

The metal-binding sites of glucose phosphates†

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In aqueous solution, the reducing sugar phosphates D-arabinose 5-phosphate, D-ribose 5-phosphate, D-fructose 1,6-bisphosphate, D-fructose 6-phosphate, D-glucose 6-phosphate and D-mannose 6-phosphate provide metal-binding sites at their glucose core on reaction with Pd^{II}(en) or M^{III}(tacn) residues (M = Ga, Co; en = ethylenediamine, tacn = 1,4,7-triazacyclononane). The individual species were detected by one- and two-dimensional NMR spectroscopy. The coordination patterns are related to the metal-binding modes of the respective parent glycoses. In detail, ribo- and arabinofuranose phosphate favour $\kappa O^{1,3}$ coordination, whereas the ketofuranose core of fructose phosphate and fructose bisphosphate provides the $\kappa O^{2,3}$ chelator thus maintaining the configuration of the respective major solution anomer. On palladium excess, D-fructose 6-phosphate is metallated twice in a unique $\kappa O^{1,3} : \kappa O^{2,4}$ metallation pattern. Dimetallation is also found for the aldohexose phosphates. A mixed glycoside-core-phosphate chelation was detected for Pd^{II}(en) and M^{III}(tacn) residues with M = Al, Ga in the pH range just above the physiological pH for the D-fructose 1,6-bisphosphate ligand. The results are discussed in relation to D-fructose-1,6-bisphosphate-metabolism in class-II aldolases.

Introduction

Biomolecules such as amino acids, nucleosides and nucleotides, as well as their oligo- and polymeric forms are, from the viewpoint of coordination chemistry, typical ambident ligands. Depending on factors such as stoichiometry, pH value and the kind of central metal, these bioligands may act as chelators of variable denticity and ligand-atom type. However, though a marked flexibility with respect to their metal-binding properties is observed, these ligands all have in common the fact that they adapt their ligand-atom pattern on the level of conformational changes: their configuration is invariable.

Reducing sugars (aldoses and ketoses = glycoses) constitute a unique bioligand class. In contrast to the well-established bioligands mentioned above, glycoses provide ligands of both adaptable configuration and conformation to a metal. Even a simple molecule such as the parent glycoside of one of the ligands in this work, the aldopentose D-arabinose, is able to provide various types of bidentate chelators to a metal, among them diol functions supplied by α - and β -D-arabinofuranose and α - and β -D-arabinopyranose.¹ Bearing in mind the interconvertibility of the various isomers, D-arabinose, though being a single substance, provides a central metal with a dynamic ligand library. This unique property is shared by glycoside derivatives whose derivatisation had not affected the glycoside's anomeric centre (C1 for an aldose, C2 for a ketose). Such reducing glycoside derivatives include glycuronic acids, deoxy glycoses, glycosamines (2-amino-2-deoxy-glycoses) and the focus of this work, the non-anomerically phosphorylated glycoside phosphates.

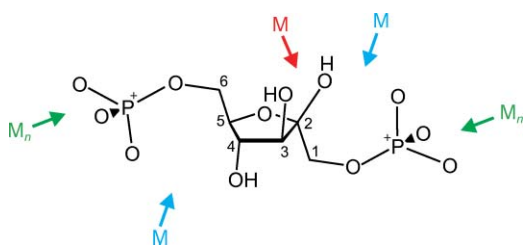
Reducing glycoside phosphates play a decisive role in all organisms' metabolism. Glucose polymers such as starch and their degradation products are converted into D-glucose 6-phosphate in the glycolytic pathway, the next step of which is the isomerisation to D-fructose 6-phosphate. Similarly, D-mannose is phosphorylated to D-mannose 6-phosphate. Both aldohexose-metabolising pathways join in the next step, the formation of D-fructose 1,6-bisphosphate.² This latter tetraanion is well-suited to demonstrate the principles of the ligand-library approach to an aldose's coordination chemistry. Restricting this introductory discussion to the bidentate chelating mode, the bisphosphate's parent glycoside, the ketohexose D-fructose, is expected to provide various *cis*-1,3 and *cis*-1,2-chelating diol functions through both the two fructofuranose (α - and β -D-Fruf) and the two fructopyranose (α - and β -D-Frup) anomers. Examples of actually observed bonding modes include the dianionic β -D-Fruf1,2H₂- $\kappa O^{1,2}$ chelator with silicon and the tetraanionic β -D-Frup2,3,4,5H₄- $\kappa O^{2,3} : \kappa O^{4,5}$ bis-chelator with boron.^{3,4}

On 1,6-bisphosphorylation, the pure hydroxy-function-based fructose ligand turns into a true ambident molecule by now bearing distinctly different functions. Thus, at first glance, the ligand properties of the parent glycoside that are diverse anyway, appear to be further complicated. However, there is a marked shrinking of the fructose's original ligand library. Since the 6-position is blocked by a phosphate function, only two of the previous four isomers of the fructose core are accessible, namely the two furanose anomers. Hence, an aqueous D-fructose 1,6-bisphosphate solution is made up of these two furanose anomers only, the minor α -D-Fruf1,6P₂ (12%) and the major β -D-Fruf1,6P₂ isomer (88%).⁵

To assess the metal-binding sites of a glycoside phosphate, the major form of D-fructose 1,6-bisphosphate, the β anomer, may be chosen as an example (Scheme 1). Various regions appear to be attractive for a metal fragment: the phosphate residues, mixed glycoside-core-phosphate chelates, and chelates that are provided by the glycoside core solely after deprotonation of a *cis*-diol function.

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Scheme 1 The potential bidentate metal-binding sites of β -D-fructofuranose 1,6-bisphosphate (in its protonation state about the physiological pH): (1) formation of a chelate with glyco-core hydroxy functions and a metal M sufficiently acidic to force deprotonation; (2) formation of eight- (left M') and seven-membered (right M') chelate rings by attack of a metal M' to a mixed glyco-core-phosphate ligand set; (3) transformation of a phosphate residue to a bridging ligand by a metal fragment M_n , as described in ref. 8 for $M_n = \text{Cu}_2$.

It might be expected that the mixed sites would not play a considerable role as a glyco-phosphate's metal-binding site since the ring sizes of the tentative chelates are rather large. Hence the left phosphate group in Scheme 1 would be, together with the 4-hydroxy function, part of an unfavourable eight-membered chelate ring. In the case of a 1-phosphorylated ketose, however, it should be noted that a less unstable seven-membered ring is possible (the right phosphate group in Scheme 1).⁶ Accordingly, the association of magnesium and zinc ions to the 1-phosphate group of D-fructose 1,6-bisphosphate has been deduced from ³¹P NMR spectra.⁷

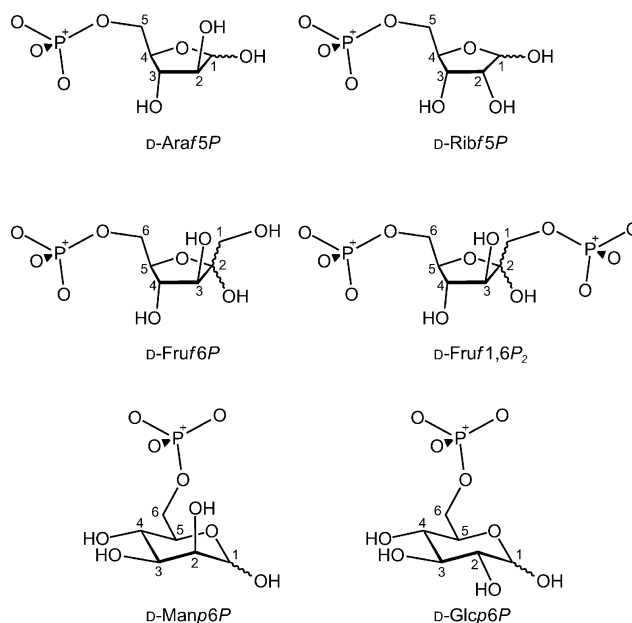
Among the factors that govern the actual binding site of an individual central metal, the interplay of the metal's Lewis acidity and the solution pH appears decisive. Hence, the design of a special phosphate-directed assembly of two moderately acidic copper(II) centres without the supply of additional base enabled the Tanase group to isolate a phosphate-bonded coordination compound of the D-Fru/1,6P₂ ligand.⁸ Neither metallation of a reducing glyco-phosphate's glyco-core nor the mixed phosphate-glyco ligation has yet been unequivocally demonstrated outside of an enzyme's active site. Even a stronger Lewis acid such as the aluminium(III) centre has been found to be exclusively phosphate-binding in a study on D-glucose 6-phosphate.⁹ However, stable metal complexes of the parent glycoses of this work have been found with di- and trivalent Lewis acidic metal centres.^{10–14}

Among the enzymes that act on sugar phosphates, class-II aldolase shows the closest relationship to the complexes of this work. Class-II aldolase cleaves D-fructose 1,6-bisphosphate to dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate. Two recent single-crystal analyses of substrate-bonding class-II aldolases are available.^{15,16} In both diffraction studies, the substrate is not captured in its furanose ground state but in an activated, open-chain keto form. A mononuclear zinc centre, whose Lewis acidity is high due to its fixation at the protein *via* neutral ligands only (three histidine residues), is chelated by O3 and O4 of the fructose core. No information is presently available about the initial binding of ground-state D-fructofuranose 1,6-bisphosphate to the metal centre.

Herein we describe the formation of metal complexes of various reducing glyco-phosphates. The metal centres have been chosen to bear sufficient Lewis acidity to make the glyco-core the ligand in an aqueous environment somewhere on the pH scale.

A specific bonding situation was adjusted by the addition of a stoichiometric amount of base needed for the deprotonation of the metal-binding hydroxy functions of the glyco-core. An example may be derived from Scheme 1. M is to be considered a fragment with three vacant coordination sites. In an attempt to bind M to the deprotonated 2,3-*cis*-diol function and one phosphate-oxygen donor, two equivalents of base were added.

