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(71) Applicants: UNIVERSITE D'AIX MARSEILLE [FR/FR]; Jardin du Pharo, 58 boulevard Charles Livon, 13007 Marseille (FR). CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE [FR/FR]; 3 rue Michel-Ange, 75016 Paris (FR).

(72) Inventors: MARQUE, Sylvain; Mazon Bise Genestelle, 07530 Antraigues-sur-Volane (FR). BUTSCHER, Teddy; Le Hameau des Pins, 13 chemin de Château Gombert, 13013 Marseille (FR). AUDRAN, Gérard; 70 chemin de St Mitre à Four de Buze, 13013 Marseille (FR). BREMOND, Paul; Résidence le Clos St Jérôme apt 36, 24 Traverse des Pâquerettes, 13014 Marseille (FR). BOSCO, Lionel; 34B boulevard Garoutte, 13012 Marseille (FR).

(74) Agents: DIAS, Sonia et al.; Gevers & Ores, 41 avenue de Friedland, 75008 Paris (FR).

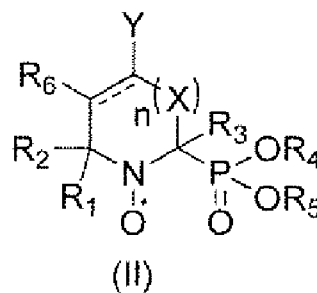
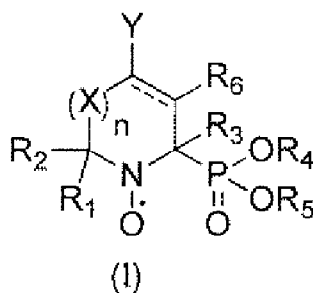
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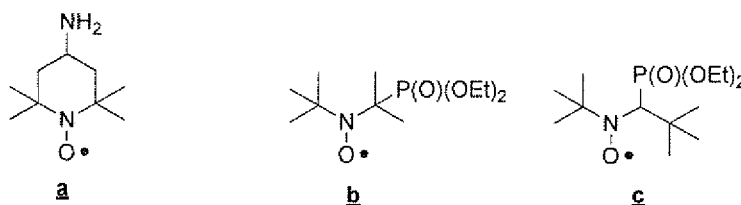
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(54) Title: DEVELOPMENT OF RADICAL PROBES HIGHLY SENSITIVE TO THE POLARITY

(57) Abstract: The present invention relates to a compound of formula (I) or (II) and its use for detecting and/or quantifying a solvent S₁ in a solvent S₂, S₁ and S₂ being distinct, wherein R₁, R₂, R₃, R₄, R₅, R₆, X, Y and n are as defined in claim 1.

DEVELOPMENT OF RADICAL PROBES HIGHLY SENSITIVE TO THE POLARITY

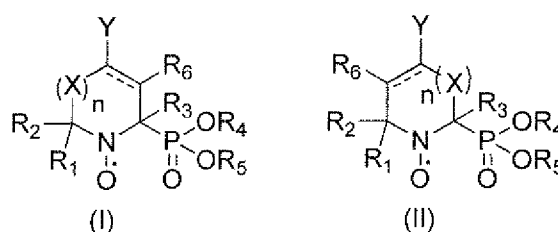
Since the seminal work of Knauer et al.^[1] on the effect of 40 solvents with 4 nitroxides, values of a_N for **a** (scheme 1) are considered as benchmark values and used for all discussion of solvent effect involving nitroxides. However, the sensitivity of nitroxides to solvent effect is rather weak, i.e. $\Delta a_N \approx 2$ G from *n*-hexane to water for **a**. However, in 1976, Il'Yasov and coll.^[2] reported a striking solvent effect (≈ 18 G) on the phosphorus hyperfine coupling constant a_P of the non-cyclic nitroxide **b**. However, this result did not raise any interest presumably because such a nitroxide was not stable. Recently,^[3] a weak effect (≈ 2 G from *n*-pentane to *N*-methyl formamide and ≈ 0.6 G from *n*-pentane to water) of the polarity of solvent on a_P for the stable non-cyclic nitroxide **c** has been reported.



It now has been discovered a class of stable betaphosphorylated nitroxides exhibiting a high sensitivity to solvent effect, that is, a significant change in the phosphorus hyperfine coupling constant (Δa_P), notably a Δa_P superior to 20 G, between two distinct solvents, herein referred as S_1 and S_2 .

Taking into account the high sensitivity (around ppb) of Electron Paramagnetic Resonance (EPR), this significant solvent effect is particularly advantageous either for detecting and/or quantifying a first solvent S_1 , for instance water, in a second solvent S_2 , for instance an organic solvent, or for investigating of changes in structure in materials when correlated to the change in polarity. As an example, a threshold of 0.1% by volume of water in THF can be detected using the betaphosphorylated nitroxides compounds according to the invention.

Thus, in one aspect, the invention relates to a compound of formula (I) or (II):

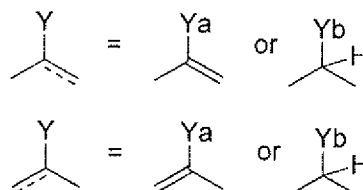


wherein:

R_1 is C_1 - C_6 alkyl optionally substituted by R_9 ;

R_2 , R_3 are independently selected from C_1 - C_6 alkyl, C_6 - C_{10} aryl, 5 to 7 membered heteroaryl optionally substituted by R_{10} ;

5 R_4 , R_5 are independently selected from C_1 - C_6 alkyl, C_6 - C_{10} aryl, 5 to 7 membered heteroaryl ;



10 Y_a is selected from C_1 - C_6 alkylcarbonyloxy, C_1 - C_6 alkoxy carbonyloxy, C_1 - C_6 alkylthiocarbonyl wherein said alkyl groups are optionally substituted by one or more F, and/or Cl, $\text{OSi}(\text{R}_{11})_3$, $\text{OSi}(\text{OR}_{11})_3$ and/or $\text{OSi}(\text{R}_{12})_3$, $\text{OSO}_2\text{R}_{13}$;

Y_b is H, OH or NHR_8 ;

X is $(\text{CH}_2)_m\text{CHR}_7(\text{CH}_2)_p$;

15 R_6 , R_7 are independently selected from H, OH, NH_2 , NHR_8 , an halogen atom, C_1 - C_6 ; alkyl, C_6 - C_{10} aryl, 5 to 7 membered heteroaryl;

R_8 is selected from H, C_1 - C_6 alkyl;

R_9 , R_{10} are each independently selected from OH, NH_2 , NHR_8 ;

R_{11} is at each occurrence independently selected from C_1 - C_6 alkyl, C_6 - C_{10} aryl;

20 R_{12} is independently F or Cl;

R_{13} is independently selected from C_1 - C_6 alkyl, C_1 - C_6 perfluoroalkyl, $(C_1$ - C_6)alkyl(C_6 - C_{10})aryl, and is notably CH_3 , CF_3 or tolyl ;

n is 0 or 1;

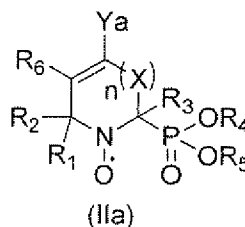
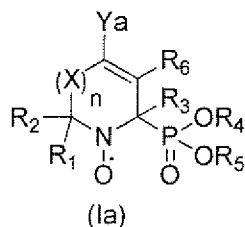
m is 0 or 1;

25 p is 0 or 1;

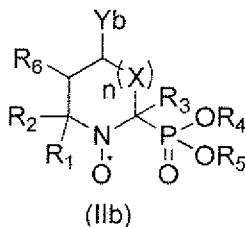
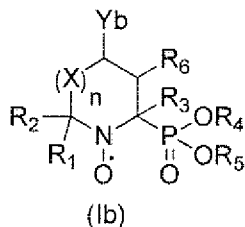
provided that when $\begin{array}{c} \text{Y} \\ | \\ \text{C} = \text{C} \end{array}$ or $\begin{array}{c} \text{Y} \\ | \\ \text{C} = \text{C} \end{array}$ is $\begin{array}{c} \text{Y}_b \\ | \\ \text{C} - \text{C} - \text{H} \end{array}$, then at least one of R_6 , R_7 ,

R_9 , Y_b is an hydrophilic group selected from OH, NH_2 , NHR_8 , said hydrophilic group being in *cis* position as regards the group $-\text{P}(=\text{O})(\text{OR}_4)(\text{OR}_5)$.

