D-hexopyranoses*

Considering the results of the transesterification reactions (see table 1), it is clear that all the lipases exert a strong selectivity towards the 4-axial position of both glucosane and mannosane. If the 4-OH stands in the equatorial position (case of the galactosane), the 2-axial position is highly preferred. This trend is observed in diacetates. For instance, in the case of mannosane in the presence of PSL, only small amounts of the diacetates were obtained and the acylation always occured at the 2-axial position. A rather curious, but easily understandable result was observed in the transesterification of galactosane in the presence of PPL and RAL. These enzymes are shown to be totally inefficient towards the galactosane acylation, but are able to induce indirectly the acetalisation of the 3- and 4-hydroxyl groups (see scheme II).

Lipases	Incubation period (days)	Conversion (%)	monoacetates (%)			diacetates (%)			triacetates (%)
			2	3	4	2,3	2,4	3,4	
Glucosane*									
PSL	10	99	-	-	62	-	24	14	-
CRL	10	99	-	-	22	-	53	20	5
PPL	10	20	-	-	80	-	-	20	-
RAL	10	31	-	-	75	-	-	25	-
Mannosane									
PSL	11	69	-	-	81	-	7	12	-
CRL	11	30	-	-	63	-	11	26	-
PPL	10	9	-	-	100	-	-	-	-
RAL	10	42	-	-	71	-	8	21	-
Galactosane									
PSL	11	12	100	-	-	-	-	-	-
CRL	12	42	36	-	-	26	38	-	-

Table 1 : *Lipase catalysed transesterification of 1,6-anhydro-β-D-hexopyranoses with vinyl acetate as an acyl donor : yields and relative percentages of the acetates.*
** data from our previous work[15].*



Scheme II : *On the role of traces of water in PPL and RAL in the case of galactosane.*

We had already noticed the undesirable effect of the remaining water in the lyophilised lipase preparations on the isomerisation of the diglycerides[20,21]. Here again, this water is responsible of a partial enzymatic hydrolysis of vinyl acetate, affording acetic acid and acetaldehyde. Acetaldehyde is able to acetalize the 3- and 4-hydroxyl groups under the catalytic action of the acetic acid. The equivalent of water liberated from this reaction is then allowed to participate again in the hydrolysis. This cycle afforded a 35% yield of the protected galactosane in the presence of PPL or RAL after 10 days of incubation. No reaction of this type was detected using glucosane and mannosane because of the unfavorable positions of the OH groups.

The results described in this paper and in our former one[15] complete the enzymatic strategies available for regioselective acylation of sugars. The hydrolysis of the triacetates of the 1,6-anhydro-β-D-gluco- and galactopyranoses studied formely by other workers afforded the regioselective synthesis of the diacetates[9-14]. Up to now the hydrolysis of the 1,6-anhydro-β-D-mannopyranose has not been studied nor has the ability of the lipases to catalyse the reverse reaction (transesterification) towards those anhydro sugars. We have shown that the regioselectivity of the lipases was the same in the synthetic as in the hydrolytic direction : the monoacetates synthesised in the former case are the complement of the diesters obtained in the latter one. Work is in progress

in our laboratory in order to introduce longer acyl chains and thus to synthesize new detergents from sugar derivatives.