The investigated reducing glyco-phosphates, all of which occur in metabolic pathways, were chosen to address the various issues of glyco-phosphate-metal interactions: (1) basic rules of glyco-core-metal chelation are derived from aldopentose 5-phosphates; (2) D-fructose 1,6-bisphosphate then shifts the focus to mixed chelates and to the physiological pH range; (3) for the related D-fructose 6-phosphate dimetallation is probed, and, finally, (4) preliminary results on the aldohexose 6-phosphates highlight their close relationship to the parent aldohexoses (Scheme 2).



Scheme 2 Formulae and symbols used for the glyco-phosphates used in this work. Top: D-arabinofuranose 5-phosphate, D-ribofuranose 5-phosphate; middle: D-fructofuranose 6-phosphate, D-fructofuranose 1,6-bisphosphate; bottom: the pyranose forms of D-mannose 6-phosphate and D-glucose 6-phosphate, the latter two aldohexose 6-phosphates being capable of forming furanose isomers, in principle. Wavy bonds indicate α/β anomericism.

Experimental

Methods and materials

All chemicals were purchased and used without further purification: disodium D-arabinose 5-phosphate, disodium D-ribose 5-phosphate dihydrate, dipotassium D-fructose 6-phosphate, dipotassium D-glucose 6-phosphate hydrate, sodium D-mannose 6-phosphate, cobalt(II) chloride hexahydrate, gallium chloride (Sigma-Aldrich), aluminium chloride hexahydrate (Fluka), deuterium oxide (Eurisotop), active charcoal, ethylenediamine (Grüssing), trisodium D-fructose 1,6-bisphosphate octahydrate

Table 1 ^{13}C NMR shifts of metal complexes of the aldopentose 5-phosphates of D-arabinose and D-ribose. $\Delta\delta$ is the difference of a shift in the presence of the metal probe and the free glucose phosphate. Note the large $\Delta\delta$ values in the case of five-membered palladacycles

		C1	C2	C3	C4	C5
[Pd(en)(α -D-Araf5P1,3H ₂ - κ O ^{1,3})] ²⁻	δ	104.4	82.4	76.8	88.1	64.6
	$\Delta\delta$	3.3 ^a	1.0	1.1 ^a	5.6	1.1
[Pd(en)(β -D-Araf5P1,2H ₂ - κ O ^{1,2})] ²⁻	δ	110.8	88.7	78.9	81.3	65.5
	$\Delta\delta$	15.5 ^a	12.5 ^a	4.5	0.6	0.9
[Pd(en)(α -D-Ribf5P1,3H ₂ - κ O ^{1,3})] ²⁻	δ	96.4	72.4	70.1	86.1	64.4
	$\Delta\delta$	0.0 ^a	1.5	-0.3 ^a	3.4	0.4
[Pd(en)(α -D-Ribf5P1,2H ₂ - κ O ^{1,2})] ²⁻	δ	111.6	82.5	71.7	79.7	64.6
	$\Delta\delta$	15.2	11.6	1.3	-3.0	0.6
[Pd(en)(β -D-Ribf5P2,3H ₂ - κ O ^{2,3})] ²⁻	δ	102.1	87.8	83.4	84.7	66.2
	$\Delta\delta$	0.8	12.3 ^a	12.5 ^a	2.7	1.4
[Ga(tacn)(α -D-Ribf5PH ₃ - κ O ^{1,2,3})] ²⁻	δ	100.6	72.8	73.7	85.2	65.1
	$\Delta\delta$	4.0 ^a	2.1 ^a	3.0 ^a	1.2	0.8
[Ga(Me ₃ tacn)(α -D-Ribf5PH ₃ - κ O ^{1,2,3})] ²⁻	δ	100.3	71.9	72.9	85.3	64.3
	$\Delta\delta$	3.7 ^a	1.3 ^a	2.3 ^a	2.3	0.1
[Co(tacn)(α -D-Ribf5PH ₃ - κ O ^{1,2,3})] ²⁻	δ	103.1	84.7	75.0	84.7	64.1
	$\Delta\delta$	3.5 ^a	13.9 ^a	2.3 ^a	0.5	1.0

^a The metal-binding site.

(Applichem), (Aldrich), palladium(II) chloride (Fluka), silver(I) oxide (VWR), 1,4,7-triazacyclononane trihydrochloride (TCI Europe), and sodium hydroxide pellets (Biesterfeld-Graën). Reactions were carried out using standard Schlenk techniques in a nitrogen atmosphere.

NMR spectroscopy

NMR spectra were recorded at room temperature on Jeol ECP 270 (^1H : 270 MHz, ^{13}C : 67.9 MHz, ^{27}Al : 70.4 MHz, ^{31}P : 109 MHz, ^{71}Ga : 82.4 MHz), Jeol ECX 400/ECP 400 (^1H : 400 MHz, $^{13}\text{C}\{^1\text{H}\}$: 100 MHz, ^{31}P : 109 MHz) and Jeol ECP 500 (^1H : 500 MHz, $^{13}\text{C}\{^1\text{H}\}$: 125 MHz) spectrometers. The signals of the deuterated solvent (^{13}C) and the residual protons therein (^1H) were used as an internal secondary reference for the chemical shift. When D₂O was used as a solvent, a small capillary tube containing [D₆]DMSO was added to the sample tube (5 mm) in order to obtain a reference signal in the ^{13}C spectra. Shift differences are given as $\delta(\text{C}_{\text{complex}}) - \delta(\text{C}_{\text{free sugar}})$. The values for the free glucose phosphates were taken from our own measurements in D₂O and were thus in neutral aqueous solution.

Syntheses

Preparation of [Pd(en)(OD)₂]. To the brown suspension of palladium(II) chloride (10.16 g, 57.4 mmol) in water (50 mL), concentrated hydrochloric acid (10 mL) was added. Two thirds of a solution of ethylenediamine (13.5 mL, 202 mmol) in water (30 mL) was added dropwise, yielding a pink precipitate. After warming to 60 °C and the addition of the remaining ethylenediamine solution, the solid dissolved. The mixture was filtrated and adjusted to pH 2 with half-concentrated hydrochloric acid. The reaction tube was stored at 0 °C for 2 h, and a yellow precipitate of [Pd(en)Cl₂] formed. The solid was filtrated and dried. The remaining solution was again adjusted to pH 2. More product precipitated in the course of 2 h. The combined yellow solids were dried in vacuum (9.95 g, 73% yield). Anal. calcd for C₂H₈N₂Cl₂Pd: C, 10.12; H, 3.40; Cl, 29.86; N, 11.80. Found: C, 10.15; H, 3.41; Cl, 29.87; N, 11.67.

Batches of [Pd(en)Cl₂] (2.00 g, 8.42 mmol) were suspended with silver(I) oxide (2.40 g, 10.41 mmol) in D₂O (16.8 mL) under a nitrogen atmosphere and the exclusion of light. The mixture was heated to 40 °C for 15 min and filtrated. The resulting clear, yellow, ca. 0.5 M solution of [Pd(en)(OD)₂] ("Pd-en") was stored at -60 °C. ^{13}C NMR (D₂O): δ [ppm] = 45.4.

Glucose phosphate solutions in Pd-en. Under ice cooling, 0.2–0.7 mmol of glucose phosphate was dissolved in a threefold amount of Pd-en (ca. 0.5 M solution in D₂O). The yellow solution was stirred for about 2 h and immediately placed under NMR investigation. The solutions were either investigated immediately or stored at -60 °C. For D-ribose 5-phosphate, solutions of 1 : 1 molar ratio were investigated as well. The respective ^{13}C NMR glucose signals are collected in Table 1.

^1H and ^{31}P NMR data of D-arabinose 5-phosphate complexes. [Pd(en)(α -D-Araf5P1,3H₂- κ O^{1,3})]²⁻: ^1H NMR (D₂O): δ [ppm] = 3.09 (s, 1H, H3), 3.40 (s, 1H, H2), 3.57–3.66 (m, 2H, H5a, H5b), 4.23 (t, 1H, H4, $^3J_{4,5}$ 5.2 Hz), 4.43 (s, 1H, H1). ^{31}P NMR (D₂O): δ [ppm] = 4.39.

[Pd(en)(β -D-Araf5P1,2H₂- κ O^{1,2})]²⁻: ^1H NMR (D₂O): δ [ppm] = 3.47 (t, 1H, H2, $^3J_{2,3}$ 3.6 Hz), 3.57–3.66 (m, 1H, H4), 4.02 (m, 2H, H5a, H5b), 4.12 (t, 1H, H3, $^3J_{3,4}$ 4.1 Hz), 4.91 (d, 1H, H1, $^3J_{1,2}$ 3.3 Hz). ^{31}P NMR (D₂O): δ [ppm] = 4.75.

^1H and ^{31}P NMR data of D-ribose 5-phosphate complexes. [Pd(en)(α -D-Ribf5P1,3H₂- κ O^{1,3})]²⁻: ^1H NMR (D₂O): δ [ppm] = 3.05 (d, 1H, H3, $^3J_{3,4}$ 5.2 Hz), 3.49–3.54 (m, 2H, H5), 3.86 (t, 1H, H2, $^3J_{2,3}$ 5.2 Hz), 4.19 (t, 1H, H4, $^3J_{4,5a}$ 5.0 Hz), 4.32 (d, 1H, H1, $^3J_{1,2}$ 4.4 Hz). ^{31}P NMR (D₂O): δ [ppm] = 4.67.

[Pd(en)(α -D-Ribf5P1,2H₂- κ O^{1,2})]²⁻: ^1H NMR (D₂O): δ [ppm] = 3.49–3.54 (m, 2H, H5), 3.56–3.57 (m, 1H, H3), 3.57 (d, 1H, H2), 4.17 (t, 1H, H4, $^3J_{4,5a}$ 5.2 Hz, $^3J_{4,5b}$ 5.0 Hz), 4.92 (d, 1H, H1, $^3J_{1,2}$ 3.3 Hz). ^{31}P NMR (D₂O): δ [ppm] = 4.67.

