Electronic Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

A Water-soluble perylene dye functionalized with a 17β-estradiol: A new fluorescent tool for steroid hormones.

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CONTENTS

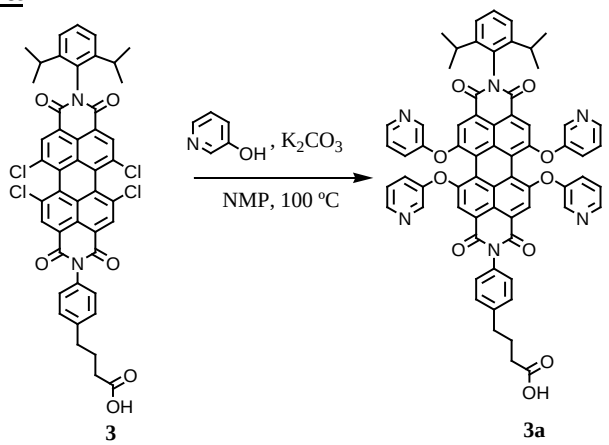
Materials and methods for the synthesis of 1 and 2.

All reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. Compound **3** was synthesized according to already published procedures.[1] Commercial TLC plates (silica gel 60 F254, SDS) were used to monitor the progress of the reaction, with spots observed under UV light at 254 and 365 nm. Column chromatography was performed with silica gel 60A (particle size 40-63 µm, SDS).

NMR spectra were taken using either a 500 MHz Bruker Avance DRX 500 or a Bruker AC-300. Ultraviolet–visible (UV-vis) absorption measurements were taken on a ThermoSpectronic Helios γ spectrophotometer. Fluorescence measurements were recorder with a Perkin Elmer LS Luminiscence spectrometer. Infrared measurements were taken with a Fourier Transform (FT-IR) ThermoNicolet model IR 200 Spectrometer in transmission mode using KBr pellets. Mass spectra were obtained either from a Bruker Reflex III matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) spectrometer using dithranol and trans-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) as matrix, or with electrospray ionization (ESI) from an API QStar Pulsar-Applied Biosystems. Elemental analyses were performed on a LECO CHNS-932 elemental analyzator.

Synthesis of PBI-1

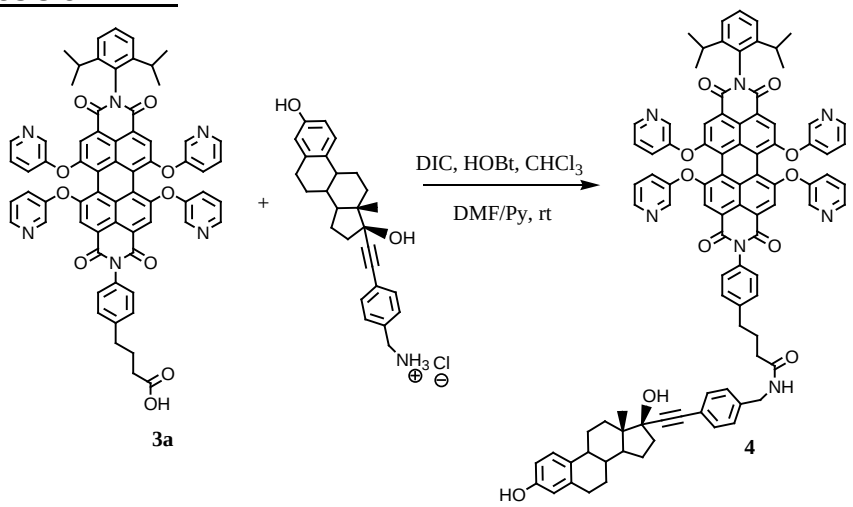
Synthesis of **PBI-3a**



PBI-3 (400 mg, 0.47 mmol), 3-hydroxypyridine (357 mg, 3.76 mmol) and anhydrous K_2CO_3 were added to anhydrous NMP (60 mL). The mixture was stirred at 100 ºC under argon for 24 h. After cooling, the reaction mixture was poured into 1M aqueous hydrochloride acid (250 mL) obtaining a red-purple solution (tetrapyridinium salt of PDI). The solution was cooled into an ice bath and aqueous $NaHCO_3$ (saturated) was added until pH 8. NaCl was added to saturation and the mixture was stirred overnight. The precipitated product was filtered under suction and then was dissolved in CH_2Cl_2/Acetone 9:1. This solution was washed twice with brine and was dried with anhydrous magnesium sulfate. The product was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 9:1), yielding 156 mg (30 %) of a red solid.[1]

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.35 (broad, 8H, H-pyridine), 8.25 (s, 2H, 2xH-PDI), 8.22 (s, 2H, 2xH-PDI), 7.45 (t, 1H, J=7.5 Hz, H-Ar), 7.33 (d, 2H, J=8.2 Hz, 2xH-Ar´), 7.34-7.26 (m, 10H, 8xH-pyridine and 2xAr-H), 7.15 (d, 2H, J=8.2 Hz, 2xH-Ar'), 2.75 (t, 2H, J=7.2 Hz, Ar-CH_2-) 2.68 (sept, 2H, J=6.9 Hz, 2xC*H*-$(CH_3)_2$), 2.39 (t, 2H, J=6.9 Hz, C*H*$_2$-CO_2H), 2.01 (m, 2H Ar-CH_2-C*H*$_2$-CH_2-CO_2H) 1.12 ppm (d,12H, J=6.9 Hz, 4xCH-(*CH*$_3$)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 176.24, 162.83, 162.63, 155.13, 155.04, 145.80, 145.74, 145.52, 142.34, 141.52, 141.47, 141.46, 141.43, 133.11, 133.08, 132.45, 130.10, 130.09, 129.72, 129.71, 129.53, 129.51, 128.22, 126.84, 126.82, 124.03, 123.71, 123.66, 121.20, 120.95, 120.84, 120.57, 120.56, 34.11, 29.14, 26.16, 24.00, 22.32 ppm. UV-vis (CH_2Cl_2), λmax/nm (log ε) = 234 (4.89), 269 (4.68), 437 (4.19), 522 (4.50), 560 (4.67). FT-IR (KBr) ν = 2963, 1708 (C=O imide), 1673 (C=O imide), 1594, 1510, 1475, 1423, 1407, 1339, 1312, 1280, 1209, 1188, 1021, 877, 804, 705 cm^{-1}. MALDI-TOF MS (DCTB): m/z = 1084 [M$^+$] Calcd for $C_{66}H_{48}N_6O_{10}.2H_2O$: C, 70.70; H, 4.67; N, 7.50. Found: C, 70.44, H, 4.58, N, 7.50.

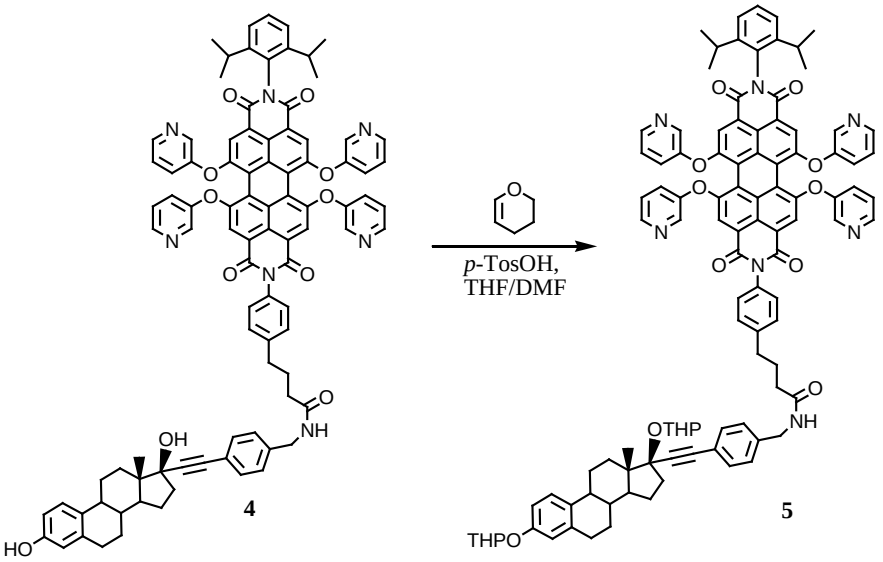
Synthesis of **PBI-4**



Perylene derivative **3a** (100 mg, 0.092 mmol), diisopropylcarbodiimide (DIC, 15 µL, 0.097 mmol) and 1-hydroxybenzotriazole (HOBt, 13.1 mg, 0.097) were were stirred in $CHCl_3$ (5 mL) for 20 min. 17α-[2'(4''-aminomethyl)phenyl]ethynylestra-1,3,5(10)-triene-3,17β-diol hydrochloride[2] (41.7 mg, 0.095 mmol) and pyridine (12.9 µL, 0.16 mmol) was dissolved in 5 mL of DMF and this solution was added to the reaction mixture .and stirred at rt under argon for 48 h. After that, $CHCl_3$ was evaporated and the residue was poured into water to remove DMF. The red precipitate was filtered off and repeatedly washed with water. The product was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 9:1), yielding 93.1 mg (70 %) of **4** as a red solid. M.p. 258 ºC.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.38 (broad, 4H, H-pyridine), 8.31 (broad, 4H, H-pyridine), 8.26 (s, 2H, 2xH-PDI), 8.21 (s, 2H, 2xH-PDI), 7.45 (t, 1H, J=7.5 Hz, H-Ar), 7.38 (d, 2H, J=8.2 Hz, 2xH-Ar´), 7.39-7.16 (m, 14H, 8xH-pyridine and 6xAr-H), 7.13 (d, 2H, J=8.2 Hz, 2xH-Ar'), 7.10 (broad, 1H, $1xAr_{estradiol}$), 6.54 (broad, 2H, $2xAr_{estradiol}$), 5.76 (t, 1H, J=5.6 Hz, -CONH-) 4.41 (d, 2H, J=5.6 Hz, -NH-*CH*$_2$-Ar), 2.91-2.55 (m, 6H, Ar-CH_2- and 2xC*H*-$(CH_3)_2$ and 2xestradiol), 2.48-1.22 (m, 17H, C*H*$_2$-CONH- and Ar-CH_2-C*H*$_2$-CH_2-CONH- and 13xestradiol) 1.12 (d,12H, J=6.9 Hz, 4xCH-(*CH*$_3$)$_2$), 0.92 ppm (s, 3H, $CH_{3estradiol}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.41, 162.83, 162.62, 155.11, 155.03, 152.03, 145.86, 145.81, 145.52, 142.24, 141.50, 141.46, 138.54, 138.13, 133.08, 133.06, 132.46, 131.91, 131.77, 130.06, 129.72, 129.49, 128.25, 127.66, 126.79, 126.73, 126.38, 124.68, 124.54, 124.04, 123.71, 123.68, 122.16, 121.24, 120.98, 120.87, 120.85, 120.68, 120.59, 115.59, 112.87, 93.13, 85.44, 80.20, 49.80, 47.69, 43.75, 43.21, 39.50, 39.01, 35.57, 34.68, 33.11, 29.72, 29.15, 27.26, 26.73, 26.50, 24.01, 22.91, 12.92 ppm. UV-vis (CH_2Cl_2), λmax/nm (log ε) = 233 (4.98), 260 (4.81), 279 (4.74), 437 (4.17), 522 (4.47), 559 (4.66). FT-IR (KBr) ν = 3306 (-OH), 2928, 2868, 1708 (C=O imide), 1672 (C=O imide), 1593, 1509, 1474, 1422, 1407, 1339, 1311, 1281, 1209, 1188, 1020, 877, 804, 705 cm^{-1}. ESI-MS (0.1% formic acid in acetonitrile): m/z = 1450,6 $[M-H_2O+H]^+$, 1468 $[M+H]^+$. Calcd for $C_{93}H_{77}N_7O_{11}.3H_2O$: C, 73.36; H, 5.49; N, 6.44. Found: C, 73.28, H, 5.48, N, 6.50.

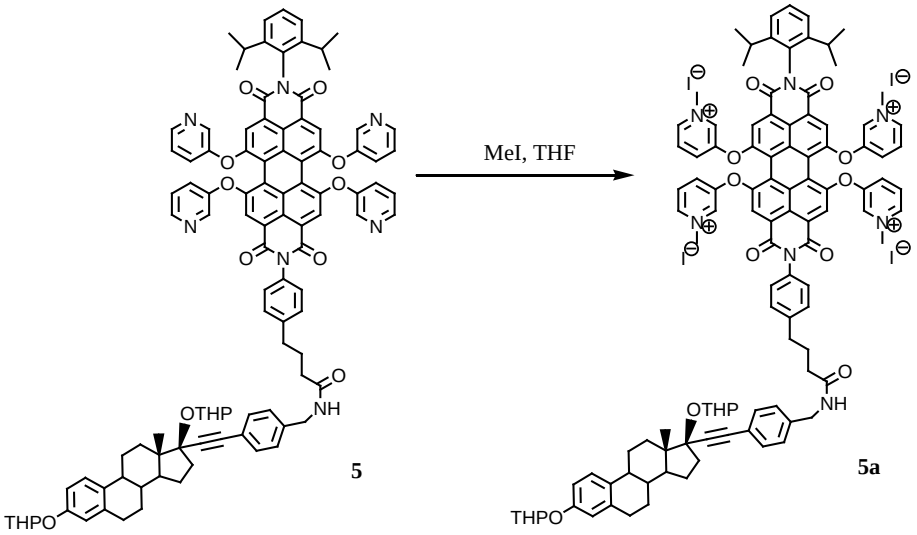
Synthesis of **PBI-5**



To a mixture of **PBI-4** (15 mg, 0.01 mmol) and *p*-toluenesulfonic acid (8.16 mg, 0.042 mmol) in dry THF (5 mL), DMF (0.5 mL) and dihydropyran (0.5 mL) were added and refluxed with vigorous stirring under argon for 24 h. After being cooled to rt, Et_3N (0.2 mL) was added to the reaction. The residue was extracted using CH_2Cl_2 and washed three times with 2 M $NaHCO_3$. The product was purified by flash chromatography (neutral alumina, CH_2Cl_2/acetone 20:4), yielding 4.7 mg (30 %) of **5** as a red solid. M.p. 226 ºC.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.38 (broad, 4H, H-pyridine), 8.32 (broad, 4H, H-pyridine), 8.26 (s, 2H, 2xH-PDI), 8.23 (s, 2H, 2xH-PDI), 7.45 (t, 1H, J=7.5 Hz, H-Ar), 7.31 (d, 2H, J=8.2 Hz, 2xH-Ar´), 7.32-7.10 (m, 17H, 8xH-pyridine and 8xAr-H and 1x$Ar_{estradiol}$), 6.83 (broad d, 1H, $Ar_{estradiol}$), 6.77 (broad s, 1H, $Ar_{estradiol}$), 5.66 (t, 1H, J=5.6 Hz, -CONH-), 5.37 (m, 1H, THP), 5.26 (m, 1H, THP), 4.42 (d, 2H, J=5.6 Hz, -NH-*CH*$_2$-Ar), 3.92 (m, 2H, THP), 3.55 (m, 2H, THP), 2.83 (m, 2H, estradiol), 2.74 (t, 2H, J=7.2 Hz, Ar-CH_2-), 2.67 (sept, 2H, J=6.9 Hz, 2xC*H*-$(CH_3)_2$), 2.31 (m, 2H, estradiol), 2.24 (t, 2H, J=6.9 Hz, C*H*$_2$-CONH-), 2.12-1.35 (m, 25H, Ar-CH_2-C*H*$_2$-CH_2-CONH- and 11xestradiol and 12xTHP) 1.12 (d,12H, J=6.9 Hz, 4xCH-$(CH_3)_2$), 0.96 and 0.95 ppm (2s, 3H, $CH_{3estradiol}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.33, 162.91, 162.73, 155.29, 155.15, 148.78, 145.95, 145.89, 145.58, 142.32, 141.68, 141.59, 139.16, 138.40, 133.72, 133.36, 131.88, 131.72, 130.12, 129.83, 129.75, 129.52, 128.89, 128.64, 128.60, 127.74, 126.79, 126.30, 125.17, 124.68, 124.09, 123.98, 123.71, 121.15, 121.03, 120.89, 120.83, 120.65, 120.59, 120.48, 115.80, 113.93, 96.07, 86.03, 85.68, 61.97, 48.75, 48.61, 43.83, 43.33, 39.17, 37.35, 35.73, 34.81, 32.29, 30.93, 30.47, 29.86, 29.78, 29.20, 29.18, 26.80, 26.74, 25.31, 24.01, 23.30, 22.49, 13.36 ppm. UV-vis (CH_2Cl_2), λmax/nm (log ε) = 233 (4.94), 260 (4.75), 278 (4.65), 438 (4.12), 522 (4.42), 559 (4.60). FT-IR (KBr) v = 2931, 2868, 1708 (C=O imide), 1674 (C=O imide), 1594, 1509, 1474, 1422, 1407, 1339, 1311, 1281, 1208, 1188, 1020, 876, 804, 706 cm^{-1}. ESI-MS (0.1% formic acid in acetone): m/z = 1450 [M-H_2O-2xTHP+H]$^+$, 1468 [M-2xTHP+H]$^+$, 1637 [M+H]$^+$. Calcd for $C_{103}H_{93}N_7O_{13}.4H_2O$: C, 72.39; H, 5.96; N, 5.74. Found: C, 72.70, H, 6.40, N, 5.35.

