Electronic supplementary information (ESI)

Chemicals and materials

All the commercial reagents: *N*-hydroxy succinimide (Acros Organics), dicyclohexyl carbodiimide (Acros Organics), amino caproic acid (Acros Organics), 3,4-dihydroxy benzoic acid (Alfa Aesar), pyrene butyric acid (Alfa Aesar), fluorescein isothiocyanate (Aldrich), and other standard organic/inorganic chemicals, solvents and materials were used without further purification. All experiments were carried out in ultrapure water (18.2 MΩ·cm; Barnstead NANOpure Diamond). Some chemicals were synthesized in-house according to published procedures. 4-(3-aminopropylaminothiocarbonylamino)fluorescein **12** was synthesized by using the literature procedure.[1] Methyl 6-aminocaproate **6** was synthesized by using the literature procedure.[2] NHS ester of 1-pyrenebutyric acid (**13**) was synthesized by using the literature procedure.[3] Carbon paper composed of carbon fibers (Spectracarb^{TM} 2050L-1050; Fuel Cell Store, TX), was used as the electrode material.

Instrumentation

Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded in BRUKER 400 MHz NMR instrument. Mass spectra were recorded in Waters Micromass Q-Tof micro mass spectrometer. All fluorescence measurements were done by using VARIAN CARY Eclipse fluorescence spectrophotometer. Electrochemical

measurements were carried out with an ECO Chemie Autolab PASTAT 10 electrochemical analyzer, using the GPES 4.9 (General Purpose Electrochemical System) software package.

Abbreviations/molecular formulas

a.u. – Arbitrary Units measured by VARIAN CARY Eclipse fluorescence spectrophotometer
FITC – Fluorescein isothiocyanate.
$CHCl_3$ – Chloroform
$SOCl_2$ – Thionyl chloride
TEA –Triethylamine
THF – Tetrahydrofuran
MeOH – Methanol
NaOH – Sodium Hydroxide
DCC – *N*,*N'*-dicyclohexylcarbodiimide
DCU – Dicyclohexylurea
NHS – *N*-hydroxysuccinimide
MeCN – Acetonitrile
DMF – *N, N*-Dimethylformamide
RT – Room temperature
Na_2SO_4 –Sodium Sulfate
^{1}H NMR – Proton nuclear magnetic resonance
^{13}C NMR – Carbon -13 nuclear magnetic resonance
RPM – Rotations per minute
FITC amine – 4-(3-aminopropylaminothiocarbonylamino)fluorescein
FITC aryl monoester ester **1a** – Thiourea, *N*-(3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-5-yl)-*N'*-3-[6-({4-[4-(1-pyrenyl)butyroyloxy]-3-hydroxybenzyoylamino}hexanoylamino)propyl]-
FITC aryl monoester ester **1b** – Thiourea, *N*-(3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-5-yl)-*N'*-3-[6-({3-[4-(1-pyrenyl)butyroyloxy]-4-hydroxybenzyoylamino}hexanoylamino)propyl]-
Pyrene FITC amide **3** – Thiourea, *N*-(3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-5-yl)-*N'*-{3-[4-(1-pyrenyl)butyroylamino]propyl-
FITC catechol **2** – Thiourea, *N*-(3',6'-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthen]-5-yl)-*N'*-3-[6-({3,4-dihydroxybenzyoylamino}hexanoylamino)propyl]-

Quantification experiments and determination of kinetics of hydrolysis of of aryl monoester FITC 1 under basic conditions.

Quantification of FITC-catechol 2 by fluorescence.

Calibration curve for dependence of fluorescence intensity from concentration of FITC-catechol **2** was done for its solutions at pH 7.2 at concentrations 5-40 nM.

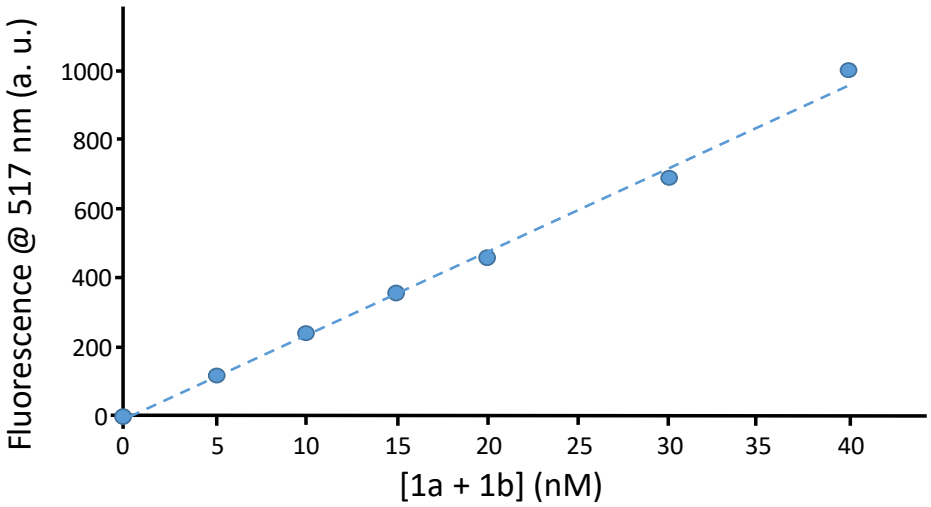


Figure **S1**

Quantification of FITC Pyrene amide 3 by fluorescence.
Calibration curve for dependence of fluorescence intensity from concentration of Pyrene FITC amide **3** was done for its solutions at pH 7.2 at concentrations 5-30 nM.

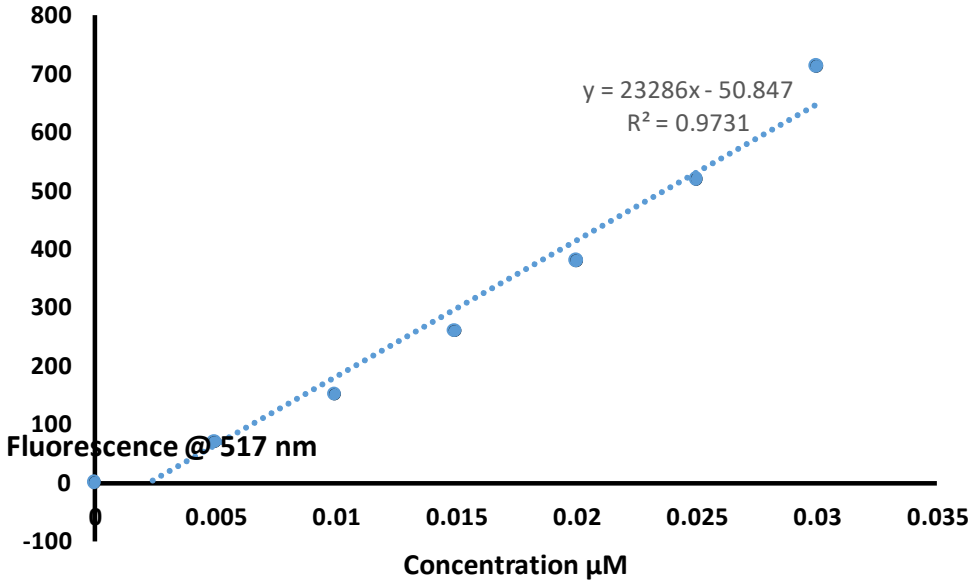


Figure **S2**

Determination of pH dependence of fluorescence intensity of FITC-catechol 2
To measure concentration of FITC-catechol **2** in solutions with pH above 7.2 the dependence of its fluorescence intensity from pH was determined and used in experiments below.

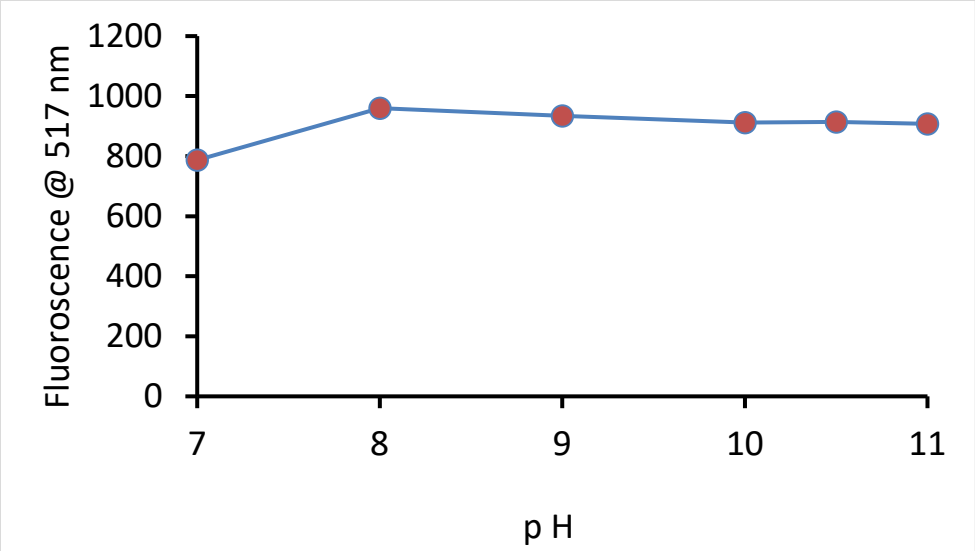


Figure **S3**

Determination of kinetics of hydrolysis of of FITC aryl monoester 1 under basic conditions in solution phase.

