Photochemical & Photobiological Sciences

Supramolecular Photochemistry of Encapsulated Caged ortho-Nitrobenzyl Triggers

Journal:	*Photochemical & Photobiological Sciences*
Manuscript ID	PP-ART-06-2019-000260
Article Type:	Paper
Date Submitted by the Author:	07-Jun-2019
Complete List of Authors:	Kamatham, Nareshbabu ; University of Miami, Chmeistry Raj, A. Mohan; University of Miami, Givens, Richard; University of Kansas, Chemistry Da Silva, Jose; University of Algarve, FCT Ramamurthy, Vaidhyanathan; University of Miami, Chemistry

Supramolecular Photochemistry of Encapsulated Caged *ortho*-Nitrobenzyl Triggers

Nareshbabu Kamatham, A. Mohan Raj, Richard S. Givens, José P. Da Silva,*
and V. Ramamurthy*

Department of Chemistry, University of Miami, Coral Gables, FL 33146, United States
Department of Chemistry, University of Kansas, Lawrence, KS 66045, United States
CCMAR - Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

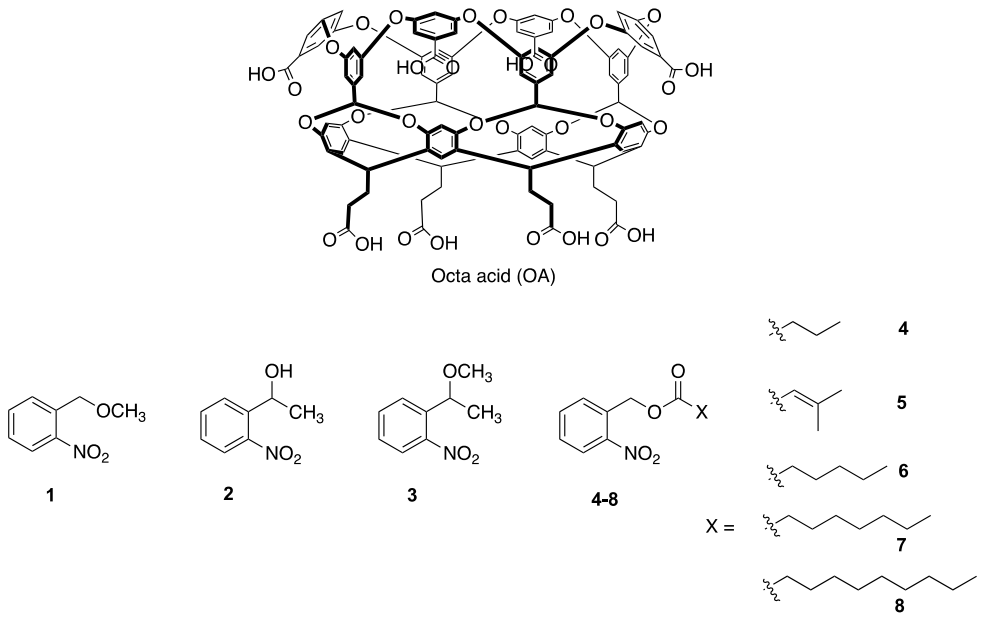
Abstract

Ortho-nitrobenzyl (oNB) triggers have been extensively used to release various molecules of interest. However, the toxicity and reactivity of the spent chromophore, *o*-nitrosobenzaldehyde, remains an unaddressed difficulty. In this study we have applied the well-established supramolecular photochemical concepts to retain the spent trigger *o*-nitrosobenzaldehyde within the organic capsule after release of water-soluble acids and alcohols. The sequestering power of organic capsules for spent chromophores during photorelease from *ortho*-nitro benzyl esters, ethers and alcohols is demonstrated with several examples.

Introduction

The protection of molecules of interest and their photorelease at chosen locations and times have been an active area of research for several decades.[1-8] A commonly adopted strategy is the use of 'phototriggers' (X-PPG) where a molecule of interest (X) is protected with a photo detachable group PPG and released at will with the help of a photon.[4] Such techniques are employed to deliver pharmaceuticals, catalysts, reagents, pheromones, fragrances, metal ions, signaling agents for inter-cell communication etc.[1] Although drug delivery and cell signaling are most effective in aqueous media, most protected molecules poorly soluble in aqueous media. Water-soluble supramolecular containers help overcome this conundrum.[9] We have used octa acid (OA, Scheme 1), a cavitand that forms capsular host-guest complexes with a wide variety of

molecules and forms a fully closed capsule around X-PPG. The 1:1 complex that the other known cavitands such as cyclodextrins, cucurbiturils and calixarenes form would expose a part of X-PPG to the media.[10] We have demonstrated the value of supramolecular concepts for the photorelease of organic acids of interest into aqueous media by encapsulating and photolyzing molecules protected by well- known PPGs such as *p*-methoxyphenacyl esters, *p*-hydroxy-phenacyl esters, 7-methoxy coumaryl-4-methyl esters and 7-diethylaminocoumaryl-4-methyl esters within OA capsule.[11-14] The advantages and disadvantages of each of these triggers prompted us to explore the most well-known and frequently applied classical triggering system, *ortho*-nitro benzyl (oNB), within OA.[15-20] In this study we establish that *ortho*-nitro benzyl systems could be included with the water soluble OA and they, depending on their size form either 2:1 or 2:2 host-guest capsular complexes. In both cases the guest is encapsulated within the capsule formed by two molecules of OA. As foreseen, irradiation resulted in the release of acid to aqueous medium and retainment of the *ortho*-nitroso compound within the capsule. Results presented highlight the value of OA in packaging the *ortho*-nitro benzyl system, solubilizing the normally insoluble X-PPG in water and releasing the protected acid upon activation with light. Details are presented below.



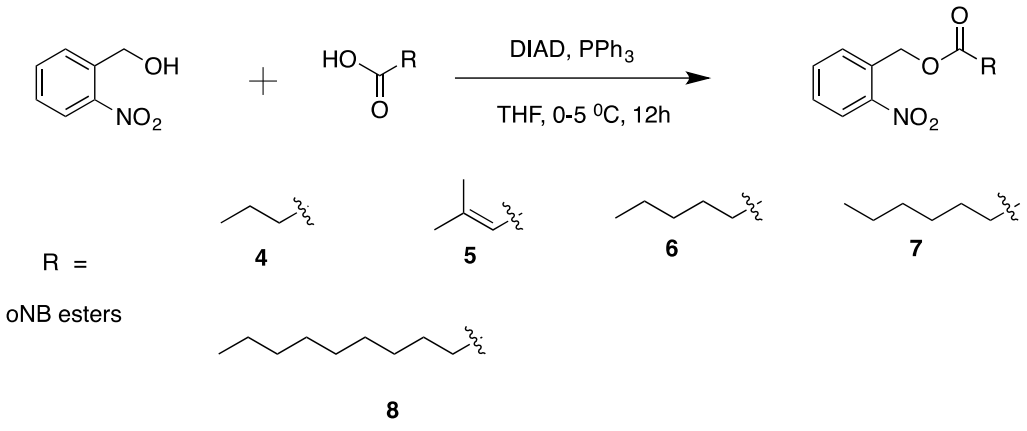
Scheme 1. Structures of water-soluble octa acid (OA) cavitand and oNB triggers (**1**-**8**).

