Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Design of novel tripyridinophane-based Eu(III) complexes as efficient luminescent labels for bioassays applications†

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General experimental

NMR , IR and Mass analyses

^{1}H and ^{13}C NMR spectra were recorded in commercially-available deuteriated solvents on a Bruker AC300, Bruker advance 400 or Bruker advance 500 spectrometer. Chemical shifts are reported in ppm, with residual protonated solvents as the internal references. IR spectra were recorded on a Nexus ThermoNicolet spectrometers High-Resolution Mass Spectra (HRMS) were recorded on a Xevo G2 QTof Waters or a QStar Elite (AppliedBiosystems, SCIEX) spectrometer operating in a positive ion mode (ESI+).

Chromatography

The analytical HPLC was performed on a Thermo Scientific Spectra system P4000 equipped with a UV 1000 detector (deuterium lamp, 190 - 350 nm).The preparative HPLC was performed on a Shimadzu LC-8A equipped with a Diode array detector Varian ProStar, Various chromatographic systems were employed for analytical and preparative HPLC.

System A: Waters XBridge RP-C18 column, 5 μm, 19 × 100 mm, flow rate 20 mL/min, eluents A (H_2O with 0.1% formic acid) – B (CH_3CN), *t* = 0 min 50% B, *t* = 17 min 100% B.

System B: Waters XBridge RP-C18 column, 300 Å, 3.5 μm, 4.6 × 100 mm, flow rate 1 mL/min, eluents A (H_2O with 0.1% formic acid) – B (CH_3CN with 0.1% formic acid), *t* = 0 min 5% B, *t* = 15 min 100% B.

System C: Waters XBridge RP-C18 column, 5 μm, 50 × 150 mm, flow rate 100 mL/min, eluents A (H_2O with 0.1% formic acid) – B (CH_3CN), *t* = 0 min 15% B, *t* = 2 min 15% B, *t* = 20 min 100% B.

System D: Waters XBridge RP-C18 column, 5 μm, 19 × 100 mm, flow rate 20 mL/min, eluents A (H_2O with 0.1% formic acid) – B (CH_3CN), *t* = 0 min 15% B, *t* = 2 min 15% B, *t* = 20 min 100% B.

System E: Waters XBridge C_{18}, 5 μm, 50 x 150 mm, flow rate 100 mL/min, eluents A (H_2O with triethylammonium acetate 25 mM) – B (CH_3CN), *t* = 0 min 2% B, *t* = 17 min 40% B.

System F: Waters Xbridge C_{18}, 300 Å, 3.5 μm, 4,6 x 100 mm, flow rate 1 mL/min, eluents A (H_2O with ammonium acetate 5 mM, pH 6.5) – B (CH_3CN),), *t* = 0 min 2% B, *t* = 15 min 40% B.

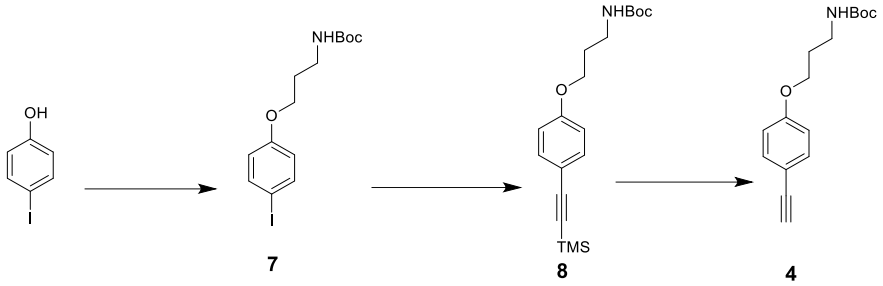
System G: Waters XBridge C_{18}, 5 μm, 19 x 100 mm, flow rate 20 mL/min, eluents A (H_2O with triethylammonium acetate 25 mM, pH 6) – B (CH_3CN), *t* = 0 min 2% B, *t* = 17 min 40% B.

Absorption spectroscopy and fluorescence spectroscopy

Absorption measurements were done with a Hewlett Packard 8453 temperature-controlled spectrometer in 10 mm quartz cuvette. Emission, excitation spectra and luminescence decays at room temperature of lanthanide complexes were measured using a Cary Eclipse spectrofluorimeter equipped with a Xenon flash lamp source and a Hamamatsu R928 photomultiplier. At liquid-nitrogen temperature, a LS-50B Perkin-Elmer spectrofluorimeter equipped with a Xenon flash lamp source, a Hamamatsu R928 photomultiplier and the low-temperature accessory n^{o} L2250136 was used. Excitation spectra were corrected for the excitation light intensity, while emission spectra were corrected for the instrument response. Lifetimes τ (uncertainty $\leq$ 5%) are made by monitoring the decay at a wavelength corresponding to the maximum intensity of the emission spectrum, following pulsed excitation. The luminescence quantum yields (uncertainty $\pm$ 10%) were determined by the method described by Haas and Stein[1] using as standard $[Ru(bpy)_3]^{2+}$ in aerated water ($\Phi = 0.04$)[2] They were measured according to conventional procedures with diluted solutions (optical density < 0.05).

Stability assessment by fluorescence spectroscopy: A 2 nM stock solution of [Eu.**L**^{a}] was prepared in HEPES buffer (0.05 M, 0.1% BSA, pH 7.4). 50 µL aliquots were added to a Greiner Costar half area 96 well plate. Each well was made up to 100 µL, by addition of 45 µL of the buffer solution and 5 µL of either water or 400 mM aqueous solutions of $CaCl_2$, $MgCl_2$, $MnCl_2$, Na_2EDTA or 8 M KF. For NaN_3, 45 µL of a 61 mM buffer solution was added and 5 µL of water. The emission intensity from each well at 620 nm was recorded on a RubyStar (BMG) plate reader using the HTRF set-up using an integration window between 60 and 460 µs, following excitation with a flash lamp at 320 nm. Each sample preparation was done in triplicate and the mean value of the intensity measurements recorded.

Synthesis of compound 4



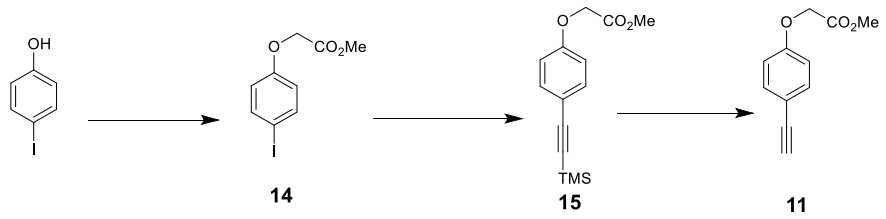
Compound 7. To a solution of 4-iodophenol (19.76 g, 90 mmol) and *N*-Boc-3-bromopropylamine (25.8 g, 108 mmol) in dry CH_3CN (400 mL) were successively added Cs_2CO_3 (44.1 g, 135 mmol) and NaI (3.38 g 22.6 mmol). The mixture was stirred at 60°C for 24 h, then cooled at room temperature and filtered off. The filtrate was concentrated under vacuum and the crude oil was purified by column chromatography on silica gel (eluent: cyclohexane/EtOAc gradient from 95/5 to 60/40 to yield compound **7** (28.8 g, 85%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56 (2H, d, J = 8.8Hz), 6.69 (2H, d, J = 8.8Hz), 4.74 (1H, bs), 4.00 (2H, t, J = 6.0Hz), 3.32 (2H, m), 1.99 (2H, m), 1.46 (9H, s) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.7, 156.0, 138.2, 116.9, 82.8, 79.2, 65.8, 38.05, 29.6, 28.4 ppm. Spectroscopy data are in agreement with those previously reported in the literature.[3]

Compound 8. A solution of compound **7** (4.0 g, 10.6 mmol) in a mixture of THF (14 mL) and triethylamine (14 mL) was degassed with argon by bubbling for 15 min. To this solution were added trimethylsilylacetylene (4.5 mL, 31.8 mmol), $PdCl_2(PPh_3)_2$ (740 mg, 1.06 mmol) and CuI (404 mg, 2.1 mmol). The mixture was irradiated with microwaves (100 W) for 30 min, then cooling to room temperature and filtered off over celite. The filtrate was concentrated under vacuum and the crude product was purified by column chromatography (eluent: cyclohexane/EtOAc 90/10 to 80/20) to give compound **8** (3.65 g, 99%) as a beige solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39 (2H, d, J = 8.6Hz), 6.80 (2H, d, J = 8.6Hz), 4.73 (1H, bs), 4.01 (2H, t, J = 6.0Hz), 3.32 (2H, m), 1.97 (2H, m), 1.44 (9H, s), 0.23 (9H, s) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.9, 155.9, 133.4, 115.3, 114.1, 105.05, 92.4, 79.1, 65.8, 37.8, 29.6, 28.5, 0.02 ppm. Spectroscopy data are in agreement with those previously reported in the literature.[3]

Compound 4. To a solution of compound **8** (10.0 g, 28.8 mmol) in dry methanol (300 mL) cooled at 0°C was added K_2CO_3 (11.9 g, 86.1 mmol). The solution was stirred at room

temperature for 24 h, then concentrated under vacuum. To the residue were added water (50 mL) and CH_2Cl_2 (200 mL). Layers were decanted and the aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL). Combined organic layers were dried over Na_2SO_4, filtered off and concentrated under vacuum to yield compound **4** (7.8 g, 98%) as a dark oil which was used in the next step without further purification.. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44 (2H, d, *J* = 8.8Hz), 6.85 (2H, d, *J* = 8.8Hz), 4.75, 1H, bs), 4.04 (2H, t, *J* = 6.0Hz), 3.35 (2H, m), 3.02 (1H, s), 2.00 (2H, m), 1.46 (9H, s) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ159.2, 156.0, 133.6, 114.45, 114.2, 83.7, 79.2, 75.9, 65.8, 38.0, 29.4, 28.4 ppm.

Synthesis of compound 11



Compound 14. To a solution of 4-iodophenol (4.4 g, 20 mmol) in acetone 25 mL were added K_2CO_3 (3.6 g, 26 mmol) and methyl bromoacetate (5.7 mL, 60 mmol). The mixture was stirred at 50°C for 12 h. After filtration, the solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2 (50 mL) and the solution was washed with water (50 mL). The organic phase was concentrated under vacuum and the crude product was purified by column chromatography on silica gel (eluent: hexanes/EtOAc 95/05) to yield compound **14** (5.65 g, 97%) as a solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ7.57 (2H, d, *J* = 8.8Hz), 6.54 (2H, d, *J* = 8.8Hz), 4.60 (2H, s), 3.80 (3H, s) ppm. Spectroscopy data are in agreement with those previously reported in the literature.[4]

Compound 15. It was prepared following literature method.[5] Yield 65%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.46 (2H, d, *J* = 8.8Hz), 6.90 (2H, d, *J* = 8.8Hz), 4.96 (2H, s), 3.68 (3H, s), 0.15 (9H, s) ppm.

Compound 11. To a solution of compound **15** (20 g, 76.2 mmol) in dry CH_2Cl_2 (20 mL) were successively added at 0°C dry MeOH (30 mL) and K_2CO_3 (15.8 g, 114 mmol). The solution

was stirred 15 min at 0°C, then 1 h at room temperature. The resulting mixture was filtered off and concentrated under vacuum. The residue was diluted with CH_2Cl_2 (100 mL) and water (80 mL) was added. Layers were decanted and the aqueous layer was extracted with CH_2Cl_3 (2 × 60 mL). The combined layers were dried over Na_2SO_4, filtered off and concentrated under vacuum to yield compound **11** (12 g, 83%) as a light brown solid which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45 (2H, d, J = 8.0Hz), 6.86 (2H, d, J = 8.0Hz), 4.65 (2H, s), 3.82 (3H, s), 3.03 (1H, s) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.0, 158.0, 133.7, 115.4, 114.6, 83.3, 76.3, 65.1, 52.3 ppm. MS (ESI+) *m/z* 191.5 (100%, $[M+H]^+$) Spectroscopy data are in agreement with those previously reported in the literature.[6]

NMR, MS and HPLC chromatograms of selected compounds

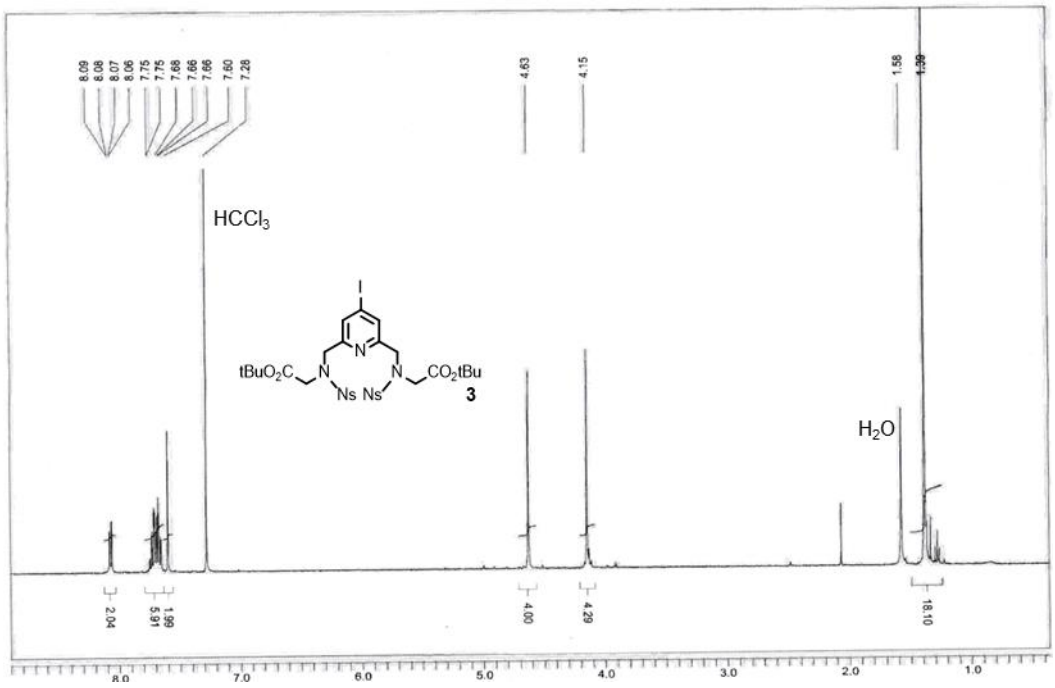


Figure S1. ^{1}H NMR (400MHz, $CDCl_3$) spectrum of compound **3**.

