Attachment of Sugar Phosphates and Nucleotide Derivatives to Pyrazolylborate-Zinc Units

Karl Weis, Michael Rombach, and Heinrich Vahrenkamp*

Institut für Anorganische und Analytische Chemie der Universität Freiburg, Albertstrasse 21, D-79104 Freiburg, Germany

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Phosphate derivatives which model intermediates of enzymatic glycoside and nucleotide transformations were reacted with the hydrolytically active zinc-hydroxide species Tp*Zn−OH (Tp* = 3,5-disubstituted tris(pyrazolyl)-borates). Depending on the substrate or the reaction conditions, either phosphate ester cleavage or deprotonation of a nucleobase NH was observed. As a result, the Tp*Zn units were attached to the phosphate part or/and to the nucleobase part of the substrate. Nucleobase adducts were found of three uridine monophosphate derivatives. Phosphate adducts were found of a hydroxyacetone monophosphate, of a ribose phosphate, and of uridine and *N*-methyluridine monophosphates. The products were identified by crystallographic and spectroscopic methods as tetrahedral Tp*Zn−X complexes. Crystals of $Tp^{Cum,Me}Zn-OPO(OC_6H_4NO_2)$(*O*-hydroxyacetone dimethyl ketal) are monoclinic, space group $P2_1/n$, with $a = 13.221(1)$ Å, $b = 13.738(1)$ Å, $c = 30.303(3)$ Å, $\beta = 99.57(1)°$, $Z = 4$; those of $Tp^{Cum,Me}Zn-OPO(OC_6H_4NO_2)$(*O*-2,3-isopropylidene-5-methylribose) are monoclinic, space group $P2_1$, with $a = 16.179(6)$, Å, $b = 16.206(5)$ Å, $c = 21.192(14)$ Å, $\beta = 101.02(5)°$, $Z = 2$; those of $Tp^{Pic,Me}Zn-(H_2O)-OPO(OC_6H_4NO_2)$(*O*-2,3-isopropylidene-5-methylribose) are triclinic, space group $P1$, with $a = 13.106(4)$ Å, $b = 13.676(5)$ Å, $c = 17.021(6)$ Å, $\alpha = 84.10(3)°$, $\beta = 88.37(3)°$, $\gamma = 62.43(3)°$, $Z = 1$.

Biomolecules bearing phosphate groups are involved in a large number of enzymatic transformations. These include not only phosphate transfer itself (e.g., from and to nucleotides, lipids, phosphotriesters, sugars, and inorganic oligophosphates) but also the metabolism of the phosphate-bearing substrates (e.g., glycosides, lipids, peptides, and inositol).[1] Quite often the enzymes effecting the transformations are metalloenzymes,[2] as exemplified by phosphatases[3] and aldolases.[4] The metals involved are typically divalent (Mg, Mn, Fe, Zn), and among them zinc plays an importante role.[5]

The biological importance of phosphate transfer, specifically by zinc enzymes, has attracted many coordination chemistry research groups. Numerous mechanistic studies[6] and model complexes[7] have been published. Similar studies related to the metabolism of phosphate-bearing substrates have been less popular among chemists so far. Thus the important mimicking of enediolate transition states in glycoside transformations,[8] e.g., by hydroxamate inhibitors,[9] is still mostly a subject of structural and medicinal biology.[10] Similarly, the coordination chemistry of sugars and sugar derivatives is an underdeveloped area,[11] and we are not aware of any work on the coordination chemistry of sugar phosphates.

We have been engaged in some aspects of the biomimetic coordination chemistry of zinc which are related to these topics. We have applied encapsulation of the metal ion by tripodal ligands to stabilize monodentate phosphate ligands.[12,13] We have made use of the hydrolytic strength of the pyrazolylborate−zinc−hydroxide complexes to cleave phosphoric acid esters and diphosphates.[14,15] We have modeled transition state analogues of glycosidic interconversions using ketoalcoholate and hydroxamate ligands,[16] and we have studied the zinc coordination

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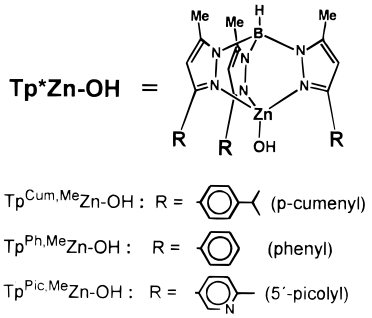
chemistry of RNA precursors and analogues thereof.[17] When phosphate or carboxylate was present in the "substrate" ligands, it was used for binding to zinc, but the phosphate-free ketoalcohols or RNA precursors used their typical donor atoms.

This paper reports on the extension of our work. In order to make the substrate models which are to be attached to zinc more natural we chose sugar phosphates and phosphate species derived thereof. The sugar of choice was ribose, a typical degradation species was hydroxyacetone, and a typical nucleotide constituent was uridine in the form of UMP derivatives. They all offer various donor functions for attachment to zinc, and the zinc−hydroxide complexes $Tp^{Cum,Me}Zn-OH$, $Tp^{Ph,Me}Zn-OH$, and $Tp^{Pic,Me}Zn-OH$ to be reacted with them could be expected to either cleave their aryl phosphate functions or deprotonate their nucleobase N−H functions.



Experimental Section

General. All experimental techniques and the standard IR and NMR equipment were as described previously.[18] The Tp*Zn−OH complexes,[19−21] bis(*p*-nitrophenyl) chlorophosphate,[22] hydroxyacetone dimethyl ketal,[23,24] 2,3-isopropylidene-5-methylribose,[25] 2′,3′-isopropylideneuridine,[26] and 2′,3′-isopropylidene-*N*-methyluridine[27] were prepared according to the published procedures. All other starting materials were obtained commercially. The solvents used were dried according to standard laboratory procedures. For the abbreviations used and for the numbering scheme, see Results and Discussion.

All *p*-nitrophenyl phosphate derivatives used as substrates were prepared from bis(*p*-nitrophenyl) chlorophosphate and the corresponding sugar derivative following procedures identical to those developed by Pfleiderer[28] and Eckstein.[29] They were fully identified. Their analytical and spectroscopic characterization is given in the Supporting Information.

