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Mixed sugar-core–phosphate chelation of D-fructose 1,6-bisphosphate with the $Re^{V}O$(tmen) metal fragment†

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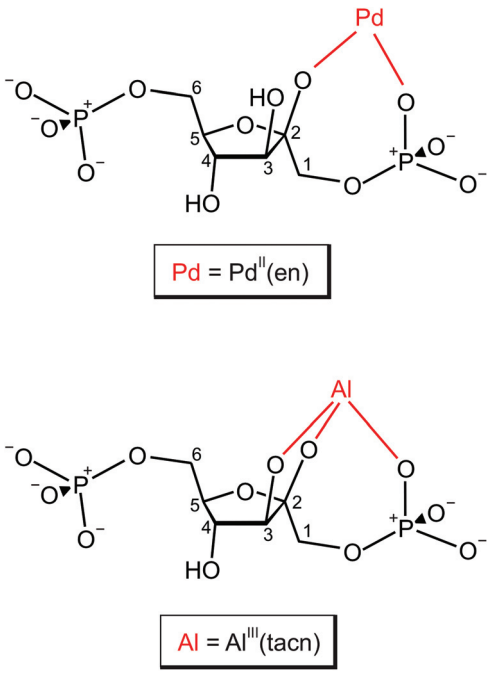
Sugar phosphates provide metal-binding sites both at their sugar core and at their phosphate group(s). Mixed sugar-core–phosphate chelation has been considered as a typical bonding mode within the physiological pH range for the central metabolite D-fructose 1,6-bisphosphate. The $Re^{V}O$(tmen) metal fragment was used to enrich this coordination type. The formation of the [ReO(tmen)(Fruf2,3H$_{-2}$1,6-P_2H_2-$\kappa^3O^{2,3,P1}$)]$^-$ monoanion was determined by NMR spectroscopy and mass spectrometry. The model compound *rac*-glycerol 1-phosphate yielded similar results in terms of NMR spectroscopy. Crystal-structure analyses of [ReO(tmen)(*rac*-Glyc2,3H$_{-2}$1*P*H-$\kappa^3O^{2,3,P}$)]·2H_2O and [ReO(phen)(*rac*-Glyc2,3H$_{-2}$1*P*H-$\kappa^3O^{2,3,P}$)]·MeOH confirmed the coordination pattern.

Introduction

Sugar phosphates play an important role in all organisms. They are ubiquitous in metabolic pathways such as the pentose phosphate pathway or glycolysis. Some reactions of these pathways are catalysed by metalloenzymes that contain divalent metal centres, namely magnesium,[1,2] iron[3] or zinc,[4–8] in their active sites. The dications are assumed to become chelated by the sugar–phosphate substrates during the catalysed reaction. Examples for such metalloenzymes include class-II aldolase,[4,5] ribulose 1,5-bisphosphate carboxylase[1,2] and glucose 6-phosphate isomerase.[6] Crystal-structure analyses of enzymes loaded with their natural substrates are available for all these examples and show the sugar phosphate in its activated form. However, information on the rules of metal chelation by sugar phosphates in their stable state is sparse.

Depending on the pH value, various sugar phosphate bonding modes appear to be accessible. In the extreme cases of alkaline and acidic solutions, either the deprotonated hydroxy functions of the sugar core chelate the metal or only the phosphate groups coordinate, respectively.[9,10] Mixed sugar-core–phosphate chelation as a typical bonding mode close to the physiological pH has been considered particularly for the significant bioligand D-fructose 1,6-bisphosphate. Thus, Scheme 1 shows the mixed bonding mode as deduced from NMR spectra for the metal fragments Pd^{II}(en) and Al^{III}(tacn) (en = ethane-1,2-diamine, tacn = 1,4,7-triazacyclononane).[9]



Scheme 1 Mixed sugar-core–phosphate ligation of D-fructose 1,6-bisphosphate in the presence of the metal fragments Pd^{II}(en) and Al^{III}(tacn) as concluded from NMR-spectroscopic experiments.