Experimental

Materials

o-Nitrobenzyl (oNB) alcohol, butyric acid, 3,3-dimethylacrylic acid, hexanoic acid, octanoic acid and decanoic acid (Sigma-Aldrich/Alfa Aeser) were used as received. Compounds **1-3** were synthesized according to reported procedures.[21, 22] The host, octa acid (OA), was synthesized following literature procedure.[23]

oNB esters **4** - **8** were synthesized by following the literature procedure as outlined in Scheme 2.[24] In a 100 mL round bottomed flask oNB alcohol (1.3 mmol), the carboxylic acid (5.2 mmol), triphenylphosphene (5.2 mmol) and 30 mL THF were added and stirred under N_2 atm to complete dissolution. The reaction mixture was cooled to 0 - 5 ^{o}C. A separately prepared solution of 1.02 mL of diisopropyl azodicarboxylate (DIAD) in 10 mL of THF was added drop wise over a 30 min period at 0 - 5 ^{o}C. The temperature was raised to room temperature and maintained to complete reaction (12 h). The reaction was followed by TLC. The solvent was then removed by volatilization yielding an oily residue. The residue was submitted to column chromatography using a silica gel column and a mixture of hexane/EtOAc (60/40) as the mobile phase. As a colored impurity co-elutes with the product, a preparative TLC was also performed to obtain the isolate pure product. Phototriggers **4** - **8** were characterized by ^{1}H NMR and electrospray ionization mass spectrometry (ESI-MS and ESI-MS/MS) (see Figs S1-S10 in ESI). The phototriggers were isolated as light brown semi solids, with the following yields. **4**: 32 %; **5**: 37%; **6**: 22 %; **7**: 31 %; **8**: 28 %.



Scheme 2. Synthetic route for of oNB esters **4** - **8**.[24]

^{1}H NMR and mass spectra (S1-S10) are included as Electronic Supporting Information (ESI). The data are summarized below:

4: ^{1}H-NMR (500 MHz, DMSO) δ: 0.88 (t, *J* = 7.5 Hz, 3H), 1.54-1.59 (m, 2H), 2.37 (t, *J* = 7 Hz, 2H), 5.41 (s, 2H), 7.61-7.68 (m, 2H), 7.78 (t, *J* = 7.5 Hz, 1H), 8.1 (d, *J* = 7.5 Hz, 1H); ESI-HRMS: Calculated for $C_{11}H_{13}NO_4Na$ $[M+Na]^+$ 246.0742 observed: 246.0752

5: ^{1}H-NMR (500 MHz, DMSO) δ: 1.91 (s, 3H), 2.120 (s, 3H), 5.42 (s, 2 H), 5.79 (s, 1H), 7.61-7.67 (m, 2H), 7.79 (t, *J* = 7.5 Hz, 1H), 8.11 (d, *J* = 8 Hz, 1H); ESI-HRMS: Calculated for $C_{12}H_{13}NO_4Na$ $[M+Na]^+$ 258.0737, observed: 258.0749

6: ^{1}H-NMR (500 MHz, DMSO) δ: 0.85 (t, *J* = 6.5 Hz, 3H), 1.23-1.28 (m, 4H), 1.51-1.57 (m, 2H), 2.37 (t, *J* = 7.5 Hz, 2H), 5.40 (s, 2H), 7.61-7.68 (m, 2H), 7.78 (t, *J* = 7.5 Hz, 1H), 8.1 (d, *J* = 7.5 Hz, 1H); ESI-HRMS: Calculated for $C_{13}H_{17}NO_4Na$ $[M+Na]^+$ 274.1050,, observed: 274.0896

7: ^{1}H-NMR (500 MHz, DMSO) δ: 0.85 (t, *J* = 6.5 Hz, 3H), 1.23-1.25 (m, 8H), 1.53-1.55 (m, 2H), 2.38 (t, *J* = 7.5 Hz, 2H), 5.40 (s, 2H), 7.61-7.68 (m, 2H), 7.78 (t, *J* = 7.5 Hz, 1H), 8.1 (d, *J* = 7.5 Hz, 1H); ESI-HRMS: Calculated for $C_{15}H_{21}NO_4Na$ $[M+Na]^+$ 302.1363, observed: 302.1352.

8: ^{1}H-NMR (500 MHz, DMSO) δ: 0.85 (t, *J* = 6.5 Hz, 3H), 1.23-1.27 (m, 2H), 1.51-1.55 (m, 2H), 2.38 (t, *J* = 7.5 Hz, 2H), 5.41 (s, 2H), 7.61-7.68 (m, 2H), 7.78 (t, *J* = 7.5 Hz, 1H), 8.1 (d, *J* = 7.5 Hz, 1H); ESI-HRMS: Calculated for $C_{17}H_{25}NO_4Na$ $[M+Na]^+$ 330.1676, observed: 330.1675.

Instrumentation

NMR studies were performed using a 500 MHz Bruker NMR. High resolution full scan ESI-MS spectra were obtained using a Bruker Daltonics microTOF QII mass spectrometer and ESI-MS/MS spectra were obtained using a Bruker Daltonics HCT *ultra* mass spectrometer. GC-MS studies were performed using a Hewlett Packard 6890N apparatus equipped with a 5973 series mass selective detector (I.E. 70 eV) and a triple quadrupole Bruker SCION TQ 456GC. LC-DAD-MS studies were performed using an Agilent Technologies 1200 Series LC, equipped with a diode array detector (DAD), and coupled to a Bruker Daltonics HCT *ultra* mass spectrometer (MS). UV spectra of triggers, products and host-guest complexes were obtained

using Shimadzu UV-3150 spectrophotometer. UV spectra of isolated products were obtained by LC-DAD.

Methods

Characterization of materials

^{1}H NMR spectra of synthesized compounds were collected at 25 ºC. For high resolution full scan ESI-MS spectra the synthesized compounds were solubilized in a mixture methanol-chloroform (50:50) containing 0.1% formic acid. The solution was continuously infused (200 μL hr^{-1}) into the source, with the help of a syringe pump (KdScientific, model 601553, USA). Typical experimental conditions were: capillary voltage, 4.5 kV; drying gas, 180 ºC at 4 L min^{-1}; nebulizer gas pressure, 0.3 bar; end plate offset -500 V. For ESI-MS/MS spectra the ions were continuously generated by infusing the compounds in acetonitrile (50 μM) at 4 μL min^{-1} into the mass spectrometer source with the help of a syringe pump (KdScientific, model 781100, USA). Typical experimental conditions were: capillary voltage, 3.5 kV; capillary exit voltage (CE), 75 V; skimmer voltage, 40 V; drying gas, 300 ºC at 6 L min^{-1}; nebulizer gas pressure, 20 psi.

Preparation of Host-Guest Complexes

Preparation of host-guest complexes for guest binding studies probed by NMR: A D_2O stock solution (600 μL) of host OA (1 mM) and sodium borate buffer (10 mM) taken in a NMR tube was titrated with the guest by sequential addition of 0.25 equ of guest (2.5 μL of a 60 mM solution in $DMSOd_6$). The complexation was achieved by shaking the NMR tube for about five minutes. ^{1}H NMR spectra were recorded at room temperature. 2:2 complex was achieved by 10 μL of guest solution to 600 μL of 1 mM OA host in 10 mM buffer.

Preparation of host-guest complexes for absorption studies: A 60 mM stock solution of each guest was prepared in DMSO, and 12 mL of 5 x 10^{-5} M of host (OA) solution was prepared at pH 8.7 using 10 mM $Na_2B_4O_7$ buffer/H_2O. The solutions of the complex were prepared by adding 5 μL of the 60 mM guest solution in DMSO-d_6 which resulted in a final guest concentration of 2.5 x 10^{-5} M for the host solution. After shaking the mixtures manually for 2 min, the UV-vis absorption spectra were recorded (Figs S11-12 in ESI).

Preparation of host-guest complexes for LC-DAD-MS studies: A 1 mM stock solution of host (OA) was prepared in 10 mM borate ($Na_2B_4O_7$) buffer aqueous solution. Stock solutions of

guests were prepared in DMSO at 10 mM concentration. The solutions of complexes contain 100 μM of guest and 200 μM of OA.

Irradiations

Photochemical studies with **1 - 3**: The NMR tube containing 1 mM host-guest complex borate buffer solution was placed in a Rayonet reactor fitted with 360 nm lamps and a cooling fan. Absorption spectra were recorded for compounds **1-3** (5×10^{-5} M) in water and also in the presence of OA. Progress of the reaction was monitored by recording absorption spectra at various times during irradiation of the samples.

Photochemical studies with **4 - 8**: A 600 μL solution of 1 mM OA (10 mM $Na_2B_4O_7$ in D_2O, pH = 8.7) was placed in an NMR tube. Then 0.5 equivalents of guest (5 μL of a 60 mM solution in DMSO-D_6) were added. After shaking the NMR tube for 5 min, the ^{1}H NMR was recorded to confirm the complex formation. The sample was irradiated with a 450 W medium pressure mercury vapor lamp (Pyrex containers, $\lambda \geq 300$ nm) and the progress of the reaction was monitored by ^{1}H NMR.