Preparations. Complex 1. A solution of $Tp^{Cum,Me}Zn-OH$ (500 mg, 0.722 mmol) in dichloromethane (40 mL) was treated with AP (160 mg, 0.362 mmol) and the mixture stirred for 16 h. The solvent was removed in vacuo. The yellowish residue was suspended in ethanol (25 mL) and treated with ultrasound for 20 min, leaving $Tp^{Cum,Me}Zn-OC_6H_4NO_2$ as a precipitate. After filtration the solvent was again removed in vacuo and the residue crystallized from acetonitrile/methanol/dichloromethane (5:1:2) at 4 °C, yielding **1** (230 mg, 64%) as colorless crystals, mp 162 °C. IR (KBr, cm^{-1}): 2558 m (BH), 1551 m, 1344 s (NO), 1261 s (PO). ^1H NMR ($CDCl_3$, δ): 0.96 [s, 3H, Me(ha)], 1.08 [d, ^3J = 6.9 Hz, 18H, Me(iPr)], 2.46 [s, 9H, Me(pz)], 2.75 [spt, ^3J = 6.9 Hz, 3H, H(iPr)], 2.88 [s, 6H, OMe], 3.34 [d, ^3J = 5.1 Hz, 2H, CH_2], 6.16 [s, 3H, H(pz)], 6.68 [d, ^3J = 9.2 Hz, 2H, Nit-(2,6)], 7.25 [d, ^3J = 8.1 Hz, 6H, Ph(3,5)], 7.54 [d, ^3J = 8.1 Hz, 6H, Ph(2,6)], 7.75 [d, ^3J = 9.2 Hz, 2H, Nit(3,5)]. ^{31}P NMR ($CDCl_3$, δ): −11.86 (t, J = 5.1 Hz). Anal. Calcd for $C_{50}H_{61}BN_7O_8PZn\cdot{}^1/_2CH_3OH$ (M_r = 1011.3): C, 59.98; H, 6.28; N, 9.70. Found: C, 59.86; H, 6.17; N, 9.57.

Complex 2a. $Tp^{Cum,Me}Zn-OH$ (500 mg, 0.722 mmol) and RP (190 mg, 0.361 mmol) in dichloromethane (40 mL) were stirred for 16 h. The solvent was removed in vacuo and the yellowish precipitate suspended in ethanol (25 mL). After treatment with ultrasound for 20 min, the yellow residue of $Tp^{Cum,Me}Zn-OC_6H_4NO_2$ was filtered off, the filtrate evaporated to dryness, and the product crystallized from acetonitrile, yielding **2a** (330 mg, 85%) as colorless crystals, mp 176 °C. IR (KBr, cm^{-1}): 2548 m (BH), 1551 s, 1343 s (NO), 1254 s (PO). ^1H NMR ($CDCl_3$, δ): 1.17 [d, ^3J = 6.9 Hz, 18H, Me(iPr)], 1.19 [s, 3H, Me(Rib-iPr)], 1.39 [s, 3H, Me(Rib-iPr)], 2.53 [s, 9H, Me(pz)], 2.85 [spt, ^3J = 6.9 Hz, 3H, H(iPr)], 3.09 [s, 3H, Me(Rib)], 3.56 [dd, ^3J = 6.6 Hz, ^3J = 5.1 Hz, 1H, C5H(a)], 3.56 [dd, ^3J = 6.6 Hz, ^3J = 5.0 Hz, 1H, C5H(b)], 3.97 [dd, ^3J = 5.1 Hz, ^3J = 5.0 Hz, 1H, C4H], 4.32 [d, ^3J = 6.0 Hz, 1H, C3H], 4.40 [d, ^3J = 6.0 Hz, 1H, C2H], 4.83 [s, 1H, C1H], 6.28 [s, 3H, H(pz)], 6.67 [d, ^3J = 9.1 Hz, 2H, Nit(2,6)], 7.31 [d, ^3J = 8.1 Hz, 6H, Ph(3,5)], 7.61 [d, ^3J = 8.1 Hz, 6H, Ph(2,6)], 7.78 [d, ^3J = 9.1 Hz, 2H, Nit(3,5)]. ^{31}P NMR ($CDCl_3$, δ): −11.56 (t, J = 6.6 Hz). Anal. Calcd for $C_{54}H_{65}BN_7O_{10}PZn$ (M_r = 1079.4): C, 60.09; H, 6.07; N, 9.09. Found: C, 59.96; H, 5.94; N, 9.13.

Complex 2b: as before from $Tp^{Ph,Me}Zn-OH$ (500 mg, 0.884 mmol) and RP (233 mg, 0.442 mmol). Yield: 308 mg (73%) of **2b** as colorless crystals, mp 164 °C. IR (KBr, cm^{-1}): 3300 m, br (H_2O), 2556 m (BH), 1547 s, 1344 s (NO), 1254 s (PO). ^1H NMR ($CDCl_3$, δ): 1.22 [s, 3H, Me(iPr)], 1.41 [s, 3H, Me(iPr)], 2.53 [s, 9H, Me(py)], 3.14 [s, 3H, H(me)], 3.51 [m, ^3J = 8.3 Hz, ^3J = 6.6 Hz, ^3J = 5.7 Hz, 2H, $C5H_2$], 3.98 [dd, ^3J = 8.3 Hz, ^3J = 6.6 Hz, 1H, C4H], 4.25 [d, ^3J = 6.0 Hz, 1H, C3H], 4.40 [d, ^3J = 6.0 Hz, 1H, C2H], 4.84 [s, 1H, C1H], 6.24 [s, 3H, H(pz)], 6.62 [d, ^3J = 9.0 Hz, 2H, Nit(2,6)], 7.25 [m, 3H, Ph(4)], 7.42 [m, 6H, Ph(3,5)], 7.63 [m, 6H, Ph(2,6)], 7.88 [d, ^3J = 9.0 Hz, 2H, Nit(3,5)]. ^{31}P NMR ($CDCl_3$, δ): −11.94 (t, J = 5.7 Hz). Anal. Calcd for $C_{45}H_{47}BN_7O_{10}PZn\cdot H_2O$ (M_r = 971.1): C, 55.66; H, 5.09; N, 10.10. Found: C, 54.98; H, 5.08; N, 10.83.

