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which should be cited to refer to this work.

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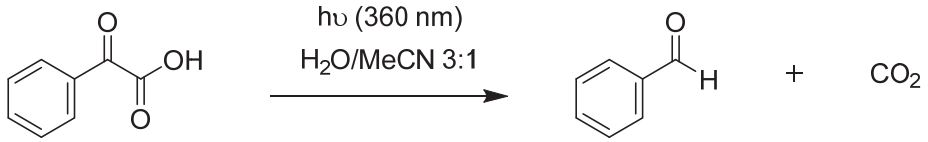
Part 1: Data Acquired by E. Janett

General Methods

Analytical photo-irradiations were performed with a LUMOS 43A photoreactor (Atlas Photonics). Preparative photo-irradiations were performed with a Rayonet photoreactor (Southern New England Ultraviolet Company). A Perkin Elmer Lambda 40 UV/Vis spectrometer was employed for the UV/Vis spectra. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on Bruker-DRX-300 or Bruker Avance DPX 500 spectrometers. All NMR spectra were recorded in CD_3CN, H_2O/D_2O 9:1, or HEPES buffer (0.1 M, pH=7.2)/D_2O 9:1, as indicated. Chemical shifts are expressed in parts per million (δ) using residual solvent protons as internal standards. Coupling constants (*J*) are reported in Hz. Splitting patterns are designated as s (singlet), d (doublet), dd (double doublet), t (triplet), dt (double triplet), q (quartet), bs (broad singlet), m (multiplet). Deuterated solvents were obtained from Cambridge Isotope Laboratory. HPLC and LC-MS was performed on an Acquity H-Class UPLC system equipped with an ESI-SQD mass spectrometer, using an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm). Method details: 0.5 mL/min, 7 mM formic acid, H_2O : acetonitrile = 0 min 95:5, 1.7 min 0:100, 4.0 min 0:100. Flash column chromatography (FC) was carried out using Brunschwig silica gel (SiO_2, 60 Å, 32-63 mesh). Mass spectra at high resolution were recorded on a Bruker 4.7T BioApex II mass spectrometer. A Bruker Tensor 27 spectrometer equipped with a golden gate was used to record IR spectra. Plotting and fitting of data was performed with Microsoft Office 2010.

Phenylglyoxylic Acid Actinometry[1]



Scheme S1. Photon-induced degradation of phenylglyoxylic acid.

General remarks

The value of the photon flux of a lamp is only valid for a precise position in the optical path and for the same photochemical reactor and cell. The solution has to absorb all the light during the whole irradiation time. At an excitation wavelength of 360 nm, a 0.05 M solution of phenylglyoxylic acid is used (optical density OD > 3). The quantum yield of the decarboxylation (see Scheme S1) has been reported to be 0.728 upon irradiation at 365 nm and 334 nm.[1]

Thus, it can be used as actinometer upon irradiation at 360 nm. All processing steps have to be executed in the dark.

Photolysis

3 mL of a 0.05 M solution of phenylglyoxylic acid in a mixture of water and acetonitrile (3:1) were put in a standard absorbance quartz cell (1 cm path length) and irradiated in a LUMOS 43A photoreactor equipped with LED lamps at 360 nm (bandwidth < 10 nm) for a given time.

Analyses

A UV/Vis absorbance spectrum was recorded every 5 minutes of irradiation. The decrease of the optical density at 390 nm (OD < 1) was monitored and used to determine the number of moles photolyzed after each irradiation (ε(360 nm) = 74 $M^{-1}cm^{-1}$). As the absorbance of the product, benzaldehyde, is negligible at 390 nm, the conversion can be determined by dividing the measured optical density by the optical density of the starting solution, and multiplying with the starting amount (in mol). The number of converted mol was plotted as a function of the time (Figure S1).

