# Electronic Supplementary Information

## Deoxyestrone-based lipofection agents with solution- and solid-state emission properties

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## CONTENT

## GENERAL INFORMATION AND CONCEPT

Commercially available chemicals were used without further purification. They were purchased from Deutero, Eurisotop, TCI, Sigma Aldrich, Fluorochem, Acros Organics, Alfa Aesar, Carbolution, abcr and Fisher Scientific. Potassium carbonate was stored in an oven at 100 °C before usage. Anhydrous THF was dried over sodium and distilled. DCM and DIPEA were dried over calcium hydride and distilled. MilliQ water was obtained through purification by *MicroPure ultrapure*-System from TKA.

Sensitive reactions were performed under an argon atmosphere using dried solvents and flame-dried glassware. Thin-layer chromatography (TLC) was used for reaction monitoring on POLYGRAM® SIL G/UV254 plates (0.2 mm) or ALUGRAM® RP-18 W/UV254 plates (0.15 mm) from Macherey-Nagel. Spots were visualized by an UV-handlamp (254 nm, 365 nm and 395 nm) or by treatment with specific stains. Flash-column chromatography was carried out under argon on silica gel MN 60 M (40–63 µm) from Macherey-Nagel or on *CombiFlash® NextGen 300+* using *RediSep Rf Gold®* normal phase silica gel (20–40 µm) from Teledyne ISCO. Purification on reverse phase was performed on the RP-18 silica gel LiChroprep from Merck (40–63 µm) with the medium pressure liquid chromatography setup PLC 2050 from Gilson. For this, *Gel ODS-AQ*-columns (12 nm/50 µm) from YMC with dimensions of 17 g (10 x 500 nm) or 120 g (25 x 50 mm) was used. Determination of purity was achieved using analytical reverse-phase high performance liquid chromatography with following setup: Dionex HPLC-system, *P680*-pump, automatic sample injection (ASI-100), *UVD-340U* UV-Detector (at 254 nm), *UltiMate 3000* compartment and *ODS-A column* (3 x 150 mm, 5 µm particle size, 12 nm pore size) type *AA12S05-1503QT* from YMC.

Sonications were conducted using *Sonorex SUPER RK 514 BH* from Bandelin Electronics. Freeze dryings were performed on *ALPHA 1-2* from Christ. For this, compounds were dispersed in distilled water and frozen in liquid nitrogen under rotation. Centrifugation was conducted on *Rotofix 32 A* from Hettich. A Canon *EOS 1100D*–Camera was used for photographs of compounds.

FT-IR spectra were measured using *IRTracer-100* (Shimadzu Corporation). High resolution mass spectra were recorded on a Bruker *maXis 4G* (*Q-TOF*) via electrospray-ionization. Samples were dissolved in dichloromethane or in methanol and injected via

flow-injection. For MS/MS-analysis, the energy of the collision-induced dissociation (CID) was set at 40 eV to induce fragmentation of the parent ion. NMR spectra were recorded on an *AVNEO400* ($^{1}$H: 400 MHz, $^{13}$C: 101 MHz, $^{19}$F: 376 MHz, $^{31}$P: 162 MHz) or an *AVHD600* spectrometer ($^{1}$H: 600 MHz, $^{13}$C: 151 MHz, $^{19}$F: 565 MHz) from Bruker. Analytical data is given with respective frequency, solvent, temperature, chemical shift δ [ppm], multiplicity, integral and assignment. The abbreviation of the fine structure is: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, tt = triplet of triplet, br = broad. Coupling constants $^{n}J_{XY}$ are given in Hertz [Hz], with *n* describing the number of bonds between coupling nuclei X and Y. The deuterated solvents used for internal referencing are given in table S1.

**Table S1: Overview of deuterated solvents used.**

| Solvent | $^{1}$H-NMR: δ [ppm] = | $^{13}$C-NMR: δ [ppm] = |
|---|---|---|
| Acetone-$d_6$ | 2.05 (quintett) | 29.92 (septett) |
| $CDCl_3$ | 7.26 (s) | 77.16 (t) |
| DCM-$d_2$ | 5.32 (t) | 53.84 (quintett) |
| DMSO-$d_6$ | 2.50 (quintett) | 39.51 (septett) |
| DMF-$d_7$ | 8.03 (s)<br>2.92 (quintett)<br>2.75 (quintett) | 163.15 (t)<br>34.89 (septett)<br>29.76 (septett) |
| Methanol-$d_4$ | 3.31 (quintett) | 49.15 (septett) |

ζ-potential was measured using Zetasizer Nano ZS (Malvern Panalytical) with HeNe-Laser (633 nm) at room temperature. Samples were measured in folded capillary cells (*DTS1070*) in triplicate.

UV-Vis spectra were measured on a Jasco V-750 double-beam spectrophotometer with baseline correction. Excitation and emission spectra were recorded on a RF-6000 spectrometer from Shimadzu Corporation or on a FLS920 from Edinburgh Instruments with 450W Xe arc lamp, single grating monochromator and coupled PMT-980 Hamamatsu photomultiplier as detector. Samples were prepared in quartz cuvettes from Hellma Analytics. Measurements were conducted at room temperature. Absolute photoluminescence quantum yields were measured with a demountable integrating sphere FLS920 from Edinburgh Instruments.

Transmission electron microscopy (TEM) images were conducted on a JEOL JEM-2200FS with 200 kV acceleration voltage. Samples were prepared dropping 5 µL of the sample solution on a 400 mesh carbon coated copper grid and letting it rest for 45 sec. before absorbing the liquid with a filter paper. Afterwards, the sample was stained with a 2% aqueous uranyl formate solution (10 µL) in the same manner, before being dried for 24 h in a desiccator over silica gel in vacuo.

## SYNTHETIC PROCEDURES

### GENERAL SYNTHETIC PROCEDURES

#### *GENERAL PROCEDURE 1 (GP1): NUCLEOPHILIC AROMATIC SUBSTITUTION*

GP1 couplings were performed based on the procedure from Dubbert *et al*.[1] The corresponding catechol (1–1.2 eq.), respective dichloro- or tetrachloroterephthalonitrile (1 eq.) and dried potassium carbonate (3.7 eq.) were suspended in anhydrous *N,N*-dimethylformamide under argon and stirred at 60 °C for 42 hours. Afterwards, the reaction was treated with hydrochloric acid (1 M) to neutral pH and diluted with water. The precipitate was collected by vacuum filtration. Increased yield was achieved by repeated extraction of mother liquor with dichloromethane with subsequent drying over magnesium sulphate and concentration. The crude product was dry mounted on *Celite® 545*, purified by column chromatography and lyophilized.

#### *GP2 PEPTIDE COUPLING AND SUBSEQUENT REMOVAL OF BOC-GROUPS*

Peptide couplings were performed based on the procedure from Zimmermann *et al.*[2] The corresponding carboxylic acid (1 eq.) was added to a Schlenk flask, which was evacuated and backfilled with argon three times. After addition of anhydrous dichloromethane and *N,N*-dimethylformamide (v/v 1/1), PyBOP (1.1 eq.) and *N*-methylmorpholine (5 eq.) the mixture was stirred for 60 min at room temperature. Then, tobramycin amine (**25**)[3] was added at 0°C. Under stirring, the mixture was allowed to reach room temperature overnight. The solvents were removed under vacuum and the crude product purified *via* column chromatography. After isolation, the BOC-protected compounds were dissolved in dichloromethane and treated with an excess of trifluoroacetic acid. The reaction progress was monitored via TLC. After completion, the mixture was concentrated *in vacuo* and purified *via* MPLC on RP-18 (water+ 0.1% trifluoroacetic acid /methanol + 0.75%

trifluoroacetic acid = 40/60 → 0/100 over 35 min., then 40 min. isocratic). After washing with cyclohexane and lyophilization, ligands were obtained as corresponding TFA salts.

## OVERVIEW FOR SYNTHESIS OF TARGET COMPOUNDS

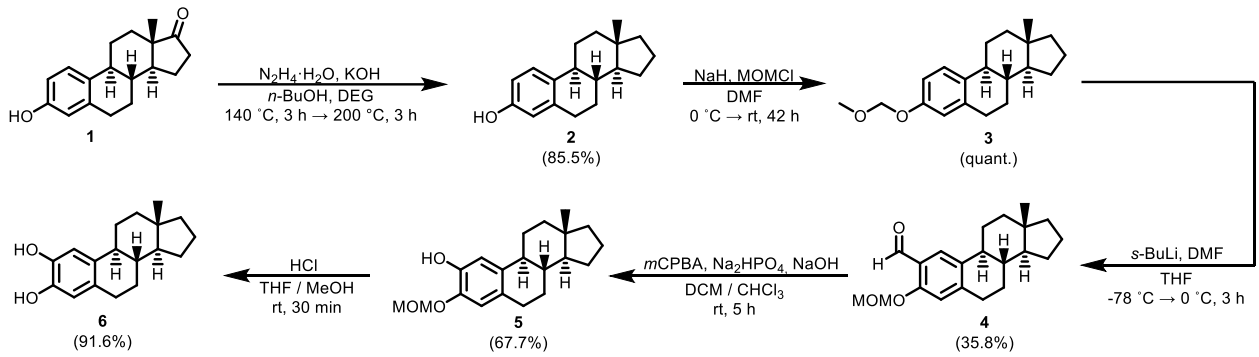



**Figure S1:** Synthesis of estrone catechol precursor **6**. Compounds **2**[4] and **3–5**[5] were synthesized according to modified literature protocols.

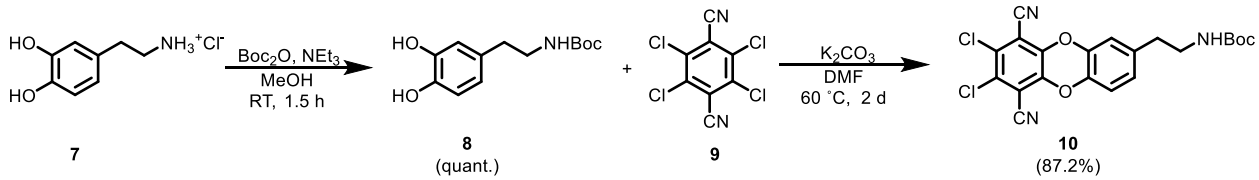



**Figure S2:** Synthesis of dopamine precursor **10**. Compound **8**[6] was synthesized according to a modified literature protocol.

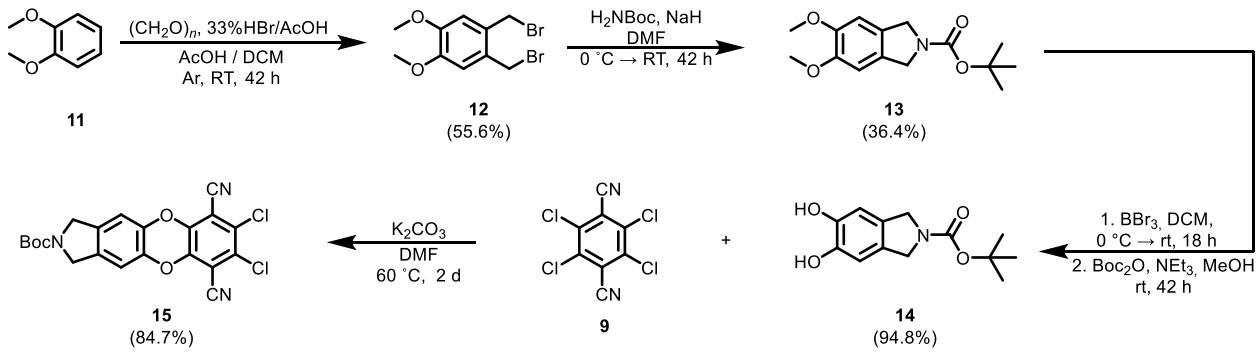



**Figure S3:** Synthesis of isoindoline precursor **15**. Compound **12**[7] was synthesized according to the respective literature protocol.

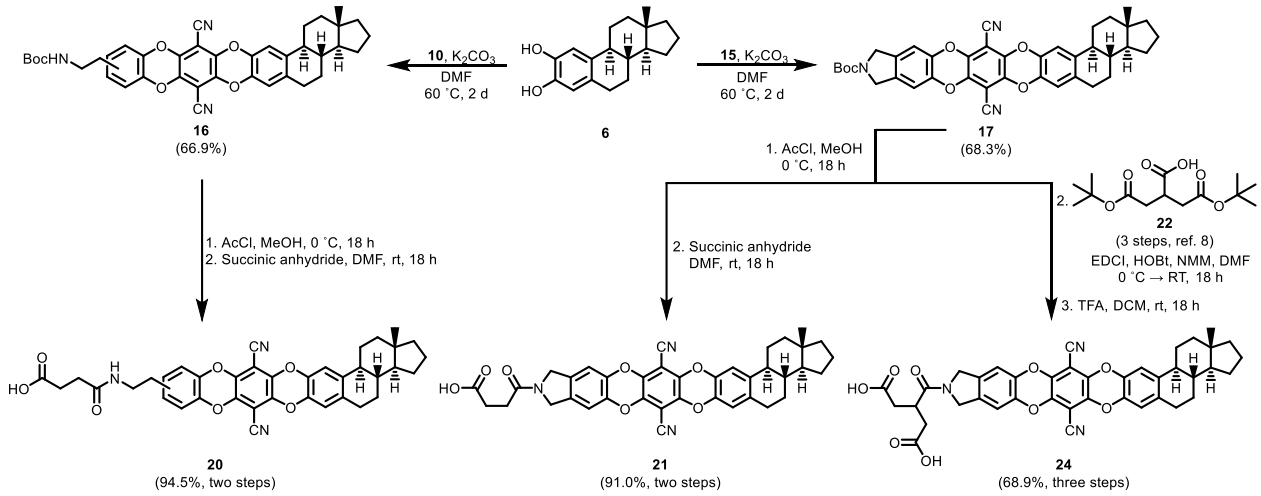



**Figure S4:** Synthesis of carboxylic acids **20–24**. Compound **22**[8] was synthesized according to the respective literature protocol.

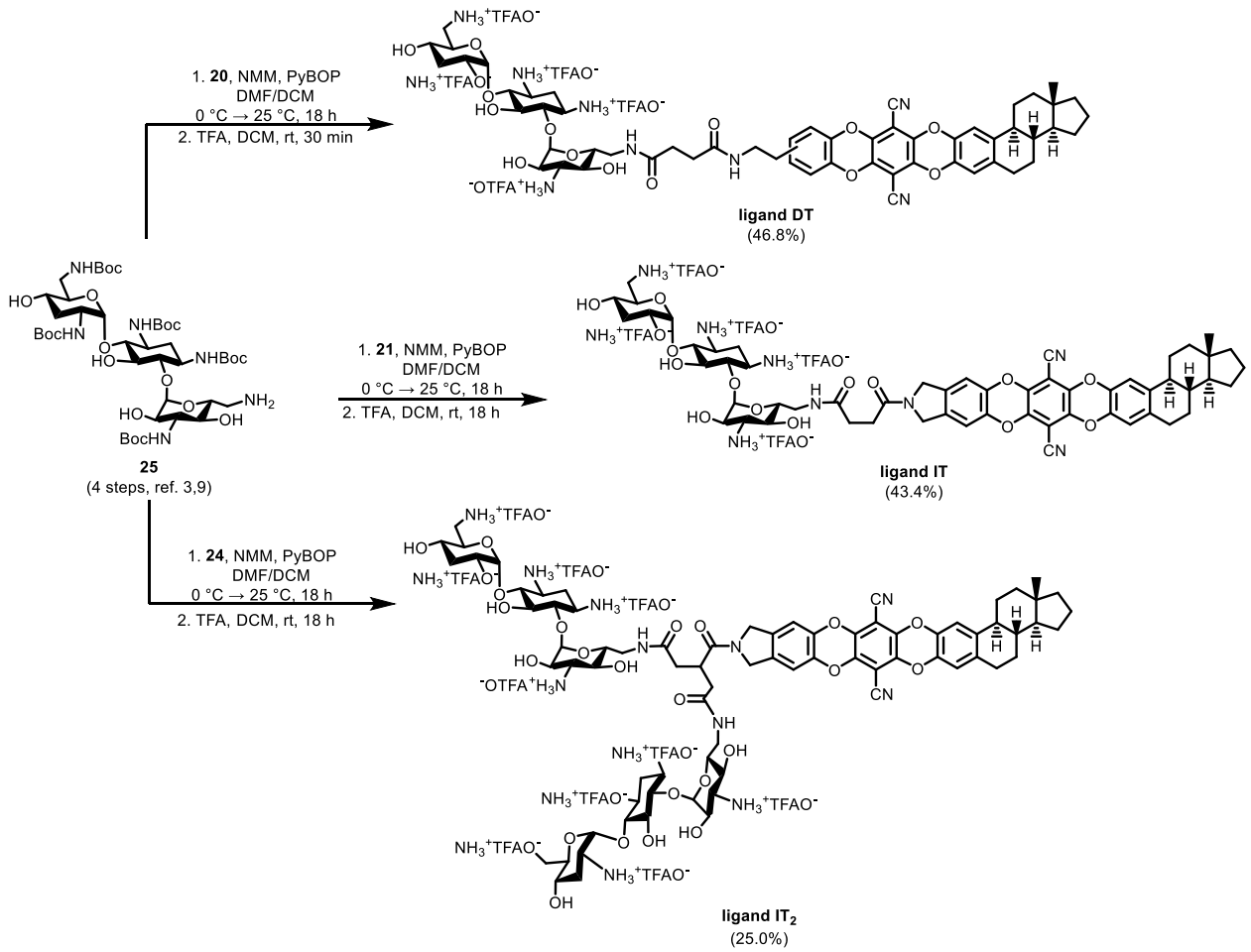
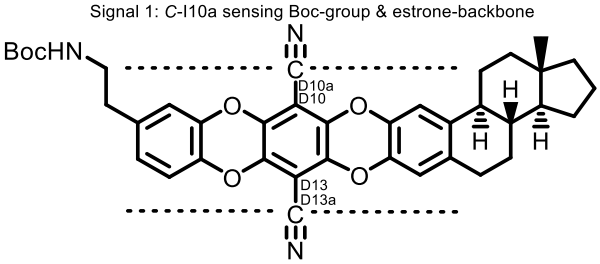



**Figure S5:** Synthesis of ligands **DT**, **IT** and **$IT_2$**. Tobramycin amine **25** was synthesized according to respective literature protocols.[3,9]

## GENERAL INFORMATION REGARDING $^{13}$C-NMR SPECTRA

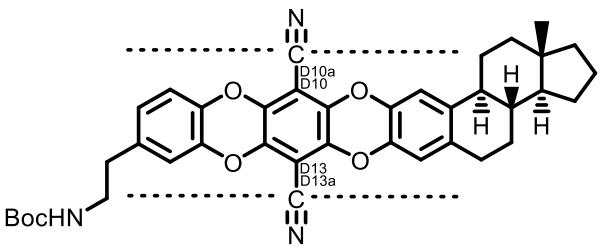
In the case of bridged dopamine derivatives **16**, **18**, **20** and **DT**, inseparable regioisomeric mixtures were obtained. With the lack of chemical information in the central terephthalonitrile core, the existence of regioisomers severely complicated $^{13}$C-NMR analysis because of unregularly pronounced signal separation or overlapping. Therefore, the number of observed signals can deviate from expectations.

Signal 1: *C*-I10a sensing Boc-group & estrone-backbone




Signal 2: *C*-I13a sensing no group

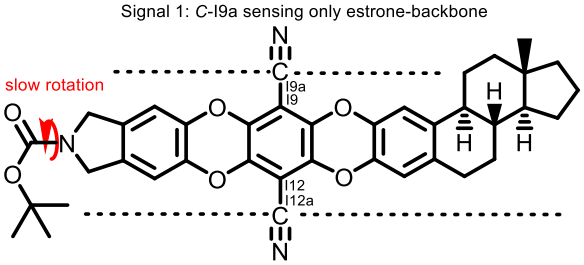
Signal 3: *C*-I10a sensing only estrone-backbone




Signal 4: *C*-D13a sensing only Boc-group

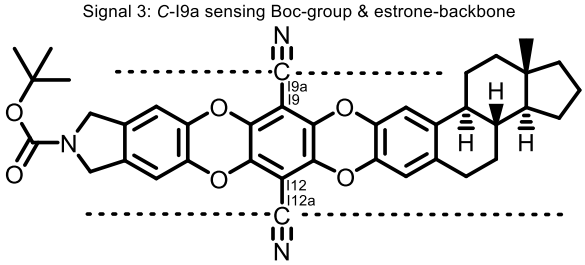
For isoindoline compounds **13**, **14**, **15**, **17**, **21**, **23**, **24**, **IT** and **IT$_2$**, slow rotation around the amide bond results in a different set of signals due to the presence of rotamers.[10] Therefore, the carbon atoms from the nitrile groups (positions I9a/I12a) and from the nitrile-bearing positions (I9/I12) each show a splitting into four signals due to spatial proximity of the symmetry-breaking BOC-group and estrone-backbone. However, in certain solvents such as DMSO and DMF, this effect is less pronounced, probably because of facilitated rotation around the amide bond.

Signal 1: *C*-I9a sensing only estrone-backbone




Signal 2: *C*-I12a sensing only Boc-group

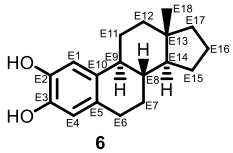
Signal 3: *C*-I9a sensing Boc-group & estrone-backbone




Signal 4: *C*-I12a sensing no group

## SYNTHESIS OF PRECURSOR

### *1.1.1.1 2-HYDROXY-17-DEOXYESTRONE (6)*




**6**

In a round-bottom flask, 2-hydroxy-3-*O*-methoxymethoxy-17-deoxyestrone (**5**, 0.444 g, 1.40 mmol, 1 eq.) was dissolved in a 1/1-mixture of tetrahydrofuran and methanol (each 15 ml) and treated with concentrated hydrochloric acid (12 M, 11.2 ml, 134 mmol, 96 eq.). The reddish mixture was stirred for 30 min. at room temperature. Crude product was extracted with dichloromethane (4 x 30 ml), concentrated over celite and purified by column chromatography on silica gel (3.3 x 5 cm, cyclohexane/ethyl acetate = 100/0 → 9/1 → 5/1 → 3/1, TLC visualisation with iron(III) chloride stain). After vacuum drying, 2-hydroxy-17-deoxyestron (**6**) was isolated as an off-white solid (0.350 g, 1.29 mmol, 91.6%). The product was stored under argon at -20°C to prevent oxidation.

**M($C_{18}H_{24}O_2$):** 272.39 g/mol.

Although compound **6** is literature known,[11] no NMR-characterization has yet been reported.

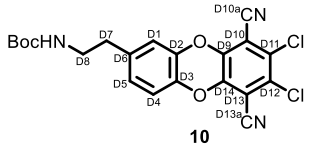
**$^1$H-NMR (400 MHz, [$D_1$]-chloroform, 298 K):** δ [ppm] =
6.82 (s, 1H, *H*-E1), 6.59 (s, 1H, *H*-E4), 5.17 (br s, 2H, O*H*), 2.83 – 2.66 (m, 2H, *H*-E6), 2.20 – 2.10 (m, 2H, *H*-E11, *H*-E9), 1.93 – 1.07 (m, 13H), 0.74 (s, 3H, *H*-E18).

**$^{13}$C{$^1$H}-NMR (101 MHz, [$D_1$]-chloroform, 298 K):** δ [ppm] =
141.3 (*C*-E2), 141.3 (*C*-E3), 134.0 (*C*-E10), 129.6 (*C*-E5), 115.7 (*C*-E4), 112.7 (*C*-E1), 53.6 (*C*-E14), 44.2 (*C*-E9), 41.2 (*C*-E13), 40.6 (*C*-E17), 39.1 (*C*-E8), 38.9 (*C*-E12), 29.2 (*C*-E6), 28.6 (*C*-E7), 26.9 (*C*-E11), 25.2 (*C*-E15), 20.6 (*C*-E16), 17.6 (*C*-E18).

**HR-MS (ESI-neg, 70 eV): m/z =**
271.1704 $[M - H]^-$, calculated for $[C_{18}H_{24}O_2 - H]^-$ = 271.1704; 543.3477 $[(M)_2 - H]^-$, calculated for $[(C_{18}H_{24}O_2)_2 - H]^-$ = 543.3480.

**IR:** $\tilde{\nu}$ [$cm^{-1}$] = 3514, 3292, 2930, 2864, 1618, 1605, 1514, 1449, 1377, 1348, 1337, 1290, 1275, 1234, 1186, 1169, 1148, 1115, 1096, 1072, 889, 876, 862.

### *1.1.1.2 tert-Butyl (2-(7,8-dichloro-6,9-dicyanodibenzo[b,e][1,4]dioxin-2-yl)ethyl)-carbamate 10*




**10**

**10** was synthesized according to GP1 using tetrachloroterephthalonitrile (**9**, 0.200 g, 0.752 mmol, 1 eq.), *N*-(*tert*-butyloxycarbonyl)dopamine (**8**, 0.191 g, 0.754 mmol, 1 eq.) and potassium carbonate (0.384 g, 2.78 mmol, 3.7 eq.) in dry *N,N*-dimethylformamide (6 ml). Column chromatography (5.5 x 60 cm, dichloromethane/acetone = 99/1) yielded product **10** as a yellow solid (0.293 g, 0.656 mmol, 87.2%).

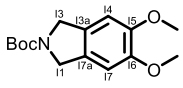
**M($C_{21}H_{17}Cl_2N_3O_4$):** 446.28 g/mol.

Spectroscopic data match literature data.[12]

**$^1$H-NMR (400 MHz, [$D_1$]-chloroform, 298 K):** δ [ppm] =
7.02 – 6.97 (m, 1H, *H*-Ar), 6.94 – 6.89 (m, 2H, *H*-Ar), 4.55 (br s, 1H, N*H*), 3.35 (m, 2H, NC$H_2$), 2.75 (t, $^3J$ = 7.0 Hz, 2H, *H*-D7), 1.44 (s, 9H, OC(C$H_3$)$_3$).

**HR-MS (ESI-pos, 70 eV): m/z =**
390.0043 [M – C($CH_3$)$_3$ + H]$^+$, calculated for [$C_{17}H_9Cl_2N_3O_4$ + H]$^+$ = 390.0043; 468.0491 [M + Na]$^+$, calculated for [$C_{21}H_{17}Cl_2N_3O_4$ + Na]$^+$ = 468.0488; 484.0228 [M + K]$^+$, calculated for [$C_{21}H_{17}Cl_2N_3O_4$ + K]$^+$ = 484.0228.

### *1.1.1.3 N-(tert-Butyloxycarbonyl)-5,6-dimethoxyisoindoline (13)*




**13**

**13** was synthesized according to a modified procedure by Bulman Page *et al.*[13] Under argon atmosphere, dibromide **12** (4.00 g, 12.3 mmol, 1 eq.) was dissolved in dry *N,N*-dimethylformamide (25 ml) and treated portionwise with sodium hydride (60% in paraffin, 2.47 g, 61.7 mmol, 5 eq.) at 0 °C. After ceased bubbling, a solution of *tert*-butyl carbamate (1.45 g, 12.3 mmol, 1 eq.) in dry *N,N*-dimethylformamide (15 ml) was added dropwise at 0 °C. After stirring for 42 hours at room temperature, the reaction was quenched by pouring on ice (200 ml). The precipitate was isolated by vacuum filtration, washed with

water (200 ml) and purified by column chromatography (5.3 x 30 cm, dichloromethane/acetone = 100/0 → 100/1 → 100/2). After lyophilization, **13** was isolated as a crystalline solid (1.25 g, 4.47 mmol, 36.4%).

**M($C_{15}H_{21}NO_4$):** 279.34 g/mol.

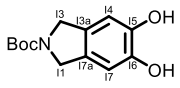
**$^1$H-NMR (400 MHz, [$D_1$]-chloroform, 298 K):** δ [ppm] =
6.75 (s, 2H, *H*-Ar), 4.60 (s, 4H, C$H_2$), 3.87 (s, 6H, OC$H_3$), 1.51 (s, 9H, C(C$H_3$)$_3$).

**$^{13}$C{$^1$H}-NMR (101 MHz, [$D_1$]-chloroform, 298 K):** δ [ppm] =
154.7 (N*C*$O_2$R), 149.1 (*C*-I6, *C*-I5), 128.8 (*C*-I3a, *C*-I4a), 105.7 (*C*-I4, *C*-I7), 79.7 (*C*(CH$_3$)$_3$), 56.2 (*C*-I1, *C*-I3), 52.4 (O*C*H$_3$), 28.7 (C(*C*H$_3$)$_3$).

