

Supporting Information

Combined Tetraphenylethylene Fluorogens with Positive Charge for Imaging

Capsule-covered Pathogens

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1、 Materials and instruments

All reagents and raw materials were purchased at analytical grade and were used directly without further purification. UV–vis absorption were measured on HITACHI U-3900H. Fluorescence spectra were obtained on a HITACHI F-4600 spectrophotometers. Centrifugation was performed using a refrigerated centrifuge (MiniMar-12K+). Bacterial fluorescence imaging was carried out using a laser scanning confocal microscope (Leica SP8).

Escherichia coli(*E.coli*), *Staphylococcus aureus*(*S.aureus*) and *Canidia albicans*(*C.albicans*) were purchased from Beijing Tiantan Hospital. *Klebsiella Pneumoniae* (*K. pneumoniae*) were from China Agricultural University. Nutrient broth and LB agar were purchased from Leagene Biotechnology. Raw 264.7 cells were purchased from Cell Resource Center, Chinese Academy of Medical Sciences & Peking Union Medical College (CAMS & PUMC), Institute of Basic Medical Sciences (IBMS), China.

2、 Synthesis of TPE-Py⁺ and TPE-Pn⁺⁺

Synthesis of TPE-Br: The synthesis method of TPE-Br (4,4'-(2-(4-bromophenyl)-2-phenylethene-1,1-diyl)bis(methoxybenzene)) has been described in the previous literature.¹The yield of TPE-Br is 88%. ¹H NMR(400 MHz, CDCl₃) δ 7.28 – 7.21 (m, 2H), 7.12 (q, J = 6.4, 6.0 Hz, 3H), 7.08 – 7.00 (m, 2H), 6.94 (ddd, J = 13.7, 8.6, 3.6 Hz, 6H), 6.73 – 6.62 (m, 4H), 3.78 (dd, J = 11.0, 1.3 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 158.28, 158.19, 158.05, 144.29, 143.79, 143.31, 140.78, 140.08, 137.91, 136.36, 136.02, 135.93, 133.04, 132.57, 132.55, 132.52, 131.36, 131.34, 130.85, 127.81, 127.66, 126.30, 126.05, 120.01, 113.21, 113.03, 112.99, 55.13, 55.09, 1.04. MALDI-TOF calcd for C₂₈H₂₃O₂Br [M + 2H]⁺ 470.09, found 469.70.

Synthesis of TPE-CHO: We successfully synthesized the TPE-CHO (4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)benzaldehyde) based on previous literature.¹ The yield of TPE-Br is 32%. ¹H NMR(400 MHz, CDCl₃) δ 9.92 (s, 1H), 7.64 (d, J = 7.9 Hz, 2H), 7.21 (d, J = 7.9 Hz, 2H), 7.15 (d, J = 6.3 Hz, 3H), 7.07 – 7.00 (m, 2H), 6.97 (dd, J = 8.8, 3.3 Hz, 4H), 6.67 (d, J = 8.3 Hz, 4H), 3.77 (d, J = 1.5 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 190.88, 157.55, 157.40, 150.26, 142.46, 141.33, 136.95, 134.60, 134.55, 132.94, 131.63, 131.57, 130.96, 130.31, 128.31, 128.16, 126.93, 125.48, 112.23, 112.04, 54.09, 54.07, 30.94, 29.12, 28.67, 22.07, 21.40, 13.18. MALDI-TOF calcd for C₂₉H₂₄O₃ [M + 2H]⁺ 420.17, found 420.80.

Synthesis of TPE-Py⁺: 1, 2-dimethylpyridin-1-ium iodide (55.95 mg, 0.24 mmol) and TPE-CHO (50.00 mg,

0.12mmol) were dissolved in anhydrous ethanol (30 mL) and a few drops of triethylamine were added into the mixture. The mixture was refluxed for 48 hours under the protection of N₂. After the reaction mixture was cooled to room temperature, the mixture was extracted three times with water and CH₂Cl₂. Then Na₂SO₄ was added to dry the mixture. The crude product was purified by a silica gel column using CH₂Cl₂/MeOH mixture (10: 1 v/v) to produce a yellow powder (23.5 mg, yield: 31%). ¹H NMR (500 MHz, Chloroform-d) δ 9.32 (d, J = 6.2 Hz, 1H), 8.34 (t, J = 8.0 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.80 (t, J = 6.9 Hz, 1H), 7.59 (d, J = 15.7 Hz, 1H), 7.49 (d, J = 7.9 Hz, 2H), 7.34 (s, 1H), 7.14 (q, J = 7.6 Hz, 5H), 7.08 – 6.91 (m, 6H), 6.67 (dd, J = 12.5, 8.3 Hz, 4H), 4.58 (s, 3H), 3.77 (s, 6H). ¹³C NMR (126 MHz, Chloroform-d) δ 158.50, 158.38, 153.29, 148.49, 146.47, 144.66, 144.42, 143.63, 141.99, 138.10, 135.86, 135.76, 132.70, 132.61, 132.30, 131.94, 131.57, 131.38, 131.24, 128.37, 127.96, 126.50, 125.43, 125.25, 115.42, 113.31, 113.06, 55.21, 55.12, 47.50. HR-MS (ESI, positive) calcd for C₃₆H₃₂NO₂ (M – I) 510.2428, found 510.2429.

Synthesis of TPE-Pn⁺⁺: The reactants 4-methyl-1-(3-(trimethylammonio)propyl) pyridin-1-ium bromide (41.89 mg, 0.12 mmol) and TPE-CHO (50.00 mg, 0.12 mmol) were dissolved in anhydrous ethanol (30 mL) using piperdine as catalyst. Then the mixture was refluxed overnight under the protection of N₂. After the mixture was cooled to room temperature, remove solvent by evaporated under reduced pressure. The crude product was purified by a silica gel column using MeOH as the eluent to give TPE -Pn⁺⁺ as a red powder(46.67 mg,yield:52%). ¹H NMR (400 MHz, Methanol-d₄) δ 8.78 (d, J = 6.7 Hz, 2H), 8.08 (d, J = 6.7 Hz, 2H), 7.78 (d, J = 16.3 Hz, 1H), 7.41 (d, J = 8.3 Hz, 2H), 7.25 (d, J = 16.2 Hz, 1H), 7.10 – 6.96 (m, 5H), 6.94 – 6.88 (m, 2H), 6.86 – 6.77 (m, 4H), 6.62 – 6.51 (m, 4H), 4.56 (t, J = 7.7 Hz, 2H), 3.62 (d, J = 0.8 Hz, 6H), 3.52 – 3.44 (m, 2H), 3.12 (s, 9H), 2.61 – 2.43 (m, 2H). ¹³C NMR (101 MHz, Methanol-d₄) δ 143.92, 132.34, 132.26, 131.86, 131.09, 127.59, 127.56, 123.93, 112.88, 112.71, 62.44, 54.20, 52.61, 48.24, 48.03, 47.81, 47.60, 47.39, 47.18, 46.96, 24.72. HR-MS (ESI, positive) calcd for C₄₁H₄₄N₂O₂ (M – 2Br)/2 298.1696, found 298.1697.

3、Synthesis of TPE-Py⁺⁺

Synthesis of TPE-2Br: The synthesis method of TPE-2Br (4, 4'-(2,2-bis(4-bromophenyl)ethene-1,1-diyl)bis(methoxybenzene)) has been described in the previous literature.² The yield of TPE-2Br is 77%. ¹H NMR (400 MHz CDCl₃, δ): 7.80–7.70 (4H, d, J = 8.4 Hz), 7.68–7.53 (4H, m), 7.53–7.44 (4H, m), 6.97–6.95 (4H, d, J = 8.7 Hz), 3.88 (6H, s). MALDI-TOF calcd for C₂₈H₂₂Br₂O₂ [M + 2H]⁺ 550.29, found 550.70.

Synthesis of TPE-2CHO: The synthesis method of TPE-2CHO (4,

4'-(2,2-bis(4-methoxyphenyl)ethene-1,1-diyl)dibenzaldehyde) has been described in the previous literature.² The yield of TPE-2CHO is 36%. ¹H NMR (400 MHz CDCl₃, δ): 9.90 (2H, s), 7.65–7.62 (4H, m), 7.17–7.15 (4H, m), 6.94–6.91 (4H, m), 6.70–6.68 (2H, d, *J* = 2.2 Hz), 6.68–6.65 (2H, d, *J* = 2.6 Hz), 3.74 (6H, s). ¹³C NMR (100 MHz CDCl₃, δ): 191.76, 158.96, 150.33, 148.52, 134.92, 134.30, 132.70, 132.65, 132.00, 129.41, 129.34, 113.35, 113.32, 113.30, 55.08. MALDI-TOF calcd for C₃₀H₂₄O₄ (M + 2H)⁺ 448.17, found 448.16.

Synthesis of TPE-Py⁺⁺: The reactants 1, 4-Dimethylpyridinium iodide (206.8 mg, 0.88 mmol) and THE-2CHO (100.00 mg, 0.24 mmol) were first dissolved in anhydrous ethanol (50 mL) and a few drops of triethylamine were added. The reaction mixture was refluxed for 48 hours under the protection of N₂. After the reaction mixture was cooled to room temperature, the mixture was extracted three times with water and CH₂Cl₂. Then Na₂SO₄ was added to dry. The crude product was purified by a silica gel column using CH₂Cl₂/MeOH mixture (15 : 1 v/v) as the eluent to produce a yellow powder (40.76 mg, yield: 21%). ¹H NMR (400 MHz, DMSO-d₆) δ 8.86 (d, *J* = 6.3 Hz, 4H), 8.21 (d, *J* = 6.7 Hz, 2H), 8.02 – 7.29 (m, 8H), 7.10 – 6.79 (m, 10H), 6.74 (d, *J* = 8.8 Hz, 4H), 4.29 (d, *J* = 19.1 Hz, 6H), 3.75 – 3.67 (m, 6H). ¹³C NMR (101 MHz, DMSO-d₆) δ 158.14, 152.40, 145.80, 145.02, 144.44, 140.11, 135.21, 133.20, 132.18, 132.08, 131.59, 127.90, 127.84, 127.78, 123.39, 113.36, 55.19, 55.02, 55.01, 47.27, 46.93. HR-MS (ESI, positive) calcd for C₄₄H₄₄N₂O₂ (M – 2I)/2 314.1539, found 314.1540.

4、Synthesis of TPE-N⁺

Synthesis of TPE-OC₃Br: The reactants 1, 3-dibromopropane (146.90 mg, 0.73 mmol), TPE-OH (4-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)phenol) (50 mg, 0.12 mmol) and K₂CO₃ (169.30 mg, 1.22 mmol) were dissolved in dry DMF (30 mL). Then the mixture was refluxed for 24 hours under the protection of N₂. After the reaction mixture was cooled to room temperature, the mixture was extracted three times with water and CH₂Cl₂. Organic phase drying with anhydrous Na₂SO₄, remove solvent by evaporated under reduced pressure. The crude product was purified by a silica gel column using PE / CH₂Cl₂ mixture (3: 1 v/v) as the eluent to produce a White powder (56.93 mg, yield 88%). ¹H NMR (400 MHz, Chloroform-*d*) δ 7.09 (d, *J* = 7.2 Hz, 3H), 7.02 (d, *J* = 7.4 Hz, 2H), 6.93 (dd, *J* = 12.8, 8.4 Hz, 6H), 6.63 (qd, *J* = 7.3, 2.5 Hz, 6H), 4.03 (t, *J* = 5.8 Hz, 2H), 3.74 (d, *J* = 8.8 Hz, 6H), 3.58 (t, *J* = 6.5 Hz, 2H), 2.28 (p, *J* = 6.2 Hz, 2H). ¹³C NMR (101 MHz, Chloroform-*d*) δ 157.94, 156.92, 144.47, 139.30, 136.96, 136.63, 136.56, 132.56, 131.42, 127.63, 126.03, 113.64, 113.06, 112.95, 65.13, 55.11, 55.08, 32.50, 30.11. MALDI-TOF calcd for C₃₁H₂₉BrO₃ (M + 2H)⁺ 528.13, found 529.90.

Synthesis of TPE-N⁺: The reactants THE-CO₃Br (100 mg, 0.19 mmol) and Me₃N (33.55 mg, 0.57 mmol)

were dissolved in dry THF (30 mL). Then the mixture was stirred at room temperature for 2 days under the protection of N₂. After the reaction was completed, the mixture was filtered and washed with an excess of THF solution to give a white solid (79.88 mg, yield 83%). ¹H NMR (400 MHz, Methanol-d₄) δ 7.12 – 7.06 (m, 3H), 7.02 – 6.97 (m, 2H), 6.95 – 6.88 (m, 6H), 6.73 – 6.62 (m, 6H), 4.06 (t, J = 5.7 Hz, 2H), 3.73 (d, J = 7.8 Hz, 6H), 3.63 – 3.56 (m, 2H), 3.20 (s, 9H), 2.28 (dq, J = 11.5, 5.6 Hz, 2H). ¹³C NMR (101 MHz, Methanol-d₄) δ 158.23, 136.47, 132.26, 132.19, 131.01, 127.30, 125.78, 113.37, 112.70, 112.64, 64.11, 54.18, 54.16, 52.31, 52.27, 52.23, 48.24, 48.03, 47.82, 47.61, 47.39, 47.18, 46.97, 22.94. HR-MS (ESI, positive) calcd for C₃₄H₃₈NO₃ (M – Br) 508.2852, found 508.2849.

