β-Phosphorus Hyperfine Coupling Constant in Nitroxide: Part 4: Titration of Water by Electron Paramagnetic Resonance

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Abstract: Recently, we showed that the phosphorus hyperfine coupling constant $a_{P\beta}$ of persistent cyclic nitroxides decreased with the normalized polarity Reichardt's constant E_T^N. Thus, we investigated the changes in $a_{P\beta}$ in binary mixtures of solvents. The sensitivity of $a_{P\beta}$ to the solvent was high enough to perform water titration in THF, 1,4-dioxane, and acetonitrile by EPR. Accuracies of a few percent were achieved.

Introduction

The magnetic properties of nitroxides,[1,2] which are pictured by the nitrogen hyperfine coupling constants a_N, are the cornerstone of many applications: nitroxides as pH-probes[3] or spin probes in Biology,[4] as agents for Dynamic Nuclear Polarization enhanced Nuclear Magnetic Resonance in Spectroscopy,[5-7] or as organic magnets in Material Sciences.[8]

Several investigations of the effect of solvents in the cybotactic region are available in the literature.[9-1213] They showed that a_N increased with increasing the normalized solvent-polarity Reichardt's constant E_T^N, as exemplified with **1•** (Figure 1).[14] Some studies showed that the hyperfine coupling constants $a_{H\beta}$[10] and $a_{F\beta}$[11] of hydrogen and fluorine at position β are weakly sensitive to the polarity of the solvent as well as to the temperature. [15] Recently, in the case of stable β-phosphorylated cyclic nitroxides **4•** - **6•** (Figure 1) as well as with the non-cyclic ones **3•**,[16] **7•**, and **8•** (Figure 1), we showed that $a_{P\beta}$ values vary weakly (**3•**, **4c•–6c•**, **7•**, and **8•**) to significantly (**4t•** - **6t•**)[§] with the solvent.

Thus, the variation in the difference in $a_{P\beta}$, $\Delta a_{P\beta}$, between organic solvents (vide infra) and water for **5c•,t•**, and **8•** was investigated for several binary mixtures. The observations reported were compared with the models developed for dyes. Moreover, the difference $\Delta a_{P\beta}$ was large enough to allow the titration of water by continuous wave Electron Paramagnetic Resonance (cw-EPR) in THF, 1,4-dioxane, and acetonitrile to be develop.

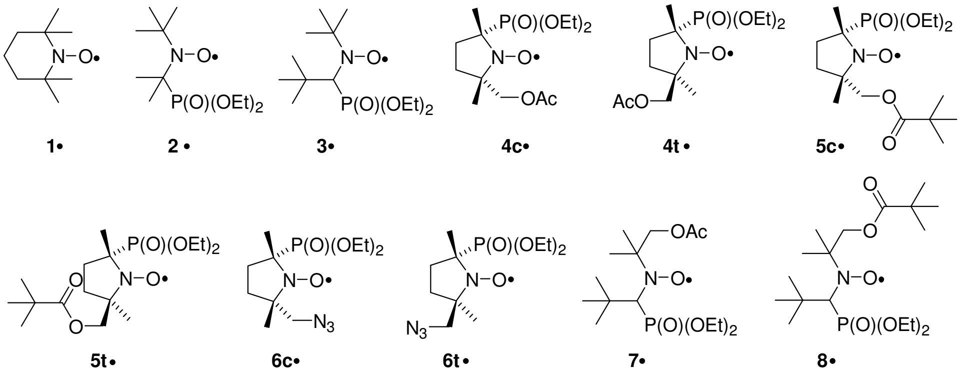

Figure 1. Nitroxides discussed in this article.

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Results

Among the nitroxides displayed in **Figure** 1, only **5c•,t•** and **8•** were investigated for the titration of water in THF, 1,4-dioxane, and acetonitrile. As expected, they exhibited the 6-line EPR signal due to the coupling between the nuclear spins of the nitrogen atom (I_N = 1) and phosphorus atom (I_P = ½) and the odd electron on the nitroxyl moiety. The nitrogen and β-phosphorus hyperfine coupling constants (hcc) a_N and $a_{\beta,P}$ are collected in Table 1.

Effect of binary mixtures of solvents.

Rosés *et al.*[17,18] developed a general model to take into account the effect of a binary mixture of solvents (S1 and S2) on an indicator *I* (Scheme 1), affording the observed physical variable *Y* through eq. (1), with Y_1 and Y_2 are the physical variables for solvent S1 and S2, respectively, with Y_{12} is the physical variable for the binary mixture, $f_{2/1}$ and $f_{12/1}$ are the preferential solvation parameters, x_2 the molar fraction of the solvent S2, and parameters *a* and *c* given by eqs. (2) and (3), respectively.

$$\mathrm{I(S1)}_m + m\mathrm{S2} \rightleftharpoons \mathrm{I(S2)}_m + m\mathrm{S1}$$
$$\mathrm{I(S1)}_m + m/2\mathrm{S2} \rightleftharpoons \mathrm{I(S12)}_m + m/2\mathrm{S1}$$