In fact, this coordination pattern was found in the enzyme 3,4-dihydroxy-2-butanone-4-phosphate synthase.[8] However, no detailed analysis of this special coordination type outside an

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enzyme is available yet. Herein we describe the mixed sugar-core–phosphate chelation of D-fructose 1,6-bisphosphate and its model glycerol 1-phosphate using the metal fragments $Re^{V}O$(tmen) and $Re^{V}O$(phen) (tmen = *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine; phen = 1,10-phenanthroline). Many studies on the $Re^{V}O$ fragment have been conducted in the last few years,[11–13] most of them dealing with the medical significance of its complexes in the field of radiopharmacy. A few studies on $Re^{V}O$ complexes with sugars or sugar analogues are also available.[12,13]

On an experimental basis, we used recently published findings on the NMR-spectroscopic trace of $Re^{V}O$–carbohydrate chelation.[12–15] Specifically, the Re^{V} centre entails a marked 'coordination-induced shift' (CIS) of about 20 ppm to the ^{13}C NMR signals of those carbon atoms bound to Re-coordinating oxygen atoms.[12] Taken together with structural information derived from $^{3}J_{H,H}$ and $^{3}J_{P,H}$ coupling constants as well as X-ray results on model compounds, the mixed sugar-core–phosphate chelation of a metal centre by a sugar phosphate was comprehensively analysed.

Experimental

Methods and materials

All chemicals were purchased and used without further purification: ammonium perrhenate (ABCR), triphenylphosphane (Acros), *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine (Sigma-Aldrich), 1,10-phenanthroline (Aldrich), deuterium oxide, methanol-d_4 (Eurisotop), trisodium D-fructose 1,6-bisphosphate octahydrate (Applichem), disodium *rac*-glycerol 1-phosphate hydrate (TCI Europe), triethylamine (Riedel-de Haën), acetone, ethanol, methanol (Fluka).

NMR spectroscopy

NMR spectra were recorded at 4 °C on Jeol ECX400/ECP 400 (^{1}H: 400 MHz; $^{13}C\{^{1}H\}$: 100 MHz; ^{31}P: 109 MHz) spectrometers. Shift differences are given as $\delta(C_{complex}) - \delta(C_{free\ sugar})$. The values for the free glycose phosphate or glycerol phosphate were taken from measurements in D_2O and were thus in neutral aqueous solution.

Species assignment

The ^{1}H, $^{31}P\{^{1}H\}$ and $^{13}C\{^{1}H\}$ NMR signals were assigned by ^{1}H–^{1}H COSY45, ^{1}H–^{13}C HMQC, and ^{31}P–^{1}H-HETCOR experiments in D_2O. To assign these signal sets to individual species, first of all, coupling constants *J* were analysed by applying a modified Karplus relationship to identify the correct anomer.[16] Afterwards, CIS values were used to assign the correct chelation site.

Syntheses

Preparation of *trans*-[$ReOCl_3(PPh_3)_2$]. The preparation was based on a published procedure.[17] Ammonium perrhenate (2.68 g, 10.0 mmol) and concentrated hydrochloric acid (20 mL) were heated in ethanol (100 mL) under reflux until the perrhenate was dissolved. After adding a solution of triphenylphosphane (15.74 g, 60.00 mmol) in hot ethanol (40 mL at 60 °C), the clear solution immediately turned into a green-yellow suspension. After 1/2 h of reflux, a yellow solid of *trans*-[$ReOCl_3(PPh_3)_2$] was filtered off and washed twice with hot ethanol and acetone. The yield was 7.52 g (90%). Anal. calcd for $C_{36}H_{31}Cl_3OP_2Re$: C, 51.90; H, 3.63; Cl, 12.77. Found: C, 52.02; H, 3.66; Cl, 12.35.

Preparation of Na[ReO(tmen)(β-D-Fru2,3H$_{-2}$1,6P_2H_2-κ$^3O^{2,3,P1}$)] (Na-1). Trisodium D-fructose 1,6-bisphosphate octahydrate (176 mg, 0.50 mmol), *trans*-[$ReOCl_3(PPh_3)_2$] (418 mg, 0.50 mmol), *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine (75.0 μL, 0.50 mmol), and NEt_3 (208 μL, 1.50 mmol) in 250 mL methanol were stirred at RT for 48 hours, yielding a clear blue solution. After the solvent was removed, the blue residue of Na-**1** was washed with acetone and dissolved in methanol-d_4 for NMR investigation.

