Supplementary Information for

Potential ^{129}Xe-NMR biosensors based on secondary and tertiary complexes of a water soluble pillar[5]arene derivative

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This Supplementary Information section contains:

1. Experimental Section.
2. Scheme 1, the synthesis of **4**, and the ^{1}H and ^{13}C NMR spectra of **3a** and its sodium (**3**c) or ammonium salts (**3**b).
3. Figures S1-S3
4. Table S1.

Experimental section

General

^1H Diffusion and ^{129}Xe-NMR measurements were performed on an 11.7T Avance III Bruker NMR spectrometer, operating at 500.13 and 139.09 MHz for ^1H and ^{129}Xe, respectively, equipped with a z-gradient system capable of producing a maximal pulsed gradient of about 500 mT/m. ^1H diffusion NMR experiments were performed using the eddy currents delays (LED) pulse sequence.[1] Sine-shaped pulse gradients, of 4 ms duration, were incremented from 7 to 322 mT/m in 10 steps, and the pulse gradient separation was 50 ms. The diffusion coefficients were extracted from:

$$ln\ I/I_0=-\gamma^2\delta^2G^2(2/\pi)^2(\Delta-\delta/4)D=-bD$$

where I and I_0 are the echo intensity, in the presence and absence of the gradient pulse, respectively, γ is the gyromagnetic ratio, G is the pulse gradient strength, $2/\pi$ is a geometrical correction factor due to the sine shape of the pulse gradients used, δ is the duration of the pulse gradient, Δ is the time interval between the leading edges of the pulse gradient used, and D is the diffusion coefficient. The diffusion coefficients were extracted from the slope of the plot of ln(I/I_0) against the b-values. All diffusion NMR data were acquired at 298K and were obtained in triplicate. The given values represent means ± the standard deviation of the means.

The ^1H and ^{13}C-NMR spectra of the synthesized materials were collected on a 9.4T Avance II Bruker NMR spectrometer operating at 400.13 and 100.6 MHz for ^1H and ^{13}C, respectively. The chemical shifts are given in ppm and the spin-spin couplings are reported in Hz. The ^1H-NMR spectra in $CDCl_3$ and D_2O were calibrated to 7.26 and 4.8 ppm, respectively (for residual $CHCl_3$ and HOD).

The ^{129}Xe NMR spectra were obtained using a 5 mm probe in 5 mm NMR tube containing the solution of the ammonium salt **(3b)** in D_2O to which a 2 mm NMR sealed tube containing xenon in $CDCl_3$ was inserted. In the ^{129}Xe NMR spectra xenon in $CDCl_3$ was used as a reference and was set to 0.0 ppm.

