Electronic Supplementary Material (ESI) for RSC Advances.


# Supplementary Information

# Antimicrobial Activities of Phosphonium Containing Polynorbornenes

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## 1. Materials

Furan, maleic anhydride, 3-Bromopropylamine hydrobromide, trimethylphosphine, triethylphosphine, tripropylphosphine, tri-tert-butyl phosphine, triphenylphosphine and tris (4-methoxyphenyl) phosphine, tetrahydrofuran, dichloromethane, petroleum ether, ethyl acetate, diethyl ether, chloroform, hexane, dimethyl sulfoxide, N,N-dimethylformamide, pentane, ethylvinyl ether, 3-bromopyridine, 2,2,2-trifluoroethanol were purchased from Aldrich and used as received. Grubbs second generation catalyst were purchased from Aldrich. Grubbs third generation catalyst [$(H_2$-Imes)(3-Br-py$)_2$-$(Cl)_2$Ru=CHPh] was freshly prepared according to the previously reported procedure.[1] All other reagents including buffers and salts were obtained from Aldrich.

## 2. Instrumentation

$^1$H NMR (500 MHz) and $^{13}$C NMR (75 MHz) spectra were recorded using a Bruker Avance III 500 MHz spectrometer. $^{31}$P NMR spectra were recorded using a The Varian Mercury VX 400 MHz BB spectrometer. The appropriate frequencies using either residual $CDCl_3$, $D_2O$ or DMSO-$d_6$ as internal reference (for $^1$H and $^{13}$C) or 85 % $H_3PO_4$ as external reference (for $^{31}$P) were applied for the analysis of NMR data. Determination of surface charge density values were recorded using Malwern Zetasizer Nano ZS (633 nm wavelength, 175° scattering angle, 172,2 toluene count rate). Viscotek GPCmax were analyzed using gel permeation chromatography (GPC) with a triple detection system. Triple detection consists of refractive index (RI), right angle light scattering (LS), and viscosimetry (VIS) detectors, which were calibrated with PEO (22 kDa standard solution. GPC analyses were performed with CATSEC 300 column at room temperature. Acetate buffer (pH = 4.8; 0.1 M AcA + 0.15 M NaOH) was used for the mobile phase and flow rate was 0.4 mL min$^{-1}$.

## 3. Synthesis

### Synthesis of compound 1

Monomer synthesis began with a Diels-Alder reaction between maleic anhydride and furan to yield exo-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride (compound 1).[2]

**Synthesis of compound 2**

Reaction of adduct 1 and 3-bromopropyl amine hydrobromide to yield 4-(3-bromopropyl) 10-oxa-4-azatricyclo[5.2.1.02,6]dec-8-ene-3,5-dione was also adapted from literature.[3] Compound 2 was purified by column chromatography using Ethyl acetate:Hexane (1:1, v/v) eluent. Yield: 51 %.

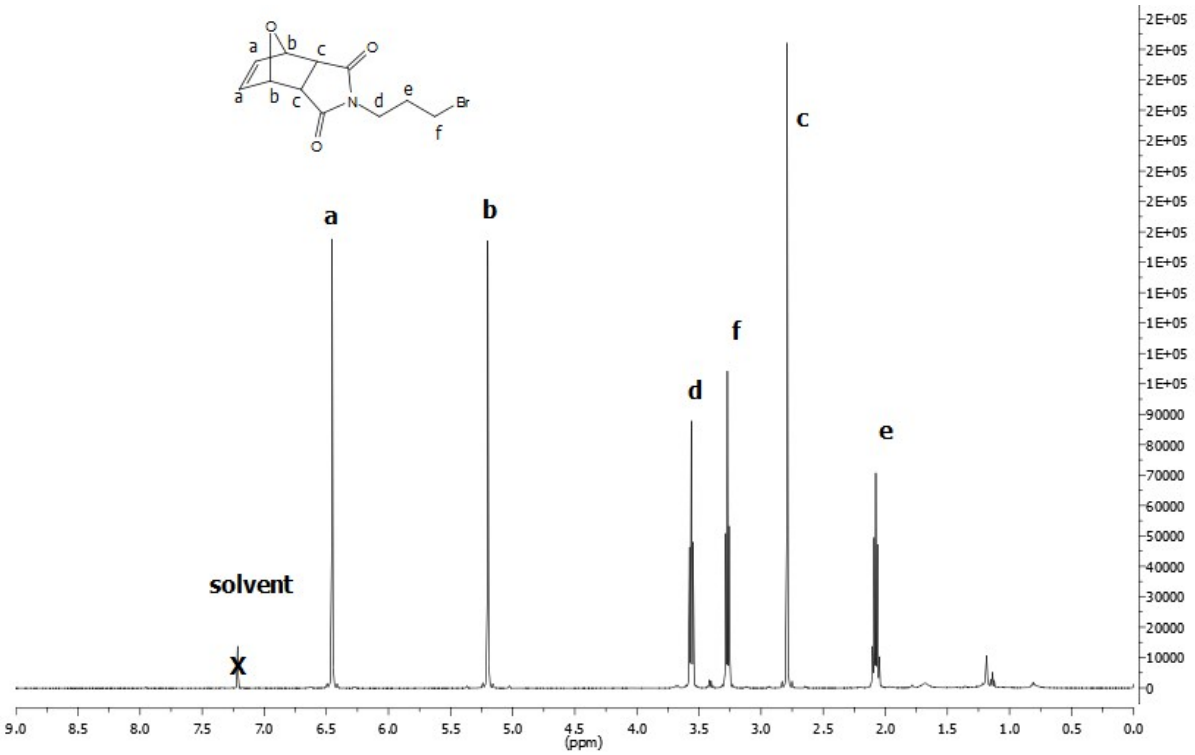


**Figure S1.** $^{1}H$ NMR spectra of compound **2** in $CDCl_3$.

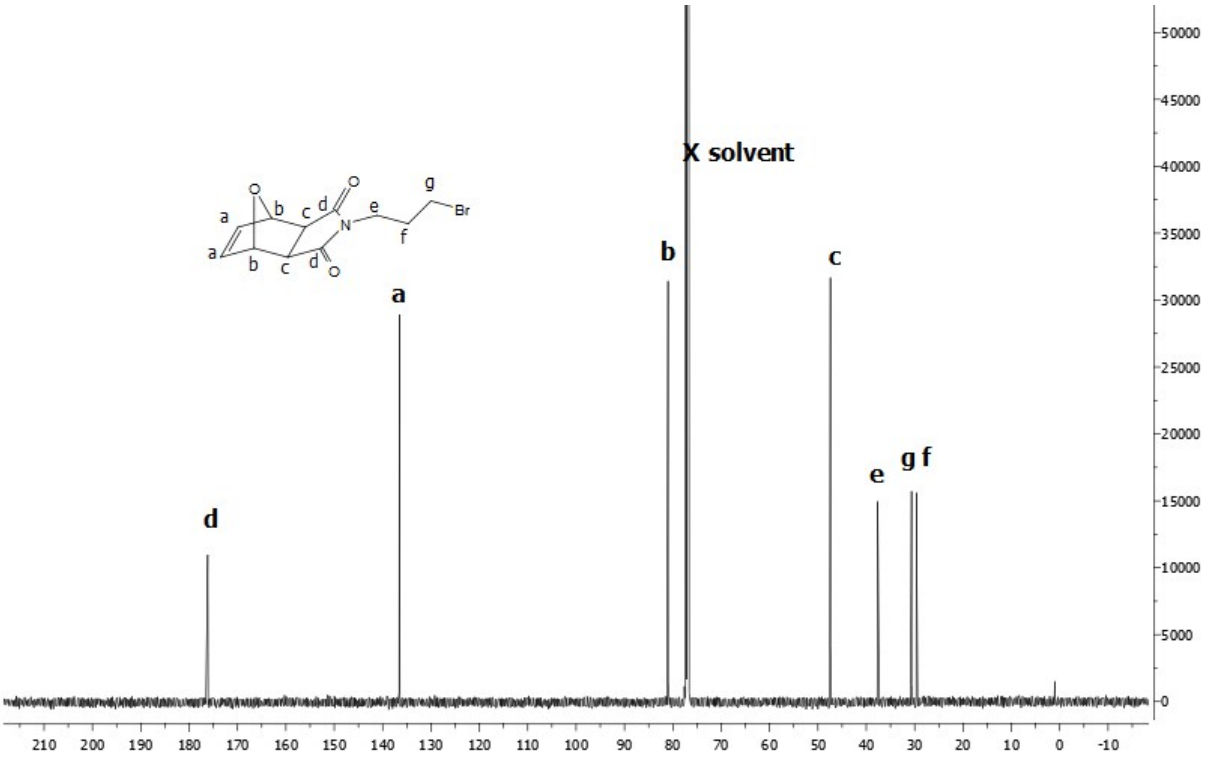


**Figure S2.** $^{13}C$ NMR spectra of compound **2** in $CDCl_3$.

**Synthesis of monomer 3a**

Compound 2 (0.5 g, 1.75 mmol) and excess amount of trimethylphosphine solution in THF (Molarity =1 mol $L^{-1}$) (5.25 mL, 5.25 mmol) was mixed in a glass vial and 5 mL THF was added to the mixture. The reaction vessel was covered with aluminium foil. The reaction was performed under nitrogen at 50°C and stirred for 24h. The product was precipitated and washed with THF. It was dried under nitrogen and stored in desiccator. Yield 80 %.

