

Molecular Design of Advanced Mechanophore Scaffolds for Triggered Release

Thesis by
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In Partial Fulfillment of the Requirements for
the degree of
Doctor of Philosophy

The Caltech logo, featuring the word "Caltech" in a bold, orange, sans-serif font, centered within a light orange rectangular background.

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ABSTRACT

In the field of polymer mechanochemistry, mechanical force is harnessed to drive productive chemical transformations within stress sensitive molecules termed as mechanophores. Mechanophores designed to release functional small molecules have garnered significant attention for their broad utility in drug delivery, catalysis, and sensing. This dissertation explores advanced molecular design strategies to expand the scope of mechanically triggered release, focusing on diversifying cargo compatibility and drastically amplifying payload capacity.

A central focus of this work is the development of a modular masked 5-aryloxy-substituted 2-furylcarbinol mechanophore. In Chapter 1, this platform is utilized to covalently conjugate an adamantylidene-phenoxy-1,2-dioxetane chemiluminophore payload. By incorporating this conjugate into poly(methyl acrylate) and poly(ethylene glycol) backbones, ultrasound-induced mechanochemical activation initiates a cascade fragmentation that generates intense green luminescence across both organic and aqueous media. Chapter 2 expands the utility of this 2-furylcarbinol architecture to enable the triggered liberation of two distinct payload molecules. By integrating a self-immolative spacer, the mechanophore successfully releases dual payloads. The specific release profile, whether simultaneous or step-wise, can be finely tuned by altering the relative attachment sites of the payloads.

While single-mechanophore architectures are highly functional, they are inherently bottlenecked by a maximum release capacity of one or two payloads per polymer chain, restricting overall material efficiency. To address this stoichiometric limitation, Chapter 3 details the development of a water-soluble multimechanophore polymer architecture with ultrahigh payload capacity. By rationally engineering both the mechanophore monomer and a cyclooctene comonomer, the resulting polymer achieves excellent aqueous solubility. Upon mechanical activation, this multi-payload design demonstrates a remarkable 106-fold enhancement in payload release efficiency in an aqueous environment relative to traditional single-mechanophore systems.

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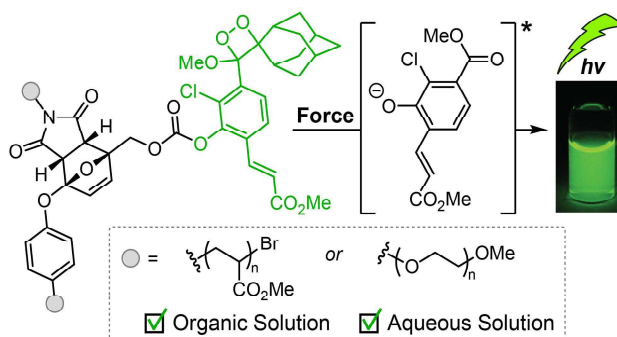
Y.-L. T. designed the research, performed the experiments, and wrote the manuscript.

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Chapter 1

MECHANICALLY TRIGGERED BRIGHT CHEMILUMINESCENCE FROM POLYMERS BY EXPLOITING A SYNERGY BETWEEN MASKED 2-FURYLCARBINOL MECHANOPHORES AND 1,2-DIOXETANE CHEMILUMINOPHORES



This chapter has been reprinted from Liu, P.; Tseng, Y.-L.; Ge, L.; Zeng, T.; Shabat, D.; Robb, M. J. *J. Am. Chem. Soc.* **2024**, *146*, 22151–22156

Mechanoluminescence, or the generation of light from materials under external force, is a powerful tool for biology and materials science. However, direct mechanoluminescence from polymers remains limited. Here, we report a novel design strategy for mechanoluminescent polymers that leverages the synergy between a masked 2-furylcarbinol mechanophore for mechanically triggered release and an adamantylidene-phenoxy-1,2-dioxetane chemiluminophore payload. Ultrasound-induced mechanochemical activation of polymers, in both organic and aqueous solutions, triggers a cascade reaction that ultimately results in bright green light emission. This novel strategy capitalizes on the modularity of the masked 2-furylcarbinol mechanophore system in combination with advances in the design of exceptionally bright and highly tunable adamantylidene-1,2-dioxetane chemiluminophores. We anticipate that this chemistry will enable diverse applications in optoelectronics, sensing, bioimaging, optogenetics, and many other areas.

1.1 Introduction

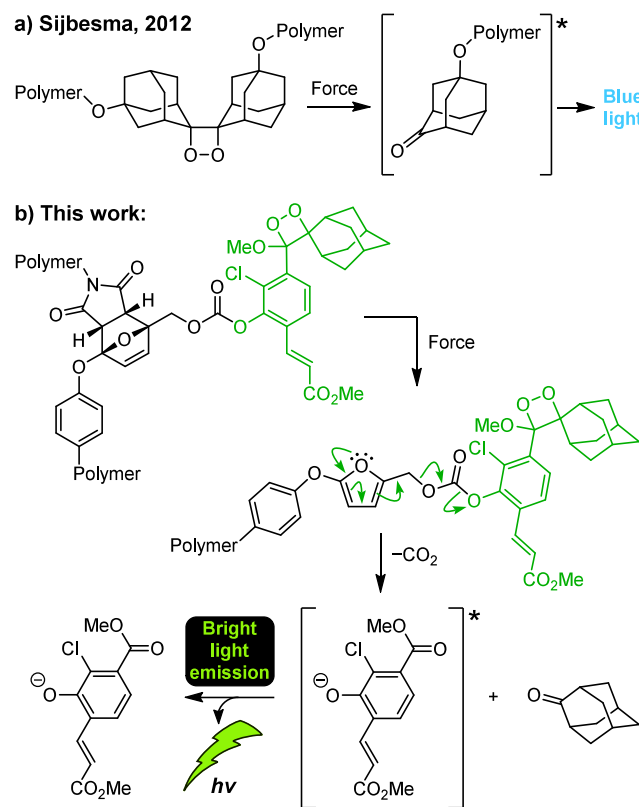
Mechanoluminescence, also known as triboluminescence, is the phenomenon of spontaneous light emission from materials in response to mechanical stimuli and has attracted significant interest since its first documented discovery in 1605¹ due to innate curiosity and myriad potential applications in optoelectronics, optogenetics, bioimaging, force-sensing, and many other areas.^{2–6} Mechanical force is transduced into light emission using a wide variety of materials and methods. Many inorganic crystals exhibit mechanoluminescence upon elastic deformation and doping a host lattice with different ions enables modulation of the mechanoluminescent properties.⁷ For instance, Wang and Hong leveraged mechanoluminescent ZnS nanoparticles doped with Ag⁺ and Co²⁺ ions as a light source for optogenetics, which were activated *in vivo* using focused ultrasound.⁸ In addition, many different types of organic compounds with mechanoluminescent properties have been developed based on a variety of mechanisms.⁹ Some propeller-shaped organic molecules with aggregation-induced emission behavior, for example, exhibit strong mechanoluminescence in the solid state.^{10,11} Mechanoluminescent polymers have also attracted recent attention.¹²

In the field of polymer mechanochemistry, mechanical force is transduced to stress-sensitive molecules termed mechanophores via covalently-bound polymer chains, resulting in specific chemical transformations.^{13–15} The infinite scope and precise construction of the molecular structure of mechanophores afforded by synthetic organic chemistry allows for programming a diverse range of functional responses including molecular release,^{16–19} coloration,^{20–23} conductivity switching,²⁴ and many others.^{25,26} In 2012, Sijbesma and coworkers published a landmark paper in which they demonstrated the mechanochemical activation of a bis-adamantyl 1,2-dioxetane compound in polymers resulting in blue light emission (**Scheme 1.1a**).²⁷ While mechanoluminescence from the bis-adamantyl dioxetane mechanophore is extremely weak with a short lifetime (< 1 s), the use of photosensitizers was shown to enhance mechanoluminescence in the solid state; however, the authors commented that light emission from sonicated solutions was too weak to be recorded.²⁷

Nevertheless, this mechanoluminescent probe has proven to be a powerful tool, enabling remarkable insights into polymer fracture mechanics at the single chain level.^{28–31} Given its impact and that more than a decade has elapsed since its discovery, it is noteworthy that Sijbesma's bis-adamantyl dioxetane remains the only chemiluminescent mechanophore reported in the literature.

Over the past several years, the Robb group has developed a platform for mechanically triggered small molecule release from polymers based on masked 2-furylcarbinol mechanophores.^{32–35} Mechanochemical activation results in the retro-Diels–Alder reaction of a furan–maleimide adduct to produce an unstable 2-furylcarbinol derivative that subsequently fragments to release a covalently-bound payload. The modularity of the system allows for the efficient release of a wide range of functionally diverse molecules from polymers, including the rapid release of phenols.^{33,34} Concurrent research in the Shabat group has pioneered the development of powerful adamantylidene-phenoxy-1,2-dioxetane chemiluminescent probes that, upon chemical deprotection of a phenol group, undergo a chemiexcitation decomposition process resulting in bright light emission both in organic and aqueous solutions.^{36–39} Specifically, incorporation of an electron-withdrawing *ortho*-substituent on Schaap's phenoxy-1,2-dioxetane results in chemiluminophores that generate up to 3,000-fold stronger light emission in aqueous solutions.³⁶ We envisaged that these two concepts could be merged to design novel mechanoluminescent polymers.

Scheme 1.1. Mechanoluminescent mechanophores.



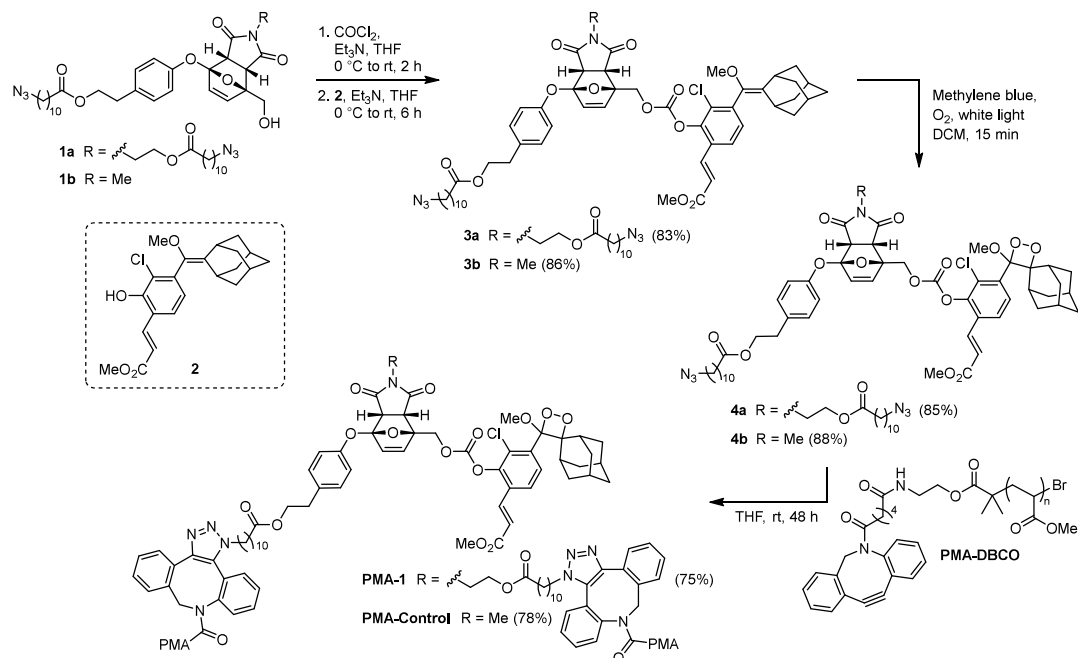
Herein, we demonstrate mechanically triggered chemiluminescence in both organic and aqueous solutions by conjugating an adamantylidene-1,2-dioxetane chemiluminophore to a masked furfuryl carbonate mechanophore covalently embedded in a polymer chain (**Scheme 1.1b**). Ultrasound-induced mechanochemical activation of the mechanophore triggers a cascade reaction that ultimately generates an electronically excited benzoate ester, which relaxes to the ground state via emission of a green photon. This study represents the only other mechanoluminescent mechanophore system reported since Sijbesma's disclosure of the bis-adamantyl 1,2-dioxetane in 2012 and the first to produce bright light emission from both organic and aqueous solutions without requiring sensitization. By combining two powerful molecular design strategies developed recently for adamantylidene-1,2-dioxetane chemiluminophores and masked 2-furylcarbinol mechanophores, we illustrate a promising new strategy for the construction of mechanoluminescent polymers for a wide range of applications.

1.2 Results and Discussion

1.2.1 Design, synthesis, and incorporation of mechanophores into poly(methyl acrylate)

We targeted a mechanophore based on a masked 5-aryloxyfurfuryl carbonate that is synthesized efficiently and was previously demonstrated to release phenol payloads rapidly upon mechanical activation (**Scheme 1.1b**).³⁴ Polymer chains are necessary to transduce force to the mechanophore, which is maximized near the chain-center during solution-phase ultrasonication as result of solvodynamic shear.⁴⁰ We pursued a click coupling approach to install the mechanophore-chemiluminophore conjugate near the center of polymer chains, based on a protocol that was used successfully for the conjugation of chemiluminescent 1,2-dioxetanes to siderophores for the detection of bacterial pathogens.⁴¹ Furan–maleimide Diels–Alder adduct **1a** with a primary alcohol for covalent payload attachment was prepared with a terminal alkyl azide group on each respective fragment (**Scheme 1.2**, see the *section 1.4.2* for details). Conjugation of adamantylidene-phenol **2** via a carbonate linkage afforded loaded mechanophore **3a** with the requisite click handles for polymer functionalization. Next, oxidation of the adamantylidene enol ether to the dioxetane generated mechanophore-

Scheme 1.2 Synthesis of poly(methyl acrylate) (PMA) polymers containing a chain-centered or chain-end masked 2-furylcarbinol mechanophore loaded with an adamantylidene-1,2-dioxetane chemiluminophore payload via SPAAC coupling.



chemiluminophore conjugate **4a**, which was covalently incorporated near the center of poly(methyl acrylate) (PMA) chains via a strain-promoted azide-alkyne cycloaddition (SPAAC) reaction⁴² with **PMA-DBCO**. Successful click coupling was confirmed by ¹H NMR spectroscopy and gel permeation chromatography (**Figures 1.1–1.4**). In addition to **PMA-1** ($M_n = 111$ kDa; $\bar{D} = 1.36$) containing a chain-centered mechanophore-chemiluminophore conjugate, we also prepared **PMA-Control** ($M_n = 125$ kDa; $\bar{D} = 1.15$)

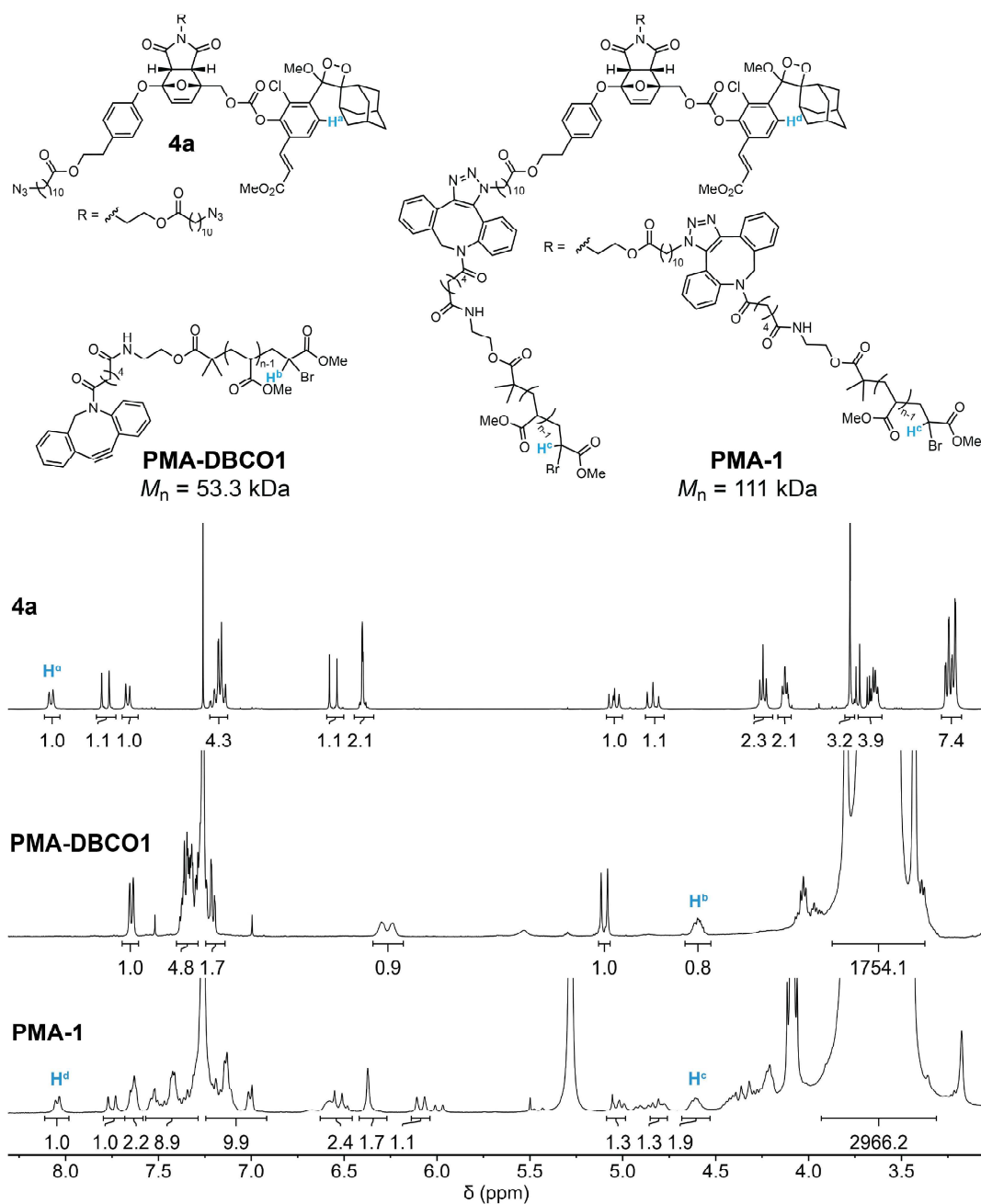


Figure 1.1. Structures and partial ^1H NMR spectra (400 MHz, CDCl_3) of **4a**, **PMA-DBCO1**, and **PMA-1**. The integration ratio of signals corresponding to protons H^c and H^d ($\sim 2:1$) indicates the successful click coupling of **4a** and **PMA-DBCO1** ($M_n = 53.3$ kDa, $D = 1.13$) to produce chain-centered polymer **PMA-1** ($M_n = 111$ kDa, $D = 1.36$). Further support is provided by GPC data in **Figure 1.3**.

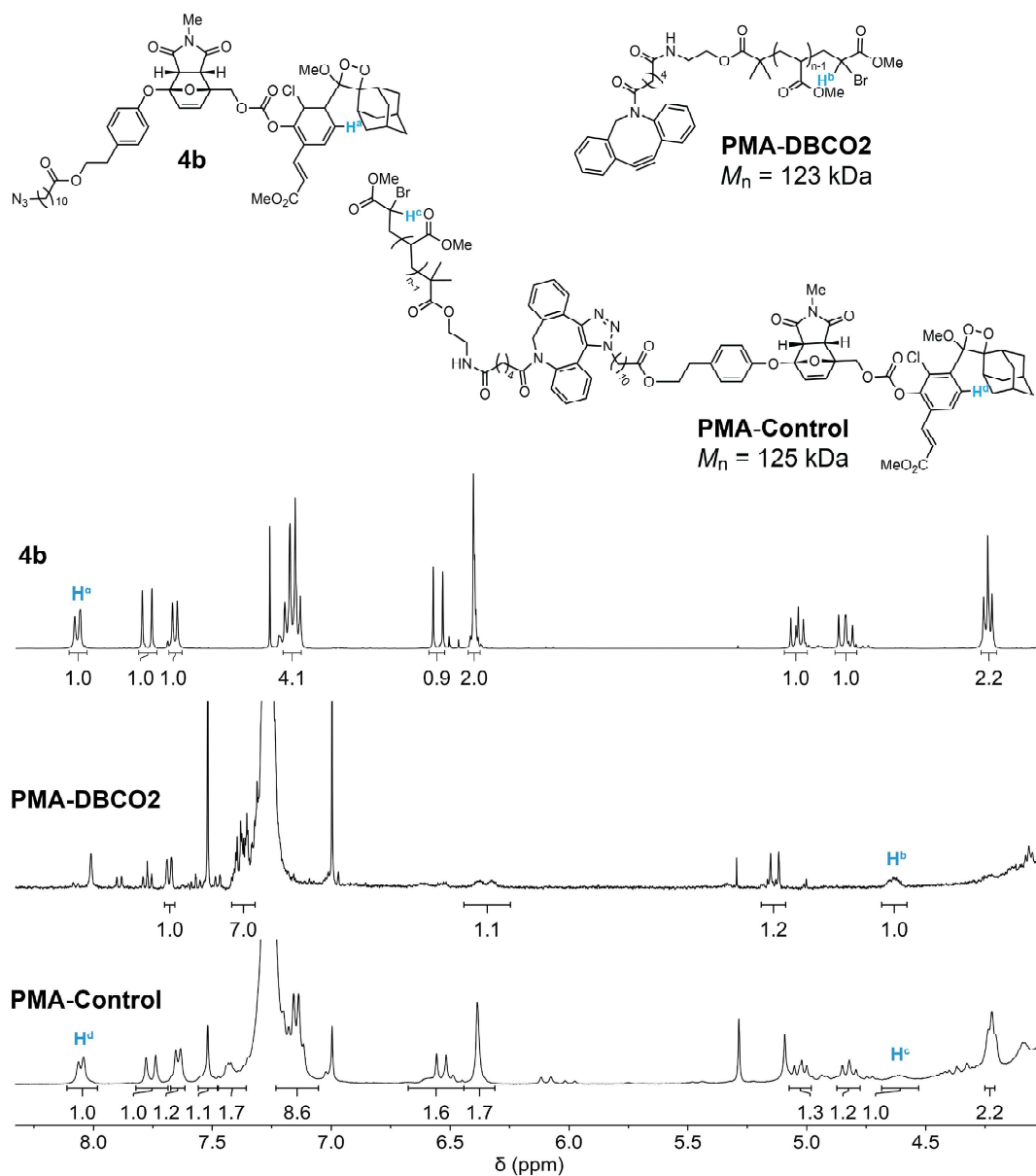


Figure 1.2. Structures and partial ^1H NMR spectra (400 MHz, CDCl_3) of **4b**, **PMA-DBCO2** and **PMA-Control**. The integration ratio of signals corresponding to protons H^c and H^d (1:1) indicates the successful click coupling of **4b** and **PMA-DBCO2** ($M_n = 123 \text{ kDa}$, $\bar{D} = 1.13$) to produce chain-end functional control polymer **PMA-Control** ($M_n = 125 \text{ kDa}$, $\bar{D} = 1.15$). Further support is provided by GPC data in **Figure 1.4**.

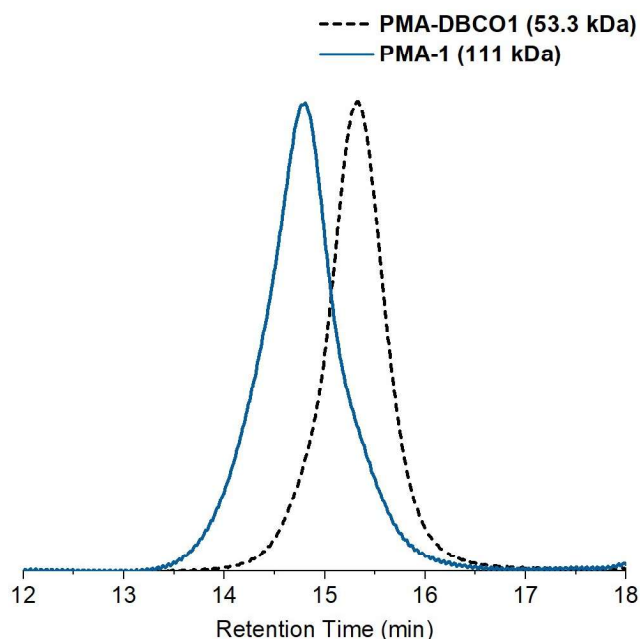


Figure 1.3. GPC chromatograms measured with a differential refractive index (dRI) detector for **PMA-DBCO1** ($M_n = 53.3$ kDa, $\mathcal{D} = 1.13$) and **PMA-1** ($M_n = 111$ kDa, $\mathcal{D} = 1.36$). The shift to shorter retention time indicates the successful click coupling of **4a** and **PMA-DBCO1** to produce chain-centered polymer **PMA-1**. The shoulder in the GPC trace of **PMA-1** at a retention time of ~ 15.5 min reflects a small amount of residual unreacted **PMA-DBCO1**, resulting in the higher apparent dispersity of **PMA-1**.

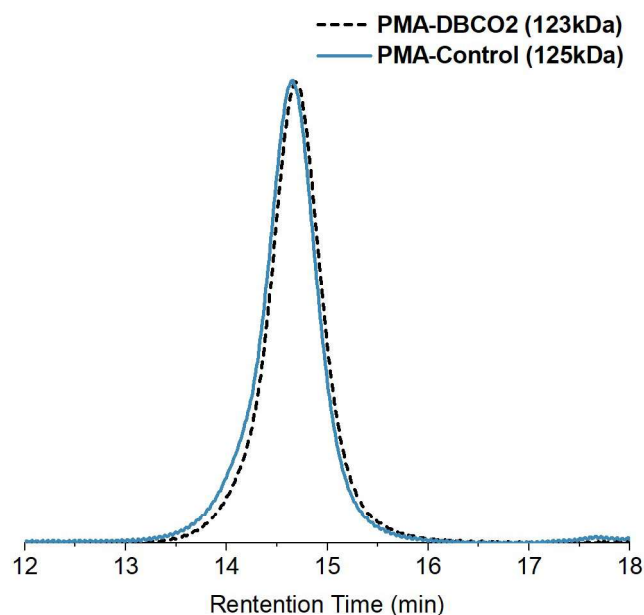


Figure 1.4. GPC chromatograms measured with a differential refractive index (dRI) detector for **PMA-DBCO2** ($M_n = 123$ kDa, $\mathcal{D} = 1.13$) and **PMA-Control** ($M_n = 125$ kDa, $\mathcal{D} = 1.15$). The slight shift to shorter retention time is consistent with the successful click coupling of **4b** and **PMA-DBCO2** to produce chain-end functional control polymer **PMA-Control**.

following a similar synthetic procedure incorporating an analogous mechanophore-chemiluminophore unit at the chain end, which is not subjected to mechanical force during ultrasonication.¹⁵

1.2.2 Investigation of mechanochemical activation and chemiluminescence in PMA

With the polymers successfully prepared, we next investigated the mechanically triggered chemiluminescence from **PMA-1** in acetonitrile (6 mg/mL) using pulsed ultrasonication (8–10 °C, 1 s on/1 s off, 8.8 W cm⁻²). Fragmentation of the mechanically unmasked furfuryl carbonate and associated cargo release occurs in polar protic environments,^{32–35} while slightly basic conditions are required to promote chemiexcitation decomposition of the chemiluminophore, which occurs via the phenoxide anion.³⁶ Therefore, aliquots (1 mL) were periodically removed from the sonicated solution and immediately combined with water (100 µL) and triethylamine (10 µL) to promote the release and chemiluminescence of the chemiluminophore prior to visual analysis and spectroscopic characterization (**Figure 1.5**). The emission of bright green light ($\lambda_{\text{em}} = 540$ nm) was observed upon ultrasonication of **PMA-1** and the intensity increased with increasing exposure to ultrasound (**Figure 1.5a**). **Figure 1.5b** illustrates photographs of the aliquots acquired in the dark, highlighting the bright green mechanoluminescence from **PMA-1** after 180 min of sonication. No chemiluminescence was observed after sonication of **PMA-1** for 180 min in anhydrous acetonitrile alone, consistent with the reactivity of the unmasked furfuryl carbonate, which only decomposes efficiently in the presence of a protic solvent.^{32–35} Likewise, no chemiluminescence was detected upon the addition of water and triethylamine to an acetonitrile solution of **PMA-1** that had not been subjected to ultrasonication. We note that partial hydrolysis of the carbonate does occur after prolonged exposure to water and triethylamine (72 h), which is to be expected for such an electron deficient aryl carbonate (**Figure 1.6**). Critically, ultrasonication of **PMA-Control** for 180 min and subsequent exposure to water and triethylamine under the same conditions does not result in significant chemiluminescence, confirming that mechanical force was responsible for light emission from **PMA-1** (**Figures 1.5a** and **1.5b**).

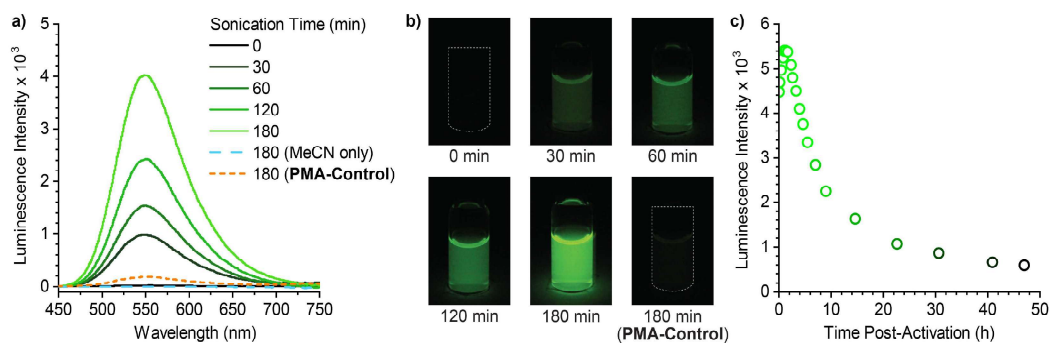


Figure 1.5. Mechanochemically triggered chemiluminescence via the molecular release of an adamantylidene-1,2-dioxetane chemiluminophore. a) Luminescence intensity from **PMA-1** (6 mg/mL in acetonitrile) subjected to varying durations of ultrasonication and immediately after the addition of water (10% v/v) and triethylamine (1% v/v). Minimal luminescence is observed without the addition of water and triethylamine, or upon ultrasonication of chain-end functional control polymer **PMA-Control**. b) Photographs acquired in the dark of the solutions corresponding to the measurements in panel (a) illustrating increasing mechanoluminescence intensity from **PMA-1** with longer exposure to ultrasound. c) Luminescence intensity at 540 nm over time from **PMA-1** measured following 180 min of ultrasonication and the addition of water and triethylamine.

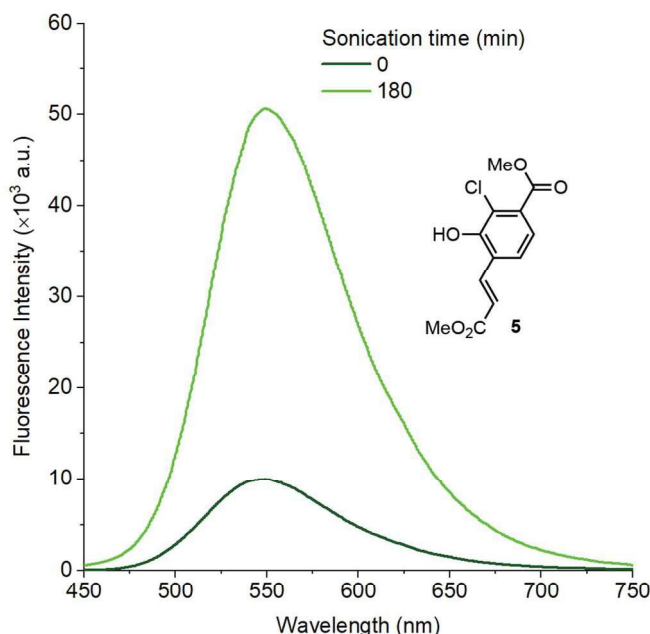


Figure 1.6. Fluorescence spectra ($\lambda_{\text{ex}} = 400$ nm) acquired from solutions of **PMA-1** (6.1 mg/mL in anhydrous MeCN) with and without sonication, and after 72 h of incubation in the presence of water (10% by volume) and triethylamine (1% by volume). Compound **5** is a terminal fluorescent product of the chemiexcitation decomposition process of the chemiluminophore (see **Scheme 1.1** in the main text). A small amount of fluorescence is observed from the unsonicated polymer solution after 72 h, indicating partial hydrolysis of the carbonate after extended exposure to water/triethylamine in acetonitrile. Significantly greater concentration of **5** is observed after sonication of **PMA-1** for 180 min, confirming successful mechanically triggered release of the chemiluminophore.

The intensity of emitted light from the solution of **PMA-1** continued to increase after cessation of sonication and the addition of water and triethylamine, reaching a maximum emission approximately 60 min later that subsequently decayed with a half-life of $t_{1/2} \sim 5$ h (**Figure 1.5c** and **Figure 1.7**). The kinetics of the process are complex due to the multistage reaction cascade involving simultaneous furfuryl carbonate fragmentation and chemiexcitation decomposition of the chemiluminophore.

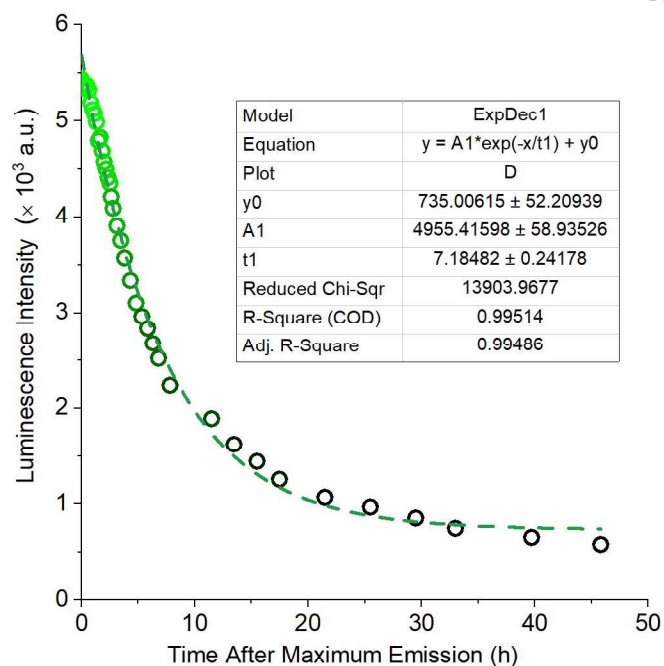


Figure 1.7. Luminescence decay at 540 nm measured after 180 min sonication of chain-centered polymer **PMA-1**, and 60 min after addition of H₂O (10% by volume) and Et₃N (1% by volume).

However, furfuryl carbonate fragmentation appears to dominate in the initial period of ~ 1 h following sonication and the addition of water and triethylamine, while the kinetics of chemiexcitation decomposition of the released adamantylidene-dioxetane chemiluminophore predominate thereafter. Finally, we note that bright green mechanoluminescence was also observed directly upon ultrasonication of **PMA-1** in a mixture of acetonitrile/water (9:1 v/v) containing 0.2% triethylamine. Under these conditions, the intensity of emitted light reached a maximum after ~ 30 min of ultrasonication (**Figure 1.8**). The kinetics of the system are even more complex with mechanophore activation also occurring concurrently with the multiple cascade fragmentation reactions. Again, control experiments demonstrated negligible chemiluminescence from the solution without sonication over a period of 5 h, or upon sonication of **PMA-Control** under the same conditions, again supporting the mechanical origin of light emission from **PMA-1** (**Figures 1.9** and **1.10**).

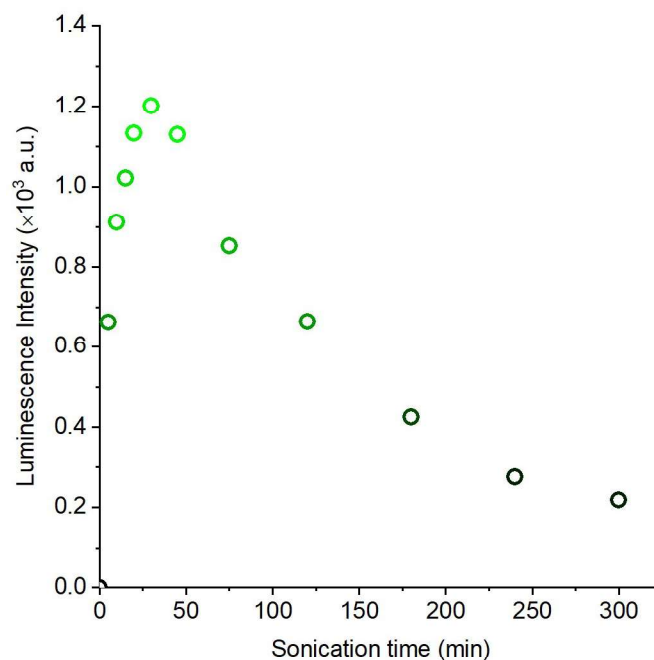


Figure 1.8. Luminescence intensity at 540 nm as a function of sonication time measured for a solution of **PMA-1** (6.0 mg/mL) in 9:1 MeCN/H₂O (v/v) containing 0.2% triethylamine. Luminescence intensity reached a maximum after approximately 30 min of sonication under these conditions.

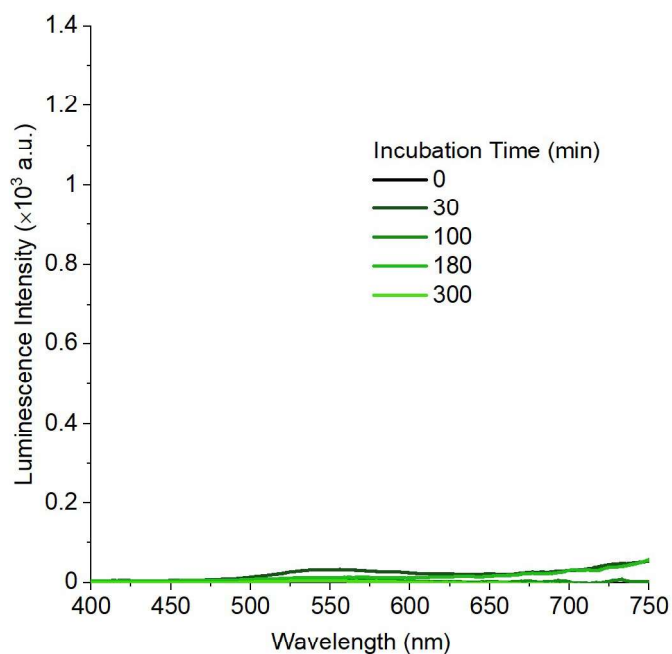


Figure 1.9. Luminescence spectra characterizing release of the chemiluminophore from **PMA-1** (6.0 mg/mL) upon incubation in 9:1 MeCN/H₂O (v/v) and 0.2% triethylamine at room temperature. Negligible luminescence is observed, illustrating the stability of the mechanophore-chemiluminophore conjugate in the absence of a mechanical trigger.

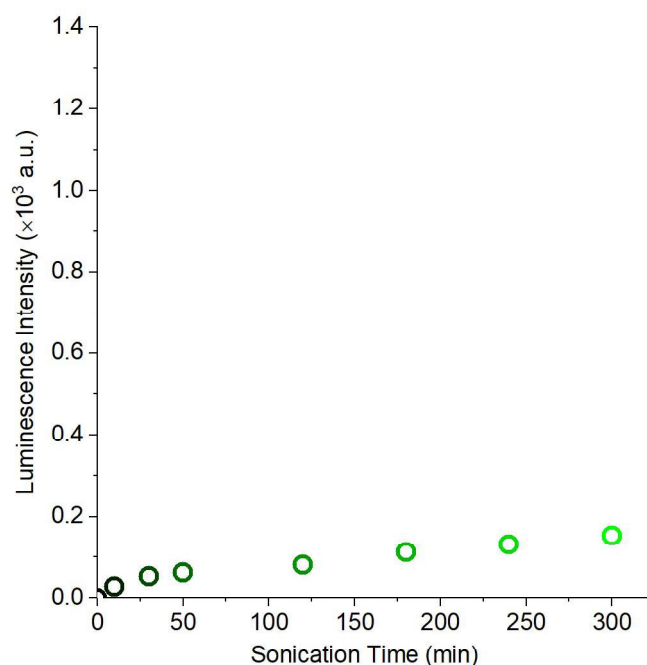


Figure 1.10. Luminescence intensity at 540 nm as a function of sonication time measured for a solution of chain-end functional control polymer **PMA-Control** (6.0 mg/mL) in 9:1 MeCN/H₂O (v/v) containing 0.2% triethylamine. Minimal luminescence is observed, supporting that the light emission from **PMA-1** is the result of mechanochemical activation.

1.2.3 Mechanochemical activation and chemiluminescence of PEG-1

There is interest in leveraging polymer mechanochemistry for biological applications,^{16,43} encouraged by recent developments in using biocompatible focused ultrasound as an external trigger for mechanophore activation.⁴⁴ Therefore, we prepared poly(ethylene glycol) (PEG) **PEG-1** incorporating a chain-centered mechanophore-chemiluminophore conjugate and investigated its mechanoluminescence in aqueous solutions (**Figure 1.11**). We followed a similar synthetic approach as that detailed above by using SPAAC coupling between azide-functional mechanophore-chemiluminophore conjugate **4a** and DBCO-terminated PEG, which was prepared in turn starting from either 30 kDa or 2 kDa NH₂-PEG-OMe (see the *section 1.4.2* for details). In addition to **PEG-1** ($M_n = 47.3$ kDa; $\mathcal{D} = 1.26$), we also synthesized oligomer **PEG-Control** ($M_n = 7.0$ kDa; $\mathcal{D} = 1.16$). In this case, both polymers contain a chain-centered mechanophore-chemiluminophore unit, but the length of **PEG-Control** was anticipated to be below the

threshold for ultrasound-induced mechanochemical activation.¹⁵ Successful click coupling in each case was confirmed by ¹H NMR spectroscopy and gel permeation chromatography (Figures 1.12–1.15).

Mechanoluminescence from **PEG-1** was investigated by subjecting a phosphate-buffered saline (PBS, pH = 7.4, 3 mg/mL) solution of the polymer to ultrasonication as employed above. Aliquots were removed at various points and characterized directly both spectroscopically and by digital photography in the dark (Figure 1.11). The generation of bright green light ($\lambda_{\text{em}} = 560$ nm) was observed upon ultrasonication with emission intensity reaching a maximum after only 5 min of ultrasonication. The maximum intensity of light emission was approximately 20% of that

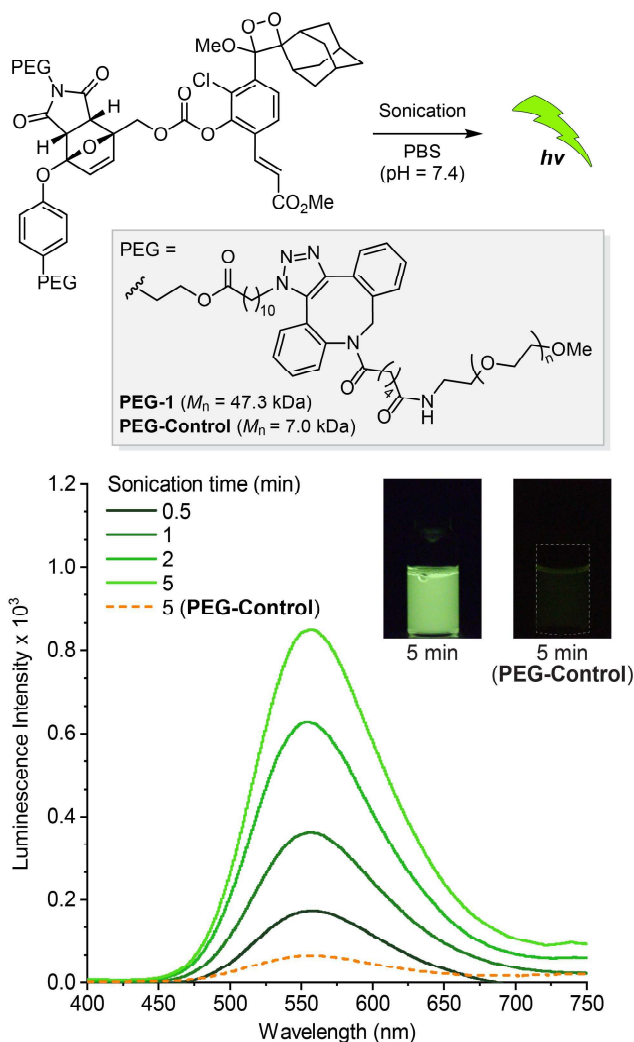
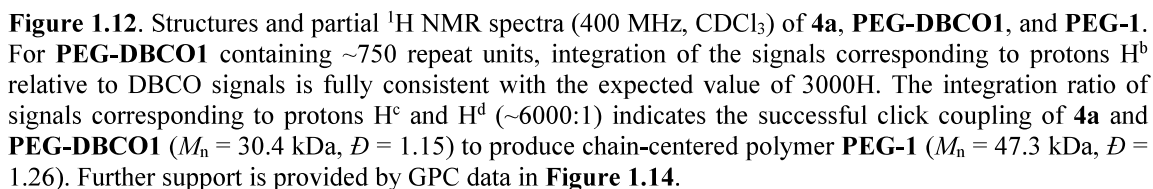


Figure 1.11. Luminescence intensity measured from solutions of **PEG-1** and **PEG-Control** (3 mg/mL) in PBS (pH = 7.4) subjected to varying durations of ultrasonication. In contrast to **PEG-1**, minimal luminescence is observed from oligomeric **PEG-Control** with a chain length below the threshold for mechanochemical activation. Insets show photographs acquired in the dark for solutions of **PEG-1** and **PEG-Control** after sonication for 5 min.

observed from **PMA-1** in acetonitrile, with an emission decay half-life that was approximately 2.5× shorter, on the order of ~2 h (Figure 1.16). This behavior is consistent with the chemiluminescence properties of similar adamantylidene-1,2-dioxetane chemiluminescence probes, which generally have lower quantum yields and exhibit shorter chemiluminescence lifetimes in aqueous media compared to organic solvents but are



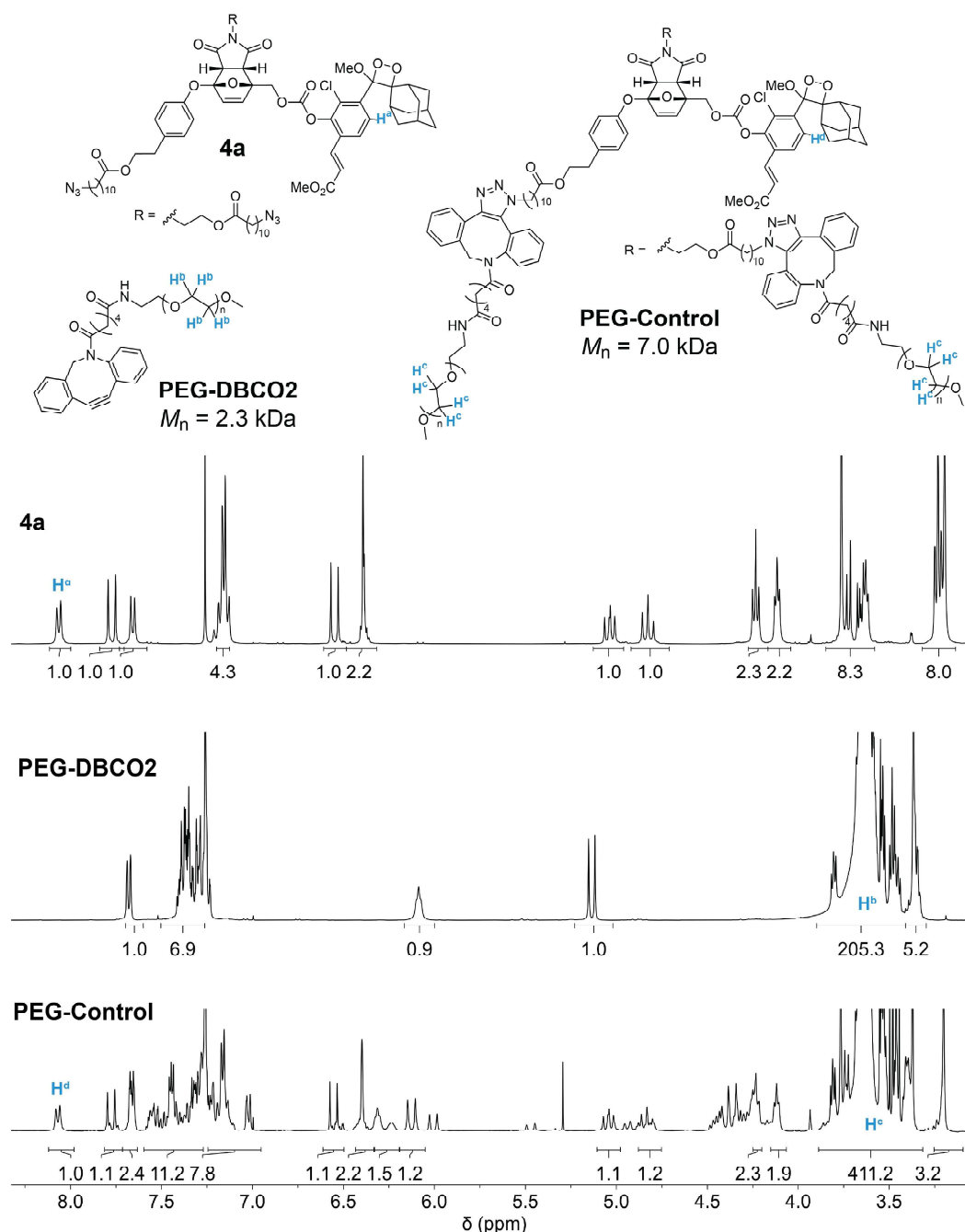


Figure 1.13. Structures and partial ^1H NMR spectra (400 MHz, CDCl_3) of **4a**, **PEG-DBCO2**, and **PEG-Control**. For **PEG-DBCO2** containing ~ 50 repeat units, integration of the signals corresponding to protons H^b relative to DBCO signals is fully consistent with the expected value of 200H. The integration ratio of signals corresponding to protons H^c and H^d ($\sim 400:1$) indicates the successful click coupling of **4a** and **PEG-DBCO2** ($M_n = 2.3$ kDa, $\bar{D} = 1.05$) to produce chain-centered control polymer **PEG-Control** ($M_n = 7.0$ kDa, $\bar{D} = 1.16$). Further support is provided by GPC data in **Figure 1.15**.

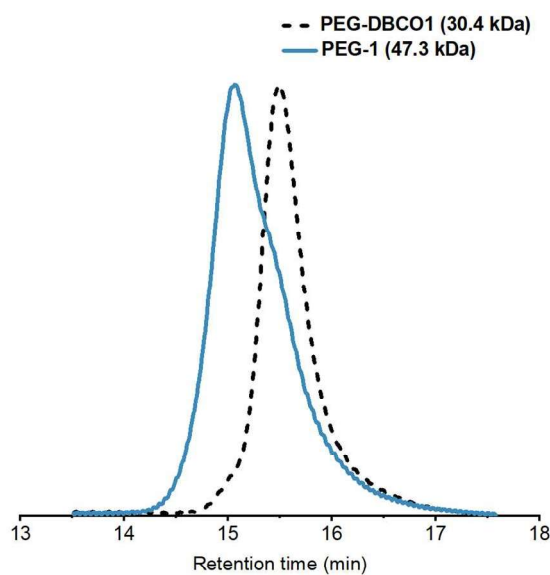


Figure 1.14. GPC chromatograms measured with a differential refractive index (dRI) detector for **PEG-DBCO1** ($M_n = 30.4$ kDa, $\mathcal{D} = 1.15$) and **PEG-1** ($M_n = 47.3$ kDa, $\mathcal{D} = 1.26$). The shift to shorter retention time indicates the successful click coupling of **4a** and **PEG-DBCO1** to produce chain-centered polymer **PEG-1**. The shoulder in the GPC trace of **PEG-1** at a retention time of ~ 15.5 min indicates a minor fraction of either residual unreacted **PEG-DBCO1** or end-functional polymer resulting from a single click reaction, both of which should be mechanochemically inactive, and is responsible for the lower average molar mass and higher dispersity of **PEG-1** compared to theoretical values.