Determination of trigger conversions yields of photoproducts

The trigger conversions were determined by LC-DAD at 320 nm. The mobile phase comprises acetonitrile (A) and water (B), both with 0.1 % of formic acid, and ethyl acetate (C). The gradient started with 52 % of A, 38 % of B and 10 % of C. The mobile phase composition was changed to 2 % of A, 73 % of B and 25 % of C in 5 minutes and kept at this composition for an additional 7 minutes. Finally, the system was allowed to return to the initial mobile phase composition (52 % of A, 38 % of B and 10 % of C) in 1 min and then stabilized for an additional 5 minutes before the next run. The flow was 0.35 ml min^{-1}. The column was a Grace C18 reversed phase LC column (10.0 cm length, 2.1 mm internal diameter, 3 μm), stabilized at 25 °C.

The yields of acid released from **4** were determined by ^{1}H NMR. A known amount of internal standard, methyl viologen (the same equivalent of the guest), was added to the complex **4**@$(OA)_2$ solution and irradiated to complete conversion. The product yields were calculated by comparison with the integration value of the methyl viologen peak as the reference. The yields of acid released for triggers **5-8** were determined by GC-MS. The samples were prepared in the following way: one mL of irradiated solution was transferred to a closed vessel. One mL of

aqueous HCl with 1.0 M concentration, was added to lower the pH and protonate the acids. Then 0.5 mL of dichloromethane was added and the mixture shaken for 2 minutes. The mixture was then centrifuged to separate the phases. The dichloromethane phase was then analyzed. For quantification the sample was spiked with known amounts of acid and the signal increase was used to determine the concentration of acids before spiking. A ZB-5MS (Phenomenex) capillary column with 30 m length, 0.25 mm internal diameter and 0.25 mm film thickness was used. The oven temperature program was 70 ºC for 1.0 min, 10 ºC-min min^{-1} increased until a final temperature of 280 ºC. The injector and the transfer line were set to 280 ºC and the injection volume was 1 μL. The acids were detected in the single ion mode by selecting the m/z values of the main fragments obtained by electron impact. For hexanoic acid a Grace AT-WAXMS column with 30 m length, 0.25 mm internal diameter and 0.25 mm film thickness was used. The oven temperature program was 80 ºC for 2.0 min, 10 ºCmin^{-1} increased until a final temperature of 260 ºC. The injector was set to 260 ºC and the injection volume was 1 μL.

The identification of major products, namely *o*-nitrosobenzaldehyde, was performed using a triple quadrupole – GC-MS by comparison of the experimental spectra with those of the library NIST 2014, 10^{th} edition. A ZB-5MS (Phenomenex) capillary column with 30 m length, 0.25 mm internal diameter and 0.25 mm film thickness was used. The oven temperature program was 45 ºC for 1.0 min, 25 ºC min^{-1} increased until a final temperature of 250 ºC The final temperature was keep for 4.8 minutes. The injector and the transfer line were set to 250 ºC and 255 ºC, respectively, and the injection volume was 1 μL.

The concentrations of product *o*-nitrosobenzaldehyde were estimated by LC-DAD using the calibration curves obtained for the trigger. The areas measured for *o*-nitrosobenzaldehyde in the LC trace at 320 nm (signal at 2.3 minutes) were multiplied by the ratio of the absorbance coefficients of trigger and product at 320 nm. The resulting value was used to estimate the concentration of *o*-nitrosobenzaldehyde using the above calibration curve.

The absorbance coefficients of triggers at 320 nm were measure by UV-Vis absorbance and the extinction coefficient of *o*-nitrosobenzaldehyde at 320 nm was obtained from Gaplovsky et. al.[22]

Results and Discussion

The study consisted of two aspects, (a) inclusion of the triggers **1- 8** with the host OA and (b) photochemical study of the host-guest complexes. The first part required us to determine the inclusion of the guests within OA and the nature of the host-guest complexes by spectral means. The inclusion of the PPG triggers **1- 8** within OA was confirmed by the ^{1}H NMR spectra of the complexes. Partial ^{1}H NMR spectra of guests included within OA (represented as guest@OA_2 for 1:2 and $guest_2$@OA_2 for 2:2 complexes respectively) are presented in Figures 1 and 2. These confirm that the signals due to aliphatic hydrogens are upfield shifted to appear between δ 1 and – 3.5 ppm, indicating the inclusion of guests within OA.[25, 26] ^{1}H NMR titration experiments (Figures S13-S18 and S20 and S22) suggested that the guest to host stoichiometry of 1:1 (or 2:2) for **1-3** and 1:2 for **4 - 8**. The 2:2 complex imply each OA capsule contains two molecules of the guest while 1:2 indicates each capsule to contain one molecule of the guest. The reason for this difference in stoichiometry has to do with the size of the guest; smaller guests **1-3** form 2:2 complexes while larger **4 - 8** form 1:2 complexes. To confirm that all guests form a capsule the diffusion constants were measured by DOSY experiments. Diffusion constant will help one to distinguish between 1:1 cavitandplex and 2:2 capsuleplex, although both have the same stoichiometry. Smaller sized cavitandplexes would be expected to have higher diffusion constants while larger capsule lexes would have lower diffusion constants. The diffusion constants measure for all seven complexes independent of whether they are 1:2 or 2:2 had closely similar constants (Table 1). The diffusion constants of all eight complexes close to 1.4 x 10^{-6} cm^2/s is lower than those for free OA (~1.9 x 10^{-6} cm^2/s) and 1:1 open cavitandplex (~1.65 x 10^{-6} cm^2/s) confirming their capsular nature).[27, 28]

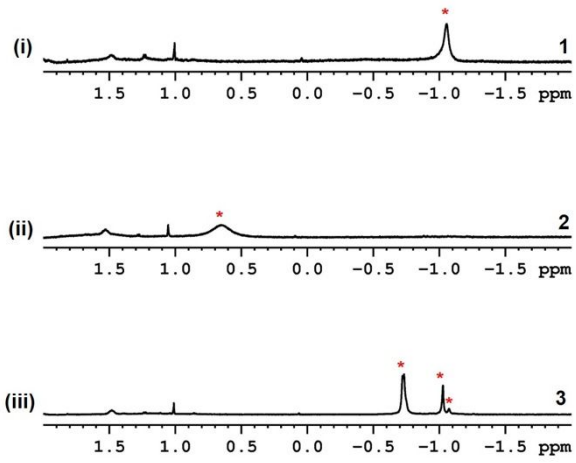


Figure 1. Partial ^{1}H NMR spectra of the OA complexes *o*-nitrobenzyl ethers **1** and **3** oNB alcohol **2**. (2:2 complexes)]: (i) **1**, (ii) **2**, (iii) **3**. "*" indicates the OA bound guest aliphatic proton signals.

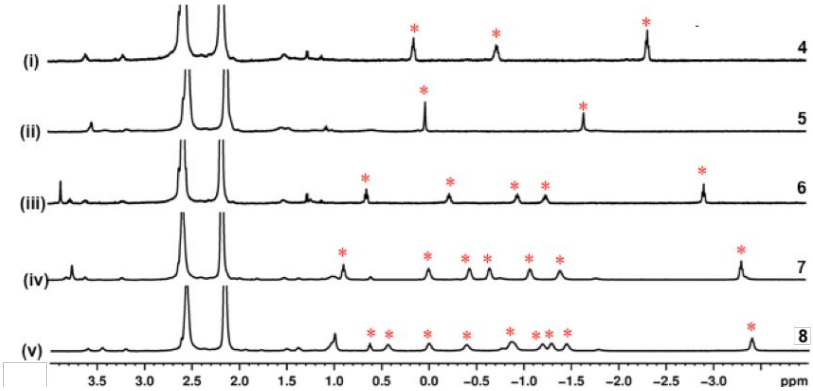


Figure 2. Selected guest region of the ^{1}H NMR spectra of the OA complexes *o*-nitrobenzyl esters **4**-**8**. (2:1 complexes)]: (i) **4**, (ii) **5**, (iii) **6**, (iv) 7, (v) **8**. "*" indicates the OA bound guest aliphatic proton signals.

