NMR Investigations of the β-Cyclodextrin Inclusion of (α-Phenyl-*N*-*tert*-butylnitrone) Analogues

David Bardelang, Jean-Louis Clément, Jean-Pierre Finet, Hakim Karoui, and Paul Tordo*

Laboratoire SREP, UMR 6517 CNRS et Universités d'Aix-Marseille 1 et 3, Case 521, 13397 Marseille Cedex 20, France

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Recently, cyclodextrin derivatives were reported to increase significantly the half-life of the spin adducts resulting from the trapping of free radicals with nitrones. To address the physicochemical characteristics of these complexes, a series of α-phenyl-*N*-*tert*-butylnitrone (PBN) analogues containing hydroxy groups on the *tert*-butyl group was prepared. The influence of the substitution on the supramolecular complexation of the nitrones with natural β-cyclodextrin was studied by NMR spectroscopy. 1D-NMR studies indicated a 1:1 stoichiometry and binding constants in the range 32 to 1075 M^{-1} depending on the substitution pattern. 2D-NMR experiments suggested a competitive inclusion of the PBN derivatives by the aryl moiety through the two accessible wide and narrow rims of the cyclodextrin.

Introduction

Cyclodextrins are constituted by three major types of cyclic oligosaccharides, formed by six (α-CD), seven (β-CD), or eight (γ-CD) glucopyranose units bonded through α-(1,4)-linkages. They are characterized by a hydrophobic truncated cone-shape that can accommodate a wide range of organic molecules leading to inclusion complexes whose properties and applications have been extensively reviewed.[1−3] These inclusion complexes have been characterized by a wide variety of techniques. Among them, EPR spectroscopy was successfully used to prove and quantify the complexation equilibrium of free radical−cyclodextrin complexes.[4] Moreover, Kotake and Janzen have reported the recognition ability of cyclodextrin for alkyl groups in nitroxides[5] and the formation of multimodal inclusion complexes with cyclodextrins in the case of stable linear nitroxides bearing alkyl and phenyl groups.[6] Recently, Lucarini et al. studied by EPR[4c] the dynamic behavior of nitroxide−cyclodextrin complexes and by NMR[7] the structure of the corresponding amine−cyclodextrin complexes in solution. In particular, they have shown that the association constant between *tert*-butylbenzylnitroxide (the PBN-H• adduct) and DM-β-CD [heptakis (2,6)-di-*O*-methyl-β-CD] is about four times greater than for the complex of the parent *tert*-butylbenzylamine with the same cyclodextrin. However, spin trapping in the presence of a cyclodextrin has been barely investigated. Luo et al.[8] have studied the trapping of carbon-centered radicals with nitroso compounds stabilized by cyclodextrins. But nitrones are more currently used to trap free radicals as they are more efficient, especially for oxygen-centered radicals in biological milieu.[9] Only a few physicochemical studies have focused on the nitrone−cyclodextrin complexes. The association constants of such complexes involving substituted α-phenyl-*N*-phenyl nitrones with β-CD were reported in a study of 1,3-dipolar cycloaddition reactions of nitrones with olefins.[10] Recently, Karoui et al. have shown that the half-life of the spin adduct formed during the trapping of superoxide by 5-diethoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO) underwent a 7-fold increase upon addition of RM-β-CD (randomly methylated β-cyclodextrin).[11] The action of biological reducing agents (glutathione peroxidase, ascorbate) on the inclusion complex formed between the DEPMPO-superoxide adduct and RM-β-CD was impeded. For example, the ESR spectra of this adduct could be recorded up to 3 min after addition of the ascorbate monoanion, instead of an instantaneous disappearance in the absence of RM-β-CD. Subsequently, Karoui and Tordo extended this study to the case of a linear nitrone, α-phenyl-*N*-*tert*-butylnitrone (PBN).[12] Thus, the use of cyclodextrins in spin trapping experiments is of potentially considerable importance to improve the capacities of nitrones for the trapping of toxic radicals, as well as for the detection of spin adducts in biological milieu. To determine the optimal pair of guest and host reagents, a number of points need to be clarified: (i) What are the structural features of the spin traps that are required for the association with the cyclodextrin host? (ii) When an inclusion complex is formed, what are the stoichiometry and association strength of the nitrone−cyclodextrin complex? (iii) Does the trapping of the free radical occur when the nitrones are outside and/or inside the cyclodextrin? (iv) What are the relative association constants of the spin traps and spin adducts with the cyclodextrin host? (v) If the trapping can take place with the nitrone included in the cyclodextrin, the knowledge of the geometry of the complex may be particularly relevant in the case of PBN derivatives, which present two sites of binding (the aryl group and the *tert*-butyl group). All these considerations led us to investigate the possible interactions occurring between model spin-traps and β-CD.

In this paper, we present NMR studies of the inclusion complexes formed between β-CD and different types of PBN derivatives. The first important point to be clarified is the determination of the stoichiometry of the complexes (formation of 1:1 or 1:2 guest−host complexes). Indeed, if the formation of 1:2 guest−host complexes is favored, the spin trap will be too embedded in the dimeric complex to permit the approach of free radicals. In contrast, if 1:1 guest−host complexes are formed, the dynamics of the association may allow the efficient contact between the spin trap and the free radical species.

* To whom correspondence should be addressed. Tel: + 33 491 632 851. Fax: + 33 491 288 758. E-mail: tordo@srepir1.univ-mrs.fr.

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TABLE 1: Synthesis of Nitrones 1−12: Reaction Conditions and Yields

entry	R-CHO (1 equiv)	R′-NO_2 (x equiv)	Zn (y equiv)	acetic acid (z equiv)	reaction time (h)	yield (%)
1	Ph-CHO	$O_2NC(CH_3)_2CH_2OH$ (1)	2	4	96	56
2	4-Me-C_6H_4-CHO	$O_2NC(CH_3)_2CH_2OH$ (2)	4	6	60	55
3	4-Et-C_6H_4-CHO	$O_2NC(CH_3)_2CH_2OH$ (0.8)	1.8	5	94	63
4	4-*i*Pr-C_6H_4-CHO	$O_2NC(CH_3)_2CH_2OH$ (2)	3	6	71	69
5	4-*t*Bu-C_6H_4-CHO	$O_2NC(CH_3)_2CH_2OH$ (1)	2	4	114	47
6	4-Ph-C_6H_4-CHO	$O_2NC(CH_3)_2CH_2OH$ (1)	2	4	116	50
7	4-MeO-C_6H_4-CHO	$O_2NC(CH_3)_2CH_2OH$ (2)	3	6	100	54
8	4-Cl-C_6H_4-CHO	$O_2NC(CH_3)_2CH_2OH$ (2)	3	6	102	64
9[a]	4-NO_2-C_6H_4-CHO	$O_2NC(CH_3)_2CH_2OH$ (2.5)	7.5	15	48 + 3.5	45
10	Ph-CHO	$O_2NCCH_3(CH_2OH)_2$ (2)	4	6	80	13
11	Ph-CHO	$O_2NC(CH_2OH)_3$ (0.8)	1.7	3.5	92	62
12	Ph-$(CH_2)_2$CHO	$O_2NC(CH_3)_2CH_2OH$ (2)	8	24	96	91

[a] Two-step synthesis.

