

## ***Supplementary Information***

### **Xanthosine 5'-monophosphate (XMP). Acid-base and metal ion-binding properties of a chameleon-like nucleotide**

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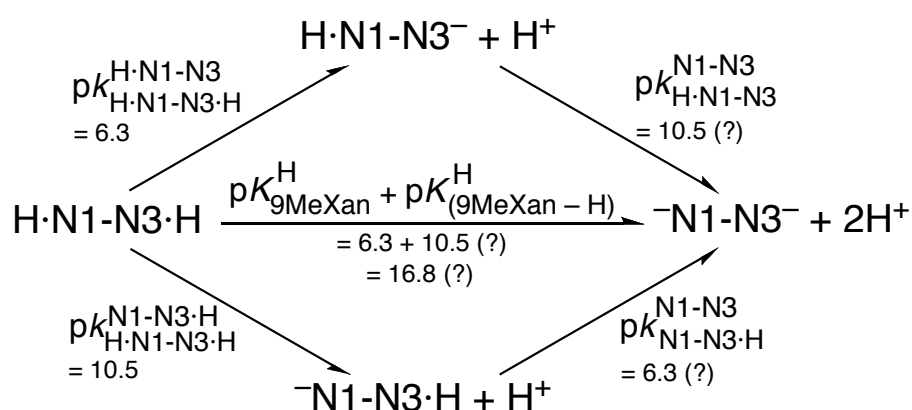
## S2.5 Evidence for intramolecular hydrogen bond formation in $(X - H \cdot MP \cdot H)^{2-}$ species

Furthermore, if the reasoning presented in the main text in Section 2.5 is correct, then the release of the proton from the  $P(O)_2(OH)^-$  group of  $(X - H \cdot MP \cdot H)^{2-}$  should be somewhat inhibited because this proton is partially involved in hydrogen bond formation with the N7/[(C6)O] site, and the extent of this inhibition should also allow an estimation for %  $(X - H \cdot MP \cdot H)_{cl}^{2-}$ . The acidity constant for this deprotonation,  $pK_{X - H \cdot MP \cdot H}^{X - H \cdot MP} = 6.40 \pm 0.04$  (see Fig. 4), needs to be corrected for the effect which the negatively charged xanthinate residue exercises on the release of the proton from the  $P(O)_2(OH)^-$  group in the open  $(X - H \cdot MP \cdot H)_{op}^{2-}$  species. However, the extent of this inhibition is difficult to assess, therefore we use the phosphate-monoprotonated nucleotide uridine 5'-monophosphate, i.e.  $H(UMP)^-$  with  $pK_{H(UMP)}^H = 6.15 \pm 0.01$ ,<sup>35</sup> and attribute in a first approximation half of the acidification of 0.25 ( $= 6.40 - 6.15$ ) to the charge effect and the other half to hydrogen bond formation which gives then  $K_{I/H} = 10^{0.125} - 1 = 0.33$  and %  $(X - H \cdot MP \cdot H)_{cl}^{2-} = 25\%$ . Moreover, comparison of  $pK_{XMP \cdot H}^{XMP} = 6.23 \pm 0.02 = pK_{H(NMP)}^H$ , where  $NMP^{2-} = AMP^{2-}$ ,  $IMP^{2-}$  and  $GMP^{2-}$  (see Fig. 4 and Table 1) with  $pK_{H(UMP)}^H = 6.15 \pm 0.01$  gives  $\Delta pK_a = 0.08 \pm 0.02$  and thus  $K_{I/H} = 10^{(0.08 \pm 0.02)} - 1 = 0.20 \pm 0.06$  and %  $(XMP \cdot H)_{cl} = \% H(NMP)_{cl} = 17 \pm 4\%$ . This is clearly again a lower limit for %  $(X - H \cdot MP \cdot H)_{cl}^{2-}$  because a negatively charged xanthinate residue is not only expected to increase somewhat the  $pK_a$  of the open isomer,  $(X - H \cdot MP \cdot H)_{op}^{2-}$  (cf. with equilibrium (22) in Section 3.5) but even more so to favor hydrogen bonding and thus the closed form (Fig. 5). Hence, taken together, all results suggest that in  $(X - H \cdot MP \cdot H)^{2-}$  the hydrogen of the  $P(O)_2(OH)^-$  group forms a hydrogen bond with the N7/[(C6)O] site, the formation degree being most likely in the order of 50%.