[Pd(en)(β -D-Ribf5P2,3H₂- κ O^{2,3})]²⁻: No assigned ^1H data available due to multiple cross-peak overlap. ^{31}P NMR (D₂O): δ [ppm] = 4.78.

^1H and ^{31}P NMR data of D-fructose 1,6-bisphosphate complexes. [Pd(en)(β -D-Fruf1,6P2,3H₂- κ O^{2,3})]⁴⁻: ^1H NMR (D₂O):

δ [ppm] = 3.44 (dd, 1H, H1a, $^2J_{1a,1b}$ 10.7 Hz, $^3J_{1a,P}$ 5.0 Hz), 3.59 (d, 1H, H3, $^3J_{3,4}$ 6.6 Hz), 3.67–3.73 (m, 1H, H5), 3.84 (dd, 1H, H1b, $^3J_{1b,P}$ 2.7 Hz), 3.88–4.04 (m, 2H, H6a, H6b), 4.4 (t, 1H, H4, $^3J_{4,5}$ 7.2 Hz). ^{31}P NMR (D_2O): δ [ppm] = 4.43, 4.79.

$[\text{Pd}(\text{en})(\alpha\text{-D-Fruf1,6P}_2\text{2,4H}_{-2}\text{-}\kappa\text{O}^{2,4})]^{4-}$: ^1H NMR (D_2O): δ [ppm] = 3.11 (s, 1H, H4), 3.60 (s, 1H, H3), 3.66–3.71 (m, 2H, H1, H6), 3.88–3.94 (m, 2H, H6 bzw. H1), 4.26 (m, 1H, H5). ^{31}P NMR (D_2O): δ [ppm] = 4.64, 5.58.

^1H and ^{31}P NMR data of D-fructose 6-phosphate complexes. $[\text{Pd}_2(\text{en})_2(\alpha\text{-D-Fruf6PH}_{-4}\text{-}\kappa\text{O}^{1,3}:\kappa\text{O}^{2,4})]^{2-}$: ^1H NMR (D_2O): δ [ppm] = 2.66 (d, 1H, H1a, $^2J_{1a,1b}$ 11.6 Hz), 2.85 (s, 1H, H3), 2.88 (d, 1H, H1b), 2.90 (s, 1H, H4), 4.35 (t, 1H, H5, $^3J_{5,6a}$ 6.0 Hz, $^3J_{5,6}$ 7.7 Hz), 4.55–4.60 (m, 2H, H6a, H6b). ^{31}P NMR (D_2O): δ [ppm] = 5.03.

$[\text{Pd}(\text{en})(\beta\text{-D-Fruf6P}_2\text{3H}_{-2}\text{-}\kappa\text{O}^{2,3})]^{2-}$: ^1H NMR (D_2O): δ [ppm] = 3.30 (d, 1H, H1a, $^2J_{1a,1b}$ 12.1 Hz), 3.49 (d, 1H, H3, $^3J_{3,4}$ 6.9 Hz), 3.61 (d, 1H, H1b), 3.58–3.62 (m, 1H, H5), 3.87–3.93 (m, 1H, H6a), 3.96–4.01 (m, 1H, H6b), 4.51 (t, 1H, H4, $^3J_{4,5}$ 7.7 Hz). ^{31}P NMR (D_2O): δ [ppm] = 4.75.

$[\text{Pd}(\text{en})(\alpha\text{-D-Fruf6P}_2\text{4H}_{-2}\text{-}\kappa\text{O}^{2,4})]^{2-}$: ^1H NMR (D_2O): δ [ppm] = 3.17 (s, 1H, H4), 3.52 (s, 1H, H3), 3.46–3.56 (m, 1H, H1a), 3.64–3.66 (m, 1H, H1b), 3.66–3.72 (m, 1H, H6a), 3.97–4.01 (m, 1H, H6b), 4.23 (t, 1H, H5, $^3J_{5,6a}$ 4.6 Hz, $^3J_{5,6b}$ 5.0 Hz). ^{31}P NMR (D_2O): δ [ppm] = 4.34.

^1H and ^{31}P NMR data of D-glucose 6-phosphate complexes. $[\text{Pd}_2(\text{en})_2(\alpha\text{-D-Glcp6PH}_{-4}\text{-}\kappa\text{O}^{1,2}:\kappa\text{O}^{3,4})]^{2-}$: ^1H NMR (D_2O): δ [ppm] = 2.66 (dd, 1H, H2, $^3J_{2,3}$ 9.0 Hz), 2.89–2.93 (m, 1H, H4), 3.60–3.64 (m, 1H, H6a), 3.72–3.76 (m, 1H, H5), 3.83–3.87 (m, 1H, H6b), 4.23–4.27 (m, 1H, H3), 4.90 (d, 1H, H1, $^3J_{1,2}$ 3.9 Hz). ^{31}P NMR (D_2O): δ [ppm] = 5.39.

$[\text{Pd}_2(\text{en})_2(\beta\text{-D-Glcp6PH}_{-4}\text{-}\kappa\text{O}^{1,2}:\kappa\text{O}^{3,4})]^{2-}$: ^1H NMR (D_2O): δ [ppm] = 2.90–2.95 (m, 1H, H4), 2.94–2.99 (m, 1H, H2, $^3J_{2,3}$ 9.1 Hz), 3.20 (t, 1H, H3, $^3J_{3,4}$ 9.1 Hz), 3.32–3.36 (m, 1H, H5), 3.54–3.58 (m, 1H, H6a), 3.83–3.87 (m, 1H, H6b), 4.25 (s, 1H, H1). ^{31}P NMR (D_2O): δ [ppm] = 5.24.

^1H and ^{31}P NMR data of D-mannose 6-phosphate complexes. $[\text{Pd}(\text{en})(\beta\text{-D-Manp6P}_1\text{2H}_{-2}\text{-}\kappa\text{O}^{1,2})]^{2-}$: ^1H NMR (D_2O): δ [ppm] = 3.08–3.12 (m, 1H, H5), 3.45 (dd, 1H, H3, $^3J_{3,4}$ 3.6 Hz), 3.65–3.66 (m, 1H, H4), 3.66–3.72 (m, 1H, H6a/b), 3.72 (d, 1H, H2, $^3J_{2,3}$ 9.9 Hz), 3.82–3.93 (m, 1H, H6a/b), 4.19 (s, 1H, H1). ^{31}P NMR (D_2O): δ [ppm] = 5.31.

$[\text{Pd}_2(\text{en})_2(\beta\text{-D-Manp6PH}_{-4}\text{-}\kappa\text{O}^{1,2}:\kappa\text{O}^{3,4})]^{2-}$: ^1H NMR (D_2O): δ [ppm] = 3.08–3.12 (m, 1H, H5), 3.19–3.23 (m, 1H, H2), 3.37 (t, 1H, H4, $^3J_{4,5}$ 9.6 Hz), 3.62–3.66 (m, 1H, H3, $^3J_{3,4}$ 9.6 Hz), 3.82–3.93 (m, 2H, H6a, H6b), 4.09 (s, 1H, H1). ^{31}P NMR (D_2O): δ [ppm] = 5.34.

$[\text{Pd}(\text{en})(\alpha\text{-D-Manp6P}_2\text{3H}_{-2}\text{-}\kappa\text{O}^{2,3})]^{2-}$: ^1H NMR (D_2O): δ [ppm] = 2.90 (dd, 1H, H3, $^3J_{3,4}$ 8.8 Hz), 3.56 (d, 1H, H2, $^3J_{2,3}$ 3.9 Hz), 3.61–3.64 (m, 1H, H5), 3.86–3.92 (m, 2H, H6), 4.13 (t, 1H, H4, $^3J_{4,5}$ 9.6 Hz), 4.75 (s, 1H, H1). ^{31}P NMR (D_2O): δ [ppm] = 5.24.

Glycose phosphate ligation to an M^{III} (tacn) residue

Aluminium and gallium complexes. $[\text{Ga}(\text{tacn})(\alpha\text{-D-Ribf5PH}_{-3}\text{-}\kappa\text{O}^{1,2,3})]^{2-}$: gallium chloride (0.035 g, 0.20 mmol), 1,4,7-triazacyclononane trihydrochloride (0.048 g, 0.20 mmol), disodium D-ribose 5-phosphate (0.055 g, 0.20 mmol) and sodium

hydroxide (0.048 g, 1.20 mmol) were dissolved in D_2O (2 mL). A colourless solution was obtained after vigorous stirring for 5 min at room temperature. ^{71}Ga NMR (82.4 MHz, H_2O): δ = 222.2 ([$\text{Ga}(\text{OH})_4^-$], 110.2. $^{31}\text{P}\{^1\text{H}\}$ NMR: δ = 3.6. $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, D_2O): ribose signals see Table 1, 42.6–41.7 (tacn). ^1H NMR (399.4 MHz, D_2O): δ = 5.28 (d, 1H, $^3J_{1,2}$ = 3.7 Hz, H1), 4.25 (t, 1H, $^3J_{4,5}$ = 5.3 Hz, H4), 4.20 (dd, 1H, $^3J_{2,1}$ = 3.7 Hz, $^3J_{2,3}$ = 5.1 Hz, H2), 3.88 (d, 1H, $^3J_{3,2}$ = 5.1 Hz, H3), 3.76–3.70 (m, 2H, H5/H5'), tacn: 3.32–3.11 (m), 2.92–2.84 (m).

Essentially the same result was obtained by the use of the N,N',N'' -trimethyl derivative of 1,4,7-triazacyclononane (^{13}C NMR resonances in Table 1).

