

A nuclear magnetic resonance study of conformational transmission in phosphorylated pyranosides

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A NUCLEAR MAGNETIC RESONANCE STUDY OF
CONFORMATIONAL TRANSMISSION IN
PHOSPHORYLATED PYRANOSIDES



N.K. DE VRIES

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
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OP GEZAG VAN DE RECTOR MAGNIFICUS, PROF.
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CHAPTER 1

Introduction

1.1 General introduction

The determination of the conformational preferences of biomolecules has always received much attention¹. It is evident that a better knowledge of the conformational properties of these molecules might assist in progress towards a proper understanding of their biological activities. The fast development in the last two decades of high-resolution NMR spectroscopic methods² and the increased use of computer calculations has greatly accelerated research in this area. It is now well possible to analyse medium-sized molecules, such as oligosaccharides³⁻⁴ and glycopeptides⁵, and also to calculate their conformational preferences⁵⁻⁷. This approach of quantum-chemical calculations, combined with conformational analysis based on NMR measurements, has recently been applied to the experimental investigation on the structural and dynamic properties of model compounds of phosphorylated biomolecules *e.g.* 5'-phosphorylated nucleosides⁸⁻⁹. On the basis of quantum-chemical calculations it was shown that a change in coordination of phosphorus from four (P^{IV}) to five (P^V) changes the conformation around the C-C bond in a P-O-C-C-O fragment from the gauche conformation to the trans conformation (g^- , in which the oxygens have maximal distance). The driving force for this conformational change is an enhanced electrostatic repulsion between the oxygen atoms in the P^V trigonal bipyramid (TBP). This concept of conformational transmission has been confirmed with NMR measurements on 5'-phosphorylated tetrahydrofurfurylsystems and 5'-phosphorylated nucleosides⁹, and recently on the 3',5' protected dinucleoside $d(T_pT)$ for which a very large change in g^- rotamer population was noted on going from P^{IV} to the P^V TBP structure (P^{IV} : $x(g^-) = 0.08$; P^V : $x(g^-) = 0.54$)¹⁰.

This thesis describes an extension of the conformational transmission effect to the field of carbohydrate chemistry, especially 6-phosphorylated pyranoside structures. The rotational motion around the exocyclic C-C bond is used as a probe for the electrostatic and stereoelectronic properties of these molecules, and the different factors which influence this rotation, e.g. electrostatic repulsion, solvent interactions, gauche and anomeric effects and sterical hindrance, are analysed.

1.2 Methods

The conformational analysis of the compounds under investigation is performed by a 200- and 300-MHz ^1H NMR study, in combination with quantum-chemical calculations. For small molecules, usually *ab initio* or MNDO calculations are performed, whereas for larger molecules the more approximate molecular mechanics¹¹ or hard-sphere calculations⁶ (HSEA) are preferred.

In this thesis, attention is focussed on conformational analysis by NMR methods, and MNDO calculations are used to support the findings. Rotamer populations are determined with a Karplus equation, which relates the vicinal proton-proton coupling constants to the torsion-angles between the coupling protons in an ethane fragment. In literature, two modified Karplus-equations are generally preferred¹²⁻¹³, because these equations have been fitted to large data sets of coupling constants. Both methods superimpose upon the angle dependency of the vicinal coupling a linear dependency on the electronegativity of the substituents. Recently, a third method has been introduced by Coluccio *e.a.*¹⁴ which allows an explicit accounting of solvent effects. In a study on conformations of carnitine and acetylcarnitine in water, the three equations were compared, and, within acceptable error, no significant differences were noted¹⁵. The modified Karplus-equation of Haasnoot *e.a.*¹² has been used throughout this thesis.

All quantum-chemical calculations are performed with the MNDO-programme, QCPE version¹⁶.

1.3 Stereoelectronic effects

In molecules which possess strong electron-withdrawing atoms, the stereoelectronic gauche and anomeric effect have an important bearing upon the conformational preferences of these molecules. The gauche effect (a pronounced preference of gauche over *trans* geometry in O-C-C-O fragments) was originally explained by quantum-chemical studies in terms of $\sigma-\sigma^*$ interactions¹⁷. Later, it was shown that these interactions only make a small contribution and that other quantum-chemical interactions determine the gauche effect¹⁸. The conformation around the exocyclic C(5)-C(6) bond of pyranosides and tetrahydropyran-2-methyl compounds is strongly influenced by this gauche effect. The *trans* rotamer of this bond only makes a significant contribution to the rotamer population when the electrostatic repulsion between the oxygen atoms of the O-C-C-O fragment is increased. This can be accomplished by either increasing the solvent polarity or by increasing the charge on the exocyclic oxygen atom by changing the coordination around phosphorus from four (P^{IV}) to five (P^V). The effect of the formation of a P^V TBP structure on the conformation around the exocyclic C-C bond is shown in the chapters 2 and 3. It is concluded that, as was found for 5'-phosphorylated nucleosides¹⁹, for the pyranosides the conformational transmission effect is also operative provided there is no sterical hindrance. The effects of solvent polarity are investigated in chapters 4 and 5.

The second stereoelectronic effect, the anomeric effect (usually defined in terms of the preference of electronegative elements for the axial configuration at the anomeric centre), is even more important. The theoretical explanation in terms of lone pair - antibonding orbital interactions²⁰ has led to the formulation of a theory of stereoelectronic control²¹, *i.e.* preferential cleavage of a tetrahedral intermediate occurs when two lone pairs are antiperiplanar to the leaving group. The role of orbital orientation in organic reactions has been shown for a variety of compounds, *e.g.* acetals²², amidines²³, phosphoramidates²⁴ and dioxaphosphorinanes²⁵. Kirby *e.a.* have suggested that knowledge of the ground state of a reacting system (and particularly of the length of the bond being broken) may give valuable mechanistic information regarding the reactivity of a system²⁶. A correlation

between the pK_a of the group at the anomeric centre and the bond length was established for pyranosides²⁷ and phosphates²⁸. In chapter 4, the influence of the pK_a and configuration of the group at the anomeric centre on the conformational preferences of the exocyclic C(5)-C(6) bond of pyranosides is analysed, i.e. the interactions between the gauche and anomeric effects in the ground state of molecules²⁹. Pyranosides are particularly suited for this study since the 4C_1 pyranoside ring has a rigid chair conformation. In principle the same interactions should be observable for furanosides and nucleosides. However, the more flexible furanoside ring alternates between two ring-puckering forms (N and S pucker). As a consequence of this difference in rigidity, the anomeric effect in pyranosides is relatively well studied, whereas for the furanosides, the $N \rightleftharpoons S$ equilibrium hampers determination of the anomeric effect since the group at the anomeric centre occupies an axial and equatorial position, alternatively. This prominent difference between pyranosides and furanosides has some important consequences for the interactions between the anomeric and gauche effect and influences the conformational properties of the respective exocyclic bonds.

In an interesting study on two uniquely modified deoxyadenosine systems it was shown that the net result of these two stereoelectronic effects determines the conformational preferences of the furanose ring in solution³⁰. Thus when the gauche and anomeric effect are cooperative, there is a strong preference for the N-pucker, whereas counteracting effects result in an $N \rightleftharpoons S$ equilibrium. For pyranose structures, where the sugar unit does not possess this flexibility, the net interaction is expressed in a different way *i.e.* in conformational changes around the exocyclic C(5)-C(6) bond. This is examined for mono- and digalactopyranosides as a function of solvent polarity, and as a function of the nature of the group at the anomeric centre. The results of this examination, given in chapters 4 and 5, have also led to the formulation of a rule for the assignment of the H(6)/H(6') resonances of galactopyranosides which can be used in combination with other methods.

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CHAPTER 2*

Configuration-dependent conformational transmission in trigonal-bipyramidal P^V compounds. Enhanced gauche(-) population around the C(5)-C(6) linkage in 6- P^V phosphorylated tetramethyl- α -D-galactopyranoside

Abstract.

A 300-MHz 1H NMR variable-temperature study of the 1'-phosphorylated trigonal-bipyramidal (TBP) tetrahydropyran-2-methyl model compound **4** is reported. For this compound, in which both the equatorial and axial sites undergoing phosphorus pseudorotation bear a tetrahydropyran-2-methyl group, a precise conformational analysis of the C(1')-C(2) bond is possible. The thermodynamic parameters which result from this analysis allow the assignment of the proton resonating at low field as H(1'). It is shown that, for the group situated axially in the P^{IV} TBP, an enhanced gauche(-) population [O(1') and O(1) *trans*-situated] occurs around C(1')-C(2) (290 K, 59%; 230 K, 74%) as compared to the corresponding P^{IV} compound **3** (298 K, 10%). The same conformational transmission is observed for 6-phosphorylated pyranosides, but in this case is found to depend upon the configuration at C(4). It appears that 6-phosphorylated methyl 2,3,4-tri-O-methyl- α -D-galactopyranoside shows a conformational change around C(5)-C(6) towards a higher g^- population, going from 6- P^{IV} to 6- P^V TBP, which is absent in the corresponding 6-phosphorylated methyl 2,3,4-tri-O-methyl- α -D-glucopyranoside. Apparently, the configuration at C(4) is jointly liable for the transmission in the P^V TBP.

* N. K. de Vries and H. M. Buck, Recl. Trav. Chim. Pays-Bas **105**, 150 (1986).

2.1 Introduction

Recent investigations of phosphorylated tetrahydrofurfuryl* systems¹ have shown that an increase in coordination of phosphorus from 5'-P^{IV} to 5'-P^V trigonal-bipyramidal (TBP) results in a change in rotamer population around C(4')-C(5') from gauche(+) (g⁺) and gauche(*trans*) (g^t) towards gauche(-) (g⁻), if the tetrahydrofurfuryl group is situated in the axial position. It has been shown that this conformational transmission is effectuated by an increased charge repulsion between the exocyclic oxygen O(5') and the endocyclic oxygen O(1'). These phosphorylated tetrahydrofurfuryl systems can be considered as simple model systems for the furanose phosphates which are important constituents of many biomolecules (e.g. 2'-deoxyribose phosphate in the DNA backbone).

Another important group of carbohydrate phosphates is that derived from the pyranoses, which play an essential role in biochemical processes² (e.g. glucose 6-phosphate and glucose 1-phosphate in carbohydrate metabolism). In a number of publications the participation of the phosphate group in enzymatic reactions has been suggested^{3,4}. However, as recently has been pointed out, little information is available on the favoured conformation of C(6) phosphate moieties in carbohydrates⁵. Therefore, the conformation around C(1')-C(2) was studied in detail for both axial and equatorial situated tetrahydropyran-2-methyl groups which were chosen as model compounds for pyranose sugars. Here also, axial location is found to be associated with a preference for the g⁻ rotamer. Furthermore, the rotamer distribution around C(5)-C(6) for 6-phosphorylated pyranosides has been determined and it has been found that the occurrence of a conformational transmission effect depends upon the configuration at C(4).

These results might explain in part the high substrate specificity of glucose 6-phosphate isomerase⁶. It is speculated that, in the reaction mechanism of this enzyme, a P^V intermediate is involved which increases the negative charge on O(6), situated in the axial position of the TBP, and allows a stabilizing hydrogen

* tetrahydrofuran-2-methyl

bond to be formed in the ring-opened glucose 6-phosphate between O(6) and O(5)-H, since the preferred conformation of glucose 6-P^V is g⁺ (see § 2.3). Such a hydrogen bond formation is not possible for galactose 6-P^V since the favoured conformation of this molecule is g⁻ rather than g⁺. It is noteworthy here that galactose 6-phosphate is an inhibitor of glucose 6-phosphate isomerase (K_i 1.7 mM)⁷.

2.2 C(1')-C(2) conformational analysis

The analysis of the conformation around the C(1')-C(2) bond, carried out using 300-MHz ¹H NMR spectroscopy, is based upon the well-known *Karplus* equation⁸ which relates the vicinal coupling constant to the torsional angle between the coupling protons. The measured coupling constants are a weighted time-average of the contribution made by three staggered rotamers, g⁺, g' and g⁻ (see Fig. 1).

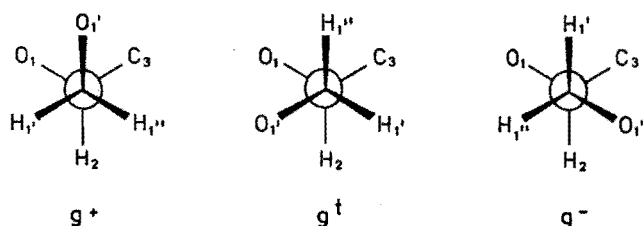


Fig. 1. Rotamers around the C(1')-C(2) bond.

For ethane fragments bearing three non-hydrogen substituents, the coupling constants corresponding to the various rotamers can be calculated with the help of an empirically generalized *Karplus* equation^{9,10}. Although in this equation proton torsion angles have been used which deviate from the angles of staggered conformations, especially for nucleosides¹¹, it is assumed that ϕ -60°, 60° and 180° for g⁺,

g^+ and g^- resp. are the correct values for tetrahydropyran-2-methyl groups. Using these calculated coupling constants corresponding to the three rotamers, together with the measured coupling constants, one obtains a set of three equations in the unknown rotamer populations $x(g^+)$, $x(g^+)$ and $x(g^-)$. This set can be transformed into a set of three equations¹² for the calculation of the conformation around C(1')-C(2). As can be seen in these equations, a correct assignment of the protons H(1') and H(1'') is necessary. Unfortunately, this remains one of the unsettled problems in the field of carbohydrate chemistry. A ¹H NMR study of deuterated pyranosides showed that the chemical shift is of no diagnostic value in differentiating between the two protons¹³. Other criteria which are often used in the NMR study of nucleosides (such as a linear inverse relationship between the chemical-shift difference and the sum of the coupling constants¹⁴, or the coupling constant ($J_{1',2}$ or $J_{1'',2}$) which increases most on raising the temperature¹⁵) are unsuitable since they imply a high g^+ population and a low g^- population. The gauche effect¹⁶ (a pronounced preference for gauche over *trans* geometry in O-C-C-O fragments) is therefore used to establish the proton assignment for the 1'-P^{IV} compounds **1** and **3**. The application of the gauche effect to the proton assignment can be rationalized on the basis of the observation that inversion of the H(1')/H(1'') assignment has little effect on the estimated g^+ rotamer population, whereas the populations of g^+ and g^- are reversed. Since g^+ [O(1') gauche to O(1)] represents an energetically more favoured state than does g^- [O(1') *trans* to O(1)], the correct proton assignment yields $x(g^+) > x(g^-)$. For the 1'-P^V TBP compounds **2** and **4**, it is uncertain whether $x(g^+)$ remains larger than $x(g^-)$ since the charge repulsion between axial exocyclic O(1') and endocyclic O(1) changes the rotamer population around C(1')-C(2) from g^+ towards g^- . The gauche effect, therefore, cannot be used for the proton assignment of the 1'-P^V compounds. However, the rotamer population of compound **4** can be obtained by calculating the enthalpy and entropy parameters, which govern the rotation around C(1')-C(2), for both possible proton assignments (see § 2.3). One assignment yields large entropy and enthalpy values, whereas the other assignment yields large entropy and small enthalpy values. Since unhindered rotational processes are enthalpy- rather than entropy-controlled, the former assignment (H(1') at low field) is chosen for **4**.

Using the thermodynamic parameters corresponding to this assignment, a conformational analysis is possible for both the axial and equatorial C(1')-C(2) bonds.

2.3 Results and Discussion

In order to obtain experimental evidence for the conformational change around C(1')-C(2) on changing the coordination of phosphorus from four to five, the 1'-P^{IV} and 1'-P^V TBP tetrahydropyran-2-methyl compounds 1-4 were synthesized (see Fig. 2).

The conformations were analysed using 300-MHz ¹H NMR spectroscopy. The spectral parameters are given in Table I. The results show a higher population of the g⁻ rotamers for the 1'-P^V TBP model compounds as compared to their P^{IV} counterparts.

The conformational change in P^V TBP compounds is only effective in ligands situated axially (*vide supra*). Since the P^V TBP compounds may undergo phosphorus pseudorotation (fast ligand permutation between axial and equatorial sides in the TBP), the actual increase in g⁻ population on going from P^{IV} to P^V TBP will be larger than is in fact measured. The true g⁻ populations for both axial and equatorial sites can be obtained from a variable-temperature ¹H NMR study of compound 4. The results of the temperature measurements are listed in Table II.

The observed rotamer populations x(i) (rotamers i: g⁺, g['] and g⁻) in Tables I and II are an average of the sum of the rotamer populations in the axial and the two equatorial sites. The determination of the eight thermodynamic parameters which govern the rotation around C(1')-C(2) allows the rotamer populations around both the equatorial and the axial bond to be calculated^{1,17}. These thermodynamic parameters are given in Table III for both possible proton assignments.

From Table III it follows that the ΔG°(g⁻,i) values for rotation around C(1')-C(2) for the tetrahydropyran-2-methyl group positioned axially are positive for both proton assignments. This means that G°_{ax}(g⁺) > G°_{ax}(g⁻) and

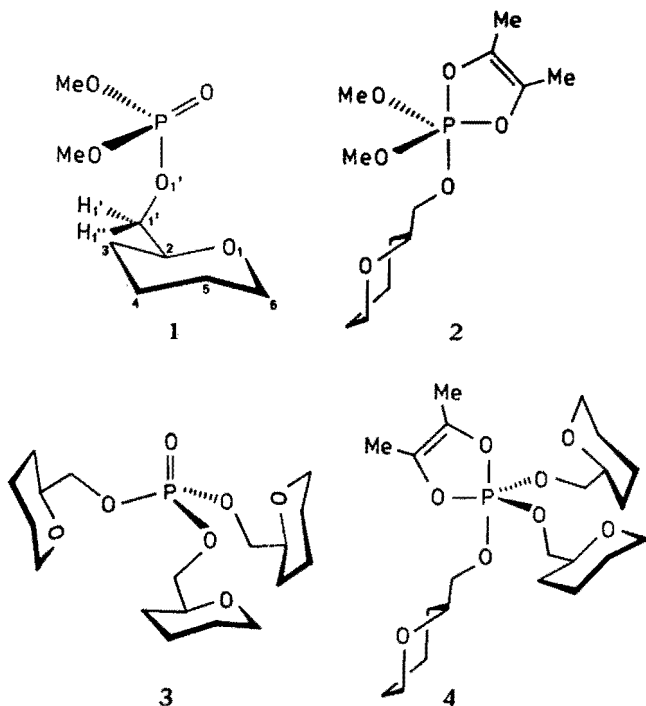


Fig. 2. Model compounds 1-4. Dominant $C(1')-C(2)$ rotamers are drawn for tetrahydropyran-2-methyl groups.

$G_{ax}^{\ominus}(g') > G_{ax}^{\ominus}(g^-)^{18}$. Thus, there is a preference for the g^- rotamer in axial location. For the compounds 1-2 and 3-4 an increase in g^- population is found on going from $1'-P^{IV}$ to $1'-P^V$ TBP (Table I). This is in agreement with the conclusion reached previously that there is a preference for the g^- rotamer in axial location since the P^{IV} ligands resemble the equatorial ligands rather than the axial ligand in the P^V TBP. The proton assignment can be determined by examining the enthalpy and entropy values $\Delta H_{ax}^{\ominus}(g^-,i)$, $\Delta S_{ax}^{\ominus}(g^-,i)$ in Table III. One assignment yields large entropy and small enthalpy values, whereas the other

Table I. $J_{1'(1'')2}$ values together with the corresponding C(1')-C(2) rotamer populations for compounds 1-4 at 300 K^a.

Compound	$J_{1'2}$ (Hz)	$J_{1''2}$ (Hz)	$x(g^+)$	$x(g')$	$x(g^-)$
1	3.73	6.48	0.37	0.53	0.10
2	6.06	5.00	0.35	0.25	0.40
3	3.73	6.27	0.40	0.50	0.10
4	5.80	5.19	0.35	0.29	0.36

^a Proton assignment for 1 and 3 [H(1') at high field] is based upon the gauche effect. The assignment for 2 and 4 [H(1') at low field] is based upon thermodynamic parameters (vide infra).

Table II. NMR parameters and C(1')-C(2) rotamer populations obtained from a variable temperature ¹H NMR study of 4.

T(K)	δ [H(1a)]	δ [H(1b)]	$J_{1a,2}$ (Hz)	$J_{1b,2}$ (Hz)
290	3.901	3.810	5.82	5.17
270	3.886	3.801	5.89	5.09
260	3.877	3.796	5.93	5.05
230	3.849	3.782	6.05	4.85

T(K)	H(1a) = H(1')			H(1b) = H(1')		
	$x(g^+)$	$x(g')$	$x(g^-)$	$x(g^+)$	$x(g')$	$x(g^-)$
290	0.350	0.285	0.365	0.330	0.385	0.285
270	0.350	0.270	0.380	0.330	0.400	0.270
260	0.350	0.265	0.385	0.330	0.405	0.265
230	0.365	0.240	0.395	0.330	0.430	0.240

assignment yields large enthalpy values. The similar compound, 2,2,2-tris(tetrahydrofurfuryloxy)-2,2-dihydro-4,5-dimethyl-1,3,2-dioxaphosphole¹, which has an unequivocal proton assignment, shows large enthalpy values $\Delta H_{ax}^\circ(g^-,i)$. It is therefore expected that the assignment H(1a) = H(1') is correct. The corresponding rotamer populations for both axial and equatorial sites are given in Table IV.

A preference for the g^- rotamer exists in the axially positioned tetrahydropyran-

Table III. Thermodynamic parameters of the C(1')-C(2) conformational equilibria for axial and equatorial tetrahydropyran-2-methyl in **4**.