Preparation of [ReO(tmen)(*rac*-Glyc2,3H$_{-2}$1*P*H-κ$^3O^{2,3,P}$)]·2H_2O (2·2H_2O). Disodium *rac*-glycerol 1-phosphate hydrate (108 mg, 0.50 mmol), *trans*-[$ReOCl_3(PPh_3)_2$] (418 mg, 0.50 mmol), *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine (75.0 μL, 0.50 mmol), and NEt_3 (139 μL, 1.00 mmol) in 250 mL methanol were stirred at RT for 48 hours, yielding a clear blue solution. After the solvent was removed, the blue residue was dissolved in acetone (40 mL). Colourless crystals of by-products such as triphenylphosphane were formed within 1 hour at RT. After the colourless crystals were removed by filtration, blue crystals of **2**·2H_2O were formed together with a small amount of further colourless crystals of by-products within 5 hours at RT. The crystals were dissolved in D_2O for NMR investigation. The yield of the mixture of blue and colourless crystals was 75 mg (*ca*. 5 mg of by-product). Due to the fact that the blue product crystals were compounded with a small amount of colourless crystals of by-products, an elementary analysis was not conducted.

Preparation of [ReO(phen)(*rac*-Glyc2,3H$_{-2}$1*P*H-κ$^3O^{2,3,P}$)]·MeOH (3·MeOH). Disodium *rac*-glycerol 1-phosphate hydrate (108 mg, 0.50 mmol), *trans*-[$ReOCl_3(PPh_3)_2$] (418 mg, 0.5 mmol), 1,10-phenanthroline (90.1 mg, 0.5 mmol), and NEt_3 (138.6 μL, 1.0 mmol) in 250 mL methanol were stirred at RT for 48 hours, yielding a clear yellow-brown solution. After the solvent was removed, the brown residue was washed with acetone and completely redissolved in methanol (80 mL). Green crystals of **3**·MeOH were formed together with a small amount of colourless crystals of by-products such as triphenylphosphane within 2 weeks at 4 °C. The yield of the mixture of green and colourless crystals was 71 mg (*ca*. 5 mg of by-product). Due to the fact that the green product crystals were compounded with a small amount of colourless crystals of by-products, an elementary analysis was not conducted.

Analytical data of product complexes. Na-**1**: ^{1}H NMR (CD_3OD): δ [ppm] = 5.32–5.53 (m, 3H, H5, H6a, H6b), 5.41 (t, 1H, H4, $^{3}J_{3,4}$ 6.7 Hz, $^{3}J_{4,5}$ 7.3 Hz), 5.77 (dd, 1H, H1a, $^{3}J_{1a,P}$ 23.8 Hz, $^{2}J_{1a,1b}$ 11.2 Hz), 5.99 (dd, 1H, H1b, $^{3}J_{1b,P}$ 7.9 Hz), 6.11 (d, 1H, H3). MS (FAB^+): *m*/*z* = 656.8 $[M - Na + 2H]^+$, 678.7 $[M + H]^+$, 700.7 $[M + Na]^+$, 757.8 $[M - Na + 2H + NEt_3]^+$, 772.8

$[M - Na + 2H + tmen]^+$. MS (FAB^-): m/z = 676.8 $[M - H]^-$, 654.8 $[M - Na]^-$, 560.7 $[M - H - tmen]^-$, 538.7 $[M - Na - tmen]^-$.

2·2H_2O: ^1H NMR (D_2O): δ [ppm] = 3.91 (dd, 1H, H3a, $^3J_{2,3}$ 6.2 Hz, $^2J_{3a,3b}$ 10.3 Hz), 4.56 (d, 1H, H3b), 4.61–4.76 (m, 3H, H1a, H1b, H2). MS (FAB^+): m/z = calculated for $C_9H_{23}O_7N_2P\,^{185}Re$ $[M + H]^+$: 487.0773, found 487.0726, calculated for $C_9H_{23}O_7N_2P^{187}Re$ $[M + H]^+$: 489.0801, found 489.0803.

3·MeOH: MS (FAB^+): m/z = calculated for $C_{15}H_{15}O_7N_2P^{185}Re$ $[M + H]^+$: 551.0147, found 551.0163, calculated for $C_{15}H_{15}O_7N_2P^{187}Re$ $[M + H]^+$: 553.0175, found 553.0220.

