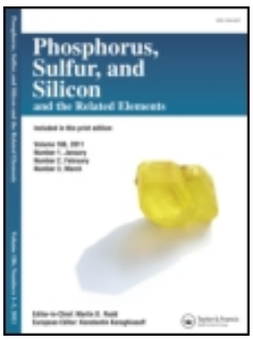

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Automated lineshape analysis of complex NMR spectra for a novel synthetic tetrafluorobisphosphonate, a potential ligand for phosphoglycerate kinase

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ABSTRACT

Tetraalkyl 1,1,3,3-tetrafluoro-2,2-dihydroxypropane-1,3-bisphosphonates were prepared. The complex $^{31}P\{^{1}H\}$- and ^{19}F-NMR spectra were analyzed as $[[A]_2X]_2$ and related systems. Modern methods of automated spectral analysis using DAISY under WIN-NMR were applied.

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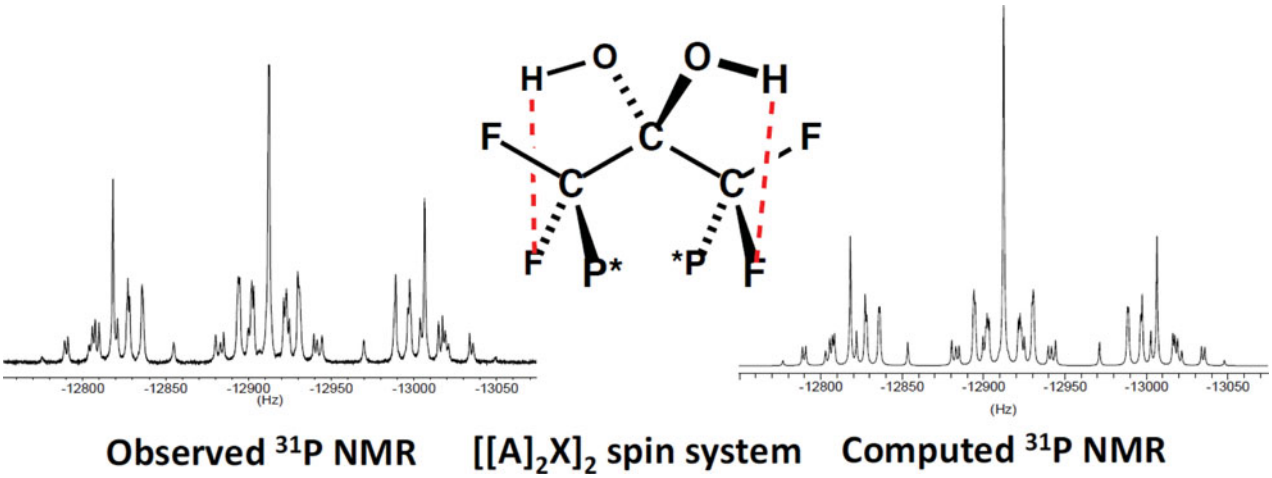
GRAPHICAL ABSTRACT



Introduction

Phosphonates have been recognized as biologically important tools, which have lead to a greater understanding of biological processes.[1] Often their application has been as transition state analogues for acyl transfer processes, and as ground state analogues for phosphoryl transfer in enzymatic reactions. Their use is based upon the principal fact that the deletion of, or replacement of, the scissile P-O-C linkage with a non-scissile P-C linker maintains the phosphoryl moiety within the molecule. Different recognition processes are achieved within the enzyme active site. Halogenation of the α-carbon has been proposed as an additional means of altering the recognition in enzyme processes[2] as a result of improving the isosteric and isoelectronic nature of the halogenated molecule.

We have synthesized a range of α,α-difluoroalkylphos phonates, which have been tested for their binding affinity toward phosphoglycerate kinase.[3,4] During the synthesis of one of these molecules we generated the tetrafluorobisphosphonates (**3**), which exhibit complex nuclear magnetic resonance (NMR) spectra. Results from spectral analysis are presented in this paper.