5. Bacterial Culture

First, a single colony of *Klebsiella pneumoniae* (*K. pneumoniae*) on the solid nutrient broth medium was transferred to 10 ml of liquid LB medium and cultured at 37 °C for 12 hours. The bacterial concentration was determined by measuring the optical density (OD₆₀₀) at 600 nm using UV Spectrophotometer. Then, the bacteria were diluted to OD₆₀₀=0.5, the bacteria were transferred to 1.5 mL EP tube. The bacteria were centrifuged at 10,000 rpm for 5 min. After centrifugation, the supernatant was removed and washed with phosphate buffer solution (PBS) for three times. The precipitate *K. pneumoniae* was suspended again in PBS for later usage. *Staphylococcus aureus* (*S.aureus*), *Escherichia coli* (*E.coli*) and *Candida albicans* (*C.albicans*) were cultured under the same conditions and operations as *K. pneumoniae*.

6. Confocal laser scanning microscopy (CLSM) characterization:

1) Bacterial imaging

For fluorescence imaging of bacteria, we first need to prepare a probe solution, which is prepared by dissolving four probes with dimethyl sulfoxide to prepare an initial concentration of 10⁻² M, and diluting them to a concentration of 20 μM using a phosphate buffer solution. Then, 200 μL of the treated bacterial solution and an equal amount of the probe solution were added to a sterilized EP tube and incubated at room temperature for 30 minutes. After that, 10 μL of the stained bacteria was transferred to clean glass slide and covered with coverslips for fixation. Images were collected using a 405 nm laser and a 100-fold mirror under confocal laser scanning microscopy.

2) Mixed bacteria imaging

Equal amounts of *E. coli* and *K. pneumoniae* were mixed in PBS. Then, 200 μL of the bacterial mixture and

an equal amount of probe TPE-N⁺ solution were added to the sterilized EP tube and incubated at room temperature for 1 h. Then, 200 uL probe TPE-Py⁺⁺ solution was added and incubated for 1h at room temperature. The bacteria were harvested by centrifugation (10000 rpm, 3 min) and they were again suspended in PBS. Then 10 μL of the stained bacterial solution was transferred to a clean glass slide and covered with coverslips for fixation. Images were collected by confocal laser scanning microscopy and excited using 405 nm laser.

7、 The infection of Raw 264.7 cells by *K.pneumoniae*:

First, 200 μl of *K. pneumoniae* that had been centrifuged was taken, and an equal amount of probe TPE-Py⁺⁺ solution (20 μM) was added. Incubate for 30 minutes at room temperature. Bacteria were harvested by centrifugation (1000 rpm, 3 min). The supernatant was removed, resuspended in 100 μL of PBS, and then mixed by adding 900 μL of DMEM. Finally, 1 mL of the prepared bacterial solution was added into the Raw 264.7 cells and incubated at 37 °C for 30 min. Images of bacterial infection at different times were collected by confocal microscopy using 405 nm laser and 40-fold mirror. The probe TPE-Pn⁺⁺ labeled *K. pneumoniae* infected the Raw 264.7 cells with the same conditions as the probe TPE-Py⁺⁺.

8、 References

1. Y. Chen, W. Ai, X. Guo, Y. Li, Y. Ma, L. Chen, H. Zhang, T. Wang, X. Zhang and Z. Wang, *Small*, 2019, **15**.
2. Y. Ma, H. Wang, S. Su, Y. Chen, Y. Li, X. Wang and Z. Wang, *Analyst*, 2019, **144**, 3381-3388.

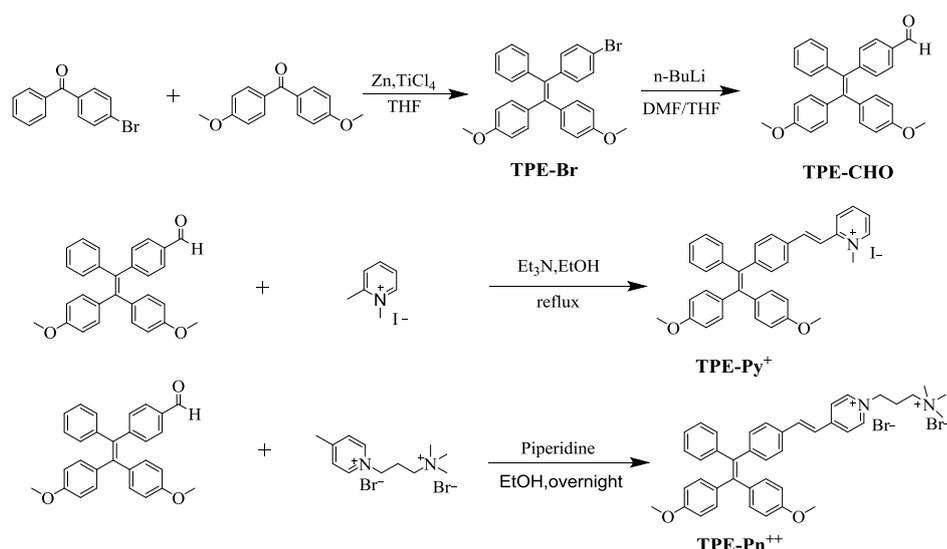
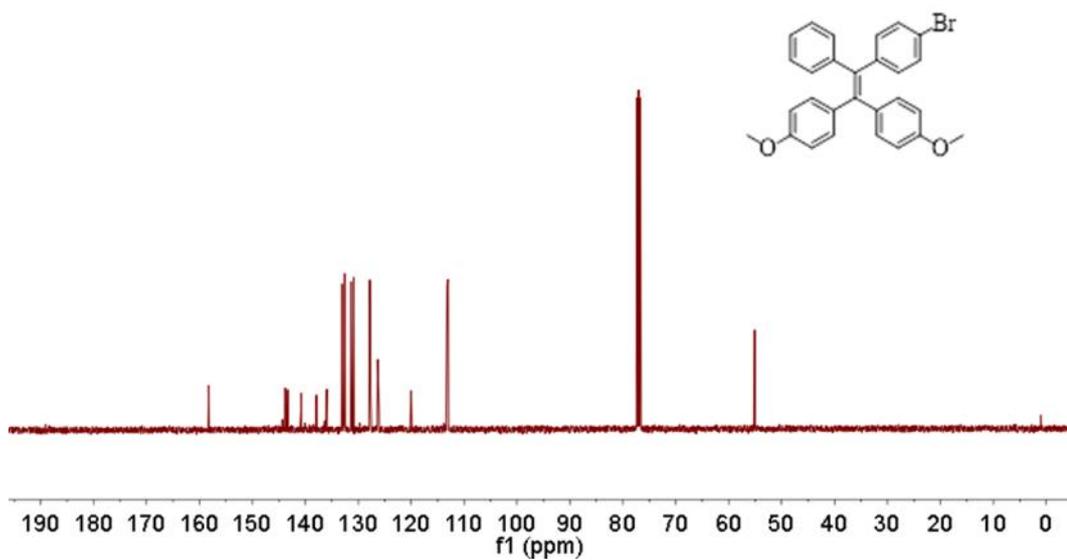
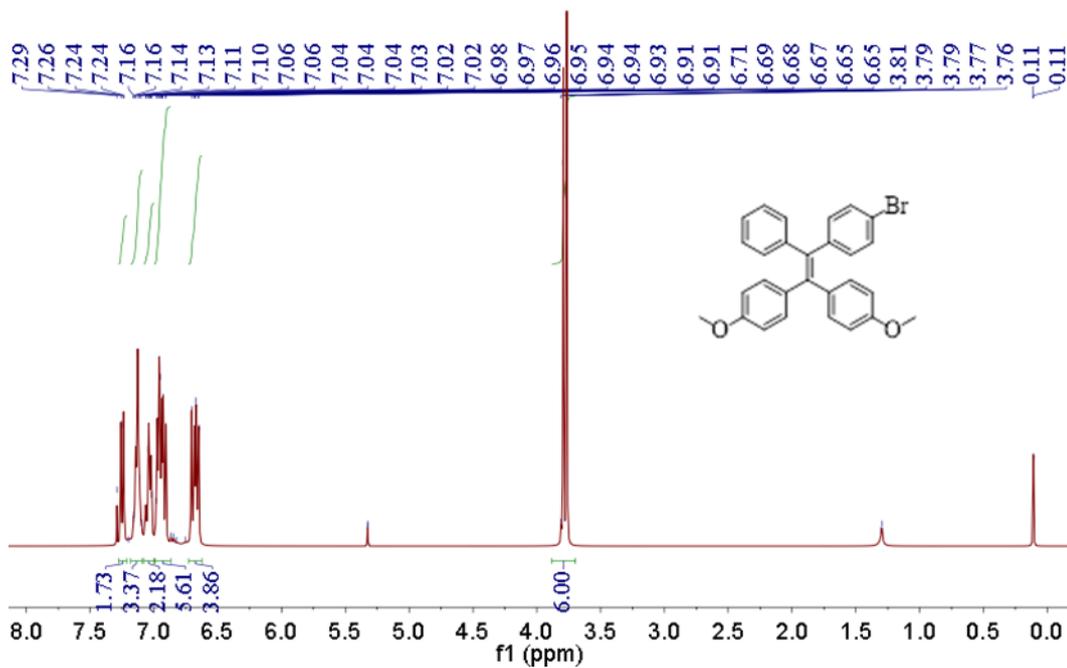


Fig S1. Synthesis routine of AIEgens TPE-Py⁺ and TPE-Pn⁺⁺.