Scheme 1. General model of solvent exchange in binary mixtures

$$Y = Y_1 + \frac{a\left(x_2^0\right)^m + c\sqrt{\left[\left(1-x_2^0\right)x_2^0\right]^m}}{\left(1-x_2^0\right)^m + f_{2/1}\left(x_2^0\right)^m + f_{12/1}\sqrt{\left[\left(1-x_2^0\right)x_2^0\right]^m}} \qquad (1)$$

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

$$c = f_{12/1}\left(Y_{12} - Y_1\right) \qquad (3)$$

$$Y = Y_1 + \frac{a\left(x_2^0\right)^2 + c\left(1-x_2^0\right)x_2^0}{\left(1-x_2^0\right)^2 + f_{2/1}\left(x_2^0\right)^2 + f_{12/1}\left(1-x_2^0\right)x_2^0} \qquad (4)$$

Rosés *et al.*[17,18] showed that the models – one step exchange and two step exchange – proposed by Connors et al.[19] correspond to the limits of their own general model with *m* = 1 for the one-step model and *m* = 2 for the two-step model. Often, these models do not hold when a synergetic effect between solvents occurs, i.e., Y_{12} is larger than Y_1 and Y_2 (vide infra). Nevertheless, they showed that the two step model was nicely described by the 5-parameter eq.

(4) – Y_1, Y_2, Y_{12}, $f_{1/2}$, $f_{12/1}$.

$$\mathrm{I(S1)} + \mathrm{S2} \rightleftharpoons \mathrm{I(S2)} + \mathrm{S1}$$

Scheme 2. One step model for the binary solvent mixture effect.

$$\mathrm{I(S1)}_2 + \mathrm{S2} \rightleftharpoons \mathrm{I(S1S2)} + \mathrm{S1}$$
$$\mathrm{I(S1S2)} + \mathrm{S2} \rightleftharpoons \mathrm{I(S2)}_2 + \mathrm{S1}$$

Scheme 3. Two-step model for the binary solvent mixture effect.

Interestingly, Rosés and coll.[20] noticed that even the one -step model is a peculiar case of the two-step model when eqs. (5) and (6) hold, implying that eq. (4) is simplified into eq. (7). Thus, it is more judicious to fit the data with the 3-parameter eq. (7) – Y_1, Y_2, and $f_{2/1}$ – when suitable (non S-shaped curve) than with the overparameterized eqs. (1) and (4), the Y_{12}, $f_{12/1}$ and $f_{12/2}$ parameters being given by eqs. (6), (5), and (8), respectively.

Therefore, Y_1, Y_2, and Y_{12} were defined as $a_{\beta,P,S1}$, $a_{\beta,P,S2}$, $a_{\beta,P,S12}$ for water as solvent S1, acetonitrile, THF, or 1,4-dioxane as solvent S2, and for the S1/S2 binary mixture with water, respectively. All $a_{\beta,P}$ measured for the binary mixtures – water/THF, water/1,4-dioxane, and water/acetonitrile – for **5•** and **8•** were fitted (Figure 2) using eq. (7) and all parameters were given by eq. (5) - (8) . They are gathered in Table 2.

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Table 1. a_N and $a_{\beta,P}$ values, volumes (%) of water,[a] and molar fractions of water x_{water} in binary mixtures of water/THF, water/14D, and water /ACN for **5c,t•** and **8•**.

%	THF							1,4-dioxane (14D)					Acetonitrile (ACN)		
		a_N (G)			$a_{\beta,P}$ (G)				a_N (G)		$a_{\beta,P}$ (G)			a_N (G)	$a_{\beta,P}$ (G)
	x_{water}	**5c•**	**5t•**	**8•**	**5c•**	**5t•**	**8•**	x_{water}	**5t•**	**8•**	**5t•**	**8•**	x_{water}	**8•**	**8•**
0	0	13.54	13.24	13.66	46.09	51.20	44.05	0	13.43	13.66	50.74	43.49	0	13.77	43.49
4	15.8	-^{b}	13.46	-^{b}	-^{b}	50.95	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}
7	25.3	-^{b}	13.39	-^{b}	-^{b}	50.43	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}	-^{b}
10	33.3	13.79	13.49	13.65	45.27	50.19	44.08	34.6	13.48	13.86	49.91	43.42	24.1	13.88	43.42
20	52.9	13.84	13.68	13.74	44.73	49.89	43.81	54.3	13.71	13.92	49.10	43.31	41.6	14.08	43.31
30	65.8	13.98	13.79	13.75	44.69	49.45	43.67	67.1	13.8	13.97	48.78	43.08	55.0	14.13	43.08
40	75.0	14.11	13.82	13.89	44.58	49.29	43.50	76.0	13.93	14.14	48.50	42.91	65.5	14.24	42.91
50	81.8	14.16	13.99	14.02	44.11	49.01	43.34	82.6	13.96	14.26	48.08	42.78	74.0	14.22	42.78
60	87.1	14.3	14.05	14.25	43.91	48.53	43.25	87.7	14.28	14.39	47.87	42.66	81.0	14.4	42.66
70	91.3	14.58	14.23	14.17	43.75	48.05	42.98	91.7	14.37	14.39	47.79	42.66	86.9	14.54	42.66
80	94.7	14.66	14.34	14.66	43.35	47.65	42.76	95.0	14.41	14.62	47.43	42.51	91.9	14.74	42.51
90	97.5	14.82	-^{b}	14.53	43.37	47.25	42.60	97.7	14.71	14.88	47.29	42.36	96.2	14.92	42.36
100	100	14.96	14.6	14.7	43.07	46.78	42.36	100	14.6	14.7	46.78	42.36	100	14.8	42.36

[a] Given in % and before solvents are mixed. See experimental section. [b] Not measured.

