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Solvent effect in β-phosphorylated nitroxides. Part 4: detection of traces of water by Electron Paramagnetic Resonance

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For decades, the nitrogene hyperfine coupling constant a_N of nitroxides has been applied to probe their environment using EPR. However, the small changes observed ($\approx$ 2 G from n-pentane to water) with the solvent polarity allow only a qualitative discussion. A stable β-phosphorylated nitroxide exhibiting a small change in a_N ($\approx$ 3 G from n-pentane to water) and a striking change ($\approx$ 25 G from n-pentane to water) in phosphorus hyperfine coupling constant a_P with the polarity of solvent was prepared and used to develop the first procedure for the titration of water in THF by EPR, down to 0.1% v/v.

Introduction

Since the seminal work of Knauer et al.[1] on solvent effects, the values of a_N for nitroxide **1** (Fig. 1) have been considered as benchmark values and used for discussions of solvent effect studies involving nitroxides. Nitroxide sensitivity to solvent effect is rather weak, i.e., $\Delta a_N \approx 2$ G from n-hexane to water for **1**. However, in 1976, Il'Yasov and coll.[2] reported a striking solvent effect ($\approx$ 18 G) on the phosphorus hyperfine coupling constant a_P of the non-cyclic nitroxide **2**. We believe that this result did not raise much interest because such a nitroxide was not stable.[§] Recently, we reported[3] a weak effect ($\approx$ 2 G from n-pentane to N-methyl formamide) of the solvent polarity on a_P for the stable non-cyclic nitroxide **3**. We investigated different types of nitroxides carrying a diethoxyphosphoryl group at position β,[4] and showed that some of them were suitable for the titration of a few percent of water in organic solvents.[5] Hereafter, we report on the stable β-phosphorylated 6-membered nitroxide **4** which exhibits a striking difference in a_P ($\Delta a_P \approx 25$ G) from n-pentane to water. The solvent effect is discussed in terms of intramolecular hydrogen bond (IHB) and chair/chair conformational equilibrium. These features are used to investigate the effect of a THF/water binary mixture and applied to the titration of water in THF down to 0.1% water by EPR.

Results and Discussion

Nitroxide **4** was prepared as reported in literature.[6] The two

diastereoisomers **4c** and **4t** (Fig. 1) with the diethoxyphosphoryl and hydroxyl groups in *cis* and *trans* relationship, respectively, were investigated separately.[†,√] The effect of 45 solvents (Table 1SI) on a_N and a_P was investigated by EPR as well as the effect of the THF/water binary mixture (Table 2SI, Fig. 2).

As expected from **1** and **2**, a_N values for **4t** increase with increasing solvent polarity given by the normalized Diromth-Reichardt constant E_T^N(Fig. 3).[a,7] Indeed, a_N values are given by the McConnell relationship[8] (eq. 1), with Q being a constant given by quantum mechanics and ρ_N the electron density on the nitrogen nucleus which depends both on the weight of each canonical forms **A** and **B** (Fig. 4)[9] – the zwitterionic form **B** is favored in polar solvent whereas form **A** is favored in the presence of electron widthdrawing groups (EWG) attached to the nitrogen atom – and on the shape of the Single Occupied Molecular Orbital SOMO – the Fermi contact term which increases with the s character of the SOMO.

$$a_N = Q \cdot \rho_N$$

(1)

The lower values of the y-intercepts ($E_T^N = 0$, Fig. 3)[ʃ] in series **1**, **2**, and **4t** are due to the increase in polarity of the nitroxides as given by the electrical Hammett constants σ_I, i.e., $\sigma_{I,1}$ = -0.03, $\sigma_{I,2}$ = 0.27 $\sigma_{I,4}$ = 0.32.[10] In general, the higher the polarity of the nitroxide, the lower the solvent effect,[11] meaning that the larger slope for **2** than for **1** denotes better accessibility3 to the nitroxyl moiety in **2** than in **1**, whereas the lower slope for **4t** is likely due to its polarity (Fig. 3).[ɟ] Deviation from the linearity at E_T^N = 0.2 is observed for **4c** with an a_N value smaller by ca. 1 G than expected from the y-intercept (Fig. 3).