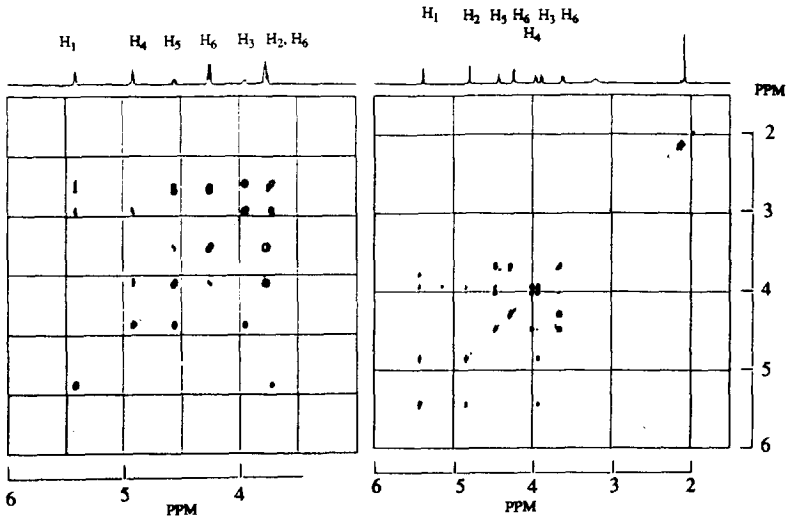


Figure 1:*Assignment of the ^1H proton NMR spectra of the 4-monoacetate of mannosane (left) and of the 2-monoacetate of galactosane (right) by means of COSY experiments- solvent : $CDCl_3$ - 500 MHz -Bruker spectrometer).*

EXPERIMENTAL :

Materials and methods : the lipases were purchased from Amano.The glucosane was supplied by Aldrich while the mannosane and the galactosane were prepared according to slightly modified procedures described in ref. 18 and 19 (see below).Vinyl acetate (from Aldrich) was used without any further purification. The course of the reactions was followed by means of TLC (precoated silica gel 60 sheets Merk F254, (see eluent for each compound). The products were separated on silica gel columns (Merk 60, 230,400 mesh). ^{13}C and ^1H NMR spectra were obtained using a Bruker WM250 spectrometer operating at 250 MHz for the proton (62,89 Mhz for the carbon). Complete analysis of the structures and assignment of each resonances was made using standard 2D sequences (COSY H-H and ^{13}C-^1H HCORR correlations) on a 500 MHz Bruker spectrometer. The optical rotations were determined with a "Optical Activity - AA10" polarimeter .

Synthesis of the 1,6-anhydro-β-D-mannopyranoses[18] : 3,3 g (18 mmol) of tosyl chloride in 15 mL of pyridine were slowly added at O°C to a solution of 3g (16,5 mmol) of mannose in 45 mL of pyridine. The solution was stirred at room temperature for four hours and the reaction was quenched by addition of 30 mL of water. The pH was then adjusted to 9 by means of 1M NaOH and the solution is stirred for one hour more. 1M HCl was then added in order to neutralise the medium (pH = 7). This mixture was concentrated under vacuum. The remaining water was extracted by means of azeotropic distillation with toluene. The solid thus obtained was extracted with hot ethyl acetate. The solvent was removed under vacuum and the crude mannosane was purified by chromatography on a silica gel column (eluent : CH_2Cl_2/CH_3OH 90/10).Yield 22%).

Synthesis of the 1,6-anhydro-β-D-galactopyranoses[19]: This compound was synthesized in two steps by a base catalysed cyclisation of the 1-phenyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside[19].

- Synthesis of the 1-phenyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside: Penta-O-acetyl-β-D-galactopyranose (3 g, 7.49 mmol), p-toluene-sulfonic acid (0.039 g) and phenol (2.58 g, 30.3 mmol) were introduced in a 50 mL flask. This solution was heated to 95°C and submitted to vacuum (15 mm Hg) . The system was allowed to react for 1,5 hour under stirring. Then, after cooling, 10,5 mL of CH_2Cl_2 were added. The organic solution , washed with 50 mL of a 0,5 M NaOH solution and with 50 mL of water, was dried with Na_2SO_4. The solvent was eliminated and the product was cristallised with 9 mL of ethanol yielding 77% of pure 1-phenyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside.
- Synthesis of the1,6-anhydro-β-D-galactose: 1-phenyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (2.52 g, 5.9 mmol) was added to a solution of NaOH (3.9 g, 97.5 mmol in 72 mL of water). The mixture was refluxed for 5 days. After cooling, a 12 M aquous HCl

solution was slowly added until the pH reached the value of 3. The water was then evaporated and the residue was extracted twice with 12 mL of a chloroform/methanol (1/1) solution. This extract was filtered and the solvents were removed. The galactosane obtained was sufficiently pure for its use in the enzymatic transesterifications. Yield = 95%.

