Supporting Information

for

PYRROC: the first functionalized cycloalkyne that facilitates isomer-free generation of organic molecules by SPAAC

Corinna Gröst and Thorsten Berg*

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Supporting Figures and Schemes

Figure S1: NMR-based Kinetic Experiments

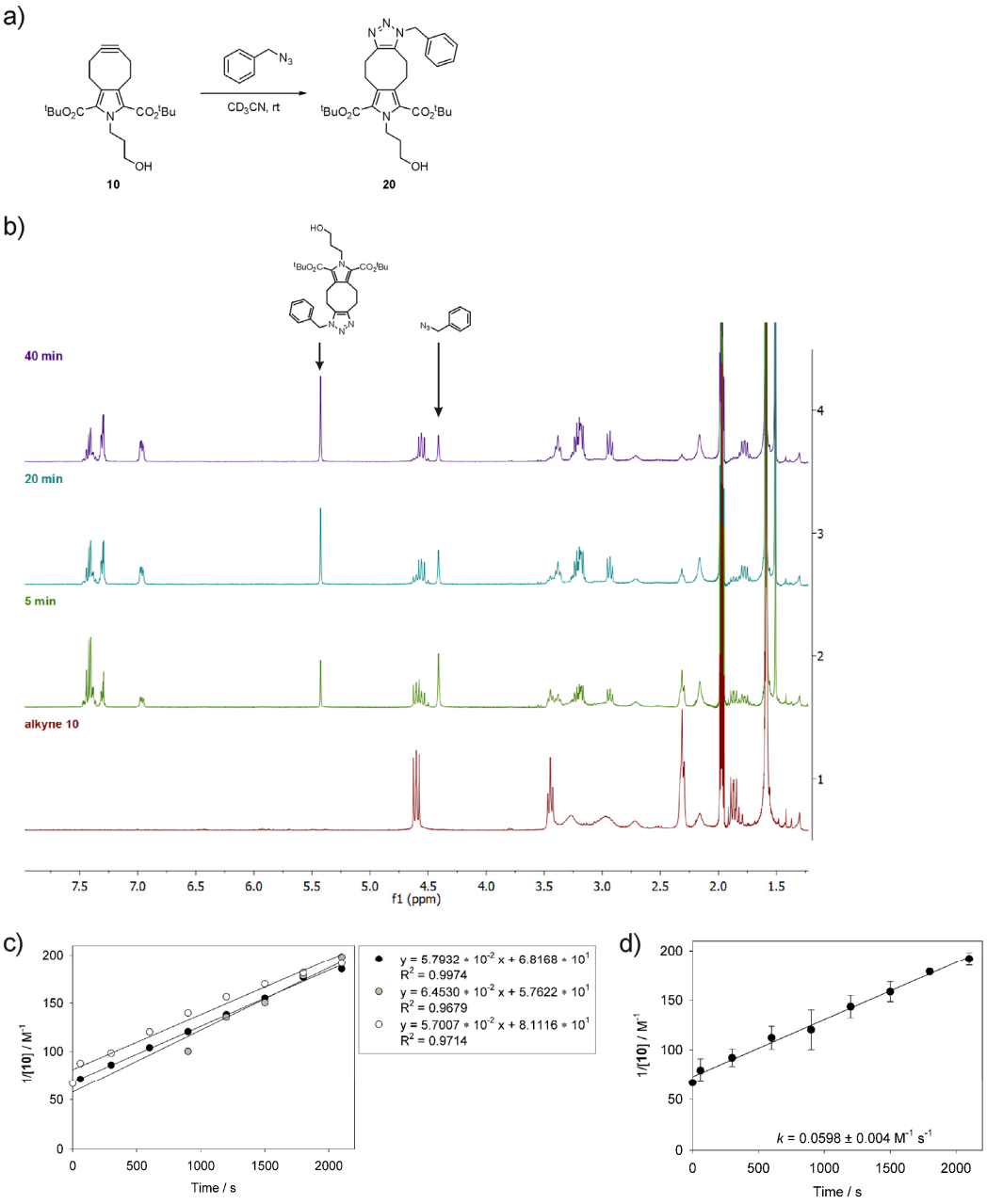


Figure S1. Determination of the second-order rate constant for the reaction between PYRROC (**10**) and benzyl azide by ^{1}H-NMR. a) Reaction scheme. b) ^{1}H-NMR spectra of **10** (bottom panel), and of the reaction between **10** and benzyl azide at the indicated time points after addition of benzyl azide (5 min, 20 min, and 40 min) (upper panels). Progress of the reactions is indicated by the decrease of the signal of the methylene protons of benzyl azide at 4.4 ppm, and the concomitant increase of the signal of the methylene protons of **20** at 5.4 ppm. c) Data from three replicate experiments are shown. The two reactants were dissolved in CD_3CN at 30 mM concentrations each and mixed together in a 1:1 ratio. The percent conversion was determined via integration of the methylene protons of benzyl azide relative to the methylene protons of the triazole product **20**. The second-order rate constant k_2 (mol^{-1} s^{-1}) for each individual experiment was determined by plotting 1/[**10**] versus time. The plot was fitted to a linear regression. The slope corresponds to k_2. d) Average values of 1/[**10**] and standard deviations from the three individual experiments as shown in c) are plotted against time. The second-order rate constant k_2 was calculated as the average of the three experiments shown in c).

Figure S2: Kinetic analysis of the reaction between PYRROC-BODIPY-FL (13) and TAMRA azide (14)

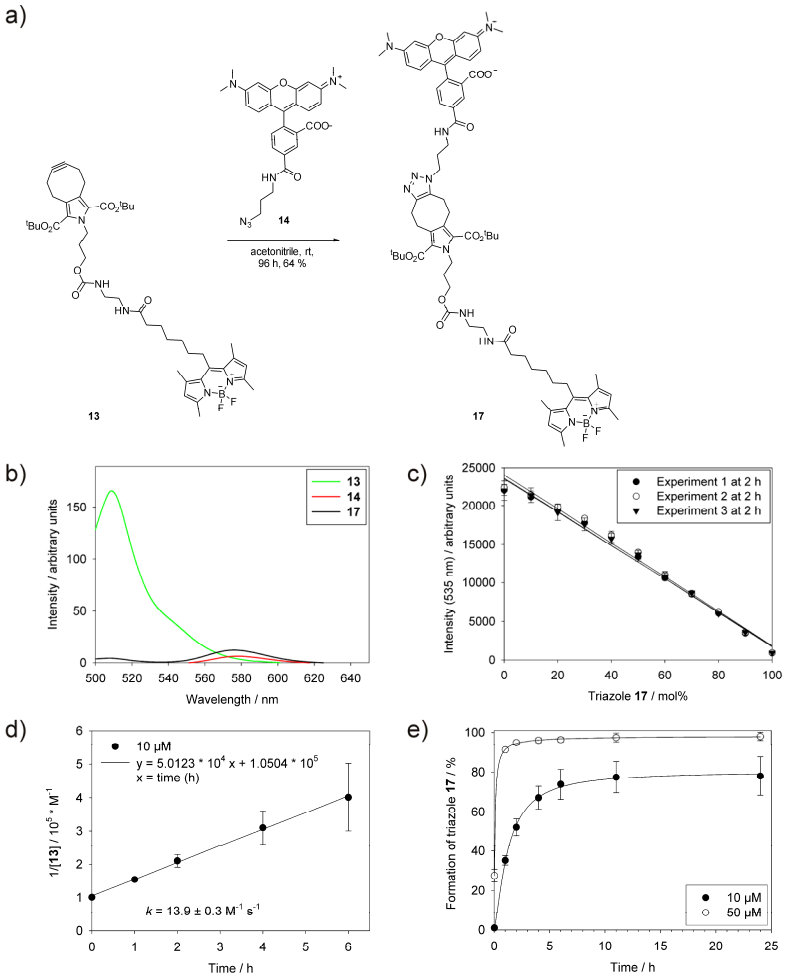


Figure S2. Determination of the second-order rate constant for the reaction of BODIPY-FL-labeled PYRROC (**13**) in reaction with 5-TAMRA azide (**14**) in PBS. a) Reaction scheme leading to triazole **17**. b) Fluorescence spectra of 10 µM **13** (light green), 10 µM 5-TAMRA azide (**14**) (red) and 10 µM triazole **17** (black) after excitation at 485 nm. Spectra were read on a JASCO fluorescence spectrometer. The conversion of **13** to **17** is characterized by a strong decrease in the fluorescence intensity of BODIPY-FL, and a comparatively minor increase in the fluorescence intensity of TAMRA, illustrating the large quantum yield of BODIPY-FL. The decrease of the BODIPY-FL emission was chosen as a read-out for further experiments. c) Standard curves mimicking the progression of the reaction between 10 µM **13** and 10 µM **14** to yield 10 µM of **17**. Fluorescence spectra of the mixtures were taken on a Tecan Infinite F500 plate reader using an excitation filter of 485 nm and an emission filter of 535 nm. The data were fit to a linear curve, which was then used to convert fluorescence intensities of the actual reaction mixtures at 535 nm to percent conversion of reactants **13** and **14** to product **17**. Each time point has its own set of standard curves. As an example, the standard curves of the three independent experiments at the 2 h time point are given. Due to different amplification factors of the two instruments used in b) and c), the scaling of the intensities on the y-axis cannot be compared between b) and c). d) Determination of the second-order rate constant using **13** and **14** at a final concentration of 10 µM of each component in PBS containing 2 % of DMSO. The percentage of conversion was determined by measuring the fluorescence intensity at 535 nm and converting to percent conversion via the standard curve of the same time point. The concentration of **13** was calculated using the equation [**13**] = 10 µM – [mol% **17** / 100 x 10] µM. The second-order rate constant k (M^{-1} s^{-1}) was determined by plotting 1/[**13**] versus time. The plot was fit to a linear regression. The slope corresponds to k. The experiment was performed in triplicate. e) Time course of the reaction between **13** and **14** to yield triazole **17** at 10 µM and 50 µM. For the experiment using 50 µM of reactants, the same procedure was carried out as illustrated in c) and d) for 10 µM. At 50 µM, the reaction was too rapid to allow for determination of the second-order rate constant.

Figure S3: Kinetic analysis of the reaction between PYRROC-BODIPY-FL (13) and BODIPY-TMR azide (15)

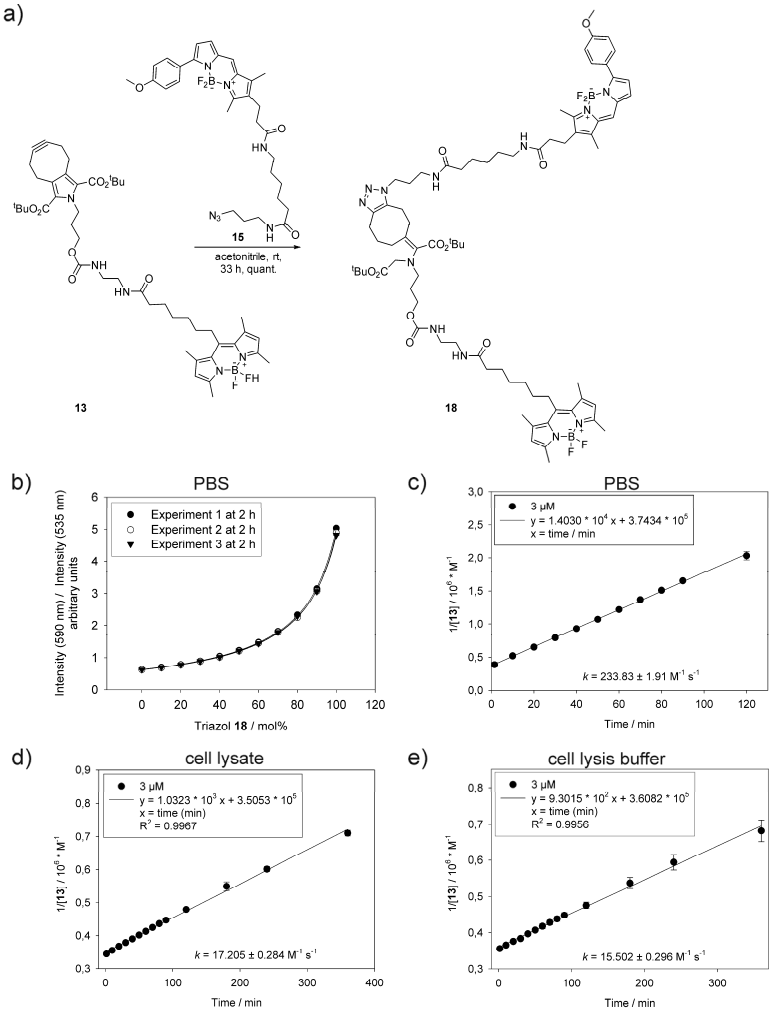


Figure S3. Determination of the second-order rate constant of the reaction between BODIPY-FL-labeled PYRROC (**13**) and BODIPY-TMR azide (**15**) in PBS. a) Reaction scheme leading to triazole **18**. b) Standard curves mimicking the progression of the reaction between 3 µM **13** and 3 µM **15** to yield 3 µM of **18**. Fluorescence spectra of the mixtures were taken on a Tecan Infinite F500 plate reader using an excitation filter of 485 nm and emission filters of 535 nm and 590 nm. The gain factors (an internal amplification factor of the instrument to allow for minimal instrument error) were adjusted to be optimal for each individual emission channel, resulting in arbitrary units of the y-axis. The ratios [Intensity (590 nm) / Intensity (535 nm)] were plotted against time. Each time point has its own set of standard curves. As an example, the standard curves of the three independent experiments at the 2 h time point are given. c) Determination of the second-order rate constant using **13** and **15** at a final concentration of 3 µM of each component in PBS containing 2 % of DMSO. The percentage of conversion was determined by measuring the fluorescence intensities at 535 nm and at 590 nm, calculating the ratio [Intensity (590 nm) / Intensity (535 nm)], and converting the ratio to percent conversion to the product **18** via the standard curve of the same time point. From this, the concentration of **13** was calculated using the equation [**13**] = 3 µM – [mol% **18** / 100 x 3] µM. The second-order rate constant k (M^{-1} s^{-1}) was determined by plotting 1/[**13**] versus time. The plot was fit to a linear regression. The slope corresponds to k. The experiment was performed in triplicate. d) Determination of the second-order rate constant using **13** and **15** at a final concentration of 3 µM of each component in cell lysates containing 2 % of DMSO. The procedures to convert fluorescence data into concentrations of **13** were analogous to those described for PBS in b). e) Determination of the second-order rate constant using **13** and **15** at a final concentration of 3 µM of each component in cell lysis buffer containing 2 % of DMSO. The procedures to convert fluorescence data into concentrations of **13** were analogous to those described for PBS in b).

Figure S4: Kinetic analysis of the reaction between PYRROC-BODIPY-FL (13) and 3-azido-7-hydroxycoumarin (16)

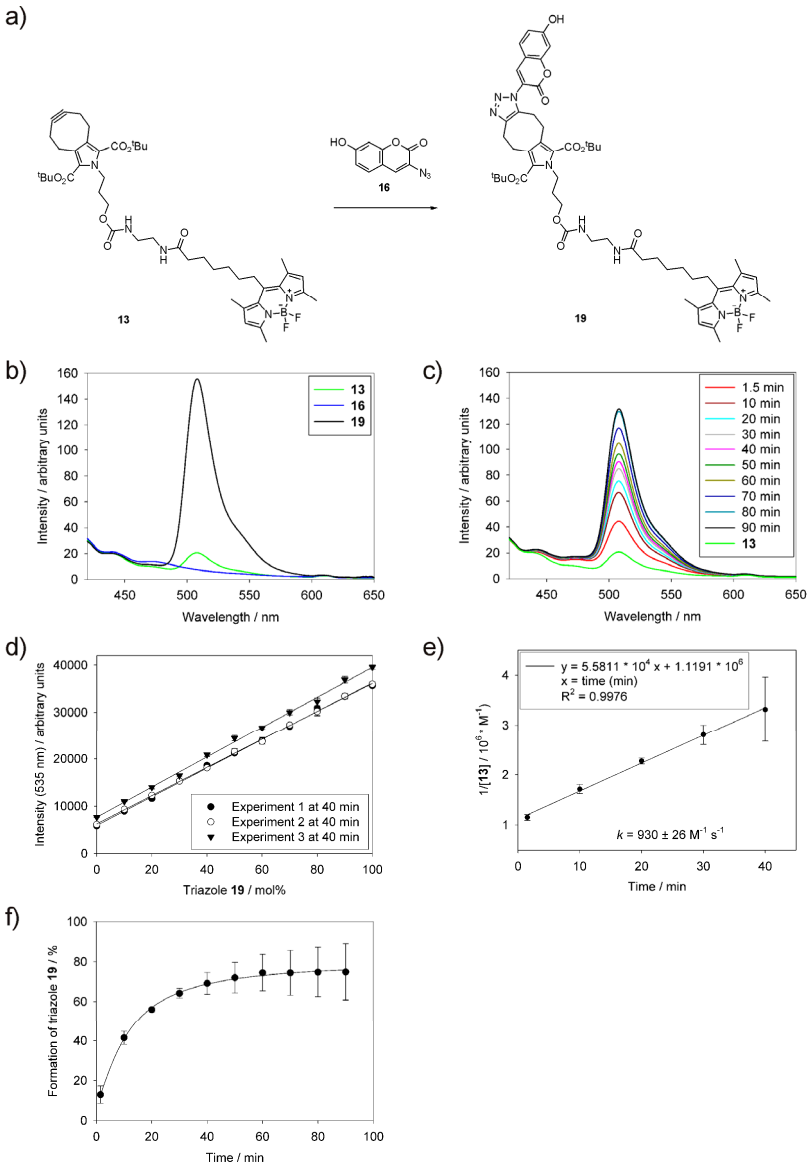


Figure S4. Determination of the second-order rate constant of **13** in reaction with 3-azido-7-hydroxycoumarin (**16**) in PBS. a) Reaction scheme leading to triazole **19**. b) Fluorescence of 1 µM **13** (light green), 1 µM **16** (blue), and 1 µM triazole **19** (black) upon excitation at 405 nm was recorded on a JASCO fluorescence spectrometer. c) Progress of the reaction using 1 µM of **13** and 1 µM of **16** was monitored on a JASCO fluorescence spectrometer. c) Standard curves mimicking the progression of the reaction between 1 µM **13** and 1 µM **16** to yield 1 µM of **19**. Fluorescence spectra of the mixtures were taken on a Tecan Infinite F500 plate reader using an excitation filter of 405 nm and an emission filter of 535 nm. The data were fit to a linear curve. Each time point has its own set of standard curves. As an example, the standard curves of the three independent experiments at the 40 min time point are given. Due to different amplification factors of the two instruments used in b),c) versus d), the scaling of the intensities on the y-axis cannot be compared between b), c) and d). e) Determination of the second-order rate constant using **13** and **16** at a final concentration of 1 µM of each component in PBS containing 2 % of DMSO. The percentage of conversion to the triazole **19** was determined by measuring the fluorescence intensity at 535 nm and converting it to percent conversion via the standard curve of the corresponding time point. The concentration of **13** was calculated using the equation [**13**] = 1 µM – [mol% **19** / 100] µM. The second-order rate constant k (M^{-1} s^{-1}) was determined by plotting 1/[**13**] versus time. The plot was fit to a linear regression. The slope corresponds to k. The experiment was performed in triplicate. f) Time course of the reaction between **13** and **16** to yield triazole **19** at 1 µM.

