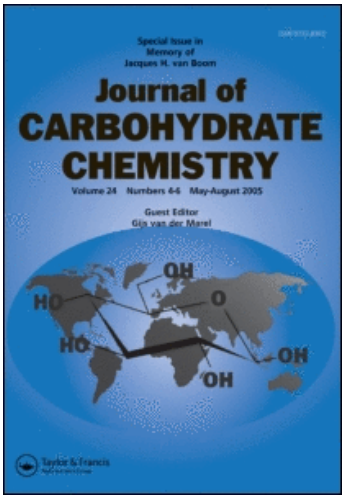


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# ON THE REGIOSELECTIVE ACYLATION OF 1,6-ANHYDRO-β-D- AND L-HEXOPYRANOSES CATALYSED BY LIPASES: STRUCTURAL BASIS AND SYNTHETIC APPLICATIONS

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## ABSTRACT

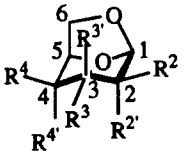
With the aim of providing new methods for the regioselective protection at the 2, 3 and 4 positions of monosaccharides, we have studied the acetylation of a class of rigid sugars: the 1,6-anhydro-β-D- and L-hexopyranoses (hexopyranosanes **D-1** to **D-5** and **L-1** to **L-5**), using vinyl acetate as an acyl donor and two common lipases,*Candida rugosa* and *Pseudomonas cepacia*, as catalysts. Our results indicate that the relative orientation of the hydroxyls governs the regioselectivity of acetylation. In the D-series, when the 3-OH is in the axial position, acetylation occurs mainly at the 4-axial OH, while the 2-axial OH is preferred when the 4-OH is equatorial. Conversely, when the 3-OH is equatorial, a strong selectivity affects the equatorial 2-OH. Compounds of the L-series were shown to be poor substrates for the lipase *Pseudomonas cepacia* except for L-galactosane for which the 2-monoacetyl ester was obtained in good yield. An attempt to rationalize the results by means of molecular modelling is also made to account for the catalytic activity of the *Candida rugosa* lipase on hexopyranosanes **1-3**.

## INTRODUCTION

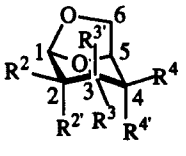
Saccharides, glycosides and glycoconjugates contain several hydroxyl groups of similar chemical reactivity and the regioselective protection of single hydroxyl function is

still a fundamental challenge for synthetic chemists. The regioselective acylation of hexopyranoses is of great interest in medicinal chemistry as conveniently protected sugars are involved in the synthesis of important di- and trisaccharides used as antitumors or anti-inflammatory drugs.[1] Long chain monoacylhexopyranoses (sucroesters) act also as surfactants in detergent formulations[2] and as emulsifiers in food.[3] Numerous chemical methods are available to achieve the selective acylation, but the processes described in the literature generally involve cumbersome and time consuming protection / deprotection steps.[4] Some previous attempts to selectively introduce an ester group in one step at the O-2, O-3 or O-4 positions of monosaccharides have resulted in complex mixtures and low yields.[5,6] Among the methods proposed, enzymatic catalysis has a particularly important place as it is able to induce stereoselective and regioselective reactions. Excellent reviews on this topic[7,8] reveal that lipases can be used with success to acylate regioselectively the C-6 OH function of furanoses and pyranoses. Nevertheless, few papers deal with the ability of the lipases to catalyse acylation of the C-2, C-3 and C-4 hydroxyl groups when the 1- and 6-hydroxy groups are already protected or absent.[9-11] In such reactions, the regioselectivity is strongly influenced either by the position (axial or equatorial) of the hydroxyl group or by the neighboring substituent. In order to have a better understanding of the factors governing the selectivity, we decided to study the behaviour of a rigid hexopyranose model in which the positions 1 and 6 were protected. In that sense, 1,6-anhydro-β-hexopyranoses (hexopyranosanes) are excellent candidates for such studies as the chair conformation is highly predominant.[12] Futhermore, the methods available to prepare the hexopyranosanes are in principle general ones, relatively easy to perform and allow the synthesis of most of the possible stereoisomers.[13-17] In addition, the literature reveals that the lipase-catalysed reactions of this class of compounds have not been extensively studied. Thus, the monodeacylation of triacyl 1,6-anhydro-β-D-glucopyranose in the presence of various lipases, has already been performed and the three possible isomeric diesters were selectively obtained in good yields when a suitable enzyme was chosen.[18-22] Similarly *Candida Rugosa* lipase (CRL) catalyses the hydrolysis of the 2-butanoate function of tributyrylgalactosane.[23] Surprisingly, it is noteworthy that the 1,6-anhydro-β-D-hexopyranoses were not used as substrates in the esterification or in the transesterification reactions. Furthermore, the studies on deacylation reactions are limited to D-glucosane and D-galactosane. In two preliminary papers, we have presented our results about the regioselective esterification of D-glucosane,[24] D-galactosane and D-mannosane[25] using vinyl acetate as an acyl donor. In this work, we have extended our study to the D-gulosane, D-allosane and to the five corresponding optical antipodes (L-series). We also report the results of a molecular modeling study in an attempt to explain, on a molecular basis, the regioselectivity of the *C. rugosa* lipase.

**Table 1.** List of the 1,6-anhydro-β-hexopyranosanes.



D-hexopyranosane



L-hexopyranosane

| compound | $R^2$ | $R^{2'}$ | $R^3$ | $R^{3'}$ | $R^4$ | $R^{4'}$ |
|---|---|---|---|---|---|---|
| **D-1, L-1** (glucosane) | H | OH | H | OH | H | OH |
| **D-2, L-2** (galactosane) | H | OH | H | OH | OH | H |
| **D-3, L-3** (mannosane) | OH | H | H | OH | H | OH |
| **D-4, L-4** (gulosane) | H | OH | OH | H | OH | H |
| **D-5, L-5** (allosane) | H | OH | OH | H | H | OH |



## RESULTS AND DISCUSSION

The acylation 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 by $^1H$ and $^{13}C$ NMR spectroscopy. All the reactions proceeded at a low rate and were stopped after 11 days of incubation. In order to avoid possible isomerisation of the esters obtained, the composition of the mixture was determined on aliquots of the reaction media by means of NMR spectroscopy. Furthermore, in order to verify the absence of acetyl group migration, we incubated the monoesters for four weeks in the presence of the lipases. In no case was a single acyl tranfer observed. This fact is notable because such isomerisations affect a number of mono and diesters derived from the triol system, and in particular for the glycerides.[26,27] The structure of synthesized esters was determined by comparison with the spectra of the 1,6-anhydro-β-hexopyranoses[28,29] and from literature data.[18,19,30] Verification of the assignments was performed by means of classical two dimensional NMR spectroscopy experiments (COSY, and C-H correlation).