MALDI-TOF,DHB,1,20180824

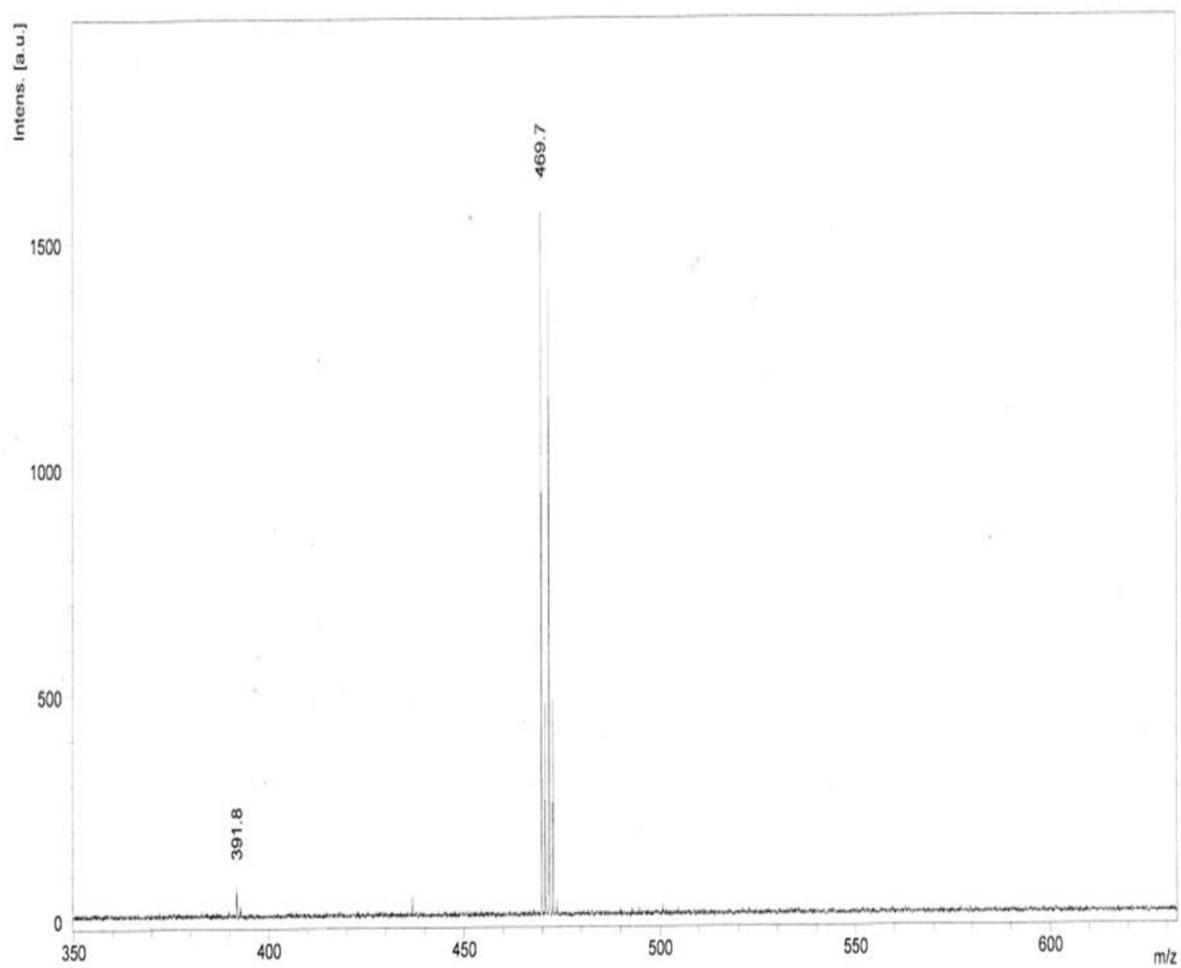
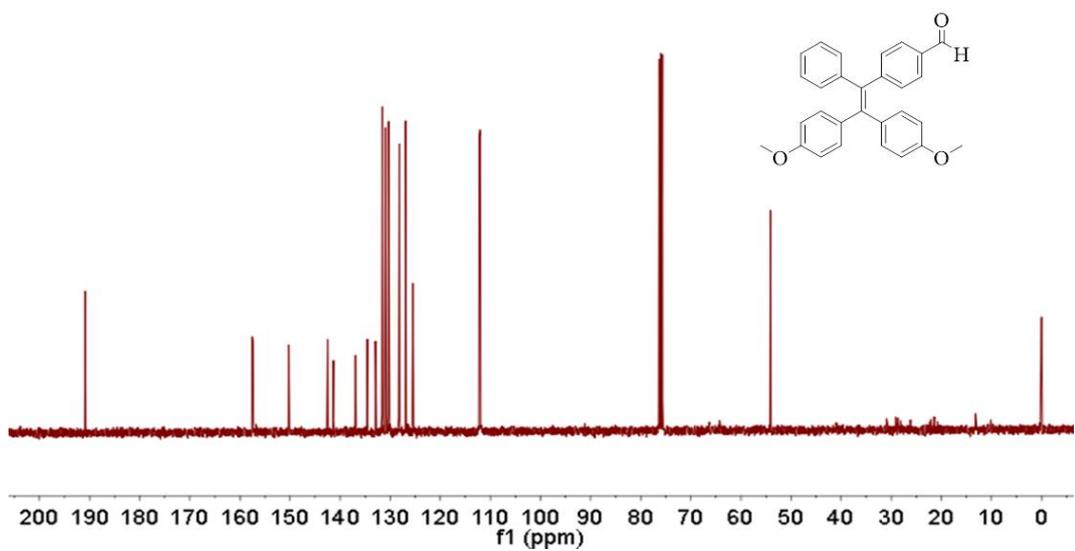
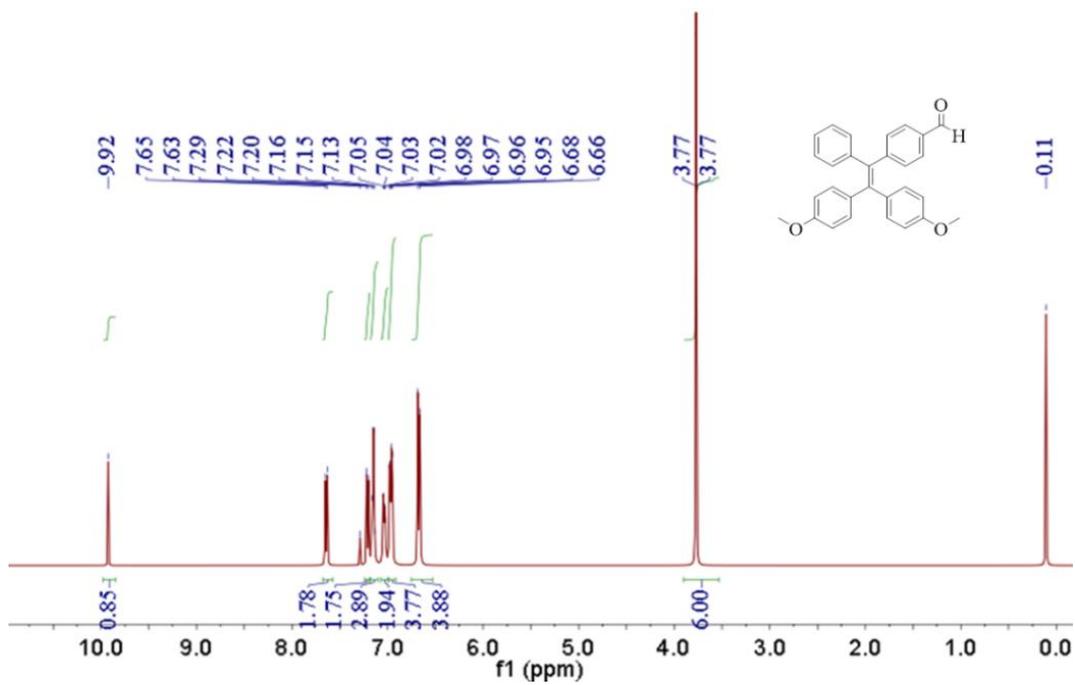


Fig S2. ^1H NMR, ^{13}C NMR and MALDI-TOF of TPE-Br.



MALDI-TOF,DHB,2,20180824

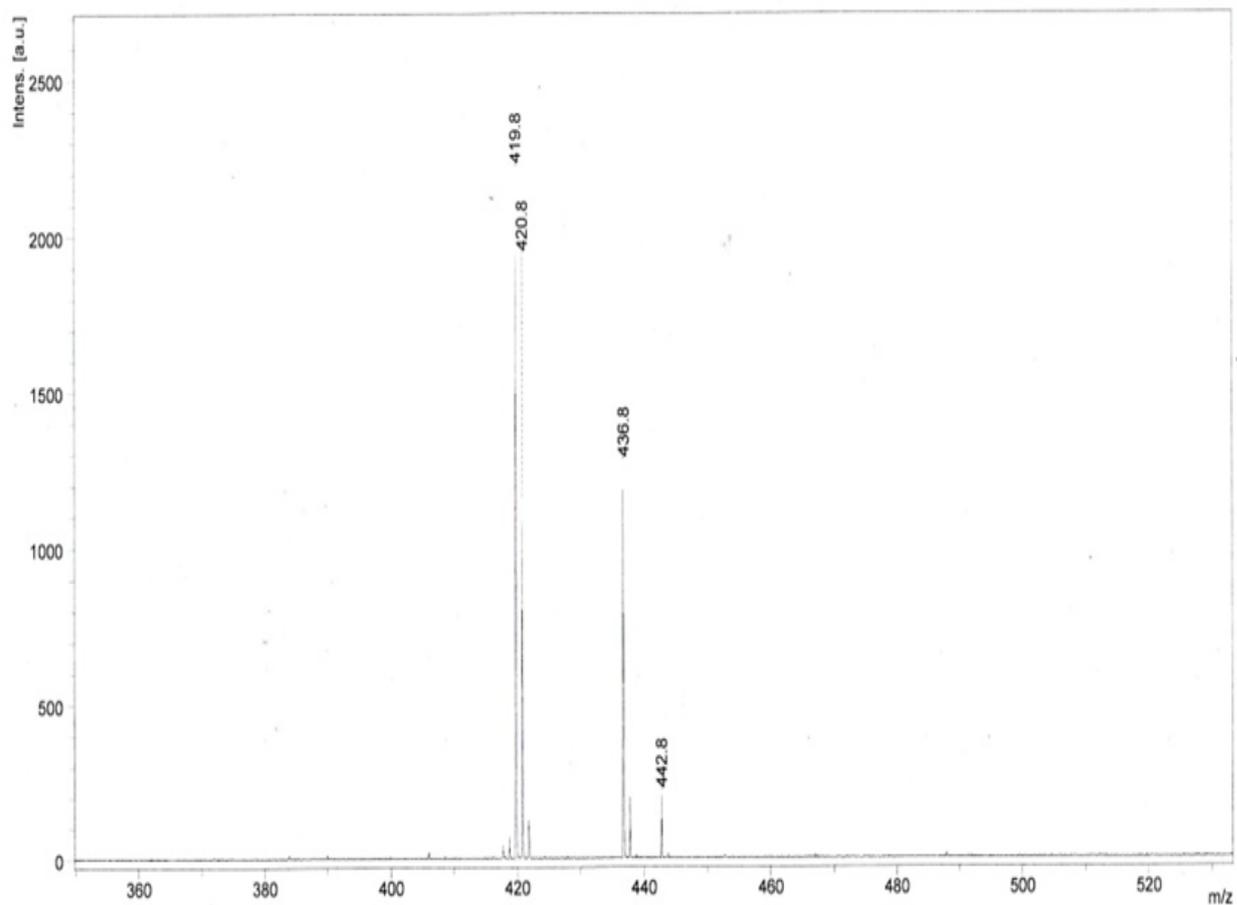
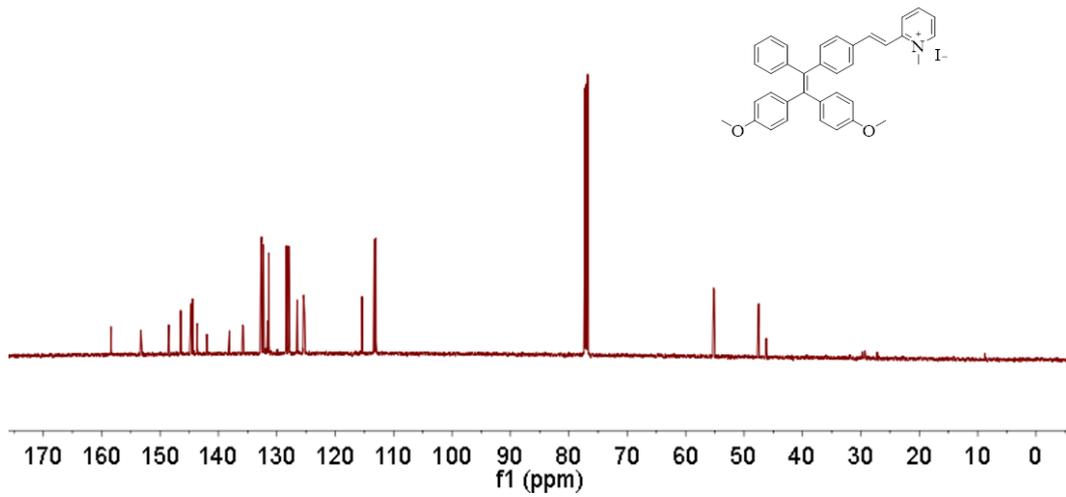
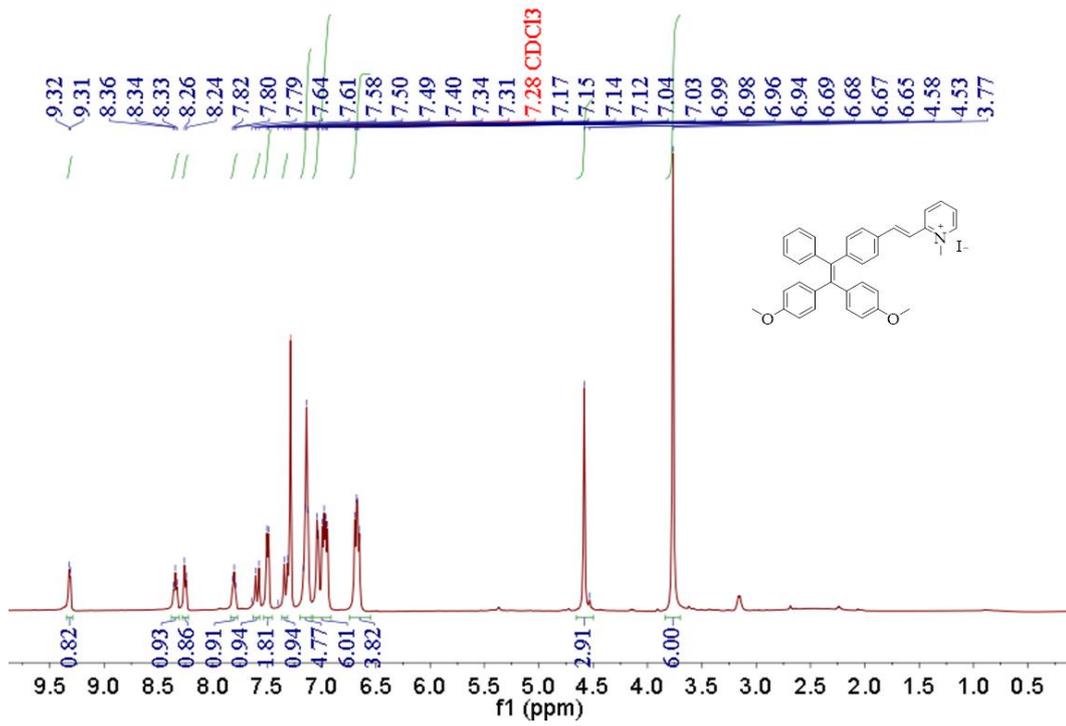


Fig S3. ^1H NMR, ^{13}C NMR and MALDI-TOF of TPE-CHO.