Introduction of adequately placed polar hydroxy substituents decreases the intermolecular interactions.[13−15] Such groups could prevent the approach of another β-cyclodextrin to a preexisting 1:1 guest−host complex, thus favoring the formation of only the 1:1 complex. To restrict the number of complexes in solution (1:1, 1:2, 2:1...), we modified the *tert*-butyl moiety by synthesizing mono-, di-, and trihydroxy derivatives of PBN.[16,17] Moreover, ortho or meta substitution on the phenyl ring destabilizes the association between the substrate and cyclodextrins.[14,18] Therefore, we modified the aryl moiety by synthesizing PBN derivatives containing a para-functionalized phenyl moiety. To study the guest−host association, the use of NMR spectroscopy was selected, as it provides specifically ^1H and ^{13}C complexation induced shifts (CIS) upon inclusion and represents a powerful tool to investigate molecular edifices in solution.[2,19] Indeed, this technique allows the characterization of the inclusion process in terms of stoichiometry,[20] association constant,[21,22] and even geometry of the complexes.[2,7]

Experimental Section

General. All chemicals and organic solvents were commercially available and were used as supplied. β-Cyclodextrin, 2-methyl-2-nitro-1,3-propanediol, 4-methylbenzaldehyde, 4-isopropylbenzaldehyde, 4-methoxybenzaldehyde, and 4-nitrobenzaldehyde were purchased from Acros Organics. 2-Methyl-2-nitropropanol, 4-chlorobenzaldehyde, 4-ethylbenzaldehyde, 4-*tert*-butylbenzaldehyde, and biphenyl-4-carboxaldehyde were from Aldrich. Trishydroxymethyl-nitromethane, benzaldehyde, and 3-phenylpropionaldehyde were from Fluka Chemika. All reactants were used as received without further purification. Crude materials were purified by flash chromatography on Merck silica gel 60 (0.040−0.063 mm). ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker DPX 300 spectrometer at 300.13 and 75.54 MHz, respectively, or a Bruker DPX 500 spectrometer at 500.13 and 125.76 MHz, respectively. Chemical shifts (δ) are reported in ppm for a solution of the nitrone in $CDCl_3$ with internal reference Me_4Si (Euriso-Top, CEA Saclay, 99.80%) or, in the case of **11**, in D_2O (Euriso-Top, CEA Saclay, 99.90%), the residual HOD signal being used as the internal reference. *J* values are reported in hertz. The assignments of NMR signals were facilitated by use of DEPT135 sequence. Melting points were measured on a Büchi B-540 apparatus and are uncorrected.

General Procedure for the Synthesis of Nitrones 1−8 and 10−12.[16] Glacial acetic acid (z equiv, see Table 1) was added dropwise to a cooled (0 °C) mixture of the appropriate 2-nitropropan-1-ol derivative (x equiv, see Table 1), the appropriate aldehyde, and zinc powder (y equiv, see Table 1) in 95% ethyl alcohol (10 mL/1 mmol of aldehyde). During the addition, the temperature was maintained below 10 °C and was allowed to reach room temperature after the end of the addition. The reaction evolution was monitored by TLC. The mixture was filtered, and the organic phase was dried over anhydrous sodium sulfate. After distillation of the organic solvents under reduced pressure, the residue was purified by flash chromatography on silica gel.

2-Methyl-2-[(phenylmethylene)amino]propan-1-ol *N*-oxide 1. Flash chromatography (CH_2Cl_2/95% EtOH, 98/2), 56%, white crystals, mp 79−80 °C (lit:[23] 76−77 °C, C_6H_6/pentane); δ_H 1.59 (s, 6H), 3.78 (s, 2H), 4.33 (s, 1H), 7.42−7.44 (m, 3H), 7.48 (s, 1H), 8.24−8.27 (m, 2H); δ_C 23.75, 69.68, 72.86, 128.4, 129.18, 130.22, 130.66, 132.19.

2-Methyl-2-[(4-methylphenylmethylene)amino]propan-1-ol *N*-oxide 2. Flash chromatography (CH_2Cl_2/Et_2O, 1/1), 55%, white solid, mp 100 °C (lit:[23] 95−96 °C, CH_2Cl_2/pentane); δ_H 1.60 (s, 6H), 2.39 (s, 3H), 3.79 (d, *J* 6, 2H), 4.35 (t, *J* 6, 1H), 7.24 (d, *J* 8, 2H), 7.44 (s, 1H), 8.16 (d, *J* 8, 2H); δ_C 21.66, 23.86, 70.08, 72.39, 127.57, 129.24, 129.27, 132.12, 141.29.

2-Methyl-2-[(4-ethylphenylmethylene)amino]propan-1-ol *N*-oxide 3. Flash chromatography (CH_2Cl_2/AcOEt, 1/1), 63%, white crystals, mp 90−92 °C; δ_H 1.25 (t, *J* 7.5, 3H), 1.60 (s, 6H), 2.68 (q, *J* 7.5, 2H), 3.79 (d, *J* 6.2, 2H), 4.38 (t, *J* 6.2, 1H), 7.27 (d, *J* 8.2, 2H), 7.45 (s, 1H), 8.18 (d, *J* 8.2, 2H); δ_C 15.25, 23.85, 28.94, 70.04, 72.39, 127.78, 128.04, 129.37, 132.10, 147.55. Calcd for $C_{13}H_{19}NO_2$ (221.30 g mol^{-1}) C, 70.56; H, 8.65; N, 6.33. Found C, 70.45; H, 8.59; N, 6.35%.

2-Methyl-2-[[4-(1-methylethyl)phenylmethylene]amino]-propan-1-ol *N*-oxide 4. Flash chromatography (CH_2Cl_2/Et_2O, 1/1), 69%, white crystals, mp 112−113 °C; δ_H 1.26 (d, *J* 7, 6H), 1.59 (s, 6H), 2.94 (h, *J* 6.9, 1H), 3.78 (s, 2H), 4.38 (s, 1H), 7.30 (d, *J* 8.3, 2H), 7.45 (s 1H), 8.18 (d, *J* 8.5, 2H); δ_C 23.68, 23.85, 34.20, 70.01, 72.43, 126.60, 127.93, 129.41, 132.08, 152.12. Calcd for $C_{14}H_{21}NO_2$ (235.33 g mol^{-1}) C, 71.45; H, 8.99; N, 5.95. Found C, 71.43; H, 8.99; N, 5.98%.