Chemical discussion

The synthesis of (**3**) is outlined in Scheme 1. Dialkyl difluoromethanephosphonate (**1**) was obtained according to a modified literature preparation.[5] Lithium diisopropylamide (LDA) was used to deprotonate (**1**) and the anion reacted with CO_2 to synthesize dialkyl phosphonodifluoroethanoic acid[6] (**2**). Appreciable quantities of dihydroxybisphosphonate (**3**) were formed during the reaction, which was isolated and recrystallized from methylene chloride (DCM)/petrol. The $^{31}P\{^{1}H\}$ and ^{19}F-NMR spectra for the tetraethyl ester (**3a**) and the tetraisopropyl ester (**3b**) are complex. In a subsequent section of this paper we

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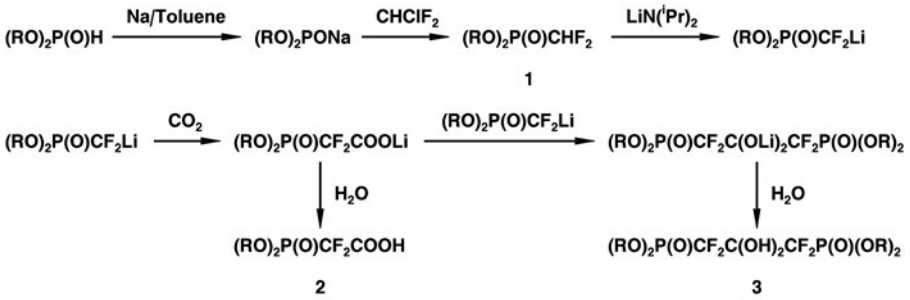


Yields	1	2	3
a: R = Et	46%	47%	6%
b: R = ^{i}Pr	95%	62%	10%

Scheme 1. Synthesis of compounds **(1)**–**(3)**.

present the results from NMR spectral analysis for tetraisopropylester (**3b**).

Sodium dialkyl phosphite reacts with chlorodifluoromethane to form dialkyl difluoromethanephosphonate[1] (**1**). Deprotonation of (**1**) with LDA yields the corresponding carbanion, which reacts with CO_2 to form primarily the lithium salt of dialkyl phosphonodifluoroethanoic acid.[2] A secondary nucleophilic attack of carbanion produces the dilithium salt of tetraalkyl 1,1,3,3-tetrafluoro-2,2-dihydroxypropane-1,3-bisphosphonate. Hydrolysis of the reaction mixture converted lithium salts into the corresponding phosphonodifluoroethanoic acid (**2**) and tetraalkyl 1,1,3,3-tetrafluoro-2,2-dihydroxypropane-1,3-bisphosphonate (**3**).

The formation of a tertiary alcohol (**4**) via a corresponding lithium salt (Scheme 2) was not observed.

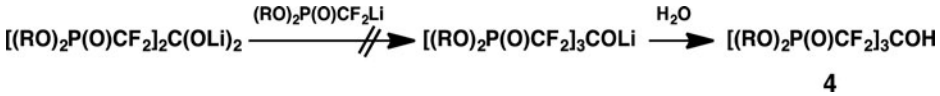
No evidence was obtained that the dihydroxy compound (**3**) was dehydrated in solution to the parent ketone, the tetraalkyl 1,1,3,3-tetrafluoro-2-oxopropane-1,3-bisphosphonate (**5**) (Scheme 3) as might be expected from literature.[7]

Experimental

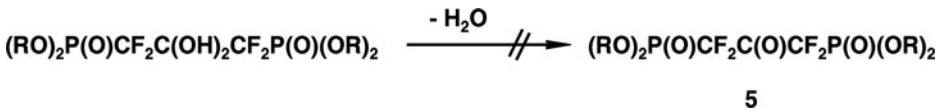
All NMR spectra of synthesis samples were recorded on a BRUKER AC-250 MHz spectrometer unless specified, where an AMX-500 MHz spectrometer was used. Toluene was distilled from calcium hydride, and tetrahydrofuran (THF) was distilled from potassium and benzophenone. Column chromatography was performed using Kieselgel 60, 230–400 mesh (Merck 9835). All other chemicals were purchased from Aldrich Chemical Company Ltd.

Diethyl difluoromethanephosphonate[5] (1a)

Diethyl phosphite (44 g, 0.32 mol, freshly distilled) was added drop-wise at RT under argon to sodium spheres (7.8 g, 0.34 mol) in toluene (250 mL, freshly distilled). The solution was further heated at reflux for 1.5 h, cooled, and transferred to a dry flask by cannula. Chlorodifluoromethane was bubbled through the solution overnight. After addition of water (300 mL), and extraction with CH_2Cl_2 (DCM, 4 × 300 mL), the organic layers were combined and dried ($MgSO_4$). The solvents were removed in vacuo to yield a colorless oil, which was purified by silica gel chromatography eluting with DCM. This gave (**1a**) as a colorless mobile oil (27.5 g, 45.6%); bp 80–90°C/5-mm Hg (lit.[1]: bp 85.6–86.5°C/12-mm Hg); R_F (DCM) 0.62; $_H$ (250 MHz, $CDCl_3$) 5.86 (1H, dt, $^2J_{PH}$ 28 Hz, $^2J_{FH}$ 48 Hz, CF_2H), 4.25 (4H, dq, $^3J_{HH}$ 7.0 Hz, 2 × CH_2), 1.31 (6H, t, $^3J_{HH}$ 7.0 Hz, 2 × CH_3); δP



Scheme 2. Formation of a tertiary alcohol is not observed.