Results and discussion

The experimental setup was chosen to mimic the tridentate bonding mode of D-fructose 1,6-bisphosphate as found in its complex with the Al^{III}(tacn) moiety (Scheme 1). Since the Re^VO fragment provides five vacant ligand-binding sites to complete the octahedral structure of the target complex, two of the five positions were blocked by a spectator ligand. Preliminary tests revealed the bidentate nitrogen chelator *N*,*N*,*N*′,*N*′-tetramethylethane-1,2-diamine (tmen) to be well-suited. The reaction of *trans*-[$ReOCl_3(PPh_3)_2$], tmen and D-fructose 1,6-bisphosphate in methanol as the solvent and NEt_3 as the base to bind the protons liberated from the sugar core's hydroxy functions led to a clear blue solution. With the solvent removed, a blue residue was obtained and analysed by mass spectroscopy. After the residue was redissolved in methanol-d_4, a blue solution was obtained and analysed by NMR spectroscopy. The ^{31}P NMR spectra are shown in Fig. 1. The ^{13}C and ^{31}P NMR chemical shifts of the resulting complex are given in Table 1.

The CIS values and the $^3J_{H,H}$ coupling constants clearly proved the coordination of β-D-fructofuranose 1,6-bisphosphate to the Re^VO(tmen) fragment through the O2 and O3 atoms of the sugar core. Hence, the CIS values of about 21 and 28 ppm were close to the range of the CIS values for diolato Re^VO complexes of methyl glycosides (19–26 ppm).[12] Moreover, the coordination of the phosphate group at C1 was validated in a ^1H-coupled ^{31}P NMR spectrum. Here, the P1 signal was found as a doublet of doublets with ^{31}P–^1H coupling constants of 23.8 and 7.9 Hz. The great difference between these two constants verified that the phosphate group was in a fixed position due to the coordination to the Re^VO(tmen) fragment. In contrast, the ^1H-coupled ^{31}P NMR spectrum of the educt showed a triplet with ^{31}P–^1H coupling constants between 3 and 5 Hz for P1 indicating the free rotation of the phosphate group. Furthermore, phosphate coordination was proven by a CIS value of −3.7 ppm for the ^{31}P NMR signal of P1. These ^{31}P-NMR-spectroscopic findings were in agreement with the results on mixed sugar-core–phosphate chelates of D-fructose 1,6-bisphosphate with Al^{III}(tacn) (CIS value of ^{31}P NMR signal: −6.7 ppm; ^{31}P–^1H coupling constants: 24.4 and 4.7 Hz).[9]

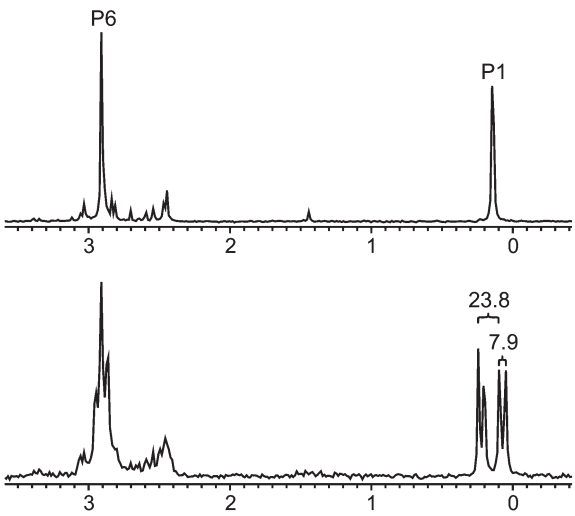
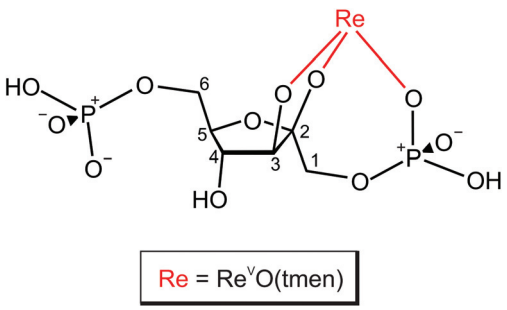


Fig. 1 The $^{31}P\{^1H\}$ NMR spectrum (top) and the ^{31}P NMR spectrum (bottom) of the blue residue obtained by evaporating the reaction solution containing the product complex anion [ReO(tmen)(Fru*f*2,3H$_{-2}$1,6P_2H$_2$-κ$^3O^{2,3,P1}$)]$^-$ (**1**) in methanol-d_4 with ^{31}P–^1H NMR coupling constants ($^3J_{P,H}$/Hz).