30 In one embodiment, the compound of formula (I) and (II) is a compound of formula (Ia) or (IIa) :



In another embodiment, the compound of formula (I) and (II) is a compound of formula (Ib) or (IIb) :



5 In a particular embodiment, n is 0.

In a further embodiment, n is 1.

In yet another embodiment, m+p is 0 or 1, notably 0.

In a further embodiment, m is 0 and p is 0.

In still a particular embodiment, Y_b is OH.

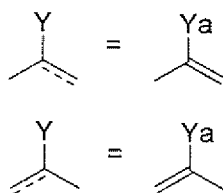
10 In an additional embodiment, R₂, R₃ are each independently a C₁-C₆ alkyl group.

In a further embodiment, R₄, R₅ are each independently a C₁-C₆ alkyl group.

In a specific embodiment, the compound of formula (Ib) is diethyl (4-hydroxy-2,6,6-trimethylpiperidin-2-yl-N-oxyl)phosphonate.

15 In a second aspect, the invention relates to the use of a compound of formula (I) or (II) as defined above for detecting and/or quantifying a solvent S₁ in a solvent S₂, S₁ and S₂ being distinct,

provided that when



20 then S₁ is water.

In one embodiment, S₁ is water and S₂ is an organic solvent.

In another embodiment, S₁ is an organic solvent and S₂ is water.

In an additional embodiment, S_1 and S_2 are each an organic solvent. Preferably, ΔE , that is the difference between the normalized Reichardt polarity Constant E_T^N between S_1 and S_2 corresponds to a $(\Delta)a_P$ larger than 4G.

In a particular embodiment, the change in the phosphorus hyperfine coupling constant of the compound of formula (I) or (II) is superior to 15 G between S_1 and S_2 .

In a further embodiment, the compound of formula (I) or (II) is used for detecting and/or quantifying a solvent S_1 crossing a polymeric film or a polymeric or biological membrane, said film or membrane having no permeability to the solvent S_2 containing the compound of formula (I) or (II).

As an example, the nitroxide compound of formula (I) may be dissolved in a non polar solvent and conversely. Then, it is possible to detect the traces of polar solvent crossing the film/membranes and conversely, using EPR as a technique of detection.

The film/membrane has no permeability to the solvent containing the nitroxide probe.

In a further embodiment, the compounds of formula (I) or (II) are used for studying or monitoring membrane porosity, film permeability, swelling of polymer, stability of materials, drug delivery.

Figures

Figure 1: EPR signal 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).

Figure 2: Plots a_N vs E_T^N for **a** (★), **b** (●), **8c** (■), and **8t** (◆). Empty symbols are for outliers.

Figure 3: Plots a_P vs E_T^N for **b** (●), **8c** (■), and **8t** (◆). Empty symbols are for outliers.

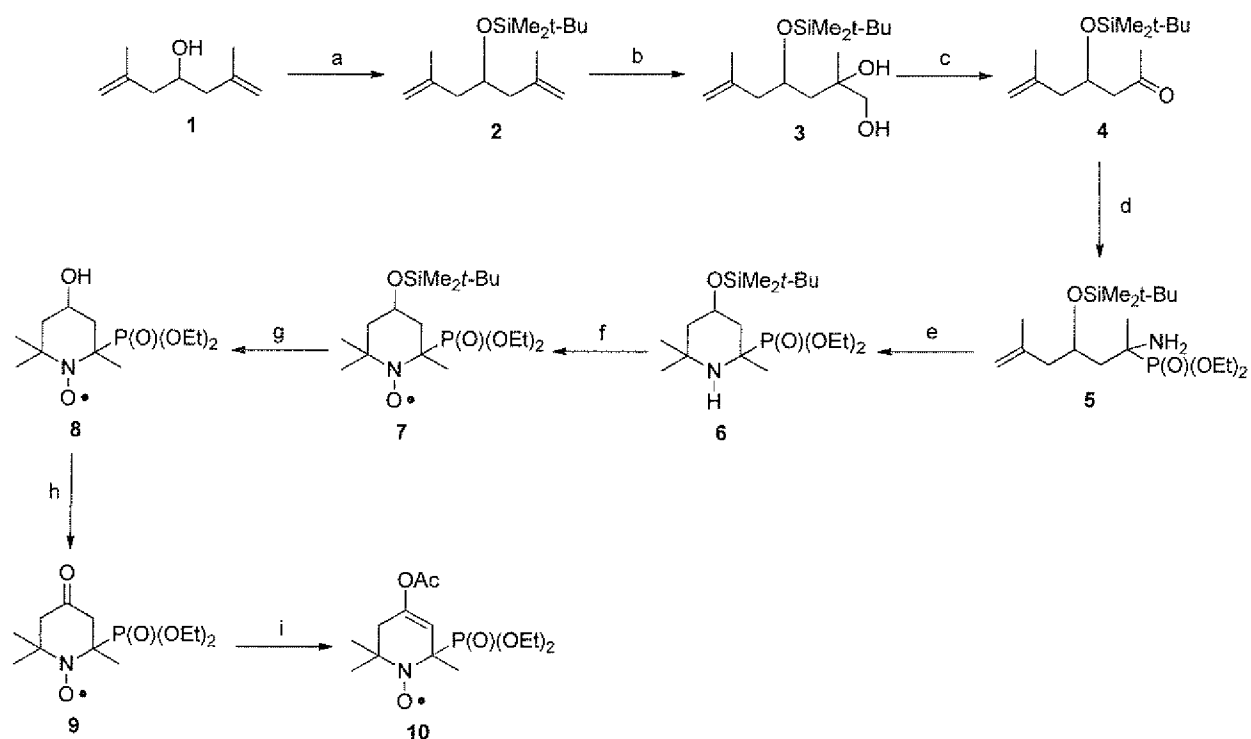
Figure 4: Plots θ vs a_P for **b** (●), **8c** (■), and **8t** (◆).

Figures 5, 6 and 7: (Figure 5) Plots x_w vs a_P for **8c**. Plots % volume of water vs a_P for **8c** (Figure 6) from pure THF to pure water and (Figure 7) from pure THF to 1% water.

Examples

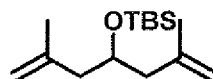
Example 1: Synthesis of diethyl (4-hydroxy-2,6,6-trimethylpiperidin-2-yl-N-oxyl)phosphonate (**8**) and diethyl (2,6,6-trimethyl-4-oxopiperidin-2-yl-N-oxyl)phosphonate (**10**)

Nitroxide **9** was prepared in **8** steps (Scheme 2) starting from the commercially available 2,6-dimethyl-4-hydroxy-hept-1,6-diene **1**. The hydroxyl function was protected with the conventional tert-butyldimethylsilyl chloride (TBDMSCl) to afford **2**. Then, one of the double bond of **2** was selectively oxidized into diol **3** with OsO₄ as oxidizing reagent. Diol **3** oxidized into ketone **4** with NaIO₄. Then, aminophosphonate **5** was obtained under condition specific for aminophosphorylation. Cyclization of **5** into **6** was performed in the presence of Hg(OAc)₂ and the crude materials was oxidized with meta-chloroperoxybenzoic acid (m-CPBA) into nitroxide **7**. The protecting group of the latter was hydrolyzed to yield **8** which was oxidized into **9**. The regioselectivity of the formation of the intracyclic double bond to afford **10** was controlled by using a bulky base, potassium hexamethyldisilazane (KHMDs), which reacted with the most accessible acidic protons which were the farer from the diethoxyphosphoryl group.



Scheme 2

a) tert-butyl((2,6-dimethylhepta-1,6-dien-4-yl)oxy)dimethylsilane (**2**).