2-Methyl-2-[[4-(1,1-dimethylethyl)phenylmethylene]amino]-propan-1-ol *N*-oxide 5. Flash chromatography (CH_2Cl_2/Et_2O, 1/1), 47%, white powder, mp 129 °C; δ_H 1.33 (s, 9H), 1.60 (s, 6H), 3.79 (d, *J* 5.7, 2H), 4.38 (t, *J* 6.0, 1H), 7.45 (s, 1H), 7.46 (d, *J* 8.5, 2H), 8.20 (d, *J* 8.3, 2H); δ_C 23.86, 31.08, 35.00, 70.05, 72.39, 125.47, 127.51, 129.14, 132.00, 154.34. Calcd for $C_{15}H_{23}NO_2$ (249.35 g mol^{-1}) C, 72.25; H, 9.30; N, 5.62. Found C, 72.19; H, 9.36; N, 5.73%.

2-Methyl-2-[(4-phenylphenylmethylene)amino]propan-1-ol *N*-oxide 6. Flash chromatography (CH_2Cl_2/95% EtOH, 98/2), 50%, light yellow powder, mp 143−144 °C; δ_H 1.63 (s, 6H), 3.82 (s, 2H), 4.23 (s, 1H), 7.38 (t, *J* 7.2, 1H), 7.46 (t, *J* 7.2, 2H), 7.53 (s, 1H), 7.63−7.66 (m, 2H), 7.68 (d, *J* 8.5, 2H), 8.34 (d, *J* 8.5, 2H); δ_C 23.83, 69.86, 72.83, 127.04, 127.87, 128.87, 129.14, 129.67, 131.85, 140.04, 143.16. Calcd for

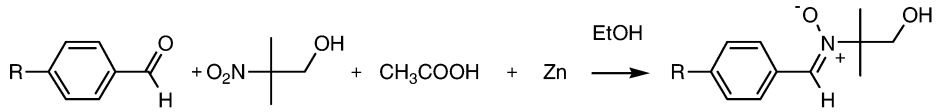


Figure 1. General route for functionalized nitrones synthesis.

$C_{17}H_{19}NO_2$ (269.34 g mol^{-1}) C, 75.81; H, 7.11; N, 5.20. Found C, 75.65; H, 7.01; N, 5.11%.

2-Methyl-2-[(4-methoxyphenylmethylene)amino]propan-1-ol *N*-oxide 7. Flash chromatography (CH_2Cl_2/95% EtOH, 19/1), 54%, white powder, mp 112−114 °C (lit:[23] 102−104 °C, EtOH); δ_H 1.59 (s, 6H), 3.79 (s, 2H), 3.86 (s, 3H), 4.49 (s, 1H), 6.94 (d, *J* 9.1, 2H), 7.40 (s, 1H), 8.26 (d, *J* 8.9, 2H); δ_C 23.82, 55.37, 70.13, 71.98, 113.86, 123.18, 131.26, 131.76, 161.31.

2-Methyl-2-[(4-chlorophenylmethylene)amino]propan-1-ol *N*-oxide 8. Flash chromatography (CH_2Cl_2/Et_2O, 1/1), 64%, white crystals, mp 125−126 °C (lit:[23] 109−111 °C, EtOH); δ_H 1.59 (s, 6H), 3.78 (d, *J* 6.3, 2H), 4.14 (t, *J* 6.3, 1H), 7.38 (d, *J* 8.7, 2H), 7.47 (s, 1H), 8.22 (d, *J* 8.7, 2H); δ_C 23.70, 69.53, 73.21, 128.70, 130.33, 131.11, 136.06.

2-Methyl-2-[(phenylmethylene)amino]-propane-1,3-diol *N*-oxide 10. Flash chromatography (CH_2Cl_2/95% EtOH, 9/1), 13%, hygroscopic white crystals, mp 83−84 °C (lit:[24] 87−88 °C, EtOH/Et_2O); δ_H 1.50 (s, 3H), 3.88−4.05 (m, 6H), 7.43−7.45 (m, 3H), 7.52 (s, 1H), 8.23−8.26 (m, 2H); δ_C 19.22, 66.71, 66.74, 75.86, 128.56, 129.61, 129.88, 131.09, 134.73.

2-(Hydroxymethyl)-2-[(phenylmethylene)amino]-propane-1,3-diol *N*-oxide 11. Flash chromatography (CH_2Cl_2/95% EtOH, 19/1), 62%, white crystals, mp 120−121 °C (lit:[24] 90−92 °C, EtOH/Et_2O); δ_H 4.03 (s, 6H), 7.54−7.60 (m, 3H), 7.81 (s, 1H), 8.24−8.27 (m, 2H); δ_C 60.68, 80.16, 129.56, 129.65, 131.18, 133.09, 141.52.

2-Methyl-*N*-(3-phenylpropylidene)amino]propan-1-ol *N*-oxide 12. Flash chromatography (AcOEt/95% EtOH, 9/1), 91%, yellow oil; δ_H 1.41 (s, 6H), 2.80−2.90 (m, 4H), 3.66 (s, 2H), 4.75 (s, 1H), 6.80 (t, *J* 5.3, 1H), 7.17−7.29 (m, 5H); δ_C 23.24, 27.95, 31.17, 69.20, 71.46, 128.16, 128.27, 128.51, 136.99, 140.25. Calcd for $C_{13}H_{19}NO_2$ (221.30 g mol^{-1}) C, 70.56; H, 8.65; N, 6.33. Found C, 70.48; H, 8.60; N, 6.29%.

2-Methyl-2-[(4-nitrophenylmethylene)amino]propan-1-ol *N*-oxide 9.[17] Glacial acetic acid (8.7 mL, 151 mmol) was added dropwise to a cooled (0 °C) solution of 2-methyl-2-nitropropan-1-ol (3 g, 25.2 mmol) and zinc powder (4.94 g, 75.5 mmol) in 95% ethyl alcohol (100 mL). During the addition, the temperature was kept below 10 °C and was allowed to reach room temperature after the end of the addition. After stirring of the sample for 48 h, the precipitate was filtered off and washed with 95% ethanol (2 × 50 mL) and chloroform (2 × 50 mL). The filtrate was then distilled under reduced pressure, and the residue was extracted with chloroform (4 × 50 mL). The combined organic extracts were concentrated to around 50 mL by distillation under reduced pressure. 4-Nitrobenzaldehyde (1.5 g, 10 mmol) was then added, and the mixture was refluxed for 3.5 h. After cooling, the reaction mixture was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude material was finally purified by flash chromatography on silica gel (CH_2Cl_2/95% EtOH, 19/1) to afford **9** as a yellow powder (1.07 g, 45%), mp 151−152 °C (lit:[23] 145−147 °C, C_6H_6); δ_H 1.63 (s, 6H), 3.52 (t, *J* 6.3, 1H), 3.82 (d, *J* 6.2, 2H), 7.64 (s, 1H), 8.26 (d, *J* 9.1, 2H), 8.42 (d, *J* 9.1, 2H); δ_C 23.78, 69.31, 74.70, 123.72, 129.41, 130.17, 134.89, 135.98.

