Lipofection with Estrone-Based Luminophores featuring Aggregation-Induced Emission Properties

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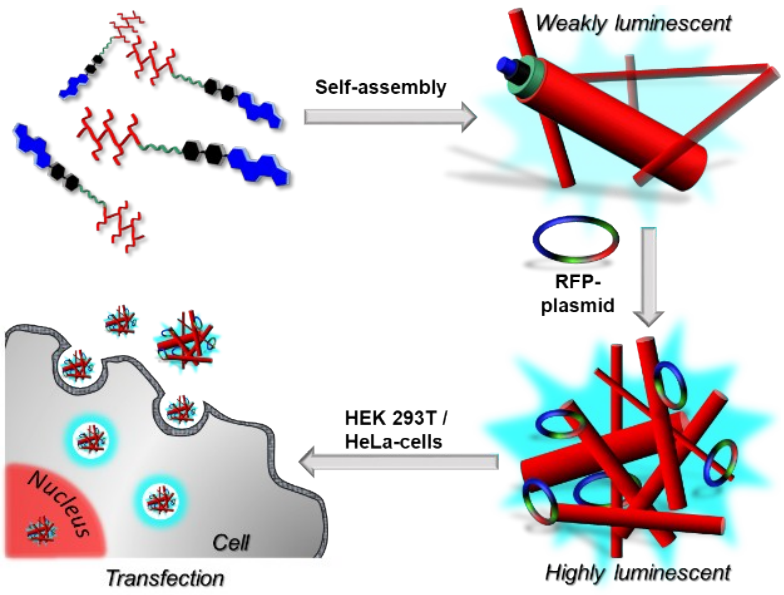
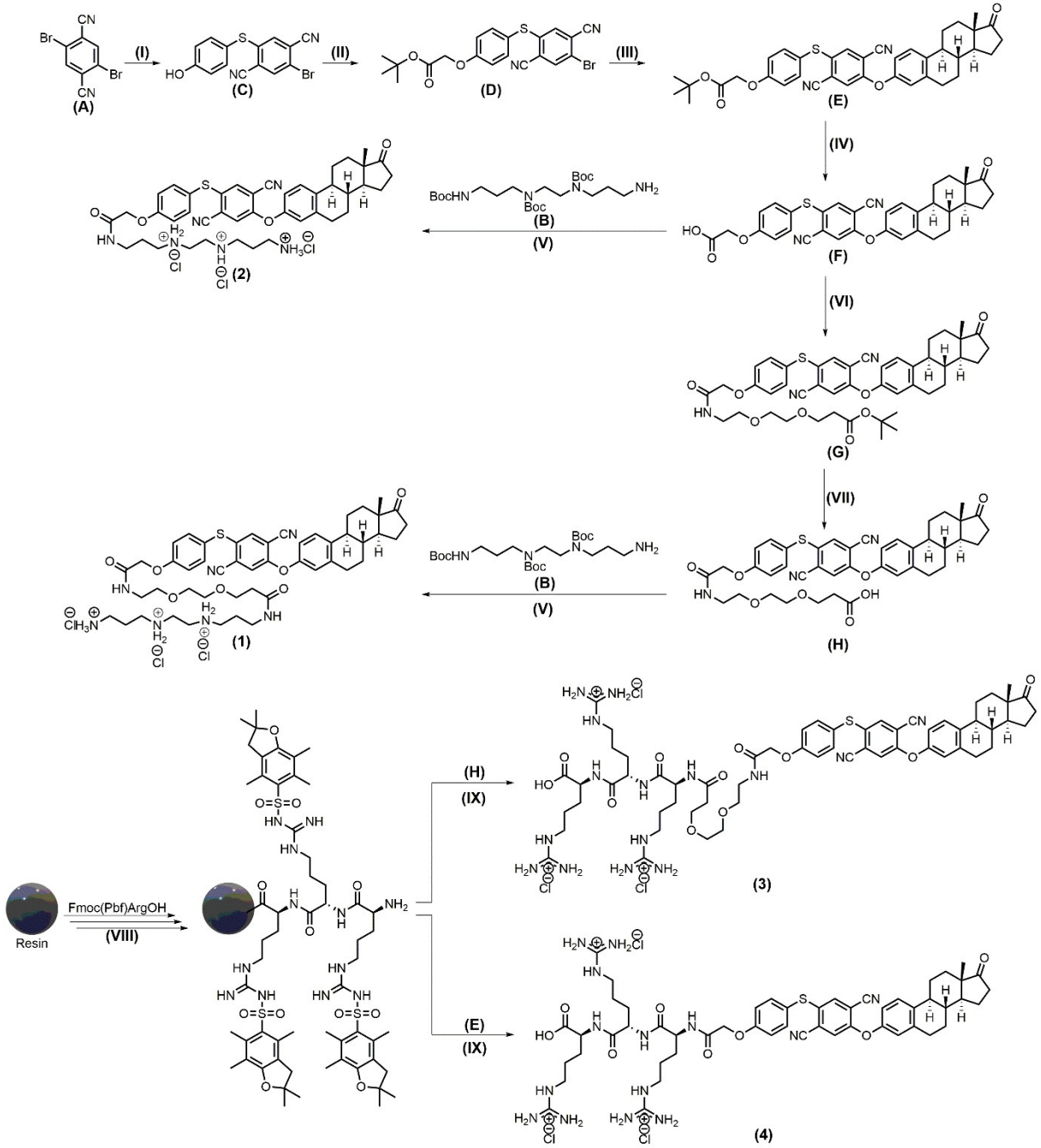
General Information and concept

Chemicals were purchased from Sigma Aldrich or from TCI Chemicals and used without further purification. Reactions were carried out using dried solvents and under an atmosphere of argon. Reactions were monitored by thin-layer chromatography (TLC), which was performed on 0.2 mm Macherey-Nagel ALUGRAM precoated silica gel aluminum sheets. Spots were visualized by treatment with basic $KMnO_4$ solution or by an UV-handlamp (254 and 365 nm). Column chromatography was carried out on silica gel 60 (0.063-0.2 mm, Merck). The used potassium carbonate was freshly ground and dried at 90 °C. Dimethylformamide was distilled and dried with molecular sieves 0,4 nm. The NMR spectra were recorded on a Bruker DMX 300 spectrometer [^{1}H: 300.16 MHz, ^{13}C: 75.47 MHz] a Bruker Avance HD 600 mit [^{1}H: 600.13 MHz, ^{13}C: 150.90 MHz] or an Bruker Avance Neo 400 [^{1}H: 400.13 MHz, ^{13}C: 100.61 MHz]. All measurements were performed at room temperature, using d_1-chloroform or d_6-DMSO as solvents. The chemical shifts are referenced relative to the residual proton signals of the solvents in the ^{1}H-NMR spectrum ($CDCl_3$: δ= 7.24 ppm, d_6-DMSO: δ= 2.50 ppm) or relative to the solvent signal in the ^{13}C-NMR spectra ($CDCl_3$: δ= 77.16 ppm, d_6-DMSO: δ= 39.51 ppm). Coupling constants (J) are reported in Hertz (Hz). High resolution mass spectrum were measured on a Bruker maXis 4G UHR-TOF or on a LTQ Orbitap LTQ XL (Thermo-Fisher Scientific, Bremen).



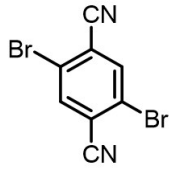
Concept: Self-assembly of AIE active amphiphiles followed by the formation of luminescent lipoplexes in the presence of plasmid DNA as well their ability to transfect cells. The schematic presentation is not to scale.

Synthesis



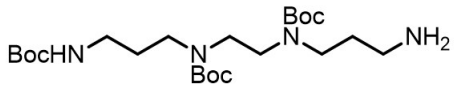
Scheme S1: Synthesis route to compound [**1**], [**2**], [**3**] and [**4**]. Reagents and conditions. **I)** 4-mercaptophenol, K_2CO_3, DMF, 50 °C, 2 h; **II)** *tert*-butyl-bromoacetate, K_2CO_3, DMF, 40 °C, 2 h; **III)** Estrone, K_2CO_3, DMF, 50 °C, 48 h; **IV)** DCM:TFA 4:1, r. t., o. n.; **V)** DCM, NMM, PyBOP, amino-triethyleneglycol-*tert*-butylester, 18 h, r. t.; **VII)** DCM:TFA 6:1, r. t., o. n.; **VIII)** 1) Resin, DCM, r. t., 2) Fmoc(Pbf)ArgOH, DIPEA, HCTU, DCM, r. t., 3 h, 3.) 1:4 piperidine:DMF, r. t., 0.5 h] 3x; **IX)** 1) [**H]** or [**E**], DIPEA, HCTU, DCM, r. t., 4 d, 2) TFA, TIS, H_2O, r. t., 4 h, 3) HCl.

Compound [A]



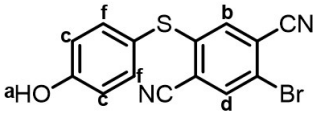
The synthesis of compound [**A**] was carried out according to a known literature procedure.[1]

Compound [B]



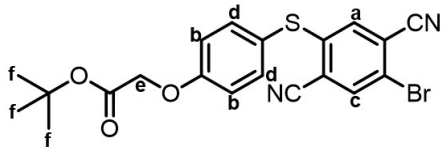
The synthesis of compound [**B**] was carried out according to a known literature procedure.[2]

Compound [C]



In a twin-neck round bottom flask 1.72 g (6,01 mmol), 2,5-dibromoterephtalodinitril [**A**], 2.76 g (19.97 mmol) and potassium carbonate were dissolved in 50 ml of DMF under an atmosphere of argon. In another flask 0.51 g (4.04 mmol) 4-mercaptophenol was dissolved in 25 ml of dry DMF. This solution was added dropwise over a period of 2 hours at 50 °C. The solution was stirred for further two hours at 50 °C. Subsequently the solution was quenched with 2 M hydrochloric acid. The yellow green precipitate was filtered. The purification was carried out by silica column chromatography using cyclohexane : ethyl acetate (8:1 → 4:1). Yield: 0.85 g (64 %), Yellow-green solid. **^{1}H-NMR** (300 MHz, DMSO-d_6) δ = 10.19 (s, 1H) **a**-H, 8.47 (s, 1H) **b**-H, 7.51-7.35 (d, *J* = 8.7 Hz, 2H) **c**-H, 7.21 (s, 1H) **d**-H, 6.98-6.82 (d, *J* = 8.7 Hz, 2H) **f**-H ppm, **^{13}C-NMR** (75 MHz, DMSO-d_6) δ = 159.7 **a**, 144.7 **b**, 137.4 **c**, 137.0 **d**, 132.0 **e**, 120.7 **f**, 119.2 **g**, 115.7 **h**, 115.9 **i**, 117.5 **j**, 114.4 **k**, 114.4 **l** ppm. **IR (neat):** v= 3344, 3086, 3011, 2926, 2856, 2785, 2706, 2659, 2362, 2324, 2253, 2228, 2112, 2083, 1891, 1648, 1608, 1583, 1495, 1455, 1433, 1369, 1338, 1280, 1266, 1230, 1204, 1166, 1147, 1088, 1050, 1011, 902, 883, 831, 693 cm^{-1}, **MS:** HR-ESI-pos (MeOH): m/z = 354.9336 (calc.: 354.9334 $[M+Na]^+$).

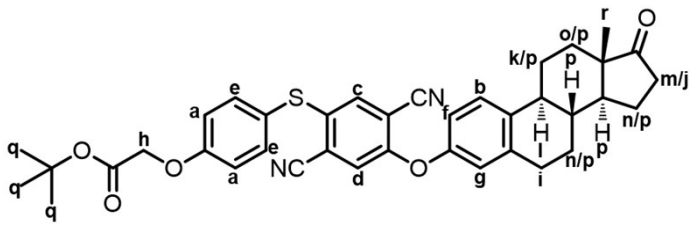
Compound [D]



In a 100 ml round bottom flask 0.61 g (1.80 mmol) of compound [**C**], 1.00 g (7.24 mmol) potassium carbonate and 0.70 mg (3.61 mmol) *tert*-butyl bromoacetate were dissolved in 15 ml of dry DMF. The solution was stirred for 4 h at 40 °C. Then the reaction was quenched with water. The white precipitate was filtered and dried in *vacuo*. Yield: 0.74 g (1.66 mmol, 93%) white solid. **^{1}H-NMR** (300 MHz, DMSO-d_6) δ = 8.50 (s, 1H) **a**-H, 7.54 (d, *J* = 8.8 Hz, 2H) **b**-H, 7.31 (s, 1H) **c**-H, 7.07 (d, J = 8.8 Hz, 2H) **d**-H, 4.75 (s, 2H) **e**-H, 1.41 (s, 9H) **e**-H ppm. **^{13}C-NMR** (75 MHz, DMSO-d_6) δ = 167.4 **a**, 159.4 **b**, 143.6 **c**, 137.6 **d**, 136.3 **e**, 132.9 **f**, 121.3 **g**, 119.3 **h**, 119.2 **i**, 116.6 **j**, 115.9 **k**, 115.3 **l**, 114.4 **m**, 81.5 **n**, 65.1 **o**, 27.6 **p** ppm. **IR (neat):** v = 3095, 3074, 2985, 2930, 2898, 2861, 2775, 2540, 2360, 2341, 2229, 2184, 2210, 1949,

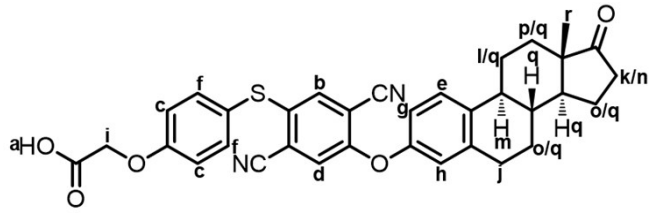
1991, 1918, 1890, 1844, 1868, 1829, 1793, 1741, 1718, 1684, 1671 1653, 1636, 1595, 1576, 1559, 1541, 1522, 1492, 1476, 1457, 1442, 1399, 1368, 1339, 1296, 1244, 1228, 1175, 1157 1109 1090, 1068, 1009 970 953, 900, 870, 859, 840, 825, 811, 758, 720, 707, 687, 652 cm^{-1}. **MS:** HR-ESI-pos (MeOH): m/z = 469.0018 (calc.: 469.0016 $[M+Na]^+$).

Compound [E]



Estrone (0.48 mg, 1,76 mmol), compound [**D**] (0.40 g, 0.90 mmol) and K_2CO_3 (0.36 g, 2.63 mmol) were dissolved under argon in 15 mL of dry DMF. This solution was heated to 50°C for 48 h followed by the addition of distilled water (20 mL). The suspension was extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to dryness. The residue was purified by silica column chromatography using cyclohexane: ethyl acetate (6:1). The product was obtained as off-white solid. Yield: 0.46 g (0.72 mmol, 80 %). **^{1}H-NMR** (600 MHz, $CDCl_3$) δ = 7.48 (d, *J* = 8.8 Hz, 2H) **a**-H, 7.36 (d, *J* = 8.5 Hz, 1H) **b**-H, 7.26 (d, *J* = 4.4 Hz, 1H) **c**-H, 7.02 (s, 1H) **d**-H, 6.98 (d, *J* = 8.8 Hz, 2H) **e**-H, 6.85 (dd, *J* = 8.5, 2.6 Hz, 1H) **f**-H, 6.81 (d, *J* = 2.5 Hz, 1H) **g**-H, 4.58 (s, 2H) **h**-H, 2.97 – 2.86 (m, 2H) **i**-H, 2.54 (dd, *J* = 19.1, 8.8 Hz, 1H) **j**-H, 2.47 – 2.41 (m, 1H) **k**-H, 2.34 (td, *J* = 11.1, 4.0 Hz, 1H) **l**-H, 2.22 – 2.13 (m, 1H) **m**-H, 2.13 – 2.04 (m, 2H) **n**-H, 2.04 – 1.98 (m, 1H) **o**-H, 1.71 – 1.48 (m, 15H) **p/q**-H, 0.97 (s, 3H) **r**-H ppm. **^{13}C-NMR** (151 MHz, $CDCl_3$) δ = 220.5 (s) **a**, 167.3 (s) **b**, 159.4 (s) **c**, 158.0 (s) **d**, 151.5 (s) **e**, 139.4 (s) **f**, 138.0 (s) **g**, 137.0 (s) **h**, 136.3 (s) **i**, 133.9 (s) **j**, 127.4 (s) **k**, 121.5 (s) **l**, 120.3 (s) **m**, 117.5 (s) **n**, 116.6 (s) **o**, 116.4 (s) **p**, 115.3 (s) **q**, 114.1 (s) **r**, 107.8 (s) **s**, 82.7 (s) **t**, 65.6 (s) **u**, 50.3 (s) **v**, 47.8 (s) **w**, 44.0 (s) **x**, 37.9 (s) **y**, 35.7 (s) **z**, 31.4 (s) **α**, 29.4 (s) **β**, 28.0 (s) **γ**, 26.1 (s) **δ**, 25.7 (s) **ε**, 21.5 (s) **ζ**, 13.8 (s) **η** ppm. **IR (ATR):** *v* = 2973 (CH aliph.), 2929(CH aliph.), 2862 (CH aliph.), 1738 (C=O), 1591, 1493, 1468, 1371, 1275, 829, 752. cm^{-1} **MS:** HR-ESI-pos (MeOH): m/z = 657.2400 (calc.: 657.2394 $[M+Na]^+$).

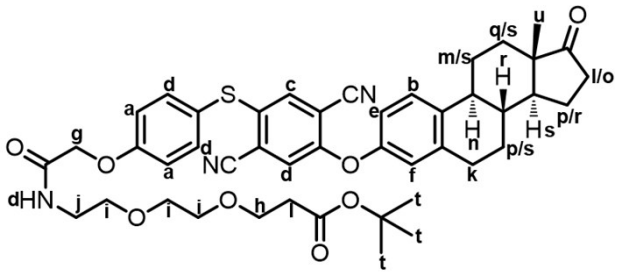
Compound [F]



To a stirred solution of compound [**E**] (0.79 g, 1.24 mmol) in 80 mL of dry dichloromethane was added 20 mL of trifluoroacetic acid. The obtained yellowish solution was stirred over night at room temperature. After that, the solution was evaporated to dryness and then re-suspended in distilled water (10 mL) and freeze-dried. Yield: 0.68 g (0.92 mmol, 92 %). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ = 13.09 (s, 1H) **a**-H, 7.72 – 7.68 (m, 1H) **b**-H, 7.53 – 7.47 (m, 2H) **c**-H, 7.45 (s, 1H) **d**-H, 7.37 (d, *J* = 8.5 Hz, 1H) **e**-H, 7.07 – 7.00 (m, 2H) **f**-H, 6.96 (dd, *J* = 8.4, 2.7 Hz, 1H) **g**-H, 6.92 (d, *J* = 2.6 Hz, 1H) **h**-H, 4.75 (s, 2H) **i**-H, 2.86 (dd, *J* = 8.7, 3.9 Hz, 2H) **j**-H, 2.48 – 2.41 (m, 1H) **k**-H, 2.32 – 2.21 (m, 1H) **l**-H, 2.13 – 2.01 (m, 1H) **m**-H, 2.01 – 1.91 (m, 2H) **n**-H, 1.81 – 1.73 (m, 1H) **o**-H, 1.64 – 1.32 (m, 6H) **p**-H, 0.84 (s, 3H) **q**-H ppm. **^{13}C-NMR** (101 MHz, DMSO-d_6) δ = 219.6 (s) **a**, 169.8 (s) **b**, 158.9 (s) **c**, 157.5 (s) **d**, 152.0 (s) **e**, 139.1 (s) **f**, 137.1

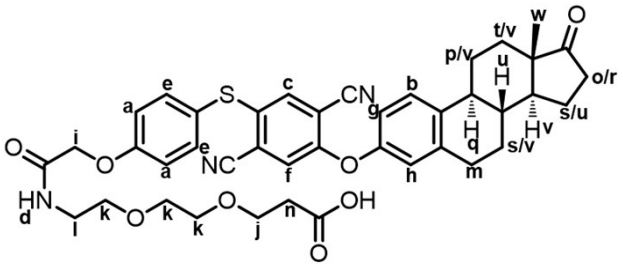
(s) **g**, 135.6 (s) **h**, 135.4 (s) **i**, 135.2 (s) **j**, 127.3 (s) **k**, 122.3 (s) **l**, 121.6 (s) **m**, 119.4 (s) **n**, 117.9 (s) **o**, 116.7 (s) **p**, 116.3 (s) **q**, 115.3 (s) **r**, 114.3 (s) **s**, 108.0 (s) **t**, 64.6 (s) **u**, 49.5 (s) **v**, 47.3 (s) **w**, 43.6 (s) **x**, 37.4 (s) **y**, 35.4 (s) **z**, 31.3 (s) **α**, 28.9 (s) **β**, 25.8 (s) **γ**, 25.3 (s) **δ**, 21.1 (s) **ε**, 13.5 (s) **ζ** ppm. **IR (ATR):** *v* = 3091 (CH arom.), 3028 (CH arom.), 2927 (CH aliph.), 2864 (CH aliph.), 2837 (CH aliph.), 1738 (C=O), 1595, 1577, 1547, 1493, 1469, 1435, 1373, 1273, 1240, 1176, 1159, 1082, 1053, 1009, 914, 825, 803, 707, 675, 665, 647, 629 cm^{-1}. **MS:** HR-ESI-neg (MeOH): m/z = 577.1826 (calcd.: 577.1803 $[M-H]^-$).