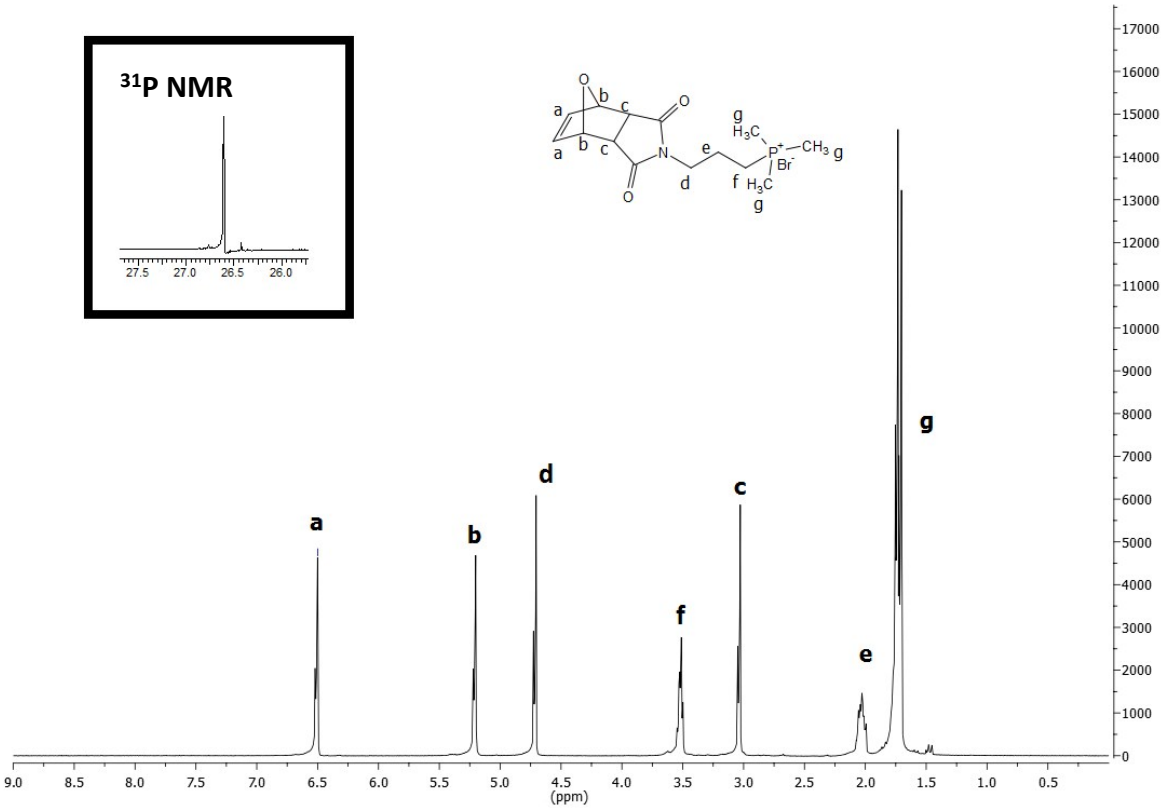


**Figure S3.** $^{1}H$ and $^{31}P$ NMR spectra of monomer **3a** in $D_2O$.

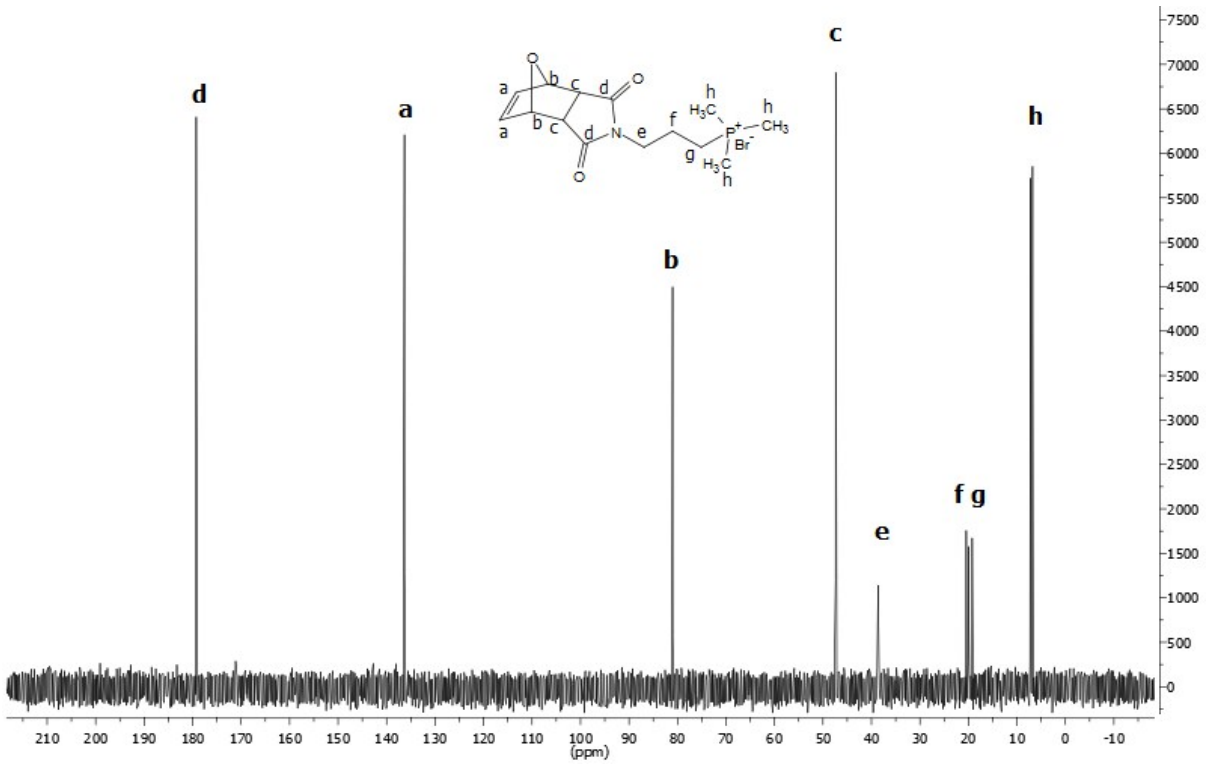


**Figure S4.** $^{13}C$ NMR spectra of monomer **3a** in $D_2O$.

**Synthesis of monomer 3b**

Compound 2 (0.5 g, 1.75 mmol) and excess amount of triethylphosphine solution in THF (M=1 mol $L^{-1}$) (5.25 mL, 5.25 mmol) was mixed in a glass vial and 5 mL THF was added to the mixture. The reaction vessel was covered with aluminium foil. The reaction was performed under nitrogen at 50°C and stirred for 24 h. Precipitation of the product in reaction vessel was observed and THF was added for further precipitation. The product was washed with THF. It was dried under nitrogen and stored in desiccator. Yield 90 %.