Figure S5: Kinetic analysis of the reaction between PYRROC (10) and 3-azido-7-hydroxycoumarin (16)

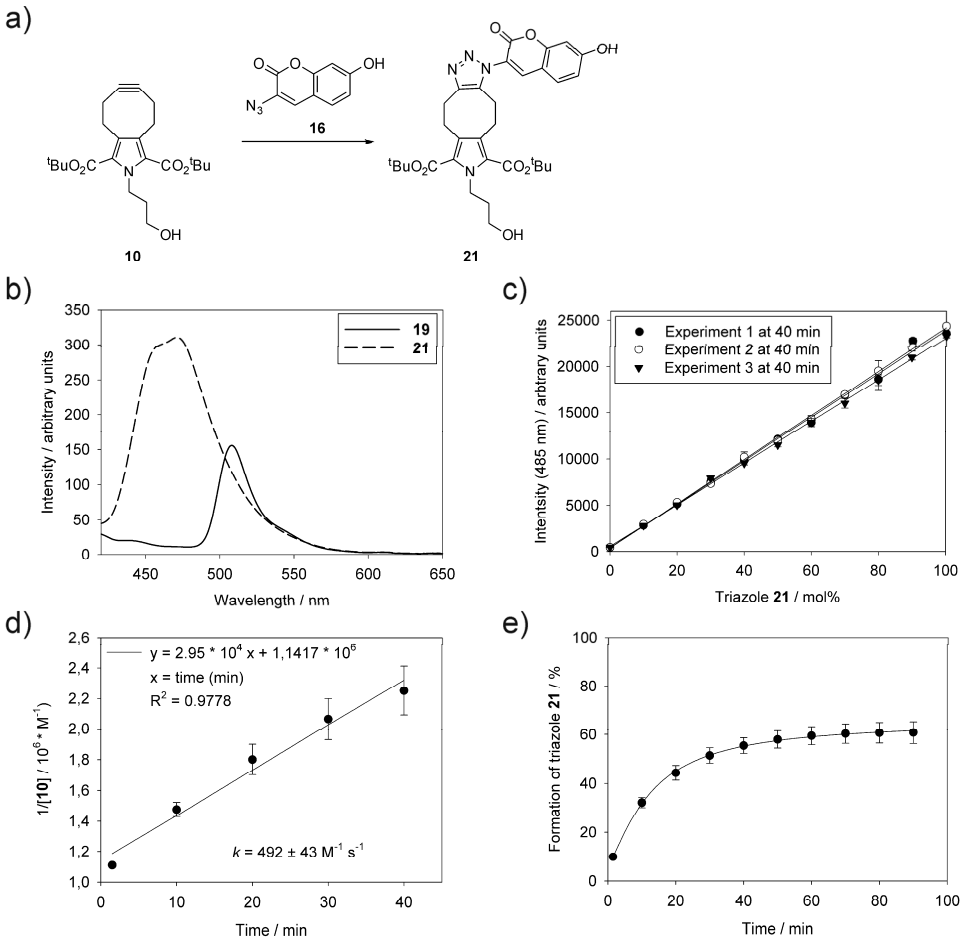
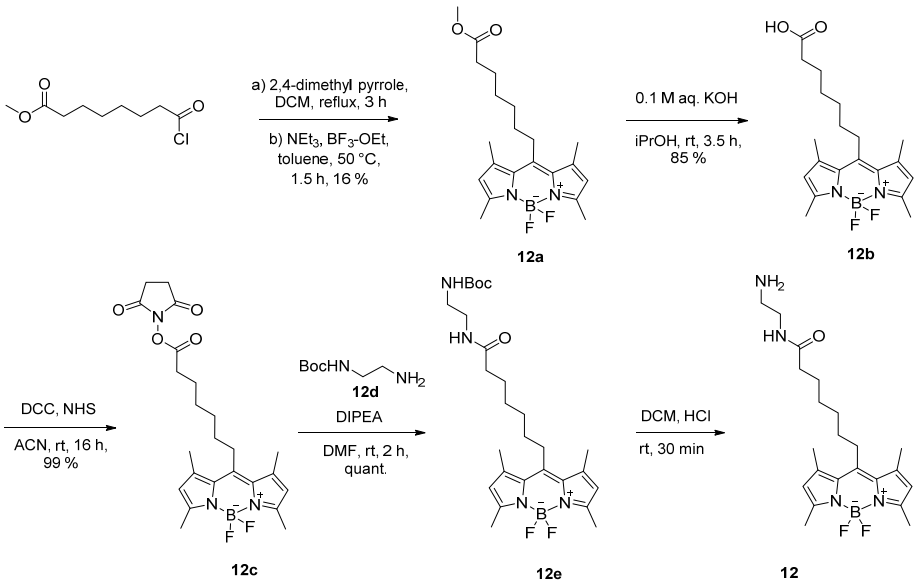


Figure S5. Determination of the second-order rate constant of **10** in reaction with 3-azido-7-hydroxycoumarin (**16**) in PBS. a) Reaction scheme leading to triazole **21**. b) Fluorescence of 1 µM triazole **19** (solid line) and 1 µM triazole **21** (dashed line) upon excitation at 405 nm was recorded on a JASCO fluorescence spectrometer. Due to the absence of FRET in **21**, it emits at shorter wavelengths than **19**. c) Standard curves mimicking the progression of the reaction between 1 µM **10** and 1 µM **16** to yield 1 µM of **21**. Due to the absence of a BODIPY-FL-labeled component, the standard curve consists of compound **16** and **21** only. Fluorescence spectra of the mixtures were taken on a Tecan Infinite F500 plate reader using an excitation filter of 405 nm and an emission filter of 485 nm. The data were fit to a linear curve. Each time point has its own set of standard curves. As an example, the standard curves of the three independent experiments at the 40 min time point are given. Due to different amplification factors of the two instruments used in b) and c), the scaling of the intensities on the y-axis cannot be compared between b) and c). d) Determination of the second-order rate constant using **10** and **16** at a final concentration of 1 µM of each component in PBS containing 2 % of DMSO. The percentage of conversion was determined by measuring the fluorescence intensity at 485 nm and converting it to percent conversion via the standard curve of the corresponding time point. The concentration of **10** was calculated using the equation [**10**] = 1 µM – [mol% **21** / 100] µM. The second-order rate constant k (M^{-1} s^{-1}) was determined by plotting 1/[**10**] versus time. The plot was fit to a linear regression. The slope corresponds to k. The experiment was performed in triplicate. e) Time course of the reaction between **10** and **16** to yield triazole **21** at 1 µM.

Scheme S1.



Scheme S1. Synthesis of fluorophore **12**.

Supporting methods

Fluorescence-based kinetic experiments

All reactions were carried out in aqueous solutions containing a final DMSO concentration of 2%. Unless stated otherwise, the reactions were carried out in PBS (phosphate buffered saline) containing 0.1% NP-40 substitute.

Composition of PBS:
138 mM NaCl
2.7 mM KCl
10 mM phosphate buffer pH 7.4

Composition of cell lysis buffer:
50 mM Tris/HCl,
150 mM NaCl,
10 mM $Na_4P_2O_7$,
10% glycerol,
1% Triton X-100,
1 mM EDTA,
pH 7.5

The following protease inhibitors are added to cell lysis buffer freshly before use:
10 mM NaF,
1 mM Na_3PO_4,
1 mM PMSF,
100 ng/ml aprotinin

The cell lysate used had a total protein concentration of 2.2 µg/µL.

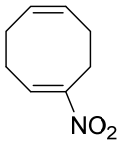
Standard curves were prepared to mimic the progress of the reaction between cycloalkyne and azide to the corresponding triazole products. The principle composition of the standard curve

is given in the table below. Since the composition of the components of the standard curve must not change over time, the BODIPY-FL ester **12a** was used instead of BODIPY-labeled PYRROC **13** as the BODIPY-FL component in the standard curves. In the standard curves used in for analyzing the kinetics between unlabeled PYRROC (**10**) and 3-azido-7-hydroxycoumarin **16**, the BODIPY-FL component was replaced with DMSO.

Conversion to triazole / mol%	BODIPY-FL component / mol%	Fluorophore azide component / mol%	Triazol / mol%
0	100	100	0
10	90	90	10
20	80	80	20
30	70	70	30
40	60	60	40
50	50	50	50
60	40	40	60
70	30	30	70
80	20	20	80
90	10	10	90
100	0	0	100

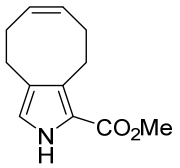
Synthesis and spectroscopic characterization of compounds

(1*E*,5*Z*)-1-nitrocycloocta-1,5-diene (**1**)



To a suspension of 1,5-cyclooctadiene (19.6 mL, 160.0 mmol), $AgNO_2$ (52.0 g, 336.0 mmol) and TEMPO (10.0 g, 64.0 mmol) in chloroform (640 mL) was added powdered molecular sieve (48.0 g). After stirring for 12 h at 70 °C, the suspension was cooled down to room temperature and filtrated. The solvent was removed in vacuo and the crude product was purified by flash column chromatography (hexane: ethyl acetate 50:1) to yield **1** (8.5 g, 55.8 mmol, 35 %) as a pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.4-2.7 (m, 6H), 3.0-3.1 (m, 2H), 5.5-5.7 (m, 2H), 7.4 (t, *J* = 6.2 Hz, 1H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 25.8, 26.5, 26.8, 27.2, 127.8, 128.8, 135,3, 151.7 ppm; IR (Nujol): $\tilde{\nu}$ = 3446 (w), 3016 (w), 2954 (m), 2927 (m), 2894 (m), 2839 (w), 1372 (m), 1668 (m), 1636 (s), 1549 (m), 1519 (s), 1483 (m), 1438 (m), 1384 (w), 1332 (s), 1279 (m), 1107 (w), 1003 (w), 918 (w), 849 (m), 820 (w), 738 (m), 723 (m), 688 (w), 661 cm^{-1} (w); HR-ESI-MS $C_8H_{11}NO_2$ calcd: 176.0682 $[M+Na]^+$, found: 176.0684.

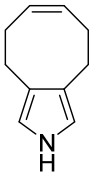
(*Z*)-methyl 4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-1-carboxylate (**2**)



(1*E*,5*Z*)-1-nitrocycloocta-1,5-diene**1** (8.2 g, 53.8 mmol) and methyl isocycanoacetate (4.9 mL, 53.8 mmol) were dissolved in THF (215 mL) at room temperature. Then DBU (8.0 ml, 53.8 mmol) was added and the reaction mixture was refluxed for 15h. Evaporation of the solvent provided the crude product, which was purified by flash column chromatography (hexane: ethyl acetate, 10:1) to give compound **2** (9.5 g, 46.1 mmol, 86 %) as a white solid.

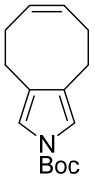
Melting point: 81°C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.5 (q, *J* = 6.4 Hz, 2H), 2.6 (q, *J* = 6.9 Hz, 2H), 2.8-3.0 (m, 2H), 3.1-3.2 (m, 2H), 3.8 (s, 3H), 5.4-5.8 (m, 2H), 6.6 (d, *J* = 2.9 Hz, 1H), 8.8 (s, 1H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 25.7, 26.1, 27.1, 29.9, 51.1, 119.1, 120.8, 125.0, 128.9, 129.5, 130.4, 161.9 ppm; IR (KBr): 3365 (s), 3006 (m), 2988 (w), 2947 (m), 2936 (m), 2913 (w), 2884 (m), 2875 (m), 2830 (w), 1682 (s), 1631 (w), 1561 (s), 1505 (m), 1479 (w), 1456 (m), 1438 (s), 1395 (m), 1350 (m), 1321 (m), 1274 (m), 1263 (m), 1250 (w), 1227 (s), 1200 (w), 1185 (w), 1159 (m), 1146 (w), 1105 (m), 1071 (s), 1039 (w), 1002 (m), 989 (w), 932 (w), 913 (m), 814 (w), 772 (m), 728 (m), 709 (m), 674 (w), 642 (w), 607 (m), 564 cm^{-1} (w); HR-ESI-MS $C_{12}H_{15}NO_2$ calcd: 228.0995 $[M+H]^+$, found: 228.0994.

(*Z*)-4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole(**3a**)



A solution of (*Z*)-methyl 4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-1-carboxylate **2** (9.5 g, 46.1 mmol) and NaOH (10.2 g, 264.7 mmol) in ethylene glycol (171 mL) is stirred at 195 °C for 45 min and cooled down to room temperature using an ice bath. The solution was partitioned between DCM and water, and the aqueous phase was extracted three times with dichloromethane. The organic phase was dried over Na_2SO_4 and the solvent was removed in vacuo. Purification by flash column chromatography (hexane:ethyl acetate, 10:1) afforded compound **3a** (6.7 g, 45.3 mmol, 98 %) as a white solid.Melting point: 55°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 2.5 (q, *J* = 5.6 Hz, 4H), 2.8-2.9 (m, 4H), 5.5-5.8 (m, 2H), 6.5 (s, 2H), 7.8 (s, 1H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 26.3, 29.1, 116.1, 122.4, 129.7 ppm; IR (KBr): $\tilde{\nu}$= 3387 (s), 3378 (s), 2979 (w), 2960 (w), 2924 (s), 2896 (s), 2876 (s), 2826 (m), 2557 (w), 1733 (w), 1697 (w), 1683 (w), 1654 (w), 1636 (w), 1558 (w), 1518 (m), 1475 (m), 1446 (w), 1433 (m), 1404 (w), 1369 (w), 1319 (w), 1291 (w), 1266 (w), 1209 (w), 1194 (w), 1171 (w), 1152 (w), 1115 (w), 1068 (s), 1020 (w), 999 (w), 975 (w), 915 (w), 888 (w), 867 (w), 800 (w), 775 (s), 724 (s), 704 (w), 664 (w), 640 (m), 610 (w), 565 (m), 542 (s), 506 (w), 438 cm^{-1} (m); HR-ESI-MS $C_{10}H_{13}N$ calcd: 170.0940 $[M+Na]^+$, found: 170.0940.

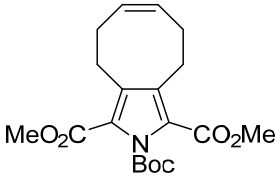
(*Z*)-*tert*-butyl 4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-2-carboxylate (**3**)



To a solution of (*Z*)-4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole **3a** (5.4 g, 36.6 mmol) in DCM (55 mL) was added triethylamine (10.1 mL, 73.2 mmol), 4-DMAP (0.9 g, 7.3 mmol) and Boc_2O (19.1 g, 87.9 mmol) at 0 °C, and the reaction mixture was stirred overnight. The solution was diluted with saturated aqueous $KHSO_4$ solution, extracted with diethylether and dried over Na_2SO_4. The solvent was removed in vacuo, and the crude product was purified by flash column chromatography (hexane: ethyl acetate, 50:1) to yield compound **3** (8.6 g, 34.7 mmol, 95 %) as a white solid. Melting point: 63°C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.6 (s, 9H), 2.4 (q, *J* = 6.0 Hz, 4H), 2.7-2.8 (m, 4H), 5.5-5.6 (m, 2H), 6.9 (s, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 26.3, 28.2, 28.4, 82.9, 117.6, 126.9, 129.8, 148.9 ppm; IR (KBr): $\tilde{\nu}$ = 3451 (m), 3020 (m), 2980 (m), 2963 (w), 2942 (m), 2922 (m), 2891 (m), 2850 (w), 2830 (m), 1734 (s), 1694 (w), 1656 (w), 1635 (w), 1541 (w), 1521 (m), 1483 (m), 1475 (m), 1460 (w), 1449 (w), 1436 (w),

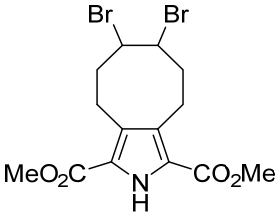
1403 (s), 1369 (s), 1342 (s), 1319 (s), 1274 (s), 1256 (s), 1241 (m), 1159 (s), 1146 (s), 1111 (w), 1029 (w), 1005 (w), 992 (s), 983 (s), 870 (w), 852 (m), 828 (m), 806 (m), 788 (m), 771 (m), 728 (m), 708 (w), 617 (w), 585 (m), 576 (w), 473 cm^{-1} (w); HR-ESI-MS $C_{15}H_{21}NO_2$ calcd: 270.1465 $[M+Na]^+$, found: 270.1462.

(*Z*)-2-*tert*-butyl 1,3-dimethyl 4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-1,2,3-tricarboxylate (**4**)



A 2 M solution of *n*-butyllithium in THF (6.4 mL, 15.8 mmol) was added to a solution of TMP (2.7 mL, 15.9 mmol) in THF (15 mL) at -78 °C and stirred for 5 min. Then (*Z*)-*tert*-butyl 4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-2-carboxylate **3** (1.5 g. 6.1 mmol) in THF (5 mL) is added and the solution is stirred for 3 h at -78 °C. Afterwards, the reaction mixture is added to methyl chloroformate (1.4 mL, 20.6 mmol) at -78 °C and again stirred for 1 h. The reaction is allowed to warm to room temperature and then quenched by the addition of saturated aqueous NH_4Cl solution and diethylether. The organic phase was washed with 1 M HCl and brine, and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by flash column chromatography (hexane:ethyl acetate, 20:1) to give compound **4** (1.7 g, 4.6 mmol, 75 %) as a white solid. Melting point: 87°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.6 (s, 9H), 2.5 (q, *J* = 6.7 Hz, 4H), 3.1 (dd, *J* = 7.8, 6.1 Hz, 4H), 3.8 (s, 6H), 5.5 (t, *J* = 4.1 Hz, 2H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 25.1, 27.5, 27.6, 51.8, 85.4, 122.9, 129.2, 131.1, 161.2 ppm; IR (KBr): $\tilde{\nu}$ = 3433 (m), 2982 (m), 2950 (s), 2881 (m), 2840 (m), 1778 (s), 1718 (s), 1667 (w), 1649 (w), 1540 (m), 1489 (m), 1474 (m), 1436 (s), 1379 (s), 1318 (w), 1257 (s), 1194 (m), 1153 (s), 1090 (s), 1038 (w), 1001 (m), 950 (m), 882 (m), 847 (s), 814 (w), 778 (m), 728 (m), 682 (w), 627 (m), 543 (w), 405 cm^{-1} (w); HR-ESI-MS $C_{19}H_{25}NO_6$ calcd: 386.1574 $[M+Na]^+$, found: 386.1575.

Dimethyl 6,7-dibromo-4,5,6,7,8,9-hexahydro-2H-cycloocta[c]pyrrole-1,3-dicarboxylate (**5**)

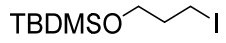


A solution of Br_2 (0.8 g, 5.2 mmol) in DCM (16.0 mL) was added to a solution of (*Z*)-2-tert-butyl 1,3-dimethyl 4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-1,2,3-tricarboxylate **4** (1.7 g, 4.6 mmol) in DCM (16.0 mL) at -78 °C. The reaction mixture was stirred for 30 min and let to come to room temperature during that time. An aqueous solution of $Na_2S_2O_3$ was added. After stirring for 10 min the crude product was extracted with DCM, dried over Na_2SO_4 and the solvent was removed *in vacuo*.

The crude product was the dissolved in DCM (24 mL), and TFA (3.7 mL, 48.4 mmol) was added at room temperature. After stirring for 30 min, saturated $NaHCO_3$ solution was added and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and the solvent removed *in vacuo* to yield the pure compound **5** (1.9 g, 4.4 mmol, 97 %) as a white solid. Melting point: 128-131 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.3-2.5 (m, 2H), 2.8-2.8 (m, 2H), 3.2-3.3 (m, 4H), 3.9 (s, 6H), 4.5-4.6 (m, 2H), 9.5 (s, 1H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 21.4, 36.3, 51.9, 57.2, 121.3, 129.7, 161.1 ppm; IR (KBr): $\tilde{\nu}$= 3439 (m), 3297 (s), 2951 (m), 2921 (m), 2852 (w), 2360 (w), 2342 (w), 1714 (s), 1557 (w),

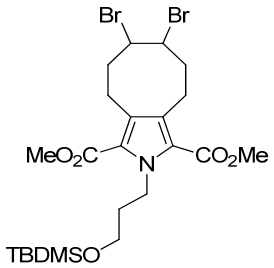
1466 (m), 1435 (s), 1352 (w), 1341 (w), 1276 (s), 1207 (m), 1195 (m), 1150 (m), 1128 (m), 1065 (w), 1049 (w), 1011 (w), 976 (m), 940 (w), 874 (w), 864 (w), 800 (w), 784 (m), 735 (w), 711 (w), 621 (w), 607 (w), 556 (w), 515 cm^{-1} (w); HR-ESI-MS $C_{14}H_{17}Br_2NO_4$ calcd: 443.9417 $[M+Na]^+$, found: 443.9419.

tert-Butyl(3-iodopropoxy)dimethylsilan(**6**)



A solution of 4-iodo-1-propanole (1.0 g, 5.4 mmol) and imidazole (1.1 g, 16.1 mmol) in DCM (8.1 mL) was stirred at room temperature for 5 minutes. Afterwards *tert*-butyldimethylsilyl chloride (1.1 g, 7.0 mmol) was added and the reaction mixture was stirred for 4 h. The mixture was partitioned between DCM and water, and the aqueous phase was extracted three times with DCM. The organic phase was dried over Na_2SO_4, and the solvent was removed in vacuo. Purification by flash column chromatography (hexane:ethyl acetate, 100:1 → 10:1) afforded compound **6** (1.6 g, 5.4 mmol, quantitative) as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.1 (s, 6H), 0.9 (s, 9H), 2.0 (ddd, *J* = 12.4, 6.5, 5.8 Hz, 2H), 3.3 (t, *J* = 6.7 Hz, 2H), 3.7 (t, *J* = 5.7 Hz, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = -5.2, 3.8, 25.8, 26.1, 36.3, 62.5 ppm; IR (Nujol): $\tilde{\nu}$= 2954 (s), 2928 (s), 2986 (m), 2857 (s), 1471 (m), 1464 (m), 1437 (w), 1424 (w), 1407 (w), 1386 (w), 1361 (w), 1351 (w), 1283 (w), 1255 (s), 1182 (m), 1138 (m), 1101 (s), 1053 (m), 1006 (w), 930 (m), 834 (s), 813 (m), 776 (w), 729 (w), 714 (w), 663 (w), 606 cm^{-1} (w); HR-ESI-MS $C_9H_{21}IOSi$ calcd: 323.0299 $[M+Na]^+$, found: 323.0298.

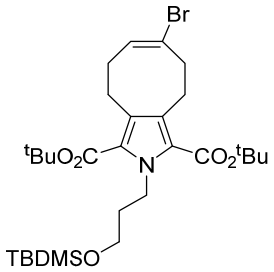
Dimethyl 6,7-dibromo-2-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-4,5,6,7,8,9-hexahydro-2H-cycloocta[c]pyrrole-1,3-dicarboxylate (**7**)



To a solution of dimethyl 6,7-dibromo-4,5,6,7,8,9-hexahydro-2H-cycloocta[c]pyrrole-1,3-dicarboxylate **5** (634.8 mg, 1.5 mmol) in DMF (15 mL) was added powdered K_2CO_3 (518.3 mg, 3.8 mmol) and *tert*-butyl(3-iodopropoxy)dimethylsilane **6** (810.7 mg, 2.7 mmol) at room temperature. The resulting suspension was then stirred at 80 °C for 1h. After cooling to room temperature, the mixture was poured into water and extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and the solvent was removed *in vacuo*. Purification was achieved by flash column chromatography (hexane: ethyl acetate, 20:1) to yield pure compound **7** (502.2 mg, 0.8 mmol, 56 %) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.1 (s, 6H), 0.9 (s, 9H), 1.8-2.0 (m, 2H), 2.3-2.5 (m, 2H), 2.7-2.9 (m, 2H), 3.1 (t, *J* = 6.3 Hz, 4H), 3.6 (t, *J* = 6.4 Hz, 2H), 3.9 (s, 6H), 4.6-4.6 (m, 2H), 4.8-4.7 (m, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = -5.2, 18.8, 21.7, 26.1, 35.4, 36.9, 44.8, 51.6, 56.2, 61.0, 124.1, 129.8, 161.9 ppm; IR (Nujol): $\tilde{\nu}$= 2952 (m), 2928 (m), 2855 (m), 1720 (s), 1700 (s), 11653 (w), 1635 (w), 1508 (w), 1471 (w), 1459 (w), 1435 (m), 1385 (w), 1362 (w), 1298 (m), 1246 (m), 1221

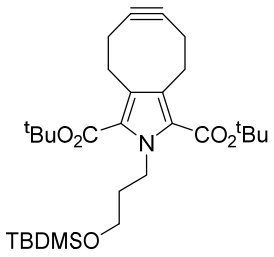
(w), 1196 (m), 1133 (m), 1095 (w), 1061 (w), 992 (w), 875 (w), 835 (m), 777 cm^{-1} (m); ESI-HRMS $C_{23}H_{37}Br_2NO_5Si$ calcd: 616.0700 $[M+Na]^+$, found: 616.0702.

