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Supporting Information

On the Tunability of Toxicity for Viologen-Derivatives as Anolyte for Neutral Aqueous Organic Redox Flow Batteries

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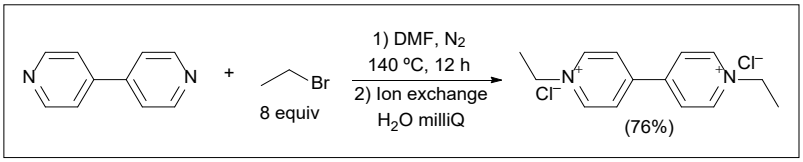
Section S1. Synthesis and characterization of viologen derivatives

Synthesis and Characterization Data of Viologen EV:

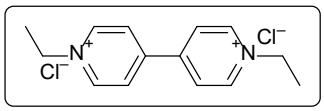
Synthesis.



EV was prepared according to a modified procedure of a reported method. [16a]

In a Schlenk flask (50 mL), 4,4'-bipyridine (1.32 g, 8.44 mmol) was dissolved in anhydrous *N*,*N*-dimethylformamide (DMF, 20 mL) under an inert nitrogen atmosphere. Then, bromoethane (13.2 g, 68 mmol) was added with stirring to the obtained solution. The reaction mixture was heated at 140 ºC with stirring for 12 h. Upon cooling to room temperature, the resulting yellow precipitate was filtered off, washed with CH_3CN and hot chloroform, and dried under vacuum. The yellow solid obtained was dissolved in H_2O Milli-Q (32 mL) and submitted to an ion-exchange chromatography with wet Amberlite IRA-900(Cl) (160 g) and H_2O Milli-Q (320 mL) as eluent. Then, the water was evaporated under reduced pressure and the desired product was dried under vacuum.

The spectral data of the compound are in good agreement with the reported data. [16a]

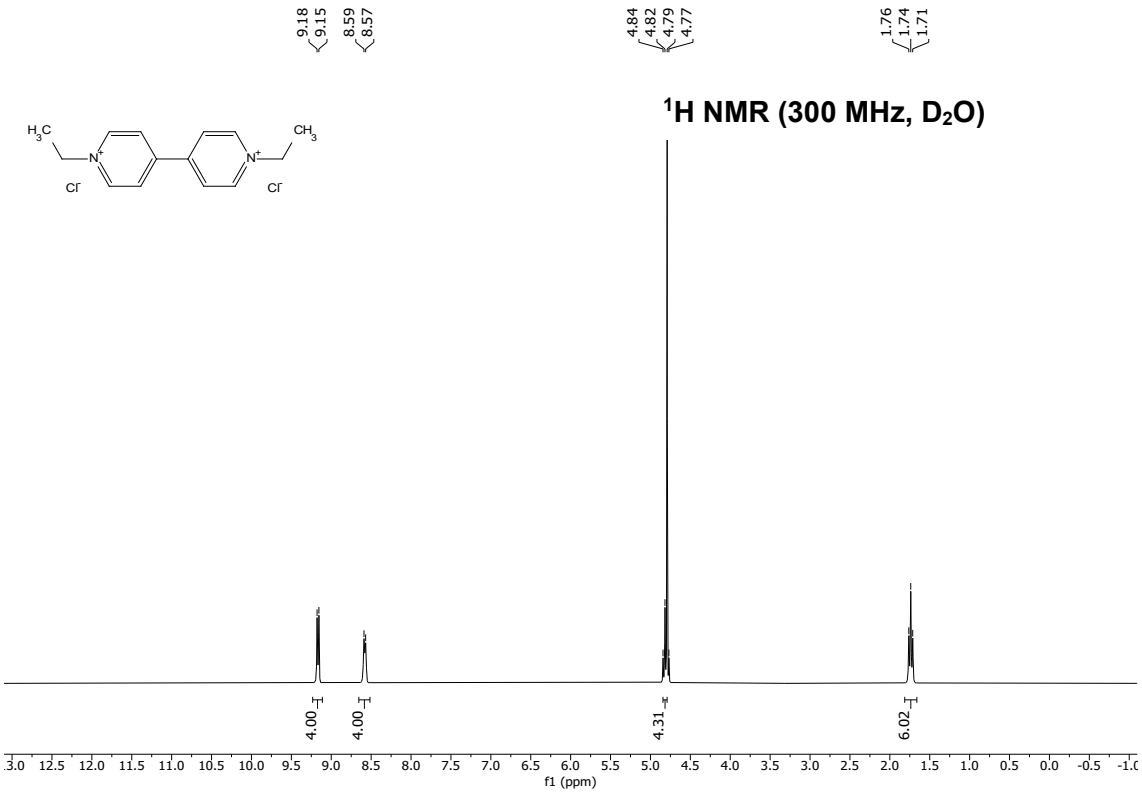


1,1'-Bis(ethyl)-4,4'-bipyridinium dichloride: Yielded (1.8 g, 76%) as an off-white solid.

^{1}H NMR (300 MHz, D_2O): δ = 9.17 (d, *J* = 6.8 Hz, 4H, Ar*H*), 8.58 (d, *J* = 6.3 Hz, 4H, Ar*H*), 4.80 (q, *J* = 7.4 Hz, 4H, C*H*$_2$), 1.74 (t, *J* = 7.4 Hz, 6H, C*H*$_3$).

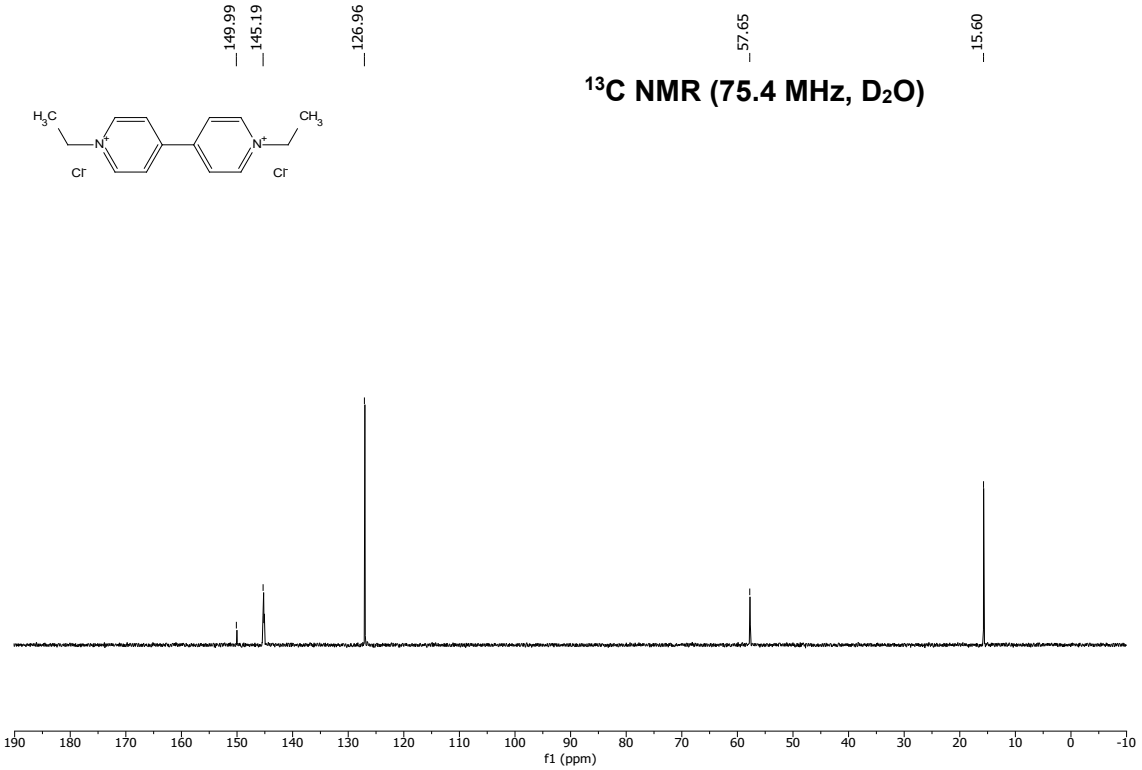
^{13}C NMR (75.4 MHz, D_2O): δ = 150.4 (2 × C), 145.2 (4 × CH), 127.2 (4 × CH), 57.7 (2 × CH_2), 15.6 (2 × CH_3).