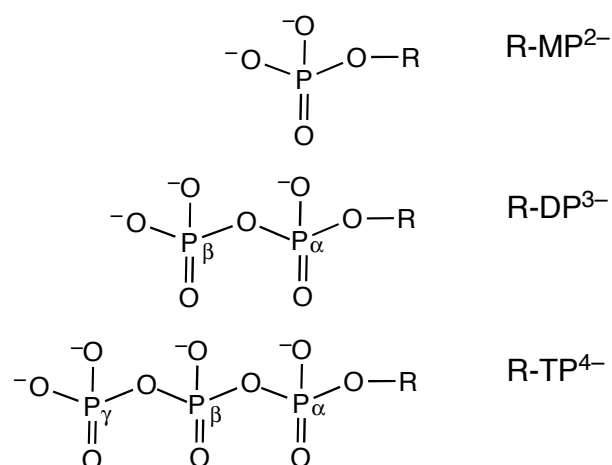
**Table S1** Summary of the various XMP species occurring in the pH range of about 0 to 12

| No <sup>a</sup> | Composition of the XMP species    | Location of the protons<br>(formation degrees in parentheses)  |
|-----------------|-----------------------------------|--|
| 1               | H <sub>3</sub> (XMP) <sup>+</sup> | (H•XMP•H <sub>2</sub> ) <sup>+</sup><br>(100%)   |
| 2               | H <sub>2</sub> (XMP)              | (H•XMP•H) <sup>±</sup> and (XMP•H <sub>2</sub> ) <sup>0</sup><br>(70%)                      (30%)    |
| 3               | H(XMP) <sup>-</sup>               | (XMP•H) <sup>-</sup> and (X – H•MP•H <sub>2</sub> ) <sup>-</sup><br>(100%)                      (0%) |
| 4               | XMP <sup>2-</sup>                 | (X – H•MP•H) <sup>2-</sup> and (XMP) <sup>2-</sup><br>(90%)                      (10%)               |
| 5               | (XMP – H) <sup>3-</sup>           | (X – H•MP) <sup>3-</sup><br>(100%)   |

<sup>a</sup> Comments: The situation regarding entries 1 and 5 is evident (Section 2.1). Regarding entry 2 see Fig. 3 and text in Section 2.3. Entry 3: because the primary proton of the P(O)(OH)<sub>2</sub> group is lost with pK<sub>a</sub> about 0.4 and since deprotonation of the xanthine residue occurs only with pK<sub>a</sub> about 5.3 (Table 1), the species (X – H•MP•H<sub>2</sub>)<sup>-</sup> does practically not form. Entry 4: rounded values from Fig. 4. Above pH 12 (XMP – 2H)<sup>4-</sup> may form due to deprotonation of the ribose residue or because both (N1)H/(N3)H sites lose their proton (see Section 2.1).



**Fig. S1** Micro acidity constant scheme for 9-methylxanthine (9MeXan), where H·N1-N3·H represents 9MeXan. Shown is the interrelation between the micro acidity constants ( $k$ ) and the macro acidity constants ( $K$ ) as well as the connection between H·N1-N3<sup>-</sup> and <sup>-</sup>N1-N3·H (see also Fig. 6) and the other species present. The arrows indicate the direction for which the acidity constants are defined. The inserted constants at the left of the scheme and at the horizontal arrow are from entries 4–6 of Table 2; the value of 10.5 for  $\text{p}K_{(9\text{MeXan} - \text{H})}^{\text{H}}$  appears to us as uncertain and is therefore given with a question mark (see also the comment to this value in footnote *c* of Table 2). However, this uncertainty has no effect on the calculations which are based on the values given at the left. Fig. S1 is a revised version of Fig. 5 in ref. 9 (*Chem. Eur. J.*) (2004); by permission of the copyright owner Wiley-VCH; Weinheim, Germany.



**Fig. S2** Chemical structures of simple monoesters of monophosphate (R-MP<sup>2-</sup>), diphosphate (R-DP<sup>3-</sup>) and triphosphate (R-TP<sup>4-</sup>). The residue R can be any organic moiety including a nucleosidyl residue which may or may not participate in metal ion binding; however, in most instances in this review R represents a noninteracting group (what is actually meant is always clear from the context).