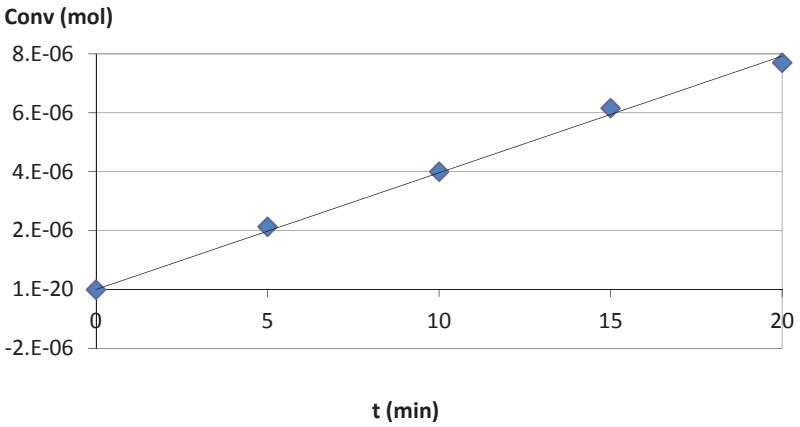
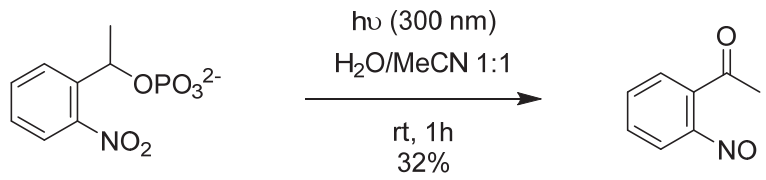


Figure S1. Amount of converted phenylglyoxylic acid (in mol) upon irradiation with a LUMOS 43A photoreactor with respect to the irradiation time. Function of the fit: conv = $3.9601 \cdot 10^{-7}$ t. $R^2 = 0.99$.

The slope of the regression line was divided by the published quantum yield of photolysis ($\Phi_{365} = 0.728$) to give a value of **$5.44 \cdot 10^{-7}$ E/min** for the photon flux of the 360 nm LED lamp of the LUMOS 43A.

Synthesis of the 1-(2-Nitrosophenyl)ethan-1-one



Scheme S2. Photouncaging of caged phosphate.

A solution of 1-(2-nitrophenyl)ethyl phosphate[2] (200 mg, 0.746 mmol) in a mixture of water and CH_3CN (1:1, 10 mL) was irradiated in a quartz reactor in a Rayonet photoreactor at 300 nm for 1 h. The solution was diluted with a saturated bicarbonate solution and extracted with CH_2Cl_2. The collected organic phases were washed with saturated bicarbonate solution, dried over $MgSO_4$, filtered, and concentrated under vacuum. Purification by flash chromatography (EtOAc/pentane 2:8) afforded 1-(2-nitrosophenyl)ethan-1-one as yellowish oil (39 mg, 0.26 mmol, 32% yield). The ^{1}H NMR spectrum showed the presence of impurities. Note, that the impurities should not play a role in our quantification method, as an internal standard was added (*vide infra*).

^{1}H NMR (300 MHz, CD_3CN) δ 7.90–7.81 (m, 1 H), 7.76–7.62 (m, 2 H), 7.14 (d, J = 8.1 Hz, 1 H), 2.64 (s, 3 H). ^{13}C NMR (75MHz, CD_3CN) δ 203.4, 164.0, 137.3, 132.5, 132.2, 128.9, 115.5, 32.2. HR-MS (ESI) *m/z* calcd. for $C_8H_7NNaO_2$ ($[M+Na]^+$) 172.03690, found 172.03742. FT-IR (golden gate, 600-4000 cm^{-1}) 2930, 2917, 2364, 1686, 1602, 1448, 1356, 1256, 956, 761, 740, 713, 630.