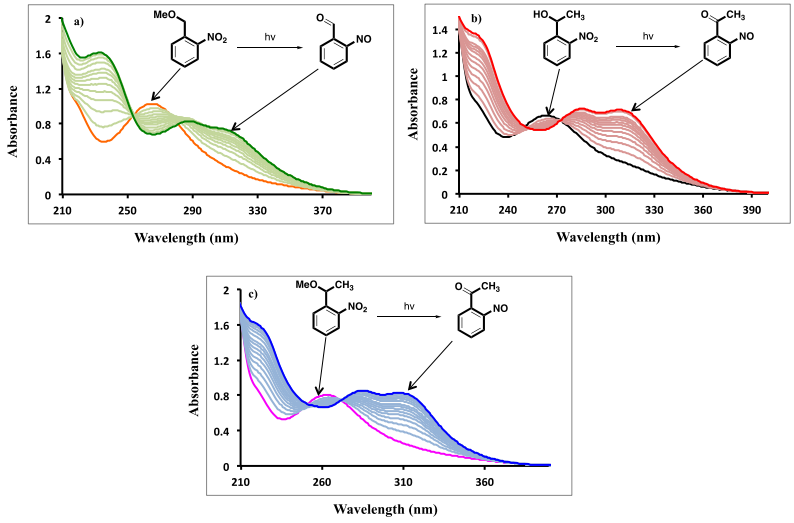
Table 1. Diffusion constant of complexes

Compound	Diffusion constant (cm^2/s)
Only OA	1.88×10^{-6}
$\mathit{1}_2$@OA_2	1.30×10^{-6}
$\mathit{2}_2$@OA_2	1.46×10^{-6}
$\mathit{3}_2$@OA_2	1.32×10^{-6}

4@OA$_2$	1.39 x 10^{-6}
5@OA$_2$	1.38 x 10^{-6}
6@OA$_2$	1.42 x 10^{-6}
7@OA$_2$	1.43 x 10^{-6}

Having confirmed that the triggers **1-8** form capsular complexes we proceeded to irradiate these either in a UV-cuvette or an NMR tube. Results of the photolysis (> 340 nm) of oNB-ethers and alcohol **1-3** are presented first. Wirz group's detailed studies on **1-3** in solution are valuable in interpreting the photobehavior of **1- 3**@OA.[21, 22, 29] Photoreactions of free and encapsulated **1-3** in water were clean and complete within 30 min. The absorption spectra recorded at regular intervals of irradiation are provided in Figures 3 and 4. Appearance of a new band with a maximum around 320 nm corresponding to *o*-nitroso-BA and *ortho*-nitroso acetophenone is consistent with literature reported values during the photolysis of **1 - 3** in solution.[21, 22, 29] The similarity between the absorption spectra observed in the presence and absence of OA suggested the photoreaction within OA to be identical to that in solution.

(a)



(b)

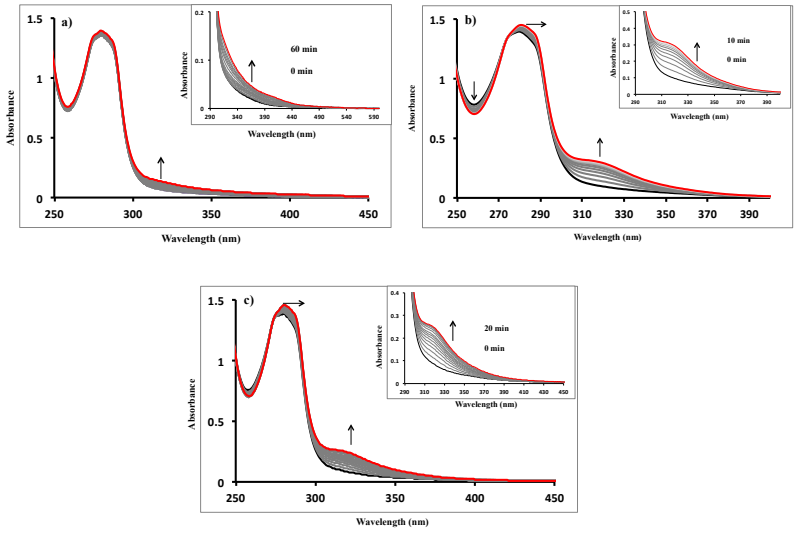
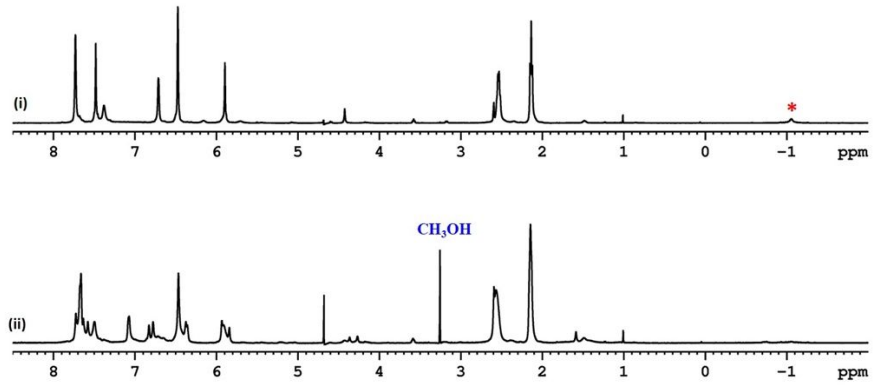


Figure 3. (a) Progress of reaction as followed by absorption spectra of upon photolysis of compounds **1**-**3** in water. Irradiation was done using Rayonet reactor (365 nm) UV lamps. (b) Progress of reaction as followed by absorption spectra of upon photolysis of a) $\mathbf{1}_2$@OA_2 ; b) $\mathbf{2}_2$@OA_2 ; c) $\mathbf{3}_2$@OA_2 in buffer. Irradiation was done using Rayonet reactor (365 nm) UV lamps.

^{1}H NMR spectra of the irradiated samples confirmed the formation of methanol in the case of **1** and **3** (Figure 4). Appearance of a signal at δ 3.25 and disappearance of the signal at δ -1.0 with **1** and **3** suggested the aqueous residence of the released methanol (Figures 5 and 6). As expected, release of water in the case of $\mathbf{2}_2$@OA_2 could not be detected by ^{1}H NMR. Locating the signals from *o*-nitroso-BAP released by **2** and **3** was easier by ^{1}H NMR spectra. Formation of *o*-nitroso product upon release of water and methanol from $\mathbf{1}_2$-$\mathbf{3}_2$@OA_2 was confirmed by a combination of ESI-MS, LC-DAD-MS and GC-MS (Figures S31-S35).

(a)



(b)

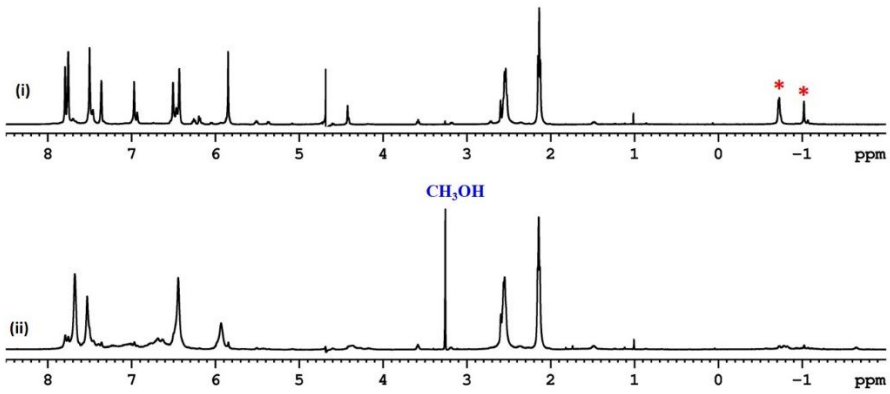


Figure 4. (a) ^{1}H NMR (500 MHz) spectra of (i) $\mathbf{1}_2$@OA_2 before irradiation; (ii) $\mathbf{1}_2$@OA_2 after 30 min irradiation. (b) ^{1}H NMR (500 MHz) spectra of (i) $\mathbf{3}_2$@OA_2 before irradiation; (ii) $\mathbf{3}_2$@OA_2 after 30 min irradiation. " * " represents the bound protons of guest **1** and **3**.