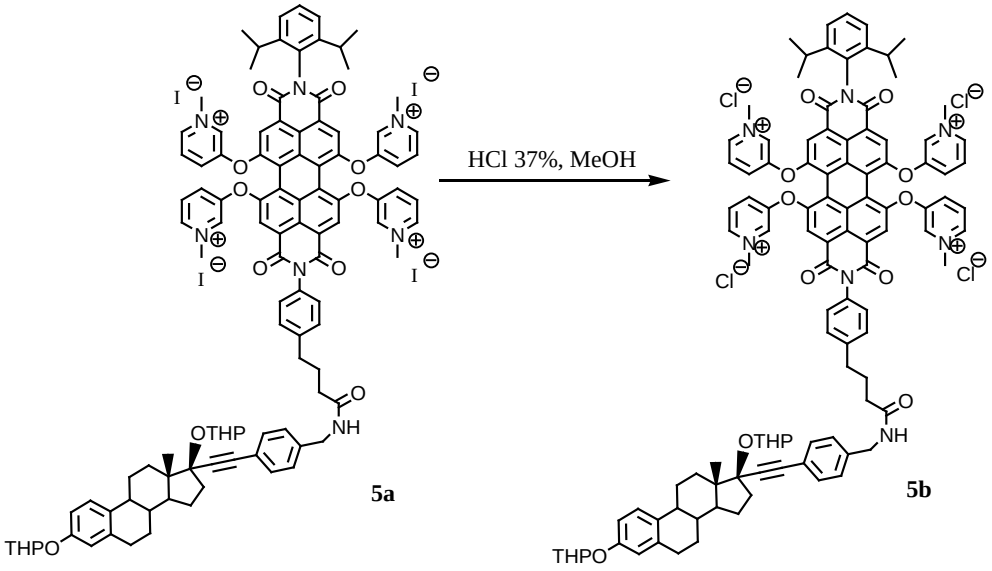
Synthesis of **PBI-5a**



Perylene **5** (3.2 mg, 0.0019 mmol) was dissolved in dry THF (2.5 mL) into a pressure flask, and MeI (2.5 mL) was added to the solution. The reaction was stirred at 80 ºC under argon for 18 h. After being cooled to rt, the obtained red precipitate was filtered off and repeatedly washed with CH_2Cl_2. Methylation of pyridine groups was monitored by NMR: M.p.> 300 ºC.

^{1}H NMR (300 MHz, CD_3OD): δ = 9.15 (broad s, 2H, 2xH-pyridinium), 9.06 (broad s, 2H, 2xH-pyridinium´), 8.68 (m, 4H,4x H-pyridinium), 8.45 (s, 2H, 2xH-PDI), 8.41 (s, 2H, 2xH-PDI), 8.43-8.32 (m, 4H, 4xH-pyridinium), 8.03 (m, 4H, 4xH-pyridinium), 7.46 (t, 1H, J=7.5 Hz, H-Ar), 7.38 (d, 2H, J=8.0 Hz, 2xH-Ar´), 7. 32 (d, 2H, J=7.5 Hz, H-Ar), 7.31 (d, 2H, J=7.9 Hz, 2xH-Ar´´), 7.26 (d, 2H, J=7.9 Hz, 2xH-Ar´´), 7.21 (d, 2H, J=8.0 Hz, 2xH-Ar'), 7.06 (d, 1H, J=9 Hz, $Ar_{estradiol}$), 6.47 (d, 1H, J=9 Hz, $Ar_{estradiol}$), 6.39 (s, 1H, $Ar_{estradiol}$), 5.17 (t, 1H, J=5.6 Hz, -CONH-), 5.00-4.70 (m, 2H, 2xTHP), 4.41 (s, 6H, 6x$CH_{3pyridinium}$), 4.40 (s, 6H, 6x$CH_{3pyridinium´}$), 4.35 (broad, 2H, -NH-*CH_2*-Ar), 3.84 (m, 1H, THP), 3.64 (m, 1H, THP), 3.54 (m, 1H, THP), 3.44 (m, 1H, THP), 2.87-2.62 (m, 6H, 2xestradiol and 2xAr-CH_2- and 2xC*H*-$(CH_3)_2$), 2.35 (m, 2H, estradiol), 2.31 (t, 2H, J=6.9 Hz, C*H_2*-CONH-), 2.20-1.26 (m, 25H, Ar-CH_2-C*H_2*-CH_2-CONH-, 11xestradiol and 12xTHP), 1.09 (d,12H, J=6.9 Hz, 4xCH-(*CH_3*)$_2$), 0.90 ppm (broad s, 3H, $CH_{3estradiol}$).

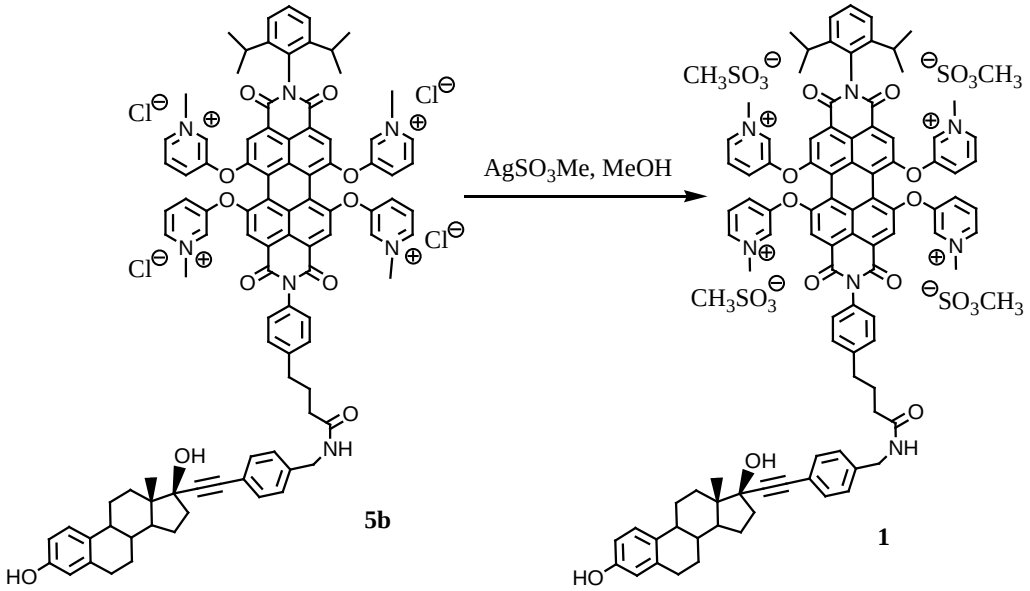
Synthesis of **PBI-5b**



PBI-5a and HCl 37% (0.5 mL) in MeOH (4mL) were stirred at rt under argon atmosphere during 24 h. The solvent was evaporated and the product was poured into ethyl ether. The obtained brown precipitate was filtered off and was repeatedly centrifuged with CH_2Cl_2/ ethyl ether. The red solid was vacuum dried. Alcohol deprotection was monitored by NMR: M.p.> 300 ºC.

^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.23 (broad s, 2H, 2xH-pyridinium), 9.20 (broad s, 2H, 2xH-pyridinium´), 8.85 (m, 4H,4x H-pyridinium), 8.43 (s, 2H, 2xH-PDI), 8.36 (s, 2H, 2xH-PDI), 8.3 (m, 4H, 4xH-pyridinium), 8.08 (m, 4H, 4xH-pyridinium), 7.46 (t, 1H, J=7.5 Hz, H-Ar), 7.40 (d, 2H, J=8.0 Hz, 2xH-Ar´), 7.37-7.19 (m, 8H, 2xH-Ar and 2xH-Ar´and 4xH-Ar´´), 7.03 (d, 1H, J=9 Hz, $Ar_{estradiol}$), 6.50 (d, 1H, J=9 Hz, $Ar_{estradiol}$), 6.46 (s, 1H, $Ar_{estradiol}$), 6.13 (t, 1H, -CONH-), 4.36 (s, 6H, 6x$CH_{3pyridinium}$), 4.33 (s, 6H, 6x$CH_{3pyridinium}$), 4.29 (broad, 2H, -NH-*CH_2*-Ar), 2.83-2.58 (m, 6H, 2xestradiol and 2xAr-CH_2- and 2xC*H*-$(CH_3)_2$), 2.39-2.12 (m, 4H, 2xestradiol and C*H_2*-CONH-), 2.00-1.20 (m, 13H, Ar-CH_2-C*H_2*-CH_2-CONH- and 11xestradiol), 1.04 (d,12H, J=6.7 Hz, 4xCH-(*CH_3*)$_2$), 0.87 ppm (broad s, 3H, $CH_{3estradiol}$).

Synthesis of **PBI-1**

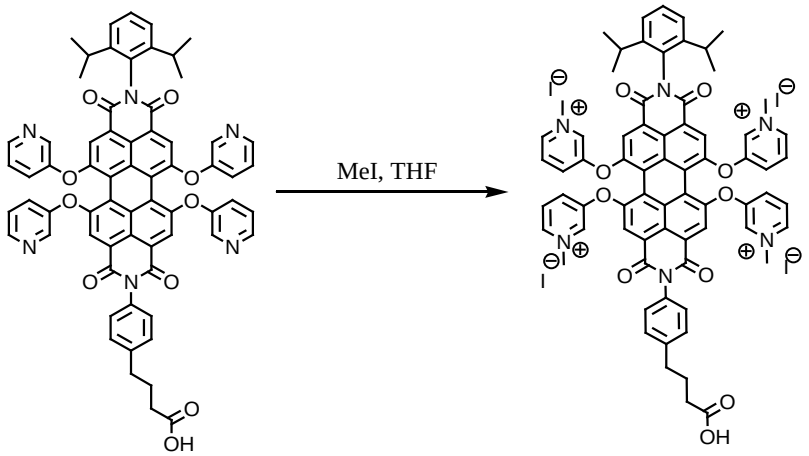


PBI-5b (2 mg, 0.0012 mmol) and silver methanesulfonate (0.98 mg, 0.0048 mmol) were added to methanol (5 mL) to form a white precipitate (silver iodide), which was removed by filtration to give a clear red solution. MeOH was evaporated and the product was centrifuged several times in CH_2Cl_2. **1** as red solid (3 mg, 80%) was obtained after evaporation of solvent. The pure product was obtained without further purification method. M.p.> 300 ºC.

^{1}H NMR (300 MHz, CD_3OD): δ = 9.03 (broad, 2H, 2xH-pyridinium), 8.98 (broad s, 2H, 2xH-pyridinium´), 8.73 (broad, 4H,4x H-pyridinium), 8.46 (s, 2H, 2xH-PDI), 8.45 (s, 2H, 2xH-PDI), 8.42-8.25 (m, 4H, 4xH-pyridinium), 8.03 (m, 4H, 4xH-pyridinium), 7.52-7.15 (m, 11H, 3xH-Ar and 4xH-Ar´and 4xH-Ar´´), 7.07 (broad, 1H, $Ar_{estradiol}$), 6.44 (broad, 2H, $Ar_{estradiol}$), 5.17 (broad t, 1H, -CONH-), 4.41 (s, 6H, 6x$CH_{3pyridinium}$), 4.40 (s, 6H, 6x$CH_{3pyridinium´}$), 4.36 (broad, 2H, -NH-*CH_2*-Ar), 2.83-2.60 (m, 6H, 2xestradiol and 2xAr-CH_2- and 2xC*H*-$(CH_3)_2$), 2.66 (s, 12H, SO_3-CH_3), 2.31 (m, 4H, 2xestradiol and C*H_2*-CONH-), 2.11-1.25 (m, 13H, Ar-CH_2-C*H_2*-CH_2-CONH- and 11xestradiol), 1.09 (d,12H, J=6.5 Hz, 4xCH-(*CH_3*)$_2$), 0.90 ppm (s, 3H, $CH_{3estradiol}$). ^{13}C NMR (75 MHz, CD_3OD): δ = 175.33, 164.10, 164.07, 157.45, 157.23, 155.80, 154.90, 154.73, 147.02, 142.93, 142.74, 142.50, 142.48, 140.60, 139.26, 139.05, 138.80, 138.52, 136.70, 135.80, 135.73, 135.68, 134.20, 134.08, 133.91, 133.15, 132.40, 131.50, 130.43, 130.20, 129.75, 129.55, 127.35, 127.30, 126.10, 125.60, 125.31, 125.15, 125.10, 123.81, 123.50, 116.52, 114.01, 96.02, 86.15, 81.13, 50.22, 47.94, 44.03, 43.56, 40.65, 39.92, 39.53, 35.86, 34.60, 34.20, 30.97, 30.37, 28.96, 27.79, 24.34, 23.15, 13.76 ppm. UV-vis (DMSO), λmax/nm (log ε) = 273 (4.62), 418 (3.86), 505 (4.18), 539 (4.33). FT-IR (KBr) ν = 3439, 2933, 1706 (C=O imide), 1666 (C=O imide), 1597, 1501, 1409, 1384, 1338, 1273, 1200, 1074, 789, 567, 535 cm^{-1}. ESI-MS (methanol): m/z = 382 [M-4x$SO_3CH_3^-$]$^{4+}$, 541 [M-3x$SO_3CH_3^-$]$^{3+}$, 858 [M-2x$SO_3CH_3^-$]$^{2+}$.ESI-HRMS (methanol): calculated for $C_{98}H_{92}N_7O_{14}S^{+3}$, 540.8852; found, 540.8802.

Synthesis of PBI-2

Synthesis of **PBI-3b**

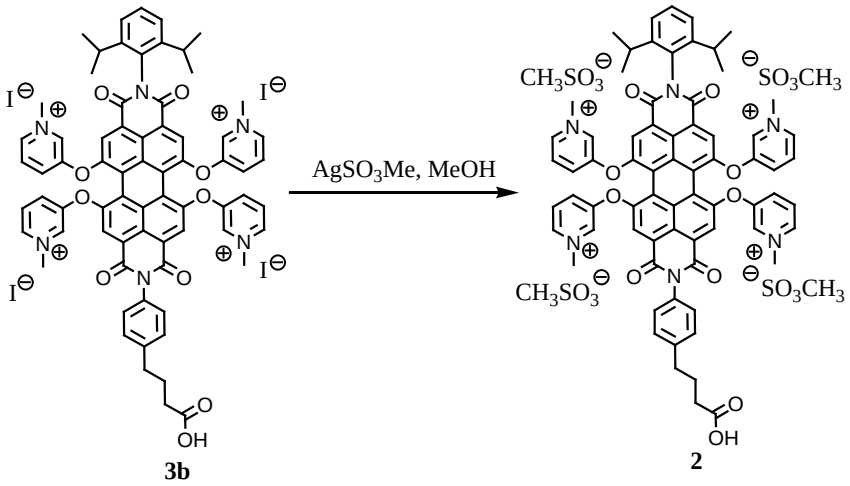


3 **3b**

The tetrapyridyloxiperylenebisimide **3** (50 mg, 0.046 mmol) was dissolved in dry THF (5 mL) into a pressure flask, and MeI (5 mL) was added to the solution. The reaction was stirred at 80 ºC under argon for 18 h. After being cooled to rt, the obtained red precipitate was filtered off and repeatedly washed with CH_2Cl_2. Methylation of pyridine groups was monitored by NMR:

^{1}H NMR (300 MHz, CD_3OD): δ = 9.11 (broad s, 2H, 2xH-pyridinium), 9.00 (broad s, 2H, 2xH-pyridinium´), 8.88 (m, 4H,4x H-pyridinium), 8.46 (s, 2H, 2xH-PDI), 8.41 (s, 2H, 2xH-PDI), 8.45-8.31 (m, 4H, 4xH-pyridinium), 8.04 (d, 2H, J=6.0 Hz, 2xH-pyridinium), 8.01 (d, 2H, J=6.0 Hz, 2xH-pyridinium´),7.45 (t, 1H, J=7.5 Hz, H-Ar), 7.35 (d, 2H, J=8.0 Hz, 2xH-Ar´), 7. 31 (d, 2H, J=7.5 Hz, H-Ar), 7.25 (d, 2H, J=8.0 Hz, 2xH-Ar´´), 4.41 (s, 6H, 6x$CH_{3pyridinium}$), 4.39 (s, 6H, 6x$CH_{3pyridinium´}$), 2.82 (sept, 2H, J=6.8 Hz, 2xC*H*-$(CH_3)_2$), 2.73 (t, 2H, J=7.5 Hz, Ar-CH_2-), 2.35 (t, 2H, J=7.3 Hz, C*H*$_2$-CO_2H), 1.95 (m, 2H Ar-CH_2-C*H*$_2$-CH_2-CO_2H), 1.09 (d,12H, J=6.8 Hz, 4xCH-(*CH*$_3$)$_2$).

