Supporting Information

An EPR and NMR study of supramolecular effects on paramagnetic interaction between a nitroxide incarcerated in a nanocapsule with a nitroxide in bulk aqueous media.

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I. Experimental Details

A. Materials

The host, octaacid (OA), was synthesized and characterized following receipt by Gibb.[1] Sodium tetraborate was purchased from Sigma-Aldrich. The diamagnetic analog of TEMPO (TCH_3) was synthesized by following patent #WO2005005388.[2] Benzophenone was linked with ^{14}N and ^{15}N labeled TEMPO, as well as diamagnetic TCH_3 via esterification by Hassner to make BP-^{14}T, BP-^{15}T and BP-$^{14}TCH_3$, respectively.[3] 4-(2,2,6,6-tetramethylpiperidine-1-N-oxyl)trimethylammonium bromide ($^{14}T\oplus$) was synthesized by following Singer and coworkers.[4] Compound $^{14}T\oplus$ was recrystallized in ethanol prior to use. 4-carboxy-TEMPO ($^{14}T\ominus$) was purchased from VWR and used as received.

Characterization for BP-$^{14}TCH_3$ is as follows:

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.24 (s, 6 H), 1.26 (s, 6 H), 1.72 (t, *J* = 11.9 Hz, 2 H), 1.97 (dd, *J* = 11.7, 4.1 Hz, 2 H), 3.64 (s, 3 H), 5.28 (m, 1 H), 7.51 (t, *J* = 8 Hz, 2 H), 7.58 (t, *J* = 7.7 Hz, 1 H), 7.63 (t, *J* = 7.4 Hz, 1 H), 7.81 (d, *J* = 7.7 Hz, 1 H), 8.24 (d, *J* = 7.8 Hz, 1 H), 8.42 (s, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.1, 33.4, 44.5, 60.4, 65.9, 68.4, 73.6, 128.8, 128.9, 130.4, 131.3, 133.2, 133.4, 134.4, 137.4, 138.3, 165.7, 196.1; FAB-MS m/z : 397 $[M+H]^+$.

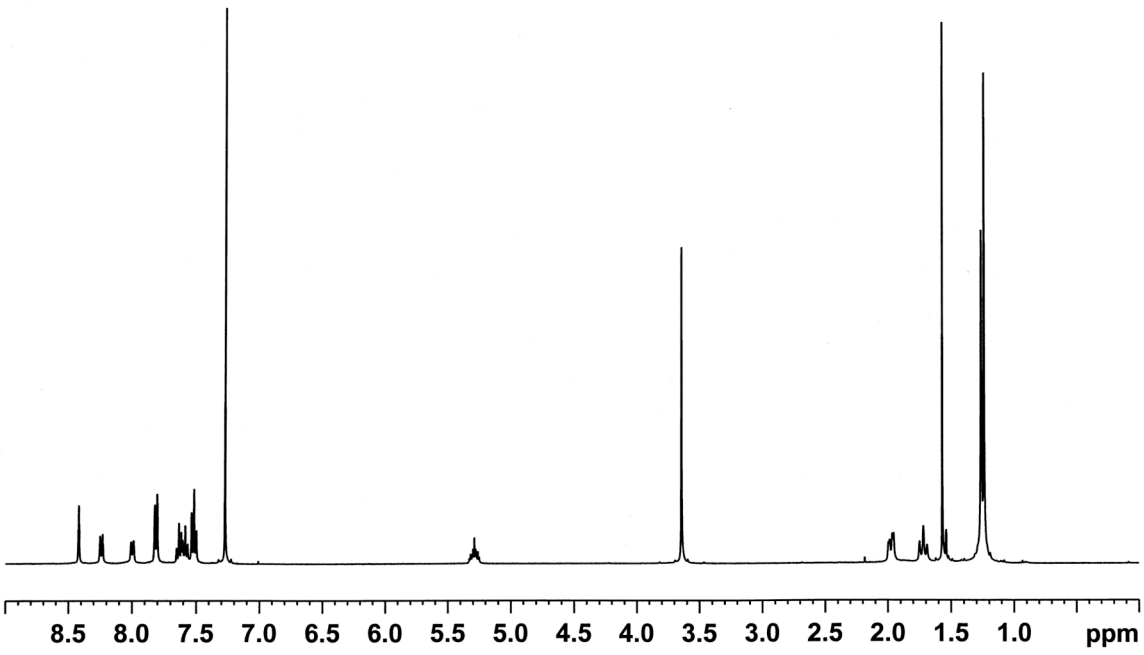

Figure S1: ^{1}H NMR spectrum of BP-$^{14}TCH_3$ (400 MHz, $CDCl_3$).