The ten stereoisomers of the hexopyranosanes examined in this work are listed in table 1.

They are numbered as **D-n** and **L-n** (n = 1 to 5) depending on the D or L structure. According to this nomenclature, the monoesters are named **D-n(m)**, **L-n(m)** and the diesters **D-n(mp)**, **L-n(mp)** where m and p define the position of the acetyl chains on the

**Table 2.** Regioselectivities induced by the lipases PSL and CRL in the acetylation reaction of D- and L-hexopyranosanes.

| substrate | Yield[a] (%) | Lipase | monoacetates (%) (2) | (3) | (4) | diacetates (%) (2,3) | (2,4) | (3,4) |
|---|---|---|---|---|---|---|---|---|
| **D-1** | 99 | CRL[b] | 0 | 0 | 22 | 0 | 53 | 20 |
| **D-1** | 99 | PSL | 0 | 0 | 62 | 0 | 24 | 14 |
| **L-1** | 35 | PSL | 49 | 0 | 33 | 13 | 5 | 0 |
| **D-2** | 42 | CRL | 36 | 0 | 0 | 26 | 38 | 0 |
| **D-2** | 12 | PSL | 100 | 0 | 0 | 0 | 0 | 0 |
| **L-2** | 62 | PSL | 79 | 0 | 0 | 0 | total % = 21 | |
| **D-3** | 30 | CRL | 0 | 0 | 63 | 0 | 11 | 26 |
| **D-3** | 69 | PSL | 0 | 0 | 81 | 0 | 7 | 12 |
| **L-3** | 40 | PSL | 50 | 13 | 25 | 0 | 6 | 6 |
| **D-4** | 37 | PSL | 0 | 0 | 100 | 0 | 0 | 0 |

With compounds **L-4**, **D-5** and **L-5**, low yields (<5%) and poor regioselectivities were observed. a.Yields obtained after 11 days of incubation b.5% of the triacetate was also synthesized.

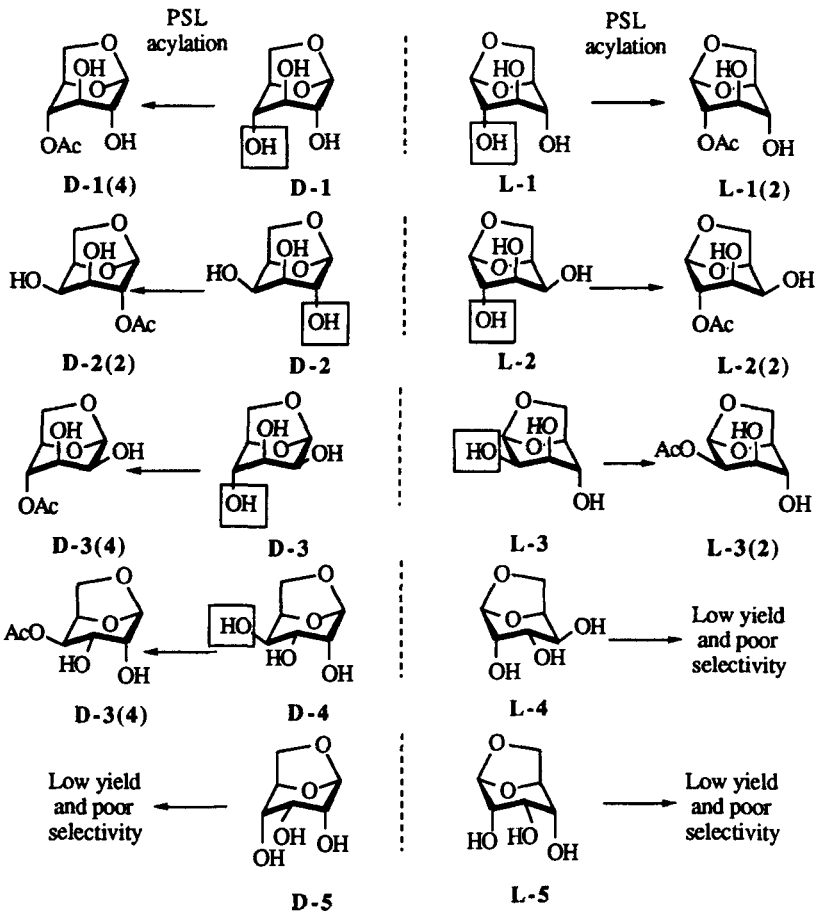


ring. For instance, the 4-monoacetyl-D-glucosane is **D-1(4)**. The results obtained are listed in table 2, they are also illustrated in Scheme 1.

In our preliminary work, we showed that the regioselectivity of the transesterification reactions was strongly dependent upon the lipase source.[24,25] Best results were obtained for glucosane and mannosane with lipase PSL (*Pseudomonas cepacia*). The use of CRL (*Candida rugosa* lipase) led to faster reactions exhibiting a similar tendency in the regioselectivity. However, as the percentages of diacetates were higher with the former, we limited the study of the transesterifications with all the hexopyranosanes to PSL.

Comparing the D- and L-series, it is clear that the D-compounds are better substrates for PSL, but the relative reactivities do not seem sufficiently different to allow an efficient kinetic resolution of the racemates. Thus, the acylation of **D-1** and **L-1** (D- and L-glucopyranose) under the same conditions lead to respective overall yields of 99% and 35%. Moreover, the case of galactosane constitutes an exception as the L enantiomer was shown to react faster than the D one.

Considering the relative reactivity of the three hydroxyl groups from a general point of view, the less reactive OH is at C-3. In addition, in the D-series, two types of compounds can be distinguished depending on the position of the 3-OH. When the 3-OH is axial a high regioselectivity is observed for the adjacent axial 4- or 2-OH. In that case,




**Scheme 1.** Enzymatic monoacetylation of the hexopyranosanes : regioselectivity induced by PSL

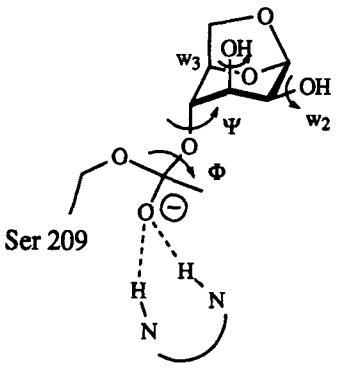
the 4-OH is always preferred (**D-1** and **D-3** give high yields respectively of monoacetates **D-1(4)** and **D-3(4)** ).