Solution of aryl monoester FITC **1** in DMSO (10 mM, 1 µL) was added to pH 11 ammonia solution (50 mM, 100 µL) and immediately a sample was drawn for analysis by mass spectrometry (ESI negetive mode). The remaing reaction mixture was left react and aliquots were drawn for mass spectrometric analysis after 5 min and 3 hours. The aryl monoester FITC 1 was completely hydrolysed after 3 hours of reaction.

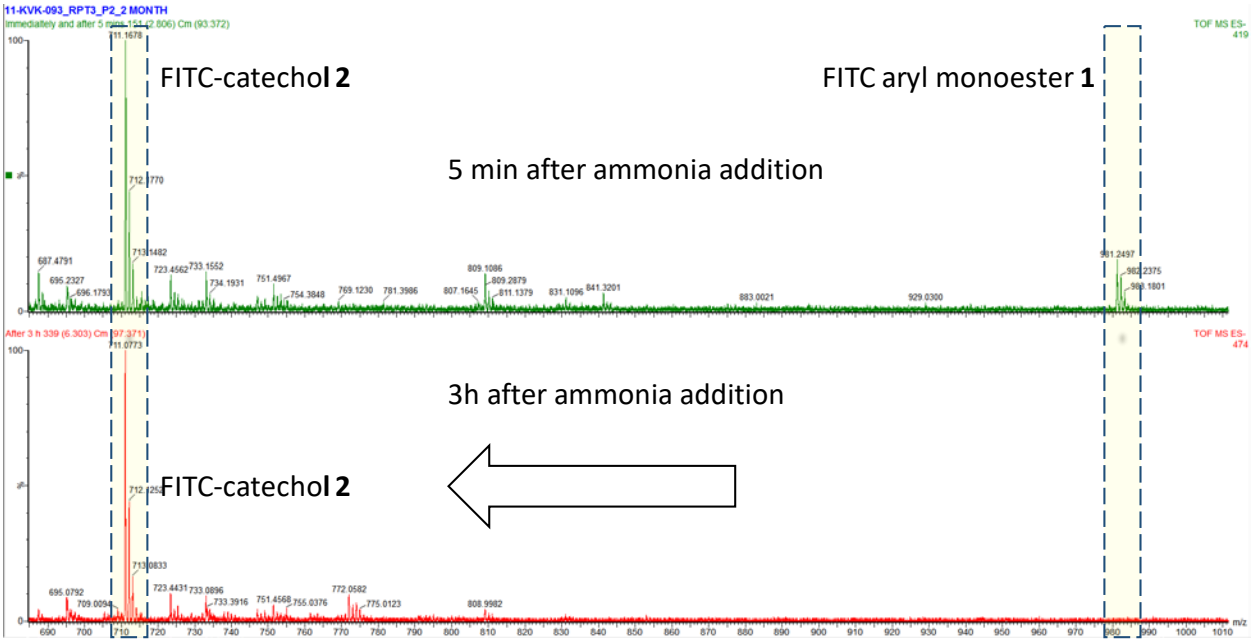


*Figure **S4**. ESI-MS (negative mode) (above) of a solution of FITC aryl monoester **1a**+**1b** (M-H⁻ 981.2192 Da) 5 min after addition of ammonia solution; (below) 3 h after addition, only M-H⁻ ion of FITC-catechol **2** at 711.0773 Da can be observed.*

Quantification of the amount of FITC aryl monoester 1a,b loaded to the graphene electrode.

Graphene-functionalized carbon fiber electrode (25 mm^2 geometrical area) was casted with 20 µL of FITC aryl monoester **1** (0.25 mM in DMSO) and left for 1 h at room temperature in dark. The electrode was rinsed with DMSO and soaked in DMSO for 2 mins. The electrode was rinsed again with DMSO and washed with phosphate buffer (3.0 mM pH 7.0) to completely remove DMSO. The electrode with adsorbed FITC aryl monoester **1** was immersed into pH 7.2 50 mM phosphate buffer (8 mL) and pH of the solution was adjusted to 11 at time 90 min. The fluorescence of the released FITC-catechol **2** in the electrolyte solution was measured after each interval by taking an aliquot (1 mL of the total 8 mL volume). After measuring the fluorescence of the aliquot (excitation at 490 nm) it was returned into the cell to continue the experiment. The measured fluorescence intensity values in a.u. were concerted into the absolute amounts of the released FITC-catechol **2** using calibration curve (Fig. S1) and adjusted for the pH and the total volume of the solution (8 mL). The calculated dependence of the total amount of FITC-catechol **2** from time is shown on Fig. S5. The total amount of the released FITC-catechol **2** after 900 min was 0.34 nanomoles.

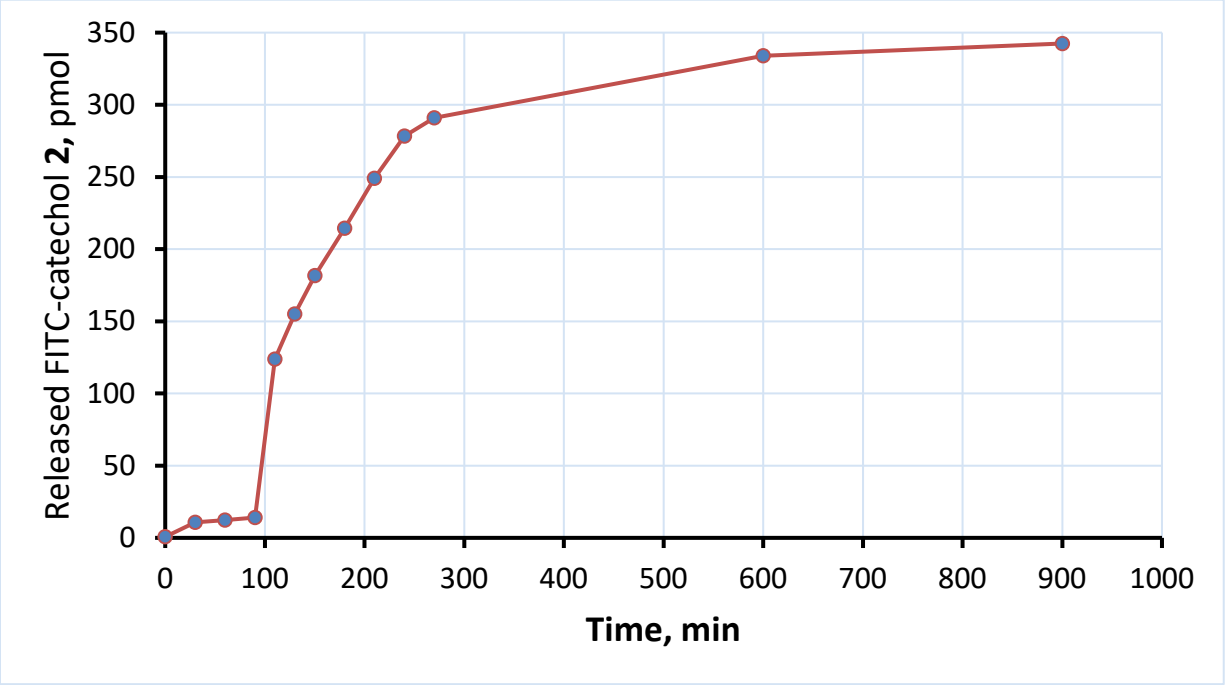


Figure S5. Estimation of amount of **FITC aryl monoester 1 (a** and **b)** adsorbed on the electrode by its fluorescence after its complete hydrolysis to FITC-catechol **2** at pH 11 after 900 min (phosphate buffer 50 mM, pH 11.0)