ESI(P),W-1,20190307

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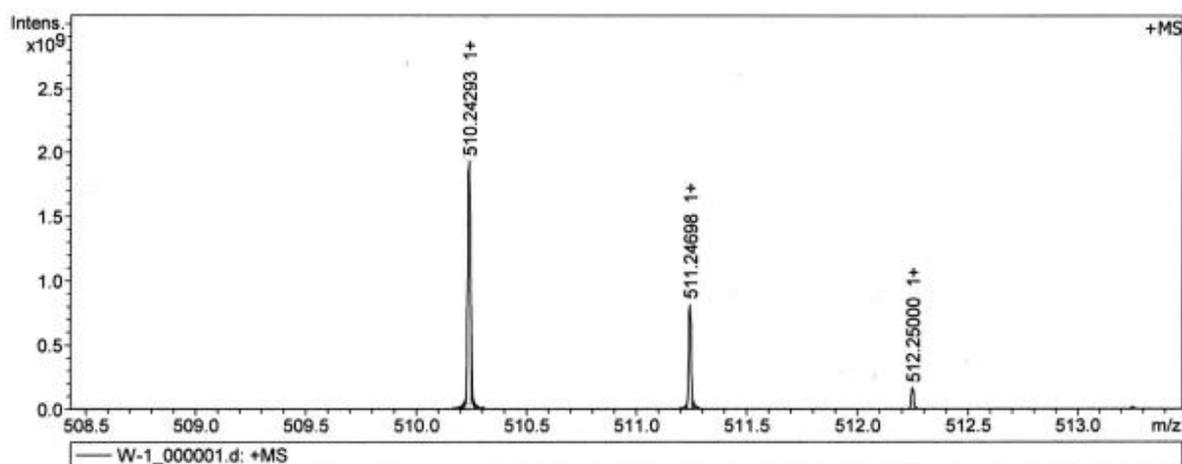
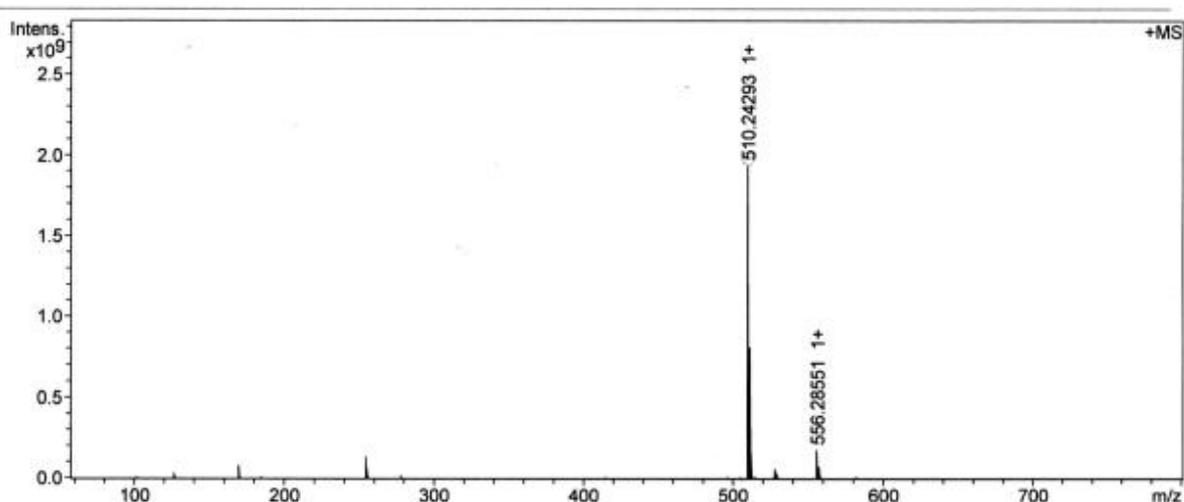
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Instrument solariX

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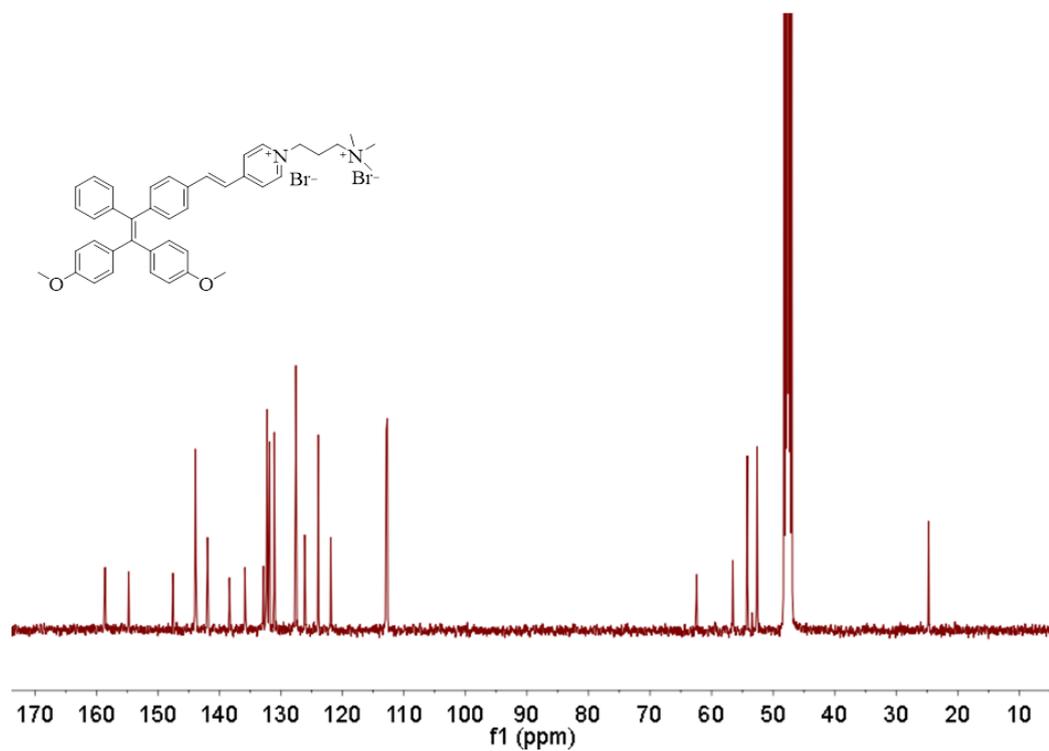
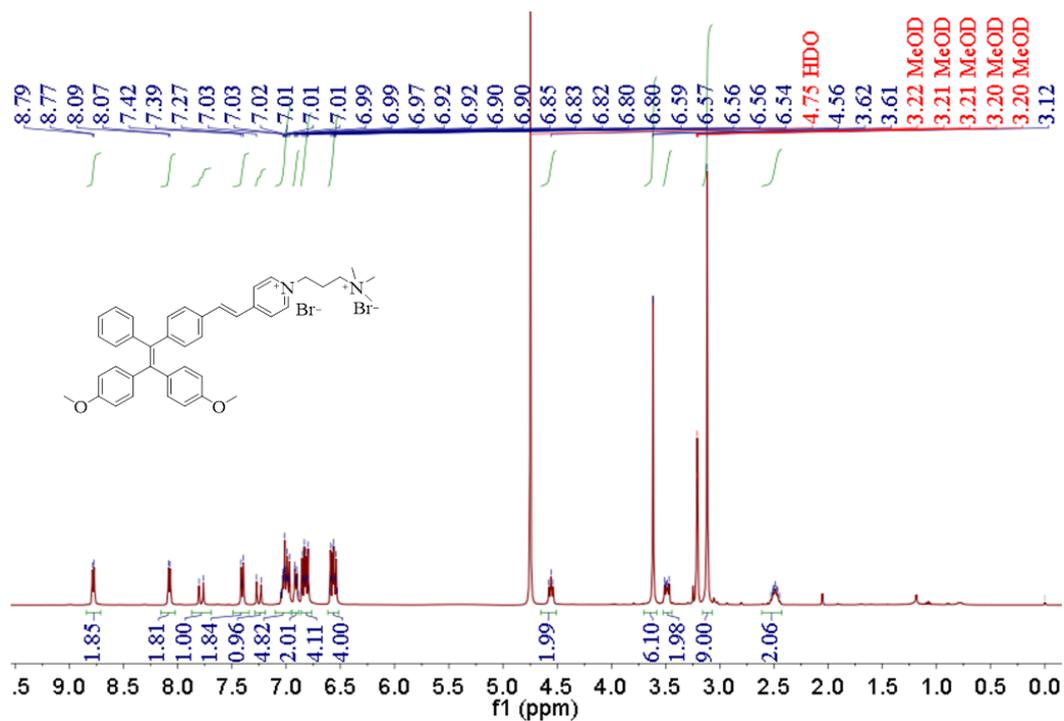
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Calibration Date Wed Mar 6 10:54:40
2019
Acquired Scans 1



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Fig S4. ¹H NMR, ¹³C NMR and high resolution mass spectrometry of TPE-Py⁺.



ESI(P),2#,20181102

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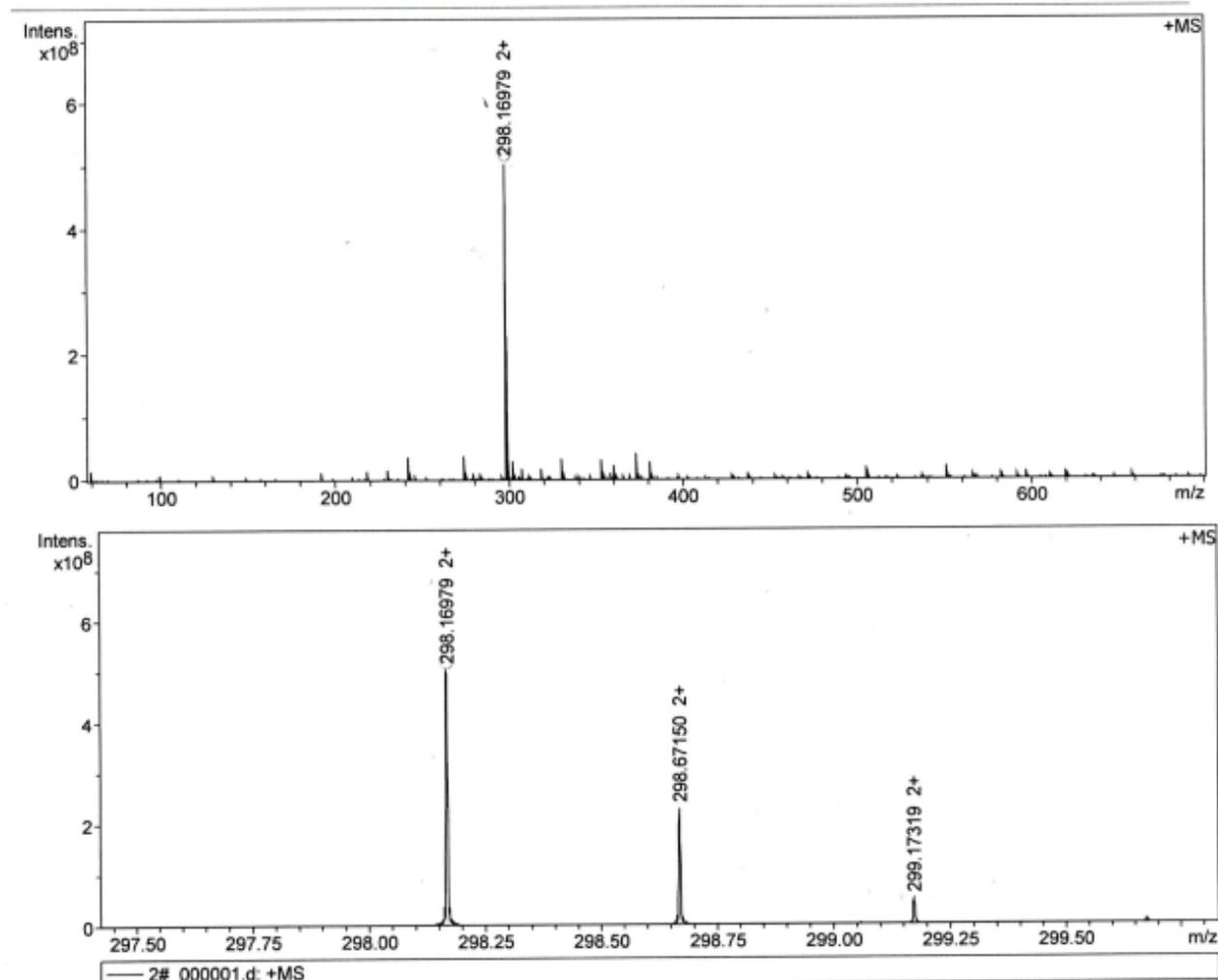
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Operator
Instrument solariX

Acquisition Parameter

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Calibration Date Tue Oct 30 08:46:32 2018
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| Meas. m/z | # | Ion Formula | Score | m/z | err [ppm] | Mean err [ppm] | mSigma | rdb | e ⁻ Conf | N-Rule |
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Fig S5. ¹H NMR, ¹³C NMR and high resolution mass spectrometry of TPE-Pn⁺⁺.