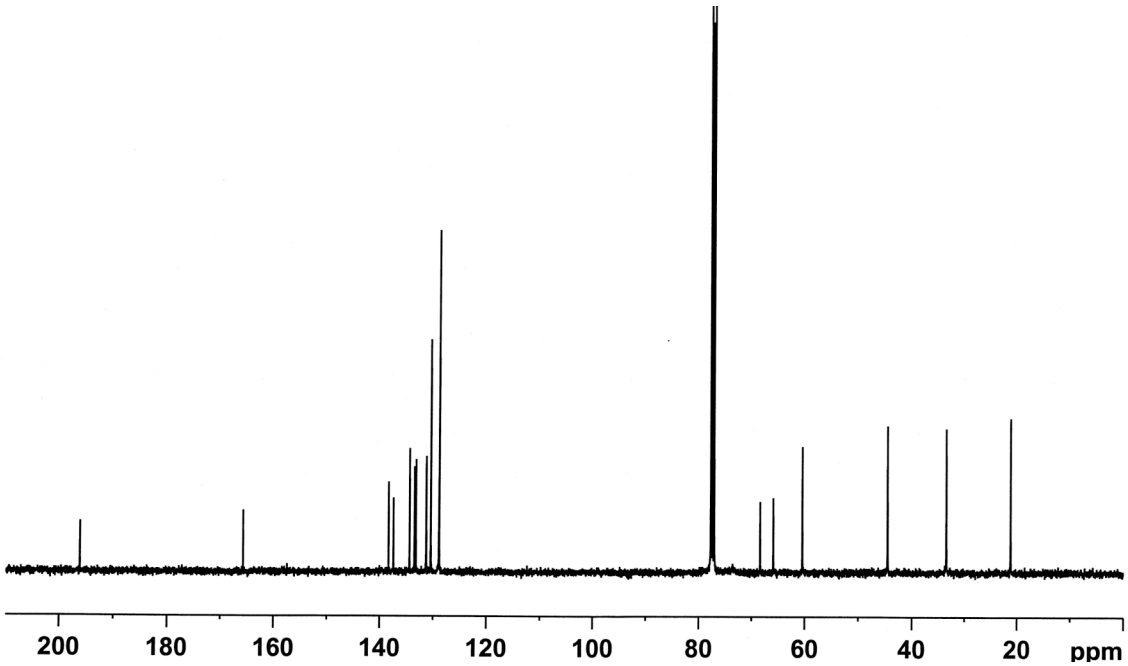

Figure S2: ^{13}C NMR spectrum of BP-$^{14}TCH_3$ (100 MHz, $CDCl_3$).

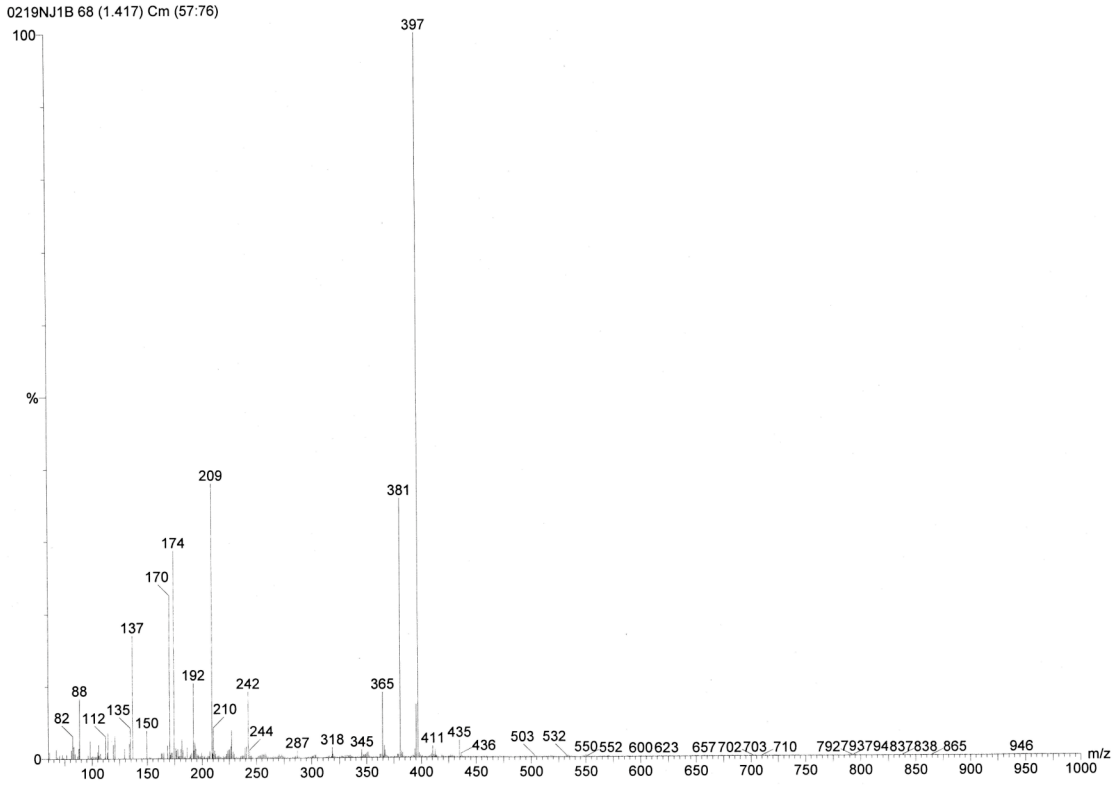

Figure S3: FAB-MS spectrum of BP-$^{14}TCH_3$.

B. Methods

NMR

600 μL of a D_2O solution of host OA (1 mM) and sodium tetraborate buffer (10 mM) was added into a NMR tube. 60 mM stock solutions of the guests were prepared in DMSO-d_6. The complexation was achieved by mixing calculated amount of guest solution to the OA and shaking the NMR tube for about 5 min (Bruker 500 MHz NMR at 27 ^oC under aerated conditions).

Diffusion experiment was carried out in Bruker 500 MHz NMR spectrometer at 27 ^oC. Data were collected by using 'stebpg1s' pulse sequence (32 scans) and processed by T_1/T_2 relaxation module in the TOPSIN 2.1 software.

EPR

EPR spectra were measured with a Bruker EMX X-band spectrometer. Samples were placed in a 1mm inner diameter pyrex tube for measurements. A 6 mM stock solution of OA was prepared in 20 mM borate buffer and an aqueous NaOH solution (30 mM) was added dropwise until pH = 9. All measurements were taken in air saturated solutions at ambient temperature (~22 °C) with a modulation frequency of 100 kHz and a microwave power of 2.012 mW. Simulations of the EPR spectra were performed using NSLS.[5,6]

Stock solutions of the internal probe BP-^{15}T, BP-^{14}T and its diamagnetic equivalent, BP-$^{14}TCH_3$ were prepared at 6 mM in $CHCl_3$. Water-soluble external probes, $^{14}T\oplus$ and $^{14}T\ominus$ were prepared at 6 mM in 20 mM borate buffer. In the case of $^{14}T\ominus$, 6 mM NaOH was added to deprotonate the carboxylic acid group.

Solutions for EPR analysis shown in Figures 1 and 2 were prepared in two steps:

a. For the complexation of internal guest with OA, 1 mM of internal guest (either BP-^{15}T, BP-^{14}T or BP-^{14}TCH$_3$) was pipetted into vial and the solvent, $CHCl_3$, was allowed to evaporate. Two equivalent of OA solution (2 mM OA in 20mM borate buffer) were added to the dry vial and the samples were allowed to shake for four hours for complexation.
b. After four hours of shaking, the spectrum of the sample containing BP-^{14}T@(OA)$_2$ was recorded (Figure 1d). To the other samples shown in (e) and (f) of Figure 1 and Figure 2, one equivalent of charged external guest (1mM), ^{14}T⊕ or ^{14}T⊖, was added and the solutions were mixed prior to acquiring the spectra.