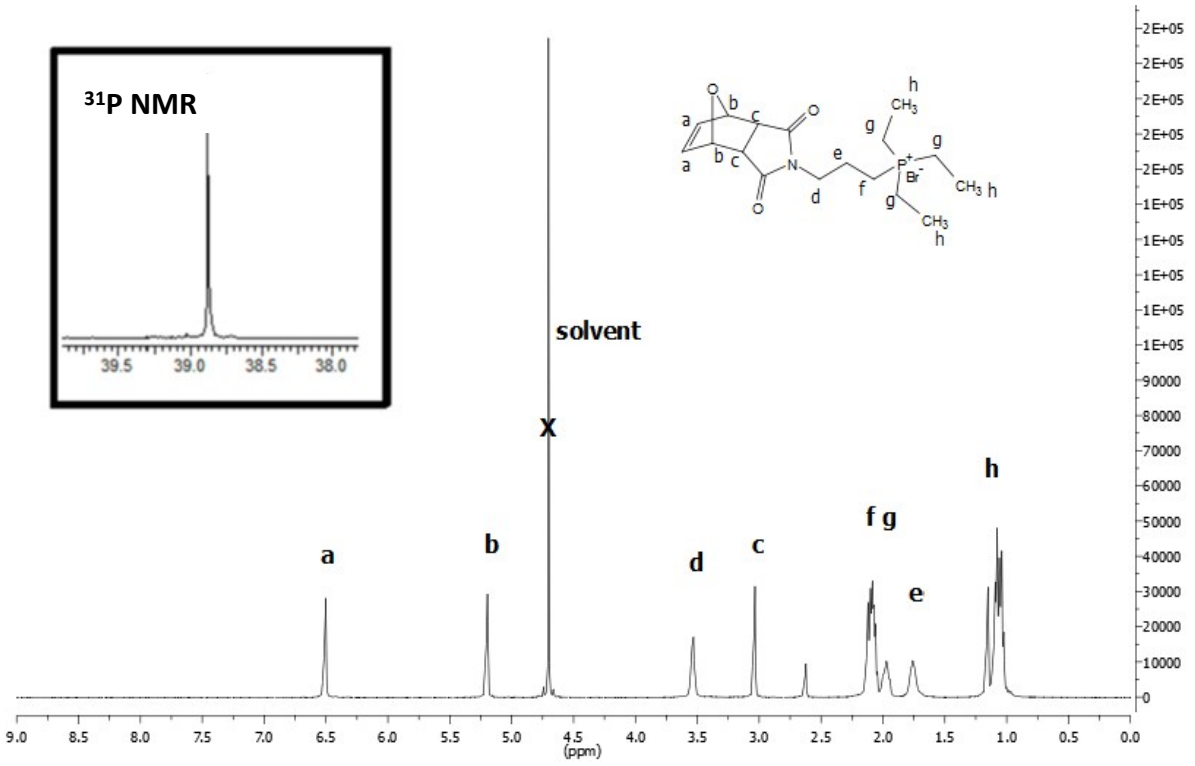


**Figure S5.** $^{1}H$ and $^{31}P$ NMR spectra of monomer **3b** in $D_2O$.

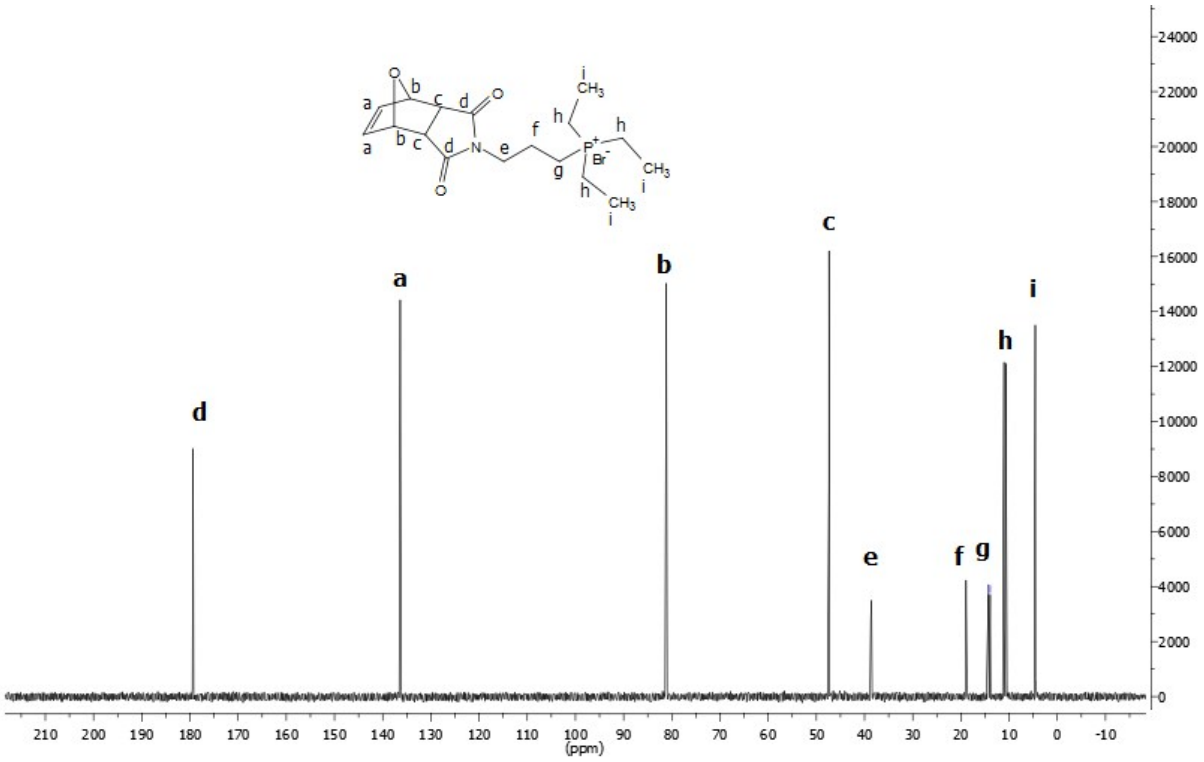


**Figure S6.** $^{13}C$ NMR spectra of monomer **3b** in $D_2O$.

**Synthesis of monomer 3c**

Compound 2 (0.5 g, 1.75 mmol) was dissolved in 8 mL THF in a glass vial and excess amount of tripropylphosphine solution in THF (d: 0,801 g $mol^{-1}$, 97 %) (1.119 mL, 0.896 mmol) was added with syringe under nitrogen. The reaction was performed at 50°C and stirred for 24 h. Precipitation was observed and the reaction vial was cooled in ice bath for further precipitation. The product was washed with diethyl ether-THF (1:1 v/v) mixture. It was dried under nitrogen and stored in desiccator. Yield 77 %.

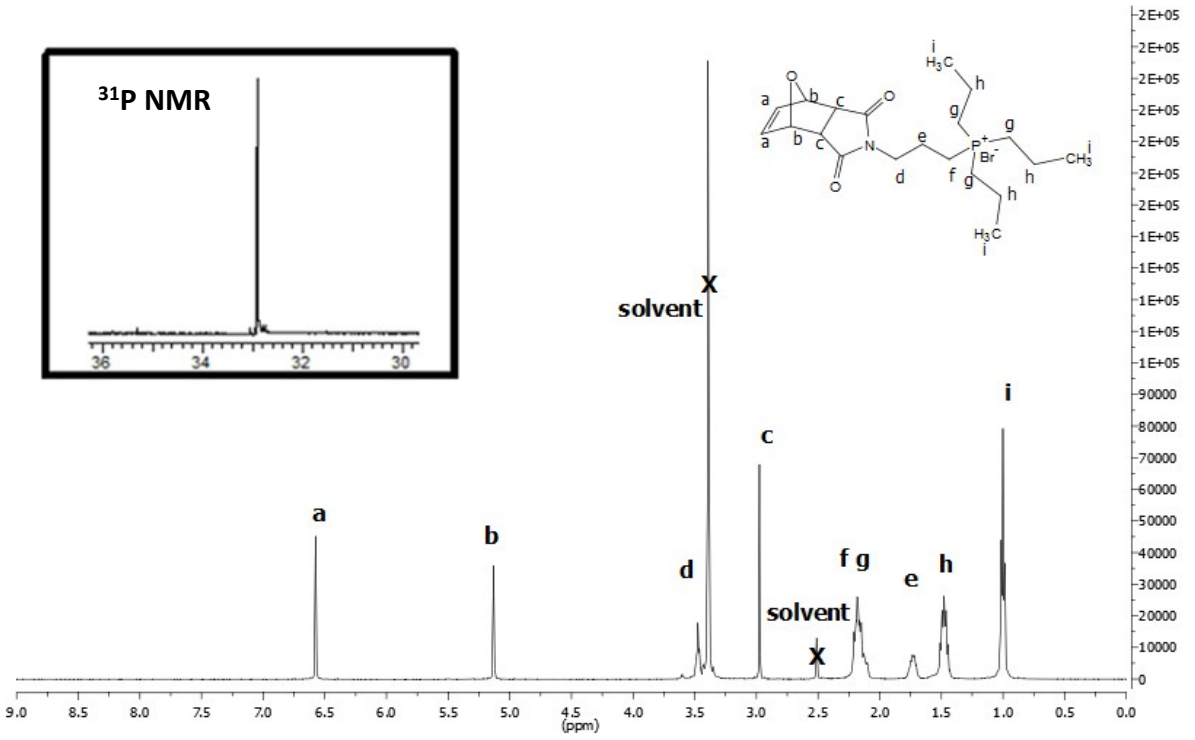


**Figure S7.** $^{1}$H and $^{31}$P NMR spectra of monomer **3c** in DMSO-$d_6$.

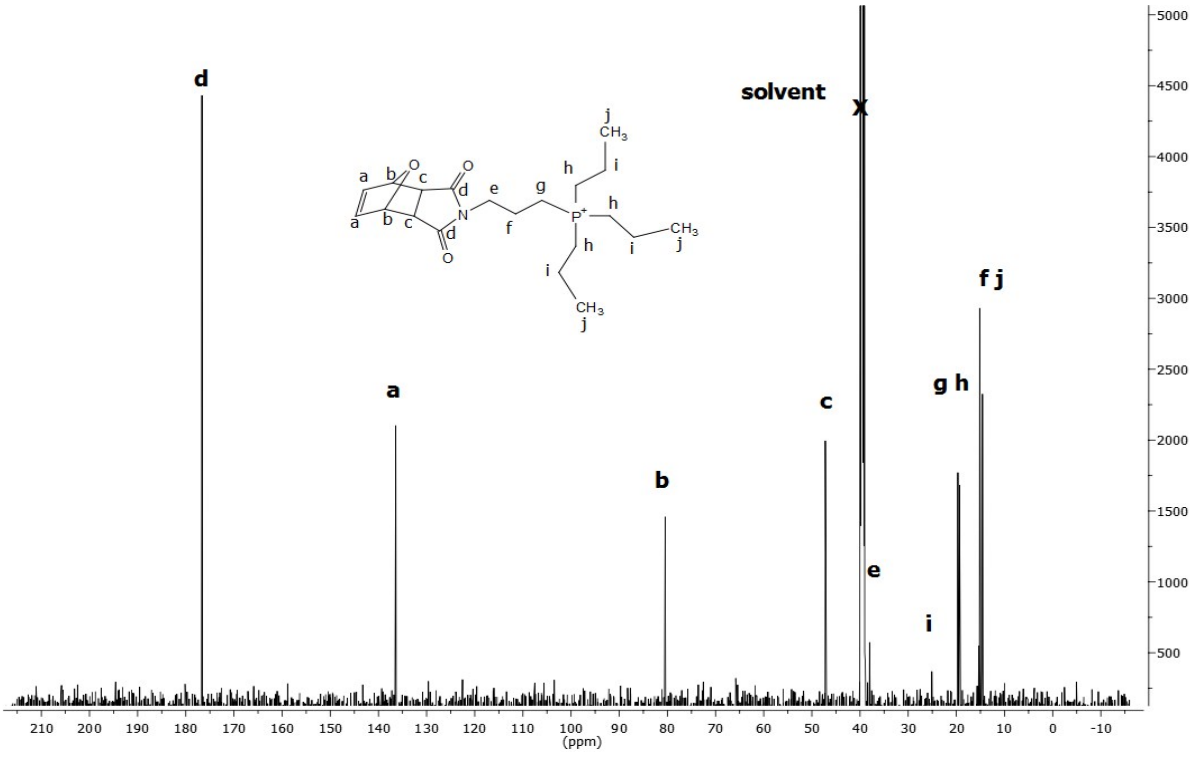


**Figure S8.** $^{13}$C NMR spectra of monomer **3c** in DMSO-$d_6$.

**Synthesis of monomer 3d**

Compound 2 (1 g, 3.5 mmol) was dissolved in 12 mL THF in a glass vial and excess amount of t-butylphosphine solution in THF (d: 0.834 g $mol^{-1}$) (3 mL) was added with syringe under nitrogen. The reaction was performed at 50°C and stirred for 24 h. Precipitation was observed and the reaction vial was cooled in ice bath for further precipitation. The product was washed with diethyl ether. It was dried under nitrogen and stored in desiccator. Yield 11 %.

