Bright, “clickable” porphyrins enable visualization of oxygenation under ambient light

SUPPORTING INFORMATION

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General information

Unless otherwise noted, solvents and reagents were purchased (Sigma Aldrich or Fisher Scientific) and used without further purification. With the exception of dry dichloromethane and tetrahydrofuran, which were freshly distilled over dehydrating reagents, all other dry solvents were purchased and supplied in bottles with molecular sieves. Thin-layer chromatography (TLC) was performed on 20 x 20 cm, glass-supported silica gel plates with fluorescent indicator (Sigma). TLC plates were visualized either under a hand-help UV lamp, or by staining with an aqueous solution of potassium permanganate. Column chromatography was performed on silica gel 60 (Alfa Aesar). NMR spectra were recorded on a Bruker 300 MHz Ultrashield magnet using Topspin software. Mass spectra were obtained on a Bruker Microflex MALDI-TOF mass spectrometer, and universal matrix (NIST database; http://www.nist.gov/mml/csd/informatics_research/maldi_recipes.cfm) was used unless noted otherwise. Quartz fluorometric cells (Starna, Inc, 1 cm optical path length) were used for the acquisition of optical spectra. Absorption spectra were recorded on a Varian Cary 100 spectrophotometer, while emission spectra were recorded on a Horiba FluoroMax fluorimeter. For all emission measurements, the absorbance of the sample at the excitation wavelength was kept below 0.1 OD. For phosphorescence quantum yield measurements, deoxygenation was achieved by bubbling Argon gas (Airgas, Grade 5.5) through the solutions of the metallated porphyrins.

Experimental techniques

Photophysical data

For the spectra displayed in Figure 1 and the calculation of the molar extinction coefficient and quantum yield values shown on Table 1, all absorption and emission spectra were recorded on a Varian Cary 100 spectrophotometer and a Horiba FluoroMax fluorimeter, respectively. For extinction coefficient (ε) measurements, two samples of each phosphor were dried in a high vacuum pump and carefully weighed. Three successive dilutions were performed for each sample, averaging a total of 6 measurements for each value. The ε values were measured using the Beer-Lambert Law ($A= \varepsilon bC$; b is the 1cm optical path length of the cuvette used), using the calculated concentrations (C) of the samples and the absorbances (A) at the reported maxima.

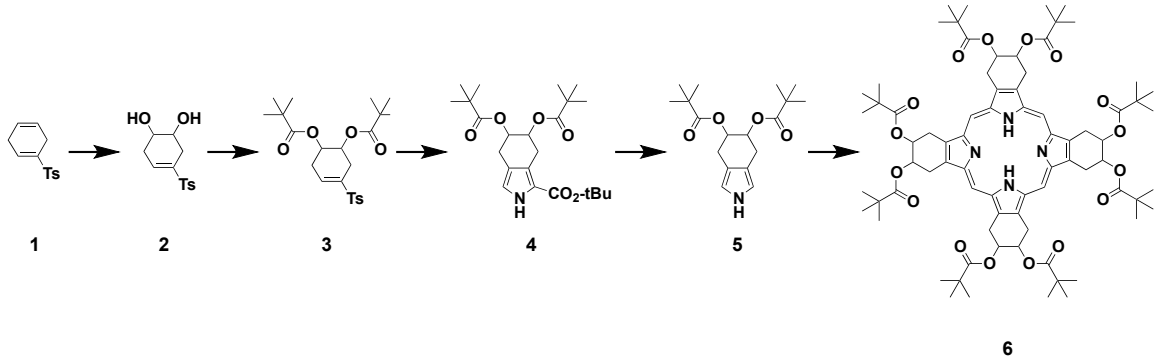
For the quantum yield (Φ) measurements, emission spectra have not been corrected for excitation fluence and detector sensitivity. However, different reference dyes were used to ensure that the phosphors were excited at and detected at

wavelengths close to those of the reference dyes. The following reference dyes were used: Rhodamine 6G (Φ=0.92 in ethanol; λ_{exc}=480nm); cresyl violet perchlorate (Φ=0.54 in ethanol; λ_{exc}=540nm); and Alexa Fluor594 (Φ=0.66 in phosphate buffered saline; λ_{exc}=590nm). The phosphors were excited at their Q-band wavelength maxima. The following formula was used for quantum yield calculations, which corrects for the differences in the optical densities of the solutions of the dyes and the refractive indices of the different solvents:

$$QY = QY_{ref} \times \frac{I \times OD_{ref} \times n^2}{I_{ref} \times OD \times n_{ref}^2}$$

where QY, I and n are the quantum yield, the area under the emission peak and the solvent refractive index, respectively, for the unknown sample. QY_{ref}, I_{ref} and n_{ref} are the corresponding parameters for the reference dye.

Synthesis



Scheme S1

Di-hydroxy sulfone 2*. The reaction does not need to be run under dry and inert conditions.* Tosyl-cyclohexadiene **1**[1] (0.5 g, 2.13 mmol) was dissolved to a 1:1 mixture of tert-butanol (25 mL) and water (25 mL), followed by the addition of potassium hexacyanoferrate (2.11 g, 6.4 mmol), potassium carbonate (0.88 g, 6.4 mmol), methanesulfonamide (0.2 g, 2.13 mmol), and potassium osmate dihydrate (0.0085 g, 0.023 mmol). The reaction mixture was stirred at room temperature overnight. Following the completion of the reaction, sodium sulfite (0.38 g, 2.98 mmol) was added and the mixture was stirred for 2 h. The aqueous layer was removed and kept separately, and portions of ethyl acetate were added to the organic layer until no more water was separated. All aqueous layers were combined with the first, and extractions with ethyl acetate were performed (3 x 15 mL). The combined organic phase was evaporated in vacuum and dried, and NMR analysis showed the white solid (0.45 g, 78%) to be sufficiently pure to be used to the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 7.64 (d, J=8.1 Hz, 2H), 7.24 (d, J=8.1 Hz, 2H), 6.81-6.78 (m, 1H), 5.71-5.54 (m, 2H), 3.82-3.78 (m,2H), 2.43-2.20 (m, 7H); ^{13}C ($CDCl_3$) δ 143.64, 136.65, 135.41, 134.28, 129.22, 127.46, 67.66, 66.78, 42.79, 30.68, 27.88, 21.01.

Pivaloyl-protected di-hydroxy sulfone 3. Diol **2** (1.85 g, 6.89 mmol) was dissolved in 38 mL of dichloromethane. DMAP (0.168 g, 1.38 mmol), pyridine (1.67 mL, 20.67 mmol) and pivaloyl chloride (2.12 mL, 17.22 mmol) were added, and the reaction mixture was stirred overnight at room temperature. TLC was performed using a 10% tetrahydrofuran/dichloromethane solvent mixture. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ and the organic layer was kept separately. The aqueous layer was extracted with dichloromethane, and the combined organic layers were washed with brine, dried over $MgSO_4$ and filtered, and the solvent was removed on a rotary evaporator. The residue was purified by chromatography on a silica column, using a gradient solvent system (5% to 30% tetrahydrofuran/dichloromethane). 1.6 g (53% yield) of a white solid was isolated. The starting diol (0.6 g) was recovered, to give approximately an 85% conversion for this reaction. ^{1}H NMR ($CDCl_3$) δ 7.72 (d, J=8.1 Hz, 2H), 7.31 (d, J=8.1 Hz, 2H), 6.98-6.96 (m, 1H), 5.16-5.12 (m, 1H), 5.09-5.04 (m, 1H), 2.72-2.37 (m, 7H), 1.10 (s, 9H), 1.06 (s, 9H); ^{13}C ($CDCl_3$) δ 176.84, 176.70, 144.00, 137.09, 135.15, 133.56, 129.36, 127.55, 66.85, 66.58, 38.19, 38.17, 28.12, 26.42, 26.37, 21.03.

tert-butoxycarbonyl pyrrole 4. *Dry tetrahydrofuran was prepared just prior to the reaction, by distillation over lithium aluminum hydride under an argon atmosphere*. A solution of tert-butyl isocyanoacetate (0.645 g, 4.57 mmol) in 13 mL dry tetrahydrofuran was slowly added to a suspension of potassium tert-butoxide (0.513 g, 4.57 mmol) in 13 mL of dry tetrahydrofuran that had been cooled down to 0°C with a water-ice bath. A solution of sulfone **3** (1.71 g, 3.92 mmol) in 10 mL tetrahydrofuran was added dropwise to the suspension at 0°C, and the reaction was allowed to warm to room temperature and was left to react for 4 h. TLC monitoring was performed using a 10% tetrahydrofuran/dichloromethane mixture. Following completion of the reaction, the solvent volume was reduced by rotary evaporation and dichloromethane was added. The organic phase was extracted with water and brine, dried over magnesium sulfate, and the solvent was evaporated. The crude mixture was purified by chromatography on a silica column, using a gradient solvent system (5% to 30% tetrahydrofuran/dichloromethane). 1.34 g (81% yield) of a white solid was isolated. ^{1}H NMR ($CDCl_3$) δ 9.34 (br.s, 1H), 6.56 (d, J=2.7 Hz), 5.27-5.15 (m, 2H), 3.11 (dd, J_1=17.4 Hz, J_2=5.1 Hz, 1H), 2.92 (dd, J_1=17.4 Hz, J_2=6.9 Hz, 1H), 2.86-2.72 (m, 2H), 1.48 (s, 9H), 1.10 (s, 9H), 1.09 (s, 9H); ^{13}C ($CDCl_3$) δ 177.20, 177.07, 160.09, 122.19, 118.67, 117.65, 116.73, 79.95, 69.25, 69.02, 38.23, 27.90, 26.52, 25.81, 24.23.

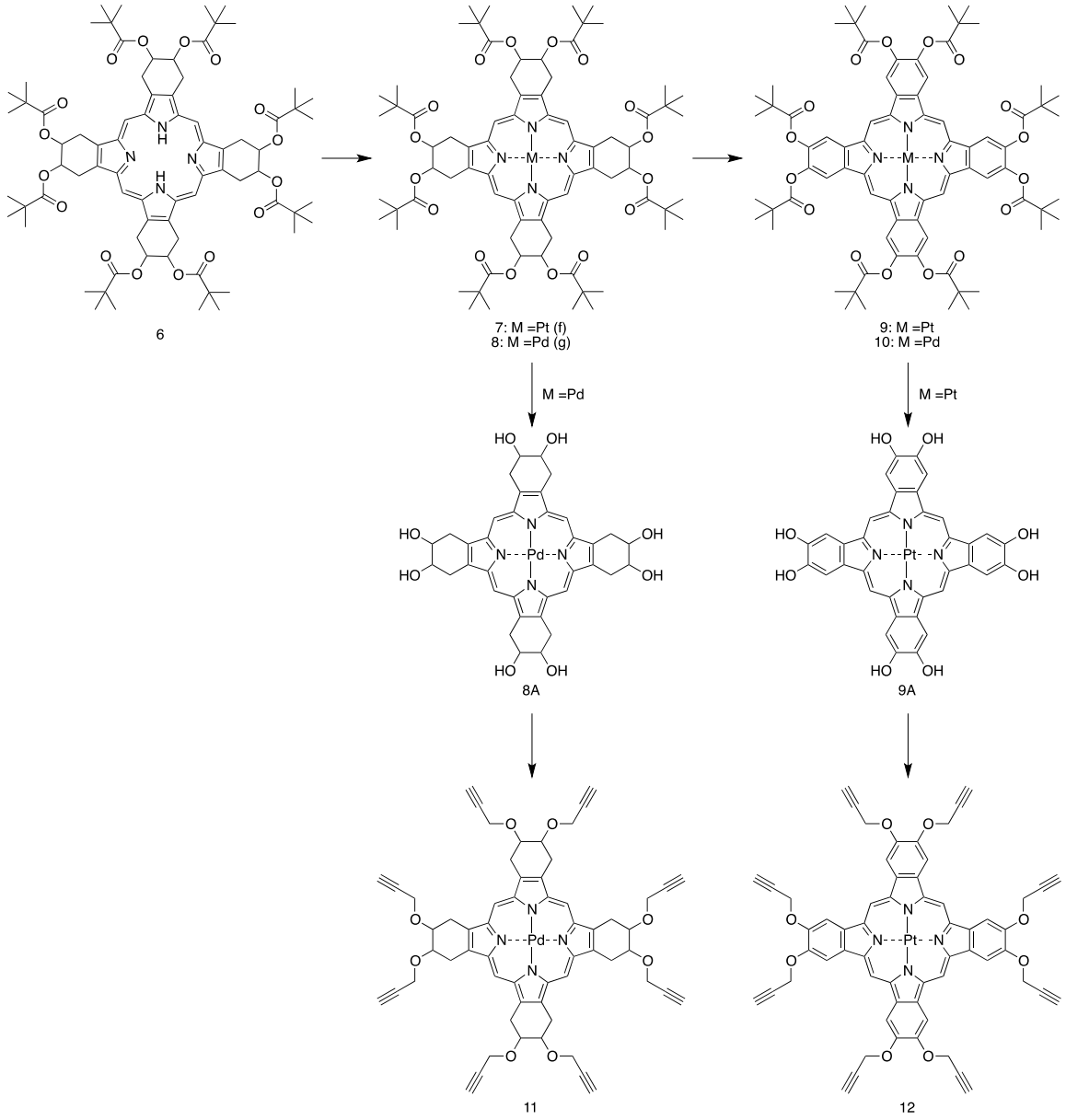
Pivaloyl protected pyrrole 5. 0.742 g (1.76 mmol) of precursor **4** in 6 mL trifluoroacetic acid were stirred in the dark for 30 min. Dichloromethane (~20 mL) was added, and the mixture was poured into ice-cold water. The organic phase was separated, washed with water, then with a 10% aqueous solution of sodium carbonate and finally with water again, and dried over $MgSO_4$. The solvent was removed by rotary evaporation. The residue was purified on a silica gel column (gradient solvent system: 100:1 to 50:1 CH_2Cl_2:THF), to afford 300 mg of a white

solid (52% yield). ^{1}H NMR ($CDCl_3$) δ 8.03 (br.s, 1H), 6.51-6.50 (m, 2H), 5.32-5.28 (m, 2H), 3.00-2.84 (m, 4H), 1.18 (m, 18H)

Notes:
TLC conditions: 100:1 CH_2Cl_2:THF. Please note that the pyrrole is not visible on the TLC plate under a UV lamp. The plate was stained using an aqueous $KMnO_4$ solution, in which the product appears as a bright, yellowish spot.

It is important that the reaction was maintained in the dark, as the pyrrole is light sensitive, and the product was handled under reduced light conditions whenever possible. The product was used quickly following the isolation, and should be stored at -20°C if necessary.