(*E*)-di-*tert*-butyl 6-bromo-2-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-1,3-dicarboxylate (**8**)



Dimethyl 6,7-dibromo-2-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-4,5,6,7,8,9-hexahydro-2H-cycloocta[c]pyrrole-1,3-dicarboxylate **7** (317.9 mg, 0.5 mmol) was dissolved in diethyl ether (1 mL) and KOtBu (10.7 mL, 10.7 mmol, 1 M in THF) was added at -10 °C. The reaction mixture was stirred for 1 h at -10°C to 10°C, and again KOtBu (2 mL, 2 mmol, 1 M in THF) was added. After stirring for another 30 min, 0.5 M HCl was added and the reaction mixture was extracted with diethyl ether. The extracts were dried over Na_2SO_4 and the solvent was evaporated in vacuo to give the crude product which was purified by flash column chromatography (hexane:ethyl acetate, 50:1). Compound **8** (209.5 mg, 0.35 mmol, 66 %) was obtained as a colourless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.1 (s, 6H), 0.9 (s, 9H), 1.6 (s, 9H), 1.6 (s, 9H), 2.0-1.9 (m, 2H), 2.5 (q, *J* = 6.9 Hz, 2H), 3.0-3.1 (m, 4H), 3.1-3.2 (m, 2H) 3.6 (t, *J* = 6.6 Hz, 2H), 4.5-4.6 (m, 2H), 6.0-5.9 (m, 1H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = -5.2, 24.9, 25.0, 26.1, 28.6, 28.9, 35.2, 38.9, 44.5, 61.4, 81.7, 81.8, 124.9, 125.2, 125.7, 127.2, 127.8, 130.6, 161.1, 161.2 ppm; IR (Nujol): $\tilde{\nu}$= 3445 (m), 2954 (s), 2929 (s), 2884 (s), 2856 (s), 2359 (w), 1715 (w), 1698 (s), 1683 (s), 1653 (m), 1558 (w), 1540 (m), 1472 (m), 1457 (m), 1427 (m), 1393 (w), 1368 (m), 1300 (m), 1252 (m), 1134 (m), 1080 (m), 1054 (m), 1006 (w), 837 (s), 779 (s), 668 (m), 602 (w), 475 (w, 418 (w), 401 cm^{-1} (w); HR-ESI-MS $C_{29}H_{48}BrNO_5Si$ calcd: 620.2377 $[M+Na]^+$, found: 620.2375.

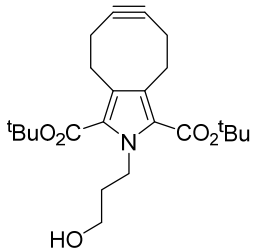
Di-*tert*-butyl (*Z*)-2-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-6,7- didehydro-4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-1,3-dicarboxylate (**9**)



To a solution of (*E*)-di-*tert*-butyl 6-bromo-2-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-1,3-dicarboxylate **8** (76.1 mg, 0.13 mmol) in diethyl ether (0.1 mL) and hexane (1 mL) was added 18-crown-6 (catalytic amount) and KOtBu (57.1 mg, 0.51 mmol) at room temperature. The suspension was then stirred at 55 °C for 20 min and 0.5 M HCl was added. The aqueous phase was extracted with diethyl ether and dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by flash column chromatography (dichloromethane) to yield compound **9** (46.4 mg, 0.1 mmol, 71 %) as a

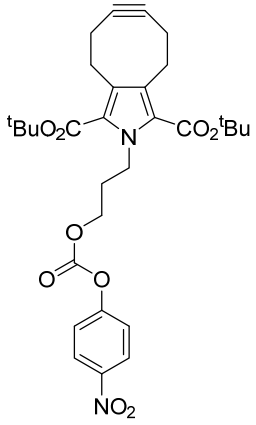
colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.1 (s, 6H), 0.9 (s, 9H), 1.6 (s, 18H), 1.9-2.1 (m, 2H), 2.2-2.5 (m, 4H), 2.8-3.1 (m, 2H), 3.1-3.4 (m, 2H) 3.7 (t, *J* = 6.6 Hz, 2H), 4.5-4.7 (m, 2H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = -5.2, 18.5, 21.9, 26.1, 28.5, 29.6, 35.2, 44.7, 61.4, 81.8, 98.8, 126.0, 129.3, 161.1 ppm; IR (Nujol): $\tilde{\nu}$= 2975 (s), 2954 (s), 2929 (s), 2857 (s), 1798 (w), 1712 (s), 1694 (s), 1536 (m), 1473 (s), 1460 (s), 1419 (m), 1392 (m), 1367 (s), 1328 (m), 1302 (s), 1255 (s), 1211 (m), 1166 (s), 1120 (s), 1035 (w), 1007 (m), 969 (m), 938 (w), 931 (w), 900 (w), 839 (s), 813 (m), 778 (s), 734 (m), 719 (w), 662 (m), 548 (m), 463 cm^{-1} (w); HR-ESI-MS $C_{29}H_{47}NO_5Si$ calcd: 518.3296 $[M+H]^+$, found: 518.3296.

Di-*tert*-butyl (Z)-2-(3-hydroxypropyl)-6,7- didehydro-4,5,8,9-tetrahydro-cycloocta[c]pyrrole-1,3-dicarboxylate (**10**)



A solution of compound **9** (40.4 mg, 0.08 mmol) and TBAF (0.08 mL, 0.08 mmol, 1 M in THF) in THF (0.6 mL) was stirred for 2 h at room temperature. Water was added and the aqueous phase was extracted with diethylether, and the combined organic phases were dried over Na_2SO_4. Evaporation of the solvent *in vacuo* gave crude compound **10** which was subjected to flash column chromatography (hexane:ethyl acetate, 5:1). Pure compound **10** (18.7 mg, 0.05 mmol, 62 %) was obtained as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.6 (s, 18H), 1.7-2.1 (m, 2H), 2.2-2.5 (m, 4H), 2.9-3.1 (m, 2H), 3.3-3.4 (m, 2H), 3.5 (t, *J* = 5.3 Hz, 2H), 4.7 (t, *J* = 6.6 Hz, 2H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 21.9, 28.6, 29.7, 35.2, 43.5, 59.4, 82.5, 98.9, 125.9, 129.6, 161.7 ppm; IR (Nujol): $\tilde{\nu}$= 3438 (m), 2976 (m), 2931 (m), 2880 (w), 1709 (s), 1694 (s), 1476 (w), 1456 (w), 1418 (w), 1392 (w), 1368 (m), 1328 (w), 1299 (m), 1252 (m), 1166 (m), 1128 (s), 1053 (w), 1017 (w), 846 (m), 786 (m), 764 (m), 461 (w), 420 (w), 411 cm^{-1} (w); HR-ESI-MS $C_{23}H_{33}NO_5$calcd: 404.2431 $[M+H]^+$, found: 404.2432.

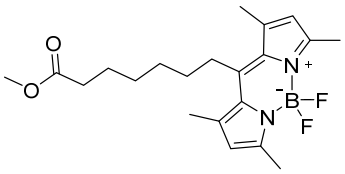
Di-*tert*-butyl (Z)-2-(3-(((4-nitrophenoxy)carbonyl)oxy)propyl)-6,7- didehydro-4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-1,3-dicarboxylate(**11**)



A solution of compound **10** (22.4 mg, 0.06 mmol), pyridine (0.01 mL, 0.14 mmol) and 4-nitrophenyl chloroformate (14.0 mg, 0.07 mmol) in DCM (1 mL) is stirred at room temperature for 1.5 h. A saturated aqueous solution of NH_4Cl is added and the phases were separated. The aqueous phase was extracted with dichloromethane, the combined phases were dried over Na_2SO_4, and the solvent was removed *in vacuo*. Flash column chromatography afforded

pure compound **11** (17.4 mg, 0.03 mmol, 95 %) as a white solid. Melting point: 87°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.6 (s, 18H), 2.2-2.5 (m, 6H), 2.8-3.1 (m, 2H), 3.2-3.4 (m, 2H), 4.4 (t, *J* = 6.2 Hz, 2H), 4.6-4.7 (m, 2H), 7.4-7.5 (m, 2H), 8.2-8.3 (m, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 21.8, 28.5, 29.7, 30.9, 44.1, 67.5, 82.1, 98.8, 122.0, 125.4, 125.9, 129.7, 145.5, 152.6, 155.9, 161.1 ppm; IR (KBr): $\tilde{\nu}$= 3440 (s), 2974 (m), 2926 (s), 2853 (m), 1769 (s), 1708 (s), 1645 (w), 1635 (w), 1617 (m), 1595 (m), 1527 (s), 1493 (w), 1474 (w), 1458 (m), 1420 (w), 1393 (w), 1368 (m), 1348 (m), 1329 (m), 1305 (m), 1254 (s), 1219 (s), 1164 (s), 1127 (s), 1055 (w), 1013 (w), 933 (w), 861 (m), 847 (m), 779 (w), 548 cm^{-1} (w); HR-ESI-MS $C_{30}H_{36}N_2O_9$ calcd: 591.2313 $[M+Na]^+$, found: 591.2310.

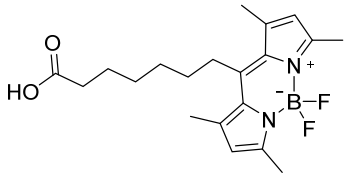
(Methyl7-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diaza-borinin-10-yl)heptanoate) (**12a**)



Synthesis of **12a** was carried out based on the published procedure.[1] In brief, to a solution of methyl-8-chloro-8-oxooctanoate (826.7 mg, 4 mmol) in DCM (40 mL) was added 2,4-dimethylpyrrol (0.8 mL, 8 mmol) at room temperature and the reaction mixture was refluxed for 3 h. The solvent was removed *in vacuo*, toluene (80 mL) and triethylamine (2.7 mL, 19.2 mmol) were added and stirred at room temperature for 15 minutes. Then, BF_3-diethyl etherate (3.3 mL, 26.8 mmol) was added and the reaction mixture was stirred at 55 °C for 1.5 h. The solution is washed with water and brine and dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography (hexane:DCM, 1:1 -> 1:4) to afford compound **12a** (608.7 mg, 1.6 mmol, 39 %) as an orange solid. Melting point: 124-126 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.3-1.6 (m, 4H), 1.6-1.7 (m, 4H), 2.3 (t, *J* = 7.4 Hz, 2H), 2.4 (s, 6H), 2.5 (s, 6H), 2.9-3.0 (m, 2H), 3.7 (s, 3H), 6.0 (s, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 14.6, 16.5, 25.0, 28.5, 29.1, 30.2, 31.9, 34.1, 51.6, 121.7, 131.6, 140.4, 146.5, 153.9, 174.1 ppm; ^{19}F-NMR (282 MHz, $CDCl_3$): δ = (-147.3)-(-147.0) (m, 2F) ppm; IR (KBr): $\tilde{\nu}$= 3435 (m), 2978 (w), 2929 (m), 2865 (w), 2101 (m), 1698 (w), 1634 (w), 1551 (s), 1510 (s), 1474 (m), 1410 (m), 1372 (m), 1308 (m), 1273 (w), 1250 (w), 1226 (m), 1203 (s), 1161 (m), 1109 (m), 1080 (m), 1063 (m), 1028 (w), 986 (m), 834 (w), 823 (w), 807 (w), 730 (w), 715 (w), 582 (w), 482 cm^{-1} (m); UV/Vis (Dichlormethan): $\lambda_{max}(\varepsilon)$ = 498 (2.657), 358 (0.503), 306 (0.803), 242 nm (1.795); HR-ESI-MS $C_{21}H_{29}BF_2N_2O_2$ calcd: 413.2182 $[M+Na]^+$, found: 413.2181.

[1] I. A. Boldyrev, J. G. Molotkovsky, *Russ. J. Bioorg. Chem.* **2006**, *32*, 78-83.

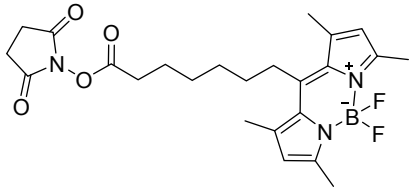
7-(5,5-Difluoro-1,3,7,9-tetramethyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanoic acid (**12b**)



Synthesis of **12b** was carried out according to the published procedure.[1] To a solution of **12a** (264.6 mg, 0.7 mmol) in *i*PrOH (100 mL) was added 0.1 M aqueous potassium hydroxide (43 mL), and the reaction mixture was stirred at room temperature for 4 h. Then, the solution was acidified with 1 M HCl and the aqoueous phase was extracted with DCM. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification by flash column chromatography (dichloromethane:methanol, 50:1 → 20:1) afforded compound **12b** (189.4 mg, 0.5 mmol, 75 %) as an orange solid. Melting point: 188-190 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.4-1.6 (m, 4H), 1.6-1.7 (m, 4H), 2.4 (t, *J* = 7.4 Hz, 2H), 2.4 (s, 6H), 2.5 (s, 6H), 2.9-3.0 (m, 2H), 6.1 (s, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 14.6, 16.5, 24.7, 28.5, 29.0, 30.1, 31.8, 34.0, 121.7, 131.5, 140.4, 146.5, 153.9, 179.7 ppm; ^{19}F-NMR (376 MHz, $CDCl_3$) δ -147.0 (ddd, *J* = 65.2, 31.4, 12.8 Hz, 2F) ppm; IR (KBr): $\tilde{\nu}$ = 3427 (m), 2929 (m), 2863 (w), 1737 (m), 1709 (m), 1635 (w), 1627 (w), 1550 (s), 1510 (s), 1475 (m), 1409 (m), 1371 (m), 1308 (m), 1226 (m), 1203 (s), 1161 (m), 1114 (w), 1081 (m), 1028 (w), 986 (s), 837 (w), 824 (w), 715 (w), 633 (w), 582 (w), 482 cm^{-1} (m); UV/Vis (dichloromethane): $\lambda_{max}(\varepsilon)$ = 498 (2.770), 361 (0.329), 306(0.543), 243 nm (1.231); HR-ESI-MS $C_{20}H_{27}BF_2N_2O_2$ calcd: 399.2026 $[M+H]^+$, found: 399.2023.

[1] I. A. Boldyrev, J. G. Molotkovsky, *Russ. J. Bioorg. Chem.* **2006**, *32*, 78-83.

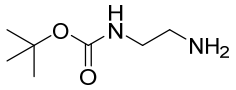
2,5-dioxopyrrolidin-1-yl-7-(5,5-difluoro-1,3,7,9-tetramethyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanoate (**12c**)



A solution **12b** (189.4 mg, 0.5 mmol) and *N*-hydroxysuccinimide (86.9 mg, 0.8 mmol) in acetonitrile (8.0 mL) was cooled down to 0 °C and a solution of dicyclohexylcarbodiimide (259.7 mg, 1.3 mmol) in acetonitrile (2.5 mL) was added. The reaction mixture was stirred for 16 h at room temperature, filtered and washed with dichloromethane. Evaporation of the solvent and purification of the crude product by flash column chromatography (hexane:ethyl acetate, 1:1) afforded compound **12c** (236.7 mg, 0.5 mmol, 99 %) as an orange solid. Melting point: 128°C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.4-1.6 (m, 4H), 1.6-1.7 (m, 2H), 1.8 (p, *J* = 7.2 Hz, 2H), 2.4 (s, 6H), 2.5 (s, 6H), 2.6 (t, *J* = 7.3 Hz, 2H), 2.8 (s, 4H), 2.9-3.0 (m, 2H), 6.1 (s, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 14.7, 16.6, 24.8, 25.8, 28.5, 28.7, 30.0, 31.0, 31.8, 121.8, 131.6, 140.5, 146.5, 154.0, 168.7, 169.3 ppm; ^{19}F-NMR (376 MHz, $CDCl_3$) δ = (-152.4)-(-152.1) (m, 2F) ppm; IR (KBr): $\tilde{\nu}$= 3434 (m), 2934 (m), 2866 (w), 1811 (m), 1784 (m), 1739 (s), 1653 (w), 1628 (w), 1550 (s), 1509 (s), 1474 (m), 1466 (m), 1437 (w), 1407 (m), 1371 (m),

1307 (m), 1270 (w), 1203 (s), 1160 (m), 1098 (m), 1078 (m), 1024 (w), 986 (m), 975 (m), 895 (w), 836 (w), 823 (w), 813 (w), 805 (w), 715 (w), 647 (w), 579 (w), 482 (m), 420 cm^{-1} (m); UV/Vis (dichloromethane): $\lambda_{max}(\varepsilon)$ = 499 (1.130), 276 nm (2.406); HR-ESI-MS $C_{24}H_{30}BF_2N_3O_4$ calcd: 496.2190 $[M+Na]^+$, found: 496.2189.

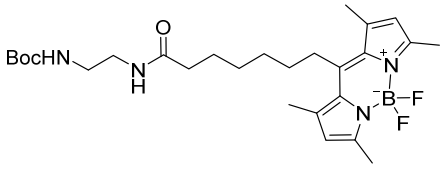
tert-butyl (2-aminoethyl)carbamate (**12d**)



A solution of ethylenediamine (0.3 mL, 40.0 mmol) in chloroform (40.0 mL) was cooled down to 0 °C and a solution of di-*tert*-butyl dicarbonate (517.4 mg, 4.0 mmol) in chloroform (20.0 mL) was added dropwise. The reaction mixture was stirred for 16 h at room temperature. The solution was washed six times with brine and once with water and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford compound **12d** (401.3 mg, 2.5 mmol, 63 %) as a colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.4 (s, 9H), 2.7 (t, *J* = 6.3 Hz, 2H), 3.1 (t, *J* = 6.3 Hz, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.7, 42.4, 44.0, 80.0 ppm; IR (Nujol): $\tilde{\nu}$ = 3354 (s), 3000 (m), 2976 (s), 2932 (s), 2870 (s), 2018 (w),1890 (w), 1685 (s), 1608 (w), 1523 (s), 1477 (m), 1456 (m), 1446 (m), 1391 (m), 1366 (s), 1344 (w), 1320 (m), 1281 (s), 1251 (s), 1169 (s), 1057 (w), 1040 (w), 1019 (w), 987 (m), 919 (w), 873 (m), 794 (m), 783 (m), 762 (m), 643 (m), 593 (w), 554 (w), 538 (w), 524 (w), 511 (w), 492 (w), 459 (w), 426 cm^{-1} (w). ESI-MS $C_{24}H_{30}BF_2N_3O_4$ calcd: 496.2190 $[M+Na]^+$, found: 496.2189.

CAS Registry Number: 57260-73-8

tert-butyl (2-(7-(5,5-difluoro-1,3,7,9-tetramethyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanamido)ethyl)carbamate (**12e**)

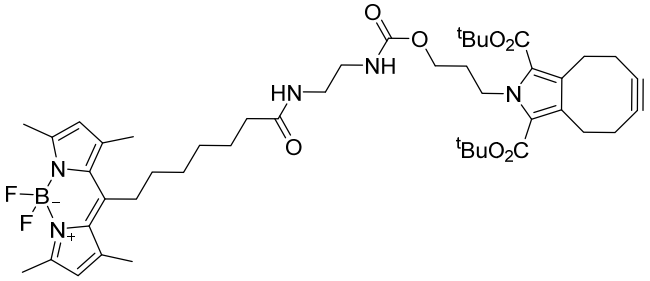


Tert-butyl (2-aminoethyl)carbamate **12d** (54.4 mg, 0.3 mmol) and DIPEA (0.1 mL, 0.6 mmol) were dissolved in DMF (1.3 mL) at room temperature and **12c** (133.9 mg, 0.3 mmol) in DMF (1.5 mL) was added. The reaction mixture is stirred for 1.5 h at room temperature. Evaporation of the solvent *in vacuo* and purification by flash column chromatography (dichloromethane:methanol, 50:1) afforded compound **12e** (178.1 mg, 0.3 mmol, quantitative) as an orange solid. Melting point: 108-110°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.3-1.6 (m, 2H), 1.4 (s, 9H), 1.6-1.8 (m, 6H), 2.2 (t, *J* = 7.5 Hz, 2H), 2.4 (s, 6H), 2.5 (s, 6H), 2.9-3.0 (m, 2H), 3.2-3.4 (m, 4H), 4.9 (s, 1H), 6.0 (s, 2H), 6.2 (s, 1H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.6, 16.5, 25.1, 25.7, 25.8, 28.5, 28.4, 29.3, 30.2, 31.9, 34.1, 36.7, 40.4, 41.1, 121.7, 131.6, 146.6, 153.9, 173.6 ppm; ^{19}F-NMR (282 MHz, $CDCl_3$) δ = (-147.3) -(-146.9) (m, 2F) ppm; IR (KBr): $\tilde{\nu}$= 3424 (s), 3000 (m), 2975 (w), 2930 (m), 2864 (w), 1693 (m),1655 (m), 1550 (s), 1511 (s), 1475 (m), 1410 (m), 1392 (w), 1366 (m), 1308 (m), 1277 (w), 1227 (w), 1202 (s), 1162 (m), 1079 (m), 1026 (m), 987 (m), 917 (w), 865 (w), 811 (w), 758 (w), 714 (w), 629 (w), 597 (w), 583 (w), 511 (w), 482 (w), 458 (w),425 cm^{-1} (w). UV/Vis (dichloromethane): λ_{max} (ε) = 499 (2.663), 475 (0.807), 360 (0.160), 306 (0.206), 243 nm (0.620); HR-ESI-MS $C_{27}H_{41}BF_2N_4O_3$ calcd: 541.3132 $[M+Na]^+$, found: 541.3131.