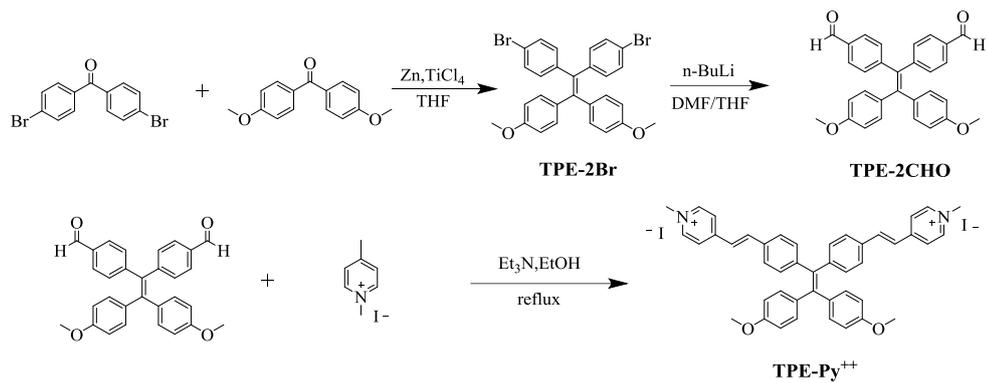
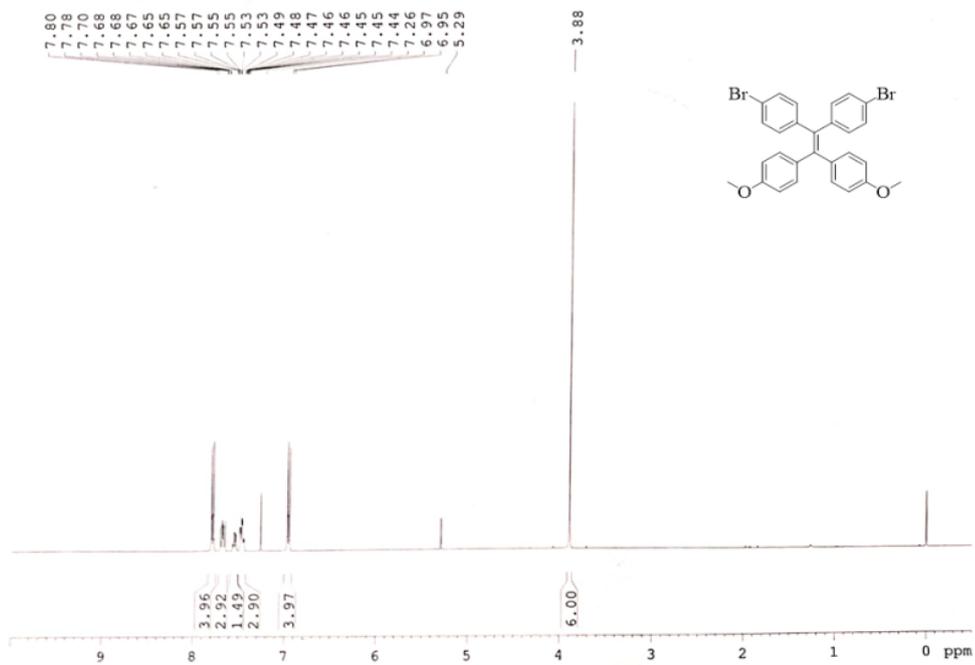
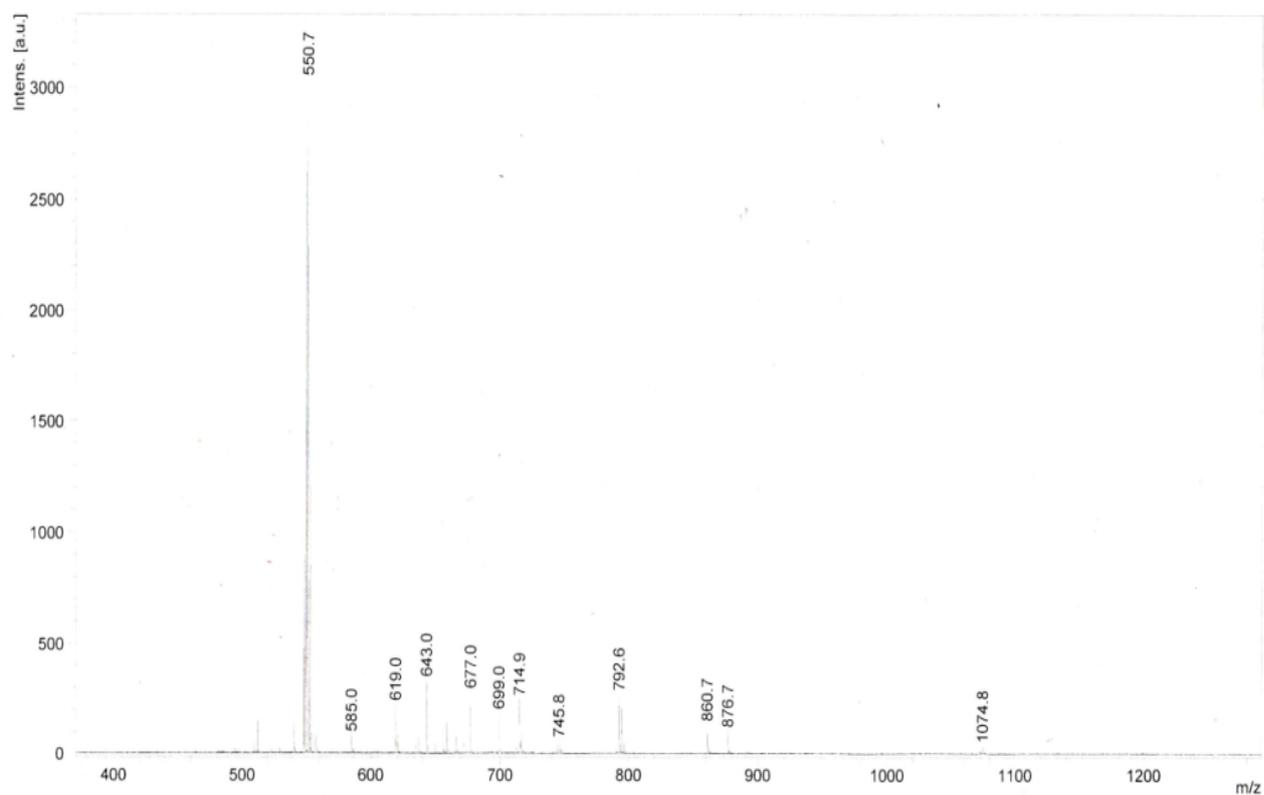
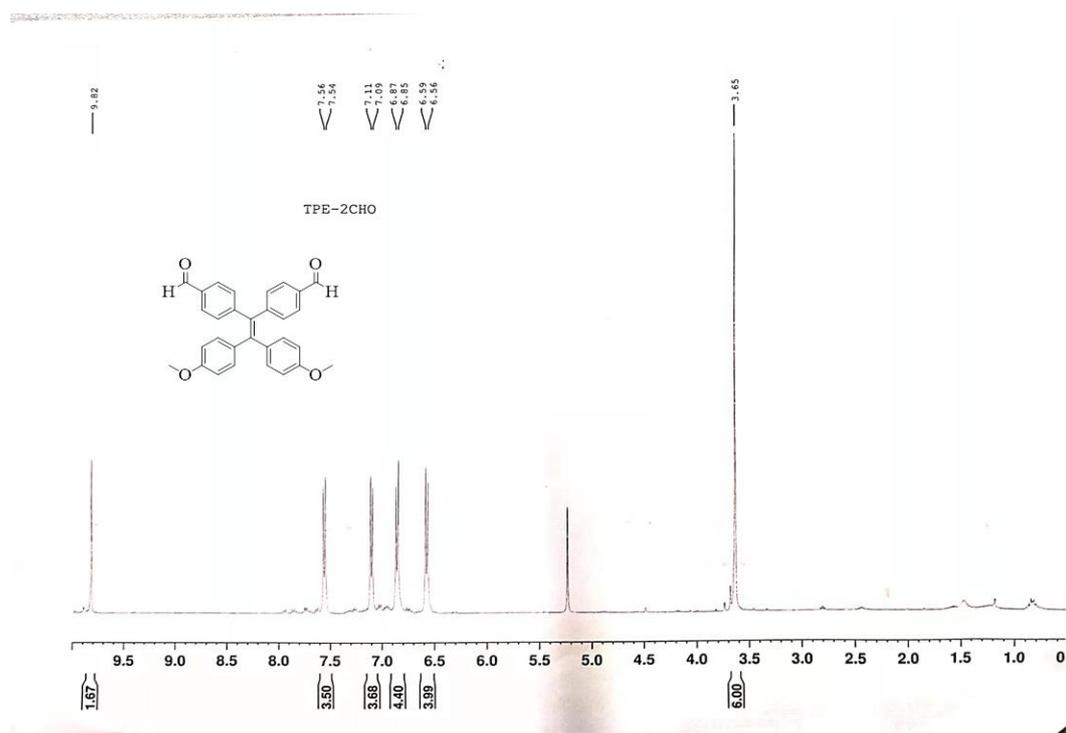
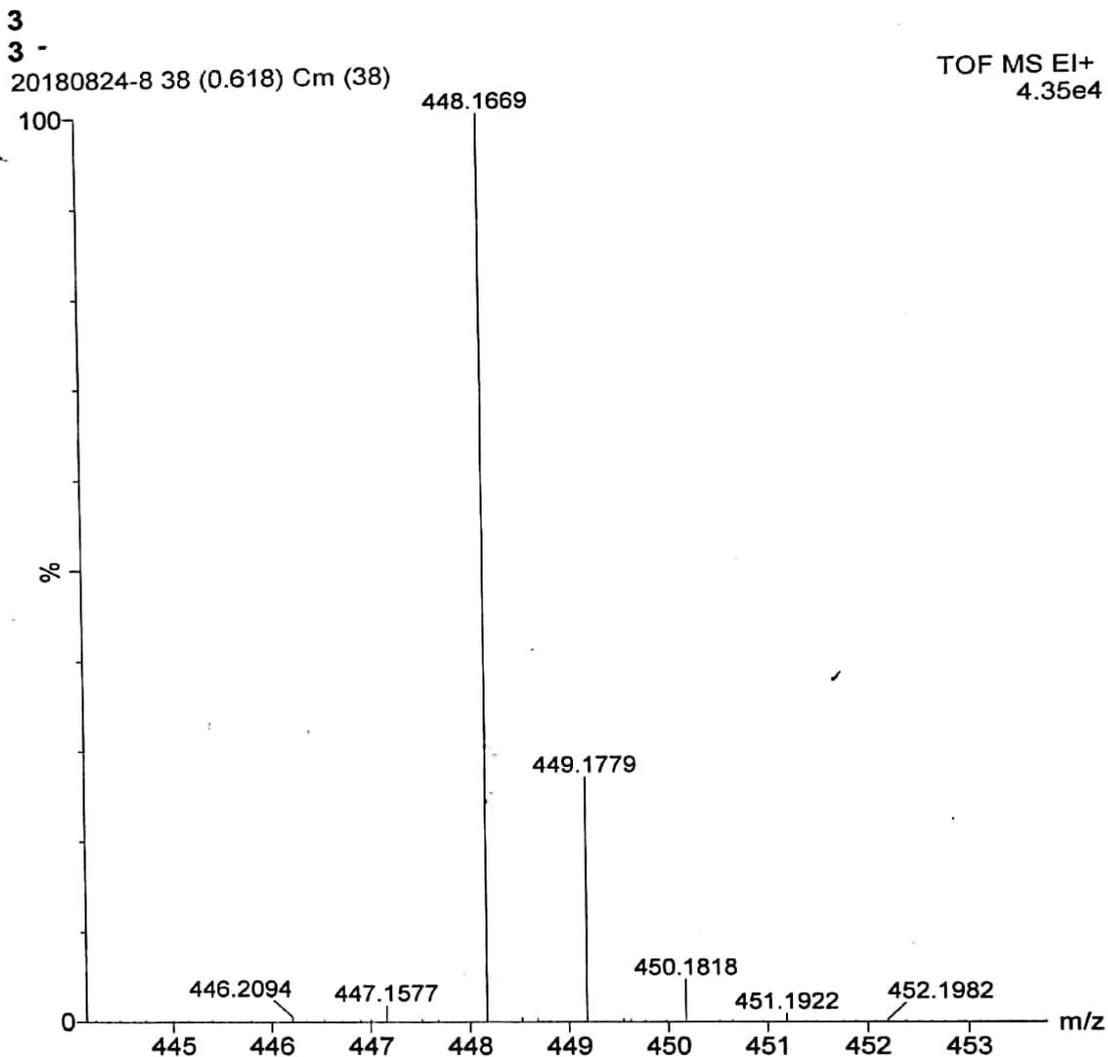


Fig S6. Synthesis routine of AIEgens TPE-Py⁺⁺.



MALDI-TOF, CCA, TPE-2Br, 20161205

Fig S7. ^1H NMR, and MALDI-TOF of TPE-2Br.