Scheme 3. Formation of a keto form is not evident.

(101.20 MHz, $CDCl_3$) 5.38 (1 P, t, $^2J_{PF}$ 91 Hz); δF (188 MHz, $CDCl_3$) –137.8 (dd, $^2J_{FH}$ 48 Hz, $^2J_{PF}$ 91 Hz).

Diethyl phosphonodifluoroethanoic acid[6] (2a) and tetraethyl 1,1,3,3-tetrafluoro-2,2-dihydroxypropane-1,3-bisphosphonate (3a)[4]

Diethyl difluoromethanephosphonate (**1a**, 4.7 g, 25 mmol) was added dropwise to a stirred solution of LDA (2M solution in heptane/THF, 18.0 mL, 35 mmol) in THF (30 mL, dry) at –70°C under nitrogen. After 20 min, CO_2 (dried over $CaCl_2$) was bubbled through the solution for 45 min at –75°C. The reaction was then left for stirring overnight and allowed to attain RT. The reaction was quenched by cautious addition of water (100 mL) and ether (100 mL), and the layers were separated. The aqueous layer was acidified with 1M H_2SO_4 (to pH 1), and extracted with EtOAc (5 × 30 mL). The combined EtOAc layers were dried (Na_2SO_4), filtered, and the solvent was removed in vacuo to give (**2a**) as a yellow oil (2.7 g, 46.5%); R_F (5% MeOH/DCM) 0.17; δ_H (250 MHz, $CDCl_3$) 9.45 (1H, bs, CO_2H), 4.1–4.3 (4H, m, 2 × CH_2), 1.3 (6H, dt, $^3J_{HH}$ 7.5 Hz, 2 × CH_3); δ_P 3.5 (t, $^2J_{PF}$ 97 Hz); δ_F –117.3 (d); *m/z* (EI) 231 (M^+).

The ether layer was dried ($MgSO_4$), filtered, and reduced in vacuo to yield an off-white oil, which solidified on standing and was recrystallized from DCM/petrol to yield (**3a**) as a white crystalline solid (0.17 g, 6.0% based on remaining (**1a**)), mp 55–58°C (Found: C, 31.43; H, 5.13. $C_{11}H_{22}F_4O_8P_2$ requires C, 31.44%; H, 5.28%); δ_H (250 MHz, $CDCl_3$) 4.25–4.45 (8H, m, 4 × CH_2), 1.4 (12H, dt, $^3J_{HH}$ 7.5 Hz, 6 × CH_2); δP (202.40 MHz, $CDCl_3$) 4.5–7.5 (m); δ_F (235.19 MHz, $CDCl_3$) –121.8 to –122.5 (m); *m/z* (CI) 402 (M^+).

Diisopropyl difluoromethanephosphonate[5] (1b)

Diisopropyl phosphite (16.6 g, 0.10 mol, freshly distilled) was added drop-wise at RT under nitrogen to sodium spheres (2.3 g, 0.10 mol) in toluene (150 mL, freshly distilled). The solution was further heated at reflux for 3 h and then cooled. Chlorodifluoromethane was bubbled through the solution for 1 h. After addition of water (200 mL) and extraction with DCM (3 × 200 mL), the organic layers were combined, dried ($MgSO_4$), and filtered. The solvents were removed in vacuo to yield a colorless mobile oil (21.0 g, 95%); bp 85–95°C/5-mm Hg (lit.[1] bp 89–90°C/12-mm Hg); R_F (DCM) 0.60; δ_H (250 MHz, $CDCl_3$) 5.80 (1H, dt, $^2J_{PH}$ 28 Hz, $^2J_{FH}$ 48 Hz, C*H*), 4.70–4.90 (2H, m, $^3J_{HH}$ 7.0 Hz, 2 × C*H*), 1.30 (12H, t, $^3J_{HH}$ 7.0 Hz, 4 × CH_3); δ_P (101.20 MHz, $CDCl_3$) 3.44 (1 P, t, $^2J_{PF}$ 92 Hz); δ_F (235.19 MHz, $CDCl_3$) –138.0 (dd).