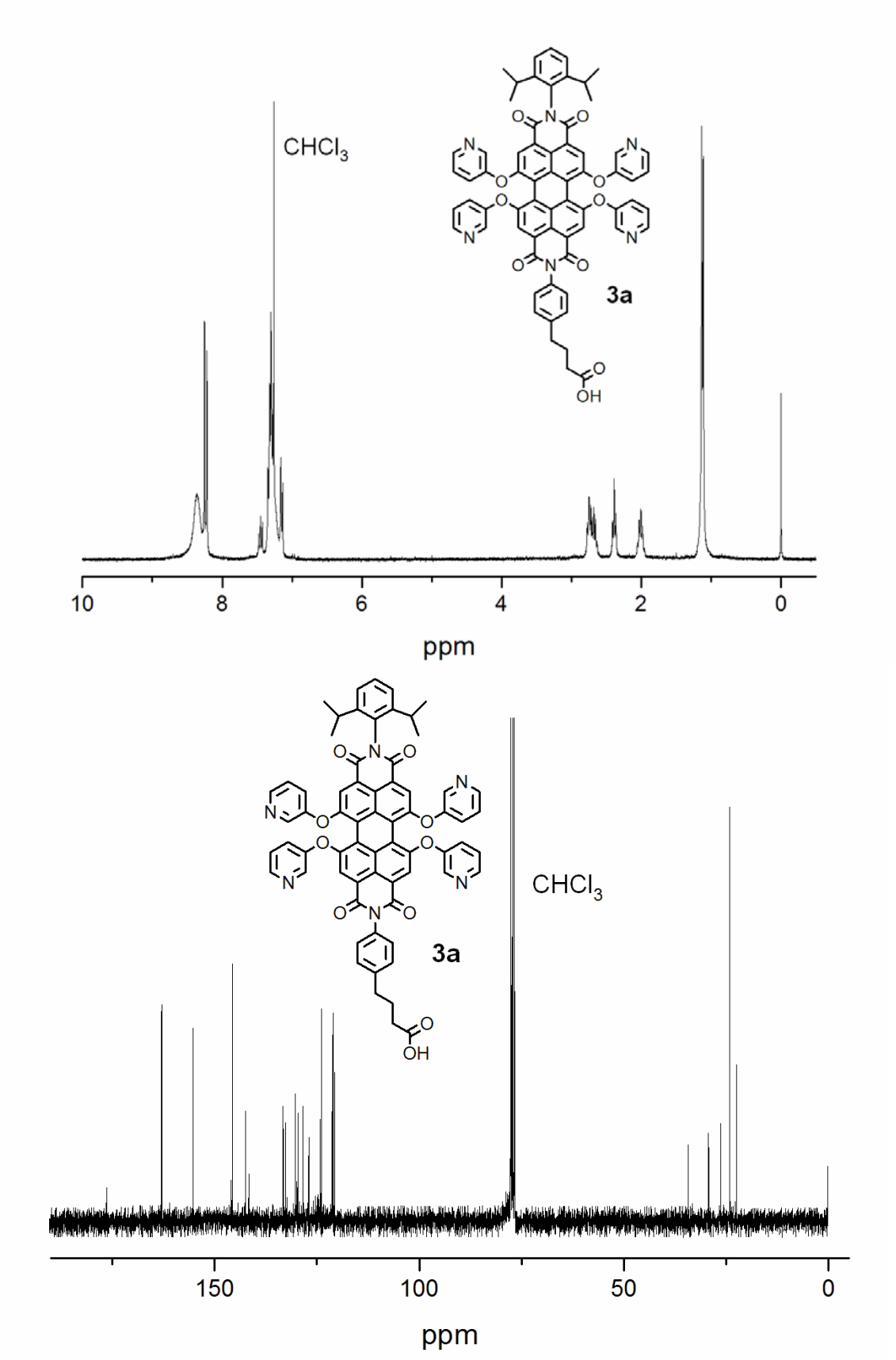
Synthesis of **PBI-2**

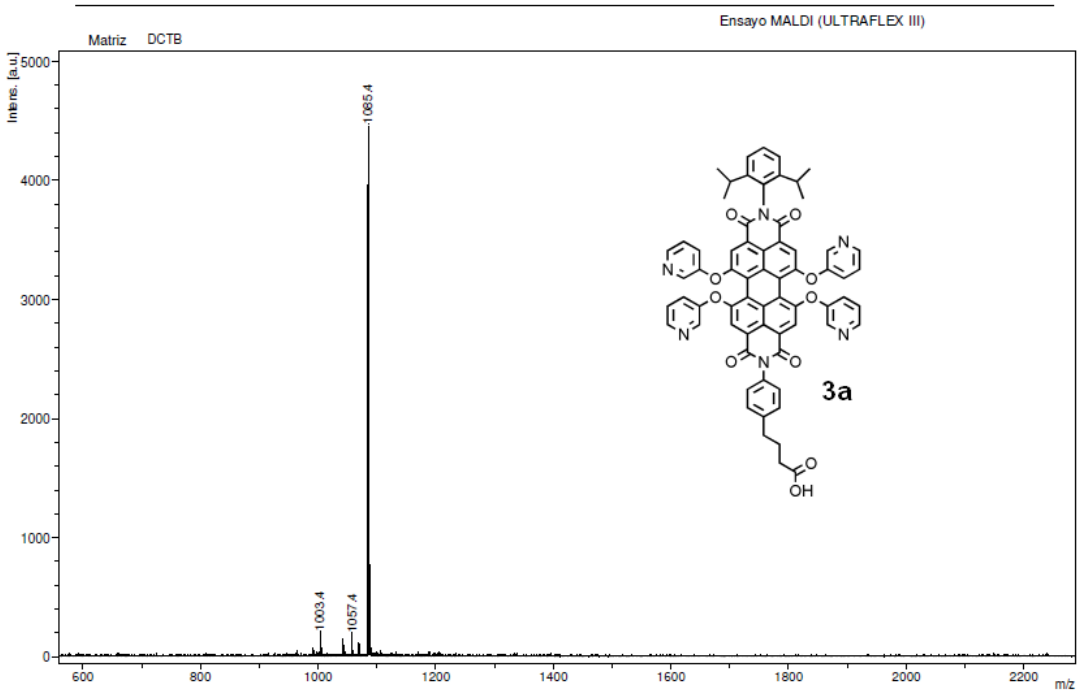


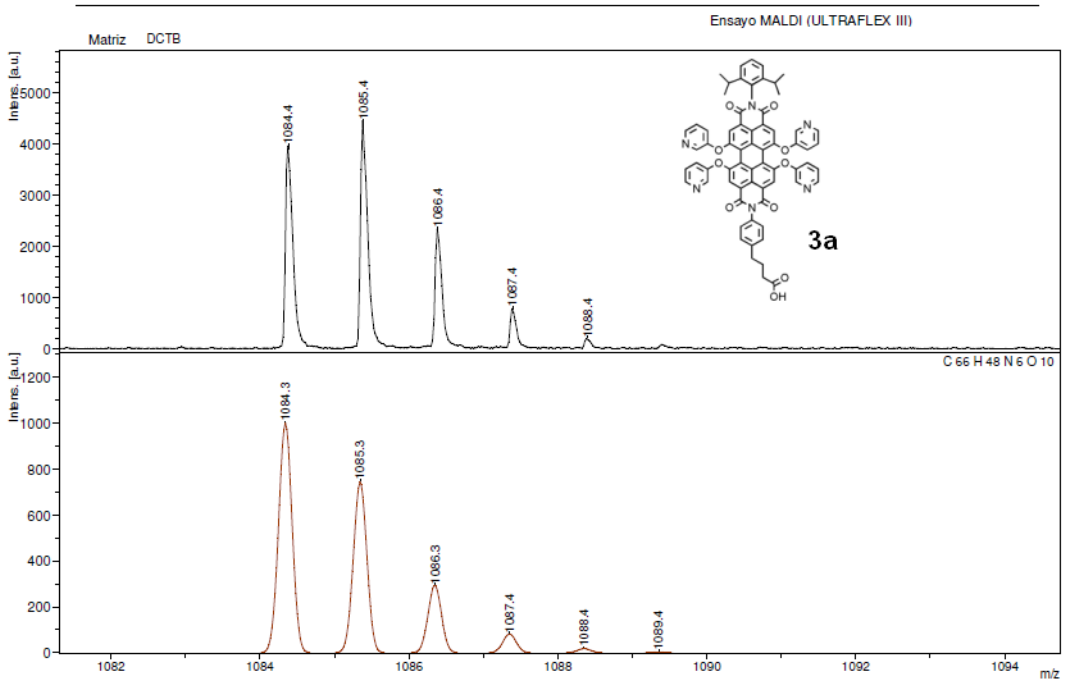
PBI-**3b** (65 mg, 0.039 mmol) and silver methanesulfonate (32 mg, 0.157 mmol) were added to methanol (10 mL) to form a white precipitate (silver iodide), which was removed by filtration to give a clear red solution. MeOH was evaporated and the product was centrifuged several times in CH_2Cl_2. A red solid (56 mg, 80%) was obtained after evaporation of solvent. The pure product **2** was obtained without further purification method.

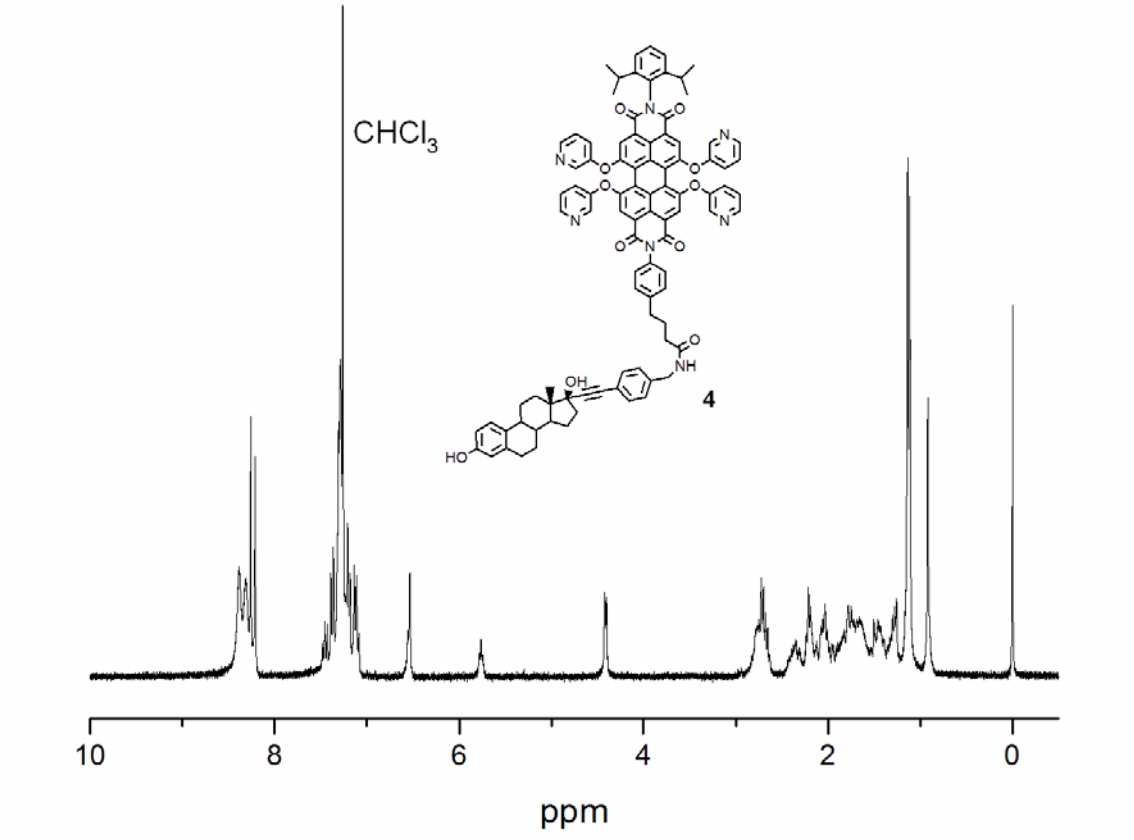
^{1}H NMR (300 MHz, CD_3OD): δ = 9.00 (m, 4H, 4xH-pyridinium), 8.72 (broad, 4H,4x H-pyridinium), 8.45 (s, 2H, 2xH-PDI), 8.40 (s, 2H, 2xH-PDI), 8.33 (m, 4H, 4xH-pyridinium), 8.03 (m, 4H, 4xH-pyridinium), 7.64 (t, 1H, J=7.5 Hz, H-Ar), 7.43-7.18 (m, 12H, 8xH-pyridine and 2xAr-H and 2xAr-H´), 7.15 (d, 2H, J=8.0 Hz, 2xH-Ar'), 4.40 (broad s, 12H, $CH_{3pyridinium}$), 2.71 (m, 4H, Ar-CH_2- and 2x*CH*-$(CH_3)_2$), 2.67 (s, 12H, SO_3-CH_3), 2.39 (m, 2H, *CH*$_2$-CO_2H), 1.99 (m, 2H Ar-CH_2-*CH*$_2$-CH_2-CO_2H), 1.09 ppm (d,12H, J=6.5 Hz, 4xCH-(*CH*$_3$)$_2$). ^{13}C NMR (75 MHz, CD_3OD): δ = 177.24, 164.18, 164.15, 157.47, 157.10, 154.70, 154.53, 147.14, 147.12, 142.90, 142.70, 142.48, 142.44, 140.57, 139.25, 139.03, 138.97, 135.80, 135.67, 135.65, 134.02, 133.99, 131.48, 130.41, 130.22, 129.81, 129.80, 127.27, 126.05, 125.34, 125.19, 125.17, 123.80, 123.48, 39.58, 35.80, 34.16, 30.31, 27.84, 24.34 ppm. UV/Vis (DMSO), λmax/nm (log ε) = 274 (4.70), 419 (3.99), 504 (4.25), 540 (4.38). FT-IR (KBr) v = 3435, 2963, 1705 (C=O imide), 1667 (C=O imide), 1596, 1501, 1409, 1339, 1274, 1198, 1109, 1076, 1060, 790, 971, 617, 567, 534 cm^{-1}. ESI-MS (methanol): m/z = 286 [M-4x$SO_3CH_3^-$]$^{4+}$, 413 [M-3x$SO_3CH_3^-$]$^{3+}$, 667 [M-2x$SO_3CH_3^-$]$^{2+}$. ESI-HRMS (methanol): calculated for $C_{72}H_{66}N_6O_{16}S_2^{+2}$, 667.1983; found, 667.2062.

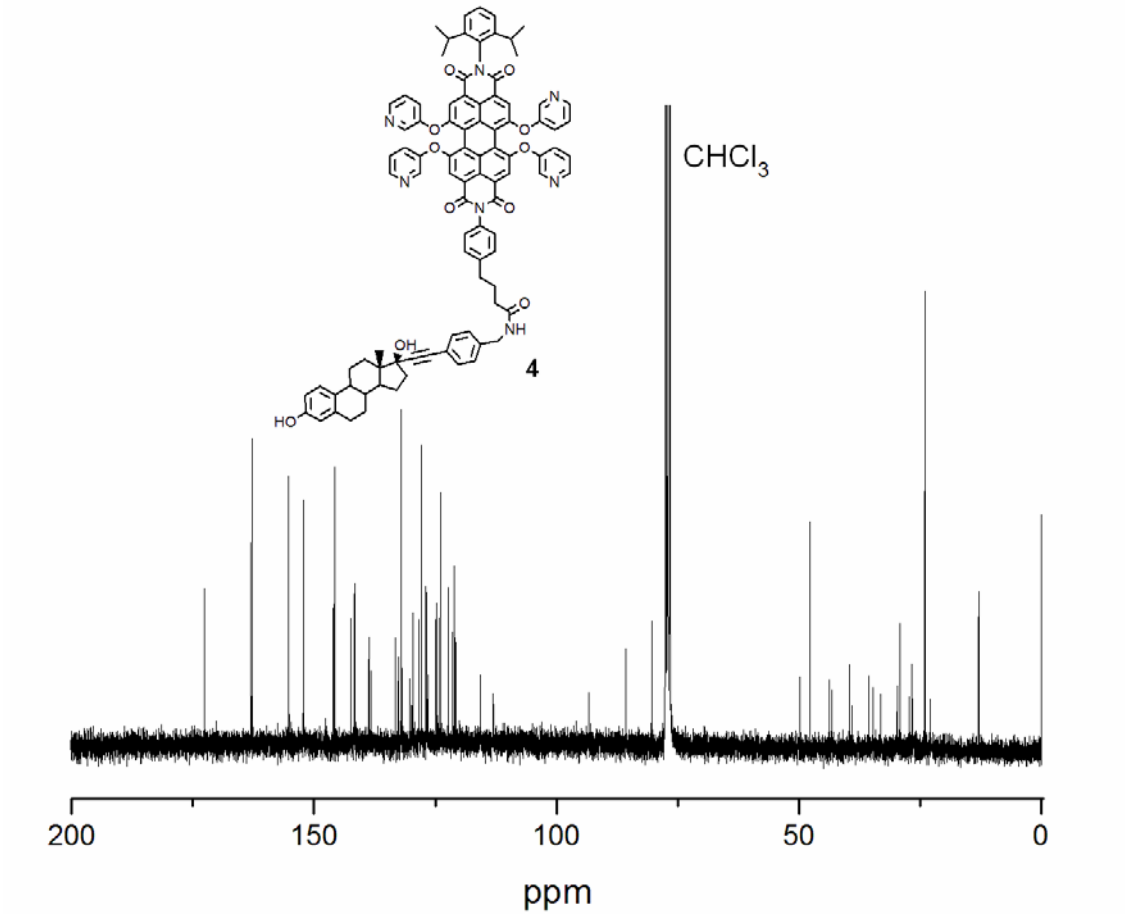
^{1}H- ,^{13}C-NMR and MS spectra of characterized compounds.