Spectra

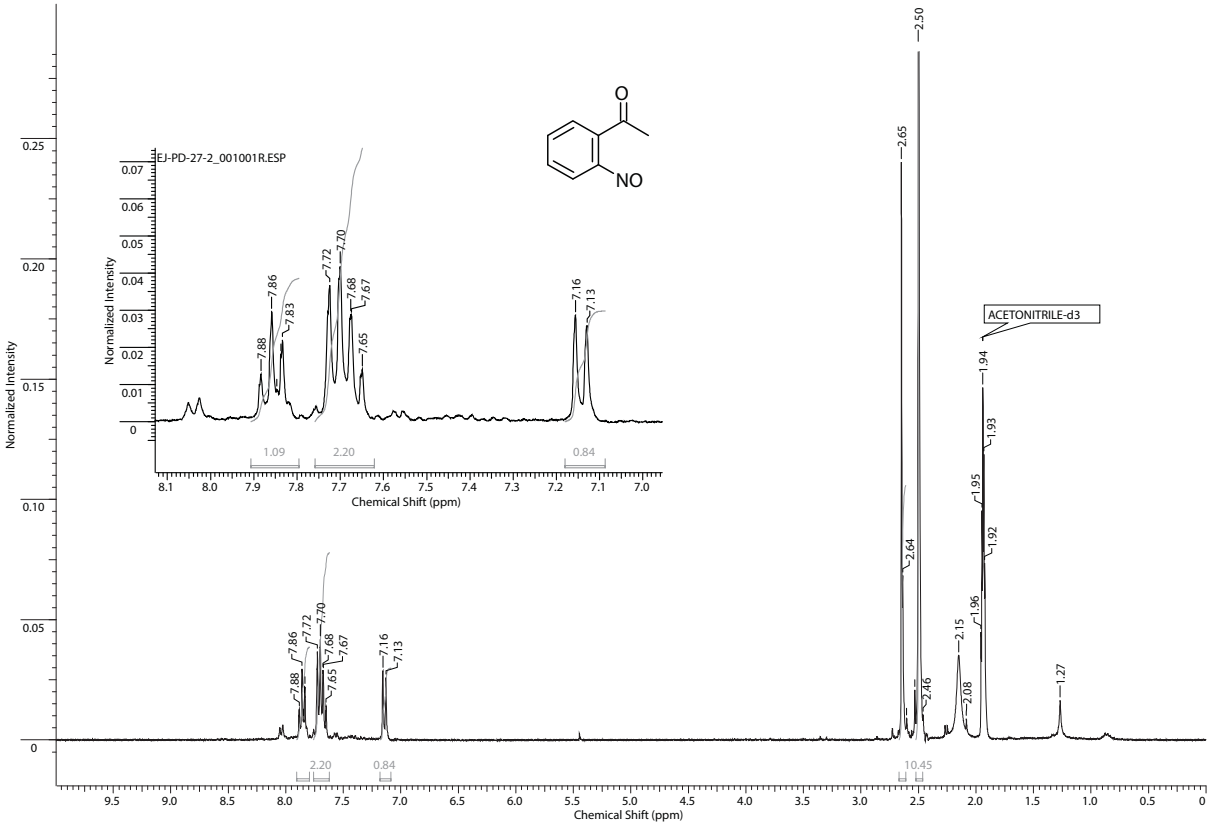


Figure S2. ^{1}H spectrum of 1-(2-nitrosophenyl)ethan-1-one in CD_3CN.