Diisopropyl phosphonodifluoroethanoic acid[6] (2b) and tetraisopropyl 1,1,3,3-tetrafluoro-2,2-dihydroxypropane-1,3-bisphosphonate (3b)[4]

Diisopropyl difluoromethanephosphonate (**1b**, 10 g, 46 mmol) was added dropwise to a stirred solution of LDA (2-M solution in heptane/THF, 25.0 mL, 50 mmol) in THF (50 mL, dry) at –75°C under nitrogen. After 1 h, CO_2 (dried over $CaCl_2$) was bubbled through the solution for 2 h at –75°C. The reaction was then left for stirring overnight and allowed to warm to RT. The reaction was quenched by cautious addition of water (150 mL) and EtOAc (200 mL), and the layers were separated. The aqueous layer was acidified with 1 M H_2SO_4 (to pH 1), and extracted with EtOAc (5 × 30 mL). The combined EtOAc layers were dried (Na_2SO_4), and the solvent was removed in vacuo to give a solid, which was recrystallized from petrol to give white crystals (**2b**) (6.5 g, 62%); mp 36–38°C; R_F (4% MeOH/DCM) 0.17; δ_H (250 MHz, $CDCl_3$) 10.00 (1H, bs, CO_2H), 4.70–4.90 (2H, m, C*H*), 1.33 (12H, d, $^3J_{HH}$ 7.5 Hz, 4 × CH_3); δ_P (101.20 MHz, $CDCl_3$) 1.6 (t, $^2J_{PF}$ 96 Hz); δ_F (235.19 MHz, $CDCl_3$) –114.3 (d); *m/z* (EI) 231 (M^+).

The initial EtOAc layer was dried ($MgSO_4$), filtered, and the solvent was removed in vacuo to yield an off-white solid and was recrystallized from DCM/petrol to yield (**3b**) as a fluffy white powder (1.2 g, 10% based on remaining (**1b**)), mp 61–62°C (Found: C, 37.95; H, 6.28. $C_{15}H_{30}F_4O_8P_2$ requires C, 37.82%; H, 6.35%); δ_H (250 MHz, $CDCl_3$) 4.80 (4H, m, C*H*), 1.37 (24H, d, $^3J_{HH}$ 7.5 Hz, CH_3); δ_P (202.40 MHz, $CDCl_3$) 2.7–4.7 (m); d_F (235.19 MHz, $CDCl_3$) –118.5 to –119.5 (m); *m/z* (CI) 402 (M^+). This product was subjected to extensive NMR spectral analytical studies presented below.

NMR spectroscopic investigations

Experimental

A sealed sample of (**3b**) as a 5% solution in $CDCl_3$ was made in the standard freeze-pump-thaw technique. $^{31}P\{^1H\}$-MR, 81 MHz, and ^{19}F-NMR, 188 MHz, spectra were measured at ambient temperature using the BRUKER AM200SY spectrometer at Düsseldorf.

The $^{31}P\{^1H\}$-NMR spectrum – the X-part of an $[[A]_2X]_2$ spin system

The experimental, proton-decoupled 81-MHz $^{31}P\{^1H\}$-NMR spectrum of tetraisopropyl 1,1,3,3-tetrafluoro-2,2-dihydroxypropanebisphosphonate, 5% (**3b**) in $CDCl_3$, is shown in Figure 1a.

The proton-decoupled $^{31}P\{^1H\}$-NMR spectrum is consistent with an $[[A]_2X]_2$ spin system (A = ^{19}F, X = ^{31}P) resulting either from a free rotating propane skeleton or a fixed conformer with two-fold symmetry as shown in a simplified Scheme 4.

Explicit rules for the spectral analysis of $[[A]_2X]_2$ spin systems were given by Harris and coworkers.[8,9] We follow this standard notation using the coupling constants and combined parameters N_{PF}, L_{PF}, N_{FF}, and L_{FF} as shown in Table 1. The experimental 81-MHz $^{31}P\{^1H\}$-NMR spectrum of (**3b**) is symmetric to the central resonance frequency ν_P. Three strong and relatively narrow lines form a triplet centered at ν_P and separated by $N_{PF} = {}^2J_{PF} + {}^4J_{PF}$. Starting from the standard values[8–13] for $^2J_{FF}$, $^4J_{FF}$, $^4J_{PP}$, $^2J_{PF}$, and $^4J_{PF}$ automated analysis and iteration for the $[[A]_2X]_2$ spin system using WINDAISY[14] running under WIN-NMR[15] yielded the NMR parameters given in Table 1 and a simulation shown in Figure 1b above.