Complex 2c: as before from $Tp^{Pic,Me}Zn-OH$ (500 mg, 0.818 mmol) and RP (215 mg, 0.409 mmol). Yield: 281 mg (70%) of **2c** as colorless crystals, mp 118 °C, which were freed from cocrystallized solvent by prolonged pumping. IR (KBr, cm^{-1}): 3430 m, br (H_2O), 2536 m (BH), 1547 m, 1342 s (NO), 1179 s (PO). ^1H NMR ($CDCl_3$, δ): 1.20 (s, 3H, Me(iPr)], 1.39 [s, 3H, Me(iPr)], 1.98 [s, 3H, CH_3(acetonitrile)], 2.42 [s, 9H, Me(py)], 2.55 [s, 9H, Me(pz)], 3.13 [s, 3H, H(me)], 3.55 [m, ^3J = 6.7 Hz, 2H, $C5H_2$], 4.00 [m, 1H, C4H], 4.35 [d, ^3J = 5.9 Hz, 1H, C3H], 4.44 [d, ^3J = 5.9 Hz, 1H C2H], 4.84 [s, 1H, C1H], 6.28 [s, 3H, H(pz)], 6.95 [d, ^3J = 9.1 Hz, 2H, Nit(2,6)], 7.23 [d, ^3J = 8.0 Hz, 3H, Py(5)], 7.93 [dd, ^3J = 8.0 Hz, ^4J = 2.2 Hz, 3H, Py(6)], 8.01 [d, ^3J = 9.1 Hz, 2H, Nit(3,5)], 8.60 [d, ^4J = 2.2 Hz, 3H, Py(2)]. ^{31}P NMR ($CDCl_3$, δ): −10.59 (t, J = 6.7 Hz). Anal. Calcd for $C_{45}H_{50}BN_{10}O_{10}PZn\cdot H_2O$ (M_r = 1016.1): C, 53.19; H, 5.16; N, 13.78. Found: C, 53.16; H, 4.90; N, 12.97.

Complex 5. $Tp^{Cum,Me}Zn-OH$ (500 mg, 0.772 mmol) and H−URP (156 mg, 0.257 mmol) in dichloromethane (50 mL) were stirred for 16 h. The solvent was removed in vacuo and the residue suspended in ethanol (25 mL). After treatment with ultrasound for 20 min, the remaining insoluble $Tp^{Cum,Me}Zn-OC_6H_4NO_2$ was filtered off. The filtrate was evacuated to dryness and the residue extracted six times with 30 mL each of pentanes. The combined organic phases were kept at −25 °C for 1 week, after which **5** (297 mg, 63%) remained as a colorless powder, mp 152 °C. IR (KBr, cm^{-1}): 2546 m (BH), 1676 m, 1647 s (CO), 1550 m, 1343 s (NO), 1277 m (PO). ^1H NMR ($CDCl_3$, δ): 1.11 [s, 3H, Me(Rib-iPr)], 1.12 [d, ^3J = 6.9 Hz, 18H, Me(a)(iPr)], 1.16 [d, ^3J = 6.9 Hz, 18H, Me(b)(iPr)], 1.26 [s, 3H, Me(Rib-iPr)], 2.49 [s, 9H, Me(a)(pz)], 2.53 [s, 9H, Me(b)(pz)], 2.79 [spt, ^3J = 6.9 Hz,

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3H, H(a)(iPr)], 2.81 [spt, ^3J = 6.9 Hz, 3H, H(b)(iPr)], 3.35 [m, ^3J = 6.1 Hz, 1H, C5′H(a)], 3.49 [m, ^3J = 6.1 Hz, 1H, C5′H(b)], 3.65 [m, 1H, C4′H], 3.81 [m, 1H, C3′H], 4.03 [m, 1H, C2′H], 4.97 [m, 1H, C1′H], 5.02 [d, ^3J = 7.8 Hz, 1H, C6H], 6.13 [s, 3H, H(a)(pz)], 6.23 [s, 3H, H(b)(pz)], 6.59 [d, ^3J = 7.8 Hz, 1H, C5H], 6.61 [d, ^3J = 9.0 Hz, 2H, Nit(2,6)], 7.10 [d, ^3J = 8.0 Hz, 6H, Ph(a)(3,5)], 7.26 [d, ^3J = 8.1 Hz, 6H, Ph(b)(3,5)], 7.49 [d, ^3J = 8.0 Hz, 6H, Ph(a)(2,6)], 7.59 [d, ^3J = 8.1 Hz, 6H, Ph(b)(2,6)], 7.87 [d, 3J = 9.0 Hz, 2H, Nit(3,5)]. ^{31}P NMR ($CDCl_3$, δ): −11.92 (t, J = 6.1 Hz). Anal. Calcd for $C_{96}H_{110}B_2N_{15}O_{11}PZn_2$ (M_r = 1833.5): C, 62.89; H, 6.05; N, 11.46; Zn, 7.13. Found: C, 62.25; H, 6.01; N, 11.34; Zn, 6.85.