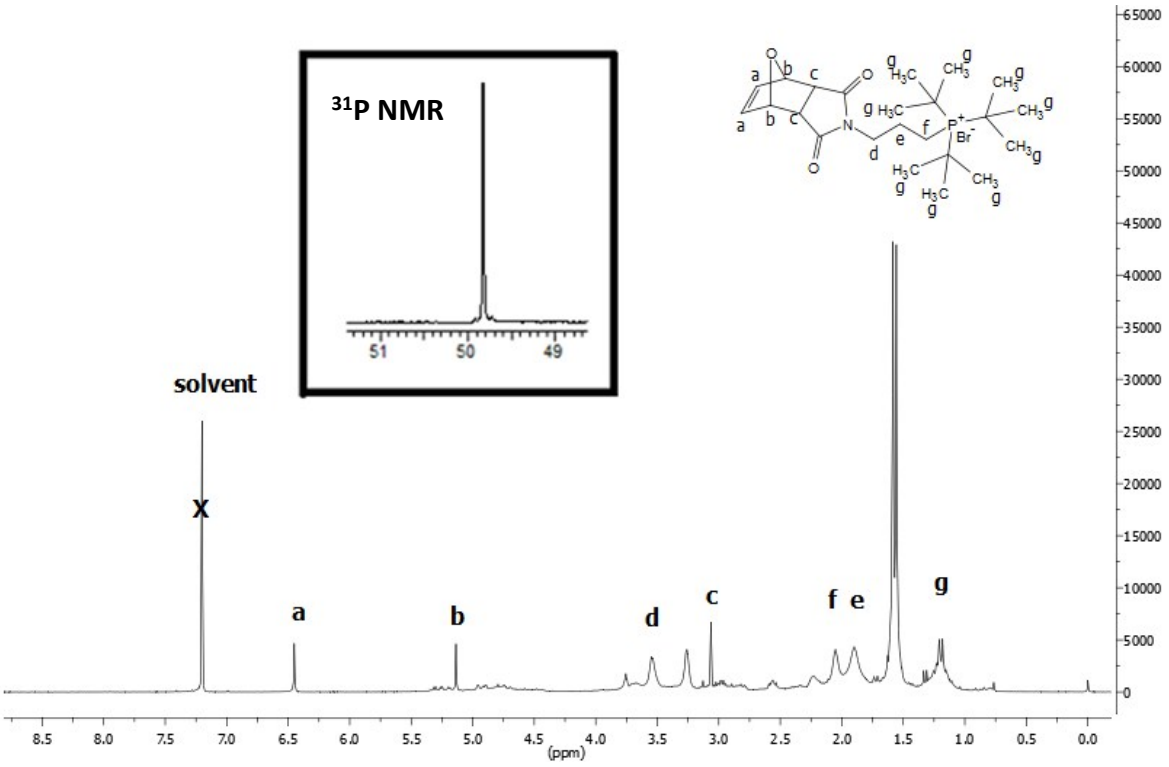


**Figure S9.** $^{1}H$ and $^{31}P$ NMR spectra of monomer **3d** in $CDCl_3$.

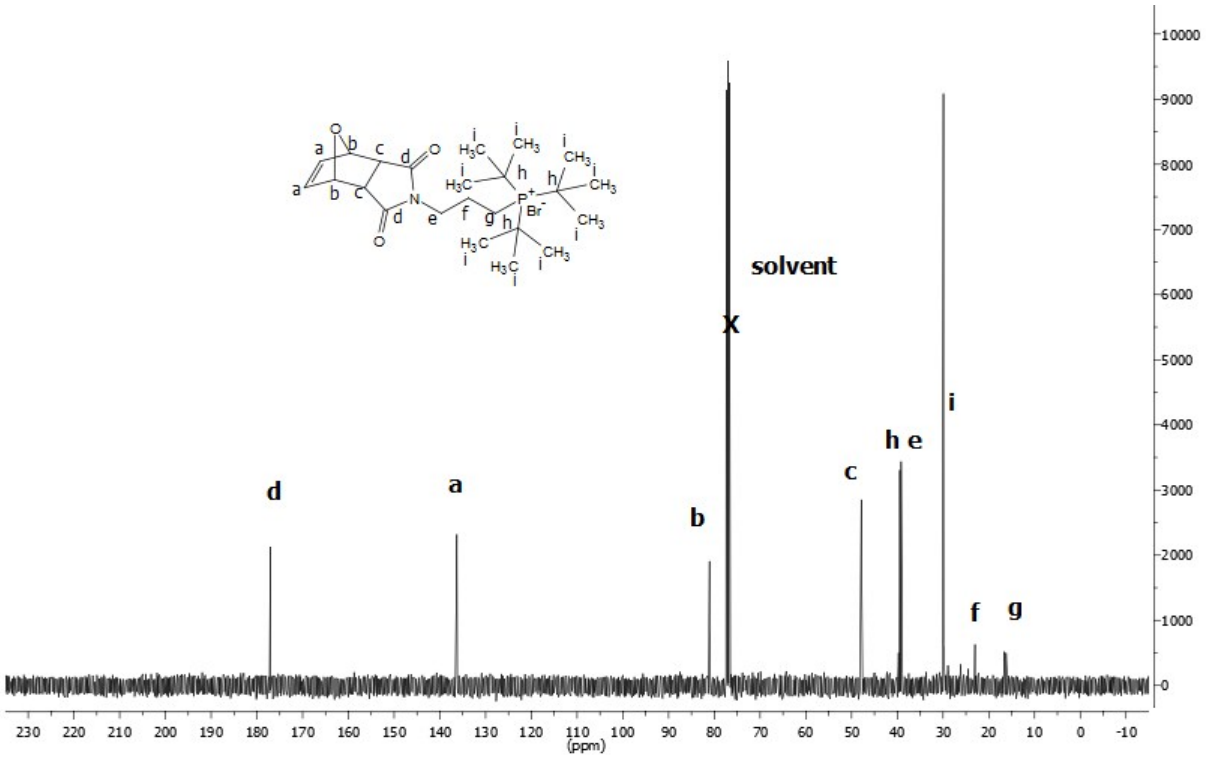


**Figure S10.** $^{13}C$ NMR spectra of monomer **3d** in $CDCl_3$.

**Synthesis of monomer 3e**

Compound 2 (0.5 g, 1.75 mmol) and excess amount of triphenylphosphine (1.376 g, 5.25 mmol) was mixed in 11 mL ethyl acetate. The reaction was performed under nitrogen in a sealed vessel at 50°C and stirred for 24 h. Precipitation of the quaternary phosphonium product in reaction vessel was observed. It was washed with ethyl acetate and THF. It was dried under nitrogen and stored in desiccator. Yield 11 %.

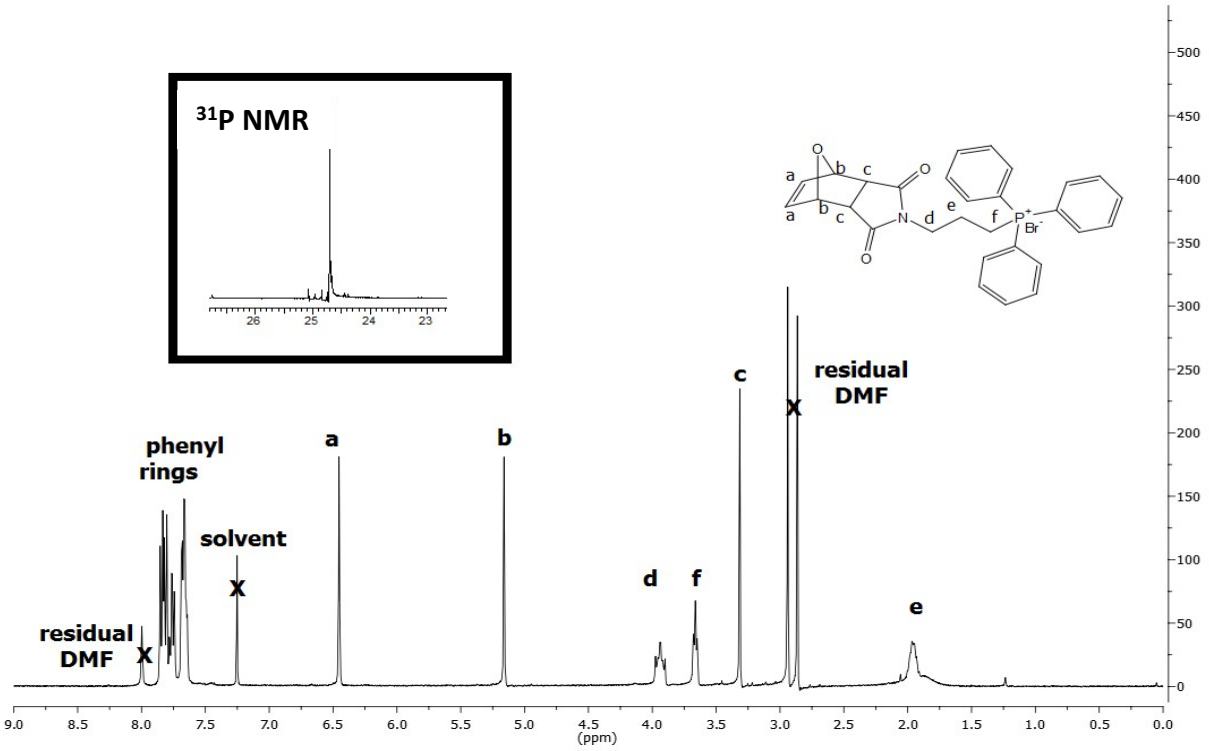


**Figure S11.** $^{1}H$ and $^{31}P$ NMR spectra of monomer **3e** in $CDCl_3$.

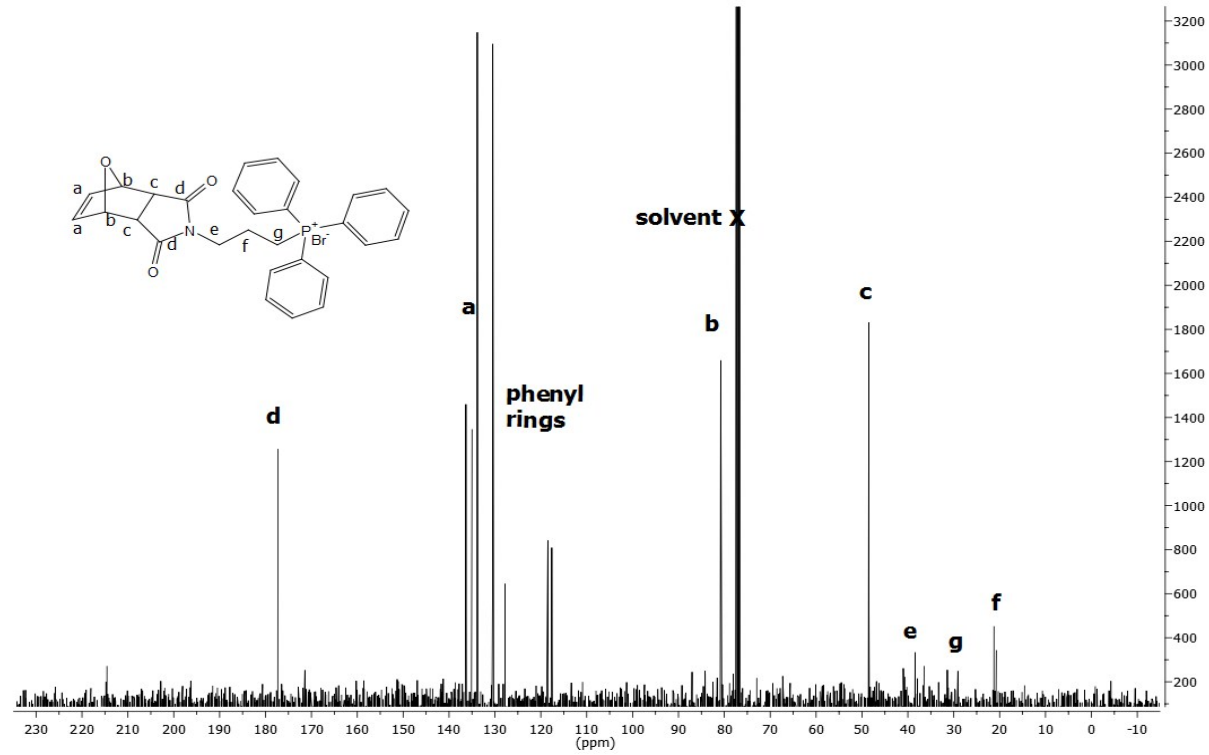


**Figure S12.** $^{13}C$ NMR spectra of monomer **3e** in $CDCl_3$.

**Synthesis of monomer 3f**

Compound 2 (0.5 g, 1.75 mmol) and excess amount of Tris(4-methoxyphenyl)phosphine (1.8487 g, 5.25 mmol) was mixed in 15 mL ethyl acetate in a round-bottom flask. The reaction was performed under nitrogen at 50°C and stirred for 24 h. The product was precipitated with diethyl ether and washed with diethyl ether-THF (1:1 v/v) mixture. It was dried under nitrogen and stored in desiccator. Yield 40 %.