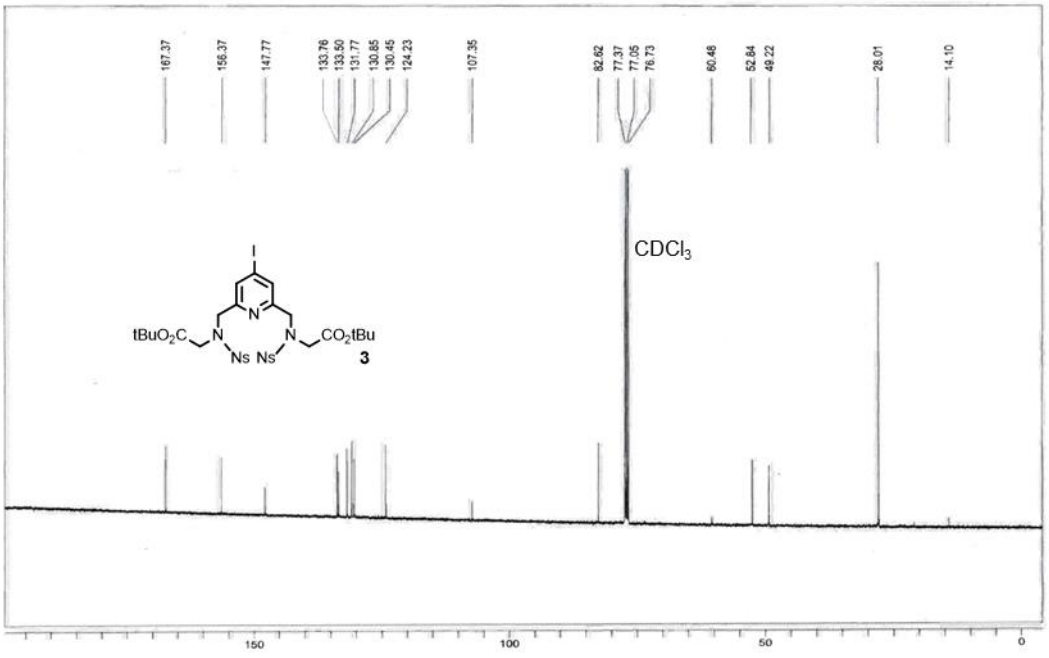


Figure S2. ^{13}C NMR (100MHz, $CDCl_3$) spectrum of compound **3**.

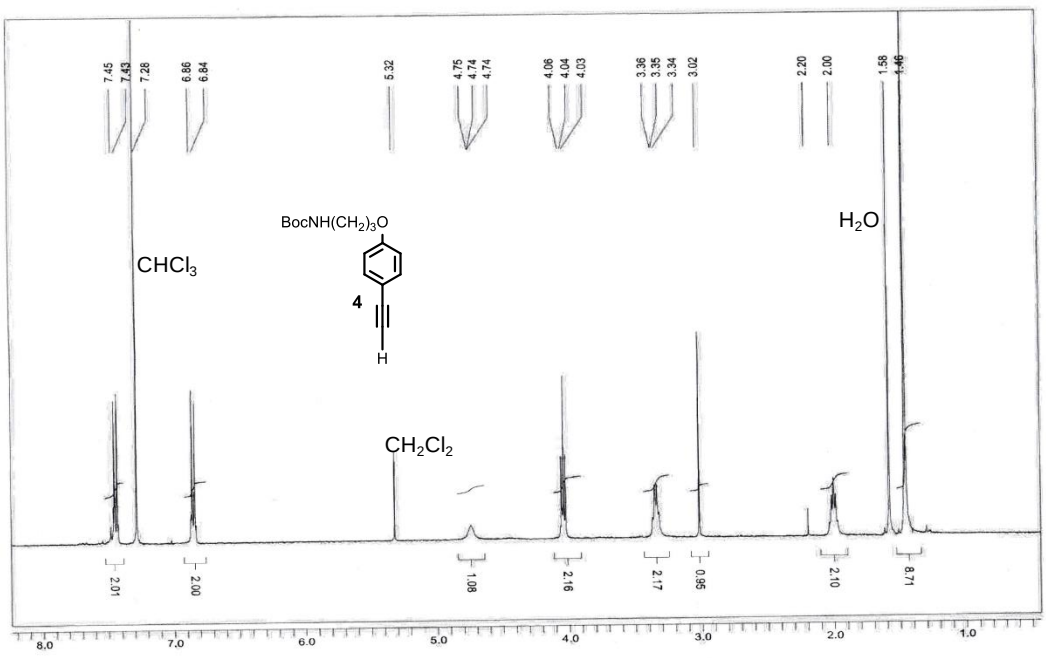


Figure S3. ^{1}H NMR (400MHz, $CDCl_3$) spectrum of compound **4**

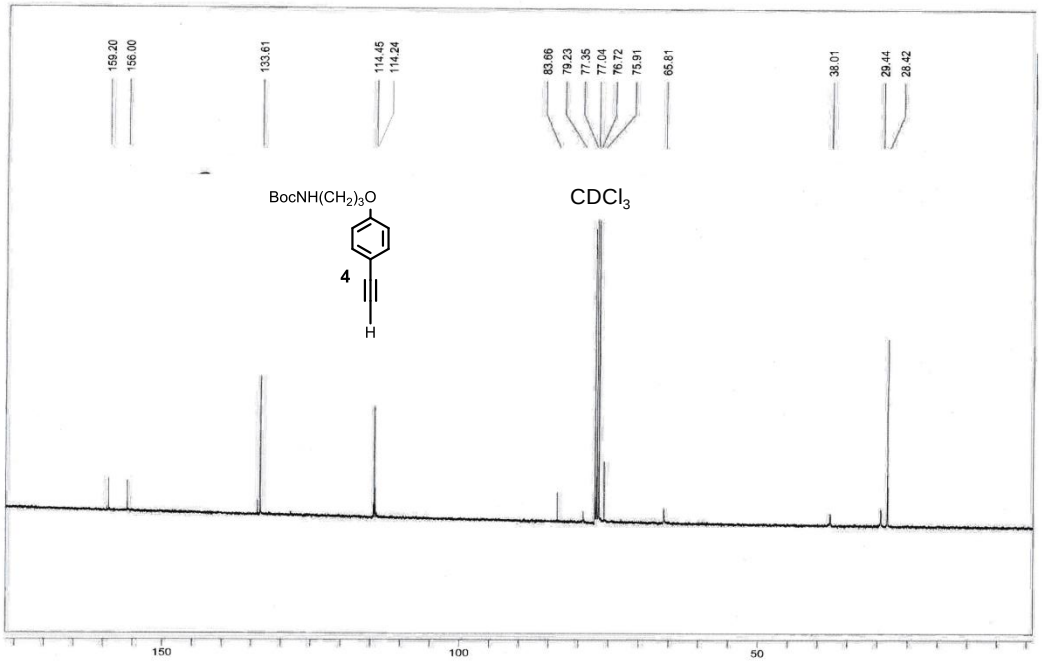


Figure S4. ^{13}C NMR (100MHz, $CDCl_3$) spectrum of compound **4**.

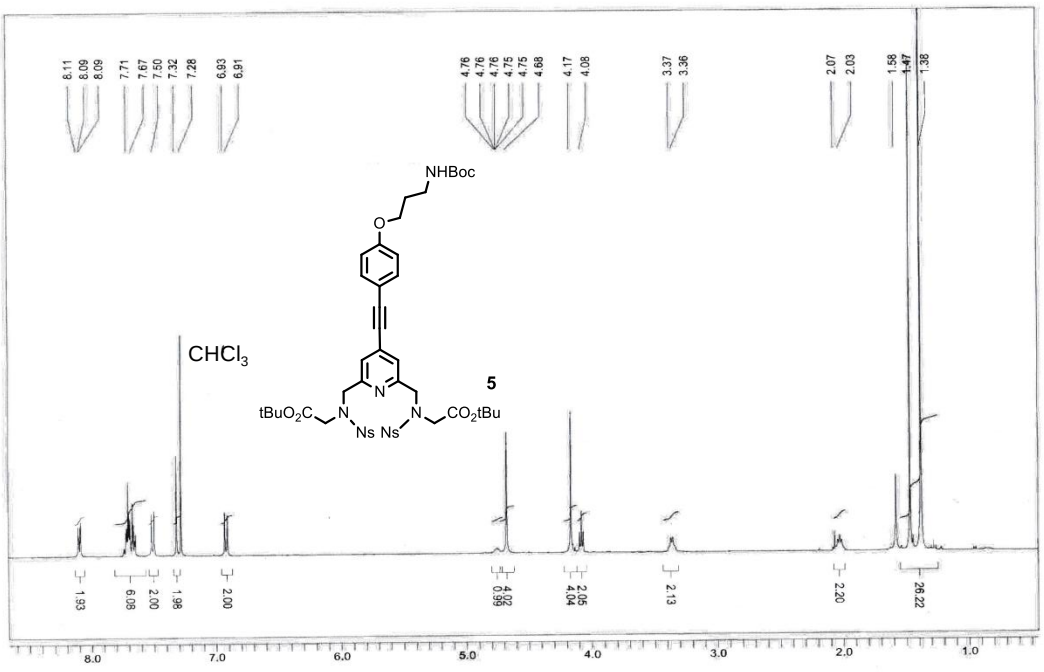


Figure S5. ^{1}H NMR (400MHz, $CDCl_3$) spectrum of compound **5**

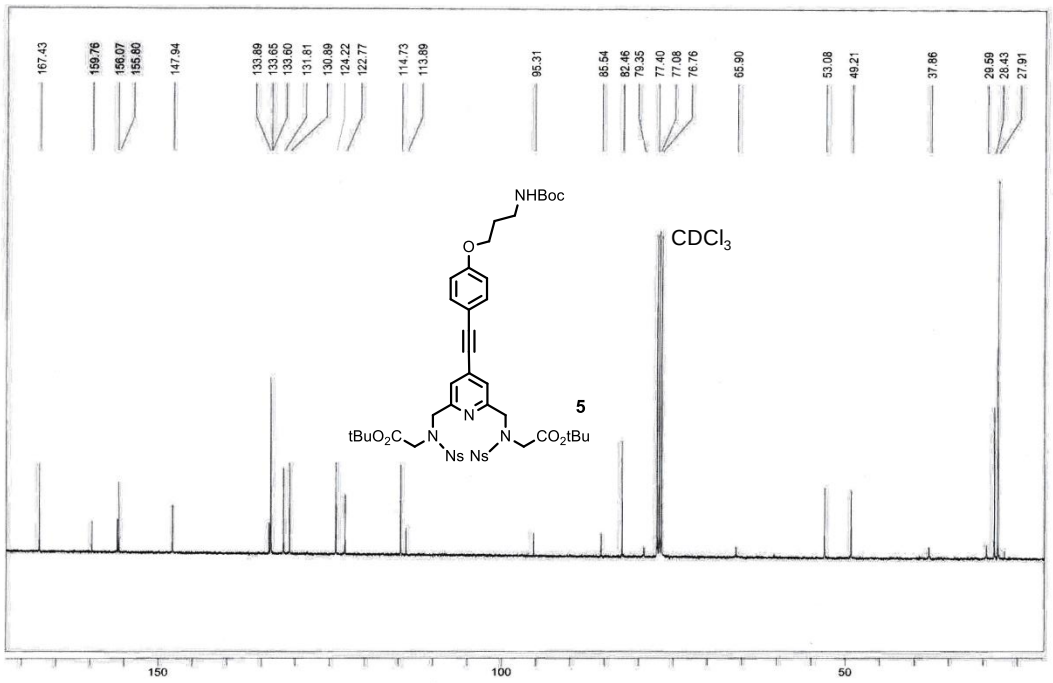


Figure S6. ^{13}C NMR (100MHz, $CDCl_3$) spectrum of compound **5**.