N-(2-aminoethyl)-7-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ4,5λ4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanamide (**12**)

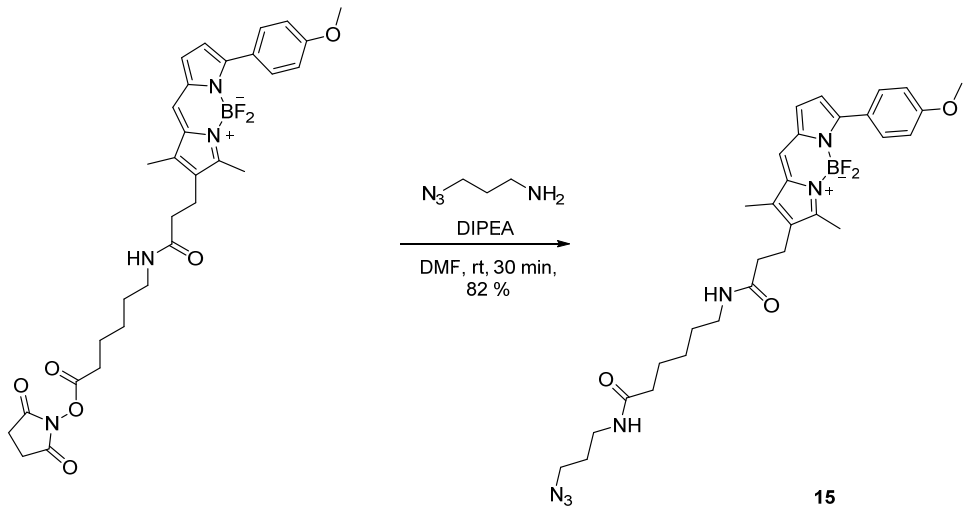
To a solution of compound **12e** (66.0 mg, 0.1 mmol) in dichloromethane (2.0 mL) were added 2mL of 2 M HCl, and the mixture was stirred for 30 minutes at room temperature. The mixture was neutralized with NaOH, extracted with diethyl ether and dried over Na_2SO_4. The solvent was removed *in vacuo*, and the crude amine (40.0 mg) was used for the next step without further purification.

Di-*tert*-butyl (*Z*)-2-(3-(((2-(7-(5,5-difluoro-1,3,7,9-tetramethyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanamido)ethyl)carbamoyl)oxy)propyl)-6,7-di-dehydro-4,5,8,9-tetrahydro-2H-cycloocta[c]pyrrole-1,3-dicarboxylate (**13**)



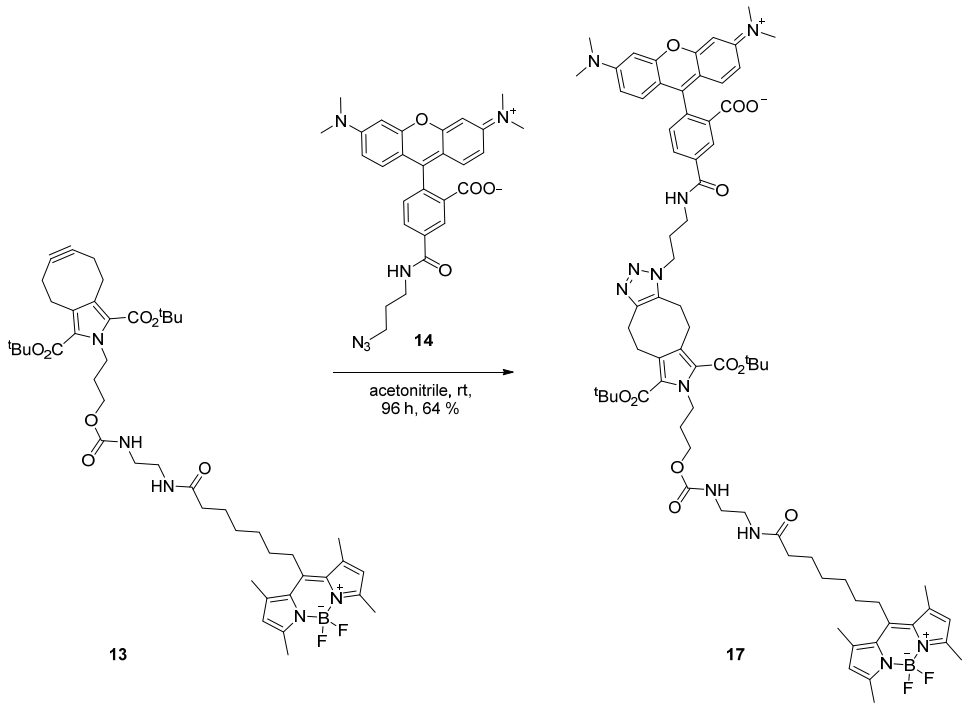
The crude amine **12** (15.0 mg, ca. 0.03 mmol) was added to a solution of compound **11** (5.7 mg, 0.01 mmol) and triethylamine (2 µL,0.01 mmol) in DMF (0.4 mL) and the mixture was stirred at room temperature for 15 min. The reaction was quenched with water, extracted with DCM and the combined organic phases were dried over Na_2SO_4. Evaporation of the solvent *in vacuo* afforded crude compound **13**, which was purified by flash column chromatography (dichloromethane:methanol, 50:1). The pure compound **13** (10.5 mg, 0.01 mmol, quantitative) was obtained as an orange solid. Melting point: 84-86 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.4-1.7 (m, 8H), 1.6 (s, 18H), 2.0-2.1 (m, 2H), 2.2 (t, *J* = 7.5 Hz, 2H), 2.3-2.4 (m, 4H), 2.4 (s, 6H), 2.5 (s, 6H), 2.9-3.0 (m, 4H), 3.2-3.3 (m, 4H), 3.3-3.4 (m, 2H), 4.0-4.2 (m, 2H), 4.6-4.7 (m, 2H), 5.0 (s, 1H), 6.0 (s, 2H), 6.2 (s, 1H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 14.6, 16.6, 21.9, 25.7, 28.5, 29.3, 29.6, 29.9, 30.3, 31.4, 31.9, 36.6, 40.8, 44.3, 63.6, 82.0, 98.9, 121.7, 125.9, 129.4, 131.6, 140.4, 146.6, 153.9, 161.2, 173.5 ppm; ^{19}F-NMR (282 MHz, $CDCl_3$): δ = (-147.4) -(-146.9) (m, 2F) ppm; IR (KBr): $\tilde{\nu}$ = 3444 (s), 2961 (m), 2927 (m), 2370 (w), 1732 (w), 1715 (m), 1698 (m), 1684 (m), 1669 (m), 1652 (s), 1647 (s), 1636 (s), 1568 (w), 1557 (m), 1551 (m), 1542 (m), 1508 (m), 1472 (m), 1457 (m), 1370 (w), 1201 (w), 1166 (m), 1127 (m), 1109 (w), 1085 (m), 986 (w), 753 (w), 624 cm^{-1} (w); HR-ESI-MS $C_{46}H_{64}BF_2N_5O_7$ calcd: 870.4759 $[M+Na]^+$, found: 870.4756.

N-(3-azidopropyl)-6-(3-(5,5-difluoro-7-(4-methoxyphenyl)-1,3-dimethyl-5*H*-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-2-yl)propanamido)hexanamid (**15**)



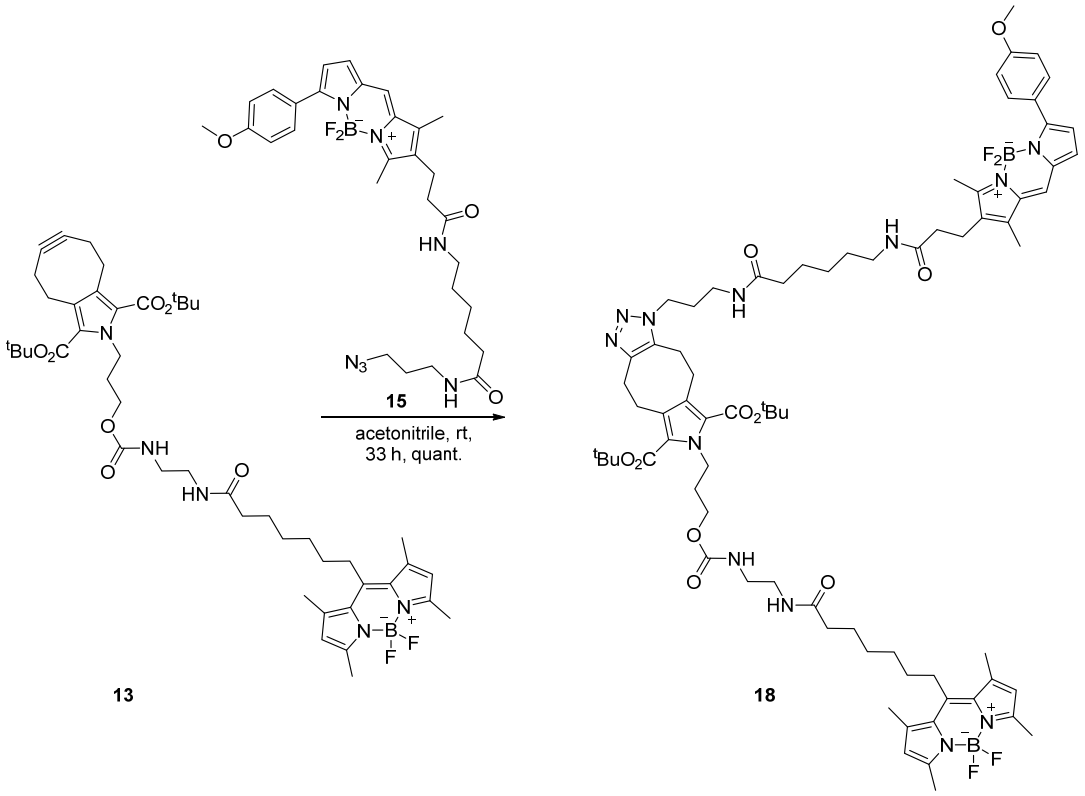
6-((4,4-difluoro-1,3-dimethyl-5-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene-2-propionyl)amino)hexanoic acid succinimidyl ester (5.0 mg, 8.2 µmol) was added to a mixture of 3-azido-1-propanamine (1.0 mg, 10.0 µmol) and DIPEA (3.0 µL, 16.4 µmol) in DMF (1.0 mL) at room temperature. After stirring for 30 minutes, water and ethyl acetate were added and the aqueous phase was extracted three times. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification of the crude product by flash column chromatography (dichloromethane:methanol, 33:1) afforded compound **15** (3.9 mg, 6.6 µmol, 82 %) as a purple solid. Melting point: 85-88 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.4 (p, *J* = 7.0 Hz, 2H), 1.5-1.6 (m, 4H), 1.7 (p, *J* = 6.7 Hz, 2H), 2.0 (t, *J* = 7.3 Hz, 2H), 2.2 (s, 3H), 2.3 (t, *J* = 7.2 Hz, 2H), 2.5 (s, 3H), 2.8 (t, *J* = 7.2 Hz, 2H), 3.1-3.3 (m, 6H), 3.9 (s, 3H), 5.5-5.7 (m, 2H), 6.6 (d, J = 4.1 Hz, 1H), 6.9-7.0 (m, 2H), 7.1 (s, 1H), 7.8-8.0 (m, 2H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 9.9, 13.4, 20.4, 25.1, 26.3, 29.0, 29.1, 29.9, 36.2, 36.7, 37.2, 39.4, 49.5, 55.5, 114.0, 118.5, 123.0, 125.6, 128.2, 130.9, 1334.6, 135.2, 140.3, 155.8, 159.7, 160.7, 171.8, 173.3 ppm; ^{19}F-NMR (282 MHz, $CDCl_3$): δ = (-139.6) -(-139.9) (m, 2F) ppm; IR (Nujol): $\tilde{\nu}$ = 3424 (s), 2924 (m), 2851 (w), 2097 (m), 1697 (w), 1646 (m), 1637 (m), 1607 (s), 1541 (w), 1526 (w), 1464 (m), 1437 (w), 1397 (w), 1385 (w), 1257 (w), 1233 (w), 1202 (m), 1182 (m), 1146 (w), 1159 (s), 1023 (s), 935 (w), 916 (w), 870 (w), 836 (w), 819 (w), 790 (w), 663 (w), 615 (w), 597 (w), 587 (w), 552 (w), 536 (w), 519 (w), 511 (w), 499 (w), 472 (w), 458 (w), 425 cm^{-1} (w); UV/Vis (dichloromethane): $\lambda_{max}(\varepsilon)$ = 548 (1.232), 387 (0.140), 338 (0.158), 274 (0.320), 233 nm (0.365); HR-ESI-MS $C_{30}H_{38}BF_2N_7O_3$ calcd: 616.2989 $[M+Na]^+$, found: 616.2987.

5-((3-(6,8-bis(*tert*-butoxycarbonyl)-7-(3-(((2-(7-(5,5-difluoro-1,3,7,9-tetramethyl-5*H*-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanamido)ethyl)carbamoyl)-oxy)propyl)-5,7,9,10-tetrahydropyrrolo[3',4':5,6]cycloocta[1,2-d][1,2,3]triazol-1(4H)-yl)-propyl)carbamoyl)-2-(6-(dimethylamino)-3-(dimethyliminio)-9,9a-dihydro-3H-xanthen-9-yl)benzoate (**17**)



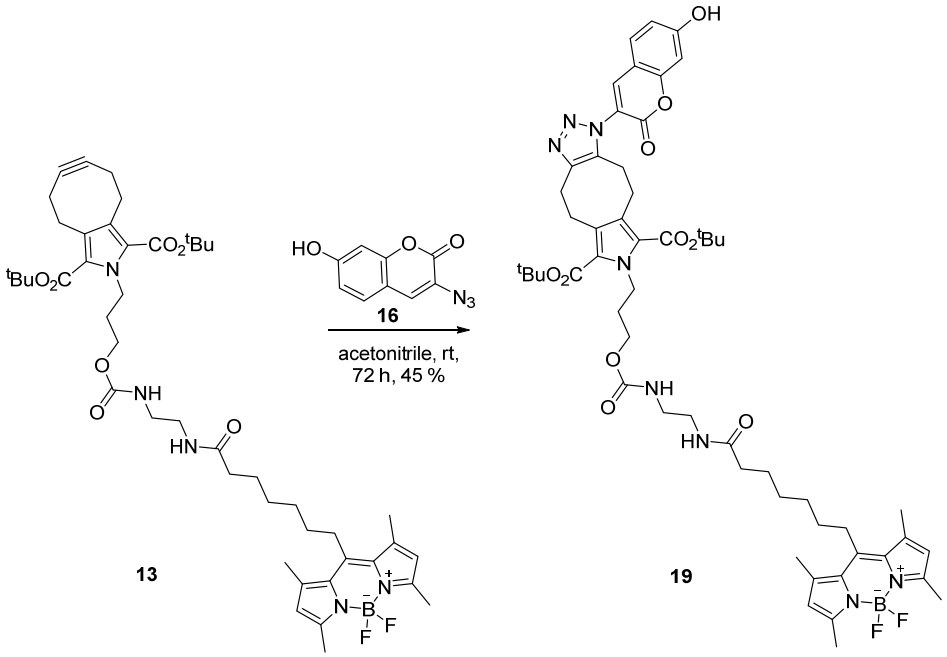
A solution of 5-TAMRA azide **14** (2.0 mg, 3.9 µmol) and **13** (3.0 mg, 3.5 µmol) in acetonitrile (0.2 mL) was stirred at room temperature for 96 h. The use of acetonitrile as solvent was preferable to water for synthesis on a preparative scale, since it allowed the use of higher concentrations of reactants. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography (dichloromethane:methanol, 10:1) to afford compound **17** (3.1 mg, 2.3 µmol, 64 %) as a purple solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.5-1.6 (m, 24H), 2.0-2.1 (m, 6H), 2.4 (s, 8H), 2.5 (s, 6H), 2.8-3.1 (m, 16H), 3.1-3.4 (m, 12H), 3.87-4.0 (m, 2H), 4.3 (t, *J* = 6.7 Hz, 2H), 4.6-4.7 (m, 3H), 4.7 (s, 1H), 5.3 (s, 1H), 6.0 (s, 2H), 6.3-6.8 (m, 7H), 8.1 (d, *J* = 8.2 Hz, 1H), 8.4 (s, 1H) ppm; HR-ESI-MS $C_{74}H_{92}BF_2N_{11}O_{11}$ calcd: 680.8592 $[M+2Na]^{2+}$, found: 680.8599.

Di-*tert*-butyl 7-(3-(((2-(7-(5,5-difluoro-1,3,7,9-tetramethyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanamido)ethyl)carbamoyl)oxy)propyl)-1-(3-(6-(3-(5,5-difluoro-7-(4-methoxyphenyl)-1,3-dimethyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diaza-borinin-2-yl)propanamido)hexanamido)propyl)-1,4,5,7,9,10-hexahydropyrrolo[3',4':5,6]-cycloocta[1,2-d][1,2,3]triazole-6,8-dicarboxylate (**18**)



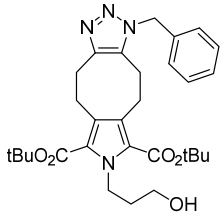
A solution of **15** (1.6 mg, 2.7 µmol) and **13** (2.1 mg, 2.5 µmol) in acetonitrile (0.1 mL) was stirred at room temperature for 33 h. The use of acetonitrile as solvent was preferable to water for synthesis on a preparative scale, since it allowed the use of higher concentrations of reactants. Then, the solvent was removed in vacuo and the crude product was purified by flash column chromatography (dichloromethane:methanol, 50:1 → 33:1). Compound **18** (3.6 mg, 2.5 µmol, quantitative) was obtained as a purple solid. ^{1}H-NMR (400 MHz, CD_3OD): δ = 1.3-1.5 (m, 12H), 1.6 (s, 9H), 1.6-1.7 (m, 13H), 1.8-1.9 (m, 4H), 2.0 (t, *J* = 7.5 Hz, 2H), 2.1-2.2 (m, 4H), 2.3 (t, *J* = 7.2 Hz, 2H), 2.4 (2x s, 2x6H), 2.5 (s, 3H), 2.7 (t, *J* = 7.2 Hz, 2H), 2.9-3.1 (m, 10H), 3.1-3.2 (m, 5H), 3.2-3.3 (m, 4H), 3.6-3.7 (m, 2H), 3.8 (s, 3H), 3.9 (t, *J* = 6.4 Hz, 2H), 4.1 (t, *J* = 7.1 Hz, 2H), 4.5 (t, *J* = 7.5 Hz, 2H), 6.1 (s, 2H), 6.5 (d, *J* = 4.1 Hz, 1H), 6.9-7.0 (m, 2H), 7.0 d, *J* = 4.1 Hz, 1H), 7.4 (s, 1H), 7.8-7.9 (m, 2H) ppm; HR-ESI-MS $C_{76}H_{102}B_2F_4N_{12}O_{10}$calcd: 1463.7856 $[M+Na]^+$, found: 1463.7863.

Di-tert-butyl 7-(3-(((2-(7-(5,5-difluoro-1,3,7,9-tetramethyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanamido)ethyl)carbamoyl)oxy)propyl)-1-(7-hydroxy-2-oxo-2H-chromen-3-yl)-1,4,5,7,9,10-hexahydropyrrolo[3',4':5,6]cycloocta[1,2-d][1,2,3]triazole-6,8-dicarboxylate(**19**)



A solution of 3-azido-7-hydroxycoumarin **16** (1.2 mg, 5.8 µmol) and **13** (4.5 mg, 5.3 µmol) in acetonitrile (0.1 mL) was stirred for 72 h at room temperature. The use of acetonitrile as solvent was preferable to water for synthesis on a preparative scale, since it allowed the use of higher concentrations of reactants. Afterwards, the solvent was removed *in vacuo*. Purification by flash column chromatography (DCM:MeOH, 50:1) afforded compound **19** (2.5 mg, 2.4 µmol, 45 %) as an orange solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.5 (s, 9H), 1.5-1.6 (m, 17H), 1.9-2.0 (m, 2H), 2.1-2.2 (m, 2H), 2.4 (s, 6H), 2.5 (s, 6H), 2.8-2.9 (m, 2H), 3.0 (t, *J* = 6.6 Hz, 2H), 3.1-3.5 (m, 10H), 3.9-4.0 (m, 2H), 4.7 (t, *J* = 6.9 Hz, 2H), 5.1 (s, 1H), 6.0 (s, 2H), 6.6-6.8 (m, 2H), 7.4 (d, *J* = 8.5 Hz, 1H), 7.9 (s, 1H) ppm; HR-ESI-MS $C_{55}H_{69}BF_2N_8O_{10}$calcd: 1051.5271 $[M+H]^+$, found: 1051.5280.

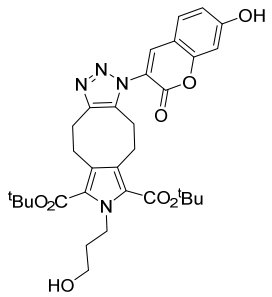
Di-tert-butyl 1-benzyl-7-(3-hydroxypropyl)-1,4,5,7,9,10-hexahydropyrrolo[3',4':5,6]cyclo-octa[1,2-d][1,2,3]triazole-6,8-dicarboxylate (**20**)



A solution of benzyl azide (3 µL, 24.0 µmol) and alkyne **10** (9.3 mg, 23.0 µmol) in acetonitrile (0.5 mL) was stirred at room temperature for 2 h. After completion of the reaction, the solvent was removed *in vacuo.* Purification by flash column chromatography (hexane:ethyl acetate, 1:1) afforded compound **20** (5.8 mg, 10.8 µmol, 47 %) as a colorless oil. Melting point: 125-127°C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.5 (s, 9H), 1.6 (s, 9H), 1.9-2.0 (m, 2H), 2.7-2.9 (m, 2H), 3.1-3.2 (m, 4H), 3.2-3.4 (m, 2H), 3.4-3.5 (m, 2H), 4.6 (t, *J* = 6.5 Hz, 2H), 5.4 (s, 2H),

6.9-7.1 (m, 2H), 7.2-7.4 (m, 3H) ppm; ^{13}C-NMR 100 MHz, $CDCl_3$): δ = 22.5, 25.6, 26.0, 28.4, 28.5, 35.0, 43.0, 52.0, 59.1, 82.3, 82.5, 124.8, 125.4, 126.9, 127.7, 128.2, 129.0, 132.7, 135.2, 144.5, 161.1, 161.5 ppm; IR (KBr): $\tilde{\nu}$ = 3443 (s), 2976 (m), 2929 (m), 1708 (s), 1687 (s), 1652 (m), 1636 (m), 1497 (w), 1473 (w), 1456 (m), 1429 (w), 1421 (w), 1391 (w), 1368 (m), 1291 (m), 1253 (m), 1167 (m), 1130 (s), 1045 (w), 850 (m), 783 (w), 739 (w), 702 cm^{-1} (w); HR-ESI-MS $C_{30}H_{40}N_4O_5$ calcd: 537.3071 $[M+H]^+$, found: 537.3070.