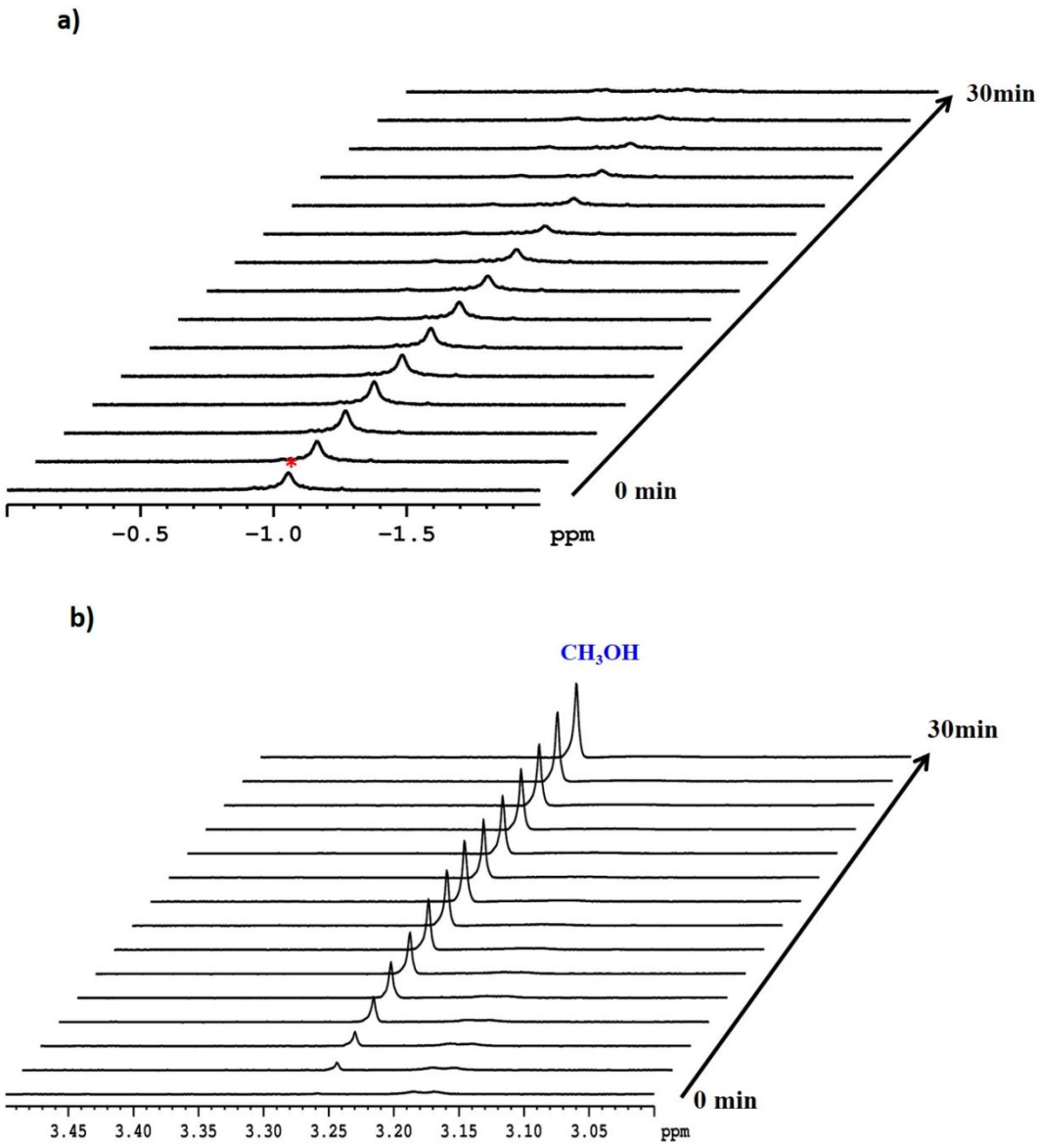
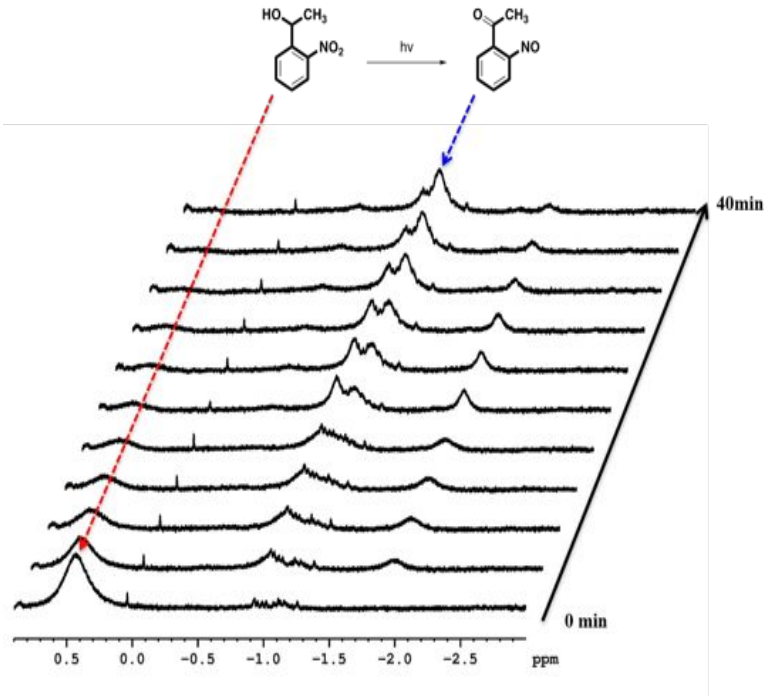
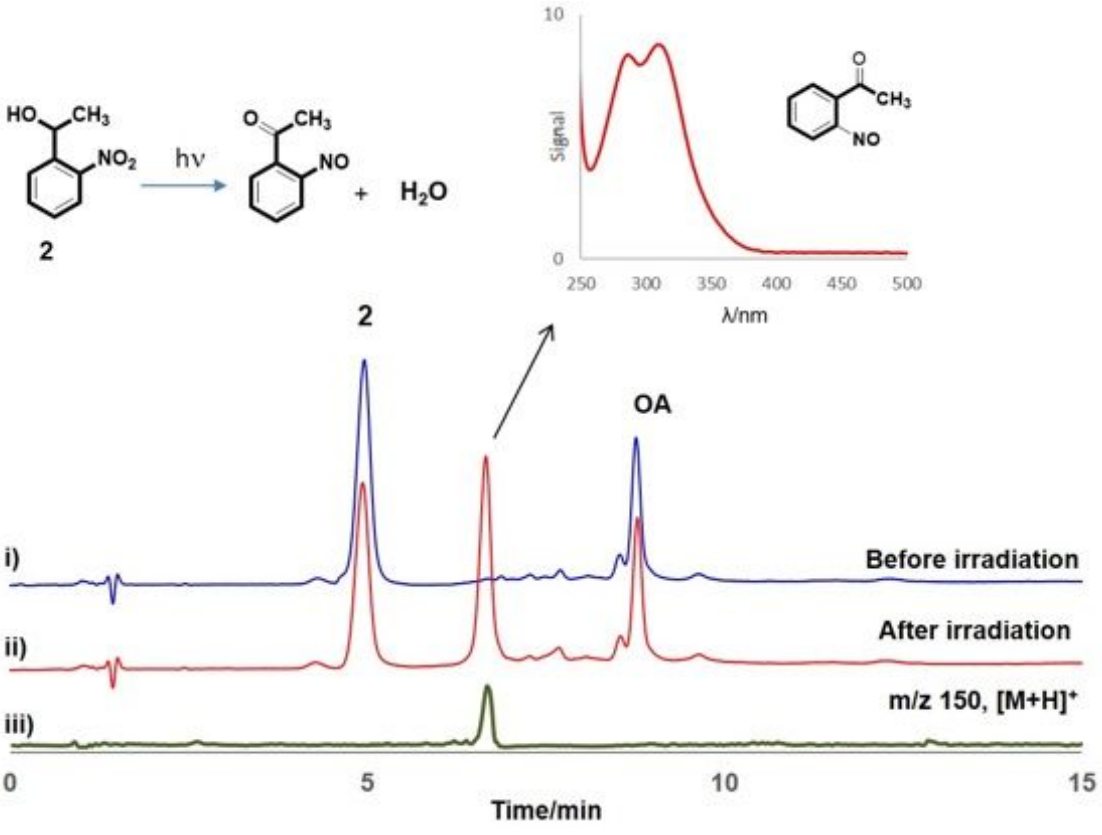