The acetylation of **D-2**, for which 4-OH is equatorial, affects the equatorial 2-OH giving only **D-2(2)** in low yields. Surprisingly, this selectivity was found again with the enantiomer **L-2** from which the ester **L-2(2)** was obtained in better yields (62%) than **D-2(2)** from **D-2**. A second class of hexopyranosane bears an equatorial 3-OH group (D- and L-gulosane and allosane). For these compounds, the enzymatic acylation proceeds generally with low yields and the selectivity is quite different: while in the former case the axial OH was preferred and particularly the C-4 one, the most reactive OH group is

equatorial at C-4. Thus gulosane **D-4** leads to the monoester **D-4(4)** with moderate yields, while allosanes **D-5** and **L-5** showed both very low reactivity and selectivity.

The advantage of the enzymatic catalysis over classical chemical methods is clearly demonstrated by a comparison with literature data. For instance, in the case of galactosane **D-2**, chemical acetylation provides a mixture of the 2,3- and 2,4-diacetates and very low yields of the 2-monoacetate.[5] Similarly, the acylation of **D-1** with various acid anhydrides and chlorides produces very low amounts of the corresponding 4-monoesters.[6b,e,f]

From these results, it is obvious that the spatial orientation of the hydroxyl groups plays an essential role in the regioselective acylation of the 1,6-anhydro-β-hexopyranoses by lipase as it has been already suggested for primary alcohols.[31] The origin of this regioselectivity is probably based on a differential binding of the corresponding transition states within the lipase active site. To test this hypothesis, we undertook molecular modelling studies of the different binding modes of the acetylated hexopyranosanes at the transition state. Kinetics measurements and inhibition studies suggested that the reaction mechanism of lipases involves acylation and deacylation steps similar to those of serine proteases.[32,33] According to this mechanism, the transacylation of hexopyranosanes proceeds first by an acylation of the enzyme resulting from the nucleophilic attack on the vinyl acetate by the Ser residue of the catalytic triad, through the formation of a first tetrahedral intermediate. Deacylation results from nucleophilic attack on the acyl-enzyme by a hexopyranosane OH, again through a tetrahedral intermediate. In both the acylation and deacylation intermediates, an oxyanion is stabilized by hydrogen bonds to the protein atom of the oxyanion hole. According to this mechanism for the transesterification, it seems clear that the regioselectivity of the reactions should be governed by a differential energy stabilization of the second transition state depending on the hexopyranosane-OH nucleophile. As a starting structure for molecular modelling, we used the known crystal structure at 2.2 Å of the *C. Rugosa* lipase complexed with a transition state analogue (CRL-(Rp)-1*R* -menthyl phosphonate).[34,35] The atoms around the tetrahedral carbon of the transition state, the oxyanion, the oxygens of the attacking hexopyranosane OH and of the active serine were positioned according to the structure of the CRL-(Rp)-1*R* -phosphonate so that all catalytically essential hydrogen bonds were formed. The methyl group was then located in the hydrophobic tunnel initially occupied by the hexyl chain of the phosphonate. The docking of the hexopyranosane ring was facilitated by the relative rigidity of the bicycle so that only four degrees of freedom had to be considered (Scheme 2). The search for energy minima was done by the combined use of a systematic variation of the two torsional angles $\phi$ and $\psi$ that determine the orientation of the hexopyranosane within the active site and a random walk approach for the orientation ($\omega_i$) of the two remaining OH groups.




**Scheme 2.** D-3 transition state corresponding to an acylation at the 4-position with the torsion angles of interest for the conformational analysis.

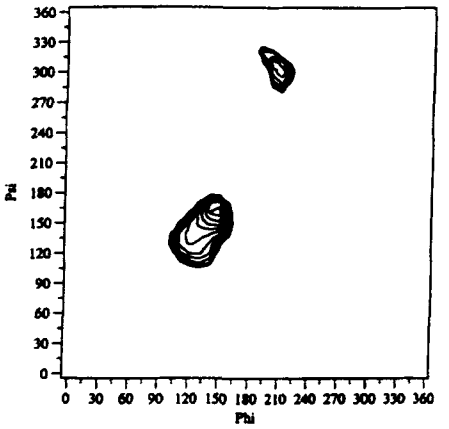



**Figure 1.** "Rigid" potential energy surface of the CRL-4-*O*-acetylmannosane transition state complex as a function of Φ and Ψ torsion angles, calculated with a 10° increment. Isoenergy contours are drawn by interpolation of 1 kcal.mol$^{-1}$.

Figure 1 represents a typical potential energy map obtained with CRL-D-3(4) and demonstrates that the low energy domain is relatively restricted. Each complex at local energy minima was then subjected to energy minimization. The calculated lowest energy conformation for the 9 different complexes (three for each of glucosane, galactosane and mannosane) was considered as a global energy minimum.

The conformations of the active site amino-acid residues exhibit minor changes during the optimization processes compared to that of starting x-ray structures. In each minimized complex, there were still strong interactions between the oxyanion of the