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Figure 1. Nitroxides discussed in this article.
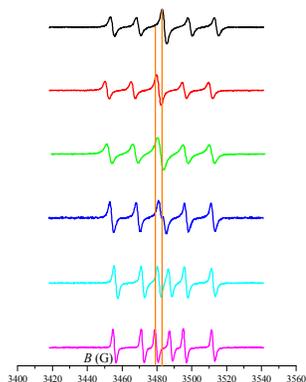
Figure 2. EPR signals, from pure THF (top) to pure water (bottom) and with different v% of water (from top to bottom, 0, 0.2, 0.6, 1.0, 2.0, and 100). Lines highlight the shift of the signal.

Figure 3. Plots a_N vs E_T^N for **1** ($\star$), **2** ($\bullet$), **4c** ($\blacksquare$), and **4t** ($\blacklozenge$). Empty symbols are for outliers. Numbers of solvent are given in SI.
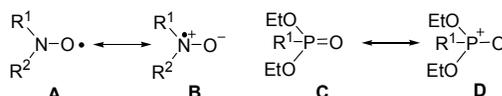
Figure 4. Canonical forms **A** and **B** of the nitroxyl moiety and **C** and **D** of the phosphoryl moiety

This unexpected decrease in a_N for $E_T^N < 0.2$ can only be ascribed to a puzzling increase in polarity in **4c** compared to **4t**. In fact, **4c** can exhibit two conformers (Fig. 5) **4cA** – diethoxyphosphoryl and hydroxyl groups in axial positions – and **4cE** – diethoxyphosphoryl and hydroxyl groups in equatorial positions. In **4cA**, an Intramolecular Hydrogen bonding (IHB) is possible through a 7-membered ring between the diethoxyphosphoryl and hydroxyl groups, the latter being favored in non H-bonding disrupting solvents for $E_T^N < 0.2$. The occurrence of IHB implies a change in the partial charge distribution in the P=O moiety favoring form **D** (Fig. 4) which in turn increases the electron widthdrawing properties of the P atom, i.e., the diethoxyphosphoryl group becomes a strong EWG, implying smaller a_N values than expected. This effect cannot be observed with **4t** as, whatever the conformations **4tA** and **4tE**, no IHB is possible (Fig. 5).

The hyperfine coupling constant of the atom at position β is given by the Heller-McConnel relationship (equation 2)[8',12] with B_0 being the constant for the transfer of the spin density through the spin polarization process (in general disregarded), B_1 being the constant for the transfer of the spin density through the hyperconjugation process, ρ_N^π the spin density on the nitrogen atom, and θ the dihedral angle between the SOMO and the C—P bond (Fig. 5).

As a consequence, an increase in a_P is expected with increasing ρ_N^π, i.e., increasing a_N, and decreasing angle θ.

$$a_{P,\beta} = B_0 + B_1 \cdot \rho_N^\pi \cdot \cos^2 \theta \quad (2)$$

Contra-intuitively, a_P for **2** and **4t** decrease when the polarity of the solvent E_T^N increases (Fig. 6),[2] i.e., when a_N increases. The effect is more marked for **2** than for **4t**, i.e., for **2** a_P changes from 40.8 G in *n*-hexane to 21.8 G in methanol whereas for **4t** it changes from 51.8 G in *n*-pentane to 47.3 G in NMF. Taking into account eq. 2, this decrease in a_P with increasing a_N implies a striking increase in θ for **2** and a small increase in θ for **4t**. In fact, polar solvents are expected to favor zwitterionic mesomeric forms **B** and **D**. Then, an increase in θ means that the phosphoryl and the nitroxyl moieties become increasingly close, favoring a stabilizing dipole – dipole interaction as depicted in the oxaphosphetane-like structure (Fig. 7).[3]