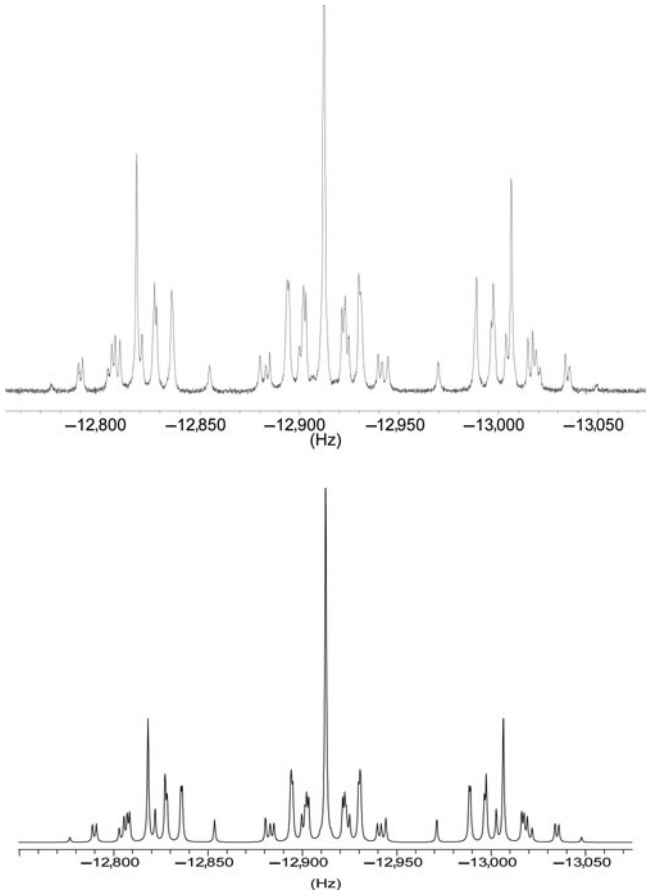
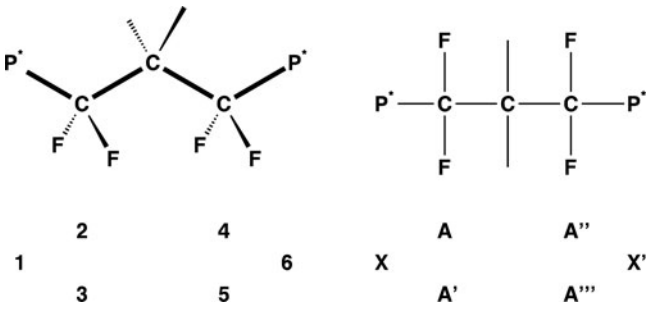


Figure 1. (a) Upper: Experimental 81-MHz $^{31}P\{^1H\}$-NMR spectrum of tetraisopropyl 1,1,3,3-tetrafluoro-2,2-dihydroxypropane-1,3-bisphosphonate (**3b**) (5%, solution in $CDCl_3$). (b) Lower: Calculated spectrum using data from Table 1.



Scheme 4. The $[[A]_2X]_2$ spin system.

Parameters given in Table 1 are consistent with results using explicit rules for the spectral analysis of $[[A]_2X]_2$ spin systems given by Harris and co-workers.[8,9] Attempts to fit the $^{31}P\{^1H\}$-MR spectrum by the more simple $[A_2X]_2$ system were unsuccessful, as expected from published theory.[8,9]

The $^{19}F\{^1H\}$-NMR spectrum – the A-part of an $[[A]_2X]_2$ spin system

We were not able to record the proton decoupled $^{19}F\{^1H\}$-NMR spectrum, consequently the complementary A-part of the $[[A]_2X]_2$ spin system, potentially serving as a proof for results given in Table 1 was not accessible.

Table 1. Resonance frequencies ν_P and coupling constants [Hz] from WINDAISY iterations based upon the experimental 81-MHz $^{31}P\{^1H\}$-MR spectrum of (**3b**). Spectral half-width of N_{PF} lines: $HWN_{PF} = 0.8$ Hz. Spectral half-width of all other lines: $HWL_{PF} = 1.0$ Hz. Resonance frequency ν_F from 188-MHz ^{19}F-NMR spectrum.