General procedure for the lipase catalysed transeterifications of the 1,6-anhydro-β-D-hexopyranoses : In a typical experiment 100 mg of the sugar is introduced into a flask with 0,5 ml of vinyl acetate and 100 mg of the lyophilized lipase preparation. The mixture is stirred at room temperature and the course of the reaction was monitored by means of TLC and ^1H NMR spectroscopy. At the end of the reaction the lipase is filtered, the excess of vinyl acetate eliminated under vacuum and the products of the reaction are separated over a silica gel column (eluent : see conditions for each product). The monoester is easily separated from the diesters which were obtained as a mixture.

4-O-acetyl-1,6-anhydro-β-D-glucopyranose : Gl-4

NMR ^1H ($CDCl_3$), 2.15 (s,3H, CH_3CO), 3.57 (m, 1H, H_2), 3.78 (m, 1H, H_3), 3.79 (m, 1H, H_{6exo}), 4.23 (d, 1H, J = 7.7Hz, H_{6endo}), 4.59 (d, 1H, J = 7.7Hz, H_5), 4.71 (s, 1H, H_4), 5.50 (s, 1H, H_1). NMR ^{13}C, 20 (q, J = 130Hz, CH3CO), 65.9 (t, J = 152Hz, C_6), 70.3 (d, J = 150Hz, C_2), 71.4 (d, J = 150Hz, C_3), 72.8 (d, J = 156Hz, C_4), 74.3 (d, J = 159Hz, C_5), 102 (d, J = 174Hz, C_1), 132 (s, CO). Anal. Calc for $C_8H_{12}O_6$: C,47.06; H,5.88. Found : C, 47.2; H,6.1, T_f = 120-123 °C. R_f = 0.37 ($MeOH/CH_2Cl_2$: 1/9). $[\alpha]_D^{22}$ -76, (c 0.25; $CHCl_3$).

2,4-di-O-acetyl-1,6-anhydro-β-D-glucopyranose : Gl-2,4

NMR ^1H ($CDCl_3$), 2.06 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 3.71 (m, 2H, H_3 et H_{6exo}), 4.13 (d, 1H, J = 7.7Hz, H_{6endo}), 4.54 (m, 3H, H_2, H_4 et H_5), 5.38 (s, 1H, H_1). NMR ^{13}C, 20.84 (q, J = 130Hz, CH_3CO), 65.50 (C_6), 69.11 (d, J = 150Hz, C_3), 72.17 et 73.07 (C_2 et C_4 ND), 73.94 (d, J = 150Hz, C_5), 99.54 (d, J = 177Hz, C_1), 170.46 (s, CO). R_f = 0.51 ($MeOH/CH_2Cl_2$: 1/9).

3,4-di-O-acetyl-1,6-anhydro-β-D-glucopyranose : Gl-3,4

NMR ^1H ($CDCl_3$), 2.03 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 3.43 (s, 1H, H_2), 3.71 (m, 1H, H_{6exo}), 4.03 (d, 1H, J = 7.7Hz, H_{6endo}), 4.54 (m, 2H, H_4 et H_5), 4.69 (s, 1H, H_3), 5.38 (s, 1H, H_1).NMR ^{13}C, 20.91 (q, J = 130Hz, CH_3CO), 65.15 (C_6), 67.86 (C_2), 70.25 (C_4), 71.82 (d, J = 160Hz, C_3), 73.56 (C_5), 101.28 (d, J = 177Hz, C_1), 170.34 (s, CO). The diacetates G-2,4 and G-3,4 were isolated as an oily mixture : Anal. Calc for $C_{10}H_{14}O_7$: C,48.78; H,5.69. Found : C, 48.6; H,5.8. R_f = 0.51 ($MeOH/CH_2Cl_2$: 1/9).

