Supplementary Material (ESI) for Photochemical & Photobiological Sciences

Studies of decarboxylation in photolysis of α-carboxy-2-nitrobenzyl (CNB) caged compounds

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Electronic Supplementary Information

Data are shown for photolysis spectra of NPE-caged monomethyl phosphate (Figure S1; page 2) and of 2-nitrobenzyl monomethyl phosphate (Figure S2, page 3). Also shown are isotope effect spectra for photolysis of the two isotopomers of **7** to show the an isotopically-sensitive carboxylate group that disappears on the same time scale as the slow phase of CO_2 release (Figure S3, page 4). Details of the synthesis of the precursor compounds for synthesis of **9** are also given (pages 5-6), together with the method used for quantitative measurement of CO_2 formation in the IR spectroscopic cells (pages 6-9).

Structures used only in the ESI are denoted by letters (**A, B** etc), while those that also appear in the main text are denoted by the numbers used therein. Numbered references refer to the text of the published paper.

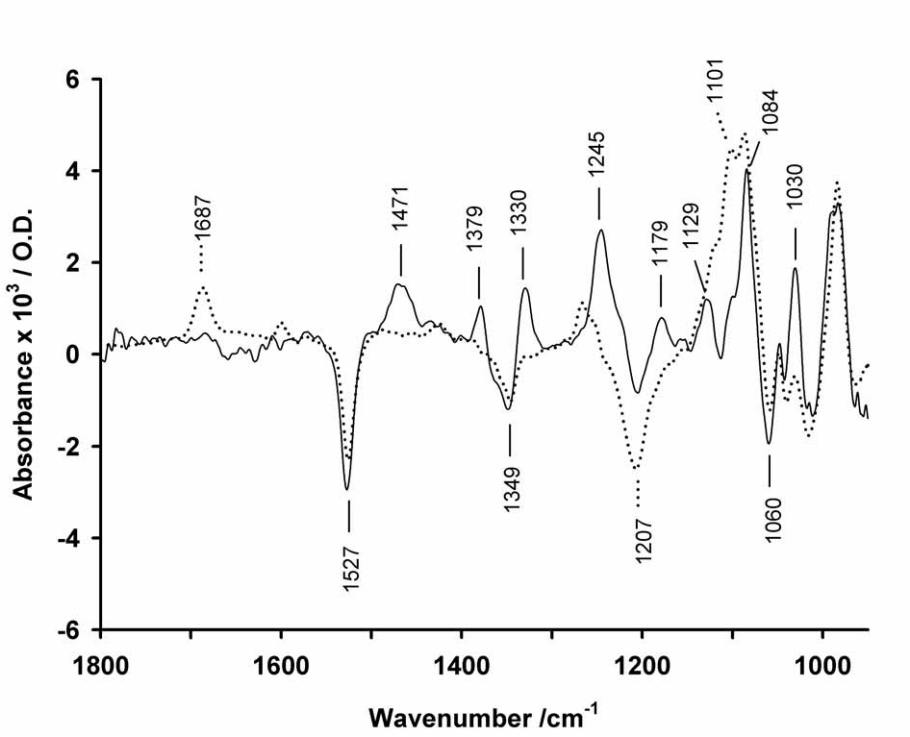
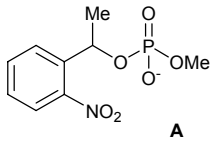


Fig. S1 Infrared difference spectra for photolysis of NPE-caged monomethyl phosphate **A**, recorded at 1 °C for an H_2O solution of the compound (100 mM) in 200 mM MOPS, pH 7.5. The time intervals are (—) 1-58 ms and (·····) 3.42-30.8 s after the photolysis flash. Negative bands in both spectra arise from functional groups in NPE-caged monomethyl phosphate **A** that disappear upon photolysis. Positive bands in the first spectrum are characteristic of the *aci*-nitro intermediate and in the second (final) spectrum of the products, 2-nitrosoacetophenone and monomethyl phosphate. The latter is characterised by the broad absorption in the region of 1100 cm^{-1}. The fine structure of this band arises from underlying negative bands in the difference spectrum.



