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Photocleavage of *o*-nitrobenzyl ether derivatives for rapid biomedical release applications

Moon Suk Kim† and Scott L. Diamond*

Institute for Medicine and Engineering, Department of Chemical and Biomolecular Engineering, 1024 Vagelos Research Laboratory, University of Pennsylvania, Philadelphia, PA 19104, USA

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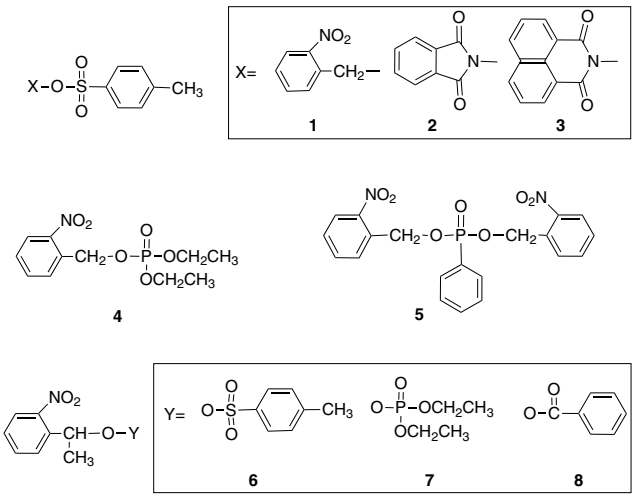
Abstract—The externally controlled cleavage of covalently linked prodrugs, proteins, or solid-phase formulation vehicles offers potential advantages for controlled drug or gene delivery. A series of *o*-nitrobenzyl ester compounds (**1**–**8**) were synthesized to allow a systematic study of photolability. The *o*-nitrobenzyl ester was strictly required for photolability, while imido esters were not photolabile. The degradation kinetics of 1-*o*-phenylethyl ester was an order of magnitude faster than that of *o*-nitrobenzyl ester. Tosylate, phosphate, and benzoate derivatives of 1-*o*-nitrophenylethyl displayed similar photolability (>80% decomposition within 10 min at 3.5 mW/cm^2 at 365 nm). *O*-*o*-Nitrobenzyl *O*′,*O*″-diethyl phosphate displayed the fastest decomposition at photoirradiation condition (3.5 mW/cm^2, 365 nm) suitable for biological systems. We report the synthesis and photo-decomposition of 1-*o*-nitrophenylethyl derivatives amenable for the creation of photolabile prodrugs or formulation particles for drug depots, DNA condensation, or tissue engineering applications.

The timed and accurate dosing of a gene or drug at a specific location in vivo or in vitro presents a challenging and diverse set of constraints.[1] Controlled delivery and release can be achieved by the use of specific external stimulation such as temperature, pH, ultrasound, electric current, or photoexcitation. Caged compounds or those with photo-cleavable linkers are able to decompose rapidly upon photoirradiation and several biological compounds have been examined.[2–5] Among them, biological compounds containing an *o*-nitrobenzyl group appear as an attractive candidate to control the release of gene and drug from a delivery formulation triggered by photoirradiation.[6–8] The photolytic mechanism of *o*-nitrobenzyl derivatives is not yet fully established, although evidence suggests the nitro group is reduced to nitroso, while the benzyl carbon is oxidized via an initiation by the attacking nitro-group oxygen.[9]

We investigated photo-cleavable delivery chemistry suitable for photoirradiation in vivo and in vitro.[10] The study of *o*-nitrobenzyl derivatives allowed substantial improvements of the rates of photocleavage. In this article, we report in detail the decomposition kinetics associated with photolysis of *o*-nitrobenzyl derivatives (Scheme 1).

A series of photo-labile compounds (**1**–**8**) were synthesized by the reaction of *p*-toluene sulfonic chloride (tosyl



Scheme 1.

Keywords: Photocleavage; *o*-Nitrobenzyl; Drug delivery.
* Corresponding author. Tel.: +1 215 573 5702; fax: +1 215 573 7227; e-mail: sld@seas.upenn.edu
† Present address: Medicinal Science Division, Korea Research Institute of Chemical Technology, PO Box 107, Yuseong, Daejeon 305-600, Republic of Korea.