Cyclohexenyl free-base porphyrin 6. In a round bottom flask equipped with a Dean-Stark apparatus and a condenser for azeotropic removal of water were added 293 mg (0.912 mmol) of pyrrole **5** in 182 mL benzene. The solution was flushed with a stream of argon, and was protected from light with aluminum foil. Formaldehyde (0.103 mL of a 37% aqueous solution) and *p*-toluenesulfonic acid monohydrate (5.7 mg, 0.03 mmol) were added, and the mixture was refluxed for 6-8 h under an argon atmosphere. After cooling to room temperature, the flask was left open in air for 2 days under continuous stirring, to allow the oxidation of the intermediate porphyrinogen into the final porphyrin. The solvent was removed on a rotary evaporator, and the residue was purified on a silica gel column (gradient solvent system: 100:1 to 10:1 CH_2Cl_2:THF). The dark red fraction was collected and evaporated to dryness, to afford 210 mg (69%) of porphyrin **7** as a dark brown-red solid. ^{1}H NMR ($CDCl_3$) δ 9.90 (br.s, 4H), 6.07 (m, 8H), 4.64-4.37 (m, 16H), 1.28 (m, 72H); MALDI-TOF *m/z* 1328.85 $(M+H)^+$, calc. 1327.64.



Scheme S2

Platinum (7) and palladium (8) porphyrins. In a round bottom flask equipped with a reflux condenser, was added 7.5 mg (0.0056 mmol) of free-base porphyrin **6** and 2 mL benzonitrile, and the solution was flushed with argon gas for 10-15 min. The solution was heated at 100°C in the dark under an argon atmosphere, and $PtCl_2$ (3 mg; 0.0113 mmol) or $PdCl_2$ (2 mg; 0.0113 mmol) were added. The temperature was increased and the mixture was refluxed under argon, protected from ambient light. Metal insertion required 15-20 min in the case of palladium porphyrin, and 8 h in the case of platinum porphyrin. The solvent was removed by distillation under reduced pressure and gentle heating (40-50°C), and the crude mixture was purified on a silica gel column (gradient solvent system: 250:1 to 100:1 CH_2Cl_2:THF). Yield of **7**: 65% (bright red solid). ^{1}H NMR ($CDCl_3$) δ 9.63 (s, 4H), 6.02-5.99 (m, 8H), 4.41-

4.20 (m, 16H), 1.26 (m, 72H); MALDI-TOF *m/z* 1522.14 (M^+), calc. 1520.70; UV-Vis (N,N-Dimethylformamide), $\lambda_{max,nm}$ (ε, $cm^{-1}M^{-1}$): 377 (250,000), 531 (55,000). Yield of **8**: 85% (red solid). ^1H NMR ($CDCl_3$) δ 9.91 (s, 4H), 6.05-6.02 (m, 8H), 4.60-4.34 (m, 16H), 1.29 (m, 72H); MALDI-TOF *m/z* 1432.58 (M^+), calc. 1432.04; UV-Vis (N,N-Dimethylformamide), $\lambda_{max,nm}$ (ε, $cm^{-1}M^{-1}$): 392 (95,000), 543 (24,000).

Platinum (9) and palladium (10) benzoporphyrins. The metallated porphyrins **7** and **8** were dissolved in dry 1,4-dioxane in a round bottom flask, under an argon atmosphere, at a concentration of $5x10^{-4}$ M. 20 eq. of DDQ were added, and the mixture was refluxed for 6-8 h in the dark. Additional portions of DDQ were added, if needed, until the reaction progressed to completion as determined by UV-Vis spectroscopy. After completion of the reaction, dioxane was removed by rotary evaporation and the residue was dissolved in dichloromethane. The resulting solution was extracted once with water, twice with 10% sodium sulfite solution, once more with water, dried over magnesium sulfate, and then the solvent was evaporated. The crude reaction products were purified on silica gel columns (gradient solvent system: 250:1 to 100:1 CH_2Cl_2:THF). Yield: 60% (blue-green solids). **9**: MALDI-TOF *m/z* 1504.75 (M^+), calc. 1504.58; UV-Vis (dichloromethane), $\lambda_{max,nm}$ (ε, $cm^{-1}M^{-1}$): 396 (95,000), 592 (89,000). **10**: MALDI-TOF *m/z* 1416.44 (M^+), calc. 1415.92; UV-Vis (dichloromethane), $\lambda_{max,nm}$ (ε, $cm^{-1}M^{-1}$): 410 (150,000), 605 (95,000).

Alkynyl-terminated Pd-porphyrin 11 and Pt-benzoporphyrin 12. *Dry dichloromethane was prepared just prior to the reaction, by distillation over lithium aluminum hydride under an argon atmosphere, and was transferred to the reaction flask using a syringe*. 0.0035 mmol of the pivaloyl-protected metalloporphyrin **8** or **9** was dissolved in 3.5 mL of dry CH_2Cl_2, and the solution was kept under an argon atmosphere. The solution was cooled to -78˚C with a dry ice/acetone bath, and 0.07 mL of DIBAL-H solution (1M in dichloromethane; 0.07 mmol) was added dropwise. The reaction mixture was allowed to warm up to room temperature and was left to react under stirring. Deprotection typically required 12 to 24 h, and completion was indicated by precipitation of the product that left a colorless supernatant solution. Completion was further confirmed by the appearance of only the octa-hydroxy porphyrin (**8A** or **9A**) peak on the MALDI-TOF MS spectrum. The reaction was quenched with methanol. After removal of the solvents, the product was resuspended in neat trifluoroacetic acid and stirred for 30 minutes. The solution was then centrifuged, and the resulting supernatant fractions were combined in a round bottom flask. The solvent was removed by rotary evaporation and the remaining residue was used for the subsequent alkylation reaction with no further purification.

The residue containing the octa-hydroxy metalloporphyrin **8A** or **9A** was dissolved in 0.250 mL of dry DMF under an argon atmosphere, the solution was cooled to 0°C with an ice/water bath and 1.7 mg of sodium hydride (60% dispersion in mineral oil; 0.042 mmol) were added in one portion. The mixture was stirred for 1 h at room

temperature, and propargyl bromide (0.005 mL of an 80% solution in toluene, 0.056 mmol), was added by syringe. It should be noted that the propargyl bromide must be fresh and colorless. If the reagent has turned yellow-brown, the reaction will not proceed. The reaction was stirred at room temperature for 48 h. Following completion of the alkylation, the solvents were removed and the residue was purified on a silica gel column (gradient solvent system: 250:1 to 100:1 CH_2Cl_2:THF). The reaction afforded a two-step yield of 35%. **8A**: MALDI-TOF *m/z* 759.58 (M^+), calc. 759.11. **9A**: MALDI-TOF *m/z* 832.06 (M^+), calc. 831.64. **11**: MALDI-TOF *m/z* 1063.88 (M^+), calc. 1063.50; UV-Vis (N,N-Dimethylformamide), $\lambda_{max,nm}$ (ε, $cm^{-1}M^{-1}$): 392 (93,000), 544 (18,000). **12**: MALDI-TOF *m/z* 1135.23 (M^+), calc. 1136.03; UV-Vis (dichloromethane), $\lambda_{max,nm}$ (ε, $cm^{-1}M^{-1}$): 398 (90,000), 595 (75,000).

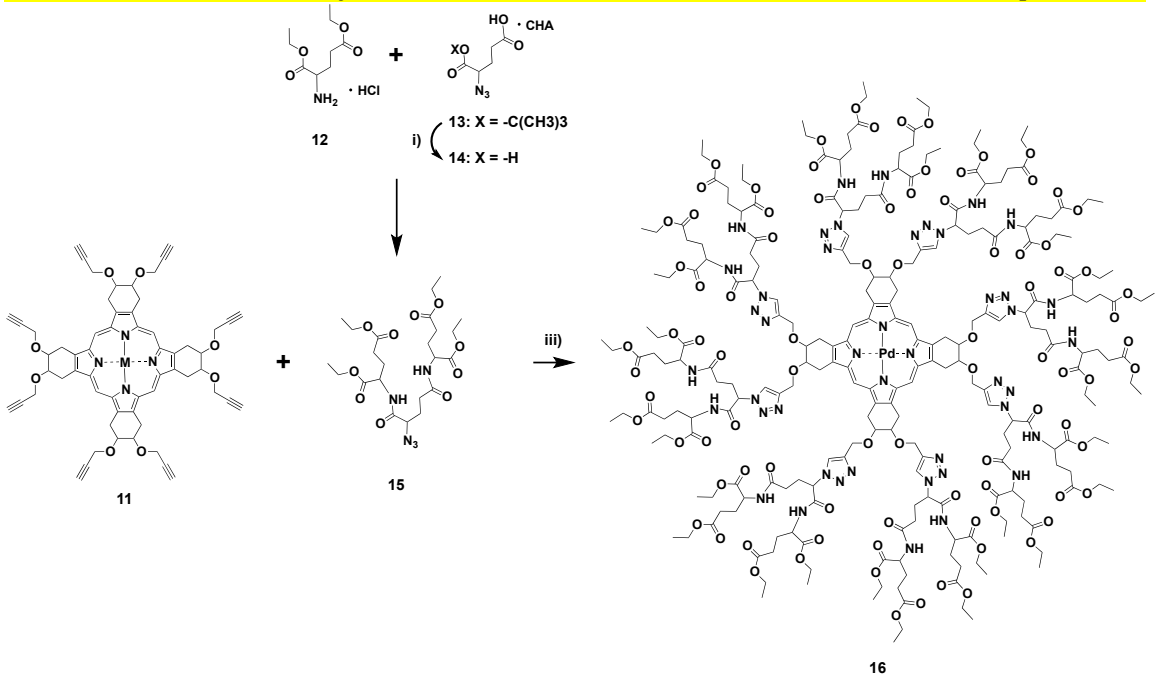
Since benzoporphyrins **9** and **10** were found to be only sparingly soluble in every available deuterated solvent, it proved nearly impossible to obtain high SNR NMR data. Nonetheless, there is overwhelming data to support the conclusion that all reported structures have been achieved.

In the case of the aromatization of cyclohexenyl porphyrins **7** and **8** to benzoporphyrins **9** and **10**, the changes in absorption and emission spectra closely follow the changes seen in the aromatization of other cyclohexenyl porphyrins, particularly the red-shifting of all major bands.[2,3] Additionally, the relative absorptivities of the Soret and Q bands of Pt benzoporphyrin **9**, unlike in the corresponding cyclohexenyl porphyrin **7**, were found to be equal, as is observed in other meso-unsubstituted Pt benzoporphyrins.[3] Furthermore, following the aromatization reaction, a change of -16 amu is clearly observed via MALDI, as would be expected if all terminal pivaloyl-protected hydroxyl groups were retained. In addition, purification of the reaction products yielded minor products whose mass spectra corresponded to elimination of exactly one pivaloyl substituent. Collectively, these data strongly suggest that this transformation did indeed yield the octa-pivaloxy benzoporphyrins **9** and **10**.

Furthermore, in the subsequent steps to form porphyrins **8A**, **9A, 11** and **12**, the changes seen by MALDI – -673 and +304 amu for the deprotection and alkylation, respectively – exactly match the changes predicted for these transformations. This further demonstrates that all peripheral groups are retained throughout all steps of this synthesis. Importantly, the characterization of these compounds by UV-Vis spectroscopy yielded spectra identical to those of the parent porphyrins **7** through **10**. Thus, it is concluded that no alteration to the porphyrin cores took place; all transformations solely affected the peripheral groups of the porphyrin. Based on this abundance of evidence, we conclude that the octa-substituted structures provided for these porphyrins throughout this synthesis are accurate.

It should be noted that, although a detailed stability study has not yet been performed, to our knowledge there is no appreciable degradation of the Clickaphors,

either as dendrimers or as core, unmodified porphyrins. All porphyrins are typically stored under Ar in a -20°C freezer, but we have found that they are stable for at least 6 months even when they are stored in the dark, in solid form at room temperature.



Scheme S3

Second generation, azido ethylglutamate 15. L-azidoglutamic acid mono-*tert*-butyl ester **13** was deprotected prior to the coupling reaction. To that end, 0.2 g (0.609 mmol) of **13** were dissolved in 2 mL of trifluoroacetic acid (TFA) and the solution was stirred for 2 h at room temperature. TFA was removed; the oily residue was dissolved in a few mL of dichloromethane and the solvent was evaporated again to remove most of the remaining TFA, and the residue was finally dried under high vacuum for a few hours.

The L-azidoglutamic acid **14** that was obtained from the deprotection reaction was dissolved in 35 mL of dry DMF under an argon atmosphere, 0.577 g (1.52 mmol) of HBTU were added to the solution and the mixture was stirred for 5 min at room temperature. 1.06 mL of DIPEA (6.09 mmol) were added to the solution in a single portion, followed immediately by 0.292 g (1.22 mmol) of L-glutamic acid diethyl ester **12**. The reaction mixture was stirred overnight, after which the solvent was removed and the residue was purified on a size exclusion column (Bio-Rad SX-1 beads) using THF as the elution solvent. The fractions containing the product, which were identified by MALDI-TOF MS, were combined and the solvent was removed, leaving 0.23 g (69%) of **15** as a white solid after drying under high vacuum. MALDI-TOF *m/z* 544.96 $(M+H)^+$, 566.95 $(M+Na)^+$, calc. 543.57.

Palladium porphyrin-dendrimer Clickaphor Red G2 16. Alkynyl-terminated porphyrin **11** (0.25 mg; 2.35×10^{-4} mmol) was dissolved in 0.1 mL of dry DMF under an argon atmosphere, and 1.57 mg (0.0028 mmol) of **15** were added. Aqueous, 5 µL

aliquots of copper sulfate and sodium ascorbate, containing 0.3 mg (0.0019 mmol) and 0.75 mg (0.0038 mmol), respectively, were added to make a solution of 10% water in DMF. The reaction mixture was heated at 75°C overnight, and was then left to react at room temperature for an additional 2 days. The DMF was evaporated and the residue was dissolved in a small volume of THF. This residue was then purified on a size exclusion column (Bio-Rad SX-1 beads) using THF as the mobile phase. The red band was collected, the solvent was evaporated and the product was dried under high vacuum. 1.15 mg (90%) of Clickaphor Red G2 **16** was obtained. MALDI-TOF *m/z* bell-shaped peak centered at ~4174, ranging from 4000 to 4500, calc. 5412.