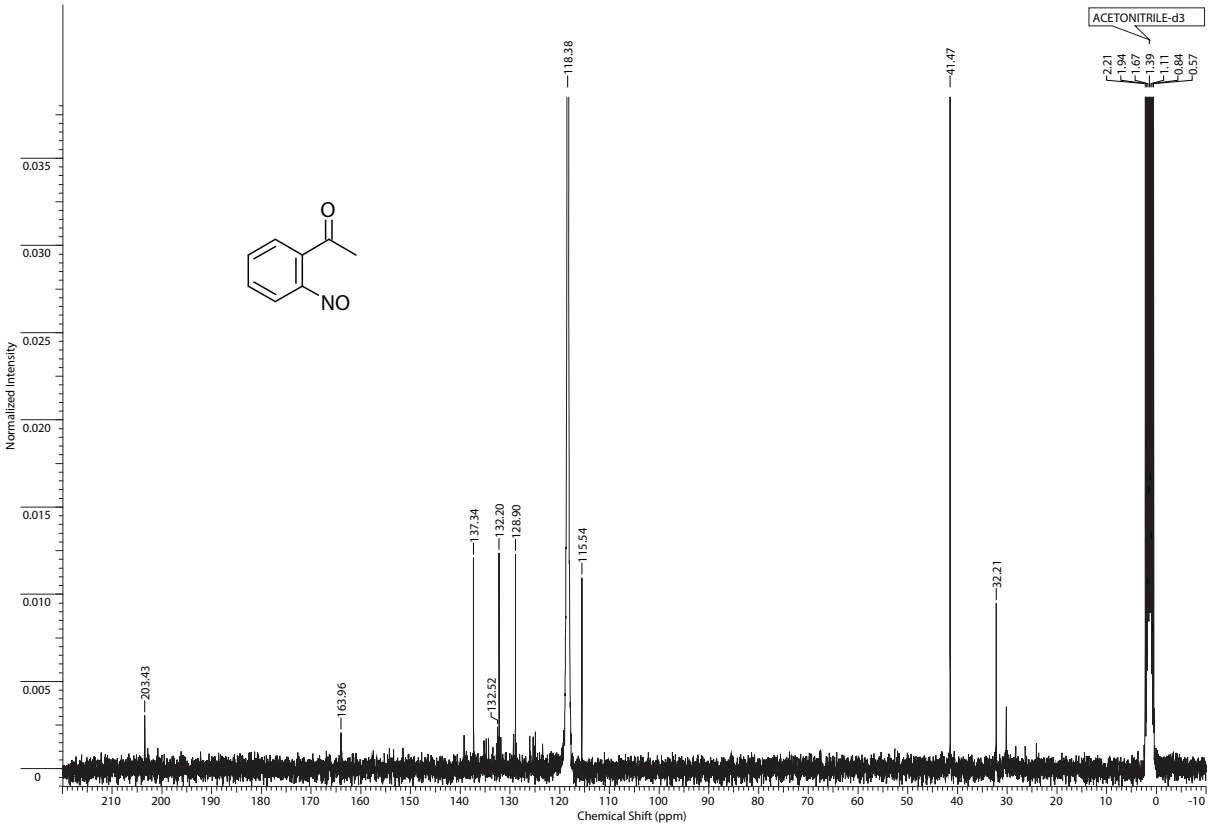


Figure S3. ^{13}C spectrum of 1-(2-nitrosophenyl)ethan-1-one in CD_3CN.

Photolysis Quantum Yield of the Caged Phosphate

Photolysis

3 mL of a 6.9 mM solution of caged phosphate in the respective solvent was put in a standard absorbance quartz cell (1 cm path length) and irradiated in a LUMOS 43A photoreactor at 360 nm for a given time. Several irradiations for different times were performed. The solvents were pure water, HEPES buffer (0.1 M, pH 7.2) or PBS buffer (0.1 M, pH 7.2), as indicated below. The solutions analyzed by NMR additionally contained 10% of D_2O and approx. 1 equiv. of ethanol (internal standard) for spectroscopic purposes. The number of converted moles per time was measured by different HPLC- and NMR-based techniques (see below). The obtained correlation between the number of converted moles and the time was divided by the previously measured photon flow ($5.44 \cdot 10^{-7}$ E/min) to give the quantum yield for the photolysis at 360 nm (Φ_{360}).

HPLC Analyses

1-(2-Nitrosophenyl)ethan-1-one HPLC Calibration Curve

To a solution of 1-(2-nitrosophenyl)ethan-1-one in CD_3CN, a known amount of DMSO was added. The mixture was analyzed by ^1H NMR and the DMSO signal used as internal standard to determine the exact concentration of 1-(2-nitrosophenyl)ethan-1-one. The concentration of 1-(2-nitrosophenyl)ethan-1-one was adjusted to 1 mM with acetonitrile, and the other three calibration solutions were prepared from it by serial dilution. The calibration solutions were analyzed by HPLC and the areas of the peaks were plotted as a function of the concentration to establish a calibration curve (Figure S4).