[1]H-NMR spectra.



^{1}H NMR (300 MHz, D_2O)

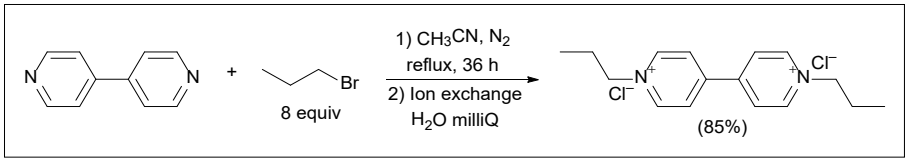
[13]C-NMR spectra.



^{13}C NMR (75.4 MHz, D_2O)

Synthesis and Characterization Data of Viologen PV:

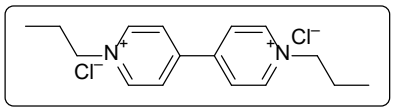
Synthesis.



PV was prepared according to a modified procedure of a reported method. [16a]

In a Schlenk flask (50 mL), 4,4'-bipyridine (3 g, 19.1 mmol) was dissolved in anhydrous acetonitrile (CH_3CN, 30 mL) under an inert nitrogen atmosphere. Then, 1-bromopropane (18.8 g, 153 mmol) was added with stirring to the obtained solution. The reaction mixture was heated under reflux (oil bath: 90 ºC) with stirring for 36 h. Upon cooling to room temperature, the resulting yellow precipitate was filtered off, washed with CH_3CN and hot chloroform, and dried under vacuum. The yellow solid obtained was dissolved in H_2O Milli-Q (80 mL) and submitted to an ion-exchange chromatography with wet Amberlite IRA-900(Cl) (415 g) and H_2O Milli-Q (950 mL) as eluent. Then, the water was evaporated under reduced pressure and the desired product was dried under vacuum.

The spectral data of the compound are in good agreement with the reported data.[16a]

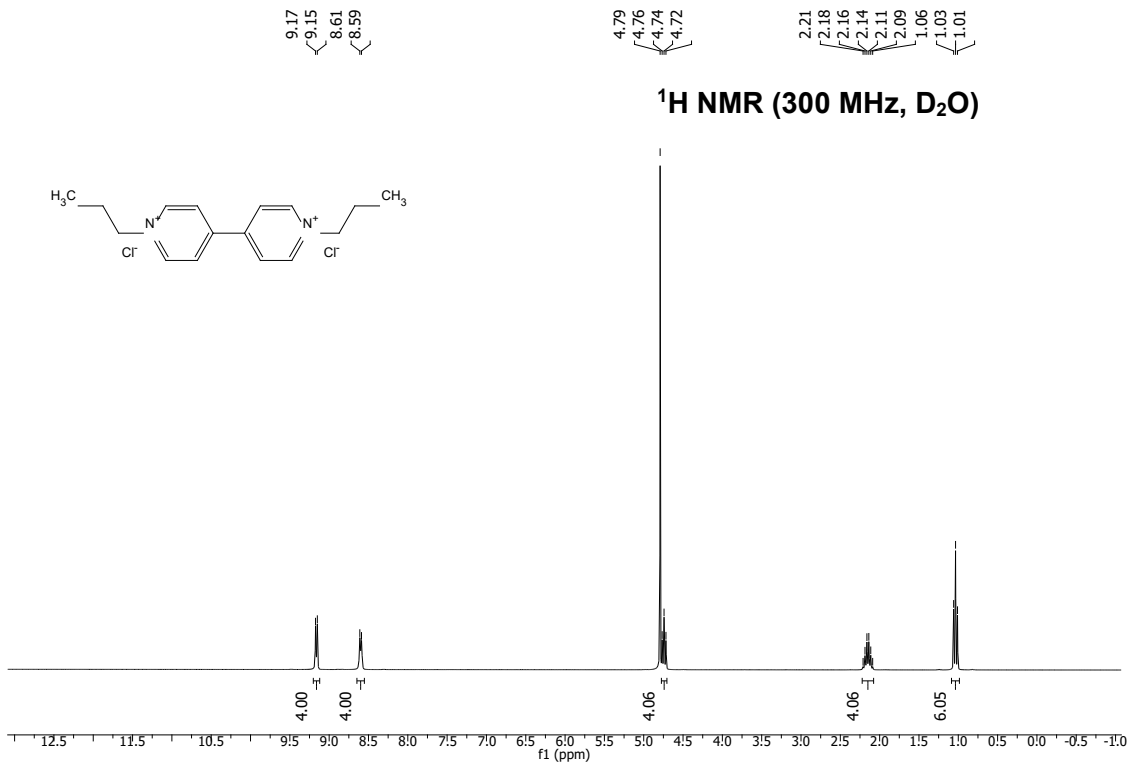


1,1'-Bis(propyl)-4,4'-bipyridinium dichloride: Yielded (5.1 g, 85%) as a grey deliquescent solid.

^{1}H NMR (300 MHz, D_2O): δ = 9.16 (d, *J* = 6.8 Hz, 4H, Ar*H*), 8.62 (d, *J* = 6.4 Hz, 4H, Ar*H*), 4.77 (t, *J* = 7.3 Hz, 4H, CH_2), 2.18 (h, *J* = 7.3 Hz, 4H, CH_2), 1.07 (t, *J* = 7.4 Hz, 6H, CH_3).