Alcohol **1** (11.5 g, 82.0 mmol), which was prepared according to the method disclosed into Breit, B. ; Breuninger, D. J. Am. Chem. Soc. 2004, 126, 10244, was dissolved

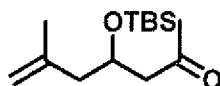
in DMF (250 mL), imidazole (17 g, 246 mmol) and *tert*-butyldimethylsilyl chloride (25 g, 164 mmol) were added, and the mixture was stirred for 5 h at 0 °C. The solution was poured into water and extracted with Et₂O. The organic layers were washed with water, brine, dried with MgSO₄, filtered, and concentrated. Column chromatography yielded 20.6 g (99%) of **2** as a colorless oil. ¹H NMR (400 MHz, CDCl₃): δ 4.77 (br s, 2H), 4.71 (br s, 2H), 3.93 (q, *J* = 6.3 Hz, 1H), 2.20-2.11 (m, 4H), 1.73 (s, 6H), 0.87 (s, 9H), 0.04 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 143.0 (2C), 113.2 (2CH₂), 69.8 (CH), 46.0 (2CH₂), 26.1 (3CH₃), 23.2 (2CH₃), 18.3 (C), -4.4 (2CH₃).

b) 4-((*tert*-butyldimethylsilyl)oxy)-2,6-dimethylhept-6-ene-1,2-diol (3**).**



Alkene **2** (4.3 g, 16.9 mmol) was dissolved in 200 ml of a mixture acetone/water (3/1 v:v), then OsO₄ (1 mL of 4% w in water) was added and the mixture was stirred for 15 min at 0 °C. After that, *N*-methylmorpholine-*N*-oxide (4.0 g, 33.8 mmol) was added and stirred for 4 h at 0 °C. The solution was poured into 10 % aqueous Na₂S₂O₃ and extracted with Et₂O. The organic layers were washed with water, brine, dried with MgSO₄, filtered, and concentrated. The crude product was obtained as a mixture of 2 diastereoisomers (ratio 2:1). Column chromatography afforded 3.38 g of **2** and 0.9 g (86%) of **3** as a yellowish oil. ¹H NMR (400 MHz, C₆D₆): δ 4.78 (br s, 1H, M), 4.76 (br s, 1H, m+M), 4.70 (br s, 1H, m), 4.26 (m, 1H, m+M), 3.78 (s, 1H, m), 3.45-3.28 (m, 2H m, 3H M), 2.37-2.27 (m, 1H, m+M), 2.22-2.07 (m, 1H M, 2H m), 1.87-1.70 (m, 2H M, 1H m), 1.65-1.50 (m, 4H M, 4H m), 1.18 (s, 3H, M), 1.14 (s, 3H, m), 0.94 (s, 9H, m + M), 0.11 (s, 3H, m), 0.09 (s, 3H, M), 0.05 (s, 3H, m+M). ¹³C NMR (75 MHz, C₆D₆): δ 142.5 (C, M), 142.0 (C, m), 113.9 (CH₂, m), 113.7 (CH₂, M), 73.0 (C, m), 72.7 (C, M), 71.0 (CH₂, m), 70.1 (CH₂, M), 69.7 (CH, m), 69.6 (CH, M), 47.9 (CH₂, m+M), 44.3 (CH₂, M), 43.1 (CH₂, m), 26.2 (CH₃, M), 26.2 (CH₃, m), 25.5 (CH₃, M), 24.0 (CH₃, m), 22.9 (CH₃, M), 22.8 (CH₃, m), 18.2 (C, M), 18.2 (C, m), -3.4 (CH₃, m), -3.5 (CH₃, M), -4.2 (CH₃, M), -4.3 (CH₃, m).

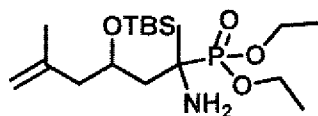
c) 4-((*tert*-butyldimethylsilyl)oxy)-6-methylhept-6-en-2-one (4**)**



Diol **3** (6.0 g, 20.8 mmol) was solved in 300 ml of a THF/water mixture (3/1 v:v) with 11.0 g (52.0 mmol) of NaIO₄ and stirred at 0 °C for 3 h. The solution was poured into water and extracted with Et₂O. The organic layers were washed with water, dried with

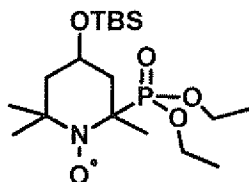
MgSO₄, filtered, and concentrated. Column chromatography yielded 5.11 g (96%) of 4 as a yellowish oil. ¹H NMR (400 MHz, CDCl₃): δ 4.78 (br s, 1H), 4.70 (br s, 1H), 4.36-4.28 (m, 1H), 2.60-2.47 (m, 2H), 2.30-2.10 (m, 2H), 2.15 (s, 3H), 1.74 (brs, 3H), 0.86 (s, 9H), 0.07 (s, 3H), 0.03 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): δ 207.9 (C), 142.3 (C), 113.7 (CH₂), 67.8 (CH), 50.5 (CH₂), 46.6 (CH₂), 31.8 (CH₃), 25.9 (3CH₃), 23.0 (CH₃), 18.05 (C), -4.5 (CH₃), -4.8 (CH₃).

d) diethyl (2-amino-4-((tert-butyldimethylsilyl)oxy)-6-methylhept-6-en-2-yl)phosphonate(5)



Ketone 4 (1.0 g, 3.9 mmol) was dissolved in 5 ml of diethylphosphite. Molecular sieves 4Å were added and the mixture was stirred under an ammoniac atmosphere for 24 h. Then, the mixture was filtered and the excess of diethylphosphite was removed under vacuum (50 °C, 2 mmHg). Column chromatography afforded 5 (550 mg, 37%) as a yellowish oil. ¹H NMR (400 MHz, CDCl₃): δ 4.77 (br s, 1H, m+M), 4.71 (br s, 1H, m+M), 4.42-4.32 (m, 1H, M), 4.27-4.20 (m, 1H, m), 4.19-4.07 (m, 4H, m+M), 2.34-2.13 (m, 2H, m+M), 1.95-1.85 (m, 1H, m+M), 1.80-1.55 (m, 6H, m+M), 1.37-1.28 (m, 9H), 0.88 (s, 9H, m+M), 0.12 (s, 3H, M), 0.12 (s, 3H, m), 0.11 (s, 3H, m), 0.11 (s, 3H, M). ³¹P NMR (162 MHz, CDCl₃): δ 31.67 (M), 30.88 (m). ¹³C NMR (75 MHz, CDCl₃): δ 142.7 (C, M), 142.2 (C, m), 113.5 (CH₂, m), 113.3 (CH₂, M), 67.8 (d, *J* = 13.2 Hz, CH, m), 67.6 (d, *J* = 10.5 Hz, CH, M), 62.5-62.1 (d overlapped, 2CH₂ m, 2CH₂ M), 52.0 (d, *J* = 155 Hz, C, m), 51.8 (d, *J* = 146 Hz, C, M), 48.16 (CH₂, M), 48.0 (CH₂, m), 42.7 (d, *J* = 3.9 Hz, CH₂), 41.9 (CH₂), 26.0 (3CH₃), 23.8 (CH₃), 22.9 (d, *J* = 2.2 Hz, CH₃, M), 22.7 (d, *J* = 2.2 Hz, CH₃, m), 18.0 (C, M), 18.0 (C, m), 16.6 (d, *J* = 5.5 Hz, CH₃), -3.6 (CH₃, m), -3.6 (CH₃, M), -4.1 (CH₃, m), -4.1 (CH₃, M).

e) diethyl(4-((tert-butyldimethylsilyl)oxy)-2,6,6-trimethylpiperidin-2-yl-N-oxyl)phosphonate (7).



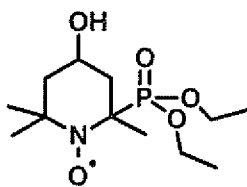
The aminophosphonate 5 (400 mg, 1.02 mmol) was dissolved in 50 mL of THF/water mixture (3/1 v:v). Hg(OAc)₂ (389 mg, 1.22 mmol) in 50 mL of the same solvent was slowly added to the aminophosphonate mixture and was stirred for 30 min at room

temperature. Then, the mixture was poured on 50 ml of aqueous solution of NaBH₄ (77 mg, 2.03 mmol) and NaOH (163 mg, 4.06 mmol), extracted with Et₂O. The organic layers were washed with brine, dried with MgSO₄, filtered, and concentrated to yield 397 mg of piperidine 6 which was used in the next step without further purifications.

A solution of piperidin 6 (900 mg, 2.29 mmol) and *m*-CPBA (1.26 g, 4.58 mmol) in DCM was stirred 2 hours at 0 °C. The solution was poured into 10 % aqueous Na₂S₂O₃ and extracted with DCM. The organic layers were washed with saturated aqueous solution of NaHCO₃, dried with MgSO₄, filtered, and concentrated. The crude product was obtained as a mixture of 2 diastereoisomers (ratio 2:1). Column chromatography yielded 685 mg of 7 (73%) as a red oil. The two diastereoisomers was separated and the major one was crystalized from Et₂O.