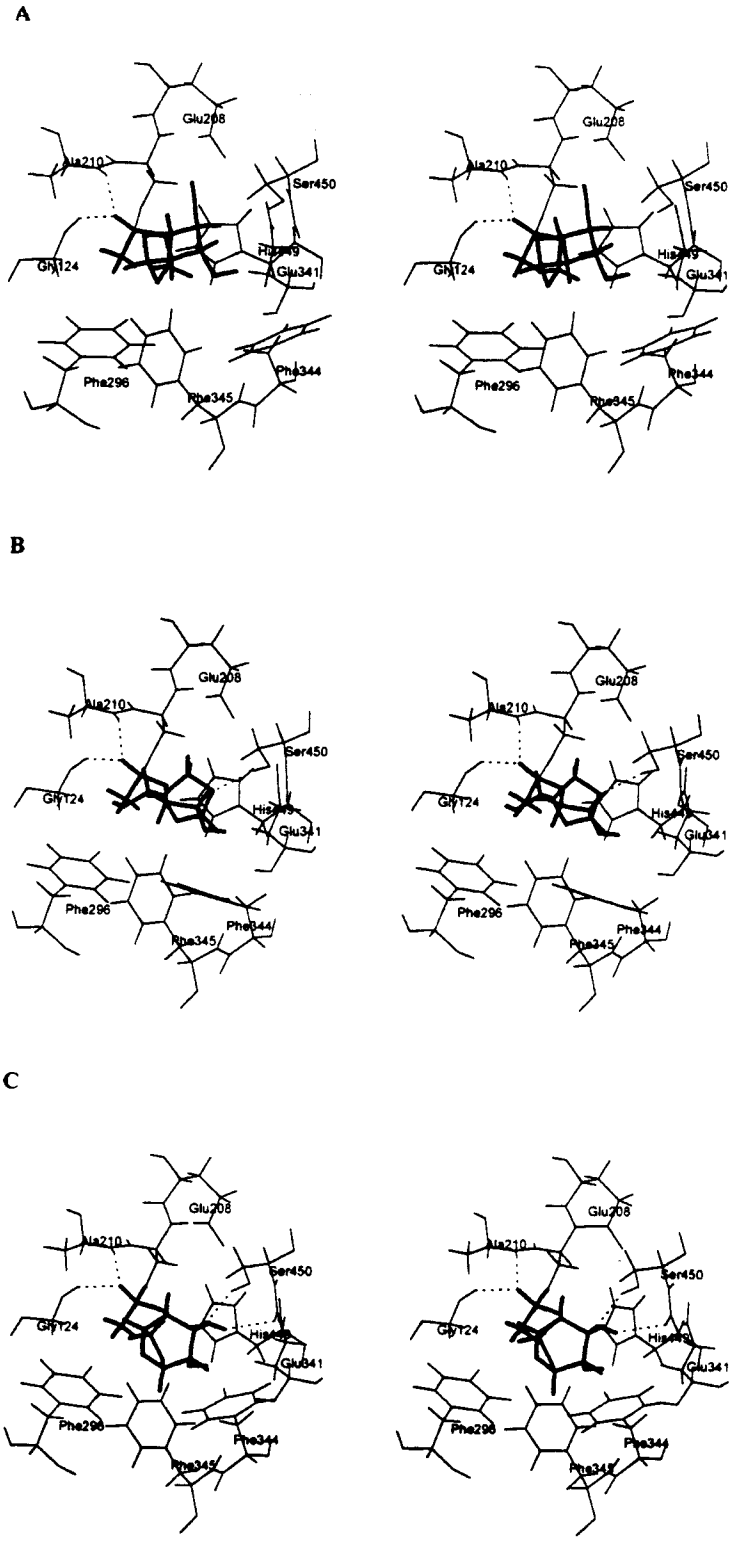


**Table 3.** Hydrogen bonds between hexopyranosane and CRL at the predicted transition state in the three possible acetylation positions.

| Compound | Donor | Acceptor | Distance (Å) D...A [a] | Angle (°) D-H...A |
|---|---|---|---|---|
| **D-1(2)** | - | - | | |
| **D-1(3)** | O-6 | Ser450 OH | 2.98 | 137 |
| **D-1(4)** | OH-3 | Ser450 OH | 2.93 | 106 |
| | His449 O | OH-3 | 3.40 | 148 |
| **D-2(2)** | OH-4 | Ser450 OH | 2.89 | 95 |
| **D-2(3)** | O-1 | Ser450 OH | 3.01 | 135 |
| **D-2(4)** | O-1 | Ser450 OH | 3.34 | 135 |
| **D-3(2)** | - | - | | |
| **D-3(3)** | OH-4 | Ser450 OH | 3.07 | 122 |
| **D-3(4)** | OH-3 | Ser450 OH | 2.95 | 125 |

a.The cut-off distance for hydrogen bonding was set at 3.5 Å.



tetrahedral intermediate and the oxyanion hole H-bonding residues of the peptide backbone NH's of Gly123 and Ala210 (Figure 2). Attempts to correlate the calculated energies of the minimized structures with the kinetics of the regioselective acylation of the hexopyranosanes were unsuccessful, probably because of the non-optimized force field parameters used in the calculations. However, the hydrogen-bonding scheme is consistent with the observed regioselectivity. This hydrogen-bond network of the optimized hexopyranosane/CRL complexes is listed in Table 3.

In each of the minimized complexes that lead to the monoesters **D-1(4)**, **D-2(2)** and **D-3(4)**, which correspond to the experimental regioselectivity, adjacent hydroxyl groups are able to form one or two hydrogen bonds to the Oγ of the Ser450 or the carbonyl oxygen of the His449. This favorable hydrogen bonding is either not present in the other computed complexes or weaker due to a larger donor-acceptor distance.

**Figure 2.** Stereoscopic representation of the lowest energy conformation of CRL-glucosane complexes at transition state: (A) **D-1(2)**; (B) **D-1(3)**; (C) **D-1(4)**. The oxyanion of the tetrahedral complexes is located in the oxyanion hole, with the negative charge on the oxygen stabilized by hydrogen-bonding with the peptide NH of Gly123 and Ala210. In the **D-1(4)** complex, two additional hydrogen bonds are indicated between the OH-3 of glucosane and the Oγ of Ser450 and the O of His449. The calculated ΔΔG values of **D-1(2)** and **D-1(3)** complexes compared to the **D-1(4)** complex were respectively 16.9 and 16.3 kcal/mol.

Furthermore, it may seem surprising that such sugar derivatives could interact and be processed within the lipase active site considering the presence of the large number of hydrophobic residues. In fact, recent three dimensional structures obtained from carbohydrate-protein complexes (such as lectins) indicate that most carbohydrate binding sites contain a high number of hydrophobic aminoacids.[36] In such structures the aromatic aminoacids pack against the apolar patch on the sugars.[37,38] Indeed, in the minimized complexes corresponding to the observed regioselectivity, the apolar part of the hexopyranosane pointed towards a hydrophobic cluster of phenylalanines, Phe296 and Phe345.

## CONCLUSION

This work shows the importance of lipases in the regioselective protection of the monosaccharides. Several authors have pointed out that the anomeric configuration plays an important role in the course of the regioselectivity induced by lipases.[39,40] Our results, obtained with the lipase catalyzed acetylation of rigid D- and L-hexopyranosanes, indicate that the relative orientation of the hydroxyls also governs the regioselectivity at the 2, 3, 4 positions. An attempt to rationalize the results is also made to account for the catalytic activity of the *Candida rugosa* lipase on hexopyranosanes **1-3** by means of molecular modelling. This structural approach suggests that regioselective acylation is governed by a preferential binding mode of the corresponding acetyl anhydropyranose at the transition state.



## EXPERIMENTAL

**Materials and methods.** The lipases were purchased from Amano. The D-glucosane was supplied by Aldrich while the other D- and L-hexopyranosanes were prepared according to slightly modified procedures described in ref. 13 and 14. All the parent sugars and vinyl acetate were purchased from Aldrich and used without further purification. The course of reactions was followed by means of TLC using precoated silica gel 60 sheets Merck F254, (see eluent for each compound). The products were separated on silica gel columns (Merck 60, 230-400 mesh). $^{13}C$ and $^{1}H$ 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 resonance was made using standard 2D sequences (COSY H-H and $^{13}C$-$^{1}H$ correlations) on a 500 MHz Bruker spectrometer. The optical rotations were determined with an "Optical Activity - AA10" polarimeter.

