Electronic Supporting Information

Suppression of spin-spin coupling in nitroxyl biradicals by supramolecular host-guest interactions

Mintu Porel,a M. Francesca Ottaviani,b Steffen Jockusch,c Nithyanandhan Jayaraj,a N. J. Turroc and V. Ramamurthya

a Department of Chemistry, University of Miami, Coral Gables, FL, 33124, USA
b Department of Geological Sciences, Chemical and Environmental Technologies (GeoTeCA) University of Urbino, Campus ex-Sogesta, Loc. Crocicchia, 61029 Urbino, Italy
c Department of Chemistry, Columbia University, New York, NY 10027, USA

Content

| Figure S1 | Dimensions of the hosts investigated | S2 |
| Figure S2 | 1\textsuperscript{H} NMR spectra of 4 (a) in CDCl\textsubscript{3} (b) in D\textsubscript{2}O and (c) 4@CB\textsubscript{8} | S3 |
| Figure S3 | EPR spectra and their simulations for (a) 4 in water, (b) 4/\textsubscript{CB8} (1:1), (c) 4/\textsubscript{CB8} (1:2), and (d) 4/\textsubscript{CB8} (1:3) | S4 |
| Figure S4 | 1\textsuperscript{H} NMR spectra of 5 (a) in D\textsubscript{2}O and (b) 5@CB\textsubscript{8} | S5 |
| Figure S5 | Partial 1\textsuperscript{H} NMR titration spectra of 5 with CB\textsubscript{8} | S6 |
| Figure S6 | EPR spectra of 3 in (a) 50 % MeOH/H\textsubscript{2}O, (b) 3/\textsubscript{CB8} in H\textsubscript{2}O (1/0.5), and (c) 3/\textsubscript{CB8} (1/1) in H\textsubscript{2}O | S7 |
| Figure S7 | EPR spectra of 1 and 2 complexes with CB\textsubscript{8} | S7 |
| Figure S8 | EPR spectra and their simulations for 3 within different hosts | S8 |
Figure S9  Comparison of hyperfine splitting and rotational correlation time of 3 in absence and presence of different hosts

Experimental Section  S10-S14

Figure S10  $^1$H NMR of 4 in CDCl$_3$  S15

Figure S11  $^1$H NMR of 5 in CDCl$_3$  S16

Figure S12  $^1$H NMR of benzyl-4-(1,2,2,6,6-pentamethyl piperidinyl)phthalate in CDCl$_3$  S17

Figure S13  $^1$H NMR of mono-4-(1,2,2,6,6-pentamethylpiperidinyl)phthalate in CDCl$_3$  S18

References  S19

Figure S1  Investigated host structures. Dimensions of the hosts refer to atom-to-atom distance and do not include Van der Walls radii.
Figure S2 ¹H NMR spectra of 4 (a) in CDCl₃ (1mM, 500 MHz) (b) in D₂O (1mM, 500 MHz) and (c) 4@CB8, (H:G = 1:1, [4] = 1 mM, (500 MHz). Aliphatic guest resonances are represented with label 1-3 and aromatic guest protons are represented with label "*". The labels ‘a’ and ‘e’ represent axial and equatorial resonances. Host resonances are represented with label “■”. The subscript ‘a’ and ‘e’ describes the ‘axial’ and ‘equatorial’ position in the piperidine moiety. The labels “♦”, “▲” and “●” represent the residual CHCl₃, DMSO and H₂O resonances respectively.
Figure S3  EPR spectra (black lines) and their simulations (red lines) for (a) 4 in water (1 mM), $A_N = 16.9$ G, $\tau_c = 0.042$ ns (b) 4/CB8 (1:1), $A_N = 17$ G, $\tau_c = 0.11$ ns and (c) 4/CB8 (1:2), $A_N = 16.9$ G, $\tau_c = 0.14$ ns and (d) 4/CB8 (1:3), $A_N = 16.9$ G, $\tau_c = 0.14$ ns.
Figure S4. Partial $^1$H NMR spectra of 5 (a) in D$_2$O (0.5 mM, 500 MHz) and (b) 5@ CB8 (H:G = 1:1, [5] = 0.5 mM, 500 MHz). The label "*" represents residual DMSO resonance. Stock solution of guest was prepared in DMSO.