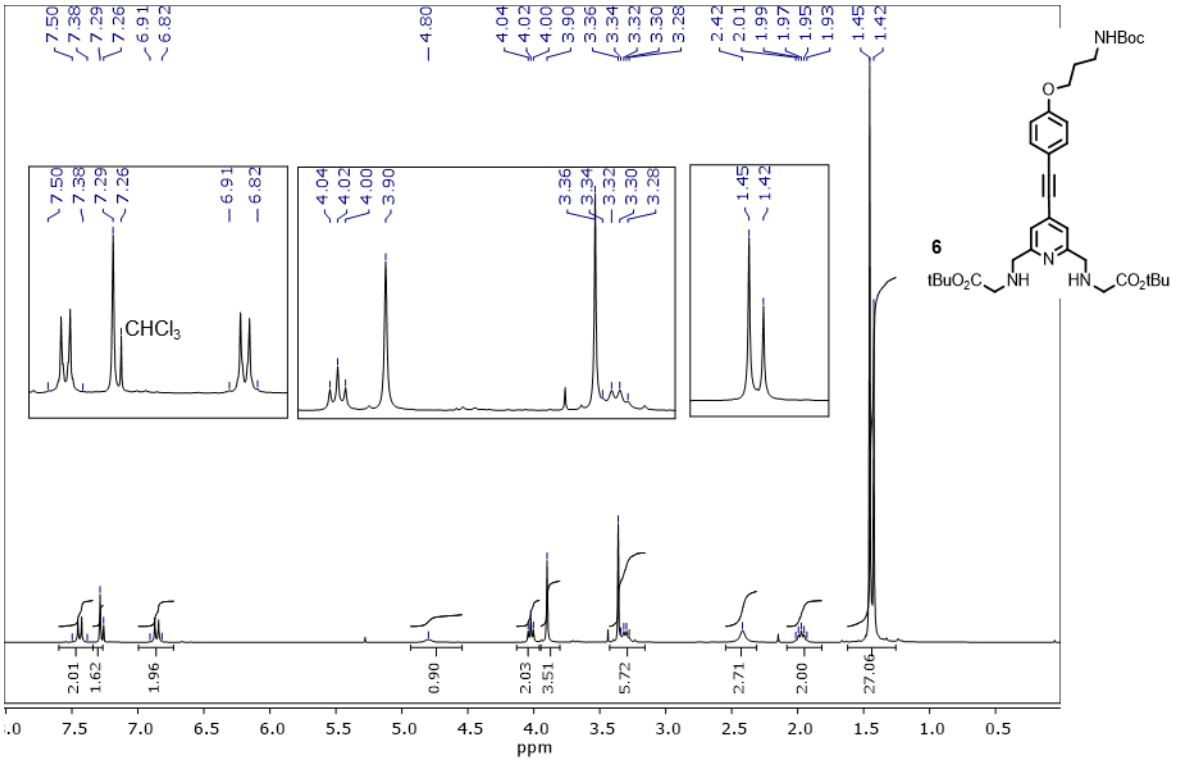


Figure S7.[1]H NMR (300MHz, $CDCl_3$) spectrum of compound **6**

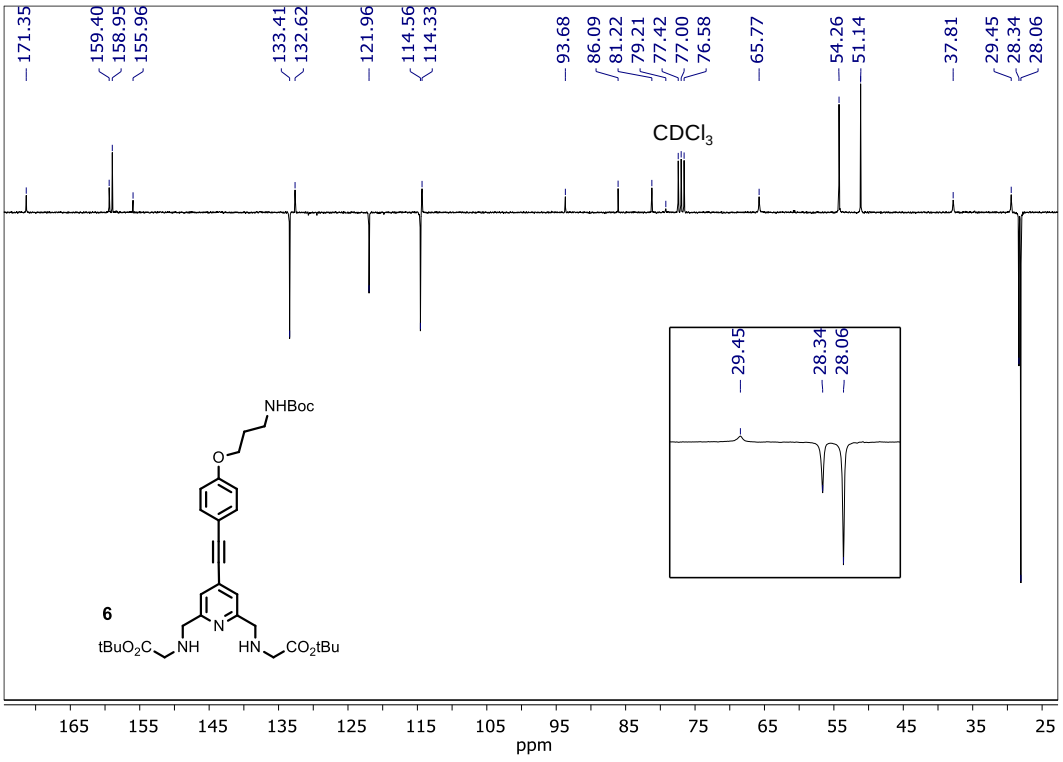
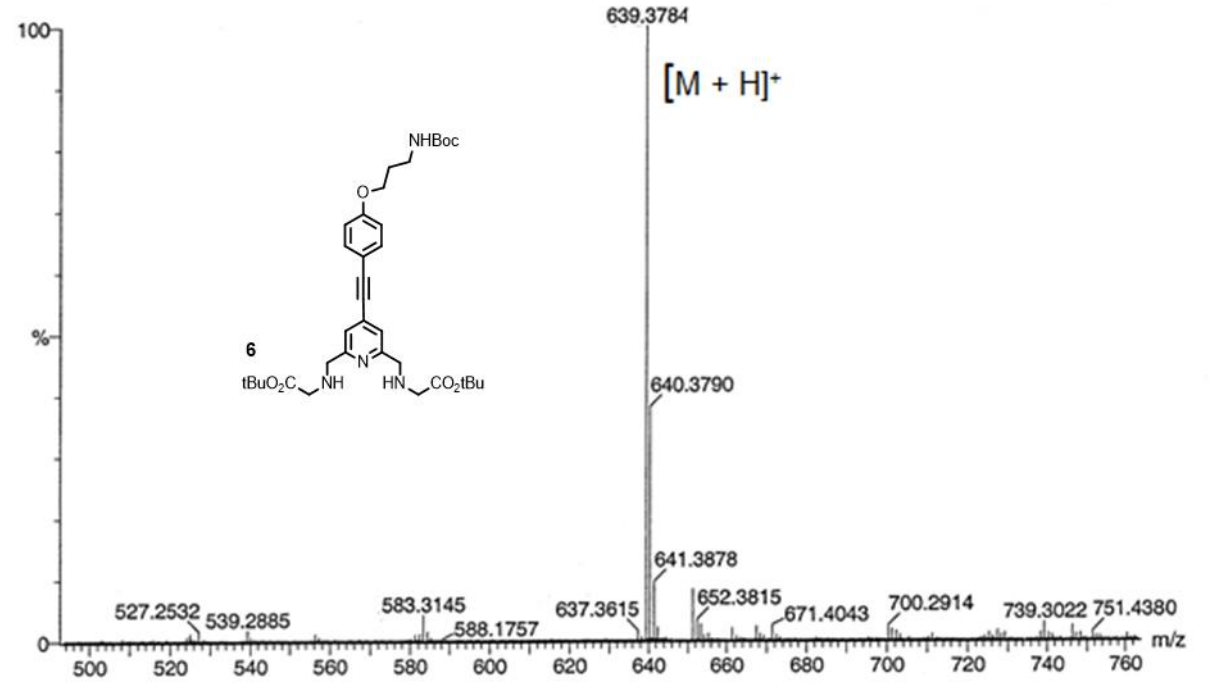


Figure S8. J-modulated [13]C NMR (75MHz, $CDCl_3$) spectrum of compound **6**.



Minimum:				-1.5		
Maximum:		3.0	5.0	70.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
639.3784	639.3758	2.6	4.1	12.5	69.0	C35 H51 N4 O7 ←
	639.3798	-1.4	-2.2	16.5	397.9	C40 H51 N2 O5

Figure S9. HRMS (ESI+) spectrum of compound **6**

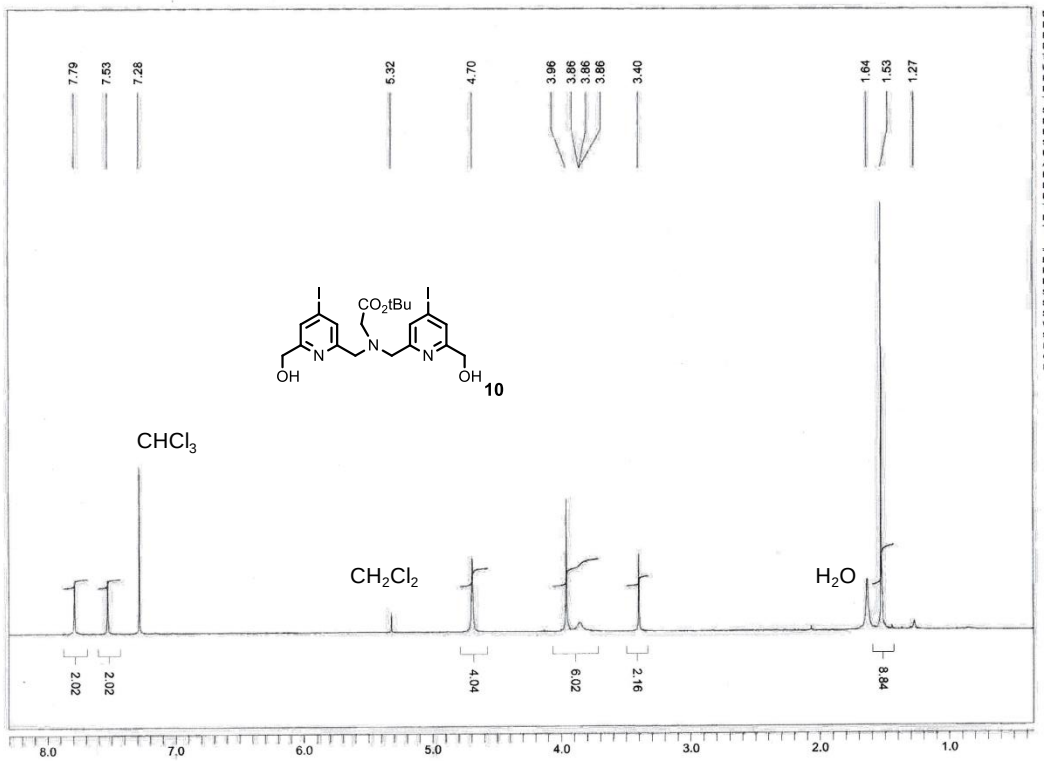


Figure S10.[1]H NMR (400MHz, $CDCl_3$) spectrum of compound **10**.

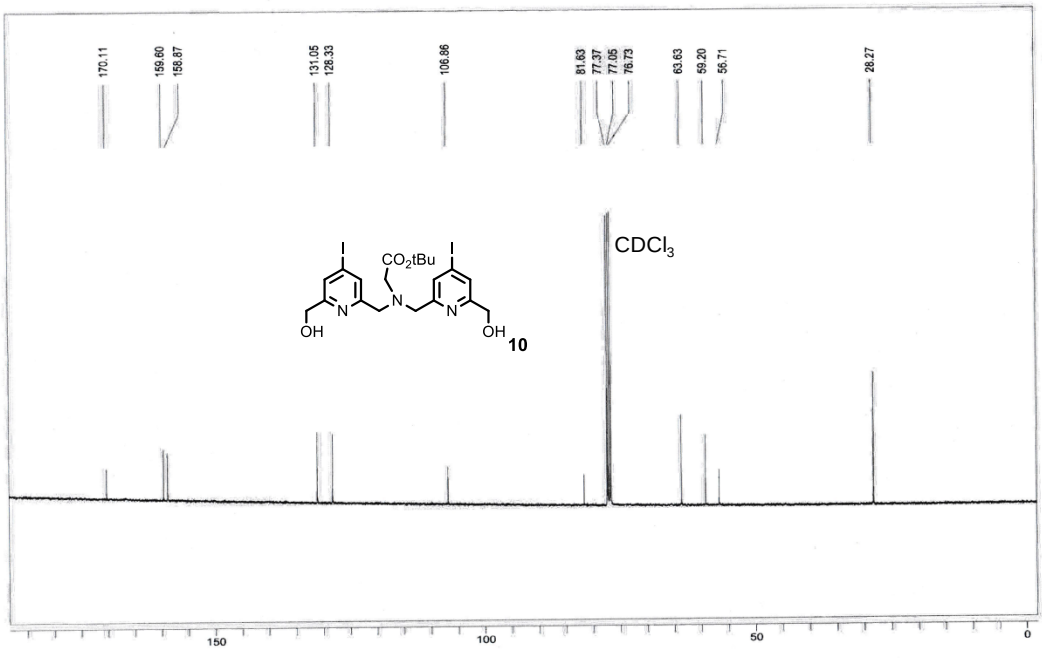


Figure S11.[13]C NMR (100MHz, $CDCl_3$) spectrum of compound **10**.

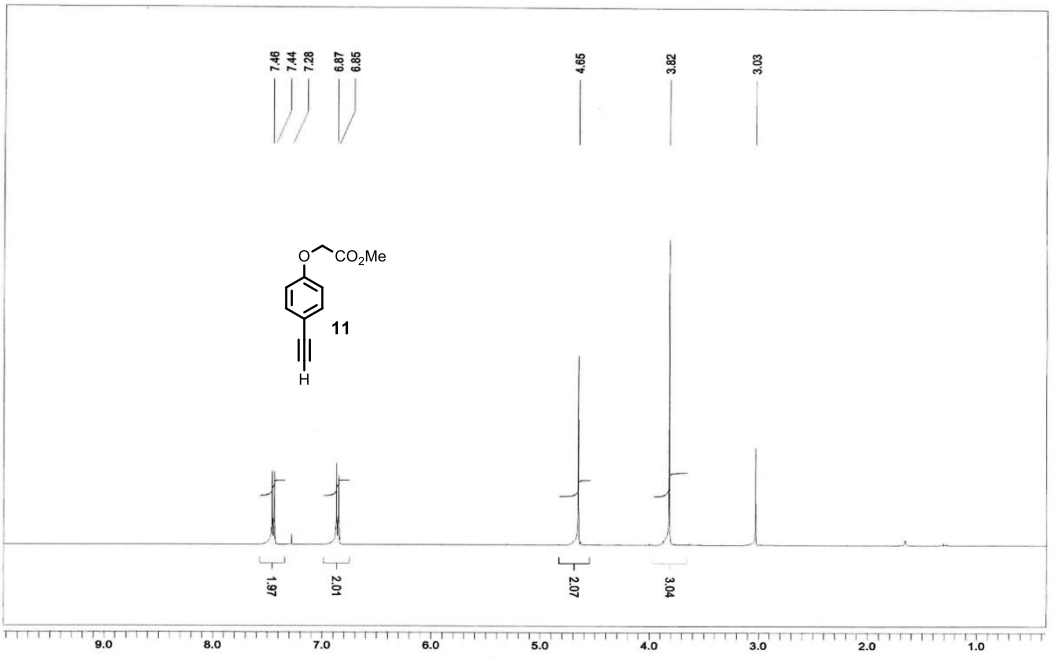


Figure S12. ^{1}H NMR (400MHz, $CDCl_3$) spectrum of compound **11**.