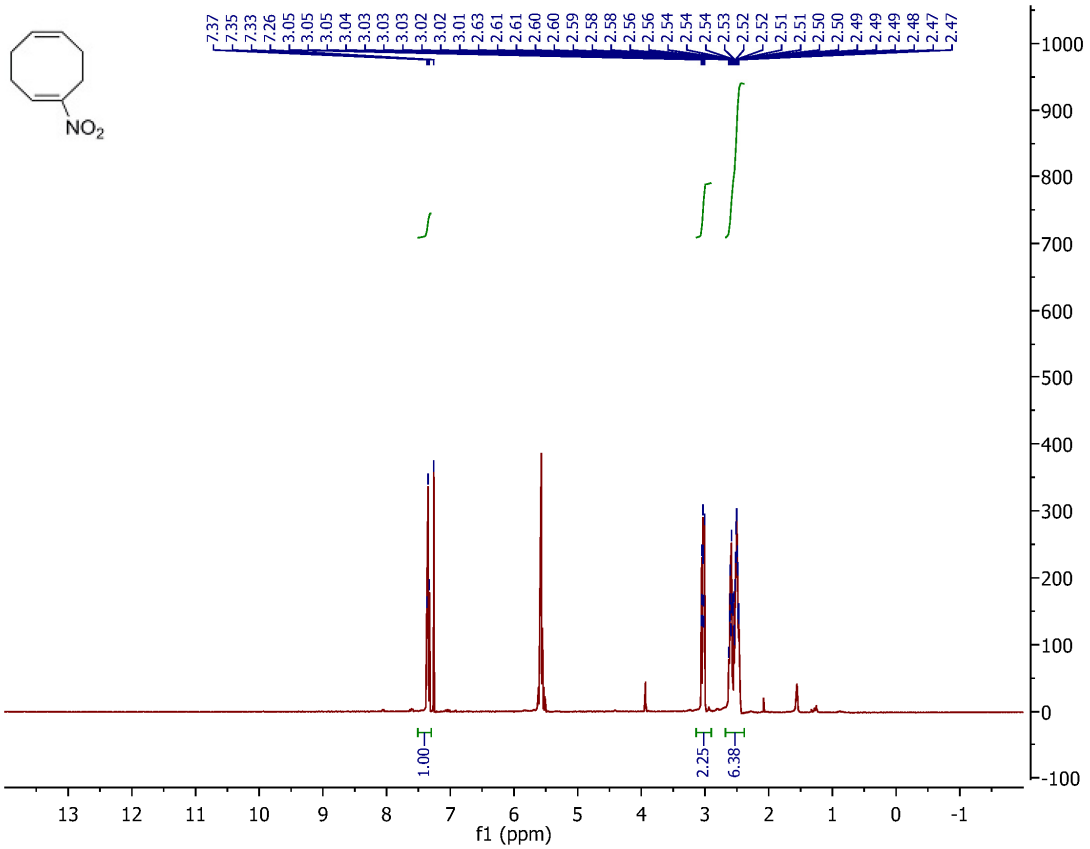
Di-tert-butyl 1-(7-hydroxy-2-oxo-2H-chromen-3-yl)-7-(3-hydroxypropyl)-1,4,5,7,9,10-hexahydropyrrolo[3',4':5,6]cycloocta[1,2-d][1,2,3]triazole-6,8-dicarboxylate (**21**)

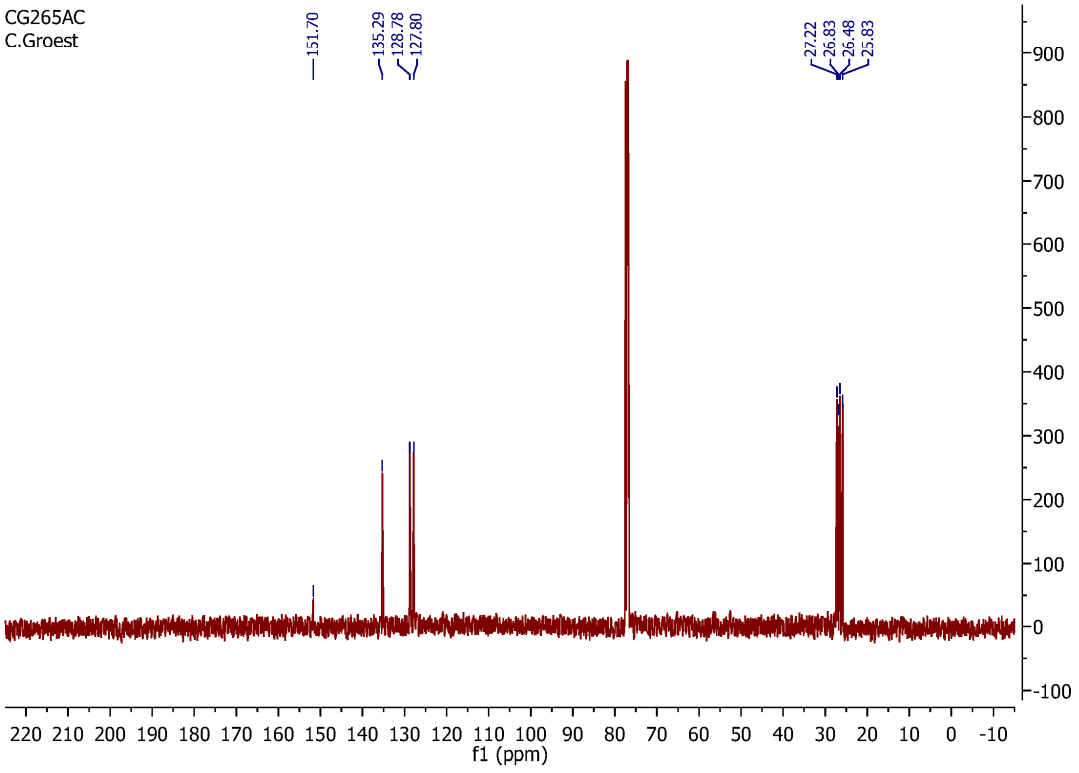


A solution of 3-azido-7-hydroxycoumarin **16** (2.8 mg, 13.6 µmol) and **10** (5.0 mg, 12.4 µmol) in acetonitrile (0.5 mL) was stirred for 96 h at room temperature. The use of acetonitrile as solvent was preferable to water for synthesis on a preparative scale, since it allowed the use of higher concentrations of reactants. Afterwards, the solvent was removed in vacuo. Purification of the crude product by flash column chromatography (dichloromethane:methanol, 100:1 → 20:1) afforded compound **20** (4.4 mg, 7.3 µmol, 59 %) as a pale white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.5 (s, 9H), 1.6 (s, 9H), 3.0 (t, *J* = 6.4 Hz, 2H), 3.2-3.3 (m, 4H), 3.3-3.4 (m, 2H), 3.4 (t, *J* = 5.5 Hz, 2H), 4.7 (t, *J* = 6.4 Hz, 2H), 6.9-7.0 (m, 2H), 7.3-7.5 (m, 1H), 7.9 (s, 1H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 22.6, 24.8, 25.0, 26.1, 28.5, 28.6, 29.9, 34.8, 43.1, 59.2, 82.7, 82.8, 110.9, 115.1, 119.3, 125.2, 125.4, 128.0, 128.6, 130.7, 136.0, 142.3, 144.2, 156.2, 157.4, 161.5, 161.6, 162.9 ppm; HR-ESI-MS $C_{32}H_{38}N_4O_8$ calcd: 629.2582 $[M+Na]^+$, found: 629.2575.