Table 2. Parameters of water (S1)/S2 binary mixtures

entry	nitroxide	solvent S2[a]	$a_{\beta,P,water}$[b,c] (G)	$a_{\beta,P,sol.}$[b,c] (G)	$a_{\beta,P,S12}$[d] (G)	$f_{2/1}$[b,c]	$f_{12/1}$[e]	$f_{12/2}$[f]	R^2[g]	χ^2[h]	N[i]	x_{iso}[j]
1	**5c•**	THF	43.17 (11) 43.07[k]	45.92 (15) 46.09[k]	45.07	2.25 (46)	3.25	1.44	0.97	0.028	11	0.33
2	**5t•**	THF	46.88 (14) 46.78[k]	50.91 (11) 51.24[k]	50.13	4.17 (63)	5.17	1.23	0.98	0.037	13	0.21
3	**5t•**	14D	47.05 (9) 46.78[k]	50.68 (13) 50.74[k]	49.40	1.83 (26)	2.83	1.55	0.98	0.022	11	0.34
4	**8•**	14D	42.32 (4) 42.30[k]	43.55 (5) 43.70[k]	43.25	3.12 (49)	4.12	1.32	0.98	0.003	11	0.24
5	**8•**	THF	42.36 (4) 42.30[k]	44.11 (4) 44.10[k]	43.86	6.00 (62)	7.00	1.17	0.99	0.003	11	0.14
6	**8•**	ACN	42.17 (4) 42.30[k]	42.98 (3) 42.90[k]	42.77	3.00 (56)	4.00	1.33	0.98	0.001	10	0.29

[a] Co-solvent S2. [b] Error given in parentheses on the last digit. [c] Given by eq. (7). [d] Given by eq. (6). [e] Given by eq. (5). [f] Given by eq. (8). [g] Square of the regression coefficient. [h] Value for the chi-square test. [i] Number of data. [j]. Molar fraction at the isosolvation point (see text). [k] Experimental hccs in pure solvent.

As quoted by Reichardt:[14] "The *iso-solvation point* x_{iso} is defined as the point describing the bulk solvent composition at which both solvents S1 and S2 participate equally in the solvation shell of the cation or anion. It has been developed[21,22-23] for NMR chemical shift δ and is given as the solvent composition at which the NMR chemical shifts lie midway between the values for the two pure solvents." Taking into account that EPR relies on the similar physics to NMR,[24,25] x_{iso} (Table 2) should hold for $a_{\beta,P}$ values and is graphically read (Figure 2) as the value corresponding to the $a_{\beta,P,xiso}$ value given by eq. (9).

Data in Figure 2 were tentatively fitted using eqs. (1) or (4), which afforded good fits exhibiting as good statistical outputs as those obtained with eq. (7). However, errors on $f_{2/1}$, $f_{12/1}$ and $a_{\beta,P,S12}$ given both by eq. (1) and (4) were as large as the estimated values!

Connors *et al.*[19] ranked the binary solvent mixtures in two families: one-step models and two step models. However, Rosés *et al.*[17] showed that this ranking makes no sense because the one-step model is only a peculiar case of the two-step model. Hence, the water/1,4-dioxane, water/THF and water/acetonitrile mixtures belong to the two-step model family, as reported by Connors *et al.*[19] for the [2,6-diphenyl-4-(2,4,6-triphenyl-1-pyridinio)phenolate] dye and their corresponding data should be described by eq.

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(4), whereas they are nicely described by eq. (6) corresponding to the one-step model (Table 2 and Figure 2). On the other hand, Rosés *et al.*[17] ranked the solvents in 3 main groups: the first group with dipolar strong hydrogen bond acceptors and poor hydrogen bond donors (α ≤ 0.2 and β ≥ 0.4), e.g., DMSO, acetone, acetonitrile; the second group with strong hydrogen bond donors (α ≥ 0.2), e.g., water, CH_2Cl_2, $CHCl_3$; and the third group with poor hydrogen bond acceptors and poor hydrogen bond donors (α ≤ 0.1 and β ≤ 0.1), e.g., *cyclo*hexane, benzene. Binary mixtures of solvents from groups 1 and 2 are prone to exhibit a synergetic effect, i.e., S1 and S2 mixed together exhibit enhanced physical properties compared to pure S1 and pure S2. In such cases, the data are better fitted with eq. (1) and in *ideal* cases, they are fitted as a two-step model (m = 2), by eq. (4). Thus, the water/THF (α = 0 and β = 0.55) and water/acetonitrile mixtures were expected to exhibit an effect described by a parabolic (synergetic) curve or an S-shaped curve (2-step model), whereas good fits were obtained with eq. (7), i.e., one-step model. To the eyes, the curve for the water/THF mixture with **5•** as indicator (Figure 2c) might be S-shaped curve although this cannot be ascertained by fitting with the suitable equations (vide supra).