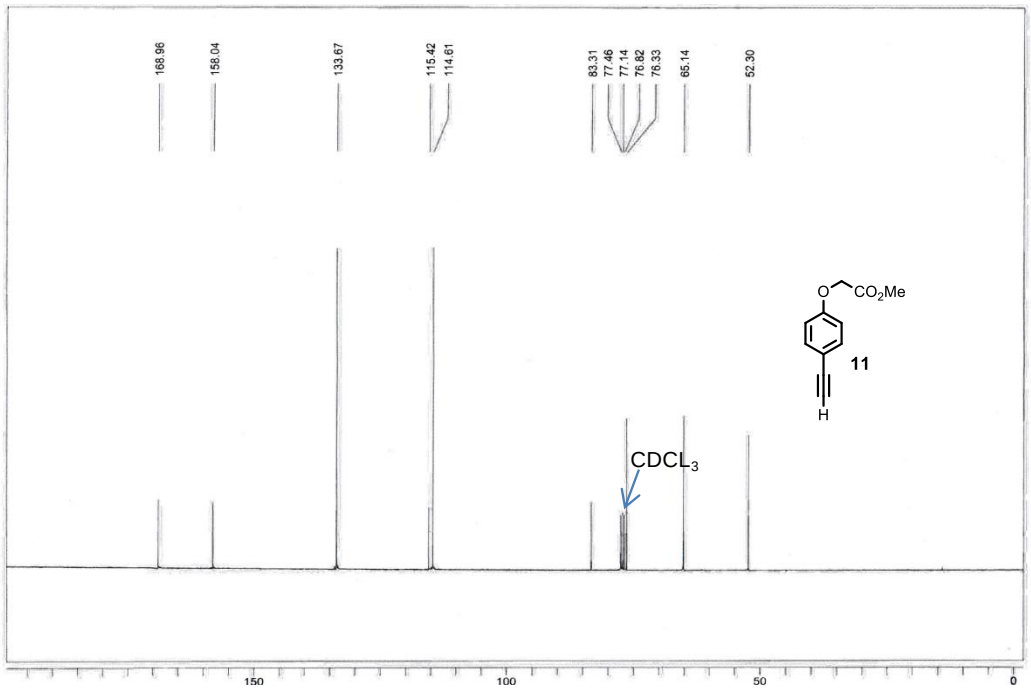


Figure S13. ^{13}C NMR (100MHz, $CDCl_3$) spectrum of compound **11**.

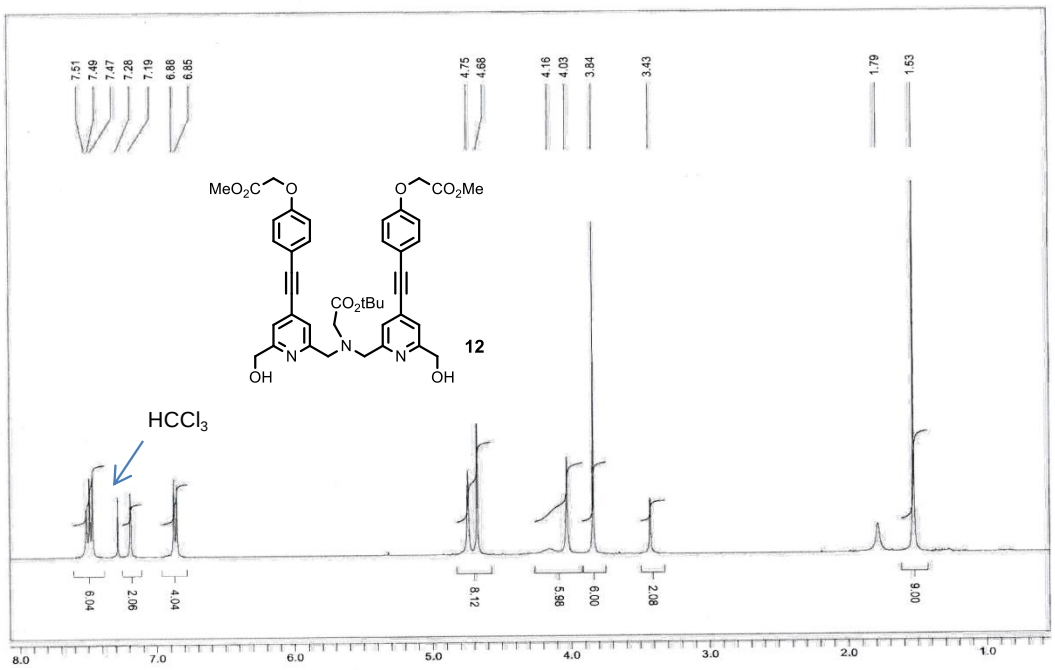


Figure S14. ^{1}H NMR (400MHz, $CDCl_3$) spectrum of compound **12**.

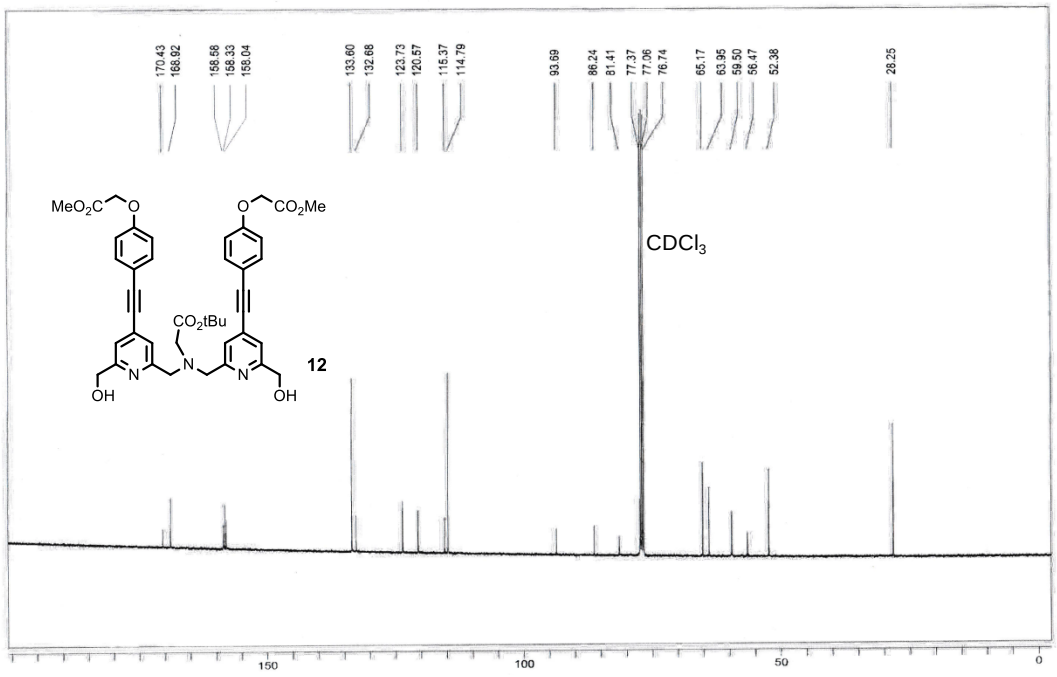


Figure S15. ^{13}C NMR (100MHz, $CDCl_3$) spectrum of compound **12**.

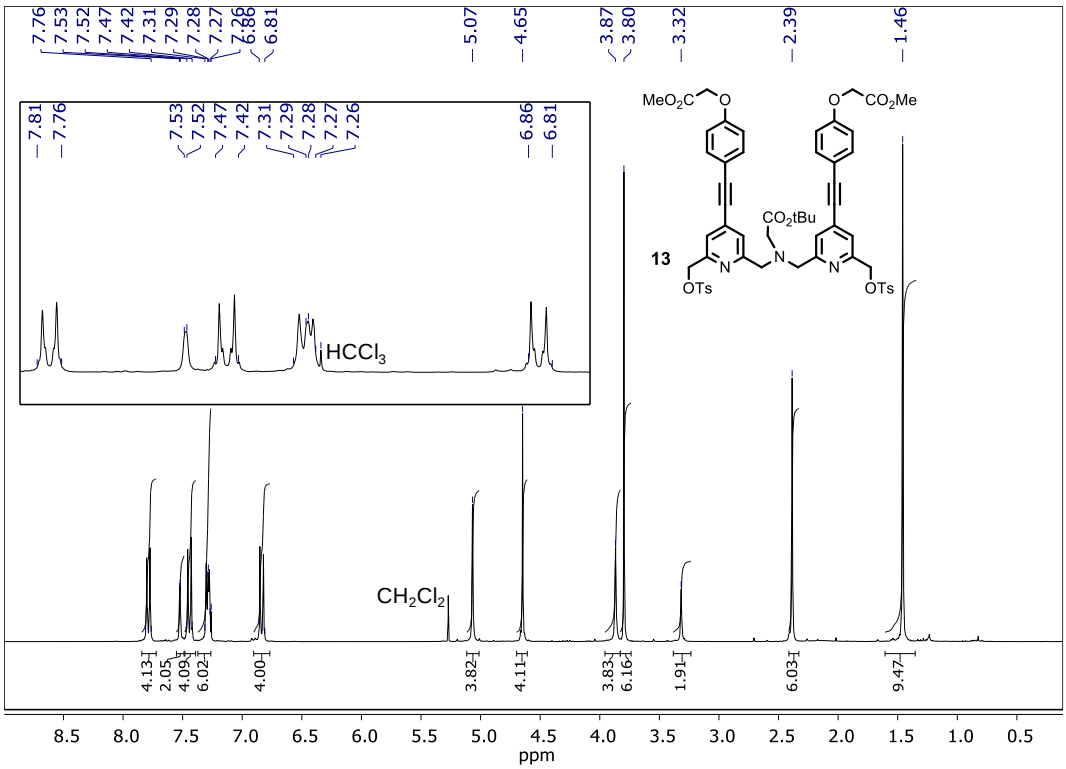


Figure S16. ^{1}H NMR (300MHz, $CDCl_3$) spectrum of compound **13**.

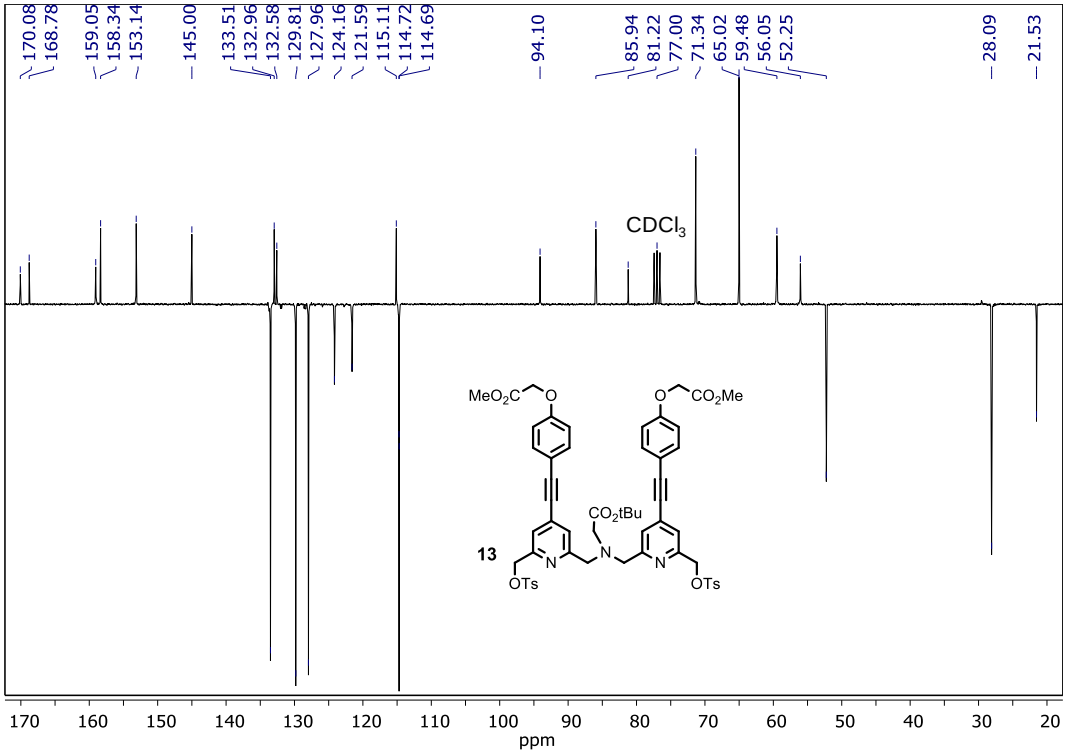
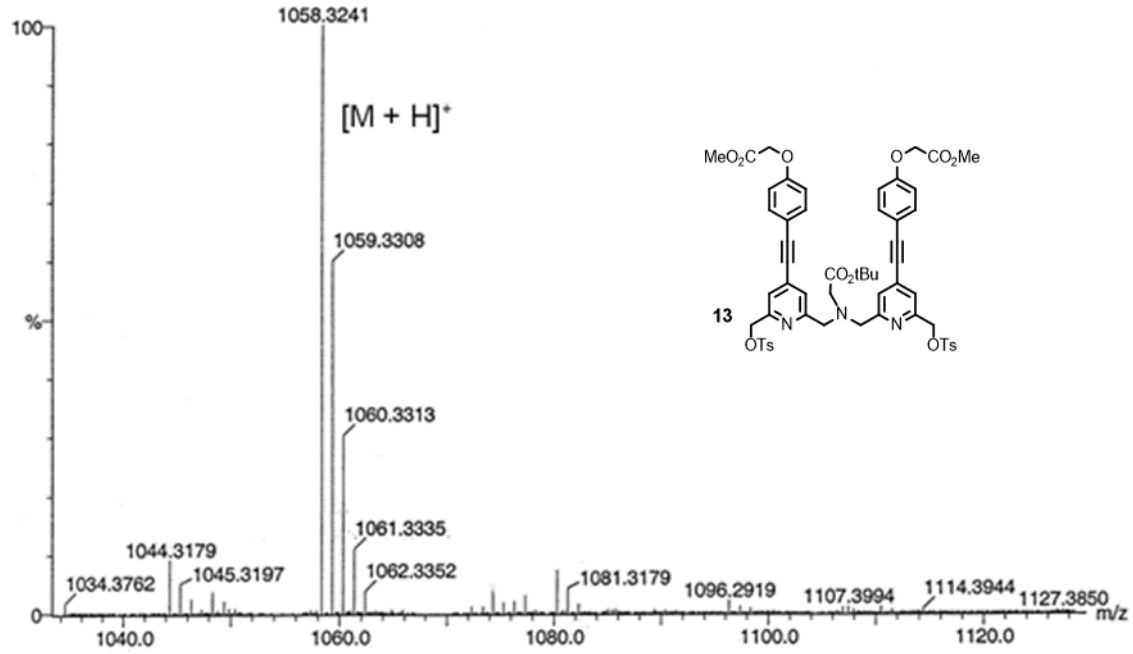


Figure S17. J-modulated ^{13}C NMR (75MHz, $CDCl_3$) spectrum of compound **13**.