Camera-based measurements

Regarding the data presented on figures 2 and 3, the image of the solutions shown in Fig. 2b was captured using a smartphone camera, while the film emission shown in Fig. 2c was recorded using a commodity color camera (Nikon) equipped with filters. Finally, the data shown in Fig. 3 was acquired using a custom-modified camera with lifetime imaging capabilities. Excitation was provided by a hand-held LED when the smartphone was used, or by a set of flash units on the cameras. Both provided broadband excitation light centered at around 400 nm, which is sufficient to excite both the fluorescent and phosphorescent chromophores.

Custom camera for luminescence imaging and quantification

Two Thyristor® electronic flash units (285-HV, Vivitar, Edison, NJ) equipped with 400/70 nm bandpass filters were used to provide a brief pulse of blue excitation light (500 μs duration) for both the oxygen-sensing phosphor (λ_{ex}= 415 nm) and the reference dye (λ_{ex}= 392 nm). A NIR CMOS camera (DCC3240N, Thorlabs, Newton, NJ) equipped with a 60 mm macrolens (Nikon, Melville, NY) was used to collect the emitted light from the sample. The phosphorescence of Clickaphor Red or Blue was captured as the "red-channel" image by placing a 650/70 nm bandpass filter or a 710 nm longpass filter, respectively, at the camera lens front. The reference dye, Coumarin 500 ($\lambda_{em,max}$= 495 nm), was imaged with a 510/25 nm bandpass filter and served as the "green-channel". The flash onset and the opening of the camera shutter were temporally coordinated by a digital delay/pulse generator (Stanford Research System, Sunnyvale, CA), so that images could be taken at arbitrary time points after the flash was triggered. Image analysis routines were performed in Matlab to generate the two-dimensional oxygen maps.

Phosphorescence lifetime τ_0 determination for pivaloyl-protected Clickaphor Red and Blue

To determine the phosphorescence lifetime under 0 mmHg oxygen, dilute dichloromethane solutions of compounds **7**, **8**, **9** or **10** were transferred into a

quartz cuvette. The solution was fully deoxygenated by bubbling argon gas through the solution for 5 min. The phosphorescence lifetime was measured using the camera system described above. Images were taken using a series of shutter delay times varying between 0 and 10 ms. Phosphorescence lifetime values were extracted using the method described in a previous publication.[4]

Oxygen-sensing paint-on bandage

The paint-on bandage mixture was formulated by mixing ethanol solutions of the Clickaphor Red G2 dendrimer (0.6 mM) and Coumarin 500 (10 mM, Exciton, Dayton, OH) with the New-Skin® liquid bandage (Prestige Brands, Tarrytown, NY) at a volumetric ratio of 2:1:10. The breathable transparent film dressing Tegaderm® (1622W, 3M, Saint Paul, MN) was used as the barrier layer covering the sensing bandage to reduce the oxygen exchange rate between the sensing bandage and room air.

System calibration using phosphorescence intensity and lifetime

The oxygen-sensing paint-on bandage was calibrated by placing a dried sensing film inside a quartz cuvette with oxygen partial pressure controlled by a gas mixing system. The oxygen content was adjusted step-wise from 160 mmHg to 0 mmHg, and monitored in real-time using a needle-based Clark electrode (Unisense, Aarhus, Denmark). The system was allowed to stabilize at each step for 5 min prior to acquisition of images.

For intensity-based calibration, a ratiometric approach was used. An 800 μs camera delay time was used while capturing the red channel (650/70 nm) image to match the experimental conditions in the *ex vivo* experiment. A corresponding green channel (510/25 nm) image was then acquired without time delay. The reference-controlled phosphorescence intensity was calculated as a red-to-green intensity ratio between the two channels and the reciprocal of this ratio was plotted over oxygen tensions to generate the intensity calibration curve.

For lifetime-based calibration, a series of red-channel (650/70 nm) phosphorescence images were taken with the delay times set between the flash and the camera exposure varying from 0 to 10 ms. The intensity over delay time plot was obtained at each pO_2, and the phosphorescence lifetime τ was extracted from the curve. The reciprocal of the lifetime τ was then plotted over oxygen tensions to generate the lifetime calibration curve.

Mapping burn wound oxygen consumption on porcine skin explants

Full-thickness burns were created on freshly excised, viable porcine skin *ex vivo* by bringing a brass rod, heated to 150°C, in contact with the surface of the skin for 15 seconds. The oxygen-sensing bandage was applied 1 h after the burn had been created, and allowed 1 min to dry in air. The bandage was also applied to an unburned skin sample that served as a control. The Tegaderm barrier layer was applied, and red- and green-channel images were taken both immediately and after 30 minutes of equilibration. Percent tissue oxygen consumption rate (%) at each image pixel was calculated by taking the difference between the initial and

equilibrium pO_2, and normalizing this value as a percentage of the maximum consumption measured in healthy skin.

Tissue oxygenation in the *ex vivo* burn models was quantified based on phosphorescence intensity or lifetime. The 800 μs camera delay time was used while capturing the red-channel image. The intensity of the red-channel image was divided by the intensity of its co-registered green-channel image and reconstructed into a ratiometric image using Matlab. 2-D tissue oxygenation maps were then obtained by correlating the intensity of the ratiometric image with pO_2 values at each image pixel, using the intensity calibration curve described above. For lifetime-based measurements, three points on each decay curve were used for curve fitting to extract the phosphorescence lifetime τ. The lifetime at each image pixel was then correlated to pO_2 using the lifetime calibration curve described above to construct the 2-D tissue oxygenation map.