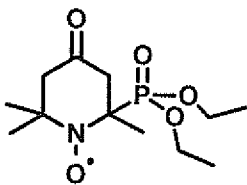
f) diethyl (4-hydroxy-2,6,6-trimethylpiperidin-2-yl-N-oxyl)phosphonate

(8)



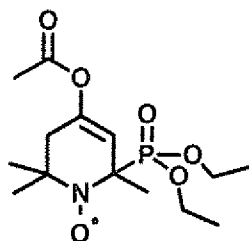
TBAF (1.0 M solution in THF, 1.47 mL, 1.47 mmol) was added dropwise to an ice-cold solution of silyl ether 7 (400 mg, 0.979 mmol) in dry THF (4 mL), under argon. The mixture was stirred 3 hours at room temperature and poured in a column of silica gel to afford 262 mg of 8 (91%) as a red oil.

g) diethyl (2,6,6-trimethyl-4-oxopiperidin-2-yl-N-oxyl)phosphonate (9)



N-methylmorpholine *N*-oxide (128 mg, 1.09 mmol, 4.0 equiv.) was added to a stirred ice-cold solution of 8 (80 mg, 0.272 mmol) with a catalytic amount of tetrapropylammonium perruthenate and powdered molecular sieves 4 Å in dry DCM (10 mL) under an argon atmosphere. After 20 min of stirring at room temperature, the reaction mixture was added to a column of silica gel to afford 70 mg of 9 (88%) as an orange solid.

h) 2-(diethoxyphosphoryl)-2,6,6-trimethyl-1,2,3,6-tetrahydropyridin-4-yl-N-oxyl acetate (10)



Ketone **9** (100 mg, 0.342 mmol) in 5 mL of dry THF was slowly added to a solution of KHMDS (1.0 M solution in THF, 0.513 mL, 0.513 mmol) in 5 mL of dry THF. The enolate formation was kept 3 hours from -80 °C to -45 °C. Then, freshly distilled Ac₂O (70 mg, 0.684 mmol) was slowly added. The mixture was stirred 2.5 hours. After that, the solution was poured on brine and extracted with EtOAc. The organic layers were dried with MgSO₄, filtered, and concentrated. Column chromatography yielded 105 mg (91%) of **10** as a red oil.

Example 2: Study of a_N^a and a_P^a values of **8c** and **8t** in various solvents

The two diastereoisomers **8c** and **8t** (Figure 1) with the diethoxyphosphoryl and hydroxyl groups in *cis* and *trans* relationships, respectively, were investigated separately. The effect of 45 solvents (Table 1) on a_N and a_P was investigated by EPR as well as the effect of the THF/water binary mixture of solvent from pure THF to pure water (Table 2) as exemplified in Figure 1.

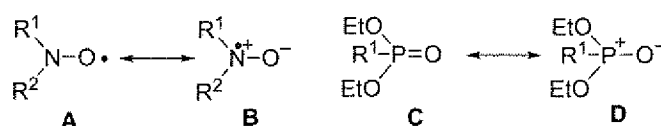
	solvent ^b	E_T^{Nc}	8c^a		8t^a	
			a_N	a_P	a_N	a_P
1	<i>n</i> -pentane	0.009	13.67	47.75	13.58	51.80
2	<i>n</i> -hexane	0.009	13.09	47.75	13.77	51.41
3	CHex	0.006	13.96	47.85	13.77	51.61
4	<i>n</i> -octane	0.012	13.58	47.95	13.67	51.70
5	benzene	0.111	14.44	40.44	14.06	50.93
6	toluene	0.099	14.25	40.92	13.96	51.12
7	<i>t</i> -BuPh	0.099	13.86	43.33	13.86	51.22
8	PhBr	0.182	14.44	40.15	14.06	50.45
9	Pyridine	0.302	15.31	25.23	14.15	49.87
10	AcPh	0.306	15.11	27.82	14.15	50.26
11	<i>t</i> -BuPH/ CH ₂ Cl ₂	$\mathcal{J}$	14.63	36.20	14.06	49.97
12	CH ₂ Cl ₂	0.309	14.73	33.89	14.15	50.26
13	DCE	0.327	14.73	33.89	14.15	50.26
14	CHCl ₃	0.259	14.83	33.79	14.25	50.83
15	CCl ₄	0.052	13.77	44.96	13.96	51.51
16	DME	0.231	14.92	27.83	13.96	50.55
17	Et ₂ O	0.117	14.73	33.31	13.96	51.22
18	<i>i</i> -Pr ₂ O	0.105	14.63	35.05	13.67	51.03
19	<i>n</i> -Bu ₂ O	0.071	14.54	33.89	13.96	50.83

20	Met-BuO	0.124	14.44	31.29	13.86	50.93
21	14D	0.164	15.02	26.96	14.15	51.03
22	THF	0.207	14.92	29.65	14.15	51.03
23	AcOEt	0.228	15.02	29.27	13.96	50.83
24	acetone	0.355	15.31	27.15	14.15	50.45
25	ACN	0.460	15.40	26.09	14.15	49.87
26	MeNO ₂	0.481	15.31	26.86	14.25	49.78
27	DMSO	0.444	15.40	24.55	14.15	50.16
28	F	0.775	15.69	23.11	14.63	48.14
29	NMF	0.722	15.50	23.11	14.44	47.27
30	DMF	0.386	15.50	25.13	14.06	50.06
31	MeOH	0.762	15.40	24.07	14.53	48.43
32	EtOH	0.654	15.40	24.26	14.54	48.72
33	TFE	0.898	15.89	24.55	14.73	51.13
34	<i>i</i> -PrOH	0.546	15.21	25.03	14.35	49.58
35	<i>n</i> -BuOH	0.586	15.31	24.55	14.35	48.43
36	<i>t</i> -BuOH	0.389	15.31	25.61	14.25	51.41
37	BnOH	0.608	15.60	24.07	14.44	49.58
38	EG	0.790	15.60	23.49	14.54	49.29
39	TEG	0.682	15.11	24.26	14.35	49.10
40	water/ MeOH	0.71	15.79	23.97	14.92	49.68
41	water	1	16.08	23.97	15.21	49.68
42	Tampon	^f	16.08	23.97	15.21	49.78
43	AcOH	0.648	15.50	24.45	14.44	49.97
44	Et ₃ N	0.043	13.78	30.91	13.86	51.61
45	<i>i</i> -Pr ₂ NH	0.145	14.70	29.56	13.86	51.22

Table 1. a_N^a and a_P^a values of **8c** and **8t** in 45 solvents^b as well as the corresponding normalized Reichardt polarity solvent constant E_T^{Nc} .

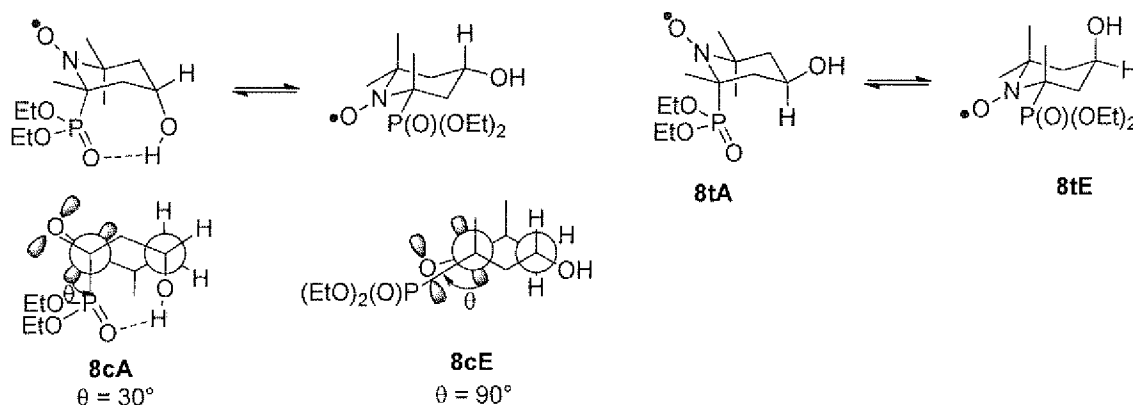
^a a_N and a_P are given in G (1 G = 0.1 mT). ^b CHex: *cyclo*-hexane, tBuPh: *tert*-butylbenzene, PhBr: bromobenzene, AcPh: acetophenone, DCE: 1,2-di-chloroethane, DME: 1,2-dimethoxyethane, 14D: 1,4-dioxane, THF: tetrahydrofuran, AcOEt: ethyl acetate, ACN: acetonitrile, DMSO: dimethylsulfoxide, F: formamide, NMF: *N*-methylformamide, DMF: *N,N*-dimethylformamide, TFE: 2,2,2-trifluoroethanol, EG: ethylene glycol, TEG: triethylene glycol, AcOH: acetic acid, BnOH: benzylic alcohol. ^c E_T^N values are given in Ref. ^[4] unless otherwise mentioned. ^f Not available.