H(1a) = H(1')	
Axial tetrahydropyran-2-methyl	Equatorial tetrahydropyran-2-methyl
$\Delta S_{ax}^{\circ} (g^-, g^+) = 13.4 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$	$\Delta S_{eq}^{\circ} (g^-, g^+) = -11.5 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$
$\Delta H_{ax}^{\circ} (g^-, g^+) = 6.1 \text{ kJ}\cdot\text{mol}^{-1}$	$\Delta H_{eq}^{\circ} (g^-, g^+) = -4.3 \text{ kJ}\cdot\text{mol}^{-1}$
$\Delta G_{ax}^{\circ} (g^-, g^+) = 2.1 \text{ kJ}\cdot\text{mol}^{-1}$	$\Delta G_{eq}^{\circ} (g^-, g^+) = -0.8 \text{ kJ}\cdot\text{mol}^{-1}$
$\Delta S_{ax}^{\circ} (g^-, g') = 14.9 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$	$\Delta S_{eq}^{\circ} (g^-, g') = -6.6 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$
$\Delta H_{ax}^{\circ} (g^-, g') = 7.2 \text{ kJ}\cdot\text{mol}^{-1}$	$\Delta H_{eq}^{\circ} (g^-, g') = -2.4 \text{ kJ}\cdot\text{mol}^{-1}$
$\Delta G_{ax}^{\circ} (g^-, g') = 2.8 \text{ kJ}\cdot\text{mol}^{-1}$	$\Delta G_{eq}^{\circ} (g^-, g') = -0.4 \text{ kJ}\cdot\text{mol}^{-1}$

H(1b) = H(1')	
Axial tetrahydropyran-2-methyl	Equatorial tetrahydropyran-2-methyl
$\Delta S_{ax}^{\circ} (g^-, g^+) = -7.6 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$	$\Delta S_{eq}^{\circ} (g^-, g^+) = -7.6 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$
$\Delta H_{ax}^{\circ} (g^-, g^+) = -0.6 \text{ kJ}\cdot\text{mol}^{-1}$	$\Delta H_{eq}^{\circ} (g^-, g^+) = -4.0 \text{ kJ}\cdot\text{mol}^{-1}$
$\Delta G_{ax}^{\circ} (g^-, g^+) = 1.7 \text{ kJ}\cdot\text{mol}^{-1}$	$\Delta G_{eq}^{\circ} (g^-, g^+) = -1.7 \text{ kJ}\cdot\text{mol}^{-1}$
$\Delta S_{ax}^{\circ} (g^-, g') = -15.8 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$	$\Delta S_{eq}^{\circ} (g^-, g') = -4.2 \text{ J}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$
$\Delta H_{ax}^{\circ} (g^-, g') = -0.4 \text{ kJ}\cdot\text{mol}^{-1}$	$\Delta H_{eq}^{\circ} (g^-, g') = -4.5 \text{ kJ}\cdot\text{mol}^{-1}$
$\Delta G_{ax}^{\circ} (g^-, g') = 4.3 \text{ kJ}\cdot\text{mol}^{-1}$	$\Delta G_{eq}^{\circ} (g^-, g') = -3.2 \text{ kJ}\cdot\text{mol}^{-1}$

Table IV. C(1')-C(2) rotamer populations for axial and equatorial tetrahydropyran-2-methyl (H(1') at low field).

T(K)	Axial			Equatorial		
	x(g ⁺)	x(g')	x(g ⁻)	x(g ⁺)	x(g')	x(g ⁻)
290	0.23	0.18	0.59	0.40	0.33	0.27
270	0.21	0.15	0.64	0.42	0.33	0.25
260	0.20	0.14	0.66	0.44	0.32	0.24
230	0.16	0.10	0.74	0.48	0.32	0.20

2-methyl group. As compared to the corresponding 1'-P^{IV} compound **3** (x(g⁻) 0.10; Table I), a large increase in g⁻ population is observed. Even without correcting for phosphorus pseudorotation (Table I), there is a clear conformational change around C(1')-C(2) on change in coordination from 1'-P^{IV} to 1'-P^V. From

the data given in Tables I and IV it follows that the assignment for 2 (H(1') at low field) must be correct. This compound resembles 4 in that a tetrahydropyran-2-methyl group alternates between the axial and equatorial sites via phosphorus pseudorotation. Thus, the measured coupling constants are a time-weighted average of the contributions made by the axial and equatorial sites. Therefore, the coupling constants resemble those of 4, *i.e.*, $J_{1',2}$ 6.06 Hz and $J_{1'',2}$ 5.00 Hz. A reversed assignment ($J_{1',2}$ 5.00 Hz, $J_{1'',2}$ 6.06 Hz) yields $x(g^-)$ 0.26. This value corresponds to a tetrahydropyran-2-methyl group permanently occupying an equatorial position (see Table IV). This is unlikely in view of the observed phosphorus pseudorotation. Whereas the concept of conformational transmission is clearly established for the tetrahydropyran-2-methyl group, it cannot *a priori* be extended to a pyranoside. Dreiding-model studies show that the substituents on the pyranose ring might interfere with rotation around the C(5)-C(6) bond. To examine the influence of a substituent at C(4) (which is nearest to O(6)) on the C(5)-C(6) rotamer population, compounds 5-8 were synthesized (see Fig. 3).

In compounds 5-6, the g^- rotamer is strongly disfavoured because of sterical hindrance between the equatorial methoxy group at C(4) and the group at O(6). An axial group at C(4) (compounds 7-8) is expected to reduce the population of the g^+ rotamer due to similar 1,3 *syn*-diaxial interactions. Studies of the C(5)-C(6) conformation of pertrimethylsilylated glycopyranosides indeed showed this dependency upon the configuration at C(4)¹⁹. It was therefore anticipated that the presence of a substituent at C(4) will also influence the conformational transmission of a 6- P^V TBP pyranoside. The spectral parameters of 5-8 are given in Table V.

The proton assignment for the P^{IV} compounds 5 and 7 is based upon the values of the coupling constants of deuterated pyranosides¹³, *i.e.* $J_{5,6}$ is smaller than $J_{5,6'}$. The corresponding rotamer populations are in agreement with expectations. A very small g^- value is found for 5 whereas for the galactopyranoside 7 a shift is observed from g^+ towards g^- compared to the unsubstituted compound 1. As can be seen from Table V, large differences exist between the glucopyranosides 5-6 and the galactopyranosides 7-8. The coupling constants $J_{5,6(6')}$ and the rotamer

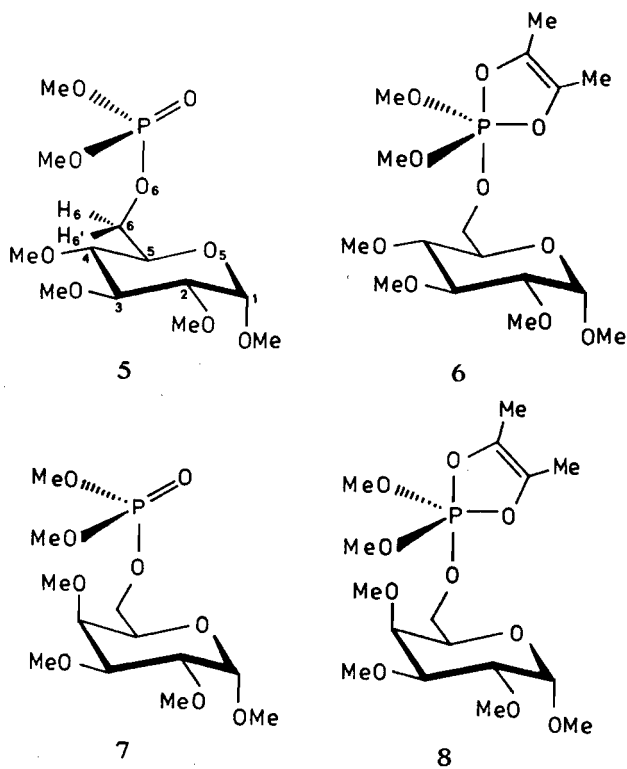


Fig. 3. Model compounds 5-8.

distribution around C(5)-C(6) remain essentially unchanged for the glucopyranoside on going from 6- P^{IV} to 6- P^V TBP. The equatorial substituent at C(4) effectively hampers rotation around C(5)-C(6) towards a larger g^- population. Model studies show that, for the galactopyranosides 7-8, the axial substituent at C(4) favours g^+ and g^- . Here, the conformational change towards larger g^- populations, on going from 6- P^{IV} to 6- P^V TBP, is possible, and this is exactly what is observed. The g^- population increases at the expense of the g^+ population, whereas the sterically unfavourable g^+ rotamer remains equally populated. As compared

Table V. Spectral parameters together with the corresponding C(5)-C(6) rotamer populations for compounds 5-8.

Compound	$\delta[\text{H}(6)]$	$\delta[\text{H}(6')]$	$J_{5,6}(\text{Hz})$	$J_{5,6'}(\text{Hz})$	$x(g^+)$	$x(g')$	$x(g^-)$
5	4.31	4.16	2.32	4.50	0.80	0.20	0.0 ^a
6	4.17	4.08	2.10	4.80	0.74	0.26	0.0 ^a
7	4.14	4.09	5.41	7.04	0.19	0.50	0.31
8	3.95	3.86	6.92	5.90	0.19	0.30	0.51

^a See ref. 20.

to the unsubstituted compounds 1-2, the g^- population is higher for both 6- P^{IV} and 6- P^V TBP compounds. In general, it can be concluded that a 6-phosphorylated pyranoside possessing an axial group at C(4) does show a conformational transmission effect on going from 6- P^{IV} to 6- P^V TBP. This effect is absent in a 6-phosphorylated pyranoside possessing an equatorial group at C(4).

2.4 Experimental

Materials. — All solvents and reagents were reagent grade. Reactions involving phosphites and dioxaphosphole (P^V) compounds were carried out under an atmosphere of dry nitrogen. All phosphites were purified on a neutral Woelm silica gel column using dry ethyl acetate as eluent.

Spectroscopy. — ^1H NMR spectra were recorded in the FT mode on a Bruker CXP-300 spectrometer at 300.1 MHz, using a 32 K point data set. Chemical shifts are given relative to the $\text{CHD}_2\text{COCD}_3$ quintet at δ 2.17 with an accuracy of 0.05 Hz. Coupling constants were measured from expansions of the patterns and were analysed using an iterative programme²¹. The accuracy of the calculated rotamer populations is about 3% (absolute). Acetone- d_6 was used as the solvent unless otherwise indicated. ^{31}P NMR spectra were recorded in the FT mode at 36.4 MHz on a Bruker HX-90R. Chemical shifts are related to 85% H_3PO_4 as external standard.

*Dimethoxy(dimethylamino) phosphine*²². — Phosphorus trichloride (0.5 mol, 69 g) was added over 30 min. to trimethyl phosphite (1 mol, 124 g) which was maintained at 60°C in a 1000-ml round-bottomed flask. The reaction mixture was then cooled to 0°C and diluted with 500 ml sodium-dried diethyl ether. Dimethylamine (3 mol, 135 g) was bubbled through the reaction mixture. After filtration of the diethylamine hydrochloride, evaporation of the diethyl ether yielded a yellowish oil, which was distilled twice at 45 mm through a 20 cm vigreux column to afford 46 g (22%) of the desired product, b.p. 51–52°C at 45 mm. ¹H NMR (C₆D₆): δ 2.63 [d, J(P,NCH₃) 8.8 Hz, 6H, NCH₃], 3.42 [d, J(P,OCH₃) 12.0 Hz, 6H, OCH₃]. ³¹P NMR (C₆D₆): δ 147.60.

Tetrahydropyran-2-methyl dimethyl phosphite. — A stirred solution of tetrahydropyran-2-methanol (20 mmol, 2.32 g) and dimethoxy(dimethylamino) phosphine (21 mmol, 2.89 g) in 5 ml of sodium dried toluene was heated for 3 h at 80°C. The solvent was evaporated and the residue was purified by column chromatography (R_f 0.67) to yield a colourless oil (5 mmol, 1 g). ¹H NMR: δ 1.23–1.92 [m, 6H, H(3), H(4) and H(5)], 3.58 [dd, 6H, OCH₃], 3.73–4.0 [m, 2H, H(2) and H(6ax)], 4.15 [m, 2H, H(1') and H(1'')], 4.42 [m, 1H, H(6eq)]. ³¹P NMR: δ 145.03.

Tetrahydropyran-2-methyl dimethyl phosphate (1). — This compound was prepared from tetrahydropyran-2-methyl dimethyl phosphite according to literature procedures¹. After removal of the solvent, the residue was distilled under reduced pressure to afford the product as a colourless liquid, b.p. 84–86°C (0.005 mm). ¹H NMR: δ 1.41–1.96 [m, 6H, H(3), H(4) and H(5)], 3.47–3.67 [m, 2H, H(2) and H(6ax)], 3.82 [d, J(P,OCH₃) 11.01 Hz, 6H, OCH₃], 4.01 [m, 2H, H(1') and H(1'')], 4.05 [m, 1H, H(6eq)]. ³¹P NMR: δ 6.59.

2,2-Dimethoxy-2-(tetrahydropyran-2-methoxy)-2,2-dihydro-4,5-dimethyl-1,3,2-dioxaphosphole (2). — An equimolar quantity of 2,3-butadione was added to a cooled solution (0°C) of tetrahydropyran-2-methyl dimethyl phosphite in dry acetone-d₆. After 3 h, ³¹P NMR spectroscopy indicated that the reaction was

complete. ^1H NMR: δ 1.36-2.0 [m, 6H, H(3), H(4) and H(5)], 1.91 [s, 6H, CH_3], 3.50 [m, 2H, H(2) and H(6ax)], 3.63 [d, $J(\text{P},\text{OCH}_3)$ 13.11 Hz, 6H, OCH_3], 3.83 [m, 2H, H(1') and H(1'')], 3.99 [m, 1H, H(6eq)]. ^{31}P NMR: δ -43.94.

Tris(tetrahydropyran-2-methyl) phosphite. — To a stirred and cooled (0°C) solution of tetrahydropyran-2-methanol (44 mmol, 5.07 g) and triethylamine (44 mmol, 4.41 g) in 250 ml of sodium-dried ether was added dropwise a solution of PCl_3 in 50 ml of dry ether. After completion of the addition (1 h), the mixture was stirred at room temperature for 3 h and refluxed for an additional hour. The mixture was cooled, the precipitated triethylamine hydrochloride was filtered off and the solvent was evaporated. This yielded a colourless oil (5.5 g) which, according to ^{31}P and ^1H NMR spectroscopy, was pure. ^1H NMR (CDCl_3): δ 1.0-2.0 [m, 18H, H(3)-H(5)], 3.1-4.2 [m, 15H, H(1'), H(2) and H(6)]. ^{31}P NMR (CDCl_3): δ 139.95.

Tris(tetrahydropyran-2-methyl) phosphate (3). — This compound was prepared from tris(tetrahydropyran-2-methyl) phosphite according to a literature procedure¹. ^1H NMR: δ 1.40-2.00 [m, 6H, H(3)-H(5)], 3.54-3.74 [m, 2H, H(2) and H(6ax)], 4.03 [m, 2H, H(1') and H(1'')], 4.10 [m, 1H, H(6eq)]. ^{31}P NMR: δ 4.32.

2,2,2-Tris(tetrahydropyran-2-methyloxy)-2,2-dihydro-4,5-dimethyl-1,3,2-dioxaphosphole (4). — This compound was prepared from tris(tetrahydropyran-2-methyl) phosphite according to the procedure described for 2. ^1H NMR: δ 1.38-1.97 [m, 6H, H(3)-H(5)], 1.92 [s, 6H, CH_3], 3.42-3.60 [m, 2H, H(2) and H(6ax)], 3.80 [m, 2H, H(1') and H(1'')], 4.04 [m, 1H, H(6eq)]. ^{31}P NMR: δ -45.44.

Methyl 6-O-(triphenylmethyl)- α -D-glucopyranoside. — This compound was prepared according to literature procedures²³. Melting point and ^1H NMR data agreed with those previously reported.

Methyl 2,3,4-tri-O-methyl-6-O-(triphenylmethyl)- α -D-glucopyranoside. — A suspension of methyl 6-O-trityl- α -D-glucopyranoside (47 mmol, 20 g) and NaH (0.83 mol, 20 g) in 500 ml DMF was stirred for 1 h. The suspension was cooled to 0°C and MeI (0.48 mol, 68 g) was added. After the addition was complete, the mixture was stirred for 5 h at 25°C. Excess NaH was destroyed with methanol and the DMF was evaporated. The residue was dissolved in 350 ml of water and the water layer was then extracted extensively with dichloromethane. The combined dichloromethane layers were dried, filtered and evaporated to yield a viscous oil. Residual DMF was removed azeotropically with p-xylene to yield an oil which solidified on standing. Recrystallization from ether/hexane yielded yellowish crystals (23 mmol, 50%), m.p. 104–106°C. ^1H NMR (CDCl_3): δ 3.2–3.6 [m, 6H, H(2)–H(6)], 3.25, 3.41, 3.51 and 3.56 [4 s, 12H, OCH_3], 4.83 [d, 1H, H(1)], 7.0–7.5 [m, 15H, ArH].

Methyl 2,3,4-tri-O-methyl- α -D-glucopyranoside. — This compound was prepared according to literature procedures²⁴ to yield a colourless oil. ^1H NMR: δ 3.1–3.9 [m, 6H, H(2)–H(6)], 3.42, 3.52, 3.57 and 3.63 [4 s, 12H, OCH_3], 4.83 [d, $J_{1,2}$ 3.5 Hz, H(1)].

Methyl 2,3,4-tri-O-methyl- α -D-glucopyranoside-6-(dimethyl phosphite). — A solution of methyl 2,3,4-tri-O-methyl- α -D-glucopyranoside (1.4 mmol, 330 mg) and dimethoxy(dimethylamino) phosphine (3.9 mmol, 550 mg) in 4 ml of dry toluene was stirred for 4 h at 80°C under a nitrogen atmosphere. Evaporation of the toluene yielded an oil which was purified by column chromatography (R_f 0.65). This yielded a colourless oil (0.3 mmol, 100 mg). ^1H NMR: δ 3.22 [dd, $J_{2,3}$ 9.58 Hz, 1H, H(2)], 3.47, 3.53, 3.63 and 3.65 [4 s, 12H, OCH_3], 3.61 [d, $J(\text{P}, \text{OCH}_3)$ 10.40 Hz, 6H, OCH_3], 3.5–3.8 [m, 3H, H(3)–H(5)], 4.08 [m, 2H, H(6)], 4.92 [d, $J_{1,2}$ 3.53 Hz, 1H, H(1)]. ^{31}P NMR: δ 145.58.

Methyl 2,3,4-tri-O-methyl- α -D-glucopyranoside-6-(dimethyl phosphate) (5). — This compound was prepared from tetramethylglucopyranoside-6-(dimethyl

phosphite) according to a literature procedure¹. After removal of the dichloromethane, a viscous oil remained which was purified on a silica gel column (Eluent: chloroform/methanol 99:1; R_f 0.40). ¹H NMR: δ 3.18 [dd, 1H, H(4)], 3.24 [dd, $J_{2,3}$ 9.58 Hz, 1H, H(2)], 3.47, 3.53, 3.63 and 3.64 [4 s, 12H, OCH₃], 3.58 [dd, $J(\text{P},\text{OCH}_3)$ 11.05 Hz, 6H, POCH₃], 4.16–4.31 [m, 2H, H(6)], 4.94 [d, $J_{1,2}$ 3.48 Hz, 1H, H(1)]. ³¹P NMR: δ 6.64.

2,2-Dimethoxy-2-(methyl 2,3,4-tri-O-methyl- α -D-glucopyranosid-6-oxy)-2,2-dihydro-4,5-dimethyl-1,3,2-dioxaphosphole (6). — This compound was prepared from tetramethylglucopyranoside-6-(dimethyl phosphite) using the method described for the preparation of 2. ¹H NMR: δ 1.92 [s, 6H, CH₃], 3.30 [dd, $J_{2,3}$ 9.60 Hz, 1H, H(2)], 3.46, 3.52, 3.62 and 3.64 [4 s, 12H, OCH₃], 3.67 [dd, $J(\text{P},\text{OCH}_3)$ 13.00 Hz, 6H, POCH₃], 3.4–3.7 [m, 3H, H(3)–H(5)], 4.08–4.17 [m, 2H, H(6)], 4.90 [d, $J_{1,2}$ 3.48 Hz, 1H, H(1)]. ³¹P NMR: δ -44.05.

Methyl 2,3,4-tri-O-methyl- α -D-galactopyranoside. — This compound was prepared according to literature procedures²⁵. Detritylation was effected using chlorotrimethylsilane/sodium iodide²⁴, to yield a yellow oil. Purification on a silica gel column yielded a colourless oil (Eluent: chloroform; R_f 0.45). ¹H NMR (CDCl₃): δ 3.4–3.9 [m, 6H, H(2)–H(6)], 3.43 [s, 3H, OCH₃], 3.53 [s, 6H, OCH₃], 3.59 [s, 3H, OCH₃], 4.87 [d, $J_{1,2}$ 2Hz, 1H, H(1)].

Methyl 2,3,4-tri-O-methyl- α -D-galactopyranoside-6-(dimethyl phosphite). — This compound was prepared from methyl 2,3,4-tri-O-methyl- α -D-galactopyranoside using the method described for the corresponding glucopyranoside (R_f 0.68). ¹H NMR: δ 3.32, 3.38, 3.44 and 3.47 [4 s, 12H, OCH₃], 3.50 [d, 6H, POCH₃], 3.2–3.6 [m, 4H, H(2)–H(5)], 3.89 [m, 2H, H(6)], 4.79 [d, 1H, H(1)]. ³¹P NMR: δ 145.19.

Methyl 2,3,4-tri-O-methyl- α -D-galactopyranoside-6-(dimethyl phosphate) (7). — This compound was prepared according to literature procedures¹ from the

corresponding phosphite. ^1H NMR: δ 3.34, 3.40, 3.46 and 3.51 [4 s, 12H, OCH_3], 3.3-3.6 [m, 4H, H(2)-H(5)], 3.75 [d, $J(\text{P},\text{OCH}_3)$ 10.92 Hz, 6H, POCH_3], 4.1-4.2 [m, 2H, H(6)], 4.83 [d, $J_{1,2}$ 2.5 Hz, 1H, H(1)]. ^{31}P NMR: δ 6.58.

2,2-Dimethoxy-2-(methyl 2,3,4-tri-O-methyl- α -D-galactopyranosid-6-oxy)-2,2-dihydro-4,5-dimethyl-1,3,2-dioxaphosphole (8). — This compound was prepared from tetramethylgalactopyranoside-6-(dimethylphosphite) using the method described for the preparation of compound 2. ^1H NMR: δ 1.80 [s, 6H, CH_3], 3.33, 3.38, 3.45 and 3.50 [4 s, 12H, OCH_3], 3.55 [dd, $J(\text{P},\text{OCH}_3)$ 13.12 Hz, 6H, POCH_3], 3.3-3.6 [m, 4H, H(2)-H(5)], 3.85-3.95 [m, 2H, H(6)], 4.76 [d, $J_{1,2}$ 2.5 Hz, 1H, H(1)]. ^{31}P NMR: -44.04.

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 $x(g^f) = -0.054 J_{1',2} + 0.104 J_{1'',2} + 0.061$
 $x(g^-) = +0.129 J_{1',2} - 0.003 J_{1'',2} - 0.364$
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$$x_{ax}(i) = \frac{\exp[-\Delta G^{\circ}_{ax}(g^{-},i)(RT)^{-1}]}{1 + \exp[-\Delta G^{\circ}_{ax}(g^{-},g^{+})(RT)^{-1}] + \exp[-\Delta G^{\circ}_{ax}(g^{-},g^{t})(RT)^{-1}]}$$

$$x_{eq}(i) = \frac{\exp[-\Delta G^{\circ}_{eq}(g^{-},i)(RT)^{-1}]}{1 + \exp[-\Delta G^{\circ}_{eq}(g^{-},g^{+})(RT)^{-1}] + \exp[-\Delta G^{\circ}_{eq}(g^{-},g^{t})(RT)^{-1}]}$$

where i denotes g^{+} , g^{t}

18. $\Delta G^{\circ}(g^{-},i) = G^{\circ}(i) - G^{\circ}(g^{-})$;
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CHAPTER 3*

Influence of electron-withdrawing substituents in the oxaphosphole ring on the axial conformational transmission in P^V compounds

Abstract.

