ACS Macro Letters
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Highest Efficiency Two-Photon Degradable Copolymer for Remote Controlled Release

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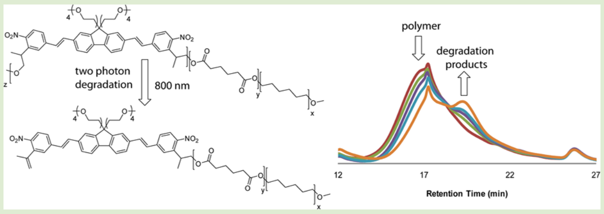
Ⓢ *Supporting Information*

ABSTRACT: To address the scarcity of polymers that degrade upon absorption of near-infrared (NIR) light, we introduce a new polymer containing moieties in its backbone capable of highly efficient NIR-triggered photocleavage. The polymer rapidly undergoes backbone scission in response to both UV–vis and near-infrared light via two-photon absorption, as revealed by gel permeation chromatography. Cleavage of photosensitive groups from the backbone is confirmed by ^{1}H NMR. These polymers were successfully formulated into particles encapsulating a dye that was released upon irradiation with UV–vis and NIR light, as indicated by changes in fluorescence characteristic of increased solvent interaction with cargo. Thus, this new polymer is readily photocleaved by UV–vis and NIR light, giving it a variety of potential applications in photopatterning and on-demand release.



Photolabile materials have diverse applications, both in industry, for example as photoresists in photolithography and patterning,[1,2] and in the biomedical field,[3] enabling precise control over biomaterial properties and drug release in living systems. For drug delivery, light-responsive nanocarriers may release cargo by several mechanisms: photoisomerization,[4−6] photothermal effects,[7] changes in hydrophobicity,[8−13] and fragmentation of the material by the photolysis of photolabile bonds.[14−21] The last method is most attractive as fragmentation allows for more complete release and easier clearance of the resulting small molecules.

To be truly useful in biological systems, photolabile materials should respond to wavelengths that penetrate deeply into cells and tissues with minimal scattering and photodamage. However, most light-responsive nanocarriers are responsive only to UV light due to its high energy, a requirement for most relevant chemical changes. Near infrared (NIR) light has better tissue penetration but generally lacks the energy required to initiate a chemical change. This challenge has been overcome with the development of materials that absorb two photons of low energy NIR light to undergo the same photoinduced change as with classical absorption of one higher energy UV photon. The selection of a photolabile group is critical to obtain a highly photosensitive material.[16]

A photoremovable group recently reported by Gug et al.[22] with a high two-photon absorption efficiency seemed to be an ideal photolabile group to engineer into polymeric materials and carriers, as it possesses an outstanding action cross-section of 5.0 Goeppert–Mayer units at 800 nm (1 GM = 10^{-50} cm^4 s photon^{-1})[22] due to its extensive conjugation. For example, systems with less conjugation, like the more commonly used 4,5-dimethoxy-2-nitrobenzyl[14] and bromo-hydroxycoumarin[16] protecting groups, have low uncaging cross sections, i.e., 0.01 GM within the 700–750 nm range[23] and 0.16 GM at 800 nm,[24] respectively, which limits the sensitivity of the polymers incorporating these photosensitive moieties.

Further, polymers incorporating bromo-hydroxycoumarin require water for uncaging,[25] which makes triggered degradation of hydrophobic assemblies (e.g., nanoparticles) difficult to achieve. We posited that a polymer bearing the photocage proposed by Gug et al.[22] would degrade upon two-photon absorption with great sensitivity. We chose to replace the 1-(3,6-dioxaheptyl) chains at the 9 position of the central fluorene with 2,5,8,11-tetraoxatetradecane to improve the aqueous solubility of the structure, important for clearance from biological systems, without decreasing the efficiency of two-photon absorption (Figure 1a). As this monomer does not allow generation of high molecular weight polymers, we pursued a copolymerization strategy with adipoyl chloride and 1,6-hexanediol (Figure 1b). This copolymerization maintained the polymer's ability to fragment into small molecules, facilitating clearance from tissues and release of a payload from polymer nanocarriers.