NMR and mass spectra

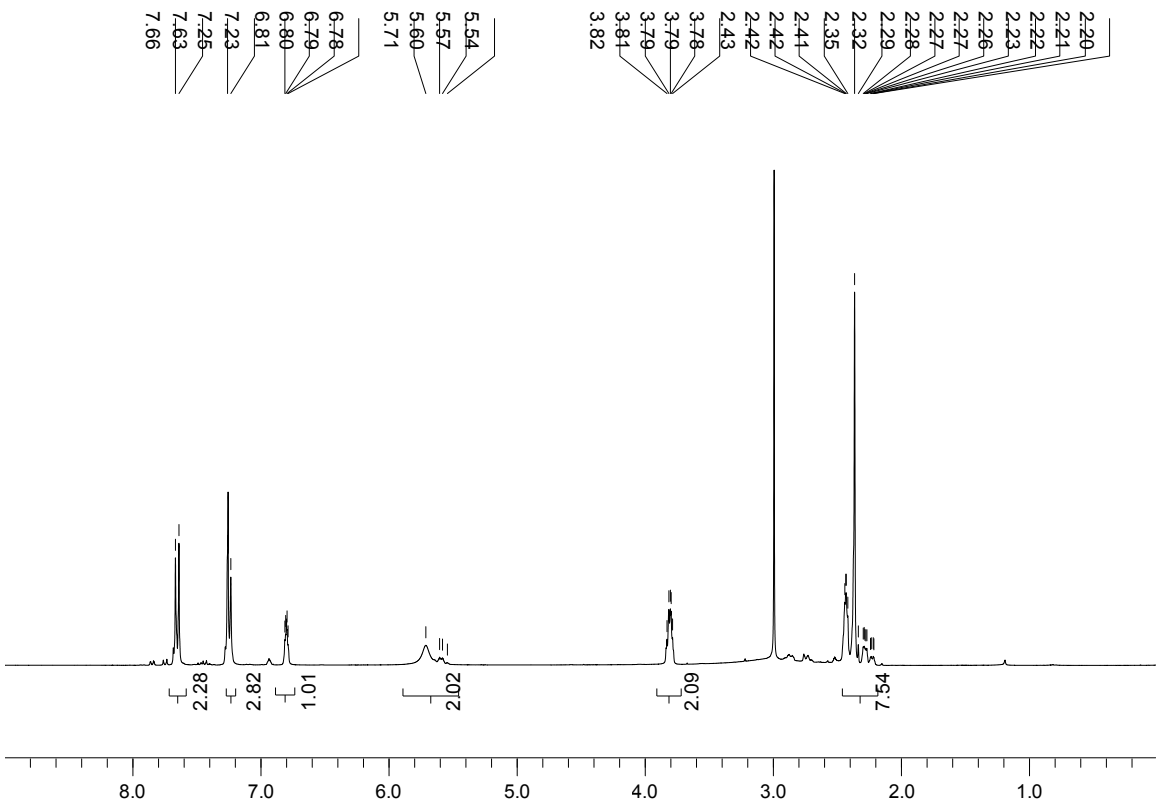


Figure S4: ^{1}H NMR spectrum of 2

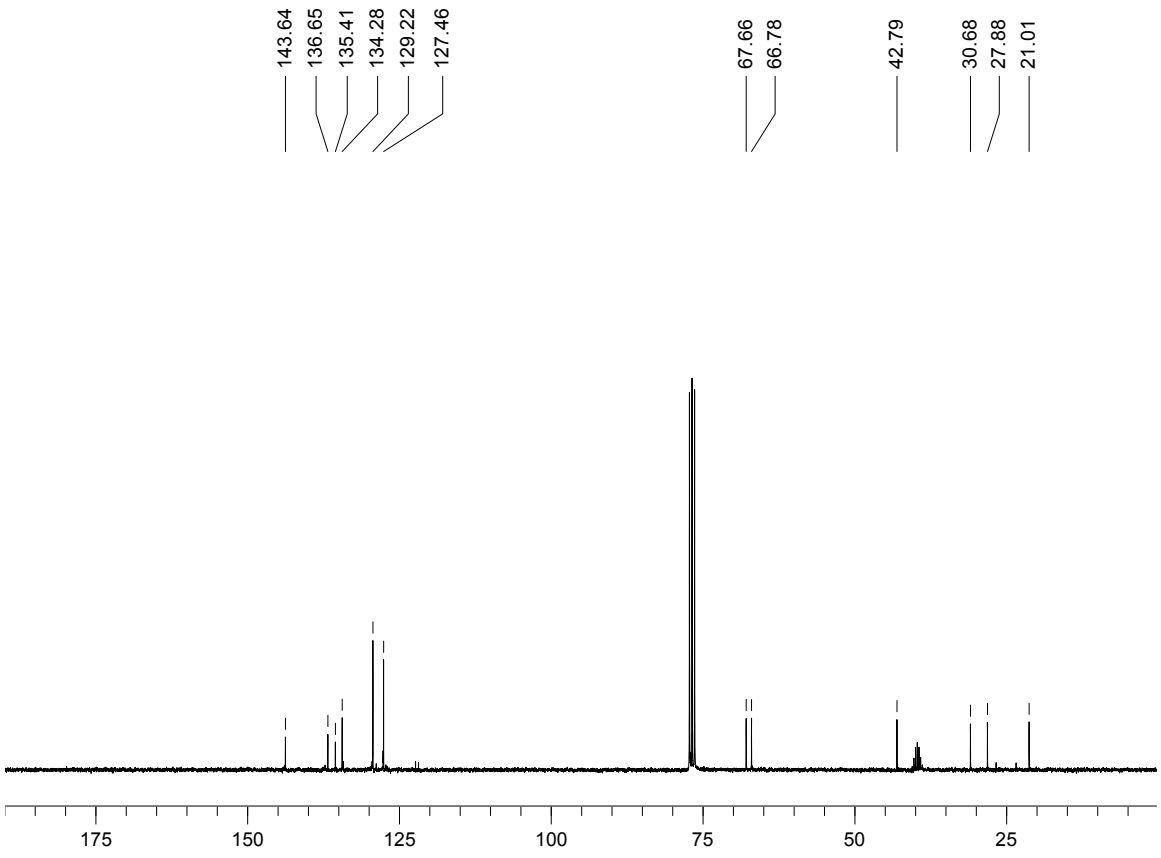


Figure S5: ^{13}C NMR spectrum of 2

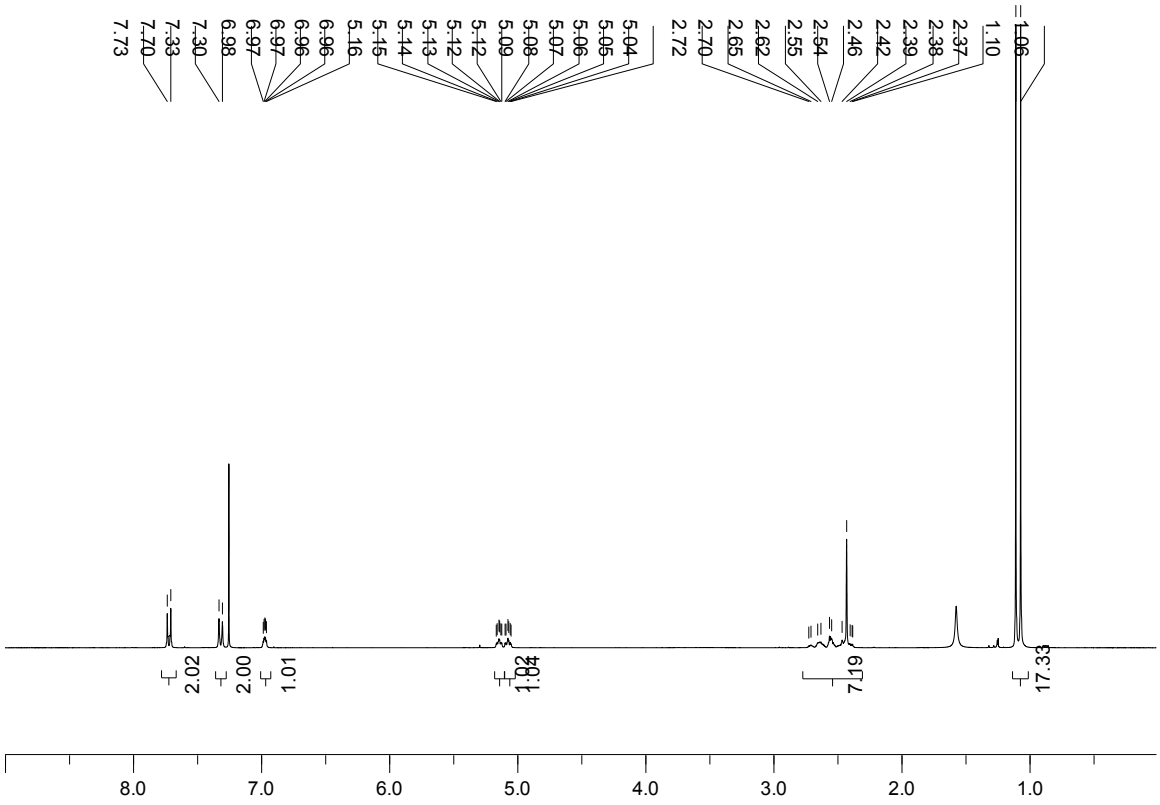


Figure S6: ^{1}H NMR spectrum of 3

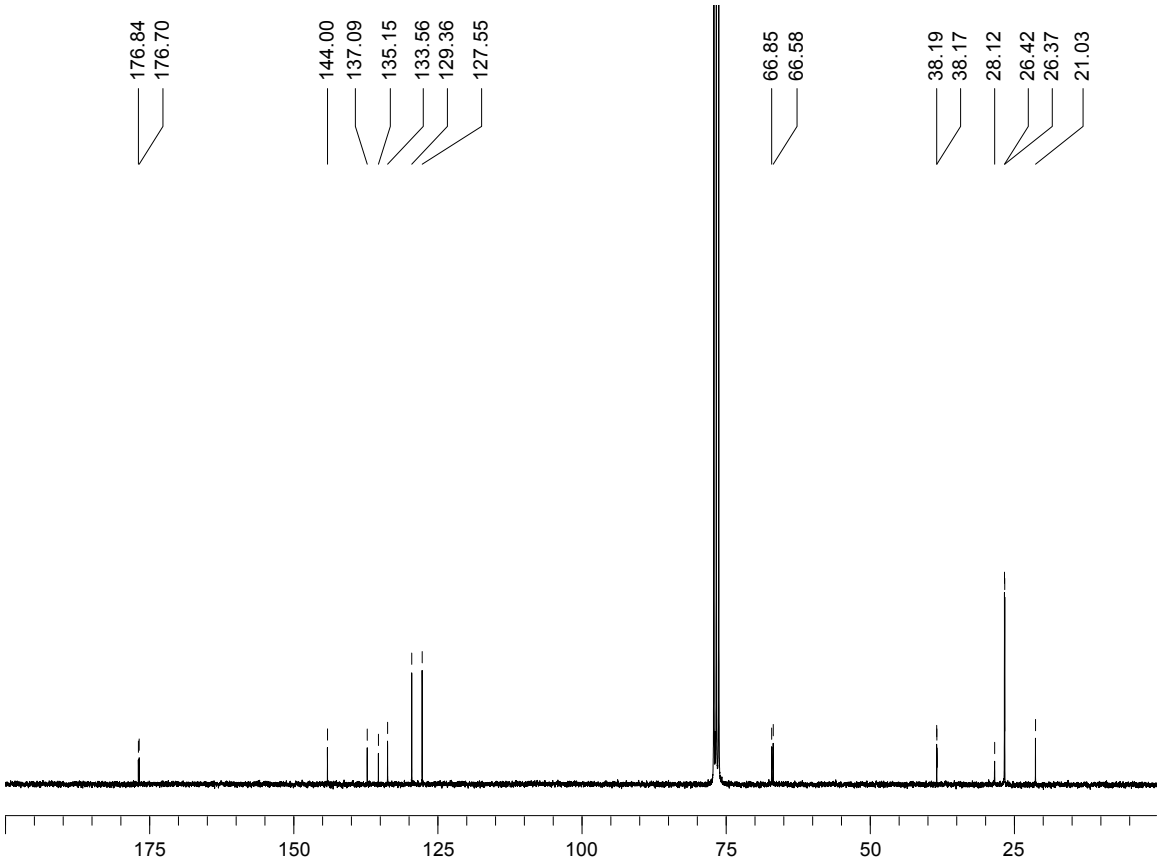


Figure S7: ^{13}C NMR spectrum of 3

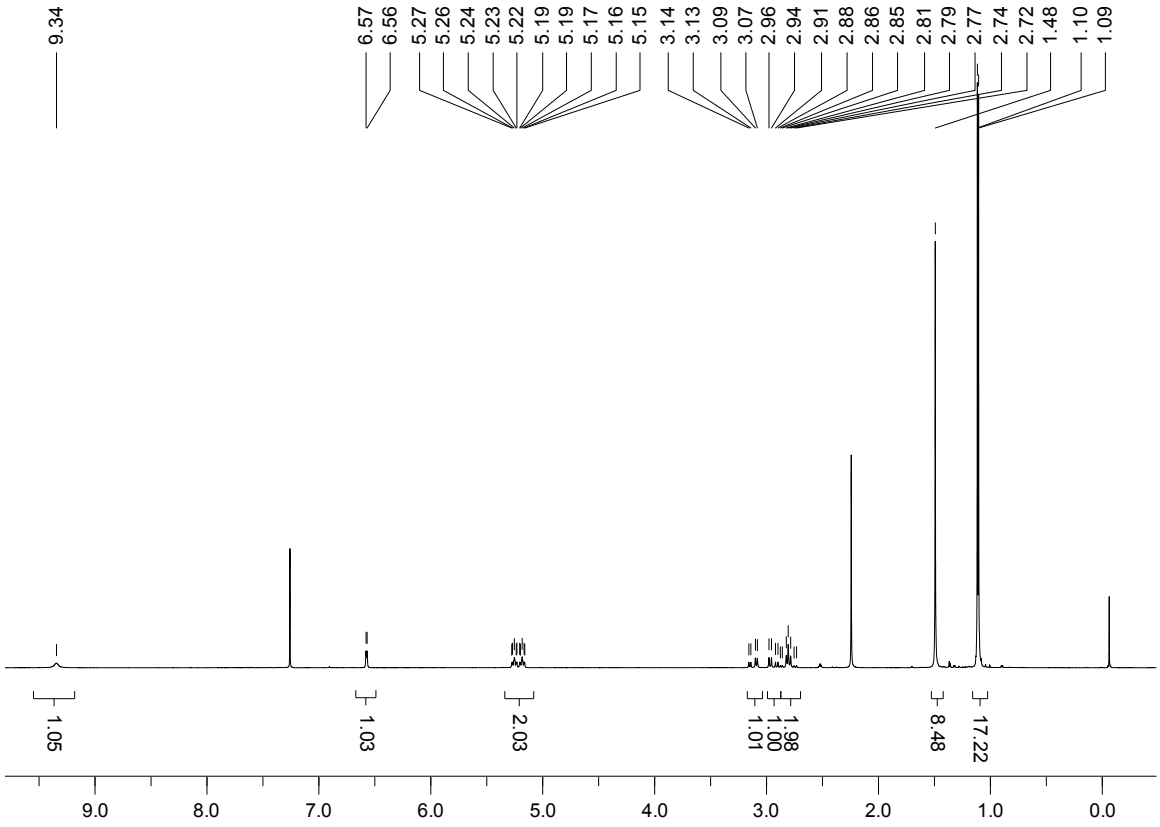


Figure S8: ^{1}H NMR spectrum of 4

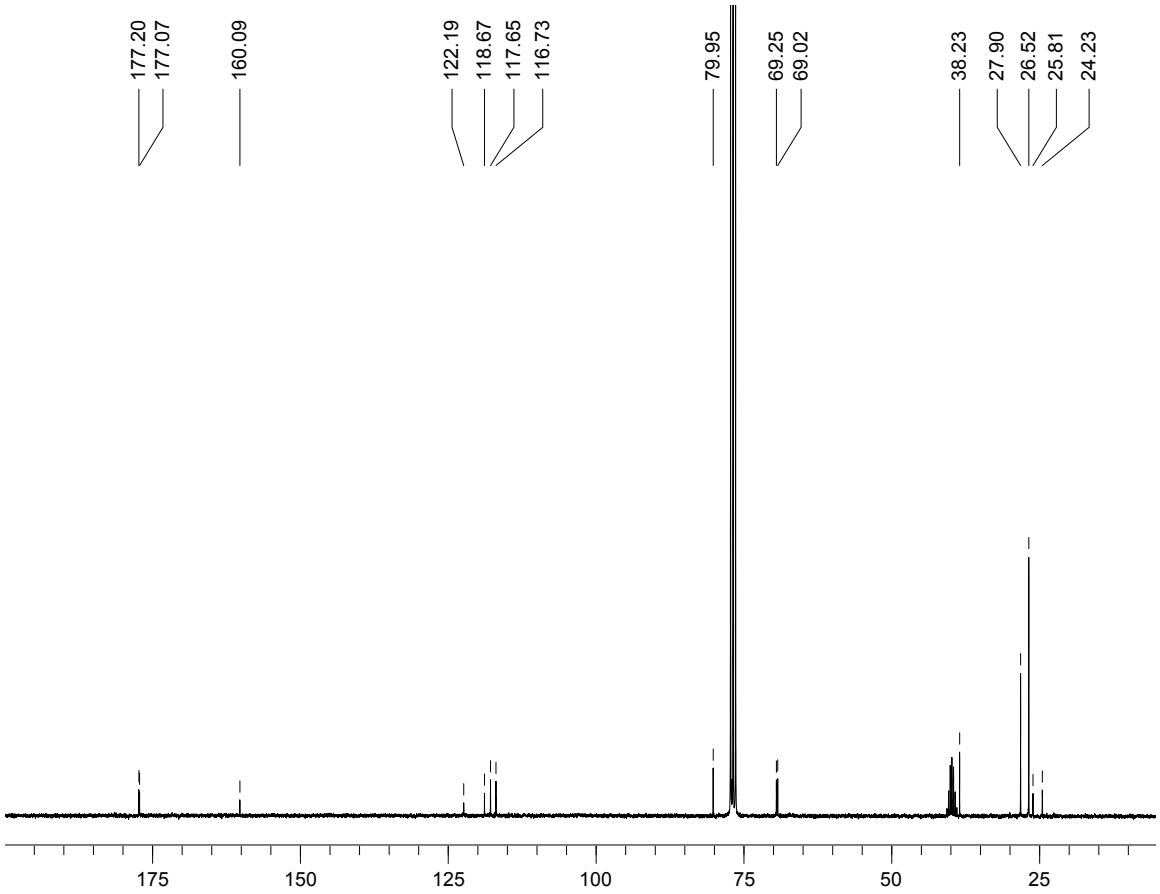


Figure S9: ^{13}C NMR spectrum of 4