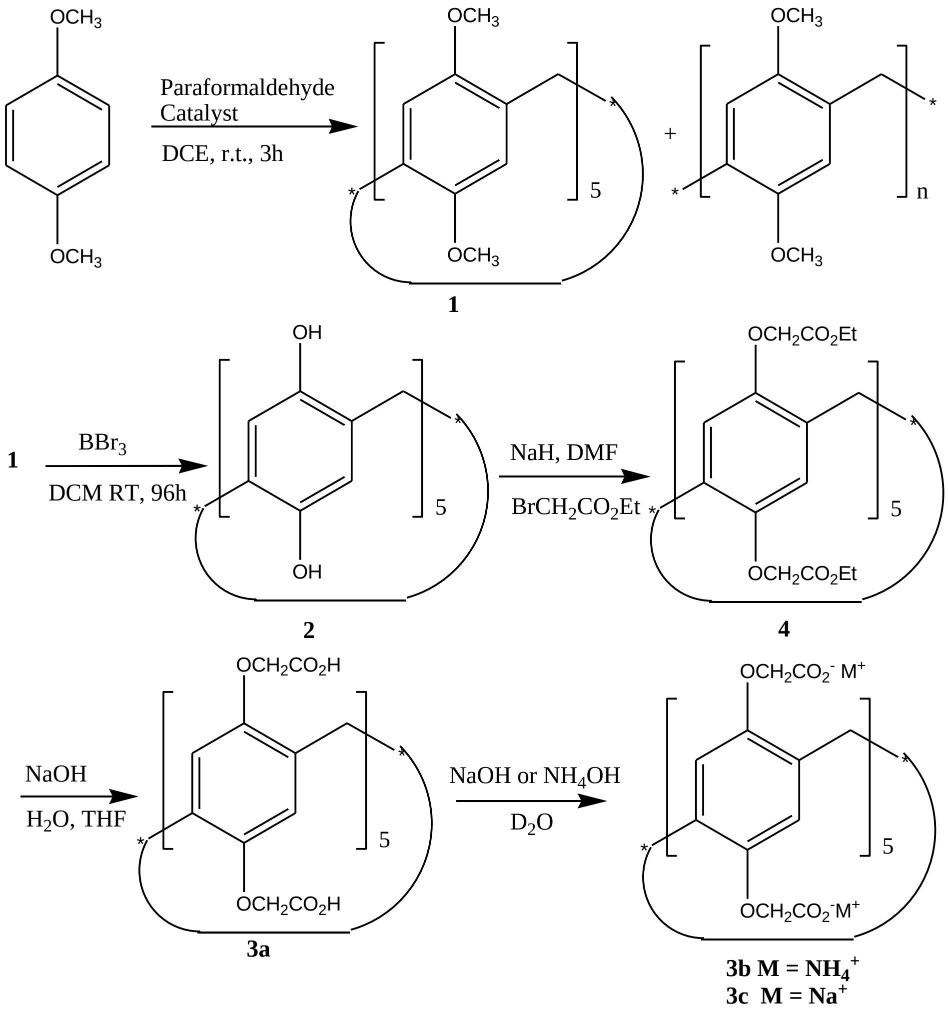
Materials

Starting materials, reagents and deuterated solvents ($CDCl_3$, D_2O) were purchased from Aldrich (Milwaukee, WI) and were used as supplied. Xenon gas was purchased from Maxima (Israel). Compounds **3a-c** were prepared according to modifications of the previously published procedure[2-3].

Synthesis and characterization of compound 3a and its salts (3b and 3c)

Dimethoxypillar[5]arene (**1**), pillar[5]arene (**2**), compound **3a** and its sodium and ammonium salts were prepared as shown in Scheme S1 according to references 2 and **3** with a slight modification only in the preparation of **4** (see below).

Scheme S1



Diethoxycarbonylmethoxy-pillar[5]arene (4)

DMF (40 mL) and THF (40 mL) were added to dry pillar[5]arene (**2**) (3.2 g, 5.2 mmol) under argon. Sodium hydride (4 g, 167 mmol) was added and the reaction mixture was stirred for 0.5 h. The anion formed gives a strong green color to the mixture. Ethyl bromoacetate (12 mL, 108 mmol) was added and the reaction mixture was heated at 60°C for 48 h. The color changed to yellow-orange. The reaction mixture was evaporated and dichloromethane (250 mL) and water (500 mL) were added to the residue. Some insoluble solid was filtered and the organic layer separated and filtered leaving an orange solid on the filter. The filtrate was evaporated. It contains the product contaminated by some derivative of ethyl bromoacetate and DMF. Distillation of most of the contaminants was performed in high vacuum (0.05 mmHg) between RT and 190^{o}C. The distillation process was stopped at that point in order to avoid overheating of the product. After cooling, acetone was added to the residue and a white solid precipitated which was then identified as diethoxycarbonylmethoxy-pillar[5]arene (**4**) (2.3 g, 1.56 mmol, Yield: 30%).