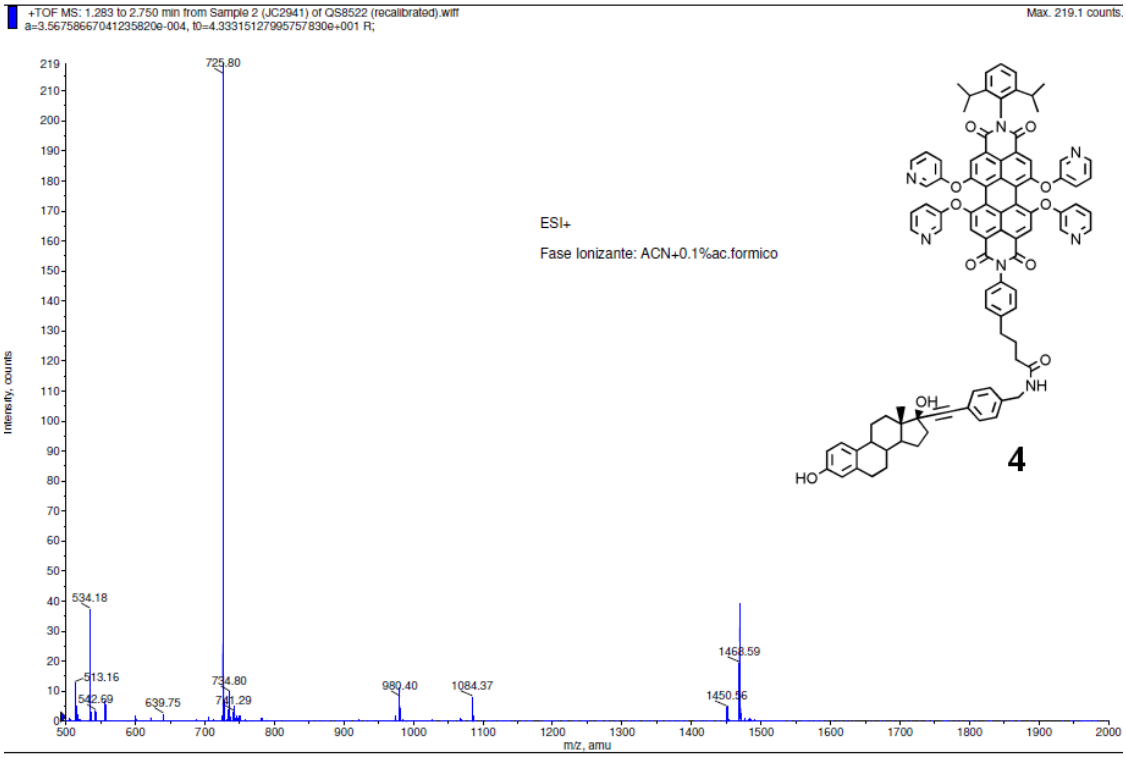


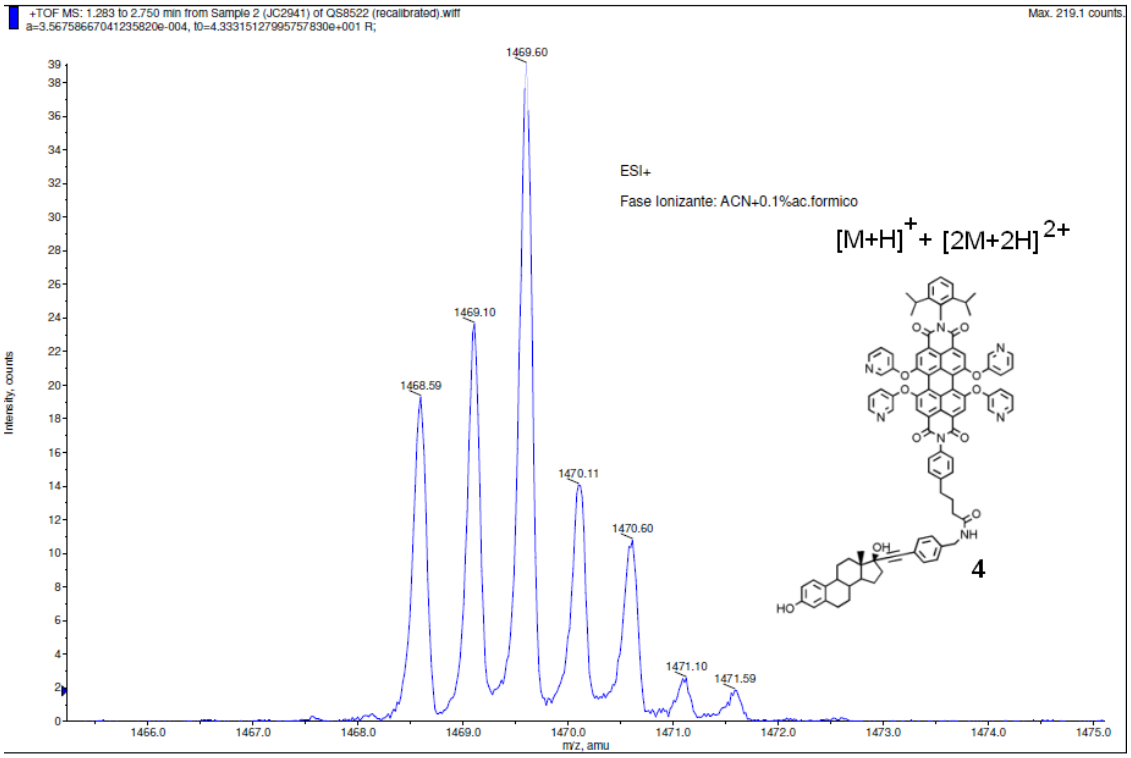

Ensayo MALDI (ULTRAFLEX III)
Matriz DCTB
Intens. [a.u.]
1065.4
1003.4
1057.4
3a
OH
m/z

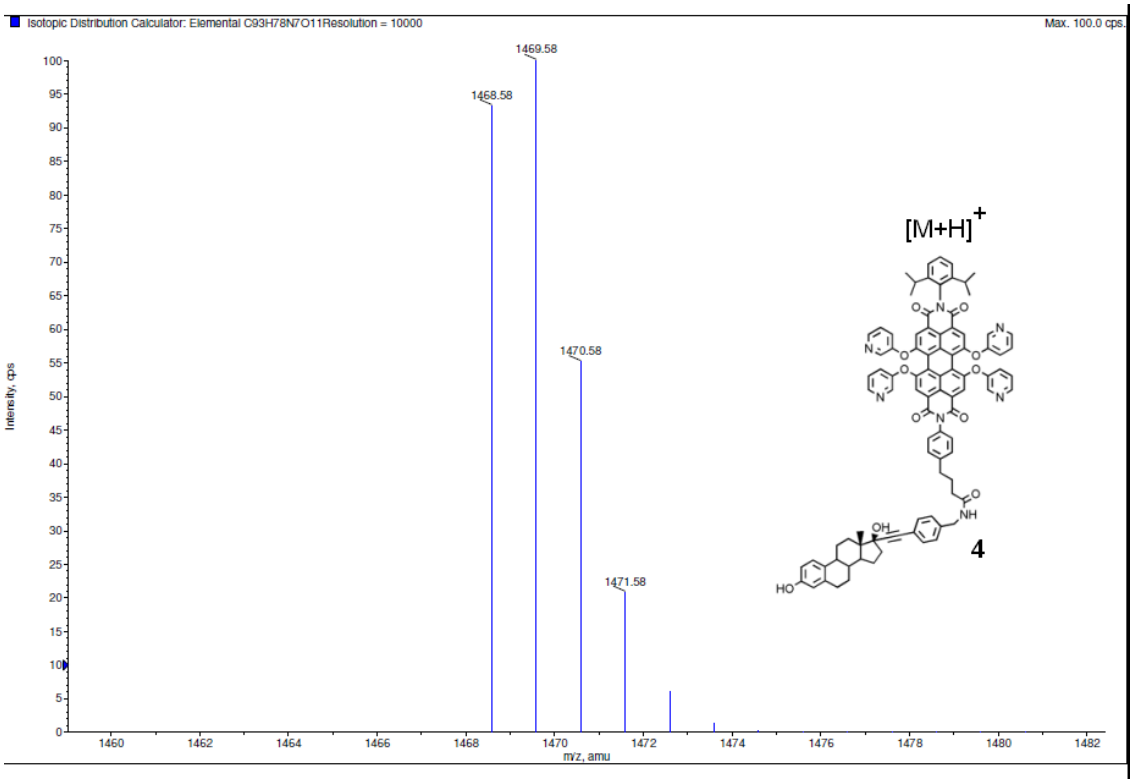

Ensayo MALDI (ULTRAFLEX III)
Matriz DCTB
Intens. [a.u.]
1084.4
1085.4
1086.4
1087.4
1088.4
3a
OH
C 66 H 48 N 6 O 10
1084.3
1085.3
1086.3
1087.4
1088.4
1089.4
m/z

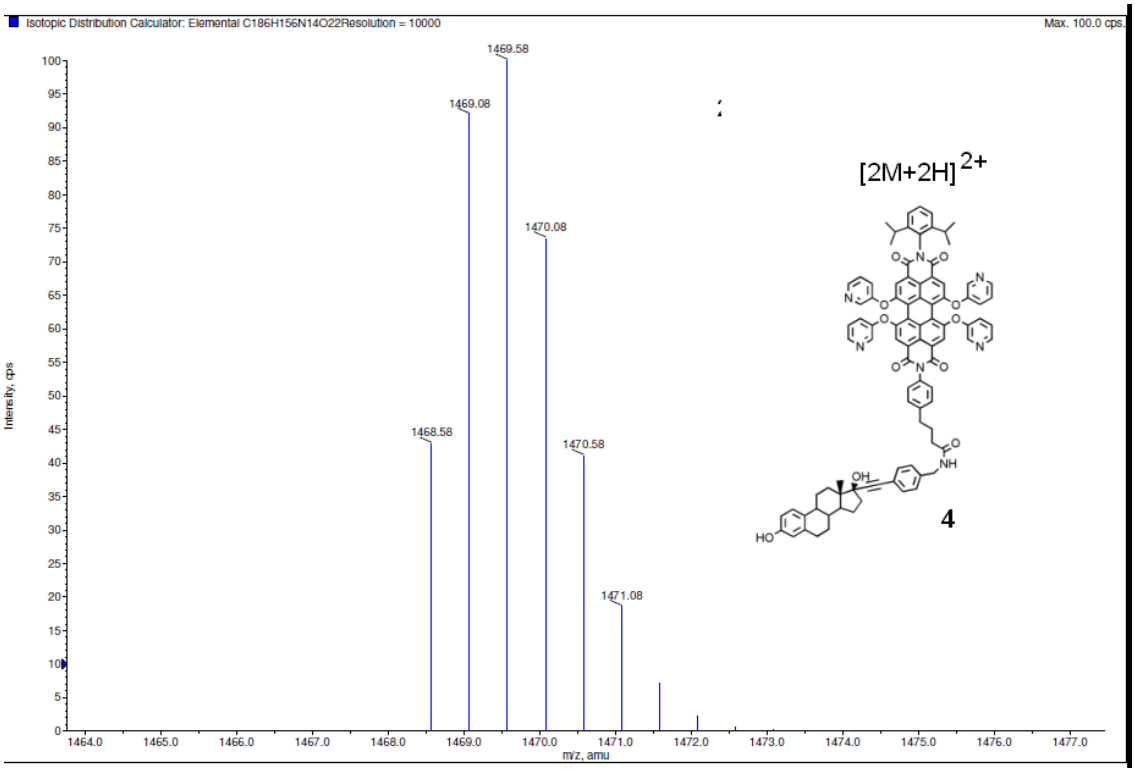


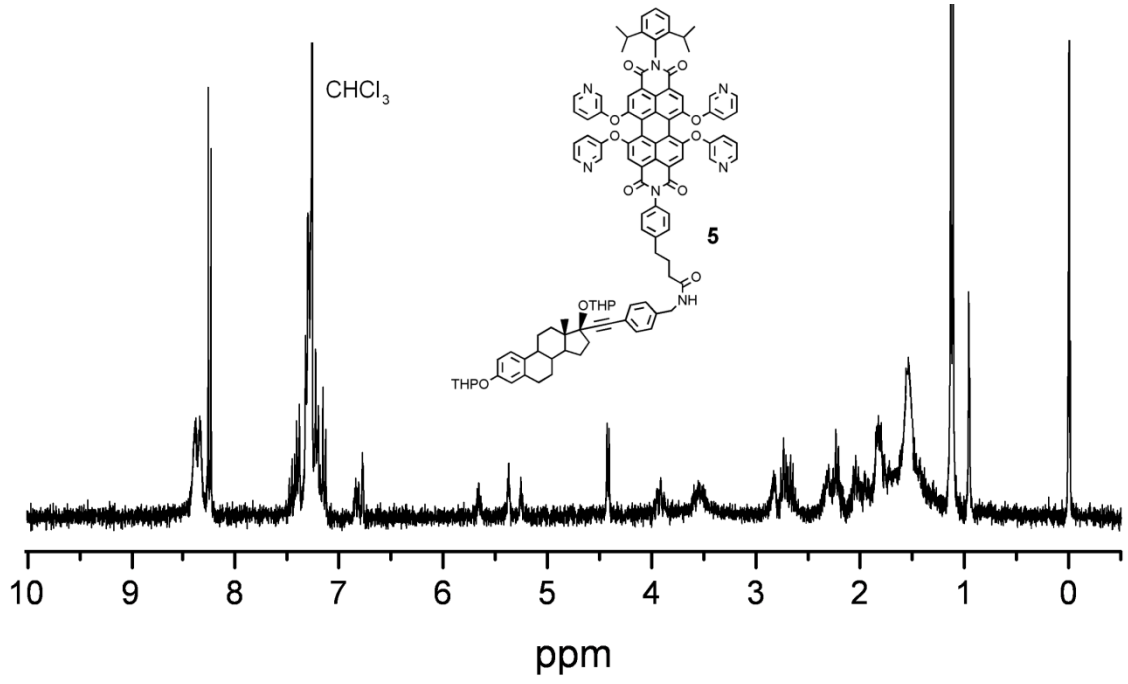


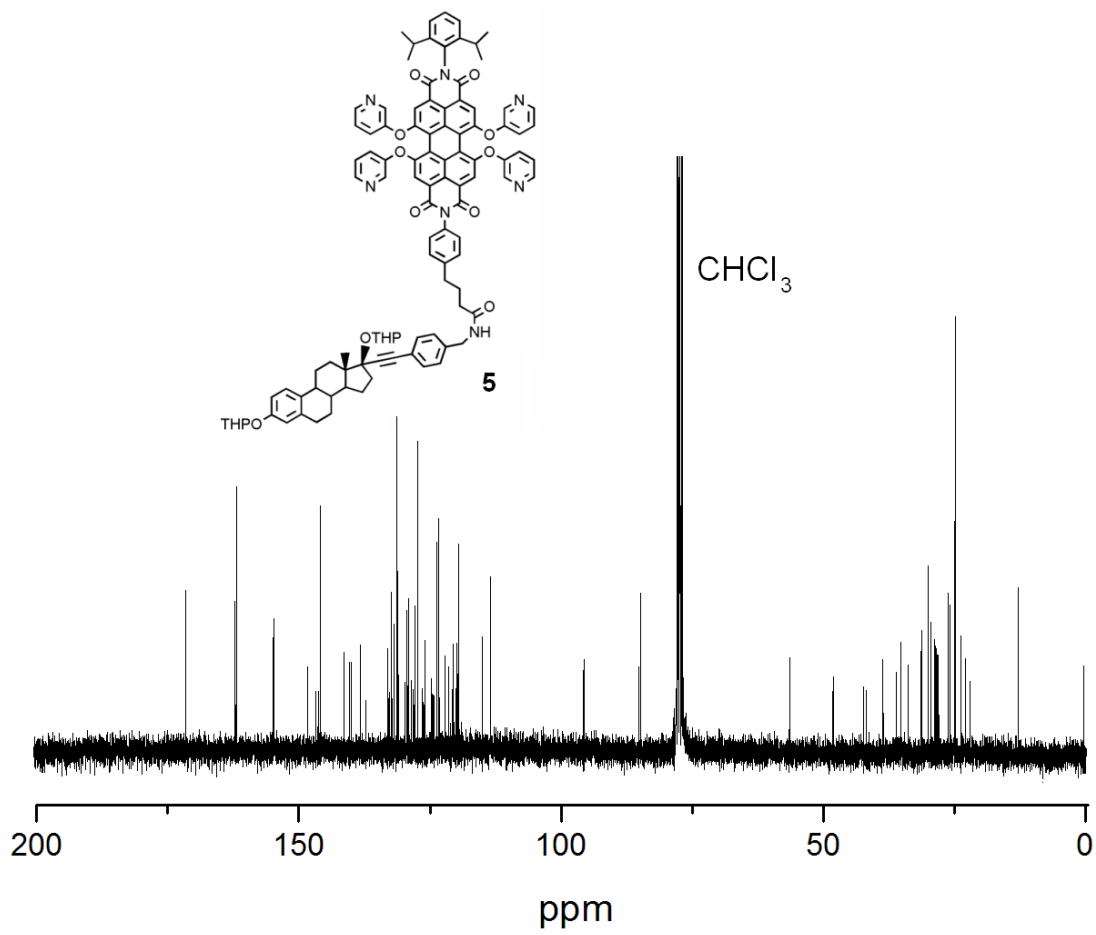

+TOF MS: 1.283 to 2.750 min from Sample 2 (JC2941) of QS8522 (recalibrated).wiff
a=3.56758667041235820e-004, t0=4.33315127995757830e+001 R;
Max. 219.1 counts.
ESI+
Fase Ionizante: ACN+0.1%ac.formico
4
Intensity, counts
m/z, amu
725.80
534.18
513.16
542.69
639.75
734.80
741.29
980.40
1084.37
1450.56
1468.59