II. Results

A. ^{1}H NMR studies:

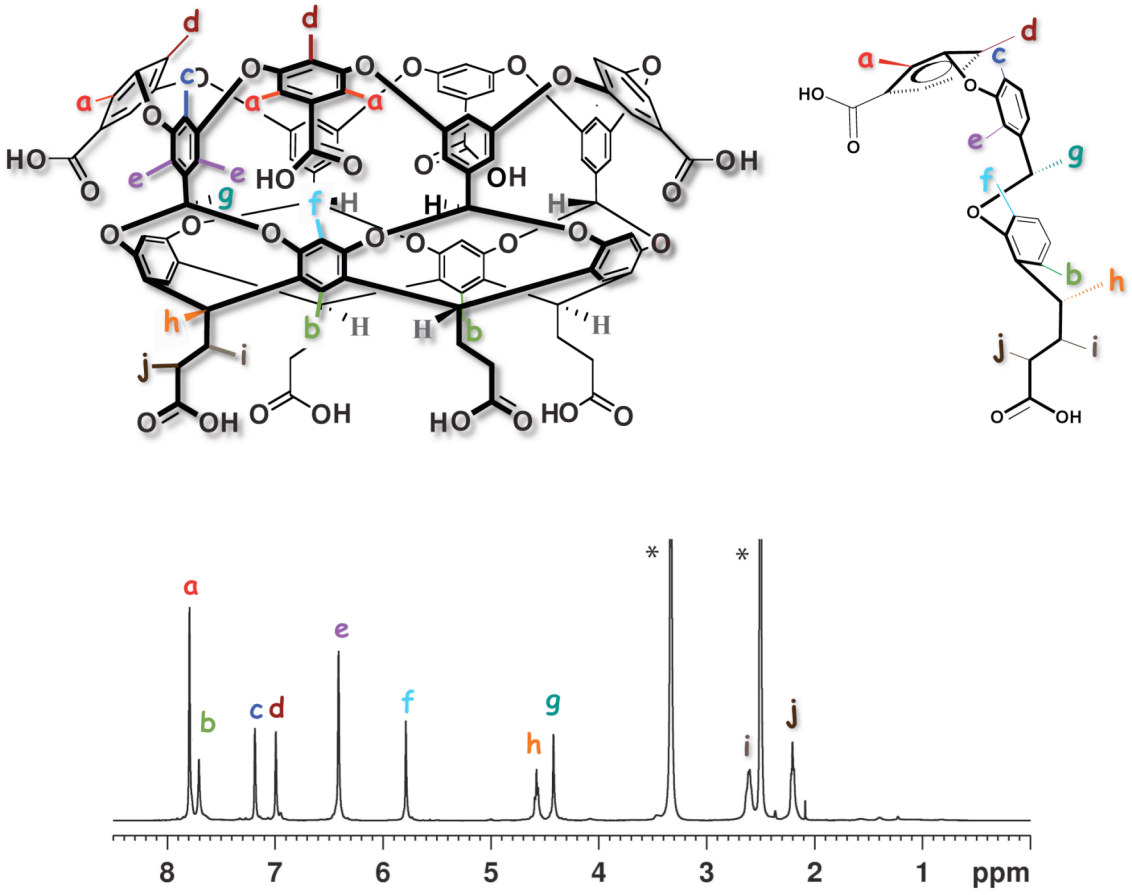

Figure S4. ^{1}H NMR spectrum of the host octa acid (500 MHz, DMSO-d_6). Resonances from residual solvents are marked with *.