Monomer **1** (Figure 1a) was synthesized in a manner similar to that previously published[22] (Scheme 1). Compound **2** was synthesized in accordance with established literature procedures[26] and was then hydroxylated using a potassium *tert*-butoxide (*t*-BuOK)/*tert*-butyl alcohol (*t*-BuOH) mixture and paraformaldehyde to form alcohol **3**. Nitrostyrene **4** was prepared via Stille coupling between **3** and tributyl(vinyl)-stannane. Next, 2,7-dibromofluorene was substituted twice at

Received: May 22, 2013
Accepted: July 3, 2013
Published: July 19, 2013

dx.doi.org/10.1021/mz400256x | *ACS Macro Lett.* 2013, 2, 683−687

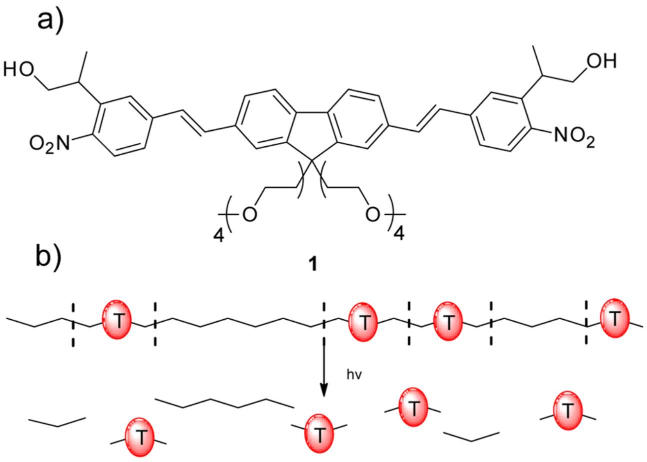


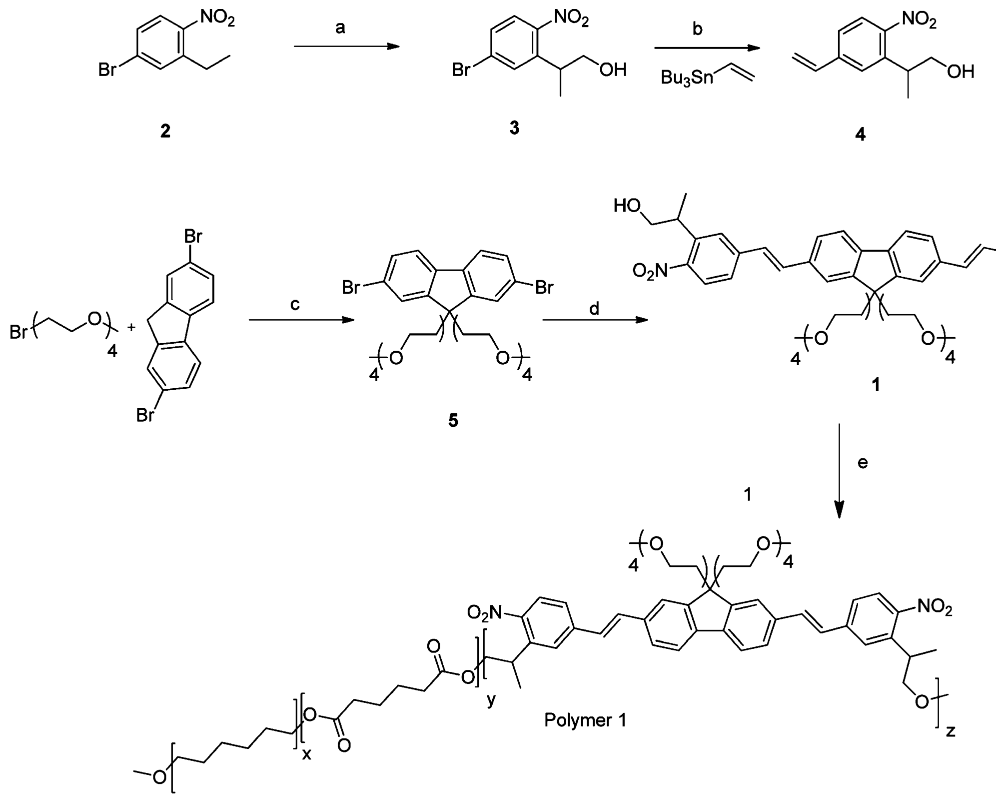
Figure 1. (a) Desired monomer **1**. (b) An illustration of the degradation of a polymer containing some photosensitive monomer in response to light.

the 9-position with 2,5,8,11-tetraoxatetradecane chains. Finally, **4** and **5** were linked via Heck coupling to form the desired monomer **1**.