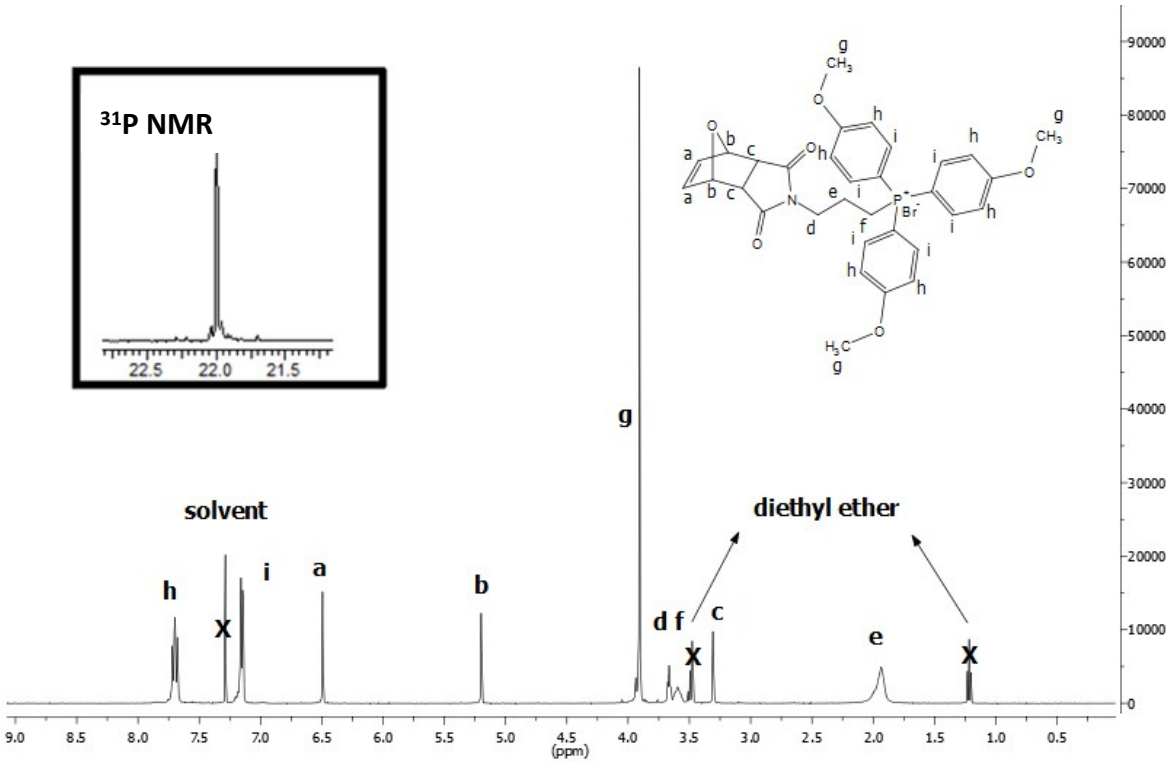


**Figure S13.** $^{1}H$ and $^{31}P$ NMR spectra of monomer **3f** in $CDCl_3$.

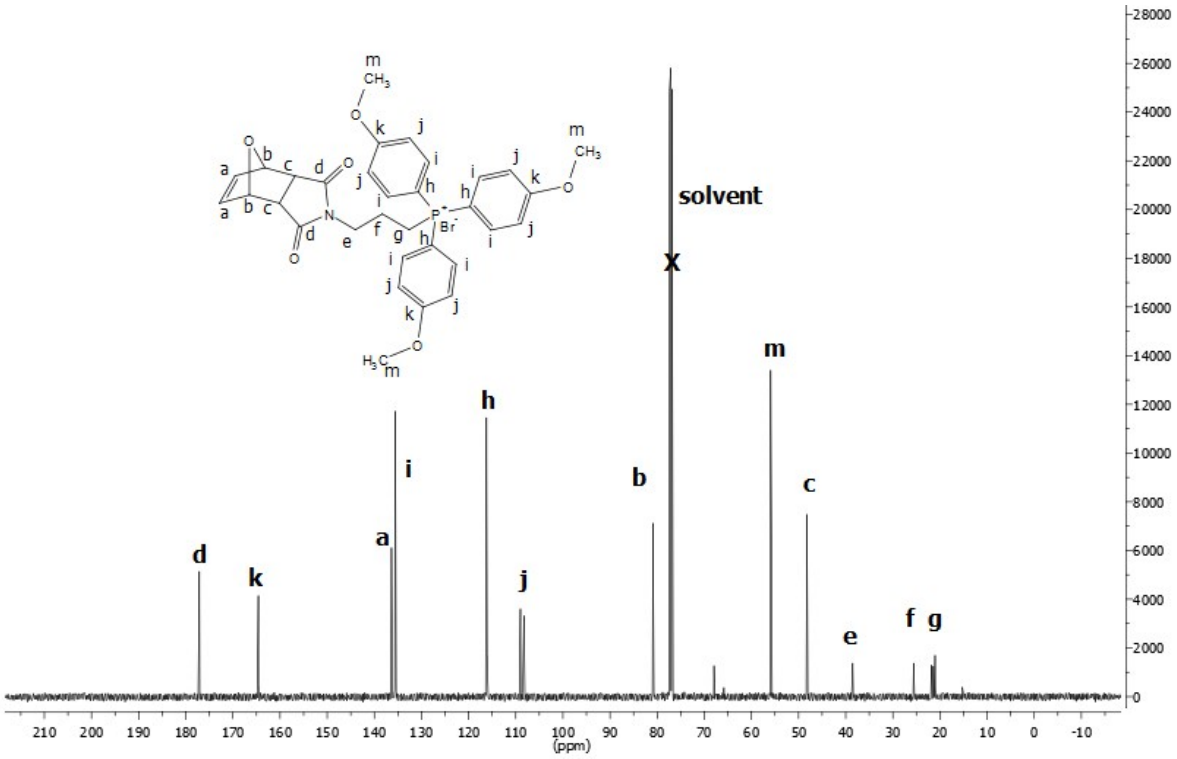


**Figure S14.** $^{13}C$ NMR spectra of monomer **3f** in $CDCl_3$.

**General Polymerization Procedure for Monomers 3a-f**

In a typical example, freshly prepared Grubbs third generation catalyst was dissolved in 0.5 mL of $CH_2Cl_2$ and added in one shot to the vigorously stirring **3e** monomer solution in 2 mL dichloromethane (DCM). The reaction mixture was stirred for 3 h at room temperature. The reaction was then terminated by an injection of 0.5 mL 30 % ethylvinyl ether (in dichloromethane). The polymer, **4e**, was precipitated and washed with diethyl ether and and dried under nitrogen. **4e** was dissolved in $CDCl_3$ for characterization. (For monomers 3a, 3b-c and 3d-f; 2,2,2-trifluoroethanol, N,N-Dimethylformamide and dichloromethane were used as solvents respectively. Polymers were precipitated and washed with either tetrahydrofuran or diethyl ether.) $^1H$ NMR spectrum of the polymers (theoretical molecular weight 3,000 g $mol^{-1}$) were given in Figure S15-S20.