	Parameters	Final	Error
$^{\nu}A$	$\nu_F = \nu_2 = \nu_3 = \nu_4 = \nu_5$	−11445.405	n.i.
$^{\nu}X$	$\nu_P = \nu_1 = \nu_6$	−12912.393	0.0030
^{J}AX	$^2J_{PF} = {}^2J_{12} = {}^2J_{13} = {}^2J_{46} = {}^2J_{56}$	94.095	0.0041
$^{J}AX'$	$^4J_{PF} = {}^4J_{15} = {}^4J_{16} = {}^4J_{23} = {}^4J_{24}$	0.062	0.0041
$^{J}AA'$	$^2J_{FF}^{gem} = {}^2J_{23}^{gem} = {}^2J_{45}^{gem}$	−220.377	n.i.
$^{J}AA''$	$^4J_{FF}^{cis} = {}^4J_{24}^{cis} = {}^4J_{35}^{cis}$ or $^4J_{FF}^{trans} = {}^4J_{25}^{trans} = {}^4J_{34}^{trans}$	33.783	0.0095
$^{J}AA'''$	$^4J_{FF}^{trans} = {}^4J_{25}^{trans} = {}^4J_{34}^{trans}$ or $^4J_{FF}^{cis} = {}^4J_{24}^{cis} = {}^4J_{35}^{cis}$	−1.403	0.0084
$^{J}XX'$	$^4J_{PP} = {}^4J_{16}$	12.393	0.0094

n.i.: Not iterated.

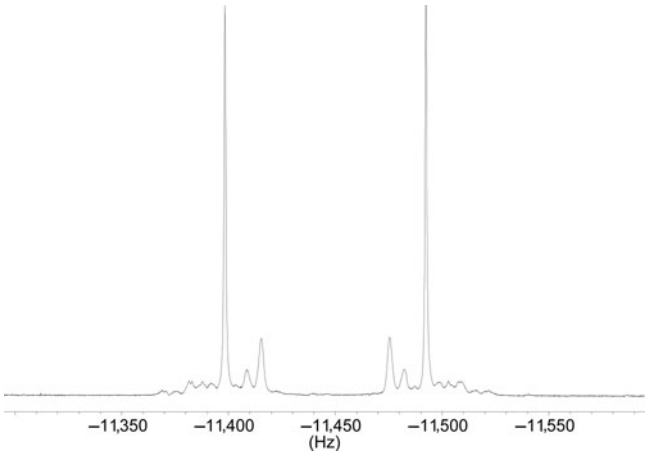
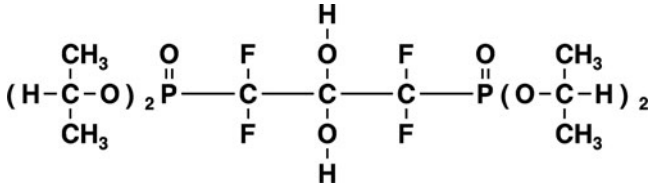


Figure 2. Experimental 188-MHz ^{19}F-NMR spectrum of tetraisopropyl 1,1,3,3-tetrafluoro-2,2-dihydroxy-propanebisphosphonate (**3b**) (5%, solution in $CDCl_3$).

Results from ^{19}F-NMR – some models for more complex spin systems

The situation in the proton-coupled total spin system is much more complex as shown in Figure 2 and Scheme 5:



Scheme 5. The total $[[A]_2K[RS_6]_2]_2$ spin system in (**3b**).

The experimental 188-MHz ^{19}F-NMR spectrum of (**3b**) is symmetric with respect to the central frequency ν_F. Two strong and relatively narrow lines ($HWN_F = 0.9$ Hz) form a doublet centered at ν_F and separated by $N_{PF} = {}^2J_{PF} + {}^4J_{PF} = 94.167$ Hz.

Model 1

Some tentative simulations and iterations using the simplified approximation for the A-part of the $[[A]_2X]_2$ spin system were not successful for both using either the LAO-PC[15] or the WIN-DAISY[14,15] programs. The N_{PF}-lines in the ^{19}F-NMR spectrum exhibit a more narrow spectral half-width (0.9 Hz)