As we aimed to incorporate a high proportion of photocleavable groups while maximizing polymer length, we varied the content of monomer **1**, producing batches of polymer with 1%, 5%, and 10% photosensitive monomer content. Weight-average molecular weights (MW) were determined by gel permeation chromotography (GPC) (Figure S2, Supporting Information) to be 68 000 Da (PDI = 1.70), 68 000 Da (PDI = 2.13), and 25 000 Da (PDI = 1.38) for polymers with 1%, 5%, and 10% content of monomer **1**, respectively, relative to poly(methyl methacrylate) standards. These PDI values were measured following removal of low molecular weight oligomers by precipitation. Incorporating greater than 10% monomer **1** led to substantially lower molecular weights (note the 43 000 Da decrease in molecular weight between the 5% and 10% polymer **1**) and a predominance of oligomers leading to generally lower yields of polymer.

To determine the amount of irradiation required to photolyze the light-sensitive bonds, we measured changes in the UV–vis absorbance spectrum of polymer **1** dissolved in tetrahydrofuran/phosphate buffer, pH 7.4 (80:20), upon different periods of irradiation. Results are reported for the polymer containing 1% monomer **1**; polymers containing 5% and 10% showed the same spectral trend. The polymer was irradiated with 320–480 nm light at 100 mW·cm^{-2} or with 800 nm light at 82.7 W·cm^{-2} (Figure 2). Both display similar decreases in absorbance at 400 nm and increases in absorbance at 450 nm upon photolysis. Although the photocage was optimized for two-photon uncaging, it still has a greater sensitivity toward one-photon absorption. As seen in Figure 2, 60 min of NIR irradiation with many times greater power density was necessary to achieve the same level of photolysis as with 30 s of irradiation with UV–vis light.

Scheme 1. Synthesis of the Desired Polymer 1^a



^{a}(a) *t*-BuOK/*t*-BuOH, paraformaldehyde, dimethyl sulfoxide (DMSO), 76%; (b)triphenylphosphine, bis(dibenzylideneacetone)palladium(0), toluene, 78%; (c) KOH/H$_2$O, DMSO, 48%; (d) **4**, Pd(OAc)$_2$, NEt$_3$, tri(*o*-tolyl)phosphine, toluene, 78%, (e) adipoyl chloride, hexane diol, dichloromethane. Quantities of monomer **1** and hexane diol were varied in the polymerization to yield polymer **1** with 1%, 5%, and 10% content of monomer.

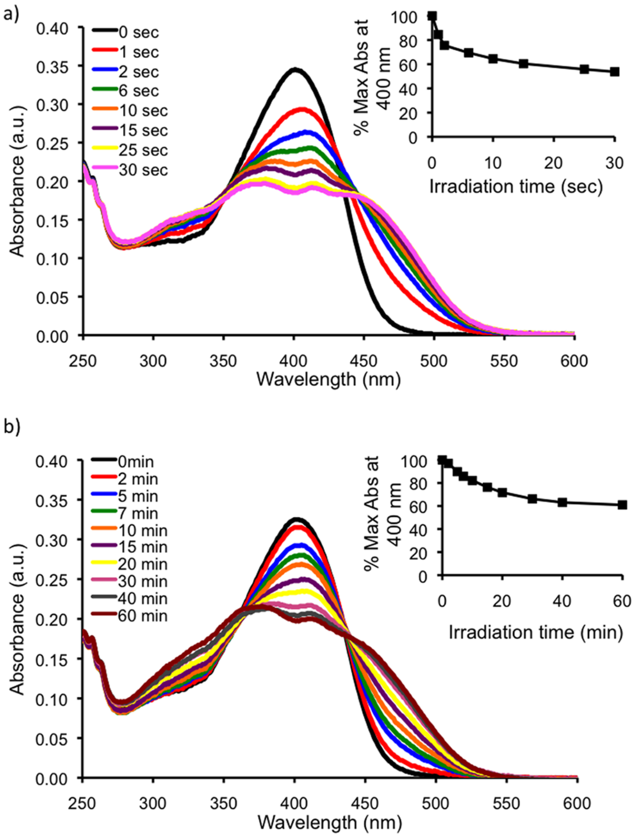


Figure 2. Polymer **1** is responsive to both UV−vis and NIR light. Absorption spectra of the 1% polymer **1** irradiated by (a) UV/vis (320−480 nm) and (b) NIR light (800 nm). Insets show the percent absorption value at 400 nm versus irradiation time.