$[\text{Al}(\text{tacn})(\beta\text{-D-Fruf1,6P}_2\text{2,3H}_{-2}\text{-}\kappa\text{O}^{2,3,P})]^{3-}$. Aluminium chloride hexahydrate (0.048 g, 0.20 mmol), 1,4,7-triazacyclononane trihydrochloride (0.048 g, 0.20 mmol), trisodium D-fructose 1,6-bisphosphate (0.110 g, 0.20 mmol) and sodium hydroxide (0.048 g, 1.20 mmol) were dissolved in D_2O (2 mL). A colourless solution was obtained after vigorous stirring for 30 min at room temperature. ^{27}Al NMR (70.4 MHz, H_2O): δ = 80.4 ([$\text{Al}(\text{OH})_4^-$], 40.0 (broad). $^{31}\text{P}\{^1\text{H}\}$ NMR (109.4 MHz, H_2O): δ = 3.8, 3.7, –2.9. ^{31}P NMR (109.4 MHz, H_2O): δ = 3.7 (t, $^3J_{\text{PH}}$ = 4.7 Hz), –2.9 (dd, $^3J_{\text{PH}}$ = 4.7 Hz, $^3J_{\text{PH}}$ = 24.7 Hz). $^{13}\text{C}\{^1\text{H}\}$ NMR (100.6 MHz, D_2O): δ = 105.3 (C2), 80.6 (C3), 79.5 (C4), 78.3 (C5), 72.3 (C1), 64.3 (C6), 44.6–43.5 (tacn). ^1H NMR (399.4 MHz, D_2O): δ = 4.10 (d, 1H, $^3J_{3,4}$ = 6.9 Hz, H3), 3.95–3.83 (m, 2H, H6), 3.77 (dd, 1H, $^3J_{4,3}$ = 6.9 Hz, $^3J_{4,5}$ = 9.1 Hz, H4), 3.65 (m, 3H, $^3J_{4,5}$ = 9.1 Hz, H1/H5).

$[\text{Ga}(\text{tacn})(\beta\text{-D-Fruf1,6P}_2\text{2,3H}_{-2}\text{-}\kappa\text{O}^{2,3,P})]^{3-}$. Gallium chloride (0.035 g, 0.20 mmol), 1,4,7-triazacyclononane trihydrochloride (0.048 g, 0.20 mmol), trisodium D-fructose 1,6-bisphosphate (0.110 g, 0.20 mmol) and sodium hydroxide (0.048 g, 1.20 mmol) were dissolved in D_2O (2 mL). A colourless solution was obtained after vigorous stirring for 15 min at room temperature. $^{31}\text{P}\{^1\text{H}\}$ NMR (161.8 MHz, D_2O): δ = 4.8 (broad), 0.6 (broad). ^{31}P NMR (161.8 MHz, D_2O): δ = 4.8 (broad), 0.6 (broad). $^{13}\text{C}\{^1\text{H}\}$ NMR (125.7 MHz, D_2O): δ = 103.0 (C2), 77.5 (C3), 77.5 (C4), 77.5 (C5), 70.4 (C1), 62.4 (C6), 41.0–40.3 (tacn). ^1H NMR (500.2 MHz, D_2O): δ = 4.07 (d, 1H, $^3J_{\text{H3,H4}}$ = 6.6 Hz, H3), 3.95–3.84 (m, 3H, H6/H6'/H1), 3.74–3.67 (m, 3H, H1'/H4/H5).

Cobalt(III) complexes. $[\text{Co}^{\text{III}}(\text{tacn})(\alpha\text{-D-Ribf5PH}_{-3}\text{-}\kappa\text{O}^{1,2,3})]^{2-}$: To a solution of cobalt(II) chloride hexahydrate (0.048 g, 0.20 mmol) in D_2O (2.0 mL), 1,4,7-triazacyclononane trihydrochloride (0.048 g, 0.20 mmol), disodium D-ribose 5-phosphate (0.055 g, 0.20 mmol) and sodium hydroxide (0.048 g, 1.2 mmol) were added under continuous stirring at room temperature. After the addition of 0.2 g active charcoal, the mixture was heated to 50 °C for 2 h under vigorous stirring. A clear pink-red solution was obtained after filtration and centrifugation. $^{13}\text{C}\{^1\text{H}\}$ NMR (100.5 MHz, D_2O): ribose signals see Table 1, tacn: 50.9–48.5. A 0.1 ppm split of the ribose-C2/C4 signal is observed by using H_2O instead of D_2O . ^1H NMR (399.8 MHz, D_2O): δ = 4.50 (d, 1H, $^3J_{\text{H1,H2}}$ = 3.30 Hz, H1), 4.04 (t, 1H, $^3J_{\text{H4,H5}}$ = 5.50 Hz, H4), 3.83–3.81 (m, 1H, H2), 3.69–3.60 (m, 2H, H5/H5'), 3.25 (d, 1H, $^3J_{\text{H3,H2}}$ = 4.12 Hz, H3), tacn: 3.35–3.30 (m), 3.19–2.95 (m), 2.83–2.63 (m).

Results and discussion

The experimental strategy

The maximum number of metal–spectator–ligand fragments that are bonded to a glucose phosphate was not initially clear. Hence, various metal : glucose-phosphate molar ratios were used to elucidate the stoichiometries of the various solution species. A typical series of spectra and their interpretation is shown below in the D-fructose 6-phosphate chapter. If a number is given for the relative amount of an individual species, it was derived by the integration of ^1H NMR spectra.

To ascertain the assignments made, basically the same experimental strategy was applied. 1D ^1H , ^{13}C , ^{31}P , DEPT-135 and 2D HMQC and COSY NMR spectra of the reaction mixtures were recorded. From the 2D spectra, peaks were assigned to their carbon atoms and to the various species. Furthermore, the characteristic ^{31}P – ^{13}C coupling supported the assignments. This coupling led to a signal splitting of carbon atoms adjacent to the phosphate group and of the ones next to those carbons. Hence, due to the presence of two terminal phosphate groups in D-fructose 1,6-bisphosphate, C1, C2, C5 and C6 showed split signals in NMR spectra for this compound. In most cases, ^1H NMR signals as well as H,H coupling constants were also accessible and were used for configurational and conformational analysis according to a modified Karplus relation.¹⁷ In addition, ^{13}C spectra were used to calculate coordination-induced shifts (CISs) which support the determination of the metal-binding site. In particular, the case of five-membered palladacycles was clearly indicated by typical CIS values of about 10 ppm downfield.

^{31}P NMR spectroscopy was used to detect the incorporation of a phosphate group into a chelate ring, a case that was observed for D-fructose 1,6-bisphosphate at pH values close to neutrality. In the absence of such rings, both the ^{31}P chemical shifts and the ^{31}P – ^1H coupling constants resembled the values of free D-fructose 1,6-bisphosphate at the respective pH.⁷ If, instead, the phosphate group contributes to a chelate ring, its characteristic rotamer distribution about the phosphate–methylene bond freezes to a conformer compatible with the chelate. To assess the P–O–C–C torsion angle in these phosphacycles, Karplus curves derived for the related case of a nucleotide's conformation about the P–O–C5–C4 axis may be used.^{18,19} However, it should be noted that a detailed conformational analysis is hampered by the fact that the torsion about the neighbouring axis, the C2–C1 bond, remains unknown (a ketose lacks a proton at its anomeric centre; hence, a useful number such as a related ^1H – ^1H coupling constant is not available).

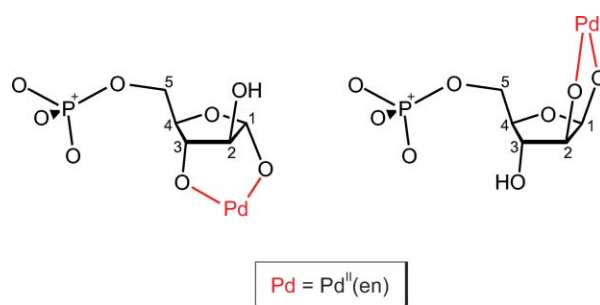
To obtain the spectroscopic results, side reactions had to be avoided. Particularly annoying reactions were observed in two cases: (1) the redox stability of palladium(II)-containing solutions may be so low that Fehling-like reactions proceeded even under cooling. Hence, only limited results were obtained for equimolar solutions of the aldohexose 6-phosphates which revealed themselves as particularly prone to palladium precipitation. (2) If the Al(tacn) fragment was reacted with a glucose phosphate and the required amount of base was added to trigger tridentate metal-binding by the glucose core, the known formation of a *N*-glycoside of tacn took place.²⁰ This side reaction was not observed with the other metal–amine–glucose combinations of this work.

Consistency checks were performed as the last step of species assignment. Thus both chemical shifts and CIS values of similar partial structure show close resemblance through the series of species. An example is given in the Conclusions section.

D-Arabinose 5-phosphate: a furanose ligand with a remote phosphate function

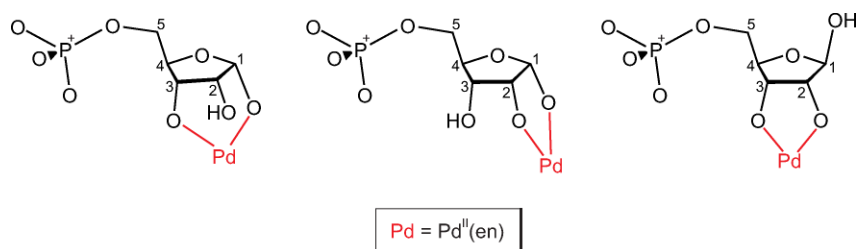
D-Arabinose 5-phosphate, in comparison to D-arabinose, is configurationally restricted to the two furanose anomers since the phosphate residue blocks the 5-position which is required for the formation of the pyranoses. The aqueous equilibrium mixture thus consists of only two significant species, the α - (57.3%) and the β -furanose (40.4%; minor species: 2.2% aldehyde hydrate and 0.2% aldehyde).⁵

Dissolution of D-arabinose 5-phosphate in excess Pd-en (molar Pd:glycose ratio of 3:1) resulted in the formation of two monometallated species (Table 1, Scheme 3). The main product (66%) was the six-ring chelate $[\text{Pd}(\text{en})(\alpha\text{-D-Araf5P1,3H}_2\text{-}\kappa\text{O}^{1,3})]^{2-}$. In terms of coupling constants, the furanose conformation was found close to 2E which resembles a contraction of the O_2 ligand set to match the binding sites of the central metal. The minor product (34%) was the five-ring chelate $[\text{Pd}(\text{en})(\beta\text{-D-Araf5P1,2H}_2\text{-}\kappa\text{O}^{1,2})]^{2-}$ with a furanose conformation between 3E and 3T_2 . Since no other signals were observed, the phosphate group thus remained uncoordinated despite the metal-excess conditions. Obviously, the formation of an eight-ring chelate such as the left one in Scheme 1 is unfavourable and the tentative dinuclear species $[\text{Pd}_2(\text{en})_2(\beta\text{-D-Araf5PH}_3\text{-}\kappa\text{O}^{1,2} : \kappa\text{O}^{3,P})]^{4-}$, which is derived from the right-most species in Scheme 3, was not detected. This statement holds also for solutions that exactly match the stoichiometry of the tentative dinuclear complex.