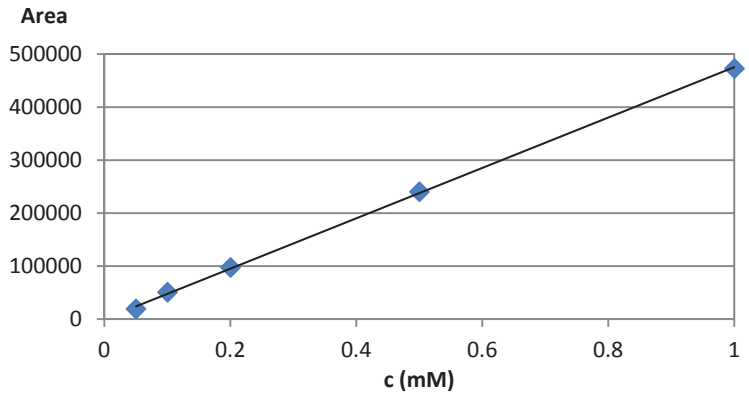


Figure S4. Calibration curve for the area in HPLC chromatograms for 1-(2-nitrosophenyl)ethane-1-one, relative to its concentration. Function of the fit: area = 475304 c. $R^2 = 0.99$.

Sample Analyses

The irradiated caged phosphate solutions in PBS or pure water were transferred into HPLC vials, diluted by a factor of two with acetonitrile and analyzed by HPLC. For every HPLC analysis the conversion was determined from the UV absorbance area of the 1-(2-nitrosophenyl)-ethan-1-one, using the calibration curve (see above) to determine the concentration of 1-(2-nitrosophenyl)-ethan-1-one in every sample (Method 1 in the article) or by considering the area of the sample analyzed after total photolysis as corresponding to 100% of conversion (Method 2 in the article). The conversion, calculated with the two methods, for the two series of measurements was plotted as a function of the time.

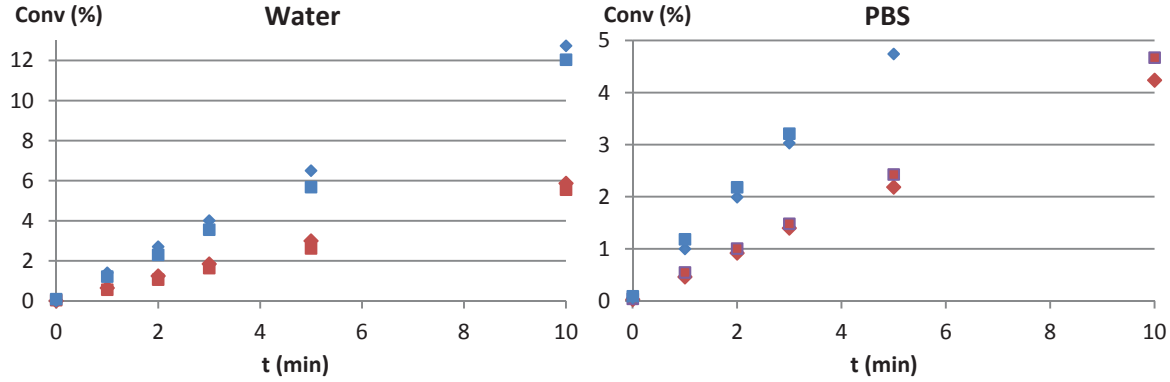


Figure S5. Conversion of caged phosphate to 1-(2-nitrosophenyl)-ethan-1-one in water (left) and PBS (right, pH=7.2). Both graphs show data points of two series of experiments (series 1: diamonds; series 2: squares), and upon analysis with Method 1 (red; slower conversion rate) and Method 2(blue; faster conversion rate).

NMR Analyses

The irradiated solutions in water or HEPES buffer (with 10% D_2O and approx. 1 equiv. ethanol) were transferred into NMR tubes and analyzed by ^{1}H NMR and ^{31}P NMR. For every sample analyzed by ^{1}H NMR, the conversion was determined by comparing the signal from the protons of the methyl group of the caged phosphate at δ=1.53 ppm with the methyl group of the internal standard (ethanol) at δ=1.17 ppm. For the ^{31}P NMR analyses, the conversion was determined by dividing the integral of the ^{31}P signal of the free phosphate at δ=19.24 ppm by the sum of the signals from the free and the caged compound at δ=19.51 ppm. The conversion was plotted as a function of the time.