With an understanding of the light sensitivity of polymer **1**, we began to study its degradation. We chose to follow the degradation of polymer containing 10% of light-sensitive monomer **1**, as it should allow the greatest degree of photocleavage and ensuing degradation. The polymer was dissolved in dimethylformamide/phosphate buffer, pH 7.4 (90:10), and solutions were irradiated with UV−vis light from 320 to 480 nm or near IR light at 800 nm. Degradation was monitored by gel permeation chromatography (Figure 3). Degradation of the 10% polymer **1** upon irradiation with UV−vis light reached a maximum after 60 s of irradiation (Figure 3a). High molecular weight polymer persists throughout the photodegradation experiment, which is likely due to the random nature of the polymerization; some long sections of polymer contain no photosensitive groups and thus do not degrade. As expected from the photolysis studies, degradation of the polymer using NIR light requires substantially longer irradiation times and greater laser power. The degradation is apparent after 15 min and progresses with further irradiation. A control polymer with 0% monomer **1** content was also irradiated in the same manner and showed no signs of degradation over the observed time period (Figure S3, Supporting Information).

Polymer degradation was also monitored by ^{1}H NMR spectroscopy upon UV−vis irradiation. A concentrated solution of polymer containing 10% monomer **1** in deuterated dichloromethane was prepared and irradiated for the prescribed times in a 1.7 mm Bruker NMR tube with 320−480 nm light (Figure 4a). As expected, the ^{1}H NMR spectra of irradiated

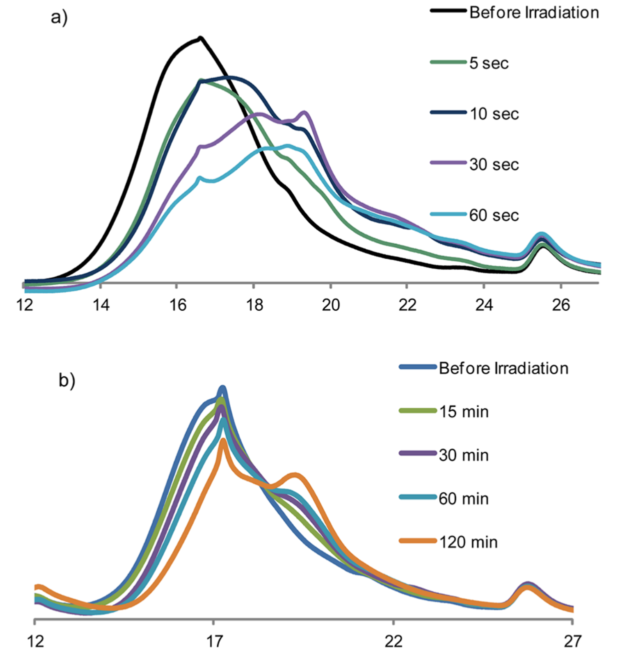


Figure 3. Polymer 1 degrades in response to both UV−vis and NIR light. (a) GPC traces of 10% polymer **1** before and after irradiation with 320−480 nm light (0−60 s). (b) GPC traces of 10% polymer **1** before and after irradiation with 800 nm light (0−120 min).