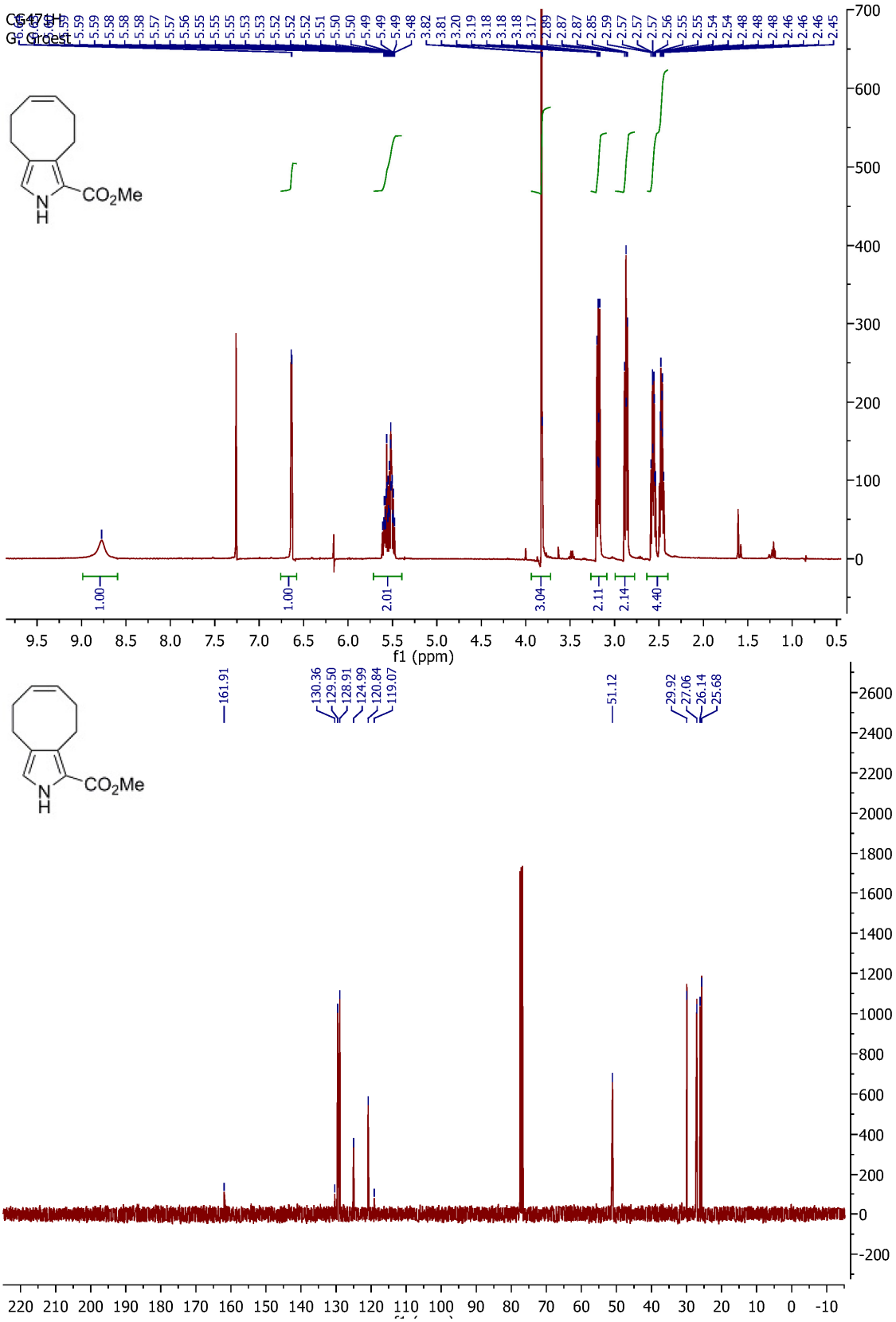
NMR spectra

^{1}H and ^{13}C-NMR of compound **1**

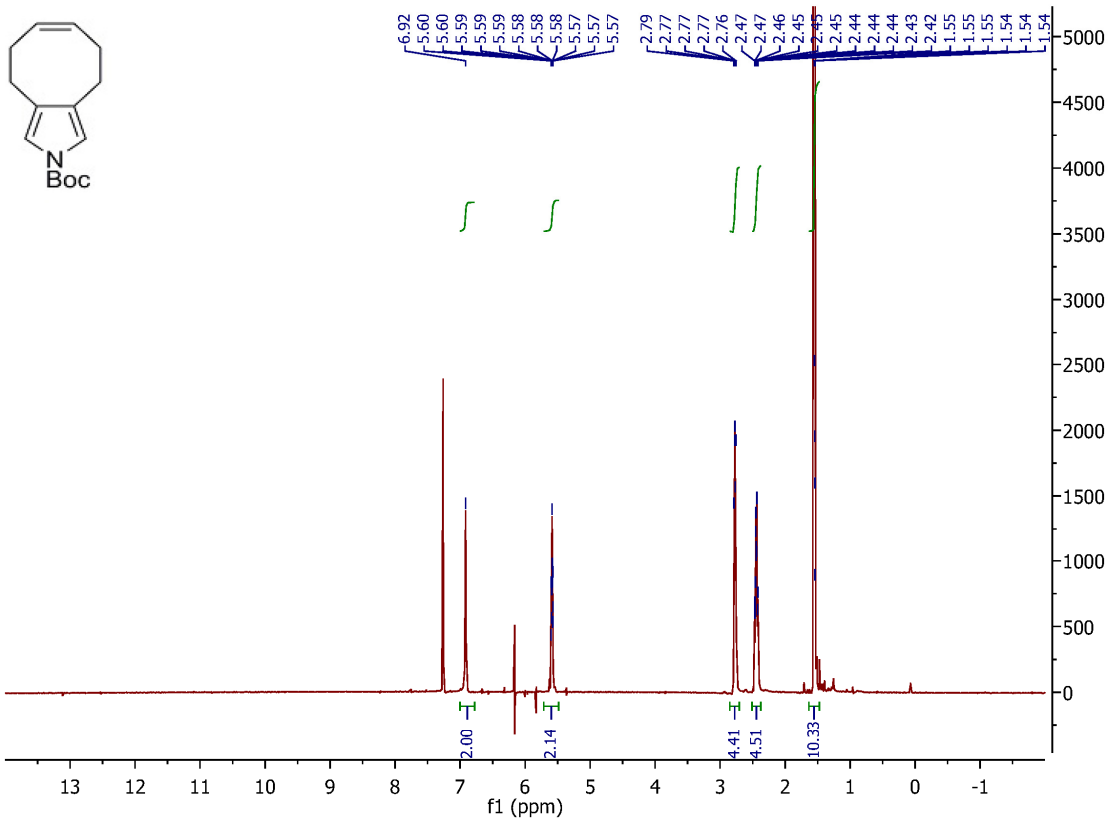


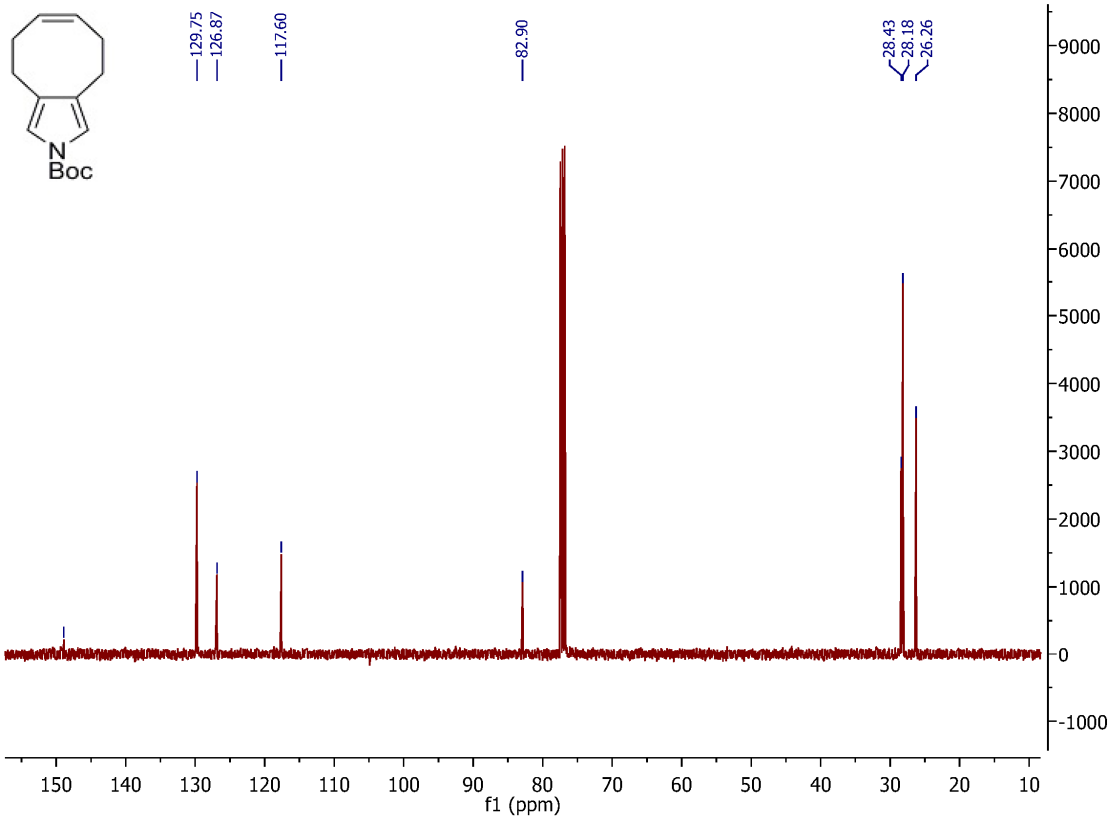


^{1}H and ^{13}C-NMR of compound **2**

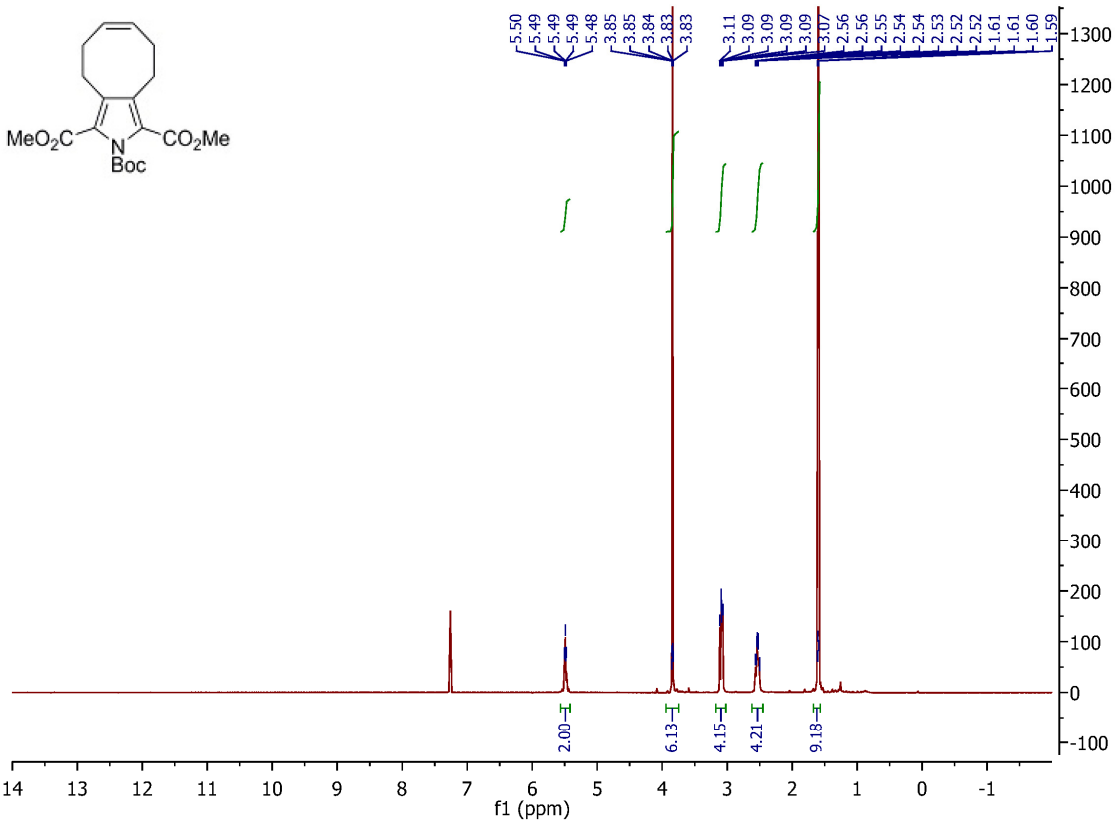


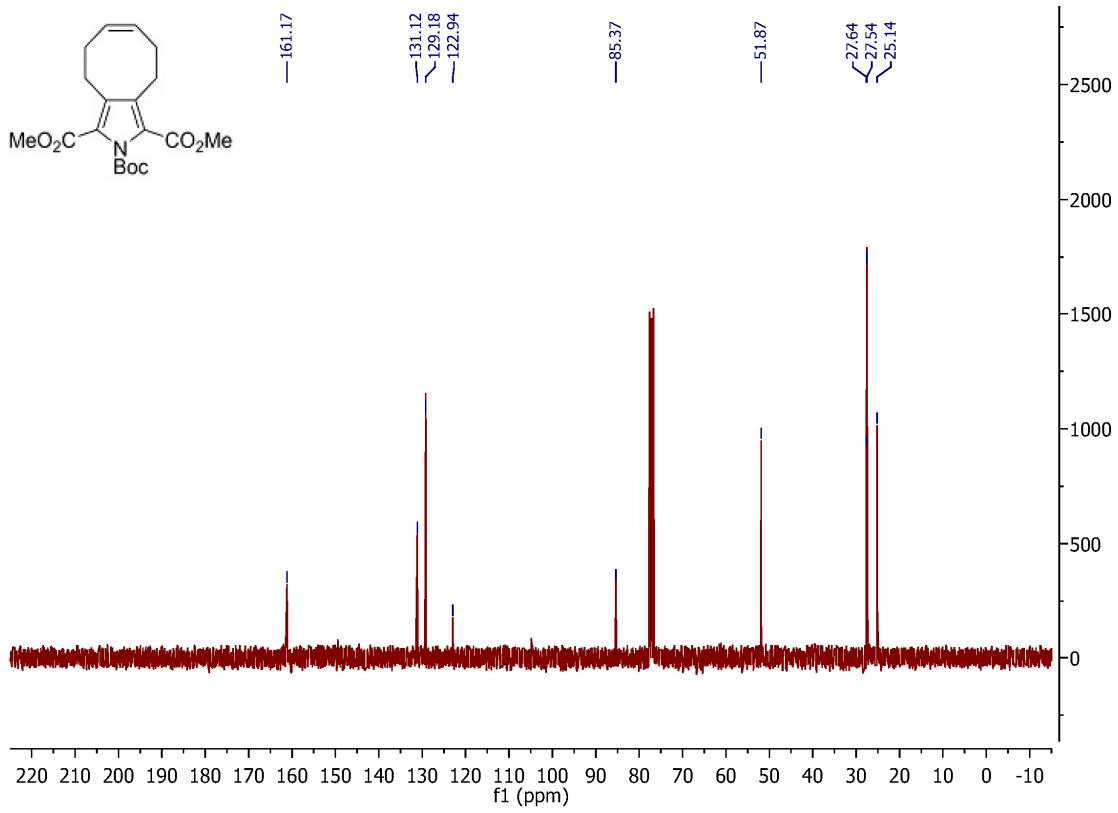
^{1}H and ^{13}C-NMR of compound **3**



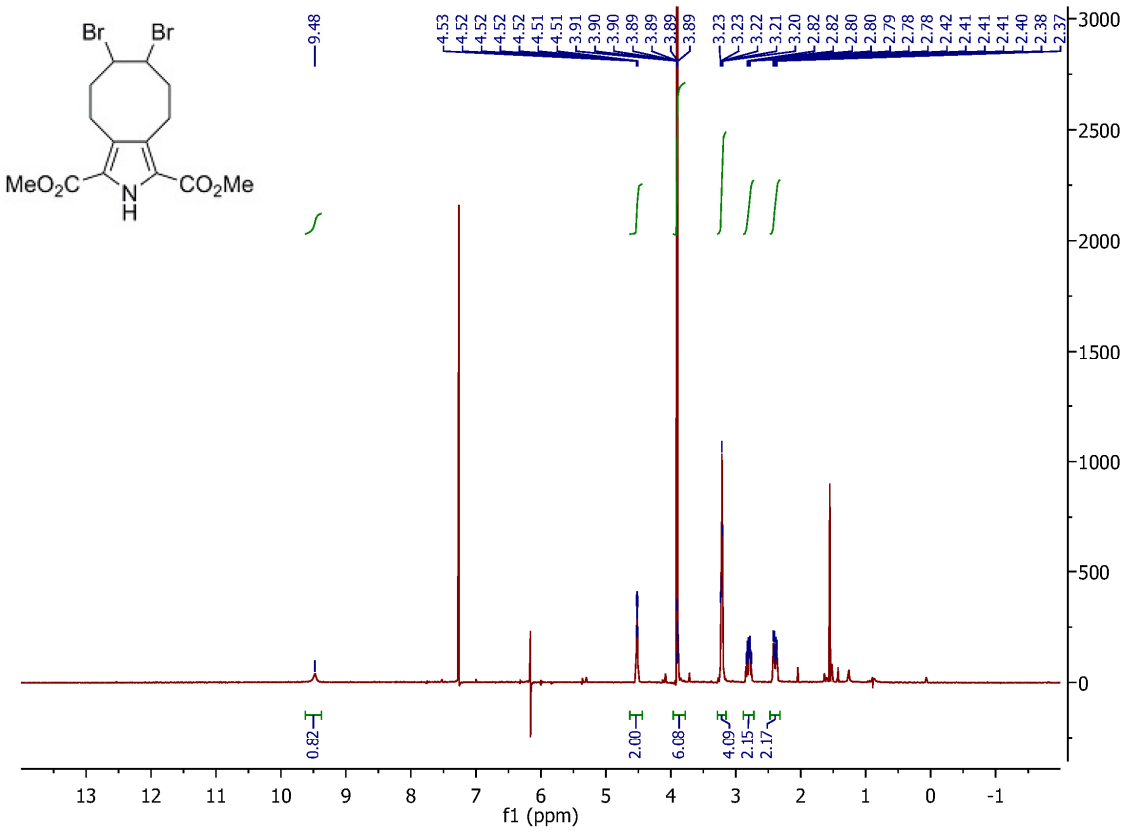


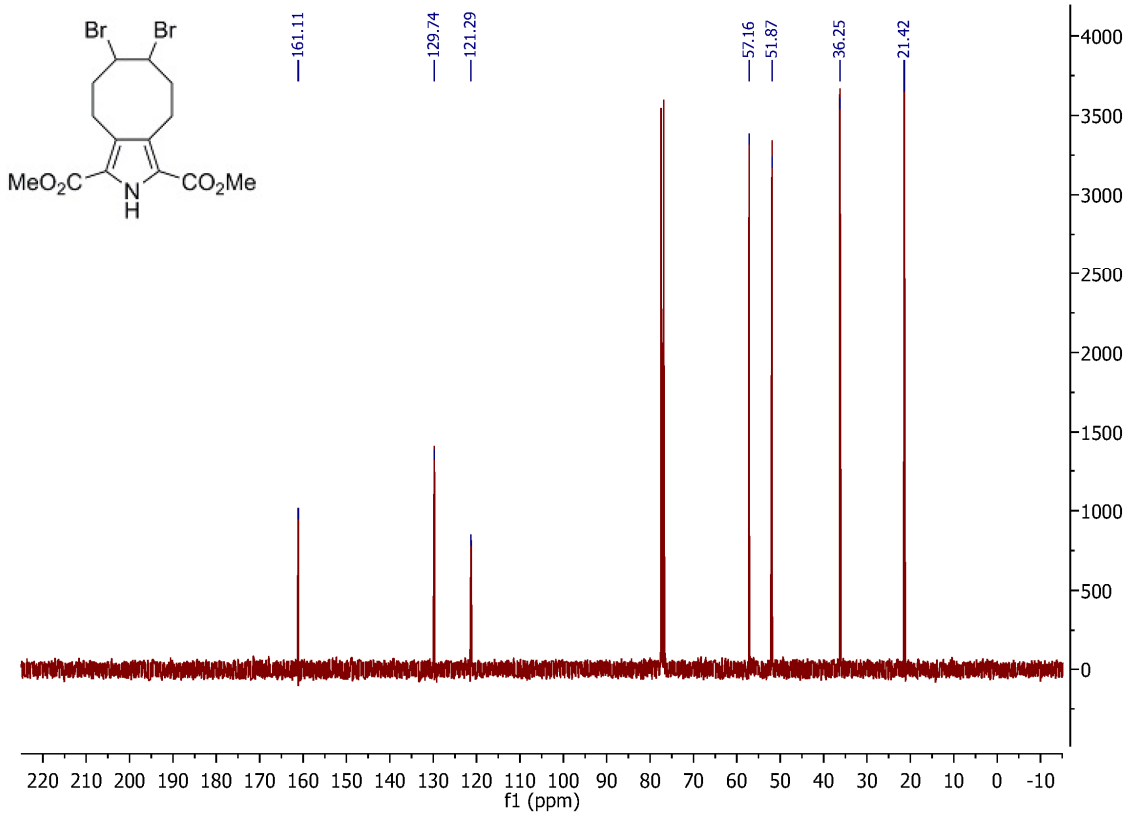
^{1}H and ^{13}C-NMR of compound **4**



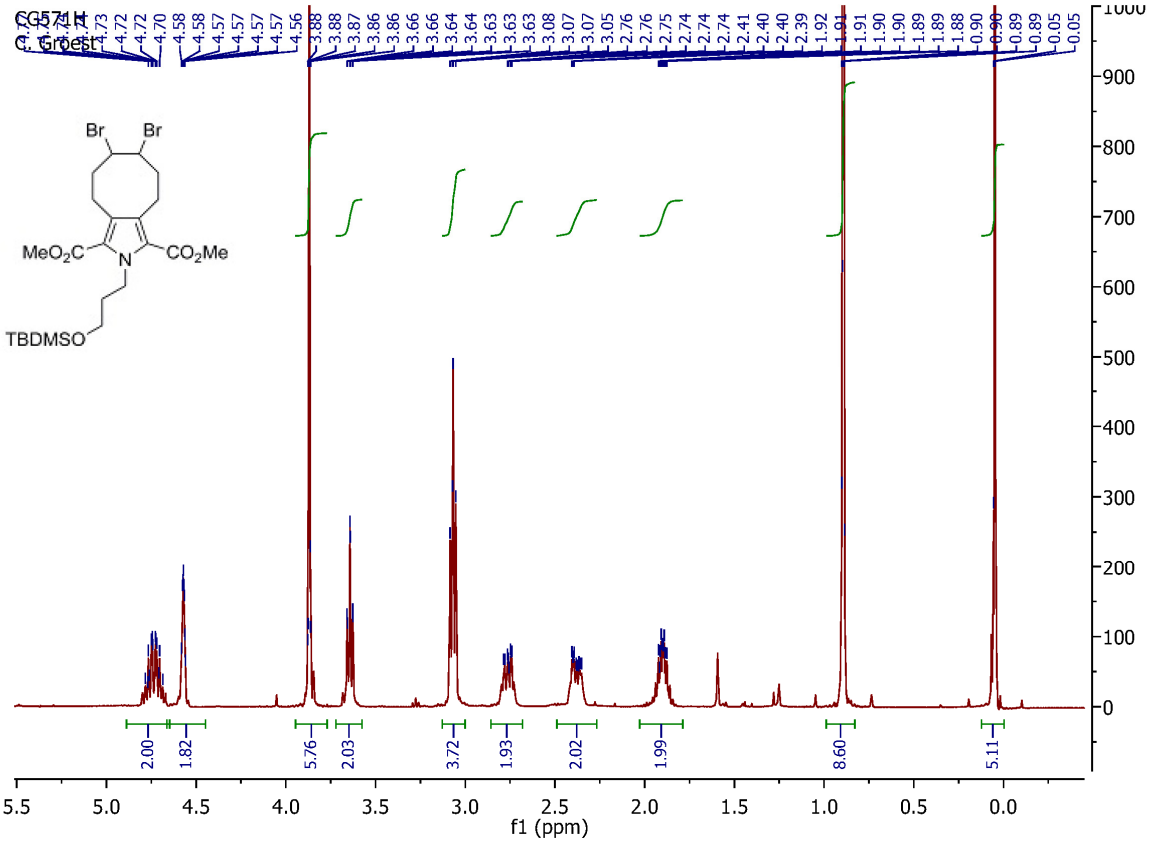


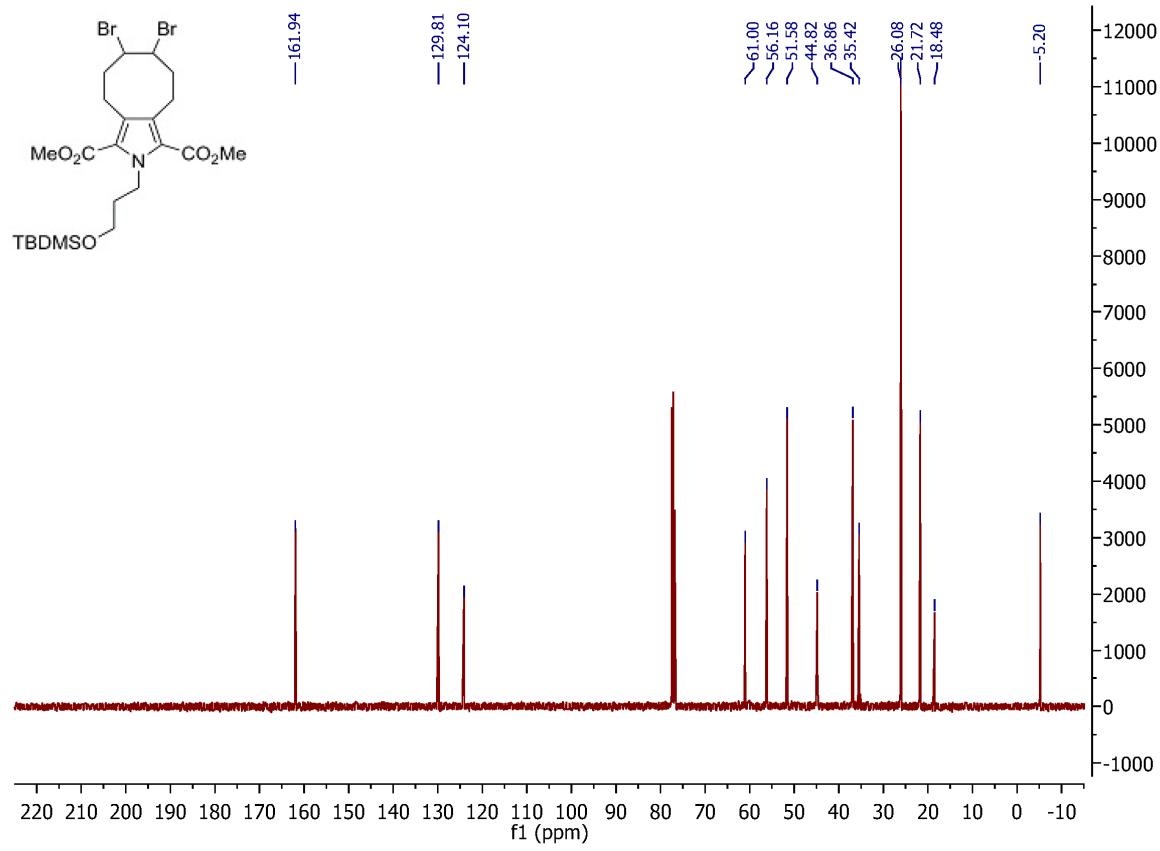