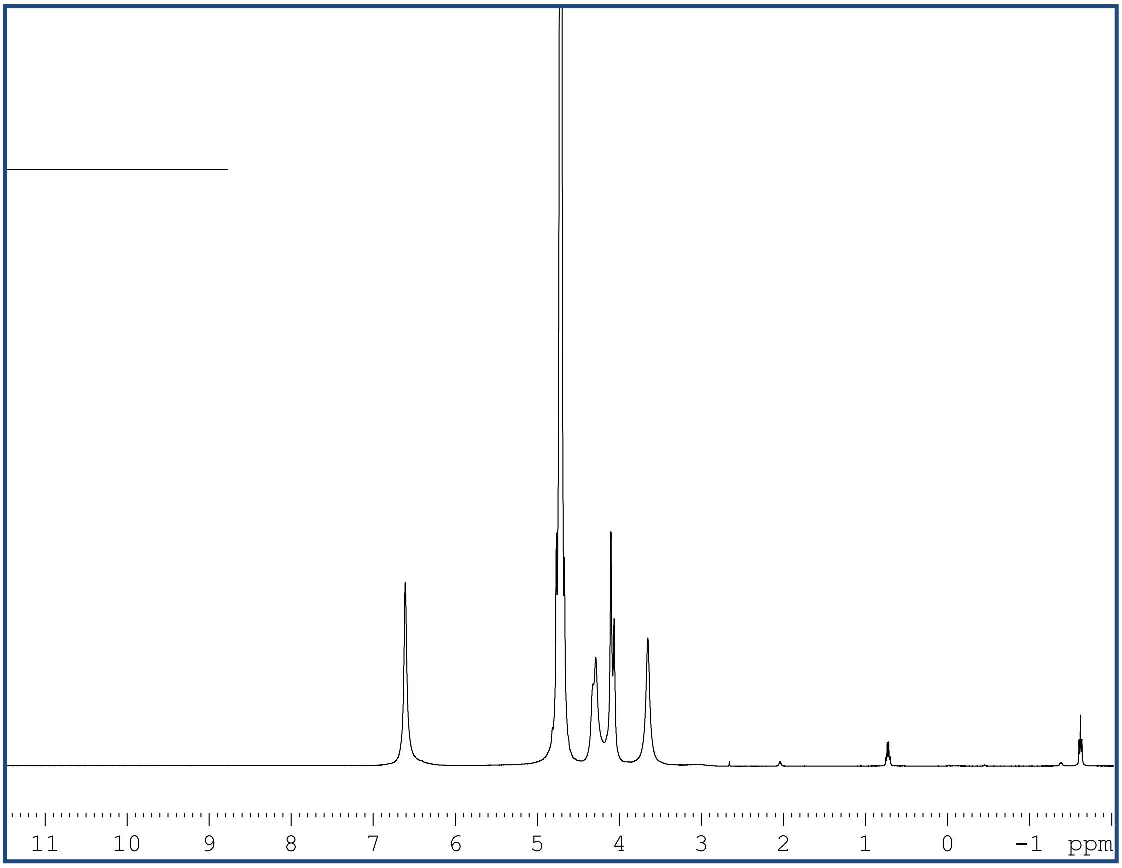
^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.04 (s, 10H, phenyl protons), 4.55 (dd, 20H, methylene protons at both rims), 4.09 (m, 20H, ethyl protons), 3.86 (s, 10H, protons from methylene bridge), 0.96 (m, 30H, ethyl protons).

Ammonium salts of 3a in D_2O.

To a suspension of **3a** (100 mg, 0.084 mmol) in D_2O (0.7mL) aqueous ammonia solution (22% w/w, 0.080 mL, 0.9 mmol) was added. A clear yellowish solution of **3b** was obtained.

^{1}H NMR (D_2O): δ 6.69 (s, 10H, phenyl protons), 4.36, 4.37 (two s, 10H, methylene protons), 4.19, 4.12 (two s, 10H, methylene protons), 3.72 (br, 10H, protons from methylene bridge).

^{13}C-NMR (D_2O): δ 178.0, 149.9, 129.2, 115.1, 68.3, 29.5.