**Synthesis of the 1,6-anhydro-β-D- or L-mannopyranoses (D-3 or L-3).**[13] 3.3 g (18 mmol) of tosyl chloride in 15 mL of pyridine were slowly added at 0 °C to a solution of 3 g (16.5 mmol) of D- or L-mannose in 45 mL of pyridine. The solution was stirred at room temperature for 4 h and the reaction was quenched by the addition of 30 mL of water. The pH was adjusted to 9 by means of 1M NaOH and the solution was stirred for 1 h. 1M HCl was added to neutralise the medium (pH = 7) and the mixture was concentrated under vacuum. The remaining water was removed 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, $R_f$=0.10). Yield 80%.

**Synthesis of other 1,6-anhydro-β-D-hexopyranoses (L-1, D-2, L-2, D-4, L-4, D-5 and L-5).**[14] The compound was synthesized in two steps by a base catalysed cyclisation of the 1-phenyl-2,3,4,6-tetra-*O* -acetyl-β-D-hexopyranoside.

- *Synthesis of the 1-phenyl-2,3,4,6-tetra-O-acetyl-β-D-hexopyranoside :* Penta-*O* -acetyl-β-D-hexopyranose (3 g, 7.49 mmol), *p* -toluenesulfonic acid (0.039 g) and phenol (2.58 g, 30.3 mmol) were introduced into a 50 mL flask. This mixture was heated to 95 °C and submitted to vacuum (15 mm Hg) . The system was allowed to react for 1.5 h with stirring, then cooled and diluted with 10.5 mL of $CH_2Cl_2$. The organic solution was washed successively with 50 mL of a 0.5 M NaOH solution and with 50 mL of water, then dried with $Na_2SO_4$. The solvent was removed and the product crystallised from 9 mL of ethanol yielding 50-80% of pure 1-phenyl-2,3,4,6-tetra-*O* -acetyl-β-D-hexopyranoside.

- *Synthesis of the 1,6-anhydro-β-D-hexopyranose.* 1-phenyl-2,3,4,6-tetra-*O*-acetyl-β-D-hexopyranoside (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 and, after cooling, a 12 M aqueous HCl solution was slowly added to a pH 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 white crystals of the hexopyranosanes thus obtained were sufficiently pure for their use in the enzymatic transesterifications. Yield = 95%.

**General procedure for the lipase catalysed transesterifications of the 1,6-anhydro-β-D-hexopyranoses.** In a typical experiment, 100 mg of the monosaccharide was introduced into a flask with 0.5 mL of vinyl acetate and 100 mg of the lyophilized lipase preparation. The mixture was 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 was filtered, the excess of vinyl acetate removed under vacuum and the products of the reaction separated using a silica gel column (eluent: see

conditions for each product). The monoester was easily separated from the diesters which were obtained as a mixture .

**4-*O*-Acetyl-1,6-anhydro-β-glucopyranoses. D-1(4) and L-1(4).** $^1H$ NMR ($CDCl_3$), 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 = 7.7 Hz, $H_{6endo}$), 4.59 (d, 1H, J = 7.7 Hz, $H_5$), 4.71 (s, 1H, $H_4$), 5.50 (s, 1H, $H_1$). NMR $^{13}C$, 20 (q, J = 130 Hz, $CH_3CO$), 65.9 (t, J = 152 Hz, $C_6$), 70.3 (d, J = 150 Hz, $C_2$), 71.4 (d, J = 150 Hz, $C_3$), 72.8 (d, J = 156 Hz, $C_4$), 74.3 (d, J = 159 Hz, $C_5$), 102 (d, J = 174 Hz, $C_1$), 132 (s, CO).

Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.88. **D-1(4).** Found: C, 47.21; H, 6.09, white solid mp 120-123 °C. $R_f$ = 0.37 ($MeOH/CH_2Cl_2$ : 1/9). $[\alpha]_D^{22}$ -76, (*c* 0.25; $CHCl_3$). **L-1(4).** Found : C, 46.89; H, 5.92, white solid mp 119-122 °C. $R_f$ = 0.37 ($MeOH/CH_2Cl_2$ : 1/9). $[\alpha]_D^{22}$ +75, (*c* 0.25; $CHCl_3$).

**2,4-Di-*O*-acetyl-1,6-anhydro-β-glucopyranoses : D-1(2,4) and L-1(2,4).** $^1H$ NMR ($CDCl_3$), 2.06 (s, 3H, $CH_3CO$), 2.08 (s, 3H, $CH_3CO$), 3.71 (m, 2H, $H_3$ and $H_{6exo}$), 4.13 (d, 1H, J = 7.7 Hz, $H_{6endo}$), 4.54 (m, 3H, $H_2$, $H_4$ and $H_5$), 5.38 (s, 1H, $H_1$). $^{13}C$ NMR, 20.84 (q, J = 130 Hz, $CH_3CO$), 65.50 ($C_6$), 69.11 (d, J = 150 Hz, $C_3$), 72.17 and 73.07 ($C_2$ and $C_4$ ND), 73.94 (d, J = 150 Hz, $C_5$), 99.54 (d, J = 177 Hz, $C_1$), 170.46 (s, CO). $R_f$ = 0.51 ($MeOH/CH_2Cl_2$ : 1/9).

**3,4-Di-*O*-acetyl-1,6-anhydro-β-glucopyranoses. D-1(3,4) and L-1(3,4).** $^1H$ NMR ($CDCl_3$), 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 = 7.7 Hz, $H_{6endo}$), 4.54 (m, 2H, $H_4$ and $H_5$), 4.69 (s, 1H, $H_3$), 5.38 (s, 1H, $H_1$). $^{13}C$ NMR, 20.91 (q, J = 130 Hz, $CH_3CO$), 65.15 ($C_6$), 67.86 ($C_2$), 70.25 ($C_4$), 71.82 (d, J = 160 Hz, $C_3$), 73.56 ($C_5$), 101.28 (d, J = 177 Hz, $C_1$), 170.34 (s, CO). The diacetates **D-1(2,4)** and **D-1(3,4)** or **L-1(2,4)** or **L-1(3,4)** were isolated as oily mixtures. $R_f$ = 0.51 ($MeOH/CH_2Cl_2$ : 1/9).

Anal. Calcd for $C_{10}H_{14}O_7$: C, 48.78; H, 5.69. **D-1(2,4)** and **D-1(3,4).** Found: C, 48.65; H, 5.78 ; **L-1(2,4)** and **L-1(3,4).** Found: C, 48.82; H, 5.79.