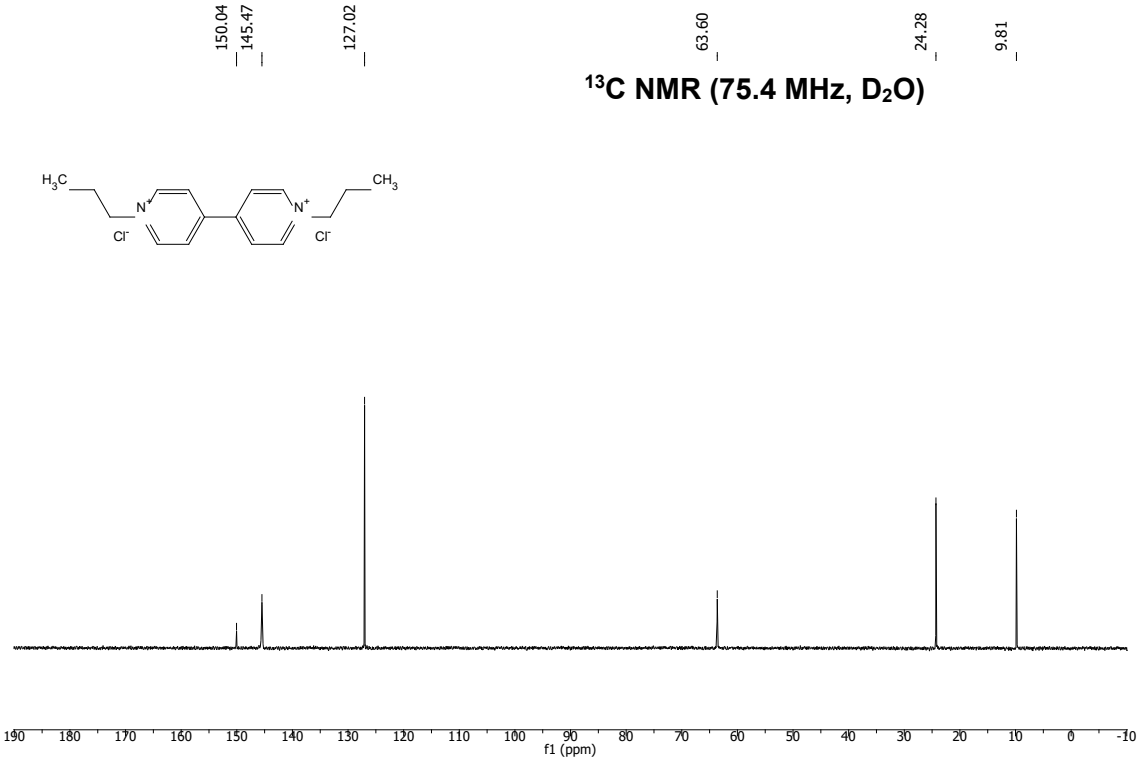
^{13}C NMR (75.4 MHz, D_2O): δ = 150.0 (2 × C), 145.5 (4 × CH), 127.0 (4 × CH), 63.6 (2 × CH_2), 24.3 (2 × CH_2), 9.8 (2 × CH_3).

[1]H-NMR spectra.



^{1}H NMR (300 MHz, D_2O)

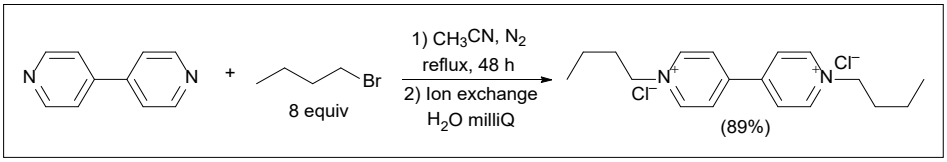
[13]C-NMR spectra.



^{13}C NMR (75.4 MHz, D_2O)

Synthesis and Characterization Data of Viologen BV:

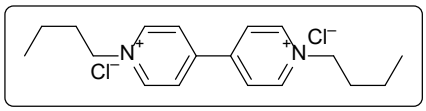
Synthesis.



BV was prepared according to a modified procedure of a reported method. [16a]

In a Schlenk flask (50 mL), 4,4'-bipyridine (3 g, 19.1 mmol) was dissolved in anhydrous acetonitrile (CH_3CN, 30 mL) under an inert nitrogen atmosphere. Then, 1-bromobutane (21 g, 153 mmol) was added with stirring to the obtained solution. The reaction mixture was heated under reflux (oil bath: 90 ºC) with stirring for 48 h. Upon cooling to room temperature, the resulting yellow precipitate was filtered off, washed with CH_3CN and hot chloroform, and dried under vacuum. The yellow solid obtained was dissolved in H_2O Milli-Q (85 mL) and submitted to an ion-exchange chromatography with wet Amberlite IRA-900(Cl) (420 g) and H_2O Milli-Q (950 mL) as eluent. Then, the water was evaporated under reduced pressure and the desired product was dried under vacuum.

The spectral data of the compound are in good agreement with the reported data. [16a]

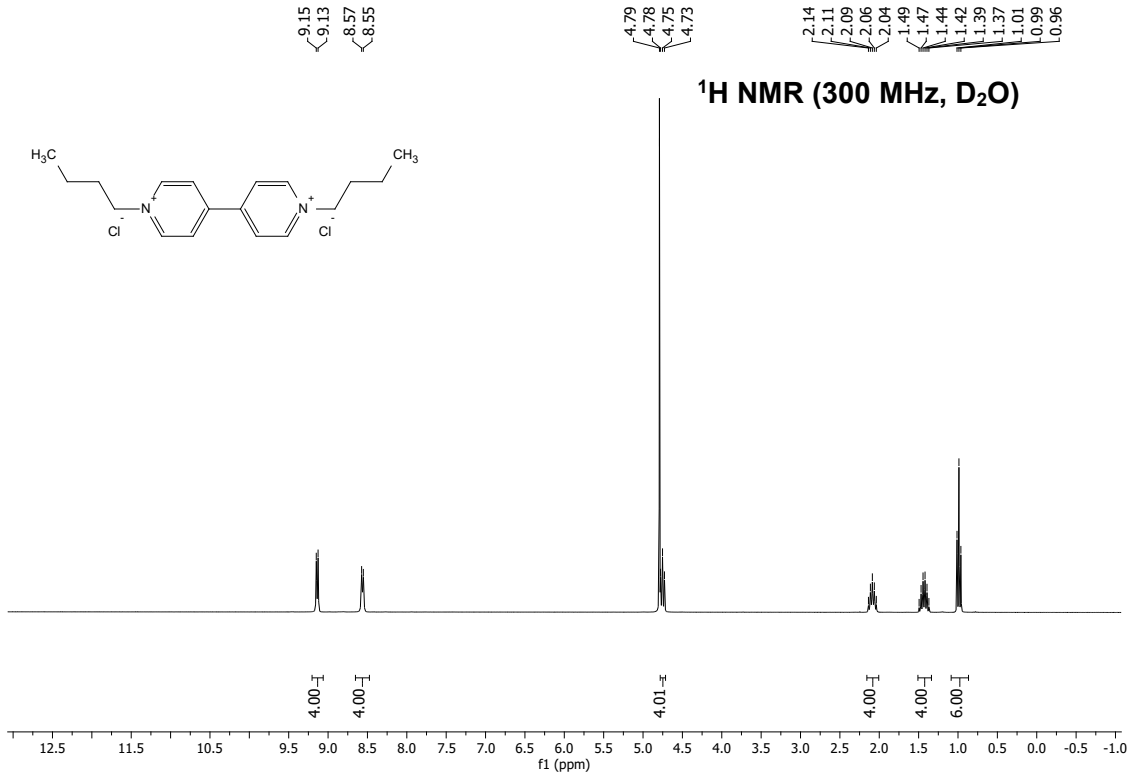


1,1'-Bis(butyl)-4,4'-bipyridinium dichloride: Yielded (5.8 g, 89%) as a brownish solid.