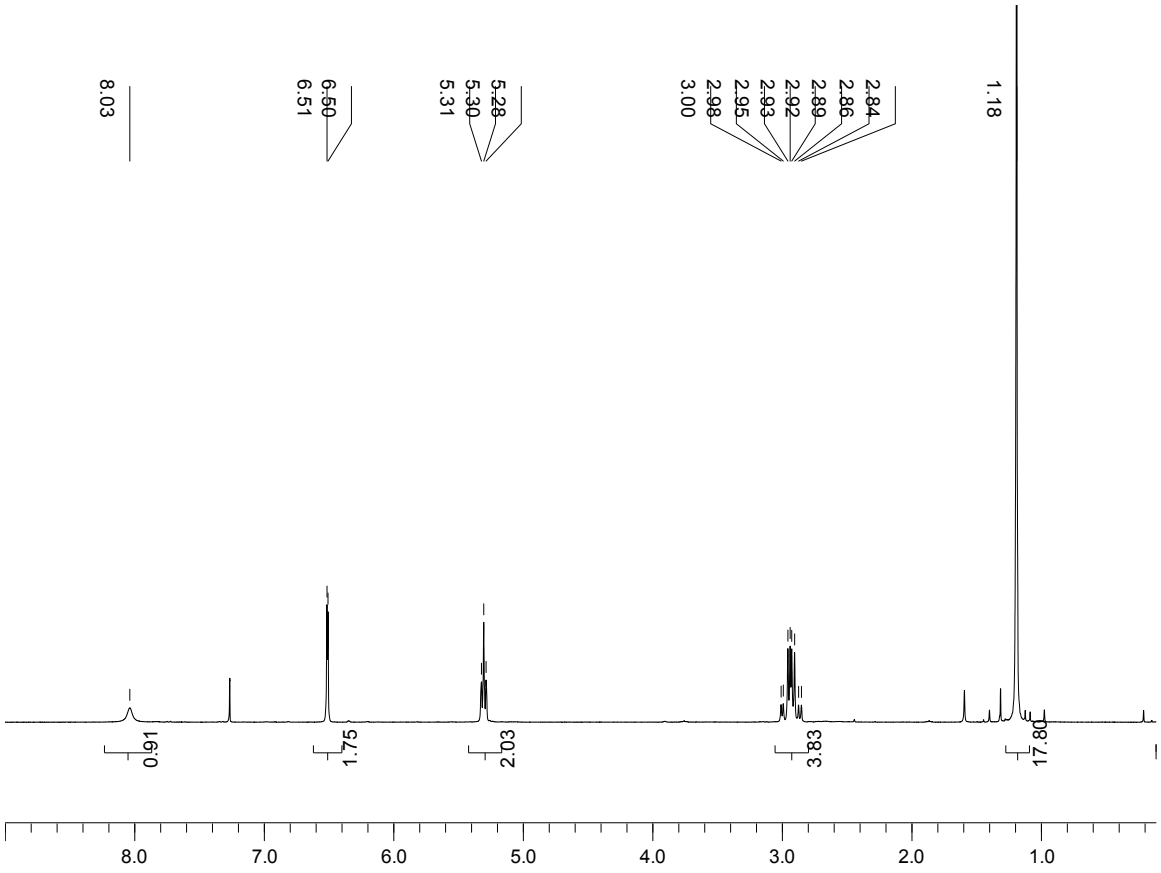


Figure S10: ^{1}H NMR spectrum of 5

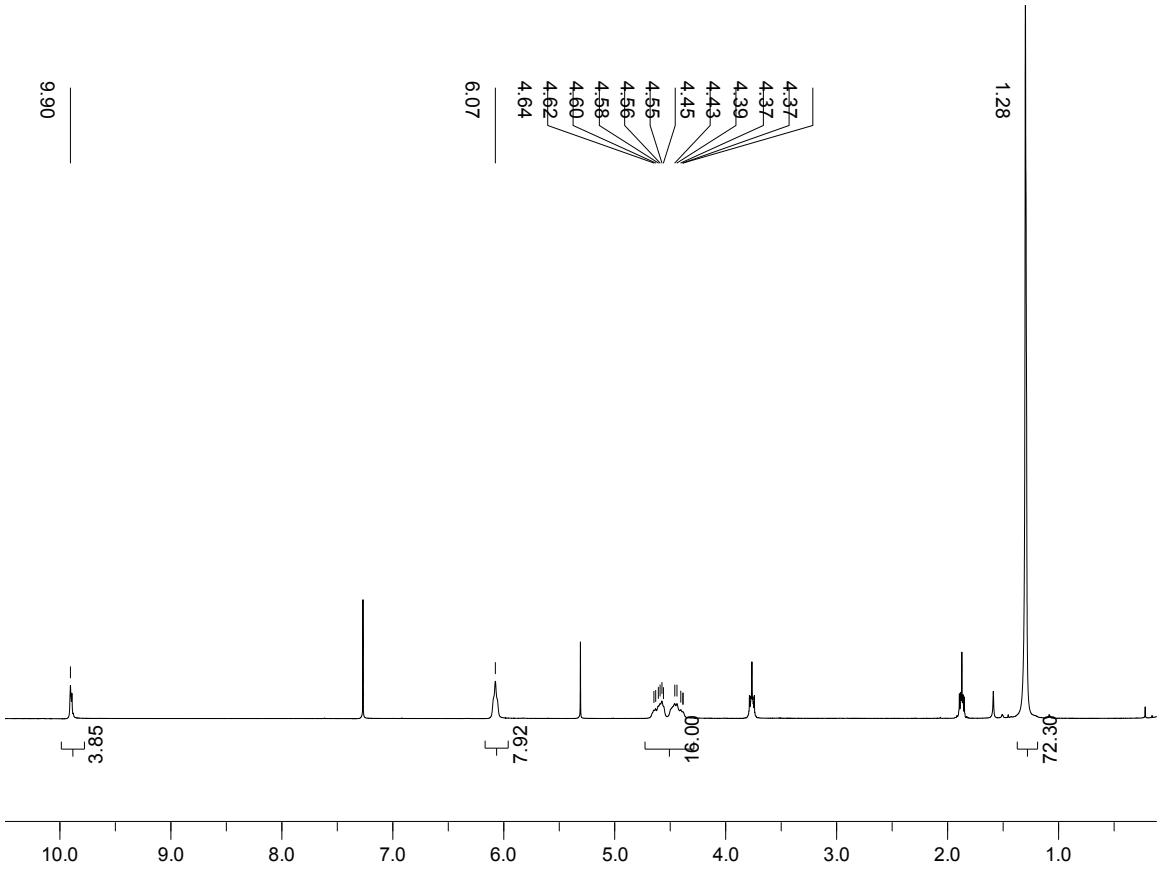


Figure S11: ^{1}H NMR spectrum of 6

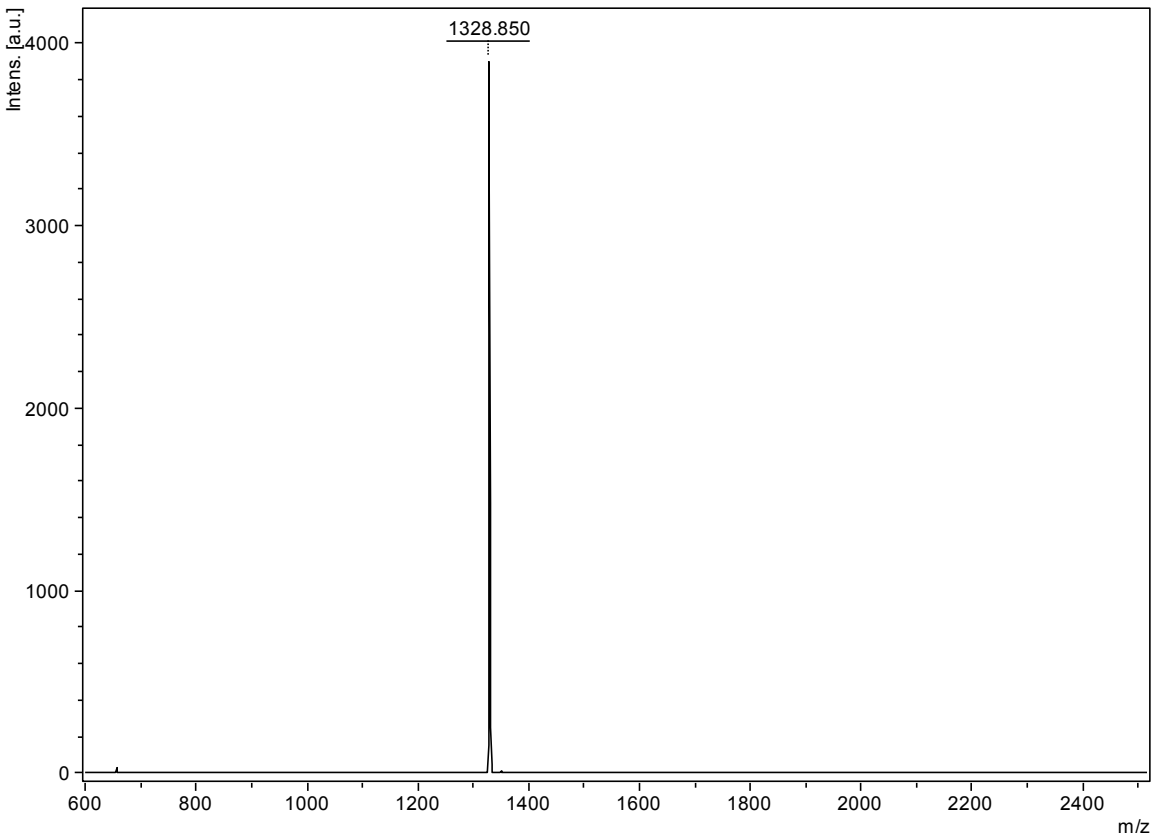


Figure S12: MALDI-TOF mass spectrum of 6

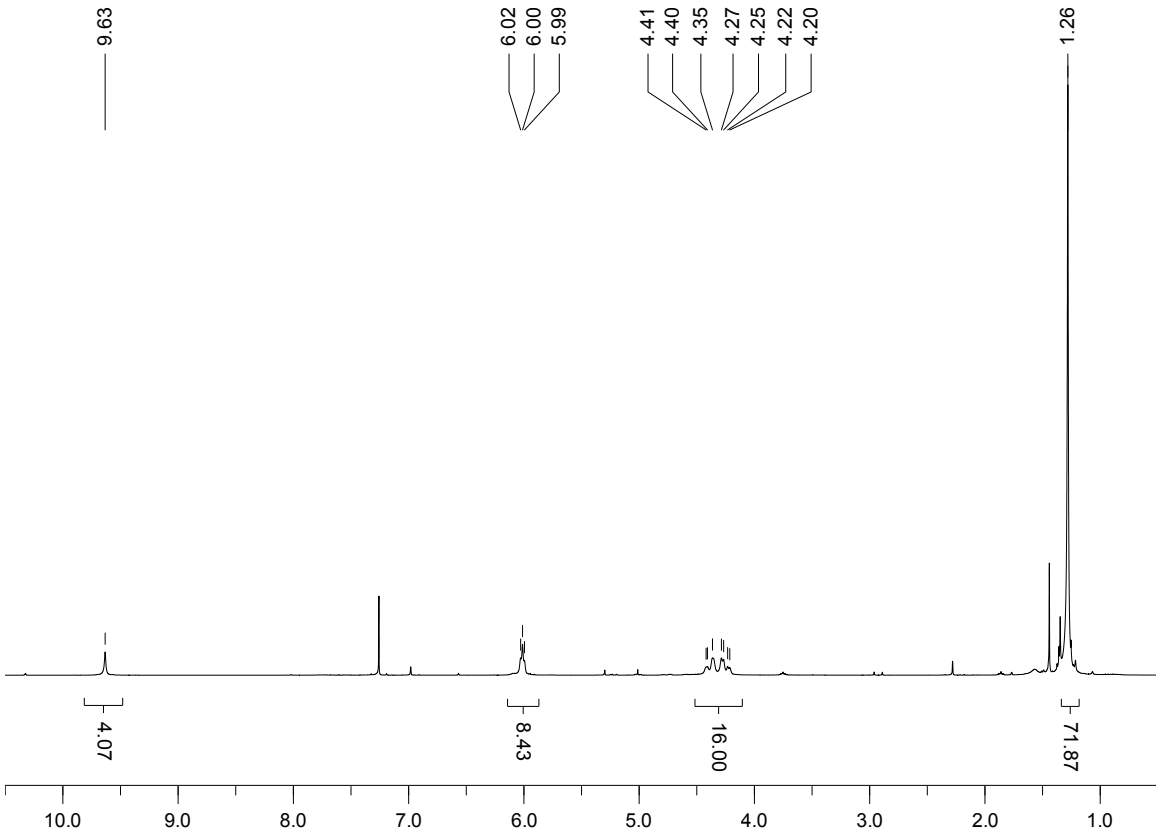


Figure S13: ^{1}H NMR spectrum of 7

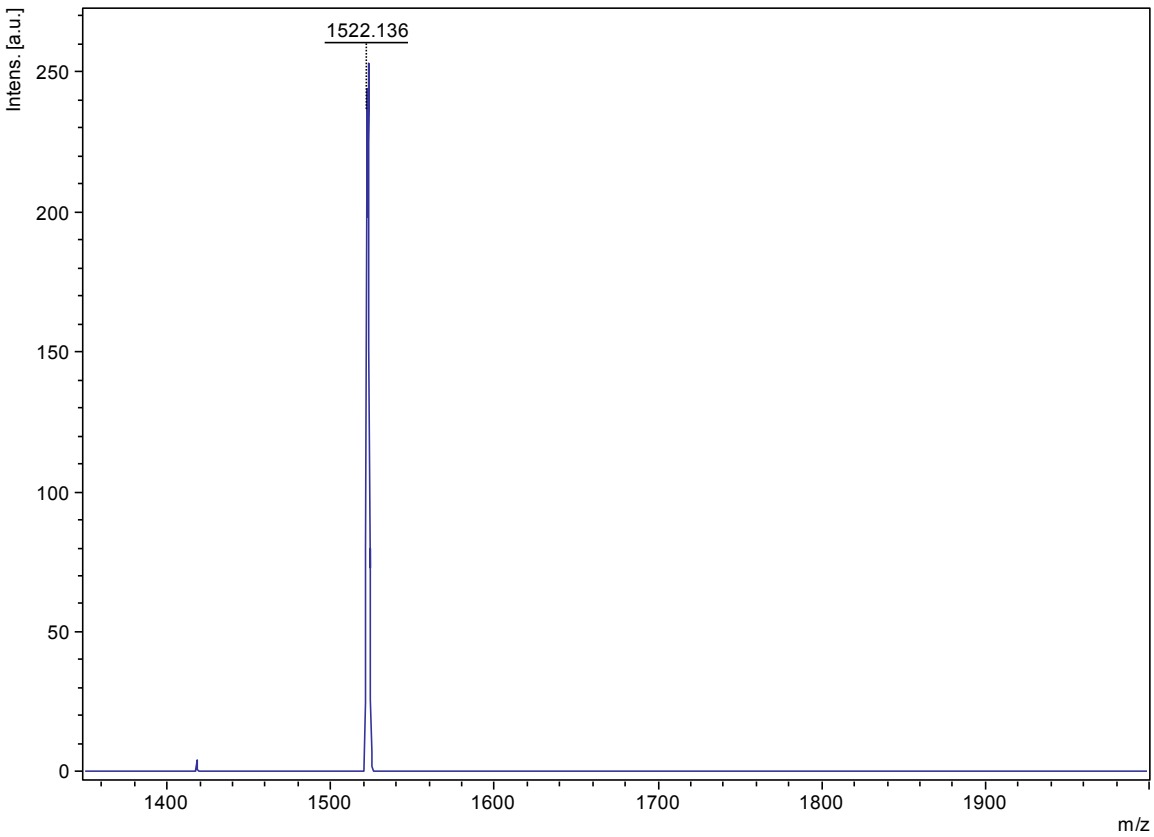


Figure S14: MALDI-TOF mass spectrum of 7

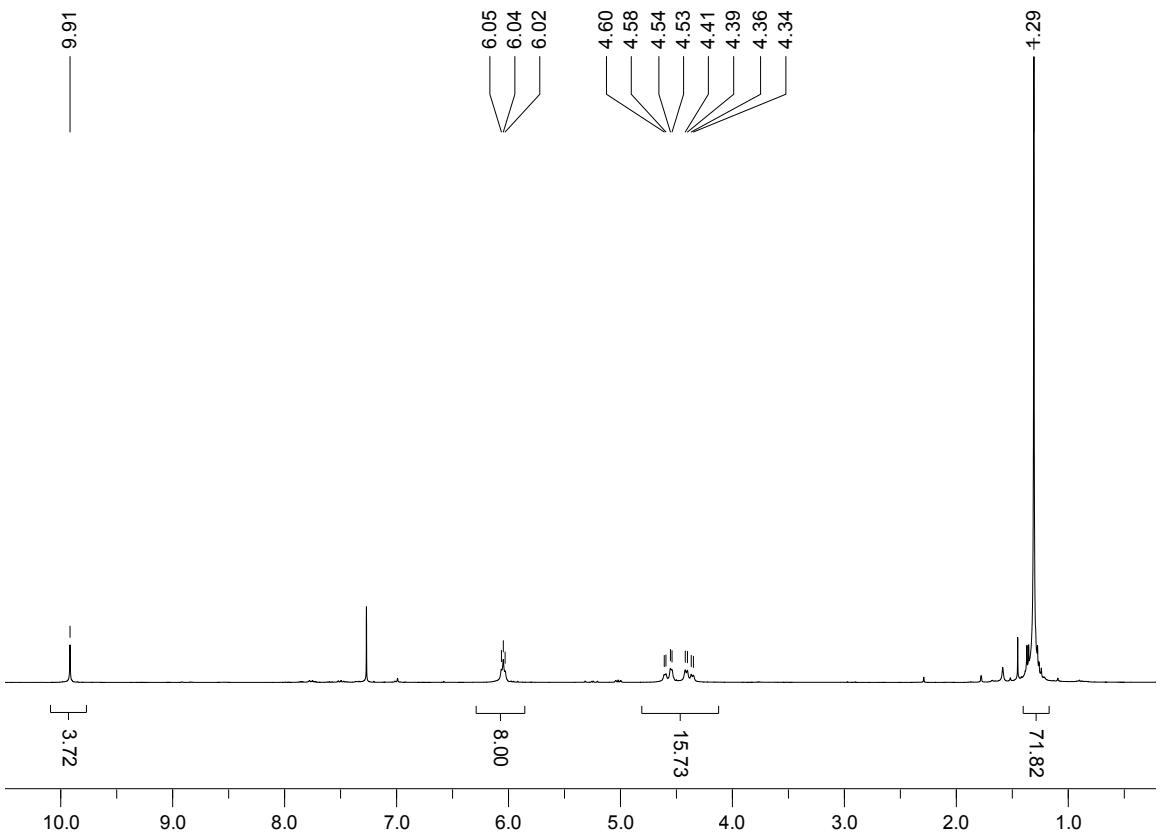


Figure S15: ^{1}H NMR spectrum of 8