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chloride), benzoyl chloride, diethyl phosphorochloridate, and phenyl phosphonic dichloride with various alcohols in the presence of an amine or sodium hydride. The structures of **1**–**8** were confirmed by ^{1}H, ^{13}C NMR, and elemental analysis.[11–19]

Photo-decomposition reactions have employed various photo-active compounds and various irradiation protocols for biochemical or chemical research.[5] The photoreactions carried out in the body tend to use photosource with comparable long wavelength ($\lambda > 300$ nm) to minimize damage to tissue.[20–22] Therefore, we have chosen *o*-nitrobenzyl group which can be activated by photoirradiation of 365 nm. First, the photoirradiation of **1**–**3** was carried out using 365 nm (1.6 mW/cm^2) for 60 min to compare photo-decomposition of *o*-nitrobenzyl and imide groups which have photoactivity. ^{1}H NMR monitored the structural changes of **1**–**3** before and after irradiation. *N*-Hydroxylphthalimide tosylate **2** and *N*-hydroxylnaphthalimide tosylate **3** exhibited no change in ^{1}H NMR after irradiation (365 nm) for 60 min, while **1** showed the integration decrease of benzyl proton and a new signal assignable to methyl proton of *p*-toluene sulfonic acid formed by decomposition of **1**. Therefore, the integration changes of ^{1}H NMR were calculated as decomposition degree of **1**.

For photolability, a strict requirement for the $-NO_2$ group was consistent with the need for the formation of nitroso intermediate to initiate the decomposition reaction. Compound **1** displayed 6%, 27%, and 43% decomposition after 60 min irradiation at 400, 365, and 300 nm (Fig. 1). On irradiation of **1** at 365 nm (1.6 mW/cm^2) in various solvents, the $\ln[C]/[C]_o$ versus time plots exhibited the expected linear decrease (Fig. 2). From the slope of the plots the values of apparent first order dissociation kinetic rate constants (k_{app}) were 8.83×10^{-4}, 1.31×10^{-4}, 1.63×10^{-4}, and 1.12×10^{-4} s^{-1}, in CD_3OD, DMSO-d_6, dioxane-d_8, and $CDCl_3$, respectively (Fig. 2).

Having verified a strict requirement for **1**, phosphate derivatives were tested for faster dissociation kinetics in relation to the tosylate of **1**–**3**. The photo-decomposition was carried out with *O*-*o*-nitrobenzyl *O*′,*O*″-diethyl phosphate **4** at 365 nm with different power (0.3, 1.6, and 3.5 mW/cm^2) for 60 min. Decomposition was determined by the integration decrease of benzyl protons in ^{1}H NMR. Compound **4** showed 84% decomposition at 3.5 mW/cm^2 and 78% at 1.6 mW/cm^2, while 40% at 0.3 mW/cm^2. We compared the photo-decomposition rate at 365 nm (1.6 mW/cm^2) of **4** (one *o*-nitrobenzyl group) with that of *O*,*O*′-di-*o*-nitrobenzylphenyl phosphonate **5** (two *o*-nitrobenzyl groups). A similar rate of decomposition was obtained for **4** and **5** (in 78% at 60 min), even though **5** showed 67% increase of absorbance at 365 nm compared with **4**.

Long-term exposures at 365 nm can damage cells or DNA in the body[21,23] so maximizing the photocleavage rate is desirable. Prior studies demonstrated that the methyl group on the benzyl position enhanced the relative cleavage kinetics by a factor of 20.[23] Therefore, a methyl group was introduced at the benzyl position to increase the decomposition rate. Figure 3 shows the UV–vis spectra of 1-*o*-nitrophenylethyl tosylate **6**, *O*-1-*o*-nitrophenylethyl *O*′,*O*″-diethyl phosphate **7**, and 1-*o*-nitrophenylethyl benzoate **8**. The absorbance order was **7** > **6** > **8** at 365 nm, but the spectra showed almost identical absorbance from 300 to 450 nm, indicating the absorbance of **6**–**8** depended mainly on the 1-*o*-nitrophenylethyl group not on the other aromatic substitutes.

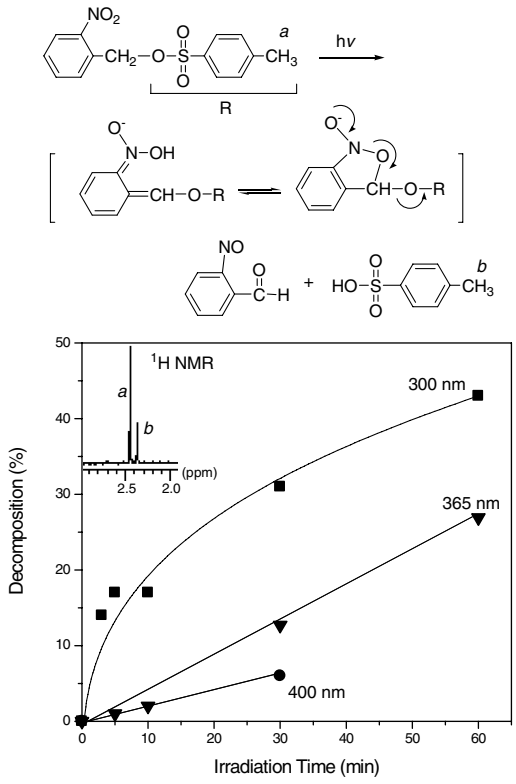

Figure 1. Decomposition–irradiation time curves of **1** in CD_3OD at 300, 365, and 400 nm.