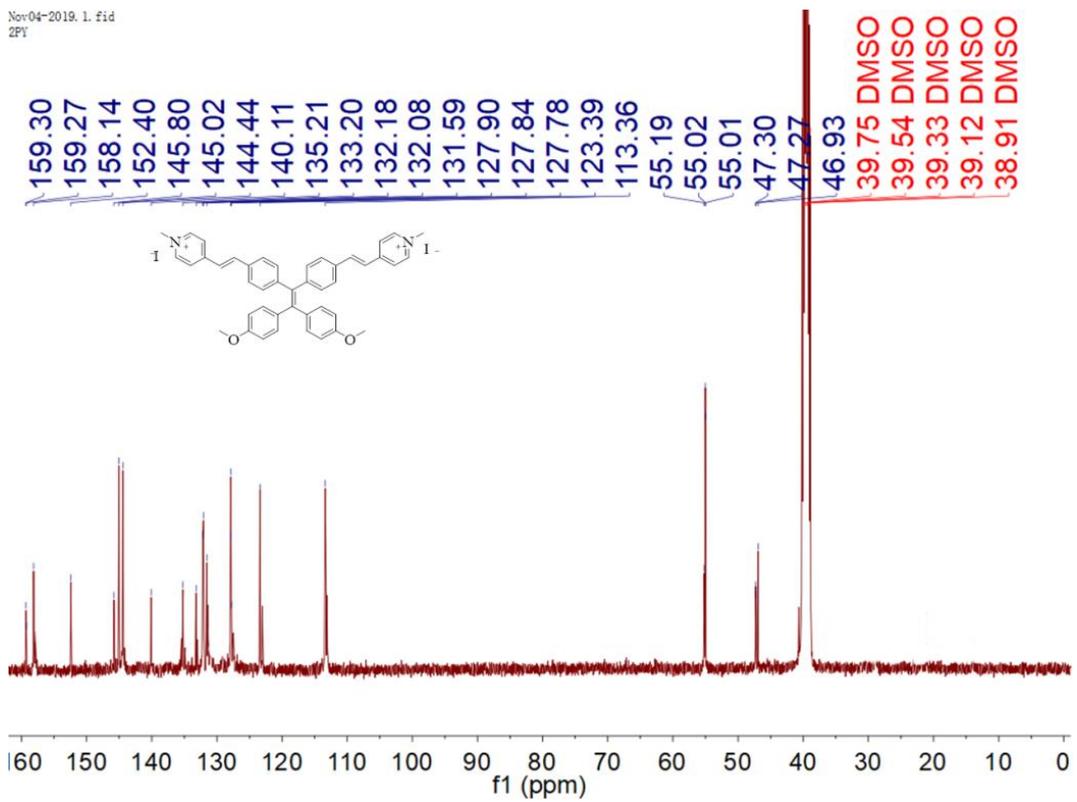
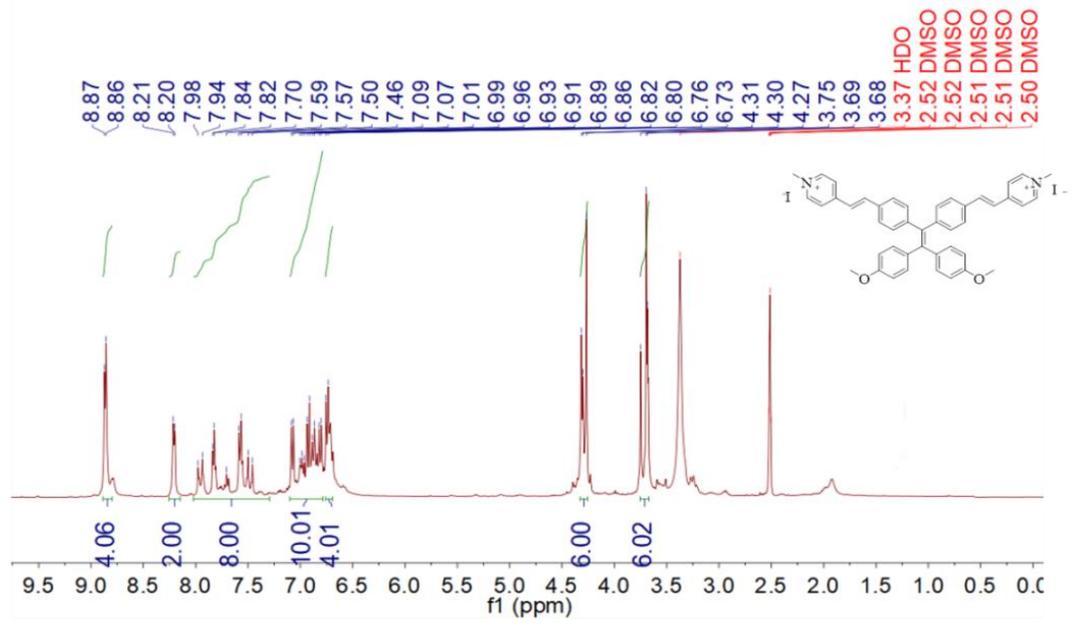
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Monoisotopic Mass, Odd and Even Electron Ions
 2 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

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|----------|--------|------------|-------|-------|------|-------|------------|--|
| Maximum: | 100.00 | | 200.0 | 100.0 | 50.0 | | | |
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| 448.1669 | 100.00 | 448.1675 | -0.6 | -1.2 | 19.0 | 1 | C30 H24 O4 | |

Fig S8. ¹H NMR, and MALDI-TOF of TPE-2CHO.



ESI(P),1,20190222

Analysis Info

Analysis Name D:\Data\ESI\2019\2019-02\0222\1_000002.d

Sample Name 1

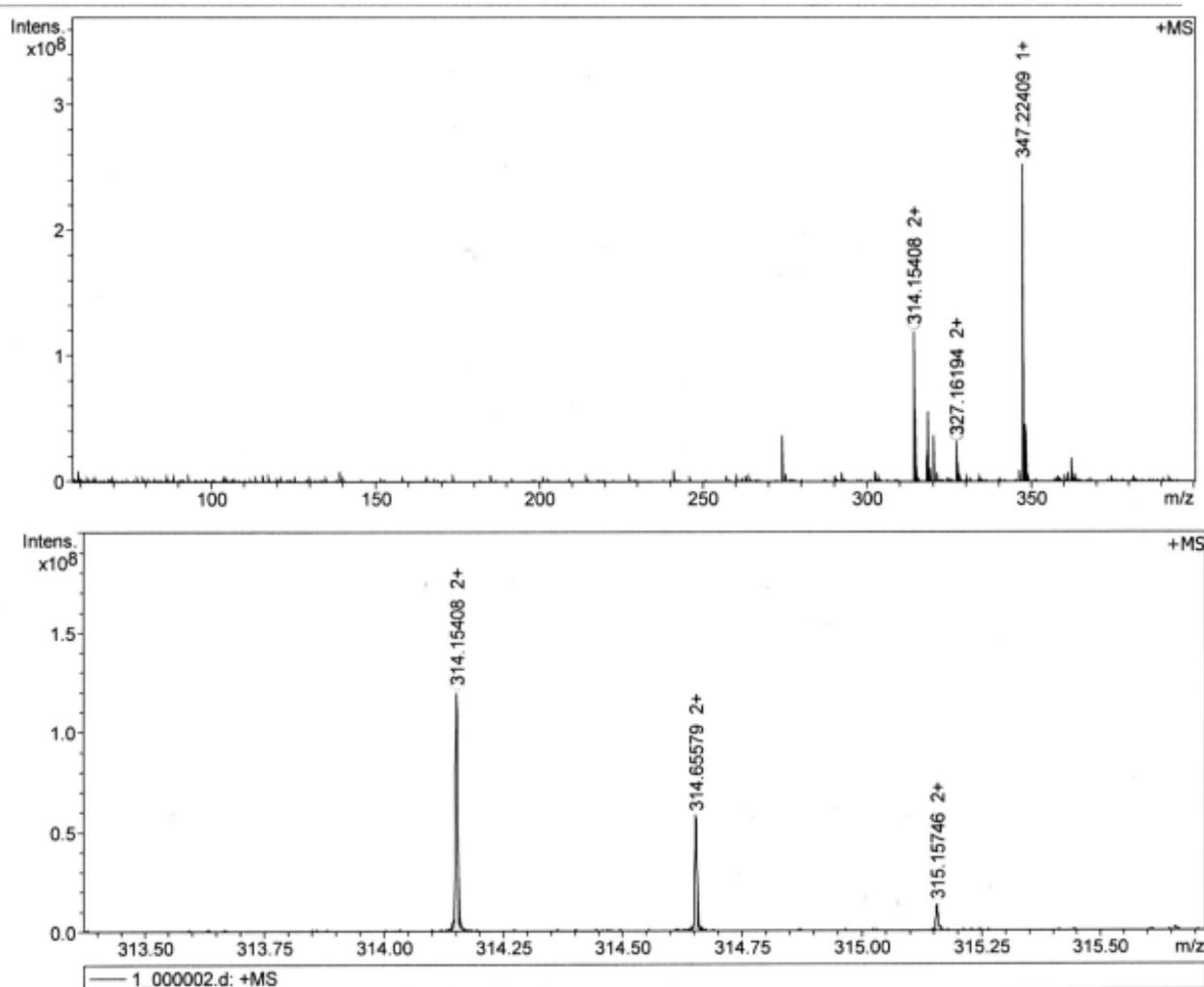
Acquisition Date 2/22/2019 11:17:27 AM

Operator
Instrument solariX

Acquisition Parameter

Polarity Positive
Broadband Low Mass 57.7 m/z
Broadband High Mass 400.0 m/z

Calibration Date Tue Feb 19 10:22:20 2019
Acquired Scans 20



| Meas. m/z | # | Ion Formula | Score | m/z | err [ppm] | Mean err [ppm] | mSigma | rdb | e ⁻ Conf | N-Rule |
|------------|---|---|--------|------------|-----------|----------------|--------|------|---------------------|--------|
| 314.154082 | 1 | C ₄₄ H ₄₀ N ₂ O ₂ | 100.00 | 314.153941 | 0.4 | -0.5 | 9.2 | 26.0 | even | ok |
| 327.161942 | 1 | C ₄₆ H ₄₂ N ₂ O ₂ | 100.00 | 327.161766 | -0.5 | -0.6 | 23.6 | 27.0 | even | ok |

Fig S9. ¹H NMR, ¹³C NMR and high resolution mass spectrometry of TPE-Py⁺⁺.

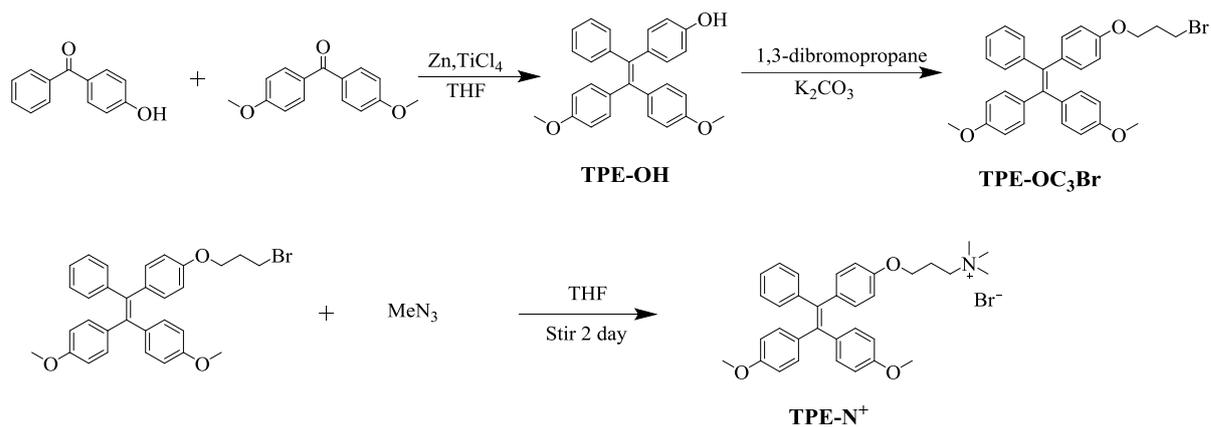
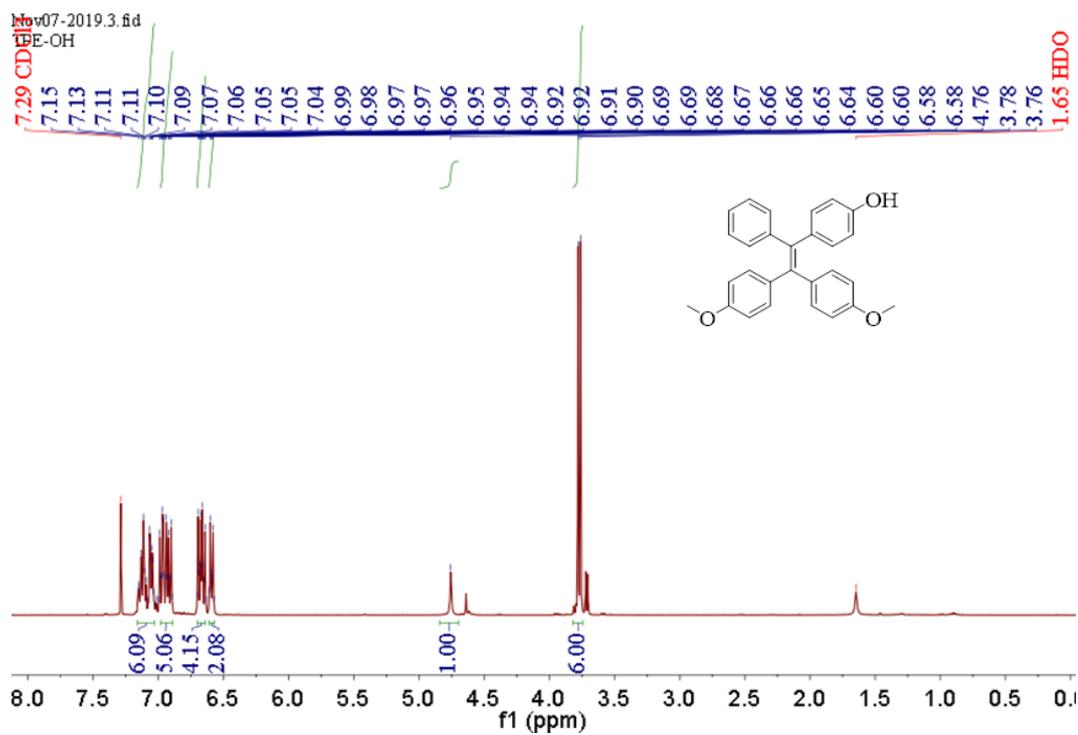


Fig S10. Synthesis routine of AIEgens TPE-N⁺.