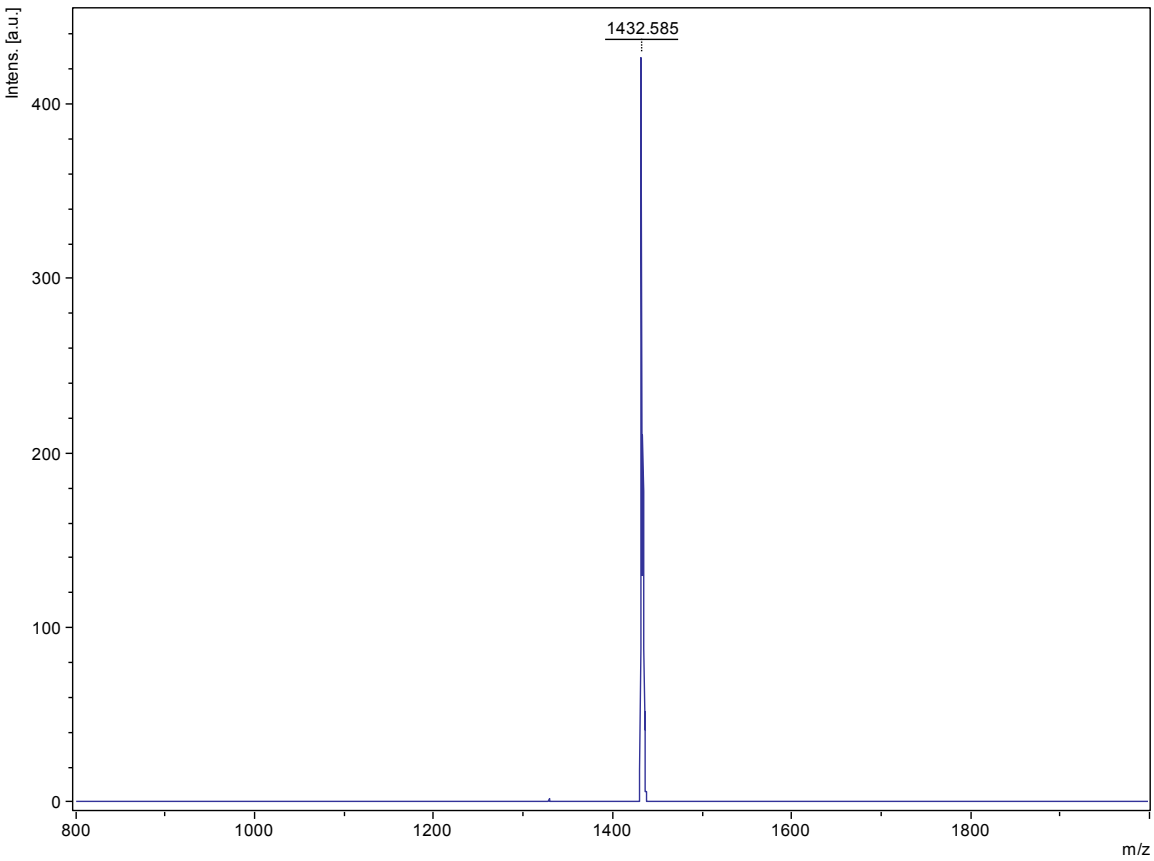


Figure S16: MALDI-TOF mass spectrum of 8

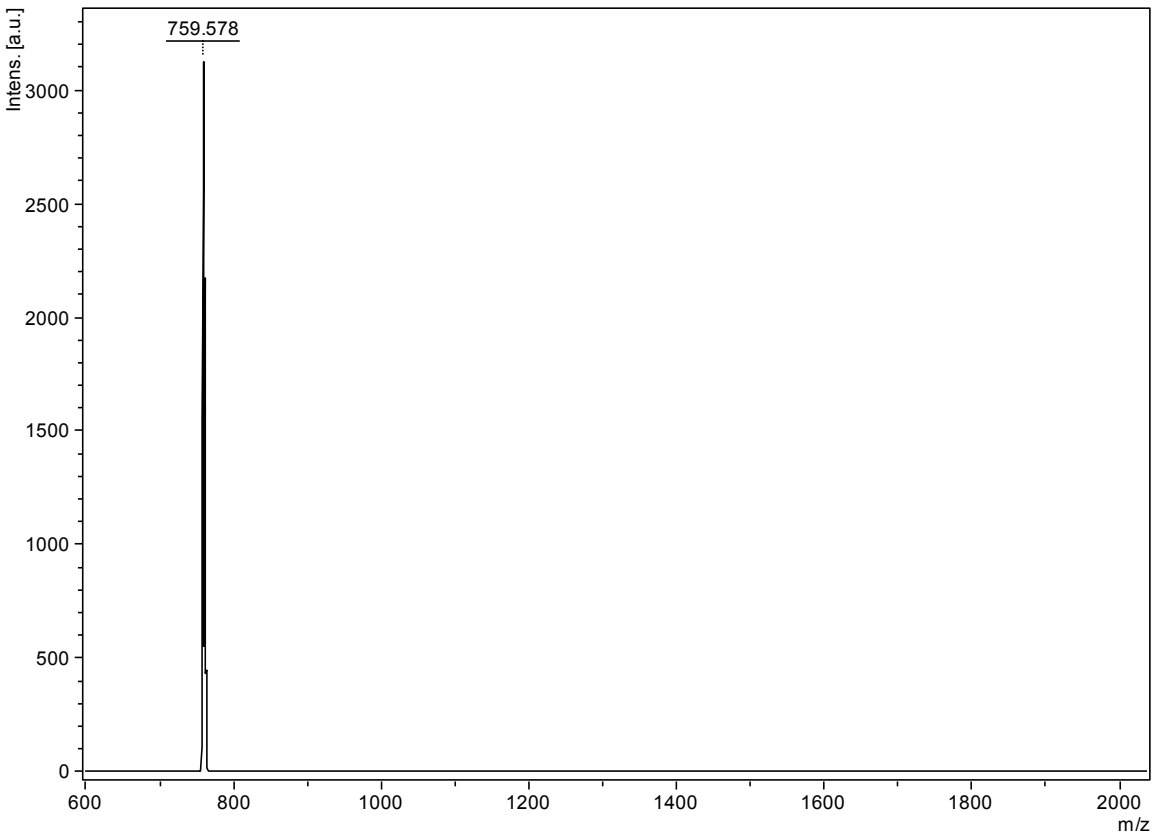


Figure S17: MALDI-TOF mass spectrum of 8A

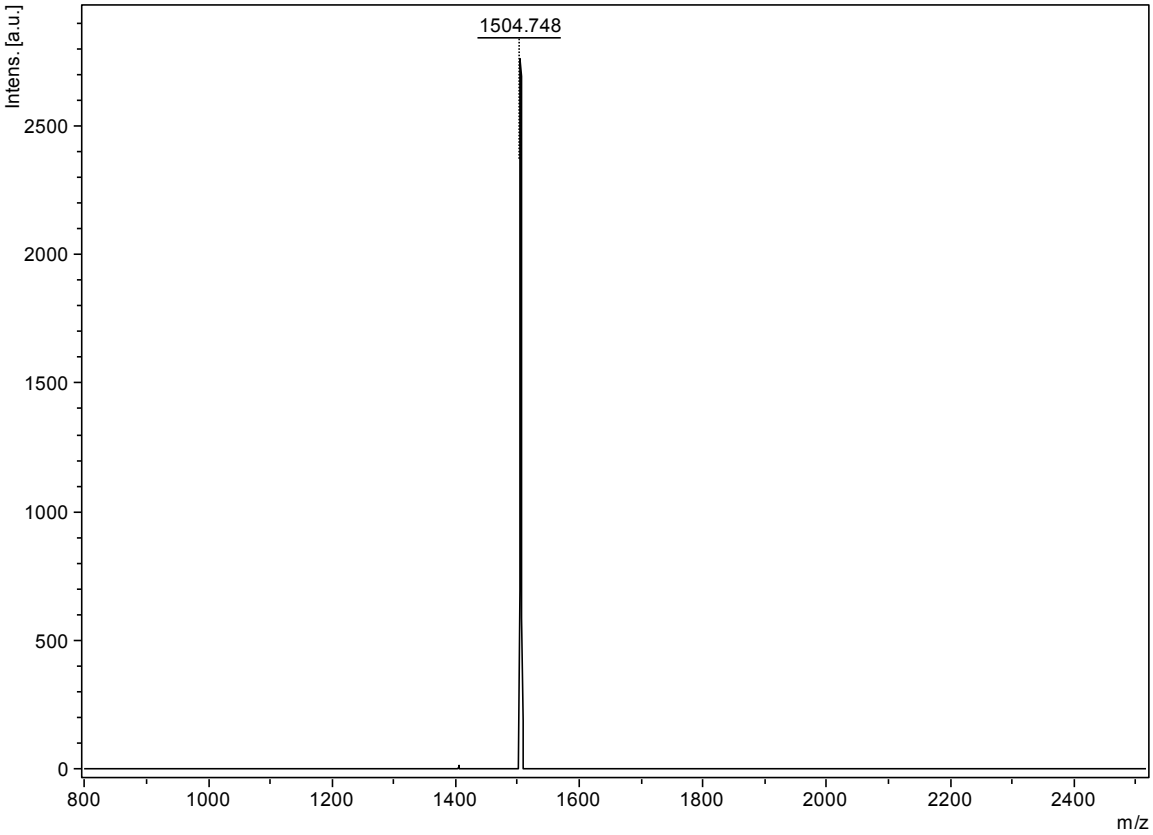


Figure S18: MALDI-TOF mass spectrum of 9

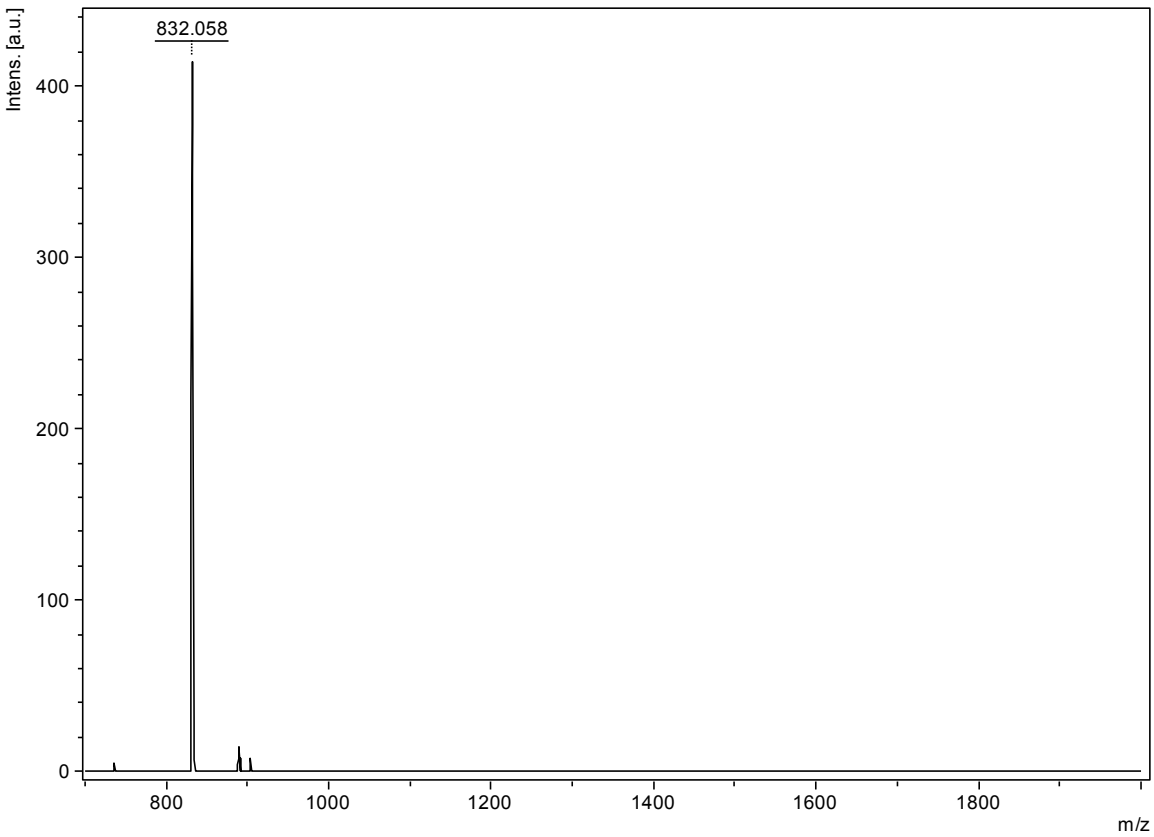


Figure S19: MALDI-TOF mass spectrum of 9A

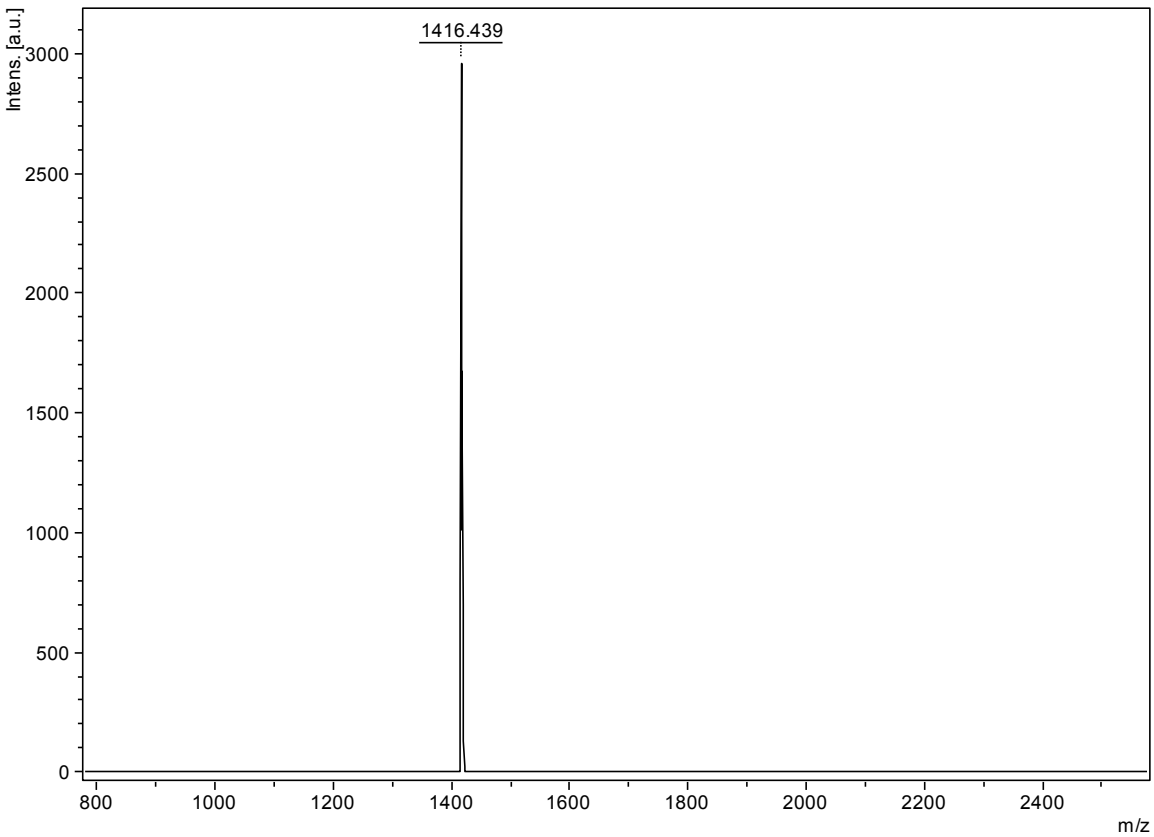


Figure S20: MALDI-TOF mass spectrum of 10

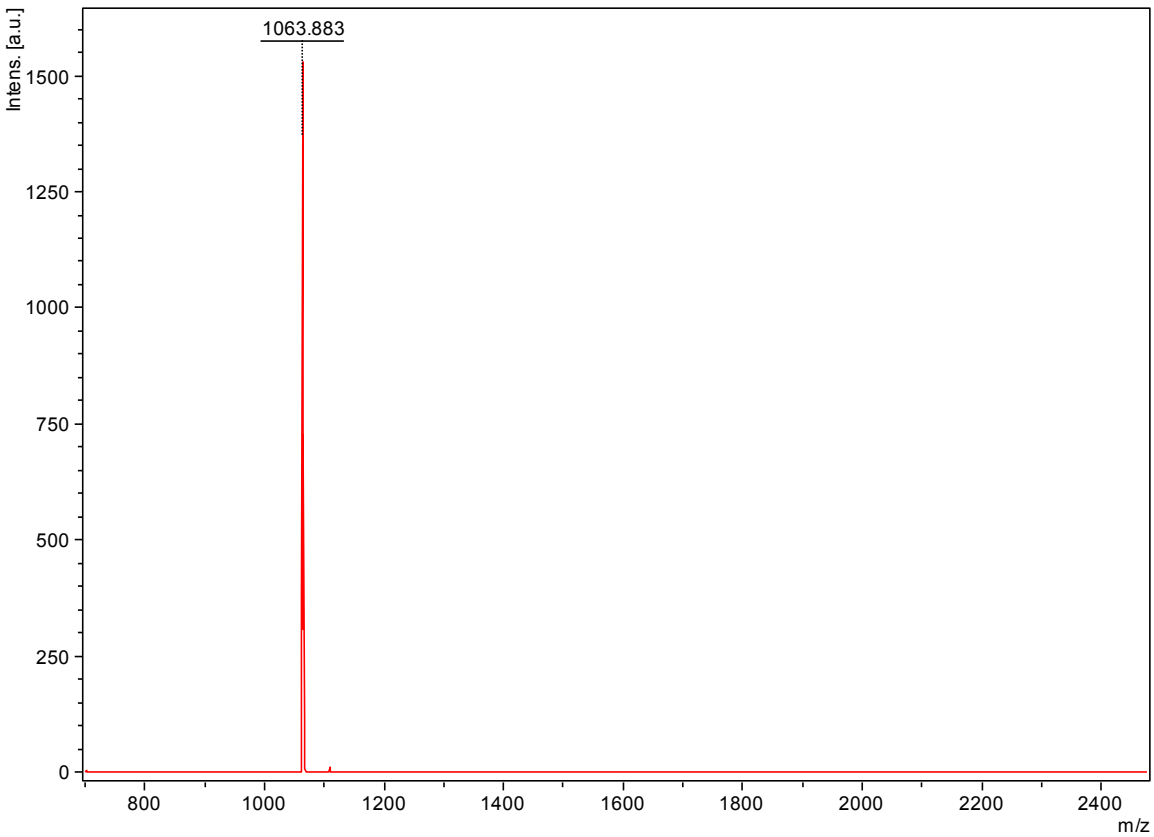


Figure S21: MALDI-TOF mass spectrum of 11