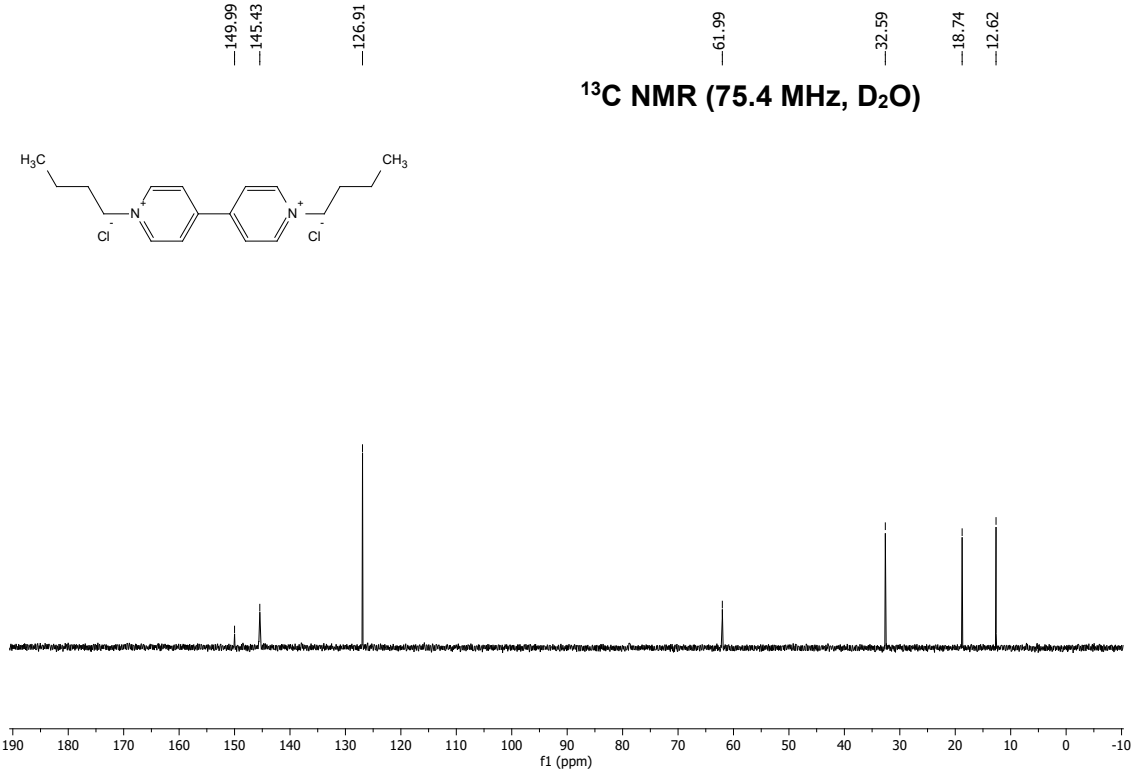
^{1}H NMR (300 MHz, D_2O): δ = 9.14 (d, *J* = 6.9 Hz, 4H, Ar*H*), 8.56 (d, *J* = 6.5 Hz, 4H, Ar*H*), 4.75 (t, *J* = 7.4 Hz, 4H, CH_2), 2.09 (p, *J* = 7.4 Hz, 4H, CH_2), 1.43 (h, *J* = 7.4 Hz, 4H, CH_2), 0.99 (t, *J* = 7.4 Hz, 6H, CH_3).

^{13}C NMR (75.4 MHz, D_2O): δ = 150.0 (2 × C), 145.4 (4 × CH), 126.9 (4 × CH), 62.0 (2 × CH_2), 32.6 (2 × CH_2), 18.7 (2 × CH_2), 12.6 (2 × CH_3).

[1]H-NMR spectra.

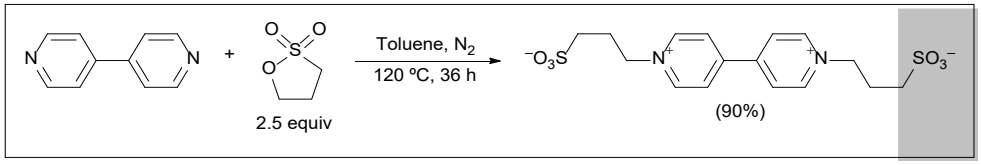


[13]C-NMR spectra.



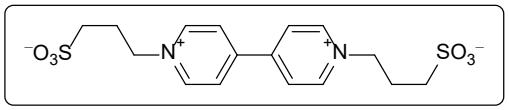
Synthesis and Characterization Data of Viologen BSPV:

Synthesis.



BSPV was prepared according to a reported procedure. [15]

In a round bottom flask (250 mL), 1,3-propanesultone (9.8 g, 80 mmol) was dissolved in anhydrous toluene (60 mL) under an inert nitrogen atmosphere and the obtained solution was heated at 110 ºC. Then, a solution of 4,4'-bipyridine (5 g, 32 mmol) in anhydrous toluene (40 mL) was added slowly with stirring. The reaction mixture was heated at reflux (oil bath, 120 ºC) with vigorous stirring for 36 h. Upon cooling to room temperature, the resulting white precipitate was filtered off, washed several times with hot MeOH and dried under vacuum.

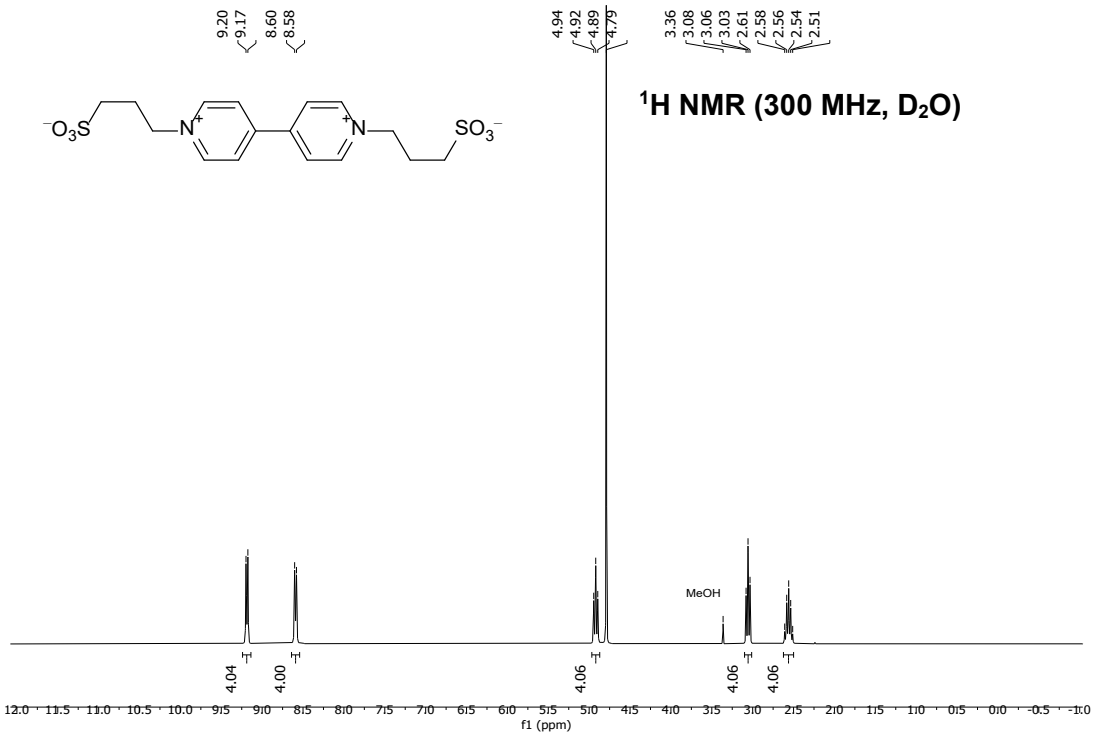


1,1'-Bis(3-sulfonatopropyl)-4,4'-bipyridinium: Yielded (11.22 g, 90%) as a white solid.