An MNDO and 300-MHz 1H NMR study of some trigonal-bipyramidal (TBP) five-coordinated phosphorus (P^V) compounds is reported. It is shown by the MNDO calculations that, in the oxaphosphole P^V TBP compounds **5a-c**, the electron distribution in the axial bonds of the TBP is affected by the electronegativity of the substituent at C(4) of the oxaphosphole ring. With increasing electronegativity of the substituent at C(4), the electron density on the axial exocyclic oxygen atom O(1') decreases whereas the electron density on the axial endocyclic atom O(1) increases. This is supported by a 1H NMR conformational analysis of the C(1')-C(2'') bond of the oxaphosphole P^V TBP compounds **6-11**. The gauche(-) rotamer fraction (O(1') and O(1'') trans situated) of these compounds, which is correlated to the electron density on O(1'), is reduced to 30% as compared to the absolute axial g^- rotamer fraction (59%) of the dioxaphosphole P^V TBP compound **13**, most likely because of the presence of the carbonyl group at C(4) of the oxaphosphole ring. So, both the 1H NMR and MNDO study show that electron withdrawing substituents on the oxaphosphole ring of P^V TBP compounds reverse the electron transfer in the axial P-O bonds of the TBP (as compared to dioxaphosphole compounds), from exocyclic O(1') towards endocyclic O(1).

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3.1 Introduction

Recently, the conformation around C(1')-C(2'') of the 1'-phosphorylated tetrahydropyran-2''-methyl (THPM) compound **1** was studied (see Fig. 1).

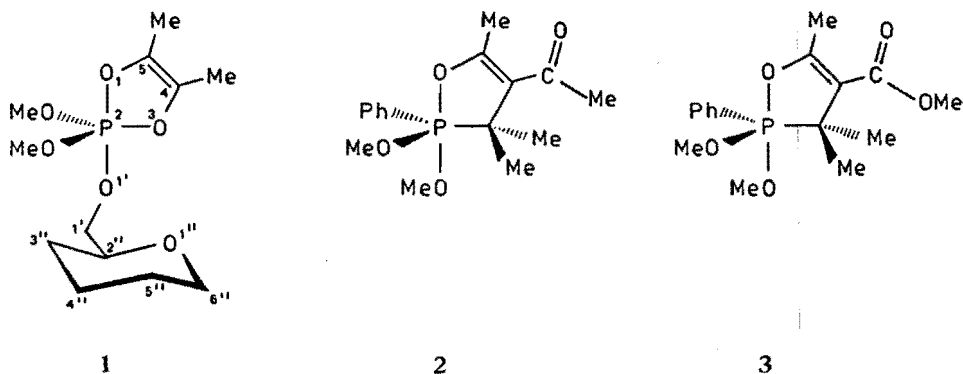


Fig. 1. Compounds 1-3.

It was shown that the fraction of the gauche(-) (g^-) rotamer (maximum distance between O(1') and O(1'')) increases to 40% on going from P^{IV} (four-coordinate phosphorus) to P^V (five-coordinate phosphorus) TBP (trigonal bipyramidal) provided that the THPM group is in axial position¹. This conformational effect can be explained by an increased electron transfer towards the axial exocyclic oxygen atom O(1') of the TBP with respect to the corresponding O(1') of the P^{IV} compounds, which results in an increased repulsion between the oxygen atoms O(1') and O(1'') (see Fig. 1). The principle of electron transfer in the axial sites of a P^V TBP appears to be of general importance. For instance, the hydrolysis of a cyclic phosphate, which is an intermediate in the hydrolysis of RNA by Ribonuclease² (see Fig. 2), proceeds via a transient P^V structure which is formed after axial nucleophilic attack on the cyclic phosphate.

The electron density on the axial exocyclic nucleophile is reduced by the opposite axial oxygen atom O(2'). This oxygen atom is specifically protonated by

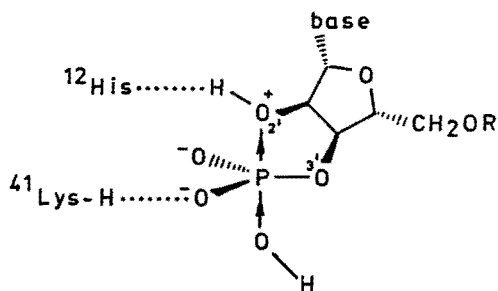


Fig. 2. Electron transfer in the axial P-O bonds of a P^V dioxaphosphole compound.

the His-12 site of the enzyme to function as an electron acceptor. Subsequently, the P-O(2') bond is broken. Similar electron transfer processes in the axial P-O bonds of the TBP were suggested to occur in the compounds 2-3 (Fig. 1)^{3,4}. In these model compounds, the carbonyl group at C(4) of the oxaphosphole ring acts as an electron acceptor, affecting the electron distribution around this ring. It is anticipated that this should result in a decreased electron density on the axial exocyclic oxygen atom O(1') (as compared to the electron density on O(1') of compound 1), with an accompanying decrease in g^- rotamer fraction. This prompted us to study the electron distribution of some P^V structures.

In this work the attention is specifically focused on electron transfer in the axial bonds of the P^V TBP systems, via MNDO calculations (compounds 4-5, Fig. 3) and a 300-MHz ^1H NMR study (compounds 6-11, Fig. 4). Note that the compounds 6-11 are particularly suited for this study since they do not show phosphorus pseudorotation, which results in a fixed location of the OR group in the axial position of the TBP. Also, the O(1')-C-C-O(1'') fragment in these molecules can be used as a probe for the electron density on the axial oxygen atom O(1') (*vide infra*). The results of the calculations which indeed show a decrease in electron density on the axial exocyclic oxygen atom O(1') (see § 3.2.1), are supported by the ^1H NMR study of the compounds 6-11. The measurements show a decrease in trans orientation between O(1') and O(1'') (g^- rotamer fraction decreases),

which is an indication of a reduced repulsion between these two oxygen atoms (see § 3.2.2).

3.2 Results and Discussion

3.2.1 MNDO calculations

Calculations were carried out with the MNDO programme, QCPE version⁵. This programme does not include d-orbital functions for phosphorus. However, a number of ab initio studies on P^V compounds revealed that the principal concept of bonding is adequately described without the introduction of d-functions⁶. Indeed, the results of our MNDO calculations for pentamethoxyphosphorane, listed in Table I, show that the values for the axial P-O bond lengths and the electron density on the axial oxygen atoms are larger than the corresponding values for the equatorial positions. This is in accordance with the TBP structure⁷. The calculated values for the P-O bond lengths are in good agreement with the values obtained from X-ray crystallographic data for pentaphenoxyphosphorane⁸. Therefore, it seems safe to assume that MNDO calculations predict the structure of P^V TBP compounds correctly.

Table I. Results of the MNDO calculations for trimethylphosphate and pentamethoxyphosphorane.

Compound	P-O bond length (Å°)	Electron density		O(ax)-P-O(eq)
		on oxygen		
Trimethylphosphate	1.604	-0.487		-
Pentamethoxyphosphorane	1.63(eq) 1.67(ax)	-0.512(eq)	-0.595(ax)	90°

For the calculations the structures of the compounds 4-5 (see Fig. 3) were selected. The non-pseudorotating P^V TBP compounds 2-3 are represented by the

Table III. Bond angles and twist angles in compounds 4-5.

	4	5a	5b	5c
P-O(1)-C(5)	114.6	115.7	117.1	116.7
O(1)-P-C(3) ^a	86.8	87.4	85.0	84.6
C(2')-P-O(1')	95.2	94.1	96.9	97.5
C(1')-O(1')-P-C(2')	63.3	59.5	60.6	61.8
C(4')-C(4)-C(5)-O(1)	179.7	180.1	179.5	179.8

^a Oxygen in compound 4.

Table IV. Electron densities in compounds 4-5.

	4	5a	5b	5c
O(1)	-0.513	-0.534	-0.546	-0.548
O(1')	-0.583	-0.617	-0.588	-0.577
C(3)	-0.333 ^a	0.095	0.138	0.148
P	1.029	0.757	0.718	0.704
C(5)	0.018	0.186	0.263	0.297
C(4)	-0.096	-0.399	-0.456	-0.454
C(4')	0.126	0.137	0.302	0.462

^a Oxygen in compound 4.

Gorenstein and Buono^{3,4}. The magnitude of this polarization depends on the nature of this substituent at C(4). Further inspection of the Tables reveals that the effects of the polarization of this π -system are transmitted through the ring, affecting the axial P-O bond lengths and the charge densities on both the axial oxygen atoms. Note that the P-O(1) bond is predicted to be longer than the P-O(1') bond. This is related to the $p\pi$ - $d\pi$ back-bonding⁹. The calculations for compound 4 show that, in the dioxaphosphole ring, the C=C bond is hardly polarized. Despite this difference in polarization between 4 and 5 there is not much difference in the calculated electron density on the axial oxygen atom O(1') of these compounds. However, within the group of compounds (5a-c), which differ only by one substituent R, there is a clear trend towards a decreasing electron density on the axial exocyclic oxygen atom O(1') with increasing polarization (increasing

electronegativity of the substituent R) of the π -system of the oxaphosphole ring.

This could be nicely demonstrated by a 300-MHz ^1H NMR study of the conformation around C(1')-C(2'') of the non-pseudorotating compounds 6-11 (Fig. 4). Three staggered rotamers (g^- and g^+ , g^t (Fig.7)) contribute to the conformation around this bond. The maximum distance between O(1') and O(1'') is reached in the g^- rotamer (trans orientation of O(1') and O(1'')). Therefore, the g^- rotamer fraction can be used as a probe for the repulsion between these two oxygen atoms. It is shown that the g^- rotamer fraction of the compounds 6-11 is decreased as compared to, for example, compound 1. This is presumably caused by the decreased electron density on the axial exocyclic oxygen atom O(1') (*vide infra*).

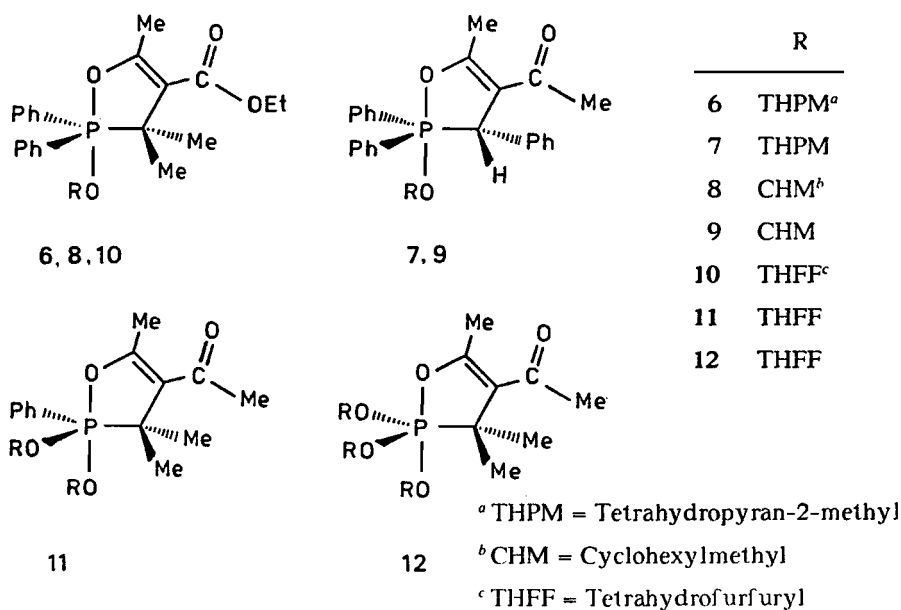


Fig. 4. Model compounds 6-12 for the ^1H NMR measurements.

3.2.2 ^1H NMR measurements

A conformational analysis was performed of the compounds 6-11 (see Fig. 4). In these compounds which do not show pseudorotation, the OR group occupies

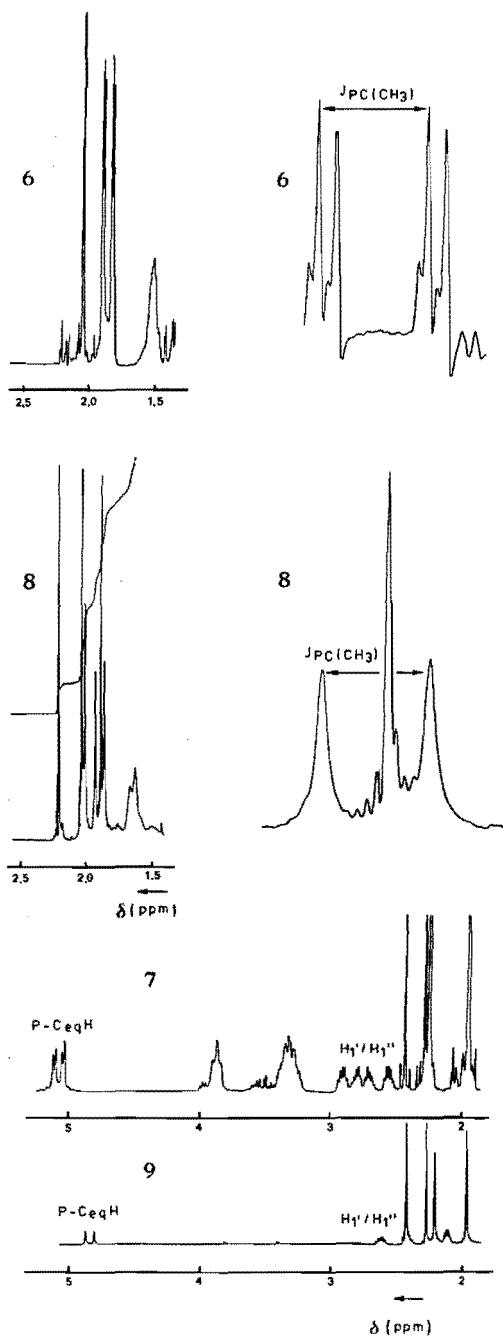


Fig. 5. 300-MHz ^1H NMR spectra of compounds 6-9.

an axial position in the TBP. This is in accordance with the apicophilicity rules¹⁰. The axial position of the OR group is confirmed by ¹H and ¹³C NMR. For compound 6, the two methyl groups at C(3) of the oxaphosphole ring are not equivalent and therefore show two doublets, due to an additional phosphorus coupling of 22 Hz³ (see Fig. 5). It is obvious that this non-equivalency cannot be attributed to an equatorial location of the THPM group since, in that case, the similar compound 8 should also show two doublets. As can be seen in Fig. 5, compound 8 only shows one doublet. Therefore, the non-equivalency has to be attributed to a different shielding of the two methyl groups by the atoms O(1') and C(3') which are located underneath the methyl groups when the THPM group is located in the axial position of the TBP¹¹. Similar arguments for 7 and 9 show that the double doublet of the hydrogen atom at C(3) of the oxaphosphole ring reduces to a doublet for compound 9 if the THPM group is in axial position (see Fig. 5). Additional evidence comes from the ¹³C measurements of the non-pseudorotating compounds 10-12. As can be seen in Fig. 6 a doublet at 67 ppm is found for 10 which is assigned to axial C(1'). For the compounds 11 and 12, downfield to the axial C(1') signal, another ¹³C signal appears which has equal and double intensity, respectively, with respect to the axial C(1') signal. This is assigned to the equatorial C(1') atoms since it was shown that equatorial carbon atoms resonate at lower field than axial carbon atoms⁴.

The rotamer populations around C(1')-C(2'') are calculated as previously¹ with the aid of an empirically generalized Karplus-relation^{12,13} which relates the measured coupling constants $J_{1',2''}$ and $J_{1'',2''}$ to the angle between H(1')/H(1'') and H(2'') (see Fig. 7).

The results of the 300-MHz ¹H NMR measurements and the rotamer fractions around C(1')-C(2'') are listed in Table V.

From a previous NMR study¹ the absolute rotamer fractions for the equatorial and axial THPM groups of compound 13 (Fig. 8) were obtained. The observed rotamer fractions in this pseudorotating compound are an average of the sum of the rotamer populations in the axial and the two equatorial sites.

With a variable-temperature ¹H NMR study the thermodynamic parameters which govern the rotation around the C(1')-C(2'') bond were determined. These

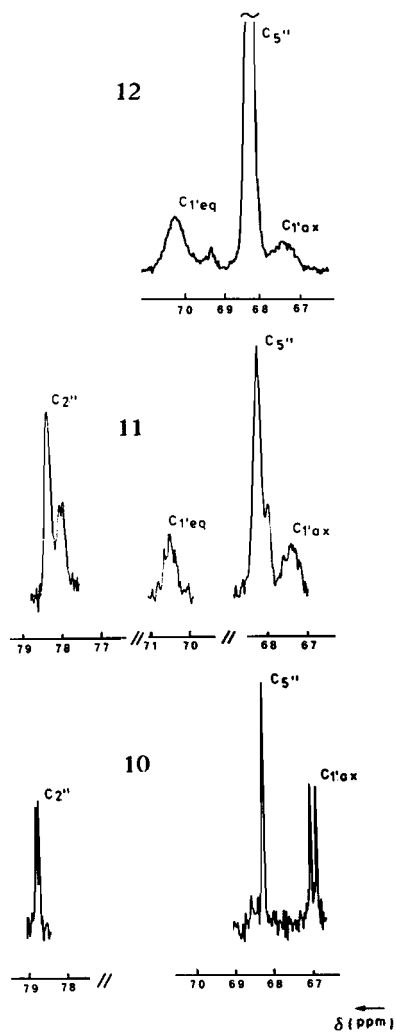


Fig. 6. ^{13}C NMR spectra of compounds 10-12.

parameters allow the calculation of the absolute rotamer fraction for the axial site ($59\% g^-$) and for the equatorial site ($27\% g^-$) of the TBP (in the corresponding P^{IV} compound 10% is found for g^-). Apparently, the absolute g^- rotamer fraction

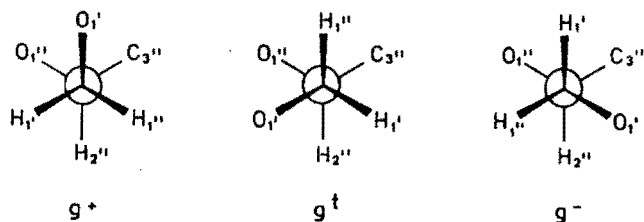


Fig. 7. Rotamers around the C(1')-C(2'') bond.

Table V. ^1H NMR parameters and the corresponding C(1')-C(2'') rotamer populations for compounds 6-11.

Compound	Solvent	$J_{1',2}$	$J_{1'',2}$	$x(g^+)$	$x(g^+)$	$x(g^-)$
6	$(\text{CD}_3)_2\text{CO}$	5.22	3.91	0.52	0.18	0.30
6	CCl_4	5.37	4.55	0.45	0.24	0.31
7 ^a	$(\text{CD}_3)_2\text{CO}$	5.20	5.15	0.40	0.31	0.29
		5.00	5.15	0.41	0.33	0.26
8	$(\text{CD}_3)_2\text{CO}$	^b	^b	-	-	-
9	CDCl_3	6.03	6.13	0.24	0.37	0.39 ^c
10	$(\text{CD}_3)_2\text{CO}$	^b	^b	-	-	-
11	CDCl_3	5.33	5.53	0.35	0.35	0.30

^aThe compounds 7 and 11 exist as a mixture of stereoisomers. For 11, the parameters of only one of the stereoisomers are shown.

^bThe coupling constants of these compounds could not be obtained due to a negligible chemical shift difference.

^cSee note 14.

of 59% for the axial site is not reached for the compounds 6-11, despite the fixed location of the OR group (see Fig. 4) in the axial site of the TBP. On addition, the g^- rotamer fraction of 6-11 is below the fraction observed for the pseudorotating compound 14 ($x(g^-) = 0.41$). Note that the only difference in the structures of 14 and 6-7 is the five-membered ring at phosphorus (and especially the substituents on the ring). This reduced g^- rotamer fraction in 6-7 cannot be attributed to an increased sterical hindrance by the substituents at C(3) of the oxaphosphole ring (see Fig. 4) because this is in contradiction with the observed random distribution about the three rotamers (see Table V). More likely, in view of the electronic

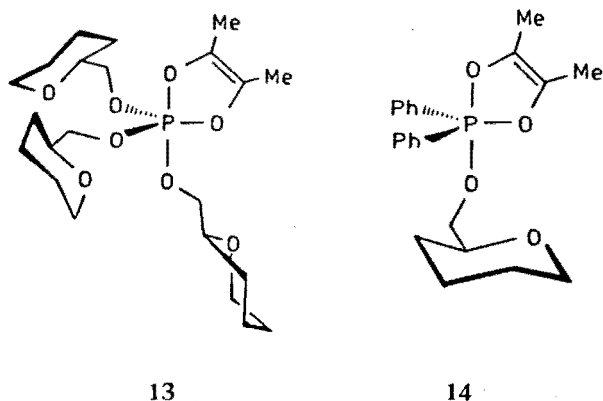


Fig. 8. Model compounds 13-14.

nature of the conformational transmission effect¹, the low g^- rotamer fractions of 6-11 must be attributed to electronic effects. These results are in good agreement with the MNDO calculations which show a clear trend towards a decreasing charge on O(1') with increasing electronegativity of the substituent R at C(4) of the oxaphosphole ring. Since the electron density on O(1') is related to the g^- rotamer fraction, the carbonyl group at C(4) may indeed have caused the decreased $x(g^-)$ value of 30% for 6-11 as compared to the absolute $x(g^-)$ value of 59% for dioxaphosphole compounds, demonstrating that the electron transfer in the axial bonds of the TBP is controlled by the electronic effects of the substituents. So, as was shown in a previous study¹, electron donation increases the electron density on axial exocyclic O(1'). This triggers the conformational transmission process towards an increased g^- rotamer fraction. Now, it is shown that electron accepting substituents reverse the electron transfer process by decreasing the electron density on the axial exocyclic oxygen atom, which results in a reduced conformational transmission effect (reduced g^- rotamer fraction).

3.3 Conclusion

It is shown by MNDO calculations that electronegative substituents at C(4) of the oxaphosphole ring change the electron density on the oxygen atoms in the axial position of the TBP. The electron density on the axial endocyclic oxygen atom is increased whereas the electron density on the axial exocyclic oxygen atom is decreased. This is supported by the conformational analysis of the oxaphosphole P^V TBP compounds 6-11. The g⁻ rotamer fraction of these compounds, which is correlated to the electron density on the axial exocyclic oxygen atom, is strongly decreased as compared to the absolute g⁻ rotamer fraction measured for dioxaphosphole P^V TBP compounds. This is probably caused by the presence of the carbonyl group at C(4) of the oxaphosphole ring.