**HR-MS (ESI-pos, 70 eV): m/z =**
224.0916 [M – C(CH$_3$)$_3$ + H]$^+$, calculated for [$C_{11}H_{13}NO_4$ + H]$^+$ = 224.0917, 280.1538 [M + H]$^+$, calculated for [$C_{15}H_{21}NO_4$ + H]$^+$ = 280.1543; 302.1361 [M + Na]$^+$, calculated for [$C_{15}H_{21}NO_4$ + Na]$^+$ = 302.1363.

**IR:** $\tilde{\nu}$ [cm$^{-1}$] = 3075, 2998, 2980, 2903, 2859, 2837, 2363, 2160, 2020, 1977, 1697, 1616, 1516, 1479, 1464, 1456, 1441, 1406, 1368, 1364, 1344, 1339, 1310, 1271, 1225, 1194, 1169, 1099, 1034, 991, 878, 864, 853, 829, 793, 772, 760, 741, 725, 627.

#### *1.1.1.4 N-(TERT-BUTYLOXYCARBONYL)-5,6-DIHYDROXYISOINDOLINE (14)[14]*




**14**

Under argon atmosphere, *N*-(*tert*-butyloxycarbonyl)-5,6-dimethoxyisoindoline (**13**, 1.18 g, 4.21 mmol, 1 eq.) was dissolved in dry dichloromethane (8 ml). Boron tribromide (2.40 ml, 25.3 mmol, 6 eq.) was carefully added at 0 °C. The mixture was stirred at room temperature overnight. After quenching with methanol (3 ml) at 0 °C, the mixture was concentrated and dried. Dry methanol (30 ml) and triethylamine (2.20 ml, 1.60 g, 15.8 mmol, 3.7 eq.) were added and the mixture dropwise treated with molten di-*tert*-butyl dicarbonate (1.70 ml, 1.62 g, 7.40 mmol, 1.7 eq). The mixture was stirred under argon atmosphere overnight at room temperature. After concentration, the crude product was dissolved in ethyl acetate (80 ml) and washed with a mixture of water (20 ml), brine (20 ml)

and hydrochloric acid (0.3 M, 20 ml). The aqueous layer was extracted four times with ethyl acetate (each 15 ml) and the organic phase dried with magnesium sulphate, filtered, and concentrated, yielding product **14** as an off-white solid (1.02 g, 4.07 mmol, 94.8%).

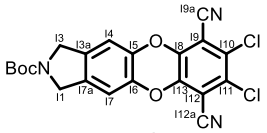
**M($C_{13}H_{17}NO_4$):** 251.28 g/mol.

Spectroscopic data match literature data.[14]

**$^1$H-NMR (400 MHz, [$D_6$]-dimethylsulfoxide, 298 K):** δ [ppm] =
8.89 (br s, 2H, O*H*), 6.65 (s, 1H, *H*-Ar), 6.65 (s, 1H, *H*-Ar), 4.40 (s, 2H, C$H_2$), 4.38 (s, 2H, C$H_2$), 1.43 (s, 9H, C(C$H_3$)$_3$).

**$^{13}$C{$^1$H}-NMR (101 MHz, [$D_6$]-dimethylsulfoxide, 298 K):** δ [ppm] =
153.6 (N*C*O$_2$R), 145.0 (*C*-I6, *C*-I5), 127.1 (*C*-I3a/*C*-I4a), 126.6 (*C*-I4a/*C*-I3a), 109.5 (*C*-I4, *C*-I7), 78.6 (*C*(CH$_3$)$_3$), 51.7 (*C*-I1/*C*-I3), 51.5 (*C*-I3/*C*-I1), 28.2 (C(*C*H$_3$)$_3$).

### *1.1.1.5 tert-Butyl 7,8-dichloro-6,9-dicyano-1H-benzo[5,6][1,4]dioxino[2,3-f]isoindole-2(3H)-carboxylate (15)*




**15**

**15** was synthesized according to GP1 using tetrachloroterephthalonitrile (**9**, 0.583 g, 2.19 mmol, 1 eq.), *N*-(*tert*-butyloxycarbonyl)-5,6-dihydroxyisoindoline (**14**, 0.595 g, 2.37 mmol, 1.1 eq.) and potassium carbonate (1.11 g, 8.05 mmol, 3.7 eq.) in dry *N,N*-dimethylformamide (10 ml). Column chromatography (6.5 x 25 cm, dichloromethane/acetone = 100/0 → 100/1 → 100/2) yielded product **15** as a yellow solid (0.825 g, 1.86 mmol, 84.7%).

**M($C_{21}H_{15}Cl_2N_3O_4$):** 444.27 g/mol.

**$^1$H-NMR (400 MHz, [$D_1$]-chloroform, 298 K):** δ [ppm] =
7.01 (s, 1H, *H*-I7/*H*-I4), 6.93 (s, 1H, *H*-I4/*H*-I7), 4.62 (s, 2H, *H*-I1/*H*-I3), 4.59 (s, 2H, *H*-I3/*H*-I1), 1.51 (s, 9H, OC(C$H_3$)$_3$).

**$^{13}$C{$^1$H}-NMR (101 MHz, [$D_1$]-chloroform, 298 K):** δ [ppm] =

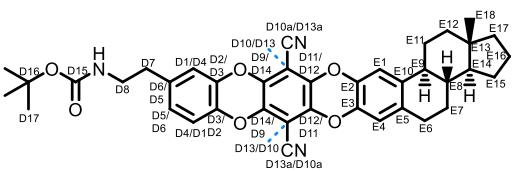
154.3 (N$CO_2$R), 144.6 (*C*q), 139.2 (*C*-I6, *C*-I5), 135.6 (*C*-I7a/*C*-I3a), 135.3 (*C*-I3a/*C*-I7a), 129.9 (*C*q), 129.9 (*C*q), 111.9 (*C*-I7/*C*-I4), 111.6 (*C*-I4/*C*-I7), 110.4 (*C*q), 110.3 (*C*q), 106.7 (*C*q), 106.6 (*C*q), 80.4 (O*C*($CH_3$)$_3$), 51.9 (*C*-I1/*C*-I3), 51.7 (*C*-I3/*C*-I1), 28.6 (OC(*C*$H_3$)$_3$).

**HR-MS (ESI-neg, 70 eV): m/z =**

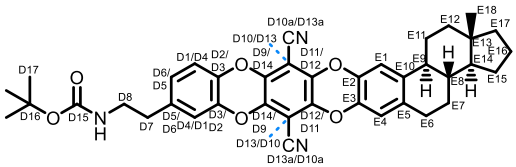
443.10446 [M - e]$^-$, calculated for [$C_{21}H_{15}Cl_2N_3O_4$ - e]$^-$ = 443.0445.

**IR:** $\tilde{\nu}$ [cm$^{-1}$] = 3046, 3009, 2984, 2922, 2872, 1977, 1676, 1584, 1493, 1437, 1410, 1368, 1356, 1319, 1294, 1279, 1254, 1163, 1123, 1065, 897, 880, 866, 822, 772, 733, 648.

### *1.1.1.6 DOPAMINE-DEOXYESTRONE ETHER 16*



**16a**



**16b**

**16** was synthesized according to GP1 using **10** (81.2 mg, 0.182 mmol, 1 eq.), **6** (53.1 mg, 0.195 mmol, 1.1 eq.) and potassium carbonate (110 mg, 0.797 mmol, 4.4 Äq.) in dry *N,N*-dimethylformamide (3 ml). Crude product was purified by column chromatography (4.5 x 18 cm, dichloromethane/acetone = 100/0 → 100/1 → 100/2) and MPLC on RP-18 (water/tetrahydrofuran = 70/30 → 0/100). After washing with *n*-hexane (4 ml) and lyophilization, product **16** (regioisomeric mixture) was isolated as a yellow solid (78.6 mg, 0.122 mmol, 66.9%).

**M($C_{39}H_{39}N_3O_6$):** 645.76 g/mol.

**$^1$H-NMR (400 MHz, [D$_1$]-chloroform, 298 K):** δ [ppm] =

6.95 – 6.90 (m, 2H, *H*-E1/4, *H*-D1/D4/D5), 6.88 – 6.83 (m, 2H, *H*-D1/D4/D5), 6.71 (d, 1H, $^5J_{HH}$ = 0.69 Hz, *H*-E4/1), 4.54 (br s, 1H, N*H*), 3.34 (m, 2H, *H*-D8), 2.81 – 2.66 (m, 4H, *H*-E6, Ar-C$H_2$), 2.22 – 2.09 (m, 2H, *H*-E9, *H*-E11), 1.93 – 1.07 (m, 22H), 0.75 (s, 3H, *H*-E18).

**$^{13}$C{$^1$H}-NMR (101 MHz, [D$_1$]-chloroform, 298 K):** δ [ppm] =

155.9 (N$CO_2$R), 139.8 (*C*q), 139.7 (*C*q), 139.7 (*C*q), 139.7 (*C*q), 139.1 (*C*q), 139.0 (*C*q), 139.0 (*C*q), 139.0 (*C*q), 138.5 (*C*q), 137.4 (*C*q), 137.2 (*C*q), 134.9 (*C*q), 126.0 (*C*H), 117.3 (*C*H), 117.1 (*C*H), 116.6 (*C*H), 113.9 (*C*H), 109.6/109.5/109.5 (*C*-D10a, *C*-D13a), 94.5/94.5/94.4/94.4 (*C*-D10, *C*-D13), 79.7 (*C*($CH_3$)$_3$), 53.6 (*C*-E14), 44.2 (*C*-E9), 41.7 (*C*-

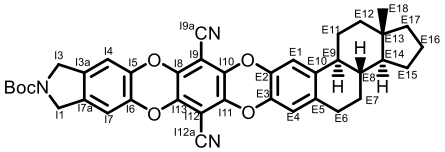
D8), 41.1 (*C*-E13), 40.6 (*C*-E17), 38.7 (*C*-E8), 38.7 (*C*-E12), 35.7 (*C*-D7) 29.2 (*C*-E6), 28.5 (C(*C*H$_3$)$_3$), 27.9 (*C*-E7), 26.8 (*C*-E11), 25.3 (*C*-E15), 20.7 (*C*-E16), 17.6 (*C*-E18).[1]

**HR-MS (ESI-pos, 70 eV): m/z =**

668.2732 [M + Na]$^+$, calculated for [$C_{39}H_{39}N_3O_6$ + Na]$^+$ = 668.2731.

**IR:** $\tilde{\nu}$ [cm$^{-1}$] = 3069, 2930, 2864, 1701, 1686, 1605, 1508, 1452, 1364, 1314, 1289, 1265, 1202, 1165, 1113, 1017, 959, 897, 864, 826, 814, 781, 750, 706, 648.

#### *1.1.1.7 ISOINDOLINE-DEOXYESTRONE ETHER 17*




**17**

**17** was synthesized according to GP1 using **10** (90.0 mg, 0.203 mmol, 1 eq.), **15** (55.0 mg, 0.219 mmol, 1.1 eq.) and potassium carbonate (100 mg, 0.724 mmol, 3.6 Äq.) in dry *N,N*-dimethylformamide (6 ml). Column chromatography (4.5 x 20 cm, dichloromethane/acetone = 100/0 → 100/1 → 100/2) yielded product **17** was isolated as a yellow solid (78.6 mg, 0.122 mmol, 66.9%).

**M($C_{39}H_{37}N_3O_6$):** 643.74 g/mol.

**$^1$H-NMR (400 MHz, [D$_1$]-chloroform, 298 K):** δ [ppm] =

6.93 (s, 1H, *H*-I4/I7), 6.93 (s, 1H, *H*-E4/E1), 6.86 (s, 1H, *H*-I7/I4), 6.71 (s, 1H, *H*-E1/E4), 4.60 (s, 2H, *H*-I4/I1), 4.57 (s, 2H, *H*-I1/I4), 2.80 – 2.73 (m, 2H, *H*-E6), 2.21 – 2.10 (m, 2H, *H*-E9, *H*-E11), 1.94 – 1.00 (m, 22H), 0.75 (s, 3H, *H*-E18).

**$^{13}$C{$^1$H}-NMR (101 MHz, [D$_1$]-chloroform, 298 K):** δ [ppm] =

154.3 (N*C*O$_2$R), 140.0 (*C*q), 139.9 (*C*q), 139.6 (*C*-I6, *C*-I5), 139.1 (*C*q), 138.9 (*C*q), 138.8 (*C*q), 137.4 (*C*q), 137.2 (*C*q), 134.9 (*C*q), 134.8 (*C*q), 134.5 (*C*q), 116.7/116.6 (*C*-E4/E1), 114.0/114.0 (*C*-E1/E4), 111.6 (*C*-I4/I7), 111.3 (*C*-I7/I4), 109.6/109.5/109.5/109.4 (*C*-I9a, *C*-I12a), 94.5/94.5/94.4/94.4 (*C*-I9, *C*-I12), 80.3 (*C*(CH$_3$)$_3$), 53.6 (*C*-E14), 52.0 (*C*-I3/1), 51.8 (*C*-I1/3), 44.2 (*C*-E9), 41.1 (*C*-E13), 40.5 (*C*-E17), 38.7 (*C*-E8), 38.7 (*C*-E12), 29.2

[1] See general information 1.1.3 regarding number of signals in $^{13}$C-NMR spectra.

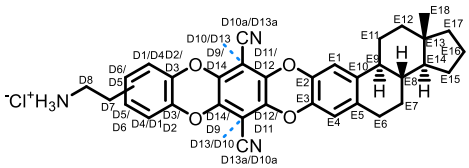
(*C*-E6), 28.6 (C(*C*H$_3$)$_3$), 27.9 (*C*-E7), 26.8 (*C*-E11), 25.3 (*C*-E15), 20.7 (*C*-E16), 17.6 (*C*-E18).[2]

**HR-MS (ESI-pos, 70 eV): m/z =**

588.2134 [M – C(CH$_3$)$_3$ + H]$^+$, calculated for [$C_{35}H_{29}N_3O_6$ + H]$^+$ = 588.2129; 666.2578 [M + Na]$^+$, calculated for [$C_{39}H_{37}N_3O_6$ + Na]$^+$ = 666.2575.

**IR:** $\tilde{\nu}$ [cm$^{-1}$] = 2928, 2868, 2239, 1701, 1607, 1504, 1452, 1393, 1366, 1354, 1306, 1288, 1265, 1163, 1111, 1014, 961, 897, 880, 854, 773, 758, 750, 733, 648.

### *1.1.1.8 BOC-DEPROTECTED DOPAMINE-DEOXYESTRONE ETHER 18*




**18**

Under argon atmosphere, **16** (59.9 mg, 92.8 µmol, 1 eq.) was suspended in dry methanol (8 ml) and dropwise treated with acetyl chloride (2.50 ml, 2.75 g, 35.0 mmol, 377 eq.) at 0 °C. The mixture was stirred for 30 min. at 0 °C and allowed to reach room temperature overnight. After concentration, **18** (regioisomeric mixture) was isolated as a yellow-coloured hydrochloric-salt (53.9 mg, 92.6 µmol, 99.8%).

**M($C_{34}H_{32}ClN_3O_4$):** 582.10 g/mol.

**$^1$H-NMR (400 MHz, [D$_6$]-dimethylsulfoxide, 298 K):** δ [ppm] =

7.94 (br s, 3H, N*H*$_3$), 7.14 – 7.09 (m, 2H, *H*-D1, *H*-D4), 6.99 (dd, $^3J_{HH}$ = 8.3 Hz, $^4J_{HH}$ = 1.5 Hz, 1H, *H*-D5), 6.92 (s, 1H, *H*-E4/1), 6.83 (s, 1H, *H*-E1/4), 3.04 (t, $^3J_{HH}$ = 7.6 Hz, 2H, *H*-D8), 2.82 (t, $^3J_{HH}$ = 7.6 Hz, 2H, *H*-D7), 2.74 – 2.65 (m, 2H, *H*-E6), 2.27 – 2.19 (m, 1H, *H*-E), 2.12 – 2.02 (m, 1H, *H*-E), 1.84 – 1.12 (m, 13H), 0.71 (s, 3H, *H*-E18).

**$^{13}$C{$^1$H}-NMR (101 MHz, [D$_6$]-dimethzylsulfoxide, 298 K):** δ [ppm] =

139.1 (*C*q), 138.9 (*C*q), 138.9 (*C*q), 138.8 (*C*q), 138.5 (*C*q), 138.5 (*C*q), 138.4 (*C*q), 138.4 (*C*q), 138.2 (*C*q), 138.0 (*C*q), 136.8 (*C*q), 136.6 (*C*q), 135.6 (*C*q), 134.4 (*C*q), 126.2 (*C*H), 117.1 (*C*H), 116.8 (*C*H), 116.3 (*C*H), 113.3 (*C*H), 109.6/109.6 (*C*-D10a, *C*-D13a), 93.3/93.3/93.3 (*C*-D10, *C*-D13), 52.9 (*C*-E14), 43.4 (*C*-E9), 40.6 (*C*-E13), 39.9 (*C*-E17,

[2] See general information 1.1.3 regarding number of signals in $^{13}$C-NMR spectra.

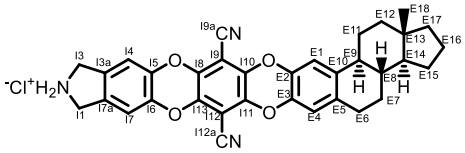
overlapping with DMSO signal), 39.3 (*C*-D8, overlapping with DMSO signal), 38.1 (*C*-E8), 38.0 (*C*-E12), 32.0 (*C*-D7), 28.2 (*C*-E6), 27.1 (*C*-E7), 26.0 (*C*-E11), 24.7 (*C*-E15), 20.2 (*C*-E16), 17.3 (*C*-E18).

**HR-MS (ESI-pos, 70 eV): m/z =**

546.2385 $[M - HCl + H]^+$, calculated for $[C_{34}H_{31}N_3O_4 + H]^+$ = 546.2387.

**IR:** $\tilde{\nu}$ [$cm^{-1}$] = 3131, 2947, 2932, 2851, 2363, 2160, 2029, 1977, 1508, 1452, 1314, 1289, 1267k 1202, 1161, 1071, 1015, 957, 870, 820, 748.

#### *1.1.1.9 BOC-DEPROTECTED ISOINDOLINE-DEOXYESTRONE ETHER 19*




**19**

Under argon atmosphere, **17** (32.0 mg, 49.7 µmol, 1 eq.) was suspended in dry methanol (6 ml) and dropwise treated with acetyl chloride (0.40 ml, 0.440 g, 3.78 mmol, 75 eq.) at 0 °C. The mixture was stirred for 30 min. at 0 °C and allowed to reach room temperature overnight. After concentration, **19** was isolated as a yellow-coloured hydrochloric-salt (28.8 mg, 49.6 µmol, 99.8%).

**M($C_{34}H_{30}ClN_3O_4$):** 580.08 g/mol.

**$^1$H-NMR (400 MHz, [D$_6$]-dimethylsulfoxide, 298 K):** δ [ppm] =

9.62 (br s, 2H, N*H*$_2$), 7.23 (s, 2H, *H*-I4, *H*-I7), 6.96 (s, 1H, *H*-E1, *H*-E4), 6.85 (s, 1H, *H*-E4/E1), 4.41 (s, 4H, *H*-I1, *H*-I3), 2.80 – 2.64 (m, 2H, *H*-E), 2.30 – 2.23 (m, 1H, *H*-E), 2.16 – 2.07 (m, 1H, *H*-E), 1.85 – 1.15 (m, 13H), 0.72 (s, 3H, *H*-E18).

**$^{13}$C{$^1$H}-NMR (101 MHz, [D$_6$]-dimethylsulfoxide, 298 K):** δ [ppm] =

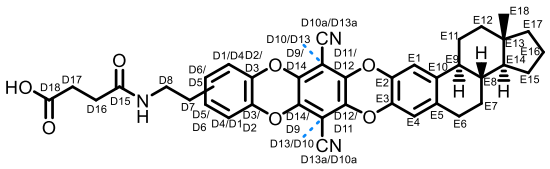
139.4 (2 *C*q), 139.1 (*C*q), 139.0 (*C*q), 138.4 (*C*q), 138.3 (*C*q), 138.2 (*C*q), 136.8 (*C*q), 136.7 (*C*q), 134.4 (*C*q), 132.4 (2 *C*q), 116.3 (*C*H), 113.4 (*C*H), 111.6 (2 *C*H), 109.7 (*C*q), 109.6 (*C*q), 93.3 (2 *C*q), 52.9 (*C*-E14), 49.8 (*C*-I3, *C*-I1), 43.5 (*C*-E9), 40.6 (*C*-E13), 39.9 (*C*-E17, overlapping with DMSO signal) 38.2 (*C*-E8), 38.1 (*C*-E12), 28.3 (*C*-E6), 27.1 (*C*-E7), 26.1 (*C*-E11), 24.7 (*C*-E15), 20.2 (*C*-E16), 17.3 (*C*-E18).

**HR-MS (ESI-pos, 70 eV): m/z =**

544.2225 [M - HCl + H]$^+$, calculated for [$C_{34}H_{29}N_3O_4$ + H]$^+$ = 543.2231.

**IR:** $\tilde{\nu}$ [cm$^{-1}$] = 3366, 2928, 2864, 2733, 2237, 1751, 1609, 1501, 1458, 1362, 1304, 1263, 1223, 1165, 1148, 1016, 895, 864, 839, 791, 746, 671, 648.

#### *1.1.1.10 DOPAMIN-DEOXYESTRON CARBOXYLIC ACID 20*



**20**

Under argon atmosphere, amine **18** (51.2 mg, 88.0 µmol, 1 eq.) was dissolved in anhydrous *N,N*-dimethylformamide (3.5 ml) and dry *N,N*-diisopropylethylamine (50.0 µl, 38.0 mg, 294 µmol, 3.3 eq.) and a solution of succinic anhydride (12.5 mg, 125 µmol, 1.4 eq.) in anhydrous *N,N*-dimethylformamide (0.5 ml) were added. The mixture was stirred overnight at room temperature. Due to incompleteness of the reaction, more succinic anhydride (8.0 mg, 79.9 µmol, 0.9 eq.) in *N,N*-dimethylformamide (0.5 ml) was added. After stirring for three more hours at room temperature, the mixture was concentrated and washed twice by centrifugation (3500 rpm, 5 min.) with water and *n*-hexane. Lyophilization yielded product **20** (regioisomeric mixture) as a yellow solid (53.8 mg, 83.3 µmol, 94.7%), which was used without further purification for the next step.

**M($C_{38}H_{35}N_3O_7$):** 645.71 g/mol.

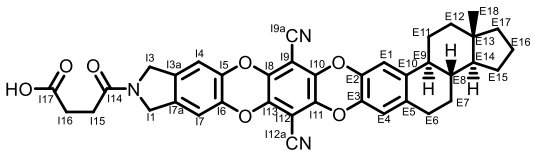
**$^1$H-NMR (600 MHz, [$D_6$]-dimethylsulfoxide, 298 K):** δ [ppm] =
12.09 (br s, 1H, COO*H*), 7.91 (t, 1H, $^3J_{HH}$ = 5.5 Hz, N*H*), 7.04 (dd, $^3J_{HH}$ = 8.3 Hz, $^4J_{HH}$ = 2.0 Hz, 1H, *H*-D5), 7.02 (s, 1H, *H*-E1/4), 6.92 (d, 1H, *H*-D4), 6.91 (s, 1H, *H*-E4/1), 6.82 (d, 1H, $^4J_{HH}$ = 1.5 Hz, *H*-D1), 3.24 (ps q, 2H, *H*-D8, overlapping with water signal), 2.76 – 2.67 (m, 2H, *H*-E6, overlapping with residual DMF signal), 2.63 (t, $^3J_{HH}$ = 7.3 Hz, 2H, *H*-D7, overlapping with DMSO satellite signal), 2.39 (t, $^3J_{HH}$ = 7.3 Hz, 2H, *H*-D16/*H*-D17, overlapping with DMSO satellite signal), 2.28 (t, $^3J_{HH}$ = 7.3 Hz, 2H, *H*-D16/*H*-D17), 2.26 – 2.21 (m, 1H, *H*-E), 2.10 – 2.04 (m, 1H, *H*-E, overlapping with acetone signal), 1.83 – 1.14 (m, 13H), 0.71 (s, 3H, *H*-E18).

**$^{13}C\{^{1}H\}$-NMR (151 MHz, [$D_6$]-dimethylsulfoxide, 298 K):** δ [ppm] =
173.9 (*C*-D18), 170.9 (*C*-D15), 138.9 (*C*q), 138.8 (*C*q), 138.8 (*C*q), 138.7 (*C*q), 138.7 (*C*q), 138.6 (*C*q), 138.6 (*C*q), 138.5 (*C*q), 138.2 (*C*q), 138.0 (*C*q), 137.5 (*C*q), 136.8 (*C*q), 136.7 (*C*q), 134.4 (*C*q), 126.0 (*C*-D4), 116.8 (*C*-E1/4), 116.5 (*C*-D5), 116.2 (*C*-D1), 113.3 (*C*-E4/1), 109.7/109.7/109.6 (*C*-D10a, *C*-D13a), 93.3/93.2 (*C*-D10, *C*-D13), 52.9 (*C*-E14), 43.4 (*C*-E9), 40.5 (*C*-E13), 39.9 (*C*-E17, overlapping with DMSO signal), 39.7 (*C*-D8, overlapping with DMSO), 38.1 (*C*-E8), 38.0 (*C*-E12), 34.1 (*C*-D7), 30.0 (*C*-D17/*C*-D16), 29.2 (*C*-D16/*C*-D17), 28.2 (*C*-E6), 27.1 (*C*-E7), 26.0 (*C*-E11), 24.7 (*C*-E15), 20.2 (*C*-E16), 17.3 (*C*-E18).

**HR-MS (ESI-pos, 70 eV): m/z =**
646.2541 $[M + H]^+$, calculated for $[C_{38}H_{35}N_3O_7 + H]^+$ = 646.2548; 668.2361 $[M + Na]^+$, calculated for $[C_{38}H_{35}N_3O_7 + Na]^+$ = 668.2367.

**IR:** $\tilde{\nu}$ [$cm^{-1}$] = 3321, 3057, 2928, 2864, 2241, 1713, 1651, 1605, 1557, 1506, 1456, 1429, 1377, 1360, 1314, 1288, 1265, 1202, 1165, 1111, 1016, 949, 897, 864, 814, 748.

#### *1.1.1.11 ISOINDOLIN-DEOXYESTRON CARBOXYLIC ACID 21*



**21**

Under argon atmosphere, amine **19** (21.9 mg, 37.8 µmol, 1 eq.) was dissolved in anhydrous *N,N*-dimethylformamide (2.0 ml) and dry *N,N*-diisopropylethylamine (40.0 µl, 30.4 mg, 235 µmol, 6.2 eq) and a solution of succinic anhydride (6.15 mg, 61.5 µmol, 1.6 eq.) in anhydrous *N,N*-dimethylformamide (0.5 ml) were added. The mixture was stirred overnight at room temperature. The mixture was concentrated and washed twice by centrifugation (4000 rpm, 10 min.) with water. Lyophilization yielded product **21** as a yellow solid (22.0 mg, 34.2 µmol, 90.5%), which was used without further purification for the next step.

**M($C_{38}H_{33}N_3O_7$):** 643.70 g/mol.

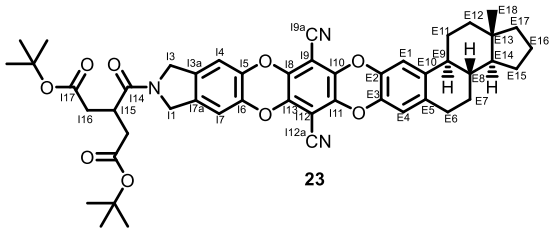
**$^1$H-NMR (600 MHz, [D$_6$]-dimethylsulfoxide, 298 K):** δ [ppm] =
12.09 (br s, 1H, COO*H*), 7.19 (s, 1H, *H*-Ar), 7.18 (s, 1H, *H*-Ar), 6.96 (s, 1H, *H*-Ar), 6.86 (s, 1H, *H*-Ar), 4.75 (s, 2H, *H*-I1/*H*-I3), 4.52 (s, 2H, *H*-I3/*H*-I1), 2.79 – 2.70 (m, 2H, *H*-E6), 2.54 (m, 4H, *H*-I15, *H*-I16, overlapping with DMSO signal), 2.30 – 2.24 (m, 1H, *H*-E), 2.14 – 2.09 (m, 1H, *H*-E), 1.85 – 1.06 (m, 13H), 0.72 (s, 3H, *H*-E18).