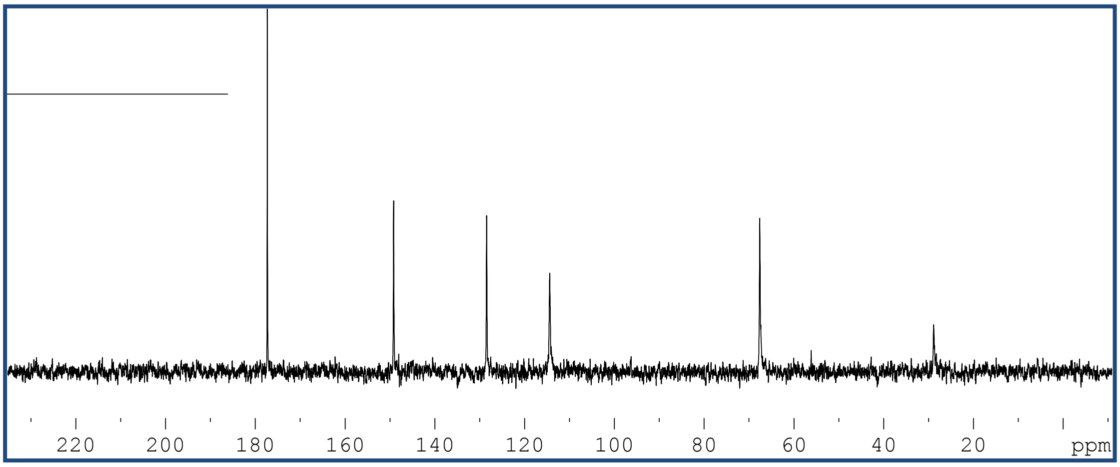


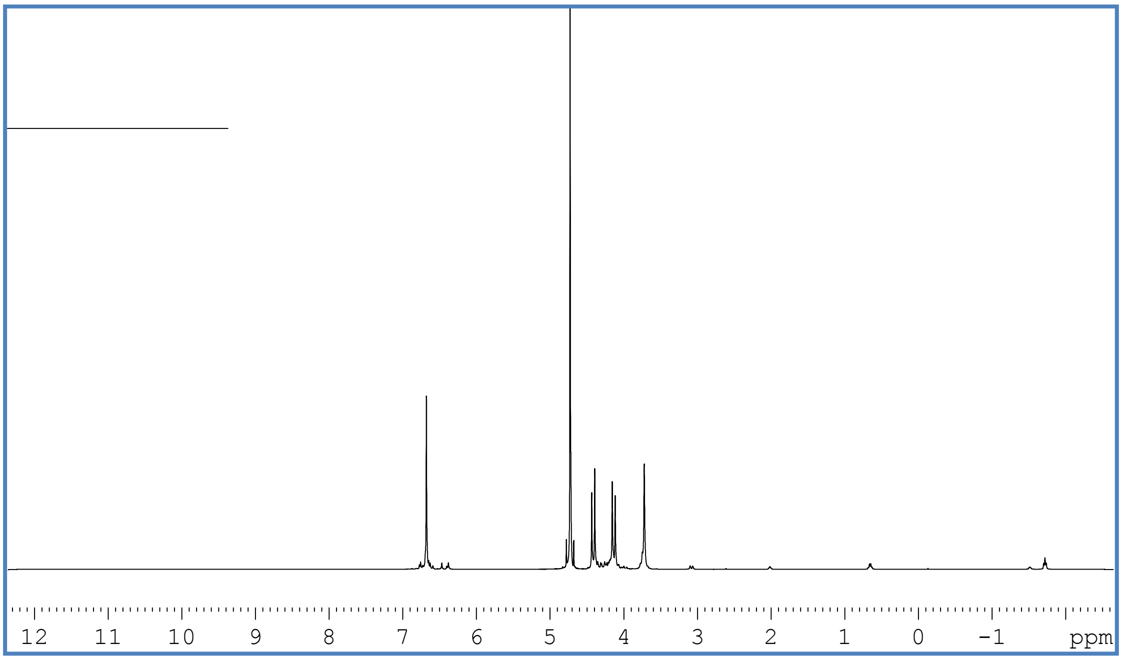
Figure S1: The ^{1}H NMR (9.4 T, 400 MHz, top) and ^{13}C NMR (9.4 T, 100 MHz, bottom) spectra of the ammonium salt of **3b** (100 mM in D_2O)

Sodium salt of of dicarboxylic acid methoxy-pillar[5]arene (3c) in D_2O.

To **3a** (100 mg, 0.084 mmol) an aqueous solution of sodium hydroxide (50 mg/mL D_2O, 0.8 mL) was added. A clear yellowish solution of **3c** was obtained.

^{1}H NMR (D_2O): δ 6.75 (s, 10H, phenyl protons), 4.49 (d, J=16 Hz, 10H, methylene protons), 4.21 (d, J=16Hz, 10H, methylene protons), 3.79 (br s, 10H, protons from methylene bridge).

^{13}C-NMR (D_2O): δ 178.2, 149.9, 129.1, 115.0 , 68.3, 29.6.