NMR Measurements. ^{1}H NMR experiments for determination of the stoichiometry and analysis of the association constant of supramolecular complexes were performed at 300.13 MHz on a Bruker DPX 300 spectrometer equipped with a QNP probe at 300 K. The nitrone and cyclodextrin were dissolved in D_2O, and the residual HOD signal (δ 4.79 ppm) was used as internal reference, to avoid competitive association processes which could take place if a different species was added as internal reference.[18]

Continuous variation method (Job's Plot): NMR samples were prepared by mixing equimolar amounts of freshly made stock solutions of β-CD and nitrone (12 mM) in different volumes leading to the desired values of the ratio $S_H = ([H]_0/([H]_0 + [G]_0)$. This technique requires the total products concentration to be kept constant (V_{tot} = 500 μL) while the molar fractions of both components vary from 0 to 1 by steps of 0.1.

NMR titrations were made considering a constant concentration of the β-CD and progressively increasing the concentration of the nitrone. The relatively poor solubility of β-CD in water (16.3 mM) and the limit of detection of the NMR signal by the apparatus led to the selection of a concentration of 3 mM of β-CD for NMR titrations of all nitrone/β-CD pairs (except PBNOH for which the β-CD concentration was 10 mM). The nitrone concentration was increased from 0.3 to 9 mM. The NMR samples were prepared by mixing adequately defined volumes of freshly made stock solutions of β-CD and nitrone. For certain pairs of host and guest molecules, the titration was performed twice.

Two-dimensional rotating frame nuclear Overhauser effect spectroscopy (ROESY) was performed on a Bruker DPX 500 spectrometer at 500.13 MHz with Cryoprobe. A Bruker standard sequence with water suppression (roesywg) was necessary to remove the signal of residual HOD and to be able to observe weak intermolecular ROE interactions. The data consisted of 32 scans collected over 4096 complex points and for a spectral width of 6010 Hz. A mixing time of 700 ms, a repetition delay of 3 s, an acquisition time of 0.682 s, and a 90° pulse width of 6.8 μs at −5 dB power attenuation were used. The data were zero-filled to 2048 × 2048 points and processed by applying a π/2 shifted Q-sine window in both dimensions. Small cross-peaks were neglected when their magnitude was close to that of noise. The NMR samples were prepared by mixing fixed volumes of freshly made β-CD and nitrone solutions to give predetermined final volume (500 μL) and final concentrations of 10 and 1 mM, respectively.

Results and Discussion

Synthesis. Nearly all the desired nitrones were prepared by one-pot synthesis,[16] which were practical and efficient in most cases. Reduction of the appropriate 2-nitropropan-1-ol derivative into the corresponding hydroxylamine by action of zinc and acetic acid was followed by direct in situ condensation with the functionalized aldehyde. Optimization of the reaction conditions showed that an excess of acetic acid led to higher yields of the nitrones. However, in the case of the 4-nitrosubstituted nitrone **9**, the one-pot synthesis cannot be used as it would require selective reduction of the nitro alcohol in the presence of nitrobenzaldehyde (Figure 2). Therefore, the nitro alcohol was first reduced at room temperature by the action of

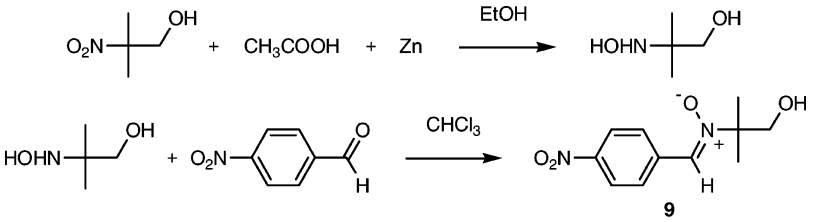
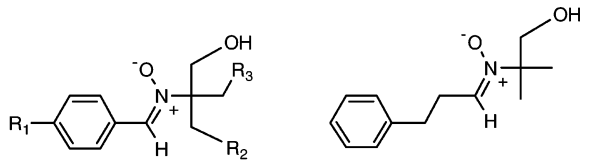


Figure 2. Two-step synthesis of nitrone **9**.



Nitrone number	Nitrone	R_1	R_2	R_3
1	PBNOH	H	H	H
2	4-Me-PBNOH	Me	H	H
3	4-Et-PBNOH	Et	H	H
4	4-*i*Pr-PBNOH	*i*-Pr	H	H
5	4-*t*Bu-PBNOH	*t*-Bu	H	H
6	4-Ph-PBNOH	Ph	H	H
7	4-MeO-PBNOH	OMe	H	H
8	4-Cl-PBNOH	Cl	H	H
9	4-NO_2-PBNOH	NO_2	H	H
10	PBNdiOH	H	OH	H
11	PBNtriOH	H	OH	OH
12	PEBNOH	-	-	-

Figure 3. Chemical structure of synthesized nitrones.

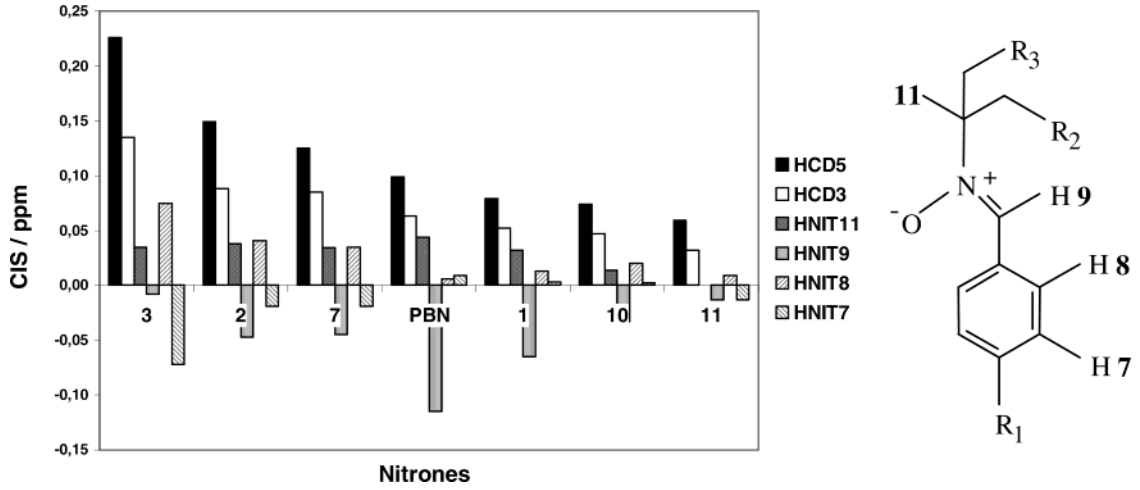
zinc and acetic acid, and the resulting crude hydroxylamine was subsequently condensed with 4-nitrobenzaldehyde under a gentle reflux. The nitrone **9** was obtained in 45% yield after flash chromatography, a yield that compares favorably with those obtained by the one-pot synthesis.[17] The structures of all the synthesized nitrones are given in Figure 3.