4-O-acetyl-1,6-anhydro-β-D-mannopyranose : M-4

NMR ^1H ($CDCl_3$), 2.10 (s, 3H, CH_3COO), 3.68 (m, 1H, J = 4.2Hz, H_2), 3.73 (d.d, 1H, J = 7.5Hz et 5.5Hz, H_{6exo}), 3.91 (d, 1H, J = 4.2Hz, H_3), 4.21 (d, 1H, J = 7.5Hz, H_{6endo}), 4.52 (d, 1H, J = 5.5Hz, H_5), 4.87 (s, 1H, H_4), 5.37 (s, 1H, H_1).

NMR ^{13}C, 20.87 (q, J = 130Hz, CH_3), 65.06 (d.d, J = 5.5 et 152Hz, C_6), 66.69 (d, J = 143Hz, C_2), 68.85 (d.d, J = 5.5 et 157Hz, C_3), 73.90 (C_4 et C_5), 101.60 (d.d, J = 3.7 et 174Hz, C_1), 170.08 (s, CO).

Anal. Calc for $C_8H_{12}O_6$: C,47.06; H,5.88. Found : C,47.0; H, 5.7.

T_f = 125-128 °C. R_f = 0.45 ($MeOH/CH_2Cl_2$: 1/9). $[\alpha]_D^{22}$ -112.5 (c1; $CHCl_3$).

2,4-di-O-acetyl-1,6-anhydro-β-D-mannopyranose : M-2,4

NMR ^1H ($CDCl_3$), 2.10 et 2.11 (2s, $2CH_3CO$), 3.76 (d.d, 1H, J = 7.5 et 5.8Hz, H_{6exo}), 3.78 (m, 1H, J = 5.9Hz, H_3), 4.04 (d.d,1H, J = 7.5Hz et 0.8Hz, H_{6endo}), 4.53 (d.d, 1H, J = 5.8 et 1.2Hz, H_5), 4.73 (t, 1H, J = 1.8Hz, H_4), 5.09 (d.d, 1H, J = 5,9 et 1,2Hz, H_2), 5,36 (t, 1H, J = 1.2Hz, H_1). NMR ^{13}C, 20.88 et 20.94 (2q, J = 2.07Hz, $2CH_3$), 65.09(C_6), 65.94 (C_3), 69.60 (C_2), 71.88 (C_4),73.61 (C_5), 101.34 (d.d, J = 3.7 et 174Hz, C_1), 169.94 et 169.88 (2s, 2CO). R_f = 0.60 ($MeOH/CH_2Cl_2$: 1/9).

3,4-di-O-acetyl-1,6-anhydro-β-D-mannopyranose : M-3,4

NMR ^1H ($CDCl_3$), 2.09 et 2.12 (2s, $2CH_3CO$), 3.74 (d.d, 1H, J = 7.4 et 5.7Hz, H_{6exo}), 4.04 (d, 1H, J =1.6Hz, H_3), 4.29 (d.d, 1H, J = 7.4 et 0.8Hz, H_{6endo}), 4.57 (d.d,1H, J =5.6 et 0.8Hz, H_5), 4.78 (d.d, 1H, J = 5.2 et 1.6Hz, H_2), 4.88 (d.d, 1H, J = 1.6Hz, H_4), 5.43 (d.d, 1H, J = 1.6Hz, H_1). NMR ^{13}C, 20.77 (2q, J = 2.07Hz, $2CH_3$), 65.38 (C_6), 68.27 (C_3), 69.40 (C_2), 73.51 (C_4), 74.18 (C_5), 99.77(d.d, J = 3.7 et 181Hz, C_1), 169.76 et 169.59 (2CO). The diacetates M-2,4 and M-3,4 were isolated as an oily mixture : Anal. Calc for $C_{10}H_{14}O_7$: C,48.78; H,5.69. Found : C,48.9; H,5.8. R_f = 0.60 ($MeOH/CH_2Cl_2$: 1/9).