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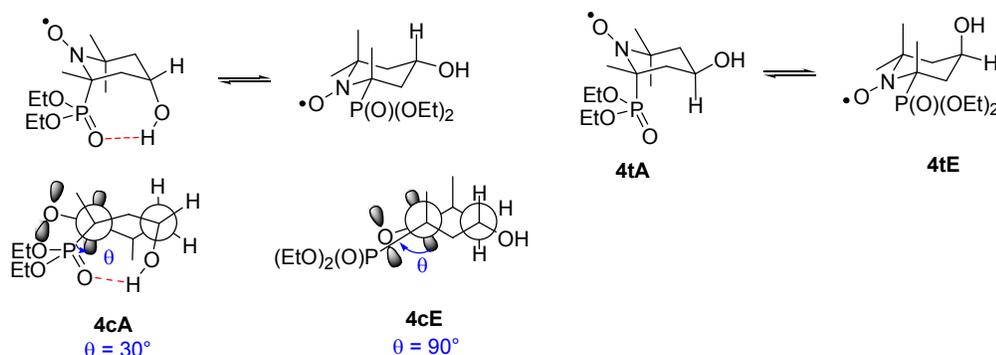
Figure 5. Conformational chair-chair equilibrium controlled by IHB in **4c** and **4t**. Dotted red line is for IHB. θ is the dihedral angle between the SOMO (gray orbitals) and the C—P bond
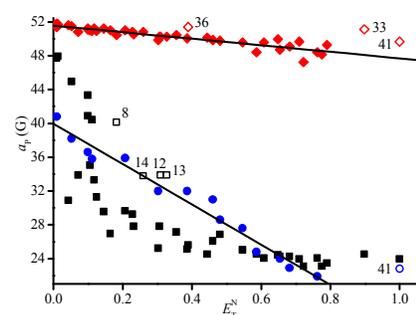
Figure 6. Plots a_P vs E_T^N for **2** (●), **4c** (■), and **4t** (◆). Empty symbols are for outliers. Numbers of solvent are given in SI.
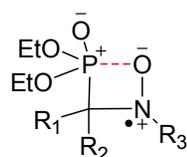
Figure 7. Oxaphosphetane-like structure.

The high a_P value in *n*-pentane for **4t** implies a small angle θ, meaning a strong hyperconjugation interaction between the SOMO and the C—P bond, implying that conformer **4tA** is the favored conformer. The small slope$^{\Omega}$ means that bond rotations are strongly impeded in **4t**.

Amazingly, **4c** exhibited the same trend as **2** and **4t** and a striking change in $\Delta a_P \approx 25$ G was observed from *n*-octane ($a_P \approx$ 48 G) to NMF ($a_P \approx 23$ G) as for **2**, except that a_P varies in a non linear manner with E_T^N. The puzzling changes in a_N (vide supra) for **4c** were assumed to be due to a conformational equilibrium between **4cA** and **4cE**, being favored in H-bonding disrupting solvents.

In apolar solvents ($E_T^N < 0.2$), the axial position of the diethoxyphosphoryl group in **4cA** maximized the overlapping between the SOMO and the C—P bond (small angle θ in Newman projections, Fig. 5) although 3 *syn*-1,3 interactions which were overbalanced by the IHB and afforded the high a_P value observed in *n*-hexane. On the other hand, in disrupting H-bonding solvents, conformer **4cE**, exhibiting only one *syn*-1,3 interaction, was favored implying the diethoxyphosphoryl group in equatorial position and, consequently, a large angle θ (Newman projections, Fig. 5) affording the small a_P values observed (Fig. 6). Angle θ can be estimated with eq. 2 for each solvent, as long as $B_1 \cdot \rho_N^{\pi}$ values are available. Assuming $B_1 \cdot \rho_N^{\pi}$ = 58 G in *n*-pentane,[13] θ_1 is given as 24° for **4c**,[‡] value very close to the value of angle θ observed in the Newman projection supporting conformation **4cA** in apolar solvents.