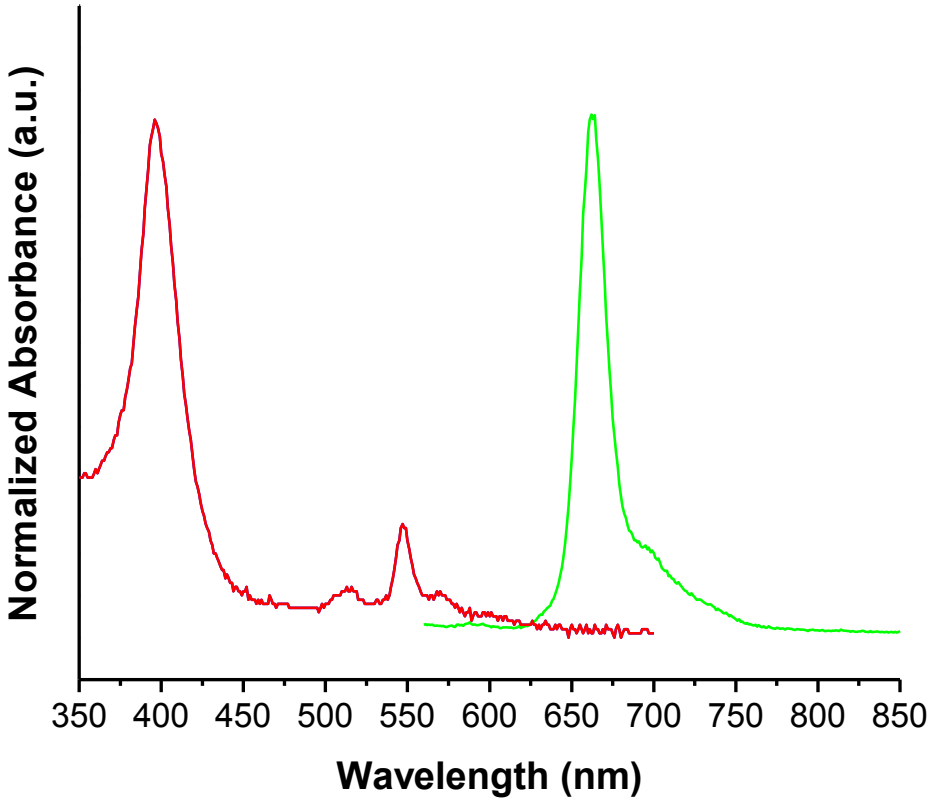


Figure S22: Normalized absorption (red) and emission (green) spectra of 11. The phosphor was excited at 542 nm for the acquisition of the emission spectrum.

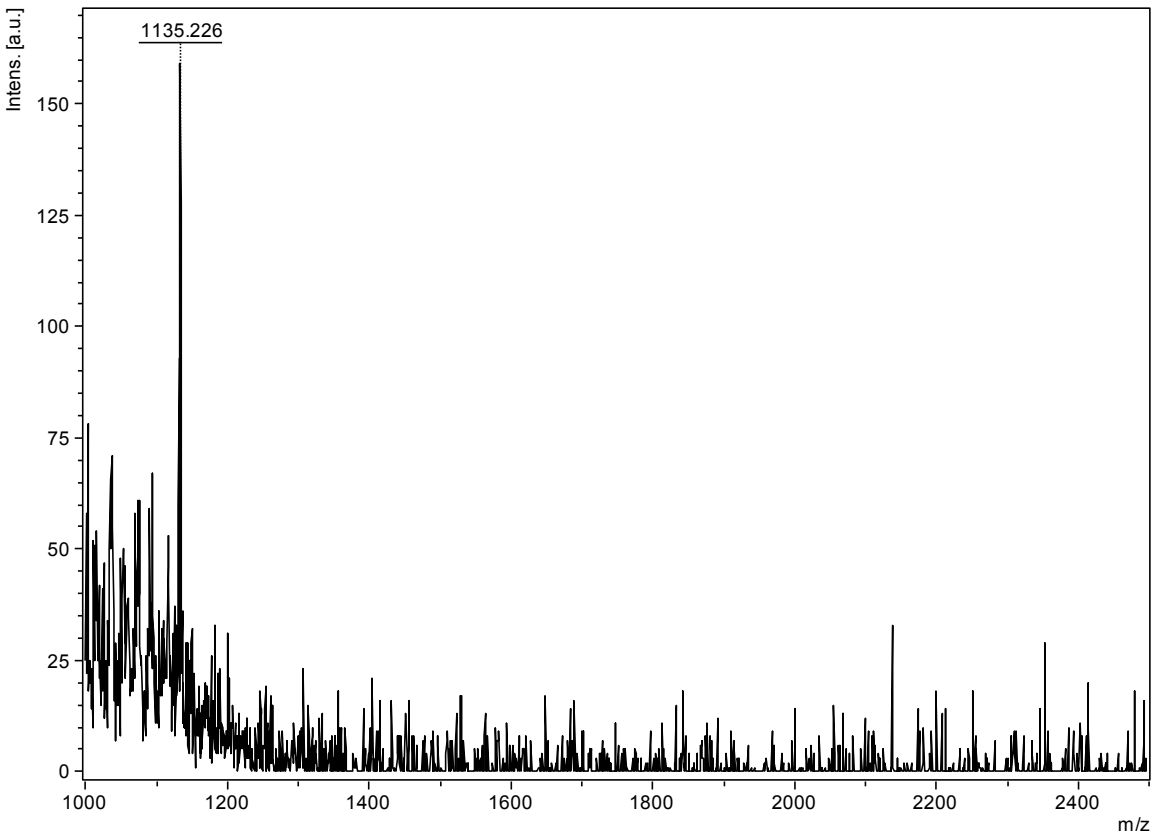


Figure S23: MALDI-TOF mass spectrum of 12

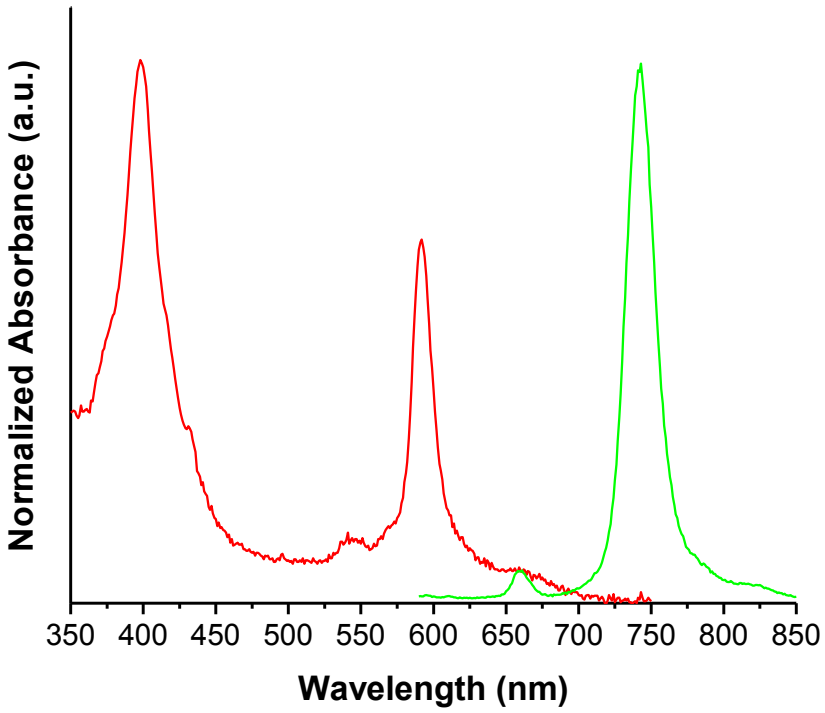


Figure S24: Normalized absorption (red) and emission (green) spectra of 12. The phosphor was excited at 590 nm for the acquisition of the emission spectrum.