Scheme 3 Products of the reaction of Pd-en with D-arabinose 5-phosphate.

Since only monometallated products were observed, a similar result was obtained on dissolution of D-arabinose 5-phosphate in an equimolar amount of Pd-en. As expected, the only difference between the spectra stems from the occurrence of unmetallated glycose phosphate in the metal-reduced batch. With its two-species metallation behaviour, D-arabinose 5-phosphate exhibits a distinctly simpler coordination chemistry towards palladium(II) than the parent glycose, D-arabinose. At varying metal supply, the latter forms no less than five metallation products, since pyranose ligands add to the $\kappa\text{O}^{1,3}$ - and $\kappa\text{O}^{1,2}$ -bonding furanoses that are common to the glycose and its 5-phosphate.^{1,12}



Scheme 4 Products of the reaction of Pd-en with D-ribose 5-phosphate.

D-Ribose 5-phosphate: bi- and tridentate glycose-core chelators

BIDENTATE PALLADIUM(II) CHELATION. D-Ribose 5-phosphate, like D-arabinose 5-phosphate, is restricted to its two furanose isomers. At aqueous equilibrium, 35.6% α - and 63.9% β -furanose were detected by NMR spectroscopy (minor species: 0.5% hydrate and 0.1% aldehyde).⁵ In contrast to D-arabinose 5-phosphate, the 2-hydroxy function is *cis* to the 1- and 3-hydroxy groups in D-ribose 5-phosphate. Hence, one more bidentate chelation mode, $\kappa O^{2,3}$, was expected.

In fact, upon the reaction of Pd-en with D-ribose 5-phosphate at a molar Pd:glycose ratio of 3:1, two major and one minor species were observed (Scheme 4). With $[\text{Pd}(\text{en})(\alpha\text{-D-Rib}/5\text{P1},3\text{H}_2\text{-}\kappa O^{1,3})]^{2-}$ (40%, conformation close to 2E) and $[\text{Pd}(\text{en})(\alpha\text{-D-Rib}/5\text{P1},2\text{H}_2\text{-}\kappa O^{1,2})]^{2-}$ (20%, conformation 2T_3 or 3T_2 [uncertainty due to missing coupling constants because of signal overlap]), one of the major and the minor species resemble the furanose- $\kappa O^{1,3}$ and furanose- $\kappa O^{1,2}$ species of the arabinose analogue. The second major species was assigned to $[\text{Pd}(\text{en})(\beta\text{-D-Rib}/5\text{P2},3\text{H}_2\text{-}\kappa O^{2,3})]^{2-}$ (40%, 3T_2). This species was not detected with the parent glycose, D-ribose, on dissolution in the related reagent Pd-chxn, an aqueous solution of $[\text{Pd}^{\text{II}}\{(R,R)\text{-chxn}\}(\text{OH})_2]$ (chxn = cyclohexane-1,2-diamine).¹ Obviously, the $\kappa O^{2,3}$ chelator gains stability with the phosphorylated glycose. The C1 and C2 signals of this species were broadened at the 3:1 molar ratio of Pd:glycose due to rapid mutarotation under these base-excess conditions. At an equimolar ratio, however, sharp signals of the major β anomer arose in addition to small signals of the minor α anomer. ${}^{13}\text{C}$ NMR shifts of the products are listed in Table 1. Particularly well resolved ${}^{13}\text{C}$ NMR spectra were obtained with the related Pd-chxn solvent. Fig. 1 (top) shows the respective spectrum. A characteristic property of the palladium probes becomes apparent, namely the ability to develop all the bidentate members of a glycose-based ligand library. In contrast, tridentate probes such as those described in the next paragraph normally find much fewer bonding partners in a glycose's equilibrium mixture.

Tridentate metal(III) chelation. The α -D-ribofuranose part of D-ribose 5-phosphate is able to cover a *fac*- O_3 position of a coordination polyhedron. This is demonstrated (1) by the reaction of the $\text{Ga}^{\text{III}}(\text{tacn})$ residue (tacn = 1,4,7-triazacyclononane) with D-ribose 5-phosphate and the required amount of base, and (2) by the reaction of cobalt(II) and tacn with D-ribose 5-phosphate and the required amount of base on exposure to air. Solutions of a pH close to 11.5 resulted. As attempted, the NMR spectra (Table 1) showed the signals of a single species each, the $[\text{M}^{\text{III}}(\text{tacn})(\alpha\text{-D-Rib}/5\text{PH}_3\text{-}\kappa O^{1,2,3})]^{2-}$ ion (Scheme 5; M = Co, Ga). It should be noted that to view a glycose solution as a dynamic ligand library

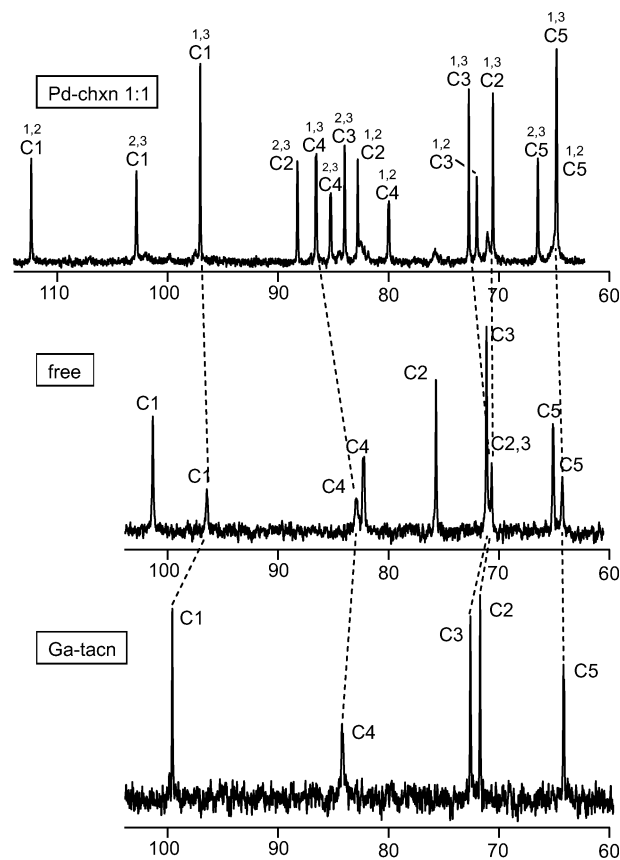


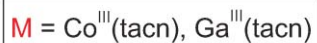
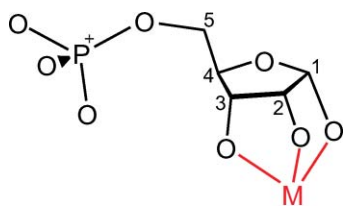
Fig. 1 Top: ${}^{13}\text{C}$ NMR spectrum of the ribose part of ribose 5-phosphate in a Pd-chxn solution at a molar Pd:D-Rib/5P ratio of 1:1. For each of the three species, the anomer and the Pd-bonding site is indicated in a short notation such as $\alpha 1,3$ for the main $[\text{Pd}^{\text{II}}\{(R,R)\text{-chxn}\}(\alpha\text{-D-Rib}/5\text{P1},3\text{H}_2\text{-}\kappa O^{1,3})]^{2-}$ species. The signals of the main species are connected to the respective signals of free $\alpha\text{-D-Rib}/5\text{P}$. Note the relatively small downfield shift of this six-ring chelate compared with the larger shifts of the two five-ring chelates. Note as well a residual amount of free ribose phosphate in the Pd-chxn solution. Middle: ${}^{13}\text{C}$ NMR spectrum of the equilibrium mixture of the minor α - and the major β -D-ribose 5-phosphate anomer. Note the splitting of the C5 and, particularly, the C4 signal due to the ${}^{31}\text{P}$ - ${}^{13}\text{C}$ coupling. Bottom: ${}^{13}\text{C}$ NMR spectrum of the ribose part of the same solution after reaction with the Ga(tacn) precursor. Note the complete transformation of the β to α anomer and the downfield shift on coordination.

is particularly useful in this case since it is the minor component of an aqueous D-ribose 5-phosphate solution which provides the ligand. On reaction with the $\text{M}^{\text{III}}(\text{tacn})$ precursors, the minor α -anomer was enriched at the expense of the major β -isomer. The mutarotation equilibrium progressively readjusts in the course of

Table 2 ^{13}C NMR shifts of the products from the reaction of various metal probes with D-fructose 1,6-bisphosphate and the hexose 6-phosphates of D-fructose, D-glucose and D-mannose (molar Pd : glycosyl ratio of 3 : 1). Note the large $\Delta\delta$ values in the case of five-membered pallada cycles

