COMMUNICATIONS

A New Method of Synthesis of Organic Magnesium Pyrophosphate Complexes

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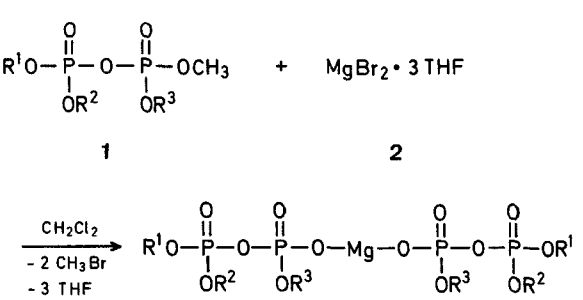
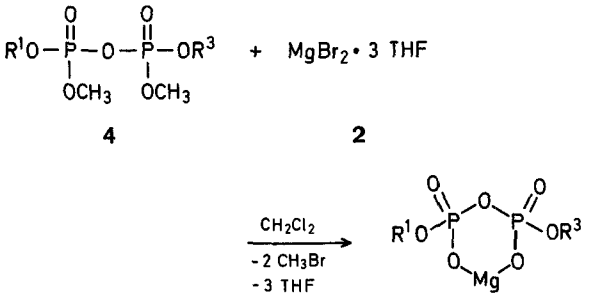
It is well known that many enzymes involved in the transfer of phosphoryl groups, (RO)(HO)P(O)—, from pyrophosphate esters to water and to biological substrates require magnesium for their activity[1,2]. These organic pyrophosphates include diesters of type $(R^1O)(HO)P_{\alpha}(O)OP_{\beta}(O)(OR^2)(OH)$, e.g., cytidine diphosphate choline (CDP-choline) and cytidine diphosphate diglycerides (CDP-diglycerides) which function as intermediates in phospholipid biosynthesis[3], as well as monoesters of type $(RO)(HO)P_{\gamma}(O)OP_{\beta}(O)(OH)OP_{\alpha}(O)(OH)_2$, e.g., nucleoside-5′-*O*-tripolyphosphates which function mainly as energized carriers of nucleoside-5′-*O*-phosphates (P_{γ}-nucleophilic attack) and of inorganic phosphate (P_{α}-nucleophilic attack)[1,2]. In spite of the importance of these processes, the literature offers no general procedures for the preparation of well defined organic magnesium pyrophosphate complexes[4]. Information on the molecular structure and the properties of such complexes is needed research directed toward the elucidation of the role played by $Mg^{2\oplus}$ ions in phosphoryl transfer reactions. Previous work has been mainly concerned with the effect of $Mg^{2\oplus}$ ions on the ^{31}P-N.M.R. signals of pyrophosphates in aqueous solutions[5,6].

We wish to describe procedures which permit the conversion of methyl esters of organic pyrophosphates into two types of magnesium pyrophosphate complexes: acyclic 1:2 complexes, **3**, shown in Scheme **A**, and cyclic 1:1 complexes, **5**, shown in Scheme **B**. The reagent used in these procedures is magnesium bromide·tris[tetrahydrofuran] (**2**)[7]. This compound is remarkably soluble in lipophilic aprotic solvents such as dichloromethane, and is endowed with powerful nucleophilic ($Br^{\ominus}$) and electrophilic ($Mg^{2\oplus}$) centers. The complexes **3** and **5** are isolated in approximately 90% yield, since the reactions are nearly quantitative and give innocuous and volatile by-products. The complexes are generated with tetrahydrofuran molecules bound to the magnesium, and can subsequently be transformed into other types of complexes by addition of appropriate uncharged ligands, e.g., water. This procedure permits a study of the coordination chemistry of the $Mg^{2\oplus}$ ion bound to organic pyrophosphates by techniques that have been applied to other types of $Mg^{2\oplus}$ complexes[7,8]. Several examples of the application of this procedure are given in the Table.



Scheme **A**



Scheme **B**

The structure of the 1:2 complexes, **3**, follows from the microanalyses and the ^{31}P- and ^{1}H-N.M.R. spectra, and from the limited reaction possibilities available to the precursor tetraester **1** when $R^2 \neq CH_3$. The assignment of the cyclic monomeric structure **5** to the 1:1 complexes is based on microanalyses and N.M.R. spectral data in all cases, and on the results of molecular weight determination in one of the complexes, magnesium P_{α},P_{β}-di-*p*-fluorophenyl dipolyphosphate dihydrate, [**5**·2 H_2O ($R^1 = R^3 = p$-F—C_6H_4)]. This complex is relatively soluble and stable in methanol solutions, where excellent agreement is obtained between the observed and the calculated M.W. values. The solubility properties of the other 1:1 complexes listed in the Table do not permit M.W. determinations, and therefore their structural assignment has been made by analogy to the *p*-fluorophenyl-derivative.

The main features of these new procedures are:

(a) The reagent, **2**, discriminates sharply between methyl and higher alkyl groups, while methyl and benzyl groups appear to have about the same degree of reactivity.