Note: In was observed that in presence of 1 eq. of CB8, -NOMe group of 5 was not upfield shifted whereas –NMe group is upfield shifted. This observation suggests that only piperidine moiety with N-Me group is encapsulated within CB8 whereas –NOMe resides in water.
Figure S5. Partial $^1$H NMR spectra of 5 (a) in D$_2$O (0.5 mM, 500 MHz) and (b) 5:CB8 (1:0.1), (c) 5:CB8 (1:0.2), (d) 5:CB8 (1:0.3), (e) 5:CB8 (1:0.4), (f) 5:CB8 (1:0.5), (g) 5:CB8 (1:0.6), (h) 5:CB8 (1:0.7), (i) 5:CB8 (1:0.8) and (j) 5:CB8 (1:1), [5] = 0.5 mM, (500 MHz). The label “*” represents residual DMSO resonance.
Figure S6. EPR spectra of (a) 3 in 50% MeOH/water, signal intensity by double integration = $1.42 \times 10^8$, (b) 3/CB8 (1/0.5) in water, signal intensity by double integration = $1.46 \times 10^8$ and (c) 3/CB8 (1/1) in water, signal intensity by double integration = $1.46 \times 10^8$, [3] = 0.1 mM.

Note: Because the signal intensities (from double integration of the EPR signal) of 3 in the absence and presence of CB8 are identical within experimental error, no nitroxy groups in 3 are “lost” due to chemical reaction in the presence of CB8. It confirms that the change of five-line EPR spectra in solution to three-line EPR spectra in presence of 1 equivalent CB8 is only because of the restriction of spin-spin exchange between two radicals upon encapsulation. Spin-spin exchange gradually decreases with increase of CB8 concentration and stopps completely in presence of 1 eq. of CB8.

Figure S7. EPR spectra (black lines) and their simulations (red lines) for (a) 2/CB8 (1:2), $A_N = 16.6$ G, $\tau_c = 0.22$ ns and (b) 1/CB8 (1:2), $A_N = 16.3$ G, $\tau_c = 0.27$ ns.
Figure S8 EPR spectrum (black lines) and its simulation (red lines) for (a) \(3/\text{CB8} \, (1:2)\), \(A_N = 16.7 \, G\), \(\tau_c = 0.24 \, ns\), (b) \(3/\text{CB7} \, (1:1)\), \(A_N = 16.9 \, G\), \(\tau_c = 0.07 \, ns\), (c) \(3/\text{CB7} \, (1:2)\), \(A_N = 16.9 \, G\), \(\tau_c = 0.06 \, ns\), (d) \(3/\text{CA8} \, (1:2)\), \(A_N = 16.9 \, G\), \(\tau_c = 0.1 \, ns\), (e) \(3/\beta\text{-CD} \, (1:2)\), \(A_N = 16.7 \, G\), \(\tau_c = 0.15 \, ns\), (f) \(3/\gamma\text{-CD} \, (1:2)\), \(A_N = 16.8 \, G\), \(\tau_c = 0.12 \, ns\), (g) \(3/\text{SDS} \, [3] = 0.1 \, mM\), \([\text{SDS}] = 200 \, mM\), \(A_N = 16.3 \, G\), \(\tau_c = 0.12 \, ns\), in the insert (44% on the total): \(\omega_{\text{exchange}} = 2 \times 10^8 \, s^{-1}\), line width = 3 G. From (a) to (f), [3]=1mM.
Figure S9: Comparison of (a) hyperfine splitting ($A_N$) and (b) rotational correlation time ($\tau_c$) of 3 in absence and presence of different hosts.
Experimental section:

Materials and Methods: Cucurbit[7]uril (CB7) and cucurbit[8]uril (CB8) were synthesized by following a published procedure\textsuperscript{1}. β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD) were purchased from Sigma-Aldrich. Calixarene[8]octa sulfonic acid (CA8) was synthesized from the published procedure\textsuperscript{2,3,4}.

Synthesis of the compounds used in the study:

Compound 1:
Terephthalic acid (0.3 g) was mixed with 4-hydroxy-2,2,6,6-tetramethyl piperidine-N-oxyl (0.8 g) and DMAP (cat.) in dry dichloromethane (20 mL) at 0 °C. A suspension of EDCI (0.83 g) in dichloromethane was added to the above reaction mixture at 0 °C, it was allowed to warm up to rt and stirred for 15 h. The reaction mixture was washed with water and dried over Na\textsubscript{2}SO\textsubscript{4} and purified by column chromatography to afford the corresponding 14N-para-14N biradical (1).