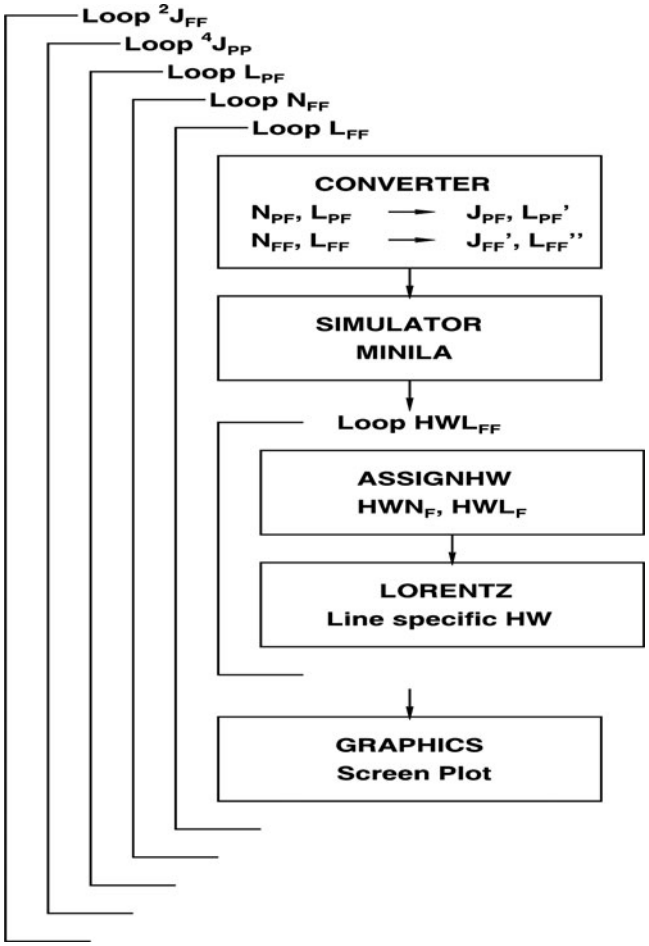


Figure 3. Flow chart diagram of PC-simulator program BLACKFILM.

and high intensities, while the remaining lines are broader (2.3 Hz) and less intense.

A useful auxiliary program BLACKFILM was designed, which allows simulation of film-like sequences of $[[A]_2X]_2$ spectra using individual line widths for N_{PF}- and the remaining (L_{PF}) lines. The basic principles of BLACKFILM are shown in Figure 3.

Directly accessible parameters: Resonance frequencies, N_{PF}, and spectral half-width of N_{PF}-lines HWN_{PF} were kept at fixed values. *The remaining parameters*: L_{PF}, N_{FF}, and L_{FF} were varied from minimal to maximal values. The individual coupling constants were generated according to:

1. $J_{PF} = (N_{PF} + L_{PF})/^2$ $J_{PF}' = (N_{PF} - L_{PF})/^2$,
2. $J_{FF} = (N_{FF} + L_{FF})/^2$ $J_{FF}' = (N_{FF} - L_{FF})/^2$,

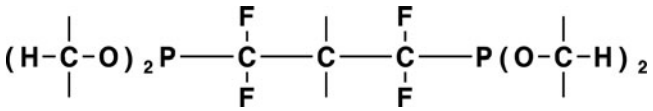
and fed to an efficient LAOCOON-type simulator MINILA.[16, 17] In addition, the spectral half-width HWL_{PF} of the remaining (non-N_{PF}) lines was looped from a minimum to a maximum value. Specific data for spectral half-width HWN_F and HWL_F were assigned and used in a line-specific Lorentz generator. The resulting Lorentz spectra were displayed on screen, producing a film-like sequence. Visual recognition (by comparison with the experimental spectrum) should lead in fortuitous cases to the best NMR meter combination. But in vain: additional line splittings occur in the experimental spectrum, not consistent with the simplified $[[A]_2X]_2$ model. Consequently, a more complex analysis of the system had to be adopted, as shown in Scheme 5.

Model 2

The total $[[A]_2K[RS_6]_2]_2$, a 34-spin system, holds the additional contributions: K from the hydroxyl protons, while the isopropoxy groups give rise to the RS_6 units.

Model 3

If J_{FH} couplings involving the hydroxyl protons and the $^4J_{POCCH}$ couplings are zero, then a simplified spin system results are shown in Scheme 6.



Scheme 6. The $[[A]_2XR_2]_2$ spin system.

Corresponding WIN-DAISY simulations based upon data derived from the proton-decoupled $^{31}P\{^1H\}$-NMR spectrum (see Table 1) were applied to the $[[A]_2XR_2]_2$ model fixing J_{POCH} to a tentative value of 5 Hz. Comparison with the experimental 188-MHz ^{19}F-NMR spectra of (**3b**) in Figure 2 showed that a typical broad line shape in the fluorine part is not explained by POCH coupling effects. But clearly this additional coupling affects the phosphorus part, as expected.