(b) The reagent attacks only one of two methyl groups situated on the *same* phosphorus atom; i.e., four-membered rings with $Mg^{2\oplus}$ ions are not produced even under forced conditions.

(c) The bromide ion reactivity is significantly different in $MgBr_2 \cdot 3THF$ (**2**) and in $(n\text{-}C_4H_9)_4NBr$, in aprotic solvents. The reagent, **2**, rapidly generates methyl bromide from both adjacent P—OCH_3 functions in a reaction that is intramolecular as shown by results of concentration-dependent studies. The intermediate, **6**, obtained after the first demethylation step appears to have the proper electronic and steric features needed for the second demethylation step. On the other hand, the ammonium bromide rapidly attacks only the first of two adjacent P—OCH_3 groups; the second demethylation is too slow to compete successfully with other reactions which result in decomposition of the monoanion intermediate.

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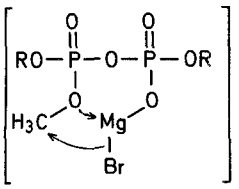

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(d) The reagent, **2**, can also be used to make $Mg^{2\oplus}$ complexes derived from methyl esters of organic tripolyphosphates, $(R^1O)(R^2O)P_\alpha(O)OP_\beta(O)(OR^3)OP_\gamma(O)(OR^4)(OCH_3)$. The $Mg^{2\oplus}$ ion can be placed at the P_β and P_γ atoms, or at the P_α and P_γ atoms, by reactions of the corresponding dimethyl-derivatives, $R^3=CH_3$ or $R^2=CH_3$, respectively. The resulting six-membered $(Mg\cdot P_\beta,P_\gamma)$ and eight-membered $(Mg\cdot P_\alpha,P_\gamma)$ complexes serve as models for the two possible types of coordinations between the metal and ATP[2, 6, 9].

Of particular significance is the observation that the coordination of the $Mg^{2\oplus}$ ion to the oxygen atoms at P_α and P_β atoms of the 1:1 complexes **5** does not increase the electrophilicity of these P-centers relative to their electrophilicity in bis-triethylammonium salts of the same pyrophosphodiesters. Solutions of **5** ($R^1=R^3=p$-F—C_6H_4) in methanol do not undergo appreciable changes after 13 days at 35°, or after 4 days at 65°, illustrating the stability of the pyrophosphate bond toward solvolyses in the Mg-complexes[10]. This absence of activation of the pyrophosphate bond by $Mg^{2\oplus}$ ions must be taken into account in formulations of models for magnesium participation in enzymatic and non-enzymatic phosphoryl-transfer reactions of pyrophosphate esters[11].

Preparation of 1:1 Mg·Pyrophosphate Complexes 5:

P_α,P_β-Di-*p*-fluorophenyl-P_α,P_β-dimethyl dipolyphosphate is prepared from *p*-fluorophenyl methyl phosphate and dicyclohexylcarbodiimide by a known procedure[12]. Separate solutions of the ester (1.76 g, 4.4 mmol in 40 ml of anhydrous dichloromethane) and $MgBr_2\cdot 3THF$[7] (1.94 g, 4.8 mmol in 40 ml of dichloromethane) are added at the same rate, over a 1 h period, to a flask containing dichloromethane (200 ml), at 20°, with efficient stirring. After 6 h at 20°, the solution is filtered through a thin bed of dry Celite to remove traces of suspended solid. The filtrate is evaporated (25°/30 torr), and the residue is dried for 6 h at 20°/0.2 torr; ^{31}P- and ^1H-N.M.R. spectra of the residue in CD_3OD reveal that it is the 1:1 complex **5** ($R^1=R^3=p$-F—C_6H_4) bound to tetrahydrofuran; yield: 2.3 g (97%). This material is conveniently stored as such, and is transformed into magnesium P_α,P_β-di-*p*-fluorophenyl dipolyphosphate dihydrate (**5**·$2H_2O$) as follows. A solution in methanol (0.86 g, 1.61 mmol in 5 ml) is filtered through a plug of glass-wool to remove traces of suspended solid. The solution is treated with water (1 ml), kept for 5 min at 20°, and evaporated (25°/30 torr). The residue is dried for 24 h at 20°/0.1 torr prior to microanalysis; yield: 0.68 g (100%). The ^1H-N.M.R. spectrum (CD_3OD) shows only aromatic and water signals.

$C_{12}H_8MgF_2O_7P_2\cdot 2H_2O$
(424.5)

calc.	C 34.0	H 2.8	Mg 5.7	P 14.6	H_2O 8.5
found	35.1	3.0	5.5	13.7	7.2

M.W. 415 (in CH_3OH)

The slightly high % C value is attributed to remaining trace amounts of tetrahydrofuran.