A

NPE-caged monomethyl phosphate

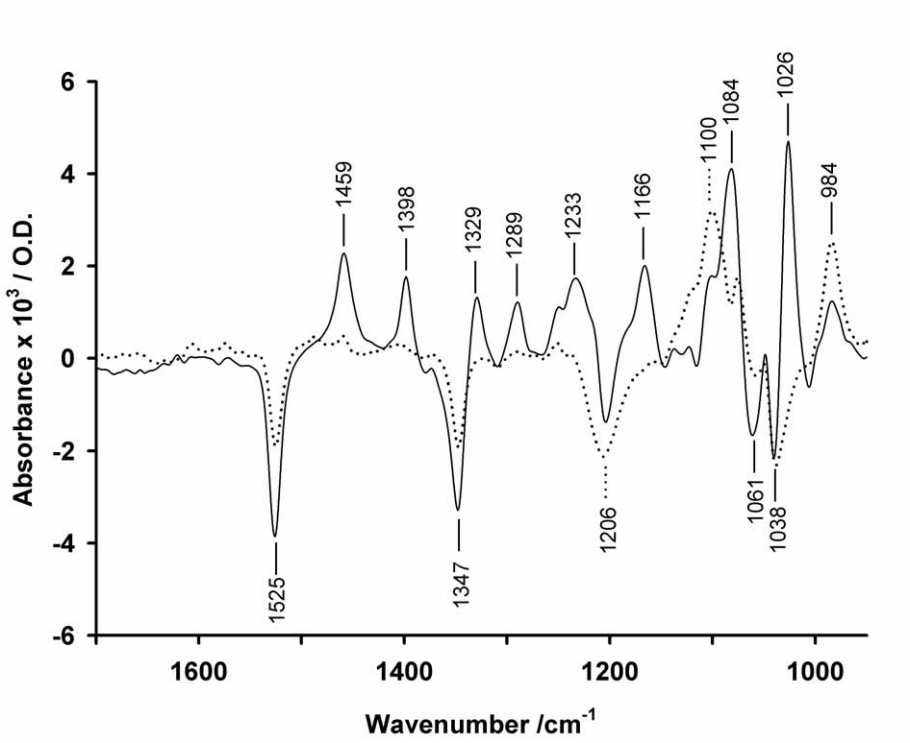
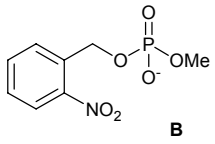


Fig. S2 Infrared difference spectra for photolysis of 2-nitrobenzyl monomethyl phosphate **B**, recorded at 1 °C for an H_2O solution of the compound (90 mM) in 200 mM MOPS, pH 7.0. The time intervals are (—) 1-899 ms and (·····) 11-21 s after the photolysis flash.