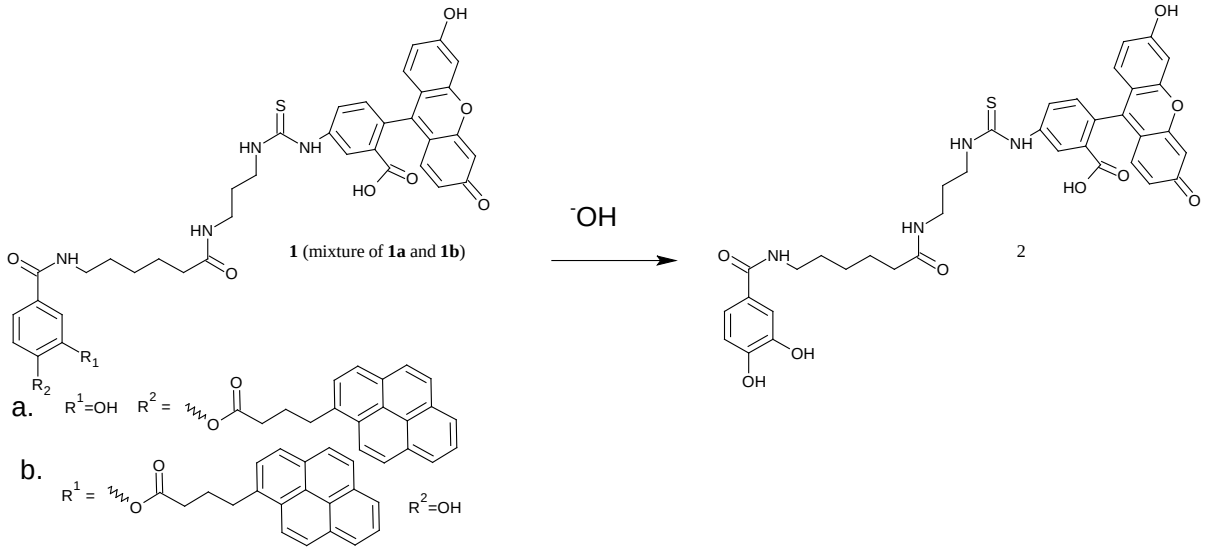
Electrochemical Experiments

Modification of the carbon fiber electrode with graphene nanosheets. The carbon fiber electrode was washed in isopropanol for 15 minutes. Cyclic voltammetry was performed from –0.5 V to +3.0 V with 4 cycles at 50 mV/s in phosphate buffer (25 mM pH 6.9) by immercing 25 mm^2 geometrical area of the electrode under stirring at 400 rpm followed by applying –1 V for 120 s in the same buffer under stirring at 400 rpm. The electrode was washed for 15 minutes in isopropanol. This procedure has been developed and extensively used in our previos studies.[4,5]

Cyclic voltammetry in the absence and presence of O_2. Graphene-functionalized carbon fiber electrode was immersed in phosphate buffer (3.0 mM – note the low buffer concentration, pH 7.2 containing 0.1 M NaCl) and then three cycles of cyclic voltammetry from 0 V to –1 V at scan rate 50 mV/s in the absence and presence of oxygen were performed. Based on the cathodic wave of the O_2 reduction, the reduction process can proceed at potentials more negative than –0.2 V.

Loading of aryl monoester FITC 1 to the electrode surface.
Graphene-functionalized carbon fiber electrode (25 mm^2 geometrical area) was casted with 20 µL of aryl monoester FITC **1** (0.25 mM in DMSO) and left for 1 h at room temperature in dark. The electrode was rinsed with DMSO and soaked in DMSO for 2 mins. The electrode was rinsed again with DMSO and washed with phosphate buffer (3.0 mM pH 7.0) to completely remove DMSO. The resultant electrode was immersed in phosphate buffer (3.0 mM, pH 7.0) for overnight under moderate shaking to wash away the excess reagent. The buffer was changed and the electrode was immersed in a phosphate buffer pH (3.0 mM, pH 7.2 containing 0.1 M NaCl) and the leakage was monitored until the leakage was stopped.

Electrochemically induced hydrolysis of aryl monoester FITC 1a,b and release of FITC-catechol 2.



The electrode with the adsorbed aryl monoester FITC **1** was placed into electrolyte solution (4 mL 3.0 mM pH 7.2 phosphate buffer containing 0.1 M NaCl). The fluorescence of the background solution was measured after each interval of the potential application (excitation wavelength: 490 nm, emission: 500-600 nm). Negative potentials (–0.4, –0.5, –0.6, –0.7 and –0.8 V) were applied in the presence of oxygen in the phosphate buffer (3.0 mM pH 7.2 containing 0.1 M NaCl) in 20 mins intervals. The fluorescence of the released FITC-catechol **2** in the electrolyte solution was measured after each interval by taking an aliquot (1 mL of the total 4 mL volume). After measuring the fluorescence of the aliquot at 517 nm (excitation at 490 nm) it was returned into the cell to continue the experiment. The measured fluorescence intensity values in a.u. were converted into the absolute amounts of the released FITC-catechol **2** using calibration curve (Fig. S1) and

adjusted for the total volume of the solution (4 mL for the release). The calculated dependence of the absolute amount of FITC-catechol **2** from time for electrode potential -0.5 V is shown on Fig. 3 (left) on the main text. It was observed that the the fluorescein electrochemically gets reduced[6] and loses fluorescence if potentials more negative than –0.5 V were applied. However, the release of FITC quinol **2** was found to be slower at potentials more positive than –0.5 V. Based on these results, the potential of –0.5 V was selected as the optimum for the electrochemical release process.

Release of FITC-catechol 2 by bulk pH change:
The electrode with the adsorbed aryl monoester FITC **1** was immersed in 50 mM phosphate buffer (4-6 mL at pH 8, 9, 10, 10.5 or 11) under gentle shaking resulting in gradual release of FITC-catechol **2**. Aliquots (1 mL) were taken from the solution at 20 mins time intervals, their fluorescence was measured at excitation 490 nm, and aliquots were then returned into the solution. The measured fluorescence intensity values in a.u. were converted into the absolute amounts of the released FITC-catechol **2** using calibration curve (Fig. S1) and adjusted for the total volume of the solution and the measured pH (Fig. S3).

Control experiments:
A set of control experiments was performed by following the exact same procedure as explained before (for electrochemical release and release by bulk pH change) but using a compound (Pyrene FITC amide **3**) which is analogous to aryl monoester FITC **1** but lacks a hydrolyzable ester bond. The fluorescence of the released Pyrene FITC amide **3** in the electrolyte solution was measured after each interval by taking an aliquot (1 mL of the total 4 mL volume). After measuring the fluorescence of the aliquot (excitation at 490 nm) it was returned into the cell to continue the experiment. The measured fluorescence intensity values in a.u. were concerted into the absolute amounts of the released Pyrene FITC amide **3** using calibration curve (Fig. **S2**) and adjusted for the total volume of the solution. The calculated dependence of the absolute amount of Pyrene FITC amide **3** from time for electrode potential -0.5 V is shown on Fig. 3 (left).

Dependency of the kinetics of electrochemically induced hydrolysis of aryl monoester FITC 1 and release of FITC-catechol 2 from concentration of oxygen.

The electrode with the adsorbed aryl monoester FITC **1** was placed into electrolyte solution (4 mL 3.0 mM pH 7.2 phosphate buffer containing 0.1 M NaCl) purged with argon. The fluorescence of the released FITC-catechol **2** in the electrolyte solution was measured after each interval by taking an aliquot (1 mL of the total 4 mL volume). After measuring the fluorescence of the aliquot (excitation at 490 nm) it was returned into the cell to continue the experiment. At time 90 min a potential -0.5 V was applied to the electrode and at time 150 min the electrochemical cell of oxygenated by air bubbling. The measured fluorescence intensity values in a.u. were concerted into the absolute amounts of the released FITC-catechol **2** using calibration curve (Fig. **S1**) and adjusted for the total volume of the solution (4 mL for the release). The calculated dependence of the absolute amount of FITC-catechol **2** from time is shown on Fig. **S6** (Curve A).
The experiment was repeated under identical conditions with electrolyte with a larger buffer capacity (50 mM instead of 3 mM). The calculated dependence of the absolute amount of FITC-catechol **2** from time is shown on Fig. **S6** (Curve B) resulting in dramatic decrease of rates of release.