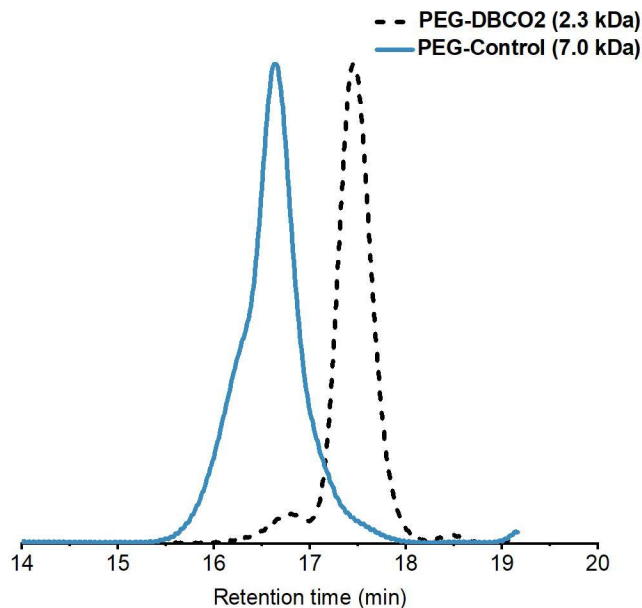


Figure 1.15. GPC chromatograms measured with a differential refractive index (dRI) detector for **PEG-DBCO2** ($M_n = 2.3$ kDa, $\mathcal{D} = 1.05$) and **PEG-Control** ($M_n = 7.0$ kDa, $\mathcal{D} = 1.16$). The peak shift to shorter retention time is consistent with the successful click coupling of **4a** and **PEG-DBCO2** to produce chain-centered control polymer **PEG-Control**. The minor peak at ~ 16.8 min in the GPC trace of **PEG-DBCO2** is derived from an impurity in the commercial PEG-NH₂ starting material and is likely accentuated in the click reaction resulting in the higher molar mass shoulder apparent in the GPC trace of **PEG-Control**.

nevertheless sufficiently bright for use in bioimaging.^{36,38,45} Negligible luminescence was observed from oligomeric **PEG-Control** upon ultrasonication of PBS solutions, supporting that mechanical force was responsible for the chemiluminescence observed from **PEG-1**. The demonstration of mechanically triggered bright chemiluminescence from PEG-based polymers in aqueous solution portends exciting opportunities for future biological applications, including in optogenetics and bioimaging by leveraging focused ultrasound to achieve precise external control of light generation.

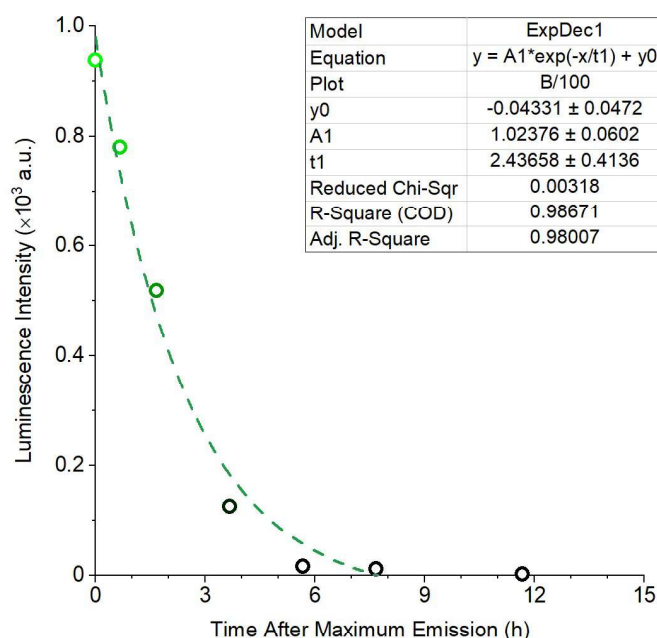


Figure 1.14. Luminescence decay at 560 nm measured for a solution of chain-centered polymer **PEG-1** (3.0 mg/mL in PBS, pH = 7.4) subjected to sonication for 5 min. Maximum emission under these conditions occurred ~0.5 h after sonication (see **Figure 1.11**). Fitting the data to an expression of simple first-order kinetics provides a half-life of ~2 h for the luminescence decay under these conditions.

1.3 Conclusions

In summary, we demonstrate mechanically triggered light emission (*i.e.*, mechanoluminescence) from polymers containing a masked 2-furylcarbinol mechanophore and an adamantylidene-1,2-dioxetane chemiluminophore payload. Ultrasound-induced mechanochemical activation triggers a reaction cascade that ultimately leads to the emission of a photon. Bright green mechanoluminescence is demonstrated from poly(methyl acrylate) and poly(ethylene glycol) polymers in organic and physiologically relevant aqueous solutions, respectively. Notably, this work represents the development of the only other chemiluminescent mechanophore since the seminal discovery of the bis-adamantyl 1,2-dioxetane mechanophore by Sijbesma in 2012. This new highly modular

system leverages the masked 2-furylcarbinol mechanophore platform for mechanically triggered molecular release in combination with exceptionally bright adamantylidene-1,2-dioxetane chemiluminophores that uniquely function in both organic and aqueous environments. We anticipate that this general strategy will be further enhanced by recent developments in the molecular design of highly tunable 1,2-dioxetane chemiluminophores with variable wavelength and temporal light emission characteristics,^{46,47} opening the door to a host of exciting applications.

1.4 Experimental Details

1.4.1 General experimental details and methods

Reagents from commercial sources were used without further purification unless otherwise stated. Methyl acrylate was passed through a short plug of basic alumina to remove inhibitor immediately prior to use. Anhydrous dichloromethane (DCM), tetrahydrofuran (THF), diethyl ether (Et₂O), acetonitrile (MeCN) and dimethylformamide (DMF) were obtained from a Pure Process Technology solvent purification system. All reactions were performed under a N₂ atmosphere unless specified otherwise. Freshly cut copper wire was immersed in 1 M HCl for 15 min, rinsed consecutively with deionized water and acetone, and then dried under a flow of N₂ prior to use. Column chromatography was performed on a Biotage Isolera system using SiliCycle SiliaSep HP or SiliaBond C18 cartridges.

NMR spectra were recorded using a 400 MHz Bruker Avance III HD with Prodigy Cryoprobe, 400 MHz Bruker Avance Neo, or Varian Inova 500 MHz spectrometers. All ¹H NMR spectra are reported in δ units, parts per million (ppm), and were measured relative to the signals for residual chloroform (7.26 ppm) in deuterated solvent. All ¹³C NMR spectra were measured in deuterated solvents and are reported in ppm relative to the signals for chloroform (77.16 ppm). Multiplicity and qualifier abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, td = triplet of doublets, ABq = AB quartet, m = multiplet, br s = broad singlet.

High resolution mass spectrometry (HR-MS) was carried out on an Agilent 6230 series time-of-flight mass spectrometer equipped with an Agilent G1958 Jet Stream Electrospray Ionization Source (ESI) or a JEOL JMS-T2000 AccuTOF GC-Alpha time-of-flight mass spectrometer interfaced with an Agilent 8890 GC system with samples analyzed by field desorption (FD).

Analytical gel permeation chromatography (GPC) was performed using an Agilent 1260 series pump equipped with two Agilent PLgel MIXED-B columns (7.5 x 300 mm), an Agilent 1200 series diode array detector, a Wyatt miniDAWN 3-angle light scattering detector, and an Optilab differential refractive index detector. The mobile phase was THF at a flow rate of 1 mL/min. Average molar masses and molar mass distributions were calculated by light scattering using a dn/dc value of 0.062 mL/g (25 °C) for poly(methyl acrylate). For poly(ethylene glycol) polymers, average dn/dc values were calculated from triplicate injections based on known sample concentration and assuming 100% mass elution (0.067 mL/g for **PEG-DBCO1** and **PEG-1**, 0.086 mL/g for **PEG-DBCO2**, and 0.098 mL/g for **PEG-Control**).

Pulsed ultrasound experiments were performed in a sound abating enclosure using a 500-watt Vibra Cell 505 liquid processor (1 s on, 1 s off, 20 kHz, 20% amplitude) equipped with a 0.5-inch diameter solid probe (part #630-0217), sonochemical adapter (part #830-00014), and a Suslick reaction vessel made by the Caltech glass shop (analogous to vessel #830-00014 from Sonics and Materials).

Luminescence spectra were recorded on a Shimadzu RF-6000 spectrofluorophotometer using a quartz microcuvette (Starna Cells 18F-Q-10-GL14-C, 10 x 2 mm). The excitation channel of the spectrometer was blocked to allow for chemiluminescence emission measurements. Chemiluminescence emission was measured in the wavelength range 400–750 nm with 20 nm emission bandwidth, 200 nm/min scan speed, and 0.1 nm data interval. For fluorescence measurements, an excitation wavelength of 400 nm was used with 3 nm excitation bandwidth, 5 nm emission bandwidth, 600 nm/min scan speed, and 0.5 nm data interval.

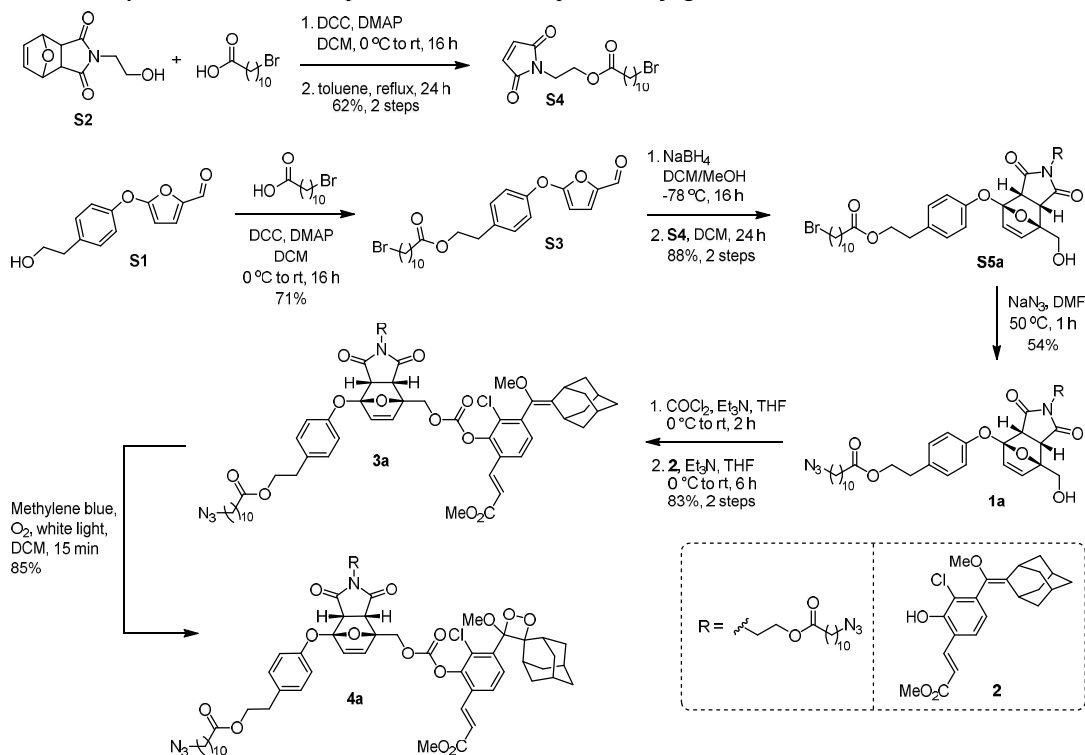
Digital photographs were acquired in a darkroom using a Canon EOS 700D digital camera equipped with a EF-S 18-55mm lens (5.6 aperture, 30 s shutter speed, ISO 3200, manual mode) or a Canon EOS Rebel SL3 digital camera equipped with a 100 mm macro lens (2.8 aperture, 30 s shutter speed, ISO 25600, manual mode). Images were cropped but otherwise used without any additional processing.

Dibenzoazacyclooctyne-carboxylic acid succinimidyl ester (DBCO-C6-NHS ester) was purchased from Broadpharm.

PEG-NH₂ was purchased from Nanosoft Polymers. Compounds **S1**,³⁴ **S2**,³⁴ **2**,³⁶ **5**,³⁶ and **S6**⁴⁸ were synthesized according to the literature procedures.

1.4.2 Synthetic details

Scheme 1.3. Synthesis of mechanophore-chemiluminophore conjugate **4a**.



4-((5-formylfuran-2-yl)oxy)phenethyl 11-bromoundecanoate (S3). A round bottom flask equipped with a stir bar was charged with **S1** (2.3 g, 10 mmol, 1.0 equiv), 11-bromoundecanoic acid (2.9 g, 11 mmol, 1.1 equiv), 4-(*N,N*-dimethylamino)pyridine (DMAP, 0.12 g, 1.0 mmol, 0.10 equiv) and dichloromethane (50 mL). The solution was

stirred to dissolve all solids and then cooled to 0 °C in an ice bath. Then dicyclohexylcarbodiimide (DCC, 3.1 g, 15 mmol, 1.5 equiv) was added in five portions. After the final addition, the solution was allowed to warm to room temperature. After stirring for 16 h, the solid precipitate was filtered off and discarded. The filtrate was diluted with diethyl ether (50 mL) and washed consecutively with 10% ammonium chloride (25 mL) and brine (25 mL). The organic layer was then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (5–20% ethyl acetate/hexane) to afford the title compound as a yellow solid (3.4 g, 71% yield).

^1H NMR (400 MHz, chloroform-*d*) δ : 9.37 (s, 1H), 7.23 (d, J = 8.7 Hz, 2H), 7.19 (d, J = 3.7 Hz, 1H), 7.11 – 7.06 (m, 2H), 5.52 (d, J = 3.7 Hz, 1H), 4.26 (t, J = 6.9 Hz, 2H), 3.38 (t, J = 6.9 Hz, 2H), 2.92 (t, J = 6.9 Hz, 2H), 2.26 (t, J = 7.5 Hz, 2H), 1.88 – 1.76 (m, 2H), 1.61 – 1.52 (m, 2H), 1.43 – 1.35 (m, 2H), 1.25 (s, 10H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, chloroform-*d*) δ : 175.5, 173.5, 162.8, 152.7, 144.7, 135.7, 130.4, 125.4, 118.9, 89.6, 64.2, 34.3, 34.1, 34.0, 32.7, 29.21, 29.16, 29.1, 28.9, 28.6, 28.0, 24.8 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{24}\text{H}_{31}\text{O}_5\text{BrNa}]^+$ ($\text{M}+\text{Na}$) $^+$, 501.1247; found, 501.1244.

2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl 11-bromoundecanoate (S4). A round bottom flask equipped with a stir bar was charged with **S2** (3.1 g, 15 mmol, 1.0 equiv), 11-bromoundecanoic acid (4.3 g, 16 mmol, 1.1 equiv), DMAP (0.18 g, 1.5 mmol, 0.10 equiv) and dichloromethane (75 mL). The solution was stirred to dissolve all reagents and cooled to 0 °C. Then, DCC (4.6 g, 22 mmol, 1.5 equiv) was added in five portions. After the final addition, the solution was allowed to warm to room temperature. After stirring for 16 h, the solid precipitate was filtered off and discarded. The filtrate was diluted with diethyl ether (50 mL) and washed consecutively with 10% ammonium chloride (25 mL) and brine (25 mL). The organic layer was then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (5–20%

ethyl acetate/hexanes) to afford a white solid, which was subsequently dissolved in toluene (60 mL) and heated to 100 °C. After 24 h, the solvent was removed under reduced pressure and the crude mixture was purified by column chromatography (5–30% ethyl acetate/hexane) to afford the title compound as a white solid (3.6 g, 62% yield over two steps).

^1H NMR (400 MHz, chloroform-*d*) δ : 6.71 (s, 2H), 4.21 (t, J = 5.3 Hz, 2H), 3.78 (t, J = 5.3 Hz, 2H), 3.39 (t, J = 6.9 Hz, 2H), 2.24 (t, J = 7.6 Hz, 2H), 1.89 – 1.78 (m, 2H), 1.59 – 1.51 (m, 2H), 1.44 – 1.35 (m, 2H), 1.26 (s, 10H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, chloroform-*d*) δ : 173.7, 170.5, 134.3, 61.3, 37.1, 34.2, 34.1, 32.9, 29.5, 29.4, 29.3, 29.2, 28.8, 28.3, 24.8 ppm.

HRMS (FD, m/z): calcd for $[\text{C}_{17}\text{H}_{26}\text{NO}_4\text{Br}]^{+*}$ (M) $^{+*}$, 387.1045; found, 387.1029.

4-(((4S,7R)-2-(2-((11-bromoundecanoyl)oxy)ethyl)-7-(hydroxymethyl)-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-4,7-epoxyisoindol-4-yl)oxy)phenethyl 11-bromoundecanoate (S5a). A flame-dried round bottom flask equipped with a stir bar was charged with **S3** (1.0 g, 2.1 mmol, 1.0 equiv), dichloromethane (10 mL), and methanol (10 mL). The solution was cooled to –78 °C in an acetone/dry ice bath before adding sodium borohydride (0.16 g, 4.2 mmol, 2.0 equiv) in three portions. The mixture was kept at –78 °C for 16 h before being quenched with 10% ammonium chloride (20 mL) and subsequently warmed to room temperature. The solution was then extracted with ethyl acetate (2 x 30 mL) and the organic phase was washed with brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, and filtered. To the filtrate was then added **S4** (0.98 g, 2.5 mmol, 1.2 equiv), which was immediately concentrated under reduced pressure until ~2 mL of viscous solution remained. The solution was then allowed to react at room temperature for 24 h, and the crude mixture was purified by column chromatography (40–50% ethyl acetate/hexane). A racemic mixture of the endo diastereomer of the title compound was isolated as a foamy white solid (1.6 g, 88% yield over two steps).

^1H NMR (400 MHz, chloroform-*d*) δ : 7.19 – 7.09 (m, 4H), 6.37 (d, J = 5.8 Hz, 1H), 6.34 (d, J = 5.8 Hz, 1H), 4.30 – 4.20 (m, 3H), 4.19 – 4.07 (m, 3H), 3.69 – 3.58 (m, 4H), 3.39 (t, J = 6.8 Hz, 4H), 2.88 (t, J = 7.1 Hz, 2H), 2.35 (t, J = 6.4 Hz, 1H), 2.29 – 2.19 (m, 4H), 1.88 – 1.78 (m, 4H), 1.62 – 1.49 (m, 4H), 1.46 – 1.35 (m, 4H), 1.30 – 1.09 (m, 20H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, chloroform-*d*) δ : 174.4, 173.7, 173.4, 173.3, 153.0, 135.5, 135.3, 134.0, 129.8, 120.5, 113.2, 86.7, 64.6, 61.3, 60.4, 50.3, 48.4, 37.6, 34.3, 34.2, 34.0, 33.9, 32.7, 29.3, 29.2, 29.1, 28.98, 28.96, 28.6, 28.1, 24.8, 24.5 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{41}\text{H}_{60}\text{Br}_2\text{NO}_9]^+ (\text{M}+\text{H})^+$, 868.2629; found, 868.2627.

4-(((4S,7R)-2-(2-((11-azidoundecanoyl)oxy)ethyl)-7-(hydroxymethyl)-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-4,7-epoxyisoindol-4-yl)oxy)phenethyl 11-azidoundecanoate (1a). A round bottom flask equipped with a stir bar was charged with **S5a** (0.71 g, 0.82 mmol, 1.0 equiv) and dimethylformamide (5.0 mL). To the homogenous solution was then added sodium azide (0.32 g, 4.9 mmol, 6.0 equiv) and the solution was stirred at 50 °C. After 1 h, the solution was cooled to room temperature and water (10 mL) was added. The mixture was extracted with ethyl acetate (3 x 10 mL) and the organic phase was washed with brine (2 x 50 mL). The organic layer was then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (10–50% ethyl acetate/hexane) to afford the title compound as a colorless oil (0.35 g, 54% yield).

^1H NMR (400 MHz, chloroform-*d*) δ : 7.15 – 7.00 (m, 4H), 6.30 (d, J = 5.8 Hz, 1H), 6.26 (d, J = 5.8 Hz, 1H), 4.23 – 3.99 (m, 6H), 3.63 – 3.56 (m, 2H), 3.54 (t, J = 5.3 Hz, 2H), 3.18 (t, J = 7.0 Hz, 4H), 3.06 (br s, 1H), 2.81 (t, J = 7.0 Hz, 2H), 2.22 – 2.14 (m, 4H), 1.55 – 1.45 (m, 8H), 1.30 – 1.17 (m, 24H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, chloroform-*d*) δ : 174.4, 173.6, 173.3, 173.2, 153.0, 135.4, 135.2, 134.0, 129.7, 120.5, 113.1, 86.7, 64.5, 61.2, 60.3, 51.3, 50.2, 48.3, 37.6, 34.2, 34.1, 33.8, 29.24, 29.15, 29.04, 28.96, 28.92, 28.90, 28.7, 26.5, 24.7, 24.5 ppm.

HRMS (ESI, m/z): calcd for $[C_{41}H_{60}N_7O_9]^+$ (M+H)⁺, 794.4447; found, 794.4451.

4-(((4S,7R)-7-((((3-(((1R,3R,5R,7S)-adamantan-2-ylidene)(methoxy)methyl)-2-chloro-6-((E)-3-methoxy-3-oxoprop-1-en-1-yl)phenoxy)carbonyl)oxy)methyl)-2-(2-((11-azido undecanoyl)oxy)ethyl)-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-4,7-epoxyisoindol-4-yl)oxy)phenethyl 11-azidoundecanoate (3a). A flame-dried Schlenk flask equipped with a stir bar was charged with compound **1a** (0.20 g, 0.25 mmol, 1.0 equiv) and dry tetrahydrofuran (1.0 mL). The solution was cooled to 0 °C in an ice bath. Dry triethylamine (0.11 mL, 0.79 mmol, 3.0 equiv) was then added, followed by phosgene solution (15 wt. % in toluene, 0.22 mL, 0.31 mmol, 1.2 equiv). After stirring at 0 °C for 2 h, a solution of compound **2** (33 mg, 85 μmol, 0.34 equiv) dissolved in dry tetrahydrofuran (1.0 mL) and dry trimethylamine (12 μL) was added. Then the mixture was allowed to warm to room temperature. After stirring for 6 h, methanol (2.0 mL) was added to quench the reaction, the mixture was concentrated under reduced pressure, and purified by column chromatography (10–50% ethyl acetate/hexane) to afford the title compound as a colorless oil (85 mg, 83% yield over two steps).

¹H NMR (400 MHz, chloroform-*d*) δ: 7.78 (d, *J* = 16.1 Hz, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.22 – 7.11 (m, 4H), 6.50 (d, *J* = 16.1 Hz, 1H), 6.41 (d, *J* = 5.8 Hz, 1H), 6.39 (d, *J* = 5.8 Hz, 1H), 5.08 – 5.03 (m, 1H), 4.86 – 4.75 (m, 1H), 4.24 (t, *J* = 7.1 Hz, 2H), 4.12 (t, *J* = 5.2 Hz, 2H), 3.77 (s, 3H), 3.74 – 3.62 (m, 4H), 3.30 (s, 3H), 3.24 (td, *J* = 7.0, 1.9 Hz, 4H), 2.89 (t, *J* = 7.1 Hz, 2H), 2.25 (dt, *J* = 17.2, 7.6 Hz, 4H), 2.09 (s, 1H), 1.98 – 1.69 (m, 13H), 1.61 – 1.52 (m, 8H), 1.34 – 1.27 (m, 24H) ppm.

¹³C{¹H} NMR (101 MHz, chloroform-*d*) δ: 173.8, 173.5, 173.4, 173.1, 166.7, 153.1, 151.9, 145.9, 138.9, 138.2, 137.0, 136.1, 134.6, 134.4, 133.4, 130.00, 129.98, 129.2, 128.8, 125.4, 121.8, 120.9, 113.5, 84.1, 66.6, 64.7, 60.5, 57.4, 52.0, 51.5, 49.9, 49.3, 39.3, 39.1, 38.7, 37.9, 37.1, 34.5, 34.3, 34.0, 33.0, 29.8, 29.5, 29.4, 29.3, 29.19, 29.16, 29.1, 28.9, 28.4, 28.2, 26.8, 25.0, 24.7 ppm.

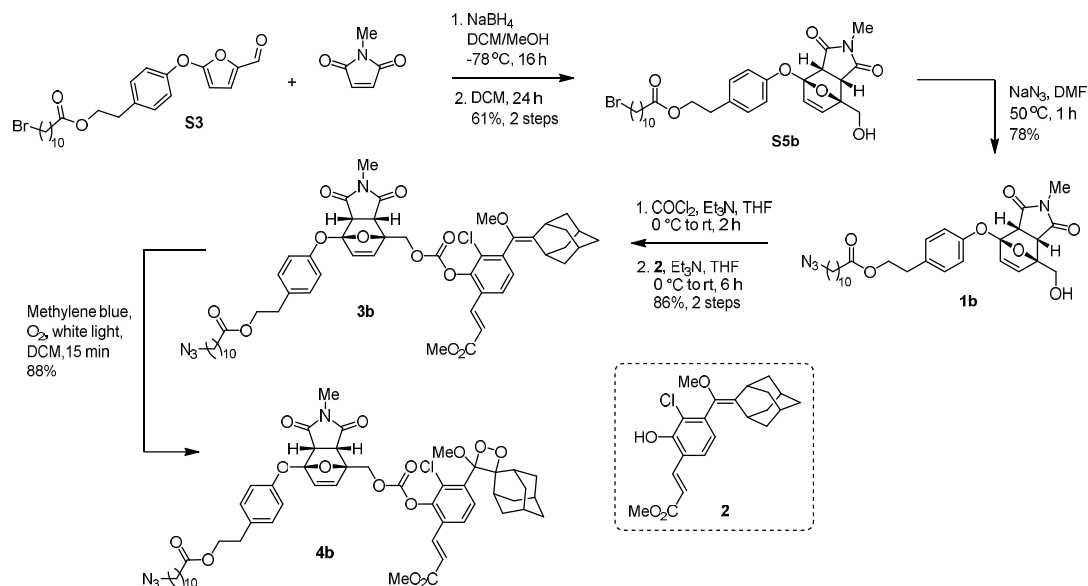
HRMS (ESI, m/z): calcd for $[C_{64}H_{83}ClN_7O_{14}]^+$ (M+H)⁺, 1208.5681; found, 1208.5678.

4-(((4S,7R)-2-(2-((11-azidoundecanoyl)oxy)ethyl)-7-(((2-chloro-6-((E)-3-methoxy-3-oxoprop-1-en-1-yl)-3-((1R,3R,5R,7R)-4'-methoxyspiro[adamantane-2,3'-[1,2]dioxetan]-4'-yl)phenoxy)carbonyl)oxy)methyl) -1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-[4,7]epoxyisoindol-4-yl)oxy)phenethyl 11-azidoundecanoate (4a). Compound **3a** (60 mg, 50 μ mol) and methylene blue (1.1 mg) were dissolved in dichloromethane (3.0 mL). Oxygen was bubbled through the solution while irradiating with white light using a Cree 19W PAR38 LED floodlamp (3000K, 1500 Lumen). The reaction was monitored by TLC. Upon completion (~15 min), the solvent was concentrated under reduced pressure. Purification by column chromatography (10–50% ethyl acetate/hexane) afforded the title compound (mixture of diastereomers) as a colorless oil (53 mg, 85% yield). Dioxetane **4a** was stored neat in a freezer protected from light and was found to be stable for several weeks. Some degradation was observed after 3 months of storage in the freezer.

^1H NMR (400 MHz, chloroform-*d*) δ : 8.06 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 16.1 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.18 – 7.11 (m, 4H), 6.55 (d, J = 16.1 Hz, 1H), 6.42 – 6.35 (m, 2H), 5.04 (t, J = 12.3 Hz, 1H), 4.83 (t, J = 12.3 Hz, 1H), 4.23 (t, J = 7.1 Hz, 2H), 4.12 (t, J = 5.3 Hz, 2H), 3.76 (s, 3H), 3.75 – 3.58 (m, 4H), 3.28 – 3.16 (m, 7H), 3.00 (s, 1H), 2.88 (t, J = 7.1 Hz, 2H), 2.31 – 2.16 (m, 5H), 1.96 (s, 1H), 1.87 – 1.51 (m, 18H), 1.47 – 1.41 (m, 1H), 1.34 – 1.27 (m, 24H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, chloroform-*d*) δ : 173.8, 173.5, 173.4, 173.1, 166.4, 153.1, 153.0, 151.8, 146.4, 136.5, 136.2, 136.1, 135.3, 134.6, 134.5, 134.43, 134.41, 131.1, 130.7, 130.0, 127.1, 125.6, 123.1, 121.0, 120.9, 113.6, 111.5, 96.43, 96.41, 84.03, 84.00, 66.7, 64.7, 60.5, 52.1, 51.5, 49.90, 49.87, 49.7, 49.2, 37.9, 36.6, 34.5, 34.3, 34.05, 34.01, 33.7, 32.5, 32.2, 31.64, 31.59, 29.5, 29.4, 29.3, 29.19, 29.16, 29.1, 28.9, 26.8, 26.2, 25.85, 25.82, 25.0, 24.7 ppm.

HRMS (ESI, m/z): calcd for $\text{C}_{64}\text{H}_{83}\text{ClN}_7\text{O}_{16}$ ($\text{M}+\text{H}$) $^+$, 1240.5579; found, 1240.5557.

Scheme 1.4. Synthesis of mechanophore-chemiluminophore conjugate **4b**.

4-(((4S,7R)-7-(hydroxymethyl)-2-methyl-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-4,7-epoxyisoindol-4-yl)oxy)phenethyl 11-bromoundecanoate (S5b). A flame-dried round bottom flask equipped with a stir bar was charged with **S3** (1.0 g, 2.1 mmol, 1.0 equiv), dichloromethane (10 mL), and methanol (10 mL). The solution was cooled to -78°C in an acetone/dry ice bath before adding sodium borohydride (0.16 g, 4.2 mmol, 2.0 equiv) in three portions. The mixture was kept at -78°C for 16 h before being quenched with 10% ammonium chloride (20 mL) and subsequently warmed to room temperature. The solution was then extracted with ethyl acetate (2 x 30 mL) and the organic phase was washed with brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, and filtered. To the filtrate was added *N*-methylmaleimide (0.47 g, 4.2 mmol, 2.0 equiv), which was then concentrated under reduced pressure until ~ 2 mL of viscous solution remained. The solution was then allowed to react at room temperature for 24 h, and the crude mixture was purified by column chromatography (40–50% ethyl acetate/hexane). A racemic mixture of the endo diastereomer of the title compound was isolated as a foamy white solid (0.76 g, 61% yield over two steps).

^1H NMR (500 MHz, chloroform-*d*) δ : 7.18 – 7.08 (m, 4H), 6.37 (d, $J = 5.7$ Hz, 1H), 6.34 (d, $J = 5.7$ Hz, 1H), 4.27 – 4.14 (m, 4H), 3.66 (s, 2H), 3.39 (t, $J = 6.9$ Hz, 2H), 2.87 (t, $J =$

7.0 Hz, 2H), 2.83 (s, 3H), 2.66 (br s, 1H), 2.26 (t, $J = 7.5$ Hz, 2H), 1.86 – 1.76 (m, 2H), 1.60 – 1.53 (m, 2H), 1.44 – 1.37 (m, 2H), 1.26 (s, 10H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (126 MHz, chloroform- d) δ : 174.8, 173.9, 173.8, 153.1, 135.6, 134.2, 129.9, 120.7, 113.3, 86.7, 64.7, 61.7, 50.6, 48.8, 34.4, 34.3, 34.2, 32.9, 29.40, 29.36, 29.2, 29.1, 28.8, 28.2, 24.94, 24.86 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{29}\text{H}_{38}\text{BrNO}_7\text{Na}]^+ (\text{M}+\text{Na})^+$, 614.1724; found, 614.1725.

4-(((4S,7R)-7-(hydroxymethyl)-2-methyl-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-4,7-epoxyisoindol-4-yl) oxy)phenethyl 11-azidoundecanoate (1b). A round bottom flask equipped with a stir bar was charged with **S5b** (0.47 g, 0.79 mmol, 1.0 equiv) and DMF (10 mL). To the homogenous solution was then added sodium azide (0.21 g, 3.2 mmol, 4.0 equiv) and the solution was stirred at 50 °C. After 1 h, the solution was cooled to room temperature and water (10 mL) was added. The mixture was extracted with ethyl acetate (3 x 10 mL) and the organic phase was washed with brine (2 x 50 mL). The organic layer was then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (10–50% ethyl acetate/hexane) to afford the title compound as a colorless oil (0.34 g, 78% yield).

^1H NMR (400 MHz, chloroform- d) δ : 7.20 – 7.10 (m, 4H), 6.39 (d, $J = 5.8$ Hz, 1H), 6.36 (d, $J = 5.8$ Hz, 1H), 4.29 – 4.14 (m, 4H), 3.71 – 3.63 (m, 2H), 3.25 (t, $J = 7.0$ Hz, 2H), 2.89 (t, $J = 7.0$ Hz, 2H), 2.85 (s, 3H), 2.27 (t, $J = 7.5$ Hz, 2H), 2.20 (br s, 1H), 1.64 – 1.54 (m, 4H), 1.36 – 1.27 (m, 12H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, chloroform- d) δ : 174.8, 173.9, 173.8, 153.1, 135.6, 135.5, 134.2, 129.9, 120.7, 113.3, 86.7, 64.7, 61.7, 51.5, 50.6, 48.8, 34.4, 34.3, 29.44, 29.35, 29.24, 29.15, 29.1, 28.9, 26.7, 24.9, 24.8 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{29}\text{H}_{39}\text{N}_4\text{O}_7]^+ (\text{M}+\text{H})^+$, 555.2813; found, 555.2839.

4-(((4S,7R)-7-((((3-(((1R,3R,5R,7S)-adamantan-2-ylidene)(methoxy)methyl)-2-chloro-6-((E)-3-methoxy-3-oxoprop-1-en-1-yl)phenoxy)carbonyl)oxy)methyl)-2-methyl-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-4,7-epoxyisoindol-4-yl)oxy)phenethyl 11-azidoundecanoate (3b). A flame-dried Schlenk flask equipped with a stir bar was charged with compound **1b** (0.20 g, 0.36 mmol, 1.0 equiv) and dry tetrahydrofuran (1.0 mL). The solution was cooled to 0 °C in an ice bath. Dry triethylamine (0.15 mL, 1.1 mmol, 3.0 equiv) was then added, followed by phosgene solution (15 wt. % in toluene, 0.31 mL, 0.43 mmol, 1.2 equiv). After stirring at 0 °C for 2 h, a solution of compound **2** (50 mg, 0.13 mmol, 0.3 equiv) dissolved in dry THF (1.0 mL) and dry trimethylamine (17 µL) was added. Then the mixture was allowed to warm to room temperature. After stirring for 6 h, methanol (2.0 mL) was added to quench the reaction, the mixture was concentrated under reduced pressure, and purified by column chromatography (10–50% ethyl acetate/hexane) to afford the title compound as a colorless oil (0.11 g, 86% yield over two steps).

¹H NMR (400 MHz, chloroform-*d*) δ : 7.78 (d, *J* = 16.1 Hz, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 1H), 7.21 – 7.12 (m, 4H), 6.50 (d, *J* = 16.1 Hz, 1H), 6.42 (d, *J* = 5.8 Hz, 1H), 6.39 (d, *J* = 5.8 Hz, 1H), 5.08 – 5.02 (m, 1H), 4.87 – 4.80 (m, 1H), 4.24 (t, *J* = 7.1 Hz, 2H), 3.77 (s, 3H), 3.75 – 3.64 (m, 2H), 3.30 (s, 3H), 3.25 (t, *J* = 7.0 Hz, 2H), 2.90 – 2.86 (m, 5H), 2.27 (t, *J* = 7.5 Hz, 2H), 2.09 (s, 1H), 1.96 – 1.68 (m, 13H), 1.63 – 1.55 (m, 4H), 1.35 – 1.27 (s, 12H) ppm.

¹³C{¹H} NMR (101 MHz, chloroform-*d*) δ : 173.9, 173.8, 173.5, 166.7, 153.1, 151.9, 145.9, 138.9, 138.2, 137.1, 136.1, 134.7, 134.4, 133.5, 130.03, 130.01, 129.2, 128.8, 125.5, 121.9, 121.0, 113.5, 84.1, 66.7, 64.8, 57.5, 52.0, 51.6, 50.1, 49.4, 39.3, 39.1, 38.7, 37.1, 34.6, 34.4, 33.0, 29.9, 29.5, 29.4, 29.3, 29.24, 29.22, 29.0, 28.4, 28.3, 26.8, 25.03, 24.96 ppm.

HRMS (ESI, *m/z*): calcd for [C₅₂H₆₂ClN₄O₁₂]⁺ (M+H)⁺, 969.4047; found, 969.4054.

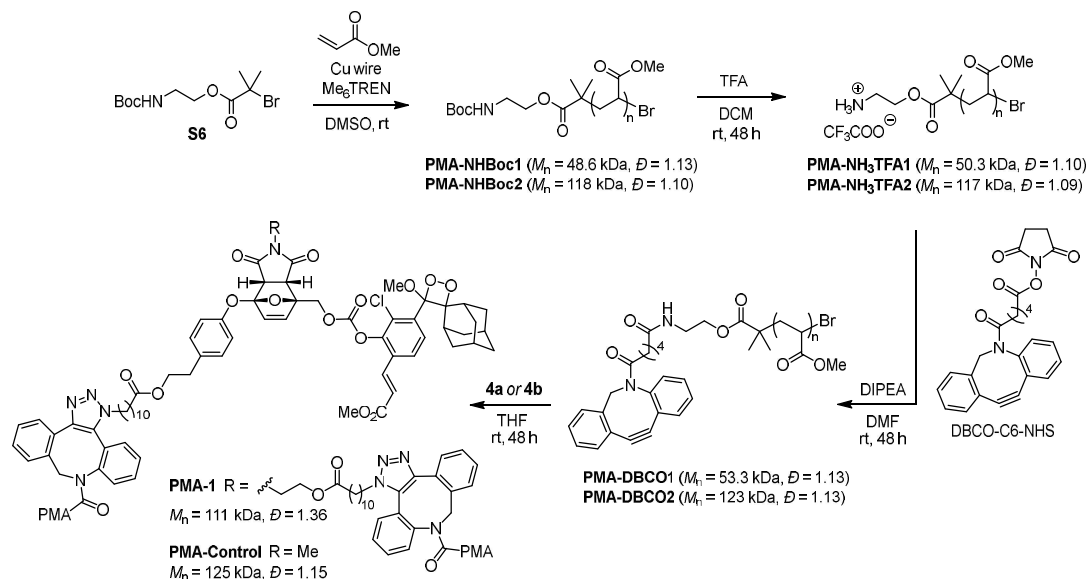
4-(((4S,7R)-7-(((2-chloro-6-((E)-3-methoxy-3-oxoprop-1-en-1-yl)-3-((1R,3R,5R,7R)-4'-methoxyspiro[adamantane-2,3'-[1,2]dioxetan]-4'-yl)phenoxy)carbonyl)oxy)methyl)-2-methyl-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-[4,7]epoxyisoindol-4-yl)oxy)phen

ethyl 11-azidoundecanoate (4b). Compound **3b** (61 mg, 63 μ mol) and methylene blue (1.1 mg) were dissolved in dichloromethane (3.0 mL). Oxygen was bubbled through the solution while irradiating with white light using a Cree 19W PAR38 LED floodlamp (3000K, 1500 Lumen). The reaction was monitored by TLC. Upon completion (~15 min), the solvent was concentrated under reduced pressure. Purification by column chromatography (10–50% ethyl acetate/hexane) afforded the title compound (mixture of diastereomers) as a colorless oil (55 mg, 88% yield). Dioxetane **4a** was stored neat in a freezer protected from light and was found to be stable for several weeks. Some degradation was observed after 3 months of storage in the freezer.

^1H NMR (400 MHz, chloroform-*d*) δ : 8.07 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 16.1 Hz, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.20 – 7.13 (m, 4H), 6.55 (d, J = 16.1 Hz, 1H), 6.42 – 6.38 (m, 2H), 5.07 – 5.00 (m, 1H), 4.87 – 4.78 (m, 1H), 4.24 (t, J = 7.1 Hz, 2H), 3.77 (s, 3H), 3.73 – 3.62 (m, 2H), 3.26 – 3.18 (m, 5H), 3.01 (br s, 1H), 2.91 – 2.82 (m, 5H), 2.27 (t, J = 7.6 Hz, 2H), 2.01 – 1.54 (m, 14H), 1.49 – 1.43 (m, 1H), 1.37 – 1.21 (m, 14H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, chloroform-*d*) δ : 173.9, 173.8, 173.4, 166.5, 153.12, 153.08, 151.8, 146.5, 136.6, 136.24, 136.16, 135.4, 134.64, 134.60, 134.42, 134.40, 131.2, 130.8, 130.0, 127.2, 125.7, 123.1, 121.0, 120.9, 113.6, 111.6, 96.49, 96.47, 84.01, 83.98, 66.7, 64.7, 52.1, 51.6, 50.1, 50.0, 49.9, 49.4, 36.6, 34.6, 34.4, 34.0, 33.7, 32.5, 32.3, 31.7, 31.6, 29.5, 29.4, 29.3, 29.23, 29.20, 28.9, 26.8, 26.2, 25.88, 25.85, 25.01, 24.97 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{52}\text{H}_{62}\text{ClN}_4\text{O}_{14}]^+$ ($\text{M}+\text{H}$) $^+$, 1001.3946; found, 1001.3947.

Scheme 1.5. Synthesis of poly(methyl acrylate) polymers **PMA-1** and **PMA-Control**

PMA-NHBoc1. Poly(methyl acrylate) with a Boc-protected amine end-group was synthesized by controlled radical polymerization following the procedure by Nguyen *et al.*⁴⁹ A 50 mL Schlenk flask equipped with a stir bar was charged with initiator **S6** (62 mg, 0.20 mmol, 1.0 equiv), methyl acrylate (11 mL, 0.12 mol, 600 equiv), freshly cut copper wire (1.0 cm length, 20 gauge), and dimethyl sulfoxide (11 mL). The flask was sealed and the solution was deoxygenated via three freeze-pump-thaw cycles, then backfilled with nitrogen. After adding tris[2-(dimethylamino)ethyl]amine (Me₆TREN, 0.11 mL, 0.40 mmol, 2.0 equiv) via microsyringe, the mixture was stirred at room temperature until the solution became sufficiently viscous, indicating that the desired monomer conversion was reached (ca. 1–2 h). The flask was then opened to atmosphere and diluted with dichloromethane (10 mL). The polymer was precipitated repeatedly into cold methanol (4 × 200 mL) and the isolated polymer was thoroughly dried under vacuum (9.8 g, 95% yield). $M_n = 48.6 \text{ kg/mol}$; $M_p = 50.2 \text{ kg/mol}$; $D = 1.13$.

PMA-NHBoc2. The same procedure as above was used to prepare a second polymer with higher molar mass. A 20 mL Schlenk flask equipped with a stir bar was charged with initiator **S6** (12 mg, 39 μmol , 1.0 equiv), methyl acrylate (5.2 mL, 58 mmol, 1500 equiv), freshly cut copper wire (1.0 cm length, 20 gauge), and dimethyl sulfoxide (5.2 mL). The flask was sealed and the solution was deoxygenated via three freeze-pump-thaw cycles, then backfilled with nitrogen. After adding tris[2-(dimethylamino)ethyl]amine (Me₆TREN, 20 μL , 75 μmol , 2.0 equiv) via microsyringe, the mixture was stirred at room temperature until the solution became sufficiently viscous, indicating that the desired monomer conversion was reached

(ca. 1–2 h). The flask was then opened to atmosphere and diluted with dichloromethane (5.0 mL). The polymer was precipitated repeatedly into cold methanol (4 x 100 mL) and the isolated polymer was thoroughly dried under vacuum (4.9 g, 98% yield). $M_n = 118$ kg/mol; $M_p = 126$ kg/mol; $D = 1.10$.

PMA-NH₃TFA1. Poly(methyl acrylate) with an ammonium trifluoroacetate end-group was prepared by deprotection of **PMA-NHBOC1**. A 50 mL round bottom flask equipped with a stir bar was charged with **PMA-NHBoc1** (9.1 g, 0.19 mmol) and dissolved in a 1:1 mixture of dichloromethane (10 mL) and trifluoroacetic acid (10 mL). The mixture was stirred for 48 h at room temperature. The polymer was then precipitated repeatedly into cold methanol (4 x 200 mL) and the isolated polymer was thoroughly dried under vacuum (8.2 g, 90% yield). $M_n = 50.3$ kg/mol; $M_p = 49.8$ kg/mol; $D = 1.10$.

PMA-NH₃TFA2. Following the same procedure as above, a 25 mL round bottom flask equipped with a stir bar was charged with **PMA-NHBoc2** (4.1 g, 35 μ mol) and dissolved in a 1:1 mixture of dichloromethane (10 mL) and trifluoroacetic acid (10 mL). The mixture was stirred for 48 h at room temperature. The polymer was precipitated repeatedly into cold methanol (4 x 100 mL) and the isolated polymer was thoroughly dried under vacuum (3.8 g, 93% yield). $M_n = 117$ kg/mol; $M_p = 124$ kg/mol; $D = 1.09$.

PMA-DBCO1. Poly(methyl acrylate) with a dibenzocyclooctyne (DBCO) end group. A 10 mL round bottom flask equipped with a stir bar was charged with **PMA-NH₃TFA1** (0.41 g, 8.2 μ mol, 1.0 equiv) and dry DMF (4.0 mL). Then *N,N*-diisopropylethylamine (DIPEA, 21 μ L, 0.12 mmol, 15 equiv) and DBCO-C6-NHS ester (20 mg, 46 μ mol, 5.6 equiv) were added sequentially. The mixture was stirred at room temperature for 48 h. Then the polymer was precipitated repeatedly into cold methanol (4 x 40 mL) and the isolated polymer was thoroughly dried under vacuum (0.31 g, 75% yield). $M_n = 53.3$ kg/mol; $M_p = 52.0$ kg/mol; $D = 1.13$.

PMA-DBCO2. Following the same procedure as above, a 10 mL round bottom flask equipped with a stir bar was charged with **PMA-NH₃TFA2** (0.30 g, 2.6 μ mol, 1.0 equiv)

and dry DMF (3.0 mL). Then *N,N*-diisopropylethylamine (7.0 μ L, 40 μ mol, 15 equiv) and DBCO-C6-NHS ester (7.0 mg, 16 μ mol, 6.2 equiv) were added sequentially. The mixture was stirred at room temperature for 48 h. Then the polymer was precipitated repeatedly into cold methanol (4 x 40 mL) and the isolated polymer was thoroughly dried under vacuum (0.23 g, 78% yield). M_n = 123 kg/mol; M_p = 126 kg/mol; $\bar{D}$ = 1.13.

PMA-1. Poly(methyl acrylate) containing a chain-centered mechanophore-chemiluminophore conjugate. A 5 mL round bottom flask equipped with a stir bar was charged with **PMA-DBCO1** (0.21 g, 3.9 μ mol, 2.1 equiv), **4a** (2.4 mg, 1.9 μ mol, 1.0 equiv), and dry THF (2.0 mL). The mixture was stirred at room temperature protected from light and the progress of the reaction was monitored by gel permeation chromatography. After completion (~48 h), the polymer was precipitated repeatedly into cold methanol (4 x 10 mL) and the isolated polymer was thoroughly dried under vacuum (0.16 g, 75% yield). M_n = 111 kg/mol; M_p = 118 kg/mol; $\bar{D}$ = 1.36.

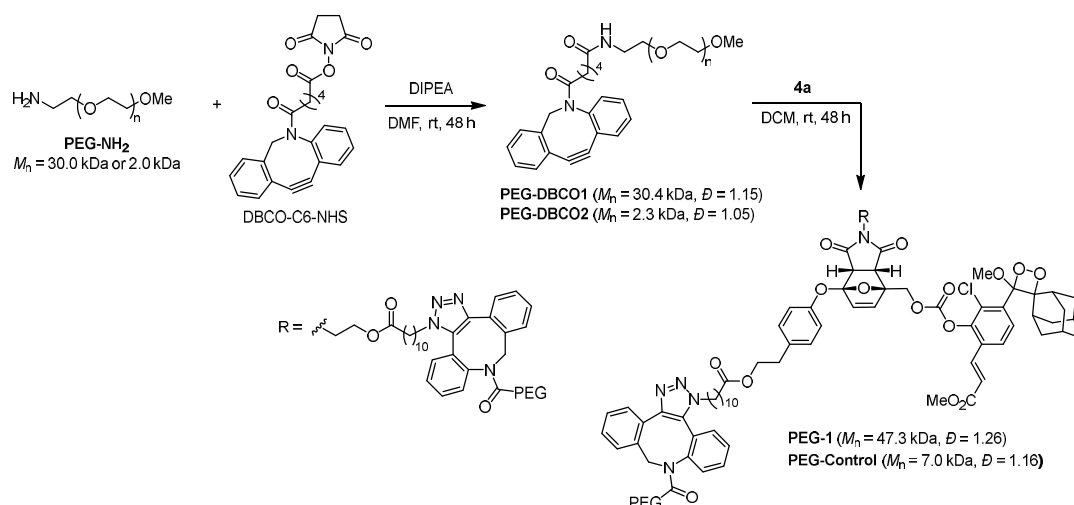
PMA-Control. Poly(methyl acrylate) containing a mechanophore-chemiluminophore conjugate at the chain end. A 5 mL round bottom flask equipped with a stir bar was charged with **PMA-DBCO2** (0.14 g, 1.1 μ mol, 1.0 equiv), **4b** (5.0 mg, 5.0 μ mol, 4.5 equiv), and dry THF (1.5 mL). The mixture was stirred at room temperature protected from light for 48 h. The polymer was then precipitated repeatedly into cold methanol (4 x 10 mL) and the isolated polymer was thoroughly dried under vacuum (0.11 g, 78% yield). M_n = 125 kg/mol; M_p = 128 kg/mol; $\bar{D}$ = 1.15.

Table 1.1. GPC characterization data for poly(methyl acrylate) (PMA) polymers.

	M_n (kDa)	M_p (kDa)	$\bar{D}$
PMA-NHBoc1	48.6	50.2	1.13
PMA-NH₃TFA1	50.3	49.8	1.10
PMA-DBCO1	53.3	52.0	1.13
PMA-1	111	118	1.36
PMA-NHBoc2	118	126	1.10

PMA-NH₃TFA2	117	124	1.09
PMA-DBCO2	123	126	1.13
PMA-Control	125	128	1.15

Scheme 1.6. Synthesis of **PEG-1** and **PEG-Control**.



PEG-DBCO1. Poly(ethylene glycol) with a dibenzocyclooctyne end group. A 20 mL scintillation vial equipped with a stir bar was charged with **PEG-NH₂** ($M_n = 30 \text{ kDa}$, 200 mg, 6.7 μmol , 1.0 equiv), *N,N*-diisopropylethylamine (17 μL , 100 μmol , 15 equiv), DBCO-C6-NHS ester (17 mg, 40 μmol , 6.0 equiv), and DMF (2.0 mL). The mixture was stirred thoroughly using a vortex mixer and the solution was stirred at room temperature for 48 h. The polymer was precipitated repeatedly into cold diethyl ether (3 x 40 mL) and the isolated polymer was thoroughly dried under vacuum (157 mg, 79% yield). $M_n = 30.4 \text{ kg/mol}$; $M_p = 28.8 \text{ kg/mol}$; $\bar{D} = 1.15$.

PEG-DBCO2. Following the same procedure as above, a 20 mL scintillation vial equipped with a stir bar was charged with **PEG-NH₂** ($M_n = 2 \text{ kDa}$, 200 mg, 0.1 mmol, 1.0 equiv), *N,N*-diisopropylethylamine (174 μL , 1.0 mmol, 10 equiv), DBCO-C6-NHS ester (198 mg, 0.46 mmol, 4.6 equiv), and DMF (4.0 mL). The mixture was stirred thoroughly using a vortex mixer and the solution was stirred at room temperature for 48 h. The polymer was

precipitated repeatedly into cold diethyl ether (3 x 40 mL) and the isolated polymer was dried under vacuum (143 mg, 63% yield). $M_n = 2.3$ kg/mol; $M_p = 2.4$ kg/mol; $\bar{D} = 1.05$.