**4-*O*-Acetyl-1,6-anhydro-β-D-mannopyranoses D-3(4) and L-3(4).** $^1H$ NMR ($CDCl_3$), 2.10 (s, 3H, $CH_3CO$), 3.68 (m, 1H, J = 4.2 Hz, $H_2$), 3.73 (dd, 1H, J = 7.5 Hz and 5.5 Hz, $H_{6exo}$), 3.91 (d, 1H, J = 4.2 Hz, $H_3$), 4.21 (d, 1H, J = 7.5 Hz, $H_{6endo}$), 4.52 (d, 1H, J = 5.5 Hz, $H_5$), 4.87 (s, 1H, $H_4$), 5.37 (s, 1H, $H_1$). $^{13}C$ NMR, 20.87 (q, J = 130 Hz, $CH_3$), 65.06 (d.d, J = 5.5 and 152 Hz, $C_6$), 66.69 (d, J = 143 Hz, $C_2$), 68.85 (dd, J = 5.5 and 157 Hz, $C_3$), 73.90 ($C_4$ and $C_5$), 101.60 (dd, J = 3.7 and 174 Hz, $C_1$), 170.08 (s, CO). $R_f$ = 0.45 ($MeOH/CH_2Cl_2$ : 1/9).

Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.88. **D-3(4).** Found : C, 47.21; H, 5,71. mp 125-128°C. $[\alpha]_D^{22}$ -112.5 (*c* 1; $CHCl_3$) .

**2-*O*-Acetyl-1,6-anhydro-β-L-mannopyranose L-3(2).** $^1$H NMR ($CDCl_3$), 2.16 (s, 3H, $CH_3CO$), 3.80 (m, 1H, $H_{6exo}$), 3.90 (m, 1H, $H_4$), 4.11 (m, 1H, $H_3$), 4.33 (d, 1H, J = 7.3 Hz, $H_{6endo}$), 4.54 (m, 1H, $H_5$), 4.85 (d, 1H, J = 4.8 Hz, $H_2$), 5.45 (s, 1H, $H_1$).

**3-*O*-Acetyl-1,6-anhydro-β-L-mannopyranose L-3(3).** $^1$H NMR ($CDCl_3$), 2.13 (s, 3H, $CH_3CO$), 3.52 (m, 1H, $H_2$), 3.80 (m, 1H, $H_{6exo}$), 3.82 - 4.40 (m, 2H, $H_4$ and $H_{6endo}$), 4.48 (m, 1H, $H_5$), 5.09 (m, 1H, $H_3$), 5.38 (d, 1H, J = 1.1 Hz, $H_1$).The three monoacetates **L3(2), L-3(3)** and **L-3(4)** were obtained as an oily mixture

Analysis of this mixture: Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.88. Found: C, 47.13; H, 5,73.

**2,4-Di-*O*-acetyl-1,6-anhydro-β-mannopyranoses D-3(2,4) and L-3(2,4).** $^1$H NMR ($CDCl_3$), 2.10 and 2.11 (2s, $2CH_3CO$), 3.76 (dd, 1H, J = 7.5 and 5.8 Hz, $H_{6exo}$), 3.78 (m, 1H, J = 5.9 Hz, $H_3$), 4.04 (dd, 1H, J = 7.5 Hz and 0.8 Hz, $H_{6endo}$), 4.53 (dd, 1H, J = 5.8 and 1.2 Hz, $H_5$), 4.73 (t, 1H, J = 1.8 Hz, $H_4$), 5.09 (dd, 1H, J = 5.9 and 1.2Hz, $H_2$), 5,36 (t, 1H, J = 1.2 Hz, $H_1$). $^{13}$C NMR, 20.88 and 20.94 (2q, J = 2.07 Hz, $2CH_3$), 65.09($C_6$), 65.94 ($C_3$), 69.60 ($C_2$), 71.88 ($C_4$), 73.61 ($C_5$), 101.34 (dd, J = 3.7 and 174 Hz, $C_1$), 169.94 and 169.88 (2s, 2CO). $R_f$ = 0.60 (MeOH/$CH_2Cl_2$ : 1/9).

**3,4-Di-*O*-acetyl-1,6-anhydro-β-mannopyranoses. D-3(3,4) and L-3(3,4).** $^1$H NMR ($CDCl_3$), 2.09 and 2.12 (2s, $2CH_3CO$), 3.74 (dd, 1H, J = 7.4 and 5.7 Hz, $H_{6exo}$), 4.04 (d, 1H, J =1.6 Hz, $H_3$), 4.29 (d.d, 1H, J = 7.4 and 0.8 Hz, $H_{6endo}$), 4.57 (dd,1H, J =5.6 and 0.8 Hz, $H_5$), 4.78 (dd, 1H, J = 5.2 and 1.6 Hz, $H_2$), 4.88 (dd, 1H, J = 1.6 Hz, $H_4$), 5.43 (dd, 1H, J = 1.6 Hz, $H_1$). $^{13}$C NMR, 20.77 (2q, J = 2.07 Hz, $2CH_3$), 65.38 ($C_6$), 68.27 ($C_3$), 69.40 ($C_2$), 73.51 ($C_4$), 74.18 ($C_5$), 99.77(dd, J = 3.7 and 181 Hz, $C_1$), 169.76 and 169.59 (2CO). $R_f$ = 0.60 (MeOH/$CH_2Cl_2$ : 1/9). The diacetates **D-3(2,4)** and **D-3(3,4)** or **L-3(2,4)** and **L-3(3,4)** were isolated as oily mixtures.

Anal. Calcd for $C_{10}H_{14}O_7$: C, 48.78; H, 5.69. Found for D-diacetates: C, 48.91; H, 5.81 and for L-diacetates : C, 48.87; H, 5.52.

**2-*O*-Acetyl-β-galactopyranoses D-2(2) and L-2(2).** $^1$H NMR ($CDCl_3$) 2.08 (s, 3H, $CH_3CO$), 3.62 (dd, 1H, J = 7.5 and 5.3 Hz, $H_{6exo}$), 3.89 (m, 1H, $H_3$) 3.95 (m, 1H, $H_4$), 4.23 (d, J = 7.7 Hz, 1H, $H_{6endo}$), 4.42 (d, 1H, J = 4.5 Hz, $H_5$), 4.8( (s, 1H, $H_2$), 5.39 (s, 1H, $H_1$). $^{13}$C NMR, 20.87 (q, J = 130 Hz, $CH_3CO$), 63.67 ($C_6$) 64.57 ($C_4$), 68.59 ($C_3$), 72.66 ($C_2$), 74.64 ($C_5$), 99.16 (d, J = 180 Hz, $C_1$), 169.94 ( CO). $R_f$ = 0.41 (MeOH/$CH_2Cl_2$ : 1/9).

Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.88. Found: **D-2(2)**, C, 47.09; H, 5.9

**L-2(2)**, C, 47.17; H, 5.73. mp 98-101 °C. **D-2(2)**: $[\alpha]_D^{22}$ +2.6 (*c* 0.38; $CHCl_3$). **L-2(2)**: $[\alpha]_D^{22}$ -2.9 (*c* 0.22; $CHCl_3$).