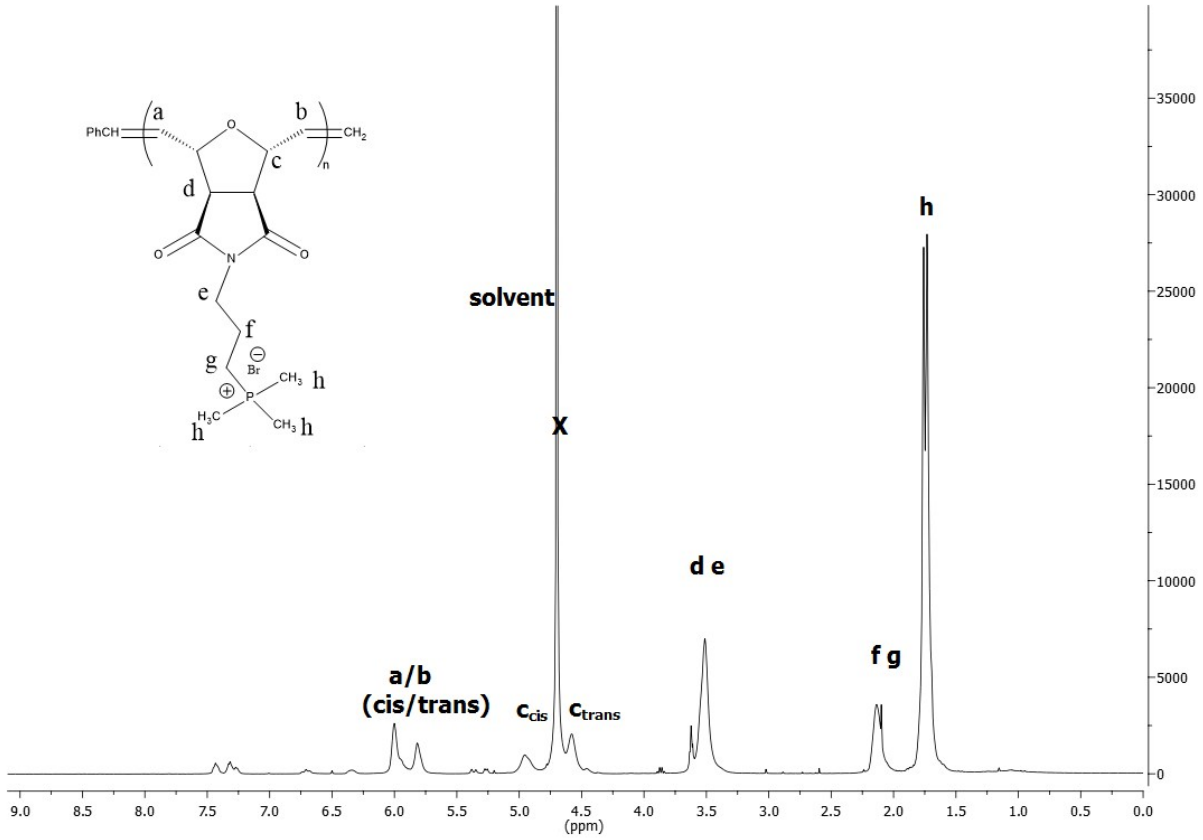


**Figure S15.** $^{1}H$ NMR spectra of **4a** in $D_2O$.

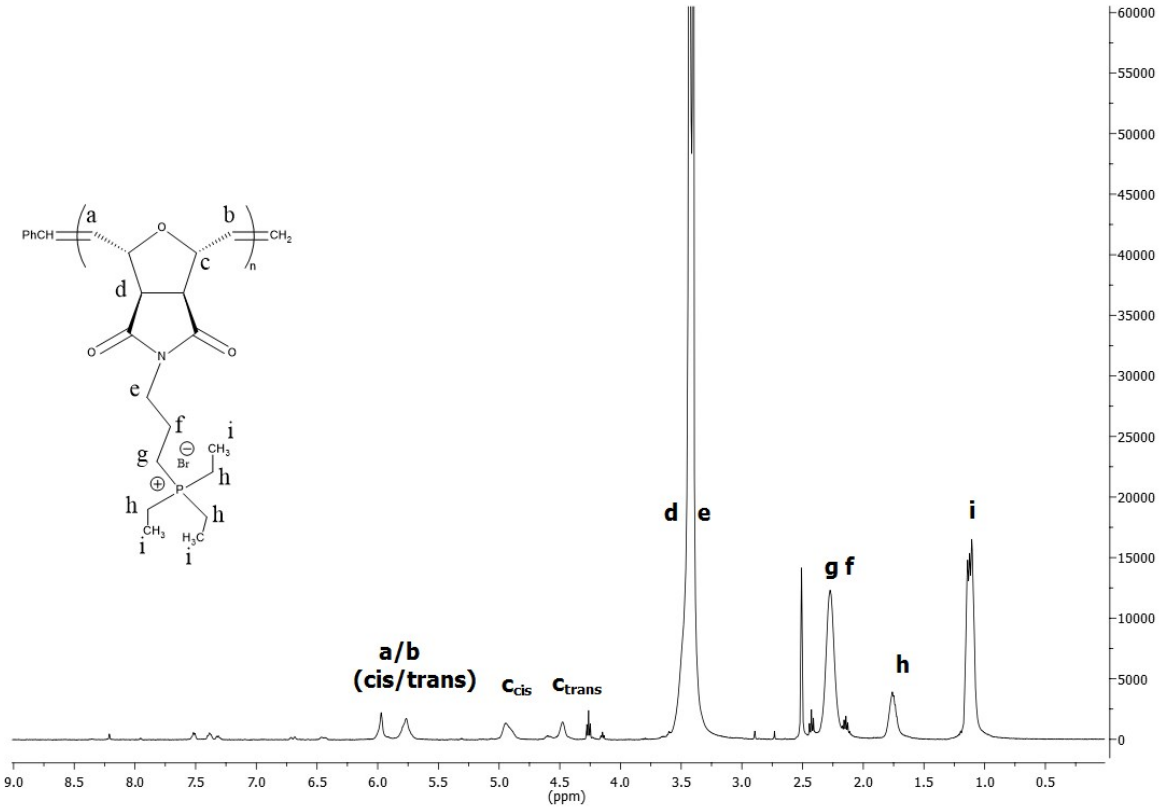


**Figure S16.** $^{1}$H NMR spectra of **4b** in DMSO-$d_6$.

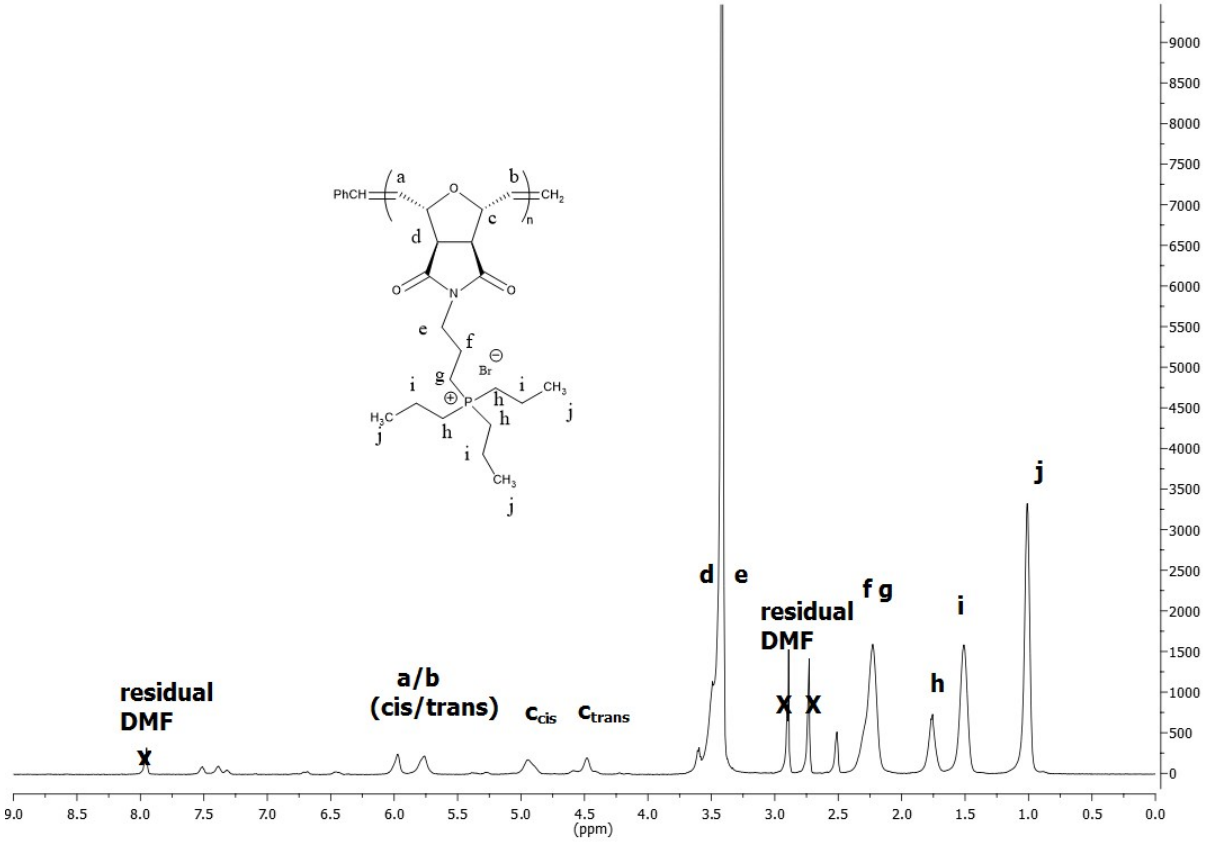


**Figure S17.** $^{1}$H NMR spectra of **4c** in DMSO-$d_6$.

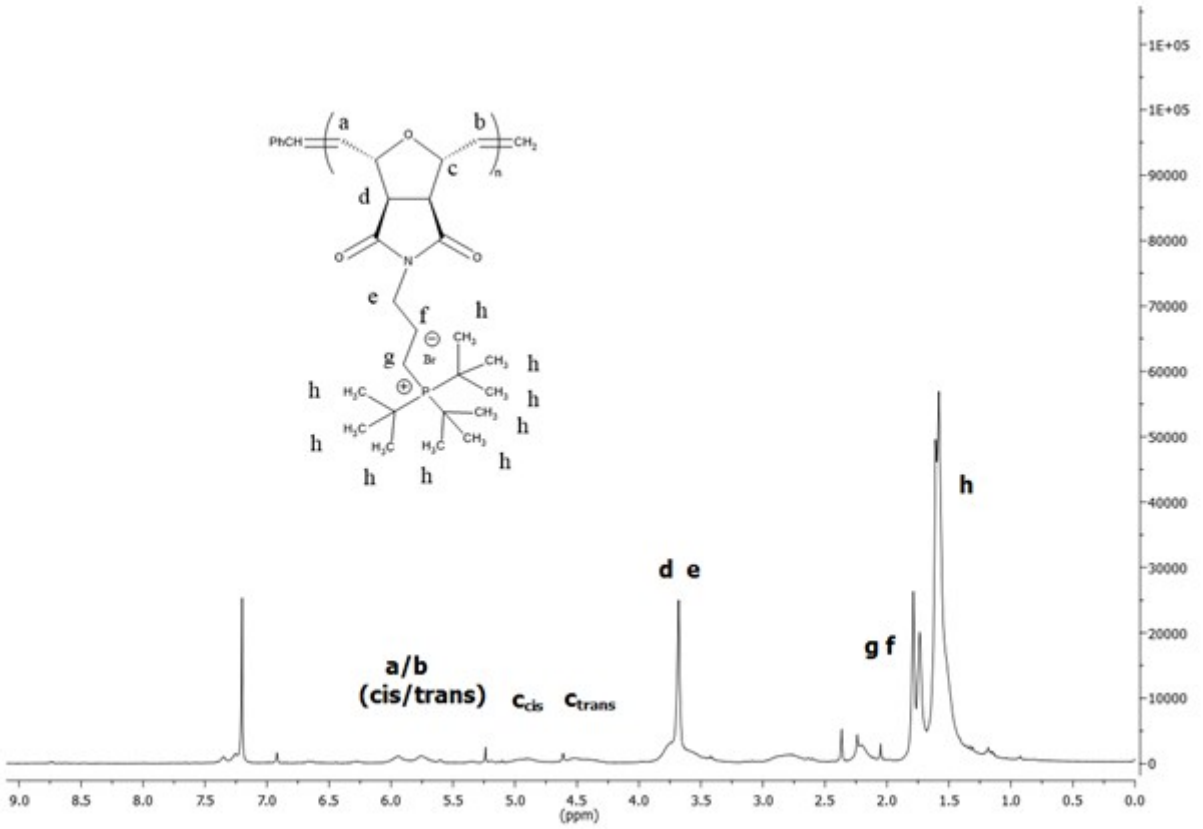


**Figure S18.** $^{1}$H NMR spectra of **4d** in $CDCl_3$.

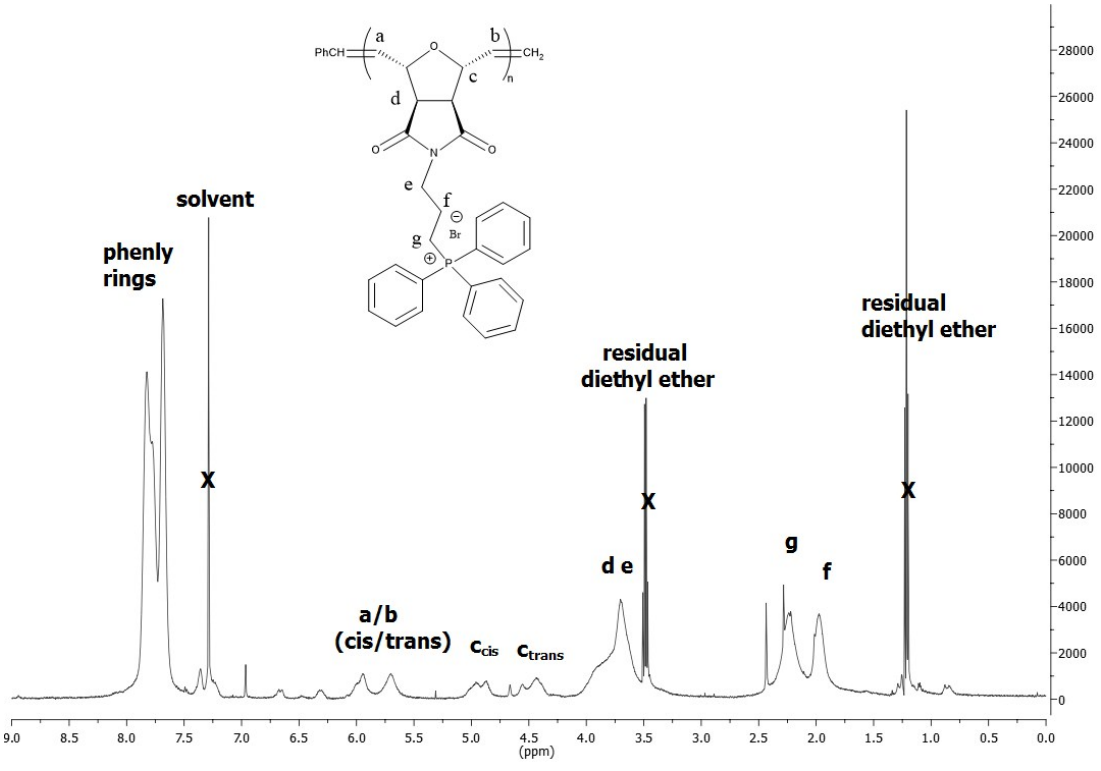


**Figure S19.** $^{1}$H NMR spectra of **4e** in $CDCl_3$.

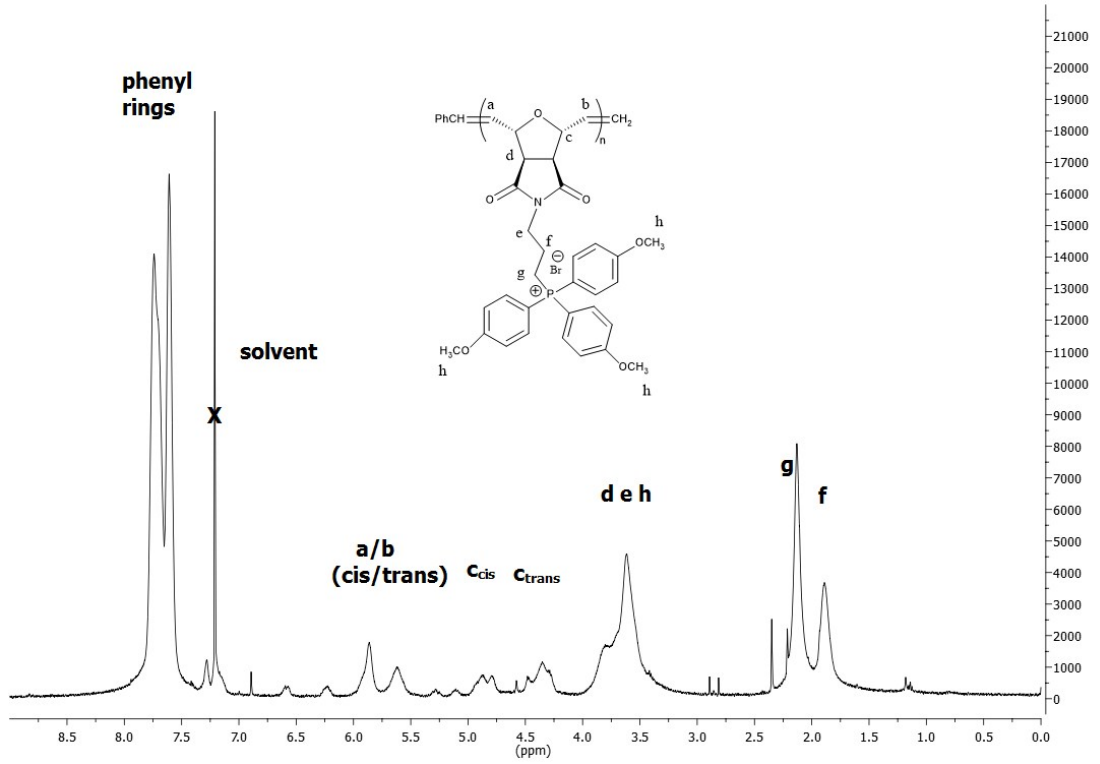


**Figure S20.** $^{1}$H NMR spectra of **4f** in $CDCl_3$.

## 4. Determination of Solution Phase Antibacterial Activity

The MIC of the cationic polymers were determined by a standard serial dilution technique with an inoculum of 5 x $10^5$ cell $mL^{-1}$ in Müller Hinton and Middle Brook Broth medium for bacteria and Sabouroud Dextrose Broth medium for fungi. Three bacteria and one fungi strains were used: *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Mycobacterium tuberculosis* (ATCC 27294) and *Candida albicans* (ATCC 10231). Polymer solutions were prepared by dissolution in water (**4a**, **4b**, **4c** and **4d_3k**) or DMSO (**4d_10k**, **4e** and **4f**) and further diluted with buffer. The concentration of the polymers ranged from 512 μg $mL^{-1}$ to 4 μg $mL^{-1}$. The MIC of the polymers was determined after 12 h of incubation at 37°C by optical density (OD) measurements. The MIC was defined as the lowest concentration at which bacterial growth was inhibited.

## 5. Determination of Hemolytic Activity

Blood sample was taken from healthy adult volunteer using vacuum tubes with sodium heparin (15 IU $mL^{-1}$). Oral consent was obtained following a brief discussion that outlined the purpose and principles of the study, including an overview of the protocols. The use of human blood was in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The experimental procedures of the present study were reviewed and approved by the Institutional Review Board (IRB), Yildiz Technical University. Polymer solutions were prepared by dissolution in water (**4a**, **4b**, **4c** and **4d_3k**) or DMSO (**4d_10k**, **4e** and **4f**) and further diluted with buffer.