PEG-1. Poly(ethylene glycol) containing a chain-centered mechanophore-chemiluminophore conjugate. A 20 mL scintillation vial equipped with a stir bar was charged with **PEG-DBCO1** (0.20 g, 6.6 μ mol, 1.9 equiv), **4a** (4.2 mg, 3.4 μ mol, 1.0 equiv), and DCM (4.0 mL). The mixture was stirred at room temperature for 48 h protected from light. The polymer was isolated by precipitation into cold diethyl ether (40 mL) and thoroughly dried under vacuum (143 mg, 71% yield). $M_n = 47.3$ kg/mol; $M_p = 63.9$ kg/mol; $\bar{D} = 1.26$.

PEG-Control. Oligo(ethylene glycol) containing a chain-centered mechanophore-chemiluminophore conjugate. A 20 mL scintillation vial equipped with a stir bar was charged with **PEG-DBCO2** (77 mg, 33 μ mol, 1.8 equiv), **4a** (24 mg, 19 μ mol, 1.0 equiv), and DCM (1.5 mL). The mixture was stirred at room temperature for 48 h protected from light. The polymer was isolated by precipitation into cold diethyl ether (40 mL) and thoroughly dried under vacuum (72 mg, 71% yield). $M_n = 7.0$ kg/mol; $M_p = 6.1$ kg/mol; $\bar{D} = 1.16$.

Table S2. GPC characterization data for poly(ethylene glycol) (PEG) polymers.

	M_n (kDa)	M_p (kDa)	$\bar{D}$	dn/dc
PEG-DBCO1	30.4	28.8	1.15	0.067
PEG-1	47.3	63.9	1.26	0.067
PEG-DBCO2	2.3	2.4	1.05	0.086
PEG-Control	7.0	6.1	1.16	0.098

1.4.3 General procedure for ultrasonication experiments

An oven-dried sonication vessel was fitted with rubber septa, placed onto the sonication probe, and allowed to cool under a stream of dry argon. The vessel was charged with a solution of the polymer (6 mg/mL, 10 mL total volume) and submerged in an ice bath. The solution was sparged continuously with argon beginning 20 min prior to sonication and for

the duration of the sonication experiment. Pulsed ultrasound (1 s on/1 s off, 20% amplitude, 20 kHz, 8.75 W/cm²) was then applied to the system. Ultrasonic intensity was calibrated using the method described by Berkowski *et al.*⁵⁰

For sonications of **PMA-1** and **PMA-Control** in anhydrous acetonitrile, aliquots of the sonicated solution (1 mL) were filtered through a 0.45 µm syringe filter followed by the addition of H₂O (0.1 ml) and Et₃N (5 µL) immediately prior to analysis.

Sonications of **PMA-1** and **PMA-Control** in 9:1 MeCN/H₂O with 0.2% triethylamine were performed according to the general procedure above with the polymer dissolved in MeCN/H₂O (9:1 v/v, 6 mg/mL, 10 mL + 20 µL triethylamine).

Sonications of **PEG-1** and **PEG-Control** were performed according to the general procedure above with the polymer dissolved in phosphate buffered saline solution (PBS, pH = 7.4, 3 mg/mL).

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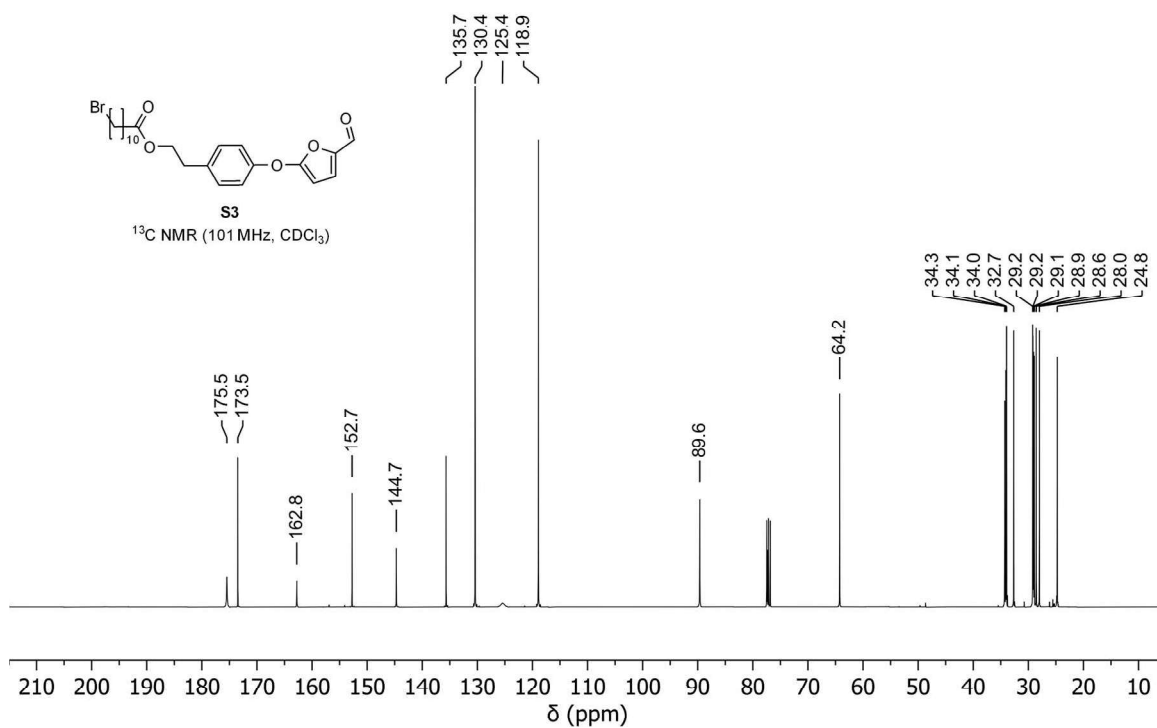
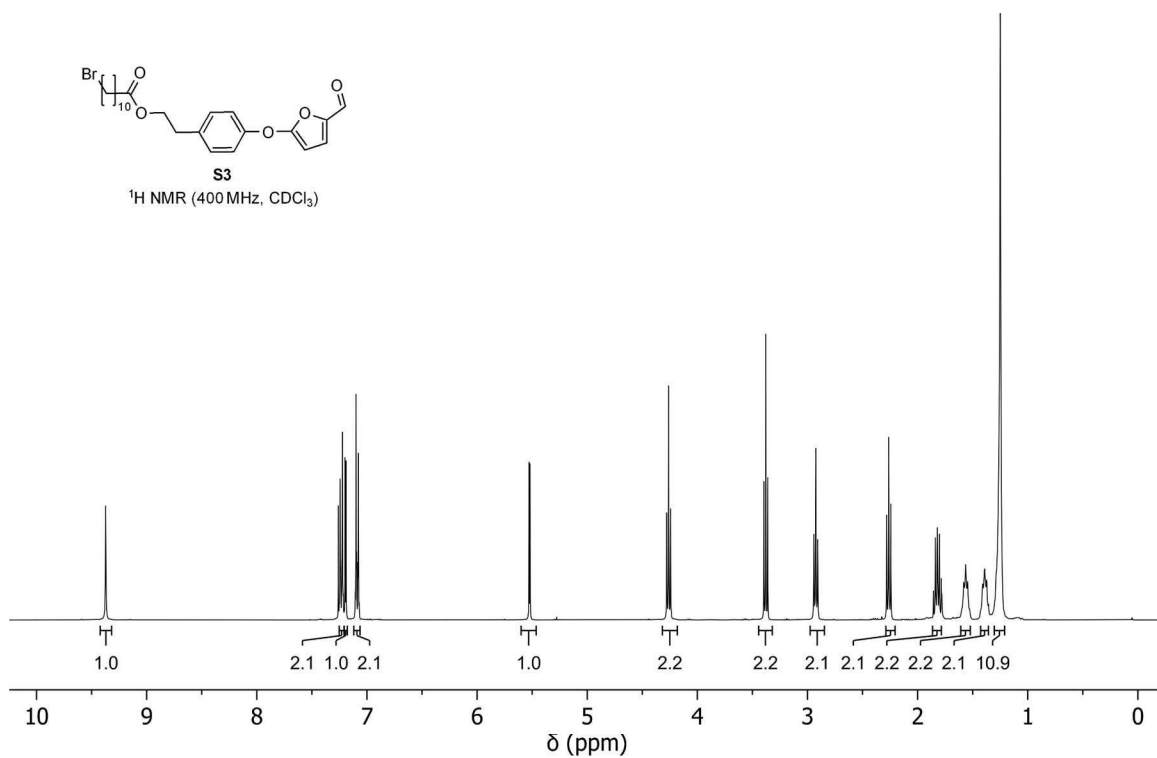
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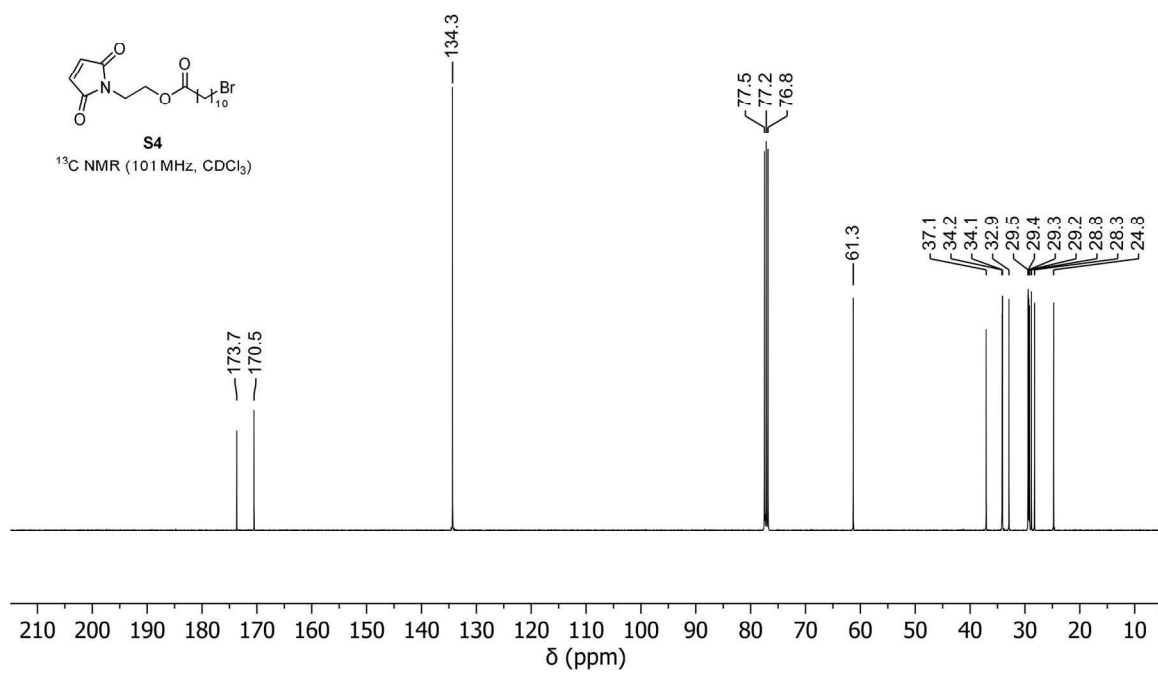
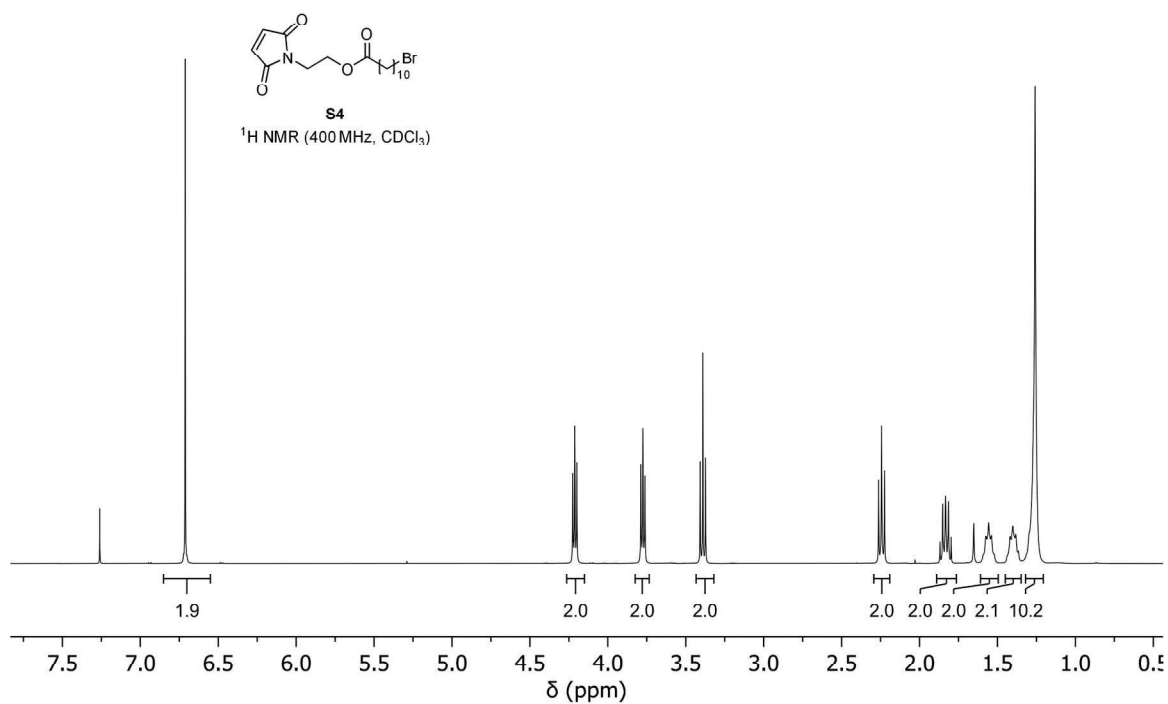
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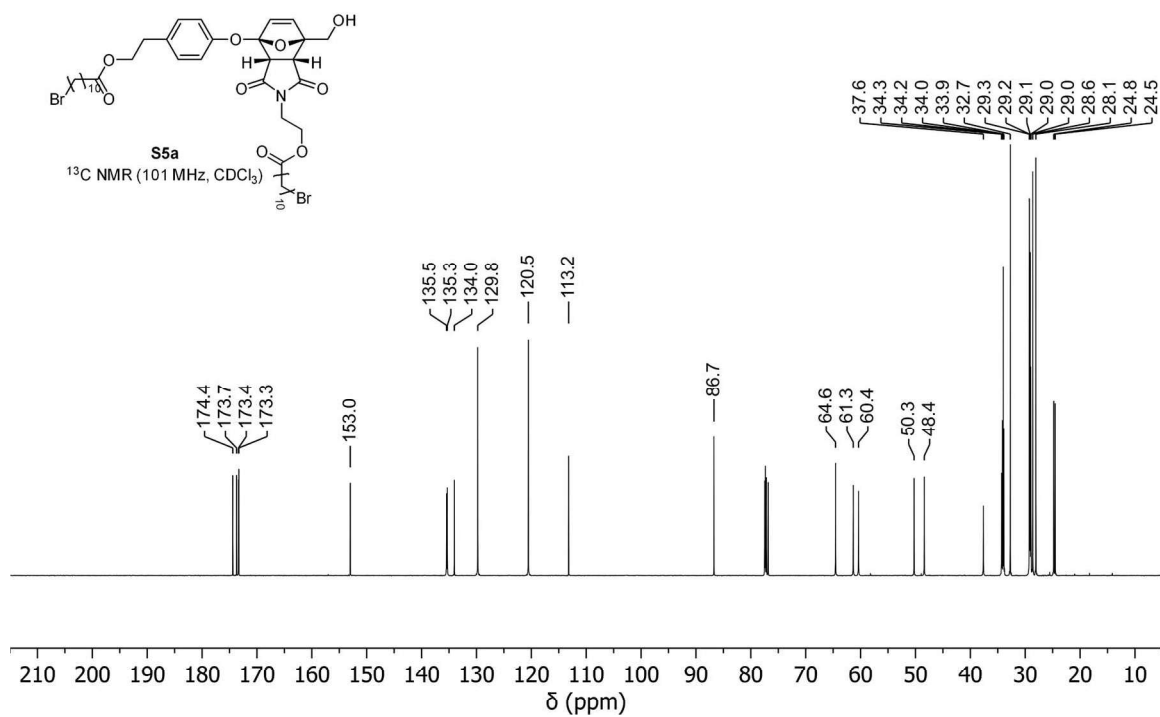
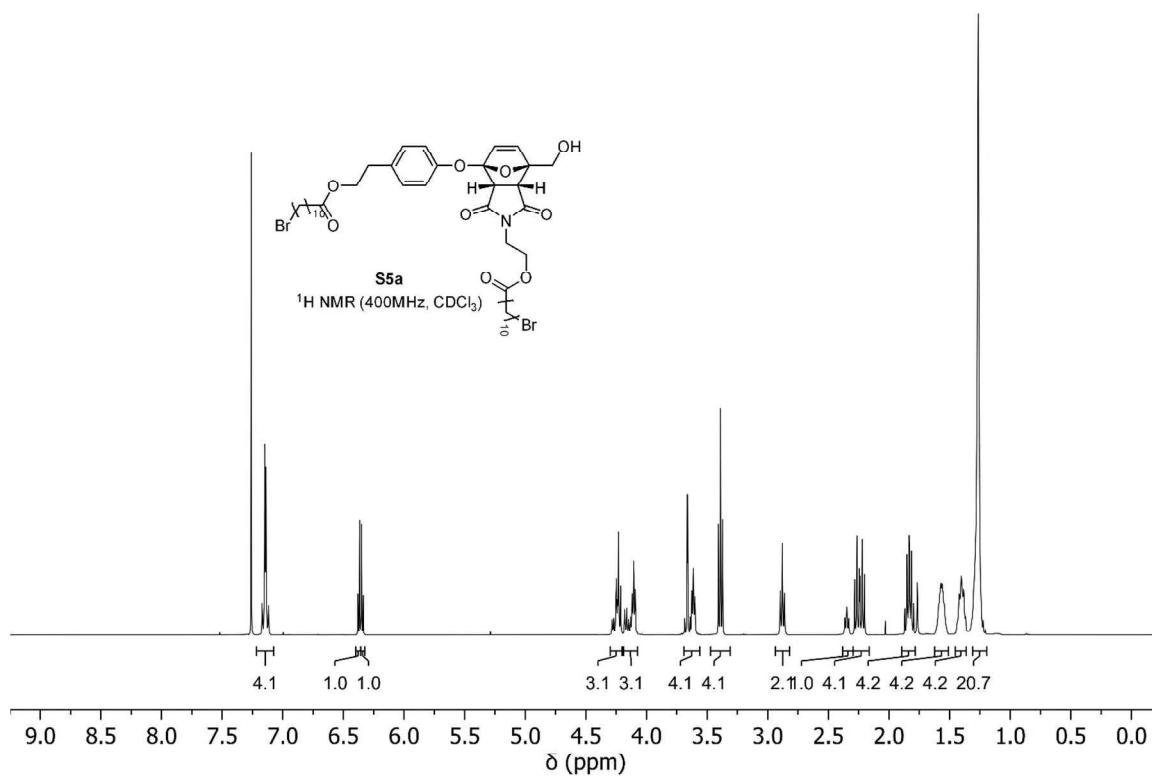
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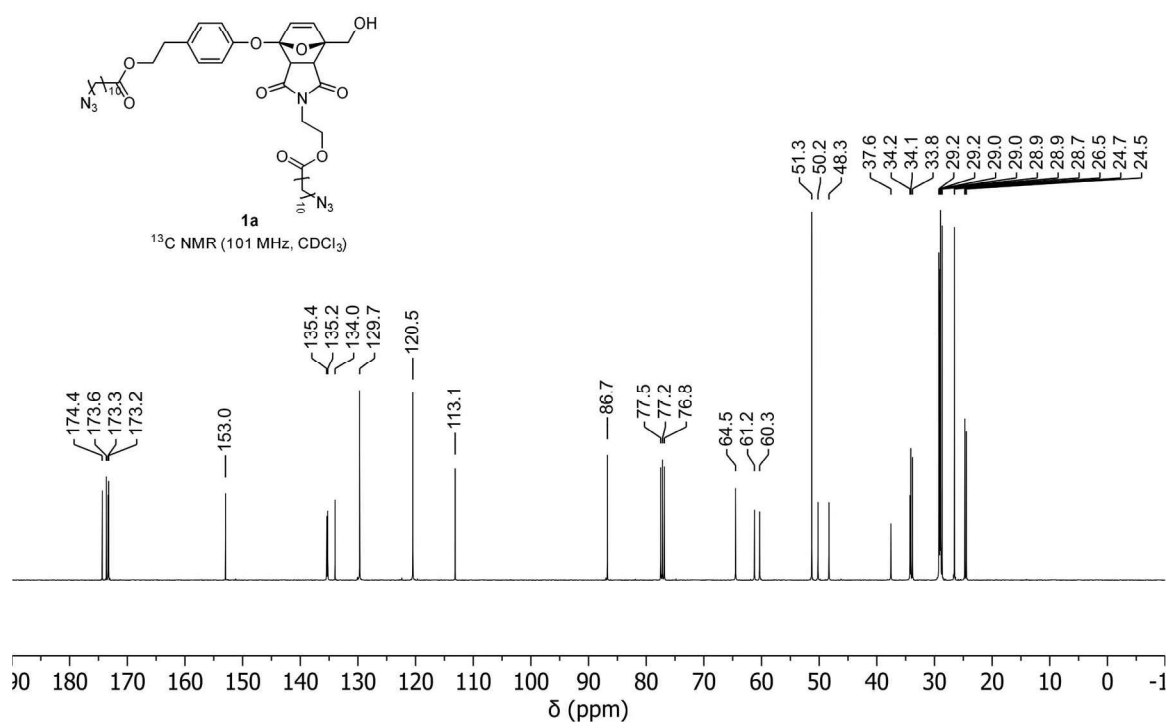
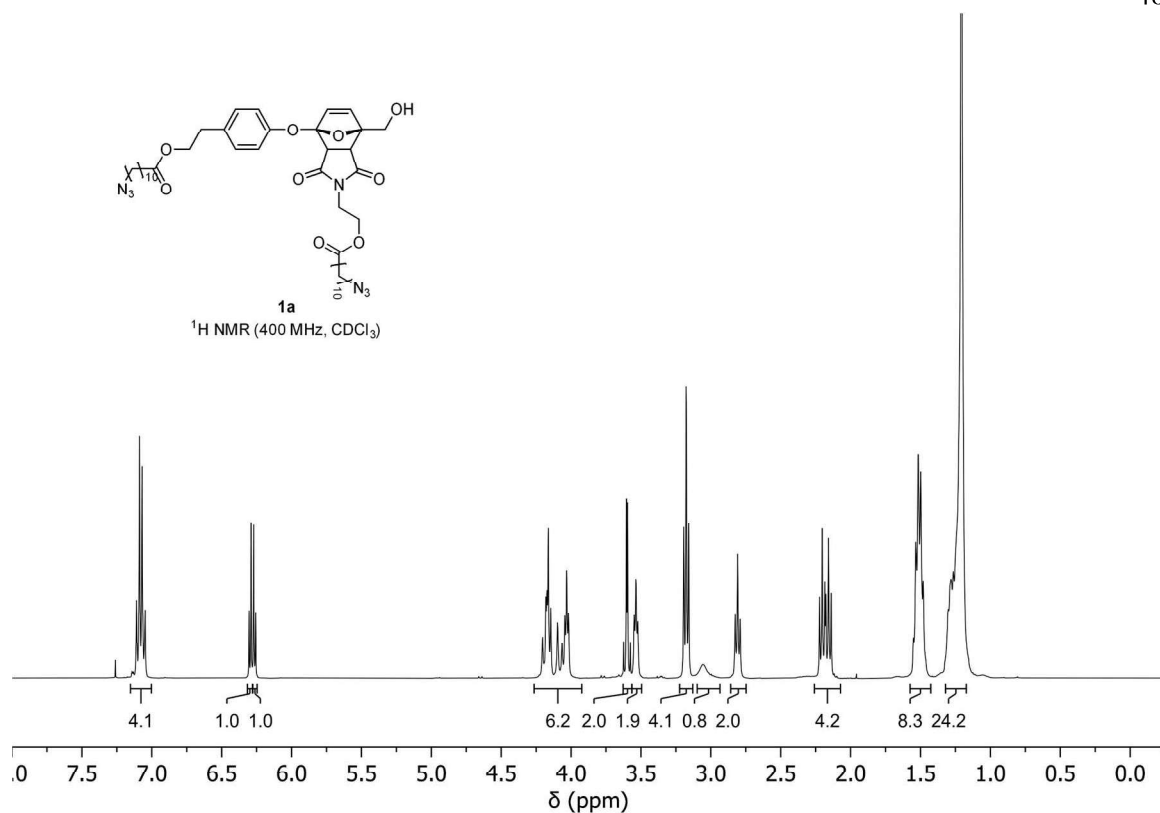
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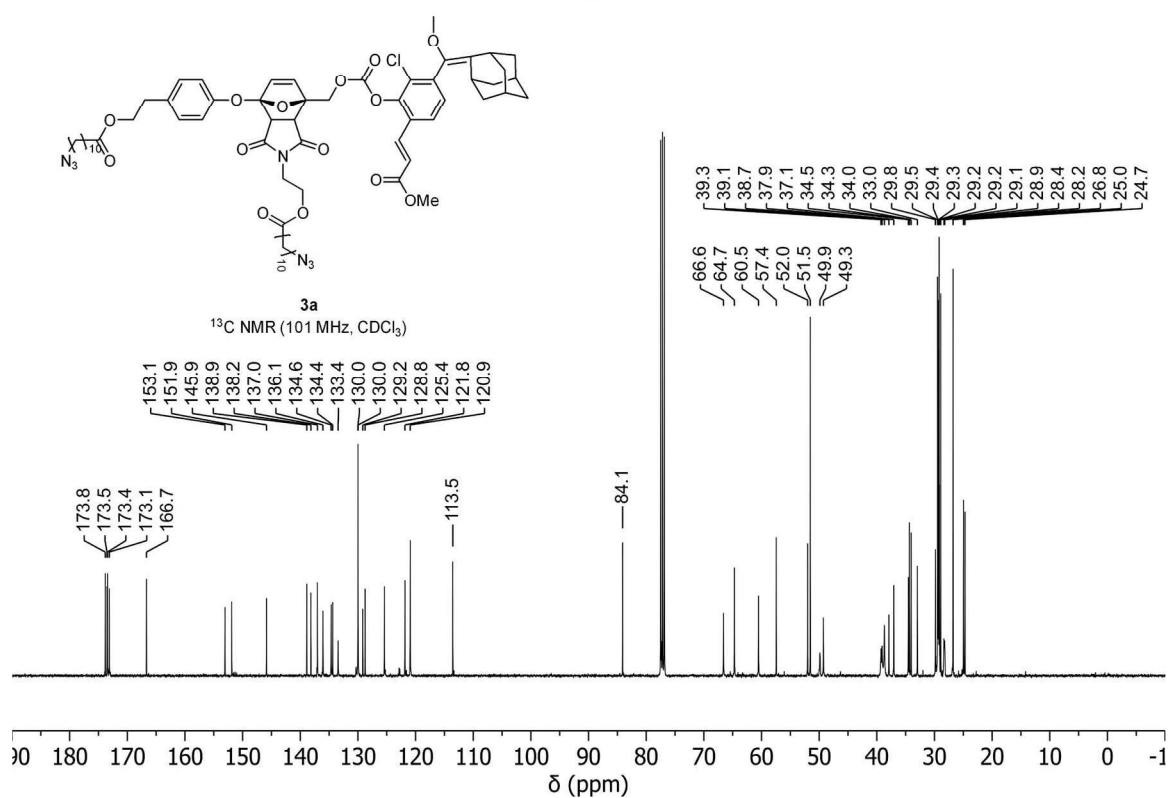
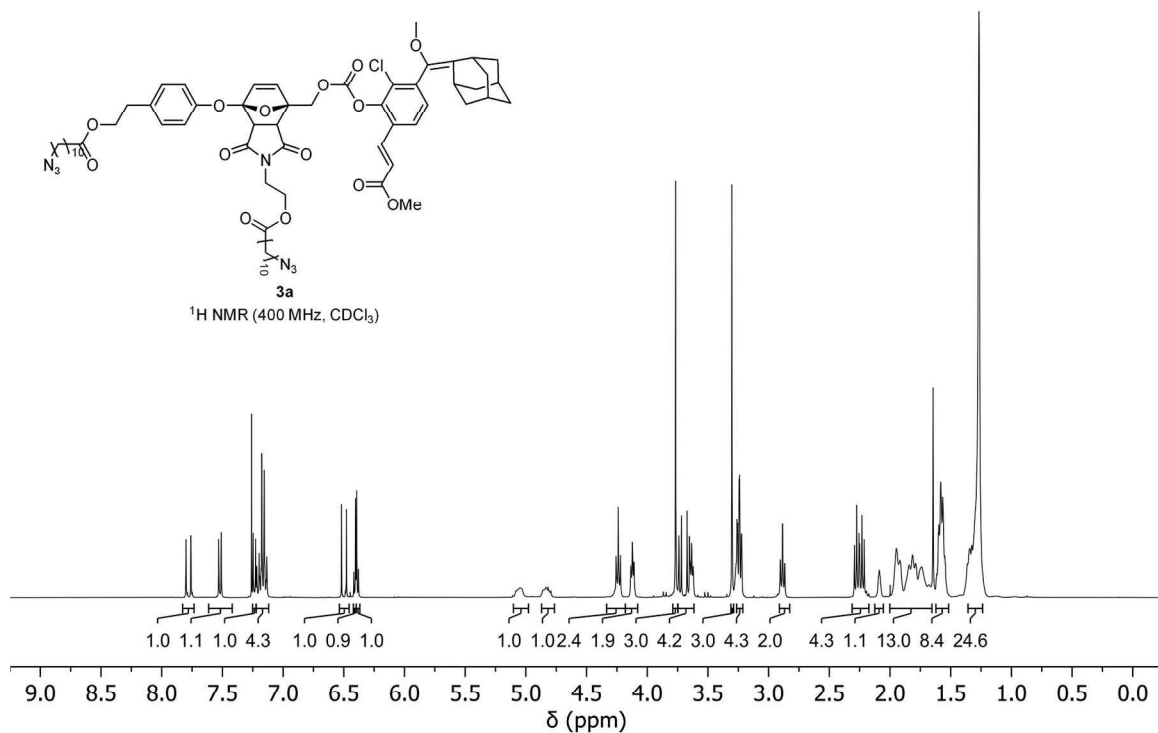
1.6 NMR Spectra

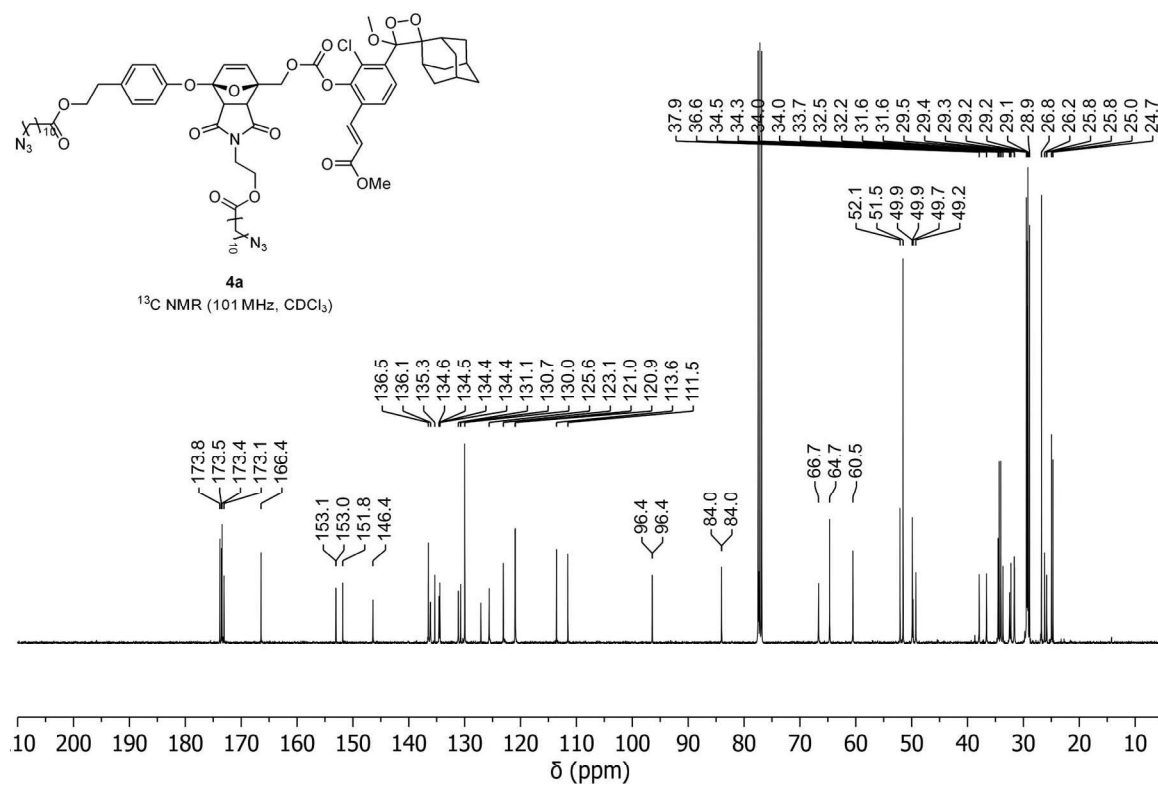
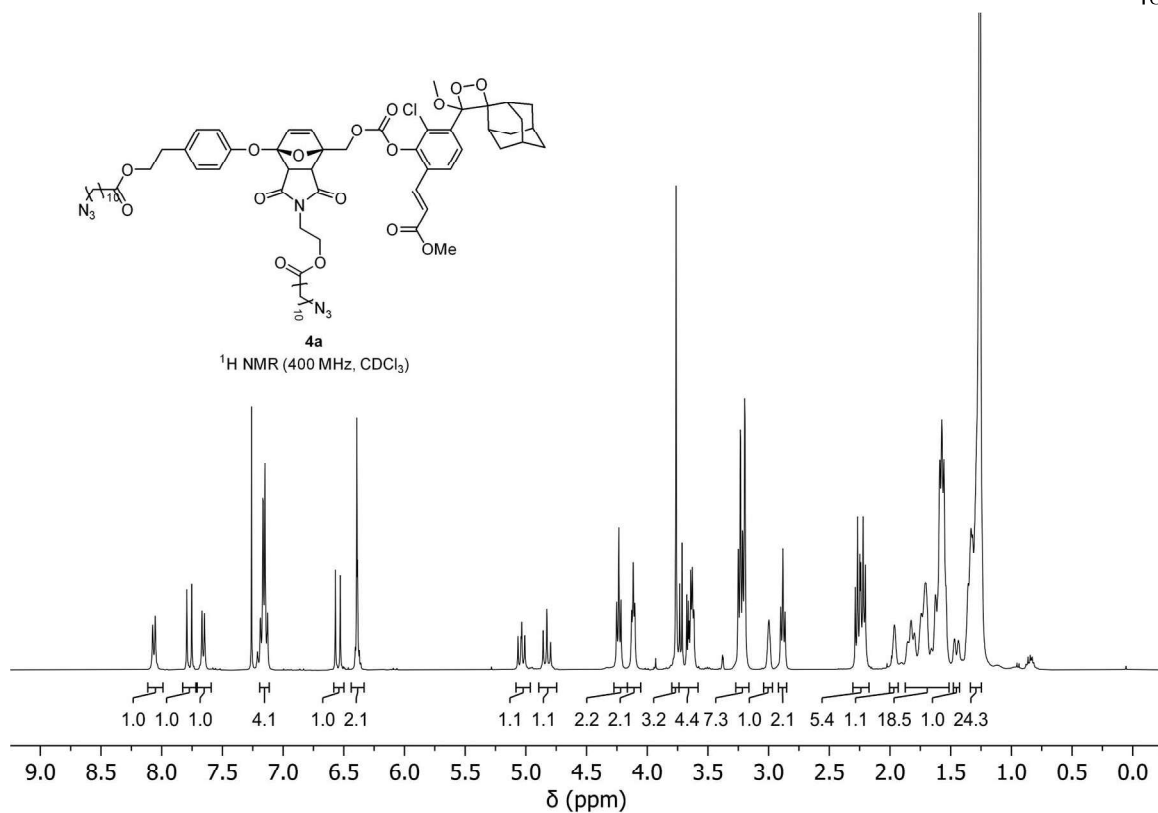


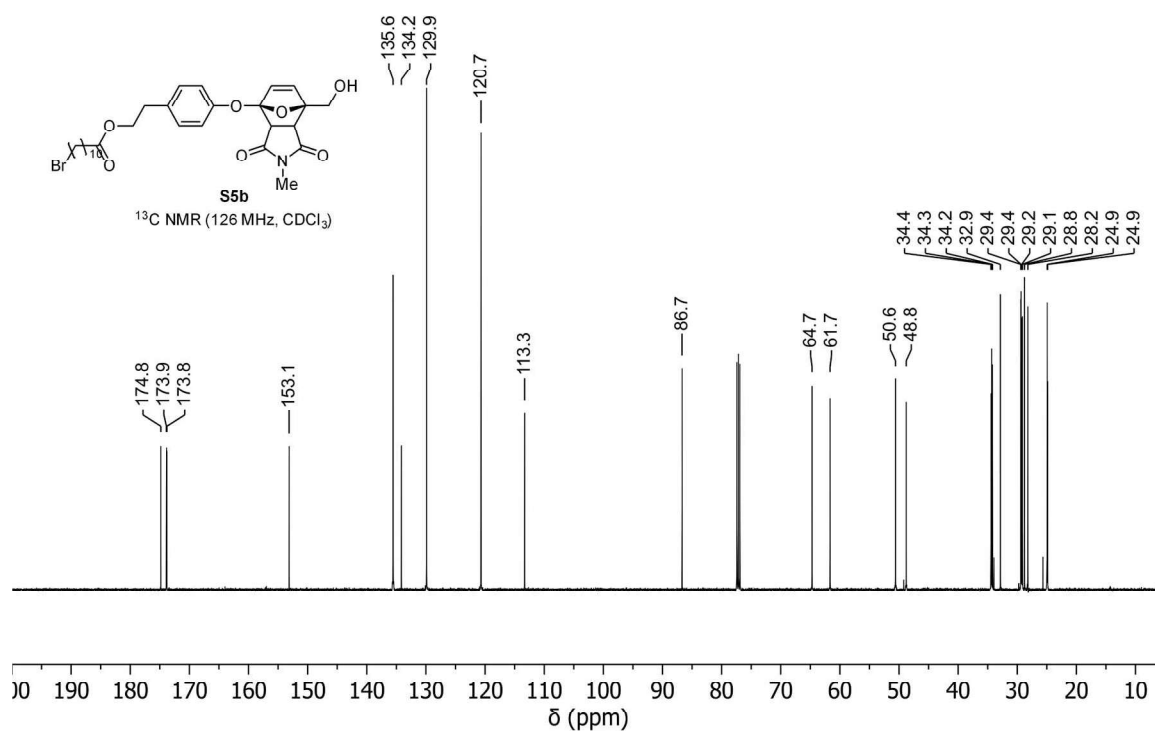
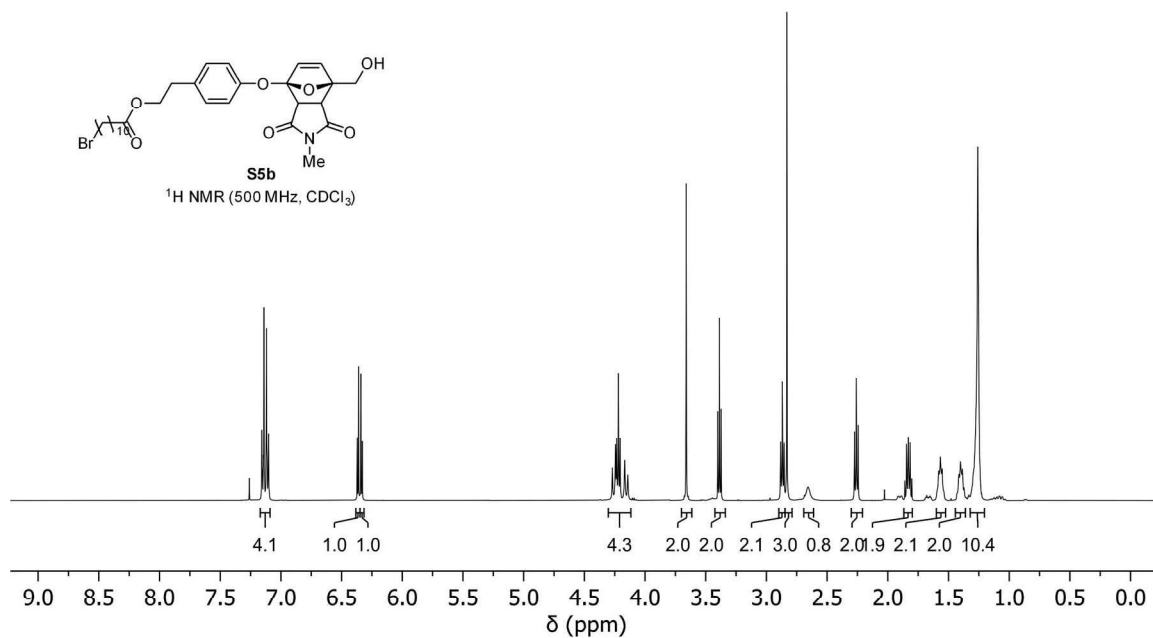


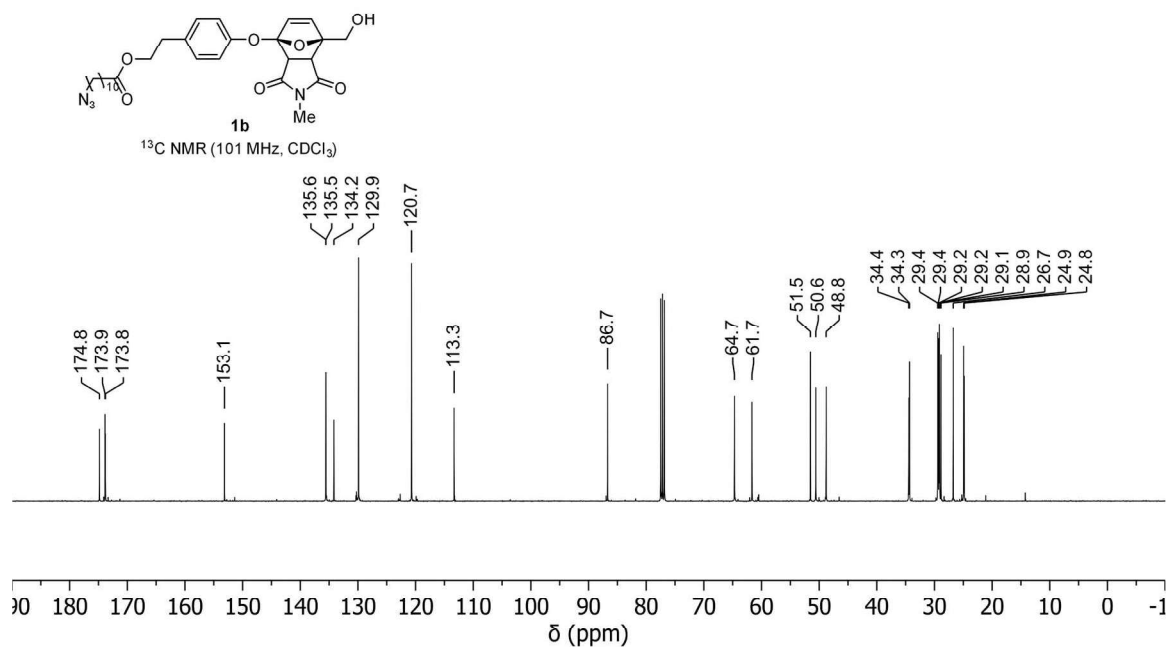
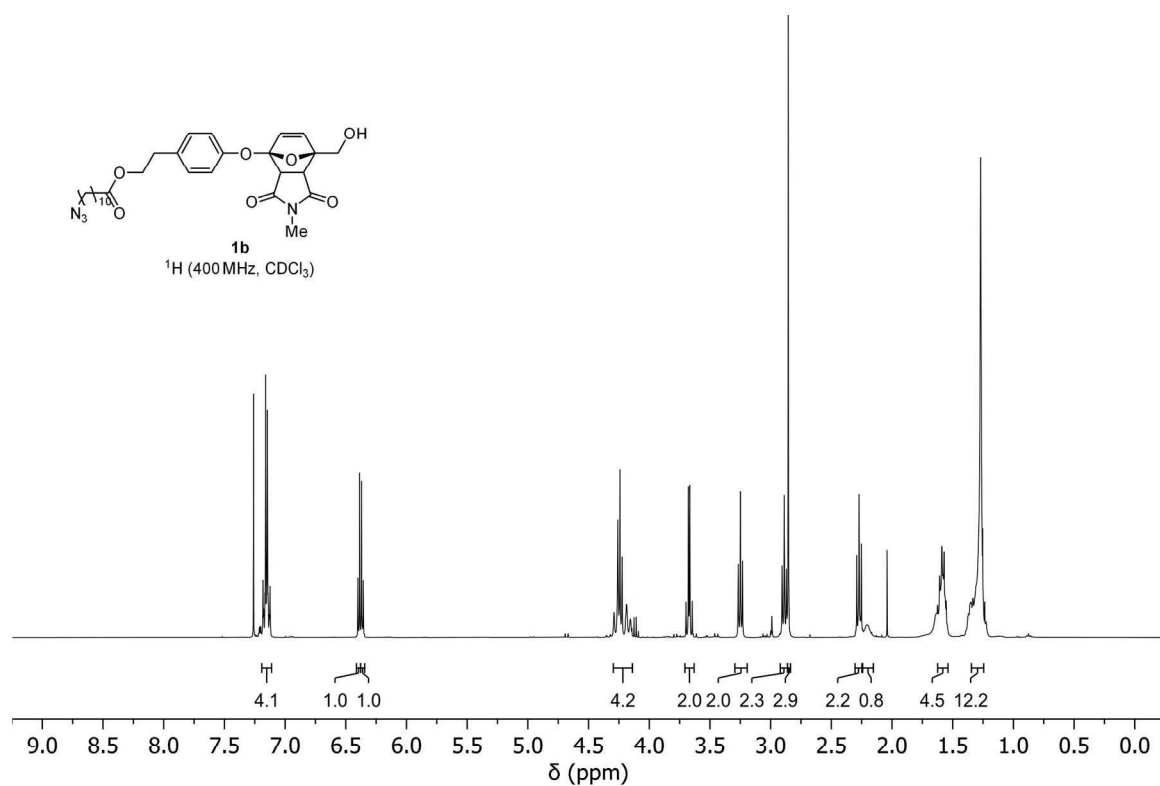


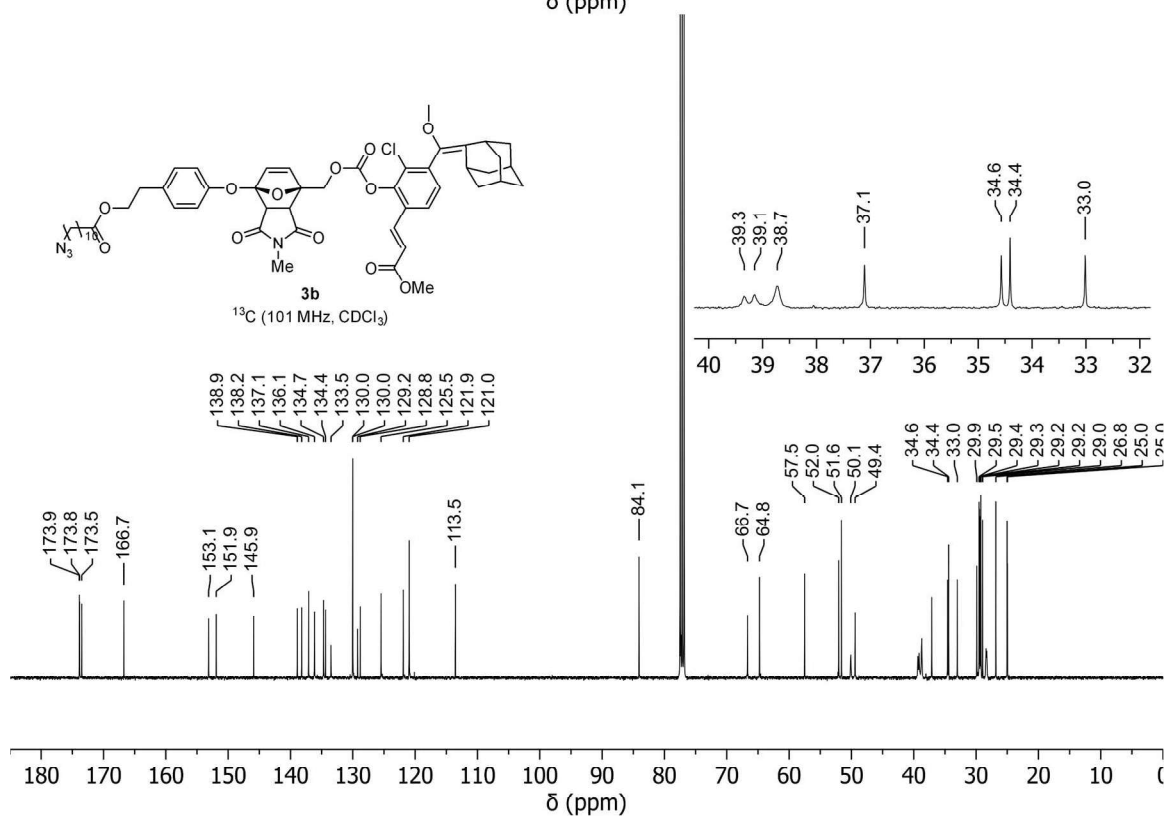
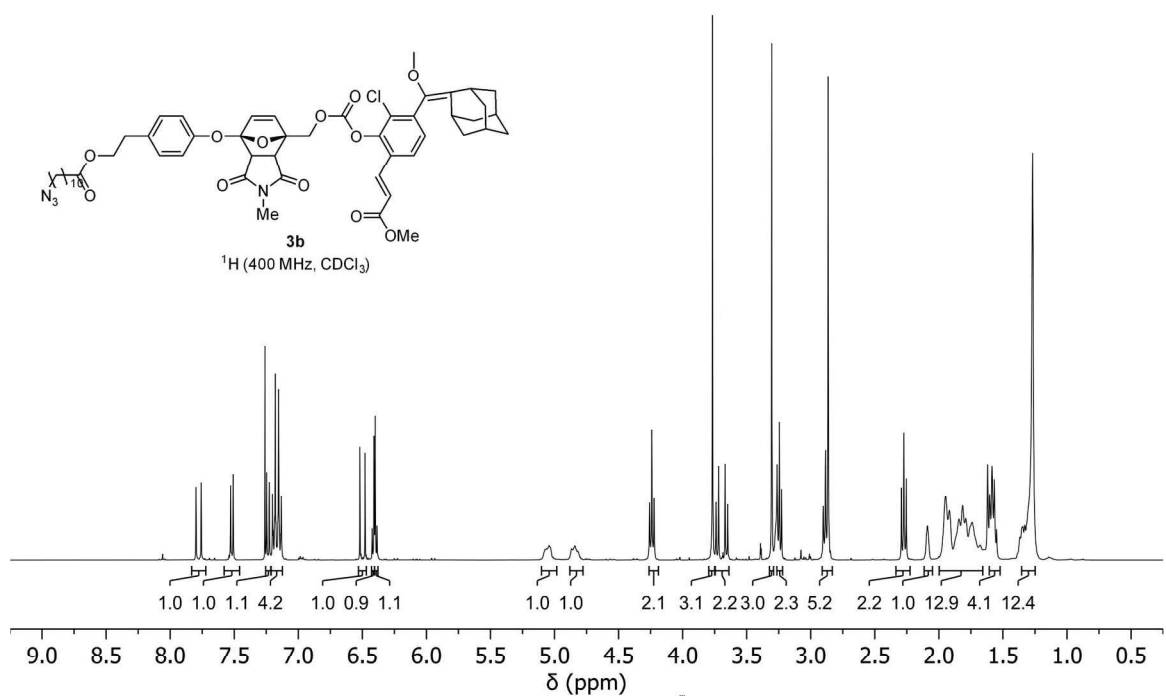


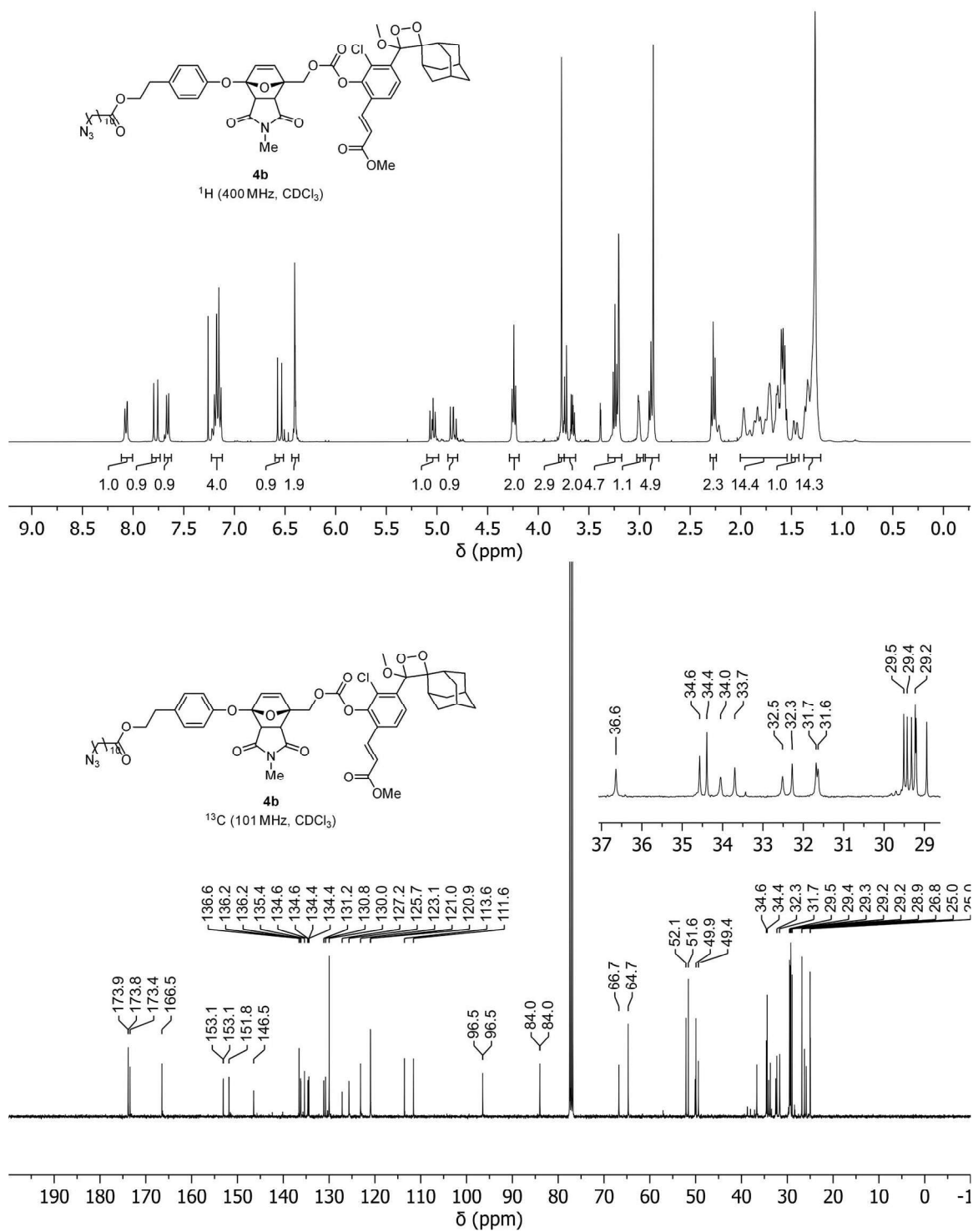






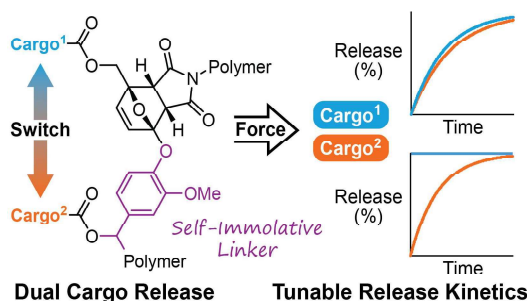






Chapter 2

INCORPORATION OF A SELF-IMMOLATIVE SPACER ENABLES MECHANICALLY TRIGGERED DUAL PAYLOAD RELEASE



This chapter has been reprinted from Tseng, Y.-L.; Zeng, T.; Robb, M. J. *Chem. Sci.* **2024**, *15*, 1472–1479.