Compound [G]



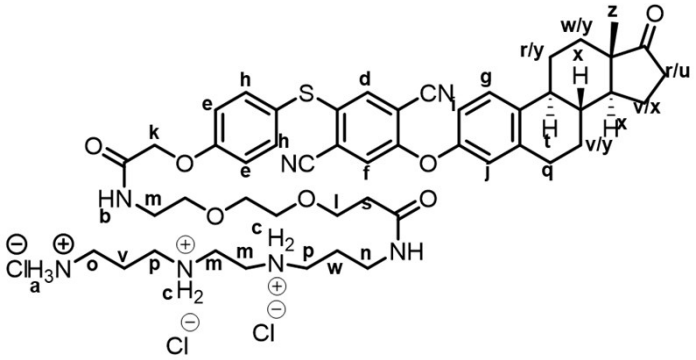
Compound [**F**] (0.40 g, 0.69 mmol) was dissolved under an atmosphere of argon in 30 mL of dry dichloromethane followed by the addition N-methylmorpholine (0.32 g, 3.18 mmol). After 1 h PyBOP (0.36 g, 0.69 mmol) and amino-triethyleneglycol-*tert*-butylester (0.12 g, 0.53 mmol) were added and the solution was stirred for further 18 h. After that, 30 mL of brine was added and the obtained solution was extracted 3 times with 20 mL of dichloromethane. The combined organic extracts were dried over $MgSO_4$ and evaporated to dryness. The residue was subjected to silica column chromatography using ethyl acetate and cyclohexane as eluent (2:1) yielding the desired compound as yellow solid. Yield: 0.41 g (0.52 mmol, 74 %). **^{1}H-NMR** (600 MHz, $CDCl_3$) δ = 7.48 (d, *J* = 8.5 Hz, 2H) **a**-H, 7.34 (d, *J* = 8.5 Hz, 1H) **b**-H, 7.25 (s, 1H) **c**-H, 7.05 – 6.97 (m, 4H) **d**-H, 6.83 (dd, *J* = 8.5, 2.5 Hz, 1H) **e**-H, 6.79 (d, *J* = 2.4 Hz, 1H) **f**-H, 4.54 (s, 2H) **g**-H, 3.71 (t, *J* = 6.5 Hz, 2H) **h**-H, 3.62 – 3.58 (m, 6H) **i**-H, 3.57 – 3.53 (m, 2H) **j**-H, 2.95 – 2.85 (m, 2H) **k**-H, 2.55 – 2.46 (m, 3H) **l**-H, 2.44 – 2.38 (m, 1H) **m**-H, 2.32 (td, *J* = 11.1, 4.0 Hz, 1H) **n**-H, 2.20 – 2.11 (m, 1H) **o**-H, 2.10 – 2.02 (m, 2H) **p**-H, 2.01 – 1.96 (m, 1H) **q**-H, 1.69 – 1.60 (m, 2H) **r**-H, 1.60 – 1.45 (m, 4H) **s**-H, 1.43 (s, 9H) **t**-H, 0.94 (s, 3H) **u**-H ppm. **^{13}C-NMR** (151 MHz, $CDCl_3$) δ = 220.7 (s) **a**, 171.0 (s) **b**, 167.6 (s) **c**, 158.8 (s) **d**, 158.4 (s) **e**, 151.7 (s) **f**, 139.6 (s) **g**, 138.3 (s) **h**, 136.7 (s) **i**, 136.5 (s) **j**, 134.3 (s) **k**, 127.7 (s) **l**, 122.7 (s) **m**, 120.5 (s) **n**, 117.7 (s) **o**, 117.0 (s) **p**, 116.7 (s) **q**, 115.4 (s) **r**, 114.3 (s) **s**, 108.0 (s) **t**, 80.7 (s) **u**, 70.4 (s) **v**, 69.8 (s) **w**, 67.5 (s) **x**, 67.0 (s) **y**, 50.5 (s) **z**, 48.0 (s) **α**, 44.2 (s) **β**, 39.0 (s) **γ**, 38.1 (s) **δ**, 36.3 (s) **ε**, 35.9 (s) **ζ**, 31.6 (s) **η**, 29.6 (s) **θ**, 28.2 (s) **ι**, 26.3 (s) **κ**, 25.9 (s) **λ**, 21.7 (s) **μ**, 14.0 (s) **ξ** ppm. **IR (ATR):** *v* = 3091 (CH arom.), 3066 (CH arom.), 3032 (CH arom.), 2925(CH aliph.), 2870(CH aliph.), 1732 (C=O ketone), 1674, 1591, 1543, 1491, 1468, 1373, 1244, 1176, 1157, 1109, 1053, 1009, 916, 883, 825, 723 cm^{-1}. **MS:** HR-ESI-pos (MeOH): m/z = 816.3304 (calc.: 816.3289 $[M+Na]^+$).

Compound [H]



To a stirred solution of compound [**G**] (0.36 g, 0.45 mmol) in 30 mL of dry dichloromethane was added 5 mL of trifluoroacetic acid. The obtained yellowish solution was stirred over night at room temperature. After that, the solution was evaporated to dryness, re-suspended in distilled water (10 mL) and freeze-dried. Yield: 0.30 g (0.52 mmol, 89%).**^{1}H-NMR** (600 MHz, $CDCl_3$) δ = 7.48 (d, *J* = 8.4 Hz, 2H) **a**-H, 7.34 (d, *J* = 8.5 Hz, 1H) **b**-H, 7.26 (s, 1H) **c**-H, 7.19 – 7.13 (m, 1H) **d**-H, 7.02 (d, *J* = 8.4 Hz, 2H) **e**-H, 7.00 (s, 1H) **f**-H, 6.83 (dd, *J* = 8.5, 2.4 Hz, 1H) **g**-H, 6.79 (d, *J* = 2.3 Hz, 1H) **h**-H, 4.58 (s, 2H) **i**-H, 3.76 (t, *J* = 6.1 Hz, 2H) **j**-H, 3.64 – 3.59 (m, 6H) **k**-H, 3.59 – 3.54 (m, 2H) **l**-H, 2.94 – 2.87 (m, 2H) **m**-H, 2.62 (t, *J* = 6.1 Hz, 2H) **n**-H, 2.52 (dd, *J* = 19.1, 8.7 Hz, 1H) **o**-H, 2.45 – 2.38 (m, 1H) **p**-H, 2.32 (td, *J* = 11.1, 4.0 Hz, 1H) **q**-H, 2.16 (dt, *J* = 18.8, 8.9 Hz, 1H) **r**-H, 2.10 – 2.01 (m, 2H) **s**-H, 2.01 – 1.95 (m, 1H) **t**-H, 1.69 – 1.61 (m, 2H) **u**-H, 1.60 – 1.44 (m, 4H) **v**-H, 0.94 (s, 3H) **w**-H ppm. **^{13}C-NMR** (151 MHz, $CDCl_3$) δ [ppm] = 220.8 (s) **a**, 174.9 (s) **b**, 168.3 (s) **c**, 158.4 (s) **d**, 158.2 (s) **e**, 151.5 (s) **f**, 139.4 (s) **g**, 138.1 (s) **h**, 136.5 (s) **i**, 136.3 (s) **j**, 134.1 (s) **k**, 127.5 (s) **l**, 122.6 (s) **m**, 120.3 (s) **n**, 117.5 (s) **o**, 116.8 (s) **p**, 116.5 (s) **q**, 115.2 (s) **r**, 114.1 (s) **s**, 107.8 (s) **t**, 70.3 (s) **u**, 69.9 (s) **u**, 69.5 (s) **u**, 67.2 (s) **v**, 66.3 (s) **w**, 50.3 (s) **x**, 47.9 (s) **y**, 44.0 (s) **z**, 39.0 (s) **α**, 37.8 (s) **β**, 35.7 (s) **γ**, 34.6 (s) **δ**, 31.4 (s) **ε**, 29.4 (s) **ζ**, 26.1 (s) **η**, 25.7 (s) **θ**, 21.5 (s) **ι**, 13.8 (s) **κ** ppm. **IR (ATR):** *v* = 3354 (NH), 3091 (CH arom.), 3060 (CH arom.), 3032 (CH arom.), 2925 (CH aliph.), 2868 (CH aliph.), 1734 (C=O ketone), 1676 C=O carboxyl), 1591,1543, 1491, 1466, 1373, 1275, 1244, 1176, 1157, 1109, 1053, 1009, 916, 883, 827 704, 631 cm^{-1}. **MS:** HR-ESI-neg (MeOH): m/z = 736.2678 (calc.: 736.2698 $[M-H]^-$).

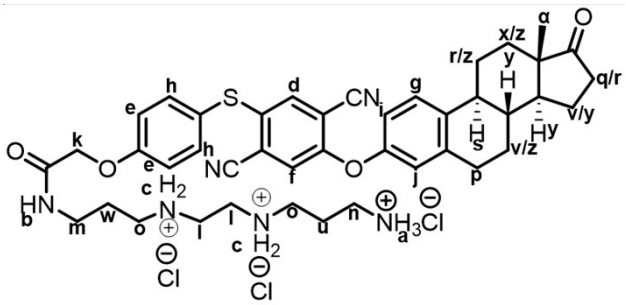
Compound [1]



To a solution of compound [**H**] (45 mg, 0.06 mmol) in 3 mL of DMF was added DIPEA (0.03 ml, 0.17 mmol).After 1 h PyBOP (70 mg, 0.13 mmol) and [**B**] (29 mg, 0.06 mmol) were added and the solution was stirred for further 16 h. Then 50 ml of H_2O were added to the reaction flask. The precipitate was filtered off and then dried on a rotary evaporator. The successful synthesis of the Boc-protected intermediate was confirmed by mass spectrometry. **MS:** HR-ESI-pos ($CHCl_3$/MeOH): m/z = 1057.5080 (calc.: 1057.5079 $[M+Na]^+$). The intermediate was dissolved in 5 ml of a 1:1 TFA: DCM mixture and stirred for one hour at room temperature. The crude product was then precipitated with Et_2O and filtered. The crude product was dried on a rotary evaporator and purified *via* MPLC (gradient 60 → 100 MeOH, 105 min.). The product was

mixed with 100 ml of a 3 molar hydrochloric acid solution. The solution was distilled off to dryness on a rotary evaporator. This procedure was repeated once. The product was then taken up in distilled water and lyophilized. An off-white solid (12 mg, 0.01 mmol, 20%) was isolated. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ [ppm] = 9.39 (bs, 3H) **a**-H, 8.21 (t, *J* = 5.7 Hz, 1H) **b**-H, 8.19 – 7.88 (m, 3H) **c**-H (note: two *sec*. amine signals were not found due to extreme broadening of the signal), 7.67 (s, 1H) **d**-H, 7.50 (d, *J* = 8.8 Hz, 2H) **e**-H, 7.44 (s, 1H) **f**-H, 7.37 (d, *J* = 8.5 Hz, 1H) **g**-H, 7.07 (d, *J* = 8.9 Hz, 2H) **h**-H, 6.94 (dd, *J* = 8.4, 2.6 Hz, 1H) **i**-H, 6.91 (d, *J* = 2.5 Hz, 1H) **j**-H, 4.55 (s, 2H) **k**-H, 3.60 (t, *J* = 6.3 Hz, 2H) **l**-H, 3.31 – .26 (m, 6H) **m**-H, 3.16 – 3.10 (m, 2H) **n**-H, 3.05 (t, *J* = 7.2 Hz, 2H) **o**-H, 2.99 – 2.89 (m, 4H) **p**-H, 2.89 – 2.81 (m, 2H) **q**-H, 2.48 – 2.35 (m, 2H) **r**-H, 2.32 (t, *J* = 6.4 Hz, 2H) **s**-H, 2.30 – 2.22 (m, 1H) **t**-H, 2.13 – 2.01 (m, 1H) **u**-H, 2.01 – 1.90 (m, 4H) **v**-H, 1.83 – 1.70 (m, 3H) **w**-H, 1.64 – 1.47 (m, 3H) **x**-H, 1.47 – 1.32 (m, 3H) **y**-H, 0.83 (s, 3H) **z**-H ppm. **^{13}C-NMR** (101 MHz, DMSO-d_6) δ = 219.9 (s) **a**, 170.8 (s) **b**, 167.6 (s) **c**, 158.9 (s) **d**, 157.6 (s) **e**, 152.1 (s) **f**, 139.3 (s) **g**, 137.3 (s) **h**, 135.6 (s) **i**, 135.6 (s) **j**, 135.3 (s) **k**, 127.5 (s) **l**, 122.4 (s) **m**, 121.9 (s) **n**, 119.5 (s) **o**, 117.9 (s) **p**, 116.8 (s) **q**, 116.6 (s) **r**, 115.4 (s) **s**, 114.4 (s) **t**, 108.1 (s) **u**, 69.6 (s) **v**, 68.9 (s) **w**, 67.0 (s) **x**, 66.8 (s) **y**, 49.7 (s) **z**, 47.4 (s) **α**, 44.9 (s) **β**, 44.2 (s) **γ**, 43.7 (s) **δ**, 43.1 (s) **ε**, 43.0 (s) **ε**, 38.4 (s) **ζ**, 37.6 (s) **η**, 36.3 (s) **θ**, 36.2 (s) **θ**, 35.7 (s) **ι**, 35.5 (s) **κ**, 31.4 (s) **λ**, 29.0 (s) **μ**, 26.1 (s) **ξ**, 25.9 (s) **ξ**, 25.4 (s) **ξ**, 23.9 (s) **π**, 21.2 (s) **ρ**, 13.6 (s) **ς** ppm. **IR (ATR):** *v* = 3329, 3091, 3060, 3028, 2927, 2866, 1734, 1649, 1591, 1545, 1491, 1468, 1373, 1277, 1246, 1176, 1157, 1084, 1053, 1009, 916, 881, 825, 754, 623 cm^{-1}. **MS:** HR-ESI-pos (MeOH/H_2O/FA): m/z = 894.4588 (calc.: 894.4582 $[M+H]^+$).

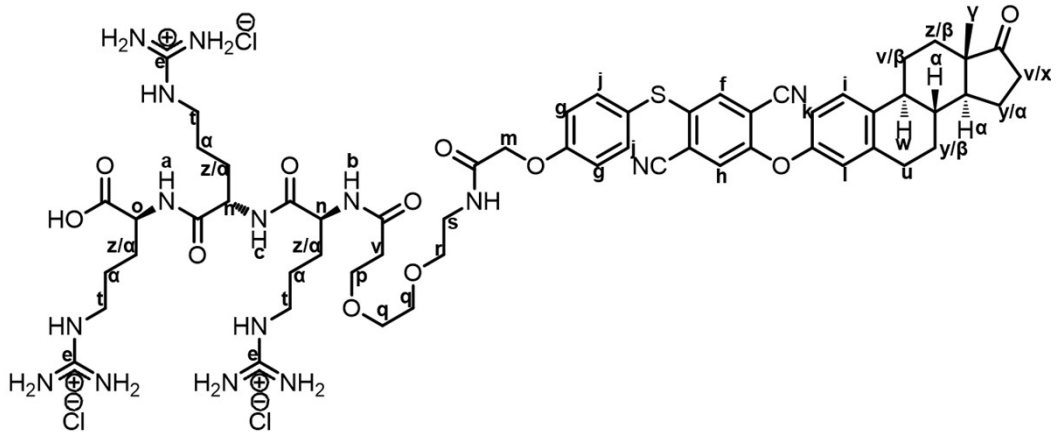
Compound [2]



To a solution of compound F (75 mg, 0.13 mmol) in 3 mL of DMF was added DIPEA (0.07 ml, 0.39 mmol). After 1 h PyBOP (130 mg, 0,25 mmol) and [**B**] (74 mg, 0.16 mmol) were added and the solution was stirred for further 16 h. Then 50 ml of H_2O were added to the reaction flask. The precipitate was filtered off and then dried on a rotary evaporator. The successful synthesis of the Boc-protected intermediate was confirmed by mass spectrometry. **MS:** HR-ESI-pos ($CHCl_3$/MeOH): m/z = 1216.5975 (calcd.: 1216.5975 $[M+Na]^+$). The intermediate was dissolved in 5 ml of a 1:1 TFA: DCM mixture and stirred for one hour at room temperature. The crude product was then precipitated with Et_2O and filtered. The crude product was dried on a rotary evaporator and purified *via* MPLC (gradient 60 → 100 MeOH, 105 min.). After cleaning, the product was mixed with 100 ml of a 3 molar hydrochloric acid solution. The solution was distilled off to dryness on a rotary evaporator. This procedure was repeated once. The product was then taken up in water and lyophilized. A slightly yellow solid (6 mg, >0.01 mmol, 5%) was isolated. **^{1}H-NMR** (600 MHz, DMSO-d_6) δ = 9.51 (bs, 3H) **a**-H, 8.44 (t, *J* = 5.5 Hz, 1H) **b**-H, 8.10 (bs, 2H) **c**-H (note: two *sec*. amine signals were not found due to extreme broadening of the signal), 7.66 (s, 1H) **d**-H, 7.51 (d, *J* = 8.5 Hz, 2H) **e**-H, 7.44 (s, 1H) **f**-H, 7.37 (d, *J* = 8.5 Hz, 1H) **g**-H, 7.10 (d, *J* = 8.5 Hz, 2H) **h**-H, 6.94 (d, *J* = 8.5 Hz, 1H) **i**-H, 6.91 (s, 1H) **j**-H, 4.56 (s, 2H) **k**-H, 3.29 (s, 4H) **l**-H, 3.26 – 3.20 (m, 2H) **m**-H, 3.06 (t, *J* = 6.9 Hz, 2H) **n**-H, 2.99 – 2.89 (m, 4H **o**-H, 2.89 – 2.80 (m, 2H) **p**-H, 2.44 (dd, *J* = 18.9, 8.4 Hz, 1H) **q**-H, 2.40 – 2.35 (m, 1H) **r**-H, 2.30 – 2.21 (m, 1H) **s**-H, 2.11 – 2.02 (m, 1H) **t**-H, 2.02 – 1.97 (m, 2H) **u**-H, 1.97 – 1.90 (m,

2H) **v**-H, 1.88 – 1.80 (m, 2H) **w**-H, 1.80 – 1.73 (m, 1H) **x**-H, 1.62 – 1.48 (m, 3H) **y**-H, 1.46 – 1.33 (m, 3H) **z**-H, 0.83 (s, 3H) **α**-H ppm. **^{13}C-NMR** (151 MHz, DMSO-d_6) δ = 167.76 (s) **b**, 158.8 (s) **c**, 157.6 (s) **d**, 152.1 (s) **e**, 139.2 (s) **f**, 137.3 (s) **g**, 135.6 (s) **h**, 135.3 (s) **i**, 127.5 (s) **j**, 122.4 (s) **k**, 121.9 (s) **l**, 119.5 (s) **m**, 117.8 (s) **n**, 116.8 (s) **o**, 116.7 (s) **p**, 115.4 (s) **q**, 114.4 (s) **r**, 108.1 (s) **s**, 67.0 (s) **t**, 49.7 (s) **u**, 47.4 (s) **v**, 44.9 (s) **w**, 44.1 (s) **x**, 43.7 (s) **y**, 43.0 (s) **z**, 43.0 (s) **z**, 37.6 (s) **α**, 36.2 (s) **β**, 35.6 (s) **γ**, 35.5 (s) **γ**, 31.4 (s) **δ**, 29.0 (s) **ε**, 26.0 (s) **ζ**, 25.8 (s) **η**, 25.4 (s) **θ**, 23.8 (s) **ι**, 21.2 (s) **κ**, 13.6 (s) **λ** ppm. **IR (ATR):** *v* = 3350 (NH), 3091 (CH arom.), 3026(CH arom.), 2929(CH aliph.), 2856 (CH aliph.), 1728 (C=O), 1655, 1593, 1543, 1491, 1468, 1373, 1279, 1246, 1176, 1157, 1053, 1009, 916, 879, 825, 752, 703 cm^{-1}. **MS:** HR-ESI-pos (MeOH/H_2O/FA): m/z = 735.3706 (calc.: 735.3687 $[M+H]^+$).

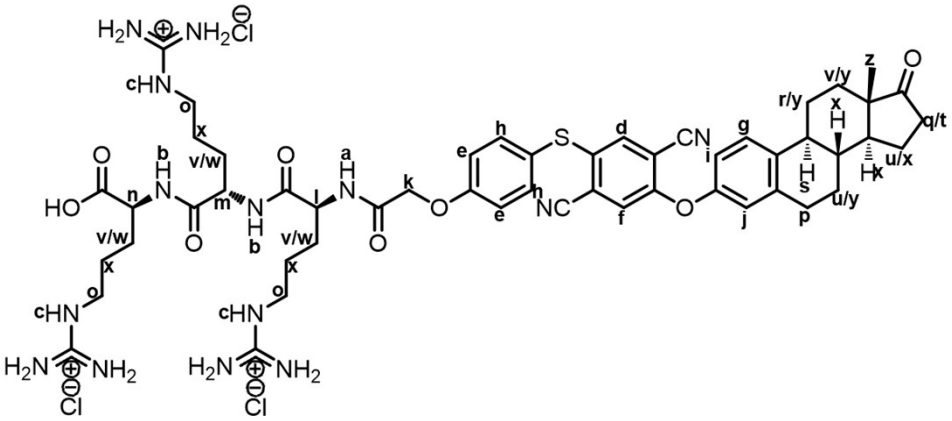
Compound [3]



The tripeptide ((Pbf)Arg)$_3$ was synthesized using standard solid phase peptide coupling conditions. The resin bound tripeptide (0.11 g, 0.07 mmol, loading 0.63 mmol/g) was suspended in dichloromethane under argon followed by the addition of DIPEA (0.25 ml, 0.14 mmol), HCTU (0.40 g, 0.10 mmol) and compound [**H**]. The mixture was shaken for 4 days at room temperature followed by filtration of the resin and washing with dichloromethane. The resin was suspended in trifluoroacetic-acid (TFA) (10 mL), triisopropylsilane (TIS) (0.25 mL) and water (0.5 mL). After shaking for 4 h, cold diethyl ether was added and the precipitate was filtered. The pure compound was dissolved in 0.1 M hydrochloric acid (5 mL) and evaporated to dryness. This procedure was repeated three times to obtain the corresponding hydrochloride. The obtained residue was further purified using HPLC. Yield: 6 mg (>0.01 mmol, 7%, based on the resin loading). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ = 8.27 (d, *J* = 7.4 Hz, 1H) **a**-H, 8.24 (t, *J* = 5.7 Hz, 1H) **b**-H, 8.20 (d, *J* = 7.8 Hz, 1H) **c**-H, 8.10 (d, *J* = 7.7 Hz, 1H) **d**-H, 7.88 – 7.78 (m, 3H) **e**-H, 7.69 (s, 1H) **f**-H, 7.51 (d, *J* = 8.8 Hz, 2H) **g**-H, 7.46 (s, 1H) **h**-H, 7.38 (d, *J* = 8.6 Hz, 1H) **i**-H, 7.08 (d, *J* = 8.8 Hz, 2H) **j**-H, 6.95 (dd, *J* = 8.5, 2.6 Hz, 1H) **k**-H, 6.92 (d, *J* = 2.5 Hz, 1H) **l**-H, 4.56 (s, 2H) **m**-H, 4.32 – 4.27 (m, 2H) **n**-H, 4.17 – 4.13 (m, 1H) **o**-H, 3.63 – 3.55 (m, 2H) **p**-H, 3.51 – 3.46 (m, 4H) **q**-H, 3.44 (t, *J* = 6.0 Hz, 2H) **r**-H, 3.29 (q, *J* = 5.9 Hz, 2H) **s**-H, 3.17 – 3.05 (m, 6H) **t**-H, 2.86 (dd, *J* = 8.7, 3.9 Hz, 2H) **u**-H, 2.48 – 2.34 (m, 4H) **v**-H, 2.31 – 2.23 (m, 1H) **w**-H, 2.12 – 2.03 (m, 1H) **x**-H, 2.00 – 1.92 (m, 2H) **y**-H, 1.80 – 1.67 (m, 4H) **z**-H, 1.66 – 1.45 (m, 12H) **α**, 1.45 – 1.34 (m, 3H) **β**, 0.84 (s, 3H) **γ** ppm. **^{13}C-NMR** (151 MHz, DMSO-d_6) δ = 219.6 (s) **a**, 173.1 (s) **b**, 171.4 (s) **c**, 171.5 (s) **c**, 170.4 (s) **d**, 167.34 (s) **e**, 158.8 (s) **f**, 157.5 (s) **g**, 157.0 (s) **h**, 152.1 (s) **i**, 139.1 (s) **j**, 137.1 (s) **k**, 135.5 (s) **l**, 135.4 (s) **l**, 135.2 (s) **m**, 127.3 (s) **n**, 122.3 (s) **o**, 121.8 (s) **p**, 119.4 (s) **q**, 117.8 (s) **r**, 116.7 (s) **s**, 116.5 (s) **t**, 115.3 (s) **u**, 114.3 (s) **v**, 108.0 (s) **w**, 69.5 (s) **x**, 69.4 (s) **x**, 68.8 (s) **y**, 66.9 (s) **z**, 66.8 (s) **α**, 52.1 (s) **β**, 51.9 (s) **β**, 51.6 (s) **γ**, 49.6 (s) **δ**, 47.3 (s) **ε**, 43.6 (s) **ζ**, 40.3 (s) **η**, 38.3 (s) **θ**, 37.4 (s) **ι**, 35.9 (s) **κ**, 35.4 (s) **λ**, 31.3 (s) **μ**, 29.1 (s) **ξ**, 29.0 (s) **ξ**, 28.9 (s) **π**, 27.9 (s) **ρ**, 25.8 (s) **ς**, 25.3 (s) **σ**, 25.2 (s) **τ**, 24.9 (s) **υ**, 21.1 (s) **φ**, 13.5 (s) **ψ** ppm. **IR (ATR):** *v* = 3329

(NH), 3261, 3167, 3060 (CH arom.), 2931 (CH aliph.), 2866 (CH aliph.), 1732 (C=O ketone), 1645 (C=O carboxylate), 1543, 1493, 1468, 1373, 1277, 1244, 1178, 1157, 1084, 1055, 1009, 916, 881, 827 cm^{-1}. **MS:** HR-ESI-pos (MeOH/H_2O/FA): m/z = 603.7982 (calc.: 603.7975 $[M+2H]^{2+}$).