$$f_{12/1} = 1 + f_{2/1} \quad (5)$$

$$Y_{12} = \frac{Y_1 + f_{2/1} Y_2}{1 + f_{2/1}} \quad (6)$$

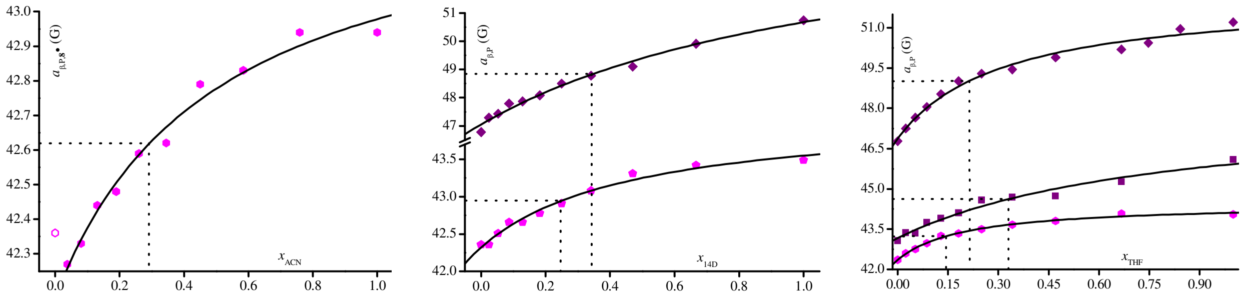
$$Y = Y_1 + \frac{a x_2^0}{\left(1 - x_2^0\right) + f_{2/1}\left(x_2^0\right)^2} \quad (7)$$

$$f_{12/2} = f_{12/1} / f_{2/1} \quad (8)$$

$$a_{\beta,\mathrm{P},x_{iso}} = a_{\beta,\mathrm{P,water}} + \frac{\left(a_{\beta,\mathrm{P,S2}} - a_{\beta,\mathrm{P,water}}\right)}{2} \quad (9)$$

Water titration in THF, 1,4-dioxane, and acetonitrile.

The titration of water using betaine dye is well known. It requires a large difference in wavelength between the two pure solvents and a high sensitivity of the probe.[26-28] As far as we know, this approach has not been developed using cw-EPR because the largest variation in a_N Δa_N = 1.8 G observed between *n*-hexane and water is too small for any applications.[#29] Indeed, the accuracy of EPR is around 0.05 G, i.e., ca. 0.3% of a_N or ca. 0.1% of $a_{\beta,P}$ to the phosphorylated nitroxides. However, a precision of 0.1 G is more likely implying accuracies of 0.5%, 1%, 2%, and 10% for Δa varying from 20 G, 10 G, 5 G, to 1 G. Besides a large Δa_N and a high accuracy on a_N (or any other physical constants), the probe must be soluble in the two solvents investigated and in the corresponding mixtures, and the solvents must be completely miscible in order to develop a reliable model. All nitroxides do not fulfil all these requirements, since Δa_N is often around 1 – 1.5 G. However, **2•** seems a very good candidate, with $\Delta a_{\beta,P}$ = 20 G between *n*-hexane and water, and $\Delta a_{\beta,P}$ = 13 G between THF and water, i.e., accuracy lower than 1%.[13,16]



(a) (b) (c)

Figure 2. Plots of $a_{\beta,P}$ vs x in (a) acetonitrile, (b) 1,4-dioxane, and (c) THF for **5c•** (■), **5t•** (◆), and **8•** (⬢). Empty symbol is for outlier. Full lines are given by eq. (7). Dotted lines are for determining x_{iso}

Table 3. EPR features of the selected nitroxides for the titration of water[a]

	5c•				5t•				8•			
Solvent	a_N	$a_{\beta,P}$	Δa_N	$\Delta a_{\beta,P}$	a_N	$a_{\beta,P}$	Δa_N	$\Delta a_{\beta,P}$	a_N	$a_{\beta,P}$	Δa_N	$\Delta a_{\beta,P}$
1,4-dioxane	13.74	45.25	1.22	2.18	13.43	50.74	1.17	*3.96*	13.66	43.75	1.04	1.39
THF	13.54	46.09	1.42	3.02	13.24	51.24	1.36	*4.46*	13.66	44.10	1.04	1.74
acetonitrile	13.61	43.65	1.35	0.58	13.86	48.33	0.74	*1.57*	13.77	42.94	0.93	0.58
water	14.96	43.07	0	0	14.60	46.78	0	0	14.70	42.36	0	0

[a] a_N, $a_{\beta,P}$, Δa_N and $\Delta a_{\beta,P}$ given in G.