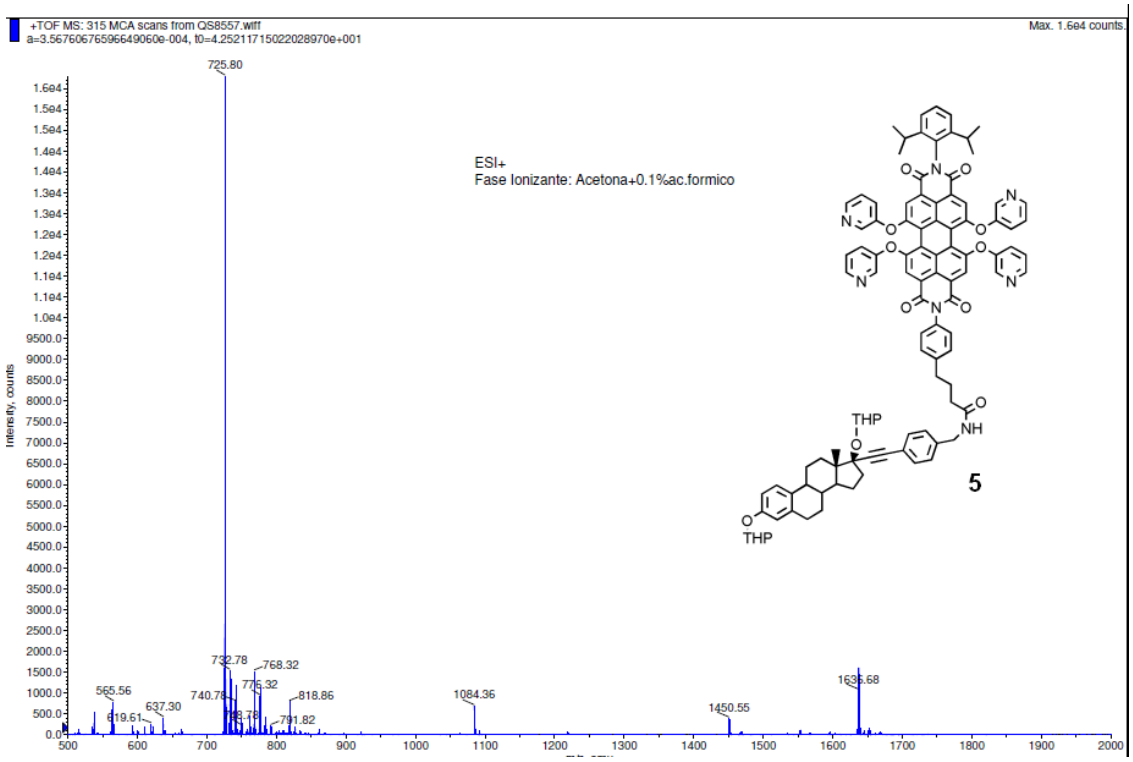

+TOF MS: 1.283 to 2.750 min from Sample 2 (JC2941) of QS8522 (recalibrated).wiff
a=3.56758667041235820e-004, t0=4.33315127995757830e+001 R;
Max. 219.1 counts.
ESI+
Fase Ionizante: ACN+0.1%ac.formico
[M+H]+ + [2M+2H]2+
4
Intensity, counts
m/z, amu
1469.60
1469.10
1468.59
1470.11
1470.60
1471.10
1471.59

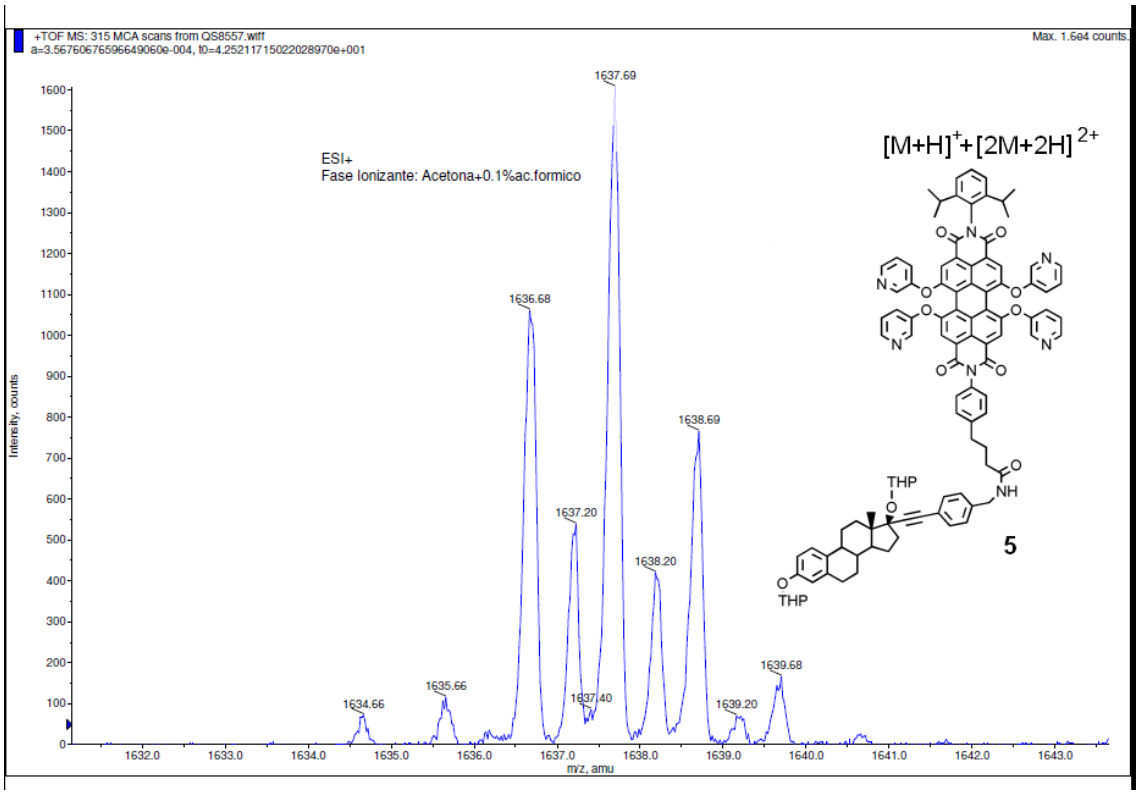

Isotopic Distribution Calculator: Elemental C93H78N7O11Resolution = 10000
Max. 100.0 cps.
1469.58
1468.58
1470.58
1471.58
[M+H]+
4
Intensity, cps
m/z, amu

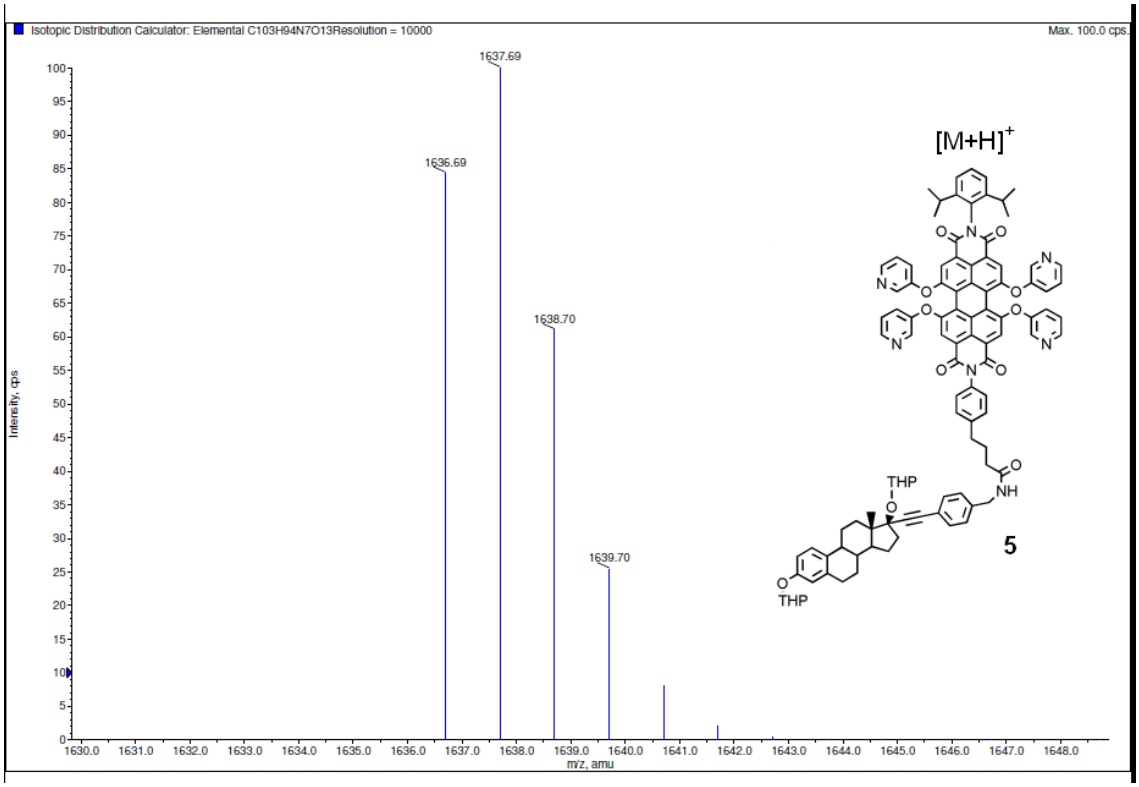

Isotopic Distribution Calculator: Elemental C186H156N14O22Resolution = 10000
Max. 100.0 cps.
1469.58
1469.08
1470.08
1468.58
1470.58
1471.08
[2M+2H]2+
4
Intensity, cps
m/z, amu

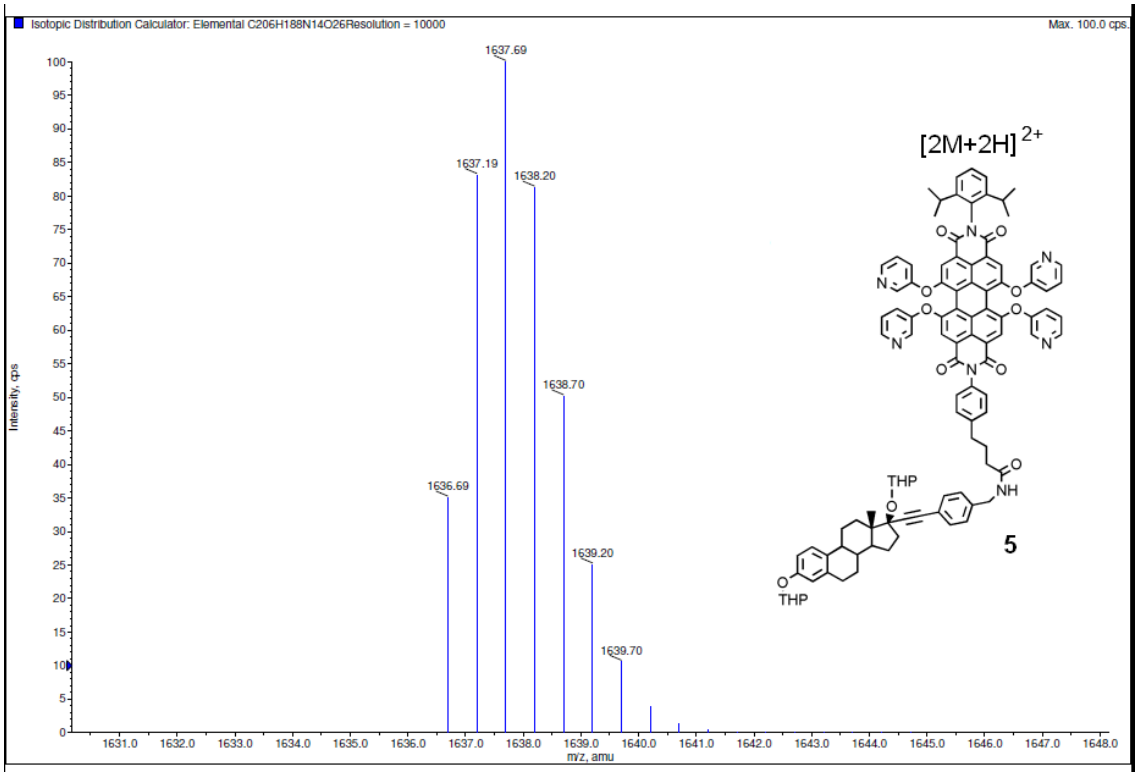

CHCl3
5
OTHP
NH
THPO
10
9
8
7
6
5
4
3
2
1
0
ppm

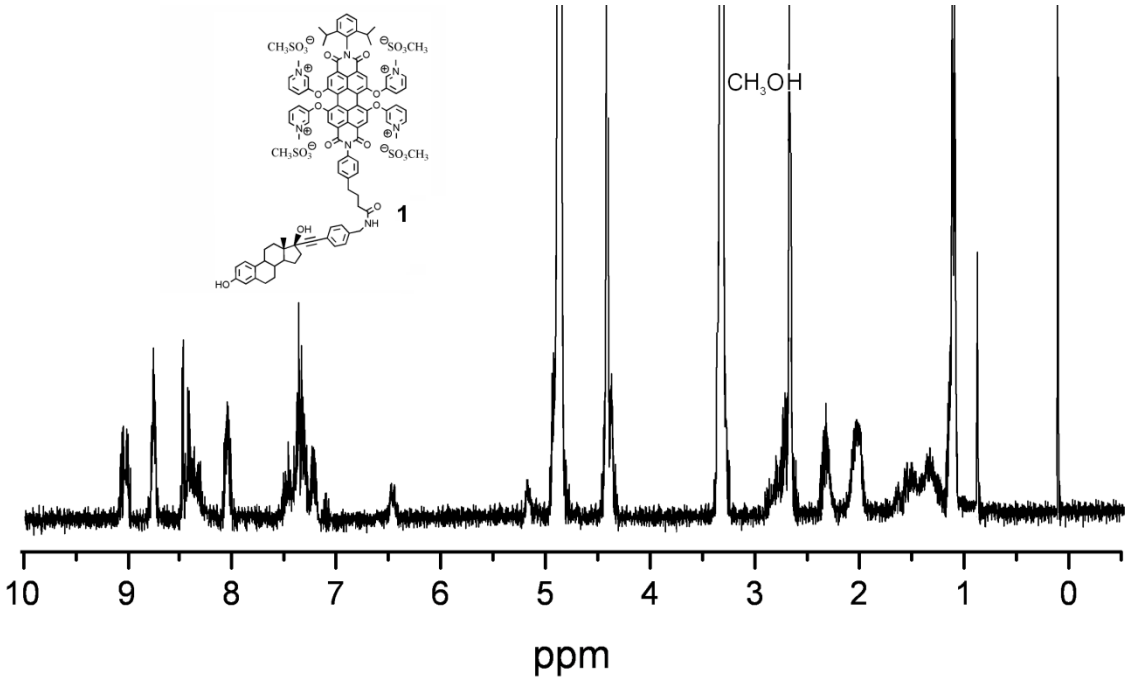

5
OTHP
NH
THPO
CHCl3
200
150
100
50
0
ppm

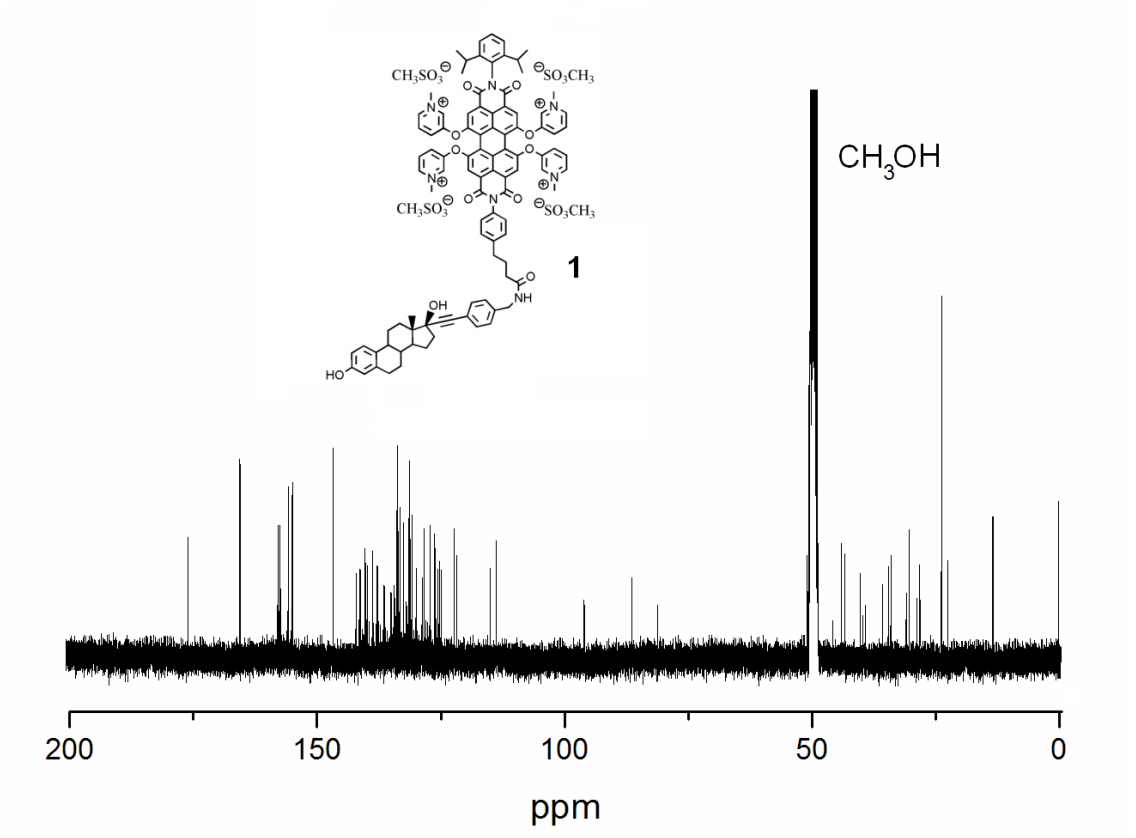

+TOF MS: 315 MCA scans from QS8557.wtf
a=3.567606765966490 60e-004, t0=4.252117150220289 70e+001
Max. 1.6e4 counts.
Intensity, counts
ESI+
Fase Ionizante: Acetona+0.1%ac.formico
THP
NH
THP
5
725.80
732.78
768.32
775.32
818.86
565.56
637.30
619.61
740.78
791.82
1084.36
1450.55
1636.68