Compound [4]



The tripeptide ((Pbf)Arg)$_3$ was synthesized using standard solid phase peptide coupling conditions. The resin bound tripeptide (0.19 mg, 0.12 mmol, loading 0.63 mmol/g) was suspended in dichloromethane under argon followed by the addition of DIPEA (0.04 ml, 0.22 mmol), HCTU (0.80 mg, 0.19 mmol) and compound [**H**] (0.10 mg, 0.17 mmol). The mixture shook for 4 days at room temperature followed by filtration of the resin and washing with dichloromethane. The resin was suspended in trifluoroacetic-acid (TFA) (20 mL), triisopropylsilane (TIS) (0.5 mL) and water (1 mL). After shaking for 4 h, cold diethyl ether was added and the precipitate was filtered. The residue was dissolved in 0.1 M hydrochloric acid (5 mL) and evaporated to dryness. This procedure was repeated three times to obtain the corresponding hydrochloride. The obtained residue was further purified using HPLC. Yield: 22 mg (0.02 mmol, 17%, based on the resin loading). **^{1}H-NMR** (600 MHz, DMSO-d_6) δ = 8.36 (d, *J* = 7.8 Hz, 1H) **a**-H, 8.33 – 8.26 (m, 2H) **b**-H, 7.92 – 7.84 (m, 3H) **c**-H, 7.68 (s, 1H) **d**-H, 7.51 (d, *J* = 8.7 Hz, 2H) **e**-H, 7.44 (s, 1H) **f**-H, 7.37 (d, *J* = 8.6 Hz, 1H) **g**-H, 7.09 (d, *J* = 8.8 Hz, 2H) **h**-H, 6.95 (dd, *J* = 8.5, 2.4 Hz, 1H) **i**-H, 6.92 (d, *J* = 2.4 Hz, 1H) **j**-H, 4.65 (dd, *J* = 25.3, 14.7 Hz, 2H) **k**-H, 4.41 – 4.35 (m, 1H) **l**-H, 4.35 – 4.27 (m, 1H) **m**-H, 4.18 – 4.11 (m, 1H) **n**-H, 3.20 – 3.04 (m, 6H) **o**-H, 2.90 – 2.81 (m, 2H) **p**-H, 2.44 (dd, *J* = 18.8, 8.5 Hz, 1H) **q**-H, 2.41 – 2.36 (m, 1H) **r**-H, 2.30 – 2.23 (m, 1H) **s**-H, 2.11 – 2.02 (m, 1H) **t**-H, 1.99 – 1.91 (m, 2H) **u**-H, 1.82 – 1.70 (m, 4H) **v**-H, 1.68 – 1.59 (m, 3H) **w**-H, 1.58 – 1.45 (m, 9H) **x**-H, 1.45 – 1.34 (m, 3H) **y**-H, 0.83 (s, 3H) **z**-H ppm. **^{13}C-NMR** (151 MHz, DMSO-d_6) δ = 219.6 (s) **a**, 173.2 (s) **b**, 171.4 (s) **c**, 171.1 (s) **d**, 167.3 (s) **e**, 158.9 (s) **f**, 157.5 (s) **g**, 157.0 (s) **h**, 152.1 (s) **i**, 139.1 (s) **j**, 137.2 (s) **k**, 135.5 (s) **l**, 135.5 (s) **m**, 135.3 (s) **n**, 127.4 (s) **o**, 122.3 (s) **p**, 121.7 (s) **q**, 119.4 (s) **r**, 117.7 (s) **s**, 116.7 (s) **t**, 116.5 (s) **u**, 115.3 (s) **v**, 114.3 (s) **w**, 108.1 (s) **x**, 66.7 (s) **y**, 52.1 (s) **z**, 52.0 (s) **z**, 51.7 (s) **α**, 49.6 (s) **β**, 47.3 (s) **γ**, 43.6 (s) **δ**, 40.3 (s) **ε**, 37.5 (s) **ζ**, 35.4 (s) **η**, 31.3 (s) **θ**, 29.1 (s) **ι**, 29.0 (s) **ι**, 28.9 (s) **κ**, 27.9 (s) **λ**, 25.8 (s) **μ**, 25.3 (s) **ξ**, 25.2 (s) **ξ**, 24.9 (s) **π**, 21.2 (s) **ρ**, 13.5 (s) **ς** ppm. **IR (ATR):** *v* = 3325 (NH), 3261, 3168, 3057 (CH arom.), 2931 (CH aliph.), 2862(CH aliph.), 1734 (C=O ketone), 1645 (C=O carboxylate), 1539, 1491, 1468, 1373, 1244, 1176, 1155, 1051, 1009, 916, 879, 825 cm^{-1}. **MS:** HR-ESI-pos (MeOH/H_2O/FA): m/z = 524.2547 (calc.: 524.2527 $[M+2H]^{2+}$).

Mass spectra of the final compounds

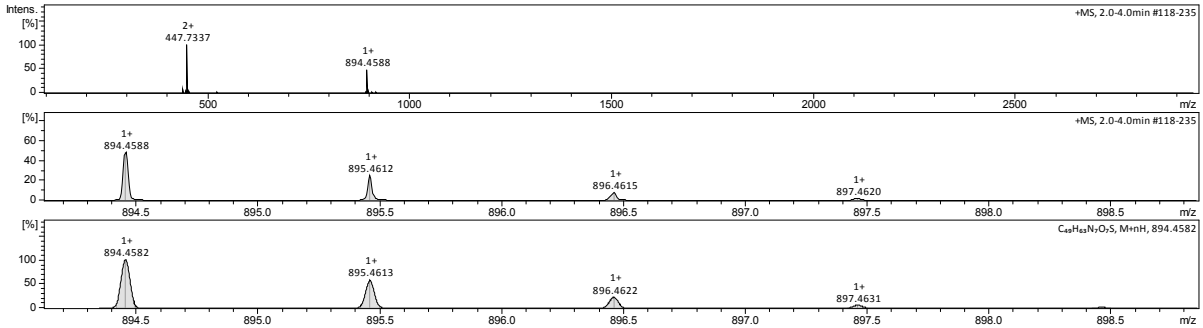


Fig. S1: HR-ESI-MS spectrum of compound [**1**]. The first spectrum shows the whole spectrum. The second is a close up. The third is the calculated spectrum.

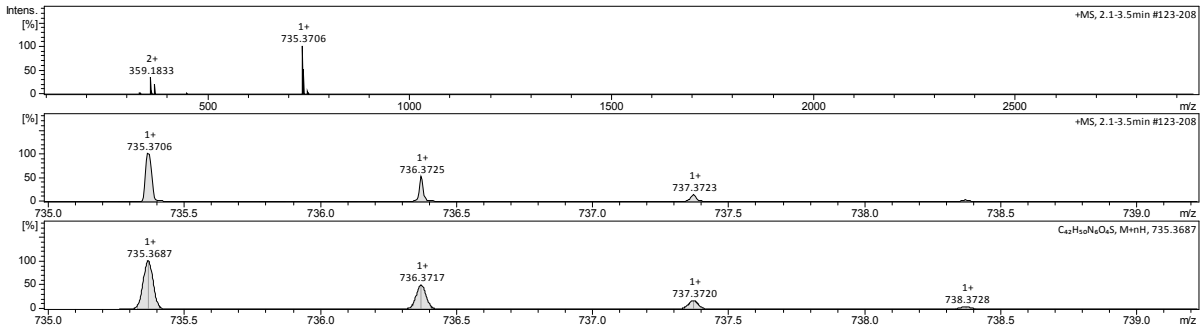


Fig. S2: HR-ESI-MS spectrum of compound [**2**]. The first spectrum shows the whole spectrum. The second is a close up. The third one is the calculated spectrum.

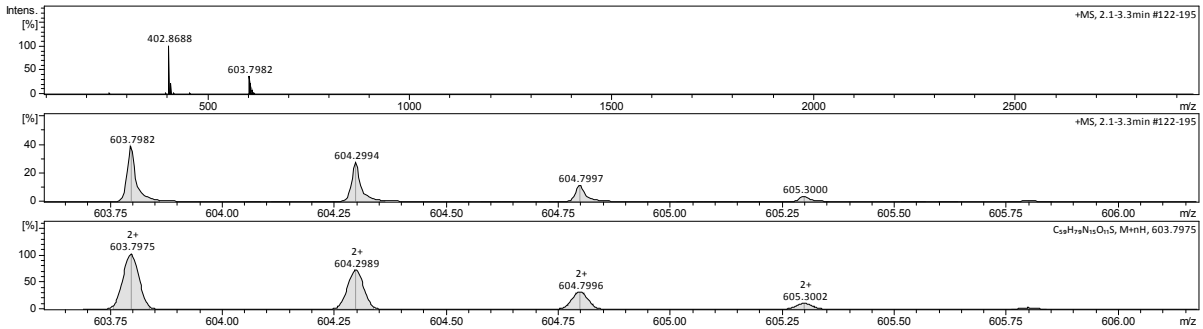


Fig. S3: HR-ESI-MS spectrum of compound [**3**]. The first spectrum shows the whole spectrum. The second is a close up. The third one is the calculated spectrum.

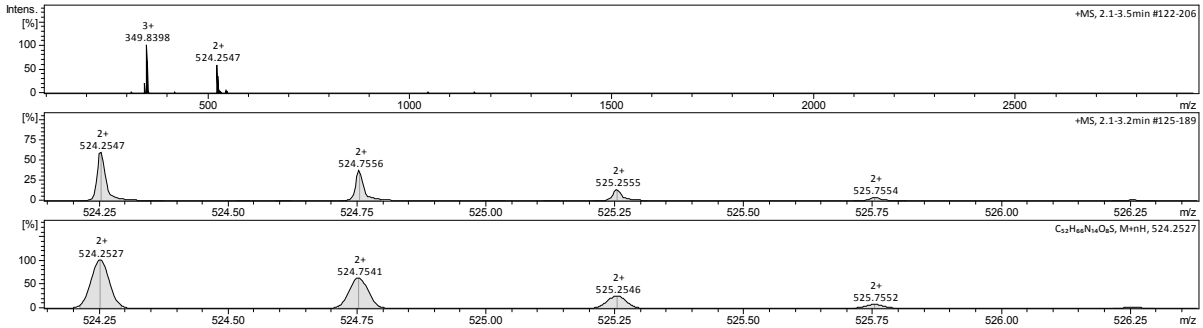


Fig. S4: HR-ESI-MS spectrum of compound [**4**]. The first spectrum shows the whole spectrum. The second is a close up. The third is the calculated spectrum.

DLS and zeta-potential measurements

Dynamic light scattering (DLS) was performed on a Malvern Zetasizer Nano ZS the incorporated HeNe laser works at a wavelength of 633 nm and uses a detector at an angle of 173° (non-invasive back scatter technology) using small volume disposable PMMA cuvettes. Measurements were recorded with 3 min equilibration time at 20 °C. Zeta potential measurements were performed on the same instruments using small volume PMMA zeta potential cuvettes.

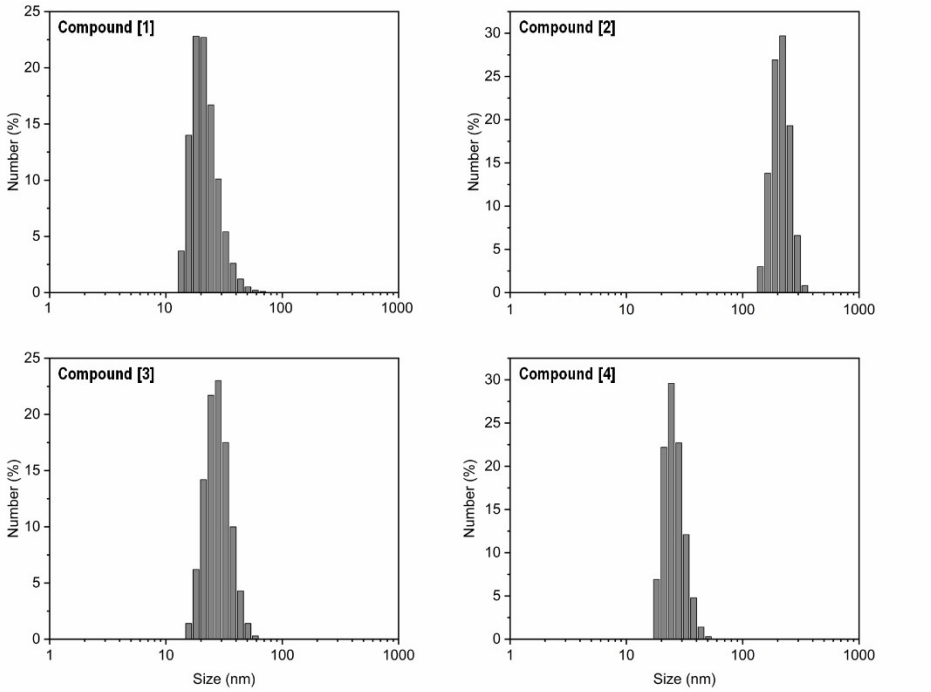


Fig. S5: DLS measurements of compounds [**1**]-[**4**] in water (c = 100 µM).

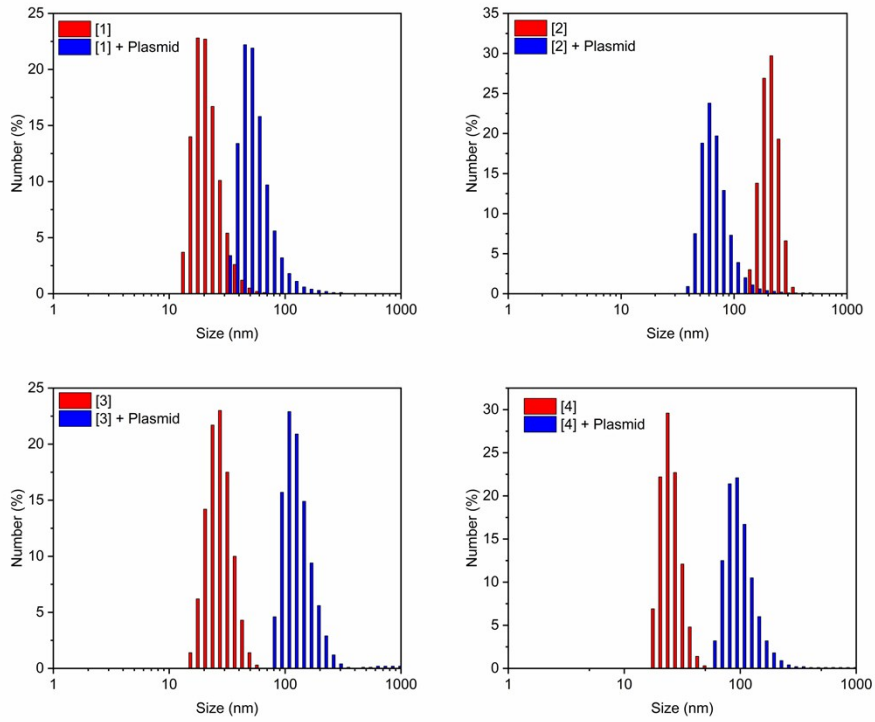


Fig. S6: DLS measurements of compound [**1**]-[**4**] in the absence and presence of RFP plasmid. c(compound) = 100 µM, c(mRFP-plasmid) = 10 µg/mL.

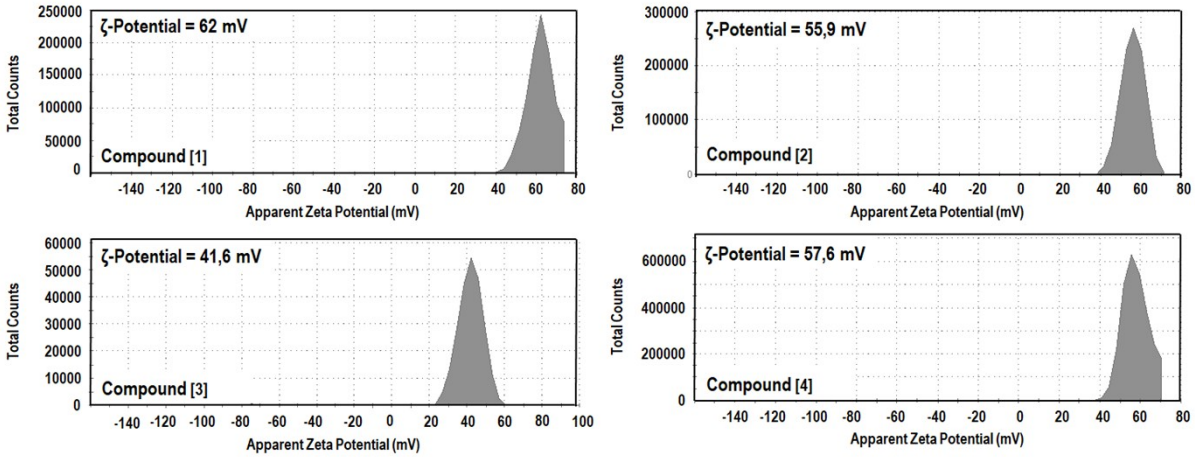


Fig. S7: Zeta-potential measurements of compounds [**1**]-[**4**] in water (c = 100 µM).

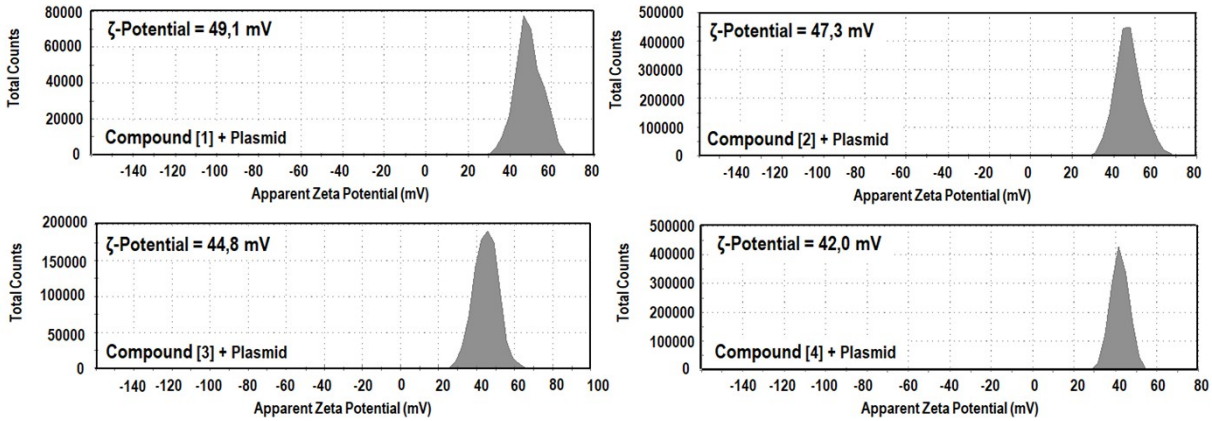


Fig. S8: Zeta-potential measurements of compounds [**1**]-[**4**] in water (c = 100 µM) in the presence of mRFP plasmid c(RFP plasmid) = 10 µg/mL.

Photophysical properties

The emission and excitation spectra were recorded on a RF-6000 from Shimadzu Corporation in Japan. The UV-Vis spectra were recorded on a Jasco V-550 from Jasco LTD in Japan Therefore we used semi-micro disposable PMMA cuvettes with dimensions 12.5mm x 12.5mm x 45 mm and a volume of 1.5 ml from Brand GmbH.

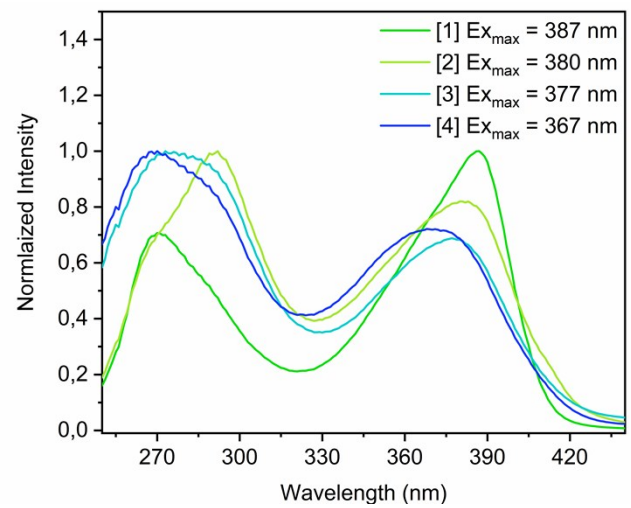


Fig. S9: Normalized excitation spectra of compounds [**1**]-[**4**] at a concentration of 100 µM, (99% H_2O/ 1 % DMSO).

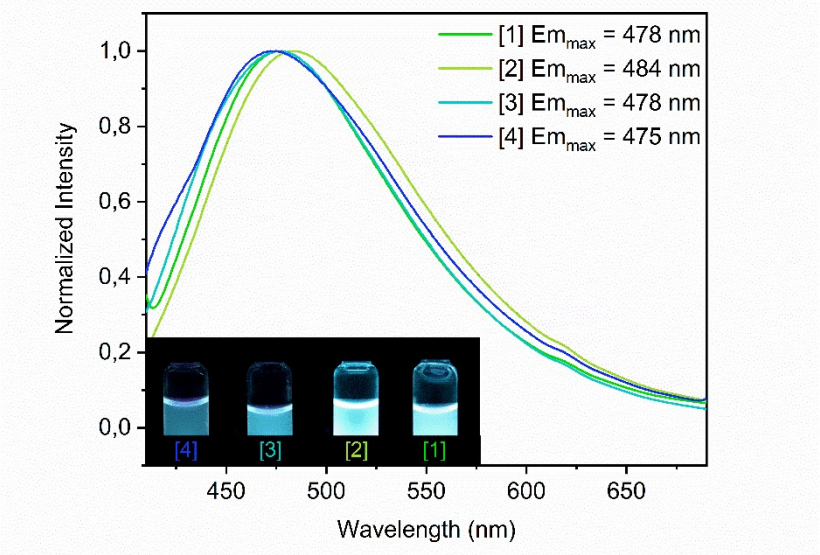


Fig. S10: Normalized emission spectra of compounds [**1**]-[**4**] at a concentration of 100 µM, (99% H_2O/ 1 % DMSO) as well as photographs pf the compounds under UV-light irradiation (λ = 365 nm).