^{1}H NMR (300 MHz, D_2O): δ = 9.19 (d, *J* = 6.8 Hz, 4H, Ar*H*), 8.59 (d, *J* = 6.8 Hz, 4H, Ar*H*), 4.92 (t, *J* = 7.4 Hz, 4H, CH_2), 3.06 (t, *J* = 7.2 Hz, 4H, CH_2), 2.56 (ap, *J* = 7.3 Hz, 4H, CH_2).

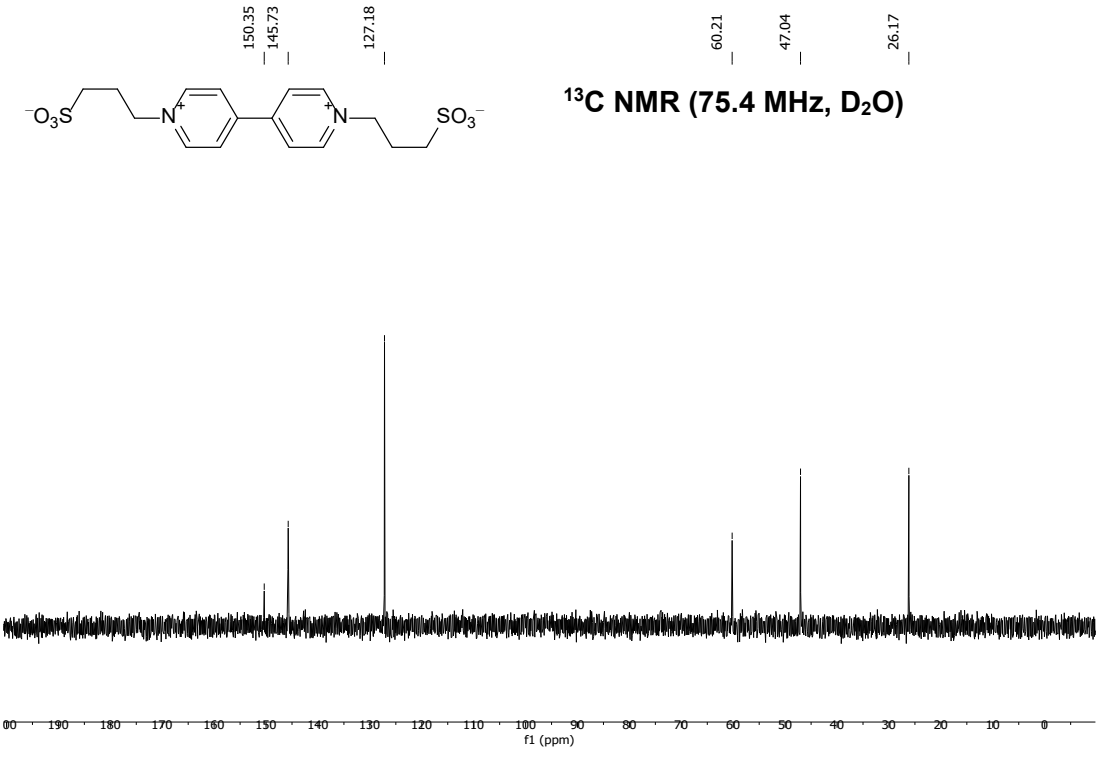
^{13}C NMR (75.4 MHz, D_2O): δ = 150.4 (2 × C), 145.7 (4 × CH), 127.2 (4 × CH), 60.2 (2 × CH_2), 47.0 (2 × CH_2), 26.2 (2 × CH_2).

[1]H-NMR spectra.



^{1}H NMR (300 MHz, D_2O)

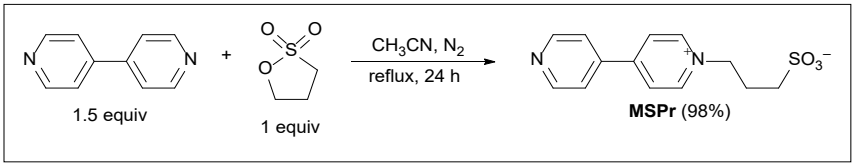
[13]C-NMR spectra.



^{13}C NMR (75.4 MHz, D_2O)

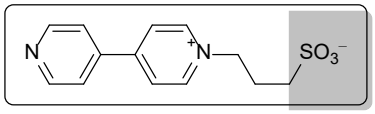
Synthesis and Characterization Data of Compound MSP:

Synthesis.



MSP was prepared according to a reported procedure. [15]

In a round bottom flask (50 mL), 4,4'-bipyridine (3 g, 19.2 mmol) was dissolved in anhydrous CH_3CN (35 mL) under an inert nitrogen atmosphere. Then, 1,3-propanesultone (1.55 g, 12.7 mmol) was added with stirring to the obtained solution. The reaction mixture was heated at reflux with stirring for 24 h. Upon cooling to room temperature, the resulting white precipitate was filtered off, washed several times with CH_3CN and dried under vacuum.