**DEPTQ-NMR (151 MHz, [D$_6$]-dimethylsulfoxide, 298 K):** δ [ppm] =
173.9 (*C*-I17), 169.8 (*C*-I14), 139.0 (*C*q), 138.9 (*C*q), 138.9 (*C*q), 138.9 (*C*q), 138.5 (*C*q), 138.5 (*C*q), 138.2 (*C*q), 136.9 (*C*q), 136.7 (*C*q), 135.5 (*C*q), 134.4 (*C*q), 134.2 (*C*q), 133.7 (*C*q), 116.3 (*C*H), 113.4 (*C*H), 111.6 (*C*H), 111.4 (*C*H), 109.8 (*C*q), 109.7 (*C*q), 93.3 (2 *C*q), 52.9 (*C*-E14), 51.3 (*C*-I4, *C*-I7), 43.5 (*C*-E9), 40.6 (*C*-E13), 39.9 (*C*-E17, overlapping with DMSO signal), 38.2 (*C*-E8), 38.1 (*C*-E12), 28.5 (*C*-I17/I16/E6), 28.3 (*C*-I16/I17/E6), 28.3 (*C*-E6/I17/I16), 27.1 (*C*-E7), 26.1 (*C*-E11), 24.7 (*C*-E15), 20.2 (*C*-E16), 17.3 (*C*-E18).

**HR-MS (ESI-pos, 70 eV): m/z =**
644.2390 $[M + H]^+$, calculated for $[C_{38}H_{33}N_3O_7 + H]^+$ = 644.2391; 666.2213 $[M + Na]^+$, calculated for $[C_{38}H_{33}N_3O_7 + Na]^+$ = 666.2211.

**IR:** $\tilde{\nu}$ [cm$^{-1}$] = 3414, 3069, 2924, 2864, 2363, 2241, 2158, 2021, 1977, 1724, 1655, 1640, 1505, 1460, 1456, 1379, 1356, 1312, 1289, 1265, 1225, 1202, 1165, 11571017, 953, 897, 862, 824, 760, 746, 704, 648.

### *1.1.1.12 ISOINDOLIN-DEOXYESTRONE DIESTER 23*




**23**

Under argon atmosphere, acid **22** (23.0 mg, 79.8 µmol, 1.5 eq.), HOBt (14.0 mg, 104 µmol, 2 eq.), EDCI (18.0 mg, 93.9 µmol, 1.8 eq.) and *N*-methylmorpholine (30.0 µl, 27.3 mg, 270 µmol, 5.2 eq.) were dissolved in anhydrous *N,N*-dimethylformamide (7.0 ml). After stirring for 30 min. at room temperature, amine **21** (30.0 mg, 51.7 µmol, 1 eq.) was added. The mixture was stirred overnight at room temperature and then concentrated. Column chromatography (3.5 x 25 cm, dichloromethane/acetone = 100/0

→ 100/1 → 100/2 → 100/5 → 100/7.5) yielded product **23** was isolated as a yellow solid (29.0 mg, 35.6 µmol, 68.9%).

**M($C_{48}H_{51}N_3O_9$):** 813.95 g/mol.

**$^1$H-NMR (400 MHz, [$D_1$]-chloroform, 298 K):** δ [ppm] =
6.94 (s, 1H, *H*-Ar), 6.93 (s, 1H, *H*-Ar), 6.91 (s, 1H, *H*-Ar), 6.72 (s, 1H, *H*-Ar), 5.03 (s, 2H, *H*-I4/I1), 4.70 (s, 2H, *H*-I1/I4), 3.35 (tt, 1H, *H*-I15), 2.80 – 2.75 (m, 2H, *H*-E6), 2.72 (dd, 2H, *H*-I16), 2.38 (dd, 2H, *H*-I16), 2.21 – 2.10 (m, 2H, *H*-E9, *H*-E11), 1.94 – 1.07 (m, 31H), 0.75 (s, 3H, *H*-E18).

**$^{13}$C{$^1$H}-NMR (101 MHz, [$D_1$]-chloroform, 298 K):** δ [ppm] =
173.0 (2 O*C*OR), 171.1 (N*C*OR), 140.0 (*C*q), 139.9 (*C*q), 139.8 (*C*q), 139.7 (*C*q), 139.1 (*C*q), 138.8 (*C*q), 138.8 (*C*q), 137.4 (*C*q), 137.2 (*C*q), 134.9 (*C*q), 134.2 (*C*q), 134.0 (*C*q), 116.6 (*C*H), 114.0 (*C*H), 111.7 (*C*H), 111.5 (*C*H), 109.5/109.5/109.5/109.4 (*C*-I9a, *C*-I12a), 94.5/94.5/94.4/94.4 (*C*-I9, *C*-I12), 81.2 (*C*($CH_3$)$_3$), 53.6 (*C*-E14), 52.4 (*C*-I1/I3), 52.0 (*C*-I3/I1), 44.2 (*C*-E9), 41.1 (*C*-E13), 40.5 (*C*-E17), 38.7 (*C*-E8), 38.7 (*C*-E12), 38.1 (*C*-I16), 35.9 (*C*-I15), 29.2 (*C*-E6), 28.2 (C(*C*$H_3$)$_3$), 27.9 (*C*-E7), 26.8 (*C*-E11), 25.3 (*C*-E15), 20.7 (*C*-E16), 17.6 (*C*-E18).[3]

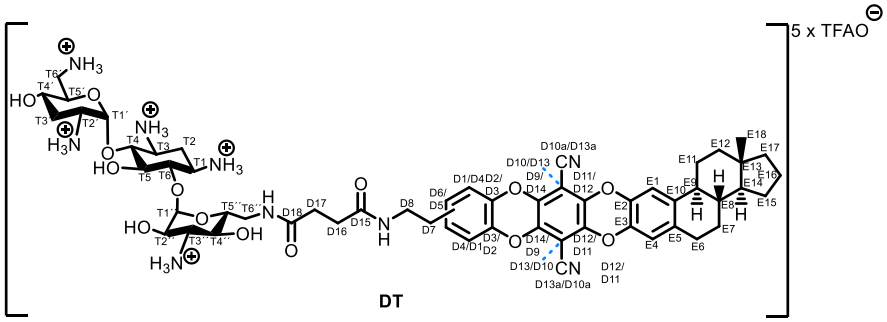
**HR-MS (ESI-pos, 70 eV): m/z =**
814.3710 $[M + H]^+$, calculated for $[C_{48}H_{51}N_3O_9 + H]^+$ = 814.3698; 836.3529 $[M + Na]^+$, calculated for $[C_{48}H_{51}N_3O_9 + Na]^+$ = 836.3518; 852.3275 $[M + K]^+$, calculated for $[C_{48}H_{51}N_3O_9 + K]^+$ = 852.3257.

**IR:** $\tilde{\nu}$ [$cm^{-1}$] = 3391, 3273, 2976, 2918, 2866, 2359, 2257, 2239, 2129, 1724, 1655, 1503, 1452, 1393, 1366, 1352, 1308, 1287, 1263, 1223, 1152, 1047, 1018, 1003, 961, 897, 864, 847, 824, 762, 745.

[3] See general information 1.1.3 regarding number of signals in $^{13}$C-NMR spectra.

## SYNTHESIS OF LIGANDS

### *1.1.1.13 LIGAND DT*




Ligand **DT** was synthesized according to GP1 using carboxylic acid **20** (34.0 mg, 52.6 µmol, 1 eq.), PyBOP (31.8 mg, 61.0 µmol, 1.2 eq.), *N*-methylmorpholine (30.8 µl, 28.0 mg, 277 µmol, 5.3 eq.) and tobramycin amine **25** (42.8 mg, 44.3 µmol, 0.84 eq.) in *N,N*-dimethylformamide and dichloromethane (each 3 ml). Column chromatography (4.6 x 12 cm, dichloromethane/acetone/methanol = 100/0/0 → 100/1/1 → 100/2/2 → 100/3/3 → 100/4/4 → 100/5/5 → 100/7.5/7.5) yielded the BOC-protected compound (65.4 mg, 41.1 µmol, 92.8%) as a yellow solid, which was characterized by mass spectrometry.

**HR-MS (ESI-pos, 70 eV): m/z =**

1594.7835 $[M + H]^+$, calculated for $[C_{81}H_{111}N_9O_{24} + H]^+$ = 1594.7815; 1616.7673 $[M + Na]^+$, calculated for $[C_{81}H_{111}N_9O_{24} + Na]^+$ = 1616.7634; 797.8948 $[M + 2H]^{2+}$, calculated for $[C_{81}H_{111}N_9O_{24} + 2H]^{2+}$ = 797.8944; 819.8775 $[M + 2Na]^{2+}$, calculated for $[C_{81}H_{111}N_9O_{24} + 2Na]^{2+}$ = 819.8763.

The BOC-protected compound (48.3 mg, 30.3 µmol, 1 eq.) was dissolved in dichloromethane (4 ml) and treated with trifluoroacetic acid (260 µl, 385 mg, 3.37 mmol, 111 eq.). The mixture was stirred 30 min. at room temperature and then concentrated. After purification (see GP1), ligand **DT** was obtained as a yellow solid (25.4 mg, 15.3 µmol, 50.4%). The product was stored under argon at 2 °C to prevent oxidation.

**M($C_{66}H_{76}F_{15}N_9O_{24}$):** 1664.35 g/mol.

**$^1$H-NMR (600 MHz, [D$_4$]-methanol, 298 K):** δ [ppm] =

6.99 (m, 3H, *H*-Ar), 6.93 (d, $J_{HH}$ = 4.4 Hz, 1H, *H*-Ar), 6.74 (d, $J_{HH}$ = 4.4 Hz, 1H, *H*-Ar), 5.92 (d, $^3J_{HH}$ = 2.9 Hz, 1H, *H*-T1´), 5.03 (d, $^3J_{HH}$ = 2.9 Hz, 1H, *H*-T1´´), 4.09 (ps t, $^3J_{HH}$ = 8.3 Hz, $^3J_{HH}$ = 8.3 Hz, 1H, *H*-T4), 3.94 (ps dt, $^3J_{HH}$ = 9.0 Hz, $^3J_{HH}$ = 3.3 Hz, 1H, *H*-T5´), 3.91 (ps

quint, $^3J_{HH}$ = 8.6 Hz, $^3J_{HH}$ = 4.5 Hz, $^3J_{HH}$ = 4.1 Hz, 1H, *H*-T5´´), 3.84 – 3.72 (m, 3H, *H*-T5, *H*-T2´´, *H*-T6), 3.60 – 3.46 (m, 6H, *H*-T6´´, *H*-T1, *H*-T3, *H*-T4, *H*-D8), 3.45 – 3.33 (m, 6H, *H*-T2´, *H*-T6´, *H*-T3´´, *H*-T4´´, *H*-D16/*H*-D17), 3.10 (dd, $^2J_{HH}$ = 13.3 Hz, $^3J_{HH}$ = 8.3 Hz, 1H, *H*-T6´), 2.80 – 2.76 (m, 2H, *H*-E6), 2.74 (t, $^3J_{HH}$ = 7.1 Hz, 2H, *H*-D7), 2.53 – 2.43 (m, 4H), 2.26 – 2.20 (m, 2H, *H*-E11, *H*-3´eq), 2.17 – 2.05 (m, 3H), 1.93 – 1.86/1.81 – 1.64/1.54 – 1.46/1.37 – 1.26/1.13 – 1.06 (5 m, 13H), 0.78 (s, 3H, *H*-E18).

**$^{13}$C{$^1$H}-NMR (151 MHz, [D$_4$]-methanol, 298 K):** δ [ppm] =

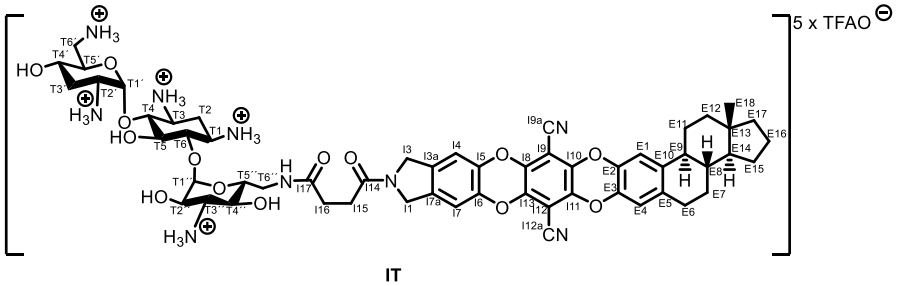
175.9 (N*C*OR), 174.9 (N*C*OR), 163.4 (q, $^2J_{CF}$ = 32.8 Hz, $CF_3CO_2H$), 141.2 (*C*q), 141.0 (*C*q), 140.9 (*C*q), 140.9 (*C*q), 140.8 (*C*q), 140.5 (*C*q), 140.4 (*C*q), 140.4 (*C*q), 140.3 (*C*q), 140.2 (*C*q), 140.2 (*C*q), 139.9 (*C*q), 139.2 (*C*q), 138.8 (*C*q), 138.7 (*C*q), 136.3 (*C*q), 136.2 (*C*q), 127.3 (*C*H), 118.3 (q, $^1J_{CF}$ = 428.3 Hz, $CF_3CO_2H$), 118.2 (*C*H), 117.9 (*C*H), 117.6/117.6 (*C*H), 114.8/114.7 (*C*H), 110.5/110.5/110.4/110.4 (2 *C*q), 102.6 (*C*H), 95.5 (*C*H), 95.4/95.4 (2 *C*q), 85.5 (*C*H), 79.0 (*C*H), 76.3 (*C*H), 73.4 (*C*H), 72.0 (*C*H), 70.3 (*C*H), 68.9 (*C*H), 66.9 (*C*H), 56.5 (*C*H), 54.9 (*C*-E14), 51.7 (*C*H), 50.3 (*C*H), 49.7 (*C*H), 45.5 (*C*-E9), 42.2 (*C*-E13), 42.0 ($CH_2$), 41.8 ($CH_2$), 41.7 (*C*-E17), 41.3 ($CH_2$), 40.2 (*C*-E8), 40.0 (*C*-E12), 35.8 ($CH_2$), 32.0 ($CH_2$), 31.9 ($CH_2$), 31.5 ($CH_2$), 30.9 ($CH_2$), 30.1 (*C*-E6), 29.0 (*C*-E7), 27.9 (*C*-E11), 26.3 (*C*-E15), 21.6 (*C*-E16), 18.1 (*C*-E18).

**HR-MS (ESI-pos, 70 eV): m/z =**

547.7634 $[M + 2H]^{2+}$, calculated for $[C_{56}H_{71}N_9O_{14} + 2H]^{2+}$ = 547.7633; 365.5110 $[M + 3H]^{3+}$, calculated for $[C_{56}H_{71}N_9O_{14} + 3H]^{3+}$ = 365.5113.

**IR:** $\tilde{\nu}$ [cm$^{-1}$] = 3392, 3088, 2930, 2870, 2243, 1981, 1674, 1634, 1549, 1510, 1460, 1433, 1377, 1360, 1312, 1288, 1271, 1200, 1130, 1051, 1016, 901, 881, 839, 799, 748, 723, 648.

### *1.1.1.14 LIGAND IT*




**IT**

Ligand **IT** was synthesized according to GP1 using carboxylic acid **21** (20.2 mg, 31.4 µmol, 1 eq), PyBOP (18.2 mg, 35.0 µmol, 1.1 eq.), *N*-methylmorpholine (18.0 µl, 16.4 mg, 162 µmol, 5.2 eq.) and tobramycin amine **25** (24.4 mg, 25.2 µmol, 0.80 eq.) in *N,N*-dimethylformamide and dichloromethane (each 1.5 ml). Column chromatography (2.5 x 16 cm, dichloromethane/acetone/methanol = 100/0/0 → 100/1/1 → 100/2/2 → 100/3/3 → 100/4/4 → 100/5/5 → 100/7.5/7.5) yielded the BOC-protected compound (27.0 mg, 17.0 µmol, 67.3%) as a yellow solid, which was characterized by mass spectrometry and used directly in the next step.

**HR-MS (ESI-pos, 70 eV): m/z =**
1592.7663 $[M + H]^+$, calculated for $[C_{81}H_{109}N_9O_{24} + H]^+$ = 1592.7658; 1614.7471 $[M + Na]^+$, calculated for $[C_{81}H_{109}N_9O_{24} + Na]^+$ = 1614.7478; 818.8690 $[M + 2Na]^{2+}$, calculated for $[C_{81}H_{109}N_9O_{24} + 2Na]^{2+}$ = 818.8685.

The BOC-protected compound (40.0 mg, 25.1 µmol, 1 eq.) was dissolved in dichloromethane (5 ml) and treated with trifluoroacetic acid (200 µl, 296 mg, 2.60 mmol, 111 eq.). The mixture was stirred 18 hours at room temperature and then concentrated. After purification (see GP1), ligand **IT** was obtained as a yellow solid (26.9 mg, 16.2 µmol, 64.5%). The product was stored under argon at 2 °C to prevent oxidation.

**M($C_{66}H_{74}F_{15}N_9O_{24}$):** 1662.33 g/mol.

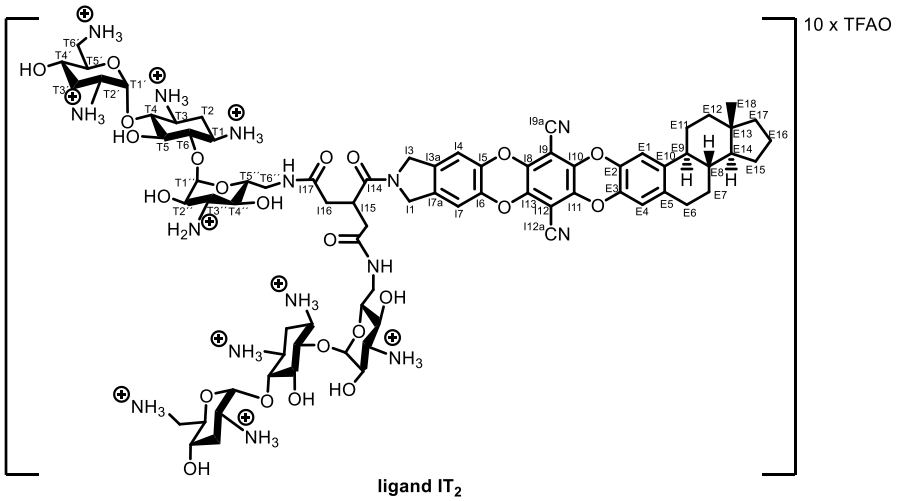
**$^1$H-NMR (600 MHz, [$D_7$]-*N,N*-dimethylformamide, 298 K):** δ [ppm] =
9.05 – 8.53 (m, 15H, RN*H*$_3$), 8.08 (t, $^3J_{HH}$ = 5.7 Hz, 1H, RN*H*), 7.92 (br s, 1H, O*H*), 7.22 (s, 1H, *H*-Ar), 7.19 (s, 1H, *H*-Ar), 7.03 (s, 1H, *H*-Ar), 6.87 (s, 1H, *H*-Ar), 6.28 (br s, 1H, O*H*), 6.21 (d, $^3J_{HH}$ = 3.7 Hz, 1H, *H*-T1´), 6.03 (br s, 1H, O*H*), 5.81 (br s, 1H, O*H*), 5.19 (d, $^3J_{HH}$ = 3.8 Hz, 1H, *H*-T1´´), 4.86 (m, 2H, *H*-I1/I3), 4.65 (m, 2H, *H*-I3/I1), 4.25 (ps t, $^3J_{HH}$ = 9.6 Hz, $^3J_{HH}$ = 9.6 Hz, 1H, *H*-T4), 4.16 (ps dt, $^3J_{HH}$ = 9.0 Hz, $^3J_{HH}$ = 2.9 Hz, 1H, *H*-T5´´/*H*-T5´), 4.03 (m, 1H, *H*-T5´/*H*-T5´´), 4.00 (dd, $^3J_{HH}$ = 10.6 Hz, $^3J_{HH}$ = 3.7 Hz, 1H, *H*-T5/*H*-T2´´), 3.93 (m, 2H, *H*-T2´´/*H*-T5, *H*-T6), 3.77 – 3.43 (m, 9H, overlapping with water signal), 3.28 (m, 1H), 3.15 (dd, $^2J_{HH}$ = 13.2 Hz, $^3J_{HH}$ = 8.6 Hz, 1H, *H*-T6´), 2.82 – 2.78 (m, 2H, *H*-E6), 2.73 (m, 2H, *H*-I15/I16, overlapping with DMF signal), 2.64 (m, 2H, *H*-I16/I15), 2.53 (m, 1H, *H*-T3´äq), 2.32 – 2.15 (m, 4H), 1.91 – 1.83/1.77 – 1.61/1.51 – 1.45/1.38 – 1.21/1.15 – 1.08 (5 m, 13H), 0.76 (s, 3H, *H*-E18).

**$^{13}C\{^{1}H\}$-NMR (151 MHz, [$D_7$]-*N,N*-dimethylformamide, 298 K):** δ [ppm] =
173.5 (N*C*OR), 171.6 (N*C*OR), 160.5 (q, $^{2}J_{CF}$ = 31.9 Hz, $CF_3CO_2H$), 140.2 (*C*q), 140.2 (2 *C*q), 140.1 (*C*q), 139.6 (*C*q), 139.5 (*C*q), 139.3 (*C*q), 138.0 (*C*q), 137.9 (*C*q), 135.4 (*C*q), 135.2 (*C*q), 134.8 (*C*q), 118.05 (q, $^{1}J_{CF}$ = 297.1 Hz, $CF_3CO_2H$), 117.0 (*C*H), 114.2 (*C*H), 112.3 (*C*H), 112.2 (*C*H), 110.3/110.3 (2 *C*q), 101.5 (*C*H), 94.4 (2 *C*q), 94.0 (*C*H), 85.1 (*C*H), 77.4 (*C*H), 75.7 (*C*H), 72.9 (*C*H), 71.2 (*C*H), 69.7 (*C*H), 68.3 (*C*H), 66.3 (*C*H), 56.5 (*C*H), 53.9 (*C*-E14), 52.4 ($CH_2$), 52.2 ($CH_2$), 51.3 (*C*H), 50.0 (*C*H), 49.3 (*C*H), 44.6 (*C*-E9), 41.8 ($CH_2$), 41.5 (*C*-E13), 40.9 (*C*-E17), 40.7 ($CH_2$), 39.2 (*C*-E8), 39.1 (*C*-E12), 31.3 ($CH_2$), 30.7 ($CH_2$), 29.6 ($CH_2$), 29.3 (*C*-E6), 28.9 ($CH_2$), 28.2 (*C*-E7), 27.1 (*C*-E11), 25.6 (*C*-E15), 20.9, (*C*-E16), 17.7 (*C*-E18).

**HR-MS (ESI-pos, 70 eV):**[4] **m/z =**
1092.5048 $[M + H]^+$, calculated for $[C_{56}H_{69}N_9O_{14} + H]^+$ = 1092.5037; 546.7567 $[M + 2H]^{2+}$, calculated for $[C_{56}H_{69}N_9O_{14} + 2H]^{2+}$ = 546.7555; 364.8391 $[M + 3H]^{3+}$, calculated for $[C_{56}H_{69}N_9O_{14} + 3H]^{3+}$ = 364.8394.

**IR:** $\tilde{\nu}$ [$cm^{-1}$] = 3377, 3059, 2930, 2868, 2234, 2245, 1674, 1628, 1537, 1504, 1454, 1356, 1308, 1288, 1265, 1200, 1132, 1051, 1016, 887, 874, 839, 799, 773, 746, 723.

### *1.1.1.15 LIGAND $IT_2$*




**ligand $IT_2$**

Diester **23** (26.0 mg, 31.9 µmol, 1 eq.) was dissolved in dichloromethane (3 ml) and treated with trifluoroacetic acid (0.40 ml, 592 mg, 5.19 mmol, 80 eq.). The mixture was

[4] Due to measurement disturbances of the TFA salt, an ion exchange with hydrochloric acid was performed to yield the corresponding chloride salt for HR-MS analysis

stirred overnight at room temperature and then concentrated. After washing with cold cyclohexane, dicarboxylic acid **24** was isolated as a yellow solid (22.4 mg, 31.9 µmol, quant.) and used without further purification in the next peptide coupling.

**HR-MS (ESI-pos, 70 eV): m/z =**

702.2456 $[M + H]^+$, calculated for $[C_{40}H_{35}N_3O_9 + H]^+$ = 702.2446; 724.2274 $[M + Na]^+$, calculated for $[C_{48}H_{51}N_3O_9 + Na]^+$ = 724.2266.

Ligand **IT₂** was synthesized according to GP1 using carboxylic acid **24** (19.0 mg, 27.1 µmol, 1 eq), PyBOP (38.0 mg, 73.0 µmol, 2.7 eq.), *N*-methylmorpholine (42.0 µl, 38.2 mg, 378 µmol, 14 eq.) and tobramycin amine **25** (51.3 mg, 53.0 µmol, 2.0 eq.) in *N,N*-dimethylformamide and dichloromethane (each 1.5 ml). Column chromatography (3.5 x 23 cm, dichloromethane/acetone/methanol = 100/0/0 → 100/1/1 → 100/2/2 → 100/3/3 → 100/4/4 → 100/5/5 → 100/7.5/7.5) yielded the BOC-protected compound (70.2 mg, 27.0 µmol, 99.7%) as a yellow solid, which was characterized by mass spectrometry and used directly in the next step.

**HR-MS (ESI-pos, 70 eV): m/z =**

1300.6546 $[M + 2H]^{2+}$, calculated for $[C_{126}H_{187}N_{15}O_{43} + 2H]^{2+}$ = 1300.6543; 1311.6450 $[M + H + Na]^{2+}$, calculated for $[C_{126}H_{187}N_{15}O_{43} + H + Na]^{2+}$ = 1311.6453; 1322.6375 $[M + 2Na]^{2+}$, calculated for $[C_{126}H_{187}N_{15}O_{43} + 2Na]^{2+}$ = 1322.6363.

The BOC-protected compound (70.0 mg, 26.9 µmol, 1 eq.) was dissolved in dichloromethane (3 ml) and treated with trifluoroacetic acid (400 µl, 592 mg, 5.19 mmol, 193 eq.). The mixture was stirred 18 hours at room temperature and then concentrated. After purification (see GP1), ligand **IT₂** was obtained as a yellow solid (18.5 mg, 6.75 µmol, 25.1%). The product was stored under argon at 2 °C to prevent oxidation.

**M($C_{96}H_{117}F_{30}N_{15}O_{43}$):** 2739.01 g/mol.

**$^1$H-NMR (600 MHz, [$D_7$]-*N,N*-dimethylformamide, 298 K):** δ [ppm] =

9.05 – 8.23 (m, >30H, RN*H*$_3$, O*H*), 8.09 (m, 1H, RN*H*), 8.06 (m, 1H, RN*H*), 7.23 (s, 1H, *H*-Ar), 7.18 (s, 1H, *H*-Ar), 7.04 (s, 1H, *H*-Ar), 6.87 (s, 1H, *H*-Ar),6.26 (br s, 2H, O*H*), 6.22 (d, 2H, *H*-T1´), 5.18 (d, $^3J_{HH}$ = 3.9 Hz, 2H, *H*-T1´´), 5.14 (m, 2H, *H*-I1/I3), 4.65 (m, 2H, *H*-I3/I1), 4.21 (dd, $^3J_{HH}$ = 10.7 Hz, $^3J_{HH}$ = 9.8 Hz, 2H, *H*-T4), 4.15 (m, 2H, *H*-T5´´/*H*-T5´), 4.03 (m, 4H, *H*-T5´/*H*-T5´´, *H*-T5/*H*-T2´´), 3.89 (m, 4H, *H*-T2´´/*H*-T5, *H*-T6), 3.76 – 3.48 (m, 18H, *H*-T6´´, *H*-T1, *H*-T3, *H*-T4´, H-T2´, H-T6´, H-T3´´, H-T4´´, overlapping with water signal),

3.42 (m, 2H), 3.31 (m, 1H), 3.22 (m, 1H), 3.16 (m, 2H), 2.81 (m, 2H, *H*-E6), 2.73 (m, 2H, *H*-I15/I16, overlapping with DMF signal), 2.65 (m, 2H, *H*-I16/I15), 2.53 (m, 2H, *H*-T3´äq), 2.32 – 2.14 (m, 5H), 1.91 – 1.84/ 1.77 – 1.61/ 1.51 – 1.45/ 1.39 – 1.29/ 1.25 – 1.20/ 1.15 – 1.08 (6 m, 13H), 0.76 (s, 3H, *H*-E18).[5]

**$^{13}C\{^{1}H\}$-NMR (151 MHz, [$D_7$]-*N,N*-dimethylformamide, 298 K):** δ [ppm] =
174.8 (N*C*OR), 172.5 (N*C*OR), 172.4 (N*C*OR), 160.6 (q, $^{2}J_{CF}$ = 31.9 Hz, $CF_3CO_2H$), 140.3 (*C*q), 140.2 (*C*q), 140.2 (2 *C*q), 139.6 (*C*q), 139.6 (*C*q), 139.3 (*C*q), 138.0 (*C*q), 137.9 (*C*q), 135.4 (*C*q), 135.3 (*C*q), 134.6 (*C*q), 118.03 (q, $^{1}J_{CF}$ = 298.1 Hz, $CF_3CO_2H$), 117.0 (*C*H), 114.2 (*C*H), 112.3 (*C*H), 112.1 (*C*H), 110.3 (2 *C*q), 101.7 (*C*H), 101.6 (*C*H), 94.4 (2 *C*q), 94.1 (*C*H), 93.7 (*C*H), 85.4 (*C*H), 85.3 (*C*H), 77.6 (*C*H), 77.2 (*C*H), 75.8 (*C*H), 75.6 (*C*H), 72.6 (*C*H), 72.3 (*C*H), 71.2 (2 *C*H), 69.8 (2 *C*H), 68.2 (*C*H), 68.2 (*C*H), 66.4 (2 *C*H), 56.7 (*C*H), 56.5 (*C*H), 53.9 (*C*-E14), 52.8 ($CH_2$), 52.4 ($CH_2$), 51.5 (*C*H), 51.4 (*C*H), 50.1 (*C*H), 49.5 (*C*H), 49.3 (*C*H), 44.6 (*C*-E9), 41.9 ($CH_2$), 41.8 ($CH_2$), 41.5 (*C*-E13), 40.9 (*C*-E17), 40.6 ($CH_2$), 39.2 (*C*-E8), 39.1 (*C*-E12), 38.5 ($CH_2$), 38.5 ($CH_2$), 36.7 (*C*H), 31.6 ($CH_2$), 31.5 ($CH_2$), 30.7 (*C*H), 29.8 ($CH_2$), 29.3 (*C*-E6), 28.2 (*C*-E7), 27.1 (*C*-E11), 25.6 (*C*-E15), 20.9, (*C*-E16), 17.7 (*C*-E18).