		C1	C2	C3	C4	C5	C6
[Pd(en)(β -D-Fruf1,6P ₂ 2,3H ₂ - κ O ^{2,3})] ⁴⁻	δ	66.4	114.7	87.5	79.7	80.9	66.2
	$\Delta\delta$	0.0	13.1 ^a	11.0 ^a	4.9	0.7	1.2
[Pd(en)(α -D-Fruf1,6P ₂ 2,4H ₂ - κ O ^{2,4})] ⁴⁻	δ	65.5	109.0	82.7	78.7	88.6	64.9
	$\Delta\delta$	0.2	3.5 ^a	0.6	1.5 ^a	6.2	0.2
[Pd ₂ (en) ₂ (α -D-Fruf6PH ₄ - κ O ^{1,3} : κ O ^{2,4})] ²⁻	δ	64.0	111.5	87.1	80.3	89.0	66.8
	$\Delta\delta$	0.7 ^a	6.6 ^a	4.9 ^a	3.9 ^a	8.1	2.9
[Pd(en)(β -D-Fruf6P ₂ 2,3H ₂ - κ O ^{2,3})] ²⁻	δ	64.2	114.7	87.4	79.6	80.6	66.0
	$\Delta\delta$	1.0	12.8 ^a	11.7 ^a	4.7	0.3	1.2
[Pd(en)(α -D-Fruf6P ₂ 2,4H ₂ - κ O ^{2,4})] ²⁻	δ	62.8	107.9	83.1	79.0	88.2	65.0
	$\Delta\delta$	-0.5	3.0 ^a	0.9	2.6 ^a	7.3	1.1
[Pd ₂ (en) ₂ (α -D-Glcp6PH ₄ - κ O ^{1,2} : κ O ^{3,4})] ²⁻	δ	102.4	83.3	86.4	78.9	74.0	63.3
	$\Delta\delta$	9.8 ^a	11.3 ^a	13.7 ^a	9.4 ^a	2.5	0.2
[Pd ₂ (en) ₂ (β -D-Glcp6PH ₄ - κ O ^{1,2} : κ O ^{3,4})] ²⁻	δ	106.0	85.2	87.1	80.3	75.9	63.8
	$\Delta\delta$	9.5 ^a	10.5 ^a	11.5 ^a	10.8 ^a	0.0	0.6
[Pd(en)(β -D-Manp6P ₁ 2H ₂ - κ O ^{1,2})] ²⁻	δ	106.5	82.7	72.3	67.2	74.9	63.7
	$\Delta\delta$	12.5 ^a	11.3 ^a	-0.4	1.0	-0.8	0.6
[Pd ₂ (en) ₂ (β -D-Manp6PH ₄ - κ O ^{1,2} : κ O ^{3,4})] ²⁻	δ	107.9	85.8	84.1	75.4	74.7	63.6
	$\Delta\delta$	13.9 ^a	14.4 ^a	11.4 ^a	9.2 ^a	-1.0	0.5
[Pd(en)(α -D-Manp6P ₂ 2,3H ₂ - κ O ^{2,3})] ²⁻	δ	94.4	81.2	79.7	70.0	71.8	64.4
	$\Delta\delta$	0.0	10.4 ^a	9.7 ^a	3.5	-0.3	1.4
[Al(tacn)(β -D-Fruf1,6P ₂ 2,3H ₃ - κ O ^{2,3,P})] ³⁻	δ	72.3	105.2	80.6	79.5	78.3	64.2
	$\Delta\delta$	6.2	5.1 ^a	5.6 ^a	4.4	0	0
[Ga(tacn)(β -D-Fruf1,6P ₂ 2,3H ₃ - κ O ^{2,3,P})] ³⁻	δ	70.4	103.0	77.5	77.5	77.5	62.4
	$\Delta\delta$	4.3	2.9 ^a	2.5 ^a	2.4	-0.9	-1.8

^a The metal-binding site.



Scheme 5 The only product of the reaction of D-ribose 5-phosphate with precursors of the M^{III}(tacn) fragment (M = Co, Ga).

the chelation reaction and, eventually, the D-ribose 5-phosphate is present as the α -configured ligand exclusively (Fig. 1).

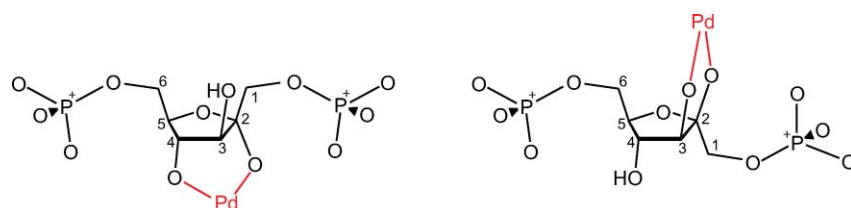
D-Fructose 1,6-bisphosphate: cooperative glycosyl-core-phosphate chelation

CHELATION BY THE GLYCOSE CORE. D-Fructose 1,6-bisphosphate has the same furanoidic backbone as D-arabinose

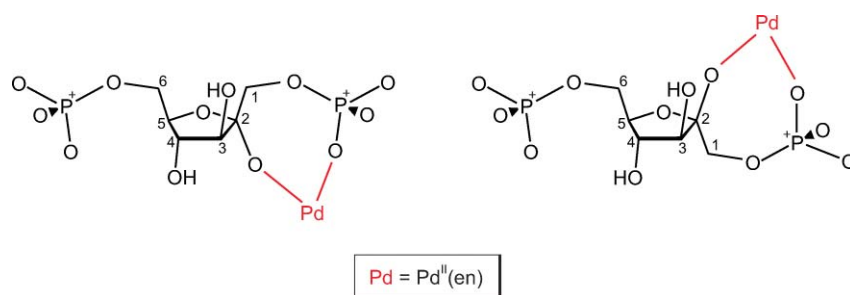
5-phosphate but carries a phosphoryloxymethyl group instead of the arabinose H1 atom. Aqueous solutions of D-fructose 1,6-bisphosphate consist of 13.1% α - and 86.0% β -furanose (minor species: 0.9% open-chain keto form).⁵ Hence, the arabinose derivative's α anomer, which is capable of providing the six-ring chelator, is no longer the major species in the free glycosyl phosphate's solution equilibrium.

The anomeric composition of the free glycosyl phosphate was nearly maintained after metallation by a threefold molar amount of Pd-en (Scheme 6). The major species was [Pd(en)(β -D-Fruf1,6P₂2,3H₂- κ O^{2,3})] (81%, conformation between ⁴E and ⁴T₃), the minor species was [Pd(en)(α -D-Fruf1,6P₂2,4H₂- κ O^{2,4})] (19%, conformation close to ³E). The chemical shifts of the compounds are collected in Table 2.

Bidentate glycosyl-core-phosphate chelation. In agreement with the bonding of only one palladium centre to the D-arabinose 5-phosphate anomers, an equimolar solution of Pd-en and the D-fructose 1,6-bisphosphate tetraanion resulted in the formation of the expected κ O^{2,3}- and the κ O^{2,4}-bonded species. It is recalled at this point that the lack of a mixed glycosyl-core-phosphate



Scheme 6 The major (right) and minor (left) products of the reaction of Pd-en with D-fructose 1,6-bisphosphate.

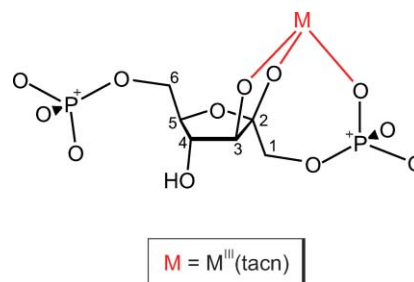


Scheme 7 Tentative formulae of two species that are related to the species in Scheme 6 by the addition of a proton to O3/O4.

chelation for arabinose and ribose 5-phosphate was connected with unsuitably large eight-membered chelate rings in these cases. In the case of a 1-phosphorylated ketose, however, a mixed ligand set would result in a less unsuitable seven-membered chelate ring. To trigger the formation of this bonding mode, one equivalent of acid was added for the reasons that have been discussed in the Introduction (in fact, Pd-en was reacted with an equivalent amount of *trisodium* D-fructose 1,6-bisphosphate). The pH value of the resulting solution was close to 8. Hence, the observed species distribution is regarded to be relevant for D-fructose 1,6-bisphosphate complexation with Lewis acids similarly strong as palladium(II) at the physiological pH. As a result, the spectroscopical analysis revealed three species: the $\kappa O^{2,3}$ - and the $\kappa O^{2,4}$ -bonded species derived above, and a third species of intermediate quantity. The signals of the latter species vanished not only on the addition of more Pd-en but also of sodium hydroxide. It thus appears reasonable to assume the participation of the 1-phosphate group to the metal-binding site of the new species since phosphate ligation does not require base addition. Arabinose 5-phosphate, being de-1-phosphoryloxymethyl-fructose 1,6-bisphosphate, did not show a third species under the same reaction conditions which demonstrates that, in fact, the 1-phosphate residue provides the metal-binding oxygen atom. These arguments led us to the formulation given in Scheme 7 which shows two anomers that exhibit seven-membered chelate rings in a mixed glucose-core-phosphate ligation. No data are available to decide which formula correctly represents the species.

³¹P NMR spectra support these conclusions. The pH 8 solution contained four types of D-fructose 1,6-bisphosphate ions: the two fructose-core-metallated species, free bisphosphate and the mixed-ligand species. Seven of the eight phosphate residues within these species are not involved in metal-binding. In agreement with their similar chemical environment, their ³¹P resonances superimposed in a range of 4 to 6 ppm. However, the resonance of the mixed-ligand species close to 10 ppm was well-separated from the group of seven. More significant, the ³¹P-¹H coupling constants markedly changed. In the case of free phosphate residues, the specific rotamer distribution of D-fructose 1,6-bisphosphate in an aqueous environment resulted in a mean ³¹P-¹H coupling constant of about 6 Hz.⁷ The respective constants of the 10 ppm signal, however, were 13 and 14 Hz. As has been noted above, the actual conformation on metal-binding was not unravelled since both the correct anomer and the rotation angle about the C2-C1 axis, which also determines the conformation, remain unknown due to the lack of an appropriate ¹H-¹H coupling in a ketose.