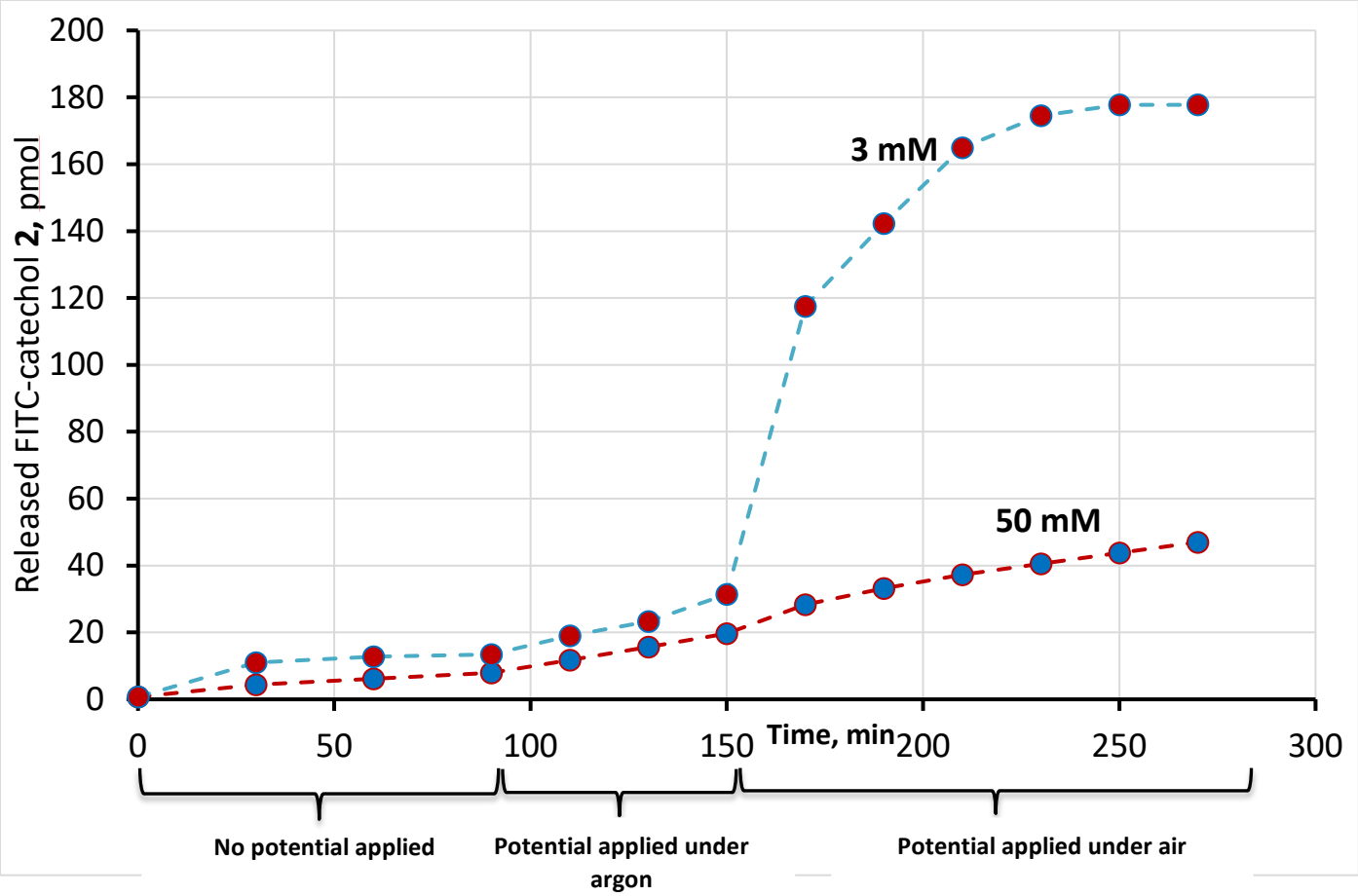
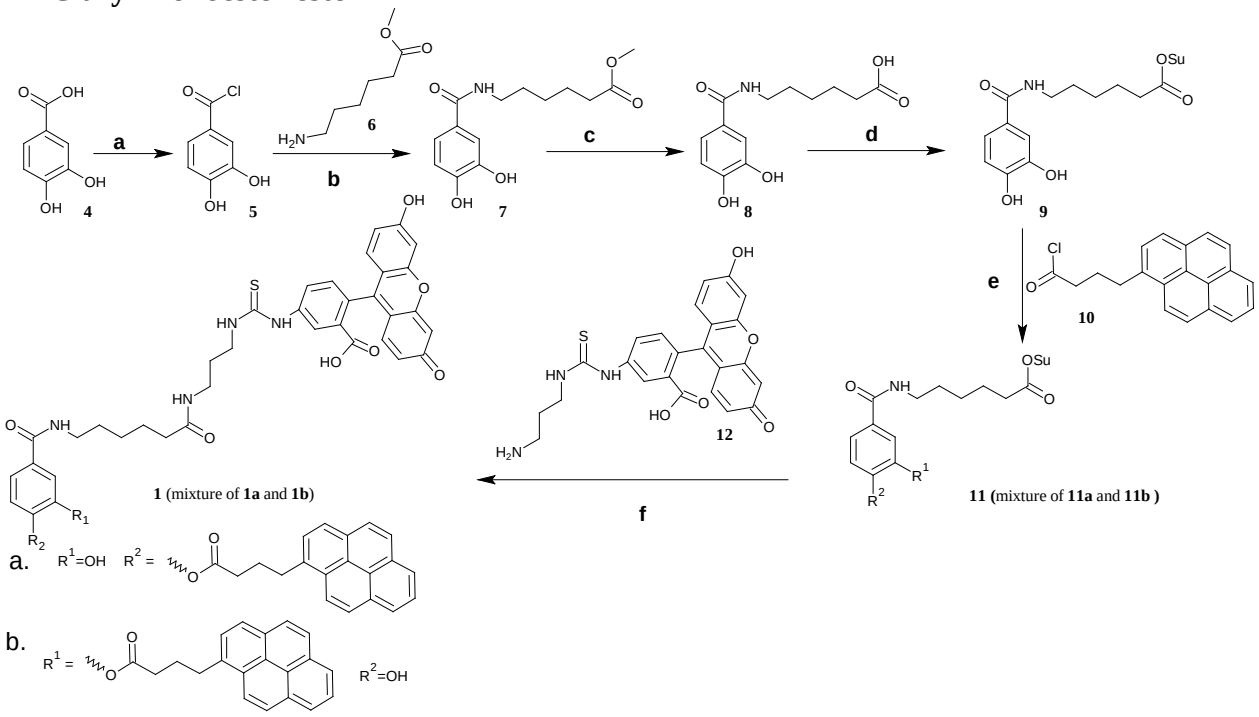


Figure S6: Electrochemical release of Quinol FITC (1a and b) in phosphate buffer pH 7.2 and concentrations 3 mM (Curve A) and 50 mM (Curve B).

Syntheses of compounds and their characterization
FITC aryl monoester ester 1



*Synthetic scheme for aryl monoester FITC reagent **1**: (a) $SOCl_2$, $CHCl_3$, 60°C, 1 h; (b) TEA, THF, room temperature, 2 h; (c) 1: 1 mixture of MeOH and water, NaOH, 0 °C to room temperature; (d) DCC, N-hydroxysuccinimide. THF, room temperature; (e) MeCN, 2,6-Lutidine, room temperature; (f) DMF-water, 2,6-Lutidine, room temperature.*

Synthesis of 3,4-dihydroxybenzoyl chloride (5). Thionyl chloride (10 mL) was added dropwise to a stirred suspension of 3,4-dihydroxybenzoic acid (3 g, 19.4 mmol) in chloroform (10 mL), followed by 2 drops of DMF. The resulting reaction mixture was stirred at 60 °C for 1 h. The reaction mixture was concentrated under reduced pressure to remove excess thionyl chloride. The residue was used without any purification and characterization.

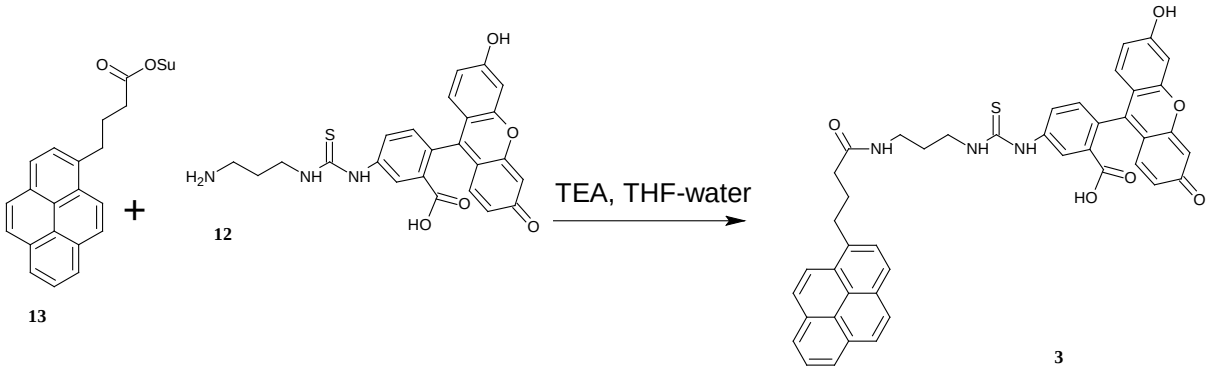
Synthesis of pyrene aryl monoester ester 11. Methyl 6-aminocaproate **6** (3.8 g; 20.9 mmol) was added to a stirred solution of 3,4-dihydroxybenzoyl chloride **5** (3 g; 17.38 mmol) in THF (30 mL) at 0–5 °C, and stirred at room temperature for 2 hrs. The reaction mixture was acidified with HCl and extracted by ethyl acetate (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to get crude methyl 6-[(3,4-dihydroxybenzoyl)amino]hexanoate (**7**). The residue was dissolved in methanol (20 mL) and a solution of sodium hydroxide (1.3 g, 3.25 mmol) was added. The resultant reaction mixture was stirred at room temperature for 3 h, acidified with dilute hydrochloric acid (HCl), and extracted by ethyl acetate (20 mL × 3 times) . The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get crude 6-[(3,4-dihydroxybenzoyl)amino]hexanoic acid (**8**). The crude acid **8** was dissolved in THF (30 mL), *N*-hydroxysuccinimide (1.35 g, 11.7 mmol) was added, followed by the addition of a solution of dicyclohexylcarbodiimide (DCC) (3.6 g, 17.4 mmol) in THF (10 mL). The resultant reaction mixture was stirred at room temperature until the reaction was complete (~2