Unfortunately, **2•** is not stable enough to allow such a type of experiment to be developed. On the other hand, all nitroxides **4•** - **8•** prepared for this article are stable at room temperature and exhibit EPR features suitable enough to perform model experiments. **5t•** was selected as model because it exhibits the largest $\Delta a_{\beta,P}$ for 1,4-dioxane and THF, **5c•** was also tested to investigate the influence of the nitroxide configuration on the solvent effect of binary

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mixtures, and **8•** was also selected as it exhibits the largest $\Delta a_{\beta,P}$ for non cyclic nitroxides (Table 3).

For each case, a nice linear dependence (eq. **(10**) and Figure 4) is observed between $a_{\beta,P}$ for **5c•,t•** and **8•** with the percent of water (%w) in 1,4-dioxane, THF, and acetonitrile (Table 4).

$$a_{\beta,P} = y_0 + \alpha_9 \cdot \%w \qquad (10)$$

Discussion

Binary solvent mixture effect.

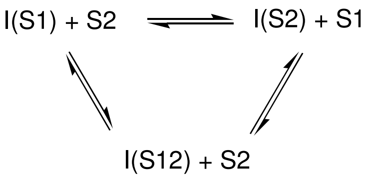
This dependence of $a_{\beta,P}$ on both the solvent and the nitroxide structure led us to investigate the effect of binary mixtures of solvents, especially water/organic solvent, in the aim of developing the titration of water in an organic solvent (vide infra). The results are discussed in the light of the model developed by Rosés *et al.*[17,18,20] (Scheme 1 and eqs. (1) - (8)). As mentioned in the results section, the results gathered in Table 1 are nicely described by a simple three-parameter model (eq. (7) and Scheme 2) rather than the complete seven-parameter model (Scheme 1 and (1)). Then, the $a_{\beta,P}$ reported in Table 2 can be discussed either as values due to the mixture of two species I(S1) and I(S2) (two species as in Scheme 1, with I for the nitroxide) or as values due to the mixture of three species I(S1), I(S2) and I(S12) (Scheme 4, with I for the nitroxide).

Table 4. Linear correlation $a_{\beta,P}$ = f(%w) for **5c,t•** and **8•** in 1,4-dioxane (14D), acetonitrile (ACN), and THF.

Eq	Solvent	Nitroxide	*y*-intercept[a]	Slope (10^{-2})[a]	R^{2b}	N^c
(10) a	THF	**5c•**	45.60 (13)	-2.7 (2)	0.95	11
(10)b	THF	**5t•**	50.88 (8)	-4.1 (2)	0.98	13
(10)c	14D	**5t•**	50.11 (18)	-3.4 (3)	0.94	11
(10)d	THF	**8•**	44.18 (7)	-1.7 (1)	0.98	11
(10)e	ACN	**8•**	42.96 (7)	-0.7 (1)	0.95	11
(10)f	14D	**8•**	43.47 (4)	-1.2 (1)	0.97	11

[a] Error given on the last digit. [b] Square of the regression coefficient. [c] Number of data.

Taking into account that $a_{\beta,PS12}$ are smaller than $a_{\beta,P,sol.}$, i.e., no synergetic effect, and very close to each other (less than 1.2 G of difference), only the one-step model (Scheme 2) will be discussed. The exchange of water molecules in the solvent cage ($f_{2/1}$) is clearly dependent on the solvent, as highlighted with **8•** (Table 2), that is, THF is more efficient at displacing water molecules from the solvation cage than 1,4-dioxane and acetonitrile. The same trend holds for **5t•**. The exchange of water molecules in the solvation cage by THF also clearly depends on the type of nitroxide, that is, the exchange is easier along series **5c•**, **5t•** and **8•**. Intuitively, **8•** is the most congested/shielded nitroxide, implying that the solvation cage should be the least efficient, i.e., the first layer of solvent is expected to be far from the nitroxyl moiety.**Error! Bookmark ot defined.** Consequently, the solvent effect should be the weakest, and, hence, water molecules are expected to be readily displaced. However, $f_{1/2}$ values cannot be correlated, even roughly, to one single parameter used for the KAT and KP relationships neither for **8•** nor for THF.[‡] Interestingly, $f_{2/1}$ and x_{iso} are correlated, that is, the higher $f_{2/1}$, the easier the solvent exchange in the cybotactic region is, the lower x_{iso} is, the more readily the effect of the water solvation is balanced. However, neither x_{iso} nor $f_{2/1}$ can be used to predict the efficiency of the nitroxide for the titration of water (vide infra).



Scheme 4. General scheme describing the three-parameter model.

Titration of water in organic solvents.

Table 3 displays EPR features suitable for water titration tested for **5c•**, **5t•**, and **8•** in THF, 1,4-dioxane, and acetonitrile. As shown in Figure 3, the EPR signal for **5t•** depends significantly on the THF/water mixture, highlighting nicely the potential of this family of nitroxides for the titration of water in an organic solvent and conversely. Nice linear plots $a_{\beta,P}$ vs % water (Figure 4) were obtained with all nitroxides and solvents. In THF, the efficiency, given by the absolute value of the slope α_9 in eq **(10)** (Table 4), increased in the series **8•**, **5c•**, and **5t•**. Thus, the best results for titration were obtained for **5t•**, although this is still far from the performance reached using the Karl-Fischer test.