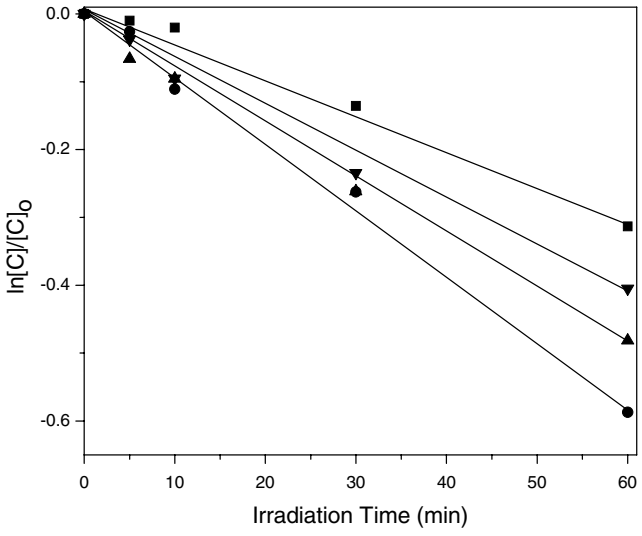

Figure 2. The plot for $\ln[C]/[C]_o$ versus irradiation time of **1** in various solvents (■) CD_3OD, (▲) DMSO-d_6, (●) dioxane-d_8, and (▼) $CDCl_3$.

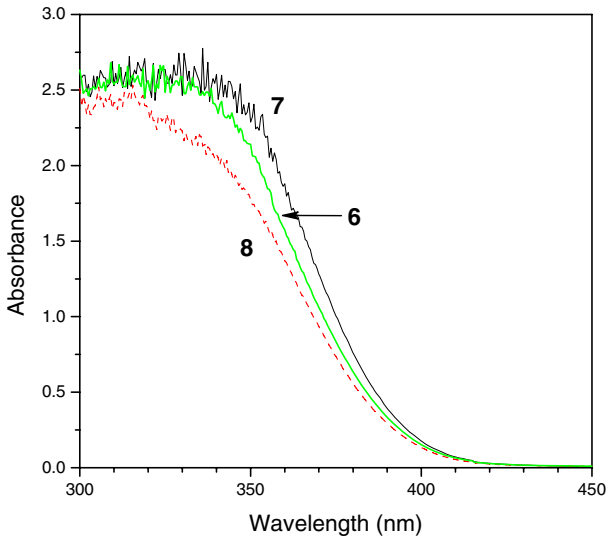

Figure 3. UV–vis spectra of **6**–**8** (4.4 mM in $CDCl_3$).

The decomposition of **6**–**8** was monitored using ^1H NMR and HPLC after irradiation. Figure 4 shows the ^1H NMR spectra of **6** before and after irradiation. In the spectrum after irradiation, integration of signals 'a' and 'b' assignable to methyl and benzyl protons decreases at 1.4 and 4.5 ppm, respectively. Meanwhile, new signals 'd' and 'e' appeared and the integration increased with irradiation time.

From the decomposition–irradiation time curves for **6**–**8**, we report the determination of the apparent first order dissociation kinetic rate constants (k_{app}) = 4.33×10^{-3} s^{-1} (**7**), 3.17×10^{-3} s^{-1} (**6**), and 3.05×10^{-3} s^{-1} (**8**). The decomposition rate of **6**–**8** showed faster decomposition than **1**–**5**. The decomposition order was **7** > **6** > **8**, consistent with the absorption strength at 365 nm (Fig. 3). This result indicated that the decomposition rate depended most strongly on the nitrobenzyl group and to a much lesser extent on the substituent groups.