h) and filtered to remove the precitiate of *N, N'*-dicylohexylurea. The organic layer was concentrated under reduced pressure to yield the crude product as a gummy oil. It was purified by silica gel column chromatography (6 % methanol in chloroform) to yield active ester **9**. A solution of pyrene butyryl chloride (**10**, 84 mg, 0.27 mmol) in chloroform (1 mL) was slowly added to a stirred ice-cooled solution of active ester **9** (200 mg; 0.55 mmol) and 2,6-lutidine (235 mg, 2.1 mmol) in acetonitrile (2 mL), and stirred at room temperature for 1 h. The reaction mixture was concetrated under reduced pressure and purified by silica gel column chromatography (60 % ethylacetate in hexane) to yield aryl monoester **11** as a mixture of isomers **11a** and **11b** (87mg, 25%). ^{1}H NMR (400 MHz, chloroform-*d* δ ppm 1.29 (d, *J*=7.05 Hz, 1 H) 1.37 (br. s., 1 H) 1.49 (br. s., 2 H) 1.60 - 1.76 (m, 2 H) 2.17 - 2.36 (m, 2 H) 2.39 - 2.55 (m, 2 H) 2.55 - 2.83 (m, 6 H) 3.32 (br. s., 2 H) 3.41 (d, *J*=6.29 Hz, 2 H) 6.58 (br. s., 1 H) 6.95 (d, *J*=7.81 Hz, 1 H) 7.40 - 7.58 (m, 1 H) 7.76 - 7.90 (m, 1 H) 7.91 - 8.21 (m, 7 H) 8.28 (t, *J*=9.57 Hz, 1 H); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 24.12, 25.45, 26.69, 28.62, 30.75, 32.45, 33.55, 39.72, 117.02, 122.27, 123.29, 124.85, 125.89, 126.74, 127.47, 128.69, 129.94, 130.83, 131.34, 135.54, 138.28, 151.32, 168.58, 169.40, 169.65, 171.96.

FITC aryl monoester ester 1.

A solution of FITC amine **12** (10 mg, 0.02 mmol) in water (200 µL) was added to a stirred ice-cooled solution of aryl monoester esters **11a** and **11b** (20 mg, 0.03 mmol) in DMF (200 µL), and the solution was stirred at room temperature for 1 h. The reaction mixture was purified by silica gel column chromatography (6-12 % methanol in chloroform) to yield FITC aryl monoester ester **1** (1 mg, 3%) as a mixture is isomers **1a** and **1b**. ^{1}H NMR (400 MHz, chloroform -*d*) δ ppm 1.07 - 1.26 (m, 4 H) 1.3-1.6 (m, 4 H) 1.90-2.10 (m, 2 H) 2.10-2.50 (m, 2 H) 2.53- 2.70 (m, 4 H) 3.10-3.20 (m, 2 H) 3.30-3.41 (m, 2 H) 3.50-3.65 (m, 2 H) 6.42 (d, *J*=6.80 Hz, 1 H) 6.50-6.60 (m, 2 H) 6.77 - 6.91 (m, 1 H) 7.00 (br. s., 1 H) 7.08-7.17 (m, 1H), 7.17 - 7.26 (m, 1 H) 7.32 - 7.53 (m, 2 H) 7.70-8.10 (m., 11 H) 8.13 - 8.31 (m, 1 H). ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm - 17.93, 23.10, 25.38, 26.89, 28.88, 29.76, 30.85, 32.64, 33.61, 36.18, 102.91, 110.49, 112.30, 112.90, 121.30, 123.4, 125.01, 125.96, 126.81, 127.56, 129.39, 130.11, 131.00, 131.49, 135.77, 138.38, 141.37, 152.11, 153.16, 156.98, 170.00, 174.94. $[M-H]^{-}$ = 981.3145 Da

Synthesis of Pyrene FITC amide 3.



*Synthetic scheme for Pyrene FITC amide **3**.*

To an ice-cooled stirred solution of NHS ester or 4-pyrenebutyric acid **13**[3](20 mg, 0.05 mmol) in THF (0.5 mL) was added a solution of triethylamine (7 mg, 0.07 mmol) and FITC amine **12** (10 mg, 0.02 mmol) in water (0.5 mL). The reaction mixture was warmed to room temperature, stirred

for 1 h, and diluted in dichloromethane (2 mL). The solution was washed with 1 M HCl, the organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to get the crude product. It was further purified by silica gel column chromatography (12% methanol in chloroform) to get the Pyrene FITC amide **3** (9 mg, 60% yield). ^{1}H NMR (400 MHz, chloroform-*d*) δ ppm 1.10-1.30 (m, 3 H) 1.62-1.85 (m, 2 H) 1.20-2.35 (br. s., 2 H) 3.10 – 3.30 (m, 2 H) 3.55-3.65 (m, 2 H) 6.5-6.80 (m, 4 H) 7.06-7.20 (m, 1 H) 7.30 - 7.42 (br. S., 1 H) 7.70 - 7.82 (m, 1 H) 7.91 - 8.50 (m, 9 H) 10.10-10.22 (br. S., 2 H); ^{13}C NMR (100 MHz, CHLOROFORM-*d*) δ ppm 25.69, 28.04, 32.73, 35.52, 36.64, 79.63, 83.46, 102.69, 110.18, 115.44, 121.33, 124.61, 125.42, 126.60, 126.97, 127.92, 129.50, 129.77, 130.89, 131.34, 137.00, 152.34, 159.92, 168.99, 173.24. $[M-H]^-$ = 732.2162 Da.

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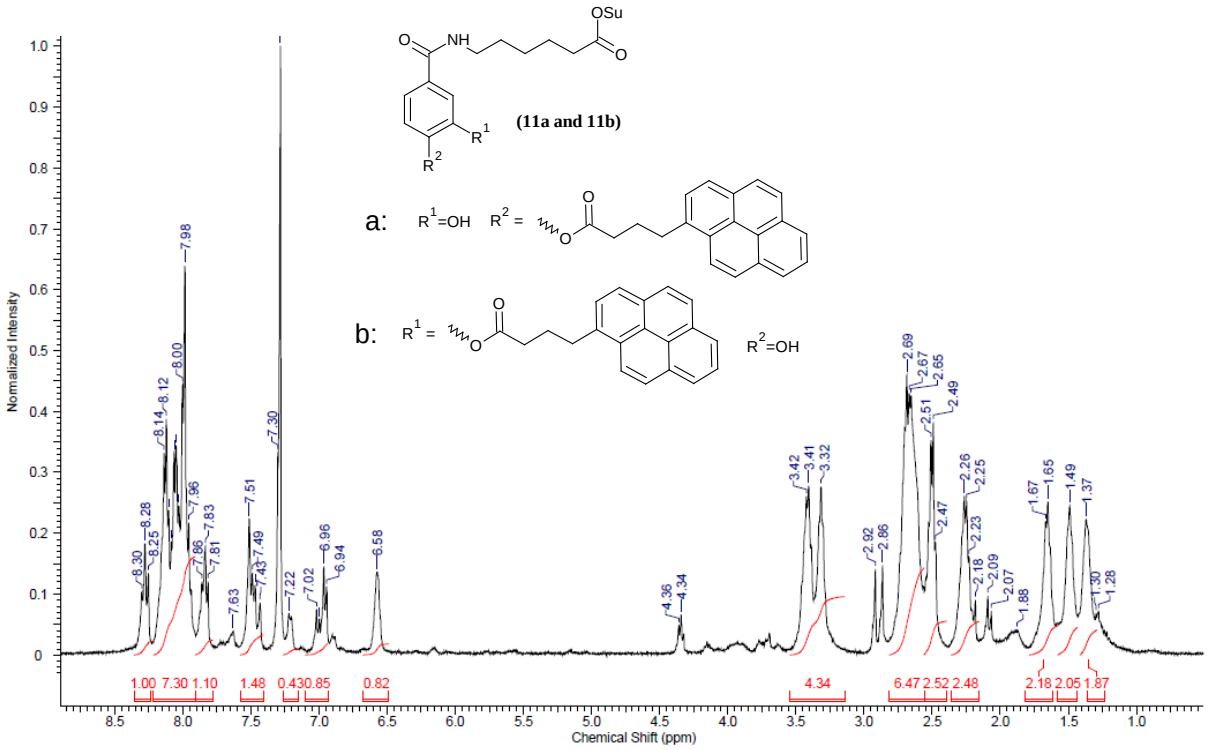
6. R. G. Compton, D. Mason and P. R. Unwin, *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases*, 1988, **84**, 483–489