**HR-MS (ESI-pos, 70 eV):**[6] **m/z =**
1598.7753 $[M + H]^+$, calculated for $[C_{76}H_{107}N_{15}O_{23} + H]^+$ = 1598.7737; 799.8919 $[M + 2H]^{2+}$, calculated for $[C_{76}H_{107}N_{15}O_{23} + 2H]^{2+}$ = 799.8905; 533.5971 $[M + 3H]^{3+}$, calculated for $[C_{76}H_{107}N_{15}O_{23} + 3H]^{3+}$ = 533.5961; 400.4492 $[M + 4H]^{4+}$, calculated for $[C_{76}H_{107}N_{15}O_{23} + 4H]^{4+}$ = 400.4489.

**IR:** $\tilde{\nu}$ [$cm^{-1}$] = 3339, 3076, 2924, 2872, 2243, 1670, 1630, 1535, 1503, 1454, 1356, 1308, 1288, 1265, 1192, 1128, 1051, 1016, 839, 799, 723.

**MS/MS analysis (ESI-pos, 40 eV): m/z =**
For MS/MS analysis (see Figure S24), parent ion [P]: $[M + 2H]^{2+}$ = 799.8919, calculated for $[C_{76}H_{107}N_{15}O_{23} + 2H]^{2+}$ = 799.8905) was fragmented. Following structural formulas are envisioned to represent the observed fragments (Figure S6):

[5] OH-signals are less observable due to fast exchange, hence integration values deviate from expected number.
[6] Due to measurement disturbances of the TFA salt, an ion exchange with hydrochloric acid was performed to yield the corresponding chlorid salt for HR-MS analysis

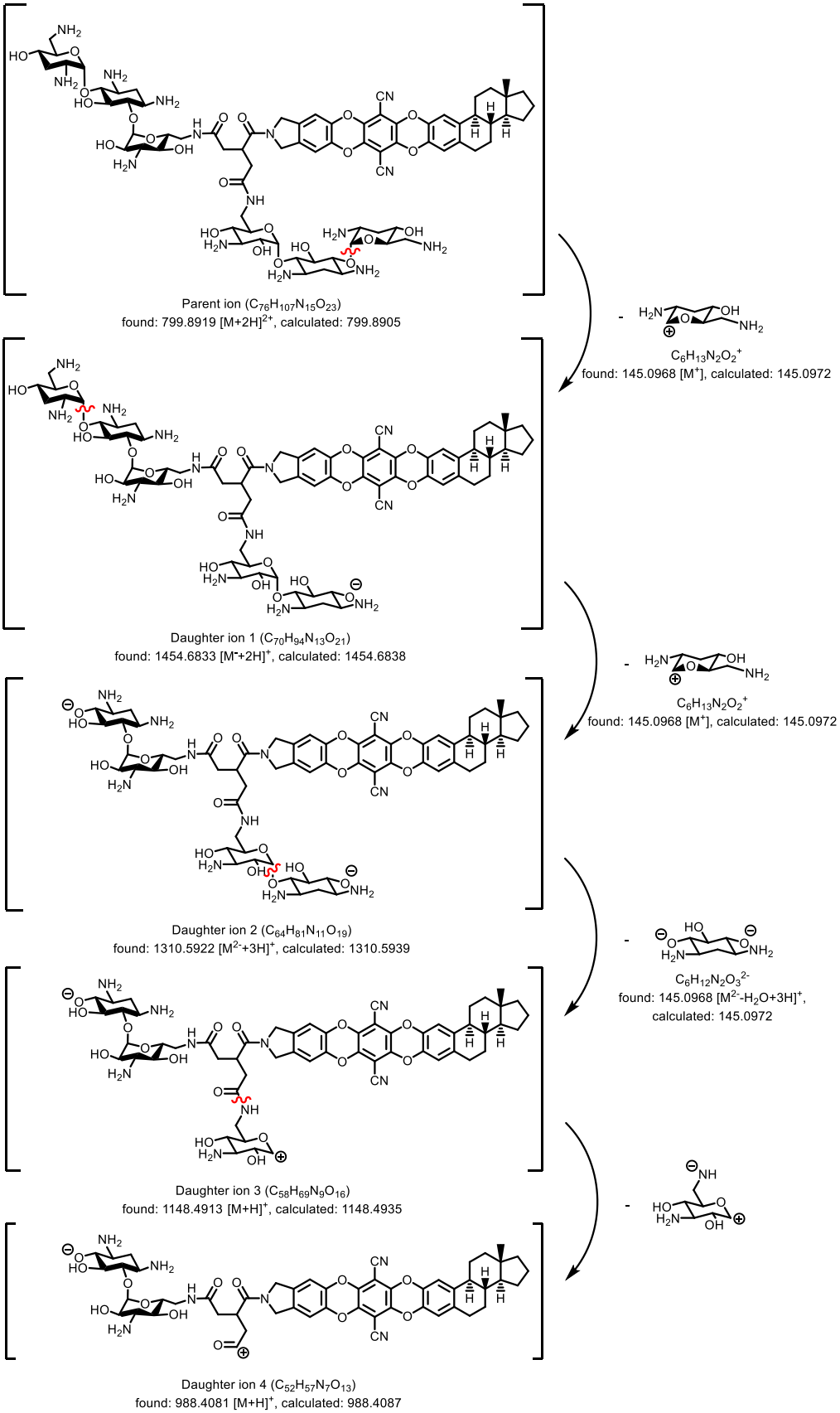



**Figure S6:** Fragments of MS/MS analysis for ligand **$IT_2$.**

## NMR SPECTRA

### *1.1.1.16 COMPOUND 6*

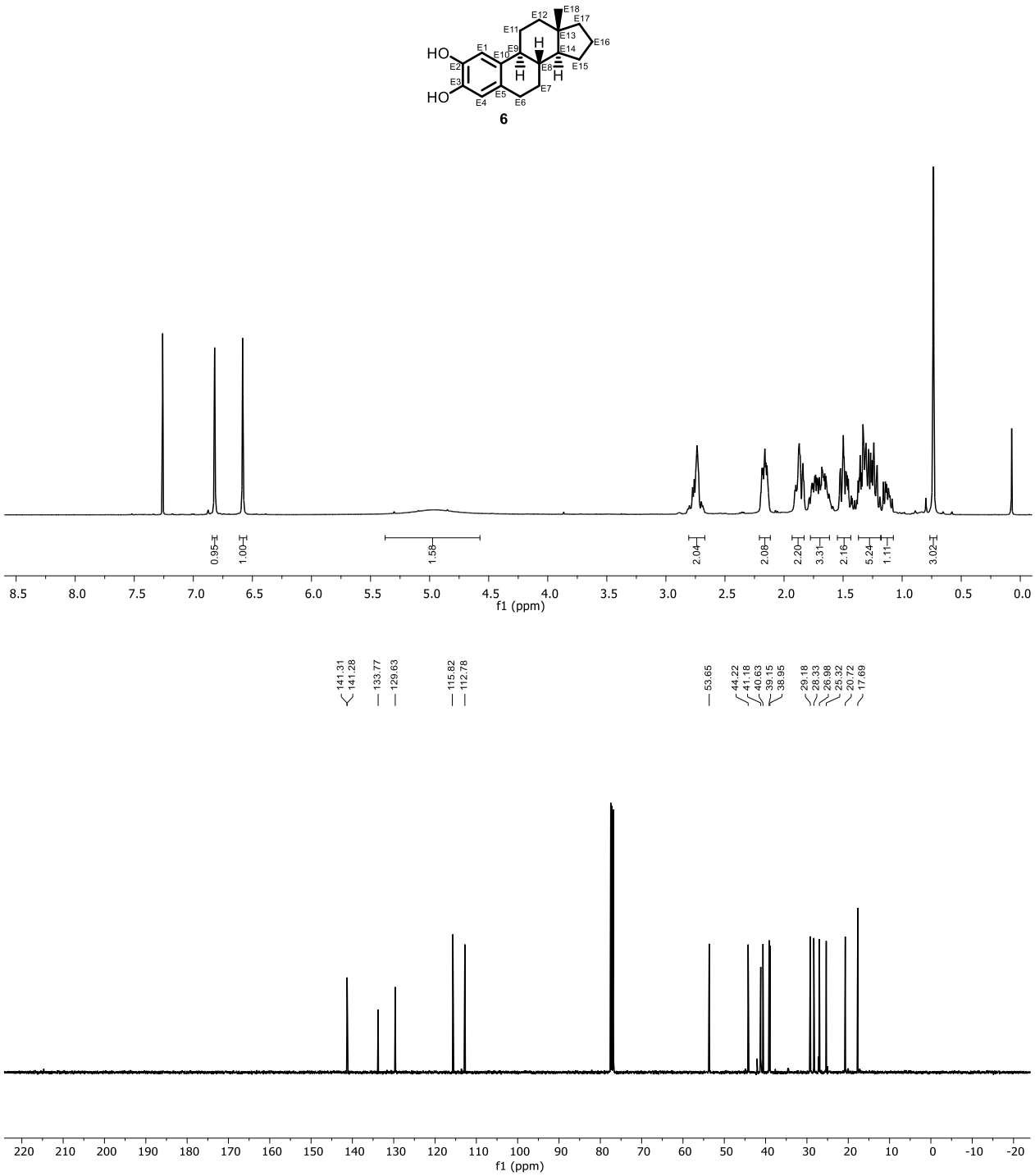



**Figure S7:** $^{1}$H- (top, 400 MHz, $CDCl_3$, 298 K) and $^{13}$C-NMR spectrum (bottom, 101 MHz, $CDCl_3$, 298 K) of compound **6**.

### *1.1.1.17 COMPOUND 13*

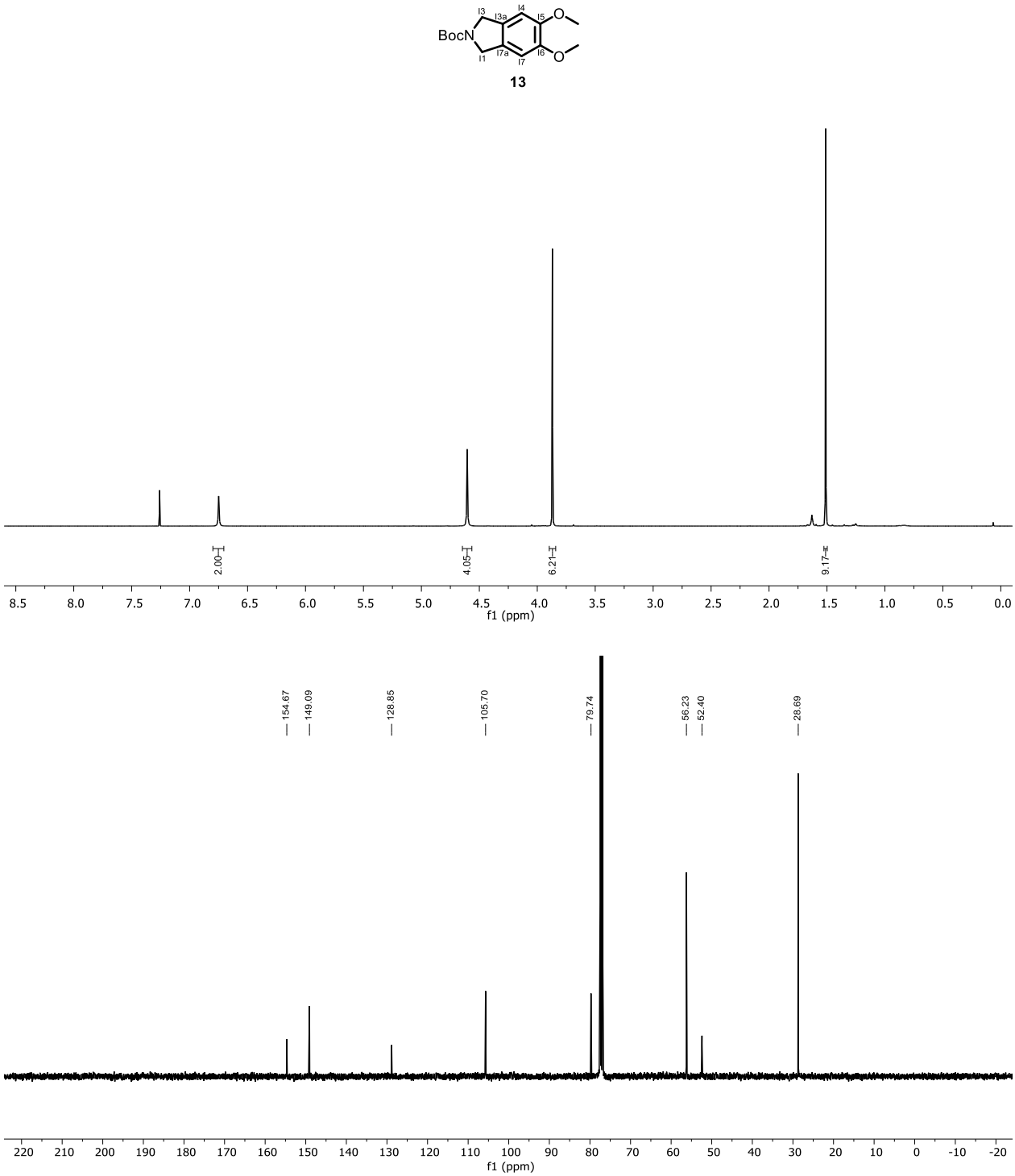



**Figure S8:** $^{1}$H- (top, 400 MHz, $CDCl_3$, 298 K) and $^{13}$C-NMR spectrum (bottom, 101 MHz, $CDCl_3$, 298 K) of compound **13**.

### *1.1.1.18 COMPOUND 14*

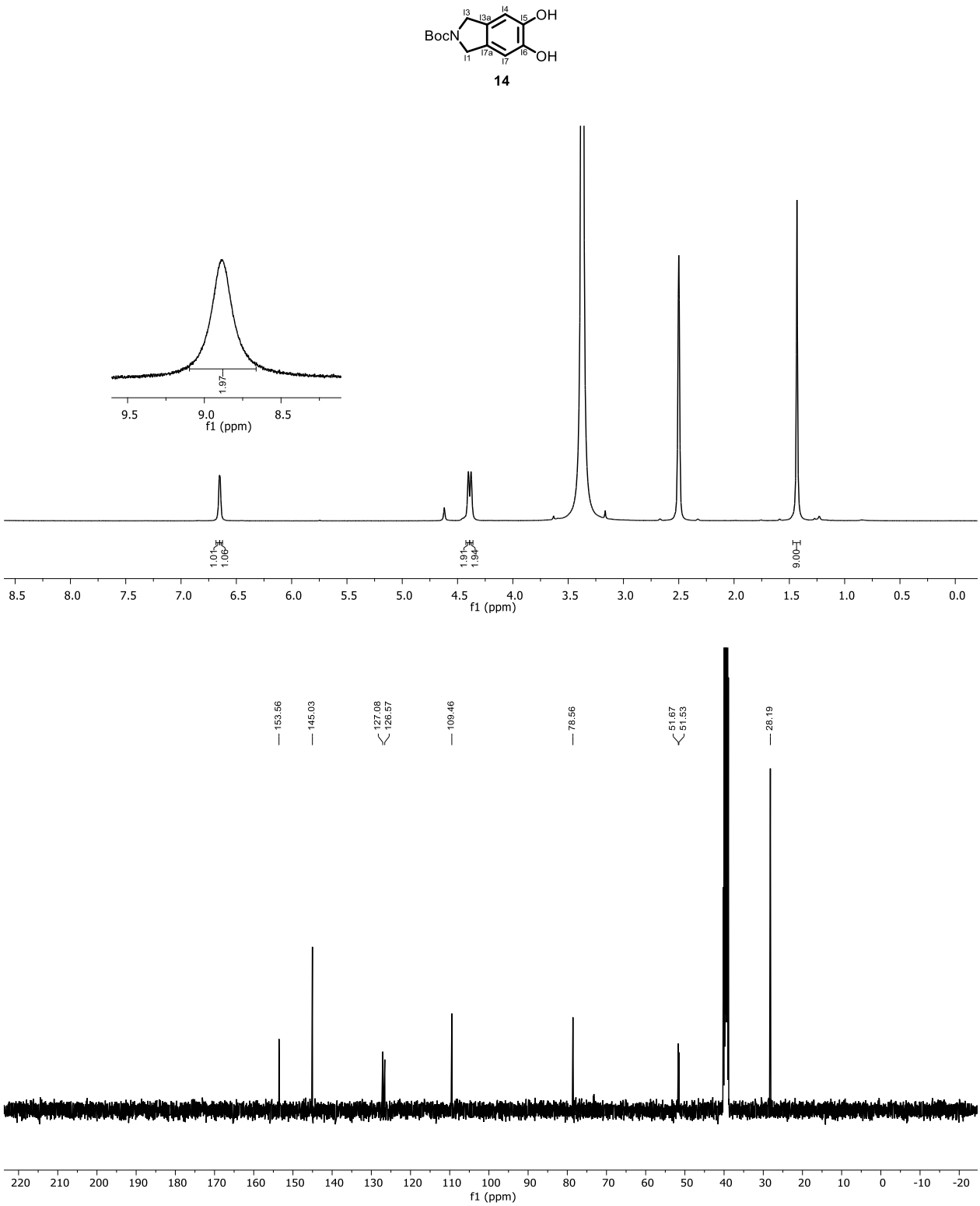
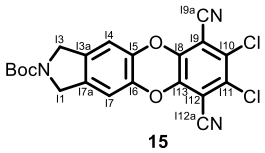



**Figure S9:** $^{1}$H- (top, 400 MHz, DMSO-$d_6$, 298 K) and $^{13}$C-NMR spectrum (bottom, 101 MHz, DMSO-$d_6$, 298 K) of compound **14**.

### *1.1.1.19 COMPOUND 15*




**15**

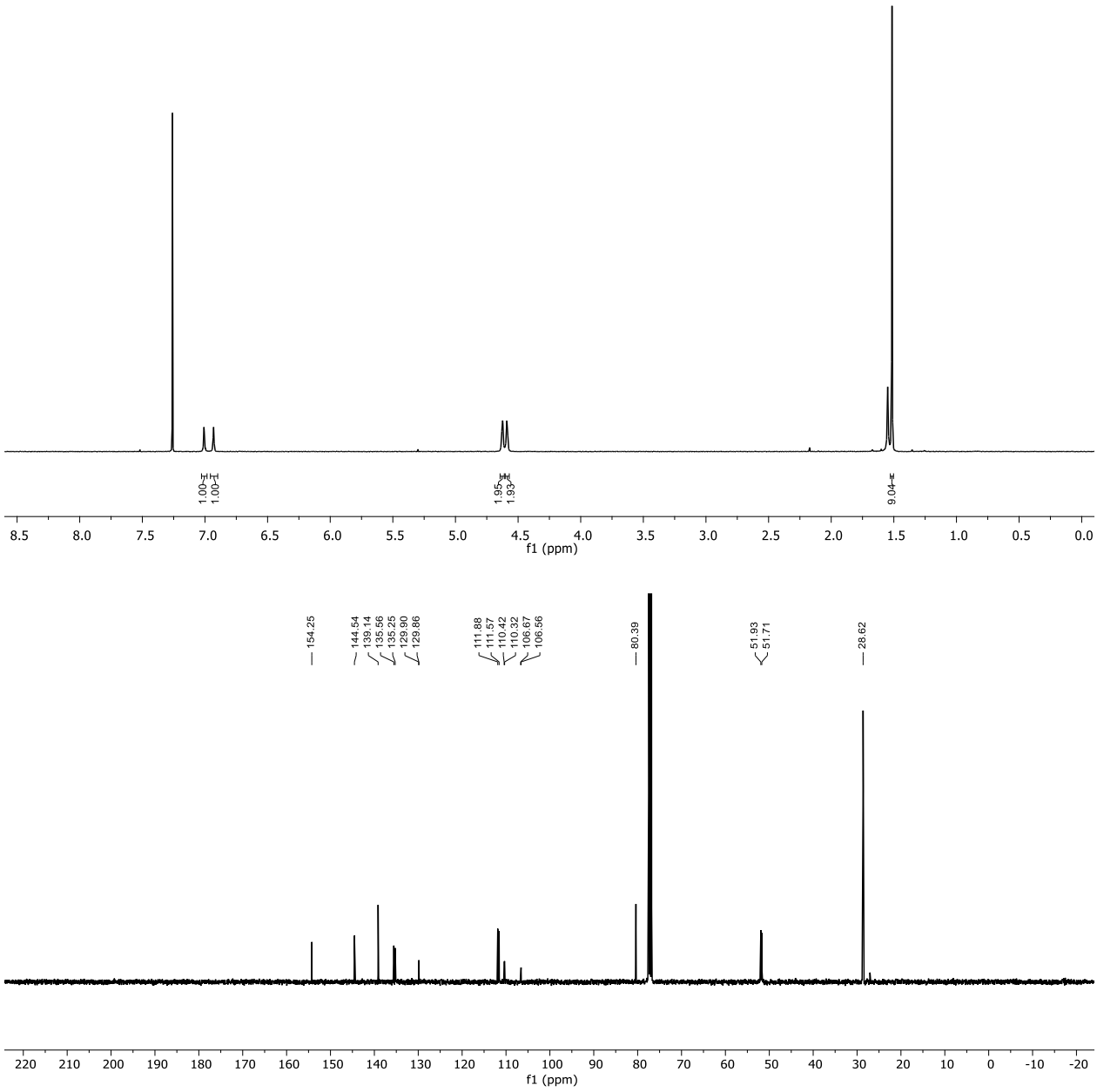
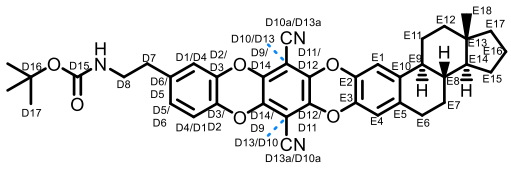
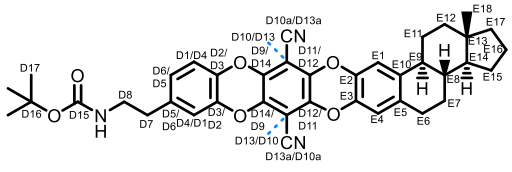



**Figure S10:** $^{1}$H- (top, 400 MHz, $CDCl_3$, 298 K) and $^{13}$C-NMR spectrum (bottom, 101 MHz, $CDCl_3$, 298 K) of compound **15**.

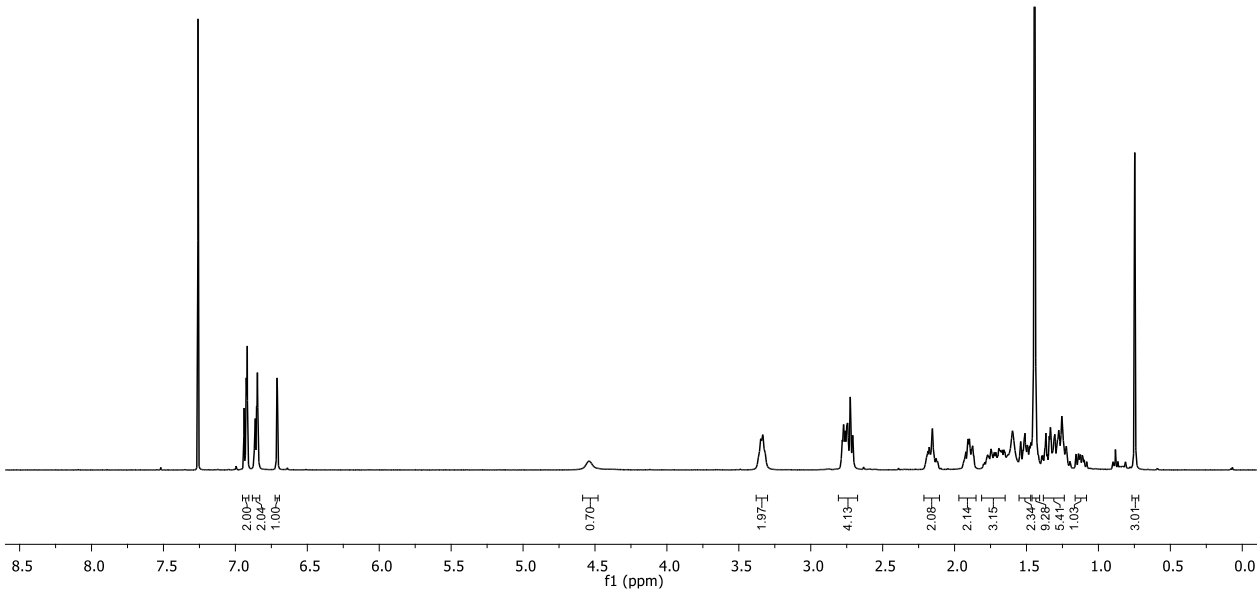
### 1.1.1.20 *COMPOUND* 16



**16a**



**16b**



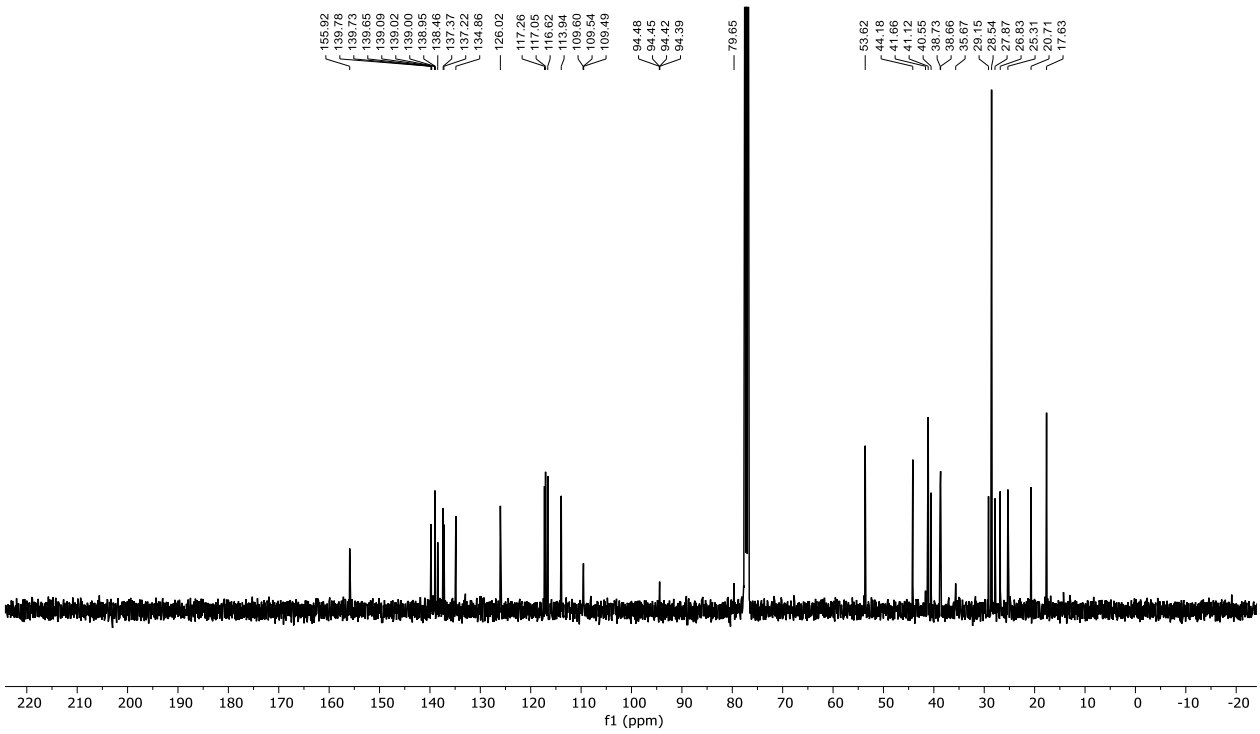
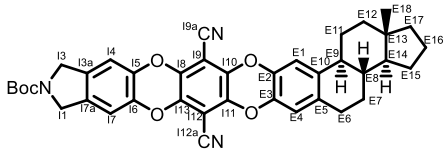




**Figure S11:** $^{1}$H- (top, 400 MHz, $CDCl_3$, 298 K) and $^{13}$C-NMR spectrum (bottom, 101 MHz, $CDCl_3$, 298 K) of compound **16**.