Conclusions

As nitroxides **5•** and **8•** are stable at room temperature, it was possible to carefully investigate the solvent effect of binary solvent mixtures with water as one of the two solvents. As far as we know, this investigation led to the development of the first titration of water in a solvent using the cw-EPR technique. We showed that it was possible to reach an accuracy of a few percent in the titration of water.[†] Probes belonging to this family of nitroxides have recently been developed by our group,[30] and such a sensitivity of $a_{\beta,P}$ to water might be applied to probe the local environment of nitroxides attached on proteins. Moreover, it might be envisioned as a possible alternative of the approach based on pulsed-EPR and developed by Dzuba and coll.[31,32]

Experimental section

All solvents were purchased from Aldrich and were dried according to the conventional procedures.[33] Nitroxides **5c,t•** were prepared according to Scheme 5. Aziridine 9c and 9t were prepared according to the literature.[30] Nitroxide **8•** was prepared by esterification of **9•**[34] (Scheme 6). ^{1}H nuclear magnetic resonance (NMR) spectra were recorded using an internal deuterium lock at ambient temperatures on the following instruments: Bruker AC400 (400 MHz) and Bruker AC300 (300 MHz). Data are presented as follows: chemical

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shift (in ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br means the signal is broad, dd = doublet of doublets), coupling constant (*J* in Hz) and integration. ^{31}P NMR spectra were recorded on a Bruker AC300 (122 MHz) and on a Bruker AC400 (162 MHz) spectrometers with complete proton decoupling. Chemical shifts (δ) were reported in ppm using residual non-deuterated solvents as internal reference.[35]

High-resolution mass spectra (HRMS) were performed on a SYNAPT G2 HDMS (Waters) spectrometer equipped with atmospheric pression ionization source (API) pneumatically assisted. Samples were ionized by positive electrospray mode as follows: electrospray tension (ISV): 2800 V ; opening tension (OR): 20 V ; nebulization gas pression (nitrogen): 800 L/h. Low resolution mass spectra were recorded on ion trap AB SCIEX 3200 QTRAP equipped with an electrospray source. The parent ion (M^+, $[M+H]^+$, $[M+Na]^+$ or $[M+NH_4]^+$) is quoted.

Analytical thin layer chromatographies (TLC) were carried out on Merck Kieselgel 60 F254 plates. Flash column chromatographies were carried out on Merck Kieselgel 60 (230-400 mesh). Solvent system: gradients of DCM/MeOH; EtOAc/EtOH.

All experiments were performed under anhydrous conditions and an inert atmosphere of argon and, except where stated, using dried apparatus and employing standard techniques for handling air-sensitive materials. All reagents were weighed and handled in air at room temperature.

*Preparation of **10t**.* A solution of aziridine **9t** (500 mg, 2.02 mmol) was dissolved in 2 mL of acetic acid. The mixture was stirred overnight at room temperature under argon. Then, the mixture was dissolved in DCM and poured on a saturated solution of $NaHCO_3$. After several extractions with DCM, the organic layer was dried with $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (DCM/MeOH gradient). The acetate **10t** was obtained as a colorless oil (385 mg, yield 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.25-4.10 (m, 4H), 3.96 (d, *J* = 10.8 Hz, 1H), 3.85 (d, *J* = 10.8 Hz, 1H), 2.45-2.28 (m, 1H), 2.09 (s, 3H), 1.93-1.55 (m, 4H), 1.40-1.28 (m, 9H), 1.20 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9 (C), 71.0 (d, *J* = 2.2 Hz,CH_2), 62.7 (d, *J* = 7.3 Hz, CH_2), 62.0 (d, *J* = 8.1 Hz, C), 61.9 (d, *J* = 8.1 Hz, CH_2), 60.3 (d, *J* = 164 Hz, C), 34.4 (d, *J* = 5.1 Hz, CH_2), 34.1 (CH_2), 25.6 (CH_3), 25.3 (d, *J* = 8.1 Hz, CH_3), 20.8 (CH_3), 16.5 (d, *J* = 5.9 Hz, CH_3), 16.4 (d, *J* = 5.9 Hz, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$): δ 30.8. HRMS (ESI) calc for $C_{13}H_{27}NO_5P^+$: 308.1621 $[M+H]^+$; found: 308.1622.

*Preparation of **11t•***. A solution of amine **10t** (366 mg, 1.20 mmol) and *m*-CPBA (1.5 eq) in DCM was stirred 1 hour at 0 °C. Then, the reaction mixture was washed with $Na_2S_2O_3$, $NaHCO_3$. After extraction with DCM, the organic layer was dried with $MgSO_4$. The solvent was evaporated and the crude product was purified by flash chromatography (AcOEt/EtOH gradient). Nitroxide **11t•** was obtained as an orange oil (360 mg, yield 94%).HRMS (ESI) calc for $C_{13}H_{26}NO_6P^{+}\bullet$: 323.1492 $[M+H]^+$; found: 323.1494.