MALDI-TOF,DHB,3,20191107

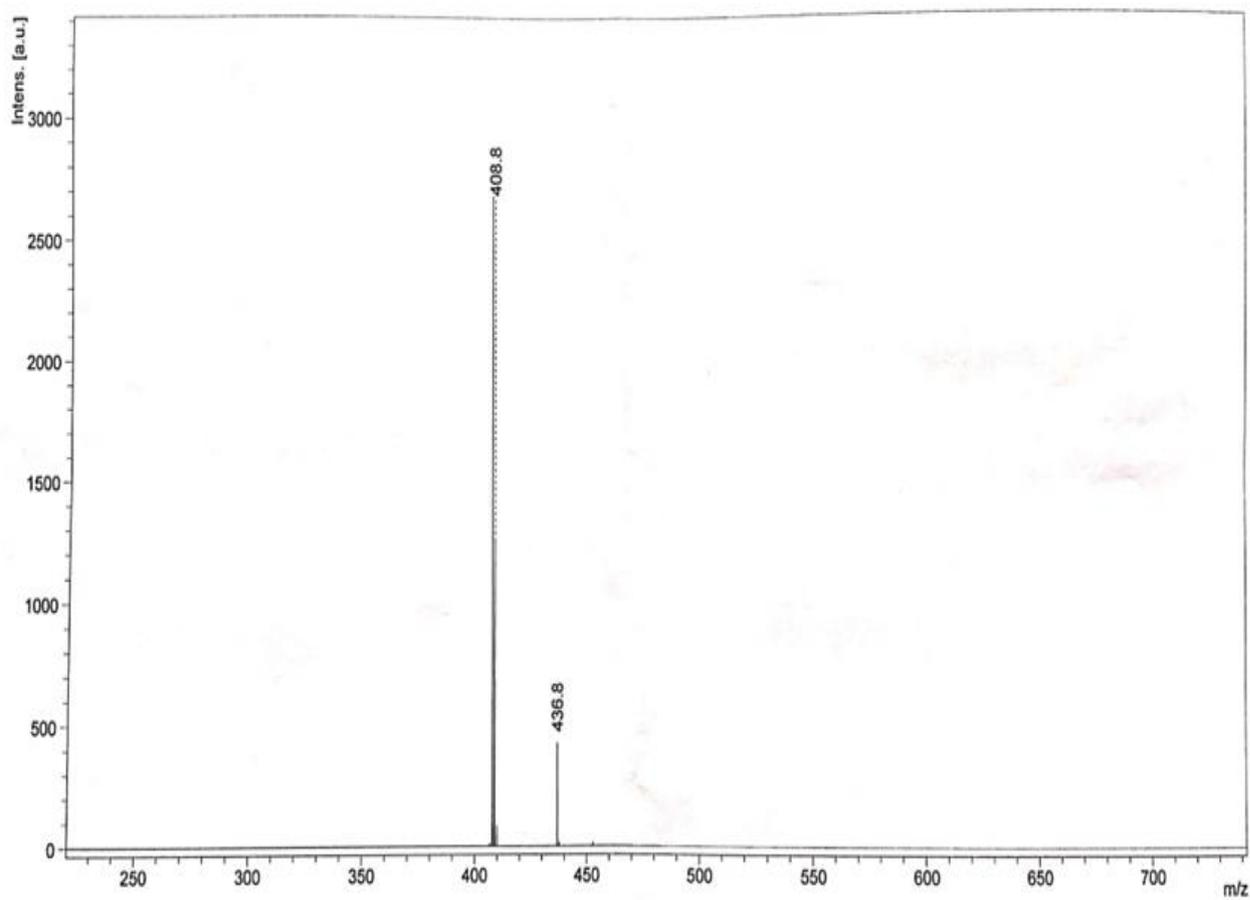
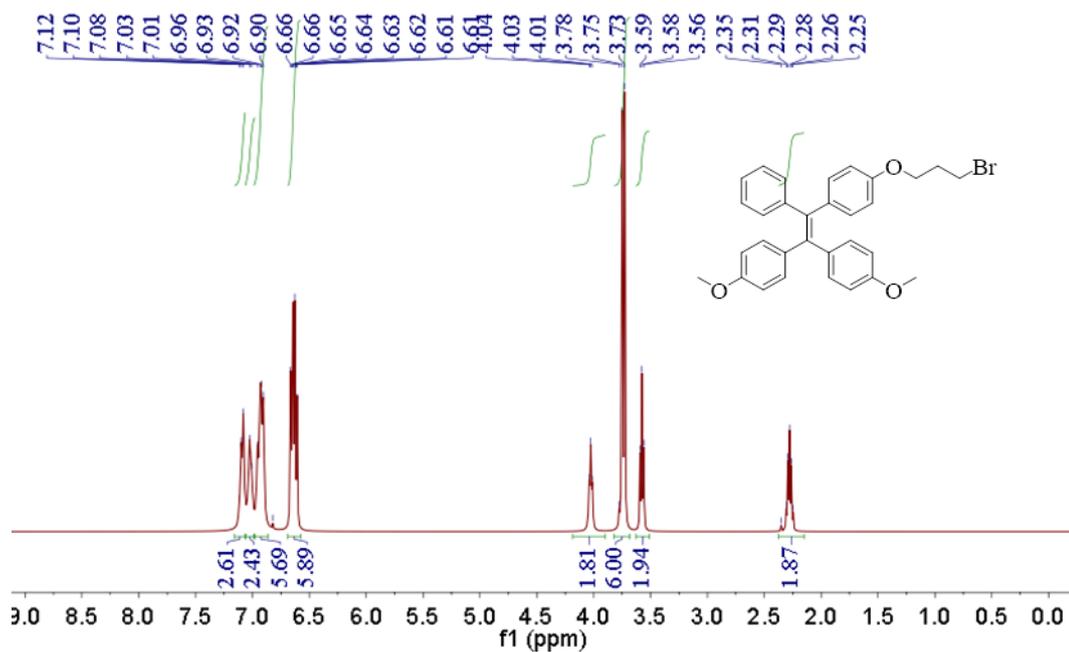


Fig S11. ¹H NMR, and MALDI-TOF of TPE-OH.



D:\DATA\2019\201911\20191107\20191107-34 210_J9\1

MALDI-TOF, DHB, 2, 20191107

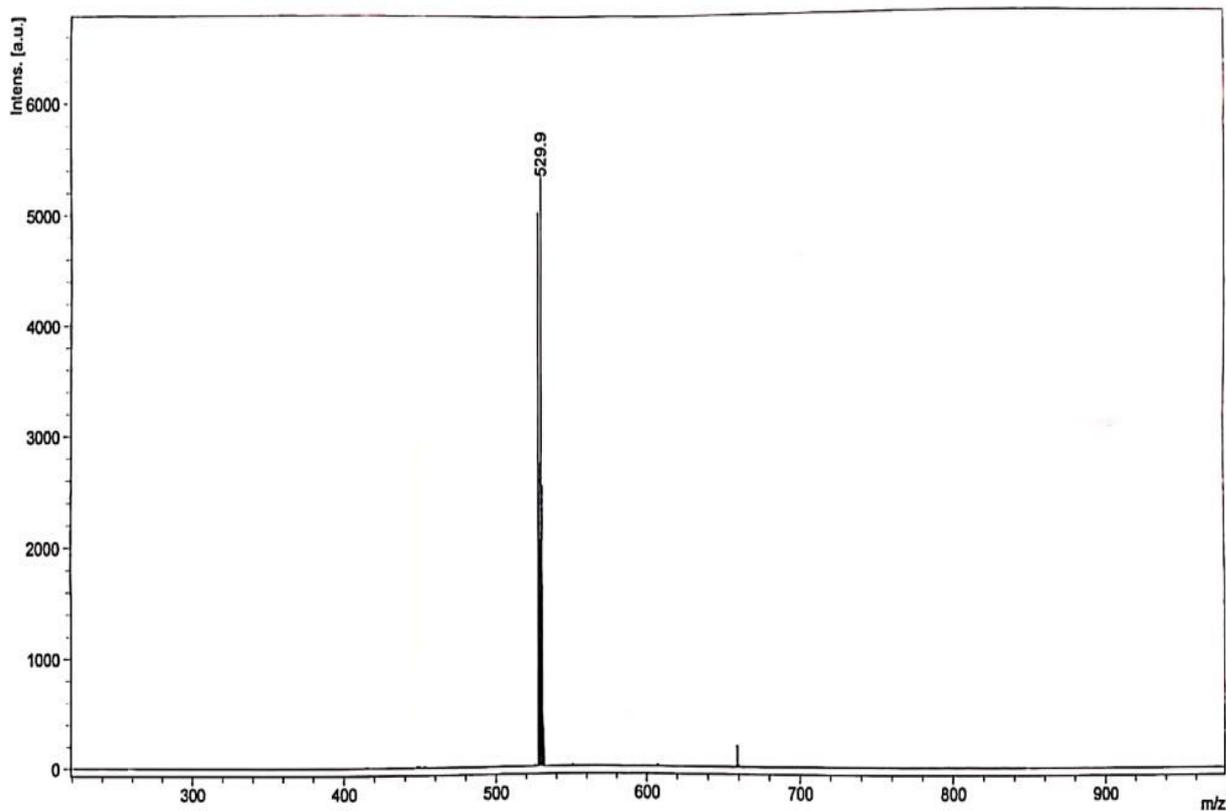
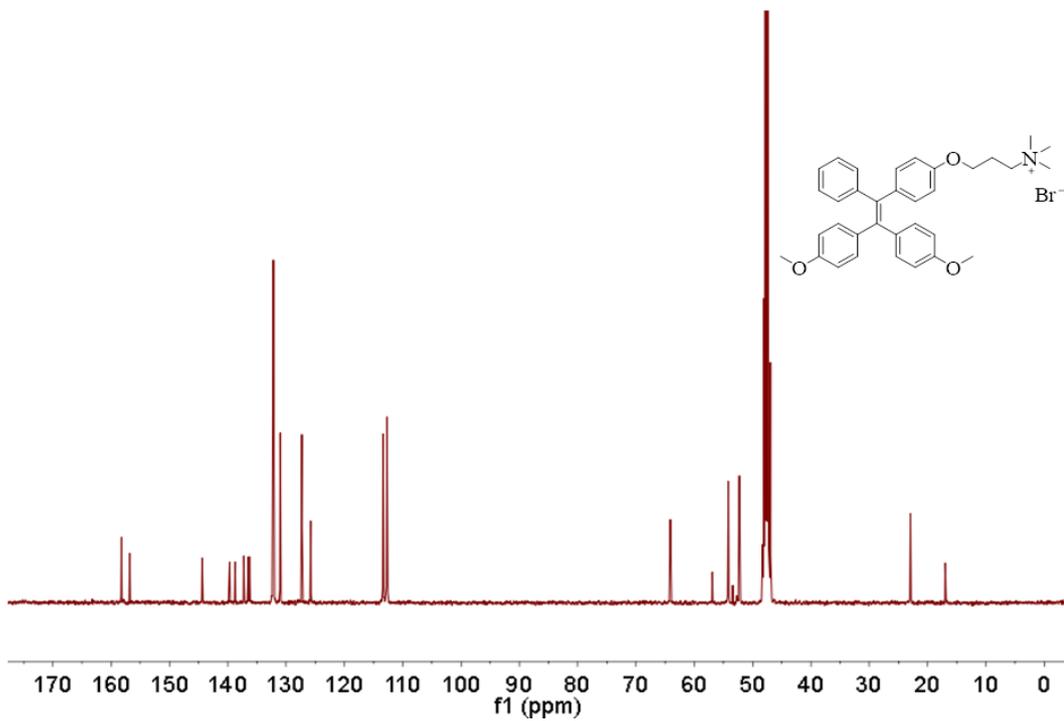
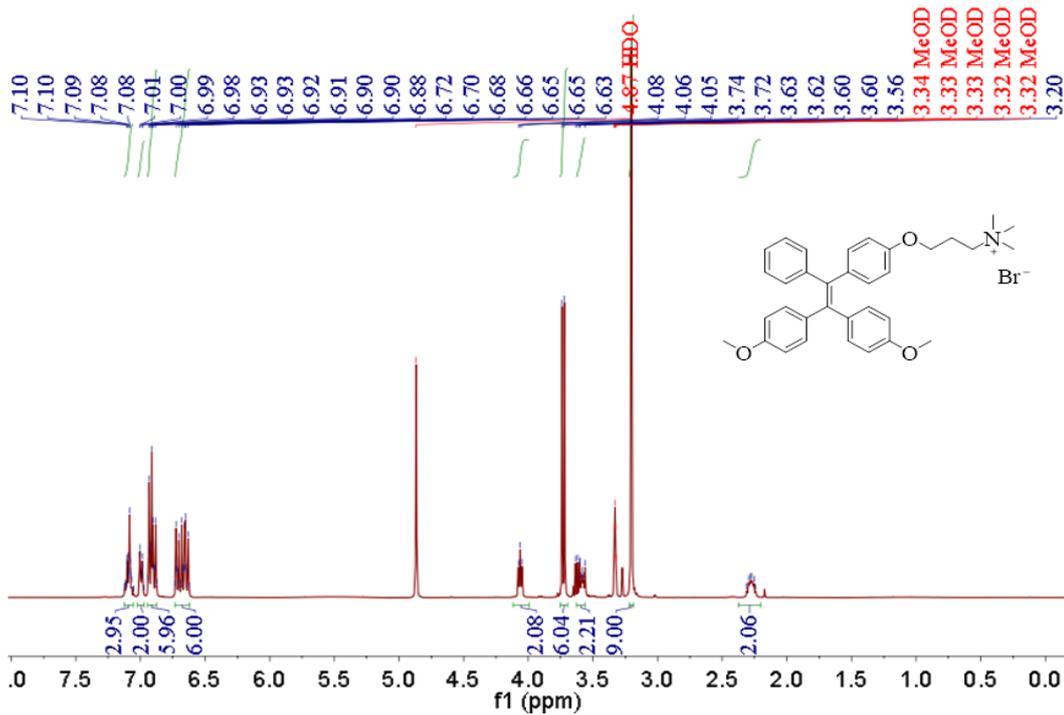