### *1.1.1.21 COMPOUND 17*




**17**

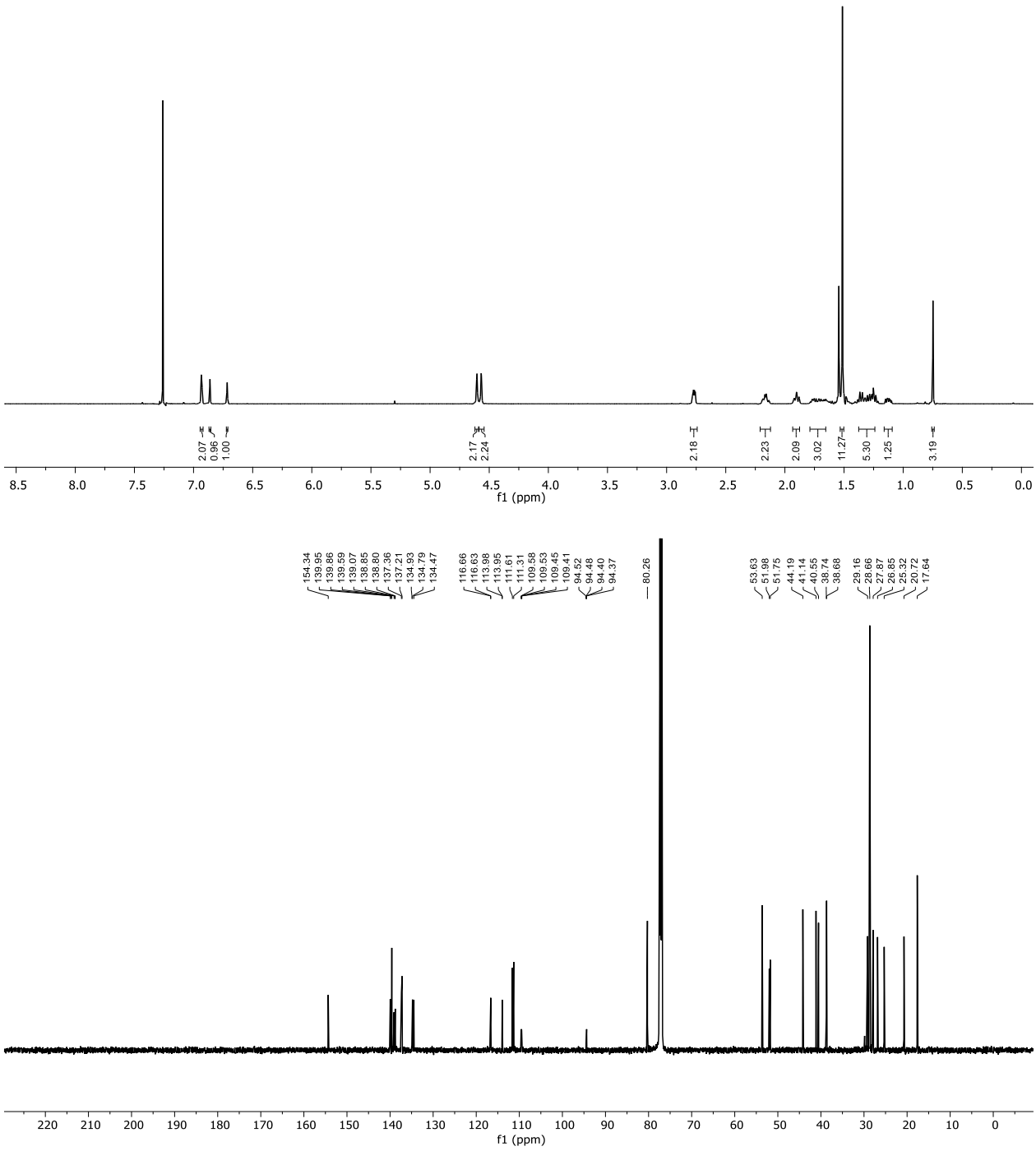
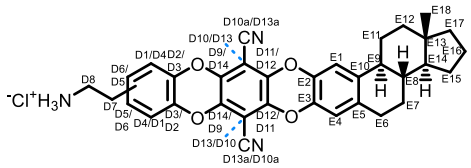
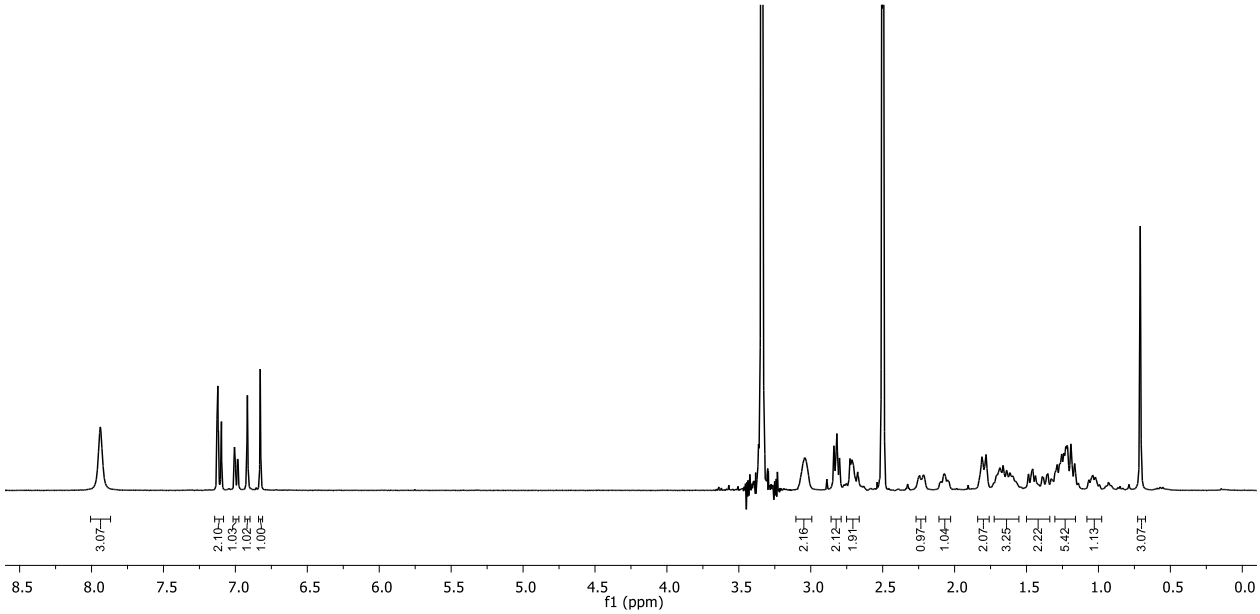



**Figure S12:** $^{1}$H- (top, 400 MHz, $CDCl_3$, 298 K) and $^{13}$C-NMR spectrum (bottom, 101 MHz, $CDCl_3$, 298 K) of compound **17**.

### *1.1.1.22 COMPOUND 18*



**18**



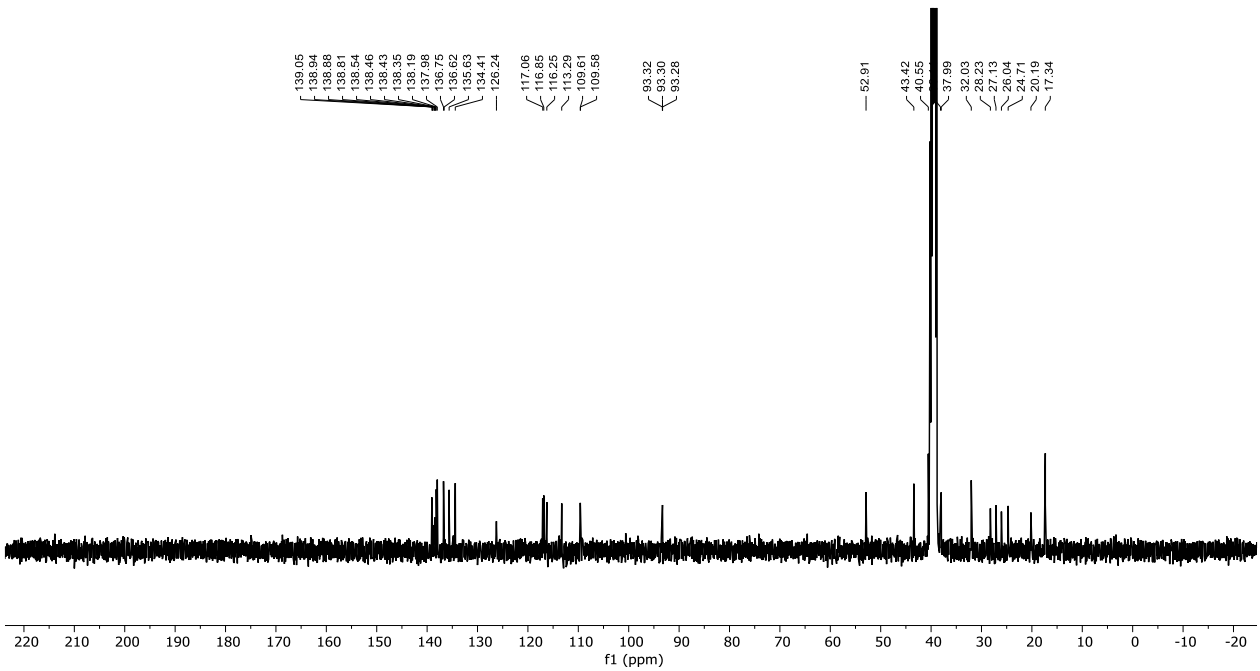




**Figure S13:** $^{1}$H- (top, 400 MHz, DMSO-$d_6$, 298 K) and $^{13}$C-NMR spectrum (bottom, 101 MHz, DMSO-$d_6$, 298 K) of compound **18**.

### *1.1.1.23 COMPOUND 19*

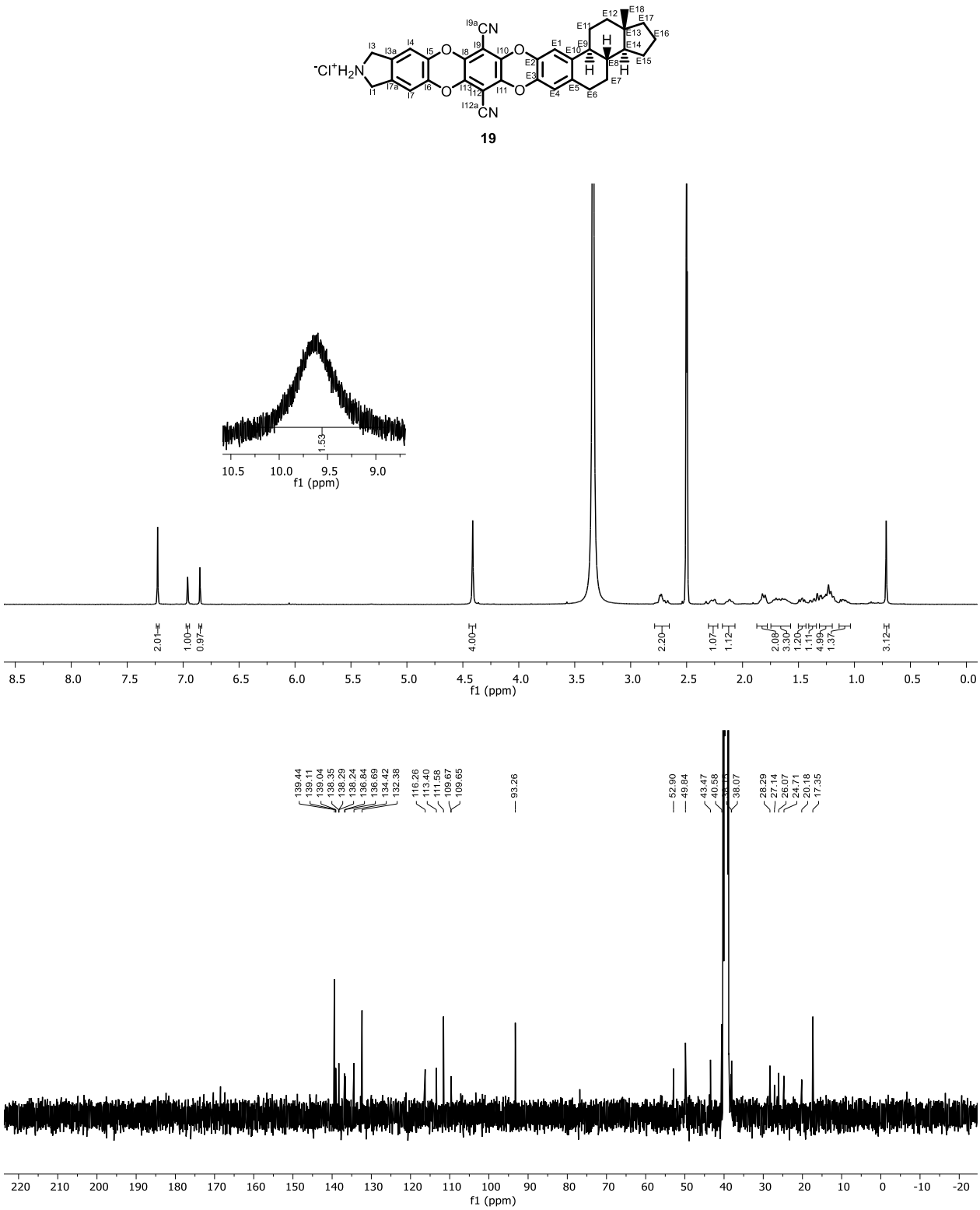



**Figure S14:** $^{1}$H- (top, 400 MHz, DMSO-$d_6$, 298 K) and $^{13}$C-NMR spectrum (bottom, 101 MHz, DMSO-$d_6$, 298 K) of compound **19**.

### *1.1.1.24 COMPOUND 20*

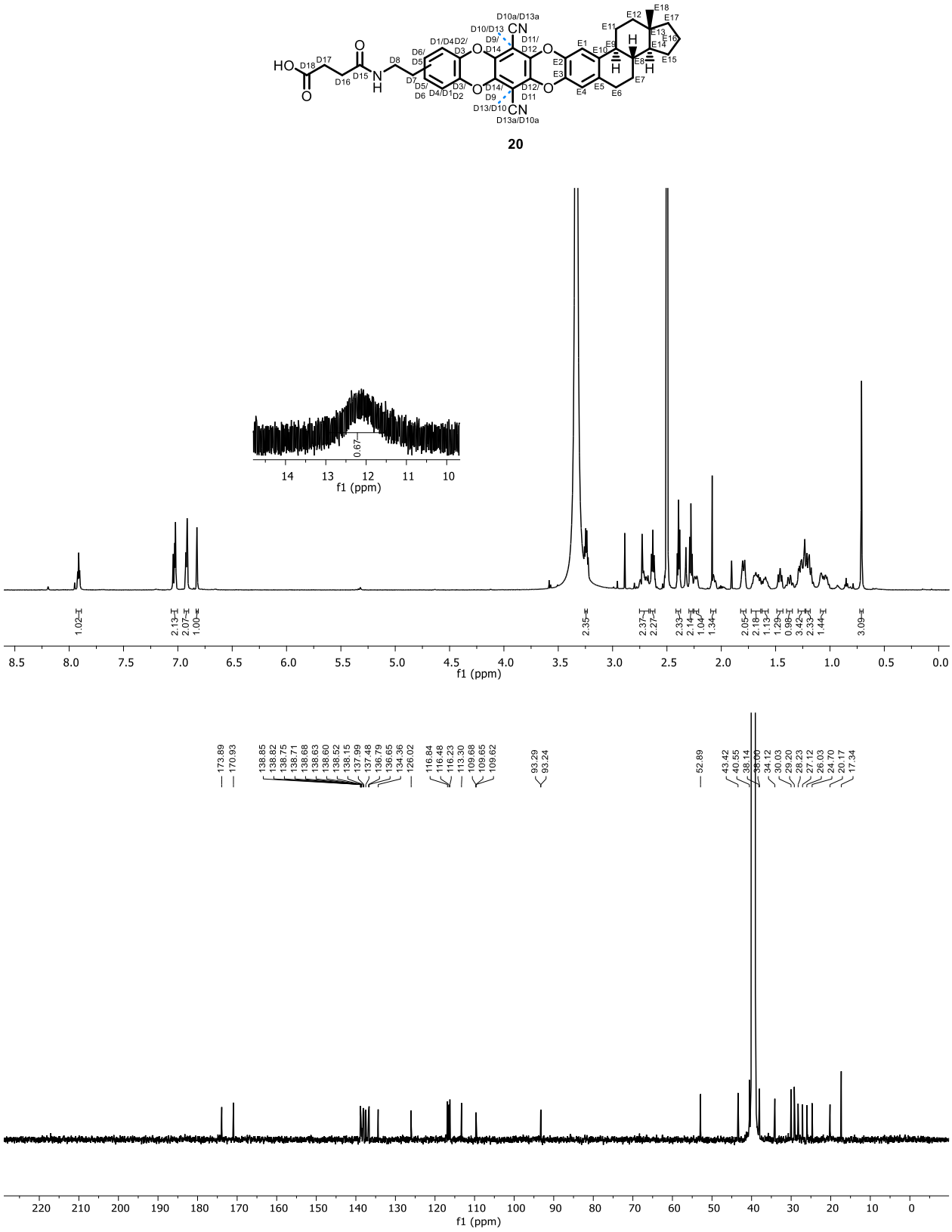



**Figure S15:** $^{1}$H- (top, 600 MHz, DMSO-$d_6$, 298 K) and $^{13}$C-NMR spectrum (bottom, 151 MHz, DMSO-$d_6$, 298 K) of compound **20**.

### *1.1.1.25 COMPOUND 21*

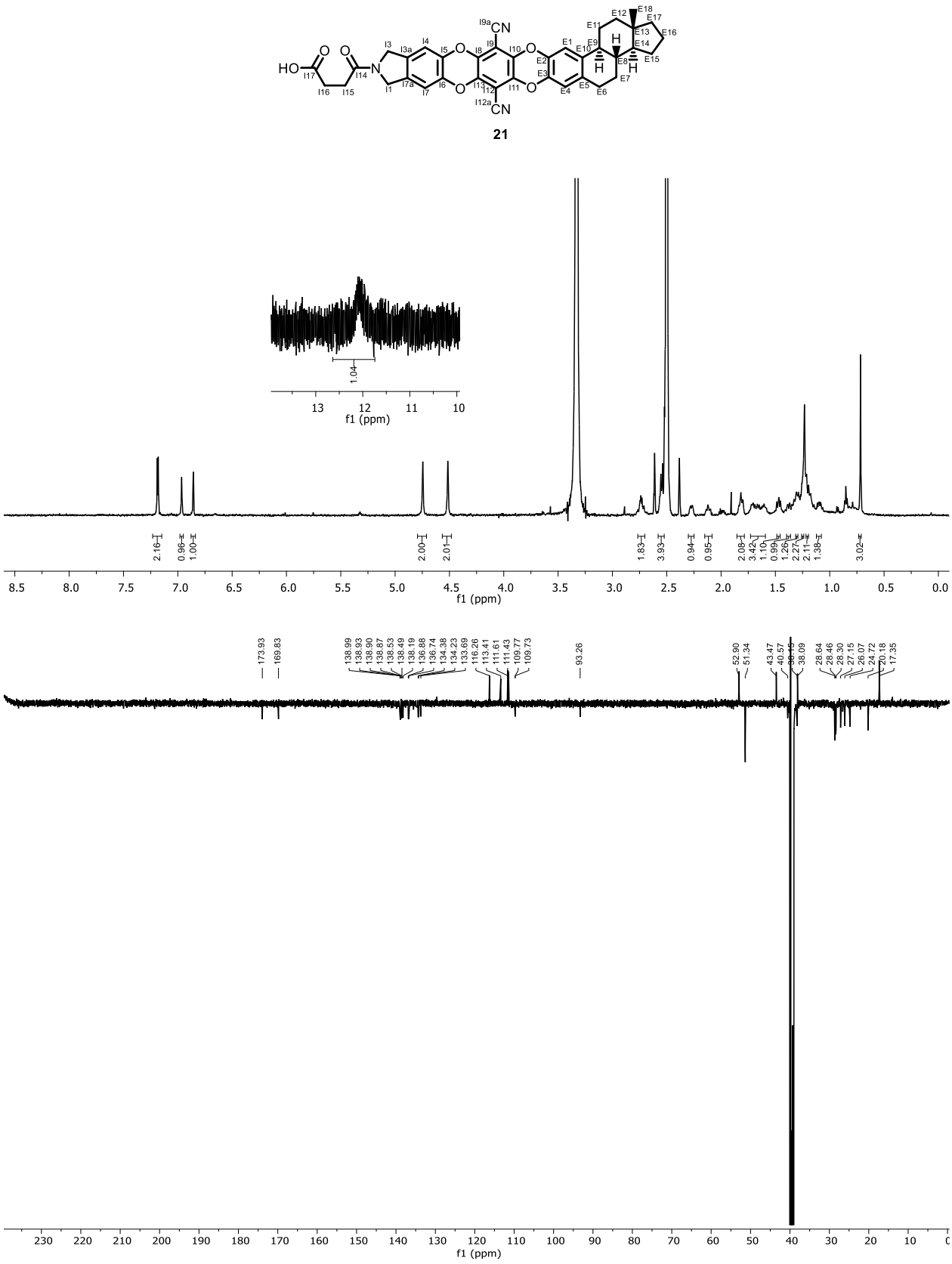



**Figure S16:** $^{1}$H- (top, 600 MHz, DMSO-$d_6$, 298 K) and DEPTQ-NMR spectrum (bottom, 151 MHz, DMSO-$d_6$, 298 K) of compound **21**.

### *1.1.1.26 COMPOUND 23*

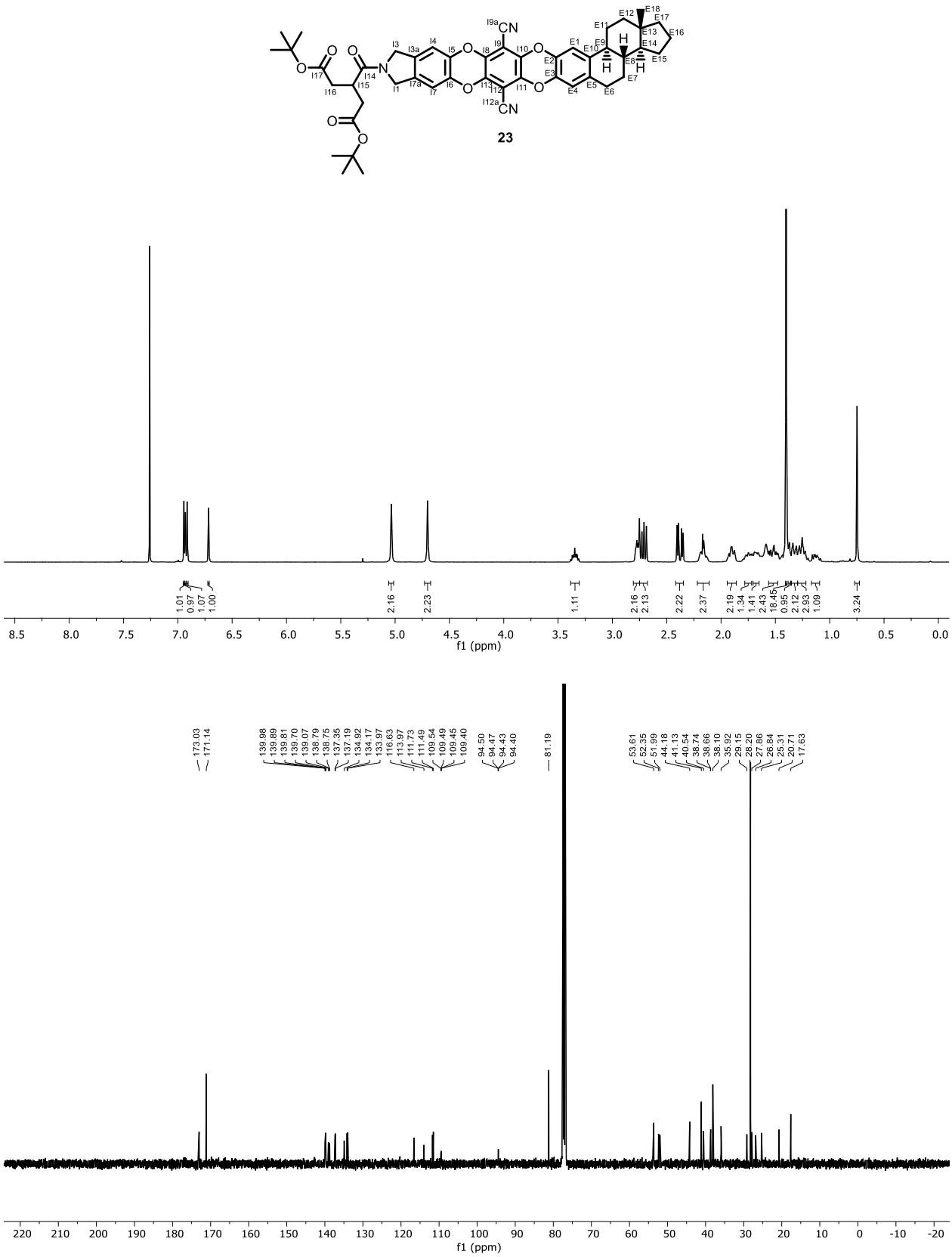



**Figure S17:** $^{1}$H- (top, 400 MHz, $CDCl_3$, 298 K) and $^{13}$C-NMR spectrum (bottom, 101 MHz, $CDCl_3$, 298 K) of compound **23**.