Complex 3a: as before from $Tp^{Cum,Me}$Zn−OH (500 mg, 0.722 mmol) and Me−URP (224 mg, 0.361 mmol) in dichloromethane (40 mL). Yield: 305 mg (72%) of **3a** as a colorless powder, mp 114 °C. IR (KBr, cm^{-1}): 2555 m (BH), 1715 m, 1673 s (CO), 1550 m, 1344 s (NO), 1251 m (PO). ^{1}H NMR ($CDCl_3$, δ): 1.14 [d, ^3J = 6.9 Hz, 18H, Me(iPr)], 1.22 [s, 3H, Me(Rib-iPr)], 1.46 [s, 3H, Me(Rib-iPr], 2.55 [s, 9H, Me(pz)], 2.80 [spt, ^3J = 6.9 Hz, 3H, H(iPr)], 3.19 [s, 3H, NCH_3], 3.38 [m, 1H, C5′H_2], 3.77 [m, 1H, C4′H], 4.12 [m, 1H, C3′H], 4.29 [m, ^3J = 2.8 Hz, 1H, C2′H], 4.94 [d, ^3J = 8.1 Hz, 1H, C6H], 5.62 [d, ^3J = 2.8 Hz, 1H, C1′H], 6.22 [s, 3H, H(pz)], 6.83 [d, ^3J = 8.1 Hz, 1H, C5H], 6.90 [d, ^3J = 9.0 Hz, 2H, Nit(2,6)], 7.28 [d, ^3J = 7.8 Hz, 6H, Ph(3,5)], 7.58 [d, ^3J = 7.8 Hz, 6H, Ph(2,6)], 7.93 [d, ^3J = 9.0 Hz, 2H, Nit(3,5)]. ^{31}P NMR ($CDCl_3$, δ): −11.98 (broad singlet). Anal. Calcd for $C_{58}H_{67}BN_9O_{11}PZn$ (M_r = 1173.4): C, 59.37; H, 5.76; N 10.75; Zn, 5.57. Found: C, 58.65; H, 5.73; N, 11.43; Zn, 5.58. MW: 1173.2 (ESI-MS).

Complex 3c: as before from $Tp^{Pic,Me}$Zn−OH (500 mg, 0.818 mmol) and Me−URP (254 mg, 0.409 mmol). Recrystallizations from 10 mL of acetonitrile and 15 mL of hexane/dichloromethane (3:11) at 0 °C yielded **3c** (464 mg, 52%) as a colorless polycrystalline material, mp 101 °C. IR (KBr, cm^{-1}): 2547 m (BH), 1716 s, 1675 m (CO), 1546 s, 1345 s (NO), 1251 s (PO). ^{1}H NMR ($CDCl_3$, δ): 1.27 [s, 3H, Me(Rib-iPr)], 1.49 [s, 3H, Me(Rib-iPr)], 2.41 [s, 9H, Me(py)], 2.58 [s, 9H, Me(pz)], 3.12 [s, 3H, NCH_3], 3.79 [m, 2H, C5′H_2], 4.02 [m, ^3J = 4.0 Hz, 1H, C4′H], 4.53 [dd, ^3J = 6.3 Hz, ^3J = 4.0 Hz, 1H, C3′H], 4.68 [dd, ^3J = 6.3 Hz, ^3J = 2.3 Hz, 1H, C2′H], 5.21 [d, ^3J = 8.0 Hz, 1H, C6H], 5.74 [d, ^3J = 2.3 Hz, 1H, C1′H], 6.30 [s, 3H, H(pz)], 7.07 [d, ^3J = 9.1 Hz, 2H, Nit(2,6)], 7.08 [d, ^3J = 8.0 Hz, 1H, C5H], 7.20 [d, ^3J = 8.0 Hz, 3H, Py(5)], 7.91 [dd, ^3J = 8.0 Hz, ^4J = 2.1 Hz, 3H, Py(6)], 8.03 [d, ^3J = 9.1 Hz, 2H, Nit(3,5)], 8.61 [d, ^4J = 2.1 Hz, 3H, Py(2)]. ^{31}P NMR ($CDCl_3$, δ): −9.68 (broad singlet). Anal. Calcd for $C_{49}H_{52}BN_{12}O_{11}PZn{\cdot}CH_2Cl_2$ (M_r = 1177.1): C, 51.02; H, 4.62; N, 14.28; Zn, 5.55. Found: C, 49.18; H, 4.77; N, 14.48; Zn, 5.17.

Complex 6. $Tp^{Cum,Me}$Zn−OH (500 mg, 0.722 mmol) and $(H-UR)_2P$ (271 mg, 0.361 mmol) in dichloromethane (40 mL) were stirred for 30 min. After filtration through a fine-porosity frit, the solution was evaporated to dryness, leaving analytically pure **6** (735 mg, 97%) as a colorless powder, mp 145 °C. IR (KBr, cm^{-1}): 2543 m (BH), 1675 m, 1649 s (CO), 1520 s, 1346 m (NO), 1294 m (PO). ^{1}H NMR ($CDCl_3$, δ): 1.21 [d, ^3J = 6.9 Hz, 18H, Me(a)(iPr)], 1.22 [d, ^3J = 6.9 Hz, 18H, Me(b)(iPr)], 1.27 [s, 6H, Me(Rib-iPr)], 1.36 [s, 3H, Me(a)(Rib-iPr)], 1.39 [s, 3H, Me(b)(Rib-iPr)], 2.51 [s, 18H, Me(pz)], 2.86 [spt, ^3J = 6.9 Hz, 6H, H(iPr)], 3.73 [m, ^3J = 8.6 Hz, 4H, C5′H_2], 3.97 [m, 2H, C4′H], 3.97 [m, 2H, C3′H], 4.32 [m, 2H, C2′H], 4.82 [s, 2H, C1′H], 5.13 [d, ^3J = 7.6 Hz, 1H, C6(a)H], 5.16 [d, ^3J = 7.6 Hz, 1H, C6(b)H], 6.13 [s, 6H, H(pz)], 6.62 [d, ^3J = 7.6 Hz, 1H, C5(b)H], 6.63 [d, ^3J = 7.6 Hz, 1H, C5(a)H], 6.96 [d, ^3J = 8.7 Hz, 2H, Nit(2,6)], 7.12 [d, ^3J = 8.1 Hz, 18H, Ph(3,5)], 7.48 [d, ^3J = 8.1 Hz, 18H, Ph(2,6)], 7.68 [d, ^3J = 8.7 Hz, 2H,Nit(3,5)]. ^{31}P NMR ($CDCl_3$, δ): −7.78 (q, J = 8.6 Hz). Anal. Calcd for $C_{108}H_{124}B_2N_{17}O_{16}PZn_2$ (M_r = 2099.7): C, 61.78; H, 5.95; N, 11.34; Zn, 6.23. Found: C, 60.99; H, 6.02; N, 11.08; Zn, 6.34.