Figure 5. Progress of reaction as followed by ^{1}H NMR (500 MHz upon photolysis of $\mathbf{1}_2$@OA_2 (a) disappearance of methyl proton; (b) formation of photoproduct (methanol) with time

(a)


HO CH3
NO2
hv
O CH3
NO
40min
0 min
0.5 0.0 -0.5 -1.0 -1.5 -2.0 -2.5
ppm


HO CH3
NO2
hν
O CH3
NO
+ H2O
2
10
Signal
0
250 300 350 400 450 500
λ/nm
2
OA
i)
Before irradiation
ii)
After irradiation
iii)
m/z 150, [M+H]+
0 5 10 15
Time/min

(b)

Figure 6. (a) Partial ^{1}H NMR (500 MHz) spectra of photoirradiation of $\mathbf{2}_2$@OA_2 monitored vs. time. (b) LC-DAD (320 nm) and LC-MS single ion (m/z 150) traces of $\mathbf{2}_2$@OA_2. i) LC-DAD trace before irradiation; ii) LC-DAD trace after 5 minutes irradiation ($\lambda > 300$ nm); iii) single ion trace at m/z 150, the expected value for the oNBAP under positive polarity ionization. The insert show the absorption spectrum taken at 6.67 minutes.

Since one of the goals of this study is to sequester the toxic photo reactive nitroso release byproduct[30-33] following release of the acid, establishing the location of *o*-nitroso-BA and *o*-nitroso-BAP was critical. Locating the ^{1}H NMR signals of the released *o*-nitroso-BAP from **2** and **3** was straight forward. Figure 6a provides partial ^{1}H NMR spectra (δ 1 to -2.5) of the irradiated $\mathbf{2}_2$@OA_2 for various time intervals. We attribute the decrease in signal at δ 0.5 due to the methyl in the reactant with time, accompanied by a corresponding increase of the new signal at δ -1.1 to the acetyl methyl of *o*-nitroso-BAP, which is confirmed by LC-MS (Figure 6b). The significant upfield shift of the methyl signal suggests the photoproduct *o*-nitroso-BAP is within the OA capsule; a signal near ~ δ 2 would be expected had it been in aqueous solution. A similar observation was made in the case of **3** (Figure S25). These findings indicate that photolysis of the OA encapsulated nitrobenzyl triggers results in the release of alcohols from the capsule and retainment of the toxic nitroso byproduct within OA. The ability to retain the unwanted and toxic nitroso product within OA provides a solution to a long-standing problem with the byproduct when using oNB triggers for delivery of a molecule of interest.

The above study with ethers was extended to oNB esters, a system studied extensively in solution. Since molecules of interest are generally protected as esters,[15] our main goal was to establish the generality of the triggering process within the water-soluble OA capsule. Simultaneously follow the nitroso moiety formation (within or outside the capsule) along with the release of the caged acid. Thus, we investigated the photorelease from encapsulated oNB esters **4**-**8**@OA_2 that release acids of different hydrophilicities (for example compare propionic acid and decanoic acid in Figures 8 and 9). As shown in Figure S12 the absorption spectra of OA and oNB esters fully overlap with a maximum at 280 nm and the latter molecules insoluble in water are solubilized by OA. The complexes were irradiated (>300 nm) in Pyrex tubes using a 450 W medium pressure a mercury lamp, a condition in which both OA and **4**-**8** would absorb

the incident light. Since OA in the excited state has been established to transfer excitation energy to the guest of lower energy,[34] we believed regardless of the light absorbing entity the reaction would occur from the excited state of oNB esters. The same triggering process is established to occur from both S_1 and T_1 of *o*-nitrobenzyl esters[35-38] We are aware the product *ortho*-nitrosobenzaldehyde is photochemically active.[39] To overcome this problem all photolysis were conducted only up to 30% conversion. We did not undertake photochemical and toxicological studies of *ortho*-nitrosobenzaldehyde.

Progress of the irradiation was followed by ^{1}H NMR, GC-MS and LC-DAD-MS. The photoreaction was clean and the corresponding acids were released in > 80 % yield for most triggers, as monitored by ^{1}H NMR and GC-MS (Table 2). Unfortunately, we could not clearly identify the peaks due to *o*-nitroso-BA by ^{1}H NMR. However, formation of *o*-nitroso-BA was detected by its characteristic absorption using a diode array detector during LC-DAD-MS analysis of the irradiated sample and further confirmed by GC-MS. HPLC traces of **4** irradiated as a free molecule and as **4**@OA_2 and their absorptions are shown in Figure 7. Assuming the absorption spectrum of the signal at 2.3 min with close resemblance to that of *o*-nitroso-BA reported in the literature[22] to be *o*-nitroso-BA, we have estimated its yield (Table 2). There is almost 1:1 correspondence between *o*-nitroso-BA and released acid. Based on the results discussed above with oNB ethers we believe it must be retained within OA. In addition to major amounts of *o*-nitroso-BA the two minor products detected by LC-DAD (signals at 7.4 and 8.5 min) have absorption characteristic of OA and *o*-nitroso-BA. We suspect these are derived via reaction between OA and the intermediates or products formed after intramolecular hydrogen abstraction.[16, 17, 36, 40]