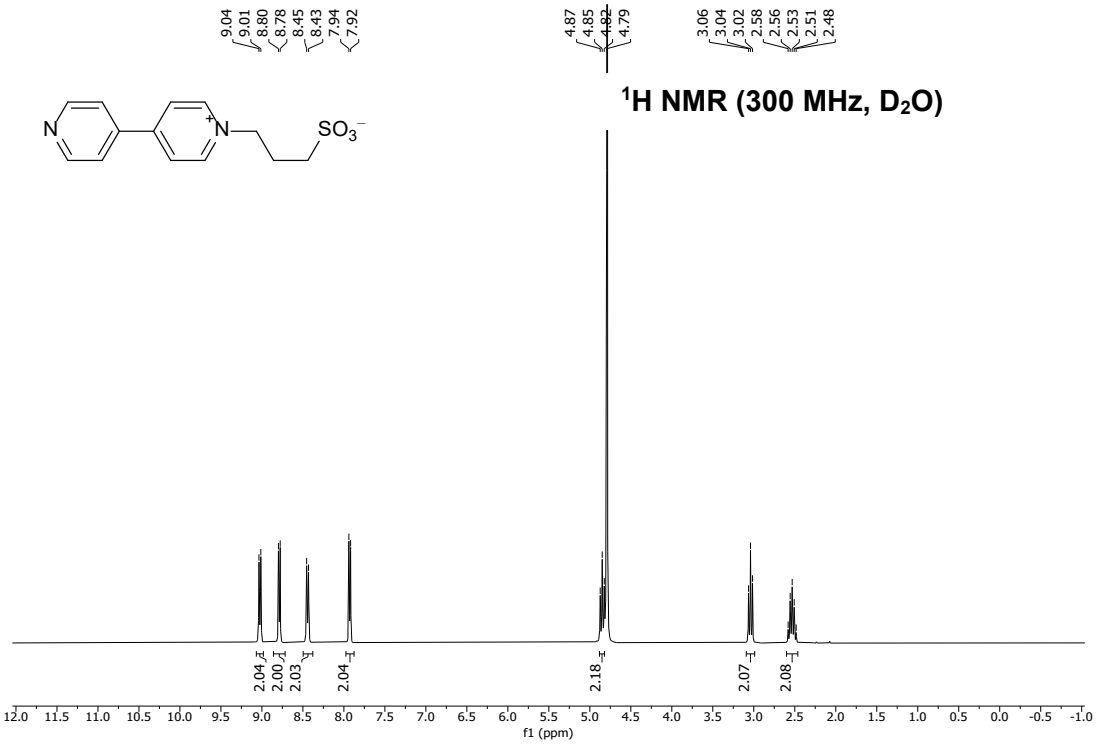


3-([4,4'-bipyridin]-1-ium-1-yl)propane-1-sulfonate: Yielded (3.45 g, 98%) as a white solid.

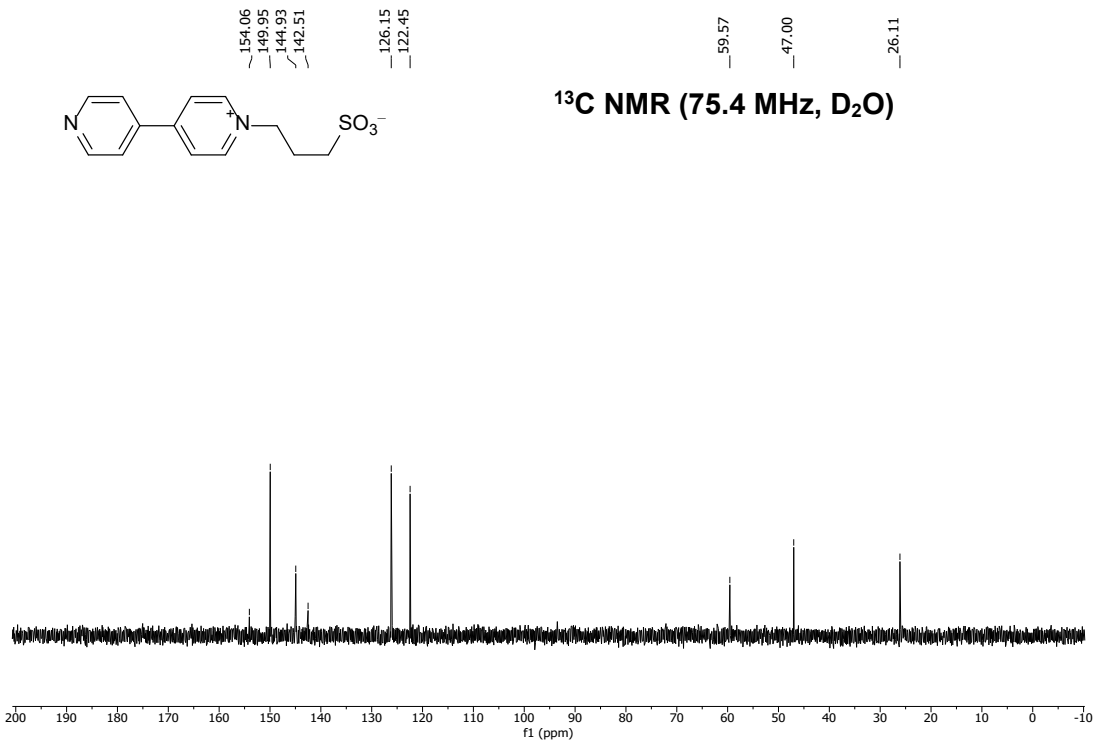
^{1}H NMR (300 MHz, D_2O): δ = 9.02 (d, *J* = 6.9 Hz, 2H, Ar*H*), 8.79 (d, *J* = 6.4 Hz, 2H, Ar*H*), 8.44 (d, *J* = 6.8 Hz, 2H, Ar*H*), 7.93 (d, *J* = 6.3 Hz, 2H, Ar*H*), 4.85 (t, *J* = 7.4 Hz, 2H, CH_2), 3.04 (t, *J* = 7.3 Hz, 2H, CH_2), 2.53 (ap, *J* = 7.3 Hz, 2H, CH_2).

^{13}C NMR (75.4 MHz, D_2O): δ = 154.1 (C), 150.0 (2 × CH), 144.9 (2 × CH), 142.5 (C), 126.2 (2 × CH), 122.5 (2 × CH), 59.8 (CH_2), 47.0 (CH_2), 26.1 (CH_2).

[1]H-NMR spectra.

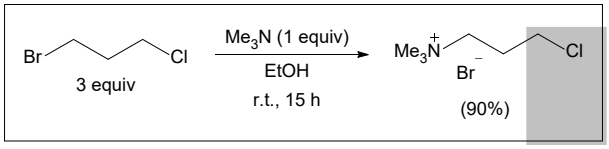


[13]C-NMR spectra.



Synthesis and Characterization Data of Viologen BTMAPV:

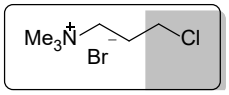
Synthesis of starting material.



TM3CPAB was prepared according to a procedure of a reported method. [16b]

In a round bottom flask (100 mL), 1-bromo-3-chloropropane (49.6 g, 315 mmol) was dissolved in a 33% solution of trimethylamine in EtOH (25 mL, 105 mmol of Me_3N). The reaction mixture was stirred at room temperature for 15 h. Then, 250 mL of Et_2O was added to the reaction mixture and the resulting white precipitate was filtered off, washed with Et_2O and dried under vacuum. This product was used in the next step without need of further purification.

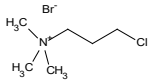
The spectral data of the compound are in good agreement with the reported data. [16b]



Trimethyl(3-chloropropyl)ammonium bromide: Yielded (61.4 g, 90%) as a white solid.

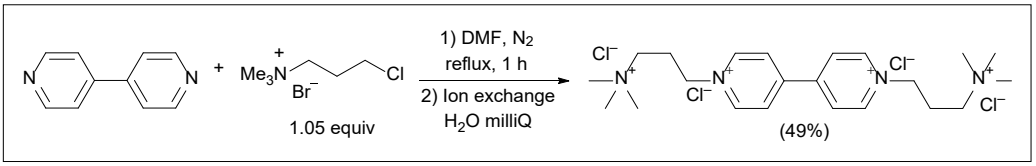
^{1}H NMR (300 MHz, D_2O): δ = 3.76 (t, *J* = 5.9 Hz, 2H, C*H*$_2$), 3.61–3.53 (m, 2H, C*H*$_2$), 3.20 (s, 3H, C*H*$_3$), 2.41–2.29 (m, 2H, C*H*$_2$).

^{1}H-NMR spectra.