Structure Determinations. Crystals of **1**, **2a**, and **2c** were obtained from the recrystallizations described above. They were sealed in glass capillaries, in the cases of **1** and **2c** together with a drop of the cocrystallized solvent. Diffraction data were recorded at 200 K with the $\omega/2\theta$ technique on a Nonius CAD4 diffractometer fitted with a molybdenum tube (Kα, λ = 0.7107 Å) and a graphite monochromator. No absorption corrections were applied. The structures were solved with direct methods and refined anisotropically with the SHELX program suite.[30] Hydrogen atoms were included with fixed distances and isotropic temperature factors 1.2 times those of their attached atoms. Parameters were refined against F^2. Drawings were produced with SCHAKAL.[31] Table 1 lists the crystallographic data.

Table 1. Crystallographic Details

	1	**2a**	**2c**
formula	$C_{50}H_{61}BN_7O_8PZn{\cdot}{}^1/_2CH_3OH$	$C_{54}H_{65}BN_7O_{10}PZn$	$C_{45}H_{50}BN_{10}O_{10}PZn{\cdot}H_2O{\cdot}2CH_3CN$
MW	1011.2	1079.3	1098.3
space group	$P2(1)/n$	$P2(1)$	$P1$
Z	4	2	1
a (Å)	13.221(1)	16.179(6)	13.106(4)
b (Å)	13.738(1)	16.206(5)	13.676(5)
c (Å)	30.303(3)	21.192(14)	17.021(6)
α (deg)	90	90	84.10(3)
β (deg)	99.569(7)	101.02(5)	88.37(3)
γ (deg)	90	90	62.43(3)
V (Å^3)	5427.7(9)	5454(5)	2689.4(2)
d(calc) [g·cm^{-3}]	1.24	1.31	1.36
d(obs) [g·cm^{-3}]	1.26	1.28	1.32
μ(Mo Kα) [mm^{-1}]	0.539	0.543	0.555
R1 (obs reflns)[a]	0.071	0.061	0.049
wR2(all reflns)[b]	0.279	0.331	0.144

[a] $R1 = \sum|F_o - F_c|/\sum F_o$. [b] $wR2 = [\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2]^{1/2}$.

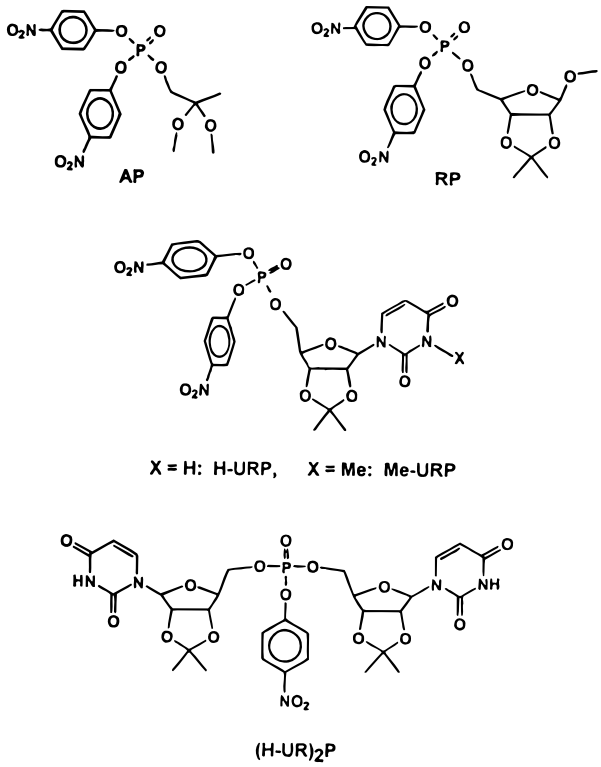
Results and Discussion

Substrates. All substrates used for reactions with the Tp*Zn−OH complexes were phosphate derivatives of sugars or sugar constituents. Preliminary experiments had shown that the unprotected sugars, that is, those with free OH groups, lead to intractable product mixtures. Therefore all of their O and OH functions were made inert by conversion to ether or acetal units. Thus the substrates had left one or two points of attack by the Tp*Zn−OH reagents: the *p*-nitrophenolate ester functions which are present in all substrates and additionally the nucleobase N−H functions in those substrates which are nucleotide derivatives.

The simplest substrate chosen, AP, is a distant relative of dihydroxyacetone phosphate. The latter results from aldolase-catalyzed degradation of ketoses to aldoses,[4,32] and its metal ion binding properties have been studied by Sigel et al.[33] We had already shown that free hydroxyacetone binds as a O,O-chelate ligand to the Tp*Zn unit.[16] Its O protection and phosphate functionalization were meant to allow modeling of the alternative mode of substrate attachment to zinc in the enzyme, that is, via zinc−phosphate interaction.[4,32] For the second substrate, RP, a simple ribose derivative was chosen. It has the phosphate units attached as in the ribonucleotides but all of its OH functions protected by ether units bearing only methyl substituents for the sake of easy NMR detection.

The remaining substrates are ribonucleotide derivatives. H−URP, a UMP analogue, bears the intended double functionality in the uridine N−H and phosphate ester units. Accordingly, Me−URP, the N-methylated derivative of H−URP, is only monofunctional again. Finally, $(H-UR)_2P$, a derivative of the non-natural bis(uridine) monophosphate, is trifunctional (two NH, one OR).

(30) Sheldrick, G. M. SHELXS-86 and SHELXL-93, Universität Göttingen, 1986 and 1993.
(31) Keller, E. SCHAKAL for Windows, Universität Freiburg 1997.
(32) Mildvan, A. S.; Kobes, R. D.; Rutter, W. J. *Biochemistry* **1971**, *10*, 1191.
(33) Liang, G.; Chen, D.; Bastian, M.; Sigel, H. *J. Am. Chem. Soc.* **1992**, *114*, 7780.