B

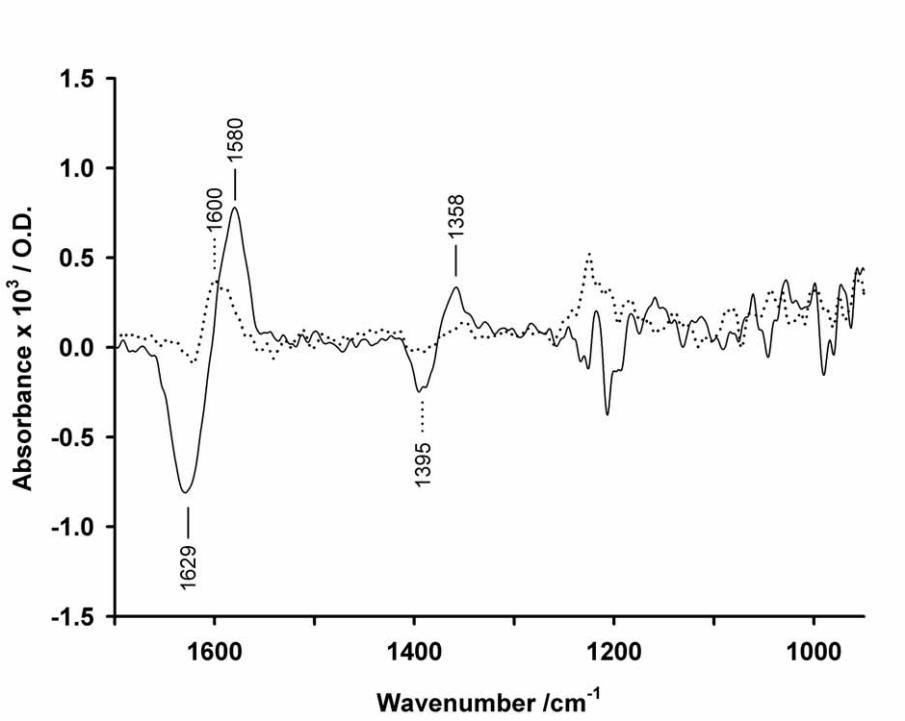
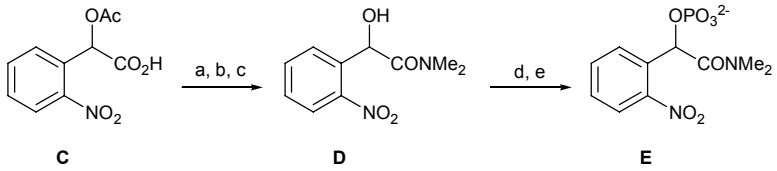


Fig. S3 Isotope effect spectra for photolysis of CNB-caged monomethyl phosphate **7** to show changes in the carbonyl vibrations that accompany the slow phase of CO_2 release, recorded in 200 mM MOPS, pH 7.0 ± 100 mM DTT. The spectrum without DTT is shown as the full line (—) and that with DTT is shown by the dotted line (·····). The spectra are obtained for each set of conditions (i.e. ± DTT) by first subtracting for each isotopomer the averaged spectra recorded in the time interval 1-670 ms from the averaged spectra recorded in the time interval 0.68-3.4 s. The time interval between the spectra covers the period of slow CO_2 release. The resulting double difference spectrum for the ^{13}C isotopomer was then subtracted from that for the ^{12}C isotopomer. The two minimum/maximum features indicate isotope shifts of two negative bands in the double difference spectra, assigned to antisymmetric and symmetric carboxylate stretching vibrations. Note that the spectrum in the presence of DTT shows no isotopically-sensitive band for a carboxylate that disappears on the slow time scale, consistent with the abolition of the slow phase of CO_2 release in the presence of DTT. The band in the isotope effect spectrum with DTT near 1600 cm^{-1} is due to a positive band in the double difference spectrum with the ^{12}C compound that is not observed for the ^{13}C isotopomer. In contrast, the band at 1580 cm^{-1} in the isotope effect spectrum without DTT is due to a negative band in the double difference spectrum with the ^{13}C isotopomer. Thus it is clear that both bands are unrelated. Note also that the amplitude

of the spectra is much smaller than the spectra of photolysis of [^{13}C]**7** (Figure 3 of the main text), consistent with the slow phase of CO_2 release being a minor pathway.

Details for synthesis of precursors of (9).



C **D** **E**

Scheme S1 Reagents: (a), *N*-hydroxysuccinimide–DCC; (b) Me_2NH; (c) Cs_2CO_3–MeOH; (d) $Et_2NP(OBu^t)_2$–1*H*-tetrazole; (e) MCPBA.