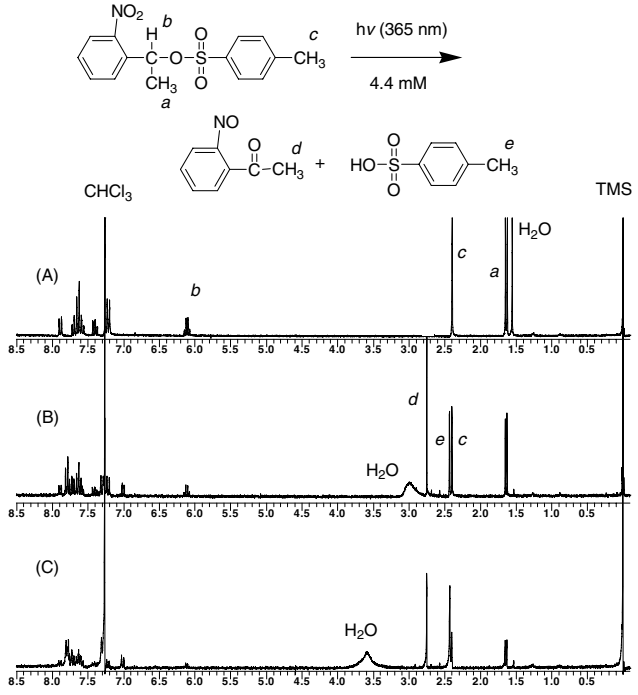

Figure 4. ^1H NMR spectra of 1-*o*-nitrophenethyl tosylate **6** before (A) and after photoirradiation for 6 min (B) and 10 min (C).

Figure 5 shows the HPLC chromatography (acetonitrile and 1% TFA aqueous [80/20, v/v]) of **6** after irradiation using 365 nm (3.5 mW/cm^2) for 0–10 min. The peak intensity of **6** decreased with increase of irradiation time, while the intensity of the new signals 'a' and 'b', assigned to *o*-nitrosoacetophenone and *p*-toluene sulfonic acid, respectively, increased (Fig. 5A). The UV spectrum of signal 'a' showed characteristic absorbance of nitrosoacetophenone at 270–350 nm (Fig. 5B).

In conclusion, the photo-decomposition of *o*-nitrobenzyl and *o*-nitrophenylethyl derivatives was carried out with various photosources and power using long wavelength UV (365 nm). The decomposition rate of *o*-nitrophenylethyl derivatives was significantly faster than the rate of

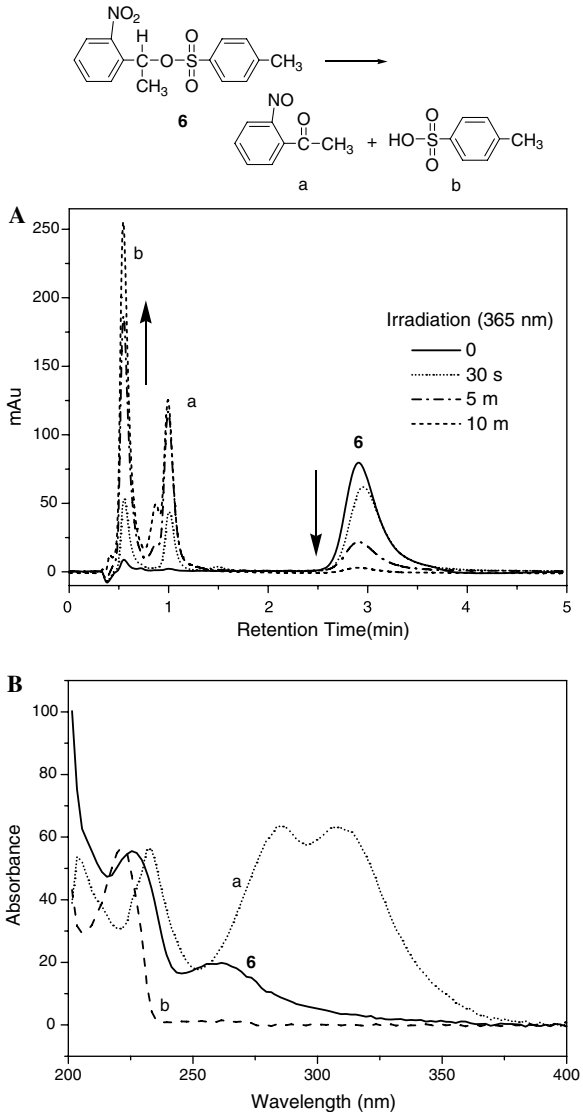

Figure 5. HPLC charts for photo-decomposition of **6** (A) and UV spectra of **6**, 'a' and 'b' (B).

o-nitrobenzyl derivatives. This detail decomposition study can serve as the basis for the design of new gene delivery system (i.e., photolytic cationic polymers) to control gene release using photoirradiation. Further research on the synthesis of gene carrier modified with *o*-nitrobenzyl derivatives for triggered extracellular or intracellular DNA release by photoirradiation is now in progress.