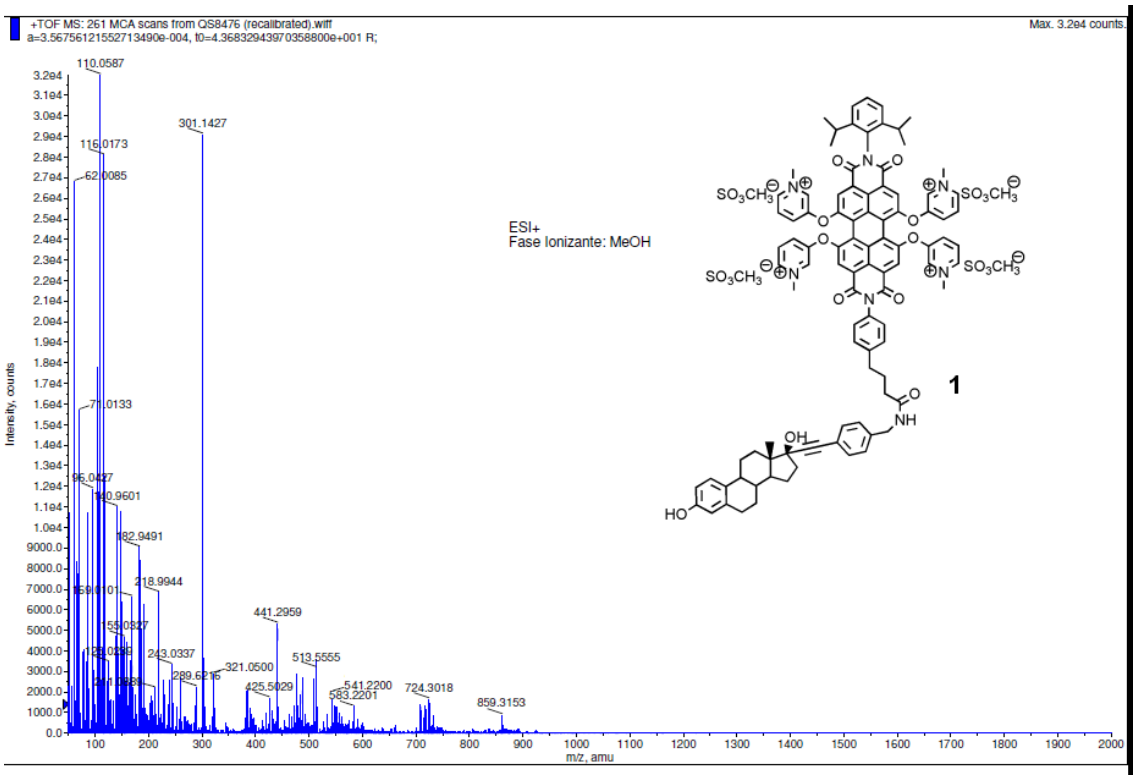

+TOF MS: 315 MCA scans from QS8557.wtf
a=3.567606765966490 60e-004, t0=4.252117150220289 70e+001
Max. 1.6e4 counts.
Intensity, counts
m/z, amu
ESI+
Fase Ionizante: Acetona+0.1%ac.formico
[M+H]+ + [2M+2H] 2+
THP
NH
THP
5
1637.69
1636.68
1638.69
1637.20
1638.20
1639.68
1635.66
1634.66
1637.40
1639.20


Isotopic Distribution Calculator: Elemental C103H94N7O13Resolution = 10000
Max. 100.0 cps.
1637.69
1636.69
1638.70
1639.70
Intensity, cps
m/z, amu
[M+H]+
THP
NH
5
THP


Isotopic Distribution Calculator: Elemental C206H188N14O26Resolution = 10000
Max. 100.0 cps.
1637.69
1637.19
1638.20
1638.70
1636.69
1639.20
1639.70
Intensity, cps
m/z, amu
[2M+2H] 2+
THP
NH
5
THP



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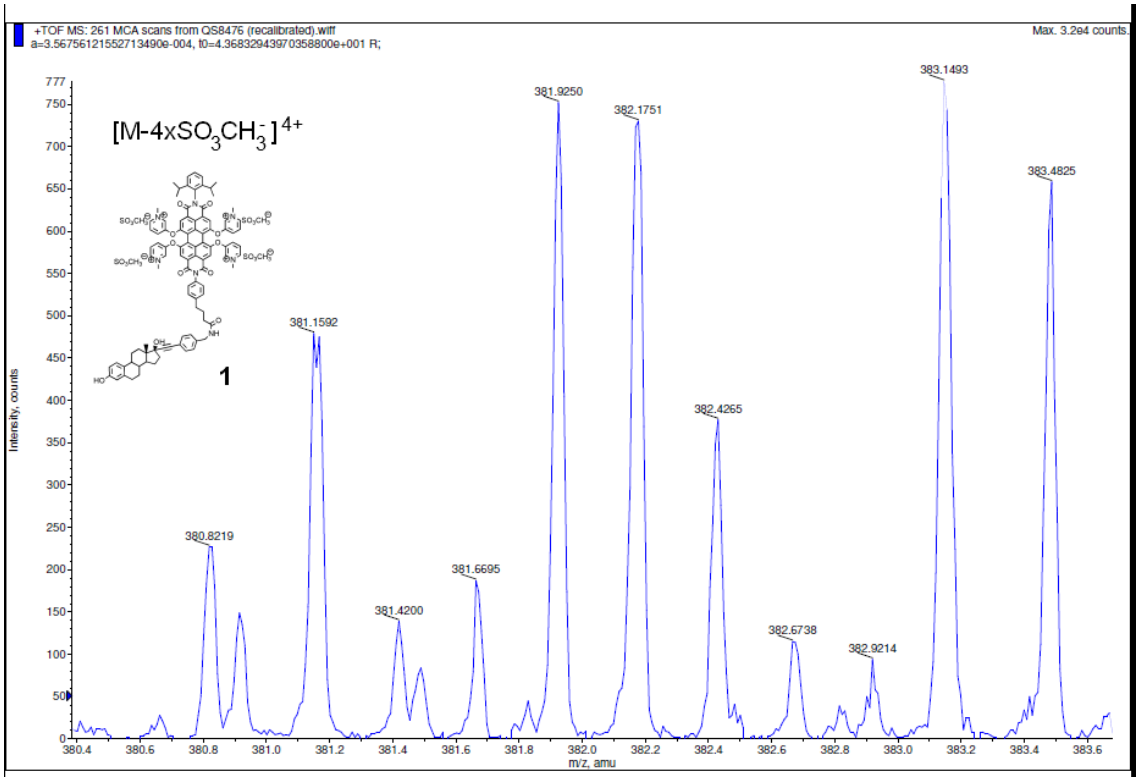
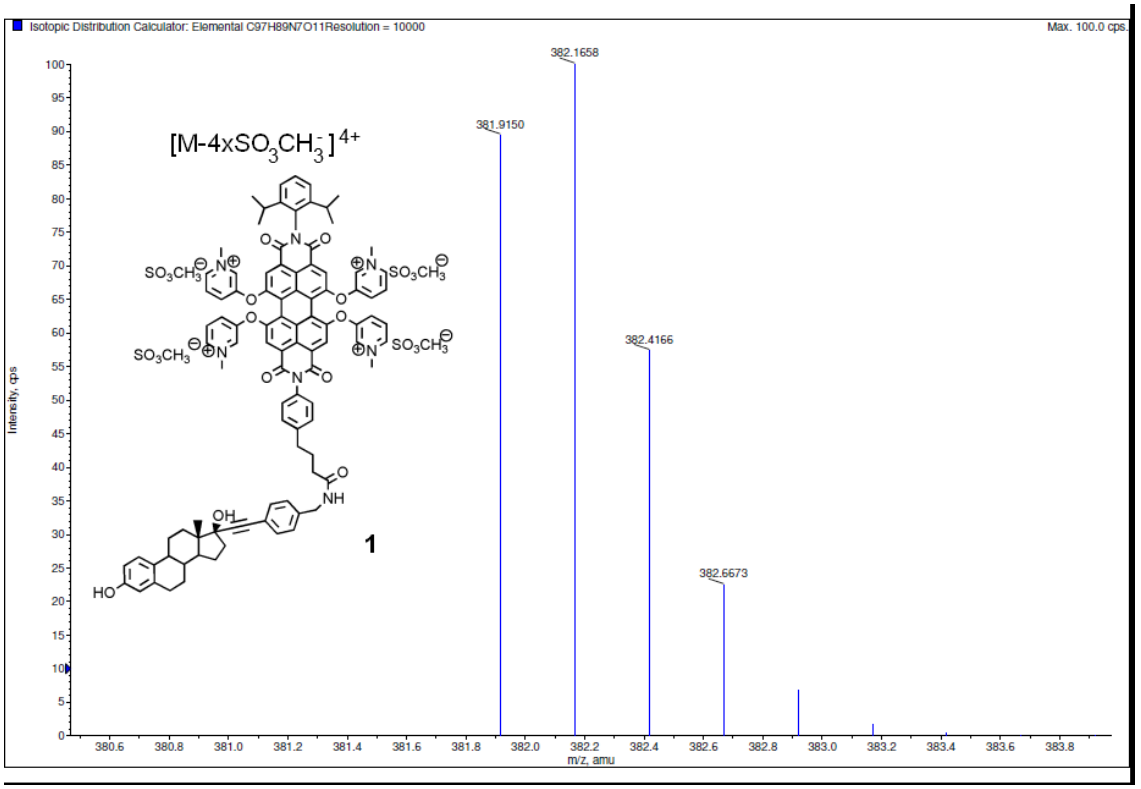
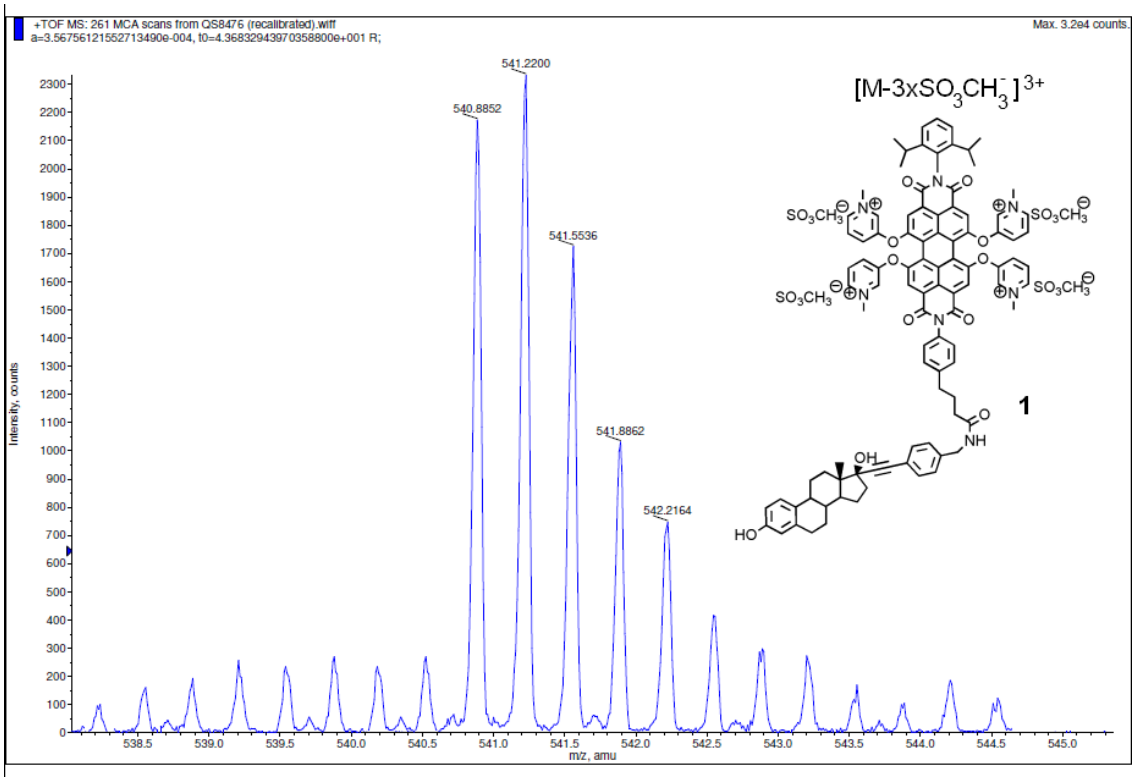
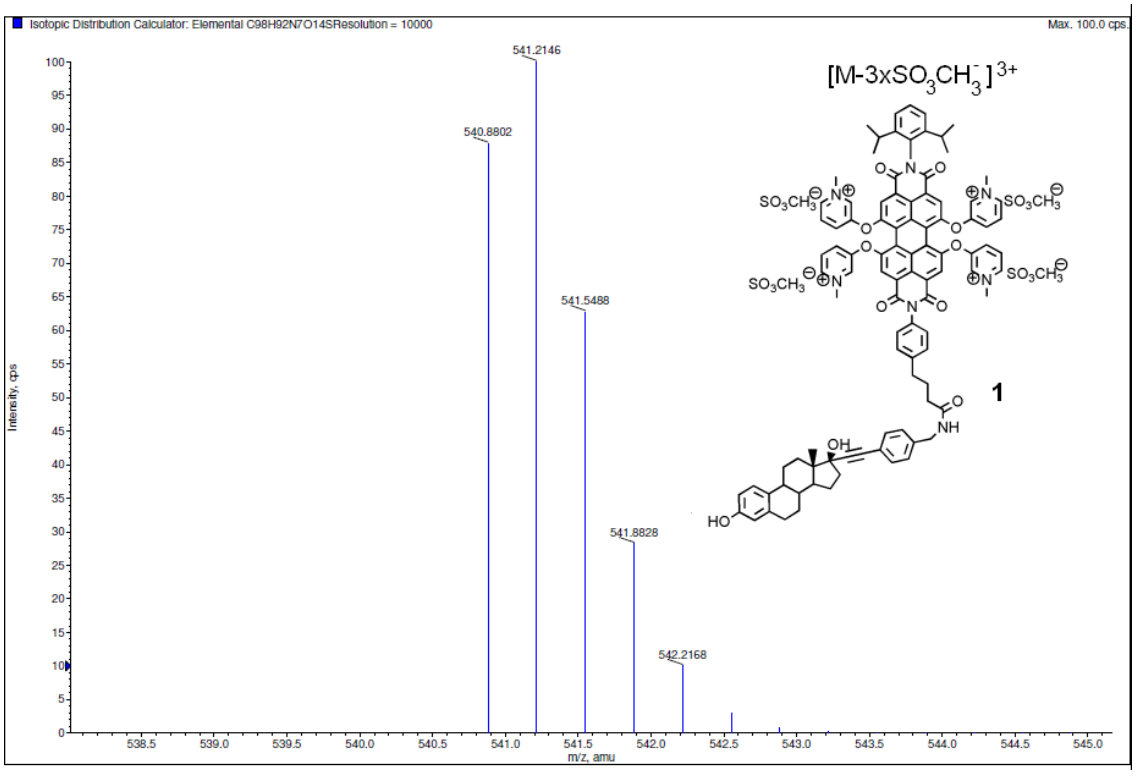
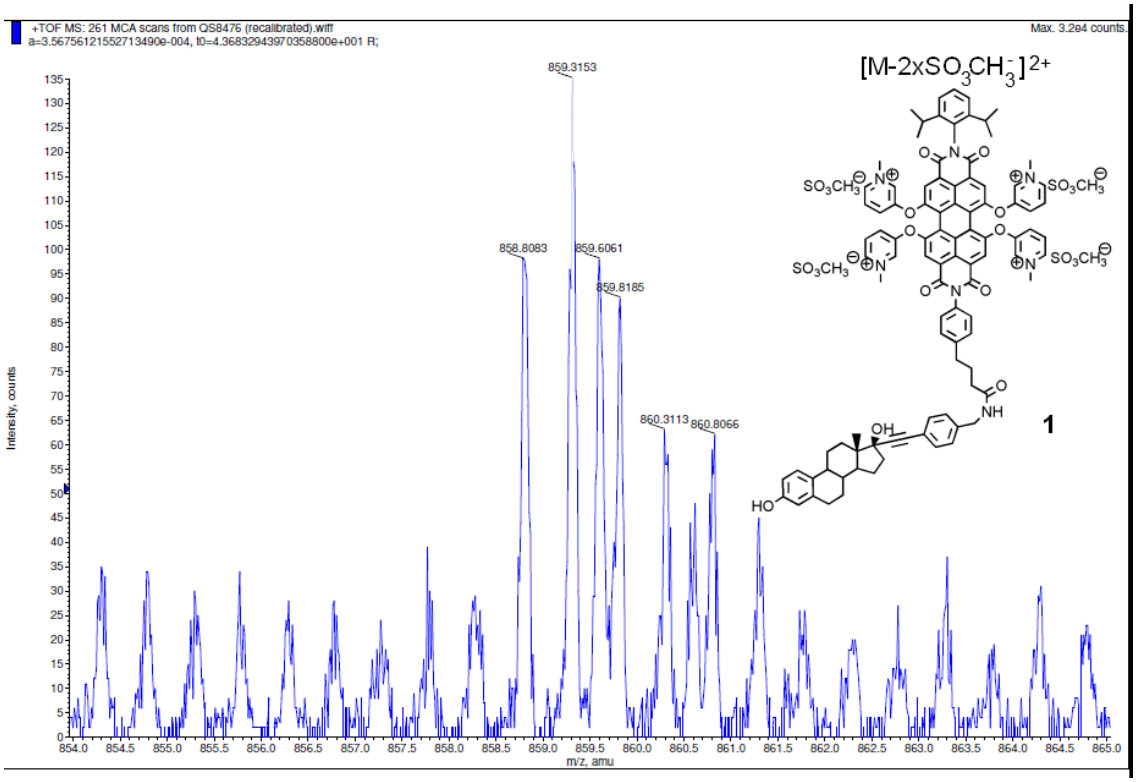
a=3.56756121552713490e-004, t0=4.36832943970358800e+001 R;
Max. 3.2e4 counts.
Intensity, counts
m/z, amu
110.0587
301.1427
116.0173
62.0085
71.0133
96.0427
140.9601
182.9491
218.9944
441.2959
513.5555
321.0500
243.0337
289.6216
425.5029
541.2200
583.2201
724.3018
859.3153
ESI+
Fase Ionizante: MeOH
1