Polymers that release functional small molecules in response to mechanical force are promising materials for a variety of applications including drug delivery, catalysis, and sensing. While many different mechanophores have been developed that enable the triggered release of a variety of small molecule payloads, most mechanophores are limited to one specific payload molecule. Here, we leverage the unique fragmentation of a 5-aryloxy-substituted 2-furylcarbinol derivative to design a novel mechanophore capable of the mechanically triggered release of two distinct cargo molecules. Critical to the mechanophore design is the incorporation of a self-immolative spacer to facilitate the release of a second payload. By varying the relative positions of each cargo molecule conjugated to the mechanophore, dual payload release occurs either concurrently or sequentially, demonstrating the ability to fine-tune the release profiles.

2.1 Introduction

Polymers that release functional small molecules in response to mechanical force are promising for a range of applications including catalysis, sensing, and drug

delivery.¹⁻³ In polymer mechanochemistry, small molecule release is accomplished by using mechanical force to drive productive chemical transformations in stress-responsive molecules termed mechanophores, which are incorporated into polymer chains to achieve force transduction.⁴⁻⁶ Mechanical force is an appealing external stimulus that is routinely applied using a variety of methods including solution-phase ultrasonication, or tension and compression in polymeric materials, which afford spatiotemporal control over mechanophore activation.⁷ In the context of molecular release, a recently reported method for activating mechanochemical reactions using biocompatible focused ultrasound under physiological conditions presents exciting opportunities for biomedical applications including drug delivery.⁸ These myriad techniques for external mechanochemical activation coupled with the excellent selectivity and control afforded by molecular structure design have attracted significant interest in the development of mechanophores for small molecule release.⁹ For example, *gem*-dichlorocyclopropane mechanophores have been designed to release HCl.^{10,11} A flex activation strategy has been devised for the liberation of a small molecule furan from an oxanorbornadiene mechanophore¹² and the release of catalytically active *N*-heterocyclic carbenes from carbodiimide adducts.¹³ Mechanophores have also been developed for the release of carbon monoxide,^{14,15} metal ions,¹⁶⁻¹⁸ and other specific small molecules.^{19,20}

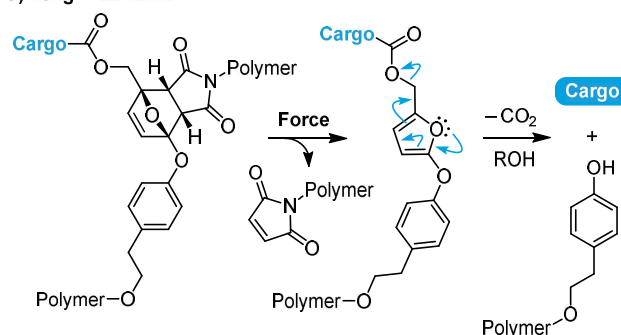
The development of mechanophores that release multiple different payloads, however, is limited. Herrmann and Göstl have developed a mechanophore platform based on a judiciously designed disulfide motif with cargo molecules attached via β -carbonate linkages.²¹ Mechanical cleavage of the disulfide bond initiates a 5-*exo-trig* cyclization with concomitant cargo release. The mechanophore can be assembled in a convergent fashion such that a distinct cargo molecule is appended via each carbonate linkage on either side of the mechanically active disulfide bond.²² Research has leveraged the bifunctional character of this platform to trigger the concurrent release of a drug molecule and a fluorescent reporter, enabling indirect

real-time tracking of drug distribution *in vitro* following *ex situ* mechanochemical activation. Combination chemotherapy is another possible application of dual payload release, in which the simultaneous delivery of two or more chemotherapeutic molecules elicits a synergistic therapeutic effect.²³ Despite the advantageous simplicity of the disulfide mechanophore platform, its susceptibility to activation via chemical or enzymatic reduction or thiol exchange reactions limits mechanochemical selectivity and payload scope has largely been limited to alcohols and phenols.²⁴

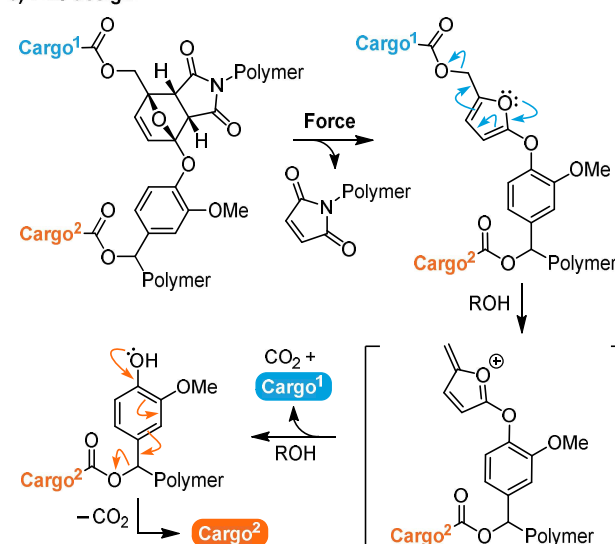
Over the past several years, our group has developed a versatile mechanophore platform enabling the mechanically triggered release of functionally diverse small molecules that relies on masked 2-furylcarbinol derivatives.^{25–28} Upon mechanochemical activation, the furan–maleimide mechanophore undergoes a formal retro-Diels–Alder reaction to reveal a thermally unstable 2-furylcarbinol derivative, which then spontaneously decomposes to release its covalently bound cargo. This general mechanophore design exhibits excellent selectivity and is capable of efficiently releasing a wide range of molecular payloads.²⁶ In 2021,

Scheme 2.1. a) Mechanically triggered small molecule release is accompanied by a secondary fragmentation pathway resulting in expulsion of a tyrosol-functionalized polymer. b) Incorporation of a self-immolative spacer enables mechanically triggered dual payload release via a multistep retro-Diels–Alder/fragmentation–decarboxylation cascade.

a) Zeng *et al.* 2021:



b) This design:



we described a mechanophore in which an aryloxy substituent is installed at the 5-position of the furan scaffold, serving both as an electron donating group to enhance molecular release and the position of polymer attachment (**Scheme 2.1a**).²⁷ The synthesis of this aryloxy-substituted mechanophore is efficient and scalable, while maintaining competency for the release of phenols and even more challenging arylamine payloads on reasonable timescales. Furthermore, we discovered a unique fragmentation pathway for the 5-aryloxy-substituted 2-furylcarbinol derivative that resulted not only in the release of the intended cargo molecule attached via a carbonate or carbamate linker, but also cleavage of the aryloxy group from the furan. We reasoned that attack of the intermediate furfuryl cation by methanol present in solution generated an ortho ester analogue that subsequently collapsed to expel the tyrosol derivative. We further hypothesized that this secondary fragmentation process could potentially be exploited to achieve dual cargo release.^{29,30}

Here, we leverage the unique fragmentation of this 5-aryloxy-substituted 2-furylcarbinol scaffold by incorporating a self-immolative spacer that enables the mechanically triggered release of two distinct cargo molecules from a single mechanophore (**Scheme 2.1b**). Upon mechanochemical activation, the furan–maleimide Diels–Alder adduct is expected to undergo a retro-[4+2] cycloaddition reaction to reveal an unstable 2-furylcarbinol derivative, which decomposes via a furfuryl cation intermediate according to the arrow pushing mechanism shown in **Scheme 1** to release the first covalently bound cargo molecule. Further fragmentation unmasks the judiciously designed phenolic spacer, which self-immolates in a 1,6-elimination process and another decarboxylation event to release the second cargo molecule. We characterize the reactivity of this novel mechanophore design through small molecule model experiments, and demonstrate mechanically triggered dual cargo release from polymers upon ultrasound-induced mechanochemical activation. Furthermore, we show that the release profiles for the two different payloads can be substantially modulated by varying their relative attachment position on the mechanophore.

We first investigated the effect of the self-immolative spacer on the decomposition of 5-aryloxy-substituted furan model compound **1** toward dual cargo release. The model compound incorporates an aryloxy substituent derived from vanillin that is capable of a 1,6-elimination mechanism upon expulsion from the furan.³¹ For comparison to our prior studies,^{26,27} we chose to study the release of fluorogenic aminocoumarin (ArNH₂ **2**) and hydroxycoumarin (ArOH **3**) as model payloads, which were connected via carbamate and carbonate linkages, respectively (**Figure 2.1**, see the section 2.4.2 for synthetic details). Preliminary experiments performed on a simpler 5-aryloxy-substituted furfuryl carbamate compound indicated that both fragmentation processes illustrated in **Scheme 2.1b** occur in close succession.²⁷ Furthermore, the release of phenols via 1,6-elimination of similar self-immolative linkers is expected to be faster than the release of an arylamine via decomposition of the furfuryl carbamate.^{27,32} Therefore we

The figure consists of two parts. The top part is a chemical reaction scheme showing the conversion of model compound **1** to products **2** and **3**. Compound **1** is a furan ring substituted with a 4-methoxyphenyl group and a 5-(2-bromo-2-methylpropanoate) group. The reaction conditions are CD₃CN/McOD (3:1) at room temperature, releasing two molecules of CO₂. The products are an arylamine (ArNH₂ **2**) and an aryl phenol (ArOH **3**).

The bottom part is a graph showing the time-dependent reaction profiles. The x-axis is Time (h) from 0 to 40. The left y-axis is Remaining Starting Material (%) from 0 to 100. The right y-axis is Cargo Release (%) from 0 to 80. The legend indicates: Furan **1** (black squares), ArNH₂ **2** (blue circles), and ArOH **3** (orange triangles). Furan **1** decreases from 100% to 0% over 40 hours. ArOH **3** increases from 0% to approximately 75% over 40 hours. ArNH₂ **2** increases from 0% to approximately 60% over 40 hours.

Time (h)	Furan 1 (%)	ArNH ₂ 2 (%)	ArOH 3 (%)
0	100	0	0
2	75	10	10
4	55	25	25
6	40	40	40
8	28	50	50
10	25	55	55
12	20	58	60
14	15	60	65
16	10	62	68
18	8	63	70
20	5	64	72
22	3	65	73
24	2	66	74
26	1	67	75
28	0	68	76
30	0	69	77
32	0	70	78
34	0	71	79
36	0	72	80
38	0	73	81
40	0	74	82

Figure 2.1. Reaction of model compound **1** in polar protic solvent at room temperature produces ArNH₂ **2** and ArOH **3** via a dual fragmentation–decarboxylation cascade. Time-dependent reaction profiles for the conversion of furan **1** and the generation of **2** and **3** characterized by ¹H NMR spectroscopy (3:1 MeCN-*d*₃/MeOD, [1]₀ = 29 mM). A rebound product is also formed under these conditions accounting for an additional ~32% of the total NH₂ **2** and OH **3** released.

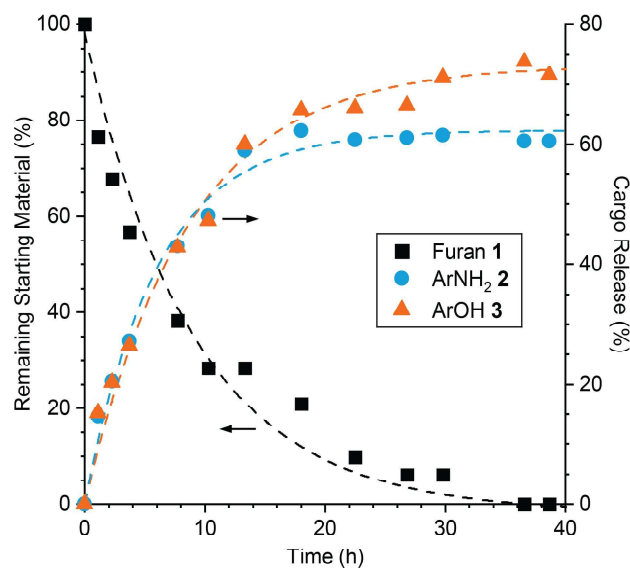
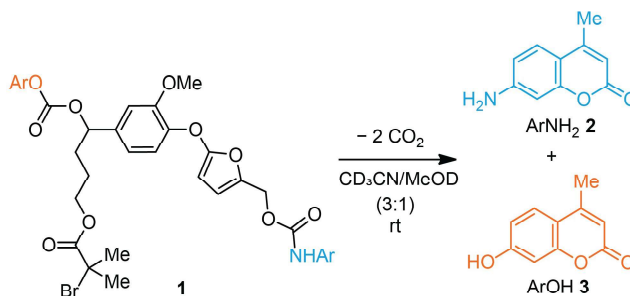


Figure 2.1. Reaction of model compound **1** in polar protic solvent at room temperature produces ArNH₂ **2** and ArOH **3** via a dual fragmentation–decarboxylation cascade. Time-dependent reaction profiles for the conversion of furan **1** and the generation of **2** and **3** characterized by ¹H NMR spectroscopy (3:1 MeCN-*d*₃/MeOD, [**1**]₀ = 29 mM). A rebound product is also formed under these conditions accounting for an additional ~32% release of ArNH₂ **2** as a result of the relatively high substrate concentration (see **Figure 2.2** for details).

anticipated that the release of ArNH₂ **2** and ArOH **3** would occur on similar timescales, with initial decomposition of the 2-furylcarbinol derivative and the release of ArNH₂ **2** being rate determining in this case.

Furfuryl carbamate **1** is transiently stable in aprotic solvents like chloroform and acetonitrile; however, the addition of methanol into an acetonitrile solution of the small molecule initiates fragmentation at room temperature resulting in the release of both payloads.²⁵ To characterize the kinetics of cargo release, model compound **1** was dissolved in CD₃CN followed by addition of CD₃OD to afford a 29 mM solution (3:1 v/v, respectively) and the reaction was monitored by ¹H NMR spectroscopy at room temperature (**Figure 2.1** and **Figure 2.2**). Model compound **1** was fully converted to products in 40 h, liberating ArNH₂ **2** in ~61% yield and ArOH **3** in ~72% yield. The release of ArNH₂ **2** and ArOH **3** follows first order kinetics with half-lives of $t_{1/2}$ = 4 and 6 h, respectively. Release kinetics are roughly 2-fold faster compared to our previously studied 5-aryloxy-substituted 2-furylcarbinol derivative, which we attribute to increased electron density on the aryloxy group due to the methoxy substituent. Another set of peaks was observed in the ¹H NMR spectrum that corresponds to the formation of a minor product resulting from rebound of the liberated nucleophilic ArNH₂ **2** onto the quinone methide derived from the self-immolative linker (**Figure 2.3**). As expected,²⁶ the formation of this rebound product is concentration dependent and is not observed in reactions with a lower substrate concentration of 20 μM (**Figure 2.4**), on the same order of the mechanophore concentration in typical mechanochemical activation experiments (*vide infra*). Taking the amount of rebound product into account, the total yield of ArNH₂ **2** is ~93% in the experiment described by **Figure 2.1**, illustrating the efficiency with which fragmentation of the furfuryl carbamate occurs. The slightly lower yield of ArOH **3** compared to ArNH₂ **2** suggests that incomplete fragmentation or other side reactions occur in the second step involving the self-immolative linker. Nevertheless, the relatively high release yields of both cargo molecules and the

similar release kinetics of ArNH₂ **2** and ArOH **3** highlight the potential of this platform for mechanically triggered dual payload release.

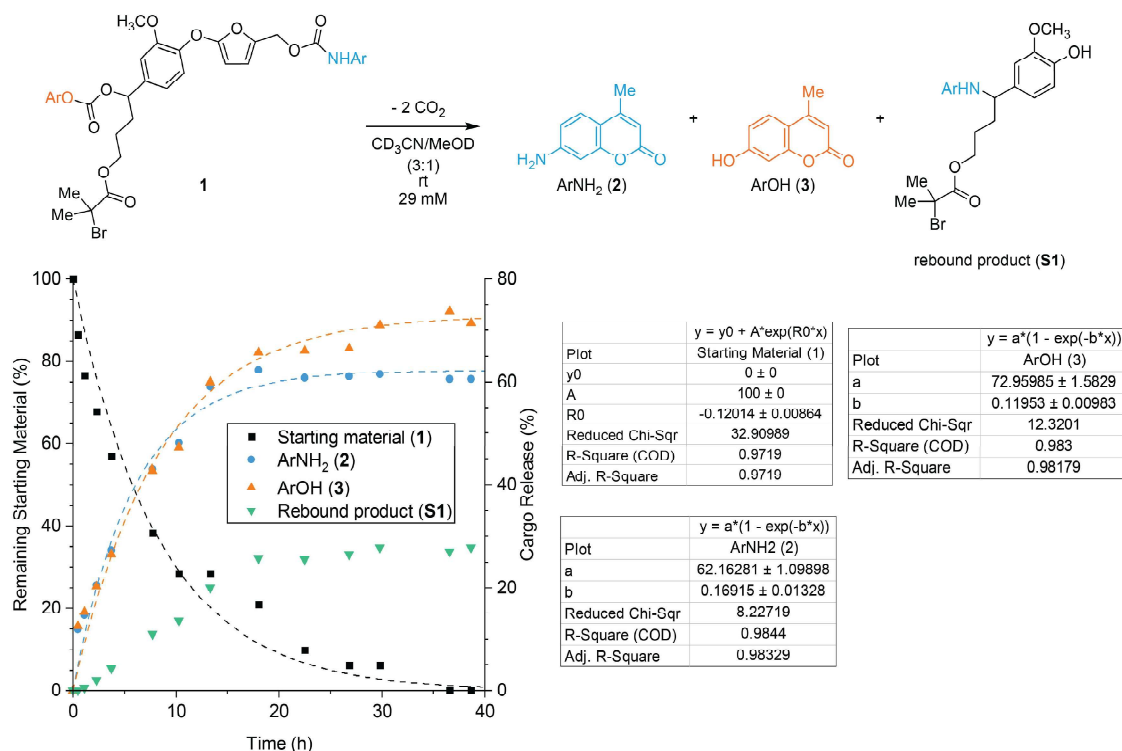


Figure 2.2. Decomposition of model compound **1** ($[1]_0 = 29$ mM) in 3:1 CD₃CN/MeOD at room temperature produces ArNH₂ **2**, ArOH **3**, and rebound product **S1**. Time-dependent reaction profiles for the conversion of **1** and the generation of **2**, **3** and **S1** were characterized by ¹H NMR spectroscopy using the signal for acetonitrile at 1.94 ppm as an internal standard (see **Figure 2.3**). The data were fit to expressions of simple first order kinetics (fitting parameters and statistics shown at right).

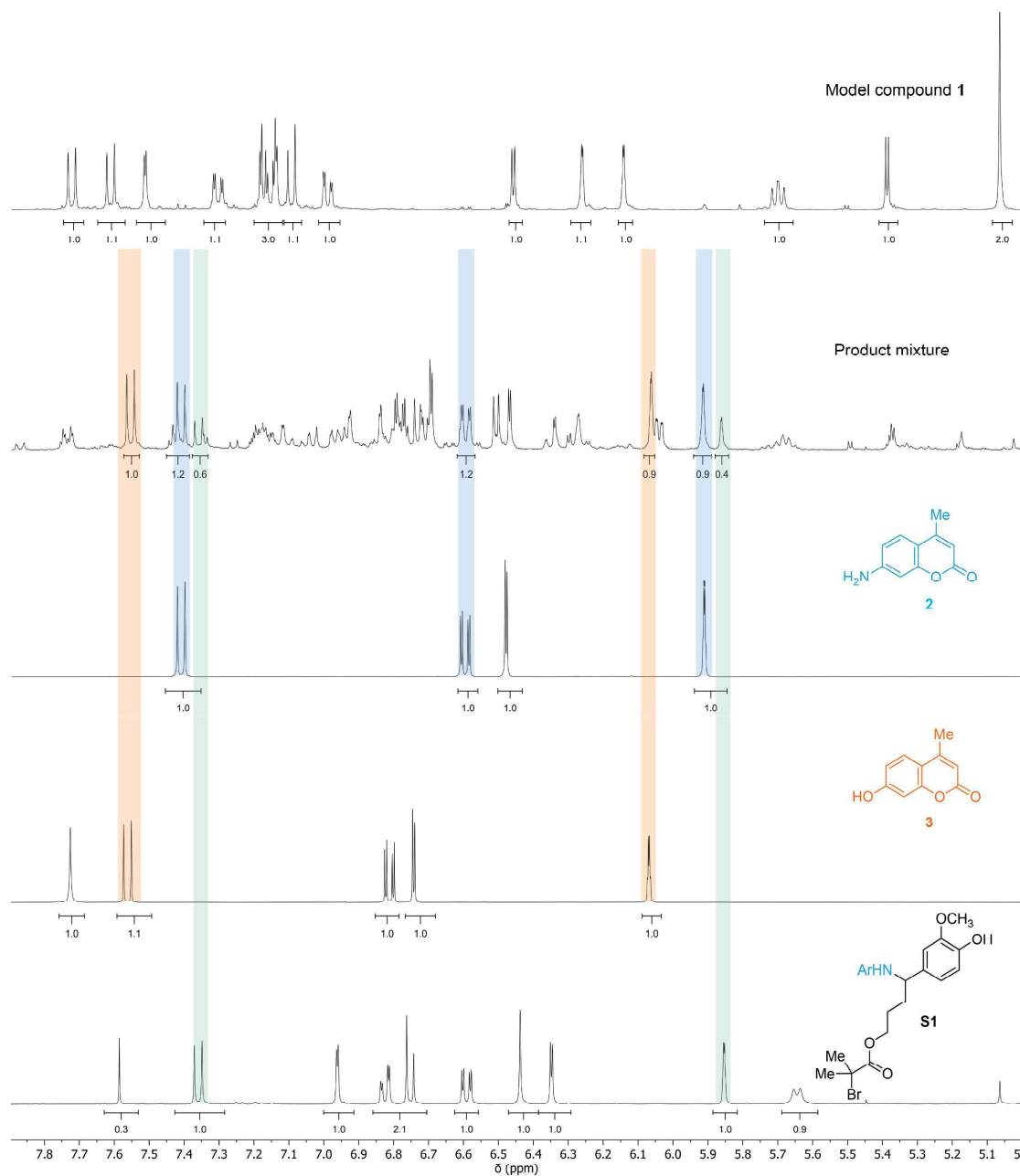


Figure 2.3. Characterization of the decomposition of model compound **1** by ^1H NMR spectroscopy (3:1 $\text{CD}_3\text{CN}/\text{MeOD}$; $[\mathbf{1}]_0 = 29 \text{ mM}$). Partial ^1H NMR spectra of starting material **1** in CD_3CN (top) compared to the product mixture after 48 h in 3:1 $\text{CD}_3\text{CN}/\text{MeOD}$ (second from top). ^1H NMR spectra of isolated compounds ArNH_2 **2**, ArOH **3**, and rebound product **S1** in CD_3CN are shown for comparison.

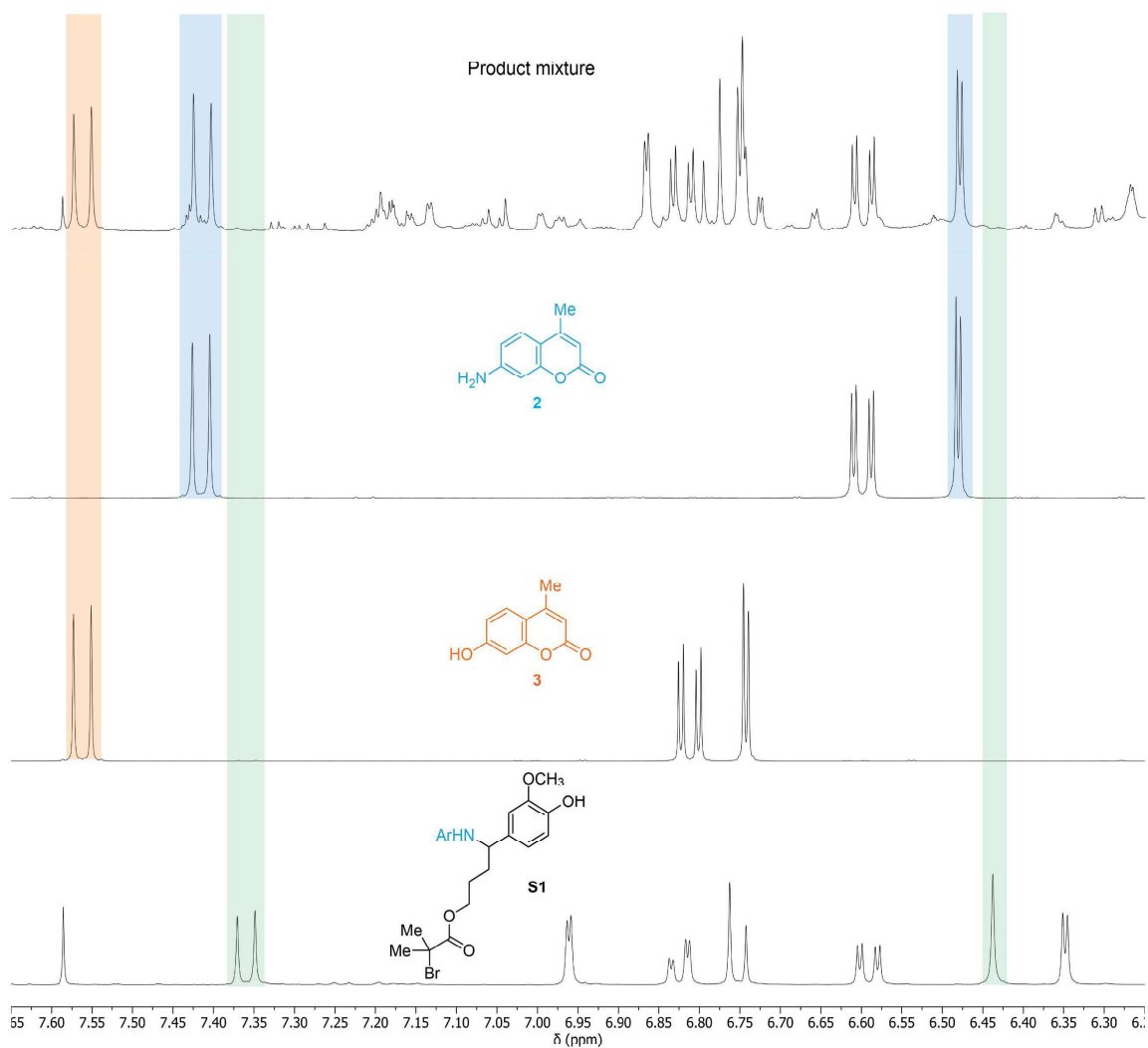


Figure 2.4. Characterization of the decomposition of model compound **1** and product distribution in 3:1 $\text{CD}_3\text{CN}/\text{MeOD}$ at lower substrate concentration ($[\mathbf{1}]_0 = 20 \mu\text{M}$). Partial ^1H NMR spectra of the product mixture after 48 h compared to isolated compounds in CD_3CN demonstrating the conversion of **1** to ArNH_2 **2** and ArOH **3**. The rebound product **S1** is not observed at this lower substrate concentration.

2.2.2 Mechanophore synthesis and incorporation into poly(methyl acrylate)

Encouraged by the reactivity of the small molecule model compound incorporating a self-immolative linker, we next set out to investigate mechanically triggered dual cargo release from a masked 2-furylcarbinol mechanophore incorporated into a polymer. Density functional calculations using the constrained geometries simulate external force (CoGEF) technique^{33,34} predict the desired retro-[4+2] cycloaddition reaction of the target furan–maleimide adduct upon mechanical extension leading to the formation of the intended 5-aryloxy-substituted 2-furylcarbinol derivative (**Figure 2.5**). Therefore, the synthesis commenced from compound **4**, which was prepared in three steps from vanillin in 50% overall yield (**Scheme 2.2**, see the *section 2.4.2* for details). Next, deprotection and nucleophilic

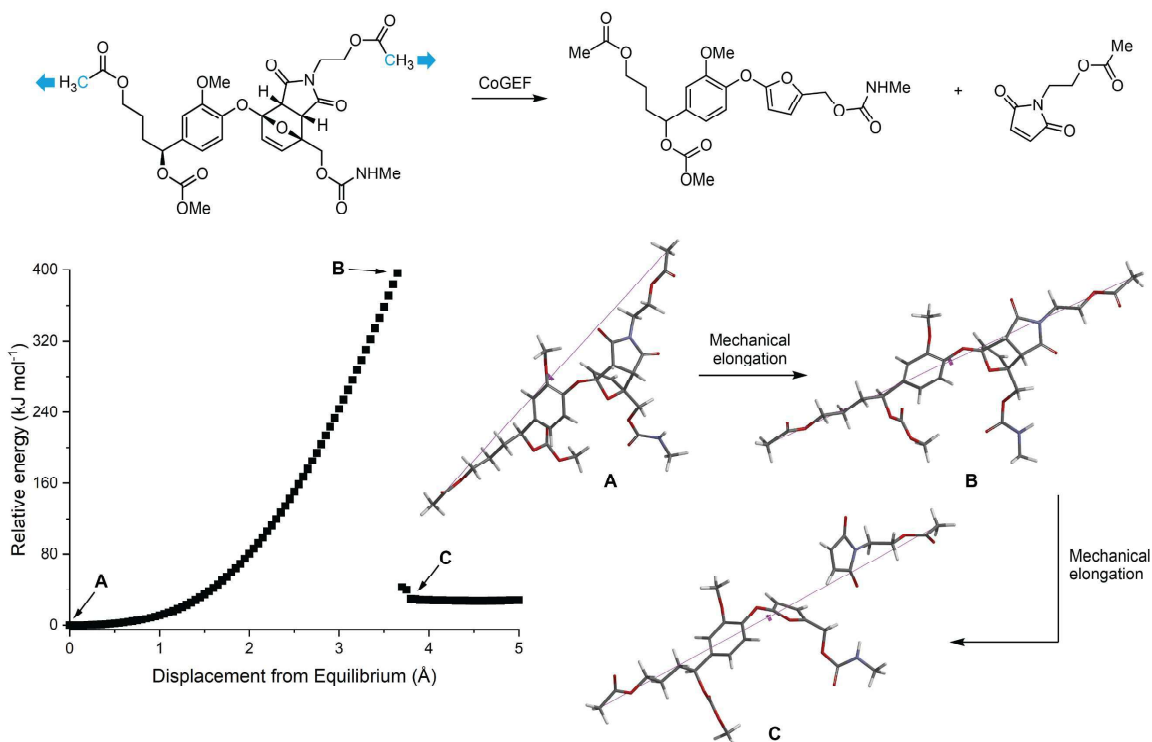
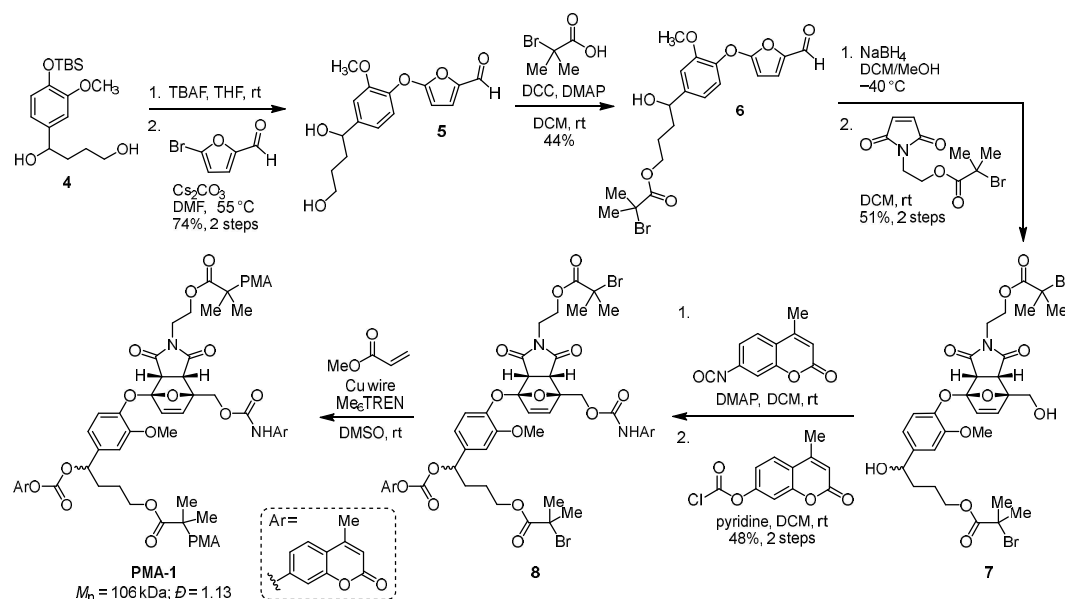


Figure 2.5. Results of density functional theory calculations performed on a truncated model of the furan–maleimide Diels–Alder adduct in polymer **PMA-1** using the constrained geometries simulate external force (CoGEF) method at the B3LYP/6-31G* level of theory. Pulling points were defined as the carbon atoms in the terminal methyl groups (colored blue) with a step size of 0.05 Å. A formal retro-Diels–Alder reaction is predicted to occur upon mechanical elongation at a maximum rupture force (F_{max}) of 4.2 nN to generate the expected 5-aryloxy-substituted furfuryl carbamate and maleimide products. The corresponding computed structures at various points of elongation indicated in the CoGEF plot are shown at right.

substitution with 5-bromofurfural afforded furaldehyde **5** in 74% yield over the two steps. Esterification of the primary alcohol proceeded via carbodiimide coupling with α -bromoisobutyric acid to produce **6** in 44% yield. Reduction of the aldehyde with sodium borohydride at $-40\text{ }^{\circ}\text{C}$ followed by a [4+2] cycloaddition reaction with a pre-functionalized maleimide dienophile at room temperature produced *endo*-Diels–Alder adduct **7** in 51% yield after isolation via silica gel chromatography. Finally, the two cargo molecules were installed in a two-step sequence, first by selective functionalization of the primary alcohol with a coumarin isocyanate followed by installation of the second cargo with a coumarin chloroformate to generate bis-initiator **8** in 48% yield, which was then employed in the controlled radical polymerization of methyl acrylate with Cu wire/ Me_6TREN in DMSO^{35} to afford **PMA-1** ($M_n = 106\text{ kDa}$; $\mathcal{D} = 1.13$) containing a chain-centered mechanophore. A similar synthetic procedure using *N*-methylmaleimide was used to prepare chain-end functional polymer **PMA-control** ($M_n = 98\text{ kDa}$; $\mathcal{D} = 1.15$, see the *section 2.4.2* for details). The electron rich aryloxy substituent imparts substantial thermal stability to Diels–Alder adduct **7**.²⁷ Heating a solution of **7** in toluene- d_8 at $70\text{ }^{\circ}\text{C}$ for 5 and 24 h results in only ~ 5 and 25% retro-Diels–Alder



Scheme 2.2. Synthesis of poly(methyl acrylate) (PMA) polymer **PMA-1** containing a chain-centered mechanophore loaded with two distinct molecular payloads.

reaction, respectively, while no retro-Diels–Alder reaction was observed at room temperature over several weeks (**Figures 2.6 and 2.7**).

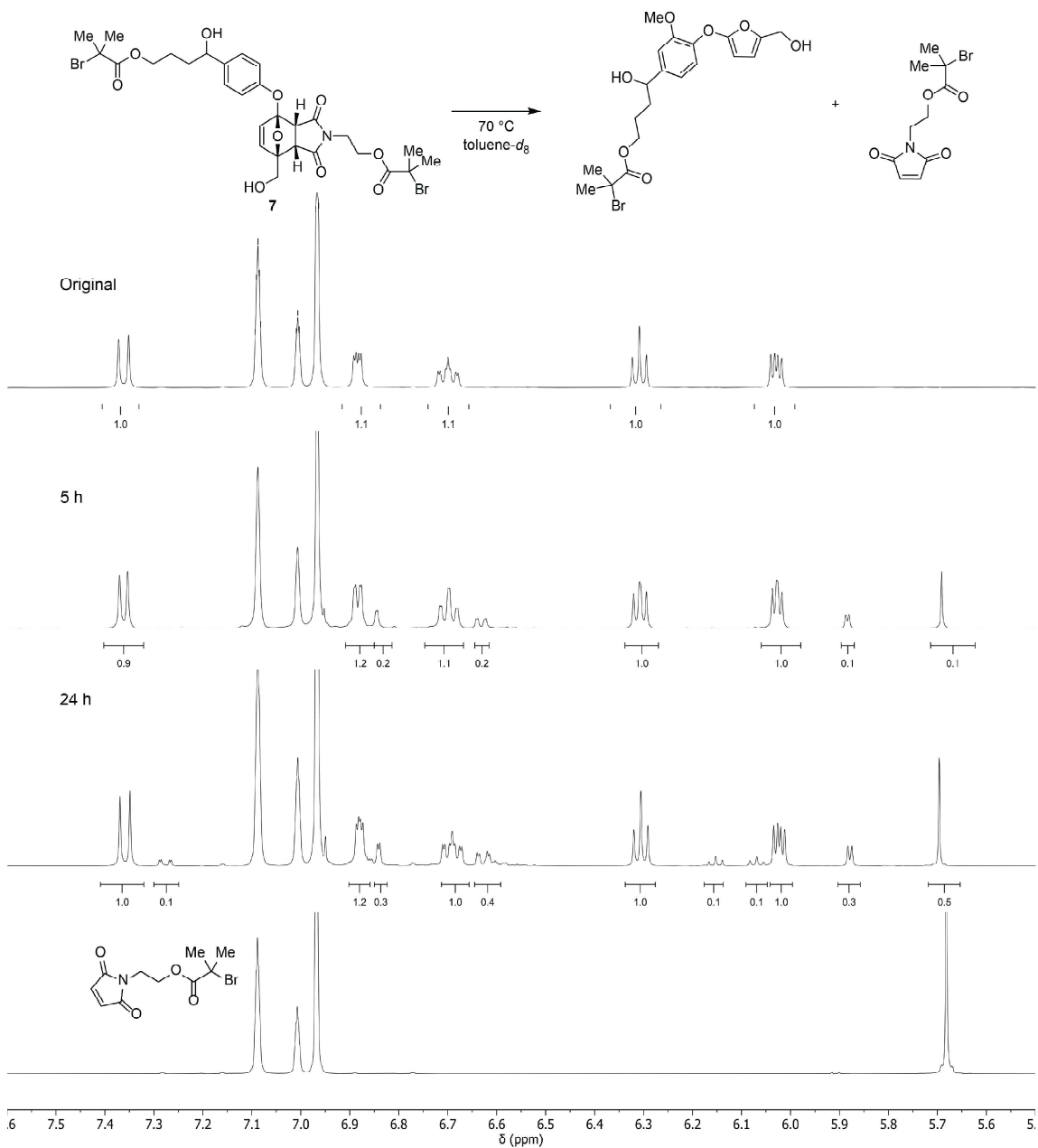


Figure 2.6. Thermal stability study of Diels–Alder adduct **7** in $\text{toluene-}d_8$ (39 mM) upon heating at 70°C . Partial ^1H NMR spectra of **7** after heating at 70°C for the indicated amount of time. Compound **7** undergoes a retro-Diels–Alder reaction upon heating at 70°C with a conversion of $\sim 5\%$ after 5 h and $\sim 25\%$ after 24 h. The partial ^1H NMR spectrum of the maleimide fragment in $\text{toluene-}d_8$ is shown at bottom for reference.

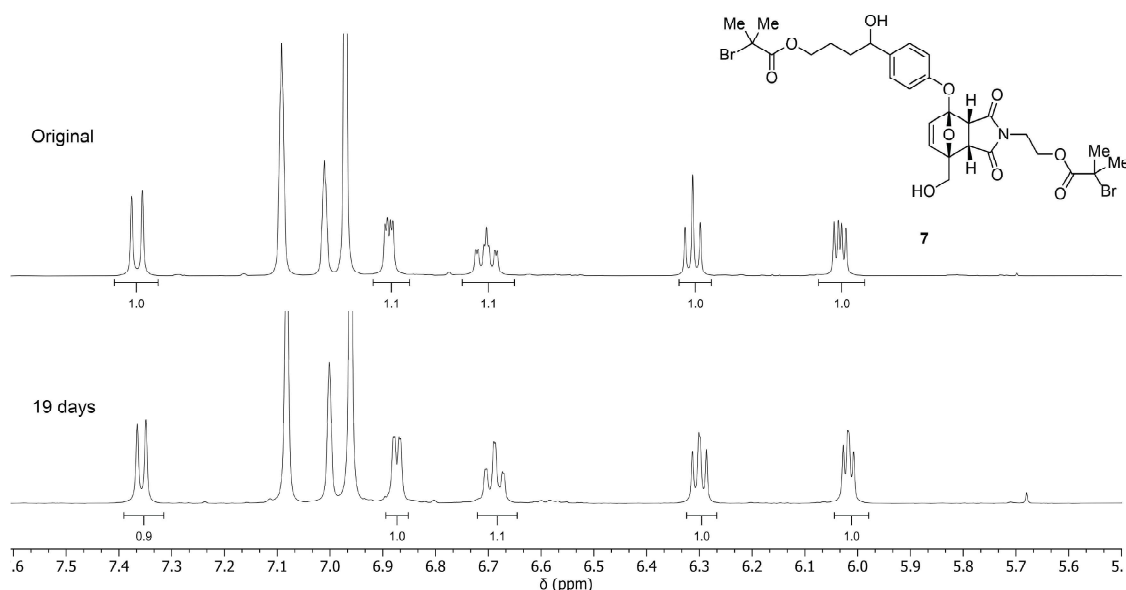


Figure 2.7. Thermal stability study of Diels–Alder adduct **7** in toluene- d_8 (39 mM) at room temperature. Partial ^1H NMR spectra of **7** after being kept at room temperature for 19 days, demonstrating minimal retro-Diels–Alder reaction over this time period. The maleimide signal at 5.68 ppm integrates to 0.04H, indicating a conversion of ~2%.

2.2.3 Characterization of mechanochemical reactivity of **PMA-1**

The mechanochemical reactivity of the mechanophore in **PMA-1** was evaluated using pulsed ultrasonication (1 s on/ 1 s off, 12–13 °C, 20 kHz, 30% amplitude, 16.4 W cm^{-2}) and cargo release was characterized by high-performance liquid chromatography (HPLC). To compare the kinetics of payload release from **PMA-1** with that from isolated small molecule model compound **1**, a 2 mg/mL solution of **PMA-1** in 3:1 MeCN/MeOH was subjected to ultrasonication for 60 min (sonication “on” time), and then cargo release was quantified at regular time intervals from aliquots of the solution incubated at room temperature (**Figure 2.8**). We note that fluorescence spectroscopy can also be used to selectively quantify the formation of ArNH_2 **2** (but not ArOH **3**), which provided similar results and additional confidence in the HPLC measurements (see the *section 2.4.4* and *2.4.5* for details). The release of ArNH_2 **2** from the mechanically unmasked 2-furylcabinol intermediate generated from **PMA-1** occurs with a half-life of $t_{1/2} = 11$ h, which is slightly shorter than our

previously reported substrate with a non-self-immolative tyrosol linker ($t_{1/2} = 15$ h),²⁷ consistent with the more electron rich aryloxy group derived from vanillin. Notably, the release of the second payload, ArOH **3**, occurs with a similar half-life of $t_{1/2} = 13$ h under these conditions. Normalized cargo release is plotted in **Figure 2.8** to highlight the relative release rates of each payload. These results are qualitatively consistent with the release kinetics measured for small molecule model compound **1** and demonstrate the rapid fragmentation–elimination–decarboxylation cascade of the self-immolative linker that follows initial furfuryl carbamate decomposition leading to the release of the first cargo, ArNH₂ **2** (see **Figure 2.1**).

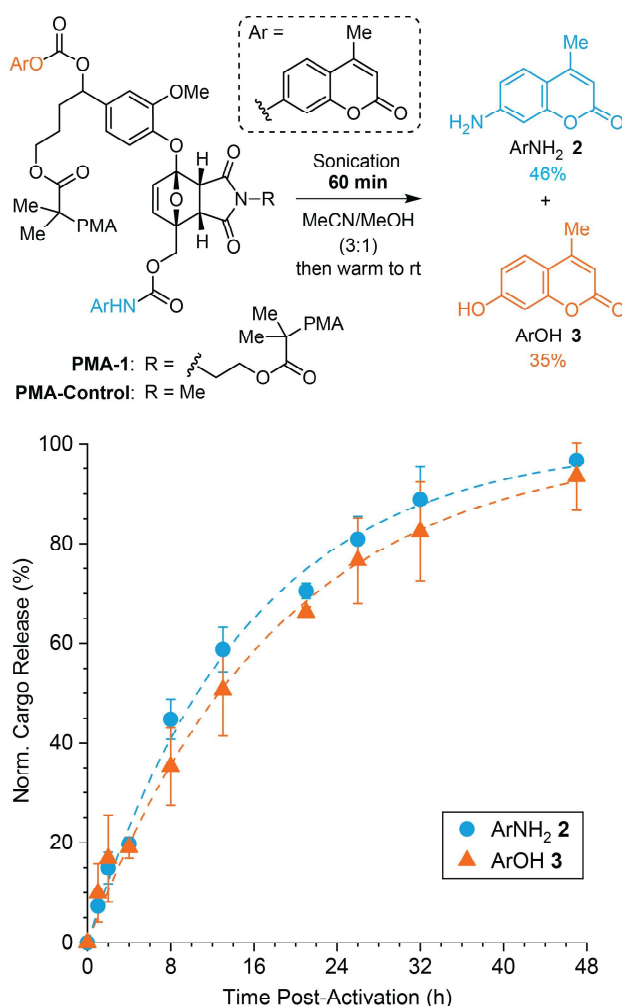


Figure 2.8. Mechanically triggered release of ArNH₂ **2** and ArOH **3** upon ultrasound-induced mechanochemical activation of **PMA-1** (2 mg/mL in 3:1 MeCN/MeOH). Cargo release was measured at room temperature beginning immediately after sonication of **PMA-1** for 60 min as characterized by quantitative HPLC. The dashed lines are fits to simple first order kinetics. The initial value was subtracted from each measurement and the data were normalized to the plateau value of the fitted curve. Error bars represent standard deviation from three replicate experiments.

Increasing the duration of ultrasonication leads to a predictable increase in payload release from **PMA-1** with increasing mechanophore conversion, achieving average yields of 64% for ArNH₂ **2** and 55% for ArOH **3** after 5 h of sonication (**Figure 2.9**). Aliquots were periodically removed from the sonicated solution of **PMA-1** over the course of the experiment and incubated at room temperature for 48

h to allow the complete fragmentation of the mechanically generated 2-furylcarbinol intermediate before quantifying cargo release by HPLC. Consistent with the experimental results for the decomposition of isolated small molecule **1**, the amount of released ArNH₂ **2** is higher than ArOH **3** at every point in the reaction, again suggesting that the second fragmentation process involving the self-immolative linker is slightly less efficient

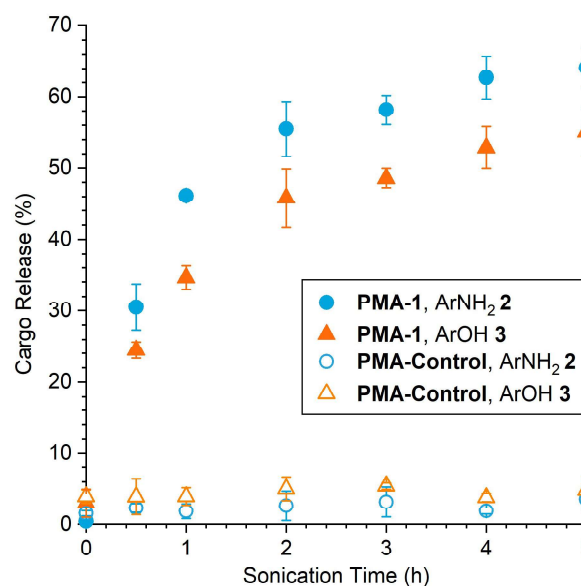


Figure 2.9. Mechanically triggered release of ArNH₂ **2** and ArOH **3** from **PMA-1** and **PMA-Control** subjected to varying durations of sonication. Cargo release was quantified by HPLC after incubation at room temperature for 48 h following sonication. Error bars represent standard deviation from three replicate experiments.

than initial fragmentation of the mechanically unmasked 2-furylcarbinol derivative. Gel permeation chromatography was also used to determine changes in molar mass resulting from polymer chain scission. Rather than the typical bimodal evolution of molar mass that is characteristic of mid-chain scission,³⁶ we observed a continuous shift in the average molar mass upon ultrasonication of **PMA-1** (**Figure 2.10**). This trend is similar to results with our original aryloxy-substituted furfuryl carbamate mechanophore,²⁷ which we attribute to the relatively broad molar mass distribution (i.e., $D > 1.1$) that increases the competition between mechanophore activation and nonspecific chain scission and ultimately results in lower mechanophore activation efficiency.^{37,38} Importantly, negligible cargo release was observed upon ultrasonication of **PMA-Control** that incorporates the cargo-loaded mechanophore at the polymer chain end, which is not subjected to mechanical force, confirming the mechanical origin of payload release from **PMA-1** (**Figure 2.9**).