$$\frac{a_{P,1}}{a_{P,n}} = \frac{a_{N,1}}{a_{N,n}} \cdot \frac{\cos^2 \theta_1}{\cos^2 \theta_n} \qquad (3)$$

Using eq. 3 and θ_1, it is possible to determine θ_n for each solvent for **2**, **4t**, and **4c** (Fig. 8). The small variation in θ ($\Delta\theta$ = 10° from *n*-hexane to water) for **4t** confirms that bond rotations are impeded in **4t** and that conformations deviate only a little from conformer **4tA** (Fig. 5). The variation $\Delta\theta$ = 20° nicely confirms the change in conformation in **2** toward the occurrence of an azaoxaphosphetane-like structure (Fig. 7) in polar and protic solvents. As expected, the value $\Delta\theta$ = 32° denotes a dramatic change in the structure of **4c** in nice agreement with the chair-chair equilibrium between conformer **4cA**, favored in apolar solvents, and **4cE**, favored in polar and protic solvents.

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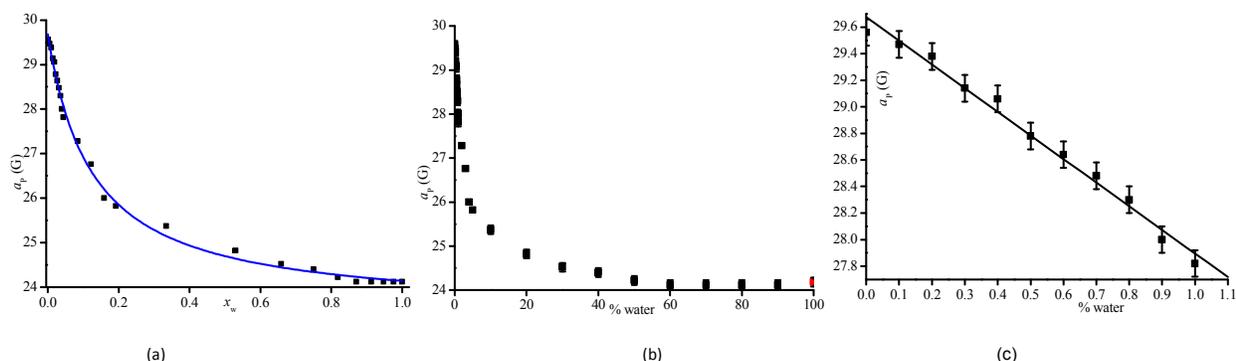
Figure 9. (a) Plots x_w (molar fraction of water) vs a_P for **4c**, blue curve is given by eq. 5. Plots % volume of water vs a_P for **4c** (b) from pure THF to pure water and (c) from pure THF to 1% water.

Interestingly, a difference Δa_P of ca. 5 G was observed between THF and water. As these two solvents are miscible in any amount, the effect of the binary mixture of solvents on a_N and a_P was investigated with the aim to develop the titration of water in solvent by EPR. As displayed in Fig. 2, EPR signals in THF (5 lines) and in water (6 lines) are very different. The EPR signal was recorded between 0 to 1% of water in THF by steps of 0.1%, and 10% to 100% of water by steps of 10% (Fig. 9). The effect of a binary mixture of solvents S1 and S2 on spectroscopic properties of the probe I has been intensively investigated using dyes. This effect is well accounted for by the simplified model (eq. 4), and the physical property Y is described as a function of the physical properties Y_1 and Y_2 in solvents S1 and S2, the preferential solvation parameters $f_{2/1}$, the initial mole fraction x_2^0 ($x_2^0 = n_2^0/(n_1^0 + n_2^0)$) of the solvent S2, and the parameter a (eqs. 5 and 6).[14,15]