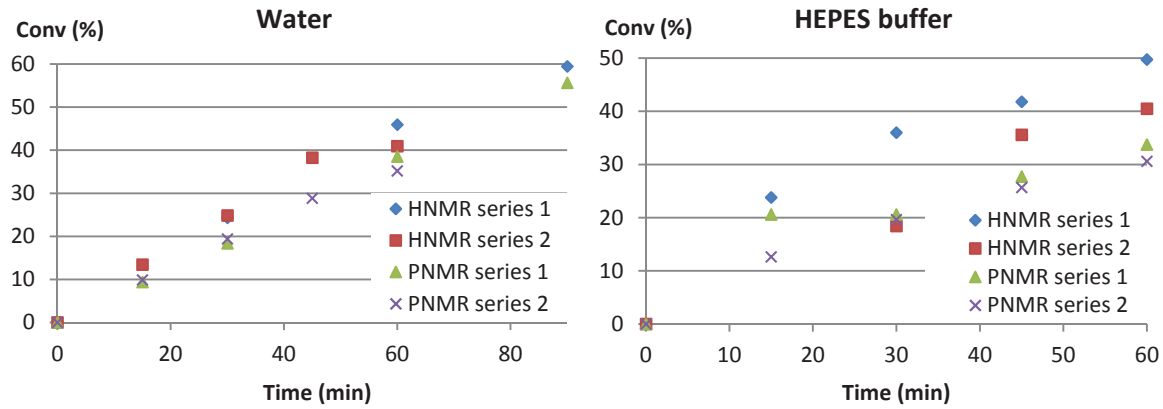


Figure S6. Conversion of caged phosphate to 1-(2-nitrosophenyl)-ethan-1-one in water (left) and HEPES buffer (right, pH=7.2). Both graphs show data points from two series of experiments (series 1 and series 2), and upon analysis with ^{1}H or ^{31}P NMR (HNMR or PNMR), as indicated.

Part 2: Data Acquired by P. Anstaett

General Methods

Analytical photo-irradiations were performed with a LUMOS 43A photoreactor (Atlas Photonics). A Perkin Elmer Lambda 40 UV/Vis spectrometer was employed for recording the UV/Vis spectra. UPLC was performed on a Waters Acquity system equipped with a PDA detector and an auto-sampler using an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm). Method details: 0.6 mL/min, H_2O + 0.1% formic acid : acetonitrile = 0 min 95:5, 0.25 min 95:5, 1.5 min 0:100, 2.0 min 0:100. Plotting and fitting of data was performed with OriginLab OriginPro 9.1.

Azobenzene Actinometry

General Remarks

Azobenzene actinometry was performed with a dilute solution of *trans*-azobenzene in methanol as described in our (P.A., A.L., G.G.) initial communication.[3]

Photolysis

trans-Azobenzene was dissolved in methanol in a low absorbing concentration (0.18 mM, OD(360 nm)=0.19). 3 mL of this solution were put in a standard absorbance quartz cell (1 cm path length) and irradiated in a LUMOS 43A photoreactor equipped with LED lamps at 360 nm (bandwidth < 10 nm) for given times.

Analyses

A UV/Vis absorbance spectrum was recorded after certain irradiation time intervals. The decreasing of the optical density at 355 nm was monitored and used to determine the number of moles photolyzed after each irradiation. The absorbance of the product, *cis*-azobenzene, being much lower than the absorbance of *trans*-azobenzene at 355 nm, the conversion can be determined for at least the first 20% of conversion by dividing the measured optical density by the optical density of the starting solution (Figure S7).