Erythrocytes were separated by centrifugation at 230 g for 10 min and washed three times with isotonic phosphate buffered saline (pH:7.0, PBS). Separated erythrocytes were re-suspended in the PBS at a hematocrit level of 0.05 L/L and mixed with each polymer solution on 96-well microplate at a concentration of 512 μg $mL^{-1}$. Then, all samples were gently shaken at 37°C for 1 hour. After incubation, the well contents were centrifuged at 230 g for 10 min and the supernatant in each well was transferred to a new plate. Absorbance of each sample was measured at 414 nm. 0.05% Triton X-100 was used as the positive control while the PBS or DMSO served as the negative control. To remove the effect of the polymer color on our

readings, absorbance of the polymer solutions (without erythrocytes) measured and substracted from final readings. The percentage of hemolysis was calculated by using the absorbance values for polymer solution (Ap), Triton X-100 (At) and PBS/DMSO (Ab) as Hemolysis (%) = [(Ap – Ab)/(At – Ab)] x100%.

## 6. DLS Assay

Stock solution of water soluble polymers (4a_3k, 4a_10k, 4b_3k, 4b_10k) were prepared in phosphate-buffered saline (PBS) (pH:7) at a concentration of 1 mg $mL^{-1}$.

Stock solution of water insoluble polymers (4e_3k, 4e_10k, 4f_3k, 4f_10k) were prepared in dimethyl sulfoxide (DMSO) at a concentration of 1 mg $mL^{-1}$. 100 µL of these stock solutions were added to 900 µL of PBS giving a final concentration of 0,1 mg/mL and zeta potentials were measured. For the assays with bacteria; one active (4e_3k) and one inactive (4b_3k) polymers were used. Bacteria solution ($10^9$ cell $mL^{-1}$) and 100 µL DMSO/ 900 µL Bacteria solution mixture were used as two different blanks, 100 µL of stock solutions (1 mg $mL^{-1}$) were diluted with 100 µL PBS to give a concentration of 0,5 mg $mL^{-1}$. Finally, 100 µL of this solution (0,5 mg $mL^{-1}$) was added to 900 µL of bacteria solution and zeta potentials were measured. Before measuring zeta potential, all polymer, bacteria and polymer-bacteria solutions were filtered through 45-µL filters.

**Results**

| | | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|---|
| Zeta Potential (mV): | 0,00161 | Peak 1: | 0,00161 | 100,0 | 5,68 |
| Zeta Deviation (mV): | 5,68 | Peak 2: | 0,00 | 0,0 | 0,00 |
| Conductivity (mS/cm): | 0,00317 | Peak 3: | 0,00 | 0,0 | 0,00 |
| Result quality | See result quality report | | | | |

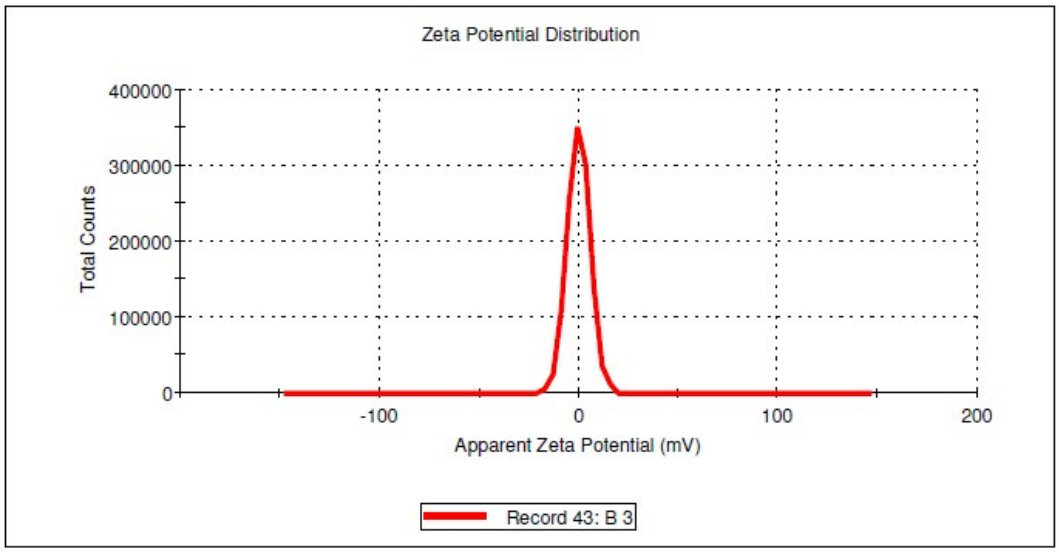


**Figure S21**. Zeta potential properties of *E.coli* in buffer.

**Results**

| | | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|---|
| Zeta Potential (mV): | 0,0437 | Peak 1: | 0,0437 | 100,0 | 3,40 |
| Zeta Deviation (mV): | 3,40 | Peak 2: | 0,00 | 0,0 | 0,00 |
| Conductivity (mS/cm): | 0,0145 | Peak 3: | 0,00 | 0,0 | 0,00 |
| Result quality | See result quality report | | | | |