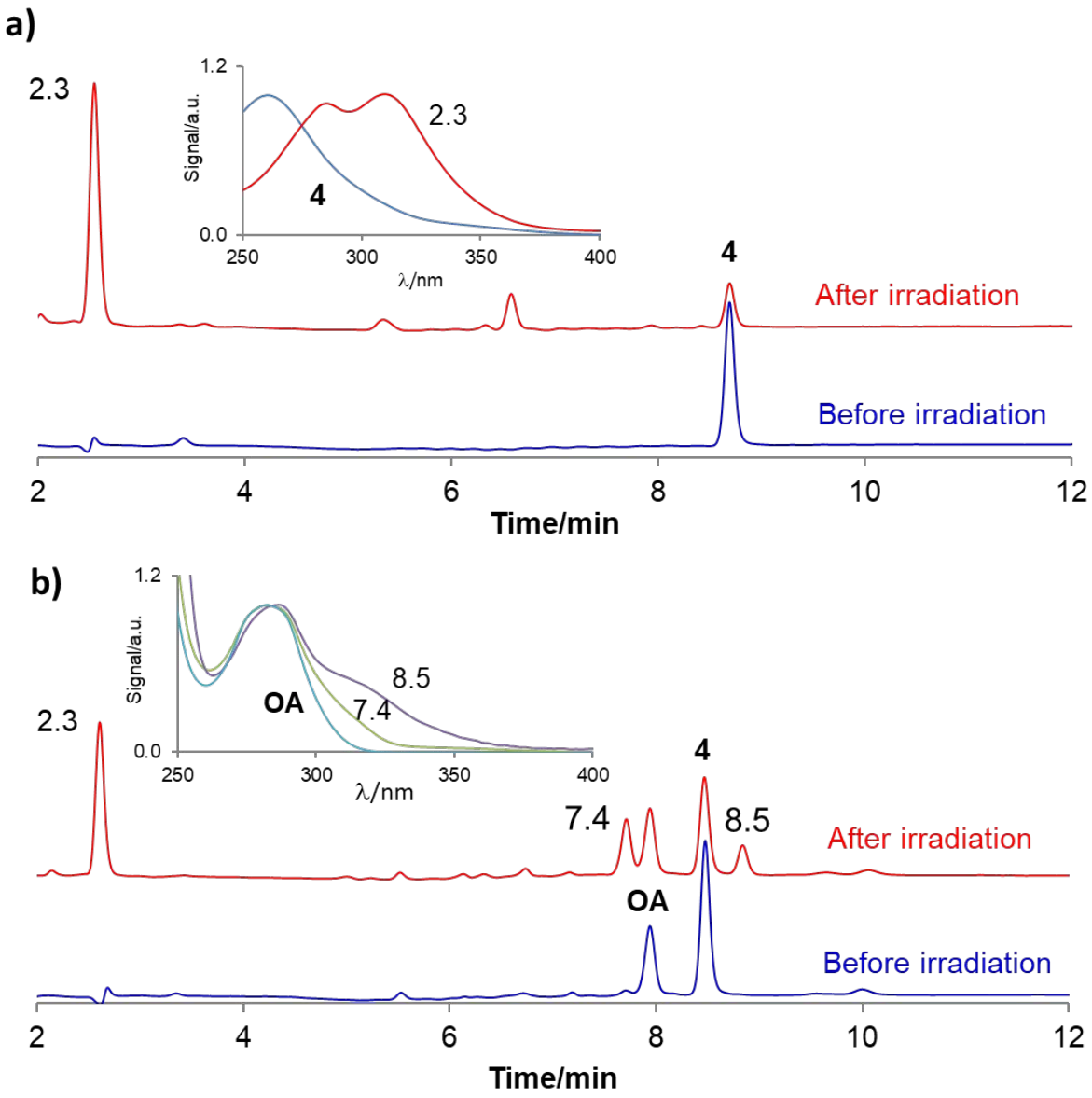


Figure 7. LC-DAD traces (320 nm) of **4**@OA_2, before and after irradiation (λ >300 nm) in aqueous medium, (a), and aqueous medium with OA, (b). The inserts show the absorption spectra taken at retention times of observed signals.

Table 2. Photoconversion of oNB phototriggers and corresponding yields of acids released in aqueous solutions containing OA (200 μM host: 100 μM guest).[a)]

Compound No	% converted	Yield of acid	Yield of nitroso benzaldehyde
4	67[b)]	91[b)]	83
5	55	65[c)]	46
6	35	83[c)]	81
7	33	79[c)]	82
8	28	81[c)]	92

[a)]After 90 minutes, Pyrex glass filter, water filter, air equilibrated. [b)] Determined by NMR. [c)] Estimated by GC-MS. The errors are ~ 15%.

We recorded ^{1}H NMR spectra of the free guest, host-guest complex, irradiated sample and the free acid to ascertain the location of the released acid in each case. The spectra for **4**@OA_2 and **8**@OA_2 are displayed in Figures 8 and 9 and for the others in SI (Figures S26-S30). Comparison of the spectra in Figure 8 (iii), (iv) and Figure 9 (iii), (iv) and (v) clearly show the released butanoic acid from **4**@OA_2 is in water while decanoic acid from **8**@OA_2 stays within OA. From the figures presented in ESI (Figures S27 and S28) it should be clear that 3,3-dimethylacrylic acid from **5**@OA_2 and hexanoic acid from **6**@OA_2 following release exit into the aqueous solution while octanoic acid (Figure S29) upon release shuttles between inside and outside the OA container.

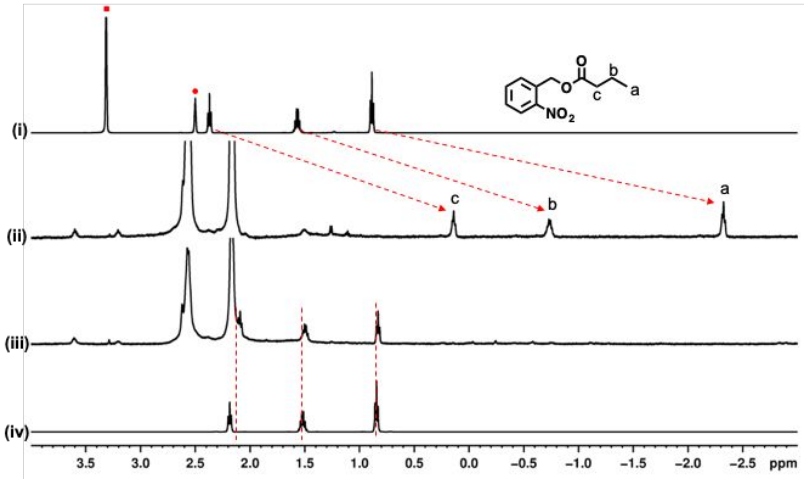


Figure 8. ^{1}H-NMR spectra (500 MHz, 10 mM $Na_2B_4O_7$ buffer/D_2O, pH = 8.7) of (i) **4** in DMSO-d_6, (ii) **4**@$(OA)_2$ ([OA] = 1 mM and [**4**] = 0.5 mM), (iii) 2.5 h irradiation of **4**@$(OA)_2$ at ($\lambda \geq 300$ nm), (iv) butyric acid in $Na_2B_4O_7$ buffer/D_2O. Symbols ■ and • indicates the residual solvent peaks of water and DMSO-d_6, respectively. "a-c" indicate the OA bound guest aliphatic proton peaks This figure should be earlier.

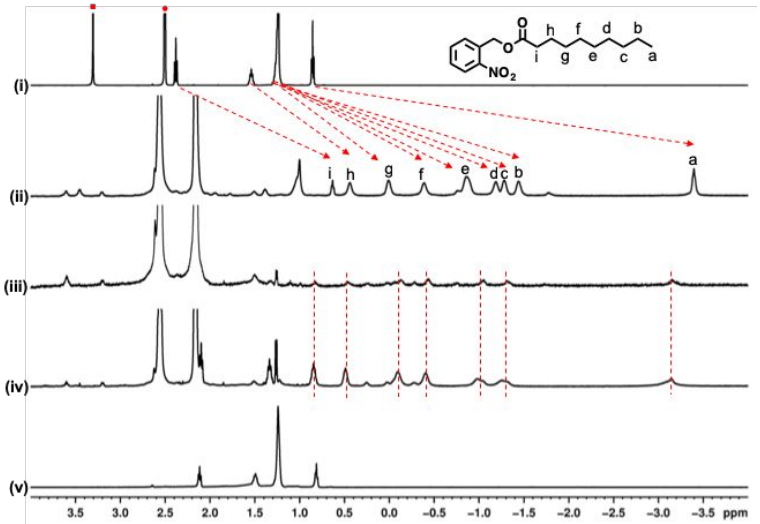


Figure 9. ^{1}H-NMR spectra (500 MHz, 10 mM $Na_2B_4O_7$ buffer/D_2O, pH = 8.7) of (i) **8** in DMSO-d_6 (ii) **8**@$(OA)_2$ ([OA] = 1 mM and [**8**] = 0.5 mM); (iii) 5 h irradiation of **8**@$(OA)_2$ at ($\lambda \geq 300$ nm); (iv) decanoic acid@OA ([OA] = 1mM, [decanoic acid] = 0.25 mM); (v) decanoic

acid in $Na_2B_4O_7$ buffer/D_2O. Symbols ■ and • indicate the residual solvent peaks of water and DMSO-d_6, respectively. "a-i" indicate the OA bound guest aliphatic proton peaks.