3.4 Experimental

Solvents and reagents were reagent grade. All reactions were carried out under an atmosphere of dry nitrogen. Phosphines and phosphites were purified by distillation, prior to their conversion to P^V compounds. ¹H NMR spectra were recorded in the FT mode on a Bruker CXP-300 spectrometer at 300.1 MHz, using a 32 K point data set. Chemical shifts are given relative to the CHD₂COD₃ quintet at δ 2.17, or relative to TMS (CDCl₃, CCl₄), with an accuracy of 0.05 Hz. Coupling constants were measured from expansions of the patterns and were analysed using an iterative programme¹⁵. The accuracy of the calculated rotamer populations is about 3% (absolute). ³¹P NMR spectra were recorded in the FT mode at 36.4 MHz on a Bruker HX-90R. Chemical shifts are related to 85% H₃PO₄ as external standard. ¹³C NMR spectra were recorded in the FT mode on a Bruker CXP-300 spectrometer at 75.3 MHz. Chemical shifts are related to the acetone-septet at 30.7 ppm or to TMS (CDCl₃). The compounds 1 and 13 were prepared according to procedures described in ref. 1.

Tetrahydropyran-2-methyloxy diphenylphosphine. — To a stirred and cooled (0°C) solution of tetrahydropyran-2-methanol (2.6 gr, 25 mmol) and triethylamine (2.53 gr, 25 mmol) in 30 ml of sodium-dried ether was added dropwise a solution of chlorodiphenyl phosphine (5.53 gr, 25 mmol) in 15 ml of ether. After completion of the addition (0.5 h), the suspension was refluxed for 2 h. The mixture was cooled, triethylamine hydrochloride was filtered off and the solvent was evaporated. This yielded a colourless oil which, according to ^{31}P and ^1H NMR spectroscopy, was pure. ^1H NMR (acetone- d_6) : δ 1.25-2.0 [m, 6H, H(3)-H(5)], 3.15-4.20 [m, 5H, H(1'), H(2) and H(6)], 7.25-7.90 [m, 10H, ArH]. ^{31}P NMR (acetone- d_6) : δ 119.74.

Cyclohexylmethyloxy diphenylphosphine. — This compound was prepared from cyclohexylmethanol and chlorodiphenyl phosphine by the method described for tetrahydropyran-2-methyloxy diphenylphosphine. Distillation (b.p. 162-166 °C, 0.3 mm) yielded a colourless oil. ^1H NMR (acetone- d_6) : δ 1.1-2.3 [m, 11H, H(1)-H(6)], 3.45-4.20 [m, 2H, H(1')], 7.3-7.9 [m, 10H, ArH]. ^{31}P NMR (acetone- d_6) : δ 116.99.

*Tetrahydrofurfuryloxy diphenylphosphine*¹⁶. — ^1H NMR (acetone- d_6) : δ 1.70-2.05 [m, 4H, H(3)-H(4)], 3.55-4.30 [m, 6H, H(1'), H(2) and H(5)], 7.20-7.70 [m, 10H, ArH]. ^{31}P NMR (acetone- d_6) : δ 119.52.

*Phenyl bis(tetrahydrofurfuryloxy) phosphine*¹⁷. — ^1H NMR (CDCl_3) : δ 1.4-2.3 [m, 8H, H(3), H(4)], 3.50-4.40 [m, 10H, H(1'), H(2) and H(5)], 7.20-7.90 [m, 5H, ArH]. ^{31}P NMR (CDCl_3) : δ 158.82, 158.77, 156.70 (ratio 2:1:1).

*Tris(tetrahydrofurfuryl) phosphite*¹⁶. — ^1H NMR (CDCl_3) : δ 1.60-2.20 [m, 12H, H(3), H(4)], 3.45-4.25 [m, 15H, H(1'), H(2) and H(5)]. ^{31}P NMR (CDCl_3) : δ 139.00.

2,2-Diphenyl-2-(tetrahydropyran-2-methyloxy)-2,2,2,3-tetrahydro-4-ethoxycarbonyl-3,3,5-trimethyl-1,2-oxaphosphole (6). — An equimolar quantity of ethyl α -isopropylidene acetoacetate¹⁸ was added to a cooled solution (0 °C) of tetrahydropyran-2-methyloxy diphenylphosphine in dry acetone. After five days, ³¹P NMR spectroscopy indicated the reaction to be complete. After several weeks, white crystals separated which were filtered and washed with a small amount of ether. ¹H NMR (acetone-d₆) : δ 1.17 [t, 3H, OEt], 1.3-1.95 [m, 6H, H(3'')-H(5'')], 1.95 [dd, 6H, CH₃-C(3), J_{PC_H} = 21 Hz], 2.03 [s, 3H, CH₃-C(5)], 2.88 [m, 2H, H(1'), H(1'')], 3.35 [m, 2H, H(2''), H(6'')_{ax}], 3.90 [m, 1H, H(6'')_{eq}], 4.07 [q, 2H, OEt], 7.4-8.0 [m, 10H, ArH]. ¹³C NMR (acetone-d₆) : δ 14.7-29.9 [C(3'')-C(5'')], 3 CH₃, CH₃-C=O], 47.64 [d, C(3), J_{CP} = 121.7 Hz], 59.35 [s, O-CH₂-C], 68.53 [d, C(1'), J_{COP} = 11.4 Hz], 69.03 [s, C(6'')], 78.26 [d, C(2''), J_{CCOP} = 5.1 Hz], 107.30 and 167.43 [C=C], 129.1-137.3 [Ar]. ³¹P NMR (acetone-d₆) : δ -28.14.

Anal. Calc. for C₂₇H₃₅O₅P : C, 68.95; H, 7.50. Found : C, 66.69; H, 7.63.

2,2,3-Triphenyl-2-(tetrahydropyran-2-methyloxy)-2,2,2,3-tetrahydro-4-acetyl-5-methyl-1,2-oxaphosphole (7). — An equimolar quantity of 2,4-pentadione-3-(phenylmethylene) was added to a cooled solution (0 °C) of tetrahydropyran-2-methyloxy diphenylphosphine in dry acetone. After one day, white crystals separated which were filtered and washed with cold acetone. ¹H NMR (acetone-d₆) : δ 1.1-1.9 [m, 6H, H(3'')-H(5'')], 1.96 [3H, CH₃-C(5)], 2.16 [3H, CH₃-C=O], 2.55-2.95 [m, 2H, H(1'), H(1'')], 3.35 [m, 2H, H(2''), H(6'')_{ax}], 3.90 [m, 1H, H(6'')_{eq}], 5.10 [dd, 1H, H(3), J_{PC_H} = 20.1 Hz], 7.2-8.0 [m, 10H, ArH]. ¹³C NMR (acetone-d₆) : δ 18.65 [CH₃-C(5)], 23.56-29.24 [C(3'')-C(5'')], 29.64 [CH₃-C=O], 50.56 [dd, C(3), J_{PC} = 114 Hz], 67.51 [dd, C(1'), J_{COP} = 9.87 Hz], 68.27 [C(6'')], 77.28 [dd, C(2''), J_{CCOP} = 4.88 Hz], 112.0 and 137.96 [C=C], 127-135 [Ar]. ³¹P NMR (acetone-d₆) : δ -26.80, -27.30.

Anal. Calc. for C₃₀H₃₃O₄P : C, 73.75; H, 6.81. Found : C, 74.32; H, 6.94.

2,2-Diphenyl-2-(cyclohexylmethyloxy)-2,2,2,3-tetrahydro-4-ethoxycarbonyl-3,3,5-trimethyl-1,2-oxaphosphole (8). — This compound was prepared from diphenylcyclohexylmethyloxy phosphine and ethyl α -isopropylidene-acetoacetate

by the procedure described for 6. ^1H NMR (acetone- d_6) : δ 0.95-1.67 [m, 11H, H(1'')-H(6'')], 1.19 [t, 3H, OEt], 1.89 [d, 6H, $\text{CH}_3\text{-C}(3)$], $J_{\text{PCH}} = 20.7$ Hz], 2.01 [s, 3H, $\text{CH}_3\text{-C}(5)$], 2.66 [m, 2H, H(1')], 4.05 [q, 2H, OEt]. ^{31}P NMR (acetone- d_6) : δ -27.23.

2,2,3-Triphenyl-2-(cyclohexylmethoxy)-2,2,2,3-tetrahydro-4-acetyl-5-methyl-1,2-oxaphosphole (9). — This compound was prepared from diphenylcyclohexylmethoxy phosphine and 2,4-pentadione-3-(phenylmethylene) by the procedure described for 7. The white solid which separated immediately was filtered and washed with cold acetone. Recrystallization from chloroform/hexane yielded white crystals. M.p. : 183.0-184.5 °C. ^1H NMR (CDCl_3) : δ 0.70-1.73 [m, 11H, H(1'')-H(6'')], 1.97 [3H, $\text{CH}_3\text{-C}(5)$], 2.19 [3H, $\text{CH}_3\text{-C=O}$], 2.60 [m, 2H, H(1')], 4.83 [d, 1H, H(3)], $J_{\text{PCH}} = 20.6$ Hz], 7.03-7.46 [m, 10H, ArH]. ^{13}C NMR (CDCl_3) : δ 19.42 [$\text{CH}_3\text{-C}(5)$], 25.93-30.07 [C(1'')-C(6''), $\text{CH}_3\text{-C=O}$], 50.21 [d, C(3)], $J_{\text{CP}} = 111.80$ Hz], 69.21 [d, C(1')], $J_{\text{COP}} = 10.40$ Hz], 111.7 and 137.1 [C=C], 127.0-134.0 [Ar]. ^{31}P NMR (CDCl_3) : δ -20.22.

2,2-Diphenyl-2-(tetrahydrofurfuryloxy)-2,2,2,3-tetrahydro-4-ethoxycarbonyl-3,3,5-trimethyl-1,2-oxaphosphole (10). — This compound was prepared from diphenyltetrahydrofurfuryloxy phosphine and ethyl α -isopropylideneacetoacetate by the procedure described for 6. ^1H NMR (acetone- d_6) : δ 1.16 [t, 3H, OEt], 1.4-1.9 [m, 4H, H(3'')-H(4'')], 1.88 [d, 6H, $\text{CH}_3\text{-C}(3)$], $J_{\text{PCH}} = 20.76$ Hz], 2.02 [s, 3H, $\text{CH}_3\text{-C}(5)$], 2.83 [m, 2H, H(1')], 3.70 [m, 2H, H(2''), H(5'')], 3.93 [m, 1H, H(5'')], 4.01 [q, 2H, OEt], 7.20-8.05 [m, 10H, ArH]. ^{13}C NMR (acetone- d_6) : δ 14.46-25.99 [C(3'')-C(5''), 3 CH_3 , $\text{CH}_3\text{-C-O}$], 46.47 [d, C(3)], $J_{\text{CP}} = 121.47$ Hz], 58.99 [s, C- $\text{CH}_2\text{-O}$], 67.05 [d, C(1')], $J_{\text{COP}} = 11.66$ Hz], 68.35 [s, C(5'')], 78.95 [d, C(2'')], $J_{\text{CCOP}} = 4.77$ Hz], 95.80 [d, C=C], 128.0-135.0 [Ar]. ^{31}P NMR (acetone- d_6) : δ -28.26.

2-Phenyl-2,2-bis(tetrahydrofurfuryloxy)-2,2,2,3-tetrahydro-4-acetyl-3,3,5-trimethyl-1,2-oxaphosphole (11). — 2,4-Pentadione-3-(1-methylethylidene) (1 eq)

was added to a solution of phenyl bis(tetrahydrofurfuryloxy) phosphine (1 eq) in dry CDCl_3 under an argon atmosphere. After 10 days at room temperature, ^{31}P NMR indicated the reaction to be nearly complete (90%). ^1H NMR (CDCl_3) : δ 1.76 [dd, 6H, $\text{CH}_3\text{-C}(3)$, $J_{\text{PCCH}} = 20.5$ Hz], 1.6-2.0 [m, 8H, $\text{H}(3'')$ - $\text{H}(4'')$], 2.12 [s, 3H, $\text{CH}_3\text{-C}(5)$], 2.21 [s, 3H, $\text{CH}_3\text{-C=O}$], 3.02-3.58 [m, 2H, $\text{H}(1')_{\text{ax}}$], 3.72-4.32 [m, 8H, $\text{H}(1')_{\text{eq}}$, $\text{H}(2'')$, $\text{H}(5'')$], 7.40-7.90 [m, 5H, ArH]. ^{13}C NMR (CDCl_3) : δ 18.5-31.3 [$\text{C}(3'')$, $\text{C}(4'')$, 4 CH_3], 45.50 [d, $\text{C}(3)$, $J_{\text{CP}} = 121$ Hz], 67.4 [m, $\text{C}(1')_{\text{ax}}$], 68.2 [$\text{C}(5'')$], 70.5 [m, $\text{C}(1')_{\text{eq}}$], 78.3 [m, $\text{C}(2'')$], 86.0 [C=C], 126.0-133.0 [Ar]. ^{31}P NMR (CDCl_3) : δ -18.45, -18.52, -18.70 (ratio 1:1:2).

2,2,2-Tris(tetrahydrofurfuryloxy)-2,2,2,3-tetrahydro-3,3,5-trimethyl-4-acetyl-1,2-oxaphosphole (12). — This compound was prepared from 2,4-pentadione-3-(1-methylethylidene) and tris(tetrahydrofurfuryl) phosphite by a literature procedure for a similar compound³ (trimethyl phosphite). ^1H NMR (CD_2Cl_2) : δ 1.50-2.05 [m, 12H, $\text{H}(3'')$ - $\text{H}(4'')$], 1.90 [6H, $\text{CH}_3\text{-C}(3)$], 2.23 [s, 3H, $\text{CH}_3\text{-C}(5)$], 2.28 [s, 3H, $\text{CH}_3\text{-C=O}$], 3.60-4.23 [m, 15H, $\text{H}(1')$, $\text{H}(2'')$, $\text{H}(5'')$]. ^{13}C NMR (CDCl_3) : δ 18.5-31.2 [$\text{C}(3'')$ - $\text{C}(4'')$], 43.8 [d, $\text{C}(3)$, $J_{\text{CP}} = 125$ Hz], 67.4 [m, $\text{C}(1')_{\text{ax}}$], 68.3 [$\text{C}(5'')$], 70.3 [$\text{C}(1')_{\text{eq}}$], 77.5 [$\text{C}(2'')$].

2,2-Diphenyl-2-(tetrahydropyran-2-methyloxy)-2,2-dihydro-4,5-dimethyl-1,3,2-dioxaphosphole (14). — An equimolar quantity of 2,3-butadione was added to a cooled solution (0 °C) of tetrahydropyran-2-methyloxy diphenyl phosphine in dry acetone- d_6 . After 2 hours, ^{31}P NMR indicated the reaction to be complete. ^1H NMR (acetone- d_6) : δ 1.38-1.69 [m, 6H, $\text{H}(3'')$ - $\text{H}(5'')$], 2.01 [s, 6H, OCH_3], 3.50-3.78 [m, 2H, $\text{H}(2'')$, $\text{H}(6'')$ ax], 4.04 [m, 1H, $\text{H}(6'')$ eq], 4.13 [m, 2H, $\text{H}(1')$], 7.40-8.05 [m, 10H, ArH]. ^{31}P NMR (acetone- d_6) : δ -23.89.

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13. $x(g^+) = -0.075 J_{1',2''} - 0.100 J_{1'',2''} + 1.303$
 $x(g^f) = -0.054 J_{1',2''} + 0.104 J_{1'',2''} + 0.061$
 $x(g^-) = 0.129 J_{1',2''} - 0.003 J_{1'',2''} - 0.364$
14. The $x(g^-)$ value of **9** deviates from the values of the other compounds because **9** doesn't possess the ring oxygen atom O(1''). Comparable values for the rotamer populations were found for 2,2-diphenyl-2-cyclopentyl-methoxy-2,2-dihydro-4,5-dimethyl-1,3,2-oxaphosphole¹⁶.

15. Panic program : Copyright, Bruker Spectrospin AG, Switzerland.
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CHAPTER 4*

Different rotamer populations around the C(5)-C(6) bond for α - and β -D-galactopyranosides through the combined interaction of the gauche and anomeric effects. A 300-MHz ^1H NMR and MNDO study

Abstract.

A 300-MHz ^1H NMR study of methyl 2,3,4-tri-O-methyl- α - (**1**) and β -D-galactopyranoside 6-dimethylphosphate (**3**) using various solvents shows that the gauche (g^+) rotamer populations about the C(5)-C(6) bond are the same in all solvents, whereas those of the gauche(trans) (g^t) and gauche(-) (g^- , O(5) and O(6) trans) are solvent dependent. The g^- population increases with decreasing polarity of the solvent which is attributed to an increased electrostatic repulsion between O(5) and O(6) in apolar solvents. The g^- population of **3** is larger than that of **1** and the same difference is observed in the corresponding compounds (**2** and **4**) which have a trigonal-bipyramidal (TBP) five-coordinated phosphorus (P^V) at position 6 and which have a higher electron density at O(6). These differences in rotamer populations are due to an effect additional to that of the coulombic effect between O(5) and O(6). That these differences are caused by a combination of the gauche and anomeric effects is supported by the finding that the g^- population increases with increasing $\text{p}K_a$ of the group at C(1). The results of the NMR measurements (in CCl_4) are reproduced fairly accurately by MNDO calculations on model systems. The solvent dependence of the rotamer population around the C(5)-C(6) bond is a good criterium for the assignment of the H(6S,6R) resonances since, for galactopyranosides, $J_{5,6S}$ increases and $J_{5,6R}$ decreases as the polarity of the solvent decreases.

* N. K. de Vries and H. M. Buck, Carbohydr. Res. **165**(1), 1 (1987).

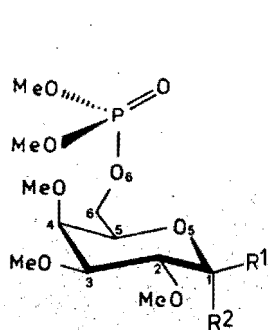
4.1 Introduction

The anomeric effect has gained widespread attention¹⁻⁷ and has been explained in several theoretical studies⁸⁻¹¹, and although the molecular orbital (MO) description of stabilizing orbital interactions is now preferred there is still some ambiguity in the interpretation of the total energies, obtained from the MO calculations. Analysis in terms of localized MO's suggests that the oxygen lone pairs are different¹², and that the anomeric effect is part of the more general gauche effect, whereas the use of canonical MO's predicts that the oxygen lone pairs are different¹³. Research in this area is still in progress, as the recent publication of two new theories shows¹⁴⁻¹⁵.

One of the major accomplishments of the MO theory is the quantitative description of the shortening of the C(1)-O(1) bond first observed in the crystal structures of pyranosides¹⁶ and attributed to the anomeric effect. Later, a correlation was noticed¹⁷ between the pK_a of the group at the anomeric centre and the lengths of the endo- [O(5)-C(1)] and exocyclic [C(1)-O(1)] bonds. As the pK_a increases the length of the O(5)-C(1) bond increases, whereas that of C(1)-O(1) bond decreases. Also, the length of the endocyclic bond of β -pyranosides where the C(1) substituent is equatorial, is larger than that in the corresponding α -anomers. These changes in bond lengths can be interpreted as a combination of coulombic and stereoelectronic effects (principally the maximalization of orbital-overlap⁸).

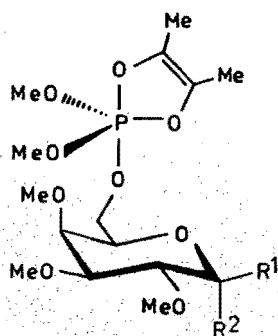
It was shown¹⁸ that the conformation around the C(5)-C(6) bond of methyl 2,3,4-tri-O-methyl- α -D-galactopyranoside-6-dimethylphosphate (**1**) changes towards a higher gauche(-) (**13**, g^-) population on going from four-coordinated phosphorus (6-P^{IV}) in **1** to the five-coordinated phosphorus (6-P^V) in **2** (see chapter 2).

The observed rotamer distributions around the C(5)-C(6) bond of **1** and **2** were also interpreted as a combination of a stereoelectronic (gauche) effect¹² and of coulombic repulsion between O(5) and O(6). In a P^V trigonal-bipyramidal structure (**2**) the electron density at O(6) is increased, thus favouring the g^- rotamer **13** where O(5) and O(6) have maximal separation. The endocyclic oxygen O(5) is



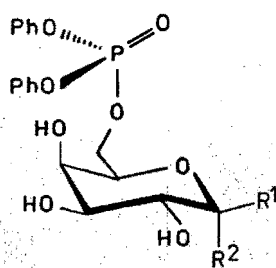
1 $R^1 = H, R^2 = OMe$

3 $R^1 = OMe, R^2 = H$



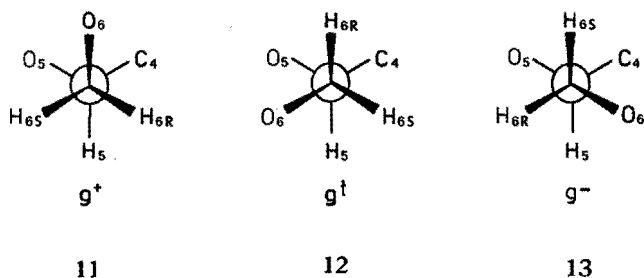
2 $R^1 = H, R^2 = OMe$

4 $R^1 = OMe, R^2 = H$



	R^1	R^2
5	H	OMe
6	H	p-OPh-NO ₂
7	OMe	H
8	OPh	H
9	p-OPh-NO ₂	H
10	o-OPh-NO ₂	H

involved in both the change in the length of the O(5)-C(1) bond and the conformational change around the C(5)-C(6) bond. We then wondered whether stereoelectronic effects of the anomeric group are transmitted via O(5), thus influencing the conformation around the C(5)-C(6) bond. We now show that in the β -anomers (3 and 4) of 1 and 2 the g^- population increases and that the conformation around the C(5)-C(6) bond reflects the pK_a of the anomeric substituent.



4.2 Results and Discussion

4.2.1 Rotamer population determination

In a 300-MHz ^1H NMR study on the galactopyranosides **3-10** the contributions of the three staggered rotamers g^+ (**11**), g' (**12**) and g^- (**13**) to the conformation around the C(5)-C(6) bond were calculated using the three equations which are based on an empirically generalized Karplus-relation¹⁹, as used in previous studies¹⁸.

$$x(g^+) = -0.075 J_{5,6S} - 0.100 J_{5,6R} + 1.303$$

$$x(g') = -0.054 J_{5,6S} + 0.104 J_{5,6R} + 0.061$$

$$x(g^-) = 0.129 J_{5,6S} - 0.003 J_{5,6R} - 0.364$$

The outcome of the calculations critically depends on a correct assignment of H(6S) and H(6R) resonances, a problem which has not yet been settled. Therefore, the assignments of H(6S,6R) were based on literature data, especially for compounds²⁰ that were specifically deuterated at C(6), and on the solvent dependence of the conformation around the C(5)-C(6) bond. For phosphorylated nucleosides and tetrahydrofuran derivatives the g^- rotamer population around the C(4)-C(5) bond increases with decreasing solvent polarity²¹ and this was attributed to an increase in electrostatic repulsion between O(5') and O(1'). A similar dependence is found for phosphorylated galactopyranosides (see § 4.2.2). In favourable cases an unambiguous assignment was possible e.g. for methyl 2,3,4-tri-O-methyl- β -D-galactopyranoside in CDCl_3 where line-broadening of the H(6S) resonance

occurs due to hydrogen-bonding of HO(6) with O(5) which results in an additional coupling with H(6S) and no coupling with H(6R) because of the unfavourable angle between HO(6) and H(6R). In Fig. 1 the conformation around the C(5)-C(6) bond is g^- ; hydrogen-bonding is also possible in the g^+ conformation (11) but this rotamer is strongly disfavoured in galactopyranosides due to 1,3-*syn*-diaxial interactions. Addition of water removes the line-broadening (see Fig. 1).