**2,4-Di-*O*-acetyl-β-galactopyranoses. D-2(2,3) and L-2(2,4).** $^1H$ NMR ($CDCl_3$), 2.09 and 2.12 (2s, 6H, $2CH_3CO$), 3.66 (m, 1H, $H_{6exo}$), 4.08 (m,1H, $H_3$), 4.36 (d, 1H, J = 7.7 Hz, $H_{6endo}$), 4.48 (t, 1H, J = 4.5 Hz, $H_5$), 4.79 (t, 1H, J = 1.5 Hz, $H_2$), 4.99 (t, 1H, J = 4.5 Hz, $H_4$), 5.43 (t, 1H, J = 1.5 Hz, $H_1$). $^{13}C$ NMR, 20.77 (q, J = 130 Hz, $2CH_3$), 64.54 ($C_6$), 67.39 ($C_3$ and $C_4$), 72.09 ($C_5$), 72.69 ($C_2$), 99.44 (d, J = 174 Hz, $C_1$), 169.76 (s, 2CO). $R_f$ = 0.56 ($MeOH/CH_2Cl_2$: 1/9).

**2,3-Di-*O*-acetyl-β-D-galactopyranoses. D-2(2,3) and L-2(2,3).** $^1H$ NMR ($CDCl_3$), 2.09 and 2.13 (2s, 6H, $CH_3CO$), 3.66 (m, 1H, $H_{6exo}$), 4.18 (t, 1H, J = 4.7 Hz, $H_4$), 4.23 (d, 1H, J =7.7 Hz, $H_{6endo}$), 4.41 (t, 1H, J =4.7 Hz, $H_5$), 4.73 (t, 1H, J = 1.5 Hz, $H_2$), 5.05 (d.d, 1H, J = 1.5 and 5.5 Hz, $H_3$), 5.36 (t, 1H, J = 1.5 Hz, $H_1$). $^{13}C$ NMR, 20.89 (q, J = 130 Hz, $2CH_3$), 63.76 ($C_6$), 64.54 ($C_4$), 69.93 ($C_3$), 70.89 ($C_2$), 73.89 ($C_5$), 98.55 (d, J = 174 Hz, $C_1$), 171.25 (s, 2CO). $R_f$ = 0.56 ($MeOH/CH_2Cl_2$: 1/9). The diacetates **D-2(2,4)** and **D-2(2,3)** or **L-2(2,4)** and **L-2(2,3)** were isolated as oily mixtures.

Anal. Calcd for $C_{10}H_{14}O_7$: C, 48.78; H, 5.69. Found for **D-2(2,4)** and **D-2(2,3)**: C, 48.59; H, 5.53 and for **L-2(2,4)** and **L-2(2,3)**: C, 48.91; H, 5.61 .

**4-*O*-Acetyl-1,6-anhydro-β-D-gulopyranose D-4(4).** $^1H$ NMR ($CDCl_3$), 2.11 (s, 3H, $CH_3CO$), 3.67 (ddd, 1H, J = 0.8, 4.7 and 7.95 Hz, $H_{6exo}$), 3.82 (dd, 1H, J = 4.76 and 8.90 Hz, $H_3$), 3.88 (dd, 1H, J = 2 and 4.76 Hz, $H_2$), 3.96 (d, 1H, J = 7.95 Hz, $H_{6endo}$), 4.51 (dd, 1H, J = 4.4 and 4.7 Hz, $H_5$), 4.93 (ddd, 1H, J = 0.8, 4.4 and 8.90 Hz, $H_4$), 5.48 (d, 1H, J = 2 Hz, $H_1$). $^{13}C$ NMR ($CDCl_3$), 21.51 (q, J =130 Hz, $CH_3$), 64.98 ($C_6$), 68.75 ($C_3$), 71.48 ($C_2$), 72.87 ($C_5$), 73.98 ($C_4$), 102.17 ($C_1$). $R_f$ = 0.45 ($MeOH/CH_2Cl_2$ (1/9).

Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.88. Found: C, 47.20; H, 5,79.

**Computational methods.** Coordinates for the starting structure of the CRL-(Rp)-1*R* menthyl phosphonate[34] complex were obtained from the Brookhaven National Laboratory Protein Data Bank.[41] All calculations were performed with a PC computer 486DX4/100 equipped with the molecular modelling software Hyperchem version 4.5 (Hypercube, Inc., 1995). Water molecules present in the initial structure derived from x-ray crystallographic studies were excluded from all calculations. For reasons of computer time, it was not possible to take into account the entire protein in the energy minimization calculations. Therefore, a region around the binding site was selected, consisting of a sphere of 15 Å radius centered on the P atom of the menthyl phosphonate complex. The protein was held rigid during all mechanics calculations except for the amino-acid side chains within 5 Å of the hexapyranosane ring. Minimizations of each complexes were

performed with the $MM^+$ Hyperchem force field, using a distance dependent dielectric constant and a nonbonded cut-off distance of 12 Å . Hexopyranosanes were drawn and minimized in vacuo in the chair conformation[12] before docking in the active site. To set up the initial structure for the conformational analysis of each transition state complex, the phosphorus and the pentanyl chain of the CRL-menthyl phosphonate complex were replaced respectively by a $sp_3$ carbon and a methyl group. The menthol ring was then replaced by the hexopyranosane ring and connected to the tetrahedral carbon according to the desired regiospecificity. Each transition state complex was then energy-minimized while keeping the protein atom rigid and fixing the reacting atoms into the desired transition state geometry. The conformational search for local energy minima was done by varying the following torsional angles for the linkage between the hexopyranosane ring and the tetrahedral carbon C*:

$$\Phi = \Theta(O_{\gamma Ser209} - C^* - O_i - C_i)$$

$$\Psi = \Theta(C^* - O_i - C_i - C_{i-1})$$

and the hydroxylic hydrogen at position i of the hexopyranosane:

$$\omega_i = \Theta(C_{i-1} - C_i - O_i - H_i)$$

The $\Phi$ and $\Psi$ torsion angles were systematically varied in steps of 10° over the whole angular range. At each step, the energy was calculated and isoenergy surfaces were then represented as a function of $\Phi$ and $\Psi$ for each complex. As the conformational energy of hexopyranosanes also depends on the orientation of the two remaining hydroxyl groups, the best orientation of these rotatable groups was determined by random variation of the dihedral angles using the Conformational Search Module of Chemplus (version 1.5, Hypercube, Inc., 1995). Three conformers corresponding to the energy minima of each CRL-transition state complex were further refined through energy minimization. The restraints were removed from all side-chain residues of the active site and the energy minimized using conjugate gradients until the maximum derivative was <0.01 kcal/(mol.Å). The conformers with the lowest energy were selected and considered to represent the global energy minimum of the complexes.