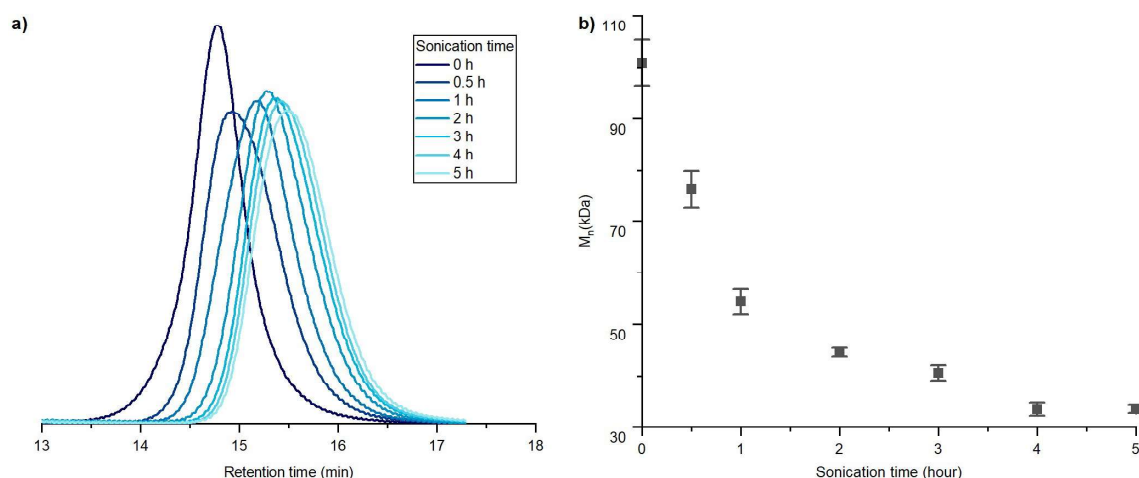


Figure 2.10. Characterization of **PMA-1** by gel permeation chromatography (GPC) during ultrasonication for 5h. a) GPC traces as a function of sonication time monitored with a refractive index (RI) detector. b) Number average molar mass (M_n) as a function of sonication time calculated using light scattering showing a steady decrease in M_n as a result of mechanochemical chain scission. Error bars represent standard deviation from three replicate trials.

2.2.4 Characterization of mechanochemical reactivity of **PMA-2**

To further probe the dual release mechanism and also demonstrate the ability to systematically modulate the kinetics of payload release, we prepared polymer **PMA-2** ($M_n = 120$ kDa, $D = 1.35$) incorporating a chain centered mechanophore in which the two cargo molecules, ArNH₂ **2** and ArOH **3**, are conjugated at opposite positions compared to **PMA-1** (**Figure 2.11**). Based on the proposed fragmentation mechanism and the pronounced difference in reactivity between the furfuryl carbonate and furfuryl carbamate,^{26,32} we would expect that the release of ArOH **3** will occur rapidly, with much slower release of the second payload, ArNH₂ **2**, conjugated to the self-immolative linker (**Figure 2.12**). A modified synthetic protocol compared to **PMA-1** was required due to challenges forming the carbonate selectively at the primary alcohol on Diels–Alder adduct **7**. Instead, ArNH₂ **2** was first installed via carbamate formation on the secondary alcohol of furfural derivative **6**, which was then carried through a similar synthesis as the one illustrated in **Scheme 2.2** (see the *section 2.4.2* for details). To evaluate the kinetics of cargo release from **PMA-2**, a 2 mg/mL polymer solution in 3:1 MeCN/MeOH was

subjected to similar ultrasonication conditions as above for 60 min and cargo release was subsequently monitored at room temperature using HPLC (Figure 2.11).

Again, normalized cargo release is plotted in Figure 2.11 to highlight the relative rates of release of each payload. The non-normalized data are provided in Figure 2.13. As anticipated, the release of ArOH 3 reached a maximum prior to the first measurement and remained essentially constant over time, indicating that furfuryl carbonate decomposition occurs essentially

immediately following mechanical activation of the mechanophore.²⁷ In contrast, the release of the second cargo, ArNH₂ 2, occurred with a significantly longer half-life of

$t_{1/2} = 18$ h. After 60 min of sonication, ArNH₂ 2 was released in 24% yield and ArOH 3 was released in 32% yield. Here, the even higher dispersity of PMA-2 compared to PMA-1 likely contributes to the diminished cargo release yields after the same extent of sonication, resulting from lower mechanophore conversion under similar conditions. We speculate that the higher dispersity may reflect the closer proximity of the carbamate group to the stereogenic center on the bis-initiator. Similar to the

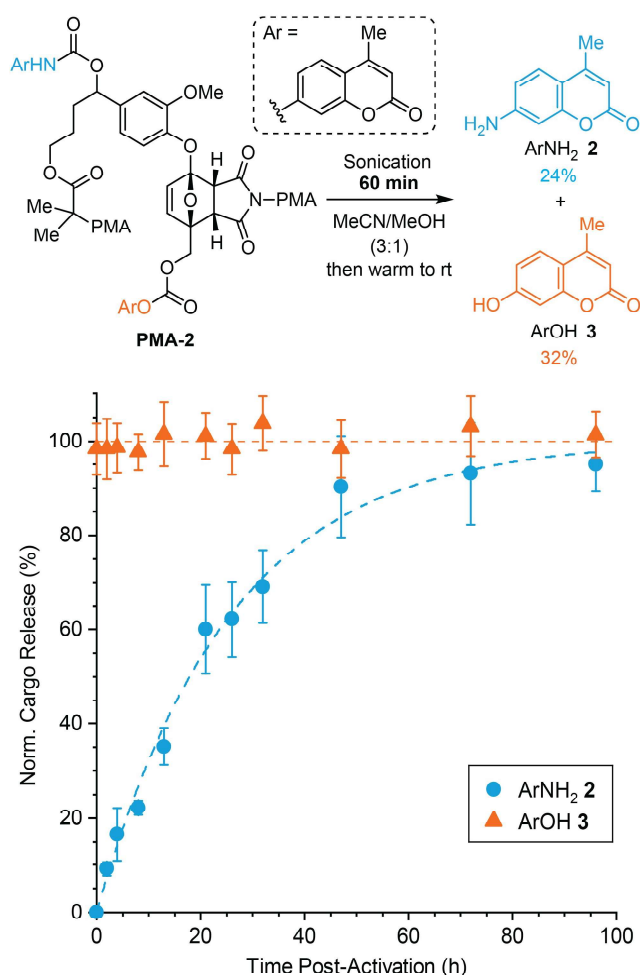


Figure 2.11. Mechanically triggered release of ArNH₂ 2 and ArOH 3 upon ultrasound-induced mechanochemical activation of PMA-2 (2 mg/mL in 3:1 MeCN/MeOH). Cargo release was measured at room temperature beginning immediately after sonication of PMA-2 for 60 min and characterized by quantitative HPLC. The data for ArNH₂ 2 were normalized to the plateau value of the fit to simple first order kinetics (dashed blue line), while the data for ArOH 3 was normalized to the average percent release across all measurements. Error bars represent standard deviation from three replicate experiments.

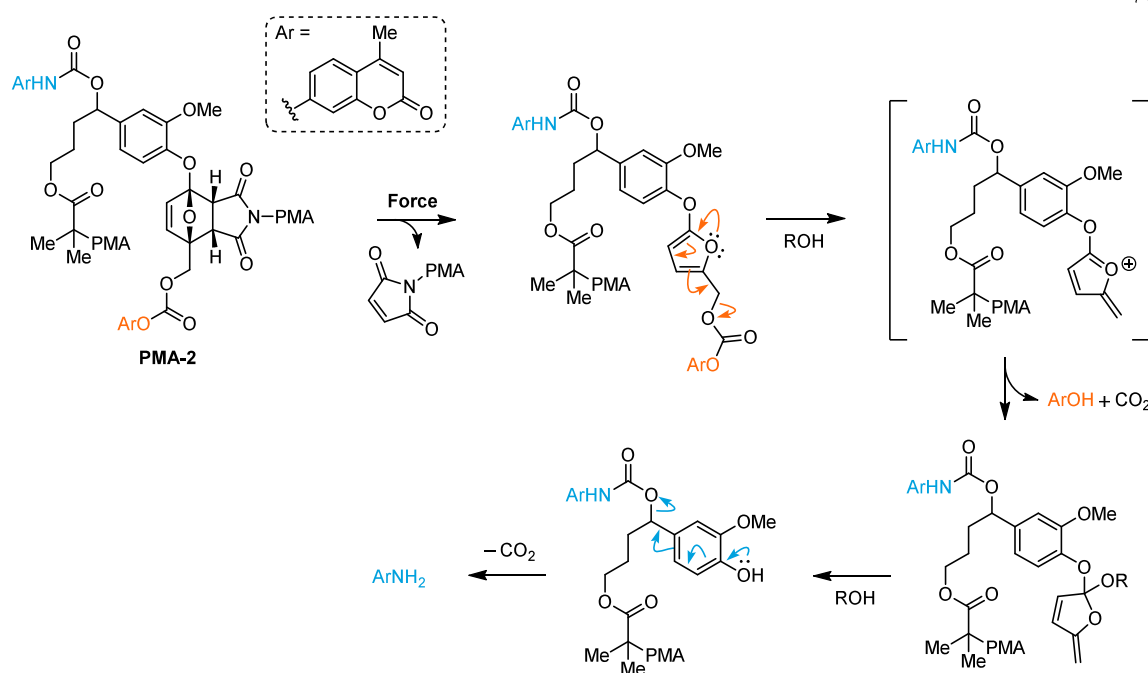


Figure 2.12. Proposed mechanism for the mechanically triggered release of ArNH_2 **2** and ArOH **3** upon ultrasound-induced mechanochemical activation of **PMA-2** in polar protic solvent via a multistep retro-Diels-Alder/fragmentation-decarboxylation cascade.

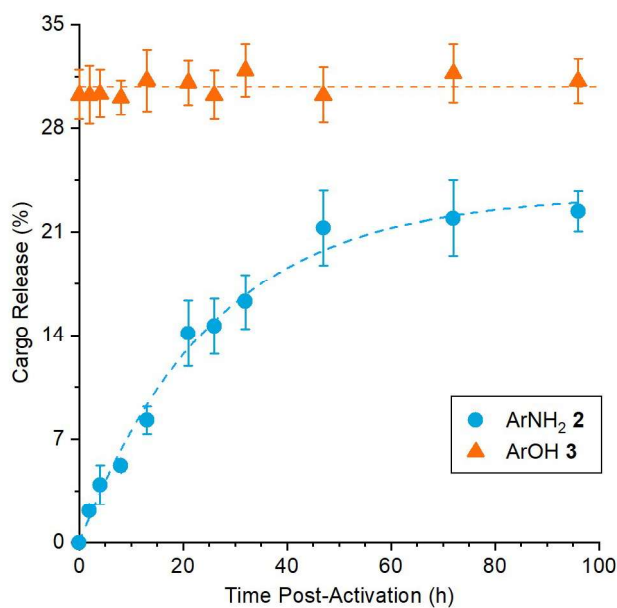


Figure 2.13. Mechanically triggered release of ArNH_2 **2** and ArOH **3** upon ultrasound-induced mechanochemical activation of **PMA-2** (2 mg/mL in 3:1 MeCN/MeOH). Cargo release was measured at room temperature beginning immediately after sonication of **PMA-2** for 60 min and characterized by quantitative HPLC. Error bars represent standard deviation from three replicate experiments. See the *section 2.4.5* for additional details.

results above, the release of the first cargo molecule from the initially generated furfuryl carbonate is again slightly more efficient than the release of the second cargo conjugated to the self-immolative linker. Notably, however, the dramatic difference in cargo release kinetics observed for **PMA-2** compared to **PMA-1** highlights the ability to fine-tune the relative release profiles based on the identity of the cargo and the attachment position on the mechanophore scaffold. Moreover, these results provide additional support for the stepwise fragmentation mechanism, indicating that expulsion of the self-immolative aryloxy group occurs after initial decomposition of the mechanically generated 2-furylcarbinol derivative. Importantly, negligible cargo release was observed after incubating **PMA-2** in 3:1 MeCN/MeOH for 96 h at room temperature (**Figure 2.14**), fully consistent with control experiments demonstrating the stability of the primary carbonate under these conditions and supporting the mechanical origin of payload release.²⁷ The ability to precisely control the relative release profiles of two different drug molecules, for example, is a unique attribute afforded by this mechanophore design, making it potentially suitable for applications in schedule-dependent combination therapies with enhanced therapeutic efficacy.^{39,40}

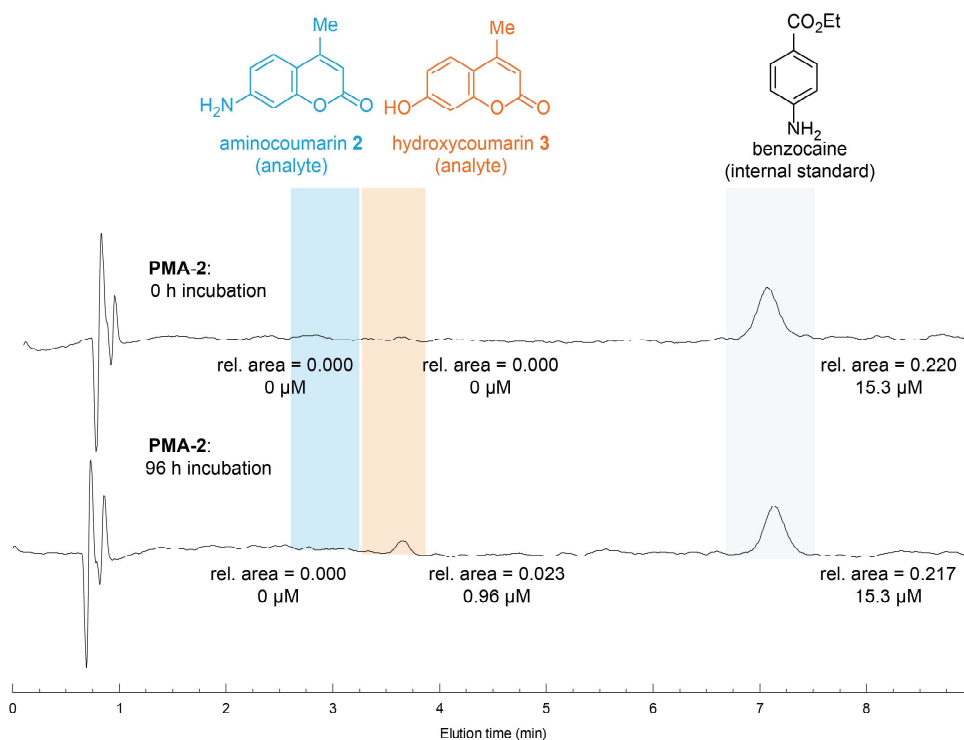


Figure 2.14. Representative HPLC chromatograms characterizing the release of ArNH₂ **2** and ArOH **3** from **PMA-2** (2 mg/mL) upon incubation in acetonitrile/methanol (3:1 v/v) at room temperature. No release of ArNH₂ **2** was observed, while < 5% release of ArOH **3** was observed after 96 h, illustrating stability of the payloads in the absence of a mechanical trigger (see the *section 2.4.5* for additional details). HPLC conditions: 22:78 MeCN/water + 0.1% AcOH, isocratic, 2 mL/min, λ = 310 nm.

2.3 Conclusions

We have designed a novel mechanophore based on a masked 5-aryloxy-substituted 2-furylcarbinol derivative incorporating a self-immolative spacer facilitating the mechanically triggered release of two distinct molecular payloads. The mechanophore design leverages a unique fragmentation pathway involving the initial decomposition of a mechanochemically unveiled 2-furylcarbinol derivative to release the first payload followed by a second fragmentation process resulting in expulsion of the aryloxy substituent. Installation of a judiciously designed aryloxy spacer capable of self-immolation via a 1,6-elimination mechanism enables the release of a second payload molecule from a single mechanophore. Notably, we demonstrate that the relative release rates of the two cargo molecules are varied over a wide range based on both the reactivity of the payload/linker group and the position

of conjugation to the mechanophore. By simply switching the attachment positions of two model payloads, mechanically triggered release occurs either concurrently or sequentially, resulting in remarkably different release profiles. The selectivity, modularity, and tunable release kinetics afforded by this mechanophore design provide a promising platform for a variety of potential applications including real-time monitoring of drug distribution, combination chemotherapy, and even dual catalysis.

2.4 Experimental Details

2.4.1 General experimental details and methods

Reagents from commercial sources were used without further purification unless otherwise stated. Methyl acrylate was passed through a short plug of basic alumina to remove inhibitor immediately prior to use. Anhydrous DCM, THF, diethyl ether, MeCN, and DMF were obtained from a Pure Process Technology solvent purification system. All reactions were performed under a N₂ atmosphere unless specified otherwise. Freshly cut copper wire was immersed in 1 M HCl for 15 min, rinsed consecutively with deionized water and acetone, and then dried under a flow of N₂ prior to use. Column chromatography was performed on a Biotage Isolera system using SiliCycle SiliaSep HP or SiliaBond C18 cartridges.

NMR spectra were recorded using a 400 MHz Bruker Avance III HD with Prodigy Cryoprobe, a 400 MHz Bruker Avance Neo, or Varian Inova 500 MHz spectrometers. All ¹H NMR spectra are reported in δ units, parts per million (ppm), and were measured relative to the signals for residual chloroform (7.26 ppm), dichloromethane (5.32 ppm), methanol (3.31 ppm), acetone (2.05 ppm), toluene (2.08 ppm), or acetonitrile (1.94 ppm) in deuterated solvent. All ¹³C NMR spectra were measured in deuterated solvents and are reported in ppm relative to the signals for chloroform (77.16 ppm), acetone (29.84 ppm), or acetonitrile (1.32 ppm). Multiplicity and qualifier abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dq = doublet of quartets, ABq = AB quartet, m = multiplet, br s = broad singlet.

High resolution mass spectra (HRMS) were obtained from an Agilent 6230 series time-of-flight mass spectrometer equipped with an Agilent G1958 Jet Stream Electrospray Ionization (ESI) Source or a JEOL JMS-T2000 GC AccuTOF GC-Alpha mass spectrometer interfaced with an Agilent 8890 GC system with samples analyzed by field desorption (FD) or field ionization (FI), which generally resulted in radical cations, $M^{+\bullet}$.

Analytical gel permeation chromatography (GPC) was performed using an Agilent 1260 series pump equipped with two Agilent PLgel MIXED-B columns (7.5 x 300 mm), an Agilent 1200 series diode array detector, a Wyatt 18-angle DAWN HELEOS light scattering detector, and an Optilab rEX differential refractive index detector. The mobile phase was THF at a flow rate of 1 mL/min. Molecular weights and molecular weight distributions were calculated by light scattering using a dn/dc value of 0.062 mL/g (25 °C) for poly(methyl acrylate).

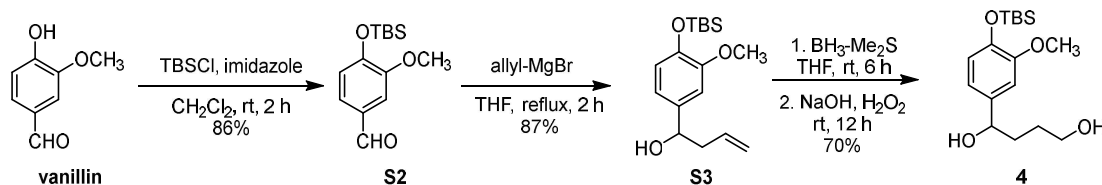
Photoluminescence spectra were recorded on a Shimadzu RF-6000 spectrofluorophotometer using a quartz microcuvette (Starna Cells 18F-Q-10-GL14-C, 10 x 2 mm). Excitation and emission slit widths were 5 nm and 3 nm, respectively.

Analytical high-performance liquid chromatography (HPLC) measurements were performed with an Agilent Eclipse XDB-C18 column (993967-902) using a multi-wavelength UV-vis detector.

Ultrasound experiments were performed inside of a sound abating enclosure using a 500 watt Vibra Cell 505 liquid processor (20 kHz) equipped with a 0.5-inch diameter solid probe (part #630-0217), sonochemical adapter (part #830-00014), and a Suslick reaction vessel made by the Caltech glass shop (analogous to vessel #830-00014 from Sonics and Materials).

2.4.2 Synthetic details

Scheme 2.3. Synthesis of Diol **4**.



4-((tert-butyldimethylsilyl)oxy)-3-methoxybenzaldehyde (S2). A two-neck round bottom flask equipped with a stir bar was charged with vanillin (10.2 g, 66.8 mmol), imidazole (11.4 g, 167 mmol), and DCM (67 mL). To the stirred solution at room temperature was added *tert*-butyldimethylsilyl chloride (TBSCl, 13.1 g, 86.8 mmol) in one portion. After 2 h, 10% NH₄Cl (30 mL) was added, the mixture was extracted with DCM (3 x 150 mL), and the combined organic phase was washed with brine (100 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (0–10% EtOAc/hexanes) to afford the title compound as a colorless oil (15.3 g, 86% yield).

TLC (25% ethyl acetate/hexane): $R_f = 0.67$

¹H NMR (400 MHz, CDCl₃) δ : 9.85 (s, 1H), 7.40 (d, $J = 1.9$ Hz, 1H), 7.37 (dd, $J = 8.0, 1.9$ Hz, 1H), 6.96 (d, $J = 8.0$ Hz, 1H), 3.87 (s, 3H), 1.00 (s, 9H), 0.19 (s, 6H) ppm.

¹³C{¹H} NMR (100 MHz, CDCl₃) δ : 191.2, 151.8, 151.5, 131.1, 126.4, 120.9, 110.3, 55.6, 25.7, 18.7, −4.4 ppm.

HRMS (ESI, m/z): calcd for [C₁₄H₂₃O₃Si]⁺ (M+H)⁺, 267.1411; found, 267.1409.

1-(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)but-3-en-1-ol (S3). A flame dried two-neck round bottom flask equipped with a stir bar and a condenser was charged with compound **S2** (313 mg, 1.18 mmol) and THF (12 mL). A solution of allyl magnesium bromide in diethyl ether (0.7 mmol/mL, 2.0 mL, 1.4 mmol) was added dropwise by syringe

and then refluxed in an oil bath for 2 h. The reaction was then cooled to 0 °C in an ice bath before deionized water (5 mL) was added. The mixture was then extracted with EtOAc (3 x 40 mL) and the combined organic phase was washed with brine (20 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (15–20% EtOAc/hexanes) to afford the title compound as a yellow oil (316 mg, 87% yield).

TLC (13% ethyl acetate/hexane): $R_f = 0.38$

¹H NMR (400 MHz, CDCl₃) δ : 6.89 (d, $J = 1.8$ Hz, 1H), 6.83 – 6.76 (m, 2H), 5.86 – 5.70 (m, 1H), 5.19 – 5.07 (m, 2H), 4.66 (t, $J = 6.5$ Hz, 1H), 3.81 (s, 3H), 2.55 – 2.46 (m, 2H), 1.98 (br s, 1H), 0.99 (s, 9H), 0.15 (s, 6H) ppm.

¹³C{¹H} NMR (500 MHz, CDCl₃) δ : 150.9, 144.3, 137.6, 134.8, 120.6, 118.2, 118.1, 109.7, 73.3, 55.5, 43.9, 25.8, 18.5, –4.6 ppm.

HRMS (EI, m/z): calcd for [C₁₇H₂₈O₃Si]⁺ (M)⁺, 308.1808; found, 308.1808.

1-(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)butane-1,4-diol (4). A flame dried two-neck round bottom flask equipped with a stir bar was charged with compound **S3** (6.20 g, 20.1 mmol) and THF (67 mL). The solution was cooled to 0 °C in an ice bath and borane dimethyl sulfide complex (2.9 mL, 30.6 mmol) was added dropwise by syringe. After stirring at room temperature for 4 h, the solution was cooled to 0 °C in an ice bath followed by the consecutive dropwise addition of 3N NaOH_(aq) (8.0 mL) and then 35% H₂O_{2 (aq)} (8.0 mL). After stirring at room temperature for 16 h, the mixture was extracted with EtOAc (3 x 400 mL) and the combined organic phase was washed with brine (200 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (40–60% EtOAc/hexanes) to afford the title compound as a white solid (4.61 g, 70% yield).

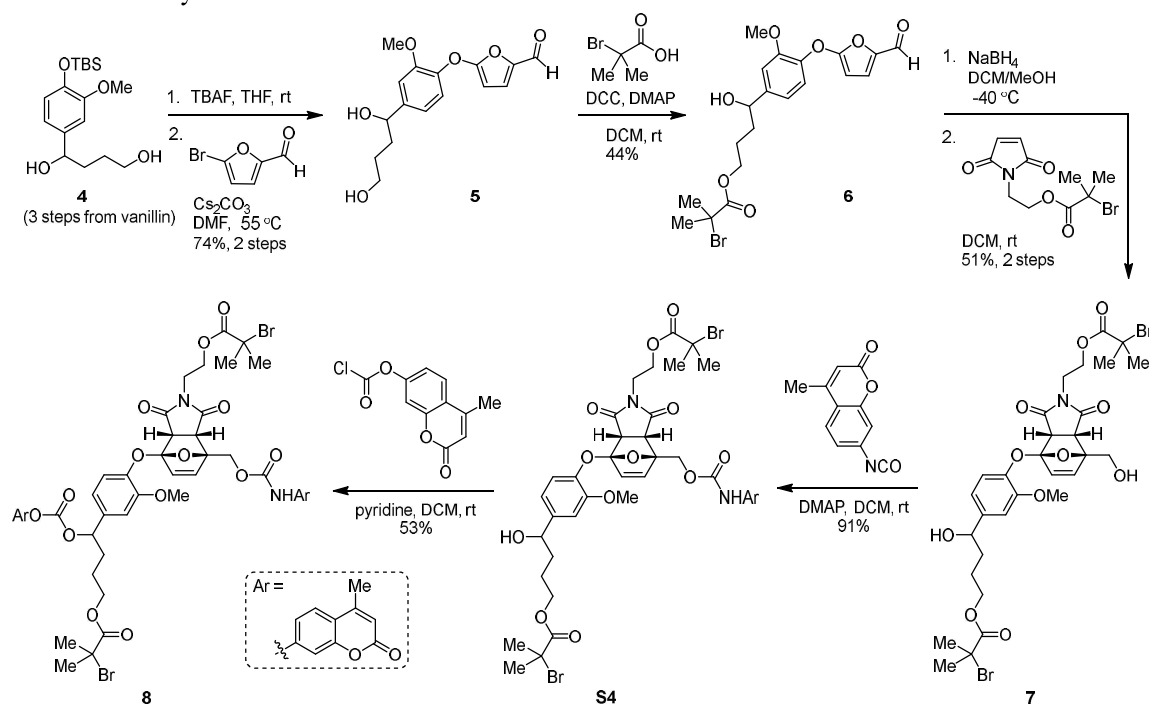
TLC (75% ethyl acetate/hexane): $R_f = 0.52$

^1H NMR (400 MHz, CDCl_3) δ : 6.87 (d, $J = 1.9$ Hz, 1H), 6.81 – 6.72 (m, 2H), 4.64 (dd, $J = 7.1, 5.6$ Hz, 1H), 3.80 (s, 3H), 3.72 – 3.58 (m, 2H), 2.98 (s, 1H), 1.89 – 1.77 (m, 2H), 1.75 – 1.59 (m, 3H), 0.99 (s, 9H), 0.14 (s, 6H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 151.0, 144.4, 138.4, 120.7, 118.2, 109.7, 74.4, 62.9, 55.6, 36.4, 29.5, 25.8, 18.6, –4.5 ppm.

HRMS (FD, m/z): calcd for $[\text{C}_{17}\text{H}_{30}\text{O}_4\text{Si}]^{++} (\text{M})^{++}$, 326.1913; found, 326.1915.

Scheme 2.4. Synthesis of Bis-Initiator **8**.



5-(4-(1,4-dihydroxybutyl)-2-methoxyphenoxy)furan-2-carbaldehyde (5). A two-neck round bottom flask equipped with a stir bar was charged with compound **4** (210 mg, 0.644 mmol), a solution of tetrabutylammonium fluoride (TBAF) in THF (1 M, 0.77 mL, 0.77 mmol), and THF (6 mL). After stirring at room temperature for 2 h, the solution was concentrated under reduced pressure. To the flask were added 5-bromo-2-furaldehyde (134 mg, 0.766 mmol), Cs_2CO_3 (312 mg, 0.957 mmol), and DMF (2 mL). The solution was then heated at 55 °C in an oil bath for 4 h. The reaction was cooled to room temperature followed by the addition of 10% NH_4Cl (20 mL). The mixture was then extracted with EtOAc (3 x 50

mL) and the combined organic phase was washed with brine (20 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (50–100% EtOAc/hexanes) to afford the title compound as a yellow solid (146 mg, 74% yield over 2 steps).

TLC (100% ethyl acetate): R_f = 0.40

¹H NMR (500 MHz, acetone-*d*₆) δ : 9.33 (s, 1H), 7.39 (d, J = 3.8 Hz, 1H), 7.28 – 7.16 (m, 2H), 7.01 (dd, J = 8.2, 1.9 Hz, 1H), 5.44 (d, J = 3.8 Hz, 1H), 4.72 (dd, J = 7.7, 5.0 Hz, 1H), 4.60 (br s, 1H), 3.83 (s, 3H), 3.76 (br s, 1H), 3.57 (t, J = 6.3 Hz, 2H), 1.86 – 1.50 (m, 4H) ppm.

¹³C{¹H} NMR (125 MHz, acetone-*d*₆) δ : 175.8, 164.7, 151.5, 147.2, 145.4, 141.6, 127.6, 121.7, 119.1, 111.8, 88.4, 73.7, 62.5, 56.3, 37.3 ppm.

HRMS (ESI, m/z): calcd for [C₁₆H₁₉O₆]⁺ (M+H)⁺, 307.1176; found, 307.1177.

4-(4-((5-formylfuran-2-yl)oxy)-3-methoxyphenyl)-4-hydroxybutyl 2-bromo-2-methyl propanoate (6). A round bottom flask equipped with a stir bar was charged with compound **5** (99.2 mg, 0.324 mmol), dicyclohexylcarbodiimide (DCC, 89.1 mg, 0.432 mmol), DMAP (8.4 mg, 0.069 mmol), and DCM (8 mL), followed by the addition of α -bromoisobutyric acid (64.3 mg, 0.385 mmol). The solution was stirred at room temperature for 2 h and then the solid precipitate was filtered off and discarded. The filtrate was diluted with EtOAc (300 mL) and washed consecutively with 10% NH₄Cl (50 mL) and brine (50 mL). The organic layer was then dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography (50–70% EtOAc/hexanes) to afford the title compound as a dark orange oil (64.9 mg, 44% yield).

TLC (67% ethyl acetate/hexanes): R_f = 0.47

¹H NMR (500 MHz, CDCl₃) δ : 9.21 (s, 1H), 7.15 (d, J = 3.8 Hz, 1H), 7.07 (d, J = 8.2 Hz, 1H), 7.02 (d, J = 1.9 Hz, 1H), 6.87 (dd, J = 8.2, 1.9 Hz, 1H), 5.34 (d, J = 3.8 Hz, 1H), 4.69

(t, $J = 5.9$ Hz, 1H), 4.21 – 4.06 (m, 2H), 3.76 (s, 3H), 3.11 (s, 1H), 1.87 (s, 6H), 1.82 – 1.63 (m, 4H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 175.4, 171.6, 163.8, 150.7, 144.4, 144.2, 141.2, 127.7, 121.0, 118.2, 110.6, 88.5, 73.1, 65.7, 56.1, 56.0, 35.3, 30.7, 24.7 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{20}\text{H}_{24}\text{BrO}_7]^+$ ($M+H$) $^+$, 455.0700; found, 455.0702.

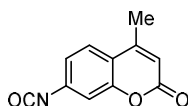
2-(4-(4-(4-((2-bromo-2-methylpropanoyl)oxy)-1-hydroxybutyl)-2-methoxyphenoxy)-7-(hydroxymethyl)-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-epoxyisoindol-2-yl)ethyl 2-bromo-2-methylpropanoate (7). A flame-dried two-neck round bottom flask equipped with a stir bar was charged with compound **6** (152 mg, 0.334 mmol), DCM (1.5 mL), and anhydrous methanol (1.5 mL). The solution was cooled to $-40\text{ }^\circ\text{C}$ in an acetonitrile/dry ice bath before adding NaBH_4 (38.1 mg, 1.00 mmol). The mixture was kept at $-40\text{ }^\circ\text{C}$ for 2 h before being quenched with 10% NH_4Cl (3 mL) and subsequently warmed to room temperature. The solution was then extracted with DCM (3 x 30 mL) and the organic phase was washed with brine (10 mL). The organic layer was dried over Na_2SO_4 , and filtered. To the filtrate was added 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl 2-bromo-2-methylpropanoate (the maleimide ester)⁴¹ (125 mg, 0.43 mmol), which was then concentrated under reduced pressure until ~ 2 mL of viscous solution remained. The mixture was then allowed to react at room temperature for 12 h. The crude reaction mixture was purified by column chromatography (50–70% EtOAc/hexanes) to afford the title compound (mixture of diastereomers) as a foamy white solid (126 mg, 51% yield).

TLC (67% ethyl acetate/hexanes): $R_f = 0.28$

^1H NMR (400 MHz, CDCl_3) δ : 7.13 (d, $J = 8.2$ Hz, 1H), 6.92 (t, $J = 1.8$ Hz, 1H), 6.74 (dt, $J = 8.2, 1.8$ Hz, 1H), 6.37 (d, $J = 5.8$ Hz, 1H), 6.33 (d, $J = 5.8$ Hz, 1H), 4.63 (t, $J = 5.7$ Hz, 1H), 4.22 – 4.03 (m, 6H), 3.86 (d, $J = 2.0$ Hz, 3H), 3.73 – 3.61 (m, 4H), 2.82 (br s, 1H), 2.56 (br s, 1H), 1.88 (s, 6H), 1.86 (s, 6H), 1.82 – 1.63 (m, 4 H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 174.5, 173.1, 171.7, 171.4, 151.60, 151.58, 142.6, 142.52, 142.48, 136.0, 135.9, 135.6, 123.2, 123.1, 117.9, 114.12, 114.09, 110.4, 110.3, 86.5, 73.5, 65.8, 62.5, 61.4, 56.4, 56.1, 55.6, 50.7, 48.81, 48.79, 37.5, 35.2, 30.78, 30.77, 30.7, 24.9 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{30}\text{H}_{38}\text{Br}_2\text{NO}_{11}]^+$ ($\text{M}+\text{H}$) $^+$, 746.0806; found, 746.0804.



7-isocyanato-4-methyl-2H-chromen-2-one (4-methylcoumarin-7-isocyanate). The starting material 7-amino-4-methyl-2H-chromen-2-one was first purified according to the literature.⁴² The substrate (5.52 g, 31.5 mmol) was dissolved in 1N HCl (100 mL) and then the solution was pipetted slowly into a 10% ammonia solution (200 mL) maintained at 0 °C in an ice bath. The precipitate was collected and the solid was recrystallized from hot ethanol to provide a tan crystalline powder (2.79 g, 15.9 mmol).

Following an adapted literature procedure,²⁶ an oven-dried two-neck round bottom flask equipped with a stir bar, an oven-dried distillation adapter, and a KOH trap was charged with purified 7-amino-4-methyl-2H-chromen-2-one (509 mg, 2.91 mmol), triphosgene (287 mg, 0.969 mmol), and DCM (42 mL) under nitrogen. **CAUTION: TRIPHOSGENE IS TOXIC. ALL OPERATIONS ARE CARRIED OUT EXCLUSIVELY INSIDE A FUME HOOD.** The suspension was cooled to 0 °C in an ice bath and then triethylamine (0.81 mL, 5.81 mmol) was added dropwise, which caused the reaction to become homogeneous. The solution was warmed to room temperature and stirred for 1 h, during which time a white precipitate formed. The solid precipitate was removed by vacuum filtration under nitrogen and discarded (see **Figure 2.15**). The filtrate was concentrated under reduce pressure, the resulting solid was suspended in anhydrous THF (60 mL), and residual insoluble solid was filtered off and discarded again using a similar apparatus as the one illustrated in **Figure 2.15**. The filtrate was concentrated under reduce pressure to afford the title compound as a white solid (527 mg, 90% yield).

TLC (50% ethyl acetate/hexanes): $R_f = 0.43$

^1H NMR (500 MHz, CDCl_3) δ : 7.55 (d, $J = 8.3$, 1H), 7.10 – 6.96 (m, 2H), 6.27 (s, 1H), 2.43 (s, 3H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 160.4, 154.3, 151.9, 136.9, 125.8, 125.7, 121.3, 118.0, 114.7, 113.1, 18.8 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{11}\text{H}_8\text{NO}_3]^+ (\text{M}+\text{H})^+$, 202.0499; found, 202.0495.

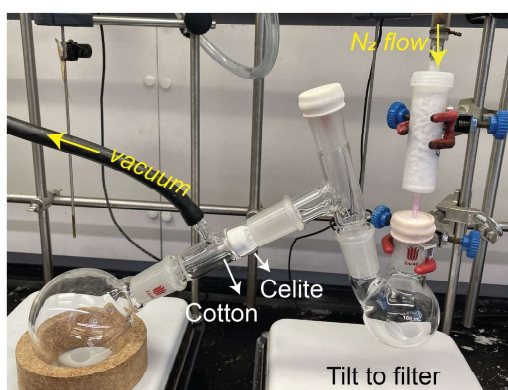


Figure 2.15. Experimental setup used in the preparation of 7-isocyanato-4-methyl-2*H*-chromen-2-one (i.e., 4-methylcoumarin-7-isocyanate).

2-(4-(4-(4-((2-bromo-2-methylpropanoyl)oxy)-1-hydroxybutyl)-2-methoxyphenoxy)-7-(((4-methyl-2-oxo-2*H*-chromen-7-yl)carbamoyl)oxy)methyl)-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2*H*-4,7-epoxyisoindol-2-yl)ethyl 2-bromo-2-methylpropanoate (S4). A round bottom flask equipped with a stir bar was charged with compound **7** (166 mg, 0.223 mmol), 4-methylcoumarin-7-isocyanate (44.8 mg, 0.223 mmol), and DCM (5 mL). The solution was cooled to 0 °C in an ice bath before adding DMAP (0.272 mg, 0.0022 mmol). The mixture was warmed to room temperature and stirred for 1 h. The mixture was then diluted with DCM (100 mL), washed with 10% NH_4Cl (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (40–60%

EtOAc/hexanes) to afford the title compound (mixture of diastereomers) as a white foamy solid (193 mg, 91% yield).

TLC (75% ethyl acetate/hexanes): $R_f = 0.34$

^1H NMR (500 MHz, CDCl_3) δ : 8.05 (s, 1H), 7.50 (d, $J = 10.3$ Hz, 3H), 7.13 (d, $J = 8.2$ Hz, 1H), 6.94 (dd, $J = 4.9, 1.9$ Hz, 1H), 6.80 – 6.67 (m, 1H), 6.45 (dd, $J = 5.8, 3.6$ Hz, 1H), 6.36 (d, $J = 5.8$ Hz, 1H), 6.15 (s, 1H), 4.74 (ABq, $\Delta\nu_{\text{AB}} = 33.5$ Hz, $J_{\text{AB}} = 10$ Hz, 2H), 4.65 (t, $J = 6.0$ Hz, 1H), 4.25 – 4.09 (m, 4H), 3.91 (d, $J = 7.9$ Hz, 1H), 3.84 – 3.79 (m, 4H), 3.77 – 3.60 (m, 2H), 2.53 (br s, 1H), 2.39 (s, 3H), 1.89 (s, 6H), 1.85 (d, $J = 3$ Hz, 6H), 1.83 – 1.66 (m, 4H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 173.8, 173.3, 171.8, 171.5, 161.3, 154.3, 152.6, 152.5, 151.54, 151.48, 142.82, 142.75, 142.4, 141.7, 135.8, 135.6, 135.5, 125.4, 123.2, 123.0, 118.0, 117.9, 115.6, 114.2, 114.1, 113.1, 110.21, 110.16, 106.3, 83.98, 83.96, 73.5, 65.8, 62.9, 62.5, 56.2, 56.1, 55.6, 50.5, 49.1, 37.6, 35.24, 35.23, 30.8, 30.7, 24.9, 18.7 ppm.

HRMS (FD, m/z): calcd for $[\text{C}_{41}\text{H}_{44}\text{Br}_2\text{N}_2\text{O}_{14}]^{++} (\text{M})^{+}$, 946.1159; found, 946.1162.

2-(4-(4-(4-((2-bromo-2-methylpropanoyl)oxy)-1-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)carbonyl)oxy)butyl)-2-methoxy phenoxy)-7-(((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)oxy)methyl)-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-epoxyisoindol-2-yl)ethyl 2-bromo-2-methylpropanoate (8). A two-neck round bottom flask equipped with a stir bar was charged with compound **S4** (98.0 mg, 0.104 mmol), DCM (2 mL), and pyridine (17 μL , 0.21 mmol) were added. The solution was cooled to 0 °C in an ice bath before adding a solution of 4-methylcoumarin-7-chloroformate²⁵ (29.6 mg, 0.124 mmol) in DCM (1 mL) dropwise. The mixture was then warmed to room temperature, and stirred for 1 h. The mixture was then diluted with DCM (100 mL), washed with 10% NH_4Cl (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (0–10% MeOH/DCM) followed by a reverse-phase chromatographic separation on a C18 column

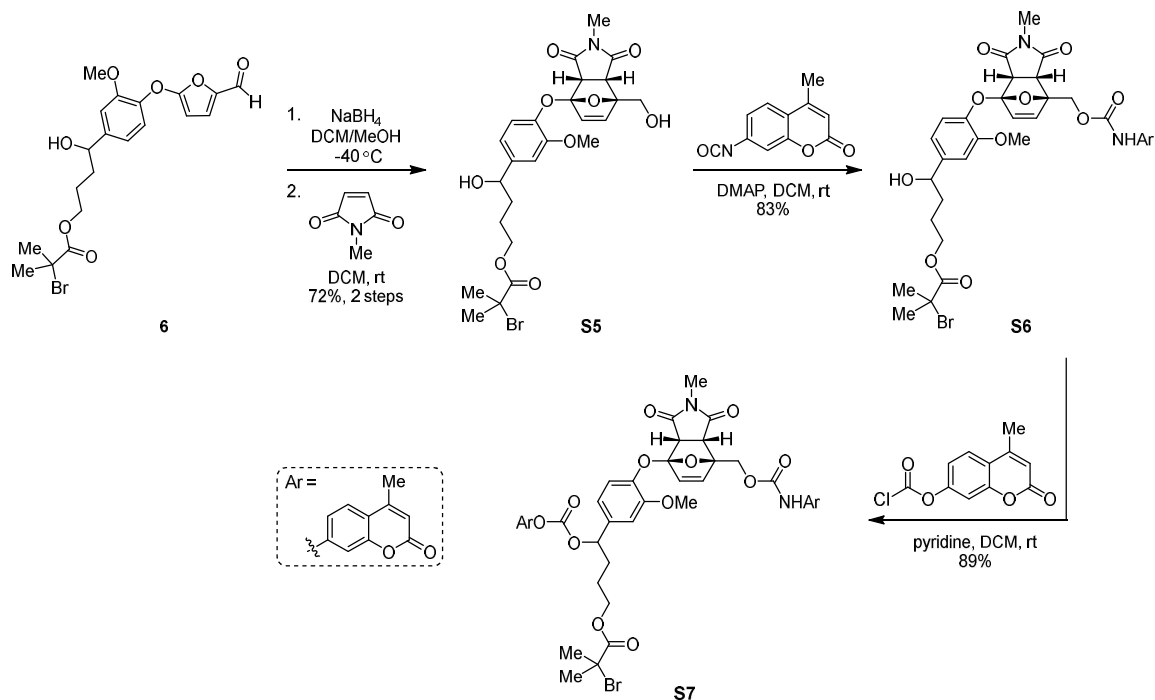
(65–85% acetonitrile/H₂O) to afford the title compound (mixture of diastereomers) as a white foamy solid (63 mg, 53% yield).

TLC (86% ethyl acetate/hexanes): $R_f = 0.25$

¹H NMR (500 MHz, CDCl₃) δ : 7.88 (d, $J = 8.4$ Hz, 1H), 7.58 – 7.49 (m, 4H), 7.22 (dd, $J = 8.3, 2.7$ Hz, 1H), 7.16 – 7.06 (m, 2H), 6.94 (dd, $J = 4.7, 2.0$ Hz, 1H), 6.86 (t, $J = 7.5$ Hz, 1H), 6.50 – 6.38 (m, 2H), 6.24 (s, 1H), 6.16 (s, 1H), 5.64 (t, $J = 6.8$ Hz, 1H), 4.79 (ABq, $\Delta\nu_{AB} = 38.7$ Hz, $J_{AB} = 12.5$ Hz, 2H), 4.27 – 4.13 (m, 4H), 3.95 (d, $J = 7.9$ Hz, 1H), 3.89 – 3.82 (m, 4H), 3.75 – 3.62 (m, 2H), 2.40 (d, $J = 4.1$ Hz, 6H), 2.19 – 2.09 (m, 1H), 2.04 – 1.95 (m, 1H), 1.91 (s, 6H), 1.86 (d, $J = 2.9$ Hz, 6H), 1.82 – 1.77 (m, 1H), 1.74 – 1.67 (m, 1H) ppm.

¹³C{¹H} NMR (125 MHz, CDCl₃) δ : 173.6, 173.3, 171.7, 171.4, 161.2, 160.4, 154.4, 154.1, 153.2, 152.50, 152.46, 152.2, 152.0, 151.7, 151.6, 143.50, 143.46, 141.6, 136.54, 136.52, 135.8, 135.6, 125.6, 125.5, 123.24, 123.21, 118.9, 118.7, 118.1, 117.3, 115.7, 114.7, 114.0, 113.2, 111.0, 110.9, 109.8, 106.3, 84.1, 80.81, 80.79, 65.1, 62.9, 62.5, 56.4, 56.0, 55.6, 50.6, 49.1, 37.7, 32.6, 30.8, 30.68, 30.67, 24.6, 18.8, 18.7 ppm.

HRMS (FD, m/z): calcd for [C₅₂H₅₀Br₂N₂O₁₈]⁺ (M)⁺, 1148.1425; found, 1148.1425.

Scheme 2.5. Synthesis of Chain-End Control Initiator **S7**.

4-hydroxy-4-(4-((7-(hydroxymethyl)-2-methyl-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-4,7-epoxyisoindol-4-yl)oxy)-3-methoxyphenyl)butyl 2-bromo-2-methylpropanoate (S5). A flame-dried two-neck round bottom flask equipped with a stir bar was charged with compound **6** (664 mg, 1.46 mmol), DCM (7 mL), and anhydrous methanol (7 mL). The solution was cooled to $-40\text{ }^{\circ}\text{C}$ in an acetonitrile/dry ice bath before adding NaBH_4 (166 mg, 4.37 mmol). The mixture was kept at $-40\text{ }^{\circ}\text{C}$ for 2 h before being quenched with 10% NH_4Cl (10 mL) and subsequently warmed to room temperature. The solution was then extracted with DCM (3 x 50 mL) and the organic phase was washed with brine (20 mL). The organic layer was dried over Na_2SO_4 , and filtered. *N*-methylmaleimide (211 mg, 1.90 mmol) was then added to the filtrate, which was concentrated under reduced pressure until ~2 mL of viscous solution remained. The mixture was then allowed to react at room temperature for 12 h. The crude reaction mixture was purified by column chromatography (0–10% MeOH/DCM) to afford the title compound (mixture of diastereomers) as a foamy pink solid (598 mg, 72% yield).

TLC (67% ethyl acetate/hexanes): $R_f = 0.23$

^1H NMR (500 MHz, CDCl_3) δ : 7.15 (d, $J = 8.1$ Hz, 1H), 6.95 (s, 1H), 6.77 (d, $J = 8.1$ Hz, 1H), 6.34 (d, $J = 5.8$ Hz, 1H), 6.30 (d, $J = 5.8$ Hz, 1H), 4.66 (t, $J = 5.8$ Hz, 1H), 4.25 – 4.05 (m, 4H), 3.90 (s, 3H), 3.73 (d, $J = 7.7$ Hz, 1H), 3.66 (d, $J = 7.7$ Hz, 1H), 2.84 (s, 3H), 2.65 (br s, 1H), 2.39 (br s, 1H), 1.91 (s, 6H), 1.86 – 1.68 (m, 4H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 175.0, 173.6, 171.8, 151.73, 151.70, 142.8, 142.51, 142.47, 136.0, 135.9, 135.6, 123.3, 123.2, 118.0, 117.9, 114.2, 114.1, 110.4, 86.4, 73.7, 65.8, 61.7, 56.4, 56.1, 50.9, 49.11, 49.09, 35.2, 30.9, 24.9 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{25}\text{H}_{31}\text{BrNO}_9]^+$ ($\text{M}+\text{H}$) $^+$, 568.1177; found, 568.1177.

4-hydroxy-4-(3-methoxy-4-((2-methyl-7-(((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)oxy)methyl)-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-4,7-epoxyisindol-4-yl)oxy)phenyl)butyl 2-bromo-2-methylpropanoate (S6). A round bottom flask equipped with a stir bar was charged with compound **S5** (338 mg, 0.596 mmol), 4-methylcoumarin-7-isocyanate (120 mg, 0.597 mmol), and DCM (12 mL). After cooling the solution to 0 °C in an ice bath, DMAP (0.728 mg, 0.006 mmol) was added and then the mixture was warmed to room temperature and stirred for 1 h. The mixture was diluted with DCM (100 mL) and washed consecutively with 10% NH_4Cl (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (0–10% MeOH/DCM) followed by a reverse-phase chromatographic separation on a C18 column (40–60% acetonitrile/ H_2O) to afford the title compound (mixture of diastereomers) as a foamy pink solid (381 mg, 83% yield).

TLC (80% ethyl acetate/hexanes): $R_f = 0.27$

^1H NMR (500 MHz, CDCl_3) δ : 8.25 (s, 1H), 7.56 – 7.38 (m, 3H), 7.11 – 7.04 (m, 1H), 6.92 (d, $J = 7.7$ Hz, 1H), 6.72 (t, $J = 8.6$ Hz, 1H), 6.35 (t, $J = 5.5$ Hz, 1H), 6.30 – 6.25 (m, 1H), 6.10 (s, 1H), 4.72 (ABq, $\Delta\nu_{\text{AB}} = 30.0$ Hz, $J_{\text{AB}} = 12.5$ Hz, 2H), 4.62 (s, 1H), 4.20 – 4.04 (m,

2H), 3.88 – 3.71 (m, 5H), 2.87 – 2.74 (m, 4H), 2.36 (s, 3H), 1.86 (s, 6H), 1.83 – 1.63 (m, 4H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 174.3, 173.8, 171.7, 161.3, 154.2, 152.7, 152.6, 151.44, 151.36, 142.8, 142.7, 142.4, 141.7, 135.6, 135.5, 135.4, 125.4, 122.8, 122.7, 117.9, 117.8, 115.4, 114.7, 114.01, 113.98, 112.9, 110.3, 110.2, 106.1, 83.89, 83.88, 73.3, 65.7, 62.8, 56.15, 56.11, 50.4, 49.1, 35.2, 30.7, 24.9, 24.8, 18.6 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{36}\text{H}_{37}\text{BrN}_2\text{NaO}_{12}]^+$ ($\text{M}+\text{Na}$) $^+$, 791.1422; found, 791.1418.