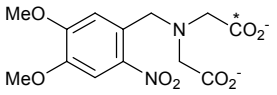
***N,N*-Dimethyl-2-hydroxy-2-(2-nitrophenyl)acetamide, D.** *N*-Hydroxy-succinimide (0.69 g, 6.0 mmol) and dicyclohexylcarbodiimide (1.13 g, 5.5 mmol) were added under nitrogen to a solution of *O*-acetyl 2-nitromandelic acid[16] **C** (1.195 g, 5.0 mmol) in acetonitrile (50 mL) and the solution was stirred for 3 h at room temperature. Dimethylamine (40% aq. solution; 1.27 mL, 10.0 mmol) was added and stirring was continued overnight. The mixture was evaporated to ~5 mL, diluted with EtOAc, washed with saturated $NaHCO_3$, H_2O and brine, dried, evaporated and purified by flash chromatography [EtOAc–light petroleum (3:2). The recovered material (1.2 g, 90%) had δ_H (90 MHz, $CDCl_3$) 8.09 (d, 1H, *J* 7.0, Ar–H), 7.42-7.71 (m, 3H, Ar-H), 7.12 (s, 1H, CH), 3.02 (s, 3H, NMe), 3.10 (s, 3H, NMe), 2.20 (s, 3H, Me). A solution of this material (1.15 g, 4.32 mmol) in methanol (13 mL) was mixed with a solution of cesium carbonate (0.07 g, 0.216 mmol) in methanol (2.5 mL) and the mixture was stirred for 0.5 hr at room temperature. The solution was diluted with EtOAc, washed with a 1:1 mixture of brine and saturated $NaHCO_3$, dried, evaporated and crystallised from CH_2Cl_2–light petroleum to give the hydroxyamide **D** (0.687 g, 71%), m.p. 134.5-136 °C; UV λ_{max} (EtOH)/ nm (ε/$M^{-1}cm^{-1}$) 264 (6000); λ_{max} [EtOH-25 mM Na phosphate, pH 7 (1:9 v/v)]/ nm (ε/$M^{-1}cm^{-1}$) 270 (5700); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (dd, *J* 7.5, 1.8, 1H,Ar-H), 7.31-7.62 (m, 3H, Ar-H), 6.00 (d, $J_{H,OH}$ 4.83, 1H, CH), 4.63 (d, 1H, OH), 2.85 (s, 3H, NMe), 3.07 (s, 3H, NMe). Anal. calcd for $C_{10}H_{12}N_2O_4$: C, 53.57; H, 5.39, N, 12.49; found: C, 53.49; H, 5.40; N, 12.46.

α-Dimethylaminocarbonyl-2-nitrobenzyl phosphate, E. Di-*tert*-butyl *N,N*-diethylphosphoramidite (0.54 g, 2.0 mmol) and 1*H*-tetrazole (0.18 g, 2.5 mmol) were added to a solution of the alcohol **D** (0.224 g, 1.0 mmol) in dry THF (8 mL) and stirred under nitrogen at room temperature for 4 h. The solution was cooled in ice and *m*-chloroperbenzoic acid (55%; 0.72 g, 2.29 mmol) in CH_2Cl_2 (10 mL) was added. The solution was stirred for 1 h and diluted with EtOAc, washed with 10% sodium metabisulfite, 1 M HCl, saturated $NaHCO_3$ and brine, dried, evaporated and purified by flash chromatography [EtOAc–chloroform (3:2)] to give an oil (0.32 g) that was stirred with trifluoroacetic acid (4 mL) for 1 h at room temperature. The solution was evaporated to a gum, dissolved in water (75 mL) and adjusted to pH 7.2 with 1 M NaOH. The aqueous solution was extracted with $CHCl_3$ and analysed by anion-exchange HPLC [mobile phase 25 mM sodium phosphate, pH 6.0–MeOH (100:13)], t_R 3.7 min, with minor components at 1.1 and 1.6 min. The former of these impurities had a retention time identical to that of the starting hydroxyl amide alcohol **D**. The solution was freeze dried for 8 h to remove $CHCl_3$, diluted with water to a conductivity of 2 mS cm^{-1} (700 mL) and fractionated by ion-exchange chromatography using a linear gradient formed from 10 and 250 mM TEAB (each 1000 mL). Analysis of fractions by anion-exchange HPLC (as above) showed that there was still some contamination by the minor impurities noted above. After the usual vacuum evaporation to remove the volatile buffer, a second round of anion-exchange chromatography (as above) using a linear gradient formed from 10 and 250 mM TEAB (each 1000 mL), combination of appropriate, clean fractions and evaporation of the volatile buffer gave the pure phosphate **E** as its triethylammonium salt (260 μmol, based on ε_{270} 5700 $M^{-1}cm^{-1}$); ^1H NMR (D_2O, 500MHz) δ 8.21 (dd, *J* 8.2, 1.3, 1H, 3-H), 7.99 (dd, *J* 7.7, 1H, 6-H), 7.87 (dt, *J* 7.5, 1.3, 1H, Ar-H), 7.63 (m, 1H, Ar-H), 6.60 (d, $J_{H,P}$ 9.7, 1H, CH), 3.01 (s, 3H, NMe), 3.42 (s, 3H, NMe); LR-MS: calcd for $(C_{10}H_{11}N_2O_7P + H)^-$: 303; found: 303.