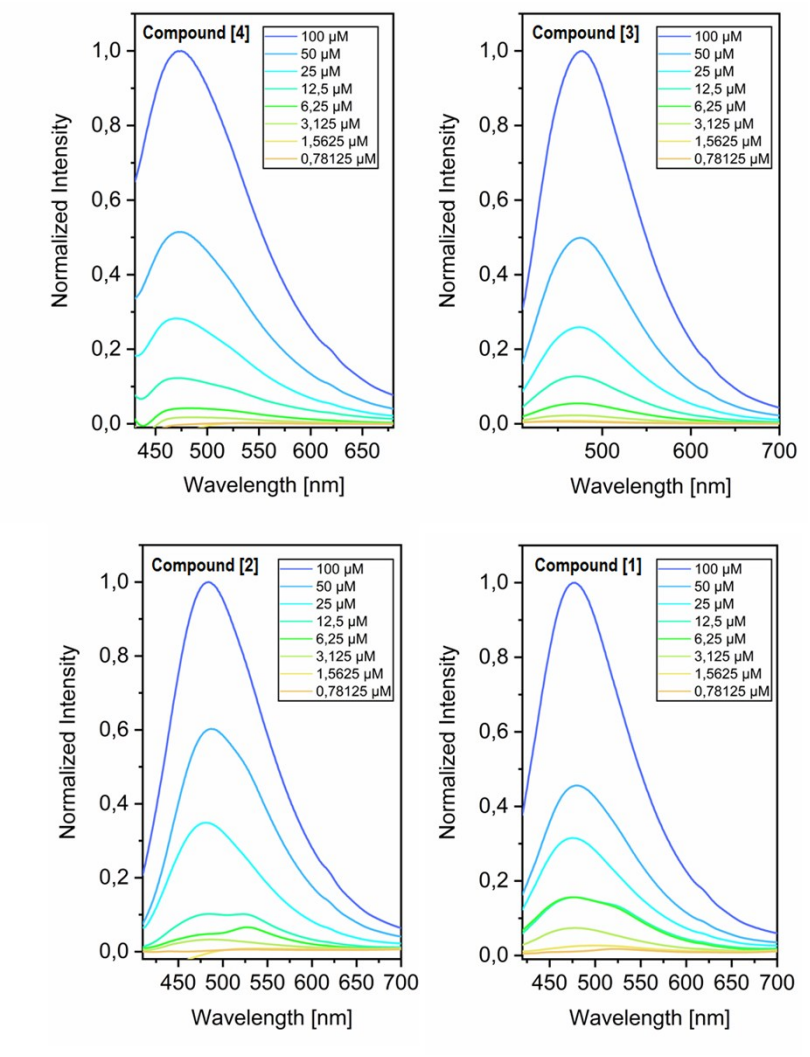


Fig. S11: Normalized emission spectra of compounds [**1**]-[**4**] with varying concentrations in water.

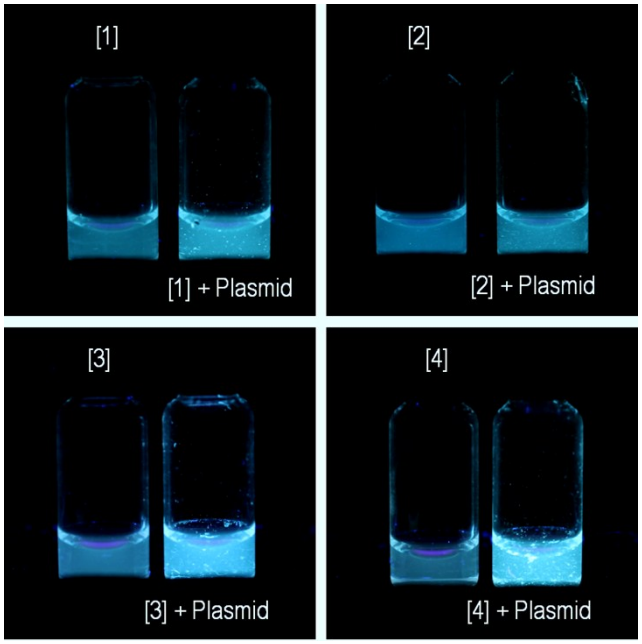


Fig. S12: Photographs of compounds [**1**]-[**4**] with and without added RFP plasmid under UV-light irradiation (λ = 365 nm). c(compound) = 0.1 mM, c(mRFP-plasmid) = 10 µg/mL.

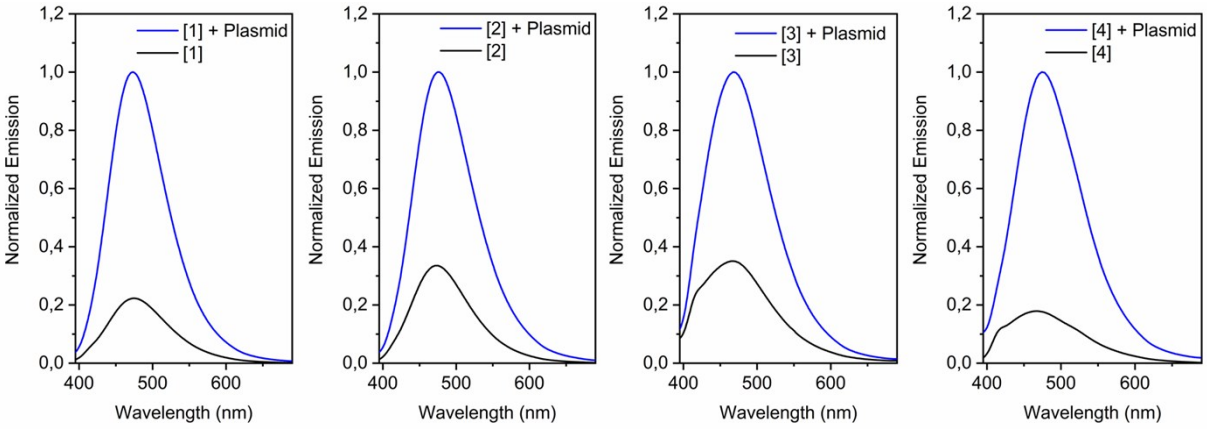


Fig. S13: Emission spectra of compound [**1**]-[**4**] with and without added RFP plasmid. c(compound) = 0.1 mM, c(mRFP-plasmid) = 10 µg/mL.

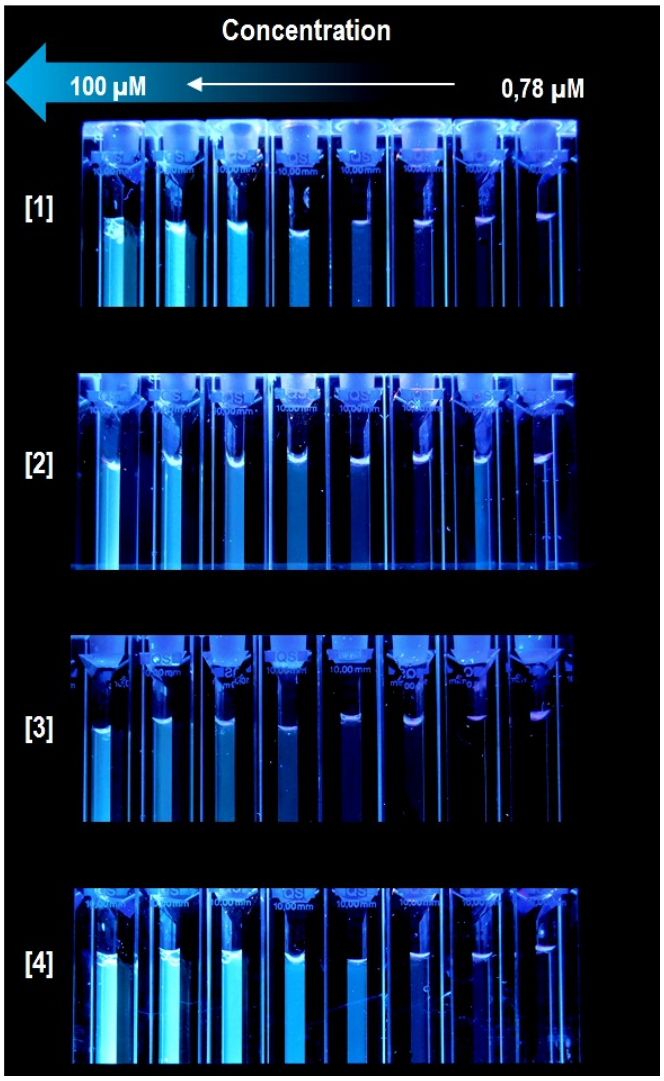


Fig. S14: Photographs of compounds [**1**]-[**4**] at different concentrations under UV-light irradiation (λ = 365 nm).

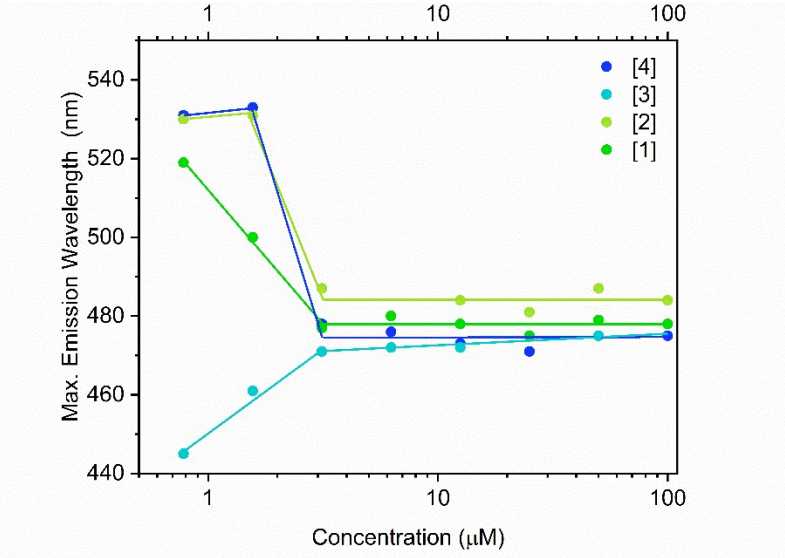


Fig. S15: Concentration dependent emission shifts of compound [**1**]-[**4**] to determine the critical aggregation concentration.

Transmission electron microscopy (TEM)

Measurements were carried out on a JEOL JEM-2200FS with 200 kV acceleration voltage. Samples were prepared by dropping 5 µL of the sample solutions on a 400 mesh carbon coated copper grid. After 45 s the remaining solution was removed with a filter paper. Afterwards the samples were stained with 2% aqueous uranyl formate solution (10 µL). At last, the grids were dried for 24 h in a desiccator over silica gel in vacuo.

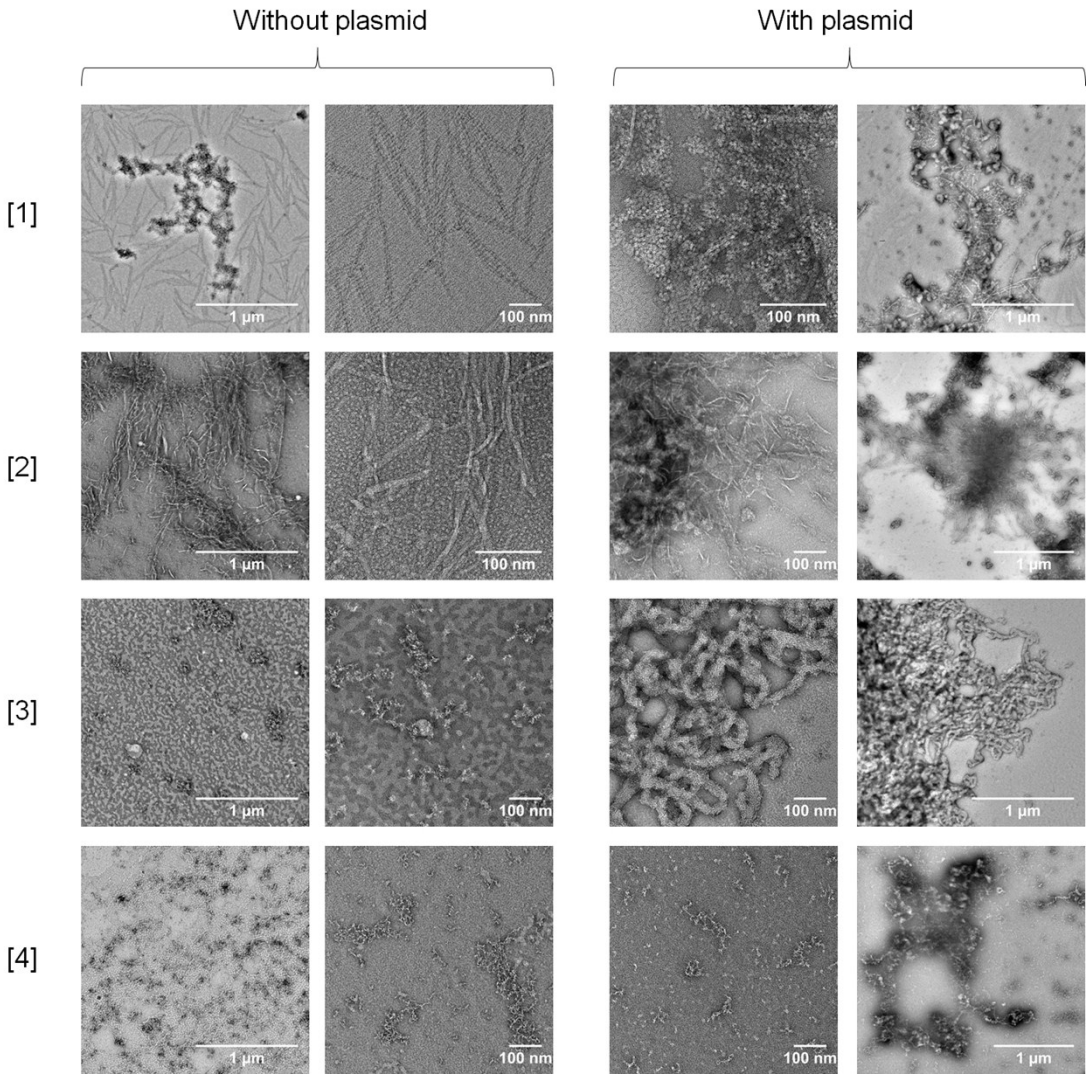


Fig. S16: TEM images of compounds [**1**]-[**4**] in the absence and presence of RFP plasmid. c(compound) = 500 µM, c(RFP plasmid) = 4 µg / 100 µL.

Single-crystal X-ray analysis

The crystals were mounted on nylon loops in inert oil. Data were collected on a Bruker AXS D8 Venture diffractometer with Photon II detector (mono-chromated $Cu_{K\alpha}$ radiation, λ = 1.54178 Å, mirco-focus source) at 100(2) K. The structures were solved by Direct Methods (SHELXS-97)[3] and refined anisotropically by full-matrix least-squares on F^2 (SHELXL-2014)[4, 5,6]. Absorption corrections were performed semi-empirically from equivalent reflections on basis of multi-scans (Bruker AXS APEX3). Hydrogen atoms were refined using a riding model

or rigid methyl groups. The long c-axis caused problems during the integration in spite of a long crystal detector distance and small frame widths. The systematic extictions for the 2_1 axis were inconclusive thus alternatively $P4_1$ was tried. This yielded a merohedral twinning (BASF 0.49(1)) and slightly lower *R*-values. However, despite vast use of restraints on the adp the displacement ellipsoids displayed unrealistic shapes thus this model was discarded. Due to the limited data quality quantitative results should be carefully scrutinised. The steroid was taken from a natural source so its enantio-purity can be taken for granted. The rather high Flack parameter is more likely caused by the poor data than by inversion twinning.
(F) crystallizes in the tetragonal space group $P4_12_12$ with one molecule plus a DMF solvent molecule in the asymmetric unit. Alternatively, a description as merohedral twin in space group $P4_1$ is possible (details see above). Bond lengths and angle are within the expected range and the overall conformation of the estrone residue remains unchanged. The overall conformation of the molecule can be roughly described as Z-shaped with the best plane of the residual groups approximately perpendicular to the plane of the central ring. The DMF is connected to the carboxyl groups by strong hydrogen bonds forming an annular motif.

CCDC-1938010 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

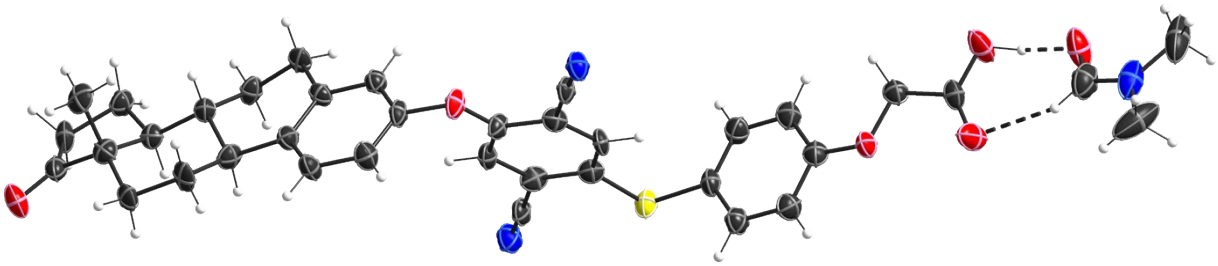
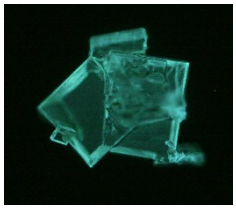


Fig. S17: Molecular structure of compound (**F**).

Identification code	**(F)**
Empirical formula	$C_{37}H_{37}N_3O_6S$
M	651.75
Crystal size [mm]	0.264 × 0.174 × 0.120
T [K]	100(2)
Crystal system	tetragonal

Space group	$P4_12_12$
a [Å]	8.0733(12)
b [Å]	8.0733(12)
c [Å]	101.060(17)
α [°]	90
β [°]	90
γ [°]	90
V [Å^3]	6587(2)
Z	8
D_{calc} [g·cm^{-3}]	1.314
μ(CuK_α [mm^{-1}])	1.295
Transmissions	0.75/0.54
F(000)	2752
Index ranges	$-9 \le h \le 10$
	$-10 \le k \le 10$
	$-127 \le l \le 127$
θ_{max} [°]	78.566
Reflections collected	148830
Independent reflections	7041
R_{int}	0.0775
Refined parameters	431
R_1 [$I > 2\sigma(I)$]	0.0985
wR_2 [all data]	0.2258
x(Flack)	0.246(6)
GooF	1.347
$\Delta\rho_{final}$ (max/min) [e·Å^{-3}]	0.365/-0.426
Photograph of the crystals under UV light irradiation λ = 365 nm	

Cell assays and microscopy

Cell culture

HEK 293T and HeLa cells were grown and maintained in DMEM media (Invitrogen) with 10 % FBS (Gibco) and 1 % Antibiotic-Antimycotic (Gibco) in a humidified 5 % CO_2 atmosphere at 37 °C.

Microscopy

For lipofection experiments, the compound (from a 40 mM DMSO stock solution) was added to water to reach the desired dilution and mixed by gentle vortexing. After approximately 5 min. the plasmid DNA was added to the mixture and vortexed again. This compound-DNA solution was finally added dropwise to the cells.

For the transfection experiment, HeLa / HEK 293T cells were seeded in ibiTreat-coated µ-Slide 8 well (ibidi). Cells were treated by adding either a solution of the compound (concentrations indicated in the figure legends) mixed with plasmid DNA (pH2B-mRFP) or Lipofectamine™ 2000 (Invitrogen) mixed with the same plasmid DNA according to the provided protocol. After 16 h of incubation, the cells were imaged with a Leica SP8 confocal laser scanning microscope (Leica) equipped with a HC PL APO 20x/0.75 CS2 objective (Leica). The compound was excited using a 405 nm diode laser, pH2B-RFP using a DPSS 561 nm laser.

For determining the transfection efficiency, Hela and HEK 293T cells were cultivated and transfected using an ibiTreat-coated µ-Slide 8 well (ibidi) like described above. After 16 h of incubation, the cells were additionally treated with 1 µM SYTO™ 61 Red Fluorescent Nucleic Acid Stain (Thermo Fisher) for 60 min. After three washing steps with DPBS the cells were imaged with a Leica SP8 confocal laser scanning microscope (Leica) equipped with a HC PL APO 20x/0.75 CS2 objective (Leica). The compound was excited using a 405 nm diode laser, pH2B-RFP using a DPSS 561 nm laser and SYTO™ 61 Red using a HeNe 633 laser. To calculate the transfection efficiency, the ratio of transfected cells (segmented using H2B-mRFP intensity) and all cells (segmented using the SYTO™ 61 Red intensity) was determined using CellProfiler™.[7]

For the localization experiment HeLa cells were seeded on ibiTreat-coated µ-Slide 8 well (ibidi). Cells were treated by adding either a solution of 48 µM [1] mixed with 0.5 µg plasmid DNA (pH2B-mRFP) and incubated with LysoTracker™ Green DND-26 (Invitrogen) according to the provided protocol. Four hours after compound treatment the cells were imaged with a Leica SP8 confocal laser scanning microscope (Leica) equipped with a HC PL APO 20x/0.75 CS2 objective (Leica). The compound was excited using a 405 nm diode laser, LysoTracker™ Green using an Argon laser, pH2B-mRFP using a DPSS 561 nm laser.

Images were generated using FIJI[8] and OMERO.[9]

CellTiter®Aqueous One Cell Proliferation Assay

The CellTiter 96® AQueous One Cell Proliferation Assay (Promega) was used to observe HeLa cell proliferation after treatment with different concentrations of the compounds (100 µM, 200 µM and 400 µM) or Lipofectamine™ 2000 (0.25 µl, 0.5 µl, 1 µl) for 24 h. The assay determines the number of viable cells per well by using the MTS tetrazolium compound (Owen's reagent) that is bioreduced by NADPH or NADH in living cells into a colored formazan.

The assay was performed according to the manufacturer's protocol by adding 20 µl of CellTiter 96® AQueous One Solution Reagent directly into each culture well of a 96 well plate and incubating for 3 h at 37 °C. Afterwards, the absorption, which is directly proportional to the number of living cells per well, was measured at 490 nm with a GloMax®-Multi plate reader (Promega).