Spectral data for FITC aryl monoester ester 1

^{1}H NMR:



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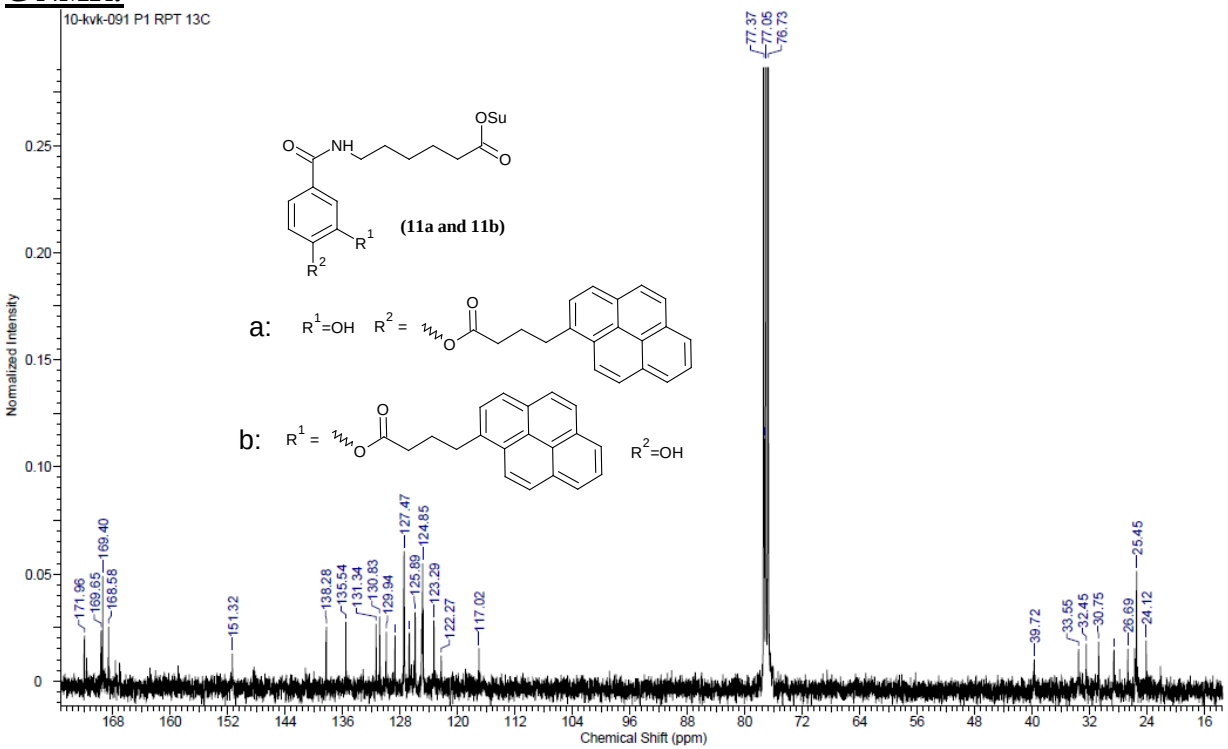

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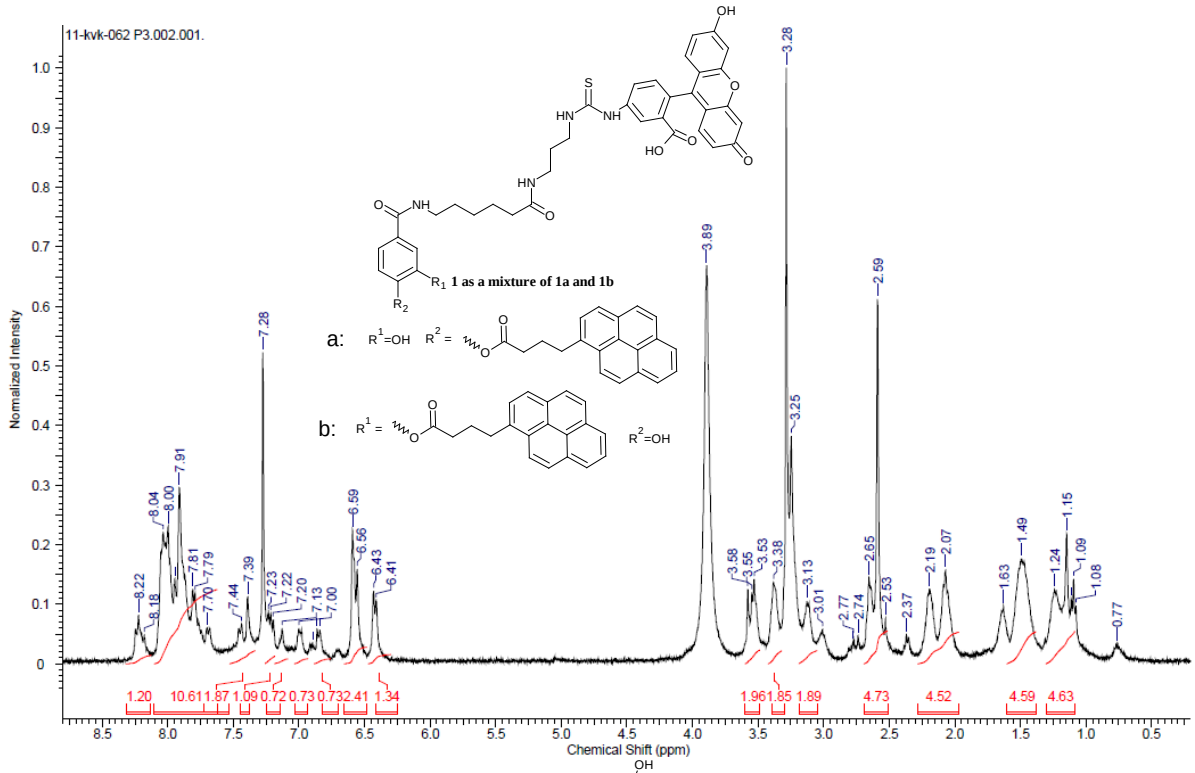
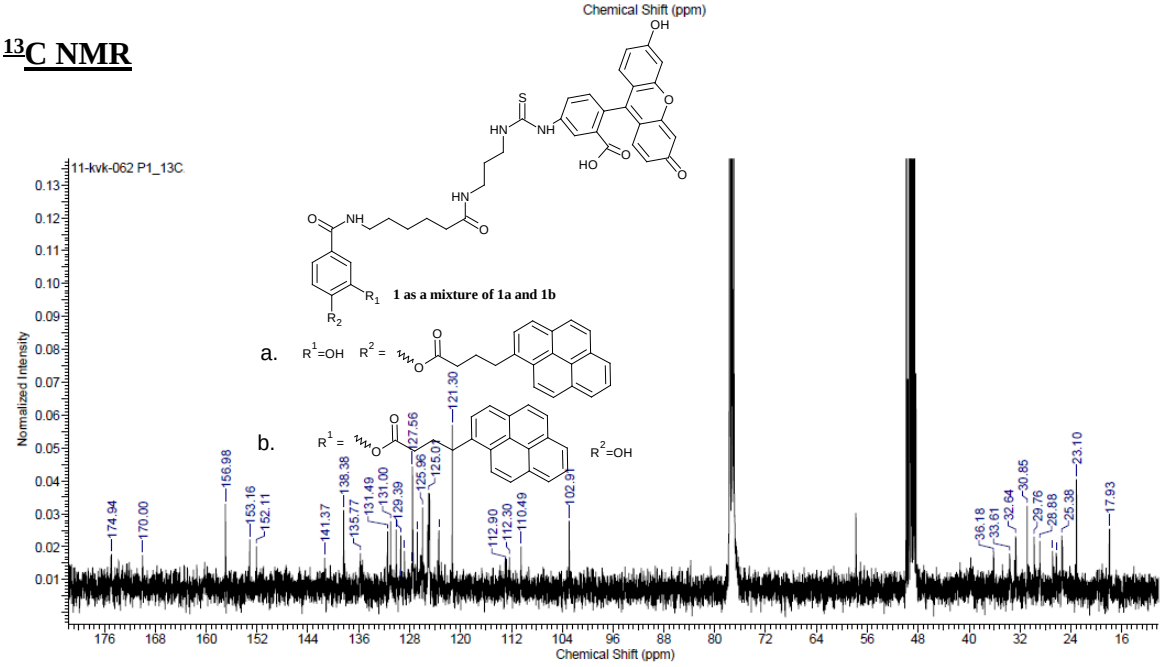
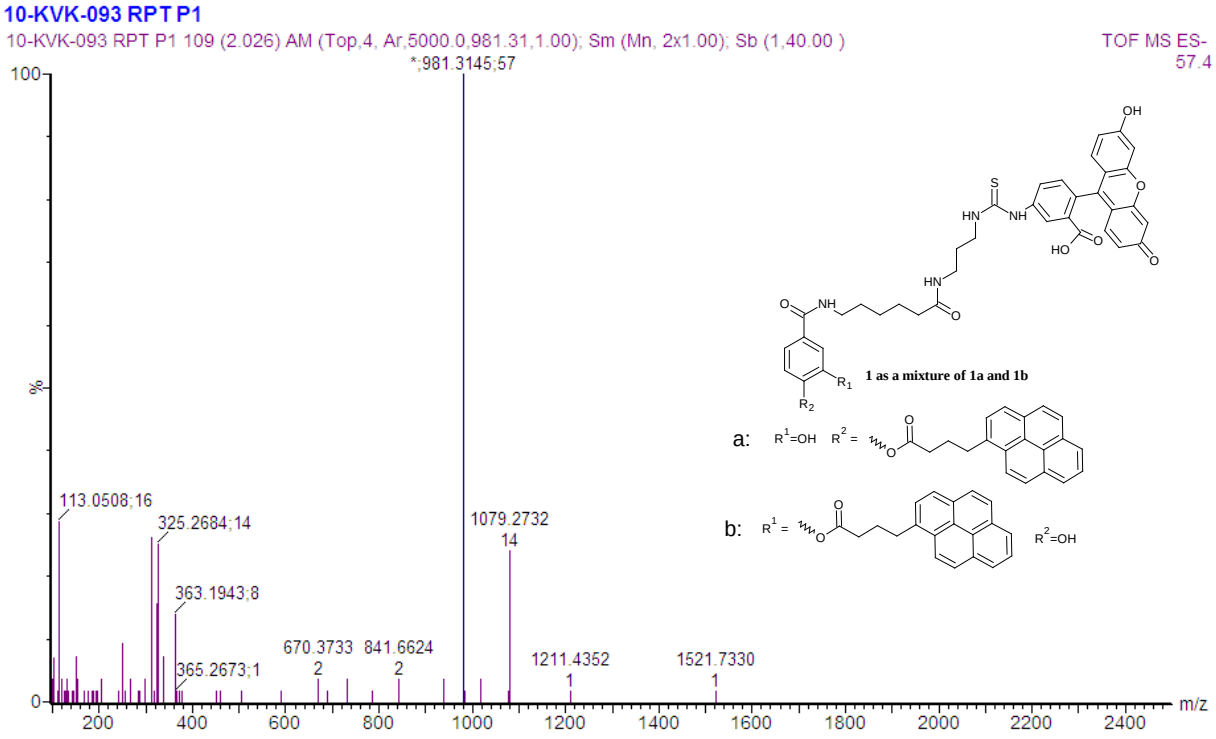