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Maximum:		3.0	5.0	70.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1058.3241	1058.3204	3.7	3.5	30.5	81.3	C56 H56 N3 O14 S2
	1058.3282	-4.1	-3.9	35.5	126.5	C58 H52 N5 O13 S
	1058.3237	0.4	0.4	25.5	129.2	C53 H60 N3 O14 S3
	1058.3278	-3.7	-3.5	29.5	283.7	C58 H60 N O12 S3
	1058.3291	-5.0	-4.7	34.5	358.8	C59 H56 N5 O8 S3

Figure S18. HRMS (ESI+) spectrum of compound **13**.

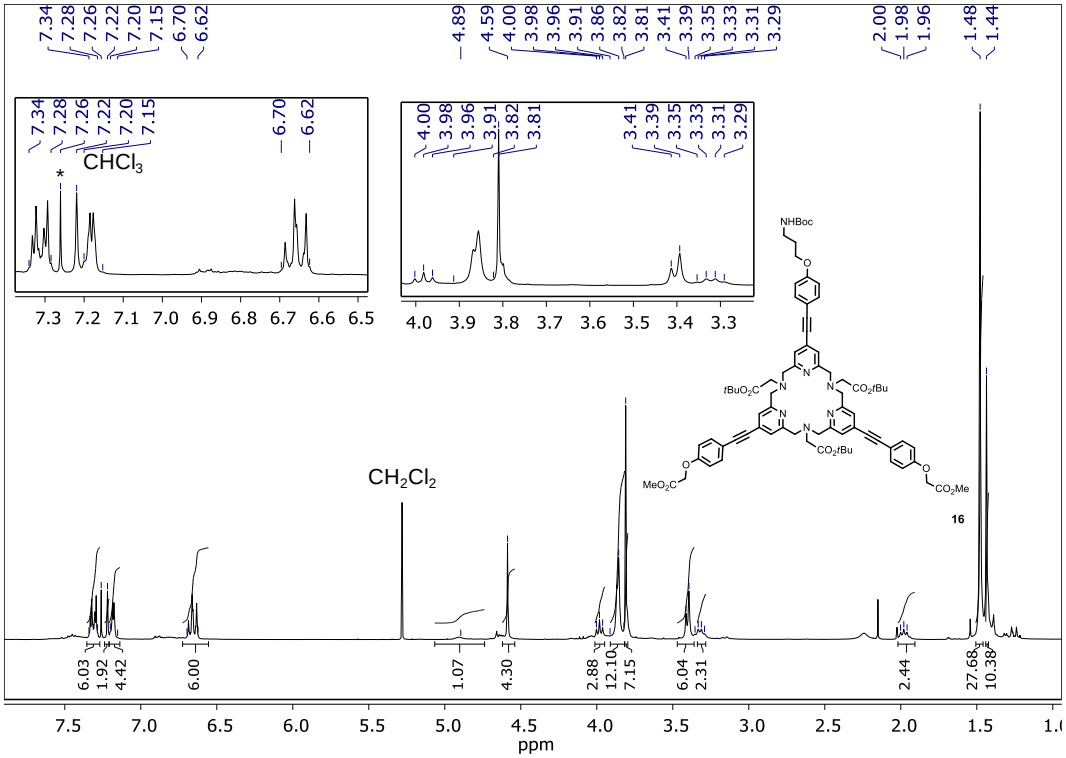


Figure S19. ^{1}H NMR (300MHz, $CDCl_3$) spectrum of compound **16**.

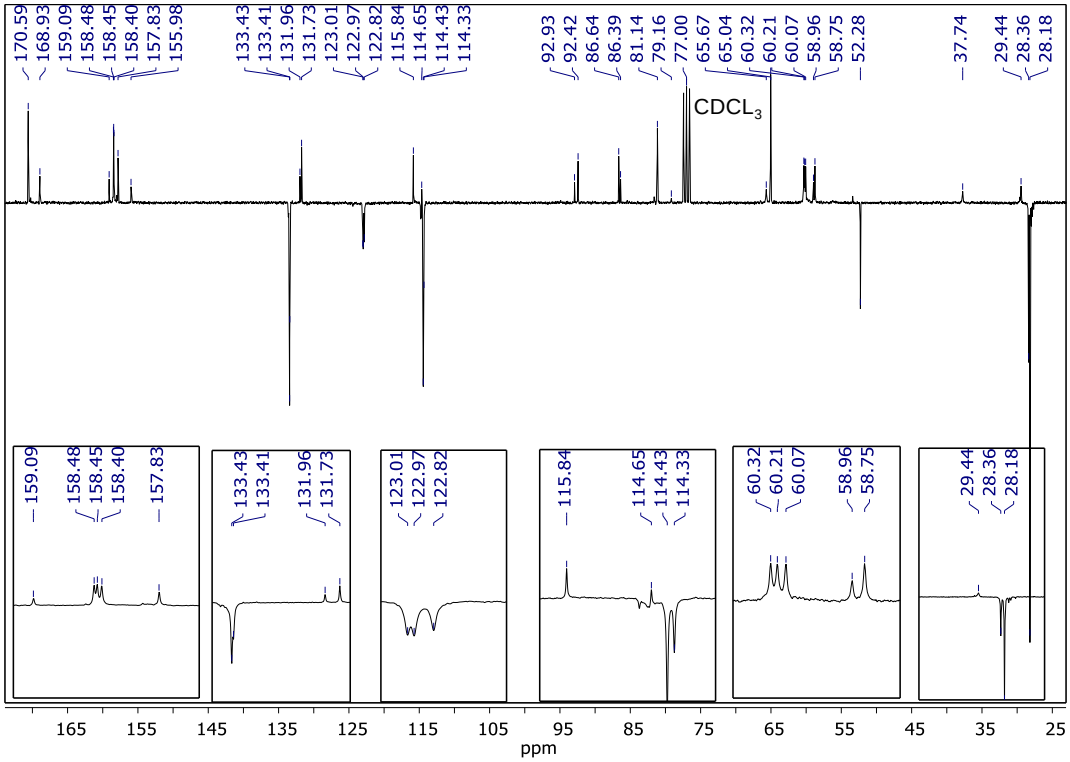


Figure S20. J-modulated ^{13}C NMR (75MHz, $CDCl_3$) spectrum of compound **16**.

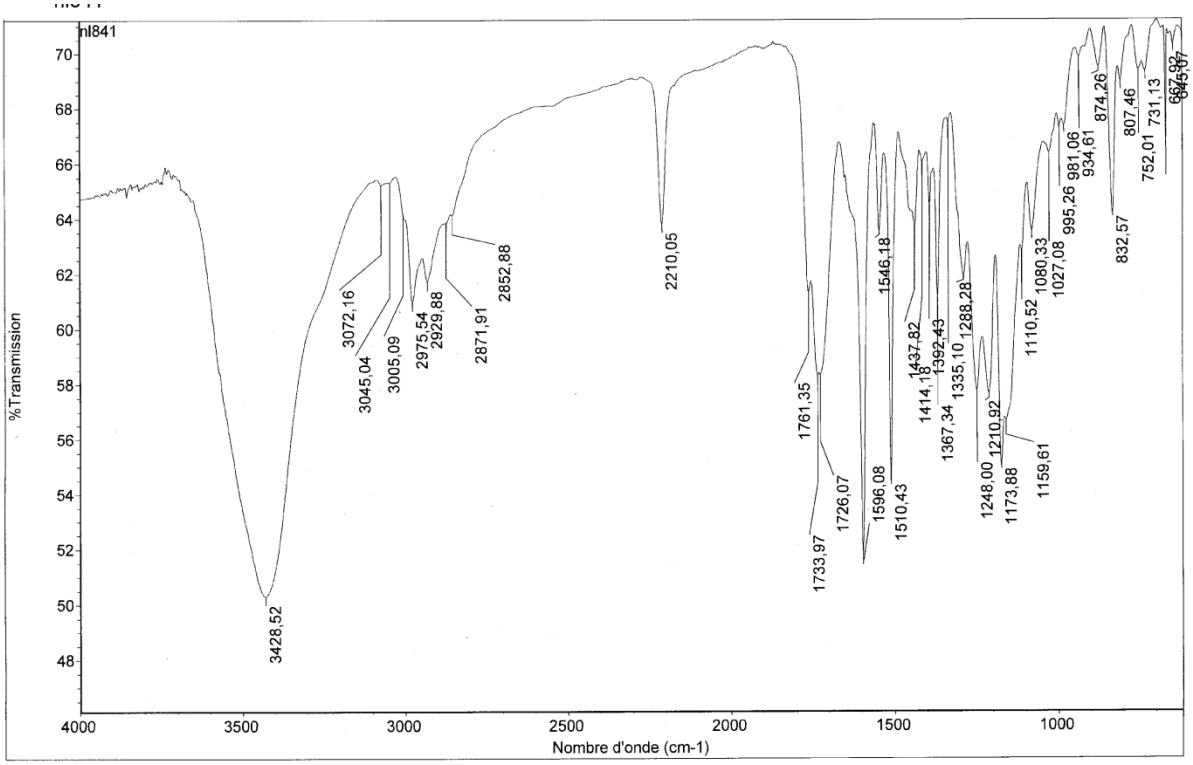
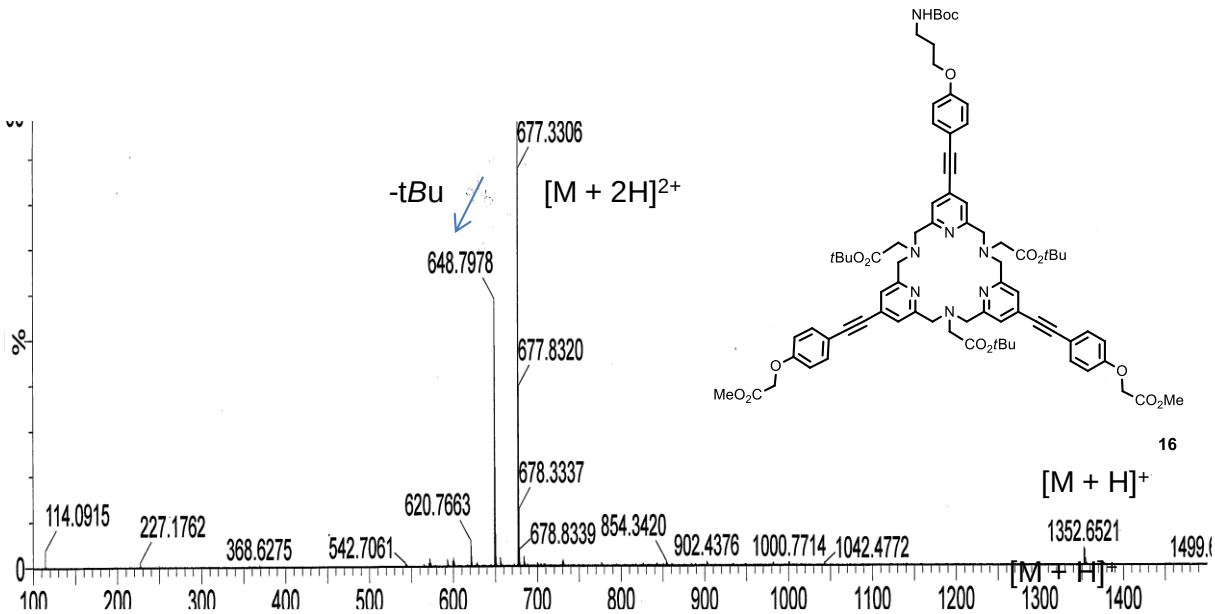


Figure S21. Infrared (neat) spectrum of compound **16**.



Minimum:				-1.5				
Maximum:		2.0	3.0	90.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
1352.6521	1352.6495	2.6	1.9	36.5	89.4	1.382	25.10	C77 H90 N7 O15
	1352.6554	-3.3	-2.4	27.5	88.5	0.492	61.14	C70 H94 N7 O20
	1352.6482	3.9	2.9	31.5	90.0	1.983	13.76	C76 H94 N3 O19

Figure S22. HRMS (ESI+) of compound **16**.