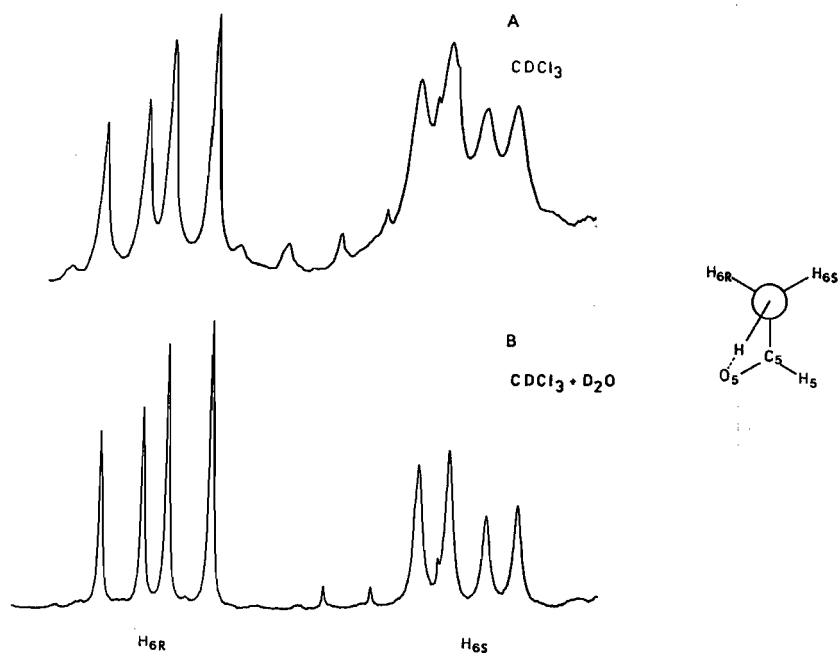


Fig. 1. $H(6R,6S)$ resonances for methyl 2,3,4-tri-*O*-methyl- β -*D*-galactopyranoside in A $CDCl_3$ and B $CDCl_3 + D_2O$.

4.2.2 Solvent dependence of the conformation around the C(5)-C(6) bond

The spectral parameters and the rotamer populations for **1** and **3** given in Table I show that the g^- rotamer population increases as the solvent polarity

decreases and that there is an accompanying decrease in the g^t population.

Table I. ^1H NMR data and the corresponding C(5)-C(6) rotamer populations.

Solvent	E_T^a	$\delta[\text{H}(6\text{S})]$	$\delta[\text{H}(6\text{R})]$	$J_{5,6\text{S}}$	$J_{5,6\text{R}}$	$x(g^+)$	$x(g^t)$	$x(g^-)$
1								
CCl_4	32.5	4.249	4.186	5.97	6.72	0.18	0.43	0.39
CDCl_3	39.1	4.181	4.167	5.79	6.84	0.18	0.46	0.36
$(\text{CD}_3)_2\text{CO}^b$	42.2	4.140	4.090	5.41	7.04	0.19	0.50	0.31
CD_3OD	55.5	4.201	4.164	4.83	7.62	0.18	0.59	0.23
D_2O	63.1	4.020	3.910	4.15	8.09	0.18	0.67	0.15
3								
CCl_4	32.5	4.272	4.201	6.52	6.12	0.20	0.34	0.46
CDCl_3	39.1	4.210	4.194	6.20	6.83	0.16	0.43	0.41
$(\text{CD}_3)_2\text{CO}$	42.2	4.247	4.209	5.61	6.90	0.19	0.47	0.34
CD_3OD^c	55.5	4.375	4.346	5.21	7.16	0.19	0.52	0.29
D_2O	63.1	4.012	3.952	4.66	8.11	0.14	0.65	0.21

^a The solvent polarity parameter E_T is based on the position of electronic spectra peaks of pyridinium-N-phenolbetaine in various solvents²².

^b See ref. 18.

^c Obtained using a Bruker 500 MHz spectrometer at the Dutch National NMR Facility (Nijmegen).

Although the g^t rotamer is more favoured energetically in polar solvents because of the gauche effect¹² (a pronounced preference of gauche over *trans* geometry in O-C-C-O fragments), in apolar solvents the g^- population increases at the cost of the g^t population because of an increased charge repulsion between O(5) and O(6). These results accord with those of Tvaroska and Kozar²³ on 2-methoxytetrahydropyran. They compared the stability of the chair conformers of MTHP, bearing an equatorially and axially oriented methoxyl group, in various solvents by using the continuum reaction field method in conjunction with quantum-chemical calculations. In this way it was shown that polar solvents stabilize conformers, which are less stable in an isolated state or in nonpolar solvents because of intramolecular electrostatic interactions. So, the *trans* conformer (equatorial OMe), which is destabilized by the anomeric effect, is stabilized by electrostatic interactions with polar solvents. The gauche conformation around C(5)-C(6),

which is destabilized by electrostatic repulsion between O(5) and O(6), is stabilized in exactly the same way by solvent-solute interactions with polar solvents (see Table I). The g^+ rotamer populations of **1** and **3**, to a good approximation, remain constant; in a theoretical study on the conformation of α - and β -glucopyranose it was shown²⁴ that the g^- rotamer is solvent dependent, despite a 1,3-*syn*-diaxial interaction of O(4) and O(6). The g^+ populations of **1** and **3** are independent of solvent polarity, despite the *gauche* orientations, since they are determined by steric factors rather than by electronic factors¹⁸.

Although the solvent dependence of the rotamer populations (g^+ , g^f , and g^-) of **1** and **3** indicates the correctness of the assignment of the H(6R,6S) resonances, the assignments were also established in a different way. Ohruj *et al.*²⁵ showed, by selective deuteration of C(6) involved in the glycosidic linkage, that for (1 \rightarrow 6)- β -linked digalactosides, H(6S) always resonated downfield of H(6R) and stated that this result was "independent of the species of the protecting groups, and even of the conformational changes about the C(5)-C(6) bond". Methyl 6-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-2,3,4-tri-O-methyl- α - and - β -D-galactopyranoside were therefore prepared. These compounds only differ from **1** and **3** in that the 6-phosphate group is replaced by a 2,3,4,6-tetra-O-acetyl-D-galactopyranose moiety. The solvent dependence of the rotamer populations around the C(5)-C(6) interglycosidic bond of these disaccharides accorded with the results of Ohruj *et al.*²⁵ (large preference for the g^f rotamer) and were virtually identical to those of the monosaccharides. Since the assignments for the disaccharides are unambiguous the assignments of the H(6R,6S) resonances for **1** and **3** (Table I) are established. The results for **1** and **3** are summarized in Fig. 2 and details for the disaccharides will be given in chapter 5.

The data in Fig. 2 show that the g^- rotamer population of the β - compound **3** is always larger than that of the α -compound **1**. This difference could reflect stereoelectronic effects, e.g. a different orientation of orbitals along the C(1)-O(5) bonds caused by the different orientation of the 1-substituent which, in turn, could influence the orbital interactions which govern the rotation around the C(5)-C(6) bond. De Bruyn and Anteunis²⁶ suggested that the different $J_{5,6S(6R)}$ values for methyl α - and β -D-galactopyranoside might be caused by mutual

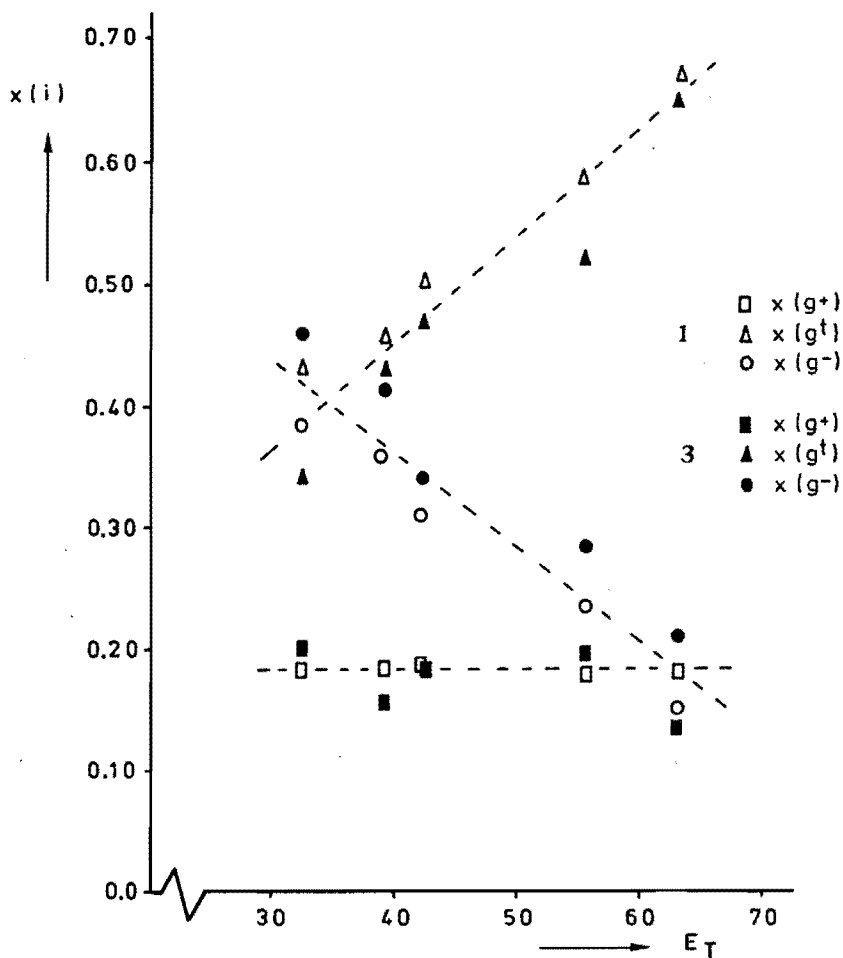
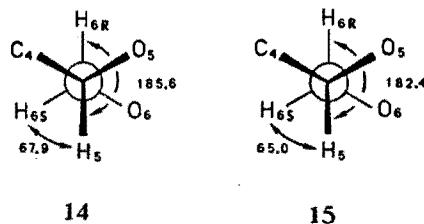


Fig. 2. Rotamer populations around the C(5)-C(6) bond of 1 and 3.

repulsion of the dipoles associated with C(6)-O(6) and C(1)-O(1) bonds. However, this dipole-dipole interaction is probably weaker than the stereoelectronic effects involved²⁷. Alternatively, the different rotamer populations of 1 and 3 might be attributed to different degrees of ring-puckering of these compounds. In general, the rings of α -pyranosides are less puckered than those of the β -

pyranosides²⁸ which results in a deviation from the proton-torsion angles (-60° , 60° and 180°) for staggered conformations which we have used in the Karplus-equation¹⁸. The neutron diffraction data for methyl α - and β -D- galactopyranoside²⁹ were used therefore to calculate two new sets of three equations, namely, α -compounds : $x(g^+) = -0.052 J_{5,6S} + 0.101 J_{5,6R} + 0.056$; $x(g^-) = 0.125 J_{5,6S} + 0.003 J_{5,6R} - 0.351$; $x(g^+) = 1 - x(g^+) - x(g^-)$; β compounds : $x(g^+) = -0.051 J_{5,6S} + 0.098 J_{5,6R} + 0.054$; $x(g^-) = 0.123 J_{5,6S} + 0.006 J_{5,6R} - 0.351$; $x(g^+) = 1 - x(g^+) - x(g^-)$. Only the proton-torsion angles for the g^+ conformation have been changed, as can be seen in the g^+ conformations 14 and 15 in which methyl α - and β -D-galactopyranoside crystallize. Because this conformer makes the largest contribution to the rotamer population around the C(5)-C(6) bond (see Table I), the effect will be most noticeable from changes in the proton-torsion angles of the g^+ rotamer.



Insertion of the measured coupling constants (Table I) into these new equations gives a new set of rotamer populations for 1 and 3 [e.g. in CD_3OD : for 1 $x(g^+) = 0.16$, $x(g^+) = 0.57$, $x(g^-) = 0.27$; for 3 $x(g^+) = 0.18$, $x(g^+) = 0.49$, $x(g^-) = 0.33$]. Although the absolute values of the rotamer populations for both 1 and 3 have changed, the difference in g^- rotamer populations between the α - and β -compound remains the same. Although this analysis is not based on the large number of crystallographic data necessary to obtain statistically reliable values for the proton-torsion angles of all rotamers around the C(5)-C(6) bond for both α - and β -compounds, it is sufficiently reliable to show that the observed differences in rotamer populations are not caused by differences in ring-puckering. This conclusion is supported by NMR measurements on a series of acetylated

methyl α - and β -D-glucopyranosides³⁰ where no difference was found in the $J_{5,6S(6R)}$ values for the α - and β -compounds, except for one compound for which there was probably hydrogen bonding between HO(6) and O(5). If the ring-puckering is the cause of the differences in rotamer populations for galactopyranosides, similar differences would be expected for glucopyranosides [different $x(g^+)$ and $x(g^t)$ values, equal $x(g^-)$ values]. Additional evidence that the difference is caused by stereoelectronic effects, and not by the ring-puckering, was obtained from the 300-MHz 1H NMR data for 5-10 which are listed in Table II.

4.2.3 Relationship between $J_{5,6S}$ and the pK_a of the group at C(1)

The g^- rotamer populations $x(g^-)$, which are largely indicated by the $J_{5,6S}$ values, are shown in Fig. 3. The changes in the length of the exo- and endocyclic bonds for the 1-O-acetyl, 1-O-phenyl and 1-O-methyl derivatives are shown in Fig. 4³¹.

Table II. 1H NMR data for 5-10.

Compound	Solvent	δ (ppm)		J(Hz)		
		H(6S)	H(6R)	5,6S	5,6R	6S,6R
5	CD ₃ OD	4.577	4.618	3.91	8.22	-10.59
6	CD ₃ OD	4.590	4.648	3.42	8.69	-10.93
7	CD ₃ OD	4.634	4.681	4.13	7.82	-10.72
8	CD ₃ OD	4.659	4.693	3.76	8.15	-10.71
8	(CD ₃) ₂ CO	4.652	4.615	4.18	7.83	-10.62
9	CD ₃ OD	4.668	4.741	3.45	8.28	-10.86
10	CD ₃ OD	4.480	4.541	3.63	8.20	-10.81
10	(CD ₃) ₂ CO	4.555	4.536	4.14	8.00	-10.73

Fig. 3 shows a clear trend towards decreasing $x(g^-)$ values with decreasing pK_a . The dotted lines in Fig. 3 are intended to show a trend and not a linear relationship although such a relationship between the pK_a and the lengths of the C-O bonds around the anomeric centre has been demonstrated¹⁷. The correlation was

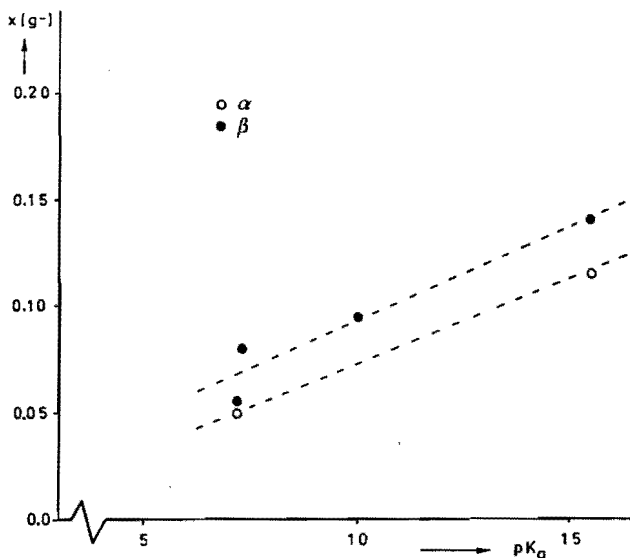


Fig. 3. The gauche(-) populations of 5-10 [pK_a 7.15: 6 (○), 9 (●); pK_a 7.17: 10 (●); pK_a 10.0: 8 (●); pK_a 15.5: 5 (○), 7 (○)].

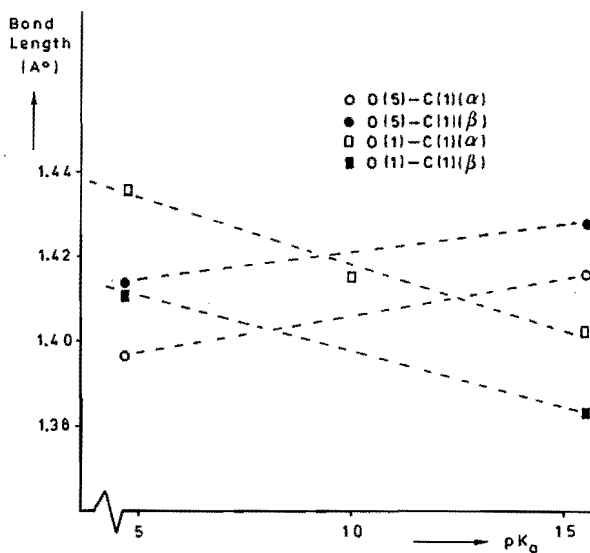
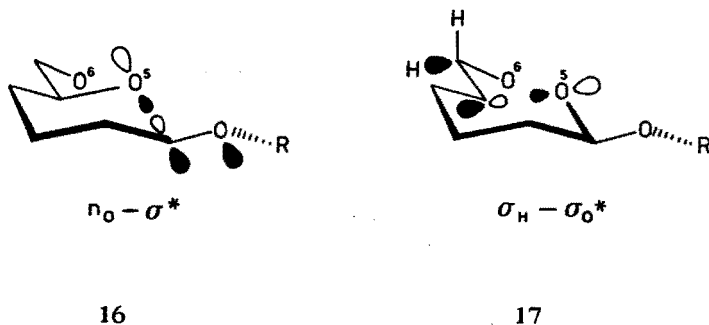


Fig. 4. Lengths of the exo- [O(1)-C(1)] and endocyclic [O(5)-C(1)] bonds of 1-O-acetyl (pK_a 4.75), 1-O-phenyl (pK_a 10.0), and 1-O-methyl (pK_a 15.5) derivatives of glycopyranosides. Values for the length of the bonds are mean values for several structures and are taken from ref. 31.

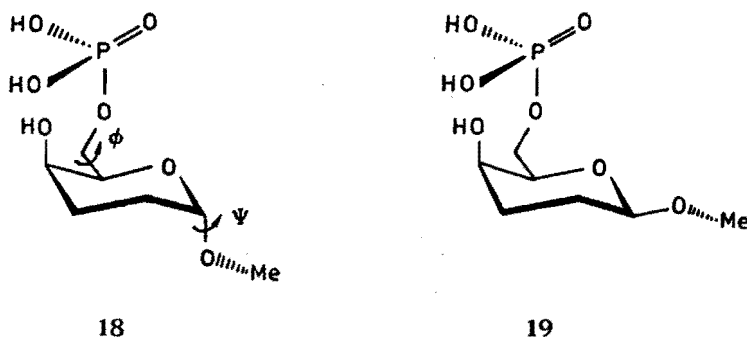
accurate for the exocyclic bond of trans-1-oxadecalin systems ($r = 0.9995$) and reasonable for the endocyclic bond ($r = 0.985$). Later, it was shown for a series of 2-aryloxytetrahydropyrans that the dependence of the bond length on the pK_a of the group at the anomeric centre is different for equatorial and axial substituents and not necessarily linear³². Regardless of the exact relationship, there is a striking resemblance between the dependence of both the lengths of the C-O bonds and $J_{5,6S(6R)}$ [and $x(g^-)$] on the pK_a (see Figs. 3 and 4). The fact that the lengths of the C-O bonds are determined by stereoelectronic interactions^{8,31} favours the suggestion that the difference in rotamer populations for α - and β -galactopyranosides also has a stereoelectronic origin. The ring-puckering cannot be responsible for the different conformations around the C(5)-C(6) bond since the J values for e.g. 8 and 9 are similar [8 (MeOD): $J_{1,2}$ 7.74, $J_{2,3}$ 9.71, $J_{3,4}$ 3.38, $J_{4,5}$ 1.07 Hz; 9 (MeOD): $J_{1,2}$ 7.80, $J_{2,3}$ 9.74, $J_{3,4}$ 3.40, $J_{4,5}$ 1.10 Hz]. Thus, there seems to be a combined interaction of the anomeric and gauche effects which governs the rotation around the C(5)-C(6) bond. This is visualized in 16 and 17 where the $n - \sigma^*$ interaction (anomeric effect) and the $\sigma_H - \sigma_{O^*}$ interaction (gauche effect in the g^t rotamer) are drawn for a β -compound.



The combined interaction of these two effects apparently results in a different stabilization of the g^t rotamer for α - and β -compounds. A more detailed description of these orbital interactions has been given by Kirby³¹.

4.2.4 MNDO calculations

The calculations were performed on **18** and **19** as models for α - and β -galactopyranoside, respectively.



The value for Ψ (C(2)-C(1)-O(1)-Me) was restricted to 180° , the most stable conformation for the exocyclic methoxy group in both the axial and equatorial conformation^{5,33}, and the energies for the staggered conformations around the C(5)-C(6) bond ($\phi = \text{C}(4)\text{-C}(5)\text{-C}(6)\text{-O}(6)$) were determined. All structures were optimized with respect to bond lengths, bond angles, and twist angles. As can be seen from the data in Table III, the anomeric effect does not lead to different electron densities on O(5) for α - and β -compounds. This finding accords with results obtained from PCILO calculations on 2-methoxytetrahydropyran³³ and STO-3G calculations on dimethoxymethane³⁴.

Hence, unlike¹⁸ **1** and **2**, the different rotamer populations around the C(5)-C(6) bonds in **1** and **3** cannot be attributed to differences in coulombic repulsion between O(5) and O(6). The relative energies of the different conformations of **18** and **19** are listed in Table IV, together with the calculated relative energies of the rotamers around the C(5)-C(6) bond of **1** and **3**.

The results in Table IV show that whereas the calculations predict the g^- rotamer to possess the lowest energy level for both α - and β -compounds, this is not found

Table III. Electron densities for **18** and **19**.

Atom	18			19		
	ϕ : -60 (g^+)	180 (g')	60 (g^-)	-60 (g^+)	180 (g')	60 (g^-)
O(5)	-0.344	-0.355	-0.342	-0.350	-0.359	-0.344
O(6)	-0.493	-0.524	-0.511	-0.495	-0.526	-0.509

Table IV. Relative energies of the rotamers around the C(5)-C(6) bond of **1**, **3**, **18**, and **19**.