Tridentate glucose-core-phosphate chelation. In order to confirm the tentative mixed-ligand set of the last subchapter, M^{III}(tacn)-based metal fragments were used. Since these fragments are selective probes of a *fac*-tridentate pattern, only a single reaction product, resulting from the exclusive coordination of the β anomer of a D-fructose 1,6-bisphosphate hexaanion, was expected in terms of chelate-ring-size considerations (Scheme 8).

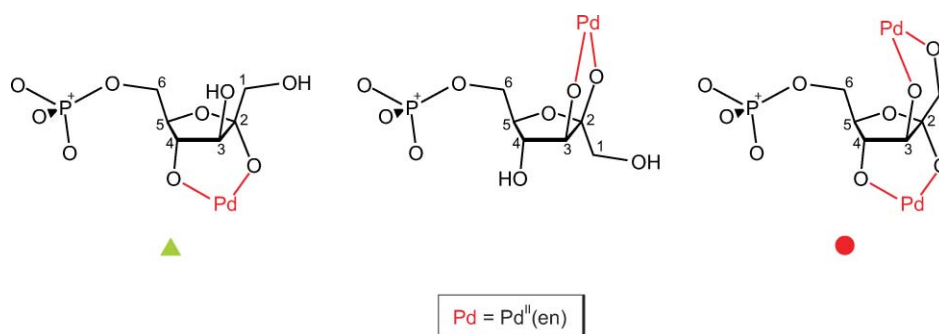


Scheme 8 The only product of the reaction of equimolar amounts of {M(tacn)}³⁺ and D-fructose 1,6-bisphosphate tetraanion, and two equivalents of base (M = Al, Ga).

In fact, the reaction of the M^{III}(tacn) fragments with D-fructose 1,6-bisphosphate and the required amount of base consistently resulted in the formation of a single species for M = Al and Ga in solutions of pH 10. (The Al^{III}-tacn probe could be included despite the N-glycosylation result with ribose 5-phosphate since the pH value is too low for the side reaction in this part of the investigation.) As attempted, the spectra showed the formation of a single species. The fixation of the 1-phosphate residue into a chelate ring became most directly apparent from the ³¹P-¹H coupling between the 1-phosphate residue and the methylene protons bonded to C1 in proton-coupled ³¹P NMR spectra of the Al-containing solutions. The coupling pattern of the 6-phosphate was in agreement with a non-chelating residue (triplet, 4.7 Hz).⁷ The signal of the 1-phosphate, however, occurred as a doublet of doublets (4.7 and 24.7 Hz) and shows, with these values, a particularly large deviation from non-chelating phosphate.

D-Fructose 6-phosphate: a dinucleating ligand

D-Fructose 6-phosphate, which consists mainly of 16.1% α - and 81.8% β -furanose at aqueous equilibrium (minor species: 2.2% open-chain keto form), resembles D-arabinose 5-phosphate in its C2/C6 part.⁵ The only difference is the hydroxymethyl function with C1 which substitutes H1 of the aldopentose analogue.



Scheme 9 Products of the reaction of Pd-en with D-fructose 6-phosphate. The symbols refer to Fig. 2.

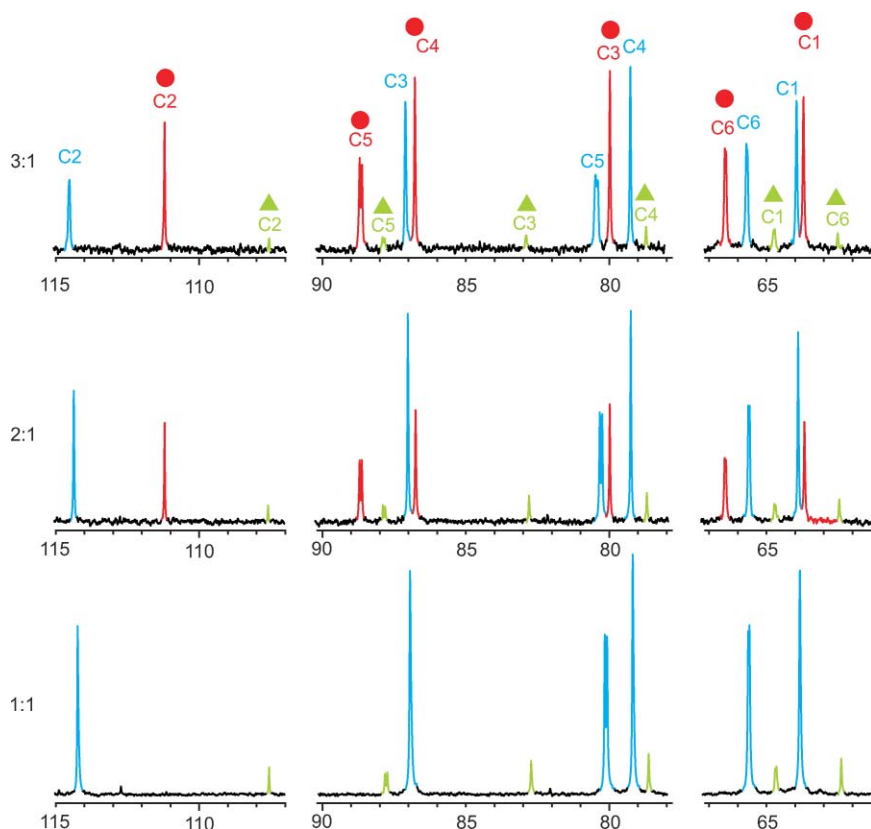


Fig. 2 ^{13}C NMR spectra of D-fructose 6-phosphate in Pd-en at molar Pd : glycose ratios of 3 : 1 (top), 2 : 1 (middle) and 1 : 1 (bottom). The symbols refer to Scheme 9. Note the splitted C5 and C6 signals as a result of ^{31}P - ^{13}C coupling.

Accordingly, the monometallation products shown in Scheme 9 were expected.

Since D-fructose 6-phosphate has four hydroxy functions at the glycose core, dimetallation seemed possible. In fact, variation of the molar ratio of palladium and glycose phosphate revealed a dimetallated product. Fig. 2, which is illustrative of the general strategy applied in this work to assure a given stoichiometry by the variation of the solution composition, shows in its bottom part—at an equimolar mixture of metal and glycose phosphate—the two signal sets that were expected for the monometallated analogues of the arabinose phosphate species. At a higher metal supply, a single dimetallated species gained significance. Its formula is depicted in Scheme 9 (right). This $[\text{Pd}_2(\text{en})_2(\alpha\text{-D-Fruf}6\text{P}H_4\text{-}\kappa\text{O}^{1,3} : \kappa\text{O}^{2,4})]^{2-}$ ion was the major species at metal excess (61% at a 3 : 1 molar ratio of palladium and glycose phosphate). The conformation of the

furanose ring was close to 3E . The major species in an equimolar solution was the $[\text{Pd}(\text{en})(\beta\text{-D-Fruf}6\text{P}2,3\text{H}_2\text{-}\kappa\text{O}^{2,3})]^{2-}$ ion (90%), the minor species the $[\text{Pd}(\text{en})(\alpha\text{-D-Fruf}6\text{P}2,4\text{H}_2\text{-}\kappa\text{O}^{2,3,4})]^{2-}$ ion (10%). The furanose conformation of the monometallated species is between 4E and 4T_3 for the former, close to 3E for the latter. Chemical shifts for all three compounds are given in Table 2. It should be noted that the CIS values of the dimetallated species reflect the unusual coordination pattern of two internested six-membered chelate rings. This motif is unprecedented as is the CIS pattern.

D-Glucose 6-phosphate and D-mannose 6-phosphate

It may be stated as an interim result that a phosphate residue reduces the number of potential ligands of a parent glycose if it

substitutes a hydroxy function at the glucose core which is needed for semiacetal formation. Hence, in all cases discussed so far, only furanose ligands had to be considered since the terminal hydroxy function of the respective glucose is essential for pyranose formation but was blocked by the phosphate residue. This is not the case for the aldohexose 6-phosphates of D-glucose and D-mannose which have both their 5- and 4-hydroxy functions free for pyranose and furanose formation. However, only first results on the ligand behaviour can be reported herein for the aldohexose 6-phosphates due to technical reasons. Among the glucose phosphates of this study, aldohexose 6-phosphate solutions in Pd-en are, by far, the most unstable with respect to palladium(II) reduction. We were able, hence, to record NMR spectra of metal-excess solutions (3:1 molar ratio of Pd and glucose phosphate), but no data are available for equimolar solutions. Keeping in mind these restrictions, the interpretation of the available data was straightforward due to the close similarity of the parent aldohexoses and their 6-phosphates.