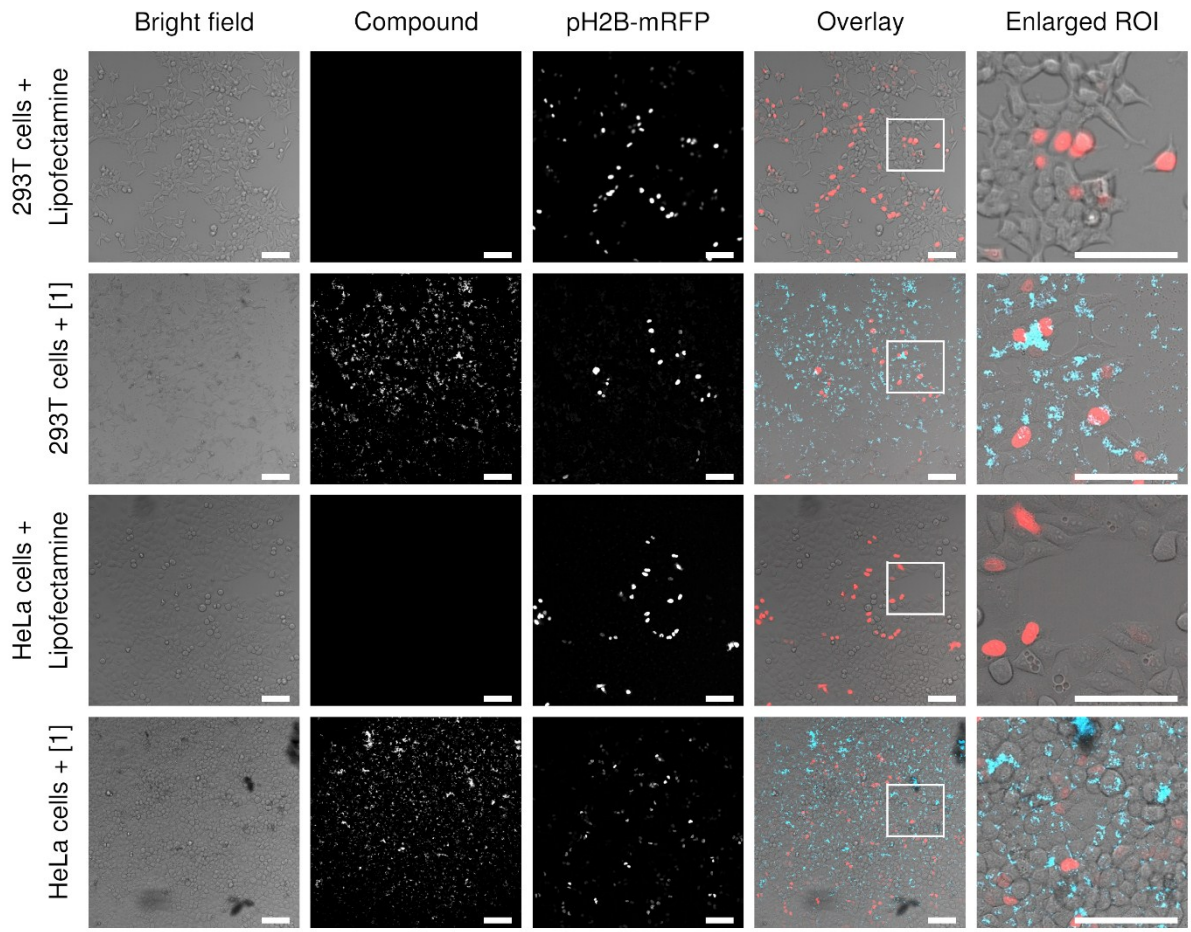


Fig. S18: Confocal images of HEK 293 T (upper panels) and HeLa cells (lower panels) 16 h after transfection of pH2B-mRFP (2.4 µg/mL; red) with Lipofectamine™ 2000 or [1] (48 µM; cyan). Scale bar: 100 µm.

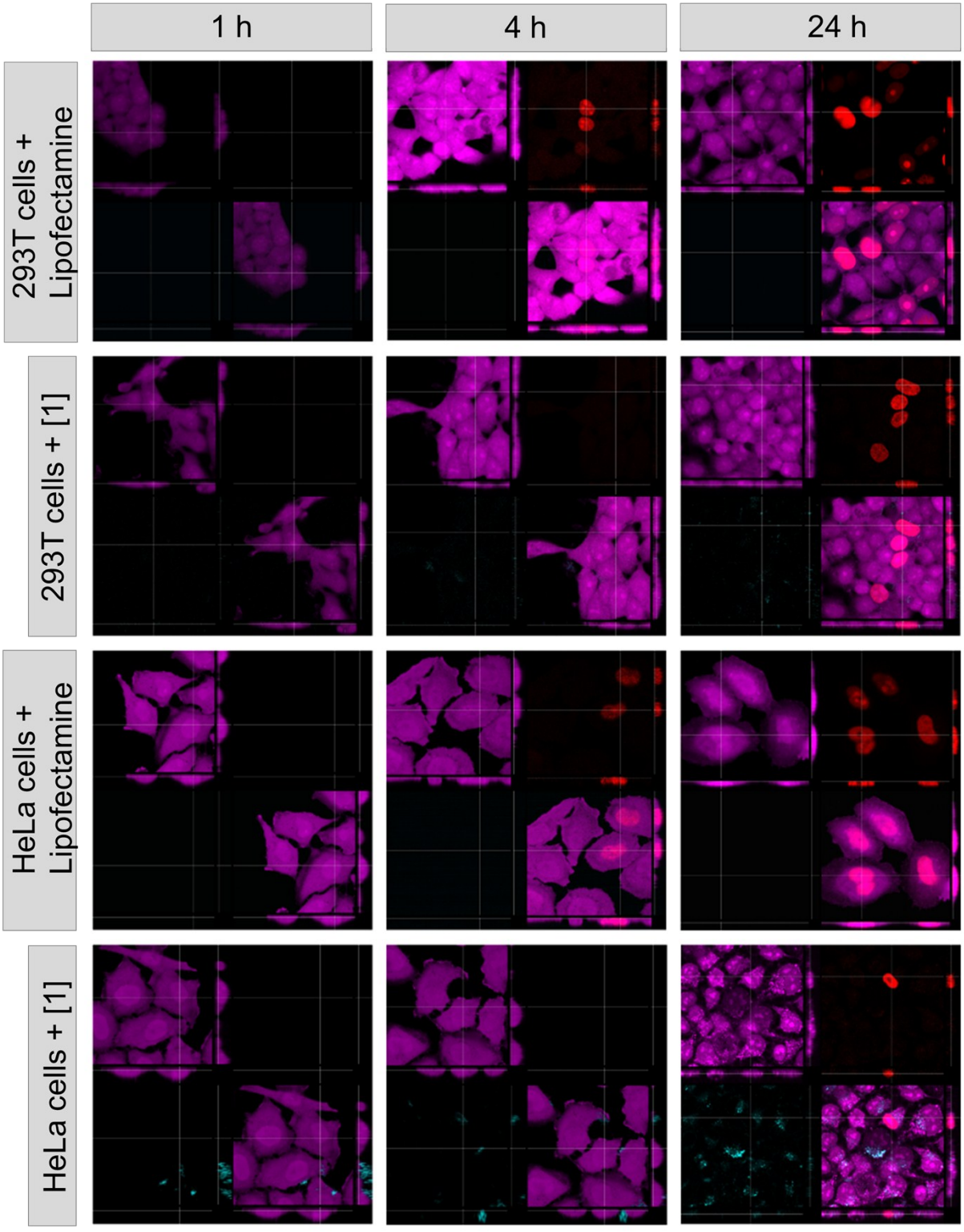


Fig. S19: 3D CLSM images of the time course of the transfection of pH2B-mRFP (red) with Lipofectamine™ 2000 or [1] (48 µM; cyan) of HEK 293T (upper panels) and HeLa cells (lower panels).

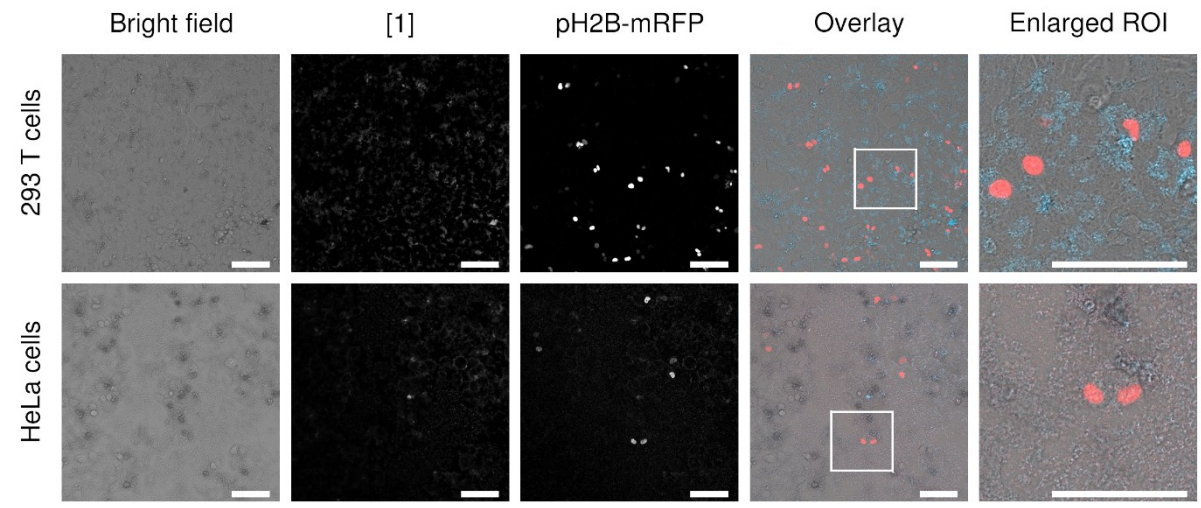


Fig. S20: Confocal images of HEK 293 T (upper panels) and HeLa cells (lower panels) 16 h after transfection of pH2B-mRFP (4.8 µg/mL; red) with [1] (190 µM; cyan). Scale bar: 100 µm.

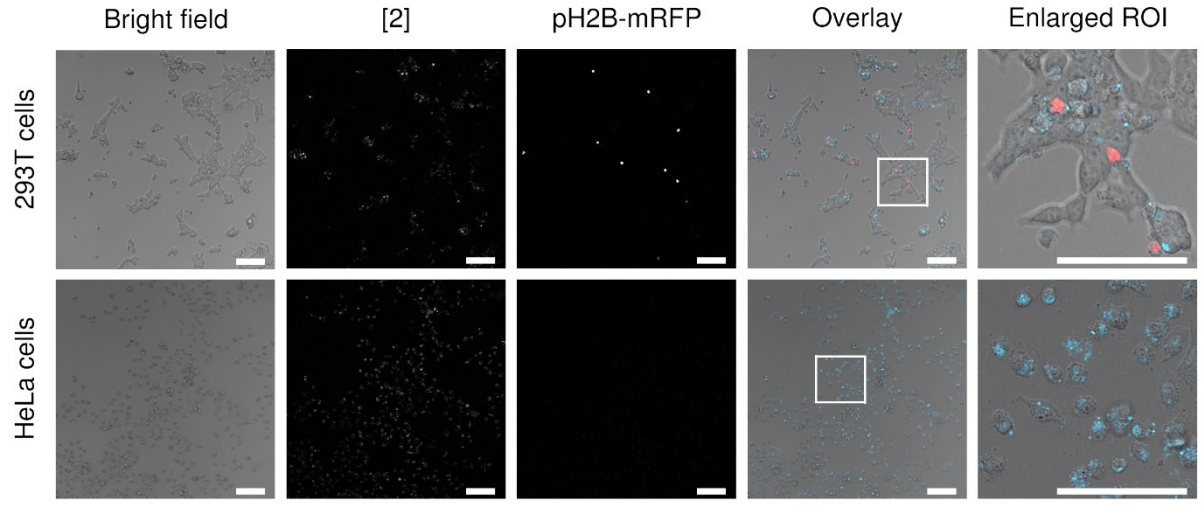


Fig. S21: Confocal images of HEK 293 T (upper panels) and HeLa cells (lower panels) 16 h after transfection of pH2B-mRFP (4.8 µg/mL; red) with [**2**] (10 µM; cyan). Scale bar: 100 µm.

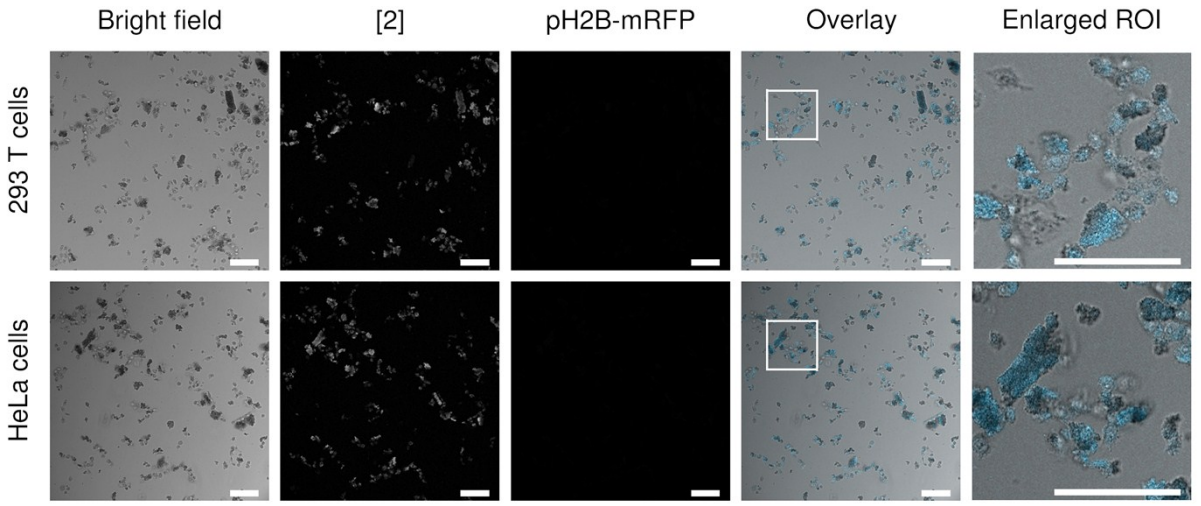


Fig. S22: Confocal images of HEK 293 T (upper panels) and HeLa cells (lower panels) 16 h after transfection of pH2B-mRFP (4.8 µg/mL; red) with [**2**] (190 µM; cyan). Scale bar: 100 µm.

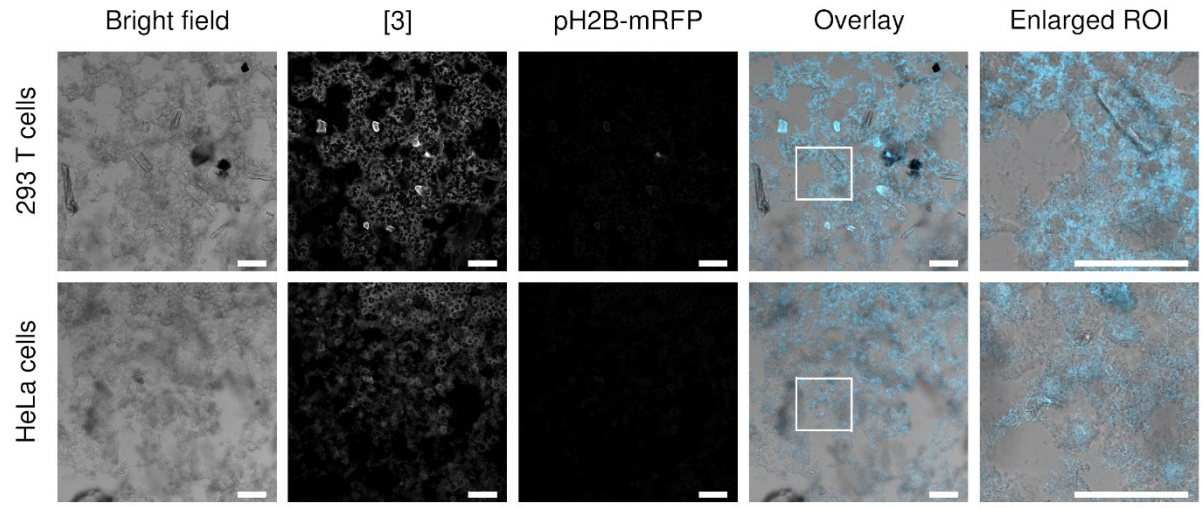


Fig. S23: Confocal images of HEK 293 T (upper panels) and HeLa cells (lower panels) 16 h after transfection of pH2B-mRFP (4.8 µg/mL; red) with [**3**] (190 µM; cyan). Scale bar: 100 µm.

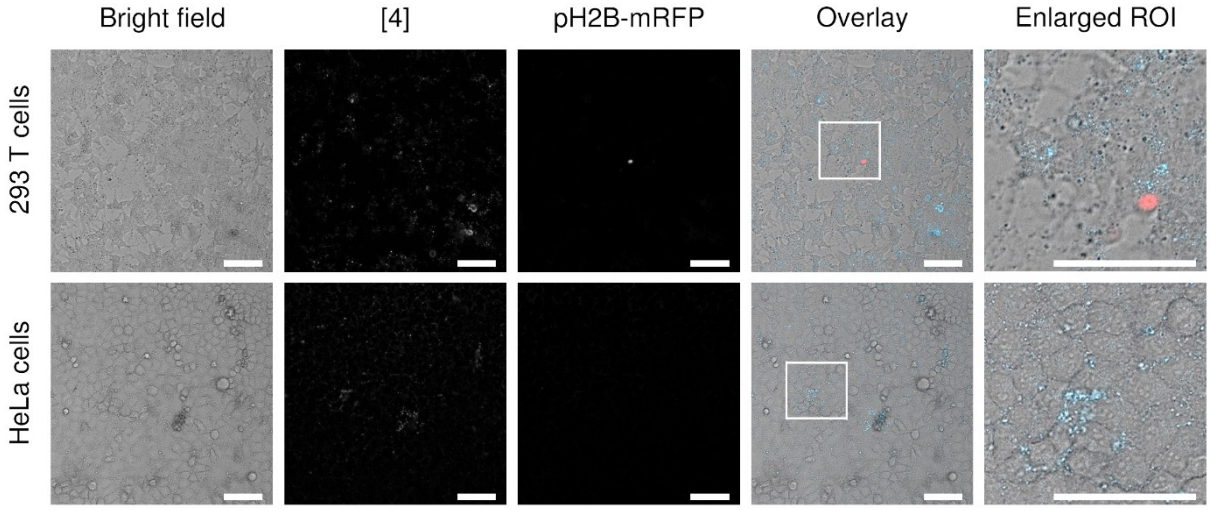


Fig. S24: Confocal images of HEK 293 T (upper panels) and HeLa cells (lower panels) 16 h after transfection of pH2B-mRFP (4.8 µg/mL; red) with [**4**] (190 µM; cyan). Scale bar: 100 µm.

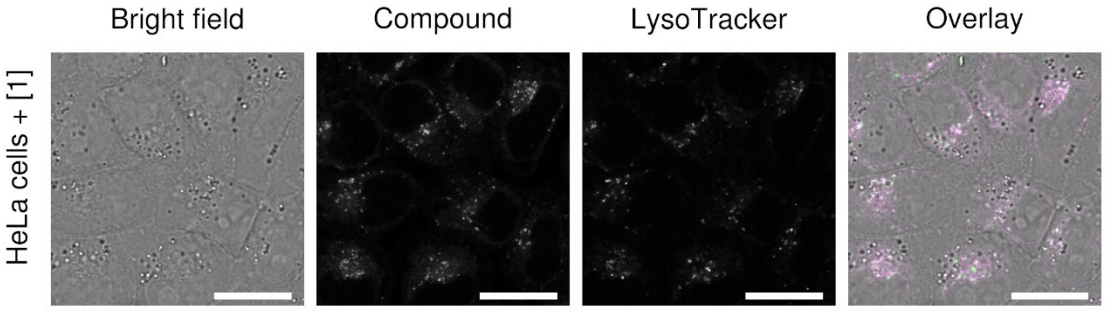


Fig. S25: Confocal images of HeLa cells 4h after incubation with [**1**] (48 µM; magenta) premixed with plasmid DNA (pH2B-mRFP) and staining with LysoTracker™ Green (green). Scale bar: 20 µm. (Concentrations: [**1**] = 48 µM, pH2B-mRFP plasmid = 2.4 µg/mL).

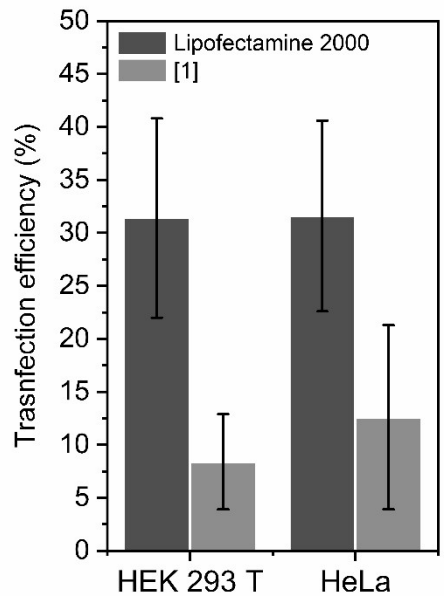


Fig. S26: Transfection efficiency of HEK293T and HeLa cells using Lipofectamine™ 2000 or compound [**1**].

NMR Spectra

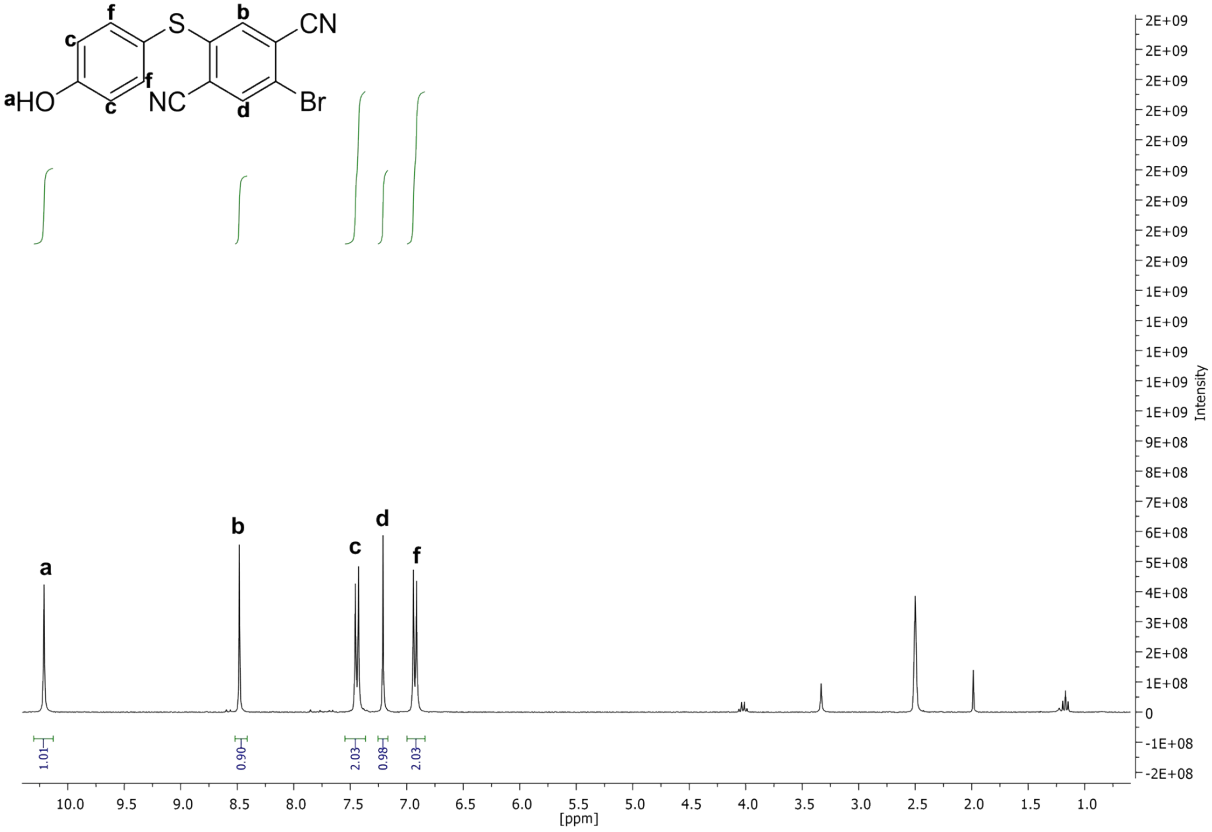


Fig. S27: ^{1}H NMR of compound **C** in DMSO-d6 (300 MHz).