^{1}H NMR (300 MHz, D_2O)

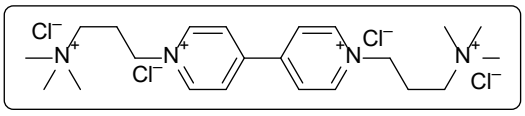
Synthesis of BTMAPV.



BTMAPV was prepared according to a modified procedure of a reported method. [16b]

In a round bottom flask (100 mL), 4,4'-bipyridine (7 g, 44.86 mmol) was mixed with trimethyl(3-chloropropyl)ammonium bromide (20.4 g, 94.2 mmol) and anhydrous *N*,*N*-dimethylformamide (DMF, 50 mL) under an inert nitrogen atmosphere. The obtained suspension was heated with vigorous stirring until the solvent started to reflux (155–160 ºC) and all solids were dissolved. Then, the reaction mixture was heated under reflux with stirring for 1 h, giving rise to a new suspension. Upon cooling to room temperature, the resulting partially solidified reaction mixture was triturated with 2-propanol (250 mL) until the dark green colour disappeared. The solid was filtrated out, washed thoroughly with 2-propanol and acetone, and dried. Then, the solid obtained was dissolved in H_2O Milli-Q (63 mL) and submitted to an ion-exchange chromatography with wet Amberlite IRA-900(Cl) (315 g) and H_2O Milli-Q (700 mL) as eluent. Then, the water was evaporated under reduced pressure and the desired product was dried under vacuum. washed with CH_3CN and hot chloroform, and dried under vacuum.

The spectral data of the compound are in good agreement with the reported data. [16b]

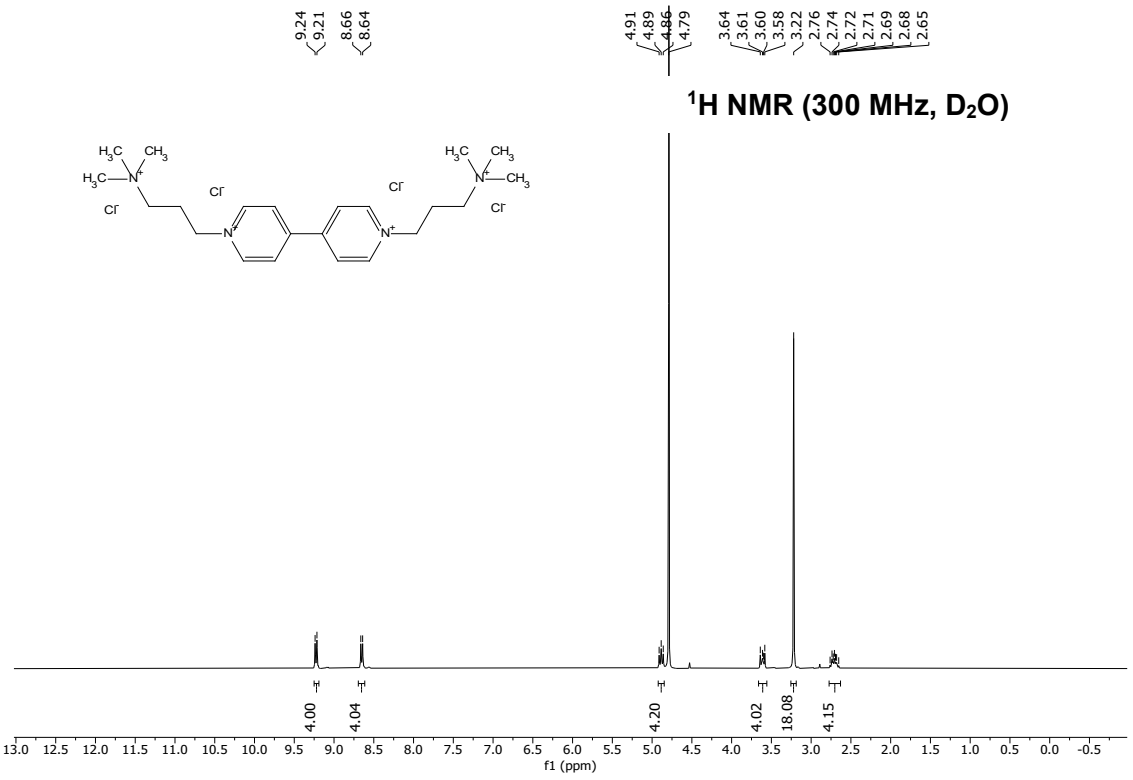


1,1'-Bis(3-(trimethylammonio)propyl))-4,4'-bipyridinium tetrachloride: Yielded (10.98 g, 49%) as an off-white solid.

^{1}H NMR (300 MHz, D_2O): δ = 9.23 (d, *J* = 7.0 Hz, 4H, Ar*H*), 8.65 (d, *J* = 6.9 Hz, 4H, Ar*H*), 4.89 (t, *J* = 7.8 Hz, 4H, CH_2), 3.66–3.56 (m, 4H, CH_2), 3.22 (s, 18H, CH_3), 2.77–2.63 (m, 2H, CH_2).

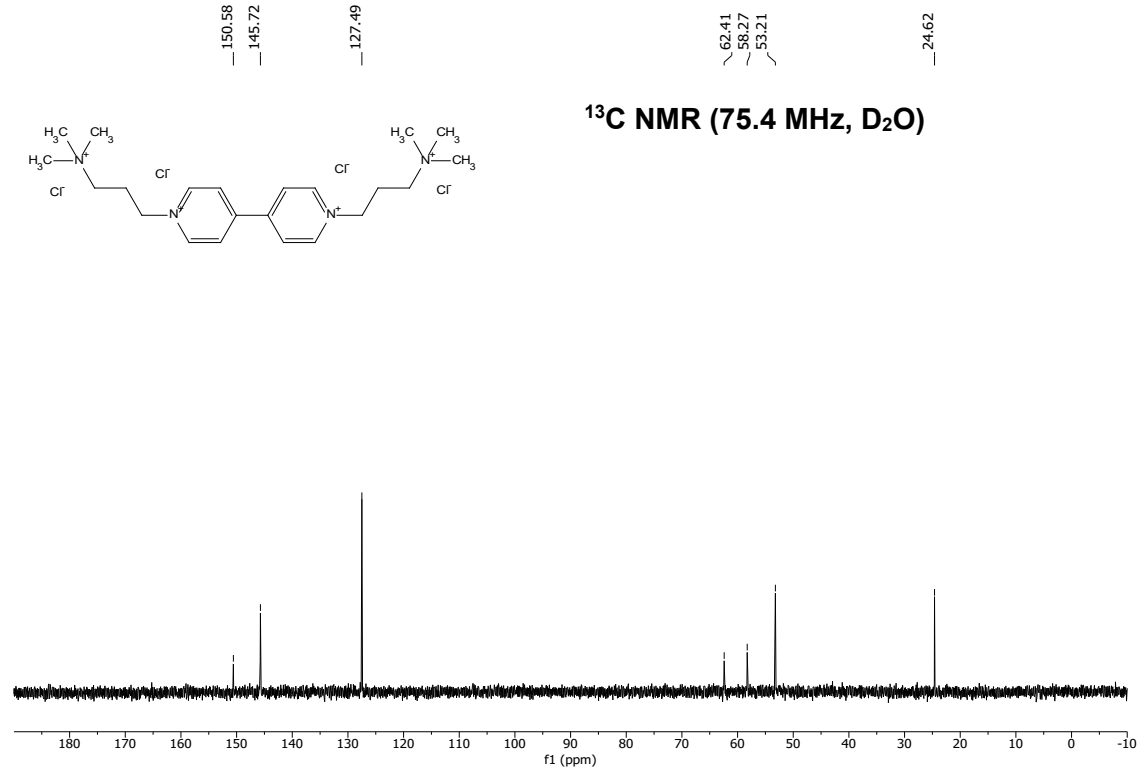
^{13}C NMR (75.4 MHz, D_2O): δ = 150.6 (2 × C), 145.7 (4 × CH), 127.5 (4 × CH), 62.4 (2 × CH_2), 58.3 (2 × CH_2), 53.2 (2 × CH_3), 24.6 (2 × CH_2).