The changes in a_N for **8c** and **8t** as well as the changes in a_P for **8t** are nicely accounted by effect recently discussed for several nitroxide models.



Scheme 2: Canonical forms A and B of the nitroxyl moiety and C and D of the phosphoryl moiety

$$a_N = Q \cdot \rho_N \quad (1)$$



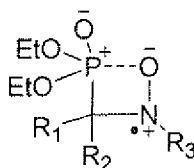
- 5 *Scheme 3 : Conformational chair-chair equilibrium controlled by IHB in 8c and 8t. Dotted line is for IHB. q is the dihedral angle between the SOMO (gray orbitals) and the C—P bond. A and E are for the diethoxyphosphoryl group in axial and equatorial positions, respectively.*

The hyperfine coupling constant of atom at the position β is given by the
 10 Heller-McConnel relationship (equation 2) with B_0 for the constant for the transfer of the spin density through the spin polarization process (in general disregarded), B_1 for 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. As consequence, an increase of a_P is expected with increasing ρ_N^π , that is, increasing a_N , and
 15 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 **8c** and **8t** decrease with increasing the polarity of the solvent E_T^N (Figure 3)^[5], i.e., when a_N increases. However, the effect is more marked for **8c** than for **8t**, i.e., for **2** a_P changes for $a_P = 40.80$ gauss in *n*-hexane to $a_P = 21.80$ in methanol
 20 whereas for **8t** it changes from $a_P = 51.80$ G in *n*-pentane to $a_P = 47.27$ G in NMF. Taking into account equation 2, this decrease of a_P with increasing a_N implies a striking increase of θ for **8c** and a small increase for **8t**. 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 moiety becomes closer and closer favoring a stabilizing dipole – dipole interaction as depicted

in the oxaphosphetane-like structure (Scheme 4). Moreover, the large a_P value in *n*-pentane for **8t** implies a small angle θ meaning a strong hyperconjugation interaction between the SOMO and the C—P bond implying that conformer **8tA** is the favored conformer. The small slope means that bond rotation are strongly impeded in **8t**.



Scheme 4. Oxaphosphetane-like structure.

Amazingly, **8c** exhibit the same trend as **b** and **8t** and a striking change of $\Delta a_P \approx 25$ G was observed from *n*-octane ($a_P = 47.95$ G) to NMF ($a_P = 23.11$ G) as for **b** except that a_P varies in an exponential fashion with E_T^N . As mentioned above, the puzzling changes in a_N for **8c** were assumed due to a conformational equilibrium between **8cA** and **8cE**, **8cE** being favored in H-bonding disrupting solvents. Then, in apolar solvents ($E_T^N < 0.2$), the axial position of the diethoxyphosphoryl group in **8cA** maximized the overlapping between the SOMO and the C—P bond (small angle θ in Newman projections in Scheme 3) although 3 *syn*-1,3 interactions which are overbalanced by the IHB and afforded the high a_P value observed in *n*-hexane. On the other hand, in disrupting H-bonding solvents, the conformer **8cE**, exhibiting only one *syn*-1,3 interaction, is favored involving the diethoxyphosphoryl group in equatorial position and, consequently, a large angle θ (Newman projections in Scheme 4) affording the small a_P values observed (Figure 3).

The angle θ can be estimated with equation 2 for each solvent as long as $B_1 \cdot \rho_N^\pi$ values are available. Thus, assuming $B_1 \cdot \rho_N^\pi = 58$ G in *n*-pentane, θ_1 is given as ca. 25° for **8c**, values very close from the angle θ observed in the Newman projection supporting the conformation **8cA** in apolar solvents. Using equation 3 and θ_1 , it was possible to determine θ_n for each solvent for **b**, **8t**, and **8c** as displayed in Figure 4. The small variation in θ ($\Delta\theta = 10^\circ$ from *n*-hexane to water) for **8t** confirms that bond rotations are impeded in **8t** and that conformations deviate only a little from the conformer **8tA** (Scheme 3). On the other hand, the variation $\Delta\theta$ of 20° confirms nicely the change of conformation in **b** toward the occurrence of oxaphosphetane-like structure (Scheme 4) in polar and protic solvents. Moreover, as expected, the value $\Delta\theta = 32^\circ$ denotes a dramatic change in structure of **8c** in

nice agreement with the chair-chair equilibrium between conformer **8cA**, favored in apolar solvents, and **8cE**, favored in polar and protic solvents.

$$\frac{a_{p,i}}{a_{p,n}} = \frac{a_{N,i}}{a_{N,n}} \cdot \frac{\cos^2 \theta_i}{\cos^2 \theta_n} \quad (3)$$

5 **Example 3:** Titration of water in THF by EPR

Interestingly, a difference Δa_p of ca. 5 G is observed between THF and water. As these two solvent are miscible in any amount, the effect of binary mixture of solvents in a_N and a_p was investigated with the aim to develop the first titration of water in solvent by EPR. As displayed in Figure 1, EPR signals in THF (5 lines) and in water (6 lines)
 10 are very different. 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% (Table 2 and Figures 5, 6 and 7). Solvent effect of binary mixture on spectroscopic properties has been intensively investigated using dyes. It has been shown that simplified the model $m = 1$, implying only the occurrence of the solvent exchange reaction is described by equation (6) and account for many solvent effect in
 15 binary mixture:

$$Y = Y_1 + \frac{a \cdot x_2^0}{(1 - x_2^0) + f_{2/1} (x_2^0)^2} \quad (6)$$

Therefore, Y_1 and Y_2 were defined as $a_{p,S1}$ and $a_{p,S2}$ for water as solvent S_2 and THF as solvent S_1 . A good fit was observed for $m = 1$ (Figure 9a) affording $a_{p,S1} = a_{p,THF} = 29.68 \pm 0.07$ G, $a_{p,S2} = a_{p,w} = 24.14 \pm 0.05$, $f_{2/1} = 9.0 \pm 0.5$, $\chi^2 = 0.017$ and $R_2 = 0.997$.
 20 Hence, m is the number of solvent molecules involved in the microsphere solvation of the nitroxide for which a_p changes, thus, only one molecule of water close to the nitroxyl moiety of **8c** is required to make a_p change. The value of $f_{2/1}$ larger than 1 means that **8c** would rather be solvated either with pure water or with some molecules of water in its cybotatic region than by pure THF.

25 The value of x_{iso} is graphically determined as 0.1 (Figure 9a) for $a_{p,xiso}$ as given by equation 7. Then, the polar effect of THF is balanced for 2.4% of water. Thus, the difference of 2.70 G for $\approx 2\%$ of water led us to investigate the potential of **8c** in the titration of water in THF as an organic solvent. It has to be mentioned that some Lucarini performed the titration of benzyl alcohol using 2,2,6,6-tetramethylpiperidin-N-oxyl radical and its ability
 30 for intermolecular H-bonding.

$$a_{P, x_w} = a_{P, \text{water}} + \frac{(a_{P, S2} - a_{P, \text{water}})}{2} \quad (7)$$

The plot % volume of water vs a_P (Figure 6) exhibits the same shape as the plot x_w vs a_P (Figure 5). 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 (Figure 7) shows a linear relationship affording an easy titration. Taking into account an error of 0.1 G, accurate titration down to 0.2% water in THF was achieved.

entry	% water	x_{water}	a_N (G)	a_P (G)
1	0	0	14.94	29.56
2	0.1	0.0045	15.05	29.47
3	0.2	0.0090	15.05	29.38
4	0.3	0.0134	15.00	29.14
5	0.4	0.0178	15.06	29.06
6	0.5	0.0221	15.04	28.78
7	0.6	0.0265	15.04	28.64
8	0.7	0.0308	15.10	28.48
9	0.8	0.0350	15.04	28.30
10	0.9	0.0393	15.04	28.00
11	1	0.0435	15.14	27.82
12	2	0.0840	15.21	27.28
13	3	0.1220	15.24	26.76
14	4	0.1580	15.34	26.00
15	5	0.1920	15.34	25.82
16	10	0.3334	15.38	25.36
17	20	0.5295	15.56	24.82
18	30	0.6586	15.62	24.52
19	40	0.7501	15.68	24.4
20	50	0.8182	15.80	24.22
21	60	0.8710	15.86	24.12
22	70	0.9131	15.98	24.12
23	80	0.9474	16.16	24.12
24	90	0.9759	16.22	24.12
25	100	1	16.22	24.18

Table 2. % water in THF, mole fraction x_{water} , nitrogen and phosphorus hyperfine coupling constants a_N and a_P , respectively, for different % of water.