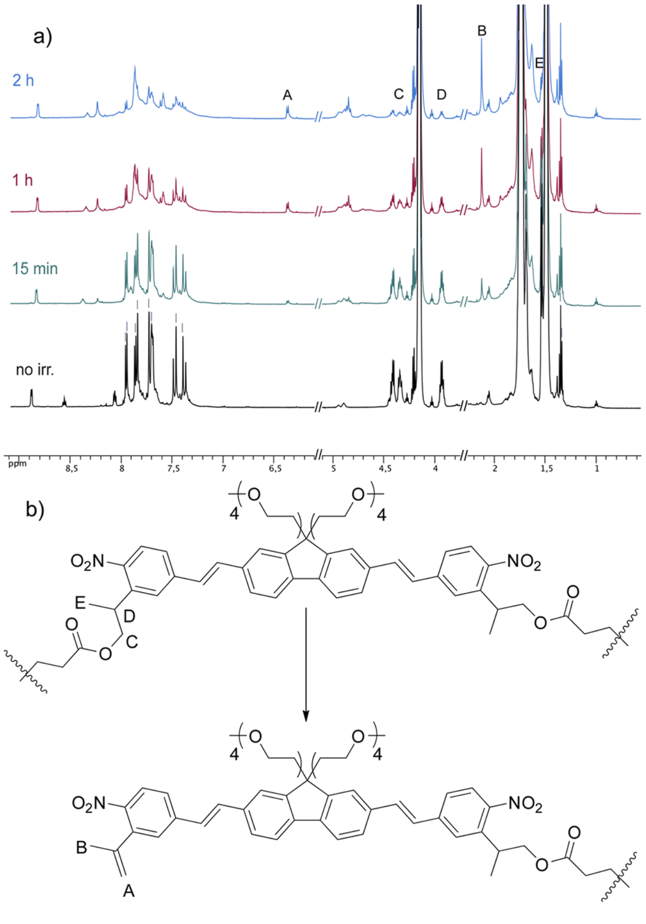


Figure 4. Photosensitive group is cleaved from the backbone upon UV irradiation. (a) ^{1}H NMR of polymer **1** (10%) in CD_2Cl_2 without irradiation and after irradiation for 15 min, 1 h, and 2 h. (b) Proposed photolysis product of irradiation of polymer **1**.

polymer show an increasing signal in the vinylic region at 6.3 ppm (A) corresponding to the expected vinylic signals of the photocleaved product (Figure 4b). The signals corresponding to the methylene protons at 4.3 and 4.2 ppm (C) and the methine proton at 3.8 ppm (D), all characteristic of the starting polymer, decrease progressively upon irradiation. The methyl doublet at 1.4 ppm (E) decreases in intensity upon irradiation, and a new singlet forms further downfield at 2.1 ppm (B) (see Figure S4, Supporting Information, for an expansion of this region). The change from doublet to singlet is consistent with loss of methine splitting of the methyl group as the polymer is irradiated, and the downfield shift is consistent with the formation of an adjacent vinyl group. The ^{1}H NMR data support degradation into the expected products upon irradiation with light.

Polymer **1** (10%) was then formulated into spherical particles of roughly 2 μm in diameter by electrospray (Figure 5a), which consists of using high voltage electricity to generate a fine aerosol of dense particles from a solution of polymer.[27] Particles encapsulated Nile Red, a hydrophobic dye that has much greater fluorescence in the hydrophobic environment of the particles than in water. Nile Red was chosen because its absorption is outside the absorbance spectrum of the photolabile group in polymer **1**. Upon polymer degradation, the dye will be quenched by either escaping the particles or entry of water into the particles facilitated by the change in hydrophobicity caused by the unmasking of carboxylic acid moieties upon photocleavage. Fluorescence quenching of the encapsulated Nile Red was monitored after irradiating the particles for 5, 15, 30, or 60 s with 320−480 nm light and after subsequent incubation. Complete quenching was reached after only 30 s of irradiation and remained unchanged even with further incubation, indicative of fast and efficient degradation of the particles and release of the payload (Figure 5b). Fluorescence quenching was similarly measured while irradiating particles with NIR light. Release was observed to progress more slowly; complete quenching of Nile Red was attained after 60 min compared to 30 s irradiation with UV−vis light. Since degradation was found to be incomplete after 60 min of irradiation with NIR light (Figure 3b), the Nile Red release experiment suggests that complete polymer degradation is not necessary to reach full payload release. Also, minimal release was observed in the absence of irradiation, which implies that the particles are stable. Quenching upon irradiation is much faster than that in the absence of irradiation, confirming that polymer degradation is responsible for cargo release.