NMR Study. Although programs for NMR titration that evaluate both the association constant and the stoichiometry exist,[13] we preferred to carry out different NMR titrations to evaluate separately this information as usually recommended.[21] Only the nitrones **1**, **2**, **3**, **7**, **10**, **11**, and **12** were sufficiently water soluble for the NMR studies. The commercially available PBN nitrone **13** was also used in these comparative studies.

Continuous Variation Method.[20,25] In the Job's Plot method, equimolar amounts of β-CD and nitrone were mixed in different volumes to realize adequately selected values of the ratio $S_H = [H]_0/([H]_0 + [G]_0)$. The absence of new peaks occurring upon titration assignable to the pure complex suggested a fast exchange process between the free and bound states on the NMR time scale.[13,26] The continuous variation method was applied in the case of the nitrones showing a good water solubility.

The two cyclodextrin protons H_3 (H_{CD3}) and H_5 (H_{CD5}), located inside the cavity of β-CD, and some protons (H_{Nit}) of the guest were shifted upon titration either upfield (CIS > 0) or downfield (CIS < 0), depending on the considered protons. These shifts proved the inclusion, and they were sufficiently large to permit the Job analysis. The most important ^{1}H NMR CIS for the selected nitrones are presented in Chart 1. These data agree with the often considered assumption that the more hydrophilic the guest, the weaker the association. However, the shifts of the protons of the *tert*-butyl moiety and of the phenyl moiety did not show any clear trend. Nevertheless, it should be mentioned that polyhydroxy PBN derivatives **10** and **11** presented only weak CIS and that 4-Et-PBNOH **3** gave the highest observed CIS. The complexation induced shifts of the nitronyl proton of the nitrones (H_{Nit9}) increased with the decreasing number of hydroxy substituents present on the *tert*-butyl moiety. The correlation between the observed CIS of H_{Nit9} and decreasing polarity, following the order: PBNtriOH > PBNdiOH > PBNOH > PBN, results from a deeper inclusion of the nitrone and a stronger interaction between H_{Nit9} and the inside cavity protons of the cyclodextrin, when the number of hydroxyl groups decreases. When alkyl groups (Me or Et, nitrones **2** and **3**, respectively), or a methoxy group (nitrone **7**),

CHART 1: Most Important ^{1}H NMR CIS Related to PBN Derivatives in Job's Analysis



are introduced on the para position of the phenyl moiety, the CIS of H_{Nit9} are reduced. This suggests a smaller depth of inclusion of the substituted phenyl inside the cavity and thus a greater availability of the nitronyl carbon toward its environment. On the other side, the absolute value of the CIS observed for all the protons interacting inside the cavity, those of the nitrone (H_{Nit7}, H_{Nit8}, and H_{Nit9}) and of the β-CD (H_{CD3} and H_{CD5}), increased with the length of the alkyl substituent on the C-4 position: (H < Me < Et). These data suggest a strong interaction between the ortho and meta nitrone protons with H_{CD3} and H_{CD5}. This observation is in line with the fact that the 4-Et-PBNOH nitrone **3**, the bulkiest and most hydrophobic of the series, appears as the most strongly complexed with the cyclodextrin. For all the studied complexes in aqueous solution, Job's diagrams of $\Delta\delta Hi \times S_{Hi}$ as a function of S_{Hi} [with $S_{Hi} = [H_i]_0/([H_i]_0 + [G_i]_0)$] showed an inflection point close to 0.5, characteristic of a 1:1 stoichiometry (Figure 4).

However, definitive conclusions on the preference of the phenyl or *tert*-butyl moiety for either the wide rim or the narrow rim of the cavity cannot be drawn from these experiments. The same impossibility applies to the evaluation of the global affinity between the nitrone and the β-cyclodextrin. The fact that the H_{CD3} and H_{CD5} protons presented the most pronounced CIS, for all the considered nitrones, showed unambiguously the inclusion and allowed the evaluation of the association constants by NMR titrations.

NMR Titrations. As the PBN analogues−β-cyclodextrin complexes were characterized by a 1:1 stoichiometry, the following equilibrium was considered for the NMR determination of the association constants:

$$H + G \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} HG$$

In this case of a fast exchange, the Macomber's formula[27] was used, and the binding constants were calculated with the following equation:

$$\delta = \delta_f - \left(\frac{\Delta\delta}{2}\right)(b - \sqrt{b^2 - 4R})$$

with

$$b = \left(1 + R + \frac{1}{KH_0}\right)$$

and

$$R = \frac{G_0}{H_0}$$

where $\Delta\delta = \delta_f - \delta_C$; H_0 = initial concentration of β-CD; G_0 = initial concentration of the guest; δ = observed chemical shift for the considered proton; δ_f = initial chemical shift for the considered proton; δ_C = chemical shift of the complexed form for the considered proton; K = association constant.

Both H_{CD5} and H_{CD3} were suitable for the determination of the association constants (Figure 5). The evolution of their chemical shifts was monitored in function of the nitrone concentration. It should be mentioned that, in this model, K and $\Delta\delta$ are parameters that must be both evaluated by a nonlinear iterative curve fitting process, since δ_C cannot be evaluated experimentally. Thus, calculations were made by considering all the experimental points (δ(Hi) and [Nitrone]), δ_f (Hi), and the fixed β-CD concentration. The fitting process gave excellent correlations ($r^2 \geq 0.994$) between experimental

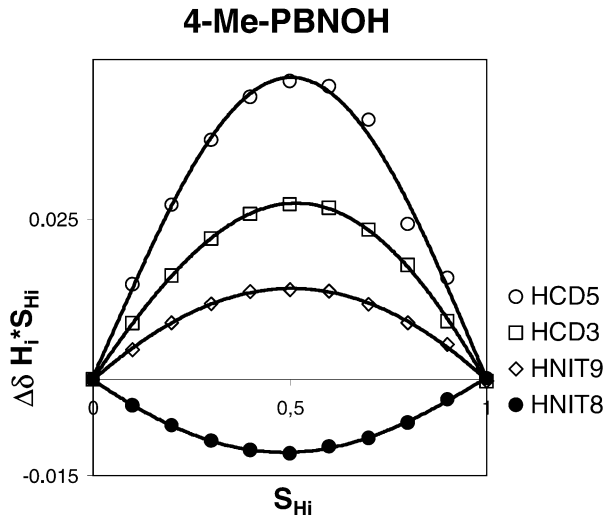


Figure 4. Job's diagrams for 4-Me-PBNOH/β-CD system.