In conclusion, an amazing and puzzling cybotactic effect in nitroxide was reported. It was controlled by the occurrence of IHB (Intramolecular Hydrogen Bonding) affording a dramatic change of 25 G from *n*-pentane to water. Interestingly, to the best of our knowledge, this is the first report that a nitroxide exhibits a behavior similar to the one observed for betaine dyes.

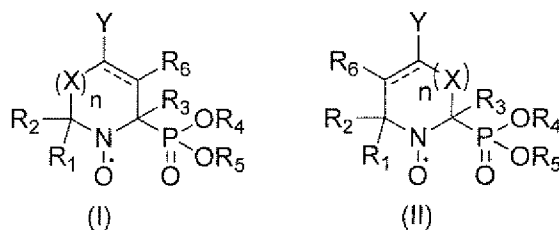
Thus, for the first time, the titration of traces of water in THF (organic solvent) was performed down to 0.1%. Obviously, this is far from matching the Karl-Fischer titration that is suitable down to the ppm range. However, knowing that EPR can be ppb sensitive, it should be possible to develop better probes reaching such a threshold. However, 5 the most interesting applications are likely in the Materials Sciences field to investigate the porosity of membranes, the stability of films in water or in organic solvents, the swelling of polymers and in Biology to investigate drug delivering via (nano)particles or micelles.

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Brémond, P.; Butscher, T, Marque, S. R. A., *Appl. Magnet. Reson.* **2015**.

Claims

1. A compound of formula (I) or (II):

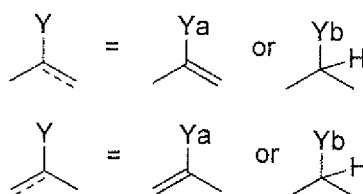


wherein:

5 R_1 is C_1 - C_6 alkyl optionally substituted by R_9 ;

R_2 , R_3 are independently selected from C_1 - C_6 alkyl, C_6 - C_{10} aryl, 5 to 7 membered heteroaryl optionally substituted by R_{10} ;

R_4 , R_5 are independently selected from C_1 - C_6 alkyl, C_6 - C_{10} aryl, 5 to 7 membered heteroaryl ;



10

Y_a is selected from C_1 - C_6 alkylcarbonyloxy, C_1 - C_6 alkoxy carbonyloxy, C_1 - C_6 alkylthiocarbonyl wherein said alkyl groups are optionally substituted by one or more F, and/or Cl ; $OSi(R_{11})_3$, $OSi(OR_{11})_3$ and/or $OSi(R_{12})_3$, OSO_2R_{13} ;

15 Y_b is H, OH or NHR_8 ;

X is $(CH_2)_mCHR_7(CH_2)_p$;

R_6 , R_7 are independently selected from H, OH, NHR_8 , an halogen atom, C_1 - C_6 alkyl, C_6 - C_{10} aryl, 5 to 7 membered heteroaryl;

R_8 is selected from H, C_1 - C_6 alkyl;

20 R_9 , R_{10} are each independently selected from OH, NHR_8 ;

R_{11} is at each occurrence independently selected from C_1 - C_6 alkyl, C_6 - C_{10} aryl;

R_{12} is independently F, Cl or C_1 - C_6 alkyl;

25 R_{13} is independently selected from C_1 - C_6 alkyl, C_1 - C_6 perfluoroalkyl, $(C_1$ - $C_6)$ alkyl(C_6 - C_{10})aryl;

n is 0 or 1;

m is 0 or 1;

then S_1 is water.

11. Use of claim 10, wherein S_1 is water and S_2 is an organic solvent.

12. Use of claim 10, wherein S_1 is an organic solvent and S_2 is water.

13. Use of claim 10, wherein S_1 and S_2 are each an organic solvent.

5 14. Use of claim 13, wherein ΔE between S_1 and S_2 corresponds to a $(\delta)a_p$ larger than 4G.

15. Use of any of claims 10 to 14, wherein the change in the phosphorus hyperfine coupling constant of the compound of formula (I) or (II) is superior to 15 G between S_1 and S_2 .

10 16. Use according to any of claims 10 to 15, wherein S_1 is a solvent crossing a polymeric film or a polymeric or biological membrane.

17. Use according to any of claims 10 to 15, for studying or monitoring membrane porosity, film permeability, swelling of polymer, stability of materials, drug delivery.