*Preparation of **12t•***. A solution of acetate **11t•** (200 mg, 0.621 mmol) in MeOH (5 mL) at O °C was treated with K_2CO_3 (129 mg, 0.931 mmol) and stirred for 1 hour. The mixture was dissolved in DCM and poured on saturated solution of $NaHCO_3$. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo.* Purification by flash chromatography (AcOEt/EtOH gradient) and recrystallization from Et_2O yielded the pure compound **12t•** as a red crystal. Yield 82% Mp = 63 °C. HRMS (ESI) calc for $C_{11}H_{24}N_4O_5P^{+}\bullet$: 281.1387 $[M+H]^+$; found: 281.1384. $C_{11}H_{23}NO_5P\bullet$ (280.3): calcd. C 47.14, H 8.27, N 5.00; found C 47.05, H 8.41, N 5.07.

*Preparation of **5t•***. To a solution of alcohol **12t•** (200 mg, 0.714 mmol) in DCM (3 mL) at room temperature under argon was added Et_3N (288 mg, 2.85 mmol), a catalytic amount of DMAP and pivaloyl chloride (172 mg, 1.43 mmol). The mixture was stirred for 1 day. Then, the mixture was poured on a saturated solution of $NaHCO_3$ and extracted with DCM. The organic layer was dried with $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by flash chromatography (AcOEt/EtOH gradient) to afford product **5t•**, 203 mg, yield 78%. HRMS (ESI) calc for $C_{16}H_{32}NO_6P^{+}\bullet$: 365.1962 $[M+H]^+$; found: 365.1962.

Molecules **10c**, **11c•**, **12c•**, and **5c•** were prepared according to the procedure described for **10t**, **11t•**, **12t•**, and **5c•**, respectively.

*Preparation of **10c**.* According to the procedure for the preparation of **10t**, aziridine **9c** (500 mg, 2.02 mmol) afforded acetate **10c** (433 mg, yield 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.3-4.05 (m, 4H), 3.98 (d, *J* = 10.3 Hz, 1H), 3.77 (d, *J* = 10.3 Hz, 1H), 2.40-2.25 (m, 1H), 2.06 (s, 3H), 1.98-1.87 (m, 1H), 1.85-1.55 (m, 3H), 1.42 (d, *J* = 15.6 Hz, 3H), 1.35-1.27 (m, 6H), 1.25 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7 (C), 70.7 (CH_2), 62.8 (d, *J* = 7.3 Hz, CH_2), 61.8 (d, *J* = 8.1 Hz, CH_2), 61.7 (d, *J* = 9.5 Hz, C), 60.4 (d, *J* = 172 Hz, C), 34.1 (d, *J* = 5.9 Hz, CH_2), 33.6 (d, *J* = 1.5 Hz, CH_2), 26.4 (CH_3), 25.3 (d, *J* = 8.1 Hz, CH_3), 20.71 (CH_3), 16.4 (d, *J* = 5.1 Hz, CH_3), 16.3 (d, *J* = 5.9 Hz, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$): δ 29.9. HRMS (ESI) calc for $C_{13}H_{27}NO_5P^+$: 308.1621 $[M+H]^+$; found: 308.1622.

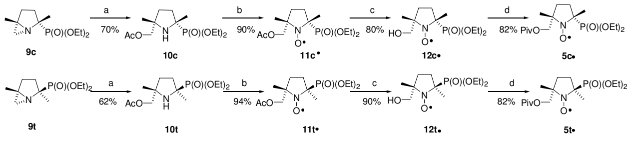
*Preparation of **11c•***. Oxidation of amine **10c** (200 mg, 0.65 mmol) afforded nitroxide **12c•** (189 mg, yield 90%). HRMS (ESI) calc for $C_{13}H_{26}NO_6P^{+}\bullet$: 323.1492 $[M+H]^+$; found: 323.1494

*Preparation of **12c•***. Saponification of **11c•**, and purification by flash chromatography (AcOEt/EtOH gradient) afforded the pure product **12c•** as a red oil. (157 mg, 90 %). HRMS (ESI) calc for $C_{11}H_{24}N_4O_5P^{+}\bullet$: 281.1387 $[M+H]^+$; found: 281.1384

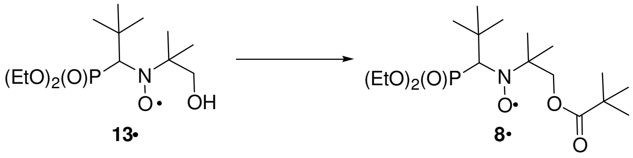
*Preparation of **5c•***. **12c•** (200 mg, 0.714 mmol) to product **5c•** (213 mg, yield 82%). HRMS (ESI) calc for $C_{16}H_{32}NO_6P^{+}\bullet$: 365.1962 $[M+H]^+$; found: 365.1962.