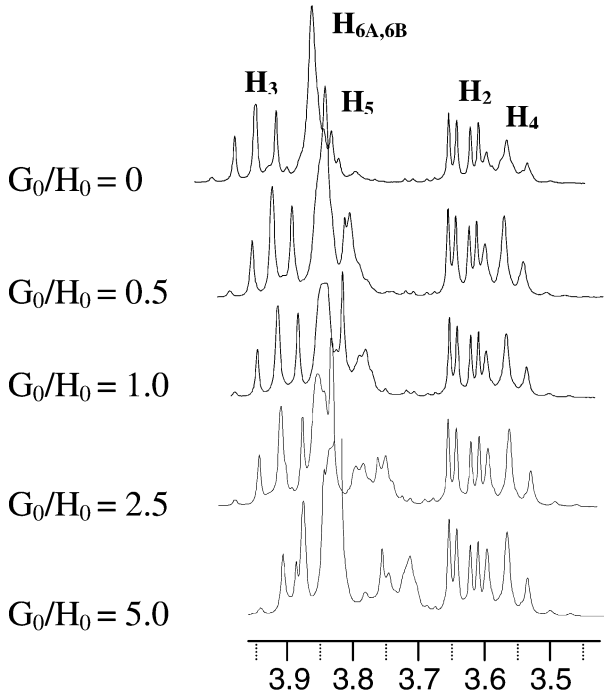


Figure 5. Selected ^{1}H NMR spectra of PBNOH/β-CD titration.

points and the calculated curves for all the nitrones except for PBNdiOH **10** ($r^2 = 0.983$ for H_{CD3}, the only useful proton), confirming the good agreement between the model and the experimental points and thus confirming the 1:1 stoichiometry (Figure 6).

The calculations were first made with the assumption that K and $\Delta\delta$ should be evaluated simultaneously. But, the $\Delta\delta$ value is determined from the fit between the mathematical model and the experimental points. Possible errors on the evaluation of the considered chemical shifts could thus lead to uncertain $\Delta\delta$ values and eventually to unreliable K values. Moreover, the simultaneous fit of K and $\Delta\delta$ parameters leads to very small estimated errors that are not significant. This procedure afforded K values that appeared very sensitive to the value of $\Delta\delta$.

To have a more precise estimation of the errors in the determination of the binding constants, the calculations were performed with fixed $\Delta\delta$ values that were incremented between each fitting procedure. Starting from the maximum experimental observed CIS (first fixed $\Delta\delta$ value) and progressively incrementing these values, the K values were eventually obtained

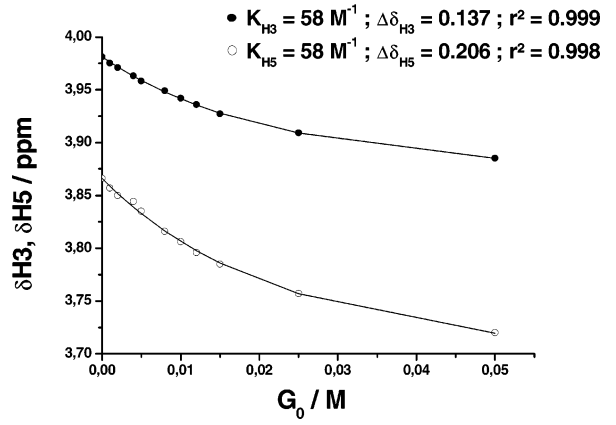


Figure 6. Plot of CIS versus concentration of nitrone **1** for PBNOH/β-CD ^{1}H NMR titration.

from the respective $\Delta\delta$ values, for both H_{CD5} and H_{CD3} protons and for each nitrone/β-CD system. The evolution of the calculated values (K from $\Delta\delta$) as a function of the obtained correlation factors (r^2) allowed the definition of the most reliable domain for K and $\Delta\delta$ values. The averaged K and $\Delta\delta$ values considering all the values exhibiting $r^2 > 0.994$ are given in Table 2 (except for PBNdiOH **10**: all the values for $r^2 = 0.983$ and for H_{CD3}). The averaged K and $\Delta\delta$ values obtained in this way are very close to those obtained by the direct and simultaneous fit of these two parameters but with a certainly more reliable estimation of the errors.

Correlation with Structural Features. The nature of the substituents present on the PBN skeleton exerts significant effects on the association constants. The presence of polar hydroxy substituents on a guest compound clearly disfavors the complexation by the hydroxy side (except for phenolic derivatives).[14] Thus, the association constant for PBNOH **1** ($K = 58$ M^{-1}) is twice smaller than the constant of the parent unsubstituted nitrone, PBN **13** ($K = 117$ M^{-1}). The nitrones PBNdiOH **10** and PBNtriOH **11** exhibited even smaller values of binding constants, ($K = 32$ and 35 M^{-1}, respectively). This trend is in good agreement with the results obtained from the continuous variation method. However, in the case of nitrone **2**, the presence of a methyl substituent in the para position of PBNOH is sufficient to restore an association constant, ($K = 98$ M^{-1}), close to that of PBN **13** ($K = 117$ M^{-1}).

Despite the limitations induced by the impossibility to take into account the activity coefficients in the equilibrium expression, the binding constants for nitrones **2**, **7**, and **1** are in fairly good agreement with the trends observed with closely related compounds.[3,18] Indeed, binding constants for 4-Me-PBNOH **2** ($K = 98$ M^{-1}) and 4-MeO-PBNOH **7** ($K = 148$ M^{-1}) are respectively approximatively twice and three times higher than for PBNOH **1** ($K = 58$ M^{-1}). The relatively high value for 4-Et-PBNOH **3** ($K = 1075$ M^{-1}) is understandable because of the higher hydrophobicity and bulk induced by the *p*-ethyl substituent.

The nitrone **12** has a different structural type as it is a phenylalkylnitrone and not an arylnitrone analogous to PBN. The association constant ($K = 235$ M^{-1}) presented the second highest value after the 4-Et-PBNOH nitrone **3**. This can be explained by the structural similarity between the fragments of the two nitrones that are embedded in the cavity. Indeed, the fragment inside the cone of the cyclodextrin is a phenylethyl group (C_6H_5-CH_2CH_2) for nitrone **12** and an ethylphenyl group (CH_3CH_2-C_6H_4) for nitrone **3**. These groups show closely related steric bulk and lipophilic character leading to the attraction for the hydrophobic cavity of the β-cyclodextrin.

In conclusion, the high differences between the values of the binding constants of the nitrones substituted on the para position of the phenyl ring suggest that this moiety is directly involved in the complexation phenomenon since the *tert*-butyl moiety remains identical (for **1**, **2**, **3**, and **7**). This observation confirms the assumed 1:1 stoichiometry and implies a complexation by the aryl side of the nitrones.