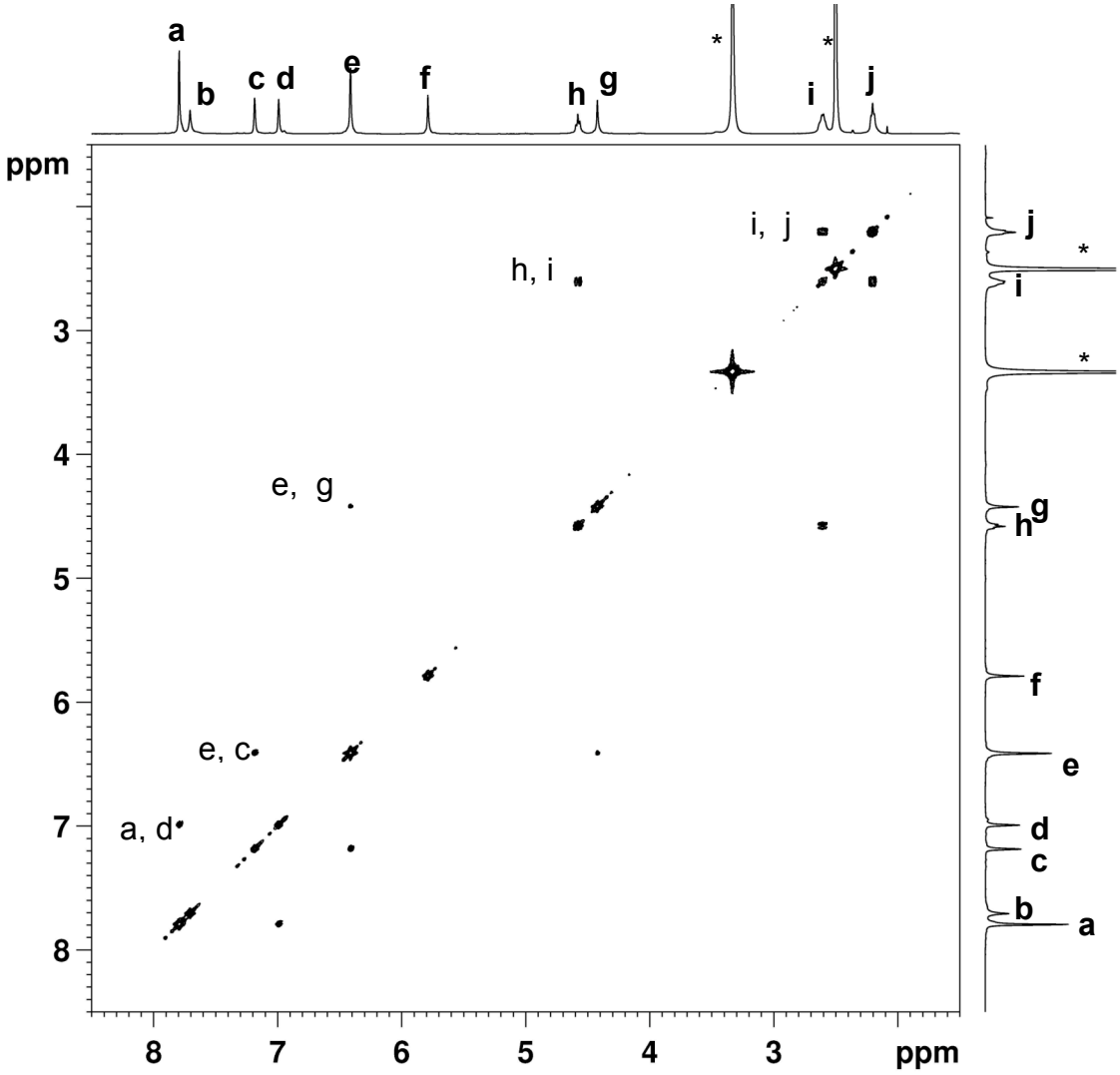

Figure S5. 2D COSY spectrum of octa acid (500 MHz, DMSO-d_6). Resonances from residual solvents are marked with *.

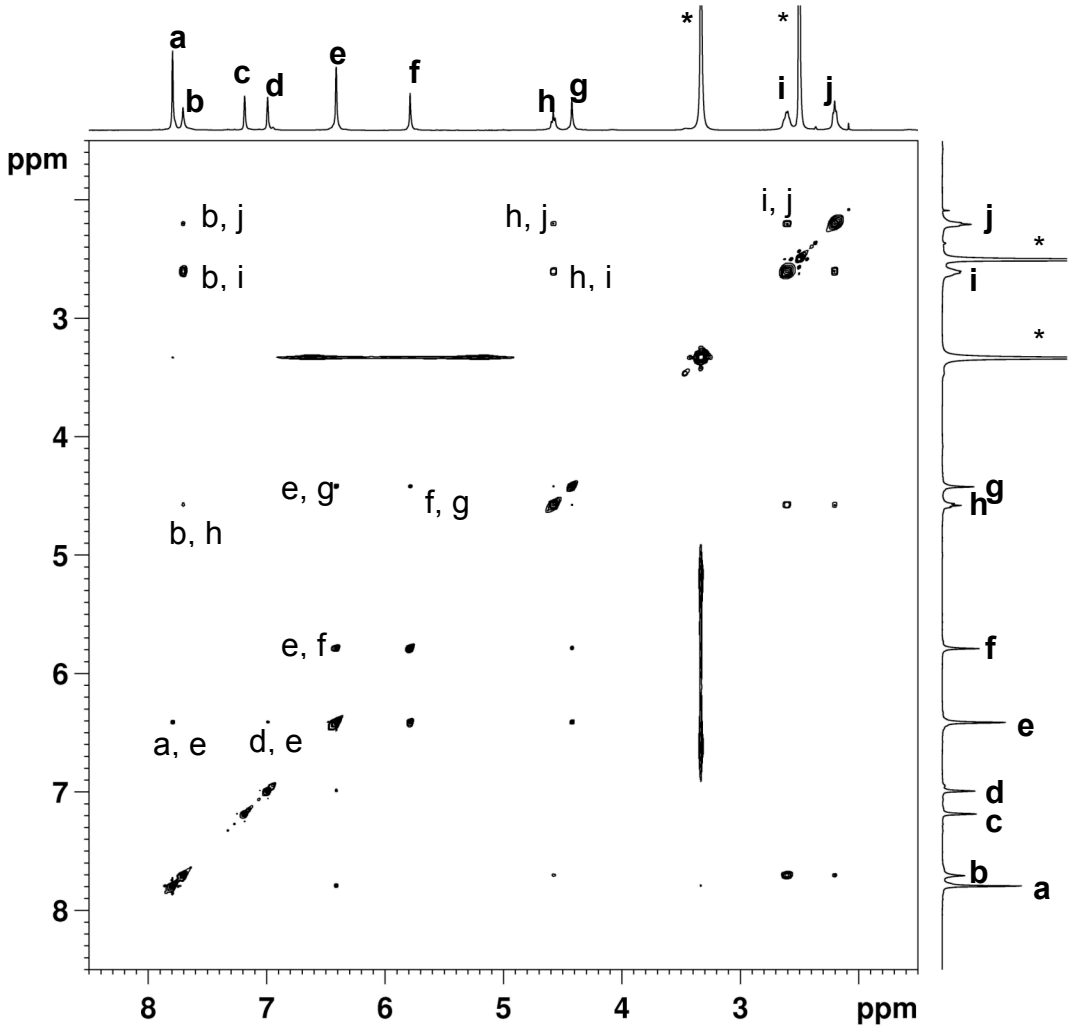

Figure S6. 2D NOESY spectrum of octa acid (500 MHz, DMSO-d_6), mixing time 300 ms. Resonances from residual solvents are marked with *.