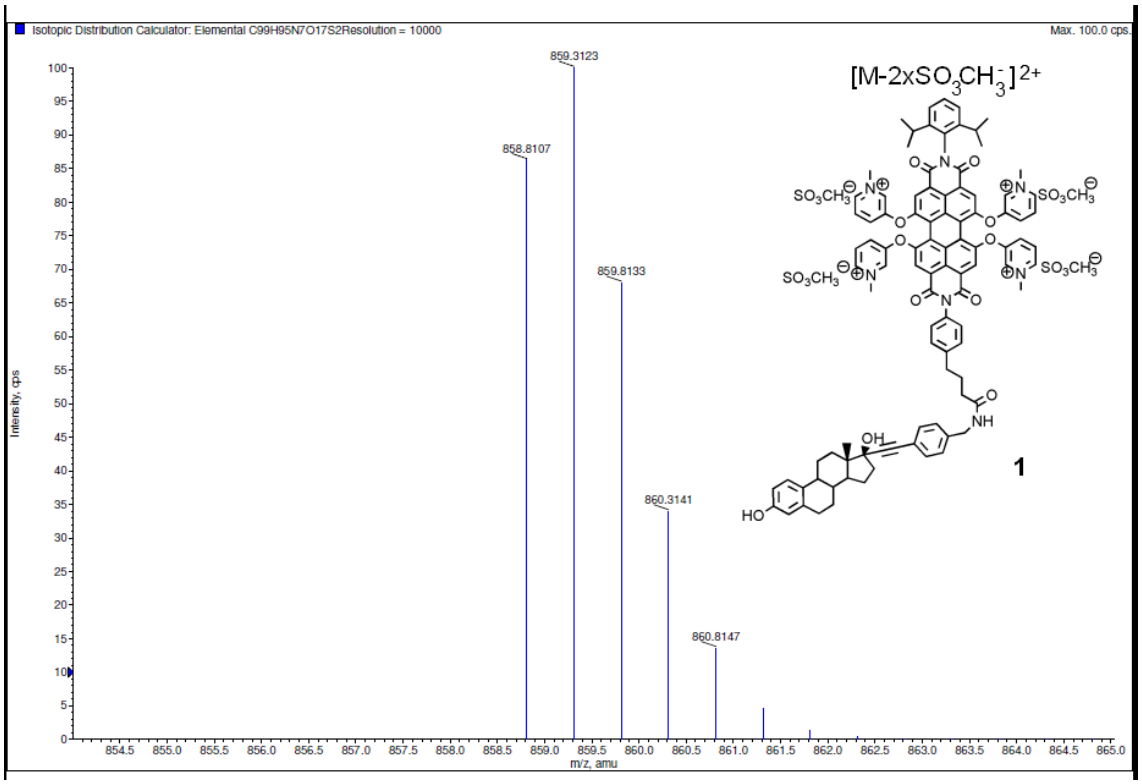

+TOF MS: 261 MCA scans from QS8476 (recalibrated).wiff
a=3.56756121552713490e-004, t0=4.36832943970358800e+001 R;
Max. 3.2e4 counts.
[M-4xSO3CH3-]4+
1
381.9250
382.1751
383.1493
383.4825
381.1592
382.4265
380.8219
381.6695
381.4200
382.6738
382.9214
Intensity, counts
m/z, amu


Isotopic Distribution Calculator: Elemental C97H89N7O11Resolution = 10000
Max. 100.0 cps.
[M-4xSO3CH3-]4+
1
382.1658
381.9150
382.4166
382.6673
Intensity, cps
m/z, amu


+TOF MS: 261 MCA scans from QS8476 (recalibrated).wiff
a=3.56756121552713490e-004, t0=4.36832943970358800e+001 R;
Max. 3.2e4 counts.
Intensity, counts
m/z, amu
541.2200
540.8852
541.5536
541.8862
542.2164
[M-3xSO3CH3-]3+
1


Isotopic Distribution Calculator: Elemental C98H92N7O14SResolution = 10000
Max. 100.0 cps.
Intensity, cps
m/z, amu
541.2146
540.8802
541.5488
541.8828
542.2168
[M-3xSO3CH3-]3+
1


+TOF MS: 261 MCA scans from QS8476 (recalibrated).wiff
a=3.567561215527134906-004, t0=4.368329439703588006+001 R;
Max. 3.2e4 counts.
Intensity, counts
m/z, amu
859.3153
858.8083
859.6061
859.8185
860.3113
860.8066
[M-2xSO3CH3-]2+
1


Isotopic Distribution Calculator: Elemental C99H95N7O17S2Resolution = 10000
Max. 100.0 cps.
Intensity, cps
m/z, amu
859.3123
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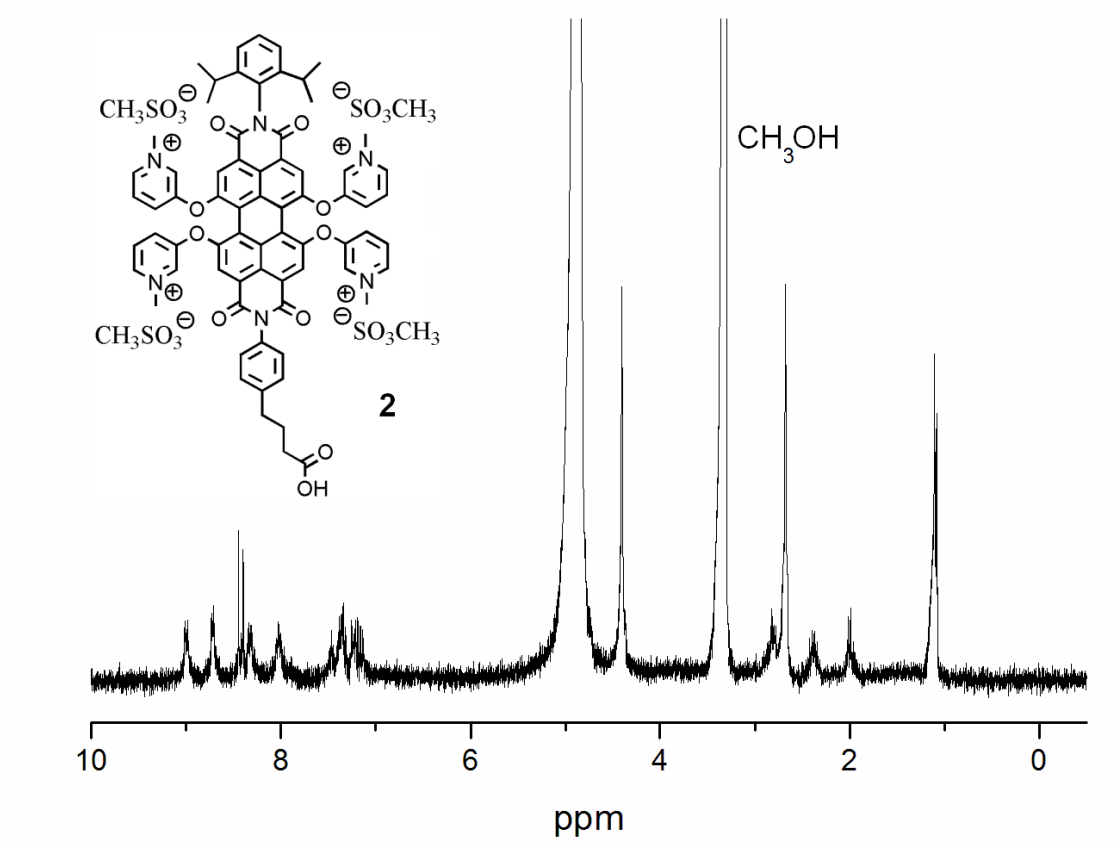
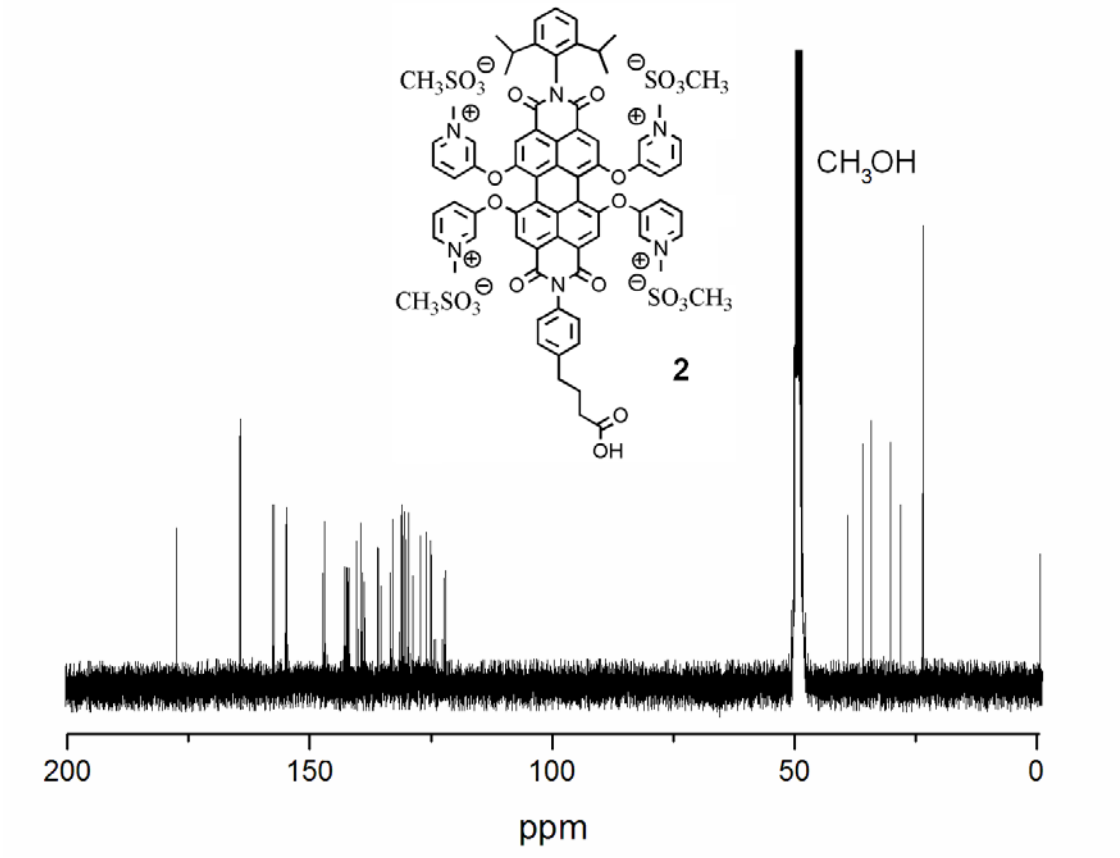
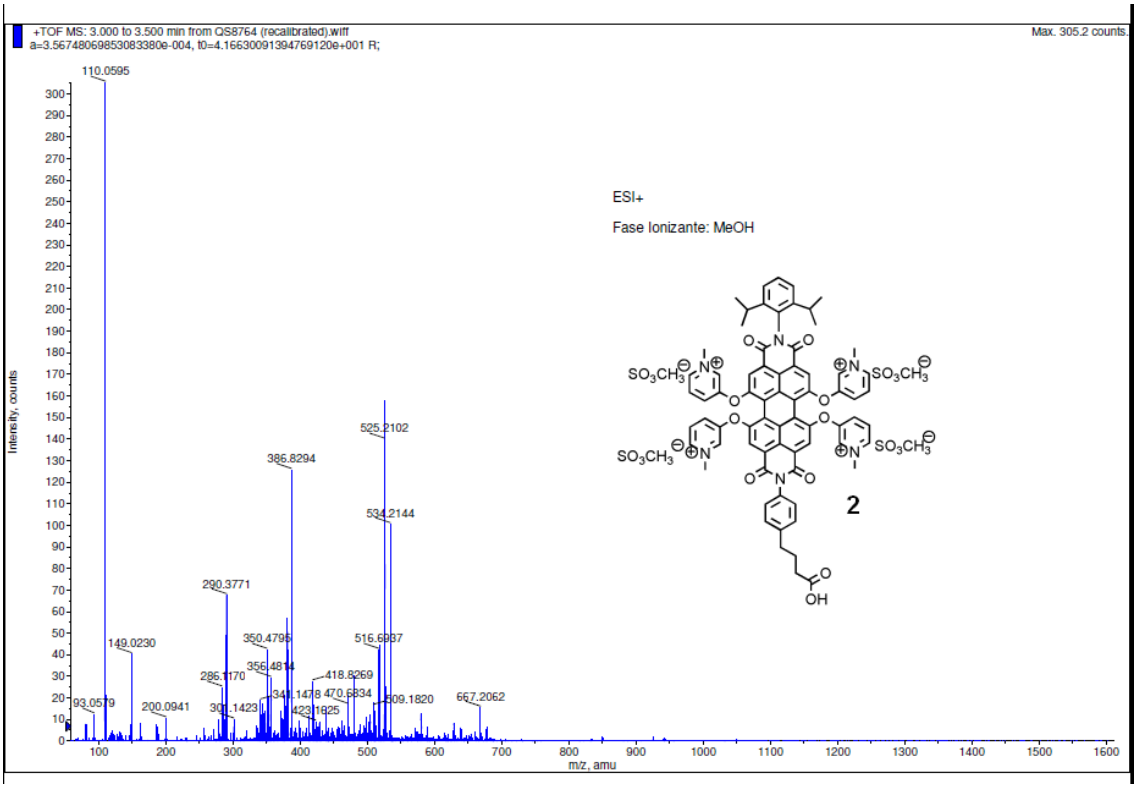
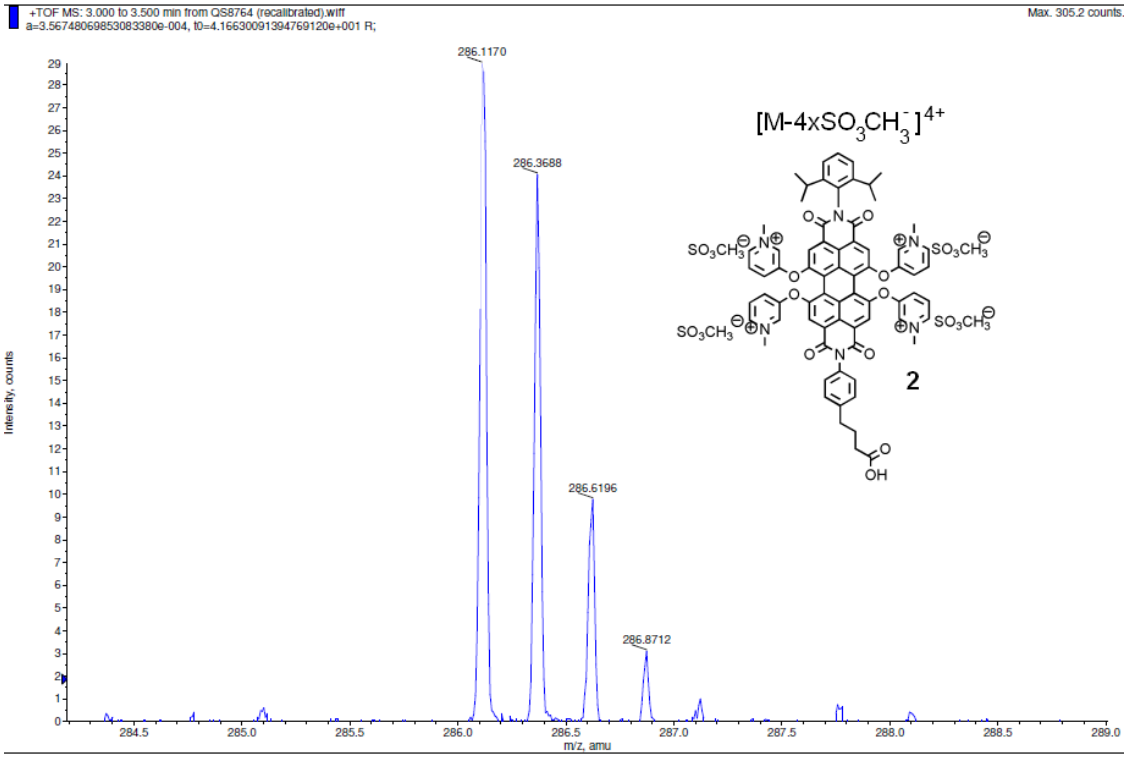
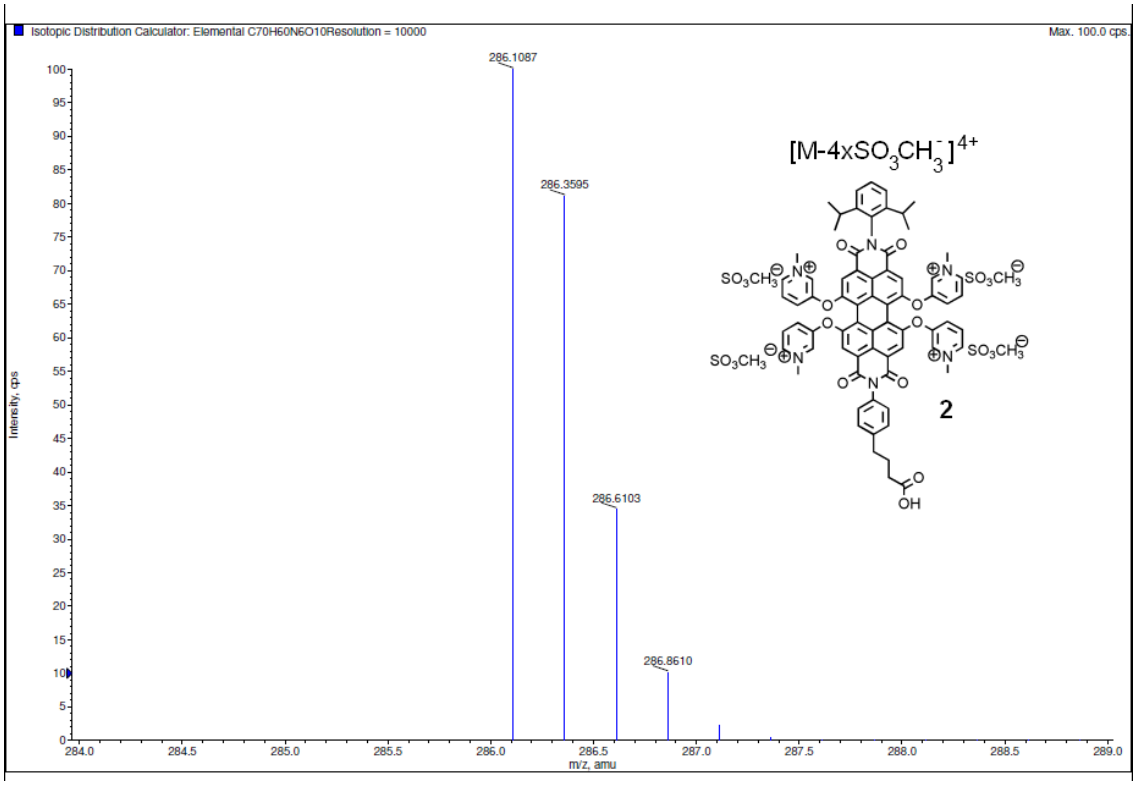
860.3141
860.8147
[M-2xSO3CH3-]2+
1