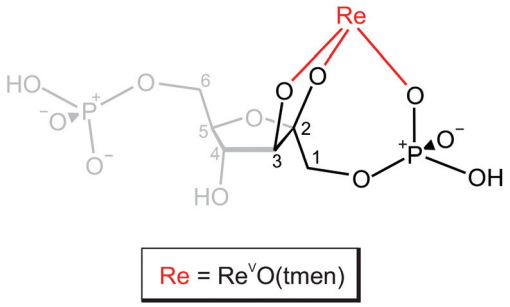
Table 1 ^{13}C and ^{31}P NMR chemical shifts (δ/ppm); $\Delta\delta$ is the difference of a shift in the presence of the metal probe and the free D-fructose 1,6-bisphosphate or glycerol 1-phosphate, respectively. Bold-printed $\Delta\delta$ values indicate the metal-binding site.

		C1	C2	C3	C4	C5	C6	P1	P6
1	δ	72.5	122.6	104.1	75.9	85.2	67.6	0.1	2.9
	$\Delta\delta$	6.5	**21.1**	**28.1**	1.4	5.1	2.2	**−3.7**	−0.8
2	δ	73.0	90.6	87.6				0.2	
	$\Delta\delta$	7.7	**18.6**	**24.7**				**−5.7**	

In fact, the reaction of *trans*-[$ReOCl_3(PPh_3)_2$] with tmen and *rac*-glycerol 1-phosphate in methanol–NEt_3 led to a clear blue solution as well. With the solvent removed, a blue residue was obtained and redissolved in acetone. After colourless crystals of PPh_3 had been removed, blue crystals of [ReO(tmen)(*rac*-Glyc2,3H$_{-2}$1*P*H-κ$^3O^{2,3,P}$)]·2H_2O (**2**·2H_2O) were formed (Fig. 2). Crystal-structure analysis (Table 2) showed that the lengths of the Re–N and Re–O bonds were in the same range as the bond



Scheme 2 The formula of **1**.



Scheme 3 Glycerol 1-phosphate and fructose 1,6-bisphosphate chelation in complexes with the $Re^{V}O$(tmen) fragment.

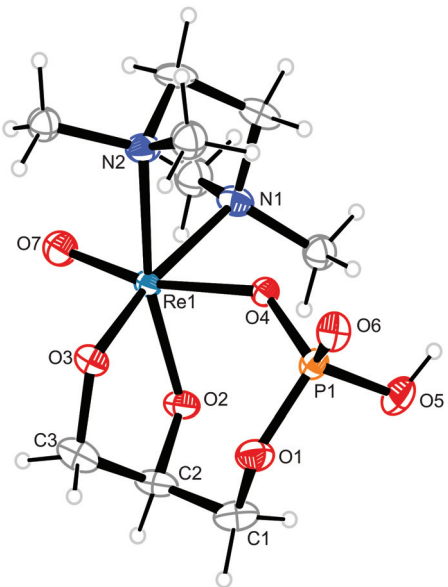


Fig. 2 Molecular structure of [ReO(tmen)(*rac*-Glyc2,3H$_{-2}$1*P*H-κ$^3O^{2,3,P}$)] (**2**) in crystals of the dihydrate (50% probability ellipsoids). Distances (Å) and angles (°): from Re1 to: O2 1.941(3), O3 1.954(3), O4 2.067(3), O7 1.678(3), N1 2.230(4), N2 2.224(4); from P1 to: O1 1.587(3), O4 1.528(3), O5 1.558(3), O6 1.483(3); O2–Re1–O3 82.26(13), O2–Re1–O4 85.24(12), O3–Re1–O4 88.40(12), O2–Re1–O7 106.18(14), O3–Re1–O7 101.51(14), N1–Re1–N2 81.69(15); O2–C2–C3–O3: −37.9(5). The O5H group forms a short hydrogen bond (O5···O91 2.522(5)) to one of the water molecules.

lengths found in crystal structures of methyl glycoside–$Re^{V}O$ complexes.[12] Regarding the Re–O(phosphate) bond, one should note that no other crystal structure containing a phosphate group coordinating to a $Re^{V}O$ centre is available yet. The only similar crystal structure available was the one of a diester of phosphoric acid coordinating to a $Re^{V}O$ centre.[18] Here, the Re–O^P distance amounted to 1.988(4) Å, shorter than the value determined for **2**.