$$I(S1) + S2 \rightleftharpoons I(S2) + S1 \quad (4)$$
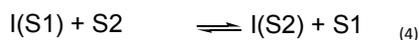
$$Y = Y_1 + \frac{a \cdot x_2^0}{\left(1 - x_2^0\right) + f_{2/1}\left(x_2^0\right)} \quad (5)$$

$$a = f_{2/1}\left(Y_2 - Y_1\right) \quad (6)$$

Therefore, Y_1 and Y_2 were defined as $a_{P,S1}$ and $a_{P,S2}$ for water as solvent S2 and THF as solvent S1, affording $a_{P,S1} = 29.7 \pm 0.1$ G, $a_{P,S2} = 24.1 \pm 0.1$, $f_{2/1} = 9.0 \pm 0.5$, $\chi^2 = 0.017$ and $R^2 = 0.997$. The value of $f_{2/1}$ larger than 1 means that **4c** would rather be solvated either with pure water or with some molecules of water in its cybotatic region than by pure THF.

Lucarini and coll.[16] performed the titration of benzyl alcohol using 2,2,6,6-tetramethylpiperidin-N-oxyl radical and its ability for intermolecular H-bonding. Thus, the difference of 2.70 G

for ≈ 2% water led us to investigate the potential of **4c** in the titration of water in THF. The plot % volume of water vs a_P (Fig. 9b) exhibits the same shape as the plot x_w (molar fraction of water) vs a_P. Obviously, the titration of *traces* (volume of THF lower than 50%) of THF in water is irrelevant. On the other hand, water in THF can be titrated accurately up to 30%, except that the relation is not linear. However, the enlargement of the zone from pure THF to 1% water (Fig. 6c) shows a linear relationship affording an easy titration down to 0.1% water in THF. Nevertheless, the titration of THF in water might be performed using other type of nitroxides.5

Notes and references

§ It was observed by spin-trapping of the diethoxyphosphoryl radical onto *tertio*butyl nitroso.

† The solvents used were of HPLC or EPR grade and from freshly opened bottles. Vials were filled with 1 ml of a 10^{-4} M stock solution in CH_2Cl_2. The solvent was removed and each vial filled with 1 ml of each of the solvent reported in Table 1SI. Then, a capillary was filled with 0.3 ml of each solution. The EPR signals were recorded on a Bruker X-band EMX machine. MW: 20 mW; gain: 2 105, amplitude modulation: 1 G, sweep time: 21 s, sweep width: 150 G. For water titration, THF was dried and freshly distilled as recommended[17] and bidistilled water was used. Binary mixtures were prepared by mixing the suitable volumes of water and of THF (Table 2SI), and then poured in vials containing nitroxide **4c**.

√ Diastereoisomers were obtained as racemic.

♫ The normalized Dimroth-Reichardt constant E_T^N described the polarizability/polarity and the hydrogen bond donor properties of the solvent, see ref. 7.

∫ y-intercepts are 15.20 (± 0.03) G, 14.15 (± 0.08) G, and 13.77 (± 0.03) G for **1**, **2**, and **4t**, respectively.

♪ Slopes are 1.55 (± 0.08), 2.13 (± 0.15), and 1.12 (± 0.07) for **1** (R^2 = 0.90, N = 44), **2** (R^2 = 0.96, N = 12), and **4t** (R^2 = 0.87, N = 43), respectively.

Ω For **4t** (R^2 = 0.83, N = 40), slope = -3. 87 (±0.27) and y-intercept = 51.57 (±0.11). For **2** (R^2 = 0.98, N = 15), slope = -24.10 (± 0.10) and y-intercept = 40.12 (± 0.48).

‡ For **4t**, θ_1 = 19° and, for **2**, θ_1 = 33°.

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