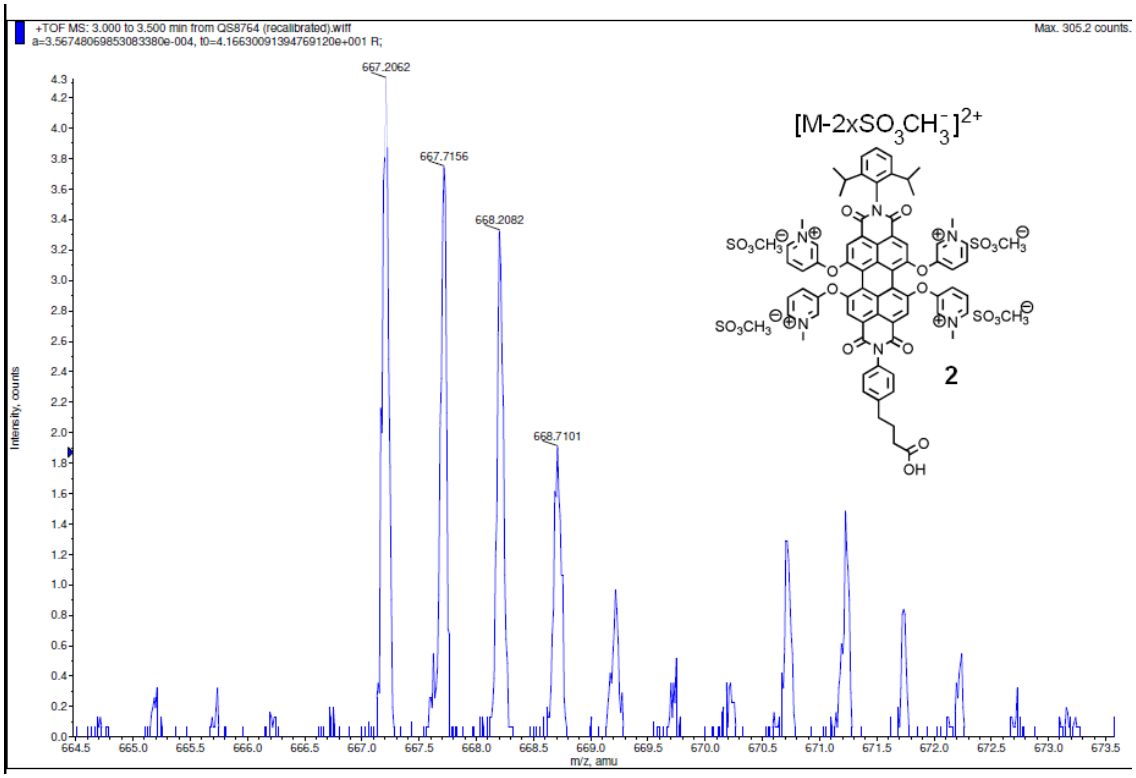

CH3SO3
SO3CH3
CH3SO3
SO3CH3
2
OH
CH3OH
10
8
6
4
2
0
ppm


CH3SO3
SO3CH3
CH3SO3
SO3CH3
2
OH
CH3OH
200
150
100
50
0
ppm


+TOF MS: 3.000 to 3.500 min from QS8764 (recalibrated).wiff
a=3.567480698530833 80e-004, t0=4.166300913947691 20e+001 R;
Max. 305.2 counts.
Intensity, counts
m/z, amu
110.0595
525.2102
386.8294
534.2144
290.3771
149.0230
350.4796
516.6937
356.4814
286.1170
418.8269
93.0579
200.0941
301.1423
341.1478
470.6834
423.1825
509.1820
667.2062
ESI+
Fase Ionizante: MeOH
2


+TOF MS: 3.000 to 3.500 min from QS8764 (recalibrated).wiff
a=3.567480698530833806-004, t0=4.166300913947691206+001 R;
Max. 305.2 counts.
286.1170
286.3688
286.6196
286.8712
[M-4xSO3CH3-]4+
2
Intensity, counts
m/z, amu


Isotopic Distribution Calculator: Elemental C70H60N6O10Resolution = 10000
Max. 100.0 cps.
286.1087
286.3595
286.6103
286.8610
[M-4xSO3CH3-]4+
2
Intensity, cps
m/z, amu


+TOF MS: 3.000 to 3.500 min from QS8764 (recalibrated).wiff
a=3.567480698530833380e-004, t0=4.166300913947691 20e+001 R;
Max. 305.2 counts.
667.2062
667.7156
668.2082
668.7101
Intensity, counts
m/z, amu
[M-2xSO3CH3-]2+
2

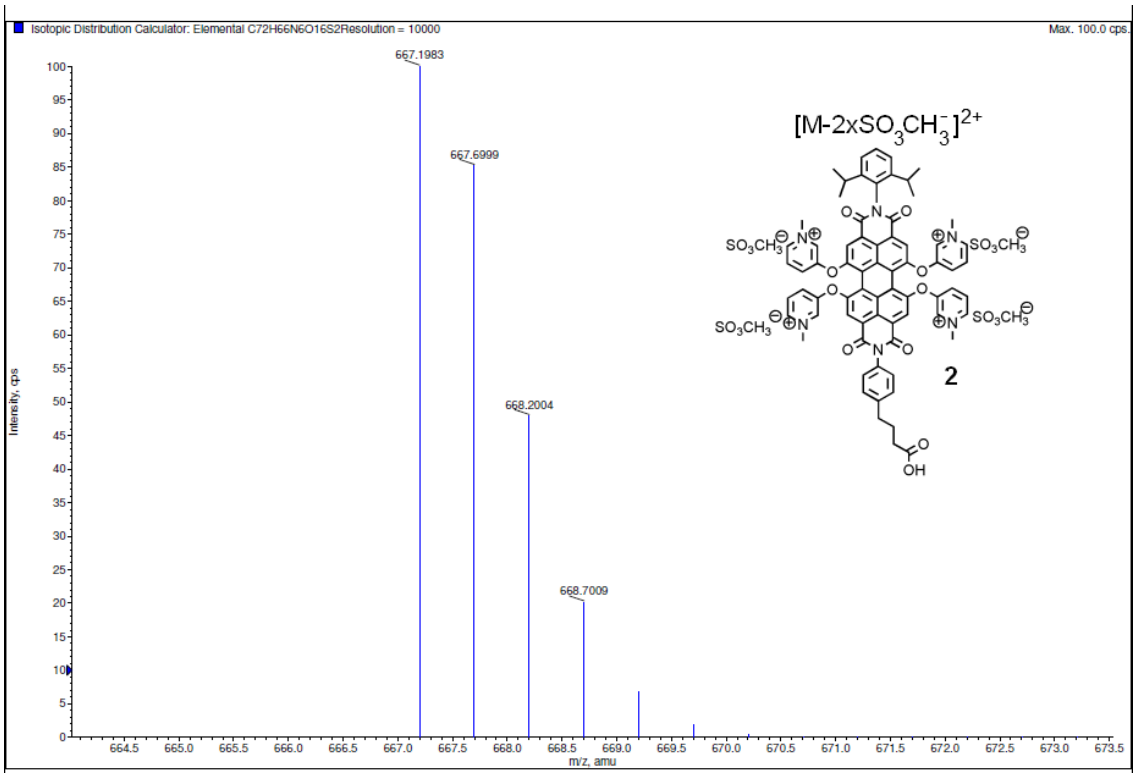
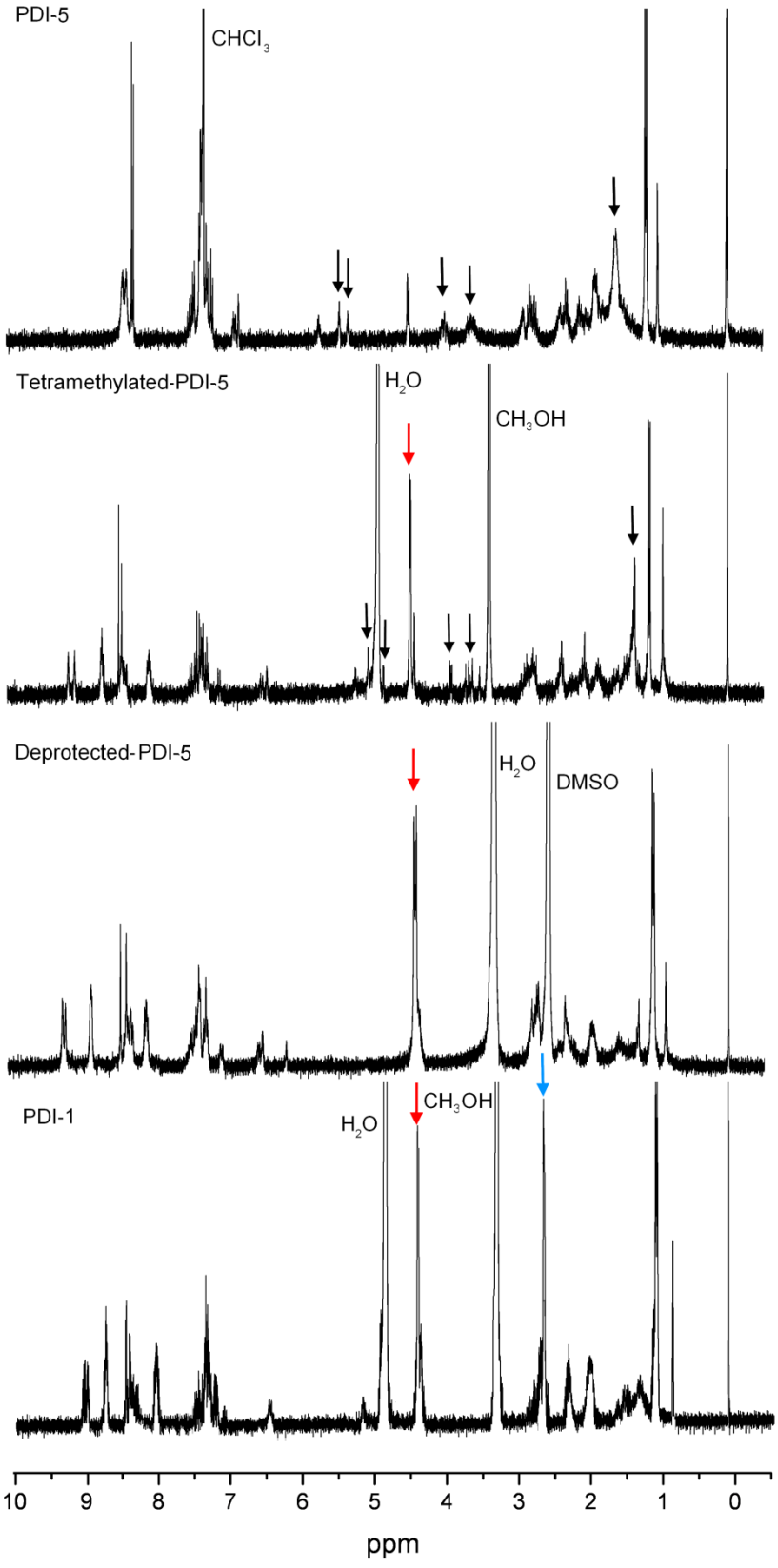

Isotopic Distribution Calculator: Elemental C72H66N6O16S2Resolution = 10000
Max. 100.0 cps.
667.1983
667.6999
668.2004
668.7009
Intensity, cps
m/z, amu
[M-2xSO3CH3-]2+
2

Figure 1. ^{1}H-NMR Monitoring to check tetramethylation, deprotection and anion exchange reactions yielding 1. Black arrows indicate the protons of the THP groups, red arrows indicate CH_3-piridinium protons, and blue arrow indicates the protons of CH_3-SO_3^-.



Materials and methods for the biological experiments.

MCF7 cells culture and Islet cells isolation

The breast cancer cell line, MCF7, is maintained in culture in Dulbecco's Modified Eagle's Medium (Sigma, Madrid, Spain) supplemented with $NaHCO_3$ 3.7g/L, 10% inactivated fetal bovine serum, 200 U/mL penicillin, 0.2 mg/mL streptomycin penicilum and 2 mM L-glutamine. One day before the experiment, around 150000 cells were plated in 12 mm covers. Pancreatic islets of Langerhans were isolated by collagenase (Sigma, Madrid, Spain) digestion as previously described.[3] Islets were dispersed into single cells with trypsin. Cells were then centrifuged and resuspended in RPMI 1640 without phenol-red (Invitrogen, Barcelona) and with 10% charcoal dextran treated fetal bovine serum (Hyclone, USA), 2 mM L-glutamine, 200 U/mL penicillin and 0.2 mg/mL streptomycin. Cells were then plated on coverslips and used within 24 hours of culture.

Cell labeling and immunocytochemistry

Cells were fixed with Bouin (Sigma, Madrid, Spain) for 2 min and then permeabilized with 0.1% Triton X-100, 5 min. For the immunostaining with the antibody against the estrogen receptor alpha (ERα), cells were fixed for 20 min with Bouin and then permeabilized for 15 min. Cells were then pretreated with 5% bovine serum albumin (BSA) for 1 h at room temperature, to block non-specific staining. Afterwards, the different perylenes, namely 1 and 2, were added to the cells in PBS with 1% BSA and incubated overnight at 4 ºC. After this, cells were washed several times with PBS, left to dry and mounted using Prolong® Gold Antifade Reagent (Invitrogen, Barcelona, Spain). For the ERα immunostaining, the anti-ERα G-20 was used at 1:100 (sc-544, Santa Cruz), followed by incubation with a secondary antibody anti-rabbit 488 (1:500, Alexa FluorR, Invitrogen). Images were obtained using a confocal Zeiss Pascal 5 microscope with Zeiss 40X and 63X objectives (numerical aperture=1.3 and 1.25 respectively). Images were analyzed using the LSM Zeiss software (Zeiss, Jena, Germany). Perylene molecules were excited using the 488nm laser line and emitted light was collected using a long-pass filter LP530.

References.

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(3) Nadal, A.; Rovira, J. M.; Laribi, O.; Leon-Quinto, T.; Andreu, E.; Ripoll, C.; Soria, B. *FASEB J* **1998**, *12*, 1341-1348.