^{1}H and ^{13}C-NMR of compound **5**



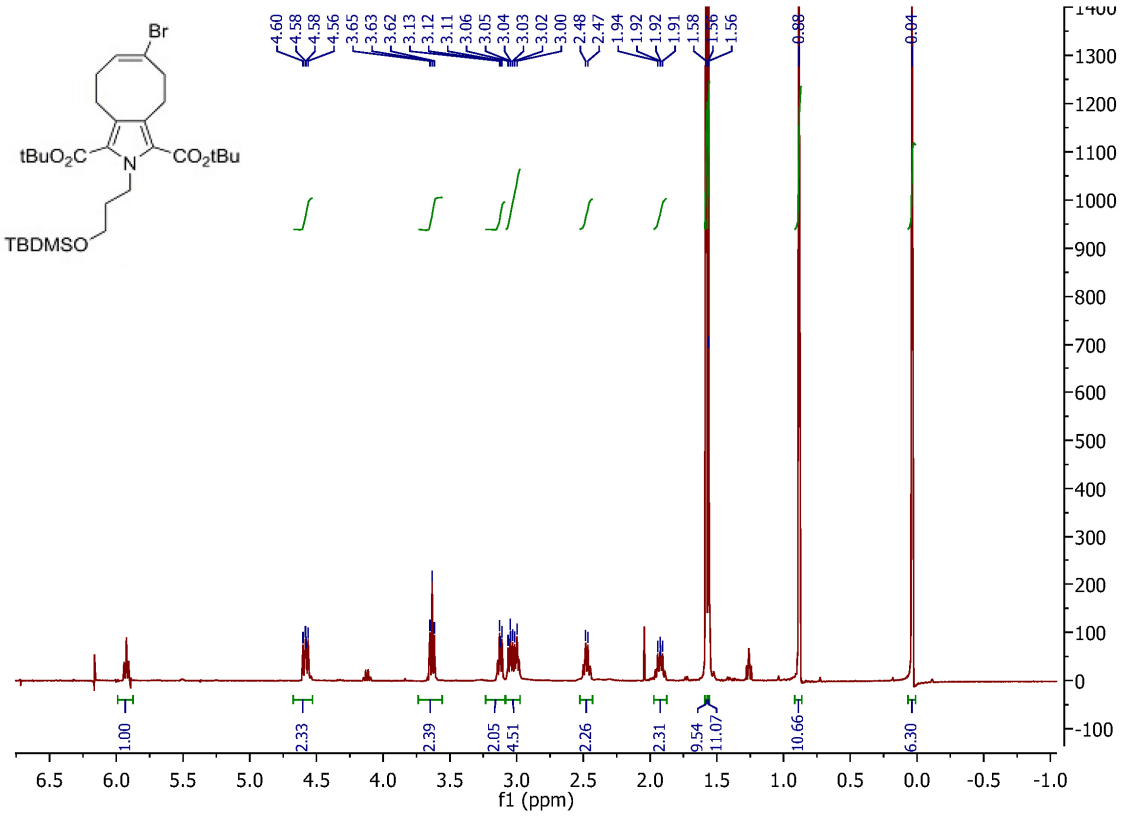


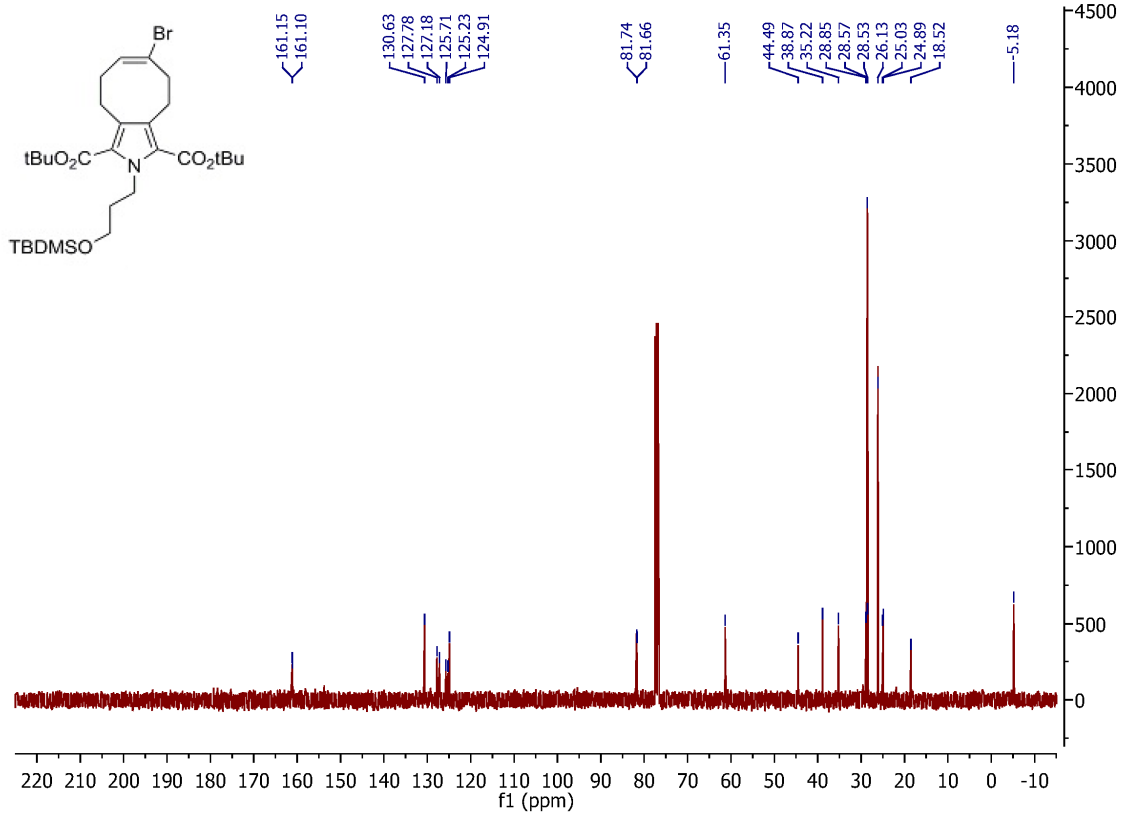
^{1}H and ^{13}C-NMR of compound **7**



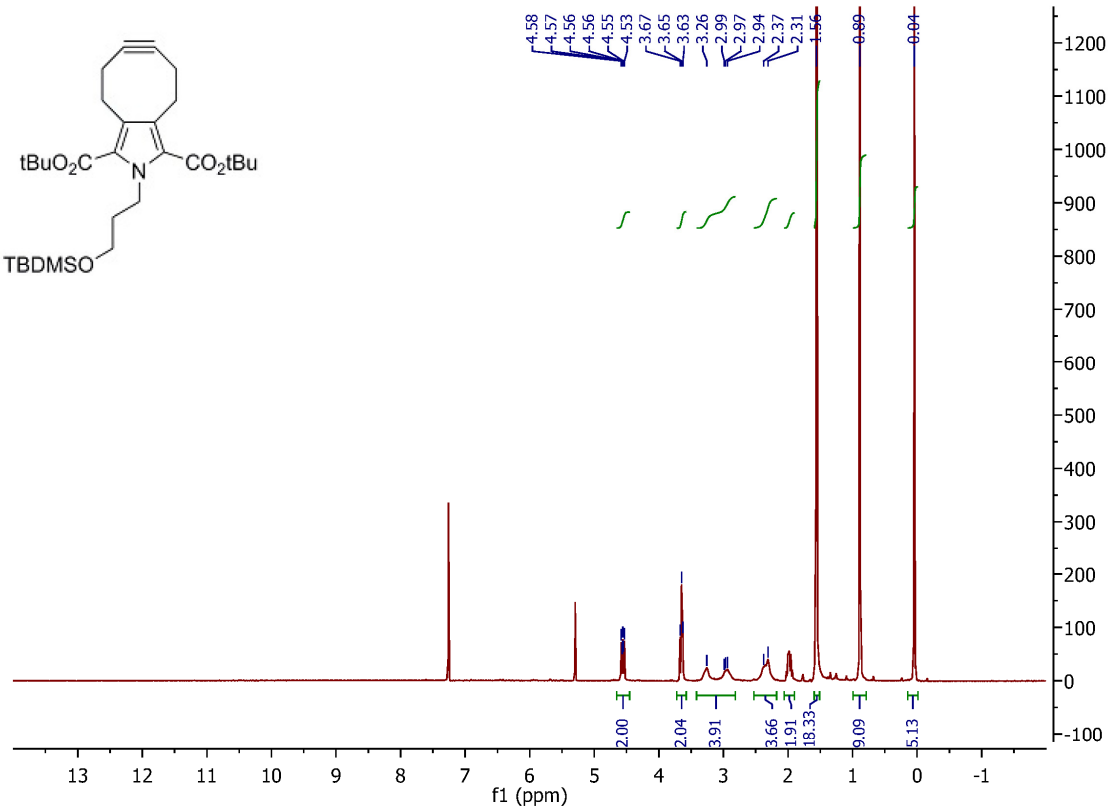


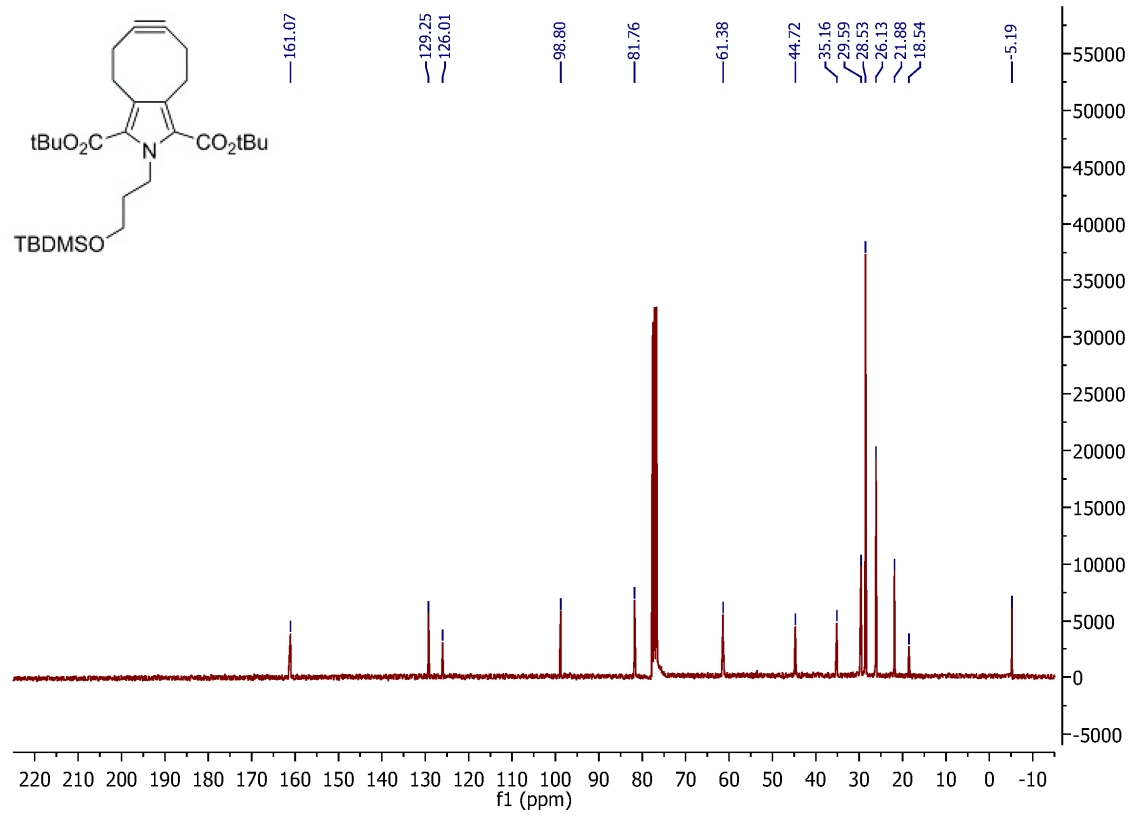
^{1}H and ^{13}C-NMR of compound **8**



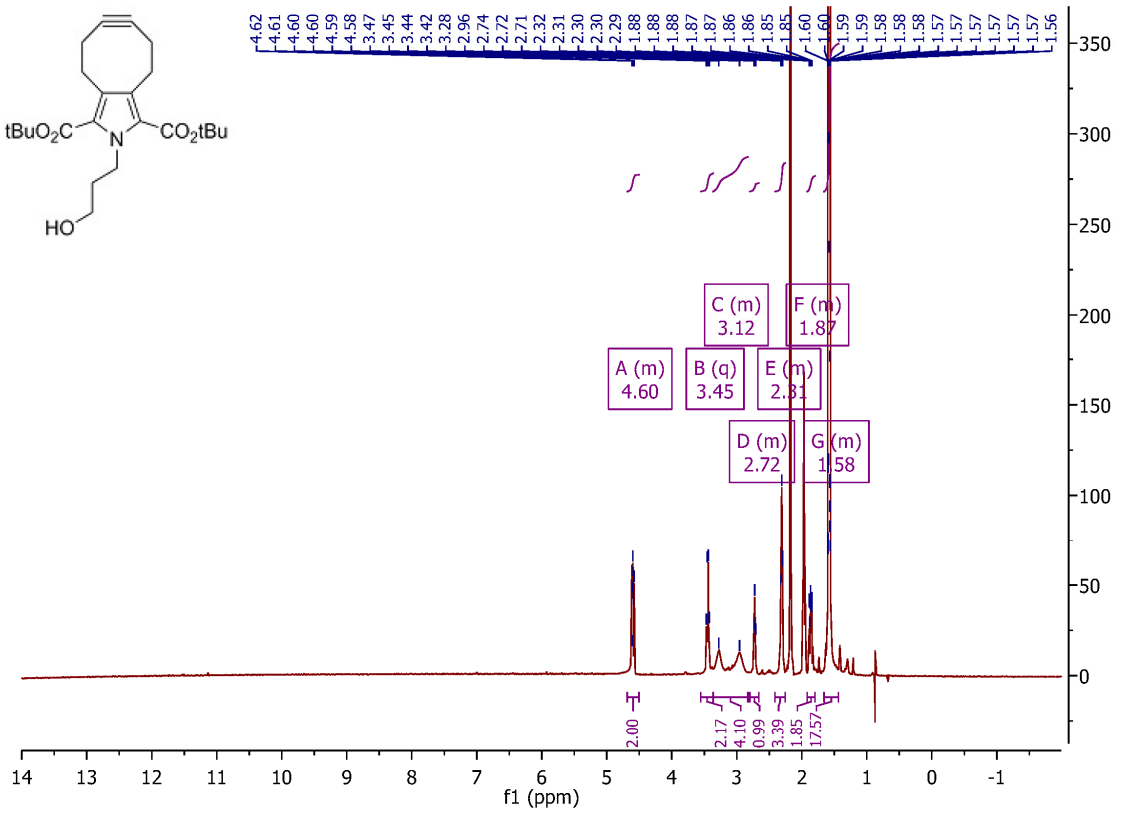


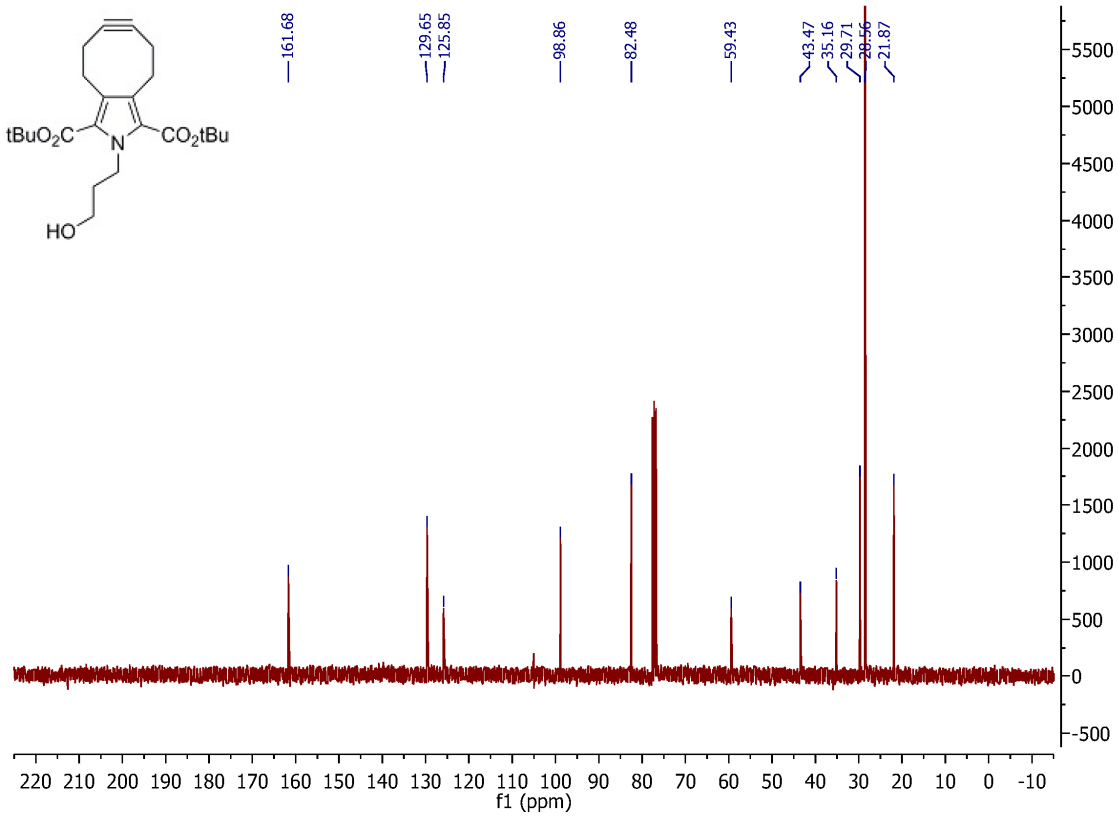
[1]H and [13]C-NMR of TBDMS-protected PYRROC (**9**)



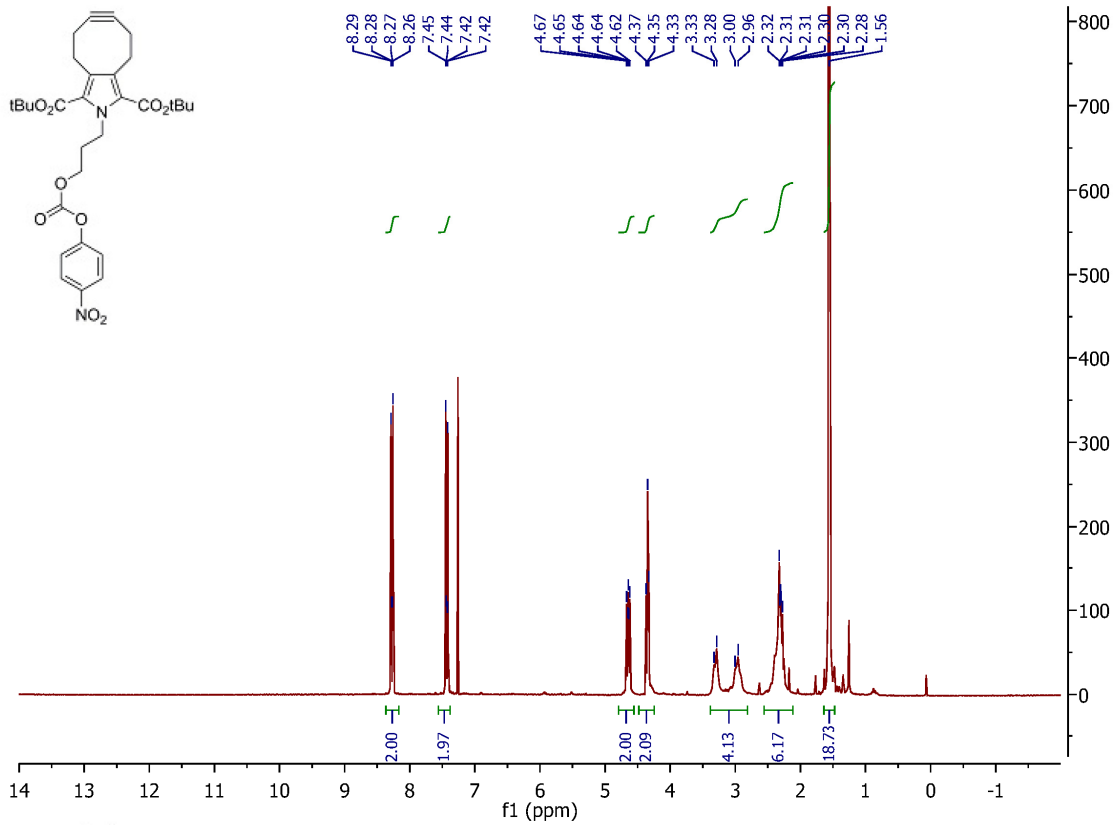


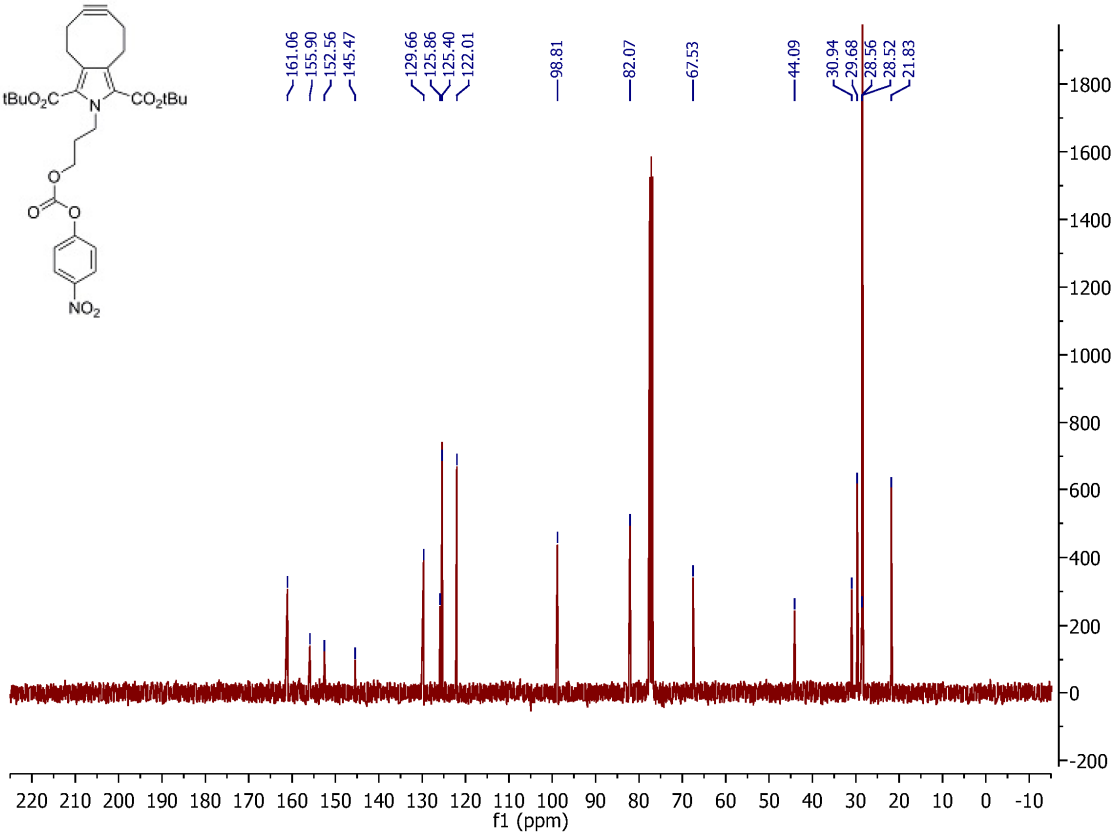
^{1}H and ^{13}C-NMR of PYRROC (**10**)