4-(3-methoxy-4-((2-methyl-7-(((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)oxy)methyl)-1,3-dioxo-1,2,3,3a,7,7a-hexahydro-4H-4,7-epoxyisoindol-4-yl)oxy)phenyl)-4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)carbonyl)oxy)butyl 2-bromo-2-methyl propanoate (S7). A two-neck round bottom flask equipped with a stir bar was charged with compound **S6** (230 mg, 0.299 mmol), DCM (4 mL), and pyridine (48 μL , 0.60 mmol). The solution was cooled to 0 °C in an ice bath before adding a solution of 4-methylcoumarin-7-chloroformate²⁵ (85.5 mg, 0.359 mmol) in DCM (2 mL) dropwise. The mixture was then warmed to room temperature and stirred for 1 h. The mixture was then diluted with DCM (100 mL), washed with 10% NH_4Cl (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (70–90% EtOAc/hexanes) to afford the title compound (mixture of diastereomers) as a foamy white solid (259 mg, 89% yield).

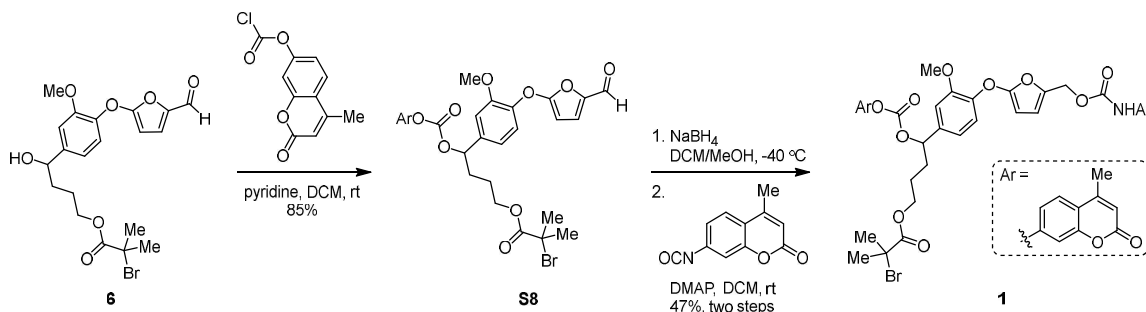
TLC (86% ethyl acetate/hexanes): R_f = 0.21

^1H NMR (400 MHz, CDCl_3) δ : 8.07 (d, J = 7.0 Hz, 1H), 7.58 – 7.46 (m, 4H), 7.19 (dd, J = 8.2, 2.4 Hz, 1H), 7.12 (t, J = 2.2 Hz, 1H), 7.08 (dt, J = 8.7, 2.6 Hz, 1H), 6.96 – 6.89 (m, 1H), 6.88 – 6.79 (m, 1H), 6.42 (dd, J = 5.8, 2.2 Hz, 1H), 6.37 (dd, J = 5.8, 1.1 Hz, 1H), 6.22 (s, 1H), 6.14 (s, 1H), 5.62 (t, J = 6.8 Hz, 1H), 4.79 (ABq, $\Delta\nu_{\text{AB}}$ = 16.0 Hz, J_{AB} = 12.0 Hz, 2H), 4.22 – 4.10 (m, 2H), 3.97 – 3.79 (m, 5H), 2.84 (s, 3H), 2.38 (d, J = 5.2 Hz, 6H), 2.16 – 2.05 (m, 1H), 2.03 – 1.93 (m, 1H), 1.89 (s, 6H), 1.82 – 1.63 (m, 2H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 174.0, 173.8, 171.5, 161.2, 160.3, 154.2, 153.9, 153.0, 152.6, 152.5, 152.08, 152.07, 152.0, 151.42, 151.38, 143.5, 143.4, 141.7, 136.23, 136.21, 135.5, 135.4, 125.6, 125.3, 122.6, 122.5, 118.7, 118.5, 117.9, 117.2, 115.4, 114.7, 114.5, 113.8, 112.9, 110.9, 110.7, 109.6, 106.0, 84.1, 80.7, 65.0, 62.7, 56.23, 56.22, 55.9, 50.5, 48.8, 32.5, 30.6, 24.9, 24.4, 18.7, 18.5 ppm.

HRMS (FD, m/z): calcd for $[\text{C}_{47}\text{H}_{43}\text{BrN}_2\text{O}_{16}]^{+*}$ (M) $^{+*}$, 970.1796; found, 970.1794.

Scheme 2.6. Synthesis of Model Compound **1**.



4-(4-((5-formylfuran-2-yl)oxy)-3-methoxyphenyl)-4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)carbonyl)oxy) butyl 2-bromo-2-methylpropanoate (S8). A two-neck round bottom flask equipped with a stir bar was charged with furaldehyde **6** (295 mg, 0.647 mmol), DCM (8 mL), and pyridine (131 μL , 1.63 mmol). The solution was cooled to 0 $^{\circ}\text{C}$ in an ice bath before adding a solution of 4-methylcoumarin-7-chloroformate²⁵ (308 mg, 1.29 mmol) in DCM (5 mL) dropwise. The mixture was then warmed to room temperature, and stirred for 1 h. Afterward, the mixture was diluted with DCM (100 mL) and washed consecutively with 10% NH_4Cl (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (35–50% EtOAc/hexanes) to afford the title compound as a foamy pink solid (364 mg, 85% yield).

TLC (50% ethyl acetate/hexanes): R_f = 0.20

^1H NMR (500 MHz, CDCl_3) δ : 9.35 (s, 1H), 7.60 (d, J = 8.7 Hz, 1H), 7.23 – 7.16 (m, 3H), 7.14 (dd, J = 8.7, 2.4 Hz, 1H), 7.05 (d, J = 2.0 Hz, 1H), 7.01 (dd, J = 8.2, 2.0 Hz, 1H), 6.26 (s, 1H), 5.70 (t, J = 6.8 Hz, 1H), 5.48 (d, J = 3.8 Hz, 1H), 4.33 – 4.16 (m, 2H), 3.84 (s, 3H),

2.43 (s, 3H), 2.23 – 2.13 (m, 1H), 2.07 – 2.00 (m, 1H), 1.92 – 1.82 (m, 7H), 1.78 – 1.72 (m, 1H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 175.6, 171.6, 163.1, 160.3, 154.1, 153.1, 152.1, 151.9, 151.0, 144.6, 142.6, 138.0, 125.9, 125.6, 121.4, 119.1, 118.0, 117.3, 114.6, 111.4, 109.7, 89.0, 80.5, 65.0, 56.2, 56.0, 32.6, 30.7, 24.5, 18.7 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{31}\text{H}_{30}\text{BrO}_{11}]^+ (\text{M}+\text{H})^+$, 657.0966; found, 657.0971.

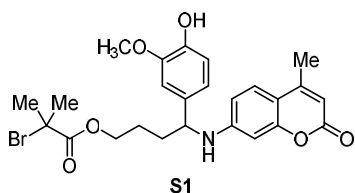
4-(3-methoxy-4-((5-(((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)oxy)methyl)furan-2-yl)oxy)phenyl)-4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)carbonyl)oxy)butyl 2-bromo-2-methylpropanoate (1). A flame-dried two-neck round bottom flask equipped with a stir bar was charged with furaldehyde **S8** (38.0 mg, 0.0578 mmol), DCM (0.5 mL), and anhydrous methanol (0.5 mL). The solution was cooled to $-40\text{ }^\circ\text{C}$ in an acetonitrile/dry ice bath before adding NaBH_4 (6.6 mg, 0.17 mmol). The mixture was kept at $-40\text{ }^\circ\text{C}$ for 2 h before being quenched with 10% NH_4Cl (5 mL) and subsequently warmed to room temperature. The solution was then extracted with DCM (3 x 50 mL) and the organic phase was washed with brine (20 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced pressure. The crude product was then combined with 4-methylcoumarin-7-isocyanate (12.0 mg, 0.060 mmol) and DCM (1 mL). The solution was cooled to $0\text{ }^\circ\text{C}$ in an ice bath before adding DMAP (0.35 mg, 0.00286 mmol). The mixture was warmed to room temperature and stirred for 1 h. The mixture was then diluted with DCM (3 mL) and filtered through celite to remove insoluble 7-amino-4-methylcoumarin. The filtrate was concentrated, dissolved in a small amount of DCM (0.3 mL), and then added into a mixture of diethyl ether (3 mL) and hexanes (6 mL) causing a white precipitate to form. The white solid was isolated using a pipette, washed with hexanes, and finally dried under high vacuum to afford the title compound (23 mg, 47% yield).

TLC (60% ethyl acetate/hexanes): $R_f = 0.33$

^1H NMR (400 MHz, CDCl_3) δ : 7.59 (d, $J = 8.7$ Hz, 1H), 7.51 (d, $J = 8.4$ Hz, 1H), 7.45 (d, $J = 2.2$ Hz, 1H), 7.35 (dd, $J = 8.8, 2.2$ Hz, 1H), 7.18 (d, $J = 2.4$ Hz, 1H), 7.13 (dd, $J = 8.7, 2.4$ Hz, 1H), 7.09 (s, 1H), 7.06 – 7.00 (m, 2H), 6.95 (dd, $J = 8.4, 2.0$ Hz, 1H), 6.42 (d, $J = 3.3$ Hz, 1H), 6.27 (s, 1H), 6.18 (s, 1H), 5.68 (t, $J = 6.8$ Hz, 1H), 5.44 (d, $J = 3.3$ Hz, 1H), 5.08 (s, 2H), 4.29 – 4.16 (m, 2H), 3.91 (s, 3H), 2.41 (d, $J = 10.1$ Hz, 6H), 2.23 – 2.11 (m, 1H), 2.07 – 1.98 (m, 1H), 1.93 (s, 6H), 1.87 – 1.81 (m, 1H), 1.78 – 1.70 (m, 1H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 171.7, 161.3, 160.5, 157.3, 154.4, 154.1, 153.2, 152.8, 152.5, 152.3, 152.0, 150.3, 145.1, 141.6, 141.2, 136.0, 125.6, 125.4, 119.1, 118.8, 118.1, 117.4, 115.5, 114.7, 114.6, 113.11, 113.07, 111.2, 109.9, 106.0, 88.6, 80.8, 65.2, 59.2, 56.3, 56.0, 32.6, 30.8, 24.6, 18.8, 18.7 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{42}\text{H}_{38}\text{BrNNaO}_{14}]^+$ ($\text{M}+\text{Na}$) $^+$, 882.1368; found, 882.1366.



4-(4-hydroxy-3-methoxyphenyl)-4-((4-methyl-2-oxo-2H-chromen-7-yl)amino)butyl 2-bromo-2-methylpropanoate (S1). Compound 1 (133.0 mg, 0.155 mmol) was dissolved in a 3:1 (v/v) mixture of acetonitrile/methanol (1 mL) and stirred at room temperature. After 48 h, the reaction mixture was concentrated under reduced pressure and the crude mixture was purified by flash chromatography (40–50% EtOAc/hexanes) followed by a reverse-phase chromatographic separation on a C18 column (55–65% acetonitrile/ H_2O) to afford the title compound as a yellow solid (5.1 mg, 6% yield).

TLC (50% ethyl acetate/hexane): $R_f = 0.33$

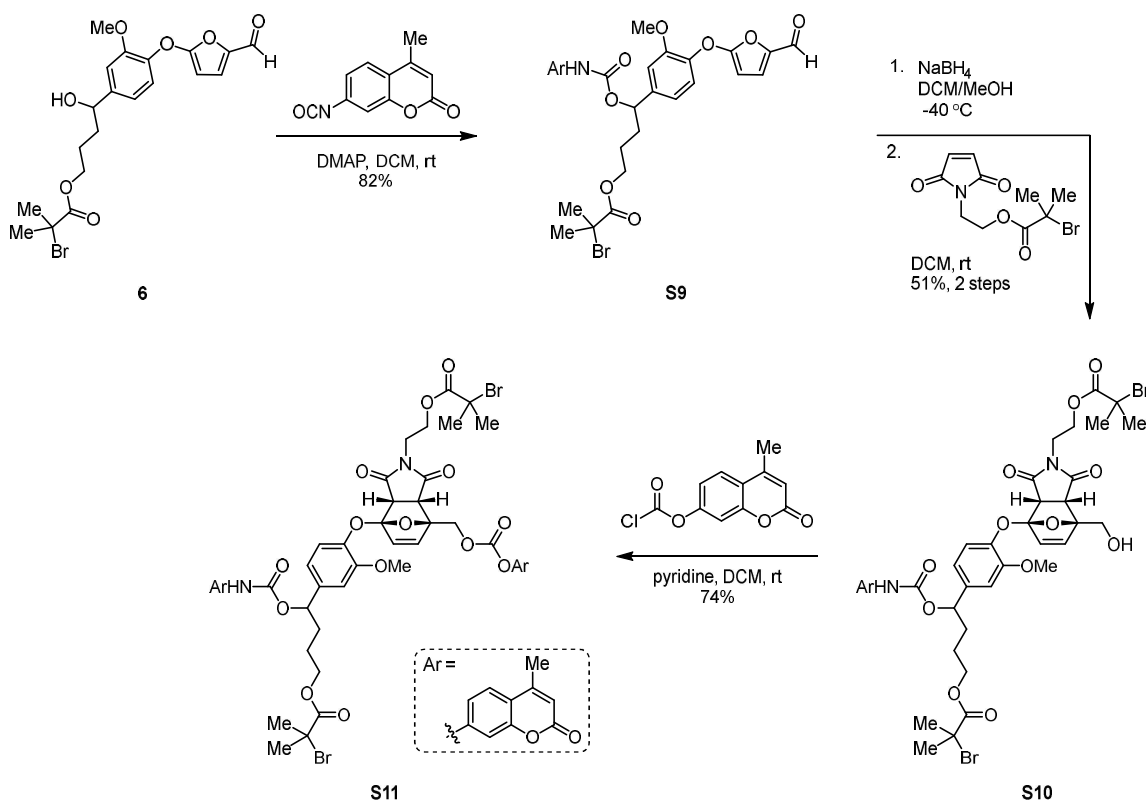
^1H NMR (400 MHz, CDCl_3) δ : 7.23 (d, $J = 8.7$ Hz, 1H), 6.80 (d, $J = 8.0$ Hz, 1H), 6.77 – 6.70 (m, 2H), 6.42 (dd, $J = 8.7, 2.3$ Hz, 1H), 6.31 (d, $J = 2.3$ Hz, 1H), 5.88 (d, $J = 1.2$ Hz, 1H),

5.49 (s, 1H), 4.55 (br s, 1H), 4.25 (t, $J = 7.2$ Hz, 1H), 4.14 (t, $J = 6.1$ Hz, 2H), 3.80 (s, 3H), 2.23 (d, $J = 1.2$ Hz, 3H), 1.96 – 1.59 (m, 10H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ : 171.8, 162.1, 155.8, 153.1, 150.6, 147.1, 145.1, 134.1, 125.5, 119.1, 114.8, 111.0, 110.9, 109.8, 108.6, 99.4, 65.4, 57.7, 56.1, 34.7, 30.9, 29.8, 25.5, 18.7 ppm.

HRMS (ESI, m/z): calcd for $[\text{C}_{25}\text{H}_{29}\text{BrNO}_6]^+$ ($\text{M}+\text{H}$) $^+$, 518.1173; found, 518.1176.

Scheme 2.7. Synthesis of Bis-Initiator **S11** with Opposite Positions of Cargo Loading.



4-(4-((5-formylfuran-2-yl)oxy)-3-methoxyphenyl)-4-(((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)oxy) butyl 2-bromo-2-methylpropanoate (S9**).** A round bottom flask equipped with a stir bar was charged with compound **6** (400 mg, 0.881 mmol), 4-methylcoumarin-7-isocyanate (265 mg, 1.318 mmol), and DCM (18 mL). The solution was cooled to 0°C in an ice bath before adding DMAP (1.1 mg, 0.0090 mmol). The mixture was

warmed to room temperature and stirred for 1 h. The mixture was then diluted with DCM (150 mL), washed with 10% NH₄Cl (20 mL), and brine (20 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (30–50% EtOAc/hexanes) to afford the title compound as a foamy white solid (474 mg, 82% yield).

TLC (60% ethyl acetate/hexanes): $R_f = 0.38$

¹H NMR (500 MHz, CDCl₃) δ : 9.30 (s, 1H), 8.05 (s, 1H), 7.54 – 7.46 (m, 3H), 7.17 (d, $J = 3.8$ Hz, 1H), 7.11 (d, $J = 8.2$ Hz, 1H), 7.02 (d, $J = 1.9$ Hz, 1H), 6.95 (dd, $J = 8.2, 1.9$ Hz, 1H), 6.14 (s, 1H), 5.76 (t, $J = 5.4$ Hz, 1H), 5.41 (d, $J = 3.8$ Hz, 1H), 4.26 – 4.09 (m, 2H), 3.80 (s, 3H), 2.38 (s, 3H), 2.09 – 1.94 (m, 1H), 1.98 – 1.88 (m, 7H), 1.86 – 1.68 (m, 2H) ppm.

¹³C{¹H} NMR (125 MHz, CDCl₃) δ : 175.5, 171.7, 163.5, 161.4, 154.3, 152.8, 152.5, 150.8, 144.5, 142.0, 141.9, 139.7, 125.4, 121.3, 118.7, 115.4, 114.6, 112.8, 111.3, 105.9, 88.8, 76.1, 65.2, 56.1, 32.8, 30.7, 24.6, 18.6 ppm.

HRMS(FD, m/z): calcd for [C₃₁H₃₀BrNO₁₀]⁺⁺ (M)⁺⁺, 655.1053; found, 655.1075.

2-(4-(4-(4-((2-bromo-2-methylpropanoyl)oxy)-1-(((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)oxy)butyl)-2-methoxyphenoxy)-7-(hydroxymethyl)-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-epoxyisoindol-2-yl)ethyl 2-bromo-2-methylpropanoate (S10). A flame-dried two-neck round bottom flask equipped with a stir bar was charged with compound **S9** (76.1 mg, 0.116 mmol), DCM (1.6 mL), and anhydrous methanol (1.6 mL). The solution was cooled to –40 °C in an acetonitrile/dry ice bath before adding NaBH₄ (13.2 mg, 0.347 mmol). The mixture was kept at –40 °C for 2 h before being quenched with 10% NH₄Cl (5 mL) and subsequently warmed to room temperature. The solution was then extracted with DCM (3 x 50 mL) and the organic phase was washed with brine (20 mL). The organic layer was dried over Na₂SO₄, and filtered. To the filtrate was then added 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl 2-bromo-2-methylpropanoate⁴¹ (the maleimide ester) (50.2 mg, 0.174 mmol), which was concentrated under reduced

pressure until ~2 mL of viscous solution remained. The mixture was then allowed to react at room temperature for 12 h. The crude mixture was purified by column chromatography (60–80% EtOAc/hexanes) to afford the title compound (mixture of diastereomers) as a colorless liquid (56 mg, 51% yield).

TLC (67% ethyl acetate/hexanes): $R_f = 0.36$

^1H NMR (500 MHz, CDCl_3) δ : 7.63 (d, $J = 2.8$ Hz, 1H), 7.53 – 7.39 (m, 3H), 7.19 (dd, $J = 8.3, 1.7$ Hz, 1H), 6.94 (t, $J = 1.7$ Hz, 1H), 6.83 (d, $J = 8.4$ Hz, 1H), 6.41 – 6.33 (m, 2H), 6.14 (d, $J = 1.4$ Hz, 1H), 5.72 (t, $J = 6.7$ Hz, 1H), 4.24 – 4.08 (m, 6H), 3.89 (s, 3H), 3.74 – 3.61 (m, 4H), 2.70 (br s, 1H), 2.36 (s, 3H), 2.07 – 1.95 (m, 2H), 1.90 (s, 6H), 1.87 (s, 6H), 1.80 – 1.64 (m, 2H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 174.4, 173.2, 171.8, 171.5, 161.4, 154.4, 152.7, 152.5, 151.6, 151.5, 143.2, 141.7, 137.70, 137.68, 136.02, 135.97, 135.6, 125.5, 123.3, 123.2, 118.5, 115.5, 114.6, 114.1, 114.0, 113.0, 111.23, 111.19, 105.9, 86.6, 86.5, 65.3, 62.5, 61.58, 61.56, 56.5, 56.1, 55.6, 50.8, 48.93, 48.87, 37.6, 32.63, 32.59, 30.79, 30.75, 30.7, 24.7, 18.7 ppm.

HRMS (FD, m/z): calcd for $[\text{C}_{41}\text{H}_{45}\text{Br}_2\text{N}_2\text{O}_{14}]^+$ ($\text{M}+\text{H}$) $^+$, 947.1237; found, 947.1230.

2-(4-(4-(4-((2-bromo-2-methylpropanoyl)oxy)-1-(((4-methyl-2-oxo-2H-chromen-7-yl)carbamoyl)oxy)butyl)-2-methoxyphenoxy)-7-((((4-methyl-2-oxo-2H-chromen-7-yl)oxy)carbonyl)oxy)methyl)-1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-epoxyisoindol-2-yl)ethyl 2-bromo-2-methylpropanoate (S11). A two-neck round bottom flask equipped with a stir bar was charged with compound **S10** (112 mg, 0.118 mmol), DCM (1.0 mL), and pyridine (20 μL , 0.25 mmol). The solution was cooled to 0 °C in an ice bath before adding a solution of 4-methylcoumarin-7-chloroformate²⁵ (36.5 mg, 0.153 mmol) in DCM (1.5 mL) dropwise. The mixture was then warmed to room temperature, and stirred for 1 h. The mixture was then diluted with DCM (100 mL), washed with 10% NH_4Cl (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4 , filtered, and concentrated under reduced

pressure. The crude material was purified by column chromatography on silica gel (50–70% EtOAc/hexanes) to afford the title compound (mixture of diastereomers) as a foamy white solid (100 mg, 74% yield).

TLC (75% ethyl acetate/hexanes): $R_f = 0.39$

^1H NMR (400 MHz, CDCl_3) δ : 8.14 (br s, 0.5H), 7.93 (br s, 0.5H), 7.61 (t, $J = 8.2$ Hz, 1H), 7.51 – 7.36 (m, 3H), 7.22 – 7.11 (m, 3H), 7.04 – 6.84 (m, 2H), 6.50 – 6.37 (m, 2H), 6.28 (dd, $J = 10.7, 1.4$ Hz, 1H), 6.11 (s, 1H), 5.77 (q, $J = 6.8$ Hz, 1H), 4.97 – 4.69 (m, 2H), 4.22 (t, $J = 5.2$ Hz, 2H), 4.18 – 4.10 (m, 2H), 3.90 (s, 3H), 3.85 – 3.63 (m, 4H), 2.48 – 2.28 (m, 6H), 2.05 – 1.85 (m, 14H), 1.81 – 1.67 (m, 2H) ppm.

$^{13}\text{C}\{^1\text{H}\}$ NMR (125 MHz, CDCl_3) δ : 173.5, 172.6, 171.7, 171.6, 171.4, 161.32, 161.25, 160.7, 160.6, 154.3, 154.0, 153.1, 152.63, 152.58, 152.57, 152.55, 152.5, 152.4, 152.3, 151.64, 151.59, 142.91, 142.88, 142.0, 141.9, 138.6, 138.3, 136.2, 136.1, 135.3, 135.19, 135.18, 125.9, 125.8, 125.33, 125.30, 123.8, 123.5, 118.3, 118.2, 118.07, 118.06, 117.9, 117.63, 117.59, 115.31, 115.26, 114.74, 114.68, 114.51, 114.48, 114.14, 114.10, 112.9, 112.8, 111.2, 111.0, 110.1, 109.92, 109.90, 105.9, 105.8, 83.7, 83.5, 76.2, 76.0, 66.2, 66.13, 66.11, 65.3, 62.50, 62.47, 56.43, 56.39, 56.1, 56.0, 55.6, 50.5, 50.3, 49.8, 49.7, 37.7, 37.64, 37.62, 33.1, 32.9, 30.73, 30.70, 30.67, 24.61, 24.58, 18.83, 18.82, 18.6 ppm.

HRMS (FD, m/z): calcd for $[\text{C}_{52}\text{H}_{50}\text{Br}_2\text{N}_2\text{O}_{18}]^{++} (\text{M})^{++}$, 1148.1425; found, 1148.1436.

General Procedure A for the Synthesis of Poly(Methyl Acrylate) (PMA) Polymers.

Polymers were synthesized by controlled radical polymerization following an adapted procedure by Nguyen *et al.*³⁵ A flame-dried Schlenk flask equipped with a stir bar was charged with the initiator, DMSO, Me_6TREN , and methyl acrylate. The flask was sealed, the solution was deoxygenated with three freeze-pump-thaw cycles, and then backfilled with nitrogen. The flask was opened briefly under a flow of N_2 , and freshly cut copper wire (1.0 cm length, 20 gauge) was added on top of the frozen mixture. The flask was resealed, evacuated for an additional 15 min, warmed to room temperature, and then backfilled with

nitrogen. The mixture was stirred at room temperature until the solution became sufficiently viscous, indicating that the desired monomer conversion was reached. Following completion of the polymerization, the flask was then opened to air and the solution was diluted with a minimal amount of DCM. The polymer was precipitated 3× into methanol cooled with dry ice and then dried under vacuum to afford the polymer. GPC traces for each polymer used in this study are shown in **Figure 2.16**.

PMA-1. Synthesized using general procedure A with initiator **8** (10.6 mg, 9.23 μmol), DMSO (1.35 mL), Me₆TREN (4.9 μL , 18 μmol), and methyl acrylate (1.35 mL, 14.9 mmol). Polymerization for 3.5 h provided the title polymer as a white solid (633 mg, 49% yield). $M_n = 106 \text{ kDa}$, $\bar{D} = 1.13$.

PMA-Control. Synthesized using general procedure A with initiator **S9** (10.0 mg, 10.3 μmol), DMSO (1.19 mL), Me₆TREN (5.5 μL , 21 μmol), and methyl acrylate (1.19 mL, 13.1 mmol). Polymerization for 3 h provided the title polymer as a white solid (683 mg, 60% yield). $M_n = 98 \text{ kDa}$, $\bar{D} = 1.15$.

PMA-2. Synthesized using general procedure A with initiator **S13** (10.8 mg, 9.41 μmol), DMSO (1.16 mL), Me₆TREN (5.0 μL , 19 μmol), and methyl acrylate (1.16 mL, 12.8 mmol). Polymerization for 1.75 h provided the title polymer as a white solid (570 mg, 52% yield). $M_n = 120 \text{ kDa}$, $\bar{D} = 1.35$.

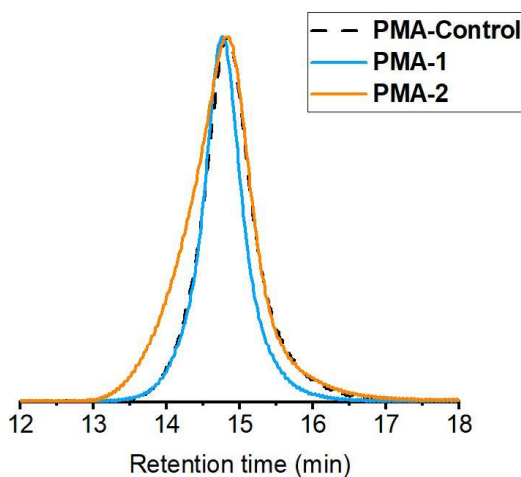


Figure 2.16. GPC traces (RI response) for the three polymers used in this study: **PMA-control** ($M_n = 98 \text{ kDa}$, $\bar{D} = 1.15$), **PMA-1** ($M_n = 106 \text{ kDa}$, $\bar{D} = 1.13$), and **PMA-2** ($M_n = 120 \text{ kDa}$, $\bar{D} = 1.35$).

2.4.3 General procedure for ultrasonication experiments

An oven-dried sonication vessel was fitted with rubber septa, placed onto the sonication probe, and allowed to cool under a stream of dry argon. The vessel was charged with a solution of the polymer in acetonitrile/methanol (3:1 v/v, 2.0 mg/mL, 20 mL) and submerged in an ice bath. The solution was sparged continuously with argon beginning 15 min prior to sonication and for the duration of the sonication experiment. Pulsed ultrasound (1 s on/1 s off, 30% amplitude, 20 kHz, 16.4 W/cm²) was then applied to the system. The solution temperature during sonication was measured to be approximately 12–13 °C. Sonicated solutions were filtered through a 0.45 µm syringe filter prior to analysis. Ultrasonic intensity was calibrated using the method described by Berkowski *et al.*³⁶

2.4.4 Characterization of aminocoumarin release using photoluminescence spectroscopy

Aliquots from the sonication experiments were added to a quartz microcuvette. The samples were then monitored with fluorescence spectroscopy at room temperature. Emission spectra were recorded using an excitation wavelength of 365 nm, which selectively excited

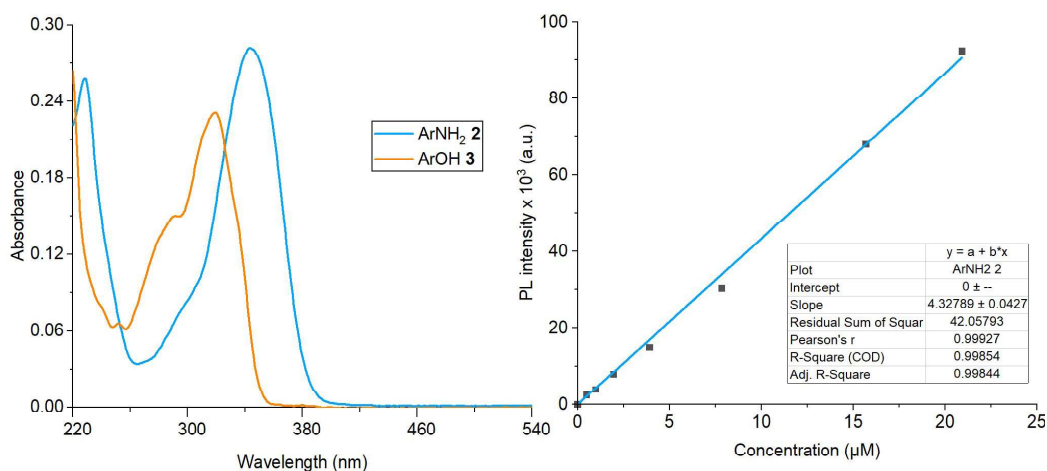


Figure 2.17. a) UV-vis absorption spectra of ArNH₂ 2 and ArOH 3 in acetonitrile/methanol (3:1 v/v). b) Calibration curve for experimental determination of the concentration of ArNH₂ 2 (λ_{ex} = 365 nm, λ_{em} = 424 nm) in acetonitrile/methanol (3:1 v/v). A linear regression of the data gives the calibration function: Y = 4328X.

aminocoumarin **2** in the presence of hydroxycoumarin **3**. A standard calibration curve was constructed to determine the concentration of aminocoumarin **2** (Figure 2.17).

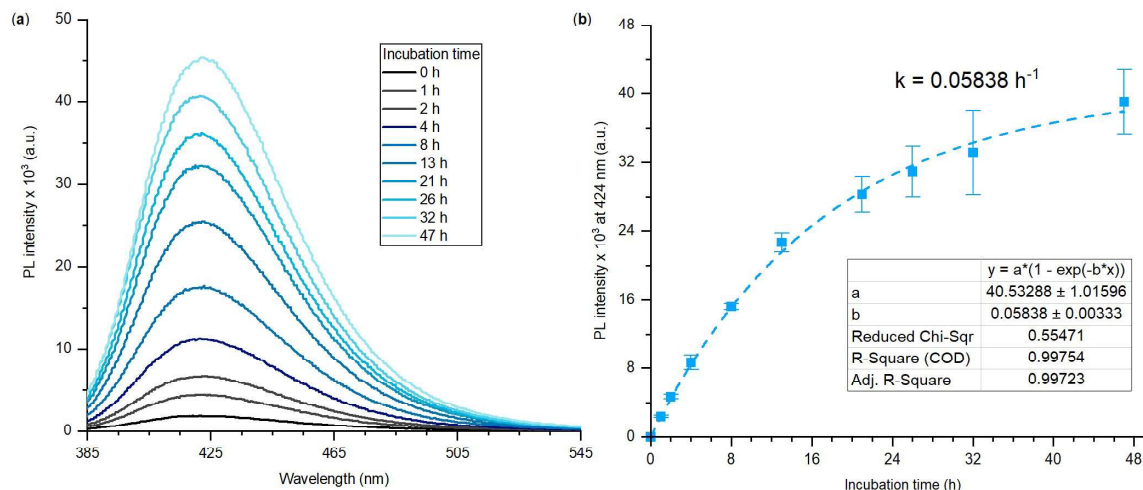


Figure 2.18. Characterization of the release of ArNH₂ **2** from a 2.0 mg/mL solution of **PMA-1** in acetonitrile/methanol (3:1 v/v) by PL spectroscopy immediately after ultrasonication for 60 min (sonication “on” time). a) Representative PL spectra of the sonicated solution at room temperature recorded at the indicated times post-sonication. b) Fluorescence intensity at 424 nm as a function of time. The background fluorescence at the start of monitoring experiment (resulting from the release of ArNH₂ **2** during ultrasonication) was subtracted from each measurement. Error bars represent standard deviation from three replicate experiments.

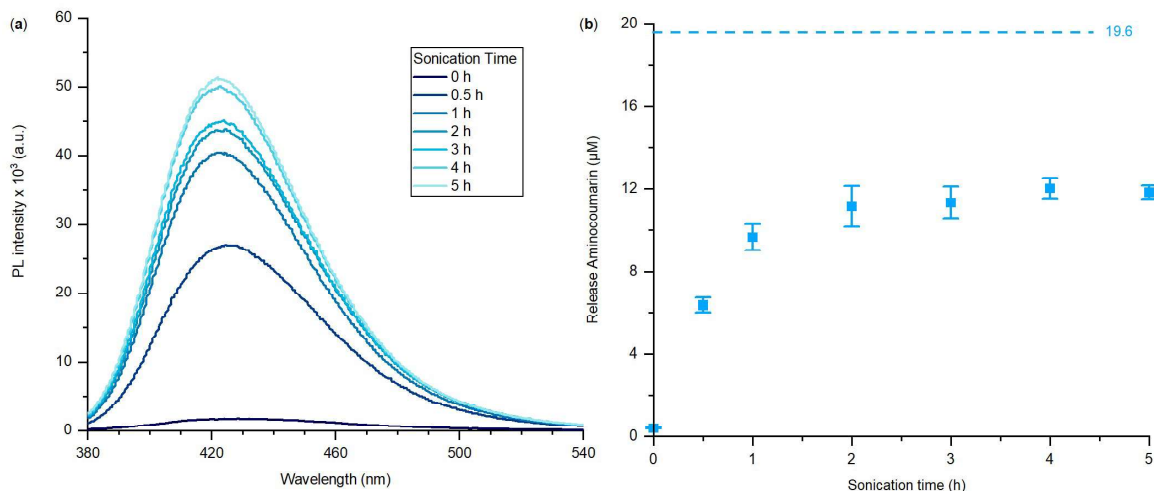


Figure 2.19. a) Representative PL spectra characterizing the release of ArNH₂ **2** from **PMA-1** (2.0 mg/mL in 3:1 MeCN/MeOH) as a function of sonication time. Aliquots were kept at room temperature for 48 h prior to analysis. $\lambda_{\text{ex}} = 365 \text{ nm}$. b) Measured concentration of ArNH₂ **2** released from **PMA-1** as a function of ultrasonication time calculated from the fluorescence intensity at 424 nm using a standard calibration curve. The theoretical concentration of ArNH₂ **2** based on 100% release from the mechanophore is 19.6 μM . Error bars represent standard deviation from three replicate experiments.

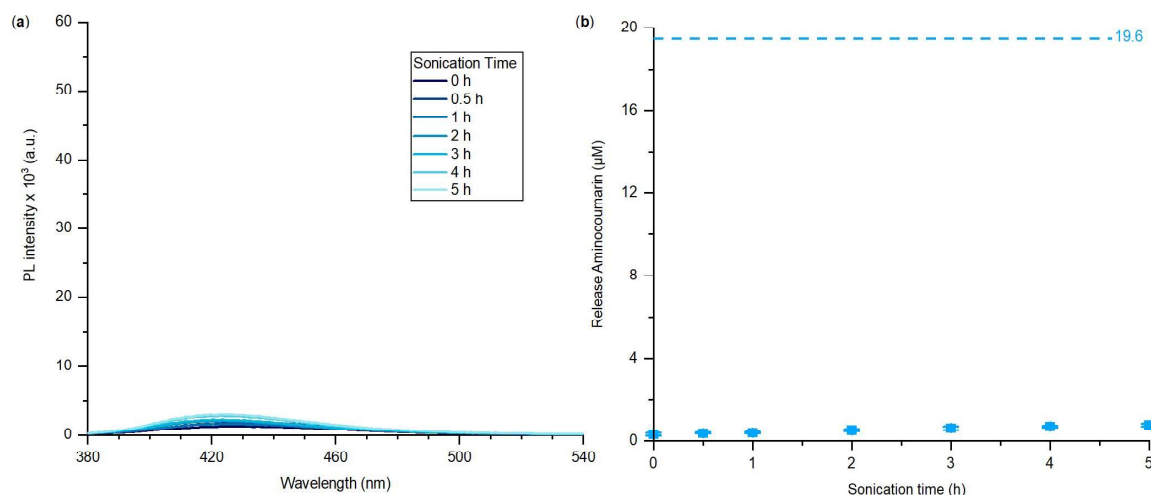


Figure 2.20. a) Representative PL spectra characterizing the release of ArNH₂ **2** from **PMA-Control** (2.0 mg/mL in 3:1 MeCN/MeOH) as a function of sonication time. Aliquots were kept at room temperature for 48 h prior to analysis. $\lambda_{\text{ex}} = 365$ nm. b) Measured concentration of ArNH₂ **2** released from **PMA-Control** as a function of ultrasonication time calculated from the fluorescence intensity at 424 nm using a standard calibration curve. The theoretical concentration of ArNH₂ **2** based on 100% release from the mechanophore is 19.6 μM. Error bars represent standard deviation from three replicate experiments.

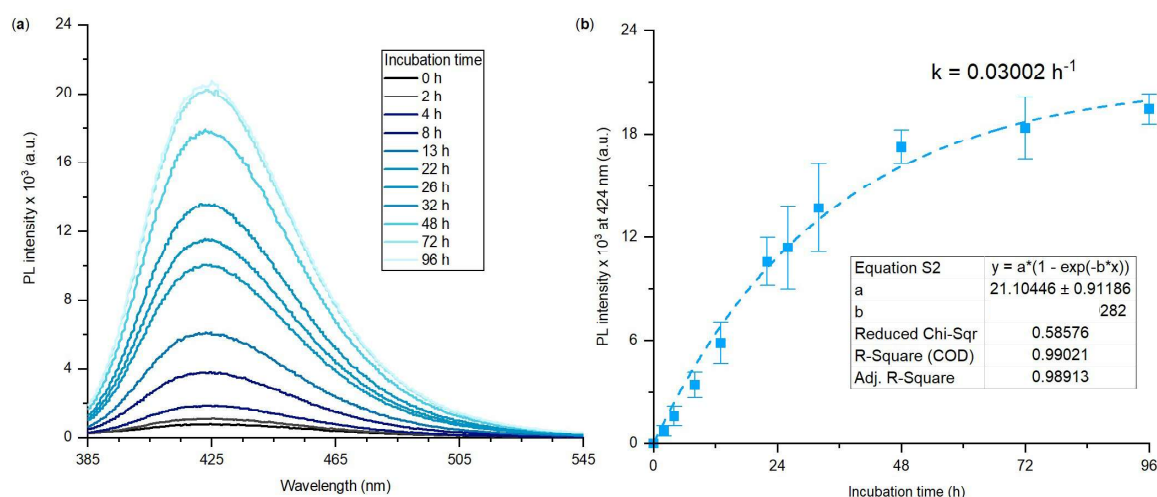


Figure 2.21. Characterization of the release of ArNH₂ **2** from a 2.0 mg/mL solution of **PMA-2** in acetonitrile/methanol (3:1 v/v) by PL spectroscopy immediately after ultrasonication for 60 min (sonication “on” time). a) Representative PL spectra of the sonicated solution at room temperature recorded at the indicated times post-sonication. b) Fluorescence intensity at 424 nm as a function of time. The background fluorescence at the start of monitoring experiment (resulting from the release of ArNH₂ **2** during ultrasonication) was subtracted from each measurement. Error bars represent standard deviation from three replicate experiments.

2.4.5. Characterization of molecular release using HPLC

Calculation of relative response factors (RRF). A standard solution with known concentrations of an internal standard (IS, benzocaine) and small molecule analytes (ArNH₂ **2** and ArOH **3**) was prepared and analyzed by HPLC equipped with a UV detector ($\lambda = 310$ nm). The RRF is calculated from the HPLC results of the standard solution using **eq. 2.1** and was determined to be 0.72 and 1.76, for ArNH₂ **2** and ArOH **3**, respectively.

$$\text{RRF} = \frac{\text{response factor of the analyte}}{\text{response factor of the IS}} = \left(\frac{\text{peak area of analyte}}{\text{concentration of analyte}} \right) / \left(\frac{\text{peak area of IS}}{\text{concentration of IS}} \right) \quad (2.1)$$

Determination of the concentration of ArNH₂ **2 and ArOH **3** released from polymers after ultrasound-induced mechanochemical activation.** Aliquots were removed during ultrasonication experiments and benzocaine (15.28 μM) was added as the internal standard. The solution was then kept at room temperature and analyzed by HPLC at various time intervals (HPLC conditions: 22:78 MeCN/water + 0.1% AcOH, 2 mL/min, $\lambda = 310$ nm). The concentration of ArNH₂ **2** and ArOH **3** (analytes) in the solution was calculated using **eq. 2.2**. A representative HPLC chromatogram is shown below in **Figure 2.22**. Data are provided below in **Tables 2.1–2.8**.

$$\text{concentration of analyte} = \frac{\text{peak area of analyte}}{\text{peak area of IS}} * \frac{1}{\text{RRF}} * \text{concentration of IS} \quad (2.2)$$

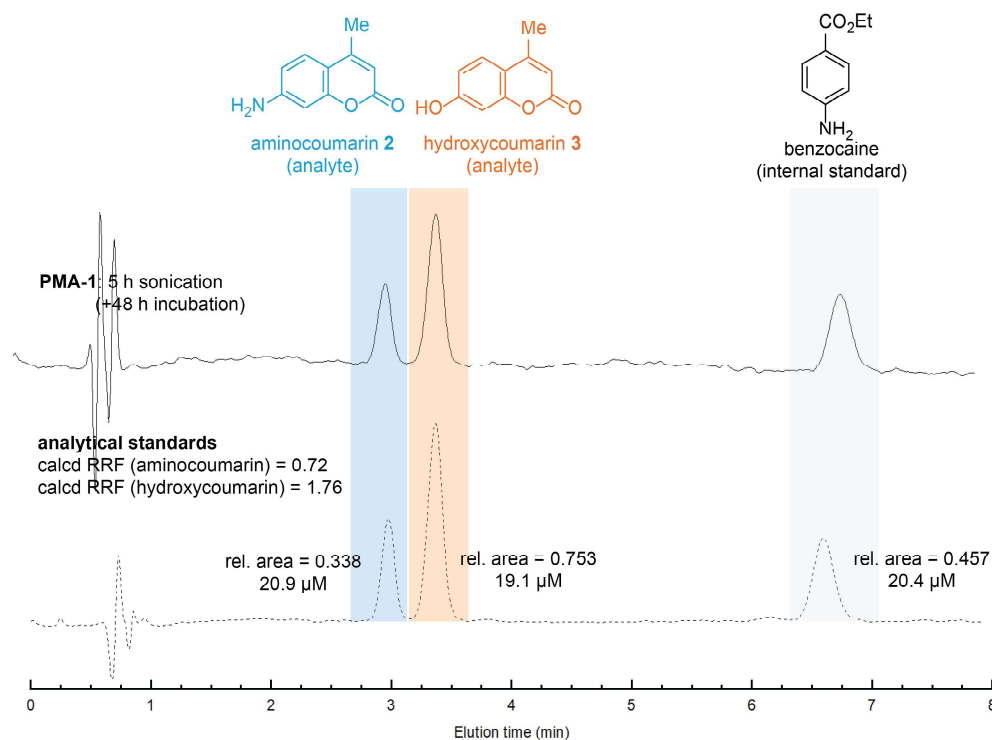


Figure 2.22. Representative HPLC chromatograms for the analysis of mechanically triggered release of ArNH₂ (**2**) and ArOH (**3**) from **PMA-1** containing a chain-centered mechanophore. HPLC conditions: 22:78 MeCN/water + 0.1% AcOH, isocratic, 2 mL/min, λ = 310 nm.

Table 2.1. Release of ArNH₂ **2** from **PMA-1** after 60 min of ultrasonication monitored by HPLC.

	Trial 1			Trial 2			Trial 3		
Time Post-Activation (h)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)
0	0.003	0.179	0.4	0.003	0.184	0.3	0.003	0.190	0.4
1	0.006	0.178	0.7	0.009	0.190	1.1	0.009	0.192	1.0
2	0.009	0.182	1.0	0.013	0.173	1.6	0.013	0.182	1.5
4	0.016	0.165	2.0	0.016	0.174	2.0	0.020	0.193	2.2
8	0.018	0.172	2.2	0.038	0.186	4.4	0.037	0.196	4.0
13	0.040	0.179	4.7	0.046	0.170	5.7	0.048	0.193	5.2
21	0.050	0.178	6.0	0.055	0.173	6.7	0.057	0.185	6.6
26	0.061	0.190	6.8	0.058	0.173	7.1	0.065	0.175	7.8
32	0.070	0.188	7.9	0.065	0.180	7.7	0.069	0.170	8.5
47	0.073	0.174	8.8	0.075	0.175	9.1	0.072	0.170	9.0

Table 2.2. Release of ArOH **3** from **PMA-1** after 60 min of ultrasonication monitored by HPLC.

	Trial 1	Trial 2	Trial 3
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Time Post-Activation (h)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)
0	0.008	0.179	0.4	0.014	0.184	0.6	0.010	0.190	0.5
1	0.011	0.178	0.5	0.022	0.190	1.0	0.023	0.192	1.0
2	0.031	0.182	1.5	0.023	0.173	1.1	0.036	0.182	1.7
4	0.039	0.165	2.0	0.035	0.174	1.8	0.041	0.193	1.9
8	0.034	0.172	1.7	0.059	0.186	2.8	0.056	0.196	2.5
13	0.070	0.179	3.4	0.068	0.170	3.4	0.086	0.193	3.9
21	0.091	0.178	4.4	0.102	0.173	5.1	0.103	0.185	4.9
26	0.108	0.190	4.9	0.106	0.173	5.3	0.110	0.175	5.5
32	0.134	0.188	6.2	0.116	0.180	5.6	0.116	0.170	5.9
47	0.133	0.174	6.6	0.130	0.175	6.4	0.134	0.170	6.9

Table 2.3. Release of ArNH₂ 2 from PMA-1 as a function of sonication time monitored by HPLC.^a

	Trial 1			Trial 2			Trial 3		
Sonication time (h)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)
0	0.001	0.218	0.1	0.001	0.179	0.1	0.001	0.179	0.1
0.5	0.062	0.198	6.7	0.044	0.172	5.4	0.050	0.180	5.9
1	0.074	0.175	9.0	0.075	0.175	9.1	0.072	0.170	9.0
2	0.094	0.196	10.1	0.093	0.181	10.9	0.097	0.177	11.6
3	0.097	0.184	11.2	0.091	0.173	11.2	0.103	0.184	11.9
4	0.115	0.190	12.9	0.095	0.173	11.7	0.103	0.177	12.3
5	0.119	0.191	13.1	0.090	0.158	12.0	0.096	0.162	12.5

^aEach sample was incubated at rt for 48 h prior to characterization

Table 2.4. Release of ArOH 3 from PMA-1 as a function of sonication time monitored by HPLC.^a

	Trial 1			Trial 2			Trial 3		
Sonication time (h)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)
0	0.026	0.218	1.0	0.008	0.179	0.4	0.007	0.179	0.4
0.5	0.106	0.198	4.7	0.093	0.172	4.7	0.105	0.180	0.5
1	0.142	0.175	7.1	0.130	0.175	6.4	0.134	0.170	0.3
2	0.182	0.196	8.1	0.195	0.181	9.4	0.194	0.177	0.8
3	0.197	0.184	9.3	0.188	0.173	9.4	0.209	0.184	1.1
4	0.238	0.190	10.9	0.194	0.173	9.8	0.213	0.177	0.3
5	0.254	0.191	11.5	0.188	0.158	10.3	0.196	0.162	1.0

^aEach sample incubated at rt for 48 h prior to characterization

Table 2.5. Release of ArNH₂ 2 from PMA-Control as a function of sonication time monitored by HPLC.^a

	Trial 1	Trial 2	Trial 3
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Sonication time (h)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)
0	0.002	0.167	0.2	0.003	0.180	0.3	0.003	0.189	0.4
0.5	0.005	0.187	0.6	0.003	0.178	0.3	0.004	0.189	0.5
1	0.005	0.182	0.6	0.002	0.169	0.2	0.003	0.207	0.3
2	0.006	0.180	0.7	0.001	0.199	0.1	0.007	0.173	0.8
3	0.005	0.192	0.6	0.002	0.190	0.2	0.009	0.184	1.1
4	0.004	0.177	0.5	0.003	0.190	0.4	0.002	0.182	0.3
5	0.006	0.192	0.7	0.004	0.182	0.4	0.009	0.196	1.0

^aEach sample incubated at rt for 48 h prior to characterization

Table 2.6. Release of ArOH 3 from PMA-Control as a function of sonication time monitored by HPLC.^a

	Trial 1			Trial 2			Trial 3		
Sonication time (h)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)
0	0.019	0.167	1.0	0.013	0.180	0.6	0.015	0.189	0.7
0.5	0.009	0.187	0.4	0.011	0.178	0.5	0.029	0.189	1.3
1	0.014	0.182	0.7	0.021	0.169	1.1	0.014	0.207	0.6
2	0.020	0.180	1.0	0.016	0.199	0.7	0.026	0.173	1.3
3	0.026	0.192	1.2	0.023	0.190	1.1	0.021	0.184	1.0
4	0.013	0.177	0.6	0.018	0.190	0.8	0.016	0.182	0.8
5	0.014	0.192	0.7	0.017	0.182	0.8	0.031	0.196	1.4

^aEach sample incubated at rt for 48 h prior to characterization

Table 2.7. Release of ArNH₂ 2 from PMA-2 after 60 min of ultrasonication monitored by HPLC.

	Trial 1			Trial 2			Trial 3		
Time Post-Activation (h)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)	Payload peak area	IS peak area	Released payload, calcd (μM)
0	0.002	0.225	0.2	0.003	0.235	0.3	0.000	0.173	0.0
2	0.005	0.207	0.5	0.009	0.252	0.8	0.004	0.175	0.4
4	0.007	0.213	0.7	0.011	0.226	1.0	0.009	0.183	1.1
8	0.012	0.214	1.2	0.013	0.215	1.3	0.010	0.185	1.1
13	0.018	0.228	1.7	0.022	0.258	1.8	0.016	0.185	1.8
22	0.028	0.224	2.7	0.030	0.224	2.9	0.029	0.186	3.3
26	0.032	0.233	2.9	0.032	0.239	2.9	0.029	0.185	3.3
32	0.034	0.215	3.3	0.036	0.240	3.1	0.031	0.183	3.6
48	0.044	0.226	4.1	0.048	0.246	4.1	0.042	0.189	4.7
72	0.048	0.243	4.2	0.049	0.241	4.3	0.043	0.188	4.9
96	0.054	0.239	4.7	0.046	0.223	4.4	0.040	0.188	4.5

Table 2.8. Release of ArOH 3 payload from PMA-2 after 60 min of ultrasonication monitored by HPLC.