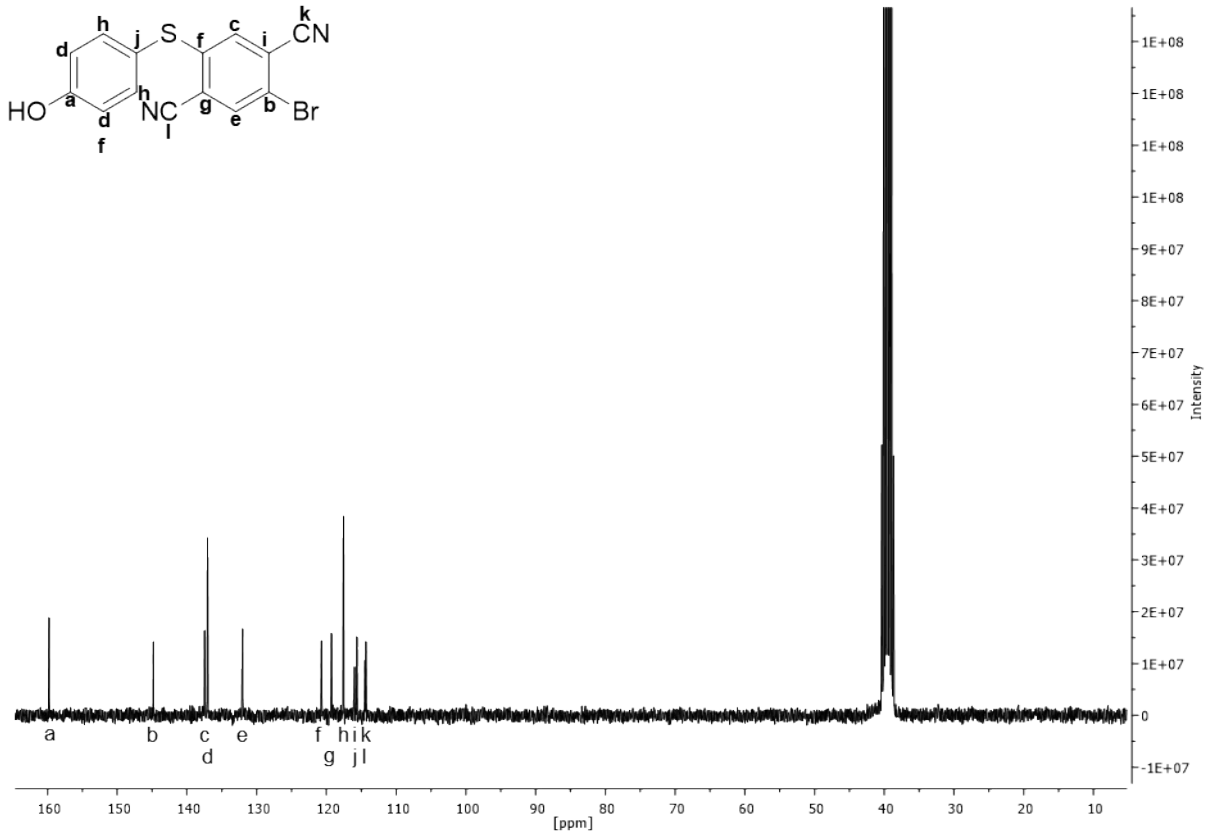


Fig. S28: ^{13}C NMR of compound **C** in DMSO-d6 (75 MHz).

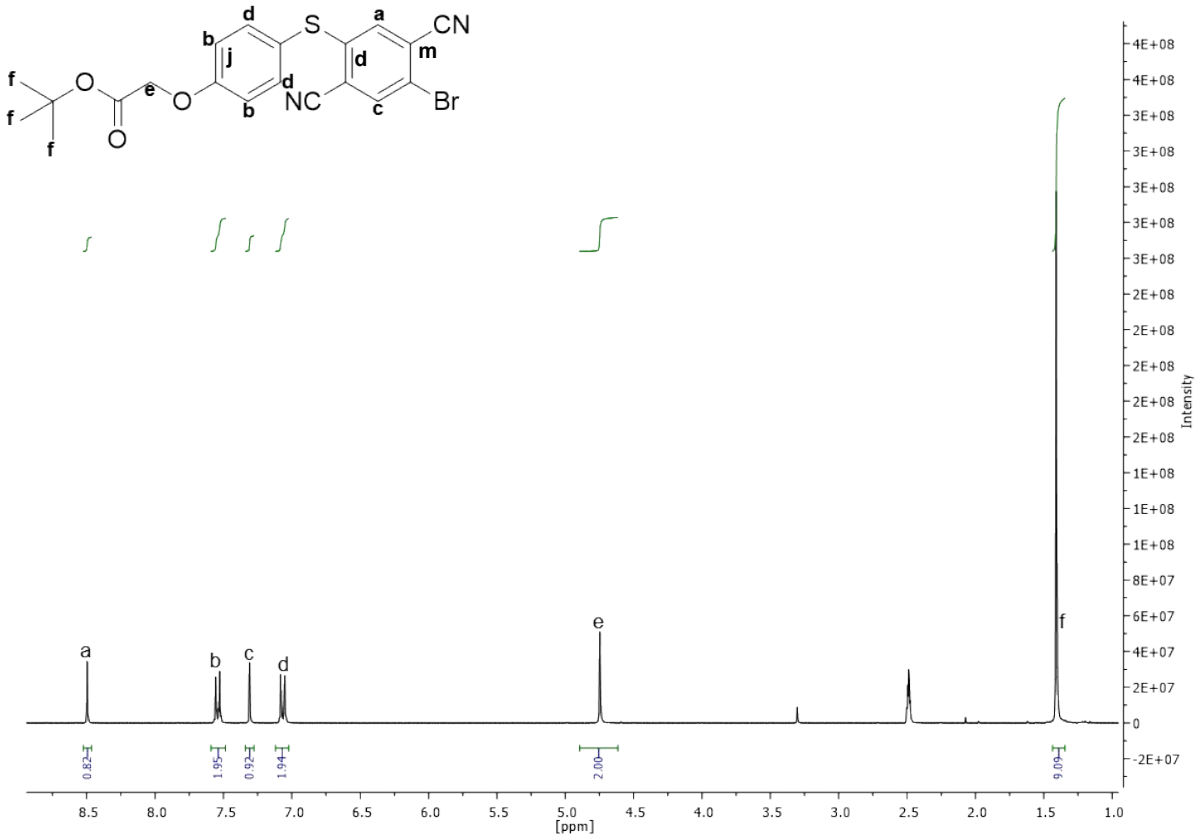


Fig. S29: ^{1}H NMR of compound **D** in DMSO-d6 (300 MHz).

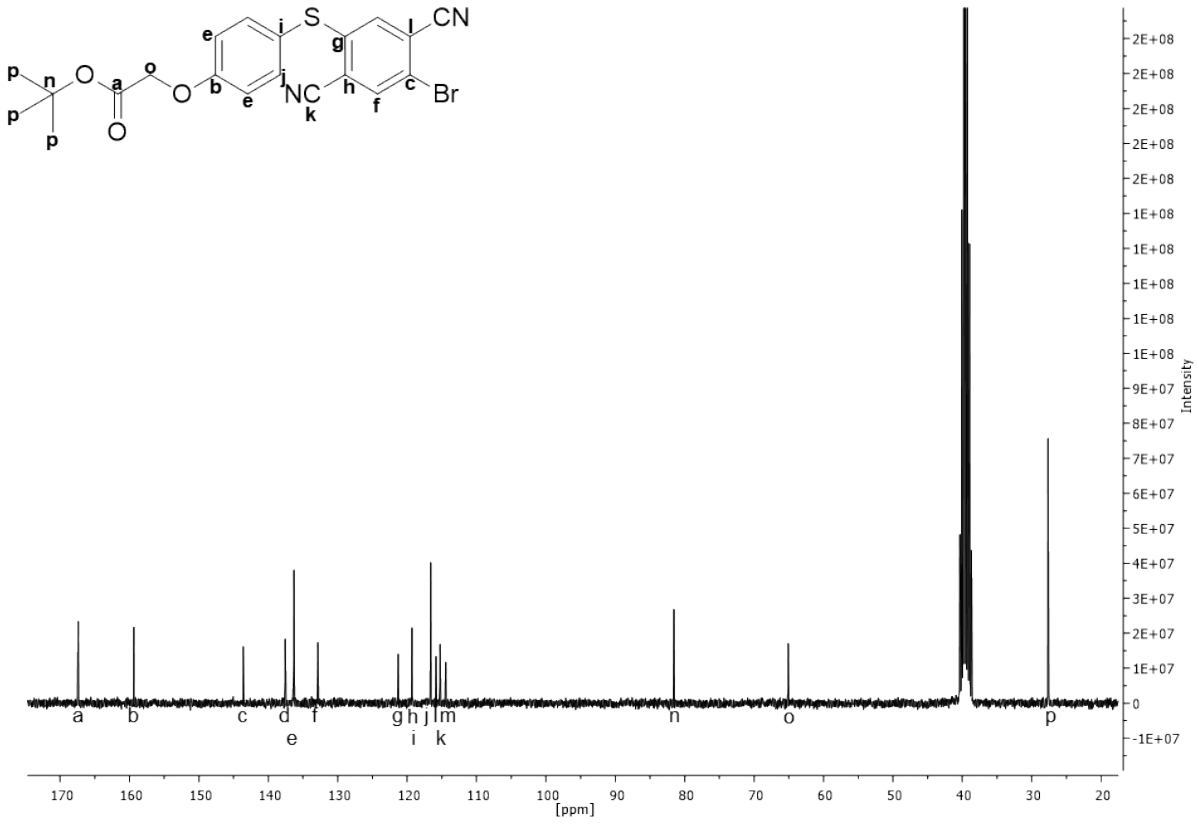


Fig. S30: ^{13}C NMR of compound **D** in DMSO-d6 (75 MHz).

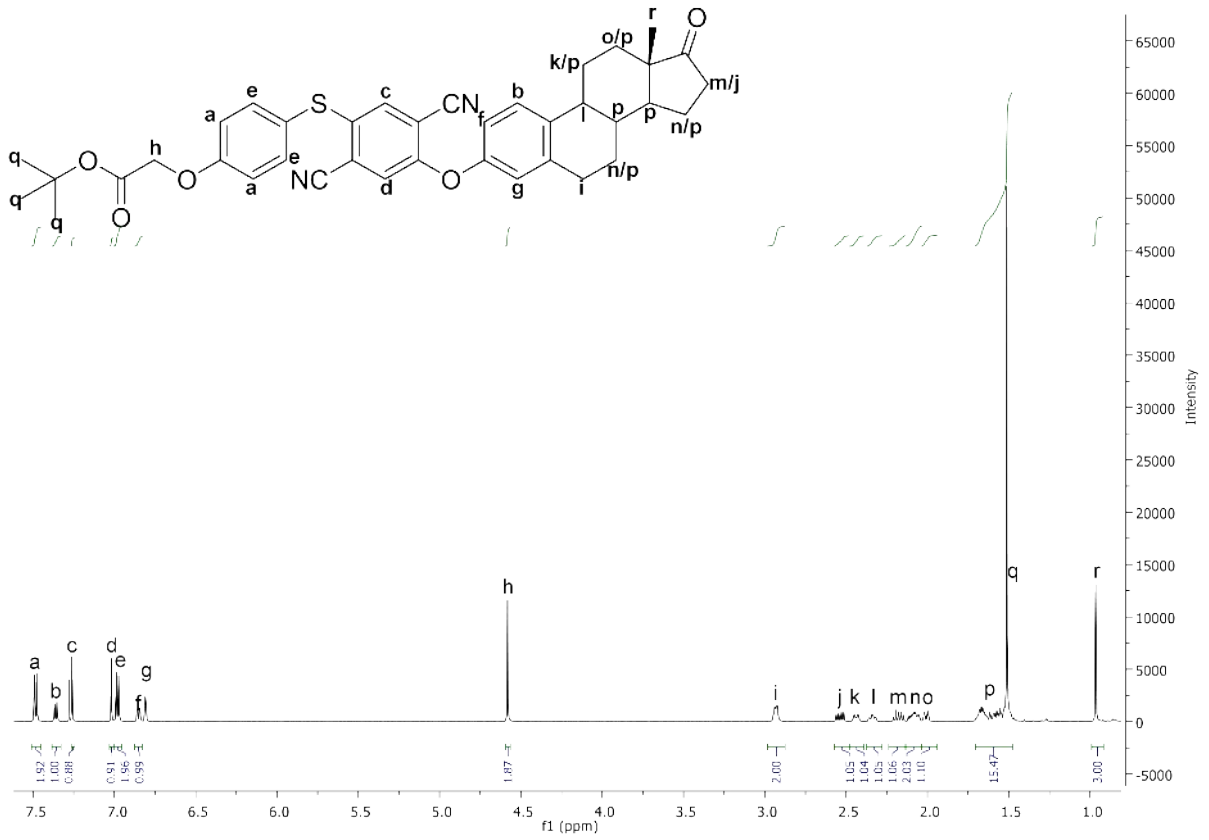


Fig. S31: ^{1}H NMR of compound **E** in $CDCl_3$ (600 MHz).

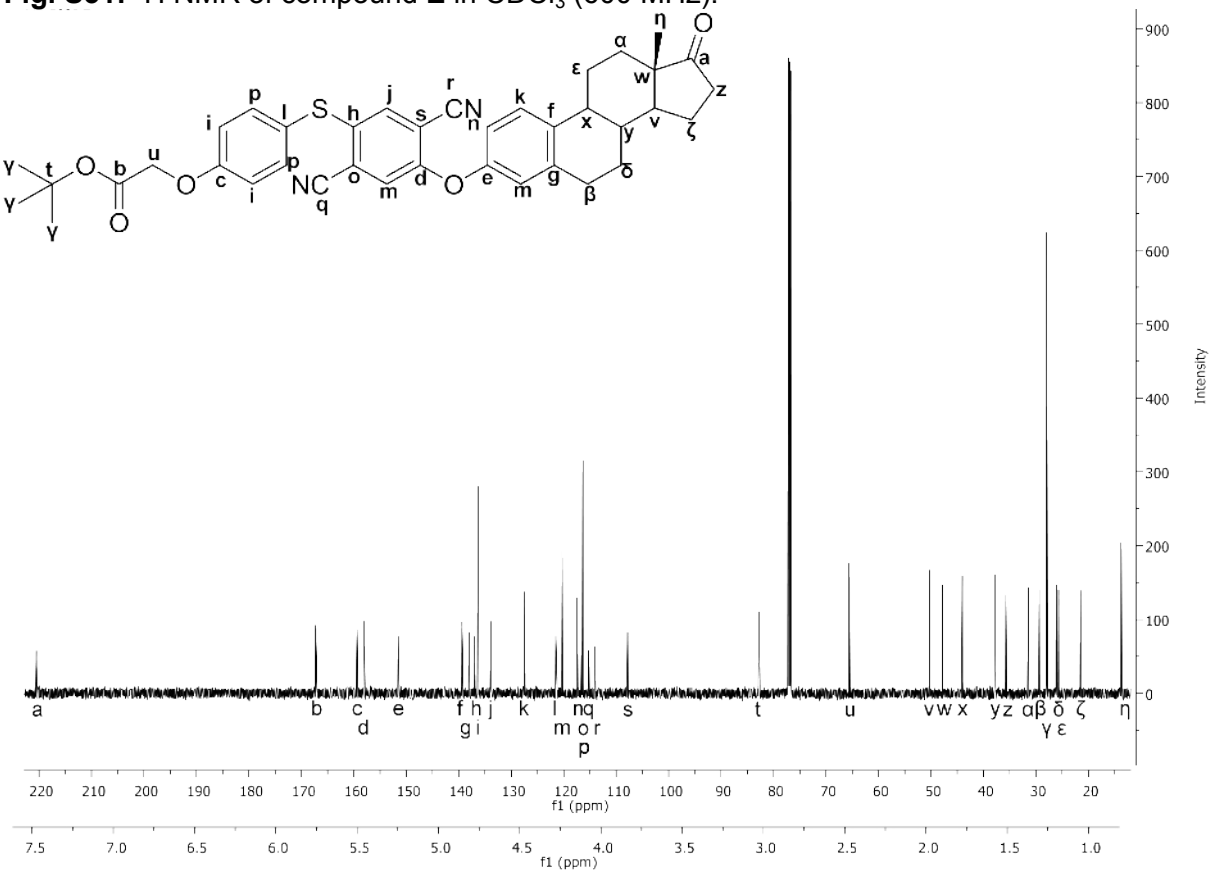


Fig. S32: ^{13}C NMR of compound **E** in $CDCl_3$ (151 MHz).

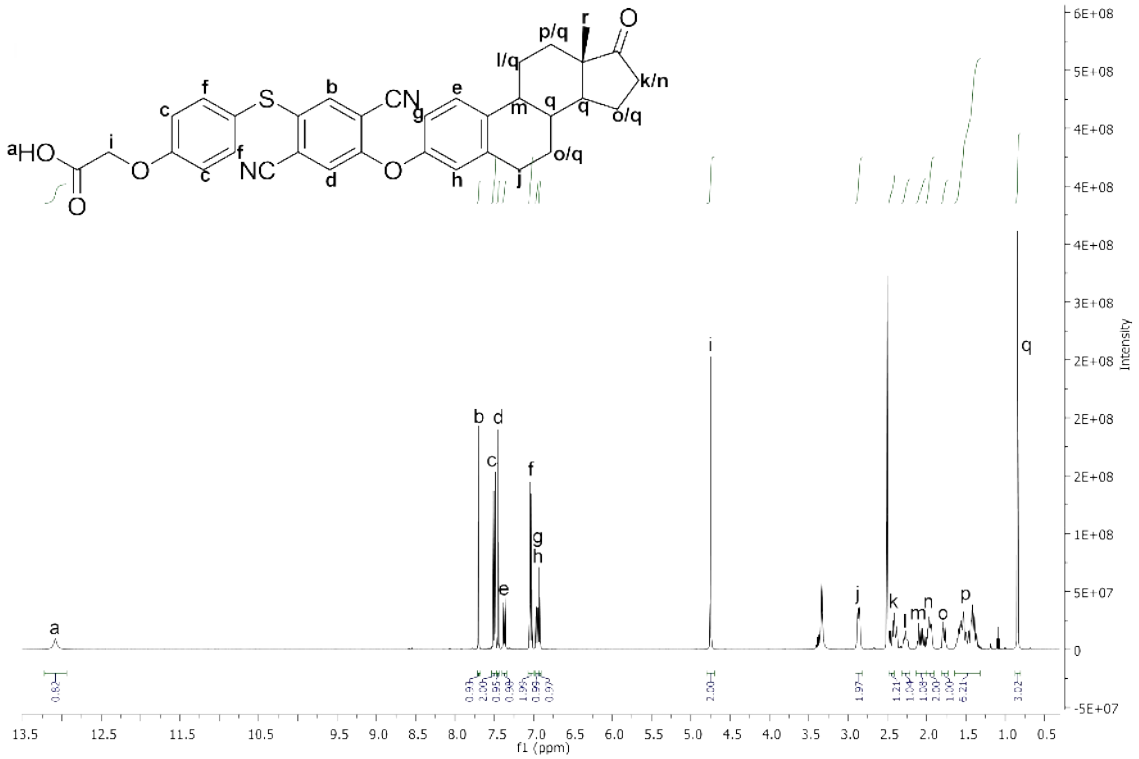


Fig. S33: ^{1}H NMR of compound **F** in DMSO-d6 (400 MHz).

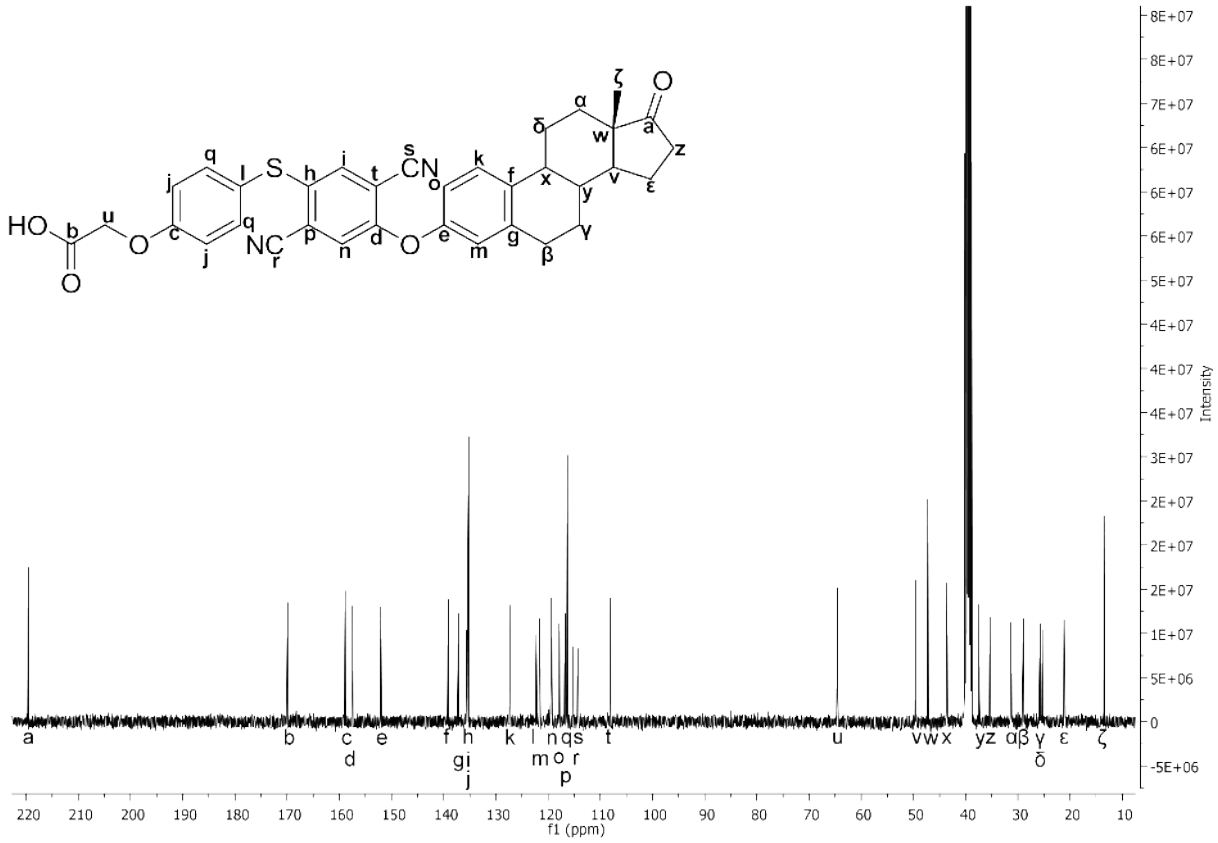


Fig. S34: ^{13}C NMR of compound **F** in DMSO-d6 (151 MHz).