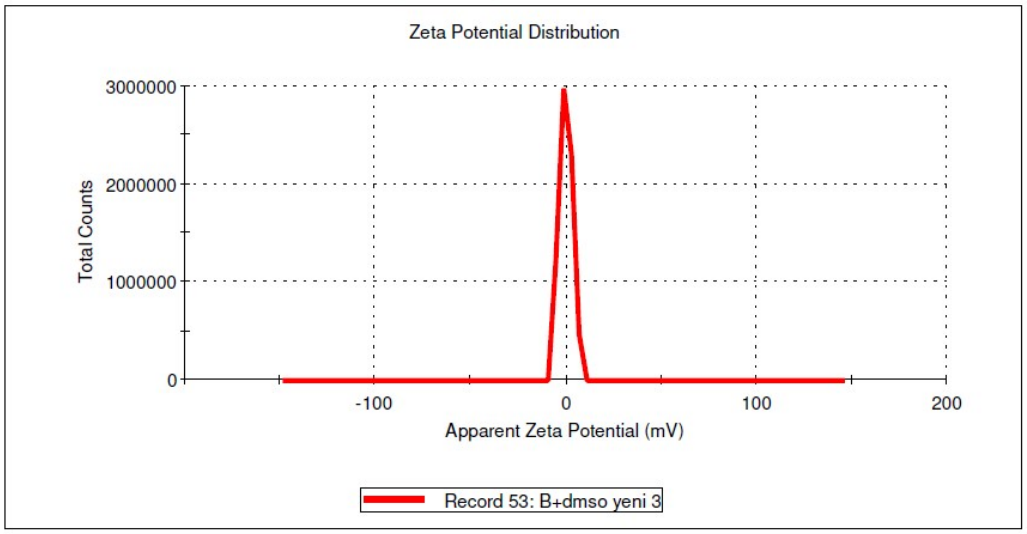


**Figure S22**. Zeta potential properties of *E.coli* in DMSO.

**Results**

| | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| **Zeta Potential (mV):** -0,0303 | **Peak 1:** | -0,0303 | 100,0 | 5,14 |
| **Zeta Deviation (mV):** 5,14 | **Peak 2:** | 0,00 | 0,0 | 0,00 |
| **Conductivity (mS/cm):** 0,00171 | **Peak 3:** | 0,00 | 0,0 | 0,00 |

**Result quality** See result quality report

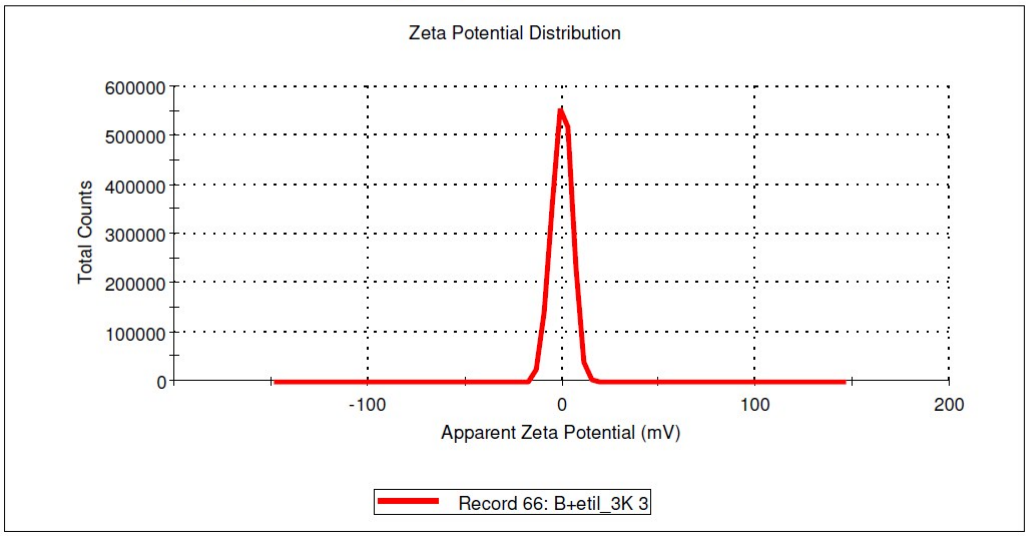


**Figure S23**. Effect of polymer **4b** on the zeta potential properties of *E.Coli*.

**Results**

| | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| **Zeta Potential (mV):** -17,1 | **Peak 1:** | -17,1 | 100,0 | 6,89 |
| **Zeta Deviation (mV):** 6,89 | **Peak 2:** | 0,00 | 0,0 | 0,00 |
| **Conductivity (mS/cm):** 4,40 | **Peak 3:** | 0,00 | 0,0 | 0,00 |

**Result quality** Good

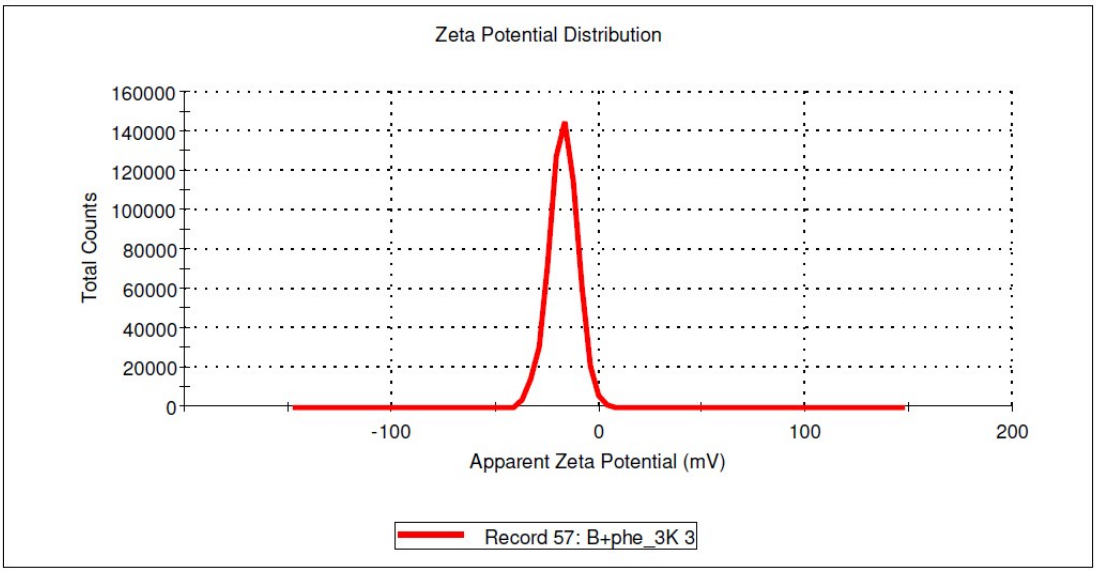


**Figure S24**. Effect of polymer **4e** on the zeta potential properties of *E.Coli*.

**Results**

| | | Mean (mV) | Area (%) | St Dev (mV) |
|---|---|---|---|---|
| Zeta Potential (mV): -15,1 | Peak 1: | -15,1 | 100,0 | 7,12 |
| Zeta Deviation (mV): 7,12 | Peak 2: | 0,00 | 0,0 | 0,00 |
| Conductivity (mS/cm): 4,14 | Peak 3: | 0,00 | 0,0 | 0,00 |
| Result quality Good | | | | |

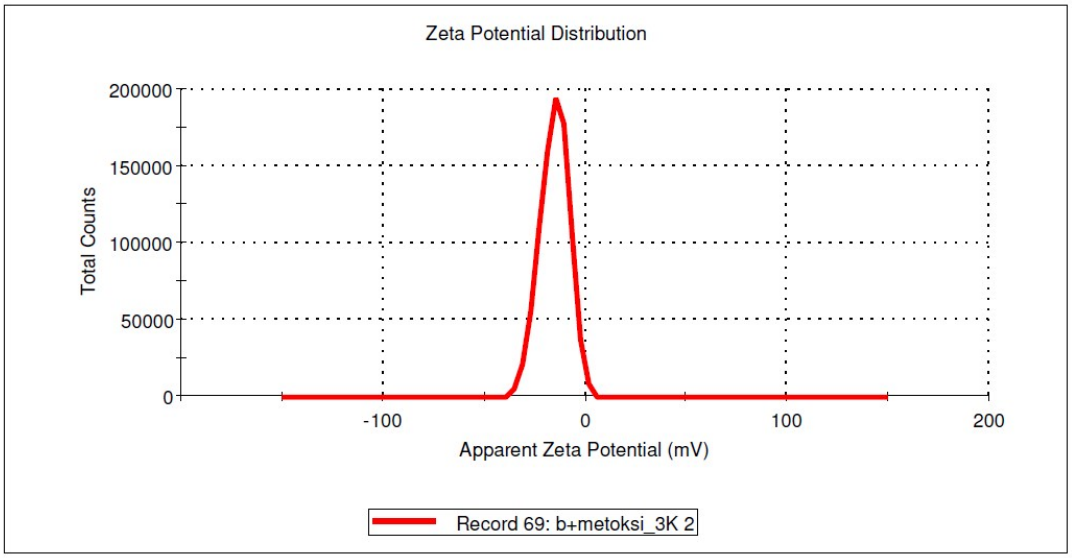


**Figure S25**. Effect of polymer **4f** on the zeta potential properties of *E.Coli*.

**References:**


1. J. A. Love, J. P. Morgan, T. M. Trnka and R. H. Grubbs, *Angewandte Chemie International Edition*, 2002, **41**, 4035-4037.
2. M. B. France, L. T. Alty and T. M. Earl, *Journal of chemical education*, 1999, **76**, 659.
3. H. S. Bazzi and H. F. Sleiman, *Macromolecules*, 2002, **35**, 9617-9620.