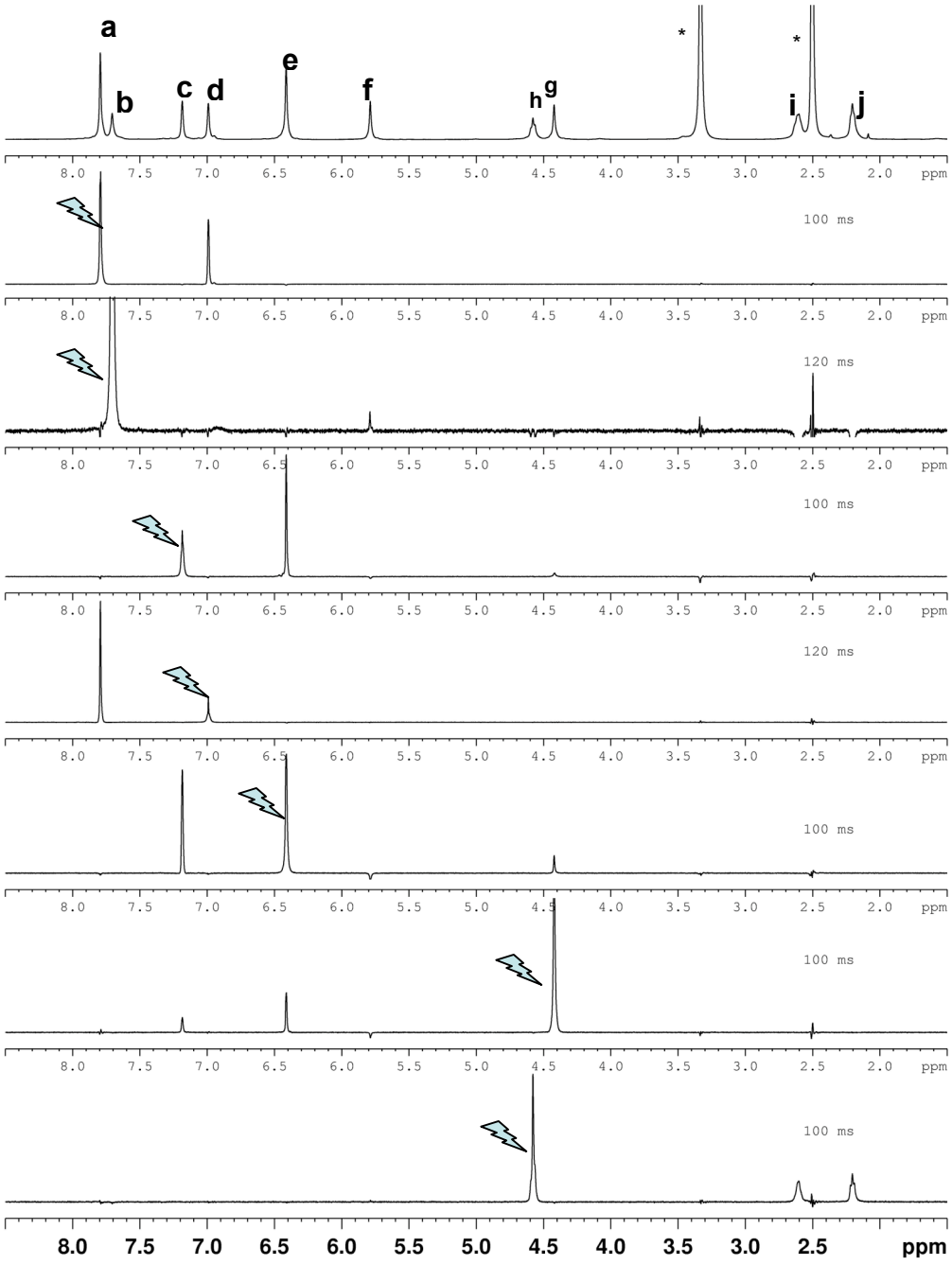

Figure S7. 1D selective TOCSY spectra of octa acid (500 MHz, DMSO-d_6). Irradiated resonances are marked with arrows. Mixing time used for each experiment is shown. Resonances from residual solvents are marked with *.

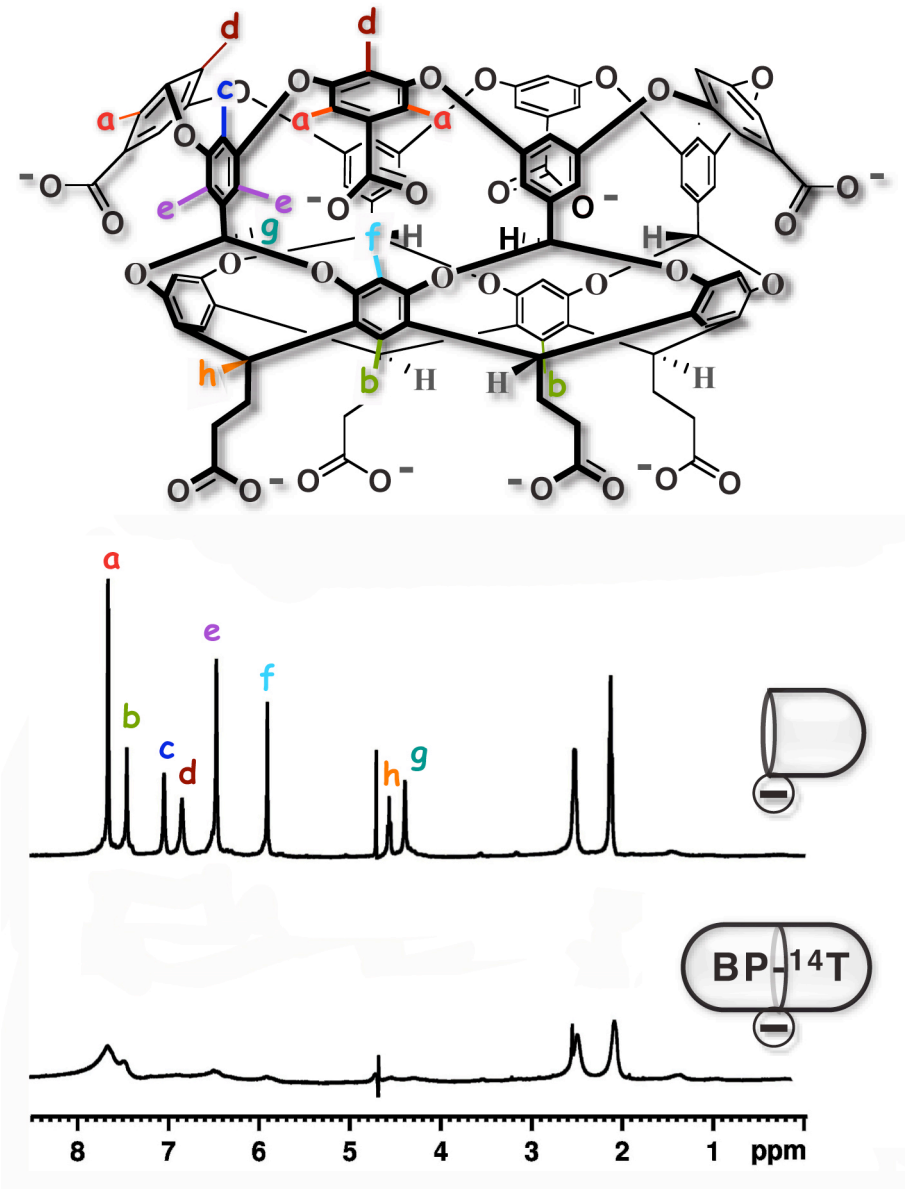

Figure S8. ^{1}H NMR spectra of octaacid (top, 1 mM in 10 mM buffered D_2O) and BP-^{14}T@$(OA)_2$.