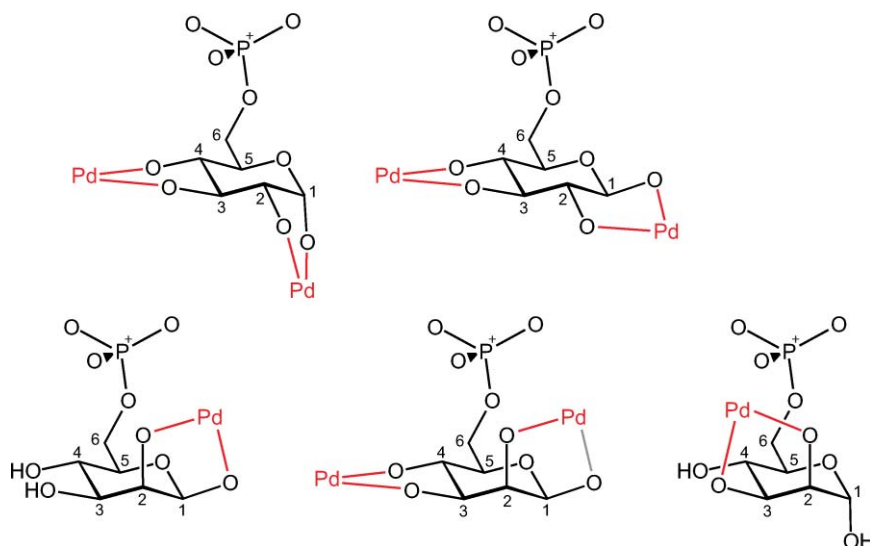
A D-glucose 6-phosphate solution thus resembles, with its equilibrium composition of 36% α - and 64% β -D-glucose 6-phosphate (from ^1H NMR spectra), the values of D-glucose (37.6% α - and 62% β -pyranose, 0.1% α - and 0.3% β -furanose).²¹ Metal excess conditions (3:1 Pd:glucose) in the Pd-chxn reagent transformed D-glucose to two dimetallated products, 65% $[\text{Pd}\{(R,R)\text{-chxn}\}(\alpha\text{-D-GlcpH}_4\text{-}\kappa\text{O}^{1,2}:\kappa\text{O}^{3,4})]$ and 35% of the respective β isomer (Scheme 10 and Table 2). Glucofuranose ligands were detectable as tentative minor species at a lower metal supply. The result for D-glucose 6-phosphate, dissolved in a threefold molar amount of Pd-en, was substantially the same. The two dimetallated species, $[\text{Pd}_2(\text{en})_2(\alpha\text{-D-Glcp6PH}_4\text{-}\kappa\text{O}^{1,2}:\kappa\text{O}^{3,4})]$ (75%) and the β analogue (25%) are depicted in Scheme 10. As for D-glucose, the additional negative charge on the O1 atom after deprotonation and bonding to palladium made the anomeric effect become more dominant, and the α anomer became enriched.

The anomeric effect dominated the metallation pattern of both D-mannose 6-phosphate and its parent glucose to a particularly

large extent due to its enhancement by the 2-axial hydroxy function of the $^4\text{C}_1$ -pyranose form. Thus the dominance of the α -pyranose in a D-mannose solution (62.2% α -D-Manp, 32.9% β -D-Manp, 0.6% α -D-Manf, 0.2% β -D-Manf) was shared by the 6-phosphate (71% α -D-Manp6P and 29% β -D-Manp6P from ^1H data).²¹ On dissolution in a threefold molar amount of Pd-en, the β -pyranose was the only anomer that was capable of forming a $\kappa\text{O}^{1,2}$ chelate due to the 2-axial substituent. However, an unusual $\kappa\text{O}^{2,3}$ ligand was also detected. Due to the anomeric effect, its 1-hydroxy function was in the α position (Scheme 10 and Table 2). The similarity with the parent glucose was recognisable. However, the dimetallated form appeared suppressed for the 6-phosphate. Thus, the three species in the bottom part of Scheme 10, $[\text{Pd}(\text{en})(\beta\text{-D-Manp6P1,2H}_2\text{-}\kappa\text{O}^{1,2})]^{2-}$, $[\text{Pd}_2(\text{en})_2(\beta\text{-D-Manp6PH}_4\text{-}\kappa\text{O}^{1,2}:\kappa\text{O}^{3,4})]^{2-}$, and $[\text{Pd}(\text{en})(\alpha\text{-D-Manp6P2,3H}_2\text{-}\kappa\text{O}^{2,3})]^{2-}$, occurred in a relative amount of 37:31:31, compared to 21:67:13 for D-mannose in Pd-chxn at the lower 2:1 molar ratio of Pd:glucose (3:1 solutions of D-mannose in Pd-chxn also exclusively showed the dimetallated species).¹ It should be noted that $\kappa\text{O}^{2,3}$ -bonded species has the same significance for both D-mannose and its 6-phosphate. In contrast, the $\kappa\text{O}^{2,3}$ -bonded ribofuranose ligand described above was found for the 5-phosphate but not for the parent glucose.

Conclusions

Glucose phosphates have been regarded as ligands for many decades. However, it was solely their phosphate function that appeared to provide the significant metal-binding site—even in the case of as strong a Lewis acid as the trivalent aluminium ion.⁹ Recent structural work on D-fructose-1,6-bisphosphate bonding to an acidic Zn(His)₃ centre in the active site of class-II aldolase, however, shifts the focus of interest to the metal-binding sites with glucose-core participation.^{15,16} We have demonstrated that for the metal centres Pd^{II}, Al^{III}, Ga^{III}, and Co^{III}, each of them bearing amine spectator ligands, in fact, two more bonding modes are accessible in aqueous solution: at a higher pH, the coordination of



Scheme 10 Products of the reaction of Pd-en with D-glucose 6-phosphate (top) and D-mannose 6-phosphate (bottom). All ligands were found as stable $^4\text{C}_1$ chairs.

solely the glucose core to the metal, and, close to the physiological pH, mixed glucose-core–phosphate chelation.

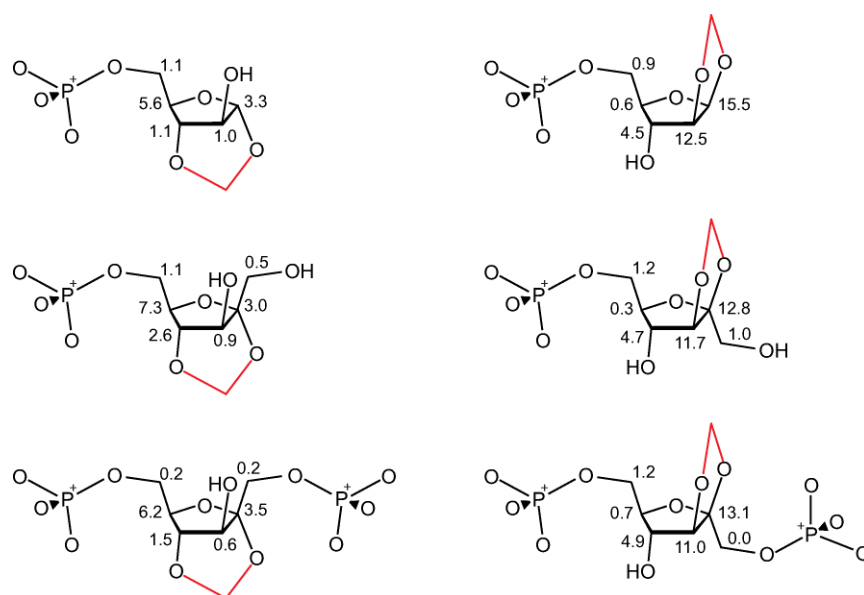
In order to optimise the glucose-core–metal interaction, we have taken advantage of a recently established concept. Reducing sugars are able to adapt their chelating functions to the requirements of a metal by adjusting both their conformation and configuration. Since the various isomers are interconvertible under the reaction conditions applied, they provide a dynamic ligand library to a metal. Among the sugar phosphates, important metabolic intermediates belong to this class of reducing glucose derivatives.

Most of them bear a phosphate function at the glucose's non-reducing terminus. Examples from this work are the aldopentose 5-phosphates and fructose 6-phosphate, where we find molecules whose δ position is phosphorylated and thus are restricted to the glucose's furanose form. In retrospect, the coordination chemistry of this subclass of reducing glucose phosphates is particularly straightforward since the phosphate residue is spatially well-separated from the glucose core. Hence, in either case we have found that the glucose core exhibits the typical coordination chemistry of the parent furanose. No mixed glucose-core–phosphate chelating has ever been observed. Taken together with the published knowledge, these ligands are expected to bind, for a given central metal, *via* their phosphate function at a low pH, and *via* their glucose core at a higher pH.

A similar separation of the phosphate function and the glucose core is given for the aldohexopyranose 6-phosphates, whose coordination chemistry is particularly close to that of the parent aldohexoses. It should be noted, however, that a closer vicinity of the phosphate group and a glucose-core hydroxy function is given for the respective furanoses that have a 5-hydroxy function adjacent to the phosphate moiety and thus might be able to establish seven-membered chelates of considerable stability. Due to the low thermal stability of the respective solutions, we were not able to further clarify this point by using a palladium-based probe.

The assumption that the seven-membered chelate rings of a mixed phosphate–glucose-core metal-binding site might be formed was substantiated for 1-phosphorylated ketoses. The most important biomolecule of this kind, D-fructose 1,6-bisphosphate, belongs to this special group of reducing glucose derivatives. On addition of the required equivalents of base, the glucose core of D-fructose 1,6-bisphosphate can be addressed as the only ligand. The same holds for a mixed glucose-core–phosphate chelation which is provoked by adding the required, smaller, equivalents of base. The latter solutions adjusted to pH values of about 8 (Pd) or 10 (Al, Ga) when the mixed bonding mode was attempted. A special and remarkable detail with respect to recent aldolase-related work should be noted for Pd^{II} chelation. The almost neutral solutions in which a mixed glucose-core–phosphate chelator was detected, contained this species not as the major component as the M^{III}(tacn) systems do. The major species in pH 8 Pd-en is the same as that of an alkaline solution, namely the only-glucose-core-bonding 2,4-furanose ligand.

A final methodological comment on the various metals might be given. The trivalent metals have been shown to use the dynamic ligand library provided by a reducing glucose or its derivatives in the sense that usually one isomer is bonded and enriched. Palladium(II), however, is different. As has been shown for the glycoses themselves, the Pd^{II} centre forms chelates of similar stability with practically all of the expected ligands, and thus displays the entire library. Using probes such as the Pd^{II}(en) fragment thus results in the accumulation of many spectral data and the possibility to correlate them. Scheme 11 shows, as an example, such a data collection which serves as an additional check of the assignments made. Cross-checking CIS values is one method of comparing NMR data. The same close relationships are obtained with the chemical shifts directly. In Scheme 11, the D-arabinofuranose core is the common partial structure that is also present in the D-fructofuranose derivatives of this work. It is



Scheme 11 CIS values of furanose ligands on bonding to palladium(II) *via* six- and five-membered chelate rings: D-arabinose 5-phosphate (top), D-fructose 6-phosphate (middle), and D-fructose 1,6-bisphosphate (bottom).

obvious that the CIS values of carbon atoms in a related chemical environment are similar.

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