Exclusive Phosphate Ester Cleavage. All substrates which have their functionality localized in the *p*-nitrophenyl phosphate units alone did react with Tp*Zn−OH quantitatively at this function. As observed before for simple aryl phosphates,[7a,14] 2 equiv of the zinc−hydroxide complex are consumed and 1 equiv of H_2O is liberated. The *p*-nitrophenol which is hydrolytically removed from the phosphate becomes attached to a Tp*Zn unit by condensation, forming a Tp*Zn−phenolate complex. This reaction is faster than the hydrolytic cleavage, which means that less than 2 equiv of Tp*Zn−OH leads to incomplete consumption of the starting substrate. The substrate derivative resulting from the hydrolytic cleavage remains attached to the Tp*Zn unit as a monodentate phosphate ligand. The reactions are unambiguous, that is, the ester function linking the sugar moiety with the phosphate unit remains untouched.

Substrate AP was reacted with $Tp^{Cum,Me}Zn$−OH, yielding complex **1**. The ribosyl phosphate RP was treated with all three Tp*Zn−OH reagents, producing complexes **2a**, **2b**, and **2c**. The N-protected uridine monophosphate Me−URP was converted by $Tp^{Cum,Me}Zn$−OH and $Tp^{Pic,Me}Zn$−OH into complexes **3a** and **3c**. In all cases the yields were good. Due to the alkylation of all OH and NH functions the compounds are completely hydrophobic and quite soluble in nonpolar organic solvents. This holds even for **2b** and **2c**, which were isolated as hydrates (see below). In the annotation chosen for the product complexes, the symbol P′ denotes the phosphate residue remaining after removal of one $OC_6H_4NO_2$ fragment, that is, AP → $OC_6H_4NO_2$ + AP′ and 2 Tp*Zn−OH + AP → Tp*Zn−$OC_6H_4NO_2$ + AP′−O−ZnTp* + H_2O.

$$\underset{\mathbf{1}}{AP'-O-ZnTp^{Cum,Me}}$$

RP′−O−$ZnTp^{R',Me}$	Me−URP′−O−$ZnTp^{R',Me}$
2a: R′ = *p*-cumenyl	**3a**: R′ = *p*-cumenyl
2b: R′ = phenyl	**3c**: R′ = 5′-picolyl
2c: R′ = 5′-picolyl	

The product complexes could be identified unambiguously by their spectra; see Experimental Section. Specifically in the ^1H NMR spectra the simple patterns of the AP′, RP′, and Me−URP′ units are present in addition to those of the Tp* ligands. Due to the embedding of the sugar phosphates between the aromatic substituents of the Tp* ligands, their ^1H NMR resonances show the upfield shifts of 0.6−1 ppm which are typical for this bonding situation.[17,19] The ^{31}P NMR resonances appear as triplets due to coupling between phosphorus and the OCH_2 protons. The IR spectra and elemental analyses of **2b** and **2c** indicate the presence of one water molecule. On the basis of our experience with Tp*Zn complexes of simpler phosphates,[14,15] we assign a coordination position at zinc to these water molecules, thereby making the zinc ion five-coordinate in a trigonal-bipyramidal environment. The identification of complexes **1**−**3** was made complete by the structure determinations of **1**, **2a**, and **2c** (see below) and by an ESI mass spectrum of **3a** showing the parent peak.

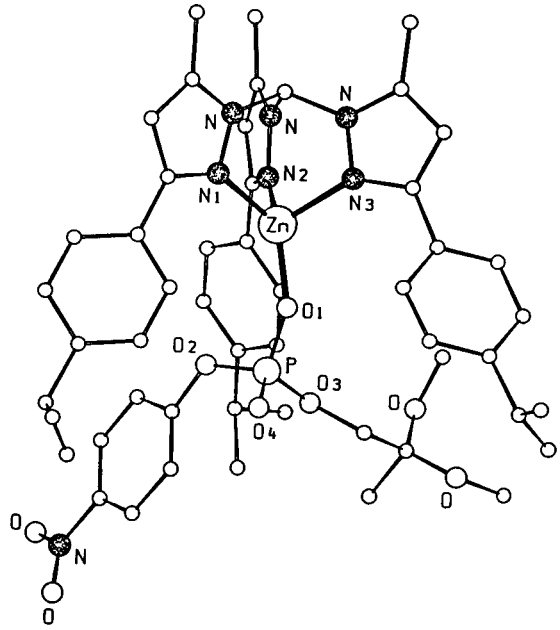


Figure 1. Molecular structure of AP′−O−$ZnTp^{Cum,Me}$ (**1**). Important bond lengths (Å) and angles (°): Zn−O1 1.862(5), Zn−N1 2.018(5), Zn−N2 2.030(5), Zn−N3 2.030(5), O1−P 1.476(5), P−O2 1.601(5), P−O3 1.574(5), P−O4 1.452(5); O1−Zn−N1 126.9(2), O1−Zn−N2 125.6(2), O1−Zn−N3 112.2(2), N1−Zn−N2 94.1(2), N1−Zn−N3 94.6(2), N2−Zn−N3 95.6(2), Zn−O1−P 154.2(3).

The structure determination of **1** (see Figure 1) suffered from a disorder problem of the acetone−dimethyl ketal fragment which had to be overcome by fixing its C−C and C−O distances at the expense of some very high thermal parameters. Otherwise the molecule is well-defined, including a methanol solvate molecule on a partially occupied position linked by a hydrogen bond to the P═O oxygen. The coordination of the zinc ion is distorted tetrahedral with the Zn−O bond displaced from the trigonal axis of the Tp* ligand toward N3. There is much precedence for this type of Tp*Zn−OX coordination and the structural features of Tp*Zn−phosphate linkage including the large Zn−O−P angle.[7a,14−17,19] It remains worth mentioning that the formation of **1** has created a chiral phosphorus center but the crystals are racemic. With respect to modeling the bonding situation of dihydroxyacetone phosphate in the active center of aldolases, the structure of **1** represents the original proposal of zinc−phosphate ligation[4a,32] while the structure of $Tp^{Cum,Me}Zn$−hydroxyacetonate[16] represents the alternative proposal of enediolate-like O,O-chelation.[4b,c]