### *1.1.1.27 LIGAND DT*

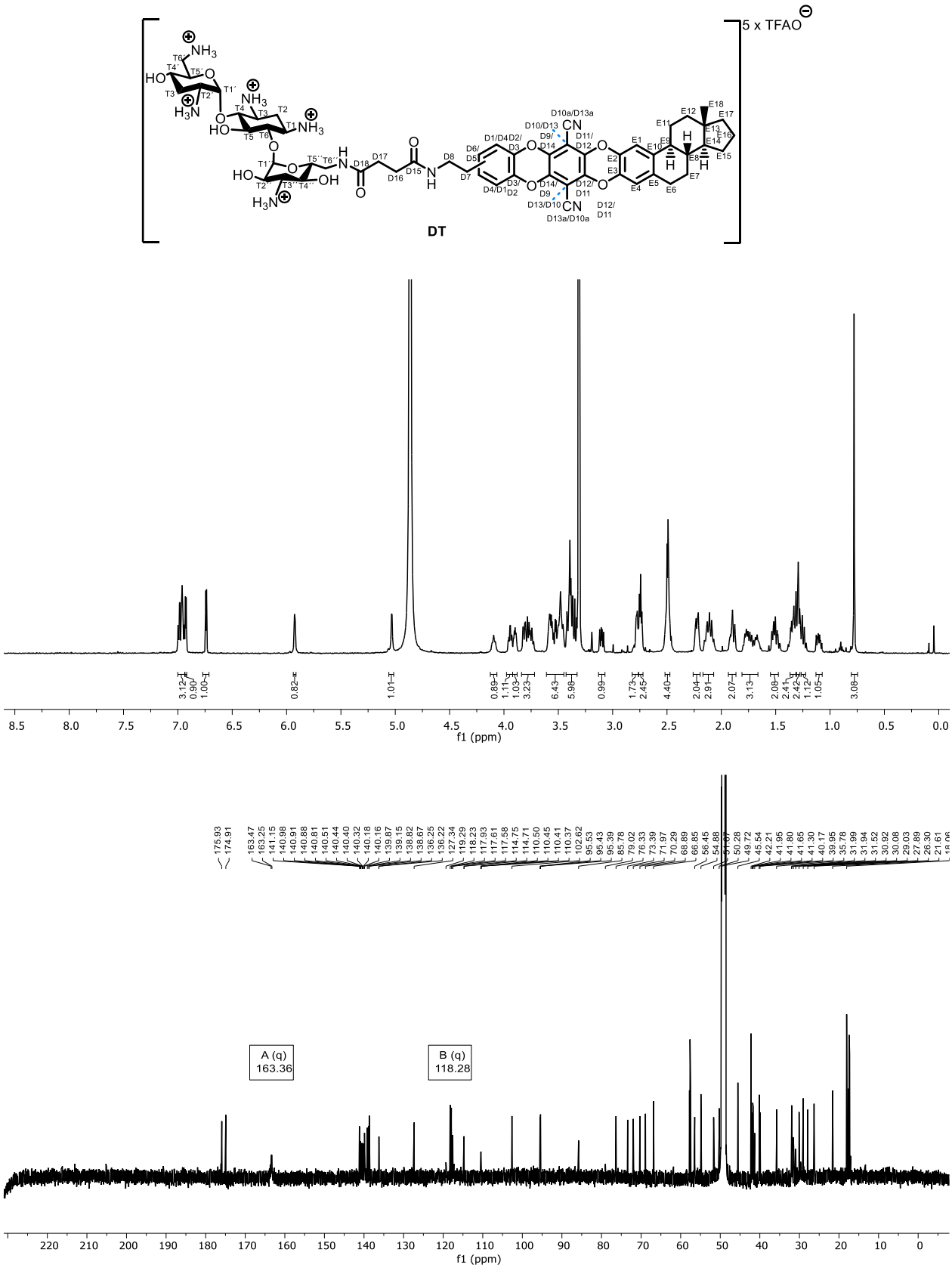



**Figure S18:** $^{1}$H- (top, 600 MHz, MeOD-d$_4$, 298 K) and $^{13}$C-NMR spectrum (bottom, 151 MHz, MeOD-d$_4$, 298 K) of ligand **DT**.

### *1.1.1.28 LIGAND IT*

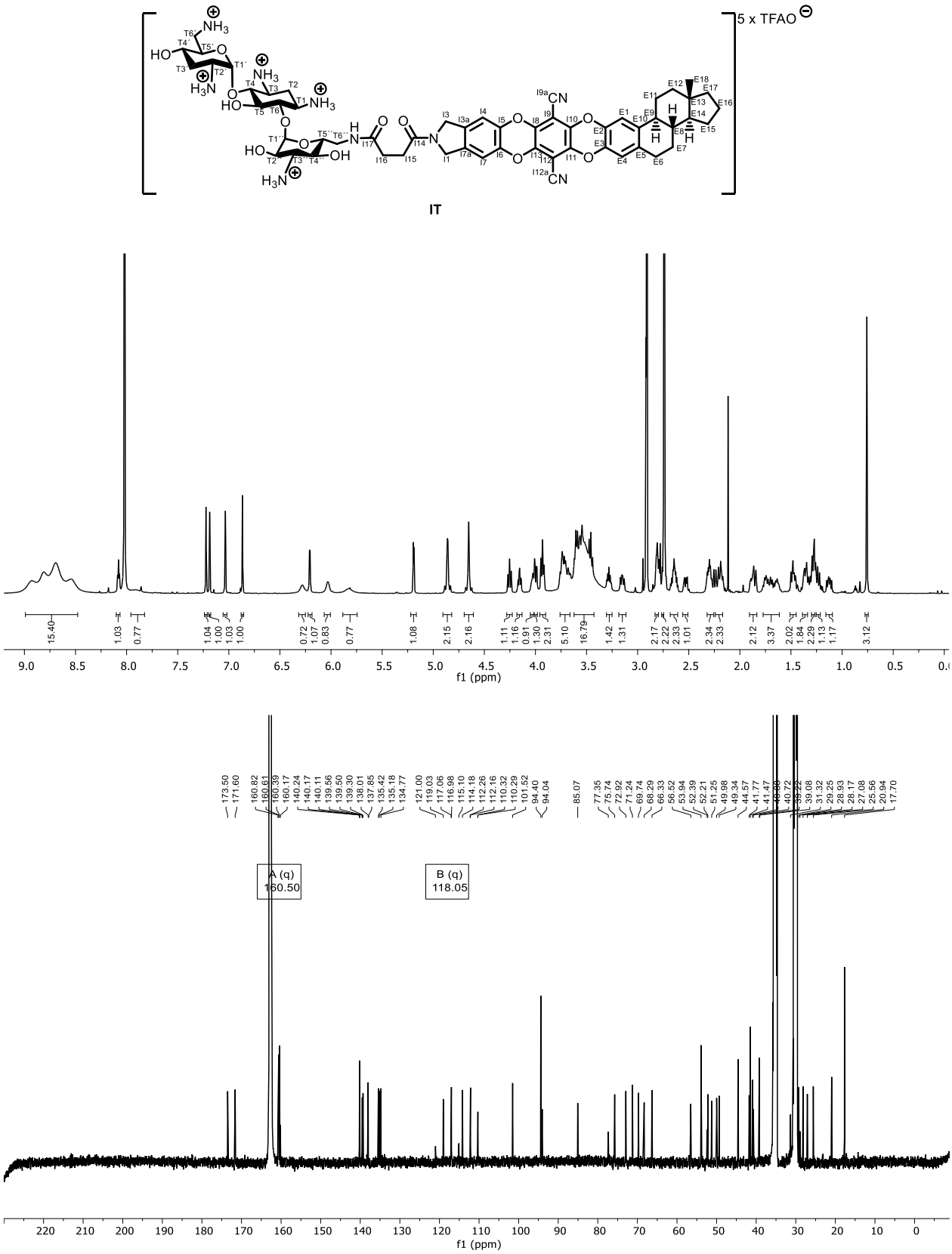
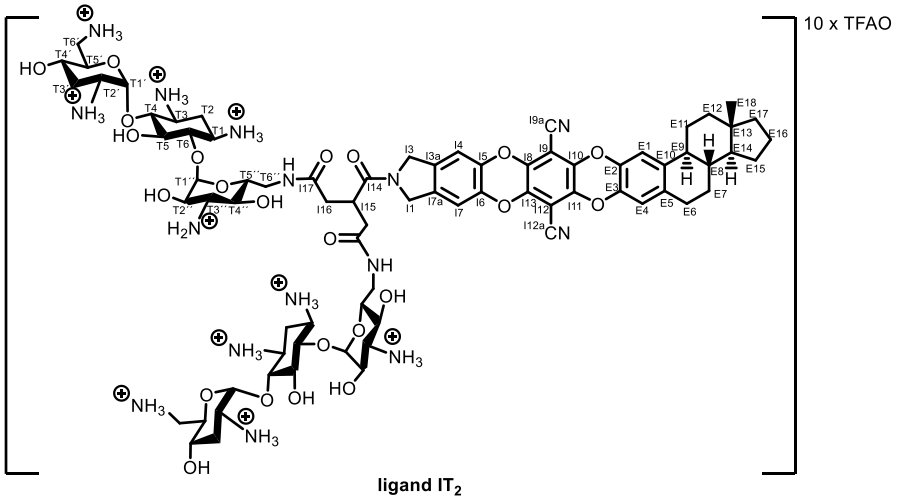
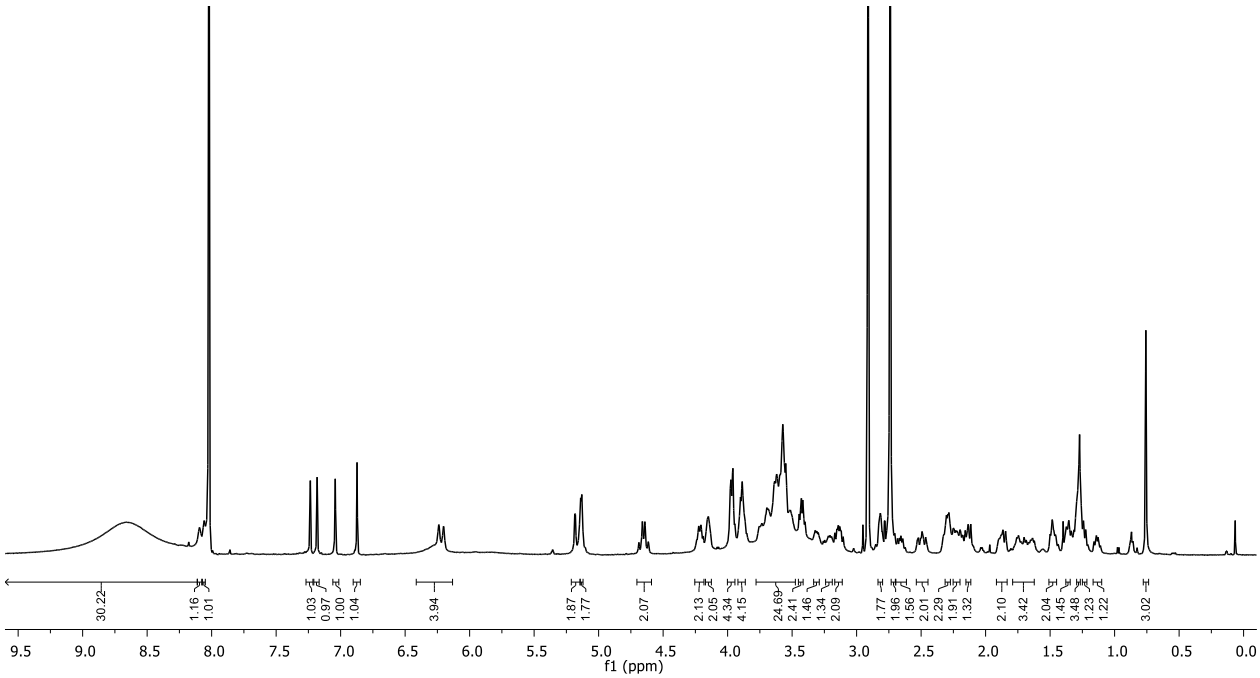



**Figure S19:** $^{1}$H- (top, 600 MHz, DMF-$d_7$, 298 K) and $^{13}$C-NMR spectrum (bottom, 151 MHz, DMF-$d_7$, 298 K) of ligand **IT**.

### 1.1.1.29 LIGAND $IT_2$




ligand $IT_2$



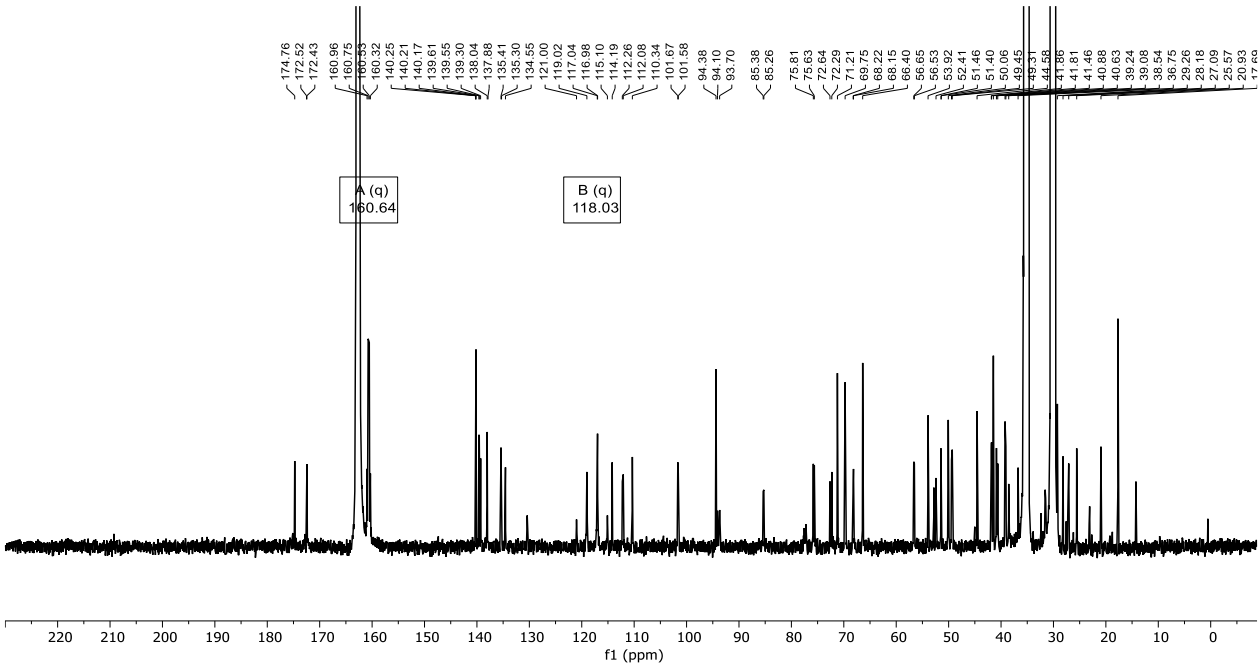



**Figure S20:** $^{1}$H- (top, 600 MHz, DMF-$d_7$, 298 K) and $^{13}$C-NMR spectrum (bottom, 151 MHz, DMF-$d_7$, 298 K) of ligand **$IT_2$**.