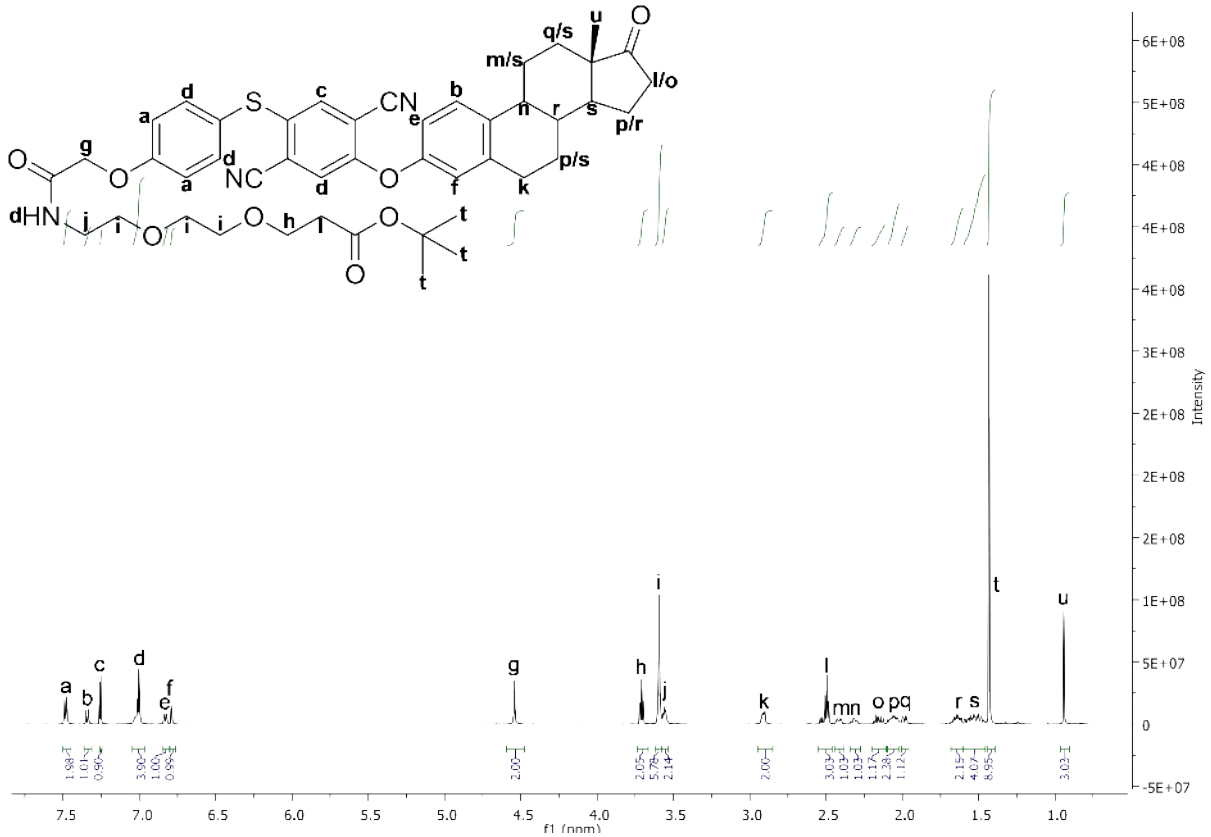


Fig. S35: ^{1}H NMR of compound **G** in $CDCl_3$ (600 MHz).

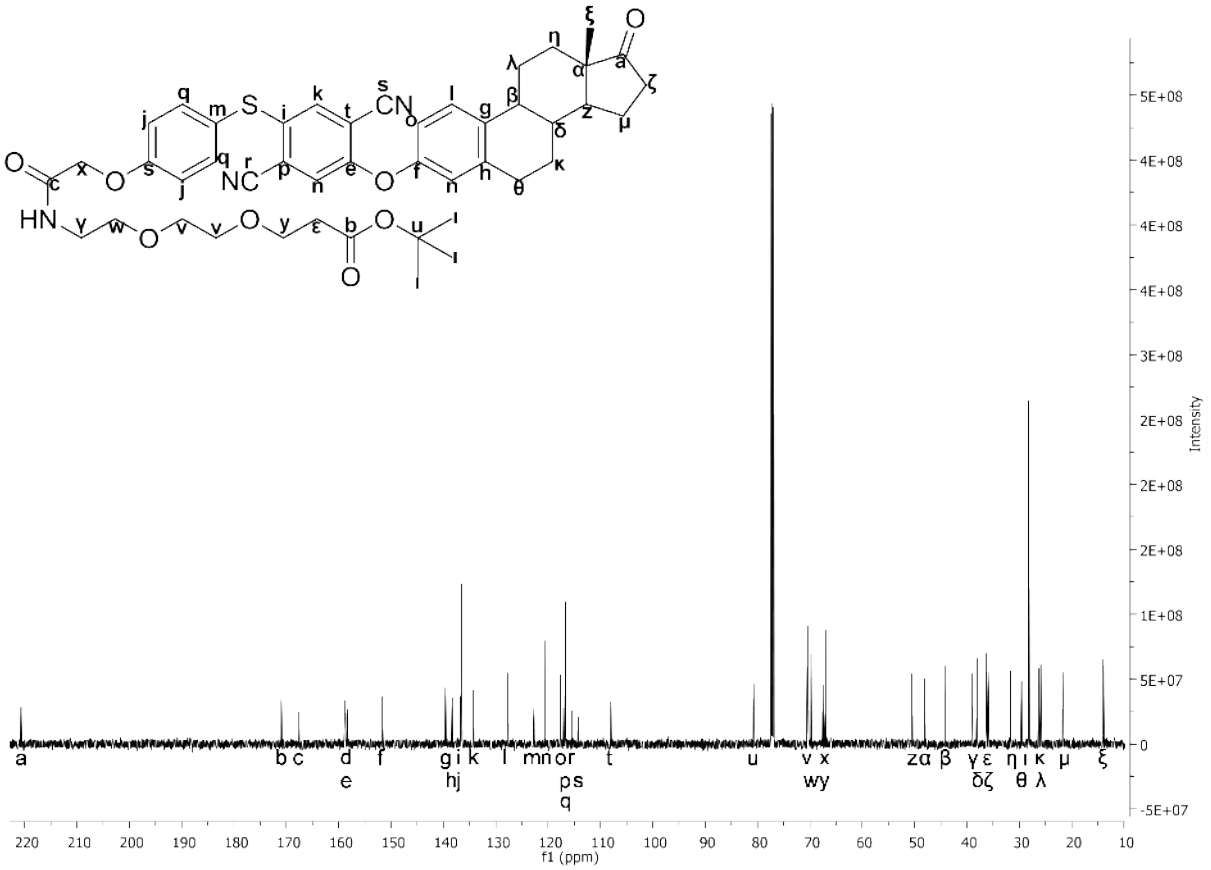


Fig. S36: ^{13}C NMR of compound **G** in $CDCl_3$ (151 MHz).

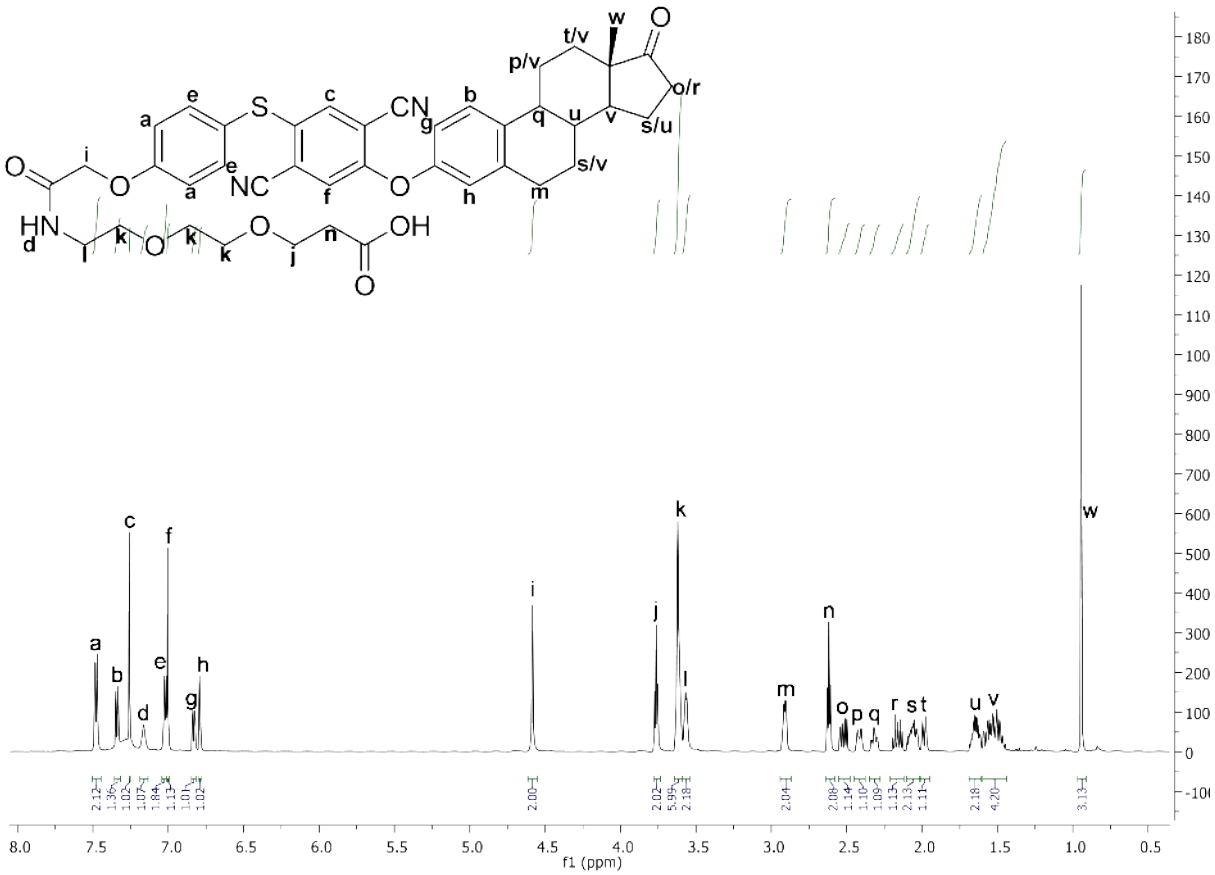


Fig. S37: ^{1}H NMR of compound **H** in $CDCl_3$ (600 MHz).

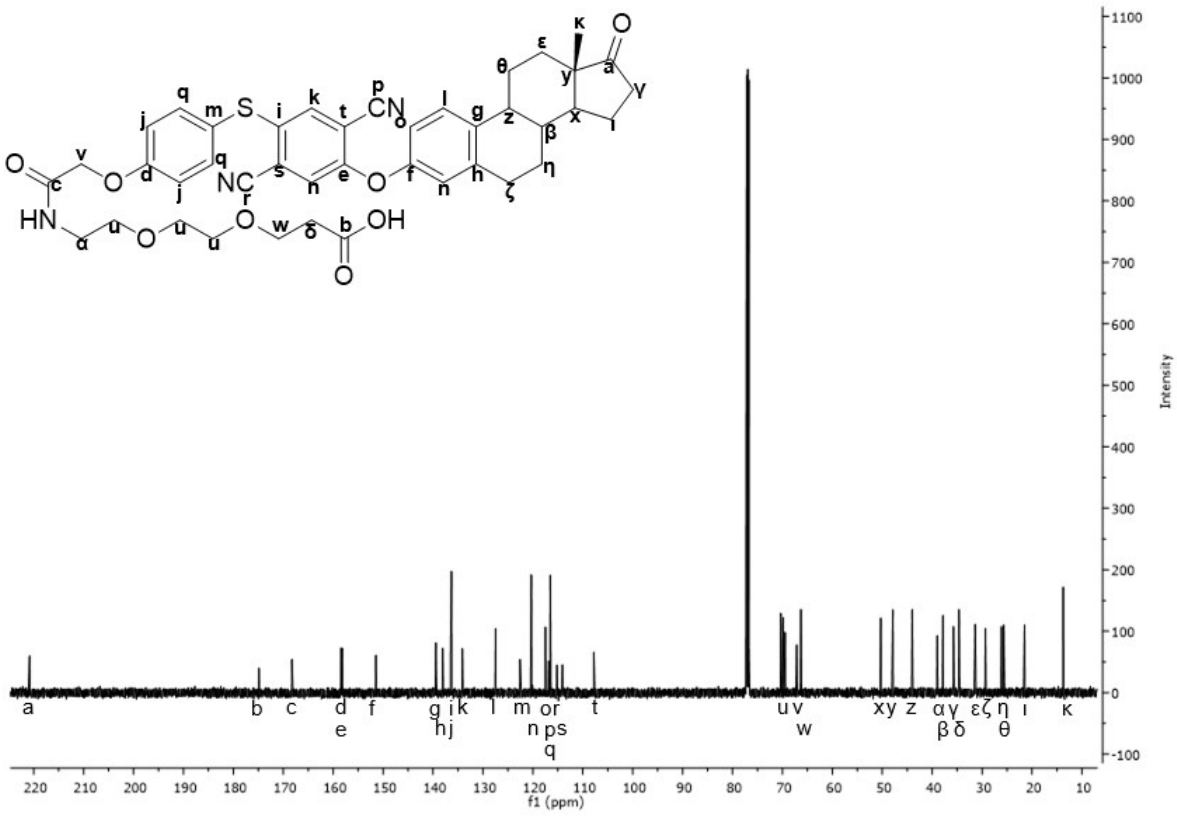


Fig. S38: ^{13}C NMR of compound **H** in $CDCl_3$ (151 MHz).

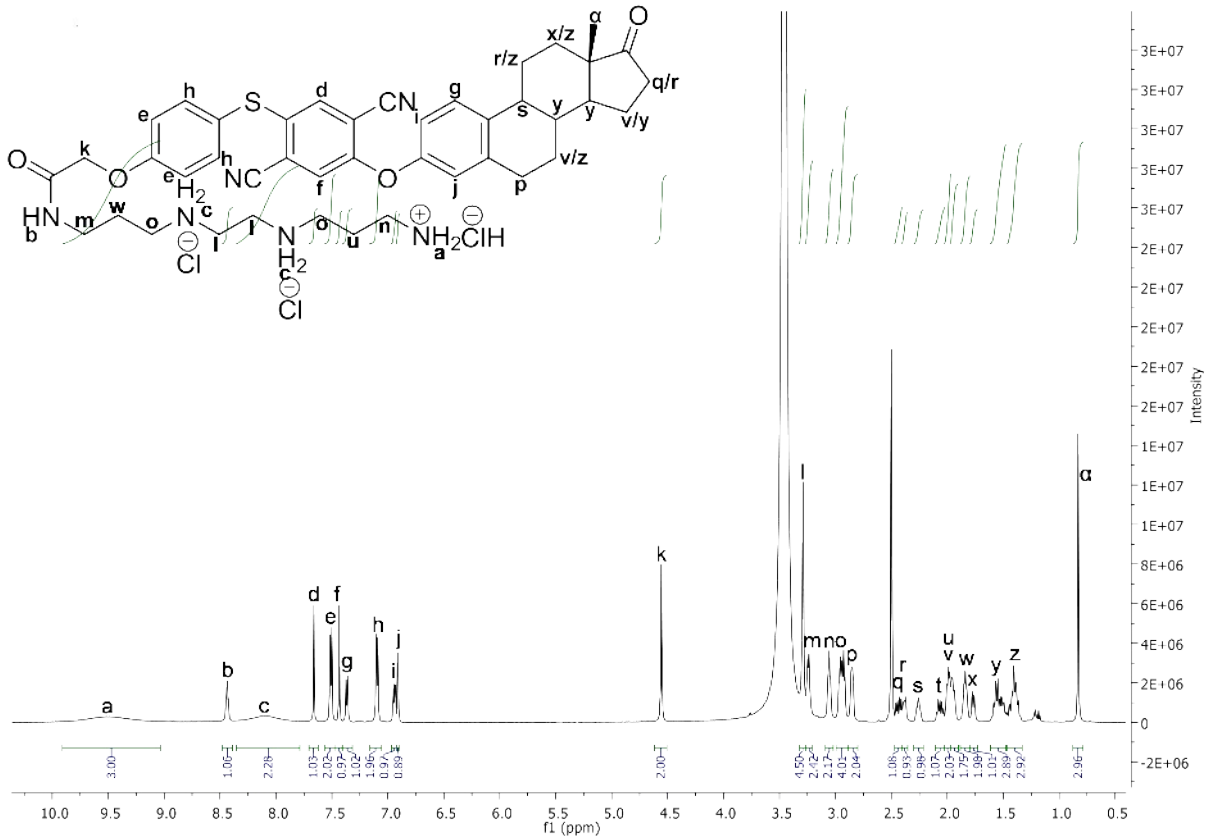


Fig. S39: ^{1}H NMR of compound **2** in DMSO-d_6 (400 MHz).

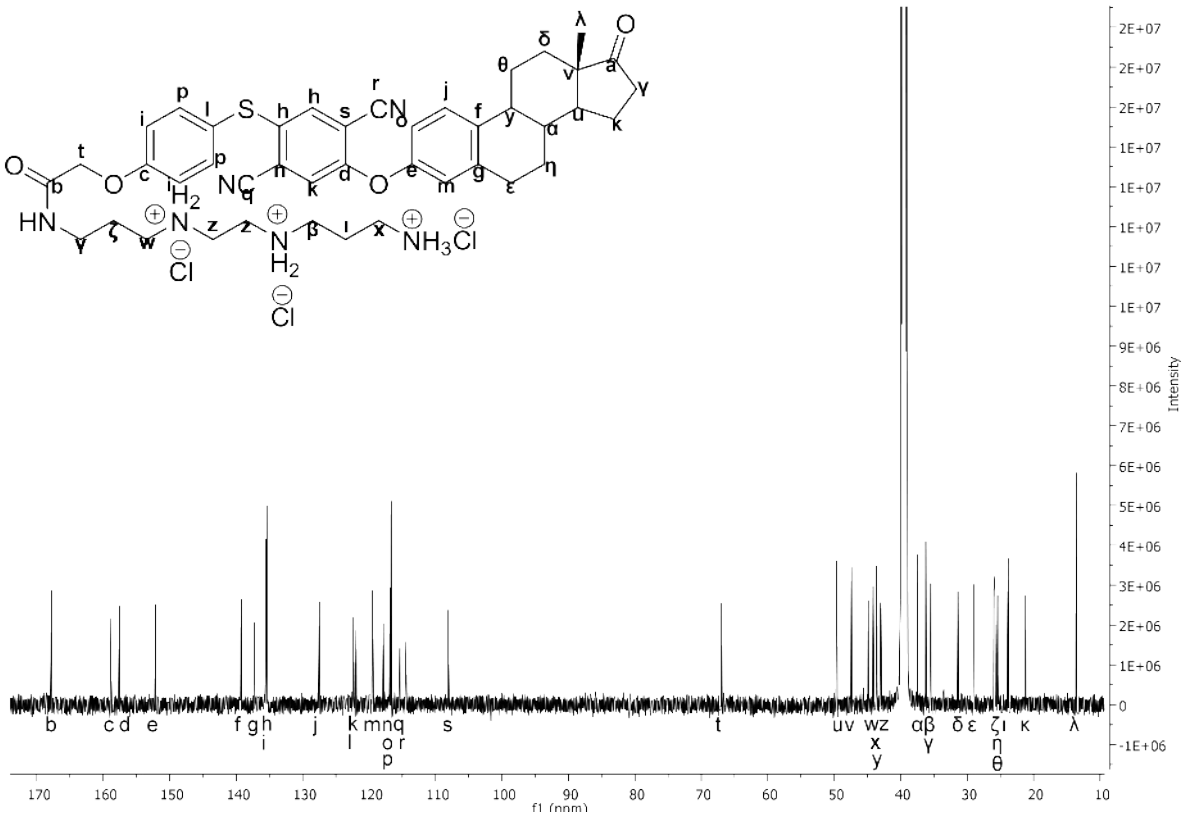


Fig. S40: ^{13}C NMR of compound 2 in DMSO-d_6 (101 MHz).

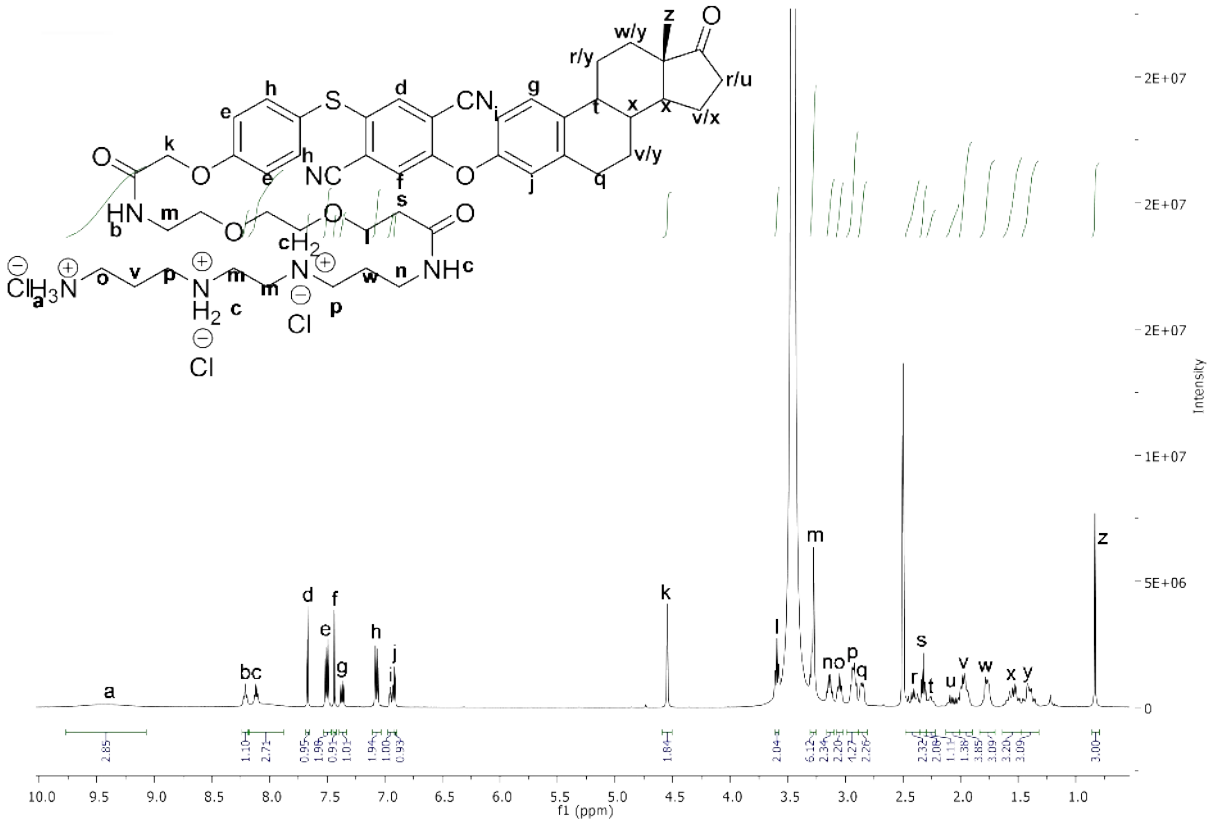


Fig. S41: ^{1}H NMR of compound **1** in DMSO-d_6 (600 MHz).