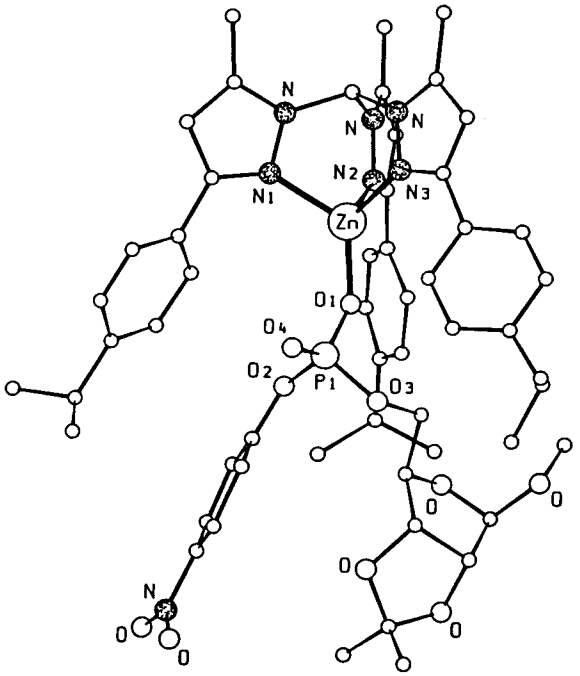


Figure 2. Molecular structure of RP′−O−ZnTp^{Cum,Me} (diastereomer with *S* configuration at phosphorus). Important bond lengths (Å) and angles (°): Zn−O1 1.88(1), Zn−N1 2.03(2), Zn−N2 2.04(2), Zn−N3 2.06(2),O1−P1 1.49(1), P1−O2 1.62(2), P1−O3 1.57(2), P1−O4 1.48-(2); O1−Zn−N1 125.1(7), O1−Zn−N2 119.1(6), O1−Zn−N3 120.3-(7), N1−Zn−N2 92.9(7), N1−Zn−N3 96.5(7), N2−Zn−N3 96.0(7), Zn−O1−P1 142.1(1).

The structure determination of **2a** (see Figure 2) revealed the non-centrosymmetric space group $P2_1$ with two independent molecules per asymmetric unit. These two molecules are the two diastereomers of **2a** with *R* and *S* configuration at phosphorus. The configurations were deduced from the known configuration of the ribosyl unit. Figure 2 shows the *S*-diastereomer. The bond lengths and angles around zinc and phosphorus are equal within 3 standard deviations for both molecules. The deviation of the Zn−O axis from the trigonal axis of the Tp* ligand is less pronounced than in **1**, and the Zn−O−P angle is less widened. But these variations fall within the range observed for the other Tp*Zn complexes with phosphate ligands.[7a,12−15] Structures of other zinc complexes with ligated ribosyl phosphates have been reported, for example, of ATP[34] and of cytidine 5′-phosphate.[35] They cannot be compared with the structure of **2a** because their nucleotide constituents use two or three P−O donors for the attachment to zinc, as do the majority of the phosphate units in the zinc−phosphate compounds structurally characterized so far.[36] In the compounds described here, the donor qualities of the phosphate diesters and the encapsulation by the Tp* ligands confine the phosphate ligands to a monodentate attachment. There is, however, no crowding in the vicinity of the zinc ion, as evidenced by the fact that among our complexes are several of the type Tp*Zn(L)(phosphate) with five-coordinate zinc bearing an additional ligand L.[14,15]

As deduced from the IR data, the molecular structure of **2c** (see Figure 3) shows five-coordinate zinc with the water ligand and one pyrazole nitrogen on the axial positions of a distorted

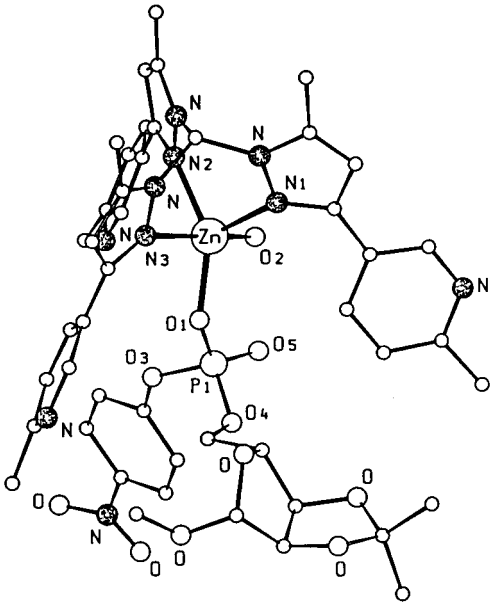


Figure 3. Molecular structure of RP′−O−ZnTp^{Pic,Me}·H_2O (diastereomer with *R* configuration at phosphorus). Important bond lengths (Å) and angles (°): Zn−O1 1.98(1), Zn−N1 2.08(1), Zn−N2 2.07(1), Zn−N3 2.19(1), Zn−O2 2.10(1), O1−P1 1.48(1), P1−O3 1.59(1), P1−O4 1.59(1), P1−O5 1.45(1); O2−Zn−N3 167.4(3), O2−Zn−O1 86.6(3), O2−Zn−N1 99.6(4), O2−Zn−N2 90.7(4), Zn−O1−P1 137.5(6).

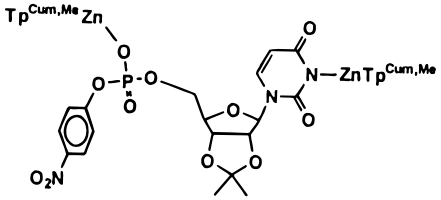
trigonal bipyramid. For the pyridine-substituted pyrazolylborates this type of zinc coordination seems to be the rule rather than the exception.[14,15,21] As for **2a**, the asymmetric unit of the crystals is composed of both diastereomers of the complex, whose bond lengths and angles do not differ significantly. The bonding features around zinc and phosphorus do not differ much from those discussed before for simpler Tp*Zn−phosphate complexes,[12−15] leading to the conclusion that the ribosyl unit has no structure-determining features here.