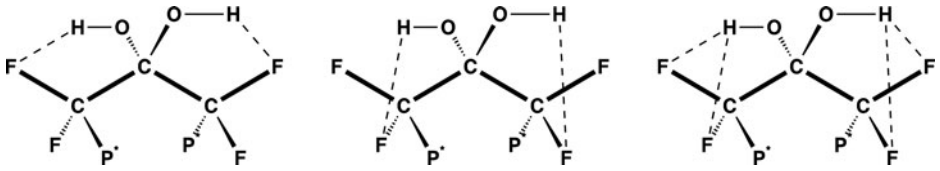
Model 4

A more likely model suggests the existence of proton fluorine couplings J_{FH}. This effect may be due to through bond interaction or via the formation of F···H-O bridges leading to FH-coupling in an $[[A]_2KX]_2$ spin system as shown in Scheme 7.

Corresponding WIN-DAISY simulations varying J_{FH} from 0–4 Hz were compared with the experimental 188-MHz ^{19}F-NMR spectra of (**3b**) in Figure 2. Indeed, the typical broad line shapes in fluorine signals are consistent with the presence of F···H coupling effects as shown in Figure 4, justifying the approximation by $[[A]_2KX]_2$. This additional coupling does not affect the simulated phosphorus-NMR spectrum.

Model 5

Another model suggests the existence of anisochronic fluorine spins. This effect may be due to the formation of F···H-O bridges leading to more fixed conformations responsible for the $[ABX]_2$ spin system shown in Scheme 7 above. Corresponding WIN-DAISY simulations varying $\Delta = \nu_A - \nu_B$ from 0–30 Hz were compared with the experimental 188-MHz ^{19}F-NMR spectra of (**3b**) in Figure 2. This anisochrony does not affect the phosphorus part, thus justifying the approximation by $[ABX]_2$. But the typical broad line shape in the fluorine part and its splitting pattern are not sufficiently explained with the assumption of anisochrony effects.



Scheme 7. The $[[A]_2KX]_2$ – induced by F-H bridges.

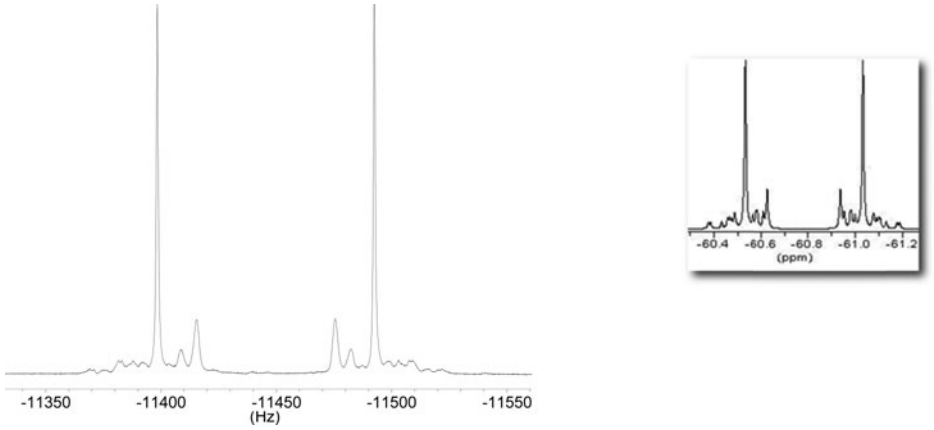


Figure 4. The closest approximation of the ^{19}F-NMR spectrum of (**3b**) from a series of $[[A]_2KX]_2$ simulations with WIN-DAISY. For data, see Table 1. $^1J_{FH} = 1$ Hz. Spectral half-width = 2 Hz.

Conclusions

The application of WIN-DAISY has solved the measured ^{31}P spectrum of tetraisopropyl 1,1,3,3-tetrafluoro-2,2-dihydroxypropane-1,3-bisphosphonate, and has significantly clarified the corresponding ^{19}F spectrum. The results of the model system are enhanced by introducing F···H-O bridges. Such bridges have often been suggested as components of the binding of fluorinated inhibitors to enzymes, although they have been difficult to verify by direct structural methods.[18] We now provide a computational basis to support such claims. The coupling constants determined in this work should facilitate the analysis of spectra recorded from other synthetic polyfluorophosphonates. WIN-DAISY has proved to be a versatile tool for simulations and iterations of complex NMR spectra. It has coped with symmetric spin systems and has enabled the development of an automated assignment of line-specific half-widths for such systems.

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