Addition of 0.5 equivalent of BP-^{14}T to the host octaacid leads to the formation of a capsular assembly of BP-^{14}T@$(OA)_2$ (1 : 2). Broadening of all the proton resonances of the host, octaacid, is observed. Particularly H_g (inside the cavity) and H_b are broadened, suggesting that the probe was bound into the deep cavity. Since the guest molecule is asymmetric, the broadening effect is greater in half of the capsular assembly and hence,

the resonances of the benzophenone bound hemisphere of the host were seen (Figure S8, bottom).

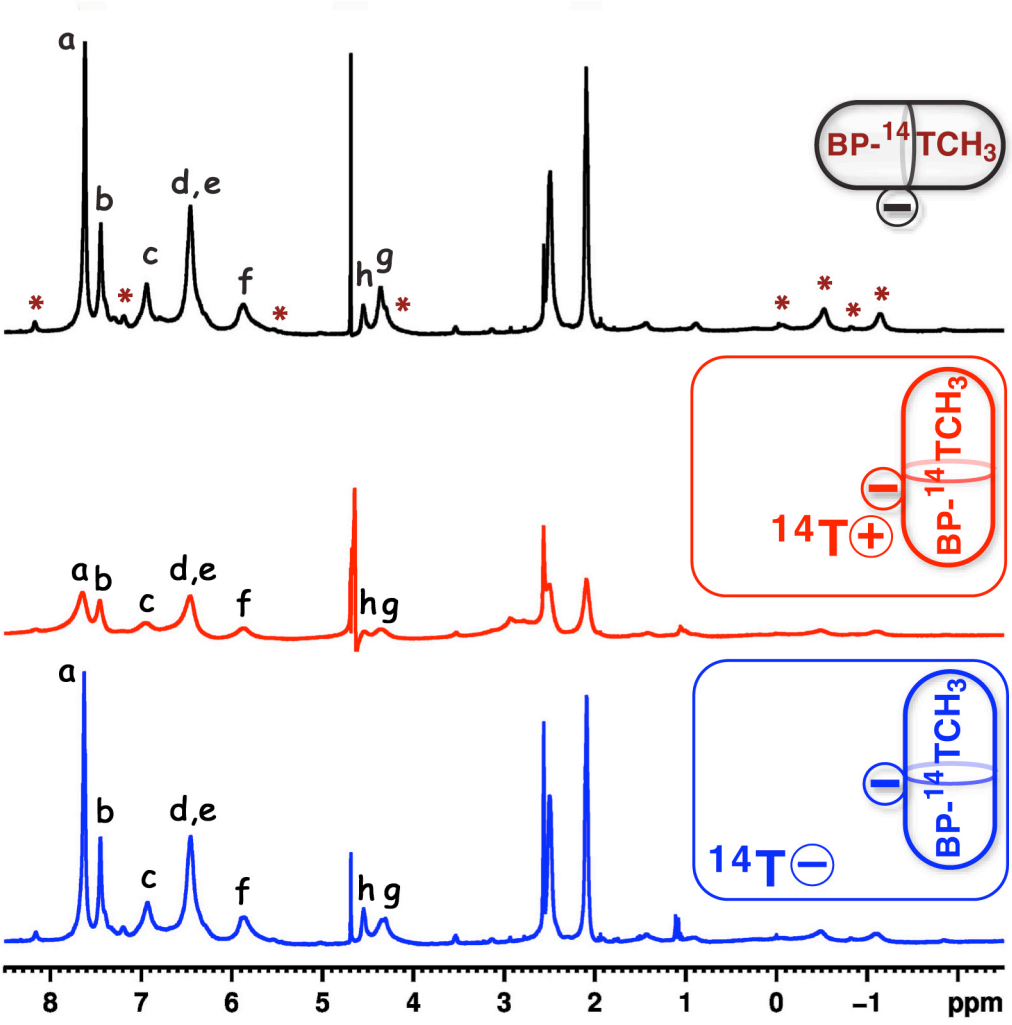

Figure S9. ^{1}H NMR spectra of octa acid (top, 1 mM in 10 mM buffered D_2O) and BP-$^{14}TCH_3$@$(OA)_2$. (*): ^{1}H NMR peaks assigned to BP-$^{14}TCH_3$@$(OA)_2$.

Upon the addition of 0.5 equivalents of diamagnetic guest, BP-$^{14}TCH_3$, upfield shifts for the methyl, methylene and bound aromatic resonances were observed (Figure S9, top, *). Incarceration of BP-$^{14}TCH_3$ resulted in the merger of H_d and H_e resonances of OA and the aromatic resonances broadened. This may be the result of the slow equilibrium between the complexed and free conformations of OA.

The diffusion constant $1.28\times10^{-6}cm^2s^{-1}$ for BP-$^{14}TCH_3$@$(OA)_2$ confirmed the formation of a 1:2 guest@host capsular assembly (diffusion constant of free OA:$1.88\times10^{-6}cm^2s^{-1}$).

Addition of cationic $^{14}T\oplus$ to the capsular assembly of BP-$^{14}TCH_3$@$(OA)_2$ led to broadening of all of the host and bound guest proton resonances (Figure S9, red) because of the paramagnetic relaxation effect (especially reducing the spin-spin relaxation time, T_2). The paramagnetic relaxation effect is less significant in the case of addition of anionic $^{14}T\ominus$ external guest to BP-$^{14}TCH_3$@$(OA)_2$ and hence, broadening of the proton resonances of host and guest was insignificant (Figure S9, blue).