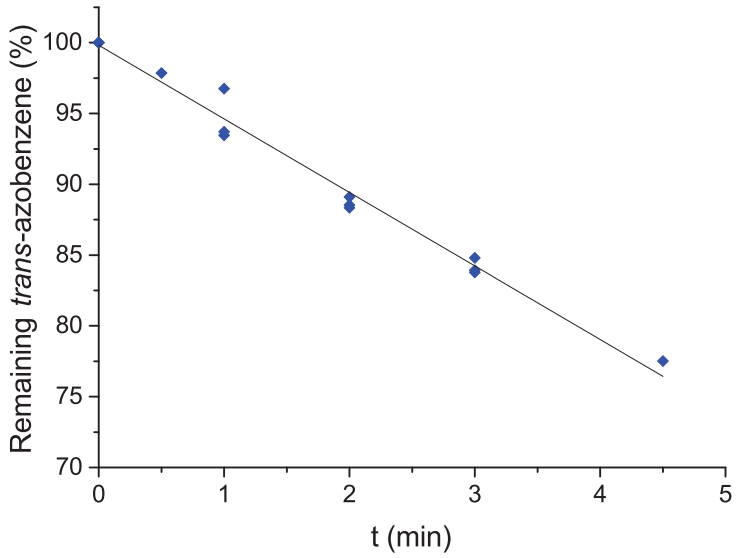


Figure S7. Photo-conversion of *trans*-azobenzene to *cis*-azobenzene, shown as a plot of remaining *trans*-azobenzene (in %) as a function of time. Function of the fit: *trans*-azobenzene = 99.81657 - 5.19469 t. R^2 = 0.99.

Photolysis Quantum Yield of the Caged Phosphate

The measurements were performed as described in our (P.A., A.L., G.G.) initial communication.[3]

3 mL of a dilute solution of caged phosphate in PBS (pH=7.2, OD(360 nm)=0.19, 0.48 mM) was put in a standard absorbance quartz cell (1 cm path length) and irradiated in a LUMOS 43A photoreactor at 360 nm for a given time. Three separately prepared solutions were irradiated for different times. 1 mL of the solutions was transferred to HPLC vials, frozen on dry ice, and kept this way until directly before measurement with the UPLC. The amount of remaining caged phosphate was determined by integrating the corresponding peak in the UV chromatogram (t_R = 0.87 min), and setting it into relation with the peak recorded for a sample without irradiation. The resultant amount of remaining caged phosphate was plotted as a function of irradiation time (Figure S8).

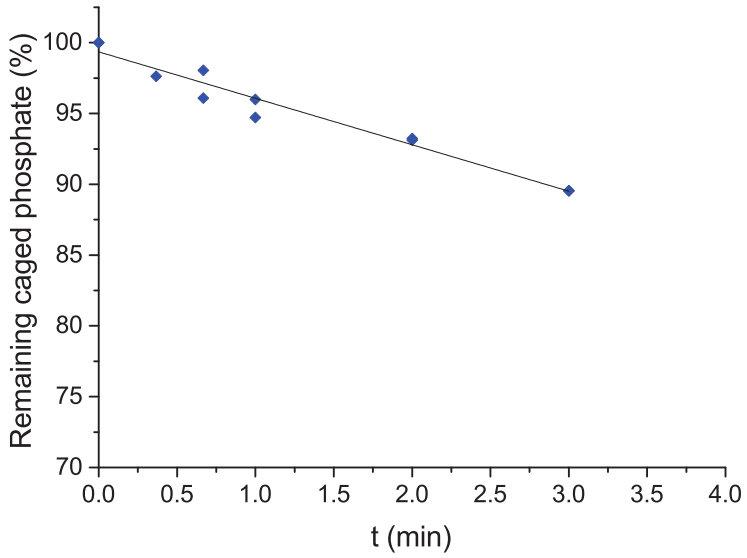


Figure S8. Photouncaging of caged phosphate, shown as a plot of remaining caged phosphate (in %) as a function of time. Function of the fit: caged phosphate = 99.35249 – 3.28013 t. R^2 = 0.97.

References

[1] A. Defoin, R. Defoin-Straatmann, K. Hildenbrand, E. Bittersmann, D. Kreft, H. J. Kuhn, *J. Photochem.* **1986**, *33*, 237–255.

[2] K. Fendler, E. Grell, M. Haubs, E. Bamberg, *EMBO J.* **1985**, *4*, 3079–3085.

[3] P. Anstaett, A. Leonidova, G. Gasser, *ChemPhysChem* **2014**, DOI: 10.1002/cphc.201402547.