*Preparation of **8•***. To a solution of alcohol **13•**[34] (300 mg, 0.97 mmol) in dichloromethane (10 mL) at room temperature under argon was added Et_3N (293 mg, 2.90 mmol), a catalytic amount of 4-dimethylaminopyridine and pivaloyl chloride (351 mg, 2.91 mmol). The mixture was stirred for 20 hours. Then, the mixture was poured on a saturated solution of $NaHCO_3$ and extracted with dichloromethane. The organic layer was dried with $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by flash chromatography (dichlomethane/MeOH gradient 1%) to afford product **8•**, 333 mg, yield 87%. HRMS (ESI) calc for $C_{18}H_{38}NO_6P^{+}$•: 395.2431 $[M+H]^{+}$; found: 395.2432.

Titration experiments. Samples were prepared at 0.5 mM nitroxide concentration in non-degassed solvents. The titration of water in THF, 1,4-dioxane, and acetonitrile as solvents was performed using the EPR parameters: gain of 2 10^{5} (72 dB for Elexsys), modulation amplitude of 1.0 G, sweep width of 150 G, sweep time of 21 s, and a power of 20 mW. Nitroxides[£] were solved in dichlomethane, which was then evaporated. The vials were filled with 1 ml of the binary mixture of solvents under investigation. Samples were measured randomly and some test solutions were prepared.[♫]



Scheme 5. Synthesis of nitroxides **5c,t•** Reagents and conditions: (a) AcOH, 24 h, r.t; (b) *m*-CPBA, CH_2Cl_2, 2 – 4 h, r.t.; (c) K_2CO_3, MeOH, 24 h, r.t.; (d) PivCl, Et_3N, DMAP, CH_2Cl_2, 24 h, r.t.



Scheme 6. Synthetis of nitroxide **8•**. Reagents and conditions: PivCl, Et_3N, CH_2Cl_2, 20 h, r.t., 87%.

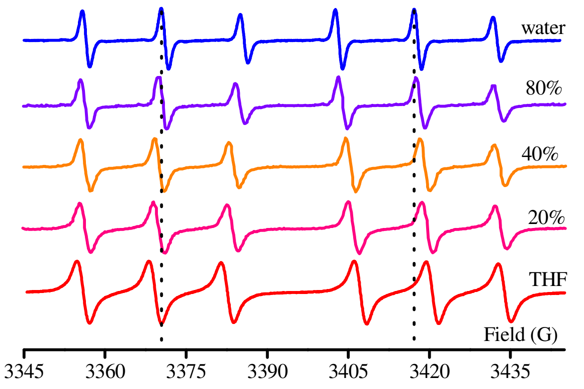


Figure 3. EPR signal for **5t•** in THF/water mixture (%) from 0:100 (top) to 100:0 (bottom). Vertical dotted lines are for visualizing the changes in $a_{P,\beta}$

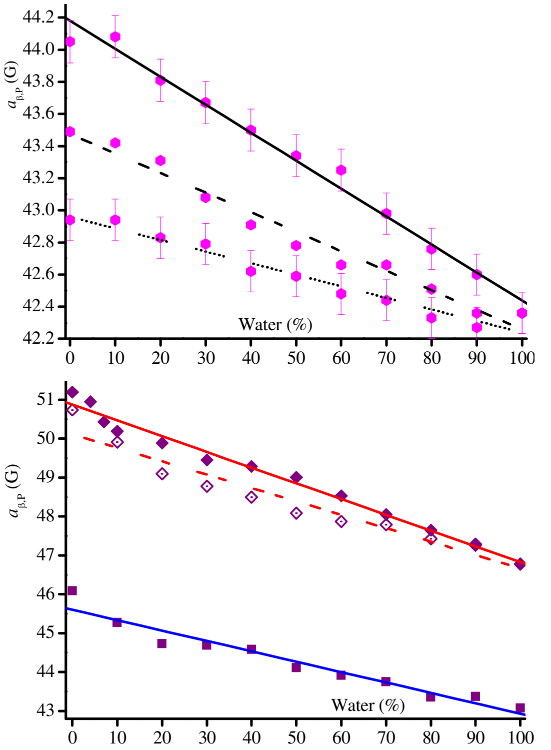


Figure 4. Plots of $a_{\beta,P}$ vs percent of water (%) in acetonitrile (····), 1,4-dioxane (--), and THF (—)with **8•** as probe (top), and with **5t•** (◆◇), and **5c•** (■) as probes (bottom).

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Notes and references

§ Despite a striking solvent effect ($\Delta a_{P\beta}$ = 18 G, see ref. 13), nitroxide **2•** was disregarded because it is weakly stable at room temperature.

\# Lucarini and coll. published similar titration using traces of benzylic alcohol in apolar solvent. See ref. 29.

‡ The set of data was too small to test multiparameter relationships.

† We are aware that an accuracy of a few percent cannot match the hundredth of a percent reached with a Karl-Fischer test or using dyes.

£ One milliliter is the sum of the volumes of the solvents used to prepare the binary mixture.

♫ These solutions were prepared by one of the co-authors and tested by another.

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