## MASS SPECTRA

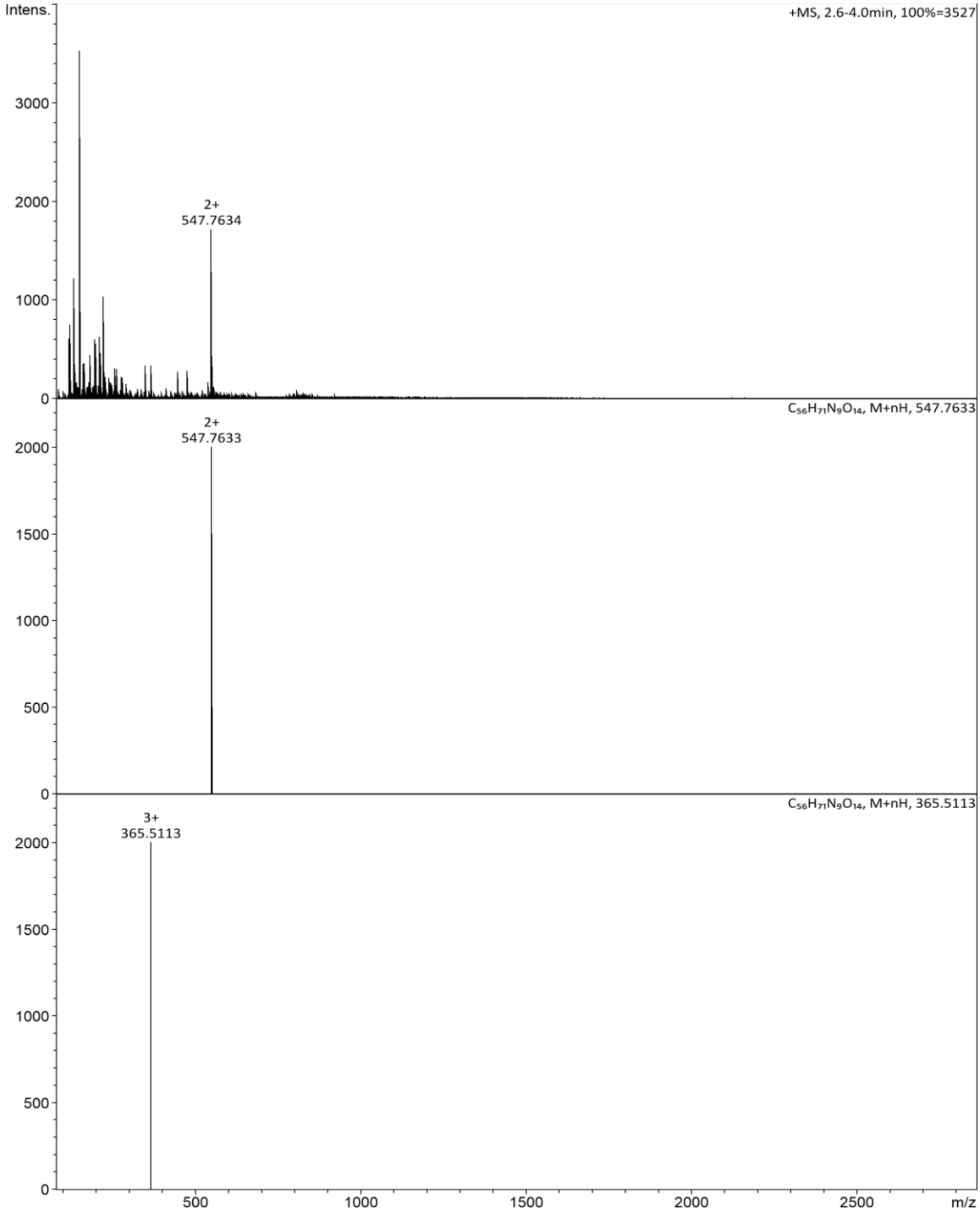



**Figure S21:** HR-MS spectrum of ligand **DT.**

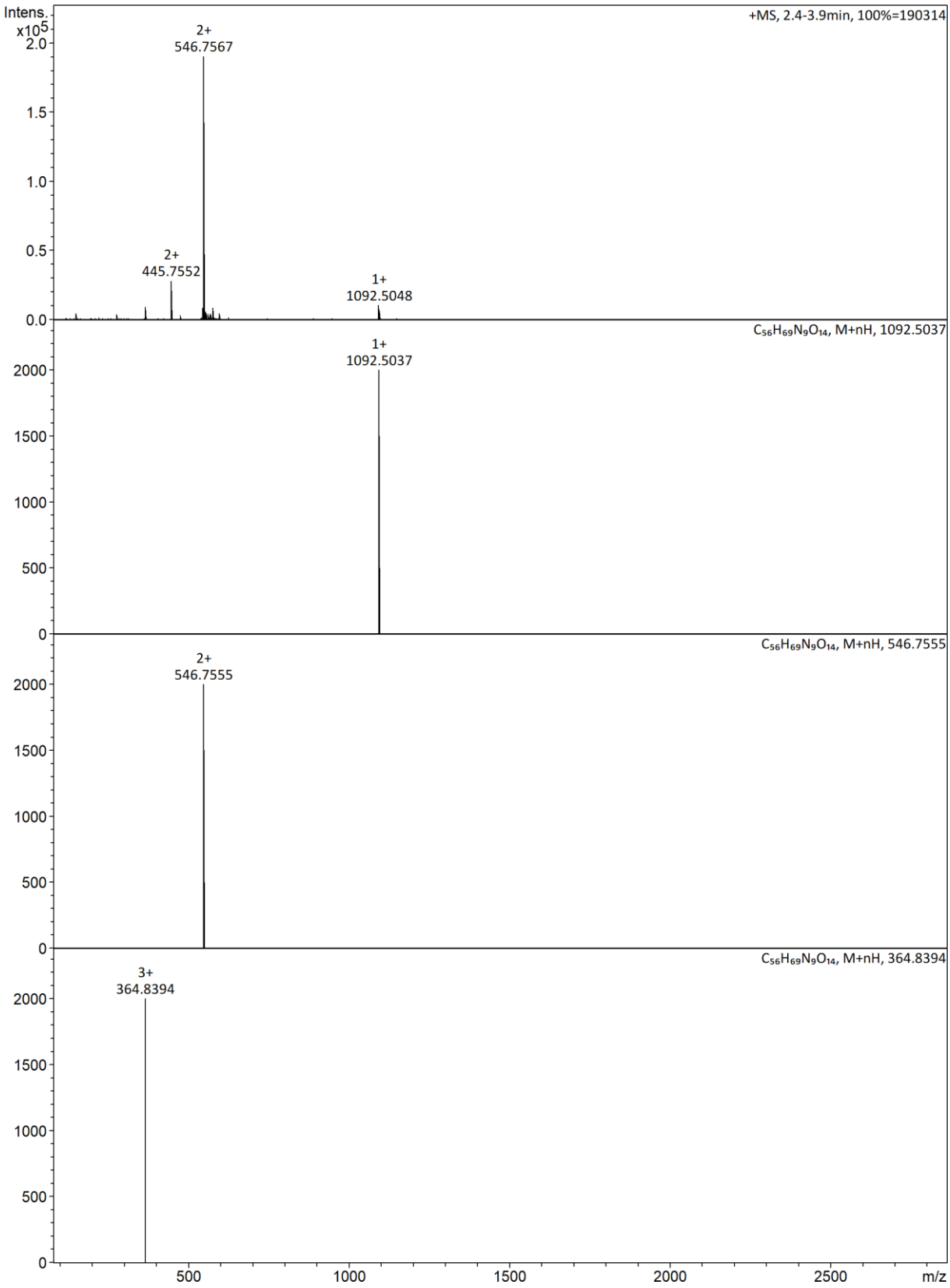



**Figure S22:** HR-MS spectrum of ligand **IT.**

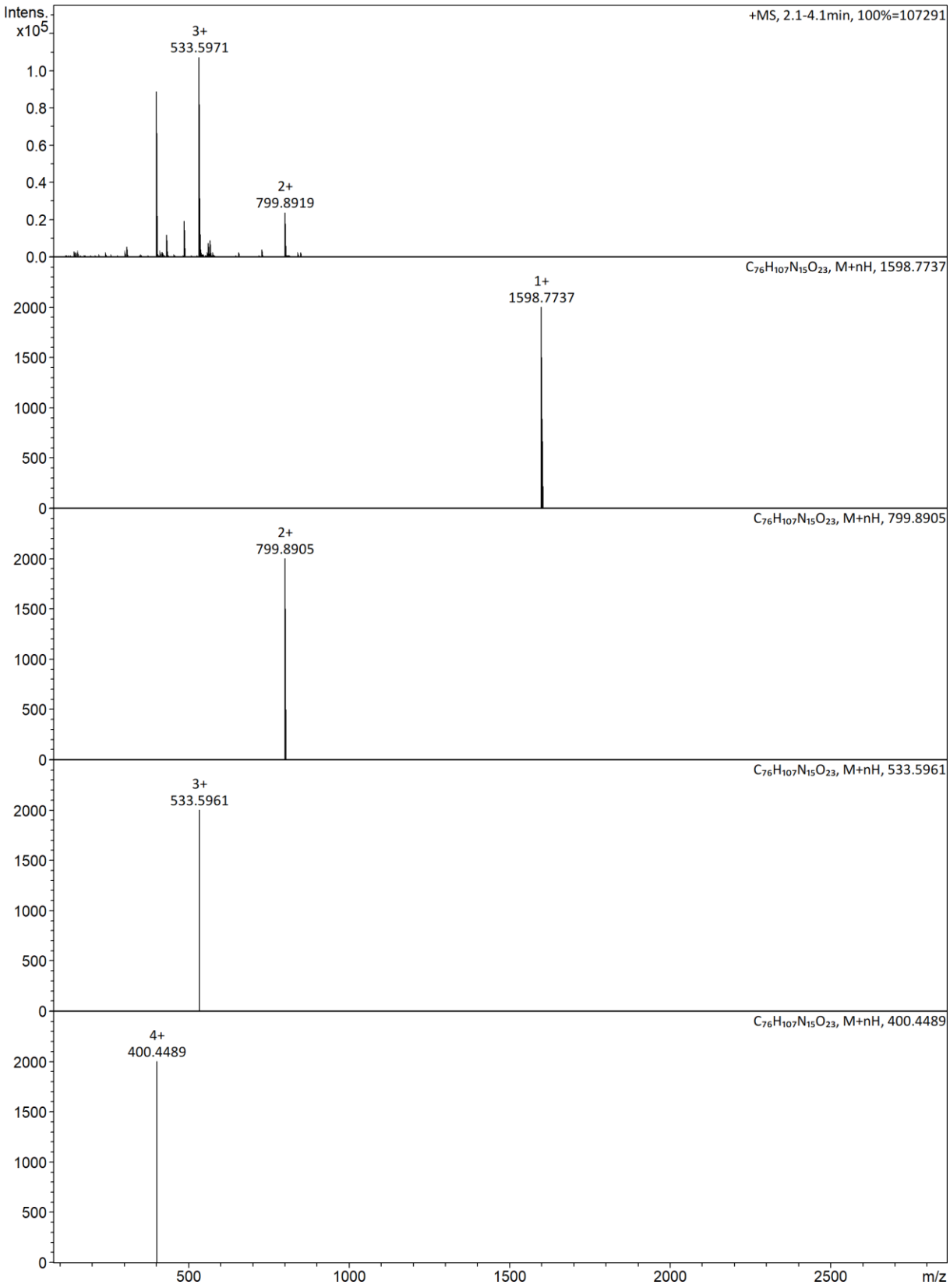



**Figure S23:** HR-MS spectrum of ligand **$IT_2$.**

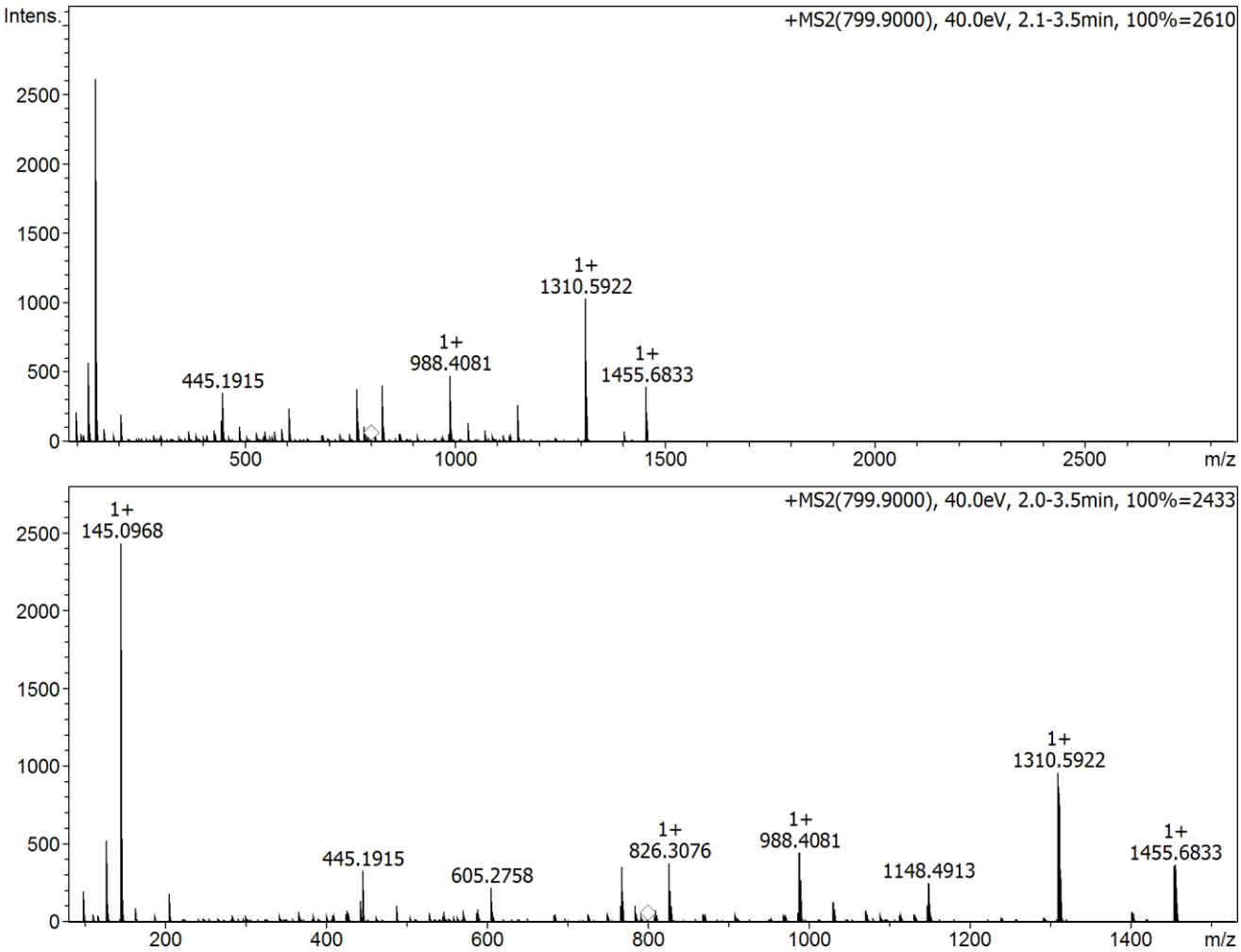



**Figure S24:** MS/MS analysis for ligand **$IT_2$.**

## HPLC RUNS

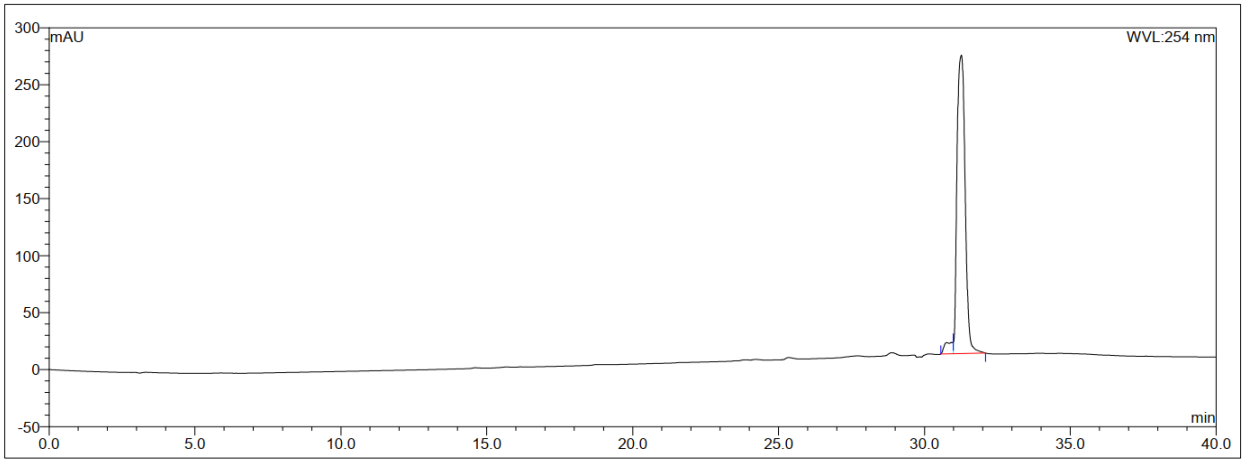



**Figure S25:** HPLC chromatogram of ligand **DT**.

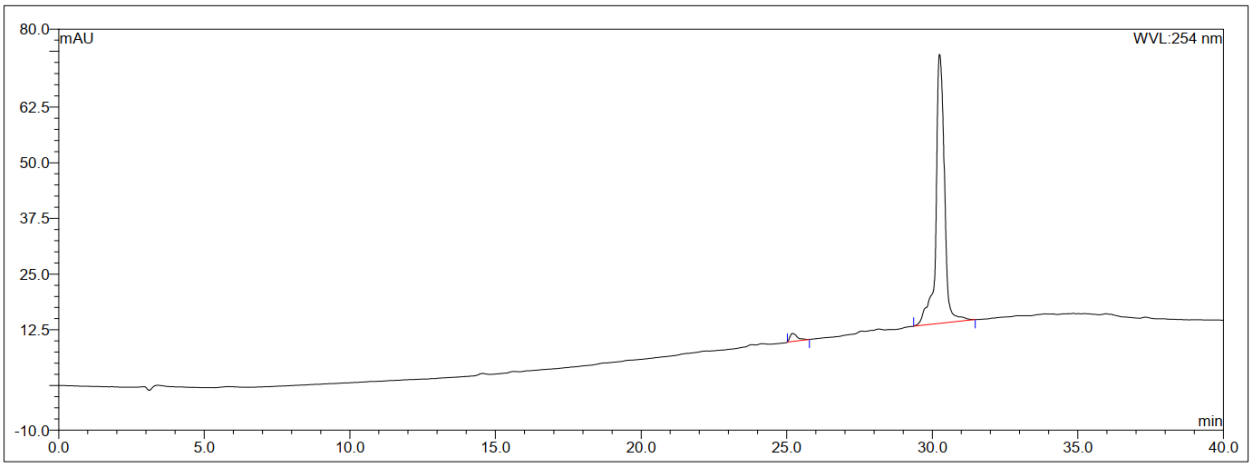



**Figure S26:** HPLC chromatogram of ligand **IT**.

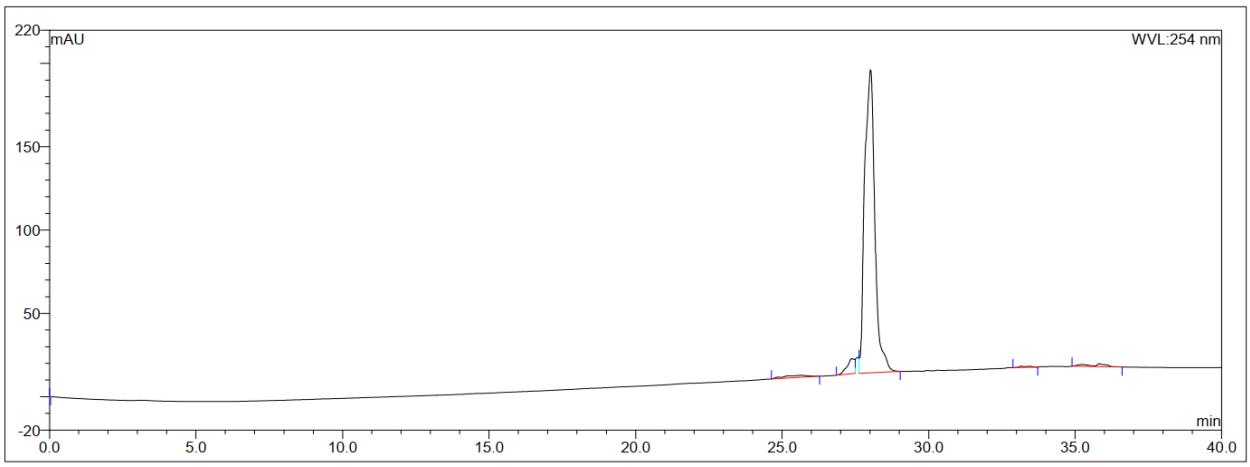



**Figure S27:** HPLC chromatogram of ligand **$IT_2$**.

## ζ-POTENTIAL

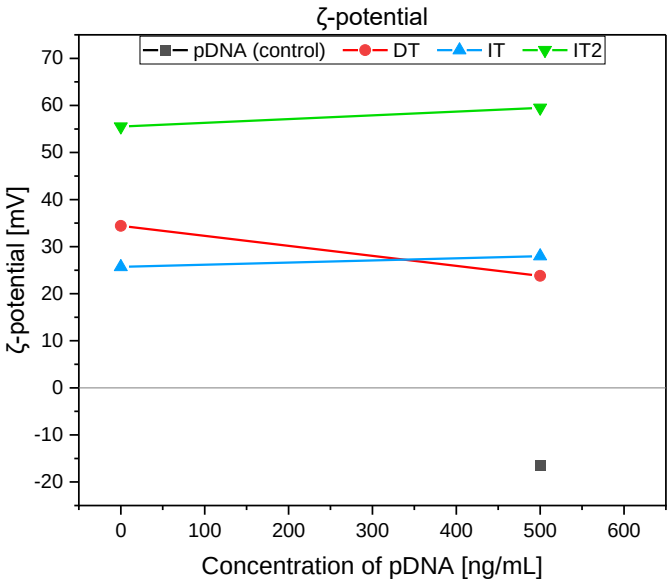



**Figure S28:** ζ-potential measurement**.**

## TEM IMAGES

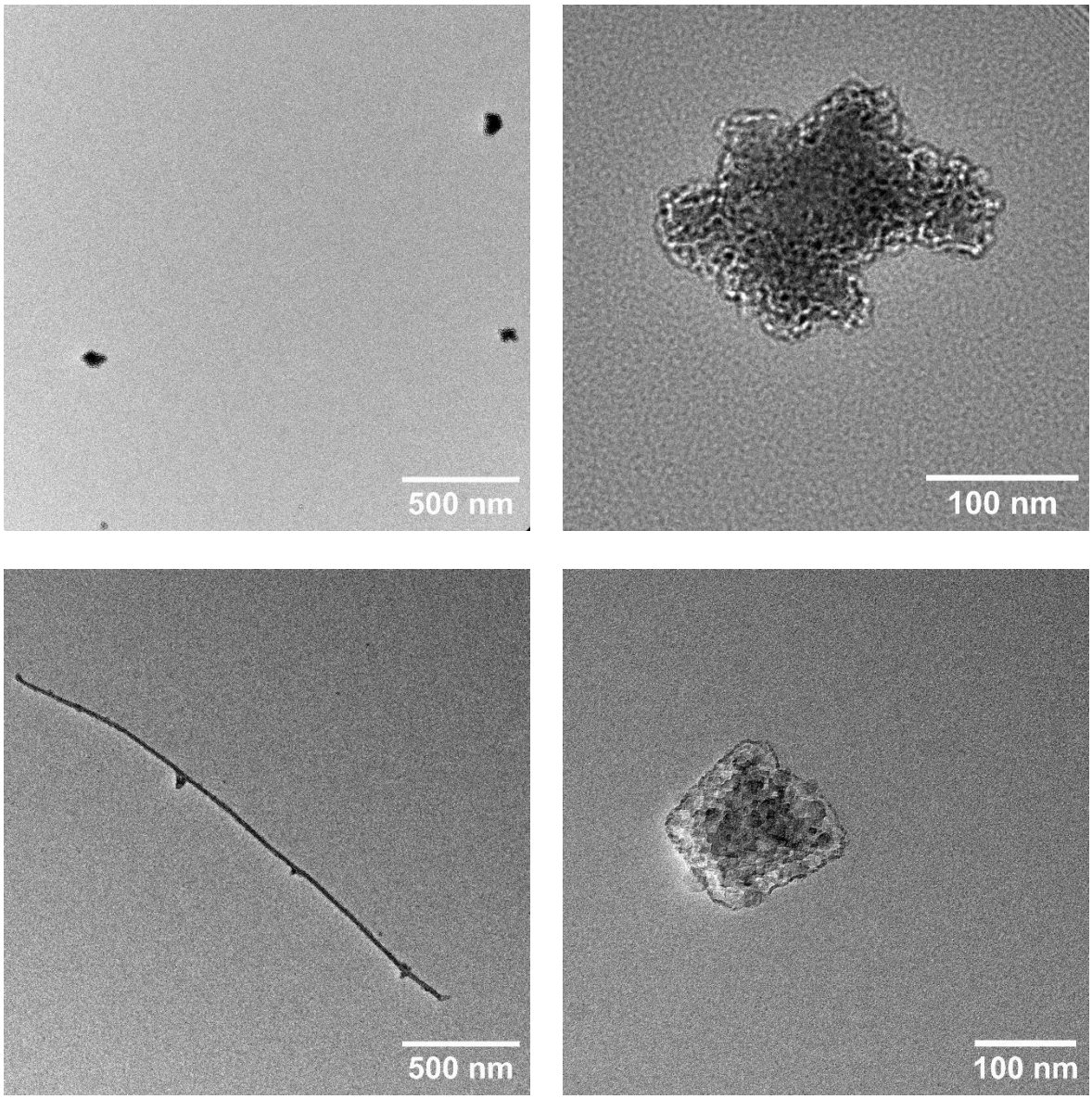



**Figure S29:** TEM images (left: overview; right: zoom) of ligand **IT** (10 µM) in water (top) and in the presence of pDNA (bottom, 500 ng/mL). The lower left picture showing rods in the presence of pDNA indicates uncomplete lipoplex formation.

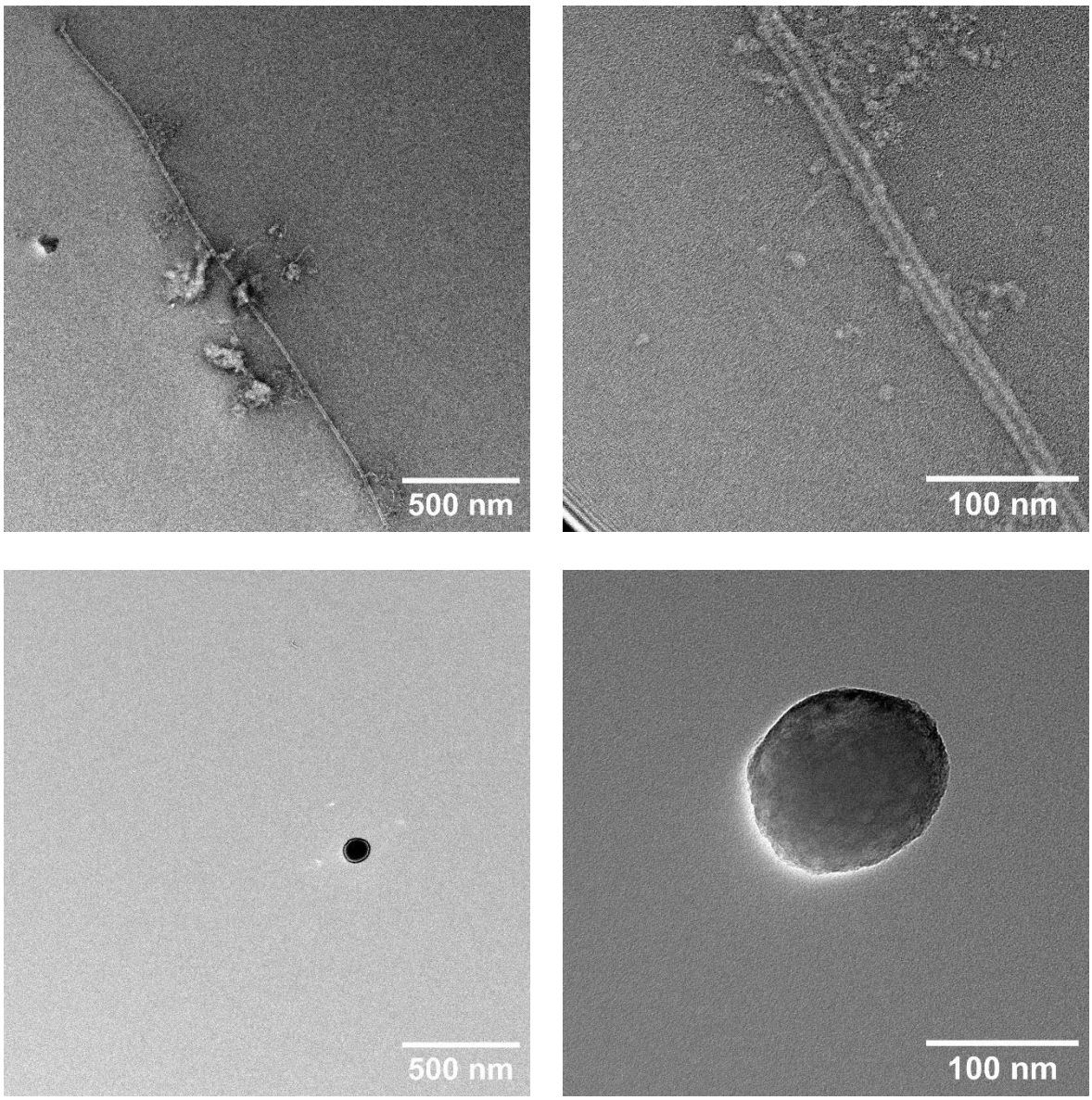



**Figure S30:** TEM images (left: overview; right: zoom) of ligand **$IT_2$** (10 µM) in water (top) and in the presence of pDNA (bottom, 500 ng/mL).

# PHOTOPHYSICS

## UV/VIS SPECTRA

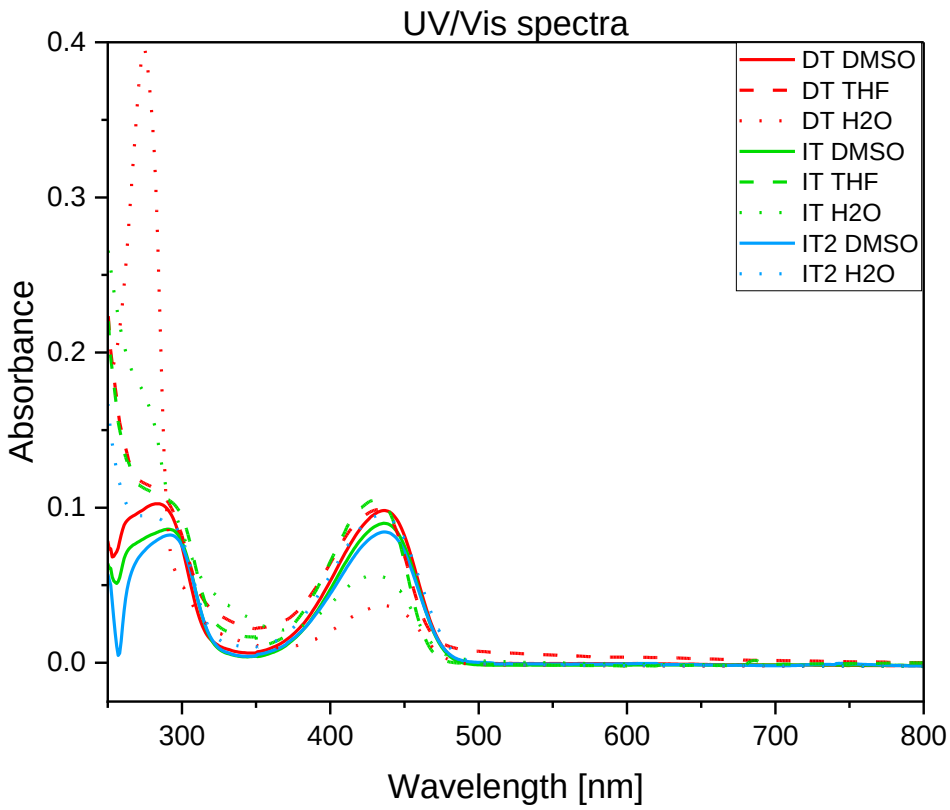



**Figure S31:** UV/Vis spectra of ligands **DT, IT** and **$IT_2$** in THF, DMSO and water.

## LUMINESCENCE SPECTRA

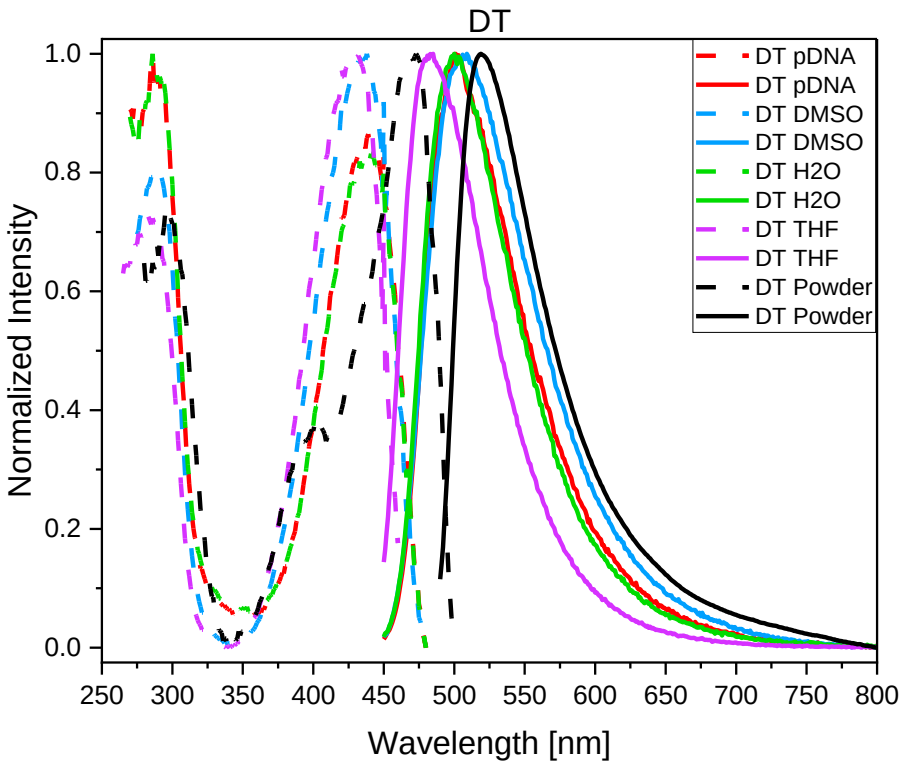



**Figure S32:** Normalized luminescence spectra of **DT** (dotted line: excitation; solid line: emission; conc. = 10 µM, conc. pDNA = 500 ng/mL).

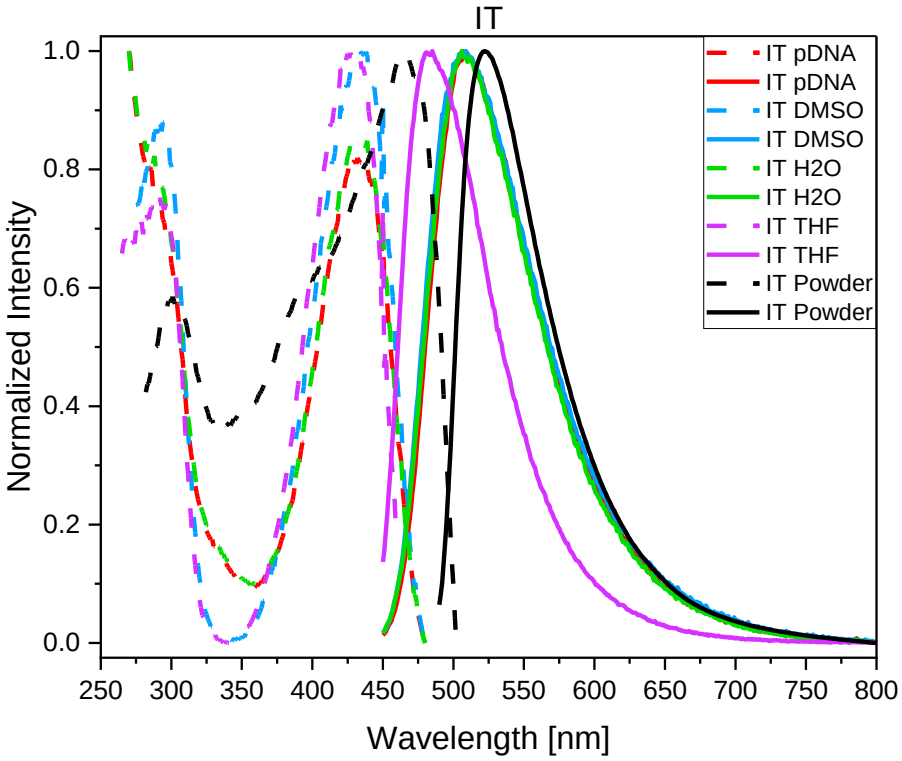



**Figure S33:** Normalized luminescence spectra of **IT** (dotted line: excitation; solid line: emission; conc. = 10 µM, conc. pDNA = 500 ng/mL).

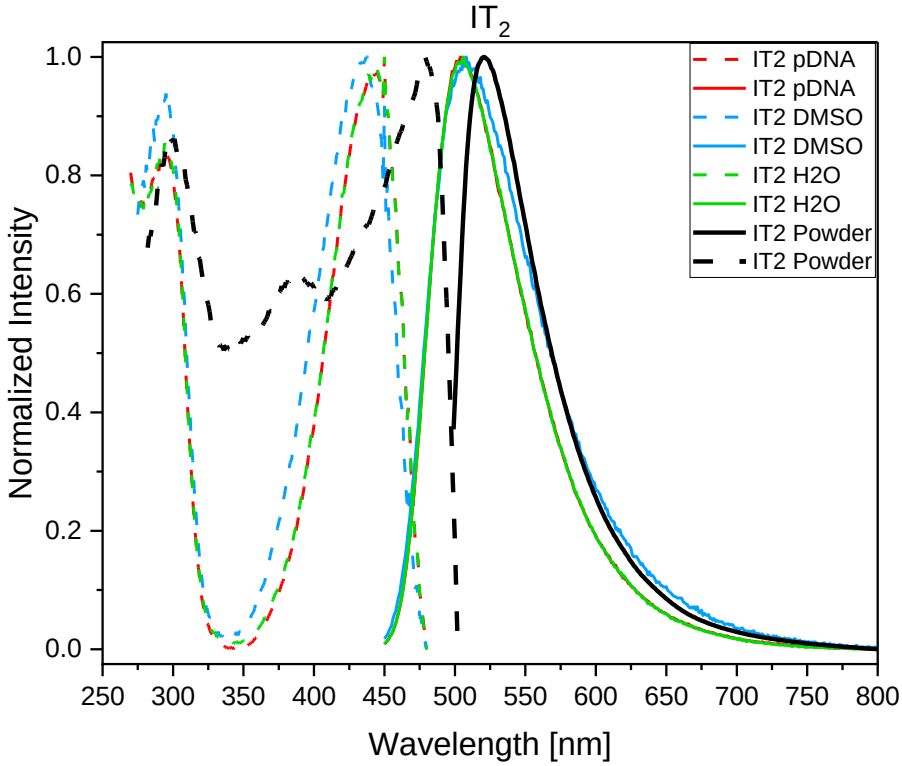



**Figure S34:** Normalized luminescence spectra of **$IT_2$** (dotted line: excitation; solid line: emission; conc. = 10 µM, conc. pDNA = 500 ng/mL).

**Table S2:** Photophysical properties of ligands **DT**, **IT** and **$IT_2$**. Stokes shift was calculated using the most bathochromically shifted absorption maximum. Quantum yields were measured using an integrating sphere (absolute method). *= Data obtained from excitation spectrum. n.d. = not determined.

| Compound | Condition | $\lambda_{abs}$ | $\lambda_{em}$ | Stokes Shift [$cm^{-1}$] | QY |
|---|---|---|---|---|---|
| **DT** | THF | 282, 433 | 485 | 2476 | 0.48 |
| | DMSO | 284, 436 | 509 | 3289 | 0.06 |
| | $H_2O$ | 275, 438 | 500 | 2831 | 0.01 |
| | pDNA | 439* | 501 | 2819 | 0.01 |
| | Triton-X | 438* | 492 | 2506 | n.d. |
| | Solid-State | 473* | 519 | 1874 | 0.04 |
| **IT** | THF | 282, 430 | 485 | 2637 | 0.56 |
| | DMSO | 290, 436 | 509 | 3289 | 0.03 |
| | $H_2O$ | 280, 428 | 506 | 3601 | 0.01 |
| | pDNA | 432* | 507 | 3425 | 0.01 |
| | Triton-X | 431* | 487 | 2668 | n.d. |
| | Solid-State | 466* | 522 | 2302 | 0.17 |
| **$IT_2$** | DMSO | 292, 437 | 508 | 3198 | 0.05 |
| | $H_2O$ | 280, 434 | 506 | 3278 | 0.05 |
| | pDNA | 445* | 504 | 2631 | 0.05 |
| | Triton-X | 441* | 495 | 2474 | n.d. |
| | Solid-State | 479* | 521 | 1683 | 0.21 |

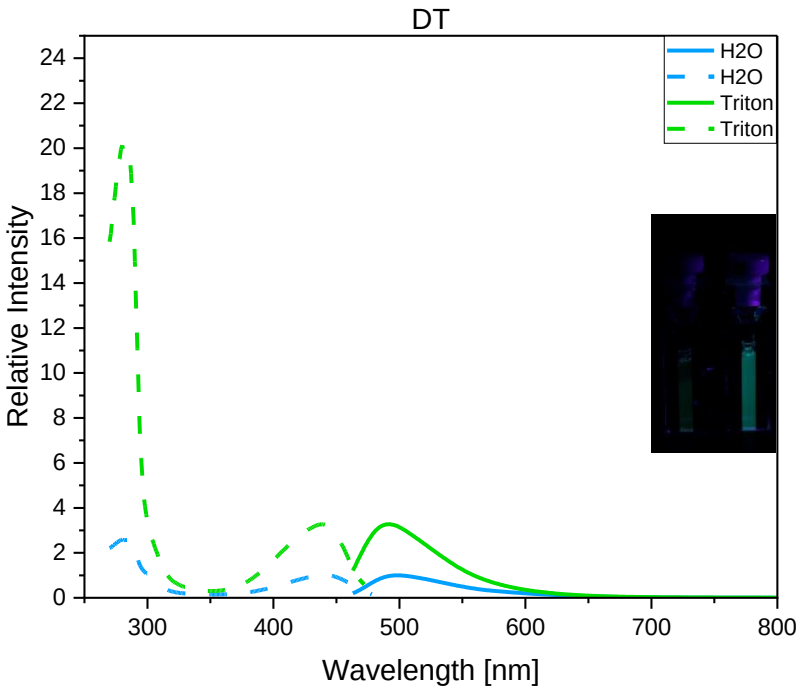



**Figure S35:** Emission enhancement of **DT** in water and in aqueous solution of Triton X-100 (compound conc. = 10 µM, Triton X-100 conc. = 500 µM). Emission maximum of **DT** in water was set to 1. Picture shows **DT** in water without (left) and with Triton-X 100 (right), irradiated with 395 nm.