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11. Compound **1**: yield 51%. ^{1}H NMR ($CDCl_3$): 8.12–7.36 (m, 8H, –2(C_6H_4)), 5.48 (s, 2H, (–CH_2–)), 2.46 (s, 3H, CH_3). Anal. Calcd for $C_{14}H_{13}NSO_5$: C, 54.71; H, 4.26, N; 4.56. Found: C; 54.68; H; 4.05, N; 4.11.
12. Compound **2**: yield 51%. ^{1}H NMR ($CDCl_3$): 7.95–7.43 (m, 8H, –(C_6H_4), –(C_6H_4)), 2.50 (s, 3H, CH_3). Anal. Calcd for $C_{15}H_{11}NSO_5$: C; 56.78, H; 3.49, N; 4.41. Found: C; 56.40; H; 3.28, N; 4.22.
13. Compound **3**: yield 75%. ^{1}H NMR ($CDCl_3$): 8.65–7.42 (m, 10H, –(C_6H_4), –($C_{12}H_6$)), 2.51 (s, 3H, CH_3). Anal. Calcd for $C_{19}H_{13}NSO_5$: C; 62.12, H; 3.57, N; 3.81. Found: C; 61.99; H; 3.30, N; 3.59.
14. Compound **4**: yield 31%. ^{1}H NMR ($CDCl_3$): 8.18–7.48 (m, 4H, –C_6H_4), 5.51 (d, J = 6.9 Hz, 2H, (–CH_2–)), 4.18 (m, 4H, 2CH_2), 1.35 (m, 6H, 2CH_3). Anal. Calcd for $C_{11}H_{16}NPO_6$: C, 45.68; H, 5.58, N; 4.84. Found: C; 45.50; H; 5.64, N; 4.29.
15. Compound **5**: yield 63%. ^{1}H NMR ($CDCl_3$): 7.91–7.46 (m, 13H, –(C_6H_5), –2($NO_2C_6H_4$)), 5.55 (m, 4H, 2(–CH_2–)). ^{13}C NMR ($CDCl_3$): 153.0, 134.0, 133.1, 131.9, 131.7, 128.8, 128.5, 124.9, 64.4. Anal. Calcd for $C_{20}H_{17}N_2PO_7$: C, 56.08; H, 4.00, N; 6.54. Found: C; 55.92; H; 3.93, N; 6.16.
16. 1-*o*-Nitrophenylethyl alcohol: yield 95%. ^{1}H NMR ($CDCl_3$): 7.83–7.32 (m, 4H, –(C_6H_4)), 5.30 (m, 1H, (–CH–)), 3.16 (br, 1H, OH), 1.46 (d, J = 6.2 Hz, CH_3).
17. Compound **6**: yield 69%. ^{1}H NMR ($CDCl_3$): 7.91–7.20 (m, 7H, –(C_6H_4), –(C_6H_3)), 6.12 (m, 1H, (–CH–)), 2.40 (s, 3H, CH_3), 1.64 (d, J = 6.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$): 144.8, 133.8, 133.1, 129.7, 128.7, 128.1, 127.7, 124.2, 75.3, 23.5, 21.5. Anal. Calcd for $C_{15}H_{15}NSO_5$: C; 56.06, H; 4.70, N; 4.36. Found: C; 56.54; H; 4.65, N; 4.07.
18. Compound **7**: yield 65%. ^{1}H NMR ($CDCl_3$): 7.88–7.34 (m, 4H, –(C_6H_4)), 5.91 (m, 1H, –CH–), 3.93 (m, 4H, 2CH_2), 1.60 (d, J = 6.2 Hz, 3H, CH_3), 1.15 (m, 6H, 2CH_3). ^{13}C NMR ($CDCl_3$): 169.4, 146.6, 137.6, 133.5, 128.4, 127.6, 124.1, 71.7, 63.7, 24.1, 15.6. Anal. Calcd for $C_{12}H_{18}NPO_6$: C; 47.53, H; 5.98, N; 4.62. Found: C; 47.20, H; 6.37, N; 4.58.
19. Compound **8**: yield 72%. ^{1}H NMR ($CDCl_3$): 8.08–7.25 (m, 9H, –(C_6H_4), –(C_6H_5)), 6.57 (m, 1H, (–CH–)), 1.79 (d, J = 6.2 Hz, CH_3). ^{13}C NMR ($CDCl_3$): 165.3, 147.6, 138.0, 133.6, 133.1, 129.5, 128.3, 127.0, 124.4, 68.7, 22.0. Anal. Calcd for $C_{15}H_{13}NO_4$: C; 66.41, H; 4.83, N; 5.16. Found: C; 66.76, H; 5.02, N; 4.86.
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