Fig S12. ¹H NMR, and MALDI-TOF of TPE-OC₃Br.



ESI(P),1,20181116

Analysis Info

Analysis Name D:\Data\ESI\2018\2018-11\1116\1_000001.d

Acquisition Date 11/16/2018 9:15:38 AM

Sample Name 1

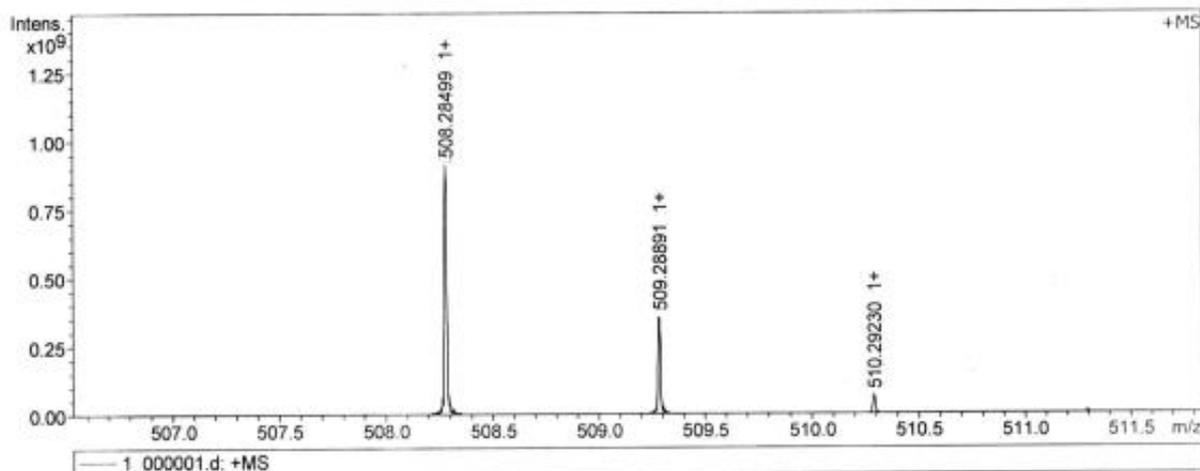
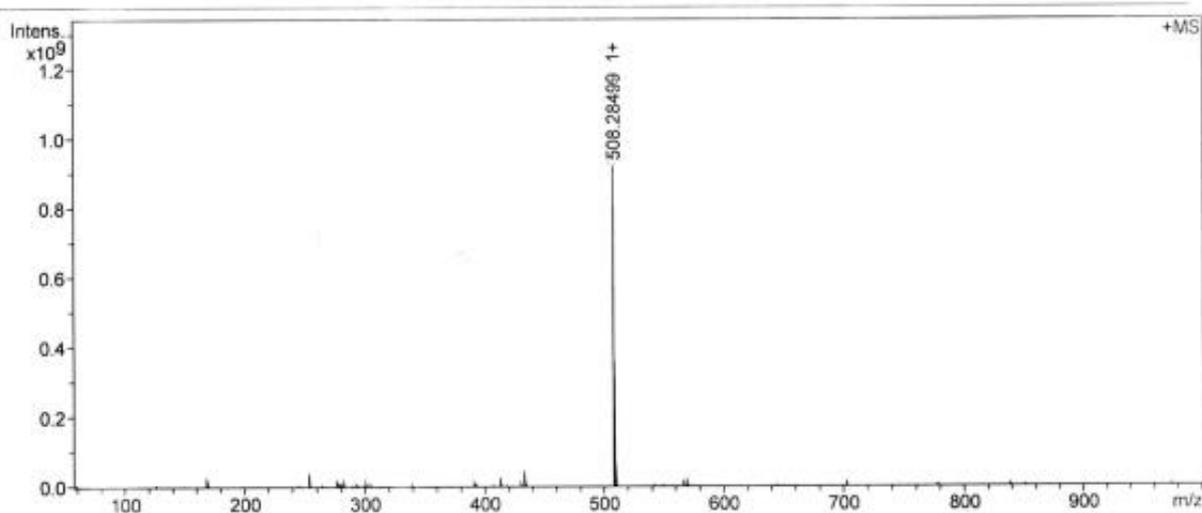
Instrument solariX

Acquisition Parameter

Acquisition Mode Single MS
Polarity Positive
Broadband Low Mass 57.7 m/z
Broadband High Mass 1000.0 m/z

Acquired Scans 1

Calibration Date Wed Nov 14 09:46:54 2018



| Meas. m/z | # | Ion Formula | Score | m/z | err [ppm] | Mean err [ppm] | mSigma | rdb | e ⁻ Conf | N-Rule |
|------------|---|---|--------|------------|-----------|----------------|--------|------|---------------------|--------|
| 508.284986 | 1 | C ₃₄ H ₃₈ NO ₃ | 100.00 | 508.284621 | 0.7 | -1.1 | 6.9 | 16.5 | even | ok |

Fig S13. ¹H NMR, ¹³C NMR and high resolution mass spectrometry of TPE-N⁺.

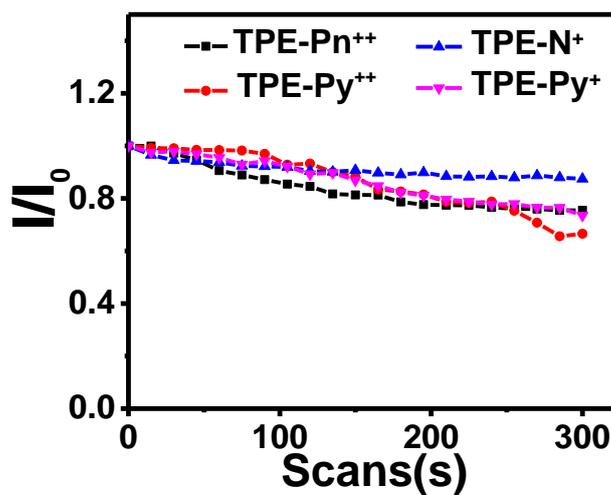


Fig S14. Photostability of PC-TPEgens (TPE-Py⁺⁺, TPE-Pn⁺⁺, TPE-Py⁺ and TPE-N⁺) under exposure to 405 nm laser with the laser intensity of 50%.

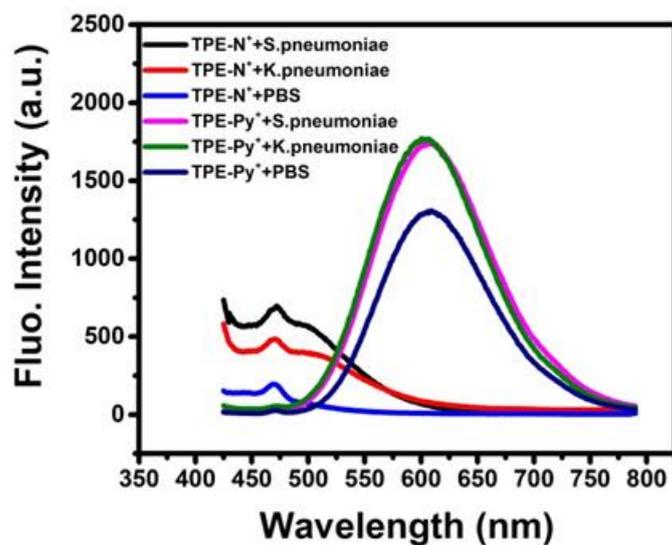


Fig S15. Fluorescent spectra of TPE-Py⁺ and TPE-N⁺ interacting with bacteria.

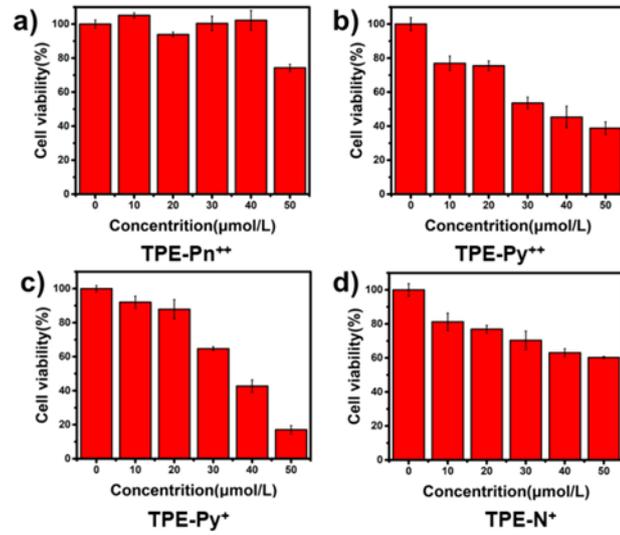


Fig S16. Cell viability of HeLa cells incubated with TPE-Py⁺⁺, TPE-Pn⁺⁺, TPE-Py⁺ and TPE-N⁺ for 24 h, respectively.

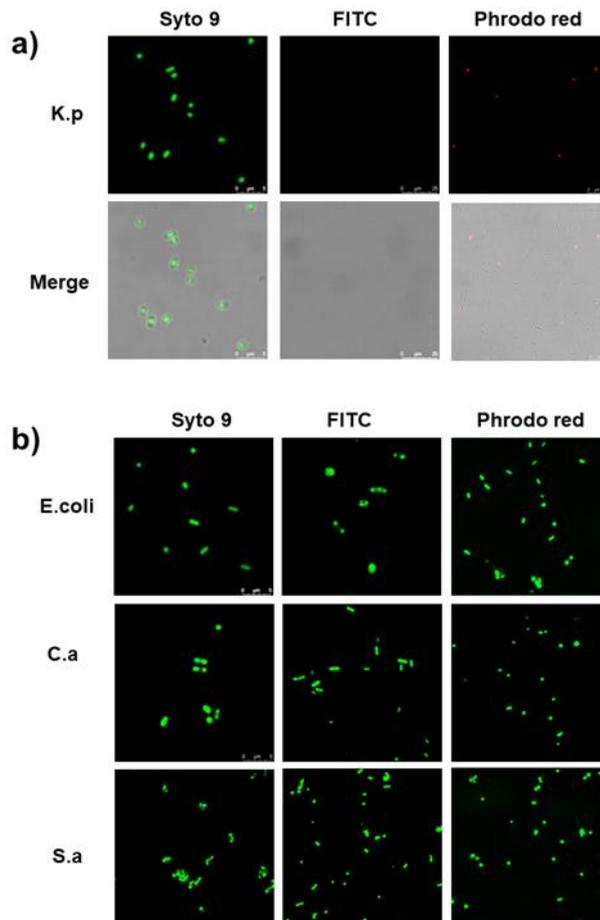


Fig S17: a) CLSM images of *K. pneumoniae* incubated with Syto 9, FITC, and Phrodo red, respectively. b) CLSM images of *E. coli*, *C. albicans*, and *S. aureus* incubated with Syto 9, FITC, and Phrodo red, respectively.