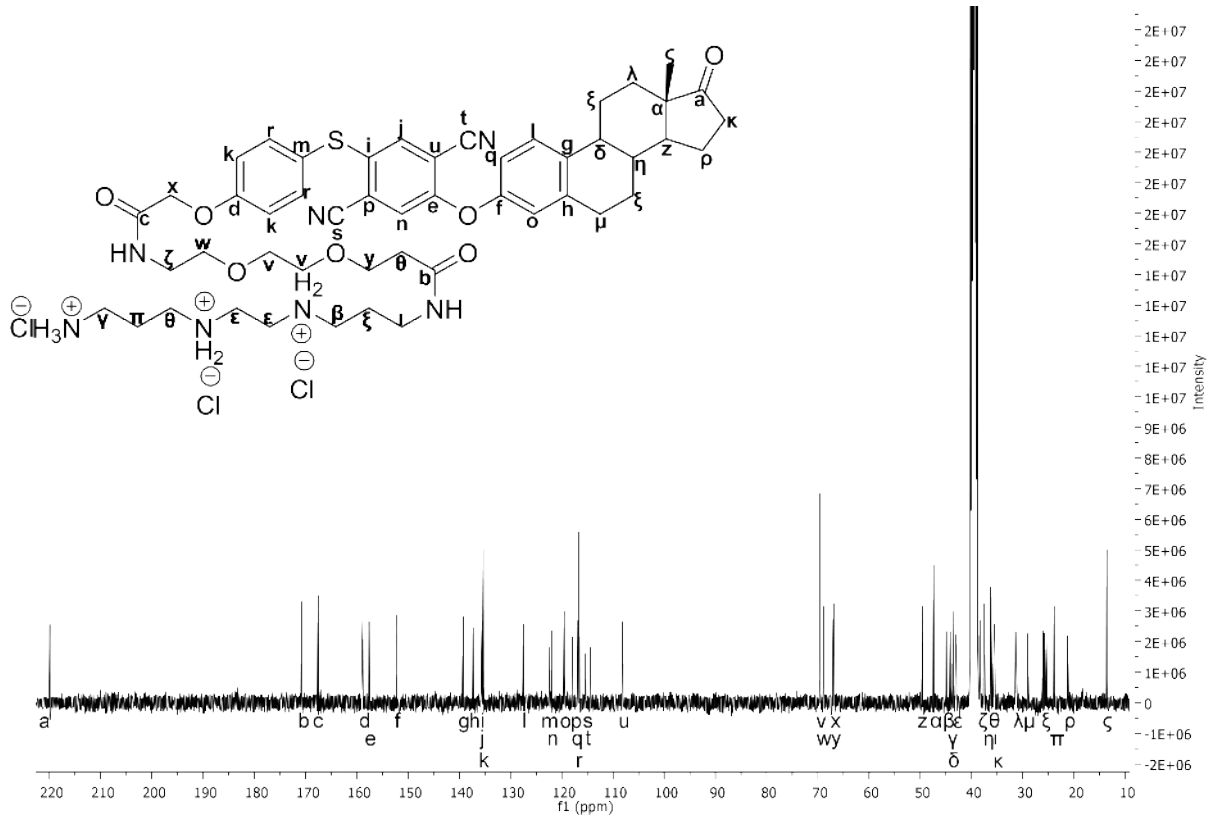


Fig. S42: ^{13}C NMR of compound **1** in DMSO-d_6 (151 MHz).

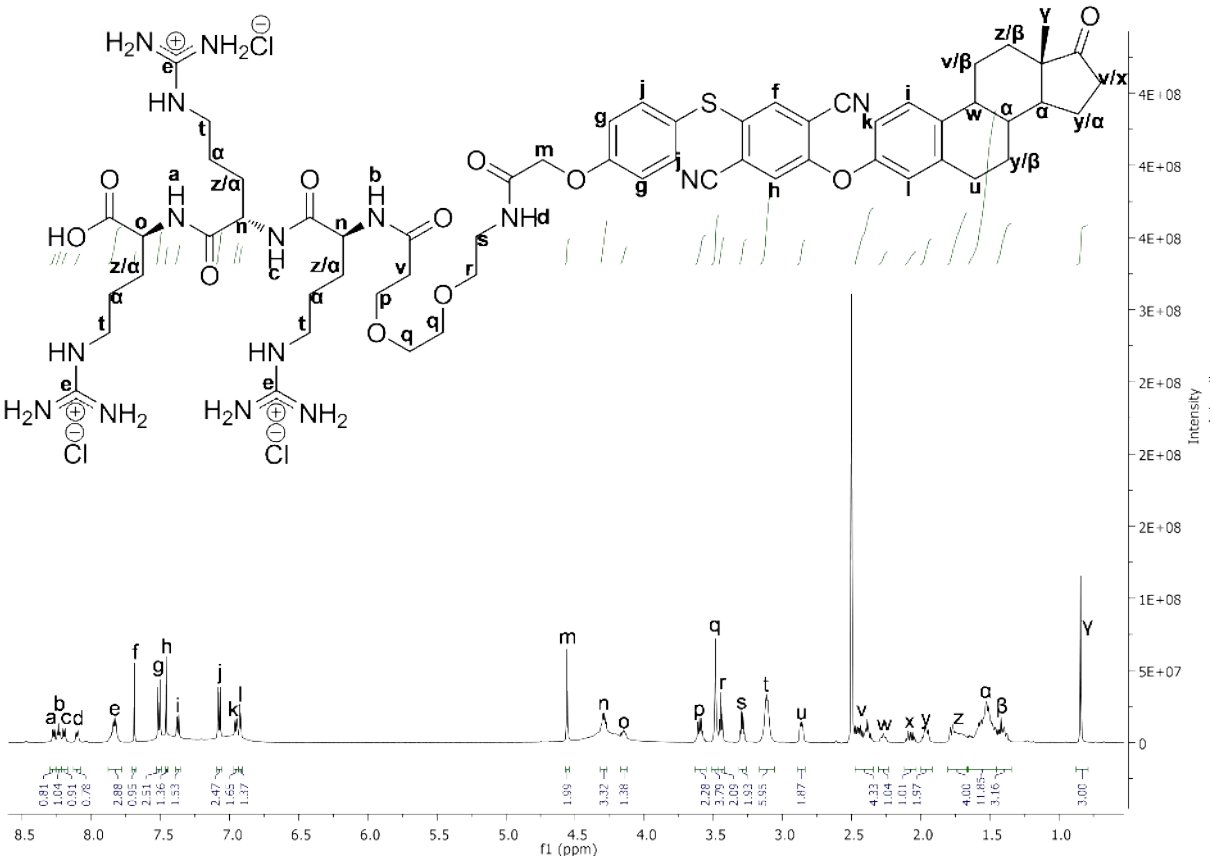


Fig. S43: ^{1}H NMR of compound **3** in DMSO-d_6 (600 MHz).

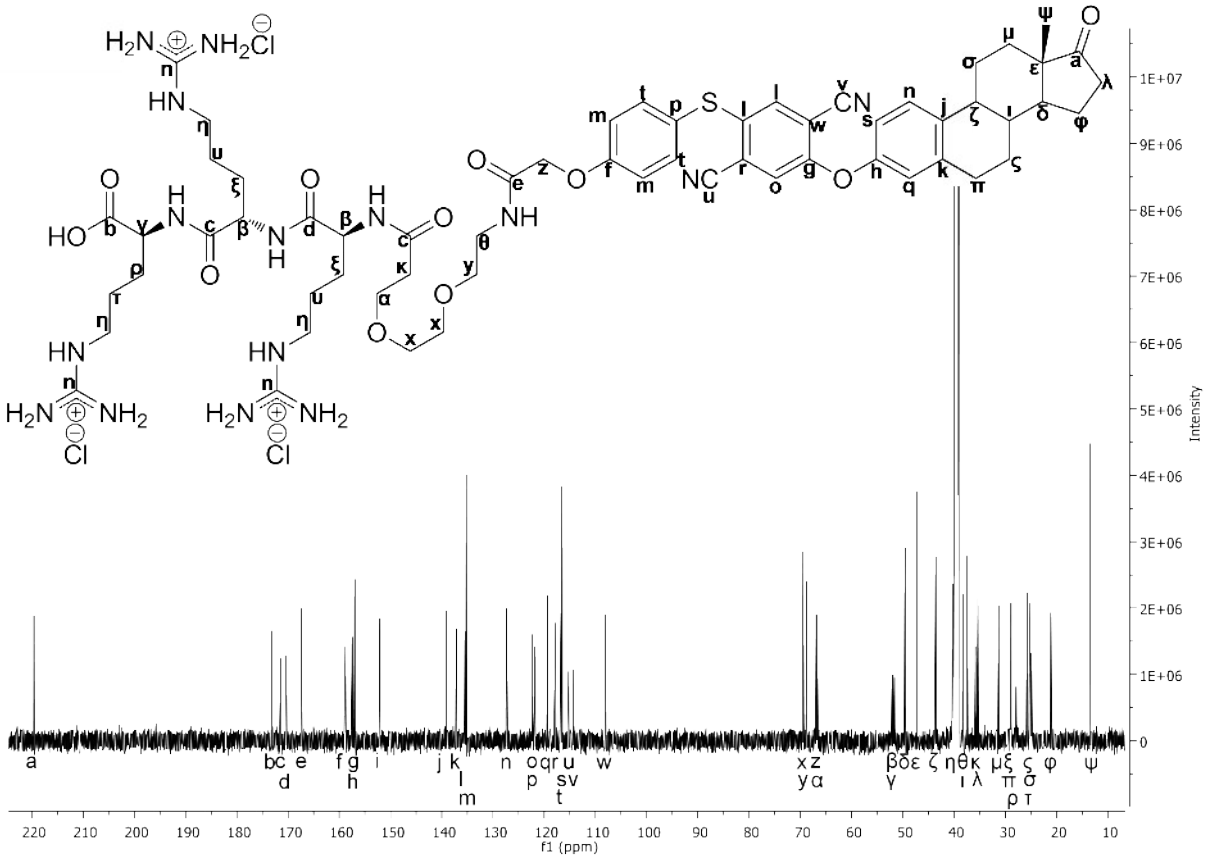


Fig. S44: ^{13}C NMR of compound **3** in DMSO-d_6 (151 MHz).

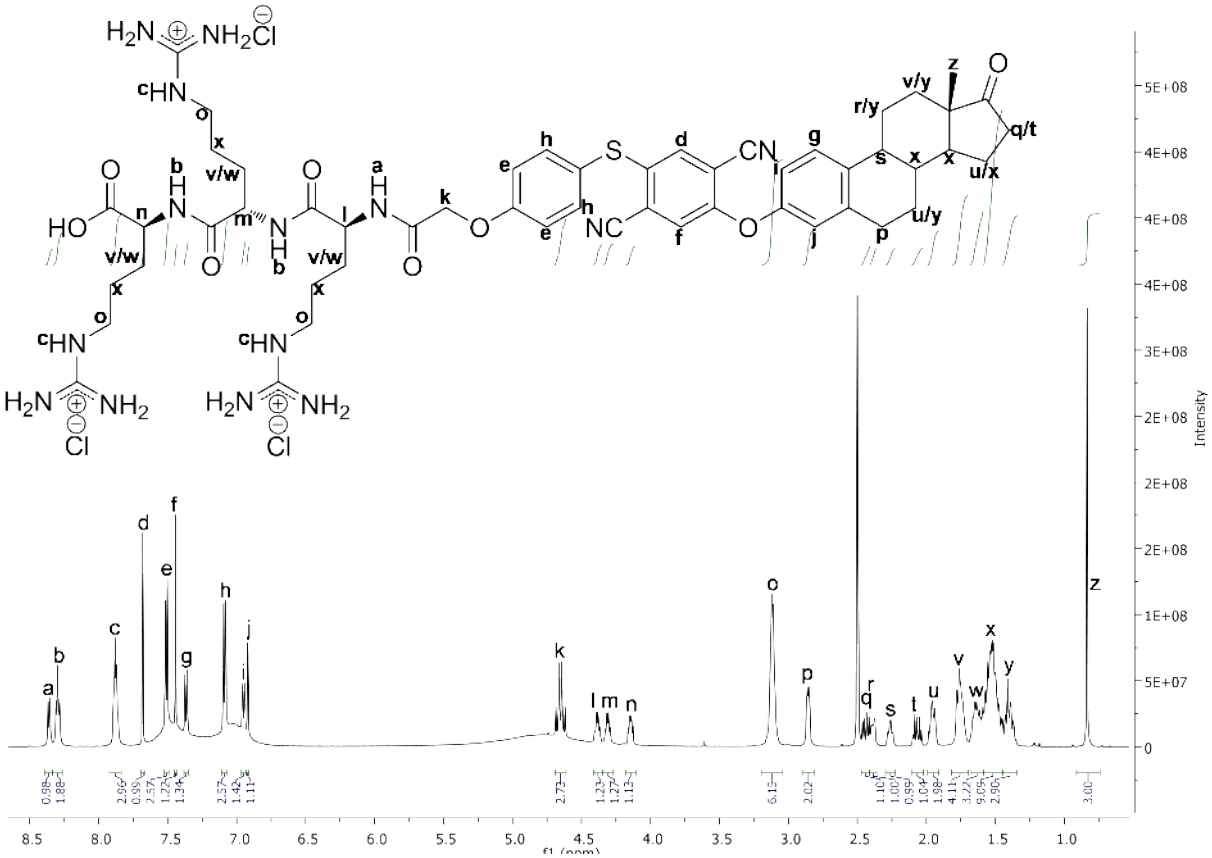


Fig. S45: ^{1}H NMR of compound **4** in DMSO-d_6 (300 MHz).

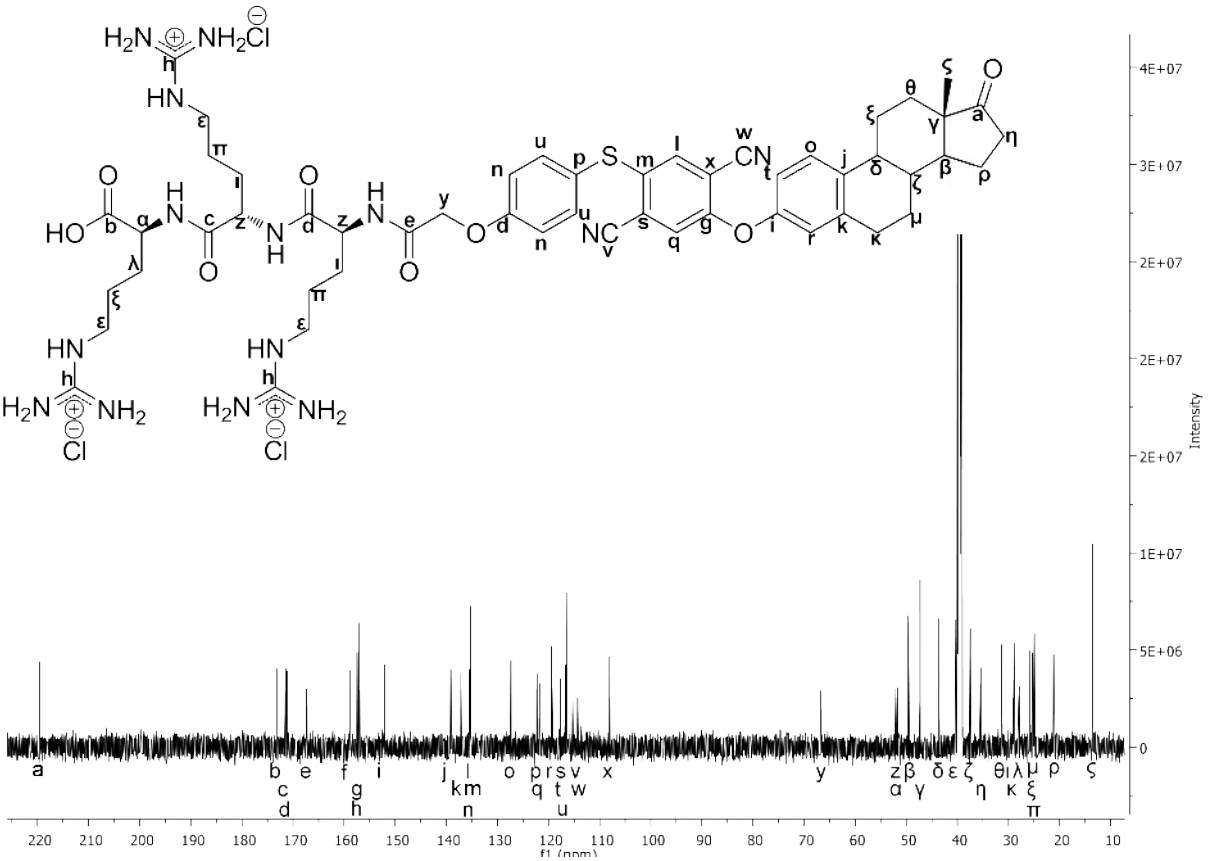


Fig. S46: ^{13}C NMR of compound **4** in DMSO-d_6 (300 MHz).

HPLC chromatograms

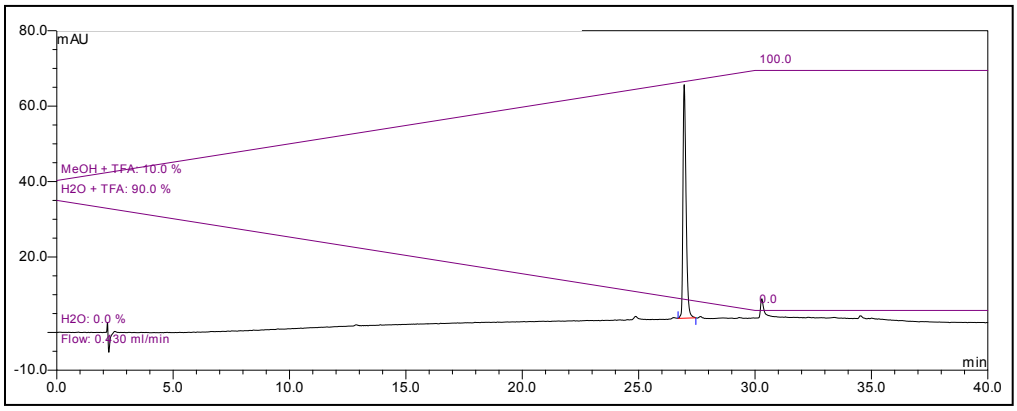


Fig. 47: HPLC chromatogram of compound **(1)**.

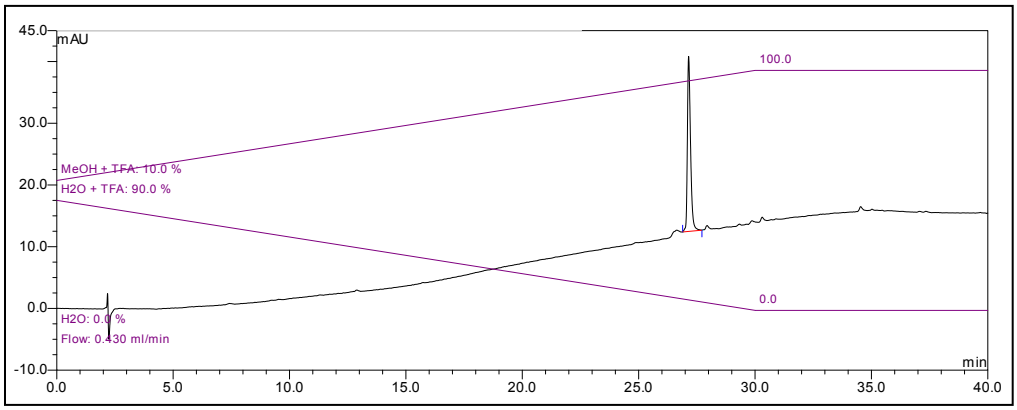


Fig. 48: HPLC chromatogram of compound **(2)**.

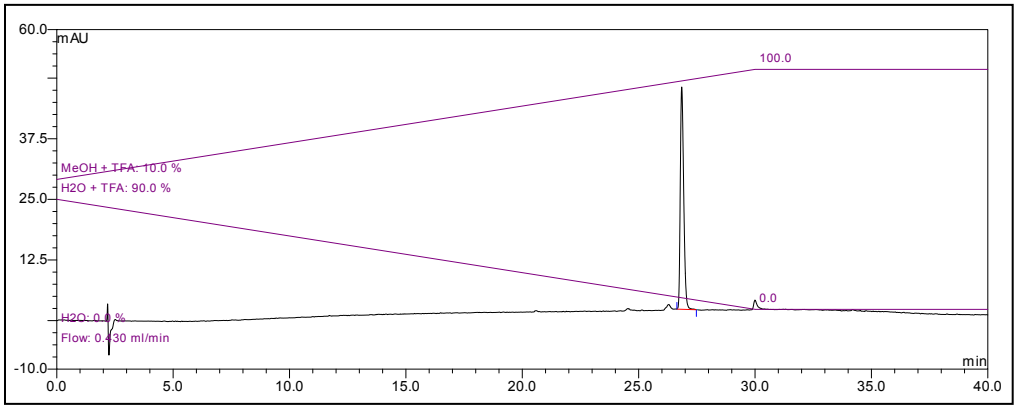


Fig. 49: HPLC chromatogram of compound **(3)**.

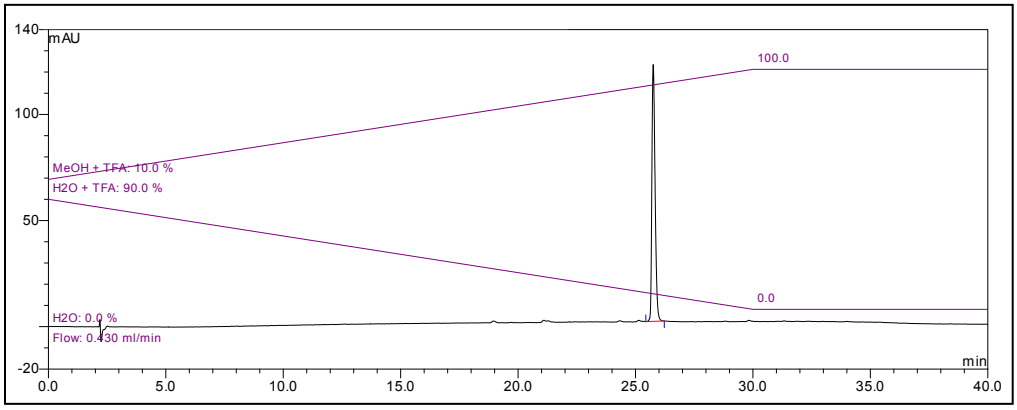


Fig. 50: HPLC chromatogram of compound **(4)**.

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