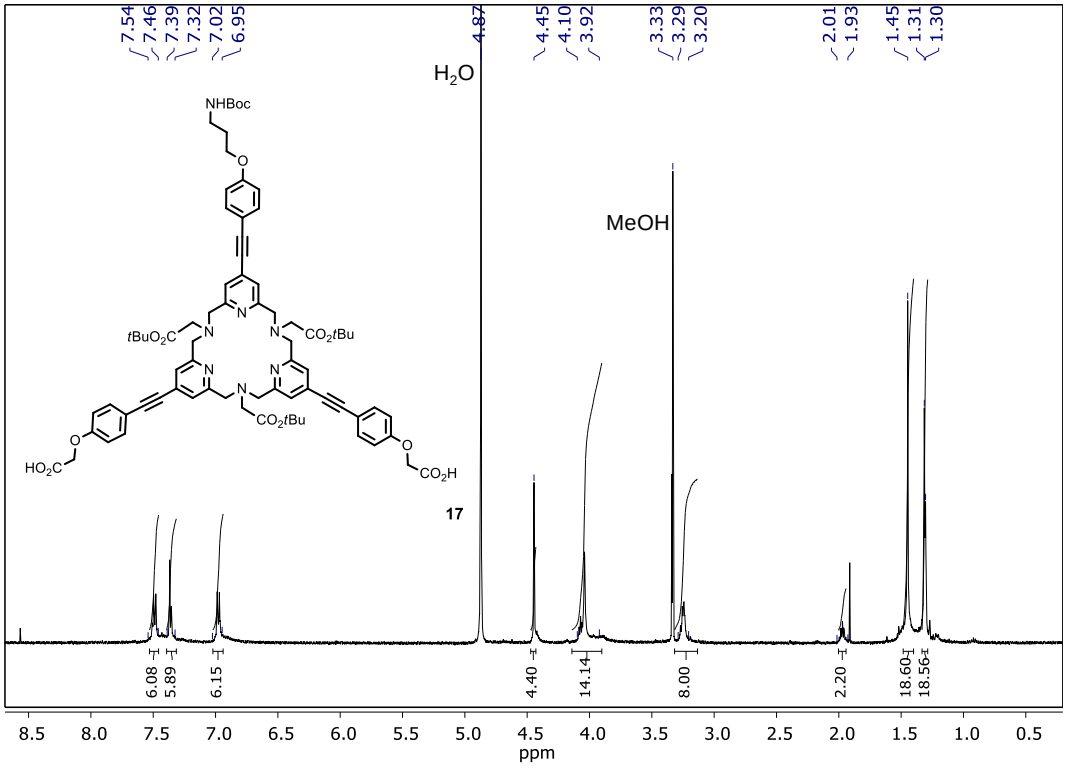


Figure S23. ^{1}H NMR (500MHz, CD_3OD) spectrum of compound **17**.

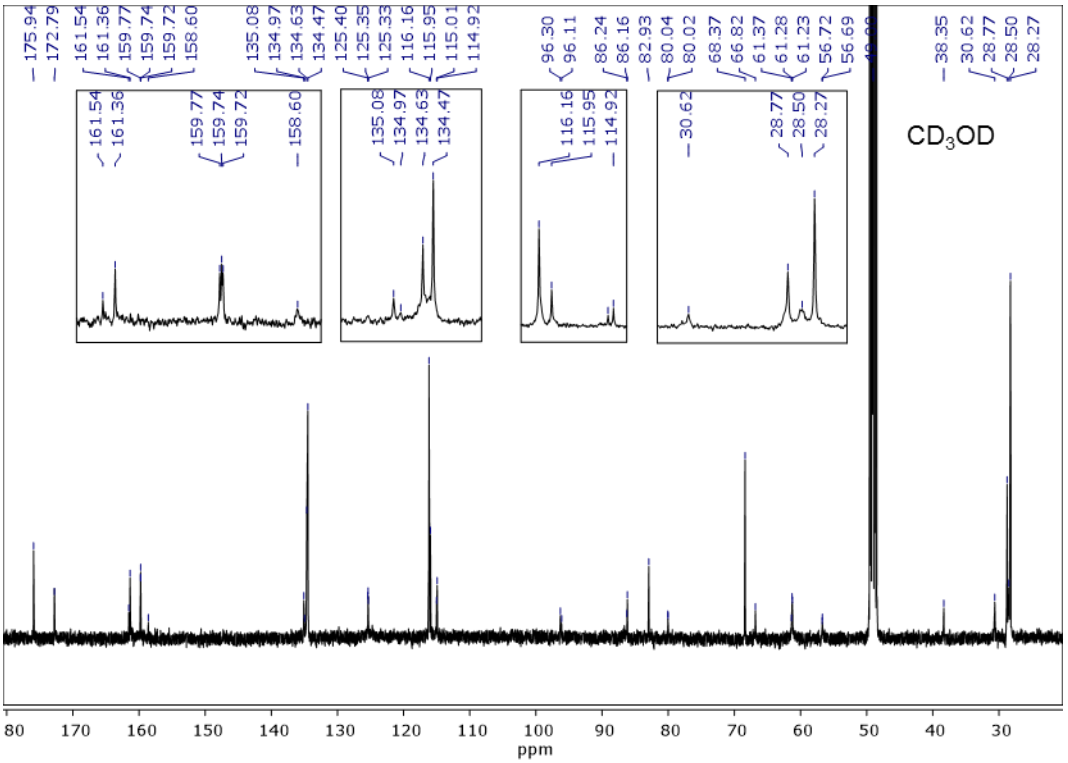


Figure S24. ^{13}C NMR (125MHz, CD_3OD) spectrum of compound **17**

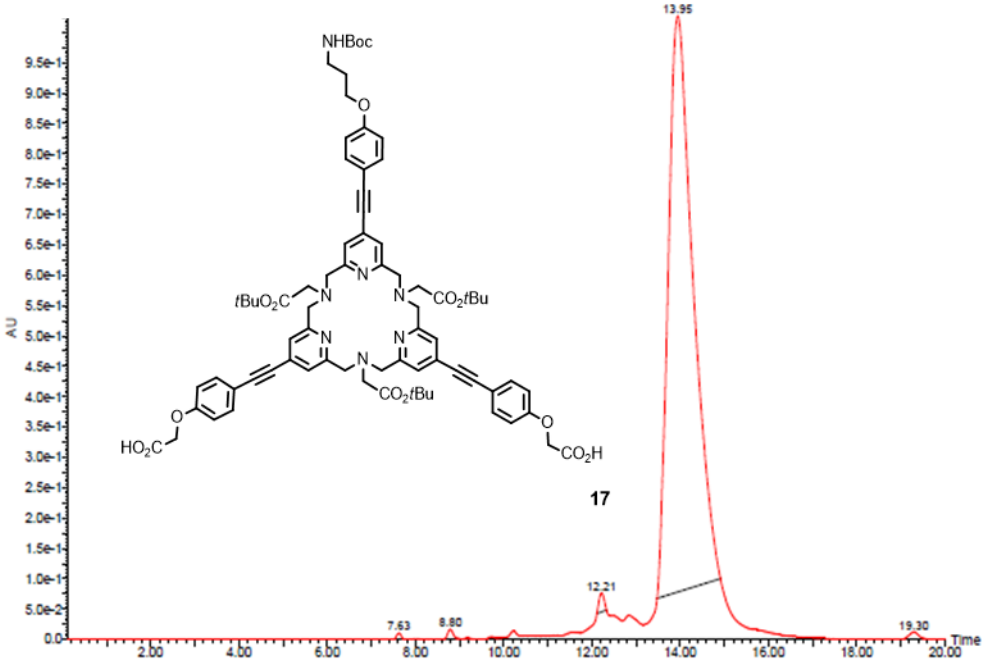


Figure S25. HPLC chromatogram of compound **17** (Conditions: Waters XBridge RP-C18 column, 300 Å, 3.5 μm, 4.6 × 100 mm, flow rate 1 mL/min, eluents A (H_2O with 0.1% formic acid) – B (CH_3CN with 0.1% formic acid), t = 0 min 5% B, t = 15 min 100% B).

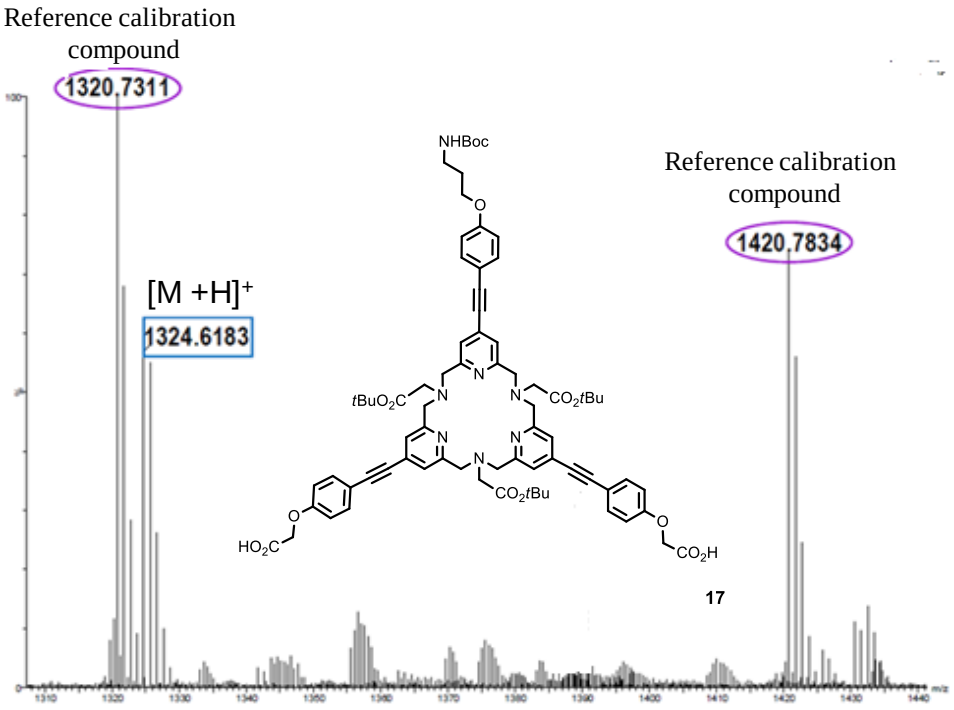


Figure S26. ESI$^+$/HRMS spectrum of compound **17**.

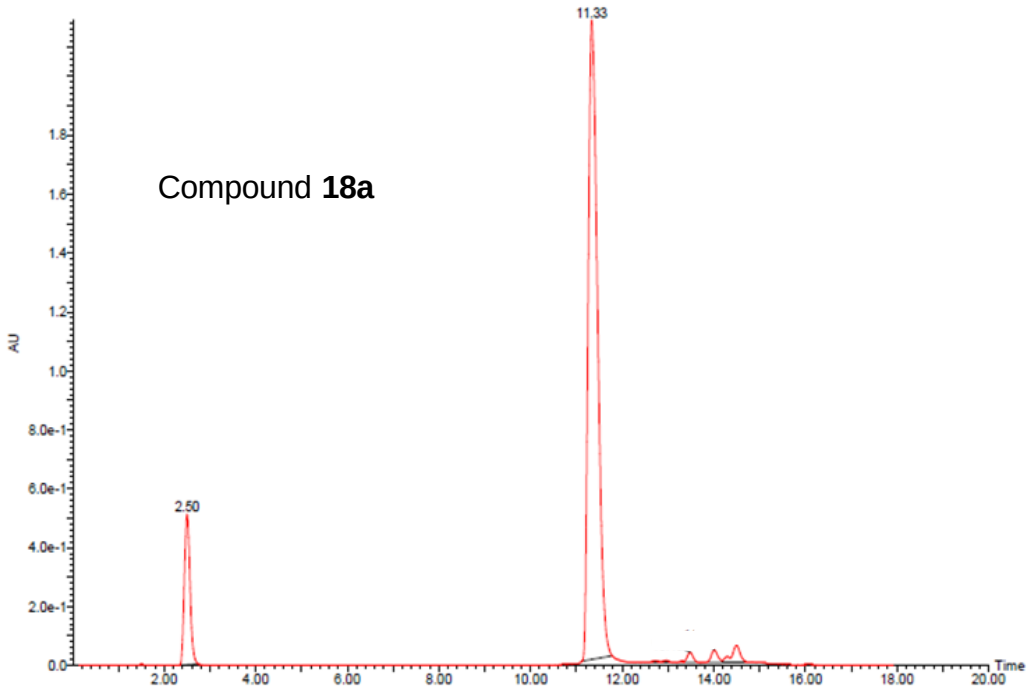


Figure S27. HPLC chromatogram of compound **18a** (Conditions: Waters XBridge RP-C18 column, 300 Å, 3.5 μm, 4.6 × 100 mm, flow rate 1 mL/min, eluents A (H_2O with 0.1% formic acid) – B (CH_3CN with 0.1% formic acid), t = 0 min 5% B, t = 15 min 100% B).

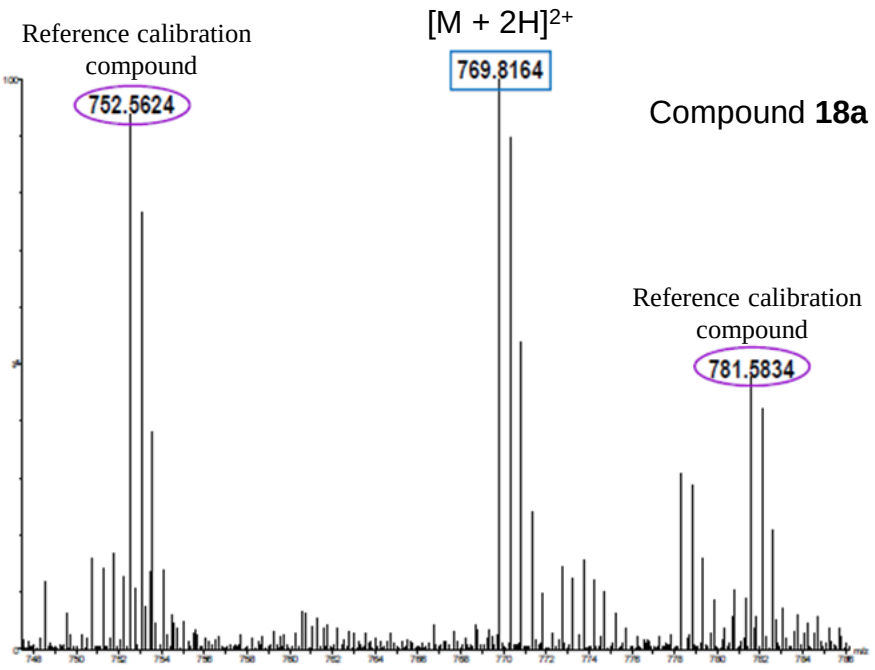


Figure S28. ESI$^+$/HRMS spectrum of compound **18a**.