Details of quantitative CO_2 measurements

Treatment of spectra. The flash photolysis experiment was performed two times on each of two to three samples of 4-nitrophenylacetate, used as CO_2 standard, [^{13}C]CNB glycine **8**, and CNB monomethyl phosphate **7**. In addition to the

experiments described in this work, spectra with [carboxy-$^{13}C_1$]*N*-(3,4-dimethoxy-6-nitrobenzyl)iminodiacetate **F**, available from previous work,[19] were used to calibrate the relative areas of the $^{12}CO_2$ and $^{13}CO_2$ bands. The relative areas reflect the relative extinction coefficients for these two bands. **F** releases $^{12}CO_2$ and $^{13}CO_2$ in equal amounts upon photolysis**.** The time-resolved spectra of different samples were averaged for each flash and spectra averaged in the following time intervals after the photolysis flash: 0.7-3.4 s (4-nitrophenylacetate and all bands except the $^{12}CO_2$ band of [^{13}C]CNB glycine **8** in MOPS) and 3.4-6.2 s (CNB monomethyl phosphate **7**, [^{13}C]CNB glycine **8** in phosphate and $^{12}CO_2$ band of [^{13}C]CNB glycine **8** in MOPS). During the evaluated time intervals, the CO_2 bands were at their maximum intensity.



F

Structure of compound **F**. The asterisk shows the position of the ^{13}C label.

Normalisation to an equal path length. The first step in quantifying the released CO_2 was to normalise all spectra to an equal optical path length. This was done by normalising all spectra to an equal water absorption using the water bands at 2141 and 1646 cm^{-1}.

Integration of CO_2 bands and correction of $^{13}CO_2$ band area. The $^{12}CO_2$ band area ^{12}A was obtained by integrating between 2346 and 2340 cm^{-1} relative to a baseline drawn between the average absorption between 2410 and 2380 cm^{-1} and between 2220 and 2120 cm^{-1}. Similarly, the $^{13}CO_2$ band was integrated between 2280 and 2274 cm^{-1}, using the same spectral ranges for the baseline points, to obtain the CO_2 band area ^{13}A.

Experiments with **F** revealed that the absorption coefficient of the $^{13}CO_2$ band at 2277 cm^{-1} is slightly smaller than that of the $^{12}CO_2$ band at 2343 cm^{-1}. In order to compare the two CO_2 band areas, one of them had therefore to be corrected and we chose the $^{13}CO_2$ band area, ^{13}A. We determined the correction factor from a spectrum obtained in the first 3.4 s after the photolysis flash averaged from experiments with three identical samples. The correction factor by which ^{13}A has to be multiplied to obtain the corrected band area ^{13c}A was 1.113. This corrected $^{13}CO_2$ band area, ^{13c}A, is

thus equal to a $^{12}CO_2$ band area, ^{12}A, if that originates from the same CO_2 concentration.