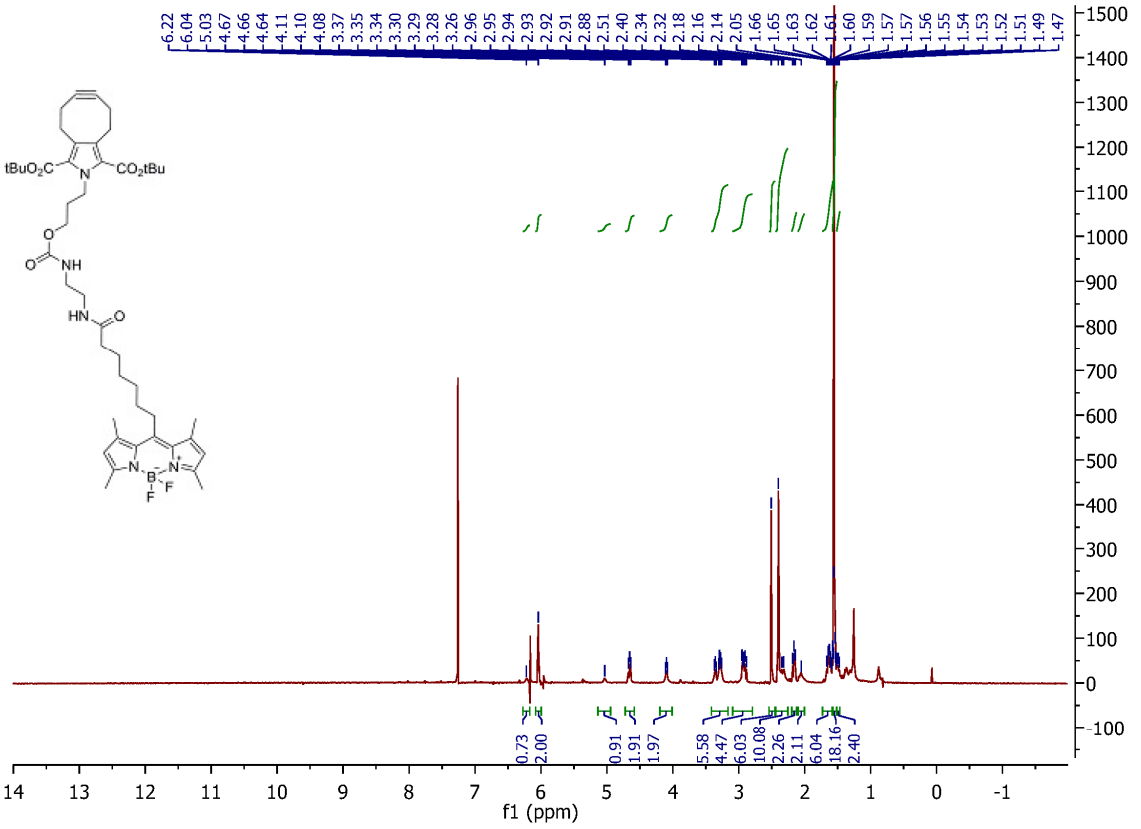


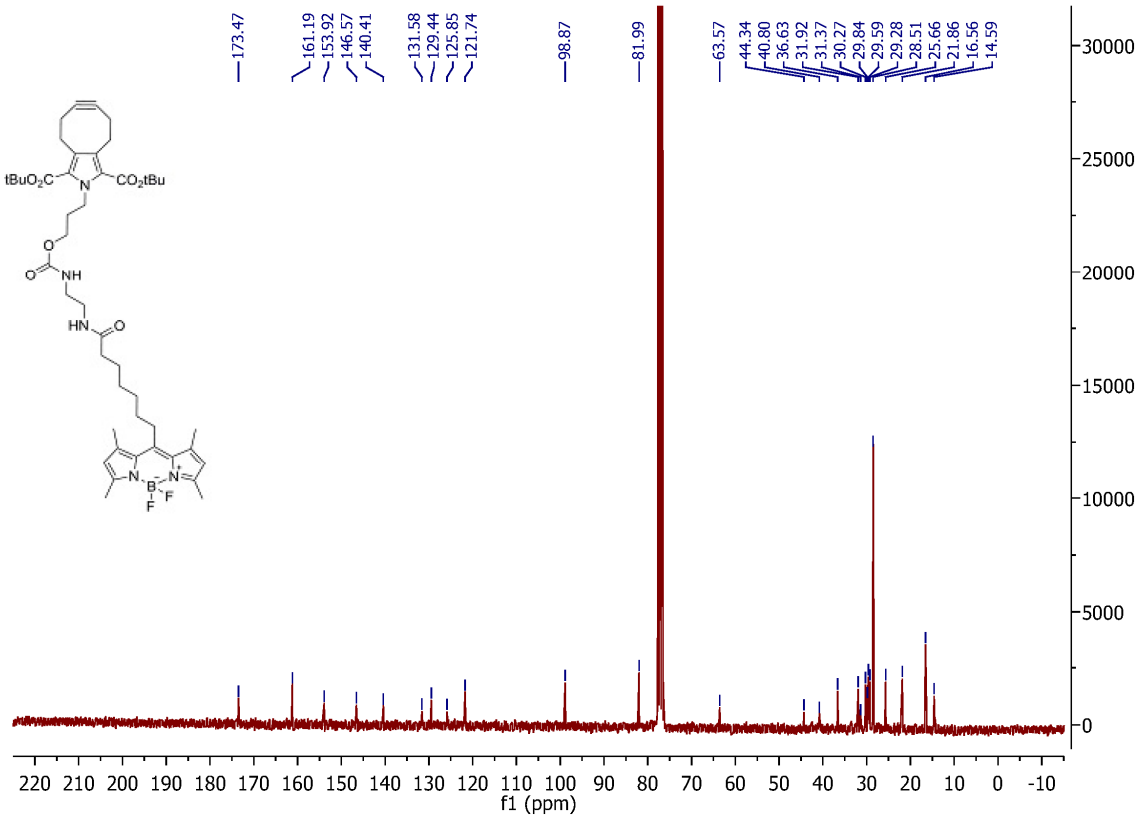
^{1}H and ^{13}C-NMR of compound **11**



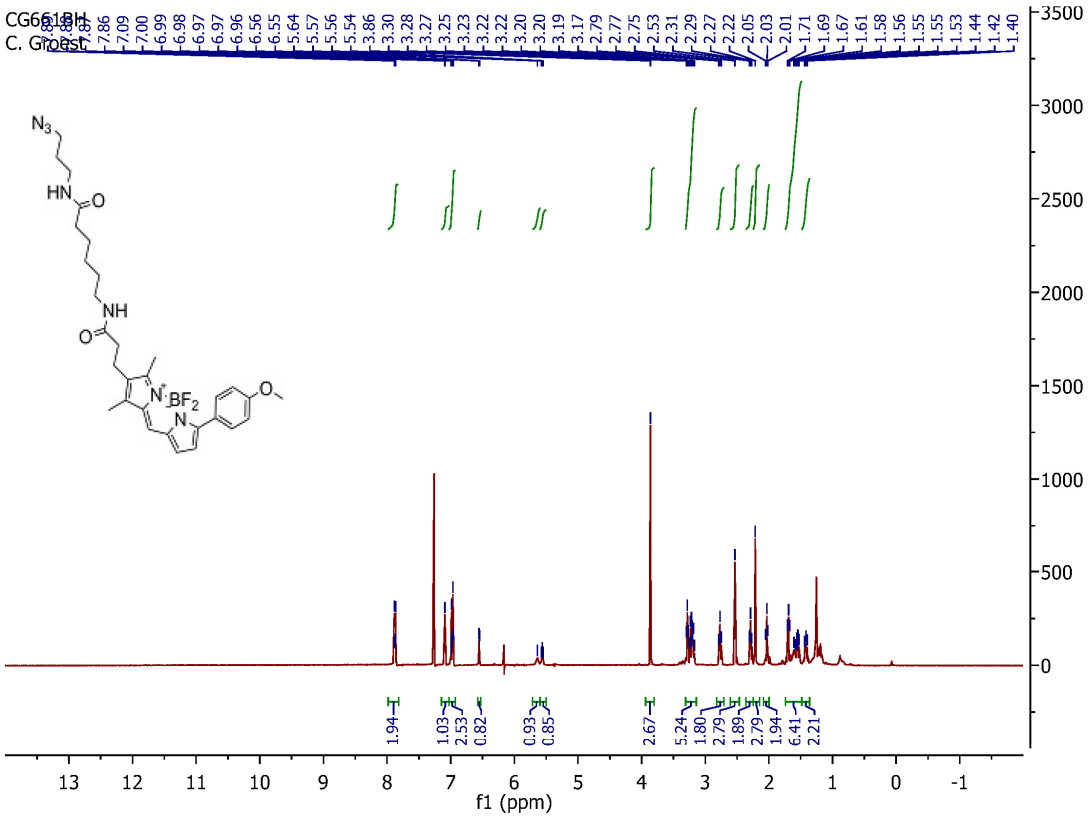


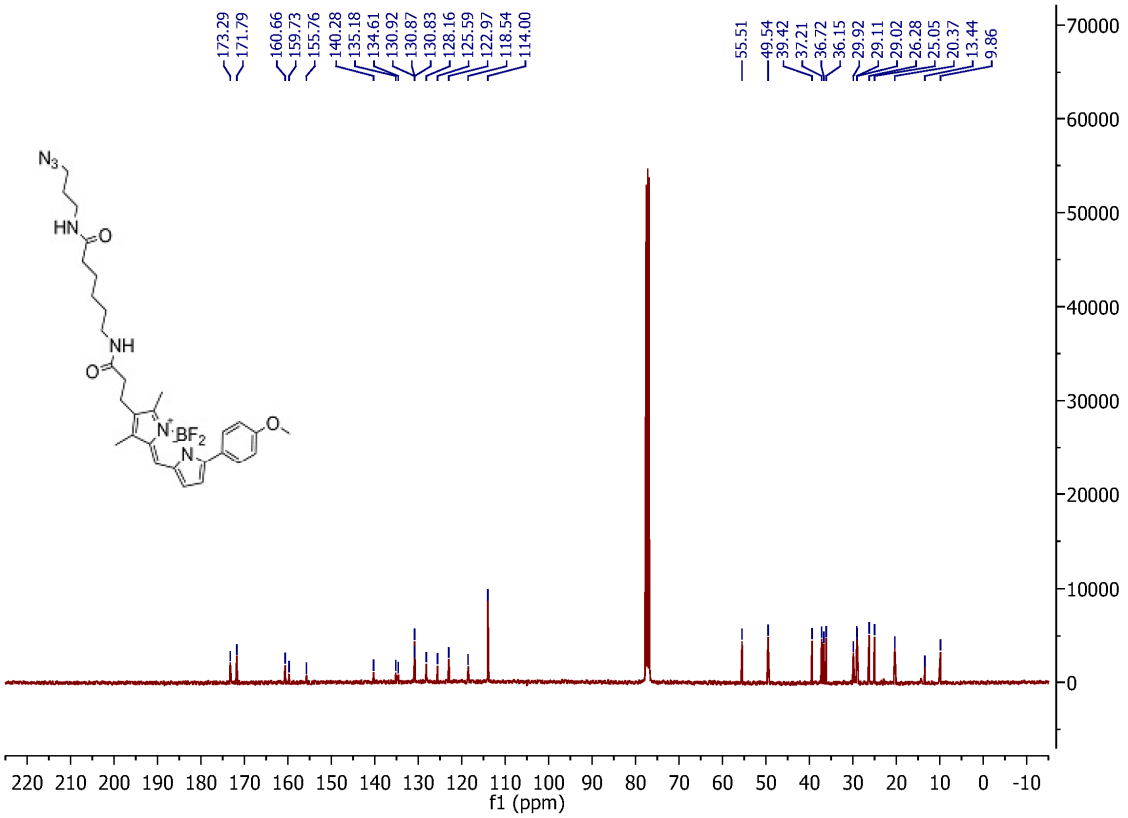
^{1}H and ^{13}C-NMR of compound **13**



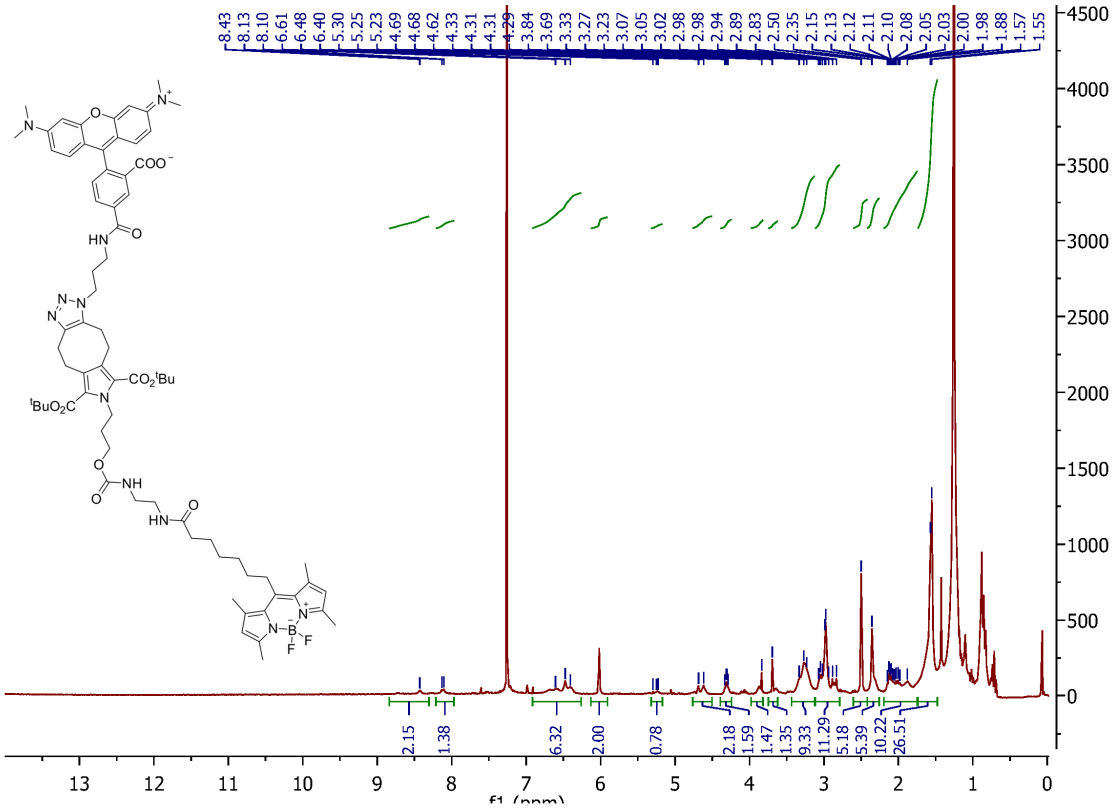


^{1}H and ^{13}C-NMR of compound **15**

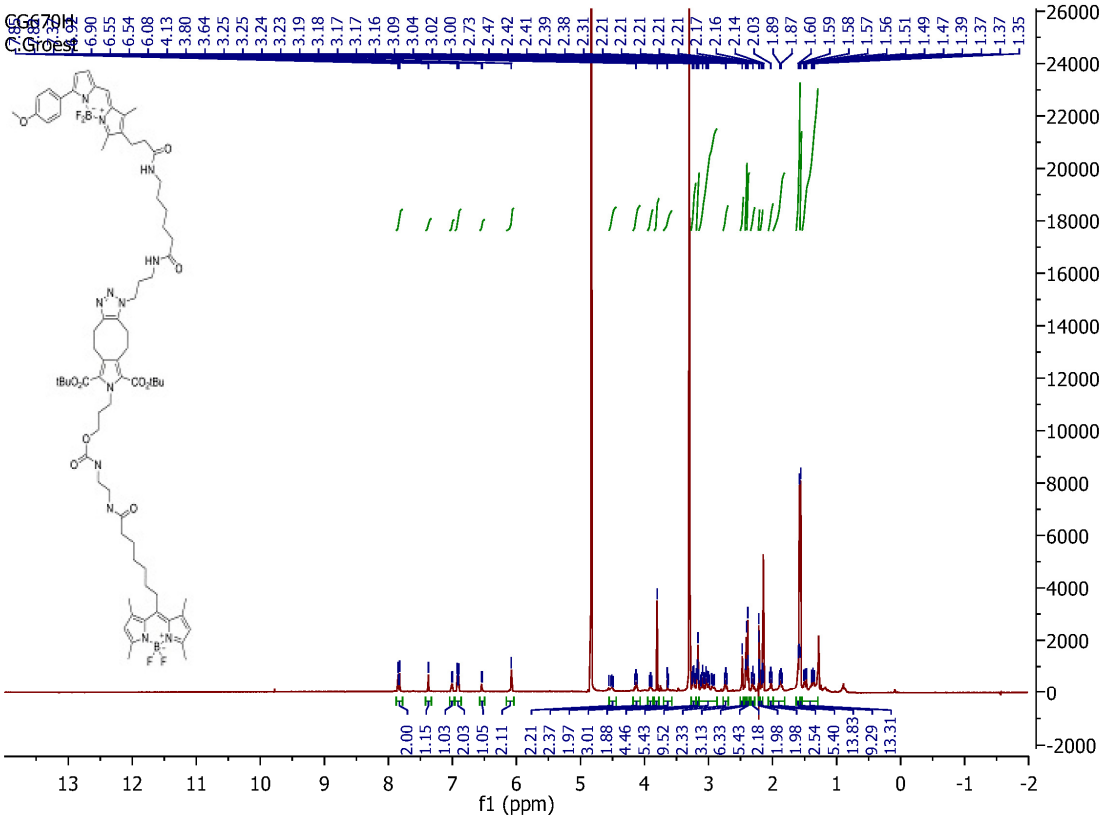




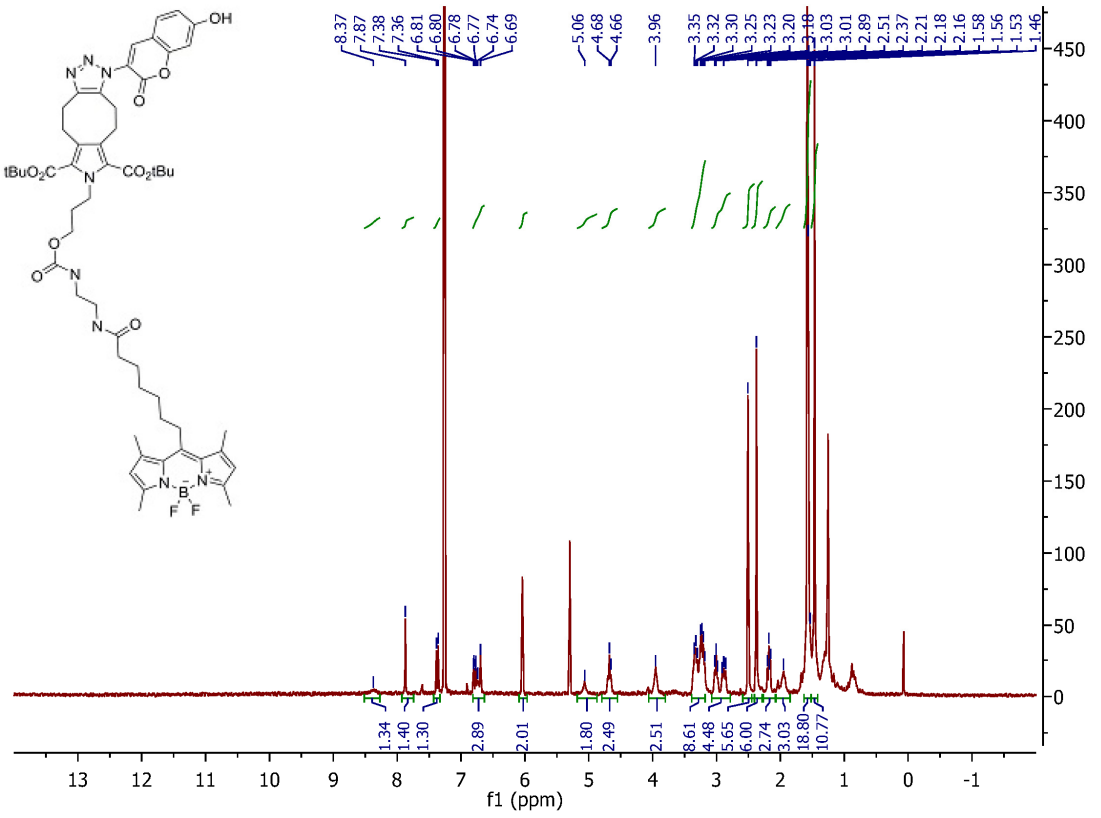
^{1}H-NMR of compound **17**



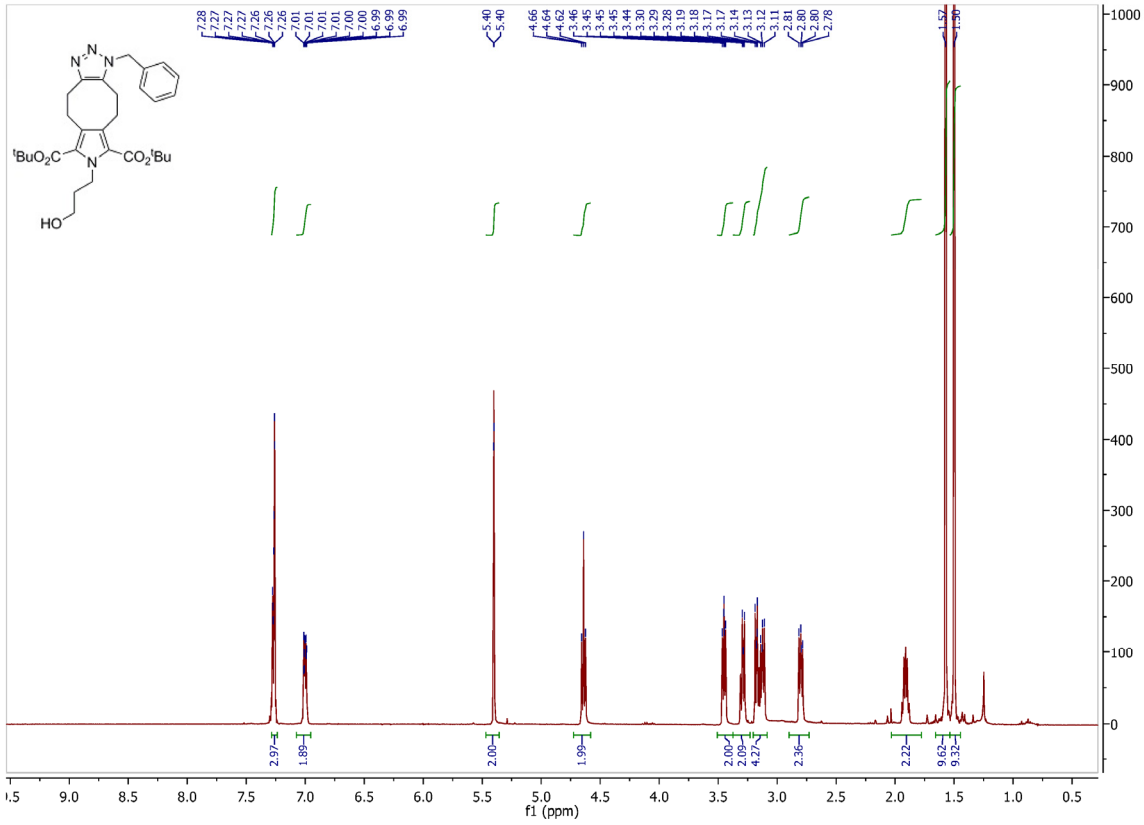
^{1}H-NMR of compound **18**



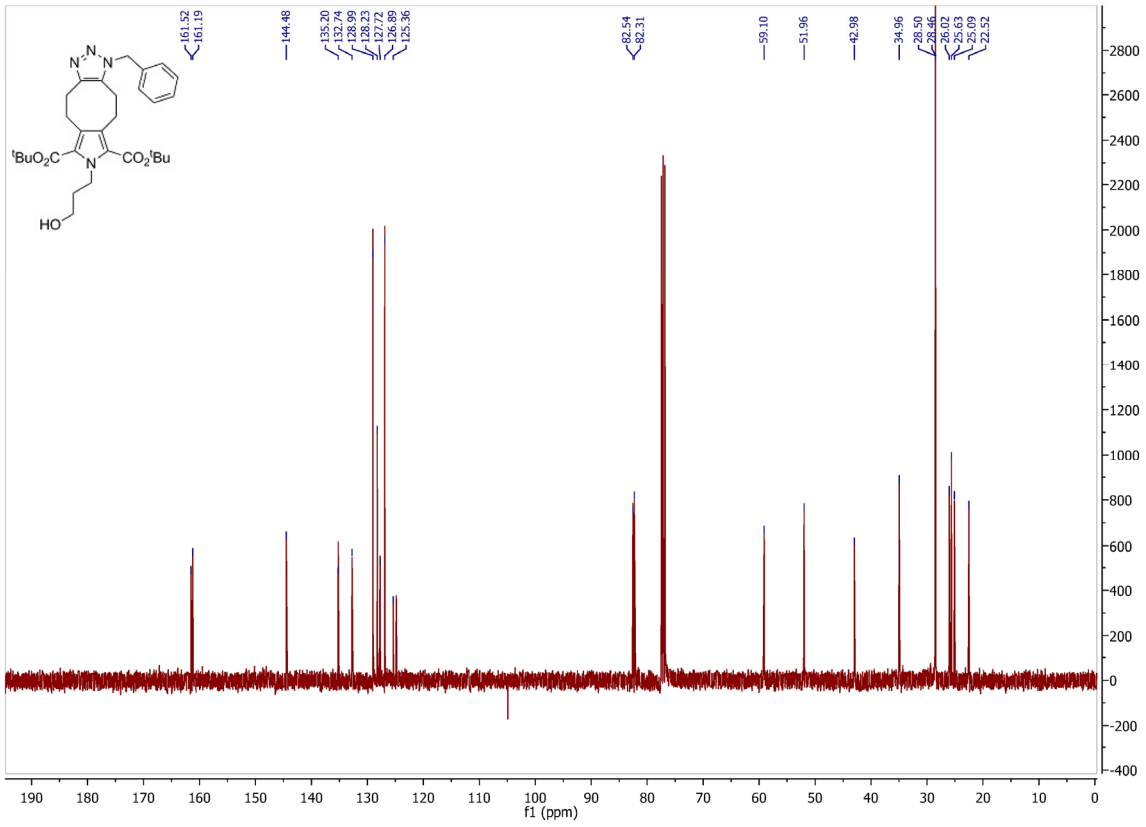
^{1}H-NMR of compound **19**



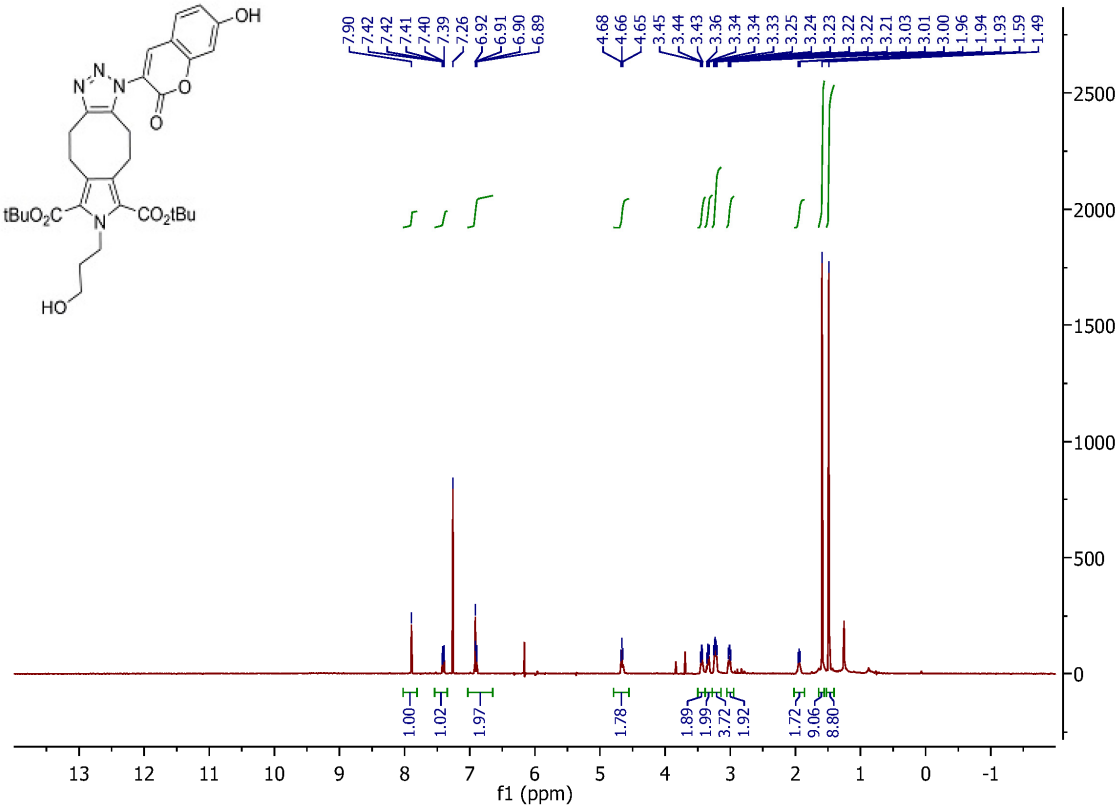
^{1}H-NMR of compound **20**



^{13}C-NMR of compound **20**



^{1}H-NMR of compound **21**



^{13}C-NMR of compound **21**