ϕ	18 ^a	19 ^a	1 ^b	3 ^b
-60 (g^+)	1.26	4.70	0.50	0.49
180 (g')	0.29	3.59	0.00	0.18
60 (g^-)	0.00	3.40	0.07	0.00

^a Energies relative to that of the g^- rotamer of **18** (in kcal.mole⁻¹).

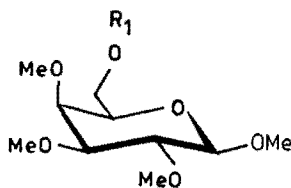
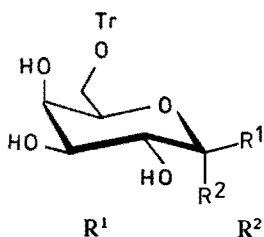
^b Free energy value (kcal.mole⁻¹) determined with the equation $\Delta G = -RT \cdot \ln K$ for **1** and **3** (CCl₄).

experimentally. The results for the β -compound **3** in CCl₄ [the calculated anomeric effects for an isolated molecule are comparable with the experimental values in CCl₄ (cf. ref. 23)] are fairly good in that they predict both the correct order for the energy levels of the rotamers and the correct differences in energy of the g^- and g' rotamers. The results for the α -compound are less good. In the calculations, the g^- rotamer is favoured whereas, experimentally, the g' rotamer is found to be more stable. However, the differences are small, and it should be kept in mind that the experimental rotamer populations are solvent dependent (see Table I), a fact that was not accounted for in the MNDO calculations. The calculations also predict a different $E(g^-) - E(g')$ value for the α - and β -compounds **18** and **19**. The difference is small but of magnitude comparable to the experimental value (0.10 kcal.mole⁻¹) (see Table IV, footnote b). Thus, although the MNDO calculations give a fairly accurate qualitative description of the experimentally observed differences in rotamer populations for α - and β -galactopyranosides, a detailed analysis of the origin of this effect is probably not possible. It is likely

that better results will be obtained with quantum-chemical calculations in combination with an investigation of the solvent effect by, for example, the continuum reaction field method²³. Recently, calculations with this method have been performed on α - and β -D-glucopyranose²⁴.

4.2.5 Influence of the substituents at positions 2-4 and 6

A 300-MHz ¹H NMR study of the galactopyranosides 20-26 was carried out in order to investigate the influence of substituents at C(2,3,4,6) on the conformation around the C(5)-C(6) bond of α - and β -galactopyranosides. The data are given in Table V and show that the substituents at C(2,3,4) strongly affect the $J_{5,6S(6R)}$ values which range from $J_{5,6S}$ 3.30 Hz (24) for hydroxyl groups to $J_{5,6S}$ 5.99 Hz (25) for methoxy groups. The $J_{5,6S}$ values were larger for the β -compounds.



20	H	OMe	25 $R^1 = \text{Tr}$
21	OMe	H	26 $R^1 = (\text{PhO})_2\text{PO}$
22	H	p-NPh-Me	
23	p-NPh-Me	H	
24	o-OPh-NO ₂	H	

The substituents at C(6) also influenced the rotamer population around the C(5)-

Table V. ^1H NMR data for 20-26.

Compound	CD_3OD					$(\text{CD}_3)_2\text{CO}$				
	$\delta(\text{ppm})$		$J(\text{Hz})$			$\delta(\text{ppm})$		$J(\text{Hz})$		
	H(6S)	H(6R)	5,6S	5,6R	6R,6S	H(6S)	H(6R)	5,6S	5,6R	6R,6S
20	3.433	3.602	4.98	7.00	-9.68	3.386	3.514	5.13	6.86	-9.33
21	3.447	3.612	5.26	6.68	-9.56	3.386	3.537	5.35	6.70	-9.26
22	3.288	-	3.85	-	-9.86	3.243	3.572	3.90	7.76	-9.50
23	3.352	3.634	4.07	7.44	-9.86	3.228	3.433	7.51	5.99	-9.19
24	3.364	3.766	3.30	8.08	-10.07	3.160	3.471	7.91	5.65	-8.73
25	3.573	3.304	5.99	7.27	-9.36	3.490	3.253	6.05	6.94	-8.85
26	-	-	-	-	-	4.530	4.515	5.39	7.02	-10.32

C(6) bond, as shown for compounds bearing the bulky trityl group (cf. 5 and 20). However, the influence is not as pronounced as that of the substituents at C(2,3,4) (cf. 3, 7 and 26). The influence of the O(6)-P^V group of the β -compound 4 on the rotamer population [$J_{5,6S}$ 7.29, $J_{5,6R}$ 6.07 Hz, $x(g^-) = 0.56$] in comparison with the P^{IV} compound 3 [$J_{5,6S}$ 5.61, $J_{5,6R}$ 6.90 Hz, $x(g^-) = 0.34$] shows that, due to an increased electron density on O(6), the g^- population increases, as found for 1 and 2¹⁸. However, more important is comparison of 4 with the corresponding α -O(6)-P^V compound 2 [$J_{5,6S}$ 6.92, $J_{5,6R}$ 5.90 Hz, $x(g^-) = 0.51$]¹⁸ which shows that the g^- population for the β -compound 4 is higher, giving a similar difference that was found for the O(6)-P^{IV} compounds 1 and 3 (see Table I). Thus, the difference in rotamer populations around the C(5)-C(6) bond for α - and β -compounds is the result of an effect additional to that of the coulombic effect between O(5) and O(6). A similar conclusion was given by Wolfe *et al.*⁸ for the variations in bond lengths in CH_2X_2 molecules. In general, it can be concluded that substituents at positions 2-4 and 6 affect the rotamer populations around the C(5)-C(6) bond, equally for α - and β -galactopyranosides, and that the difference between α - and β -compounds is caused, presumably, by a combination of the gauche and anomeric effects.

4.2.6 Assignment of the H(6S) and H(6R) resonances

Specific deuteration at C(6) enables assignment of the individual H(6S,6R) resonances³⁵ but there are few examples in literature for galactopyranosides. For methyl α -D-galactopyranoside and its tetrabenzoate²⁰ the resonance of H(6R) is downfield of that of H(6S). As can be seen from the data in Tables II and V this situation is consistent with the assignment for all α - and β -compounds bearing hydroxyl groups at C(2,3,4) (5-10 and 20-24), except for 8 and 10 in $(\text{CD}_3)_2\text{CO}$. Although the reason for two exceptions is not certain it is probably attributable to an interaction with the solvent or a specific intramolecular interaction (e.g. the reversal in the order of H(6S) and H(6R) chemical shifts for 4,6-di-O-acetyl hexopyranoses³⁰). The assignments in Tables II and V also accord with the algebraic relationship between $J_{6R,6S}$ and the g^- rotamer population. An increase of $J_{6R,6S}$ reflects³⁶ an increase in amount of the g^- rotamer [e.g. in CD_3OD : 7 $J_{6S,6R}$ -10.72 Hz, $x(g^-) = 0.14$; 25 $J_{6R,6S}$ -9.36 Hz, $x(g^-) = 0.39$]. The compounds (1, 3, 25 and 26) bearing a methoxy group at C(2,3,4) show a reversed pattern, with H(6S) resonating downfield of H(6R) (see Tables I and V). The value of $J_{5,6S}$ is smaller than that of $J_{5,6R}$ and the g^- rotamer population is smaller than the g^+ population. Then, according to the "syn-upfield rule"³⁷, H(6R) should resonate upfield of H(6S) which accords with experimental results. The apparent conflict with the "syn-upfield rule" for compounds with HO(4) axial was explained²⁶ by a superimposed deshielding effect of quasi-syn-axial O(4). Assuming that the assignments of H(6S) and H(6R) are correct, it can be seen from the data in Tables I, II, and V that the values for $J_{5,6S}$ tend to be larger in the less polar solvents. For $J_{5,6R}$ the reverse is true. Therefore, the solvent dependence of $J_{5,6R(6S)}$ of galactopyranosides seems to be a suitable criterion for the assignment of the H(6S) resonances which can be used in combination with other criteria.

4.3 Summary and Conclusions

It is shown by 300-MHz ^1H NMR measurements that the rotamer population distribution around C(5)-C(6) for galactopyranosides is the result of intramolecular interactions (gauche and anomeric effect) and solute-solvent interactions. The rotamer population depends on the nature and the configuration of the substituent at C(1) of the pyranoside ring. The g^- rotamer population increases with increasing $\text{p}K_a$ of the substituent at C(1), and is larger for β -compounds as compared to α -compounds. A similar dependence was found for the bond length changes around the anomeric centre but the presented method of conformational analysis with NMR has several advantages. The analysis is easier and faster, compared to röntgen diffraction measurements, and it provides a dynamic view of the interactions instead of the static view in crystal structures.

4.4 Experimental

Compounds 1-2¹⁸, 20¹⁸, and 21³⁸ were prepared according to literature procedures and 25 was prepared according to the procedure for the corresponding α -compound¹⁸. The spectral data and melting points were in accordance with the expected structures. ^1H -NMR spectra (300.1 MHz) were recorded in the FT mode using a Bruker CXP-300 spectrometer and a 32 K data set. Chemical shifts are given relative to the $\text{CHD}_2\text{COCD}_3$ quintet at δ 2.17, the CHD_2OD quintet at δ 3.49, or Me_4Si (CDCl_3) with an accuracy of ± 0.05 Hz. Coupling constants in Tables I, II, and V were measured from expansions of the patterns with an accuracy of ± 0.1 Hz and analysed using an iterative programme³⁹. ^{31}P -NMR spectra (36.4 MHz) were recorded in the FT mode using a Bruker HX-90R spectrometer. Chemical shifts are related to external aqueous 85% H_3PO_4 . ^{13}C -NMR spectra (22.6 MHz) were recorded in the FT mode using a Bruker HX-90R spectrometer. Chemical shifts are related to the acetone-septet (δ 30.7) or to Me_4Si (CDCl_3).

Melting points were determined on a Mettler FP2 and are uncorrected. Optical rotations were determined using an AA10 polarimeter (Optical Activity Ltd.).

Methyl 2,3,4-tri-O-methyl-β-D-galactopyranoside. — Prepared from **25** by detritylation with chlorotrimethylsilane/sodiumiodide⁴⁰, this compound had m.p. 72–74.5° (from ether), $[\alpha]_D -20.5^\circ$ (c 1, methanol). ¹H NMR (CDCl₃): δ 3.17 [dd, J_{2,3} 9.70, J_{3,4} 3.01 Hz, H(3)], 3.33 [dd, J_{1,2} 7.42, J_{2,3} 9.70 Hz, H(2)], 3.43 [m, H(5)], 3.49 [dd, J_{3,4} 3.01, J_{4,5} 1.0 Hz, H(4)], 3.527, 3.536, 3.564, and 3.589 [4 s, 4 OMe], 3.754 [m, H(6S)], 3.922 [m, H(6R)], 4.18 [d, J_{1,2} 7.42 Hz, H(1)]. ¹³C NMR: δ 57.8–62.2 [4 OCH₃], 63.09 [C(6)], 75.7, 76.7, 81.6, and 85.2 [C(2,3,4,5)], 105.7 [C(1β)].

Methyl 2,3,4-tri-O-methyl-β-D-galactopyranoside-6-dimethylphosphate (3). — This compound was prepared by phosphorylation of methyl 2,3,4-tri-O-methyl-β-D-galactopyranoside with dimethoxychlorophosphine and subsequent oxidation with ozone⁴¹. Column chromatography (CHCl₃-MeOH 95:5, R_F 0.57) gave the product as a viscous oil, $[\alpha]_D -9^\circ$ (c 0.7, methanol). ¹H NMR [(CD₃)₂CO]: δ 3.25 [dd, J_{1,2} 7.59, J_{2,3} 9.61 Hz, H(2)], 3.36 [dd, J_{2,3} 9.61, J_{3,4} 2.98 Hz, H(3)], 3.56 and 3.70 [2 d, J_{P,OMe} 11.10 Hz, POMe], 3.60–3.87 [4 s, 4 OMe], 4.209 [m, H(6R)], 4.247 [m, H(6S)], 4.27 [d, J_{1,2} 7.59 Hz, H(1)]. ¹³C NMR: δ 57.53, 59.28, 59.41, 61.49, 62.00, and 62.06 [6 OCH₃], 67.74 [J_{P,OC} 5.2 Hz, C(6)], 74.48 [J_{P,OCC} 7.9 Hz, C(5)], 76.71, 82.37, 85.68 [C(2,3,4)], 106.19 [C(1β)]. ³¹P NMR: δ 6.39.

Anal. Calc. for C₁₂H₂₅O₉P: C, 41.86; H, 7.32. Found: C, 41.55, H, 7.77.

2,2-Dihydro-2,2-dimethoxy-4,5-dimethyl-2-(methyl 2,3,4-tri-O-methyl-β-D-galactopyranosid-6-yloxy)-1,3,2-dioxaphosphole (4). — This compound, prepared¹⁸ from the phosphite (see **3**) was hygroscopic and could only be characterized by ¹H- and ³¹P-NMR spectroscopy. ¹H NMR [(CD₃)₂CO]: δ 1.93 [s, 6 H, Me], 3.24 [dd, J_{1,2} 7.14, J_{2,3} 9.70 Hz, H(2)], 3.30 [dd, J_{2,3} 9.70, J_{3,4} 2.97 Hz, H(3)], 3.57 and 3.62 [2 d, J_{P,OMe} 13.19 Hz, POMe], 3.54–3.73 [4 s, 4 OMe], 4.04 [m, H(6S) and H(6R)], 4.22 [d, J_{1,2} 7.14 Hz, H(1)]. ³¹P NMR: δ -43.97.

Compounds 5-10. These compounds were prepared according to a literature procedure⁴². A typical example is outlined. To a solution of phenyl β -D-galactopyranoside (0.45 g) in dry pyridine (50 ml) at 0° was added slowly a solution of 0.5 equiv. of diphenylphosphochloridate in dry pyridine (5 ml), and after 5 h more (0.5 equiv.) was added. The mixture was stirred at 25° for 15 h, then concentrated under reduced pressure, residual pyridine was removed by co-evaporation with toluene, and the resulting oil was purified by column chromatography (ethyl acetate, R_F 0.25). The resulting oil was triturated with ether to yield phenyl β -D-galactopyranoside-6-diphenylphosphate (8) as a white powder (60 mg), m.p. 92-94.5°, $[\alpha]_D$ -25° (c 0.85, methanol). ¹H NMR [(CD₃)₂CO plus 1 drop of MeOD to remove OH couplings]: δ 3.80 [dd, $J_{2,3}$ 9.49, $J_{3,4}$ 3.42 Hz, H(3)], 3.95 [dd, $J_{1,2}$ 7.68, $J_{2,3}$ 9.49 Hz, H(2)], 4.10 [dd, $J_{3,4}$ 3.42, $J_{4,5}$ 1.20 Hz, H(4)], 4.22 [m, H(5)], 4.615 [m, H(6R)], 4.652 [m, H(6S)], 5.08 [d, $J_{1,2}$ 7.68 Hz, H(1)], 7.1-7.6 [m, ArH]. ¹³C NMR: δ , 69.46 [C(4,6)], 71.79 and 74.16 [C(2,3)], 74.89 [C(5)], $J_{P,OCC}$ 7.2 Hz], 102.29 [C(1 β)], 116.0-130.0 [aromatic]. ³¹P NMR: δ -6.52.

Anal. Calc. for C₂₄H₂₅O₉P: C, 59.02; H, 5.16. Found: C, 59.17; H, 5.06.

Methyl α -D-galactopyranoside-6-diphenylphosphate (5). — M.p. 134-138.5°, $[\alpha]_D$ 26° (c 0.2, methanol).

p-Nitrophenyl α -D-galactopyranoside-6-diphenylphosphate (6). — M.p. 110-114°, $[\alpha]_D$ 153° (c 0.7, methanol).

Methyl β -D-galactopyranoside-6-diphenylphosphate (7). — Obtained as a viscous oil, $[\alpha]_D$ -4.5° (c 0.5, methanol).

p-Nitrophenyl β -D-galactopyranoside-6-diphenylphosphate (9). — M.p. 112-114.5°, $[\alpha]_D$ -56° (c 0.4, methanol).

o-Nitrophenyl β -D-galactopyranoside-6-diphenylphosphate (10). — M.p. 107-109°, $[\alpha]_D$ -36 (c 0.6, methanol).

N-(*p*-Methylphenyl) 6-*O*-trityl- $\alpha\beta$ -galactopyranosylamine (22 and 23). — These compounds were prepared by boiling under reflux a solution of 6-*O*-trityl-*D*-galactopyranose and *p*-toluidine in ethanol for 4 h. The resulting mixture was cooled to 0°, the product was collected, and recrystallized from ether-hexane to yield white crystals, m.p. 116–118° which were shown by ¹H-NMR spectroscopy to be a 1:4 $\alpha\beta$ -mixture. Because the H(6*S*,6*R*) signals of the α - and β -compound were clearly separated (see Table V) no further purification was attempted. The assignments of signals were proved by selective decoupling experiments. ¹H NMR [(CD₃)₂CO]: δ 3.243 [m, H(6*S* α)], 3.281 [m, H(6*S* β)], 3.529 [H(6*R* β)], 3.572 [H(6*R* α)], 3.73 [dd, H(2 β)], 3.91 [m, *J*_{4,5} 1.14 Hz, H(5 β)], 3.95 [dd, H(4 β)], 4.14 [dd, *J*_{1,2} 5.25, *J*_{2,3} 9.26 Hz, H(2 α)], 4.24 [m, H(5 α)], 4.63 [d, *J*_{1,2} 8.38 Hz, H(1 β)], 5.18 [d, *J*_{1,2} 5.25 Hz, H(1 α)], 6.8–7.6 [m, ArH].

Anal. Calc. for C₃₂H₃₃NO₅: C, 75.12; H, 6.50; N, 2.74. Found: C, 74.61; H, 6.60; N, 2.52.

o-Nitrophenyl 6-*O*-trityl- β -*D*-galactopyranoside (24). — *o*-Nitrophenyl β -*D*-galactopyranoside was stirred with 1 equiv. of trityl chloride in dry pyridine for 3 days at 25°. Evaporation of the solvent yielded a white powder which was purified by column chromatography (CHCl₃-MeOH 95:5, *R_F* 0.22), and then had m.p. 104–107.5° (from ether-hexane), [α]_D -35° (c 0.7, methanol). ¹H NMR [(CD₃)₂CO]: δ 3.345 [dd, H(6*S*)], 3.682 [dd, H(6*R*)], 3.78 [dd, *J*_{2,3} 9.43, *J*_{3,4} 3.45 Hz, H(3)], 3.96 [dd, *J*_{3,4} 3.45, *J*_{4,5} 1.15 Hz, H(4)], 3.98 [dd, *J*_{1,2} 7.68, *J*_{2,3} 9.43 Hz, H(2)], 4.16 [m, H(5)], 5.23 [d, *J*_{1,2} 7.68 Hz, H(1)], 7.2–7.9 [ArH]. ¹³C NMR: δ 65.7 [C(6)], 71.0, 72.6, 75.4, and 76.7 [C-(2,3,4,5)], 103.2 [C(1 β)], 119.2–146.0 [aromatic].

Anal. Calc. for C₂₇H₂₉NO₈: C, 68.45; H, 5.38; N, 2.57. Found: C, 68.03; H, 5.87; N, 2.16.

Methyl 2,3,4-tri-O-methyl- β -D-galactopyranoside-6-diphenylphosphate (26). — To a solution of methyl 2,3,4-tri-*O*-methyl- β -*D*-galactopyranoside (50 mg) and triethylamine (22 mg) in ether (10 ml) was added a solution of

diphenylphosphochloridate in ether (3 ml). The mixture was stirred for 4 days at 25°, then concentrated. Column chromatography (ethyl acetate) of the oily residue gave 26 $[\alpha]_D -6^\circ$ (c 1.2, methanol). $^1\text{H NMR}$ [$(\text{CD}_3)_2\text{CO}$]: δ 3.26–3.33 [m, H(2,3)], 3.52, 3.56, 3.57, and 3.59 [4 s, 4 OMe], 3.78 [dd, $J_{3,4}$ 2.62, $J_{4,5}$ 1.18 Hz, H(4)], 3.85 [m, H(5)], 4.515 [m, H(6R)], 4.530 [m, H(6S)], 4.27 [d, $J_{1,2}$ 7.36 Hz, H(1)], 7.3–7.6 [m, ArH]. $^{13}\text{C NMR}$: δ 56.96, 58.94, 60.91, and 61.39 [4 s, 4 OMe], 68.77 [d, $J_{P,OC}$ 6 Hz, C(6)], 73.98 [d, $J_{P,OC}$ 7 Hz, C(5)], 76.33, 82.07, and 85.41 [C(2,3,4)], 106.22 [C(1 β)], 121.85–153.3 [aromatic]. $^{31}\text{P NMR}$: δ -6.70.

Anal. Calc. for $\text{C}_{22}\text{H}_{29}\text{O}_9\text{P}$: C, 56.41; H, 6.24. Found: C, 56.31; H, 6.68.

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CHAPTER 5*

Solvent dependence of the rotamer population around the interglycosidic C(5)-C(6) bond of (1→6)- β -linked digalactopyranosides

Abstract.

A 300-MHz ^1H NMR study of the rotamer population around the interglycosidic C(5)-C(6) bond of methyl 2,3,4-tri-O-methyl-6-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)- β - (1) and - α -D-galactopyranoside (2) is reported. The existence of the (1→6)- β -linkage in these compounds is established by homo- and heteronuclear 2-D NMR techniques. It is shown that the dependence on solvent polarity of the population around the C(5)-C(6) bond for the disaccharides is almost identical to that for monosaccharides. The influences of stereoelectronic effects and solute-solvent interactions on the conformational properties of the interglycosidic linkage are discussed briefly.

* N. K. de Vries and H. M. Buck, Recl. Trav. Chim. Pays-Bas **106**, 453 (1987).

5.1 Introduction

The glycosidic linkage, which consists of two electronegative atoms linked to an anomeric carbon atom, is an important molecular segment in carbohydrates. The properties of this linkage have led to the formulation of the anomeric and exoanomeric effect^{1,2}. These effects which markedly affect conformational preferences, have been investigated extensively, both experimentally^{3,4} and theoretically^{5,6}. Recently, the conformation around C(5)-C(6) of 6-phosphorylated galactopyranosides was studied as a function of the configuration of the group at the anomeric centre⁷. It was shown that, both for four and five coordinated phosphorus compounds, an equatorial configuration (β) results in a larger gauche ($-$) population ($x(g^-)$, O(5) and O(6) trans situated) as compared to an axial configuration (α). This was attributed to an interaction of two stereoelectronic effects, the gauche⁸ and the anomeric effect. The fact that $x(g^-)_\beta - x(g^-)_\alpha$ is equal for four and five coordinated phosphorus compounds, shows that this difference is an additional effect superposed upon the effects of coulombic repulsion. Also, it was found that the $x(g^-)$ value increases with decreasing solvent polarity which was attributed to an increased electrostatic repulsion between O(5) and O(6). These observations appeared to be independent of the nature and size of the group at O(6).