Determination of the concentration of photolysed material. For each compound, several bands were used to determine the photolysis yield. The band areas obtained with flash n (A_n) are proportional to the concentration of caged compound before the flash, c_n. The ratio A_{n+1}/A_n is thus equal to c_{n+1}/c_n, i.e. the concentration before flash n+1 divided by the concentration before flash n. Since $c_{n+1} = c_n - \Delta c_n$ where Δc_n is the concentration of caged compound photolysed in flash n, the experimentally determined ratio c_{n+1}/c_n is equal to $1 - \Delta c_n/c_n$ where $\Delta c_n/c_n$ is the photolysis yield. From the two subsequent photolysis experiments done on one sample, the A_2/A_1 ratios were calculated for the different bands and averaged. This gave the photolysis yield $1 - A_2/A_1$. The concentration of photolysed material was then obtained from the photolysis yield and the starting concentration of the photosensitive compound before the first flash.

Relating CO_2 band area to CO_2 concentration. It is assumed that photolysis of 4-nitrophenylacetate produces CO_2 with 100% efficiency since, based on product identification studies, this is the only pathway for consumption of the starting compound.[35] This compound was therefore used to relate the integrated CO_2 band area to the concentration of CO_2. From the known concentration of photolysed material (determined as described above), and the CO_2 band area, the band area for 1 mM $^{12}CO_2$ was determined to be 6.681×10^{-3} mM^{-1} cm^{-1} ± 20% at our standard path length of 5-6 μm, as estimated from the water absorption.

The CO_2 concentration(s) formed from **7** and **8** was determined from the CO_2 band area obtained for the first flash for each compound and the calibration described above. The CO_2 concentration was then related to the concentration of photolysed material. 35 ± 14% (phosphate buffer) and 57 ± 14% (MOPS) of the photolysed [^{13}C]CNB monomethyl phosphate **7** molecules produce a CO_2 molecule. A comparison of spectra normalised to the $\nu_{as}(NO_2)$ band near 1530 cm^{-1} confirmed for **7** that CO_2 production in MOPS buffer was~140% of that in phosphate buffer.

$^{12}CO_2$ production upon product release from [^{13}C]CNB glycine **8** was 33 ± 14% in phosphate buffer and 24 ± 11% in MOPS buffer and $^{13}CO_2$ production from decarboxylation of the α-carboxylate was 35 ± 15% in phosphate and 48 ± 21% in

MOPS. Comparison of $^{12}CO_2$ and $^{13}CO_2$ bands confirmed that $^{12}CO_2$ and $^{13}CO_2$ were produced in nearly equal amount in phosphate buffer but that in MOPS buffer $^{13}CO_2$ production was twice that of $^{12}CO_2$. Comparison of spectra normalised to the $\nu_{as}(NO_2)$ band near 1528 cm^{-1} confirmed further that $^{12}CO_2$ production in phosphate was ~140% of that in MOPS and $^{13}CO_2$ production in phosphate was ~70% of that in MOPS.

In these measurements, the large error comes from two sources: the calibration has a standard deviation of about 20% as has the photolysis yield. If the relative CO_2 production between two compounds or two conditions is compared (i.e. the CO_2 production of A is 1.4 times larger than that of B), the only source of error is the photolysis yield and the numbers are accurate to within 20%. They are supported by the direct comparison of spectra as discussed above. For the absolute amount of CO_2 errors in photolysis yield and calibration contribute and the numbers have a total relative error of 40% (i.e. 100 ± 40%, 50 ± 20%, 25 ± 10% etc.). Thus the above quantification of the produced CO_2 yields reliable relative amounts although the absolute error is considerable.