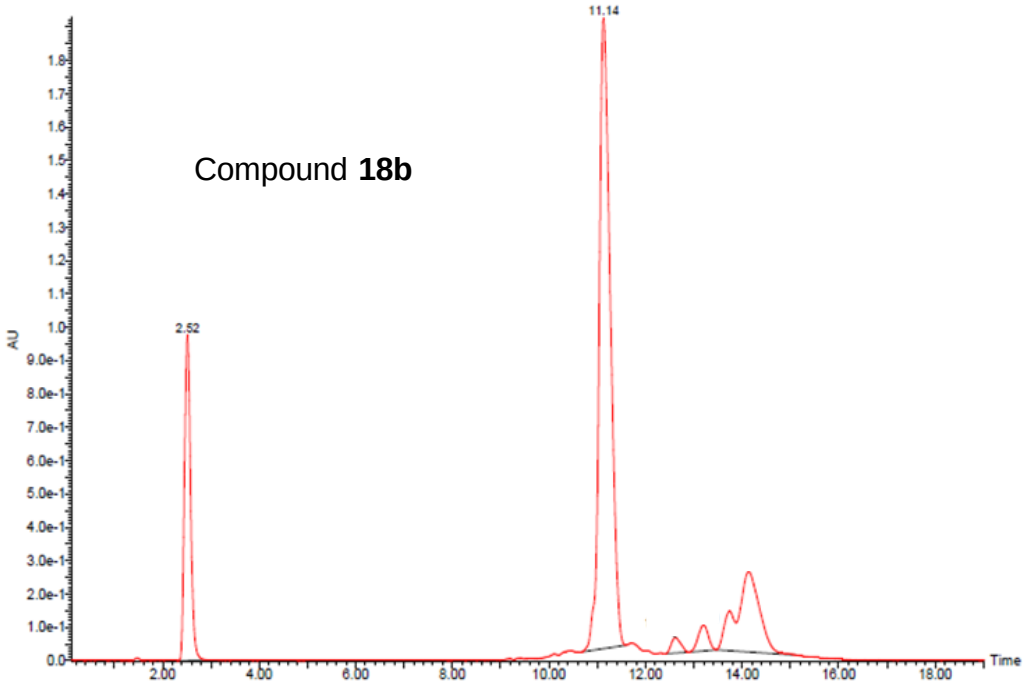


Figure S29. HPLC chromatogram of compound **18b** (Conditions: Waters XBridge RP-C18 column, 300 Å, 3.5 μm, 4.6 × 100 mm, flow rate 1 mL/min, eluents A (H_2O with 0.1% formic acid) – B (CH_3CN with 0.1% formic acid), *t* = 0 min 5% B, *t* = 15 min 100% B).

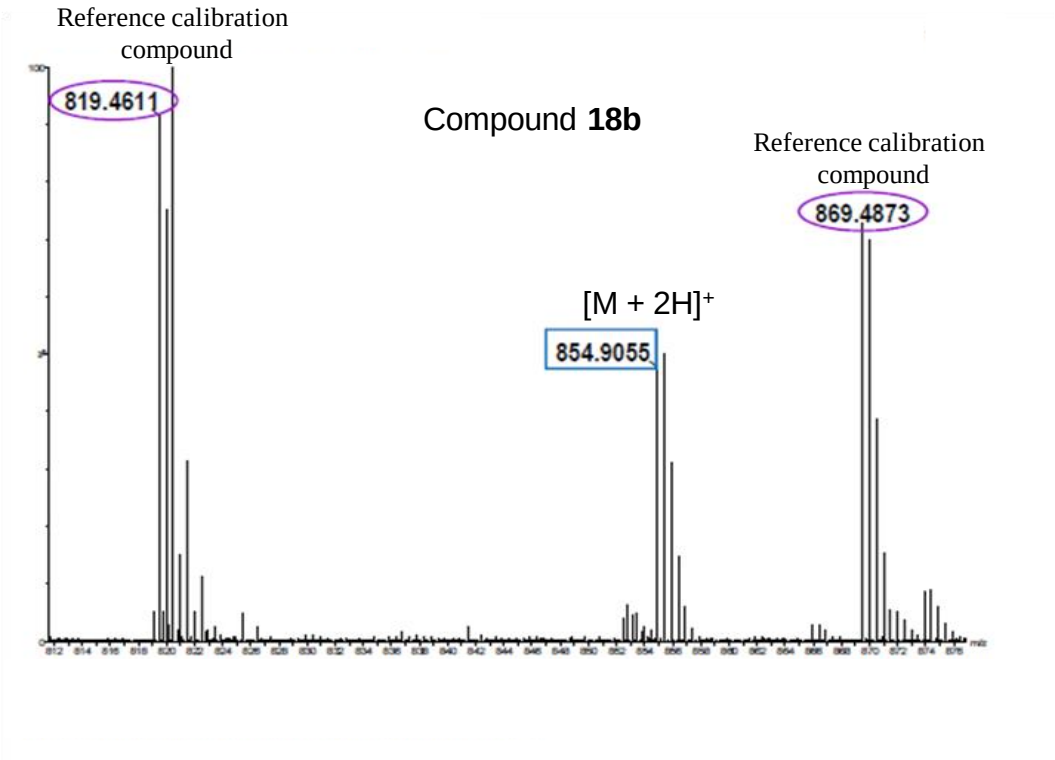


Figure S30. ESI$^+$/HRMS spectrum of compound **18b**.

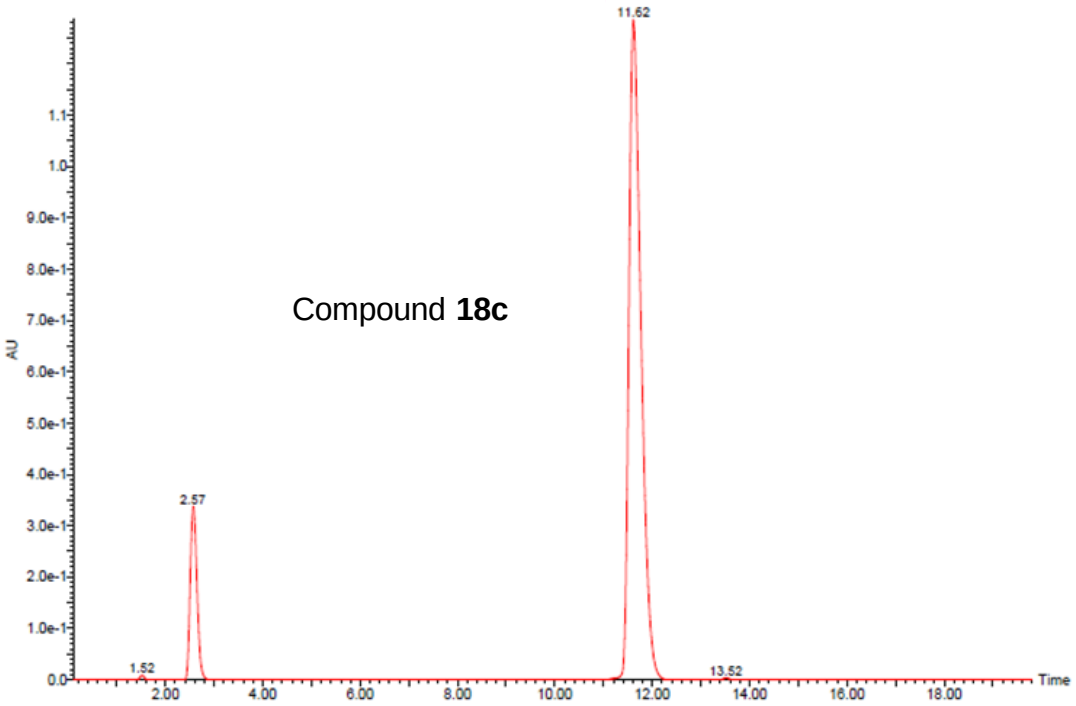


Figure S31. HPLC chromatogram of compound **18c** (Conditions: Waters XBridge RP-C18 column, 300 Å, 3.5 μm, 4.6 × 100 mm, flow rate 1 mL/min, eluents A (H_2O with 0.1% formic acid) – B (CH_3CN with 0.1% formic acid), t = 0 min 5% B, t = 15 min 100% B).

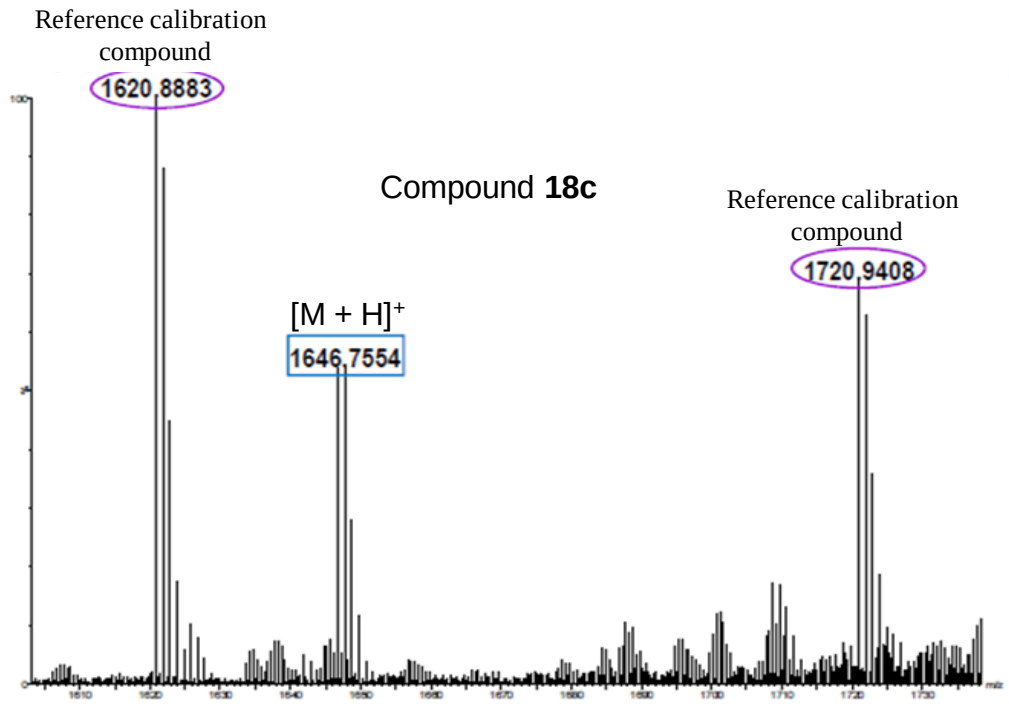


Figure S32. ESI$^+$/HRMS spectrum of compound **18c**.

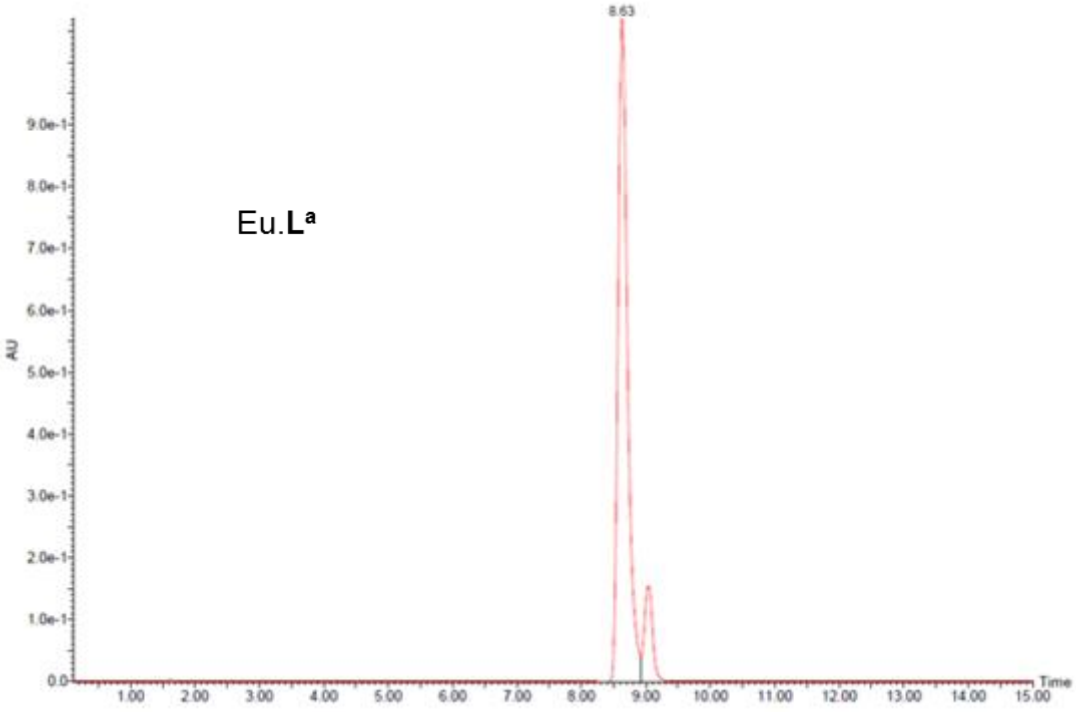


Figure S33. HPLC chromatogram of Eu.**L**^{a} (Conditions: Waters Xbridge C_{18}, 300 Å, 3.5 µm, 4,6 x 100 mm, flow rate 1 mL/min, eluents A (H_2O with ammonium acetate 5 mM, pH 6.5), – B (CH_3CN), t = 0 min 2% B, t = 15 min 40% B).