2-O-acetyl-β-D-galactopyranose : Ga-4

NMR ^1H ($CDCl_3$), 2.08 (s, 3H, CH_3CO), 3.62 (d.d, 1H, J = 7.5 et 5.3Hz, H_{6exo}), 3.89 (m, 1H, H_3), 3.95 (m, 1H, H_4), 4.23 (d, J = 7.7Hz, 1H, H_{6endo}), 4.42 (d, 1H, J = 4.5Hz, H_5), 4.80 (s, 1H, H_2), 5.39 (s, 1H, H_1). NMR ^{13}C, 20.87 (q, J = 130Hz, CH_3CO), 63.67 (C_6), 64.57 (C_4), 68.59 (C_3), 72.66 (C_2), 74.64 (C_5), 99.16 (d, J = 180Hz, C_1), 169.94 (s, CO). Anal. Calc for $C_8H_{12}O_6$: C,47.06; H,5.88. Found : C,47.1; H, 5.9. T_f = 98-101°C. R_f = 0.41 ($MeOH/CH_2Cl_2$: 1/9). $[\alpha]_D^{22}$ +2.6 (c 0.38; $CHCl_3$).

2,4-di-O-acetyl-β-D-galactopyranose : Ga-2,4

NMR ^1H ($CDCl_3$), 2.09 et 2.12 (2s, 6H, $2CH_3CO$), 3.66 (m, 1H, H_{6exo}), 4.08 (m,1H, H_3), 4.36 (d, 1H, J = 7.7Hz, H_{6endo}), 4.48 (t, 1H, J = 4.5Hz, H_5), 4.79 (t, 1H, J = 1.5Hz, H_2), 4.99 (t, 1H, J = 4.5Hz, H_4), 5.43 (t, 1H, J = 1.5Hz, H_1).

NMR ^{13}C, 20.77 (q, J = 130Hz, $2CH_3$), 64.54 (C_6), 67.39 (C_3 et C_4), 72.09 (C_5), 72.69 (C_2), 99.44 (d, J = 174Hz, C_1), 169.76 (s, 2CO). R_f = 0.56 ($MeOH/CH_2Cl_2$: 1/9).

2,3-di-O-acetyl-β-D-galactopyranose : Ga-2,3
NMR ^1H (CDCl3), 2.09 et 2.13 (2s, 6H, CH_3CO), 3.66 (m, 1H, H_{6exo}), 4.18 (t, 1H, J = 4.7Hz, H_4), 4.23 (d, 1H, J =7.7Hz, H_{6endo}), 4.41 (t, 1H, J =4.7Hz, H_5), 4.73 (t, 1H, J = 1.5Hz, H_2), 5.05 (d.d, 1H, J = 1.5 et 5.5Hz, H_3), 5.36 (t, 1H, J = 1.5Hz, H_1). NMR ^{13}C, 20.89 (q, J = 130Hz, $2CH_3$), 63.76 (C_6), 64.54 (C_4), 69.93 (C_3), 70.89 (C_2), 73.89 (C_5), 98.55 (d, J = 174Hz, C_1), 171.25 (s, 2CO). Rf = 0.56 ($MeOH/CH_2Cl_2$: 1/9). The diacetates Ga-2,4 and Ga-2,3 were isolated as an oily mixture : Anal. Calc for $C_{10}H_{14}O_7$: C,48.78; H,5.69. Found : C,48.6; H,5.5.
2,3-acetal of galactosane
NMR ^1H ($CDCl_3$), 1.43 (s, 3H, CH_3), 3.58 (d.d, 1H, J = 5.5 et 7.5Hz, H_{6exo}), 3.89 (s.e, 1H, H_2), 3.96 (d, 1H, J = 7Hz, H_3), 4.10 (d, 1H, J = 7.5Hz, H_{6endo}), 4.36 (d.d, 1H, J = 5.5Hz, H_4), 4.46 (d.d, 1H, J = 5.5Hz, H_5), 4.98 (q, 1H, J = 4.9Hz, CH-CH_3), 5.36 (s, 1H, H_1). Anal. Calc for $C_8H_{12}O_5$: C,51.06; H,6.38. Found : C, 50.9; H, 6.3.

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