In the crystals of **2**·2H_2O, the phosphate group was monoprotonated just as the phosphate groups in the fructose 1,6-bisphosphate complex were in terms of mass spectrometry. Mass-spectrometric analysis of **2** confirmed the crystallographic result by showing signals only for the mono-protonated form. Solutions of the blue crystals of **2** in D_2O (pH ~ 5) were investigated by NMR spectroscopy. The ^{13}C NMR

Table 2 Crystal-structure analyses

	2·2H_2O	**3**·MeOH
Net formula	$C_9H_{26}N_2O_9PRe$	$C_{16}H_{18}N_2O_8Pre$
M_r/g mol^{-1}	523.492	583.504
Crystal size/mm	0.13 × 0.10 × 0.03	0.11 × 0.07 × 0.02
T/K	173(2)	173(2)
Radiation	Mo-Kα	Mo-Kα
Diffractometer	KappaCCD	Oxford XCalibur
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
a/Å	14.1114(5)	10.2979(5)
b/Å	7.5592(5)	9.0749(5)
c/Å	16.5961(5)	19.4763(7)
β(°)	111.0465(5)	91.399(3)
V/Å^3	1652.22(13)	1819.56(14)
Z	4	4
D_c/g cm^{-3}	2.10454(17)	2.13006(16)
μ/mm^{-1}	7.496	6.816
Absorption correction	Multi-scan	Multi-scan
Transmission factor range	0.411–0.799	0.570–0.873
Refls measured	27 719	6341
R_{int}	0.0501	0.0837
Mean $\sigma(I)/I$	0.0330	0.1187
θ range	3.15–27.61	4.18–26.31
Observed refls	3372	2717
x, y(weighting scheme)	0.0029, 6.6091	0.0093, 0
Refls in refinement	3808	3673
Parameters	220	258
Restraints	6	1
$R(F_{obs})$	0.0267	0.0474
$R_w(F^2)$	0.0549	0.1017
S	1.089	0.938
Shift/error$_{max}$	0.001	0.001
Max electron density/e Å^{-3}	1.834	2.571[a]
Min electron density/e Å^{-3}	−1.116	−2.382

[a] The maximum electron density is located at a distance of 0.98 Å to Re.

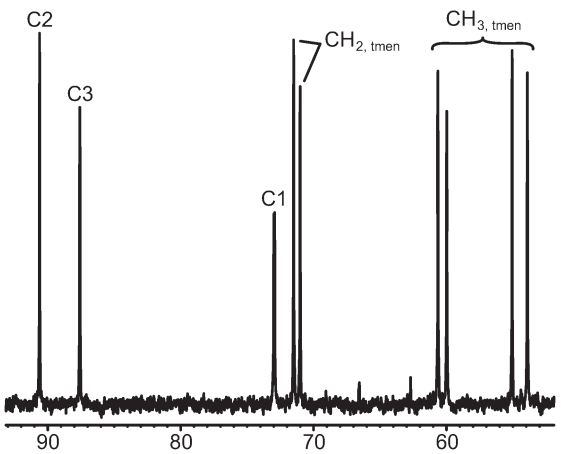


Fig. 3 ^{13}C NMR spectrum of [ReO(tmen)(*rac*-Glyc2,3H$_{-2}$1*P*H-κ$^3O^{2,3,P}$)] (**2**) in D_2O measured at 4 °C.

spectrum is shown in Fig. 3. The ^{13}C and ^{31}P NMR chemical shifts are given in Table 1.