Zinc−Nucleobase Interactions. When the N-unprotected UMP derivative H−URP is reacted with Tp^{Cum,Me}Zn−OH, the first point of interaction is the uracil N−H function. The resulting complex **4** having zinc bound to N3 of the uracil moiety, which we have described before in the context of zinc−nucleobase interactions,[17] is very labile and can only be isolated when applying exactly a 1:1 stoichiometry and mild reaction conditions. Its thermal degradation leads to a reaction mixture containing complex **5**. **5** was isolated in good yields when 3 equiv of Tp^{Cum,Me}Zn−OH per equivalent of H−URP was applied. **5**, which is considerably more stable than **4**, contains Tp^{Cum,Me}Zn units attached to both termini of the nucleotide derivative.

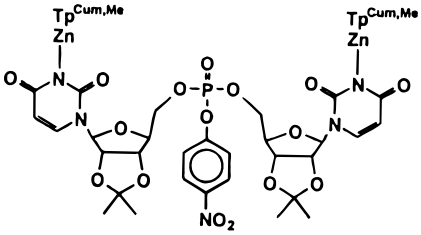
Tp^{Cum,Me}Zn−URP **4** Tp^{Cum,Me}Zn−URP′−O−ZnTp^{Cum,Me} **5**

In an attempt to attach three Tp*Zn units, the bis(nucleotide) derivative $(H−UR)_2P$ was reacted with Tp^{Cum,Me}Zn−OH. But irrespective of the stoichiometric ratio or the reaction temperature, the only isolable product was complex **6**. **6** has its two Tp*Zn units bound only to the two N3 nitrogens of uracil. Hydrolytic removal of the one remaining *p*-nitrophenolate group by Tp^{Cum,Me}Zn−OH has been found not to be possible, presumably for steric reasons. The formation of **6** confirms the observations made with **4** and **5**, namely, that in these nucleotides the hydrolytically active zinc compound has at least

(34) Orioli, P.; Cini, R.; Donati, D.; Mangani, S. *J. Am. Chem. Soc.* **1981**, *103*, 4446.
(35) Aoki, K. *Biochim. Biophys. Acta* **1976**, *447*, 379.
(36) The Cambridge Crystallographic Data File contains 70 structures of compounds with Zn−O−P linkages.



5



6

Figure 4. Proposed structures of complexes **5** and **6**.

a kinetic preference to attack the nucleobase rather than the phosphate.

$$\underset{\mathbf{6}}{Tp^{Cum,Me}Zn-UR-P-RU-ZnTp^{Cum,Me}}$$

Complexes **5** and **6** are again quite soluble in all organic solvents, which has so far prevented X-ray quality crystals from being obtained. Nevertheless their identification based on their spectroscopic data and previous experience is unambiguous. Their ^{1}H NMR data (see Experimental Section) show all expected resonances in the right positions and intensity ratios. For **5** two sets of $Tp^{Cum,Me}$ resonances are observed, for **6** one set with double intensity. The phosphate-attached part of **5** corresponds to complex **3a**, and the uracil-attached part corresponds to the isopropylideneuridine−$ZnTp^{Cum,Me}$ complex.[17] The ^{31}P NMR spectra show attachment of the phosphate to one ribosyl unit for **5** (triplet) and to two ribosyl units for **6** (quintet due to coupling with two equivalent OCH_2 units). Most informative are the IR spectra in the ν(CO) region which reflect the bonding situation of the uracil moiety. H−URP and $(H-UR)_2P$ show a single absorption at 1695 cm^{-1}. In complexes **4**, **5**, and **6** this absorption is split and lowered by 20 and 50 cm^{-1} to ca. 1675 and ca. 1648 cm^{-1}. In contrast the ν(CO) absorptions of Me−URP (1709 and 1665 cm^{-1}) appear almost unshifted in complexes **3a** (1715 and 1673 cm^{-1}) and **3c** (1716 and 1675 cm^{-1}), in accord with the nonquestionable attachment of zinc to phosphate in these complexes. For the sake of clarity Figure 4 shows the proposed molecular constitutions of **5** and **6** based on this information.

Conclusions

This work has confirmed the considerations on which it was based. The hydrolytic activity of the three Tp*Zn−OH complexes used is strong enough to cleave off one *p*-nitrophenolate from the sugar phosphate and nucleotide derivatives used as substrates. In all cases where this happens, it results in Tp*Zn−$OPO(OX)_2$ complexes of the corresponding sugar phosphate species. The hydrolytic cleavage occurs exclusively for the *p*-nitrophenolate and not for the ribosyl phosphate function.

In those cases where the substrates contain an unmasked nitrogen donor function in their nucleobases (H−URP and $(H-UR)_2P$), the first point of attachment to zinc is this nucleobase donor (via condensation between N−H and Zn−OH). Only subsequently does phosphate ester cleavage occur, and in the case of $(H-UR)_2P$ it is completely inhibited. With respect to glycoside and nucleotide transformations, this means that it cannot be stated a priori where and how the catalytically active metal ion of an enzyme interacts with the substrate.

All evidence obtained for these and related molecular zinc phosphate complexes of tripodal ligands points to the fact that the phosphate-containing substrate is bound to zinc in a strictly monodentate fashion, be it via its O(phosphate) or N(nucleobase) donor functions and irrespective of the coordination number of zinc. Again with respect to enzymatic catalysis this asks for a correlation with the fact that many hydrolytic, specifically phosphate-transferring, enzymes contain several metal ions.[3] One proposal resulting from our observations might be that the substrates, for example, nucleotides or RNA, need not only multiple activation of their phosphate parts but also or alternatively multiple attachment via their phosphate and/or nucleobase donors. Complex **5** may serve as an example for this.

In order to put our observations and conclusions on a firmer basis, this work has to be extended to other nucleotide derivatives and to be supported by mechanistic studies. Preliminary investigations indicate that the cleavage reactions work, though less cleanly, for AMP derivatives and that the ester hydrolysis is a bimolecular process, being first order in phosphate as well as in zinc−hydroxide complex.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We are obliged to Dr. M. Ruf for discussions and technical support and to Dr. W. Deck and B. Müller for measurements.

Supporting Information Available: Spectroscopic and analytical characterization of the five phosphate derivatives used as substrates and fully labeled ORTEP plots for **1**, **2a**, and **2c** (6 pages). Three crystallographic files, in CIF format, are available on the Internet only. Ordering and access information is given on any current masthead page.

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