2D-NMR ROESY Experiments. Precise knowledge on the availability of the nitrone function in aqueous medium is a factor that can be of utmost importance for the use of these complexes in spin trapping experiments. The ROESY experiments were performed with the four nitrones PBN **13**, PBNOH **1**, 4-Me-PBNOH **2**, and 4-Et-PBNOH **3** in the presence of β-CD. All the considered supramolecular systems exhibited the same kind of spectrum. A partial ROESY spectrum of the PBN/β-CD system is presented in Figure 7 and is representative of the four systems that were studied. Cross-peaks related to spatial interactions between the aromatic and vinylic protons of the guest and the β-CD inside-cavity protons clearly appeared.

To attribute unambiguously the protons H-5, H-6A, and H-6B of the β-cyclodextrin region, a HMQC spectrum (Figure 8) of the PBN/β-CD and PBNOH/β-CD systems was performed in the same conditions as those used for the ROESY spectrum. This assignment is important since the shift that could occur upon complexation could lead to an inversion in the attributions of the protons of the pure β-CD NMR spectrum. The HMQC experiment led to the precise determination of the ^{1}H NMR spectrum region of H_{CD5} and $H_{CD6A,6B}$, which were essentially superimposed and the attribution of the large ROE interactions to both H_{CD5} and $H_{CD6A,6B}$ protons. Moreover, in the case of PBNOH **1**, the HMQC spectrum distinguished clearly the meta and para protons of the nitrone, and precised unambiguously the ROE interactions between the meta nitrone protons and the H_{CD3}, H_{CD5}, and $H_{CD6A,6B}$ protons. In the case of PBN **13**, ROE interactions between the meta protons of the nitrone and the H_{CD3}, H_{CD5}, and $H_{CD6A,6B}$ protons of the β-cyclodextrin were clearly observed as well as a weak interaction between the para proton of the nitrone and only the $H_{CD6A,6B}$ protons of the β-cyclodextrin.

The ROE cross-peaks were again characteristic of a phenyl-in inclusion. However two cross-peaks between the nitronyl H_{Nit9} proton and the H_{CD5} and H_{CD3} protons of the β-cyclodextrin were present. These two cross-peaks can result either from (i) the formation of two different phenyl-in nitrone complexes (one complex with the phenyl moiety embedded in the cavity from the wide rim and the other complex with the phenyl ring embedded in the cavity from the narrow rim), or from (ii) rapid moves of the substrate inside the cavity so that the vinylic proton could in turn approach the two inside cavity protons. This latter explanation is not relevant because of the absence of cross-peaks between the protons of the *tert*-butyl substituent and the β-cyclodextrin inside cavity protons, which should be observed in the conformation with the vinylic nitronyl proton deeply embedded in the cavity so as to interact with the H_{CD5} protons. On the other hand, the former possibility is more likely because of the very small difference of cavity diameter between the wide and narrow rims (6.5 and 6 Å, respectively). Therefore, we consider that the nitrones enter the β-cyclodextrin by its phenyl side and through the two accessible rims of the cavity. Recently, Funasaki et al.[28] have described a method for the determination of the relative ratio between two kinds of 1:1 complexes. However, at the present stage of our investigations, such a

TABLE 2: Calculated Values of Association Constants and Maximum CIS for All Considered Nitrone/β-CD Systems

nitrone number	nitrone structure	K H_{CD5} (M^{-1})	$\Delta\delta$ H_{CD5} (ppm)	K H_{CD3} (M^{-1})	$\Delta\delta$ H_{CD3} (ppm)	K^a (M^{-1})
3	4-Et-PBNOH	1162 (184)	0.231 (0.007)	988 (177)	0.146 (0.006)	1075 (184)
12	PEBNOH	224 (69)	0.240 (0.032)	245 (65)	0.135 (0.014)	235 (69)
7	4-MeO-PBNOH	132 (34)	0.237 (0.032)	164 (54)	0.149 (0.023)	148 (54)
13	PBN	135 (20)	0.132 (0.010)	98 (9)	0.126 (0.007)	117 (16)
2	4-Me-PBNOH	112 (24)	0.280 (0.035)	84 (29)	0.196 (0.041)	98 (29)
1	PBNOH	58 (9)	0.206 (0.014)	58 (11)	0.137 (0.011)	58 (11)
11	PBNtriOH	30 (11)	0.307 (0.081)	39 (12)	0.156 (0.033)	35 (12)
10	PBNdiOH	*b*	*b*	32 (13)	0.186 (0.050)	32 (13)

[a] Average calculated value between data derived from H_{CD5} and H_{CD3}. [b] The fitting procedure did not converge.

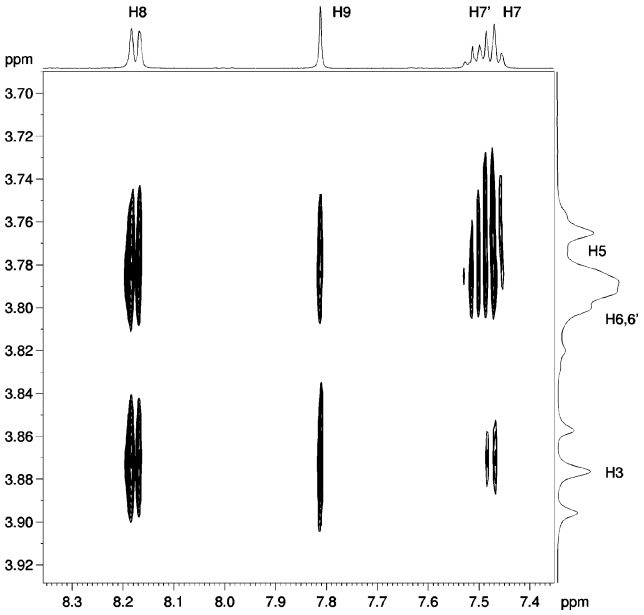


Figure 7. Part of the 500 MHz ROESY spectrum of PBN/β-CD illustrating intermolecular interactions between the protons of the phenyl moiety of the nitrone and protons of β-cyclodextrin situated inside of the cavity.

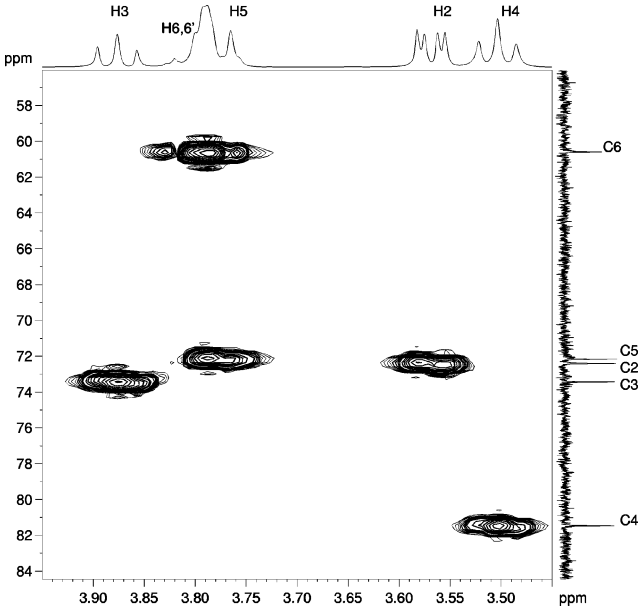


Figure 8. Part of the 500 MHz HMQC spectrum of PBN/β-CD determining the precise ^{1}H NMR signals attribution of the β-cyclodextrin protons.