B. EPR

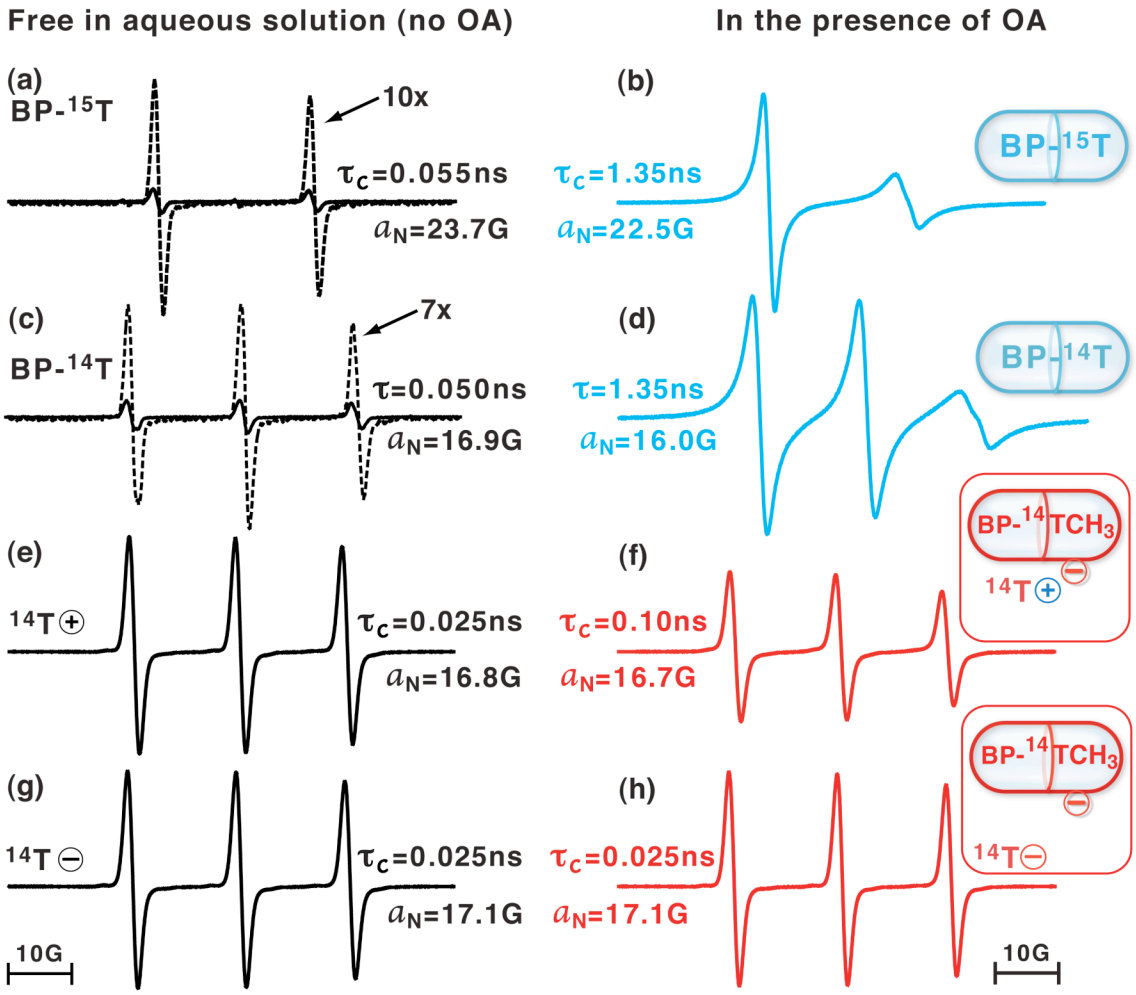

Figure S10. EPR spectra of paramagnetic guests in the absence (left) and presence (right) of OA (2 mM) in aqueous buffer solution at pH = 9 at 22°C. (b, d-h): [guest]] = 1mM; (a,c): [guest] < 0.1mM due to low solubility. The dashed spectra (a,c) were scaled by a factor of 10 and 7, respectively. The correlation times (τ_c) and hyperfine couplings (a_N) derived from simulations are shown.

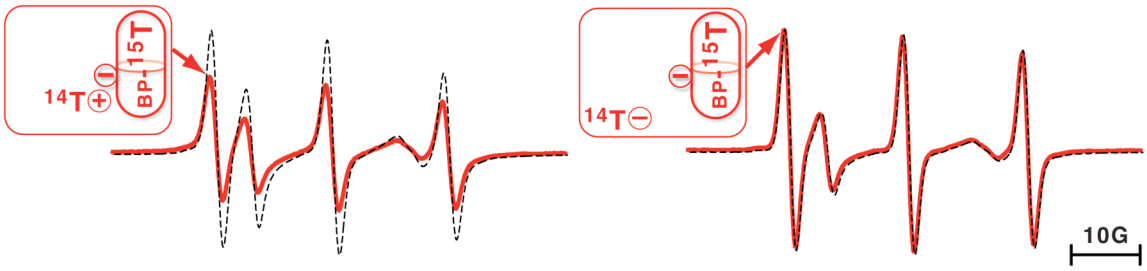

Figure S11. The EPR spectra corresponding to Figure 2 prior to integration. EPR spectra of BP-^{15}T@(OA)$_2$ in the presence (red lines) of positively charged external guest (left) and negatively charged external guest (right). The dashed black lines represent the sum of the EPR spectra of BP-^{15}T@(OA)$_2$ in the absence of external guest and the EPR spectrum of the external guest in the presence of diamagnetic BP-^{14}TCH$_3$@(OA)$_2$ (left ^{14}T⊕ or right ^{14}T⊖). [guest] = 1 mM, [OA] = 2 mM.

C. Simulation

Table S1. Correlation times, τ_c, hyperfine coupling constants, a_N of systems in aqueous solution at room temperature.