Therefore, it was anticipated that these effects could also be noticeable in (1 \rightarrow 6)-linked disaccharides. Now, a study is reported of the conformation around the C(5)-C(6) interglycosidic linkage of the β -linked disaccharides 1-2 (see Fig. 1). It will be shown that the conformation around C(5)-C(6) of these disaccharides shows similar features as the previously reported monosaccharides⁷. The results might assist in the study of the structure of (1 \rightarrow 6)-linked oligosaccharides of D-galactopyranoside. These compounds are currently synthesized by several groups in connection with studies on the modes of binding of ligands to immunoglobulins^{9,10} and antibodies¹¹. It was argued that¹⁰, even though the angle around the C(1')-O(1') bond might be determined, the rotation around O(1')-C(6) and C(5)-C(6) would allow too many possible conformations. The conformational preference around C(5)-C(6) can now be estimated on the basis of this study, *e.g.*

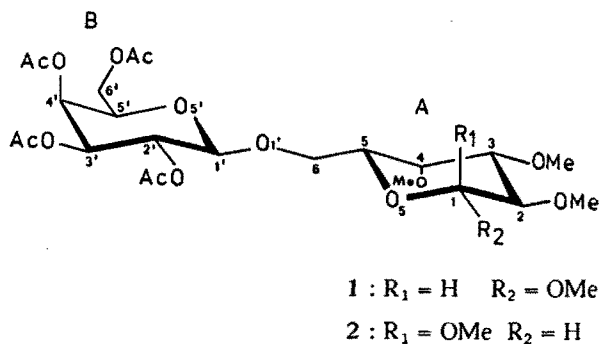


Fig. 1. *Compounds 1-2. A and B denote the permethylated and acetylated galactopyranoside ring respectively.*

the postulated conformation for galactopyranosyl-(1→6)- β -D-galactose in water (gauche(trans) around C(5)-C(6) from considerations of least orbital-overlap¹²) is supported by the NMR measurements.

5.2 Results and Discussion

5.2.1 NMR measurements

The structure of the β -D-(1→6)-disaccharides 1-2 (Fig. 1) was established through ¹H NMR (vide infra) and ¹³C NMR measurements. Fig. 2 shows the development of the assignments of the lines in the ¹³C spectrum of 1-2 with the aid of 3-5. Compounds 3-4 (see Fig. 2) are model compounds for the galactoside-ring A, and compound 5 for ring B.

As can be seen, the C(1)/C(1') chemical shifts of 1 are typical of a β -D-Me- β -D-(1→6)-disaccharide, whereas those of 2 are of a α -D-Me- β -D-(1→6)-disaccharide. The upfield shift of C(5) (1.6 ppm) and the downfield shift of C(6)

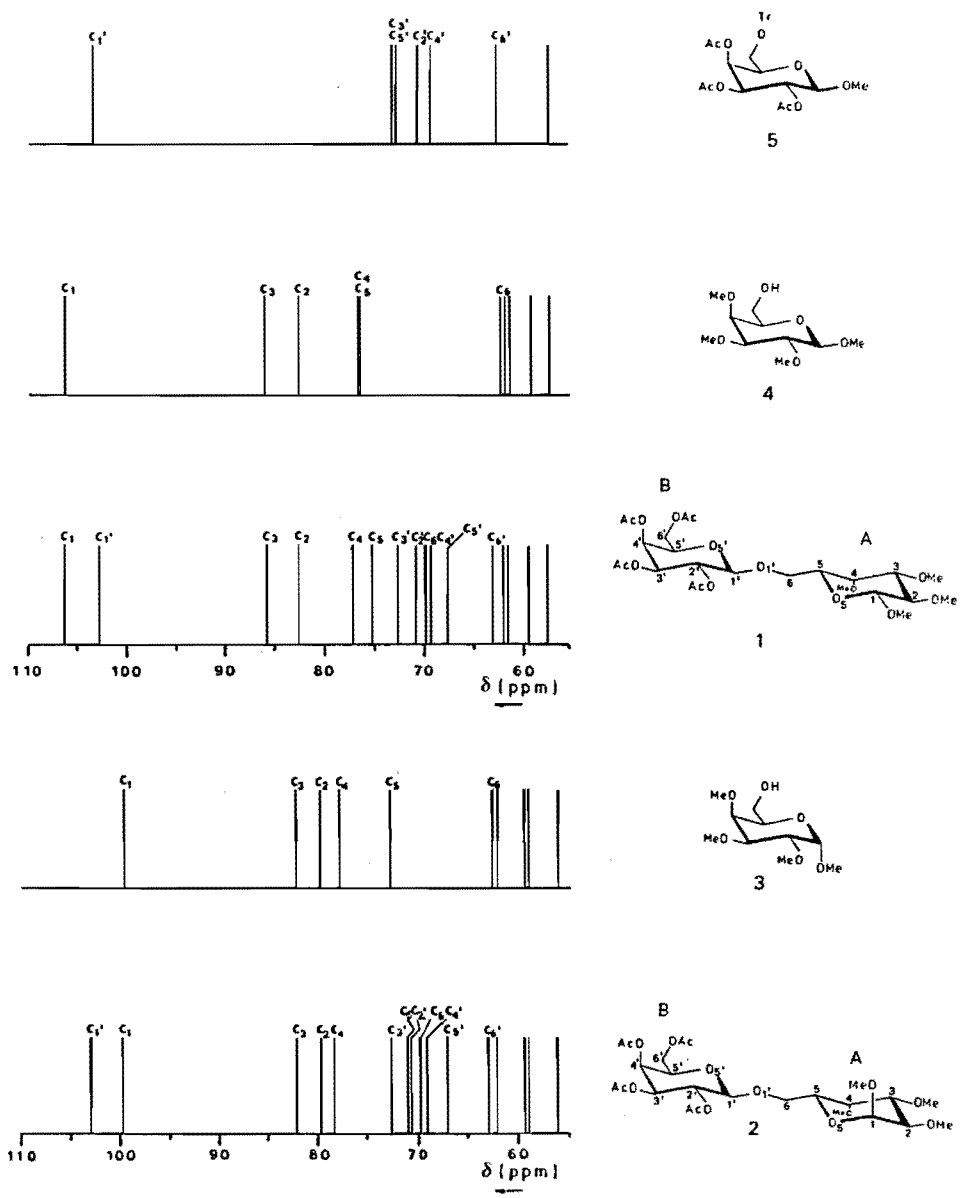


Fig. 2. Development of the assignment of lines in the ^{13}C NMR spectra of the disaccharides 1-2 with the aid of the spectra of the monosaccharides 3-5. Unassigned lines are methoxy resonances.

(ca. 7.5 ppm) in the spectra of 1-2, as compared to 3-4, is also in accordance with the formation of a glycosidic linkage^{9,13}. The ¹³C resonances of 1-5 are determined with the aid of the assigned spectra of α - and β -D-pentamethylgalactoside¹⁴ (CD₃CN) and methyl 2,3,4-tri-O-acetyl-6-O-trityl- β -D-galactopyranoside (CDCl₃)^{15,16}, and the assignment has been confirmed with a 2-D heteronuclear ¹H-¹³C chemical shift correlation experiment (see Fig. 3)¹⁷.

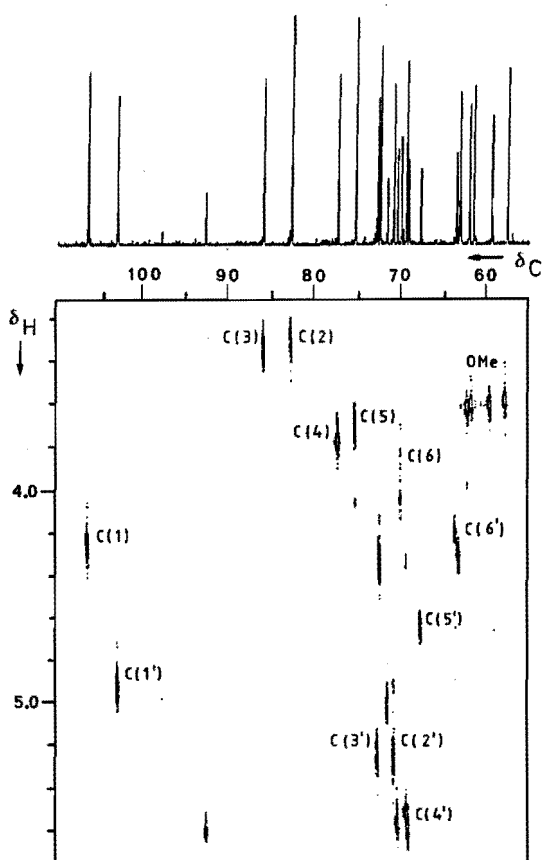


Fig. 3. ¹H-¹³C chemical shift correlation spectrum of 1 in acetone-d₆.

The conformational analysis of the C(5)-C(6) bond was performed by a 300-MHz ¹H NMR study on the compounds 1-2. The contribution of the three staggered

rotamers g^+ , g^t and g^- (see Fig. 4) to the conformation around C(5)-C(6) is calculated¹⁸ from the coupling constants $J_{5,6A(5,6A')}$ with the aid of an empirically generalized Karplus-relation¹⁹, which was also used in previous studies^{7,20}.

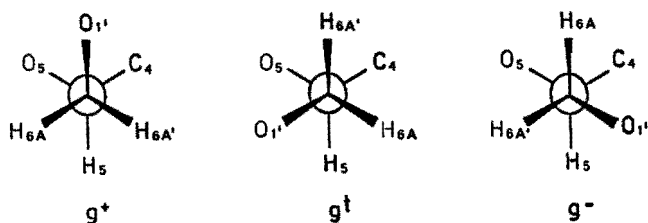


Fig. 4. Staggered rotamers around the C(5)-C(6) bond.

The coupling constants were obtained from expansions of the spectral patterns, and analysed with the simulation program Panic²¹. A typical example of the proton resonances of the permethylated galactosidering A is shown in Fig. 5.

The proton resonances of the acetylated ring B appear more downfield except for H(6B)/H(6B'). As can be seen in Fig. 5, the resonances of these protons are in close proximity to those of H(6A)/H(6A'). Because it is of crucial importance for an accurate analysis of the C(5)-C(6) linkage to assign these resonances correctly, two 2-D homonuclear correlation spectroscopy experiments (COSY) were performed. First, the proton-proton connectivities within the galactopyranoside residues A and B were established with a conventional COSY measurement²². Then, a delayed COSY experiment²³ was performed which emphasizes long-range couplings. This second experiment can be used to prove the interresidue linkage between C(1') and C(6) (*vide infra*). The assignments were further supported by (1-D) proton-decoupling experiments.

5.2.2 Assignment of H(6A) and H(6A')

The COSY-90 contour plot of **1** is shown in Fig. 6.

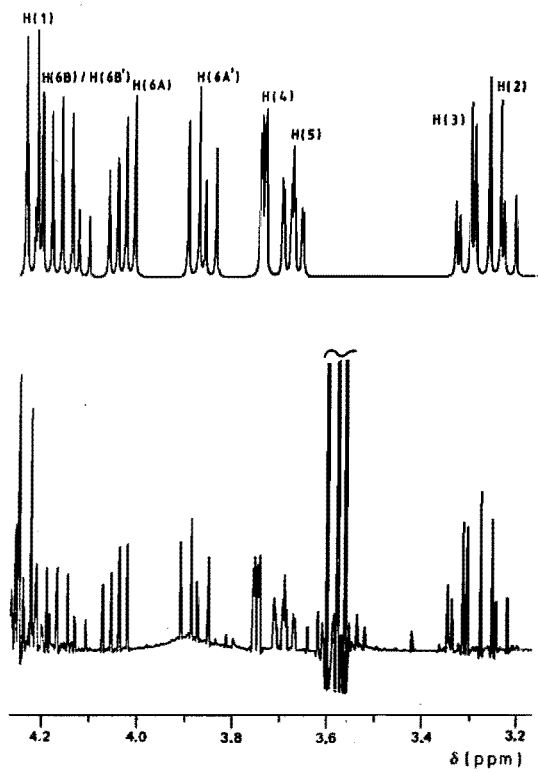


Fig. 5. The highfield region (3.15–4.25 ppm) of the 300-MHz ^1H NMR resolution enhanced spectrum of **1** in acetone- d_6 (lower trace) and the computer-simulated spectrum (upper trace).

Evaluation of this plot shows that the upfield H(6)/H(6') resonances at 3.85–4.05 ppm are those of H(6A)/H(6A'), whereas the signals at 4.10–4.25 ppm belong to H(6B)/H(6B'). The existence of the (1→6)-glycosidic linkage was established via the four-bond interglycosidic coupling between H(1') and H(6A)/H(6A') (see Fig. 7). This long-range coupling can be emphasized by introducing a fixed delay in the pulse sequence of the COSY experiment²⁴. This technique was first used for carbohydrates by Liptak to establish the α -D-(1→4)-glycosidic linkage in a trisaccharide²⁵.

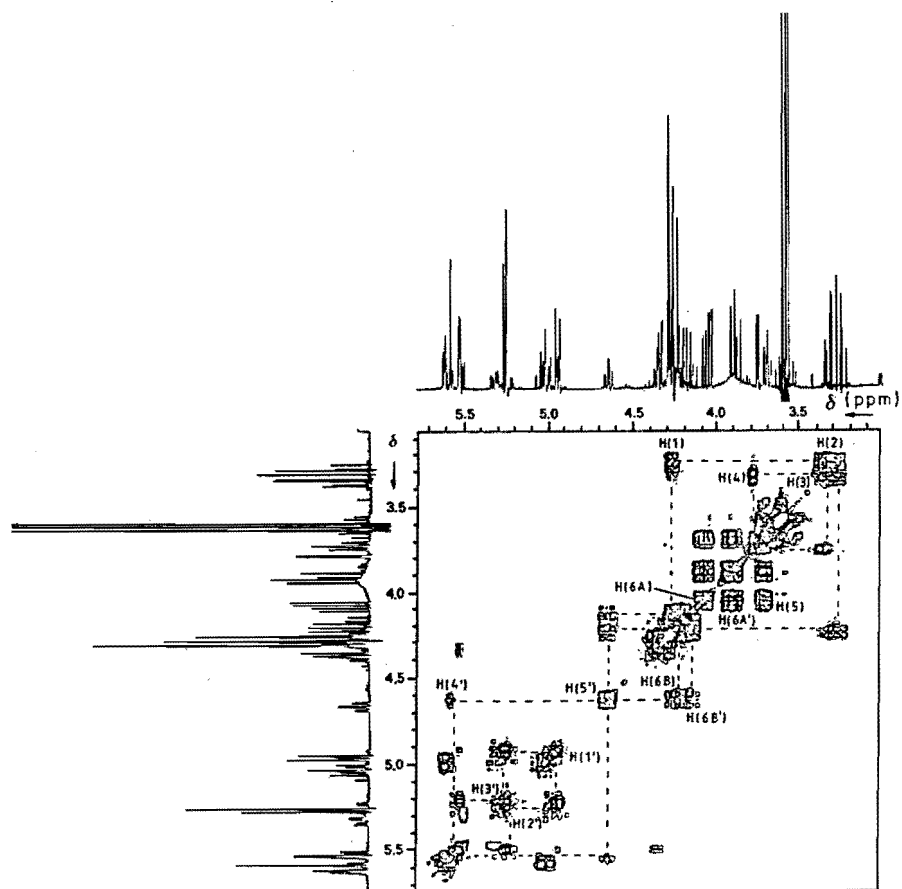


Fig. 6. Contour plot of a 200-MHz COSY-90 spectrum of **1** in acetone- d_6 . Dashed lines have been added to indicate some of the correlations. The corresponding region of the one-dimensional ^1H NMR spectrum is shown at the sides. Details on the data acquisition are given in the experimental section.

An enlarged portion of the contour plot of **1** (Fig. 7) is shown in Fig. 8, together with a projection of the signals on the f_2 axis. Both projections of the H(6A) signals clearly show the coupling with the other H(6A) signal, with H(4) and H(5), and also with H(1') (doublet at δ 4.92 ppm). This proves, in combination with

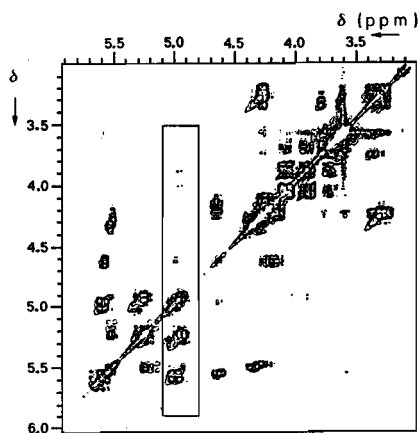


Fig. 7. Contour plot of a 200-MHz ^1H delayed COSY spectrum of **1** in acetone- d_6 . Details are given in the experimental section.

the ^{13}C NMR data, the existence of a β -(1 \rightarrow 6)-linkage in **1-2**.

The measured coupling constants $J_{5,6A(5,6A')}$ are listed in Table I, together with the calculated rotamer populations around the C(5)-C(6) linkage. As one can see in the equations¹⁸, the outcome of the calculated populations also depends on the assignment of the protons H(6A) and H(6A') (e.g. H(6A) downfield or upfield of H(6A')). This assignment provided no difficulties, since it was observed by Ohrui in a study of α - and β -linked (1 \rightarrow 6)-disaccharides, specifically deuterated at C(6), that H(6A) always resonated downfield of H(6A') in case of β -linkage²⁶. This observation was independent of the species of the protecting groups on the sugar rings, and, more important, it was also independent of the conformational changes around the C(5)-C(6) bond. The consequences for the conformation of the glycosidic linkage will be discussed later (*vide infra*).

5.2.3 Solvent dependence of the rotamer population around C(5)-C(6)

The spectral parameters and the rotamer populations of the compounds **1-2** are listed in Table I.

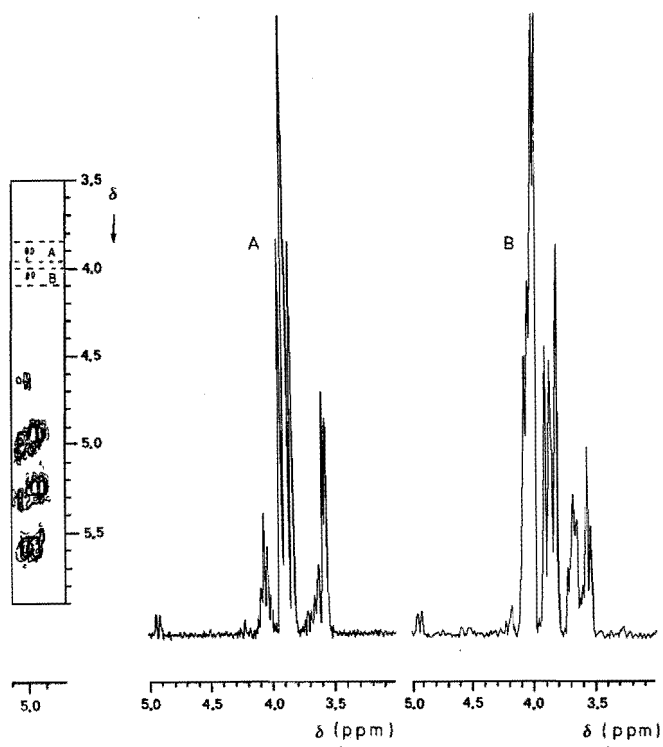


Fig. 8. Expanded $H(1')$ region of the delayed COSY spectrum in Fig. 7, and a projection onto the f_2 axis, taken between 3.85 and 3.95 ppm (A, $H(6A')$) and between 4.00 and 4.10 ppm (B, $H(6A)$).

The results of the conformational analysis clearly show an increasing g^- rotamer population with decreasing solvent polarity, and an accompanying decrease in g^+ rotamer population. The g^+ rotamer remains almost equally populated in all solvents because of 1,3-*syn*-diaxial interactions of $O(1')$ with $O(4)$. The increase in g^- rotamer population (maximum distance between $O(5)$ and $O(1')$) is in accordance with an increased charge repulsion between $O(5)$ and $O(1')$ in apolar solvents. The same explanation was given for monosaccharides in experimental⁷ and theoretical⁵ studies. The results for the disaccharides 1-2 and for the monosaccharide methyl-2,3,4-tri-*O*-methyl-6-*O*-(dimethylphosphate)- β -D-galacto-

Table I. Spectral parameters and rotamer populations of 1-2.

solvent	E_T^{27}	$\delta(H(6A))$	$\delta(H(6A'))$	$J_{5,6A}$	$J_{5,6A'}$	$x(g^+)$	$x(g')$	$x(g^-)$
1								
CCl ₄	32.5	3.88	3.63	6.08	6.26	0.22	0.38	0.40
C ₆ D ₆	34.5	4.06	3.85	5.51	6.56	0.23	0.44	0.33
CDCl ₃	39.1	4.00	3.84	5.24	7.02	0.21	0.51	0.28
(CD ₃) ₂ CO	42.2	4.04	3.88	5.39	6.91	0.20	0.49	0.31
CD ₃ CN	46.0	3.86	3.73	4.83	7.33	0.20	0.56	0.24
D ₂ O	63.1	4.06	3.88	3.98	8.19	0.18	0.69	0.13
2								
CCl ₄	32.5	3.85	3.53	5.00	6.77	0.25	0.49	0.26
(CD ₃) ₂ CO	42.2	4.06	3.81	4.84	7.35	0.20	0.56	0.24
D ₂ O	63.1	3.95	3.82	3.40	8.33	0.21	0.74	0.05

pyranoside⁷ are compared in Fig. 9. It is apparent that the solvent dependence of the g^- population of the mono- and disaccharides shows a similar trend.