^{13}C NMR



ESI-MS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10-KVK-093 RPT P1 109 (2.026) AM (Top,4, Ar,5000.0,981.31,1.00); Sm (Mn, 2x1.00); Sb (1,40.00)
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2
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14
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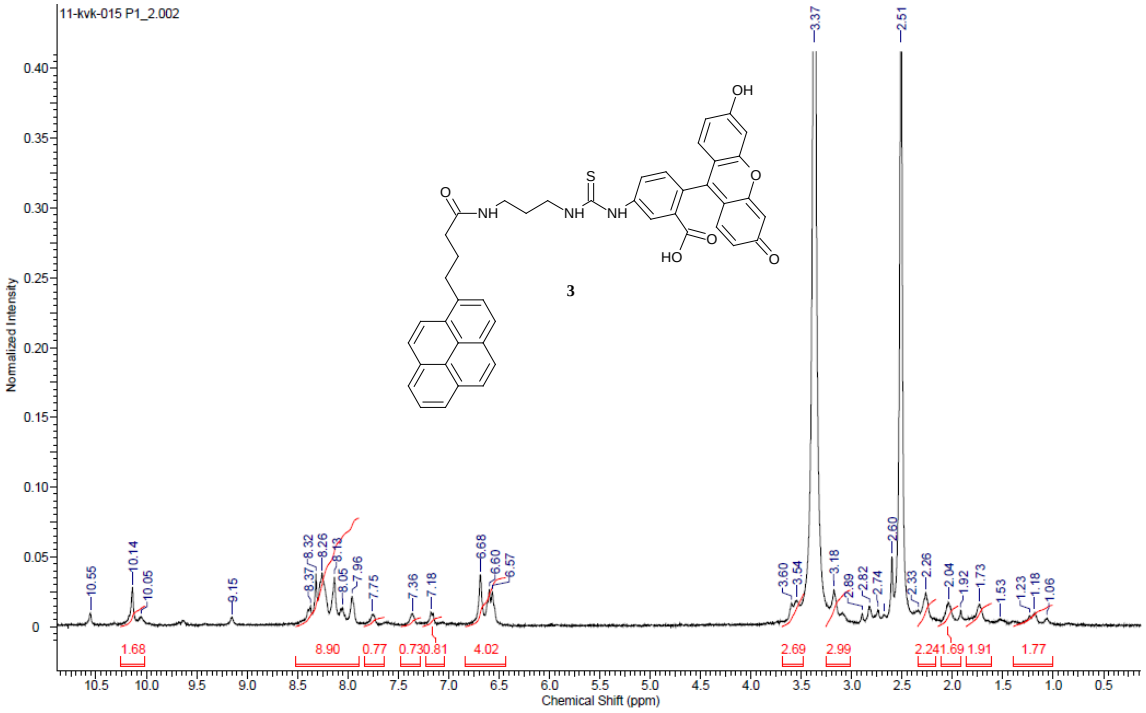
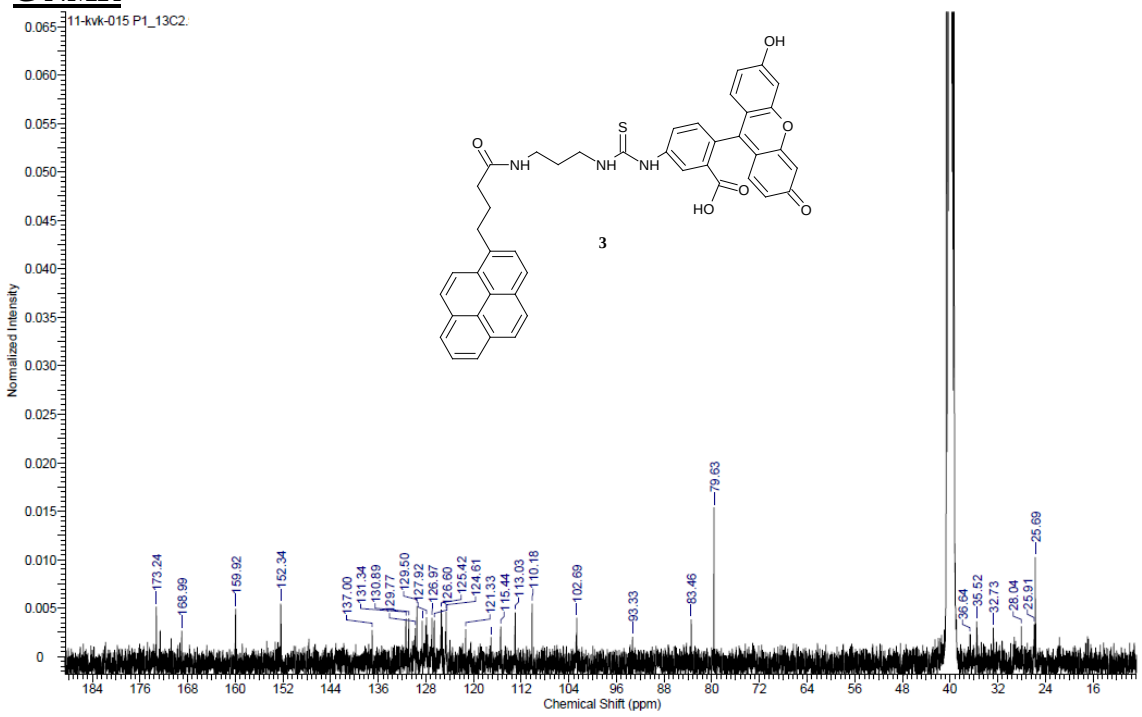
1
1521.7330
1
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
m/z
1 as a mixture of 1a and 1b
a:
b:

Spectral data for Pyrene FITC amide 3

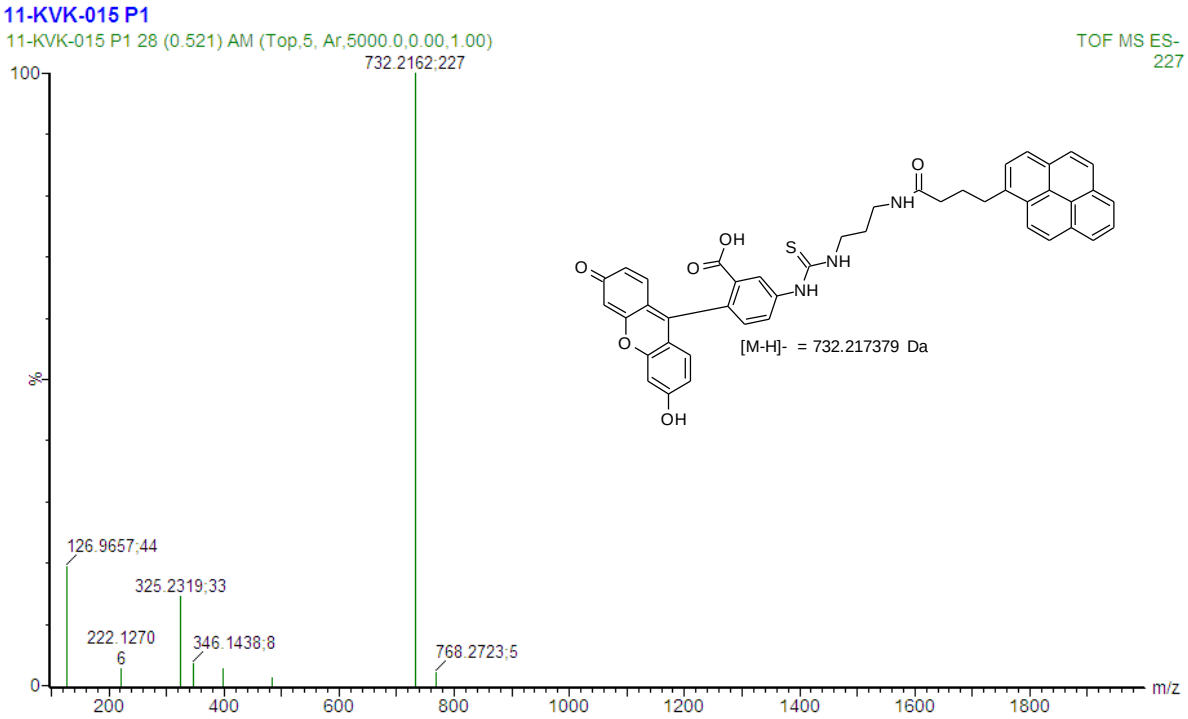
^{1}H NMR



^{13}C NMR

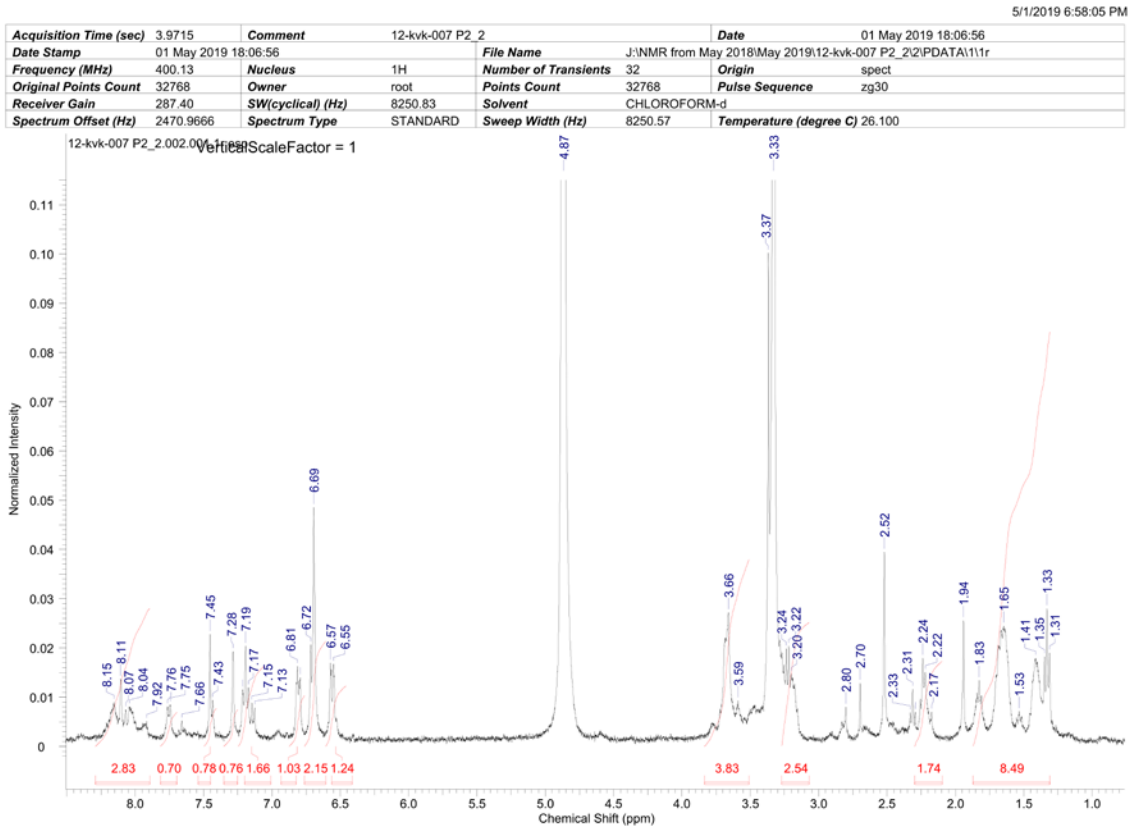


ESI-MS

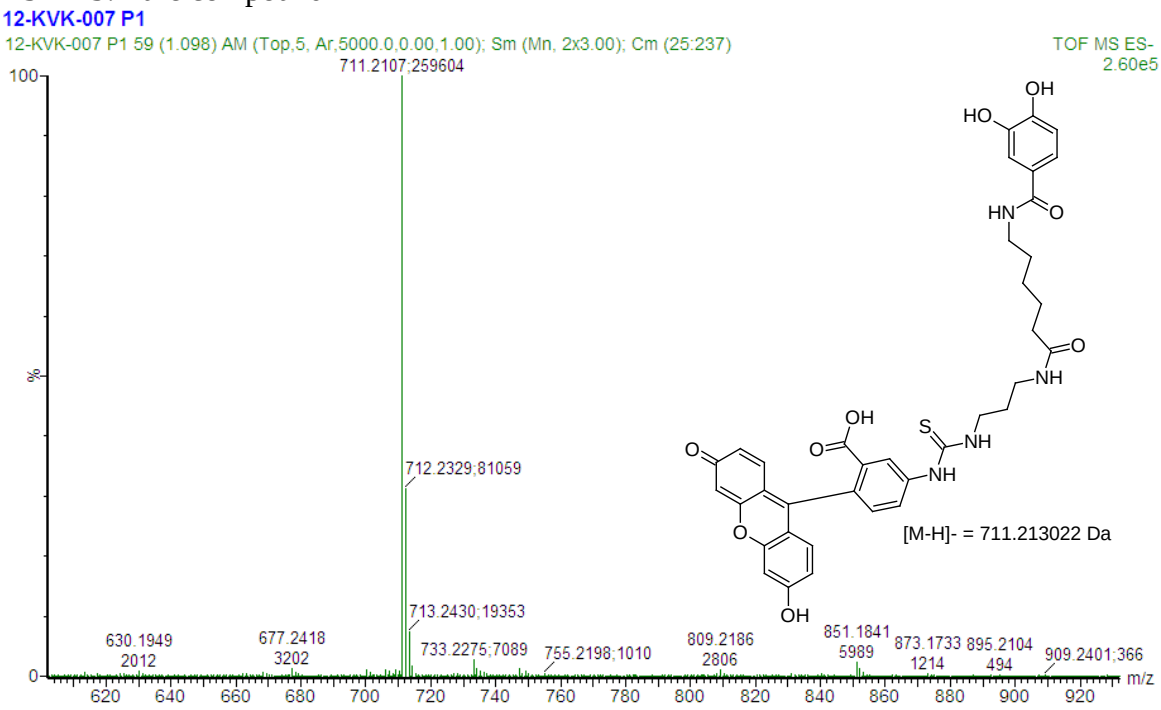


Spectral data for FITC catechol 2

^{1}H NMR



ESI-MS: Pure compound



ESI-MS, FITC catechol 2 after release