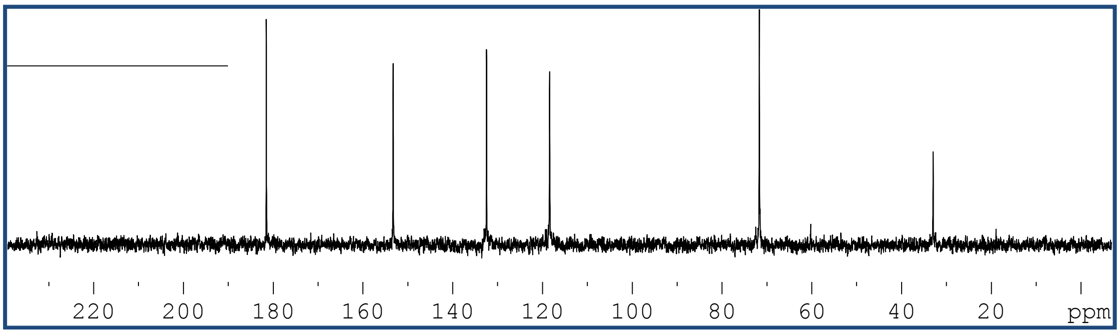


Figure S2: The ^{1}H NMR (400 MHz, top) and ^{13}C NMR (100 MHz, bottom) spectra of the sodium salt of **3c** (120 mM in D_2O)

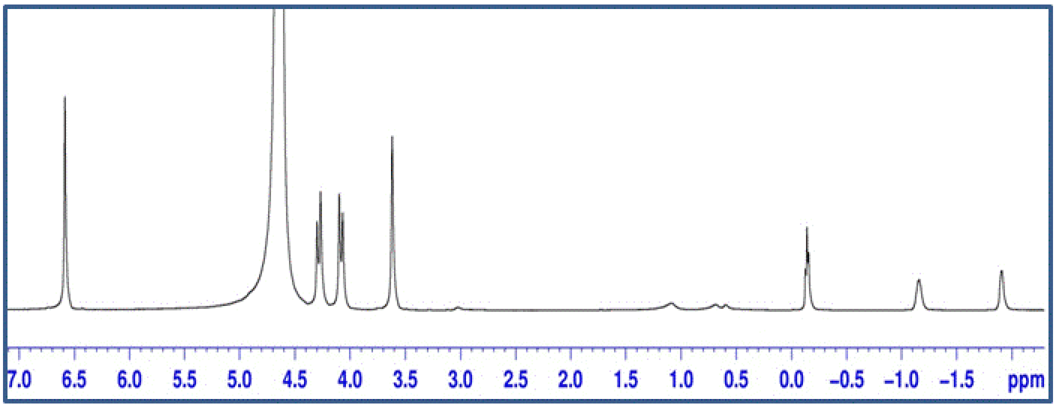


Figure S3: The ^{1}H NMR spectra of **3b** (500MHz, 100 mM in D_2O) in the presence of n-hexane. Note the resonances appearing at high field (-0.1, -1.1 and -2.0 ppm), representing the threaded n-hexane.

Table S1. Diffusion coefficients, along with their standard deviations, obtained for the different peaks of the solution shown in the Figure S3 and for the D_2O solution of **3b** (no hexane added).

δ [ppm]	**Diffusion coefficients [x10^{-5}cm^{2}/sec]**	
-1.9 **Hexane - in**	0.16 ± 0.01	----
-1.1 **Hexane - in**	0.16 ± 0.01	----
-0.1 **Hexane - in**	0.16 ± 0.01	----
0.2 **Hexane - out**	0.56 ± 0.01	----
0.7 **Hexane - out**	0.57 ± 0.01	----
1.3 **Hexane - out**	0.57 ± 0.01	----
3.6 **PA (3)**	0.16 ± 0.01[a]	0.16 ± 0.01[b]
4.1 **PA (3)**	0.16 ± 0.01[a]	0.16 ± 0.01[b]
4.3 **PA (3)**	0.16 ± 0.01[a]	0.16 ± 0.01[b]
4.8 **water**	1.48 ± 0.01[a]	1.42 ± 0.01[b]
6.8 **PA (3)**	0.16 ± 0.01[a]	0.16 ± 0.01[b]

[a] **3** in D_2O [100mM].in the presence of n-hexane.

[b] **3** in D_2O [100mM].

References

1. Gibbs, S. J.; Johnson, C. S. Jr., *J. Magn. Reson*., **1991**, *93*, 395-402
2. Ogoshi, T.; Aoki, T., Kitajima, K.; Fujinami, S.; Yamagishi, T.; Nakamoto, Y., *J. Org. Chem.*, **2011**, 76, 328-33.
3. Ogoshi, T.; Hashizume, M.; Yamagishi, T. A.; Nakamoto, Y., *Chem. Comm.* **2010,** 46, 3708-3710.