quantitative assessment of the relative weight of the two complexes was not deemed necessary, as we were essentially interested by the values of the association constants which are

TABLE 3: Intermolecular ROE Cross-Peaks[a] between Nitrone and Cyclodextrin Protons

nitrone	H_{Nit7}	H_{Nit8}	H_{Nit9}
PBN 13			
H_{CD3}	+	+++	+++
$H_{CD5,6A,6B}$	+++	+++	++
PBNOH 1			
H_{CD3}	++	++	++
$H_{CD5,6A,6B}$	++	++	++
4-Me-PBNOH 2			
H_{CD3}	++	+++	++
$H_{CD5,6A,6B}$	++	+++	++
4-Et-PBNOH 3			
H_{CD3}	++	+++	+
$H_{CD5,6A,6B}$	++	+++	+

[a] The relative strength of cross-peaks is indicated by +++ (strong), ++ (medium), and + (weak).

critical for the ultimate goal of our study, the trapping of free radicals with complexation of the spin traps, and their spin adducts through dynamic processes. Schneider et al.[29] have suggested the similar existence of two geometries in the case of a tripeptide substrate complexed with substituted amino cyclodextrins with estimation of the depth of inclusion based on the cross-peak intensities of probe protons. In the case of our nitrone−cyclodextrin complexes, the presence of the supplementary H_{Nit9} proton behaving like a probe related to the depth of inclusion of the phenyl part on one hand, and the absence of spatial interactions between the *tert*-butyl part and the inside cyclodextrin cavity protons on the other hand, are clearly in favor of the existence of two types of 1:1 complexes. Similar types of competitive inclusion by the wide and the narrow rims of the cyclodextrin were also reported by Salvatierra et al.[30] The fast equilibrium experienced between both complexed and isolated molecules could explain the reliable stoichiometries as well as binding constants calculations despite the presence of two different complexes in solution. Thus, the average geometry clearly appeared in our NMR experiments because of the fast equilibrium phenomenon. The less intense cross-peaks appearing in the case of PBNOH **1** are probably due to the smaller binding constant since the accumulation time was the same for all our experiments. The results of a qualitative analysis of cross-peaks between guest and host protons are reported in Table 3.

In the case of the phenyl *tert*-butyl nitrone **13**, the experimental $\Delta\delta$ observed in the continuous variation method showed strong interactions between the H_{CD3}, H_{CD5} protons of the β-cyclodextrin and the nitronyl and *tert*-butyl protons of PBN, suggesting the possibility of a *tert*-butyl-in complexation in which the nitronyl function is deeply inserted into the CD cavity. But on the other hand, the ROESY experiments suggested a phenyl-in interaction with β-CD. Moreover, the stronger interactions in the ROESY spectrum between H_{Nit7} and H_{CD5} on one hand and between H_{Nit9} and H_{CD3} on the other hand suggested more favorable interactions between the phenyl moiety and the

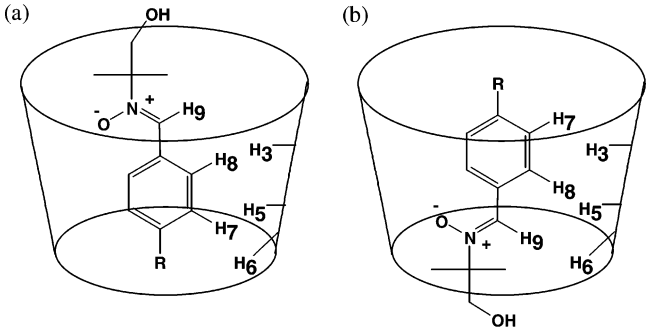


Figure 9. Proposed structure of 4-R-PBNOH/β-CD inclusion complexes with complexation through the wide rim (a) or the narrow rim (b).

narrow rim of the cyclodextrin and for the *tert*-butyl side with the wide rim. A similar structure was suggested by Lucarini et al.[7] for the DM-β-CD complex of *tert*-butylbenzylamine in which the aromatic moiety was located near the narrow rim of DM-β-CD, whereas the *tert*-butyl moiety was located near the wide rim.

In the case of 4-R-PBNOH (with R = H **1**, Me **2**, Et **3**), both experimental $\Delta\delta$ and ROESY experiments showed strong interactions between the cyclodextrin H_3 and H_5 protons and the aromatic and vinylic protons of the nitrones, suggesting only a phenyl-in complexation in which the nitronyl function is less inserted in the cavity. H_{Nit9} interacted with both H_{CD5} and H_{CD3} in the same way proving the equally likely presence of two complexes in solution. The increasing interactions between H_{Nit9} and β-CD protons in the series 4-Et-PBNOH, 4-Me-PBNOH, and PBNOH suggest, as previously deduced from the continuous variation method studies (Chart 1), a deeper inclusion of the nitronyl carbon inside the hydrophobic cavity in the case of PBNOH. Indeed, the stronger interactions of the 4-ethylphenyl moiety with the β-CD cavity in the case of 4-Et-PBNOH **3** are confirmed by the presence of more intense cross-peaks between H_{Nit8} and H_{Nit7} with the inside cavity protons while H_{Nit9} interacted less intensely than in the other cases. The geometry of the two inclusion complexes for the 4-Me-PBNOH/β-CD system as deduced by NMR is presented in Figure 9.

Finally, the results obtained by 1D-NMR both with $\Delta\delta$ derived from Job's analysis and binding constants from NMR titrations are in good agreement with the picture deduced from 2D-NMR experiments.

Conclusion

PBN and all the new related PBN derivatives form inclusion complexes with β-cyclodextrin that are characterized by a 1:1 stoichiometry. The value of the association constant of PBN was 117 M^{-1}. Chemical modifications led to significant effects on the association properties and for the investigated series of PBN analogues, most of the binding constants ranged from 32 to 235 M^{-1}, except for the 4-Et-PBNOH, which exhibited a constant of 1075 M^{-1}. Introduction of hydroxy substituents on the *tert*-butyl group decreased the association with the cyclodextrin. But subsequent addition of a methyl group in the para position of the aryl moiety was sufficient to restore a binding constant close to that of PBN. 2D-NMR experiments suggested the presence of two types of inclusion complexes, the aryl moiety of the nitrone being included in the cyclodextrin through the two accessible rims. In the applications of spin trapping in the presence of cyclodextrins, the knowledge of the binding constants for the inclusion complexes formed by the cyclodextrins with either the spin traps or the spin adducts will be very important to guide the choice of reagents for the optimal experimental conditions.

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