	Trial 1			Trial 2			Trial 3		
Time Post-Activation (h)	Payload peak area	IS peak area	Released payload, calcd (μ M)	Payload peak area	IS peak area	Released payload, calcd (μ M)	Payload peak area	IS peak area	Released payload, calcd (μ M)
0	0.144	0.225	5.6	0.169	0.235	6.2	0.120	0.173	6.0
2	0.131	0.207	5.5	0.178	0.252	6.2	0.124	0.175	6.2
4	0.141	0.213	5.8	0.164	0.226	6.3	0.122	0.183	5.8
8	0.140	0.214	5.7	0.151	0.215	6.1	0.126	0.185	5.9
13	0.154	0.228	5.9	0.196	0.258	6.6	0.126	0.185	5.9
22	0.152	0.224	5.9	0.166	0.224	6.4	0.128	0.186	6.0
26	0.150	0.233	5.6	0.172	0.239	6.3	0.126	0.185	5.9
32	0.147	0.215	5.9	0.184	0.240	6.6	0.131	0.183	6.2
48	0.144	0.226	5.5	0.178	0.246	6.3	0.131	0.189	6.0
72	0.164	0.243	5.9	0.183	0.241	6.6	0.134	0.188	6.2
96	0.160	0.239	5.8	0.165	0.223	6.4	0.133	0.188	6.1

2.4.6. DFT (CoGEF) calculations

CoGEF calculations were performed using Spartan '18 Parallel Suite according to previously reported methods.^{33,34} Ground state energies were calculated using DFT at the B3LYP/6-31G* level of theory. Starting from the equilibrium geometry of the unconstrained molecule (relative energy = 0 kJ/mol), the distance between the carbon atoms in the terminal methyl groups of the truncated structure was increased in increments of 0.05 Å and the energy was minimized at each step. The maximum force associated with the retro-Diels–Alder reaction was calculated from the slope of the curve immediately prior to bond cleavage.

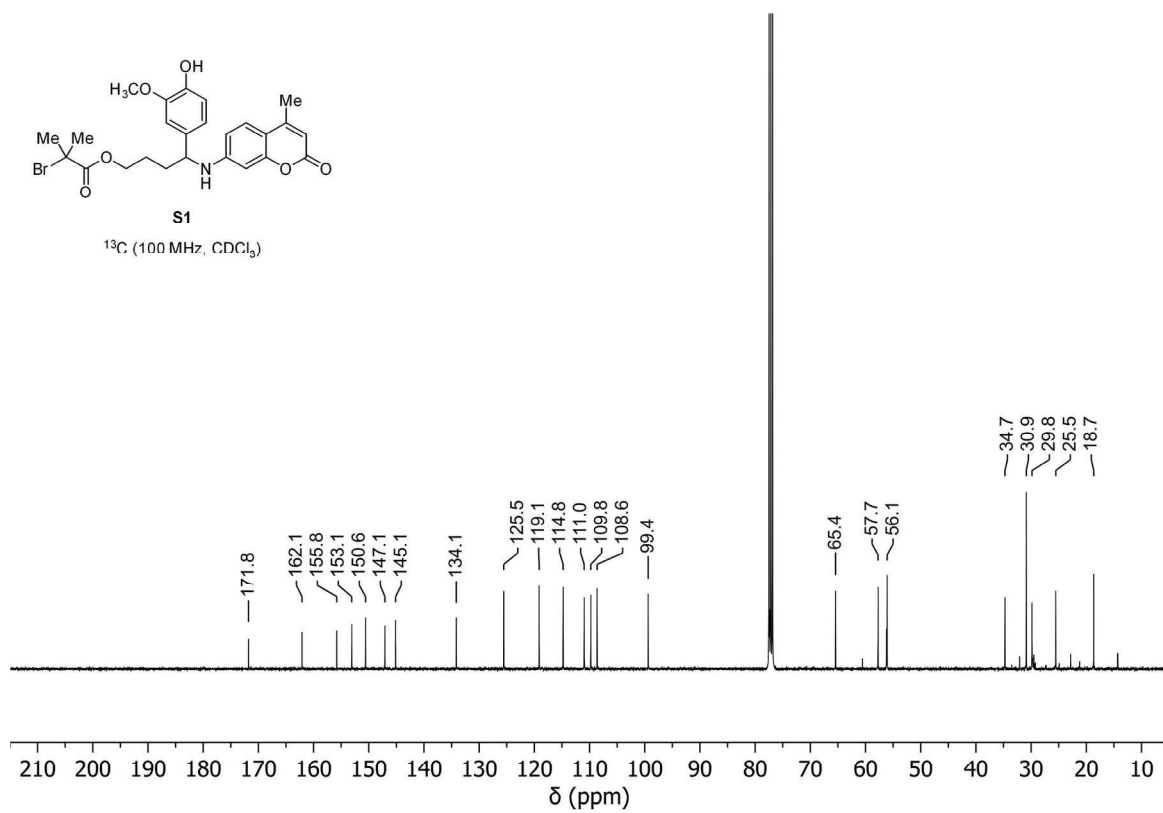
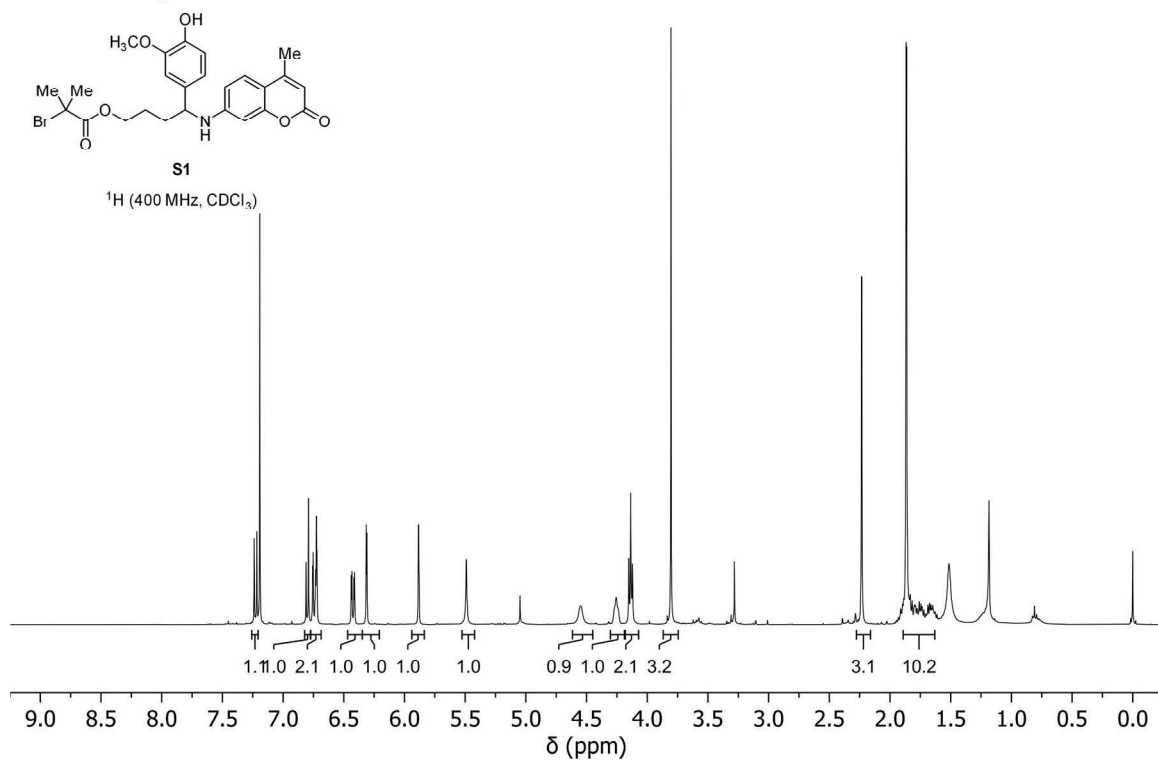
2.5 References

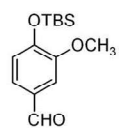
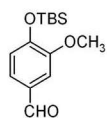
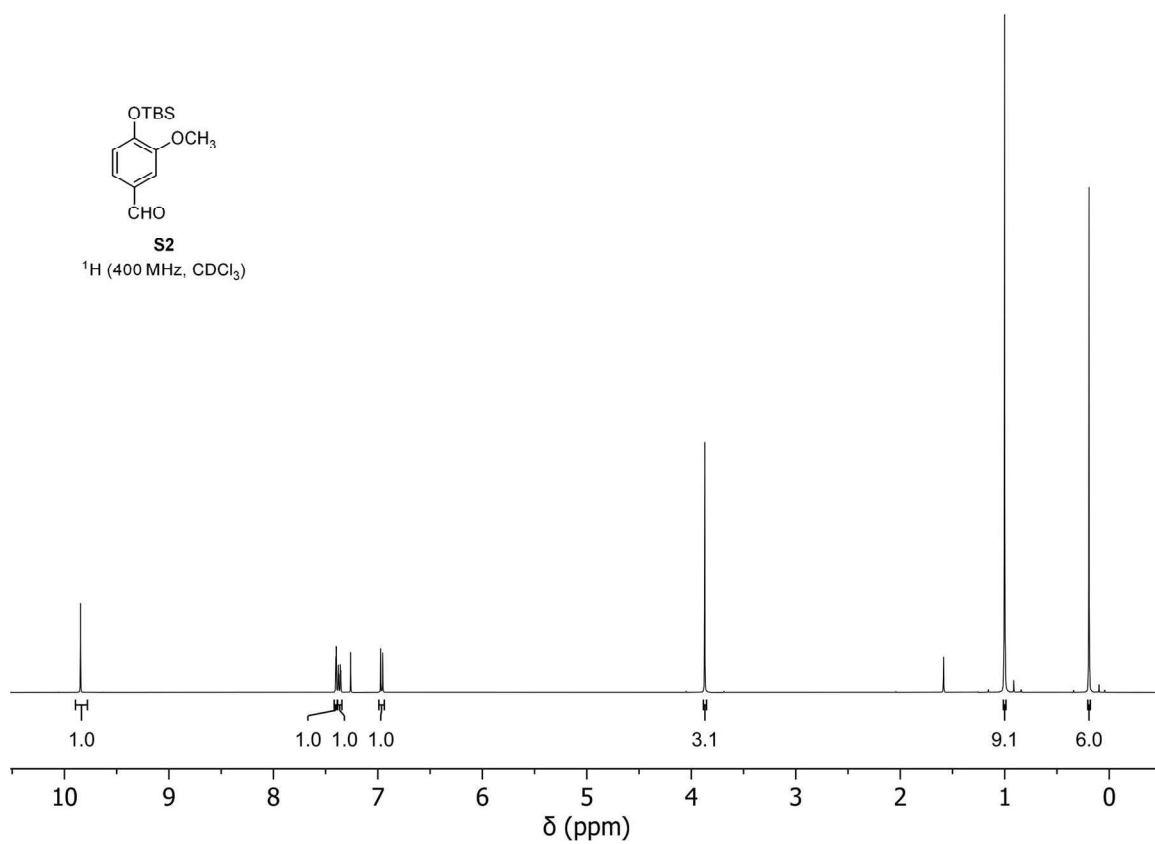
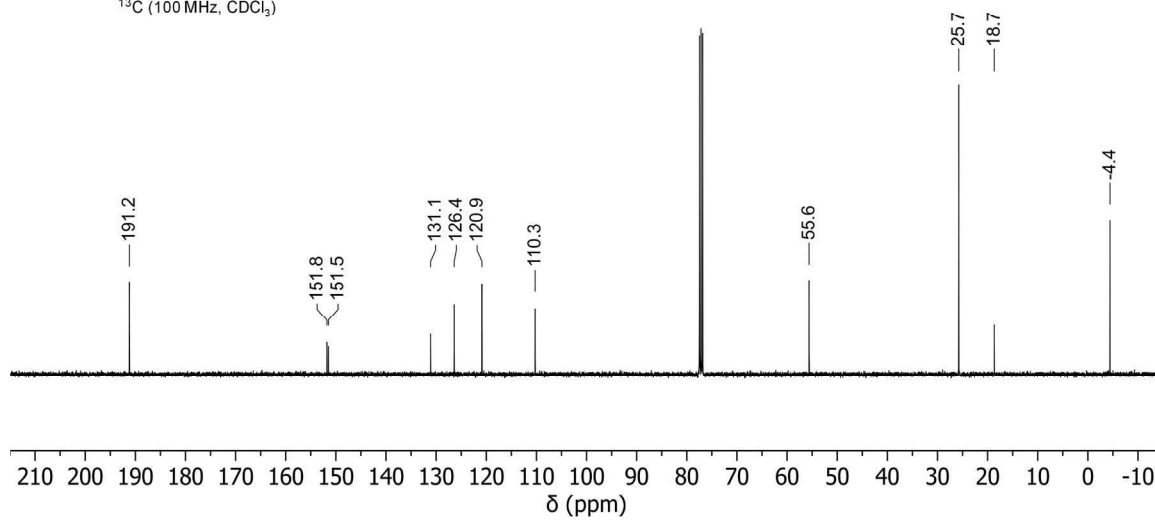
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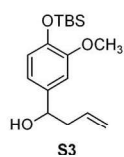
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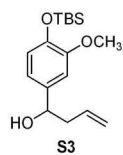
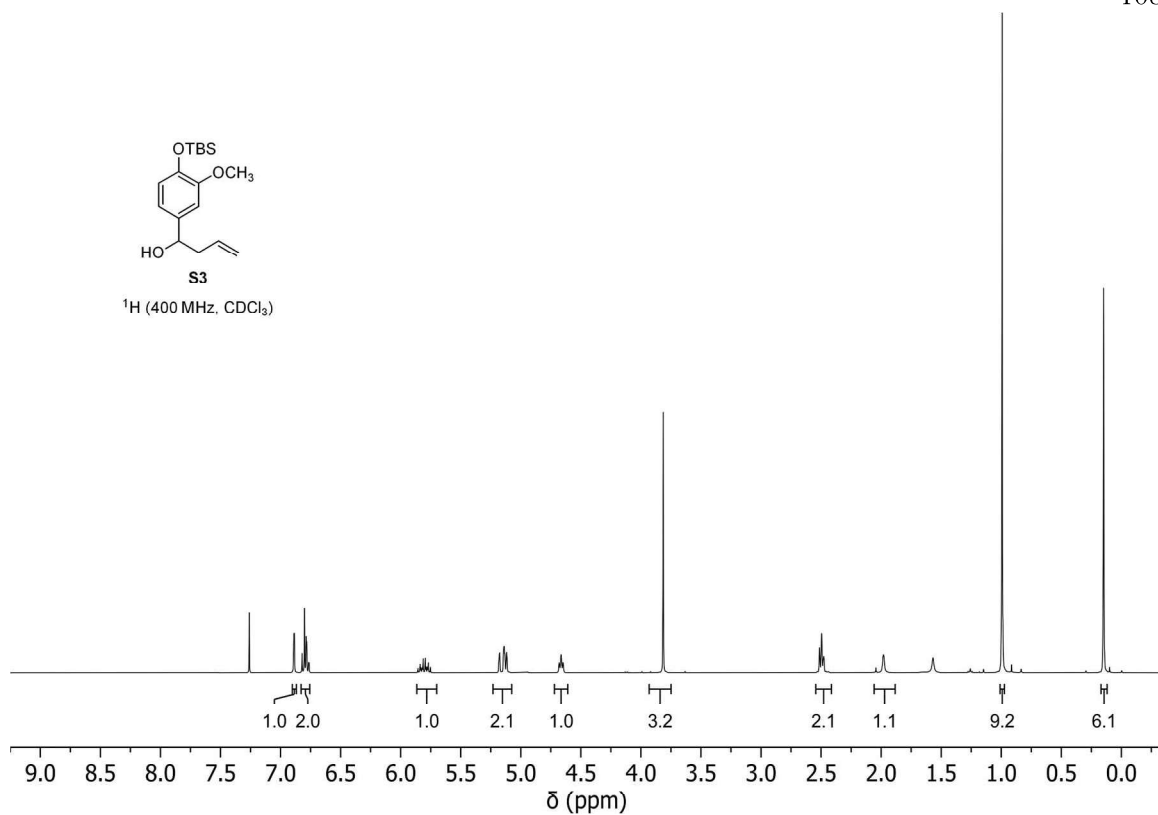
2.6 NMR Spectra



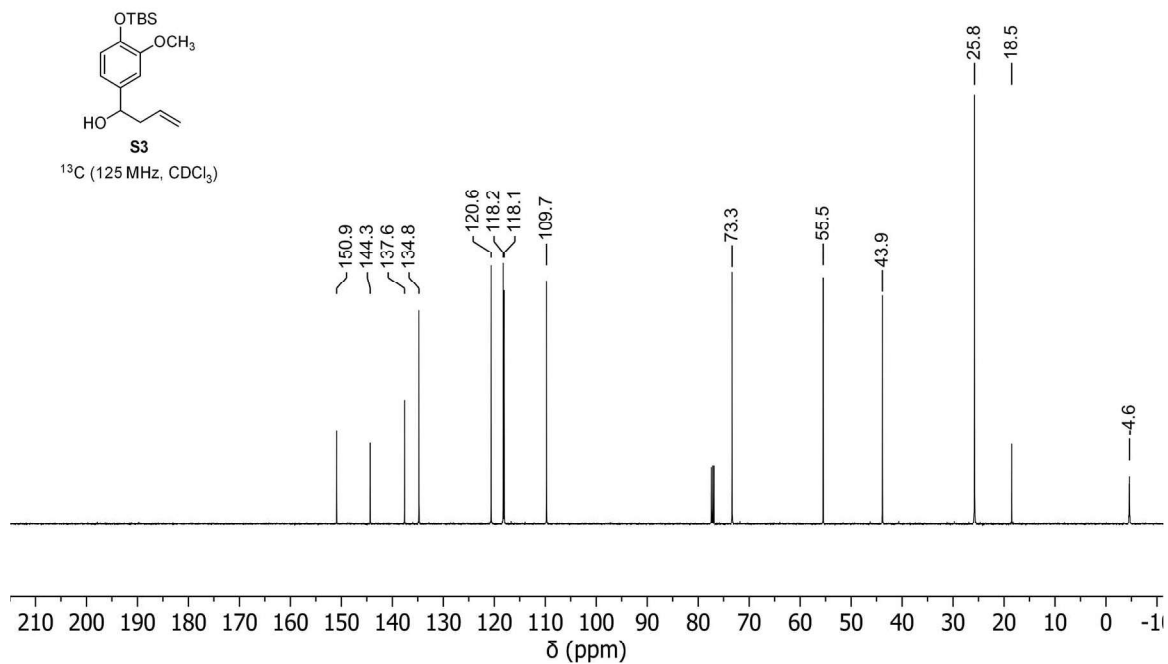
**S2**¹H (400 MHz, CDCl₃)**S2**¹³C (100 MHz, CDCl₃)

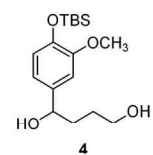
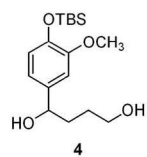
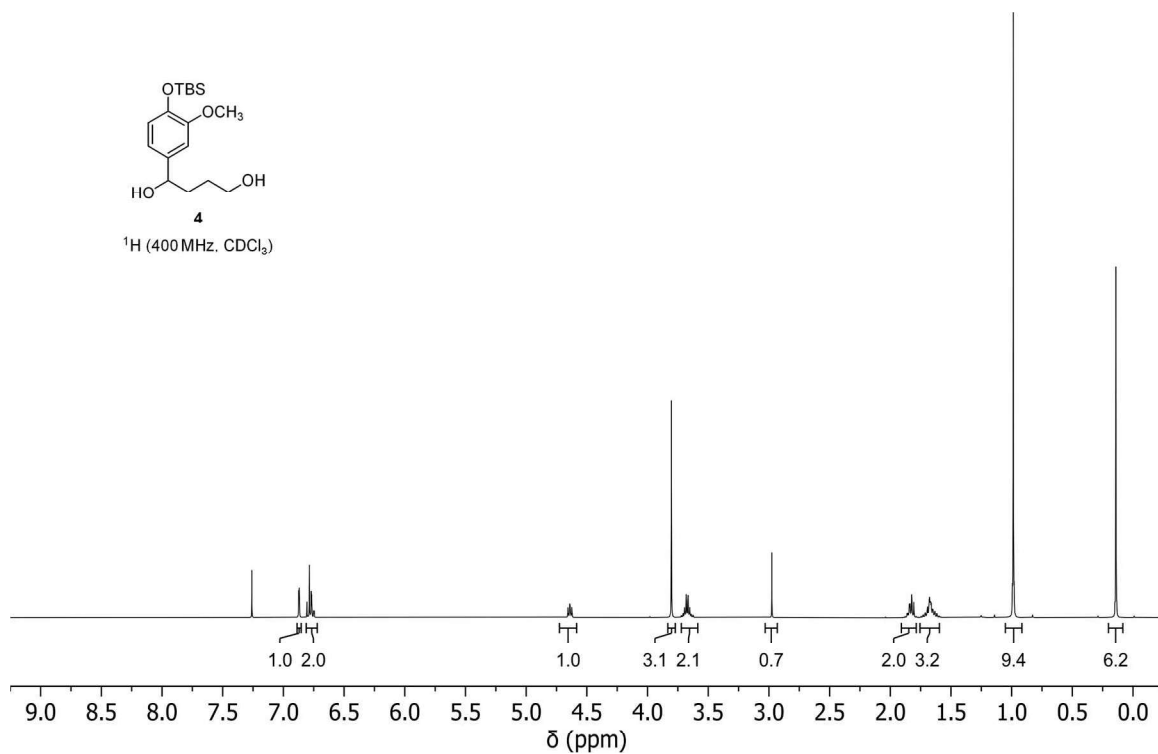
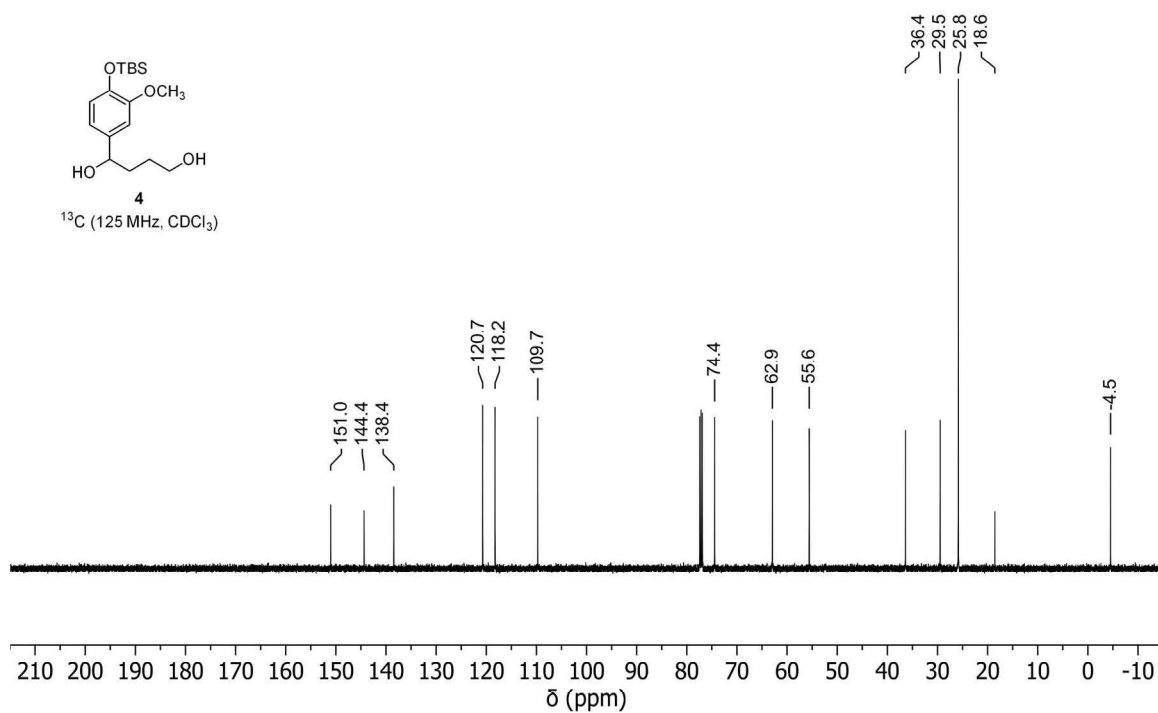


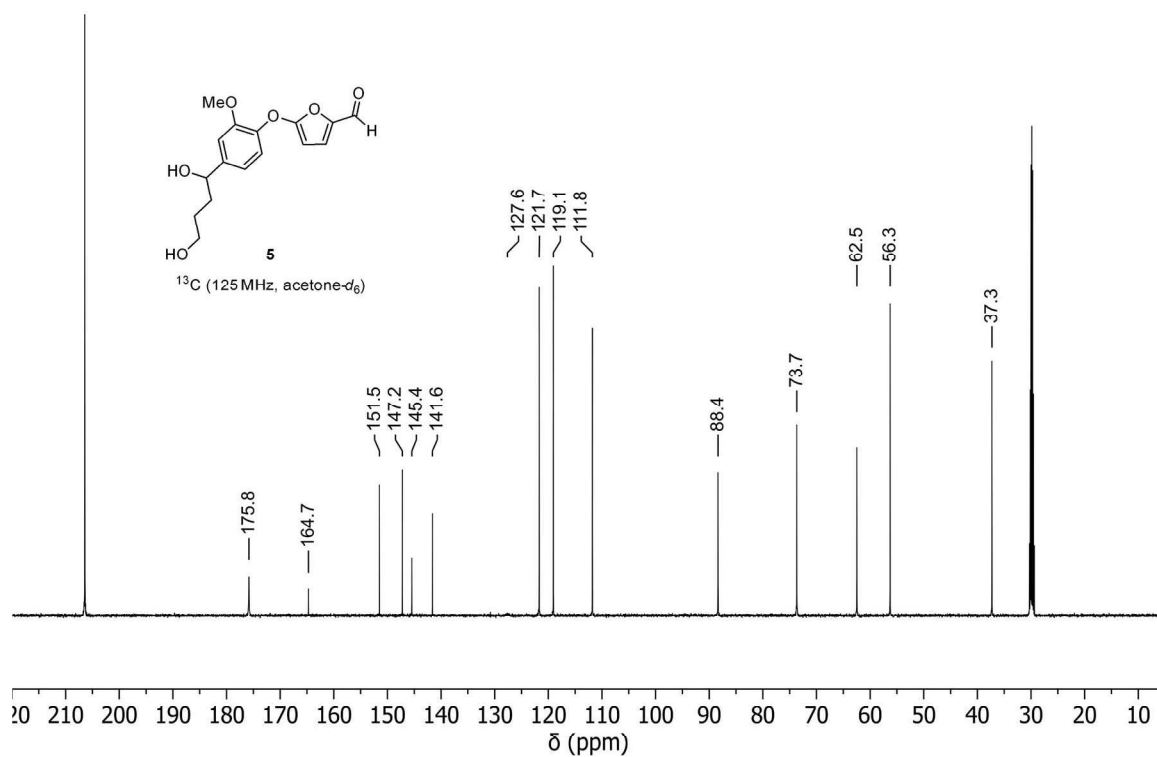
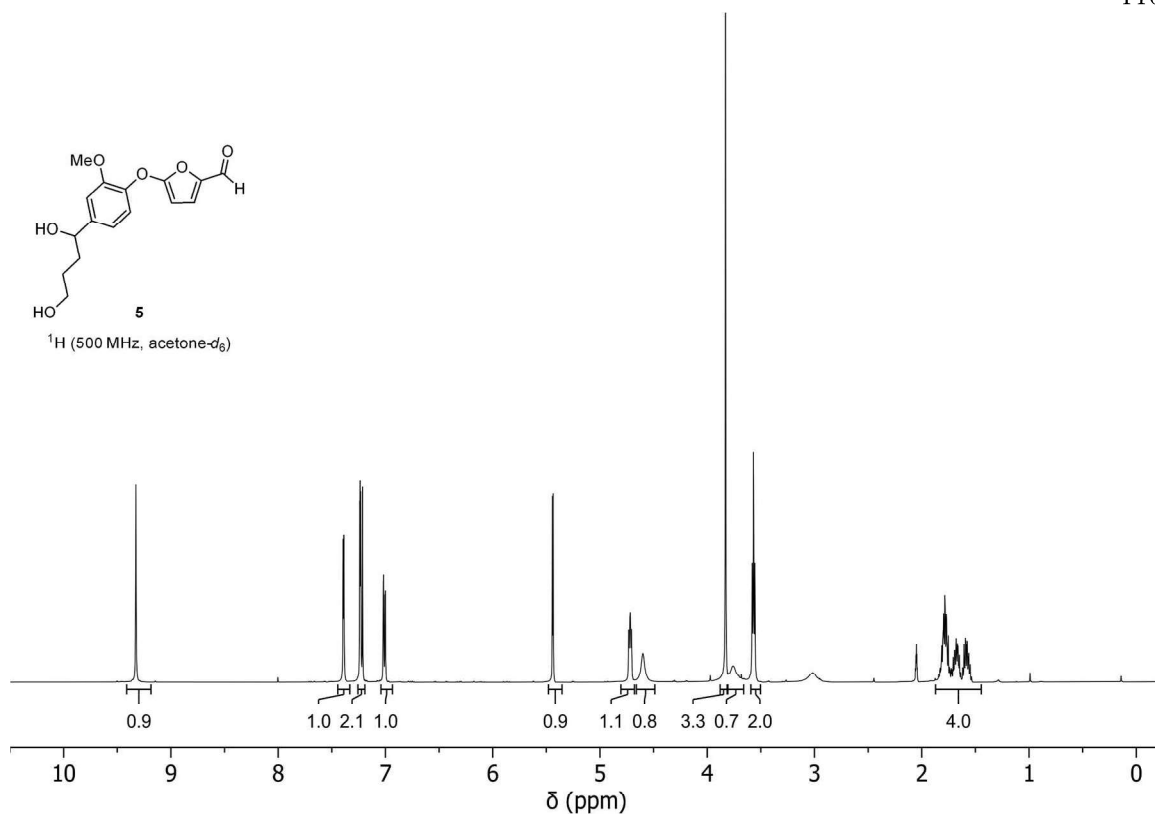
^1H (400 MHz, CDCl_3)

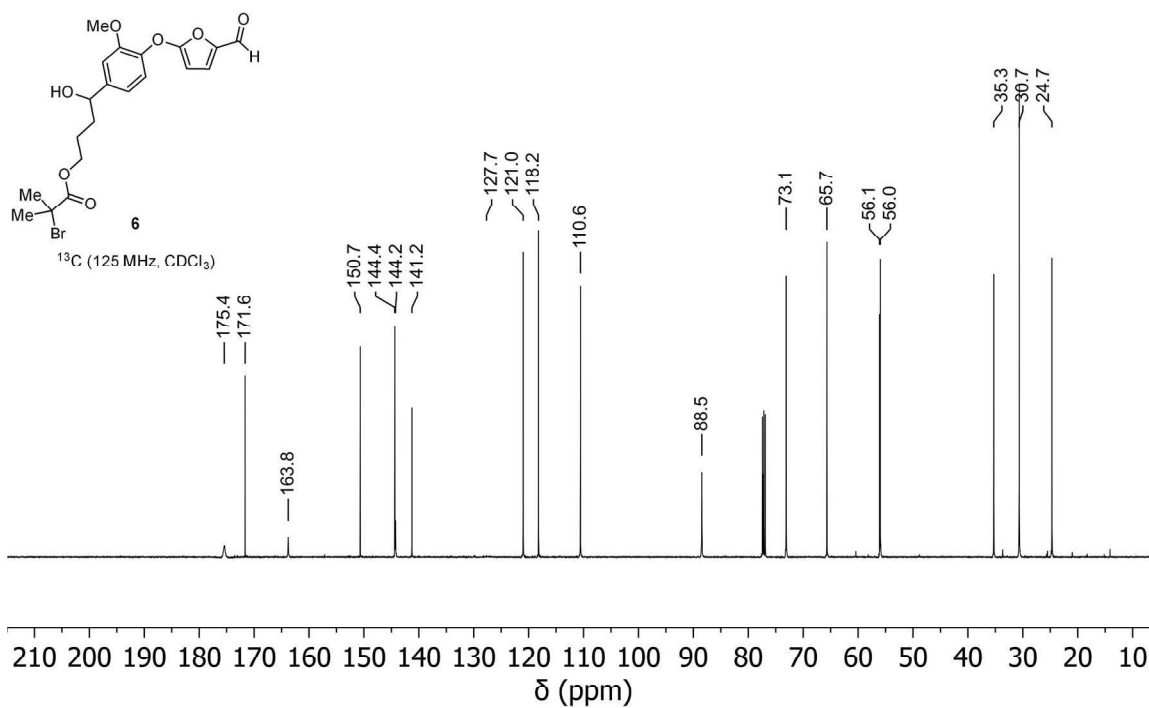
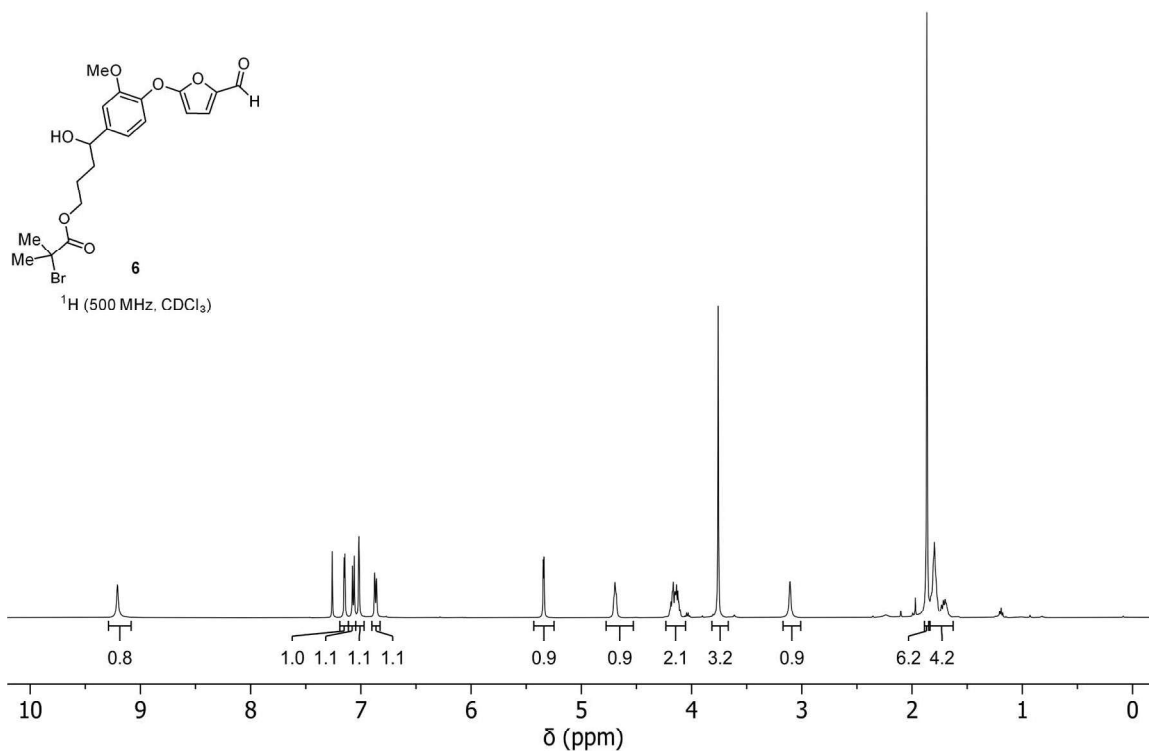


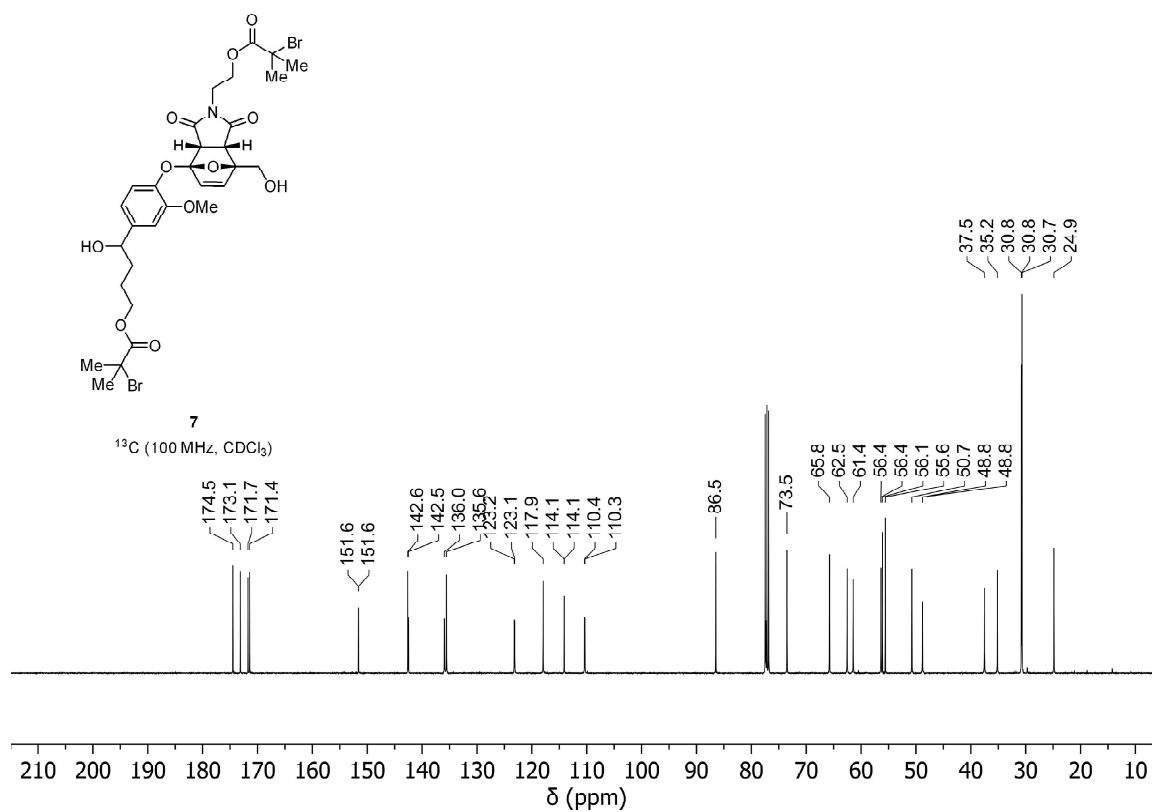
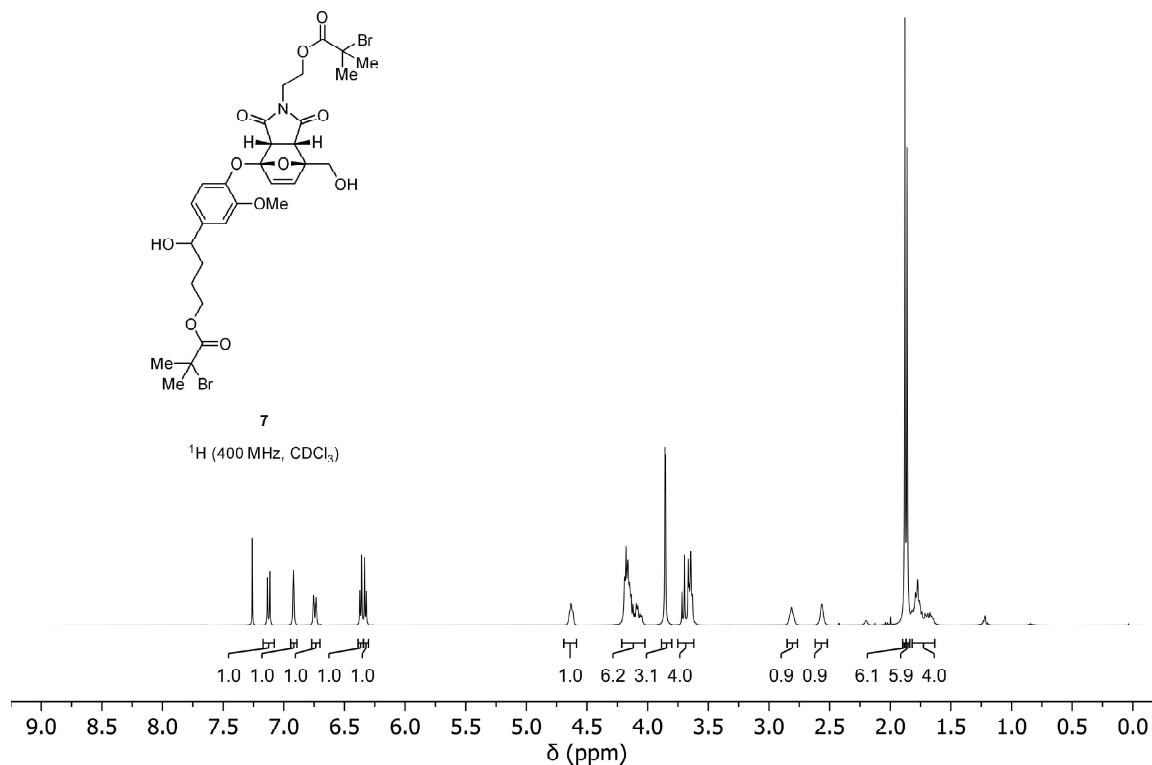
^{13}C (125 MHz, CDCl_3)

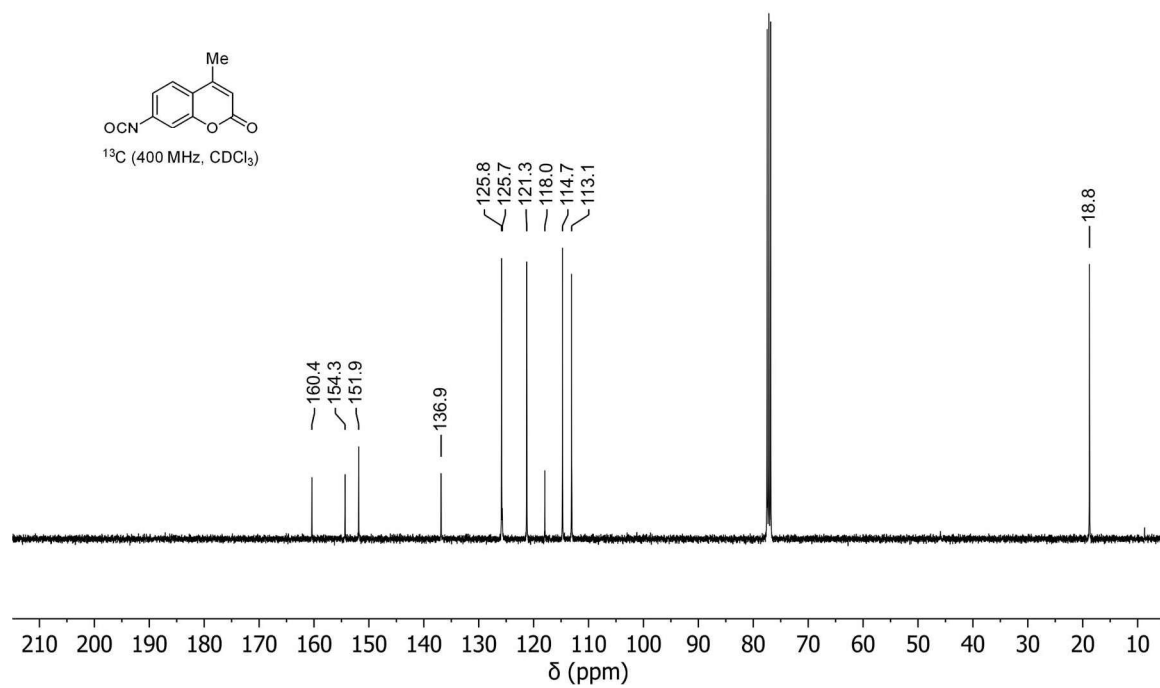
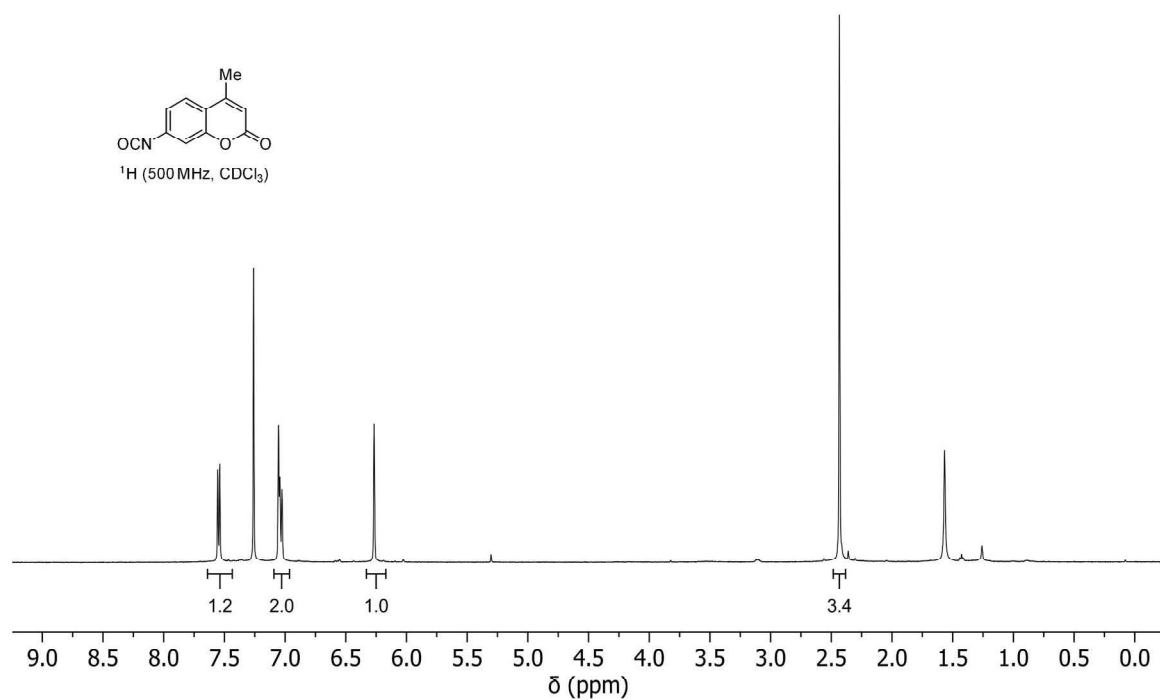


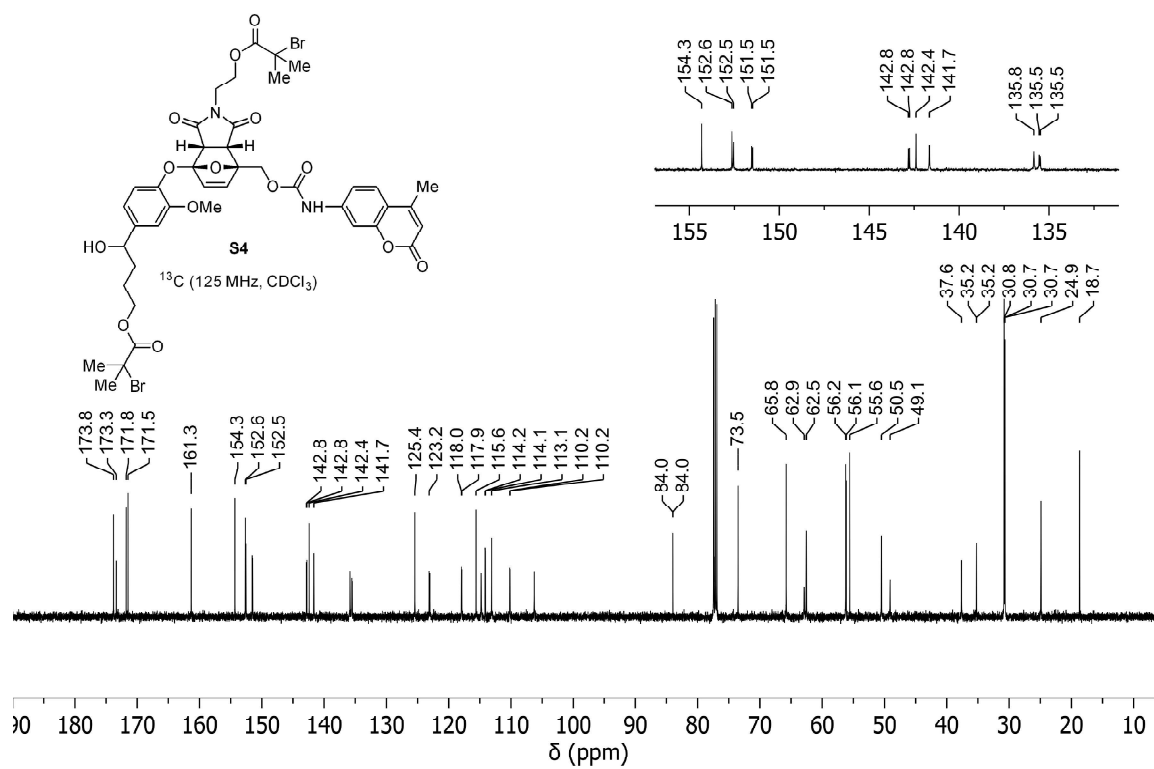
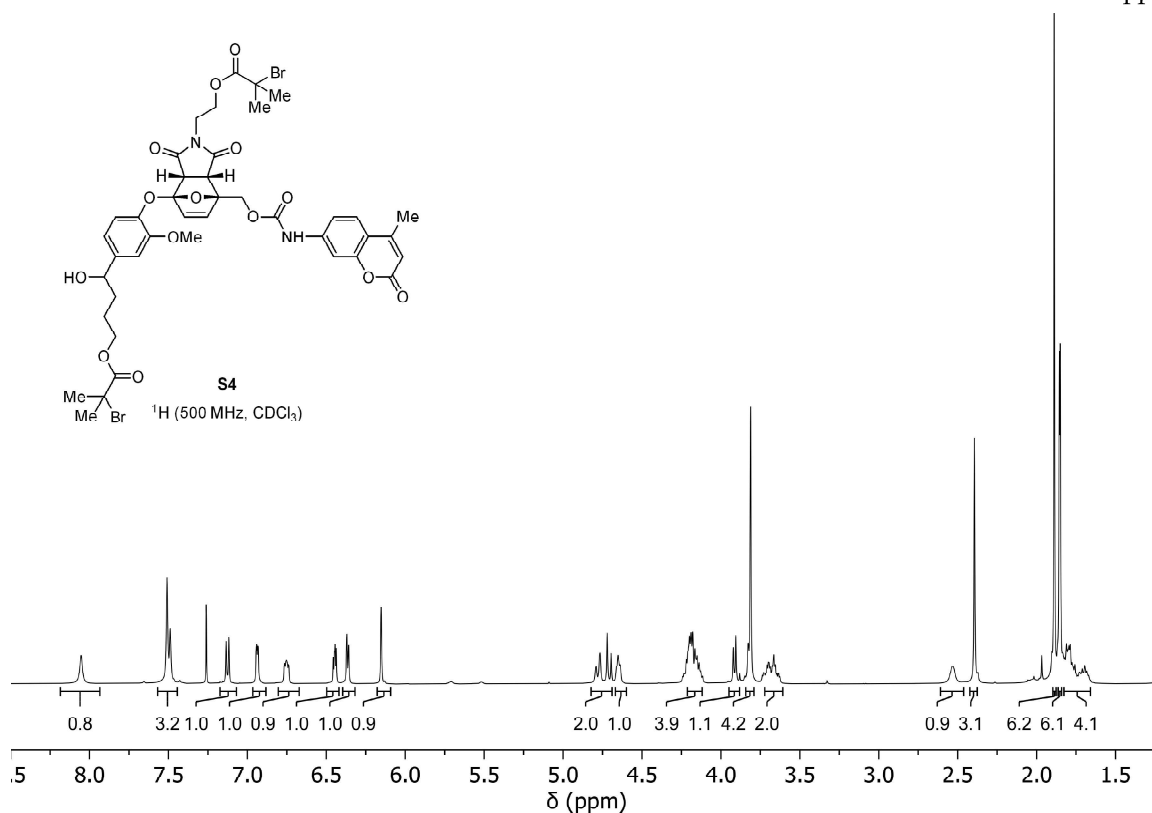
 ^1H (400 MHz, CDCl_3) ^{13}C (125 MHz, CDCl_3)