Summary

Several of our recent studies have demonstrated the value of encapsulating X-PPG molecules where PPGs are derived from substituted acetophenones and coumarins.[11-14] The current study has explored a fifth and the most popular PPG, the *ortho*-nitro benzyl system. The major advantage of the supramolecular encapsulation approach is that the water insoluble PPG protected substrates could be solubilized and the molecules of interest be released into water. Additional advantage is that the main reaction of photorelease from the excited PPG occurs within the capsule, which minimizes the potential of quenching by exogenous quenchers such as oxygen as well as reaction of any very reactive and short-lived intermediates that might be initially generated. With certain PPG's, we have observed reactions of OA with reactive intermediates that remain within the OA capsule, thus encapsulating additional unwanted byproducts of the photorelease process.[12, 14] These attributes along with the demonstrated ease of synthesis of the oNB protecting group are distinct advantages in the release of drugs at the required place. We recognize that these advantageous properties are significantly limited by the capacity of the OA when the size of guest exceeds the size that the OA can contain. None the less, the established 'proof of principle' of the supramolecular photorelease strategy has potential for delivering hydrophobic, reactive reagents of interest in aqueous media. This underexplored supramolecular strategy for reagent delivery to a remote, desired location where the spent, unwanted, often times toxic trigger retains within the delivering capsule will have far reaching applications toward discovery of larger, water-soluble capsular hosts.

Electronic Supplementary Information

Experimental procedures, ^{1}H NMR, UV and ESI-MS spectra for all new compounds. Irradiation procedures, ^{1}H NMR titration spectra of host-guest complexes, progress of photoreactions as monitored by ^{1}H NMR, LC-DAD-MS.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgements

The National Science Foundation (CHE-1807729) and Kansas University Endowment Association respectively are thanked by VR and RSG for financial support. JPDS thanks Portuguese national funds from FCT - Foundation for Science and Technology through project UID/Multi/04326/2019, and from the operational programs CRESC Algarve 2020 and COMPETE 2020 through project EMBRC.PT ALG-01-0145-FEDER-022121.

References

1. P. Klan, T. Solomek, C. G. Bochet, A. Blanc, R. Givens, M. Rubina, V. Popik, A. Kostikov and J. Wirz, *Chem. Rev.*, 2013, **113**, 119-191.
2. D. D. Young and A. Deiters, *Org. Bimol. Chem.*, 2007, **5**, 999-1005.
3. G. Mayer and A. Heckel, *Angew. Chem. Int. Ed.*, 2006, **45**, 4900-4921.
4. M. Goeldner and R. Givens, *Dynamic Studies in Biology: Phototriggers, Photoswitches and Caged Molecules*, Wiley-VCH, New York, 2005.
5. A. P. Pelliccioli and J. Wirz, *Photochem. Photobiol. Sci.*, 2002, **1**, 441-458.
6. M. Abe, Y. Chitosea, S. Jakkampudia, P. T. T. Thuya, Q. Lina, B. T. Vana, A. Yamadaa, R. Oyamaa, M. Sasakia and C. Katan, *Synthesis*, 2017, **49**, 3337-3346.
7. C. Bochet, *J. chem. Soc., Perkin Trans. 1*, 2002, 125-142.
8. R. S. Givens, M. Rubina and J. Wirz, *Photochem. Photobiol.*, 2012, **11**, 472-488.
9. J. Vazquez, M. A. Romero, R. N. Dsouza and U. Pischel, *Chem. Commun.*, 2016, **52**, 6245-6248.
10. U. H. Brinker and J.-L. Mieusset, eds., *Molecular Encapsulation*, John Wiley & Sons, Chichester, 2010.
11. P. Jagadesan, J. P. Da Silva, R. S. Givens and V. Ramamurthy, *Org. Lett.*, 2015, **17**, 1276-1279.
12. N. Jayaraj, P. Jagadesan, S. R. Samanta, J. P. Da Silva and V. Ramamurthy, *Org. Lett.*, 2015, **17**, 1276–1279.
13. N. Kamatham, J. P. Da Silva, R. S. Givens and V. Ramamurthy, *Org. Lett.*, 2017, **19**, 3588-3591.

14. N. Kamatham, D. C. Mendes, J. P. Da Silva, R. S. Givens and V. Ramamurthy, *Org. Lett.*, 2016, **18**, 5480-5483.
15. J. E. T. Corrie, in *Dynamic Studies in Biology*, eds. M. Goeldner and R. Givens, Wiley-VCH Verlag GmbH & Co., Weinheim, 2003, ch. 1.1, pp. 1-28.
16. J. E. T. Corrie and D. R. Trentham, in *Biological Applications of Switches*, ed. H. Morrison, John Wiley & Sons, Inc., New York, 1993, vol. 2, ch. 5, pp. 243-305.
17. J. A. McCray and D. R. Trentham, *Annu. Rev. Biophys. Chem.*, 1989, **18**, 239-270.
18. P. De Mayo and S. T. Reid, *Q. Rev. Chem. Soc.*, 1961, **15**, 393-417.
19. J. A. Barltrop, P. J. Plant and P. Schofield, *Chem. Commun.*, 1966, 822-823.
20. A. Patchornik, B. Amit and R. B. Woodward, *J. Am. Chem. Soc.*, 1970, **92**, 6333–6335.
21. Y. V. Il'ichev, M. A. Schworer and J. Wirz, *J. Am. Chem. Soc.*, 2004, **126**, 4581-4595.
22. M. Gaplovsky, Y. V. Il'ichev, Y. Kamdzhilov, S. V. Kombarova, M. Mac, M. A. Schworer and J. Wirz, *Photochem. Photobiol. Sci.*, 2005, **4**, 33-42.
23. C. L. D. Gibb and B. C. Gibb, *J. Am. Chem. Soc.*, 2004, **126**, 11408-11409.
24. G. Appendino, A. Minassi, N. Daddario, F. Bianchi and G. C. Tron, *Org. Lett.*, 2002, **4**, 3839-3841.
25. M. Porel, N. Jayaraj, L. S. Kaanumalle, M. V. S. N. Maddipatla, A. Parthasarathy and V. Ramamurthy, *Langmuir*, 2009, **25**, 3473-3481.
26. N. Jayaraj, S. Jockusch, L. S. Kaanumalle, N. J. Turro and V. Ramamurthy, *Can. J. Chem.* , 2011, **89**, 203-213.
27. R. Choudhury, A. Barman, R. Prabhakar and V. Ramamurthy, *J. Phys. Chem. B*, 2013, **117**, 398-407.
28. N. Jayaraj, Y. Zhao, A. Parthasarathy, M. Porel, R. S. H. Liu and V. Ramamurthy, *Langmuir*, 2009, **25**, 10575-10586.
29. M. Schwörer and J. Wirz, *Helv. Chim. Acta*, 2001, **84**, 1441-1458.
30. M. C. Pirrung, Y. R. Lee, K. Park and J. B. Springer, *J. Org. Chem.*, 1999, **64**, 5042-5047.
31. D. Beaudoin and J. D. Wuest, *Chem. Rev.*, 2016, **116**, 258-286.
32. I. Biljan and H. Vanˇcik, *Crystals*, 2017, **7**, 376, 371-315.
33. H. Tomioka, N. Ichikawa and K. Komatsu, *J. Am. Chem. Soc.*, 1992, **114**, 8045-8053.

34. P. Jagadesan, B. Mondal, A. Parthasarathy, V. Jayathirtha Rao and V. Ramamurthy, *Org. Lett.*, 2013, **15**, 1326-1329.
35. T. Schmierer, S. Laimgruber, K. Haiser, K. Kiewisch, J. Neugebauer and P. Gilch, *Phys. Chem. Chem. Phys.*, 2010, **12**, 15653-15664.
36. R. W. Yip, Y. X. Wen, D. Gravel, R. Giasson and D. K. Sharma, *J. Phys. Chem.*, 1991, **95**, 6078-6081.
37. T. Schmierer, F. Bley, K. Schaper and P. Gilch, *J. Photochem. Photobiol. A*, 2011, **217**, 363-368.
38. R. W. Yip, D. K. Sharma, R. Glasson and D. Gravel, *J. Phys. Chem.*, 1985, **89**, 5328-5330.
39. H. A. Morrison, in *The Chemistry of Nitro and Nitroso Groups*, ed. H. Feuer, John Wiley and Sons,Inc., New York, 1969, pp. 165-213.
40. T. Šolomek, S. Mercier, T. Bally and C. G. Bochet, *Photochem. Photobiol. Sci.*, 2012, **11**, 548-555.