Entry	System	τ_c (ns)	$\langle a_N \rangle$ (Gauss)
1	BP-^{15}T	0.055	23.7*
2	BP-^{15}T@$(OA)_2$	1.35	22.5*
3	BP-^{14}T	0.050	16.9
4	BP-^{14}T@$(OA)_2$	1.35	16.0
5	^{14}T⊕	0.025	16.8
6	BP-$^{14}TCH_3$@$(OA)_2$ + ^{14}T⊕	0.10	16.7
7	^{14}T⊖	0.025	17.1
8	BP-$^{14}TCH_3$@$(OA)_2$ + ^{14}T⊖	0.025	17.1
9	BP-^{15}T@$(OA)_2$ + ^{14}T⊕:		
	BP-^{15}T@$(OA)_2$	1.23	22.6*
	^{14}T⊕	0.10	16.7
10	BP-^{15}T@$(OA)_2$ + ^{14}T⊖:		
	BP-^{15}T@$(OA)_2$	1.35	22.6*
	^{14}T⊖	0.033	17.1

***** The higher values of a_N for BP-^{15}T compared to BP-^{14}T are due to the different isotopes [^{14}N (I= 1/2); ^{15}N (I=1)]

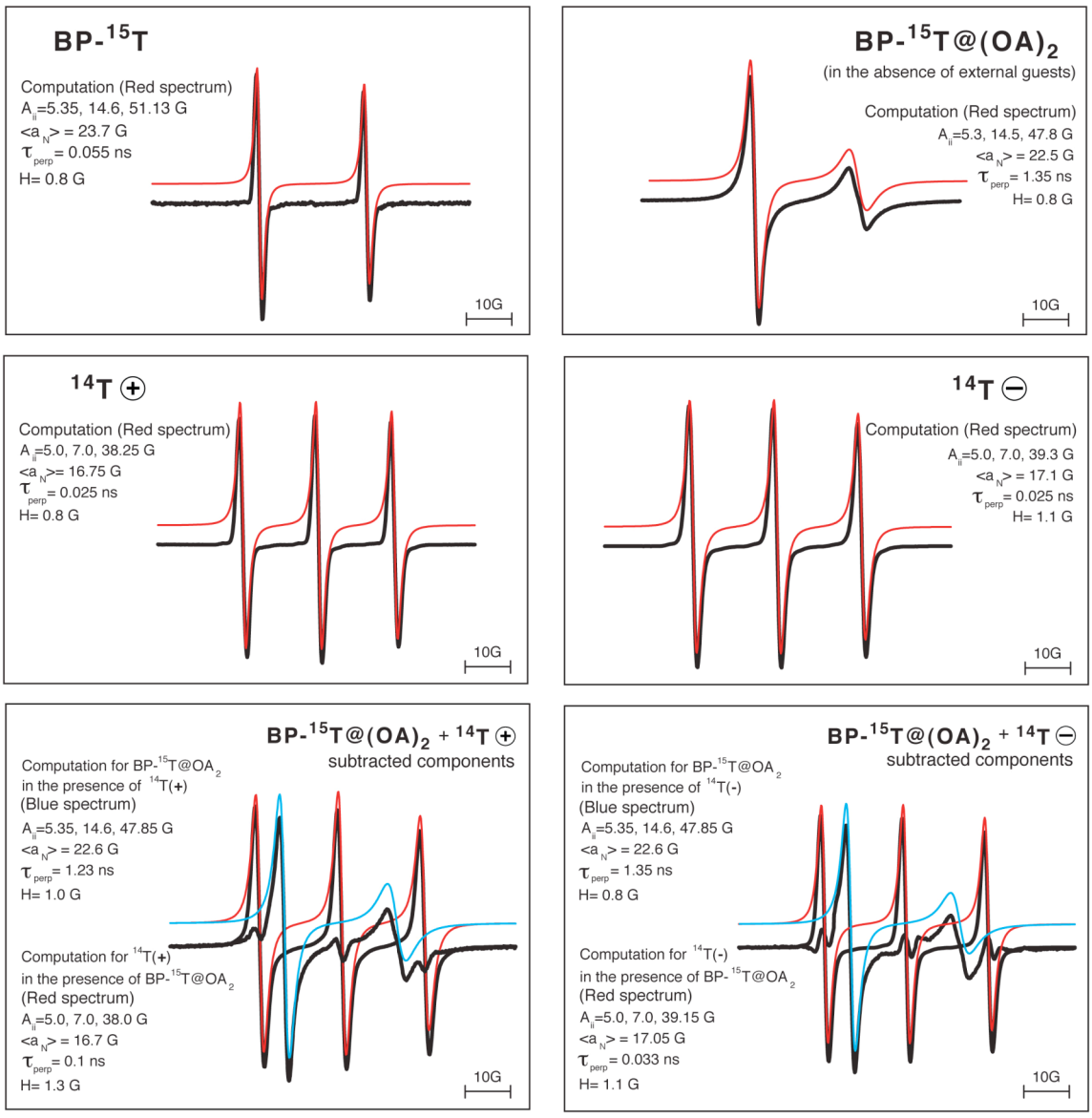

Figure S12. Experimental and simulated EPR spectra for determination of the correlation times, τ_C and hyperfine coupling constants, a_N presented in Figure 1 and Table S1.

References:

(1) Gibb, C. L. D.; Gibb, B. C. *J. Am. Chem. Soc.* **2004**, *126*, 11408-11409.
(2) Peter, G. J.; Edward, D. R. In *International Search Report*; C07D211/94; C07D211/00 ed.; Organization, W. I. P., Ed. US, 2005, p 57.
(3) Hassner, A.; Alexanian, V. *Tetrahedron Letters* **1978**, *19*, 4475-4478.
(4) Kwan, C. L.; Atik, S.; Singer, L. A. *J. Am. Chem. Soc.* **1978**, *100*, 4783-4786.
(5) Budil, D. E.; Lee, S.; Saxena, S.; Freed, J. H. *Journal of Magnetic Resonance, Series A* **1996**, *120*, 155-189.
(6) Earle, K. A.; Budil, D. E. In *Advanced ESR Methods in Polymer Research*; Schlick, S., Ed.; John Wiley & Sons, Inc.: 2006, p 53-81.