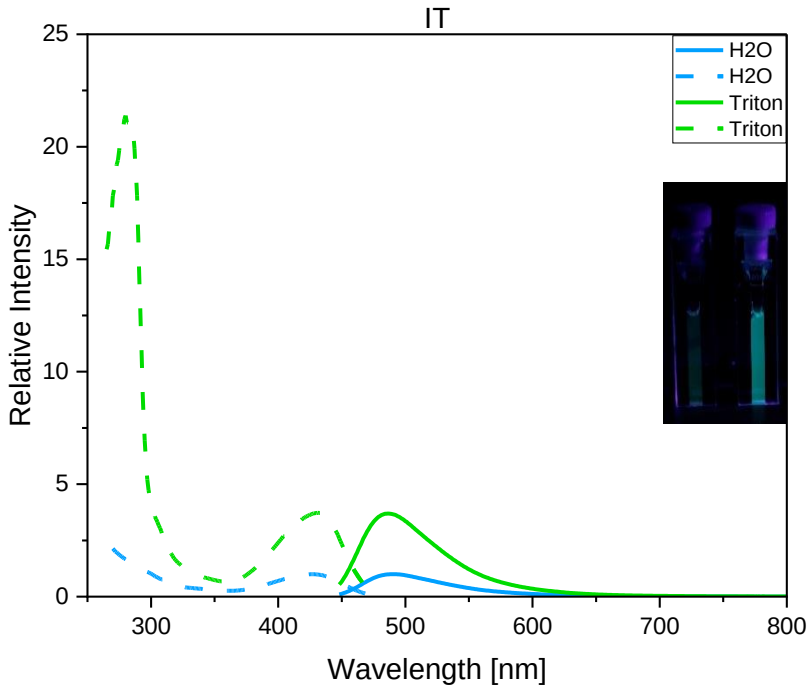



**Figure S36:** Emission enhancement of **IT** in water and in aqueous solution of Triton X-100 (compound conc. = 10 µM, Triton X-100 conc. = 500 µM). Emission maximum of **IT** in water was set to 1. Picture shows **IT** in water without (left) and with Triton-X 100 (right), irradiated with 395 nm.

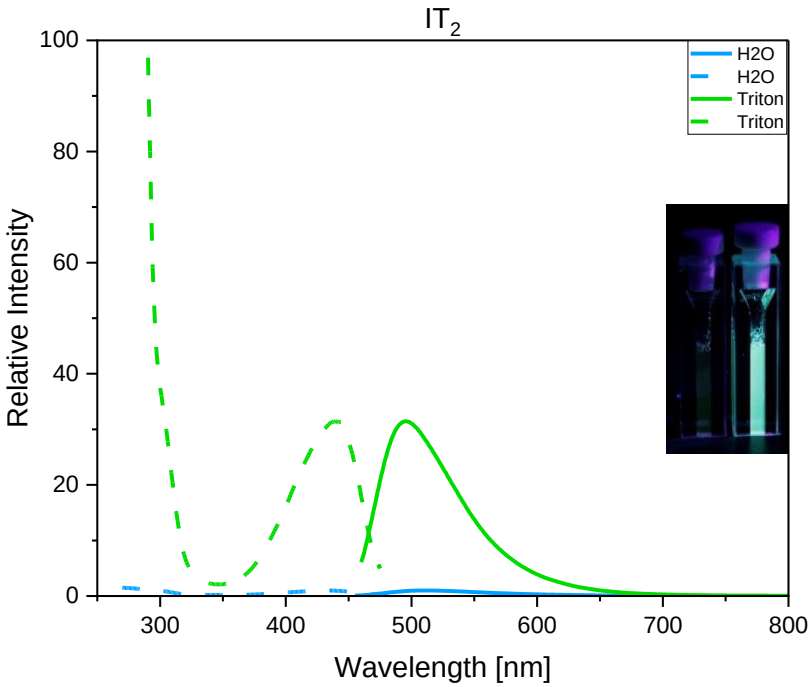



**Figure S37:** Emission enhancement of **$IT_2$** in water and in aqueous solution of Triton X-100 (compound conc. = 10 µM, Triton X-100 conc. = 500 µM). Emission maximum of **$IT_2$** in water was set to 1. Picture shows **$IT_2$** in water without (left) and with Triton-X 100 (right), irradiated with 395 nm.

## PHOTOGRAPHS

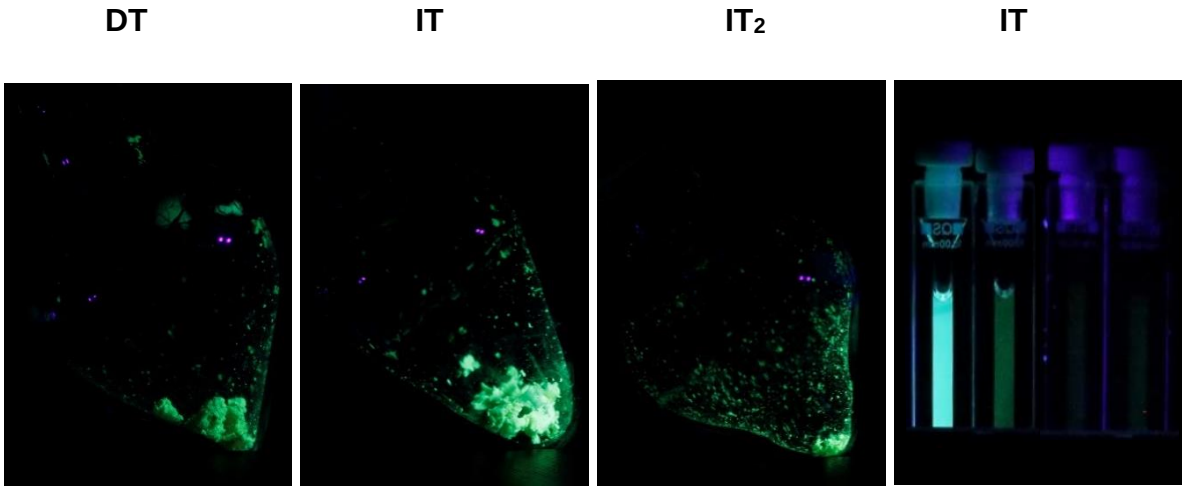



**Figure S38:** Photographs of compounds **DT**, **IT** and **IT₂** (f.l.t.r.) in the solid-state (irradiation with 395 nm) and in solution (10 µM, f.l.t.r.: THF, DMSO, $H_2O$, $H_2O$ + pDNA) for **IT**.

## CELL ASSAYS AND MICROSCOPY

The cell culturing, transfection study, co-localisation, microscopy and MTS cell proliferation assays were performed according to literature with slightly modified procedures.

### CELL CULTURE

The eukaryotic cell lines HeLa and HEK 293T were cultivated in DMEM growth medium (Invitrogen) with 10% FBS (Gibco) and 1% Antibiotic-Antimycotic (Gibco) at 37 °C, 5% $CO_2$ and 90% relative humidity.

### TRANSFECTION AND MICROSCOPY

Compound samples for transfection were diluted with water from a 40 mM DMSO stock solution to the respective concentration. After mixing, the described amount of pDNA (coding for the fusion-protein H2B-mRFP) was added. This mixture was gently added to the cultivated HeLa or HEK 293T cells.

For this, the corresponding cells were seeded in 8 well µ-slides (ibiTreat-coated, Ibidi) and treated with the transfection mixture of the ligands or the commercially available transfection reagent Lipofectamine® 2000 (Invitrogen, 1 µL) as control.

24 h after transfection the samples were imaged using a Leica SP8X Falcon confocal laser scanning microscope (Leica) using a HC PL APO 20x/0.75 CS2 objective (Leica). The compounds were excited using a 405 nm diode laser and pH2B-mRFP using 561 nm light of a white light laser.

For calculating the transfection efficiency cells were treated like described above. Additionally, 1 h before imaging the cells were stained with CellTracker™ Deep Red (1 µM, Thermo Fisher). The staining with CellTracker™ Deep Red was excited using 633 nm light of the white light laser. The transfection efficiency was determined by the ratio of transfected cells (segmented using H2BmRFP intensity, $\lambda_{em}$ =: 607 nm, detection window =: 575–620 nm) and all cells (segmented using CellTracker™ Deep Red intensity, $\lambda_{em}$ (CellTracker™ Deep Red) = 660 nm, see Fig. S13) using CellProfiler™. In total ≥ 1159 HEK 293T and ≥ 2058 HeLa cells were analysed.

To examine the co-localization of the ligands with the lysosomes, HeLa cells were seeded on ibiTreat-coated µ-Slide 8 well (ibidi). Afterwards, the cells were treated with 10 µM of the respective compound mixed with 500 ng plasmid DNA (pH2B-mRFP). Prior to microscopy, lysosomes were stained with LysoTracker™ Deep Red (Invitrogen) according to the manufacturer's protocol. 2 hours after compound treatment, the cells were imaged with a Leica SP8X Falcon confocal laser scanning microscope (Leica) equipped with a HC PL APO 20x/0.75 CS2 objective (Leica). The ligands were excited using a 405 nm diode laser, LysoTracker™ deep red using 633 nm light of the white light laser and pH2B-mRFP using 561 nm light of the white light laser.

Images were generated using FIJI[15] and OMERO.[16]

### MTS Cell Proliferation Assay (CellTiter®AQueous One)

The cytotoxicity of the ligands was examined using a MTS Cell Proliferation Assay (CellTiter 96® AQueous One, Promega). For this, HeLa cells were treated with different concentrations of the compounds (0.05 µM - 400 µM) for 24 h. The number of viable cells per well was determined by adding 20 µl of the MTS tetrazolium compound (CellTiter 96® AQueous One Solution Reagent) that is bioreduced by NADPH or NADH in living cells into a colored formazan. After 1 hour of incubation at 37 °C, the absorption of the metabolite, which is directly proportional to the number of living cells per well, was measured at 490 nm with a GloMax®-Multi plate reader (Promega).

### Gel retardation assay

To determine the strength of the lipoplex formation different concentrations of the three compounds (DT, IT and $IT_2$) were tested in a gel retardation assay. For this experiment 500 ng of DNA was preincubated with 0, 1, 10 or 100 µM of the compound for 30 min. After this Gel Loading Dye, Purple(6x) (New England BioLabs) was added and the samples were electrophoresed on 1% (w/w) agarose gel containing ethidium bromide in TAE buffer for 45 min at 120 V. The gel was documented using the UV illumination of an Fusion FX7 (Vilber Lourmat).

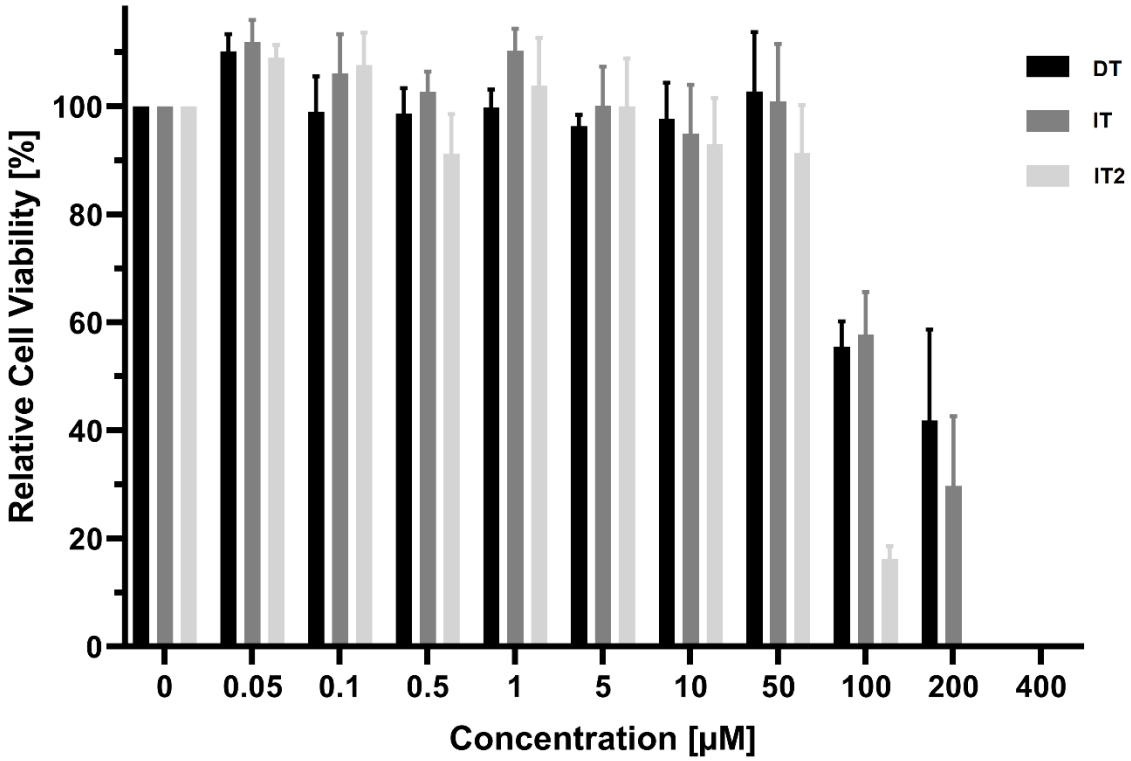



**Figure S39:** 24 h toxicity study (MTS cell proliferation assay) investigating concentration-dependent effects of **DT**, **IT** and **$IT_2$** on the viability of HeLa cells.

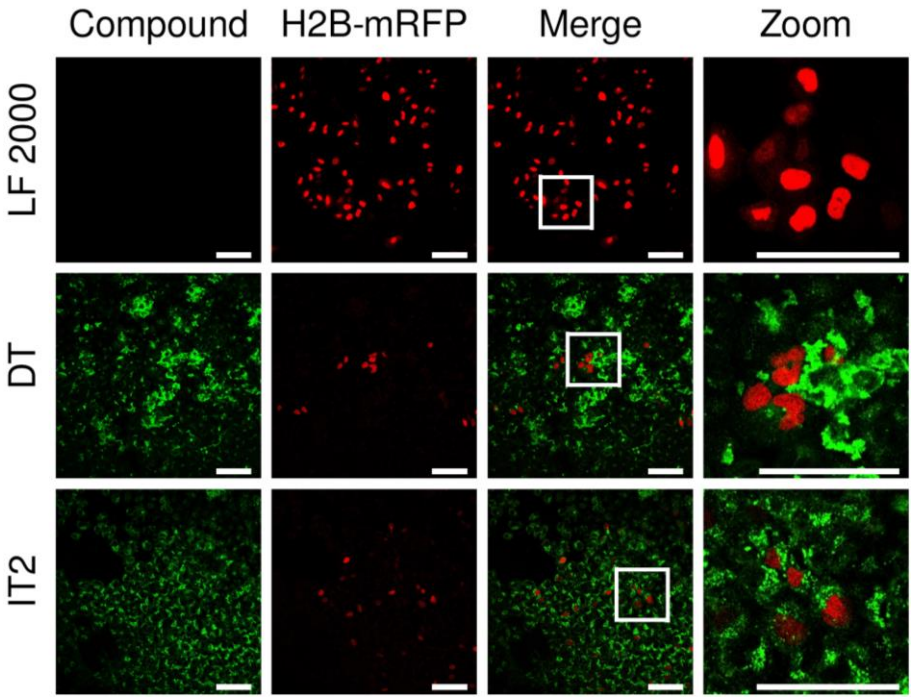



**Figure S40:** Confocal images of HeLa cells after transfection of pH2B-mRFP with Lipofectamine™ 2000 (top) as control, ligand **DT** (middle) or ligand **$IT_2$** (bottom). Scale bar: 100 µm.

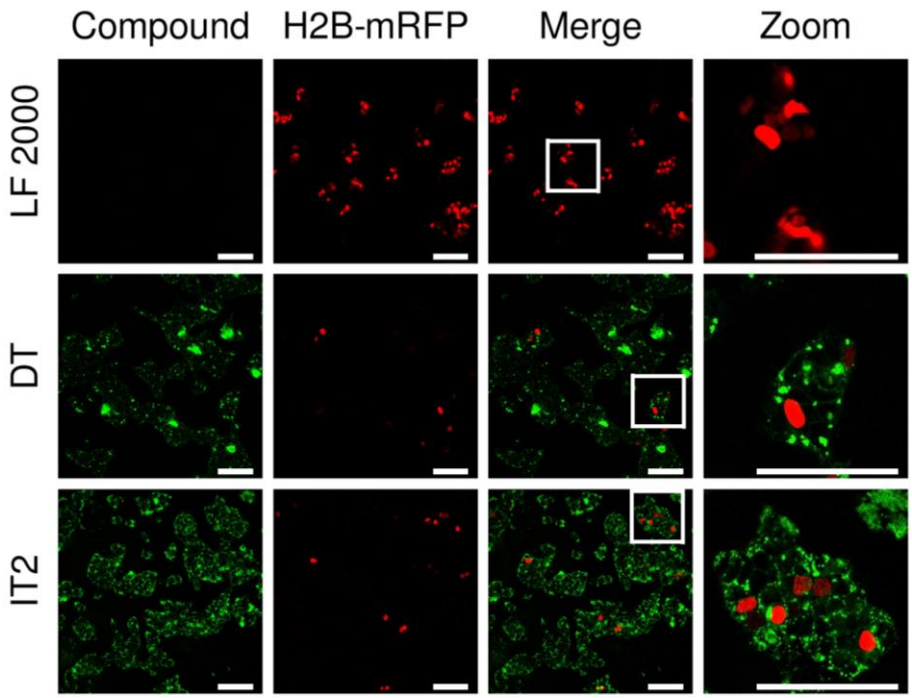



**Figure S41:** Confocal images of HEK 293T cells after transfection of pH2B-mRFP with Lipofectamine™ 2000 (top) as control, ligand **DT** (middle) or ligand **$IT_2$** (bottom). Scale bar: 100 µm.

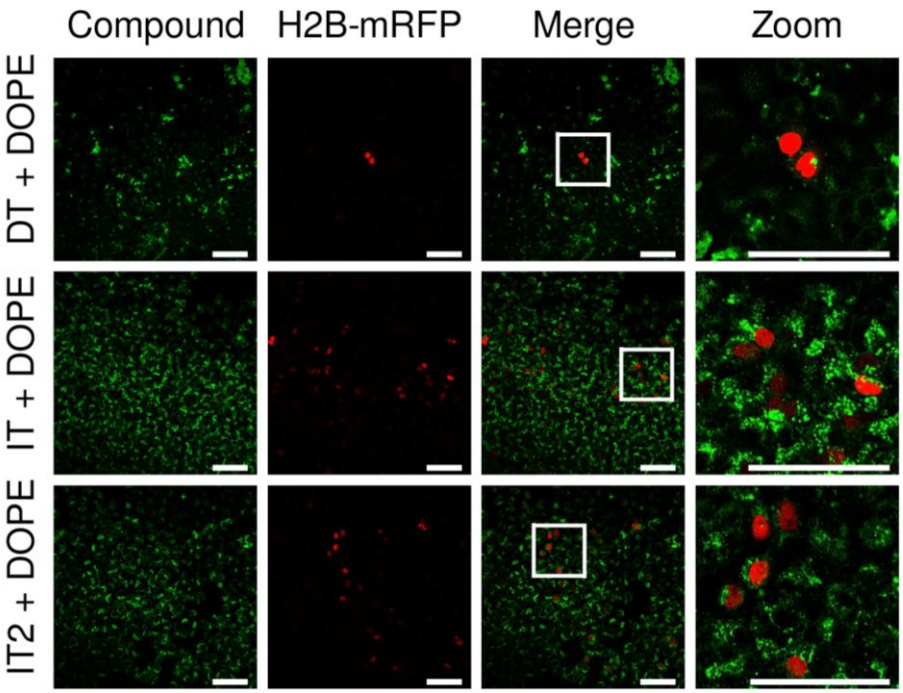



**Figure S42:** Confocal images of HeLa cells after transfection of pH2B-mRFP with ligand **DT** (top), **IT** (middle) or ligand **$IT_2$** (bottom) in the presence of DOPE (10 µM each). Scale bar: 100 µm.

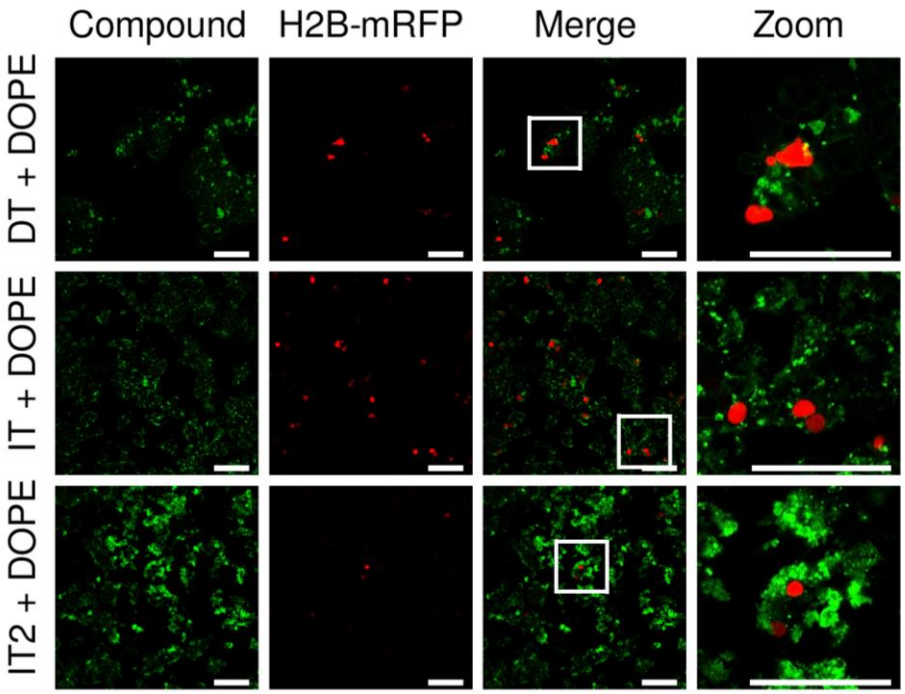



**Figure S43:** Confocal images of HEK 293T cells after transfection of pH2B-mRFP with ligand **DT** (top), **IT** (middle) or ligand **$IT_2$** (bottom) in the presence of DOPE (10 µM each). Scale bar: 100 µm.

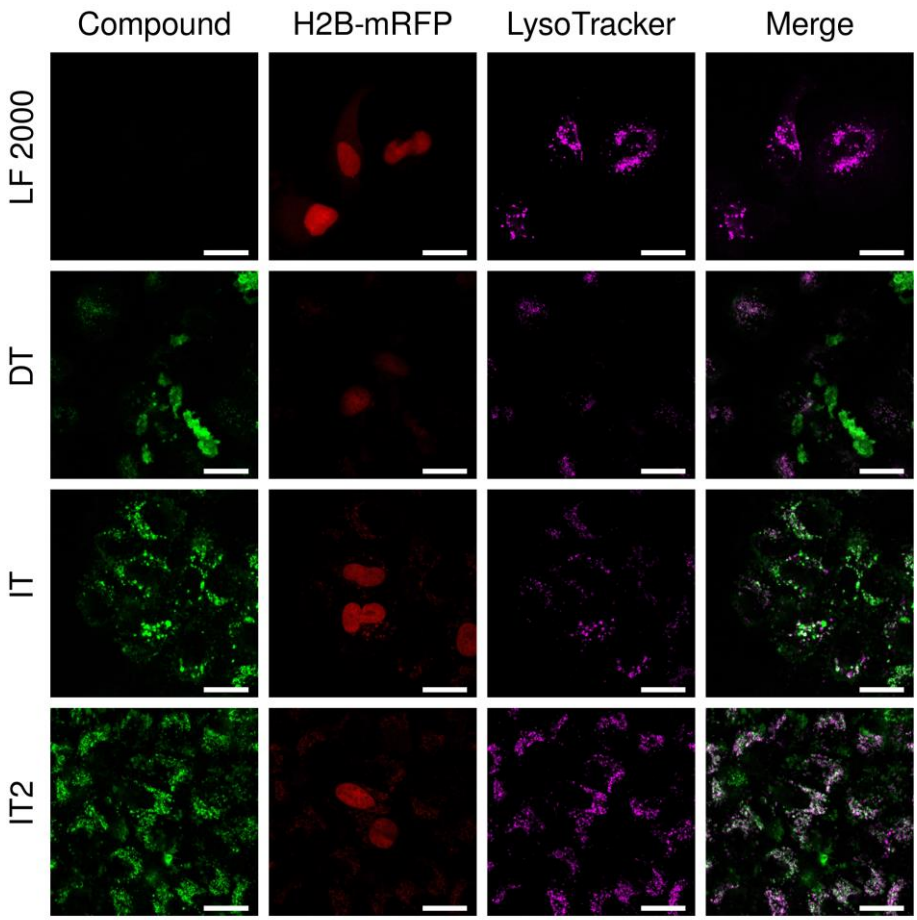



**Figure S44:** Confocal images of HeLa cells after treatment with LysoTracker™ deep red and transfection of pH2B-mRFP with Lipofectamine™ 2000 (top) as control, ligand **DT**, ligand **IT** or ligand **$IT_2$** (bottom). Scale bar: 25 µm.

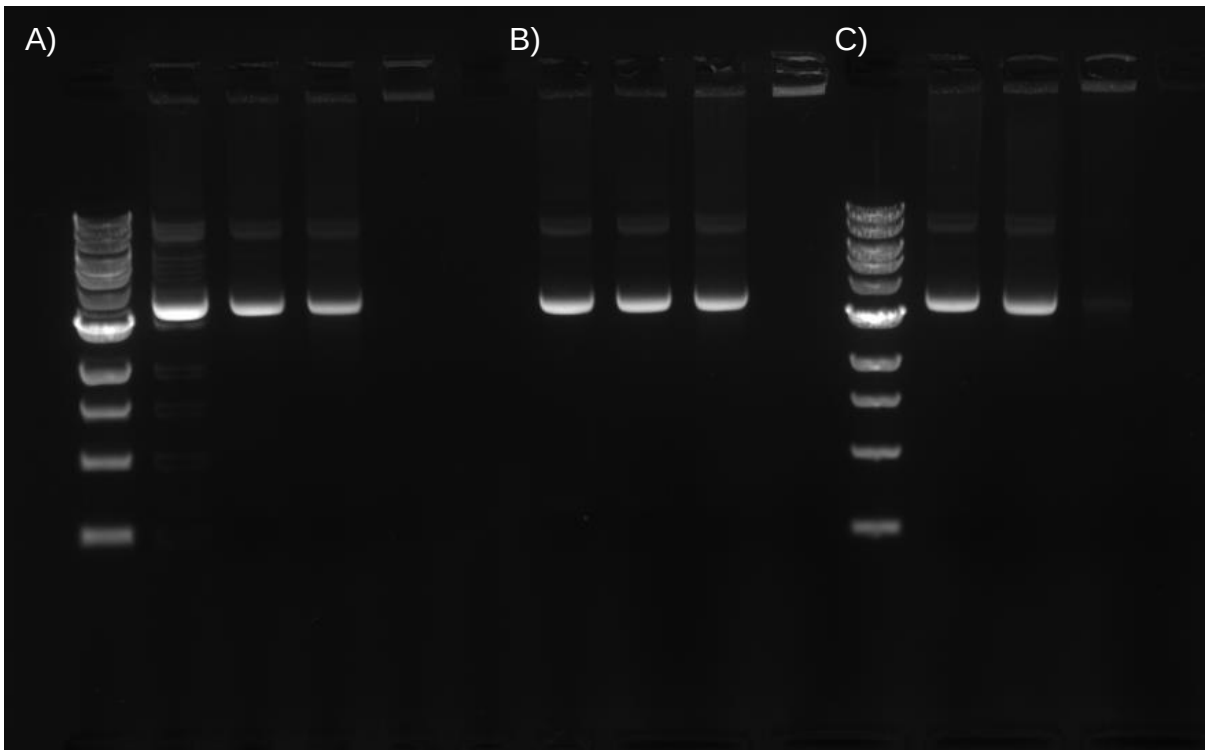



**Figure S45:** Gel retardation assay with different concentrations of compounds **DT** (A), **IT** (B) and **$IT_2$** (C).

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