FAB-MS (m/z): 475 (M +H)\textsuperscript{+}

By comparing the EPR signal intensity (double integrating the EPR spectra) of the known concentration of the compound against a standard solution of 4-hydroxy TEMPO (1 mM in EtOH), the purity was ascertained.

Compound 2: A similar procedure was followed as described for compound 1 with isophthalic acid to afford the 14N-meta-14N biradical (2).

Compound 3: 14N-ortho-14N biradical (3) was synthesized by following the procedure reported in the literature.\textsuperscript{5}
Compounds 4 and 5:

Scheme S1. Synthesis of compounds 4 and 5.
1,2,2,6,6-Pentamethylpiperidine-4-ol (A):

2,2,6,6-Tetramethylpiperidine-4-ol (1.5 g) was mixed with 2 mL of formalin (37 % formaldehyde) solution and 0.5 mL of formic acid and refluxed for 7 h. The solution was poured in to ice and the solution was made basic with KOH and extracted with diethyl ether to afford the required 1,2,2,6,6-pentamethylpiperidine-4-ol (A) in 85 % yield.

$^1$H NMR (500 MHz, CDCl$_3$) δ: 1.01 (6 H, s), 1.15 (6 H, s), 1.36 (dd, 2 H), 1.83 (d, 2 H), 2.22 (3 H, s) 3.91 (1 H, m); $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 20.54, 28.04, 33.28, 50.06, 55.34, 63.91. GC-MS (m/z): 171 (M$^+$, 10 %), 156 (M-15, 100 %).

Mono-benzyl-phthalate (B):

A solution containing 10 mmol of benzylalcohol and phthalicanhydride in 10 mL of pyridine and 50 mL of benzene was heated at 100 °C for 2 h. The reaction mixture was cooled and poured in to ice-water mixture, acidified with conc. HCl and extracted with CHCl$_3$ and washed with 5% aqueous sodium carbonate solution. The aqueous solution was further acidified with conc. HCl and extracted with CHCl$_3$ to afford the required mono-benzyl-phthalate in quantitative yield.

$^1$H NMR (500 MHz, CDCl$_3$) δ: 5.37 (2 H, s), 7.29 – 7.9 (9 H, m); $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 67.9, 128.45, 128.56, 128.63, 128.87, 129.9, 129.97, 130.94, 132.36, 133.24, 135.24, 167.95, 172.32.

Benzyl-4-(1,2,2,6,6-pentamethylpiperidinyl) phthalate (C): Mono-benzyl-phthalate (0.68 g) was mixed with 1,2,2,6,6-pentamethylpiperidine-4-ol (0.35 g) and DMAP (cat.) in dry dichloromethane (20 mL) at 0 °C. A suspension of EDCI (0.55 g) in dichloromethane was added to the above reaction mixture at 0 °C, it was allowed to warm up to rt and stirred for 15 h. The reaction mixture was diluted with chloroform, washed with water and dried over Na$_2$SO$_4$. The crude reaction mixture was purified by column chromatography to afford the product C in 60 % yield.

$^1$H NMR (500 MHz, CDCl$_3$) δ: 1.09 (6 H, s), 1.17 (6 H, s), 1.70 (dd, 2 H), 1.94 (d, 2 H), 2.26 (3 H, s), 5.26 (1 H, m), 5.37 (2H, s), 7.36-7.73 (9 H, m); $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 20.71, 28.02, 33.25, 45.58, 55.34, 67.43, 69.38.
Mono-4-(1,2,2,6,6-pentamethylpiperidinyl)phthalate (D): The compound C was dissolved in THF and degassed with nitrogen for 15 min, charged with Pd-C (5 % on carbon, 10 % by weight) cautiously and purged with hydrogen gas for 1 h, Pd-C was removed by filtration over celite pad and the solution was concentrated to give the product in quantitative yield.

\[^{1}\text{H}\text{ NMR (500 MHz, CDCl}_3\text{): 1.38 (6 H, s), 1.56 (6 H, s), 2.26 (dd, 2 H), 2.44 (dd, 2 H), 2.68 (3 H, s), 5.34 (1 H, m), 7.36-7.77 (4 H, m); ^{13}\text{C NMR (125 MHz, CDCl}_3\text{): 22.72, 28.96, 41.99, 46.27, 63.59, 65.95, 129.06, 129.18, 131.53, 133.52, 134.04, 139.08, 169.14, 171.12.}\]