[1]H-NMR spectra.



^{1}H NMR (300 MHz, D_2O)

[13]C-NMR spectra.



^{13}C NMR (75.4 MHz, D_2O)

Section S2. Cyclic Voltammogram of 3-([4,4'-bipyridin]-1-ium-1-yl)propane-1-sulfonate

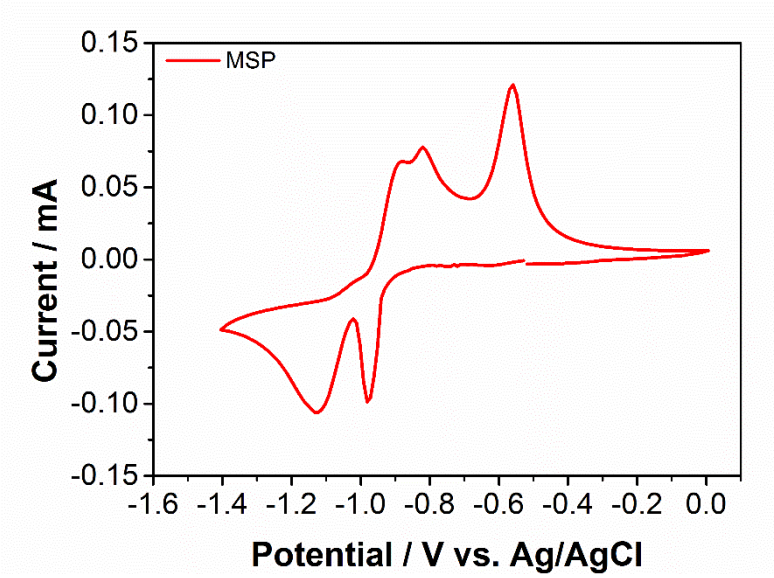


Figure S1. Cyclic voltammogram of 25 mM MSP in 1 M KCl at 10 mVs^{-1} on a glassy carbon electrode.

Section S3. Assessment of cytotoxicity and toxicity in the yeast S. cerevisiae for potassium ferrocyanide.

Determination of potassium ferrocyanide toxicity in the A549 human cell line

The potential cytotoxic effects of potassium ferrocyanide was assessed in A549 cells, using the MV as benchmark. The viability of this human cell line after 24 h of exposure to 5 and 20 mM of MV and potassium ferrocyanide was determined by the neutral red uptake assay, described above. **Figure S2** shows the results obtained. Cells that were exposed to MV showed a statistically significant reduction in viability at both concentrations tested, being this effect more noticeable at the highest concentration tested. Conversely, exposure to potassium ferrocyanide did not generate any harmful effect on cell viability at the concentrations tested, showing in both cases viability values similar to the control.

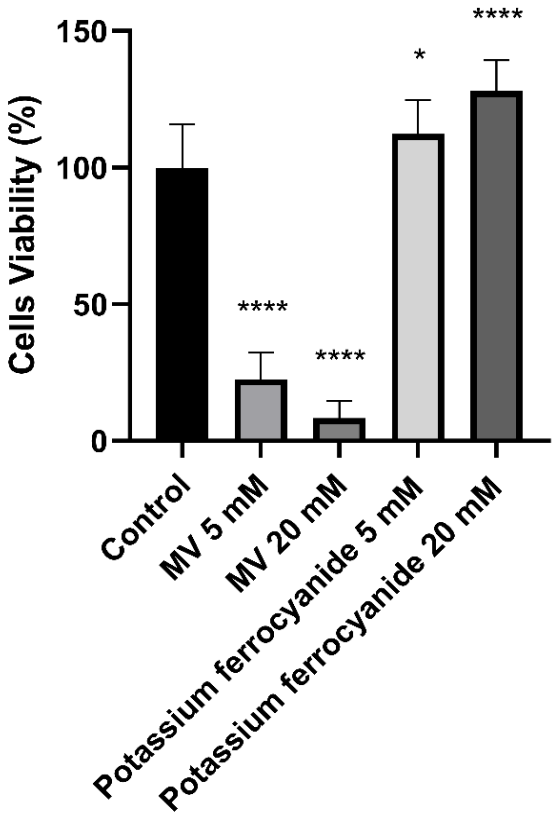


Figure S2. Viability of A549 cells (Neutral red assay) treated with metthyl viologen and potassium ferrocyanide at 5 and 20 mM. Results are expressed as % of control (untreated cells). Data represent the mean (± standard deviation, SD) of at least 6 biological replicates obtained in two independent experiments. Differences were established using a One-way ANOVA followed by Dunnet *post hoc* test to compare every mean with the control and considered significant at $P \leq 0.05$. *$P \leq 0.05$, ***$P \leq 0.0001$.

Determination of potassium ferrocyanide toxicity in the yeast *S. cerevisiae*

The toxicological potential of potassium ferrocyanide on single-celled fungi was evaluated on the yeast *S. cerevisiae* using the plate-drop method described above. As shown in **Figure S3**, critical effects on the viability of *S. cerevisiae* were observed after 48 h of exposure to 2 mM MV. However, no detrimental effects were observed after exposure to 2 mM potassium ferrocyanide, showing yeast growth similar as the control.

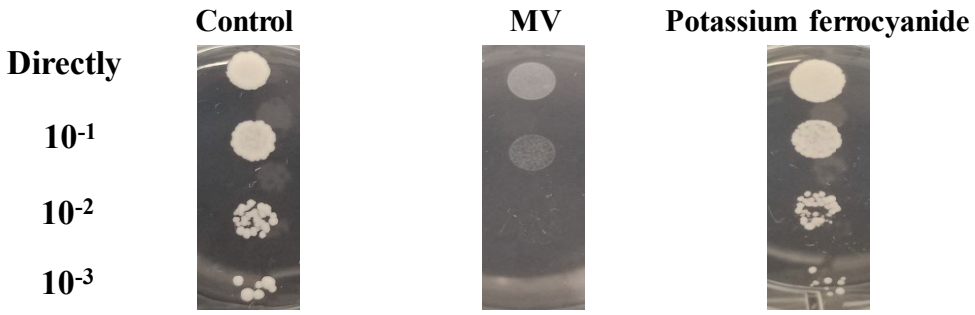


Figure S3. Viability of *S. cerevisiae* cells (drop plate method) exposed at 2 mM of viologen and potassium ferrocyanide.