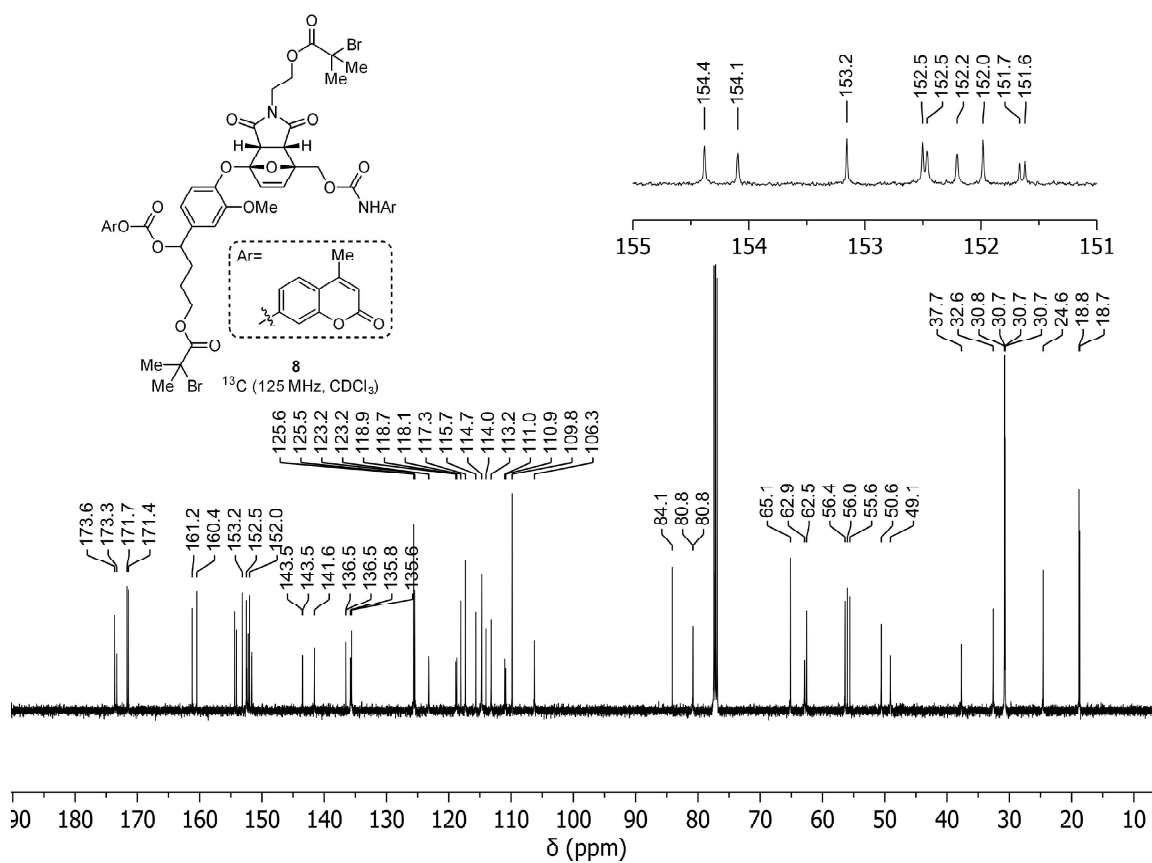
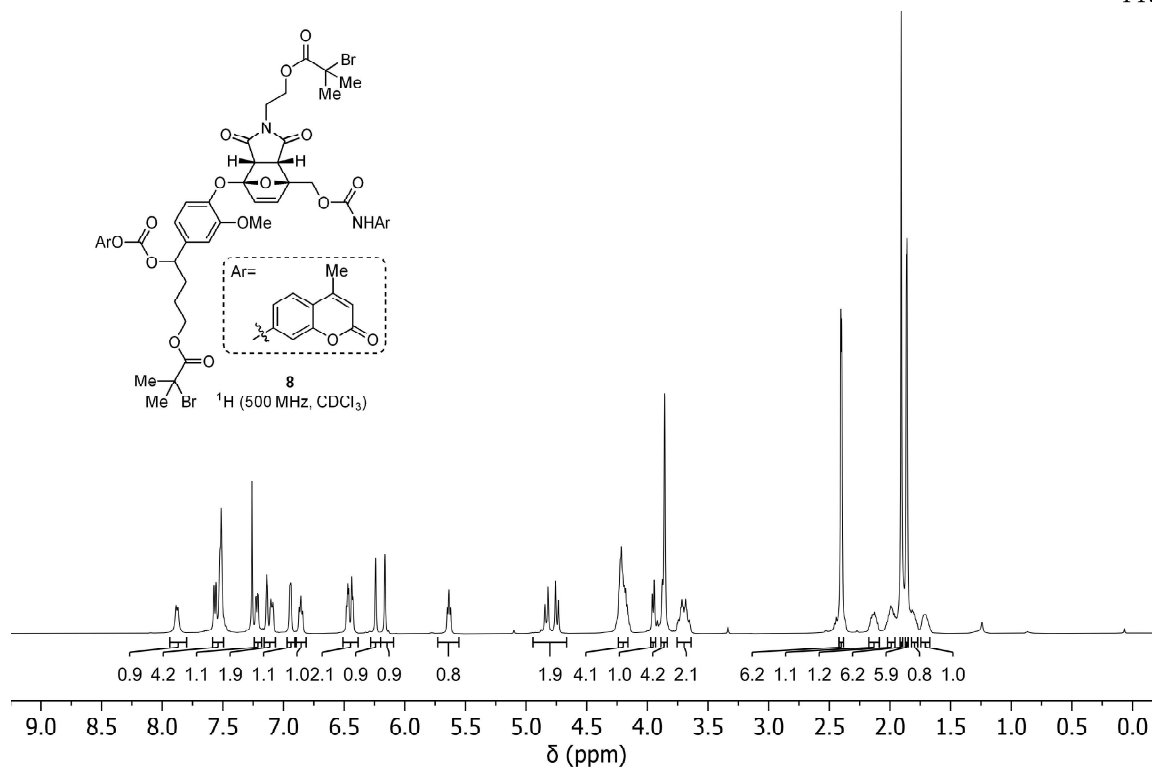


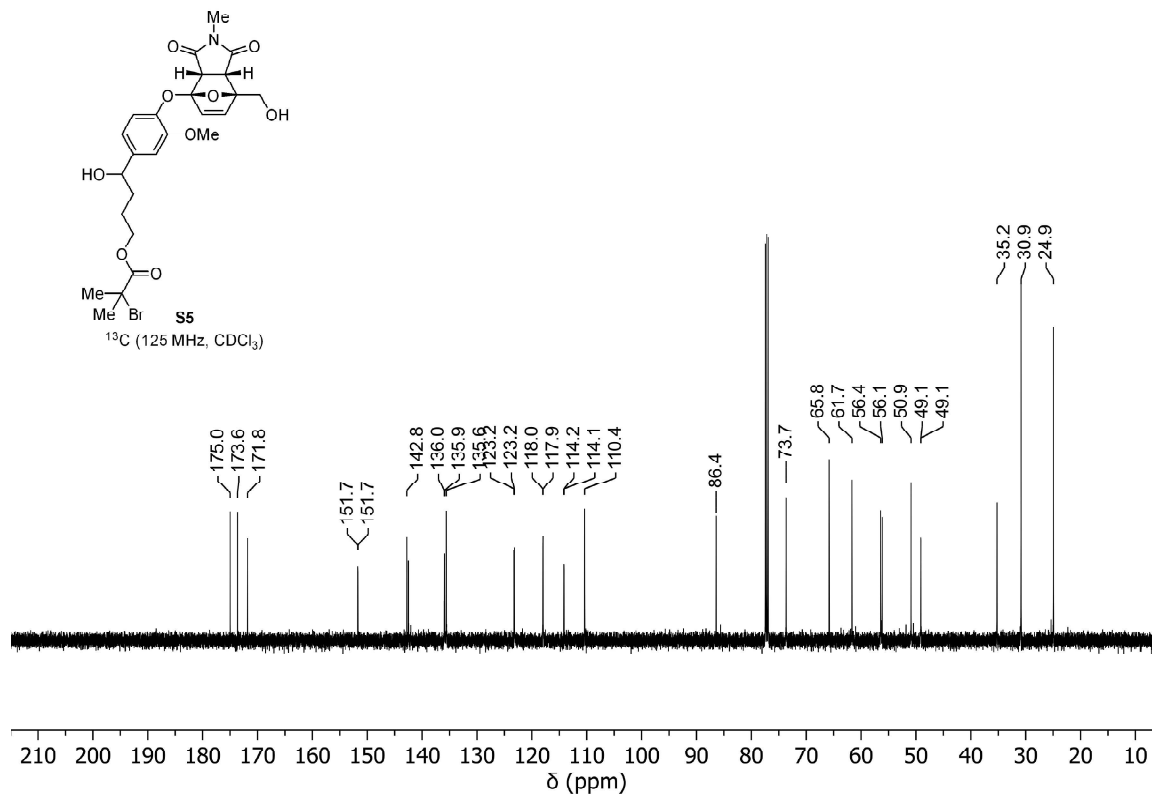
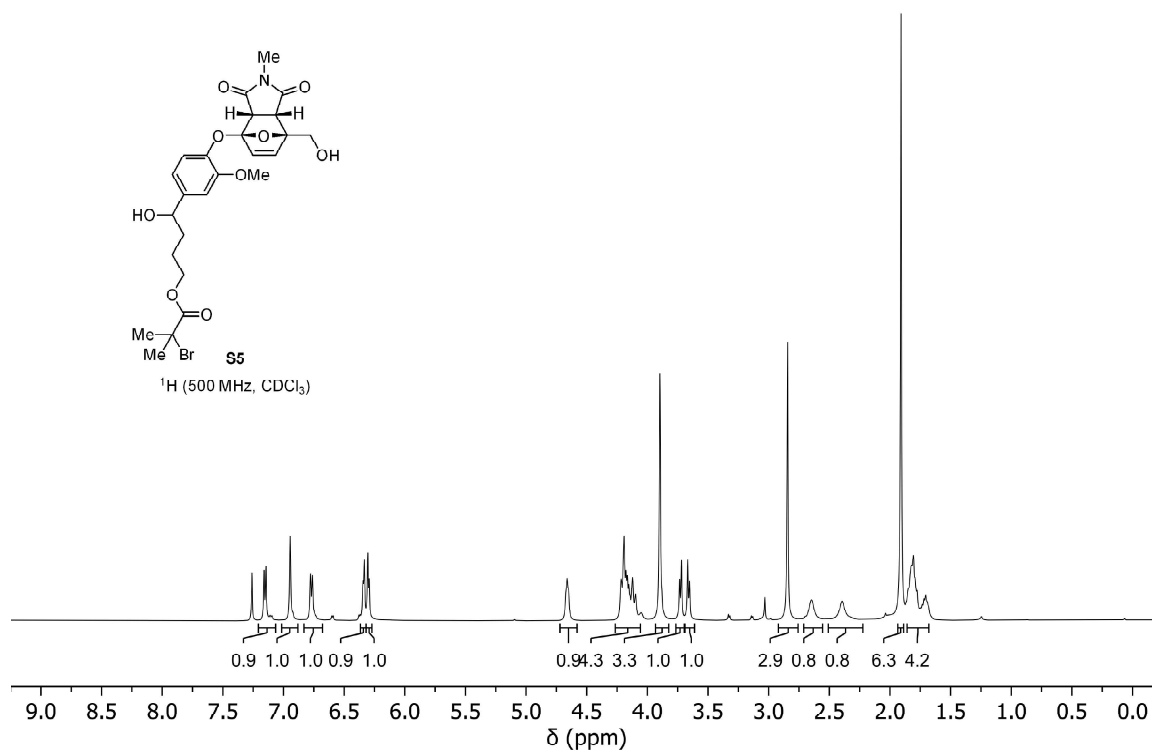


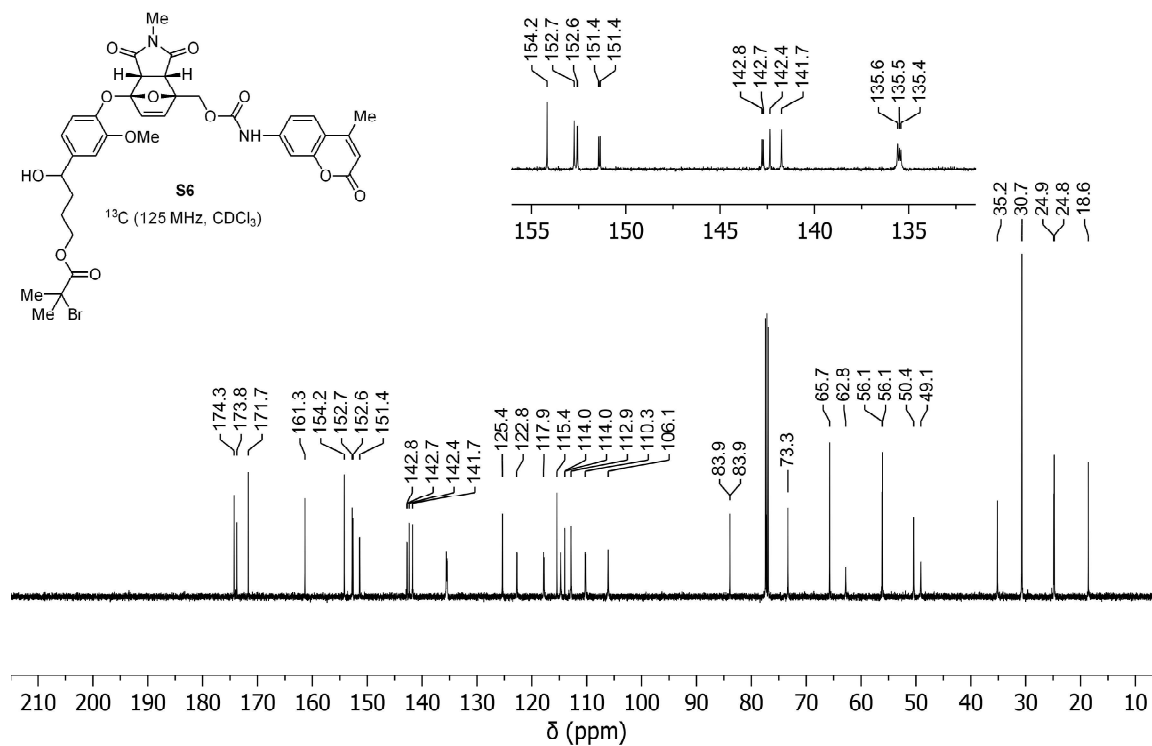
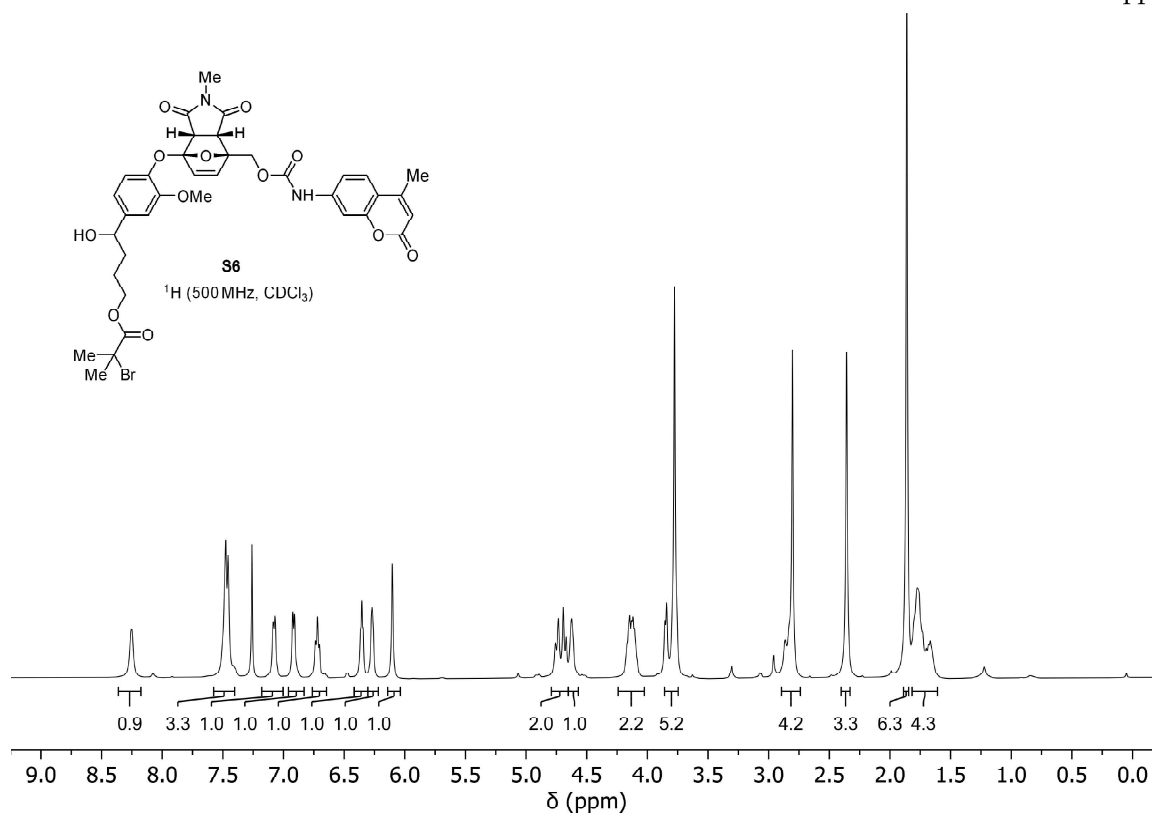


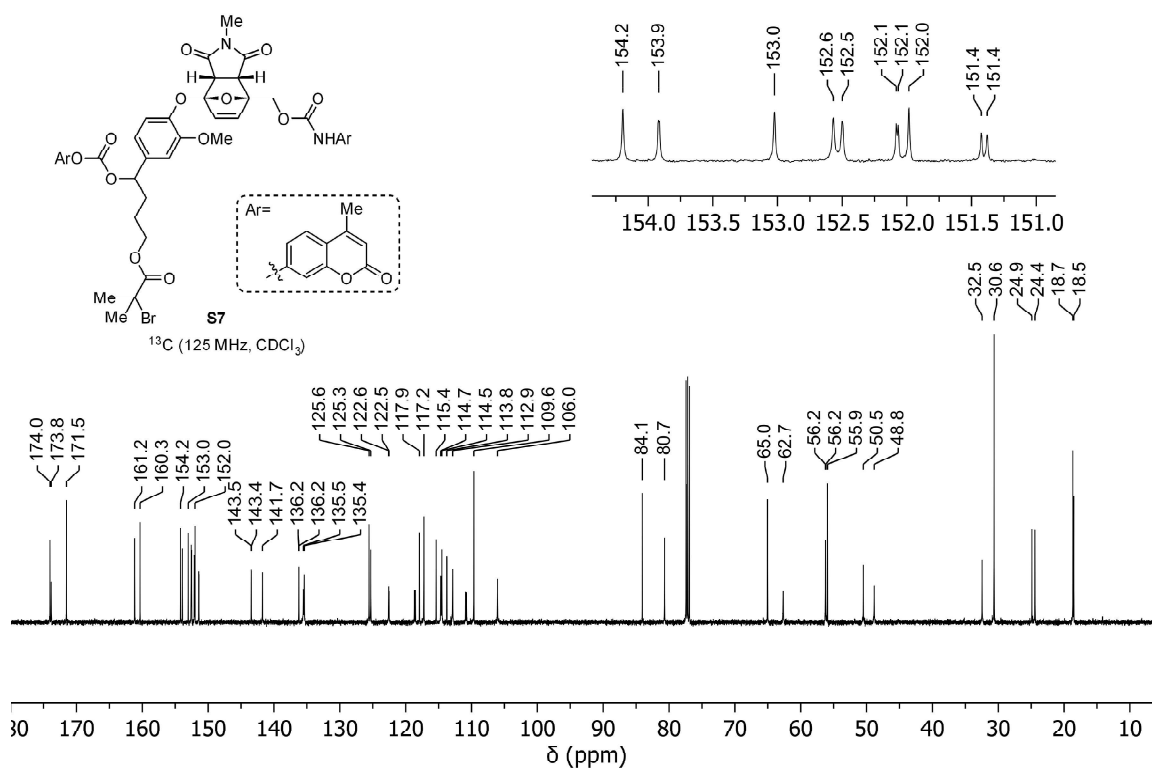
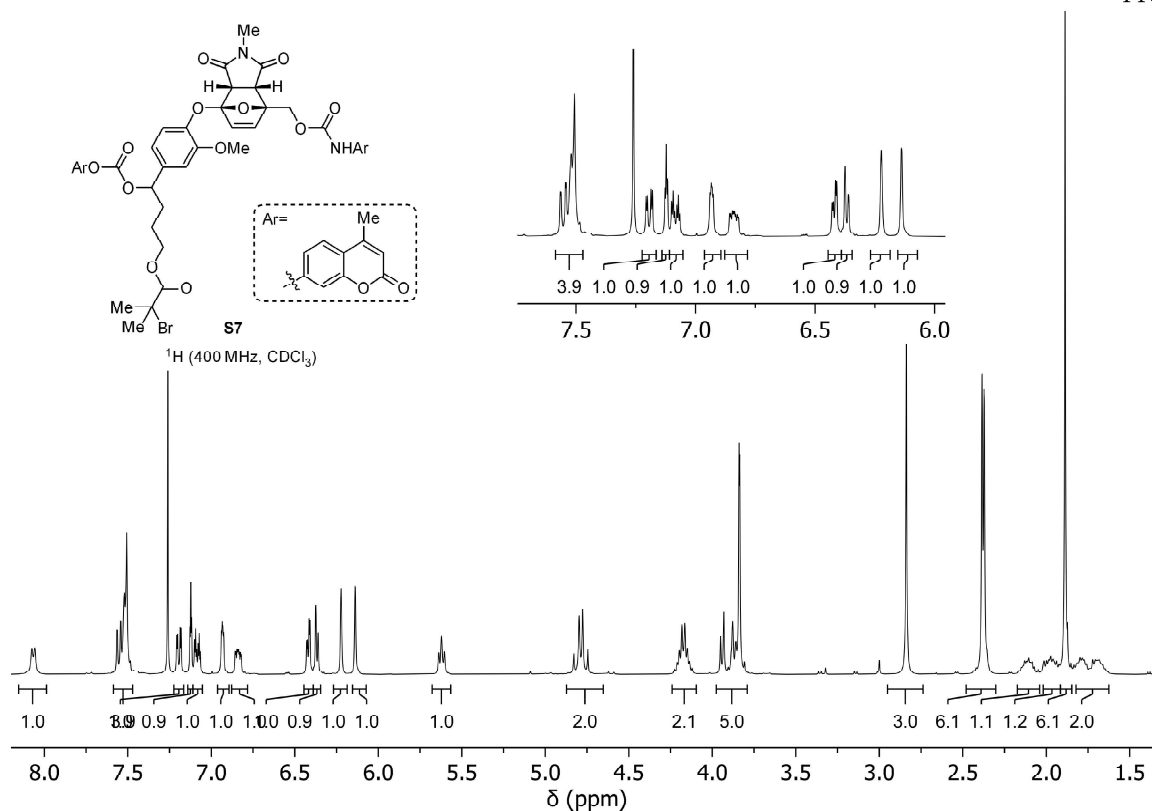


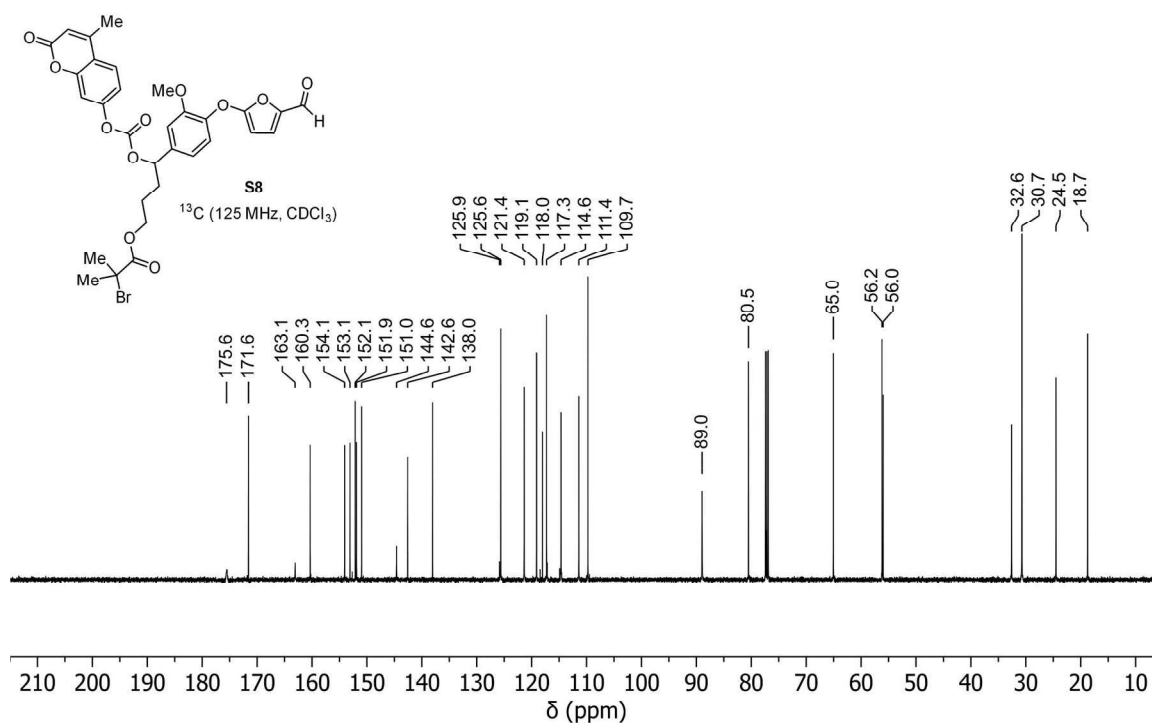
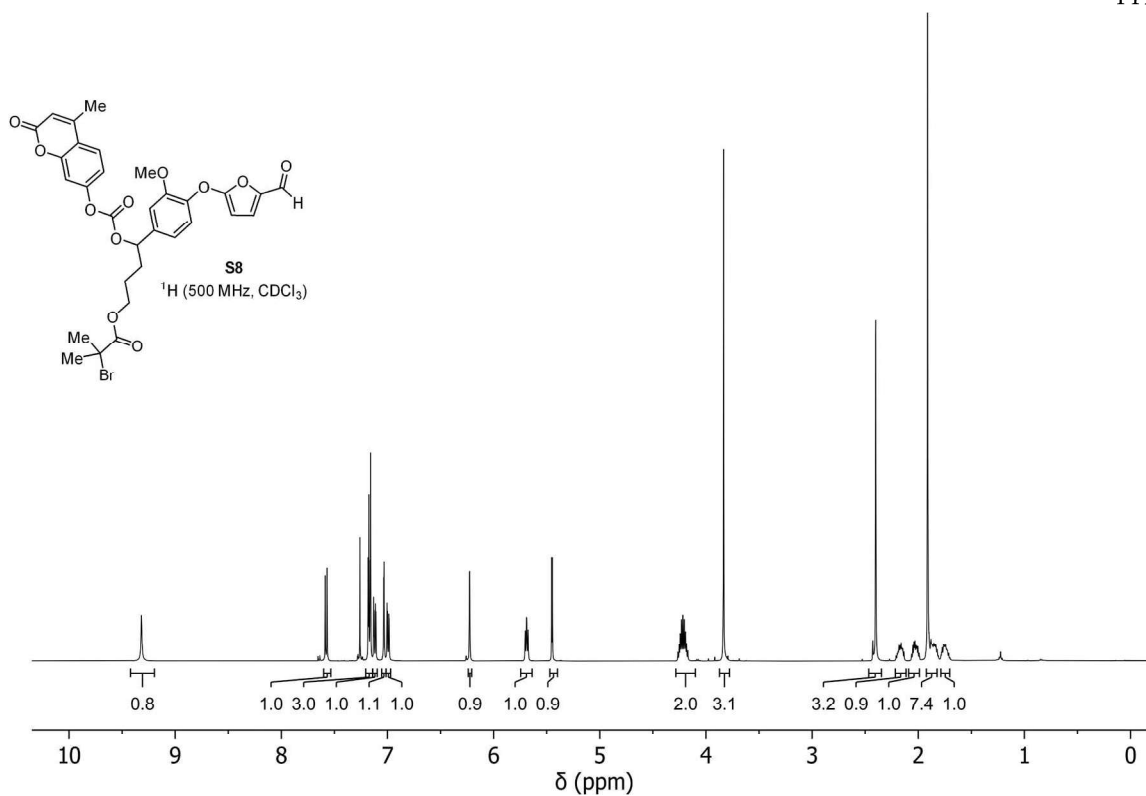


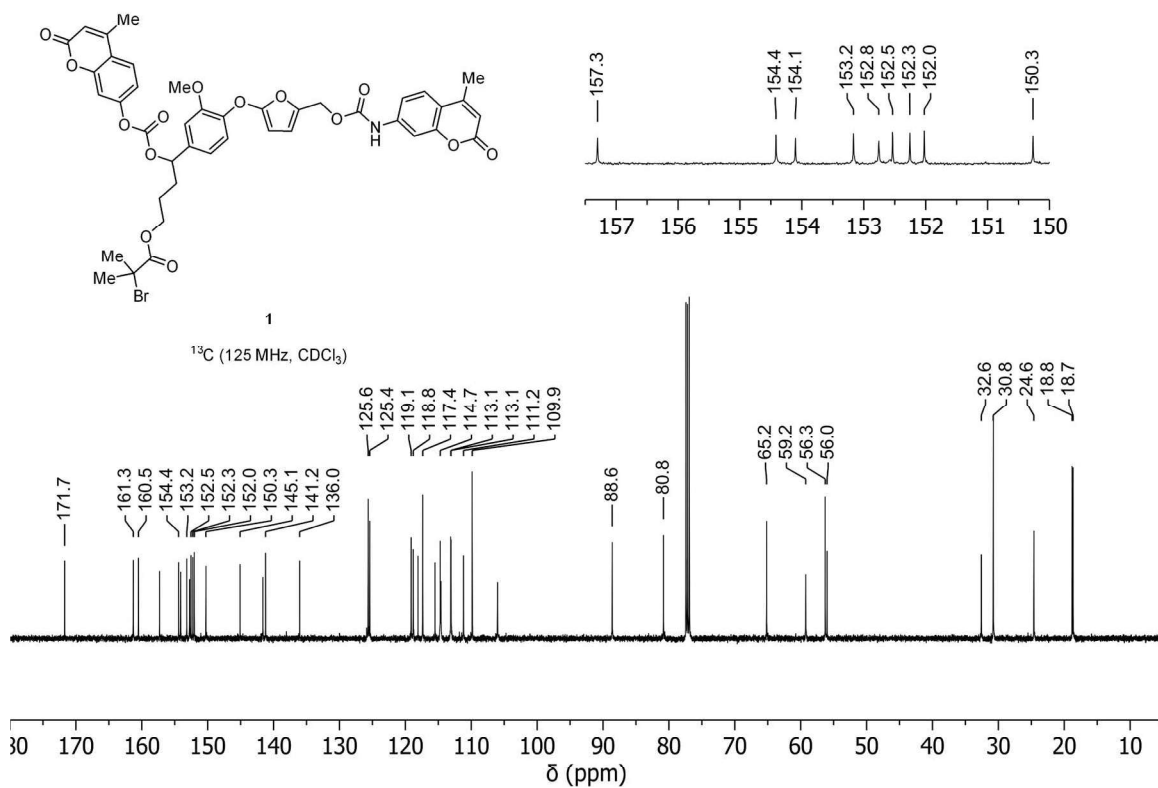
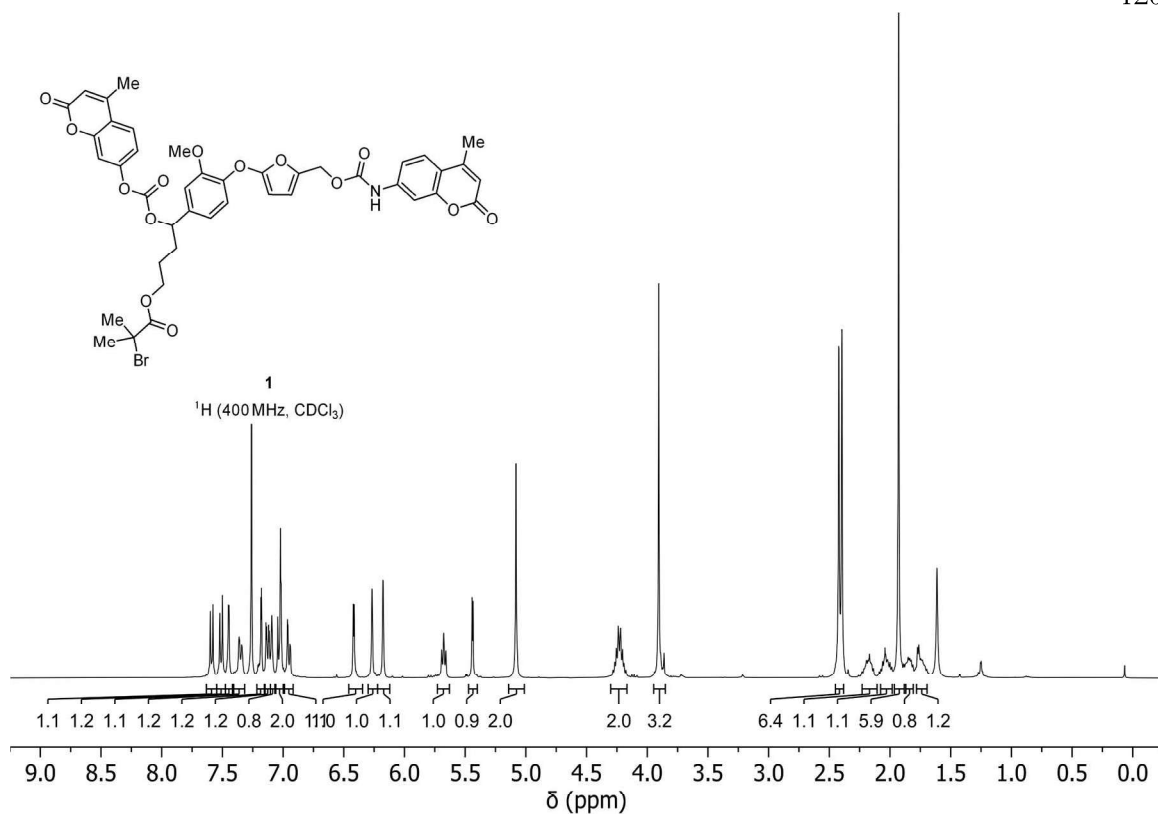


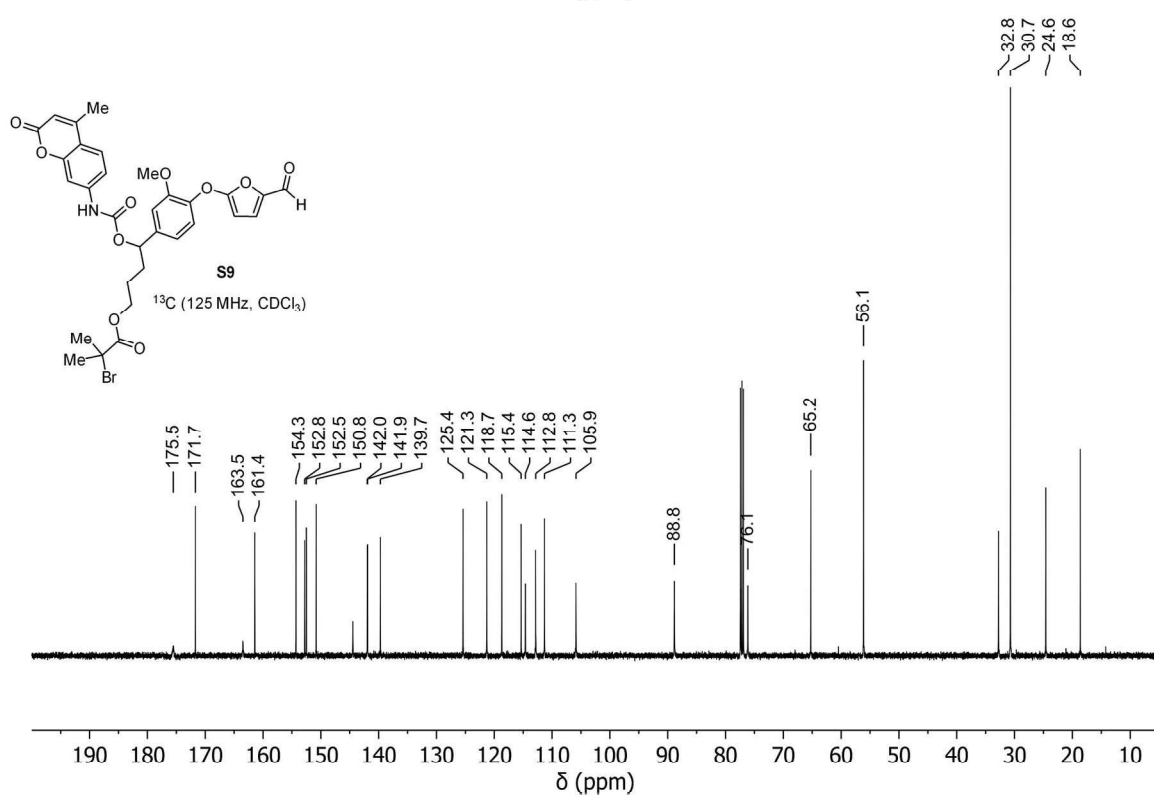
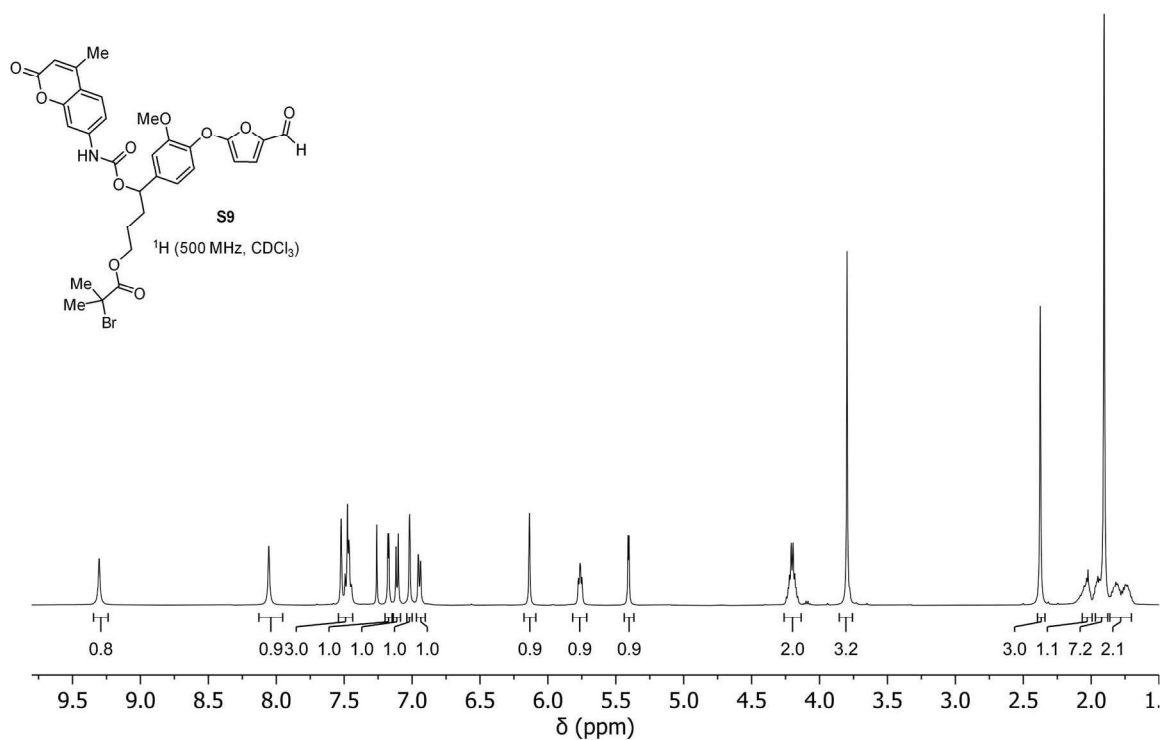


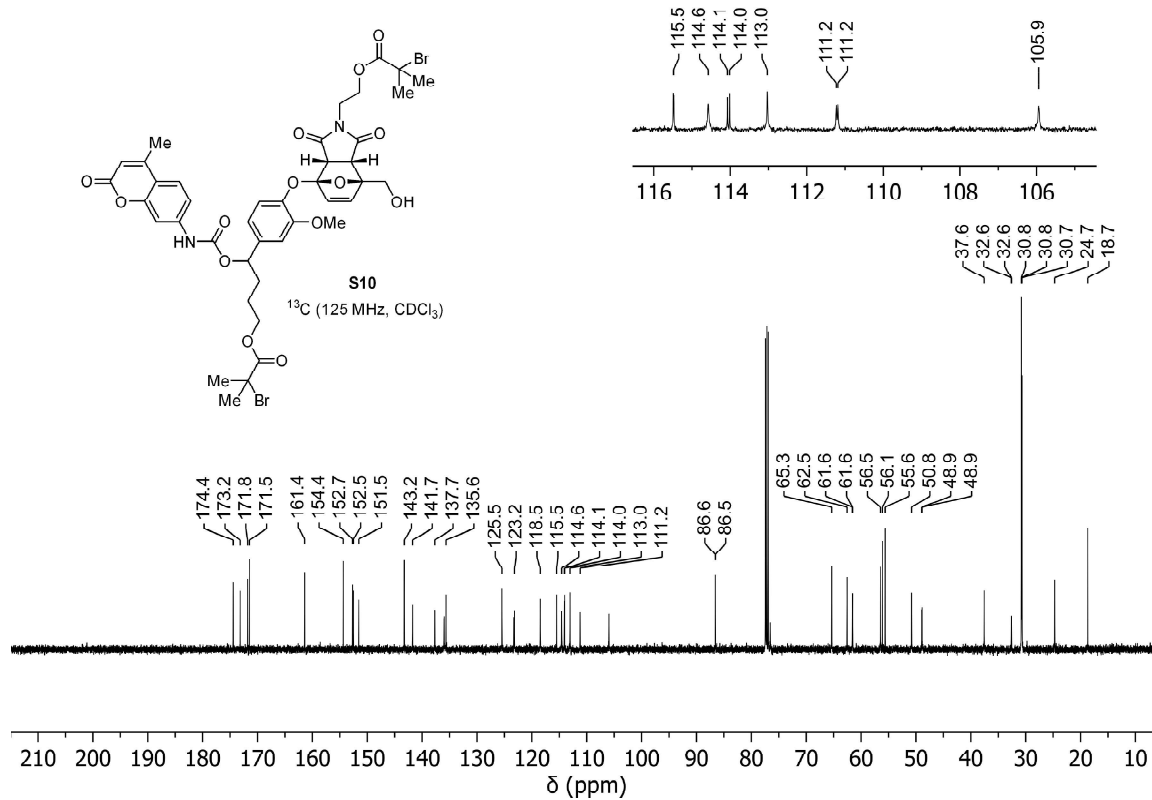
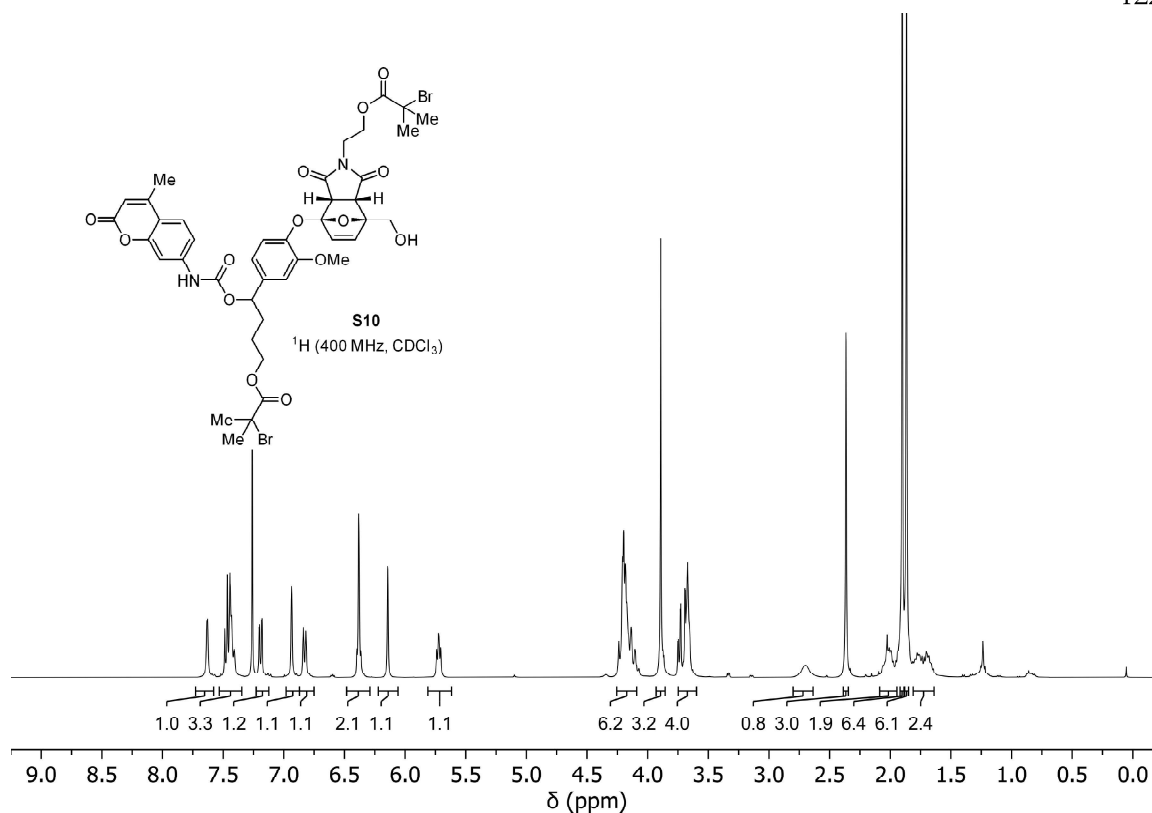


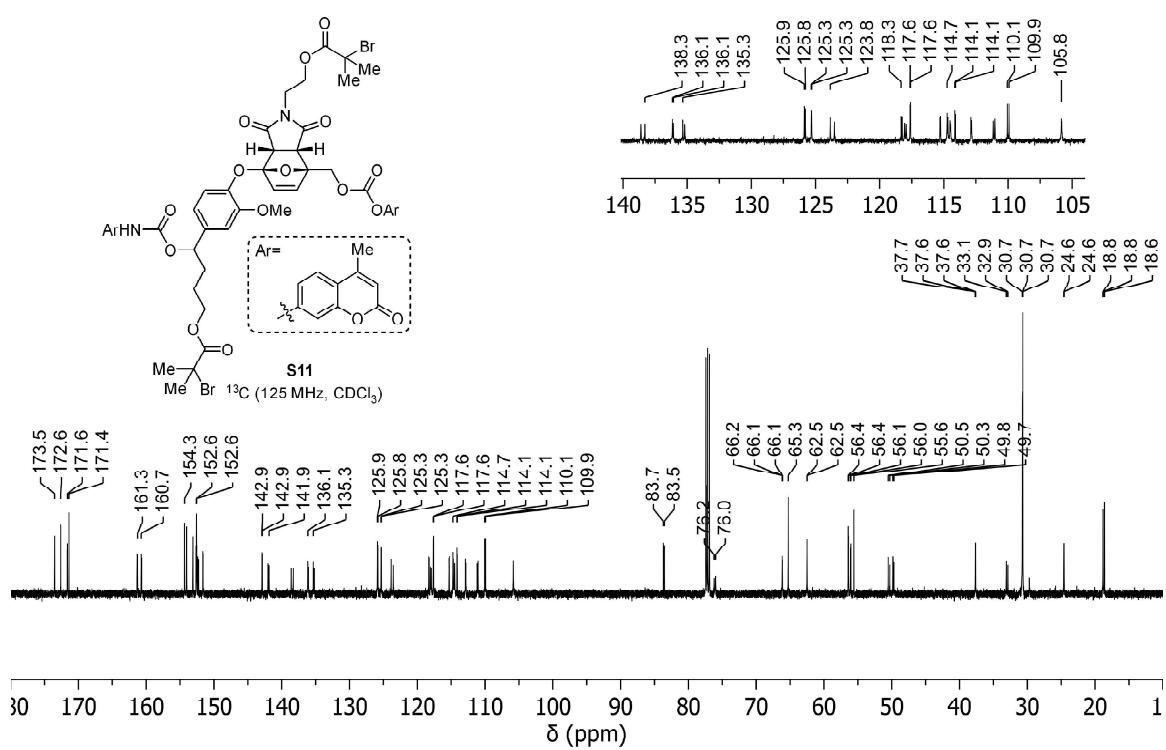
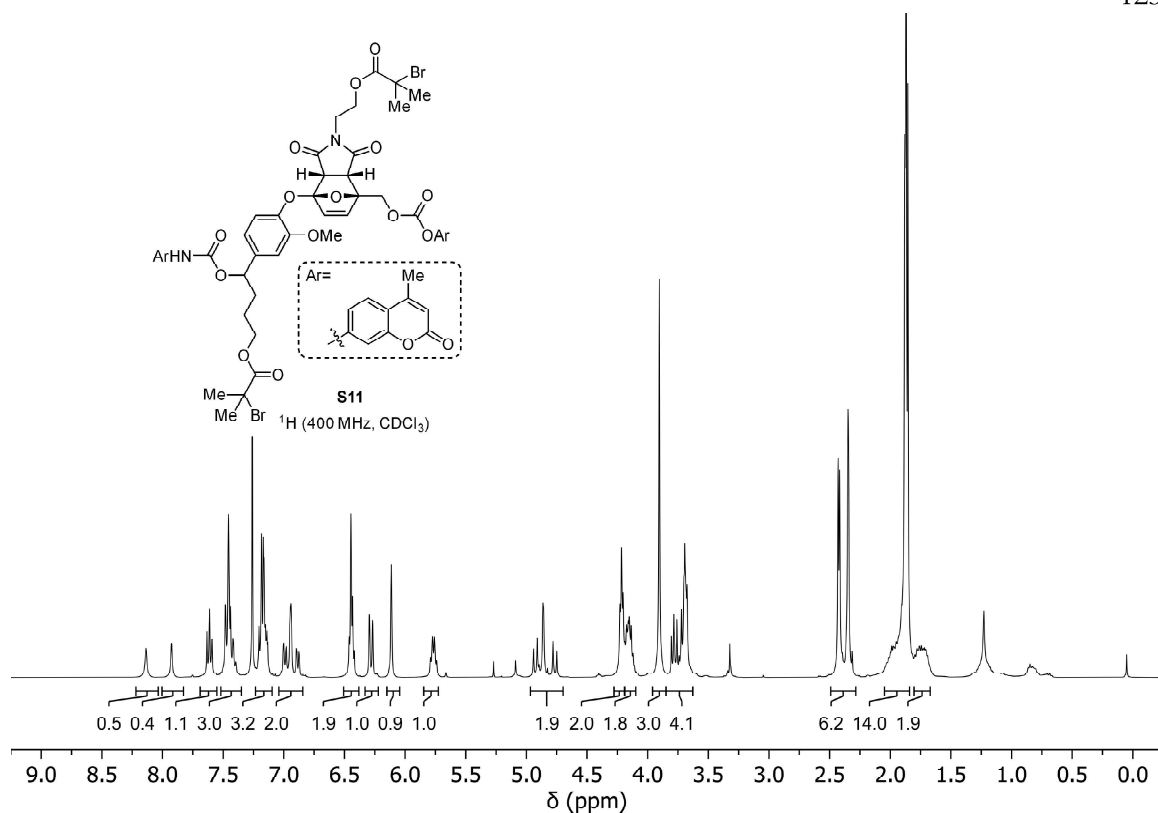












This chapter is temporarily embargoed.