On the basis of these results, three important conclusions can be drawn:

i) The presence of a large group (2,3,4,6-O-tetraacetylgalactopyranoside) attached to O(6) of tetramethyl- β -D-galactopyranoside does not influence the solvent dependence of the conformation around the C(5)-C(6) bond.

ii) Since the monosaccharide shows the same solvent dependence as the disaccharides 1-2, and the assignment for H(6A)/H(6A') of 1-2 is unambiguous²⁶, the assignment for H(6A)/H(6A') of the monosaccharide (which was based on this solvent dependence⁷) must also be correct.

iii) The influence of the configuration of the group at C(1) on the conformation around C(5)-C(6) is also noticeable for the disaccharides 1-2. Thus, the g^- population for the β -compound 1 is larger over the entire solvent range than the g^- population for the corresponding α -compound 2 (see Table I and Fig. 9). This is possibly attributable to an interaction of the gauche⁸ ($\sigma_H - \sigma_O^*$) and anomeric effect ($n - \sigma_O^*$) via the ring oxygen atom O(5), as was argued earlier⁷. A similar through-bond interaction (lone pair of the oxygen atom at C(6') with the C=O⁺ - π bond at the anomeric centre) has recently been proposed to explain the observed

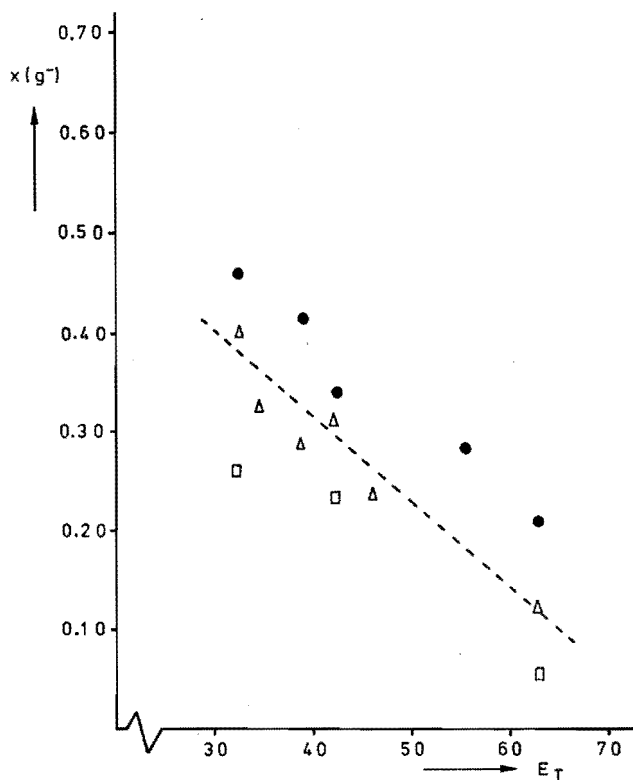


Fig. 9. Relationship between the solvent polarity and the gauche(-) populations around the C(5)-C(6) bond of 1(Δ), 2(\square) and methyl-2,3,4-tri-O-methyl-6-O-(dimethylphosphate)- β -D-galactopyranoside (\bullet)⁷. This relationship, which is accentuated by the dashed line, is not necessarily linear⁷.

β/α ratio in the synthesis of disaccharides²⁸.

It is interesting to discuss the conformation around the C(1')-O(1')-C(6)-C(5) bonds (see Fig. 10) on the basis of these results.

The torsion angle ϕ is largely determined by the exoanomeric effect. Theoretical studies have shown that, even in polar solvents, the gauche conformer will be preferred along the C(1')-O(1') bond⁵. Measurements of $^{13}\text{C} - ^1\text{H}$ coupling constants in ^{13}C enriched (1 \rightarrow 4)-linked disaccharides²⁹ and values for ϕ in crystal

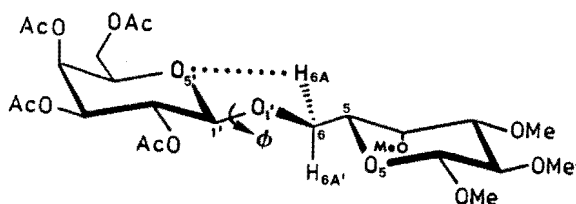


Fig. 10. *Deshielding of H(6A) by O(5')*.

structures of (1→6)-linked disaccharides³⁰ also correspond with an angle of approximately -60° . This relatively rigid structure along C(1')-O(1') might explain in part the observed downfield shift of H(6A) relative to H(6A') in β -D-(1→6)-disaccharides²⁶, since, in the gauche conformation ($\phi = -60^\circ$), H(6A) will be closer to O(5') than H(6A'), and subsequently will be more deshielded (see Fig. 10). This downfield shift is noticeable in all solvents and is independent of rotation around C(5)-C(6) (see Table I). Determination of the torsion angle along the O(1')-C(6) bond is not possible with $^3J_{HH}$ coupling constants. However, the observed flexibility of rotation around C(5)-C(6), the study of Dreiding models and hard-sphere calculations¹¹ indicate that this torsion angle will be in the region 120° - 240° . Finally, the conformation around the C(5)-C(6) bond has been determined accurately in this study. It is governed by solute-solvent interactions and by intramolecular interactions (combined interaction of gauche and anomeric effect). In apolar solvents, g^+ and g^- are about equally populated, whereas in water, the normal solvent for unsubstituted di- and oligosaccharides, the g^+ conformer will be strongly preferred. This preference will be even more pronounced for C(1) α -substituted compounds (see Table I). In conclusion, it is shown that the basic conformational properties of the C(5)-C(6) bond of β -D-(1→6)-linked disaccharides are already observable in the corresponding monosaccharides. The conformation around the (1→6)-linkage of digalactosides is largely determined by stereoelectronic effects ((*exo*-) anomeric effect, gauche effect), and is to some extent influenced by solute-solvent interactions.

5.3 Experimental

Spectroscopy — ^1H NMR spectra were recorded in the FT mode on a Bruker CXP-300 spectrometer at 300.1 MHz, using a 32 K point data set. Chemical shifts are given relative to TMS(CDCl_3 , CCl_4 , C_6D_6), relative to the $\text{CHD}_2\text{COCD}_3$ quintet at δ 2.17, relative to the CHD_2CN quintet at δ 1.95 or relative to 3-(trimethylsilyl)-propanesulfonic acid, sodium salt(D_2O) with an accuracy of 0.05 Hz. Coupling constants were taken from expansions of the patterns with an accuracy of 0.1 Hz and they were analysed with an iterative programme²¹. The calculated rotamer populations are given with an accuracy of about 3% (absolute). ^{13}C NMR spectra were recorded in the FT mode on a Bruker AC-200 spectrometer at 50.32 MHz, using a 16 K point data set. Chemical shifts are given relative to the acetone-septet(δ 30.70). The COSY spectra were recorded on a Bruker AC-200. The twodimensional map is composed of 1 K \times 512 data point spectra, each incremented by 0.83 ms; 16 transients were taken for each t_1 increment. The spectral widths were 600 Hz in ω_1 and 1200 Hz in ω_2 . Phase cycling for quadrature detection in both dimensions was used. A 2 s relaxation delay was allowed between each pulse sequence. For the delayed COSY experiment an extra delay of 0.4 s was inserted before evolution and the 0.427 s detection period. Data were multiplied with a sine-bell function, zero filled to 2 K \times 1 K and then Fourier transformed and symmetrized. The expansions in Fig. 8 were taken from the unsymmetrized spectra. The heteronuclear chemical shift correlation experiment was also performed on a Bruker AC-200. The two-dimensional map is composed of 1 K \times 128 data points with 256 transients for each t_1 increment. Spectral widths were 1200 Hz in ω_1 and 4400 Hz in ω_2 . Gaussian weighting was used in both dimensions. The final data matrix was 2 K \times 1 K.

Synthesis — Compounds **3**¹⁵, **4**⁷ and **5**²⁰(see Fig. 2) were synthesized according to literature procedures. Compounds **1** and **2** were also synthesized according to a literature procedure³¹ from tetraacetylgalactopyranosylbromide (SIGMA) and compounds **4** and **5** respectively. They were purified by column chromatography (dichloromethane : acetone 97:3 \rightarrow 90:10). Compound **1** (R_f = 0.03) and **2** (R_f =

0.05) both eluted together with some unreacted tetraacetylgalactopyranose (15% according to ^1H NMR).

1 and 2 were characterized by ^1H and ^{13}C NMR.

Methyl 2,3,4-tri-O-methyl-6-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)- β -D-galactopyranoside (1) — ^1H NMR (acetone- d_6) : δ 2.04–2.36 [4 s, 12H, OAc], 3.25 [m, $J_{1,2}$ 7.45 Hz, H(2)], 3.32 [m, $J_{2,3}$ 9.67 Hz, H(3)], 3.69 [m, $J_{4,5}$ 1.09 Hz, H(5)], 3.75 [m, $J_{3,4}$ 2.88 Hz, H(4)], 3.55–3.60 [4 s, 12H, OCH₃], 3.88 [m, $J_{5,6A'}$ 6.91 Hz, $J_{6,6'}$ -10.73 Hz, H(6A')], 4.04 [m, $J_{5,6A}$ 5.39 Hz, H(6A)], 4.10–4.25 [m, $J_{5,6B}$ 6.42;6.73 Hz, $J_{6,6'}$ -11.14 Hz, H(6B)/(6B')], 4.23 [d, H(1)], 4.62 [m, $J_{4,5}$ 0.87 Hz, H(5')], 4.92 [d, $J_{1',2'}$ 7.90 Hz, H(1')], 5.20–5.25 [m, 2H, H(2')/H(3')], 5.53 [m, H(4')]. ^{13}C NMR (acetone- d_6) : δ 63,02 [C(6')], 67.54 [C(5')], 69.16 [C(4')], 70.24 [C(6)], 71.24 [C(2')], 72.53 [C(3')], 75.08 [C(5)], 77.10 [C(4)], 82.45 [C(2)], 85.72 [C(3)], 102.7 [C(1')], 106.16 [C(1)].

Methyl 2,3,4-tri-O-methyl-6-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)- α -D-galactopyranoside (2) — ^1H NMR (acetone- d_6) : δ 2.04–2.36 [4 s, 12H, OAc], 3.46–3.60 [4 s, 12H, OCH₃], 3.60 [m, $J_{1,2}$ 2.60 Hz, H(2)], 3.62 [m, H(3)], 3.808 [m, $J_{3,4}$ 0.90 Hz, H(4)], 3.81 [m, $J_{5,6A'}$ 7.35 Hz, H(6A')], 3.92 [m, H(5)], 4.51 [m, $J_{5,6A}$ 4.84 Hz, $J_{6,6'}$ -10.46 Hz, H(6A)], 4.14–4.21 [m, $J_{5,6B}$ 6.78; 6.54 Hz, $J_{6,6'}$ -11.13 Hz, H(6B)/H(6B')], 4.53 [m, H(5')], 4.888 [d, $J_{1',2'}$ 7.85 Hz, H(1')], 4.90 [d, $J_{1,2}$ 2.60 Hz, H(1)], 5.23–5.25 [m, H(2')/H(3')], 5.54 [m, $J_{3',4'}$ 1.60 Hz, H(4')]. ^{13}C NMR (acetone- d_6) : δ 62.97 [C(6')], 67.04 [C(5')], 69.18 [C(4')], 69.88 [C(6)], 70.69 [C(2')], 71.06 [C(5)], 72.22; 72.46 [C(3')], 78.33 [C(4)], 79.70 [C(2)], 82.09 [C(3)], 99.65 [C(1)], 102.92 [C(1')].

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 $x(g^+) = -0.054 J_{5,6A} + 0.104 J_{5,6A'} + 0.061$
 $x(g^+) = +0.129 J_{5,6A} - 0.003 J_{5,6A'} - 0.364$
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SUMMARY

This thesis describes an experimental NMR study, combined with MNDO calculations, on the conformational preferences of the exocyclic bond of 6-phosphorylated pyranosides and tetrahydropyran-2-methyl compounds. The various factors influencing the rotamer population distribution around this O-C-C-O fragment, e.g. solvent polarity, stereoelectronic effects and coordination of phosphorus, are analysed.

In chapter 2 it is shown that a change in coordination of phosphorus from four (P^{IV}) to five (P^V) induces a conformational change around the C(1')-C(2) bond of the tetrahydropyran-2-methyl group towards the g^- rotamer (O(1') and O(1) trans-situated), provided that this group is situated axially in the P^V trigonal-bipyramidal (TBP) structure. This was proven by a variable-temperature study on a P^V compound which yielded the thermodynamic parameters governing the rotation around the C(1')-C(2) bond. This also allowed, for the P^V compounds, the assignment of the proton resonating at low field as H(1'). The assignment of the H(1')/H(1'') resonances for the P^{IV} compounds was established on the basis of the gauche effect. A similar conformational transmission effect around the C(5)-C(6) bond was found for galactopyranosides, but not for glucopyranosides due to 1,3-syn-diaxial interactions.

The influence of electron-withdrawing substituents at C(4) of the oxaphosphole ring of P^V TBP compounds on the axial exocyclic oxygen atom is shown in chapter 3. MNDO calculations reveal that an increase in electronegativity of these substituents decreases the electron density on this atom. This results in a decreased g^- rotamer fraction ($x(g^-)$) as compared to the dioxaphosphole compounds of chapter 2.

An increase in solvent polarity also causes a decrease in g^- rotamer fraction around the C(5)-C(6) bond of 6-phosphorylated galactopyranosides (chapter 4). Also, $x(g^-)$ depends on the configuration of the group at the anomeric centre. It is shown that in all solvents, the $x(g^-)$ value for α -compounds is smaller than for

β -compounds. This is observed both for P^{IV} and P^V compounds which proves that this difference is caused by an effect additional to that of electrostatic repulsion between O(5) and O(6), presumably by an interaction between the orbitals governing the gauche and anomeric effect. This is supported by a decreasing g^- population with decreasing pK_a of the group at the anomeric centre. The results of the NMR measurements (in CCl_4) are reproduced fairly accurately by MNDO calculations.

In chapter 5 it is shown that the dependence on solvent polarity of the rotamer population around the C(5)-C(6) interglycosidic bond of digalactopyranosides is almost identical to that for monogalactopyranosides. This establishes the assignment of the H(6R)/H(6S) resonances for the monogalactopyranosides since the corresponding assignment for the digalactopyranosides is unambiguous. The solvent dependence of the rotamer population distribution around the C(5)-C(6) bond can be used as a new criterium for the assignment of the H(6R)/H(6S) resonances in galactopyranosides ($J_{5,6}$ increases with decreasing solvent polarity).

SAMENVATTING

Dit proefschrift beschrijft een experimentele NMR studie, gecombineerd met MNDO berekeningen, naar de voorkeursconformaties van de exocyclische binding van 6-gefosforyleerde pyranosiden en tetrahydropyraan-2-methyl verbindingen. De verschillende factoren die de rotameerpopulatie-verdeling rond dit O-C-C-O fragment beïnvloeden, zoals bijvoorbeeld de polariteit van het oplosmiddel, stereo-elektronische effecten en het coördinatie getal van fosfor, zijn geanalyseerd.

In hoofdstuk 2 wordt aangetoond dat een coördinatieverandering rond fosfor van vier (P^{IV}) naar vijf (P^V) een conformatieverandering rond de C(1')-C(2) binding van de tetrahydropyraan-2-methyl groep veroorzaakt naar de g^- rotameer (O(1') en O(1) trans georiënteerd), mits deze groep een axiale positie in de P^V trigonaal-bipyramidale (TBP) structuur inneemt. Dit werd bewezen met een temperatuur-afhankelijke studie aan een P^V verbinding die de thermodynamische parameters opleverde die bepalend zijn voor rotatie rond C(1')-C(2). Hierdoor kon ook, voor P^V verbindingen, de protonresonantie bij laag veld aan H(1') toegekend worden. De toekenning van de H(1')/H(1'') resonanties van de P^{IV} verbindingen is gevestigd op basis van het gauche effect. Voor galactopyranosiden is een vergelijkbaar conformatietransmissie-effect rond de C(5)-C(6) binding gevonden, maar niet voor glucopyranosiden vanwege 1,3-syn-diaxiale interacties.

De invloed van elektronen-zuigende substituenten aan C(4) van de oxafosfoleen ring van P^V TBP verbindingen op het axiale exocyclische zuurstofatoom wordt aangetoond in hoofdstuk 3. MNDO berekeningen tonen aan dat de elektronendichtheid op dit atoom wordt verminderd met toenemende elektronegativiteit van deze substituenten. Dit resulteert in een verlaagde g^- rotameer fractie ($x(g^-)$) vergeleken met de P^V verbindingen in hoofdstuk 2.

Een verhoogde polariteit van het oplosmiddel veroorzaakt ook een vermindering in de g^- rotameer fractie rond de C(5)-C(6) binding van 6-gefosforyleerde galactopyranosiden (hoofdstuk 4). Bovendien hangt $x(g^-)$ af van de configuratie van de groep aan het anomere centrum. Er wordt aangetoond dat de waarde voor

$x(g^-)$ in alle oplosmiddelen lager is voor α -verbindingen dan voor β -verbindingen. Dit wordt waargenomen voor zowel P^{IV} als voor P^V verbindingen, wat bewijst dat dit verschil veroorzaakt wordt door een extra effect naast de elektrostatische afstoting tussen O(5) en O(6). Vermoedelijk is dit een interactie tussen de orbitalen die het gauche en anomere effect bepalen. Dit wordt ondersteund door de dalende g^- populatie met dalend pK_a van de groep aan het anomere centrum. De resultaten van de NMR metingen (in CCl_4) worden redelijk nauwkeurig gereproduceerd door MNDO berekeningen.

In hoofdstuk 5 wordt aangetoond dat de rotameerpopulatie rond de interglycoside C(5)-C(6) binding van digalactopyranosiden op identieke wijze afhankelijk is van de polariteit van het oplosmiddel als gevonden is voor monogalactopyranosiden. Dit legt de toekenning van de H(6R)/H(6S) resonanties vast voor de monogalactopyranosiden, aangezien de overeenkomstige toekenning voor de digalactopyranosiden eenduidig is. De afhankelijkheid van de rotameerpopulatie-verdeling rond de C(5)-C(6) binding van de oplosmiddelpolariteit kan gebruikt worden als een nieuw criterium voor de toekenning van de H(6R)/H(6S) resonanties in galactopyranosiden ($J_{5,6}$ stijgt met dalende polariteit van het oplosmiddel).

CURRICULUM VITAE

van Nanne Koen de Vries, geboren op 9 juli 1960

Na het behalen van het atheneum-B diploma aan het Gemeentelijk Lyceum te Eindhoven in 1978 werd in september van dat jaar begonnen met de studie voor scheikundig ingenieur aan de Technische Hogeschool Eindhoven.

Het afstudeerwerk werd verricht bij de vakgroep Organische Chemie onder leiding van prof. dr. H. M. Buck. In februari 1984 werd het ingenieursexamen met lof afgelegd.

Het onderzoek, beschreven in dit proefschrift, werd gestart op 1 maart 1984 en stond onder leiding van prof. dr. H. M. Buck. Vanaf 1 maart 1984 tot 1 maart 1986 was de schrijver van dit proefschrift als wetenschappelijk assistent in dienst van de Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek, en vanaf 1 maart 1986 tot 1 april 1987 bij de TU Eindhoven. Sedert 1 april 1987 is hij in dienst van DSM te Geleen.

NAWOORD

Aan de totstandkoming van dit proefschrift hebben velen hun medewerking verleend op theoretisch, synthetisch en technisch gebied, waarvoor ik hen zeer erkentelijk ben. Voorts wil ik mijn kamergenoten ir. Henk de Keijzer, ir. Peter de Kok, ir. Berry Meulendijks en ir. Michel Verhoeven bedanken voor de goede samenwerking en de prettige werksfeer. Prof. dr. H. M. Buck wil ik bedanken voor zijn inzet en vertrouwen, waardoor het schrijven van dit proefschrift mogelijk werd. Voor de vormgeving van het proefschrift ben ik dank verschuldigd aan mr. Inge de Vries voor het typewerk, aan mevr. P. Meyer voor de correcties en aan Henk Eding voor de tekeningen en de lay-out. Een speciaal woord van dank gaat tenslotte uit naar mijn ouders voor hun steun en belangstelling.

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Stellingen

1. De bepaling van de kristalstructuur van het trinatriumzout van β -D-fructofuranose 1,6-difosfaat octahydraat door Cerrini et al. heeft niet geleid tot nieuwe inzichten betreffende de ruimtelijke structuur van dit molecuul, vergeleken met de resultaten van Narendra et al.

N. Narendra, T. P. Seshadri en M. A. Viswamitra, *Acta Cryst.* **C41**, 31 (1985).
S. Cerrini, V. M. Coiro, D. Lamba en G. M. Bisso, *Carbohydr. Res.* **147**, 183 (1986).

2. De bewering van Juaristi et al., dat 3p-3d electron-donatie van zwavel naar fosfor bijdraagt tot de axiale voorkeur van de 2-difenyfosfinoylgroep in 1,3-dithianen, wordt niet weerlegd door de constatering van Mikolajczyk et al. dat een trifenyfosfoniumgroep een voorkeur bezit voor de equatoriale conformatie.

E. Juaristi, L. Valle, B. Valenzuela en M. A. Aguilar, *J. Am. Chem. Soc.* **108**, 2000 (1986).
M. Mikolajczyk, P. Graczyk en P. Balczewski, *Tetrahedron Lett.* **28**, 573 (1987).

3. De hogere populatie van de gauche-conformatie rond de C(2)-C(3) binding van acetylcarnitine, vergeleken met carnitine, kan niet alleen toegeschreven worden aan een versterking van het gauche-effect ten gevolge van een meer polaire C(3)-O(3) binding.

W. J. Colucci, R. D. Gandour en E. A. Mooberry, *J. Am. Chem. Soc.* **108**, 7141 (1986).

4. De conclusie van Haw en Johnson dat hun ^{13}C CP/MAS studie weinig inzicht verschaft in de details van het uithardingsproces van harsen ten gevolge van een te lage signaal-ruis verhouding per tijdseenheid en onvoldoende resolutie, is in tegenspraak met hun suggestie dat deze methode toepassing zou kunnen vinden bij de bestudering van de gelvorming van silicaatoplossingen.

J. F. Haw en N. A. Johnson, *Anal. Chem.* **58**, 3254 (1986).

5. Het feit dat de giftigheid van een waterige propyleenoxide oplossing vermindert in de tijd, is een onvoldoende reden om deze alkylerende verbinding aan te bevelen als een chemisch sterilisatiemiddel.

H. Sato, T. Kidaki en M. Hori, *Int. J. Artif. Organs* 8, 109 (1985).

6. De ^1H NMR toekenning van de protonen aan het exocyclische C(6) atoom in methyl β -D-galactopyranoside door de Bruyn en Anteunis is onjuist.

A. de Bruyn en M. Anteunis, *Carbohydr. Res.* 47, 311 (1976).

7. De ontwikkeling van een expertsysteem dat ruimtelijke structuuropheldering van molekulen op basis van kernspinresonantietechnieken en molecular modelling combineert, verdient gestimuleerd te worden.

8. Bij de ontwikkeling en toepassing van kunststoffen dient een hogere prioriteit te worden toegekend aan de veiligheidsaspecten, gezien de potentiële gevaren van smeulende en brandende kunststoffen.

E. G. Butcher en A. C. Parnell, *Smoke control in fire safety design*, E & F. N. Spon, London, 1979.

9. Een conformatieanalyse die uitsluitend gebaseerd is op de chemische verschuiving van protonen, biedt onvoldoende nauwkeurigheid.

C. P. J. Glaudemans, E. Zisis en M. E. Jolley, *Carbohydr. Res.* 40, 129 (1975).

10. Het verdient aanbeveling om de definitie in het woordenboek van het woord "tijdelijk" te wijzigen in "voor beperkte tijd geldig, met stilzwijgende mogelijkheid tot onbeperkte verlenging", als het gebruikt wordt in de context van een belastingmaatregel.