15

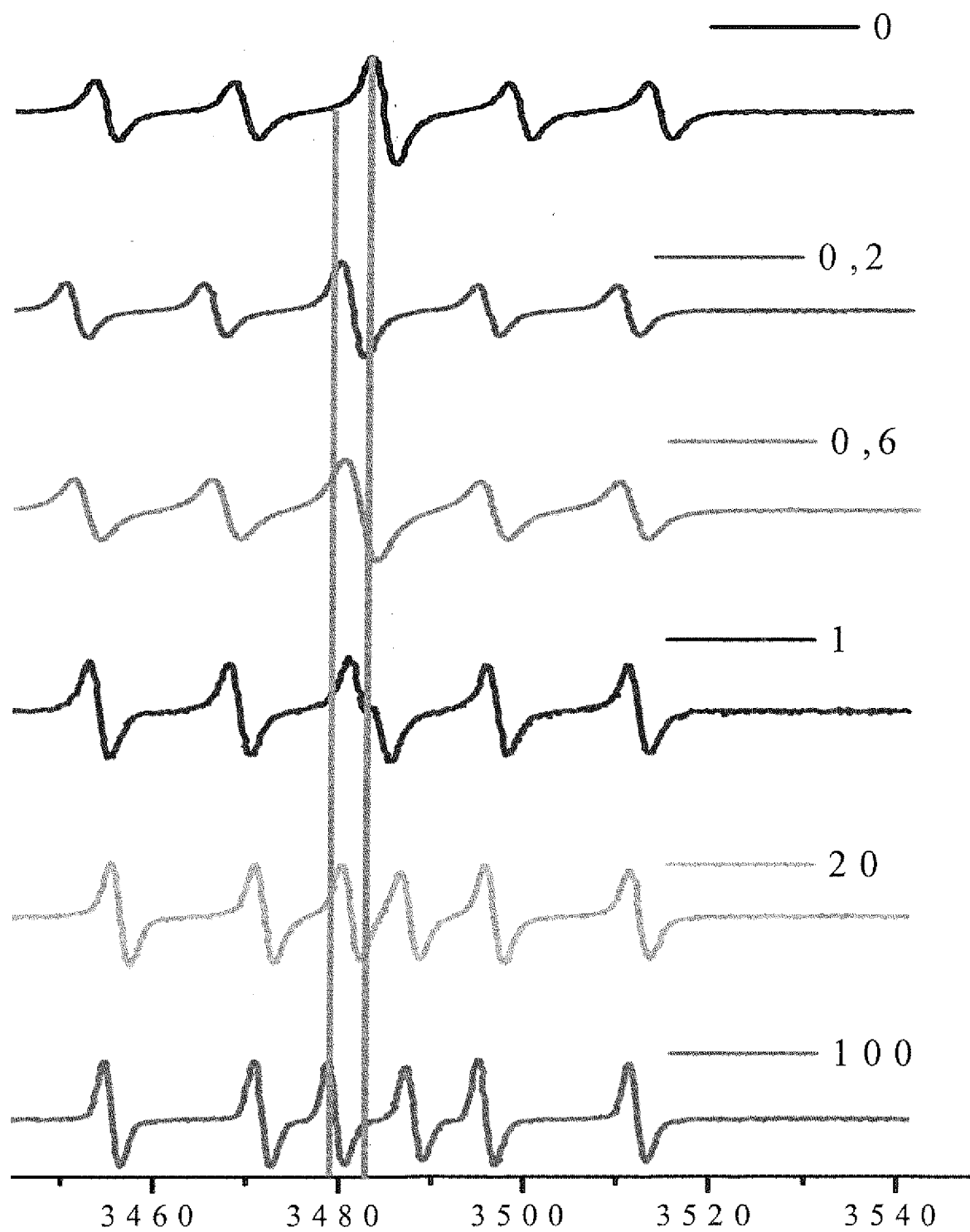


FIGURE 1

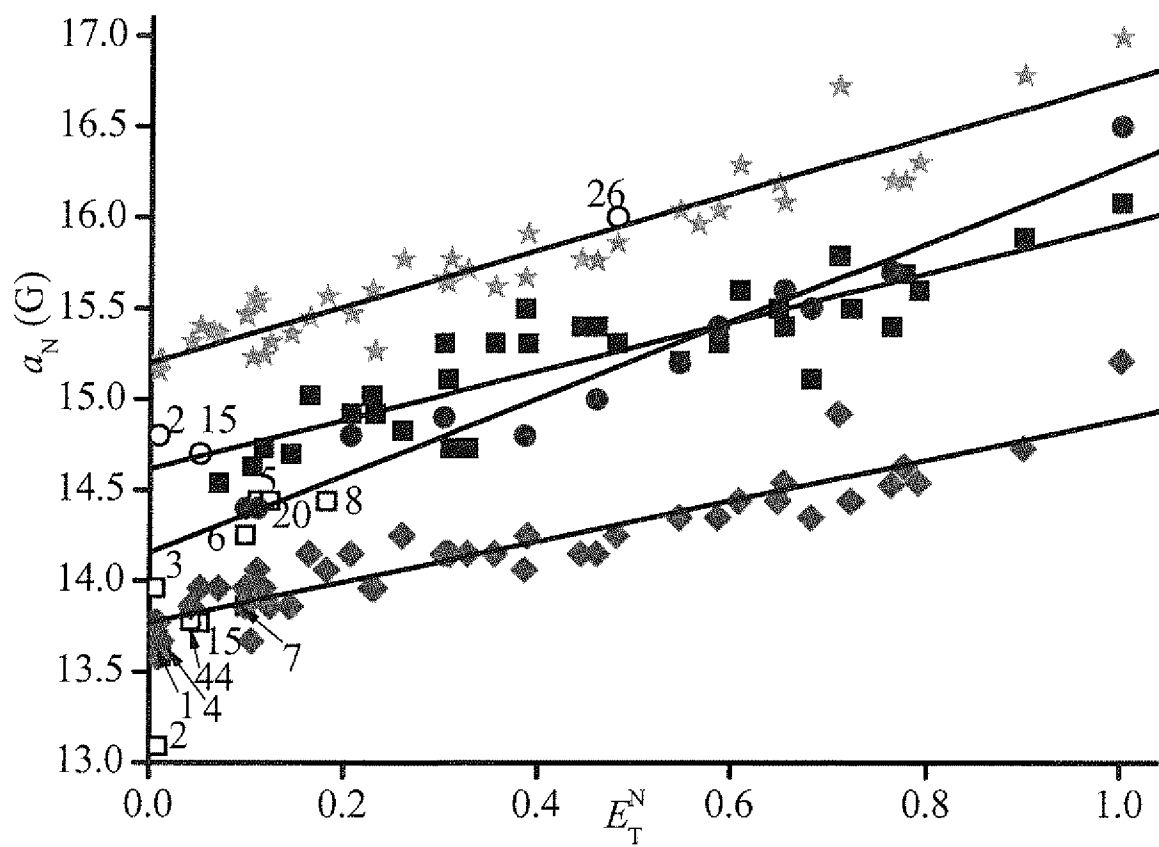


FIGURE 2

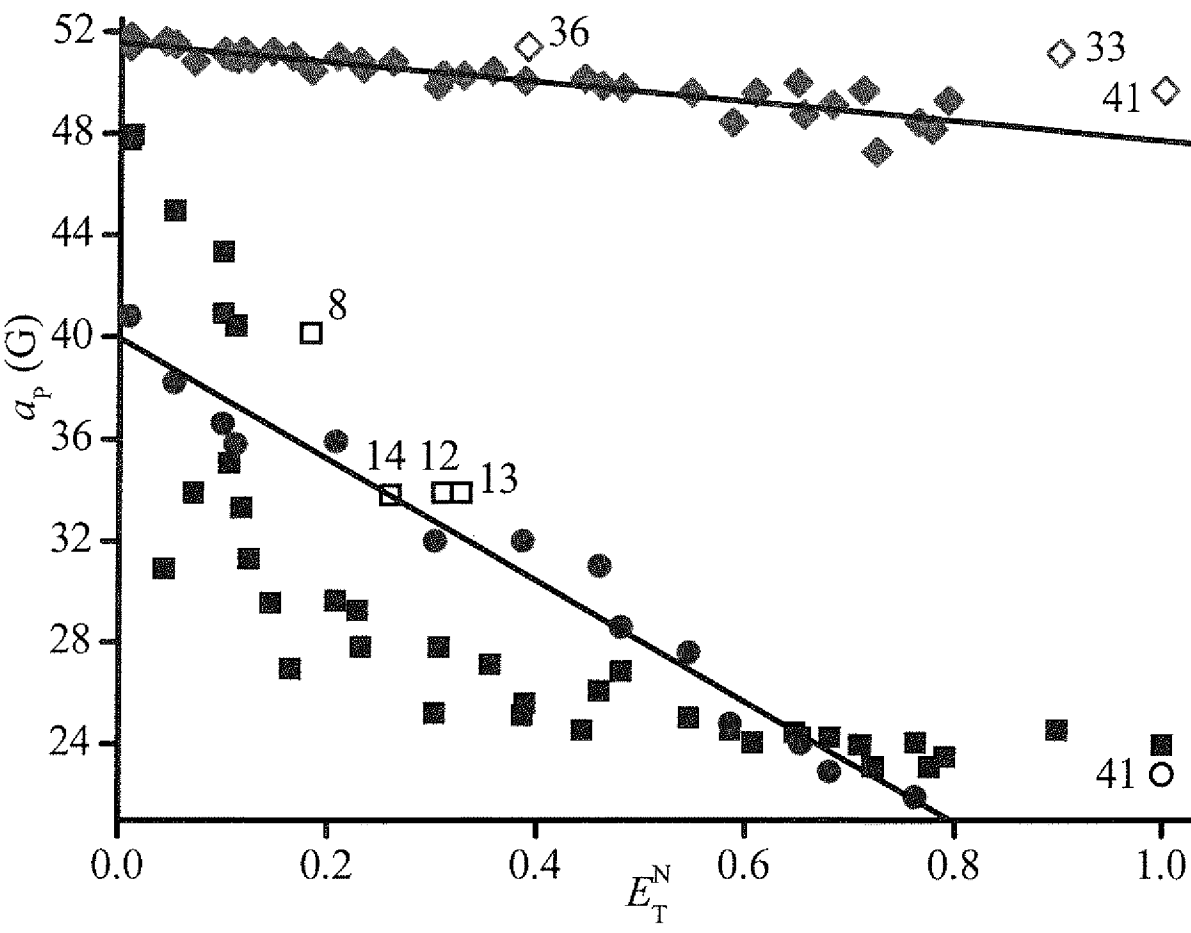


FIGURE 3

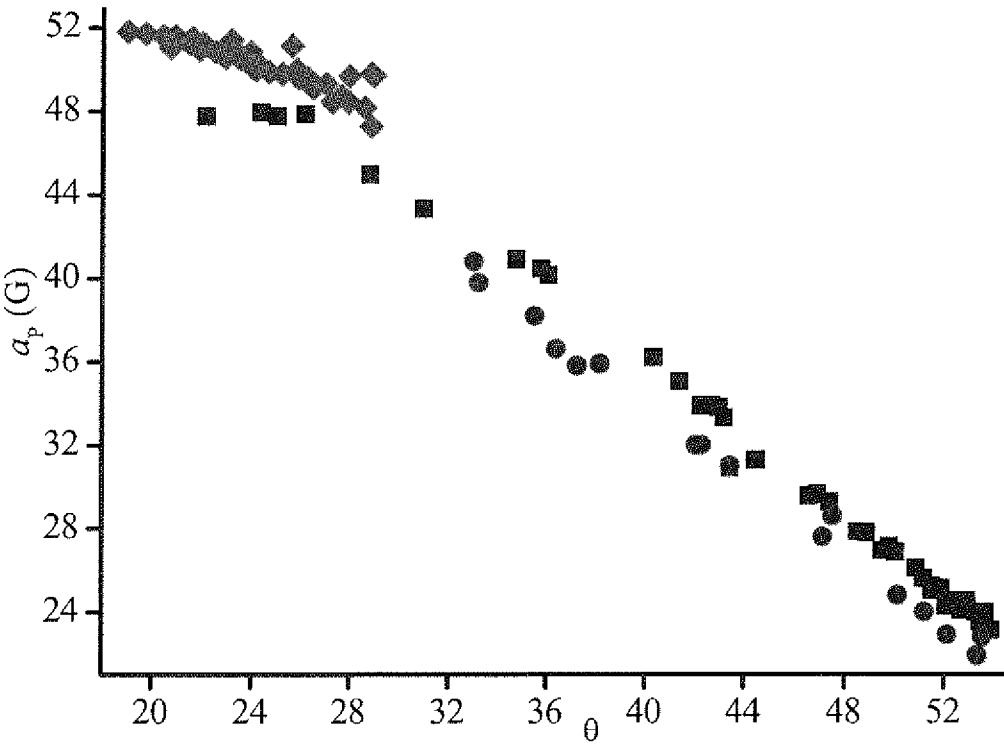


FIGURE 4

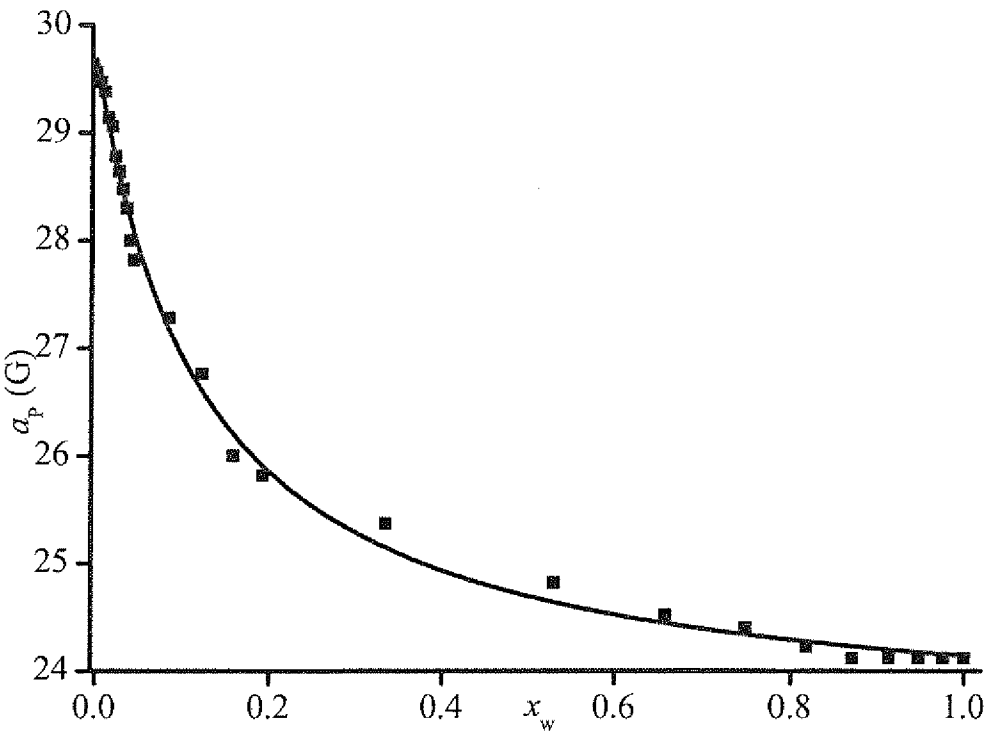


FIGURE 5

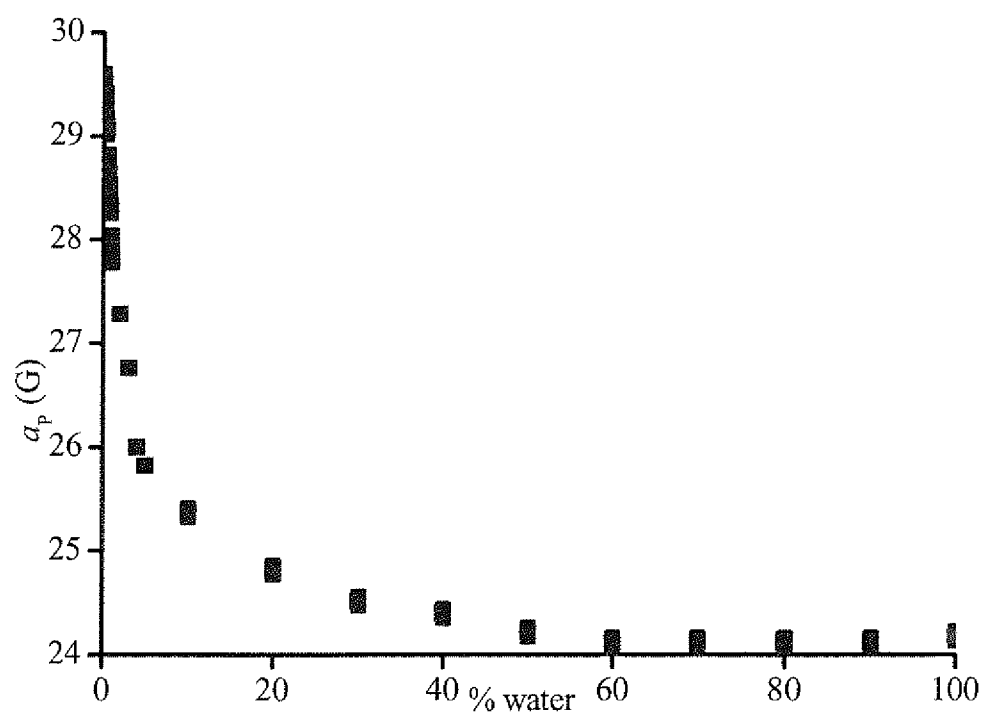


FIGURE 6

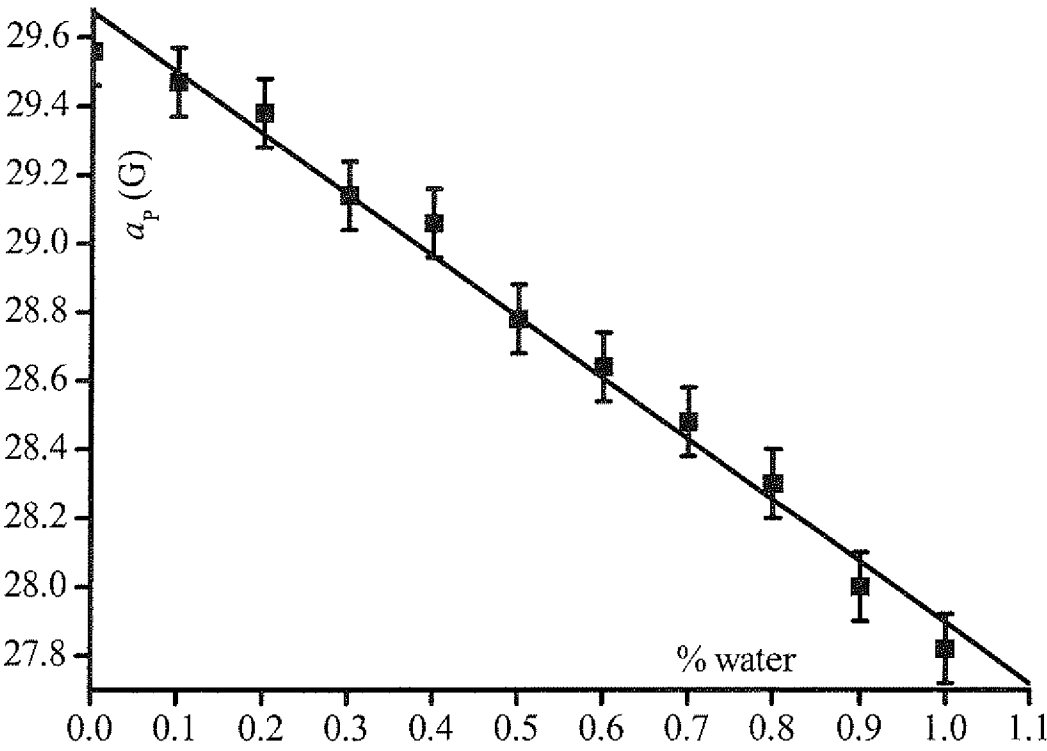


FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/066208A. CLASSIFICATION OF SUBJECT MATTER
INV. C07F9/59
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GÉRARD AUDRAN ET AL: "Solvent Effect in [beta]-Phosphorylated Nitroxides: Model Nitroxides", APPLIED MAGNETIC RESONANCE., vol. 46, no. 12, 24 February 2015 (2015-02-24), pages 1333-1342, XP055232777, AU ISSN: 0937-9347, DOI: 10.1007/s00723-015-0649-4 cited in the application abstract compound 5 tables 1-3 -----	1-17



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

9 September 2016

Date of mailing of the international search report

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Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

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Eberhard, Michael