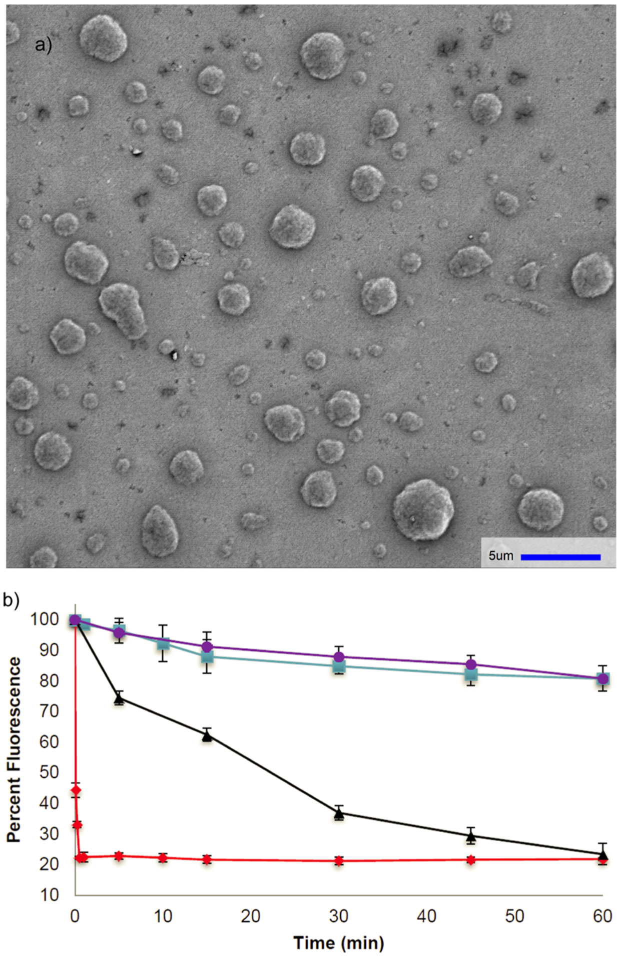


Figure 5. Particles of polymer **1** become porous or release cargo upon UV irradiation. (a) SEM image of particles composed of polymer **1** (10%) formulated by electrospray. (b) Fluorescence quenching of Nile Red encapsulated in polymeric nanoparticles. Red diamonds, sample irradiated with UV for one minute, then maintained at 37 °C; blue squares, sample incubated at 37 °C; black triangles, particles irradiated at 800 nm at 20 °C; purple circles, particles maintained at 20 °C.

In conclusion, we have developed a new photosensitive polymer sensitive to both UV/visible light and NIR laser light. By incorporating an efficient two-photon absorbing photocage within the polymer architecture, a polymer with a substantially larger two-photon uncaging action cross-section than most previously reported polymers was developed. This photocage is cleaved upon absorption within the hydrophobic microenvironment of the microcarrier, thus overcoming the main challenge to application of the previously reported bromo-hydroxycoumarin-containing polymers.[25] Although the polymerization initially proved challenging, copolymerization with a second diol led to high yield of polymer **1** that maintained the desired properties. Polymer **1** degrades in response to both UV/visible light and NIR laser light, as demonstrated by GPC. The photodegradation products of polymer **1** were identified by ^{1}H NMR spectroscopy. Polymer **1** was formulated into microparticles to encapsulate and release a payload, demonstrating polymer **1**'s potential in drug delivery. To achieve more complete degradation, we are pursuing polymers incorporating newer photoremovable structures that cleave more efficiently upon absorption.[28]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and characterization data for the synthesis of the described polymers, procedure for nanoparticle formulation, and expanded ^{1}H NMR spectrum for degradation study. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the NIH Director's New Innovator Award 1DP2OD006499-01 and a King Abdulaziz City for Science and Technology center grant to the Center of Excellence in Nanomedicine at UC San Diego. We thank the NIH New Innovator Award (DP 2OD006499) and KACST (through the KACST-UCSD Center of Excellence in Nanomedicine) for funding. We also thank Shivanjali Joshi-Barr and Cathryn McFearin for their intellectual contributions. NMR spectra were collected at the UCSD Skaggs School of Pharmacy and Pharmaceutical Sciences NMR Facility.

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