The CIS values found were in agreement with the crystallographically determined κ$^2O^2$,O^3 coordination of glycerol 1-phosphate to the $Re^{V}O$(tmen) moiety. The coordination of the phosphate group was characterised by a doublet of doublets in

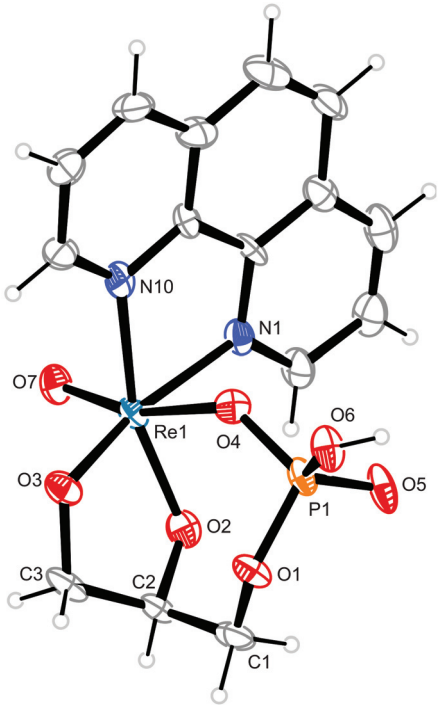


Fig. 4 Structure of [ReO(phen)(*rac*-Glyc2,3H$_{-2}$1*P*H-κ^3*O*^{2,3,P})] (**3**) in crystals of the methanol adduct (50% probability ellipsoids). Distances (Å) and angles (°): from Re1 to: O2 1.917(6), O3 1.957(6), O4 2.102(6), O7 1.690(6), N1 2.129(6), N10 2.158(7); from P1 to: O1 1.583(6), O4 1.527(6), O5 1.490(7), O6 1.558(7); O2–Re1–O3 82.7(3), O2–Re1–O4 88.2(2), O3–Re1–O4 85.5(2), O2–Re1–O7 106.8(3), O3–Re1–O7 103.8(3), N1–Re1–N10 77.7(3); O2–C2–C3–O3 −32.2(9). Centrosymmetric dimers form *via* two short hydrogen bonds (O6···O5^{i} and O6^{i}···O5 2.495(8); ^{i}−*x*, −*y*, −*z*) in a carbonic-acid-dimer-typical way.

the ^{1}H-coupled ^{31}P NMR spectrum with ^{31}P–^{1}H coupling constants of 23.8 and 6.9 Hz. Furthermore, the phosphate coordination caused a CIS value for the ^{31}P NMR signal of −5.7 ppm. The similarity of these values with the results of the D-fructose 1,6-bisphosphate experiments is obvious.

Further evidence that the mixed sugar-core–phosphate bonding mode can be reliably addressed was obtained with 1,10-phenanthroline (phen) as the spectator ligand. Though phen proved unsuitable in terms of the solubility of the obtained products as a prerequisite for NMR analysis, another crystal-structure analysis succeeded (Table 2). Hence, green crystals of [ReO(phen)(*rac*-Glyc2,3H$_{-2}$1*P*H-κ^3*O*^{2,3,P})]·MeOH (**3**·MeOH) were isolated from a solution of *trans*-[ReOCl$_3$(PPh$_3$)$_2$] with phen, *rac*-glycerol 1-phosphate in methanol–NEt$_3$ (Fig. 4).

Again, the phosphate group was found to be mono-protonated by high-resolution mass spectroscopy. Furthermore, the similarity of the two crystal structures was obvious in terms of bond lengths and angles (Fig. 2, Fig. 4).

Conclusions

Mixed sugar-core–phosphate chelation may be considered as an initial bonding mode of D-fructose 1,6-bisphosphate to a metal centre close to the physiological pH. The metrical and spectroscopic characteristics of the tridentate mode of this bonding type were analysed by X-ray diffraction, various NMR techniques and mass spectrometry by using Re^{V}O(N$_2$) metal fragments as the probes (N$_2$ = tmen, phen). Experiments with the D-fructose 1,6-bisphosphate model *rac*-glycerol 1-phosphate confirmed the extension of the stable five-membered chelates as found in analogous methyl-glycoside complexes by seven-membered, phosphate-containing chelate rings. Hence, **2** and **3** are the first crystalline complexes that feature this special coordination pattern. The close similarity of the NMR-spectroscopic and mass-spectrometric results on both D-fructose 1,6-bisphosphate and glycerol 1-phosphate chelates confirms the similarity of the metal centres' bonding situation in both environments.

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