4-(2,2,6,6-tetramethylpiperidine-N-oxyl)-4-(1,2,2,6,6-pentamethylpiperidinyl)phthalate (4): Compound D (0.22 g) was mixed with 4-hydroxy TEMPO (0.16 g,) and DMAP (cat.) in dry dichloromethane (15 mL) at 0 °C. A suspension of EDCI (0.19 g) in dichloromethane was added to the above reaction mixture at 0 °C and stirred at rt for 15 h, washed with water and dried over Na\textsubscript{2}SO\textsubscript{4}. The crude reaction mixture was purified by column chromatography to afford the product 4 in 50 % yield. FAB-MS (m/z): 473 (M+) By comparing the EPR signal intensity (double integrating the EPR spectra) of the known concentration of the compound against a standard solution of 4-hydroxy TEMPO (1 mM in EtOH), the purity was ascertained.

\[^{1}\text{H}\text{ NMR (500 MHz, CDCl}_3\text{): 1.14 (6 H, s), 1.2 (3 H, s), 1.62 (2H, s), 2.0 (2H, s), 5.32 (1H, s), 7.57 (2H, s), 7.76 (2H, s).}\]

4-(1-Methoxy-2,2,6,6-tetramethylpiperidinyl)-4-(1,2,2,6,6-pentamethylpiperidinyl)-phthalate (5): Compound D (0.3 g) was mixed with compound 1-Methoxy-2,2,6,6-tetramethylpiperidin-4-ol (E) (0.19 g) and DMAP (cat.) in dry dichloromethane (15 mL) at 0 °C.\textsuperscript{6} A suspension of EDCI (0.23 g) in dichloromethane was added to the above reaction mixture at 0 °C and stirred at rt for 15 h, washed with water and dried over Na\textsubscript{2}SO\textsubscript{4}. The crude reaction mixture was purified by column chromatography to afford the product 5 in 37 % yield.

\[^{1}\text{H}\text{ NMR (500 MHz, CDCl}_3\text{): 1.14 (6 H, s), 1.2 (3 H, s), 1.14 (6 H, s), 1.23 (6 H, s), 1.24 (6 H, s), 1.63 (4H, m), 2.0 (4H, dd), 2.27 (3H, s), 3.62 (3H, s), 5.28 (2H, m), 7.52}\]
(2H, s), 7.69 (2H, s); $^{13}$C NMR (125 MHz, CDCl$_3$) δ: 21.09, 21.18, 28.45, 30.11, 33.49, 33.69, 44.35, 46.16, 55.71, 60.41, 65.89, 68.55, 69.63, 76.99, 129.22, 131.21, 131.3, 133.02, 167.46

**Preparation of host/guest complex:** Stock solution (20 mM) of guest was prepared in CHCl$_3$. Host stock solution (5 mM) was prepared in H$_2$O. Required amount of guest solution in CHCl$_3$ was added in a vial and the solvent was evaporated by shaking in a mechanical shaker. Then calculated amount of host solution and water were added and shaken by the mechanical shaker for 15 h. The same procedure was adopted for all guests.

**EPR study:** EPR spectra were recorded at room temperature in Bruker EMX spectrometer at 9.5 GHz (X band) employing 100 KHz of field modulation frequency. Spectrometer setting: Power, 1.997 mW; amplitude modulation, 0.50 G; time constant, 163.84 ms; conversion time, 163.84 ms. Samples were loaded to quartz (CFQ) EPR tubes from Wilmad LabGlass (2 mm OD, 0.5 mm wall thickness, 10 cm height) for the EPR experiments.

**Protocol for NMR study:** A $^1$H NMR spectrum of 600 µL of 1 mM CB8 in D$_2$O was recorded. To this solution 1 equivalent of guest (10 µL of 60 mM solution of 4 in DMSO) was added. The mixture was shaken well for about 5 min and the spectra were recorded.
Figure S10 $^1$H NMR of 4 in CDCl$_3$ (500 MHz)
Figure S11 $^1$H NMR of 5 in CDCl$_3$ (500 MHz)
Figure S12 $^1$H NMR of benzyl-4-(1,2,2,6,6-pentamethylpiperidinyl)phthalate (C) in CDCl$_3$ (500 MHz).
Figure S13 $^1$H NMR of mono-4-(1,2,2,6,6-pentamethyldipiperidinyl) phthalate (D) in CDCl$_3$ (500 MHz).
References