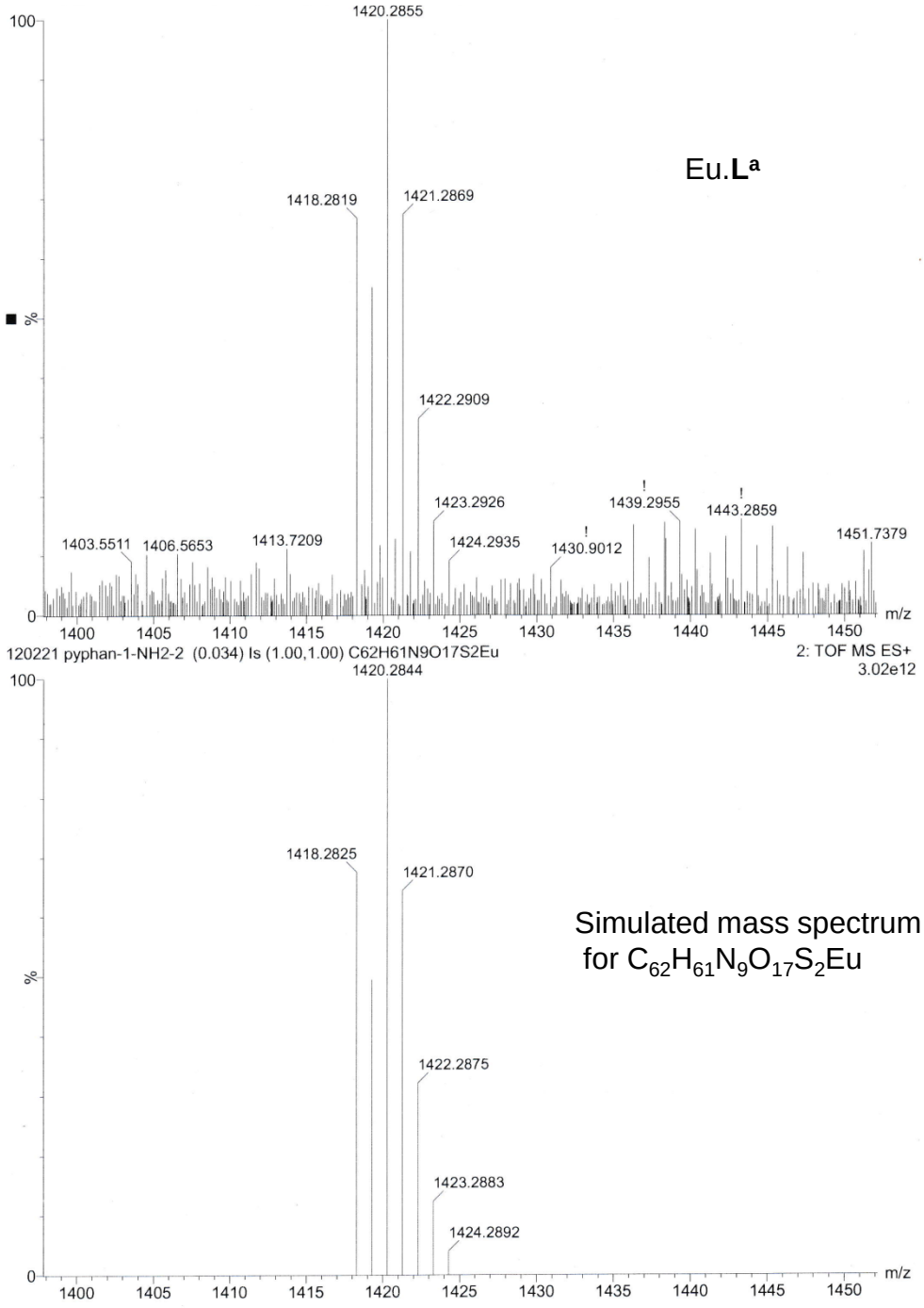


Figure S34. ESI$^+$/HRMS spectrum (measured and calculated pattern) of Eu.**L**^{a} complex

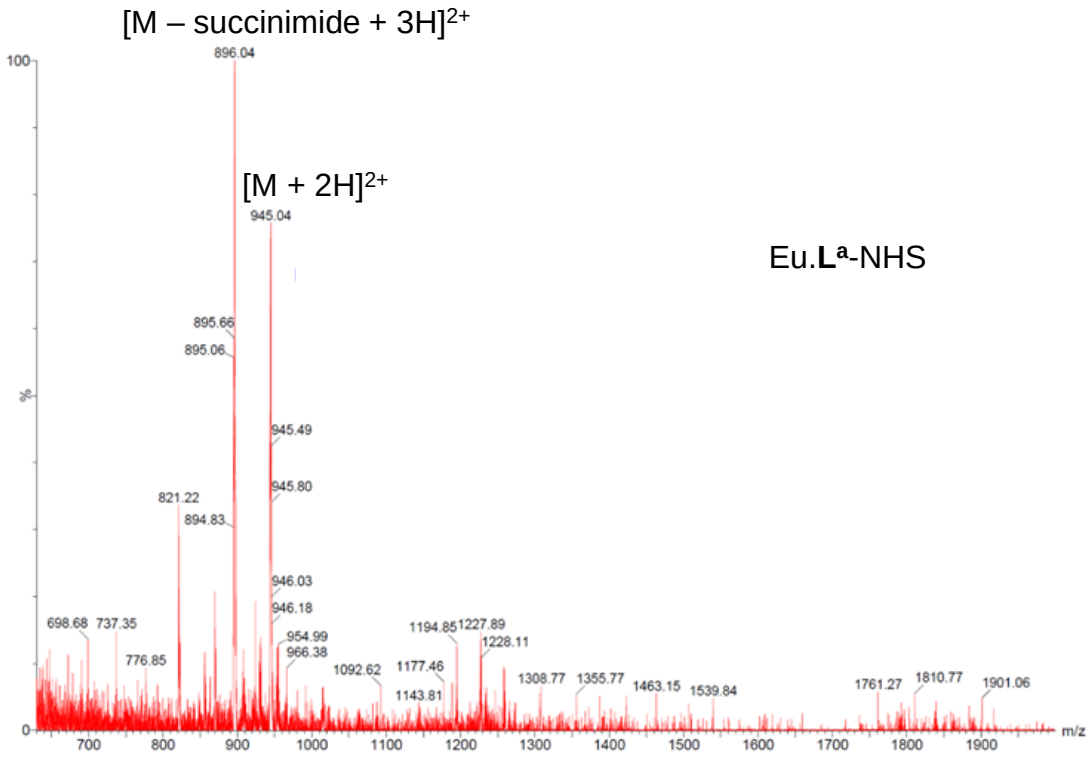


Figure S35. ESI$^+$/MS spectrum of compound Eu.**L^a**-NHS.

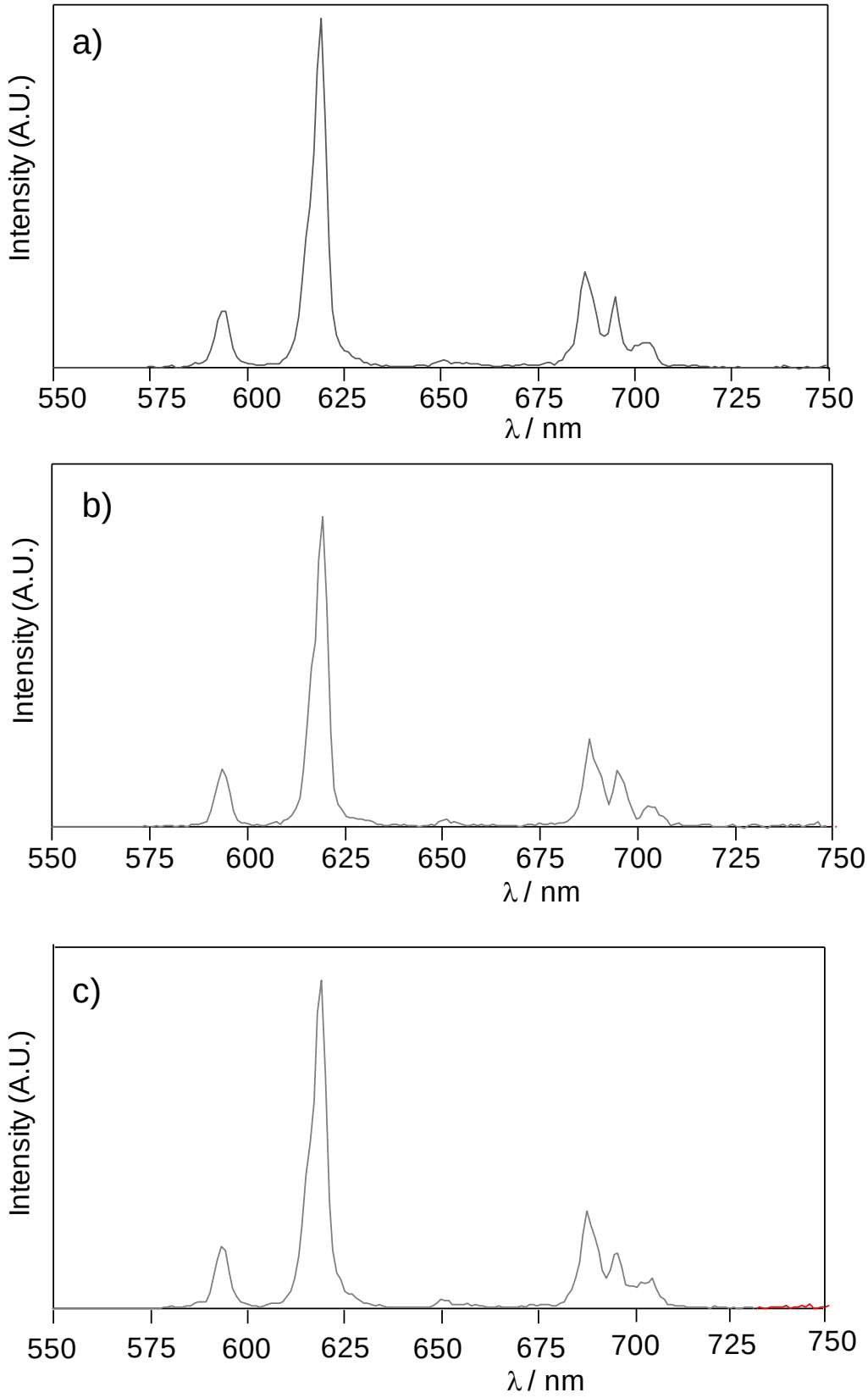


Figure S36. Corrected emission spectrum of Eu.**L**^{a} (a), Eu.**L**^{b} (b) and Eu**L**^{c} (c) in Tris buffer (pH 7.4) at 298 K, λ_{exc} = 337 nm, and excitation / emission band passes 5 / 2.5 nm.

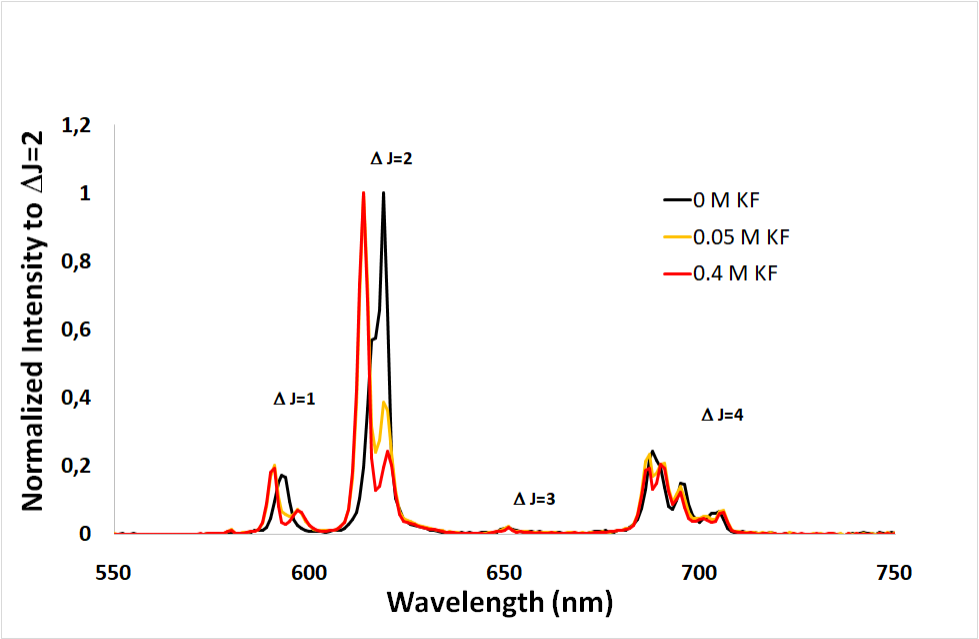


Figure S37. Spectral changes of Eu.**L**[a] upon addition of KF. Spectra were recorded in a 0.05 M Phosphate buffer at pH7.0 + 0.1% BSA (λ_{exc} = 337 nm, excitation and emission band passes 20 and 1.5 nm, respectively). The highest intensity bands were set at 1 to exemplify the changes in the ΔJ=2 band.

Table S1. Measured and **c**alculated photophysical parameters for Eu.**L**^{a}, Eu.**L**^{b} and Eu.**L**^{c} complexes in Tris buffer (pH 7.4) at 298 K.

	Eu.**L**^{a}	Eu.**L**^{b}	Eu.**L**^{c}
$I_{\Sigma Fj} / I_F$	10.1	10.1	10.1
k_r (s^{-1})	349	348	348
k_{obs} (s^{-1})	1470	1449	1408
Φ_{Eu} (%)	24.0	24.0	24.7
η_{sens} (%)	59.0	54.0	59.5
Φ_{ov} (%)	14.0	13.0	14.7
Σk_{nr} (s^{-1})	1121	1101	1060

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