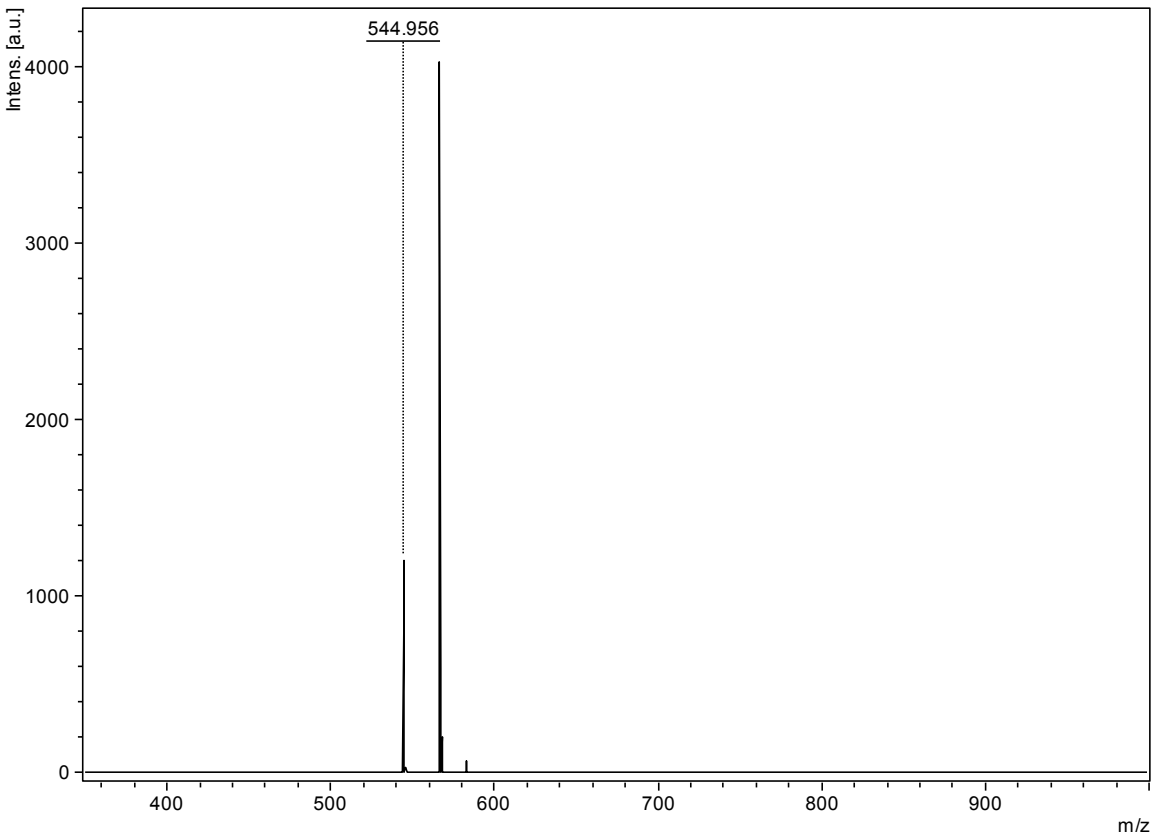


Figure S25: MALDI-TOF mass spectrum of 15

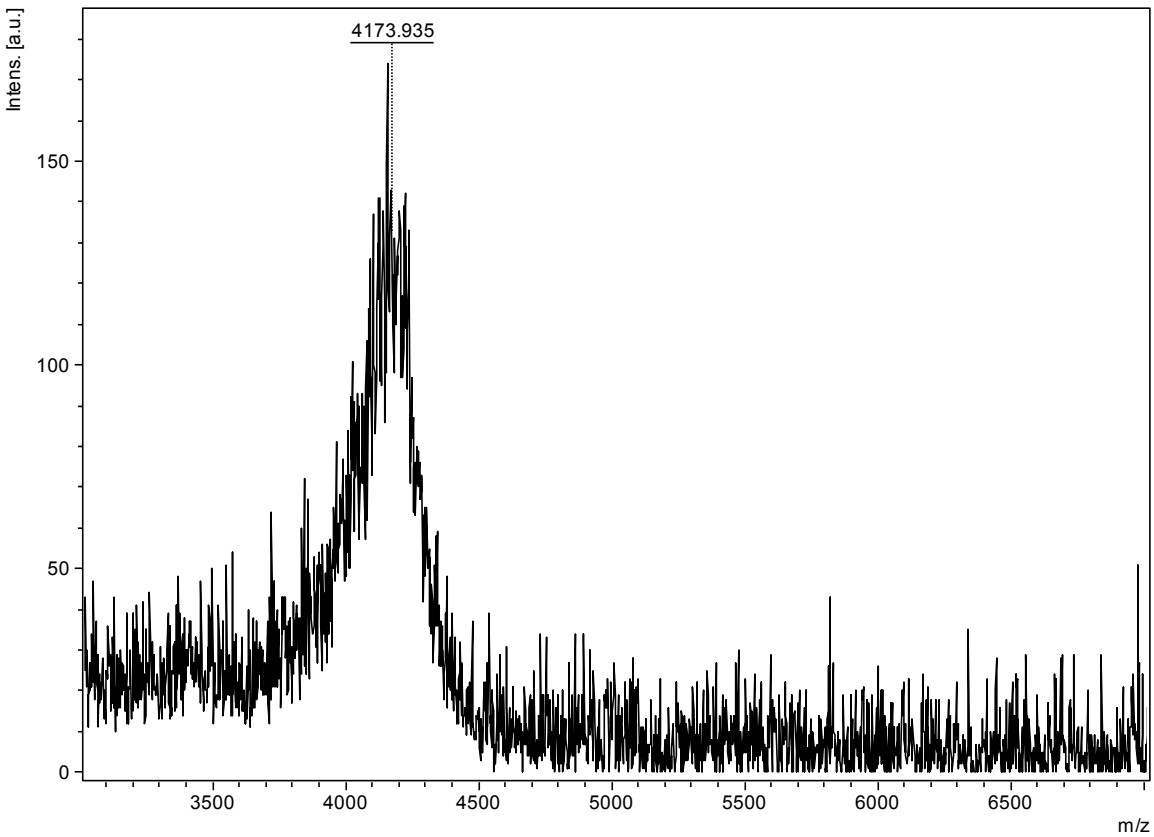


Figure S26: MALDI-TOF mass spectrum of 16

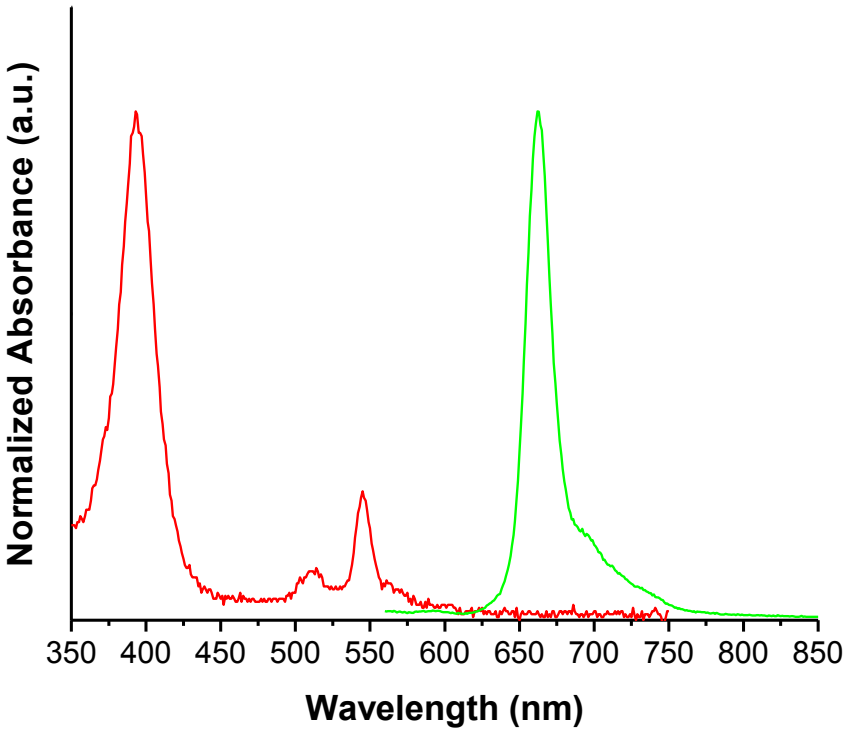


Figure S27: Normalized absorption (red) and emission (green) spectra of 16. The phosphor was excited at 540 nm for the acquisition of the emission spectrum.

Calibration and decay curves

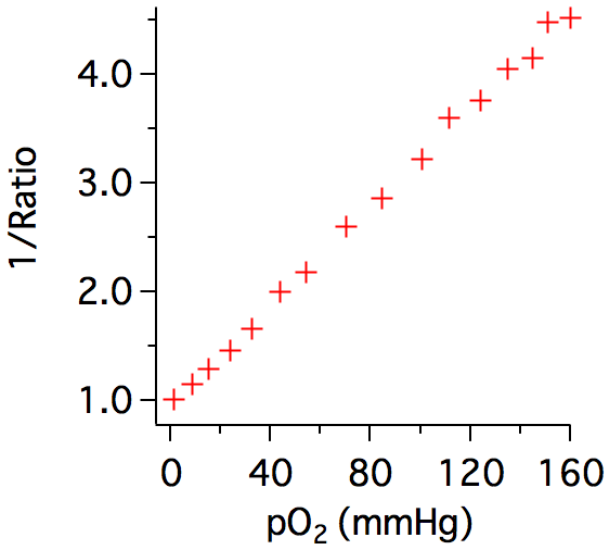


Figure S28. Calibrating the oxygen-sensing bandage: system calibration curve based on phosphorescence intensity. Phosphorescent intensity is expressed as red-to-green channel intensity ratio.

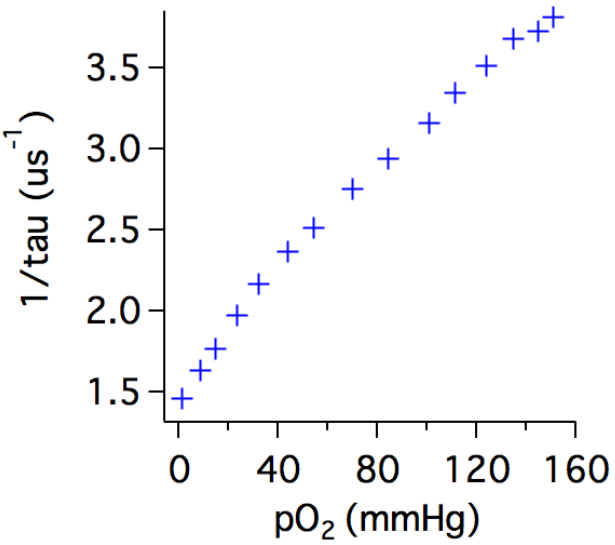


Figure S29. Calibrating the oxygen-sensing bandage: system calibration curve based on phosphorescence lifetime.

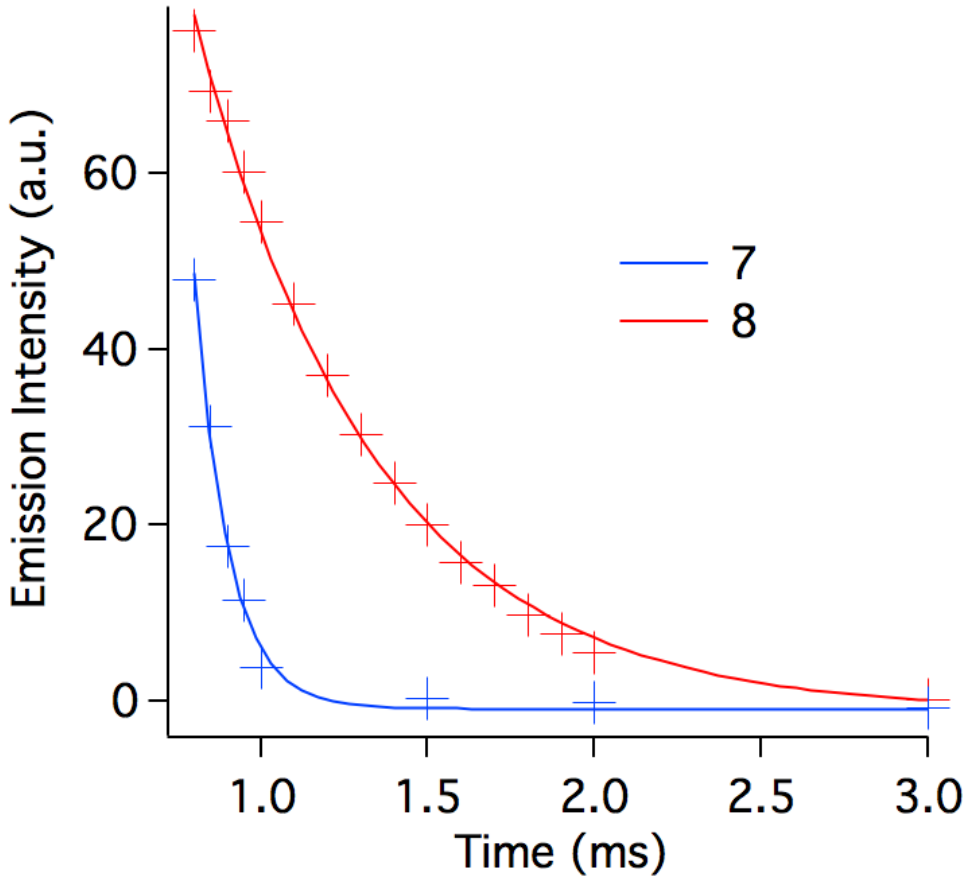


Figure S30. Plot of phosphorescence intensity vs. camera delay time for pivaloyl-terminated compounds **7** and **8** measured at 0 mmHg pO_2 by the custom camera.

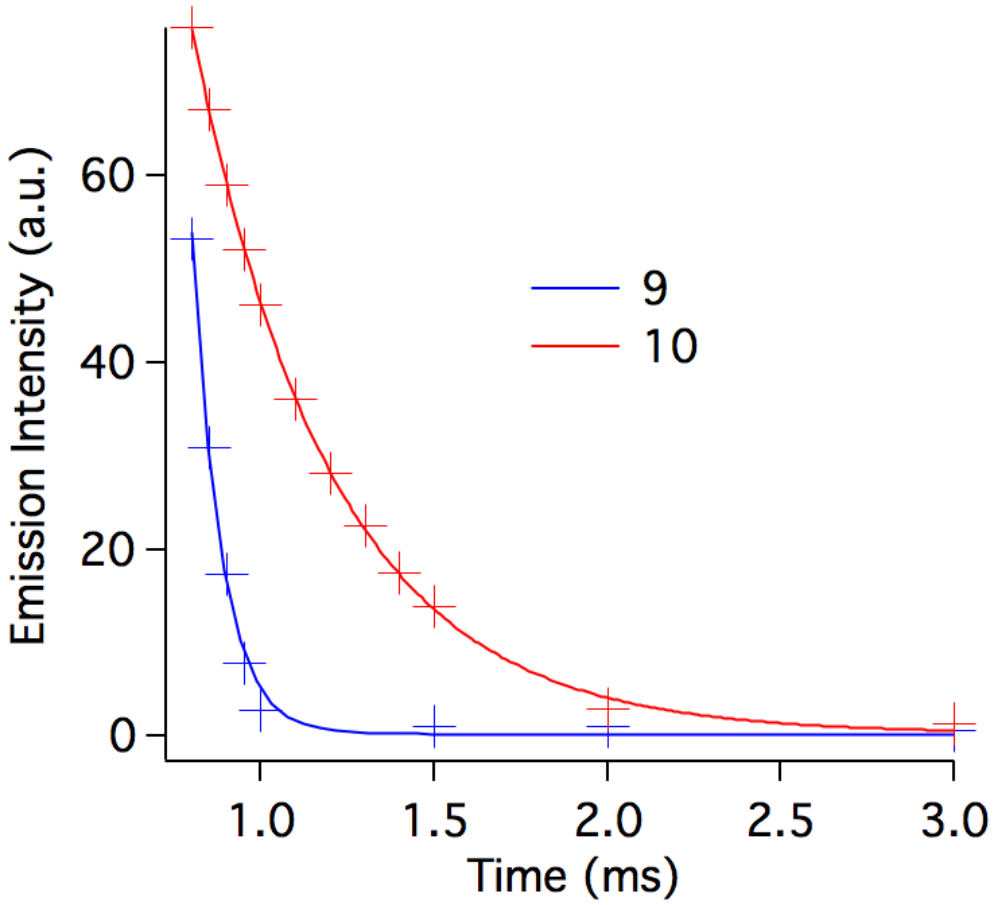


Figure S31. Plot of phosphorescence intensity vs. camera delay time for pivaloyl-terminated compounds **9** and **10** measured at 0 mmHg pO_2 by the custom camera.

References

(1) M. A. Filatov, A. V. Cheprakov, I. P. Beletskaya, *Eur. J. Org. Chem.* **2007**, *2007*, 3468-3475.

(2) O. S. Finikova, A. V. Cheprakov, I. P. Beletskaya, P. J. Carroll, S. A. Vinogradov, *J. Org. Chem.* **2004**, *69*, 522-535.

(3) O. S. Finikova, A. V. Cheprakov, S. A. Vinogradov, *J. Org. Chem.* **2005**, *70*, 9562-9572.

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