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## REFERENCES

1. P.J. Garegg, *Acc. Chem. Res.*, **25**, 575 (1992).

2. M.J. Donnelly and J. D. Bu'lock, *J. Am. Oil Chem. Soc*, **65** , 284 (1988).
3. J.P. Gibbons and C.J. Swanson, *J. Am. Oil Chem. Soc.*, **36**, 553 (1959).
4. T.W. Greene and P.G.M. Wuts, in *Protective Groups in Organic Synthesis*, 2nd ed Wiley and Sons, New York, 1991.
5. M. E. Chacon-Fuertes and M. Martin-Lomas, *Carbohydr. Res.*, **42**, C4-C5 (1975).
6. a) T.B. Grindley and R. Thangarasa, *Carbohydr. Res.*, **172**, 311 (1988).
   b) J.M. MacLeod, L.R. Schroeder and P.A. Seib, *Carbohydr. Res.*, **30**, 337, (1973).
   c) D. Shapiro, A.J. Acher, Y. Rabinsohn and A. Diver-Haber, *J. Org. Chem.*, **36**, 832 (1971).
   d) M. Cerny, J. Stanek and J. Pacàk, *Collect. Czech. Chem. Comm.*, **34**, 849 (1969).
   e) M. Cerny, V. Gut and J. Pacàk, *Collect. Czech. Chem. Comm.*, **26**, 2542 (1961).
   f) P.A. Seib, *Carbohydr. Res.*, **8**, 101 (1968).
7. D.G. Drueckhammer, W.J. Hennen, C.F. Pederson, R.L.Barbas, C.M. Gautheron, T. Krach and C.H. Wong, *Synthesis*, 499 (1991).
8. H. Waldmann and D. Sebastian, *Chem. Rev.*, **94** , 911 (1994).
9. D. Colombo, F. Ronchetti and L. Toma, *Tetrahedron*, **47**, 103 (1991).
10. M.J. Chinn, G. Lacazio, D.G. Spackman, N.J. Turner and S.M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 661 (1992).
11. R. Lopez, E. Montero, F. Sanchez, J. Canada and A. Fernandez-Mayoralas, *J. Org. Chem.*, **59** , 7027 (1994).
12. T.B. Grindley, A. Cude, J. Kralovic and R. Thangarasa, in *Levoglucosenone and Levoglucosans, Chemistry and Applications*; Z.J. Witczak Ed., ATL Press, Inc., Science Publishers, Mount Prospect, II, USA, 1994; pp 147-164.
13. M. Cerny and J. Stanek, *J. Adv. Carbohydr. Chem. Biochem.*, **34**, 23 (1977).
14. M.A. Zattola, R. Alonso and B. Fraser-Reid, *J. Org. Chem.*, **54**, 6123 (1989).
15. M. Georges and B. Fraser-Reid, *Carbohydr. Res.*, **127**, 162 (1984).
16. P.A. Gent, R. Gigg and A.A. Penglis, *J. Chem. Soc., Perkin Trans. 1*, 1395 (1976).
17. M. Kloosterman, J.G. Weijnen, N.K. De Vries, J. Mentech, I. Caron, G. Descotes, H.E. Schœmaker and E.M. Meijer, *J. Carbohydr. Chem.*, **8**, 693 (1989).
18. J. Zemek, S. Kucàr and D. Anderle, *Collect. Czech. Chem. Comm.*, **53**, 1851 (1988).
19. J. Zemek, S. Kucàr and D. Anderle, *Collect. Czech. Chem. Comm.*, **52**, 2347 (1987).
20. R. Csuk and B.J. Glänzer, *Z. Naturforsch.*, **43b** , 1355 (1988).
21. M. Kloosterman, M.P. De Nijs, J.G. Weijnen, H.E. Schœmaker and E.M. Meijer, *J. Carbohydr. Chem.*, **8** , 333 (1989).
22. E.W. Holla, V. Sinwell and W. Klaffke, *Synlett.*, 413 (1992).
23. A. Ballesteros, M. Bernabe, C. Cruzado, M. Martin-Lomas and C. Otero, *Tetrahedron*, **45**, 7077 (1989).
24. C. Chon, A. Heisler, N. Junot, F. Levayer and C. Rabiller, *Tetrahedron: Asymmetry*, **4**, 2441 (1993).
25. N. Junot, J.C. Meslin and C. Rabiller, *Tetrahedron: Asymmetry*, **6**, 1387 (1995).
26. A. Heisler, C. Rabiller and L. Hublin, *Biotechnol. Lett.*, **13**, 327 (1991).
27. C. Rabiller, A. Heisler and G. Hägele in *Progress in Biotechnology*, Vol. 8, J. Tramper *et al*, Eds.; Elsevier: Amsterdam, 1992, p 283.
28. K. Heyns and J. Weyer, *Liebigs Ann. Chem.*, **718**, 224 (1968).
29. R.G.S. Ritchie, N. Cyr and A.S. Perlin, *Can. J. Chem.*, **54**, 2301 (1976).
30. D.D. Ward and F. Shafizadeh, *Carbohydr. Res.*, **108**, 71 (1982).
31. A.N.E. Weissfloch and R.J. Kazlauskas, *J. Org. Chem.*, **60**, 6959 (1985).
32. C. Chapus, M. Sémériva, C. Bovier-Lapierre and P. Desnuelle, *Biochemistry*, **15**, 4980 (1976).

33. C. Chapus and M. Sémériva, *Biochemistry*, **15**, 4988 (1976).
34. P. Grochulski, F. Bouthillier, R.J. Kaslauskas, A.N. Serreqi, J.D. Schrag, E. Ziomek and M. Cygler, *Biochemistry*, **33**, 3494 (1994).
35. M. Cygler, P. Grochulski R.J. Kaslauskas, J.D. Schrag, F. Bouthillier, R. Byron, A.N. Serreqi and A.K. Gupta, *J. Am. Chem. Soc*, **116**, 3180 (1994).
36. W.I. Weis and K. Drickamer, *Annu. Rev. Biochem.*, **65**, 441 (1996).
37. C.S. Wright, *J. Mol. Biol.*, **215**, 635 (1990).
38. N.K. Vias, *Curr. Opin.Struct. Biol.*, **1**, 732 (1991).
39. L. Panza, M. Luisetti, E. Crociati and S. Riva, *J. Carbohydr. Chem.*, **12**, 125 (1993).
40. G. Iacazio and S.M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1099 (1993).
41. F.C. Bernstein, T.F. Koetzle, G.J.B. Williams, E.F. Meyer, M.D. Brice, J.R. Rodgers, O. Kennard, T. Shimanouchi and M. Tasumi, *J. Mol. Biol.*, **112**, 535 (1977).