The 1:1 complex, **5**·$2H_2O$ (0.21 g, 0.5 mmol; $R^1=R^3=p$-F—C_6H_4), is transformed into bis-triethylammonium P_α,P_β-di-*p*-fluorophenyl dipolyphosphate by passing a methanol solution of the complex through a BioRad AG 50W-X8 cation exchange resin in the $(C_2H_5)_3NH^\oplus$ form, eluting with methanol, and evaporating the solvent at 20°/0.1 torr; yield: 0.28 g (100%). The triethylammonium pyrophosphate has $\delta_P=-17.2$ ppm, and has the expected ratio of aromatic/CH_3 protons (4/9) in the ^1H-N.M.R. spectrum (in $CDCl_3$).

The other 1:1 complexes, **5**, listed in the Table are obtained by an analogous procedure. The microanalyses of the anhydrous tetrahydrofuran-free complexes are performed on samples treated for 30 min with benzene at 50°, followed by drying for 24 h at 20°/0.1 torr; the analyses agree with the calculated values: C, ±0.5; H, ±0.5; Mg, ±0.3; P, ±0.3. ^1H-N.M.R. spectra in DMSO-d_6/$CDCl_3$, 90/10, confirm the absence of tetrahydrofuran and water in the complexes.

Table. Preparation of Magnesium Pyrophosphate Complexes **3** and **5**

Pyrophosphate					Magnesium Pyrophosphate Complex				
No.	R^1	R^2	R^3	^{31}P-N.M.R. ($CDCl_3$)[a] δ [ppm]	No.	Type	Yield [%]	Molecular formula[b]	^{31}P-N.M.R. (DMSO-d_6/$CDCl_3$, 9:1)[a] δ [ppm]
1a	C_2H_5	C_2H_5	CH_3	−13.0; −10.7	**3a**	1:2	88	$C_{10}H_{26}MgO_{14}P_4$ (518.5)	~ −12
4b	C_6H_5	CH_3	C_6H_5	−17.3	**3b**	1:2[c]	96	$C_{26}H_{26}MgO_{14}P_4$ (710.7)	−15.6; −19.6
4b	C_6H_5	CH_3	C_6H_5	−17.3	**5b**	1:1	95	$C_{12}H_{10}MgO_7P_2$ (352.5)	−18.8
4c	p-F—C_6H_4	CH_3	p-F—C_6H_4	−17.2	**5c**	1:1	97	$C_{12}H_8F_2MgO_7P_2\cdot 2H_2O$ (424.5)	−18.8[d]
4d	n-C_8H_{17}	CH_3	n-C_8H_{17}	−11.6	**5d**	1:1	95	$C_{16}H_{34}MgO_7P_2$ (424.7)	−16.4
4e	n-$C_{14}H_{29}$	CH_3	n-$C_{14}H_{29}$	−11.6	**5e**	1:1	96	$C_{28}H_{58}MgO_7P_2$ (593.0)	−16.5

[a] ^{31}P-N.M.R. (85% aqueous H_3PO_4 as external standard); positive values are downfield from the standard.

[b] All products (except **5c**, see text) gave satisfactory microanalyses.

[c] ^1H-N.M.R.: δ [ppm]: 3.77 (doublet, $J_{HP}=12$ Hz; CH_3O) and 7.25 (multiplet; aromatics) in 6:20 proportion in DMSO-d_6/$CDCl_3$ 9:1 (TMS as internal standard).

[d] Dihydrate, **5c**·$2H_2O$ in CD_3OD.

Preparation of 1:2 Mg·Pyrophosphate Complexes 3:
P_α,P_γ-Diethyl-P_β,P_β-dimethyl dipolyphosphate is prepared from diethyl phosphorochloridate and *N*-methylpyridinium dimethyl phosphate by known procedures[13]. A dichloromethane solution (20 ml) containing $MgBr_2 \cdot 3THF$ (1 mol-equiv.) is added, over a 5 min period, to a dichloromethane solution (4 ml) containing the tetraester (2 mol-equiv.), at 25°, with stirring. The final solution is 0.1 molar with respect to the tetraester. After 12 h at 25°, the solvent is evaporated, the residue is stirred for 30 min with benzene at 50°, and the mixture is cooled to 25° and filtered. The complex is dried for 24 h at 20°/0.1 torr; yield: 88%. The ^{1}H-N.M.R. spectrum of magnesium bis[P_α,P_γ-diethyl-P_β-methyl dipolyphosphate] shows the signals due to C_2H_5- and CH_3-groups in the expected ratio (in DMSO-d_6/$CDCl_3$, 90/10).

$C_{10}H_{26}MgO_{14}P_4$	calc.	C 23.2	H 5.0	Mg 4.6	P 23.9
(518.5)	found	23.0	4.9	4.7	24.0

The same procedure applied to P_α,P_β-diphenyl-P_γ,P_β-dimethyl dipolyphosphate gives magnesium bis[P_α,P_β-diphenyl-P_γ-methyl dipolyphosphate], with the expected ratio of $2C_6H_5:CH_3$ in the ^{1}H-N.M.R. spectrum (DMSO-d_6/$CDCl_3$, 90/10).

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